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Supporting information for article:

Experimental phasing with vanadium and application to nucleotide-binding membrane proteins

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Table S1 Comparison between processing and phasing statistics of two RNAse A datasets collected at λ = 2.2604 Å and λ = 1.7711 Å.

Data collection	RNAse A 5485 eV	RNAse A 7000 eV
Beamline	DLS-I23	DLS-I23
Space group	*C*2	*C*2
Unit cell (Å, º)	a=100.4 b=32.9, c=72.5 α=γ=90, β=90.4	a=100.4 b=32.9, c=72.5 α=γ=90, β=90.4
Number of degrees, number of crystals	360°, 1	360°, 1
Wavelength (Å)	2.2604	1.7711
Resolution (Å)	72.44-1.47 (1.50-1.47)	72.47-1.30 (1.32-1.30)
Number of unique reflections	34664 (1007)	54052 (2365)
Completeness (%)	86.5 (51.0)	92.3 (81)
Completeness (%) ellipsoidal		
Multiplicity	4.2 (2.1)	4.6 (4)
<I/σ(I)>	21.3 (2.8)	17.8 (1.6)
Rmerge (%)	3.4 (18.8)	3.5 (61.7)
Rpim (%)	2.4 (18.8)	2.4 (51.4)
CC1/2 highest resolution shell	0.88	0.68
Anomalous completeness	77.0 (47.7)	84.6 (70.8)
Anomalous multiplicity	2.0 (1.1)	2.2 (2.1)
Mid-Slope of Anom Normal Probability	1.59	1.19
Phasing (Crank2)		
Substructure CFOM	53.5	43.5
Mean phasing FOM	0.25	0.19
FOM after density modification	60.8	51.3
FOM after initial automatic model building	0.89	0.92
Number of residues built after automatic model building/number of fragments	246/4	239/5
Rwork/Rfree after model building	27.4/29.6	28.7/30.8

Table S2 Vanadium-containing ligands deposited in the PDB as of 2019.

Values in parentheses are the number of membrane proteins complexed with vanadium derivatives.

Ligand identifier	Ligand name	Number of PDB Entries (membrane proteins)
VO4	TRIOXIDO-OXO-VANADIUM	96 (4)
VN4	OXIDO(DIOXO)VANADIUM	12 (1)
AOV	ADP ORTHOVANADATE	8 (2)
AD9	ADP METAVANADATE	7
VN3	TRIOXOVANADIUM	4
UVC	URIDINE-2',3'-VANADATE	3
V5A	ADENOSINE-5'-VANADATE	3
V	VANADIUM ION	3
AVC	ADENOSINE-5'-MONOPHOSPHATE-2',3'-VANADATE	2
V7O	META VANADATE	2
V4O	CYCLO-TETRAMETAVANADATE	2 (1)
SVA	SERINE VANADATE	1
VG1	ALPHA-D-GLUCOSE-1-PHOSPHATE-6-VANADATE	1
VO3	TETRAMETAVANADATE	1
BVA	TRIHYDROXY[(N-HYDROXYBENZAMIDATO)OXO]VANADATE	1
FV1	DIHYDROXY{[(2R,3S)-3-METHYLOXIRAN-2-YL]PHOSPHONATO-KAPPAO}OXOVANADIUM	1
AV2	ADENOSINE-5'-DIPHOSPHATE-2',3'-VANADATE	1
VA3	TRIVANADATE	1
8P8	C Fe7 S8 V	1
AIV	HYDROXY(OXO)BIS(PYRIDINE-2-CARBOXYLATO-KAPPA~2~N,O)VANADIUM(3+)	1
D6N	FeV	1

Table S3 Effect of the multiplicity on the structure solution of SERCA

12 datasets (360° each) were processed separately and incrementally merged in XSCALE (Kabsch, 2010). The reflection files were converted to mtz files using Aimless (Evans & Murshudov, 2013) and fed to CRANK2 (Skubak & Pannu, 2013). The table shows that at least 4 datasets are necessary to obtain a starting model.

Number of datasets	Overall SigAno/Anomalous correlation (XSCALE)	Multiplicity (Aimless)	Mean phasing FOM	Density modification FOM	FOM after initial model building	Number of building cycles	% of residues built against sequence/ against built
1	0.84/11	6.5	0.151	48.5	0.51	50	4/4
2	0.89/14	13	0.144	42.1	0.49	50	2/2
3	0.93/17	19.4	0.148	41.5	0.51	50	5/5
4	0.97/19	25.8	0.164	54.2	0.81	22	49/56
5	1.01/21	32.3	0.173	46.5	0.87	41	67/77
6	1.05/23	38.8	0.175	54.1	0.77	15	53/57
7	1.08/25	45.2	0.196	56.4	0.8	14	71/77
8	1.11/27	51.7	0.192	57.6	0.8	14	61/65
9	1.14/30	58.1	0.175	46.9	0.79	16	36/58
10	1.16/30	64.6	0.192	57.3	0.85	17	53/59
11	1.18/30	71	0.199	57.5	0.85	15	40/63
12	1.21/34	77.4	0.182	57.5	0.88	16	58/63

