Aromatic-aromatic interactions as an intrinsic modulator of amyloid formation

https://doi.org/10.17952/37EPS.2024.P2290

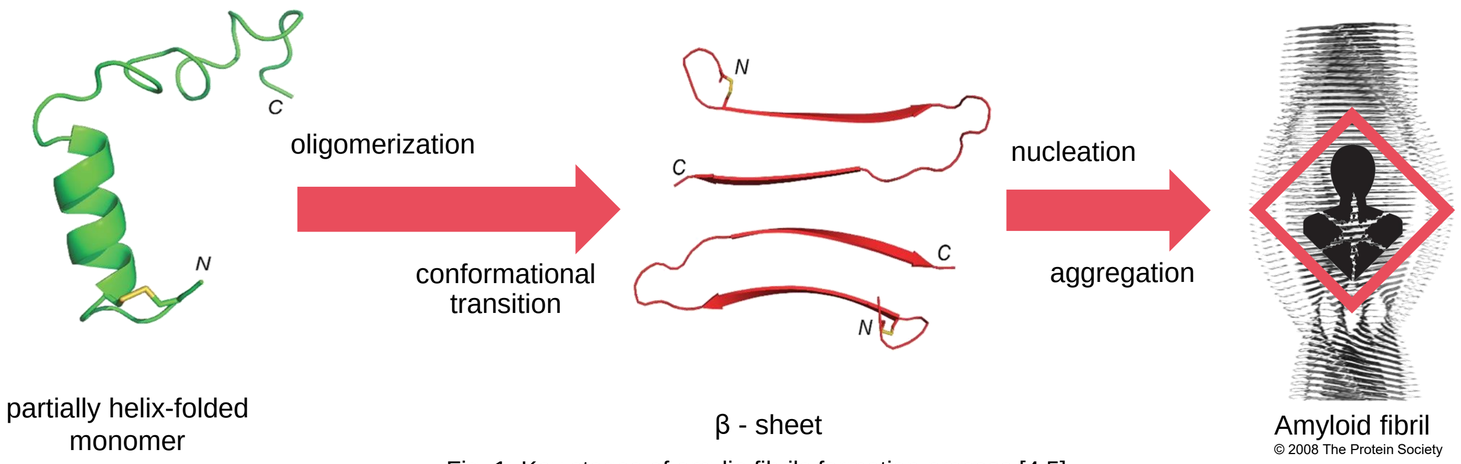
Artem Pavlov, Beate Koksch

Freie Universität Berlin, Arnimallee 20, 14195 Berlin

artem.pavlov@fu-berlin.de

Introduction

Amylin (hIAPP) sequence: H_2N–KCNTATCATQ RLANFLVHSS NNFGAILSST NVGSNTY–C(=O)–NH_2



partially helix-folded monomer

β - sheet

Fig. 1: Key stages of amylin fibrils formation process [4,5].

- Amyloid fibrils formed by amylin play a crucial role in the development of type 2 diabetes [1].
- The formation of these structures depends on the peptide's amino acid sequence with its internal propensities to the particular secondary structures and specific interactions involved in the process [2].
- Aromatic-aromatic interactions contribution remains debated [3].

Aim and Methods

This study examines how substituting aromatic residues in the amylin peptide influences amyloid fibril formation kinetics. Prior studies involving fluorinated Phe and Leu highlighted the importance of hydrophobics and secondary structure propensity. By selectively altering these contributions, the research aims to isolate and analyze the role of aromatic-aromatic interactions in amyloidogenesis, while maintaining other structural aspects of the peptide.

