

Project title: LightForce

Project number: K333/2020

Executive Summary

In LightForce, Leibniz-INM, Leibniz-DWI and ICS (University Strassbourg) envisioned light-responsive, mechanoactuated hydrogels as biophysical platforms to apply forces to cells and study mechanobiology contexts. During the project duration, the partners developed (i) synthetic rotary motors that can be coupled to materials as mechanically active components, (ii) modulators to regulate force ranges and force application rates, and (iii) mechanoactuated biomaterial platforms to quantify force fields and responses of living cells combined with atomic and traction force microscopy methodologies. The different parts have converged into advanced interfaces to study the reversible mechanoactuation performance and mechanobiological responses (talin protein accumulation, F-actin dynamics and cell traction forces). 4 postdoctoral researchers had the opportunity to closely collaborate between the groups, were exposed to interdisciplinary and international research and supported developing their scientific profiles and career.

1. Achievement of objectives and milestones

Designing mechanoactive molecules based on synthetic molecular machines as elementary building blocks of actuation (WP1). The **ICS** group has maintained continuous supply of key motor molecules to partners at **INM** for experiments in **WP2** and **WP4** throughout the project. To improve efficiency of the molecular machines, the **ICS** group performed a study in the gel state showing the importance of the light intensity, concentration of motor, and molecular weight of the polymer (*Angew. Chem.* 2023). In a complementary approach, **ICS** demonstrated the limit torque (10 pN.nm) and force that can be produced by the rotary motor by studying the relaxation of figure-of-8 motor polymer conjugates as discrete objects in solution (*J. Am Chem. Soc.* 2022). These results are important to understand relaxation events in the mechanotransduction experiments performed at **INM** in **WP4**.

Quantification of forces and relaxation times in nanomechanical experiments (WP2). Towards experiments with single motor molecules, we refined the surface chemistry protocols for attachment of motor molecules by means of Cu-based click chemistry. Single microbeads were linked to single motor molecules by means of DNA origami molecules. We successfully recorded thousands of force-extension curves for microbeads tethered to the surface by single motor molecules or by not rotating control molecules of equal linker attachment, which were both received from **ICS** within the project. The characteristic non-linear force-extension characteristic confirmed that the experiment addressed the entropic force regime of stretching the DNA linkers and, therefore, has the sensitivity to detect the expected force of single motors (~5 pN). We attempted to measure light-activated pulling forces of the single motors against the liquid flow in the microfluidic cell in two different setups, one optimized for massive parallel observation of hundreds of motors and one optimized for local, controlled irradiation. Unfortunately, no significant light-activated forces could be detected on the noise and drift background. We speculate that the entanglement of two linkers attached to one single motor may not create enough force for detection. Possibly, the entanglement of only two parallel linkers may even increase their effective persistence length and thus lead to no pulling force at all. Note that the force effects shown in other configurations of this project involve ensembles of molecular motors and an entanglement of linkers in local network or loops.

We engaged in an intensive exchange with ICS towards the design of homogeneous gels based on tetra-PEG building blocks and symmetrically functionalized molecular motors. The synthesis of the molecular functions was still under development at the end of this current project.

New molecular modulator (WP3). By addressing limitations of conventional diarylethenes (DAEs), such as poor aqueous solubility and slow thermal cycloreversion, DWI successfully developed a novel diaryltriazolium (DAT⁺) photoswitch with exceptional properties to be used as modulator in combination with the molecular motor. Using the modularity of click chemistry, a diaryltriazole (DAT) scaffold was synthesized with high yield. Initial characterization revealed promising properties yet some limitations. While DAT showed a significant redshift in the absorption of the closed isomer and exhibited millisecond-scale thermal ring-opening, it suffered from limited solubility in water and moderate fatigue resistance. To overcome these limitations, we introduced an alkylation step that converted DAT into its corresponding triazolium salt (DAT⁺). This structural modification proved transformative. The closed form of DAT⁺ exhibited a remarkable absorption maximum at 904 nm, well within the biological optical window. Moreover, its thermal half-life decreased to as little as 10 ms in polar solvents, setting a new benchmark for cycloreversion speed among diarylethene-derived systems. Crucially, DAT⁺ demonstrated excellent water solubility (>1 mM) and superior fatigue resistance. Its high aqueous solubility further positioned it as a strong candidate for biological applications. Computational analysis supported the experimental findings (in collaboration with Jun.-Prof. Christoph Bannwarth, RWTH Aachen University), revealing that the enhanced photophysical properties of DAT⁺ stem from a reduced HOMO–LUMO gap and lower activation barriers for ring-opening (*Angew. Chem.* 2024).

