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**Supporting information for article:**

**Against the odds? *De novo* structure determination of a pilin with two cysteine residues by sulfur SAD**

**Manuela Gorgel, Andreas Bøggild, Jakob Jensen Ulstrup, Manfred S. Weiss, Uwe Müller, Poul Nissen and Thomas Boesen**

### **S1. Cloning, expression, purification and crystallization**

Details of the sample preparation are described elsewhere. In short, residues 29-136 of Pil<sub>Bac1</sub> (a type IV pilin in a gene cluster with a **pilin** with a **Bactofilin** motif) were expressed with an N-terminal His-Tag and a TEV protease cleavage site in *E. coli*. Pil<sub>Bac1</sub> was then purified by a step nickel-affinity chromatography and a size exclusion step.

Pil<sub>Bac1</sub> was crystallized at 15 mg/ml in a hanging drop vapour diffusion experiment in a 2:1 protein:reservoir ratio against a reservoir solution containing 26% (w/v) PEG 8,000, 0.1 M CHES pH 8.6, 0.15 M ammonium sulfate (subset A) and 28% (w/v) PEG 8000, 0.1 M TAPS pH 8.0, 0.15 M ammonium sulfate (subset B), both at 19°C. Rod shaped crystals appeared after a few days with final dimensions of 70x70x700 µm<sup>3</sup>, and were mounted in nylon loops and were flash-cooled in liquid nitrogen without the addition of any cryo-protectant.

### **Table S1** Structures determined by S-SAD and deposited in the PDB

The multiplicity was either taken directly from the corresponding publication or, if not available, from the PDB annotation. Yet, not for all structures a multiplicity could be provided. We have noted that the multiplicity was reported differently for different structures and it has not always been stated whether the multiplicity refers to processing with keeping I+ and I- separately or not; therefore, there will be a given uncertainty. The Bijvoet ratio was calculated as described in section 3.3. We have taken the number of atoms the structure was refined against for Np and the number of anomalous scatterers that were deposited in the PDB as the number of anomalous scatterers. CoA: Acetyl-Coenzyme A; Cu-Kα: Kα edge for Cu; D1D: 4S,5S-1,2-dithiane-4,5-diol; FMN: Flavin-mononucleotide; MES: 2-N-morpholio-ethanesulfonic acid; MPO: 3-N-morpholio-propane sulfonic acid; NBB: N-nutyl-benzene-sulfonamide; SR: Synchrotron Radiation.

Year	Protein	Uniprot	S from sequen- ce built	atoms from refine- ment	PDB	Other scatterers in the crystal	Wavelength [Å]	X-ray source	Space group	Multiplicity	Bijvoet ratio	Reference
1981	Crambin	P01542	6	327	1CRN		1.5418	Cu-Ka	P2 <sub>1</sub>		0.016	Hendrickson W. A. & Teeter M. M. (1981). Nature 290, 107-113.
2001	Thiostrepton	POC8P8	5	114	1E9W		1.5418	Cu-Ka	P4 <sub>3</sub> 2 <sub>1</sub> 2	37	0.025	Bond. C. S., Shaw. M. P., Alphey. M. S. & Hunter. W.N. (2001). Acta Cryst. D57, 755-758.
2000	Obelin	Q27709	8	1557	1EL4	1Cl	1.7400	SR	P6 <sub>2</sub>	6	0.012	Liu. Z.J., Vysotski. E.S., Chen. C.J., Rose. J.P., Lee. J. & Wang. B.C. (2000). Protein Sci. 9, 2085-2093.
2002	IGF2R Domain 11	P11717	11	1011	1GP0	1SO <sub>4</sub>	1.7100	SR	P4 <sub>3</sub> 2 <sub>1</sub> 2	33	0.016	Brown. J., Esnouf. R. M., Jones. M. A., Linnell. J., Harlos. K., Hassan. A. B. & Jones. E. Y. (2002). EMBO J. 21,1054–1062.
2003	Lima bean trypsin inhibitor	P01056	14	424	1H34		1.5418	Cu-Ka	I2 <sub>1</sub> 3	85	0.021	Debreczeni J.E., Bunkóczy G., Girmann B. & Sheldrick. G.M. (2003). Acta Cryst. D59, 393 - 395.
2001	Apocrustacya nin A1	P80029	12	2902	1H91	Xe	2.0000	SR	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	15	0.012	Cianci. M., Rizkallah. P. J., Olczak. A., Raftery. J., Chayen. N. E., Zagalsky. P. F. & Helliwell. J. R. (2001). Acta Cryst. D57, 1219–1229.
2001	α- crustacyanin C1	P80029	14	2920	1I4U	2 SO <sub>4</sub>	1.7700	SR	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	11	0.011	Gordon. E. J., Leonard. G. A., McSweeney. S. & Zagalsky. P. F. (2001). Acta Cryst. D57, 1230-1237.
1999	Lysozyme	P00689	10	1010	1LZ8	8 Cl, (1 Na)	1.54	SR	P 4 <sub>3</sub> 2 <sub>1</sub> 2	23	0.017	Dauter,Z., Dauter, M., de La

