Supplemental Figure Legends

Fig. S1. Electron density maps of GCaMP2 structures. Portions of the $2F_o$-F_c electron density map corresponding to the chromophore-containing central helix of the cpEGFP domain, contoured at 1.5 σ, are displayed on a stick representation of this region of each structure. The rest of the cpEGFP barrel is displayed as semitransparent ribbons for context.

Fig. S2. Estimation of intracellular [GCaMP2]. The mean fluorescence intensity of purified GCaMP2 (red) and GCaMP2 expressing neurons (black) at 10% and 20% laser power. Dark current was subtracted from each measurement. The eight recorded neurons have a range of estimated intracellular GCaMP2 concentrations of 6.25μM to 16.15μM, a mean of 10.04μM and standard deviation of 3.30μM.

Fig. S3. SEC of a concentration series of Ca^{2+}-saturated GCaMP2 demonstrating the relative proportion of dimeric species as a function of total GCaMP2 concentration. The maximum peak height of the monomer peak was used to normalize each chromatogram. Total GCaMP2 concentrations are displayed to the right.

Fig. S4. SEC analysis of GCaMP2 mutants designed to disrupt dimerization. The maximum peak height of the monomer peak was used to normalize each chromatogram. The full normalized monomer peaks are displayed in the inset. Mutations analyzed are labeled at right. Refer to Supplemental Table 2 for details of each dimer disruption mutation tested.

Fig. S5. Topology diagram of GCaMP2 structure. Secondary structure elements are colored by domain and labeled. The calcium-binding sites of CaM are indicated by asterisks. The chromophore of cpEGFP is shown as black lines in the middle of helix α2.

Fig. S6. Sequence of GCaMP2 with both GCaMP2 numbering (top) and numbering according to EGFP, CaM and M13-peptide (bottom). Sequence of EGF, CaM and M13-domains is in italics, pRSET expression tag and linker sequence is in regular font style. Extent of GCaMP2 domains is indicated above the sequence.

Fig. S7. CaM-cpEGFP domain interfaces. A semitransparent surface in purple (for intramolecular contacts) or green (from intermolecular contacts in the dimer) is displayed for atoms of the cpEGFP domain within 4 Å of the CaM domain. The cpEGFP domain of each structure is displayed as a grey cartoon, with the chromophore represented as sticks.

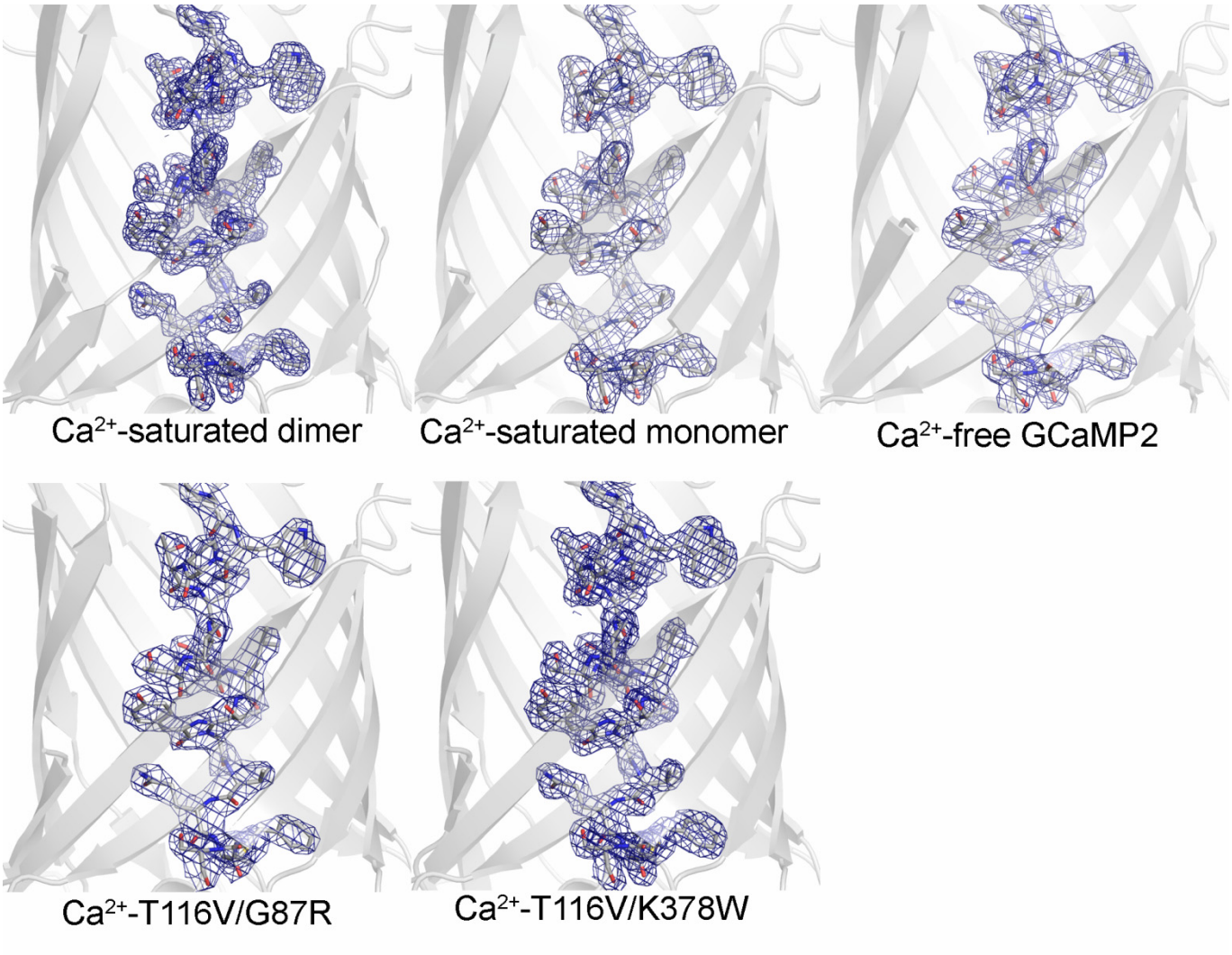
Fig. S8. Comparison of GCaMP2 domains with previous structures. (*A*) The cpEGFP domain of each of the GCaMP2 structures determined here is represented as blue ribbons superimposed on the original GFP structure (PDB ID: 1EMA, green). (*B*) The CaM-M13 domain of each of the Ca^{2+}-saturated dimer (orange) and Ca^{2+}-saturated monomer (blue) structures are shown superimposed on the original CaM-M13 structure (PDB ID: 1CDL).

