Supporting Information

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SI Materials and Methods

DNA Construct and Protein Production. DNA sequences for the wild type and all four designs were synthesized by GenScript USA and cloned into the pQE-80L vector as 6×-His-maltose-binding protein (MBP) fusions as described previously (1). All proteins were expressed in BL21(DE3) pLysS cells induced with 0.3 mM IPTG overnight at 18 °C. The proteins were purified by immobilized-nickel affinity chromatography and then cleaved from 6×-His-MBP with tobacco etch virus protease. The cleaved proteins were again subjected to immobilized-nickel affinity chromatography to trap the 6×-His-MBP. Flow-through from the nickel column was then further purified with size-exclusion chromatography (Superdex 75) in buffer A (20 mM MES, pH 6.0, and 150 mM NaCl). Protein concentration was quantified based on absorbance and a predicted extinction coefficient (ExPASy; ProtParam) of 8,480 M^{-1} cm^{-1} for wild type and βdimer4, 13,980 M^{-1} cm^{-1} for βdimer1, 12,490 M^{-1} cm^{-1} for βdimer2, and 9,970 M^{-1} cm^{-1} for βdimer3.

Multiangle Light Scattering. Samples of βdimer1, βdimer3, and the wild-type protein were concentrated to approximately 300 µM (4 mg/mL) in buffer A and injected onto a WTC-030S5 size-exclusion column (Wyatt Technologies) connected to a multiangle light scattering instrument (DAWN HELEOS II; Wyatt Technologies) and a refractometer (OPTILAB rEX; Wyatt Technologies). Molecular mass of particles in a single elution peak was calculated based light scattering data using the ASTRA software package (Wyatt Technologies).

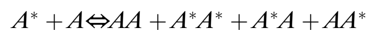
Analytical Ultracentrifugation Sedimentation Equilibrium. Sedimentation equilibrium experiments were performed using a Beckman XL-I analytical ultracentrifuge using six-sector cells and an An-50 Ti rotor. Samples of wild-type protein, βdimer1, and βdimer3, at concentrations of 20, 40, and 60 µM in buffer A, were spun at 46,400 × g until equilibrium was reached. Absorbance measurements at 280 nm were taken every 2 h. The absorbance offset was found by meniscus depletion after spinning the samples at 163,300 × g for 6 h. The sedimentation equilibrium data were analyzed with XL-I data analysis software.

The homodimer dissociation constant was measured in a similar fashion to the method outlined above. The βdimer1 was placed at in the sample cells at concentrations of 0.8, 1.5, and 2.0 µM in 20 mM KH_2PO_4, pH 7.0 and 150 mM NaCl. The absorbance was measured at 215 nm to obtain readings sufficiently above background to reliably fit the data. The data were analyzed using a monomer-dimer equilibrium model.

Fluorescence Polarization Assay. A variant of βdimer1 with the mutation S62C was produced for labeling with thiol reactive Bodipy (507/545)-iodoacetamide (Molecular Probes). The labeling procedure was performed as previously described (1). Buffer A supplemented with 5 mM β-mercaptoethanol was used as the binding buffer for the titrations. Bodipy-labeled βdimer1, at a concentration of 2 nM, was placed in a 1-cm path length cuvette and titrated with unlabeled protein. The change in fluorescence polarization was measured using a Jobin Yvon Horiba Spex FluoroLog-3 instrument (Jobin Yvon, Inc.). The data were analyzed according to a homodimerization model (given below) and fit with Prism (GraphPad Software).

Homodimerization Fluorescence Polarization Fitting Procedure. We derived a homodimerization model to be used when fitting the fluorescence polarization data. This model accounts for the interaction of a protein A with itself in its labeled (A^*) and unlabeled states (A). The model was derived as follows where P is the total amount of protein in a given state:

$$A + A \Leftrightarrow AA$$



$$A^* + A \Leftrightarrow AA + A^*A^* + A^*A + AA^*$$

$$K_d = \frac{[A]^2}{[AA]}$$

$$[P_{total}] = [A^*_{total}] + [A_{total}]$$

$$[P_{monomer}] = [A^*_{monomer}] + [A_{monomer}]$$

$$[P_{dimer}] = [A^*A^*] + [A^*A] + [AA^*] + [AA]$$

$$[P_{dimer}] = \frac{[P_{total}] - [P_{monomer}]}{2}.$$

Solving for the total concentration of monomeric protein gives

$$[P_{monomer}] = \frac{-K_d + \sqrt{K_d^2 + 8[P_{total}]K_d}}{4}.$$

Any change in signal seen would come from association of a labeled and unlabeled protein. We assume that the interactions between labeled and labeled is negligible because labeled protein is present in low concentrations.

