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Assembly of a Vectorially-Oriented Four-Helix Bundle at the Air/Water Interface via Directed Electrostatic Interactions

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Beamline(s): X22B

Introduction: Alpha-helical bundles can provide a molecular scaffolding for binding specific prosthetic groups at desired locations within the structure of synthetic peptides, permitting *de novo* design of building blocks for materials that mimic some of the functions of biological proteins. The solid phase synthetic techniques used to make the peptides limit their length to about 60 amino acids, only long enough for di- α -helical units. The alkane chains that we attach to one end of the di- α -helices help them form monolayers at the air/water interface that are vectorially-oriented and stable under high applied surface pressure, but constrain the insoluble peptides to all have the same orientation with respect to each other (a parallel configuration), and results in liquid-like in-plane ordering of the di- α -helices. The soluble di- α -helices without the hydrocarbon chains form unstable monolayers, but in solution spontaneously associate into four-helix bundles in the anti-parallel orientation. We investigated whether a mixture of soluble and insoluble peptide di- α -helices could result in a stable monolayer of four-helix bundles at the air/water interface.

Methods and Materials: We measured x-ray reflectivity from a series of monolayers using the Liquid Surface Spectrometer at X22B with a Langmuir trough on the sample stage. The monolayers were composed of a phospholipid, DLPE, that acted as a place-holder, and different amounts of two different pairs of soluble/insoluble peptides. The first peptide had an alternating distribution of lys (+ charged) and glu (-) residues on its exterior, while the second peptide had only lys on the exterior of one helix and glu on the exterior of the other helix, promoting a favorable electrostatic interaction between dihelices. Comparison of the electron density distribution, $\rho(z)$, for binary mixtures of DLPE and the insoluble peptide and $\rho(z)$ for ternary mixtures of DLPE, the insoluble peptide and the soluble peptide showed whether or not the soluble component was retained when high surface pressure was applied (figure 1).

Results and Conclusions: The electron density distributions obtained from fitting slab models to the reflectivity data indicate that only in the system with the directed electrostatic interactions, the soluble component is retained in the ternary mixed monolayers at high pressure with an approximately 1:1 stoichiometry (Figure 2, bottom), indicating the formation of 4-helix bundles at the interface.

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References: See our article in the upcoming *Langmuir* special issue on The Biomolecular Interface (in press), and references therein.

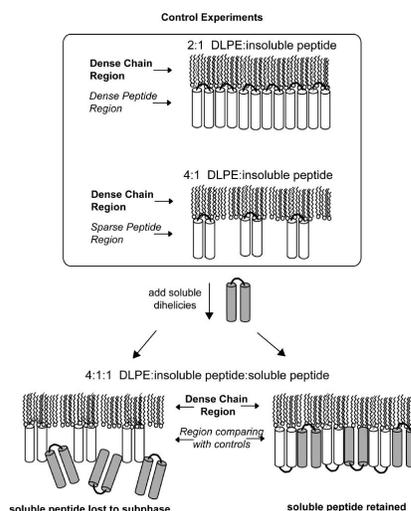


Figure 1: The experimental plan.

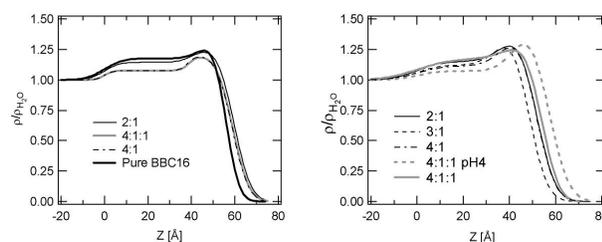


Figure 2: Slab model electron density distributions constructed from best fits to the reflectivity data. In the system without directed electrostatic interactions (left), the electron density of the peptide region ($0 < z < 40$ Å) for the ternary mixture (gray) is as sparse as for the 4:1 binary mixture (dash-dot) – the soluble component is lost. The ternary mixture in the system with directed electrostatic interactions (right) has a dense peptide region at neutral pH (gray), but not at pH 4 (dashed gray) – the soluble component is retained at neutral pH, when both glu and lys are charged.