Application for IF-BM32 GM CRG Beam Time

Title of proposal:
Grazing incidence x-ray diffraction study of β-sheet peptide monolayers a the air/water interface.

Co-Proposers: P. Guenoun, C. Chevallard, J. Daillant, A. Mitraki and D. Luzet

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GUENOUN Patrick
LIONS Laboratory - Service de Chimie Moléculaire
CEA/Saclay - Bât. 125
F-91191 Gif-sur-Yvette Cedex – France
E-mail : pguenoun@cea.fr
Phone : +33 (0)1 69 08 74 33
Fax : +33 (0)1 69 08 87 86

CHEVALLARD Corinne
LIONS Laboratory - Service de Chimie Moléculaire
CEA/Saclay - Bât. 125
F-91191 Gif-sur-Yvette Cedex – France
E-mail : chevalla@drecam.saclay.cea.fr
Phone : +33 (0)1 69 08 74 33
Fax : +33 (0)1 69 08 87 86

DAILLANT Jean
LURE - Bât. 209D
Centre Universitaire Paris Sud
BP 34
F-91898 Orsay Cedex – France
E-mail : daillant@lure.u-psud.fr
Phone : +33 (0)1 64 46 82 25
Fax : +33 (0)1 64 46 41 02

MITRAKI Anna
IBS – Laboratoire de Biophysique Moléculaire
41 rue Jules Horowitz
F-38027 Grenoble cedex 1 – France
E-mail : mitraki@ibs.fr
Phone : +33 (0)4 38 78 41 37
Fax : +33 (0)4 38 78 54 94

LUZET Daniel
LIONS Laboratory - Service de Chimie Moléculaire
CEA/Saclay - Bât. 125
F-91191 Gif-sur-Yvette Cedex – France
E-mail : luzet@drecam.cea.fr
Phone : +33 (0)1 69 08 72 60
Fax : +33 (0)1 69 08 87 86

Aims of the experiment and scientific background

Peptide self-assembling properties offer very exciting perspectives for the design of new materials with a predictable and tunable structure along with controlled functionalities [Leiserowitz, Hecht, etc.]. Peptides can actually form highly organized supramolecular architectures, so-called quaternary structures, which depend on the secondary conformation of the brick element (α-helix or β-sheet peptide conformation). As an example, peptides and proteins with a β-sheet conformation in solution can exhibit quaternary structures such as filaments, ribbons, or fibrils [Lashuel et al., 2000].

Understanding the conditions (primary sequence, pH, ionic strength of the surrounding environment, etc.) that favour the emergence of one specific structure is a challenging question, owing to its implications for material science as well as for the study of many biological processes. Thus, amyloid and prion deseases are ascribed to the conformational change of some protein, which makes the latter self-assemble into insoluble quaternary structures [van Raaij et al., 1999]. Organic matrix-mediated biomineralization also implies the self-assemblage of organic macromolecules, leading to the formation of a template for mineral deposition [Mann, 2001]: in mollusc shells, the crystallization of calcium carbonate occurs in contact with a 2D assembly of highly acidic macromolecules (glycoproteins) in a β-sheet conformation.

These glycoproteins partially exhibit an alternate hydrophilic/hydrophobic amino-acid sequence and recent progress in the design of *de novo* peptides has clearly evidenced the link between this amino-acid primary sequence and the peptide secondary structure. Hecht and coll. have designed *de novo* peptides with a strict periodic alternance of hydrophilic and hydrophobic amino-acid residues, which generically develop a β-sheet conformation in solution [Xiong et al., 1995] and further evolve toward cross-β amyloid fibrils, in which β-strands run perpendicularly to the fibril axis [West et al., 1999].