												Fortelle, E., Bricogne, G. & Sheldrick, G.M. (1999). <i>J. Mol. Biol.</i> 289, 83-92.
2002	F53F4.3 CAP-Gly	Q20728	4	727	1LPL		1.7400	SR	P6 <sub>1</sub> 2 2	17	0.011	Li. S., Finley. J., Liu. Z. J., Qiu. S. H., Chen. H., Luan. C. H., Carson. M., Tsao. J., Johnson. D., Lin. G., Zhao. J., Thomas. W., Nagy. L. A., Sha. B., DeLucas. L.J., Wang. B.-C. & Luo. M. (2002). <i>J. Biol. Chem.</i> 277, 48596-48601.
2003	PF1951	Q8TZN6	8	2340	1NNH	(1 Na)	2.2900	Cr-Ka	I4 <sub>1</sub>		0.014	To be published.
2002	Tryparedoxin form I	O77093	14	2343	1O6J		1.7700	SR	P4 <sub>1</sub> 2 <sub>1</sub> 2	13	0.012	Micossi. E., Hunter. W. N. & Leonard. G. A. (2002). <i>Acta Cryst. D</i> 57, 21-28.
2002	Tryparedoxin form II	O77093	15	2363	1O81	1SO <sub>4</sub>	1.7700	SR	P4 <sub>2</sub> 2 <sub>1</sub> 2	15	0.012	Micossi. E., Hunter. W. N. & Leonard. G. A. (2002). <i>Acta Cryst. D</i> 57, 21-28.
2003	Viscotoxin A3	P01538	13	681	1OKH	2 PO <sub>4</sub> , 1 SO <sub>4</sub>	1.5418	Cu-Ka	P2 <sub>1</sub> 2 <sub>1</sub> 2	72	0.023	Debreczeni. J. E., Girmann. B., Zeeck. A., Kraetzner. R. & Sheldrick. G.M. (2003). <i>Acta Cryst. D</i> 59, 2125-2132.
2003	PRRSV	Q9YJ11	4	894	1P65		1.7400	SR	P3 <sub>2</sub> 2 1	20	0.010	Doan. D. N. P. & Dokland. T. (2003). <i>Structure</i> 11, 1445-1451.
2004	Sso10a	Q5W1E8	7	729	1R7J		2.2900	Cr-Ka	P2 <sub>1</sub> 2 <sub>1</sub> 2	13	0.024	Chen. L., Chen. L. R., Zhou. X. E., Wang. Y., Kahsai. M. A., Clark. A. T. Edmondson. S. P., Liu. Z. J., Rose. J. P., Wang. B.-C., Meehan. E. J. & Shriver. J. W. (2007). <i>J. Mol. Biol.</i> , 341, 73-91.
2006	PA1204	Q9I4D4	4	1335	1RTT	1SO <sub>4</sub>	1.7	SR	I2 2 2	12	0.008	Agarwal. R., Bonnano. J. B., Burley. S. K. & Swaminathan.

												S. (2006) Acta Cryst. D62, 383-391.
2005	Acl- Phospholipase A2	P49121	17	977	1S8I	ISO <sub>4</sub>	1.5440	SR	P4 <sub>1</sub> 2 <sub>1</sub> 2	15	0.016	Ambrosio. A.L.B., Nonato. M.C., Selistre de Araujo. H.S., Arni. R., Ward. R.J., Ownby. C.L., de Souzam D.H.F. & Garratt. R.C. (2005). J.Biol. Chem. 280, 7326-7335.
2004	Dsrc	O28055	5	941	1SAU		1.9000	SR	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	10; 40	0.013	Weiss. M. S., Mander. G., Hedderich. R., Diederichs. K., Ermler. U. & Warkentin. E. (2004). Acta Cryst. D60, 686-695.
2004	Coproporphyrinogen iii oxidase	P11353	8	1759	1TKI		2.2900	Cr-Ka	P4 <sub>1</sub> 2 <sub>1</sub> 2	11	0.016	Phillips. J. D., Whitby. F. G. Warby. C. A., Labbe. P., Yang. C., Pflugrath. J. W., Ferrara. J. D., Robinson. H., Kushner. J. P. & Hill. C. P. (2004). J. Biol. Chem. 279, 38960-38968.
2004	Cophoryn Oxidase	P11353	20	5286	1TKL		2.2900	Cr-Ka	P4 <sub>1</sub> 2 <sub>1</sub> 2	11	0.015	Phillips L.D., Whitby F.G., Warby C.A., Labbe P., Yang C., Pflugrath J.W., Ferrara J.D., Robinson H., Kushner J.P., Hill C.P. (2004) J. Biol. Chem. 279,38960-38968.
2002	CAP-GLY domain of F53F4.3	Q20728	5	734	1TOV	ISO <sub>4</sub>	1.7400	SR	P6 <sub>1</sub> 2 2	17	0.012	Li. S., Finley. J., Liu. Z.J., Qiu. S.H., Luan. C.H., Carson. M., Tsao.J., Johnson. D., Lim. G., Zhao. J., Thomas. W., Nagy. L.A., Sha. B., Delucas. L.J., Wang. B.C. & Luo. M. (2002), JBC 277. 48596-48601.
2004	Putative glycine	Q9KQ45	20	2651	1U8S		1.7000	SR	H3		0.012	Kumaran. D. & Swaminathan. S. (2004). To be published.

	cleavage system transcriptional repressor											
2004	Bubble protein	P83799	8	452	1UOY		1.5418	Cu-Ka	C2 2 2 <sub>1</sub>	14	0.016	Olsen. J. G., Flensburg. C., Olsen. O., Bricogne. G. & Henriksen. A. (2004). Acta Cryst, D60, 250-255.
2004	Q15691	Q15691	12	2054	1VKA		2.2900	Cr-Ka	P1 2 <sub>1</sub> 1		0.018	Liu. Z.-J., Tempel. W., Schubot. F. D., Shah. A., Rose. J. P., Richardson. D. C., Richardson. J. S. & Wang. B.- C. (2004). To be published.
2005	Aig-2 like protein (a2ld1. ggatc. mgc7867)	Q923B0	8	1188	1VKB		1.7426	SR	P6 <sub>5</sub>	60	0.012	Klock. H.E., Schwarzenbacher. R., Xu. Q., McMullan. D., Abdubek. P., Ambing. E., Axelrod. H., Biorac. T., Canaves. J.M., Chiu. H.J., Deacon. A.M., DiDonato. M., Elslinger. M.A., Godzik. A., Grittini. C., Grzechnik. S.K., Hale. J., Hampton. E., Hand. G.W., Haugen. J., Hornsby. M., Jaroszewski. L., Koesema. E., Kreusch. A., Kuhn. P., Miller. M.D., Moy. K., Nigoghossian. E., Paulsen. J., Quijano. K., Reyes. R., Rife. C., Sims. E., Spraggon. G., Stevens. R.C., van den Bedem. H., Velasquez. J., Vincent. J., White. A., Wolf. G., Hodgson. K.O., Wooley. J., Lesley. S.A. & Wilson. I.A. (2005). Proteins 61, 1132-1136.

