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Report on SC-2031

Structure of complex networks of actin filaments: bundle phases and composite networks

The aim of the first series of experiments was, to determine under which experimental conditions the microsctructure of differently crosslinked actin network can be obtained by SAXS measurements. To obtain information about the microstructure of F-actin networks and bundles other groups used highly concentrated actin pellets. The experimental procedure to obtain these pellets might distort the samples and network structure. The high quality of our samples, and the high brilliance of the instrument, allowed us to obtain excellent structural data on actin networks that were several orders of magnitude less concentrated. Already in this first series of experiments exciting insights into the organization of actin bundles were obtained.

In a first series of experiments, we tried to reproduce experiments already published by the group of C. Safinya on highly concentrated networks crosslinked by α-actinin (1). Moreover, we wanted to shed light on the strong temperature dependence of the network organization as it was reported for the mechanical properties before (2). The general form of the observed diffraction data, in particular the appearance of a broad peak at q~0.3nm^{-1}, is in excellent agreement with the published data (Fig. 1A). By reducing the temperature, the structural rearrangement indicated by the formation of the peak is slightly increased as predicted by rheological measurements (Fig. 1B). Although this is in agreement with previous measurements, α-actinin crosslinked actin networks are very inhomogeneous. Both fluorescence imaging and electron microscopy show a composite network phase, where bundles and a network are simultaneously present. This makes a quantitative data analysis very difficult, and we are currently investigating how such phases can be analysed. We therefore decided to concentrate on experiments on more well defined systems, instead of trying higher actin concentrations.

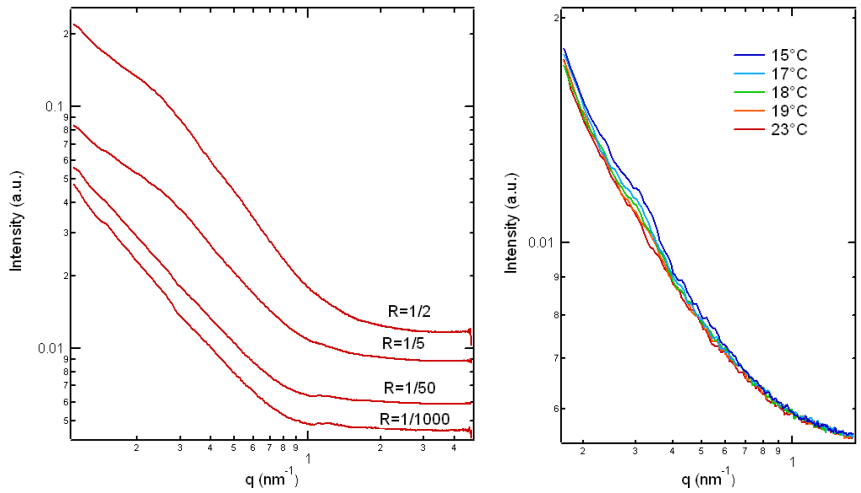

Fig. 1 (A) Circularly averaged diffraction data for increasing α-actinin concentration at c_a=0.8mg/ml at room temperature. For R=0.2 a broad peak appears at q~0.3nm^{-1}.
(B) Temperature dependence of a network with R_a=0.1 and c_a=0.8mg/ml

In a second series of experiments we investigated a purely bundled phase of fascin-crosslinked networks – a system in which we already studied the mechanical properties in detail (3,4). The actin bundles partially align in the capillary which makes it possible to extract data on both the packing order and the filaments structure (Fig. 2B). The index diffraction peaks along q_r unambiguously indicate hexagonal F-actin packing, the reciprocal lattice vectors up to q_{30} are clearly visible (Fig. 2A). The lattice constant of $4\pi q_{10}/\sqrt{3}$ =12.4 nm remains the same for all fascin and actin concentrations. Assuming a F-

actin diameter of 7.5 nm this corresponds to a surface-to-surface distance of 4.9 nm which is in good agreement with the size of the fascin molecule. The obtained data on this system is surprisingly similar to data published right after our experiments at ID02 on espin crosslinked actin networks (5). Though espin is much smaller, has a different binding affinity and binding site, the hexagonal packing is the same.

