Design and synthesis of helicoidal constrained peptides to inhibit the amyloid aggregation by stabilizing the monomer

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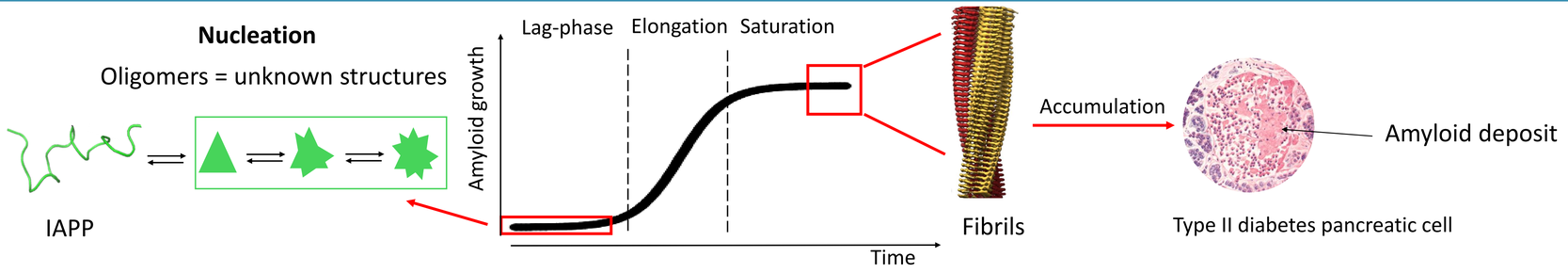
Introduction

Islet Amyloid Polypeptide (IAPP)
- Peptide hormone of 37 residues
- Secreted by β-pancreatic cells into random coil secondary structure
- Cytotoxic oligomers

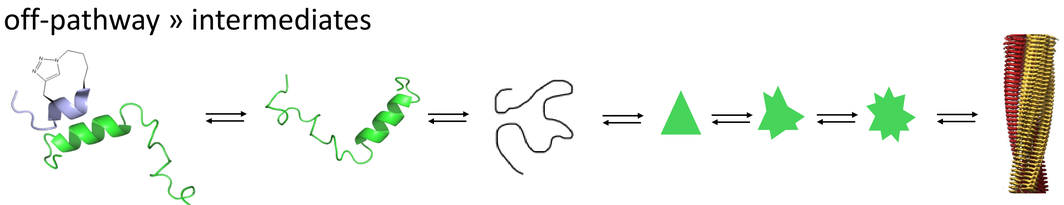


- Major component of amyloid deposits
- Amyloid deposits linked to organ dysfunction

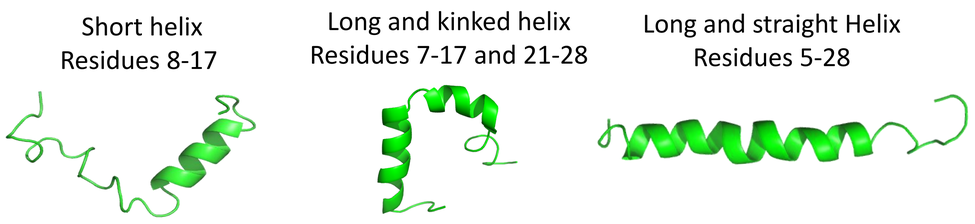
Problem : aggregation mechanism not well understood

Objectives and rational design

Hypothesis: Stabilizing the monomer by targeting the helicoidal structure produces « off-pathway » intermediates



- Helicoidal conformation of IAPP

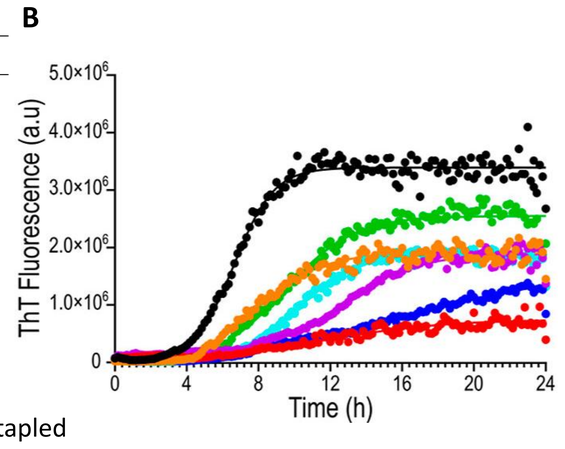


IAPP full length analogues were developed and showed inhibition of the aggregation processus

