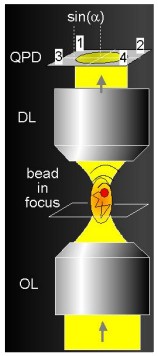
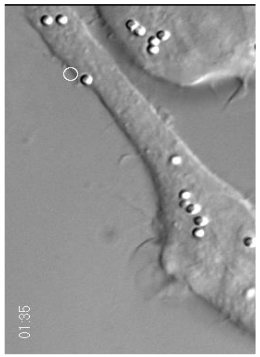
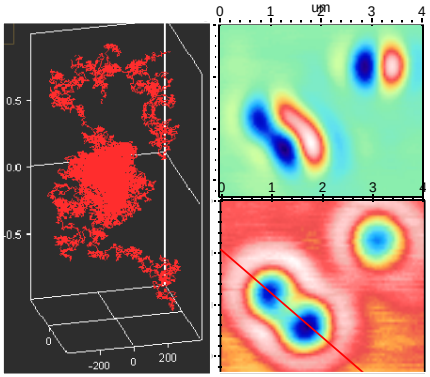
Alexander Rohrbach

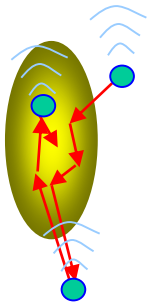
The group of Bio- and Nano-Photonics:

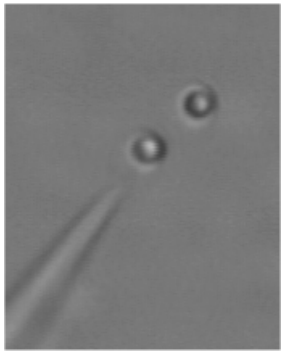
What we do in Biophysics











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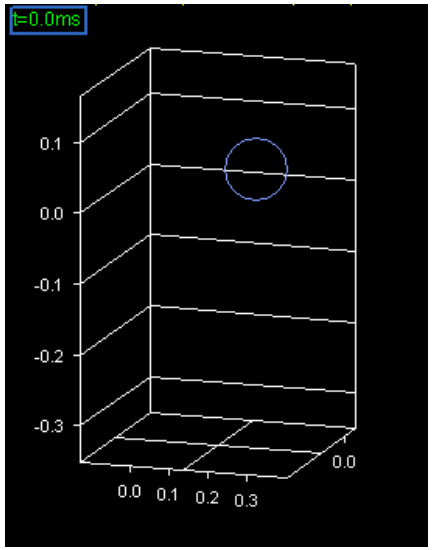
Department of Microsystems Engineering (IMTEK)
University of Freiburg, Germany

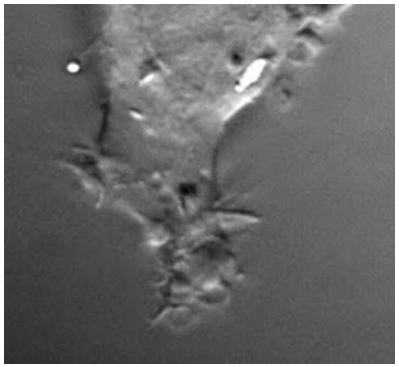
Thanks to my group in Freiburg

and to:

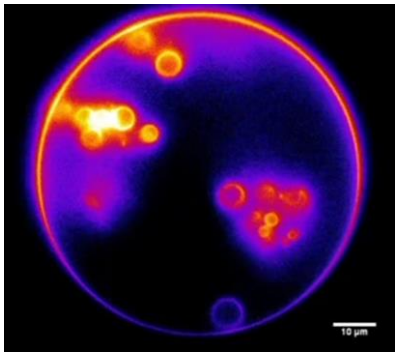
Fluctuation based nano-mechanics of biological systems



