CELL-FREE PROTEIN PRODUCTION – THE DEVELOPMENT OF AN *IN VITRO* EXPRESSION SYSTEM FOR (CYANO)BACTERIA

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Background - Cyanobacteria

Cyanobacteria provide a promising approach for industrial biotechnology and regenerative energy through the synthesis of high-value chemicals or biofuel or clean, solar-driven hydrogen gas production. Basic and applied research facilitates the understanding and engineering of cyanobacteria towards such a "green" biotechnology, with a special emphasis on synthetic biology (www.cyanofactory.eu).

Today, despite advances giving a deeper understanding of the underlying biochemical mechanisms and special cyanobacterial characteristics, our abilities to develop and engineer cyanobacteria in a synthetic biology sense are still limited (1; 2).

Background – *In Vitro* Expression

Cell-free protein expression systems are in use since the 1950s to elucidate protein synthesis and cell biological mechanisms but only recently became of high interest for research and application, in particular within the emerging field of synthetic biology. Such systems allow for a comparable easiness to monitor and modify reaction conditions, and are independent of restrictions usually imposed on *in vivo* protein expression systems. There exist several implementations of such systems, which are based on either crude cell lysate of pro- or eukaryotic origin, or reconstituted from purified components (3).

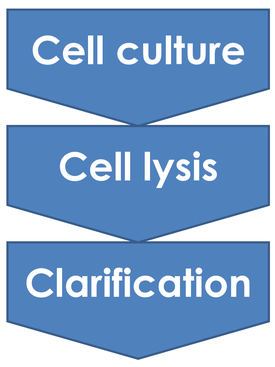
Motivation

Current cyanobacterial research focuses mainly on *in vivo* or *in silico* methodology, a cyanobacterial *in vitro* expression platform combining transcription and translation reaction is not established to our knowledge. Such a platform would e.g. enable characterization and engineering of (synthetic) parts comparably faster than the *in vivo* approaches currently existing. Moreover, it would potentially allow for the design, building and testing of whole synthetic metabolism and pathway engineering in a cyanobacterial environment.

Conception and Methodology

Aim of the project is the establishment of a reliable and cheap *in house* protocol for an *E. coli* and subsequently cyanobacterial *in vitro* protein expression system, with emphasis on (**A**) crude extract generation (4; 5), (**B**) expression template design, (**C**) an easy-to-handle *in vitro* protein synthesis reaction (5; 6) and suitable detection assays.

A



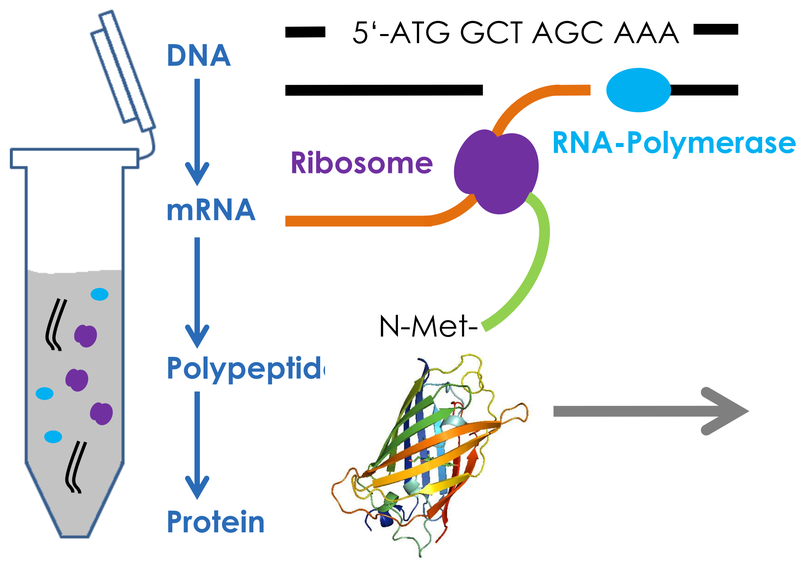
- Organism: *E. coli*
- Medium: 2xYTPG
- Method: Sonication (4; 5)
- Device: Bandelin HD2200
- Method: Centrifugation
- Conditions: 4°C, 12000g

B

1. T7-Promotor
2. RBS
3. MCS / GFP-His-tag
4. T7-Terminator

- Methods:
  Cloning
  PCR
  Gibson-Assembly

C



Reaction conditions (5; 6):

30°C **4-6h**

Reaction components:

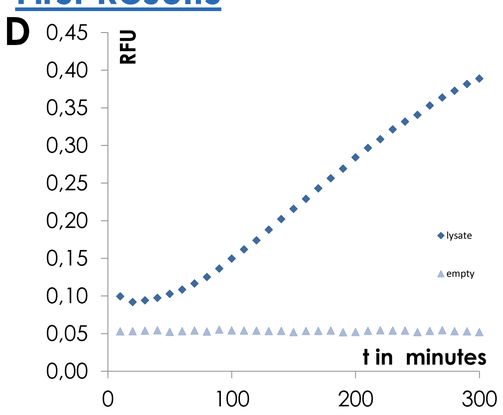
- Testing and validation vs. RTS 100 *E. coli* HY kit (5Prime)

Detection:

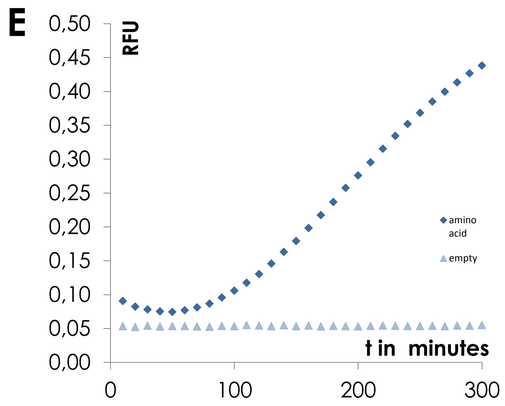
- Real-time vs. End-point
- Optical vs. Immunological

First Results

D



E



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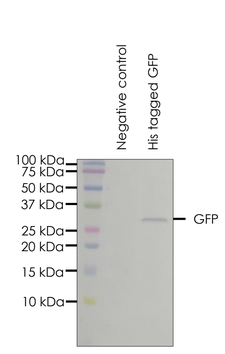

Exemplary real-time detection of fluorescence indicating *in vitro* protein synthesis of GFP employing self-prepared (**D**) lysate, (**E**) amino acid mixture in the MiniOpticon Realtime PCR system (Biorad, Germany). Other chemicals from RTS 100 *E. coli* HY kit (5Prime). (**F**) Westernblot detecting 6xHis-tag with the HIS.H8 antibody (1:2000, Thermo-Fisher).

Discussion and prospect

An in house protocol for *in vitro* protein expression based on *E. coli* lysate generated by sonication is currently being developed. Adequate detection assays based on real-time monitoring and SDS-Page/Western Blot have already been established. After validation and optimization of the *E. coli* system, experiences shall be transferred to Cyanobacteria.

In vitro synthesized GFP
In 1.5 ml reaction tube

References

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