The M13 peptide, as well as the N-terminal and C-terminal lobes of CaM are labeled, and the inter-lobe linker of CaM is indicated with an asterisk.

Fig. S9. Excitation, emission and absorbance scans of GCaMP2, GCaMP2-T116V and the mutants described in Table S4 and Figure 3. All of the GCaMP2 mutants also contain the T116V mutation. Scans are normalized to the largest peak in the spectrum.

Fig. S10. Stereoview comparison of the M13-cpEGFP linker in Ca^{2+}-saturated monomer (purple), Ca^{2+}-saturated dimer (cyan), and Ca^{2+}-free (green) GCaMP2. The Leu61 and Glu61 sidechains of the M13-cpEGFP linker are represented as sticks. Glu61 (denoted by asterisk) is hydrogen bonded (dashed black line) to the backbone amide nitrogen of Arg81 (also represented as sticks).

Fig. S1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Ca2+-saturated monomer
Ca2+-free GCaMP2
Ca2+-T116V/G87R
Ca2+-T116V/K378W

Fig. S2

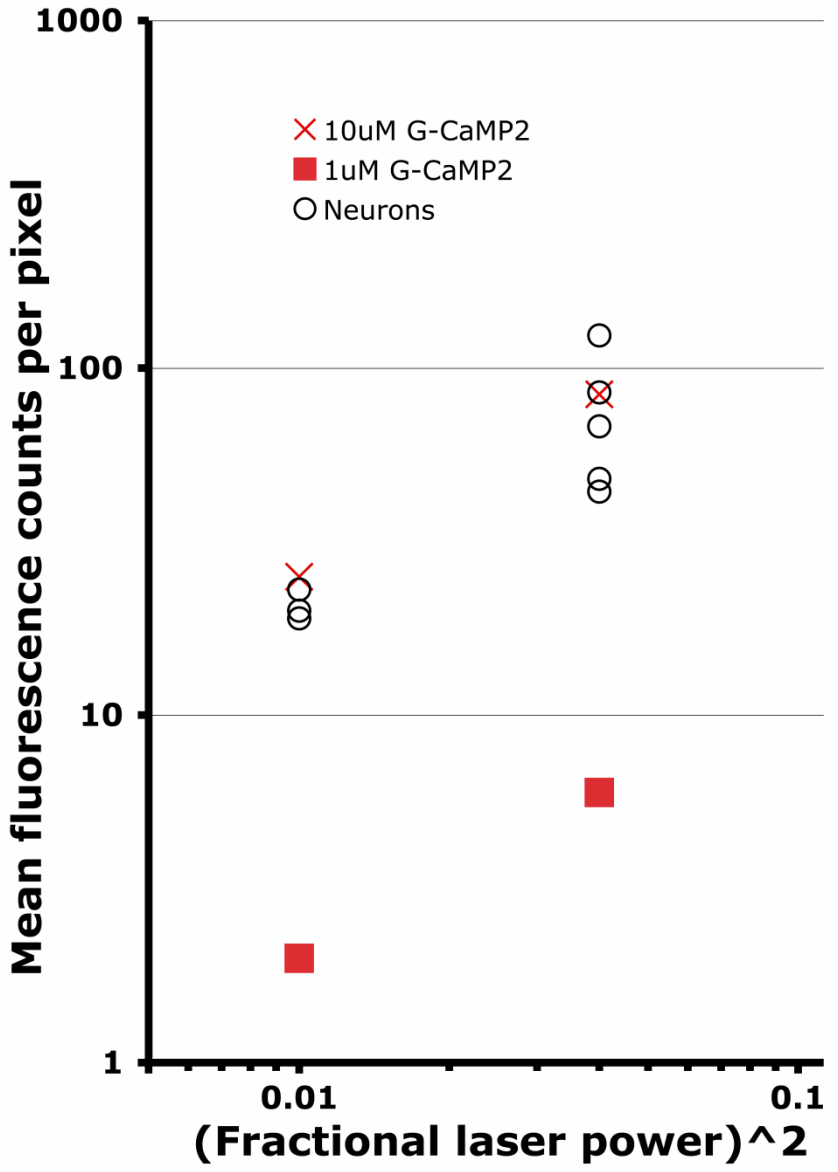
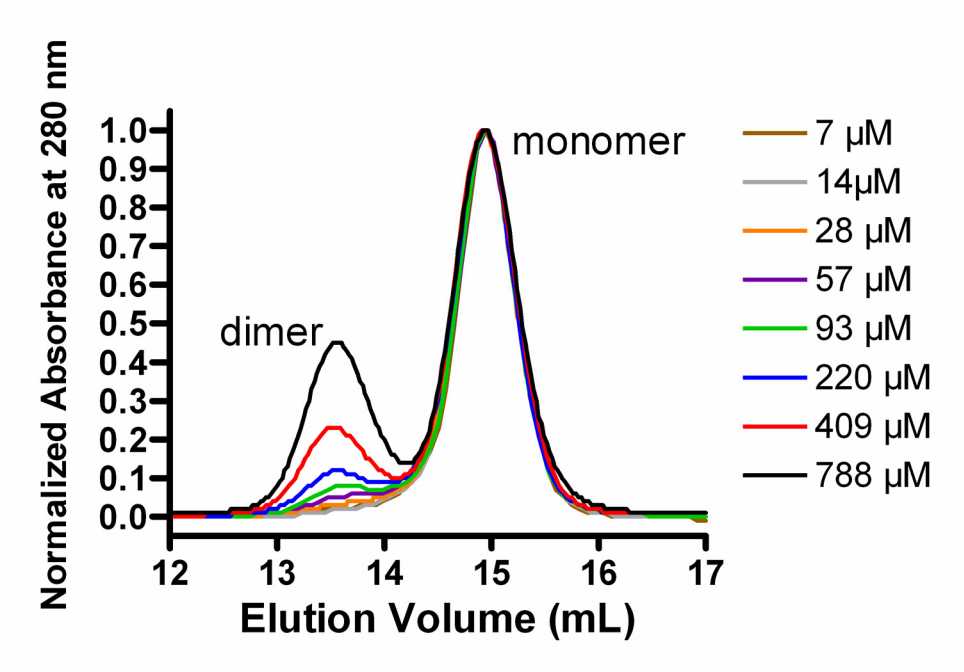

