Supplementary Material (ESI) for Journal of Materials Chemistry

ELECTRONIC SUPPORTING INFORMATION

Second-Order Nonlinear Optical Properties of Fluorescent Proteins for Second-Harmonic Imaging

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Addition to Materials and methods

Expression and purification of recombinant proteins

The fluorescent proteins were obtained by heterologous overexpression in *Escherichia coli* and purification. Plasmids coding for EGFP and DsRed were used as described in Cotlet *et al.* (1). The gene coding for EYFP was cloned from pEYFP (Clontech) into pBAD/HisA in a similar way as described in Cotlet *et al.* The fluorescent proteins were expressed in *E. coli* Top-10 cells which were cultured in 4 l LB-medium supplemented with ampicillin (100 mg/l). Expression of the proteins was induced by 0.2% arabinose at an optical density of 0.5 at 600 nm. The cells were harvested after 12 hours and a cell lyste was obtained. The fluorescent proteins, carrying an N-terminal His-tag, were purified under native conditions by Ni-affinity chromatography (1 ml HisTrap columns, GE Healthcare) according to the manufacturers recommended protocol and concentrated using a Vivaspin concentrator (cutoff 5000 Da). The final pH was 7.3. The proteins were checked for purity and mass by SDS-PAGE and mass spectrometry (Fig. S1). While the method used for MS suggests DsRed to be mono- and dimeric, a gel filtration experiment in the buffer used for HRS measurements demonstrates a tetrameric form of DsRed. The concentration of the samples used for HRS measurements was determined using the extinction coefficient of the chromophore at the wavelength of maximal absorption (listed in Table S1).

Table S1: Concentrations of proteins based on the extinction coefficient ε_{max} at the wavelength λ_{max} maximal absorption Abs_{max} of the chromophore (2).

	EGFP	EYFP	DsRed
λ_{max} (nm)	488	513	558
ε_{max} ($M^{-1}cm^{-1}$)	56000	83400	35000
Abs_{max}	1,0458	2,1387	1,2199
Distance (cm)	0,2	0,2	0,2
Concentration (μM)	93,4	128	174

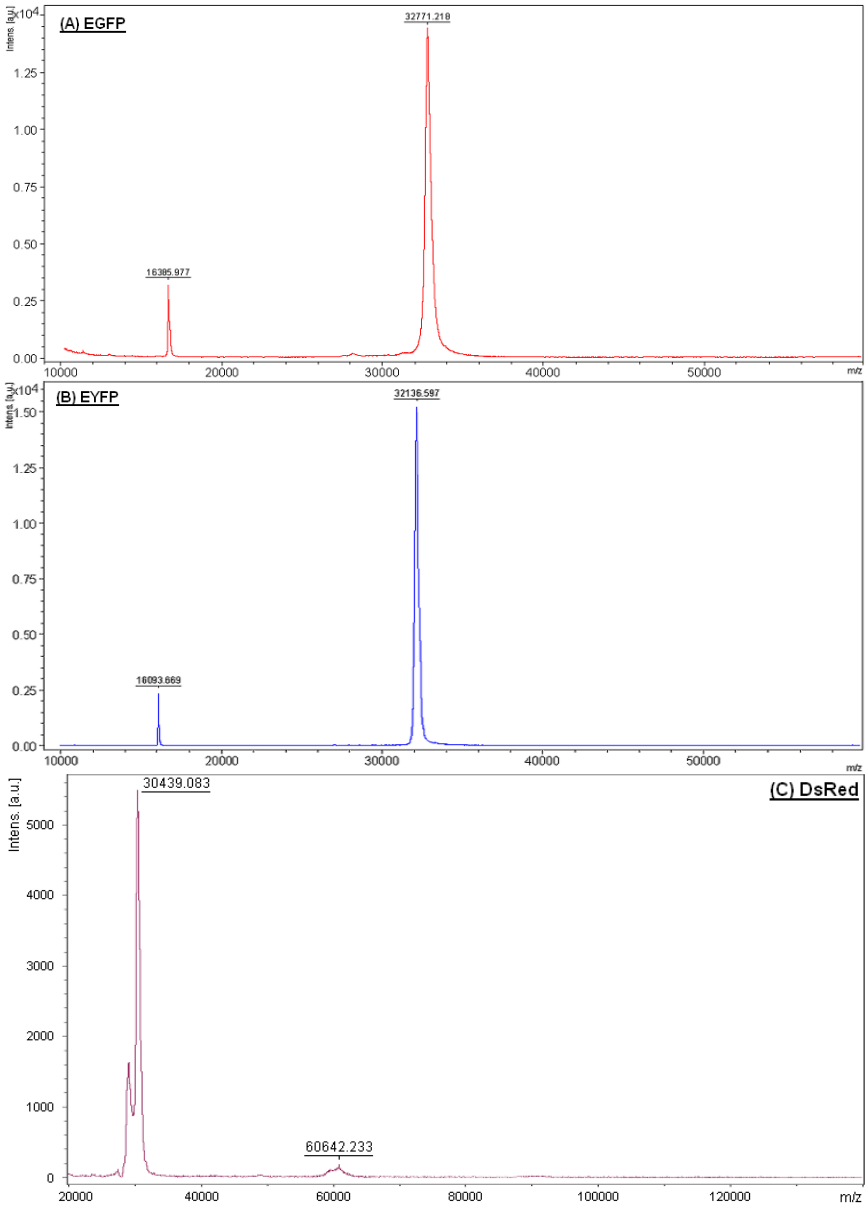

Figure S1: Mass spectrometry of (A) EGFP, (B) EYFP and (C) DsRed. The MALDI-TOF measurements were performed on a Bruker Ultraflex II mass spectrometer. Sinapinic acid was used as matrix and all measurements were carried out in positive linear ion mode, with a selected mass range of (A) and (B) 10 – 60 kDa, and (C) 20 – 140 kDa.

References

1. M. Cotlet, J. Hofkens, S. Habuchi, G. Dirix, M. Van Guyse, J. Michiels, J. Vanderleyden, F. C. De Schryver, *Proc Natl Acad Sci U S A*, 2001, **98,** 14398
2. N.C. Shaner, P.A. Steinbach, R.Y. Tsien, *Nat Methods*, 2005, **2**, 905