Monolith Protocol MO-P-059

Tris-NTA – His_6-Peptide

The tris-NTA/His-tag system comprises one of the smallest high-affinity recognition elements known to date. This interaction is based on the capacity of the histidine's imidazole groups to form coordinative bonds with transition metal ions such as Ni(II). Chelators such as nitrilotriacetic acid (NTA) stably bind Ni(II) ions via three oxygen atoms and one nitrogen atom. The two remaining coordination sites of Ni(II) can each bind one histidine moiety of a His-tag, yielding a molecular binding affinity of ~10 µM. Tris-NTA is comprised of three NTA moieties coupled to a cyclic scaffold and thus can simultaneously bind six Histidine residues of a His_6-tag, yielding nanomolar binding affinity and a well-defined 1:1 stoichiometry.

peptide – small molecule interaction | His_6-tag

A1. Target/Fluorescent Molecule

Tri-nitrilotriacetic acid, complexed with Ni(II) ions (Tris-NTA)

A2. Molecule Class/Organism

Chelating agent

A3. Sequence/Formula

N/A

A4. Purification Strategy/Source

Monolith His-Tag Labeling Kit RED-tris-NTA 2nd Generation (MO-L018, NanoTemper Technologies GmbH)

A5. Stock Concentration/Stock Buffer

125 nmol lyophilized powder

A6. Molecular Weight/Extinction Coefficient

195,000 $M^{-1}cm^{-1}$ (ε_{650})

A7. Dilution Buffer

20 mM HEPES, pH 7.4, 150 mM NaCl, 0.01% Pluronic® F-127

A8. Labeling Strategy

Dye-conjugate

A9. Labeling Procedure

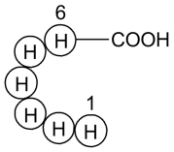
1. Suspend 125 pmol RED-tris-NTA Dye 2nd Generation in 25 µL of dilution buffer to obtain a 5 µM dye solution.
2. Mix 198 µL of dilution buffer with 2 µL dye (5 µM) to obtain 200 µL of a 50 nM dye solution.
3. Mix 16 µL of 50 nM dye with 184 µL of dilution buffer to obtain 200 µL of a 4 nM dye solution.

A10. Labeling Efficiency

100%

B1. Ligand/Non-Fluorescent Binding Partner

His_6-peptide



B2. Molecule Class/Organism

Peptide

B3. Sequence/Formula

HHHHHH

B4. Purification Strategy/Source

APExBIO
A6006

B5. Stock Concentration/Stock Buffer

5 mg
Lyophilized powder

B6. Molecular Weight/Extinction Coefficient

840.8 Da
45,100 $M^{-1}cm^{-1}$ (ε_{205})[1]

[1] Calculated from amino acid sequence (Anthis et al., Protein Science 2013, 22, 851–858).

B7. Serial Dilution Preparation

1. Prepare a 10 µM His_6-peptide stock solution by dissolving the lyophilized peptide into ddH_2O. Verify the concentration spectroscopically using an extinction coefficient of 45,100 $M^{-1}cm^{-1}$ (ε_{205}).
2. Prepare a PCR-rack with 16 PCR tubes. Mix 8 µL of 10 µM His_6-peptide and 12 µL of dilution buffer in tube **1** to obtain 20 µL of a 4 µM His_6-peptide solution. Then, mix 80 µL of ddH_2O and 120 µL of dilution buffer in a separate tube and add 10 µL of this solution 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 RED-tris-NTA Dye 2nd Generation (4 nM) to each tube from **16** to **1** and mix by pipetting.
5. Incubate for 45 minutes at room temperature in the dark before loading capillaries.

C. Applied Quality Checks

N/A

D1. MST System/Capillaries

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

D2. MST Software

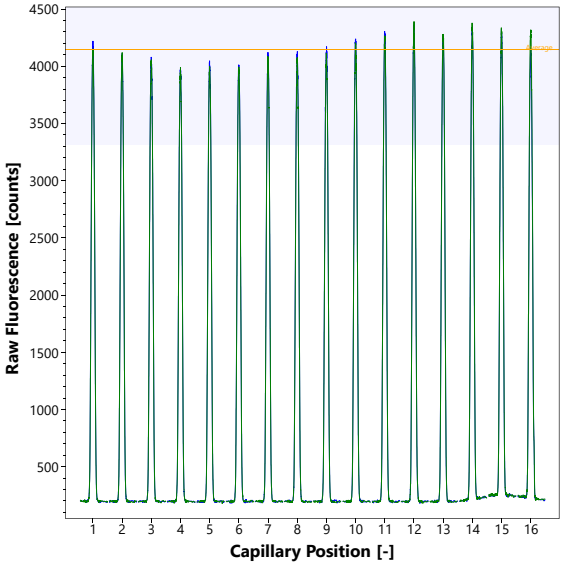
MO.Control v1.6 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

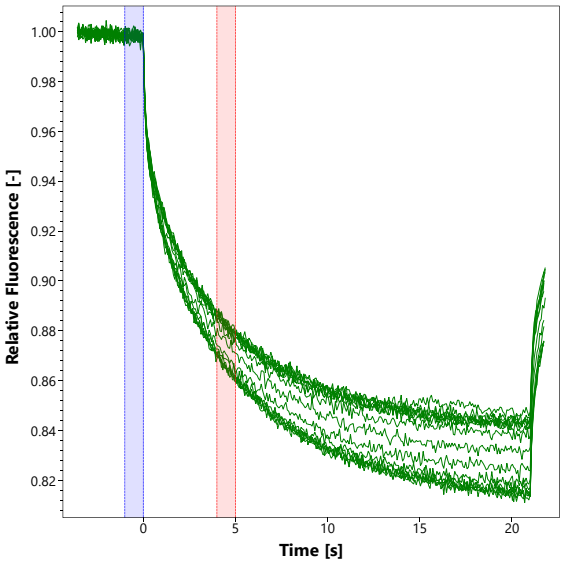
D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

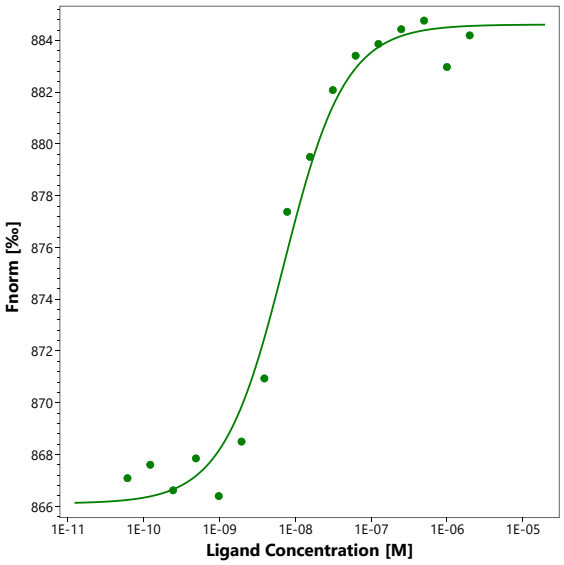
16 mM HEPES, pH 7.4, 120 mM NaCl, 0.008% Pluronic® F-127
2 nM Tris-NTA | 2 µM – 61 pM His_6-peptide | 22°C | medium MST power | 20% excitation power

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

K_d = 6.1 nM







D5. Reference Results/Supporting Results

K_d = 20 ± 10 nM Isothermal Titration Calorimetry (ITC)
Lata et al., J. Am. Chem. Soc. 2005, 127, 10205-10215

E. Contributors

Andreas Langer[2]

[2] NanoTemper Technologies GmbH, München, Germany | nanotempertech.com