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Supplementary Materials for

Engineered chemotaxis core signaling units indicate a constrained kinase-off state

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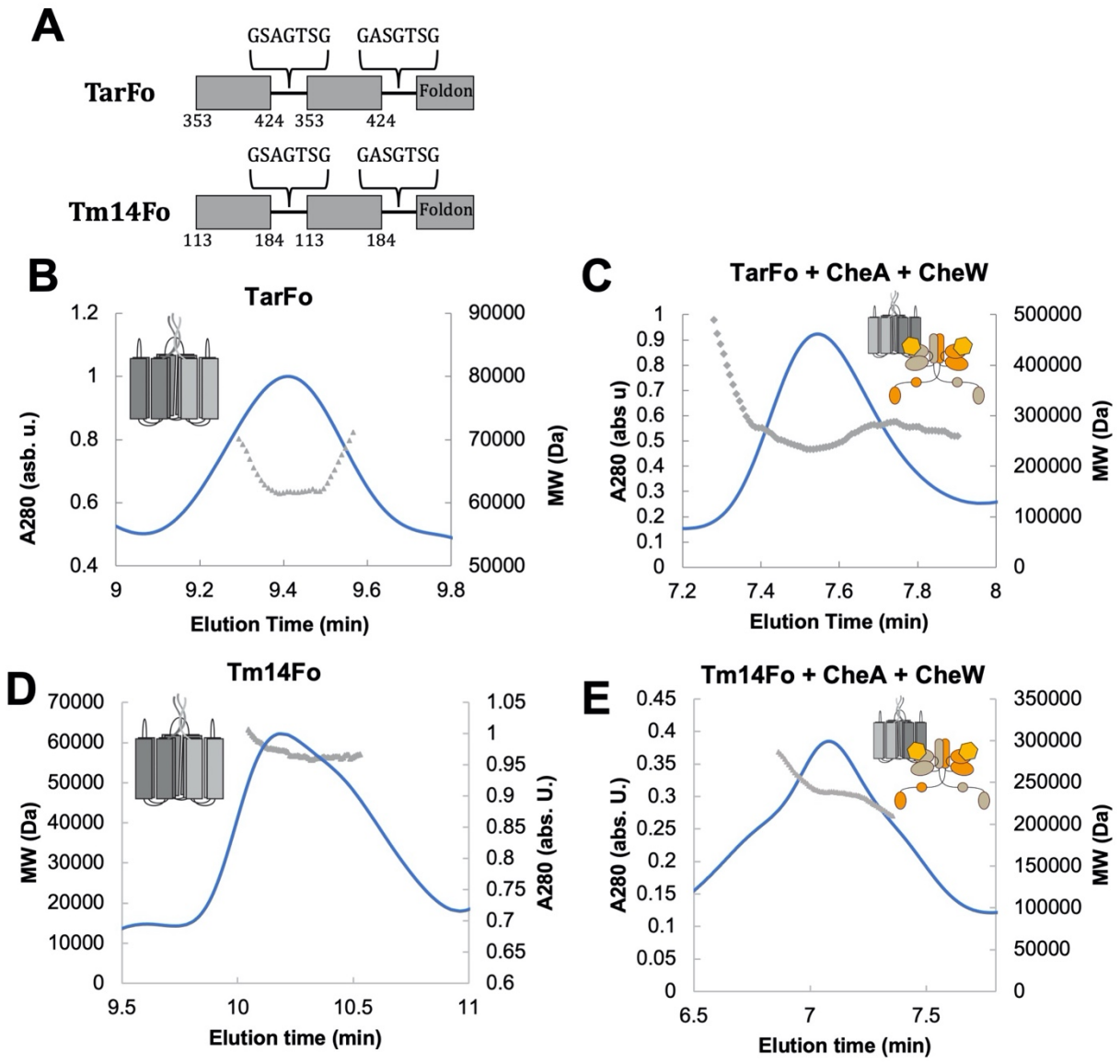

Fig. S1. Preliminary purification and characterization of receptor foldons and their complexes. (A) Subunit fusion and linker design of the foldons. The foldons were engineered to produce a pre-formed trimer-of-(single-chain)-dimer arrangement. Receptor regions surrounding the protein interaction regions (PIRs) of the receptor cytoplasmic domains were fused to form a single-chained dimer that is capped with an N-terminal trimerization motif from T4-phage fibritin. **(B)** SEC-MALS trace of the Tar foldon; MW (60 kDa) matches expectations for a trimer **(C)** SEC-MALS trace of TarFO with *Thermotoga maritima* (*Tm*) CheA and CheW produces high molecular-weight complexes. **(D)** SEC-MALS trace of the Tm14 foldon; MW 60 kDa also corresponds to a trimer-of-single-chain-dimers and **(E)** SEC-MALS trace of Tm14 FO with Tm CheA and CheW.