2004	YkuL	O31698	15	2136	1YAV	1 SO <sub>4</sub>	1.7000	SR	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	42	0.012	Kumaran. D. & Swaminathan. S. (2004). To be published.
2005	AF1432 (APC5600)	O28840	18	4023	1YNB		2.2900	Cr-Ka	C2		0.017	Dong. A., Skarina. T., Savchenko. A., Pai. E.F. & Edwards. A. (2005). To be published.
2005	APC5556	Q9I2R0	18	2234	1YOC		2.2900	Cr-Ka	C2 2 2 <sub>1</sub>		0.022	Dong. A., Evdokimova. E., Kudritskam. M., Zhang. R.G., Yakunin. A., Pai. E., Edwards. A. & Savchenko. A. (2005). To be published.
2005	AF0625	O29630	7	2261	1YQE	2P	2.2900	Cr-Ka	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>		0.015	Liu. Y., Skarina. T., Dong. A., Kudritskam. M., Savchenko. A., Pai. E.F. & Edwards. A. (2005). To be published.
2005	Mud1 UBA	Q10256	2	520	1Z96		1.7712	SR	P61 2 2	44	0.010	Trempe. J.-F., Brown. N.R., Lowe. E.D., Gordon. C., Campbell. I.D., Noble. M.E.M. & Endicott. J.A. (2005). EMBO J. 24, 3178-3189.
2006	Ptr-ToxA	P78737	4	828	1ZLD	2 SO <sub>4</sub>	1.5418	Cu-Ka	P2 <sub>1</sub> 3	63	0.008	Sarma. G. N. & Karplus. P. A. (2006). Acta Cryst. D62, 707-716.
2005	PA1268	Q9I476	10	2386	2AZP		2.2900	Cr-Ka	I2 2 2	12	0.016	Liu. Y., Gorodichtenskaia. E., Skarina. T., Yang. C., Joachimiak. A., Edwards. A., Pai. E.F. & Savchenko. A. (2005). To be published.
2006	Fibronectin	P02751	11	708	2CG6	1 SO <sub>4</sub>	1.7710	SR	P4 <sub>1</sub> 2 <sub>1</sub> 2	42	0.019	Rudino-Pinera. E., Ravelli. R.B.G., Sheldrick. G.M., Nanao. M.H., Korostelev. V.V., Werner. J.M., Schwarz-Linek. U., Potts. J.R. & Garman. E.F. (2007). J. Mol. Biol. 368, 833-

												844.
2006	Lam16A	Q874E3	13	2247	2CL2		1.7750	SR	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	20	0.012	Vasur. J., Kawai. R., Larsson. A. M., Igarashi. K., Sandgren. M., Dameyima. M. & Stahlberg. J. (2006). Acta Cryst. D62, 1422-1429.
2005	TT0570 (changed to TTHA1634)	Q5SHU6	22	9772	2D5W		2.2900	Cr-Ka	P2 <sub>1</sub> 2 <sub>1</sub> 2	26	0.011	Watanabe. N., Kitago. Y., Tanaka. I., Wang. J., Gu. Y., Zheng. C. & Fan. H. (2005). Acta Cryst. D61, 1533-1540
2006	SCO7518	Q93J02	8	5590	2DG8		2.2900	Cr-Ka	P2 <sub>1</sub>	14	0.009	Hayashi. T., Watanabe. N., Tanaka. Y., Sakai. N., Tamura. T., Yao. M. & Tanaka. I. (2006). To be published.
2009	FKBP35 (PFL2275c)	Q8I4V8	8	2504	2FBN		2.2900	Cr-Ka	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	8	0.013	Alag. R., Bharatham. N., Dong. A., Hills. T., Harikishore. A., Widjaja. A. A., Shochat. S. G., Hui. R. & Yoon. H. S. (2009) Protein Sci. 18, 2115-2124.
2006	DIPP-1	O95989	5	1137	2FVV	1 SO <sub>4</sub> , 1Cl, 6P	1.5418	Cu-Ka	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	6	0.011	Thorsell. A.G., Persson. C., Graslund. S., Hammarstrom. M., Busam. R.D. & Hallberg. B.M. (2009). Proteins 77, 242-246.
2007	hARH3	Q9NX46	15	2597	2G4K	2 Mg, 2 Cl	2.0000	SR	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	12	0.016	Mueller-Dieckmann. C., Panjekar. S., Schmidt. A., Mueller. S., Kuper. J., Geerlof. A., Wilmanns. M., Singh. R. K., Tucker. P. A. & Weiss. M. S. (2007). Acta Cryst. D63, 366-380.
2007	HNL	P52704	14	2087	2G4L	5SO <sub>4</sub> , 1 Cl	2.0000	SR	C2 2 2 <sub>1</sub>	12	0.017	Mueller-Dieckmann. C., Panjekar. S., Schmidt. A., Mueller. S., Kuper. J., Geerlof.



												A., Wilmanns. M., Singh. R. K., Tucker. P. A. & Weiss. M. S. (2007). Acta Cryst. D63, 366-380.
2007	Insulin	P01315	6	403	2G4M		2.0000	SR	I <sub>2</sub> 3	35	0.027	Mueller-Dieckmann. C., Panjekar. S., Schmidt. A., Mueller. S., Kuper. J., Geerlof. A., Wilmanns. M., Singh. R. K., Tucker. P. A. & Weiss. M. S. (2007). Acta Cryst. D63, 366-380.
2007	LeuB	P9WKK8	26	9988	2G4O	2 SO <sub>4</sub> , 6 Cl	2.0000	SR	P <sub>2</sub> 2 <sub>1</sub> 2 <sub>1</sub>	12	0.011	Mueller-Dieckmann. C., Panjekar. S., Schmidt. A., Mueller. S., Kuper. J., Geerlof. A., Wilmanns. M., Singh. R. K., Tucker. P. A. & Weiss. M. S. (2007). Acta Cryst. D63, 366-380.
2007	MogA	O53877	6	3165	2G4R	1 Cl	2.0000	SR	P <sub>2</sub> <sub>1</sub>	6	0.009	Mueller-Dieckmann. C., Panjekar. S., Schmidt. A., Mueller. S., Kuper. J., Geerlof. A., Wilmanns. M., Singh. R. K., Tucker. P. A. & Weiss. M. S. (2007). Acta Cryst. D63, 366-380.
2007	NBR1 PB1	Q14596	4	692	2G4S	1 Cl	2.0000	SR	P <sub>6</sub> <sub>3</sub> 2 2	34	0.017	Mueller-Dieckmann. C., Panjekar. S., Schmidt. A., Mueller. S., Kuper. J., Geerlof. A., Wilmanns. M., Singh. R. K., Tucker. P. A. & Weiss. M. S. (2007). Acta Cryst. D63, 366-380.
2007	PPE	P00772	12	1833	2G4T	2 SO <sub>4</sub> , 1 Na	2.0000	SR	P <sub>2</sub> 2 <sub>1</sub> 2 <sub>1</sub>	12	0.015	Mueller-Dieckmann. C., Panjekar. S., Schmidt. A.,

