Table 1: Crystallographic Data, Phasing, and Refinement

Crystal[1]	a (Å)	b (Å)	c (Å)	λ (Å)	d_{min} (Å)	#Observed reflections	#Unique reflections	Completeness (%)	$<I>/<\sigma_I>$	$R_{sym}{}^{2}$ (%)
Native	100.54	112.71	200.77	1.0332	2.4	309,408	42,980	99.1 (98.8)	17.4	7.9 (32.9)
Iodine	100.44	112.77	199.00	1.0332	3.0	81,645	42,094	96.2 (89.1)	12.0	6.7 (46.2)
SeMet Sn1	98.72	111.07	198.84	0.9797	3.0	160,622	46,665	99.7 (99.4)	17.0	8.1 (37.7)
SeMet Sx	100.11	113.13	198.93							
λ_1 (low energy remote)				1.0688	3.2	120,963	35,261	97.9 (97.9)	16.9	6.4 (26.9)
λ_2 (inflection point)				0.9801	3.2	110,188	35,008	97.3 (95.9)	13.0	7.9 (32.0)
λ_3 (anomalous peak)				0.9797	3.2	112,147	35,072	97.3 (96.1)	13.9	7.3 (32.0)
SeMet Sn1Sn2	99.88	112.95	198.64							
λ_1 (low energy remote)				1.0688	3.0	137,029	42,024	96.5 (86.3)	16.7	6.0 (32.4)
λ_2 (inflection point)				0.9800	3.0	129,455	41,257	95.1 (82.2)	13.8	7.2 (38.5)
λ_3 (anomalous peak)				0.9795	3.0	129,052	41,449	95.0 (81.6)	12.6	7.5 (42.0)
SeMet Sn1Sn2Sx	99.94	112.79	198.61							
λ_1 (low energy remote)				1.0688	3.3	115,718	31,945	97.8 (98.5)	14.7	8.1 (28.3)
λ_2 (inflection point)				0.9800	3.3	113,142	31,613	97.3 (97.9)	13.8	10.4 (35.3)
λ_3 (anomalous peak)				0.9795	3.3	111,867	31,456	97.4 (97.7)	11.3	10.8 (38.0)

Diffraction Ratios[3]

	Sx λ_1	Sx λ_2	Sx λ_3
Sx λ_1	0.038	0.059	0.063

Sx λ_2	0.050	0.047
Sx λ_3		0.066

Diffraction Ratios[3]

	Sn1Sn2 λ_1	Sn1Sn2 λ_2	Sn1Sn2 λ_3
Sn1Sn2 λ_1	0.035	0.073	0.072
Sn1Sn2 λ_2		0.050	0.050
Sn1Sn2 λ_3			0.086

Diffraction Ratios[3]

	Sn1Sn2Sx λ_1	Sn1Sn2Sx λ_2	Sn1Sn2Sx λ_3
Sn1Sn2Sx λ_1	0.050	0.090	0.094
Sn1Sn2Sx λ_2		0.067	0.066
Sn1Sn2Sx λ_3			0.106

Diffraction Ratios[4]

	Native
Iodine	0.2300
SeMet Sn1	0.263
SeMet Sx (λ_1)	0.259
SeMet Sn1Sn1 (λ_1)	0.235
SeMet Sn1Sn2Sx (λ_1)	0.242

Table 1, continued

	Sx	Sn1Sn2	Sn1Sn2Sx	combined
Number of sites	15	31	46	
MAD phasing power[5] ($\lambda_1 \rightarrow \lambda_1^-$)	0.17	0.20	0.22	
MAD phasing power ($\lambda_1 \rightarrow \lambda_2^+$)	0.71	1.16	1.30	
MAD phasing power ($\lambda_1 \rightarrow \lambda_2^-$)	0.74	1.18	1.25	
MAD phasing power ($\lambda_1 \rightarrow \lambda_3^+$)	0.77	1.04	1.13	
MAD phasing power ($\lambda_1 \rightarrow \lambda_3^-$)	0.84	1.15	1.22	
FOM[6]	0.45	0.53	0.57	0.61

	Iodine	All combined
Number of sites	6	
Isomorphous phasing power[5]	0.82	
Anomalous phasing power[5]	0.18	
FOM	0.27	0.62

Resolution (Å)	50-5.17	4.1	3.59	3.26	3.02	2.85	2.70	2.59	2.49	2.40	50-2.4
R-Value[7]	.268	.221	.231	.257	.263	.283	.283	.295	.313	.353	.265
Free R-Value[8]	.301	.272	.270	.321	.311	.318	.308	.307	.329	.389	.303

Resolution (Å)	50-5.17	4.1	3.59	3.26	3.00	50-3.0
Phase difference[9] (°)	33.5	29.4	37.0	47.7	59.7	42.1

Table 2: Synaptic Fusion Complex Layers[1]

