Supporting Information

Kim and Sauer 10.1073/pnas.1204791109

SI Materials and Methods

Various *degP* mutations were introduced into *Escherichia coli* by two-step recombination as described in Davis et al. (1). For the first step (replacing the *degP* gene with a cassette encoding selectable and counter selectable markers), the mPheS-kan^R allele was amplified from pJD141 with two primers (dP_ss_for1 and dP_rev1). Cells (*E. coli* W3110) carrying pSIM5 (2) were grown in LB broth to OD_{600} 0.3∼0.5 at 30 °C, and then incubated for ∼ 20 min at 42 °C for induction of λ-RED recombination proteins. Cells were then cooled on ice, harvested, washed with cold water, and finally resuspended in cold 10% (vol/vol) glycerol. The PCR product carrying the mPheS-kan^R cassette was electroporated into cells, which were recovered, grown in Super Optimal broth with Catabolite repression (SOC) for 6∼12 h at 30 °C, and plated on LB agar plus kanamycin (30 μg/mL) at 30 °C. Successful recombination was confirmed by resistance to kanamycin, sensitivity to 16 mM ρ-chlorophenylalanine (ρ-Cl-Phe), and the size difference of PCR products amplified using primers flanking the chromosomal *degP* allele.

For the second step of recombination (replacing the mPheS-kan^R cassette with a mutant *degP* gene), primers insP_ss-for1 in combination with insP_rev2 (for SK330 and SK338) or insP_rev3 (for SK326) were used to amplify DNA fragments containing mutant *degP* genes from plasmids pSK473 (for SK326), pSK534 (for SK330), or pSK566 (for SK338). To obtain the DNA fragment for the in-frame deletion of protein-coding sequences (the *ΔdegP* strain), two primers (dP_orf-for and dP_orf-rev) were extended using each other as templates. Recombineering was performed as described, except for using SK324 as recipient cells and plating on YEG (0.5% yeast extract, 1% NaCl, and 0.4% glucose) agar/ρ-Cl-Phe (16 mM). Successful recombination was confirmed by resistance to ρ-Cl-Phe, sensitivity to kanamycin, and DNA sequencing of chromosomal *degP* locus. Cells were also tested for the removal of pSIM5 (Cm^R) on LB agar/chloramphenicol (10 μg/mL), and, if necessary, cells were restreaked on LB agar plate and grown at 37 °C to remove pSIM5.

1. Davis JH, Baker TA, Sauer RT (2011) Small-molecule control of protein degradation using split adaptors. *ACS Chem Biol* 6:1205–1213.

2. Datta S, Costantino N, Court DL (2006) A set of recombineering plasmids for gram-negative bacteria. *Gene* 379:109–115.

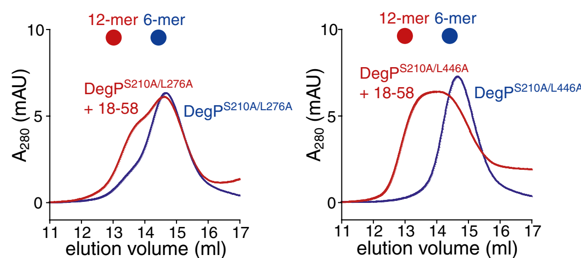

Fig. S1. The L276A and L446A mutations destabilize but do not prevent cage formation. Gel-filtration chromatograms of DegP$^{S210A/L276A}$ and DegP$^{S210A/L446A}$ in the absence and presence of 18–58. Blue dots mark the elution position of DegP^{S210A}; red dots mark the elution position of DegP^{S210A} plus 18–58.

