Monolith Protocol MO-P-071

Zn(II)-Mediated Dimerization of His-Tags

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). The presence of high concentrations of other transition metals such as Co(II), Cu(II) or Zn(II), however, can interfere with the tris-NTA/His-tag interaction. Zn(II), for example, has been described to mediate the dimerization of His-tagged proteins at low micromolar concentrations.

peptide – ion interaction | dimerization | His_6-tag | competitive assay

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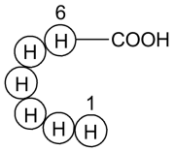
A1. Target/Fluorescent Molecule

His_6-peptide



A2. Molecule Class/Organism

Peptide

A3. Sequence/Formula

HHHHHH

A4. Purification Strategy/Source

APExBIO
A6006

A5. Stock Concentration/Stock Buffer

5 mg
Lyophilized powder

A6. 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).

A7. Dilution Buffer

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

A8. Labeling Strategy

Monolith His-Tag Labeling Kit RED-tris-NTA 2nd Generation (MO-L018, NanoTemper Technologies GmbH)
1* 125 pmol RED-tris-NTA Dye 2nd Generation

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. 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}).
3. Mix 4 µL of 10 µM His_6-peptide and 2 µL of the RED-tris-NTA Dye 2nd Generation (5 µM) with 194 µL of dilution buffer to obtain 200 µL of a 200 nM His_6-peptide, 50 nM dye solution.
4. Incubate for 30 minutes at room temperature in the dark.

A10. Labeling Efficiency

N/A

B1. Ligand/Non-Fluorescent Binding Partner

Zn^{2+}

B2. Molecule Class/Organism

Divalent cation

B3. Sequence/Formula

$ZnCl_2$

B4. Purification Strategy/Source

Carl Roth GmbH
T887.1

B5. Stock Concentration/Stock Buffer

Powdered

B6. Molecular Weight/Extinction Coefficient

136.28 Da

B7. Serial Dilution Preparation

1. Dissolve 0.34 g of $ZnCl_2$ in 10 mL of ddH_2O to obtain a 250 mM $ZnCl_2$ stock solution. Add 4 drops of HCl (1.2 M) to acidify the solution and completely dissolve all $ZnCl_2$.
2. Mix 4 µL of the 250 mM $ZnCl_2$ stock with 496 µL of ddH_2O to obtain a 500 µL of a 2 mM $ZnCl_2$ solution.
3. Prepare a PCR-rack with 16 PCR tubes. Mix 2 µL of the 2 mM $ZnCl_2$ solution with 18 µL of ddH_2O in tube **1**. Then, add 10 µL of ddH_2O to tubes **2** to **16**.
4. 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.
5. Add 10 µL of labeled His_6-peptide (200 nM) to each tube from **16** to **1** and mix by pipetting.
6. Incubate for 30 minutes at room temperature in the dark before loading capillaries.

B8. EDTA Control

1. Prepare a PCR-rack with 16 fresh PCR tubes. Mix 2 µL of the 2 mM $ZnCl_2$ solution with 18 µL of ddH_2O in tube **1**. Then, add 10 µL of ddH_2O to 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. Mix 10 µL of a 1 mM buffered EDTA solution (pH ~7 – 8) with 184 µL of dilution buffer. Then, add 4 µL of 10 µM His_6-peptide and 2 µL of the RED-tris-NTA Dye 2nd Generation (5 µM) to obtain 200 µL of a 200 nM His_6-peptide, 50 nM dye, 50 µM EDTA solution.
4. Add 10 µL of this solution to each tube from **16** to **1** and mix by pipetting.
5. Incubate for 30 minutes at room temperature in the dark before loading capillaries.

D1. MST System/Capillaries

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

D2. MST Software

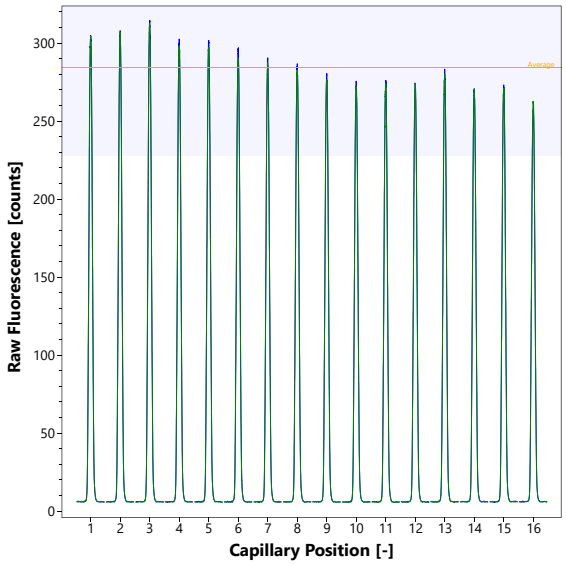
MO.Control v1.6 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

