

Novel genetically encoded bright positive calcium indicator NCaMP7 based on the mNeonGreen fluorescent protein

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Table S1. *In vitro* properties for the purified derivatives of NCaMP7 indicator with different calcium affinities.

Properties		Proteins					
		NCaMP4		NCaMP9		NCaMP10	
		apo	sat	apo	sat	apo	sat
Abs/Exc maximum (nm)		400/400	505/508	403/402	509/512	403/408	509/512
Emission maximum (nm)		520		520	522	520	522
Quantum yield ^a		0.079±0.002	0.53±0.03	0.047±0.003	0.51±0.03	0.056±0.002	0.52±0.03
ϵ (mM ⁻¹ cm ⁻¹) ^b		43.3±5.9	101.0±4.0	51.2±4.8	106.5±6.2	46.0±6.2	112.0±5.5
Brightness (%) ^c		11	166	7.4	170	8.1	182
$\Delta F/F$ (fold)	0 mM Mg ²⁺	32±5		99±73		61±16	
	1 mM Mg ²⁺	8.9±0.2		29±5		15±1	
pK _a		5.38±0.03 6.84±0.04	5.88±0.16	5.43±0.09 6.58±0.15	6.04±0.30	5.44±0.09 6.61±0.1	6.15±0.29
K _d (nM) ^d	0 mM Mg ²⁺	66±4 [n=1.89±0.14]		104±7 [n=1.9±0.2]		204±16 [n=1.7±0.2]	
	1 mM Mg ²⁺	82±6 [n=1.47±0.12]		173±14 [n=1.4±0.2]		306±14 [n=1.36±0.08]	
k _{obs} (s ⁻¹) ^e		1.46 ± 0.01		0.35 ± 0.01		0.59 ± 0.01	
k _{off} (s ⁻¹) ^f	k ₁ (contrib., %)	0.54 ± 0.01		0.74 ± 0.01 (68 ± 1)		1.20 ± 0.01 (83 ± 1)	
	k ₂ (contrib., %)			0.07 ± 0.01 (32 ± 1)		0.12 ± 0.01 (17 ± 1)	
t _{1/2} (s) ^g		1.3 ± 0.1		1.6 ± 0.1		0.75 ± 0.04	

^a EGFP (QY=0.60 ^{ref. [1]}) and mTagBFP2 (QY=0.64 ^{ref. [2]}) were used as reference standards for 505-509- and 400-404-nm absorbing states, respectively. ^b Extinction coefficient was determined by alkaline denaturation. ^c Brightness was normalized to EGFP having QY of 0.60 and extinction coefficient of 55.3±3.6 mM⁻¹cm⁻¹. ^d Hill coefficient is shown in square brackets. ^e The observed association rates were determined at 300 nM Ca²⁺ concentration from association kinetics curves (ESI Figure S2). GCaMP6f and GCaMP6s had k_{obs} value of 1.28 ± 0.03 and 0.49 ± 0.05 sec⁻¹, respectively. ^f k_{off} values were estimated from calcium dissociation curves (ESI Figure S2) using mono or double exponential decay fitting with individual exponent contributions shown in the brackets. GCaMP6f and GCaMP6s had k_{off} values of 1.89 ± 0.01 and 0.69±0.01 s⁻¹, respectively. ^g GCaMP6f and GCaMP6s had t_{off} values of 0.37±0.04 and 1.01±0.04 s, respectively.

Table S2. Calcium association kinetics: k_{obs} rate constants at 700 and 1000 nM calcium concentration.

Indicator	k_{obs} (700 nM Ca^{2+}), s^{-1}		k_{obs} (1000 nM Ca^{2+}), s^{-1}	
	k_1 (contrib., %)	k_2 (contrib., %)	k_1 (contrib., %)	k_2 (contrib., %)
NCaMP4	5.15 ± 0.02		19.7 ± 0.3	
NCaMP7	2.1 ± 0.2 (88 $\pm 3\%$)	0.3 ± 0.1 (12 $\pm 3\%$)	7.0 ± 0.1 (72 $\pm 2\%$)	1.4 ± 0.1 (28 $\pm 2\%$)
NCaMP9	1.86 ± 0.03 (83 $\pm 4\%$)	0.38 ± 0.08 (17 $\pm 4\%$)	13.4 ± 0.9 (70 $\pm 10\%$)	4.7 ± 0.6 (30 $\pm 10\%$)
NCaMP10	1.35 ± 0.01		2.3 ± 0.1 (85 $\pm 10\%$)	1.4 ± 0.5 (15 $\pm 10\%$)
GCaMP6s	2.28 ± 0.01		5.99 ± 0.02	
GCaMP6f	6.36 ± 0.02		17.9 ± 0.2	

The observed association rates were determined at given Ca^{2+} concentrations from association kinetics curves (ESI Figure S2).

Table S3. Data collection, processing and refinement.

Data collection	
Diffraction source	K4.4 beamline, NRC "Kurchatov Institute"
Wavelength (Å)	0.79272
Temperature (K)	100
Detector	CCD
Crystal-to-detector distance (mm)	120.00
Rotation range per image (°)	1.0
Total rotation range (°)	276
Space group	P2 ₁ 2 ₁ 2 ₁
<i>a</i> , <i>b</i> , <i>c</i> (Å)	61.90; 65.70; 93.02
α , β , γ (°)	90.0
Average mosaicity (°)	0.124
Unique reflections	38947 (8372)
Resolution range (Å)	50.0-1.75 (1.90-1.75)
Completeness (%)	100.0 (100.0)
Average redundancy	11.4 (11.5)
$\langle I/\sigma(I) \rangle$	14.5 (3.4)
R _{meas} (%)	16.5 (88.1)
CC _{1/2}	99.8 (85.0)
Refinement	
<i>R</i> _{fact} (%)	16.3
<i>R</i> _{free} (%)	19.3
Bonds (Å)	0.01
Angles (°)	1.81
<i>Ramachandran plot</i>	
Most favoured (%)	98.4
Allowed (%)	1.6
<i>No. atoms</i>	
Protein	3066
Water	357
Chromophore	19
Calcium ion	4
Other ligands	20
<i>B-factors</i> (Å ²)	
Protein	13.06
Water	25.31
Chromophore	14.55
Calcium ion	11.40
Other ligands	22.70

Values in parenthesis are for the highest-resolution shell.

Table S4. Comparison of the NCaMP7 and its variants response to the addition of ionomycin in HeLa cells.

Indicator in fusion	$\Delta F/F_0$	Relative $\Delta F/F_0$ ^a	Relative brightness ^a	N cells
NES-jRGECO1a-P2A-NCaMP7	10.0±2.4 (p=0.8016) ^b	3.2±0.9 (p=0.0079) ^c	1.1±0.2 (p=0.6667) ^b	5
NES-jRGECO1a-P2A-NCaMP4	2.0±0.6 (p=0.0079) ^b	1.0±0.5 (p=0.6032) ^c	1.3±0.4 (p=0.4127) ^b	
NES-jRGECO1a-P2A-NCaMP9	22.5±3.3 (p=0.0079) ^b	7.3±0.8 (p=0.0079) ^c	1.0±0.2 (p=0.8889) ^b	
NES-jRGECO1a-P2A-NCaMP10	19.6±1.7 (p=0.0159) ^b	7.2±0.9 (p=0.0079) ^c	1.4±0.2 (p=0.0238) ^b	
NES-jRGECO1a-P2A-GCaMP6s	12.2±3.1	4.4±1.2 (p=0.0079) ^c	1.0±0.3	

HeLa cells transiently co-expressing green NCaMPs and red NES-jRGECO1a indicators were imaged in DMEM medium supplemented with 20 mM HEPES, pH 7.40, 10% FBS, Glutamine, 50 U/ml penicillin, and 50 U/ml streptomycin before and after addition of 2.5 μ M ionomycin till indicator fluorescence reached plateau. Errors correspond to standard deviations. Data were averaged across 5 cells.

^a For estimation of relative $\Delta F/F_0$ and brightness values, the maximal $\Delta F/F_0$ values and maximal green fluorescence of calcium indicators were normalized to the maximal $\Delta F/F_0$ values and maximal red fluorescence of jRGECO1a red calcium indicator co-expressing in the same cell. Relative brightness was additionally normalized to the relative brightness of GCaMP6s.

^b p value shows statistical difference between the respective values for NCaMPs and GCaMP6s control indicator.

^c p value shows statistical difference between the respective values for NCaMPs/GCaMP6s and jRGECO1a control indicator.

Table S5. Characteristics of calcium responses to external field stimulation of neurons co-expressing the green NCaMPs and GCaMP6s indicators with control red RGECO1 GECI in dissociated neuronal culture.

Indicator	$\Delta F/F_0/1AP$, % ^b	N cells
NCaMP7	40±9 (p<0.0001)	8
NCaMP4	19±13 (p=0.2494)	5
NCaMP9	36±9 (p<0.0001)	8
NCaMP10	13±18 (p=0.0953)	9
GCaMP6s ^a	13±8	44

Neurons expressing NES-R-GECO1-P2A-NCaMPs were stimulated and imaged on DIV 7th using confocal microscope and electric stimulator as described earlier [3]. Neuronal cultures were transfected on DIV 4th using calcium-phosphate approach. Errors correspond to standard deviations. Data were averaged across 5-44 cells.

^a Neurons co-expressing the GCaMP6s and NES-R-GECO1 indicators were imaged and stimulated on DIV 14-20th. Neuronal cultures were transduced on DIV 4th with the mixture of rAAVs viral particles carrying GCaMP6s and NES-R-GECO1.

^b The $\Delta F/F$ values normalized to 1AP were calculated as a slope of the dependence of $\Delta F/F$ responses vs a number of APs (ESI Figure S7). A number of APs was calculated according to the $\Delta F/F$ response of red R-GECO1 indicator co-expressing in the same cell, assuming that its $\Delta F/F$ value of 4±1% corresponds to 1AP and has linear dependence from a number of APs [4]. p value shows statistical difference between the respective values for NCaMPs and GCaMP6s control indicator.

Table S6. Comparison of calcium responses in neurons expressing NCaMPs indicators in hippocampus of freely moving mice registered with nVista HD miniscope *in vivo*.

