

UNIVERSITÉ DU QUÉBEC À MONTRÉAL

MODULATION DE L'AUTO-ASSEMBLAGE DE PEPTIDES EN
NANOSTRUCTURES AMYLOÏDES À DES FINS D'APPLICATIONS
BIOMÉDICALES

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DÉDICACE

À l'âme de mon cher beau père

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LISTE DES ABRÉVIATIONS

AFM	Microscopie à force atomique
ANS	Acide 8-anilino-1-naphthalènesulfonique
ARN	Acide ribonucléique
A β	Amyloïde- β
CAC	Concentration critique d'agrégation
CD	Spectroscopie de dichroïsme circulaire
CGRP	Peptides associés aux gènes de la calcitonine
CPEB	Isoforme spécifique aux neurones du protéine de liaison aux éléments de polyadénylation cytoplasmique (Prion).
CR	Rouge de Congo
Cryo-EM	Microscopie électronique cryogénique
CT	calcitonine
DAPI	4',6-Diamidino-2-phenylindole dihydrochloride
DIEA	Diisopropylethylamine
DC	Cellule dendritique
DLS	Diffusion dynamique de la lumière (Dynamic light scattering)
DMEM	Milieu de culture Dulbecco's modified Eagle
DMF	<i>N,N</i> -diméthylformamide
DMSO	Diméthylsulfoxyde
dsDNA	double stranded DNA
FBS	Sérum de veau fœtal
FlAsH	Fluorogenic probe fluorescein arsenical hairpin
FTIR	Spectroscopie infrarouge à transformée de Fourier
GAGs	Glycosaminoglycanes
HCTU	<i>o</i> -(1H-6-Chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluoro-phosphate
HFIP	Hexafluoroisopropanol

IAPP	Islet amyloid polypeptide
IPECs	polyelectrolyte complexes
LC-ESI-TOF	Chromatographie en phase liquide couplée à la spectroscopie de masse avec source electrospray et analyseur en temps de vol
M2e	Ectodomaine de la protéine M2 du virus influenza
MeCN	Acétonitrile
MeOH	Méthanol
PDB	Protein data bank
PICUP	Photochemical-Induced Cross-Linking
PrP	Protéine Prion
RAMP	Receptor activity-modifying protein
RMN	Résonance magnétique nucléaire
ROS	Espèces oxygénées réactives
RP-HPLC	Chromatographie liquide à haute performance en phase inverse
RPMI	Milieu de culture Roswell Park Memorial Institute
SDS-PAGE	Électrophorèse sur gel de polyacrylamide en présence de Sérum de veau foetal
SPPS	Synthèse peptidique sur support solide
ssRMN	Résonance magnétique nucléaire à l'état solide
T2D	Diabète de type 2
TCEP	Tris(2-carboxyethyl)phosphine
TEM	Microscopie électronique à transmission
TFA	Acide trifluoroacétique
THF	Tétrahydrofurane
ThT	Thioflavine T
TLR	Récepteur de type Toll
XRD	Diffraction des rayons X
α -syn	α -synucléine

RÉSUMÉ

Les fibres amyloïdes sont des nanostructures biologiques caractérisées par une structure quaternaire organisée en feuillets- β -croisés et impliquées dans de nombreux processus physiologiques et diverses pathologies. En raison de leurs propriétés mécaniques, chimiques et biologiques intéressantes, les assemblages amyloïdes sont de plus en plus exploités en tant que matériaux bio-inspirés pour diverses applications en biotechnologie et nanomédecine. Le fort potentiel d'exploitation de ces nanostructures protéiques encourage à poursuivre les études afin d'élucider les bases fondamentales du processus d'autoassemblage hautement complexe qui conduit à une diversité morphologique des amyloïdes. Néanmoins, sous contexte pathologique, le processus d'autoassemblage peut générer des protéospèces transitoires causant diverses pathologies dégénératives, et ceci demeure un grand défi à relever pour la conception rationnelle de nouveaux agents thérapeutiques efficaces. C'est dans ce contexte que s'inscrit l'objectif global de ce projet de thèse de doctorat visant à explorer les contributions structurales et conformationnelles guidant le processus d'autoassemblage et à concevoir de nouvelles nanostructures pour des applications ciblées. Premièrement, inspiré de la directionnalité et de la fonctionnalisation des assemblages amyloïdes, nous avons évalué la capacité de séquences amyloïdes à guider l'autoassemblage de systèmes π -conjugués en nanostructures fibrillaires semi-conductrices et biocompatibles. En exploitant la séquence amyloïdogénique 20-29 de l'islet amyloid polypeptide (I_{10}), nous avons préparé via un contrôle stœchiométrique, deux peptides amyloïdes π -conjugués, *i.e.* l'un symétrique et l'autre asymétrique, capables de s'autoassembler en solution aqueuse sous forme de nanofilaments longs et linéaires caractérisés par une organisation quaternaire riche en feuillets- β -croisés. Avec une signature de semi-conducteurs et une fluorescence modérée, ces nanofilaments robustes dont la formation était guidée par la séquence peptidique se sont révélés biocompatibles et optoélectroniques. Deuxièmement, nous avons exploité les paramètres physico-chimiques et structuraux modulant la morphologie et la taille de nanostructures amyloïdes afin de comprendre les bases moléculaires de leurs propriétés immunomodulatrices. En modifiant les extrémités *N*- et *C*-terminales du peptide I_{10} , nous avons démontré qu'il existe une relation étroite entre ces modifications et la morphologie / taille des assemblages amyloïdes résultants. Ainsi, cette modulation fine de l'autoassemblage a permis de révéler que l'organisation supramoléculaire est essentielle pour engager la signalisation cellulaire des TLR2/TLR6. En outre, l'activation des récepteurs de l'immunité innée TLR2/TLR6 était associée à la densité de charges positives à la surface des nanostructures peptidiques. Troisièmement, considérant que le processus d'assemblage peut générer des espèces protéotoxiques étant à la source de plusieurs pathologies, nous avons examiné les effets de nanostructures d'ADN sur le processus d'autoassemblage de peptides amyloïdes et sur

les propriétés cytotoxiques des diverses structures quaternaires engendrées. L'utilisation de différentes nanostructures d'ADN double brin, linéaire (1D), triangulaire (2D) et tétraédrique (3D), a permis d'observer que l'ADN peut interagir via son squelette phosphate chargé négativement avec les charges positives de l'IAPP, les piégeant ainsi sous la forme d'un complexe interpolyélectrolytique sphérique dans une conformation désordonnée non amyloïdogène et non toxique. En conclusion, les résultats de cette thèse mettent en évidence les contributions structurales et fonctionnelles guidant le processus d'autoassemblage des amyloïdes en contexte physiologique ou pathologique, et ouvrent de nouvelles perspectives vers la conception rationnelle de nanostructures bio-inspirées pour des applications ciblées en théranostique, prophylaxie et opto-bioélectronique.

Mots clés : Fibres amyloïdes, Autoassemblage, Peptides, Systèmes π -conjugués, Islet amyloid polypeptide, Récepteur TLR2/TLR6, Nanostructures d'ADN

CHAPITRE I

INTRODUCTION

1.1 Les assemblages biologiques comme source d'inspiration aux nanomatériaux

Les organismes vivants sont capables d'élaborer des nanostructures complexes et élégantes affichant des fonctions bien spécifiques en utilisant des processus d'autoassemblage *bottom-up*. Reproduire le concept de base des organismes vivants est ainsi une démarche qui suscite un intérêt croissant de la communauté scientifique afin d'élaborer de nouveaux matériaux bio-inspirés ou biomimétiques. La fibroïne de soie d'araignée est l'un des exemples de biomimétisme phares. Avec ses propriétés mécaniques, d'endurance et structurelles exceptionnelles, cette protéine fibreuse émerge dans diverses applications telles que l'ingénierie tissulaire, les applications biomédicales, ainsi que les dispositifs optiques et électriques (Rahaiee *et al.*, 2019). En outre, la chitine, issue principalement de la carapace des crustacés, est un polysaccharide qui forme des matériaux multi-échelles allant de petits oligomères, de nanocristaux en forme de bâtonnets, de nanofibres plus longues à des assemblages hiérarchiques de nanofibres. En raison de sa biocompatibilité et biodégradabilité, ce biopolymère et ses dérivés trouvent de nombreuses applications biomédicales et environnementales (Lee *et al.*, 2023). De même, les nanofibres de collagène jouent un rôle dominant dans le maintien de l'intégrité biologique et structurale de divers tissus et organes, notamment les os, la peau, les tendons, les vaisseaux sanguins et le cartilage. Les nanofibres de collagène artificielles sont de plus en plus importantes dans de nombreuses applications d'ingénierie tissulaire où ces échafaudages moléculaires favorisent la croissance et la prolifération cellulaire (Kolácná *et al.*, 2007). Un autre exemple intéressant pouvant servir d'inspiration au biomimétisme consiste aux

nanostructures amyloïdes. Les fibrilles amyloïdes sont des matériaux biologiques que l'on retrouve dans presque tous les organismes vivants. En raison de leurs propriétés mécaniques uniques, leur stabilité et leur biocompatibilité, les structures amyloïdes sont de plus en plus considérées comme des éléments de base pour la conception et la construction de nouveaux biomatériaux prometteurs en biotechnologie et nanomédecine (Lai *et al.*, 2021). Par exemple, des assemblages protéiques de structures amyloïdes ont été utilisés pour fabriquer des supercondensateurs pour stocker l'énergie (Adler-Abramovich *et al.*, 2009; Hu. *et al.*, 2018), des plateformes de détection d'agents pathogènes (De la Rica *et al.*, 2008) ou encore des hydrogels pour des applications en médecine régénérative.

1.2 Fibres amyloïdes

1.2.1 Structures des fibres amyloïdes

Les amyloïdes sont des fibrilles non branchées et non ramifiées de largeur de 7 à 12 nm et de longueur variables (Makin et Serpell, 2005). Chaque fibre est constituée de 2 à 6 protofilaments qui s'enroulent ou s'associent latéralement sous forme de ruban (Goldsbury *et al.*, 2011; Makin et Serpell, 2005). Ces suprastructures sont stabilisées par des interactions intramoléculaires entre les brins- β qui s'alignent de manière orthogonale vis-à-vis l'axe de la fibre pour aboutir à une structure en feuillets- β -croisés (Figure 1.1). Cette caractéristique confère plus de résistance à la protéolyse et une importante insolubilité à toutes les formes amyloïdes. Les brins β se forment grâce à des liaisons de type hydrogène impliquant principalement le squelette peptidique, tandis que les interactions entre les feuillets- β dépendent fortement de la nature des chaînes latérales et de leurs caractéristiques, telles que la taille, la charges, etc. (Knowles *et al.*, 2014). Des analyses de diffraction par rayons X ont révélé deux pics de réflexion caractéristiques de 4,7 Å et d'environ 8-10 Å, définissant respectivement les espacements inter-brins et inter-feuillets (Figure 1.1) (Eanes et Glenner, 1968; Harrison *et al.*, 2007; Makin *et al.*, 2005; Makin et Serpell, 2005).

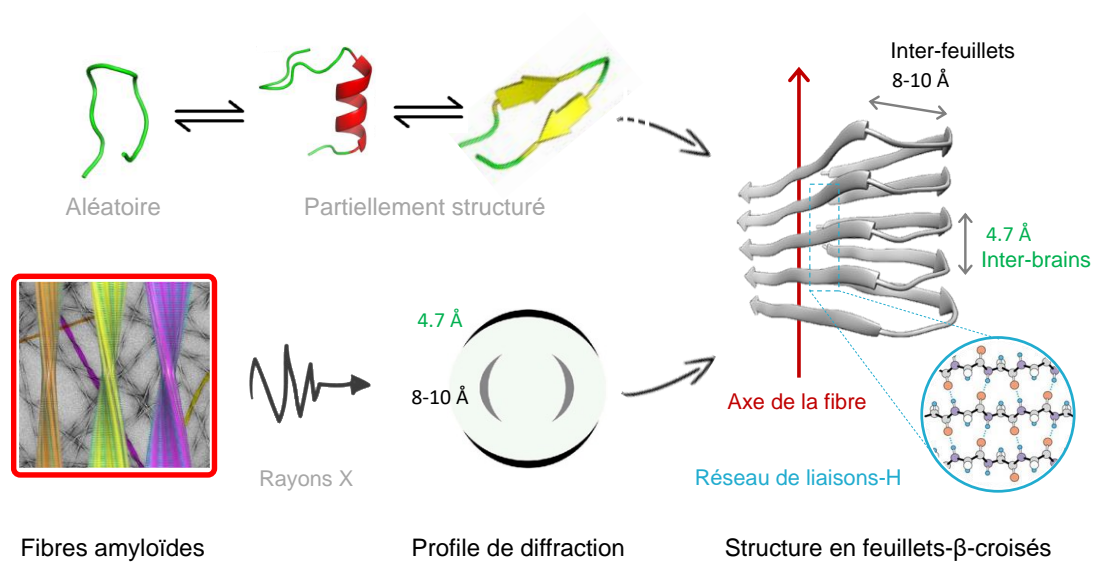


Figure 1.1 Organisation moléculaire et structure des fibres amyloïdes.

Les fibrilles amyloïdes partagent tous une structure quaternaire riche en feuillets β -croisés (*cross- β -sheet*) résultant d'un croisement parallèle ou antiparallèle de brins- β (*β -stands*) malgré les différences évidentes de leurs séquences natives (Figure 1.2) (Benzinger *et al.*, 1998; Eanes et Glenner, 1968; Qiang *et al.*, 2012; Sunde *et al.*, 1997). Les structures riches en feuillets β -croisés forment des sites de liaison avec certains colorants, tels que le rouge de Congo (CR) et la thioflavine T (ThT) (Biancalana et Koide, 2010). Bien que son mécanisme d'interaction avec les fibrilles amyloïdes reste mal connu, la ThT est devenue l'une des sondes moléculaires les plus largement utilisée pour la coloration sélective et l'identification rapide des fibrilles amyloïdes *in vivo* et *in vitro* (LeVine, 1999; Naiki *et al.*, 1989; Sabaté et Saupe, 2007). De même, les fibres amyloïdes peuvent être visualisées par microscopie à force atomique (AFM) ou microscopie électronique à transmission (TEM).


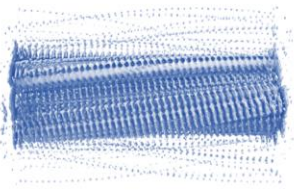
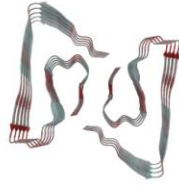



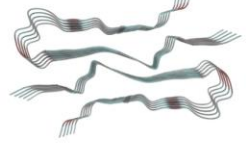






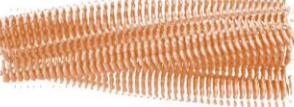
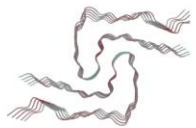

Structure 3D des fibrilles		Feuillet- β	Monomère	Peptide
Vue le long de l'axe Z	Vue le long de l'axe Y	Vue le long de l'axe Z	3D	Références
				β-amyloïde « 1-42 » EMD-3851 PDB-5OQV <i>Gremer et al. 2017</i>
				Prion « Variant M129 » EMD-20900 PDB-6UUR <i>Glynn et al. 2020</i>
				hIAPP « Amyline » EMD-21410 PDB-6VW2 <i>Cao et al. 2020</i>
				α-synucléine « Tordue » EMD-7619 PDB-6CU8 <i>Li et al. 2018</i>

Figure 1.2 Modèles atomiques de différents nanofilaments amyloïdes.

Il est admis qu'une séquence polypeptidique précise et précurseur peut engendrer une grande diversité morphologique de fibrilles (Peelaerts *et al.*, 2015; Qiang *et al.*, 2017). Cette hétérogénéité est le fruit de variations qui peuvent survenir au niveau de la conformation des blocs de construction, ce qui semble être le cas pour les protéines prions (Igel *et al.*, 2023), ou au niveau de l'assemblage des protofilaments, comme décrit pour l' α -synucléine (α -Syn) humaine (Li *et al.*, 2018). En effet, des analyses par cryo-microscopie électronique (cryo-EM) ont révélé récemment que l' α -synucléine (α -Syn) humaine d'origine recombinante s'autoassemble sous forme de fibrilles polymorphes avec deux espèces prédominantes sous forme de tige et torsade (Figure 1.3). Les modèles atomiques (à une résolution de 3,7 Å) ont révélé que les deux

polymorphes partagent la même structure de noyau, mais diffèrent par leurs interfaces inter-protofilaments conduisant ainsi à différentes morphologies avec des torsions hélicoïdales distinctes le long de l'axe des fibrilles (Li *et al.*, 2018). De même, les différents points de contact des deux unités asymétriques opposées expliquent la différence d'enroulement des deux protofilaments dans les deux polymorphes. Ce polymorphisme a été aussi observé dans des fibrilles de la protéine tau provenant d'un patient atteint de maladie d'Alzheimer, où des analyses par cryo-EM ont révélé que cette hétérogénéité résulte d'une combinaison d'empilement de brins- β classiques et de structures β -hélices (Fitzpatrick *et al.*, 2017). Ces modèles en β -hélices résultent d'un enroulement d'un ou plusieurs feuillets β étendus autour d'un noyau creux de façon hélicoïdale et les brins adjacents de l'hélice sont reliés par des liaisons hydrogène. Ces structures en β -hélices ont été également observées dans des assemblages de protéines prions Sup35 et PrP (Govaerts *et al.*, 2004; Krishnan et Lindquist, 2005; Wille *et al.*, 2009).

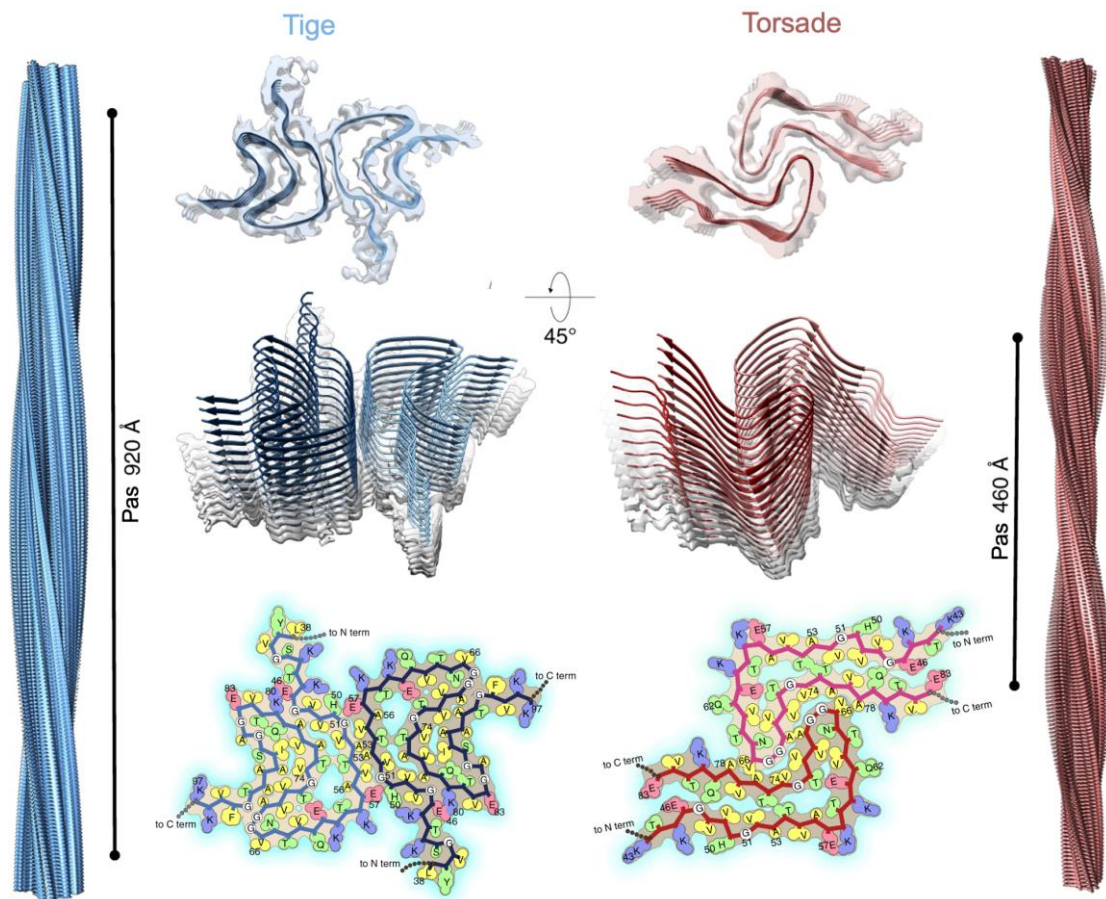


Figure 1.3 Modèles atomiques des polymorphes aSyn obtenus par Cryo-EM. Modèles 3D obtenus par cryo-EM des polymorphes sous forme de tige (à gauche) et torsadé (à droite) avec des vues sous deux angles différents (panneaux supérieurs) et les interfaces d'interactions entre les noyaux de protofilament dans les deux polymorphes aSyn (panneaux inférieurs) vus vers le bas de l'axe des fibrilles. Les résidus sont colorés par hydrophobicité (jaune : hydrophobe ; vert : polaire ; rouge : charge négative ; bleu : charge positive). Adapté de (Li *et al.*, 2018)

1.2.2 Mécanismes de formation des fibres amyloïdes

De la biosynthèse jusqu'à la dégradation finale, les protéines peuvent avoir plusieurs voies et états d'agrégation dans un système vivant. Avec une tendance intrinsèque à se convertir de leurs états fonctionnels natifs en agrégats amyloïdes insolubles, ces chaînes polypeptidiques peuvent adopter divers états d'équilibre conformationnel au cours de leur processus d'agrégation (Chiti et Dobson, 2017). Ainsi, plusieurs

mécanismes d'agrégation et plusieurs voies ont été décrits en fonction de la séquence protéique, des états conformationnels adoptés par le monomère amyloïdogène et des conditions expérimentales tels que la température, le pH, la concentration en protéines et l'effets du solvant (Chiti *et al.*, 2000).

Le mécanisme général de formation des fibres amyloïdes est souvent associé à un processus d'autoassemblage nucléé, présentant typiquement une cinétique de propagation sigmoïdale qui se déroule en trois étapes (Figure 1.4) (Ferrone *et al.*, 1999; Iadanza *et al.*, 2018; Morris *et al.*, 2009). Il s'agit d'un mécanisme nucléation primaire, préférentiel à des concentrations de protéines relativement faibles, favorisant la présence d'espèces monomères en solution. En effet, la formation des fibres amyloïdes adopte un mécanisme de type nucléation/propagation débutant par une phase rapide de nucléation, dite de latence, qui consiste en un effondrement hydrophobe des monomères protéiques puis leur réarrangement pour former des noyaux en feuillets- β indispensables pour accélérer le processus d'agrégation (Serio *et al.*, 2000). La durée de cette phase de latence dépend de plusieurs facteurs tels que les conditions d'agrégation ainsi que la séquence primaire du peptide, où il a été montré que des mutations peuvent même conduire à une élimination complète de cette phase (Pedersen *et al.*, 2004; Uversky. *et al.*, 2002). On peut également s'affranchir de cette phase de latence en utilisant la technique d'ensemencement « seeding » qui consiste à introduire en solution des fibrilles pré-formées et/ou des noyaux capables d'initier l'agrégation fibrillaire des protéines/peptides (Cohen. *et al.*, 2011; Hao *et al.*, 2019; Li *et al.*, 2022). La seconde étape est une phase exponentielle de propagation où le noyau accélère l'agrégation par addition d'espèces monomériques et/ou oligomériques en formant des structures en feuillets- β -croisées résultant d'une interdigitation de feuillets- β (Horwich *et al.*, 2002). La dernière est une phase stationnaire caractérisée par un plateau, ou équilibre, indiquant la formation de fibrilles matures (Chiti et Dobson, 2006; Serio *et al.*, 2000). Deux types de mécanismes nucléation primaire ont été décrits : homogènes et hétérogènes. Le premier décrit l'agrégation de monomères dans une solution globale, tandis que le second implique l'association des sous-unités de monomères à la surface

d'un objet différent, tel que la paroi interne d'un récipient (Basu *et al.*, 2013; Kuelto *et al.*, 2008; Perevozchikova *et al.*, 2015), d'autres protéines ou peptides (Zhang, J. et Liu, 2003), des bicouches phospholipidiques (Chauhan *et al.*, 2000; Ege et Lee, 2004; Galvagnion *et al.*, 2015; Zhengjian *et al.*, 2018), ou l'interface air-eau (Campioni *et al.*, 2014; Li, Shanghao et Leblanc, 2014; Pavlova *et al.*, 2016).

Il a été observé dans plusieurs cas la présence de multiples oligomères hétérogènes et des espèces intermédiaires transitoires au cours de la formation des fibrilles que les mécanismes de polymérisation nucléée ne peuvent expliquer. Dans ces cas, un mécanisme de conversion conformationnelle nucléée a été proposé. Il s'agit d'une autre variante de polymérisation nucléée qui explique mieux la cinétique sigmoïdale (Lee *et al.*, 2011; Serio *et al.*, 2000). Selon ce modèle, les monomères solubles sont initialement en équilibre rapide avec les oligomères solubles non structurés (Scheibel et Lindquist, 2001; Serio *et al.*, 2000). Au cours de la première phase d'agrégation, ces oligomères solubles vont subir une réorganisation conformationnelle progressive pour générer des noyaux β -croisés dynamiques, mettant fin à la phase de latence et déclenche la fibrillation qui se déroule rapidement à mesure que les noyaux recrutent et convertissent les monomères et les oligomères solubles en forme β -croisée aux extrémités des fibrilles en croissance (Krishnan *et al.*, 2012; Krishnan et Lindquist, 2005; Scheibel *et al.*, 2004; Scheibel et Lindquist, 2001; Serio *et al.*, 2000). Ce type de nucléation a été notamment observé chez des variants A β (Fu *et al.*, 2015; Lee *et al.*, 2011), la protéine prion (Sup35) (Serio *et al.*, 2000), la polyglutamine (Bhattacharyya *et al.*, 2005) et lysozyme (Hill *et al.*, 2009).

Une fois les fibres formées, un second processus peut se mettre en place pour former et catalyser de nouveaux agrégats ou fibres (Figure 1.4). Il s'agit d'une fragmentation des fibrilles (un processus indépendant du monomère), qui libère de nouvelles extrémités de fibrilles en croissance, et donc la formation et la propagation des graines (Colby *et al.*, 2009; Cushman *et al.*, 2010; Shorter, 2010) ou d'une nucléation secondaire catalysée par les fibrilles (un processus dépendant du monomère) où la

Wang, Juan *et al.*, 2016; Yuan *et al.*, 2017). Ainsi, la liaison hydrogène est l'une des principales interactions impliquées dans le processus d'autoassemblage des peptides amyloïdes (Stephanopoulos *et al.*, 2013). Cette interaction entre les différents squelettes peptidiques formant le feuillet- β est à l'origine de la rigidité et la résistance des nanostructures amyloïdes formées (Knowles *et al.*, 2007). L'autre type d'interaction non covalente gouvernant le processus d'autoassemblage est les forces électrostatiques basées sur des attractions coulombiennes entre des charges opposées des différents résidus (Faul et Antonietti, 2003; Knowles *et al.*, 2007; Rehm et Schmuck, 2010). Les forces hydrophobes et les empilements π - π entre les résidus aromatiques sont moins directionnelles, mais sont significativement améliorées dans l'eau en raison de la faible solubilité des groupements associés (Knowles *et al.*, 2007; Stanković *et al.*, 2020). Les interactions de van der Waals sont également associées au processus d'autoassemblage mais pas à la force motrice dominante (Israelachvili *et al.*, 2011).

Il a été observé que la séquence amyloïdogénique 20-29 de l'IAPP (SNNFGAILSS ; I₁₀) peut s'auto-assembler sous forme de fibrilles torsadées polymorphes caractérisées par la présence à la fois de formes parallèles et antiparallèles (Madine *et al.*, 2008). Des simulations de dynamique moléculaire ont révélé que les symétries antiparallèles sont structurellement les plus stables en raison du nombre plus important de liaisons hydrogène et des interactions électrostatiques amplifiées (Berryman *et al.*, 2011). Une autre étude sur une série de dérivés peptidiques préparés par incorporation de résidus chargés à l'extrémité *N*-terminale du segment I₁₀ a également montré l'effet des interactions électrostatiques sur le processus d'autoassemblage et la morphologie des assemblages résultants (Zottig *et al.*, 2019). En particulier, des nanostructures sous forme de nanotiges uniformes ont été obtenues en ajoutant des résidus positifs à l'extrémité *N*-terminale du segment peptidique, où une corrélation a été observée entre la longueur de ces tiges et le nombre de charges. Cela a été attribué à une limitation de la torsion du cœur en feuillets- β -croisés induite par des répulsions électrostatiques entre les résidus de charges positives, conduisant ainsi à un contrôle unique sur la taille de

ces assemblages cytocompatibles (Zottig *et al.*, 2019). La relation entre les interactions électrostatiques et la morphologie des assemblages amyloïdes a été encore étudiée sur des nanostructures auto-assemblées à partir du segment 16-22 du peptide β -amyloïde (KLVFFAE) et son dérivé coiffé aux extrémités *N*- et *C*-terminales (Ac-KLVFFAE-NH₂) (Tao *et al.*, 2011). Il a été démontré que le coiffage à l'extrémité *N*-terminale réduit la charge et les répulsions électrostatiques entre les résidus lysine chargés positivement, ce qui favorise une transformation des nanofibrilles en nanobandes plates où la liaison hydrogène entre les brins β s'étend dans une direction axiale alors que les interactions hydrophobes et les empilements π - π favorisaient l'attraction face à face entre les feuillettes- β . Une simulation de dynamique moléculaire a été ensuite réalisée pour étudier les effets de substituant des deux résidus phénylalanine (FF) en positions 19 et 20 par des acides aminés non naturels sur la morphologie des assemblages amyloïdes (Wang *et al.*, 2016). Il a été démontré que les nanobandes plates et larges du peptide Ac-KLVFFAE-NH₂ ont été transformés en nanotubes minces lorsque les résidus FF ont été remplacés par des cyclohexylalanines, ce qui a éliminé les empilements π - π latéraux alors que les interactions hydrophobes et l'encombrement stérique ont été légèrement améliorés. Par ailleurs, les nanobandes plates se sont transformés en nanofibrilles plus fines et tordues après remplacement des deux résidus FF par des phénylglycines, en raison d'un encombrement stérique beaucoup plus fort à la suite d'une rotation limitée du cycle aromatique.

À l'aide de dérivés peptidiques, conçus à partir du segment I₁₀ de l'IAPP acétylé à l'extrémité *N*-terminale (Ac-NNFGAILSS), il a été démontré que la substitution de la sérine en position *C*-terminale par une thréonine induit une transformation des fibrilles amyloïdes de rubans torsadés en structures non torsadées, où le groupe méthyle de la chaîne latérale semble être responsable du changement morphologique (Wang *et al.*, 2017). Cet effet a été encore vérifié en substituant la sérine par la valine et l'alanine et en étendant la longueur de la séquence, ce qui a été attribué à la restriction de la liberté conformationnelle et une augmentation des interactions hydrophobes et liaisons hydrogène. Par ailleurs, le peptide non coiffé a formé des structures non torsadées

(Figure 1.5), suggérant une formation accrue de feuillets- β , ce qui peut être expliqué par des interactions électrostatiques entre $-\text{NH}_2$ et $-\text{COOH}$ des extrémités N - et C -terminales. En résumé, la fibrillation et la diversité morphologique des assemblages peptidiques sont dépendantes de la séquence (Bera *et al.*, 2019; Godin *et al.*, 2019).

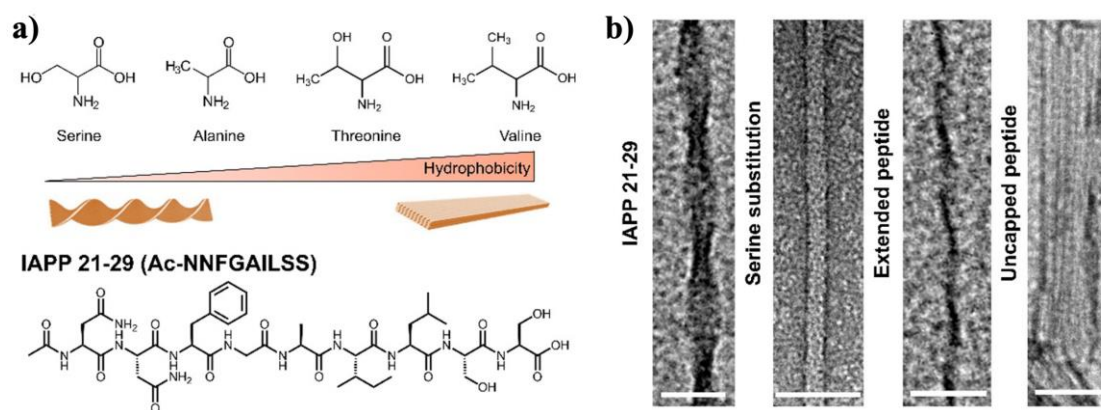


Figure 1.5 Relation entre la nature/taille de la séquence amyloïde et les changements morphologiques. (a) Séquence amyloïdogène 21-29 (Ac-NNFGAILSS) de l'IAPP avec les différentes modifications (b) Images TEM des fibrilles polymorphes (rubans tordus et planaires) de différentes tailles (de gauche à droite : Ac-NNFGAILSS, Ac-NNFGAILTT, Ac-SSNFGAILSS, H-SSNFGAILSS). Barre d'échelle : 50 nm. Adapté de (Wang *et al.*, 2017).

Les paramètres tels que la température, la concentration, la présence d'ions métalliques, le pH du milieu et la nature du solvant, sont des facteurs critiques pour contrôler la thermodynamique des assemblages polypeptidiques et moduler de ce fait la morphologie et les fonctionnalités des suprastructures résultantes (Lai *et al.*, 2021; Mandal *et al.*, 2014; Pellarin *et al.*, 2010; Wang *et al.*, 2016; Yuan *et al.*, 2017). En effet, les températures élevées aident à renforcer les effets hydrophobes, alors qu'elles peuvent induire une rupture ou une faiblesse des liaisons hydrogène, comme il a été révélé avec un heptapeptide conçu *de novo* (GNNQQNY) qui s'auto-assemble sous forme d'un cristal α -hélicoïdal croisé métastable à basse température (4 °C), mais qui se transforme en fibrilles amyloïdes riches en structures β -croisées

thermodynamiquement stables en chauffant à 30 °C et 50 °C (Figure 1.8) (Mondal *et al.*, 2019).

Les forces des interactions électrostatiques et des liaisons hydrogène sont aussi affectées par les changements de pH, comme il a été démontré avec le fragment 17-42 du peptide A β qui s'agrège plus rapidement lorsque le pH est supérieur au point isoélectrique (pI) (Kobayashi *et al.*, 2015). Des études théoriques ont montré qu'un pont ionique intermoléculaire se formait entre Asp₂₃ et Lys₂₈ dans cette gamme de pH supérieur au pI, ce qui peut être à l'origine de la stabilité des fibrilles A β et des oligomères. D'ailleurs, aucune agrégation du peptide A β ₁₋₄₂ n'a été observée expérimentalement à pH 9,5, ce qui peut être expliqué par une neutralisation du groupe amine de la chaîne latérale de la Lys₂₈. De même, l'agrégation du peptide IAPP dépend également du pH où il a été révélé que le résidu His₁₈ agissait comme un interrupteur électrostatique ralentissant la fibrillation dans son état protoné (Jha *et al.*, 2014).

Le processus d'autoassemblage des peptides amyloïdes dépend largement des forces ioniques. Par exemple, des simulations de dynamique moléculaire réalisées sur la séquence 37-42 d'A β dans une solution saline, ainsi que des expérimentations menées sur l'IAPP dans la plage de 20 à 600 mM de NaCl à pH 8,0 ont montré que les voies dominantes d'agrégation varient de manière significative par l'ajout de NaCl (Marek *et al.*, 2012; Yang et Gao, 2015). De plus, le processus d'autoassemblage des peptides amyloïdes ainsi que la morphologie des assemblages résultants semblent être dépendants de la concentration des espèces monomériques présentes (Figure 1.6), où des rubans hélicoïdaux formés par des peptides en feuillet- β seraient transformés en rubans torsadés (rubans doubles), en fibrilles (empilements de rubans torsadés) et en fibres (fibrilles torsadées) en présence d'une concentration croissante du peptide (Aggeli *et al.*, 2001).

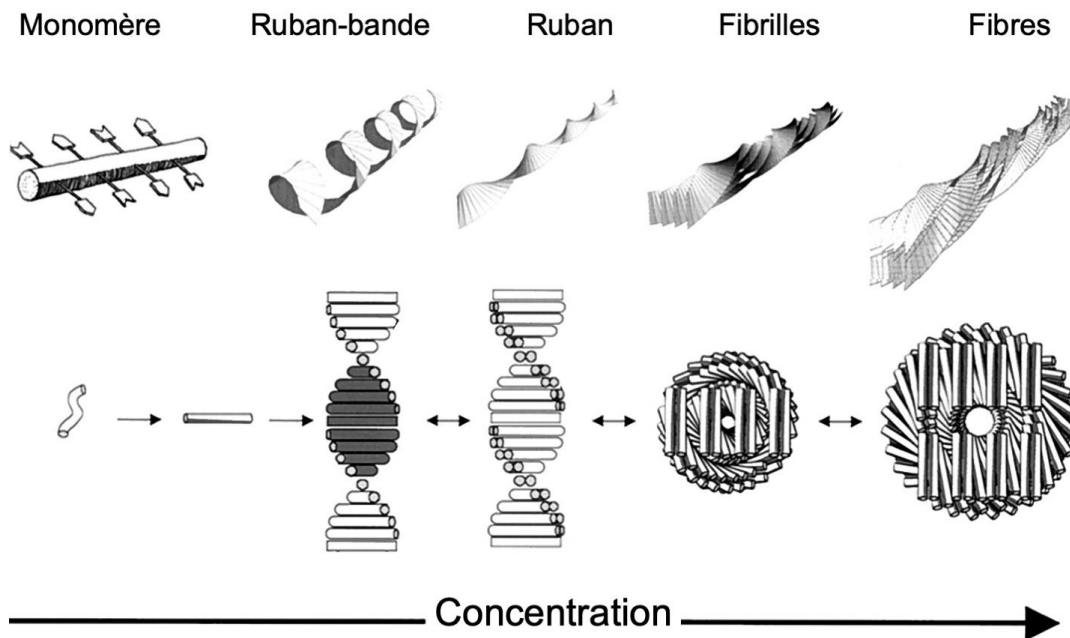


Figure 1.6 Effet de la concentration sur la morphologie des assemblages amyloïdes. Adapté de (Aggeli *et al.*, 2001).

Les ions métalliques sont aussi impliqués dans les dépôts amyloïdes où plusieurs études sur l'autoassemblage de peptide amyloïde induit par la coordination métallique ont été rapportées (Bousejra-ElGarah *et al.*, 2011; De Gregorio *et al.*, 2019; Wallin *et al.*, 2020; Wallin *et al.*, 2016). Par exemple, l'ajout de Zn^{2+} peut augmenter les taux relatifs de nucléation et de propagation de la séquence 10-21 du peptide $A\beta$ (YEVHHQKLVFFA) suite à des interactions inter-feuillets médiées par des Zn^{2+} , impliquant à la fois l' His_{13} et l' His_{14} (Morgan *et al.*, 2002). Il a été de même rapporté que la variation de la concentration des ions Zn^{2+} peut modifier considérablement la cinétique d'autoassemblage et la morphologie des assemblages formés. En résumé, le contrôle combiné de la thermodynamique et de la cinétique d'autoassemblage des amyloïdes joue un rôle clé dans la modulation de la morphologie et des fonctionnalités des assemblages résultants (Yuan *et al.*, 2017).

1.2.4 Modulateurs de l'auto-assemblage amyloïde

Les fibres amyloïdes et/ou les précurseurs monomériques ont aussi une capacité intrinsèque à interagir avec divers biopolymères, notamment les polyanions glycosaminoglycanes (Castillo *et al.*, 1999; Iannuzzi *et al.*, 2015; Quittot *et al.*, 2017), les membranes composées de phospholipides et les chaînes d'acides nucléiques (Ginsberg *et al.*, 1998; Marcinkiewicz *et al.*, 2002). Cet effet corrèle avec le fait que des polyélectrolytes chargés sont virtuellement toujours retrouvés dans des dépôts amyloïdes extraits de patients (Calamai *et al.*, 2006). Par exemple, des acides nucléiques ont été détectés dans des enchevêtrements neurofibrillaires et des inclusions intracellulaires principalement composées de la protéine tau, ainsi que dans des plaques séniles composées de peptides A β extraits de tissu cérébral de patients atteint de la maladie d'Alzheimer (Ginsberg *et al.*, 1998). De plus, il a été démontré que l'ARN est capable de stimuler l'agrégation de la protéine tau (Kampers *et al.*, 1996), tandis que l'ADN double brin peut favoriser la fibrillation de l' α -synucléine (Cherny *et al.*, 2004). De même, il a été démontré que des ARN ribosomiaux intergéniques non codants sont impliqués dans les transitions de phases intracellulaires pour la formation de corps amyloïdes permettant aux cellules d'entrer dans un état dormance en réponse à un stress (Audas *et al.*, 2016). Cela a été attribué principalement aux interactions électrostatiques impliquant les charges négatives des polyélectrolytes et les résidus chargés positivement des chaînes latérales de la protéine agrégée, ce qui a fourni de nouvelles informations sur les dépôts amyloïdes catalysés par des polyanions (Iannuzzi *et al.*, 2015). Basé sur ces données, une courte séquence d'ADN double brin a été utilisée pour diriger sélectivement l'assemblage de peptides amyloïdes sous forme de nanotubes multilamellaires (Rha *et al.*, 2020). Les lamelles individuelles formant les nanotubes peptide/ADN sont composées de monocouches chargées négativement d'ADN qui couvrent, via des interactions électrostatiques complémentaires, la surface chargée positivement de peptides autoassemblés sous forme de feuilletts β antiparallèles (Figure 1.7 A-C) (Rha *et al.*, 2020). Une autre étude a démontré que l'ADN simple brin est capable non seulement d'inhiber l'agrégation du peptide A β , mais également de

défaire les fibrilles préformées via des interactions électrostatiques, conduisant ainsi à la formation de complexes interpolyélectrolytes pour lesquels le peptide amyloïde s'enfouit au cœur de la sphère formée (Figure 1.7 D) (Abraham *et al.*, 2014). En résumé, la compréhension des mécanismes d'interaction entre les peptides amyloïdes et les polyanions, notamment les acides nucléiques, fournit de nouvelles informations sur leur implication dans l'étiologie des amyloïdoses et ouvre la voie non seulement vers le développement de nouveaux traitements efficaces contre ces pathologies mais également pour la conception de matériaux hybrides ayant des propriétés attrayantes telles qu'une stabilité métabolique améliorée, un contrôle précis de la géométrie, une biocompatibilité et une biodégradabilité élevées (Menon *et al.*, 2023).

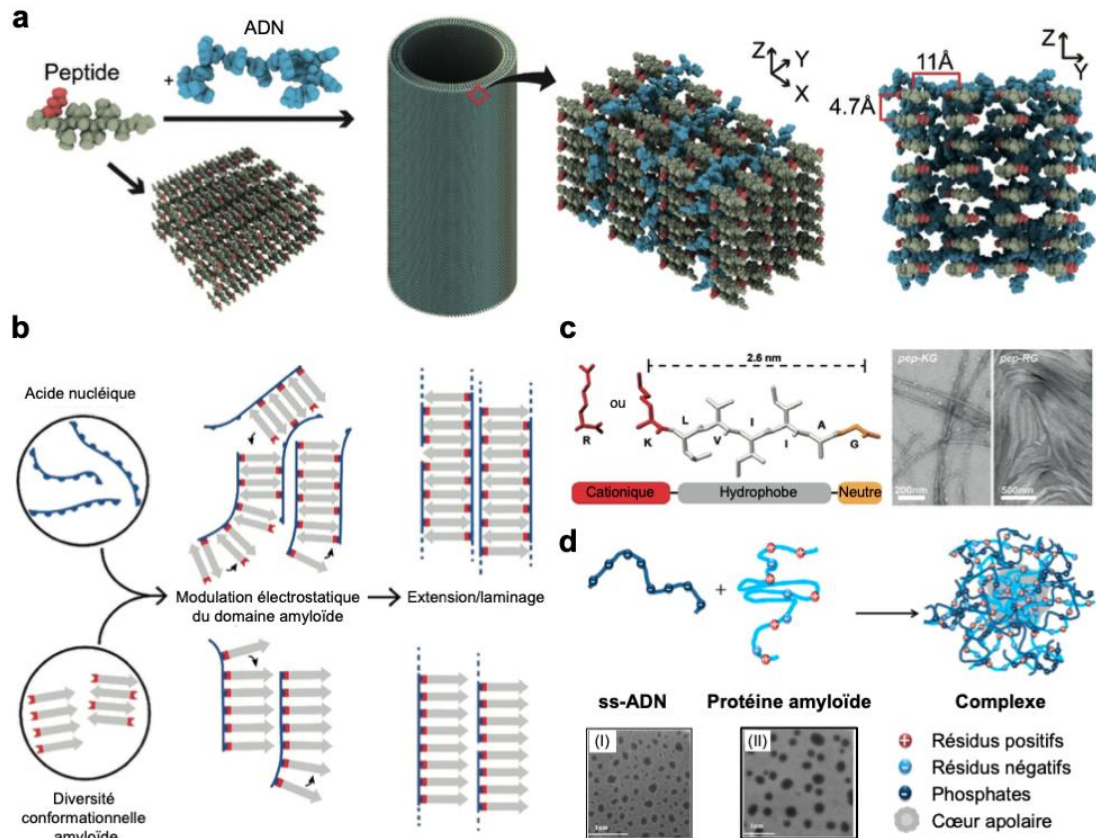


Figure 1.7 A) Modèles structurels de co-assemblages peptide et ADN. B) Les peptides s'assemblent sous forme de feuillets- β parallèles et antiparallèles et sous forme de nanotubes multilamellaires en présence d'ADN (5'-TCTGAG-3'). Les monocouches β -croisées constituent les lamelles individuelles des nanotubes peptide/ADN, l'ADN passive la surface β -croisées chargée positivement. B) Modèle d'assemblage peptide/acides nucléiques. Les peptides chargés positivement forment des phases cationiques capables d'interagir de façon complémentaire avec les polyanions d'acide nucléique. Les acides nucléiques peuvent guider sélectivement les feuillets- β en deux co-assemblages distincts. C) Structure primaire des peptides et caractérisation des assemblages par TEM. D) Mécanisme avec images TEM de l'inhibition/désagrégation de fibrilles peptidiques A β 42 par la formation d'un complexe interpolyélectrolytique entre le peptide et les séquences nucléotidiques. Les images TEM montrent (I) des complexes formés lorsque A β 42 (10 μ M) a été co-incubé avec 50 μ M d'ADN (Inhibition de la genèse des fibrilles) et (II) des complexes formés par désassemblage de fibres préformés d'A β 42 (10 μ M) après incubation avec 50 μ M d'ADN. Adapté de (Abraham *et al.*, 2014; Rha *et al.*, 2020)

1.3 Amyloïdes pathogéniques

1.3.1 Maladies associées aux amyloïdes

Les amyloïdoses résultent de dépôts amyloïdes altérant le fonctionnement normal de différentes cellules et provoquant ainsi plus de 50 types de pathologies humaines, parmi lesquelles on peut citer la maladie d'Alzheimer, la maladie de Parkinson, la maladie de Creutzfeldt-Jakob, la maladie de Huntington et le diabète de type II, associées respectivement par l'agrégation du peptide A β) de l' α -synucléine, de la protéine prion, de la protéine huntingtine et de l'amyline (Tableau 1.1) (Chiti et Dobson, 2006; Hajipour *et al.*, 2017; Harrison *et al.*, 2007; Rajasekhar et Govindaraju, 2018). Ces amyloïdoses sont liées à un mauvais repliement des protéines/peptides solubles fonctionnelles vers des fibrilles amyloïdes ordonnées qui s'accumulent progressivement sous forme d'inclusions au niveau intracellulaire ou sous forme de plaques au niveau extracellulaire (Buxbaum *et al.*, 2022; Selkoe *et al.*, 2003).

Tableau 1.1 Exemples de protéine/peptide formant des fibrilles de type amyloïde ayant des rôles pathologiques.

Pathologies	Protéine/Peptide	Résidus ^a	Structure native
Maladie d'Alzheimer	Peptide amyloïde- β (A β)	40 ou 42 ^b	Non structuré
Maladie de Creutzfeldt-Jakob	Protéine Prion (PrP)	208	Partiellement en hélice α
Maladie de Parkinson	α -synucléine	140	Non structuré
Maladie de Huntington	Huntingtin Polyglutamique	~103 - 187 ^b	Non structuré
Amylose de l'hémodialyse	β 2-microglobuline	99	Feuillet β
Amylose systématique sénile	Transthyrétine (TTR)	127	Feuillet β
Diabète de type II	Amyline (IAPP)	37	Non structuré
Amylose au point d'injection	Insuline	(30 + 21) ^c	Hélice α
Amylose auriculaire	Calcitonin	32	Non structuré

a Nombre d'acide aminés de la séquence primaire.

b Des fragments de différentes longueurs ont été rapportés pour les fibrilles *ex vivo*.

c Longueurs des chaînes A et B reliées par un pont disulfure

1.3.2 Autoassemblage amyloïde et mécanisme de toxicité

Historiquement, on suppose que les fibrilles matures sont seules responsables de la dégénérescence tissulaire observée lors des différentes amyloïdoses. Cependant, plusieurs structures intermédiaires entrent en jeu dans la cascade amyloïde et la communauté scientifique tente toujours de repérer lesquelles sont responsables de la cytotoxicité observée. Plusieurs études ont montré que les principaux responsables des dommages cellulaires seraient les espèces intermédiaires solubles formées durant l'interconversion d'une forme native, provenant de diverses protéines, en une forme fibrillaire (Huang et Liu, 2020; Niyangoda *et al.*, 2021; Sengupta *et al.*, 2016). Quoique les mécanismes de toxicité associés aux oligomères soient encore mal connus, la nocivité précoce de ces espèces semble résulter d'une capacité à interagir avec les membranes cellulaires en perturbant leur fonctionnement (Figure 1.8), produisant ainsi des espèces oxygénées réactives (ROS) et provoquant l'apoptose (Ahmad *et al.*, 2017; Cheignon *et al.*, 2018; Han *et al.*, 2017). En effet, un changement conformationnel conduit à la formation de précurseurs possédant une structure amyloïde β -croisée et pourvus d'une surface hydrophobe qui augmente potentiellement les interactions aberrantes avec d'autres composants cellulaires dont, entre autres, les membranes plasmiques (Fusco *et al.*, 2017; Vivoli *et al.*, 2019). Ces interactions peuvent causer de la toxicité en modifiant la fonction des canaux calciques voltage-dépendants et des protéines membranaires (Angelova *et al.*, 2016; De Felice *et al.*, 2007; Hou *et al.*, 2007; Hsieh *et al.*, 2006; Pellistri *et al.*, 2008), ou encore via la formation de pores ce qui induit une perte de l'intégrité de la bicouche lipidique et l'entrée d'ions calcium dans la cellule (Bode *et al.*, 2017; Caughey et Lansbury, 2003; Fusco *et al.*, 2017; Kawahara, 2004; Kourie et Shorthouse, 2000; Wong *et al.*, 2018). Par conséquent, des espèces réactives de l'oxygène (ROS) sont alors produites suite à une activation des pompes ATPases du réticulum endoplasmique et une augmentation du métabolisme cellulaire, ce qui libère le cytochrome c, qui provoque l'activation de la caspase 12, responsable de l'apoptose intrinsèque (Squier *et al.*, 2001). Les espèces oligomériques s'assemblent en fibrilles amyloïde matures pour lesquelles les régions hydrophobes peuvent se

dissimuler au cœur des fibres ce qui explique, en partie, la réduction de leur effet délétère. Les oligomères et protofibrilles formés, ou résultant d'une décomposition de fibrilles, peuvent pénétrer dans les cellules, en passant directement la bicouche lipidique de la membrane plasmique, ou par endocytose, et subir une élongation en fibrille mature qui se dépose à l'intérieur de la cellule et induisent l'autophagie. Par ailleurs, les fibrilles matures peuvent induire une accumulation anormale ce qui induit une apoptose extrinsèque (Bucciantini *et al.*, 2012).

