Supplementary Information.

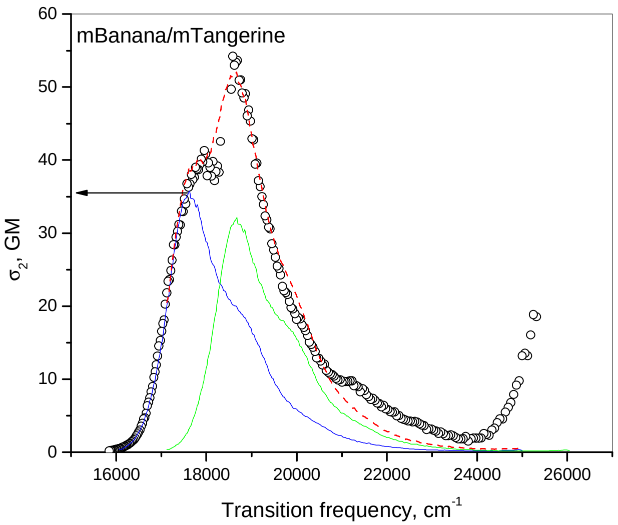
Supplementary Table. One-photon absorption, fluorescence, two-photon absorption, and photobleaching properties of red fluorescent proteins.

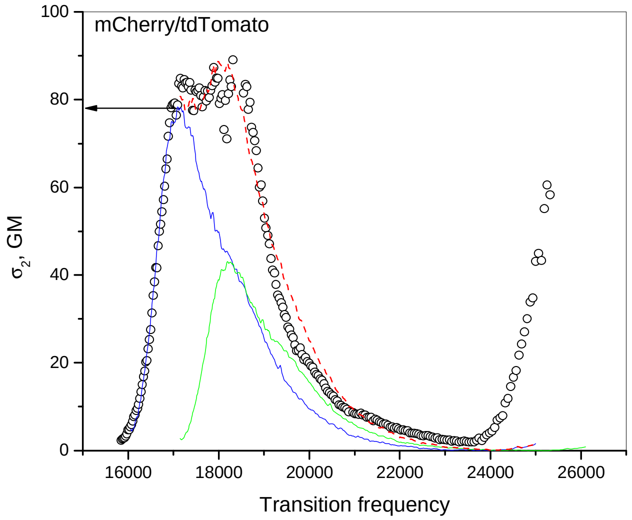
No.	Protein	λ_{abs} nm	$\varepsilon(\bar{\nu}_0)$ $10^3\ M^{-1}\ cm^{-1}$	$\sigma_1(532)$ $10^{-16}\ cm^2$	λ_{fl} nm	τ ns	ϕ_{fl}	$\sigma_2(\bar{\nu}_0)$ GM	$\Delta\mu$ D	ϕ_p	k_1 ($10^4\ s^{-1}$)	E_P kcal/mol	E_I kcal/mol
1	mTangerine	567[1]	**50**	1.01	584[1]	2.53[1]	0.22[1]	2.35	1.1	2.1×10^{-4}	8.3	14.0	10.2
			35[1]										
		568[2]	*38*[2]		*585*[2]		*0.30*[2]						
2	mStrawberry	576[1]	**66**	1.33	596[1]	2.20[1]	0.34[1]	4.7	1.4	4.5×10^{-5}	2.0	13.7	10.5
			60[1]										
		574[2]	*90*[2]		*596*[2]		*0.29*[2]						
						2.1[3]							
		575[4]	*98*[4]		*594*[4]	*2.04*[4]	*0.35*[4]						
3	mCherry	589[1]	**71**	0.78	611[1]	1.49[1]	0.24[1]	14	2.2	1.3×10^{-5}	0.88	12.8	11.4
			74[1]										
		587[2]	*72*[2]		*610*[2]		*0.22*[2]						
			78[5]			*1.4*[5]							
						1.6[3]							
		587[6]	*71*[6]		*610*[6]	*1.55*[6]	*0.22*[6]						
		585[7]	*66.4*[7]		*609*[7]		*0.23*[7]						
		588[8]	*101*[8]		*611*[8]								
		586[4]	*97*[4]		*606*[4]	*1.87*[4]	*0.16*[4]						
4	mPlum	590[1]	**62**	0.78	644[1]	0.87[1]	0.13[1]	15	2.6	3.8×10^{-6}	0.44	12.4	11.8
			65[1]										
		590[9]	*41*[9]		*649*[9]		*0.10*[9]						
		583[4]	*75*[4]		*633*[4]		*0.08*[4]						
			143[10]										
		587[11]	*29.3*[11]		*649*[11]		*0.10*[11]						
			22[5]										
5	mCherry pH11	566[12]	**66**	1.52	594[12]	1.94	0.28	30	3.6	1.4×10^{-5}	0.74	11.3	12.9
			72[13]										

