Supplementary Video 1-2: Movement of the N2-N2 dimer interfaces. Animations showing linear interpolations between the conformations of two TrkA protomers forming the two N2-N2 interfaces in the TrkA-TrkH complex structure and the TrkA structure. The beginning and end states are aligned on both N2 subdomains. The coordinates of the intermediate states for all animations were calculated by linear interpolation with the program LSQMAN. The intermediate states in linear interpolations are not physically realistic and should not be used for drawing mechanistic conclusions.

Supplementary Video 3: Movement of the N1-N1 dimer interface. An animation showing a linear interpolation between the conformations of two TrkA protomers forming an N1-N1 interface in the TrkA-TrkH complex structure and the TrkA structure. The beginning and end states are aligned on both N1 subdomains.

Supplementary Video 4: Conformational change of the TrkA protomer. An animation showing a linear interpolation between the conformations of one TrkA protomer in the TrkA-TrkH complex structure and the TrkA structure. The beginning and end states are aligned on their N1 subdomains.

Supplementary Video 5: Conformational change of the TrkA tetramer. An animation showing a linear interpolation between the conformations of the TrkA tetramer in the TrkA-TrkH complex structure and the TrkA structure. The beginning and end states are aligned on their N1 and N2 subdomains. The yellow spheres represent residues that form salt bridges with TrkH in the structure of the complex.

Supplementary Table 1: Amplitudes of three current levels.

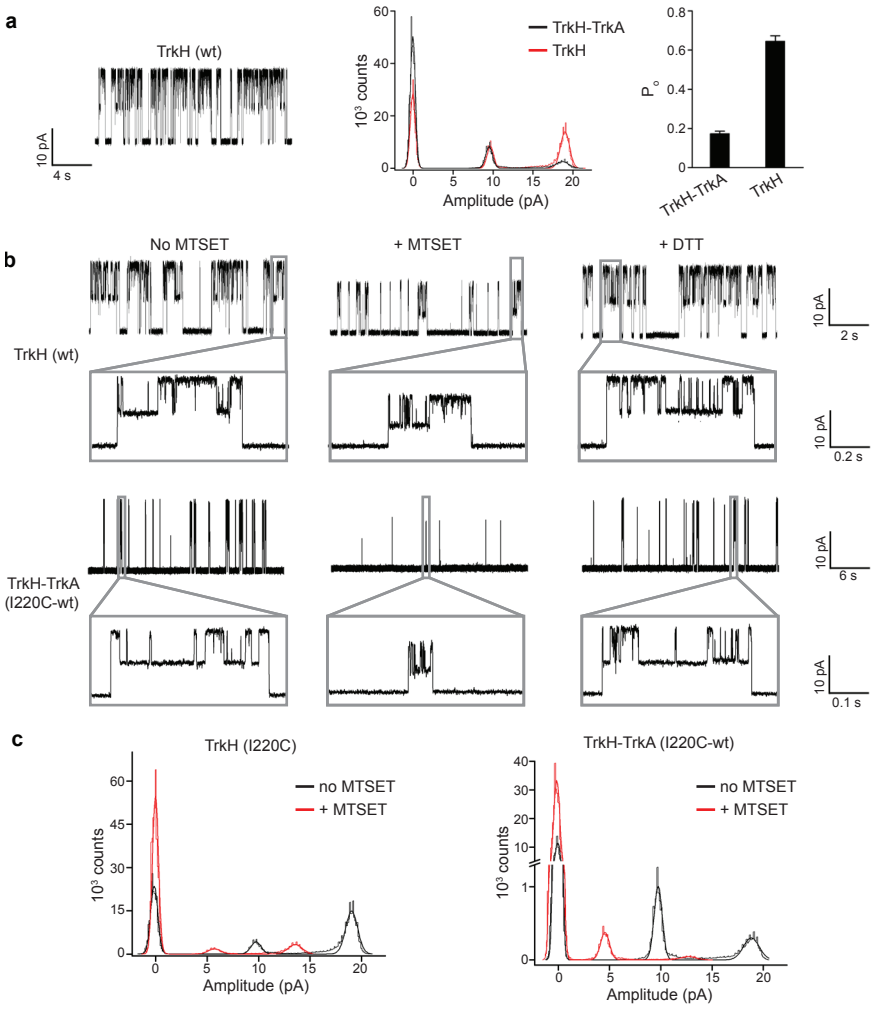
	Level 0 (pA)	Level 1 (pA)	Level 2 (pA)
TrkA- TrkH WT			
200 K	0.01 ± 0.01‡	9.5 ± 0.01	18.7 ± 0.03
200 Na *	0.001 ± 0.01	3.9 ± 0.01	12.5 ± 0.03
200 Li *	-0.02 ± 0.01	4.0 ± 0.01	12.7 ± 0.03
TrkH WT	0.01 ± 0.01	9.6 ± 0.01	18.8 ± 0.02
TrkH I220C			
before MTSET	0.03 ± 0.01	9.7 ± 0.01	18.9 ± 0.02
with MTSET	-0.02 ± 0.01	5.6 ± 0.02	13.6 ± 0.03
TrkA-TrkH I220C			
before MTSET	-0.02 ± 0.01	9.7 ± 0.01	18.8 ± 0.03
with MTSET	-0.03 ± 0.01	4.6 ± 0.01	12.7 ± 0.04

‡Values were obtained from fitting all-point amplitude histograms using a Gaussion function.
*Indicates that the current was recorded with 200 mM K^+ in the pipette solution and 200 mM of Na^+ or Li^+ in the bath solution. All other recordings were performed under symmetrical 200 mM KCl.

