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Sabine Szunerits, Amar Abderrahmani, Rabah Boukherroub. Nanoparticles and Nanocolloidal Carbon: Will They Be the Next Antidiabetic Class That Targets Fibrillation and Aggregation of Human Islet Amyloid Polypeptide in Type 2 Diabetes?. *Accounts of Chemical Research*, 2022, Published as part of the *Accounts of Chemical Research* special issue “Chemistry of Advanced Carbon Materials”, 55 (20), pp.2869-2881. 10.1021/acs.accounts.2c00415 . hal-03795926

HAL Id: hal-03795926

<https://hal.science/hal-03795926>

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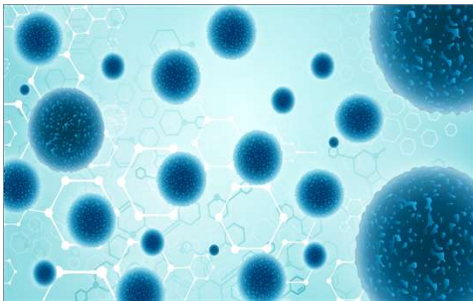
Nanoparticles and nanocolloidal carbon: Will they be the next antidiabetic class that targets fibrillation and aggregation of human islet amyloid polypeptide in type 2 diabetes?

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CONSPECTUS



Nanotechnology is revolutionizing human medicine. Nanoparticles (NPs) are currently used for treating various cancers, developing vaccines and imaging, and other promises offered by NPs might come true soon. Thanks to the interplay between NPs and proteins, there are more and more evidences supporting the role of NPs for treating amyloid-based diseases. NPs can induce some conformational changes of the adsorbed protein molecules *via* various molecular interactions, leading to inhibition of aggregation and fibrillation of several and different amyloid proteins. Though a depth understanding of such interactions between NPs and amyloid structure is still lacking, the inhibition of protein aggregation by NPs represents a new generation of innovative and effective medicines to combat metabolic diseases such as type 2 diabetes (T2D). Here, we lay out advances made in the field of T2D notably for optimizing protein aggregation inhibition strategies. This account covers discussions about the current understanding of β -cells, the insulin producing cells within the pancreas, under diabetic conditions notably increased glucose and fatty acid levels, and the implication of these conditions on the formation of human islet amyloid polypeptide (hIAPP) amylin oligomers and aggregates. Owing to the great potential of carbon nanostructures to interfere with protein aggregation, an important part of this article will be devoted to the state of the art of therapeutic options in the form of emerging nanomaterials-based amyloidosis inhibitors. Our group has recently made some substantial progress in this regard by

investigating the impact of glucose and fatty acid concentrations on hIAPP aggregation and β -cells toxicity. Furthermore, the great potential of carbon nanocolloids in reversing hIAPP aggregation under diabetic conditions will be highlighted as the approach has been validated on β -cell cultures from rats. We hope that this account will evoke new ideas and concepts in this regard. I give some lead references below on pancreatic beta-cell aspects and carbon quantum dots for managing diabetics and nanomedicine related aspects, a topic of interest in our laboratory .

KEY REFERENCES

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- Barras, A.; Sauvage, F. de Hoon, I.; Braeckmans, K.; Hua, D.; Buvat, G.; Fraire, J. C.; Lethien, C., Sebag, J.; Harrington, M.; Abderrahmani, A.; Boukherroub, R.; De Smedt, S.; Szunerits, S. Carbon quantum dots as a dual platform for the inhibition and light-based destruction of collagen fibers: implications for the treatment of eye floaters. *Nanoscale Horiz.*, **2021**, 6, 449-461.³ *This work reports the potential of carbon quantum dots to inhibit the formation of collagen aggregates and destroy vitreous opacities for the first time.*
- Voronova, A.; Barras, A.; Plaisance V.; Pawlovski, V.; Boukherroub, R.; Abderrahmani, A.; Szunerits, S. Anti-aggregation effect of carbon quantum dots on the diabetogenic and beta-cell cytotoxic amylin and beta amyloid heterocomplex. *Nanoscale* **2022**, DOI: 10.1039/D2NR03173F.⁴ *Clear evidence is provided that the diabetogenic environment of pancreatic islets directly participates to the formation of complex protein aggregates, which are toxic for β -cells. Carbon nanocolloids efficiently prevent the formation of cell toxic heterocomplexes.*

INTRODUCTION

Diabetes, a metabolic disease defined by high blood sugar, is one of the deadliest chronic diseases worldwide.⁵ The current treatment of type 1 diabetics (T1D) consists of insulin replacement by subcutaneous injection of human recombinant insulin. Some patients with uncontrolled diabetes by insulin treatment can be eligible for islet or pancreas transplantation. This therapeutic strategy is highly efficient for achieving long-term glycemic control, but it remains marginal as, often, pancreas of two or three donors are required for one recipient. Type 2 diabetes (T2D) is characterized by a loss of insulin sensitivity referred as “insulin resistance”. Age, obesity, bad dietary habits, lack of physical activity and gestational diabetes all are risk factors for insulin resistance (**Figure 1a**). T2D develops when β -cells fail to adapt their insulin production to insulin resistance (**Figure 1b**).¹ Although there are treatments that lower glycemia in the first years, none of the current treatments is able to achieve a long-term glycemic control. Hyperglycemia promotes in addition the formation of pancreatic islet amyloid deposition, and has become a pathological hallmark of T2D.^{1, 6} Developing efficient drugs that prevent amyloid formation and preserve β -cell functions are sought after, with a limited number of small therapeutic molecules been currently considered in clinical trials.⁷⁻⁸ An increasing amount of work indicates the interest of nanomaterials for helping in the inhibition of β -cell loss via interaction with cytotoxic amyloid fibrils.

The major component of islet amyloid is the human islet amyloid polypeptide (hIAPP) amylin, co-secreted with insulin from β -cells. There is growing evidence, that islet amyloid is formed by the heteromeric oligomerization of hIAPP with other proteins such as A β 42, the peptide amyloid that is responsible of the amyloid plaque and neuronal loss in Alzheimer disease.^{4, 9-12} Islet β -cells express the amyloid precursor protein (APP) and the enzymatic machinery that generates the amyloidogenic A β 42. The formed heterocomplex (hIAPP|A β 42) is even more cytotoxic than the single oligomers^{4, 9} and requires the development of inhibitors that efficiently prevent the formation of homomeric and heteromeric complexes. Such inhibitors that prevent and stop β -cell dysfunction and destruction are still unmet medical dreams. The application of nanomaterials- and nanomedicine-derived approaches to limit protein aggregation in the context of T2D has been investigated by us lately and is the base for this account article.⁴ While nanomedicine remains challenging, it holds great potential for the future of diabetes management and, for the moment, the suggested benefits in diabetic care seem to outweigh the possible pitfalls.² Inputs from technology-driven medicine for diabetes are expected from chemistry through the synthesis of novel drug formulations, including the development of oral therapeutic protein delivery such as insulin and GLP-1¹³⁻¹⁴ as well as alternative diabetic drug delivery routes reported by us¹⁵⁻¹⁹ and

others.²⁰ Another critical promise from T2D targeted nanomedicine is the addition of therapeutic concepts capable of inhibiting islet amyloid formation into the antidiabetic drug arsenal. Before reporting the advances on this direction and what nanostructures add to the field, it is important to understand the mechanisms underlying the decrease of β -cell mass first.

Along with inflammation, chronic hyperlipidemia and hyperglycemia (**Figure 1c**), amyloid deposits play a central role in β -cell mass destruction. To face insulin resistance, healthy β -cell will secrete larger amount of insulin alongside hIAPP, which may exceed the synthesis, folding and trafficking capacity of the Endoplasmic Reticulum (ER), leading to the formation of toxic hIAPP oligomers. To prevent ER stress-induced apoptosis, the elimination of misfolded and oligomerized hIAPP fibrils is a strategic option. In addition, amyloidogenic hIAPP toxicity is linked to islet inflammation and macrophage infiltration as hIAPP aggregation triggers activation of the nucleotide-binding domain leucine-rich repeat and pyrin domain containing receptor 3 (NLPR3), leading to the production of proinflammatory cytokine interleukin 1β .

