LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN GRK2062

WHAT'S GOING ON

in the Research Training Group Molecular Principles of Synthetic Biology

NEWSLETTER #4 2017

FOCUS ON RESEARCH

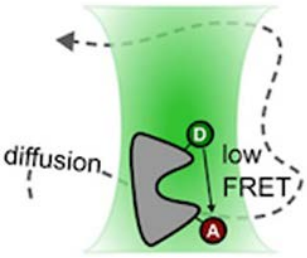

Figure 1. *Schematic respresentation of intramolecular smFRET analysis of diffusing particles to assess conformational states of proteins.*

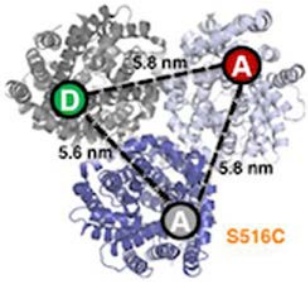

Figure 2. *Crystal structure of the secondary transporter BetP with a particular set of labels and associated distances using in a novel smFRET assays dubbed "caged FRET".*

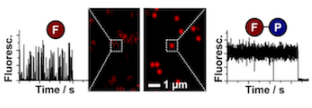

Figure 3. *Illustration of effects of covalently-linked photostabilizer molecules on the photophysics of organic fluorophores using single-molecule fluorescence microscopy. While the photophysical properties of*

Prof. Dr. Thorben Cordes

C3 / Area C Synthetic Systems

Physical and Synthetic Biology

The Cordes group specializes in the development and application of novel spectroscopy and imaging techniques that allow to map structure and function of biomolecules and (bio)chemical processes in space and time. For this the group uses a combination of optical techniques (single-molecule fluorescence spectroscopy & super-resolution imaging) with nanoscale sensors, i.e., fluorescent probes.

Molecular mechanisms of membrane transport

Active membrane transport proteins play crucial roles in numerous cellular processes. Despite their importance, all proposed molecular models for transport are based on indirect evidence due to the inability of classical biophysical and biochemical techniques to directly visualize structural dynamics. The group is using single-molecule tools to decipher the molecular mechanisms of transport of these complex machines directly.

Probe development, spectroscopy, biolabelling and novel biophysical assays

Fluorescence emission has evolved to an indispensable tool in the life sciences. Fluorescent probes intrinsically suffer from transient excursions to dark states limiting signal height and stability as well as from irreversible photochemical destruction (“photobleaching”) that restricts their observation time. The Cordes lab is developing novel fluorophores with self-healing or other functional properties imparted by the covalent linkage of e.g., photostabilizers to the fluorophore. These vastly improved properties have proven to be crucial for advanced fluorescence applications and super-resolution microscopy (STED & STORM). Current projects focus on optimization of chemical linkage of photostabilizer-dye conjugates and their application in live-cells and advancing the information content of biophysical assays.

single dye molecules show uncontrolled blinking and fast bleaching (left) the photostabilizer-dye conjugate has a stable and constant emission for pro-longed time periods.

GRK2062 PUBLICATION

ACS Synthetic Biology 2017

A Versatile Toolbox for the Control of Protein Levels using *N*ε-acetyl-L-lysine Dependent Amber Suppression

Wolfram Volkwein, Christopher Maier, Ralph Krafczyk, Kirsten Jung and Jürgen Lassak