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2007	RNase A	P61823	25	1908	2G4W	1 SO <sub>4</sub> , 1 Cl	2.0000	SR	C2	6	0.022	Mueller-Dieckmann. C., Panjekar. S., Schmidt. A., Mueller. S., Kuper. J., Geerlof. A., Wilmanns. M., Singh. R. K., Tucker. P. A. & Weiss. M. S. (2007). Acta Cryst. D63, 366-380.
2007	Thaumatococin	Q8RVT0	17	1562	2G4Y		2.0000	SR	P4 <sub>1</sub> 2 <sub>1</sub> 2	25	0.020	Mueller-Dieckmann. C., Panjekar. S., Schmidt. A., Mueller. S., Kuper. J., Geerlof. A., Wilmanns. M., Singh. R. K., Tucker. P. A. & Weiss. M. S. (2007). Acta Cryst. D63, 366-380.
2007	Trypsin P1	P35049	7	1554	2G51	3 Cl	2.0000	SR	P1	3	0.015	Mueller-Dieckmann. C., Panjekar. S., Schmidt. A., Mueller. S., Kuper. J., Geerlof. A., Wilmanns. M., Singh. R. K., Tucker. P. A. & Weiss. M. S. (2007). Acta Cryst. D63, 366-380.
2006	AR2 (VEGF-E)	P52584	46	2963	2GNN	2 SO <sub>4</sub> , 3Cl	1.6984	SR	P4 <sub>1</sub> 2 2	15	0.019	Pieren. M., Prota. A.E., Ruch. C., Kostrewa. D., Wagner. A., Biedermann. K., Winkler. F.K. & Ballmer-Hofer. K. (2006). J. Biol. Chem. 281, 19578-19587.
2006	ATU299 (APC5867)	A9CI22	10	1585	2HLY		2.2900	Cr-Ka	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>		0.019	Dong. A., Xu. X., Zheng. H., Kim. Y., Edwards. A.M.,

												Joachimiak. A. & Savchenko. A. (2006), to be published.
2007	h phosphatase (DUSP9)	Q99956	3	1162	2HXP	1 PO <sub>4</sub>	1.7000	SR	P32	11	0.008	Almo. S.C., Bonanno. J.B., Sauder. J.M., Emtage. S., Dilorenzo. T.P., Malashkevich. V., Wasserman. S.R., Swaminathan. S., Eswaramoorthy. S., Agarwal. R., Kumaran. D., Madegowda. M., Ragumani. S., Patskovsky. Y., Alvarado. J., Ramagopal. U.A., Faber-Barata. J., Chance. M.R., Sali. A., Fiser. A., Zhang. Z.Y., Lawrence. D.S. & Burley. S.K. (2007). <i>J. Struct. Funct. Genom.</i> 8, 121-140.
2006	RSP_3880	Q3HKK5	26	5817	2HZG		1.74300	SR	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	13	0.016	Ramagopal. U. A., Toro. R. & Almo. S.C. (2006). To be published.
2007	Titin (A168-A169)	Q8WZ42	4	1526	2ILL	2 Cl	2.0000	SR	I2 2 2	12	0.013	Mueller-Dieckmann. C., Panjekar. S., Schmidt. A., Mueller. S., Kuper. J., Geerlof. A., Wilmanns. M., Singh. R. K., Tucker. P. A. & Weiss. M. S. (2007). <i>Acta Cryst.</i> D63, 366-380.
2007	CD5 domain III	P05127	8	789	2JA4		1.8000	SR	P3 <sub>2</sub> 2 1	52	0.016	Rodamilans. B., Munoz. I.G., Bragado-Nilsson. E., Sarrias. M.R., Padila. O., Blanco. F.J., Lozano. F. & Montoya. G. (2007). <i>J. Biol. Chem.</i> 282, 12669-12677.
2008	6th ORF of R. blasitica	P05449	26	4041	2NXV		1.8000	SR	P2 <sub>1</sub> 2 <sub>1</sub> 2	54	0.013	Enroth. C. & Ake. S. (2008). <i>BBA</i> 1784, 379-384.

	operon											
2008	Glucocorticoid induced TNF receptor ligand	Q7TS55	12	1982	2QDN		1.7433	SR	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	21	0.011	Chattopadhyay. K., Ramagopal. U.A., Brenowitz. M., Nathenson. S.G. & Almo. S.C. (2008). Proc. Natl. Acad. Sci. USA 105, 635-640.
2007	AF1382	O28889	4	714	2QVO		1.9000	SR	P4 <sub>2</sub>		0.013	Zhu. J., Zhao. M., Fu. Z.-Q., Yang. H., Chang. J., Hao. X., Chen. L., Liu. Z.J., Rose. J.P. & Wang. B.C. (2007). To be published.
2007	SCO5068	Q9ADD9	6	2457	2REK		2.2900	Cr-Ka	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>		0.012	Dong. A., Xu. X., Gu. J., Edwards. A.m., Joachimiak. A. & Svachenko. A. (2007). To be published
2007	TP0655	O83661	12	2604	2V84	5Cl, 2MES	1.5418	Cu-Ka	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	12	0.010	Machius. M., Brautigam. C. A. Tomchick. D. R. Ward. P., Otwinowski. Z., Blevins. J. S., Deka. R. K. & Norgard. M. V. (2007). J. Mol. Biol. 373, 681-694.
2009	PPIG	Q13427	9	1434	2WFJ	2 Mg, 1 Cl	1.5418	Cu-Ka	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>		0.010	Stegmann. C.M., Seeliger. D., Sheldrick. G.M., DeGroot. B-L- & Wahl. M.C. (2009). Angew. Chem. Int. Ed. Engl. 48, 5207.
2010	GFP	P42212	6	1821	2WUR		1.5418	Cu-Ka	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	20	0.007	Shinobu. A., Palm. G.J., Schierbeek. A.J. & Agmon. N. (2010). J. Am. Che. Soc. 132:11093.
2011	HP0721	O25423	7	802	2XRH		1.9070	SR	P 3 <sub>1</sub> 2 1	19	0.016	Cioci. G., Terradit. L., Dian. C., Mueller-Dieckman. C. & Leonard. G.(2011). Proteins 79, 1678-1681

