Supplement to Stein et al.: "Helical extension of the neuronal SNARE complex into the membrane"

Supplementary Figure S1

a, Solvent flattened, single-wavelength anomalous dispersion electron density map (gray mesh) of the synaptic SNARE complex with its two linker regions and TMRs contoured at the 1 σ level, superimposed on a Cα plot of the final model.

b, Stereo-view of an anomalous difference Fourier map (magenta mesh) contoured at the 4.5 σ level, showing the selenium positions of the selenomethionines in syntaxin 1A. Selenomethionine side chains are shown as sticks.

c, Stereo-close-up view of **b** showing the positions of the selenomethionines in the SNARE motif of syntaxin 1A.

Supplementary Figure S2

a, Final $2F_o$-F_c electron density (gray mesh) of the monoclinic crystal form contoured at the 1.5 σ level, covering one synaptic SNARE complex.

b, Final $2F_o$-F_c electron density (gray mesh) around the characteristic zero layer at the 1.5 σ level. Hydrogen bonds are indicated as dashed lines. Arginine residue R56 in synaptobrevin 2 and the three glutamine residues Q226 (syntaxin 1A), Q53 (SN1 (N)) and Q174 (SN2 (C)) are shown as sticks using the same colour code as in figure 2.

c, $2F_o$-F_c electron density (gray mesh) of the orthorhombic crystal form contoured at 1.5 σ level, covering one synaptic SNARE complex.

Supplementary figure S3

a, X-shaped assembly of four synaptic SNARE complexes (I-IV) in the C2 structure. The SNARE complexes are shown as ribbons using the same colour code as in figure 2. The lower panel is rotated 90° about the horizontal axis as indicated.

b, X-shaped assembly of four synaptic SNARE complexes (I-IV) in the $I2_12_12_1$ structure. Complexes II and III are in the same orientation as complexes II and III of panel **a**.

