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Crystallization of the focal adhesion kinase targeting (FAT) domain in a primitive orthorhombic space group

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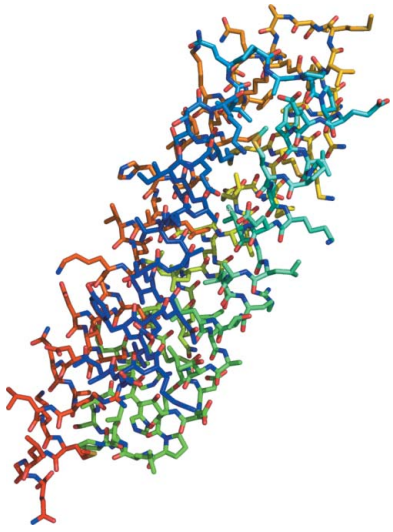


X-ray diffraction data from the targeting (FAT) domain of focal adhesion kinase (FAK) were collected from a single crystal that diffracted to 1.99 Å resolution and reduced to the primitive orthorhombic lattice. A single molecule was predicted to be present in the asymmetric unit based on the Matthews coefficient. The data were phased using molecular-replacement methods using an existing model of the FAK FAT domain. All structures of human focal adhesion kinase FAT domains solved to date have been solved in a *C*-centered orthorhombic space group.

1. Introduction

Focal adhesion kinase is a tyrosine kinase that is involved in cell adhesion, migration and signaling (Frisch *et al.*, 1996; Gilmore & Romer, 1996; Shen & Schaller, 1999) and is known to be over-expressed in a diverse set of cancers prior to metastasis (Weiner *et al.*, 1993). There is evidence that focal adhesion kinase plays a key role in cell motility (Cary *et al.*, 1996) and regulation of cellular apoptosis (Kurenova *et al.*, 2004). Focal adhesion kinase consists of 879 residues and exhibits three distinct structural domains: the FERM domain, the kinase domain and the focal adhesion targeting (FAT) domain. The FAT domain is both necessary and sufficient for both cell adhesion-dependent regulation and downstream signaling (Shen & Schaller, 1999). Targeting this domain may provide the basis for a novel therapy to treat a diverse set of malignancies. In this paper, we describe methods of crystallizing and phasing a novel form of the FAT domain of FAK in a primitive orthorhombic space group.

2. Materials and methods

2.1. Purification of FAK

The construct was transformed into *Escherichia coli* BL21 (DE3) strain for IPTG-induced expression. A 1 l culture was grown to an OD_{600} of 0.6 at 310 K and 500 m*M* IPTG was added to a final concentration of 1 m*M*. The culture was grown for an additional 5 h at 310 K. Cells were lysed, sonicated and centrifuged prior to purification using GST-agarose beads. The FAT domain of FAK was cleaved from GST using thrombin according to an established protocol (Waas & Dalby, 2007). Cleavage with thrombin resulted in a polypeptide consisting of a six-amino-acid spacer, GSPEFR, followed by human FAK beginning at residue 919. Electron density was not present for the spacer residues and was present beginning at residue 921 of human FAK. Residual GST was removed using a cation-exchange column (HiPrep SP/XL 16/10 column). Cleaved protein was loaded in 25 m*M* sodium phosphate buffer pH 5.5 and eluted with a step gradient using 25 m*M* and 1.0 *M* NaCl. Fractions containing the FAT domain of FAK were dialyzed into 10 m*M* Tris pH 8.0, 50 m*M* NaCl and concentrated to 10 mg ml^{-1} using a centrifugal filter device (5 kDa molecular-weight cutoff; Millipore).