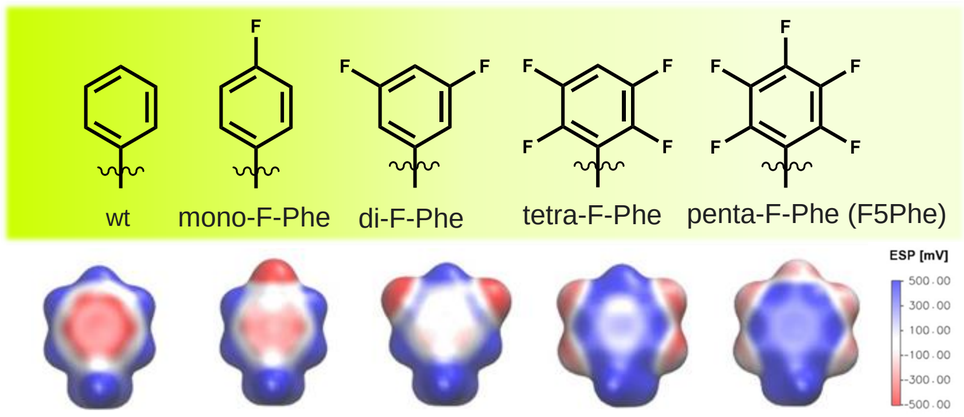


Fig. 2: Aromatic ring fragment of Phe variants of our **first set** amylin analogues and its electrostatic potential maps [6].

Position with penta-F-Phe	15	23	37
peptide 15F	✓		
peptide 23F		✓	
peptide 37F			✓
peptide 15/23F	✓	✓	
peptide 23/37F		✓	✓
peptide 15/37F	✓		✓

Tables of the peptide **second set** with individual and pair substitution of Phe/Tyr with pentafluoro-Phe.

Two sets of full-length amylin variants were obtained: first one – with different fluoro-Phe analogues inside the hydrophobic core of the sequence (NFGAIL); second one – contains individual and pair substitution of Phe/Tyr with pentafluoro-Phe.

State of the art

Short-fragment model studies stated:

- ↑Hydrophobicity of Phe analogues → ↑rate of fibril formation.
- Amyloidogenesis independence of specific π-stacking geometries, and by extension, aromaticity.[6]

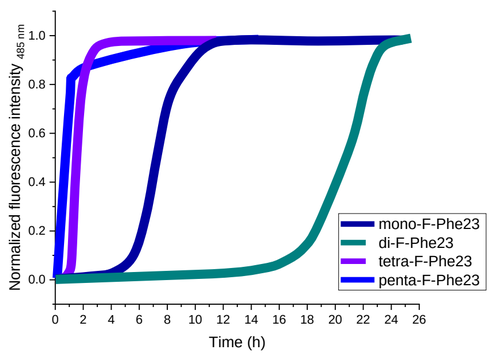


Fig. 3: Thioflavin T assay of fibrils formation kinetics of NFGAIL and its Phe_{23} variants [6]. (peptide conc. 3/4 mmol, pH 7.4)

Phe/Tyr → Leu amylin mutants model studies:

- Shift from discussing aromatic-aromatic interactions
- Highlight the importance of α-helix and β-sheet propensities of amino acids, which influence oligomerization and β-sheet formation as key steps in the fibril formation process [3].

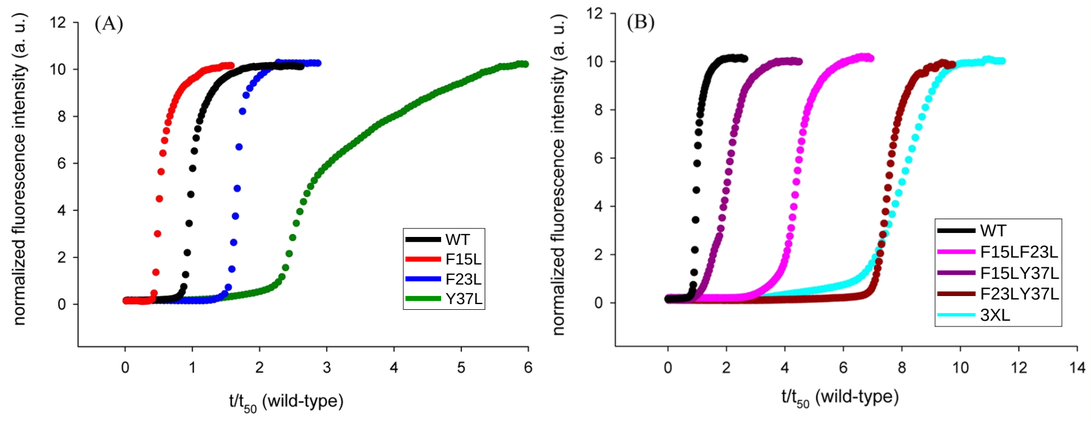


Fig. 4: Thioflavin T assay of fibril formation kinetics of amylin Phe/Tyr → Leu mutants [3]. (peptide conc. 16 μmol, pH 7.4)

Results

Full-length model probing the hydrophobic core:

- ↑ Aromatic-aromatic interactions → ↓rate of fibril formation.
- Amyloidogenesis independence of hydrophobicity changes at the Phe_{23}.

