SI Text

Preparation of GFPm

Oligonucleotides for site-directed mutagenesis All oligonucleotides were synthesized at Qiagen (Valencia, CA) on a scale of 10 nmol.

GFP-1: 5'-CATCACGGATCCATGAGTAAAGGAGAAGAACTTTTCACTGG-3'

GFP-2: 5'-CTAATTAAGCTTCTATTTGTAGAGCTCATCCATGCCATG-3'

GFP-3: 5'-CAACATTGAAGATGGTTCCGTTCAACTAGCAG-3'

GFP-4: 5'-CTGCTAGTTGAACGGAACCATCTTCAATGTTG-3'

GFP-5: 5'-CTTGTCACTACTTTCGGTTATGGTGTTCAATGC-3'

GFP-6: 5'- GCATTGAACACCATAACCGAAAGTAGTGACAAG -3'

GFP-7: 5'-GGTGTTCAATGCTTTGCGCGTTATCCGGATC-3'

GFP-8: 5'-GATCCGGATAACGCGCAAAGCATTGAACACC-3'

***Addition of two restriction sites, BamHI* and *HindIII*, and removal of the internal BamHI restriction site.** CLONTECH GFP-UV vector, containing the "cycle-3" variant was used as a template for two PCR steps by using *Pfu*
DNA polymerase (Stratagene). The first fragment was obtained by PCR with GFP-1 and GFP-4. The second fragment was obtained by PCR with GFP-2 and GFP-3. The two fragments were purified on a 2% agarose gel (QIAquick gel extraction kit, Qiagen) and assembled by PCR. The PCR products were purified on a 2% agarose gel.

S65G mutation.
The purified PCR product was used as a template to mutate amino acid residue 65 from serine to glycine. Following the same procedure as above, two fragments were generated by PCR with GFP-1 and GFP-6, or with GFP-2 and GFP-5. The fragments were purified on a 2% agarose gel and assembled by PCR. The PCR products were purified on a 2% agarose gel.

S72A mutation. A DNA fragment bearing terminal BamHI
and HindIII sites and the S65G mutation, was used as a template for PCR to mutate position 72 to glycine. One fragment was generated by PCR with GFP-1 and GFP-8; the other with GFP-2 and GFP-7. The two fragments were assembled by PCR and purified on a 2% agarose gel.

Construction of the expression plasmid.
The DNA fragment encoding GFPm was digested with BamHI (Roche) and HindIII (Roche) and ligated into expression plasmid pQE-80L (Qiagen) using T4 DNA ligase (New England Biolabs). The resulting plasmid was designated pQE-80L/GFPm.

Equilibrium acid denaturation (1, 2)

Tryptophan fluorescence was measured at 320 nm with excitation at 295 nm after protein samples were equilibrated in 100 mM buffer containing 100 mM NaCl at room temperature. The buffers used were Hepes (pH 8.0, 7.5, and 7.0), Mes (pH 6.5, 6.0, and 5.5), and acetate (pH 5.0, 4.5, and 4.0).

Sedimentation velocity analysis

Three protein samples (GFPm-L, 11.3.3-L, and 11.3.3-T) were equilibrated with PBS by passage through PD-10 columns. Sedimentation velocity analysis was performed at the National Analytical Ultracentrifugation Facility at the University of Connecticut by using a Beckman XL-I Analytical Ultracentrifuge at 20°C. The concentrations of protein samples were 0.125, 0.25, and 0.5 mg/ml. The rotor was accelerated to 55,000 rpm, and interference scans were acquired at 1-minute intervals for 4 h. The data were analyzed by using the program DcDt+ (3) to obtain normalized g(s*) vs. s* plots (SI Fig. 11). Analysis of the sedimentation velocity runs on GFPm-L showed it exists in a reversible monomer-dimer self-association; K_d was calculated as 23 μM by global fitting using Sedphat (4). 11.3.3-L and 11.3.3-T are present largely as dimers in the concentration range studied. The program Sedfit (version 9.3) (5) indicated small concentrations of tetramer (≈8% and ≈3% for 11.3.3-L and 11.3.3-T, respectively).

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2. Enoki S, Saeki K, Maki K, Kuwajima K (2004) *Biochemistry* 43:14238-14248.

3. Philo JS (2000) *Anal Biochem* 279:151-163.

4. Schuck P (2003) *Anal Biochem* 320:104-124.

5. Schuck P (2000) *Biophys J* 78:1606-1619.