Monolith Protocol MO-P-062

Lysozyme – Aptamer (competitive assay)

Lysozyme is an enzyme that prevents bacterial infections by attacking peptidoglycan, a component of certain bacterial cell walls. Peptidoglycan is composed of the repeating amino sugars N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM), which are crosslinked by peptide bridges. Lysozyme hydrolyzes the bond between NAG and NAM, increasing the bacteria's permeability and causing the bacteria to burst. It is widely distributed in plants and animals. The majority of the lysozyme used in research is purified from hen egg whites. The DNA aptamer for lysozyme binds lysozyme with high affinity.

protein – DNA interaction | aptamer

A1. Target/Fluorescent Molecule

Lysozyme
uniprot.org/uniprot/B8YK79

A2. Molecule Class/Organism

Glycoside hydrolase
Gallus gallus (Chicken)

A3. Sequence/Formula

KVFGRCELAA AMKRHGLDNY RGYSLGNWVC AAKFESNFNT QATNRNTDGS TDYGILQINS RWWCNDGRTP GSRNLCNIPC
SALLSSDITA SVNCAKKIVS DGNGMNAWVA WRNRCKGTDV QAWIRGCRL

A4. Purification Strategy/Source

Sigma-Aldrich GmbH
L6876

A5. Stock Concentration/Stock Buffer

32 µg lyophilized powder

A6. Molecular Weight/Extinction Coefficient

14.3 kDa
37,970 $M^{-1}cm^{-1}$ (ε_{280})

A7. Dilution Buffer

25 mM Histidine buffer, pH 5.0, 0.01% Pluronic® F-127

A8. Labeling Strategy

Self-competition assay[1] with Cy5-labeled lysozyme-binding aptamer (Cy5-LBA)
(metabion international AG)

5' Cy5 TTT TTT ATC AGG GCT AAA GAG TGC AGA GTT ACT TAG 3'

A9. Labeling Procedure

N/A

A10. Labeling Efficiency

N/A

B1. Ligand/Non-Fluorescent Binding Partner

Lysozyme-binding aptamer (LBA)

B2. Molecule Class/Organism

DNA aptamer

B3. Sequence/Formula

5' ATC AGG GCT AAA GAG TGC AGA GTT ACT TAG 3'

B4. Purification Strategy/Source

metabion international AG

B5. Stock Concentration/Stock Buffer

100 µM
ddH_2O

B6. Molecular Weight/Extinction Coefficient

11,678 Da
356,800 $M^{-1}cm^{-1}$ (ε_{260})

[1] NHS-labeling of lysozyme interferes with aptamer binding due to lysines in the binding interface, while lysozyme at high concentrations tends to form fibrils or aggregates. A self-competition assay with unlabeled LBA allows to work with a low concentration of lysozyme while avoiding the need to label lysozyme with a fluorescent dye.

B7. Serial Dilution Preparation

Direct binding assay

1. Resuspend 32 µg lysozyme in 112 µL of dilution buffer to obtain a 20 µM solution.
2. Mix 2 µL of 100 µM Cy5-LBA with 98 µL of dilution buffer to obtain 100 µL of a 2 µM Cy5-LBA solution.
3. Mix 4 µL of 2 µM Cy5-LBA with 196 µL of dilution buffer to obtain 200 µL of a 40 nM Cy5-LBA solution.
4. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of the 20 µM lysozyme solution into tube **1**. Then, transfer 10 µL of dilution buffer into tubes **2** to **16**.
5. 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.
6. Add 10 µL of 40 nM Cy5-LBA to each tube from **16** to **1** and mix by pipetting.
7. Incubate for 5 minutes at room temperature in the dark before loading capillaries.