A. Biological cellular systems



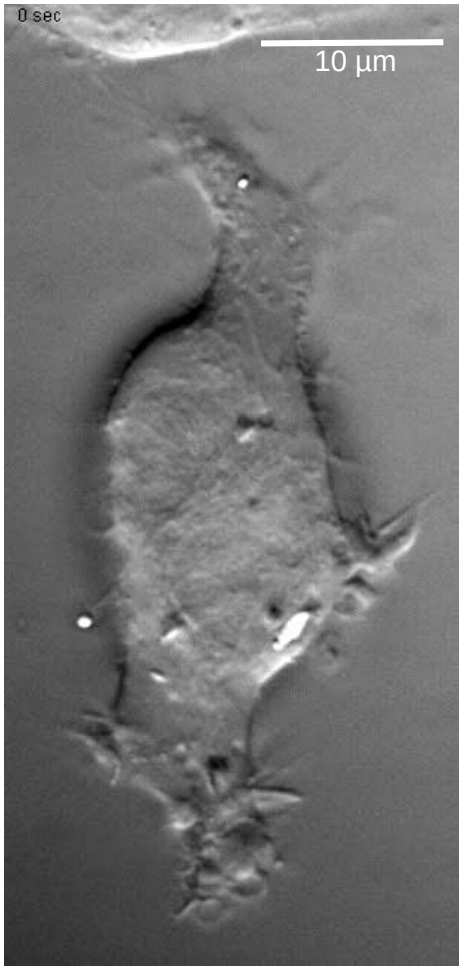
B. Minimal and biomimetic systems



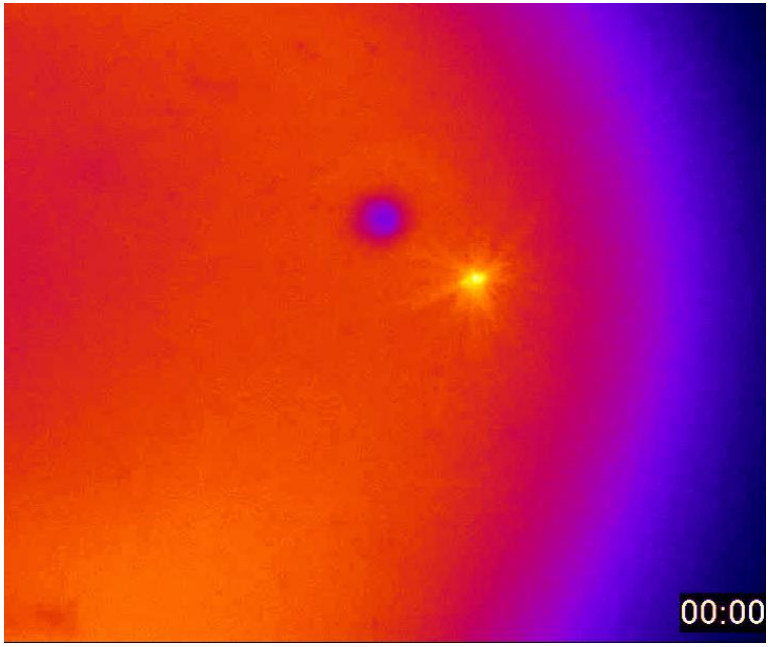
1. The fascination of living and dynamic systems

Complex and bio-mimetic system from our lab: What drives motion and generates forces ?

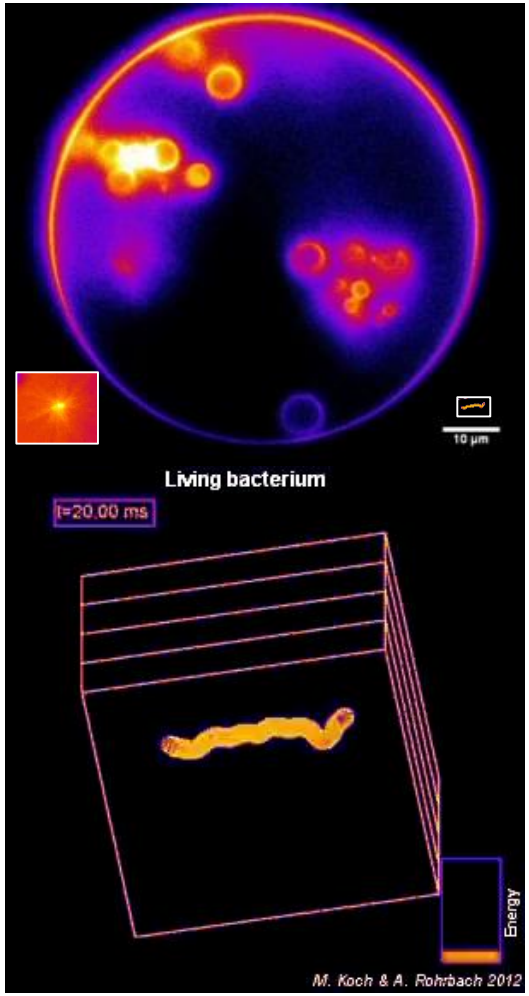
Active macrophage



Microtubule aster

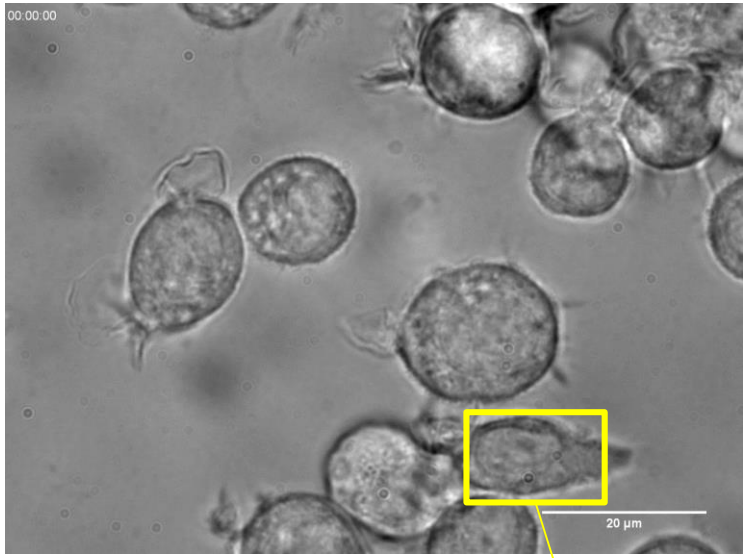


Giant uni-lamellar vesicles (GUV)