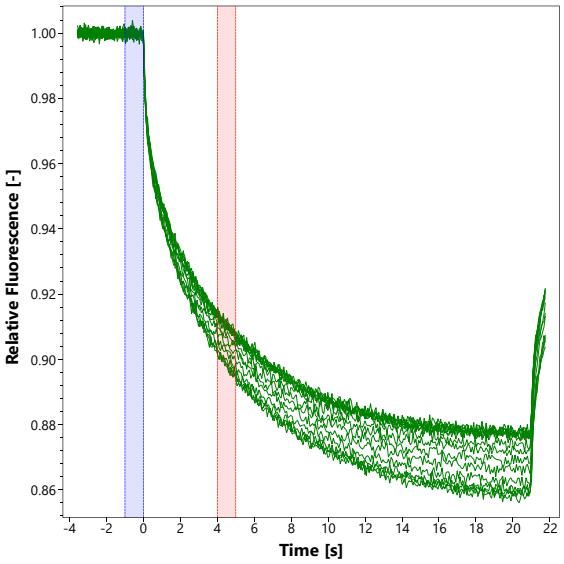
D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

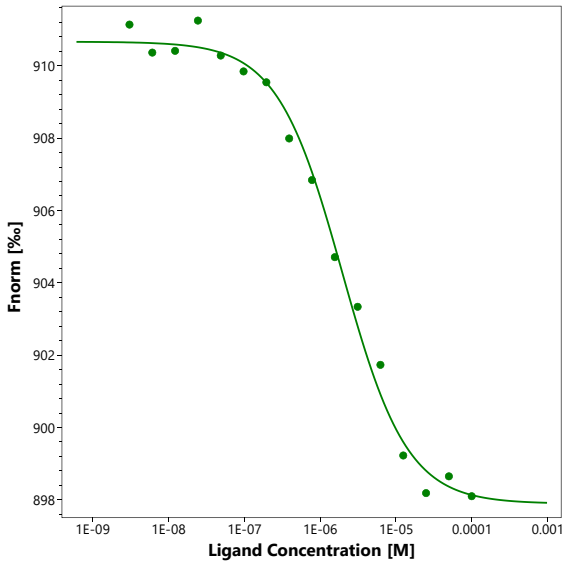
10 mM HEPES, pH 7.4, 75 mM NaCl, 0.005% Pluronic® F-127
100 nM His_6-peptide | 100 µM – 3.05 nM $ZnCl_2$ | 22°C | medium MST power | 40% excitation power

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

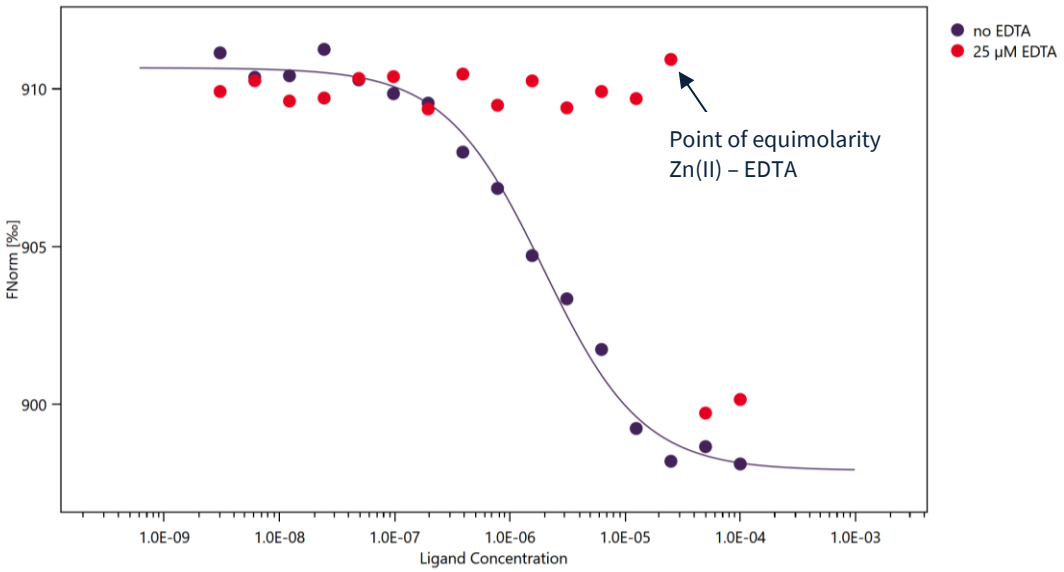
EC_{50} = 1.9 µM







EDTA control (25 µM final concentration in the assay):



D5. Reference Results/Supporting Results

Zn(II)-induced dimerization of His-tags

$K_A = 7.7 \cdot 10^{10}\ M^{-2}$ Fluorescence Spectroscopy

Evers et al., Protein Engineering, Design & Selection 21 (8), 529–536, 2008

E. Contributors

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