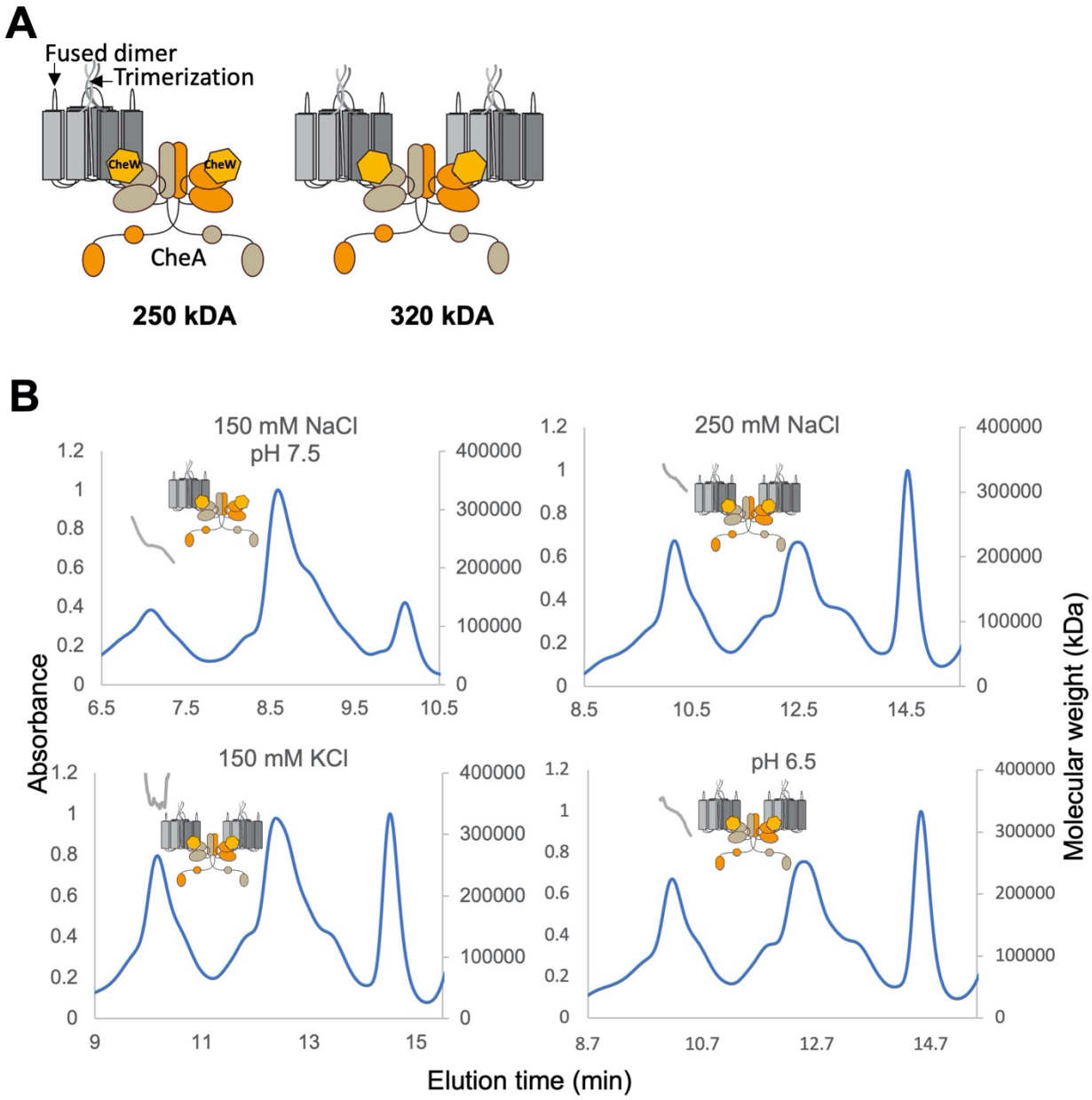

Fig. S2. Buffer conditions that encourage interactions among TarFo, CheA and CheW. (A) The foldons primarily form two particles in solution: MW = 250 kDa or MW = 320 kDa, the latter consistent with the size of a core signaling unit. **(B)** SEC-MALS traces of ternary complexes under various buffer conditions. Larger complexes (320 kDa) are present with KCl and lower pH.