2. The fascination of measurements

Migration and inter-cell communication of J774 macrophages



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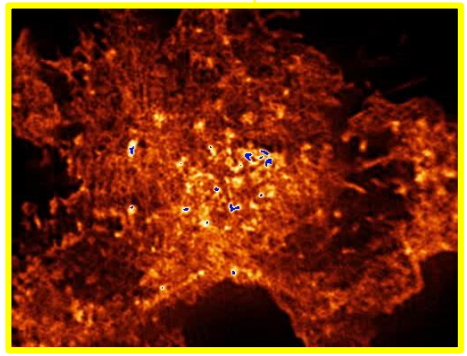
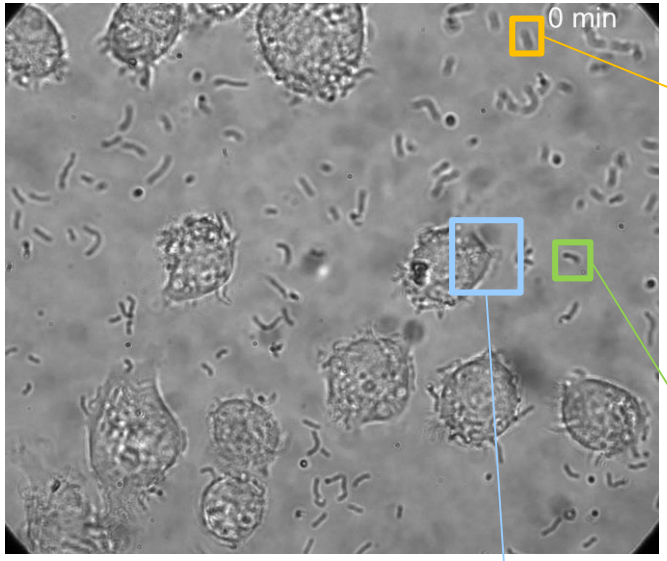
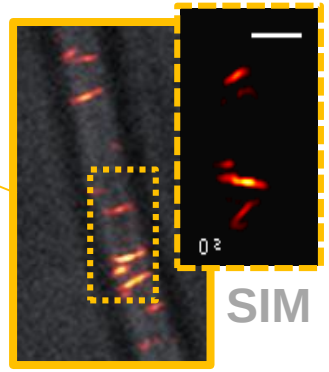


Uptake of *B.Subtilis* bacteria by J774 macrophages





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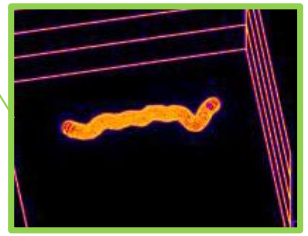
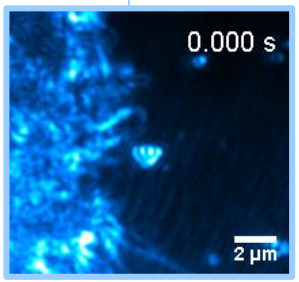


Interferometry

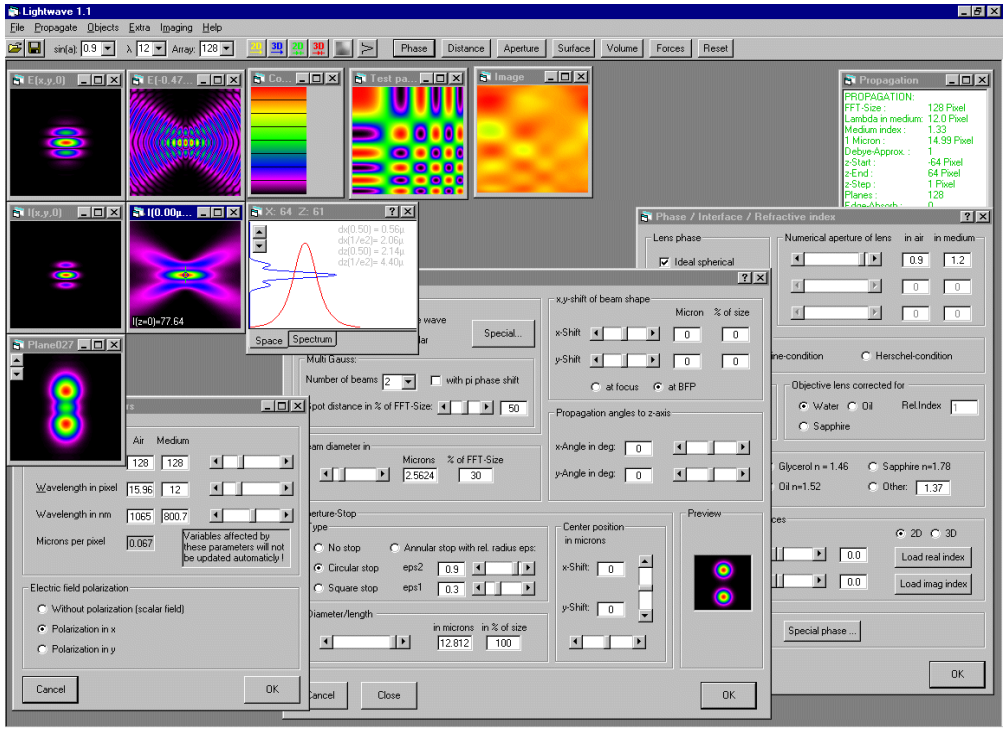
ROCS-M



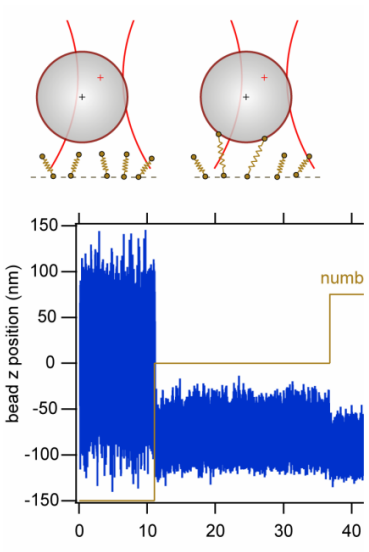
3. The fascination of theory and Simulations

