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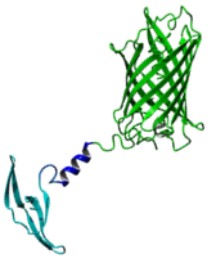
Bachelor/Master Thesis

Engineering of eGFP with reduced non-specific binding to various surfaces.



Background

For successful directed evolution campaigns of adhesion promoting (AP) peptides, it is necessary to develop robust high-throughput screening system. One such approach is based on using eGFP as fluorescent reporter in AP-fusion construct [1-3]. Our experiments, however, reveal that eGFP itself adheres to some surfaces (i.e. polymers) causing high-background values that affect binding peptide selection process.

Aim of the study: Engineer eGFP molecule with reduced binding to several hydrophobic polymer surfaces.

Work Packages:

-computational modelling of protein structure

-SSM/SDM of eGFP based on computational modelling data to reduce the hydrophobic patch of eGFP

-Screening of produced eGFP variants to evaluate the binding to hydrophobic surfaces, selection of the variants with lower adhesion to surfaces

-Comparison of binding properties of selected variants using surface plasmon resonance (SPR) or quartz crystal microbalance (QCM)

-Optionally: Generation of 5 AP-eGFP fusion constructs using optimized eGFP variants to demonstrate better suitability of newly developed fusion constructs for AP-screening

Methods:

- Plasmid preparation
- Primer design, SDM, SSM
- Cloning
- Expression in microtiter plates & flasks
- DNA- and protein analytics
- Microtiter plate-based screening assays
- Fluorescent microscopy
- SPR or QCM

Language: English

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Literature:

1. Rübsam, K., et al., *Anchor peptides: A green and versatile method for polypropylene functionalization.* Polymer, 2017. **116**: p. 124-132.
2. Dedisch, S., et al., *Matter-tag: A universal immobilization platform for enzymes on polymers, metals, and silicon-based materials.* Biotechnology and Bioengineering, 2020. **117**(1): p. 49-61.
3. Rübsam, K., et al., *Directed evolution of polypropylene and polystyrene binding peptides.* Biotechnology and Bioengineering, 2018. **115**(2): p. 321-330.