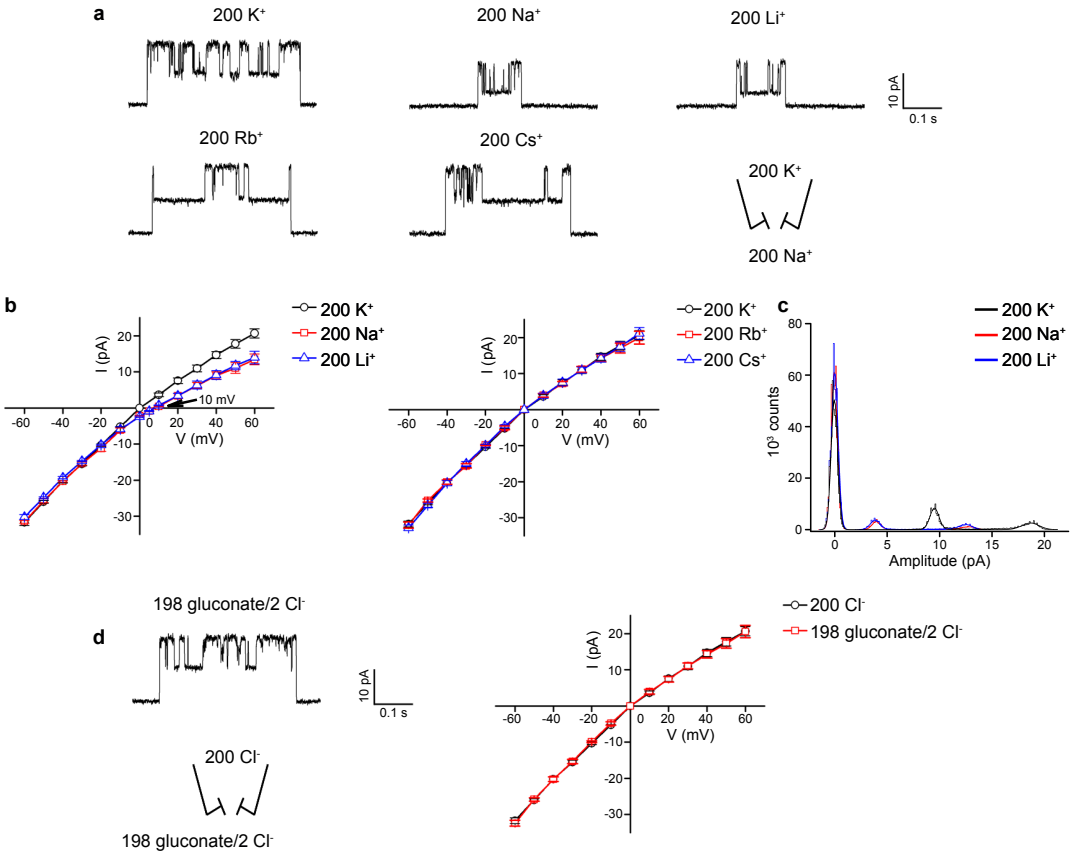
Supplementary Table 2: Data collection, phasing, and refinement statistics.

	TrkH-TrkA w/NADH, Ta_6Br_{12}	TrkA w/ATPγS
Data collection		
Space group	$P2_1$	$P6_122$
Cell Dimensions		
a, b, c (Å)	133.73, 146.63, 163.67	109.32, 109.32, 337.66
α, β, γ (°)	90.0, 99.32, 90.0	90.0, 90.0, 120.0
Resolution (Å)	3.8 (3.87-3.80)*	3.05 (3.10-3.05)
R_{sym} or R_{merge}	0.144 (0.814)	0.103 (0.889)
I/σ(I)	8.6 (2.68)	20.47 (2.75)
Completeness (%)	99.7 (98.7)	100 (100)
Refinement		
Resolution (Å)	3.8 (3.86-3.80)	3.05 (3.17-3.05)
No. reflections	61260 (141)	23674 (2536)
Completeness (%)	99.5 (97.0)	99.9 (99.5)
R_{work}/ R_{free}	23.2/28.0	21.1/27.2
No. atoms		
Protein	28063	6990
Solvent	—	11
Ligand/ion	504	131
B-factors		
Protein	89.2	85.6
Solvent	—	52.9
Ligand/ion	113.3	95.8
R.m.s deviations		
Bond lengths (Å)	0.005	0.006
Bond angles (°)	1.242	1.021