		566[14]			*594*[14]								
6	mTangerine/mCherry	588	**52**	0.72	610	1.67	0.14	27	3.8	1.5x10^{-5}	0.91	11.1	13.1
7	DsRed2	562[1] *563*[5] *558*[2] *558*[15] *559*[16] *555*[17]	**106** *86*[1] *65*[5] *75*[2] *150*[15]	1.83	587[1] *587*[5] *583*[2] *583*[15] *586*[16] *586*[17]	3.44[1] *3.67*[16] *3.60*[17] *3.3*[18] *3.6*[19] *3.6*[19]	0.71[1] *0.55*[5] *0.79*[2] *0.7*[15]	58	3.9	7.1x10^{-5}	2.1	11.0	13.2
8	mStrawberry pH11	551 *548*[14]	**72**	1.90	567 *565*[14]	2.77	0.39	40	4.0	5.2x10^{-5}	1.9	10.9	13.3
9	mBanana/mTangerine	569	**57**	1.14	587	1.49	0.16	36	4.3	6.1x10^{-5}	4.1	10.6	13.6
10	mStrawberry/DsRed2	584	**54**	0.82	606	2.07	0.18	36	4.3	4.8x10^{-5}	2.3	10.6	13.6
11	mBanana	541[1] *540*[2]	**82** *73*[1] *6.0*[2]	2.71	555[1] *553*[2]	3.23[1]	0.69[1] 0.70	63	4.8	3.1x10^{-4}	9.7	10.0	14.2
12	tdTomato/DsRed2	568	**52**	1.02	591	2.78	0.25	43	4.9	5.1x10^{-5}	18	9.9	14.3
13	mCherry/tdTomato	586	**85**	1.28	608	0.29	0.029	78	5.0	2.7x10^{-5}	9.2	9.8	14.4

One-photon absorption maximum wavelength (λ_{abs}), extinction coefficient ($\varepsilon(\bar{\nu}_0)$), cross section at 532 nm ($\sigma_1(532)$); fluorescence maximum wavelength (λ_{fl}), lifetime (τ), and quantum yield (ϕ_{fl}); two-photon absorption cross section $\sigma_2(\bar{\nu}_0)$, corresponding to the maximum of the long-wavelength fitting component (see main text and Supplementary Figs 1-13), change of permanent dipole moment upon excitation $\Delta\mu$; quantum efficiency of the first step of photoblecahing, ϕ_p, monomolecular rate constant of the first step of photoblecahing , k_1, and the activation energies for the P- and I-rotations in the excite state, E_P and E_I, respectively. The first line for each protein corresponds to the values obtained either in this paper or in[1] and used for the interpretation. Other available literature data are presented in the next rows and shown by italic. All extinction coefficients are obtained by alkaline denaturation method, except for those from refs[1,13], which were obtained by using the Strickler-Berg method[1].

Supplementary Fig. 1. Two-photon absorption spectra of red FPs. For each protein, the two-photon absorption spectrum (open circles) is fitted to the sum of two one-photon excitation spectra (red dashed line): one non-shifted (blue line) with variable amplitude and another – shifted to higher frequencies (green line) with variable shift and amplitude (see Methods for details). The black arrow depicts the value of two-photon cross section $\sigma_2(\bar{\nu}_0)$ which was used in calculation of the permanent dipole moments difference.





