

Structural dynamics and pH-regulated membrane binding of the TRAF2-NC trimer

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The supplementary materials concern:

Figure S1. SDS-PAGE of TRAF2-NC.

Figure S2. Superdex 200 Calibration Curves.

Figure S3. Per-residue confidence profile of the AlphaFold-derived TRAF2-NC model

Figure S4. Per-residue predicted pKa values.

Figure S5. Chi-squared values of individual trajectory frames along the three unbiased simulation replicas with respect to experimental SAXS data (computed by Pepsi-SAXS).

Table S1. Screening of buffer conditions by thermal stability assays.

Table S2. Assigned protonation states of histidine residues in the TRAF2-NC model.

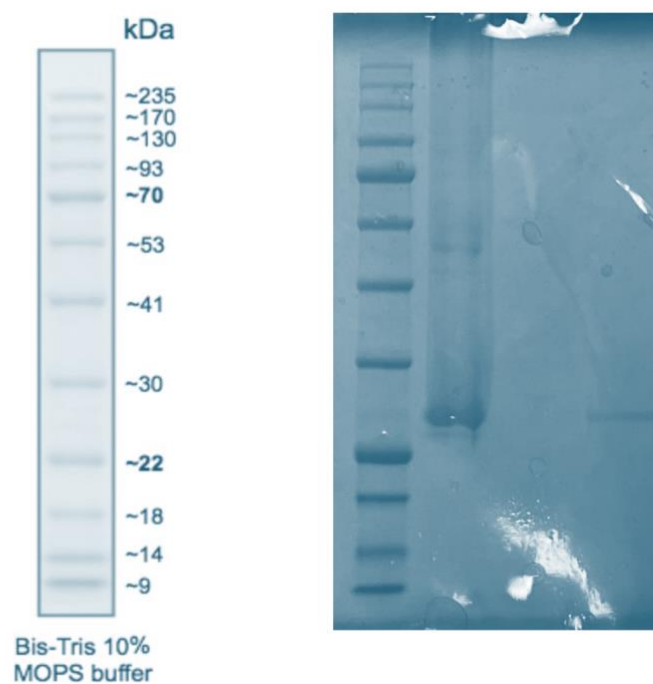


Figure S1. SDS-PAGE of TRAF2-NC. Lane 1: marker; Lane 2: TRAF2-NC after Ni-NTA; Lane 4: TRAF2-NC after Superdex 200.