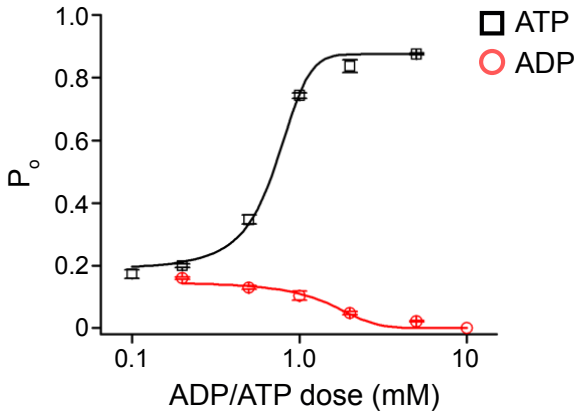
*Numbers in parentheses indicate values for the highest resolution shell.



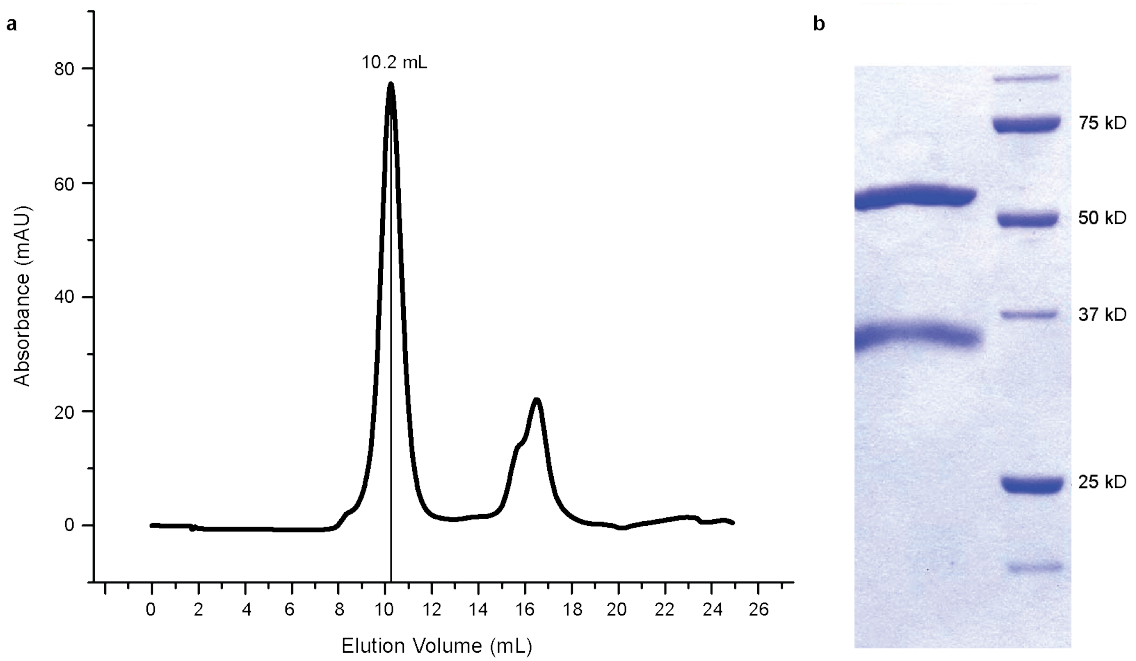
Supplementary Figure 1: Single-channel activities of TrkH only and the TrkH-TrkA complex. (a) Left, single-channel currents through TrkH only were recorded at +50 mV in symmetrical 200 mM KCl. Middle, the amplitude histogram for recordings through TrkH only and the TrkH-TrkA complex. Right, the open probability (P_o) from TrkH only and the TrkH-TrkA complex. Error bars are s.e.m of three single-channel patches. **(b)** Current traces of TrkH (I220C) only and the TrkH (I220C)-TrkA complex were recorded at +50 mV without MTSET (left), with 1 mM MTSET (middle), and 20 mM DTT (right). The dotted boxes demarcate the bursts shown. **(c)** All-point amplitude histograms for the recordings on TrkH (I220C) only (left) and the TrkH (I220C) TrkA complex (right).



