SUPPLEMENTARY INFORMATION

Dimerization controls activation of kinase-dependent functions of FAK at focal adhesions

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Supplementary Methods

Surface Plasmon Resonance (SPR)

GST-FERM or FAT was covalently coupled to a CM5 sensor chip (GE Healthcare) via its primary amine groups. The carboxymethylated dextran surface was activated by injection of a mixture of 0.2 M 1-ethyl-3- (3-dimethylaminopropyl) and 0.05 M N-hydroxysuccinimide. Binding assays were performed at 25°C with a Biacore 3000 (Biacore, Inc., IFR83 core facility) with 100 mM HEPES, pH 7.4, containing 150 mM NaCl, 3 mM EDTA and 0.005% (vol/vol) P20 surfactant (GE Healthcare) as the running buffer. GST-FERM, FAT, and His<sub>6</sub>-FAK were injected at various concentrations and at a 5 µl/min flow rate. The surface was regenerated with a 30 sec pulse of 10 mM glycine chloride, pH 2.0, with a flow rate of 30 µl/min or 50 mM NaOH, 1 M NaCl with a flow rate of 10 µl/min. Identical injections over a blank surface were done in parallel and they gave a value of 0 RU. The association time was 5 min with a flow rate of 5 µl/min and dissociation was observed during 10 min with the same flow rate before the surface was regenerated. Kinetic evaluation and K<sub>d</sub> determination were done with the manufacturer’s software (BIAevaluation version 4, Biacore). The kinetic evaluation curves were fitted by using five to seven different protein concentrations.

Isothermal Titration Calorimetry (ITC)

For the FERM-FAT affinity measurements, recombinant proteins were dialyzed in degassed buffer (20 mM HEPES at pH 7.5, 150 mM NaCl, 2 mM βME, and 2 mM EDTA) and FERM–FAT titrations were performed on an iTC200 MicroCalorimeter (MicroCal). To do so, 90 µM FAT (in the 40 µl syringe) was injected in 2-3 µl injections into 9 µM FERM (in the 200 µl cell) at 25°C. Control experiments consisted in injecting 90 µM FAT into the buffer from the last dialysis step.

Analytical Ultracentrifugation (AUC)

Sedimentation equilibrium AUC was performed at 20 °C using a Beckman XL-I instrument with an AnTi60 rotor equipped with sample cells using Epon double sector centerpieces and sapphire windows. A protein sample (175 µl) containing 30 µM FERM protein dissolved in 20
mM Tris-HCl, 200 mM NaCl, 2 mM DTT and 2 mM EDTA at pH 8.0 was centrifuged for 48 hours at three rotor speeds: 9000 r.p.m., 14000 r.p.m. and 17000 r.p.m. The protein partial specific volume, solvent density and solvent viscosity were calculated using Sednterp 1.09 (Laue et al. 1992). Data analysis was performed using Sedphat 10.40 (Schuck 2003). The sedimentation equilibrium profiles were globally fitted to a monomer-dimer equilibrium model using mass conservation and rotor stretch restraints.

**X Ray Crystallography**

hFERM31-405;F85L,W181G crystals were obtained at room temperature using the hanging drop method by mixing 1 l of protein solution at 9 mg/ml and 1 l of reservoir solution containing 15-19% PEG 10,000, 0.1M Ammonium citrate, 0.1M Bis-Tris, pH 5.5. Crystals were cryoprotected in 25% (v/v) glycerol and flash-cooled to 100K. Data up to 2.8 Å-resolution were collected at the Advanced Light Source, beamline 8.3.1. The structure was determined by molecular replacement using avian FERM (2AL6). The model will be deposited in the PDB.