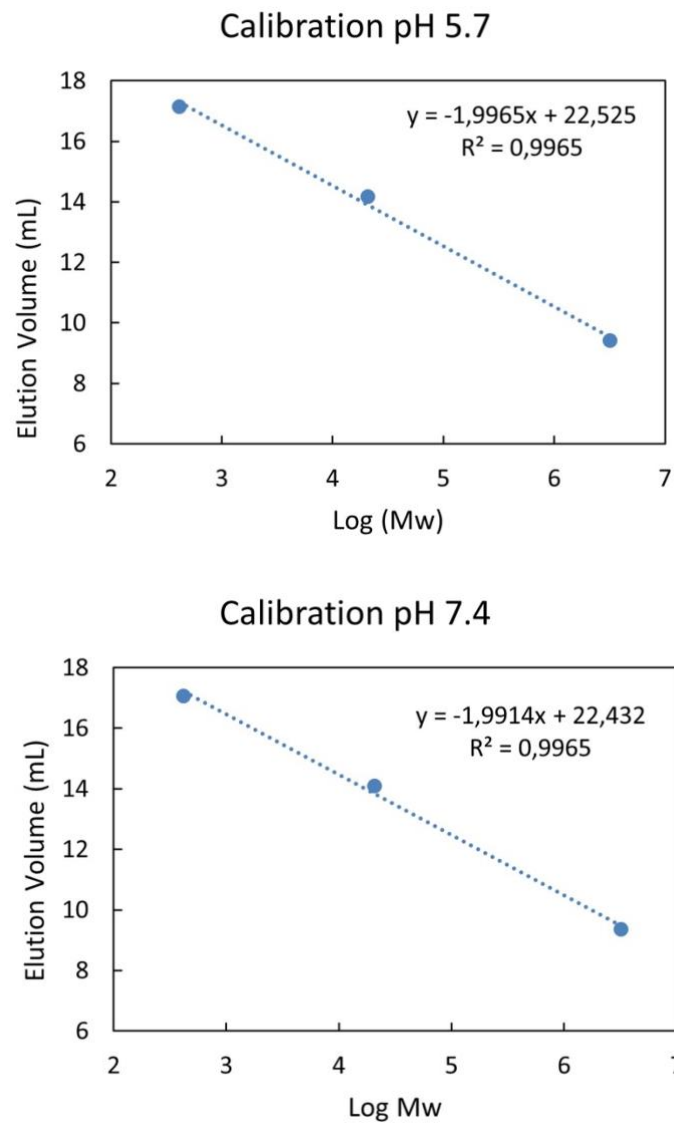


Figure S2. Superdex 200 Calibration Curves. Calibration curves using in a Superdex 200 increase 10/300 gl (Cytiva ®) at 8 °C with a flow rate of 0.8 ml/min: A) at pH=5.7 in the buffer 45 mM KH₂PO₄ pH 5.7, 500 mM NaCl, 10 mM DTT; B) at pH=7.4 in the buffer 50 mM BIS-TRIS propane pH 7.4, 200 mM NaCl, 10 mM DTT.

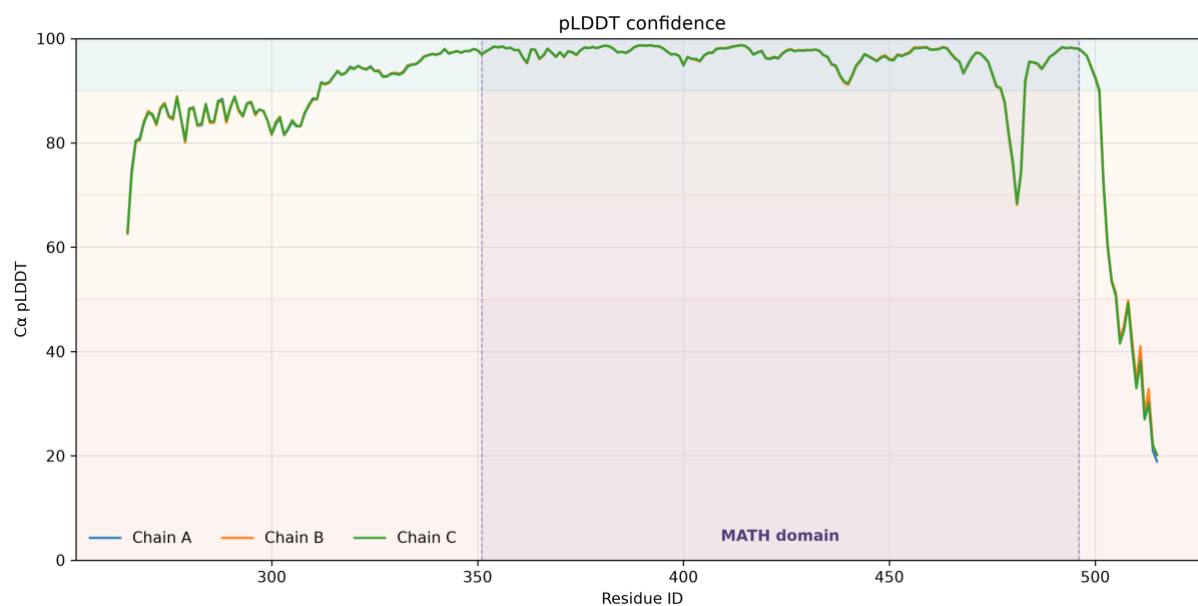


Figure S3. Per-residue confidence profile of the AlphaFold-derived TRAF2-NC model. Per-residue confidence profile of the AlphaFold3-derived TRAF2-NC model. Residue number is plotted on the x -axis and the AlphaFold predicted Local Distance Difference Test (pLDDT) score on the y -axis. Higher pLDDT values indicate greater confidence in the predicted local structure.

