

→ Mechano(Bio)Chemistry

MAX PLANCK RESEARCH GROUP

MECHANO(BIO)CHEMISTRY



From a Materials Science perspective, biological systems are highly sophisticated smart materials. They are stimuli-responsive and possess impressive self-reporting and self-healing properties. One example of such a multifunctional biomaterial is the extracellular matrix (ECM) that forms the interface between cells as well as between cells and surfaces. The ECM combines structural, mechanical support with biochemical signalling. Both of these functions are often fulfilled by one and the same molecule that has evolved to translate a (bio)chemical into a mechanical signal and *vice versa*.

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Biological materials in general, and the ECM in particular, are consequently an important source of inspiration for materials scientists who aim at integrating several different functions within synthetic materials. Towards this goal, we are currently focussing on four different topics: 1) Understanding the mechanical properties of the molecular building blocks of the ECM and the different strategies of converting (bio)chemical and mechanical information. 2) Design of artificial building blocks that mimic the function of ECM components, especially molecular force sensors. 3) Design of smart materials with force sensing properties. 4) Development of novel measurement techniques that allow for measuring molecular forces *in situ* both at the ensemble and at the single molecule level.

Characterization of Mechanical Building Blocks

Fibrillar proteins forming superhelical structures are important components of both the cytoskeleton and the ECM. Whereas the intracellular cytoskeletal components are mechanically well characterized, much less is known about their extracellular counterparts, *e.g.* collagen. Using synthetic superhelical peptide fragments, we aim to characterize the mechanical stability of these structural proteins at the molecular level (collaboration with Dr. L. Bertinetti and Dr. A. Masic, Biomaterials). Using single molecule force spectroscopy, we aim to investigate how length, sequence composition, assembly state and pulling direction affect the mechanical response of these important ECM components. In such a well-controlled experiment we are further able to systematically investigate the influence of molecular binding events on helix stability. We expect that these experiments will provide direct molecular insights into the interplay between the biochemical environment and structural stability.

Often ECM proteins do not only possess mechanical function. More importantly, they are equipped with specialized domains that are able to convert a mechanical into a biochemical signal. These so-called molecular force sensors (**Fig. 1**) undergo a conformational change that alters their function. In the ECM, force sensing often involves the exposure of cryptic binding sites or cryptic catalytic sites that become exposed following a mechanical stimulus. With the goal of understanding the force-sensing properties of these ECM proteins, we aim to develop novel techniques that allow

us to screen for cryptic sites in ECM proteins and to subsequently characterize their structure-function relationships (see below).

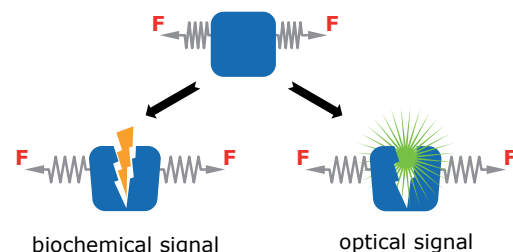


Fig. 1: Molecular force sensors convert mechanical signals into a different readout signal. a) Natural force sensors generate biochemical signals. b) Synthetic force sensors provide an 'optical signal' that can be detected with spectroscopic techniques.

Design of Synthetic Molecular Force Sensors

Inspired by the function of different ECM components, especially the molecular force sensors, we aim at developing synthetic molecules with force sensing properties. In contrast to natural force sensors, which convert the mechanical stimulus into a biochemical signal, we will equip these force sensors with an optical readout signal (**Fig. 1**). In this way, we will be able to detect the response of the sensor molecule with fluorescence microscopy techniques [1]. Depending on the desired application, different force sensor designs are possible (**Fig. 2**).

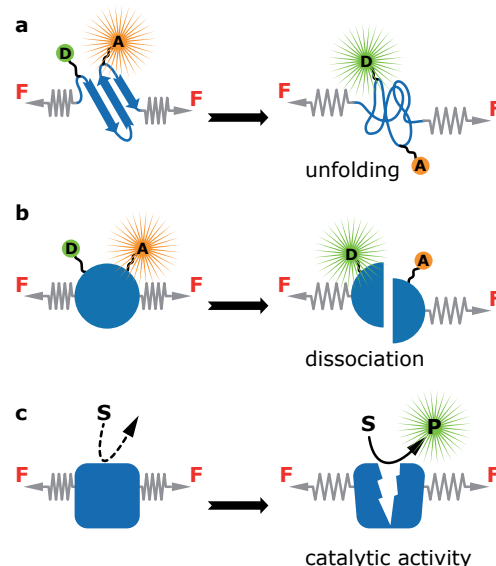


Fig. 2: Design principles of synthetic molecular force sensors. a) Monitoring the mechanical unfolding of a protein domain or a synthetic polymer using a FRET reporter system. b) FRET-based detection of the force-induced dissociation of a molecular interaction. c) Activation of a cryptic catalytic site.