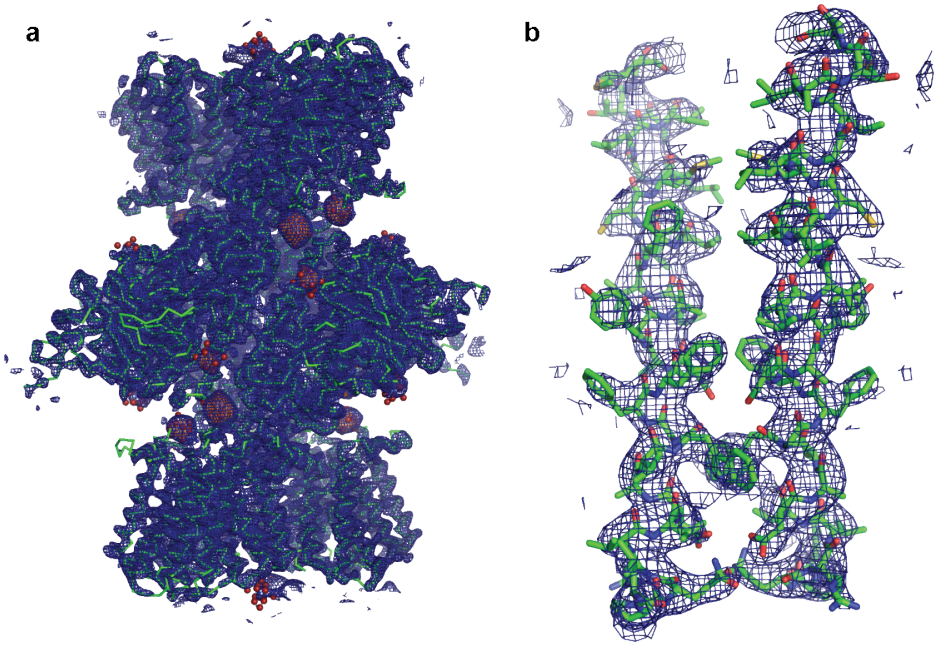
Supplementary Figure 2: The ion selectivity of the TrkH-TrkA complex under bi-ionic conditions. (a) Single channel traces of the TrkH-TrkA complex were recorded at +50 mV with 200 mM K^+ in the pipette solution and 200 mM of the indicated cations in the bath solution. The inset shows the conditions for perfusion of 200 mM Na^+ to the intracellular side of the patch. **(b)** The open-channel current-voltage relationship of TrkH-TrkA under different bi-ionic conditions. The arrow indicates that the reversal potential with 200 Na^+ or 200 Li^+ in the bath solution was shifted by about 10 mV. **(c)** All-point amplitude histograms for recordings with 200 mM K^+, 200 mM Na^+ or 200 Li^+ in the bath solution. **(d)** Left, single channel traces of TrkH-TrkA recorded at +50 mV with 200 mM KCl in the pipette solution and 198 mM potassium gluconate with 2 mM KCl in the bath solution. Right, the open-channel current-voltage relationship under low chloride conditions.



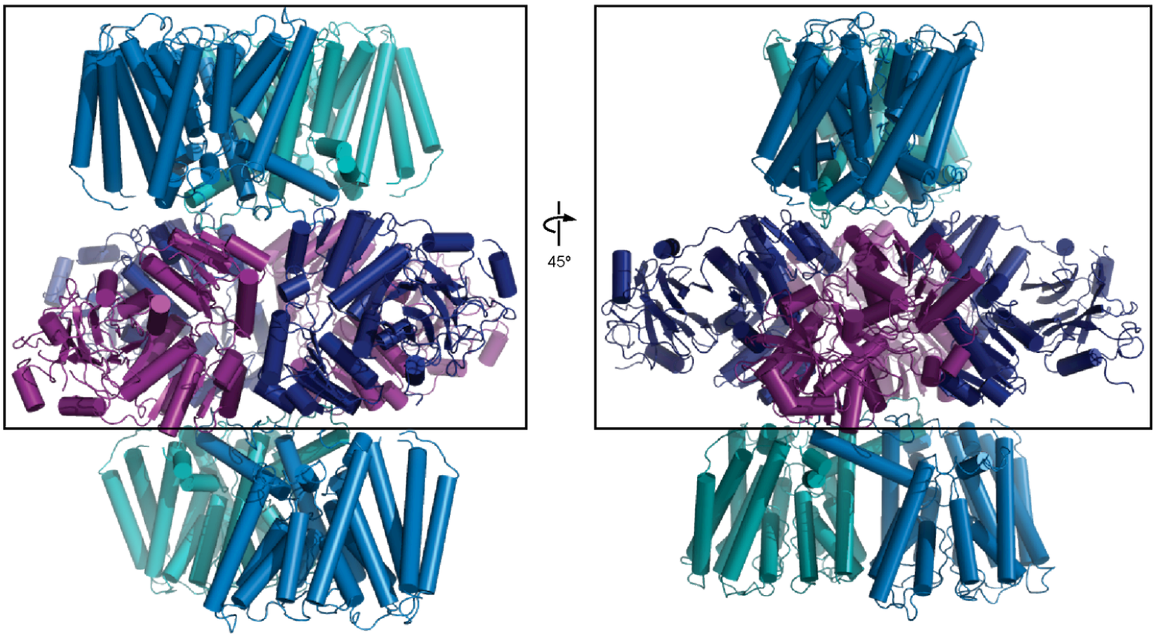
Supplementary Figure 3: ATP and ADP regulate TrkH channel activities in a dose-dependent manner. The open probability (P_o) of the TrkH-TrkA complex after perfusion of different concentrations of ATP or ADP is shown.



