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Anti-SMT3 nanoCLAMP P972 Characterization and SMT3-A1(Resin) Performance

June 25, 2023

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- SMT3-A1(Resin) performance

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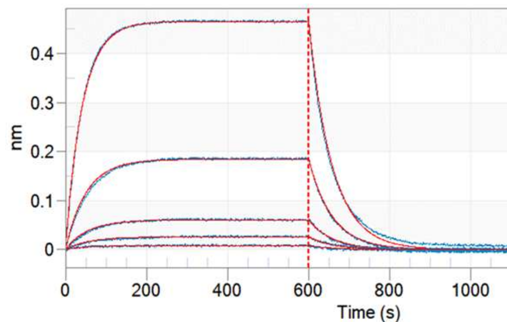
- SUMO Tag Affinity Resin performance data

P972: 232 nM K_D for SMT3 by biolayer interferometry

Biolayer interferometry



Parameter	Value
Ligand	P972 (biotinylated)
Analyte	SUMO-NusA
Analyte concentrations	88.9, 29.6, 9.88, 3.29, 1.1 nM
K_D	232 nM
k_a	7.46×10^4 $M^{-1}s^{-1}$
k_d	1.73×10^{-2} s^{-1}

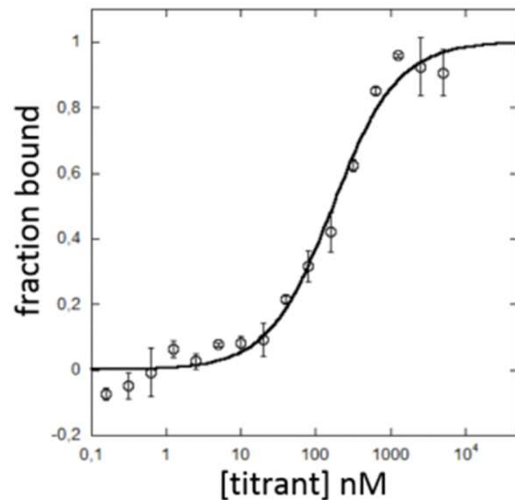
Methods

“P972” is also referred to as “SMT3-A1.”

Kinetic analysis of interactions between the biotinylated nanoCLAMP and SUMO-NusA fusion was carried out on an OctetRed96 using SAX streptavidin coated sensor tips. The tips were transferred first to buffer (150 mM NaCl, 20 mM MOPS pH 6.5, 1 mM $CaCl_2$, 1% BSA) for 300 sec, then to biotinylated SUMO at 2 mg/mL in buffer for 180 sec, then to buffer for 300 sec, then to 4 dilutions of the nanoCLAMP in buffer (association) for 200 or 600 sec, then to buffer (dissociation) for 400 - 500 sec. The cells were constantly vortexing at 1000 rpm at rm temp. The sensorgrams were fit to a 1:1 model and K_D calculated using global fit analysis.

P972: 163 nM K_D for SMT3 by microscale thermophoresis

Microscale thermophoresis



Binding	Binding event detected
Kd (tech 1+2)	163.4 ± 27.4 nM
Amplitude (tech 1+2)	22.71 fluorescence units
R2	0.985

Methods

Analysis of the interaction between P972 and SMT3-GFP (SUMO-GFP) by microscale thermophoresis. Binding of SMT3-A1 to SMT3-GFP fusion protein was quantified in PBS-T (0.05% Tween). SMT3-GFP was held at 10 nM while the concentration of SMT3-A1 was varied from 5 µM to 0.153 nM while the migration of the fluorescent protein was measured upon local heating using a Monolith NT.115 Pico with 20% Laser Power, 15% LED power, at 25°C

