2016-2017

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

Protein Stability at Interfaces

Context

One of the prime objectives in the development of protein therapeutics, like monoclonal antibodies, is to ensure their stability. During the expression, purification, storage and administration, therapeutic proteins are exposed to varying material surfaces and interfaces, all of which can potentially lead to protein adsorption and sometimes to aggregation. It is well documented that interfaces between air and liquid or air-liquid and material surfaces are involved in protein aggregation. Our team has recently demonstrated, using a wetting-dewetting experiment in a microchannel, that insulin amyloid aggregates form and accumulate at a dynamic triple air-liquid-solid interface (Figure 1).

Protein aggregation at interfaces is triggered by enhanced local concentrations and concomittant stresses, like for example shear stress due to agitation or partial dehydration due to varying exposure to air. These phenomena favour intermolecular interactions which lead to the formation of aggregation nuclei at the very site of the interface. Once established, these nuclei then trigger the rapid accumulation of protein aggregates.

While the accumulation of aggregates is sufficiently well characterized, the initial steps of aggregation nuclei formation at interfaces remain more elusive.

We propose to visualize and characterize the onset of protein aggregation at interfaces combining Surface Enhanced Ellipsometry Contrast (SEEC) imaging with fluorescence microscopy. Moreover we have identified a set of small peptides that adsorb on hydrophobic surfaces and enhance nuclei formation locally at the solid-liquid interface. These tools enable us to precisely control the location of nuclei aggregation and to follow their formation and accumulation *in situ* with time.

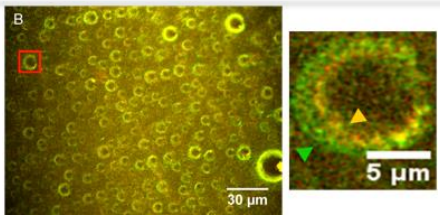

Fig. 1: Accumulation of insulin amyloid aggregates as concentric rings around a droplet with time (ThT fluorescence)

Project

SEEC uses engineered glass surfaces that allow to precisely control the surface reflection properties and therefore to optimize the contrast of nanometric objects deposited on them. It can be used in liquid and in dry conditions (air) and is ideally suited to study protein adsorption at surfaces. Thus aggregation nuclei, typically containing several protein monomers, can be visualized using SEEC under the condition that the position of the aggregation nuclei on the surface is known.

Using surface-adsorbed peptides, that are known to trigger nuclei formation locally, we will target nuclei formation to specific sites on the surface and follow their formation in time using SEEC. Once nuclei trigger the accumulation of bigger aggregates, these can be visualized using conformation-sensitive dyes, like Thioflavin T, a marker of amyloid aggregates. These experiments will be performed using insulin as a model protein and a microfluidic setup to create and control the interface between a hydrophobic surface, the protein solution and air.

The expected results will strengthen our knowledge on protein behavior at interfaces and allow for the first time to visualize aggregation nuclei *in situ* at interfaces. This knowledge will be crucial in our mastering of protein stability at interfaces and can serve in the elaboration of improved production protocols and delivery practices of therapeutic preparations.

Competences

The student should have an educational background in physics or physical instrumentation and some basic knowledge in biomolecules. Good oral and written English is an asset.

Time and place

Feb-July 2017 at the LMGP in Grenoble, France (http://www.lmgp.grenoble-inp.fr/)

Internship stipend : 554€ per month

Contact

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