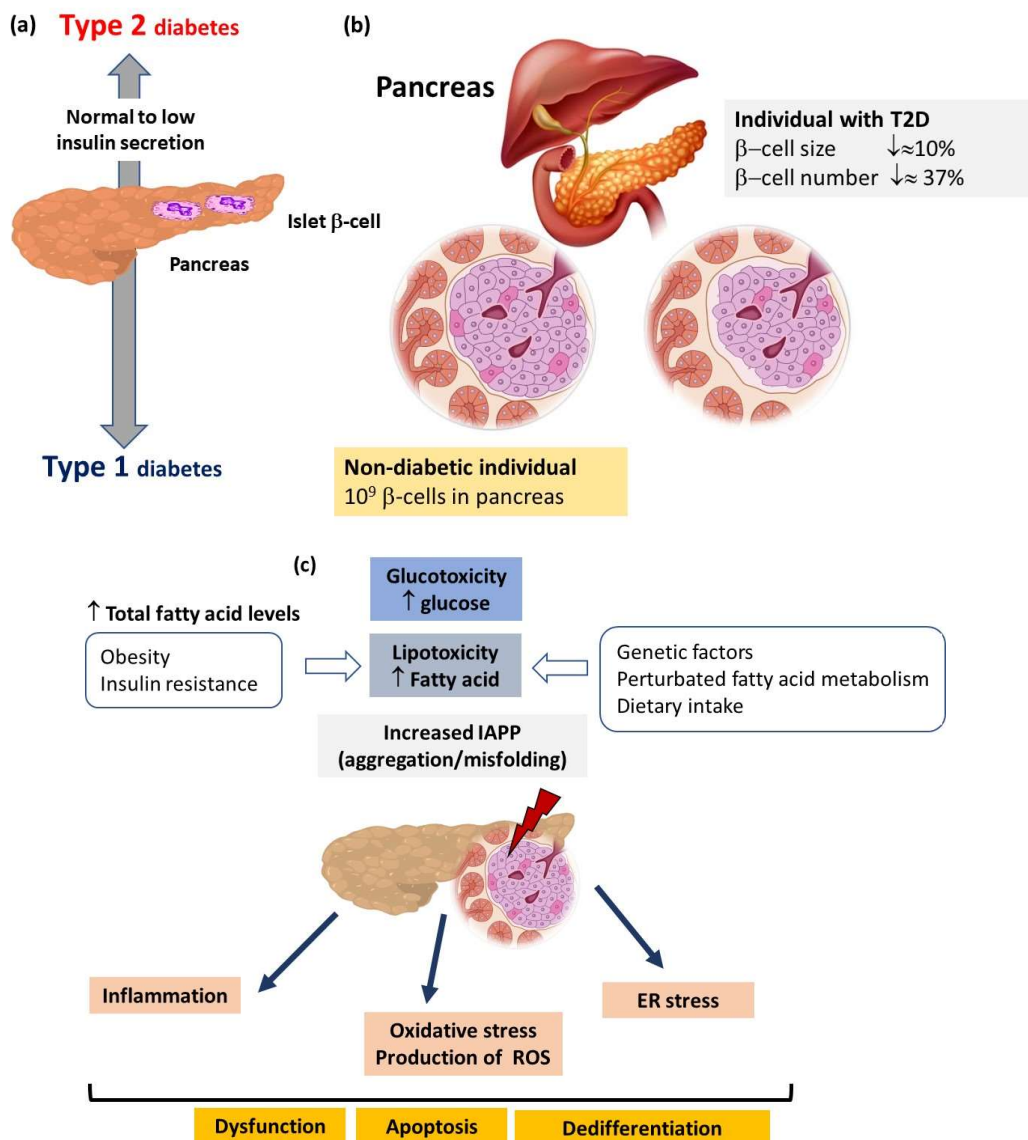


Figure 1: Pathophysiology of type 2 diabetes (T2D): (a) Severity of β -cell dysfunction between T1D and T2D diabetes. (b) β -cell mass for non-diabetic individuals *versus* individuals with T2D. (c) Molecular mechanism involved in pancreatic β -cell loss under T2D prone situations: Increased hIAPP levels with aggregation or misfolding, inflammation, lipotoxicity and glucotoxicity

Human islet amyloid polypeptide: atomic structures and cytotoxicity

hIAPP, a 37-residue peptide hormone that is stored in soluble form at millimolar concentrations in the β -cell from islets of Langerhans before being released into the bloodstream, is a hydrophobic polypeptide containing several positively charged residues such as Lys-1, Arg-11 at and below the physiological pH (**Figure 2a**). The net positive charge of hIAPP is important for interactions with negatively charged, non-physiological model membranes and for interactions with sulfated proteoglycans of the extracellular matrix. In non-diabetic patients, its circulation concentration ranges from 1.6 to 20 pM,²¹ but changes of the physiological environment, once secreted into the blood, and overproduction of hIAPP can promote hIAPP fibrillation (**Figures 2b,c**). Structural changes of hIAPP can be followed by atomic force microscopy (**Figure 2d**) with the width of most hIAPP fibrils ranging between 8 and 12 nm.²² Interestingly, there is currently only one natural genetic mutation of hIAPP reported so far, the S20G variant,²³ associated with early-onset T2D and more aggregation-prone compared with wild-type hIAPP.²⁴

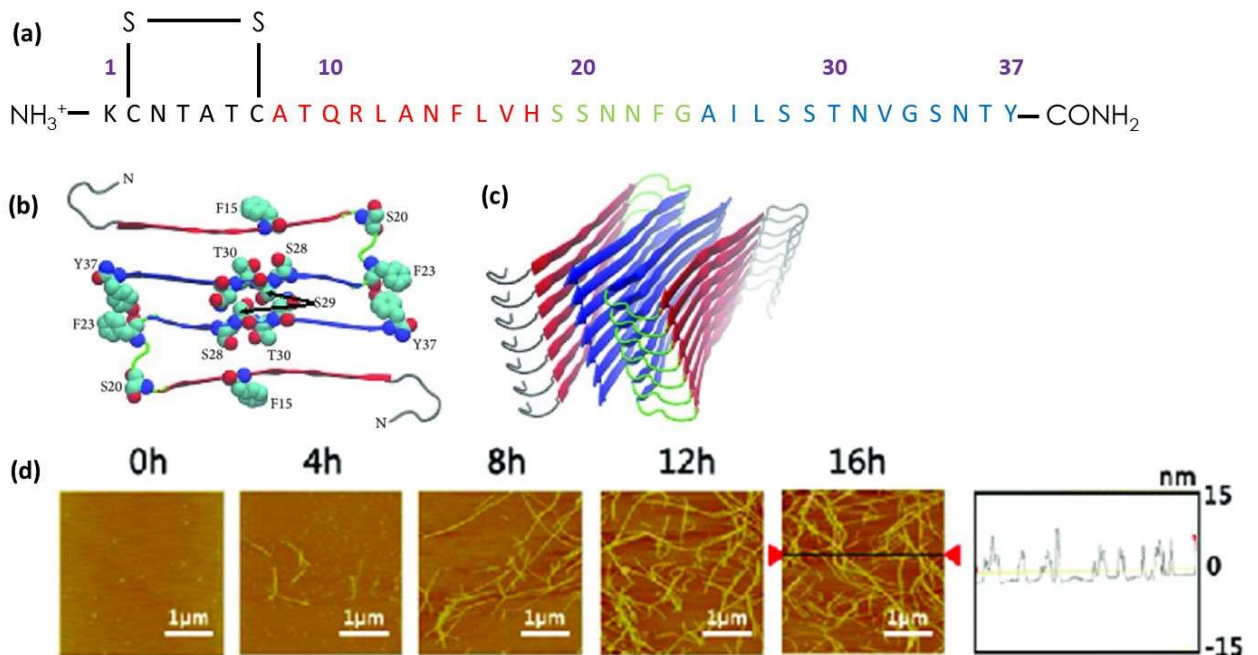


Figure 2: Morphology and structure of human islet amyloid polypeptide (hIAPP): (a) Primary sequence of full-length hIAPP. color-code: red: sequence found in the first β -strand in the fibril; blue: sequence found in the second β -strand, green: ones located in the partially-ordered

loop that connects the two strands. The first seven residues are believed to be outside of the structured core of the fibril and are color-coded black. (b) Top down view of the model with several key residues. The N-termini are labeled. (c) View rotated by 90° showing the arrangement of the two stacks as a ribbon diagram. (Reproduced with permission from ref. 25. Copyright 2016 the authors. Published by Hindawi under a Creative Commons Attribution 3.0 Unported License (CC BY 3.0). (d) AFM images of hIAPP (25 μM) captured at different time points with height analysis of the final amyloid fibrils (Reproduced with permission from ref. 26. Copyright 2017 American Chemical Society.)