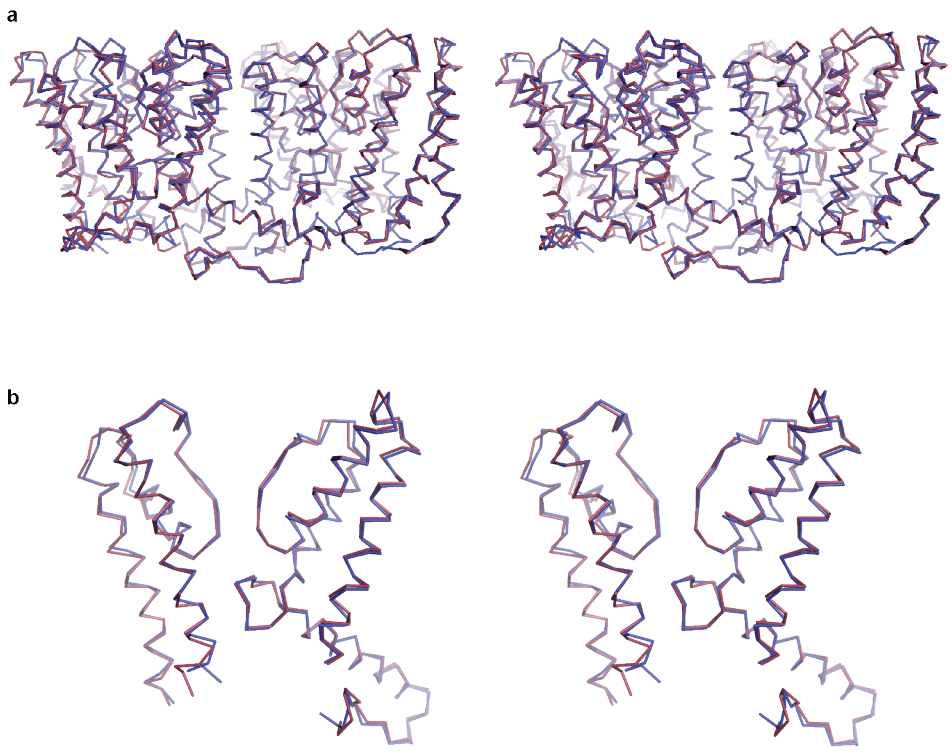
Supplementary Figure 4: Purification of the TrkH-TrkA complex. (a) Elution profile of the TrkH-TrkA complex during size exclusion chromatography, after a previous step purifying the His tagged-TrkH-TrkA complex on a Co^{2+} IMAC column **(b)** SDS PAGE gel of the 10.2 mL peak in panel **a**. The molecular weights of TrkH and TrkA are both ~50 kD. The anomalous position of TrkH at 37 kD is typical for membrane proteins.



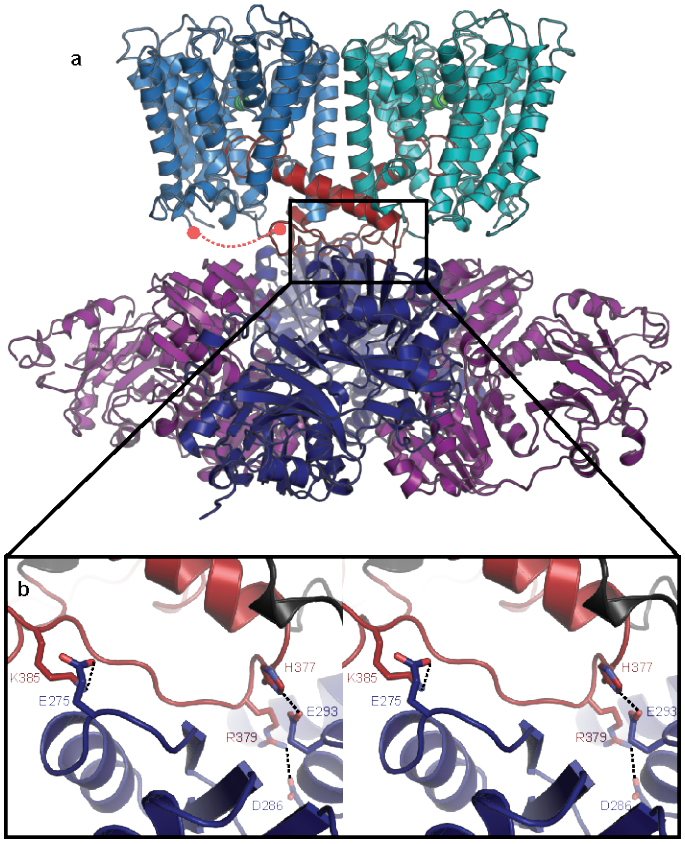
Supplementary Figure 5: 3.8 Å structure of the TrkH-TrkA complex. (a) Kicked $2F_o$-F_c electron density maps contoured at 1.5 σ (blue mesh), and kicked anomalous difference maps contoured at 4.0 σ (orange mesh) are shown overlaid on a ribbon representation of the asymmetric unit from the TrkH-TrkA structure. Tantalum bromide clusters are shown as orange and red spheres. **(b)** A closer view of the kicked $2F_o$-F_c electron density maps contoured at 1.5 σ is shown for two helices forming the dimer interface in TrkH.



