C2: Development of mechanically responsive sensors

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Presentations

- 7 oral IRTG
- 7 posters IRTG
- 4 oral other
- 2 posters other

Publications

[1] J. Davila, A. Chassepot, J. Longo et al., *JACS*, 2012, *134*, 83–86.

[2] C. Rios, J. Longo et al., Angewandte Chemie 2014, *submitted.*

[3] J.longo et al., Langmuir 2014, *in preparation.*

Other activities

•Participation in Science Days

•Participation in 8th Workshop „Molecular Interactions" (Berlin)

•Stay of one Week in the Institute for Biochemistry (Freiburg)

•Scientific exchange with IS2M (Mulhouse)

Motivation

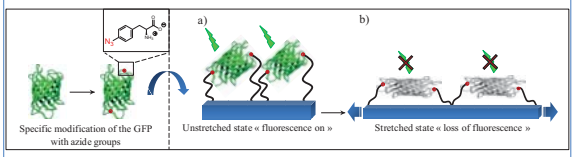
In nature, all living cells face mechanical forces and transform these into sets of biochemical signals. The mechanisms by which mechanical forces eventually lead to biochemical and molecular responses often remains undefined. It is suggested that partial protein unfolding under cell-derived forces exposes active sites. This concept can be used to develop protein. The goal of our IRTG project was to design mechanoresponsive materials that transform a mechanical stress into a chemical signal by mimicking the physical processes used by nature, namely based on protein conformational changes.

To develop a "mechanosensor", two parts of works are included.

• Biological site (Group Schiller): Modify proteins at two well-defined positions. For the site-specific covalent immobilization onto a stretchable material, we modify proteins with genetically encoded unnatural amino acid.

• Material site (Group Schaaf): creation of a mechanically responsive material. The sensor is based on the site-selective immobilization of a protein onto a stretchable material. Stretching the material, allows manipulating the protein in several ways. It can expose the active site for a potential reaction, change its function or properties or a binding partner, and allows us to modulate the biochemical reactivity by mechanical stimuli.

Strategy

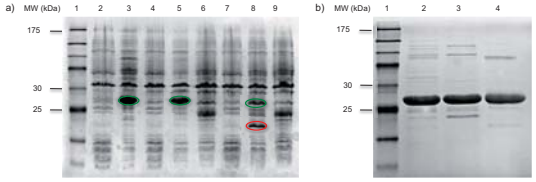


-Figure 1: Schematic representation of a mechano sensor. Pull the matrix induce lose the fluorescence (switch-off) and release the matrix recover the fluorescence (switch-on). GFP: green fluorescent protein.

Methods and results

Modify GFP with two site-specifiy functions

To graft the protein covalently onto the networks for stretching, we need to modifiy the protein at two defined positions. Utilizing an orthogonal *M. jannaschii* tRNA/tRNA-synthetase pair, we inserted unnatural amino acids (*p*-azidophenylalanine, *p*AzF), e.g. with an azide side chain into proteins in E. Coli. This azide functional group is used to bind the protein onto the surface of a Polymer-Network using the Huisgen-Azide-Alkyne cycloaddition ("click-reaction").



-Figure 2: a) SDS-PAGE analysis of expressions test. Lane 1 molecular weight standards (kDa), lane 3 wild-type GFP as reference, lane 5 modified GFP with mono-functional azide at position 39, lane 8 modified GFP with bi-functional azide at positions 39 and 182. Lane 2, 4, 7 are non-induced test, lane 6, 9 are the tests in absence of *p*AzF, in these tests no GFP was produced. The expressed full-length GFP variants are marked in green, and truncated GFP182Stop as by-product is marked in red. b) SDS-PAGE analysis of wild-type GFP and modified GFP after purification with Ni-NTA column. Lane 1 molecular weight standards, lane 2 wild-type GFP as reference lane 3 modified GFP with mono-functional azide at position 39 (GFPY39*p*AzF), lane 4 modified GFP with bi-functional azide at positions 39 and 182 (GFPY39*p*AzF&Y182*p*AzF).