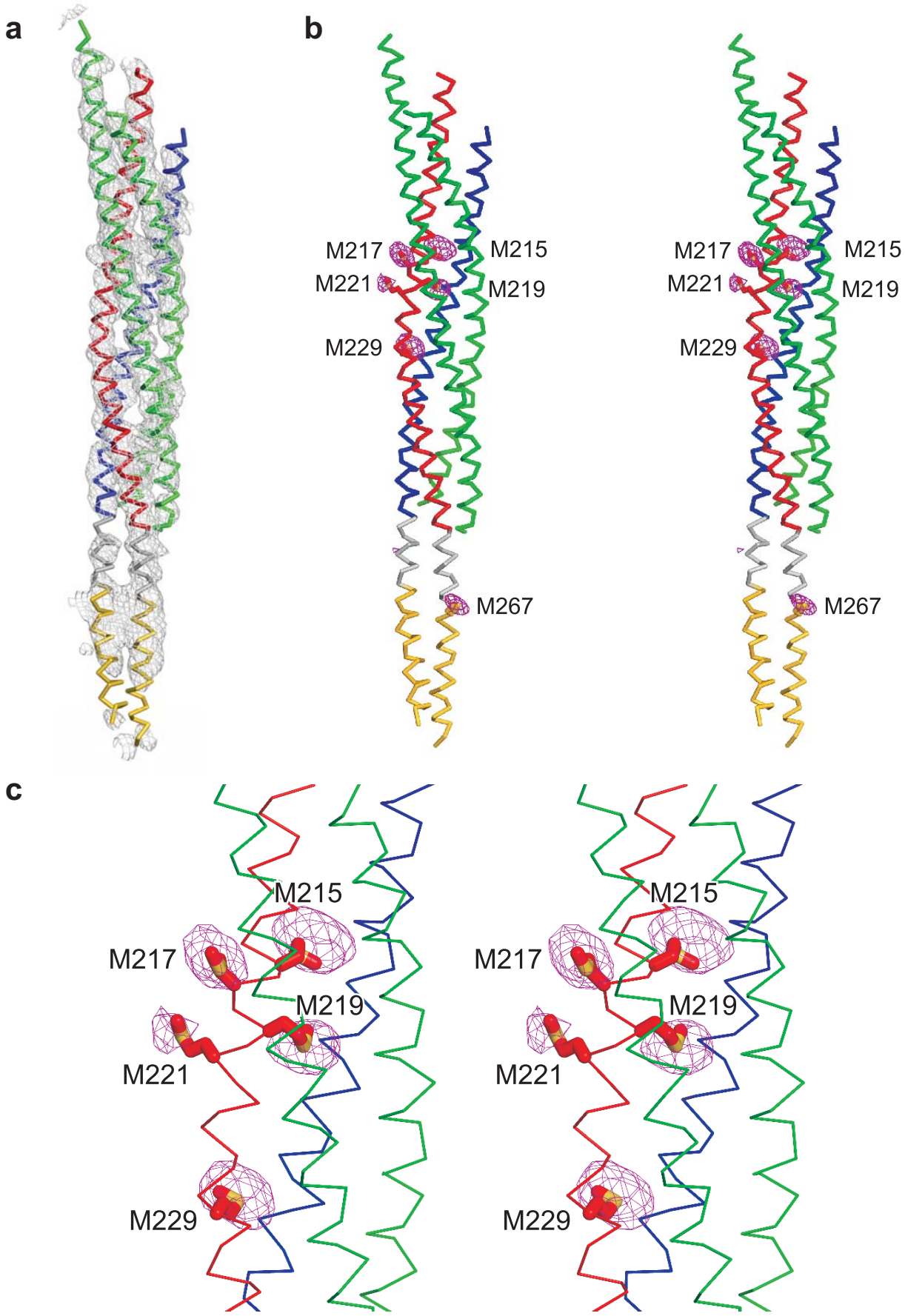
Supplementary figure S4

a, Stereo-view (Cα plot) of an overlay of the synaptic SNARE complexes from the monoclinic form (black and TMRs in dark yellow) and the orthorhombic form (light gray and TMRs in lighter yellow). Deviations are seen only at the very C-terminus of synaptobrevin 2. The overlay was calculated with SSM Superpose [1].
b, Rms deviations between C_α-atoms of syntaxin 1A and synaptobrevin 2 in the two crystal forms.