In parallel, DWI investigated the role of thiophene oxidation on the thermal properties of our photoswitch. Increasing the oxidation state from thiophene to its respective sulfoxide and sulfone systematically increased the thermal half-life without significantly affecting the redshift or solubility. In follow-up work, this system will be optimized for high emission and application as a fluorescent reporter for super-resolution microscopy.

To demonstrate dynamic force regulation with dual-light irradiation, a synthetic strategy to integrate the molecular motor and the modulator was developed by ICS. This required achievement of two key steps: synthesis of a new unsymmetric modulator and coupling to the molecular motor. Failure in the initial synthesis pathway for the modulator conducted to a modification of its structure. A new synthetic strategy was established involving a diarylethene moiety bearing orthogonal alcohol-protecting groups: a silyl ether and a benzyl ether. Optimisation of the reaction conditions afforded the desired product in 9 steps synthesis. Unfortunately, deprotection of the benzyl protecting groups never worked, forcing us to a change from benzyl ether to tetrahydropyranyl (THP) ether. Finally, selective deprotection of silyl or THP ether confirmed the obtention of unsymmetric modulator. Despite this final achievement, the coupling of the motor and modulator could not be concluded within the duration of LightForce project.

Biophysical experimentation platforms to study motor-driven force application on cell membrane receptors and simultaneous quantification of cell forces and mechanotransduction (WP4). Following up on our work on a synthetic biointerface for opto-actuation of individual integrins (*Nature Comm.* 2021), we expanded this platform to a light-regulated mechanoactuated hydrogel substrate to enable simultaneous force application to membrane integrins engaged in focal adhesions while simultaneously imaging intracellular force-responsive processes and quantifying cell traction forces in real time. We validated the methodology by monitoring talin protein recruitment, F-actin dynamics, and cellular traction forces alongside motor-based actuation. We demonstrated that the actuation of FAs promotes localized recruitment of talin and enhances the overall polymerization of F-actin within the cell.

With our platform we could reveal that local integrin activation could partially propagate to other regions of the cell, resulting in a reversible decrease in overall cellular traction force. We observed an unexpected decrease in traction force with mechanoactuation, which suggests a biophysical limit to integrin-mediated mechanotransduction, where there is an optimal range of force that promotes adhesion, and exceeding this range undermines the stability of adhesive contacts. We also monitored the cellular response after turning off illumination to assess the potential reversibility of the mechano-actuated cellular response. We demonstrated a positive and reversible correlation between the responses of talin and F-actin to the force exerted on the FAs. A collaborative article between INM, ICS and Prof. A García (GeorgiaTech, USA) with these successful results is close to final form and will be sent for review soon.

INM established the molecular tension sensing methodology from Salaita's Lab (GeorgiaTech, USA) to quantify the forces applied by the molecular motor to the integrins on the cell membrane. For this purpose, patterns of gold nanoparticles were formed on PEGylated layers and modified with molecular tension sensors, motor and cell adhesive ligand. We were able to calibrate the fluorescence signal to motor-induced forces. Using cells with intracellular FRET-based talin tension sensor from Prof. Carsten Grashoff (U Münster), we are now correlating the external and intracellular forces. A manuscript is currently in preparation at INM but it will need a few more months to finalize it.