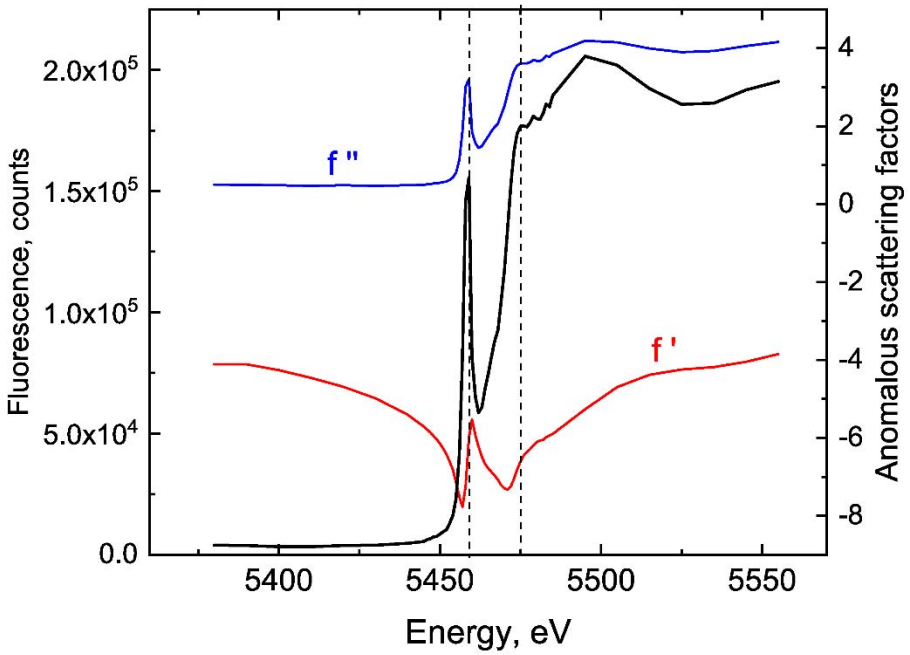

Figure S1 Vanadium K-edge XAS spectra of sodium ortho-vanadate taken on the Diamond I23 beamline (black line). The dashed lines show the pre-edge peak (E = 5459 eV) and the peak (E = 5475 eV with f_0 = 23 e^-, f' = -6.6 e^- and f'' =3.6 e^-) as determined by CHOOCH (Evans & Pettifer, 2001).

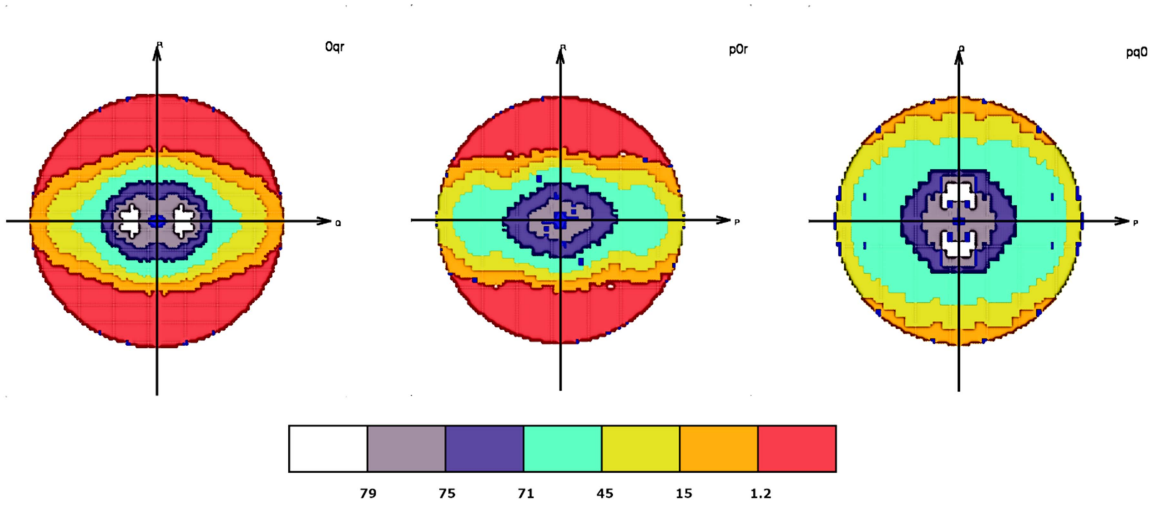

Figure S2 Intensity data in 3D reciprocal space for McjD. The local mean value of $I/\sigma(I)$ for each reciprocal lattice point within the sphere of observation is calculated by STARANISO and colour-coded: the blue points represent unobserved data, the red points represent observed data falling below the 1.2σ threshold for cut-off, and the other colours represent successively higher values of the mean $I/\sigma(I)$ as shown by the colour bar.