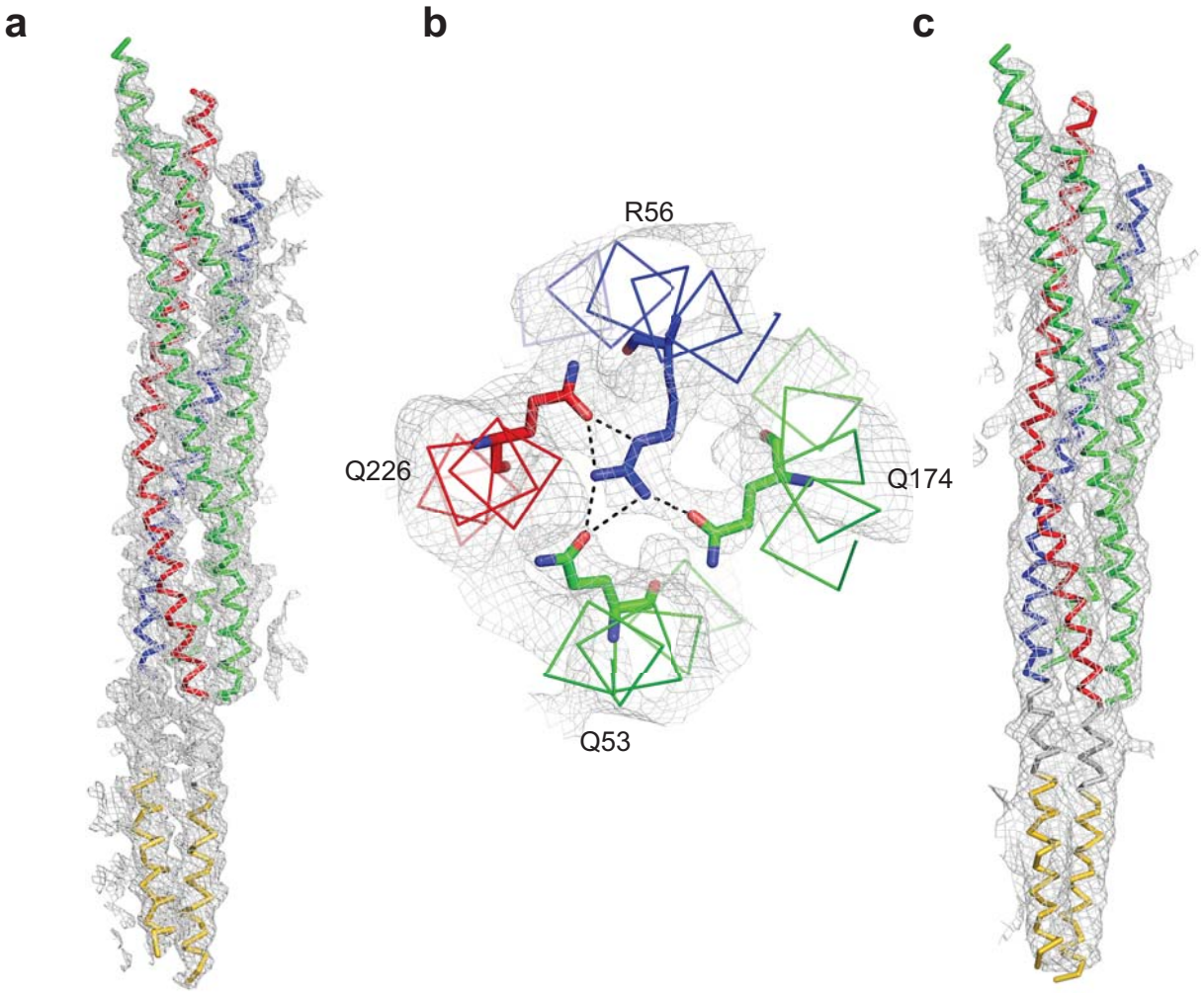
Reference to the supplement

[1] Krissinel, E. and Henrick, K., Secondary-structure matching (SSM), a new tool for fast protein structure alignment in three dimensions. *Acta Crystallogr D Biol Crystallogr* **60** (Pt 12 Pt 1), 2256 (2004).