$$[A^*A] = [AA^*] = [P_{dimer}]\frac{[A^*_{total}] + [A_{total}]}{[P_{total}]}$$

This model is then written to fit the change in polarization:

$$Pol_{obs} = (Pol_{max} - Pol_{min})\frac{[A^*A] + [AA^*]}{[A_{total}]} - Pol_{min}.$$

A protocol to fit data with this model in Prism is available upon request.

Crystallization and Structure Refinement. Crystallization of βdimer1 was performed using the hanging-drop vapor diffusion method at 20 °C. Crystals formed after one week in a drop consisting of 2 µL of βdimer1 (7 mg/mL in buffer A) and 1 µL of well solution [100 mM sodium acetate, pH 5.0, 6% (vol/vol) isopropanol, 20% (wt/vol) PEG 8000]. Prior to data collection, crystals were cryoprotected by transferring them into well solution supplemented with 15% (vol/vol) ethylene glycol before plunging them into liquid nitrogen. The crystals diffracted X-rays to a minimum Bragg spacing of about 1.0 Å, exhibited the symmetry of space group $P2_1$ with cell parameters of $a = 50.6$ Å, $b = 44.3$ Å, $c = 53.0$, $\beta = 91.91°$, and contained two molecules in the asymmetric unit (solvent content, 44%). Diffraction data were collected at 100 K at a wavelength of 0.91840 Å at the Advanced Proton Source General Medicine and Cancer institutes Colla-

borative Access Team 23IDB beamline. The diffraction data were indexed and reduced using HKL2000 (2).

The structure of βdimer1 was determined by molecular replacement using the program Phaser (3); the computationally designed dimer of βdimer1 was used as a search model. Iterative rounds of refinement were conducted with REFMAC (4) and PHENIX (5), interspersed with manual adjustments to the model using the program Coot (6). The final model contains two molecules in the asymmetric unit with all residues defined in the electron density, except for residues 23–26 in both molecules.

1. Jha RK, et al. (2010) Computational design of a PAK1 binding protein. *J Mol Biol* 400:257–270.
2. Otwinowski Z, Minor W (1997) Processing of X-ray diffraction data collected in oscillation mode. *Methods Enzymol* 276:307–326.
3. McCoy AJ, et al. (2007) Phaser crystallographic software. *J Appl Crystallogr* 40:658–674.
4. Murshudov GN, et al. (2011) REFMAC5 for the refinement of macromolecular crystal structures. *Acta Crystallogr D Biol Crystallogr* 67:355–367.
5. Adams PD, et al. (2010) PHENIX: A comprehensive Python-based system for macromolecular structure solution. *Acta Crystallogr D Biol Crystallogr* 66:213–221.
6. Emsley P, Cowtan K (2004) Coot: Model-building tools for molecular graphics. *Acta Crystallogr D Biol Crystallogr* 60:2126–2132.

