Universität Basel

Swiss Nanoscience Institute

SNI SWISS NANOSCIENCE INSTITUTE
EINE INITIATIVE DER UNIVERSITÄT BASEL UND DES KANTONS AARGAU

Project P1503 Watching Giant Multienzymes at Work using High-Speed AFM

Main proposer: **Timm Maier,** Biozentrum, University of Basel, Switzerland, **timm.maier@unibas.ch**
Co-proposer: **Roderick Lim,** Biozentrum and the Swiss Nanoscience Institute, University, of Basel, **roderick.lim@unibas.ch**

Multienzymes are biological nanofactories that integrate all functional components required for multiple catalytic steps into a single protein assembly. Carrier-protein-based multienzymes (CPMEs) tether a nascent product to a mobile carrier protein domain, which supports the synthesis of insoluble or complex products such as fatty acids [1, 2] and polyketides [3-6], the most successful class of natural products in drug discovery. In human biology, CPMEs are involved in committed steps of metabolism, e.g. in lipid or sterol biosynthesis, with close links to cancerogenesis. A key characteristic of the 10-20nm sized multienzymes is their highly dynamic protein architecture. First, the carrier domains are flexibly tethered on linkers of 10 - 30 amino acids length. Second, most CPMEs feature large-scale motions of individual domains or multidomain regions. The fundamental nature of overall conformational changes is just emerging and has recently been supported by structural and functional studies [7-9].

T. Maier has strongly contributed to studies of multienzymes organization by revealing the core architecture of a 2.6MDa fungal multienzyme [1]; uncovering the architecture of mammalian fatty acid synthase [2, 10]; deciphering evolutionary origins of the complex multienzyme architectures [11]. However, revealing principles of multienzyme function requires an understanding of the interplay between catalysis and nanomechanical properties.

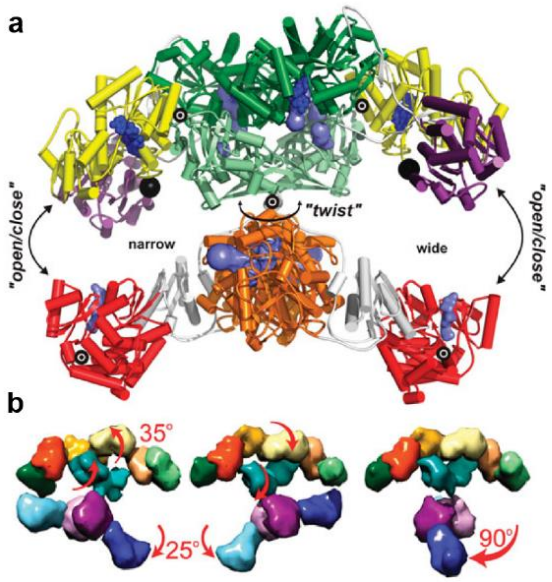

Fig. 1: The dynamic multienzyme architecture of animal fatty acid synthase. (a) Crystal Structure of porcine fatty acid synthase (FAS). FAS is a head-to-head dimer containing two times five catalytic domains for fatty acid elongation. It catalyzes more than 40 reaction steps for the biosynthesis of fatty acids from carbohydrate derived precursors. FAS is segregated into two halves, which based on the function of the respective catalytic domains are referred to as the condensing (lower) and the modifying (upper) region. Structural anaylsis suggested potential hinge regions. (b) Electron microscopy analysis experimentally confirmed large scale motions of the condensing relative to the modifying region, as well as conformational changes within the modifying region and suggested a coupling of large-scale dynamics to the enzymatic state of FAS. (modified from Maier et al., Quart. Rev. Biophys, 2010)

In this project**, we will develop and optimize strategies for immobilizing multienzymes and apply state-of-the-art HSAFM to directly visualize their functional dynamics at sub-100 ms time-scales.** Specifically, we will study fatty acids synthases[10] as an established multienzyme system as well as carboxylases [12-14]. Dynamic HSAFM data will be integrated with high-resolution structural data from X-ray crystallography and electron microscopy as well as simulations and single-molecule fluorescence analysis. Importantly, this project will lead to a quantitative multi-scale model that describes how multienzyme function derives from the complex interplay between catalysis and nanomechanics.

Open questions include:

(i) What is the time-scale of large-scale motions in a resting state?
(ii) Are protein dynamics influenced by the presence of substrate under turnover conditions?
(iii) Can different modes of structural changes be distinguished?
(iv) Does trapping at a specific step stabilize a particular conformation?

This study takes advantage of established expertise and experimental capabilities in both collaborating labs and offers an excellent opportunity for an interdisciplinary PhD project at the interface of nanoscience, single-molecule biophysics and structural biology.

Maier Lab: Eukaryotic protein production, protein purification, enzymatic characterization of multi-enzymes, chemical crosslinking mass spectrometry (XL-MS), X-ray crystallography of giant multienzymes, electron microscopy, chemo-enzymatic trapping

Lim Lab: High speed-AFM (HS-AFM), scanning electron microscopy (SEM)-based fabrication of HSAFM carbon-nanotube tips, AFM for force spectroscopy, surface plasmon resonance for testing immobilization strategies, fluorescence microscopy, single particle tracking, quantitative physical modeling

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