Monolith Protocol MO-P-034

Ricin A Chain – P1-P2 Heterodimer

The ribosomal stalk also known as P-stalk is a pentameric complex composed of universally conserved proteins. The stalk is part of the GTPase Associated Centre (GAC) and represents one of its main functional elements. The role of the P-stalk within GAC is to recruit translational GTPases, such as elongation factor 2 (EF2) and stimulation of factor-dependent GTP hydrolysis. Interestingly, a family of ribosomal inactivating proteins (RIP) engages with P-stalk to activate its mechanism of inhibition. For example, the interaction of Ricin (type II RIP) with ribosomal stalk leads to depurination of a sarcin-ricin loop and translation stalling.

protein – protein interaction | ribosomal proteins | P-stalk | ricin | P1-P2

A1. Target/Fluorescent Molecule

Ricin A chain (RTA)
uniprot.org/uniprot/P02879

A2. Molecule Class/Organism

Toxin subunit
Ricinus communis (Castor Bean)

A3. Sequence/Formula

```
IFPKQYPIIN FTTAGATVQS YTNFIRAVRG RLTTGADVRH EIPVLPNRVG LPINQRFILV ELSNHAELSV TLALDVTNAY
VVGYRAGNSA YFFHPDNQED AEAITHLFTD VQNRYTFAFG GNYDRLEQLA GNLRENIELG NGPLEEAISA LYYYSTGGTQ
LPTLARSFII CIQMISEAAR FQYIEGEMRT RIRYNRRSAP DPSVITLENS WGRLSTAIQE SNQGAFASPI QLQRRNGSKF
SVYDVSILIP IIALMVYRCA PPPSSQF
```

A4. Purification Strategy/Source

RTA was expressed in Escherichia coli BL21(DE3)RIL cells and N-terminal 10xHis-tagged recombinant RTA was purified using Ni-NTA agarose from QIAGEN (Valencia, CA, USA). The protein showed a single band on SDS-PAGE by Coomassie Brilliant Blue R-250 staining and by immunoblot analysis and was active in in vitro binding assays.

A5. Stock Concentration/Stock Buffer

1.26 mg/mL | 42 µM
50 mM Tris-HCl, pH 7.8, 150 mM NaCl, 10 mM $MgCl_2$

A6. Molecular Weight/Extinction Coefficient

29.9 kDa
26,485 $M^{-1}cm^{-1}$ (ε_{280})

A7. Dilution Buffer

Phosphate-buffered saline (PBS, pH 7.4), 0.05% TWEEN® 20

A8. Labeling Strategy

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

A9. Labeling Procedure

1. Suspend 125 pmol RED-tris-NTA 2nd Generation Dye in 25 µL of dilution buffer to obtain a 5 µM dye solution.
2. Prepare a 20 nM dye solution by mixing 2 µL of dye (5 µM) and 498 µL of dilution buffer.
3. Prepare a 200 nM RTA solution by mixing 2.4 µL of 42 µM RTA and 498 µL of dilution buffer.
4. Mix 500 µL of RTA (200 nM) with 500 µL of dye (20 nM).
5. Incubate for 30 minutes at room temperature.
6. Centrifuge for 10 min at 4°C and 15 000 × g.

A10. Labeling Efficiency

N/A

B1. Ligand/Non-Fluorescent Binding Partner

P1-P2 heterodimer
uniprot.org/uniprot/P05386 | uniprot.org/uniprot/P05387

B2. Molecule Class/Organism

Ribosomal protein
Homo sapiens (Human)

B3. Sequence/Formula

P1

```
MASVSELACI YSALILHDDE VTVTEDKINA LIKAAGVNVE PFWPGLFAKA LANVNIGSLI CNVGAGGPAP AAGAAPAGGP
APSTAAAPAE EKKVEAKKEE SEESDDDMGF GLFD
```

P2

```
MRYVASYLLA ALGGNSSPSA KDIKKILDSV GIEADDDRLN KVISELNGKN IEDVIAQGIG KLASVPAGGA VAVSAAPGSA
APAAGSAPAA AEEKKDEKKE ESEESDDDMG FGLFD
```