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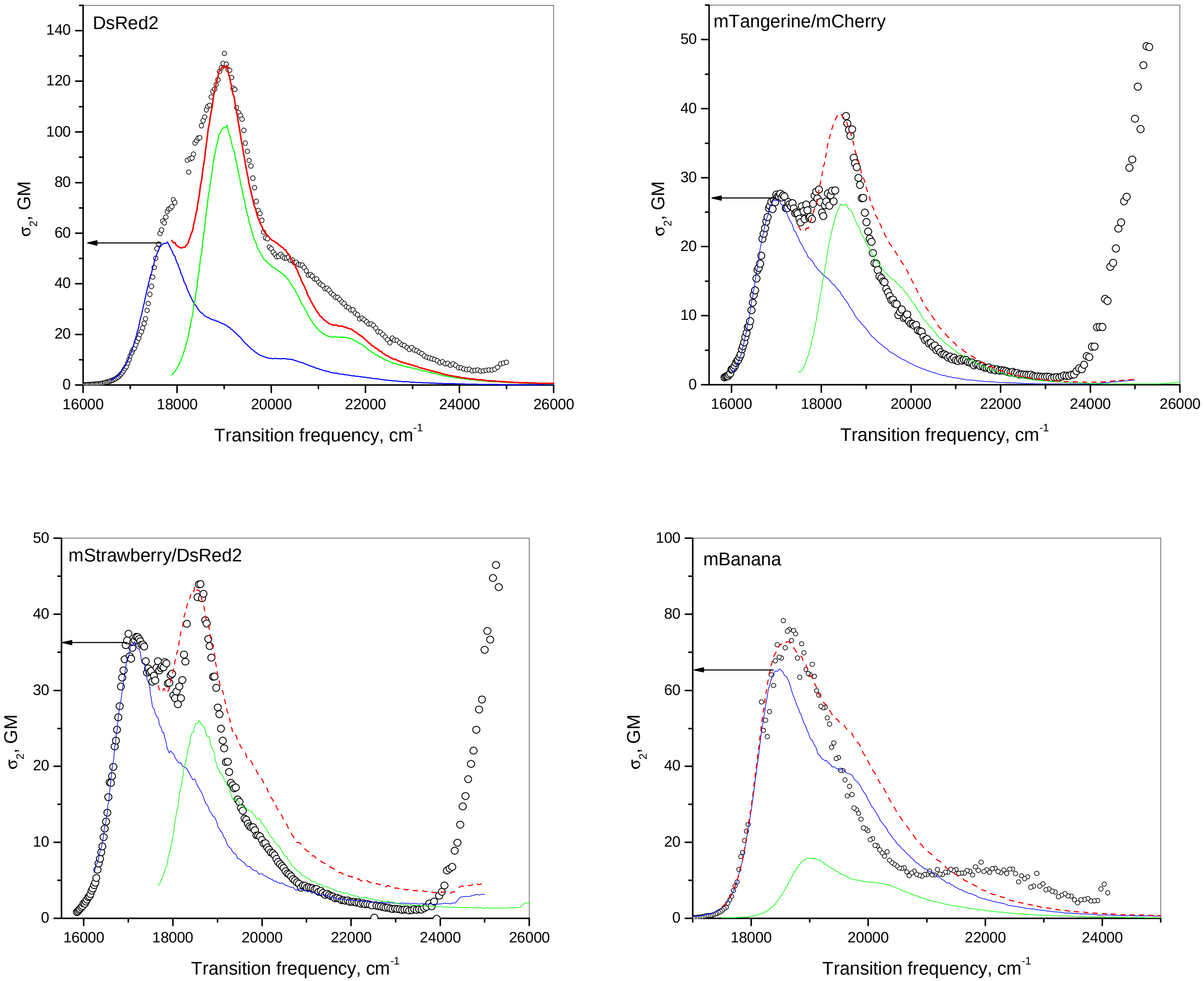
