Selection of Phage Displayed Peptides for Biosensor-Based Detection of Viruses

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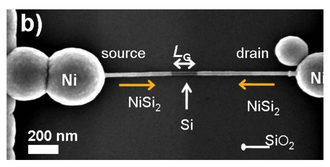
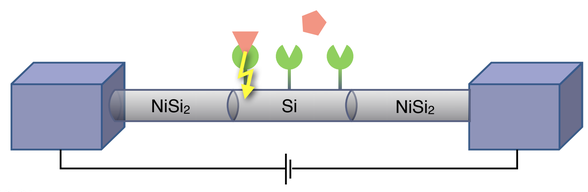
Motivation

Why Nanowire-based Biosensing?

- early diagnosis of infections vitally important to inititate sanitary and therapeutic actions



$NiSi_2$ nanowire-based FET [1]

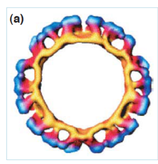


- nanowires:
  → 1D nanostructure
  → high surface-to-volume ratio
  → binding of target molecule → change in electron transport properties
  → sensitive and real-time detection of analytes

Why Phage Displayed Peptides as Receptors?

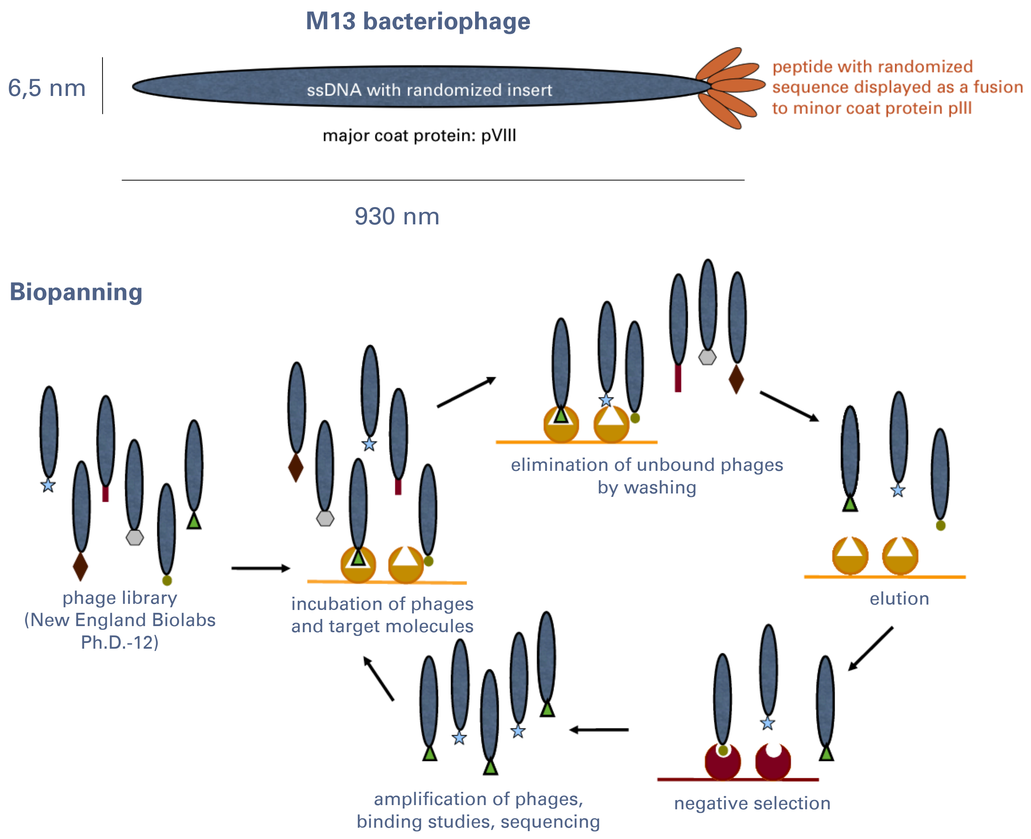
- can be developed for almost every potential target molecule
- very small (12 amino acids)
- stable under varying conditions
- highly affine and specific

→ analyte: human norovirus (genogroup GGII) P2 subdomain of capsid protein (blue)



norovirus VLP [2]

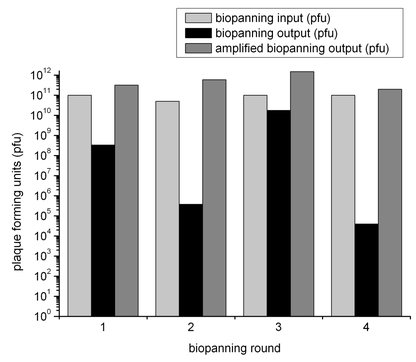
Experimental Outline



critical parameters:

- concentration of target molecule
- stringency of washing
- elution
- negative selection
- amplification

Biopanning

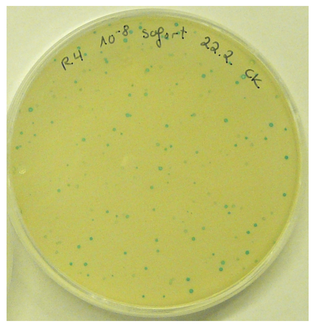


Immobilization of target molecules during biopanning

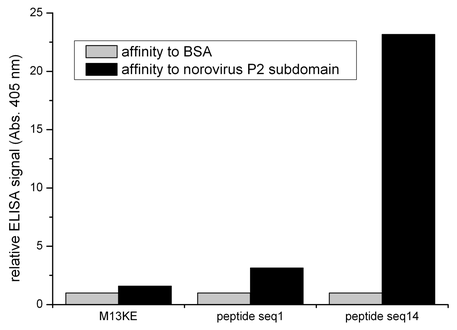
- round 1 and 3: His SpinTrap column
  → elution: imidazole
- round 2 and 4: Nunc MaxiSorp 96 well plate
  → elution: pH↓ + ultrasound

Characterization of Individual Phage Clones

Isolation and amplification of individual phage plaques

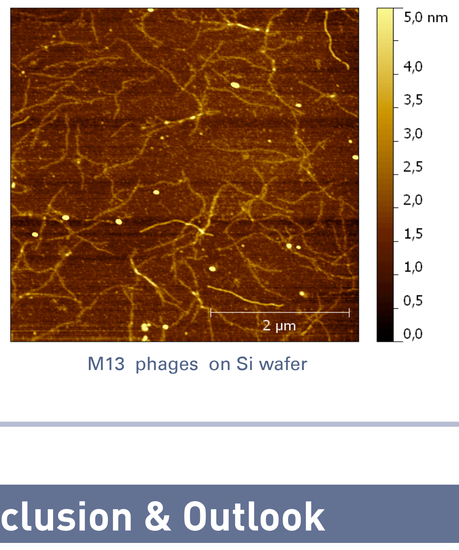


ELISA after round 4

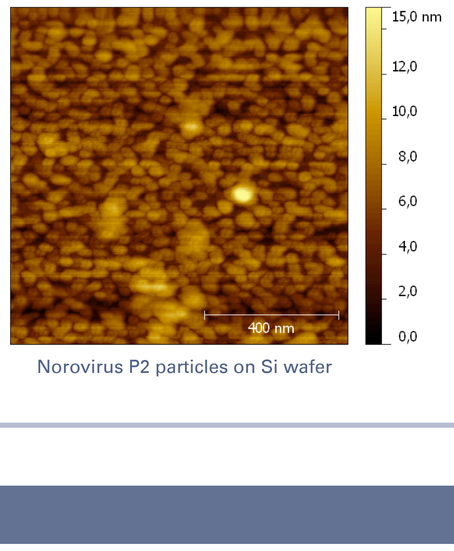


→ ELISA signal strongly dependent on phage concentration and binding affinity, but reproducibility has to be improved

AFM Imaging of Phages and Analytes

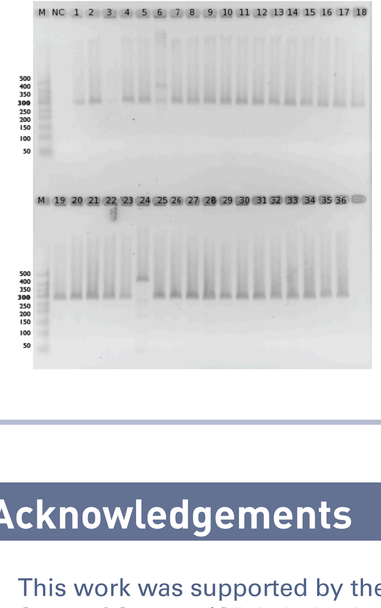


M13 phages on Si wafer



Norovirus P2 particles on Si wafer

Sequencing of Phage-Displayed Peptides



- PCR and gel electrophoresis of enriched phage library inserts
- sequencing result:
  35 of 36 sequences analyzable
  23 different sequences
  1 most prominent sequence: seq1 (25%)

Conclusion & Outlook

→ further binding studies of most promising candidates
→ biopanning against other virus proteins and virus like particles
→ immobilization of peptides for nanowire-based biosensing

References and Contact

[1] W.M. Weber et al., Nanotechnology 2008, NANO '08. 8th IEEE Conference on, 580-581
[2] M. Tan et al., Trends in Microbiology 2005, vol. 13 (6), 285-293

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