Despite being first identified as the amyloidogenic peptide associated with T2D more than 30 years ago,²⁷ *in vitro* studies revealed that hIAPP at micromolar concentrations can readily fibrillate into toxic amyloid aggregates within hours and follows a characteristic nucleation-dependent polymerisation process (**Figure 3a**).²⁸ Once a critical quantity of fibers is formed, aggregation is accelerated by further nucleation on existing aggregate populations, leading to exponential growth of the fibrils until saturation. The relative contribution of the different processes in amyloid fibril are dependent on protein sequence, pH, time-, temperature- and concentration.²⁹⁻³⁰ The team of Vaux provided lately a better understanding of hIAPP aggregation through hydrogelation of the amyloidogenic hIAPP.³¹ The development of novel “chemical tools” to study hIAPP aggregation of wild-type hIAPP and its more amyloidogenic natural variant S20G was lately the focus of a study by the team of Foster and Radford.³² They reported the discovery of two structurally distinct small molecule modulators of hIAPP assembly, one delaying the aggregation of wt hIAPP, but not S20G, while the other enhances the rate of aggregation of both variants at substoichiometric concentrations.³² While earlier studies ascribed hIAPP amyloids as the toxic species,^{28, 33} recent studies pointed oligomers as the causative toxic agent for β-cell loss.³⁴⁻³⁵ The ambiguities around hIAPP toxicity stems from the difficult isolation of hIAPP oligomers from monomers as well as the complex intra- and extra-cellular environment of hIAPP where fatty acids, lipids and proteins are present in abundance. Studies on the hIAPP-protein interactions under physiological relevant conditions are scarce and almost fully lacking.³⁶ We added to this discussion by testing the cytotoxicity of hIAPP on rat insulinoma (INS-1) in the presence of normal and elevated glucose concentrations (**Figures 3 c, d**) and in a mixture of 11 mM of glucose and 0.5 mM of palmitate as, elevated lipid concentrations can accelerate islet amyloid fibrillation.³⁷ Already fresh hIAPP causes significant cell death (**Figure 3b**). Next to hyperglycemia, elevated concentrations in saturated fatty acids are important for hIAPP to aggregate as seen in **Figures 3b**. While investigating the effect of the fatty acid, palmitate, on hIAPP aggregation, it was noticed that insulin mRNA levels decreased by about 50% after treatment with fibrillated hIAPP with increased

membrane permeability, as validated by LDH leakage. However, it remains unclear if hIAPP oligomerization and fibrillation are directly or indirectly triggered by this environment.

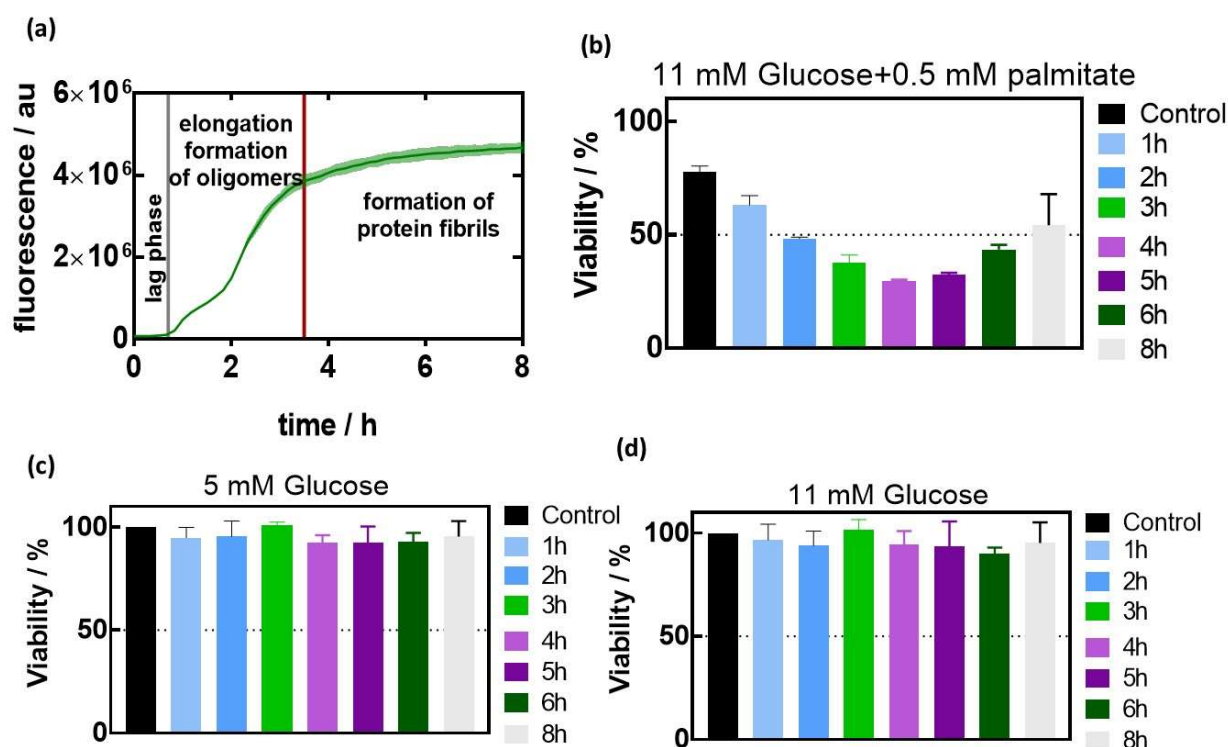


Figure 3: hIAPP aggregation and its effect on cell viability under diabetic and non-diabetic conditions: (a) Elementary molecular events of pathological protein aggregation. The green curve corresponds to a time-dependent hIAPP (20 μ M) aggregation at 37 $^{\circ}$ C followed by Thioflavin-T fluorescence intensity. Effects of hIAPP (20 μ M) fibrillation time on viability of INS-1 cells in the presence of (b) 11 mM glucose and 0.5 mM palmitate, (c) 5 mM glucose and (d) 11 mM glucose.

Next to glucose and palmitate, the aggregation behavior of hIAPP is also influenced by the presence of equally structured proteins, notably β -amyloid from A β 42 (**Figure 4**). Bharadwaj et al.⁹ showed that hIAPP:A β 42 complexes have a 3-fold increased neural cell death. Indeed, different to hIAPP, A β 42 does not aggregate over a time span of 24 h (**Figure 4a**), while the addition of hIAPP (10 %) to A β 42 showed fluorescence at the starting point, indicating that some oligomers might have formed already (**Figure 4a**). There is only a slight increase in fluorescence after 20 h, being much lower than for hIAPP alone. At an equal concentration ratio of hIAPP and A β 42, significant aggregation occurs after a lag time of 14 h, approaching the fluorescence level of hIAPP alone. The addition of glucose to hIAPP:A β 42 (1:1) accelerates strongly the fibrillation of the protein aggregates, while the presence of palmitate results in lower fibrillated fiber content. These results indicate clearly that the interplay between hIAPP, A β 42, palmitate and glucose is a

complex process. Using rat insulinoma (INS-1) cells as model, the toxicity of hIAPP, A β 42 and hIAPP:A β 42 heterocomplexes under diabetic and non-diabetic conditions was evaluated (**Figure 4b**). hIAPP alone had no toxic effect at 5 and 11 mM. In the presence of 11 mM glucose, oligomerized A β 42 and the hIAPP:A β 42 heterocomplex significantly affected the viability of INS-1 cells. Cytotoxicity induced by the oligomerized A β 42 at 11 mM glucose was further amplified by palmitate and confirms that amyloids generation and toxicity can only happen under diabetogenic condition including high glucose concentration and saturated fatty acids. The hIAPP:A β 42 heterocomplex was clearly found to be more cytotoxic than the single oligomers and/or aggregates of hIAPP on INS-1 rat cells. This unveil the critical role of the A β 42 oligomers/aggregates and support the hypothesis that A β 42 oligomers/aggregates are a critical component within the amyloid deposit found in islets of patients with T2D.