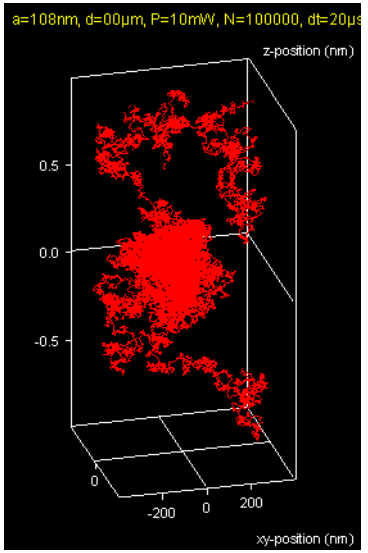
We develop the simulation software and theories ourselves!

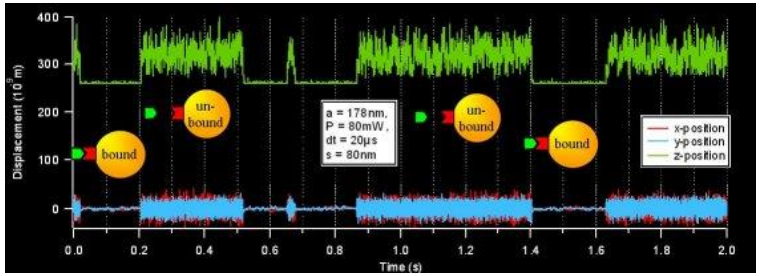
Electrodynamics simulations
in light propagation, scattering, optical forces and 3D imaging



Brownian dynamics simulations
for molecular motors, diffusion in optical potential landscapes and binding dynamics







Two research lines

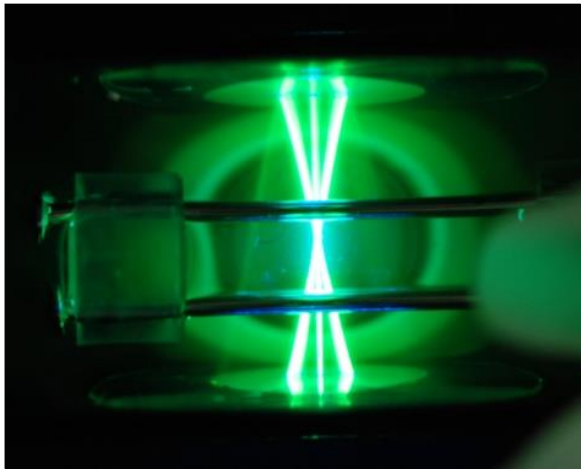
and two research strategies: Experiments & Simulations

1. Optics Line

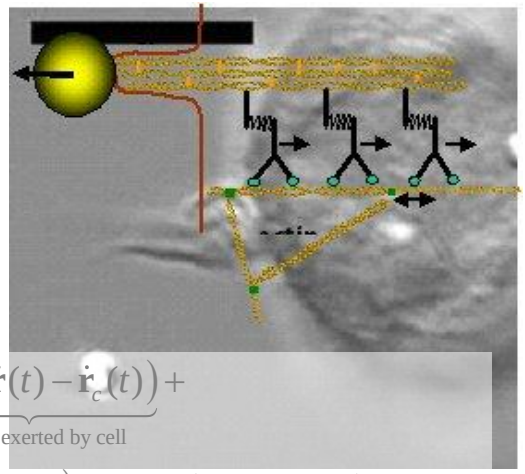
Experiments & Simulations

2. Nano-bio Line

Experiments & Simulations



$$H_{obj}\left(\mathbf{k}_{\perp}, y_0, \mu_{sca}\right) = \sum_{j=0}^{\infty} c_j\left(y_0, \mu_{sca}\right) \cdot exp\left(-\tfrac{1}{3}\mathbf{k}_{\perp}^2 j \tfrac{y_0^2}{g^2}\right)$$



$$\underbrace{\gamma_b \dot{\mathbf{r}}(t)}_{\text{friction in solution}} + \underbrace{\hat{\gamma}_c(t)\left(\dot{\mathbf{r}}(t) - \dot{\mathbf{r}}_c(t)\right)}_{\text{friction exerted by cell}} +$$

$$+ \underbrace{\hat{\kappa}_{opt}\left(\mathbf{r}(t) - \mathbf{r}_{trap}(t)\right)}_{\text{optical force}} + \underbrace{\hat{\kappa}_c(t)\left(\mathbf{r}(t) - \mathbf{r}_c(t)\right)}_{\text{binding to the cell}} = \mathbf{F}_{th}(t)$$

3 Research areas

Experiments and simulations:

1. **Microscopy**: fast super-resolution and lightsheet microscopy
2. **Optical trapping & particle tracking:** induce interactions
3. **Biophysics**: Fast mechanics of cells and biomimetic systems