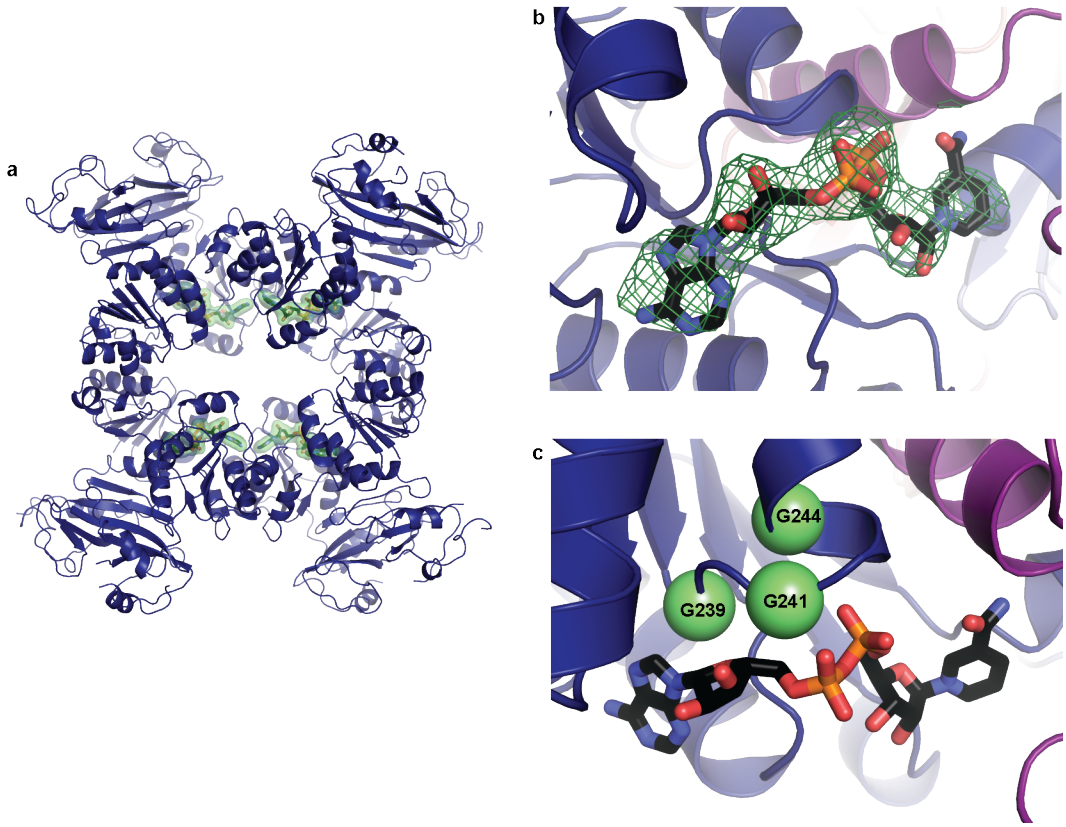
Supplementary Figure 6: Crystal packing in the TrkH-TrkA complex structure. The four TrkH subunits and four TrkA subunits comprising one asymmetric unit in the structure of the TrkH-TrkA complex are shown from two orientations in a cartoon representation. The black rectangle marks two TrkH subunits and four TrkA subunits, likely forming one biological unit.



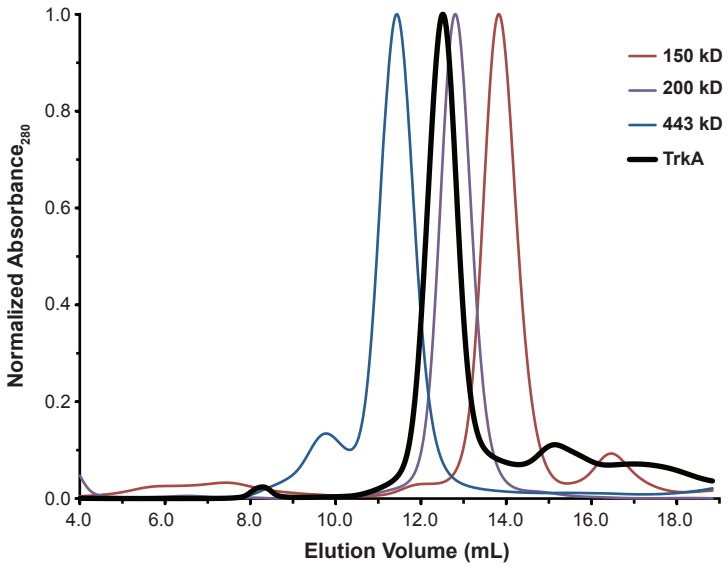
Supplementary Figure 7: Comparison of TrkH in the isolated TrkH and TrkH-TrkA complex structures. (a) Stereo-image of the superposition of the TrkH dimer from the TrkH-TrkA complex structure (red) with the structure of isolated TrkH from PDB 3PJZ (blue). **(b)** Stereo-image of the superposition of domains D1 and D3, including the intramembrane loop and D3M2b, from the TrkH-TrkA complex structure (red) with the structure of isolated TrkH from PDB 3PJZ (blue).