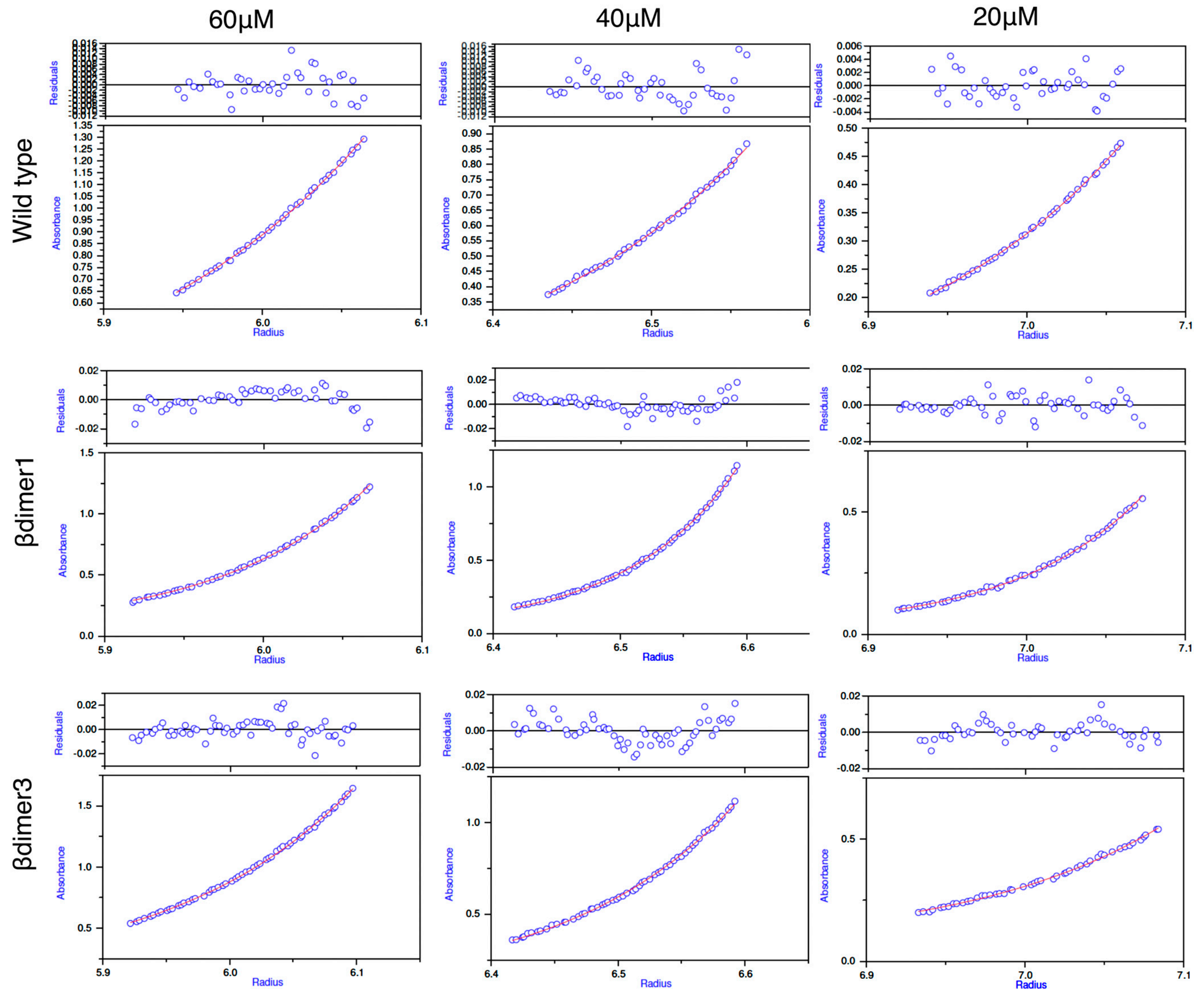


Fig. S1. Determination of molecular mass of wild type, βdimer1, and βdimer3 by analytical ultracentrifugation sedimentation equilibrium. A single-species model was fit to the data for the wild-type protein, βdimer1, and βdimer3. Data from all three concentrations (20, 40, and 60 μM) were used in fitting to find the molecular mass. The molecular mass was 12 kDa for the wild type, 26 kDa for βdimer1, and 16 kDa for βdimer3.

Fig. S2. Determination of βdimer1 dimer dissociation constant by analytical ultracentrifugation sedimentation equilibrium. Absorbance was measured at 215 nm for βdimer1 at concentrations of 2 (*A*), 1.5 (*B*), and 0.8 μM (*C*). Data from all three concentrations were pooled and fit according to a self-association model with the molecular mass of the monomer assumed to be 13,628 Da. The fit produced a K_a of 9.0 in absorbance units, which can be converted to concentration units with the equation

$$K_a(\mathrm{M}) = K_a(\mathrm{Abs})^*(\varepsilon^* l/2),$$

where l is the path length (1.2 cm) and the extinction coefficient (ε) is 192,100 $M^{-1}\ cm^{-1}$. The extinction coefficient at 215 nm was found experimentally by performing serial dilutions of a known concentration βdimer1, based on absorbance at 280 nm, and measuring the absorbance at 215 nm. Using this information the dimer association constant of βdimer1 (K_a) is found to be 1.04×10^6 M. The dimer dissociation constant is 0.96 μM.

Fig. S3. Comparison of interacting β-strands in βdimer1 chain A (cyan) to the wild-type structure (2A7B, orange). Residues between S103 (Y103 in βdimer1) to A109 are shown in sticks. Only small perturbations in the backbone are observed. The backbone atom rmsd between βdimer1 chain A and the wild-type structure is 0.3 Å for this range.