A

Peptide	Sequence
IAPP	KCNTA TCA**T**Q RL**A**NF L**V**HS**S** **N**NF**G**AIL**S**ST NVGSNTY
c[Pra^{9},AzK^{13}]IAPP	KCNTA TCAPraQ RLAzKNF LVHSS NNFGAILSST NVGSNTY
c[Pra^{13},AzK^{17}]IAPP	KCNTA TCATQ RLPraNF LAzKHSS NNFGAILSST NVGSNTY
c[Pra^{17},AzK^{21}]IAPP	KCNTA TCATQ RLANF LPraHSS AzKNFGAILSST NVGSNTY
c[Pra^{20},AzK^{24}]IAPP	KCNTA TCATQ RLANF LVHSPra NNFAzKAILSST NVGSNTY
c[Pra^{24},AzK^{28}]IAPP	KCNTA TCATQ RLANF LVHSS NNFPraAILAzKST NVGSNTY
bis c[Pra^{13},AzK^{17}] [Pra^{24},AzK^{28}]IAPP	KCNTA TCATQ RLPraNFLAzKHSS NNFPraAILAzKST NVGSNTY

B



(A) Design of an helicoidal library derivatives of IAPP intramolecularly stapled
(B) Determination of their ability to inhibit IAPP aggregation

Objective :
Aim at identifying determine the smallest portion necessary to maintain amyloid inhibition of the helicoidal IAPP segment

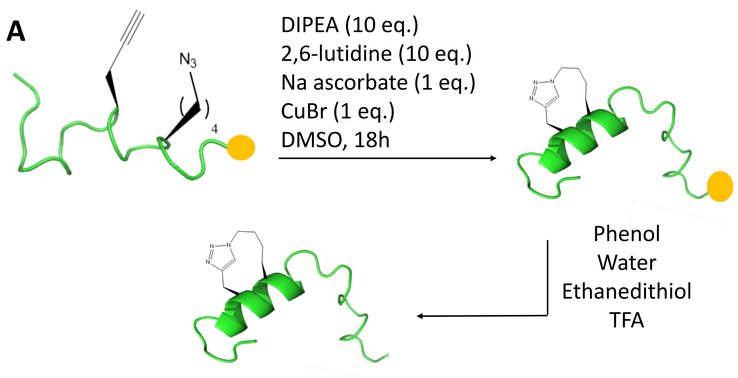
Methodology and results

1. Conception of the truncated stapled IAPP derivatives

Peptide	Sequence
IAPP	KCNTA TCATQ RL**A**NF L**V**HS**S** NNF**G**AILSST NVGSNTY
c[Pra^{20},AzK^{24}]IAPP(18-28)	HSPraNNFAzKAILS
c[Pra^{13},AzK^{17}]IAPP(11-21)	RLPraNFLAzKHSSN
c[Pra^{20},AzK^{24}]IAPP(11-28)	RLANFLVHSPraNNFAzKAILS
c[Pra^{13},AzK^{17}]IAPP(11-28)	RLPraNFLAzKHSSNNFGAILS
c[Pra^{20},AzK^{24}]IAPP(18-37)	HSPraNNFAzKAILSST NVGSNTY
c[Pra^{13},AzK^{17}]IAPP	KCNTA TCATQ RLPraNFLAzKHSSNNFGAILSST NVGSNTY
c[Pra^{20},AzK^{24}]IAPP	KCNTA TCATQ RLANFLVHSPraNNFAzKAILSST NVGSNTY

2. Synthesis and side-chain to side-chain cyclisation

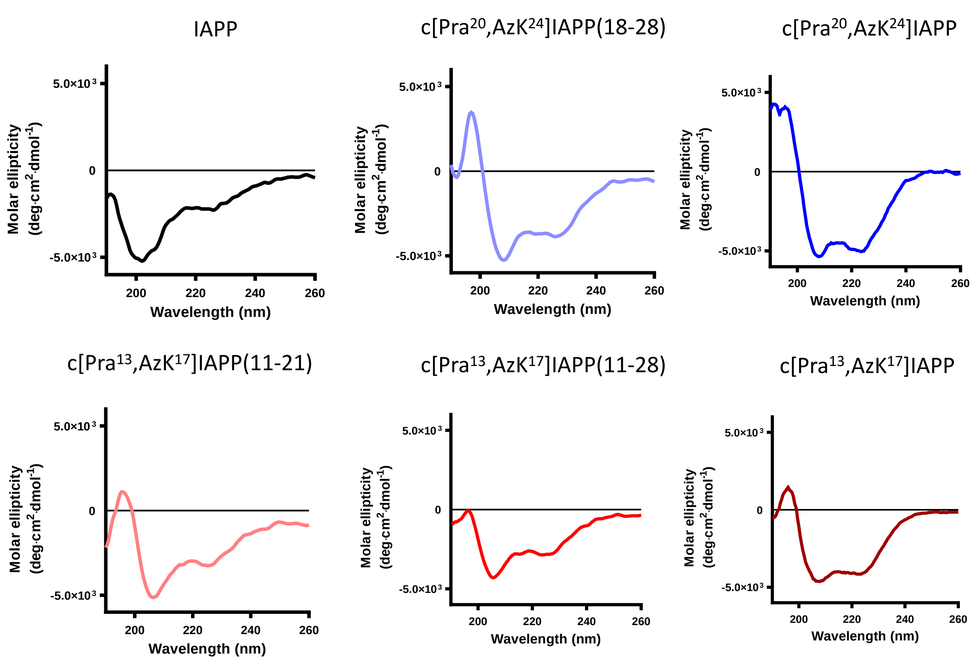
Staple formed between an intramolecular azide and an alkyne



