Supplemental Materials

Molecular Biology of the Cell

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Materials and Methods

Crystallization, data collection, and solving the crystal structure of Erk variants

Crystals of Erk2(WT) and Erk2(R65S) proteins were obtained by the microbatch method using the ORYX6 (Douglas Instruments Ltd.) automated crystallization system. Crystals appeared under two main conditions. The first contained protein solution (2.3-2.5 mg/ml) and 61 mM Ammonium Sulfate, 100mM Bis Tris pH 7.0, 12-15% (v/v) PEG 3350. The second contained 2.0-3.75 mg/ml protein, 61 mM Ammonium Sulfate, 100mM Bis Tris pH 7.0, 12-18% (v/v) PEG 4000. For data collection, crystals were soaked in cryoprotectant solution containing the crystallization solution and 15% glycerol and mounted on a MiTeGen cryoloop. Crystallographic data for Erk2(WT) and Erk2(R65S) were collected at 100 K using an Oxford Cryostream cooling device from a single crystal on an ADSC Quantum 315R CCD detector with an oscillation range of 1.0º at beam lines ID23-1 and ID14-4 at the European Synchrotron Radiation Facility (ESRF), Grenoble, France. The crystals belonged to the monoclinic space group $P2_1$ with one Erk2 molecule in the asymmetric unit. Data were integrated, reduced and scaled using the HKL suite (Otwinowski and Minor, 1997). Crystals of the Erk2(WT) and Erk2(R65S) complexed with the ATP analogue AMP-PNP (Adenosine 5′-(β,γ-imido)triphosphate tetralithium salt hydrate) (Sigma A2647) were obtained via soaking the compound into the crystals. For that crystals were transferred into a solution containing either 100mM Tris pH 7.4, 2mM $MgCl_2$, 10mM AMP-PNP for 4 hours, or 100mM Bis Tris pH 7.0, 60mM Ammonium Sulfate, 18% PEG 3350, 2mM $MgCl_2$, 10mM AMP-PNP for 16-18 hours. Prior to data collection crystals of the AMP-PNP complex were treated with a

cryoprotectant solution, similar to that described above, and subsequently flash frozen. Crystallographic data were collected at the ESRF at ID14-4. Data were integrated and scaled using the HKL suite. The structures of all proteins were solved via molecular replacement methods using Molrep (Murshudov *et al.*, 1997) implemented in CCP4i suite (Potterton *et al.*, 2003) using the Erk2 structure (1ERK) as the search model after removing all solvent molecules. Following molecular replacement, the models were refined using rigid body and then restrained options REFMAC5 (Murshudov *et al.*, 1997). Solvent molecules were added utilizing ARP/WARP (Lamzin and Wilson, 1993). The models were fitted into electron-density maps using the graphics program Coot (Emsley and Cowtan, 2004).

Table 1a: Data collection and current refinement statistics for Erk2(WT), and Erk2(R65S)

	Erk2	**Erk2(R65S)**
ESRF beamline	ID23-1	ID29
Wavelength (Å)	0.98	0.93
RCSB entry	4S31	4S2Z
Space Group	$P2_1$	$P2_1$
Unit Cell Parameters (Å)	a=48.8 b=70.2 c=60.7 β=109.6º	a=48.8 b=70.1 c=61.0 β=109.7º
Resolution range (last resolution shell)	50.0-1.45 (1.48-1.45)	50-1.48 (1.53-1.48)
Unique Reflections	66,668	60,954
Redundancy	3.7	4.3
Rsym(I)[a] (%)	5.0 (65.4)	5.7 (47.2)
Completeness	97.2 (95.3)	96.2 (84.3)
I/σ	40.9 (1.9)	44.4 (1.7)
Number of protein atoms	2823	2811
Number of solvent atoms	210	324
R-factor	20.8	19.5
R-free[b]	23.9	22.1
Protein	30.2	34.6
Solvent	34.2	42.4
Bond Length (Å)	0.013	0.014
Bond Angle (º)	1.63	1.71
Favored (%)	96.5	95.0
Allowed (%)	3.5	5.0
Disallowed (%)	0.0	0.0

[a]Rsym(I)=□|I-<I>|/□I., [b]Test set consists of 5% for all data.

Table 1b: Data collection and refinement statistics for Erk2(WT), and Erk2(R65S) in complex with PNP AMP-(ANP).

	Erk2-ANP	**Erk2(R65S)-ANP**
ESRF beamline	ID14-4	ID14-4
Wavelength (Å)	0.94	0.94
RCSB entry	4S32	4S33
Space Group	$P2_1$	$P2_1$
Unit Cell Parameters (Å)	a=48.96 b=70.0 c=59.8 □=109.0º	a=48.8 b=70.4 c=61.0 □=109.3º
Resolution range (last resolution shell)	50-1.35 (1.37-1.35)	50-1.48 (1.51-1.48)
Unique Reflections	83034	62605
Redundancy	4.7	4.5
Rsym(I)[a] (%)	6.0 (77.2)	4.4 (69.8)
Completeness	98.3 (97.0)	98.0 (96.0)
I/σ	51.6 (1.8)	41.6 (1.7)
Number of protein atoms	2818	2811
Number of solvent atoms	212	222
Number of ANP-PNP atoms	23	27
Number of sulfate atoms	5	5
Number of Mg atoms	1	1
R-factor	21.1	19.6
R-free[b]	24.1	23.9
Protein	32.1	30.8
Solvent	37.5	36.3
ANP-PNP	40.1	30.5
Sulfate	31.6	42.0
Mg	35.3	29.6
Bond Length (Å)	0.013	0.014
Bond Angle (º)	1.54	1.76
Favored (%)	96.5	95.3
Allowed (%)	3.5	4.7
Disallowed (%)	0.0	0.0

[a]Rsym(I)=□|I-<I>|/□I., [b]Test set consists of 5% for all data.

References

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