

convolution which deals comfortably with the situation where broad diffraction spots lie close to the meridian or the centre of the pattern.

## Conclusion

If the traditional form of the Lorentz correction is applied to diffraction spots on a point by point basis, a good approximation to the profile calculation described above is obtained when the spots are not too close to the meridian. However, this method of correcting spot intensities breaks down at the meridian and works poorly where broad spots lie close to the meridian. The method employed by LSQINT naturally applies to these situations and also provides an easy way of scaling together diffraction spots of different widths, possibly from different structures within the same specimen.

## References

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### 3-D Reconstruction from Fibre X-ray Diffraction Patterns: Myosin-Decorated Actin Filaments

J.J.Harford<sup>1</sup>, R.C.Denny<sup>1</sup>, E.Morris<sup>2</sup>, R.Mendelson<sup>3</sup> and J.M.Squire<sup>1</sup>

<sup>1</sup> Biophysics Section, Blackett Laboratory, Imperial College, London;

<sup>2</sup> Biochemistry Department, Imperial College, London

<sup>3</sup> Cardiovascular Research Institute, UCSF, San Francisco.

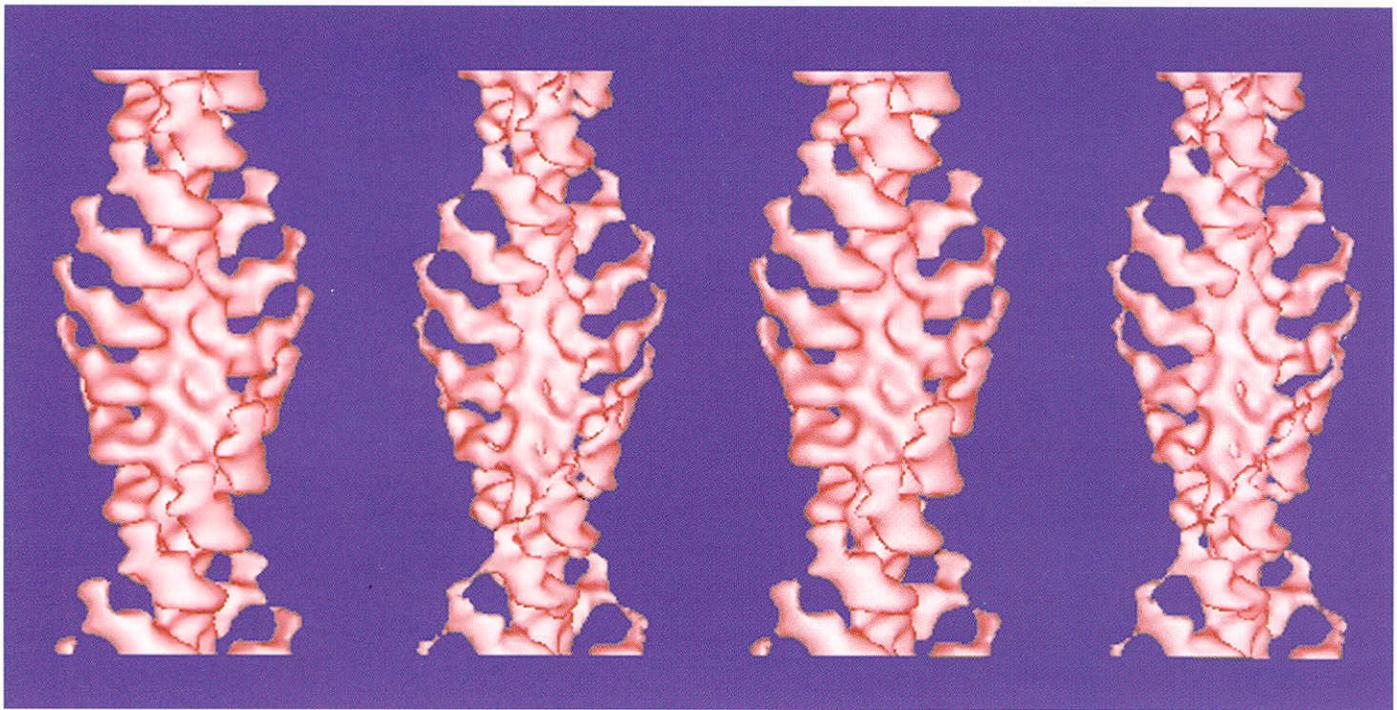
Helical biological particles such as actin filaments have been studied for many years by electron microscopy and their structures have been determined to about 20Å to 30Å resolution by 3-D reconstruction from single images [1,2]. A big advantage of electron micrographs is that they are real space images of the objects being studied. If one uses methods of reconstruction based on Fourier transforms computed from digitized images, then both amplitude and phase information can be obtained. A single view of a helical object contains many images of the repeating unit on the helix but

with different rotations around the helix axis. Therefore, a single view is sufficient to reconstruct the full helix in 3-dimensions. The main problems with electron microscopy of such biological filaments (often contrasted by using negative staining methods) are that the resolution is usually limited to about 20Å and the amplitude data are uncertain because of the contrast transfer function of the electron microscope.

