Molecular Structures and High Resolution TEM

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High resolution cryo-EM of protein polymers: new insights into evolutionary divergence

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Helical polymers are ubiquitous in biology for the reason that helical symmetry arises from the simplest bonding rule between any two asymmetric units. If two identical copies of the same protein have a favorable interaction, in general this interaction can be repeated many times forming a polymer in which every subunit is in an identical environment. Most protein polymers can never be crystallized, since only a helix with exactly 2, 3, 4 or 6 subunits per turn is compatible with a crystal space group symmetry. Electron microscopy is therefore the method of choice for looking at protein and protein-nucleic acid polymers. In principle, low-resolution EM reconstructions can be combined with high resolution crystal or NMR structures to generate pseudo-atomic models of polymers of interest. However, when the resolution is limited to 20 Å or worse, interpretation of such density maps can be both ambiguous and problematic [1]. Recent advances in cryo-EM have now made it possible to routinely achieve sub-nanometer resolutions for a large variety of helical polymers. But there are a number of obstacles to imaging such polymers at the highest resolutions. One is that in the absence of something like a space group (present in a crystal) to maintain long-range order, all helical polymers will show cumulative disorder [2;3]. The only question is what is the scale of this disorder, or what is the persistence length for helical order. A number of years ago we introduced a method for surmounting the lack of long range order, called the Iterative Helical Real Space Reconstruction approach [4], that is now the main technique used for three-dimensional reconstruction of helical polymers by EM. Examples will be given from a number of systems to show how near-atomic resolution, even from fairly disordered and variable polymers, can now be achieved by cryo-EM and image processing. Most of the results have been obtained very recently and are not yet published. The polymers include actin [5], MDA5-RNA filaments [6], filaments formed by the pyrin domain (PYD) of ASC [7], and the adhesion filaments from *I. hospitalis* [8] (Figure 1). In the case of the PYD filaments we have achieved a resolution of 3.8 Å, allowing us to position quite a few of the larger amino acid sidechains. This is a great advance, as all EM structures currently deposited in the EMDB at better than 4.0 Å resolution have been viruses. We can now show that a resolution where the polypeptide chain can be reliably traced can be achieved with very thin protein polymers (~ 85 Å) that have a variable twist.

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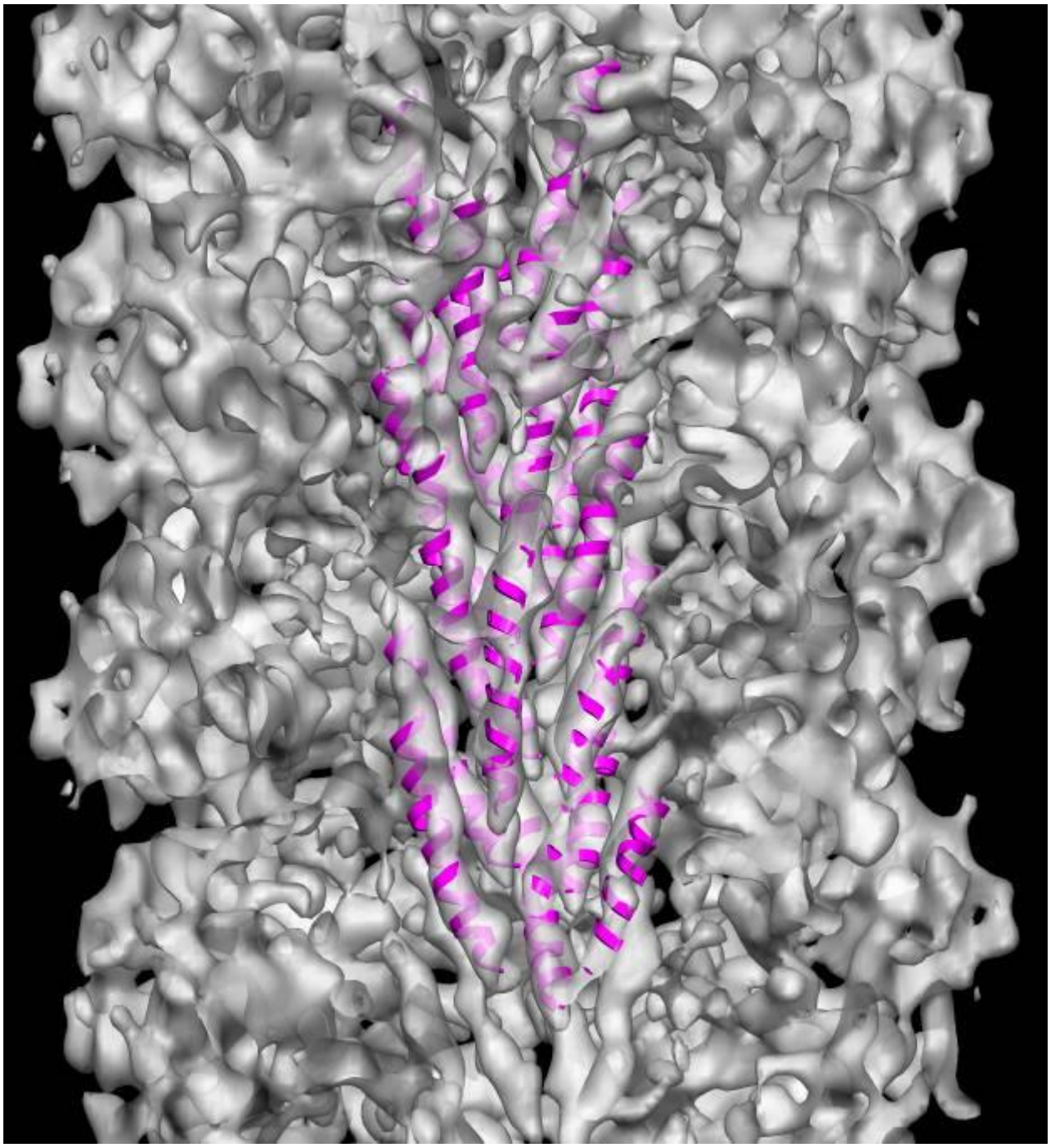

Figure 1. The N-terminal α-helices in the adhesion filaments of *I. hospitalis* can be positioned quite nicely into a three-dimensional cryo-EM reconstruction. As the resolution of such EM reconstructions improves one might expect to be able to fully trace the protein's polypeptide chain.