2.2. Preparation of crystals

Focal adhesion kinase FAT domain crystals were grown using the hanging-drop vapor-diffusion method (McPherson, 1999). 4 µl drops

consisting of 2 µl 10 mg ml^{-1} protein solution and 2 µl well solution were micropipetted on siliconized slides and inverted over wells containing a 700 µl reservoir of well solution. The protein was initially screened using Hampton Crystal Screens 1 and 2 and protein crystals were observed in 0.2 *M* zinc acetate dihydrate, 0.1 *M* sodium cacodylate trihydrate pH 6.5, 18%(*w*/*v*) polyethylene glycol 8000. After optimization, maximally sized crystals of the FAK FAT domain were obtained in approximately 21 d under the following conditions: 0.4 *M* zinc acetate, 0.1 *M* sodium cacodylate pH 6.4 and 23%(*w*/*v*) polyethylene glycol 8000. All crystals were grown at 295 K.

2.3. X-ray data collection

Data collection was perfomed on beamline X6A of the National Synchrotron Light Source at Brookhaven National Laboratory, Upton, New York, USA. An Oxford cryosystem was used to flash-freeze the crystals in gasous nitrogen in order to minimize radiation damage from the high-intensity X-rays. The diffractometer on beamline X6A was a single-axis CrystalLogic coupled with an ADSC 210 CCD detector and a Kappa goniometer head. X-ray wavelengths were filtered using a Si(111) channel-cut monochromator and focused with an Oxford Danfysik toroidal mirror.

The selected FAK FAT-domain crystal was cryoprotected in mineral oil and flash-cooled prior to data collection. Data collection proceeded at a wavelength of 0.9322 Å over 150° of rotation, with each 1° frame consisting of 60 s X-ray exposure. *HKL*-2000 (Otwinowski & Minor, 1997) was used to process and scale the resulting 150 frames of data and *XPREP* (Sheldrick, 1991) was used to analyze the resulting list of intensities and confirm the space group. The *Matthews Probability Calculator* (Kantardjieff & Rupp, 2003; Matthews, 1968) was used to predict the number of molecules in the asymmetric unit based on the unit-cell volume and the molecular weight of the protein.

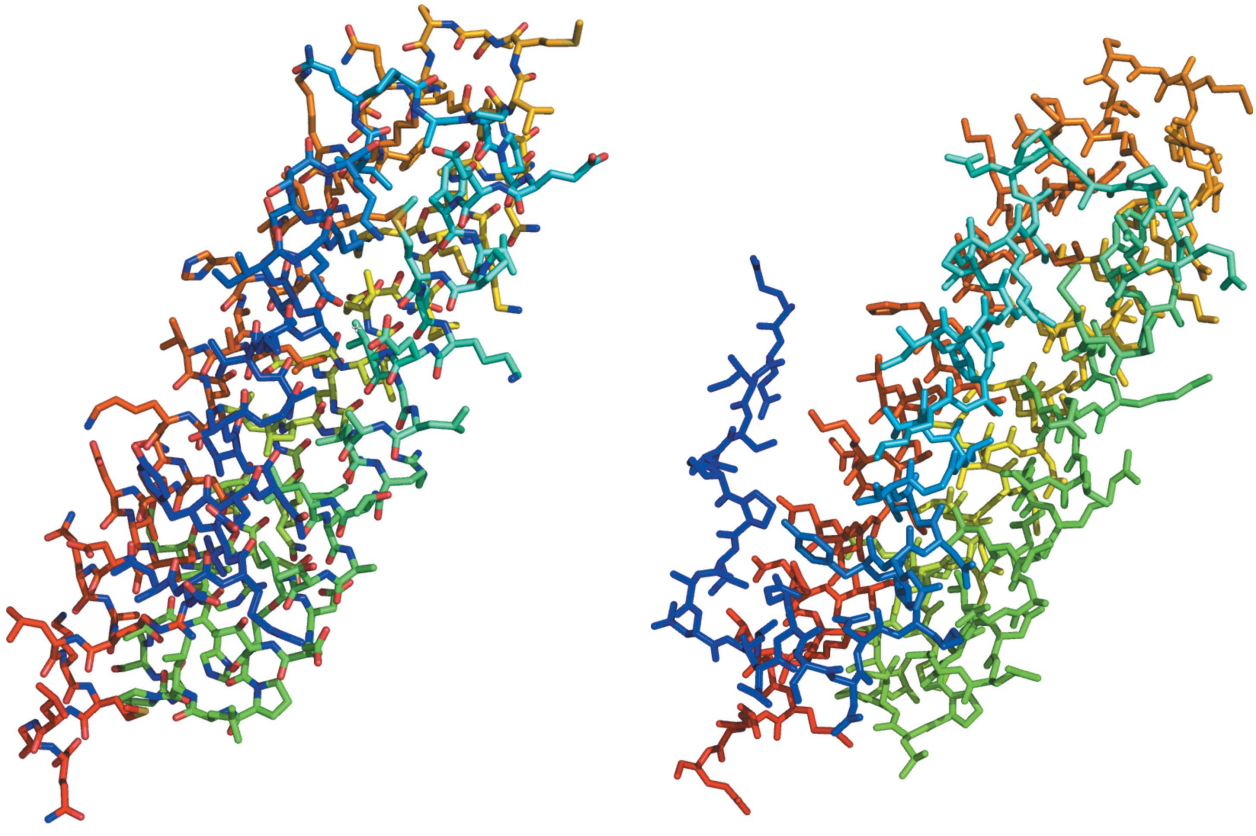
Table 1
Data-collection and reduction statistics.

