doi:10.1017/S1431927615002676 Paper No. 0188 Microsc. Microanal. 21 (Suppl 3), 2015

Cryo-EM Methods for the Structural Analysis of Biomimetic Materials based on Peptides and Proteins

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Molecular self-assembly is a fundamental principle of life, with cells having mastered this process to encode incredible diversity of function. Sequence-specific biological macromolecules (i.e., proteins and nucleic acids) interact with very high selectivity within the nanometer to micrometer size regime to create the complex cellular machinery that performs the physico-chemical functions associated with metabolism, signal transduction, replication, and differentiation in living systems. These complex biological machines arise from self-assembly of structurally complementary biomolecules (protomers) on the basis of structural features programmed into polypeptide and polynucleotide sequences at the molecular level. As a consequence of the near-absolute control of macromolecular architecture that results from such sequence specificity, biological structural platforms may have advantages for the creation of well-defined supramolecular assemblies in comparison to synthetic systems, at least at the current state of development for the latter. Thus, the conceptual design of synthetic nano-scale systems can derive significant information from structural investigations of biologically derived supramolecular assemblies and, conversely, biological structural motifs present an attractive target for the synthesis of artificial nano-scale systems on the basis of relationships between sequence and supramolecular structure that have been established for native biological assemblies. Sequence-specific biological materials represent conceptual and structural prototypes for the design of artificial smart materials and will continue to be at the forefront of efforts to develop materials for specialized applications beyond those observed in the native biological context.

However, structurally ordered supramolecular materials on the nanometer length-scale are the most challenging to rationally construct and the most difficult to structurally analyze. Cryo-EM (HRSEM and TEM) methods have provided the means to understand the mechanism of interaction between structural elements that guide the formation of higher order structure and, ultimately, functional properties within these biomimetic materials. Structural information from complementary methods (solid-state NMR measurements, small-angle X-ray scattering, and X-ray fiber diffraction) can place structural constraints on the EM analysis, which can assist in the development of structural models facilitate an understanding of the synthetic assemblies. In addition, recent advances in cryo-EM analysis have permitted, in combination with molecular modelling, direct access to near atomic resolution structural information for naturally occurring protein assemblies [1]. Several examples of cryo-EM structural analysis of self-assembled protein- and peptide-based materials are described, which, in combination with complementary methods, allowed the construction of structural models on different length scales that have provided insight into the mechanism of self-assembly.

The first example focuses on the creation of synthetic protein-based materials that mimic the self-assembly behavior and elastomeric mechanical response of native elastin. Protein engineering was employed to create synthetic elastin derivatives that comprised blocks of different sequence that self-assembled reversibly from aqueous solutions into protein nanoparticles [2] and nano-textured hydrogels [3]. Cryo-HRSEM of the resultant materials indicated that the nano-scale structural features recapitulated those of native elastic tissue (Figure 1). Chemical sequence control afforded non-native

sequences that mimicked native elastin assembly, but could be tailored for specific applications in controlled release and tissue engineering.

The second example describes the application of cryo-electron microscopy and helical reconstruction to the structural analysis of helical assemblies derived from short, synthetic peptide sequences [4]. The structures of two assemblies were determined at near atomic-level resolution (Figure 2). Surprisingly, despite very small changes in peptide sequence, profound structural differences were observed between assemblies derived from the respective peptides. Mutagenesis studies indicated that substitutions corresponding to one or two amino acids were sufficient to interconvert the structures of two assemblies. These studies suggest that cryo-EM structural analysis may provide answers to fundamental questions of acute significance to biomaterials science: from understanding of the functional roles of protein assemblies in biology to shedding light on the robustness of protein quaternary structure in sequence space.

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[5] The author acknowledges funding from the NIH, NSF, and DOE. Elizabeth Wright, Ed Egelman, and Rob Apkarian are acknowledged for essential contributions to the EM analysis in this research.

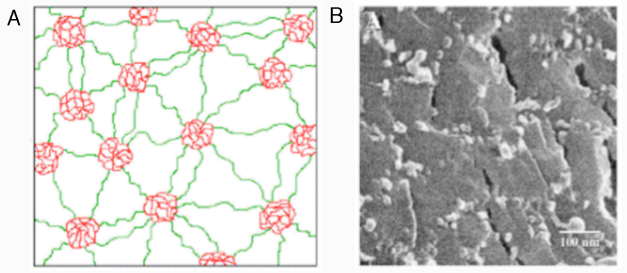

Figure 1. A. Schematic representation of an elastin-mimetic hydrogel. B. Cryo-HRSEM image of the corresponding hydrogel.

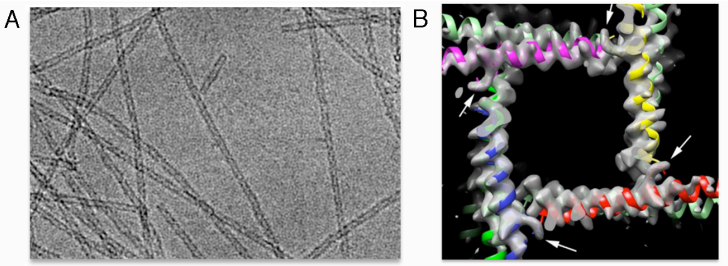

Figure 2. A. Cryo-EM image of a helical assembly derived from a synthetic coiled-coil peptide. B. Cross-section of the atomic resolution structure of the corresponding assembly indicating helix-helix interaction that are critical for its stability.

https://doi.org/10.1017/S1431927615002676 Published online by Cambridge University Press