Thermodynamic coupling between neighboring binding sites in homo-oligomeric ligand sensing proteins from mass resolved ligand dependent population distributions

Weicheng Li[‡], Andrew Norris[‡], Katie Lichtenthal, Skyler Kelly, Melody L. Holmquist, Elihu C. Ihms, Paul Gollnick, Vicki H Wysocki*, Mark P. Foster*

[1]Department of Chemistry and Biochemistry, The Ohio State University, Columbus, Ohio, 43210, United States [2]Department of Biological Sciences, University at Buffalo, State University of New York, Buffalo, New York 14260, United States [3]VPPL, Vaccine Research Center, NIAID, NIH, Vaccine Pilot Plant (VPP), Frederick, Gaithersburg, Maryland 20878, United States
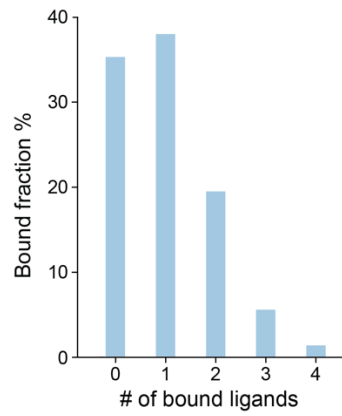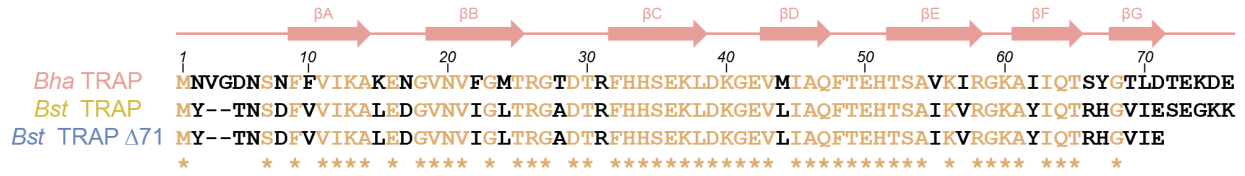
Supporting Information

Figure S1. Predicted ligand bound populations of 12mer protein at the saturation level of 1/12 = 8.3% via binomial distribution.

A   Comparisons of wild-type sequences

| | βA | βB | βC | βD | βE | βF | βG |

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                1        10        20        30        40        50        60        70
Bha TRAP   MNVGDNSNFFVIKAKENGVNVFGMTRGTDTRFHHSEKLDKGEVMIAQFTEHTSAVKIRGKAIIQTSYGTLDTEKDE
Bst  TRAP  MY--TNSDFVVIKALEDGVNVIGLTRGADTRFHHSEKLDKGEVLIAQFTEHTSAIKVRGKAYIQTRHGVIESEGKK
Bst  TRAP Δ71  MY--TNSDFVVIKALEDGVNVIGLTRGADTRFHHSEKLDKGEVLIAQFTEHTSAIKVRGKAYIQTRHGVIE
              *     * * **** * **** * *** ** *********** ********** * **** *** *
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B   Expected protein sequence for expression and purification

Bha TRAP WT

Expression

MNVGDNSNFFVIKAKENGVNVFGMTRGTDTRFHHSEKLDKGEVMIAQFTEHTSAVKIRGKAIIQTSYGTLDTEKDE**ENLYFQG**HHHHHH

Post-purification (expected protomer mass: 9287.38 Da)

MNVGDNSNFFVIKAKENGVNVFGMTRGTDTRFHHSEKLDKGEVMIAQFTEHTSAVKIRGKAIIQTSYGTLDTEKDE**ENLYFQ**

Bst TRAP

Expresion & post-purification (expected protomer mass: 8237.32 Da)

MYTNSDFVVIKALEDGVNVIGLTRGADTRFHHSEKLDKGEVLIAQFTEHTSAIKVRGKAYIQTRHGVIESEGKK

Bst TRAP Δ71

Expresion & post-purification (expected protomer mass: 7712.77 Da)