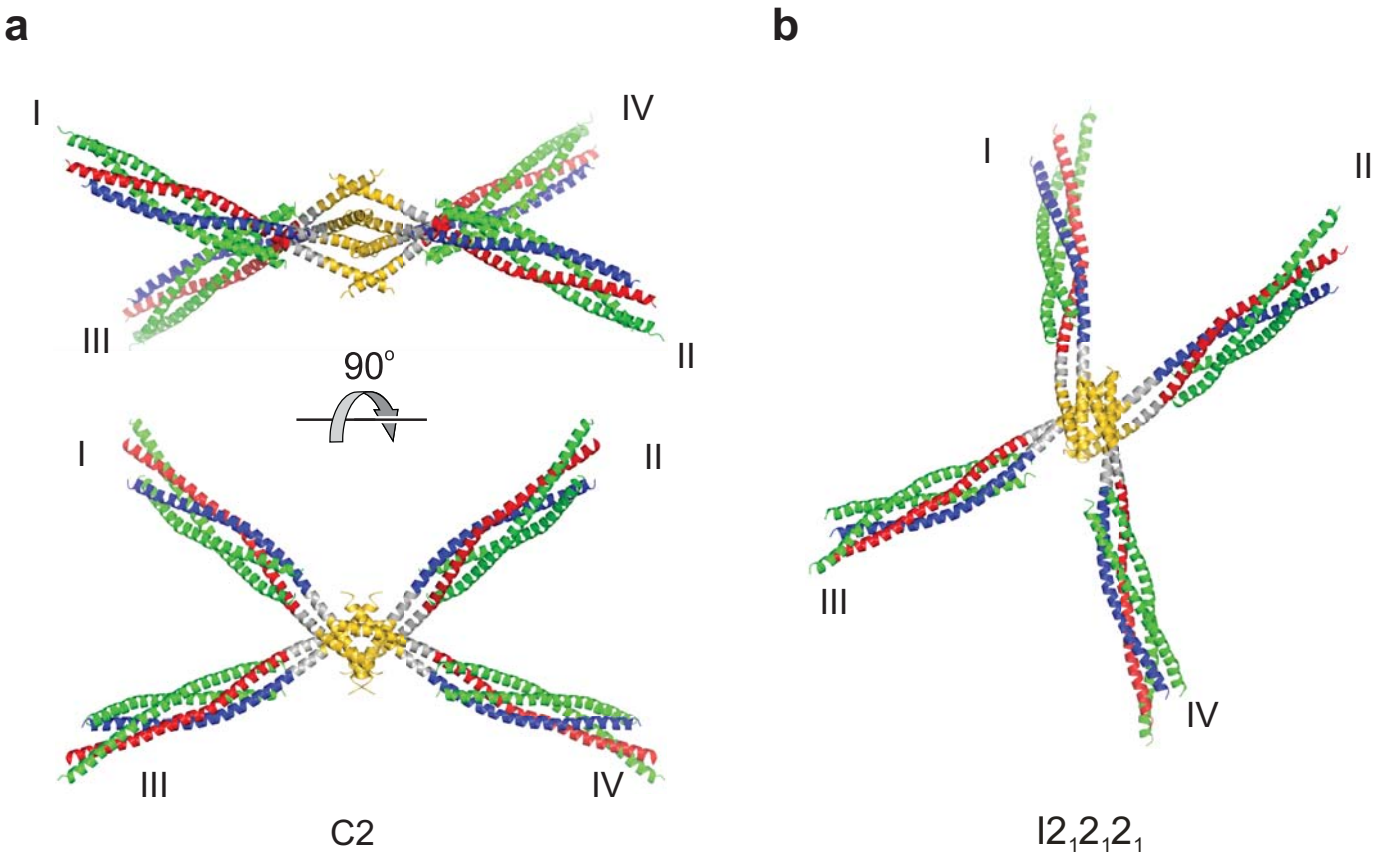
Jahn, Supplementary Figure S1



Jahn, Supplementary Figure S2

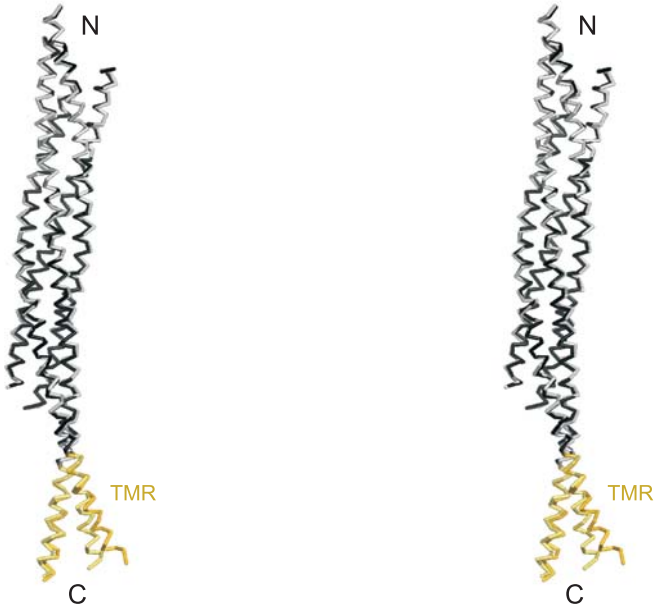


Jahn, Supplementary Figure S3

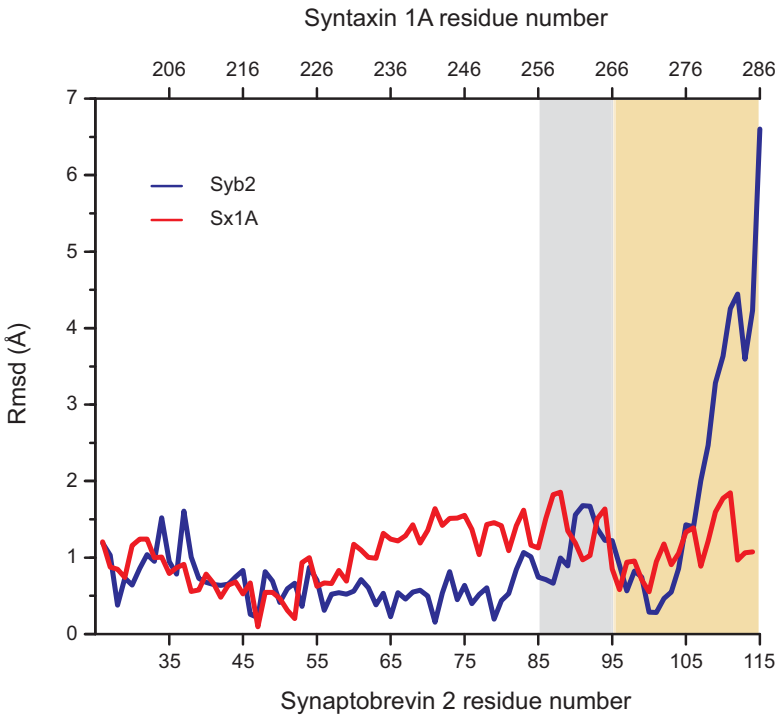


Jahn, Supplementary Figure S4

a



b



Supplementary table 1: Crystallographic Data and Refinement

Data Collection			
	Native 1	**SeMet**	**Native 2**
Wavelength (Å)	0.9791	0.9780	0.9789
Temperature (K)	100	100	100
Space Group	C2	C2	$I2_12_12_1$
Unit Cell Parameters (Å, °)	a = 265.4	a = 272.03	a = 109.9
	b = 135.5	b = 135.93	b = 215.7
	c = 58.7	c = 59.42	c = 262.8
	β = 96.3	β = 98.6	
Resolution (Å)	50.0 - 3.4	50.0 - 4.3	50.0 – 4.8
	(3.52 - 3.40)[a]	(4.37 - 4.30)	(4.88 – 4.80)
Reflections			
Unique	28140 (2332)	14610 (728)	16049 (797)
Completeness (%)	99.8 (100)	99.8 (99.6)	99.9 (99.9)
Redundancy	6.9 (6.9)	7.7 (7.5)	7.0 (6.6)
I/σ(I)	15.1 (1.8)	13.6 (1.7)	11.1 (2.1)
R_{sym}(I)[b]	0.072 (0.574)	0.063 (0.610)	0.112 (0.510)
Phasing			
Resolution (Å)		50.0 - 4.3	
Heavy Atom Sites		12	
Correlation Coefficients[c]			
SHELXD CC/CC_{weak}		42.5/19.6	
SHELXE $CC_{overall}$		34.0	
CC_{free} Left/Right Hand		65.6/58.2	
FOM[d]		0.58	
Refinement			
Resolution (Å)	44.2 - 3.40		49.9 – 4.8
	(3.52 - 3.40)		(5.17 – 4.80)
Reflections			
Number	27960 (2415)		13184 (2171)
Completeness (%)	98.2 (89.0)		84.2 (74.0)
Test Set (%)	5.1		8.0
R_{work}[e]	0.239 (0.416)		0.304 (0.342)
R_{free}[e]	0.271 (0.485)		0.332 (0.434)