Properties	Indicators			
	NCaMP4	NCaMP7	NCaMP9	NCaMP10
Number of mice	2	1	2	1
Number of active cells ^a	220±180	51	236±176	112
Number of spikes detected	1491	202	1049	1050
Rise half-time, s ^b	0.9±0.6	0.9±0.7	1.2±0.8	0.8±0.6
Decay half-time, s ^b	2.3±1.3	2.1±1.2	2.5±1.2	2.0±1.2
Peak $\Delta F/F_0$ ^c	0.012±0.008	0.017±0.015	0.013±0.009	0.016±0.014
SNR ^d	4.8±2.7	8.7±6.6	4.6±2.7	5.1±2.0

^a Cell was considered as active, if at least one spike exceeding 4MAD threshold was registered.

^b Rise and decay half-times were calculated as time intervals between the peak of the mean spike and half-peak at front and back slopes of the spike.

^c Peak $\Delta F/F_0$ was calculated as $(F-F_0)/F_0$, where F_0 is the baseline fluorescence signal averaged over the whole period of imaging.

^d Signal-to-noise ratio (SNR) was quantified as peak $\Delta F/F_0$ response over median absolute deviation (MAD), calculated for each cell over the whole period of imaging.

^{b-d} Mean values ± standard error of mean are given. Analysis of calcium imaging data was performed as previously described [3].

Table S7. Comparison of calcium responses in neurons expressing NCaMP7 and GCaMP6s indicators in hippocampus of freely moving mice registered with nVista HD miniscope *in vivo*.

Properties	Indicators	
	NCaMP7	GCaMP6s
A number of mice	2	4
A number of active cells ^a	230	436
A number of spikes detected	325	316
Rise half-time, s ^b	0.95±0.52 (p<0.0001)	0.76±0.49
Decay half-time, s ^b	3.0±0.9 (p<0.0001)	2.5±0.9
Peak $\Delta F/F_0$ ^c	1.9±1.6 (p=0.0615)	2.1±1.8

^a Only spikes exceeding 4MAD threshold and not less than 50% of maximum value of the trace were taken into account.

^b Rise and decay half-times were calculated as time intervals between the peak of the mean spike and half-peak at front and back slopes of the spike.

^c Peak $\Delta F/F_0$ is given in MIN1PIPE units, which are $\Delta F/F_0$ units after normalization and registration.

^{b-c} Mean values ± standard error of mean are given. Analysis of calcium imaging data was performed as previously described [3].

p value shows statistical difference between the respective values for NCaMP7 and GCaMP6s control indicator.

Table S8. List of primers.

Primer	Primer sequence (5'-3')
Neon-BglII-2	CGAGATCTATGGTCTCAAAGGGAGAGGAG
Neon-EcoRI-r2	GCGAATTCCTACTTATACAGCTCGTCCATGC
NEON-M13:	GACAAATTCCTGACAGCCNNSNNSNSTCATCACGTCGTAAGTGG.
Neon-M13-r:	CCACTTACGACGTGATGASNNSNNSNNGGCTGTCAGGGAATTTGTC
M13-CaM	CTATAGGTCGGCTGAGCTCAGGCGGTAGCGACCAACTGACTGAAGAG
M13-CaM-r	CTCTTCAGTCAGTTGGTCGCTACCGCCTGAGCTCAGCCGACCTATAG
CaM-Neon	CAAATGATGACAGCGAAGNNSNNSNNSGCTGACTGGTGTAGATCC
CaM-Neon-r	GGATCTACACCAGTCAGCSNNSNNSNNTTCGCTGTCATCATTTG
Neon-CaM	GACAAATTCCTGACAGCCNNSNNSNNSGACCAACTGACTGAAGAGC
Neon-CaM-r	GCTCTTCAGTCAGTTGGTCSNNSNNSNNGGCTGTCAGGGAATTTGTC
CaM-M13	CAAATGATGACAGCGAAGGGCGGTAGCTCATCACGTCGTAAGTGG
CaM-M13-r	CCACTTACGACGTGATGAGCTACCGCCCTTCGCTGTCATCATTTG
M13-Neon	CTATAGGTCGGCTGAGCTCANNNSNNSNNSGCTGACTGGTGTAGATCC
M13-Neon-r	GGATCTACACCAGTCAGCSNNSNNSNNTGAGCTCAGCCGACCTATAG
GCaMP-BglII	CGAGATCTGCTAGCATGGTCGACTCATCACGTC
GCaMP-EcoRI-HindIII-r	GTCAAGCTTGAATTCCTACTTCGCTGTCATCATTTG

	1	10	20	30	40	50	60
Library	MVSKG	EEDN	MASL	PATHE	LHIF	GSING	VDFD
NCaMP7	MVSKG	EEDN	MASL	PATHE	LHIF	GSING	VDFD
NCaMP4	MVSKG	EEDN	MASL	PATHE	LHIF	GSING	VDFD
NCaMP9	MVSKG	EEDN	MASL	PATHE	LHIF	GSING	VDFD
NCaMP10	MVSKG	EEDN	MASL	PATHE	LHIF	GSING	VDFD
	61	70	80	90	100	110	120
Library	WILVP	HIGY	GFHQ	YLPY	PDGMS	PFQA	AMVD
NCaMP7	WILVP	HIGY	GFHQ	YLPY	PDGMS	PFQA	AMVD
NCaMP4	WILVP	HIGY	GFHQ	YLPY	PDGMS	PFQA	AMVD
NCaMP9	WILVP	HIGY	GFHQ	YLPY	PDGMS	PFQA	AMVD
NCaMP10	WILVP	HIGY	GFHQ	YLPY	PDGMS	PFQA	AMVD
	121	130	140	150	160	170	180
Library	IKGEA	QVEG	TGFP	ADGPM	VTNSL	TAXXD	QLTEE
NCaMP7	IKGEA	QVEG	TGFP	ADGPM	VTNSL	TAXXD	QLTEE
NCaMP4	IKGEA	QVEG	TGFP	ADGPM	VTNSL	TAXXD	QLTEE
NCaMP9	IKGEA	QVEG	TGFP	ADGPM	VTNSL	TAXXD	QLTEE
NCaMP10	IKGEA	QVEG	TGFP	ADGPM	VTNSL	TAXXD	QLTEE
	181	190	200	210	220	230	240
Library	TVMRSL	GQNP	TEAEL	QDMINE	VDAD	GDGT	IDFP
NCaMP7	TVMRSL	GQNP	TEAEL	QDMINE	VDAD	GDGT	IDFP
NCaMP4	TVMRSL	GQNP	TEAEL	QDMINE	VDAD	GDGT	IDFP
NCaMP9	TVMRSL	GQNP	TEAEL	QDMINE	VDAD	GDGT	IDFP
NCaMP10	TVMRSL	GQNP	TEAEL	QDMINE	VDAD	GDGT	IDFP
	241	250	260	270	280	290	300
Library	KDNGY	IGAAE	LRHVM	TNLGE	KLTD	EEVDE	MIREA
NCaMP7	KDNGY	IGAAE	LRHVM	TNLGE	KLTD	EEVDE	MIREA
NCaMP4	KDNGY	IGAAE	LRHVM	TNLGE	KLTD	EEVDE	MIREA
NCaMP9	KDNGY	IGAAE	LRHVM	TNLGE	KLTD	EEVDE	MIREA
NCaMP10	KDNGY	IGAAE	LRHVM	TNLGE	KLTD	EEVDE	MIREA
	301	310	320	330	340	350	360
Library	GSSSR	RKWN	KAGHA	VRAIG	RLSS	XXXA	DWC
NCaMP7	GSSSR	RKWN	KAGHA	VRAIG	RLSS	XXXA	DWC
NCaMP4	GSSSR	RKWN	KAGHA	VRAIG	RLSS	XXXA	DWC
NCaMP9	GSSSR	RKWN	KAGHA	VRAIG	RLSS	XXXA	DWC
NCaMP10	GSSSR	RKWN	KAGHA	VRAIG	RLSS	XXXA	DWC
	361	370	380	390	400	410	
Library	TARIT	YTF	AKP	MAAN	YLKN	QPMY	VFRK
NCaMP7	TARIT	YTF	AKP	MAAN	YLKN	QPMY	VFRK
NCaMP4	TARIT	YTF	AKP	MAAN	YLKN	QPMY	VFRK
NCaMP9	TARIT	YTF	AKP	MAAN	YLKN	QPMY	VFRK
NCaMP10	TARIT	YTF	AKP	MAAN	YLKN	QPMY	VFRK

Figure S1. Alignment of the amino acid sequences for the original library and NCaMP calcium indicators. Alignment numbering follows that of NCaMPs. Residues from fluorescent part buried in β -can are highlighted with green. Residues that are forming chromophore are underlined. Mutations in NCaMPs related to the original library including linkers between fluorescent and calcium-binding parts are highlighted in red. Residues that are forming Ca^{2+} -binding loops are highlighted in cyan. Calcium-coordinating residues are selected with asterisks.