MYTNSDFVVIKALEDGVNVIGLTRGADTRFHHSEKLDKGEVLIAQFTEHTSAIKVRGKAYIQTRHGVIE

Figure S2. Sequence alignment of TRAP protomers from *Bacillus halodurans* (*Bha*), *Bacillus stearothermophilus* (*Bst*), and *Bst Δ71*-TRAP. (A) The identical residues are labeled with asterisk (*) symbol. Secondary structural elements are from the crystal structure of *Bha* TRAP (PDB 3zzl). (B) Expected protein sequence for expressions and post-purifications with the expected mass.
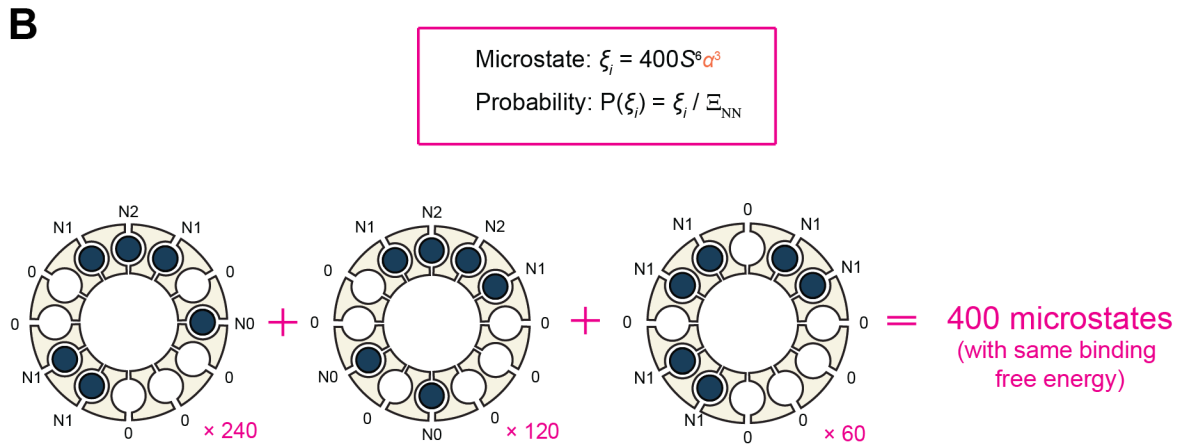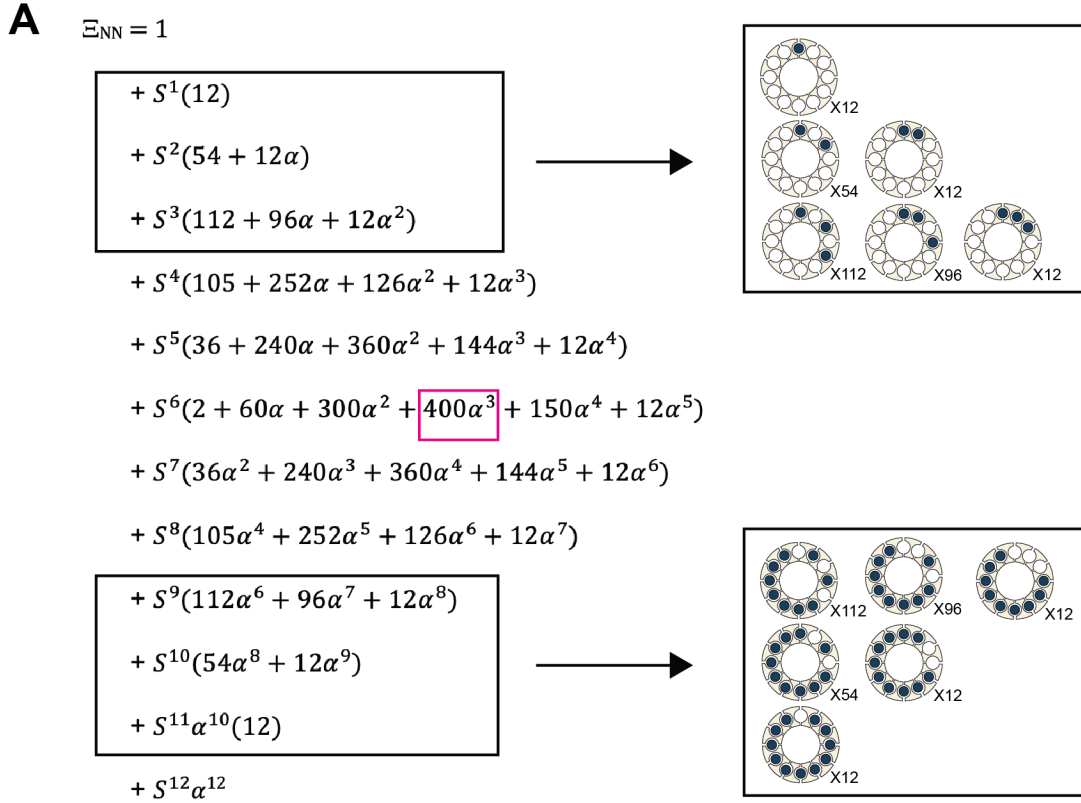
A

