## **Refining helical structures using Frealix**

<u>Alexis Rohou<sup>1</sup></u>\*, Elena Zehr<sup>2</sup>, Joseph Pogliano<sup>3</sup>, David Agard<sup>2</sup> et Nikolaus Grigorieff<sup>1</sup>

<sup>1</sup>HHMI Janelia Research Campus, Asburn, Virginia, USA <sup>2</sup>University of California, San Francisco 94158, USA <sup>3</sup>University of California, San Diego, La Jolla 92093, USA

Frealix is software designed to refine helical structures calculated from images of frozenhydrated protein filaments. It can process filament images following the single-particle paradigm or incorporate a number of *a priori* restraints derived from mechanical considerations, which allow it to use arbitrarily short segments for alignment and thereby out-perform single-particle algorithms in cases of high curvature and low signal-to-noise ratios.

In addition to the macromolecular structure of the helical subunit, Frealix refines a 3D representation of each filament in the dataset. In aggregate, these "metadata" describing filament shape, deformations etc. make it possible to test the predictions of models (such as the worm-like chain model) against in vitro observables.

We present an overview of the program, some recently implemented features, and 3D reconstructions in the 3.5-to-5.5-Å range recently obtained with Frealix.