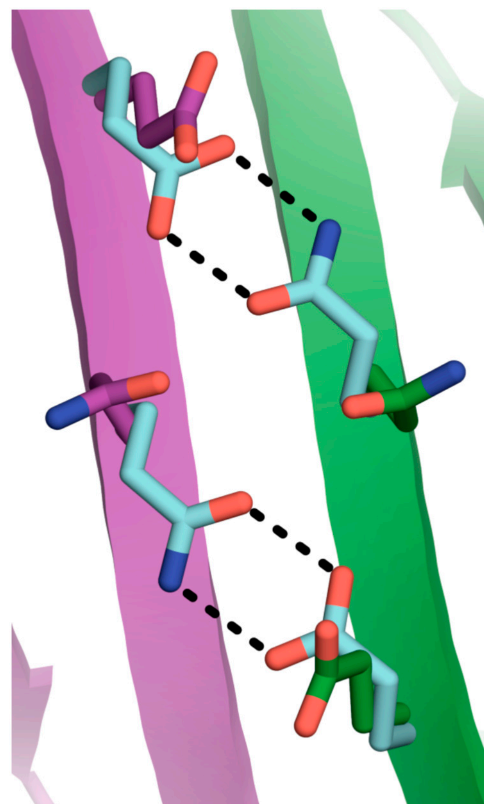


Fig. S4. The crystal structure reveals side-chain interactions between Q106 and E108 on the solvent accessible side of the interacting β-strands in βdimer1 that were not modeled in the design. The computational model (purple and green) predicts no interaction between these side chains, whereas the crystal structure (cyan) indicates a head-on pairing of Q106 and E108. Black dashed lines represent hydrogen-bond interactions. It is likely that E108 is protonated (βdimer1 crystals were grown at pH 5.0). A protonated variant of glutamate was not considered in the computational design protocol.

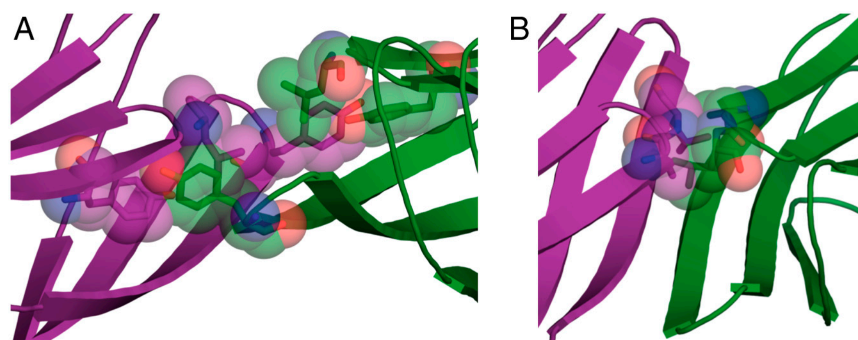


Fig. S5. Clashes prevent register shift of βdimer1. (*A*) A register shift of βdimer1 to make fewer backbone–backbone contacts is prevented by the introduction of clashes of Y8 and L11 on one chain with Y103 on the matching chain. (*B*) A register shift in the opposite direction would be disfavored by clashes of L11 on one strand with L11 on the symmetric strand. These assemblies were made by manually moving one of the chains to the next register of backbone–backbone hydrogen-bond contacts.

Table S1. Data collection and refinement statistics

	βdimer1
Data collection	
Space group	$P12_11$
Cell dimensions	
a, *b*, *c*, Å	50.64, 44.25, 53.02
α, β, γ, °	90.00, 91.91, 90.00
Resolution, Å	23.14-1.09 (1.10-1.09)*
R_{merge}	0.053 (0.331)
I/σ	33.1 (1.9)
Completeness, %	92.6 (44.6)
Redundancy	3.6 (1.8)
Refinement	
Resolution, Å	23.14-1.09
No. reflections	90,300
R_{work}/R_{free}	0.159/0.181
No. atoms	
Protein	2,087
Ligand/ion	22
Water	314
B factors, Å^2	
Protein	14.2
Ligand/ion	23.5
Water	24.5
rms deviations	
Bond lengths, Å	0.011
Bond angles, °	1.421
Ramachandran statistics	
Most favored, %/no.	98.2/220
Additionally allowed, %/no.	1.8/4
Generally allowed, %/no.	0/0
Disallowed, %/no.	0/0

*Values in parentheses are for highest-resolution shell.