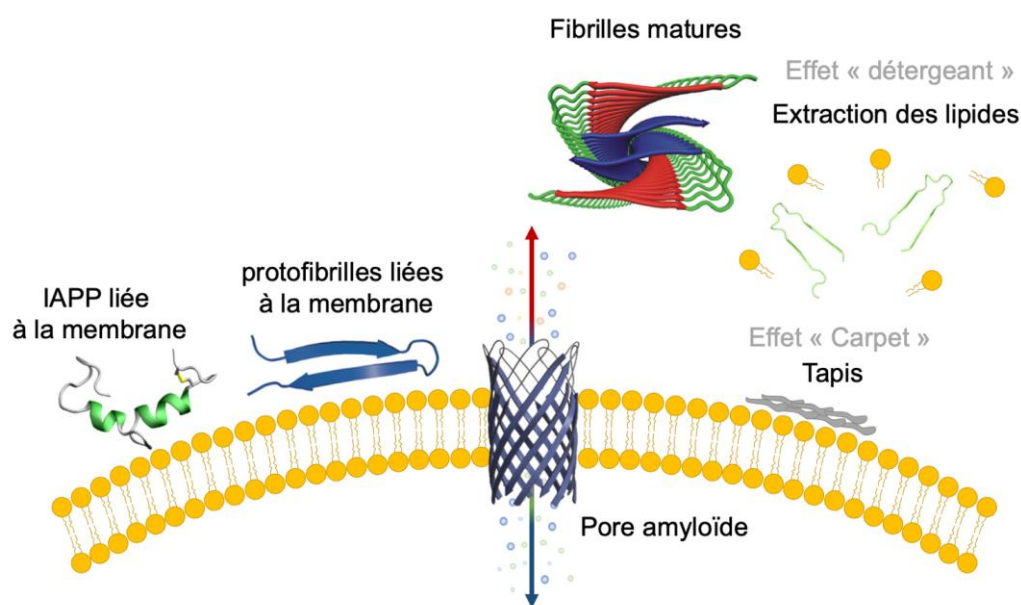


Figure 1.8 Représentation schématique des différents mécanismes de perturbation membranaire médiée par les amyloïdes. Les flèches indiquent le passage des ions à travers le pore amyloïde. Adapté de (Sciacca *et al.*, 2021).

1.3.3 Diabète de type II et islet amyloid polypeptide

Le diabète de type 2 (T2D) est une pathologie endocrinienne entraînant une augmentation de la néoglucogénèse hépatique à la suite d'une résistance des cellules périphériques à l'activité de l'insuline (Lee et Pervaiz, 2007; Westermark *et al.*, 1987). En réponse à l'insulinorésistance, la production hépatique de glucose augmente et la capacité du pancréas à produire de l'insuline diminue progressivement menant ainsi à

une perte des cellules β des îlots de Langerhans et à l'apparition d'hyperglycémie. L'une des caractéristiques de cette pathologie est le dépôt d'agrégats localisés au niveau des îlots de Langerhans pancréatiques (Montane *et al.*, 2012). Ces dépôts sont composés majoritairement de l'*islet amyloid polypeptide* (IAPP), ou amyline, une hormone composée de 37 résidus et qui est co-sécrétée avec l'insuline par les cellules β pancréatiques (Bhowmick *et al.*, 2022; Clark *et al.*, 1988; Lukinius *et al.*, 1989; Westermark *et al.*, 1987). Avec un pont disulfure entre les cystéines 2 et 7, la séquence peptidique de l'IAPP, amidée à l'extrémité C-terminale, est semblable à celles des peptides de la famille des calcitonines (*calcitonin gene related peptide* ; CGRP) (Johnson *et al.*, 1992) (Tableau 1.2). La fonction principale de l'IAPP n'est toujours pas clairement élucidée. Néanmoins, il est connu que sa co-sécrétion avec l'insuline par les cellules β pancréatiques supporte un rôle dans le maintien de l'homéostasie du glucose. D'autres rôles physiologiques de l'IAPP ont été proposés, notamment l'inhibition de la sécrétion d'insuline, le contrôle de la vidange gastrique et la satiété, ainsi que l'inhibition de la libération de glucagon (Akter *et al.*, 2016; Westermark *et al.*, 2011). Toutefois, une surexpression de l'IAPP peut être un facteur diabétogène suite à une inhibition excessive de la sécrétion de l'insuline (Ahrén *et al.*, 1998). L'IAPP se lie au récepteur de la calcitonine (CT) avec une affinité améliorée lorsque ce dernier forme un complexe avec les RAMP (*receptor activity-modifying protein*) (McLatchie *et al.*, 1998). L'IAPP est exprimé chez tous les mammifères, mais sa capacité à former des dépôts amyloïdes dépend aux variations inter-espèces de la séquence peptidique. Plusieurs études comparatives de séquence de l'IAPP issues de différentes espèces animales ainsi que des études *in vitro* effectuées sur différents fragments synthétiques de l'IAPP ont permis d'identifier trois segments amyloïdogéniques (8-20, 20-29 et 30-37) essentiels pour la formation de l'amyloïde (O'Brien *et al.*, 1994; Westermark *et al.*, 2011). Les segments 8-20 et 30-37 sont hautement conservés entre les espèces. Les IAPP provenant du rat et de la souris sont identiques, mais contrairement aux autres espèces leurs régions 20-29 sont riches en prolines ce qui prévient l'agrégation en fibrilles (Höppener *et al.*, 2002) (Tableau 1.2).

Tableau 1.2 Séquences primaires des CGRP et de l'IAPP humain et de différentes espèces. Adapté de (Akter *et al.*, 2016)

	1	10	20	30
CGRP1 humain	: A C D T A T C V T	: H R L A G L L S R S	: G G V V K N N F V P	: T N V G S K A F
CGRP2 humain	: A C D T A T C V T	: H R L A G L L S R S	: G G M V K S N F V P	: T N V G S K A F
IAPP				
Humain*	: K C N T A T C A T	: Q R L A N F L V H S	: S N N F G A I L S S	: T N V G S N T Y
Singe*	: K C N T A T C A T	: Q R L A N F L V R S	: S N N F G T I L S S	: T N V G S D T Y
Vache	: K C G T A T C E T	: Q R L A N F L A P S	: S N K L G A I F S P	: T K M G S N T Y
Chat*	: K C N T A T C A T	: Q R L A N F L I R S	: S N N L G A I L S P	: T N V G S N T Y
Chien	: K C N T A T C A T	: Q R L A N F L V R T	: S N N L G A I L S P	: T N V G S N T Y
Rat	: K C N T A T C A T	: Q R L A N F L V R S	: S N N L G P V L P P	: T N V G S N T Y
Souris	: K C N T A T C A T	: Q R L A N F L V R S	: S N N L G P V L P P	: T N V G S N T Y
Hamster	: K C N T A T C A T	: Q R L A N F L V H S	: N N N L G P V L S P	: T N V G S N T Y

* Amyloïdogénique

- Les résidus qui diffèrent de la séquence IAPP humaine sont en rouge.

- Les séquences matures biologiquement actives ont toutes un pont disulfure entre Cys-2 et Cys-7 et une extrémité C-terminale amidée

Sous son état monomérique et soluble, l'IAPP humain présente un ensemble conformationnel majoritairement désordonné, bien que des conformations ordonnées peuvent se former de façon transitoire (Figure 1.9) (Williamson et Miranker, 2007). En effet, l'IAPP est constitué de plusieurs acides aminés, tels que Gly, Ser, Ala, Asn et Thr, qui maintiennent le peptide non structuré à l'état natif. Le segment 8 à 22 du domaine *N*-terminal a un caractère modérément amphipathique, typique d'une hélice soluble ou liée à la membrane (Apostolidou *et al.*, 2008). D'autres caractéristiques du peptide incluent une liaison disulfure intramoléculaire entre les résidus Cys 2 et Cys 7 ainsi qu'une extrémité *C*-terminale amidée (Uversky *et al.*, 2011; Williams *et al.*, 2000). Le peptide IAPP humain est hautement hydrophobe, mais aussi légèrement cationique au pH physiologique en raison de la présence de trois charges positives (Lys1, Arg11 et His18).

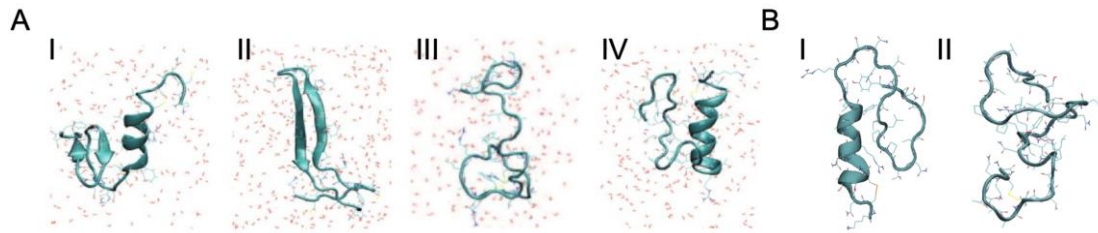


Figure 1.9 Modèles structuraux des différentes conformations secondaires de l'IAPP. (A) IAPP humain : (I) hélice α , (II) feuillet- β antiparallèle et (III) conformation aléatoire. Adapté de (Reddy, Wang, Singh, *et al.*, 2010) (B) Conformations représentatives de IAPP de rat : (I) hélice α et (II) aléatoire. Adapté de (Reddy, Wang, Lin, *et al.*, 2010)

Plusieurs modèles structuraux ont été proposés pour décrire en détail la structure quaternaire des fibrilles IAPP. Un des modèles proposé suggère une conformation sous forme « S » à trois brins β alternés de deux coudes en positions 17-19 et 28-29 et d'une séquence non structurée contenant un pont-disulfure à l'extrémité *N*-terminale (Figure 1.10 A) (Kajava *et al.*, 2005). Par ailleurs, des analyses par RMN à l'état solide ont révélé que les unités de base formant les protofibrilles IAPP adoptent une structure sous forme « U » à deux brins- β reliés par un coude en positions 18-27 (Figure 1.10 B-C) (Bedrood *et al.*, 2012; Luca *et al.*, 2007). Plusieurs études *in silico* ont également démontré une conformation sous forme « U » (Liang *et al.*, 2013; Wang *et al.*, 2011; Wiltzius *et al.*, 2008). Des études de dynamique moléculaire *all-atom*, basées sur des données expérimentales de RMN à l'état solide et de microscopie électronique, ont révélé un polymorphisme des oligomères/fibrilles de l'IAPP (Zhao *et al.*, 2011). Les trois polymorphes résultant de cette étude présentent une symétrie à 3-plis le long de l'axe de croissance des fibrilles, pour laquelle des sous-unités sous formes « U » s'empilent de façon perpendiculaire à l'axe de la fibrille. Les fibrilles hélicoïdales formées s'enroulent en triplet dans symétrie triangulaire autour d'un noyau hydrophobe et avec des périodicités différentes, similaire à une conformation A β triples obtenue par analyses RMN (Zhao *et al.*, 2011).

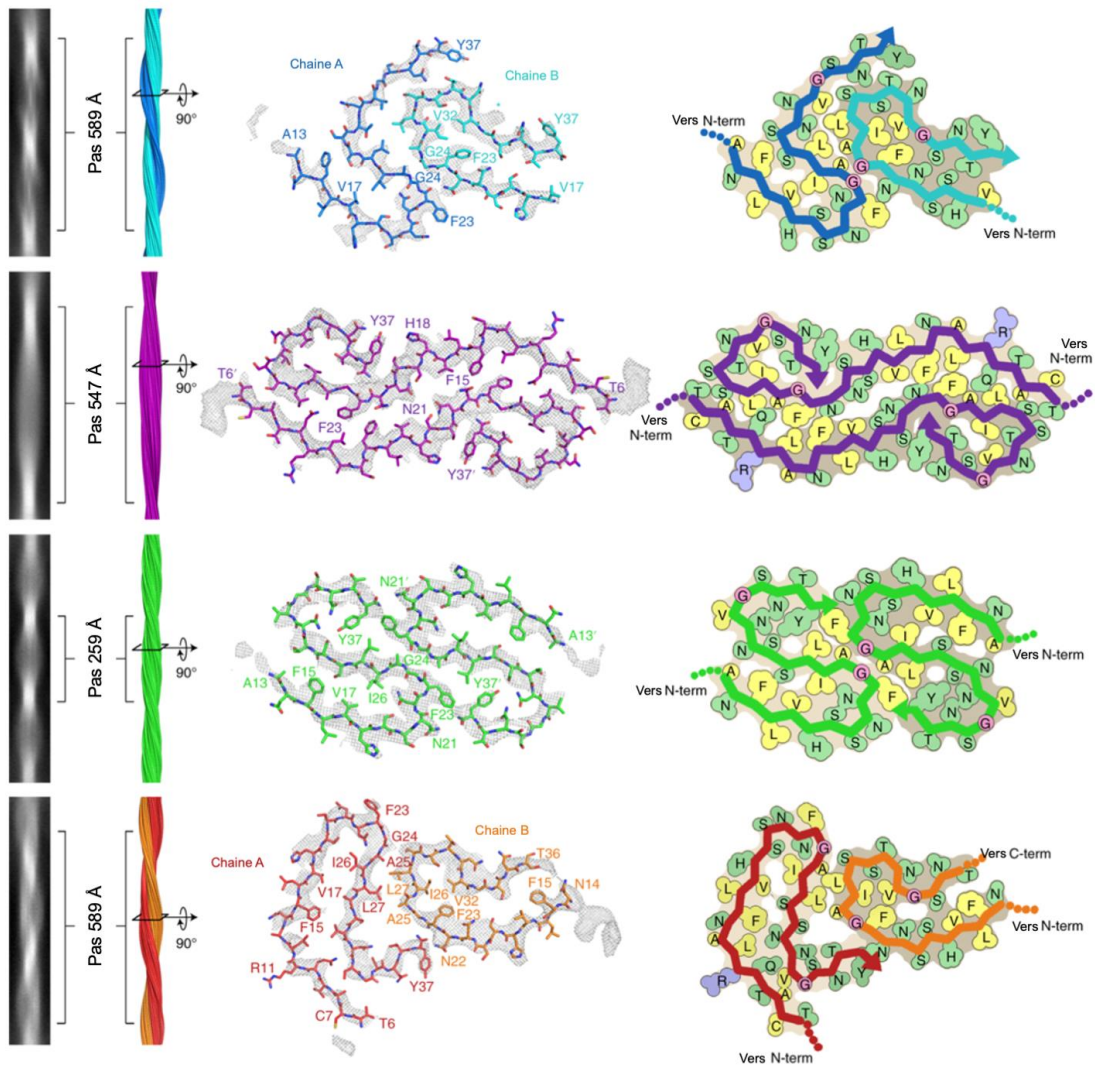


Figure 1.11 À l'extrême gauche : classes 2D représentatives de quatre morphologies distinctes des fibrilles IAPP. Milieu gauche : reconstructions des fibrilles mises à l'échelle pour correspondre aux classes 2D avec le pas de chaque morphologie. Au milieu à droite : modèle atomique et densité d'une couche transversale de chaque morphologie. Tout à droite : modèle schématique de chaque morphologie. Les résidus hydrophobes sont représentés en jaune, les polaires en vert, les glycines en rose et les arginines en bleu, term = terminal. Adapté de (Cao *et al.*, 2021).

1.3.4 Approches thérapeutiques pour lutter contre les amyloïdoses

La stabilisation de l'état natif des protéines semble être l'approche la plus prometteuse pour le développement de traitements efficaces contre les maladies associées aux amyloïdoses. Ceci a été illustré par le Tafamidis, un médicament cliniquement approuvé pour bloquer l'agrégation de la transthyrétine. (Bulawa *et al.*, 2012). Toutefois, cette stratégie est difficilement applicable pour lutter contre l'agrégation de polypeptides amyloïdogènes intrinsèquement désordonnés, tels que le peptide amyloïde- β (A β), l' α -synucléine et l'IAPP. Ainsi, des petites molécules, telles que les polyphénols naturels (Nedumpully-Govindan *et al.*, 2016) et de courtes séquences peptidiques avec des motifs d'auto-reconnaissance (Kanchi et Dasmahapatra, 2021; Wang *et al.*, 2014), ont montré une efficacité intéressante pour inhiber ou déstabiliser *in vitro* la fibrillation amyloïde bien que leur application clinique reste difficile à atteindre. Récemment, des immunothérapies basées sur des anticorps, capables de se lier aux dépôts amyloïdes et les éliminer, ont été introduites en clinique (Bateman *et al.*, 2022; Lee *et al.*, 2016; Nuvolone *et al.*, 2022). Par ailleurs, leur efficacité pour améliorer la qualité de vie des patients a été un peu limitée et des problèmes de sécurité ont été également soulevés (Pernecky *et al.*, 2023).

1.4 Amyloïdes fonctionnels

Depuis les deux dernières décennies, plusieurs structures amyloïdes contribuant à la physiologie normale des cellules, ont été identifiées chez la majorité des organismes vivants, allant des bactéries aux mammifères, y compris chez l'homme (Tableau 1.3) (Knowles et Buehler, 2011; Knowles et Mezzenga., 2016; Otzen et Riek, 2019). Parmi ceux-ci figurent les fibrilles formées par la protéine Pmel17 dans les mélanosomes de vertébrés, qui agissent comme une matrice pour la polymérisation de la mélanine qui fait intervenir des intermédiaires hautement toxiques (Hu *et al.*, 2011; McGlinchey *et al.*, 2011). De même, il a été observé que le stockage d'hormones peptidiques dans les granules sécrétoires de mammifères implique la formation de dépôts amyloïdes

hautement réversibles (Badtke *et al.*, 2009; Maji *et al.*, 2009). Finalement, les protéines de type Curli, principales composantes des biofilms bactériens, constituent l'exemple d'amyloïde fonctionnel le plus connu (Chapman *et al.*, 2002). Plusieurs études ont révélé la présence et l'importance des assemblages amyloïdes dans un large éventail de bactéries (Akbej et Andreasen, 2022; Barnhart et Chapman, 2006; Bieler *et al.*, 2005; Otzen et Riek, 2019; Sawyer *et al.*, 2011; Sønderby *et al.*, 2022).

Tableau 1.3 Exemples de protéine/peptide formant des fibrilles de type amyloïde ayant des rôles physiologiques.

Protéine	Organisme	Rôle physiologique	Références
Pmel17	Homo sapiens	Former, à l'intérieur des mélanosomes, des stries fibreuses sur lesquelles se forment des granules de mélanine.	(Berson <i>et al.</i> , 2003)
Curli	<i>Escherichia coli</i> (Bactérie)	Pour coloniser les surfaces inertes et assurer la liaison aux protéines hôtes.	(Chapman <i>et al.</i> , 2002)
Spidroïne	<i>Nephila edulis</i> (Araignée)	Pour former les fibres de soie de la toile.	(Römer et Scheibel, 2008)
CPEB*	<i>Aplisia californica</i> (Escargot marin)	Promouvoir le maintien à long terme des changements synaptiques associés au stockage en mémoire.	(Si <i>et al.</i> , 2003)

* Isoforme spécifique aux neurones du protéine de liaison aux éléments de polyadénylation cytoplasmique (Prion).

1.5 Matériaux bioinspirés des structures amyloïdes

En raison de leurs programmabilité structurale, polyvalence fonctionnelle, biocompatibilité et facilité de synthèse à grande échelle, les peptides d'autoassemblage amyloïdogéniques sont de plus en plus exploités comme bloc de construction offrant une grande diversité de propriétés chimiques, physiques, structurales, mécaniques et fonctionnelles (Adler-Abramovich *et al.*, 2014; Ulijn et Smith, 2008). Ainsi, les peptides amyloïdes ont été exploités pour concevoir et développer une panoplie de nanostructures ordonnées avec des caractéristiques structurelles contrôlables tels que les nanofibres (Hauser *et al.*, 2011; Xu. *et al.*, 2018), les nanotubes (Reches et Gazit, 2003), les nanosphères (Childers *et al.*, 2010; Krysmann *et al.*, 2008; Li *et al.*, 2016; Li *et al.*, 2014; Lu *et al.*, 2003; Reches et Gazit, 2004), les nanorubans (Adamcik *et al.*, 2016; Castelletto *et al.*, 2009) et les hydrogels (De Leon *et al.*, 2016; Loo *et al.*, 2014; Marchesan *et al.*, 2012; Mondal *et al.*, 2020; Yadav *et al.*, 2020). Récemment, l'intérêt s'est accru autour des applications biomédicales, biotechnologiques et nanotechnologiques polyvalentes de ces architectures fonctionnelles (Knowles et Mezzenga., 2016; Lai *et al.*, 2021; Wei *et al.*, 2017) .

1.5.1 Applications biomédicales

Les nanomatériaux amyloïdes sont de plus en plus exploités ces dernières décennies dans le domaine biomédical (Qi *et al.*, 2018), notamment la délivrance des médicaments (Panda et Chauhan, 2014; Wang *et al.*, 2019), l'ingénierie tissulaire (Panda et Chauhan, 2014), la bioimagerie (Lapshina *et al.*, 2019; Ni *et al.*, 2019) et la vaccination (Al-Halifa *et al.*, 2019; Hamley *et al.*, 2022; Zottig *et al.*, 2020). En effet, les peptides amyloïdes ont attiré une attention particulière en raison de leurs autoassemblage dynamique, diversité structurale et biocompatibilité, permettant de les exploiter comme bloc de construction de nanomatériaux capables de réduire la toxicité des médicaments, amélioré leur efficacité thérapeutique, contrôler leur voie d'administration et prolonger leur demi-vie *in vivo* (Adler-Abramovich *et al.*, 2014;

Ulijn et Smith, 2008). Par exemple, un promédicament peptidique, composé de quatre fragments, dont la camptothécine (CPT) comme chimiomédicament hydrophobe, le peptide LVFF comme motif d'autoassemblage, le peptide GFLG clivable par une enzyme et une chaîne hydrophile de polyéthylène glycol (PEG) lié au peptide d'adhésion RGD au niveau terminal. L'hybride résultant CPT-LVFF-GFLG-PEG-RGD s'auto-assemble en nanoparticules injectables par voie intraveineuse, puis s'accumule sous forme de nanofibrilles par action de la cathepsine B surexprimées dans les tumeurs. La perte de la partie hydrophile (PEG-RGD) déclenche une transformation morphologique autocatalytique et la libération prolongée de médicaments ce qui induit une inhibition tumorale efficace (Cheng *et al.*, 2019). Dans un autre exemple, les polypeptides amyloïdes sensibles au pH jouent également un rôle important dans l'administration de médicaments assistée par des nanomatériaux peptidiques. Ceci a été exploité pour développer un système de délivrance non invasif du vert d'indocyanine (ICG) et de la doxorubicine (DOX) encapsulés dans des nanofibres peptidiques auto-assemblées à partir de domaines centraux d'A β (Yang *et al.*, 2017). Le peptide de base est composé d'un motif bis-pyrène (BP), une séquence KLVFF, une chaîne PEG hydrophile et un motif peptidique sensible au pH (His6). Il a été révélé que le passage d'un pH 7,4 à 6,5 déclenche une transformation morphologique des nanoparticules auto-assemblées en nanofibres à feuillet- β . Il a été ensuite démontré que cette propriété d'autoassemblage sensible au pH est exploitable dans un microenvironnement tumoral acide, pour déclencher une accumulation accrue dans la tumeur ciblée *in vivo*. En parallèle, l'ICG et la DOX encapsulés à l'intérieur des nanofibres exercent, respectivement, un effet photothermique et une chimiotoxicité hautement efficaces pour la régression tumorale (Yang *et al.*, 2017).

En raison de leurs propriétés mécaniques et structurales uniques, les fibrilles amyloïdes deviennent de plus en plus des échafaudages attrayants pour des applications en ingénierie tissulaire (Reynolds *et al.*, 2019). Contrairement aux systèmes polymères conventionnels, ces nanostructures peptidiques offrent une morphologie nanofibrillaire requise par la matrice extracellulaire native. En effet, des réseaux à base d'assemblages

amyloïdes ont été formés en tant qu'échafaudages pour encapsuler des cellules souches, favorisant ainsi leur prolifération et leur différenciation, et encourager la migration et la régénération de cellules ou de tissus sains (Das *et al.*, 2018). La construction de matrice extracellulaire artificielle est une stratégie prometteuse pour le succès de l'ingénierie tissulaire en offrant un soutien physique et en encourageant les cellules à remplir leur fonction et leur migration. Les fibres amyloïdes sont capables d'imiter la morphologie nanofibrillaire de matrice extracellulaire et peuvent être modulées pour se lier aux cellules. A cet effet, une séquence peptidique (RGD-YIGSR) dérivée de la laminine (LN) a été fonctionnalisée avec un fragment amyloïde (KLVFF) et conjugué à une unité fluorescente de bis-pyrène (BP) (Hu *et al.*, 2017). Les nanoparticules ainsi résultantes sont capables de se lier spécifiquement aux intégrines et aux récepteurs LN des cellules tumorales via les ligands RGD et YIGSR respectivement, et simultanément s'auto-assembler sous forme de nanofibrilles induites par les interactions ligand-récepteur. D'une part, ce biomatériau est entré en compétition avec la matrice extracellulaire naturelle pour les sites de liaison sur les cellules, inhibant ainsi la liaison cellule-matrice extracellulaire et modifiant le microenvironnement des cellules tumorales. D'autre part, la capacité de ce biomatériau de s'auto-assembler *in situ* sous forme d'un réseau de nanofibres mimant la matrice extracellulaire permet de former une barrière du transfert cellulaire.

En raison de leurs propriétés de fonctionnalisation, ainsi que leurs biocompatibilité et biodégradation, les nanomatériaux à base de peptides amyloïdes sont de plus en plus exploités dans des plateformes de biocapteurs pour la détection ou l'imagerie avec des sensibilités et des spécificités améliorées (Al-Halifa *et al.*, 2019; Men *et al.*, 2009; Wang *et al.*, 2014). En effet, une plate-forme de nanofibres glycopeptidiques a été développée pour l'analyse de l'interaction entre A β et l'acide sialique ainsi que pour une détection efficace d'A β très sensible (Lei *et al.*, 2019). Pour ce faire, plusieurs acides sialiques ont été exposés à la surface d'une nanofibre peptidique auto-assemblée à partir d'un glycopeptide résultant d'une conjugaison par chimie-click entre un fragment 16-23 d'A β (Ac-KLVFFAL-NH₂) et l'acide sialique, ce qui a grandement

amélioré la spécificité et la sélectivité de liaison du peptide à $A\beta_{1-40}$. De plus, un système d'activation de la fluorescence à base d'un fragment $A\beta$ marqué par un fluorophore carboxyfluorescein (FAM) a permis de détecter et surveiller le processus d'agrégation du peptide $A\beta_{1-40}$ (Lei *et al.*, 2019).

D'autres travaux ont démontré que la séquence peptidique protégée au niveau du groupement amine en *N*-terminal (Fmoc-KLVFF) peut s'auto-assembler sous forme de nanoparticules fluorescentes suite à une coordination du zinc avec les résidus FF ainsi que des empilement π - π améliorés en présence du groupement protecteur Fmoc (Liu *et al.*, 2021). Il a été ensuite révélé que ces nanoparticules peuvent être utilisées pour la détection et quantification des différentes espèces $A\beta$ dans un environnement biologique complexe. Un autre exemple d'une plateforme à base de nanofibres fluorescentes sensibles à l'environnement, avec une capacité de bioimagerie spécifique et sensible, a été générée par l'autoassemblage d'une séquence peptidique composée principalement du 4-nitro-2,1,3-benzoxadiazole (NBD) comme fluorophore sensible à l'environnement, d'un peptide auto-assemblant (FFY) et d'un tripeptide de liaison au cuivre (GGH) (Cai *et al.*, 2014).

Il a été démontré que les nanofibres fluorescentes de NBD-FFYEEGGH peuvent se lier spécifiquement au Cu^{2+} , conduisant ainsi à la formation de nanofibres allongées avec une extinction de fluorescence ce qui a été exploité pour la détection de Cu^{2+} *in vitro* et dans les cellules. L'ajout d'un lien peptidique (DEVD) entre le peptide auto-assemblé FFY et le tripeptide GGH a permis de générer des nanofibres capables de surveiller l'activité de la caspase-3 par restauration de la fluorescence à la suite d'une libération du peptide de liaison Cu^{2+} par clivage enzymatique. En raison de l'absorption cellulaire améliorée et de la mobilité réduite au sein des cellules, cette plate-forme pourrait fournir une imagerie non invasive et en temps réel de l'activité enzymatique *in situ*. Enfin, un biocapteur électrochimique à ions métalliques a été développé par immobilisation de nanofibres peptidiques auto-assemblées à partir d'une séquence riche en cystéine sur une électrode en or pour la détection des ions cuivre en solution

(Viguier *et al.*, 2011). Il a été, de même, démontré qu'il est possible de réutiliser ce capteur après sa régénération dans un état sans cuivre.

1.5.1.1 Structure amyloïde comme plate-forme de livraison d'antigènes

La vaccination vise à déclencher principalement une réponse immunitaire contre les agents pathogènes envahisseurs. A cet effet, deux réponses immunitaires distinctes peuvent avoir lieu au niveau de l'organisme hôte. La première est une réponse immunitaire innée qui consiste à une reconnaissance des agents pathogènes par les macrophages et les cellules dendritiques via des récepteurs de reconnaissance de motifs moléculaires (PRR ; pattern recognition receptor), tels que les récepteurs de type toll (TLR ; toll-like receptors). Les cellules vont alors s'activer, ce qui déclenche une cascade d'événements, notamment la phagocytose, pour éradiquer les agents pathogènes. Par ailleurs, si l'hôte n'est plus capable d'éliminer les agents pathogènes par son système immunitaire inné, il peut réagir en développant une réponse immunitaire adaptative. Ce système adaptatif implique des lymphocytes T et B agissant par reconnaissance spécifique des antigènes présentés par l'agent pathogène. Le système immunitaire mémorise alors les empreintes des agents envahisseurs ce qui aide à activer l'immunité adaptative et prévenir, de ce fait, l'infection provoquée par des agents pathogènes. A cet effet, des nanovaccins à base de suprastructures décorés à leur surface par des antigènes capables de déclencher une réponse immunitaire et répondre, par leurs caractéristiques uniques, aux limitations associées à l'utilisation de vaccins conventionnels (vivants et inactivés), tels que retour à la virulence, l'inactivation incomplète et des réponses immunitaires parfois faibles. Ainsi, les fibrilles amyloïdes ont été récemment exploitées pour améliorer l'immunogénicité intrinsèque des nanomatériaux biocompatibles et biodégradables et développer des plateformes vaccinales pour la livraison d'antigènes (Hamley, 2022). Contrairement aux peptides monomériques peu immunogènes et dont l'épitope est soumis à une dégradation protéolytique, les assemblages peptidiques sont plus stables, résistants à la protéolyse et peuvent porter de multiples copies de déterminants antigéniques, ce qui peut

déclencher une réponse immunitaire plus forte. Certains peptides d'autoassemblage tels que Q11 (QQKFQFQFEQQ) et KFE8 (FKFEFKFE) ont été exploités récemment en tant que nanostructures adjuvantes pour la conception de nanovaccins sous-unitaires (Chesson *et al.*, 2018; Rudra *et al.*, 2012; Rudra *et al.*, 2010). Les adjuvants sont des molécules immunostimulantes capables d'améliorer la réponse immunitaire d'un antigène sans présenter par elle-même une activité antigénique. Des études immunologiques ont révélé que les nanostructures fibrillaires en feuillets β -croisés décorées en surface avec des déterminants immunogènes des cellules B et/ou T sont immunostimulantes et conduisent à des titres élevés d'anticorps contre les antigènes présentés (Huang *et al.*, 2012; Rudra *et al.*, 2010). De plus, il a été rapporté que le peptide amyloïde I₁₀, dérivé du domaine amyloïdeogène de l'IAPP, attaché à l'épitope immunogène E₂EP₃ de la glycoprotéine E₂ du virus Chikungunya ou à un épitope de la protéine matricielle de la grippe A (M2e) par un court espaceur flexible, peut activer les voies de signalisation NF- κ B sans affecter de manière significative la viabilité cellulaire dans une lignée cellulaire exprimant les récepteurs hétérodimère TLR2/TLR6 (Al-Halifa *et al.*, 2020). Une stratégie de co-assemblage a permis de préparer des vaccins multicomposants sous forme de nanofilaments peptidiques à feuillets- β -croisés exposant à leur surface un épitope M2e et un agoniste des TLR (Bricha *et al.*, 2023). En plus de leur effet protecteur contre la protéolyse de l'antigène incorporé et leur dépôt au site d'injection, qui semble être l'effet adjuvant potentiel, la structure quaternaire en feuillet- β -croisé des vaccins à base de fibrilles active également la réponse immunitaire innée via l'inflammasome. En revanche, l'utilisation de fibrilles longues riche en feuillet- β croisées dans la vaccination reste limitée en raison de leur polymorphisme et la difficulté à contrôler le processus d'autoassemblage.

1.5.2 Applications en nanotechnologie

Outre les applications mentionnées auparavant, les fibrilles amyloïdes ont été utilisées pour la fabrication d'autres nanomatériaux fonctionnels bioinspirés pour des applications en nanotechnologie. La conjugaison *in situ* de nanotubes à base de peptide

autoassemblé (FF) avec des complexes de lanthanides a permis de générer des nanotubes photoluminescents (Ryu *et al.*, 2009). Ceci résulte d'un effet synergique entre les nanotubes peptidiques et le photosensibilisateur via un mécanisme de transfert d'énergie en cascade. Les fibrilles amyloïdes ont également trouvé des applications importantes dans les sciences de l'environnement, tel qu'illustré par des fibrilles amyloïdes à base de β -lactoglobuline utilisées pour former des membranes hybrides capables de purifier des eaux usées (Bolisetty et Mezzenga, 2016). En plus de la possibilité de les recycler, ces membranes ont montré une capacité à éliminer sélectivement les ions métalliques lourds. D'autres nano-biosorbants à base de fibrilles amyloïdes de lysozyme ont également montré un effet efficace pour éliminer des colorants polluants dans l'eau (Leung *et al.*, 2015). Substituées par de éthylènediamine, pour réduire leur teneur en carboxyle, ces fibrilles ont montré une capacité d'adsorption des ions toxiques de chrome (Cr^{VI}). Ces études ouvrent la porte vers le développement de nouveaux matériaux à base de fibrilles amyloïdes fonctionnalisés pour éliminer des polluants dans le traitement des eaux usées.

1.5.2.1 Matériaux optoélectroniques

Inspirée par la grande efficacité du transport de charge dans les systèmes biologiques et la conductivité unique des nanostructures peptidiques, l'incorporation de nouveaux nanomatériaux peptidiques hybrides dans des nanodispositifs électroniques a été suggérée (Tu *et al.*, 2013). Des nanofils conducteurs de type noyau-coquille ont été préparés à base d'une matrice de nanofibrilles amyloïdes sur laquelle vient s'adsorber, via des interactions hydrophobes, une polyaniline comme polymère conducteur (Meier *et al.*, 2015). La conductivité des fibrilles hybrides revêtues s'est avérée largement améliorée, ce qui a montré que les fibrilles amyloïdes fournissent un excellent support structural pour le dépôt dirigé de polymères conducteurs, et ouvre la porte vers une nouvelle génération de dispositifs microélectroniques. Inspiré des pili conducteurs de la bactérie *Geobacter*, un biofilm conducteur a été récemment fabriqué en introduisant des acides aminés aromatiques dans une séquence de la protéine Curli (Dorval *et al.*,

2018; Lovley *et al.*, 2011; Nguyen *et al.*, 2014). Les résidus aromatiques sont de plus en plus incorporés dans des séquences amyloïdogéniques pour aider leur autoassemblage et stabiliser les suprastructures résultantes. Ces noyaux aromatiques, lorsqu'ils sont correctement alignés, peuvent former des nuages d'électrons π délocalisés sur une longue distance et devenir conducteurs (Kalyoncu *et al.*, 2017; Walker *et al.*, 2018). De la même façon, des mutants de la protéine CsgA ont été conçus *in silico*, où il a été encore démontré expérimentalement que l'incorporation de noyaux aromatiques peut améliorer efficacement la conductivité du biofilm formé (Dorval *et al.*, 2018). Plusieurs autres études ont exploité les fibrilles amyloïdes, co-assemblées avec des polymères conjugués luminescents ou incorporant des complexes organométalliques phosphorescents, pour fabriquer des diodes électroluminescentes organiques (Herland *et al.*, 2005; Solin et Inganäs, 2012; Tanaka *et al.*, 2008). D'autres études ont exploité les peptides d'autoassemblage en feuillets- β pour diriger l'assemblage de systèmes π -conjugués sous forme de nanostructures robustes et hautement ordonnées ce qui a aidé à améliorer leurs propriétés optoélectroniques telles que le transport de charge en faveur des nuages π délocalisés sur une longue distance (Ardoña et Tovar, 2015; Chen *et al.*, 2015; Khalily *et al.*, 2017; Krieg *et al.*, 2016; Singha *et al.*, 2019). Par exemple, le perylène diimide (PDI), un semiconducteur organique, a été conjugué symétriquement avec à un heptapeptide court (IKHLSVN) dérivé d'une interface β -continue de la protéine bovine peroxirédoxine-3, pour améliorer son hydrosolubilité et guider son autoassemblage en suprastructures organiques semi-conductrices (Eakins *et al.*, 2015).

1.6 Problématique et objectifs

L'amyloïde est une structure quaternaire organisée, stable et non ramifiée, composée d'unités répétitives de brins β à partir de monomères polypeptidiques auto-assemblés via un réseau de liaisons hydrogène intermoléculaires (Mukhopadhyay *et al.*, 2014). Historiquement, ces agrégats protéiques ont été étudiés dans le cadre de diverses pathologies dégénératives, où des protéines solubles s'agrègent suite à un repliement anormal pour former des dépôts amyloïdes insolubles qui s'accumulent dans les

organes, perturbant ainsi leur bon fonctionnement (Harrison *et al.*, 2007). Néanmoins, des assemblages amyloïdes fonctionnels ont été découverts chez la majorité des espèces et ceux-ci sont impliqués dans de nombreux processus physiologiques essentiels (Fioriti *et al.*, 2015; Fowler *et al.*, 2006; Maji *et al.*, 2009). Ces découvertes combinées aux propriétés mécaniques, chimiques et biologiques intéressantes des structures amyloïdes ont conduit les chercheurs à entrevoir l'utilisation de celles-ci en tant que matériaux bio-inspirés pour des applications en vaccinologie (Al-Halifa *et al.*, 2020), en biodétection (Hauser *et al.*, 2014), en régénération tissulaire (Reynolds, 2019), ou pour des installations photovoltaïques (Barrau *et al.*, 2008). Ces exemples démontrent le potentiel d'applications de ces nanostructures protéiques et encouragent à poursuivre les investigations pour élucider les bases mécanistiques et structurales de l'autoassemblage et de la diversité morphologique des amyloïdes. Néanmoins, le processus d'assemblage peut générer des espèces protéotoxiques transitoires ayant des effets délétères sur les systèmes biologiques, et ceci demeure un défi à relever pour le développement rationnel de stratégies thérapeutiques et prophylactiques. C'est dans ce contexte que s'inscrit l'objectif global de cette thèse de doctorat qui vise à explorer les contributions structurales et fonctionnelles guidant le processus d'autoassemblage et à concevoir de nouvelles nanostructures pour des applications ciblées. Pour ce faire, les trois objectifs spécifiques suivants seront adressés :

1. Étudier la capacité de séquences amyloïdes à guider l'autoassemblage de systèmes π -conjugués en nanostructures fibrillaires semi-conductrices et biocompatibles.
2. Exploiter les paramètres physico-chimiques et structuraux qui modulent la morphologie et la taille de nanostructures amyloïdes afin de comprendre les bases moléculaires de leurs propriétés immunomodulatrices.
3. Évaluer les effets de nanostructures d'ADN sur le processus d'autoassemblage de peptides amyloïdes et sur les propriétés cytotoxiques des différentes structures quaternaires engendrées.

CHAPITRE II

SEMICONDUCTIVE AND BIOCOMPARIBLE NANOFIBRILS FROM THE SELF-ASSEMBLY OF AMYLOID π -CONJUGATED PEPTIDES

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Contribution des auteurs :

Nadjib Kihal : Conception du projet, exécution des expériences (synthèse, purification et caractérisation des molécules), résonance magnétique nucléaire, microscopie à force atomique, spectroscopie de fluorescence, spectroscopie UV-Visible, spectroscopie de dichroïsme circulaire, spectroscopie FTIR, diffraction des rayons-X, AFM-conductive, test de viabilité cellulaire (résazurine et LIVE/DEAD), microscopie confocale, analyses des résultats, préparation des figures, rédaction de l'article.

Mélanie Côté-Cyr : Exécution des expériences (microscopie électronique à transmission)

Ali Nazemi : Supervision du projet, rédaction et correction de l'article.

Steve Bourgault : Supervision du projet, rédaction et correction de l'article.

2.1 Résumé

En raison de leur capacité d'autoassemblage sous forme de nanostructures organisées, les peptides amyloïdes peuvent servir de bloc de construction pour la conception de matériaux semi-conducteurs biocompatibles. Dans cette étude, des peptides amyloïdes π -conjugués, symétriques et asymétriques, ont été préparés par condensation du pérylène diimide (PDI) avec une séquence amyloïdogène naturelle dérivée de l'islet amyloid polypeptide (IAPP). Ces PDI-bioconjugués se sont auto-assemblés sous forme de nanofilaments longs et linéaires en solution aqueuse, caractérisés par une organisation quaternaire riche en feuillets- β croisés. Les courbes de courant-tension ont montré une signature claire de semi-conducteurs tandis que les analyses cellulaires ont révélé une cytocompatibilité et une application potentielle en microscopie à fluorescence. Bien que l'incorporation d'une seule séquence amyloïde se soit avérée suffisante pour guider l'autoassemblage en fibrilles organisées, l'incorporation de deux séquences peptidiques aux positions imides du PDI a considérablement amélioré la conductivité des films à base de nanofibrilles. Dans l'ensemble, cette étude a exposé une nouvelle stratégie basée sur un peptide amyloïdogène pour guider l'autoassemblage de systèmes π -conjugués sous forme de nanofilaments robustes, biocompatibles et optoélectroniques.

2.2 Abstract

Owing to their capacity to self-assemble into organized nanostructures, amyloid polypeptides can serve as scaffolds for the design of biocompatible semiconductive materials. Herein, symmetric and asymmetric amyloid π -conjugated peptides were prepared through condensation of perylene diimide (PDI) with a natural amyloidogenic sequence derived from the islet amyloid polypeptide. These PDI-bioconjugates assembled into long and linear nanofilaments in aqueous solution, which were characterized by a cross- β -sheet quaternary organization. Current-voltage curves exhibited a clear signature of semiconductors whereas the cellular assays revealed cytocompatibility and potential application in fluorescence microscopy. Although the incorporation of a single amyloid peptide appeared sufficient to drive the self-assembly into organized fibrils, the incorporation of two peptide sequences at the PDI's imide positions significantly enhanced the conductivity of nanofibril-based films. Overall, this study exposes a novel strategy based on amyloidogenic peptide to guide the self-assembly of π -conjugated systems into robust, biocompatible, and optoelectronic nanofilaments.

2.3 Introduction

Bioelectronics aims at developing tools and approaches to sense, stimulate and/or manipulate biological systems by combining knowledge from electronics and biology (Ohayon et Inal, 2020; Simon *et al.*, 2016). Semi-conductive nanodevices have shown great potential and applications in clinical neuroengineering (Sunwoo *et al.*, 2020), health monitoring and disease diagnosis (Lee *et al.*, 2021), wearable and implantable biomedical devices (Choi *et al.*, 2019; Sunwoo *et al.*, 2021), and in precision therapy (Wu *et al.*, 2017). Numerous bioelectronic devices are composed of π -conjugated organic semiconducting nanomaterials, which allow very effective charge transfer (Hoeben *et al.*, 2005). Among π -conjugated systems, perylene diimide (PDI)-derived molecules have received a great deal of attention for their optoelectronic, physicochemical, and/or structural properties that can be modulated by the modification of their substituents (Huang *et al.*, 2011; Kihal *et al.*, 2022). In addition to their chemical robustness and thermal stability, PDIs are known for their propensity to self-assemble into diverse supramolecular architectures that modulate their spectroscopic and semiconductive properties. While PDI self-assembly is mainly driven by π - π stacking interactions, it can be dramatically altered by environmental conditions, such as solvent polarity, pH, concentration, and temperature as well as the chemical nature and the position of their substituents (Son *et al.*, 2014). Notwithstanding that PDI-based nanostructures have been exploited as pigments (Greene *et al.*, 2009), photovoltaics (Zhang *et al.*, 2022), dye lasers (Sadrai et Bird, 1984), and sensors (Chen *et al.*, 2020), their use in bioelectronics has been marginally investigated. This is partly due to poor solubility of PDI derivatives in aqueous media which precludes their self-assembly into organized semiconductive nanostructures and their use in numerous biological systems (Huang *et al.*, 2011; Würthner, 2004). To address this limitation, substitutions have been reported to enhance the hydrosolubility of PDIs and to modulate their assembly (Sun *et al.*, 2016). Particularly, substituents in *imide* position are well-suited to control solubility in aqueous solutions, while having limited effects on the electronic properties of PDIs due to the presence of nodes at the

HOMO and LUMO of imide nitrogens (Hartnett *et al.*, 2015; Würthner, 2004). At the same time, the chemical nature of the imide substituents has a significant impact on the self-assembly behavior and the morphology of the resulting assemblies, ultimately affecting semiconducting properties of the PDI-conjugated nanostructures (Chen *et al.*, 2015; Huang *et al.*, 2011; Würthner, 2004). Over the last two decades, conjugation of biomacromolecules, such as short peptides and DNA oligonucleotides, to PDI units has been investigated to promote solubility in aqueous medium and to modulate self-assembly into well-ordered hybrid nanomaterials, ultimately guiding function (Chen *et al.*, 2015; Kihal *et al.*, 2022; Würthner *et al.*, 2016). In fact, biomacromolecules are well-known for their unique capacity to self-associate into distinct nanoarchitectures, while being biocompatible and easily functionalizable. Such properties can be harnessed to design bioelectronic nanodevices.

Recent discoveries indicating that proteinaceous amyloid fibrils perform vital biological activities in host organisms have encouraged their potential use in life-inspired biomaterials, nanodevices and matrices (Gazit, 2014; Mankar *et al.*, 2011). Whereas amyloids have been historically associated with pathological states, (Chiti et Dobson, 2017) advances in the molecular understanding of protein misfolding disorders have shown that amyloid deposits likely play a protective role by sequestering toxic oligomers (Bemporad et Chiti, 2012; Chiti et Dobson, 2017). Moreover, today, functional amyloids have been identified in almost all species, from bacteria to mammals (Audas *et al.*, 2016; Maji *et al.*, 2009; McGlinchey *et al.*, 2009). In addition, synthetic amyloidogenic peptides are particularly attractive owing to their ease of synthesis and functionalization, programmable aggregation, biocompatibility and degradability (Al-Halifa *et al.*, 2019; Chen et Zou, 2019). Amyloid assemblies are structurally organized in a cross- β -sheet quaternary conformation, in which individual β -strands are running perpendicular to the fibril axis and the β -sheets are tightly packed on one another (Tycko et Wickner, 2013). At the mesoscopic level, prototypical amyloid fibrils, with a diameter of 4 to 15 nm, are organized hierarchically with 2 to 10 protofilaments than can either be linearly packed or helically twisted (Wei *et al.*,

2017). Particularly, the spontaneous self-recognition of amyloidogenic peptide sequences into well organized, stable, and biocompatible nanostructures appears attractive to template the self-assembly of π -conjugated molecules into robust and semiconductive nanofilaments under aqueous, biologically relevant conditions.

So far, amino acids and very short peptides have been employed as directing groups to guide the self-assembly of PDI moieties (Bai *et al.*, 2014; Eakins *et al.*, 2014; Eakins, Pandey, *et al.*, 2015; Eakins, Wojciechowski, *et al.*, 2015; Panda *et al.*, 2021; Wei *et al.*, 2019). For instance, the PDI core was symmetrically conjugated with the FF self-assembling dipeptide, and the resulting PDI-[FF]₂ nanofibers exhibited superior semiconducting properties in comparison to their corresponding nanospheres and nanorings (Ahmed *et al.*, 2018; Ahmed *et al.*, 2017). Interestingly, PDI was conjugated to a short heptapeptide (IKHLSVN) derived from a β -continuous interface of the bovine peroxiredoxin-3 protein, to increase its hydrosolubility and to guide its self-assembly into organic semiconductive nanostructures (Eakins *et al.*, 2015). However, owing to the reversibility of the assembly process and to their low thermodynamic stability, the β -sheet networks formed through β -continuous interfaces are known to dissociate upon dilution (Valéry *et al.*, 2013). These properties retaining from their parent β -continuous interfaces can limit their use as stable semiconductive nanostructures. Conjugation of π -conjugated systems on robust self-assembling macromolecules could lead to the identification of stable nanomaterials exhibiting long-range order, ultimately favoring efficient intermolecular charge delocalization of the π -electron cloud and enhancing charge mobility. The present study aims at investigating the use of a natural amyloidogenic peptide sequence to guide the self-assembly of PDI molecules into highly ordered biocompatible semiconductive nanofilaments. We suggest that the programmable assembly of amyloid peptide scaffolds, through a delicate balance of hydrophobic interactions and hydrogen bonding, could confer robust directionality to the formation of stable π -conjugated nanosystems. To the best of our knowledge, this is the first example of PDI functionalization of a self-assembling amyloid peptide. Our results revealed that PDI

conjugation to the amyloid peptide does not only ensure solubility of π -conjugated molecules in aqueous solution, but also directs long-range spatial organization of π -conjugates into optoelectronic materials.

2.4 Materials and methods

2.4.1 Materials

Fmoc-Gly-OH ($\geq 98\%$), Fmoc-L-Ser(tBu)-OH ($\geq 98.0\%$), Fmoc-L-Ala-OH (98%), Fmoc-L-Phe-OH (98%), Fmoc-L-Ile-OH ($\geq 98\%$), Fmoc-L-Leu-OH ($\geq 98\%$), Fmoc-L-Lys(Boc)-OH ($\geq 98\%$), Fmoc-L-Asn(Trt)-OH ($\geq 98\%$), *O*-(1*H*-6-Chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HCTU) and Fmoc-6-Ahx-OH were purchased from Matrix Innovation. Perylene-3,4,9,10-tetracarboxylic dianhydride ($\geq 97\%$), *N,N*-diisopropylethylamine (DIPEA), trifluoroacetic acid (99%), imidazole ($\geq 99\%$) were purchased from Sigma-Aldrich. Piperidine was purchased from A&C American Chemicals Ltd. All solvents were of analytical grade and were purchased from Sigma-Aldrich. Deionized water was used to prepare buffer solution (pH 7.4). All solvents, reagents and other chemicals were used as received without further purification.

2.4.2 Synthesis, Purification and Characterization

Synthesis of the asymmetric (PDI-I₁₀) and symmetric (PDI-[I₁₀]₂) PDI-substituted peptide derivatives was achieved via two steps. First, condensation of 3,4,9,10-perylenetetracarboxylic dianhydride (PTCDA) with L-leucine (L) in molten imidazole led to a PDI derivative harboring two leucine residues at the imide positions (Xu *et al.*, 2007). The resulting PDI-[L]₂ was characterized by ¹H-NMR spectroscopy (300 MHz, DMSO-*d*₆): δ 8.51-8.27 (m, 8H), 5.45 (t, 2H), 2.13 (m, 4H), 1.66 (m, 2H), 1.02 (dd, 12H). Second, the PDI-peptide derivatives were obtained by coupling PDI-[L]₂ to the N-terminal amine group of the peptidyl-resin (AhxKKGSGSSNNFGAILSS) in *N,N*-

dimethylformamide (DMF) overnight at room temperature (Figure 2.1). The peptide sequence was initially elongated by means of standard Fmoc solid-phase peptide synthesis (SPPS) using HCTU and DIPEA coupling strategy. For the bi-substituted symmetric PDI-peptide (PDI-[I₁₀]₂), 0.5 eq of PDI-[L]₂ was coupled to the *N*-terminus of the peptide elongated on a Rink amide resin having a moderate loading of 0.55 mmol/g. For mono-substituted asymmetric PDI-peptide (PDI-I₁₀), 12 eq of PDI-[L]₂ was used for the coupling to a Rink amide peptidyl-resin with low loading (0.4 mmol/g). After acidic cleavage from the solid support using a cocktail of trifluoroacetic acid (TFA)/phenol/H₂O and precipitation in ethyl ether, peptide derivatives were purified using reverse-phase high-performance liquid chromatography (HPLC). Peptide identity was confirmed by liquid chromatography coupled with high-resolution mass spectrometry (Figures S2-S3), as previously described (Babych *et al.*, 2018). Desired fractions of PDI-conjugates amyloid peptide, with a purity higher than 95%, were collected, combined, and lyophilized.