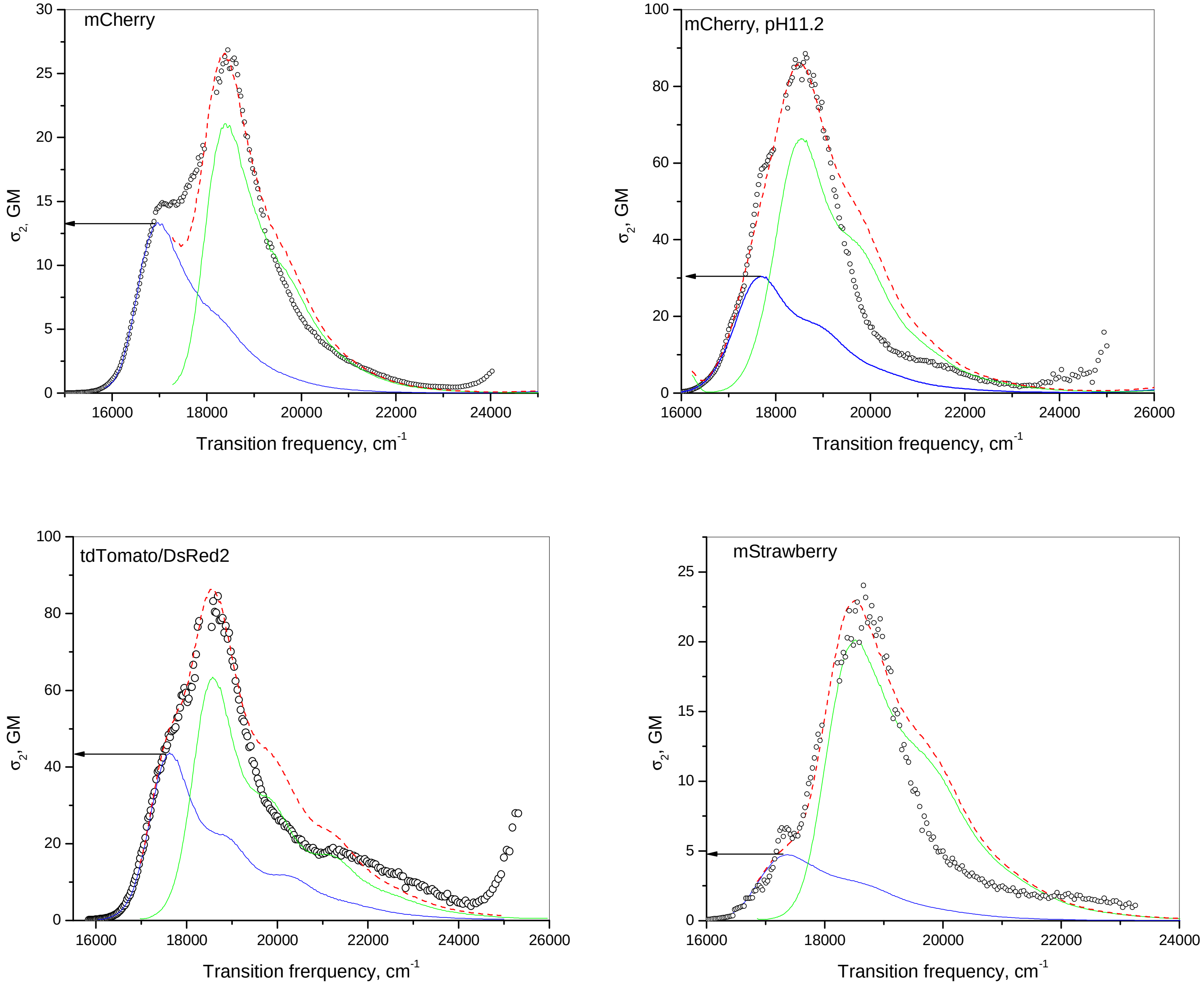
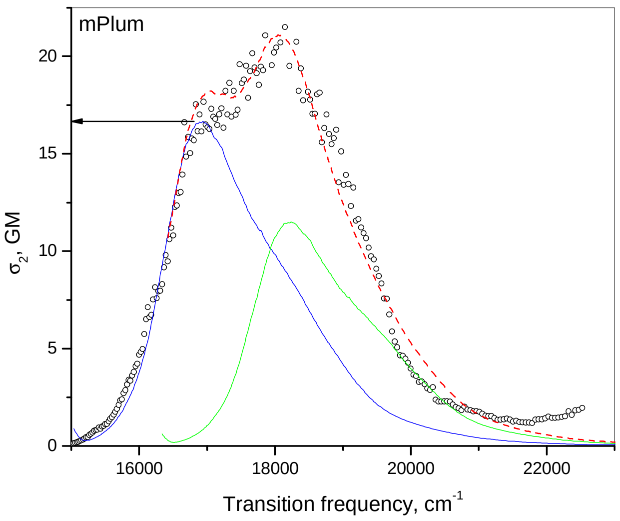
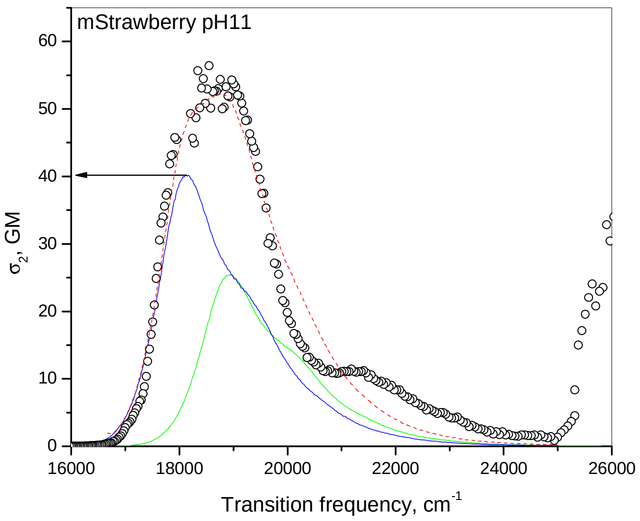