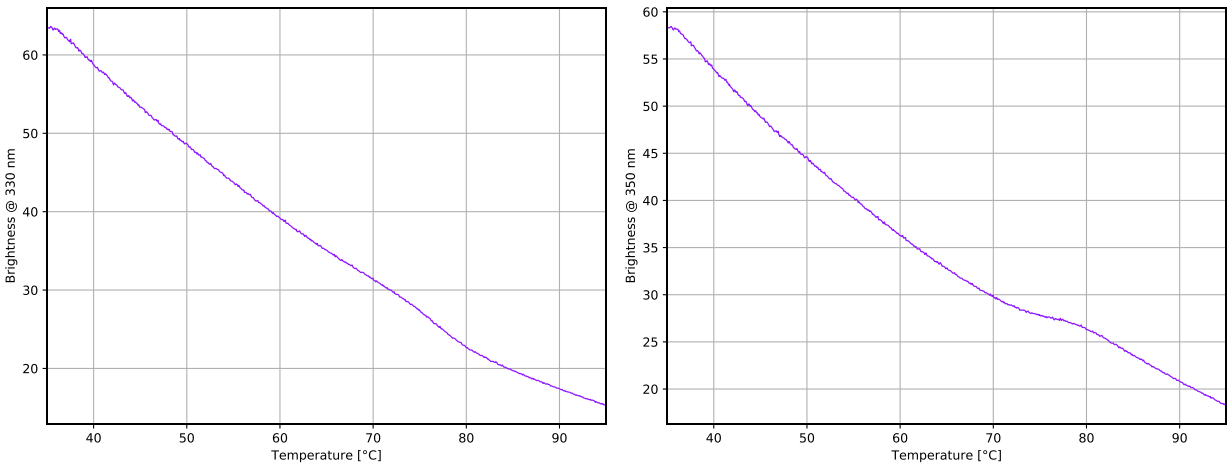
Self-competition assay

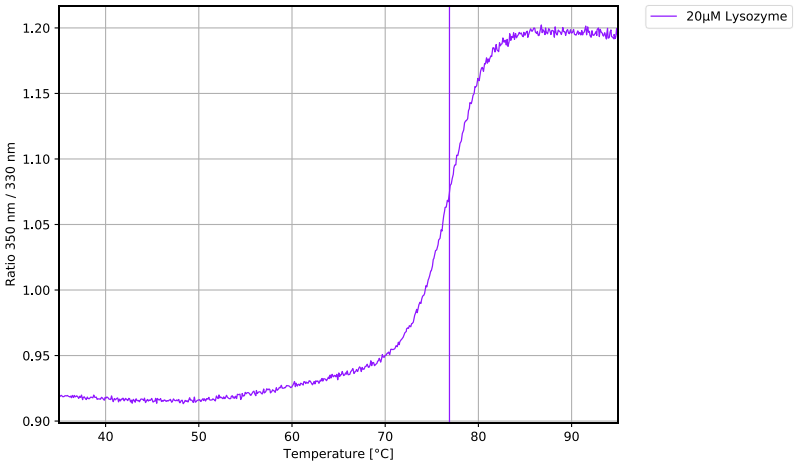
1. Mix 6 µL of 2 µM Cy5-LBA with 194 µL of dilution buffer to obtain 200 µL of a 60 nM Cy5-LBA solution.
2. Prepare a PCR-rack with 16 new PCR tubes. Mix 3.6 µL of a 100 µM LBA solution with 16.4 µL of dilution buffer in tube **1**. Then, transfer 10 µL of dilution buffer into tubes **2** to **16**.
3. 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.
4. Add 10 µL of 60 nM Cy5-LBA to each tube from **16** to **1** and mix by pipetting.
5. Mix 2 µL of 20 µM lysozyme with 18 µL of dilution buffer to obtain 20 µL of a 2 µM lysozyme solution.
6. Mix 6 µL of 2 µM lysozyme with 194 µL of dilution buffer to obtain 200 µL of a 60 nM lysozyme solution.
7. Add 10 µL of 60 nM lysozyme to each tube from **16** to **1** and mix by pipetting.
8. Incubate for 5 minutes at room temperature in the dark before loading capillaries.

C. Applied Quality Checks

Validation of the structural integrity of lysozyme using Tycho NT.6:
nanotempertech.com/tycho

Lysozyme	10 µL of 20 µM lysozyme	T_i = 76.9°C





D1. MST System/Capillaries

Monolith NT.115 Red (NanoTemper Technologies GmbH)
Premium Capillaries Monolith NT.115 (MO-K025, NanoTemper Technologies GmbH)

D2. MST Software

MO.Control v1.6 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

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

Direct binding assay

25 mM Histidine buffer, pH 5.0, 0.01% Pluronic® F-127
20 nM Cy5-LBA | 10 µM – 305 pM lysozyme | 22°C | medium MST power | 20% excitation power

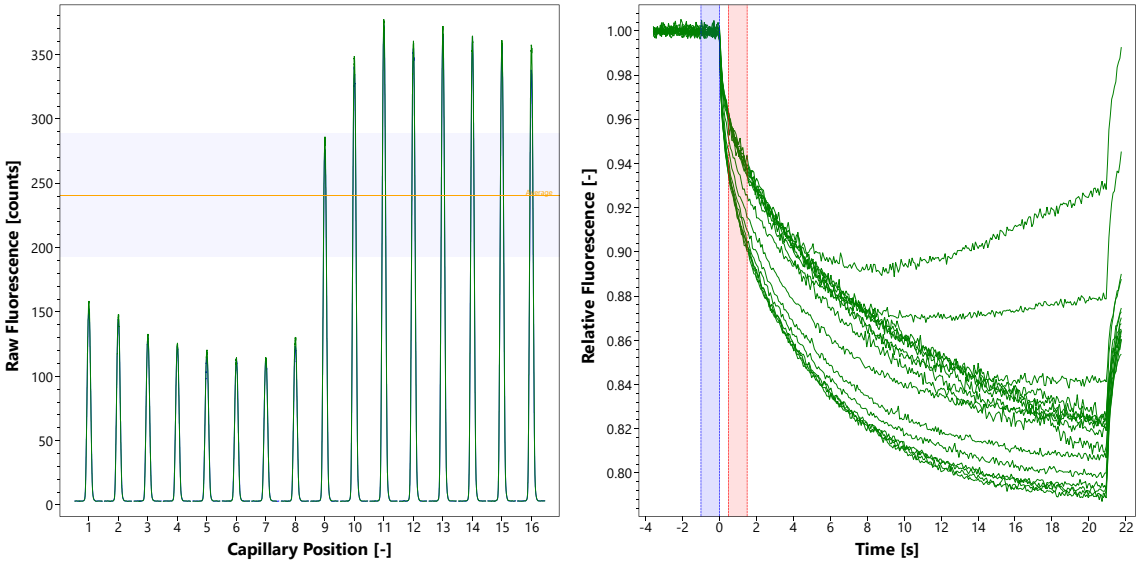
Self-competition assay

25 mM Histidine buffer, pH 5.0, 0.01% Pluronic® F-127
20 nM lysozyme, 20 nM Cy5-LBA | 6 µM – 183 pM LBA | 22°C | medium MST power | 20% excitation power