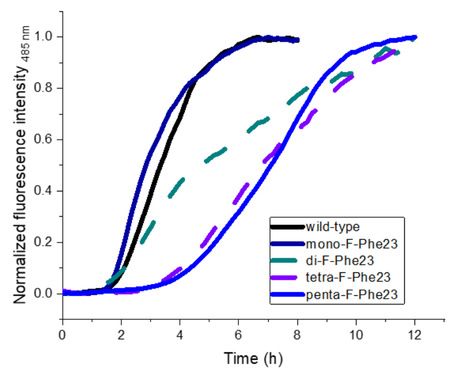


Fig. 5: Thioflavin T assay of fibrils formation kinetics of hIAPP and its Phe_{23} variants. (peptide conc. 15 μmol, pH 7.4)

Phe/Tyr → F5Phe amylin mutants model studies:

- Challenge the α-helix propensity of AA_{15} as a key factor in amyloidogenesis enhancement.
- Reveal complex interplay where certain aromatic interactions enhance, disrupt, or compensate for each other to amyloidogenesis.

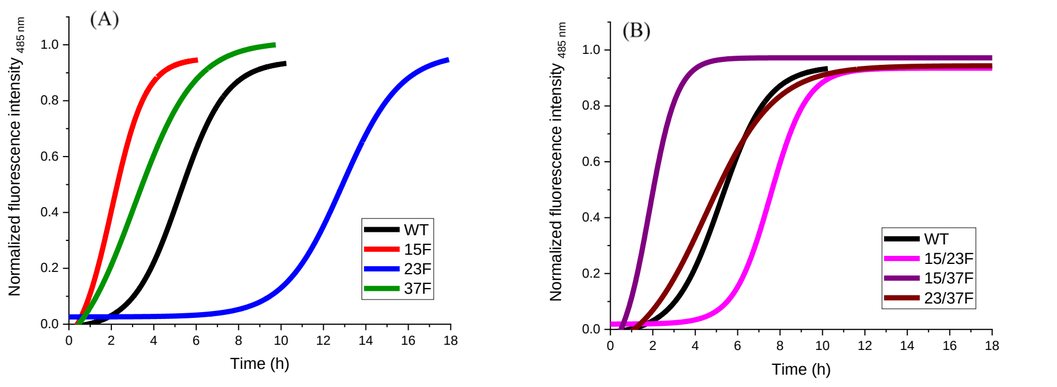


Fig.6 : Thioflavin T assay of fibril formation kinetics of amylin Phe/Tyr → F5Phe analogues (peptide conc. 15 μmol, pH 7.4)

Conclusions and Outlook

- Our study demonstrated distinct effects of aromatic-aromatic interactions on amyloidogenesis, highlighting its critical role.
- Substitution of Phe/Tyr with pentafluoro-Phe, which has reversed electrostatic potential of the ring, favors π-stacking with natural aromatic rings. In case of double substitution experiments, it emphasizes the divergent contribution of aromatic-aromatic interactions to amyloidogenesis Fig.6 (B).
- Based on the obtained data and the comparative analysis of previous studies we hypothesize new features of **molecular mechanism** specifically: Phe_{23} with Tyr_{37} may engage in specific π-stacking interactions leading to amyloid formation, meanwhile, Phe_{15} acts as a stabilizer, potentially preventing this process. This hypothesis opens up new avenues for further investigation in future studies.

References

[1] Clark, A. et al., The Lancet, **1987**, 330, 231-234
[2] Gai Liu, et al., J. Am. Chem. Soc., **2010** 132 (51), 18223-18232
[3] Ling-Hsien Tu, D.P. Raleigh, Biochemistry, **2013**, 52, 2, 333–342
[4] D. C Rodriguez. et al., eLife, **2017**, 6:e31226
[5] J.J. Wiltzius, et al. Protein Sci, **2008**, 17 (9), 1467-1474
[6] S. Chowdhary, et al., ChemBioChem, **2020**, 21, 3544-3554

Acknowledgments: We gratefully acknowledge financial support by the DFG 1114 and assistance of DFG CoreFacility BioSupraMol, especially the director of the NMR department Dr. Mario Schubert.