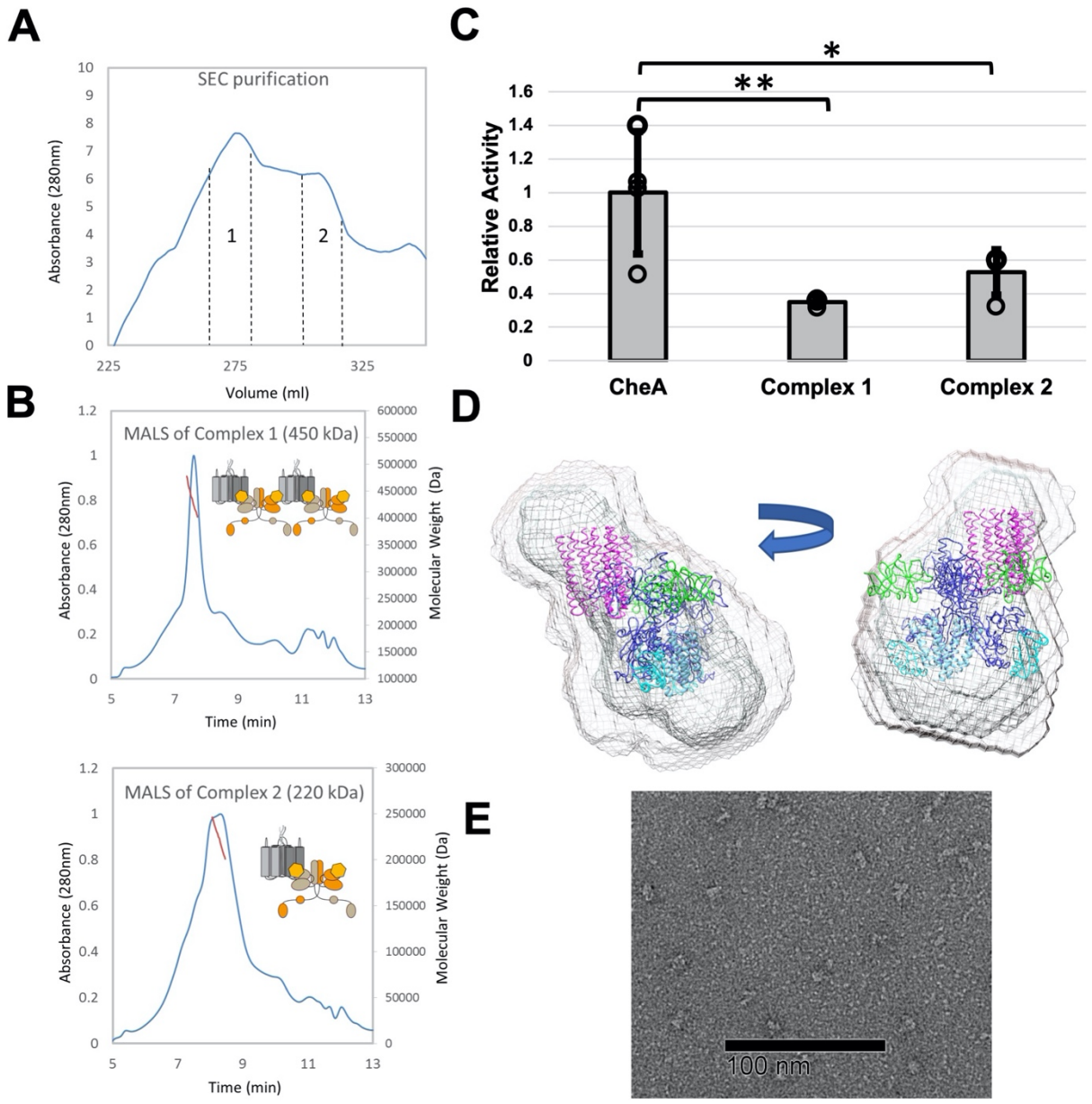

Fig. S3. Properties of the Tm14Fo complexes with *Tm* CheA and *Tm* CheW. (A) Size-exclusion chromatography of a mixture of Tm14 foldon with CheA and CheW produces two high-molecular weight peaks (denoted 1 and 2). **(B)** SEC-MALS experiments with complexes in Peak 1 denote particles of MW that corresponds to four CheA subunits, 4 CheW subunits and two receptor foldons. SEC-MALS of Peak 2 indicates that the particles consist of one CheA dimer with two CheW subunits and one receptor foldon. **(C)** ^{32}P-ATP initial autophosphorylation activity of free CheA and complexes 1 and 2. Data taken at 3-6 min when product accumulation is linear. Mean values ± s shown for N = 4 (* $P < 0.05$, ** $P < 0.01$, one-way ANOVA, Tukey post hoc comparison). The 450 kDa complex deactivates CheA ~3 fold and the 220 kDa complex deactivates CheA ~1.5 fold; CheA subunit concentration was kept constant. **(D)** SEC-SAXS derived molecular envelope of the complexes in Peak 1 superimposed with a complex of TarFO:CheA(P3P4P5):CheW based on crystal structures and array models to gauge spatial extent. **(E)** Negative-stain electron microscopy of particles in Peak 1 shows monodispersed particles of around 100 Å in diameter. Black scale bar is 100 nm.

