

SUPPLEMENTAL METHODS:

The unfolding of iRFP713 in a crowded milieu

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1. Protein expression and purification.

The iRFP713 genes were amplified and cloned into a pBAD/His-B vector (Invitrogen, Carlsbad, CA, USA) using BglII and EcoRI sites. LMG194 host cells (Invitrogen, Carlsbad, CA, USA) were co-transformed by pWA23h plasmid for the expression of heme oxygenase under the rhamnose promoter [1] and pBAD/His-B plasmid encoding iRFP713 and its variants with polyhistidine tags on the N-termini. Bacterial cells were grown in RM medium supplemented with ampicillin and kanamycin. The expression of heme oxygenase was initiated first by 0.02% rhamnose. After incubation of cell culture for 5 h at 37 °C the expression of the target protein was induced by 0.002% arabinose followed by the incubation of cell culture for 12 h at 37 °C and for 24 h at 18 °C. Proteins were purified with affinity chromatography on a Ni-NTA agarose column (GE Healthcare, Chicago, IL, USA). The Ni-NTA elution buffer contained 100 mM EDTA instead of imidazole. The elution buffer was exchanged to PBS buffer by dialysis. The final purification was achieved with ion-exchange chromatography on a MonoQ column (GE Healthcare, Chicago, IL, USA). The apoform of iRFP713 was expressed in LMG194 cells. The overnight LMG194 culture was grown for 2–3 h at 37 °C; then protein synthesis was induced by 0.002% arabinose. The subsequent steps of expression and purification of protein in apoform were the same as for proteins in holoform.

2. Determination of volume fraction of crowders

The density of the solution containing PEG-8000 at different concentration.

w^I	c (g/ml)	ρ^I (g cm ⁻³)
0.5	0.5431	1.0862
0.4	0.42684	1.0671
0.3	0.31461	1.0487
0.2	0.20634	1.0317
0.1	0.10148	1.0148
0	0	0.99707

¹ Data are taken from [2].

The partial specific volume was calculated on the basis of a linear approximation of the dependence of the density of the solution on the concentration of PEG-8000 according to the equation:

$$\rho = \rho_0 + (1 - v\rho_0)c \quad (1),$$

where ρ_0 is the density of the solvent in the absence of crowding agent, v is the partial specific volume and c is the concentration of a crowder.

References

1. Shcherbakova DM, Verkhusha VV (2013) Near-infrared fluorescent proteins for multicolor in vivo imaging. *Nat Methods* 10: 751-754. doi: 10.1038/nmeth.2521.
2. Gonzalez-Tello P, Camacho F, Blazquez G (1994) Density and Viscosity of Concentrated Aqueous Solutions of Polyethylene Glycol. *J Chem Eng Data* 39: 611-614. doi: 10.1021/je00015a050.