Abstract

The analysis of the function of essential genes *in vivo* depends on the ability to experimentally modulate levels of their protein products. Current methods to address this are based on transcriptional or post-transcriptional regulation of mRNAs, but approaches based on the exploitation of translation regulation have so far been neglected. Here we describe a toolbox, based on amber suppression in the presence of *N*ε-acetyl-l-lysine (AcK), for translational tuning of protein output. We chose the highly sensitive luminescence system LuxCDABE as a reporter and incorporated a UAG stop codon into the gene for the reductase subunit LuxC. The system was used to measure and compare the effects of AcK- and *N*ε-(*tert*-butoxycarbonyl)-l-lysine (BocK) dependent amber suppression in *Escherichia coli*. We also demonstrate here that, in combination with transcriptional regulation, the system allows protein production to be either totally repressed or gradually adjusted. To identify sequence motifs that provide improved translational regulation, we varied the sequence context of the amber codon and found that insertion of two preceding prolines drastically decreases luminescence. In addition, using LacZ as a reporter, we demonstrated that a strain encoding a variant with a Pro-Pro amber motif can only grow on lactose when AcK is supplied, thus confirming the tight translational regulation of protein output. In parallel, we constructed an *E. coli* strain that carries an isopropyl β-d-1-thiogalactopyranoside (IPTG)-inducible version of the AcK-tRNA synthetase (AcKRS) gene on the chromosome, thus preventing mischarging of noncognate substrates. Subsequently, a diaminopimelic acid auxotrophic mutant (Δ*dapA*) was generated demonstrating the potential of this strain in regulating essential gene products. Furthermore, we assembled a set of vectors based

on the broad-host-range pBBR *ori* that enable the AcK-dependent amber suppression system to control protein output not only in *E. coli*, but also in *Salmonella enterica* and *Vibrio cholerae*.

Full text http://dx.doi.org/10.1021/acssynbio.7b00048

NEW MEMBERS

PhD-students

Zhenghuan Guo, M.Sc., started his PhD study in June 2017. Supervised by Jürgen Lassak he is focusing on "Novel protein deoxyhexose modifications in bacteria: Evolution and Function of EF-P rhamnosylation".

Rebecca Mächtel, M.Sc., finished her master studies at the Technische Universität München. She joined the Cordes lab in July 2017 for her PhD study "Sensitive fluorescence monitoring of ATP turnover and small molecule transport".

FAREWELLS

After submitting her dissertation in May 2017 **Chiara Gandini** has joined the Rothamsted Research Institute near London. Chiara was working as PhD-student in the Leister lab. We wish her all the best for her future career and of course we stay in touch!

EVENTS

Joint Retreat 2017 - ICMSE Pre-conference and ICMSE main conference

Date: August 26-27, 2017 and August 27-29, 2017
Location: Uni Basel, Switzerland
Arrival: We will start by bus on August 25 at 12:00 pm at

München Ostbahnhof, next stop is LMU Biocenter in Martinsried (bus stop "LMU Martinsried") at 12:45 pm. Basel will be reached around 6:30 pm.
Accommodation: Hotel Spalentor (about 4 min to walk to the conference location).

Program: On Saturday, August 26 our joint Retreat will start at 9 am with a talk of Benjamin Davis followed by sessions of the FMS Research Center.
In the afternoon, Vincent Noireaux who was invited by our Junior Researchers Committee will give a talk about "Engineering and testing gene circuits with a cell-free TXTL toolbox". After that Tobias Härtel will give an overview about research topics of our Research Training Group. The following GRK2062 junior researchers have been elected to present their talks afterwards: Fridtjof Brauns, Mona Dotzler, Matthäus Schwarz-Schilling, Christopher Scheidler.
On Sunday, after a talk of Roy Bar-Ziv, we will get deeper insights into research areas of NCCR Molecular Systems Engeneering.

ICMSE main conference will start on Sunday, August 27 at 6 pm. A tentative schedule can be found here. There will be a bus transfer back to Munich in the evening of August 29, 2017.

Upcoming Transferable Skills Courses

For women only: **Führungswerkstatt** by Dr. Brigitte Winkler
The course will run on October 17, 2017 at LMU Biocenter.
Please note: the course is held in German, but contributions in English are welcome.
For details of the program and registration please contact grk2062@bio.lmu.de

Effective Visual Communication for Scientists, Seyens
If created properly, graphics are the most effective way to explain complex ideas in the shortest amount of time, attract audience and raise credibility. Nevertheless, researchers aren't trained in visual communication in the traditional PhD curricula and are supposed to acquire these skills by themselves. This workshop uses a hands-on approach to help researchers visually present their own research through various means of scientific communication. The course will run on the 29th and 30th of November 2017 in room N01.012 at BMC in Martinsried.
For registration please contact grk2062@bio.lmu.de

Scientific Writing, Science Craft
This course will run on the 22nd and 23rd of February 2018 at LMU Biocenter. For a full description please click here.