1000
100
10
1
Mean fluorescence counts per pixel
10uM G-CaMP2
1uM G-CaMP2
Neurons
0.01
0.1
(Fractional laser power)^2

Fig. S3


Normalized Absorbance at 280 nm
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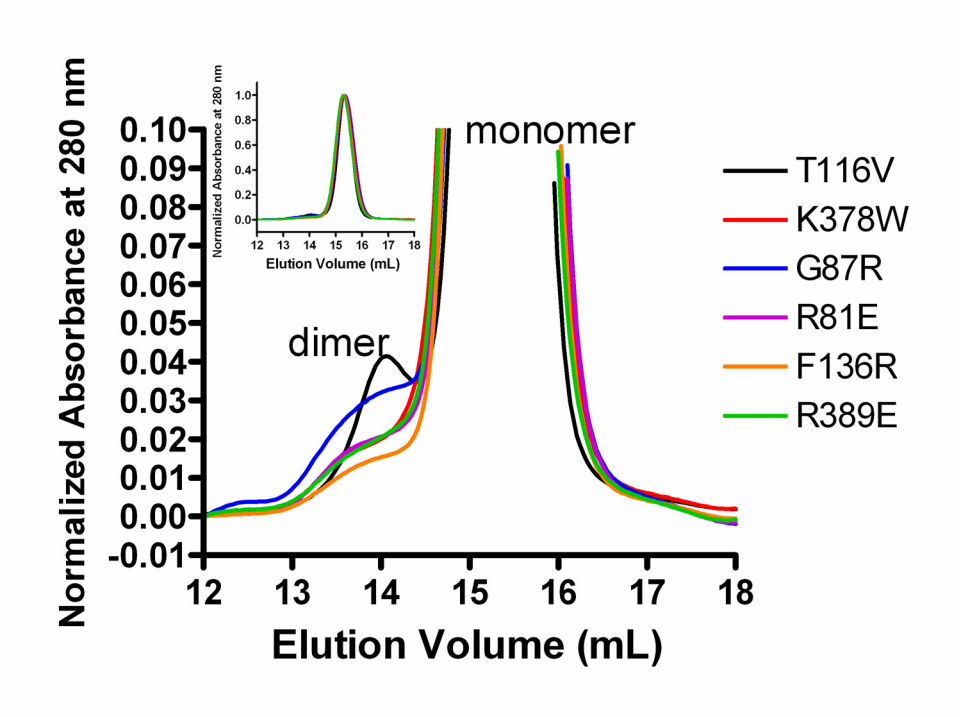
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
12
13
14
15
16
17
Elution Volume (mL)
dimer
monomer
7 μM
14μM
28 μM
57 μM
93 μM
220 μM
409 μM
788 μM

Fig. S4


Normalized Absorbance at 280 nm
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0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
-0.01
12
13
14
15
16
17
18
Elution Volume (mL)
dimer
monomer
T116V
K378W
G87R
R81E
F136R
R389E
Normalized Absorbance at 280 nm
1.0
0.8
0.6
0.4
0.2
0.0
12
13
14
15
16
17
18
Elution Volume (mL)

