Factory- on- a- Chip

Temporal and spatial control of protein synthesis by in vitro recombination inside picolitre reactors

Divya Ail, Stanley Dinesh Chandradoss, Anna Grushina, Daniel Herde, Deepikaa Menon, Tomasz Sadowski, Arnab Sen, Priyanka Sharma.
Kaj Bernhardt, Salvatore Loguercio, Eugene Petrov, Loic Royer, Ilaria Visco, Prof. Petra Schwille, Prof. Francis Stewart.
Biotechnology Center (BIOTEC), Dresden University of Technology, Tatzberg 47/49, D-01307 Dresden, Germany

iGEM Jamboree 2009
31st Oct - 2nd Nov
MIT Boston

Introduction

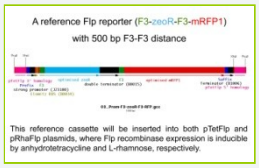
Organizing matter on micro- and nano-scale requires specialized tools that control how components are assembled, when and where they are added, and how the product is transported. Our factory-on-a-chip is designed to do just that.

In this project we conduct a two-step assembly process of biological and metallic components in microvesicles of picolitre reaction volumes. Production and packaging in picolitre reactors allows for precise control of all components of the product. The reactors are vesicles moving through a microfluidic chamber. A genetic timer determines the onset of transcription and marks the beginning of the production process. It is a genetic circuit based on Flp recombinase [1]. The protein we express is GFP tagged with a sequence which is known to nucleate silver nanoparticles.

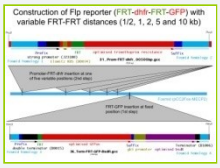
Combining a transcription-translation system with protein coding genes and a recombination logic inside microdroplets provides spatial control. Moreover, in the microfluidic chamber we can pinpoint the beginning of synthesis, and easily track and isolate the droplets.

Flp Recombinase based timer

The Flp recombinase recognizes FRT sites, and we designed our construct such that when the Flp (controlled by an inducible promoter) is expressed and the FRT sites are excised, our reporter is expressed. Expression of the reporter can be observed in a time dependent manner. Through this part of our project we aimed at characterizing the dependence of recombination efficiency of Flp recombinase on the FRT site distances [2]. To achieve this the FRT sites were placed at varying distance on a fosmid backbone. A fosmid was selected because large distances of 2kb, 5kb and 10kb would be possible.



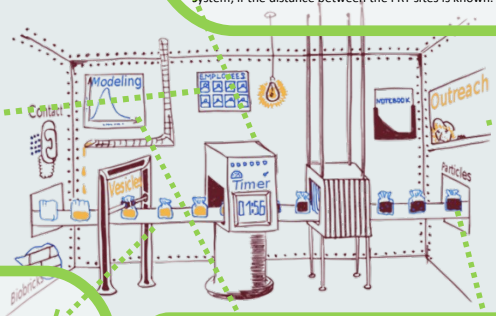
The Flp reporter that was used to test how the recombination efficiency will vary depending on the level of Flp expression



The reporter and one FRT site is inserted at a fixed site and a second FRT site is placed at variable distances in the fosmid backbone.

Characterizing the recombination efficiency and time required for reporter expression in relation to the FRT distances would enable us to design a Flp genetic timer, wherein we would be able to predict when the reporter expression would occur in our system, if the distance between the FRT sites is known.

iGEM team BIOTEC-Dresden



Outreach

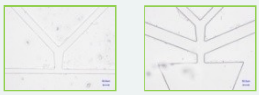
In our community outreach program we cooperated with a local high school – the St. Benno-Gymnasium Dresden – and invited a group of 13 students aged 11 to 18 to our iGEM lab. We believe this program was beneficial for all involved. For most students this was their first contact to the profession of science. Learning about science in the setting of iGEM is fun and can motivate interested youth to develop their scientific skills.

We encouraged our visitors to ask any questions they might have. And ask they did. The students showed a remarkable talent to inquire all essential problems of the matter. Explaining our project to them reminded us of the "big picture" and provided us with new perspectives.

Students visiting our iGEM lab

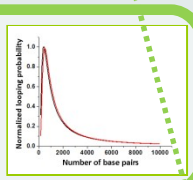
Microfluidic system: Packaging, Trafficking and Monitoring

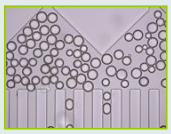
A microfluidic system is the assembly line of our factory-on-a-chip. It feeds the raw materials and surfactant-enriched oil into the packaging chamber, where they are compartmentalized into spherical droplets . Further downstream it acts as a conveyor belt propelled by the constant supply of the fluids at controllable rates. The transparent material of the system – polydimethylsiloxane (PDMS) – allows for permanent monitoring of the packages and their content moving along the conveyor belt by light microscopy. At the end of the conveyor belt the packages can be conveniently delivered into a tube.



Microfluidic chamber with T shaped and V shaped junctions

DNA Looping Probability (simulation)



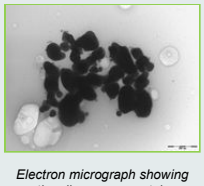


Formation of droplets in the channels

Silver Nanoparticles

The production of proteins tagged with silver nanoparticles was inspired by the silver resistance mechanism of *Pseudomonas stutzerii*. This bacterium reduces silver ions to silver nanoparticles rendering them harmless to its metabolism. In our biomimetic approach we tried to use the 12 amino acid sequence (AG4) that has been shown to nucleate silver nanoparticles [3]. We tested this mechanism *in vitro* by incubating the synthesized AG4 peptide with silver nitrate and a reducing agent. We produced a variety of silver nanoparticles between 20 and 100 nm in diameter and measured their characteristic absorption.

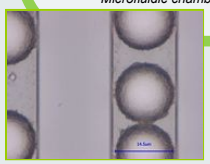
Now we are ready to apply this procedure to a GFP tagged with AG4 expressed inside the picolitre reactors.



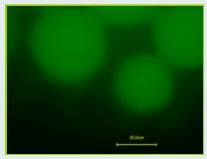
Electron micrograph showing the silver nanocrystals

Microdroplets: Packaging in Style

The microdroplets created in the packaging chamber are aqueous droplets stabilized with a surfactant (Span 80). They can be loaded with a commercial *in-vitro* transcription-translation kit, vectors and other water-soluble components. Protein expression in microdroplets rather than in bacteria offers a number of advantages. Droplets can be loaded with only the necessary reaction components. Toxicity of raw materials or of the product to the „host" is not an issue. Product isolation is easy. Unlike bacteria microdroplets cannot interact with environmental ecosystem, they cannot replicate and they are not infectious. There are also advantages over mere *in vitro* expression. The compartmentalisation of the reaction volume by many picolitre reactors and their ordered movement through the microfluidic system allow to localize and monitor single droplets containing the reaction in a defined state.



The microdroplets in the channels are of uniform size and shape.



microdroplets loaded with alexa488 illuminating water filled vesicles

References:

[1] Qiao, J., et al., (2009). J Mol. Bio. 390, 579-594.
[2] Ringrose L., et al., (1999). EMBO J. 18, 6630–6641.
[3] Naik.R.R.,et al.,(2002). Nat. Mater. 1,169-172.
[4] Stewart et al., (1999). EMBO J. 18, 6630-6641.