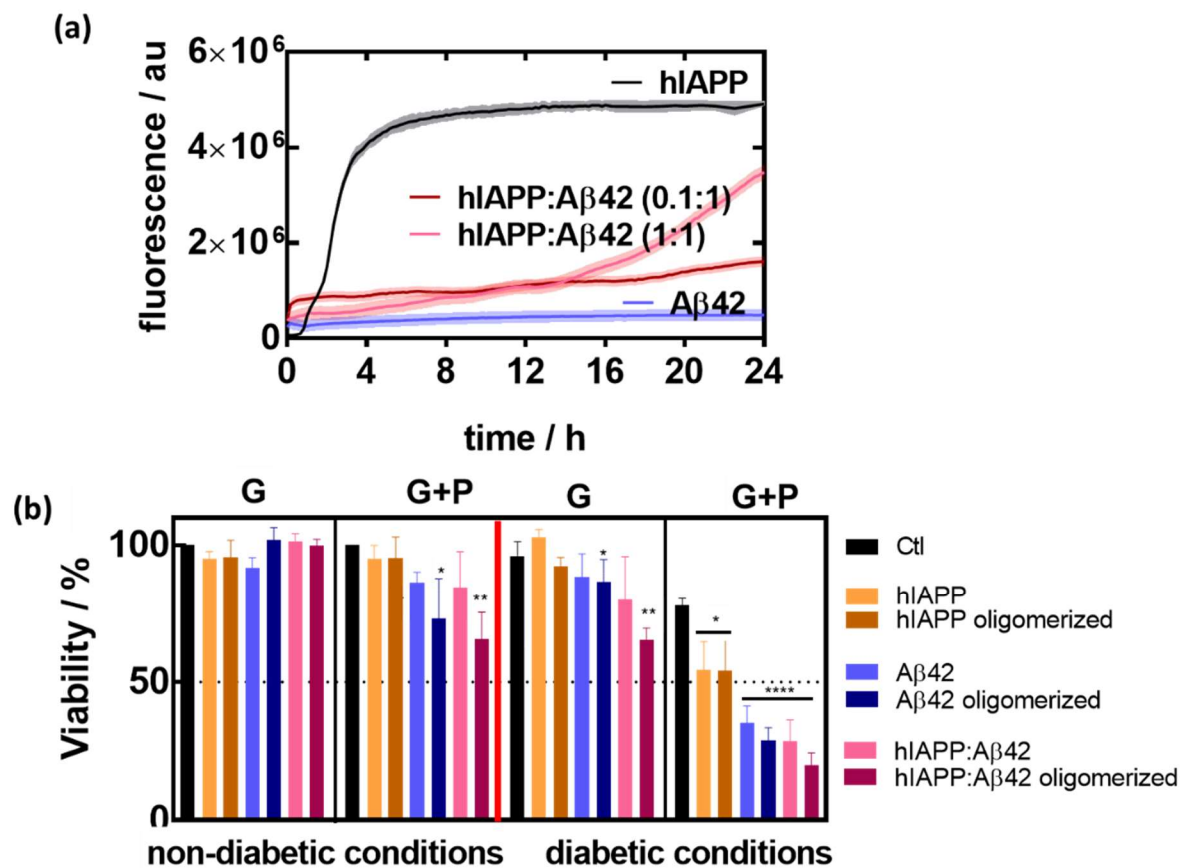


Figure 4: hIAPP aggregation in the presence of A β 42 under diabetic conditions: (a) Thioflavin-T fluorescence intensity as a function of time of hIAPP (20 μ M), A β 42 (20 μ M) and two ratios of hIAPP:A β 42. (b) Viability of rat INS-1 cells cultured in a medium containing 0.1% (vol/vol) of BSA and non-diabetic conditions: 5 mM glucose (G) and 5 mM glucose+0.5 mM (G+P) palmitate or diabetic conditions: 11 mM glucose (G) or 11 mM+0.5 mM palmitate (G+P), in the absence (Ctl) or presence of either fresh (non-oligomerized) or aggregated (6 h, 37°C) hIAPP

(20 μ M), A β 42 (20 μ M) and hIAPP:A β 42 mixtures: INS-1 cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, 1% sodium pyruvate, 50 μ M β -mercaptoethanol, seeded at a density of 10^5 cells/well in a 96-well plate and grown for 24 h before assay. The cell viability was evaluated using resazurin cell viability assay. The results are expressed as the mean \pm SEM of at least 3 independent samples for each group. * p <0.123 (n.s); ** p <0.033; *** p <0.0021; **** p <0.0002, one-way ANOVA test against control, different between A β 42 and A β 42 oligomerized and hIAPP:A β 42 and hIAPP:A β 42 oligomerized is n.s.

Prevention of amylin β -sheets and their aggregation: from molecular inhibitors to nanostructures

With the better understanding of cytotoxic species, the development of effective therapeutics to prevent protein aggregation-related diseases started remains at an early stage.³⁸ Several studies have examined the anti-amyloid mechanisms of small molecules such as epigallocatechin gallate,³⁹ resveratrol,⁴⁰ curcumin,⁴¹ or anle145c.⁴² These molecules mitigate amyloidosis with encouraging results *in vitro*, but with moderate success *in vivo* in mice models and abysmal outcomes in human trials. Peptide inhibitors are believed to be of limited use as they have the tendency to aggregate themselves and have rather low water solubility.⁴³ Polyphenol inhibitors are more effective due to the formation of hIAPP-polyphenol hydrogen bonds and π - π stacking combined with hydrophobic interactions responsible for solubilizing formed oligomers.⁴⁴ Just as large quantities of aggregates are toxic, large doses of inhibitors themselves are toxic as well, suggesting the following question: what is the balance between the degree of inhibition and the toxicity of the inhibitor. Killian and co-workers investigated more closely INS-1 and MJS cells after incubation with and saw that the percentage of dead cells decreased significantly with anle145c in a concentration-dependent manner to about 7%, after which even higher concentrations of anle145c did not result in improved cell viability.

In the last decades, extensive efforts have been put into the development and use of nanomaterials for protein aggregation inhibition. While a large range of issues, such as ethics, market size, possibility of upscaling etc. must be addressed in the implementation of these nanostructures for practical applications, the benefits of employing nanoparticles are mainly due to several key characteristics of nanostructures such as increased solubility and superior bioavailability of drugs when encapsulated in nanostructures, good biocompatibility and biodegradability of the materials as well as their small (< 100 nm) dimensions. Encapsulation and stabilization of monomeric hIAPP

in hydrophobic cavities such as OH-terminated polyamyloidoamine dendrimers was one of the first strategies to inhibit hIAPP fibrillation.⁴⁴ As the hydrophobicity of the hIAPP amyloid protein is increasing from the monomer form to the protofibrils and then decreases as fibrils are formed, the toxicity of the aggregated species rises and falls accordingly. The use of anionic α -lactalbumin-modified Au NPs was studied by Javed et al. as hIAPP inhibitor (**Figure 5a**) with the hypothesis that the intrinsically disordered hIAPP could bind many co-localized proteins (**Table 1**). β -sheet stacking between hIAPP and the β -lactoglobulin “corona” of the Au NPs eliminated indeed hIAPP toxicity, owing to electrostatic interactions of the negatively charged AuNPs/ β -lactoglobulin nanocomposites with the positively charged hIAPP.⁴⁵