Values in parentheses are for the highest resolution shell.

No. of frames	150
Crystal-to-detector distance (mm)	180
Exposure (s)	60
Wavelength (Å)	0.9322
Unique reflections	8673
Redundancy	5.9 (6.1)
Resolution range (Å)	30.0–1.99 (2.01–1.99)
Space group	$P2_12_12_1$
Unit-cell parameters (Å, °)	a = 48.246, b = 50.289, c = 49.532, $\alpha = \beta = \gamma$ = 90.0
Oscillation step (°)	1
Mosaicity (°)	0.534
$I/\sigma(I)$	20.6 (3.0)
Reflections >3σ (%)	75.5 (42.3)
Completeness (%)	100.0 (100.0)
Unit-cell volume (Å^3)	120177.2
V_M (Å^3 Da^{-1})	2.27
Solvent (%)	46
No. of molecules in ASU	1

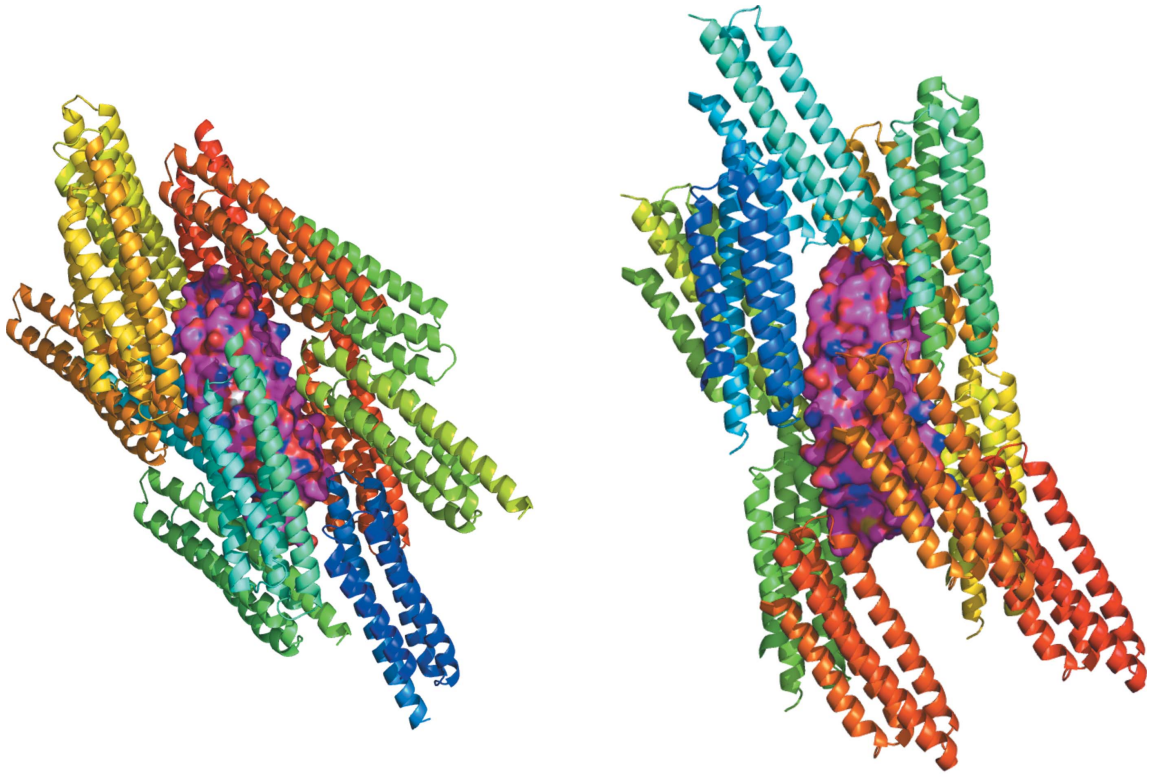
2.4. Phasing

Phasing of the resulting data set was performed by molecular replacement using the *CCP*4 program *MOLREP* (Collaborative Computational Project, Number 4, 1994) to a resolution of 1.99 Å. A



(*a*) (*b*)

Figure 1
(*a*) Primitive orthorhombic focal adhesion kinase FAT domain (unrefined) from this experiment; (*b*) *C*-centered orthorhombic FAK FAT domain (PDB code 1k04) containing the N-terminal loop region. Images created with *PyMOL* (DeLano, 2002).



(*a*) (*b*)

Figure 2
Crystal packing of (*a*) the human FAK FAT domain in a primitive orthorhombic space group ($P2_12_12_1$) and (*b*) the mouse FAK FAT domain in a *C*-centered monoclinic space group (*C*2; PDB code 1k40). Images created with *PyMOL*.

solution was identified which gives rotation and translation function peaks 11.34/σ and 20.75/σ, respectively. The top solution for the rotation and translation function yielded a solution with an initial *R* factor of 0.414 and score (defined by product of correlation coefficient and maximum value of packing function) of 0.707.

3. Results and discussion

