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Structure of the Acrosomal Bundle, a Biological Machine, at 9.5 Å Resolution

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Limulus sperm contains an organelle called the acrosomal bundle, composed of actin filaments crosslinked by scruin. It is bent and twisted into a coil around the base of the nucleus. When activated, the bundle uncoils and fully extends to ~60 μm in a few seconds. This spring-like action is powered by stored mechanical energy, and does not require the action of motor proteins. Our 9.5 Å electron cryomicroscopic structure [1,2] of the extended crystalline bundle shows that the orientation of actin subunits deviates widely from that in a "standard" F-actin filament. This variability is probably induced by the scruin in order to rigidly pack the actin-scruin filaments into the bundle in the extended state and suggests a mechanism for the transition between coiled and extended states.

Since there are 14 crystallographically independent actin and scruin molecules in the asymmetric unit of this P21 crystal, there is an opportunity to perform cross-correlation on the asymmetric unit. We took advantage of this, and did several kinds of cross-correlation search on the map, both an azimuthal and axial (2D) search about the filament axis using iterative density averages as the search object, and a full (6D) search with *foldhunter* [3], using the actin crystallographic coordinates. This operation yields two kinds of critically useful information. (1) Averaging the densities based on the cross-correlation orientations and positions was used to obtain a preliminary model for the scruin domains, and a detailed average of the actin molecules, which revealed what the average actin looks like in the bundle. (2) The orientation information itself can be used to reveal the relationships among the molecules in the bundle, both for the actin - to show the deviations from a “perfect” helix - and for the scruin – to show how it packs to create the filament-filament interactions that stabilize the bundle and allow for its biological activity. Each of these is discussed separately.

(1) Correlation averaging the actin density using foldhunter results is shown in Fig. 1a and b. The density showed several helices which are actually present in the actin structure, and the side view (Fig. 1b) reveals a “hydrophobic plug”, but not exactly in the position predicted by Holmes [4] from fiber diffraction data (shown in red ribbon drawing). The subdomains of actin are in a slightly closed conformation (Fig. 1c), characteristic of nucleotide-bound forms of actin. The scruin domain correlation averages derived from the 2D search (not shown) are consistent with a beta-propeller type structure, which was predicted from sequence analysis [5].

(2) The orientations of the actin molecules derived from the cross-correlation show a deviation from the standard actin helix (Fig. 2a). These deviations may be coordinated, but each is different from its neighbors. These deviations are likely due to the influence of the scruin to which the actins are bound. The packing in the bundle is shown schematically in Fig. 2b. One scruin domain (E, in green) is closely associated with the actin filament, and tracks the actin distortion closely. The other domain (S, in blue) appears responsible for the major filament-filament crosslinking and crystal packing, and as such carries a larger distortion from the canonical helix. The implications for the dynamics of the extension of the acrosomal bundle in its biological context are discussed [6].

