Monolith Protocol MO-P-046

Influenza Virus X31 – Inhibitor Coated Nanoparticle

Seasonal influenza infections affect up to 15% of the human population and high mutation rates limit the use of available antiviral agents. Enveloped viruses such as influenza A bind to host cell surfaces in a multivalent binding manner. Inhibiting this multivalent binding is known to reduce the viral infection since decades. Thus, the design of such inhibitors that can inhibit this mechanism and thereby reducing the viral infection is a promising strategy in drug development. In the following, the affinity of dendritic polyglycerols (dPG) decorated with the antiviral peptide PeB^{GF} against intact influenza A virus particles of the strain X31 was determined using MST.

virus – small molecule interaction | therapeutics

A1. Target/Fluorescent Molecule

X31 (Influenza type A) particles

A2. Molecule Class/Organism

Virus particles
X31 (reassortant) Influenza strain/Aichi/2/68 H3N2

A3. Sequence/Formula

N/A

A4. Purification Strategy/Source

The virus was harvested from allantoic fluid of embryonated chicken eggs, clarified by centrifugation (300 × g, 10 min) and then concentrated by ultracentrifugation (100 000 × g, 1 h) through a 20% sucrose cushion. Thereafter, the virus particles were resuspended in TNE buffer (10 mM Tris, pH 7.4, 100 mM NaCl, 1 mM EDTA) and centrifuged through a 20-60 % sucrose density gradient. The virus band was collected and concentrated by ultracentrifugation (100 000 × g, 4 h). Thereafter, the virus band was collected and concentrated again, if necessary. Finally, the virus particles were dialyzed against DPBS for 2 h, the DPBS was changed and the dialysis was continued over night at 4°C. Before the binding assays, the virus was inactivated (for safety reasons) with UV light for 5 min on ice.

A5. Stock Concentration/Stock Buffer

4 mg/mL protein amount from BCA assay
Dulbecco's Phosphate buffered saline (DPBS), pH 7.4

A6. Molecular Weight/Extinction Coefficient

~100 MDa

A7. Dilution Buffer

Dulbecco's Phosphate buffered saline (DPBS), pH 7.4

A8. Labeling Strategy

Labeling with octadecyl rhodamine B chloride (R18)[1]

A9. Labeling Procedure

The envelope (1 mg/mL envelope proteins, measured with the BSA kit from Pierce) of the viruses was labeled with 20 µM R18 for 30 min under gentle shaking at room temperature. Unbound R18 was removed by centrifuging the sample at 20,000 × g for 5 min. A small portion of the labeled viruses was diluted 1:4 in DPBS to measure the fluorescence intensity. Thereafter, the dilution of the labeled viruses was customized to get the recommended fluorescence counts. The labeled virus was stored on ice (dark) until use. To remove virus aggregates, the suspension was filtered through a 0.45 µm filter (Millipore).

A10. Labeling Efficiency

N/A

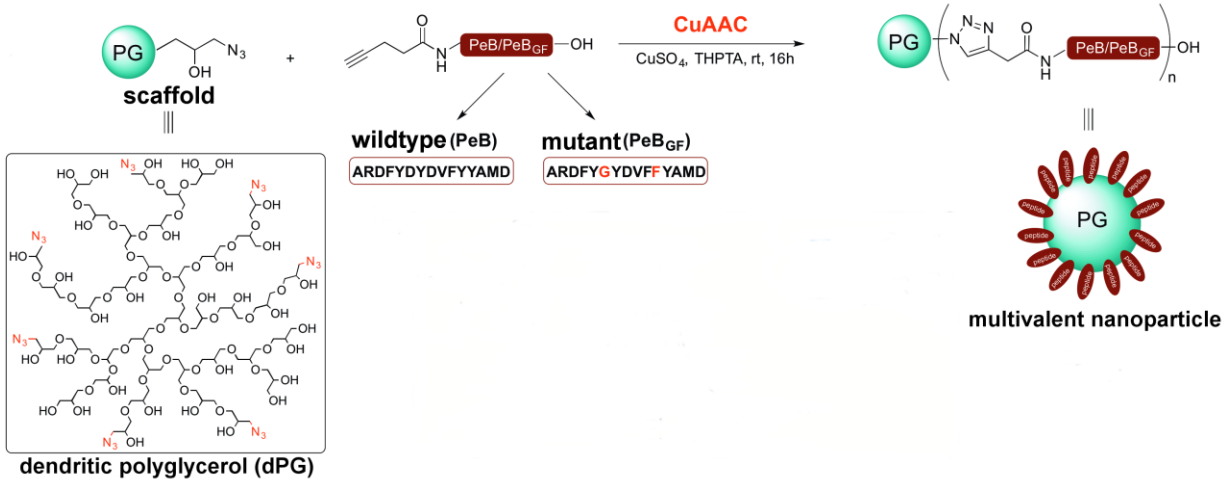
B1. Ligand/Non-Fluorescent Binding Partner

Antibody derived peptide PeB^{GF} (inhibitor) conjugated to nanoparticles

B2. Molecule Class/Organism

Multivalent peptide-nanoparticle conjugate

B3. Sequence/Formula



[1] The lipophilic octadecyl rhodamine B binds to membranes with the fluorophore at the aqueous interface and the alkyl tail protruding into the lipid interior.