Fig. S5

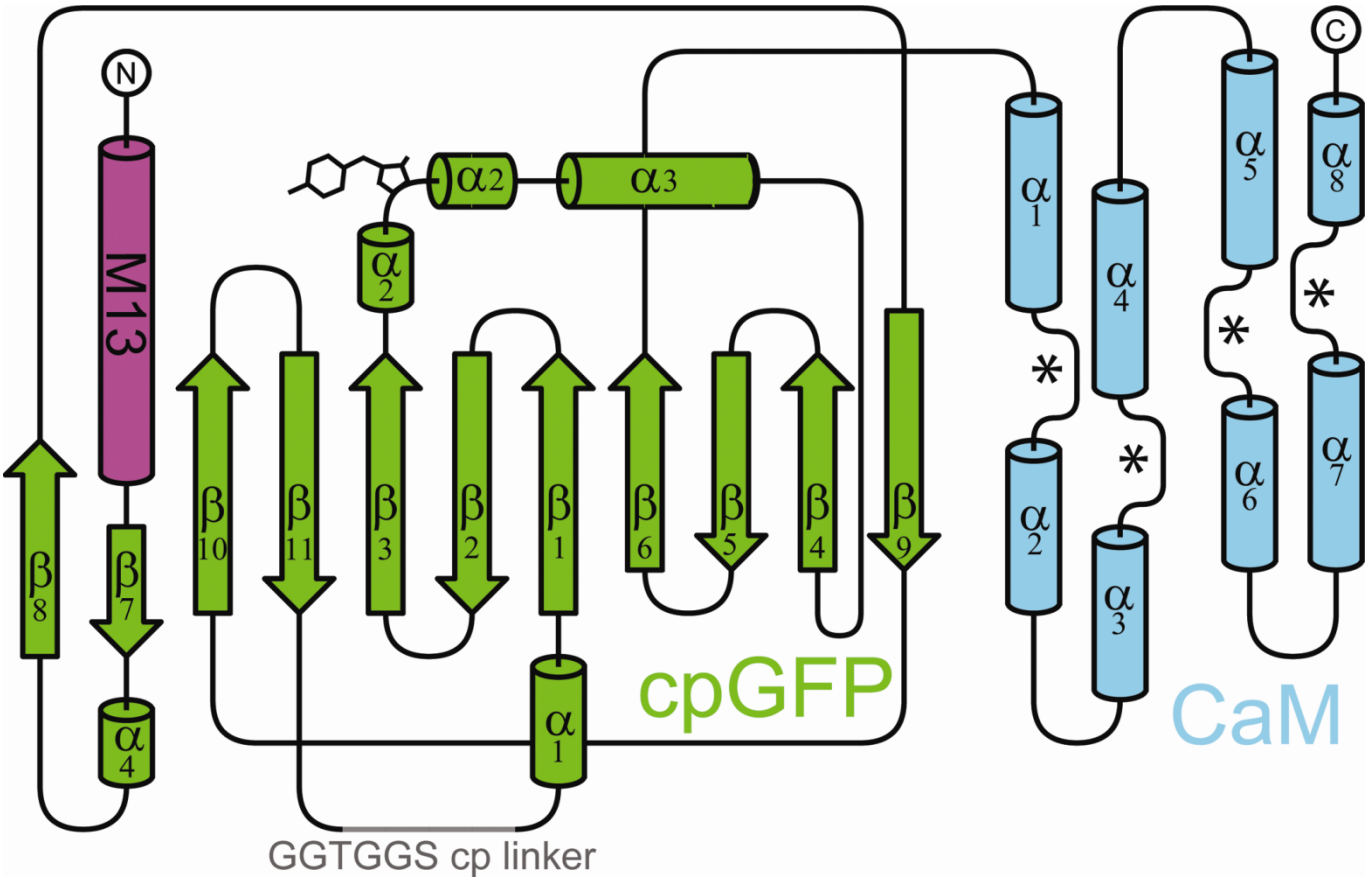
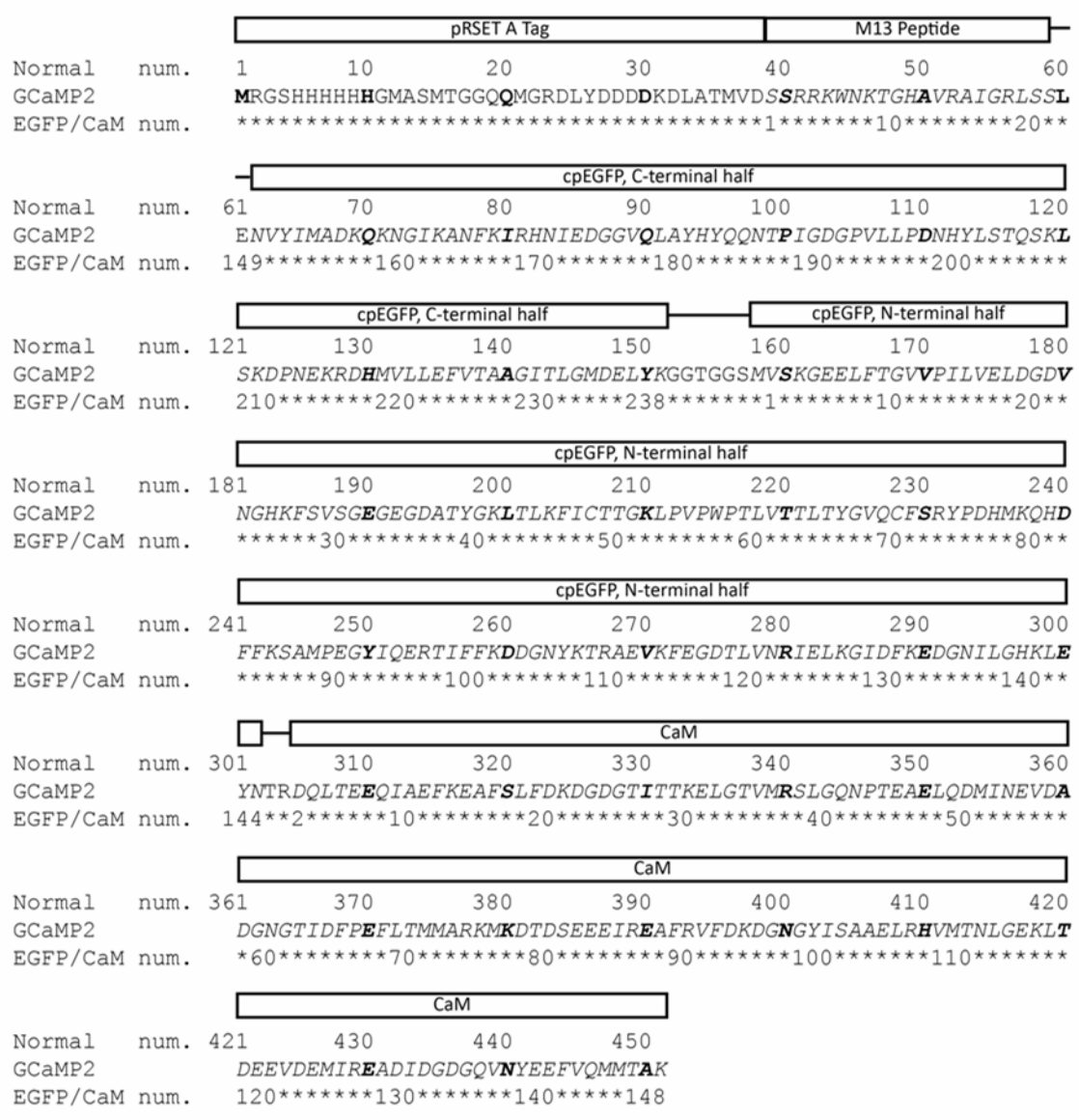

N
C
M13
α2
α3
α2
α1
α4
α5
α8
α6
α7
α3
β8
β7
β10
β11
β3
β2
β1
β6
β5
β4
β9
α1
cpGFP
CaM
GGTGGS cp linker