2.4.3 Self-assembly of Amyloid π -conjugated Peptides

Peptide solutions were prepared by dispersing the lyophilised powder into selected solvent at a self-assembling concentration of 500 μ M. Peptide mixtures were incubated for one week (168 h) at room temperature (RT) and under constant circular agitation at 40 rpm.

2.4.4 Nuclear Magnetic Resonance Spectroscopy (NMR)

¹H-NMR spectra were recorded in DMSO-*d*₆ using a Bruker-300 UltraShield NMR spectrometer (300 MHz). Chemical shifts are given in parts per million (ppm) and reported in delta (δ) units.

2.4.5 UV-visible Spectroscopy (UV-vis)

UV-vis absorption spectra were recorded from 400 to 700 nm on Mandel UV-1280 spectrophotometer. The light path length was 10 mm, and the internal step was set to 1 nm. Samples were prepared by diluting the initial PDI-peptide stock solutions to 50 μM in the corresponding solvent.

2.4.6 Degree of Aggregated Molecules (α_{agg})

The α_{agg} is estimated based on the following equation

$$\alpha_{agg} = \frac{A_i - A_m}{A_a - A_m} \quad (1)$$

where A_i is the $A_{0 \rightarrow 0}$ at the given time, A_m is the $A_{0 \rightarrow 0}$ at monomeric state, approximately taken at the time-zero, and A_a is the absorbance in the aggregation state after one-week incubation. (Echue *et al.*, 2015)

2.4.7 Fluorescence Spectroscopy (FL)

Fluorescence measurements were carried out at RT on a Photon Technology International (PTI) steady-state spectrofluorometer (QuantaMaster 40) and operated with PTI's FelixGX software with light pathlength of 3 mm. Excitation wavelength was set at 504 nm and the emission was recorded from 520 to 700 nm. Peptide solutions were diluted to 50 μM in the corresponding solvent.

2.4.8 Circular Dichroism Spectroscopy (CD)

CD spectra were recorded from 190 to 260 nm on Jasco J-815 spectrophotometer at room temperature, with light pathlength of 1 or 2 mm. Peptide solutions were diluted in water to reach a concentration of 50 μM . Resulting CD spectra were smoothed using a smoothing moving windows of 13 data points through Savitsky-Golay algorithm.

2.4.9 Critical Aggregation Concentration (CAC)

The CAC was determined using the pyrene fluorescent dye.(Babych *et al.*, 2021) Initially, an ethanolic pyrene stock solution (1 mM) was diluted in the corresponding buffer. Peptides were respectively solubilized in the pyrene aqueous solution, keeping pyrene concentration constant at 2 μ M. The excitation wavelength was set at 335 nm and the emission spectra was monitored from 350 to 450 nm. CAC was determined by plotting the ratio of the intensities of the first emission band at 373 nm and the third emission band at 384 nm (I_1/I_3), as function of peptide concentration in logarithmic scale.

2.4.10 Atomic Force Microscopy (AFM)

Peptide solutions were first diluted to reach a final concentration of 50 μ M in 1% acetic acid and were immediately spotted onto a freshly cleaved mica surface. Acetic acid was added just prior deposition to facilitate the adsorption of the peptide assemblies on the mica surface. Excess liquid was removed by wicking, washed twice with deionized water, and air-dried overnight. AFM images were acquired using a Bruker (MultiMode 8) equipped with a Nanoscope E controller. Samples were scanned in scanasyst-air mode using a silicon nitride AFM tip (2–12 nm tip radius, $k = 0.4$ N/m) under the ambient conditions. Images were analysed using Gwyddion v2.59.

2.4.11 Transmission Electron Microscopy (TEM)

Peptide solutions were diluted to a final concentration of 50 μ M and loaded onto 400-mesh copper grids. Excess liquid was removed, and samples were negatively stained with 1.5% uranyl formate and air-dried overnight. TEM analyses were performed using

FEI Tecnai G2 Spirit Twin transmission electron microscope operated at 120 kV and equipped with a Gatan Ultrascan 4000 4k x 4k CCD camera system.

2.4.12 Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy

PDI-peptide assemblies obtained after 168 h incubation were isolated by centrifugation and dried with nitrogen stream. ATR-FTIR spectra were recorded using a Nicolet Magna 560 spectrometer equipped with a nitrogen-cooled MCT detector.

2.4.13 X-ray Diffraction (XRD)

PDI-peptide solutions (500 μM), incubated for one week, were deposited on an X-ray diffraction lamella and dried with nitrogen stream. Powder XRD measurements (current = 40 mA and voltage = 40 mV) were performed using a Bruker X-ray diffractometer (D8 Advance). Diffractograms were analyzed using X'pert data software and interplanar spacings were determined from powder raw pattern (2θ), that satisfies the conditions of Bragg's law.

2.4.14 Electronic Measurements

Interdigitated electrodes (5 μm parallel bands with 3 μm intra-band spacing) were photolithographically patterned onto a silicon wafer with 100 nm Au and a 10 nm Ti adhesion layer deposited by electron beam evaporation and using a shadow mask. Prior to use, devices were sonicated in acetone, isopropanol, and ultrapure water. 5 μL of peptide solutions diluted at 50 μM were deposited onto cleaned surface of interdigitated electrodes and dried overnight. Dried films were rinsed with ultrapure water to remove salts, dried under N_2 flow and I - V measurements were performed with conductive AFM using a Bruker (MultiMode 8) with Nanoscope E controller in TUNA mode with doped diamond-coated electrical probes (Antimony (n) doped Si, 100-150 nm tip radius, $k =$

0.2 N/m). Scans were first done in contact mode with a 0 V bias to determine the position of the edge of the electrode. The current response of the nanofibers was monitored in ± 10 V bias range. As results, *I-V* curve for each PDI-conjugate was constructed by median current values at a specific voltage applied on definite area of nanofibers-based films.

2.4.15 Cell Viability Assays

Chinese hamster ovary cells K1 (CHO-K1) cells were cultured in black wall clear bottom 96-well plates at a density of 7.5 k cells per well in Ham's F12 media containing 10% of fetal bovine serum (FBS), 10 kUI/mL penicillin, 10 kUI/mL streptomycin and 2 mM L-glutamine (Quittot, N. *et al.*, 2021). After 48 h incubation in a humidified atmosphere of 5% CO₂ and 95% air at 37 °C, cells were treated by the direct addition of peptide nanofilaments (from 0.1 to 10 μ M). Cells were incubated for 24 h, and cellular viability was evaluated using the resazurin metabolic assay. Cell viability was determined in % from the fluorescence ratio of peptide-treated sample to vehicle-treated control (20 mM Tris-Buffer, pH 7.4). Data of a least three independent assays achieved in triplicate were averaged and expressed as the mean \pm standard deviation (SD). For LIVE/DEAD assays, CHO-K1 cells were seeded in 8-well plates at a density of 25 k cells per well for 48 h before treatments. Cells were treated with 10 μ M peptide assemblies and then incubated for 24 h. Fluorescent microscopy analysis were performed 45 min after addition of 4 μ M ethidium homodimer-1 and 2 μ M calcein-AM reagent solutions. ImageJ software was used for images analysis.

2.4.16 Confocal Microscopy

CHO-K1 cells were seeded in 8-well plates at a density of 50 k cells/well for 48 h before treatment. Cells were then treated with 10 μ M peptide assemblies and then incubated for 6 hours. After removing the culture media, cells were extensively washed

with PBS, and fixed with 4% paraformaldehyde for 10 min. Cells were washed twice with PBS and the plasma membrane and nucleus were respectively stained with 1 U/mL of dilinoleyloxacarbocyanine perchlorate (*FAST DiO*TM) and 100 ng/mL of 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) for 25 min. Cells were washed two times with PBS, and a confocal Nikon microscope with a 60X oil immersion objective was used for fluorescence analysis. Nikon imaging software was used for image acquisition and analysis.

2.5 Results and discussion

2.5.1 Design and Synthesis of PDI-conjugated Self-assembling Amyloid Peptides

Figure 2.1 illustrates the design of amyloid π -conjugated peptides and their directional self-assembly into nanofilaments. We took advantage of the high self-recognition propensity of the central 20-29 region of the islet amyloid polypeptide (*I*₁₀) to guide PDI organization into ordered conductive and biocompatible nanofilaments. We have previously reported that the *I*₁₀ sequence (SSNFGAILSS) has a very robust capacity to self-assemble into polymorphic twisted unbranched fibrils characterized by a cross- β -sheet quaternary structure, which tolerates functionalization of 18 to 22-mer peptide antigens (Al-Halifa *et al.*, 2020; Babych *et al.*, 2018). A flexible tetrapeptide linker (GSGS) was added at the N-terminus of the amyloid core followed by two charged lysine (KK) residues. The KK motif was added to promote solubility in aqueous solution whereas the GSGS linker, which is commonly used in protein engineering (Arslan *et al.*, 2022), was incorporated in order that the PDI does not interfere with the self-assembly process driven by the amyloid segment. The PDI unit (PDI-[L]₂) previously functionalized in solution by a leucine at both imide positions (Figure S2.1), was attached to the peptidyl-resin by means of a short aliphatic 6-amino hexanoic acid (Ahx) linker to provide a low steric hindrance between the PDI core and the self-recognition sequence. This rational design provides water-soluble π -conjugated peptides, which should readily self-assemble into ordered nanofilaments

allowing long-range intermolecular charge delocalization of π -electron cloud. Hybrid π -conjugated peptides were obtained by conjugating the PDI-[L]₂ derivative to the elongated peptidyl resin, leading to an asymmetric (PDI-I₁₀) or a symmetric (PDI-[I₁₀]₂) structure (Figure 2.1). To control the stoichiometry of PDI coupling to the peptidyl-resin, we employed different loadings of the solid support as well as varying equivalents of PDI-[L]₂. PDI-I₁₀ derivative was easily obtained through a stoichiometric control using 12 eq of PDI-[L]₂ and a Rink amide resin with a moderate loading of 0.4 mmol/g. In contrast to strategies commonly used to obtain asymmetric PDI-derivatives involving partial hydrolysis of PDI or PTCDA (Kihal *et al.*, 2022; Nagao *et al.*, 1997), this innovative strategy to access a stoichiometric control is straightforward and led exclusively to the target mono-substituted PDI. The symmetric conjugate PDI-[I₁₀]₂ was obtained by employing 0.5 eq of PDI-[L]₂ and a Rink amide support with a higher loading of 0.55 mmol/g, favoring concurrent conjugation of PDI to two elongated peptides. This novel approach to gain stoichiometric control by means of resin loading and PDI-reagent equivalents constitutes a straightforward strategy for introducing peptides at one, or both, sides of PDIs.

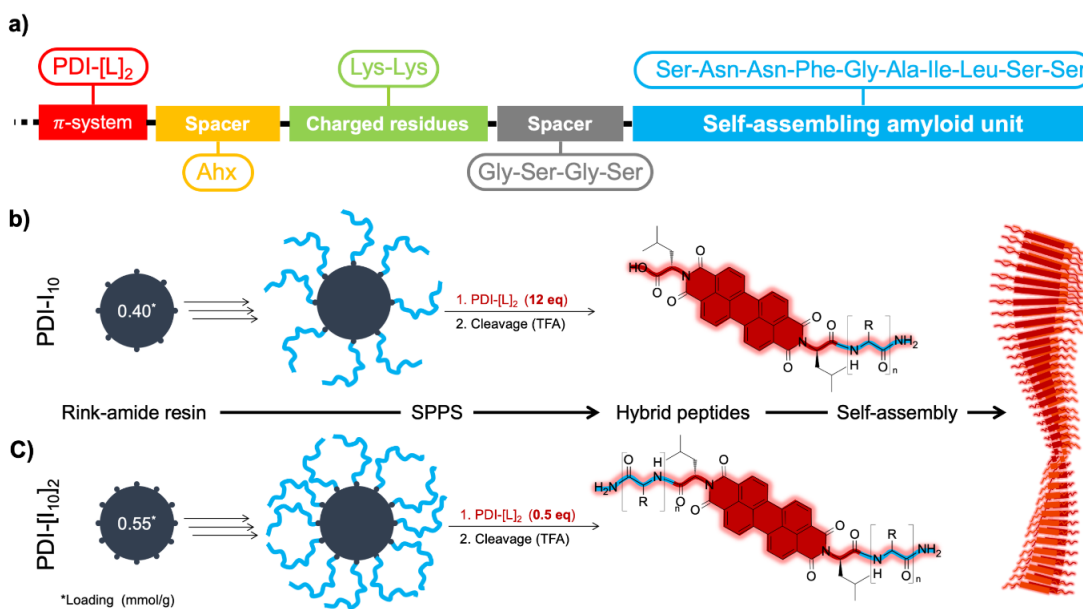


Figure 2.1 Design and synthesis of PDI-conjugated amyloid peptides. (a) Schematic representation of peptide sequence, including the amyloid segment, a GSGS spacer and two charged residues. The PDI molecule is attached to *N*-terminus of peptide sequence via an Ahx linker. (b,c) Schematic representation of the design and synthesis of PDI-conjugated amyloid peptides and their self-assembly into cross- β -sheet nanofilaments. (b) PDI-I₁₀ and (c) PDI-[I₁₀]₂. (b, c). Synthesis and cleavage conditions: 1. PDI-[L]₂/DIPEA/HCTU/DMF; 2. TFA/ Phenol/H₂O. SPPS: Solid phase peptide synthesis.

2.5.2 Self-assembly of PDI-[I₁₀]₂ Symmetric Derivative Under Different Conditions

We initially evaluated the self-assembly of PDI-[I₁₀]₂ in different solvents (Figure 2.2), as the nature of the solvent can dramatically modulate the process of PDI aggregation as well as the resulting supramolecular architectures (Ahmed *et al.*, 2018; Kihal *et al.*, 2022). Except for H₂O and dimethyl sulfoxide (DMSO), the symmetric π -conjugated peptide has very limited or no solubility in other polar protic or aprotic solvents, such as methanol (MeOH), acetonitrile (MeCN), and tetrahydrofuran (THF). However, it became soluble when these organic solvents were mixed with 50% water. This is associated with the known poor solubility of long peptide chains in organic solvents, such as the 32-residue long PDI-[I₁₀]₂ peptide, for which solubility generally requires water. Absorption and emission spectra profiles of PDIs are known to be sensitive to

the interchromophore distance and orientation of the PDI cores (Hestand et Spano, 2018; Würthner *et al.*, 2016). It is also worth noting that absorption and emission spectra of PDIs are highly sensitive to the polarity, viscosity, polarizability, and dielectric constant of solvent (Durga *et al.*, 2020). For this purpose, A_{0-0}/A_{0-1} ratio of the dual transition bands, where A_{0-0} and A_{0-1} are the absorption corresponding to the $0 \rightarrow 0$ and $0 \rightarrow 1$ vibronic transitions from ground to first excited singlet states ($S_{0 \rightarrow 1}$) (Hestand et Spano, 2018), is widely regarded as an indicator of the aggregation of PDI moiety. The large A_{0-0}/A_{0-1} ratio (~ 1.60) for PDI-[I₁₀]₂ in DMSO (Table S2.1) suggests the presence of PDIs in their monomeric state (Shang *et al.*, 2022; Wang, X. *et al.*, 2017). The monomeric nature of this symmetric PDI-peptide in DMSO was also confirmed by the corresponding strong emission spectra which is a mirror image with the absorption one, in accordance with the Franck-Condon principle (Figure S2.4) (Zhang et Hoshino, 2014). Additionally, TEM and AFM analyses revealed the absence of nanostructures upon 168 h incubation, and no visual quenching of the fluorescence was observed. Similarly, no spectral change was noticed in presence of 50% THF and MeCN aqueous mixtures (Figure 2.2a, S2.5a and S2.5b), and no visual fluorescence extinction (Figure 2.2b), or aggregates (Figure 2c), were observed after one week of incubation. In sharp contrast, in 50% MeOH and DMSO aqueous mixtures, formation of aggregates of varying morphologies (Figures 2.2c, S2.5c and S2.5d) was accompanied with reversal of the vibronic A_{0-0} and A_{0-1} bands (Figure 2.2a), and a drastic fluorescence quenching after one-week of incubation (Figure 2.2b). In DMSO/H₂O, precipitates were noticed by naked eyes upon incubation, as also exposed by the light scattering at 600 nm of the large precipitates formed in the solution (Figure 2.2b), whereas no precipitates could be observed in all the other solvent systems. Interestingly, nanofibers were accessible by using water as the sole self-assembly medium (Figure 2.2) and these filaments remain soluble over incubation time. Such self-assembly condition, *i.e.* excluding the use of organic co-solvents, is highly desirable not only in the context of the development of conductive ordered nanostructures but also opens the door towards their safe use in biomedical applications. For this purpose, we next studied the kinetics of fibrillization in an aqueous solution.

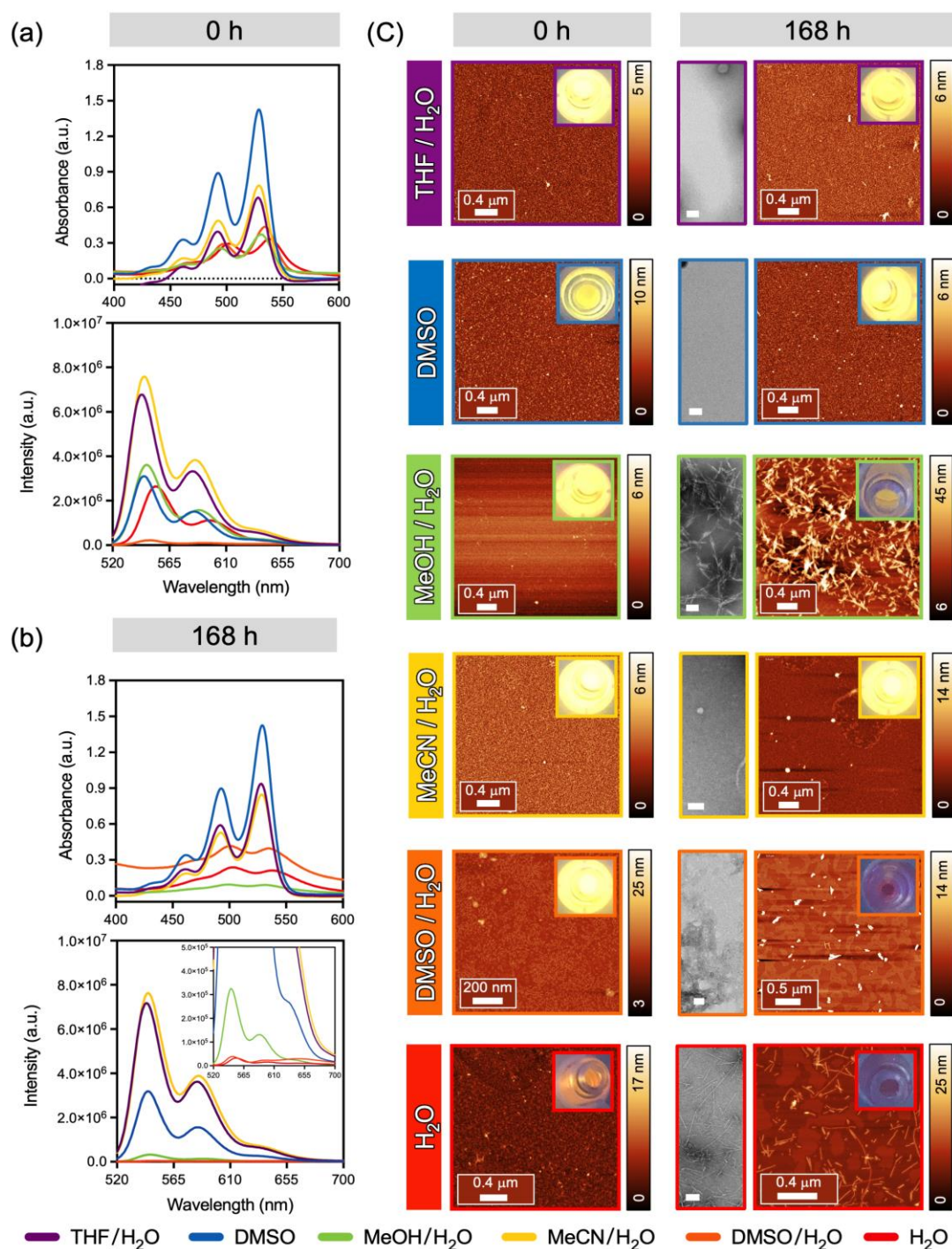


Figure 2.2 Self-assembly of symmetric PDI-[I₁₀]₂ peptide under different conditions. UV-vis spectra (top) and emission spectra (bottom) of PDI-[I₁₀]₂ in different solvents at (a) 0 h and (b) after 168 h incubation. (c) AFM images at 0 h and TEM (left) and AFM (right) images at 168 h of PDI-[I₁₀]₂. The scale bar for TEM images is 100 nm. The inset showed in (c) represent the digital photos of the samples taken under irradiation of a 365 nm UV light.

2.5.3 Kinetics of Self-assembly of the Symmetric PDI-[I₁₀]₂ Peptide in Aqueous Solution

Next, we investigated the kinetics of self-assembly of PDI-[I₁₀]₂ in Tris-HCl buffer at pH 7.4 to gain mechanistic insights and to elucidate when the self-assembly process reaches equilibrium. First, UV-vis absorbance and fluorescence measurements were periodically performed during the incubation of PDI-[I₁₀]₂ in Tris-HCl buffer (pH 7.4) under constant agitation to follow the appearance of intramolecular π - π -stacking of PDI moieties. Considering our previous work with the I₁₀ sequence (Zottig *et al.*, 2019) and that agitation is consistently used in order to accelerate kinetics of aggregation and to improve the reproducibility of experimental results (Giehm *et al.*, 2011), self-assembly was conducted under constant rotatory agitation to reach the equilibrium phase of amyloid formation within a reasonable timeframe. Initially, the absorption bands centred at 539 and 502 nm, respectively, corresponding to the 0 \rightarrow 0 and 0 \rightarrow 1 transitions, have a Franck-Condon ratio of 1.03 favoring the S₀ \rightarrow S₁ transition (Wang *et al.*, 2017; Bag *et al.*, 2019). This ratio indicates that in aqueous buffer, PDI-[I₁₀]₂ unimers are initially in dynamic equilibrium with their H-type assembled oligomeric species (Bag *et al.*, 2019). The absorption spectra of PDI-[I₁₀]₂ showed a reversal intensity of the vibronic 0 \rightarrow 0 and 0 \rightarrow 1 bands over the incubation time (Figure 2.3a), confirming a transition from soluble monomers or small oligomers to extended aggregates characterized by H-type cofacial π - π stacking of PDI units, which is essential to form conductive networks within the material (Giaino *et al.*, 2008; Hartnett *et al.*, 2015). The corresponding fluorescence spectra further supported the presence of unimers or small aggregates with a well-resolved vibronic structure at time-zero, and the conversion into H-aggregates over time (Figure 2.3b). This distinct spectral characteristic has been used as a tool to assess the organization mode of PDI molecules into H- or J-type aggregates (Clark *et al.*, 2007). Such intramolecular interactions became more enhanced over time, supported by an increase of α_{agg} and a decrease in the A₀₋₀/A₀₋₁ ratio of PDI-[I₁₀]₂ in Tris-HCl buffer (Figure S2.7), and further verified by the decrease of the strong emission band intensity at \sim 552 nm over time (Figure

2.3c). As shown in Figure 2.3c, these spectroscopic transitions as a function of time for this symmetric bio-conjugate, fitted very well to a sigmoidal curve characteristic of amyloid formation (Echue *et al.*, 2015). Unfortunately, we could not evaluate the kinetics of peptide-PDI self-assembly using the amyloid-sensitive dye thioflavin T (ThT) (Godin *et al.*, 2019; Sebastiao *et al.*, 2017), as its fluorescence overlaps with PDI emission. Thus, kinetics of aggregation was evaluated by AFM imaging at 0 h and after 24 h, 48 h, 72 h, and 1-week of incubation, where the formation of a much denser network of filaments over incubation time was observed (Figure 2.3d).

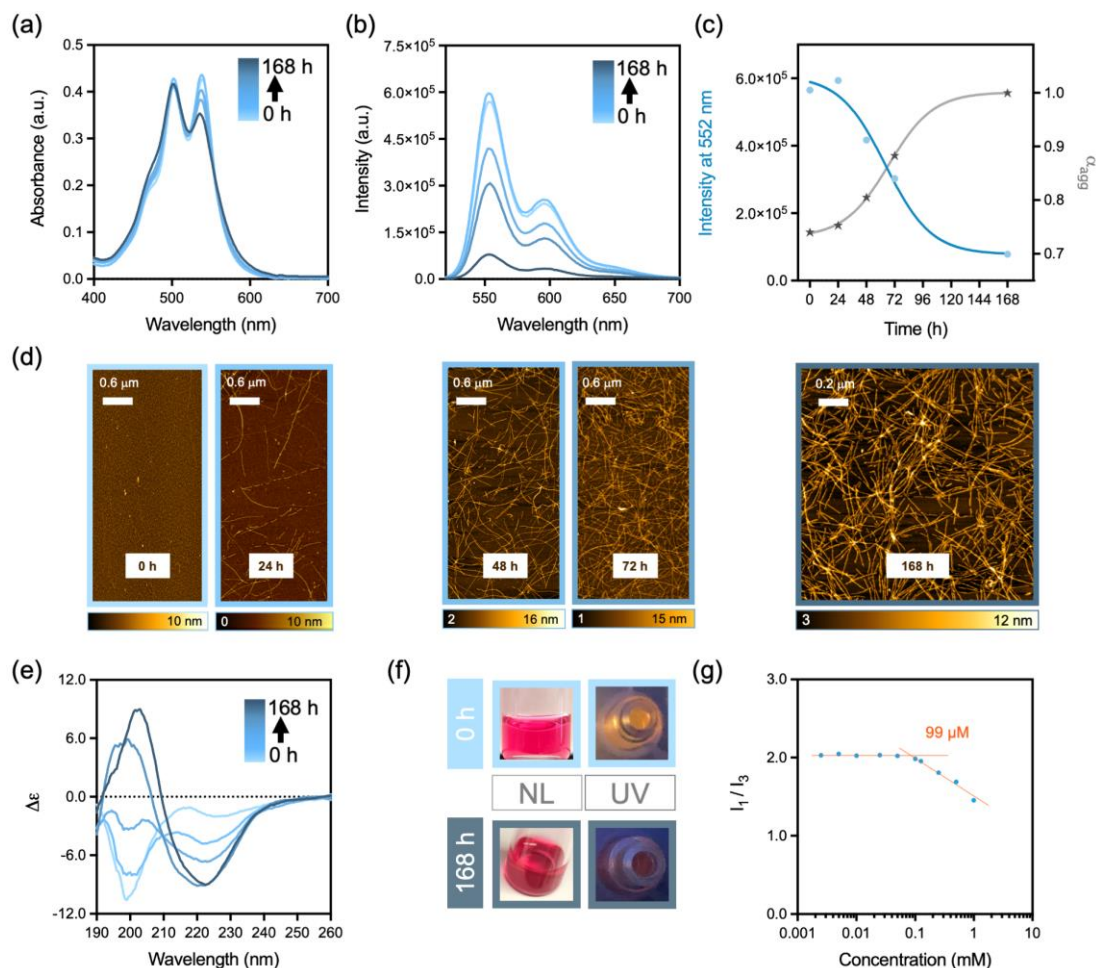


Figure 2.3 Kinetics of self-assembly and critical aggregation concentration of PDI-[I₁₀]₂. (a) UV-vis spectra, and (b) fluorescence emission spectra. The graphics of (c) represent the ratio of emission intensity at 552 nm and degree of aggregated molecules over time. (d) AFM images of PDI-[I₁₀]₂, and (e) far-UV CD spectra. (a-e) PDI-[I₁₀]₂ was incubated in Tris Buffer, pH 7.4, at a concentration of 500 μ M and under continuous agitation at room temperature, and the analysis were conducted at 0 h and after 24 h, 48 h, 72 h, and 1 week of incubation. The inset of (f) represent the digital photos of the samples taken under natural light (NL) and UV irradiation at 365 nm of light at 0 h and after 1-week incubation. (g) Determination of CAC for PDI-[I₁₀]₂ by pyrene fluorescence.

To gain further insight into the kinetics of self-assembly and to evaluate if this process is indeed guided by the amyloidogenic peptide sequence through directional hydrogen bonding, far UV circular dichroism (CD) spectroscopy was employed. Immediately after the resuspension of PDI-[I₁₀]₂ in Tris-HCl buffer (0 h), a CD spectrum

characterized with a single minimum at 202 nm (Figure 2.3e), representative of a random coil secondary structure, was observed. After 24 h incubation, a CD spectrum with two minima at 202 and 225 nm was observed, indicative of a partial transition into ordered secondary structure. Upon prolonged incubation, a clear transition in the spectra characterized with a single minimum at around 225 nm, typical for β -sheet rich secondary structure, was observed. An evident transition occurred during self-assembly and a clear isosbestic point could be observed (Figure 2.3e), indicative of a two-state conformational conversion of the π -conjugated peptide from random coil secondary structure to β -sheet rich amyloid. In contrast, the PDI-[L]₂ molecule without the amyloidogenic sequence promptly aggregated in small amorphous clumps (Figure S2.6), suggesting that the I₁₀ peptide drives the self-assembly process into organized nanofilaments. Particularly, ellipticity value at 202 nm shown in Figure S2.7 represents the structural transition of symmetric derivative over incubation time. Furthermore, all curves obtained from CD, FL and UV-vis analysis fitted very well to an amyloid typical sigmoidal curve, also supporting the fact that the peptide amyloidogenic unit controls self-assembly. Additionally, the formation of assemblies could be visually observed by fluorescence, as exemplified by the notable quenching of fluorescence after 1-week of incubation (Figure 2.3f). Finally, we evaluated the critical aggregation concentration (CAC) of PDI-[I₁₀]₂ using pyrene, a probe that is sensitive to the polarity of the local environment (Cao *et al.*, 2017). A sharp transition from unimers to assemblies was observed when the concentration exceeded 99 μ M (Figure 2.3g).

2.5.4 Molecular Architecture of Symmetric PDI-[I₁₀]₂ Nanofilaments

Fourier transform infrared spectroscopy (FTIR) was used to probe the secondary structure within the nanoassemblies by recording the amide I (1700–1600 cm^{-1}), amide II (1510 and 1580 cm^{-1}) and amide III (near 1290 cm^{-1}) regions of the spectrum (Mallamace *et al.*, 2015; Parker *et al.*, 1971). Attenuated total reflectance (ATR) allowed us to characterize the assembled nanofilaments at low concentration. It has

been previously reported that amyloid fibrils derived from IAPP₂₀₋₂₉ contain both parallel and antiparallel β -strands and this could be at the origin of the observed macroscopic polymorphism (Zottig *et al.*, 2019). Analysis of the amide I region allows to differentiate between parallel and antiparallel β -sheet (Miyazawa et Blout, 1961). For antiparallel β -sheet, the amide I region usually displays two typical peaks at $\sim 1630\text{ cm}^{-1}$ and at $\sim 1695\text{ cm}^{-1}$ (Chirgadze et Nevskaya, 1976a). However, in the parallel β -sheet organization, only one characteristic peak is observed at around 1630 cm^{-1} (Chirgadze et Nevskaya, 1976b). FTIR spectra obtained for PDI-[I₁₀]₂ assemblies were characterized by amide I, amide II and amide III peaks at 1630 , 1550 and 1290 cm^{-1} , respectively (Figure 2.4a). The peak at 1630 cm^{-1} is suggestive of a parallel β -sheet secondary organization. Although no clear band at 1695 cm^{-1} was detected, a shoulder peak at around 1656 cm^{-1} was noticed, which could be associated with some heterogeneity of the secondary structure and/or with presence of traces of trifluoroacetate (CF_3COO^-) salts (Horvat *et al.*, 1997). This proposed parallel orientation of β -sheets fits well with the absorption and fluorescence emission spectra of PDI, which were prototypical of stable π - π stacking interactions between adjacent PDIs.

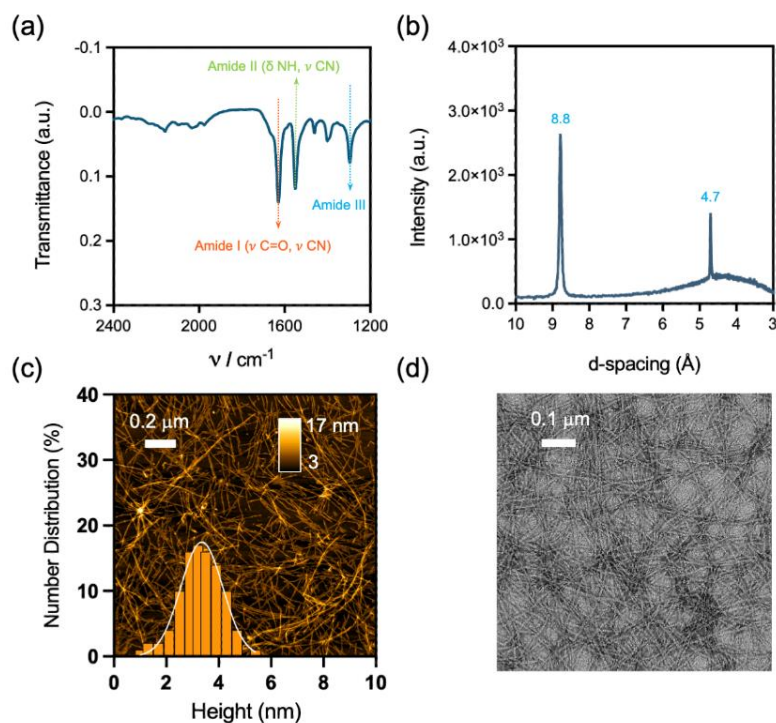


Figure 2.4 Molecular organization of PDI-[I₁₀]₂ nanostructures. (a) FTIR spectrum and (b) XRD patterns of PDI-[I₁₀]₂ powder dried under nitrogen. (c) AFM and (d) TEM images with quantification of width of nanofilaments. (a-d) PDI-[I₁₀]₂ was assembled in Tris Buffer, pH 7.4, at a concentration of 500 μM and under continuous agitation at room temperature for 1 week.

To confirm the cross- β -sheet suprastructure organization of the fibrils, we employed powder X-ray diffraction (XRD) (Morris et Serpell, 2012). The characteristic X-ray diffraction pattern of cross- β amyloid fibrils has two characteristic signals, a sharp reflection at 4.7 Å spacing along the meridian (vertical) in the fibril axis and an diffuse reflection at between 8-10 Å spacing along the equator (horizontal), perpendicular to the fibril axis. XRD analysis of PDI-[I₁₀]₂ assemblies revealed a diffraction pattern characterized with two sharp Bragg reflections peaks corresponding to 4.7 and 8.8 Å periodic spacing (Figure 2.4b), indicating that the presence of the PDI core did not modify the molecular packing at atomistic level of amyloid fibrils. These typical reflection peaks observed at 4.7 and 8.8 Å for PDI-[I₁₀]₂ assemblies reflect the inter-strand and the inter-sheet spacing characterizing the cross- β structure, respectively (Del

Pozo-Yauner *et al.*, 2015). AFM and TEM images revealed a homogenous distribution of long, unbranched and interwind nanofilaments with an average diameter of 3.27 ± 0.60 nm (Figures 2.4c and 2.4d). As shown in Figure S2.9a, the charged residues and the spacers connecting I₁₀ sequence to PDI had a minimal effect on fibril organization, with I₁₀ peptide showing a dense network of unbranched filaments.

2.5.5 Self-assembly of the Asymmetric PDI-I₁₀ Peptide in Aqueous Solution

Having access to an asymmetric peptide-conjugated PDI through stoichiometric control of conjugation on solid support, we then studied the self-assembly of PDI-I₁₀ in aqueous buffer. Asymmetric PDI-conjugated I₁₀ showed a reversal intensity of the vibronic 0→0 and 0→1 bands (Figure 2.5a), accompanied with decrease of fluorescence emission over the incubation time (Figure 2.5b), as observed for the symmetric PDI-[I₁₀]₂. In addition, the A_{0→0}/A_{0→1} ratio decreased over incubation time (Figure S2.7), indicating that the aqueous buffer promotes the transition from unimers, or small oligomeric species, to well-organized extended H-aggregates. These observations also demonstrate that a single self-assembling amyloid peptide at the imide position is sufficient to drive the organized aggregation of PDI moieties. Particularly, similar kinetics of self-assembly were also verified by the increase of α_{agg} and the decrease of the strong emission band intensity at ~552 nm over time (Figure 2.5c), that fitted very well to a sigmoidal curve characteristic of amyloid formation. AFM imaging allowed us to observe the formation of a network of filamentous assemblies, although short protofibrils could also be observed on the mica after 168 h incubation (Figure 2.5d). In addition, ultraviolet range CD analysis revealed a secondary conformational transition of the peptide backbone from a random coil to a β -sheet structure (Figure 2.5e and S2.7). Furthermore, the notable quenching of fluorescence after 1-week of incubation could be a visual signature of the PDI-I₁₀ assemblies' formation (Figure 2.5f). Finally, the CAC measurements of PDI-I₁₀ showed a sharp transition from unimers to assemblies when the concentration exceeded 109 μ M (Figure 2.5g).

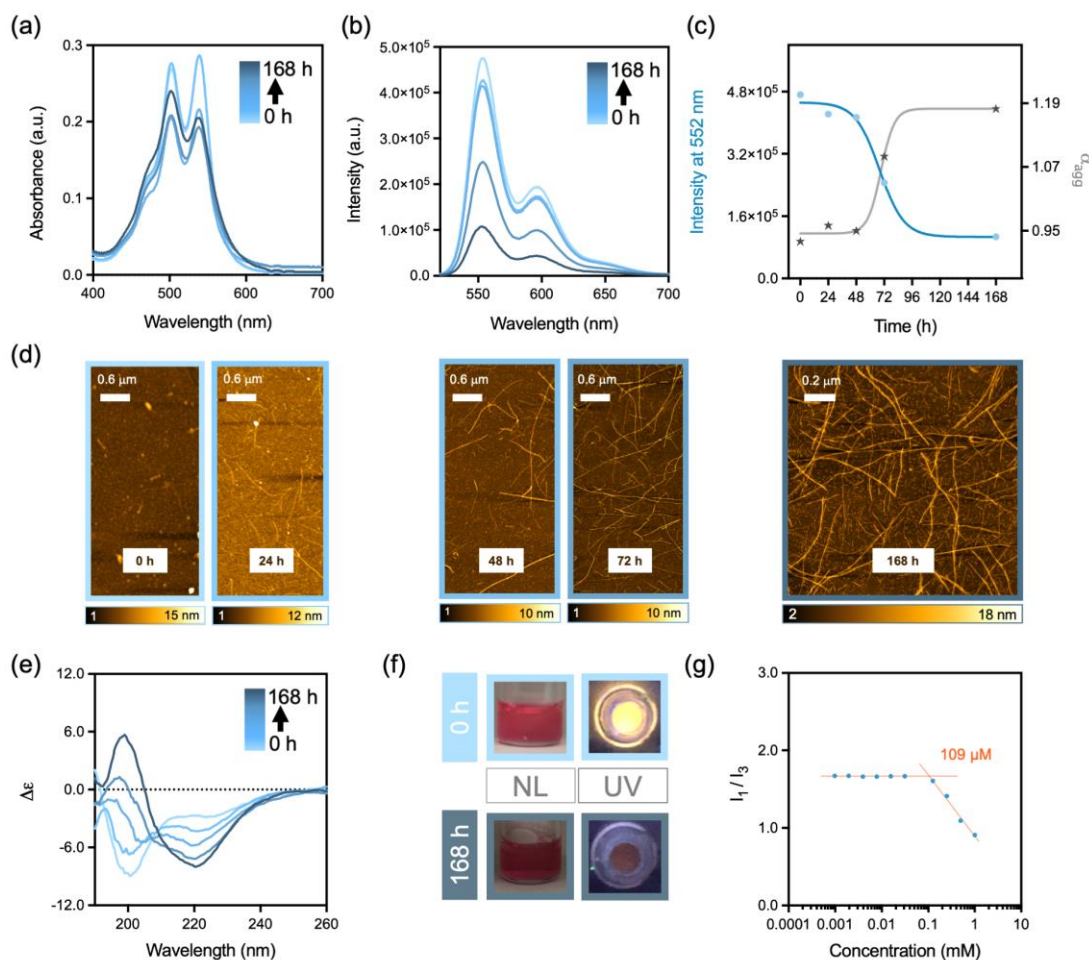


Figure 2.5 Kinetics of self-assembly and critical aggregation concentration of PDI-I₁₀. (a) UV-vis spectra, and (b) fluorescence emission spectra. The graphics of (c) represent the ratio of emission intensity at 552 nm and degree of aggregated molecules over time. (d) AFM images of PDI-I₁₀, and (e) ultraviolet range CD spectra. (a-e) PDI-I₁₀ was incubated in Tris Buffer, pH 7.4, at a concentration of 500 μM and under continuous agitation at room temperature, and the analysis were conducted at 0 h and after 24 h, 48 h, 72 h, and 1 week of incubation. The inset of (f) represent the digital photos of the samples taken under natural light (NL) and UV irradiation at 365 nm of light at 0 h and after 1-week incubation. (g) Determination of CAC for PDI-I₁₀ by pyrene fluorescence.

ATR-FTIR analyses revealed a characteristic amide I band at 1630 cm^{-1} , with the absence of a characteristic band of an antiparallel β -sheet at 1695 cm^{-1} (Figure 2.6a), suggesting a parallel orientation of the β -sheets. Nonetheless, as observed for PDI-[I₁₀]₂

filaments, a shoulder at $\sim 1656\text{ cm}^{-1}$ was detected, which could relate to traces of CF_3COO^- salts. The parallel orientation for the PDI-peptide assemblies is likely supported by a balanced contribution of directional peptide hydrogen bonding and weak π - π stacking interactions between adjacent PDIs. XRD of PDI-I₁₀ assemblies revealed two reflection peaks at 4.7 and 8.8 Å, characterizing the cross- β structure (Figure 2.6c). In contrast to PDI-[I₁₀]₂, AFM and TEM analyses revealed a heterogeneous distribution of short protofibril-like assemblies and long nanofilaments for the PDI-I₁₀ derivative (Figure 2.6c and 2.6d). While the average height of PDI-I₁₀-derived nanostructures is $3.42 \pm 0.98\text{ nm}$, two distinctive populations could be observed; the very short protofibrils showed an average height of $2.87 \pm 0.64\text{ nm}$, whereas the long nanofilaments exhibited an average height of $4.92 \pm 0.57\text{ nm}$ (Figure 2.6c). The discrepancy in the height of these two populations of assemblies suggest that the long PDI-I₁₀ fibrils could be organized with two protofilaments, allowing the PDIs to be buried from the aqueous solution within the fibrils. In contrast, the short protofibrils could be constituted of a single stack and these protofibrils cannot grow without associating with another protofibrils because of the thermodynamic penalty imposed by the aqueous solvent exposition of the PDI hydrophobic molecule. Such organization and cooperative self-assembly has already been proposed for asymmetrical PDI nanostructures (van der Weegen *et al.*, 2013). Regarding the PDI-[I₁₀]₂ conjugate that showed a homogenous distribution of long and linear nanofilaments with an average height of $3.27 \pm 0.59\text{ nm}$ (Figure 2.4), these fibrils could be organized with a single stack of peptides and the resulting protofilament helically twisting around its central axis. This supramolecular organization, which has been proposed previously for symmetrical PDIs bisubstituted with short peptides or branched alkyl chains (Görl *et al.*, 2012), would allow the PDI core to be buried from the water.

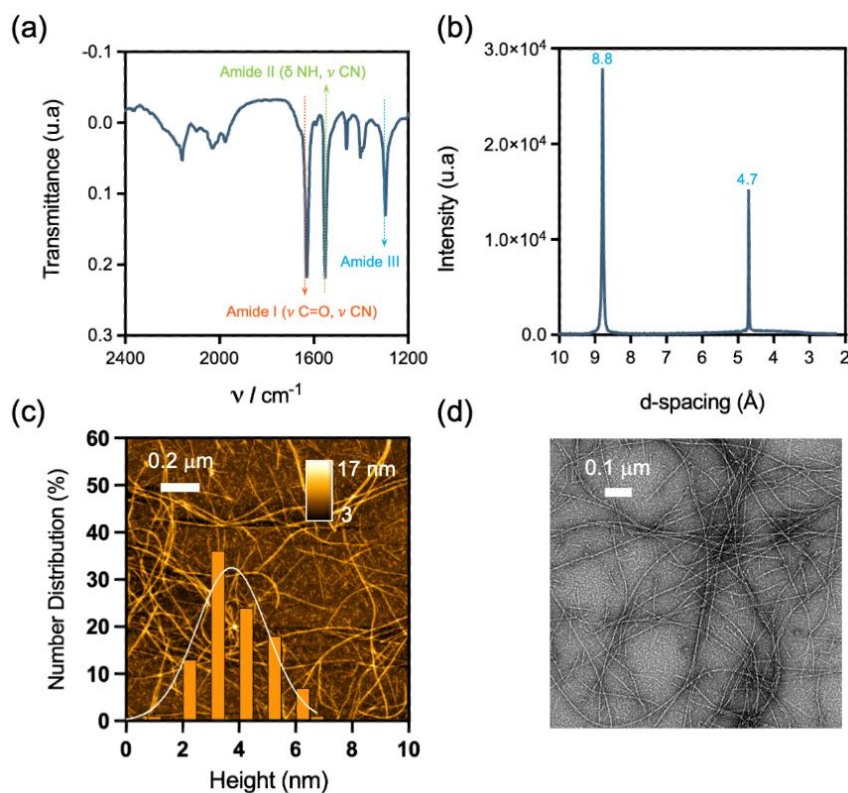


Figure 2.6 Molecular organization of PDI-I₁₀ nanofilaments. (a) FTIR spectrum and (b) XRD patterns of PDI-I₁₀ powder dried under nitrogen. (c) AFM and (d) TEM images with quantification of width of nanofilaments. (a-d) PDI-I₁₀ was assembled in Tris Buffer, pH 7.4, at a concentration of 500 μM and under continuous agitation at room temperature for 1 week.

2.5.6 PDI-functionalized Amyloid Fibrils are Cytocompatible and Can Be Imaged by Fluorescence Microscopy

Bioelectronic nanomaterials are expected to provide biocompatibility with living organisms for safe use in (nano)biomedicine. Historically, amyloid structures have been associated and studied in the context of protein misfolding disorders pathologies. Nevertheless, it is now generally assumed that cell degeneration in these diseases is associated with oligomeric intermediates and several functional amyloid fibrils have been identified (Jackson et Hewitt, 2017; Kaye et Lasagna-Reeves, 2013). Whereas PDI derivatives functionalized with amino acids or very short peptide sequences have demonstrated low cytotoxicity (Kim, Y. O. *et al.*, 2018; Muthuraj *et al.*, 2015), the

cytocompatibility of PDI-conjugated proteinaceous nanofilaments have never been evaluated so far. Accordingly, we evaluated the cytocompatibility of the PDI-I₁₀ and PDI-[I₁₀]₂ semiconductive nanofilaments using CHO-K1 cells. First, to assess potential cytotoxicity, cells were treated with increasing concentrations of pre-assembled PDI-I₁₀ and PDI-[I₁₀]₂ nanofilaments for 24 h and metabolic activity of living cells was measured by resazurin reduction. Results revealed that PDI-I₁₀ and PDI-[I₁₀]₂ assemblies were fully cytocompatible within the evaluated concentration range (Figure 2.7a). It is worth mentioning that amyloid fibrils, such as those assembled from I₁₀ peptides, are highly stable and do not dissociate, or disassemble, upon their dilution at low concentrations (Zottig *et al.*, 2020). In fact, we confirmed the stability of the pre-assembled nanofilaments upon 1 week incubation in culture media at a concentration of 10 μ M by AFM imaging (Figure S2.8). Moreover, no toxicity was observed using the LIVE/DEAD assays (Figure 2.7b), as CHO-K1 cells treated with 10 μ M pre-assembled fibrils showed a similar green/red cell ratio to the vehicle treated control. Ethidium homodimer-1 red fluorescence correlates with the loss of cell membrane integrity that occurs late in the cell death process, while calcein-AM green fluorescence is associated with intracellular esterase activity of living cells.

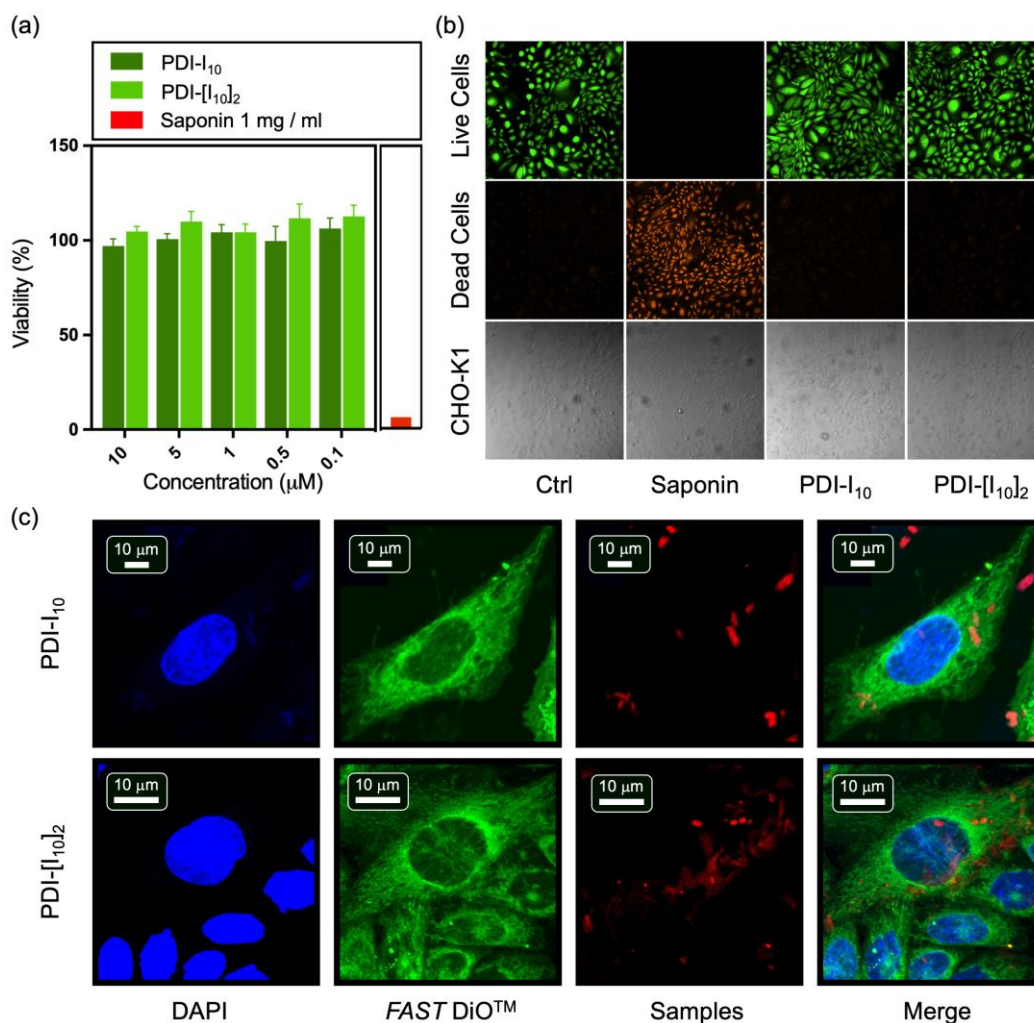


Figure 2.7 Cytocompatibility and fluorescence imaging of PDI-functionalized nanofilaments. (a) CHO-K1 cells were incubated for 24 h with increasing concentrations of pre-assembled nanofilaments, or Saponin (1 mg/ml), and resazurin was used to measure metabolic activity. (b) CHO-K1 cells were incubated for 24 h with 10 μM of PDI-I₁₀ or PDI-[I₁₀]₂ pre-assembled nanofilaments and cell viability was evaluated by staining with calcein AM (green; live cells) and ethidium homodimer (red; dead cells). (c) Confocal microscopy images of CHO-K1 cells after 6 h incubation with 10 μM of PDI-I₁₀ (top) and PDI-[I₁₀]₂ (bottom) assemblies (red: fibrils, blue: DAPI, and green: FAST DiOTM).