References

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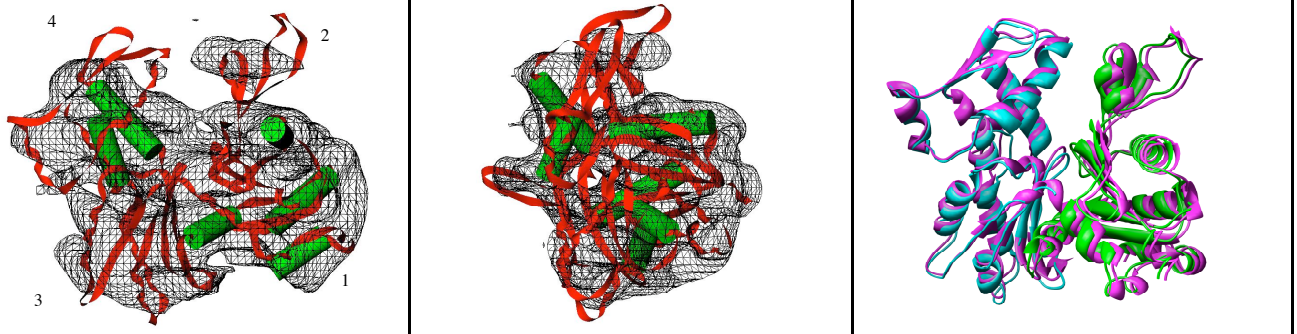
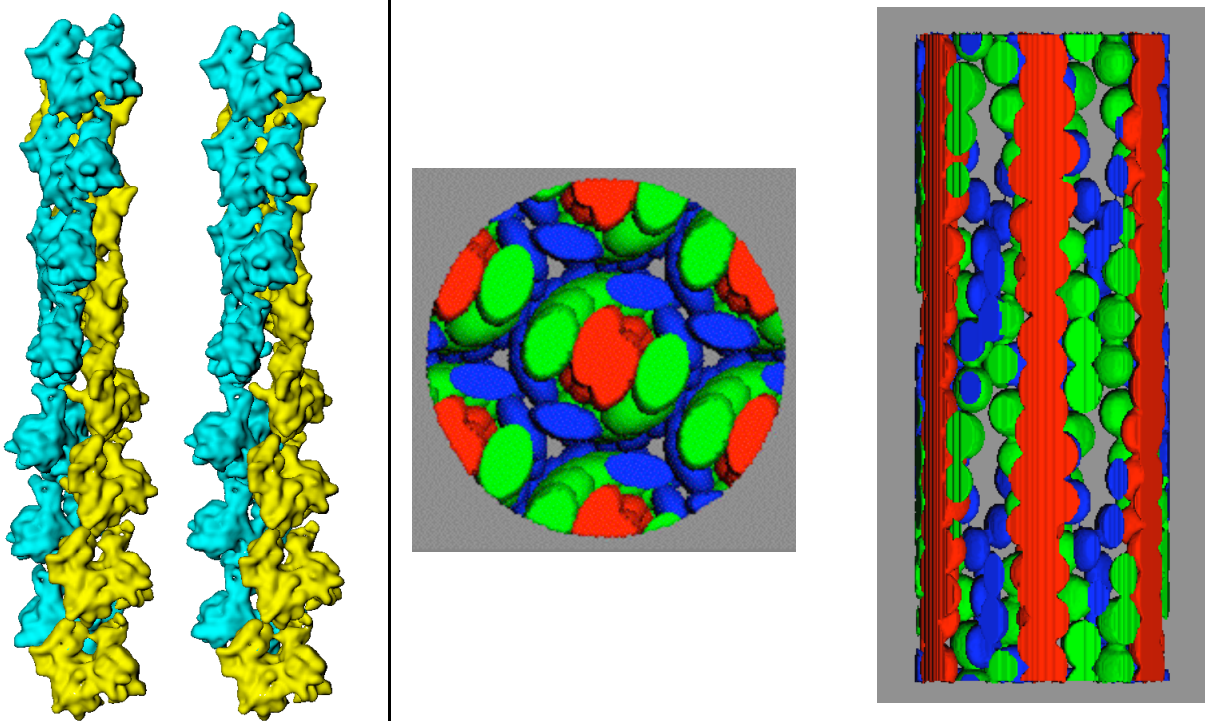
a b c



1 a. *Foldhunter* correlation average density of actin in wireframe, helices found in the density maps in green, Holmes filamentous actin model in red ribbon. Subdomains 1 through 4 are indicated. b. Side view showing hydrophobic plug on the left. c. Separate correlation searches for subdomains 1 and 2 (green) and 3 and 4 (cyan) show that the acrosomal actin is slightly more closed than Holmes model (purple).

a b



2 a. left - Holmes canonical actin helix, right – acrosomal actin, showing deviations from perfect helicity. b. Schematic view of actin (red), scruin E domain (green) and scruin S domain (blue) in top view (left) and side view (right).

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