EMBL Australia
Single Molecule Science

Special seminar
Kirby Seminar Room, Level 6, Wallace Wurth Building
11am-12pm, Friday 28th February 2020

UNSW AUSTRALIA

Prof. Jörg Fitter
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The Cell Motility & Mechanobiology group and SoMS are proud to host Prof. Jörg Fitter for a special seminar at 11am-12pm, February 28th 2020.

Single-molecule studies on structure, dynamics and interactions of proteins: mechanisms and tool development

Abstract Cell-free protein synthesis systems offer enormous opportunities for the application of single-molecule techniques. For example, by employing confocal two-color coincidence detection (TCCD) the fraction of active ribosomes in a population, as well as their productivity can be determined [1]. Based on a further development of the TCCD method, we monitored the subunit dissociation of 70S ribosomes for translation initiation in a cell free transcription/translation assay and unraveled a previously undetermined second initiation pathway in bacterial protein synthesis [2]. In a further approach we extended single-molecule FRET (smFRET) studies, which typically require bright organic fluorescent dyes, to genetically encoded FRET-based biosensors with two different fluorescent proteins employed as donor/acceptor pair. The obtained smFRET data provides valuable insights into the sensor performance and thereby helps to understand and to optimise the design of FRET-based sensors [3]. In a last example we investigated unfolding/folding transitions in a multi-domain protein. For phosphyglycerate kinase, a two-domain protein, we mapped several intra-molecular distances upon chemical denaturation to identify folding intermediates [4]. A key in producing suitable FRET samples for multiple distances is to make use of new fluorescent labelling strategies, for example by employing unnatural amino acids in cell-free protein synthesis [5].

References

1. N. Kempf et al., Scientific Reports, 7: 46753, (2017)
2. H. Höfig et al., Communications Biology, 2: 459, (2019)
3. H. Höfig et al., ACS Sensors, 3: 1462-1470, (2018)
4. M. Cerminara et al., Biophysical Journal, in press (2020)
5. M. Sadoine et al., Analytical Chemistry, 89: 11278-11285, (2017)

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