Supporting Information

Hoepker et al. 10.1073/pnas.1424021112

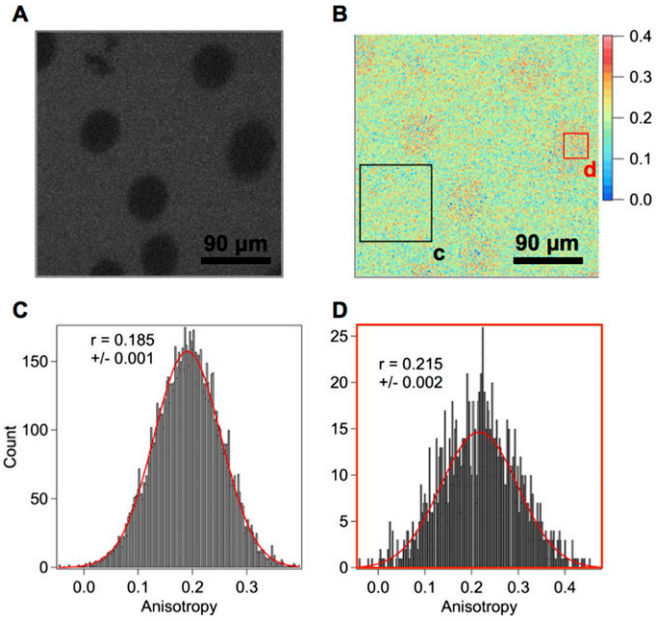

Fig. S1. Fluorescence intensity and anisotropy image of unlabeled agarose beads with 10 μM His-tagged LUMP in buffer at 20 °C. (*A*) Fluorescence intensity image of unlabeled agarose bead showing no intensity at the bead. (*B*) FA image obtained from P- and S-polarized images with a G-factor of 0.78. (*C*) Anisotropy distribution of Box c in *B*. The anisotropy of 0.185 matches the anisotropy obtained from SLM-AB2 fluorometer measurement of His-LUMP. For comparison, LUMP without the His-tag measures 0.166, as referenced earlier. (*D*) Anisotropy distribution of Box d in *B*.

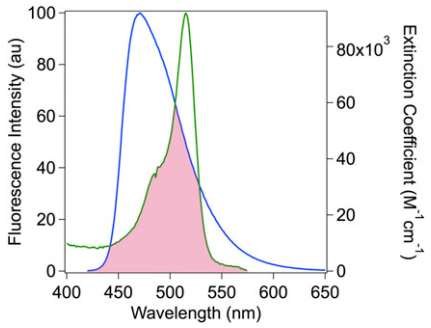

Fig. S2. Peak-normalized absorption spectrum of Venus (green trace) superimposed on fluorescence emission spectrum of LUMP (blue trace) in aqueous buffer [20 mM Hepes (pH 7.9), 150 mM NaCl] at 20 °C. The shaded area indicates the overlap integral $J(\lambda)$ that is used to calculate the Förster radius R_0 with a MATLAB (MathWorks) routine from Fluortools.com. The maximum absorbance of Venus is set at its extinction coefficient: 92,200 $M^{-1}{\cdot}cm^{-1}$. The maximum emission of LUMP is normalized at 100 au.

Table S1. Amino acid sequences of LUMP constructs

Protein	Amino acid sequence
LUMP	MGSSHHHHHHDYDIPTTENLYFQ//GHMFRGIVQGRGVIRSISKSEDSQRHGIAFPEGMFQLVDVDTVMLVNGCSLTVVRILGDMVYFDIDQALGT-TTFDGLKEGDQVNLEIHPKFGEVVGRGGLTGNIKGTALVAAIEENDAGFSVLIDIPKGLAENLTVKDDIGIDGISLPITDMSDSIITLNYSRDLLASTN-IASLAKDVKVNVEILNEW
LUMP-GBD	MGSSHHHHHHDYDIPTTENLYFQ//GHMGLSAQDISQPLQNSFIHTGHGDSDPRHCWGFPDRIDELYLGNGSGSASFRGIVQGRGVIRSISKSEDSQR-HGIAFPEGMFQLVDVDTVMLVNGCSLTVVRILGDMVYFDIDQALGTTTFDGLKEGDQVNLEIHPKFGEVVGRGGLTGNIKGTALVAAIEENDAGF-SVLIDIPKGLAENLTVKDDIGIDGISLPITDMSDSIITLNYSRDLLASTNIASLAKDVKVNVEILNEW
Venus-LUMP	MGSSHHHHHHDYDIPTTENLYFQ//GHMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTFGYGLM-CFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIK-VNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSYQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYKASFRGIVQGRGVIRSISKS-EDSQRHGIAFPEGMFQLVDVDTVMLVNGCSLTVVRILGDMVYFDIDQALGTTTFDGLKEGDQVNLEIHPKFGEVVGRGGLTGNIKGTALVAAIE-ENDAGFSVLIDIPKGLAENLTVKDDIGIDGISLPITDMSDSIITLNYSRDLLASTNIASLAKDVKVNVEILNEW
Venus-thrombin-LUMP	MGSSHHHHHHDYDIPTTENLYFQ//GHMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTFGYGLMC-FARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKV-NFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSYQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYKASLVPRGSRGSFRGIVQGRG-VIRSISKSEDSQRHGIAFPEGMFQLVDVDTVMLVNGCSLTVVRILGDMVYFDIDQALGTTTFDGLKEGDQVNLEIHPKFGEVVGRGGLTGNIKGT-ALVAAIEENDAGFSVLIDIPKGLAENLTVKDDIGIDGISLPITDMSDSIITLNYSRDLLASTNIASLAKDVKVNVEILNEW

All proteins were purified with the affinity tag removed with TEV protease. The cut site is denoted by the symbol //.

Table S2. Fluorescence lifetime measurements

Protein	A_1	τ_1 (ns)	A_2	τ_2 (ns)
LUMP	0.28 ± 0.011	6.3 ± 0.2	0.65 ± 0.01	14.91 ± 0.09
LUMP-GBD	0.37 ± 0.007	6.0 ± 0.1	0.55 ± 0.01	13.15 ± 0.06
Venus-LUMP	0.93 ± 0.002	4.17 ± 0.01	0.04 ± 0.002	12.9 ± 0.3

Pre-exponential factor A and fluorescence lifetime τ with SEs. The average fluorescence lifetimes are calculated from the two fluorescent components above as described in *Methods*.

Table S3. Average fluorescence lifetimes (τ_{avg}s)

Protein	τ_{avg} (ns)
LUMP	13.6
LUMP-GBD	11.5
Venus-LUMP	5.20
Venus-thrombin-LUMP	6.64