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### **Assembly of a Vectorially-Oriented Four-Helix Bundle at the Air/Water Interface**

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Beamline(s): X22B

**Introduction:** Alpha-helical bundles can provide a structural framework for binding specific prosthetic groups at selected locations within the structure of an artificial peptide thereby designed to mimic a number of functions exhibited by biological proteins, including vectorial electron transfer. The first family of these artificial peptides was based on amphipathic di-helices (each helix possessing two faces, each composed of either nonpolar or polar residues) which self-assembled in aqueous solution to form four-helix bundles with a polar exterior and nonpolar interior and thought to be of *anti* (or antiparallel) topology[1]. In order to realize any potential device applications of these designed artificial peptides, it is necessary to vectorially orient an ensemble of such peptides, for example at an interface. Thus, the di-helices were rendered amphiphilic via the covalent attachment of a C16 hydrocarbon chain to their amino terminus[2]. Electron density profiles derived from specular X-ray reflectivity showed that these amphiphilic di-helices could be vectorially-oriented at an air-water interface in a Langmuir monolayer at relatively high surface pressures, either in pure form or in binary mixtures with phospholipids, with their helical axes normal to the interface[3]. However, off-specular x-ray reflectivity indicated that these di-helices remained as such at the interface, namely they did not associate to form four-helix bundles[3] possibly because their amphiphilicity would require such bundles to be of the *syn* (or parallel) topology. As a result, we have attempted to form four-helix bundles vectorially-oriented at the air water interface via a 1:1 association of the amphiphilic di-helices and their water-soluble counterparts in 1:4 binary mixtures with a diacyl phospholipid.

**Methods and Materials:** Monolayers were spread in a custom-built Langmuir trough mounted on the sample stage of the Liquid Surface Spectrometer of X22B and x-ray specular reflectivity data were collected as described elsewhere[3].

**Results and Conclusions:** We have employed the electron density profiles derived from X-ray reflectivity to demonstrate that this is indeed possible when the association between di-helices is directed via designed attractive electrostatic interactions between the polar faces of the amphipathic helices, namely when the polar face of one amphipathic helix in the di-helix is composed of positively charged polar residues while the polar face of the other amphipathic helix is composed of negatively charged residues[4]. This association to form the four-helix bundle is pH-dependent as expected and does not occur in the absence of the directed attractive electrostatic interactions, namely when the polar faces of the amphipathic helices in the di-helix are each composed of equal numbers of positively and negatively charged residues. Off-specular x-ray reflectivity will be used to further characterize the vectorially-oriented four-helix bundles and neutron reflectivity, employing selected perdeuterated residues, will be used to assess the *syn* vs. *anti* topology of the bundles.

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