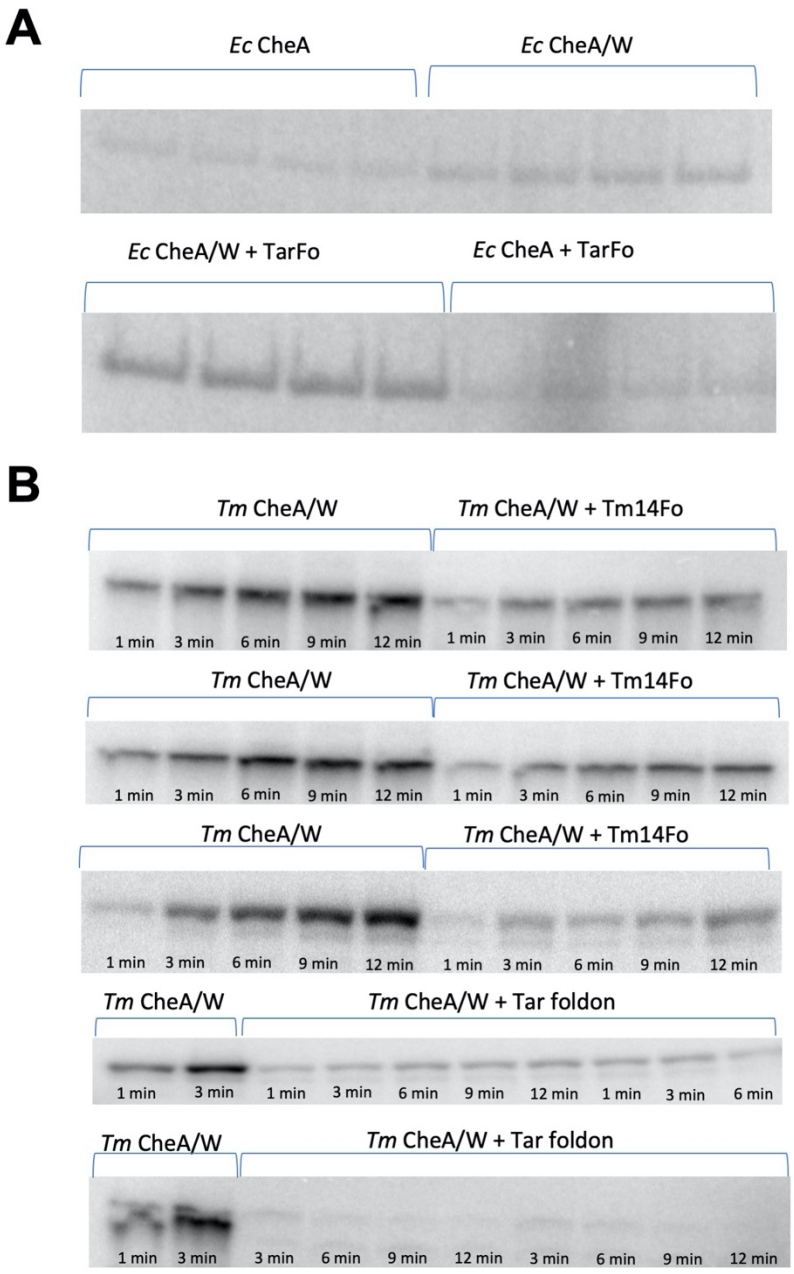

Fig. S4. Autoradiographs of the CheA autophosphorylation experiments from Fig. 1. (A) Radioisotope assays with *Ec* CheA. Assays performed in parallel and imaged together for comparison of CheW-dependent activation of free Ec CheA and Ec CheA in signaling units. **(B)** Radioisotope assays with *Tm* CheA.

Foldon Variant	Tar WT	Tm14 WT	Tar R391A	Tar R386F	Tar R386W	Tm14 F395W
Purification quantity	6 mg	7 mg	N/A	20 mg	5 mg	7 mg
Oligomeric state	Trimer	Trimer	X	Trimer/ dimer	Monomer	Trimer
Complex formation	Yes	Yes	X	Defective	No	Yes
CheA modulation	20-fold deactivation	2-fold deactivation	X	1.5-fold deactivation	No effect	2-fold deactivation

Fig. S5. Properties of single-residue foldon variants. Summary of the amounts of foldon proteins produced from 8 L *E. coli* expression, their oligomeric states, capability to form complexes with CheA and CheW and ability to deactivate CheA autophosphorylation.