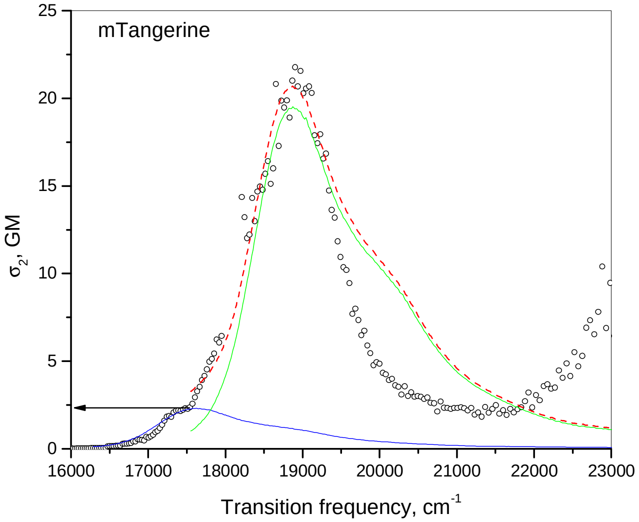
DsRed2
mTangerine/mCherry
mStrawberry/DsRed2
mBanana
σ2, GM
Transition frequency, cm-1



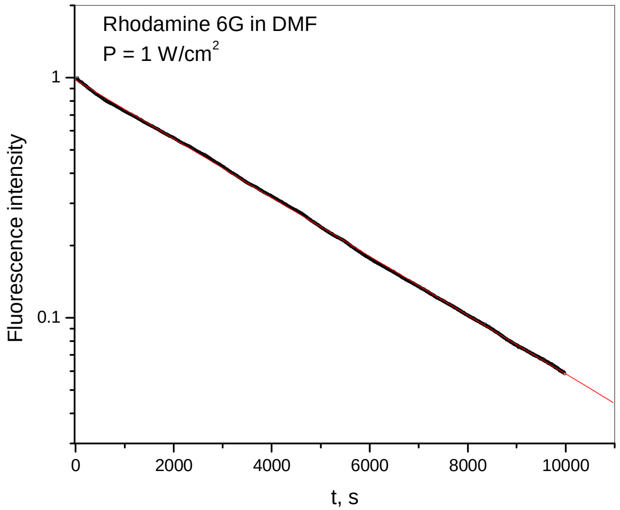
mCherry
0
5
10
15
20
25
30
σ2, GM
16000
18000
20000
22000
24000
Transition frequency, cm-1
mCherry, pH11.2
0
20
40
60
80
100
σ2, GM
16000
18000
20000
22000
24000
26000
Transition frequency, cm-1
tdTomato/DsRed2
0
20
40
60
80
100
σ2, GM
16000
18000
20000
22000
24000
26000
Transition frerquency, cm-1
mStrawberry
0
5
10
15
20
25
σ2, GM
16000
18000
20000
22000
24000
Transition frequency, cm-1


mPlum
σ2, GM
Transition frequency, cm-1
0
5
10
15
20
16000
18000
20000
22000


mStrawberry pH11
σ2, GM
Transition frequency, cm-1
0
10
20
30
40
50
60
16000
18000
20000
22000
24000
26000


mTangerine
σ2, GM
Transition frequency, cm-1
0
5
10
15
20
25
16000
17000
18000
19000
20000
21000
22000
23000

Supplementary Fig. 2. Fluorescence intensity (symbols) versus time in course of photobleaching of Rhodamine 6G in dimethylformamide with the cw laser at 532 nm, P = 1 W/cm^2. The mono-exponential fit (with zero background) is shown by red line.



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