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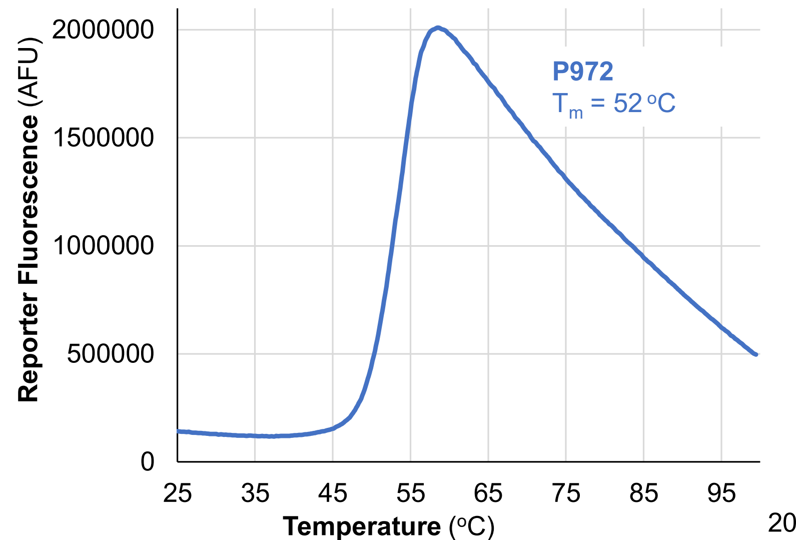
P972: T_m of 52°C by differential scanning fluorimetry

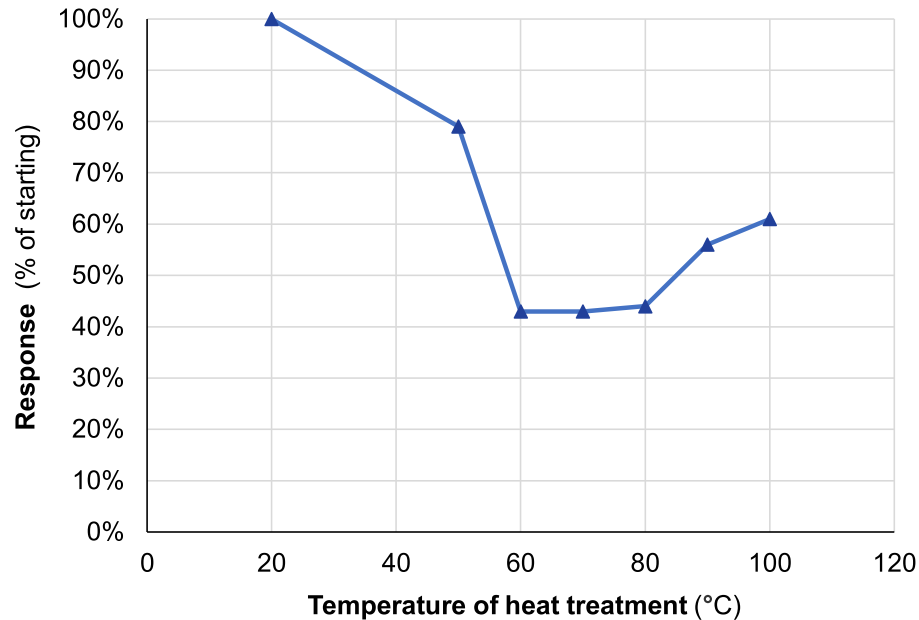
Differential scanning fluorimetry



Methods

The melting temperature of purified nanoCLAMP was determined using the GloMelt Thermal Shift Protein Stability Kit (Biotium) per manufacturer's instructions. Purified nanoCLAMPs were adjusted to 1 mg/mL in 150 mM NaCl, 20 mM MOPS pH 6.5, 1 mM $CaCl_2$ and diluted with an equal volume of 2X GloMelt (Biotium). The sample was aliquoted to 386 well plate and sealed with optical film. The plate was then heated in a Quantstudio 5 qPCR machine using the setting for SYBR Green reporter with no passive reference. The heating profile was 25°C for 2 min; ramp at 0.05°C /sec to 99°C; 99°C for 2 min. T_m is defined as the inflection point in the unfolding curve.

P972: T_{50} of 58°C by differential scanning fluorimetry



Methods

nanoCLAMPs were subjected to heat treatment at the specified temperatures for 5 min. and then cooled and centrifuged. The supernatant was tested for binding activity by biolayer interferometry. The percent of starting response was measured as the amplitude of binding divided by that obtained by the control sample (held at 20°C during heat treatments). The T_{50} is defined as the temperature of the 5 min. heat challenge after which 50% of activity is lost.

