

Table S1. Data Collection and Refinement Statistics, Related to Figure 2

Data collection	
Energy (eV)	12,682.5
Resolution range (Å)	44.6 – 2.75 (2.80 – 2.75)
Unique reflections	49,988 (1,399)
Multiplicity	3.6 (3.3)
Data completeness (%)	98.1 (99.2)
R_{merge} (%) ^a	6.6 (74.0)
$I/\sigma(I)$	19.7 (1.5)
Wilson B-value (Å ²)	75.4
Refinement statistics	
Resolution range (Å)	44.6 – 2.75 (2.80 – 2.75)
No. of reflections $R_{\text{work}}/R_{\text{free}}$	49,937/2,484 (1,839/98)
Data completeness (%)	98.1 (69.0)
Atoms (non-H protein/ATP/Ca ²⁺)	11,864/93/3
R_{work} (%)	21.9 (38.4)
R_{free} (%)	27.2 (40.5)
RMSD bond length (Å)	0.004
RMSD bond angle (°)	0.66
Mean B-value (Å ²) (protein/ATP/Ca ²⁺)	93.4/63.0/77.8
Ramachandran plot (%) (favored/additional/disallowed) ^b	96.4/3.4/0.2
Maximum likelihood coordinate error	0.46
Missing residues, by chain	A: 335-338, 475-484; B: 332-338, 476-484; C: 1-5, 44-49, 325-326, 375; D: 1-4, 41-49, 375; E: 1-5, 39-54, 66-68, 375.

^a $R_{\text{merge}} = 100 \frac{\sum_h \sum_i |I_{h,i} - \langle I_h \rangle|}{\sum_h \sum_i I_{h,i}}$, where the outer sum (h) is over the unique reflections and the inner sum (i) is over the set of independent observations of each unique reflection.

^bAs defined by the validation suite MolProbity (Davis, I.W., Leaver-Fay, A., Chen, V.B., Block, J.N., Kapral, G.J., Wang, X., Murray, L.W., Arendall, W.B., Snoeyink, J., Richardson, J.S. and Richardson, D.C. (2007) MolProbity: all-atom contacts and structure validation for proteins and nucleic acids. *Nucleic Acids Res.* **35**, W375-W383.).

Data for the outermost shell are given in parentheses. The resolution limit stated for the structure is consistent with the resolution of the data used for structure refinement. The data completeness for the resolution shells was 99+% for all shells except for the final one (from 2.8 – 2.75 Å, the completeness was 69%, see table included below), and was 98.1% overall. Inclusion of this final shell of data improved both the appearance of the electron density map and the final refinement statistics, especially the Ramachandran plot. Inclusion of data with an average $I/\sigma(I) = 1.5$, even with a merging R-factor upwards of 99%, is considered acceptable if there is sufficient information content (Karplus and Diederichs).

Table S2. Actin Polymerization Activity of VopL Mutants, Related to Figure 5

VopL Construct	$t_{1/2}$ (s) at 50 nM
Actin control	1352 +/- 51
Wild type	41 +/- 10
Arm mutants	
K323E / R347E / R354E	300 +/- 12
D326G / V327G / P333G	132 +/- 6
Base mutants	
K421A / Y425A / R428D	112 +/- 17
Y425A / R428D	98 +/- 5
E408K / D413K / E417A	64 +/- 6