$\Xi_{NN} = 1$

$+ S^1(12)$

$+ S^2(54 + 12\alpha)$

$+ S^3(112 + 96\alpha + 12\alpha^2)$

$+ S^4(105 + 252\alpha + 126\alpha^2 + 12\alpha^3)$

$+ S^5(36 + 240\alpha + 360\alpha^2 + 144\alpha^3 + 12\alpha^4)$

$+ S^6(2 + 60\alpha + 300\alpha^2 + \boxed{400\alpha^3} + 150\alpha^4 + 12\alpha^5)$

$+ S^7(36\alpha^2 + 240\alpha^3 + 360\alpha^4 + 144\alpha^5 + 12\alpha^6)$

$+ S^8(105\alpha^4 + 252\alpha^5 + 126\alpha^6 + 12\alpha^7)$

$+ S^9(112\alpha^6 + 96\alpha^7 + 12\alpha^8)$

$+ S^{10}(54\alpha^8 + 12\alpha^9)$

$+ S^{11}\alpha^{10}(12)$

$+ S^{12}\alpha^{12}$

B

Microstate: $\xi_i = 400S^6\alpha^3$

Probability: $P(\xi_i) = \xi_i / \Xi_{NN}$

$\times 240$ $+$ $\times 120$ $+$ $\times 60$ $=$ **400 microstates**
(with same binding free energy)

Figure S3. Partition function from nearest neighbor (NN) model for cyclic homo-12mer protein, and example configurations. (A) Total degeneracies for each macro-state with m bound ligands are $W(n, m) = \binom{m}{n} = \frac{m!}{n!(m-n)}$, where m is the total number of sites, and n the number of occupied sites. Example configurations are shown for binding with 1, 2, 3, 9, 10 or 11 ligands (rectangles). (B) Detailed nearest-neighbor model illustration of the configurations that give rise to the term $400\times S^6\alpha^3$ (pink rectangle) in the partition function.
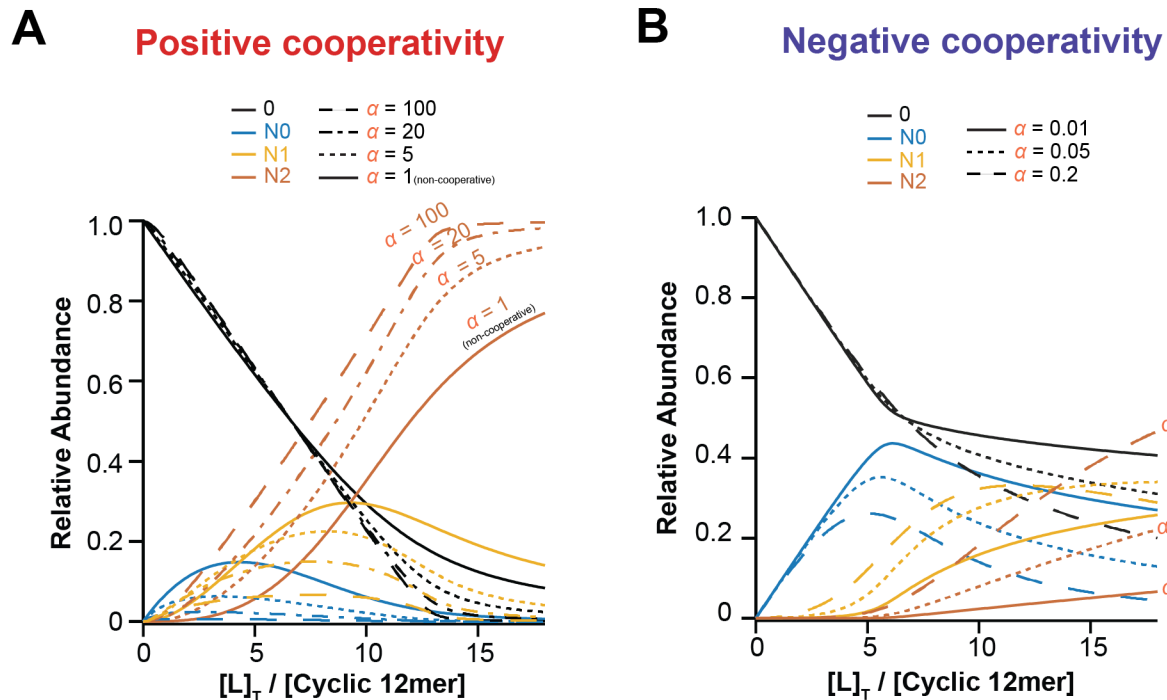
A **Positive cooperativity**