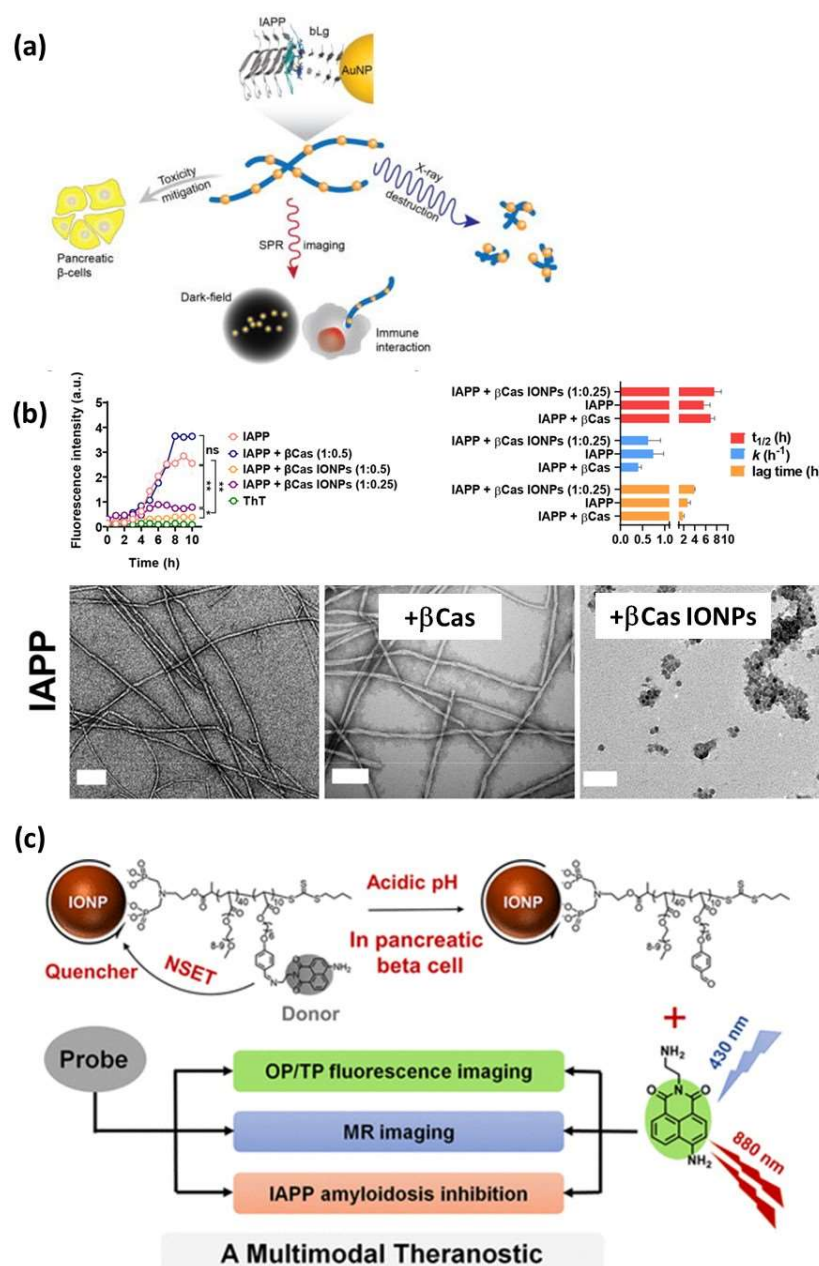


Figure 5: hIAPP amyloidosis mitigation strategies: (a) Protein corona concept on gold nanoparticles modified with α -lactoglobulin amyloid (Reproduced with permission from ref. 46.

Copyright 2017 American Chemical Society). (b) Inhibitory effects of β Cas-modified IONPs on hIAPP fibrillation (50 μ M) in the presence and absence of β Cas (25 μ M) and β Cas-modified IONPs as monitored by a ThT fluorescence kinetic assay. ThT (100 μ M) was used as the control. The data points shown are the mean values ($n = 3$) \pm SEM. Bar graphs of $t_{1/2}$, lag time, and apparent aggregation constant (k) values over time (h) for hIAPP in the presence and absence of β Cas and β Cas-IONPs as well as TEM images of hIAPP + β Cas, and hIAPP + β Cas-IONP (Reproduced with permission from ref. 47. Copyright 2021 American Chemical Society). (c) IOPNs conjugated with acid hydrolysable 2-photon dye and their use for fluorescent, MIR imaging as well as amyloidosis inhibition strategies (Reproduced with permission from ref. 48. Copyright 2020 American Chemical Society).

Gold-based nanomaterials have the strengths of being chemically inert, having tunable local surface plasmon resonance (LSPR) absorption, a property which allows to localize them in animal tissue with its LSPR signal as demonstrated on the brain sections of the larvae using β Cas AuNPs.⁴⁹ While the use of magnetic nanoparticles instead of microbeads is very promising to increase of mass transfer for target molecule diffusion in the separation processes,⁵⁰ in the context of protein fibrillation magnetic nanostructures are of the same standing as Au NPs. Indeed, in parallel to β Cas AuNPs,⁴⁹ Ke and co-workers showed lately the inhibition potential of Janus iron oxide nanoparticles coated with β -casein of 50 ± 24 nm in size and zeta-potential of -31 ± 5 mV.⁴⁷ With a hIAPP half-time aggregation rate of 6.9 h in the presence of the nanostructures, being larger than for hIAPP alone (5.6 h), there was evidence for a prolonged elongation with the formation of amorphous aggregates, as evidenced by TEM imaging (**Figure 5b**). The utilization of magnetic nanoparticles conjugated with a 2-photon dye polymer converting into a fluorescence probe upon acid hydrolyzation in pancreatic β -cells was proposed as hIAPP fibrillation inhibitor, allowing in parallel β -mass imaging *in vitro* and *ex vivo* (**Figure 5c**).⁴⁸ Chiral silica nanoribbons were in addition proposed to mitigate hIAPP amyloidogenic *in vivo*,⁵¹ underlying the potential of employing chiral nanostructures against the mesoscopic enantioselective amyloid proteins and their associated diseases. While these different examples were directed toward hIAPP inhibition, not all nanostructures are capable to achieve inhibition. The best example is that reported on star-shaped polymers where hIAPP fibrillation was even increased mostly due to further interaction with the hydrophobic shell of the polymer stars.⁵²

Table 1: Studies on using nanomaterials for inhibiting hIAPP fibrillation

Nanomaterial	Size charge	Protein inhibition mechanism	Ref.
β Cas-IONP	35.1 \pm 11.8 nm -24.8 \pm 4.4 mV	prolonged elongation phase with the formation of amorphous aggregates	47
IONP@PEG-b-PFPHA-NaP	6.20 nm	Formation of peptide-probe aggregate	48
Chiral (L/R) silica nanoribbons	19.5-44.7 nm Pitch size: 90 nm -22 to -23 mV	R-SiO ₂ more potent due to fibrillization along the perpendicular direction and higher hIAPP seeding on nanostructure	51
star-shaped poly(2-hydroxyethyl acrylate) (PHEA)	12 nm ζ =negative	PHEA arms serve as rodlike scaffolds for hIAPP binding and accelerates hIAPP aggregation by increased local peptide concentration. Accelerated protein fibrillation circumvent accumulation of toxic intermediates.	52
citrate -Au NPs	10 nm ζ =negative	Competition between peptide-peptide and peptide-surface interactions	53
citrate -Au NPs	5-20 nm ζ =-22 to -25 mV	metal-binding sequence motif in the hydrophilic hIAPP domain strongly interacts with the Au surface in both the monomeric and fibrillar states.	54
citrate-Ag NPs PEI-Ag NPs PEG-IONP PC-IONP	20-50 nm ζ =-35.0 (citrate-Ag NPs) ζ =+40.3 (PEI-Ag NPs) 20-50 nm ζ =-21.1 (PEG-IONP) ζ =-11.1 (PC-IONP)	PEI-Au NPs: electrostatic repulsion of hIAPP Citrate-Ag NPs: fibril shortening due to strong electrostatic interactions PC-IONP: fibril softening and looping due to dipolar interactions of zwitterionic PC moieties and polar IAPP monomers	55
polyamyloidoamine dendrimers	ζ -10.5 \pm 1.51 mV	stabilization of monomeric hIAPP in hydrophobic cavities of the dendrimer	44
GO nanosheets	irregularly shaped flakes each of \sim 2 μ m in size ζ =-42.0 \pm 5.6 mV	strong binding of hIAPP to GO driven by hydrogen bonding, aromatic stacking and strong peptide-GO binding efficiently inhibited hIAPP self-association and aggregation	56
GQDs Formed electrochemically	3.6 \pm 0.3 nm size ζ =-20.6 mV	Intercalation of GQDS into hIAPP fibrils during aggregation as well as adsorption of unfolded hIAPP onto GO. hydrophobic and hydrophilic residues of hIAPP are partitioned onto oxidized and non-oxidized region of GQDs with the positively charged residues (e.g. arg11) in hIAPP forming salt-	57

