

Abstract of research activity:

My research focuses on several parallel topics:

- Actin-based movement/dynamic of cellular protrusions.
- Collective behaviors of motor proteins.
- Active gels: self-organization phenomena in the cytoskeleton.
- Mechano-sensor proteins

The cell cytoskeleton is an active network that controls the morphologies and the mechanical properties of cells and provides a scaffold for many cellular processes. The cytoskeleton is a three-dimensional protein network of polar elastic filaments (i.e., actin filaments and microtubules) constantly remodeling via the action of a large number of actin binding proteins (ABP's) interacting with the cytoskeletal filaments. As a consequence, *in vivo*, actin filaments (F-actin) rarely exist as isolated single filaments but instead associate into bundles or networks, in concert with these ABP's to support various cellular processes such as motility, division, and adhesion. The cytoskeletal networks are intrinsically active materials driven away from equilibrium due to ATP hydrolysis. These networks resemble in many aspects polymer solutions or gels. Therefore, they are referred as "active gels". Their major difference from passive polymer solutions is their internal activity resulting from filaments' polymerization/depolymerization kinetics or from the action of molecular motors aggregates which act as active crosslinkers, constantly breaking and reforming. These small motor aggregates can generate relative movement between filaments, giving rise to complex self-organization dynamics resulting in a rich variety of structures.

My main focus is the investigation of cellular processes such as cell movement and division using simplified and controlled *in vitro* model systems. It is extremely difficult to investigate mechanisms of such complex cellular processes in living cells. *In vivo*, the number of participants is vast and their concentrations are unknown and continuously varying. The approach that I am adopting is to identify the minimal set of components which enable a certain cellular activity and to study it *in vitro* under controlled conditions. So far, such model systems were found successful in reconstituting propulsion and movement of endosomes and pathogens by actin polymerization.

Numerous cellular processes are driven and controlled by the action of motor proteins. One important class of motor proteins is myosin II. Myosin II motors self-assemble into small motor aggregates and work cooperatively to generate force and motion at the molecular level. These motors control the dynamic self-organization of various cytoskeletal structures and are implicated in processes such as cellular division (formation of a contractile ring) and motility. Myosin II motors also involved in the formation of contractile substructures such as stress fibers and dense active networks; these structures provide the cell with mechanical resistance and reinforce it against mechanical deformations.

My motivation is to develop successful *in vitro* systems able of reproducing fundamental cellular processes involving myosin II motors. The main difficulty that prevented other researchers from realizing such systems, that we have solved, was the characterization of the essential set of proteins. We found that the minimal set of

proteins includes myosin II motors, actin and the bundling protein, fascin. Using this model system we demonstrated that myosin II motors can reorganize actin into large scale patterns, but only in the presence of the bundling protein fascin (Fig. 1)¹. We found that although this system is extremely simplified (i.e., containing only three purified proteins), it can generate a wide range of *active* processes and rich dynamics, many of them not predicted by current theoretical models. The results of this study were published in *J. Physical Biology* and according to the referee's comments, "*it will certainly be of very high interest to the biological physics community and it represents a breakthrough in the field of simple in vitro actin systems*".

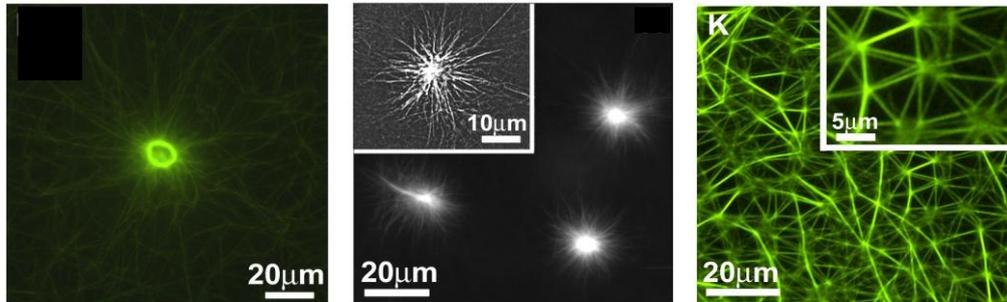


Fig. 1. Actin self-organization visualized by fluorescence microscopy. Composition: $[G\text{-actin}] = [A] = 21 \mu\text{m}$, and variable ratios of $[myosin]/[G\text{-actin}]$ ($[M]/[A]$) and $[fascin]/[G\text{-actin}]$ ($[F]/[A]$). a) Rings: $[M]/[A] = 0.03$ and $[F]/[A] = 0.05$. b) Asters: $[M]/[A] = 0.1$ and $[F]/[A] = 0.1$. c) Active network: $[M]/[A] = 0.05$ and $[F]/[A] = 0.14$.