Legend: — 0 — N0 — N1 — N2 — – α = 100 — · – α = 20 ···· α = 5 — α = 1 (non-cooperative)

Y-axis: **Relative Abundance** (0, 0.2, 0.4, 0.6, 0.8, 1.0)

X-axis: **[L]$_T$ / [Cyclic 12mer]** (0, 5, 10, 15)

Curve labels: α = 100, α = 20, α = 5, α = 1 (non-cooperative)

B **Negative cooperativity**

Legend: — 0 — N0 — N1 — N2 — α = 0.01 ···· α = 0.05 — – α = 0.2

Y-axis: **Relative Abundance** (0, 0.2, 0.4, 0.6, 0.8, 1.0)

X-axis: **[L]$_T$ / [Cyclic 12mer]** (0, 5, 10, 15)

Curve labels: α = 0.01, α = 0.05, α = 0.01

Figure S4. Relative abundance of the four microscopic binding configurations as a function of total ligand concentration as predicted by the nearest-neighbor (NN) model under different extents of cooperativity (α); simulation parameters were: (A) Cooperativity strengths α = 1, 5, 20, 100; the non-cooperative case corresponds to α = 1. (B) Negative cooperativity, with α = 0.2, 0.05, 0.01. The micro-states are colored differently: empty (black), N0 (blue), N1 (yellow), N2 (brown).
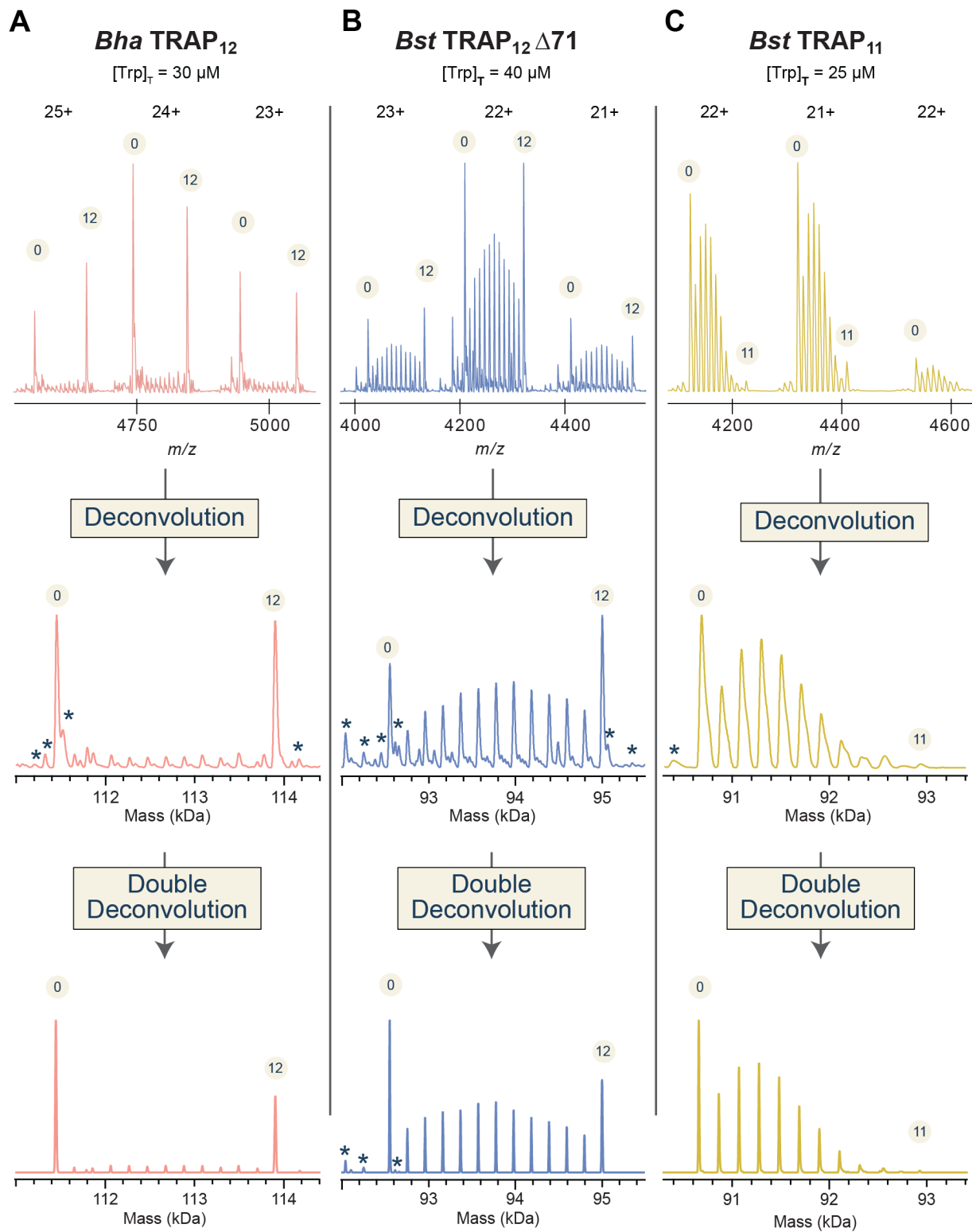
Figure S5. Representative deconvolution processes by Deconvolution and DoubleDec method, sequentially, via UniDec deconvolution program. Only three charge states are shown on the top m/z spectra for each TRAP proteins, followed by the deconvoluted spectra, and double-