Next, as the PDI-conjugated amyloid fibrils showed moderate fluorescence intensities, we were intrigued to evaluate if their interactions with cells could be visualized by fluorescence confocal laser scanning microscopy. As shown in Figures 2.7c and S2.9, PDI-functionalized fibrils could be observed in red (*ex*: 488 nm; *em*: 700 nm) and were localized at the cell surface as well as in the cytoplasm. The distribution of red cytoplasmic puncta revealed that these nanofilaments could be internalized, likely by endocytosis, a typical pathway for nanoparticles (Couceiro *et al.*, 2015; Manzanares et Ceña, 2020). As previously reported for amyloid fibrils, it is possible that PDI-I₁₀ and PDI-[I₁₀]₂ fibrils are taken up by cells via micropinocytosis (Jin *et al.*, 2016). Thus, the moderate fluorescence emission of the assemblies seems sufficient for fluorescence imaging and combined with their cytocompatibility, PDI-functionalized amyloid fibrils could be employed for live-cell imaging experiments in combination with applications in bioelectronics.

2.5.7 Nanofilaments Assembled From PDI-conjugated Amyloid Peptide Show Robust Semiconducting Properties

Electrical measurements were conducted on nanofiber-based films deposited on interdigitated gold electrodes. Interdigitated gold electrodes have been previously used for measuring the electrical conductivity of nanofiber-based films (Creasey *et al.*, 2019). Conductive atomic force microscopy (C-AFM) was used to map the conductive surface of nanofiber-based films formed with pre-assembled PDI-I₁₀ and PDI-[I₁₀]₂ in aqueous solution. The C-AFM is a scanning probe technique that simultaneously measures the topography and the electric current flow with nanoscale resolution by scanning the surface with a conductive material coated tip as nanoelectrode at an applied bias voltage. In addition, current-voltage (*I-V*) measurements on an area of nanofiber-based films were collected to investigate the ohmic behavior. Surface topography revealed a uniform distribution of peptide-based nanofilaments on the interdigitated electrode surface (Figure 2.8a and 2.8b). High conductivity ($I > 1.0$ nA) was measured for symmetric PDI-[I₁₀]₂ peptide-based film at 10 V scanning voltage

(Figure 2.8c). In sharp contrast, low conductivity ($I < 1.0$ nA) was shown at the same scanning voltage for the film formed from the asymmetric PDI-I₁₀ peptide (Figure 2.8d). It is worth mentioning that high conductivity of assemblies depends on their ordered structure that leads to high charge carrier delocalization (Qu *et al.*, 2018). The homogenous distribution of nanofilament-based film of symmetric PDI-[I₁₀]₂ could be associated with a topographically higher area with higher charge carrier delocalization, leading to high conductivity. The conductance of PDI-peptide bioconjugate assemblies, could be explained by two different hypotheses, π - π stacking of aromatic ring, and electron hopping (Schneebeli *et al.*, 2011; Shipps *et al.*, 2021). Amyloid π -conjugated peptides are not expected to establish long-range π - π stacking periodicity necessary to enable delocalization along the length of supramolecular structure (Ing *et al.*, 2018). Hence, we suggest that electron transfer may be the result of both short-range π - π interactions and electron hopping between stacks. Current-voltage curves recorded for both hybrid peptides displayed non-Ohmic S-shape (Figures 2.8c and 2.8d), often observed for organic semiconductors (Creasey *et al.*, 2019). In addition, current images show a higher current response for PDI-[I₁₀]₂ film compared to that of the PDI-I₁₀ film (Figures 2.8e and 2.8f), and the S-shape for PDI-[I₁₀]₂ *I-V* curve was more pronounced than the corresponding PDI-I₁₀ *I-V* curve, indicative of an increase in conductivity. Considering the discrepancy in the distribution and dispersity of the macroscopic architectures observed between PDI-[I₁₀]₂ and PDI-I₁₀ (Figures 2.4c and 2.4d vs Figures 2.6c and 2.6d), the homogeneous distribution of long and linear nanofilaments is likely associated with the high conductivity observed for PDI-[I₁₀]₂ in comparison to PDI-I₁₀ assemblies. Besides, fibrils assembled from peptide sequence without the PDI unit. *i.e.* I₁₀, appeared to undergo degradation after application of voltage (± 1 V) (Figure S2.10). On the other hand, the films formed by PDI-[I₁₀]₂ and PDI-I₁₀ fibrils exhibited higher stabilities, even after application of higher voltages (± 10 V) (Figures S2.11 and S2.12). Accordingly, the PDI core not only enhances the conductivity of the resulting nanofilaments, but also significantly improves their stability under applied voltage.

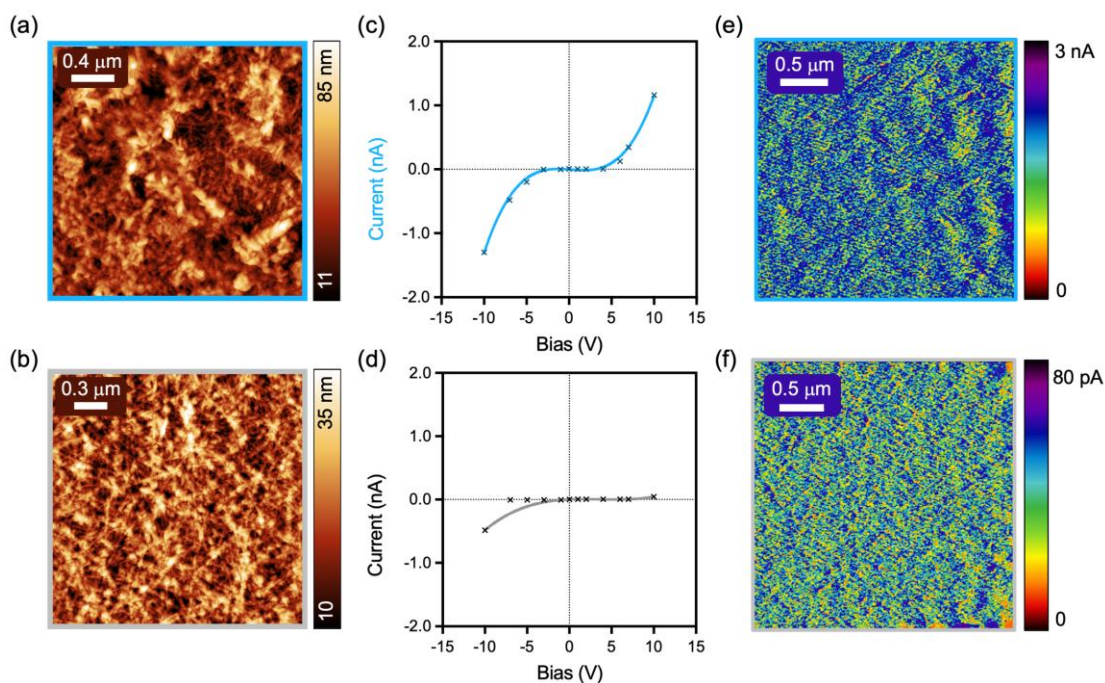


Figure 2.8 C-AFM Analysis of hybrid π -conjugated systems. Surface topography of (a) PDI-[I₁₀]₂ and (b) PDI-I₁₀ fibrils deposited onto interdigitated electrodes. Current-voltage characteristics of (c) PDI-[I₁₀]₂ and (d) PDI-I₁₀, the scanning voltages are ± 10 V. Current image of (e) PDI-[I₁₀]₂ and (f) PDI-I₁₀, the scanning voltage 10 V.

2.6 Conclusion

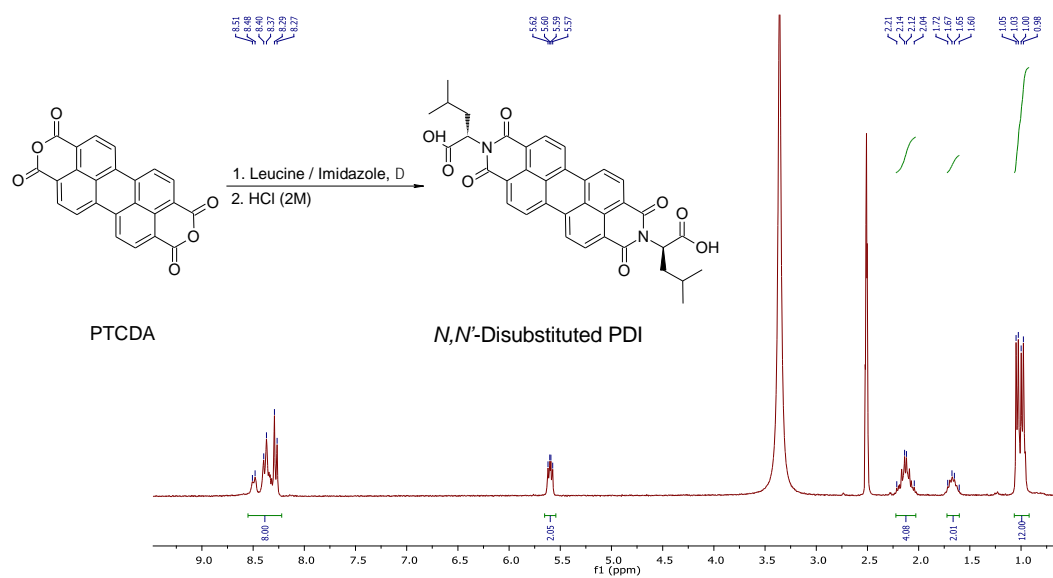
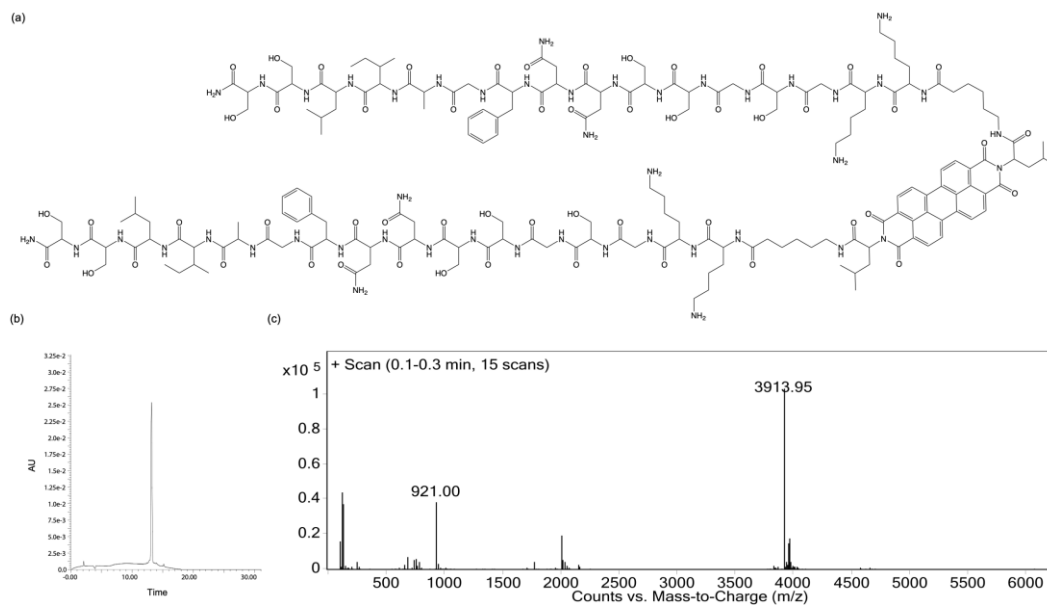
Overall, this study revealed for the first time that amyloid self-assembling peptides can be harnessed to guide the self-assembly of a π -conjugated PDI systems, which constitutes an efficient strategy to conceive programmable and biocompatible nanofilaments with robust optoelectronic properties. A new approach was implemented to easily access symmetric and asymmetric PDI derivatives using solid support methodology. Taking advantage of the directional hydrogen bonding involved in peptide self-recognition, compared to π - π stacking interactions, the self-assembly of these hybrid biomolecules was mainly driven by the amyloid core, although the PDI moieties could also participate in this process. It was also observed that a single peptide at the imide positions is sufficient to drive the assembly into nanofilaments, but not sufficient to form a homogeneous distribution of long and linear nanofilaments that

would favor, as observed with PDI-[I₁₀]₂ assembly, a long-range intermolecular charge carrier delocalization of the π -electron cloud and improve their semiconductive properties. Moreover, PDI-I₁₀ and PDI-[I₁₀]₂ assemblies were fully cytocompatible and could be observed by fluorescence confocal microscopy. Considering that the mechanisms of self-assembly of amyloidogenic peptides and the resulting supramolecular architectures are highly sensitive to the environment, it would be interesting to further study the self-recognition process of amyloid-PDI conjugates under different conditions to ultimately modulate the bioelectronic properties of these assemblies. These novel optoelectronic biomaterials can be exploited in numerous applications, including the conception of implantable, or wearable bioelectronic interfacing devices for treatments of chronic conditions or diseases states.

2.7 Acknowledgments

This research was funded by the Natural Sciences and Engineering Research Council of Canada (NSERC) grants RGPIN-2018-06209 to S.B. and RGPIN-2018-05799 to A.N. N.K. acknowledges a scholarship from the Center of Excellence in Research on Orphan Diseases-Fondation Courtois (CERMO-FC) and M.C-C is a recipient of a Vanier Canada graduate scholarship.

2.8 Supporting information

Figure S2.1 Synthesis and ^1H NMR Spectrum of PDI-[L] $_2$ (300 MHz, DMSO- d_6).Figure S2.2 (a) Molecular structure (b) HPLC and (c) MS analysis of PDI-[I $_{10}$] $_2$.

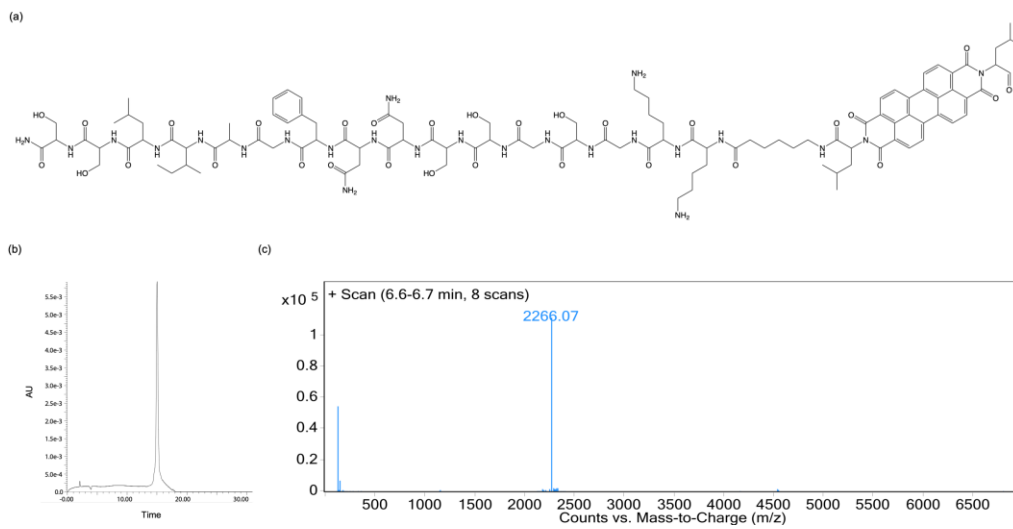


Figure S2.3 (a) Molecular structure, (b) HPLC and (c) MS analysis of PDI-I₁₀.

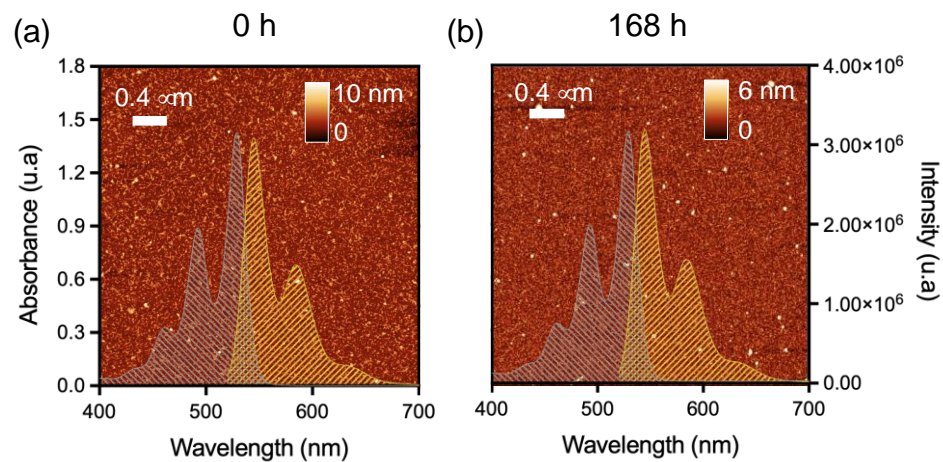


Figure S2.4 UV-vis spectra (gray), emission spectra (yellow) and AFM images of PDI-[I₁₀]₂ in DMSO at (a) 0 h, and after (b) 1-week incubation at room temperature at 500 μ M.

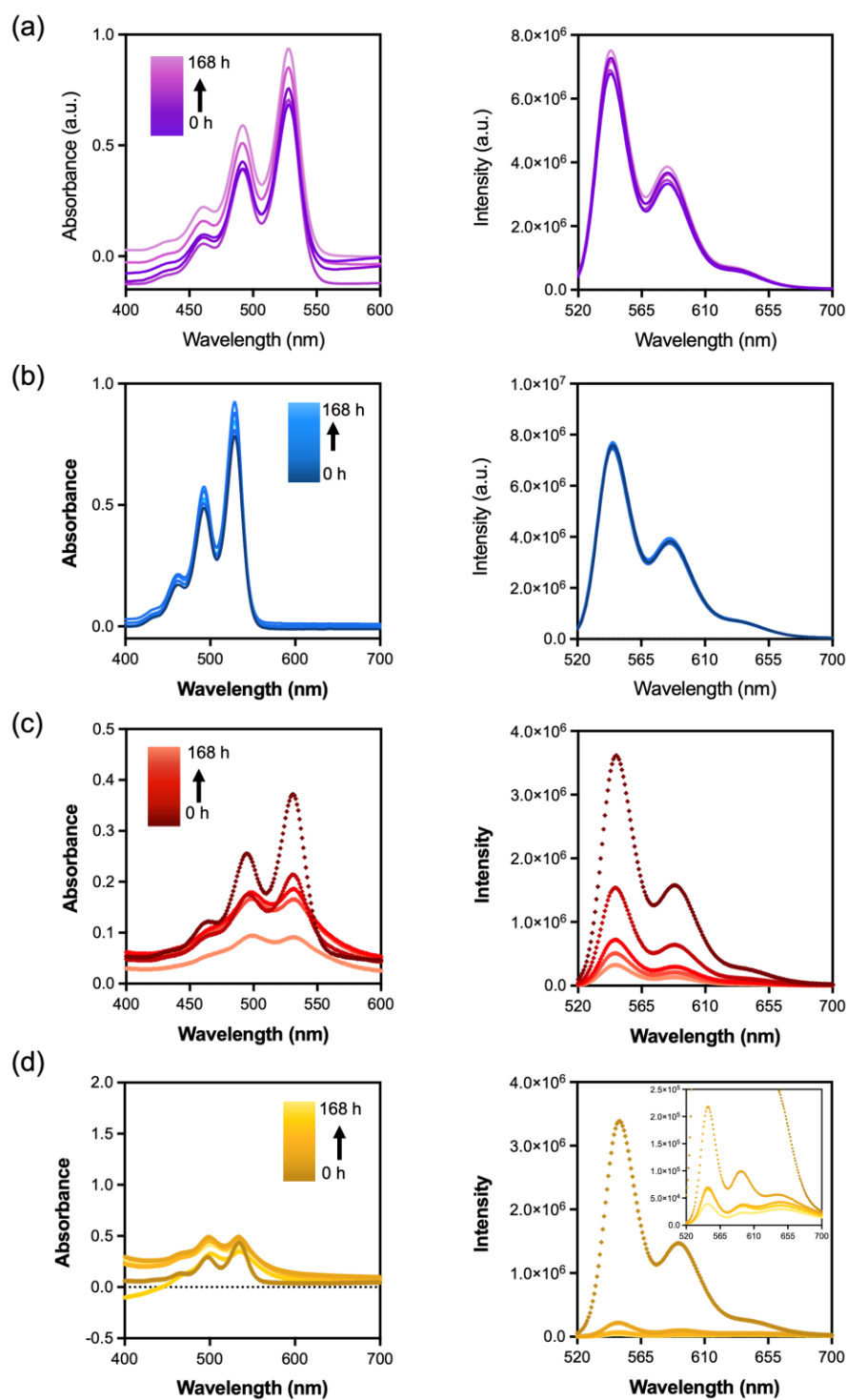


Figure S2.5 UV-vis (left), and fluorescence emission (right) spectra (excitation at 504 nm) of PDI-[I₁₀]₂ incubated at 500 μM in (a) THF, (b) MeCN, (c) MeOH and (d) DMSO water mixtures, at 0 h, and after 24 h, 48 h, 72 h, and 1-week incubation.

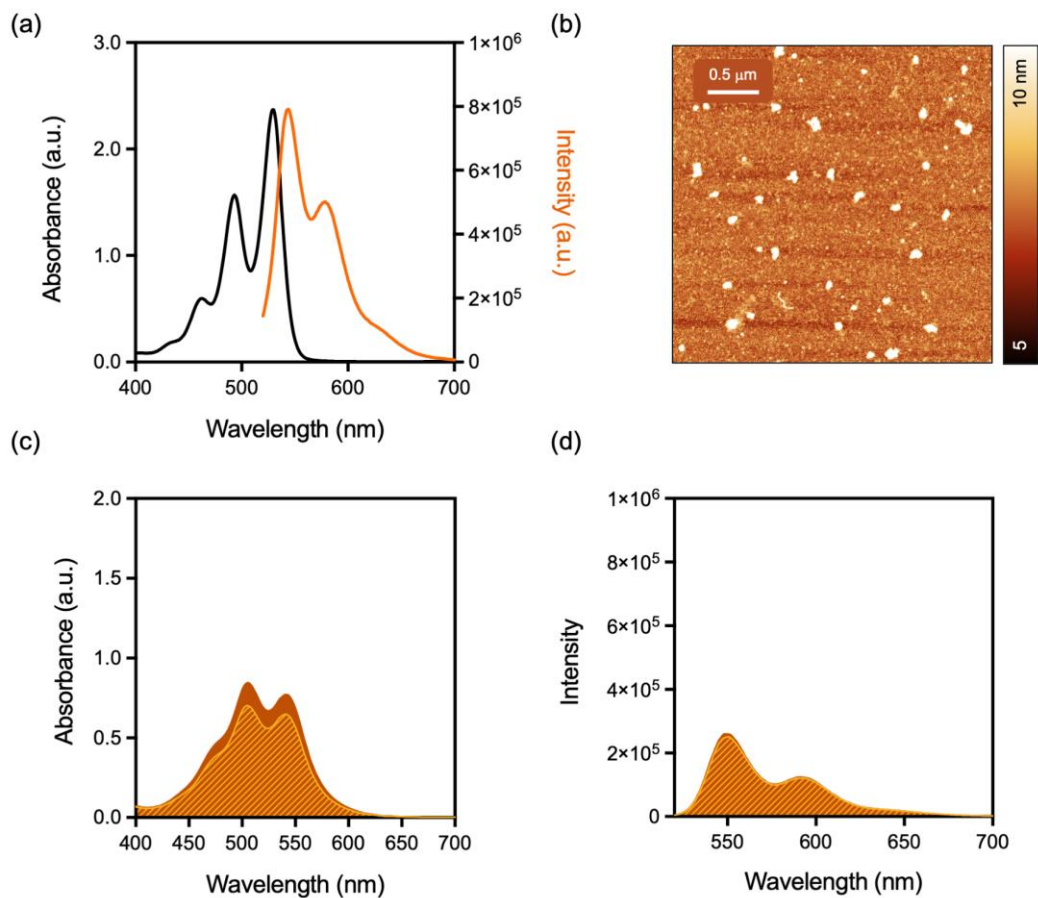


Figure S2.6 (a) UV-vis (dark) and emission (orange) spectra of PDI-[L]₂ in DMSO at 500 μM . (b) AFM imaging after 24 h incubation of PDI-[L]₂ at 500 μM , in the Tris-HCl buffer. (c) UV-vis and (d) emission spectra of PDI-[L]₂ in the Tris-HCl buffer at 0 h (Solid), and after 24 h (Hatched) incubation at room temperature at 500 μM .

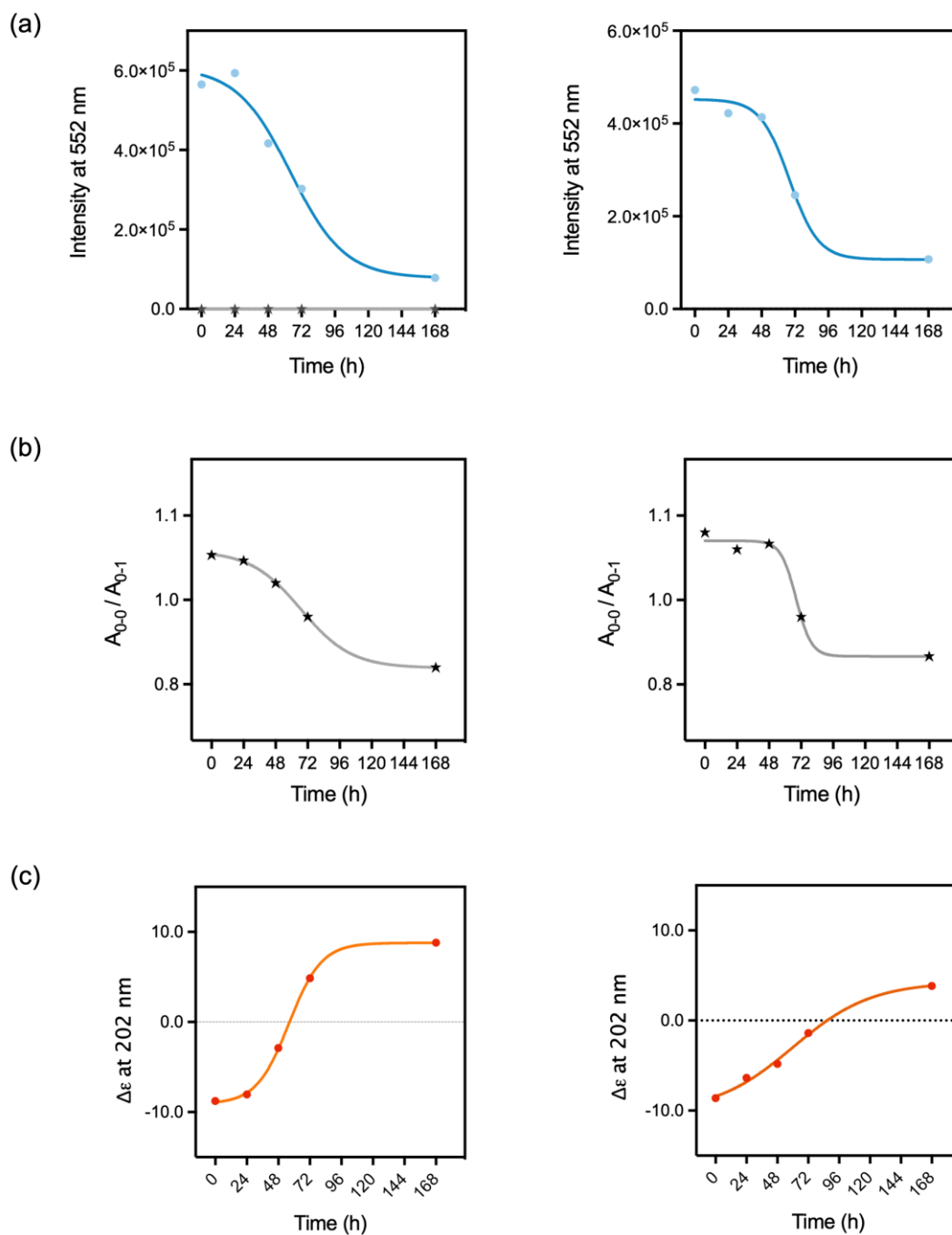


Figure S2.7 Graphics representing (a, b) the emission intensity at 552 nm, (c, d) the ratio of absorbance at bands 0–0 and 0–1, and (e, f) molar ellipticity at 202 nm of PDI-[I₁₀]₂ (left) PDI-I₁₀ (right) over time.

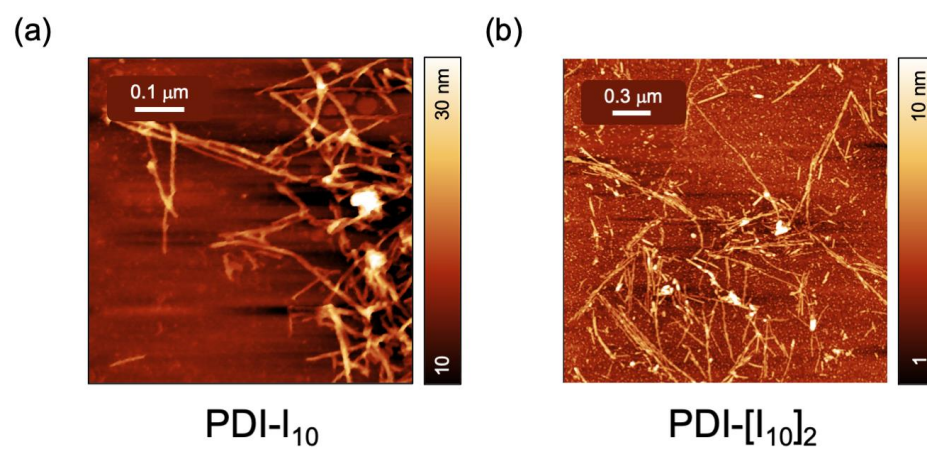


Figure S2.8 AFM images of pre-assembled (a) PDI-I₁₀ and (b) PDI-[I₁₀]₂ obtained after 1 week of incubation in cell culture media at room temperature at a concentration of 10 μM.

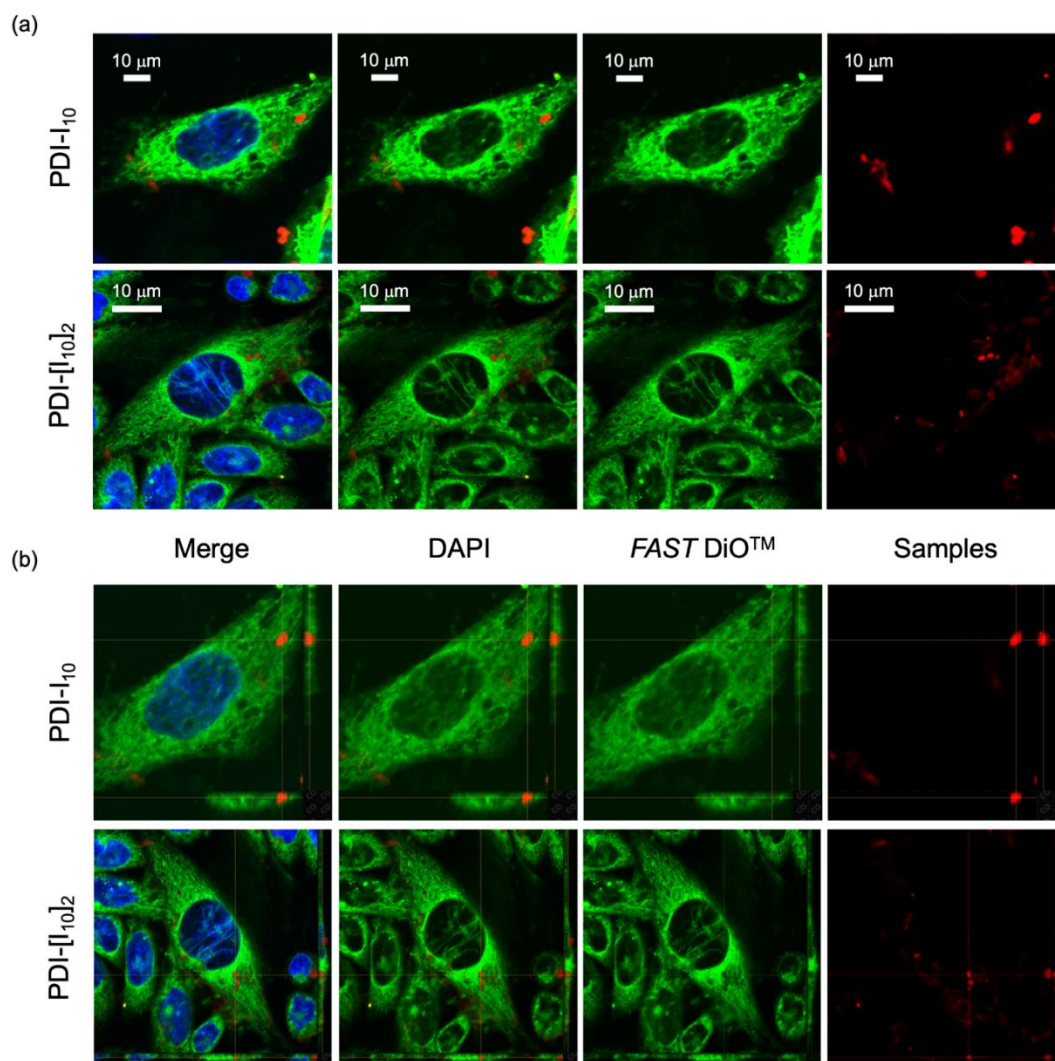


Figure S2.9 Confocal microscopy images. (a) Single cross-section and (b) Z-stack projections and orthogonal views of confocal laser scanning confocal microscopy image of CHO-K1 cells after 6 h incubation with 10 μ M of PDI-I₁₀ (top) and PDI-[I₁₀]₂ (bottom) assemblies (red: fibrils, blue: DAPI, and green: FAST DiO™).

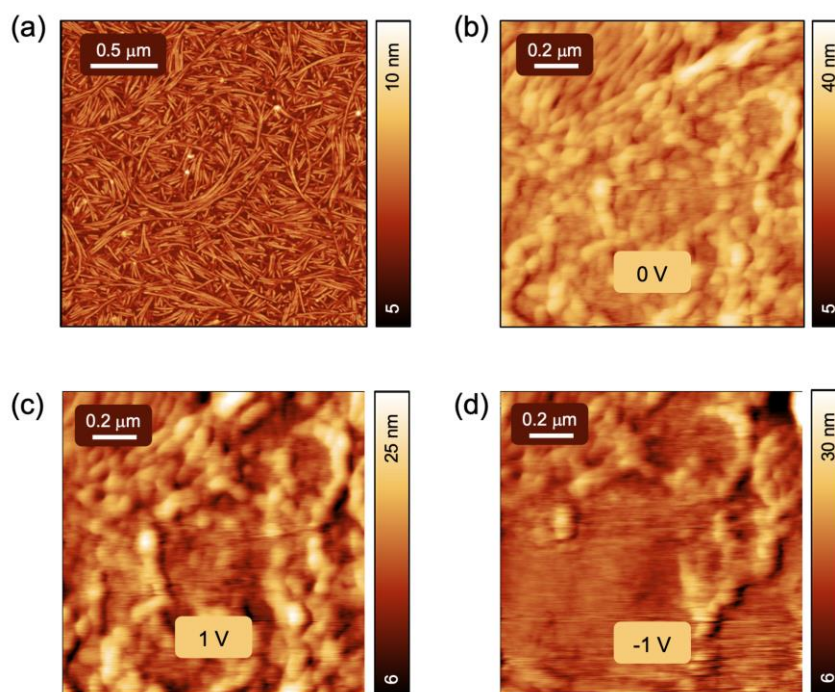


Figure S2.10 (a) AFM imaging after 1-week incubation of I₁₀ peptide sequence at 500 μM, in the Tris-HCl buffer. Surface topography of peptide fibrils deposited onto interdigitated electrodes before (b) and after (c, d) applying voltage.

Table S2.1 Photophysical properties of PDI-[L]₂ in different solvents.

Solvent	λ_{max}^{FL} (nm)	λ_{max}^{Abs} (nm)	I ₀₋₀ /I ₁₋₀	A ₀₋₀ /A ₀₋₁
H ₂ O/THF	543	528	2.04	1,73
DMSO	545	529	2.05	1.60
H ₂ O/MeOH	547	531	2.29	1.46
H ₂ O/MeCN	545	529	1.98	1.61
H ₂ O/DMSO	549	534	2.19	1.04
H ₂ O	554	539	2.38	1.13
TrisHCl buffer	555	539	2,34	1,03

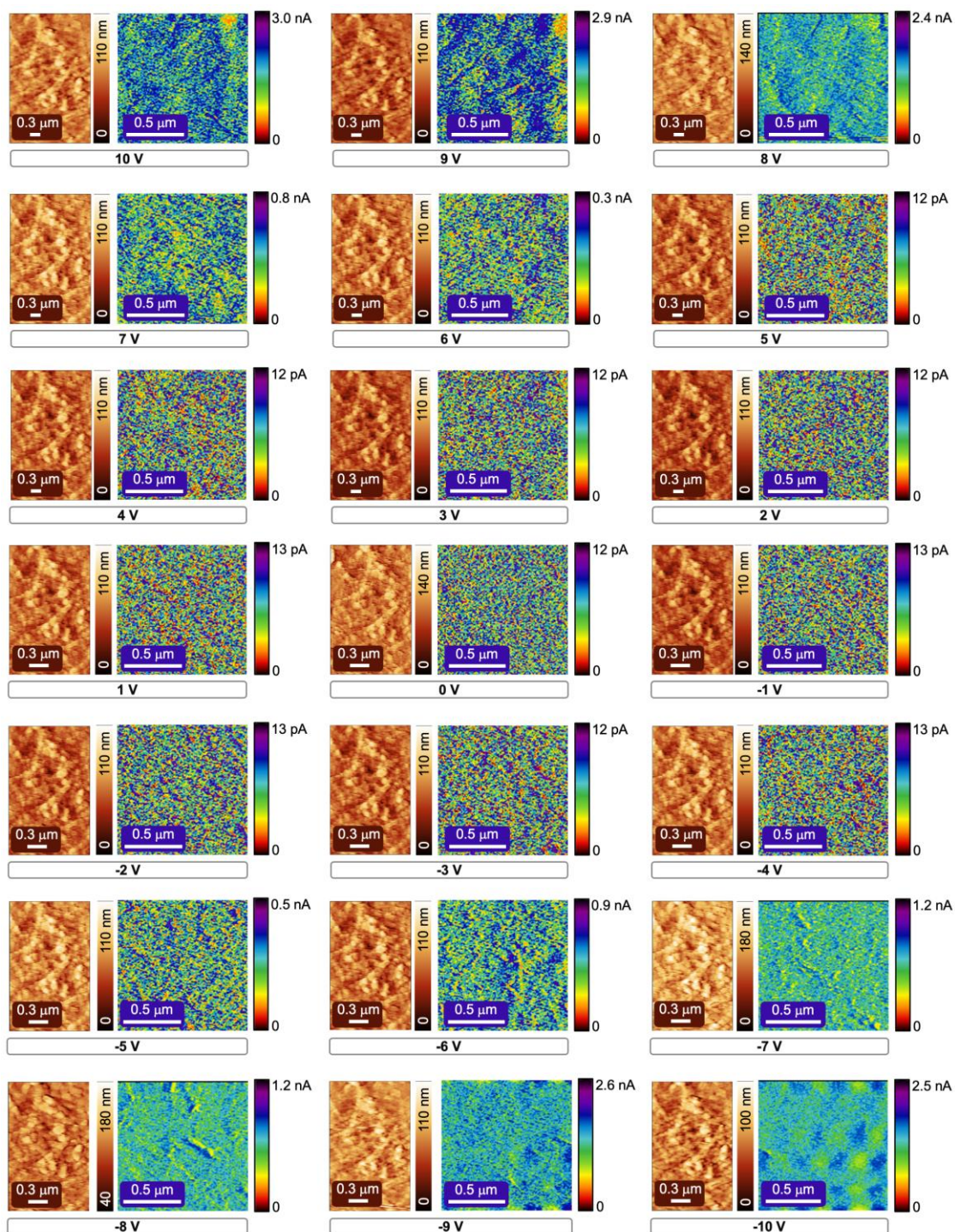


Figure S2.11 Surface topography (right) and C-AFM images (left) of PDI-[I₁₀]₂ fibrils deposited onto interdigitated electrodes, the scanning voltages are ± 10 V.

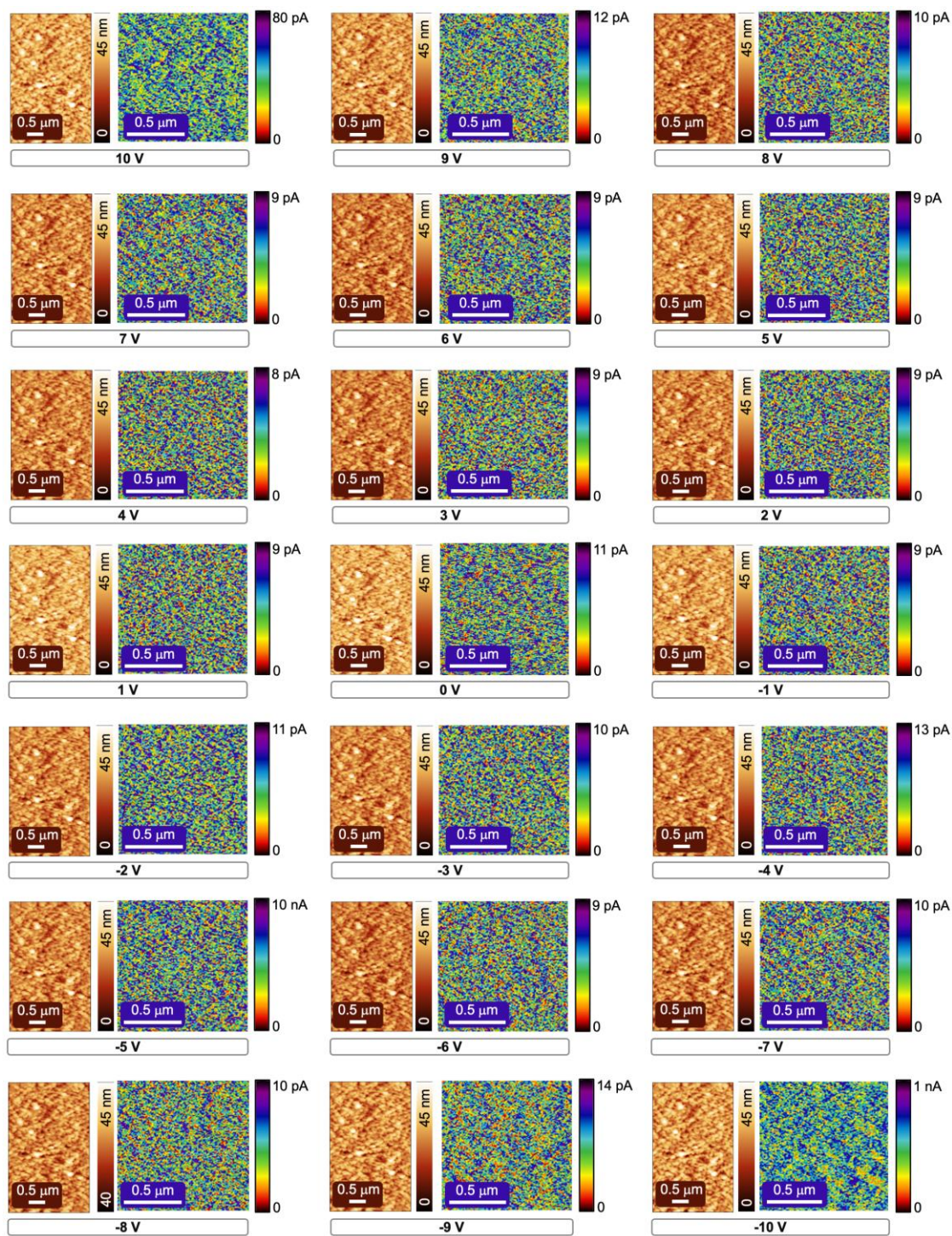


Figure S2.12 Surface topography (right) and C-AFM images (left) of PDI-I₁₀ fibrils deposited onto interdigitated electrodes, the scanning voltages are ± 10 V.

CHAPITRE III

EFFECT OF TERMINAL CAPPING OF β -SHEET PEPTIDES ON SELF-ASSEMBLY AND ACTIVATION OF TOLL-LIKE RECEPTOR 2/6 BY AMYLOID-LIKE NANOSTRUCTURES

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Manuscrit en préparation pour soumission au journal *Soft Matter*

Contribution des auteurs :

Nadjib Kihal : Conception du projet, exécution des expériences (synthèse, purification et caractérisation des peptides), microscopie à force atomique, spectroscopie de fluorescence, spectroscopie de dichroïsme circulaire, spectroscopie FTIR, tests de viabilité cellulaire, tests d'activation des TLR2/TLR6, analyses des résultats, préparation des figures, rédaction de l'article

Margaryta Babych : Exécution des expériences (synthèse, purification et caractérisation des peptides)

Marie-Jeanne Archambault : Tests de viabilité cellulaire

Ali Nazemi : Supervision du projet, rédaction et correction de l'article

Steve Bourgault : Supervision du projet, rédaction et correction de l'article

3.1 Résumé

Reconnues pour leur capacité à activer le système immunitaire inné, les fibrilles amyloïdes peuvent être exploitées en tant que système de délivrance d'antigènes auto-adjuvant pour la conception de vaccins. Parmi les mécanismes responsables de ces effets immunostimulants, l'activation du récepteur hétérodimérique Toll-like-receptor 2/6 (TLR2/TLR6) par des assemblages protéiques riches en feuillets- β -croisés semble critique. Néanmoins, le manque de contrôle du processus de formation des amyloïdes et la polydispersité typique des nanostructures résultantes rendent l'élucidation des bases moléculaires de l'activation des TLR2/TLR6 par les assemblages amyloïdes très difficile. Dans ce contexte, nous avons exploité les effets des modifications en positions *N*- et *C*-terminales d'un court peptide- β dérivé de l'islet amyloid polypeptide (I₁₀) pour étudier les relations entre la morphologie/propriétés physicochimiques des assemblages amyloïdes et leur activité agoniste des TLR2/TLR6. Les substitutions en positions *N*- et *C*-terminales du cœur d'autoassemblage I₁₀ ont permis d'obtenir une diversité de nanostructures, notamment des nanofilaments sous forme de ruban, des nanotiges de différentes longueurs ainsi que des fibrilles plates et torsadées. Ces préparations homogènes et cytocompatibles d'assemblages peptidiques ont montré différentes capacités d'activer le TLR2/TLR6, qui étaient liées à la charge exposée à la surface et/ou à la longueur et à la morphologie des nanostructures. Dans l'ensemble, cette étude a exposé le fort effet modulateur du coiffage électrostatique aux extrémités *N*- et *C*-terminales sur l'autoassemblage d'un peptide- β et a conduit à une meilleure compréhension à l'échelle moléculaire des effets immunomodulateurs des nanoplateformes amyloïdes pour la livraison d'antigènes.

3.2 Abstract

Amyloid fibrils are known for their capacity to activate the innate immune system, which can be exploited for their usage as self-adjuvanted antigen delivery system for vaccine design. Among the mechanisms responsible for these immunostimulatory effects, the activation of the heterodimeric toll-like receptor 2/6 (TLR2/TLR6) by cross- β -sheet proteinaceous assemblies appears essential. Nonetheless, the lack of control over the process of amyloid formation and the typical polydispersity of the resulting nanostructures makes it very challenging to elucidate the molecular basis of TLR2/TLR6 engagement by amyloid assemblies. In this context, we harnessed the effects of *N*- and *C*-terminal modifications of a short 10-mer β -peptide derived from the islet amyloid polypeptide (I_{10}) to investigate the relationships between the morphology/physicochemical properties of amyloid assemblies and TLR2/TLR6-agonist activity. *N*- and *C*-terminal chemical substitutions of the I_{10} self-assembling core allowed the controlled formation of a diversity of nanostructures, including belt-like filaments, nanorods of different length as well as flat and twisted fibrils. These cytocompatible homogenous preparations of peptide assemblies showed different potencies to activate TLR2/TLR6, which was related to the charge exposed on the surface and/or the length and morphology of the nanostructures. Overall, this study further exposes the strong modulatory effect of *N*- and *C*-terminal electrostatic capping of β -peptides on self-assembly and offers molecular insights into the immunomodulatory effects of amyloid-based nanoscaffold for antigen delivery.

3.3 Introduction

Due to their high propensity to self-assemble into organized nanostructures, polypeptide chains constitute key molecular building blocks for the design of biomimetic soft materials for a wide range of potential applications (Hamley, 2019; Wang *et al.*, 2020), from bioimaging and biosensing (Reitermann *et al.*, 1989) to drug (Lai *et al.*, 2023) and antigen (Lamontagne *et al.*, 2022) delivery. Ordered protein assemblies show interesting properties, such as defined hierarchical supramolecular structures, biocompatibility, mechanical strength, biodegradability and, most often, intrinsic functionalities (Edwards-Gayle et Hamley, 2017). Several bio-inspired proteinaceous materials have been studied over the last decades, including spider's silk fibers (Bini *et al.*, 2004; Trancik *et al.*, 2006), byssus from bivalve mollusc (Waite *et al.*, 1992) and amyloid fibrils (Al-Halifa *et al.*, 2019; Knowles et Mezzenga., 2016). Amyloids are ubiquitous ordered protein assemblies, characterized by a cross- β -sheet quaternary structure, which is stabilized by intermolecular hydrogen bonding networks between β -stands that are oriented perpendicularly to the fibril axis (Chuang *et al.*, 2018; Fitzpatrick *et al.*, 2013; Mukhopadhyay *et al.*, 2014; Wetzel *et al.*, 2006). While amyloids have been initially discovered for their association with various human diseases, including Alzheimer's disease and type II diabetes (T2D) (Benson *et al.*, 2020; Dobson, 2003), recent studies have also demonstrated that amyloid fibrils play vital biological functions such as melanosome biogenesis, long-term memory and storage of peptide hormones (Chuang *et al.*, 2018; Fowler, Douglas *et al.*, 2007). The (bio)stability, robust mechanical properties and highly ordered structure of amyloid fibrils have prompted researchers to exploit these as protein-based materials for cell adhesion/culture, drug release, electronic devices, sensors, and nanovaccines (Hamley, 2019; Mankar *et al.*, 2011). However, amyloid nanomaterials are known for their high supramolecular polymorphism, which arises from the delicate interplay between a number of factors; including the conditions under which the self-assembly occurs (pH, ionic strength, temperature, agitation, sonication) and the multiple interactions driving aggregation (hydrophobics, hydrogen bonding, electrostatics, π - π stacking) (Seuring *et*

al., 2017). While the diversity of suprastructures that can be engendered constitutes an advantage to construct a variety of macromolecular assemblies, this intrinsic heterogeneity that is a characteristic of materials built by a bottom-up approach, limits the controlled assembly into precise ordered nanoplatfoms with tailored function(s). Over the last ten years, several studies have reported that amyloid fibrils constitute efficient antigen delivery systems for subunit vaccines due their intrinsic immunomodulatory properties (Lamontagne *et al.*, 2023). For instance, unbranched cross- β -sheet filaments assembled from the short fibrillizing peptide Q11 (QQKFQFQFEQQ) and decorated with known B- and/or T-cell epitopes, induced high levels of epitope-specific antibodies in mice (Pompano *et al.*, 2014; Rudra *et al.*, 2012; Rudra *et al.*, 2012; Rudra *et al.*, 2010; Wen *et al.*, 2016). Similarly, we reported the preparation of synthetic fibrillar vaccines based on a 10-mer β -sheet sequence (I₁₀) derived from the islet amyloid polypeptide (IAPP). The E2EP3 epitope from chikungunya virus and the M2e epitope from influenza A virus were respectively attached to the I₁₀ amyloidogenic core and the chimeric peptides spontaneously assembled into cytocompatible fibrils that induced a robust IgG response against the grafted epitopes upon immunization (Al-Halifa *et al.*, 2020; Babych *et al.*, 2018; Zottig *et al.*, 2021). The immunostimulating properties of cross- β assemblies likely resides in a combination of biological mechanisms, such as the induction of a depot effect at the injection site, increase uptake by antigen presenting cells (APCs), increase metabolic stability and stimulation of immune cells (Al-Halifa *et al.*, 2019). Interestingly, amyloid fibrils have been shown to activate the cytosolic NLRP3 inflammasome and the toll-like receptor 2 (TLR2), stimulating APC maturation and production of cytokines (Al-Halifa *et al.*, 2020; Lamontagne *et al.*, 2023; Rapsinski *et al.*, 2015; Tukul *et al.*, 2010; Tukul *et al.*, 2009). TLRs are transmembrane receptors that are found primarily on innate immunity cells, such as monocytes, macrophages, or dendritic cells, and serve as part of the body's first line of defense against infectious diseases (El-Zayat *et al.*, 2019; Wicherska-Pawłowska *et al.*, 2021). Specifically, the heterodimeric TLR2/TLR6 is activated by diacyl-lipopeptides, which have been employed as adjuvants in numerous vaccine studies (Ignacio *et al.*, 2018). While the

intrinsic capacity of amyloid fibrils to activate TLR2/TLR6 is paramount for their use as self-adjuvanted antigen delivery nanosystem, the molecular basis of the recognition of proteinaceous cross- β filaments by TLR2/TLR6 heterodimers remains elusive. Particularly, the lack of control over the process of amyloid formation and the polydispersity of the resulting filaments makes it very challenging to draw any relationships between the TLR-agonist activity of cross- β fibrils and their morphology/structure and physicochemical properties.

