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 $\lambda = 2.2604$ Å and $\lambda = 1.7711$ Å.

| Data collection | RNAse A 5485 eV | RNAse A 7000 eV |
|-----------------|-----------------|-----------------|
| Beamline        | DLS-I23         | DLS-I23         |
| Space group     | C2              | C2              |
| Unit cell (Å, °) | $a=100.4, b=32.9, c=72.5$ $\alpha=\gamma=90, \beta=90.4$ | $a=100.4, b=32.9, c=72.5$ $\alpha=\gamma=90, \beta=90.4$ |
| 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)         |
| $\langle I/\sigma(I) \rangle$ | 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 META-VANADATE                              | 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               | TRIH YDROXY][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/A (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 $\sigma$ respectively.