deconvoluted spectra. Apo and holo states are labeled with circled 0 and 12 (or 11), respectively. Protein proteoforms are labeled with asterisk (*) symbol. (A) *Bha* TRAP mixed with 30 µM Trp. (B) *Bst* TRAP Δ71 mixed with 40 µM Trp, and (C) *Bst* TRAP mixed with 25 µM Trp. Protein concentrations can be found in Table S3.
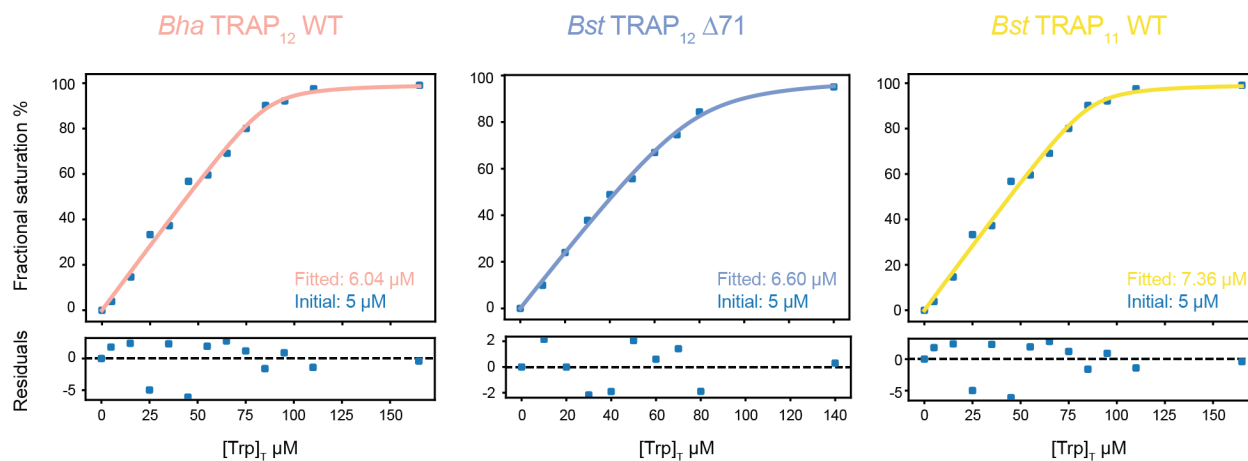
Figure S6. Representative concentration corrections of native MS data using mass balance equation.
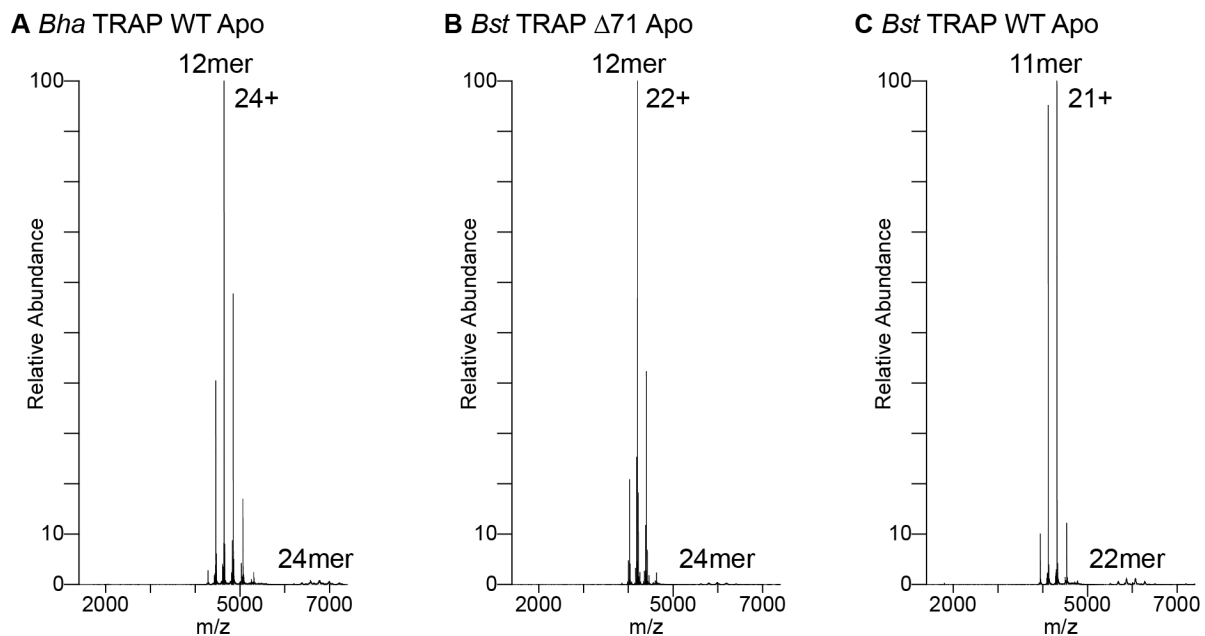
