Parallel β-sheet assemblies at interfaces

R. Sneer, M. J. Weygand[1], K. Kjaer[1], D. A. Tirrell[2] and H. Rapaport

Department of Biotechnology Engineering, Ben-Gurion University of the Negev, 84105 Beer-Sheva, Israel

[1] Department of Materials Research, Risø National Laboratory, DK-4000 Roskilde, Denmark

[2]Division of Chemical Engineering California Institute of Technology, Pasadena, CA 91125

Polypeptide assemblies may exhibit various topologies that are of interest for nanometer-scale surface patterning and its potential applications. The success in designing such ordered molecular architectures entails control over peptide conformations and intermolecular interactions. At the air water interface peptides composed of alternating hydrophilic and hydrophobic amino acids tend to adopt β-sheet structures[1] yet the repetitive nature of these peptides promotes as well non-specific intermolecular aggregation. The system presented here was designed to favour the formation of parallel β-sheet packing at interfaces. It consists of two amphiphilic peptides, P_A: CH_3CO-Pro-Cys-Phe-Ser-Phe-Lys-Phe-Glu-Pro-NH_2, and peptide P_B, which is identical to P_A but for Glu and Lys reversed in their positions along the strand (Figure 1a). Two cross-strand pair interactions between the oppositely charged Glu and Lys residues were expected to support the hydrogen-bonded parallel arrangement of neighbouring P_A and P_B peptides[2]. These envisaged electrostatic interactions between the complementary charges entail registry of adjacent strands. Hence, Pro residues that previously have been shown to encourage strand juxtaposition at interfaces [1] were placed at the peptide termini.

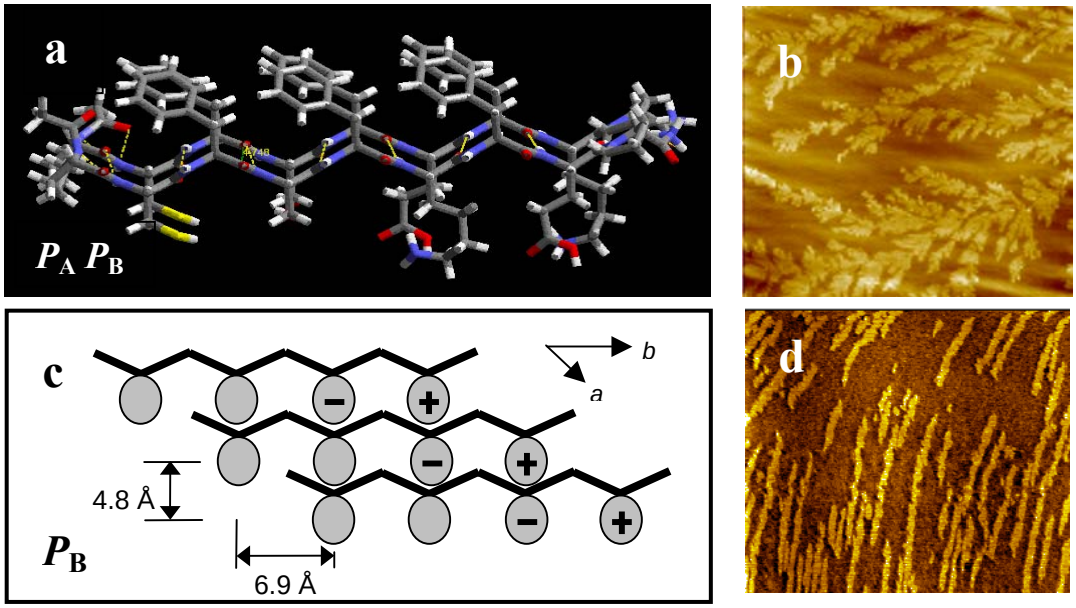

Figure 1: The β-sheet peptides P_A and P_B in parallel orientation **a)** and topography AFM image of the P_AP_B monolayer on mica **b)**. The assembly of the P_B peptide **c)** and its monolayer AFM topography image **d)**.

Grazing incidence X-ray diffraction (GIXD) measurements at the air-water interface indicate marked differences in the structures of the P_A and P_B single-component assemblies. The P_A peptide monolayer yields only a Bragg peak at q_{xy} =1.309 $Å^{-1}$, corresponding to the 4.80 Å spacing characteristic of the interstrand β-sheet structure (Bragg peak not shown). GIXD measurements of the peptide P_B monolayer at the air-water interface show a 4.77 Å spacing Bragg peak at q_{xy} = 1.317 $Å^{-1}$ and an additional peak at q_{xy} = 0.159 $Å^{-1}$ corresponding to 39.5 Å spacing (Figure 2a-b). An estimated length of the nine residues β-strand [1] with free amine and carboxy termini would be ~3.45 × 9 = ~31 Å. The acetyl terminus adds ~3 Å in length, resulting in a ~5 Å difference between this estimate and the observed spacing. The GIXD pattern of peptide P_B could be rationalized by a model presented schematically in Figure 1c. This model incorporates a ~ 6.9 Å offset (along the *b* direction) between neighbouring parallel P_B strands. In this arrangement Glu (negatively charged) may form cross-strand pairs with Lys (positively charged) on the neighbouring strands along the 4.77 Å spacing. This offset is likely to repeat in a ribbon extending along the *a* axis (Figure 1c).