2013	PA1645	Q9I380	17	2839	2XU8	11 SO <sub>4</sub>	1.6000	SR	I4 2 2	73	0.010	Moynie. L., Schnell. R., McMahon. S.A., Sandalova. T., Boulkerou. W.A., Schmidberger. J.W., Alphey. M.S., Cukier. C., Duthie. F., Kopec. J., Liu. H., Jacewicz. A., Hunter. W.N., Naismith. J.H. & Schneider. G. (2013) Acta Cryst. F69, 25-34.
2011	DNTR Inducer Binding Domain	Q7WT50	12	1704	2Y7P		1.8200	SR	P 3 <sub>1</sub> 2 1	41	0.013	Devesse. L., Smirnova. I., Lonneborg. R., Kapp. U., Brzenisnki. P., Leonard. G.A. & Dian. C. (2011). Mol. Microbiol. 81, 354-367
2011	Lectin SML2	P81860	105	6025	2YIL	7CL. 3SO <sub>4</sub>	2.0000	SR	P 2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	18	0.026	Mueller. J.J., Weiss. M.S. & Heinemann. U. (2011). Acta Cryst. D67, 936-944.
2006	PH1780	O59416	8	1788	2YZQ		2.2900	Cr-Ka	C2		0.017	Kanagawa. M., Minami. Y., Watanabe. N., Yokoyama. S. & Kuramitsu. S. (2007). To be published.
2007	TTHA1012	Q5SJJ6	2	1334	2YZY		2.2900	Cr-Ka	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>		0.009	Ebihara. A., Watanabe. N., Yokoyama. S. & Kuramitsu. S. (2007). To be published.
2008	TetR transcription factor regulator SCO0332	Q9RK47	4	2881	2ZB9		2.2900	Cr-Ka	P2 <sub>1</sub>	7	0.009	Okada. U., Kondo. K., Hayashi. T., Watanabe. N., Yao. M., Tamura. T. & Tanaka. I. (2008). Acta Cryst. D64. 198-205.
2009	TetR transcription factor regulator SCO7815	Q9FBX0	4	1574	2ZCX		2.2900	Cr-Ka	P4 <sub>1</sub> 2 <sub>1</sub> 2	27	0.012	Okada. U., Kondo. K., Hayasi. T., Watanabe. N., Yao. M., Tamura. T. & Tanaka. I. (2007). To be published.

2004	Pheromone binding protein	Q8WRW5	11	927	3BJH	1NBB	1.7700	SR	C2 2 2 <sub>1</sub>	35	0.017	Lartigue. A., Gruez. A., Briand. L., Blon. F., Walsh. M., Pernollet. J. C., Tegoni. M. & Cambillau. C. (2004). J. Biol. Chem. 279, 4459-4464.
2008	AF1514	O28758	4	682	3C0F		2.2900	Cr-Ka	P4 <sub>1</sub> 2 <sub>1</sub> 2	50	0.018	Li. Y., Bahti. P., Shaw. N., Song. G., Chen. S., Zhang. X., Zhang. M., Chen. C., Yin. J., Zhu. J.-Y., Zhang. H., Che. D., Xu. H., Abbas. A., Wang., B.-C. & Liu. Z.-J. (2008). Proteins 4, 2109-2113.
2008	Viscotoxin A1	D0VWT3	12	672	3C8P		1.5418	Cu-Ka	P4 <sub>3</sub> 2 <sub>1</sub> 2	54	0.016	Pal. A., Debreczeni. J. E., Sewana. M., Gruene. T., Kahle. B., Zeeck. A. & Sheldrick. G. M. (2008). Acta Cryst. D64, 985-992.
2008	All3740 (HETL)	Q8YQS9	6	1798	3DU1		1.5418	Cu-Ka	I2 2 2	50	0.007	Ni. S., Sheldrick. G.M., Benning. M.M. & Kennedy. M.A. (2008). J.Struct. Biol. 165, 47-52.
2008	FeoA	D0VWU5	20	2145	3E19	1 PO <sub>4</sub>	1.9000	SR	C2	23	0.009	Hughes. R. C., Li. Y., Wang. B.-C., Liu. Z.-J. & Ng. J.D. (2011). To be published.
2008	Npun_R1517	B2IZS7	3	611	3E56		1.5418	Cu-Ka	P4 <sub>3</sub> 2 <sub>1</sub> 2	106	0.008	Ni. S., Benning. M.M., Smola. M.J., Feldmann. E.A. & Kennedy. M.A. (2008). Proteins 74, 794-798.
2009	HEWL	P00698	10	1000	3EXD		1.4600	SR	P4 <sub>3</sub> 2 <sub>1</sub> 2	25	0.011	Guimares. B.G., Sanfelici. L., Neuenschwander. R.T., Rodrigues. F., Grizolli. W.C., Raulik. M.A., Piton. J.R., Meyer. B.C., Nascimento. A.S. & Polikarpov. I. (2009) J.

