Development of self-assembling building blocks for creation of filamentous nanostructures

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Research interest of the Bio-Nanosystems Laboratory is focused on the exploitation of structural and functional principles of self-assembling biological systems for applications in bio-nanotechnology.

The aim of this project is to furnish various proteins with polymerization ability and to use these self-assembling units to build filamentous nanostructures.

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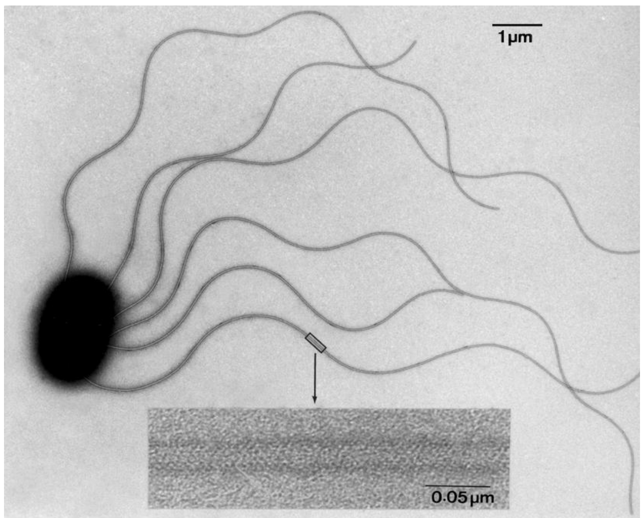
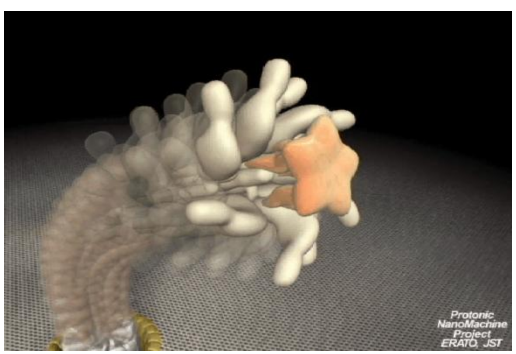
Creation of fusion constructs with the polymerizable protein, flagellin.

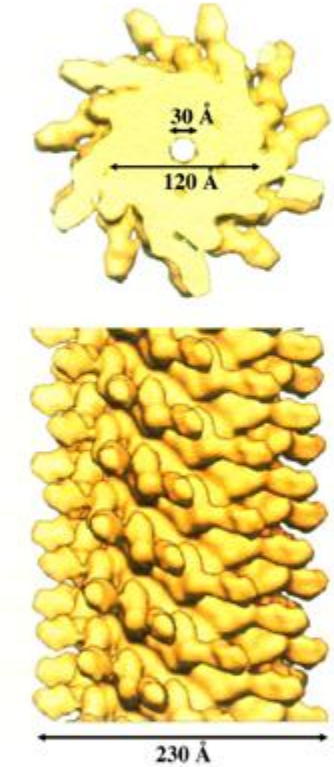
Bacterial flagella

(the locomotion organelles of bacteria)

A self-assembling biological system







The flagellar filament:

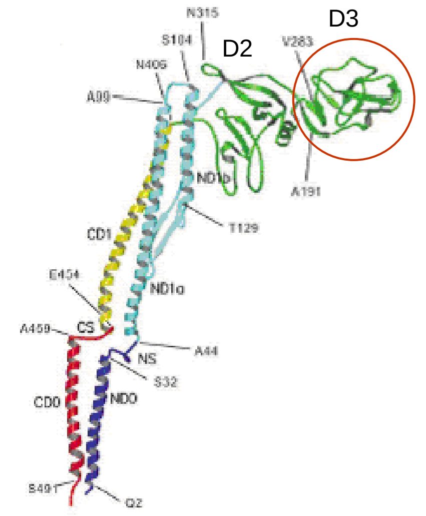
- *~10^4 flagellin subunits*
- *length: 5-20 μm*
- *diameter: 23 nm*

