POSTERS

Monday 11 September 13:00–15:00

Synthetic Biology

P.1.1-001
Optogenestat –mini photo bioreactors for *in vivo,* real time characterization and and evolutionary tuning of bacterial optogenetic circuits

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Gene circuits have been dynamically characterized by using chemical effectors molecules, which has been limited by diffusion of effector across the cellular membrane. Optical method for creating on-demand protein signals in live cells would bypass limitations and, in principle, enable the dynamical characterization of virtually any gene circuit that responds to changes in protein concentration. Current standard protocol to characterize such optogenetic circuit using flow cytometer is tedious, labor intensive and cumbersome. So far very limited data is available for different growth conditions. In this work, we engineer a bioreactor of working volume ~10 mL specifically designed for optogenetic characterization of light sensing *E. coli*. We develop an integrated bioreactor that uses optogenetic control for quantitative, up/down control of gene expression and monitors the measurement of the relevant parameters during the microbial growth. The optical density, fluorescence detection for green fluorescence protein reporter as well as the input stimuli is provided by light emitting diode (LED) at multiplexed wavelength. The light sensing *E. Coli* harbors a synthetic two component system (TCS) circuit from CcaS-CcaR system used in the chromatic adaption of cyanobacteria *synechocystis* PCC 6803. The sensor histidine kinase CcaS is produced in a green-absorbing ground state. Absorption of green light flips CcaS to a kinase active red-absorbing state that phosphorylates the response regulator CcaR, which then binds to the *cpcG2* promoter and activates transcription. We aslo evolutionary tuning of such TCS by serial dilution transfer to reveal the interdependence of growth (fitness) and gene expression. With light illumination for photosynthesis, such bioreactors can also be used for characterization of synthetic genetic circuit of photosynthetic microorganisms such as *P. tricornutum*.

P.1.1-002
Light-induced pancreatic β-cell proliferation through endogenous opsin signaling

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Diabetes is a disease characterized by the loss of function and number of healthy pancreatic β-cells, with no permanent cure yet. A key therapeutic concept is based on regenerating β-cell mass by islet transplantation or inducing proliferation of β-cells by growth factors, respectively. These therapies, however, are either highly invasive or often non-specific and thus potentially carcinogenic. Here we propose an alternative approach based on light induced proliferation of β-cells that is less invasive and increases the spatio-temporal precision, potentially circumventing previous limitations.

Using RT-PCR, we found that several opsins, especially panopsin, and to lower levels melanopsin and rhodopsin, are present in rodent and human pancreatic islets. To test whether these opsins have a functional role, we analyzed the response of primary murine and human pancreatic islets to illumination with blue-green light. By using a nucleotide incorporation assay we determined the percentage of proliferating cells in primary human and murine islets, which showed increased proliferation after illumination compared to unilluminated control groups. Illumination resulted in elevated activation of the major proliferative MAPK/Erk and anti-apoptotic PI3K/Akt pathways, as determined by Erk1/2 and Akt phosphorylation levels using Western blot. Furthermore, we optimized the illumination protocol regarding wavelength- and intensity-dependence of proliferative pathway activation.

Taken together, our results show that pancreatic β-cell proliferation can be induced with high spatial and temporal precision using visible light, without addition of exogenous factors or gene transfer, indicating a potential mechanism for a novel therapeutic strategy.

P.1.1-003
Directed evolution of cellobiose dehydrogenase from *Phanerochaete chrysosporium* in yeast *Saccharomyces cerevisiae* for increased activity

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Cellobiose dehydrogenase (CDH) gene from *Phanerochaete chrysosporium* has been cloned in yeast *Saccharomyces cerevisiae* for extracellular expression. Gene library was constructed using random mutagenesis by error-prone PCR. Obtained library was screened with microtiter plate assay using modified DCIP assay. Several mutants were found that have higher kcat compared to wild-type enzyme. Both mutants and wild type expressed in yeast showed broad band in SDS electrophoresis due to high glycosylation level. Obtained mutants could be useful in lactobionic acid production and biosensor manufacturing.

P.1.1-004
Expanding the genetic code of a phototrophic organism

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The photoautotrophic fresh water cyanobacterium *S. elongatus* is widely used as a chassis for biotechnological applications as well as a photosynthetic bacterial model. In this study, a method has been established to expand the genetic code of this cyanobacterium thereby enabling the incorporation of unnatural amino acids into proteins. This was achieved through UAG stop codon suppression, using archaeal pyrrolysyl orthogonal translation system. We demonstrate incorporation of unnatural amino acids into green fluorescent protein with 20 ± 3.5% suppression efficiency. The introduced components were shown to be orthogonal to the host translational machinery. In addition, we observed

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