Single crystals of the focal adhesion kinase FAT domain were used for data collection. A single crystal diffracted to 1.99 Å and was indexed in a primitive orthorhombic space group, with unit-cell parameters $a = 48.246$, $b = 50.289$, $c = 49.532$ Å. The unit cell belonged to space group $P2_12_12_1$. The Matthews coefficient was computed using the *Matthews Probability Calculator* as 2.27 Å^3 Da^{-1}, indicating the presence of a single molecule in the asymmetric unit. The solvent content was predicted to be 46% with a unit-cell volume of 120 177.2 Å^3 and a molecular weight of 14 244.71 Da (Table 1).

The structure of human focal adhesion kinase FAT domain has previously been solved in a *C*-centered orthorhombic space group ($C222_1$). The original model used for phasing by molecular replacement was PDB entry 1k04 (Arold *et al.*, 2002). Molecular-replacement methods yielded the single predicted molecule in the asymmetric unit. As shown in Fig. 1, the 15-residue loop region (residues 906–920) of the FAK FAT domain was omitted from the protein in this experiment. It could therefore be suggested that this N-terminal loop region of the $C222_1$ FAK FAT domain determined the crystal space group, as this region could sterically prevent the tight packing seen in the primitive orthorhombic crystal structure. Another human FAK FAT domain (PDB code 1k05; Arold *et al.*, 2002) also crystallized in space group $C222_1$ with this N-terminal loop. It should be noted that the mouse FAK FAT domain, which is 98% identical to the human homolog, crystallized in space group *C*2 in the absence of this loop region (Fig. 2; Hayashi *et al.*, 2002).

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