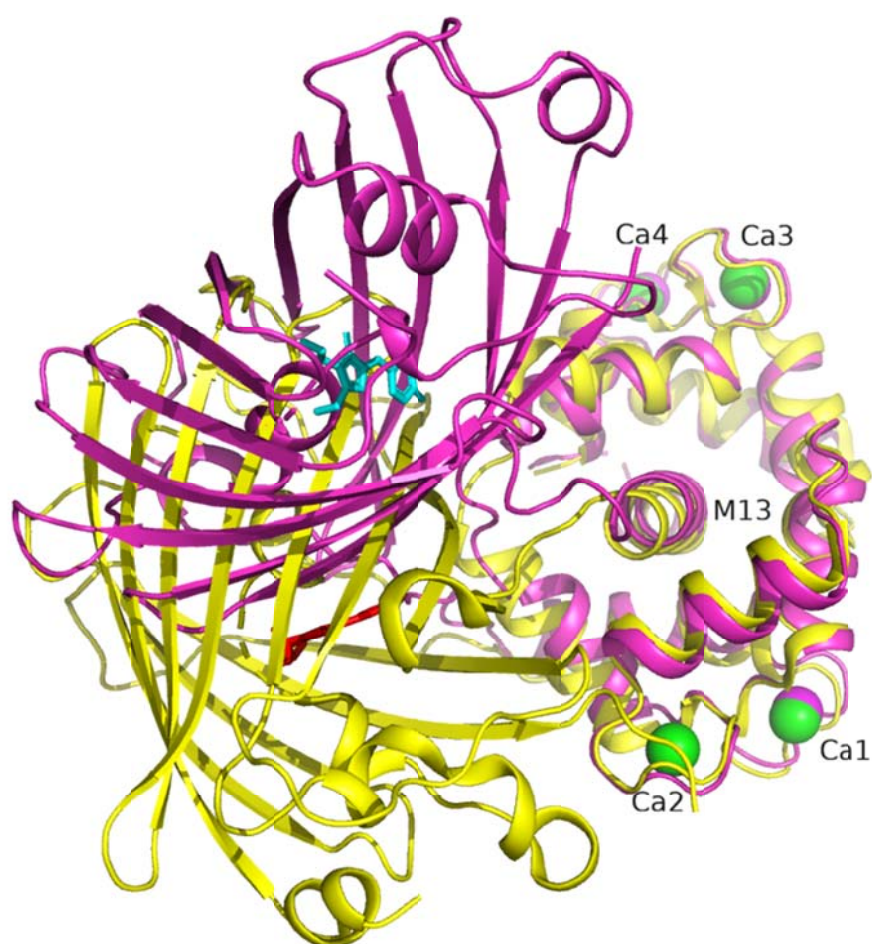


Figure S2. Superposition of NCaMP7 (PDB ID - 6XW2, in magenta) and GCaMP6m (PDB ID 3WLD, in yellow) structures. Chromophores for NCaMP7 and GCaMP6m are shown in cyan and red colors, respectively. Calcium ions for NCaMP7 and GCaMP6m are in magenta and green. M13 peptide is labeled as well as calcium ions. Structures are superposed by their calmodulin domains.

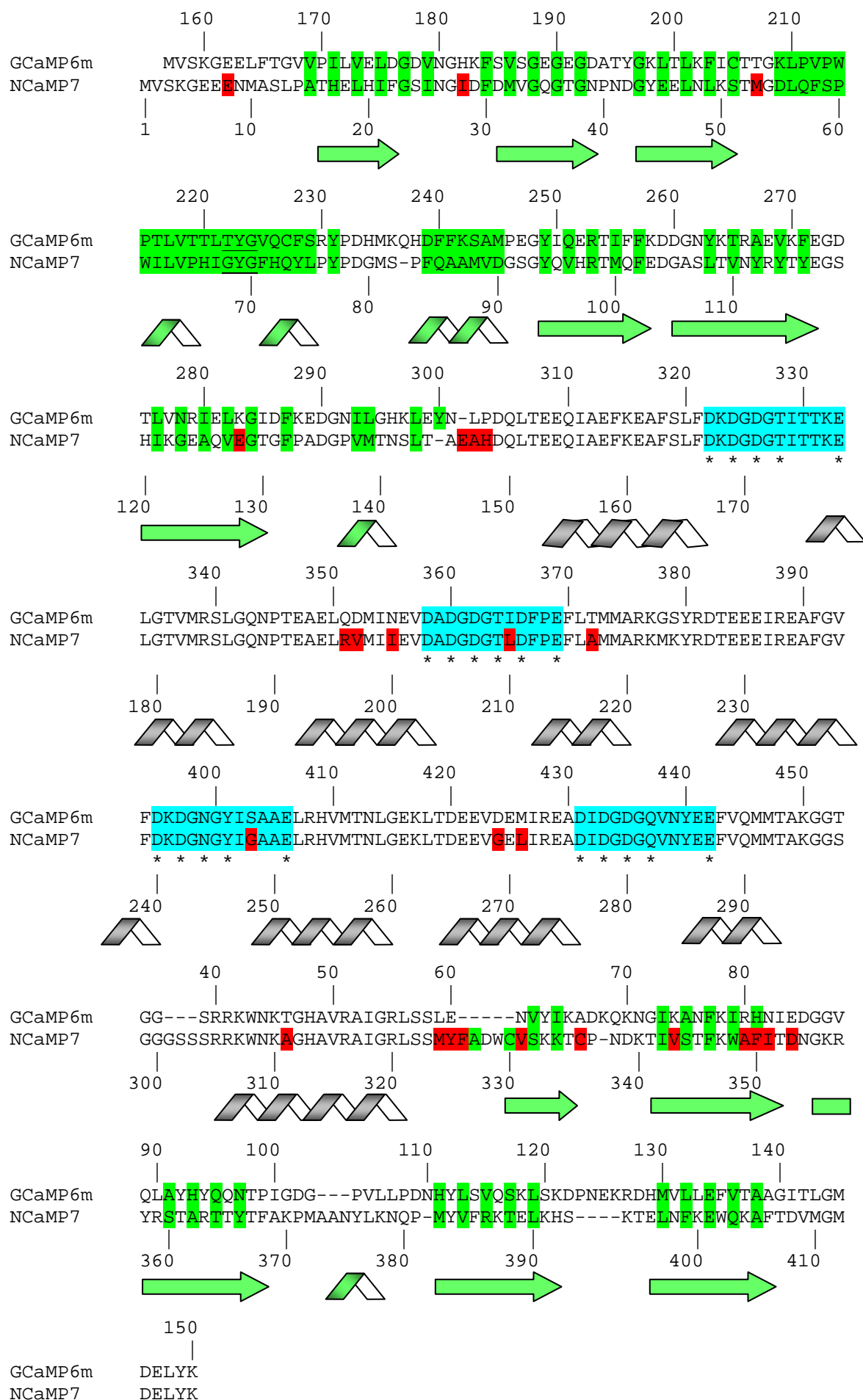


Figure S3. Alignment of the amino acid sequences for the GCaMP6m and NCaMP7 fluorescent proteins. Alignment numbering follows that of GCaMP6m (top line) and NCaMP7 (bottom line). Residues from fluorescent part buried in β -can are highlighted with green. Residues that are forming chromophore are underlined. Mutations in NCaMP7 appeared during protein engineering including linkers between fluorescent and calcium-binding parts are highlighted in red. Residues that are forming Ca^{2+} -binding loops are highlighted in cyan. Calcium-coordinating residues are selected with asterisks. Secondary structure is presented for the NCaMP7 indicator based on its X-ray data.

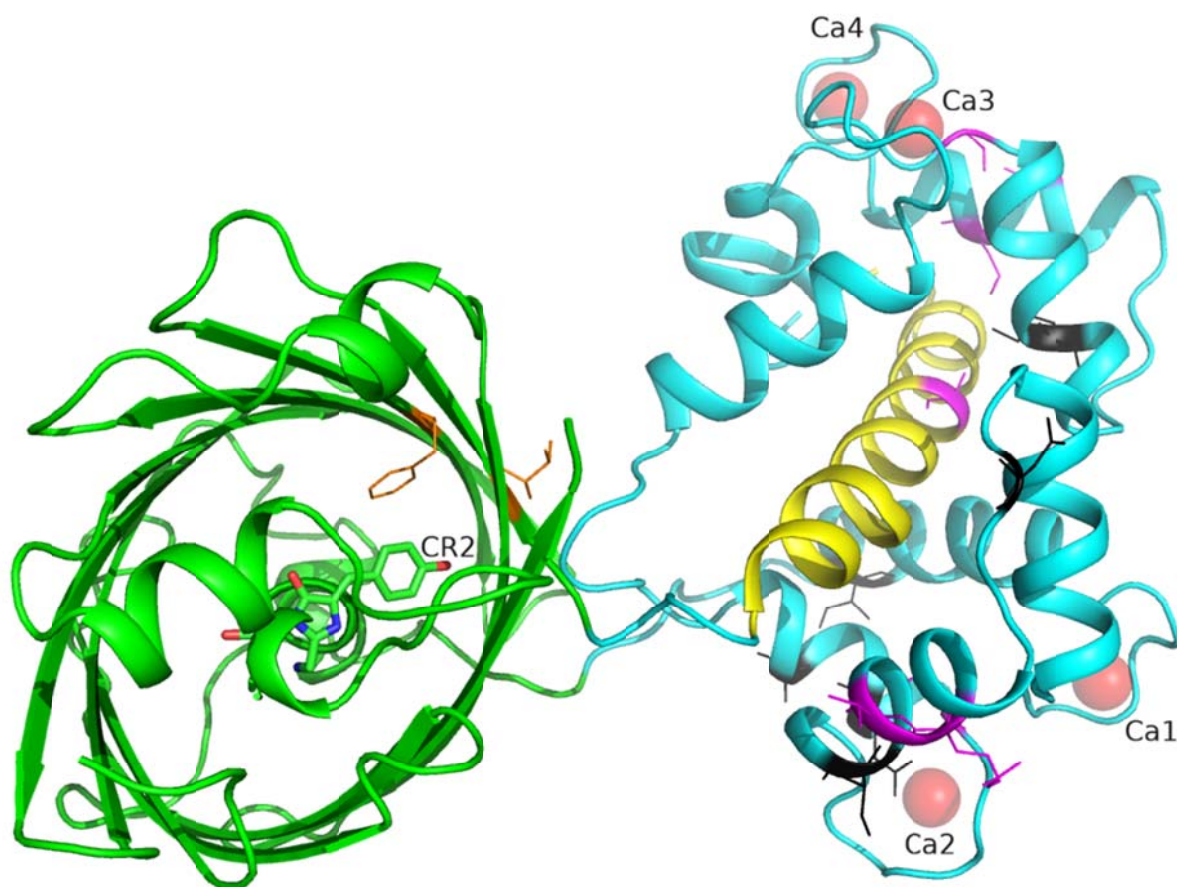


Figure S4. Overview of the mutations found during mutagenesis of NCaMPs on the crystal structure of the NCaMP7 indicator (PDB ID - 6XW2). Cartoon representation of NCaMP7 crystal structure with mutations in fluorescent domain close to the chromophore (within 6Å, in orange), common mutation in CaM and M13-peptide calcium-binding domain (in magenta) and mutations unique to each of NCaMPs (in black).