		bridges with the carboxylic acid and polar residues of GQDs	
Fluorine-modified GQDs	2.38±0.04 nm	electronegative F functional group enhances electrostatic hydrophobic π - π stacking interactions, resulting in increased binding ability to hIAPP and formation of short thin fibrils and amorphous aggregates.	⁵⁸
amphiphilic CQDs from citric acid and <i>o</i> -phenylenediamine	3.4 nm±1.0 nm ζ =-15.3 mV	Binding of hIAPP to surface of CQDs with either hydrophilic or hydrophobic surface as binding pattern.	⁵⁹
CQDs formed by “polymerization” and “carbonization” using sucrose and oleic acid	2.4 ± 0.9 nm ζ =-15.3 mV	CQDs cannot inhibit oligomers to form ‘protofibrils’ or fibrils, but significantly decreases the rate of normal protein monomers transforming into oligomers	⁶⁰
CQDs from Victorian brown coal	2.8 nm ζ =-44.6±7.9 mV	CQDs targets the amyloidogenic and C-terminus region of hIAPP	⁶¹
CQDs derived from glucosamine with ethylenediamine as passivating agent	16.8 ± 6.7 nm ζ =+32.5 mV	Inhibition under diabetic conditions due to electrostatic repulsion	⁴
CQDs from amino acid tyrosine under hydrothermal conditions	d=5 ± 2 nm	The pronounced inhibitory effect is traced to the display of ubiquitous aromatic residues upon the C-dots' surface, mimicking the anti-fibril and anti-toxic activity of natural polyphenolic compounds.	⁶²

d=diameter, β Cas= β -casein; IONP= iron oxide nanoparticle; ζ =zeta-potential: GO=graphene oxide; GQDs: graphene quantum dots (GQDs); CQDs: Carbon quantum dots.

Potential of carbon nanoparticles and carbon colloids to inhibit hIAPP fibrillation

While it has emerged from the results presented so far that a key to amyloidosis inhibition is to disrupt protein-protein association by promoting nanoparticle-protein interactions, the choice of the nanomaterials was sporadic in the early stage.⁵³ The use of nanomaterials with an aromatic network such as graphene oxide (GO), graphene quantum dots (GQDs), carbon nanocolloids, hydroxylated fullerenes, etc.⁵⁶ are expected to be ideal candidates for hIAPP aggregation inhibition (Table 1). Numerical computation of tetramer and octamer hIAPP with diverse carbon nanoparticles (graphene, single-wall carbon nanotubes (SWCNTs), fullerene C₆₀) revealed that peptides strongly adsorb onto graphene and SWCNTs, while C₆₀ prevents aggregation to a lesser extent.⁶³ The highly curved fullerene surface exhibits reduced efficiency for π -stacking, but promotes increased peptide dynamics. The low water solubility of fullerenes and SWCNTs has to be overcome to make these nanomaterials of practical use. Hydroxylated carbon nanomaterials

such as GO nanosheets are alternatives. One of the first studies in this direction is the work by Ding and co-workers using GO as hIAPP amyloid fibrillation inhibitor.⁵⁶ GO nanosheets exhibit strong binding to hIAPP driven by hydrogen bonding and aromatic stacking and the strong peptide–GO binding efficiently inhibited hIAPP self-association and aggregation. About 30% of the residues, mostly those near the N-terminal, formed an α -helix, while about 50% of the residues formed random coils resulting in efficient protection of β -cell.⁵⁶

The efficiency of graphene quantum dots (GQDs), derivatives of graphene displaying the same physico-chemical features,⁶⁴⁻⁶⁵ against hIAPP aggregation and toxicity *in vivo* was underlined by Wang et al.⁵⁷ The addition of GQDs ($500 \mu\text{g mL}^{-1}$) to hIAPP ($25 \mu\text{M}$) solutions resulted in a strongly reduced persistence and contour lengths of the hIAPP fibers. Using DMD simulations, in the presence of GQDs, the hIAPP monomers are absorbed onto the nanosheet surface transforming the ordered helical and β -sheet structures of hIAPP into random coils. The hydrophobic and hydrophilic residues of hIAPP were portioned onto oxidized and non-oxidized region of GQDs with the positively charged residues (e.g. arg11) in hIAPP forming salt-bridges with the carboxylic acid and polar residues of GQDs. Fluorine-modified GQDs (FGQDs) of 2.38 ± 0.04 nm in size were proposed by Yousaf et al. as hIAPP aggregation inhibitors⁵⁸ The highly electronegative F functional group was expected to enhance electrostatic interactions as well as hydrophobic and π - π stacking interactions, resulting in increased binding ability of FGQDs.

Next to graphene-based nanostructures, of carbon quantum dots (CQDs) (also called carbogenic dots or carbon nano-dots) have been investigated more recently in connection with hIAPP fibrillation inhibition. CQDs are an emerging subset of nanomaterials, defined by characteristic sizes of < 10 nm and a carbon core that is functionalized by various groups at the surface. Elucidating the origin and key factors controlling their properties such as toxicity and dispersibility remains an outstanding challenge. The inhibitory effect of CQDs relies on the delicate balance of hydrophobic interaction, aromatic stacking, and electrostatic interaction and explain why different carbon-nanoparticles display similar interaction mechanism with proteins but have different inhibitory effects (**Figure 6, Table 1**).

