Cloning and purification protocol for NZGFP, NGFP, CGFP, and CZGFP

The NZGFP, NGFP, CGFP and CZGFP coding DNA were obtained by PCR amplification of the GFP (sg100) plasmid template, provided by G. J. Palm, using appropriate primers. The DNA fragments were cut with NheI/BamH1 and ligated into the pET11a vector. The DNA sequences of the NZGFP, NGFP, CGFP, and CZGFP containing clones were verified by dideoxyoligonucleotide sequencing at the Keck facility at Yale. The protein products were overexpressed in BL21(DE3) cells at 37 °C without IPTG induction. The cells were lysed by sonication and the proteins were individually purified by passage over 2 successive Q-sepharose columns and then over a Gel-filtration column. Fractions containing our protein of interest, as determined by SDS-PAGE, were pooled and dialyzed against 2mM DTT, 10 mM Tris.HCl buffer at pH 7.2. Final purified yields of proteins were between 10-20 mg/ L. Protein molecular weights were verified by MALDI mass spectrometry to within 0.05% of the calculated molecular weight. Amino acid analysis of the proteins established the correct compositions and protein concentrations for further biophysical studies.

Amino acid sequences of NGFP, NZGFP, CGFP, and CZGFP.

Leucine zippers are in bold and linker regions underlined. Note the 6 residue linker between the C-terminal of NGFP and NZ and the 4 residue linker between CGFP and CZ.

NGFP

MASKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTLCYGVQCFS
RYPDHMKRHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYN
YNHNVLIMADKQ*GGSGSG*

NZGFP

MASKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTLCYGVQCFS
RYPDHMKRHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYN
YNHNVLIMADKQ*GGSGSG***ALKKELQANKKELAQLKWELQALKKELAQ**

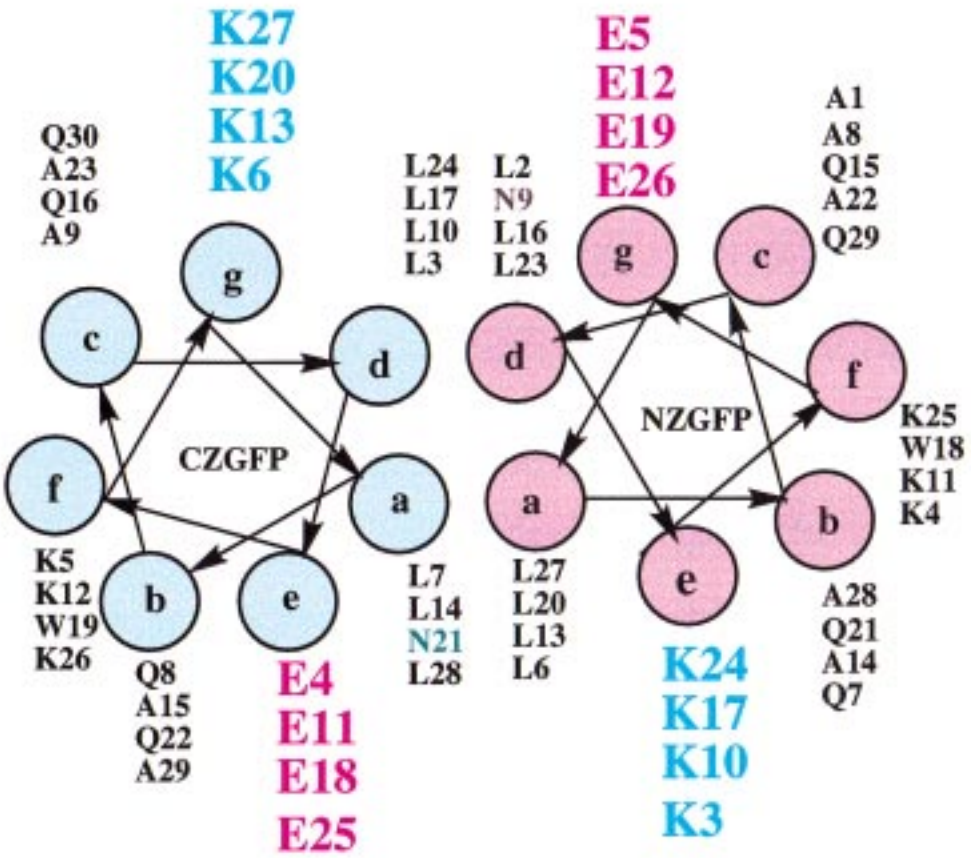
CGFP

MAS*GGSG*KNGIKVNFKTRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLE
FVTAAGITHGMDELYN

CZGFP

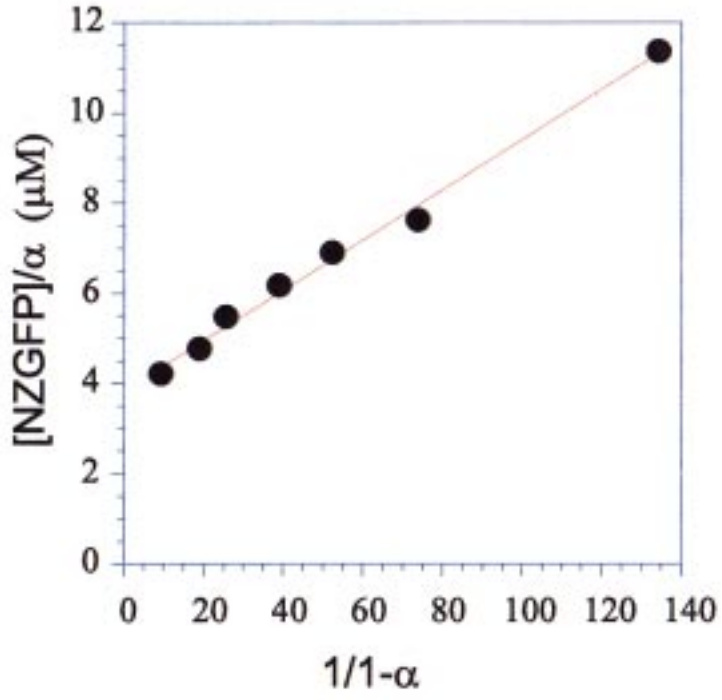
MAS**EQLEKKLQALEKKLAQLEWKNQALEKKLAQ***GGSG*KNGIKVNFKTRHNIEDGSVQLADHYQQNTPIG
DGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITHGMDELYN

Helical wheel representation of NZ and CZ coiled coils in an antiparallel orientation.



α-analysis of fluorescence binding data from Figure 2.

Reference (13) Bagshaw, C. R.; Harris, D. A.; Spectrophotometry and spectrofluorimetry: A practical approach; IRL Press, Washington, 1987, pp 91-113.



Linear regression of data between 45 to 98% of saturation.
The y-intercept yields the total concentration of ligand binding sites on CZGFP.

—— y = 3.8418 + 0.055715x R= 0.99647

Since the concentration of CZGFP is 4 μM, the stoichiometry of NZGFP:CZGFP = 3.8418/4.0 = 0.96 or 1.