												Synchr. Rad. 16, 69-75.
2010	ATV ORF131	Q3V4Q3	3	778	3FAJ	3 Cl	2.0000	SR	P6 <sub>1</sub>	24	0.019	Goulet. A., Vestergaard. G., Felisberto-Rodrigues. C., Campanacci. V., Garrett. R. A., Cambillau. C. & Ortiz-Lombardia. M. (2010). Acta Cryst. D66, 304-308.
2009	Pldb2	Q3TCN2	22	4289	3FBX	0.05 Xe	1.9000	SR	C2	23	0.014	Lakomek. K., Dickmanns. A., Mueller. U., Kollmann. K., Deuschl. F., Berndt. A., Luebke. T. & Ficner. R. (2009). Acta Cryst. D65, 220-228.
2010	Lipase	Q9HFW6	23	3876	3G7N	5 SO <sub>4</sub>	1.9000	SR	P4 <sub>3</sub> 2 <sub>1</sub> 2	20	0.013	Bian. C., Yuan. C., Chen. L., Meehan. E. J., Jiang. L., Huang. Z., Lin. L. & Huang. M. (2010). Proteins 78, 1601-1605.
2009	Iodotyrosine deiodinase	Q9DCX8	7	1484	3GB5	1 PO <sub>4</sub> , 1 FMN	1.6530	SR	P3 <sub>1</sub> 2 <sub>1</sub>	14	0.010	Thomas. S.R., McTamney. P.M., Adler. J.M., Laronde-Leblanc. N. & Rokita. S.E. (2009). J. Biol. Chem. 284, 19659-19667.
2009	BH3703	Q9K6M5	11	2957	3I0T	1 SO <sub>4</sub>	2.26864	SR	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	7	0.015	Ramagopal. U. A., Toro. R., Wasserman. S., Burley. S. K. & Almo. S. C. (2009). tbp
2009	PRKAR1A	P00514	3	425	3IM3		1.9000	SR	P6 <sub>2</sub> 2 <sub>2</sub>	52	0.015	Sarma. G.N., Kinderman. F.S., Kim. C., von Daake. S., Chen. L., Wang. B.C. & Taylor. S.S. (2010). Structure 18, 155-166.
2009	P1 protein (rabies)	Q0GBY3	2	742	3L32		1.7710	SR	I4 <sub>1</sub> 2 <sub>2</sub>	44	0.008	Ivanov. I., Crepin. T., Jamin. M. & Ruigrok. R.W.H. (2010). J. Virol. 84, 3707-3710.
2012	ORF AF1383	O288889	4	740	3O3K		1.9000	SR	P4 <sub>2</sub>	25	0.013	Zhu J.-Y., Fu. Z.Q., Chen. L., Xu. H., Chrzas. J., Rose. J. &

												Wang, B.C. (2012) Acta Cryst D68, 1242-1252.
2012	Tdr domain 3	Q9H7E2	3	447	3PMT		1.5400	Cu-Ka	P6 <sub>5</sub>	20	0.010	Liu K., Guo Y., Liu H., Bian C., Lam R., Liu Y., Mackenzie F., Rojas L.A., Reinberg D., Bedford M.T., Xu R.M., Min J.- (2012). Plos One 7:e30375-e30375.
2012	A654L	O41136	24	3240	3QB8	2 CoA	1.7000	SR	P 3 <sub>1</sub>	77	0.013	Charlop-Powers. Z., Zhou. M.-M., Jakoncic. J., Gurnon. J. & Van Etten. J. (2011). To be published.
2012	Phosphorylation domain of ryanodine receptor	P11716	4	1530	3RQR		2.2900	Cr-Ka	P4 <sub>3</sub> 2 <sub>1</sub> 2	9	0.012	Sharma P., Ishiyama. N. . Nair. U., Li. W., Dong. A., Miyake. T., Wilson. A., Ryan. T., MacLennan. D.H., Kislinger. T., Ikura. M., Dhe-Paganon. S. & Gramolini. A.O. (2012). FEBS J. 279, 3952-3964.
2012	CysZ	Q5QUJ8	21	3926	3TX3	4 Cl, 1 SO <sub>4</sub>	1.7432	SR	C 1 2 1	50	0.012	Liu. Q., Dahmane. T., Zhang. Z., Assur. Z., Brasch. J., Shapiro. L., Mancina. F. & Hendrickson. W. (2012) Science 336: 1033-1037.
2012	TNFRSF21	O75509	21	1229	3U3Q		2.0000	SR	P 6 <sub>1</sub> 2 2	39	0.025	Ru. H., Zhao. L., Ding. W., Jiao. L., Shaw. N., Liang. W., Zhang. L., Hung. L.W., Matsugaki. N., Wakatsuki. S. & Liu. Z.J. (2012) Acta Cryst D 6, 521-530.
2012	TNFRSF21	O75510	21	1229	3U3V		2.7000	SR	P 6 <sub>1</sub> 2 2	25	0.041	Ru. H., Zhao. L., Ding. W., Jiao. L., Shaw. N., Liang. W., Zhang. L., Hung. L.W., Matsugaki. N., Wakatsuki. S. &



												Liu. Z.J. (2012) Acta Cryst D 6, 521-530.
2011	HPAR17	Q9Y237	15	808	3UI5	4 SO <sub>4</sub> , D1D	1.5418	Cu-Ka	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	30	0.016	Mueller.J.W., Link. N.M., Matena. A., Hoppstock. L., Ruppel. A., Bayer. P. & Blankenfeldt. W. (2011). J.AM.CHEM.SOC. 133, 20096-20099.
2012	RPA2292 (HK9s)	Q6N7G5	3	1002	3VA9	1 Cl	1.7432	SR	I 4 <sub>1</sub> 2 2	150	0.008	Liu. Q., Dahmane. T., Zhang. Z., Assur. Z., Brasch. J., Shapiro. L., Mancina. F. & Hendrickson. W. (2012) Science 336, 1033-1037.
2013	CofA	Q59393	5	1322	3VOR		1.5000	SR	P2 <sub>1</sub>	7	0.007	Fukakukusa S., Kawahara. K., Nakamura. S., Iwashita. T., Baba. S., Nishimura. M., Kobayashi. Y., Honda. T., Iida. T., Taniguci. T. & Ohkubo. T. (2012) ActA Cryst D68, 1418-1429
2014	h Prostaglandin E2Synthase 1	O14684	9	1353	4BPM	Glutathione	2.0660	SR	H3 2		0.016	Li. D., Howe. N., Dukkupati. A., Shah. S.T.A., Bax. B.D., Edge. C., Bridges. A., Hardwicke. P., Singh. O.M.P., Giblin. G., Pautsch. A., Pfau. R., Schnapp. G., Wang. M., Olieric. V. & Caffrey. M. (2014). Cryst. Griwth Des. 14, 2034-2047.
2013	LpNTPDaseI	Q5ZUA2	32	5734	4BRM	4 SO <sub>4</sub> , 2 Cl	1.9000	SR	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	14	0.014	Zebisch. M., Krauss. M., Sxhaefer. P., Lauble. P. & Straeter. N. (2013). ActA Cryst F69, 257-262
2012	PSAP	P07602	9	926	4DDJ		2.2900	Cr-Ka	P 6 <sub>1</sub> 2 2	49	0.024	Popovic. K., Holyoake. J.,