In this context, the objective of the present study was to harness the effect of *N*- and *C*-terminal electrostatic capping of self-assembling β -peptides on the morphology and physicochemical properties of cross- β assemblies to gain molecular insights regarding the stimulation of innate immunity by amyloid assemblies through TLR2/TLR6 activation. It has been previously shown by us (Zottig *et al.*, 2019) and others (Andreasen *et al.*, 2014; Arya *et al.*, 2020; Hajiraissi *et al.*, 2019; Pizzi *et al.*, 2017) that modifications of the *N*- or *C*-terminus of amyloidogenic peptides dramatically affect the length, mesoscopic architecture and/or the intrinsic polymorphism of the resulting cross- β assemblies. Accordingly, a small peptide library derived from the I₁₀ sequence was prepared and self-assembly was characterized using a combination of biophysical approaches. Whereas the monomeric/pre-fibrillar peptides could not activate the TLR2/TLR6, assemblies were able, to different extent, to engage membrane receptors. Overall, this study further reinforces the modulatory effect of *N*- and *C*-terminal modifications on peptide nanostructures and provides preliminary structure-activity relationships regarding the activation of TLR2/TLR6 by cross- β amyloid assemblies.

3.4 Materials and methods

Peptides were prepared by means of standard Fmoc solid-phase peptide synthesis using 2-(6-chloro-1-*H*-benzotriazol-1-yl)-1,1,3,3-tetramethylammoniumhexafluorophosphate (HCTU) as a coupling agent on a trityl chloride resin, or rink amide resin (0.4 mmol/g).

After acidic cleavage from the solid support using trifluoroacetic acid (TFA) and precipitation with ethyl ether, peptide derivatives were purified using reverse-phase high-performance liquid chromatography (HPLC) using a Waters 2489. Peptide identity was confirmed by liquid chromatography coupled with high-resolution mass spectrometry on an Agilent 1200 series (See Figure S3.1 and S3.2 in the supporting information). Desired fractions, with a purity higher than 95%, were collected, combined, and lyophilized. Peptides were kept dried at -20°C until their use.

3.4.1 Self-assembly of I₁₀ peptides

Peptide solutions were prepared by dispersing the lyophilised powder into 20 mM Tris-HCl buffer (pH 7.4) at a concentration of 1 mM, and then incubated for one week at room temperature (RT) and under constant circular agitation (40 rpm).

3.4.2 Fluorescence Spectroscopy

Thioflavin-T (ThT) and 8-anilinonaphtalene-1-sulfonic (ANS) fluorescence measurements were carried out at RT on a PTI steady-state spectrofluorometer and operated with PTI's FelixGX software with light pathlength of 3 mm. The final concentrations of ThT and ANS were kept constant to 40 and 50 μM , respectively. For ThT, the excitation wavelength was set at 440 nm and the emission spectra were recorded from 450 to 550 nm. For ANS, the excitation wavelength was set at 355 nm and the emission spectra were recorded from 385 to 585 nm. All peptide samples were diluted at 50 μM in 20 mM Tris-HCl buffer (pH 7.4) and fluorescence analyses were performed in triplicate. Representative data are presented.

3.4.3 Far-UV Circular Dichroism Spectroscopy

CD spectra were collected, from 260 to 190 nm, on Jasco J-815 spectrophotometer at RT, in a 2 mm quartz cuvette. All peptide solutions were diluted in corresponding

buffer to reach a final concentration of 50 μM . Curves were smoothed using Savitsky-Golay algorithm with 13 data points of smoothing moving window, and 20 mM Tris-HCl buffer was used for data baseline correction.

3.4.4 Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy

Peptide assemblies obtained after one week of incubation in 20 mM Tris-HCl buffer (pH 7.4) were isolated by lyophilization. ATR-FTIR spectra of lyophilized peptides were recorded using a Nicolet Magna 560 spectrometer equipped with a nitrogen-cooled MCT detector.

3.4.5 Atomic Force Microscopy

Peptide mixtures were first diluted to reach a final concentration of 50 μM in 1% acetic acid and immediately spotted onto a freshly cleaved mica. The excess of liquid was eliminated by wicking, and the samples were washed twice with deionized water before being air-dried overnight. AFM images were recorded on Bruker (MultiMode 8) using Nanoscope E controller. All peptide samples were scanned, under the ambient conditions, using silicon nitride AFM tip (2–12 nm tip radius, $k = 0.4 \text{ N/m}$) in scanasyst-air mode. Images were examined using Gwyddion v2.59.

3.4.6 Evaluation of Cellular Viability

DC.2.4 cells were cultured in black wall clear bottom 96-well plates at a density of 30 k cells per well in RPMI-1640 medium, supplemented with 25 mM HEPES buffer solution, 0.05 mM β -mercaptoethanol, 2 mM L-Glutamine and 10% (v/v) FBS, then incubated for 24 h, in a humidified atmosphere of 5% CO_2 and 95% air at 37 $^\circ\text{C}$, in presence of different concentrations (from 6.25 to 100 μM) of monomeric/pre-fibrillar (0 h pre-incubation) and assembled (1 week pre-incubation) I_{10} peptides. Cellular

viability was evaluated using the resazurin metabolic assay and cell viability (in %) was calculated from the fluorescence ratio of treated sample to buffer control-treated cells. Data of a least three independent assays achieved in triplicate were averaged and expressed as the mean \pm standard deviation (S.D.).

3.4.7 Evaluation of TLR Activation

HEK-Blue hTLR2/TLR6 cells were cultured in DMEM medium supplemented with 2 mM L-glutamine, 4.5 g/L glucose, 10% (v/v) fetal bovine serum, 100 μ g/mL streptomycin, 100 U/mL penicillin, 100 μ g/mL normocin, and 1 \times HEK-Blue selection reagent. When confluence reached 50 to 80%, HEK-Blue hTLR2/TLR6 cells were seeded at a density of 280 000 cells/mL in a 96-well plate (100 μ L per well) in HEK-Blue detection medium, which contains a SEAP color substrate, in the presence of equal volumes of peptide mixtures or buffer vehicle control. Monomers and/or pre-fibrillar species (*i.e.* 0 h of self-assembly) were evaluated at a single concentration of 100 μ M whereas the nanostructures (1 week of self-assembly) were evaluated at concentrations ranging from 3.125 to 100 μ M (equivalent to monomeric peptide). The positive control Pam₂CSK₄ was used at 10 ng/mL. After 16 h incubation at 37 °C in 5% CO₂, the absorbance was measured at 630 nm as an indicator of SEAP activity associated with NF- κ B activation. The experiments were carried at least three times in triplicate, and the data were averaged and expressed as mean \pm S.E.M.

3.5 Results and discussion

3.5.1 Design and synthesis of self-assembling β -sheet peptides

Amyloid nanomaterials are known for their high polymorphism and for the inherent difficulty of controlling the final nanostructures from the polypeptide sequence, which ultimately limits the understanding of the molecular basis of TLR engagement by amyloid fibrils. Previous studies have shown that modifications, or capping, at the *N*-

and C-terminus of peptide dramatically affect the architecture and/or the polydispersity of the resulting cross- β assemblies (Andreasen *et al.*, 2014; Arya *et al.*, 2020; Hajiraissi *et al.*, 2019; Pizzi *et al.*, 2017; Zottig *et al.*, 2019), representing a promising opportunity to control the final morphology. In this study, we harnessed the modulatory effect of N- and C-terminal modifications of self-assembling β -peptides on amyloid formation to uncover potential structure-activity relationships (SAR) regarding the capacity of amyloid fibrils to stimulate TLR2/TLR6. The central 20-29 region (SNNFGAILSS) of the islet amyloid polypeptide (IAPP, amylin) was selected as the amyloidogenic core given that we have previously reported that divergent morphology could be obtained by electrostatic capping (Zottig *et al.*, 2019). Moreover, the self-assembly of I₁₀-derived peptides tolerates functionalization with different antigenic epitopes and the resulting fibrils behave as efficient self-adjuvanted nanovaccine platforms (Al-Halifa *et al.*, 2020; Babych *et al.*, 2018). A flexible tetrapeptide linker (GSGS), commonly used in protein engineering (Arslan *et al.*, 2022), was incorporated at the N-terminus of the I₁₀ core followed, or not, by the positively charged Lys (Figure 3.1). As previously reported (Zottig *et al.*, 2019), the positive charges at the N-terminus have significant effects on the final morphologies, which will allow us to probe the molecular basis of TLR activation by cross- β assemblies. Additionally, to expand the diversity landscape of the supramolecular architecture obtained from a single self-assembling β -peptide core sequence, C-terminal capping, *i.e.* C-amidation, was also applied. All peptides were prepared by elongation on solid support based on Fmoc chemistry, as previously reported (Kihal *et al.*, 2023), before being purified and characterized by HPLC and ESI-TOF-MS, respectively.

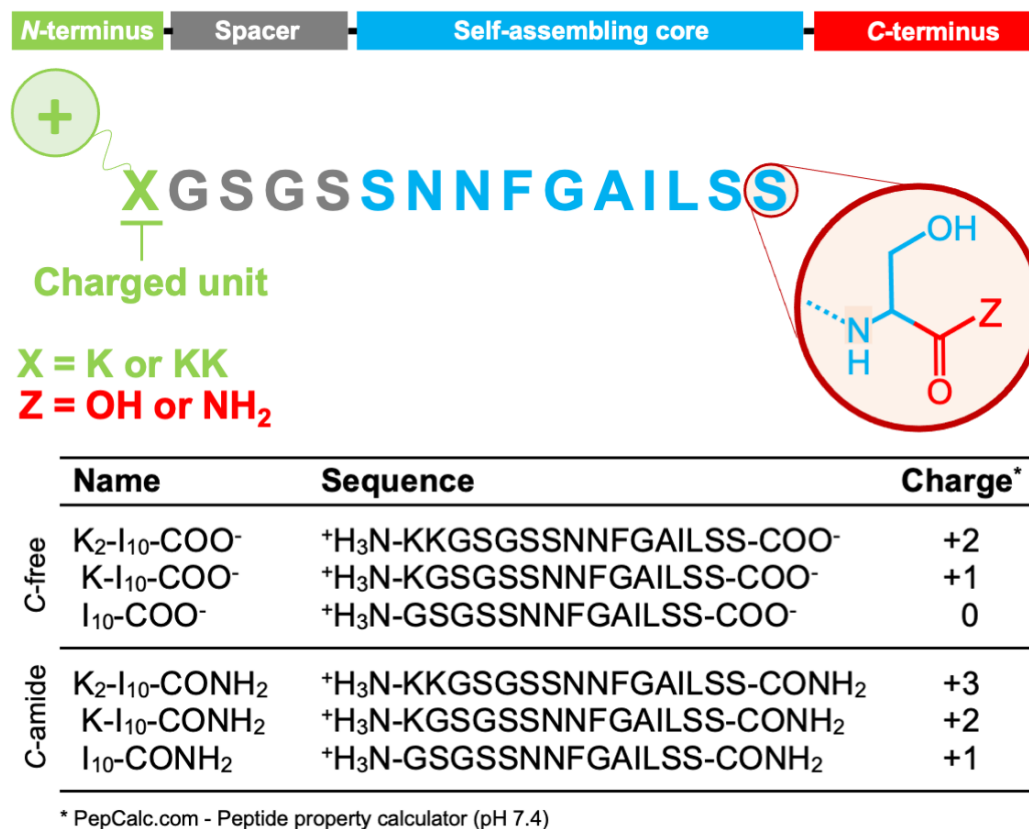


Figure 3.1 Design of N- and/or C-terminal modified I₁₀ derivatives. I₁₀ amyloidogenic self-assembling core (blue) with flexible linker (gray), N-terminal charged capping unit (green) and C-terminal group (red). Peptide charges are determined at pH 7.4.

3.5.2 Chemical modifications at the N- and C-terminus of the amyloid core modulate the morphology of the nanostructures

We initially evaluated the kinetics of peptide self-assembly to determine the time necessary to reach the equilibrium phase. Lyophilised I₁₀ derivatives were resuspended in 20 mM Tris-HCl buffer (pH 7.4) at a final concentration of 1 mM and incubated under constant agitation at RT for up to 1 week, and peptide solutions were periodically analyzed using the amyloid-sensitive dye ThT (Sebastiao *et al.*, 2017; Xue *et al.*, 2017). For most peptides, ThT fluorescence reached a plateau before 72 h incubation, (See Figure S3.3 in the supporting information). In agreement with our previous work (Al-Halifa *et al.*, 2020), no increase of ThT fluorescence was observed for the positively

charged peptides K₂-I₁₀-CONH₂ and K-I₁₀-CONH₂, even after 1 week incubation. This observation could be associated with the inhibition of ThT binding by electrostatic repulsion from the surface charge of the assemblies, as the ThT molecule contains a positive charge on its thiazole ring. Nevertheless, all peptides were assembled for 1 week under constant agitation before moving forward with biophysical and biological analyses of the generated assemblies.

AFM analysis was conducted to scrutinize the morphological variances within the I₁₀-based assemblies. Terminal capping impacted considerably the final morphology, with the resulting assemblies showing different shapes, such as belt-like structures (I₁₀-CONH₂), nanorods of different length (K₂-I₁₀-COO⁻, K₂-I₁₀-CONH₂, K-I₁₀-CONH₂), as well as flat (I₁₀-COO⁻) and twisted (K-I₁₀-COO⁻) fibrils (Figure 3.2). As previously reported, positive capping units at the *N*-terminus of I₁₀ peptide affects the final morphology and drive self-assembly into uniform nanorods (Zottig *et al.*, 2019). Peptide with *C*-terminal amide capping group and free *N*-terminus (I₁₀-CONH₂) self-assembled into short belt-like fibrils with an average length of 107.6 ± 95.5 nm. In contrast, the incorporation of a capping charge of +2 (K-I₁₀-CONH₂) and +3 (K₂-I₁₀-CONH₂) at the *N*-terminus induced self-assembly into uniform nanorods of average length of 63.0 ± 14.3 nm and 20.4 ± 5.5 nm, respectively. These data further emphasize that the length of assemblies can be, to some extent, modulated by the electrostatic repulsive forces at the *N*-terminus of an amyloidogenic sequence (Zottig *et al.*, 2019). However, no clear correlation could be observed between the net charge of the positive capping unit and the length of the assemblies for the *C*-carboxylated I₁₀ peptides. In fact, the I₁₀-COO⁻ peptide assembled into flat fibrils, whereas K-I₁₀-COO⁻ formed twisted fibrils with a pitch of ~ 137 nm. Both peptides form long fibrils greater than 1 μ m in length. Besides, very short and uniform (20.6 ± 8.0 nm) nanorods were obtained with the K₂-I₁₀-COO⁻ peptide. In addition, the height for the assemblies was affected by *N*- and *C*-terminal alterations. For instance, an average height of 2.79 ± 0.17 nm was obtained for K₂-I₁₀-CONH₂ whereas the K-I₁₀-CONH₂ and I₁₀-CONH₂ peptides respectively assembled into structures with height of 3.74 ± 0.31 nm and 7.58 ± 0.27

nm. It is worth noting that detailed examination of AFM images revealed that the obtained preparations of peptide assemblies showed a somewhat uniform distribution and homogeneity, which is usually difficult to reach with amyloid fibrils. Overall, these data expose that a diversity of nanostructure morphologies can be obtained from a single amyloid sequence simply by terminal modifications, which can be harnessed to probe the molecular basis of TLR2/TLR6 activation by cross- β assemblies.

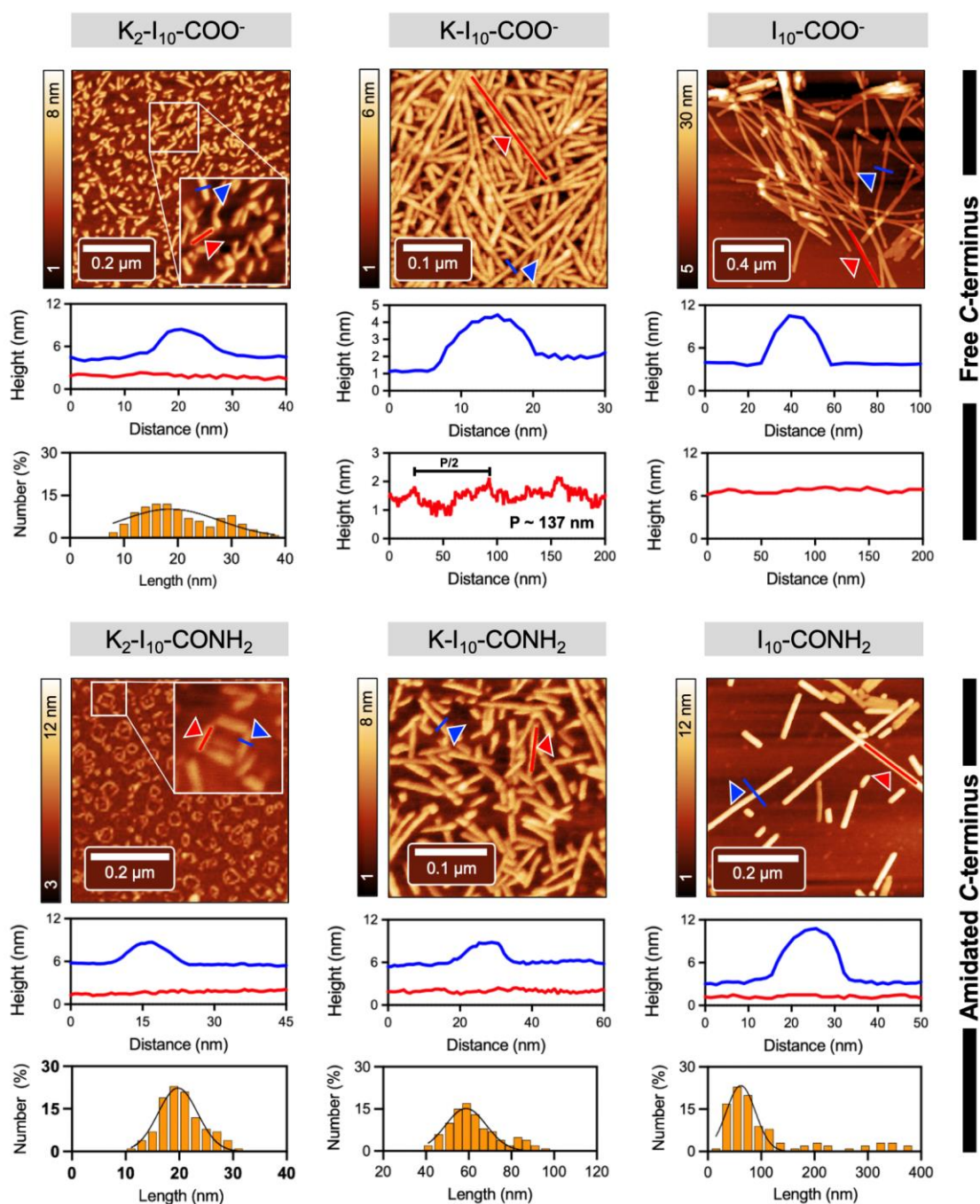


Figure 3.2 Morphology analysis of I_{10} derivatives. AFM images (top) and topography analysis (down) of assemblies formed by free and amidated C-terminus modified I_{10} peptides. The images were acquired after 1 week of incubation in 20 mM Tris-HCl buffer (pH 7.4) at a concentration of 1 mM and under continuous rotatory agitation at RT.

3.5.3 Molecular organization within I₁₀ assemblies

Next, the assemblies were characterized by far-UV CD and FTIR spectroscopy to gain insights regarding the peptide molecular arrangement within the nanostructures. Immediately after their solubilization in 20 mM Tris-HCl (0 h), all peptides, excepted for the I₁₀-CONH₂ derivative, showed a CD spectrum characteristic of a random coil secondary structure with a single minimum at around 195 nm (Figure 3.3). Interestingly, for the C-amidated I₁₀ peptides, a signal characteristic of a β -sheet could be observed at time 0 h, perhaps indicative of very fast aggregation upon resuspension of the lyophilised powder. A significant conformational transition from a predominantly random coil to a β -sheet-rich structures was observed for all three C-terminus peptides (I₁₀-COO⁻, K-I₁₀-COO⁻, K₂-I₁₀-COO⁻). However, the C-amidated peptides K-I₁₀-CONH₂ and K₂-I₁₀-CONH₂ did not show any significant changes in their CD spectra upon 1 week of incubation, although well defined nanostructures could be observed by AFM. These unusually CD spectra for peptide assemblies have also been previously observed by us (Zottig *et al.*, 2019).

The secondary structure within assemblies was further probed using FTIR spectroscopy by recording the amide I region (1700-1600 cm⁻¹), which can also differentiate parallel and antiparallel β -sheets (Miyazawa et Blout, 1961). All FTIR spectra were characterized by a sharp amide I peak at ~1630 cm⁻¹ (Figure 3.3), a clear hallmark of a β -sheet-rich secondary structure. The appearance of a second characteristic peak at around ~ 1681 cm⁻¹ for I₁₀-COO⁻ and I₁₀-CONH₂ could be associated with the presence of antiparallel structures (Chirgadze et Nevskaya, 1976a, 1976b). In contrast, no distinctive band at ~1680-1685 cm⁻¹ could be detected for the nanostructures assembled from peptides with positive charges at the N- terminus, whether the C-terminus is capped or not (K₂-I₁₀-COO⁻, K₂-I₁₀-CONH₂, K-I₁₀-COO⁻, K-I₁₀-CONH₂), indicative of a parallel β -sheet secondary organization (Chirgadze et Nevskaya, 1976a, 1976b). The shoulder peak at around 1670 cm⁻¹ could be attributed to some heterogeneity of the secondary structure, *i.e.* presence of random coil segment,

and/or with presence of traces of TFA salts (Horvat *et al.*, 1997). Overall, these spectroscopic observations suggest the presence of an amyloid-like β -sheet-rich quaternary organization for all assemblies, although the secondary arrangement within these assemblies differed substantially (Zottig *et al.*, 2019), which translates into the distinctive mesoscopic organizations.

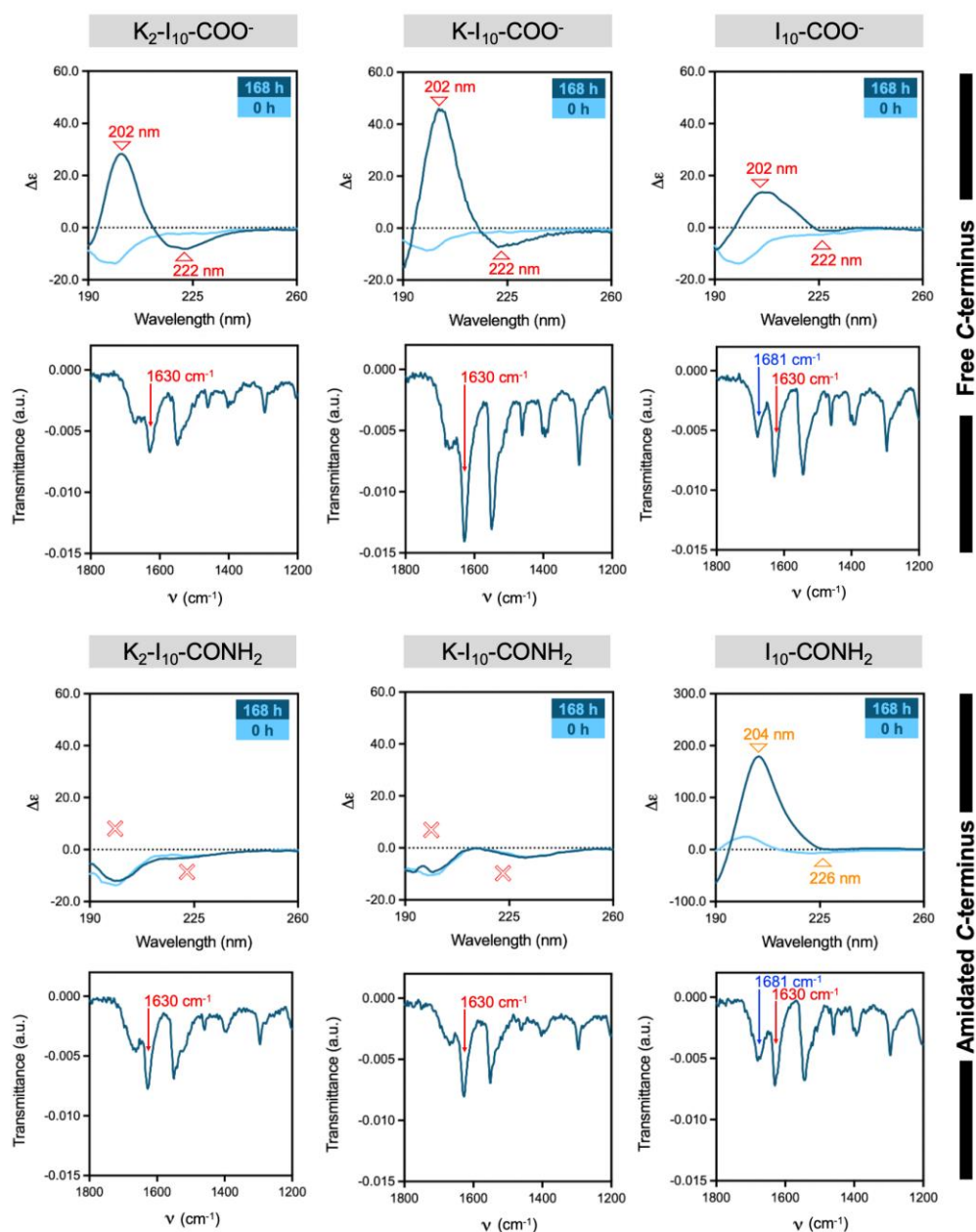


Figure 3.3 Spectroscopic analysis of peptide secondary organization. Far-UV CD (top) and FTIR (down) spectra of I₁₀ peptides. For CD, data were acquired at 0 h and after 1 week of incubation in Tris-HCl buffer (pH 7.4) at RT, whereas for FTIR, only the signal of the nanostructures obtained after 168 h incubation were acquired.

3.5.4 Investigating the cross- β -sheet quaternary conformation with thioflavin T fluorescence

The amyloid cross- β -sheet quaternary conformation was probed by measuring ThT fluorescence. Unexpectedly, although that aggregates could be observed for all peptides by AFM upon 168 h incubation and that characteristic signals of β -sheet were inferred from FTIR analysis, some peptides showed no increase of ThT fluorescence upon 1 week incubation (Figure 3.4). In fact, strong ThT signals were observed for K-I₁₀-COO⁻, I₁₀-COO⁻ and I₁₀-CONH₂ upon assembly. Besides, the two I₁₀ peptides with no N-terminal lysine, showed an instantaneous increase in ThT fluorescence upon their resuspension in Tris-HCl buffer (0 h), which could be attributed to the higher hydrophobicity that promptly drives self-association into cross- β -sheet structure. In sharp contrast, no increase in ThT fluorescence was observed for K₂-I₁₀-CONH₂, K-I₁₀-CONH₂ and K₂-I₁₀-COO⁻ upon 1 week of incubation. The positively charged surface, resulting from N-terminal lysine incorporation, could inhibit the ThT binding through electrostatic repulsion, as this probe carries a thiazole positive charged ring (Arad *et al.*, 2020; Zottig *et al.*, 2019).

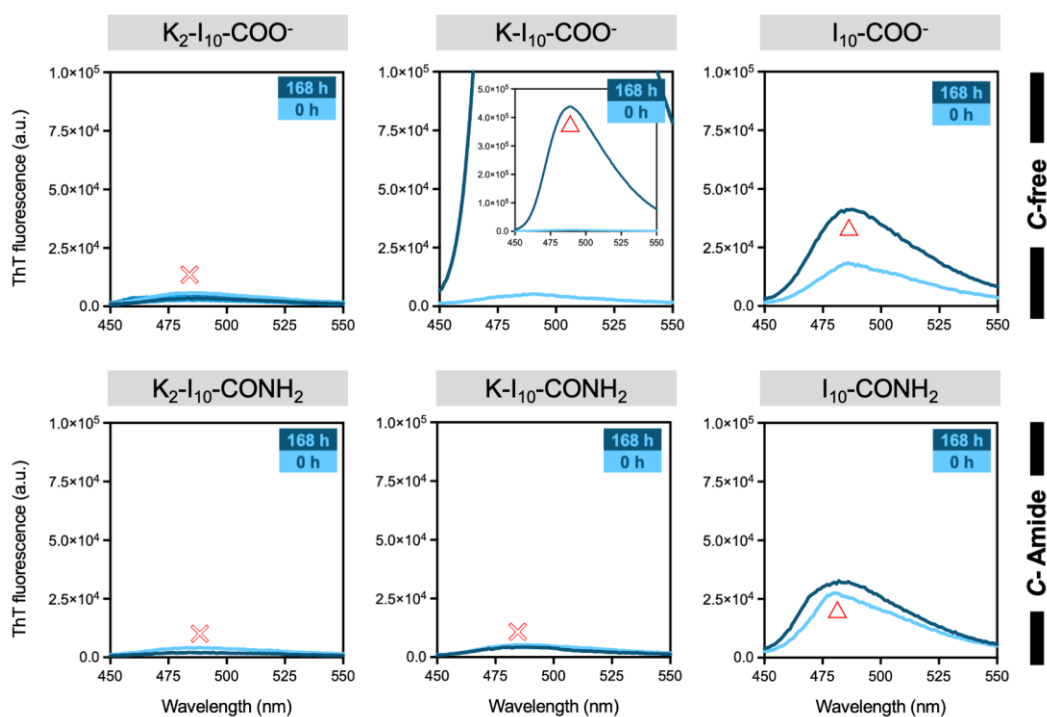


Figure 3.4 ThT fluorescence for amidated and free C-terminus modified I₁₀ peptides. The data were acquired at 0 h and after 1 week of incubation in 20 mM Tris-HCl buffer (pH 7.4) at RT.

3.5.5 Relationships between the morphology of I₁₀ assemblies and their capacity to engage TLR2/TLR6

By modulating the process of self-assembly through the incorporation of *N*- and *C*-terminal modifications, we prepared a small library of amyloid-like nanostructures with a diversity of morphology and conformational properties, while having the same amyloidogenic core sequence (I₁₀). Before moving with the evaluation of the activation of the innate immune receptor TLR2/TLR6, we evaluated the cytocompatibility of these nanostructures. As a matter of fact, although several functional amyloids have been recently identified, amyloid fibrils have been historically associated with protein misfolding diseases (Jackson et Hewitt, 2017; Kaye et Lasagna-Reeves, 2013). Accordingly, we first verified the cytocompatibility of monomeric and assembled I₁₀ peptides using dendritic cells (DC.2.4). Cells were treated with increasing concentrations (from 6.25 to 100 μ M) of monomeric and pre-assembled modified I₁₀

peptides for 24 h and metabolic activity of living cells was measured by resazurin reduction. Results revealed that all monomeric peptides (0 h of assembly) and their assemblies (168 h of assembly) were fully cytocompatible within the concentration range (See Figure S3.4 in the supporting information). Full-length IAPP, used as a positive control of cytotoxicity, was toxic to dendritic cells under its monomeric/pre-fibrillar forms, whereas no toxicity was observed for the fibrillated form of IAPP.

Having access to a set of structural divergent fully cytocompatible amyloid-like nanostructures, we next probed how the morphology, molecular organization and/or size of peptide assemblies affect their capacity to activate the heterodimeric TLR2/TLR6. HEK-Blue hTLR2/TLR6 cells, which overexpress this receptor in addition to a NF- κ B-inducible SEAP (secreted embryonic alkaline phosphatase) reporter-gene, were incubated for 16 h with increasing concentrations of the different assemblies, and NF- κ B activation was evaluated by measuring the absorbance at 630 nm. Interestingly, K₂-I₁₀-CONH₂, K-I₁₀-CONH₂, I₁₀-COO⁻ and I₁₀-CONH₂ assemblies readily activated the TLR2/TLR6, while the K₂-I₁₀-COO⁻ and K-I₁₀-COO⁻ nanostructures, obtained after 7 days incubation were significantly less active (Figure 3.5). Notably, all corresponding monomers, except for the I₁₀-CONH₂ peptide, were inactive to induce NF- κ B activation, indicating that the supramolecular organization is critical to engage TLR2/TLR6 cellular signaling. The activity of I₁₀-CONH₂ without prior pre-incubation could be associated with a quasi-instantaneous assembly as revealed by ThT analysis. In addition, the increase of activity observed for K₂-I₁₀-CONH₂ (50% of Pam₂CSK₄ at 100 μ M) and K-I₁₀-CONH₂ (85% of Pam₂CSK₄ at 100 μ M) assemblies (Table 3.1), compared to their carboxylated counterparts, could be associated with the density of positive charge on the surface. To further investigate the importance of the positive charges for TLR2/TLR6 activation, I₁₀-based assemblies with negatively charged surface were prepared from the E-I₁₀-CONH₂ and evaluated (Figure 3.5). The results indicated that the negatively charged E-I₁₀-CONH₂ cross- β fibrils could not activate TLR2/TLR6 (See Figure S3.5 in the supporting information),

suggesting that not only the quaternary organization is critical but also the surface charges.

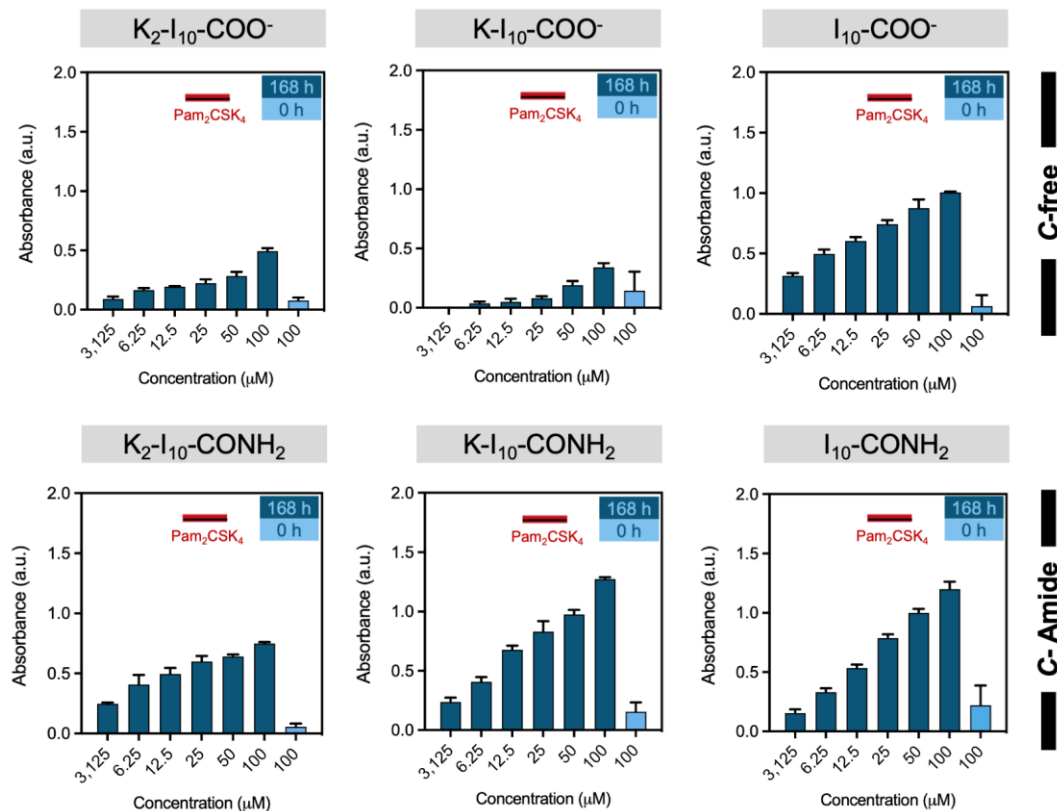


Figure 3.5 TLR Activation with I₁₀ derivatives and their assemblies. HEK-Blue hTLR2/TLR6 cells were incubated for 16 h with increasing concentrations of pre-assembled modified I₁₀ peptides, or with 100 μM of monomeric (0 h) peptides. The positive control Pam₂CSK₄ was used at 10 ng/mL.

Tableau 3.1 Comparative overview of experimental results of amyloid-like assemblies.

Name	Morphology ^a	ThT	CD minima (nm)	FTIR	Length ^b (nm)	Height ^c (nm)	TLR2/6 ^e (%)
K ₂ -I ₁₀ -COO ⁻	Nanorods	+	222	Parallel	20.6 ± 8.0	3.29 ± 0.55	33
K-I ₁₀ -COO ⁻	Twisted fibrils	+++	222	Parallel	-	3.34 ± 0.24	23
I ₁₀ -COO ⁻	Flat fibrils	++	222	Anti-parallel	-	7.25 ± 0.47	68
K ₂ -I ₁₀ -CONH ₂	Nanorods	-	198	Parallel	20.4 ± 5.5	2.79 ± 0.17	50
K-I ₁₀ -CONH ₂	Nanorods	-	198	Parallel	63.0 ± 14.3	3.74 ± 0.31	85
I ₁₀ -CONH ₂	Belt-like	++	226	Anti-parallel	107.6 ± 95.5	7.58 ± 0.27	81

a, b, and c) AFM, e) TLR activation. The (-) and (+) signs are used to compare the extent of fibrillation (ThT), secondary structure changes (CD), and TLR activation (TLR). (-) stands for no change, (+ to +++) stands for an appreciable to a large extent of change.

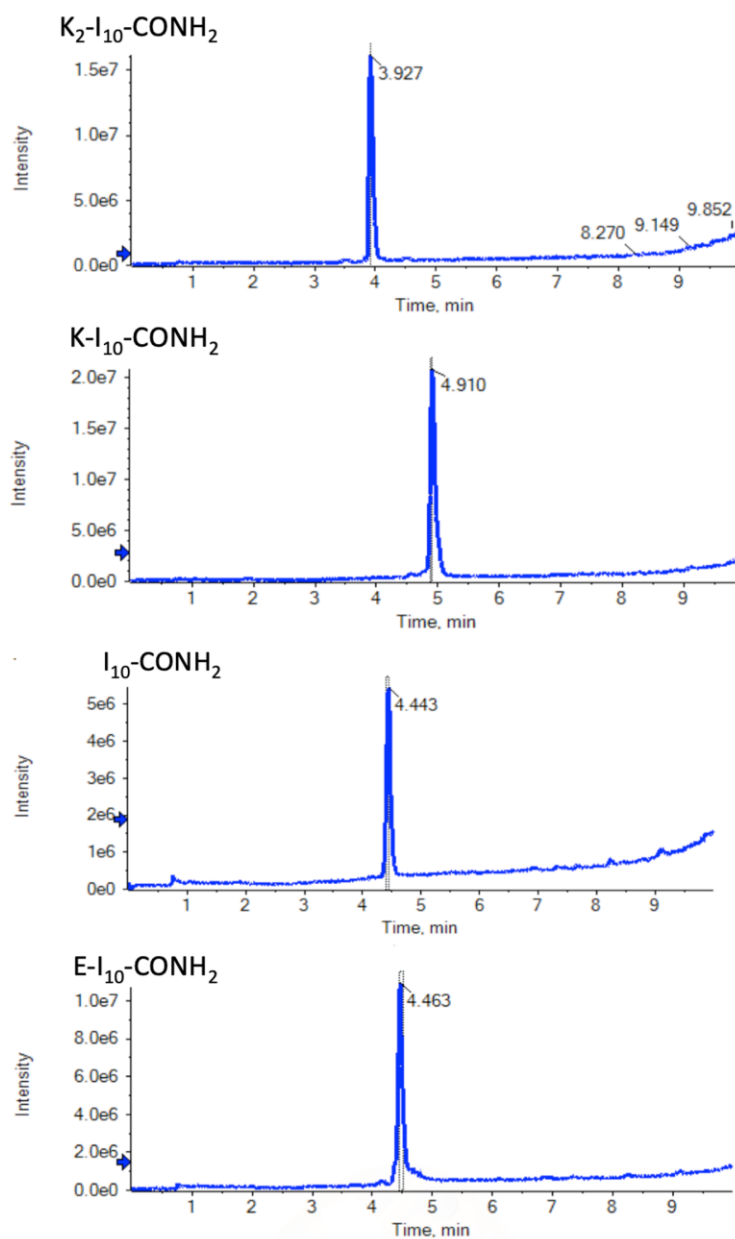
3.6 Conclusion

Herein, electrostatic modifications at the *N*- and/or *C*-terminal of amyloid β -sheet-forming sequence was harnessed to guide the self-assembly and to modulate morphology of the resulting assemblies. Taking advantage of the directional hydrogen bonding involved in peptide self-recognition, as well as the electrostatic repulsive forces at the *N*- and *C*-extremities of amyloid β -sheet-forming sequence, morphology and polymorphism of the resulting amyloid-like assemblies were driven to a diversity of architecture. Moreover, modified I₁₀ peptides and their assemblies were fully cytocompatible and activated, to different extent, the TLR2/TLR6. It was also observed that positive surface charge is an important factor to activate TLR2 by amyloid-like nanostructures. Considering the structural diversity of all the assemblies generated, it would be interesting to further conduct molecular dynamics studies to ultimately understand the structure-activity relationships regarding the activation of TLR2/TLR6 by cross- β assemblies.

3.7 Acknowledgements

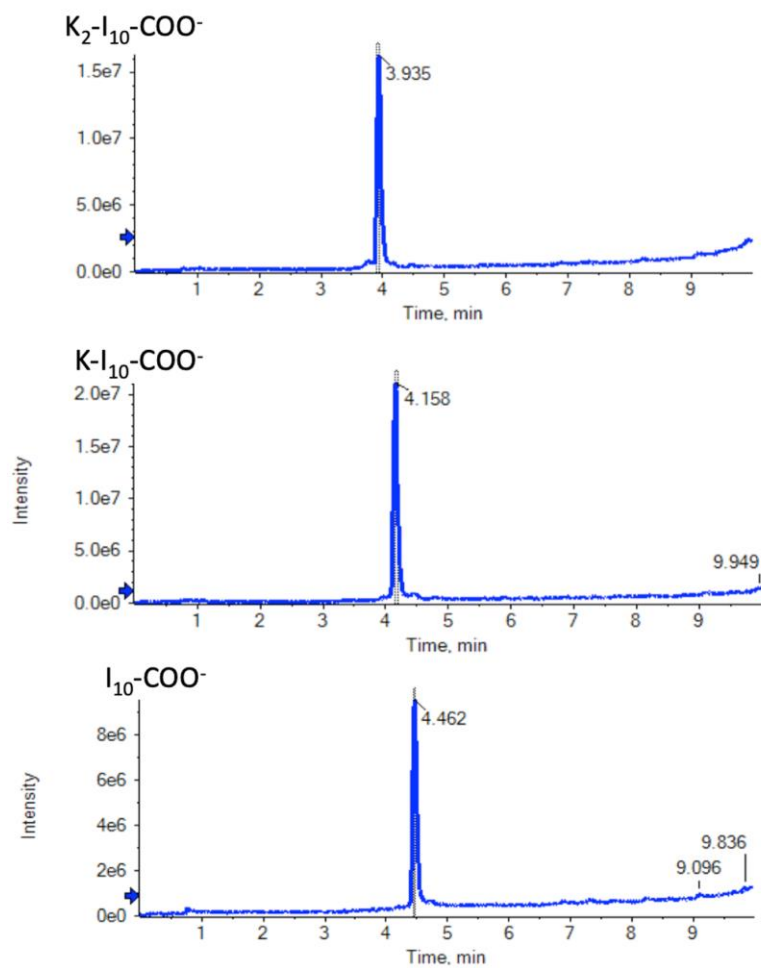
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3.8 Supporting information



Peptides	Formula	Exact mass	Calculated m/z	RT (min)	PPM Error	Observed m/z
K ₂ -I ₁₀ -CONH ₂	C ₆₅ H ₁₀₉ N ₂₁ O ₂₃	1551.8005	776.9075	3.92	0.1	776.9076
K-I ₁₀ -CONH ₂	C ₅₉ H ₉₇ N ₁₉ O ₂₂	1423.7056	712.8601	4.91	0.1	712.8602
I ₁₀ -CONH ₂	C ₅₃ H ₈₅ N ₁₇ O ₂₁	1295.6106	648.8126	4.43	0.6	648.813
E-I ₁₀ -CONH ₂	C ₅₈ H ₉₂ N ₁₈ O ₂₄	1424.6532	713.3339	4.46	0.8	713.3345

Figure S3.1 HPLC and MS analysis of all amidated C-terminus I₁₀ peptides.



Peptides	Formula	Exact mass	Calculated m/z	RT (min)	PPM Error	Observed m/z
K ₂ -I ₁₀ -COO ⁻	C ₆₅ H ₁₀₈ N ₂₀ O ₂₄	1552.7845	777.3995	3.93	1.7	777.4009
K-I ₁₀ -COO ⁻	C ₅₉ H ₉₆ N ₁₈ O ₂₃	1424.6896	713.3521	4.15	0.8	713.3526
I ₁₀ -COO ⁻	C ₅₃ H ₈₄ N ₁₆ O ₂₂	1296.5946	649.3046	4.45	-1	649.304

Figure S3.2 HPLC and MS analysis of all free C-terminus I₁₀ peptides.

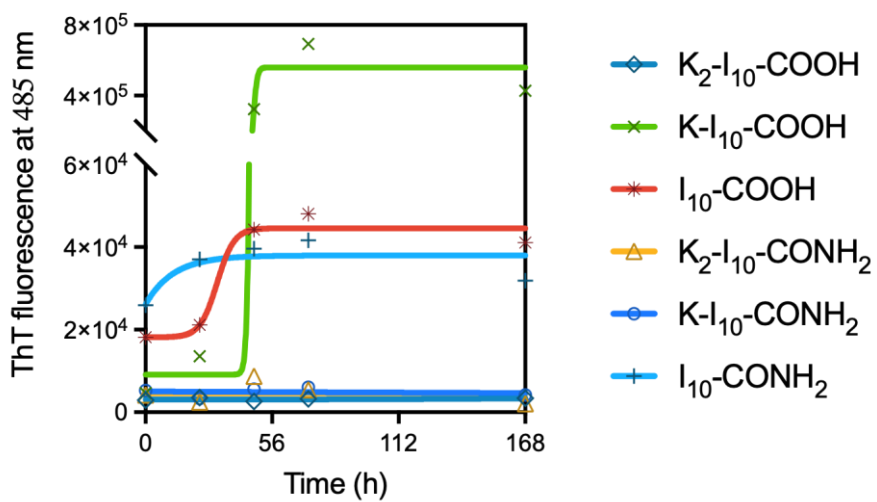


Figure S3.3 Kinetic self-assembly of modified I₁₀ derivatives. Peptides were incubated in Tris Buffer, pH 7.4, at a concentration of 1 mM and under continuous agitation at room temperature, and the analysis were conducted at 0 h and after 24 h, 48 h, 72 h, and 1 week of incubation.

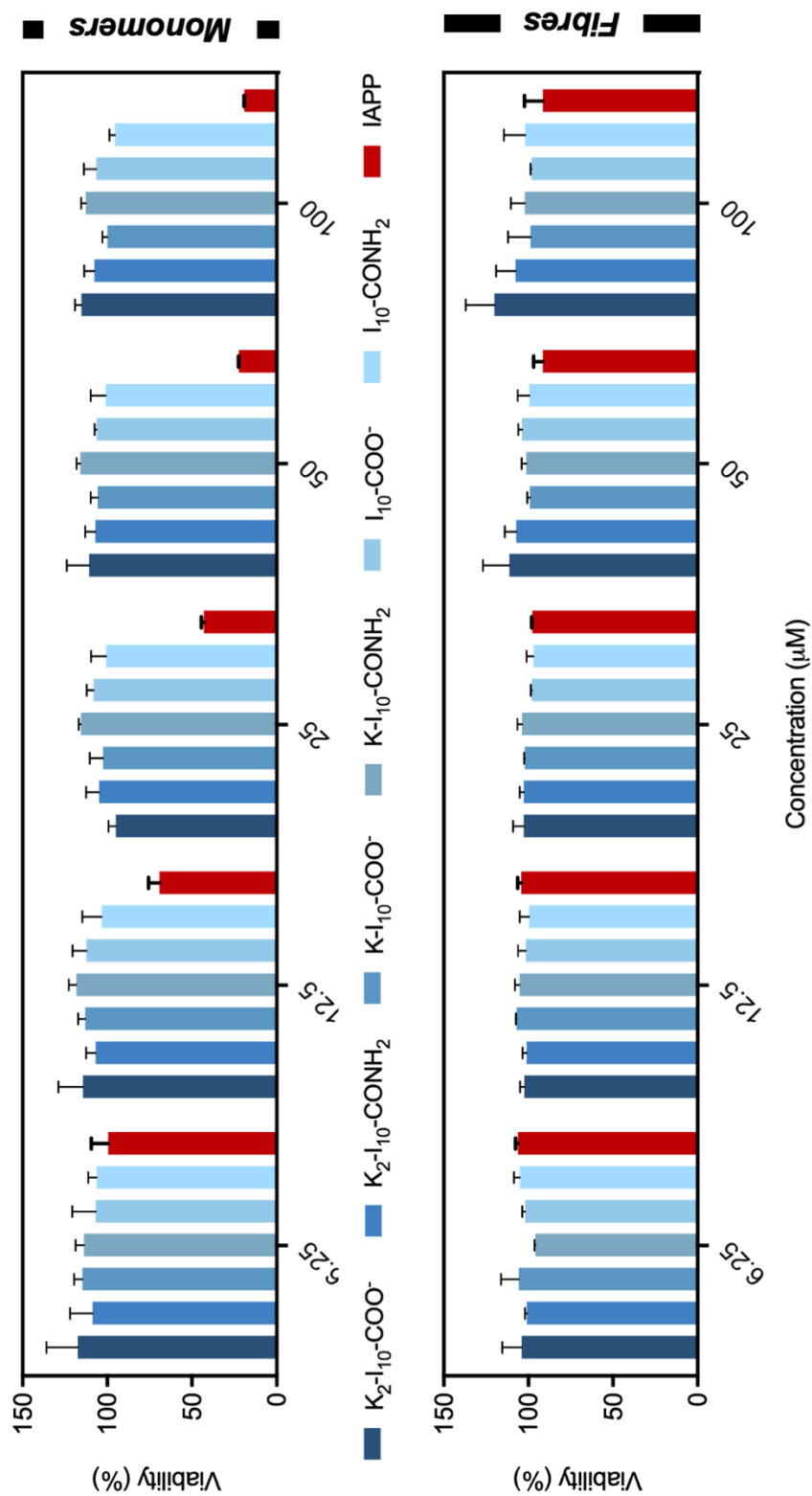


Figure S3.4 Cytocompatibility of monomeric and fibrillar peptides. DC.2.4 cells were incubated for 24 h with increasing concentrations of monomeric (0 h) or assembled (168 h) peptides, and viability was measured by means of the resazurin-based metabolic activity. Data represent the means \pm S.E.M. of at least three individual experiments performed in triplicate.

