A synthetic receptor architecture for the detection of pathogens with small peptides

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Objectives

- development of a specific detection unit for influenza virus
- optimization of the peptide arrangement for signal amplification
- design of a detection platform and immobilisation method

background

- the membrane protein hemagglutinin (HA) is located on the surface of influenza virus type A
- Matsubara et al. [1] identified HA-binding peptides (Tab. 1 bottom)
- multiple simultaneous interactions are relevant for the attachment – also for the influenza host cell interaction and detection [2]

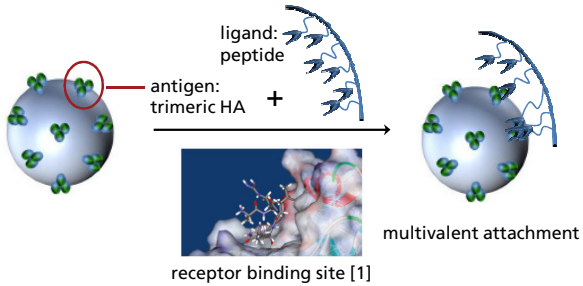


Fig. 1 cartoon demonstrating the binding of influenza virus to a multivalent peptide receptor; docking simulation of the complex between peptide S1 and the receptor binding site of HA

micelle characterization

→ peptides were coupled to palmitic acid
→ micelle formation in solution (Fig.2)

- determination of the critical micelle concentration (CMC) with drop and bubble shape tensiometer (Fig. 3)
- measurements of micelle size via dynamic light scattering (DLS)

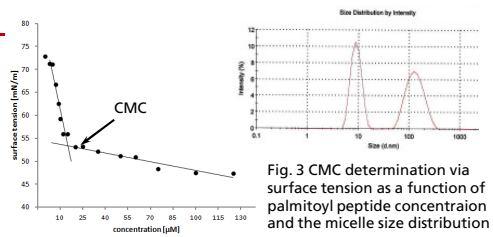


Fig. 3 CMC determination via surface tension as a function of palmitoyl peptide concentraion and the micelle size distribution

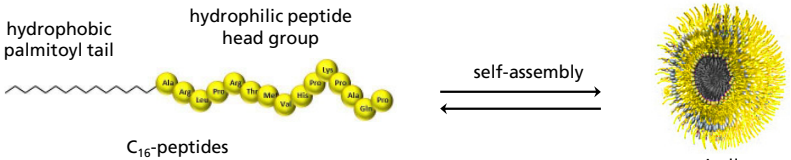


Fig. 2 schematic presentation of the micellar peptide assembly

binding studies by SPR

- immobilisation of the antigen: hemagglutinin protein H5 HA1 domain via amine coupling
- analyte: peptide micelle buffer solution

→ 100fold signal amplification in comparison to the single peptide (Fig. 4)
→inhibition test with the single peptide (Fig. 5) → specificity of the palmitoyl-peptide H5 interaction

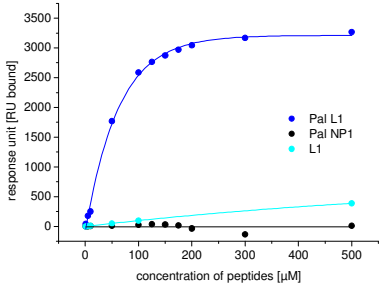


Fig. 4 binding curve of Pal L1 and single L1 and the negative control (Pal NP1) as a function of the peptide concentration

Tab. 1 peptide samples

palmitoyl peptides	sequence
Pal L1	C_{16}-ARLPRTMVHPKPAQP
Pal M1	C_{16}-ARLPRTMV
Pal S1	C_{16}-ARLPR
Pal NP1	C_{16}-GSWGEW
non-palmitoyl peptides	**sequence**
L1 (large)	ARLPRTMVHPKPAQP
M1 (middle)	ARLPRTMV
S1 (short)	ARLPR
NP1 (negative control)	GSWGEW

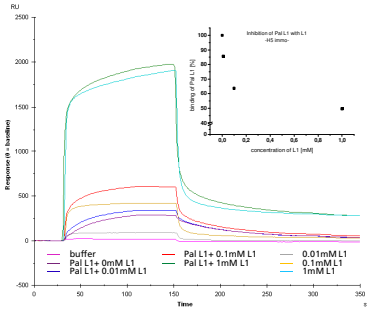
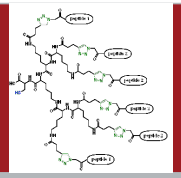


Fig. 5 sensorgram of the inhibition test of Pal L1 with L1, up to 50 % inhibition at 1 mM L1

Outlook

- assembly of a lysine dendron to provide the multivalent architecture
- arrangement of peptides in selected distances by click chemistry
- immobilisation on a SPR chip for kinetic studies



[1] T. Matsubara et al., J Med Chem 53:4441-4449
[2] M. Whitesides et al., Angew Chem 37:2754-2794

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