Main characteristics of flagellin

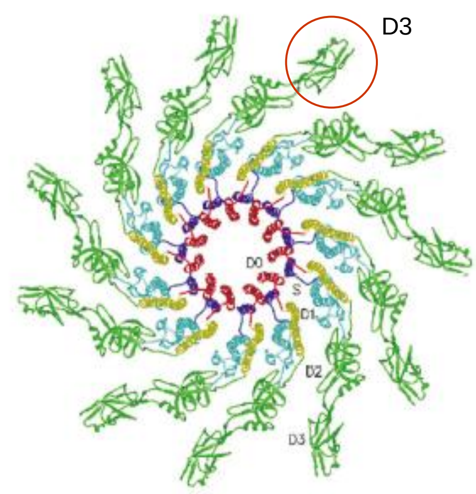
❑ *Flagellin (FliC) is capable of polymerization*

❑ *Terminal regions of flagellin are responsible for subunit interactions in the filament core*

❑ *The D3 domain is situated on the filament surface and not involved in filament formation*



The structure of flagellin subunit



Subunit arrangement within the filament

Objective

The concept in this project is to replace the hypervariable D3 domain of flagellin with suitable monomeric proteins, and to use these chimeric flagellins to build tubular nanostructures.

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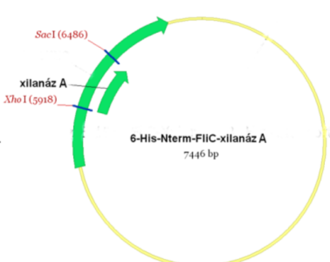
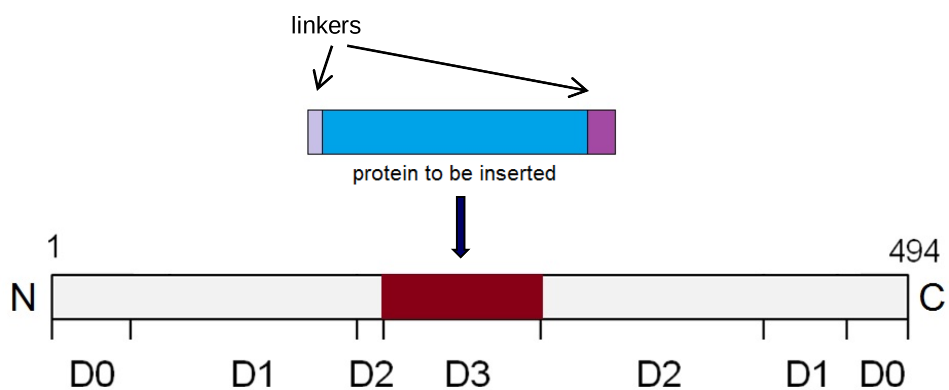
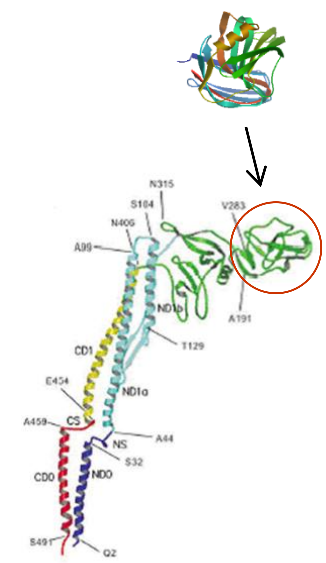
- ❑ *flagellin-GFP reporter subunits*
- ❑ *flagellin-enzyme fusion constructs (flagzymes)*
- ❑ *flagellin-based binding proteins*



fusion gene construction





Development of flagellin-based fusion contructs

Steps:

✓*Structural modeling of fusion partners, linker design*

✓*Creation of the fusion gene*

✓*Bacterial protein expression, purification*

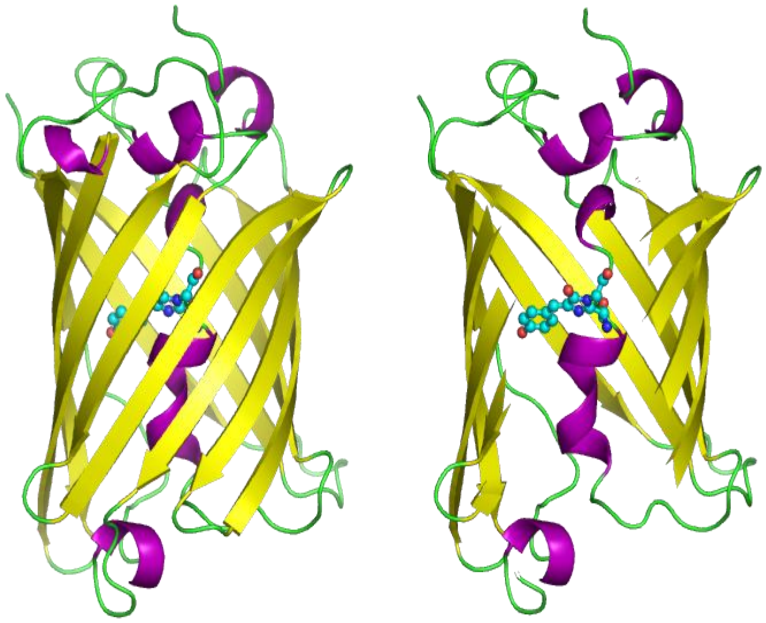
✓*Functional characterization of the fusion protein*

✓ *Demonstration of polymerization ability*

The FliC-sfGFP reporter subunit

The Green Fluorescent Protein (GFP)

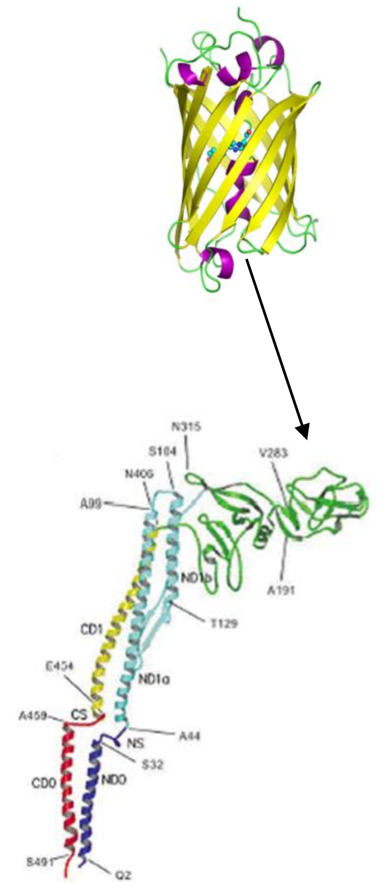
- 238 amino acids
- β-barrel architecture
- Ser-Tyr-Gly chromophore → green fluorescence
- Excitation maximum: 495 nm
- Emission maximum: 508 nm
- *sfGFP*: a GFP mutant with enhanced folding ability

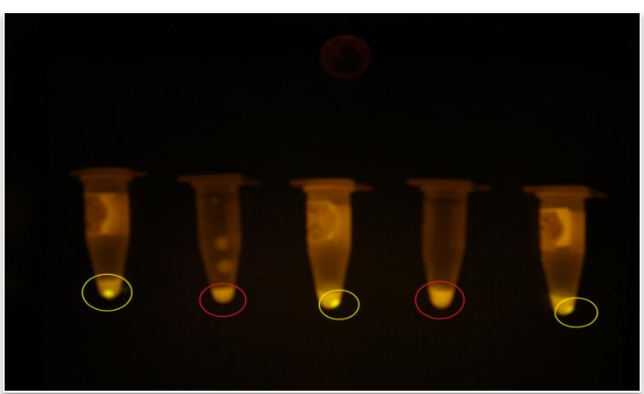


Design, expression and purification of FliC-sfGFP

- ❑ *Replacement of D3 by sfGFP (applied linkers: LEGS, EL)*
- ❑ *Cloning in pET23b plasmid (C-terminal His_6-tag)*
- ❑ *Protein expression in E.coli host cells*
- ❑ *Purification by Ni-affinity chromatography*