Linear and non-linear Light-sheet Microscopy with self-reconstructing beams

Cell mechanics of particle uptake

Binding mechanisms of viruses

Assembling nano-bricks with optical forces

Fluorescence and label-free super-resolution microscopy

Bacterial cytoskeleton dynamics and cell wall repair

Optical force based cell sorter and rotator

Dynamics of helical bacteria in a light tube

Surface scanning with optically trapped probes

Particle uptake in giant uni-lamellar vesicles

The control of the bacterial cell wall synthesis

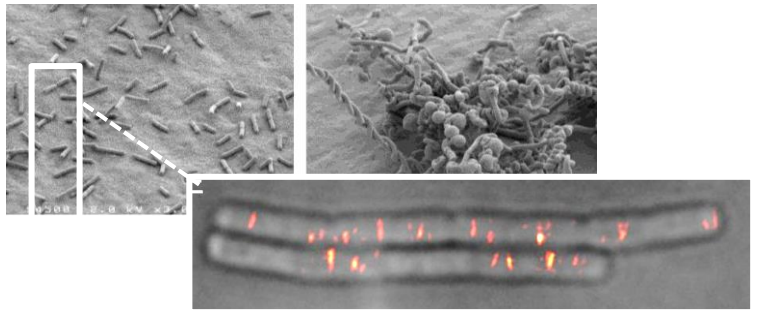
Background: Bacteria are powerful, often dangerous species on this planet. But, their stiff, hardly penetrable cell wall is still not understood. The cytoskeletal protein MreB is an essential component of the bacterial cell shape generation system. MreB interacts with cell wall synthesis machines, which produce peptidoglycan (PG) strands in hardly resolvable patterns. Inside living Bacillus subtilis cells MreB forms moving filamentous structures of variable lengths.

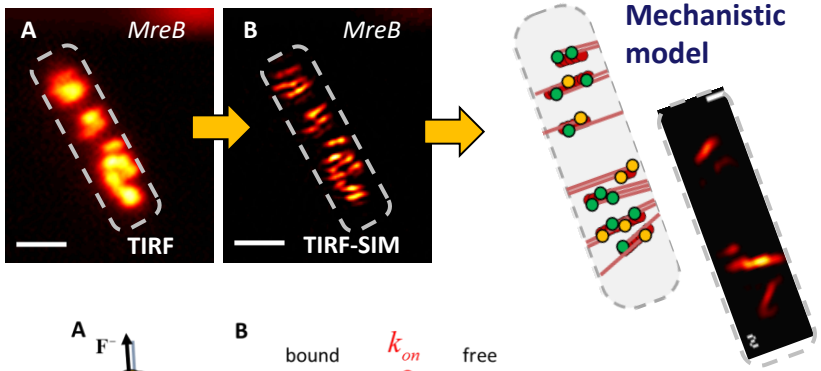
Problem: What is the role of the MreB filaments in the context of cell wall synthesis and reorganization? How does the dynamics of MReB filaments change under various forms of stress and under molecular constraints. How does the cell wall react to antibiotics?

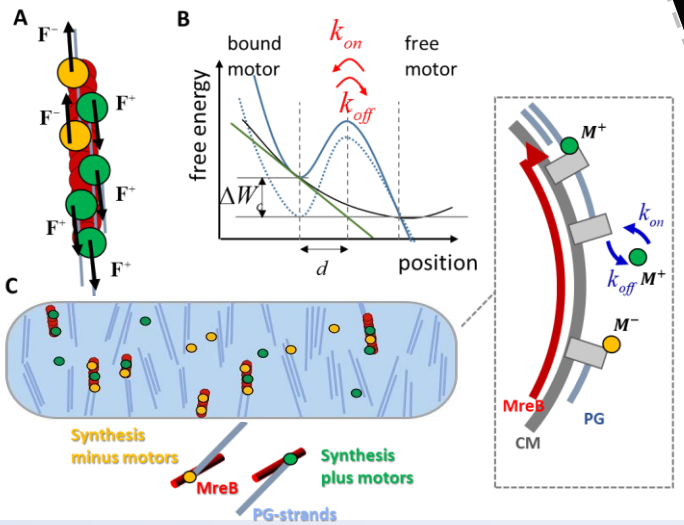
Approach: We use fast super-resolution fluorescence microscopy based on TIRF-SIM to measure the strange dynamics of MreB filaments in B. Subtitlis. We develop mechanistic and mathematical model consistent with all observations.

Computer model: The force F_c transporting MreB filaments depends on the numbers $n_{+/-}$ of synthesis motors elongating PG strands in positive (green) and negative (yellow) direction at velocities v_B. v_F is the transport velocity of the filament. F_s is the motor stall force.

$$v(n_+,n_-)=\begin{cases}\frac{n_+-n_-}{n_+/v_F+n_-/v_B}\\ \frac{n_+-n_-}{n_+/v_B+n_-/v_F}\\ 0\end{cases}\qquad F_C(n_+,n_-)=\begin{cases}F_S\,n_+\,n_-\frac{v_B+v_F}{v_B\,n_-+v_F\,n_+} & \text{for } n_+>n_-\\ F_S\,n_+\,n_-\frac{v_F+v_B}{v_F\,n_-+v_B\,n_+} & \text{for } n_+<n_-\\ F_s\,n & \text{for } n_+=n_-=n\end{cases}$$

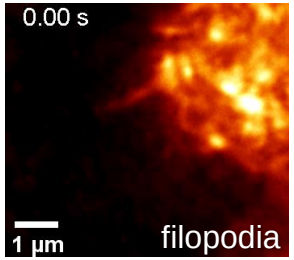






The nano-mechanics of filopodia tips-
investigated by Photonic Force Microscopy

Background: Phagocytosis is a central mechanism in our immune system used by cells (macrophages) to take up bacteria and other particles. Often this is mediated by thin protrusions, called filopodia.



