Supplemental Material

Supplementary Figure 1. Small angle x-ray scattering (SAXS) data of the Rb pocket domain demonstrate that solution conformations are consistent with crystal structures both in the absence and presence of E2F[TD]. To test the sensitivity of the SAXS data to conformational changes in Rb, a normal modes analysis was used to generate realistic models of altered structures. The calculated scattering curves from these models demonstrate the sensitivity of SAXS to even small conformational changes and here provide additional support that the solution and crystal structures are similar. (A) Experimental scattering profile for the crystallization pocket construct after cleavage with thrombin (light blue squares with error bars) and comparison to model curves calculated from the crystallographic model using normal modes analysis (grey lines). The calculated scattering curves agree with the data with Chi squared values ranging from 0.7 (yellow line) and 2.0. The curve calculated from the crystal structure itself (blue) agrees with a Chi squared of 1.0. (B) Aligned structures of the pocket domain resulting from normal modes analysis of the unliganded crystal structure. The extreme conformations of each mode are shown (grey) along with the conformation that best fits the SAXS data (yellow) and the crystal structure (blue). (C) Experimental scattering profile for the uncut crystallization pocket construct bound by E2F[TD] (light pink circles with error bars) and comparison to model curves calculated from the crystallographic model (PDB: 1N4M) using normal modes analysis (grey lines). The calculated scattering curves agree with the data with Chi squared values ranging from 1.6 (yellow line) and 2.9. The curve calculated from the crystal structure itself (red) agrees with a Chi squared of 1.7. (D) Aligned structures resulting from normal modes analysis of the pocket-E2F[TD] complex crystal structure. The extreme conformations of each mode are shown (grey) along with the conformation that best fits the SAXS data (yellow) and the crystal structure (pink).

Supplementary Figure 2. Comparison of lattice packing in Rb crystal structures. The variability of position and extent of surface area involved in crystal contacts suggests that crystal environment does not significantly contribute to the observed similarity between unliganded and ligand-bound Rb structures. Models of Rb unliganded (A and B, blue) and bound to the E2F[TD] (C, pink) and E7 LxCxE peptides (D, gold) are shown. A and B correspond to the two molecules in the asymmetric unit of the unliganded crystal. Atoms involved in crystal contacts are shown as spheres. Crystal contacts were analyzed using the CCP4 program AREAIMOL.[1]

Supplementary Methods

Protein samples were prepared for SAXS by loading onto a Superdex200 size-exclusion chromatography column equilibrated in 100 mM NaCl, 25 mM Tris, 0.4 mM TCEP, and 2% glycerol (pH 8.0). In each case, the peak fraction was collected and used directly for scattering experiments. The concentration of the sample cut with thrombin was 3 mg/mL, and the concentration of the uncut sample with E2F[TD] was 8 mg/mL. The E2F2 transactivation domain (409-427) was added at a 3-fold molar excess prior to the Superdex200 column (K_d ~ 100 nM). SAXS data were collected on Beamline 12.3.1 at the Advanced Light Source (Lawrence Berkeley National Laboratories).[2] The scattering vector q = $4\pi\sin\theta/\lambda$, in which 2θ is the scattering angle and λ is the x-ray wavelength. Complete scattering profiles were constructed as described by merging large q data from

long exposures (6 sec) with small q data from short exposures (0.5 sec) on diluted samples.[2,3] The radius of gyration determined for each sample was similar for three different dilutions, indicating that the protein is monodisperse in the conditions used for data collection.[3] Normal modes analysis was performed using the ElNemo (Elastic Network Model) webserver with five modes and default values for the amplitudes of perturbation.[4] The resulting structures used for the calculation of model scattering curves corresponded to the extreme perturbations for each mode. Scattering profiles were calculated from model structures and fit to the experimental data using the FoXS web server.[5] The Chi squared value is calculated as

$$\sum_i \left((I_{exp}(q_i) - cI_m(q_i)) / \sigma_{exp}(q_i) \right)$$

in which I_{exp} and I_m are the experimental and calculated scattering intensities respectively, σ_{exp} is the experimental error for each measurement, and c is a constant overall scaling factor.

Supplemental References

1. Bailey S. The Ccp4 Suite - Programs for Protein Crystallography. Acta Crystallographica Section D-Biological Crystallography 1994;50:760-763.
2. Hura GL, Menon AL, Hammel M, Rambo RP, Poole FL, 2nd, Tsutakawa SE, Jenney FE, Jr., Classen S, Frankel KA, Hopkins RC, Yang SJ, Scott JW, Dillard BD, Adams MW, Tainer JA. Robust, high-throughput solution structural analyses by small angle X-ray scattering (SAXS). Nat Methods 2009;6(8):606-612.
3. Putnam CD, Hammel M, Hura GL, Tainer JA. X-ray solution scattering (SAXS) combined with crystallography and computation: defining accurate macromolecular structures, conformations and assemblies in solution. Q Rev Biophys 2007;40(3):191-285.
4. Suhre K, Sanejouand YH. ElNemo: a normal mode web server for protein movement analysis and the generation of templates for molecular replacement. Nucleic Acids Research 2004;32:W610-W614.
5. Schneidman-Duhovny D, Hammel M, Sali A. FoXS: a web server for rapid computation and fitting of SAXS profiles. Nucleic Acids Res;38(Web Server issue):W540-544.