sfGFP





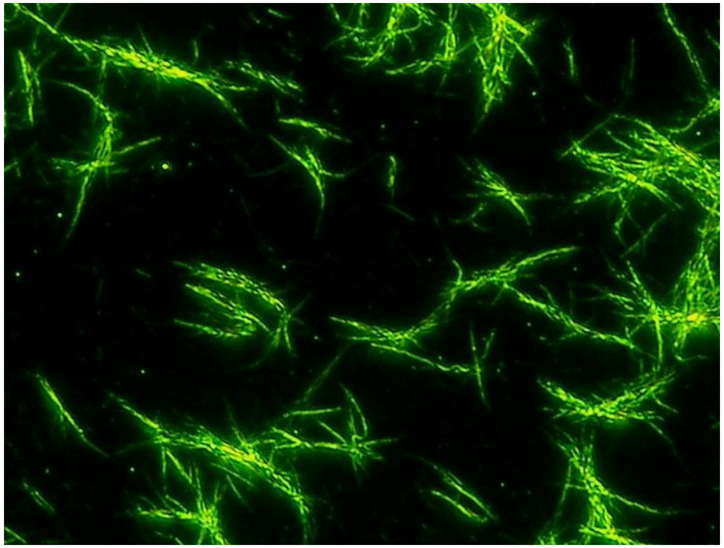
Protein expression trials

Ni-NTA purification

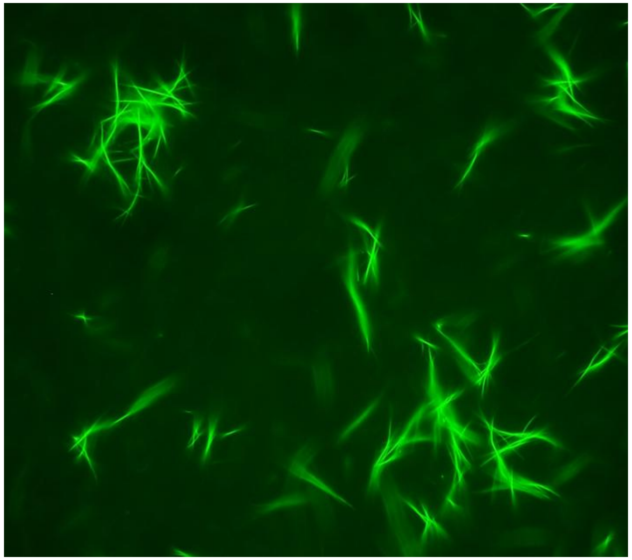
FliC-sfGFP filaments

- *In vitro filament formation was induced by ammonium sulfate (0.4M)*
- *FliC-sfGFP readily formed long stable filaments*
- *Copolymers with wild type flagellin were also obtained*

Dark-field microscopy:



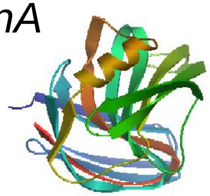
Fluorescence microscopy:

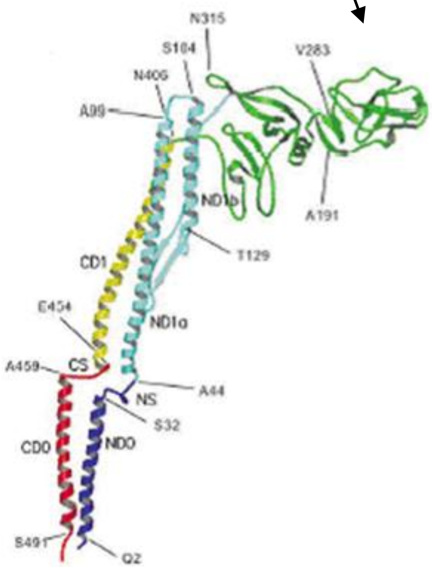


The FliC-Xylanase A fusion protein

Development of the FliC-XynA flagzyme

XynA

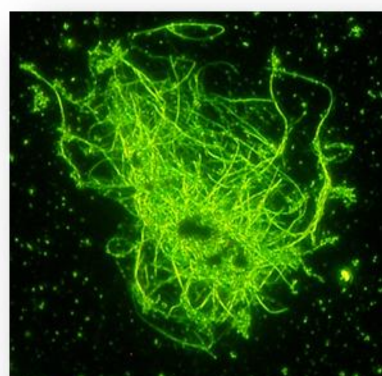




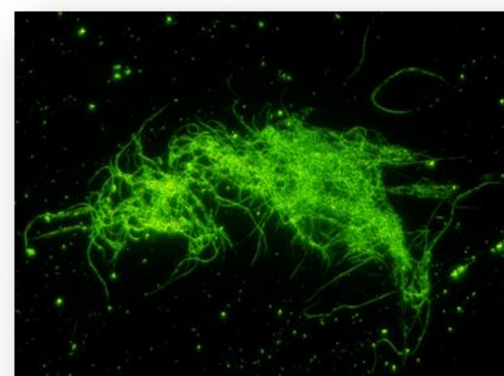
XynA: xylanase A (B.subtilis;185 aa)

Function: xylan degradation

- ❑ *FliC-XynA is capable of polymerization*
- ❑ *FliC-XynA is enzymatically active*



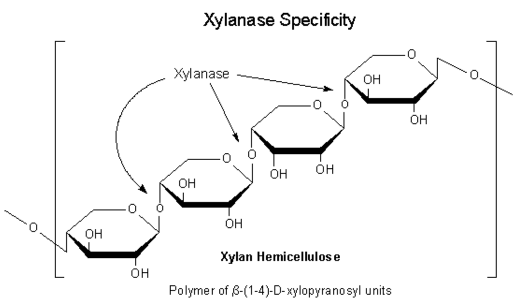
FliC-XynA polymer (0.4M AS)



Flagellin:FliC-XynA (1:1) mixed copolymer (0.4M AS)