Figure S7. Native mass spectra of the TRAP samples used in this study. *Bha* TRAP (left), *Bst* TRAP Δ71 (middle), and *Bst* TRAP (right) are all at ~ 6 μM ring (Table S3) and in the apo state. The most abundant charge state distribution in each spectrum is the expected oligomeric state for each TRAP sample (labeled the oligomeric state and the most intense charge state). The masses for these oligomers are as follows: *Bha* $TRAP_{12}$ 111444 Da expected and 111453 Da observed, *Bst* $TRAP_{12}$ Δ71 92552 Da expected and 92560 Da observed, *Bst* $TRAP_{11}$ 90662 Da expected and 90670 Da observed. In each spectrum a low relative abundance of "double donut" (24mer or 22mer) is observed.
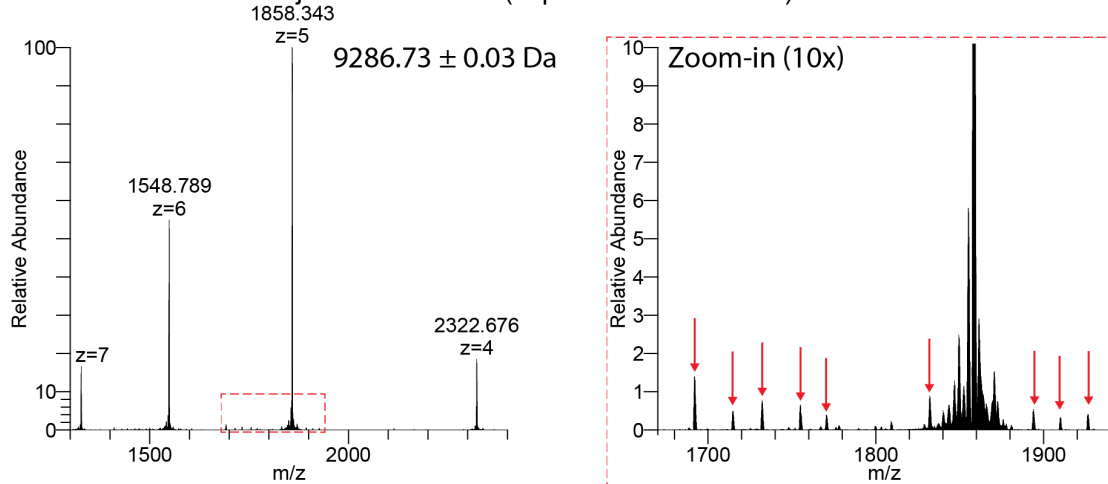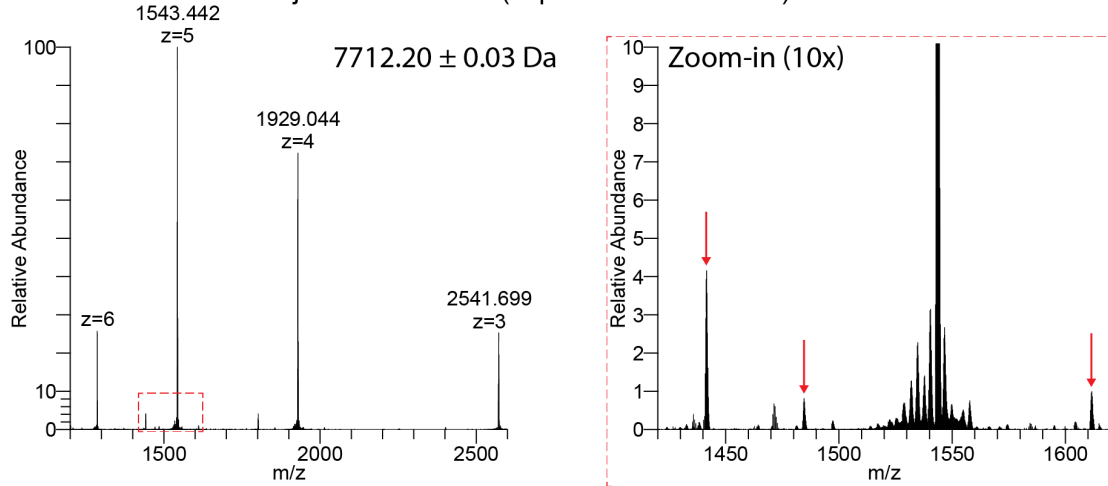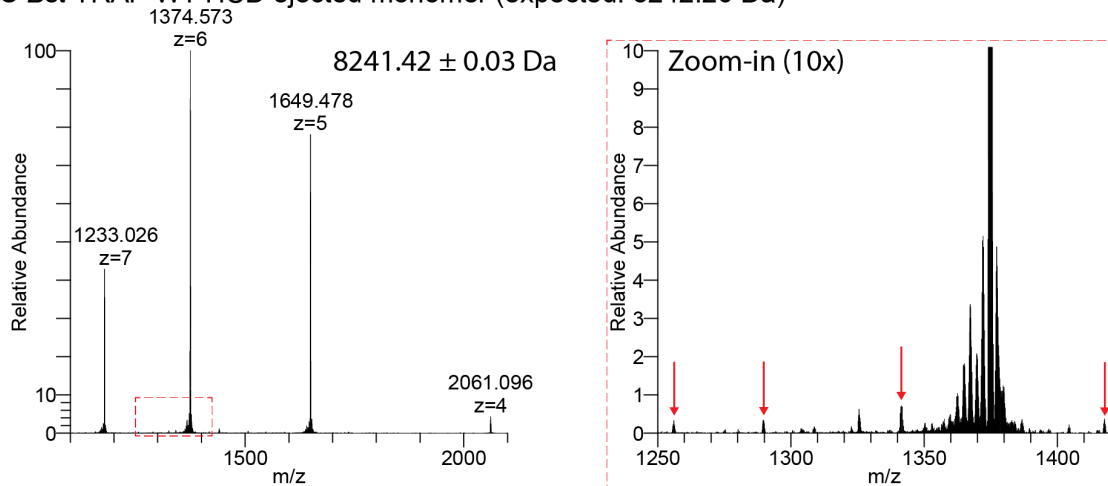
Figure S8. Dissociation (HCD) spectra (left) of (A) *Bha* TRAP, (B) *Bst* TRAP Δ71, and (C) *Bst* TRAP. Zoomed in spectra (right) have red arrows pointing to large mass proteoforms (greater or less than 100 Da).