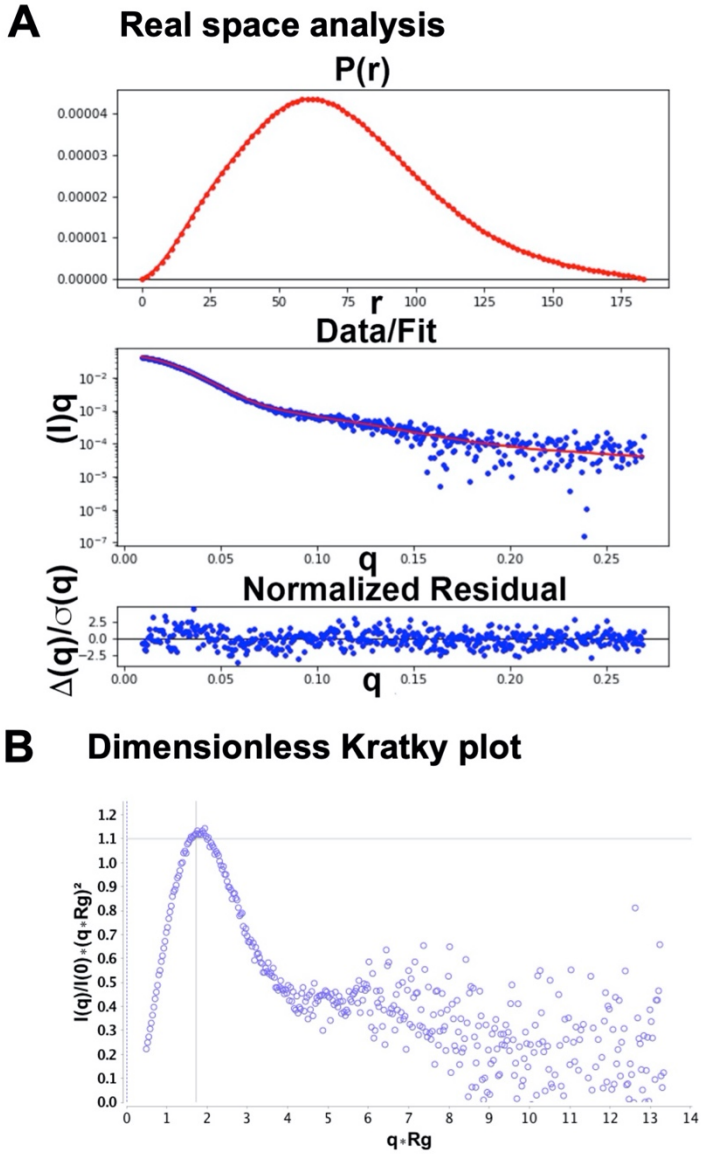

Fig. S6. SEC-SAXS data of the cross-linked foldon complex (A) Real-space analysis of the particle by GNOM after SVD decomposition of the SEC-SAXS profile (Volume of Correlation MW = 305 kDa; Porod volume MW = 328 kDa; DAMAVER MW = 339 kDa, X2 = 1.34 Dmax = 201 Å Radius of Gyration (Rg) = 55.4 Å, Normalized Spatial Discrepancy (NSD) = 1.34; Gunier Rg = 55.4 Å; GNOM Rg = 53.8 Å). **(B)** Dimensionless Kratky plot of the cross-linked complex from ScÅtter indicate a globular shape of the particle. An intersection of the x and y variables at the cross-hairs of the plot indicate globular particles.

P1

Residue no.	Residue no.	Distance	Reference PDB	Fragment 1	Fragment 2
72	87	13.5, 12.1	2LD6, 1TQG	CHTLENILD[K]AR	D[K]IF
72	122	9.4	2LD6	CHTLENILD[K]AR	SDTI[K]SF
61	72	16.9	1TQG	SSMA[K]L	CHTLENILD[K]AR

P2

Residue no.	Residue no.	Distance	Reference PDB	Fragment 1	Fragment 2
174	207	13.6	1U0S	N[K]GFK	[K]CEVVR
192	202	16.9	1U0S	[K]SAR	H[K]LEEL
202	207	11.0	1U0S	H[K]LEEL	[K]CEVVR
174	202	21.4	1U0S	N[K]GF	H[K]LEEL
182	192	14.6	1U0S	I[K]VILKEGTQL	[K]SAR

P3

Residue no.	Residue no.	Distance	Reference PDB	Fragment 1	Fragment 2
325	330	14.0, 16.4*	1B3Q	ETL[K]KY	ILETL[K]ELDESLSHL
326	330	15.6, 13.6*	1B3Q	ILETLK[K]Y	NI[K]ELDESLSHL

*Measurements are from inter-subunit separations in the P3 dimer

P4

Residue no.	Residue no.	Distance	Reference PDB	Fragment 1	Fragment 2
466	496	16.3	2CH4	KAIE[K]GLIDESK	STKE[K]VSEVSGR
462	496	17.2	2CH4	[K]AIEK	E[K]VSEVSGR
424	458	17.8	2CH4	IA[K]GKPPIGTL	HEGNNVVIEVEDDGRGIDKE[K]IIR
374	418	14	2CH4	DLA[K]K	RNAIDHGIEP[K]EER
375	496	20.6	2CH4	[K]MNKEVNF	STKE[K]VSEVSGR
456	496	24.8	2CH4	HEGNNVVIEVEDDGRGID[K]EKIIRK	STKE[K]VSEVSGR
458	496	21.5	2CH4	HEGNNVVIEVEDDGRGIDKE[K]IIRK	STKE[K]VSEVSGR
418	496	31.0	2CH4	NAIDHGIEP[K]EER	STKE[K]VSEVSGR
424	456	19.8	2CH4	IA[K]GKPPIGTL	SARHEGNNVVIEVEDDGRGID[K]EKIIR
456	462	10.6	2CH4	SARHEGNNVVIEVEDDGRGID[K]EKIIR	[K]AIEK
458	462	6.1	2CH4	HEGNNVVIEVEDDGRGIDKE[K]IIR	[K]AIEK

P5

Residue no.	Residue no.	Distance	Reference PDB	Fragment 1	Fragment 2
571	604	11.4	2CH4	SIS[K]EDIQRVQDRDVIVIRGEVIPVY	EVLQIEH[K]EELEEMEAVIVR
638	642	14.2	2CH4	GIVVDDLLGQDDIVI[K]SL	G[K]VF
550	604	12.6	2CH4	V[K]VNNLVY	EVLQIEH[K]EELEEMEAVIVR
642	648	14.7	2CH4	SLG[K]VF	SEV[K]EF

P3 to P5

Residue no.	Residue no.	Distance	Reference PDB	Fragment 1	Fragment 2
325	648	39.1	1B3Q	[K]KY	SEV[K]EF
330	604	>40	N/A	NI[K]ELDESLSHL	QIEH[K]EELEEMEAVIVR
330	648	48.4	1B3Q	NI[K]ELDESLSHL	SEV[K]EF
330	550	40.5	1B3Q	NI[K]ELDESLSHL	V[K]VNNLVY

P4 to P5

Residue no.	Residue no.	Distance	Reference PDB	Fragment 1	Fragment 2
473	648	22.4	1B3Q	GLIDES[K]AATL	SEV[K]EF
473	621	14.4	1B3Q	KAIEKGLIDES[K]AATL	VGNR[K]Y

Fig. S7. Inter-domain cross-links in free CheA. Cross-linked peptides in yellow are found in the free kinase, but not the foldon complex. For individual domains, distancs are given for intra-subunit separations between Lysine residues (Cb-to-Cb) as found in the noted crystal or NMR Protein Data Bank structures. In the case of P3, asterisks indicate the inter-subunit distance of the dimer. For cross-links between domains, the closest distance in the respective crystal structures are shown.