**SAXS Analysis**

Data were collected at beamline X33 at DESY, EMBL (Hamburg, Germany) at 10°C, λ = 1.5 Å. Data analysis, *ab initio* shape calculations, and rigid-body refinement were performed using the ATSAS program suite (Konarev et al. 2006). FAK samples were centrifuged at 100,000 g for 6 min and supplemented with 5 mM DTT before x-ray exposure. Only FAK concentrations of 0.5-1 mg/ml were used, due to FAK’s tendency to aggregate at higher concentrations. We recorded SAXS curves for momentum transfer values of \( q = 4\pi \sin \theta / \lambda \) between 0.05–5.0 nm\(^{-1}\). Since the presence of protease inhibitors hampered the determination of FAK concentrations by precise spectroscopic techniques, we had to use the less-precise Bradford assays. Because of the resulting uncertainty in the FAK concentration, the extrapolated zero-angle scattering intensity, \( I_0 \), value of the measurements was not used to deduce the oligomeric state of FAK. The Guinier region of the SAXS plot showed only a minor sign of aggregation and indicated a radius of gyration \( (R_g) \) of about 8.25 nm. Subsequent analysis with GNOM using data for a range of \( q = 0.12–1.56 \text{ nm}^{-1} \) yielded a maximum diameter \( (D_m) \) of 26 nm and an \( R_g \) of 8 nm.
We used DAMMIF to obtain *ab initio* sphere models of FAK in solution, because DAMMIF reconstructions do not require information about the number of residues of the solute species. Twenty individual *ab initio* shape calculations with DAMMIF gave similar elongated structures (Figure S1A), with a normalized spatial discrepancy [NSD] = 0.94 ± 0.034. For the *ab initio* shape reconstruction, we used an iterative procedure in which 20 individual DAMMIF envelopes were calculated and then aligned and averaged. The space occupied by the ensemble of aligned structures was then used as a starting volume for a subsequent round of 20 DAMMIF models. The procedure converged after the fourth iteration from the initial averaged shape (Supplementary Figure 3a, $\chi = 1.5$) into the shape shown in Supplementary Figure S3b ($\chi = 1.01$).

Although clearly similar in shape to the FAK FERM-kinase dimer constructed based on the crystal structure of the FAK FERM-kinase fragment (Lietal. et al. 2007), the SAXS envelope appeared to be slightly larger than the crystallographic dimer. Shrinking of the SAXS shape yielded a very good fit of the two FERM-kinase fragments into the two halves of the SAXS envelope (Figure 4a). As the size of an object in SAXS is determined by the Guinier region of the SAXS pattern, we reasoned that residual aggregation in the sample had tailed out into the Guinier region, leading to a model that was slightly bigger than the FAK species in solution. Our attempts to eliminate the residual aggregation of FAK (by varying the purification protocol, SAXS beamline used, SAXS sample preparation and buffer conditions) failed to yield improved experimental data. Because aggregation mostly affects the very low resolution of the SAXS pattern, we produced another series of *ab initio* models from data that were cut off below a resolution of $q = 0.283$ nm$^{-1}$. After iterative averaging with DAMMIF, the resulting shape showed the same features as the bead model produced using $q = 0.12$–1.56 nm$^{-1}$, however its size corresponded to that of the crystallographic FAK-FERM dimer (data not shown). This supported the possibility that the SAXS pattern below a resolution of $q = 0.283$ nm$^{-1}$ was affected by residual aggregation, which resulted in a slight overestimation of the size of the species in solution. Consequently, data between $q = 0.283$ and 1.56 nm$^{-1}$ were used for full-length FAK reconstructions (using BUNCH) to exclude artifacts due to residual aggregation (Supplementary Figure S3c).

BUNCH runs were also performed assuming only monomeric FAK. However, these runs failed to yield models that fitted the data well. In particular, even when put into an
extended conformation, the monomeric models were too small to fit the SAXS data. To investigate whether a monomer-dimer mixture would fit the SAXS data better, we used the program OLIGOMER (Konarev et al. 2006) to estimate the monomer-dimer content. Using as an input assembled monomeric and dimeric models of FAK as well as a monomeric model that was produced by BUNCH with FAT in a detached position, OLIGOMER determined that the SAXS sample consisted of 93 % dimeric FAK and only 7 % monomeric (open) FAK.

**Construction of FAK Models Correlated with Experimental Rs**

Ten models were built for each state (FAK monomer or dimer with FAT and/or kinase domain bound to FERM or freely protruding in the solvent) using EOM and BUNCH (Konarev et al. 2006), and their Rs and radius of gyration (Rg) were calculated through the bead shell modeling technique with HYDROPRO (Garcia De La Torre et al. 2000) and with CRY Sol (Konarev et al. 2006), respectively. The particle hydrated volume was calculated from the molecular weight of the monomer or dimer, with partial specific volume (0.74 cm³/g) and estimated hydration (0.41 g water/g protein) obtained from the amino acid sequence by the SEDNTERP software (Laue et al. 1992). The radius of the atomic element in HYDROPRO was fitted in order to adjust the volume of the model to that calculated previously for the whole particle.

**References for Supplementary Methods**