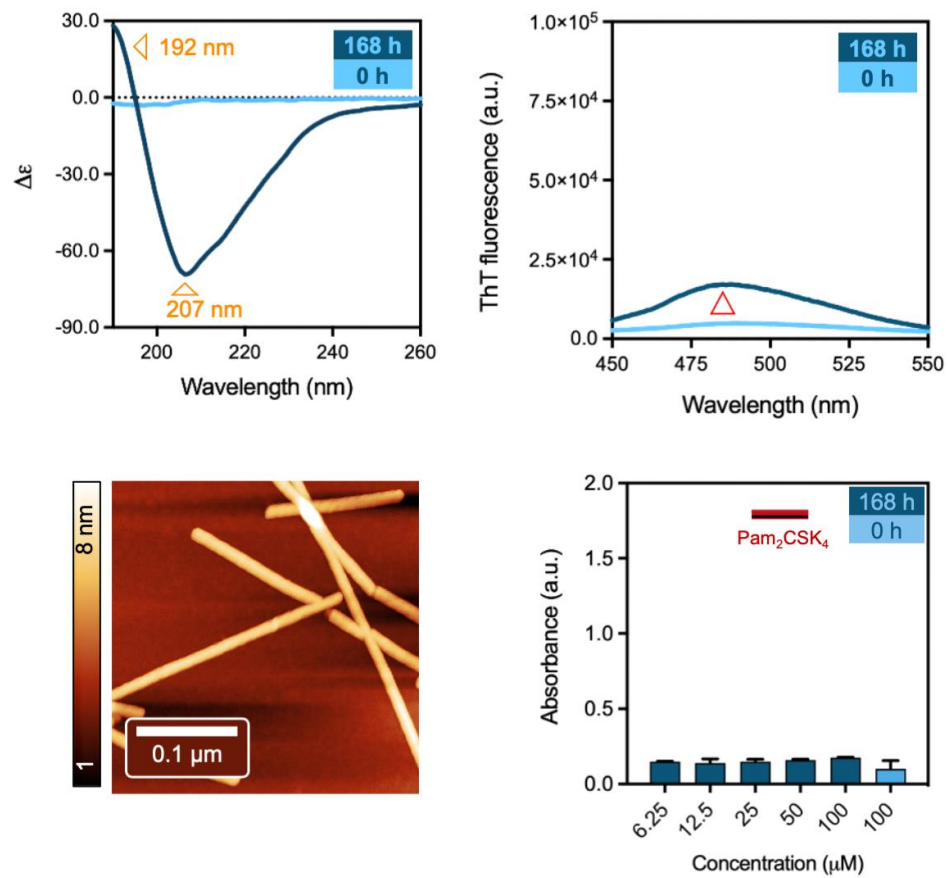


Figure S3.5 Biophysical analysis (CD, ThT and AFM, respectively) and TLR activation of E-I₁₀-CONH₂.

CHAPITRE IV

DNA NANOSTRUCTURES PREVENT THE FORMATION OF AND CONVERT TOXIC AMYLOID PROTEOSPECIES INTO CYTocompatible SPHERICAL COMPLEXES

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Contribution des auteurs :

Nadjib Kihal : Conception du projet, exécution des expériences (synthèse, purification et caractérisation des peptides, microscopie à force atomique, spectroscopie de fluorescence, spectroscopie UV-Visible, spectroscopie de dichroïsme circulaire, spectroscopie FTIR, cinétique d'autoassemblage (ThT et FLAsH), diffusion dynamique de la lumière, potentiel-Zeta, tests de viabilité cellulaire, tests de stabilité, dot-blot et PICUP), analyses des résultats, préparation des figures, rédaction de l'article.

Phuong Trang Nguyen : Exécution des expériences (synthèse, purification et caractérisation des peptides, tests de stabilité, dot-blot et PICUP, LDH, LIVE/DEADE).

Ali Nazemi : Supervision du projet, rédaction et correction de l'article.

Andrea A. Greschner : Conception du projet, exécution des expériences (assemblage, purification et caractérisation des nanostructures d'ADN).

Marc A. Gauthier : Supervision du projet, rédaction et correction de l'article.

Steve Bourgault : Supervision du projet, rédaction et correction de l'article.

4.1 Résumé

Le dépôt d'agrégats protéiques insolubles sous forme de fibrilles amyloïdes est associé à de nombreuses maladies. Le développement d'approches thérapeutiques qui bloquent la formation d'amyloïdes et qui préviennent la dégénérescence cellulaire demeure un grand défi. Cela est dû à la complexité du processus d'agrégation des protéines, qui implique un ensemble infini de conformations et de structures quaternaires. Il a été démontré que les biopolymères polyanioniques, tels que les glycosaminoglycanes (GAG) et les acides ribonucléiques (ARN), sont capables de moduler l'autoassemblage des polypeptides amyloïdogènes et réduisent de ce fait la toxicité induite par la formation des espèces oligomériques et/ou pré-fibrillaires. Dans cette étude, nous avons évalué l'effet de nanostructures d'ADN double brin (ADNdb) sur l'autoassemblage des amyloïdes et la désagrégation des fibrilles, ainsi que la cytotoxicité associée à ces processus. En utilisant l'islet amyloid polypeptide (IAPP) comme modèle de protéine amyloïdogénique, dont l'accumulation tissulaire est une caractéristique du diabète de type 2, nous avons démontré que les nanostructures d'ADN inhibent l'autoassemblage de l'IAPP en induisant la formation de complexes sphériques dans lesquels les peptides adoptent une conformation aléatoire. Particulièrement, les nanostructures d'ADN ont montré une forte capacité à désassembler les fibrilles amyloïdes thermodynamiquement stables de l'IAPP pour former des complexes solubles ADN/peptides. Non seulement ces complexes sphériques étaient entièrement cytocompatibles, mais les nanostructures d'ADN ont piégé instantanément des espèces oligomériques solubles hautement toxiques en les convertissant en complexes binaires non toxiques. Dans l'ensemble, ces résultats ont exposé les effets modulateurs de l'ADNdb sur les voies amyloïdogènes, et ces nanoéchafaudages d'ADN pourraient être utilisés comme source d'inspiration pour la conception de molécules destinées à lutter contre les troubles liés à l'amyloïde.

4.2 Abstract

The deposition of insoluble proteinaceous aggregates in the form of amyloid fibrils within the extracellular space of tissues is associated with numerous diseases. The development of therapeutic approaches that arrest amyloid formation and prevent cellular degeneration remains very challenging. This is due to the complexity of the process of protein aggregation, which encompasses an infinite array of conformations and quaternary structures. Polyanionic biopolymers, such as glycosaminoglycans and RNAs, have been shown to modulate the self-assembly of amyloidogenic polypeptides and to reduce the toxicity induced by the formation of oligomeric and/or pre-fibrillar proteospecies. In this study, we evaluated the effects of double stranded DNA (dsDNA) nanostructures (1D, 2D, 3D) on amyloid self-assembly, fibril disaggregation, and the cytotoxicity associated with these processes. By using the islet amyloid polypeptide (IAPP) as an amyloidogenic protein model whose tissue accumulation is a hallmark of type 2 diabetes, we observed that dsDNA nanostructures inhibit IAPP amyloid self-assembly by inducing the formation of spherical complexes in which the peptides adopted a random coil conformation. Intriguingly, the DNA nanostructures showed persistent ability to disassemble thermodynamically stable IAPP amyloid fibrils into nanoscale DNA/peptide entities. These spherical complexes were not only fully compatible to pancreatic cells, but dsDNA nanostructures also avidly trapped highly toxic soluble oligomeric species and converted them into non-toxic binary complexes. Overall, these results exposed the modulatory effects of dsDNA on amyloidogenic pathways, and these DNA nanoscaffolds could ultimately be used as a source of inspiration for the design of molecules to fight amyloid-related disorders.

4.3 Introduction

Protein aggregation into ordered amyloid structures and subsequent tissue deposition are associated with numerous human diseases, including Alzheimer's disease (AD), systemic amyloidoses and type II diabetes (T2D) (Benson *et al.*, 2020; Mukhopadhyay *et al.*, 2014). While the protein precursors that misfold into insoluble amyloid aggregates share no sequence nor native state structure homology, the resulting unbranched fibrils share a stable cross- β -sheet quaternary conformation in which monomer β -strands run perpendicular to the fibril axis (Chuang *et al.*, 2018; Mukhopadhyay *et al.*, 2014). While the amyloid hypothesis (*i.e.*, that protein aggregation causes tissue degeneration) is supported by compelling experimental and clinical evidence, the development of therapeutic approaches that interfere with the amyloidogenic cascade and prevent the resulting pathology is still in its infancy. Among strategies that have been used to circumvent amyloidogenesis, the stabilization of the protein native state appears to be the most promising approach. This has been exemplified by the clinically-approved drug Tafamidis, which arrests transthyretin aggregation by inhibiting the dissociation of the tetramer into misfolding-prone monomers (Bulawa *et al.*, 2012). Nonetheless, this strategy is based on pharmacological chaperoning and is difficult to apply to amyloidogenic polypeptides that are intrinsically disordered, such as the amyloid- β (A β) peptide and α -synuclein. Moreover, the inhibition of amyloid growth and/or the destabilization of the cross- β -sheet quaternary structure by small molecules, such as natural polyphenols (Nedumpully-Govindan *et al.*, 2016) and short peptides with self-recognition motifs (Kanchi et Dasmahapatra, 2021; Wang *et al.*, 2014), have shown interesting efficacy *in vitro*, though their clinical translation remains elusive. More recently, passive immunotherapies based on antibodies that bind and remove amyloid deposits have been introduced into the clinics (Bateman *et al.*, 2022; Lee *et al.*, 2016; Nuvolone *et al.*, 2022), although their efficacy to improve the quality of life of patients has been somewhat limited and safety concerns have been raised (Pernecky *et al.*, 2023). Thus,

it is still critical to identify (bio)molecules that can interact with the large ensemble of proteospecies generated throughout the amyloidogenic cascade and convert them into non-toxic structures that can be cleared by the host organism.

Polyanionic biopolymers, such as glycosaminoglycans (GAGs), RNAs, and dendrimers, have been shown to promote the self-assembly of amyloidogenic proteins and to reduce the toxicity associated with the formation of oligomeric and/or pre-fibrillar species (Jha *et al.*, 2011; Nguyen *et al.*, 2016; Pepys *et al.*, 2002; Saridaki *et al.*, 2012). However, GAGs are well-known to be associated with virtually all amyloids extracted from patients, where they contribute to the accumulation and persistence of protein deposits (Quittot *et al.*, 2017). Interestingly, short single-stranded DNA (ssDNA) sequences were reported to not only inhibit A β peptide self-assembly, but also to disassemble pre-formed fibrils through electrostatic interactions, leading to the formation of inter polyelectrolyte complexes (IPECs) (Abraham *et al.*, 2014). Similarly, an *in silico* study has suggested that ssDNA could disassemble A β peptides through the electrostatic interactions between the DNA phosphate backbone and positively-charged residues (Dutta et Mattaparthi, 2017). Although these preliminary works appear promising to support the design of amyloid modulators based on ssDNA, the toxicity of these IPECs was not evaluated and ssDNAs are known for their poor stability and high promiscuity (Messaoudi *et al.*, 2019).

The pancreatic deposition of the islet amyloid polypeptide (IAPP) is a hallmark of T2D and correlates closely with disease progression. IAPP, or amylin, is a 37-residue glucomodulatory hormone that is co-expressed and co-secreted with insulin by pancreatic β -cells (Moore et Cooper, 1991). Owing to its primary structure encompassing numerous hydrophobic residues, several sidechains that are prone to H-bonding, and only two charged residues (Figure 4.1a), IAPP is a highly aggregation-prone peptide. IAPP amyloid self-assembly is described as a nucleated polymerization

in which the rate-limiting step is the formation of a high energy nucleus followed by the rapid elongation of the protofilaments (Chatani *et al.*, 2015; Zhang, X. *et al.*, 2020). This process gives rise to a diversity of quaternary structures, each being characterized by conformational polydispersity (Liu *et al.*, 2010). Monomeric IAPP is not cytotoxic *per se*, but induces cell death by assembling into transient soluble oligomers and prefibrillar species that damage the plasma membrane and trigger oxidative stress and apoptosis (Kakinen *et al.*, 2019; Raimundo *et al.*, 2020) (Figure 4.1b). While mature IAPP amyloid fibrils assembled *in vitro* are usually non-toxic to cells (Moore et Cooper, 1991), massive deposition of amyloid plaques in the islets of Langerhans causes inflammatory stress and dysfunction of insulin-secreting cells, contributing to the pathogenesis of T2D (Moore et Cooper, 1991). Thus, therapeutic interventions targeting IAPP-associated pathogenicity should not only inhibit the formation of toxic pre-fibrillar species, but also promote the elimination/clearance of pre-formed amyloid fibrils. However, in its pre-fibrillar states, IAPP shows a large conformational ensemble that complexifies the design of molecules that stabilise the peptide in a non-aggregating form (Hebda *et al.*, 2009; Kaffy *et al.*, 2020). Moreover, amyloid deposits are highly stable and resistant to proteolytic and lysosomal degradation, which precludes their elimination from the afflicted tissues.

Inspired by the initial work regarding the effect of DNA on A β self-assembly, herein we evaluated the effect of small DNA-based nanostructures on IAPP amyloidogenesis and on the cytotoxicity of associated proteospecies. While short ssDNAs have been proven to interact with A β peptides, studies regarding dsDNA have been limited to large genomic DNA systems and shorter dsDNA strands, and small DNA nanostructures have not to be evaluated so far in this context. Biophysical analyses revealed that unlike other polyanionic biopolymers that accelerate amyloid self-assembly, 1D-, 2D-, and 3D-DNA nanostructures (Figure 4.1c) fully inhibit fibril formation. Interestingly, DNA nanostructures efficiently disassembled stable amyloid fibrils and trapped highly toxic oligomers, converting both quaternary species into

cytocompatible and degradable complexes. In addition, the 1D-, 2D-, and 3D-DNA nanostructures were designed such that IAPP peptide fragments responsible for nucleation could be precisely positioned along each structure to ultimately offer some specificity of interaction. Overall, this study highlights the modulatory effects of DNA nanostructures on amyloid formation and suggests that DNA scaffolds could be a source of inspiration for the design of molecular tools to fight protein aggregation disorders.

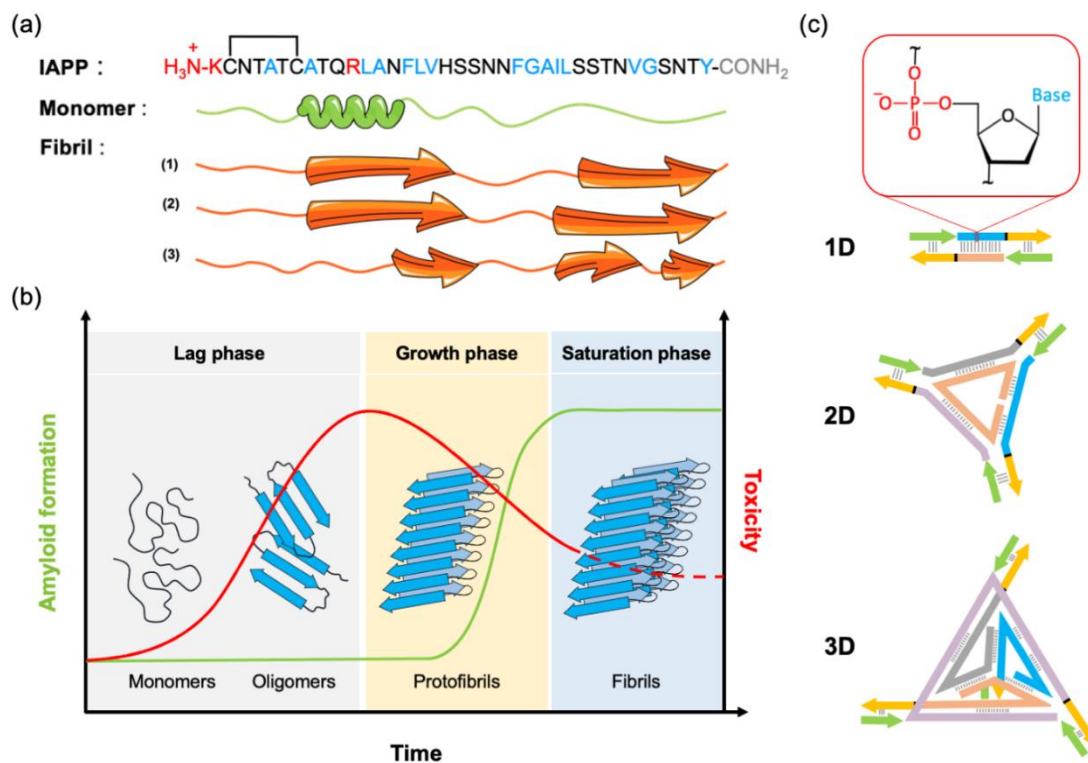


Figure 4.1 IAPP structures and self-assembly as well as the design of DNA nanostructures to interact with them. (a) Schematic representation of IAPP sequence, with hydrophobic residues in blue and positively charged residues in red. The disulfide bond between residues Cys² and Cys⁷ is indicated. Secondary structures of its monomeric (Rodriguez Camargo *et al.*, 2017) and fibrillar states as determined by (1) solution-state NMR (Luca *et al.*, 2007) or (2) Cryo-EM (Röder *et al.*, 2020). (b) Illustration of the self-assembly kinetics of IAPP (green line) alongside the corresponding toxicity profile (red) of the different quaternary species generated. (c)

Schematic representation of 1D, 2D, and 3D-DNA nanostructures evaluated in this work (Inset: negatively-charged phosphate group of the DNA backbone).

4.4 Methods

4.4.1 Materials

Fmoc-protected amino acids and *O*-(1*H*-6-chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HCTU) were purchased from Matrix Innovation. Piperidine was purchased from A&C American Chemicals Ltd. Tris(hydroxymethyl)aminomethane, magnesium chloride, ammonium persulfate, glacial acetic acid, and tetramethylethylenediamine were purchased from Bioshop Canada. Unlabeled DNA strands were purchased from idtDNA. Maleimide-labeled DNA strands were purchased from GeneLink. TAMg buffer was made in-house (40 mM Tris, 12.5 mM MgCl₂·6H₂O, 20 mM glacial acetic acid, pH 7.8). GelRed stain was purchased from VWR Canada. Precast 4-15% PAGE gels were obtained from BioRad. All solvents were of analytical grade and were purchased from Sigma-Aldrich. Deionized water was used to prepare buffer solution (pH 7.4). All solvents, reagents, and other chemicals were used as received without further purification.

4.4.2 Peptide Synthesis, Purification, and Characterization

IAPP was synthesised by means of standard Fmoc solid-phase peptide synthesis (SPPS) then purified using reverse-phase high-performance liquid chromatography (RP-HPLC), as previously described (Nguyen *et al.*, 2017). Formation of the C²–C⁷ disulfide bridge was achieved by oxidation in dimethyl sulfoxide (DMSO) at room temperature (RT). The assembled IAPP was purified by RP-HPLC and analyzed by high-resolution mass spectrometry using an Agilent 1200 series HPLC coupled to a electrospray time-of-flight mass spectrometer (ESI-TOF) (Figure S4.1). Peptide

fractions, with a purity higher than 95% were combined and lyophilized. Subsequently, aliquots of monomerized IAPP peptide were prepared by treating the lyophilized powder in 100% hexafluoro-2-propanol (HFIP) to a concentration of 1 mM. The solution was sonicated for 30 min, filtered through a 0.22 μm hydrophilic polypropylene filter and lyophilized. The lyophilized IAPP powder was solubilized again in HFIP at the same concentration, sonicated for 30 min, and then lyophilized a second time. Monomerized samples were kept in the frozen dried state at $-80\text{ }^{\circ}\text{C}$ for a maximum period of 4 weeks.

4.4.3 Design and Assembly of DNA Nanostructures

Linear DNA nanostructures (DNA-1D) consisted of a 39 base pair complementary region (Table S4.1), followed by a four-thymine spacer, and a 15-base addressable overhang. The overhang permits the incorporation of overhang complements (OC, Table S4.1). Unmodified OC strands are used to complete the double-stranded structures bearing no ligands. For peptide-guided complexing studies, the OC stranded was conjugated to a short fragment of IAPP (20-29). The sequences of the pairing region and the overhang were generated using CANADA 2.0 (Feldkamp *et al*, 2010), a random DNA sequence generator. Sequences were then cross-analyzed for unwanted binding using idtDNA's Oligoanalyzer tool, based on the work of Allawi (Allawi *et SantaLucia*, 1997). Final sequences were ordered from idtDNA. Assembly of the desired structure was verified using polyacrylamide gel electrophoresis (Figure S4.2, 0.002 nmol of structure per well, pre-annealed from $90\text{-}23.3\text{ }^{\circ}\text{C}$ over the course of 16 h; 80 V, 90 min, 4–15% polyacrylamide). The DNA triangle (DNA-2D) is a novel design created to provide a 2D structure with 3 overhangs (Figure 4.1 and Table S4.1). Built around a central (TriGreen) strand, three strands with regions complementary to both the TriGreen and each other were designed using CANADA2.0 to generate unique sequences. Final sequences were chosen based on cross-analyses for undesired binding.

The final structure resembles a triangle with 3 arms, each possessing a structural binding region and an overhang region. Once the complementary sequences were selected, the structure was modeled using GIDEON (Allawi et SantaLucia, 1997), a program for DNA nanostructure design. GIDEON was used to determine if extra non-binding bases (thymines) were required at each vertex. Extra bases allow for continuation of the DNA helix, releasing strain between the main triangle and each arm. These bases are indicated in black in Figure S4.1 and Table S4.1. Assembly of the desired structure was based on the predicted design and migration via PAGE. Each strand was added in a stepwise manner, to ensure that changes in mobility were as expected (Figure S4.2, 0.00435 nmol of structure per well, pre-annealed from 90-23.3 °C over the course of 16 h; 80 V, 90 min, 4–15% polyacrylamide). The DNA tetrahedron (DNA-3D) was adapted from the design of Dong, et al (Dong *et al.*, 2015), with the addition of a four-thymine linker and the addressable overhang at each vertex. To verify assembly, each strand was added in a stepwise manner to ensure that changes in mobility were as expected (Figure S4.2, 0.00435 nmol of structure per well, pre-annealed from 90-23.3 °C over the course of 16 h; 80 V, 90 min, 4–15% polyacrylamide.). Each DNA strand was quantified by absorbance at 260 nm prior to use. For each structure, sufficient DNA was added to reach a final concentration of 14 μ M in TAMg buffer. The overhang regions were blocked with appropriate amounts of OC, creating fully duplexed structures. The molar ratio of OC per assembly was dependant on the structure. For the linear structure, the ratio was 2:1 (OC: structure). For the triangle, the ratio was 3:1. For tetrahedron the ratio was 4:1. Due to the prevalence of oligomer formation at high 2D and 3D DNA concentrations, the stock solutions of each structure were diluted 10 \times with water prior to assembly. The stock was mixed, then separated into PCR tubes. Using a PCR machine, the tubes were heating to 90°C and then cooled to 23.3°C over the course of 16 h. Samples were recombined and lyophilized to dryness, then resuspended to the initial volume using sterile water. Assembly was verified using polyacrylamide gel electrophoresis (80 V, 75 min).

4.4.4 Preparation of peptide-labeled DNA (OC-Pep) structures

Maleimide-labeled DNA strands (OC-mal) arrive as protected maleimido-2,5-dimethylfural cycloadducts. Maleimide deprotection was performed using a microwave deprotection strategy (Sánchez *et al.*, 2011). Briefly, the cycloadduct was deprotected in 1:1 MeOH:H₂O solvent with a final DNA concentration of 25 µM in 200 µL. This mix was placed in a CEM microwave reactor. A ‘dynamic’ method was prepared (T = 90 °C, Power = 300 W). With this method, the temperature was maintained at T = 90 °C by varying the power. The buildup of pressure kept the sample from boiling, and PowerMax was enabled to provide cooling via compressed air. The reaction was run for 90 minutes. Once the mixture had cooled, it was transferred to an Eppendorf tube and dried using a SpeedVac. For conjugation to the maleimide, an IAPP peptide fragment containing an additional Cys with free thiol was synthesized (CKGSGSSNNFGAILSS), purified using RP-HPLC and analyzed by liquid chromatography coupled with high-resolution mass spectrometry (Figure S4.3). The conjugation reaction was then performed in TAMg buffer, with 20 µM of OC-mal and 40 µM of peptide (1:2 ratio). The reaction mixture was placed on a shaker overnight at room temperature. To ensure conjugation of the peptide to OC-mal, crude reaction product was evaluated by LC-MS, with a gradient of 20-60% acetonitrile + 0.1% trifluoroacetic acid over 25 min (flow rate 1 ml/min, Agilent Zorbax 300SB-C18, 5 µm, 4.6 x 150 mm column, electrospray ionization). Three peaks were identified through a combination of liquid chromatography controls and mass calculations: OC-mal eluted at 14.5 min, peptide eluted at 4.2 min, and OC-Pep eluted at 15.5 min. Analysis of peak areas revealed no leftover OC-mal, so no further purification was performed. Free peptides may have been present but are minimal in comparison to the large amounts of peptide added for subsequent experiments. Polyacrylamide gel electrophoresis (0.024

nmol DNA-Pep per lane, 90 min at 80 V) confirmed hybridization of OC-peptide to the OC portions of the linear structure.

4.4.5 Preparation of IAPP quaternary structures

IAPP quaternary structures were prepared by dissolving the lyophilized and monomerized peptide into 20 mM Tris-HCl buffer (pH 7.4) at 75 μ M. The freshly prepared solution was used immediately as mainly monomeric species (IAPP_M), incubated under fully quiescent conditions at RT for 3 h to form oligomeric and pre-fibrillar species (IAPP_O), or incubated for 48 h to generate amyloid fibrils (IAPP_F). Solutions were then diluted to reach a final concentration of 25 μ M of IAPP to which were added (or not) 1.6 μ M of DNA nanostructures.

4.4.6 UV-visible Spectroscopy

Mandel UV-1280 spectrophotometer was used to record UV-vis absorption spectra from 190-390 nm. The light pathlength was 10 mm, and the internal step was set to 1 nm.

4.4.7 Fluorescence Spectroscopy

Fluorescence measurements were carried out at RT on a PTI Photon Technology International steady state spectrofluorometer (QuantaMaster 40) and operated with PTI's FelixGX software with light pathlength of 3 mm. For thioflavin T (ThT), used at concentration of 40 μ M, the excitation wavelength was set at 440 nm and the emission was recorded from 450-550 nm. For 8-anilino-1-naphthalene-sulfonic (ANS), used at concentration of 100 μ M, the excitation wavelength was set at 355 nm and the emission spectra were recorded from 385-585 nm. For fluorescein arsenical hairpin (FlAsH),

used at concentration of 0.5 μM , and supplemented with 125 μM dithiol British anti-lewisite (BAL) and 500 μM tris(2-carboxyethyl)phosphine (TCEP), the excitation wavelength was set at 508 nm and the emission spectra were recorded from 460-540 nm. For intrinsic fluorescence spectroscopy, the tyrosine fluorescence spectra were recorded from 290-360 nm with an excitation wavelength of 274 nm. The intrinsic fluorescence of IAPP Y³⁷ in the absence of DNA was assigned as F_0 , and the intrinsic fluorescence of tyrosine in the presence of DNA was assigned as F . The extent of the change in the fluorescence intensity was ascribed to $(F_0 - F)/F_0$.

4.4.8 Kinetics of IAPP Fibrillization

Lyophilized peptide was dissolved in 20 mM Tris-HCl, pH 7.4 with or without 1.6 μM DNA. Final IAPP concentration was 25 μM in presence of 0.5 μM FIAsh (supplemented with 125 μM BAL and 500 μM TCEP). Kinetics assays were performed in triplicate using black-wall sealed 96-well microplates with clear bottom and non-binding surfaces. Fluorescence measurements were taken from the bottom of the microplate every 10 min and under fully quiescent conditions at 25 °C using a Molecular Devices Spectramax i3 plate reader, with an initial shaking for 5 s (linear shaking, 2 mm amplitude, 654 rpm). The excitation wavelength was set to 508 nm and the emission was recorded at 533 nm. The kinetics data from triplicates were fitted to a Boltzmann sigmoidal function (1):

$$F = \frac{F_{min} + (F_{max} - F_{min})}{1 + e^{-(t - t_{1/2})/k_{app}}} \quad (1)$$

where F_{min} and F_{max} are, respectively, the minimum and maximum fluorescence intensities, $t_{1/2}$ is the time to reach half-maximum fluorescence intensity, and k_{app} is the apparent first-order rate constant. The lag time was calculated as $t_{1/2} - 2k_{app}$. Data of a

least three independent assays achieved in triplicate were averaged and expressed as the mean \pm standard deviation (S.D.).

4.4.9 Far-UV Circular Dichroism Spectroscopy

CD spectra were collected on Jasco J-815 spectrophotometer at RT, in a 2-mm quartz cuvette. Peptide solutions were diluted in buffer to reach concentration of 25 μ M, and the CD spectra were collected from 260-190 nm. Curves were smoothed using a smoothing moving window of 13 data points through the Savitsky-Golay algorithm.

4.4.10 Dynamic Light Scattering

Size measurements were performed using the Malvern, Zetasizer Nano S90 (Malvern Panalytical, UK) containing a 4 mW helium-neon laser with a wavelength of 633 nm and a scattering angle of 90°. Each measurement corresponded to a triplicate of 10 runs per analysis performed at 25°C. The results were presented as number distribution (%) of particles versus hydrodynamic radius (nm) calculated from the Malvern software.

4.4.11 Zeta Potential

Measurements were carried out using a zeta potential analyzer (BrookHaven Instrument Corp., ZetaPlus / BI-PALS). Each measurement corresponds to a triplicate of 10 runs per analysis.

4.4.12 Atomic Force Microscopy

IAPP solutions, with and without DNA nanostructures, were spotted onto a freshly peeled mica surface. Excess liquid was removed by wicking, then the surface was

rinsed twice with deionized water. After being air-dried overnight, AFM imaging was conducted using a Bruker (MultiMode 8) microscope equipped with a Nanoscope E controller. To characterize the morphology of the resulting complexes, samples were scanned in scanasyst-air mode using a silicon nitride AFM tip (2–12 nm tip radius, $k = 0.4$ N/m) under ambient conditions. Gwyddion v2.59 was used to analyse images.

4.4.13 Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy

IAPP incubated for up to 72 h in 20 mM Tris-HCl, pH 7.4 with, or without, of 1D-, 2D-, or 3D-DNA were lyophilized and ATR-FTIR spectra were recorded from the solid-state sample using a Nicolet Magna 560 spectrometer equipped with a nitrogen-cooled MCT detector.

4.4.14 Photochemical-Induced Cross-Linking

An IAPP₀ solution (75 μ M) was treated with cross-linking solution (70 μ M tris-(bipyridyl)Ru(II) and 1.4 mM ammonium persulfate) and then illuminated with a 150 W incandescent bulb for 5 s. Subsequently, the reaction was quenched with 1 M dithiothreitol (DTT), oligomers were separated by SDS-PAGE electrophoresis (10% Tris-tricine gel) and revealed using silver staining.

4.4.15 Dot Blot Analysis

IAPP₀ solution (10 μ L at 75 μ M) was applied to a nitrocellulose membrane, was allowed to air dry, and then blocked in 5% non-fat dried milk in Tris buffered saline with Tween-20 (TBS-T) for 30 min. The membrane was incubated with an anti-oligomer rabbit polyclonal primary antibody (A11) for 2 h and then washed three times in TBS-T. The membrane was incubated for 1 h with an HRP-conjugated secondary antibody, diluted at 1:10 000 in 5% non-fat dried milk in TBS-T. Finally, after three

washes of the blot in TBS-T, the signal from the secondary antibody was detected by enhanced chemiluminescence.

4.4.16 Proteolytic Digestion by Proteinase K.

Stability against proteolysis was assessed by subjecting the IAPP fibrils and IAPP_F/DNA-1D complexes to 120U/mL of proteinase K for 1h and digestion was evaluated by Matrix Assisted Laser Desorption Ionization - Time of Flight (MALDI-TOF) mass spectrometry.

4.4.17 Cell Viability Assays

Rat pancreatic β -cell line (INS-1E) were cultured in black wall clear bottom 96-well plates treated for tissue culture at a density of 25 k cells/well in RPMI-1640 media containing 10% of fetal bovine serum (FBS), 100 UI/mL penicillin, 100 mg/mL streptomycin, 10 mM HEPES, 1 mM sodium pyruvate, 50 mM mercaptoethanol, and 2 mM L-glutamine.(Nguyen, Phuong Trang *et al.*, 2021) After 48 h incubation in a humidified atmosphere of 5% CO₂ and 95% air at 37 °C, cells were treated by the direct addition of IAPP_O (25 μ M), with or without the co-addition of 1.6 μ M 1D, 2D, or 3D DNA nanostructures, and then incubated for 5 h. For the evaluation of the cytotoxicity of the IAPP-DNA complexes, obtained by preincubation of IAPP (75 μ M) with DNAs (4.8 μ M) in 20 mM Tris-HCl buffer (pH 7.4) for 72 h, cells were treated with 25 μ M of IAPP/DNA complexes and then incubated for 24 h. Cellular viability was evaluated using the resazurin metabolic assay and determined in % from the fluorescence ratio of peptide-treated sample to vehicle-treated control (20 mM Tris-Buffer, pH 7.4). Data of a least three independent assays achieved in triplicate were averaged and expressed as the mean \pm standard error of mean (S.E.M.). For Live/Dead assays, INS-1E cells were plated in 12-well plates at density of 150 000 cells/well for 48 h before treatment.

Fluorescent microscopy analyses were performed after the incubation with 4 μM of ethidium homodimer-1 and 2 μM of calcein-AM for 45 min incubation.

4.4.18 Lactate Dehydrogenase (LDH) Release Assay

INS-1E cells were seeded in 96-well plates at 25 000 cells/well. After 48 h incubation, cells were treated by direct addition of IAPP_M, IAPP_O, IAPP_F (25 μM), with or without the co-addition of 1.6 μM linear DNA nanostructures. Cell media (50 μl) was collected after 6h of treatment and incubated with the reaction mixture from the Pierce LDH Cytotoxicity Detection Kit for 30 min at room temperature. Reaction was stopped and absorbance was measured from 490 nm to 680 nm. To determine LDH activity, the absorbance at 680 nm (background) was subtracted from the 490 nm absorbance before calculation. Percentage of LDH leakage was calculated:

$$\% \text{ LDH leakage} = \frac{\text{LDH activity}_{\text{peptide-treated}} - \text{LDH activity}_0}{\text{LDH activity}_{\text{max}} - \text{LDH activity}_0} \times 100$$

where LDH activity₀ is the absorbance measured in absence of peptide and LDH activity_{max} is the absorbance measured for cells treated with lysis buffer. Data of at least three individual experiments performed with different lot of peptides were averaged and expressed as the mean \pm S.E.M.

4.5 Results and discussion

4.5.1 DNA Nanostructures Modulate IAPP Self-Assembly

Owing to their programmability and addressability, DNA molecules have emerged as versatile nanoscale materials for numerous biomedical applications including vectors for targeted drug delivery as well as sensors for diagnostics and theranostics (Mathur

et al., 2023). Interestingly, short ssDNAs have been shown to be potent modulators of amyloid formation of the A β peptide (Abraham *et al.*, 2014). However, ssDNA is highly flexible and less thermodynamically stable compared to double-stranded DNA (dsDNA), especially those assembled into 2D and 3D architectures (Pal et Levy, 2019). In this context, the present study explored a new paradigm to modulate IAPP amyloid formation via a DNA-mediated scavenging mechanism. Through rational design of DNA sequences, almost any desired shape can be constructed with nanometer scale precision (Kim, H. *et al.*, 2014). Firstly, small and ordered 1D, 2D, and 3D dsDNA nanostructures were assembled from individual DNA strands (Table S4.1 and Figure 4.1). The linear dsDNA strand used in this study were longer compared to the ssDNA previously used (Abraham *et al.*, 2014) to ensure that all regions remained stably hybridized during the increased temperatures required for *in vitro* cell studies, especially the overhang regions. The DNA tetrahedron is based on a common motif that has been used in multiple *in-vitro* and *in vivo* studies (Ma *et al.*, 2022). The 2D structure is an original design, created to have approximately the same number of base pairs (and polyanions) as the tetrahedron. DNA stability over 1-week under experimental conditions was confirmed by UV-vis spectroscopy (Figure S4.4).

Monomerized IAPP (IAPP_M) was incubated in the presence or absence of unconjugated DNA nanostructures in Tris-buffer, pH 7.4 at room temperature under fully quiescent conditions. The kinetics of IAPP fibrillization were evaluated over time by means of fluorescence spectroscopy in a microplate reader. Although ThT fluorescence is usually used to probe amyloid formation with good selectivity (Naiki *et al.*, 1989), ThT exhibits significant emission in presence of DNA nanostructures (Hanczyc *et al.*, 2021) that overlapped with amyloid binding (Figure S4.5), precluding the use of this method. Thus, we used an alternative strategy based on the fluorogenic FIAsh dye to probe the kinetics of IAPP self-assembly in the presence of DNA nanostructures. FIAsh is a derivative of fluorescein whose fluorescence quantum yield is dramatically amplified upon its binding to a tetracysteine motif. Accordingly, under reducing conditions that

do not affect IAPP fibrillization, a non-contiguous tetracysteine FAsH-binding site is formed upon the convergence of *N*-terminal domains that each contain a Cys²-Cys⁷ motif (Figure 4.1a), leading to a fluorescent signal (Quittot *et al.*, 2018). Thus, this method is well-suited to investigate the kinetics of IAPP aggregation by monitoring the formation of quaternary complexes in the presence of DNA. As shown in Figure 4.2a, a prototypical sigmoidal growth curve was obtained for IAPP in the absence of DNA by monitoring FAsH fluorescence. The extracted lag-time of 3.3 ± 0.1 h and half-time ($t_{1/2}$) of 5.0 ± 0.2 h were within the range of the kinetics data obtained using ThT fluorescence under these conditions (Figure 4.2a). In sharp contrast, incubation of 25 μ M IAPP in the presence of 1.6 μ M of linear (1D), triangular (2D), or tetrahedral (3D) dsDNA nanostructures, induced a sharp increase of FAsH fluorescence with the absence of any observable lag phase (Figures 4.2b and 4.2c). This observation indicates that the dsDNA nanostructures accelerate the formation of the split-tetracysteine motif through the convergence of the *N*-terminal domain of IAPP molecules. IAPP is a positively charged peptide displaying a net charge of +3 at physiological pH (*N*-terminus, Lys¹, Arg¹¹; Figure 4.1a), likely favoring its binding through electrostatic interactions to the negatively charged phosphate backbone of DNA. Thus, DNA nanostructures likely potentiate IAPP self-assembly by an electrostatic-type mechanism (De Carufel *et al.*, 2013; Motamedi-Shad *et al.*, 2009), in which the negatively charged DNA interacts with the positively charged *N*-terminal domain of IAPP, increasing local concentration into clusters that allow intermolecular FAsH binding. No significant differences were observed between the kinetics of IAPP assembly in the presence of the different DNA nanostructures. Nevertheless, a trend in the maximum intensity of FAsH could be observed as $3D < 2D < 1D$, suggesting an influence of the structure of DNA on the morphology of the assemblies formed in the presence of IAPP, with greater convergence of the *N*-termini of IAPP than in the absence of DNA.

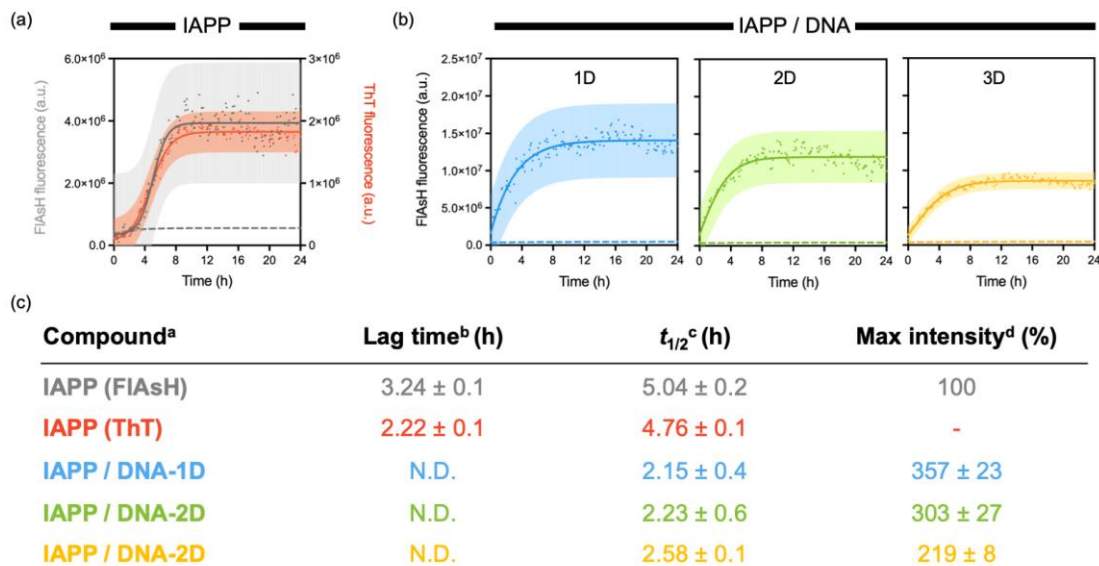


Figure 4.2 Effects of dsDNA nanostructures on the kinetics of IAPP fibrillation. IAPP (25 μ M) was incubated in 20 mM Tris, pH 7.4, at 25°C in the (a) absence or in the presence of 1.6 μ M of (b) 1D, (c) 2D and (d) 3D DNAs. FIAsh and ThT (for IAPP) fluorescence was measured every 10 min, with excitation / emission at 508 / 533 nm and 440 / 585 nm, respectively. Dashed lines represent (a) control vehicle buffer and (b) DNA alone (absence of IAPP).

Next, we further evaluated the ability of DNA nanostructures to modulate IAPP self-assembly by performing biophysical analyses and AFM imaging after prolonged incubation of IAPP with DNA. CD spectroscopy revealed that IAPP alone, *i.e.* in absence of DNAs, exhibits a random coil secondary structure with a negative band at 202 nm immediately after its solubilization (0 h), which shifted towards a β -sheet-rich conformation after 72 h of incubation (Figure S4.6a). This secondary structure conversion is associated with a sharp increase in ThT fluorescence (Figure S4.6b), indicative of the formation of cross- β -sheet quaternary organization (Sebastiao *et al.*, 2017). Similarly, FIAsh and ANS fluorescence respectively revealed the gathering of adjacent *N*-terminal regions and the formation of hydrophobic clusters upon 72 h incubation (Figure 4.3a, 4.3b and Figures S4.6c, S4.6d). Curiously, little change to FIAsh intensity was observed in the presence of the 2D and 3D DNA nanostructures,

despite changes observed by other techniques. For IAPP, the formation of amyloid fibrils was confirmed by AFM, with images showing a network of typical fibrils with two major distinctive morphologies: flat ribbons and twisted filaments (Figure 4.3c and Figure S4.6e). This morphological heterogeneity is typical for IAPP fibrils assembled *in vitro* (Luca *et al.*, 2007). Because of their high background signal, CD spectroscopy and ThT fluorescence could not be performed in the presence of DNA nanostructures. Remarkably, incubation of 1D, 2D, or 3D dsDNA nanostructures with monomerized IAPP for 72 h led to the formation of spherical assemblies with the total absence of fibrillar aggregates (Figure 4.3c), indicating that these entities inhibit IAPP fibril formation by influencing the mechanism of self-assembly. These spherical aggregates were characterized by the presence of solvent-accessible hydrophobic patches, as observed with the sharp increase in the intensity of ANS fluorescence (Figure 4.3b). DLS analyses revealed that the DNA/peptide complexes exhibit a hydrodynamic radius between 300-400 nm, independently of the identity of the DNA nanostructure. These are much smaller than the IAPP structures formed in the absence of DNA (Figure 4.3d and Figure S4.7). Next, we probed if this effect, probably mediated through electrostatic interactions, could occur in presence of a physiologically relevant ionic strength. Thus, monomeric IAPP was incubated in presence of DNA nanostructures in 20 mM Tris, pH 7.4, supplemented with 150 mM NaCl. Results revealed the formation of similar spherical complexes with total absence of amyloid fibrils after 3 days incubation (Figure S4.8), indicating that IAPP-DNA interactions can occur even in presence of a physiologically relevant concentration of salts. Finally, we evaluated if this inhibitory effect is affected upon the functionalization of the DNA nanostructures aiming at offering some specificity of interaction. To do so, the nucleating core of IAPP, the region 20-29 (I₁₀), was covalently attached to the flanking region of the 1D dsDNA through a maleimido-Cys coupling strategy and the resulting peptide-labeled DNA structure was incubated in presence of IAPP. Interestingly, the same effect on IAPP amyloid formation was observed in presence of 1D DNA functionalized with the I₁₀ peptide (Figure S4.9), indicating that dsDNA can be functionalized, while maintaining

its inhibitory effect on amyloid self-assembly. Overall, these results revealed that linear, triangular, and tetrahedral dsDNA promote the self-assembly of IAPP_M into spherical structures, preventing the formation of amyloid fibrils. This observation is consistent with a previous study showing that short ssDNA inhibits the amyloid aggregation of the A β peptide (Abraham *et al.*, 2014).

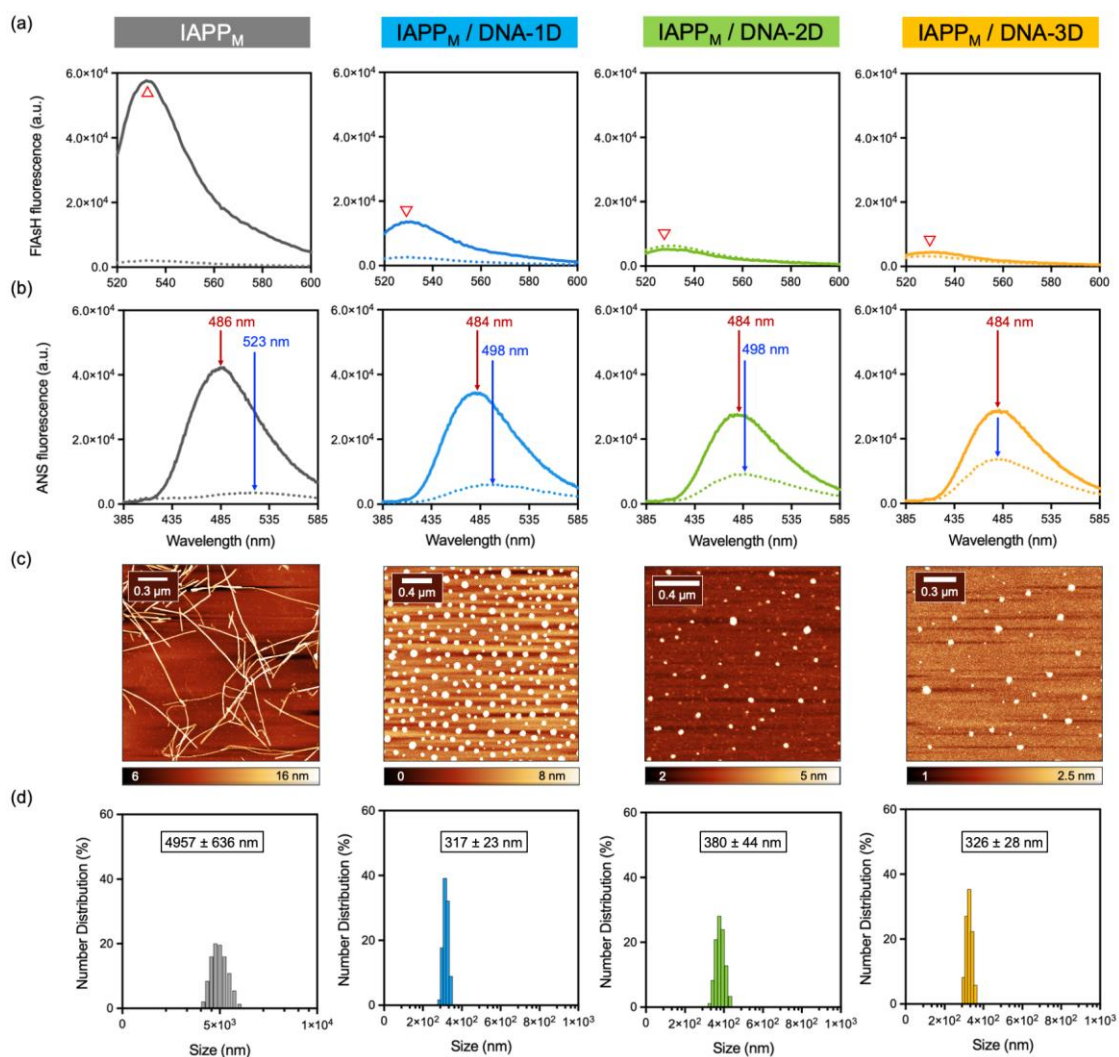


Figure 4.3 DNA nanostructures inhibit IAPP fibrillization by promoting the formation of spherical assemblies. (a) FIAsh and (b) ANS fluorescence spectra of IAPP_M without (left) and with (right) different DNA nanostructures at 0 h (dashed line) and after up to 72 h incubation (solid line). (c) AFM images and (d) DLS number distributions after 72 h incubation.

72 h incubation of IAPP_M (left) and mixtures of IAPP_M and DNA nanostructures (right). The latter are identified by labels at the top of each column.

4.5.2 DNA nanostructures convert oligomeric cytotoxic proteospecies into spherical complexes

Considering that transient prefibrillar assemblies are the most toxic species of the amyloid pathway, we investigated the effects of DNA nanostructures on pre-assembled oligomers. First, time-resolved toxicity of IAPP proteospecies was evaluated by monitoring the viability of INS-1E cells after incubation with proteospecies pre-assembled for different times (Quittot *et al.*, 2021). This will allow us to identify the mixture of IAPP assemblies having the highest cytotoxic potential that will be used for evaluating the effect of DNA nanostructures. As shown in Figure 4.4a, IAPP cytotoxicity correlated with self-assembly time with the oligomers and pre-fibrillar proteospecies, obtained after 3 h incubation at RT being the most toxic (15% viability of control cells). Accordingly, IAPP oligomers (IAPP_O) were prepared by incubating the monomerized peptide for 3 h under fully quiescent conditions (20 mM Tris-HCl, pH 7.4, concentration of 75 μM, RT) before being exposed to DNA nanostructures. The formation of oligomers/pre-fibrillar species under these conditions was confirmed by ThT and ANS fluorescence, CD spectroscopy, AFM, dot blot analysis, and photochemical cross-linking (PICUP). Predominantly random coil conformation in the CD spectra (*i.e.*, sharp band at 202 nm and weak signal at 222 nm) alongside weak ThT fluorescence were observed (Figure S4.6a and S4.6b). Moreover, dot blot analysis conducted with the oligomer-specific A11 antibody (Nguyen *et al.*, 2021) and SDS analysis upon PICUP confirmed the formation of oligomeric assemblies (Figure 4.4b and 4.4c).

As expected, when this heterogeneous mixture of prefibrillar aggregates was left to incubate for an additional 72 h in the absence of DNA, a dense network of fibrils and positive ANS and FIAsh signals were obtained (Figure 4.4d and 4.4e). Strikingly, incubation of pre-assembled IAPP_O with 1D-, 2D- or 3D-DNA nanostructures led to the formation of spherical aggregates with complete absence of fibrils (Figure 4.4f). These assemblies closely resemble those obtained by the incubation of IAPP_M with the DNA nanostructures (Figure 4.3d). Moreover, immediately after mixing the DNA nanostructures with IAPP_O solutions, *i.e.* experimental dead time of around 4 min, a strong increase of ANS fluorescence was observed, which likely correlates with the quasi-instantaneous binding of the oligomeric species to DNA, which generates a high density of hydrophobic patches (Figure 4.4e). Over 72 h incubation, these DNA-peptide clusters likely rearrange into micellar interpolyelectrolyte complexes in which the hydrophobic IAPP core is buried inside the micelle and the DNA polyanions sequestered to the solvent-exposed shell, as inferred by the decrease of ANS fluorescence. Similarly, FIAsh fluorescence, which reports on the convergence of *N*-terminal domains of adjacent IAPP molecules, also increased immediately upon the addition of the DNA nanostructures to the IAPP_O solutions, before subsequently decreasing over time (Figure 4.4e). Again, this suggests a rapid clustering of IAPP prefibrillar species onto the DNA nanostructures, leading to the formation of complexes. The height of these clusters observed by AFM imaging and the hydrodynamic diameter by DLS were within the range of those observed for the IAPP_M-DNA complexes. Overall, these results indicate that dsDNA nanostructures avidly interact with highly cytotoxic IAPP oligomeric species, shifting the amyloid pathway towards the formation of spherical complexes.

