Supporting Materials and Methods

Synthesis of GFP-cI. We used GFP–cI covalent dimers to verify the characteristic fluorescence patterns of GFP dimers. The dimeric GFP-cI fusion protein, where the Tyr→Cys substitution at position 88 was shown by Sauer *et al.* to stabilize the Lambda repressor dimer by forming disulfide bonds between the subunits of the N-terminal domain (1), was constructed by mutating a copy of cI on plasmid pDT831 (gift of B. J. Egan, Princeton University, Princeton) using the QuikChange site-directed mutagenesis kit (Stratagene). Existence of the correct mutation (TAT→TGT in codon 88) was verified by sequencing. The mutant cI gene was then inserted back into the bacteriophage by recombination (see ref. 2).

cI (both wild-type and Cys-88 alleles) was amplified from pDT831 by PCR and cloned into the BamHI site of expression vector pPROTet.E133 (Clontech, BD Biosciences) to yield pPROTet133:CI. Plasmid DNA samples with inserts of the correct size were purified and checked for cI expression by standard immunity tests using superinfection of plasmid-carrying strains with wild-type λ, λvir, and λ_{imm}434 (3). GFPmut3 (4) was fused at the N terminus by amplifying from plasmid pKEN2 (gift of P. Wolanin, Princeton University, Princeton) and cloning into the SalI site of pPROTet133:CI upstream of the cI sequence. All cloning steps were performed by using standard methods (5). The product was checked again for immunity. At this stage, the fusion was also checked by DNA sequencing and Western blotting.

The fusion, which carries a N terminus histidine–asparagine hexamer (HN_6) tag, was used to purify the fusion protein on a cobalt column according to the manufacturer's protocol. The purified protein was then tested for *in vitro* activity by standard gel-shift assays (5) using a 200-bp λ fragment containing all three right operator sequences labeled at the 5[prime] end with either ^{32}P or Alexa Fluor 488. The band-shift assay exhibited the expected dependence on protein concentration, and binding could be competed by excess unlabeled fragment but not by nonspecific competitor. Finally, the structure of the GFP-cICys-88 dimer was verified by polyacrylamide gel electrophoresis in denaturing gels in

the presence and absence of DTT, a strong reducing agent. As expected (6), the purified GFP–cICys-88 dimer comigrated with the GFP–cI monomer in the presence of DTT (apparent molecular mass, 55 kDa). In nonreducing gels the GFP–cICys-88 fusion migrated with an apparent mass of 110 kDa, the GFP–cI fusion, an apparent mass of 55 kDa. These are the expected molecular weights of the monomer and Cys-88–GFP fusion products.

Microscope/Camera Efficiency. The collection efficiency η of our microscope/camera system is

$$\eta = \eta_{NA} \times T_{obj} \times T_{dic} \times T_{ef} \times T_{tl} \times T_{cw} \times QE_{CCD} = 0.33 \times 0.9 \times 0.85 \times 0.9 \times 0.9 \times 0.9 \times 0.35 = 6.4\%,$$

where $\eta_{NA} = 0.33$ is the collection efficiency of the objective as calculated from the photon collection solid angle obtained from numerical aperture 1.45 of the objective, $T_{obj} \approx 0.9$ is the transmittance of the objective lens, $T_{dic} = 0.85$ is the transmittance of the dichroic beam splitter at 525 nm, $T_{ef} \approx 0.9$ is the transmission efficiency of the emission band-pass filter centered at 525 nm, $T_{tl} \approx 0.9$ is the transmittance of the projection tube lens, $T_{cw} \approx 0.9$ is the transmission efficiency of the camera window, and $QE_{CCD} = 35\%$ is the quantum yield of the camera at 525 nm. Among these parameters, an estimation of 90% was used for transmittance of the objective lens, the projection lens, and the camera window.

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