Appendix

Materials and Methods

1. Preparation of Crystals of CHK2 bound to CCT241533

CCT241533 was resuspended in 100% DMSO to give a 50 mM solution. A 10 mM 'stock solution' was then generated from this by dilution in a buffer containing 100 mM HEPES-NaOH pH 7.5 and DMSO (at varying concentrations between 0 and 25 % (v/v), sufficient to keep the compound in solution). Crystals of CHK2 bound to inhibitor were produced using 25 µl of purified CHK2 kinase domain (CHK2-KD), at a concentration of 13.5 mg/ml mixed with 1 to 2.5 µl of the compound 'stock solution'. This complex was crystallised at 4˚C, in hanging-drop vapor diffusion experiments, by mixing 1 µl of the complex with 1 µl of precipitant containing 0.1M HEPES-NaOH pH 7.5, 0.2 M $Mg(NO_3)_2$, 10 % v/v ethylene glycol, 10 mM DTT and 8 to 16 % w/v PEG 3350. Streak-seeding into drops containing low concentrations of PEG 3350 (8% - 12%) was necessary to produce crystals of sufficient quality for data collection. Crystals were cryo-protected by a rapid swipe through a solution containing 0.1 M HEPES-NaOH pH 7.5, 0.2 $Mg(NO_3)_2$, 0.2 M NaCl, 10 mM DTT and 20% v/v ethylene glycol, before being plunge-frozen in liquid nitrogen.

Diffraction data were collected at the Diamond Light Source (Didcot, UK) on station I03. Data were collected from a single crystal at 100 K. Diffraction images were integrated using MOSFLM (1) and reduced/scaled using programs from the CCP4 suite (2). Structures were solved by molecular replacement using

PHASER (3) with the X-ray structure of CHK2-KD (PDB: 2CN5) as a search model. Difference maps were used to identify and model the position of the bound compound. Iterative cycles of refinement using the PHENIX suite (4) and manual model building in Coot (5) produced the final model. Crystallographic statistics are given in Table S3.

References

1. Leslie A. MOSFLM Users Guide. Cambridge, UK.: MRC Laboratory of Molecular Biology.; 1995.
2. The CCP4 suite: programs for protein crystallography. Acta Crystallogr D Biol Crystallogr 1994; 50: 760-3.
3. McCoy AJ, Grosse-Kunstleve RW, Storoni LC, Read RJ. Likelihood-enhanced fast translation functions. Acta Crystallogr D Biol Crystallogr 2005; 61: 458-64.
4. Adams PD, Grosse-Kunstleve RW, Hung LW, et al. PHENIX: building new software for automated crystallographic structure determination. Acta Crystallogr D Biol Crystallogr 2002; 58: 1948-54.
5. Emsley P, Cowtan K. Coot: model-building tools for molecular graphics. Acta Crystallogr D Biol Crystallogr 2004; 60: 2126-32.