ESU (Å)[f]	0.86		3.23
Contents of A.U.[g]			
Protein Molecules/Residues/Atoms	8/654/5243		8/654/5242
Ligand Molecules/Atoms	11/79		-
Mean B-Factors (Å^2)			
Protein	174.2		289.3
Ligand	202.9		-
Ramachandran Plot[g] (%)			
Favored	97.0		95.9
Outliers	0.16		0
Rmsd[i] **from Target Geometry**			
Bond Lengths (Å)	0.003		0.004
Bond Angles (°)	0.626		0.696
PDB ID	3HD7		3HD9

[a] Data for the highest resolution shell in parentheses

[b] $R_{sym}(I) = \Sigma_{hkl}\Sigma_i |I_i(hkl) - \langle I(hkl)\rangle| / \Sigma_{hkl}\Sigma_i |I_i(hkl)|$; for n independent reflections and i observations of a given

reflection; <I(hkl)> – average intensity of the i observations

[c] CC = $[\Sigma wE_oE_c\Sigma w - \Sigma wE_o\Sigma wE_c]/\{[\Sigma wE_o^2\Sigma_w - (\Sigma wE_o)^2]\ [\Sigma wE_c^2\Sigma w - (\Sigma wE_c)^2]\}^{½}$; w – weight (see http://shelx.uni-ac.gwdg.de/SHELX/shelx_de.pdf for full definitions).

[d] FOM – figure of merit = $|F(hkl)_{best}| / |F(hkl)|$; $\mathbf{F}(hkl)_{best} = \Sigma_\alpha P(\alpha)\mathbf{F}_{hkl}(\alpha) / \Sigma_\alpha P(\alpha)$

[e] $R = \Sigma_{hkl} ||F_{obs}| - |F_{calc}|| / \Sigma_{hkl} |F_{obs}|$; R_{work} – hkl ∉ T; R_{free} – hkl ∈ T; T – test set

[f] ESU – estimated overall coordinate error based on maximum likelihood

[g] A.U. – asymmetric unit

[h] Calculated with MolProbity (http://molprobity.biochem.duke.edu/)

[i] Rmsd – root-mean-square deviation