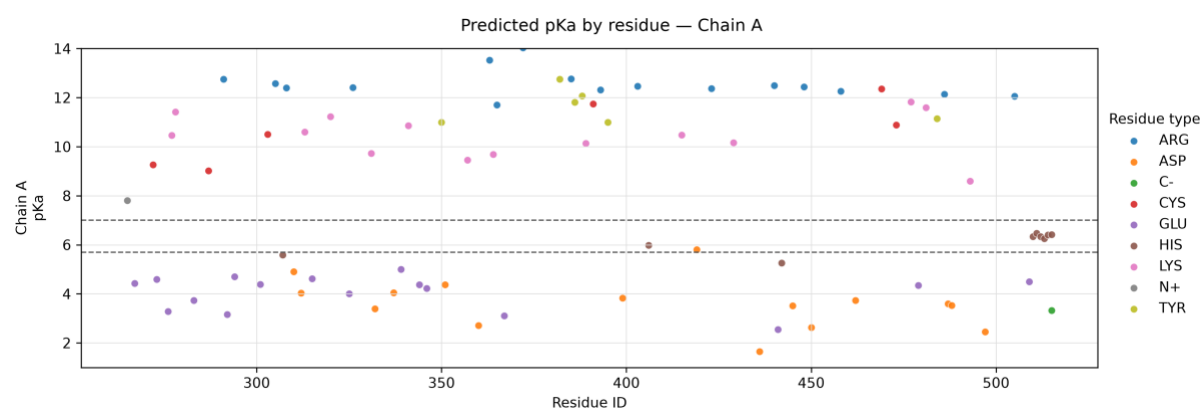


Figure S4. Per-residue predicted pKa values. No significant protonation changes are expected between pH 7 and pH 5.7 beyond the HIS-tag region.