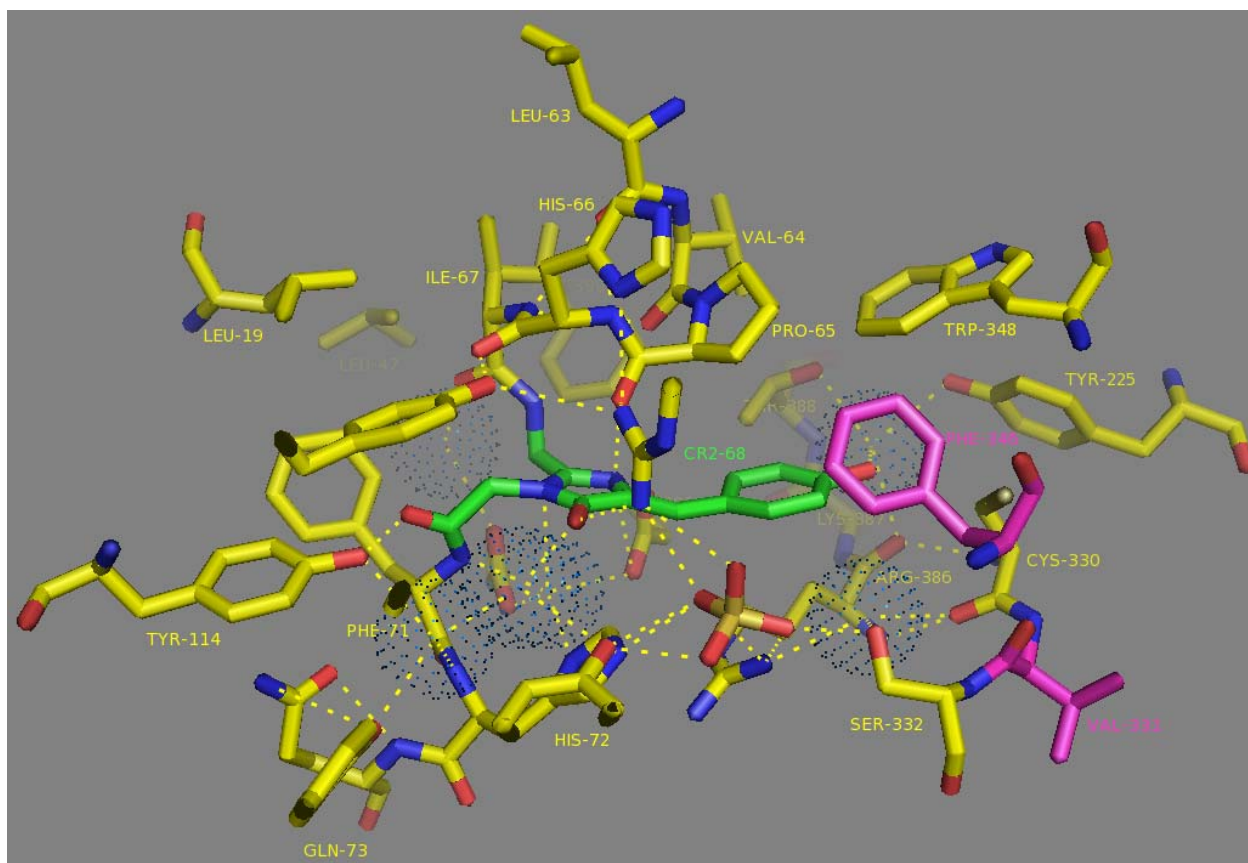


Figure S5. 6.0Å chromophore environment according to NCaMP7 X-ray structure (PDB ID - 6XW2). Chromophore, 6.0Å-surrounding residues, water molecules as dots and V331, F346 residues are shown in green, yellow, blue and magenta colors, respectively. H-bonds are shown as dash lines.

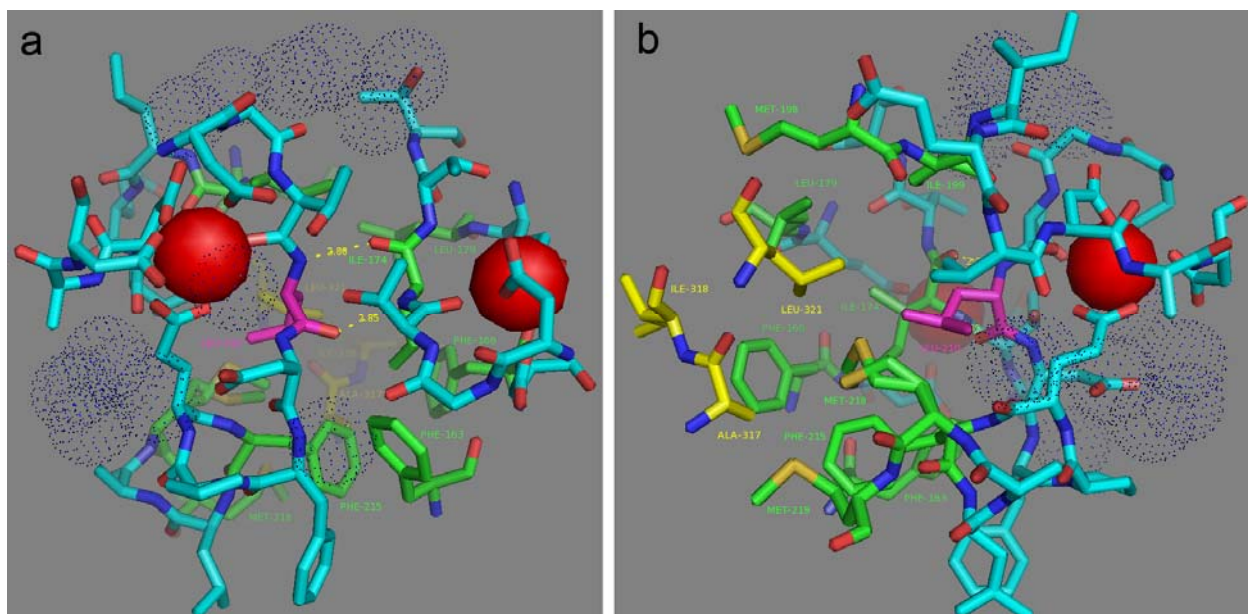


Figure S6. L210 residue hydrogen (a) and hydrophobic (b) contacts according to NCaMP7 X-ray structure (PDB ID - 6XW2). (a) Two H-bonds between L210 (in magenta) and I174 residues are shown as dash lines. (b) Side group of L210 residue (in magenta) is exposed to the hydrophobic pocket which is formed by residues from M13-peptide (A317, I318, and L321, in yellow) and CaM (EF1 and EF2 hands and adjacent to them amino acids) (F163, F166, I174, L179, I199, L210, F215, M218, and M219, in green). Calcium ions and water molecules are shown as red spheres and blue dots, respectively.

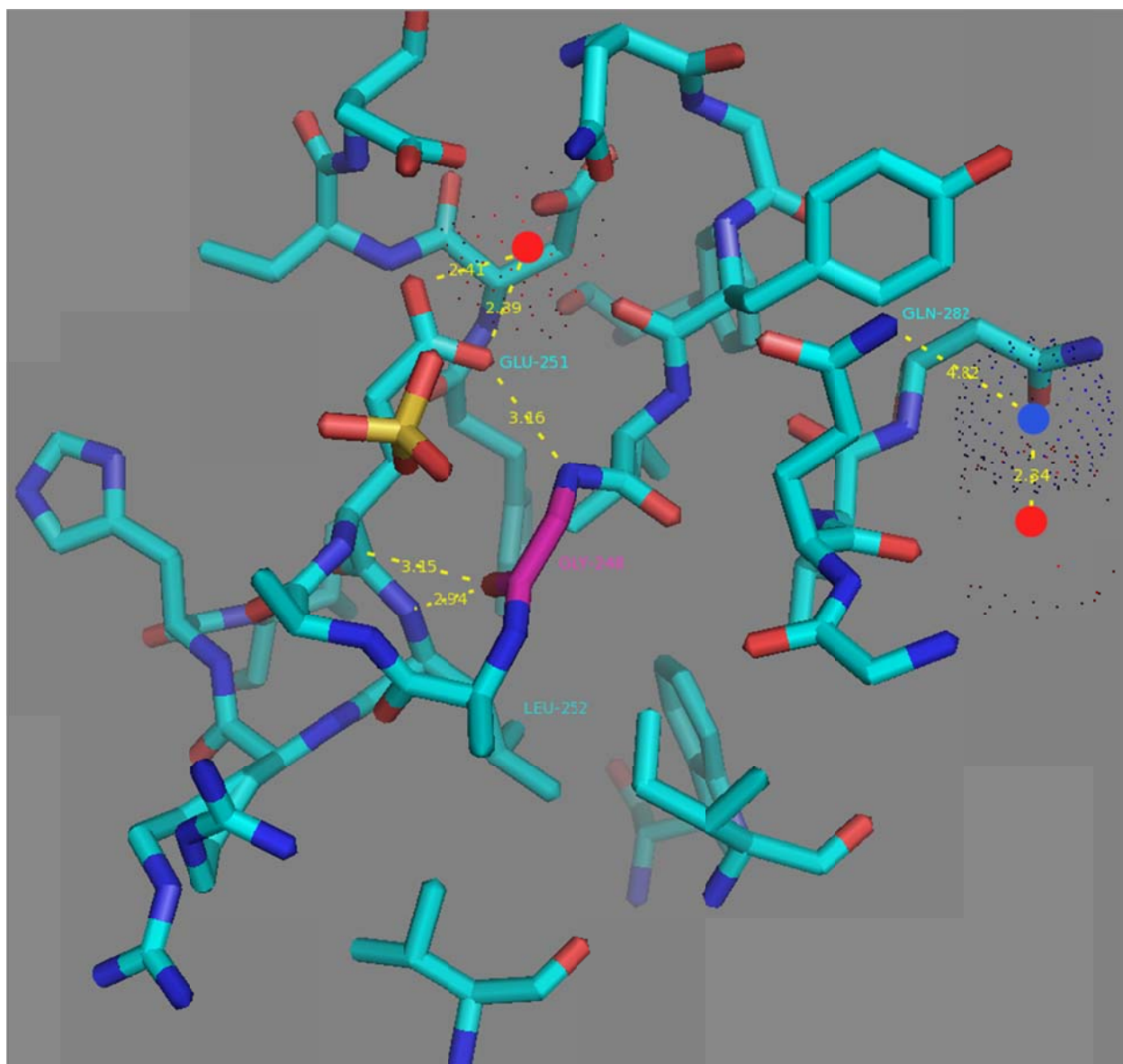


Figure S7. G248 residue surrounding according to NCaMP7 X-ray structure (PDB ID - 6XW2). H-bonds are shown as dash lines between G248 (in magenta) and E251, E251 and calcium ion from EF3 (as red dots), G248 and L252, Q282 and water molecule (as blue dots), water molecule and calcium ion from EF4 (as red dots).