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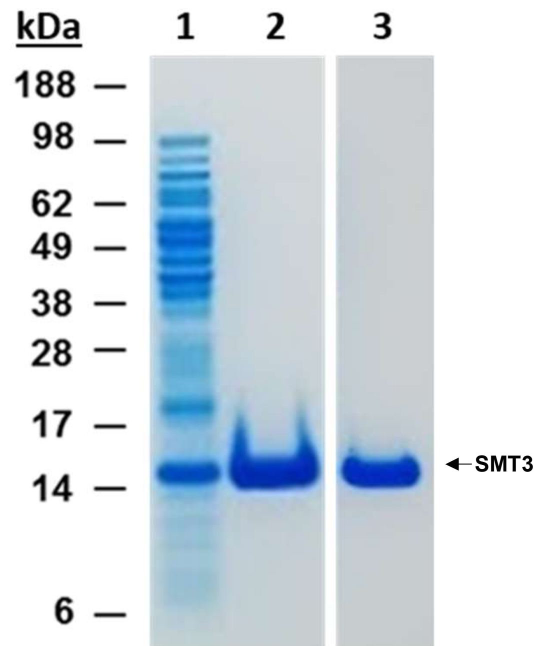
  - Purification of SUMOstar from *E. coli* lysate
  - Limited resistance to 0.1 M NaOH

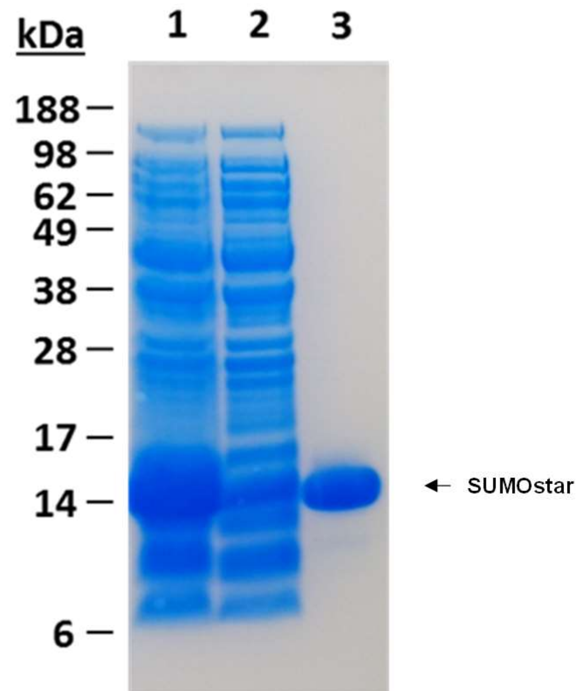
SMT3-A1(Resin): Purification of SMT3 from *E. coli* lysate



Methods

SDS-PAGE analysis of one step purification of SMT3 (yeast SUMO) from E.coli BL21DE3 whole cell lysates using SMT3-A1(Resin) affinity resin. SMT3-A1(Resin) consists of nanoCLAMP P972 coupled to 6% cross-linked agarose. *E.coli* were lysed with BPER (Thermo), cleared by centrifugation, and diluted with PBS to 1.2 mg total protein/ml. The lysate was spiked with recombinant antigen to 0.1 mg/ml in 1.5 ml and incubated with 10 µl (packed vol) SMT3-A1(Resin). Beads were washed with PBS and eluted with polyol elution buffer at near neutral pH. Lysates and eluants were analyzed on 12% SDS-PAGE in reducing SDS sample buffer.
Lane 1: SMT3-spiked lysate containing 10 µg total protein.
Lane 2: 6.5 µl of polyol eluant from the affinity resin, from a total of 125 µl total elution.
Lane 3: same as lane 2, except TCA precipitated prior to SDS-PAGE analysis

SMT3-A1(Resin): Purification of SUMOstar from *E. coli* lysate



Methods

SDS-PAGE analysis of one step purification of SUMOstar from E. coli BL21DE3 whole cell lysate using SMT3-A1(Resin) affinity resin. *E. coli* overexpressing SUMOstar were lysed by sonication, cleared by centrifugation, and incubated with 1.8 ml (packed vol) SMT3-A1(Resin) for 1 h at 4°C. Beads were washed with PBS and eluted with polyol elution buffer. The eluate was buffer exchanged into Mops buffered saline, pH 7.4. Lysates and eluates were analyzed on 12% SDS-PAGE in reducing SDS sample buffer.