												Pomes. R. & Prive. G.G. (2012). Proc.Natl.Acad.Sci.USA 109: 2908-2912
2012	GAIN and HormR doamins of CIRL1/Latrophilin (CL1)	O88917	25	2893	4DLQ	8 SO <sub>4</sub>	2.0000	SR	P3 <sub>1</sub> 2 1	22	0.018	Arac. D., Boucard. A.A., Bolliger. M.F., Nguyen. J., Soltis. S.,Sudhof. T.C., & Brunger. A.T. (2012). EMBO J. 31, 1364-1378.
2013	CXL1	F1Q6N2	7	552	4HED		2.2900	Cr-Ka	P6 <sub>1</sub> 2 2	14	0.027	Rajasekaran D., Fan. C., Meng. W., Pflugrath. J.W. & Lolis. E.J. (2013) Proteins 82 (5): 708-716.
2014	Glutaredoxin 1 Pf	Q9NLB2	6	852	4HJM	MPO	1.5400	Cu-Ka	P3 <sub>2</sub> 2 1	19	0.010	Yogavel. M., Tripathi. T., Gupta. A., Bandy. M.M., Rahlfs. S., Becker. K., Belrhali. H. & Sharma. A. (2014). Acta Cryst D 70, 91-100.
2013	CNOT1	A5YKK6	9	1619	4J8S		1.5400	Cu-Ka	P2 <sub>1</sub> 3	12	0.009	Fabian. M.R., Frank. F., Rouya. C., Siddiqui. N., Lai. W.S., Karetnikov. A., Blackshear. P.J., Nagar. B. & Sonenberg. N. (2013). Nat. Struct. Mol. Biol. 20, 735-739.
2013	plant defence barwin like protein	U5HK42	6	962	4JP6		1.5400	Cu-Ka	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	52	0.009	Huet. J., Teinkela Mbosso. E.J., Soror. S., Meyer. F., Looze. Y., Wintjens. R. & Wohlkonig. A. (2013). Acta Cryst. D69, 2017-2026.
2014	YetJ	O31539	9	1633	4TJQ	2Ca, 3Cl	2.1	SR	P6 <sub>5</sub> 2 2	535.8	0.024	Liu, Q., Guo, Y., Chang, C., Cai, Z., Assur, Z., Mancina, F., Greene, M.I. & Hendrickson, W.A. (2014). Acta Cryst. D. 70, 2544-2557.

2014	ThiT	S5L6I0	16	1418	4TKR		2.1	SR	P3 <sub>1</sub> 2 <sub>1</sub>	140.2	0.021	Liu, Q., Guo, Y., Chang, C., Cai, Z., Assur, Z., Mancina, F., Greene, M.I. & Hendrickson, W.A. (2014). Acta Cryst. D. 70, 2544-2557.
2014	EGFR kinase domain	P00533	17	2451	4TKS		2.1	SR	I2 3	254.1	0.017	Liu, Q., Guo, Y., Chang, C., Cai, Z., Assur, Z., Mancina, F., Greene, M.I. & Hendrickson, W.A. (2014). Acta Cryst. D. 70, 2544-2557.

**Table S2** Refinement statistics for the structure solution from data set B1-B3

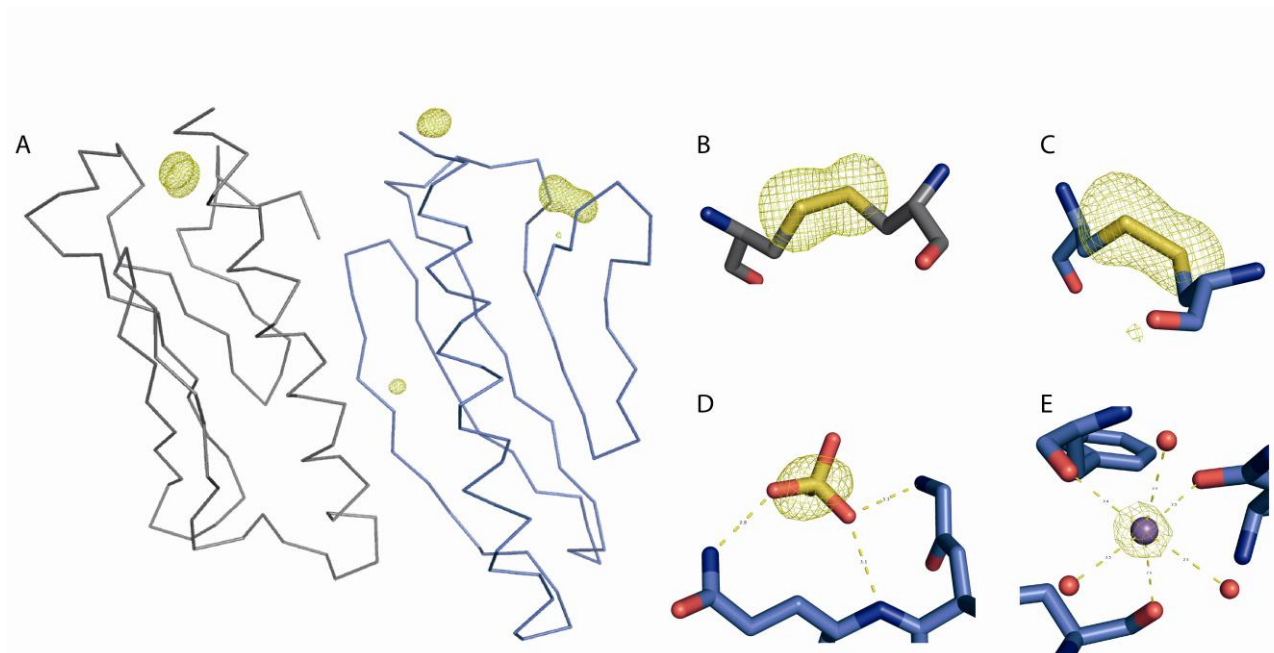
Data were refined using Phenix.Refine (Afonine *et al.*, 2012) and the model was built in Coot (Emsley *et al.*, 2010).