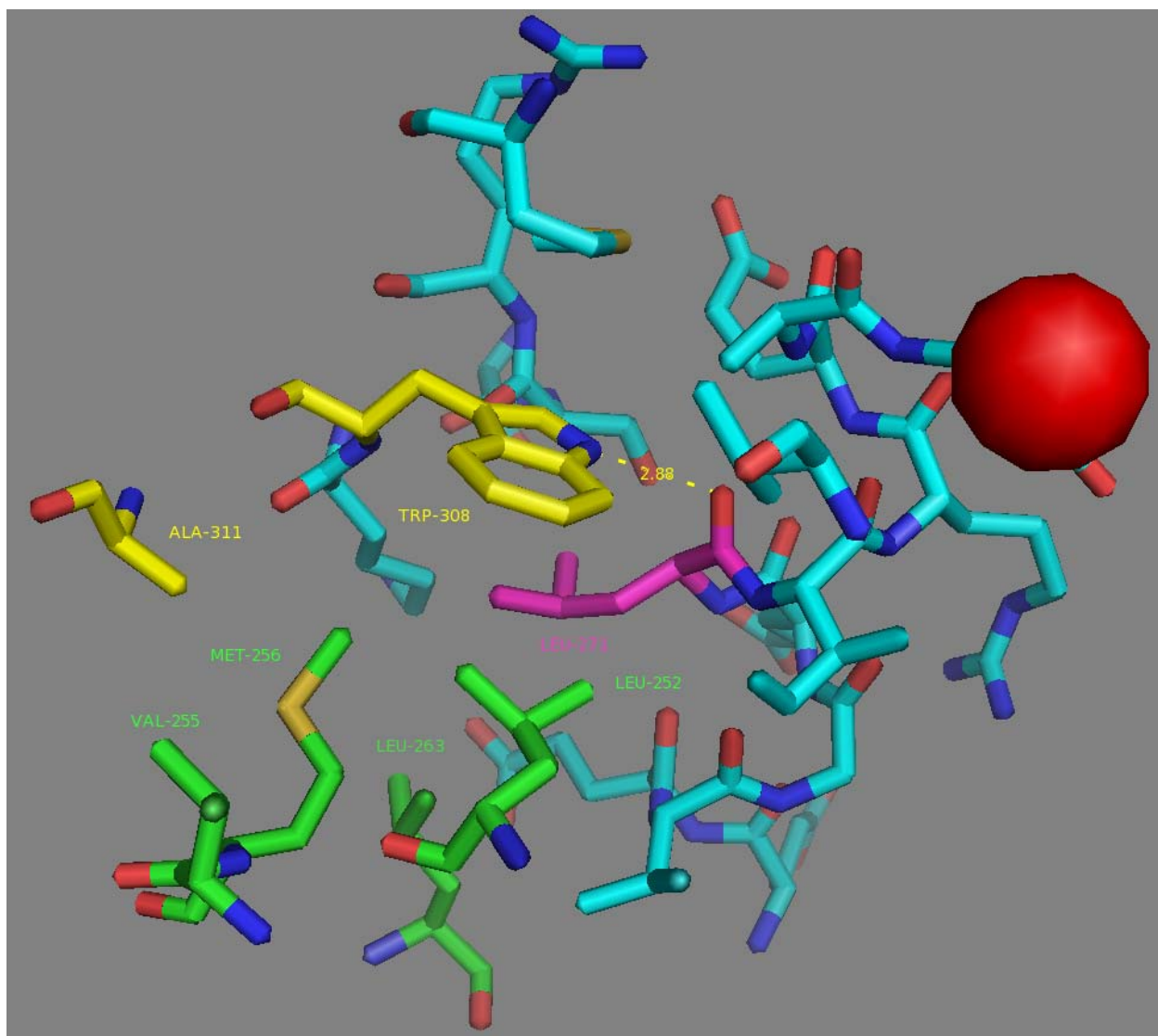


Figure S8. L271 residue surrounding according to NCaMP7 X-ray structure (PDB ID - 6XW2). H-bond is shown as dash lines between L271 (in magenta) and W308. E251 Calcium ion from EF4 is shown as red sphere. Side group of L271 residue (in magenta) is exposed to the hydrophobic pocket which is formed by residues from M13-peptide (A311 and W30, in yellow) and CaM (L252, V255, M256, L263 and L271, in green).

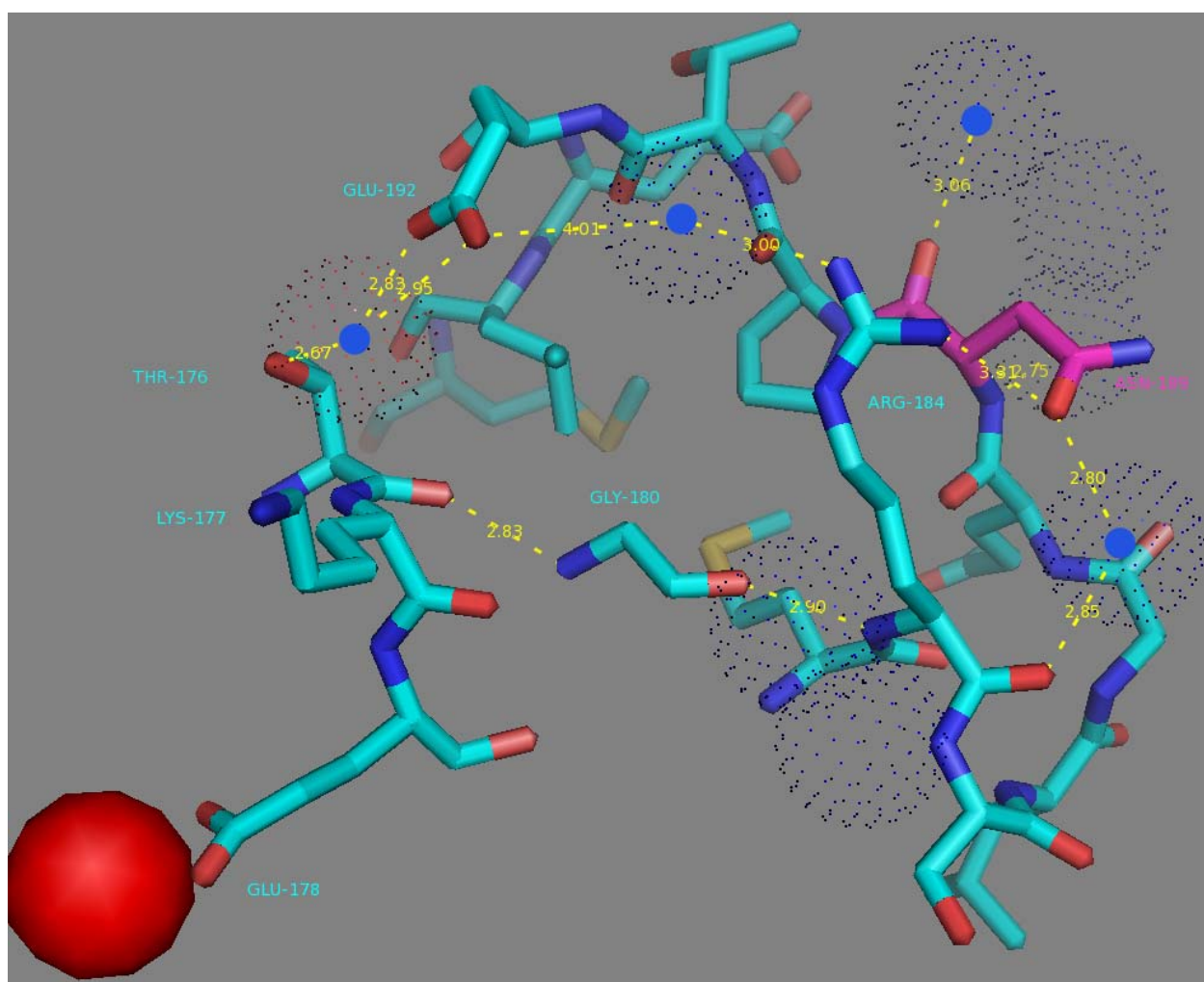


Figure S9. N189 residue surrounding and H-bonds network to the T176 residue from EF1 calcium coordinating center according to NCaMP7 X-ray structure (PDB ID - 6XW2). H-bonds network is shown as dash lines between N189 (in magenta), R184, water molecules (in blue dots), E192, G180 and T176. Calcium ion from EF1 is shown as red sphere.