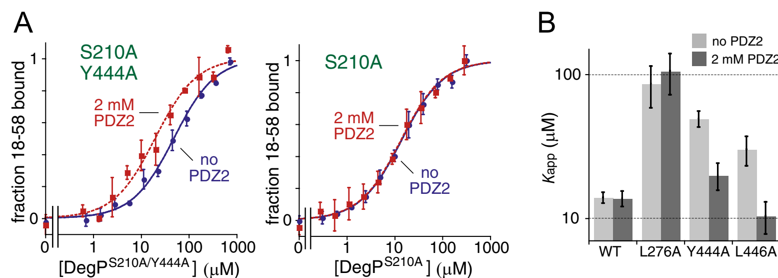

Fig. S2. PDZ1–PDZ2′ interactions enhance substrate binding. (*A*) Addition of 2 mM PDZ2 domain enhanced substrate binding to DegP$^{S210A/Y444A}$ (*Left*), but not to DegP^{S210A} (*Right*). Binding of ^{flC}18–58 was monitored by changes in fluorescence anisotropy. The curves are fits to a hyperbolic equation. Error bars are averages ±1 SD (n = 3). (*B*) The 2 mM PDZ2 decreased K_{app} for ^{flC}18–58 binding to DegP$^{S210A/Y444A}$ and DegP$^{S210A/L446A}$, but not to DegP^{S210A} and DegP$^{S210A/L276A}$, indicating that the affinity-enhancing effects depend on PDZ1–PDZ2′ interactions. Bars represent the error of fitting to hyperbolic binding curves, as shown in Fig. S2*A*.

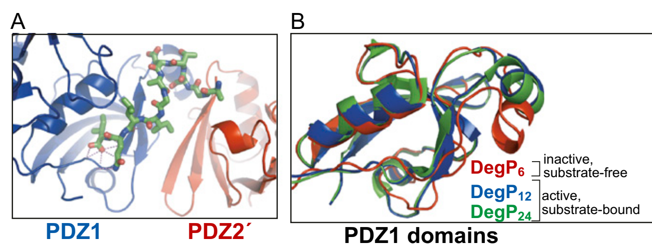

Fig. S3. Structural mechanisms that might link substrate binding to enhanced PDZ1–PDZ2′ interactions. (*A*) The C terminus of the 18–58 substrate (green; stick representation) binds to the PDZ1 domain (blue; cartoon representation) in chain C of the dodecamer (PDB ID code 3OTP), but some substrate residues are also very near the neighboring PDZ2′ domain (red; cartoon representation). Contacts of this type might couple substrate binding to cage formation. (*B*) PDZ1 domains in substrate-bound cages (blue, 3OTP; green, 3CS0) have slightly different conformations than a PDZ1 domain in a substrate-free hexamer (red; 1KY9). The blue and green domains superimpose with an rmsd of ~0.24 Å for 364 main-chain atoms, whereas the blue and red domains superimpose with an rmsd of ~1.6 Å for 309 main-chain atoms.

Table S1. Strains, plasmids, and primers for genetic study

Name	Description
Strains	
W3110	Wild type
SK324	W3110 *degP::mPheS-kan*^{R}
SK345	W3110 Δ*degP(orf)*
SK326	W3110 *degP(Y444A)*
SK330	W3110 *degP(*Δ*linker)*
SK338	W3110 *degP(+20_linker)*
Plasmids	
pSIM5 (2)	Carries lambda RED recombination genes, pSC101*ts* origin
pJD141	carries *mPheS-kan*^{R}
p7	Overexpression of wild-type DegP with N-terminal His-tag
pSK473	Overexpression of DegP(Y444A) with N-terminal His-tag
pSK534	Overexpression of DegP(Δlinker) with N-terminal His-tag
pSK566	Overexpression of DegP(+20_linker) with N-terminal His-tag
Primers for recombineering	
dP_ss_for1	GAGTTTAGGTTTGGCGTTATCTCCGCTCTCTGCAACGGCGgaattcgcggccgcttctag
dP_rev1	GGAGAACCCCTTCCCGTTTTCAGGAAGGGGTTGAGGGAGActgcagcggccgctactagt
insP_ss-for1	GAGTTTAGGTTTGGCGTTATCTCCGCTCTCTGCAACGGCGgctgagacttcttcagcaac
insP_rev2	GAACCCCTTCCCGTTTTCAGGAAGGGGTTGAGGGAGAttactgcattaacaggtagatgg
insP_rev3	GAACCCCTTCCCGTTTTCAGGAAGGGGTTGAGGGAGAttactgcattaacagggcgatggtgctgt
dP_orf-for	GAAGAACACAGCAATTTTGCGTTATCTGTTAATCGAGACTGAAATAC TCTCCCTCAACCC
dP_orf-rev	ATTGTAAGGAGAACCCCTTCCCGTTTTCAGGAAGGGGTTGAGGGAGA GTATTTCAGTCTC

Sequences in uppercase are homologous to the *E. coli degP* locus; sequences in lowercase are used for amplification from plasmids.