Refinement statistics	
$R_{\text{work}}$	0.1794
$R_{\text{free}}$	0.2201
Number of non-hydrogen atoms modelled	1534
No. of protein atoms	1403
No. of ligand atoms	6
No. of solvent molecules	125
Protein residues	178
R.m.s. deviations from ideal values	
Bonds (Å)	0.006
Angles (°)	1.02
Ramachandran favoured (%)	98
Ramachandran outliers (%)	0
Clash Score	1.82
Average B-factor	35.70
Average B-factor for protein	35.20
Average B-factor for ligands	75.70
Average B-factor for solvent	38.50

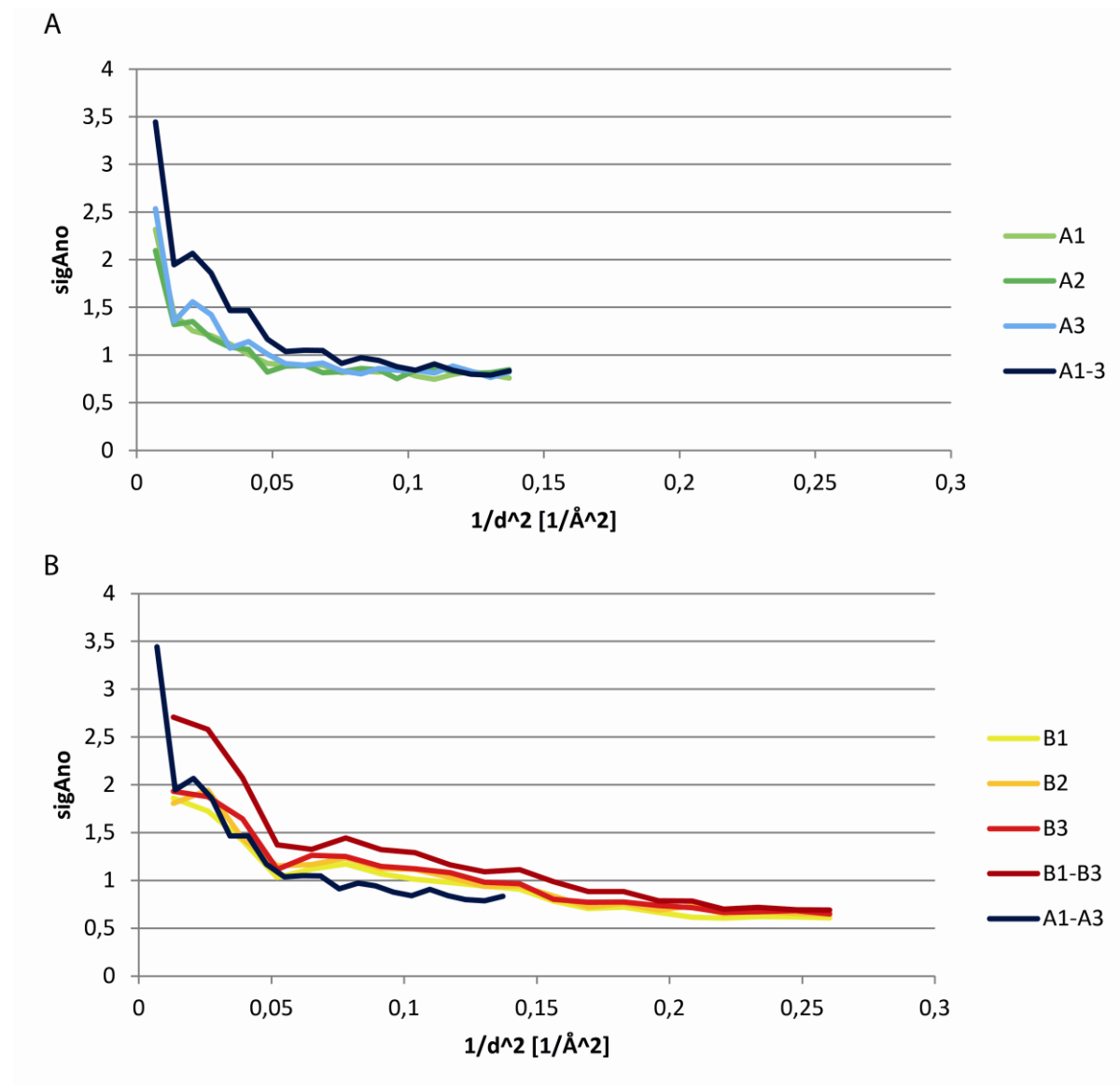
**Table S3** Relative anomalous peak heights in  $\sigma$ 

Anomalous difference maps were calculated from the different data sets using model phases from the final structure in Phenix.Maps from the Phenix GUI (Adams *et al.*, 2010). The peak heights were investigated in Coot (Emsley *et al.*, 2010).

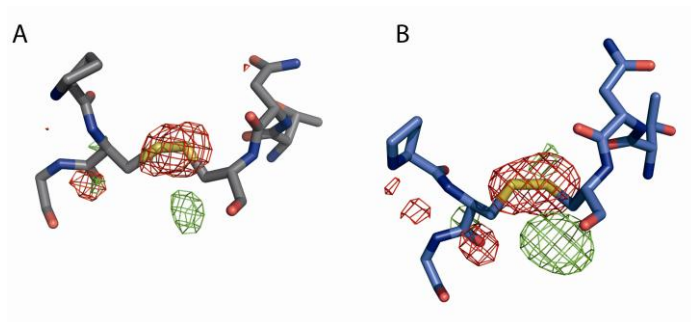
	A1	A2	A3	A1-3	B1	B2	B3	B1-3
SSA	10.8	11.5	12.8	19.1	16.6	20.3	22.1	26.0
SSB	9.4	9.6	11.8	14.9	13.6	17.3	19.1	21.5
SO4	6.2	6.6	6.1	9.9	10.2	9.5	10.2	12.7
Broken SSB					7.9	4.7		6.0
Na					6.9	4.9	5.3	6.5



**Figure S1** Anomalous peaks of data set B1-B3 against the final model. The map is contoured at a level of  $5\sigma$ . A: Overview over all 6 identified peaks. Chain A is shown in grey and chain B in blue. B: Disulfide bridge in chain A. C: Disulfide bridge in chain B. A peak for the broken cysteine bridge is clearly visible. D: Sulfate ion bound to the N-terminus at chain B. Hydrogen bridges are shown in dashed lines. E:  $\text{Na}^+$  ion. The ion is octahedrally coordinated by oxygens only. The average coordination distance is  $2.5 \text{ \AA}$ .



**Figure S2** SigAno plotted against  $1/d^2$ . A: Data sets A1, A2, A3 and A1-A3. B: Data Sets B1, B2, B3, B1-B3 and A1-3.



**Figure S3** Difference maps between the first and the second 180° degrees of data set B3 around the disulfide bridges from chain A (A) and B(B). The map is contoured at  $4\sigma$ .