Overall impact of the collaborative project: The molecular tools developed and applied in this project are unique but also very sophisticated. They require enormous parallel experimental effort and very long interdisciplinary collaboration, only possible through simultaneous funding of the groups. With our results, we are pushing the boundaries of chemical design and biophysical experimentation. The high visibility of the reported and expected publications from this work confirms the originality and relevance of our scientific approach. The partners were able to progress in the development of the experimental tools individually within the group and in the integration through the collaboration.

Changes in financial planning. In LightForce, personnel costs were the largest expenditure item for all partners, totalling €671,262.97 of the total project budget of €832,900 (net total). The actual personnel expenses incurred slightly deviated from the requested and approved budget for this item. The requested budget for all partners exceeded the 'Other direct costs' category, due to higher-than-estimated expenditure on consumables. This additional requirement could be offset in a cost-neutral manner, as expenditure on 'Travel' and 'Publications' during the project was lower than planned.

2. Activities and obstacles

The activity in the Consortium is specified in the previous section and followed by the detailed plan in WPs of the original proposal. Some parts of the project demanded more dedication than expected and delayed the completion of some of the proposed objectives, as detailed in this section. Nevertheless, the partners are highly interested in maintaining the cooperation and expect most of the open points to be completed in the future.

Following INM request, ICS attempted to attach polymer chains of defined lengths and incorporating succinimide ester groups (WP1) for the generation of highly regular 3D networks that could be mechanically tested (WP2) and applied for cell studies (Task 4.2). At first, coupling reaction was attempted using [3+2] copper-catalyzed cycloaddition with tetra-azide molecular motor, forming a mix of mono-, bis-, tri- and tetra-substituted motor. Unfortunately, difficulties were encountered during the purification process of these molecules. Although optimization of the reaction to majorly form the tetra-substituted product was done by changing different parameters like temperature, copper source, solvent, microwave, time, number of equivalents, we never succeed to purify the tetra substituted molecule. A second strategy

involving cyclooctyne moiety was also unsuccessful. The synthesis of the modulator molecule was successfully achieved, but it required far more effort than planned (WP3).

Although the coupling of the unsymmetric modulator to the motor could not be completed by DWI and ICS within the project time, the groups will continue working on this development in the future.

At INM, the quantification of forces by traction force microscopy in 3D actuated microgels could not be attempted (Task 4.2) since the 3D networks for cell encapsulation could not be developed on time. Instead, the molecular tension probes planned to be implemented in Task 4.2 were successfully integrated with previous generations of motor/PEG conjugates in 2D cell culture models to measure cell applied traction forces in real time and complemented Task 4.1.

3. Results and successes

Publications

- 3 collaborative papers are in advanced stage of preparation to be submitted in 2025.
- Diaryltriazolium Photoswitch: Reaching a Millisecond Cycloreversion with High Stability and NIR Absorption. D Kolarski, P Steinbach, C Bannwarth, K Klaue, **S Hecht**. Angew. Chem. Int. Ed. 2024, 63, e202318015
- Bending actuation of hydrogels through rotation of light-driven molecular motors. A Perrot, W Wang, E Buhler, E Moulin, **N Giuseppone**, Angew. Chem. Int. Ed. 2023, 10.1002/anie.202300263
- Light-Driven Molecular Whirligig. C Gao, A Vargas Jentzsch, E Moulin, **N Giuseppone**. J. Am. Chem. Soc. 2022, 144, 9845–9852
- Optoregulated force application to cellular receptors using molecular motors. Y Zheng, MKL Han, R Zhao, J Blass, J Zhang, DW Zhou, JR Colard-Ilté, D Datler, M Hoth, AJ García, B Qu, **R. Bennewitz, N. Giuseppone, A. de Campo**. Nature Comm. 2021, 12, 3580

Presentations at scientific conferences

Only the contributions of early career researchers from LightForce are listed below. For information on the contributions of the PIs, please refer to the corresponding table.