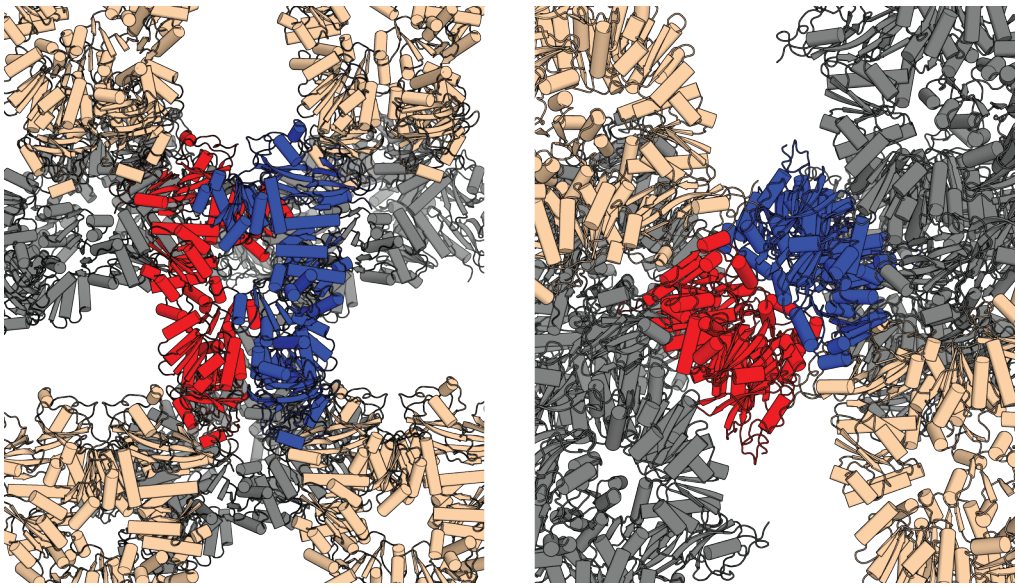
Supplementary Figure 8: Interface of the TrkH-TrkA complex. (a) The TrkH-TrkA complex viewed from within the plane of the membrane. Helix D3M2b in each TrkH subunit is highlighted in red. The approximate location of the unresolved loop between D1 and D2 is marked with a red dashed line **(b)** A close-up stereo-view of the boxed region in panel **a**. Residues forming salt bridges between TrkH and TrkA are shown as sticks.



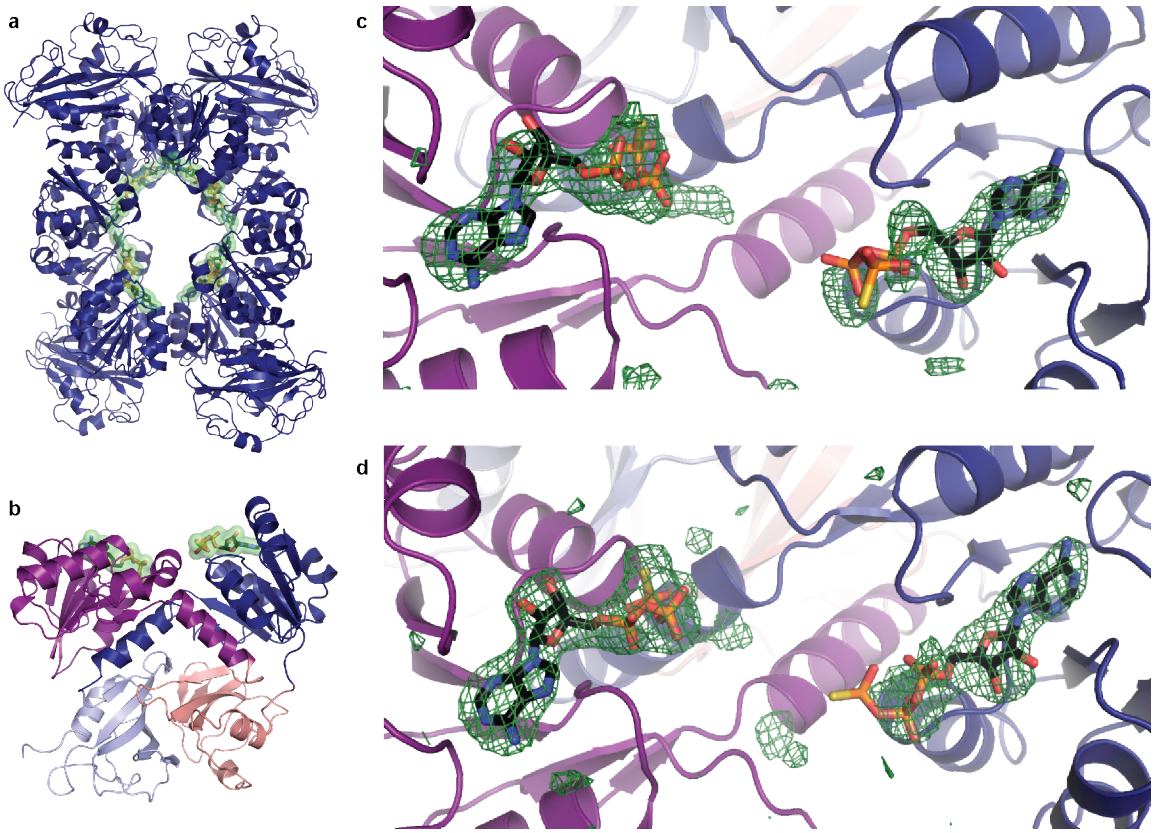
Supplementary Figure 9: NADH-binding sites in the structure of the TrkH-TrkA complex. (a) Structure of the TrkA tetramer from the TrkH-TrkA complex shown with the four NADH binding sites highlighted in green. **(b)** The NADH binding site from one TrkH protomer shown with the likelihood-weighted F_o-F_c maps calculated without NADH in green, contoured at 3 σ. **(c)** The α-carbons of the glycine residues from the conserved GXGXXG motif are shown as green spheres in the NADH binding site.