Lane 1: Lysate (16 ul) containing overexpressed SUMOstar.

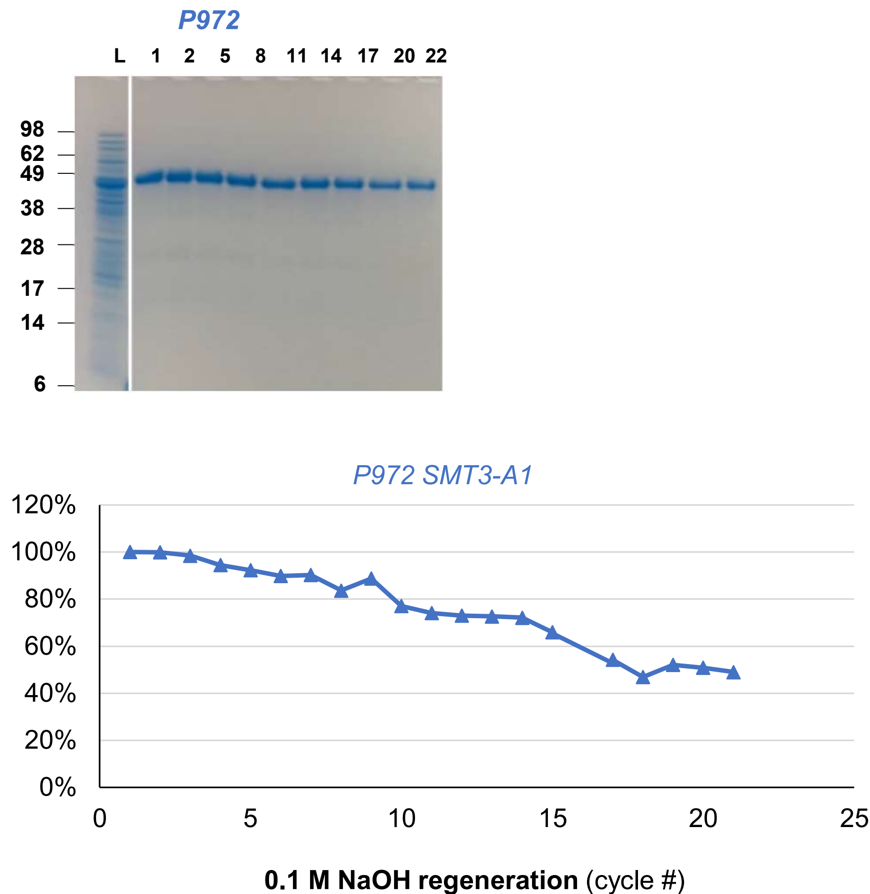
Lane 2: Flow-through (16 ul) post-incubation on 10 ul SMT3-A1(Resin)

Lane 3: 10 ug of buffer-exchanged eluate from SMT3-A1(Resin), from a total of 6.76 mg total elution.

Note

This protocol was scaled up to accommodate lysate from 100 ml E. coli expression culture

P972: Limited resistance to NaOH (80% capacity maintained after 10 cycles of 0.1 M NaOH)



Methods

SMT3-A1(Resin) was used to purify a SUMO-GFP fusion from a crude *E. coli* lysate. Each cycle consisted of a lysate sample load, wash, elution with 3M Imidazole (collected), wash, 0.1 M NaOH regeneration (10 min contact time per cycle), and a refolding wash for 5 min. The target protein in the eluate was quantified by fluorescence spectroscopy and the percent yield calculated by dividing the fluorescence by the initial eluate fluorescence. The purity of the eluted target from each cycle was assessed by SDS-PAGE and stained with Coomassie. Cycle number is shown for each lane, L = Load. The prominent band in the eluates of each gel is SUMO-GFP (42 kD).

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