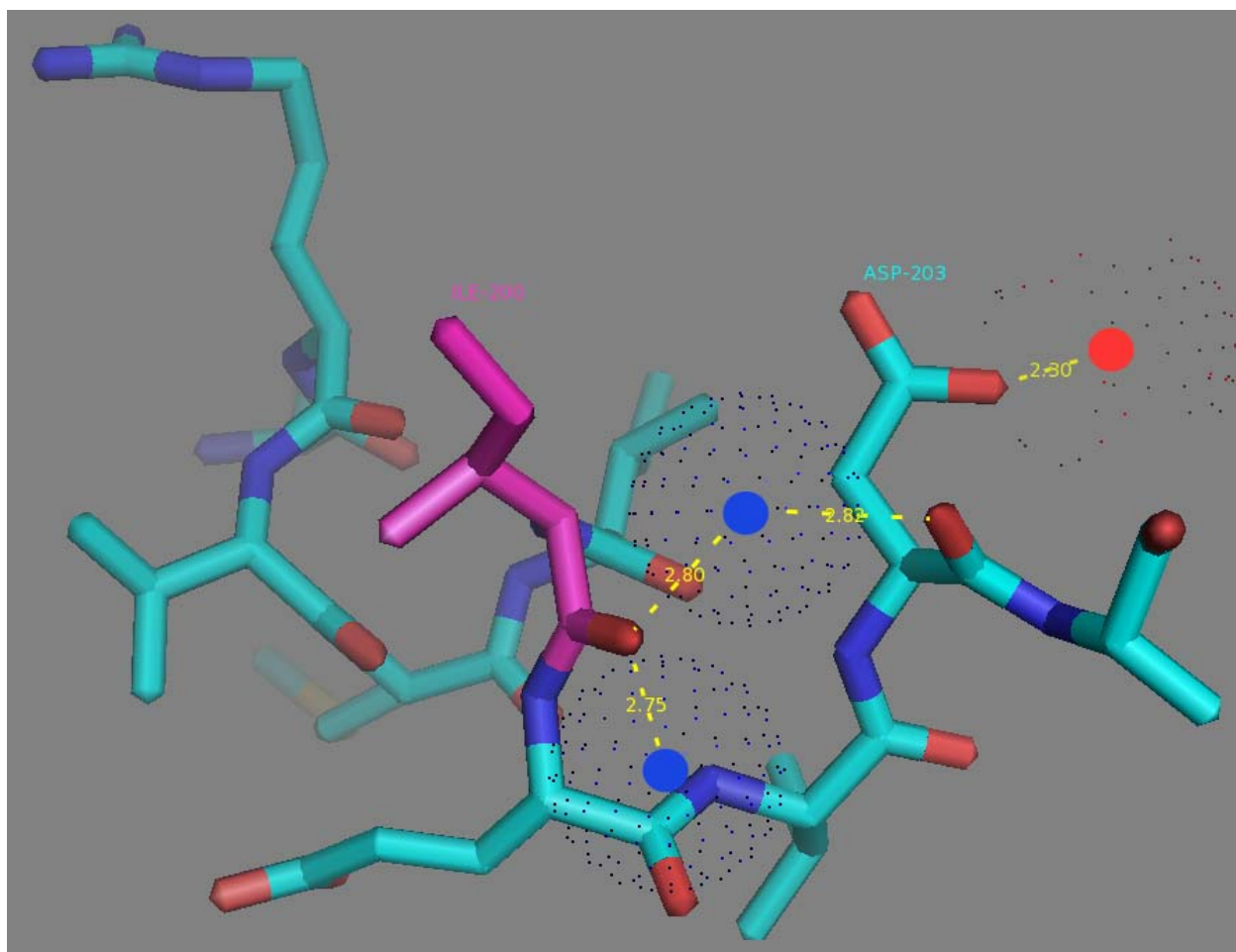


Figure S10. I200 residue surrounding and H-bonds network to the D203 residue from EF2 calcium coordinating center according to NCaMP7 X-ray structure (PDB ID - 6XW2). H-bonds network is shown as dash lines between I200 (in magenta), water molecules (in blue dots), E203 and calcium ion from EF2 (red sphere).

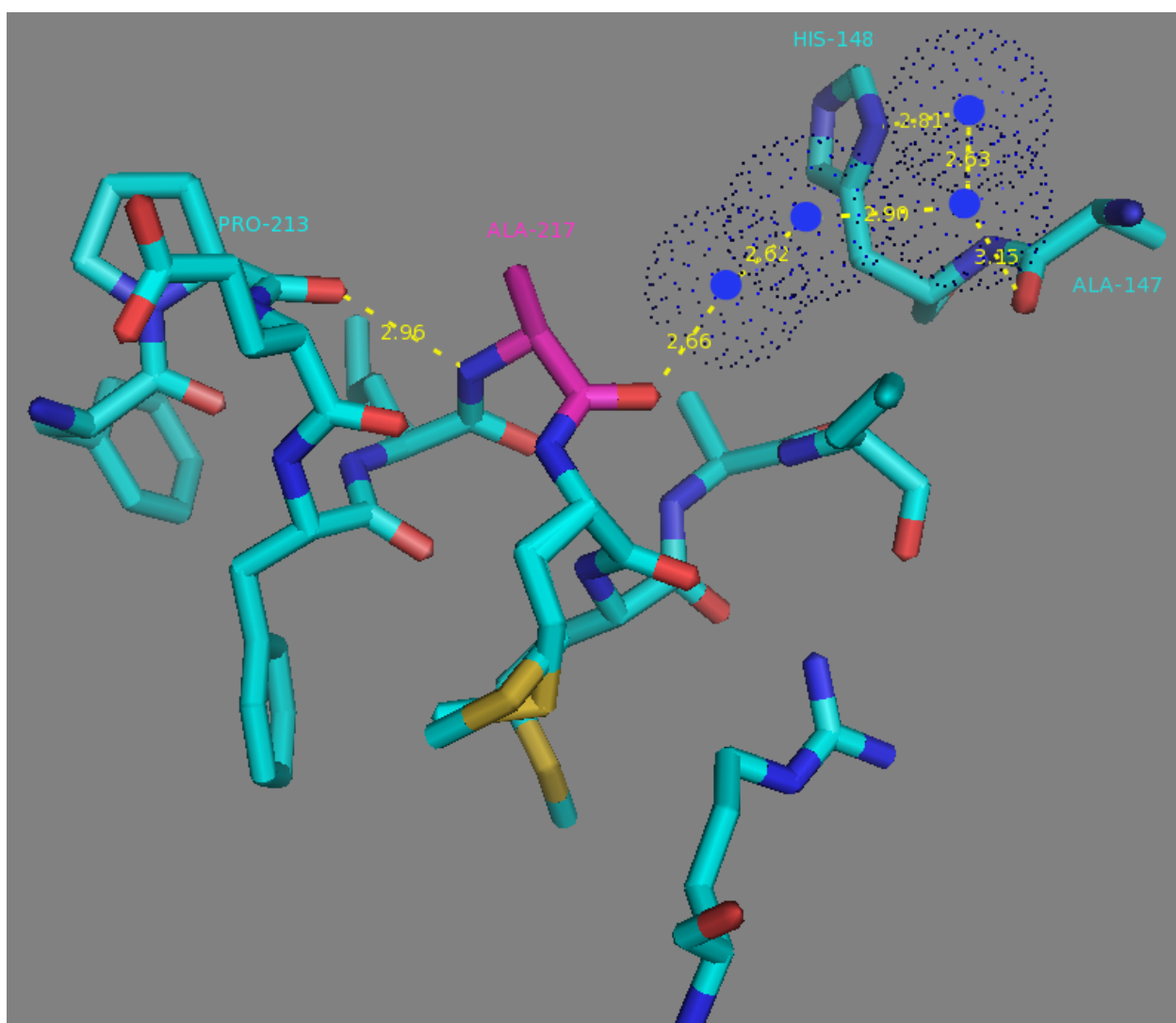


Figure S11. A217 residue surrounding and H-bonds network to the A147 and H148 residues from linker between fluorescent and calcium-binding domains according to NCaMP7 X-ray structure (PDB ID - 6XW2). H-bonds network is shown as dash lines between A217 (in magenta), water molecules (in blue dots), P213, A147 and H148.

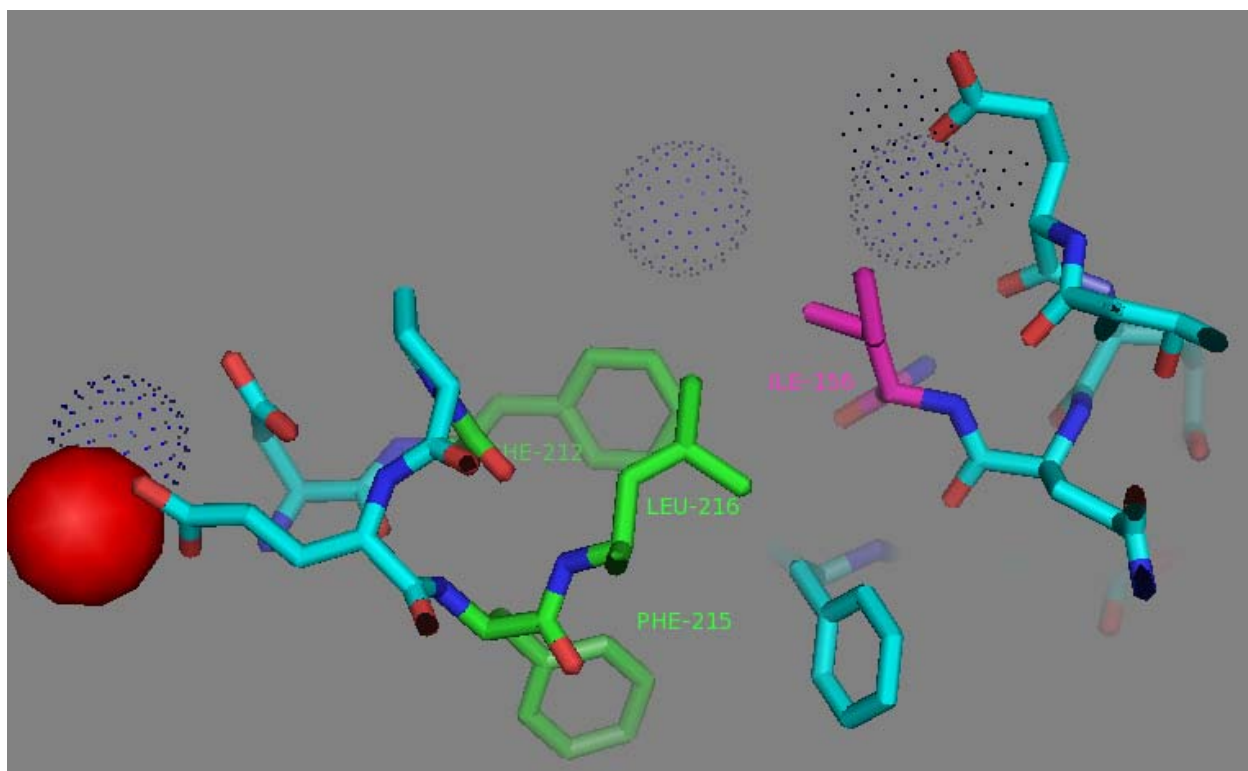


Figure S12. I156 residue surrounding and network to the residues in EF2 coordinating calcium ion according to NCaMP7 X-ray structure (PDB ID - 6XW2). Calcium ion from EF2 is shown as red sphere. Side group of I156 residue (in magenta) is exposed to the hydrophobic pocket which is formed by residues from EF2 (F212, in green) and adjacent to EF2 amino acids (F215 and L216, in green). Water molecules are shown in blue dots.