Fig. S6


pRSET A Tag
M13 Peptide
Normal num. 1 10 20 30 40 50 60
GCaMP2 MRGSHHHHHHGMASMTGGQQMGRDLYDDDDKDLATMVDSSRRKWNKTGHAVRAIGRLSSL
EGFP/CaM num. ************************************1*******10********20**
cpEGFP, C-terminal half
Normal num. 61 70 80 90 100 110 120
GCaMP2 ENVYIMADKQKNGIKANFKIRHNIEDGGVQLAYHYQQNTPIGDGPVLLPDNHYLSTQSKL
EGFP/CaM num. 149********160*******170*******180*******190*******200*******
cpEGFP, C-terminal half
cpEGFP, N-terminal half
Normal num. 121 130 140 150 160 170 180
GCaMP2 SKDPNEKRDHMVLLEFVTAAGITLGMDELYKGGTGGSMVSKGEELFTGVVPILVELDGDV
EGFP/CaM num. 210*******220*******230*****238*******1*******10********20**
cpEGFP, N-terminal half
Normal num. 181 190 200 210 220 230 240
GCaMP2 NGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHMKQHD
EGFP/CaM num. ******30********40********50********60********70********80**
cpEGFP, N-terminal half
Normal num. 241 250 260 270 280 290 300
GCaMP2 FFKSAMPEGYIQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLE
EGFP/CaM num. ******90*******100*******110*******120*******130*******140**
CaM
Normal num. 301 310 320 330 340 350 360
GCaMP2 YNTRDQLTEEQIAEFKEAFSLFDKDGDGTITTKELGTVMRSLGQNPTEAELQDMINEVDA
EGFP/CaM num. 144**2******10********20********30********40********50*******
CaM
Normal num. 361 370 380 390 400 410 420
GCaMP2 DGNGTIDFPEFLTMMARKMKDTDSEEEIREAFRVFDKDGNGYISAAELRHVMTNLGEKLT
EGFP/CaM num. *60********70********80********90*******100*******110*******
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Normal num. 421 430 440 450
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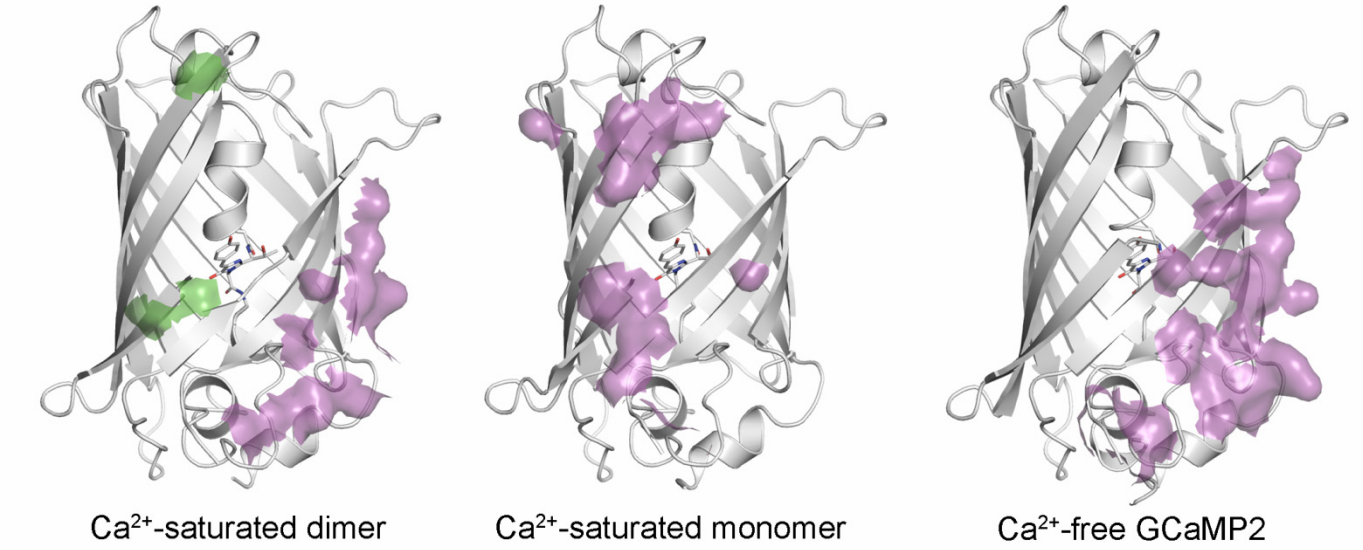
EGFP/CaM num. 120*******130*******140*****148

