Supporting Information

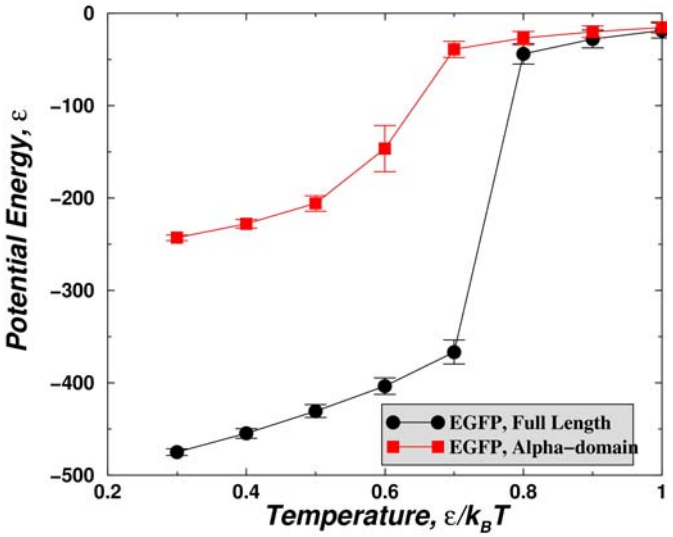

Fig. 5. Full-length EGFP is more stable than its large fragment. The graph shows folding thermodynamics of the large EGFP fragment (also known as alpha domain) as compared to full-length EGFP; it is clear that the latter has a higher transition temperature T_F. Evidently, the increase in stability is a result of interactions between the large and small EGFP fragments (see below). Thus, the presence of the smaller EGFP domain substantially stabilizes the fold of the full-size protein. Folded EGFP structure: x-ray structure (Protein Data Bank ID code 1c4f; S65T GFP mutant, pH 4.6). We consider this conformation as a native EGFP fold because differences between this structure and some other EGFP x-ray structures are small. For instance, the rms deviation between PDB entry 1c4f and PDB entry 1emg (S65T GFP mutant, pH 8.0) is only 0.18 Å.

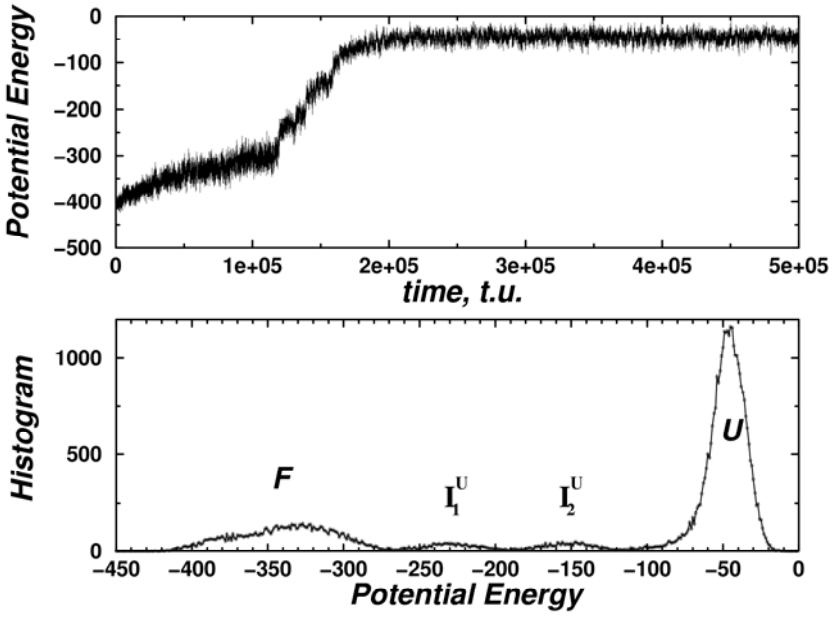

Fig. 6. Full-length EGFP has two folding-unfolding intermediate states. These two graphs show the quasi-equilibrium unfolding of EGFP studied by DMD simulations with gradual heating by using the Berendsen's thermostat (1). Starting from the folded state, the temperature of the protein system is slowly increased from $T_l = 0.6 < T_F$ to $T_h = 0.8 > T_F$. We performed 10 unfolding simulations of EGFP. (*Upper*) A typical trajectory is shown. (*Lower*) Two intermediate states, I_1^U and I_2^U, are observed along the averaged unfolding pathway between folded (*F*) and unfolded (*U*) conformations. Similar results were obtained for 10 quasi-equilibrium EGFP folding simulations starting from the unfolded state.