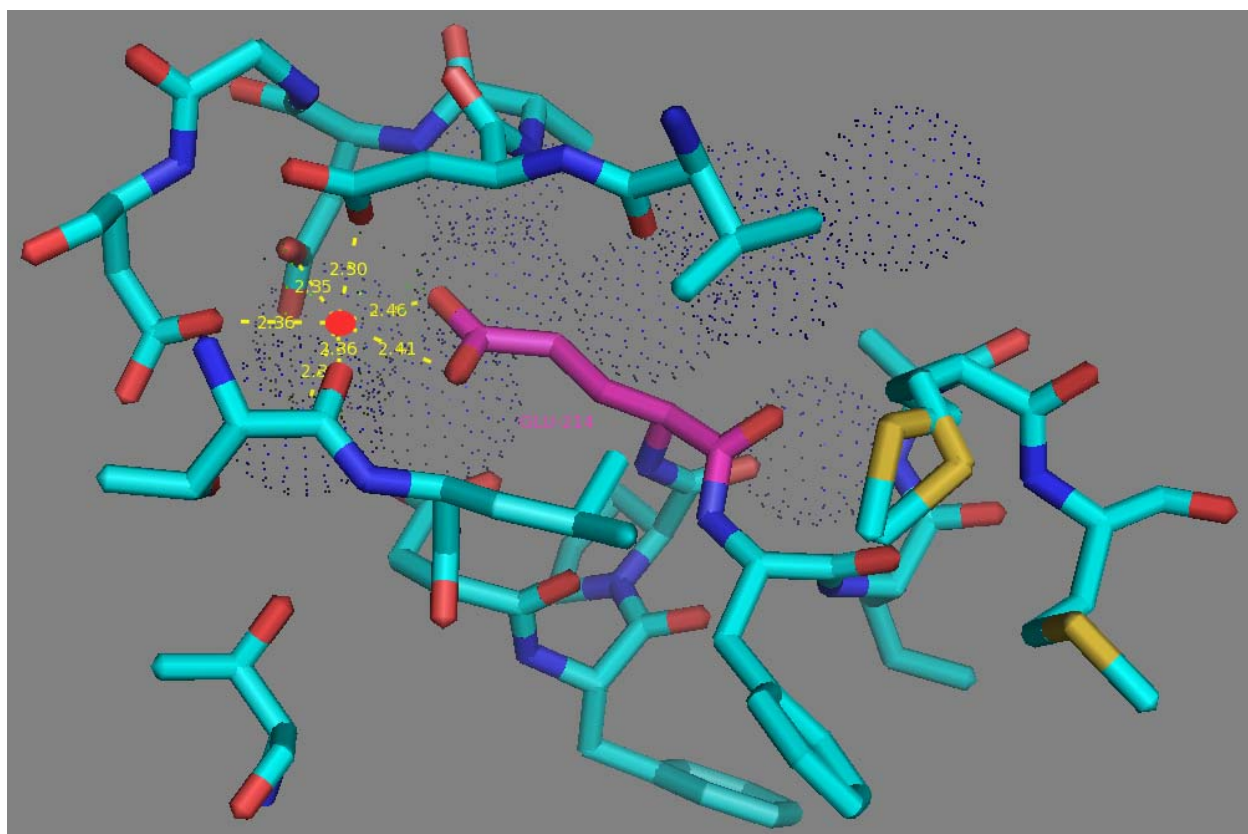


Figure S13. E214 residue surrounding and calcium coordination sphere in EF2 hand according to NCaMP7 X-ray structure (PDB ID - 6XW2). Side group of E214 residue (in magenta) forms two H-bonds with calcium ion (as red dots) from EF2. Water molecules are shown as blue dots.

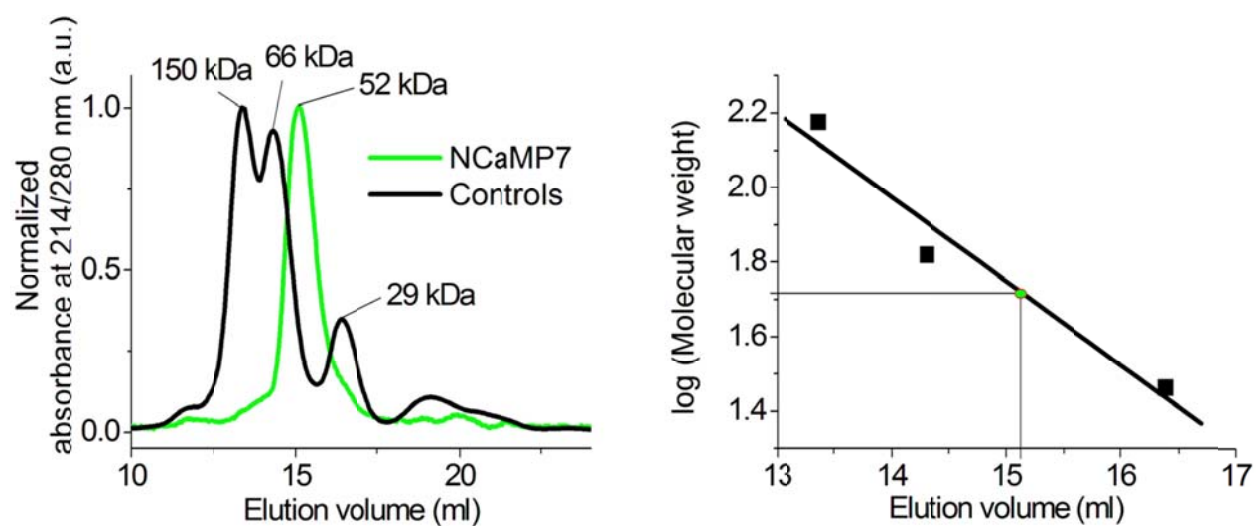


Figure S14. Fast protein liquid chromatography of NCaMP7. NCaMP7 (1.7 mg/ml) was eluted in 40 mM Tris-HCl (pH 7.5) and 200 mM NaCl buffer supplemented with 5 mM CaCl_2 . The molecular weight of NCaMP7 was determined from a linear regression of the dependence of logarithm of control molecular weights vs elution volume.

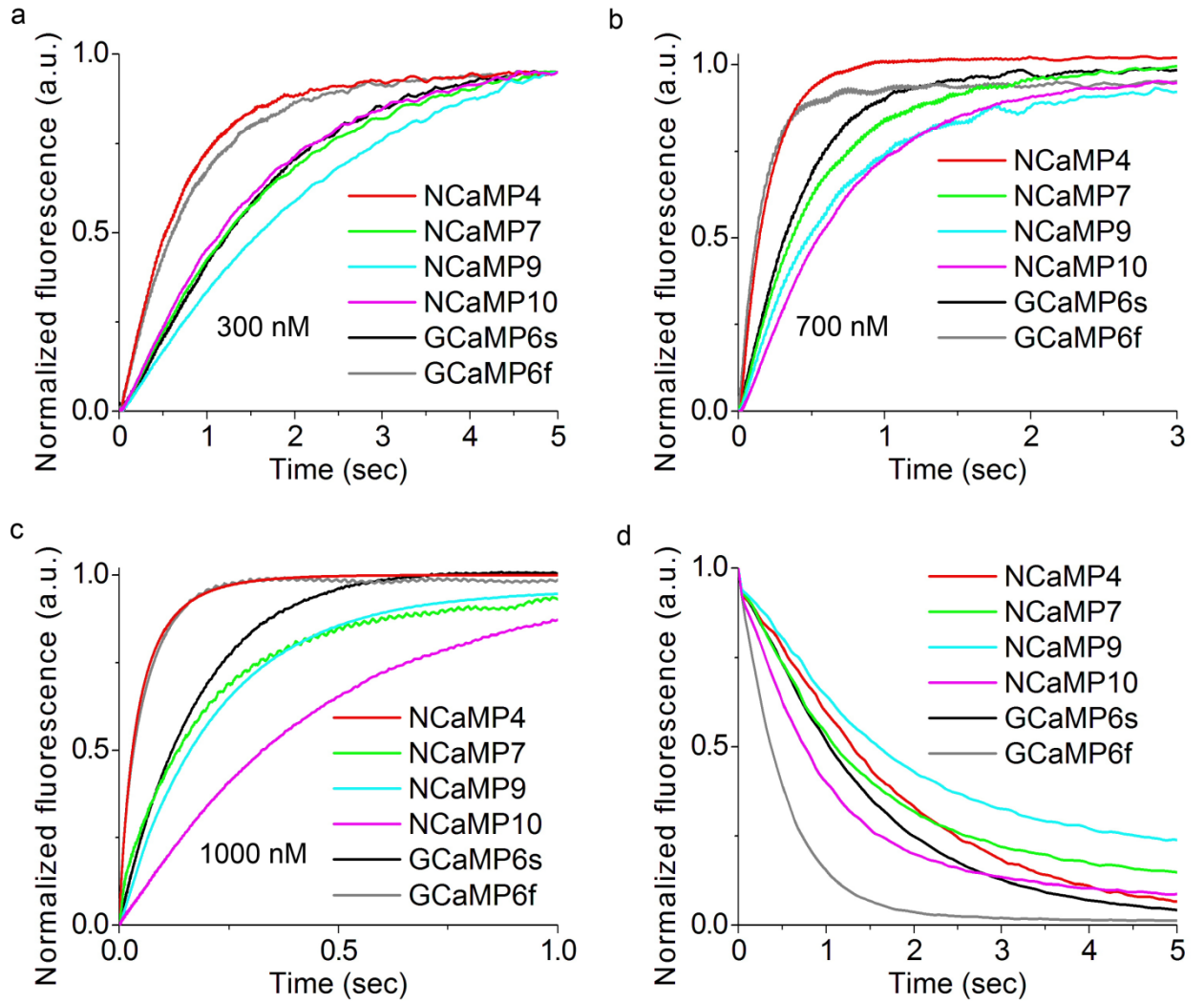


Figure S15. Calcium-association and -dissociation kinetics for the NCaMP7 indicator, its variants, GCaMP6s and GCaMP6f investigated using stopped-flow fluorimetry. (a)-(c) Calcium-association kinetics curves for given GECIs were recorded at 300, 700 and 1000 nM Ca^{2+} -free final concentration. (d) Calcium-dissociation kinetics for respective GECIs was recorded at 1000 nM Ca^{2+} -free starting concentration. Three replicates were averaged for analysis.