Problem: The interactions of membrane, cytoskeleton and molecular motors during initial particle contact, filopodia retraction (and uptake) is only little understood.

Approach: We use 3D optical trapping and tracking of 1µm beads and approach them to the filopodium tip. Temporal and spatial analysis of the particle fluctuations at 1 MHz reveal biophysical processes on a molecular scale. Computer models for collective cluster (un-) binding help to understand molecular mechanism inside filopodia tips.

Langevin equation of bead motion at tip

$$\underbrace{\left(\gamma_{bd}\frac{\partial}{\partial t}+\kappa_{opt}\right)\mathbf{r}(t)}_{\text{trapped bead}}-\underbrace{\left(\gamma_{tip}\frac{\partial}{\partial t}+\kappa_{tip}\right)(\mathbf{r}(t)-\mathbf{R})}_{\text{filopodial tip connection}}+\underbrace{\left(\gamma_{tip}\frac{\partial}{\partial t}+\kappa_{tip}\right)\mathbf{r}_{BB}(t)}_{\text{backbone pulling}}=\underbrace{F_{th}(t)}_{\text{thermal noise}}$$

Life-time dependent mean bead retraction velocity under force

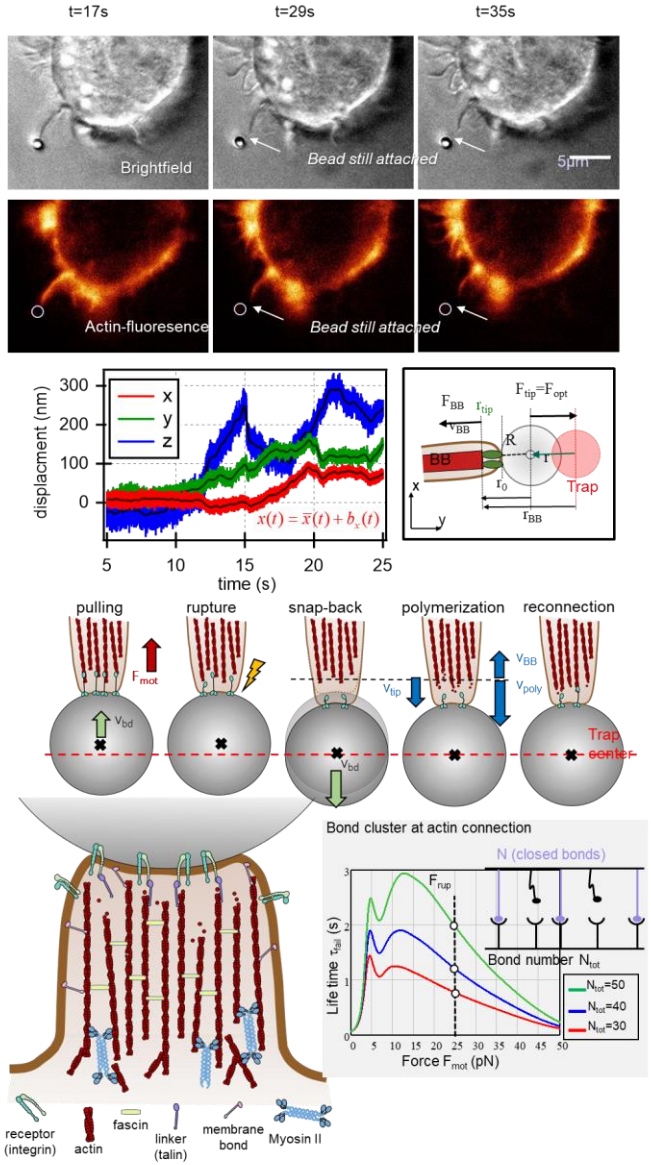
$$\bar{v}_{bd}(F)=\bar{v}_{BB}\cdot\frac{\tau_{on}(F)}{\tau_{on}(F)+\tau_{off}}=\bar{v}_{BB}\cdot\frac{\tau_{on}(F)}{\tau_{on}(F)+x_{gap}/\bar{v}_{tip}}$$

Mechanical parameters $\kappa_{tip,j}(t_L)=\kappa_{tot,j}(t_L)-\kappa_{opt,j}$ $\gamma_{tip,j}(t_L)=\gamma_{tot,j}(t_L)-\gamma_{bd,j}$ obtained from autocorrelations AC for each time window.

$$AC(\tau,t_L)=\frac{k_BT}{\kappa_j(t_L)}\cdot exp\left(-\tau\cdot\frac{\kappa_{tot,j}(t_L)}{\gamma_{tot,j}(t_L)}\right)$$

Lifetime of integrin bond cluster for N_{tot} bonds with un-/re-binding rates u and v under pulling force F_{mot}

$$\tau_{fail}(N_{tot},F)=\sum_{n=1}^{N_s}\left(\frac{1}{u(n,F)}+\sum_{m=n+1}^{N_{tot}}\left(\frac{1}{u(n,F)}\prod_{k=n}^{m-1}\frac{v(k)}{u(k,F)}\right)\right)$$



Particle-cell interaction on a thermal noise level

Background: Among all the external perturbations that a cell is consistently exposed to, mechanical interactions are of paramount importance in physics, biology, medicine and pharmacy.