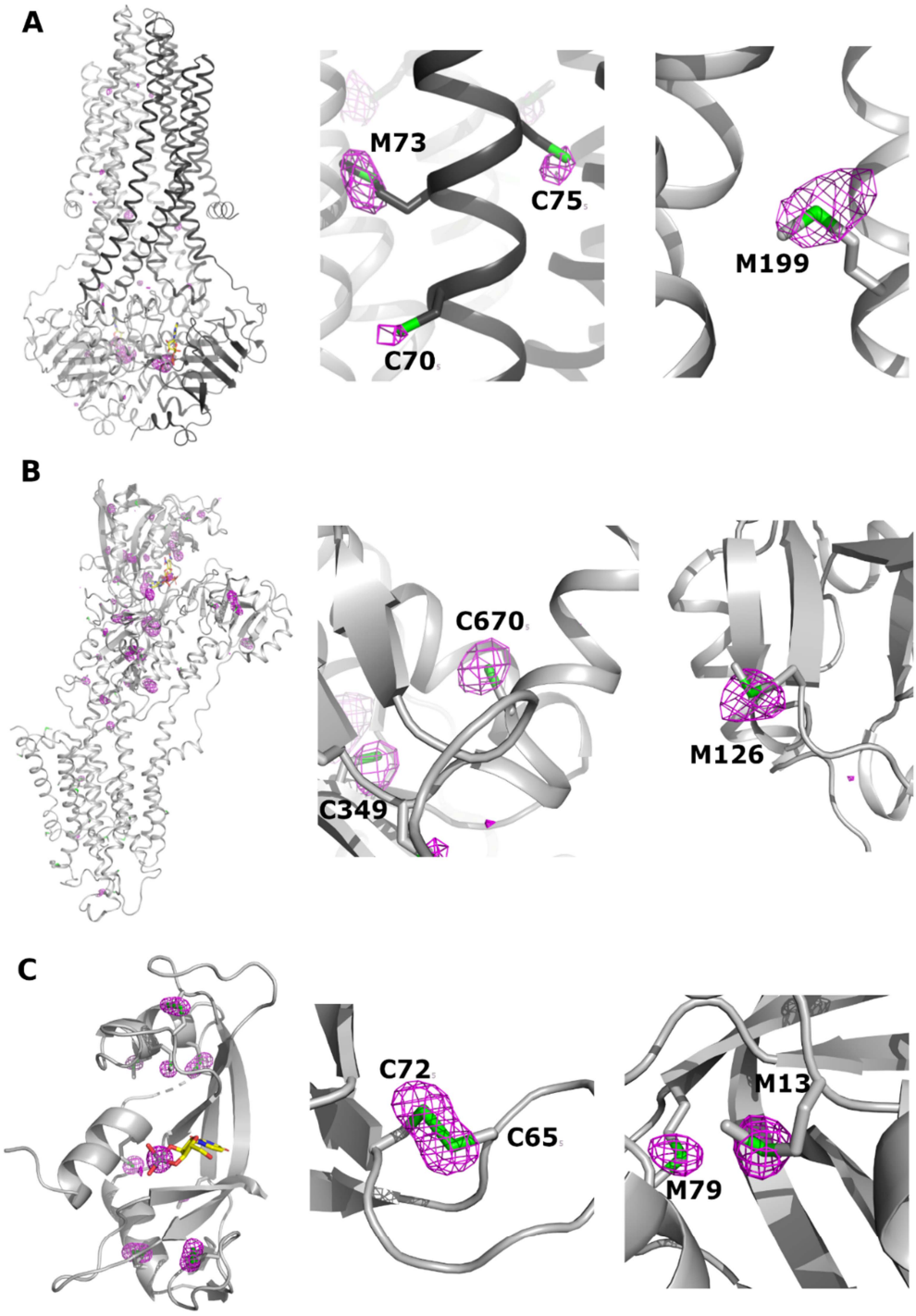

Figure S3 Examples of peaks in phased anomalous difference maps corresponding to sulphur atoms (magenta) calculated with ANODE (Thorn & Sheldrick, 2011). McjD (A), SERCA (B) and RNAse A (C) sulphur peaks are contoured at 3.5, 5 and 3.5 σ respectively.