2015-2016

Internship proposal (Master 2 or final internship) at the LMGP

# Effect of (LK)nL peptides on Insulin aggregation kinetics

## Context

Protein amyloid aggregates are involved in many devastating human diseases such as Alzheimer's or diabetes. Amyloidal aggregation of proteins starts when aggregative nuclei form, which then promote rapid local accumulation of proteins. This accumulation leads to the formation of fibers resulting from a beta-sheet stacking of successive proteins. For insulin, amyloid aggregation is triggered by the presence of hydrophobic material surfaces, which efficiently stabilize aggregation nuclei and therefore promote aggregation. The formation of surface-bound aggregation nuclei can be accelerated using small peptides, like (LK)nL peptides of varying length, which adsorb in beta-sheet conformation on hydrophobic surfaces[1-2]. Their cooperative and stable surface-adsorption at largely sub-stoichiometric concentrations compared to insulin, drive the formation of peptide/protein clusters, on which insulin incorporation is facilitated and hence fiber formation accelerated. On the other hand, (LK)nL peptides, at slightly higher albeit still sub-stoichiometric concentrations, can also delay insulin aggregation. This inhibitory effect does not rely on surface-adsorbed peptides but rather on an excess of peptides in solution.

We aim at understanding the molecular interactions between the peptides and insulin, involved in both the pro- and anti-aggregative properties of the same peptides. Fluorescently labeled versions of the $(LK)_5L$ peptide and insulin will be used as well as chemical cross-linking and mass spectrometry to determine peptide/protein contacts and analyze the underlying molecular mechanisms.

## Project

A TAMRA-labeled $(LK)_5L$ peptide, exhibiting the same dual accelerating and inhibiting effects on insulin aggregation as its non-labeled parent peptide, has been obtained. It will be used to image the formation of surface-bound peptide patches and their evolution into aggregation nuclei. Its stable surface-adsorption[2] will allow following these phenomena as a function of incubation time along the maturation of nuclei.

The TAMRA-labeled $(LK)_5L$ peptide and FITC-insulin will be used to probe peptide-insulin interactions using fluorescence energy transfer (FRET). These interactions will be probed for the acceleratory and inhibitory mode of the (LK)nL peptides which are likely to reveal different underlying mechanisms. Competition studies for their integration into active sites on surface-bound aggregation nuclei will be performed using peptide and insulin.

The (LK)nL peptides will also be used in a chemical crosslinking approach, aiming at isolating different peptide/peptide and peptide/protein complexes during the aggregation of inulin. We will particularly focus on complexes formed at different stages during the maturation of surface-bound aggregation nuclei. The purification and analysis of these complexes by mass spectrometry should allow to reveal the hitherto unknown stoechiometry and positioning of the peptides/proteins in these surface-bound complexes.

## Competences

The student should have an educational background in chemistry or biochemistry, in particular protein biochemistry. Experience in fluorescence measurements or mass spectrometry are a plus. Good oral and written English is an asset.

## Time and place

Feb-July 2016 at the LMGP in Grenoble, France

Web site of the lab: http://www.lmgp.grenoble-inp.fr/

**PhD possible** : Yes, provided that funding can be recruited

**Internship stipend** : 554€ per month

## Contact

Marianne Weidenhaupt Phelma Minatec LMGP office M1-07; marianne.weidenhaupt@grenoble-inp.fr

## References

[1]Nault L, Vendrely C, Brechet Y, Bruckert F and Weidenhaupt M. Peptides that Form Beta-Sheets on Hydrophobic Surfaces Accelerate Surface-Induced Insulin Amyloidal Aggregation. 2013, *FEBS Lett* **587**, 1281.

[2]Chouchane K, Vendrely C, Amari M, Moreaux K, Bruckert F and Weidenhaupt M. Dual effect of (LK)nL Peptides on the Onset of Insulin Amyloid Fiber Formation at Hydrophobic Surfaces. 2015 J Phys Chem B **119**, 10543.