(A) Formation of the intramolecular triazole with click reaction
(B) Validation of the disappearance of the azide group with FTIR between before (red curve) and after (blue curve) the click reaction

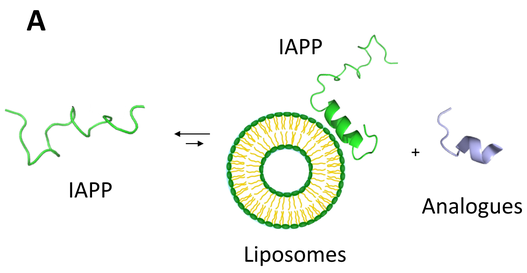
3. Secondary structure validation by circular dichroism

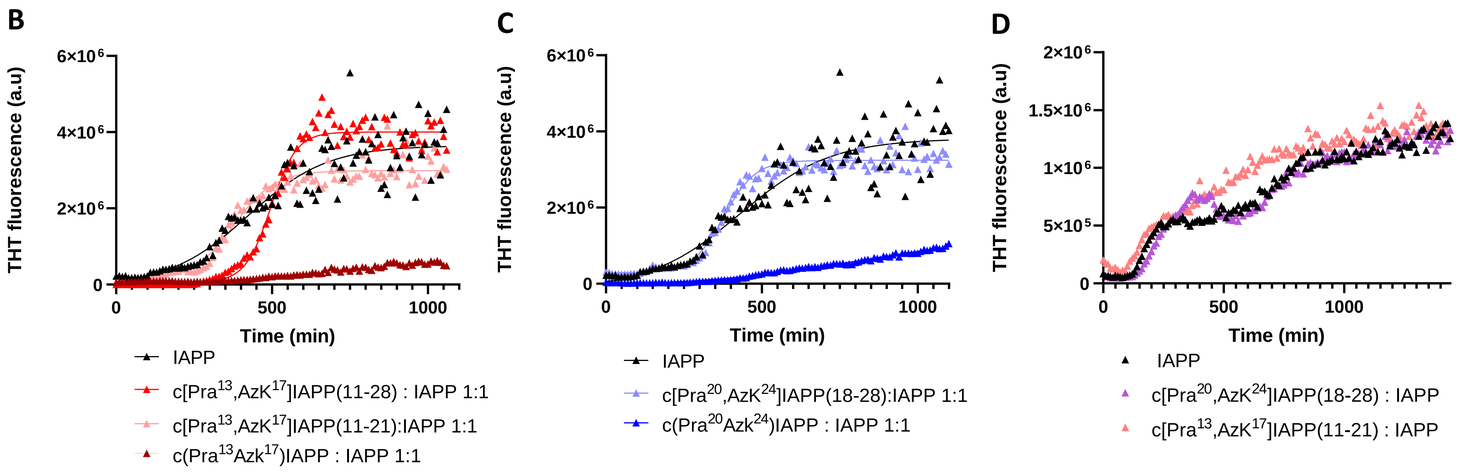
Determination of the secondary structure with circular dichroism showing α-helix conformations at t=0 min except for IAPP, which is in random coil



4. Kinetics of aggregation

Fibril formation can be followed using the fluorescent probe Thioflavin T (ThT)
(A) in presence of liposomes, IAPP naturally adopts an helicoidal conformation
(B) analogues are incubated at 1:1 ratio with IAPP for the 13-17 stapled librairies
(C) analogues are incubated at 1:1 ratio with IAPP for the 20-24 stapled librairies
(D) Inhibition test with IAPP, stapled analogues and in presence of LUVs





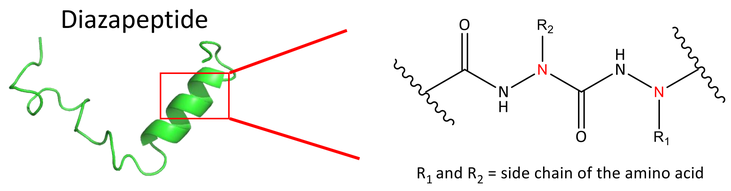
Conclusion and perspectives

- Full length of IAPP is needed to inhibit the aggregation of IAPP by means of helical stabilization.

Hypothesis : the substitution of the amino acids in the sequence disturbs the recognition phenomenon.

- As an alternative, diazapeptide will be tested as potential inhibitors.
- Understanding the aggregation mechanism of IAPP can give clues to understand other amyloid aggregation processes.

Diazapeptide



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