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

Direct binding assay

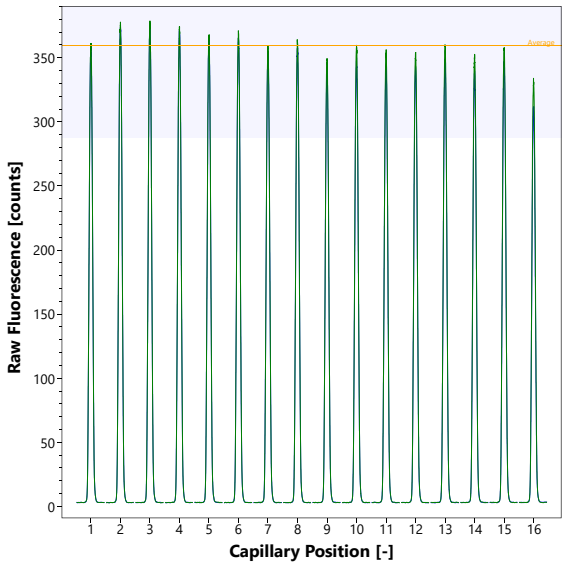
$K_d = N/A^2$

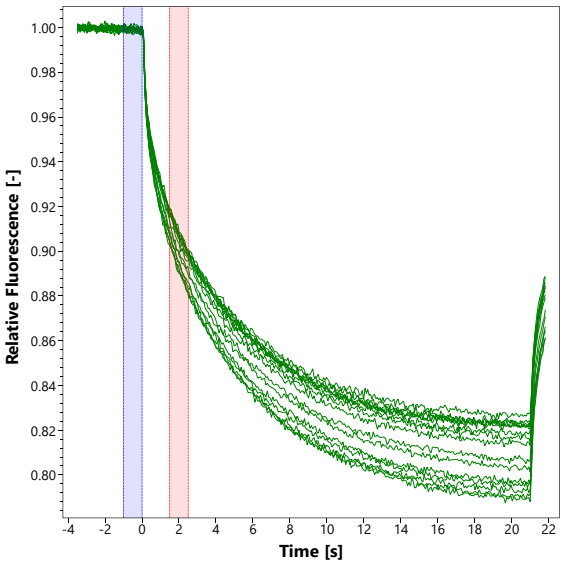


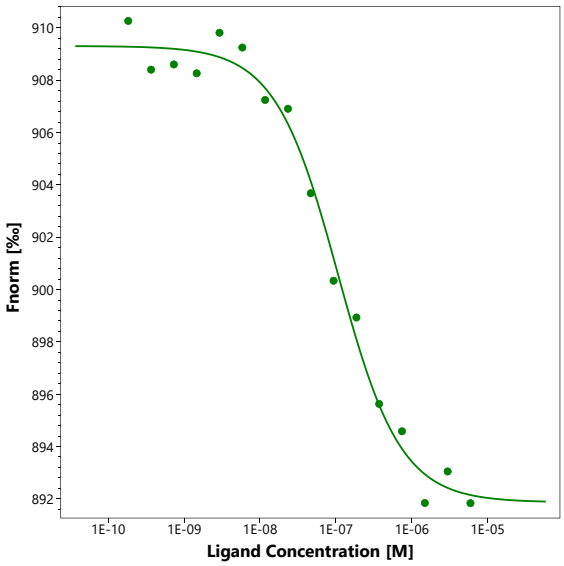
[2] Binding cannot be evaluated in the direct binding assay due to strong ligand-induced fluorescence changes that may be caused by lysozyme aggregation or fibrillation at high concentration. Therefore, an intermediate concentration of 20 nM (capillary #10) is chosen to form a complex between Cy5-LBA and lysozyme. Next, unlabeled LBA is added in a self-competition assay format to compete with Cy5-LBA for lysozyme binding.

Self-competition assay

EC_{50} = 97.2 nM | K_i = 53.0 nM[3]







D5. Reference Results/Supporting Results

K_d = 49.5 nM Frontal analysis continuous microchip electrophoresis (FACMCE)
Girardot et al., Journal of Chromatography A, 1218 (2011) 4052–4058

E. Contributors

Andreas Langer[4]

[3] For calculation of K_i, it was assumed that the affinities of lysozyme for LBA and Cy5-LBA are identical (see also the NanoTemper 'FAQ Competitive Binding Assay').
[4] NanoTemper Technologies GmbH, München, Germany | nanotempertech.com