Name	RADOSLAV ENCHEV
Position	Group Leader (1st 6)
Year joined *(Crick or founder institute)*	2018

Career History

2003 – 2006 BSc in Molecular Biotechnology, Heidelberg University
2006 – 2010 PhD in Structural Biology, Institute of Cancer Research, London
2010 – 2011 Postdoctoral Associate, Institute of Cancer Research, London
2011 – 2016 Postdoctoral Associate, ETH Zurich
2016 – 2018 Project Leader and Lecturer (“Oberassistent”), ETH Zurich

Major Awards, Honours and Prizes

Membership of external committees, editorial boards, review panels, SABs etc

Lab Name	***Visual Biochemistry Laboratory***

Research programme and achievements

Molecular biology research aims to obtain a comprehensive mechanistic description of cellular physiology. In general, the best-case outcome of most biochemical studies is the structural and kinetic characterisation of their underlying chemical processes. Since no single experimental technique can directly deliver such information in a broadly applicable manner, structural studies of usually static macromolecules are complemented by kinetic characterisation and molecular dynamics simulations. In practice progress is often impeded by the typical requirement for large amounts of labelled or crystallised samples. Moreover, the widely-used strategy of using mutants or crosslinking probes to stabilise otherwise short-lived catalytic intermediates may be prone to artefacts and precludes time-resolved tracking of molecular events.

My group is developing and applying a method that largely overcomes these limitations by allowing the direct observation of biochemical processes at atomic spatial- and milliseconds time-resolution by combining microfluidics and electron cryomicroscopy (cryo-EM). We recently published a prototype that reproducibly prepares cryo-EM samples, improves the quality of the sample relative to standard methods and allows monitoring of the progression of a biochemical process over three orders of magnitude of

time (tens to thousands of milliseconds)[1]. It consists of a syringe pump driven microfluidic chip that ensures mixing and incubation of two samples, a nozzle which uses gas to spray the sample as a thin plume onto a sample grid and plunging into liquid ethane. The reactants are subjected to subsequent 3D structure determination by cryo-EM and single particle analysis. Iterating the procedure at increasing incubation times after sample mixing allows the visualisation of a biochemical binding and/or enzymatic reaction as a time-lapse “movie”.

An engineering subgroup in the lab has substantially improved the published workflow and has nearly completed a next-generation device. It achieves higher quality sample preparation with sub-microlitre sample amounts, owing to complete automation through an on-board computer, elaborate valving and use of additive manufacturing for integration of the microfluidic chip and a modified nozzle. We anticipate that these advances will greatly accelerate the adoption of this new standard in preparing samples for cryo-EM and single particle analysis, enabling off-equilibrium structural studies and facilitating the technology’s spread to academic and industrial applications.

Naturally, we are applying our method to fundamental biological questions. The core biological interests of the group are two-fold: elucidating the structural basis for the molecular mechanisms of DNA double strand break repair by homologous recombination and the regulation of ubiquitin signalling.

Genome integrity is essential for organismal health and reproduction. However, the chemical environment and cellular metabolism frequently cause DNA damage, counteracted by multiple repair pathways. Homologous recombination repair (HRR) is responsible for the nearly error-free restoration of DNA double-strand breaks and is of tremendous importance to human health and gene technology and therapy.

Although many HRR components are amenable to *in vitro* analysis, their functionally relevant interactions are often transient and recalcitrant to conventional trapping techniques. This limits the insights of structural studies and impedes the development of better-targeted diagnostics and therapy. The key questions we are pursuing revolve around uncovering the structural and kinetic basis for specificity during Rad51-mediated homology search. We have already demonstrated the utility of time-resolved cryo-EM to visualise and quantify the ATP-dependent growth of filaments of the bacterial recombinase RecA on ssDNA. We are now focusing on applying the method to study microhomology search intermediates by elucidating structural specificity determinants and directly correlating them to kinetic behaviour. We will extend these studies to human Rad51 and paralogues, and comprehensively describe the defining biochemical step of homologous recombination.

Ubiquitination is a versatile signalling mechanism in eukaryotes, which regulates most aspects of cellular metabolism. Ubiquitination itself is a tightly regulated process and the biochemical pathways regulating its specificity and signalling outcomes present promising drug targets. Cullin-RING E3 ligases (CRLs) comprise nearly half of all cellular ubiquitin ligases. All CRLs are regulated by only a few factors which collectively reshape the cellular CRL pool and enable various rapid adaptive cellular responses to internal and external cues. Despite a wealth of biochemical and cellular data, how a specific CRL is assembled following a signalling event remains poorly understood because the underlying biochemical events are either too short-lived and/or mediated by very low affinity interactions to be easily tractable by conventional structural determination techniques. Current projects in the lab are using time-resolved cryo-EM analysis to elucidate the

detailed mechanisms of CSN-mediated CRL deneddylation and CAND1-mediated exchange of CRL substrate receptors.

Research outputs

Mäeots M.E., Lee B., Nans A., Jeong, S.G., Esfahani M.M.N., Ding S., Smith D.J., Lee C.S., Lee S., Peter M., Enchev R.I. (2020) ***Modular microfluidics enables kinetic insight from time-resolved cryo-EM*****. Nat. Commun. 11, 1–14. DOI: 10.1038/s41467-020-17230-4**
Cryo-EM has the potential to study any native conformation of a macromolecule. However, the sample preparation time is high, compared to the timescale of most protein interactions and conformational changes. In this paper, we established a robust method of time-resolved cryo-EM sample preparation. We produced high-quality samples for microscopy while speeding up the process of making them by several orders of magnitude. This allowed samples to be collected within 30ms of the initiation of a biochemical reaction, within the timeframe of many critically important and interesting processes. This enables a whole new class of experiments in structural biology research.

Mosadeghi R., Reichermeier K.M., Winkler M., Schreiber A., Reitsma J.M., Zhang Y., Stengel F., Cao J., Kim M., Sweredoski M.J., Hess S., Leitner A., Aebersold R., Peter M., Deshaies R.J and Enchev R. I. (2016) ***Structural and kinetic analysis of CSN activation and the cullin–RING ubiquitin ligase deneddylation cycle*****. eLife, 10.7554/eLife.12102. DOI: 10.7554/eLife.12102**
Cullin–RING ubiquitin E3 ligases (CRL) comprise one of the largest families of eukaryotic regulatory enzymes and control most cellular processes. They are activated by neddylation, covalent attachment of the ubiquitin-like protein Nedd8. This modification is reversed by a large molecular machine, the COP9 signalosome (CSN). This paper presents a comprehensive kinetic analysis of CSN-CRL binding and de-neddylation and elucidated their structural underpinnings. CSN uses a complex induced-fit mechanism to evolve a very high affinity catalytic intermediate, which rapidly dissociates after catalysis. This mechanism shapes the entire cellular CRL network, enabling an incredibly rapid adaptive response to a changing environment.