P1

Residue no.	Residue no.	Distance	Reference PDB	Fragment 1	Fragment 2
72	87	13.5, 12.1	2LD6, 1TQG	ENILD[K]AR	ITSDLLD[K]IF
72	122	9.4	2LD6	LCHTLENILD[K]AR	SDTI[K]SF
79	122	14.4	2LD6	NSEI[K]ITSDLLDK	SDTI[K]SF

P2

Residue no.	Residue no.	Distance	Reference PDB	Fragment 1	Fragment 2
174	207	13.6	1U0S	N[K]GFK	[K]CEVVR
177	241	11.6	1U0S	GF[K]TFY	VISPVDLE[K]LSEAL
202	241	11.6	1U0S	LVFH[K]LEEL	VISPVDLE[K]LSEAL
207	241	12.5	1U0S	LEEL[K]CEVVR	VISPVDLE[K]LSEAL
186	192	9.9	1U0S	[K]EGTQL	[K]SAR

Note: residue K174 is not present in 1U0S so measurements were taken from residue G175.

P3

Residue no.	Residue no.	Distance	Reference PDB	Fragment 1	Fragment 2
325	330	14.0, 16.4*	1B3Q	ILETL[K]K	YNI[K]ELDESLSHL

*Measurements are from inter-subunit separations in the P3 dimer

P4

Residue no.	Residue no.	Distance	Reference PDB	Fragment 1	Fragment 2
374	418	14.0	2CH4	DLA[K]K	NAIDHGIEP[K]EER
375	496	20.6	2CH4	[K]MNK	E[K]VSEVSGR
424	458	17.8	2CH4	IA[K]GKPPIGTL	HEGNNVVIEVEDDGRGIDKE[K]IIR
456	496	24.8	2CH4	E[K]VSEVSGR	HEGNNVVIEVEDDGRGID[K]EKIIR
458	496	21.5	2CH4	HEGNNVVIEVEDDGRGIDKE[K]IIR	E[K]VSEVSGR
462	496	17.2	2CH4	[K]AIEK	E[K]VSEVSGR
466	496	16.3	2CH4	AIE[K]GLIDESK	E[K]VSEVSGR
424	527	10.0	2CH4	IA[K]GKPPIGTL	NVVESLNGSISIESE[K]DKGTK
424	529	10.2	2CH4	IA[K]GKPPIGTL	NVVESLNGSISIESEKD[K]GTK
426	527	13.1	2CH4	IAKG[K]PPIGTLIL	NVVESLNGSISIESE[K]DKGTK

P5

Residue no.	Residue no.	Distance	Reference PDB	Fragment 1	Fragment 2
571	604	12.9	3UR1	SIS[K]EDIQR	QIEH[K]EELEEMEAVIVR
638	642	13.1	3UR1	GIVVDDLLGQDDIVI[K]SL	G[K]VF

P4 to P5

Residue no.	Residue no.	Distance	Reference PDB	Fragment 1	Fragment 2
473	648	22.4	1B3Q	GLIDES[K]AATL	VFSEV[K]EF
511	648	24.0	3UR1	GVGMDVV[K]NVVESLNGSISIESEKDK	VFSEV[K]EF

P1 to P2

Residue no.	Residue no.	Distance	Reference PDB	Fragment 1	Fragment 2
61	241	N/A	-	SSMA[K]L	VISPVDLE[K]LSEAL
87	241	N/A	-	D[K]IF	VISPVDLE[K]LSEAL
122	186	N/A	-	SDTI[K]SF	VIL[K]EGTQLK

P1 to P4

Residue no.	Residue no.	Distance	Reference PDB	Fragment 1	Fragment 2
29	418	N/A	-	ELE[K]NPEDMELINEAFR	NAIDHGIEP[K]EER
87	375	N/A	-	ITSDLLD[K]IF	[K]MNK
87	418	N/A	-	D[K]IF	NAIDHGIEP[K]EER
87	496	N/A	-	ITSDLLD[K]IF	E[K]VSEVSGR

P2 to P4

Residue no.	Residue no.	Distance	Reference PDB	Fragment 1	Fragment 2
174	418	N/A	-	N[K]GF	NAIDHGIEP[K]EER
177	418	N/A	-	[K]TF	NAIDHGIEP[K]EER
177	496	N/A	-	[K]TF	E[K]VSEVSGR
186	496	N/A	-	[K]EGTQLK	E[K]VSEVSGR
241	496	N/A	-	VISPVDLE[K]LSEAL	E[K]VSEVSGR

Fig. S8. Inter-domain cross-links in the receptor foldon complex. Cross-linked peptides in yellow are found in the foldon complexes, but not the free kinase. For individual domains, distancs are given for intra-subunit separations between Lysine residues (Cb-to-Cb) as found in the noted crystal or NMR Protein Data Bank structures. In the case of P3, asterisks indicate the inter-subunit distance of the dimer. For cross-links between domains, the closest distance in the respective crystal structures are shown