1. Berendsen, H. J. C., Postma, J. P. M., van Gunsteren, W. F., Di Nola, A. & Haak, J. R. (1984) *J. Chem. Phys.* **81,** 3684–3690.

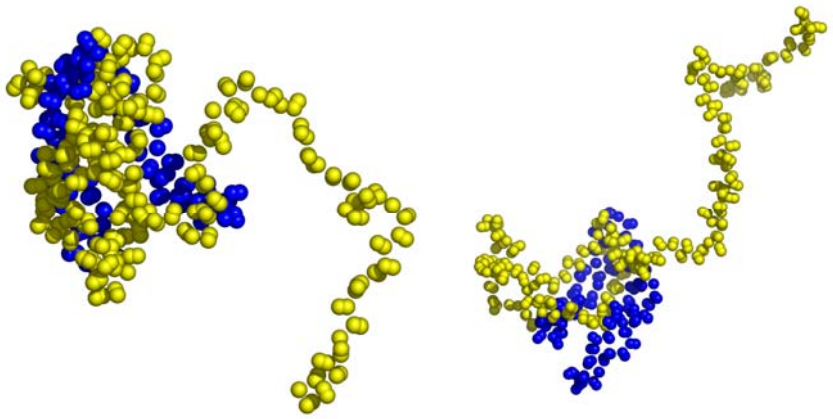

Fig. 7. EGFP folding-unfolding intermediates. Unfolding intermediate I_1^U (left snapshot) corresponds to the unfurling of the N-terminal β-strands, and the second unfolding intermediate I_2^U (right snapshot) corresponds to the unfolding of almost the entire larger EGFP domain (yellow color) with its C-terminal interacting with the smaller EGFP domain (blue color). Similar snapshots were obtained for the two analogous intermediates observed in the reverse order in quasi-equilibrium EGFP-folding simulations.

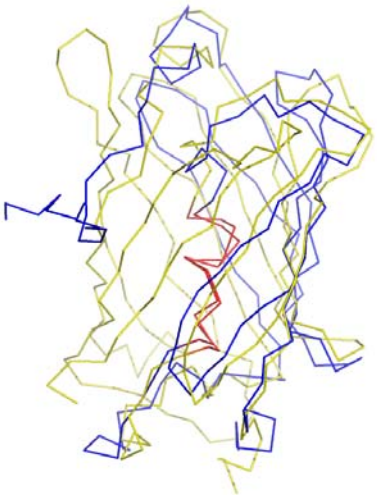

Fig. 8. Backbone alignment of one DMD-folded large EGFP fragment (blue) and the full-length EGFP (yellow). Here, the chromophore-forming residues of both polypeptides are shown in red. The arrangements of the chromophore-forming amino acids in the full-size EGFP and within its large fragment are essentially the same.

Table 1. Primers used to PCR-amplify coding sequences of large and small EGFP fragments and biotinylated oligonucleotides tagged to these fragments

Primer or oligonucleotide	**Sequence**
Large EGFP fragment with C-terminal cysteine	
Primer α_dir	5′-AGTTTCTAGAATGGTGAGCAAGGGCG
Primer α-CYS_rev	5′-ATCGCTCGAGTTAGCACTGCTTGTCGGCCATG
biotinylated oligo 1	biotin-5′-CGACTGCGTTAGCATGTGTTG
Small EGFP fragment with N-terminal cysteine	
Primer β-CYS_dir	5′-ATCGGATATCATGTGCAAGAACGGCATCAAGGTG
Primer β_rev	5′-ATCGCTCGAGTTACTTGTACAGCTCGTCC
biotinylated oligo 2	5′-CAACACATGCTAACGCAGTCG-biotin