Fig. S7



Ca^{2+}-saturated dimer

Ca^{2+}-saturated monomer

Ca^{2+}-free GCaMP2

Fig. S8

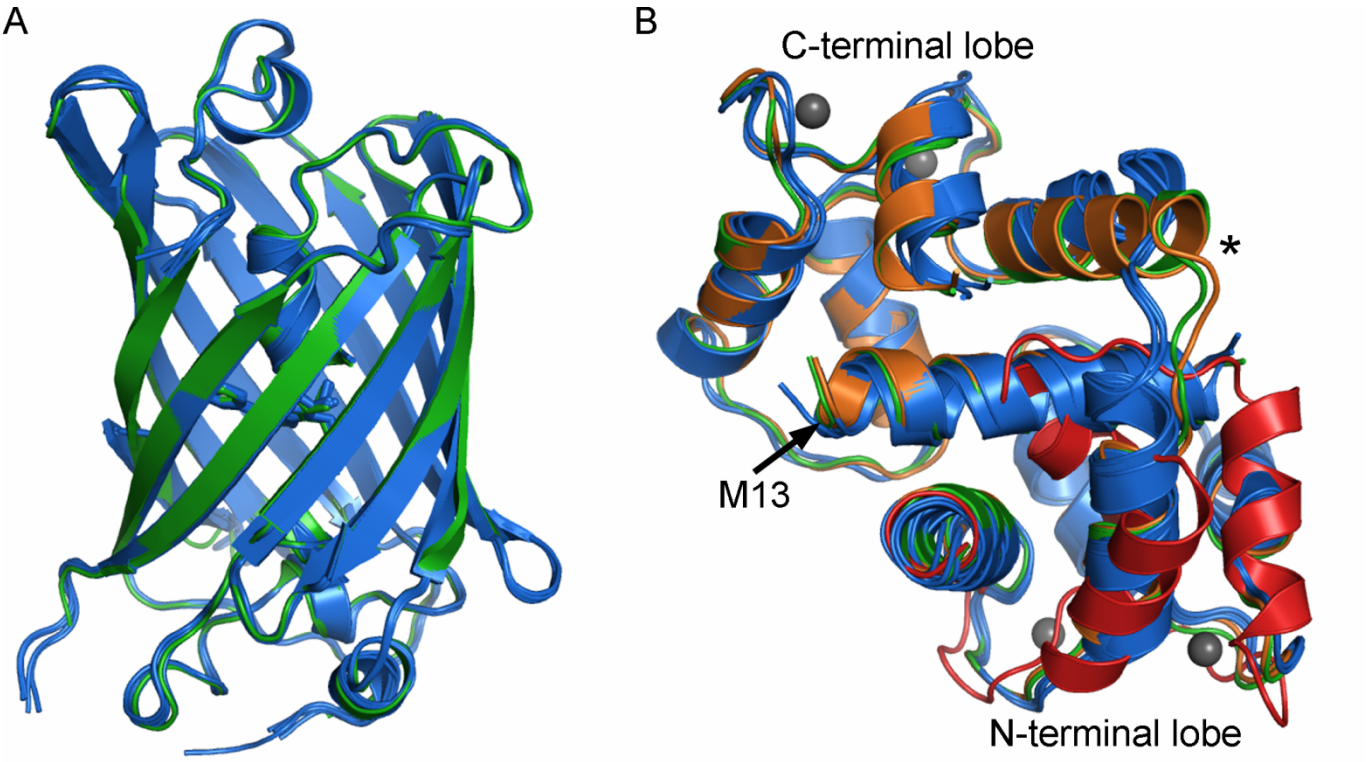
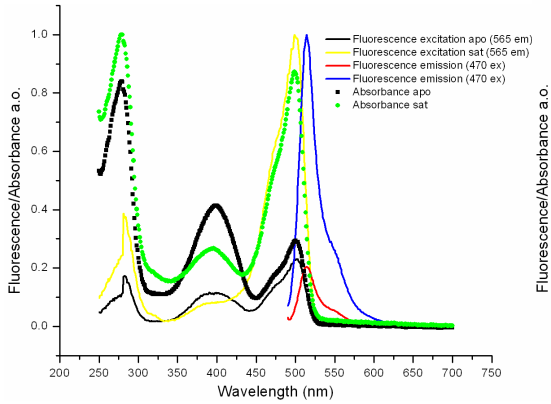
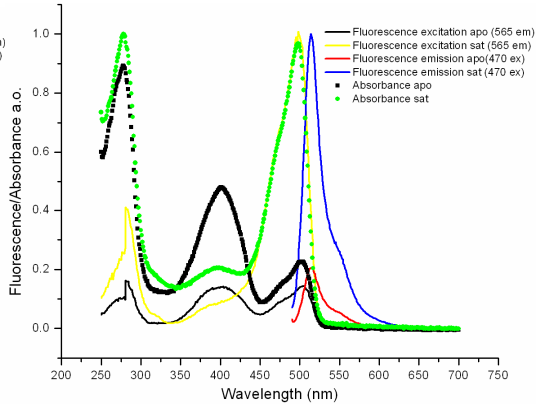


Fig. S9

T303W



T303Y



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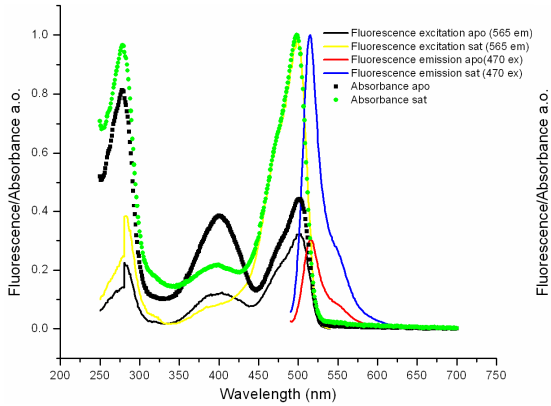

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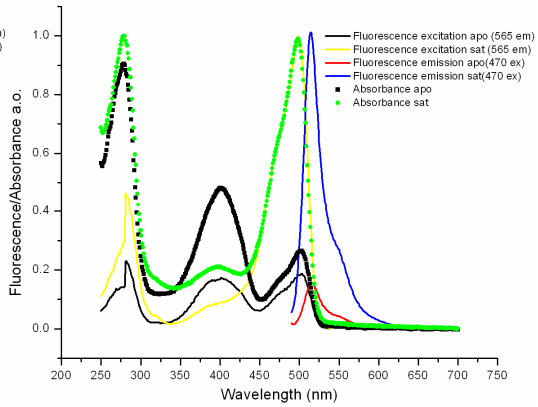

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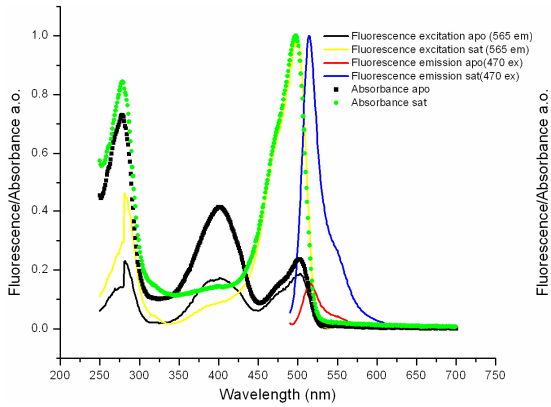