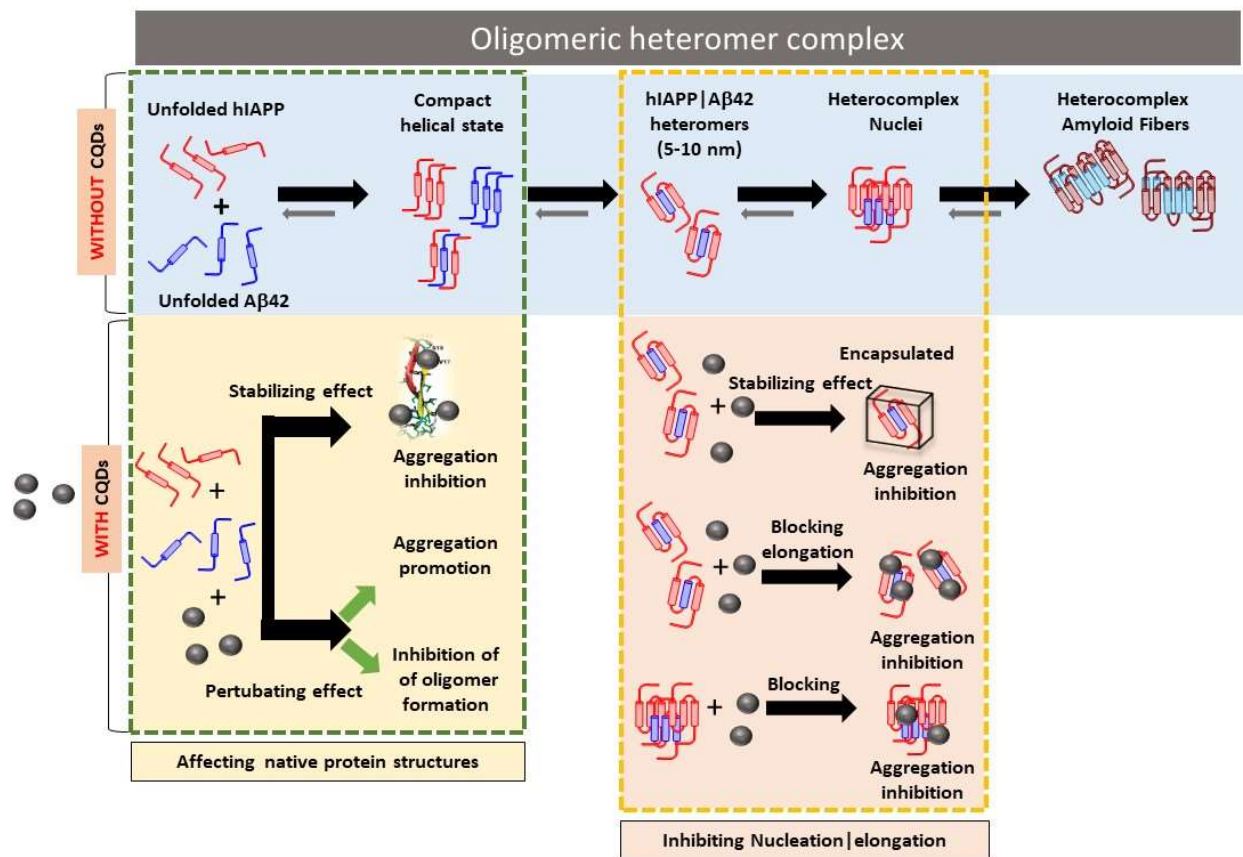


Figure 6. Various interaction mechanisms of hIAPP aggregation in the presence of CQDs and other proteins, notable A β 42.

Other factors, such as surface modification and size effect, play a critical role but their influence on hIAPP fibrillation inhibition is not directly drawn still. In the case of amino acid tyrosine-derived CQDs,⁶² the pronounced inhibitory effect is related to the display of ubiquitous aromatic residues on the CQDs's surface, mimicking the anti-fibril and anti-toxic activity of natural polyphenolic. Amphiphilic CQDs, as proposed by the group of Leblanc, inhibited the overexpression of hIAPP and A β 42.⁵⁹ The CQDs, synthesized by an ultrasonication-mediated methodology using citric acid and *o*-phenylenediamine as precursors, had an average diameter size of 3 nm with pending primary amine and carboxyl groups. These particles were able to cross the blood-brain-barrier of 5-day old wild-type zebrafish by passive diffusion due to the amphiphilicity of the particles, and enter cells to inhibit the overexpression of hIAPP and β -amyloid. While the inhibition of human insulin fibrillation was the focus of the work by Yang et al.⁶⁰ by using CQDs, derived from polymerization and carbonization of sucrose and oleic acid, Koppel et al. investigated the mitigation of CQDs on hIAPP amyloses, including the catalytic effect of lipopolysaccharide (LPS) on IAPP.⁶¹ CQDs, produced using Victorian brown coal under oxidation conditions, resulted in particles of about 2 nm in size. Reduction in β -sheet content occurred for IAPP in the presence of CQDs and change in morphology.

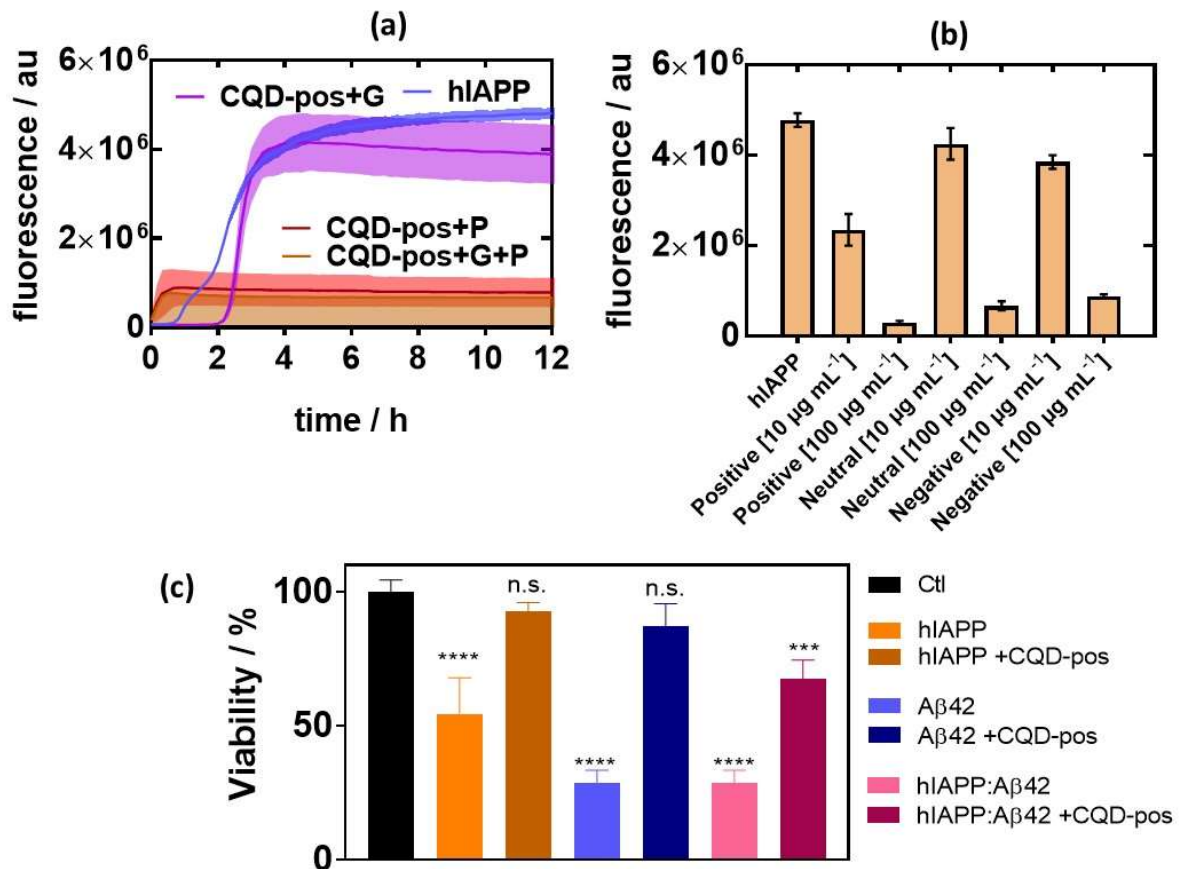


Figure 7: Carbon quantum dots for hIAPP aggregation inhibition: (a) Effect of glucose (G, 11 mM) and palmitate (P, 500 µM) and a mixture of both on hIAPP fibrillation (20 µM) in the presence of 10 µg mL⁻¹ CQD-pos. (b) Comparison of ThT fluorescence intensities after 12 h for hIAPP (control) and in the presence of glucose (11 mM) and palmitate (0.5 mM) and CQDs of different charges at 10 and 100 µg mL⁻¹. (c) Viability of INS-1 cells treated with fresh and aggregated (6 h, 37°C) hIAPP (20 µM), Aβ42 (20 µM) and hIAPP:Aβ42 mixture (1:1) in the presence of glucose (11 mM) and palmitate (0.5 mM) with and without 100 µg mL⁻¹ CQDs-pos. The results are expressed as the mean ± SEM of at least 3 independent samples for each group. *p<0.123 (n.s); **p<0.033; ***p<0.0021; ****p<0.0002, one-way ANOVA test against control.