JOURNAL CLUB

Science 18 June 2016

News

Scientists want to replace lab workhorse *E. coli* with the world's fastest-growing bacterium

Ben Panko

One month after being outed as the co-organizer of a controversial project to synthesize the human genome, Harvard University geneticist George Church is now looking to rock the microbiology world. In a preprint article published online on bioRxiv, he and colleagues propose an alternative to the workhorse research organism *Escherichia coli*. They argue that scientific experiments are unnecessarily prolonged by waiting for *E. coli* to grow and reproduce. As a new model prokaryote—and a potential maker of medicines and other compounds— the team offers *Vibrio natriegens*, a salt marsh denizen that is the fastest-growing bacterium known.

Since its discovery 131 years ago, *E. coli* has become the go-to bacterium for fundamental explorations of microbiology, analyses of gene function, and much more. *E. coli*'s favored status comes from its ease of cultivation and its safety—the four strains commonly used as model organisms have adapted well to growing in laboratories over decades of use, and they have lost their ability to infect the human intestines. They also reproduce relatively quickly, doubling in number every 20 minutes in their ideal growth medium. But the biggest factor driving their popularity is inertia—*E. coli* is a reliably known bacterium with a long history of documentation.

“We use *E. coli* just because we know the most about it,” says Harvard geneticist Henry Lee, who collaborated with Church on the *Vibrio* proposal. Coming from the field of electrical engineering, Lee was dismayed at how much time was spent in genomics research simply waiting for things to grow. This drove him to look for a better alternative, and he landed on *V. natriegens*, a bacterium that doubles in number every 10 minutes in ideal conditions, compared to twice the time in *E. coli* and 12 to 16 hours in the bacterium that causes tuberculosis.

V. natriegens shares a genus with *Vibrio cholerae*, the bacterium behind cholera. However, there's no evidence *V. natriegens* itself is harmful to people, Lee says. During

testing, it was not susceptible to the same viruses, known as bacteriophages, that cause other *Vibrio* bacterium to produce the cholera toxins.

To encourage *V. natriegens'* adoption and testing by other labs, Lee and his team have sequenced its full genome for the first time, making it—along with a list of tested growing conditions for the bacterium—public. They've also developed a version of the CRISPR genome-editing system that works on the bacterium. “We want to develop tools that would make it a drop in, turn-key alternative for *E. coli*,” Lee says.

The microbiology world seems cautiously intrigued by Lee and Church's proposal. Columbia University biologist Harris Wang, who uses *E. coli* to study synthetic biology and genomes, says that further research needs to explore how stable the organism's genome will be over generations of experimentation and how the salty growing conditions of *V. natriegens* might affect extracting purified DNA from the microbes, a common step in many studies. “Nonetheless, I think it's an exciting area to explore and certainly a useful platform if these and other considerations are addressed in the future,” Wang says.

Biologist Richard Lenski at Michigan State University in East Lansing, who has charted the long-term evolution of a batch of *E. coli* for more than 28 years, also sees promise in *V. natriegens*, though he's unsure how much researchers will actually benefit from its reproductive prowess. “I don't know whether most research and applications are really limited by rapid growth, once you're already down into the range where cultures easily replace themselves in a day,” Lenski says. “But time will tell, and it will certainly be interesting to learn more about this organism.”

Related articles:

Nature Methods 13, 849–851 (2016): *Vibrio natriegens* as a fast-growing host for molecular biology

ACS Synth. Biol., June 2017: Multiplex Genome Editing by Natural Transformation (MuGENT) for Synthetic Biology in *Vibrio natriegens*

MISCELLANEOUS

Responsible Research - Toolbox

On July 20, about 250 participants took part in a great event engaging in good scientific conduct especially focusing on reproducible research.

As part of the take home message the organizers and speakers put together additional information about **Good Scientific Practice** in the form of a toolbox.

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