Conformational dynamics and protein function studied by hpFRET

Supervisors / institute:

Main supervisor: Claus Seidel, Lehrstuhl für molekulare physikalische Chemie, HHU
Co-supervisor: Karl-Erich Jaeger, IMET, HHU

Project background and description:

Background. Proteins are inherently plastic molecules, whose function often critically depends on equilibria between different molecular conformations (conformers).Thus, experimental tools and a rigorous understanding of the relation between protein structure, dynamics and function should be worked out. We developed new robust concepts of filtered Fluorescence Correlation Spectroscopy (fFCS) to study the conformational dynamics of proteins in a wide time range (50 ns -10 ms) using fluorescence anisotropy and/or Förster resonance energy transfer (FRET).

Main goal. In this project the Seidel group will collaborate with the group of Karl-Erich Jaeger to study the molecular mechanisms of the membrane chaperone Lif (lipase-specific foldase) for which it has been shown that is interacting with proteins of the large Sec complex located at inner membrane of Pseudomonas aeruginosa. The Sec-machinery accomplishes the translocation of lipase LipA through the inner membrane and at the same time the foldase converts these lipases into their enzymatically active conformation. We want to study the conformational changes of the chaperone Lif during the conversion of its substrate, an inactive lipase, into an enzymatically active conformation by an as yet unknown molecular mechanism. High-precision Förster-Resonance Energy Transfer (hp-FRET) will be applied to characterize the binding mode as well as the conformational transitions of the lipase LipA and its foldase (Lif) of Pseudomonas aeruginosa in their isolated forms and during complex formation, folding and catalysis. Moreover, we will use FCS and fFCS to measure the affinities and elucidate the molecular mechanism of interaction of the foldase with the bacterial membrane bound secretion complex. The results of single molecule fluorescence measurements will be combined with molecular dynamics simulations (Holger Gohlke) and NMR measurements (Dieter Willbold).

Aims of the project:

Subproject 1. Deciphering the structural basis for the function and the corresponding conformational changes of Lif.
Subproject 2. Analysis of the conformational changes during folding of LipA.
Subproject 3. Monitoring complexation of LipA and the kinetics of folding in artificial membrane systems.
Subproject 4. Characterization of the interactions of Lif and the SecEYG complex of *P. aeruginosa*.

About collaboration and networking

Molecular biology, biochemistry and fluorescence labelling will be carried out in collaboration with the group of C. Karl-Erich Jaeger, the biophysical studies are done in the Seidel group, techniques complementing the FRET measurements will be performed as well: molecular dynamics simulations (H. Gohlke), NMR measurements (D. Willbold).

Requirements:

- Master degree in biotechnology, biochemistry, chemistry or physics.
- Experience in fluorescence spectroscopy would be useful (not mandatory).

Additional information:

www.mpc.uni-duesseldorf.de
An overview on the applied fluorescence techniques can be found in: Sisamakis, E., Valeri, A., Kalinin, S., Rothwell, P. J., Seidel, C. A. M. Accurate single-molecule FRET studies using multiparameter fluorescence detection. Methods Enzymol. **475**, 455-514 (2010).