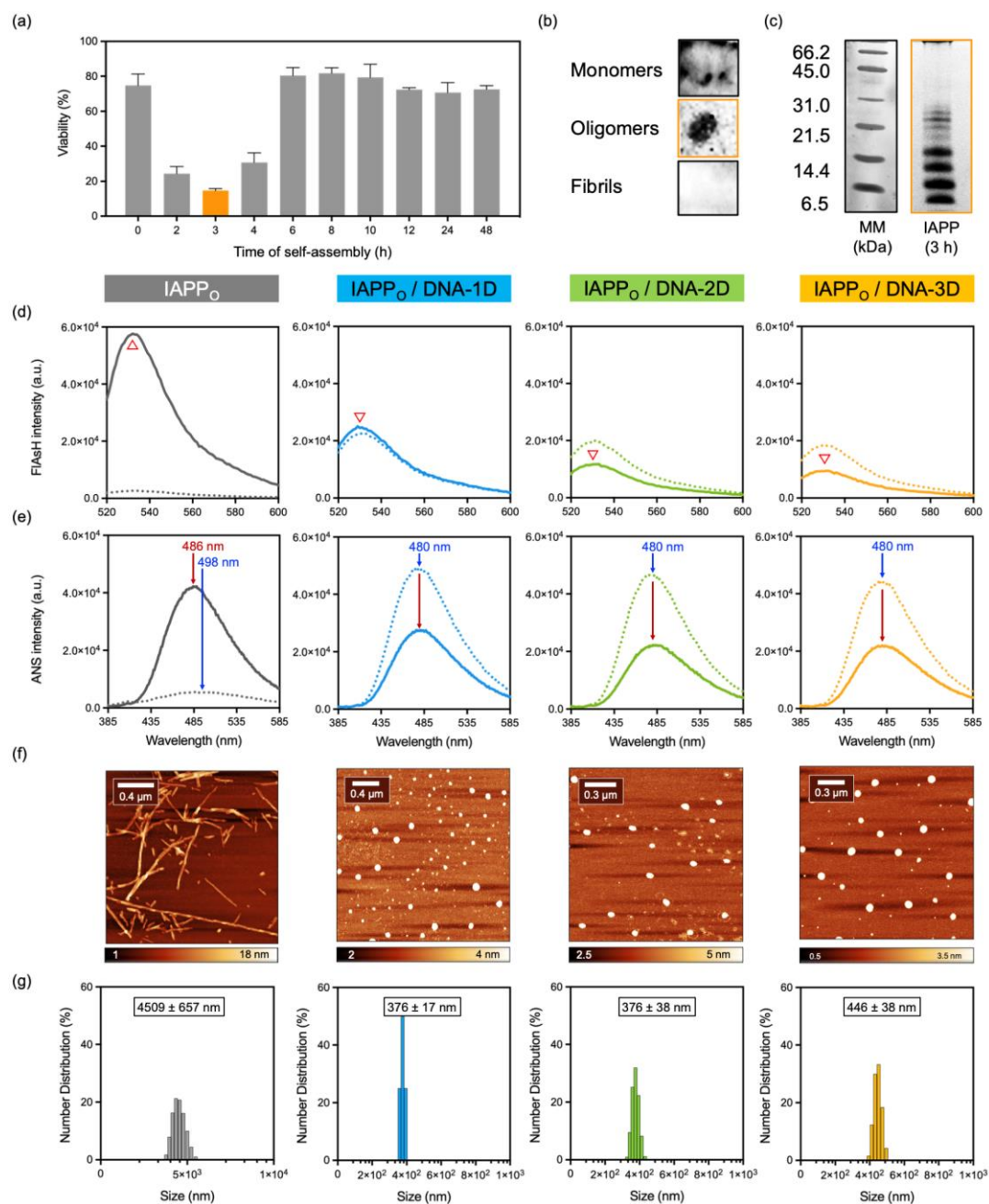


Figure 4.4 DNA nanostructures interact with IAPP pre-fibrillar oligomeric species. (a) Time-resolved cytotoxicity of pre-assembled IAPP evaluated by measuring the metabolic activity of INS-1E cells treated with 25 μ M peptides after 0-48 h pre-incubation in 20 mM Tris-HCl buffer (pH 7.4). (b) Dot blot assay with A11 antibody and (c) SDS-PAGE analysis after PICUP. (d) FIAsh and (e) ANS fluorescence spectra of IAPP₀ without (left) and with (right) different DNA nanostructures after 0 h (dashed line) and 72 h incubation (solid line). (f) AFM imaging and (g) DLS analysis after 72

h incubation in 20 mM Tris-HCl buffer of IAPP_O (left) and IAPP_O/DNA mixtures (right). The latter are identified by labels at the top of each column.

4.5.3 dsDNA nanostructures disassemble stable amyloid fibrils into spherical complexes

Amyloid fibrils are highly stable structures that are resistant to thermal denaturation, chemical treatment with mild denaturants as well as proteolytic degradation (Morel *et al.*, 2010). This stability results in the life-long and massive accumulation of amyloid deposits *in vivo*, which ultimately causes tissue dysfunction despite the low cytotoxicity of individual organized fibrils (Yoshiike *et al.*, 2007). Astonishingly, it was reported that short ssDNA could disassemble A β fibrils into IPECs (Abraham *et al.*, 2014). Accordingly, we investigated the effects of dsDNA nanostructures on the stability of pre-assembled mature IAPP amyloid fibrils (IAPP_F). Fibrils were prepared by incubating the monomerized peptide for 48 h in 20 mM Tris-HCl pH 7.4 under quiescent conditions at a concentration of 75 μ M, a procedure known to reach the saturation phase (Nguyen *et al.*, 2021). The formation of amyloid fibrils was confirmed by ThT fluorescence, CD spectroscopy, and AFM imaging (Figure S4.6). These fibrils were characterized by intense FIAsh and ANS fluorescence signals, respectively resulting from the convergence of *N*-terminal domains and the presence of hydrophobic domains (Figures 4.5a and 4.5b). These amyloid fibrils also resisted to thermal denaturation and proteolytic degradation by proteinase K, as confirmed by AFM imaging, CD spectroscopy and MALDI-TOF mass spectrometry (Figure S4.10 and S4.11). As expected, incubation of these pre-assembled fibrils in absence of DNA for an additional 72 h did not significantly alter FIAsh or ANS fluorescence, as well as the morphology of the fibrils (Figure 4.5). In sharp contrast, the presence of 1.6 μ M of either 1D-, 2D-, or 3D- DNA nanostructures in the IAPP_F mixture led to the total disappearance of the fibrillar aggregates and the formation of spherical nanostructures (Figures 4.5c and 4.5d), with similar shapes and sizes to those reported for IAPP_M (Figure 4.3) and IAPP_O (Figure 4.4). This mesoscopic morphological conversion was accompanied by a slight decrease of FIAsh and ANS fluorescence (Figures 4.5a and

4.5b). The mixtures obtained after DNA incubation were incubated in presence of proteinase K to evaluate if the peptide can be digested when incorporated into these spherical complexes. As observed by MALDI-TOF mass spectrometry, after incubation with the protease, IAPP within the DNA/peptide complexes were digested to similar extent to IAPP_M, in contrast to IAPP_F that were fully resistant to enzymatic hydrolysis (Figure S4.11). These observations indicate that DNA nanostructures not only have the capacity to interact with monomeric and oligomeric IAPP proteospecies but can also disassemble thermodynamically stable and proteolytic resistant amyloid fibrils into biodegradable spherical aggregates.

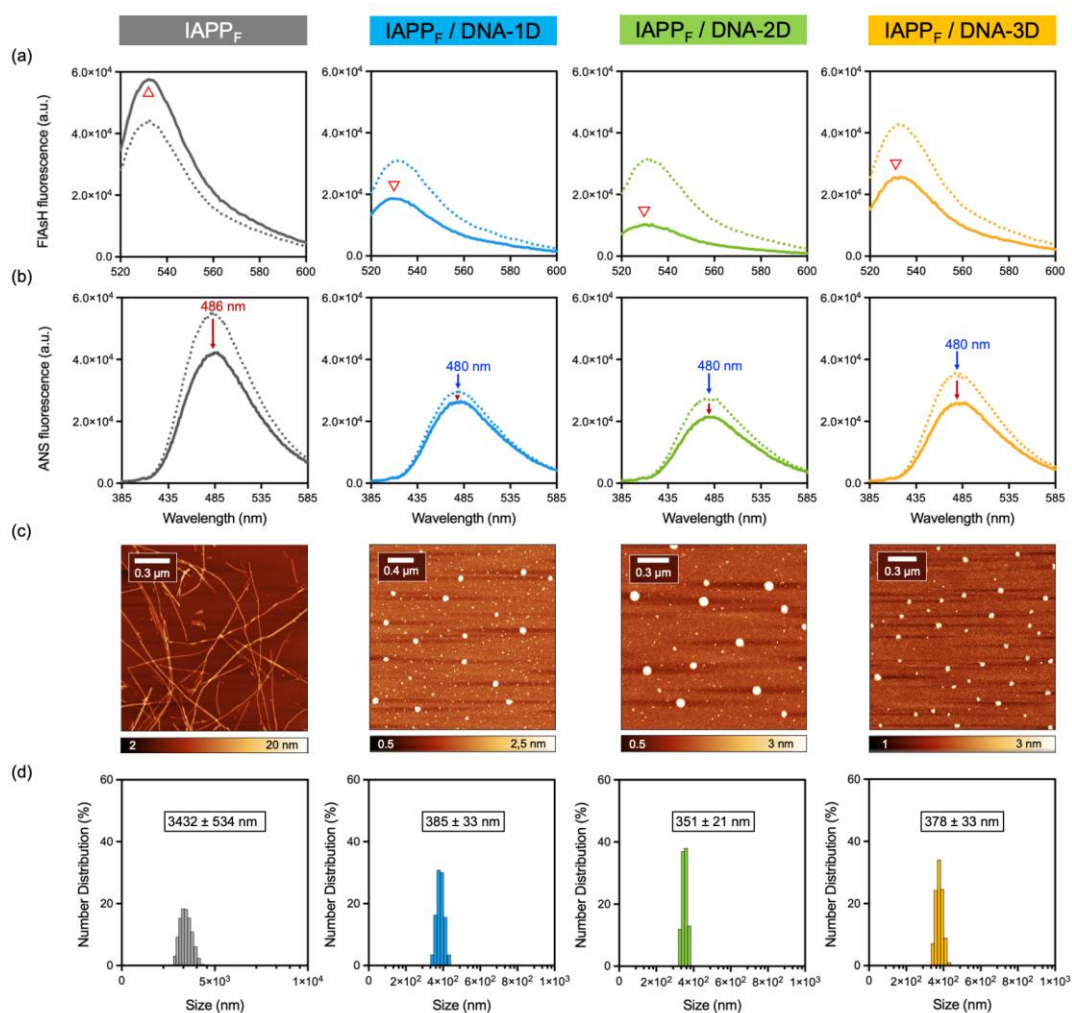


Figure 4.5 DNAs disassemble IAPP amyloid fibrils into spherical complexes. (a) FLAsH and (b) ANS fluorescence spectra of IAPP_F without (left) and with (right) the DNA nanostructures at 0 h (dashed line) and after up to 72 h incubation (solid line). (c) AFM imaging and (d) DLS analysis after 72 h incubation of IAPP_F (left) and mixtures of IAPP_F and DNA nanostructures (right). The latter are identified by labels at the top of each column.

4.5.4 DNA binding promotes the random coil conformation of IAPP

Results obtained so far revealed that the interaction of IAPP with DNA nanostructures induces the formation of spherical complexes, regardless of the peptide's initial secondary or quaternary structure (*i.e.*, random coiled monomers, heterogeneous oligomers/prefibrillar species, and organized β -sheet-rich amyloid fibrils). In fact, for all IAPP assembly states, the resulting spherical structures upon the incubation with DNA nanostructures showed similar characteristics in terms of morphology, hydrophobicity, size, and peptide clustering. IAPP-dsDNA interactions were further investigated by monitoring changes to the spectroscopic properties of IAPP and DNA molecules. As described above, monomers, oligomers, and fibrils were obtained by dispersing the lyophilized and monomerized IAPP into 20 mM Tris-HCl buffer (pH 7.4) at 75 μ M. Each solution was then diluted to reach a final concentration of 25 μ M of IAPP in the absence or in the presence of 1.6 μ M of DNA nanostructure. As observed in Figure 4.6, in the absence of peptide, the UV-Vis absorption spectrum of dsDNA showed a broad band in the UV region with a maximum absorption at 260 nm that does not overlap with the absorption maximum of IAPP species (Figure S4.12). To assess the direct interaction between DNA and the proteospecies, the UV-Vis absorption spectra of IAPP/DNA mixtures were scrutinized for shifts of the position of the maximum bands (Sirajuddin *et al.*, 2013). The spectra of the 1D-, 2D-, and 3D-DNA nanostructures in the presence of IAPP_M, IAPP_O, and IAPP_F species were characterized by hyperchromism and slight bathochromism (red shift) (Figure 4.6 and Figure S4.13). Combined with zeta potential measurements (Figure S4.14), these observations indicate direct binding of dsDNA to the different peptide proteospecies.

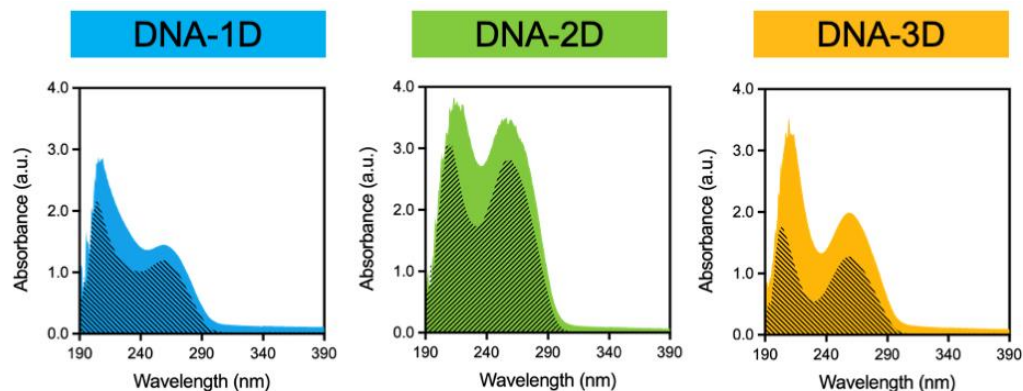


Figure 4.6 DNA nanostructures interact with IAPP species. UV-vis spectra of 1D-, 2D-, and 3D- DNA nanostructures without (hatched area) and with (shaded area) IAPP_M after 72 h incubation in 20 mM Tris-HCl buffer (pH 7.4). The nanostructures tested are identified by labels at the top of each column.

Since CD spectroscopy could not be adequately used due to the strong signal of DNA, ATR-FTIR spectroscopy was employed to gain insights into the secondary structure of IAPP species and to monitor conformational changes associated with the interaction of the peptide with DNA nanostructures. The amide III region of the spectrum (~ 1175 - 1310 cm^{-1}) is mainly associated with CN stretching and NH bending vibrations of the amide group and is highly sensitive to the secondary structure of the polypeptide chain (*i.e.*, α -helix, β -sheet, β -turn, and random coil) (Cai et Singh, 1999; Stani *et al.*, 2020). In particular, this region does not overlap with the spectrum of DNA, contrary to the amide I region, nor does it overlap with the absorption of water (Bal *et al.*, 1993; Cai et Singh, 2004; Stani *et al.*, 2020). Figure 4.7a shows the FTIR spectra of IAPP species obtained during the different phases of the fibrillization process. The spectrum of IAPP_M was characterized by a random-coil secondary structure with band at 1209 cm^{-1} and a shoulder at $\sim 1186\text{ cm}^{-1}$. The FTIR spectrum of IAPP_O was characterized by the same band at 1209 cm^{-1} with a shoulder at $\sim 1186\text{ cm}^{-1}$ as well as a new band at 1226 cm^{-1} corresponding to the oligomeric transition state from random-coil to β -sheet secondary structure. In contrast, the FTIR spectrum of IAPP_F displayed a clear signature of a β -sheet secondary structure with a characteristic band at 1226 cm^{-1} and

a shoulder at $\sim 1181 \text{ cm}^{-1}$. In the presence of DNA nanostructures, no band was observed at 1226 cm^{-1} for any of the IAPP-DNA mixtures, and all FTIR spectra were indicative of a random coil peptide secondary structure (Figure 4.7 and Figure S4.15). Next, we harnessed the intrinsic fluorescence of IAPP to further investigate the interaction of DNA nanostructures with IAPP species. As shown in Figure S4.16, the fluorescence intensity associated with the emission of Tyr³⁷ was significantly quenched when DNA nanostructures were added to the solutions, further confirming the binding of IAPP to DNA. Overall, these results suggest a templating-based mechanism, in which the negatively charged phosphate backbone of DNA interacts with the positive charges of IAPP, possibly trapping them as a micellar interpolyelectrolyte complex in a disordered non-amyloidogenic conformation.

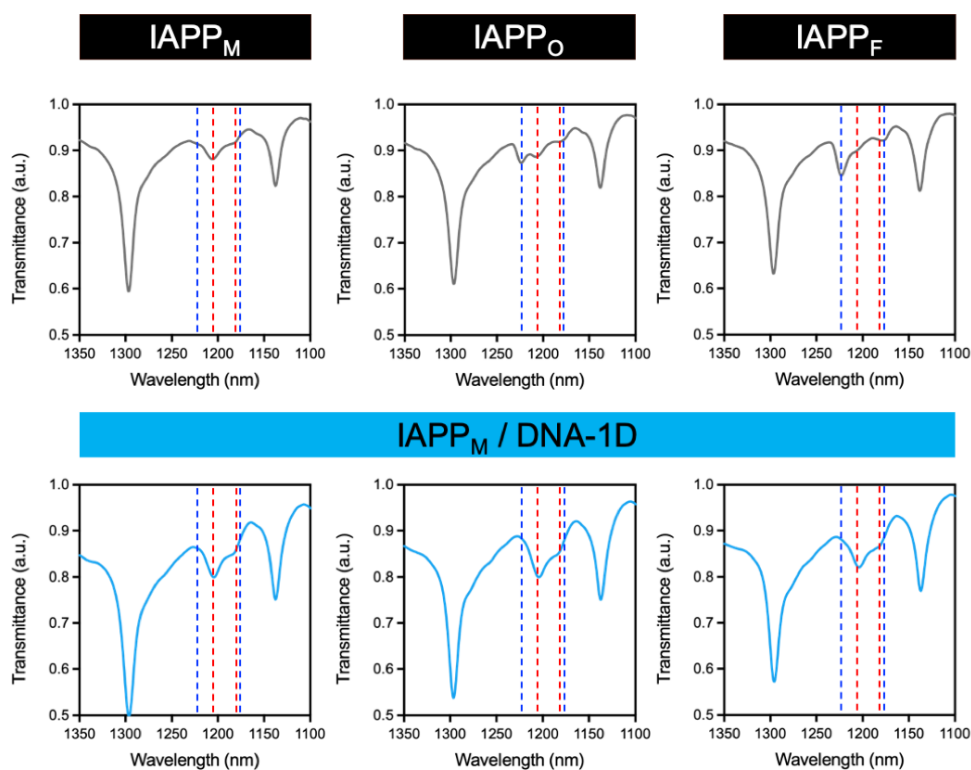


Figure 4.7 DNA nanostructures promote the random coil conformation of IAPP. ATR-FTIR spectra of IAPP_M, IAPP_O, and IAPP_F without (top) and with (bottom) different

DNA nanostructures after 72 h incubation in 20 mM Tris-HCl buffer (pH 7.4). The nanostructures tested are identified by labels at the right of each row.

4.5.5 DNA nanostructures prevent IAPP cytotoxicity by generating non-toxic complexes

We evaluated the cytocompatibility of the spherical IAPP-DNA complexes using INS-1E pancreatic cells, a model commonly used to study IAPP-associated toxicity. While the incubation of INS-1E cells with 25 μM IAPP_M for 24 h reduced their viability by 60%, the pre-formed IAPP-DNA complexes (*i.e.*, 25 μM IAPP and 1.6 μM DNA incubated for 72 h), did not affect cellular viability, nor did the DNA nanostructures alone (Figure 4.8a). For instance, pre-formed spherical complexes assembled from IAPP_M and DNA-1D revealed over 95% viability of the treated control cells. Next, the activity of the cytoplasmic enzyme LDH, a common indicator of plasma membrane permeabilization, in presence of DNA-1D, IAPP-DNA-1D complexes or IAPP alone was measured. The LDH activity measured in the cell media after treatment for 6 h indicated that both IAPP_M and IAPP_O avidly disrupt the plasma membrane (Figure 4.8b). In contrast, DNA-1D alone and preformed IAPP-DNA-1D complexes induced a lower LDH activity. It is known that pre-fibrillar oligomers are the most toxic IAPP species and contribute to pancreatic β -cell death in T2D (Abedini *et al.*, 2016; Wei *et al.*, 2011). Accordingly, we evaluated if the DNA nanostructures could avidly trap pre-assembled toxic oligomers by converting them into cytocompatible complexes. As described above, IAPP was pre-incubated at 75 μM in 20 mM Tris-HCl for 3 h to obtain IAPP_O, and these proteospecies reduced cellular viability close to 10% after 5 h incubation with the cells (Figure 4.8c). Strikingly, co-addition of 1.6 μM DNAs in the cell culture fully abrogated the cytotoxicity of IAPP_O. This result was confirmed by fluorescence microscopy analysis using the LIVE/DEAD assay, with the IAPP_O/DNA-1D showing quasi-absence of red-labelled cells (Figure 4.8d). This observation indicates that DNA nanostructures promptly interact with toxic oligomers in complex

biological media containing high concentration of a diversity of biomolecules, including those from FBS, and convert them into non-toxic proteospecies.

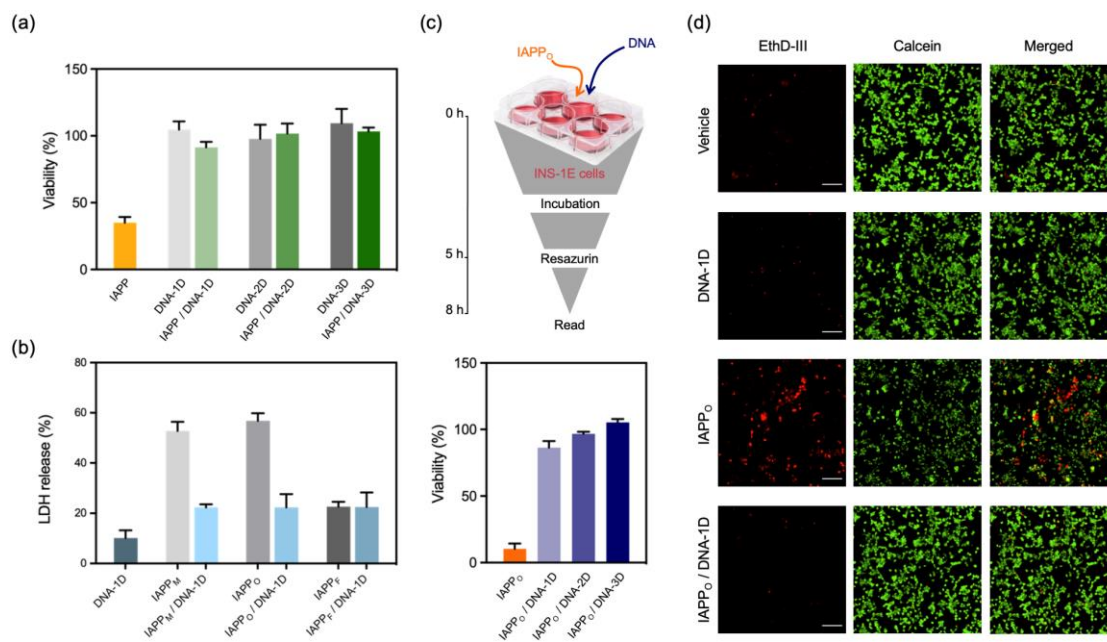


Figure 4.8 DNAs abrogates IAPP-associated cytotoxicity. (a) Viability of INS-1E cells evaluated by measuring the metabolic activity after 24 h treatment with IAPP_M, IAPP-DNA complexes, or DNA alone. (b) Membrane perturbation evaluated by LDH release from INS-1E cells incubated with DNA-1D, IAPP-DNA-1D complexes, or IAPP alone. (c) Viability of INS-1E cells incubated for 5 h with pre-assembled IAPP_O in the absence or presence of DNA nanostructures. (a,c) Cell viability was determined by the reduction of resazurin and expressed as a percentage of the viability of cells treated with vehicle (buffer). (d) INS-1E cells were incubated for 24 h with DNA-1D, IAPP_O-DNA-1D complexes, or IAPP_O alone, and cell viability was evaluated by staining with calcein AM (green; live cells) and ethidium homodimer (red; dead cells). In panels a, b and c data represent the means \pm S.E.M. of at least three individual experiments performed in triplicate.

4.6 Conclusion

The present work demonstrates the ability of linear (1D), triangular (2D), and tetrahedral (3D) dsDNA nanostructures to fully inhibit IAPP amyloid fibril formation

as well as their unique capacity to disassemble rapidly and efficiently fibrillar IAPP quaternary species. Importantly, these DNA nanostructures efficiently trapped highly toxic oligomers (even in complex biological media) and fully abrogated their cytotoxicity by converting them into cytocompatible and enzymatically degradable spherical complexes. The effects observed on IAPP amyloidogenic pathway and associated toxicity were independent of the architecture of the DNA nanostructure and were unaffected by peptide ligands appended to the dsDNA nanostructures, which could ultimately be exploited for theranostics usage. While the mechanism(s) underlying these observations remain(s) to be established, the efficient capacity to sequester highly toxic and metastable amyloid oligomers/pre-fibrillar species in complex media containing FBS and high concentration of salts and biomolecules is remarkable and points to the need to further probe interactions of amyloid peptides and circulating DNA in relation to amyloid-associated diseases. The results obtained herein suggest that the dsDNA nanostructures rapidly interact with IAPP proteospecies via, most likely, electrostatic interactions, leading to a conformational shift from a cross- β -sheet organization to random coil structure. The lack of a distinctive difference between the 1D/2D/3D nanostructures seems to suggest that penetration of the nanostructure into the peptide assembly is unlikely. Nevertheless, adsorption of the nanostructures to the surface of the aggregate could impose a stress on the assembly, altering it sufficiently to increase its dynamics sufficiently to promote a change in quaternary morphology. Indeed, the FIAsh experiments seem to suggest that the convergence of the N-termini of the peptides is closely similar in the fibrillar (cross- β -sheet) and spherical (random-coil) forms of the aggregates. This study also provides insight into the design of DNA-based entities that could potentially modulate the aggregation of amyloid peptides *in vivo*, either for therapeutic purposes or for fundamental studies on these diseases.

4.7 Acknowledgments

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4.8 Supporting information

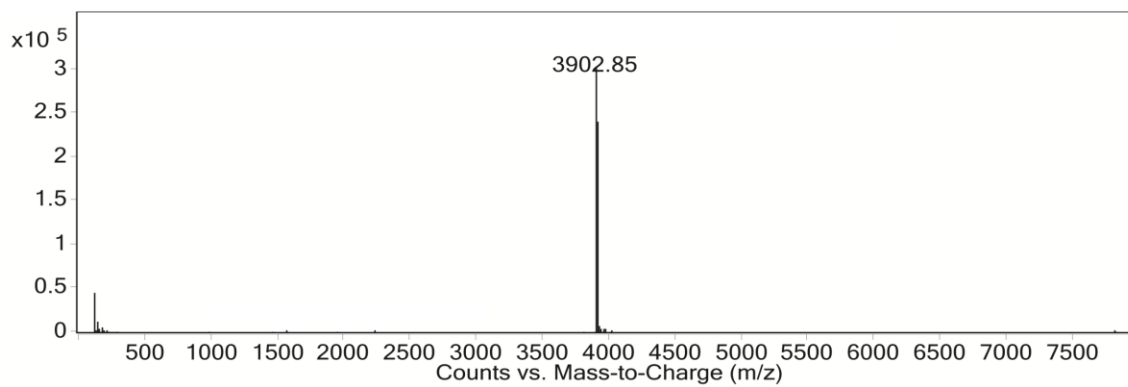
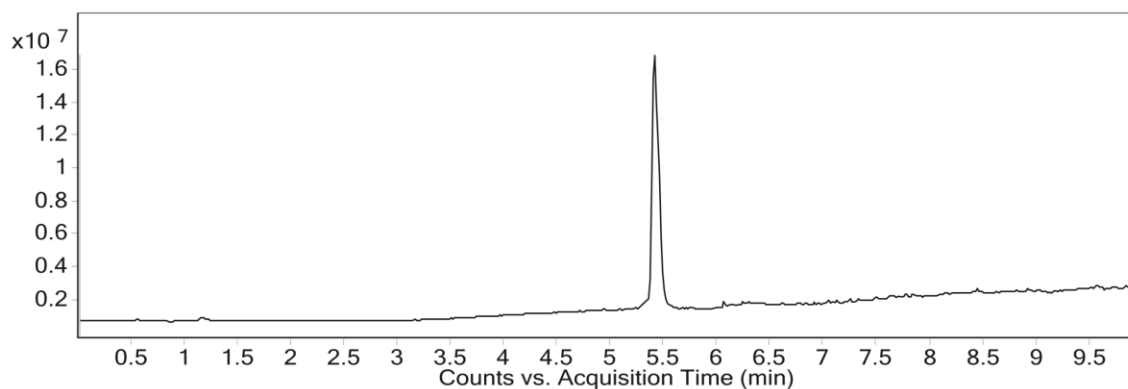


Figure S4.1. (a) HPLC and (b) ESI-MS analysis of purified synthetic IAPP peptide.

Table S4.1 DNA strands used in the assembly of the 1D (linear), 2D (triangle), and 3D (tetrahedron) structures. Colors correspond to the structures in Figure 4.1 of the main manuscript. Black indicates non-binding thymines inserted to aid in assembly. All structures are fully duplexed DNA, except for thymine linkers.

Structure	Strand Name	Sequence (5' – 3')
Linear	Lin1	GGCTGATTCTATTGAGGTATCCCCACGAGAGTAAGACATTTTT CCCGCAGATGACTAA
	Lin2	ATGTCTTACTCTCGTGGGGATACCTCAATAGAATCAGCCTTTT CCCGCAGATGACTAA
Triangle	Tri1	GACCGTAACTCTCTGATACTGGATCGACGTACCATCACAGCAG TTATTAGAGGATCACGTTAAATTGAGGCTCTATTCGAGTACAG GCTAATGATGTAATTCTTTTGGACCAGAGCAAGACGACA
	Tri2	CTAACGGCCTCCGGTTTTGTATCAGAGAGTTACGGTCTGTCTG TCTTGCTCTGGTCCTTAGCAGGCCCATCGATTTTCCCGCAGATGACTAA
	Tri3	TGCATGGGCTGCTAGAATTACATCATTAGCCTGTACTCGAATA GAGCCTCAATTTATACTATCGGTCCCTATTTCCCGCAGATGACTAA
	Tri4	TAGGGACCGATAGTATTACGTGATCCTTAATAACTGCTGTGATG GTACGTCGATCCATACCGGAGGCCGTTAGTTTTCCCGCAGATGACTAA
Tetrahedron	Tet1	ACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAA GAGCCGCCATAGTATTTCCCGCAGATGACTAA
	Tet2	TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCCA GGGTCCAATACTTTTCCCGCAGATGACTAA
	Tet3	TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTAT GGCGGCTCTTTTCCCGCAGATGACTAA
	Tet4	TTCAGACTTAGGAATGTGCTTCCACGTAGTGTCGTTTGATTGGA CCCTCGCATTTTCCCGCAGATGACTAA
Overhang Complement	OC	TTAGTCATCTGCGGG

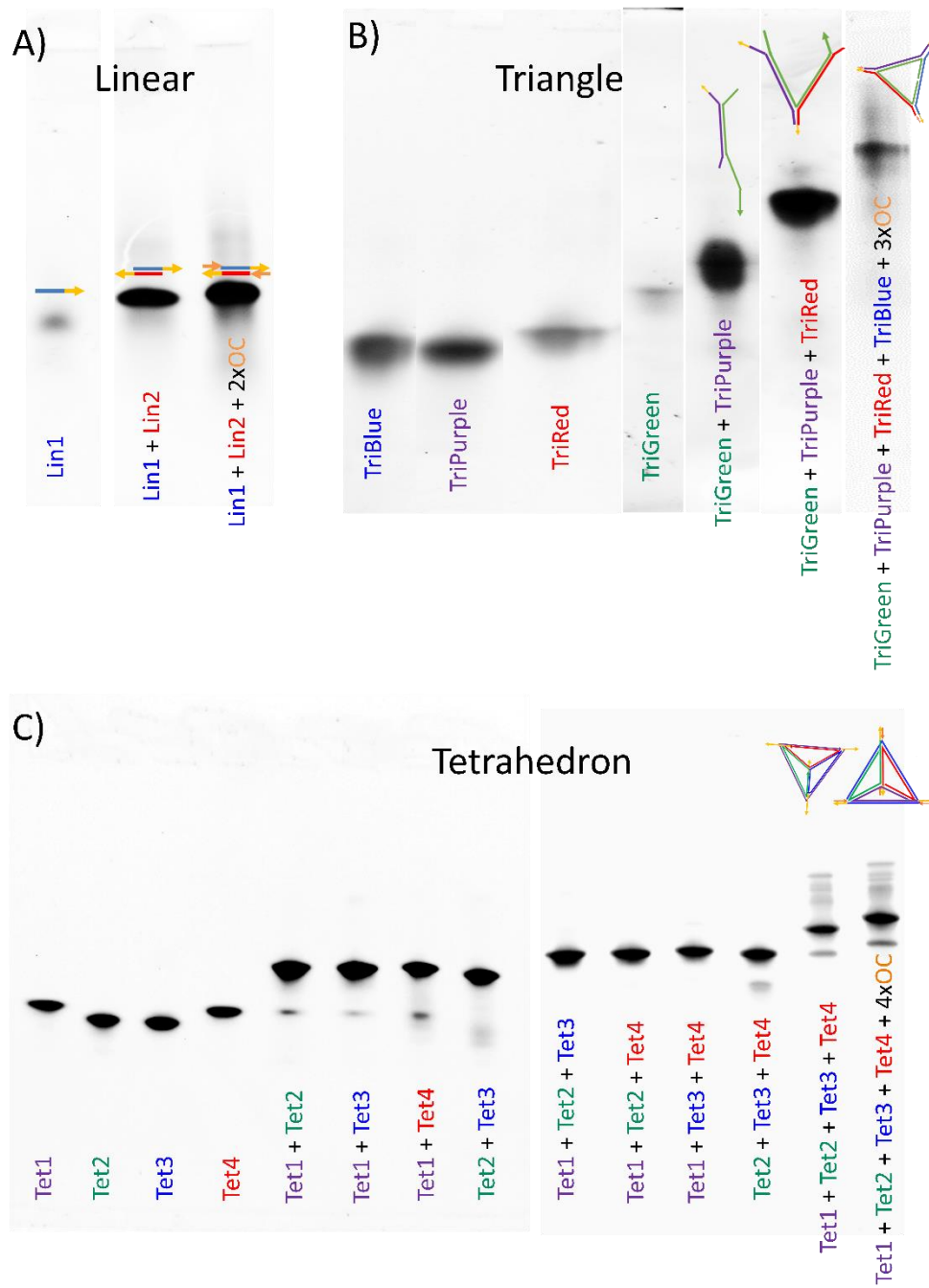


Figure S4.2 Assembly of the linear (a), triangle (b), and tetrahedron (c) structures, visualized by gel electrophoresis. All gels were stained with GelRed.

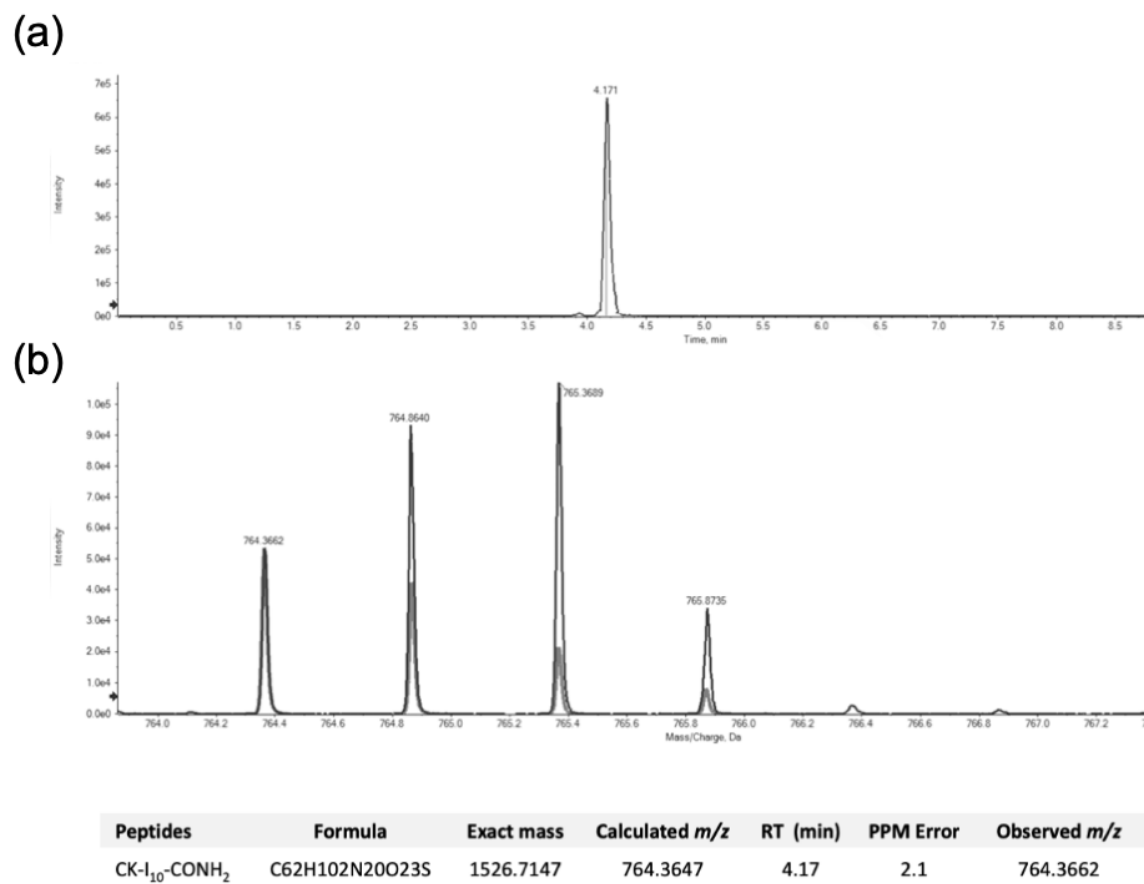


Figure S4.3 (a) HPLC and (b) ESI-MS analysis of purified synthetic IAPP peptide fragment containing an additional Cys with free thiol.

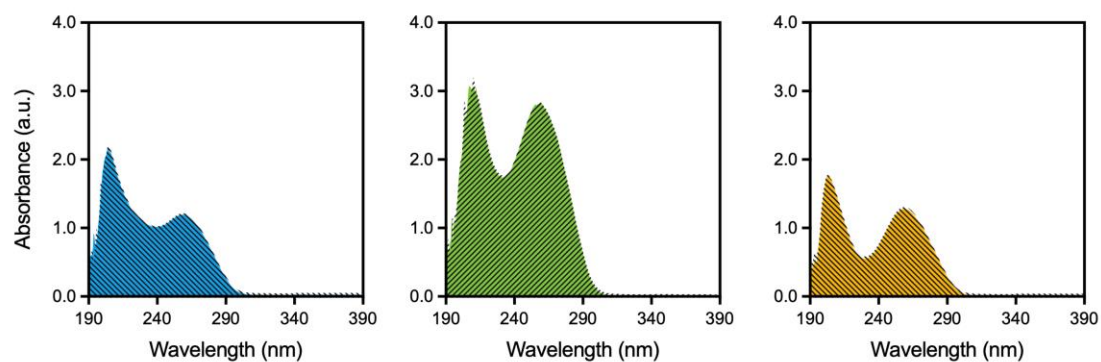


Figure S4.4 DNA stability evaluated by comparing UV spectra of (a) 1D-DNA, (b) 2D-DNA and (c) 3D-DNA in Tris-HCL buffer, pH 7.4 at 0 h (shaded area), and after 1-week incubation (hatched area) at RT.

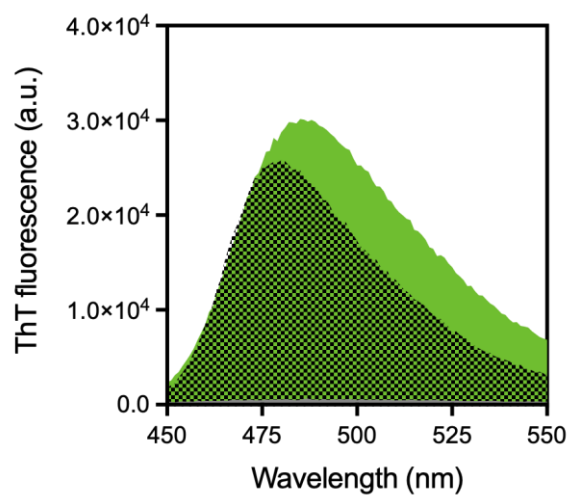


Figure S4.5 Overlapping ThT fluorescence spectra of 2D-DNA (shaded area) and IAPP fibrils (hatched area) at RT.

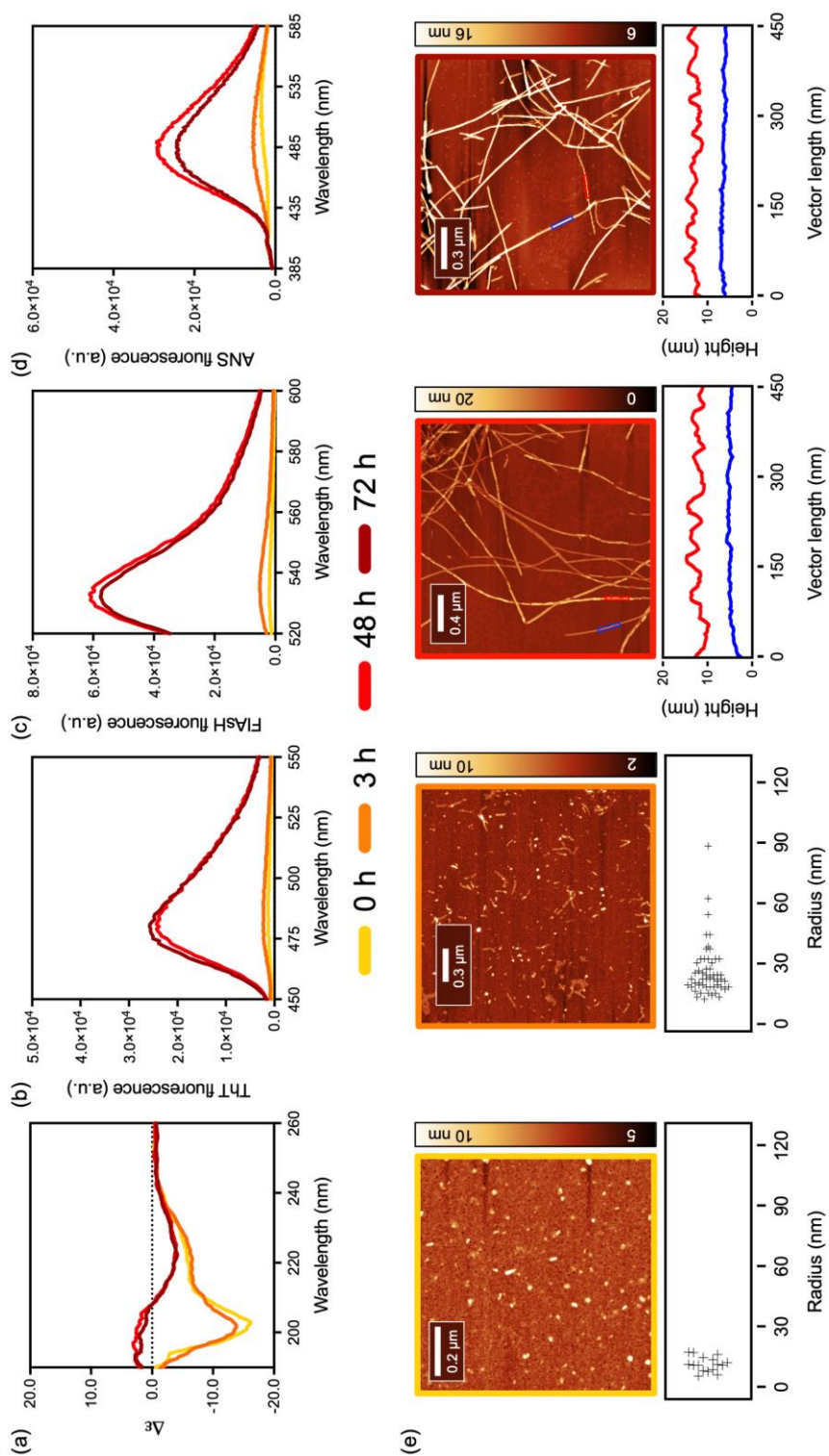


Figure S4.6 Evaluation of IAPP self-assembly by (a) far-UV CD spectroscopy, (b) ThT, (c) FIAsH and (d) ANS fluorescence and (e) AFM imaging upon incubation in 10 mM Tris-HCl, pH 7.4 at RT. (e) Hydrodynamic radius distribution at time 0 h and time 3 h and line height profile of the fibrils to showing differences in morphology.

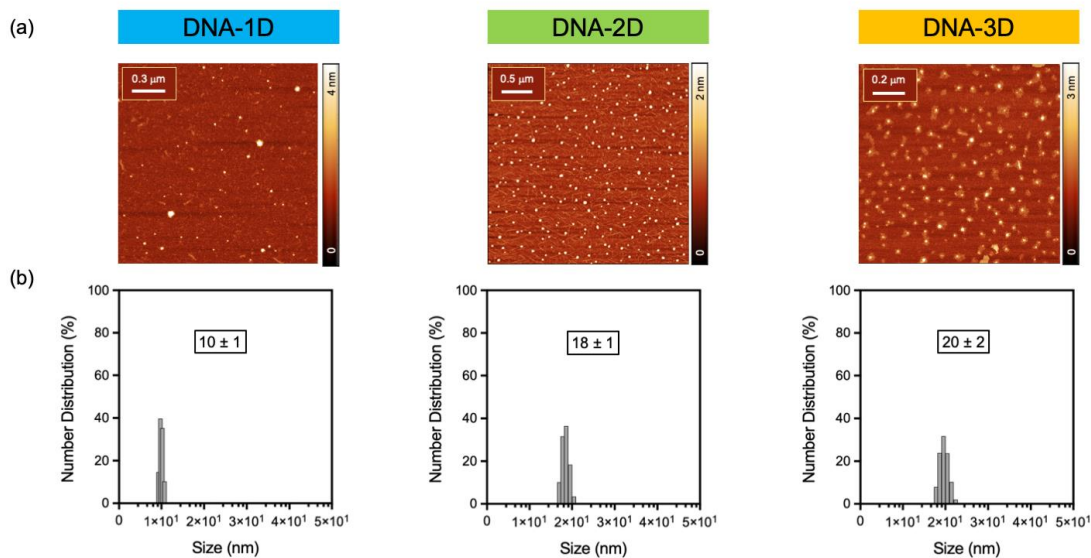


Figure S4.7 (a) AFM images and (b) DLS number distribution of 1D, 2D and 3D DNA nanostructures alone.

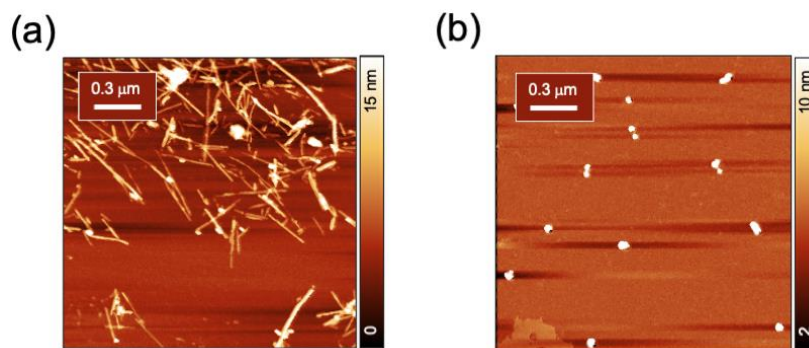


Figure S4.8 Formation of spherical IAPP-DNA complexes in presence of a physiologically relevant ionic strength. AFM imaging of (a) IAPP_M and (b) IAPP in presence of DNA-1D after 72 h incubated in 20 mM Tris, pH 7.4, supplemented with 150 mM NaCl.

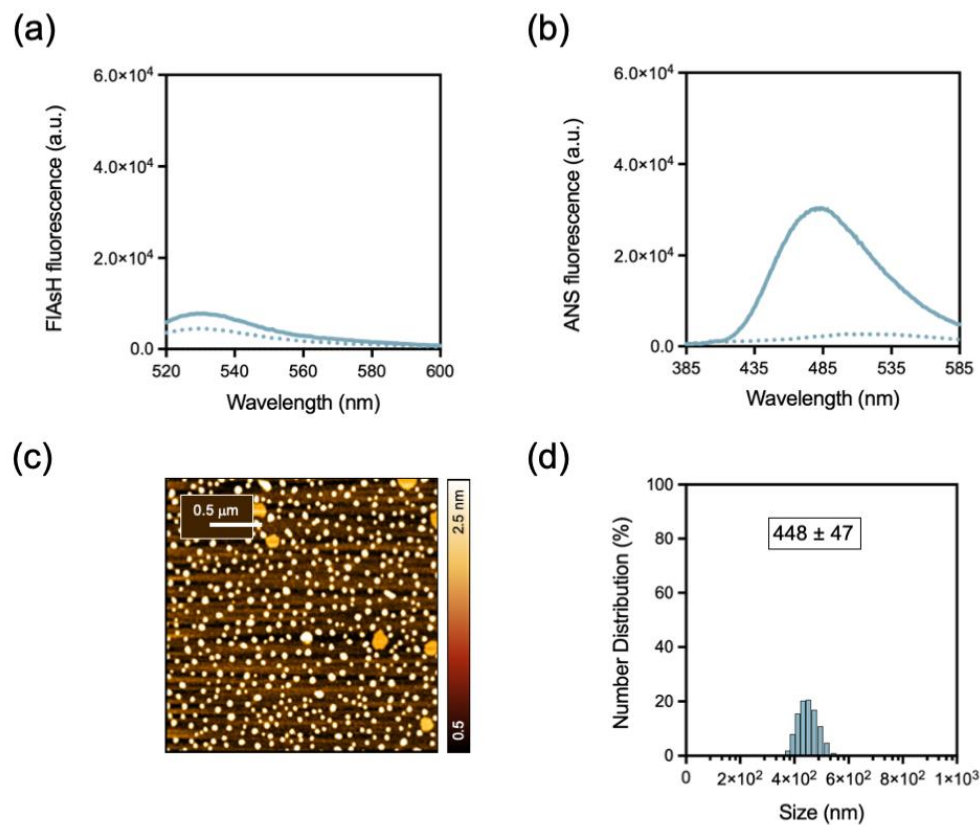


Figure S4.9 DNA-I₁₀ nanostructures inhibit IAPP fibril formation. (a) FIAsH and (b) ANS fluorescence spectra of IAPP_M incubated with DNA-I₁₀ nanostructures at 0 h (dashed line) and after 72 h incubation (solid line). (c) AFM images and (d) DLS number distributions after 72 h incubation of mixtures of IAPP_M and DNA-I₁₀ nanostructures.

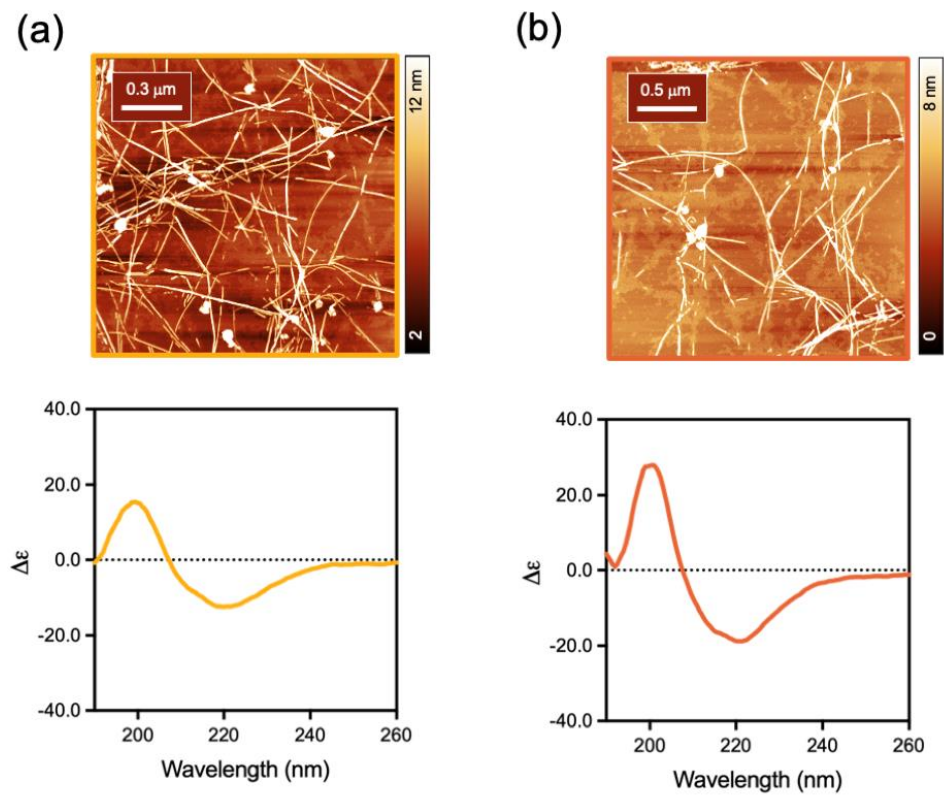


Figure S4.10 Stability of amyloid fibrils evaluated by thermal denaturation monitored by AFM imaging (top) and far-UV CD spectroscopy (down) at (a) 25 °C and (b) 90 °C. IAPP_F were incubated at the indicated temperature for 1 h before analysis.