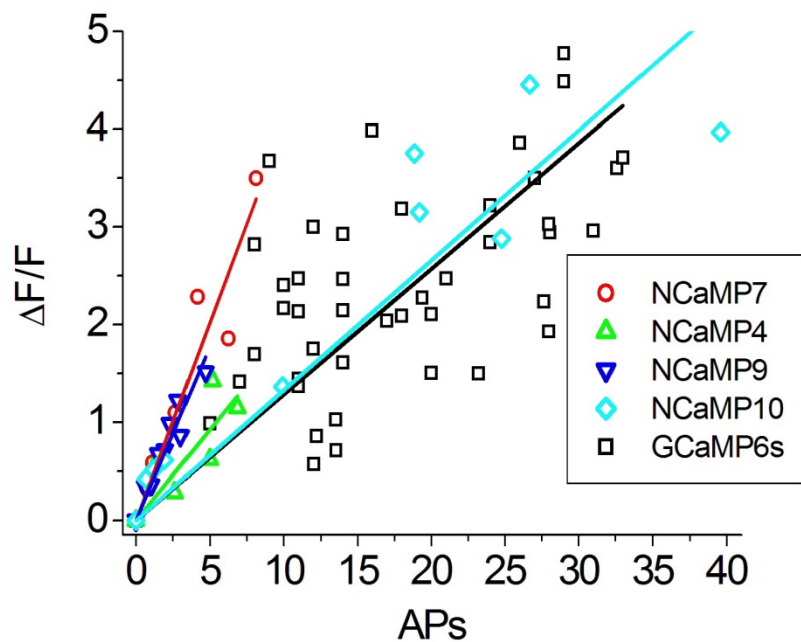


Figure S16. Comparison of responses of green NCaMPs and GCaMP6s indicators normalized to the response of the control red R-GECO1 GECI to external field stimulation of neurons co-expressing the GECIs in dissociated neuronal culture. The numeric values of the slopes calculated from the dependences of $\Delta F/F$ over a number of APs are listed in ESI Table S5.

NES-NCaMP7-stop gene:

ATGCTTCAACTTCCTCCTCTTGAACGTCTTACTCTTTTCGAGATCTATGGTCTCAAAGGGAGAGGAG
GAAAACATGGCTAGTCTGCCTGCTACCCACGAACTGCATATTTTTGGCTCCATAAATGGCATCGA
TTTCGACATGGTAGGCCAAGGCACCGGTAATCCAAACGACGGCTATGAGGAGCTGAACCTGAAG
TCCACCATGGGCGATCTGCAGTTCAGTCCCTGGATCCTGGTGCCGCACATTGGGTACGGGTTTCA
CCAGTATCTTCCCTATCCAGACGGGATGTCACCATTTCAGGCTGCAATGGTCGACGGGTCCGGAT
ACCAAGTGCACCGCACTATGCAGTTTGAGGACGGTGCTCACTGACCGTGAATTACAGATACAC
CTACGAAGGATCACATATCAAAGGGGAAGCTCAGGTGGAGGGCACTGGGTTCCCAGCTGACGG
CCCTGTGATGACAAATTCCTGACAGCCGAGGCCACGACCAGCTGACTGAAGAGCAGATCGCT
GAATTTAAAGAGGCTTTCTCCCTATTTGACAAGGACGGGGATGGGACAATAACAACCAAGGAGC
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TGAAGTAGATGCCGACGGTGACGGCACACTCGACTTCCCTGAGTTCCTGGCAATGATGGCAAGA
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CCAATGGCCGCAAATTACCTCAAAAATCAGCCGATGTATGTCTTTTCGGAAAACAGAGCTGAAAC
ATTCTAAGACCGAACTGAATTTTAAAGAGTGGCAAAGGCCTTTACGGACGTGATGGGCATGGA
CGAGCTGTATAAGTAA

NES-NCaMP4-stop gene:

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CCAGTATCTTCCCTATCCAGACGGGATGTCACCATTTCAGGCTGCAATGGTCGACGGGTCCGGAT
ATCAAGTGCACCGCACTATGCAGTTTGAGGACGGTGCTTCACTGACCGTGAATTACAGATACACC
TACGAAGGATCACATATCAAAGGGGAAGCTCAGGTGGAGGGCACTGGGTTCCCAGCTGACGGC
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NES-NCaMP9-stop gene:

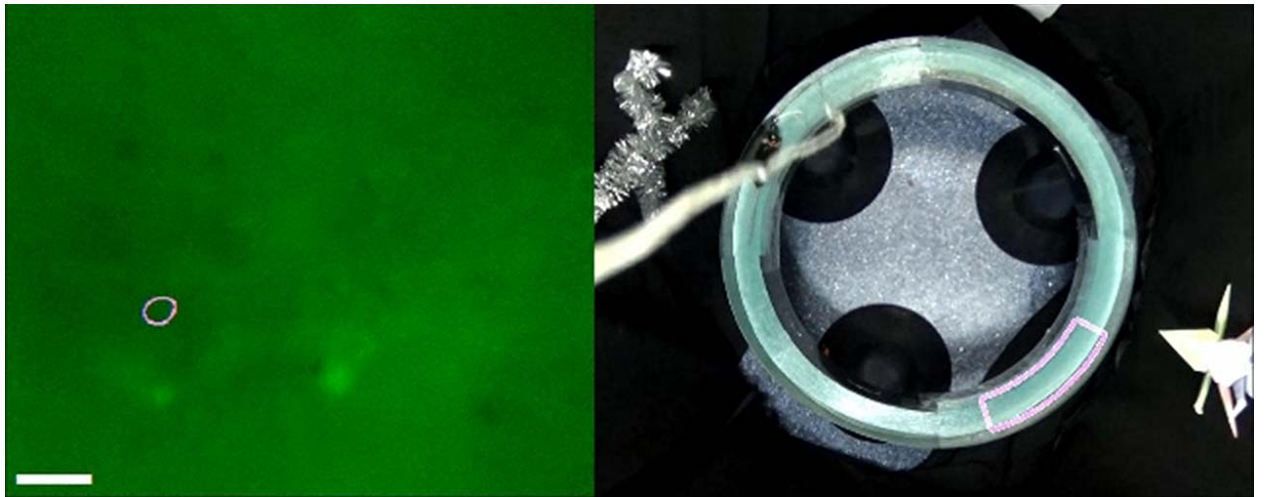
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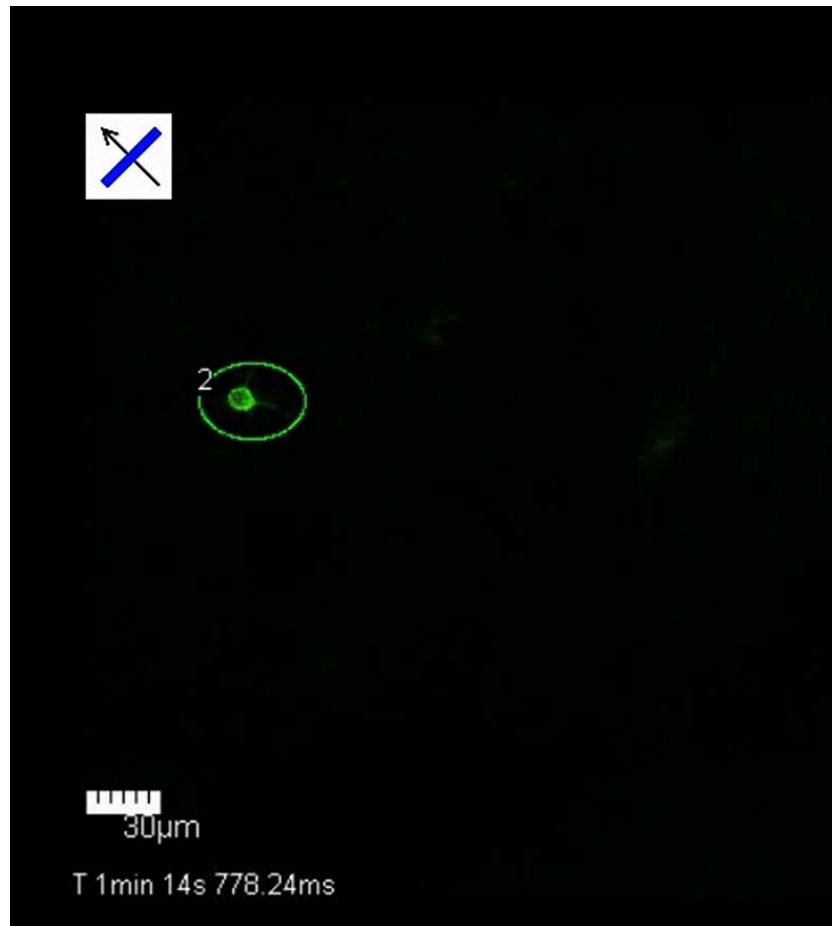
NES-NCaMP10-stop gene:

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 GAGCTGTATAAGTAA

Figure S17. Nucleotide sequences for NES-NCaMPs indicators. NES – nuclei exclusion signal.



Video S1. *In vivo* specific neuronal place cell activity in the CA1 area of hippocampus of mouse visualized with the NCaMP7 calcium indicator and nVista HD miniscope synchronized with mouse position. A video of specific neuronal place cell calcium activity in CA1 area of mouse hippocampus displayed as $\Delta F/F$ changes in fluorescence at 20 Hz frame rate (left) synchronized with mouse movement in O-shaped track (right). GLP 1040 lens probe was used. Video is shown for a time from 4.5 to 6.5 min. Scalebar, 100 μm . The identified place cell is marked by circle and corresponds to the cell shown in Main text, Figure 5e.



Video S2. *In vivo* moving grating-evoked neuronal calcium activity in the mouse V1 visual cortex at a 104 μm depth as visualized with the NCaMP7 calcium indicator and two-photon microscopy. Single frame for the time of 1m14s778.24ms is shown. Neurons specifically activated by the moving grating visual stimulus (shown as blue; the direction of grating is shown with black arrow) are selected with green circle. This field of view corresponds to the neuron 2 shown in the main text, Figure 5b.

Supplementary references

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4. Wu, J.; Abdelfattah, A. S.; Miraucourt, L. S.; Kutsarova, E.; Ruangkittisakul, A.; Zhou, H.; Ballanyi, K.; Wicks, G.; Drobizhev, M.; Rebane, A.; Ruthazer, E. S.; Campbell, R. E., A long Stokes shift red fluorescent Ca(2+) indicator protein for two-photon and ratiometric imaging. *Nat Commun* **2014**, 5, 5262.