Refining helical structures using Frealix

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1. INTRODUCTION

Treating segments of protein filaments as independent « single particles » allows for the structure determination of deformed helical assemblies but, without *a priori* restraints on the alignment of these segments, it is difficult to obtain reliable estimates for their exact position and orientation and it becomes necessary to use longer segments. Our software, Frealix [1], models protein filaments in three dimensions as deformable semiflexible rods and uses this mechanical model to determine alignment parameters simultaneously for all segments. Uniquely among helical image-processing algorithms, this makes it possible to use arbitrarily short segments during analysis.

2. RESULTS

Benchmarking : TMV & amyloid-β fibrils

During development Frealix was tested against two previously-described datasets: amyloid-β (1-40) and tobacco mosaic virus (TMV), both recorded on film. In both cases, Frealix matched the performance of Spider scripts. In the case of amyloid fibrils, Frealix was able to process all fibrils, regardless of curvature. When processing highly-curved filaments (which had been discarded in previous studies), Frealix outperformed single-particle processing as implemented in Frealign (Figure 1).

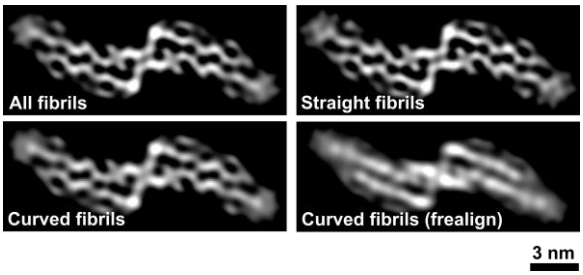
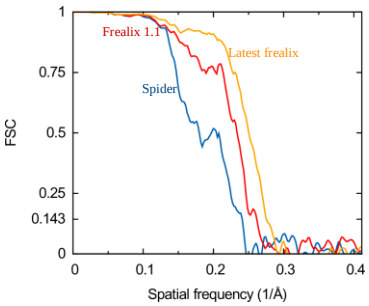


Figure 1. Cross-sections through amyloid-β fibril reconstructions. A set of 450 filaments selected from film micrographs were processed to give a reconstruction at ~ 7.5 Å (top left). The 188 straightest (top right) or most bent (bottom left) filaments were processed to give reconstructions at 8.0-8.3 Å. The same subset of curved fibrils was further refined by Frealign for 15 iterations, during which significant divergence of the refinement and misalignments were observed, leading to a smeared reconstruction (bottom right).

Phage tubulin (PhuZ) filaments

490 PhuZ filaments [2] taken from K2 micrographs were selected using 2D classification in Relion and then processed using Spider, to a resolution of ~ 4.2 Å (blue FSC curve, below). Initial refinement using Frealix (release version 1.1) gave a resolution of ~ 3.8 Å (red). Using newly-implemented features in Frealix such as subframe motion correction, and new in-house programs for frame alignment (unblur) and CTF estimation (ctffind4), the map was further improved at all frequencies. The current resolution is 3.6 Å (yellow FSC curve).



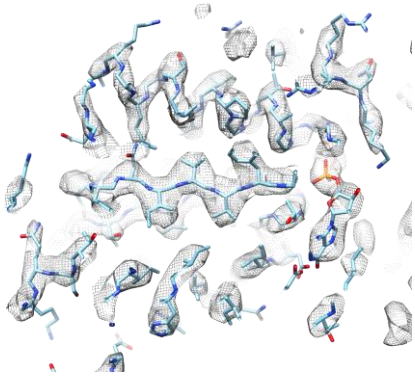


Figure 2. PhuZ filament in complex with GMPCPP were refined and reconstructed by Frealix to a final resolution of 3.6 Å

3. CONCLUSION

We have developed software which enables structure determination even from the most challenging, highly deformed filamentous specimens (e.g. amyloid fibrils) and performs well at high resolution when dealing with

more favorable protein filaments (e.g. PhuZ). We are currently implementing maximum likelihood-based multi-reference refinement to allow us to analyse helical heterogeneity and solve multiple structures from datasets.

REFERENCES

[1] Rohou, A., Grigorieff, N., 2014. Frealix: Model-based refinement of helical filament structures from electron micrographs. J. Struct. Biol. 186, 234–44.

[2] Zehr, E., Kraemer, J., Erb, M.L., Coker, J.K.C., Montabana, E., Pogliano, J., Agard, D., 2014. The Structure and Assembly Mechanism of a Novel Three-Stranded Tubulin Filament that Centers Phage DNA. Structure 22, 539–48.