This work received the journal cover page.



In addition to being implicated in actin cytoskeleton remodeling, myosin II motors also play major roles in other dynamic processes, i.e., muscle contraction, the oscillatory motion of myofibrils, and beating of insect wings. It has been proposed that the cooperative action of large number of motors is at the origin of this oscillatory motion. While directionality in individual motors stems mostly from their internal structure, the motion generated by a large number of motors working cooperatively is still poorly understood. Our motivation was to establish a simple experimental assay

that can reconstitute oscillatory behaviors resulting from the action of collection of motors. We use a motility assay with actin bundles consisting of short filamentous segments with randomly alternating polarities. These actin tracks exhibit bidirectional (oscillatory) motion when subjected to the action of a large number of myosin II motors (Figs. 2,3)². Analysis of the motion revealed no dependence of the characteristic reversal time on the size of the moving bundle. This observation is contradictory to previous theoretical calculations predicting an exponential increase of time reversal with bundle size. We presented a modified version of this model that takes into account the elastic energy due to the stretching of the actin track by the myosin II motors. The new model yielded a very good quantitative agreement with the experimental results.

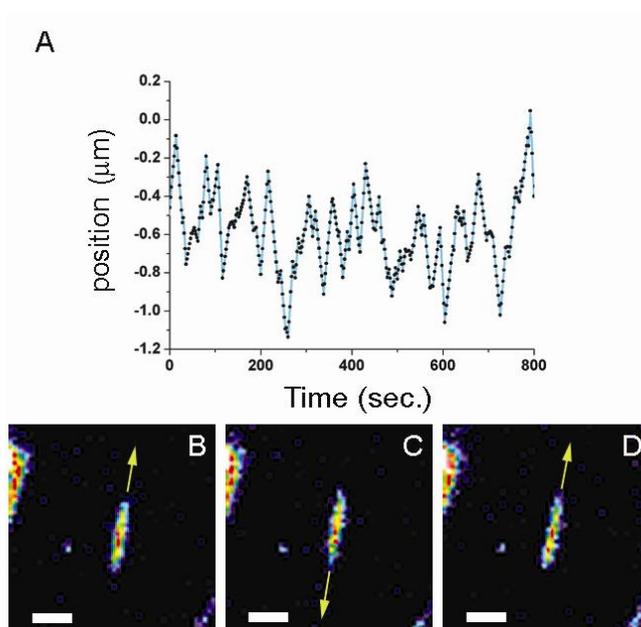


Fig. 2. (A) Position of a bundle over a time interval of 800 sec. The time interval between the consecutive data points is 2 sec. (B-D) Pseudocolor images of the actin bundle. The yellow arrows indicate the instantaneous direction of motion of the bundle. Bar size is 5 μm

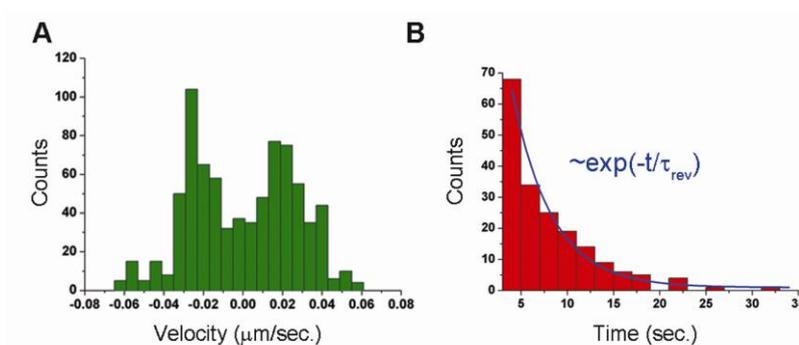


Fig. 3. (A) Velocity histogram of the bundle whose motion is shown in Fig. 2, exhibiting a clear bimodal distribution. (B) Distribution of the reversal time for the same bundle. The distribution is fitted by a single exponential decay

function with a characteristic reversal time: $\tau_{rev} \sim 3$ sec.

References:

1. F. Backouche, L. Haviv, D. Groswasser, and **A. Bernheim-Groswasser**, Active gels: dynamics of patterning and self-organization, *Physical Biology* **3**, 264 (2006). Including cover page.
2. B. Gilboa, D. Gillo, O. Farago, and **A. Bernheim-Groswasser**, Bidirectional cooperative motion of myosin II motors on actin tracks with randomly alternating polarities (submitted revised manuscript to *Biophys. J.*).