Problem: To understand the molecular principles that govern cellular reactions to an approaching particle, we need to understand its thermal motion on different timescales. While interactions can be visible on one timescale, they can be completely invisible on another.

Approach: We use 3D optical trapping and tracking of 1µm beads and approach them to cell periphery. Spatio-temporal analysis of the particle fluctuations at 2 MHz reveal biophysical processes on a molecular scale.

Langevin equation at extracellular matrix (ECM) with bead position $b_j(t)$, $j = x,y,z$. *Viscous drag* $\gamma(t)$ *and stiffness* $\kappa(t)$

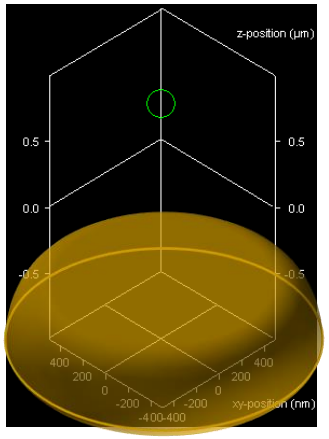
$$\gamma_{m,j}(d)\cdot\dot{b}_j(t)+\kappa_{tot,j}(d)\cdot b_j(t)=F_{th,j}(t)$$

Frequency spectrum of bead positions using response theory for N serially coupled elements, response function $\alpha_n(\omega,d)$

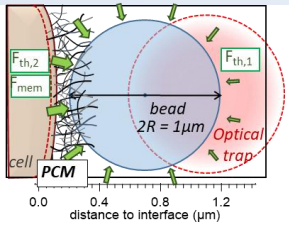
$$\tilde{b}_j(\omega,d)=\sum_{n=1}^{N}\alpha_{nj}(\omega,d)\cdot\tilde{F}_{th,j}(\omega)=\frac{\tilde{F}_{th,j}(\omega)}{6\pi R\cdot G_j(\omega,d)}$$

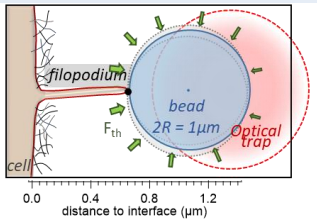
Frequency dependent complex shear modulus of bead coupling to ECM at different cell distances *d*

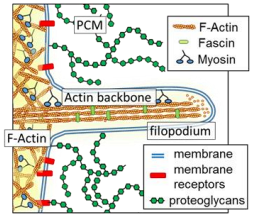
$$G_j(\omega,d)=\frac{1}{6\pi R}\left(\frac{1}{(\kappa_{opt}+\kappa_{0,j})+i\omega\cdot(\gamma_m+\gamma_{0,j})}+\frac{1}{\kappa_{1,j}+i\omega\cdot\gamma_{1,j}}\right)^{-1}$$

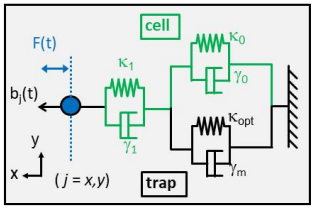


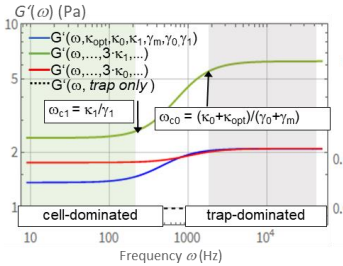
Particle binding to cell
1000 x slow motion

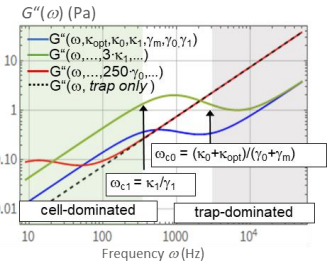


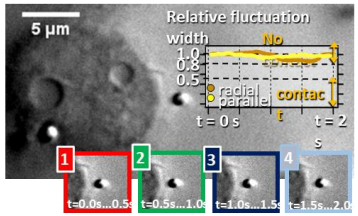


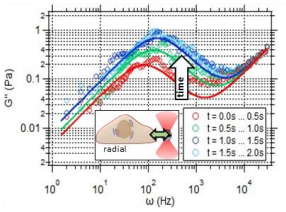


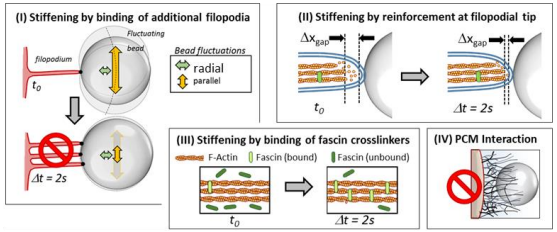






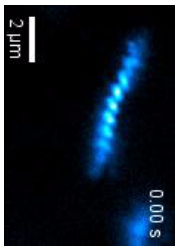






Molecular-mechanics inside helical bacteria – investigated by scanning optical traps

Background: The world smallest cell, a 200nm thin, helically shaped bacterium undergoes fast shape changes in 3D. Observation: correlative switching between different deformation states. We assume molecular shortening and relaxation of protein chains with velocities $v_{k1}(c)$ inside the cell.