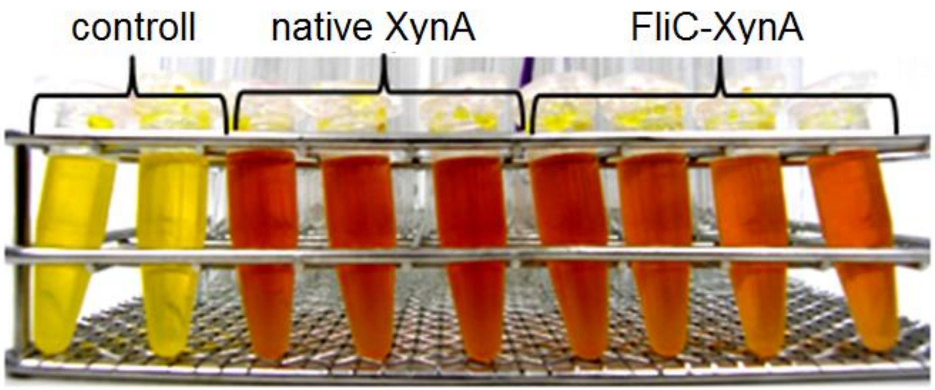
Enzyme activity of FliC-XynA

Degradation of xylan



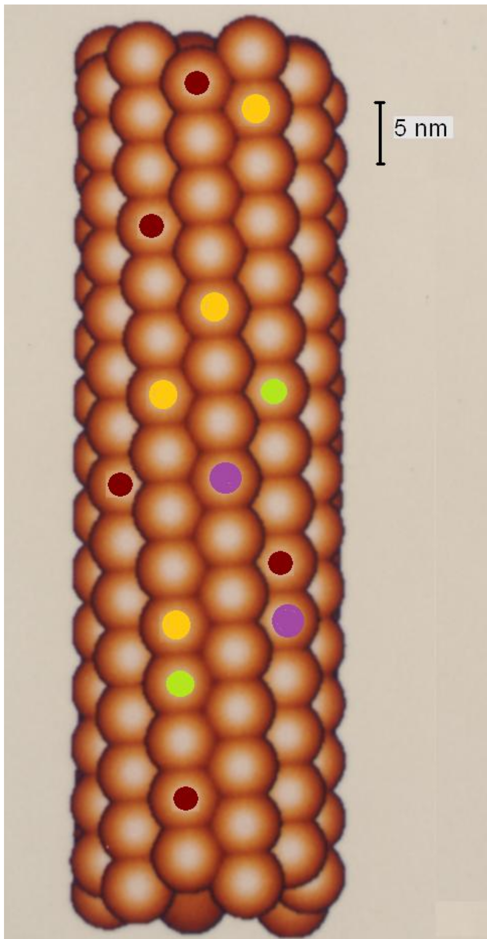
Deliberated D-xylane monomers interact with dinitro-salicylic acid

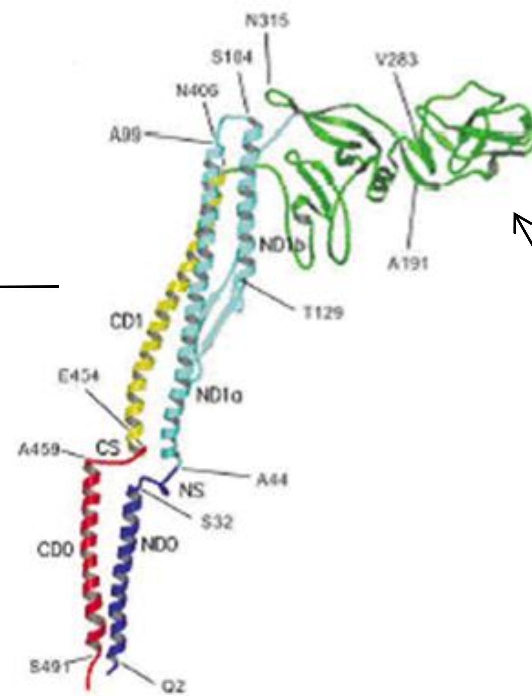
Colour reaction: from yellow to orange
(λ=575 nm)

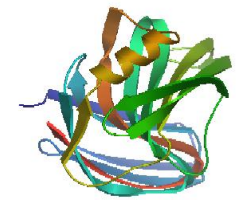


Sample	OD_{575}	D-xylose deliberated (μg/ml)
native xylanase A	0,176	26
FliC(XynA) monomer	0,153	22
FliC/FliC(XynA) copolymer 2:1	0,156	23
FliC/FliC(XynA) copolymer 1:1	0,163	25

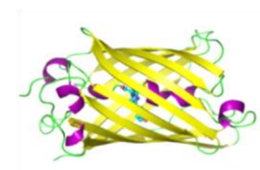
Flagellin-based building blocks to create filamentous assemblies