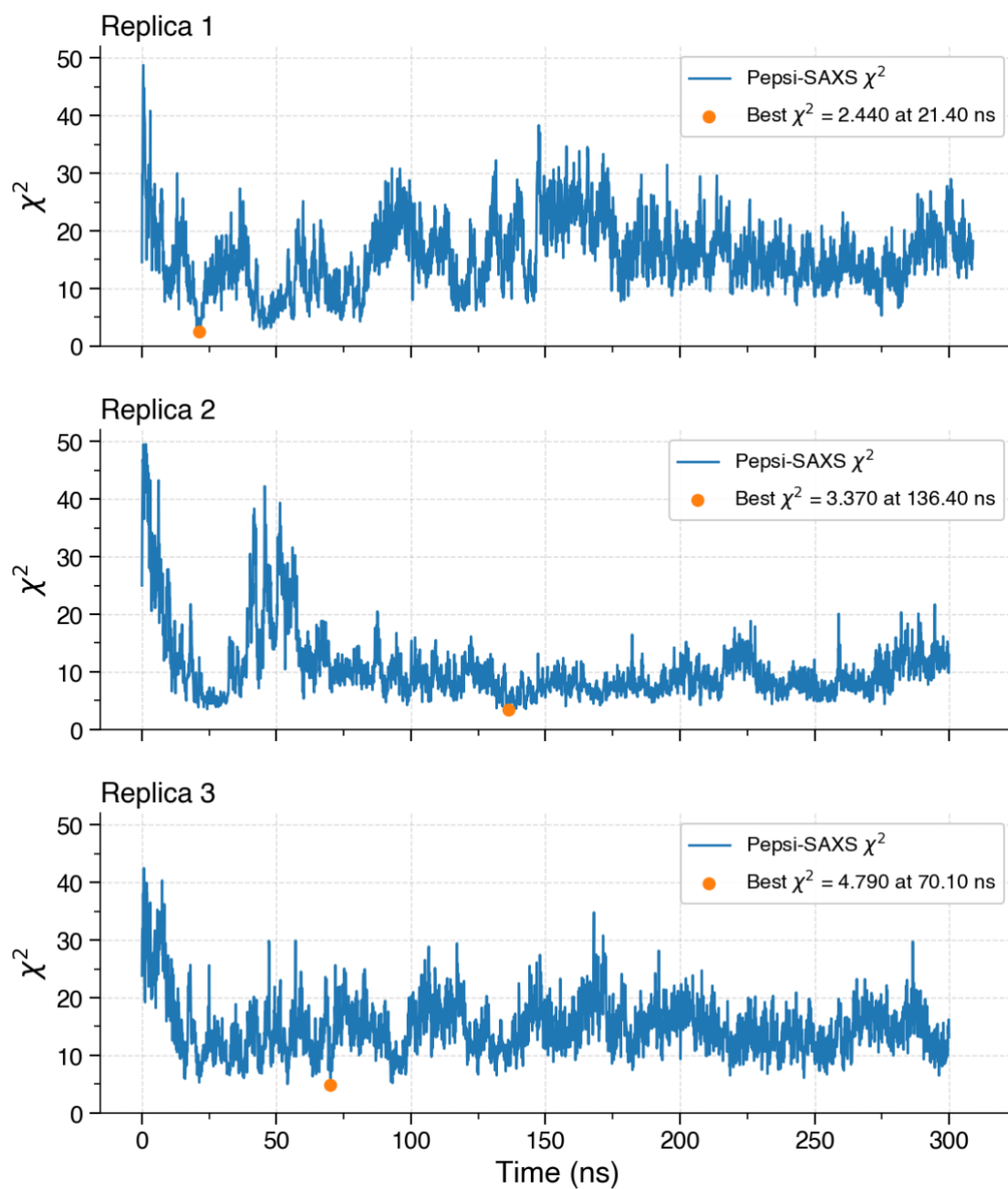


Figure S5. Chi-squared values of individual trajectory frames along the three unbiased simulation replicas with respect to experimental SAXS data (computed by Pepsi-SAXS). Minima are highlighted.

Table S1. Screening of buffer conditions by thermal stability assays.

<i>0.1 M</i>	<i>pH</i>	<i>NaCl [M]</i> *				
		<i>0</i>	<i>0.1</i>	<i>0.2</i>	<i>0.3</i>	<i>0.5</i>
<i>Sodium Citrate</i>	<i>4.0</i>	46.3	45.2	44.3	43.5	41.8
<i>Potassium Phosphate</i>	<i>5.7</i>	53.7	53.1	52.6	52.1	51.5
<i>MES</i>	<i>6.0</i>	52.1	51.9	51.5	51.7	51.0
<i>Bis Tris Propane</i>	<i>7.4</i>	55.3	54.9	54.5	54.4	53.8
<i>Tris-HCl</i>	<i>8.0</i>	55.7	55.1	54.8	54.4	54.0
<i>Tris-HCl</i>	<i>9.0</i>	54.6	54.1	53.7	53.3	52.8

*The experimental error is based on three different measurements and is approximately ± 0.1 – 0.3 .

Table S2. Assigned protonation states of histidine residues in the TRAF2-NC model.

Residue	Chain	PROPKA3- predicted pKa	Assigned state	Protonated ring nitrogen
His307	A	5.59	HID	Nδ1
His406	A	5.98	HID	Nδ1
His442	A	5.26	HIE	Nε2
His510*	A	6.34	HID	Nδ1
His511*	A	6.47	HID	Nδ1
His512*	A	6.33	HID	Nδ1
His513*	A	6.26	HID	Nδ1
His514*	A	6.40	HID	Nδ1
His515*	A	6.42	HID	Nδ1
His307	B	6.06	HID	Nδ1
His406	B	5.98	HID	Nδ1
His442	B	5.23	HIE	Nε2
His510*	B	6.36	HID	Nδ1
His511*	B	6.46	HID	Nδ1
His512*	B	6.32	HID	Nδ1
His513*	B	6.25	HID	Nδ1
His514*	B	6.40	HID	Nδ1
His515*	B	6.41	HID	Nδ1
His307	C	5.36	HID	Nδ1
His406	C	5.98	HID	Nδ1
His442	C	5.23	HIE	Nε2
His510*	C	6.34	HID	Nδ1
His511*	C	6.46	HID	Nδ1
His512*	C	6.34	HID	Nδ1
His513*	C	6.26	HID	Nδ1
His514*	C	6.40	HID	Nδ1
His515*	C	6.41	HID	Nδ1

HID and HIE denote neutral histidine tautomers protonated at Nδ1 and Nε2, respectively; HIP denotes the positively charged form protonated at both ring nitrogens. No HIP residues were assigned in this model. * indicates residues in the His-tag.