Monolith Protocol MO-P-061

Reversible Quenching of Red Fluorophores by TCEP

Tris(2-carboxyethyl)phosphine (TCEP) is a widely used reducing agent for the reduction of disulfide bonds on proteins. Some red fluorophores, including Cy5 and Alexa 647, are strongly quenched by TCEP. The quenching is caused by a reversible addition of TCEP to the dye with a dissociation constant in the mM range. To avoid interference in Monolith binding assays, TCEP should only be used at sub-mM concentrations.

small molecule – small molecule | quenching

A1. Target/Fluorescent Molecule

RED-tris-NTA Dye 2nd Generation

A2. Molecule Class/Organism

Fluorescent dye

A3. Sequence/Formula

N/A

A4. Purification Strategy/Source

Monolith RED-tris-NTA 2nd Generation labeling kit MO-L018, NanoTemper Technologies GmbH

A5. Stock Concentration/Stock Buffer

125 pmol lyophilized powder

A6. Molecular Weight/Extinction Coefficient

N/A

A7. Dilution Buffer

20 mM HEPES pH 7.4, 150 mM NaCl, 0.005% TWEEN® 20

A8. Labeling Strategy

N/A

A9. Labeling Procedure

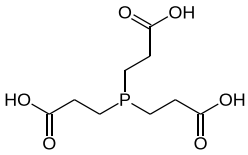
1. Suspend 125 pmol RED-tris-NTA Dye 2nd Generation in 25 µL of dilution buffer to obtain a 5 µM dye solution.
2. Mix 198 µL of dilution buffer with 2 µL dye (5 µM) to obtain 200 µL of a 50 nM dye solution.

A10. Labeling Efficiency

100%

B1. Ligand/Non-Fluorescent Binding Partner

Tris(2-carboxyethyl)phosphine (TCEP)



B2. Molecule Class/Organism

Reducing agent

B3. Sequence/Formula

$C_9H_{15}O_6P$

B4. Purification Strategy/Source

Sigma-Aldrich GmbH
C4706

B5. Stock Concentration/Stock Buffer

0.5 M
pH 7.0 (aqueous solution; pH adjusted with ammonium hydroxide)

B6. Molecular Weight/Extinction Coefficient

286.65 Da

B7. Serial Dilution Preparation

1. Mix 10 µL of 0.5 M TCEP with 15 µL of dilution buffer to obtain 25 µL of a 200 mM TCEP solution.
2. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of the 200 mM TCEP solution into 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 RED-tris-NTA 2nd Generation dye (50 nM) to each tube from **16** to **1** and mix by pipetting.
5. Incubate for 5 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

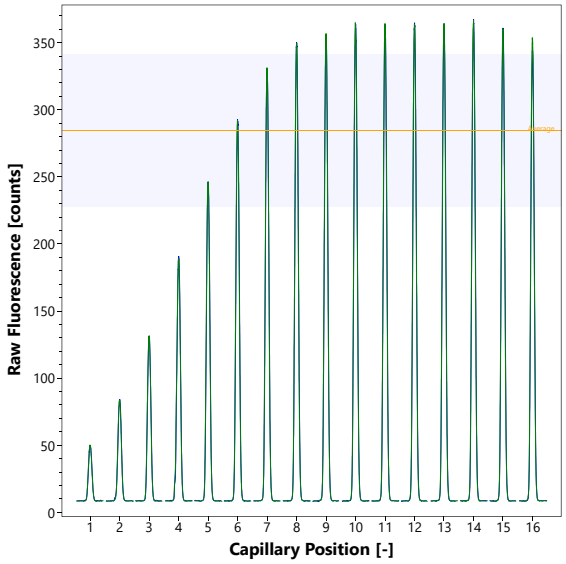
MO.Control v1.6 (NanoTemper Technologies GmbH)
https://nanotempertech.com/monolith-mo-control-software/

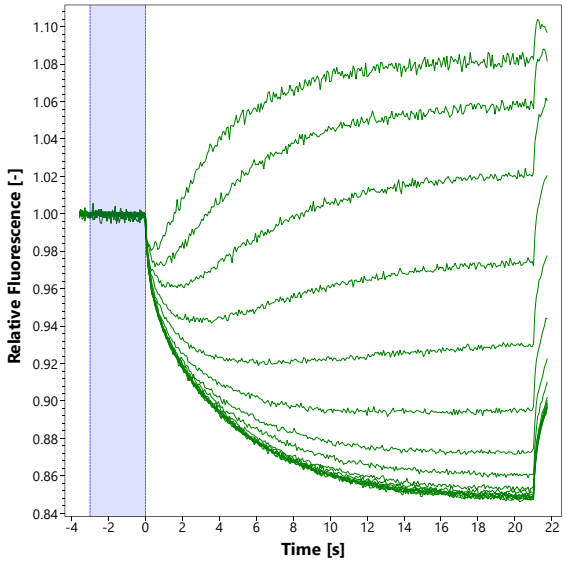
D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

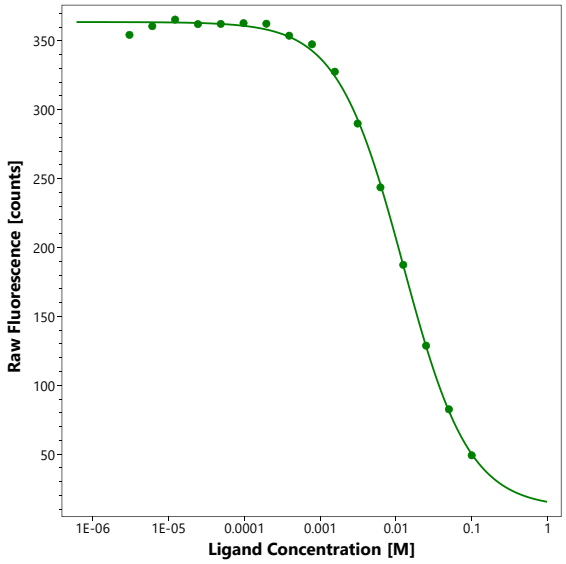
20 mM HEPES pH 7.4, 150 mM NaCl, 0.005% TWEEN® 20
25 nM dye | 100 mM – 3.05 µM TCEP | 25°C | medium MST power | 40% excitation power

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

K_d = 12.5 mM







D5. Reference Results/Supporting Results

The cyanine dye Cy5 and several of its structural relatives are reversibly quenched by the phosphine TCEP (tris(2-carboxyethyl)phosphine). The quenching reaction occurs by 1,4-addition of the phosphine to the polymethine bridge of Cy5 to form a covalent adduct.
Vaughan et al., J Am Chem Soc 135(4), 1197–1200 (2013)

E. Contributors

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