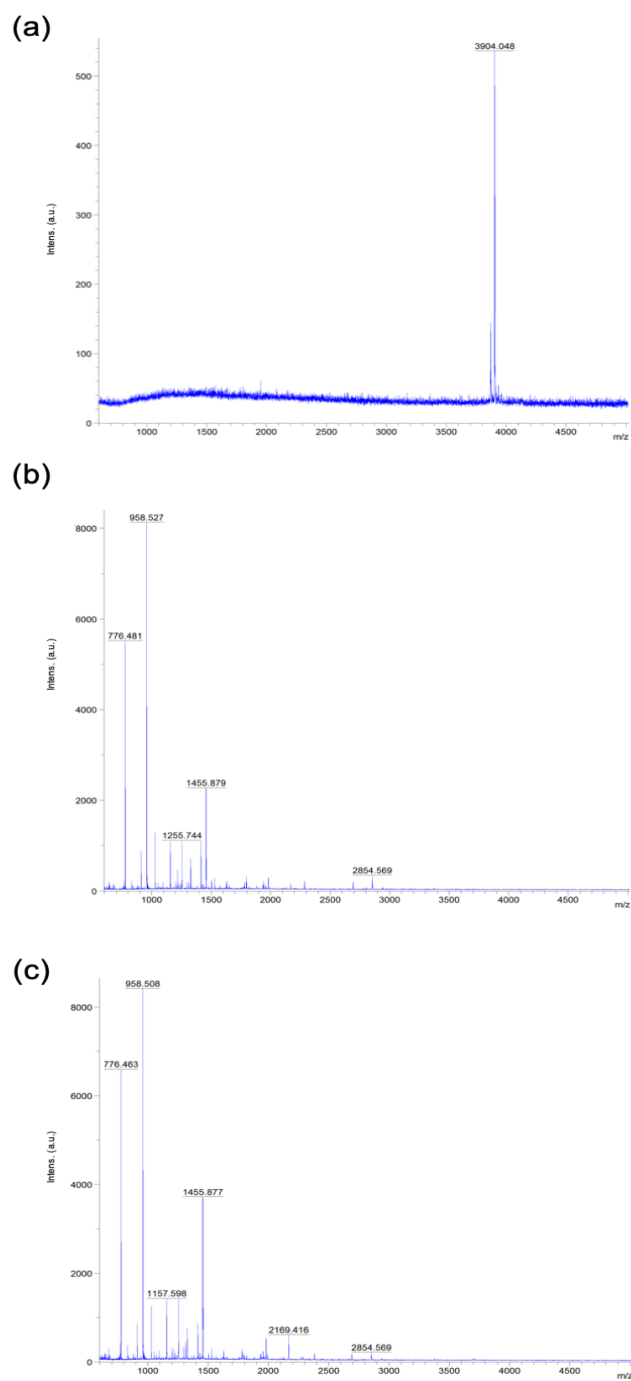


Figure S4.11 Proteolytic degradation by proteinase K of (a) IAPP_F, (b) IAPP_M and (c) preformed IAPP-DNA-1D complex evaluated by MALDI-TOF spectrometry.

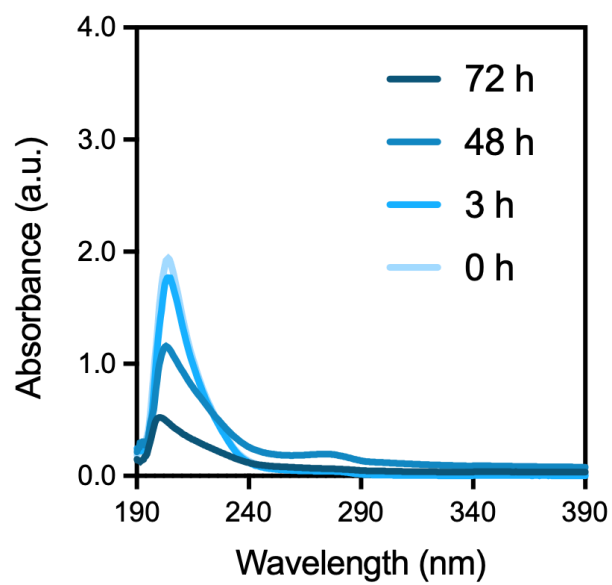


Figure S4.12 UV-vis spectra of IAPP at 0 h, 3 h, 48 h and 72 h incubation in 10 mM Tris-HCl, pH 7.4 under quiescent conditions.

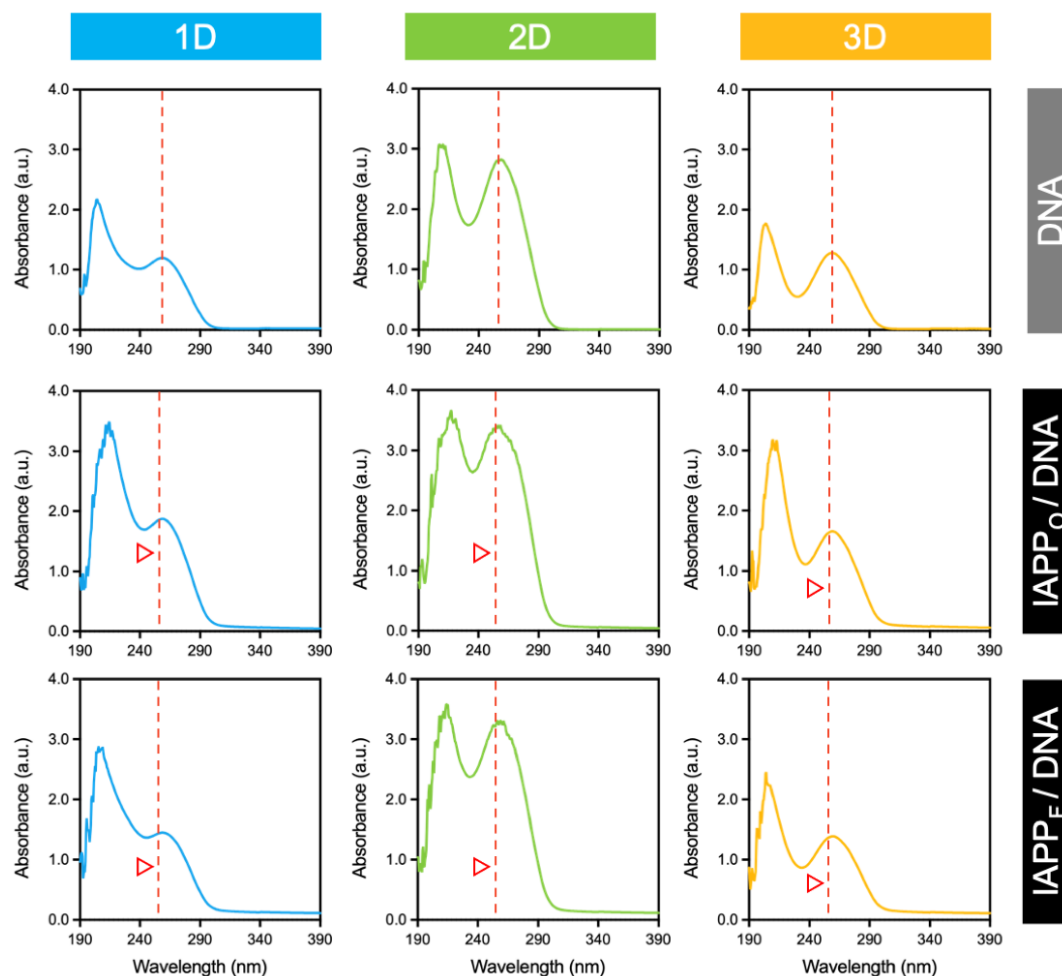


Figure S4.13 DNA nanostructures interact with IAPP species. UV-vis spectra of 1D-, 2D-, and 3D- DNA nanostructures without (top) and with (bottom) IAPP_O, and IAPP_F after 72 h incubation in 20 mM Tris-HCl buffer (pH 7.4). The nanostructures tested are identified by labels at the top of each column.

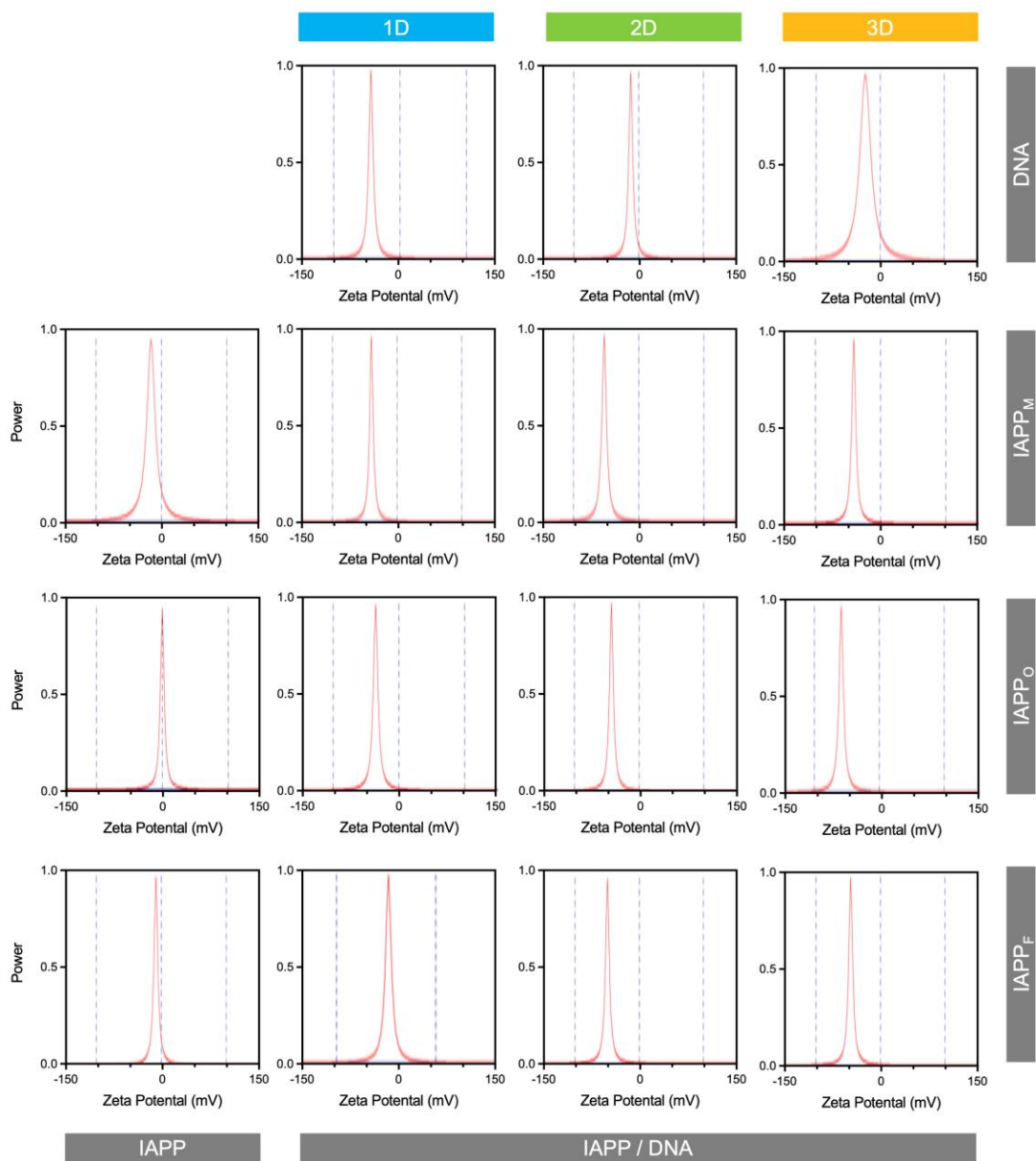


Figure S4.14 Zeta potential measured at 0 h (IAPP_M, DNAs) and after 3 h (IAPP_O), 48 h (IAPP_F) and 72 h (IAPP/DNA mixtures) incubation in 10 mM Tris-HCl, pH 7.4.

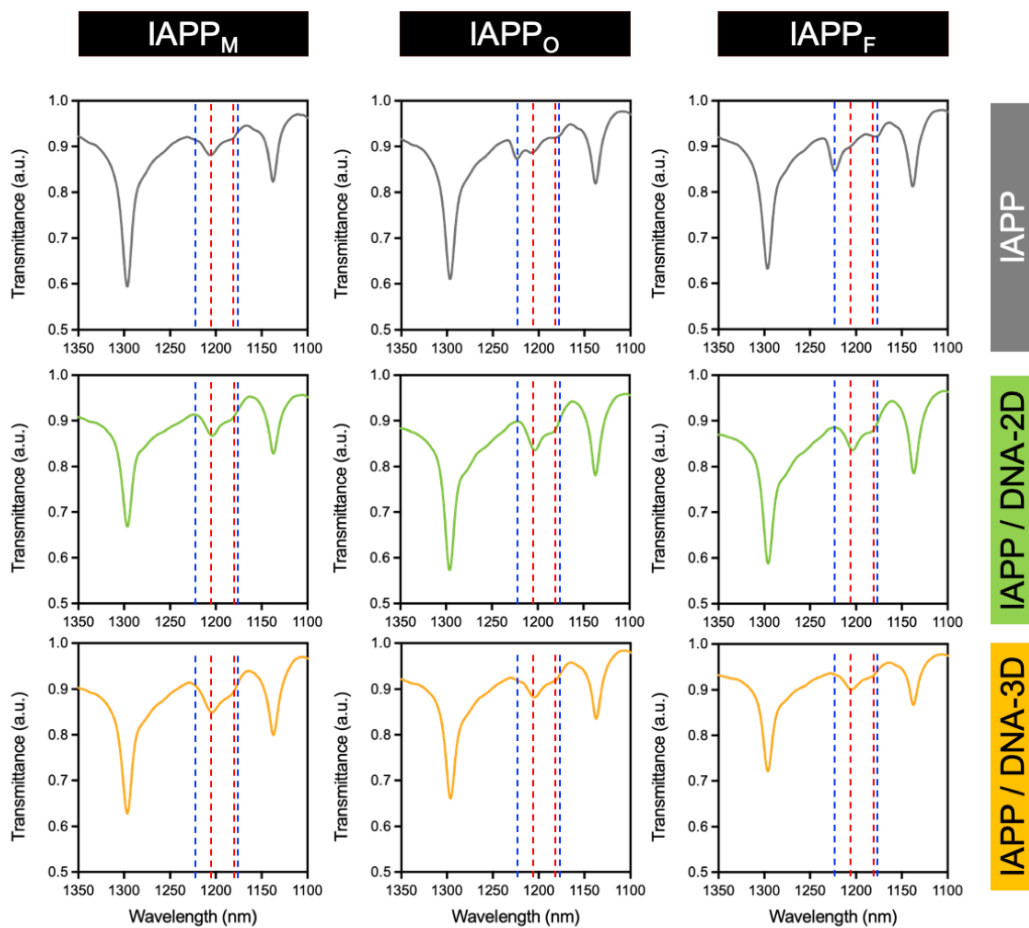


Figure S4.15 DNA nanostructures promote the random coil conformation of IAPP. ATR-FTIR spectra of IAPP_O and IAPP_F without (top) and with (bottom) different DNA nanostructures after 72 h incubation in 20 mM Tris-HCl buffer (pH 7.4). The nanostructures tested are identified by labels at the right of each row.

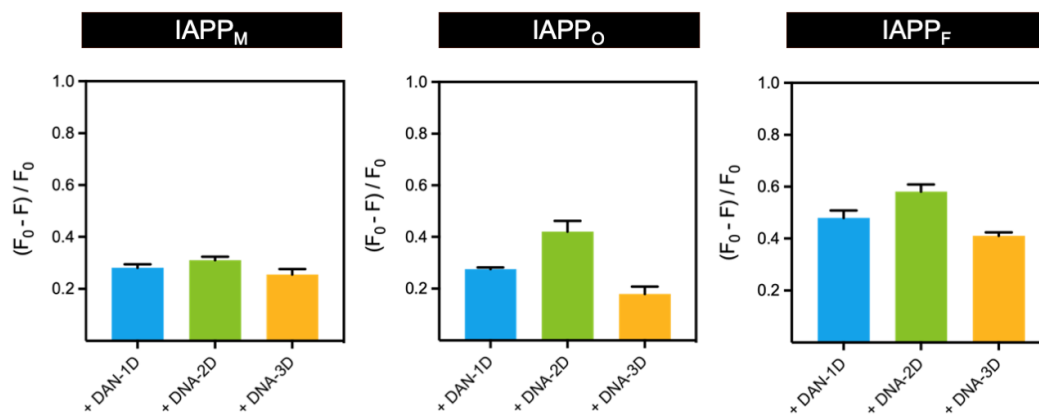


Figure S4.16 Interaction of IAPP with DNA nanostructures inferred from intrinsic fluorescence of IAPP Y³⁷, evaluated after 72 h incubation of the IAPP_M, IAPP_O or IAPP_F with DNAs.

CHAPITRE V

DISCUSSION

Les amyloïdes fonctionnels ont été découverts chez la majorité des espèces où ils sont impliqués dans de nombreux processus physiologiques essentiels (Fioriti *et al.*, 2015; Fowler, D. M. *et al.*, 2006). Caractérisés par une structure quaternaire organisée, non ramifiée et stable, les assemblages amyloïdes résultent d'un empilement d'unités répétitives de brins β en protofilaments qui s'associent latéralement sous forme de fibrilles biocompatibles avec des propriétés mécaniques uniques (Maji *et al.*, 2009; Mukhopadhyay *et al.*, 2014). A cet effet, les amyloïdes sont de plus en plus exploités comme matériaux bioinspirés pour développer de nouvelles suprastructures pour des applications en biotechnologie et nanomédecine (Lai *et al.*, 2021). Le potentiel de ces nanostructures protéiques encourage à poursuivre les investigations pour élucider les bases mécanistiques et structurales de l'autoassemblage et de la diversité morphologique des amyloïdes. Dans ce contexte, le premier objectif de cette thèse était d'étudier la capacité de séquences amyloïdes à guider l'autoassemblage de systèmes π -conjugués en nanostructures fibrillaires semi-conductrices et biocompatibles. De même le second objectif visait à déterminer et exploiter les paramètres physico-chimiques et structuraux modulant la morphologie et la taille des nanostructures amyloïdes afin de comprendre les bases moléculaires de leurs propriétés immunomodultrices. D'autre part, le processus d'assemblage des amyloïdes peut générer des espèces protéotoxiques transitoires ayant des effets néfastes sur les systèmes biologiques, ce qui semble être à l'origine de diverses pathologies dégénératives (Harrison *et al.*, 2007). Ce phénomène demeure un grand défi à relever pour la conception rationnelle de stratégies thérapeutiques efficaces et sécuritaires. À cet effet, le troisième et dernier objectif de cette thèse visait à évaluer l'effet de nanostructures d'ADN sur le processus

d'autoassemblage de peptides amyloïdes et sur les propriétés cytotoxiques des différentes structures quaternaires engendrées.

5.1 Autoassemblage de peptides amyloïdes π -conjugués pour concevoir des nanofibrilles semi-conductives et biocompatibles.

Les systèmes π -conjugués sont de plus en plus exploités sous forme de nanomatériaux organiques semi-conducteurs dans des dispositifs bioélectroniques (Hoeben *et al.*, 2005). Le pérylène diimide (PDI) et ses dérivés sont parmi les systèmes π -conjugués qui ont attiré une attention particulière en raison de leurs propriétés optoélectroniques, physicochimiques et structurales particulières ainsi que leur capacité à s'auto-associer sous diverses architectures supramoléculaires (Huang *et al.*, 2011; Kihal *et al.*, 2022). Néanmoins, ces dérivés de PDI sont peu exploités en bioélectronique en raison de leur faible solubilité dans les milieux aqueux ce qui empêche de contrôler leur autoassemblage sous forme de nanostructures semiconductrices souhaitées (Huang *et al.*, 2011; Würthner, 2004). Toutefois, plusieurs modulations structurales ont été adressées pour remédier à ces limitations. Par exemple, des acides aminés et de courtes séquences peptidiques ont été employés pour guider l'assemblage de PDI (Bai *et al.*, 2014; Eakins *et al.*, 2014; Eakins *et al.*, 2015; Eakins *et al.*, 2015; Panda *et al.*, 2021; Wei *et al.*, 2019). Ainsi, une interface β -continue (IKHLSVN), dérivée de la protéine bovine 3-peroxyredoxine, a été conjuguée au PDI non seulement pour améliorer l'hydrosolubilité des blocs hybrides résultants, mais également pour guider l'assemblage en nanostructures organiques semi-conductives (Eakins, *et al.*, 2015). Néanmoins, les assemblages issus d'interfaces β -continues souffrent d'une faible stabilité thermodynamique et se dissocient par dilution (Valéry *et al.*, 2013), ce qui limite leurs exploitation comme nanostructures semi-conductives. Dans notre étude, par la conjugaison du PDI avec un peptide amyloïdogénique naturel nous avons pu guider l'assemblage des PDI hybrides vers des suprastructures hautement ordonnées, biocompatibles et semiconductives. Ce résultat suggère que l'autoassemblage programmable de la séquence amyloïde, à travers une balance des interactions

hydrophobes et liaisons hydrogène, confère une robustesse directionnelle vers la formation de nanosystèmes π -conjugués stables.

Les peptides amyloïdogéniques sont particulièrement attractifs en raison de leur synthèse facile, leur fonctionnalisation, leur agrégation programmable, ainsi que leur biocompatibilité et dégradabilité (Al-Halifa *et al.*, 2019; Chen et Zou, 2019). A cet effet, nous avons exploité la haute capacité d'auto-reconnaissance de la région centrale 20-29 de l'IAPP (I₁₀) pour guider l'agrégation de PDI sous forme de nanofilaments ordonnés, biocompatibles et conductives. Nous avons déjà démontré que le peptide I₁₀ (SSNFGAILSS) est une séquence amyloïdogénique fonctionnalisable capable de s'autoassembler sous forme de structures fibreuses très stables et non ramifiées caractérisées par une structure quaternaire riche en feuillets- β -croisés (Al-Halifa *et al.*, 2020; Babych *et al.*, 2018).

Initialement, nous avons préparé par synthèse peptidique sur support solide un dérivé symétrique PDI-[I₁₀]₂. Vu que la nature du solvant peut affecter grandement le processus d'autoassemblage de PDI et l'architecture des suprastructures résultantes, nous avons initialement étudié l'autoassemblage du dérivé PDI-[I₁₀]₂ dans différents solvants. A l'exception de l'eau (H₂O) et le diméthylsulfoxyde (DMSO), le dérivé PDI-[I₁₀]₂ est faiblement soluble ou complètement insoluble dans des solvants aprotiques polaires ou aprotique, comme le méthanol (MeOH), l'acétonitrile (MeCN) et tétrahydrofurane (THF). Par ailleurs, ce dérivé devient soluble dans un mélange à 50% de solvant organique/H₂O. Ceci est attribué à la solubilité limitée de la longue chaîne peptidique (32-résidus, incluent les espaceurs) dans des solvants organiques, qui nécessite généralement de l'eau. De plus, nous avons observé des nanofilaments hydrosolubles seulement dans l'H₂O, sans même l'utilisation de co-solvants organiques, ce qui ouvre la porte non seulement vers le développement de nanostructures ordonnées et conductives, mais également pour leur application biomédicale. A cet effet, nous avons étudié la cinétique de fibrillation du dérivé PDI-

[I₁₀]₂ dans un milieu aqueux, pour mieux comprendre le mécanisme de formation des fibrilles et le temps d'autoassemblage nécessaire pour atteindre l'équilibre.

Avec une cinétique sigmoïdale caractéristique de la formation amyloïde (Echue *et al.*, 2015; Godin *et al.*, 2019), le dérivé PDI-[I₁₀]₂ passe d'un état de monomères ou de petits oligomères à un état d'agrégats de type H via des interactions π co-faciales des unités PDI, indispensables pour former un réseau conducteur (Giaino *et al.*, 2008; Hartnett *et al.*, 2015). Nonobstant la fluorescence chevauchante de PDI avec la ThT, souvent utilisée pour suivre la cinétique d'assemblage des peptides amyloïdes (Sebastiao *et al.*, 2017), les analyses AFM ont révélé une formation de nanofilaments de plus en plus dense au cours du temps. De même, les analyses UV-vis et de fluorescence ont révélé une organisation spatiale des unités PDI dans le processus d'autoassemblage via des interactions π - π . En outre, les analyses CD ont confirmé par la présence d'un point isobestique, indiquant une nette transition d'une conformation aléatoire vers une structure secondaire ordonnée riche en feuillets- β , que c'est la séquence peptidique qui dirige le processus d'autoassemblage via des liaisons hydrogène directionnelles. D'ailleurs, le PDI-[L]₂, dépourvu de séquence peptidique, s'agrège sous forme de petits agrégats amorphes sous conditions aqueuses.

Bien que, des assemblages I₁₀ contiennent à la fois des brins- β parallèles et antiparallèle à l'origine probablement du polymorphisme observé (Zottig *et al.*, 2019), nous avons démontré par des analyses FTIR et DRX que les nanofilaments formés adoptent une organisation parallèle de feuillets- β -croisés. Cette orientation en feuillets- β parallèles concorde bien avec les spectres d'absorption et d'émission, révélant ainsi un empilement π stable entre les molécules de PDI adjacentes.

Après avoir démontré qu'il est possible de préparer le dérivé asymétrique PDI-I₁₀ via un contrôle stœchiométrique par synthèse peptidique sur support solide, nous avons étudié l'autoassemblage de ce dernier dans un tampon aqueux où nous avons encore

observé une transition des unimères, ou petites espèces oligomériques, vers des agrégats H étendus bien organisés. Ces résultats ont révélé qu'une seule séquence peptidique en position imide est suffisante pour guider l'autoassemblage des unités PDI-peptide.

L'extinction notable de la fluorescence après une semaine d'incubation des deux dérivés pourrait être une signature visuelle de la formation des assemblages PDI-I₁₀ et PDI-[I₁₀]₂. Avec une cinétique de conversion conformationnelle d'autoassemblage comparable à celle observée pour le dérivé symétrique, les images AFM et TEM ont révélé une distribution hétérogène de courtes protofibrilles et de longs nanofilaments d'un diamètre moyen de 2.87 ± 0.64 nm et 4.92 ± 0.57 nm, respectivement. La différence de diamètre des deux populations suggère que les longues fibrilles de PDI-I₁₀ résultent d'une association de deux protofilaments, permettant ainsi aux PDI de s'enfuir de la solution aqueuse vers le cœur des fibrilles. Cependant, les courtes protofibrilles proviendraient d'un empilement individuel de protofilaments qui ne peuvent pas croître sans s'associer ensemble en raison de la pénalité thermodynamique imposée par l'exposition au solvant aqueux de l'unité PDI hydrophobe. Une telle organisation via un autoassemblage coopératif a déjà été proposée pour des nanostructures PDI asymétriques (van der Weegen *et al.*, 2013). Concernant le dérivé PDI-[I₁₀]₂ qui présentait une distribution homogène de longs nanofilaments linéaires d'un diamètre moyen de $3,27 \pm 0,59$ nm, ces fibrilles pourraient être organisées avec un seul empilement de peptides et le protofilament résultant s'enroulerait en hélice autour de son axe central. Cette organisation supramoléculaire, proposée pour des PDI symétriques bisubstitués par des courts peptides ou des chaînes alkyles ramifiées (Görl *et al.*, 2012), permettrait au noyau PDI de s'enfuir hors de l'eau.

Les essais de viabilité cellulaire ont montré que les assemblages de PDI-I₁₀ et PDI-[L]₂ sont complètement cytocompatibles et stables même après dilution en bas de la concentration critique d'agrégation (10 μ M). De plus, avec une intensité modérée de fluorescence, les fibrilles PDI-I₁₀ et PDI-[L]₂ ont pu être observées par microscopie

confocale (ex : 488 nm ; em : 700 nm) et ont été localisées à la surface cellulaire ainsi que dans le cytoplasme. Ces nanofilaments sont probablement internalisés par endocytose qui est une voie typique pour les nanoparticules (Couceiro *et al.*, 2015; Manzanares et Ceña, 2020). Comme indiqué précédemment pour les fibrilles amyloïdes, il est possible que les fibrilles PDI-I₁₀ et PDI-[I₁₀]₂ soient capturées par les cellules via les micropinocytoses (Jin *et al.*, 2016). Ainsi, l'émission modérée de ces assemblages cytocompatibles semble suffisante pour les exploiter dans des expériences d'imagerie de cellules vivantes en combinaison avec des applications en bioélectronique.

Enfin, nous avons démontré par des mesures électriques sur des films à base de nanofibrilles déposés sur des électrodes en or interdigitalisées que ces nanofilaments présentent des propriétés semi-conductrices robustes. Ce type d'électrodes a déjà été utilisé pour des mesures de conductivité électrique de films à base de nanofibres (Creasey *et al.*, 2019). De plus, une étude comparative a révélé une conductivité élevée ($I > 1,0$ nA) pour un film à base de fibrilles PDI-[I₁₀]₂ à une tension de balayage de 10 V, contrairement au film formé de fibrilles PDI-I₁₀ qui a démontré une faible conductivité ($I < 1,0$ nA) à la même tension de balayage. Il convient de mentionner que la conductivité élevée des assemblages dépend de leur structure ordonnée qui conduit à une délocalisation à longue distance des charges (Qu *et al.*, 2018). Ceci est probablement en relation avec la distribution homogène des nanofilaments longs et linéaires observés pour des nanofilaments de PDI-[I₁₀]₂, conduisant ainsi à une zone topographiquement plus élevée. La conductance des assemblages bioconjugués PDI-peptide pourrait être expliquée par deux hypothèses différentes, l'empilement π des cycles aromatiques et le saut d'électrons (Schneebeli *et al.*, 2011; Shipps *et al.*, 2021). Les peptides amyloïdes π -conjugués ne devraient pas établir une périodicité d'empilement π à longue distance, nécessaire pour permettre la délocalisation le long de la structure supramoléculaire (Ing *et al.*, 2018). Par conséquent, nous suggérons que le transfert d'électrons peut être le résultat à la fois d'interactions π à courte distance et de sauts d'électrons entre les empilements. De même, les courbes courant-tension

enregistrées présentaient une forme en S non-ohmique, souvent observée pour les semi-conducteurs organiques (Creasey *et al.*, 2019). En outre, les fibrilles assemblées à partir d'une séquence peptidique I₁₀ sans PDI semble subir une dégradation après l'application d'une tension de ± 1 V, alors que les films formés par des fibrilles PDI-[I₁₀]₂ et PDI-I₁₀ présentaient des stabilités plus élevées, même après l'application de tensions plus élevées de ± 10 V. En conséquence, le noyau PDI améliore non seulement la conductivité des nanofilaments résultants, mais améliore également considérablement leur stabilité sous tension appliquée. En résumé, la conjugaison de PDI aux peptides amyloïdes permet de diriger son organisation à longue distance pour former des nanomatériaux optoélectroniques. Ces résultats encourageants ouvrent la porte vers la compréhension des mécanismes gouvernant le processus d'autoassemblage amyloïdes et la morphologie des assemblages résultants pour une conception théranostique ciblée.

5.2 Effet du coiffage électrostatique terminal des peptides en feuillets- β sur l'autoassemblage et l'activation du récepteur Toll-like 2/6 par des nanostructures de type amyloïde.

Les fibrilles amyloïdes sont connues pour leur capacité à activer le récepteur Toll-like 2 (TLR2), un récepteur transmembranaire présent principalement sur les cellules de l'immunité innée, qui fait partie de la première ligne de défense de l'organisme contre les maladies infectieuses. Cependant, les nanomatériaux amyloïdes sont connus pour leur polymorphisme supramoléculaire élevé (Seuring *et al.*, 2017), résultant de l'interaction délicate entre un certain nombre de facteurs ; y compris les conditions dans lesquelles l'autoassemblage se produit et la complexité des interactions multiples conduisant à l'agrégation. Si la diversité supramoléculaire des amyloïdes peut être considéré comme un avantage pour construire une grande variété d'assemblages macromoléculaires, cette hétérogénéité intrinsèque caractéristique limite le contrôle précis de leur autoassemblage sous formes nanoplateformes ordonnées pour des fonctions adaptées. Dans ce contexte, nous avons exploité l'effet du coiffage électrostatique aux extrémités N- et C-terminales de peptides amyloïdogéniques sur la

morphologie, la taille et les propriétés physicochimiques des assemblages en feuillets- β -croisés afin de déterminer les relations entre la morphologie/structure et la stimulation de l'immunité innée via l'activation du TLR2.

Une petite bibliothèque de peptides dérivés de la séquence I₁₀ a été préparée et l'autoassemblage a été caractérisé à l'aide d'une combinaison d'approches biophysiques. Ainsi, nous avons démontré que le coiffage électrostatique en positions *N*- et *C*-terminales du peptide I₁₀ conduit à la formation contrôlée d'une diversité de nanostructures, notamment des nanofilaments sous forme de ruban, des nanotiges de différentes longueurs ainsi que des fibrilles plates et torsadées. Il a été déjà démontré auparavant que les modifications des extrémités *N*- ou *C*-terminales de peptides amyloïdogéniques affectent considérablement la longueur, l'architecture mésoscopique et/ou le polymorphisme intrinsèque des assemblages- β résultants (Andreasen *et al.*, 2014; Arya *et al.*, 2020; Hajiraissi *et al.*, 2019; Pizzi *et al.*, 2017; Zottig *et al.*, 2019). Les analyses spectroscopiques suggèrent la présence d'une organisation quaternaire de type amyloïde riche en feuillets- β pour tous les assemblages. De plus, un examen détaillé des images AFM a révélé que les d'assemblages peptidiques obtenus présentaient une homogénéité et une distribution uniforme, généralement difficiles à atteindre avec les fibrilles amyloïdes. Comme indiqué précédemment (Zottig *et al.*, 2019), les unités de coiffage positives à l'extrémité *N*-terminale du peptide I₁₀ affectent la morphologie finale et conduisent à l'auto-assemblage en nanotiges uniformes. Ce qui laisse conclure que la longueur des assemblages peut être, dans une certaine mesure, modulée par les forces de répulsion électrostatiques à l'extrémité *N*-terminale de la séquence I₁₀. Cependant, aucune corrélation claire n'a pu être observée entre la charge nette de l'unité de coiffage positive et la longueur des assemblages pour les peptides I₁₀ carboxylés en *C*-terminal.

Les essais de viabilité cellulaire ont montré que tous les dérivés I₁₀ monomériques et leurs assemblages étaient entièrement cytocompatibles pour les cellules dendritiques, contrairement à l'IAPP, utilisé comme contrôle positif, qui s'est révélé toxique sous ses

formes monomériques/préfibrillaires, alors qu'aucune toxicité n'a été observée pour sa forme fibrillaire. D'autre part, tous les dérivés monomériques, à l'exception du peptide I₁₀-CONH₂, étaient inactifs pour induire l'activation de NF-κB, ce qui indique que l'organisation supramoléculaire est essentielle pour engager la signalisation cellulaire TLR2/TLR6. L'activité du dérivé I₁₀-CONH₂ sans préincubation préalable pourrait être associée à un assemblage quasi-instantané, comme le suggère l'analyse de la fluorescence de la ThT. De plus, l'augmentation de l'activité observée pour les assemblages K₂-I₁₀-CONH₂ (50% de Pam₂CSK₄ at 100μM) et K-I₁₀-CONH₂ (85% de Pam₂CSK₄ at 100μM) par rapport à leurs homologues carboxylés, pourrait être associée à la densité de charge positive à la surface. (Arad *et al.*, 2020; Zottig *et al.*, 2019). Pour étudier plus en détail l'importance des charges positives pour l'activation de TLR2/TLR6, des assemblages à base du peptide I₁₀ avec une surface chargée négativement ont été préparés à partir du E-I₁₀-CONH₂ et évalués. Les résultats ont indiqué que les fibrilles croisées E-I₁₀-CONH₂ chargées négativement ne pouvaient pas activer TLR2/TLR6, ce qui suggère que non seulement l'organisation quaternaire est critique, mais également les charges à la surface. En résumé, cette étude renforce l'effet modulateur des modifications en positions N- et C-terminales de peptides amyloïdogéniques et fournit des relations structure-activité préliminaires concernant l'activation de TLR2 par des assemblages-β. Cet effet de charge est intéressant et peut être une source d'inspiration pour le développement d'approches thérapeutiques efficaces pour lutter contre les amyloïdoses.

5.3 Les nanostructures d'ADN empêchent la formation et convertissent les protéospèces amyloïdes toxiques en complexes sphériques cytocompatibles

Les dépôts amyloïdes sont des agrégats ou fibrilles protéiques insolubles qui s'accumulent dans l'espace extracellulaire des tissus et ce processus a été longuement associé à de nombreuses maladies, tel que le diabète de type 2. L'une des caractéristiques de cette pathologie est le dépôt d'agrégats localisés au niveau des îlots de Langerhans pancréatiques (Montane *et al.*, 2012). Ces dépôts sont composés

majoritairement de l'IAPP (Bhowmick *et al.*, 2022; Clark *et al.*, 1988; Lukinius *et al.*, 1989; Westermark *et al.*, 1987). En raison de sa structure primaire comprenant de nombreux résidus hydrophobes, plusieurs chaînes latérales à l'origine de liaisons H et seulement deux résidus chargés, l'IAPP présente une forte tendance à s'agréger sous forme de fibrilles torsadées caractérisées par une polydispersité conformationnelle (Liu *et al.*, 2010). L'IAPP sous sa forme monomérique n'est pas cytotoxique en soi, mais induit la mort cellulaire en s'assemblant en oligomères solubles transitoires et en espèces préfibrillaires qui endommagent la membrane plasmique et déclenchent, entre autres, le stress oxydatif et l'apoptose (Kakinen *et al.*, 2019; Raimundo *et al.*, 2020). Bien que les fibrilles amyloïdes d'IAPP matures assemblées *in vitro* sont généralement non toxiques pour les cellules (Moore et Cooper, 1991), le dépôt massif de plaques amyloïdes dans les îlots de Langerhans provoque un stress inflammatoire et un dysfonctionnement des cellules sécrétrices d'insuline, contribuant à la pathogenèse du diabète de type 2 (Moore et Cooper, 1991). Ainsi, les interventions thérapeutiques ciblant la pathogénicité associée à l'IAPP devraient non seulement inhiber la formation d'espèces préfibrillaires toxiques, mais également favoriser l'élimination/clairance des fibrilles amyloïdes préformées. Cependant, dans ses états pré-fibrillaires, l'IAPP présente une diversité conformationnelle qui complique la conception de molécules capables de stabiliser le peptide sous une forme non agrégée. A cet effet, le développement d'approches thérapeutiques efficaces pour inhiber la formation d'amyloïde et prévenir la dégénérescence cellulaire reste toujours un grand défi, dû essentiellement à la complexité du processus d'agrégation des amyloïdes regroupant une série infinie de structures quaternaires. Il est intéressant à noter que de courtes séquences d'ADN simple brin se sont révélées de puissants modulateurs de la fibrillation du peptide A β (Abraham *et al.*, 2014). Cependant, cet ADN simple brin est très flexible et est moins stable thermodynamiquement comparé à l'ADN double brin, en particulier ceux assemblés dans des architectures 2D et 3D (Messaoudi *et al.*, 2019; Pal et Levy, 2019). Dans ce contexte, la présente étude vise à explorer un nouveau paradigme pour moduler la formation d'amyloïde IAPP via un mécanisme de recouvrement médié par l'ADN.

Initialement, nous avons évalué la cinétique d'agrégation de l'IAPP en absence et en présence des différentes nanostructures d'ADN en utilisant le FIASH comme sonde fluorogénique alternative à la ThT (Quittot *et al.*, 2018). Cette méthode, qui repose sur la formation d'un motif tétracystéine non-contigu, est bien adaptée pour étudier la cinétique de la fibrilisation de l'IAPP en surveillant la formation de complexes quaternaires en présence de nanostructures d'ADN puisqu'il a été déjà démontré que la ThT présente une émission significative en présence de ces derniers (Hanczyc *et al.*, 2021). Une courbe de croissance sigmoïdale prototypique a été obtenue pour l'IAPP en l'absence d'ADN. En revanche, l'incubation de l'IAPP en présence de nanostructures d'ADN linéaires (1D), triangulaires (2D) et tétraédriques (3D) a induit une forte augmentation de la fluorescence du FIASH avec une absence de la phase de latence, ce qui indique que les nanostructures d'ADN accélèrent la convergence du domaine *N*-terminal des molécules IAPP. L'IAPP est un peptide chargé positivement (+3) à pH physiologique, favorisant probablement sa liaison via des interactions électrostatiques aux groupements phosphates chargés négativement du squelette d'ADN (De Carufel *et al.*, 2013; Motamedi-Shad *et al.*, 2009).

Nous avons ensuite évalué la capacité des nanostructures d'ADN à moduler les espèces monomériques (IAPP_M), oligomériques (IAPP_O) et fibrillaires (IAPP_F) de l'IAPP via des analyses biophysiques et imagerie AFM après incubation prolongée de ces dernières avec les différentes nanostructures d'ADN. Les résultats obtenus ont montré que l'incubation de l'IAPP_M en présence des différentes nanostructures d'ADN conduit à la formation d'assemblages sphériques avec absence totale d'agrégats fibrillaires, indiquant que ces entités inhibent la formation de fibrilles en affectant leur mécanisme d'autoassemblage. Ces complexes IAPP_M-ADN sont beaucoup plus petits que les structures d'IAPP formées en l'absence d'ADN, ce qui révèle que ces nanostructures d'ADN linéaires, triangulaires et tétraédriques favorisent l'autoassemblage de l'IAPP monomérique en structures sphériques, empêchant ainsi la formation de fibrilles amyloïdes. Cette observation est cohérente avec une étude précédente montrant qu'une

courte séquence d'ADN simple brin inhibe l'agrégation amyloïde du peptide A β (Abraham *et al.*, 2014).

Nous avons de même observé que les nanostructures d'ADN convertissent les protéospèces oligomériques hautement cytotoxiques en complexes sphériques cytocompatibles d'une taille comparable celle obtenue avec les complexes IAPP_M-ADN. Considérant que les assemblages préfibrillaires transitoires sont les espèces les plus toxiques de la voie amyloïde ; nous avons étudié les effets des nanostructures d'ADN sur des oligomères pré-assemblés. Étonnamment, des complexes semblables à ceux obtenus avec l'IAPP_M sont obtenus suite à l'incubation de l'IAPP_O en présence des différentes nanostructures d'ADN. De plus, immédiatement après avoir mélangé les nanostructures d'ADN avec l'IAPP_O, une forte augmentation de la fluorescence ANS est observée, ce qui est probablement en corrélation avec la liaison quasi-instantanée des espèces oligomériques à l'ADN, ce qui génère une forte densité de surfaces hydrophobes. Après 72 h, ces assemblages IAPP_O-ADN se réorganisent en complexes interpolyélectrolytes micellaires pour lesquels le noyau hydrophobe de l'IAPP est enfoui au cœur de la micelle et les polyanions d'ADN s'étalent sur la surface exposée au solvant, comme le déduit la diminution de la fluorescence de l'ANS.

Nous avons ensuite étudié l'effet des nanostructures d'ADN sur la stabilité des fibrilles IAPP matures préassemblées (Nguyen *et al.*, 2021). Il est bien connu que les fibrilles amyloïdes sont des suprastructures très stables qui résistent à la dégradation protéolytique ainsi qu'à la dénaturation thermique (Morel *et al.*, 2010). Cette stabilité se traduit par une accumulation massive et permanente de dépôts amyloïdes *in vivo*, ce qui provoque un dysfonctionnement des tissus malgré la faible cytotoxicité des fibrilles isolées (Yoshiike *et al.*, 2007). A cet effet, et après avoir confirmé que les fibrilles IAPP_F résistent à la dénaturation thermique et à la dégradation protéolytique par la protéinase K, nous avons évalué leur stabilité après 72 h d'incubation en présence de nanostructures d'ADN. Ces dernières sont capables de désassembler complètement les fibrilles IAPP_F stables et forment des complexes de forme et taille similaires à celles

observées avec IAPP_M et IAPP_O. Il a été déjà rapporté qu'une courte séquence d'ADN simple brin désassemble les fibrilles A β (Abraham *et al.*, 2014).

De plus, nous avons démontré que les différentes nanostructures d'ADN sont capables d'interagir avec l'IAPP et de former des complexes sphériques de caractéristiques similaires en termes de morphologie, d'hydrophobicité, de taille et de regroupement de peptides sous forme secondaire aléatoire, quelle que soit les structures secondaire et quaternaire initiales du peptide. Dans l'ensemble, ces résultats suggèrent un mécanisme basé sur un modèle dans lequel le squelette phosphate chargé négativement de l'ADN interagit avec les charges positives de l'IAPP, les piégeant éventuellement sous la forme d'un complexe interpolyélectrolytique sphérique dans une conformation désordonnée non amyloïdogène.

Enfin, nous avons démontré que les différentes nanostructures d'ADN préviennent la cytotoxicité de l'IAPP en générant des complexes non toxiques. Les nanostructures d'ADN seules et les complexes IAPP_M-ADN n'ont pas affecté la viabilité cellulaire. Mieux encore, l'application d'oligomères toxiques simultanément à un traitement aux nanostructures d'ADN a complètement supprimé la cytotoxicité associée. Il est bien connu que les oligomères préfibrillaires sont les espèces IAPP les plus toxiques et contribuent à la mort des cellules β -pancréatiques dans le diabète de type 2 (Abedini *et al.*, 2016; Wei *et al.*, 2011). Ces observations indiquent que les nanostructures d'ADN interagissent rapidement avec des oligomères toxiques dans des milieux biologiques complexes en les convertissant en protéoespèces non toxiques. En résumé, cette étude a démontré l'effet modulateur de nanostructures d'ADN sur la formation amyloïde, ce qui constitue une source d'inspiration potentielle pour la conception d'outils moléculaires permettant de lutter contre les troubles liés à l'agrégation des protéines.

5.4 Conclusion et perspectives

Les travaux de cette thèse indiquent que les peptides amyloïdogéniques peuvent être moduler soit pour générer, dans un contexte physiologique, des assemblages fonctionnels, soit, dans un contexte pathologique, pour inhiber la formation de protéoespèces toxiques. A cet effet, ces travaux ont révélé pour la première fois que les peptides amyloïdes auto-assemblés peuvent être exploités pour guider l'autoassemblage de systèmes π -conjugués (PDI), ce qui constitue une stratégie efficace pour concevoir des nanofilaments programmables et biocompatibles dotés de propriétés optoélectroniques robustes. Une nouvelle approche a été mise en œuvre pour accéder facilement aux dérivés PDI symétriques et asymétriques en utilisant une méthodologie de synthèse peptidique sur support solide. Tirant parti de la liaison hydrogène directionnelle impliquée dans l'auto-reconnaissance des peptides, par rapport aux interactions d'empilement π , l'autoassemblage de ces biomolécules hybrides était principalement piloté par la séquence amyloïde, bien que les PDI semblent également participer à ce processus. Il a été également observé qu'un seul peptide aux positions imides est suffisant pour conduire l'assemblage en nanofilaments, mais pas suffisant pour former une distribution homogène de nanofilaments longs et linéaires qui favoriserait, comme observé avec l'assemblages symétriques, une délocalisation de charge à longue distance du nuage d'électrons π et une amélioration de leurs propriétés semi-conductrices. De plus, les deux assemblages étaient entièrement cytocompatibles et pouvaient être observés par microscopie confocale à fluorescence. Considérant que les mécanismes d'autoassemblage des peptides amyloïdogéniques et les architectures supramoléculaires résultantes sont très sensibles à l'environnement, il serait intéressant d'étudier plus en profondeur le processus d'auto-reconnaissance des conjugués amyloïde-PDI dans différentes conditions pour mieux moduler les propriétés bioélectroniques de ces derniers. Ces nouveaux biomatériaux optoélectroniques peuvent être exploités dans de nombreuses applications, notamment la conception de dispositifs d'interface bioélectroniques implantables ou portables. Encore dans un contexte physiologique, des modifications

électrostatiques au niveau des extrémités *N*- et/ou *C*-terminales du court peptide amyloïde ont été exploitées pour guider l'auto-assemblage et moduler la morphologie des assemblages résultants. En tirant profit de la liaison hydrogène directionnelle impliquée dans l'auto-reconnaissance des peptides, ainsi que des répulsions électrostatiques aux extrémités *N*- et/ou *C*-terminales, la morphologie et le polymorphisme des assemblages de type amyloïde résultants ont été déterminés. De plus, les dérivés I₁₀ modifiés et leurs assemblages étaient entièrement cytocompatibles et activaient, à des degrés variables, les TLR2/TLR6. De même, la charge positive à la surface semble être un facteur important pour activer les TLR2 par des nanostructures de type amyloïde. Compte tenu de la diversité structurelle de tous les assemblages générés, il serait très intéressant d'entreprendre des études de dynamique moléculaire pour mieux comprendre les relations structure-activité concernant l'activation de TLR2/TLR6 par des assemblages en feuillets- β .

Dans un contexte pathologique, le processus d'assemblage des peptides amyloïdogéniques peut générer des espèces protéotoxiques transitoires ayant des effets délétères sur les systèmes biologiques. Ainsi, nos travaux ont démontré la capacité des nanostructures d'ADN à inhiber complètement la formation de fibrilles amyloïdes de l'IAPP ainsi que leur capacité unique à désassembler rapidement et efficacement les espèces fibrillaires. Il est de même important de noter que ces nanostructures d'ADN sont capables de piéger efficacement les oligomères hautement toxiques (même dans des milieux biologiques complexes) et de supprimer totalement leur cytotoxicité en les convertissant en complexes sphériques cytocompatibles. De plus, les effets observés sur l'IAPP étaient indépendants de l'architecture des nanostructures d'ADN et n'étaient pas affectés par les ligands ajoutés à l'ADN. Bien que le ou les mécanismes sous-jacents à ces observations restent à établir, la perturbation efficace des oligomères dans des milieux complexes contenant du FBS, une concentration élevée de sels et diverses biomolécules, est remarquable et souligne la nécessité d'examiner davantage les interactions entre les peptides amyloïdes et l'ADN circulant, en relation avec les maladies associées à l'amyloïde. Les résultats obtenus suggèrent que les nanostructures

d'ADN double brin interagissent rapidement avec les protéoespèces d'IAPP via, très probablement, des interactions électrostatiques, conduisant à un éventuel changement morphologique d'une organisation riche en feuillets- β -croisés vers une structure aléatoire. Aucune différence significative entre les différentes nanostructures d'ADN n'a été observé, ce qui nous laisse suggérer que la pénétration des nanostructures dans l'assemblage peptidique est peu probable. Néanmoins, l'adsorption des nanostructures à la surface des agrégats pourrait imposer une contrainte sur les assemblages et favoriser un changement morphologique. En effet, les expériences FIAsh semblent suggérer que la convergence des extrémités *N*-terminales des peptides est très similaire dans les formes fibrillaires (feuillets- β -croisés) et sphériques (structures aléatoires). Ainsi, ces résultats pourraient être une source d'inspiration pour la conception de systèmes à base d'ADN capables de moduler, *in vivo*, le processus d'agrégation amyloïdes pour des fins thérapeutiques, où encore pour des études fondamentales sur les maladies associées.

Finalement, compte tenu des ambiguïtés qui entourent encore les mécanismes d'autoassemblage et les diverses structures moléculaires des nanofilaments (peptides hybrides π -conjugués ; coiffage électrostatique) et des complexes sphériques IAPP-ADN, il serait très intéressant d'étudier par dynamique moléculaire l'ensemble de ces assemblages amyloïdes pour une meilleure compréhension des relations structure/morphologie-activité.

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