B4. Purification Strategy/Source

Recombinant human P1 and P2 proteins, truncated forms, were expressed in Escherichia coli BL21(DE3)RIL cells and purified using a procedure described previously for the yeast P proteins. The P1-P2 heterocomplex was prepared following the denaturation/renaturation procedure established for the yeast P protein complex.[1]

B5. Stock Concentration/Stock Buffer

0.5 mg/mL | 115 µM
50 mM Tris-HCl, pH 7.8, 150 mM NaCl, 10 mM $MgCl_2$

B6. Molecular Weight/Extinction Coefficient

23.2 kDa
10,095 $M^{-1}cm^{-1}$ (ε_{280})

B7. Serial Dilution Preparation

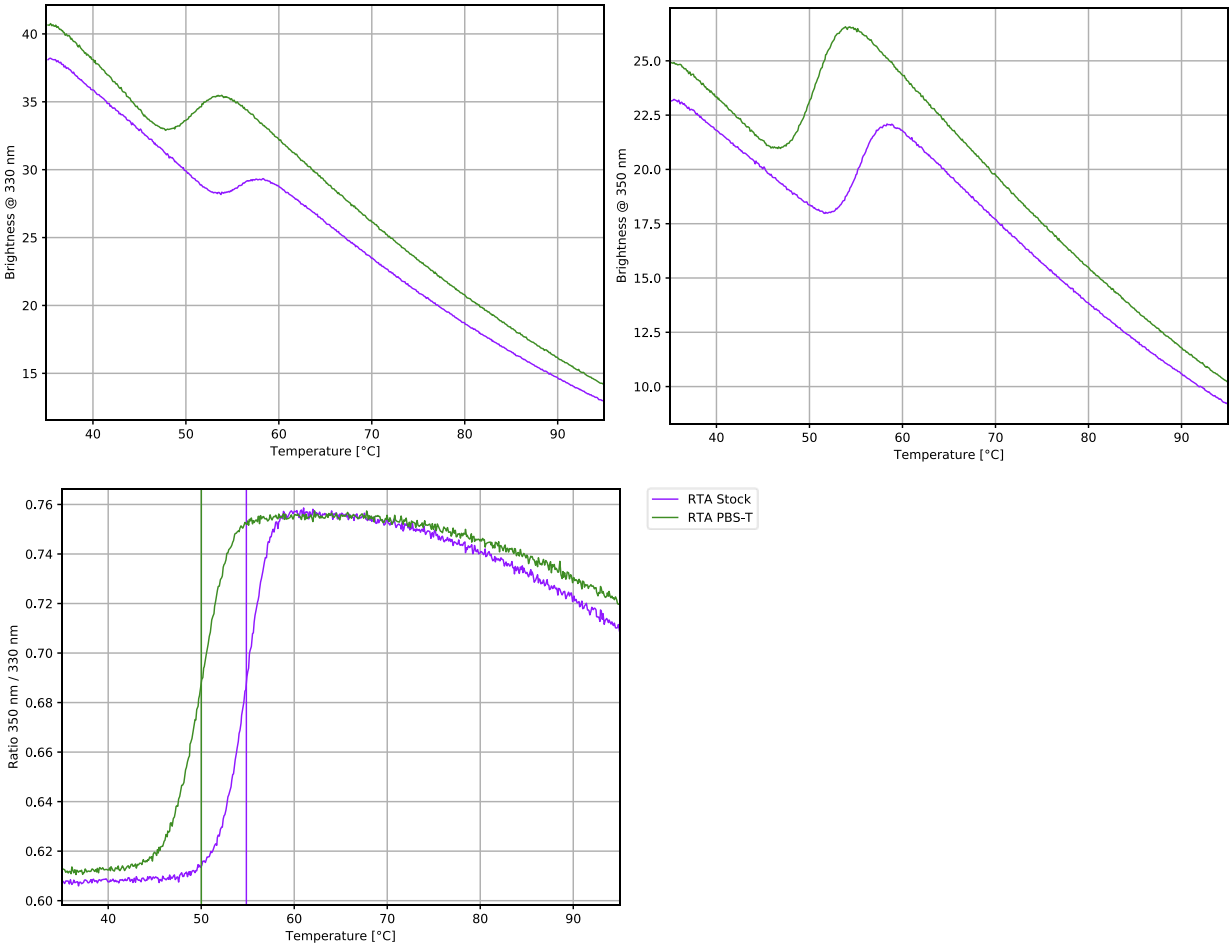
1. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of the 115 µM P1-P2 solution into tube **1**. Then, transfer 10 µL of stock buffer into tubes **2** to **16**.
2. Prepare a 1:1 serial dilution by transferring 10 µL from tube to tube. Mix carefully by pipetting up and down. Remember to discard 10 µL from tube **16** to get an equal volume of 10 µL for all samples.
3. Add 10 µL of 100 nM labeled RTA 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.

