DNA coding beyond triplets...

“revolutionizing synthetic biology”

The principle:

The main idea of our project is to use the DNA sequence to represent a program consisting of a series of blocks, which determine the arrangement of DNA binding domains along the double helix. Each of those DNA-binding proteins is fused to a different functional protein. Therefore, the sequence of target motifs encoded by the DNA program defines the arrangement of functional proteins along with the ordering of DNA-binding factors.

Project steps:

Selection of DNA binding domains
Verification of binding of multiple domains
Assembly of biosynthetic pathway

1. DNA binding domains

Which ones to choose?

Several classes of DNA binding proteins exist in nature, e.g. zinc finger family of proteins, TAL effectors, different transcription factors and endo/exonucleases. Zinc finger domains (ZNF) were selected because of their modularity and the potential to create practically unlimited number of sequence specific DNA binding domains and availability of >700 characterized zinc fingers.

Demonstration of specific binding of zinc fingers to DNA

We prepared six zinc fingers and determined their binding to the cognate DNA using three methods: EMSA (electrophoretic mobility shift assay), SPR (surface plasmon resonance) and repression of β-galactosidase activity.

2. Oscillators based on ZNFs

A repressor is an oscillator initially based on three repressors connected into a cyclic feedback loop. The only experimentally tested repressor contains three bacterial repressors: LacI, TetR and Bcl. Our selection of zinc fingers as artificial repressors provides the building blocks to construct new repressilators, including an extended number of genes in the cycle. We modelled repressilators based on 3, 4 and 5 zinc fingers and performed deterministic and stochastic simulations.

Circular repression scheme. The figure presents the oscillator where five zinc fingers are used in a similar topology as the original repressor. Only odd number of zinc fingers produces oscillations. One of the zinc fingers also represses the reporter gene. All promoters are activated by the orthogonal 17 TAL DNA polymerase and each of the promotors is located a binding site for the preceding zinc finger in the cycle.

3. Detection of binding of multiple DNA binding domains

To determine binding of several fusion proteins, we exploited the phenomenon of the FRET effect ( Förster resonance energy transfer), a non-radiative energy transfer between two fluorophores. We demonstrated binding of several zinc finger domains with reconstitution of split fluorescent proteins YFP and CFP, and FRET effect in mammalian cells. Our results clearly demonstrate that DNA program sequence can be used as a scaffold for four chimeric proteins.

4. DNA-guided biosynthetic pathways

We constructed enzymes of the violacin biosynthetic pathway, fused to DNA binding proteins, and designed the scaffold DNA molecule - a DNA program. Zinc finger binding sequences were arranged on program DNA in a way that enabled arrangement of chimeric enzymes in the correct order. We also designed a scrambled variant of DNA program, where all zinc finger binding sequences were still present but were not arranged in a correct order. The yield of violacin was improved 6-fold in the presence of program DNA in comparison to scrambled or no program DNA. In addition, formation of a side product deoxychromoviridin was significantly decreased.

Main achievements:
- invented a new platform of DNA-guided protein scaffold
- performed deterministic and stochastic simulations of a repressor based on synthetic repressors demonstrating new features and potentials for experimental realization
- proved simultaneous binding of four zinc finger proteins to adjacent sites on program DNA by FRET
- designed chimeric violacin biosynthetic enzymes with added zinc finger DNA binding domains
- improved the yield of violacin in the presence of a DNA program 6-fold and suppressed formation of side reaction product deoxychromoviridin

Conclusions and future perspectives:

Sequential ordering of functional fusion proteins on a DNA scaffold represents a new technological platform for constructing complex biosynthetic pathways with multiple (>10) enzymes and improving yield and direction of biosynthetic flux.

Furthermore, this approach has potentials for information processing. This principle extends the biological paradigm of DNA as a carrier of information.

Aims and goals:

- characterize binding of DNA binding proteins (zinc fingers) and demonstrate their binding could be defined by the order of DNA target elements on DNA program sequence and later use them in an application, such as biosynthesis
- demonstrate the advantages of using artificial repressors to extend the functionality of genetic oscillators

Protein scaffold has been used to increase the proximity of biosynthetic enzymes. However, this approach does not lead to an ordered assembly.