Two-dimensional self-assemblies of such peptides have also been considered recently [Xu et al, 2001; Rapaport et al., 2000 & 2002; Bekele et al., 1999]. Various alternate sequences of hydrophilic/hydrophobic natural or non-natural amino-acids were shown to exhibit an anti-parallel β-sheet structure when deposited at the air-water interface (hydrophobic residues towards air, and hydrophilic ones towards water). These amphiphilic β-sheets further assemble via hydrogen bonding into a crystalline monolayer. This assembly could be used as a scaffold for CdS deposition [Bekele et al., 1999].Early studies of peptide monolayer at an air/water interface employed FTIR spectroscopy and circular dichroism techniques to provide evidence of the β-sheet formation [Bekele et al., 1999; Xu et al, 2001]. However, these methods do not allow to specify the degree of crystallinity of the monolayer. Recent studies [Rapaport et al., 2000 & 2002] have therefore used grazing incidence x-ray diffraction to provide a true structural characterization of the monolayer: crystallinity order (one-dimensional – ribbons – or two-dimensional crystalline order), coherence length of the crystalline domains, type of molecular packing.

In all the aforementioned studies, only strict amphiphilic alternate peptides were investigated though recently, Mitraki and coll. have observed the cross-β amyloid fibril formation for a 41-amino-acid designed peptide corresponding to the shaft sequence of the human adenovirus Ad2 protein [Mitraki et al., 2002] and lacking the strict alternance of hydrophilic/hydrophobic amino-acids. A similar result was obtained with the shorter peptide LSFD (12 amino-acids)

derived from the same protein (unpublished result). FTIR spectroscopy and circular dichroism studies indicated for both peptides a secondary anti-parallel β-sheet conformation in solution.

Our proposal aims at understanding how variations in the alternance of the hydrophilic-hydrophobic sequence perturbs the 2D order of such peptides. We will first study the self-assembling properties of the LSFD peptide at the air/water interface, since this peptide is also a candidate for developping a 2D template for controlled calcium carbonate mineralization. Preliminary FTIR spectroscopy studies strongly suggest an anti-parallel β-sheet structure for this peptide when spread at the air/water interface.

Using grazing incidence x-ray diffraction (GIXD), we wish now to specify in more details the 2D structure of the LSFD monolayer at the air/water interface. Such a study will help to address the following questions: is the difference in hydrophobicity of the constituent amino-acids sufficient to induce a β-sheet two-dimensional ordering at the air/water interface? If so, does the monolayer exhibit some crystalline (1D or 2D) order? What is the molecular packing? Is it possible to induce structural transitions by compressing the monolayer?

A similar and comparative study will be carried out on a 12-amino-acid strictly alternated peptide in order to compare the 2D crystalline orderings and the aforementioned possible structural transitions. The salt content of the subphase as well as the pH value will be varied to determine their influence on the self-assembly for both peptides.

Experimental method

We wish to carry out grazing-incidence x-ray diffraction experiments using the french CRG-IF BM32 beamline at the European Synchrotron Radiation Facility (Grenoble, France). The peptide film will be deposited at the surface of a Langmuir trough. Experiments will be performed at 5°C in order to compare our results to those taken from the literature [Rapaport et al., 2000 & 2002]. The monochromatic x-ray beam will be adjusted to impinge the surface at an incident angle equal to 0.85 α_c, where α_c is the critical angle for total internal reflection. To reduce the scattering by the air above the monolayer, a flux of He gas will be maintained inside the Langmuir trough.
From the q_{xy} position of the Bragg peaks, we will deduce the parameters of the lattice repeat distances of the monolayer. In particular, one expects a Bragg peak at 1.329 $Å^{-1}$ corresponding to a repeat distance of 4.7 Å, characteristic of crystalline anti-parallel β-sheet systems [Lotz et al., 1971]. The full width at half maximum of the Bragg peaks will yield the lateral 2D crystalline coherence length. Finally, the width of the Bragg rod profile along q_z will provide a measure of the film thickness.

The GIXD measurements will be performed on both peptide monolayer (LSFD and 12aa alternated peptide), for 3 different compression states, 3 different values of the subphase ionic content and 3 values of pH. Considering the time-consuming procedures that are the initial beam alignment, the apparatus cleaning and the monolayer equilibration in-between any two experiments with different subphase, we request an experimental time of 15 shifts.

Results expected

The GIXD experiments described above should confirm the possibility of an anti-parallel β-sheet structure for the two peptides when spread at the air/water interface. This study should help us to specify the best conditions (pH, ionic strength) for getting a 2D crystalline β-sheet monolayer with large coherence lengths in the two in-plane directions. These conditions will be further used to process the controlled mineralization of calcium carbonate using the peptide monolayer as a template.

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