Along the *b* direction strands may be related by a two-fold symmetry axis (normal to the *ab* plane) that apposes C-termini amides at distances favourable for hydrogen bonding. The unit cell describing this structure, a = 8.4, b = 69.5 Å, γ = 145.3º, pseudo *p*2 symmetry with two P_B peptides positioned along the *b* axis, yields a length of ~35 Å per peptide which is in reasonable agreement with the above estimated value. In such a lattice the q_{xy} = 0.159 Å^{-1} Bragg peak indexes (0,1). The absence of the (0,2) peak and an assumed weakening of the (0,1) peak may be rationalized by disorder along *b* axis, due to equal probability of a 6.9 Å offset either along the +*b* or -*b* directions, as confirmed by very preliminary diffraction pattern calculations.

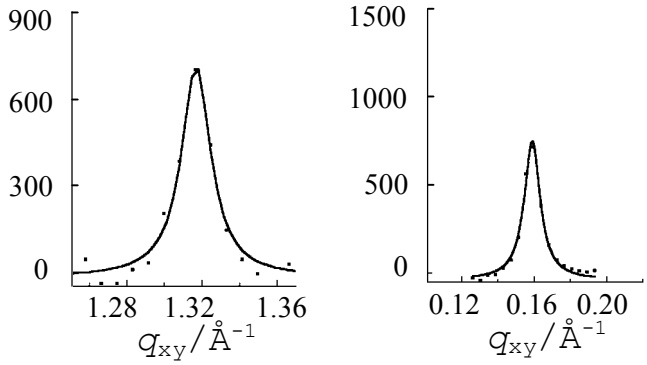

Figure 2: The measured GIXD pattern I(q_{xy}) of peptide P_B. Bragg peaks at 1.317 and 0.159 Å^{-1} correspond to spacings d = 4.77 and 39.50 Å respectively.

GIXD patterns of the P_AP_B film (a 1:1 molar ratio mixture of P_A and P_B) show a peak at q_{xy} = 1.319 Å^{-1} and 4.76 Å spacing (not shown), characteristic as mentioned above, of the interstrand hydrogen bonded spacing. Another peak at q_{xy} = 1.51 Å^{-1} corresponding to 4.16 Å, could be attributed to a subcell generated by the pleated peptide backbones (a = 4.82, b = 6.9 Å, γ = 97.3º) in which the 4.76 Å and the 4.16 Å are attributed to (0,1) and (1,-1) spacings. The absence of a peak corresponding to P_AP_B registry along the *b* direction may be the result of interfering P_B - P_B and P_A - P_A interactions, likely to adopt the 6.9 Å offset, and thus leading to disorder along the *b* direction.

The Cys residues included in the P_A and P_B sequences may allow disulfide bond formation between neighbouring peptides that are juxtaposed in the β-sheet parallel mode. Disulfide bridge formation was induced by injection of iodine into the subphase of a Langmuir peptide monolayer (at 145 Å^2/molecule). Samples of each of the three-peptide monolayers were collected from the air aqueous solution interface and analyzed by MALDI. A distinct peak at 2274.4 m/z corresponding to twice the molecular weight of either P_A or P_B (m = 1142) indicated disulfide bridge formation only in the P_AP_B film thus supporting the model of P_A and P_B strands packed in the parallel arrangement. These peptide films were also transferred from the air-water interface onto freshly cleaved mica slides by the Langmuir-Blodgett (LB) technique and scanned by atomic force microscopy. Images of P_A show two-dimensional domains roughly round-edged, ~ 10 Å in height and of variable sizes (not shown). P_B films display elongated tape-like assemblies ~ 60-130 nm wide, ~ 8 Å in height and up to 4 mm in length (Figure 1d). These shapes reflect the prevailing growth along the direction of cross pair strand electrostatic and interstrand hydrogen bond interactions. The P_AP_B film exhibits fractal-like two-dimensional structure (Figure 1b) that may be the result of the complex assembly behaviours of the two-component mixture, as described above.

References

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[2] R. Sneer, M. J. Weygand, K. Kjaer, D. A. Tirrell, and H. Rapaport, ChemPhysChem 5, 747 (2004).

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