In collaboration with Dr. John Dunlop (Biomaterials) we will, for example, use these sensors for measuring the forces that cells are able to exert on their environment. In this project we will immobilize integrin ligands to a solid surface via a range of dissociation-based force sensors (**Fig. 2b**) that are calibrated for different rupture forces. The sensor will remain intact if the cell-generated forces are lower than the rupture force of the sensor and the cells will be able to grow. If, on the other hand, the cell-generated forces will exceed the rupture force of the sensor, it will break, leading to a change in the FRET signal. In this way, we will be able to determine the critical force range that determines the interaction between integrins and their specific ligands at the molecular level directly in the cell culture system.

Design of Synthetic Stimuli-Responsive Materials

Utilizing the knowledge generated in the above projects, we will then combine the mechanically characterized structural ECM building blocks with synthetic molecular force sensors (**Fig. 3**). This will deliver a novel ECM mimic with unique force sensing properties. Depending on the location of the force sensors, we will be able to monitor the force distribution in the material itself (**Fig. 3a, b**). Alternatively, using a similar strategy as described above, a readout of the forces that act between cells and the matrix will become possible in a 3D cell culture environment (**Fig. 3c**).

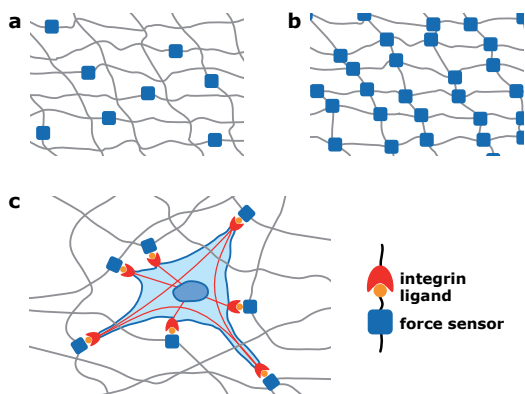


Fig. 3: Strategies for the assembly of synthetic materials with mechanosensing properties. a) Intrachain force sensors. b) Mechanosensitive crosslinkers. c) Force sensors for measuring cell-material interactions.

Development of Characterization Techniques

The identification and characterization of natural and synthetic force sensors requires a set of integrated measurement techniques that combine an activity readout with a possibility for mechanical manipulation [1]. Synthetic force sensors need to be calibrated to directly relate the force applied on the sensor molecule to the optical signal. This calibration requires a single molecule approach to provide a defined stoichiometry and to ensure that exactly one molecular force sensor is measured. We will achieve this with the integration

of atomic force microscopy with a total internal reflection fluorescence (TIRF) detection scheme. This allows us to perform single molecule force spectroscopy while following the response of the force sensor optically (**Fig. 4a**).

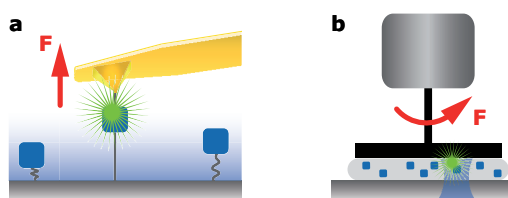


Fig. 4: Combining mechanical characterization techniques with fluorescence microscopy. a) Integrated AFM-TIRF setup. b) Confocal rheology.

Once the molecular force sensors are integrated into the material, again methods need to be developed that allow a defined mechanical manipulation of the material while following the response of the force sensors with a fluorescence measurement. A commonly applied technique for the characterization of (protein-based) polymeric materials is rheology, which applies a defined shear stress to the material. Combining a rheology setup with confocal microscopy will allow us to follow the behaviour of the force sensors as a function of the applied stress (**Fig. 4b**). Such a measurement can be performed with a high concentration of force sensors providing information about the global material response. Reducing the sensor concentration will eventually allow the localization and observation of individual sensor molecules providing unique information about the local force distribution in the material.

Lastly, we will apply these techniques not only to our synthetic systems, but also to the 'natural' ECM and its components. We will develop confocal rheology as a new screening method for identifying cryptic binding sites or catalytic sites in the ECM. Once a new cryptic site has been found, the single molecule approach will allow for a detailed characterization of its molecular mechanism. In summary, using an integrated, interdisciplinary approach, we aim to understand the interplay between mechanics and biochemistry in the ECM and to utilize these principles for designing novel stimuli-responsive materials that report on their mechanical state down to the level of their molecular building blocks.

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