$$\left\langle k_{1b\pm} \right\rangle = \frac{k_0}{M} \sum_{m=1}^{M} e^{\pm\frac{1}{2}\beta\left((\mu_T - G_0)\cdot a^{m-1} - G_0\right)}$$

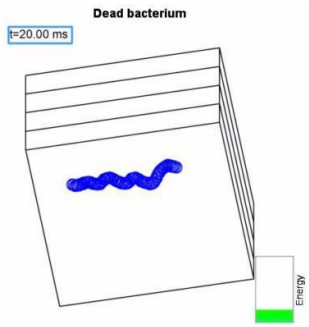
$$v_{k1}(c_r) = \frac{L_C}{N_{Fib}}\left(\left\langle k_{1b+}(c_r)\right\rangle - \left\langle k_{1b-}(c_r)\right\rangle\right)$$

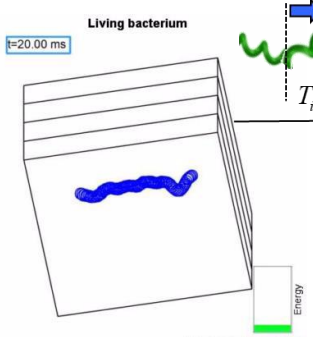
Problem: The transfer of energy inside the cell body leading to coordinated (allosteric) switching M elements of a protein chain is hardly understood (q = switch efficiency). Stochastic computer models help to estimate $M \le \frac{1}{\ln(q)} \cdot \ln\left(\frac{G_A}{G_L + \ln(c_r) - G_0}\right)$ from free energies derived from measured transition times. This allows to reconstruct basic locomotion principles invented by nature.

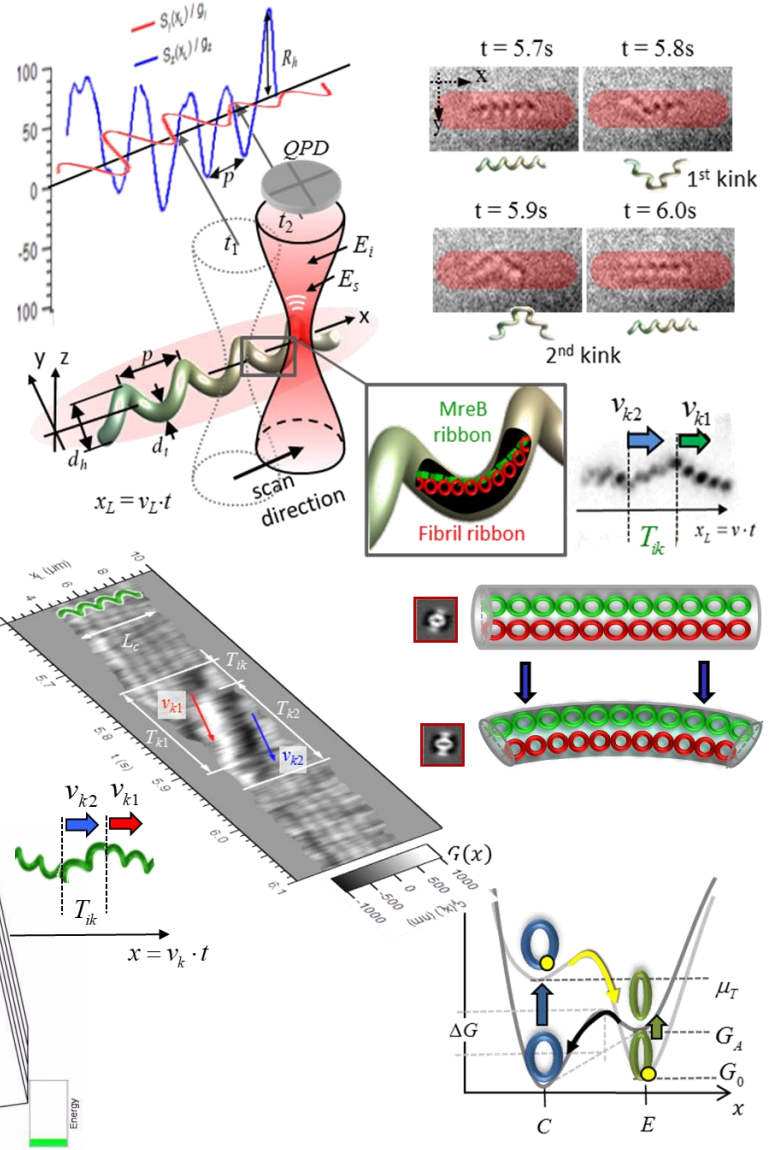
Approach: Interferometrically measured changes of the shape **c**(x) from tiny optical phase delays

$$A \cdot \Delta\varphi_s(\mathbf{c}) \approx \mathrm{Re}\left\{\tilde{\mathbf{E}}_i \cdot \tilde{\mathbf{E}}_s^*(\mathbf{c})\right\} \approx \left|\tilde{\mathbf{E}}_i\right| \cdot \left|\tilde{\mathbf{E}}_s^*(\mathbf{c})\right| \cdot \mathrm{Re}\left\{\exp\left(i\varphi_s - i\varphi_i(\mathbf{c})\right)\right\}$$

reveal deformations of the bacterium and energy changes, which differ under external forces.







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