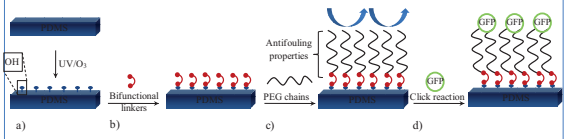
Chracterization of with Azid modified GFPs

The fidelity of the incorporation of p-azidophenylalanine was characterised with Electrospray ionization mass spectrometry (ESI-MS).

Protein	Sequence (26-42)	M (cal.)	M (found)
GFP	K.FSVSGEGEGDATYGK.L	1502,6525	1502,6525
GFP-Y39*p*AzF	K.FSVSGEGEGDATY*GK.L	1502,6525	1527,6620
GFP-Y39*p*AzF-Y182*p*AzF	K.FSVSGEGEGDATY*GK.L	1502,6525	1527,6620

-Tab. 1: List of the modified peptide fragments obtained after digestion with trypsin. Y* = mutaion of Y to *p*AzF

PDMS substrate functionalization (Polydimethylsiloxan)

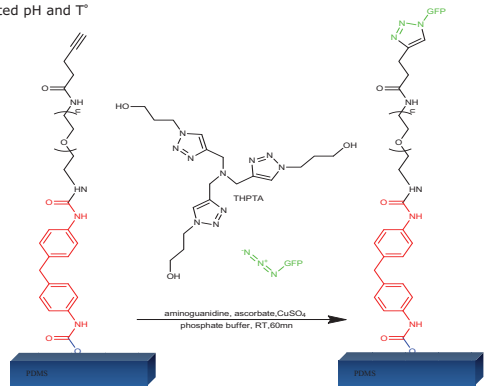


-Figure 3: Schematic representation of the different parts of the project corresponding to (a) oxidation of the surface; (b) protein grafting through click-chemistry and to avoid non-specific protein adsorption

GFP coupling through „Click Chemistry"

Proteins are fragile and have to stay in specific environment to avoid degradation, thus soft conditions of reaction are needed:

- Use of ligands (THPTA)
- adjusted pH and T°

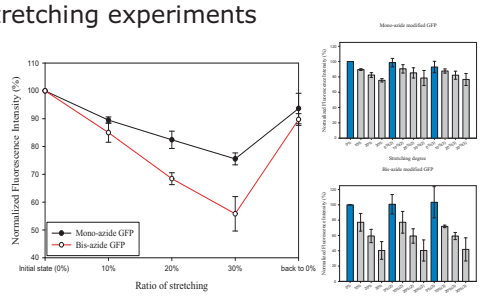


Fluorescence analyses

Sample	Fluorescence Intensity (a.u.)
(a) MDI functionalization + GFP 1H	2196
(a) PEG 3000 functionalization + GFP 1H	227
(a) PEG 3000 functionalization + click GFP 1H	1229

- Elastomeric substrate
- Step-by-step functionalization
- Homogenous modification
- Antifouling properties
- No degradation of GFP

Stretching experiments



One observes a decrease of the fluorescence for both the bis and mono modified GFP. Yet, for the bis-functionalized GFP the fluorescence decrease is about 60% of the initial fluorescence whereas it is only 20% for the mono-functionalized GFP. The decrease for the mono-functionalized GFP can be attributed to a decrease of the overall GFP surface density by stretching as determined above. These results indicate that the fluorescence of the stretched bis-functionalized GFP decreases strongly by stretching the substrate. The larger decrease of fluorescence of the bis-modified GFP compared to the mono-modified GFP can be attributed to the deformation of the GFP itself leading to important modifications in its fluorescence.

Conclusions & outlook

In this project we proved the possibility of putting out of shape a biomacromolecule grafted in a material such as GFP, through stretching constraints. We were able to "visualize" this conformational modification by changes in its fluorescence properties coming from physical modification of its chromophore environment.

In the next works, we planned to adapt this concept to enzymatic active films. by covalently grafting enzymes onto stretchable substrates. Stretching such substrates should induce changes in the conformation of grafted enzymes and lead to changes in their enzymatic activity.