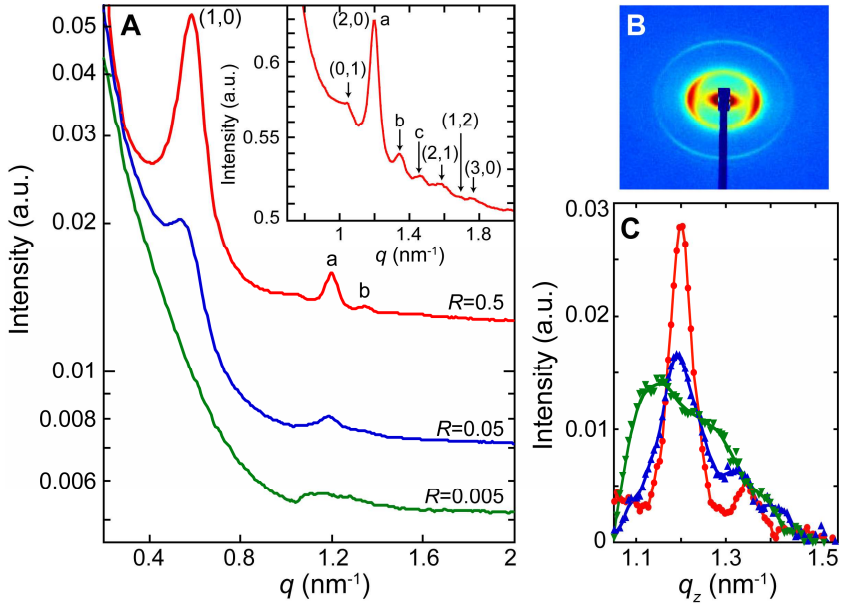

Fig. 2 (A) Circularly averaged diffraction data for different fascin concentrations and c_a=2 mg/ml. For R≥0.01 peaks related to hexagonal packing of filaments appear. A magnification of the diffraction at R=0.5 is shown in the inset. The peaks belonging to the layer lines are indexed a, b and c. **(B)** A typical 2D diffraction pattern of a partially aligned F-actin/fascin network for R=0.5 and c_A= 2mg/ml. **(C)** Angularly averaged wedges along the axial direction q_z. Peaks corresponding to the helical structure of the actin filaments shift with R. (R=0.005 ▼, R=0.05 ▲, R=0.5 ●)

Although we used much lower actin concentrations (c_a=2 mg/ml) we were able to extract the helical symmetry of the individual actin filaments in the bundle from the diffraction intensity along q_z. By analyzing peaks corresponding to the 6th and 7th layer line (Fig. 2A, a,b,c) we observe change in filament twist with increasing crosslinker concentration (Fig. 2C). Using complementary mesoscopic fluorescence experiments we were able to show that a finite equilibrium bundle size exists. Combining both findings, we proposed a new model for the regulation of bundle sizes *in vitro* and *in vivo*, a process of fundamental importance for the proper function of cells. We attached the manuscript which is currently under revision at Nature (6).

In the experiments shown above the length of the actin filaments was not controlled; filaments can be up to 100 µm long. Cells tightly control the filament length and in vivo filaments are typically 1-2 µm. To investigate the influence of the filament length on the network structure we shortened the filaments to an average length of approximately 2µm by addition of gelsolin. As illustrated in Fig. 3 we could not observe any change neither in bundle thickness nor in helical twist.

To observe actin bundles in fluorescence microscopy we bind fluorescently labelled phalloidin to the F-actin. The addition of phalloidin does not affect the packing or helical twist of filaments in the bundle (Fig. 3).

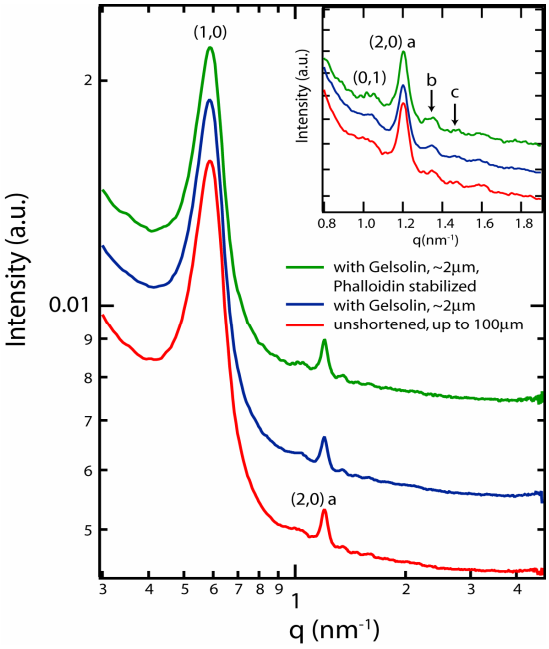

Fig. 3 Circularly averaged diffraction pattern in dependence of the filament length for c_a=1.6mg/ml. The peaks belonging to the helical symmetry are labeled with a,b,c.

Concomitant with the change in helical twist, the slope of the diffraction pattern at low q increases from an initial ~q^{-1} to ~q^{-3} (Fig. 4). The q^{-1} scaling observed for pure actin or very low fascin/actin ratios can be attributed to the approximately cylindrical shape of the actin filaments. The change in slope corresponds to changes in the network structure which have to be further analysed.

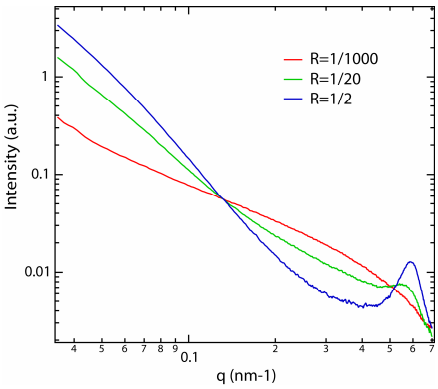

Fig. 4 Circularly averaged diffraction pattern for different fascin concentration at c_a=1.6mg/ml. The slope at low q-values gradually increases with increasing fascin concentration

Although information about the molecular organisation of actin bundles starts to become available, relatively little is known about the initial process of bundle formation. Using the fascin/actin system we investigated the polymerization and build up of higher order structures in the beam. The diffraction peaks that are indicative of both filament and bundle structure are already observable a few minutes after injection. The process of bundle formation is surprisingly fast. To observe the rapidly evolving structure an automated injection system would be mandatory.

In summary, already the first scattering experiments on our system gave exciting new insights into the size regulation mechanism and organization of cytoskeletal bundles. The obtained results are very encouraging to be followed up with even more physiological relevant systems of mixtures of crosslinkers – especially the similarity of the espin and fascin system is striking and fluorescence imaging indicates already that such mixture give rise to completely new structures. Also the time dependence of structure formation will need further investigations using a rapid mixing device or lowering the temperature.

References

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