Layer	Heptad position	Residues				Rmsd from planarity (Å)	Deviation from square geometry (°)	Tilt angle (°)
		Sb	Sx	Sn1	Sn2			
-7	a	Leu32	Ile202	Thr29	Leu150	.09 (.06)	14.37 (2.22)	10.65 (1.27)
-6	d	Thr35	Leu205	Met32	Val153	.20 (.03)	12.11 (1.05)	8.43 (1.28)
-5	a	Val39	Ile209	Val36	Ile157	.26 (.12)	9.86 (1.48)	10.30 (1.90)
-4	d	Val42	Leu212	Ser39	Leu160	.53 (.11)	14.80 (2.23)	9.20 (2.00)
-3	a	Met46	Phe216	Gly43	Ala164	.42 (.04)	12.52 (0.88)	10.94 (1.14)
-2	d	Asn49	Met219	Thr46	Met167	.23 (.09)	15.99 (0.98)	9.73 (1.31)
-1	a	Val53	Val223	Leu50	Ile171	.12 (.08)	7.56 (1.51)	10.00 (1.15)
0	d	Arg56	Gln226	Gln53	Gln174	.05 (.04)	9.72 (1.18)	10.67 (0.67)
+1	a	Leu60	Ile230	Leu57	Ile178	.13 (.03)	6.37 (0.52)	12.07 (1.03)
+2	d	Leu63	Ile233	Val60	Ile181	.07 (.05)	6.88 (0.85)	10.42 (0.84)
+3	a	Ala67	Val237	Met64	Ala185	.50 (.12)	6.89 (1.50)	12.06 (1.23)
+4	d	Leu70	Ala240	Ile67	Asn188	.43 (.07)	2.88 (0.76)	13.60 (1.39)
+5	a	Ala74	Val244	Met71	Ile192	.40 (.09)	18.81 (0.82)	11.32 (0.94)
+6	d	Phe77	Ala247	Ala74	Ala195	.24 (.04)	7.79 (1.50)	10.51 (0.73)
+7	a	Ala81	Thr251	Leu78	Ala199	.37 (.12)	9.69 (1.00)	8.33 (2.82)
+8	d	Leu84	Ala254	Leu81	Met202	.33 (.21)	21.52 (2.42)	8.78 (1.58)
GCN4						.11 (0.06)	6.26 (2.75)	1.05 (0.90)

Table Captions

Table 1: [1]Sn1Sn2: Synaptic fusion complex with SeMet labels on both Sn1 and Sn2.
Sn1Sn2Sx: Synaptic fusion complex with SeMet labels on Sn1, Sn2, and Sx. Values in parentheses are for the highest resolution bins. [2]$R_{sym} = \Sigma_{\mathbf{h}}\Sigma_i|I_i(\mathbf{h}) - \langle I(\mathbf{h})\rangle / \Sigma_{\mathbf{h}}\langle I(\mathbf{h})\rangle$ where $I_i(\mathbf{h})$ is the i-th measurement and $\langle I(\mathbf{h})\rangle$ is the weighted mean of all measurements of $I(\mathbf{h})$ for Miller indices **h**. [3]Values are $\langle(\Delta|\mathbf{F}|)^2\rangle^{1/2}/\langle|\mathbf{F}|^2\rangle^{1/2}$, where $\Delta|\mathbf{F}|$ is the dispersive (off-diagonal elements), or Bijvoet difference (diagonal elements), computed to d_{min} = 4 Å resolution. [4]Values are $\langle(\Delta|\mathbf{F}|)^2\rangle^{1/2}/\langle|\mathbf{F}|^2\rangle^{1/2}$, where $\Delta|\mathbf{F}|$ is the isomorphous difference, computed to d_{min}=4 Å resolution. [5]Phasing power is defined as $[\langle|\mathbf{F}_D\text{-}\mathbf{F}_N|^2\rangle/\int_\phi P(\phi)(||\mathbf{F}_N|e^{i\phi}+\Delta\mathbf{F}_h|-|\mathbf{F}_D|)^2 d\phi]^{1/2}$ where $P(\phi)$ is the experimental phase probability distribution. For the isomorphous phasing power, $\mathbf{F}_N$ and $\mathbf{F}_D$ are the structure factor amplitudes of the native data and derivative data, respectively, and $\mathbf{F}_h$ are the heavy atom structure factors. For the MAD phasing power, $\mathbf{F}_N$ corresponds to the structure factors at the reference wavelength λ_1, $\mathbf{F}_D$ corresponds to the structure factors at wavelength λ_i (indicated by a superscript "+") or its Friedel mate (indicated by a superscript "-"), and $\mathbf{F}_h$ is the difference in heavy atom structure factors between the two wavelengths. For the anomalous phasing power, $\mathbf{F}_N$ corresponds to the structure factors of the derivative, $\mathbf{F}_D$ corresponds to that of its Friedel mate, and $\mathbf{F}_h$ is twice the anomalous components of the heavy atom structure factors. The quantities were computed to the corresponding diffraction limits for the MAD data sets, and to d_{min} =3.4 Å for the iodine dataset. [6] Figure of merit. [7]$R = \Sigma(|\mathbf{F}_{obs}|-k|\mathbf{F}_{calc}|)/\Sigma|\mathbf{F}_{obs}|$. [8]Free R value [46] is the R value obtained for a test set of reflections consisting of a random set of 10 % of the diffraction data not used during refinement or σ_A -value calculations [47]. [9] Unweighted phase difference between model phases and phase obtained by combination of the Sx, Sn1Sn2, and Sn1Sn2Sx MAD phases followed by density modification.

Table 2: [1]Layers are defined by the Cα carbons that are closest to the center of the four helix bundle. These layers form a heptad repeat type pattern. Geometrical parameters are computed for the "a" and "d" layers of the synaptic fusion complex in the region of the complex that forms a four helix bundle (Fig. 2c). Layer numbering is centered at the ionic layer. Rmsd from planarity is defined as the rmsd distance from the four Cα positions of the particular layer to the layer plane obtained by least-squares fit to the Cα positions. Tilt angle is defined as the angle of the normal of the layer plane to the helical bundle axis. Deviation from square geometry is the maximum deviation from 90° of the angles formed by the four Cα positions. Numbers in parenthesis are the standard deviations of the corresponding parameters obtained from the three synaptic fusion complexes in the asymmetric unit. The entire bundle has an overall average of 3.55 residues per turn. For comparison, the values for the GCN4-tetrameric mutant [20] are listed using the four inner most

leucine/isoleucine layers.