[1] See Grela et al., J Biochem 143, 169-177 (2008) and Tchorzewski et al., Protein Expr. Purif. 15, 40-47 (1999) for further details.

C. Applied Quality Checks

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

Unlabeled, in stock buffer	10 µL of 3.3 µM RTA in stock buffer	T_i = 54.9°C
Unlabeled, in PBS-T	10 µL of 3.3 µM RTA in PBS-T	T_i = 50.0°C



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

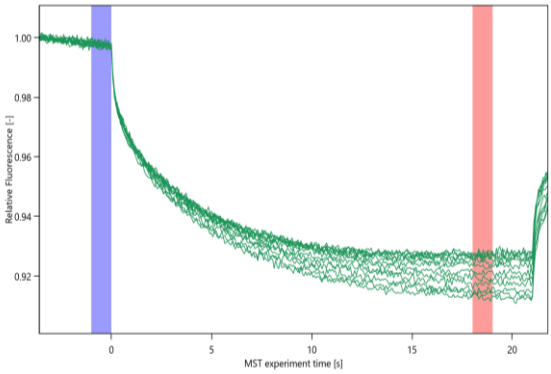
MO.Control v1.6 (NanoTemper Technologies GmbH)
https://nanotempertech.com/monolith-mo-control-software/

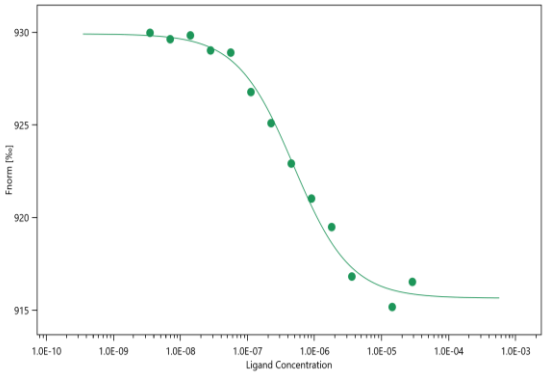
D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

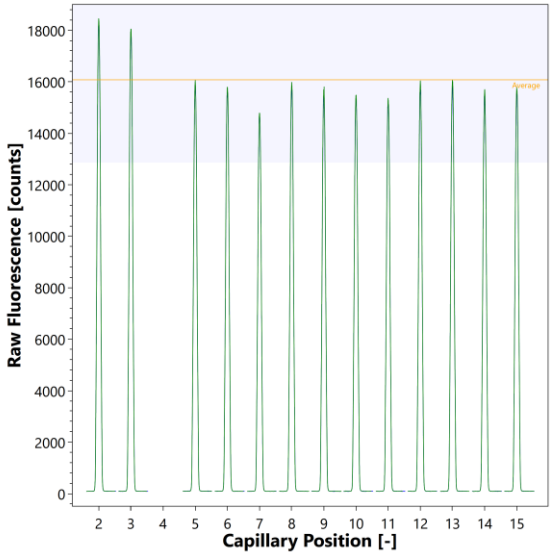
25 mM Tris-HCl, PBS-T, pH 7.4-7.8, 5 mM $MgCl_2$, 0.025% TWEEN® 20
50 nM RTA | 45.5 µM – 1.39 nM P1-P2 | 25°C | low MST power | 20% excitation power

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

K_d = 447 nM







D5. Reference Results/Supporting Results

K_d = 21 nM Apparent K_d, BioLayer Interferometry (BLI)
Grela et al., Scientific Reports 7, 5608 (2017)

E. Contributors

Przemysław Grela[2], Patrycja Horbowicz-Drożdżal[2], Jakub Nowak[3]

[2] Department of Molecular Biology, Maria Curie Sklodowska University, Lublin, Poland
[3] NanoTemper Technologies Sp. z.o.o., Kraków, Poland | nanotempertech.com