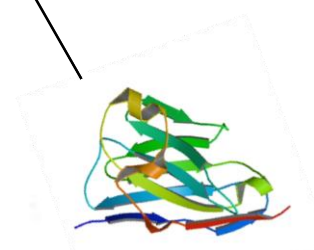




Xyn A



sfGFP

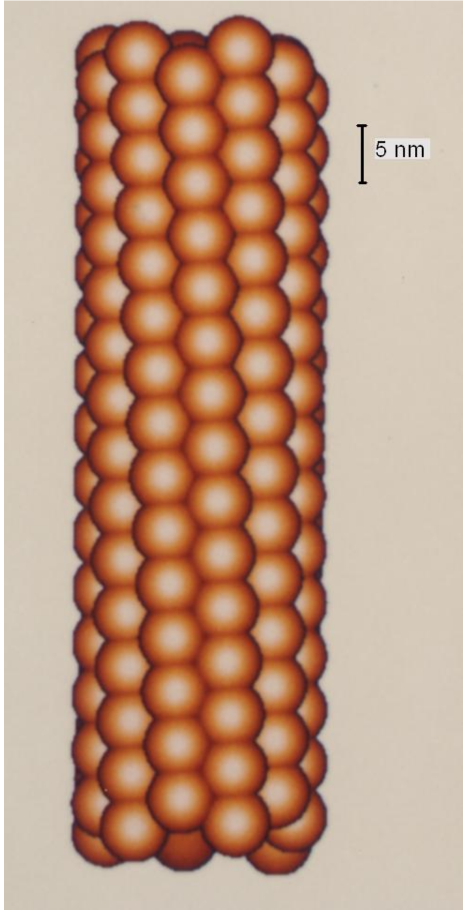


single domain antibody

Why to use flagellin-based filamentous assemblies?

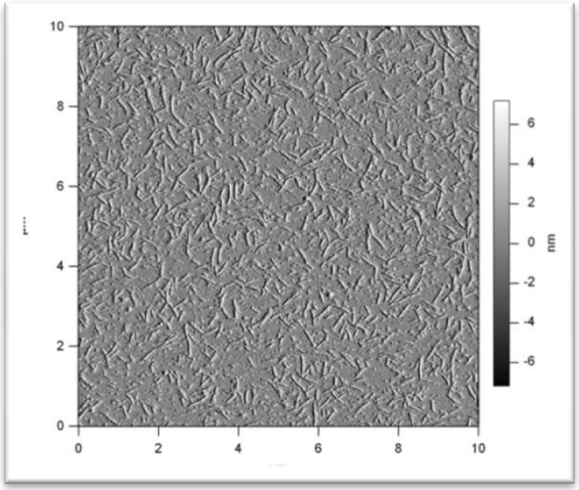
Advantages:

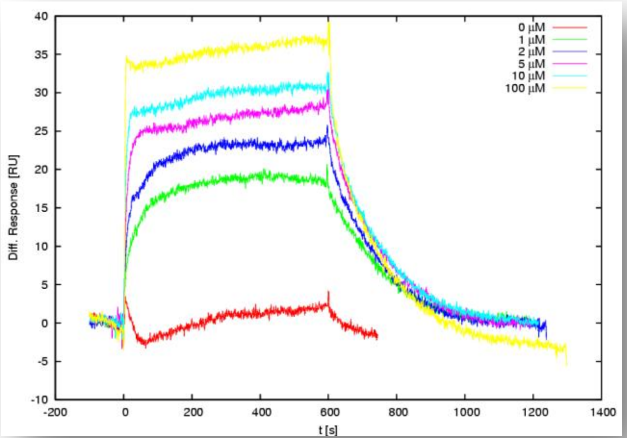
- *Stability*
- *High binding/functional site density*
- *Identical local environments of subunits (no matrix required)*
- *Homopolymers, mixed or block copolymers can be formed*



Potential applications

- *Sensing layers → medical diagnostics, environmental monitoring*
- *Multienzyme complexes → biotechnology*
- *Functional nanoparticles → nanomedicine*





Summary

- Polymerization ability can be introduced into various proteins by producing fusions with flagellin
- Flagellin-based fusion proteins may serve as building blocks in creation of filamentous nanostructures with useful properties

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