B4. Purification Strategy/Source

Preparation of the peptide functionalized polyglycerols (nanoparticles) were as previously described.[2]

B5. Stock Concentration/Stock Buffer

4 mM peptide concentration
Dulbecco's Phosphate buffered saline (DPBS), pH 7.4

B6. Molecular Weight/Extinction Coefficient

~1.3 MDa ($dPG_{340}PeB^{GF}_{10}$)

B7. Serial Dilution Preparation

1. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of 250 µM PeB^{GF} solution into tube **1**. Then, transfer 10 µL of dilution buffer into tubes **2** to **16**.
2. Prepare a 1:1 serial dilution by transferring 10 µL from tube to tube. Mix carefully by pipetting up and down. Remember to discard 10 µL from tube **16** to get an equal volume of 10 µL for all samples.
3. Add 10 µL of 0.01 nM (4 HAU/ 10 µl) X31-R18 to each tube from **16** to **1** and mix by pipetting.
4. Incubate for 5 minutes at room temperature in the dark before loading capillaries.

D1. MST System/Capillaries

Monolith NT.115 Green (NanoTemper Technologies GmbH)
Capillaries Monolith NT.115 (MO-K022, NanoTemper Technologies GmbH)

D2. MST Software

MO.Affinity Analysis v2.3 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith/#monolith-software

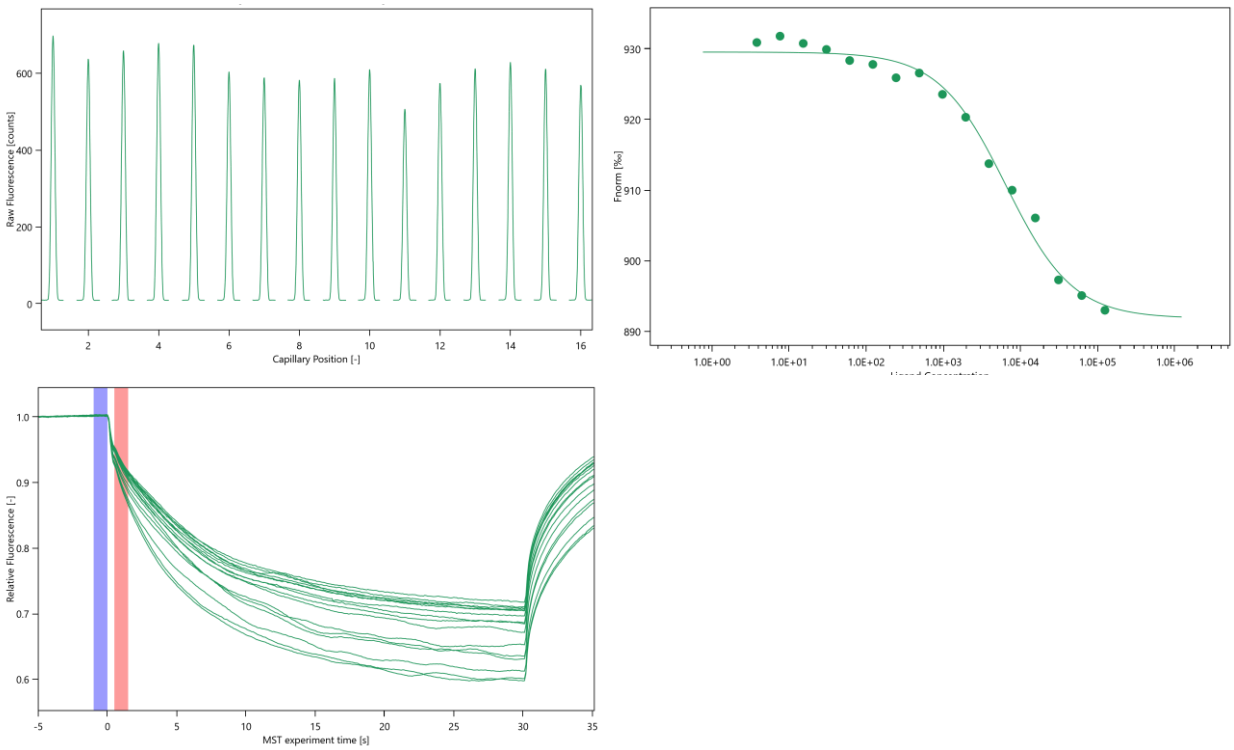
D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

Dulbecco's Phosphate buffered saline (DPBS), pH 7.4
0.01 nM X31 | 120 µM – 37 nM PeB^{GF} | 22°C | high MST power | 100% excitation power

[2] See Lauster et al., Angew. Chem 56 (2017) 5931-5936 for further details.

D4. MST Results (Capillary Scan/Time Traces/Dose Response)

$K_{d,app}$ = 3.1 µM peptide (6.8 nM multivalent nanoparticles) Lauster et al., Angew. Chem 56 (2017) 5931-5936



D5. Reference Results/Supporting Results

N/A

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