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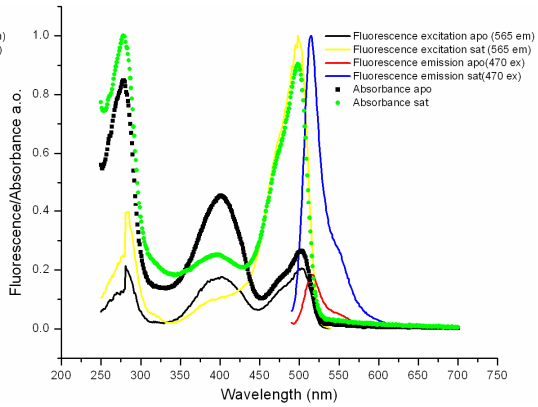

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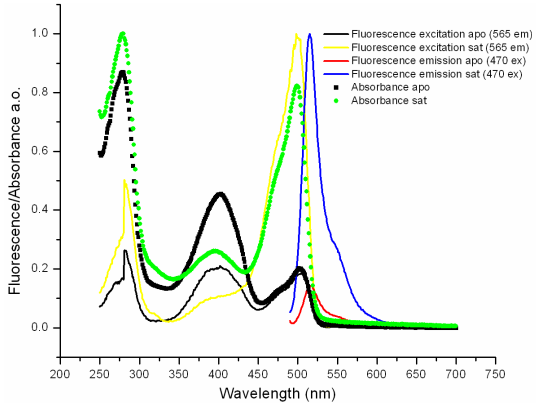

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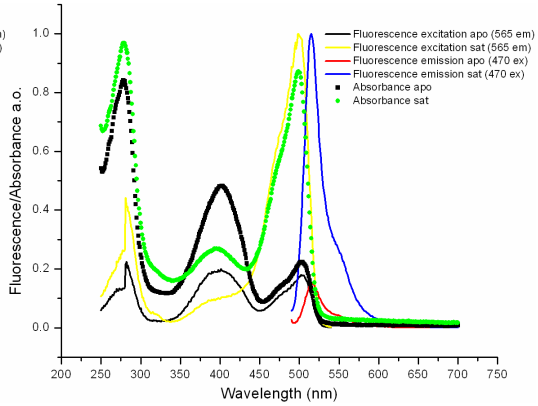

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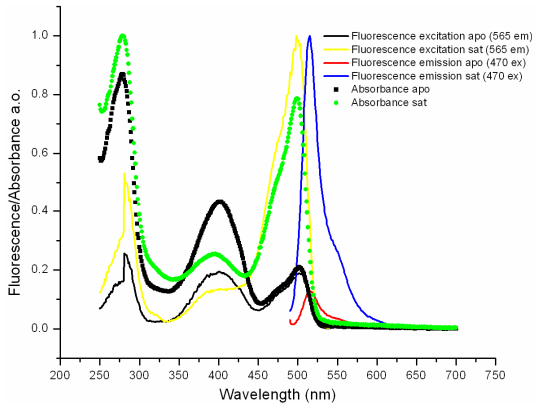

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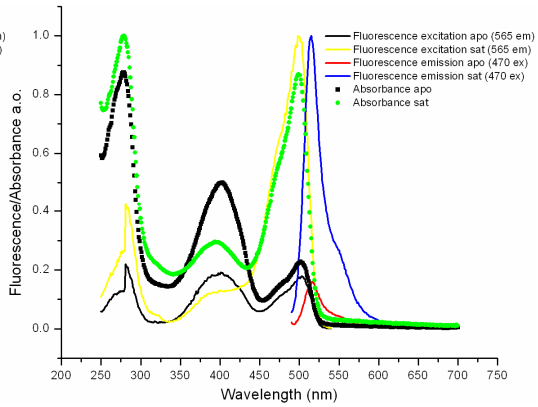

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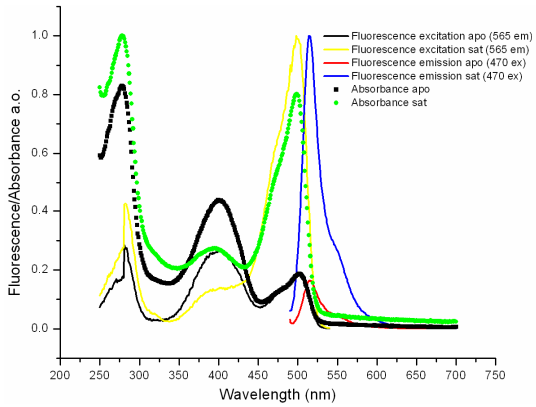

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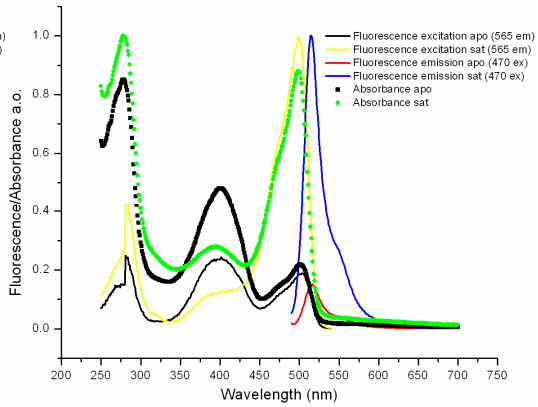

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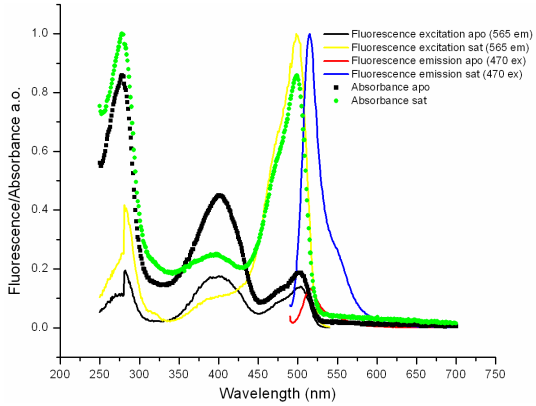