X-ray fibre diffraction studies from equivalent systems can have the advantage that the diffraction information can extend to about 10Å and beyond and, when properly stripped, the layer-line data should be reliable. However, unlike electron microscopy data, there is little phase information and one cannot directly compute a 3-D reconstruction. This paper discusses the combination, to 27Å resolution, of amplitudes from fibre X-ray diffraction patterns of actin filaments labelled with myosin heads (myosin S1) and phases determined from electron microscopy. It also shows how the structure might be refined to a resolution of at least 13Å, far beyond current electron microscopy data, by modelling using the lower resolution reconstruction shown here as a starting point.

During muscle contraction, force and movement are supposed to be produced by the interaction of the globular heads of myosin molecules with adjacent, helical, actin filaments. The myosin heads are known as ATPases; they bind and can hydrolyse the molecule adenosine triphosphate (ATP) to adenosine diphosphate (ADP) and inorganic phosphate (Pi). This hydrolysis is associated with the release of free energy which is utilised to drive the generation of force and movement. The myosin ATPase is rather slow unless the myosin heads are interacting with actin filaments. In the absence of ATP or ADP, the myosin heads become rigidly attached to actin in the so-called rigor complex. This occurs on death when we stop making ATP and our muscles become 'cross-linked', hence stiff (*rigor mortis* sets in), by the permanent binding of myosin heads to actin.

The myosin molecule is a long (1500Å) coiled-coil  $\alpha$ -helical rod on one end of which are two myosin heads. The myosin heads can be separated from the rod by proteolysis, yielding individual, isolated, heads known as myosin subfragment-1 (S1). Such myosin S1 molecules can attach to isolated actin filaments in the absence of ATP to form so-called 'decorated' actin filaments. These have a



**Figure 1:** Stereo pairs of 3-dimensional reconstructions of actin filaments 'decorated' with myosin heads using (left) electron micrograph data alone [2] and (right) X-ray fibre diffraction amplitudes combined with electron micrograph phases. The axial (cross-over) repeat of the actin filament is about 360 Å

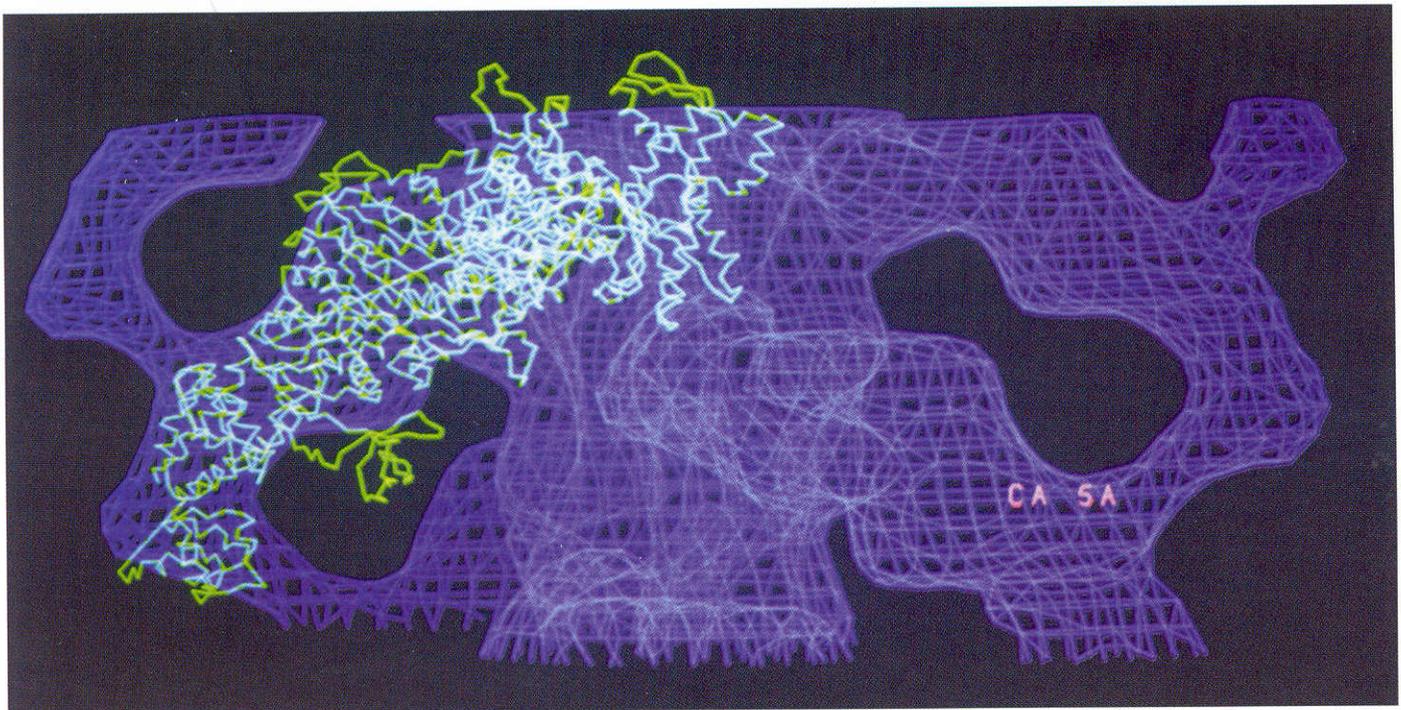
characteristic pointed appearance; the heads all tilt in the same direction, towards one end of the actin filament. This can be seen in stereo as Figure 1 (left) which is a 3-D reconstruction of decorated actin based solely on electron micrograph data and published by Milligan and his colleagues [2].