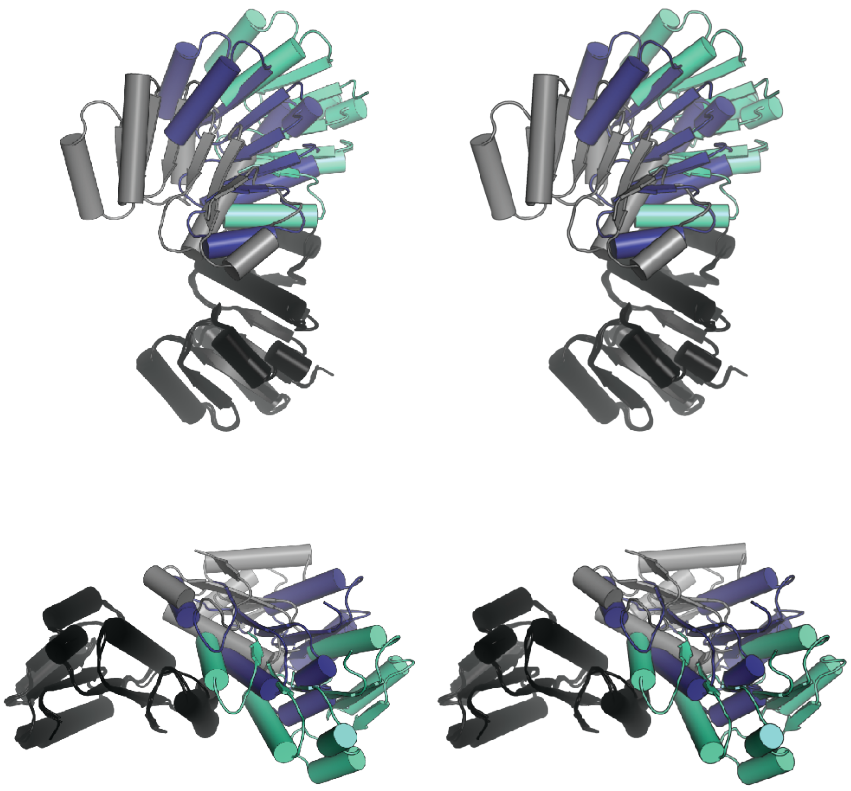
Supplementary Figure 10: TrkA forms a tetramer in solution. The elution profile of purified TrkA from size-exclusion chromatography is shown compared to the profiles of various proteins of known size run on the same column. The size of an individual TrkA protomer is ~50 kD. The protein standards used were yeast alcohol dehydrogenase (150 kD), sweet potato ß-amylase (200 kD), and horse apoferritin (433 kD).



Supplementary Figure 11: Crystal packing in the isolated TrkA structure. A region of the crystal lattice in the TrkA-only structure is shown from two views. TrkA crystallized in the $P6_122$ space group with two protomers in the asymmetric unit (red); the other two subunits of the tetramer are generated by crystallographic symmetry axes (blue).



Supplementary Figure 12: ATPγS-binding sites in the structure of isolated TrkA. (a) Structure of the isolated TrkA tetramer shown with the eight ATPγS binding sites highlighted in green. **(b)** Structure of a protomer from the TrkA tetramer, colored by subdomain, shown with the two bound ATPγS molecules highlighted in green. **(c-d)** The ATPγS binding sites in protomer A (**c**) and protomer B (**d**) shown with the likelihood-weighted F_o-F_c maps calculated without ATPγS in green, contoured at 3 σ.



Supplementary Figure 13: Motion of the N2-N2 interface. Two stereoviews of the superposition of an N2-N2 interface from the TrkH-TrkA complex structure (gray), and both N2-N2 interfaces in the TrkA-only (blue or cyan) structures. The alignment was based on the domains colored black in both structures.