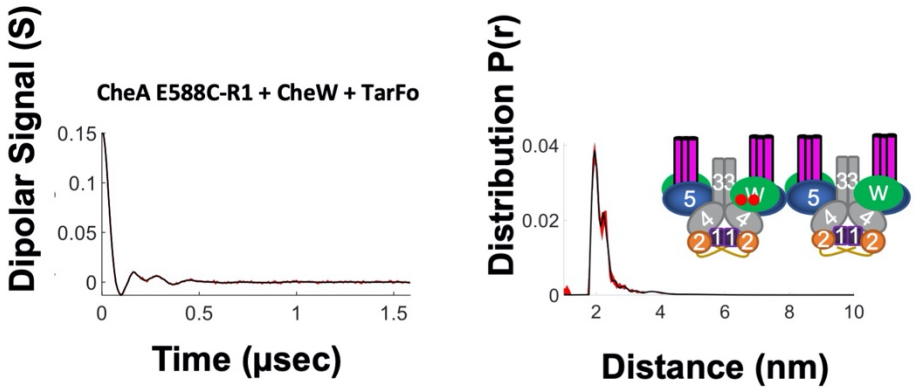

Fig. S9. PDS of *Tm* CheA E588C-R1 with CheW and TarFo. Base-line corrected time domain data (left) before (red) and after (black) wavelet denoising and resulting distance distributions (right, black) with error bounds (red). Schematic shows known CheA self-association through its P5 domains in a manner that would bring opposing 588C-R1 sites in close proximity (shown by red dots in the schematic). In absence of other spin sites, the close 588C-R1 positions dominate the PDS signal.