Another approach to solving this kind of structure is to soak whole muscle with exogenous myosin S1 in the absence of nucleotide so that the muscle itself then provides an oriented fibre of decorated actin filaments. The X-ray diffraction pattern in Figure 2, recorded on line 16.1 at the CCLRC Daresbury SRS using image plates, shows a very rich layer-line pattern (extending axially to about 27Å resolution) as stripped using CCP13 programs. The amplitudes from this stripped pattern were then combined with the phases from Milligan's electron microscopy to give the 3-D reconstruction shown in stereo as Figure 1 (right). This structure therefore has the same resolution as the em reconstruction and it has made use of some em data and some X-ray fibre diffraction data. The work is preliminary so far, but in the end, when fully processed, the X-ray amplitudes should be more reliable than the electron microscopy values. Figure 3 shows that the X-ray reconstruction is quite good because here the known structures of the actin monomer and the myosin head, both determined to atomic resolution by protein crystallography [3,4], have been fitted into the reconstruction. The shapes agree quite well.



**Figure 2:** X-ray fibre diffraction pattern (fibre axis vertical) from skinned plaice fin muscle into which a solution of myosin S1 heads has been soaked in the absence of ATP. This effectively produces an oriented fibre of decorated actin filaments as in Figure 1. The myosin head preparation was from chicken pectoralis muscle myosin proteolytically cleaved using the enzyme chymotrypsin. The fibre diffraction data, recorded on image plates on line 16.1 at the CCLRC Daresbury SRS, were stripped using CCP13 software and the displayed image is after background subtraction and layer-line fitting. The outer edge of the pattern corresponds to a resolution of about 25 Å.

So far so good, but layer-lines in the X-ray fibre diffraction pattern shown in Figure 2 actually extend to an axial resolution of at least 13Å (not shown);



**Figure 3:** The same 3-D reconstruction as shown in the right hand image in Figure 1, but here represented as a chicken-wire surface using FRODO and into which  $\alpha$ -carbon positions in the actin monomer [4] and the myosin head [3] have been fitted. The individual molecular shapes fit the density distribution quite well, but both the reconstruction and the fit have yet to be optimised.

way beyond the current resolution limit ( $27\text{\AA}$ ) of the electron microscopy. How can the decorated actin structure be solved to this higher resolution? In fact, a similar procedure to that used to model the low-angle X-ray diffraction data from myosin filaments in muscle [5,6] can be used once again here. The positioning of the myosin head and actin in Figure 3 provides a good starting point from which to try to refine the structure to be compatible with the X-ray layer-line amplitudes to  $13\text{\AA}$  resolution. As in the case of the myosin filaments, the positions of the actin monomer and myosin head can be parameterized, as can more subtle features such as the relative positions of the four sub-domains of the actin monomer and the relative positions of different parts of each myosin head. These parameters can then be refined by a simulated annealing search, together with local minimization, using the structure in Figure 3 as an initial guess. It is evident that the medium resolution combination of electron microscope and X-ray data, followed by high resolution refinement, is a very powerful approach to solving high resolution fibre diffraction data from biological systems.

#### Acknowledgements

We are indebted to the NCD staff at the Daresbury Laboratory, particularly Liz Towns-Andrews, for their unfailing help, to the BBSRC for grant support,

and to the protein crystallography group at Imperial College for access to their Evans and Sutherland workstation running FRODO.

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