Table S1 Quadratic single-site binding analysis of circular dichroism (CD) binding data on Trp-TRAP bindings

	$K_{d,app}$ (μM)	Amplitude (%)
Bha TRAP$_{12}$	14.6 ± 1.8	128.9 ± 6.4
Bst TRAP$_{12}$ Δ71	4.0 ± 0.4	100.6 ± 1.8
Bst TRAP$_{11}$	3.0 ± 0.6	95.6 ± 3.3

Parameters were obtained by fitting CD binding data (37˚C) of Trp-TRAP interactions against the quadratic binding equation (see methods). Values are average ± standard deviation of triplicates for each CD binding data. $K_{d,app}$ is the apparent dissociation constant; *Amplitude* is the proportionality between fractional saturation Y and the signal change.

Table S2 Thermodynamic parameters for phenomenological model of Trp cooperativity at 25°C

	Modes		n	ΔH (kcal mol⁻¹)	ΔG (kcal mol⁻¹)	K_d (μM)
Bha TRAP$_{12}$	Two-sites	Site 1	0.7 ± 0.1	-49.1 ± 3.2	-7.8 ± 0.1	1.8 ± 0.3
		Site 2	11.4 ± 0.2	-8.3 ± 0.2	-8.3 ± 0.1	0.8 ± 0.1
Bst TRAP$_{12}$ Δ71	One-site		12.00 ± 0.02	-18.07 ± 0.07	-9.60 ± 0.04	0.09 ± 0.02
Bst TRAP$_{11}$	One-site		11.05 ± 0.03	-11.47 ± 0.05	-9.16 ± 0.05	0.19 ± 0.02

Parameters were obtained by fitting ITC datasets (25°C) of Trp-TRAP titrations against one-site or two-sites phenomenological binding model. Confidence intervals were obtained by bootstrapping with 200 replicates. *Modes* indicates the type of the phenomenological model. *n* represents fitted binding sites. Dissociation constant from $\Delta G = RT$ ln K_d at 25°C.

Table S3 Corrected [TRAP$_{ring}$] and K_d via mass balance equation of *DoubleDec*-processed native MS data

	Initial Conc. (µM)	Fitted Conc. (µM)	Std. Error	K_d (µM)	Std. Error
Bha TRAP$_{12}$	5	6.04	0.48	0.25	0.95
Bst TRAP$_{12}$ Δ71	5	6.60	0.56	3.12	1.98
Bst TRAP$_{11}$	5	7.36	0.76	0.87	3.09

Initial concentrations were obtained from protein extinction coefficients at 280 nm. Fitted concentrations were obtained by fitting fractional saturation of Trp binding sites against mass balance equation (see methods) with the assumption of 1-to-1 ratio of Trp binding sites and Trp. Standard errors were estimated using the fits from three replicates of native MS titration.