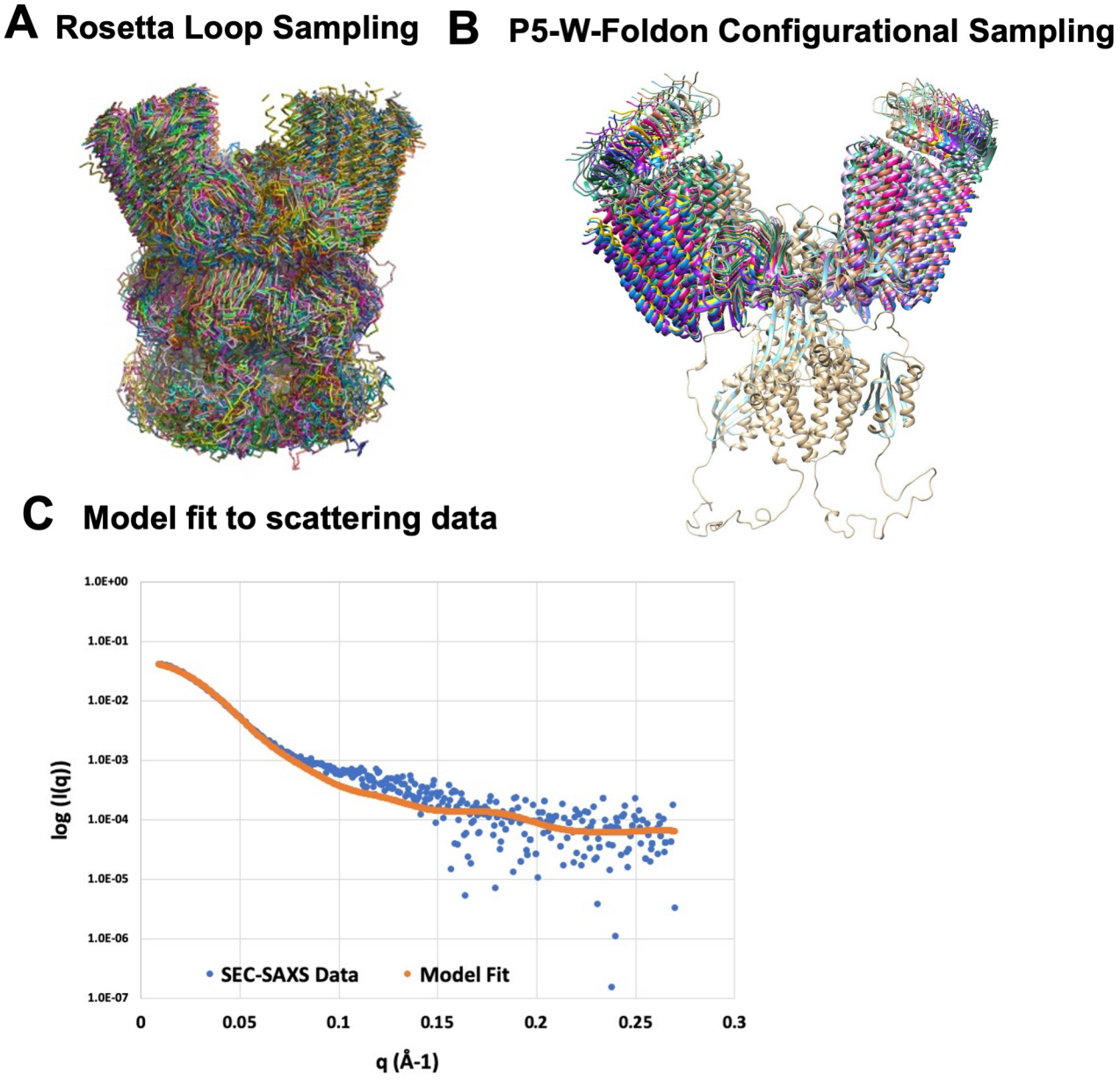

Fig. S10. Modeling of the foldon complex. (A) Overlay of Cα traces representing conformations sampled in Rosetta under constraints from PDS and cross-linking. Conformations were evaluated for their agreement with SAXS data and low Rosetta energy scores. **(B)** Conformational models generated by considering flexibility of L4. The highest scoring model in (A) was subjected to loop perturbation about the P4-P5 hinge (L4), while treating P5-CheW-receptor foldon units as rigid units on each CheA subunit, and then subsequently evaluated against the SAXS data. **(C)** Highest scoring model ($X^2 = 4.5$) fit to the solution x-ray scattering data.

Atom Pair 1		Atom Pair 2		PDS Distance (Å)	Model 10 (Å)	Model 41 (Å)
Molecule (Chain)	Atom	Molecule (Chain)	Atom			
ATP (A)	PG 673	ATP (B)	PG 673	34	40	40
CheA (A)	CG 387	CheA (B)	CG 387	42	52	50
CheA (A)	CG 588	ATP (A)	PG 673	50	52	53
CheA (B)	CG 588	ATP (B)	PG 673	50	56	55
CheA (A)	CA 12	ATP (A)	PA 673	30	31	32
CheA (B)	CA 12	ATP (B)	PA 673	30	32	30

Atom Pair 1		Atom Pair 2		Cross-linking Distance (Å)	Model 10 (Å)	Model 41 (Å)
Molecule (Chain)	Atom	Molecule (Chain)	Atom			
CheA (A)	CA 87	CheA (A)	CA 496	20±7	26	25
CheA (B)	CA 87	CheA (B)	CA 496	20±7	26	22
CheA (A)	CA 87	CheA (B)	CA 375	20±7	17	18
CheA (B)	CA 87	CheA (A)	CA 375	20±7	19	20
CheA (A)	CA 29	CheA (B)	CA 418	20±7	29	28
CheA (B)	CA 29	CheA (A)	CA 418	20±7	28	30
CheA (A)	CA 87	CheA (B)	CA 418	20±7	30	30
CheA (B)	CA 87	CheA (A)	CA 418	20±7	32	30
CheA (A)	CA 177	CheA (A)	CA 496	20±7	24	31
CheA (B)	CA 177	CheA (B)	CA 496	20±7	26	24
CheA (A)	CA 186	CheA (A)	CA 496	20±7	27	13
CheA (B)	CA 186	CheA (B)	CA 496	20±7	17	23
CheA (A)	CA 241	CheA (A)	CA 496	20±7	15	22
CheA (B)	CA 241	CheA (B)	CA 496	20±7	17	23
CheA (A)	CA 51	CheA (A)	CA 241	20±7	24	26
CheA (B)	CA 51	CheA (B)	CA 241	20±7	25	28
CheA (A)	CA 87	CheA (A)	CA 241	20±7	21	29
CheA (B)	CA 87	CheA (B)	CA 241	20±7	24	27

Fig. S11. Summary of model restraints in Rosetta refinements. Restraints are derived from PDS measured distances and unique DSSO cross-links formed in the foldon-receptor complexes. Model 10 represents the model with the lowest Rosetta energy (-5863 Rosetta Energy Units (REUs)); model 41 represents the model with the closest agreement to the SAXS data (-5680 REU).

Table S1. Real Space SAXS parameters generated from SEC-SAXS for foldon complexes.

Sample	Porod Vol. (Å^3)	Rg (Å)	Dmax (Å)	NSD	Chi^2	MW (kDa)
Δ289:CheW (*Tm*)	266k	42.6	145	0.78	1.23	161
Δ289:CheW:Tm14fo (*Tm*)	235k	45.95	142	0.78	1.16	190
CheAfl (*Tm*)	514k	60.6	263	0.59	1.22	250
CheAfl:CheW (*Tm*)	588k	57.9	238	0.74	1.16	300
CheAfl:CheW:Tm14fo (*Tm*)	796k	61.1	239	0.65	1.01	355
CheAfl:CheW:Tarfo (*Ec*)	804k	58.9	226	0.58	0.95	347
Cross-linked CheAfl:CheW:Tarfo (*Tm*)	796k	55.4	201	1.24	1.47	339

Porod Vol.: Porod volume. Rg: Radius of gyration. Dmax: The maximum distance between two points of the particle. NSD: Normalized spatial discrepancy: a measure of the similarity among three-dimensional envelopes generated from the SAXS data. MW: Molecular weight of the particle calculated from the Porod volume.

Table S2. List of PDS distance restraints for Rosetta modeling.

Atom Pair 1		Atom Pair 2		EPR Distance (Å)
Molecule (Chain)	Atom	Molecule (Chain)	Atom	
ATP (A)	PG 673	ACP (B)	PG 673	34
CheA (A)	CG 387	CheA (B)	CG 387	42
CheA (A)	CG 588	ACP (A)	PG 673	50
CheA (B)	CG 588	ACP (B)	PG 673	50