While several approaches have been explored to reduce aggregation-induced hIAPP cytotoxicity,⁴⁴ rarely diabetic conditions i.e. high glucose and fatty acid levels been considered in these studies. hIAPP aggregation occurs however only when islets are exposed to diabetes environment.⁶⁶ Our team provided lately valuable data to this discussion by investigating the effect of different carbon nanocolloids on hIAPP aggregation under diabetic conditions.⁴ Three types of CDQs were prepared using the microwave-assisted hydrothermal method: (i) positively charged CQD-pos ($\zeta=+32.5$ mV)³ using glucosamine as a carbon precursor with ethylenediamine as a passivating agent, (ii) neutral CQD-neut via mixing glucose and hydrofluoric acid to catalyze dehydration

reaction,⁵⁸ (iii) negatively charged CQD-neg ($\zeta=-24.1\pm 1.6$ mV) using glucosamine as a precursor and β -alanine as a surface passivating agent.⁴ All particles were of spherical shape with an average particle size determined by TEM of 16.8 ± 6.7 nm (CQD-pos), 22.3 ± 5.7 nm (CQD-neut) and 20.8 ± 6.6 nm (CQD-neg). CQD-pos of $10 \mu\text{g mL}^{-1}$ slowed and reduced the amplitude of hIAPP fibrillation (**Figure 7a**) at high glucose levels (11 mM), but showed almost complete aggregation inhibition in the presence of a fatty acid such as palmitate. The positively charged CQDs are more effective than other CQDs structures (**Figure 7b**) under diabetic conditions, indicating a priori that the inhibitory effect occurs through electrostatic repulsion between CQD-pos and hIAPP, as reported by others using PEI-modified Ag NPs with a zeta potential of +40.0 mV.⁵⁵ When added to INS-1 cells, single oligomers are toxic for β -cells in the presence of glucose and palmitate. The cytotoxicity was more prominent for the A β 42 oligomers and the heterodimer hIAPP:A β 42 complex when compared to hIAPP alone. This confirms that heterocomplex formation between hIAPP and A β 42 is of high cellular toxicity under diabetic conditions. When A β 42 and the heterodimer hIAPP:A β 42 aggregation was inhibited by CQD-pos, the toxicity could be efficiently alleviated (**Figure 7c**). Motivated by these findings, the possibility to disaggregate formed fibrils was assessed in addition. Rapid decrease in ThT fluorescence following the addition of CQD-pos (**Figure 8a**) in a concentration-dependent manner was observed. The ability of CQDs to disintegrate hIAPP aggregates was further confirmed by atomic force microscopy (AFM) of the fibers before and after disaggregation (**Figure 8b**) where instead of hIAPP fibrils of diameter ≈ 100 nm, small aggregates were observed.

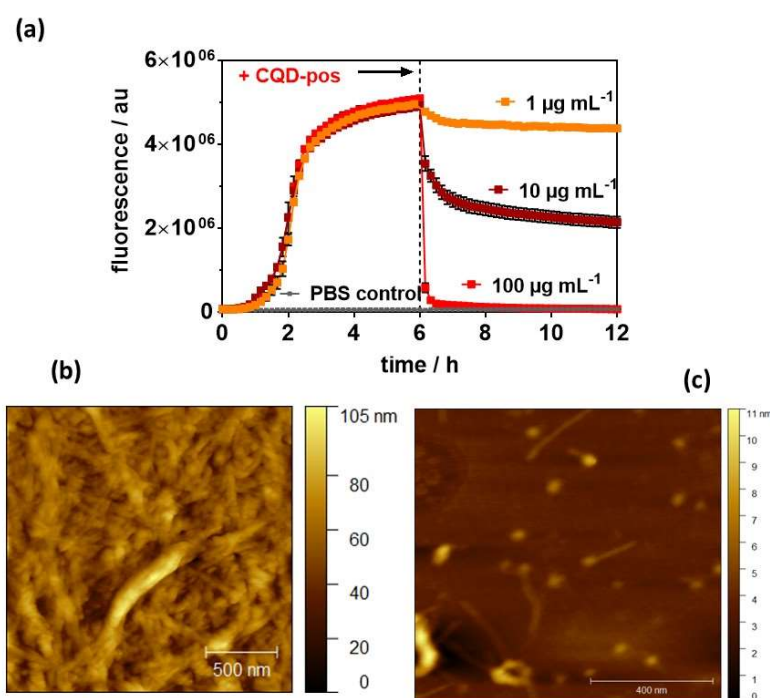


Figure 8. Disaggregation efficiency of CQDs on fibrillated hIAPP: (a) Change in ThT fluorescence intensity after the addition of different concentrations of CQD-pos. (b) AFM image of hIAPP aggregates. (c) AFM image of hIAPP aggregates after addition of CQD-pos ($100 \mu\text{g mL}^{-1}$) for 6 h.

CONCLUSION and PERSPECTIVES

Over the last years, despite the intensive research investigations for understanding the molecular and cellular basis of the loss of functional beta-cell mass in T2D, the mechanisms underlying the generation of islet amyloids are not fully elucidated. Before its secretion into the bloodstream, hIAPP is stored inside β -cell granules at millimolar concentrations for hours without formation of toxic aggregates in healthy individual. The low pH of 5.5 in the β -cell granules is believed to be responsible for the increase in hIAPP aggregation lag time and hIAPP stabilization in its monomeric form. This is due to the electrostatic repulsion between hIAPP with protonated histidine 18 that inhibits self-association of hIAPP at low pH. Only under diabetic conditions, corresponding to a glucose levels $> 10 \text{ mM}$ and high saturated fatty acid levels of $> 0.5 \text{ mM}$, hIAPP oligomerization and fibrillation are initiated. To counteract the misfolding and aggregation of hIAPP, which result in β -cell dismiss, nanoparticle-hIAPP interactions have proven to be highly effective. Even though, a clinically-viable hIAPP inhibitor is still lacking, the question was brought by Wang and Relight as early as 2014 if A β inhibitors are effective against hIAPP.⁶⁷ Their study clearly underlined no activity of several A β inhibitors on hIAPP aggregation, and might have opened the field of nanomedicine for nanoparticle-based inhibitor screening. The nanoparticles-hIAPP formation is a multifactorial process and not only depends on the characteristics of the nanostructures, but also on the medium. As the hydrophobicity of the hIAPP amyloid protein is increasing and evolving from monomer to protofibrils and then decreases as fibrils are formed, encapsulation and stabilization of monomeric hIAPP in and onto hydrophobic nanostructures have been proposed. With the advancement in the field and in the understanding of the importance of considering diabetic conditions when performing such screening methods, CQDs formed from different synthetic precursors have proven strong potential as efficient hIAPP inhibitors as well as therapeutic agents counteracting the formation of hIAPP aggregates. The in-depth physiochemical characterization of the inhibitory properties have now to be implemented in *in vivo* experiments. This is possible as exemplified by the recent findings reporting that CQDs efficiently suppress the fibrillation and disaggregate of A β 42 fibrils in mice model of Alzheimer's disease.⁶⁸ Therefore, it is possible to inject the CQDs in transgenic humanized mice model overexpressing

hIAPP in β -cells.⁶⁹⁻⁷⁰ In this model, islet amyloids concomitantly develop with hyperglycemia when mice are fed with a high fat diet.⁷⁰ The efficiency of CQDs for reducing amyloids and improving insulin secretion and glycemia can therefore be investigated.

While the use of these nanostructures might open new ways for T2D treatment, nanomedicine in the form of therapeutic nanoparticle formulations is a relatively new developing science with a range of issues which have to be addressed in their commercial development including ethical, biological and cost-related issues. Some of the proposed strategies are only applicable when the toxicity of the nanocolloids on human health and the environment are under control. There is a compelling need to address the safety issue and toxicity in parallel to efficacy of nanocarbon materials not only *in vitro* and *in vivo* but also at the clinical trial scale. This will allow evaluation if the engineered nanocarbon colloids meet the expected hopes in a medical setting. *In vitro* assays assessing the effects of the nanostructure on cellular activity (e.g. mitochondrial function, reactive oxidative stress, membrane stress) have revealed that the cytotoxicity of nanocarbon materials are dependent on numerous factors such as morphology, concentration, and exposure time. These variations can be attributed to variable aspects such as the differing internalization mechanisms between cell types. Different to carbon nanotubes, with generally higher cytotoxic effects compared to other structures within the carbon family,⁷¹ CQDs seem to be favorable for biological applications due to their good water dispersibility and limited cytotoxicity *in vitro*, as shown in the account work and related literatures.³⁻⁴ *In vivo* toxicity is the next logical step following cellular level studies to be implemented. CQDs injected in female BALB/C mice were studied over three months.⁷² The 20 mg/kg dose used did not show any toxicity signs, with no weight loss or mortality. Liver function markers and hematology markers indicated normal results relative to the control, with accumulation in the reticuloendothelial system (RES) organs, while early accumulation was seen in the kidney. Comparable results were recently observed by us.⁷³ More studies on rats and primates are still required to get a full and better understanding of their effect on human health and can lead to a concrete pathway for the implementation of the next antidiabetic class that targets fibrillation and aggregation of human islet amyloid polypeptide in type 2 diabetes?

The authors declare no competing financial interest

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