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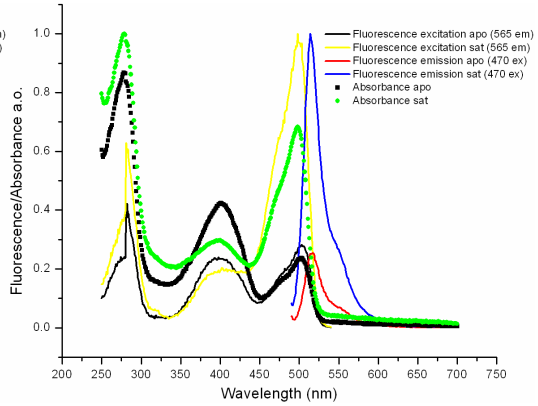

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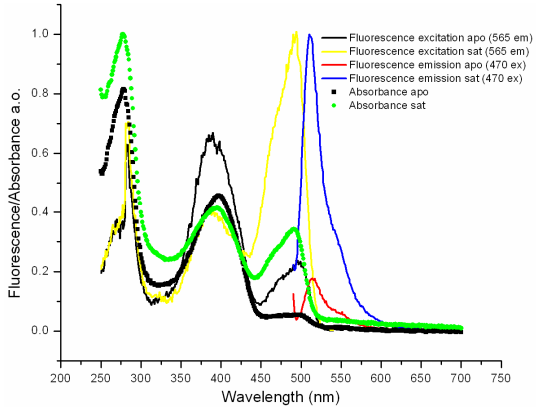

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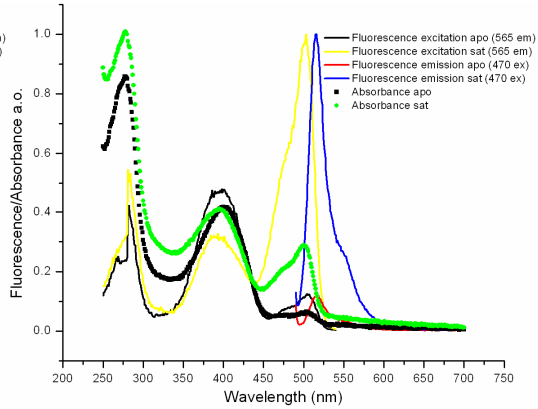

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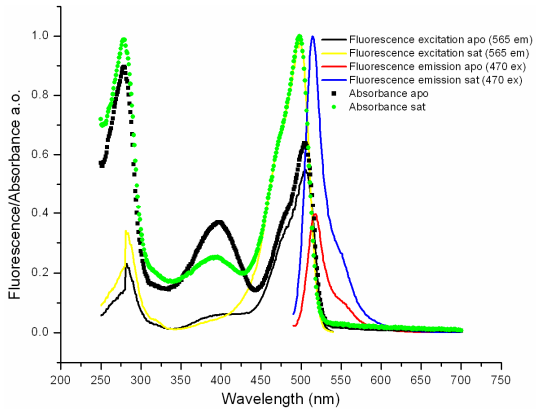

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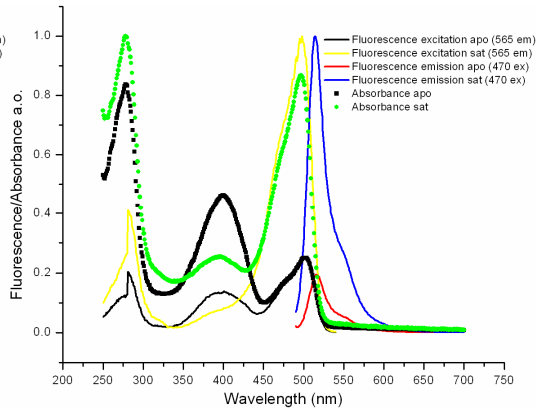

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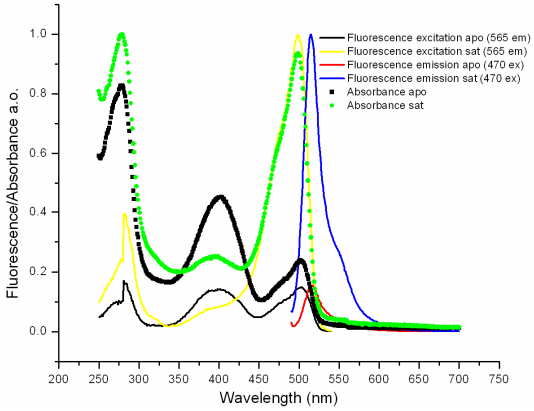

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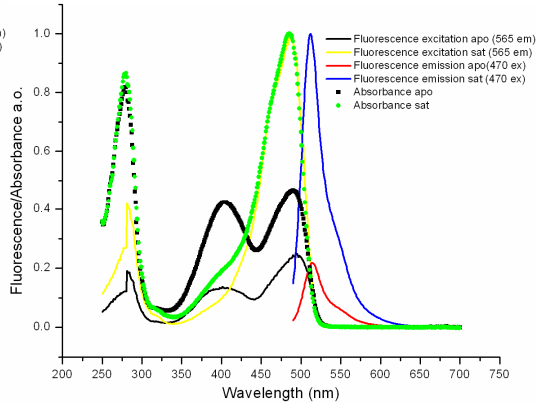

8-EF GCaMP2

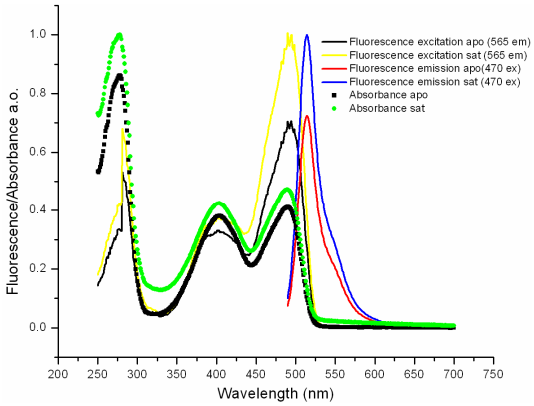

Fig. S10