- **Inga Melnyk** (poster). "Nanomechanics of molecular motor molecules linked by self-assembled DNA building blocks". German Biophysical Society Meeting 2022, 25 Sept. - 28 Sept. 2022, in Konstanz, Germany.
- **Inga Melnyk** (poster). Nanomechanics of molecular motors and self-assembled DNA building blocks. GRC Multiscale Mechanochemistry and Mechanobiology, 31.07-5.08.2022 in California, USA.
- **Rinku Kumar** (poster) Characterization of motor functionality in live cell condition Cell Physics 2021. 29th Sept – 1st Oct 2021.
- **Rinku Kumar** (poster) Synthetic cellular microenvironment to study interactions at Bio interface SFB 1027 status seminar, Saarland, 1st Dec 2022.
- **Rinku Kumar** (poster) Cellular mechano-activation at molecular level using opto regulated nano-machine. Mechanocontrol Summer School IBEC Barcelona Spain 27th Sept – 1st Oct 2022.
- **Rinku Kumar** (poster) Cellular mechanoactuation at molecular level using opto-regulated nano machine CellPhysics SFB 1027 Saarland 10th Oct 2023.
- **Rinku Kumar** (poster) Mechano-actuation of integrin by light regulated Motor. MERLN 10 Year symposium, Maastricht Netherland, 16-18 Oct 2024
- **Adrien Bessaguet** (oral presentation) "Synthesis of new motor-modulator unit to control the mechanical force", Young researchers' day, october 12th 2023, University of Strasbourg, France.

- **Hosoowi Lee** (oral presentation) "Synthesis and characterisation of a new ideal network of molecular motors" Young researchers' day, octobere 12th 2023, University of Strasbourg, France

Theses and dissertations

- **Eleni Sachs**: "Synthesis and Characterisation of Iminium Ions as Room Temperature Photoswitches to Control Dynamic Covalent Chemical Bonds", Doctoral Thesis, RWTH Aachen University, 2025.

4. Equal opportunities, career development and internationalisation

The participating institutions have measures in place to support gender, internationalization and to support early career scientists, in accordance with Leibniz Standards. The involved early career scientists in this proposal were mainly at postdoctoral level to accelerate the interaction between the groups. Dr. Anil Kumar Dasanna (initially involved), Dr. I Melnyk (female, german, ukrainian) and Dr. R Kumar (male, indian) worked at INM and the two later continue working at INM in this project for a few more months. Dr. D Kolarski (male, Serbian) worked at DWI and moved on to another postdoctoral position at the MPI for Multidisciplinary Sciences in Göttingen, where he aims to further develop his DAT platform into probes for super-resolution microscopy. Following Dr. Kolarski's departure, E Sachs (female, German) was involved in the project, working on alternative photoswitches. She has recently graduated from RWTH Aachen University. Dr. Hosoowi Lee (female, South Korea) and Dr. Adrien Bessaguet (male, France) worked at ICS. Dr. Bessaguet continues working at ICS.

5. Structures and collaboration

The young scientist runs the project and leads communication and collaboration within the Consortium. They met with all PIs at yearly status meetings, ran virtually during the Corona pandemic, and in person afterwards.

The partners at INM and ICS collaborate with Prof. Andrés García (GeorgiaTech) for the study of cell responses to mechanoactuation using the rotary molecular motors. This collaboration was reinforced through a joint Marie Curie Fellow (Dr. Marc Fernández) who spent the Fellowship between GeorgiaTech and INM in 2021-23. Dr. Fernández was appointed Ass. Prof. at Queen Mary University London after his stay at INM. INM also collaborated with Prof. Carsten Grasshoff (Münster), who provided cell lines with labeled mechanosensory proteins. Through the experiments with F-actin developed in this project, Dr. Kumar established a collaboration with Prof. Heiko Rieger (U Saarland) for modelling actin dynamics. DWI collaborated with Jun.-Prof. Christoph Bannwarth (RWTH Aachen University) to rationalize various experimental findings by computational methods.

6. Quality assurance

The three participant institutions and groups have their own internal procedures for quality assurance, good scientific praxis, data management and open access publication which are in line with the guidelines from Leibniz Association.

7. Additional resources

Not relevant.

8. Outlook

The partners plan to continue the cooperation and progress in the further integration of the molecular tools into advanced platforms for mechanobiology experimentation in 2D and 3D.