Correlating Calcium Binding, Förster Resonance Energy Transfer, and Conformational Change in the Biosensor TN-XXL

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Supporting Material

Supplementary Table 1: Fluorescence decay parameters for truncation and "Amber"-like constructs.

	Ca^{2+} conc. [nM]	α_1 [%]	τ_1 [ns]	α_2 [%]	τ_2 [ns]	α_3 [%]	τ_3 [ns]	τ_{ave} [ns]
ECFP	"Ca^{2+} free"	38	0.720	20	2.00	42	3.87	2.31
	"high Ca^{2+}"	37	0.735	20	1.98	44	3.87	2.35
TN-XXL ΔcpCit	"Ca^{2+} free"	34	0.718	25	2.15	41	3.97	2.40
	"high Ca^{2+}"	33	0.704	22	2.10	45	3.95	2.48
TN-XXL cpCit°	"Ca^{2+} free"	32	0.722	26	2.00	41	3.92	2.38
	"high Ca^{2+}"	33	0.719	25	1.97	42	3.85	2.36
cpCitrine	"Ca^{2+} free"	100	3.56	--	--	--	--	3.56
	"high Ca^{2+}"	100	3.57	--	--	--	--	3.57
TN-XXL ΔECFP	"Ca^{2+} free"	100	3.45	--	--	--	--	3.45
	"high Ca^{2+}"	100	3.37	--	--	--	--	3.37
TN-XXL ECFP°	"Ca^{2+} free"	100	3.50	--	--	--	--	3.50
	"high Ca^{2+}"	100	3.27	--	--	--	--	3.27

Supplementary Table 2: Fluorescence decay parameters in TN-XXL.

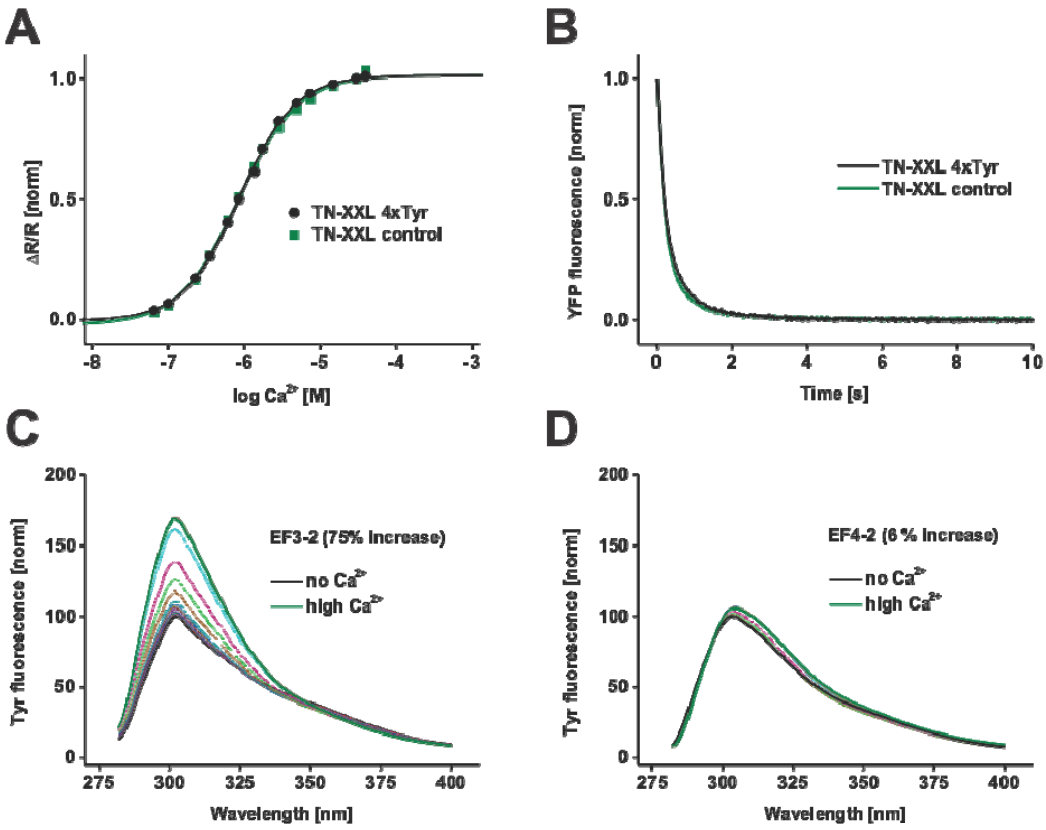
	fit	α_1 [%]	τ_1 [ns]	α_2 [%]	τ_2 [ns]	α_3 [%]	τ_3 [ns]	χ^2	τ_{ave} [ns]
TN-XXL cpCit°	bi-exp	47	1.16	---	---	53	3.76	1.202	2.53
„Ca^{2+} free“	**tri-exp**	**30**	**0.762**	**25**	**1.97 (fix)**	**45**	**3.96**	**1.197**	**2.49**
TN-XXL cpCit°	bi-exp	47	1.14	---	---	53	3.70	1.193	2.50
“high Ca^{2+}”	**tri-exp**	**32**	**0.738**	**24**	**1.97 (fix)**	**44**	**3.88**	**1.123**	**2.414**
TN-XXL	bi-exp	52	1.11	---	---	48	3.51	1.202	2.26
“Ca^{2+} free“	**tri-exp**	**36**	**0.705**	**30**	**1.97 (fix)**	**34**	**3.73**	**1.121**	**2.11**
TN-XXL	bi-exp	71	0.758	---	---	29	3.24	1.476	1.47
“high Ca^{2+}“	**tri-exp**	**64**	**0.511**	**24**	**1.97 (fix)**	**12**	**3.79**	**1.206**	**1.26**

Donor excitation wavelength: 440 nm
Donor emission wavelength: 470 nm

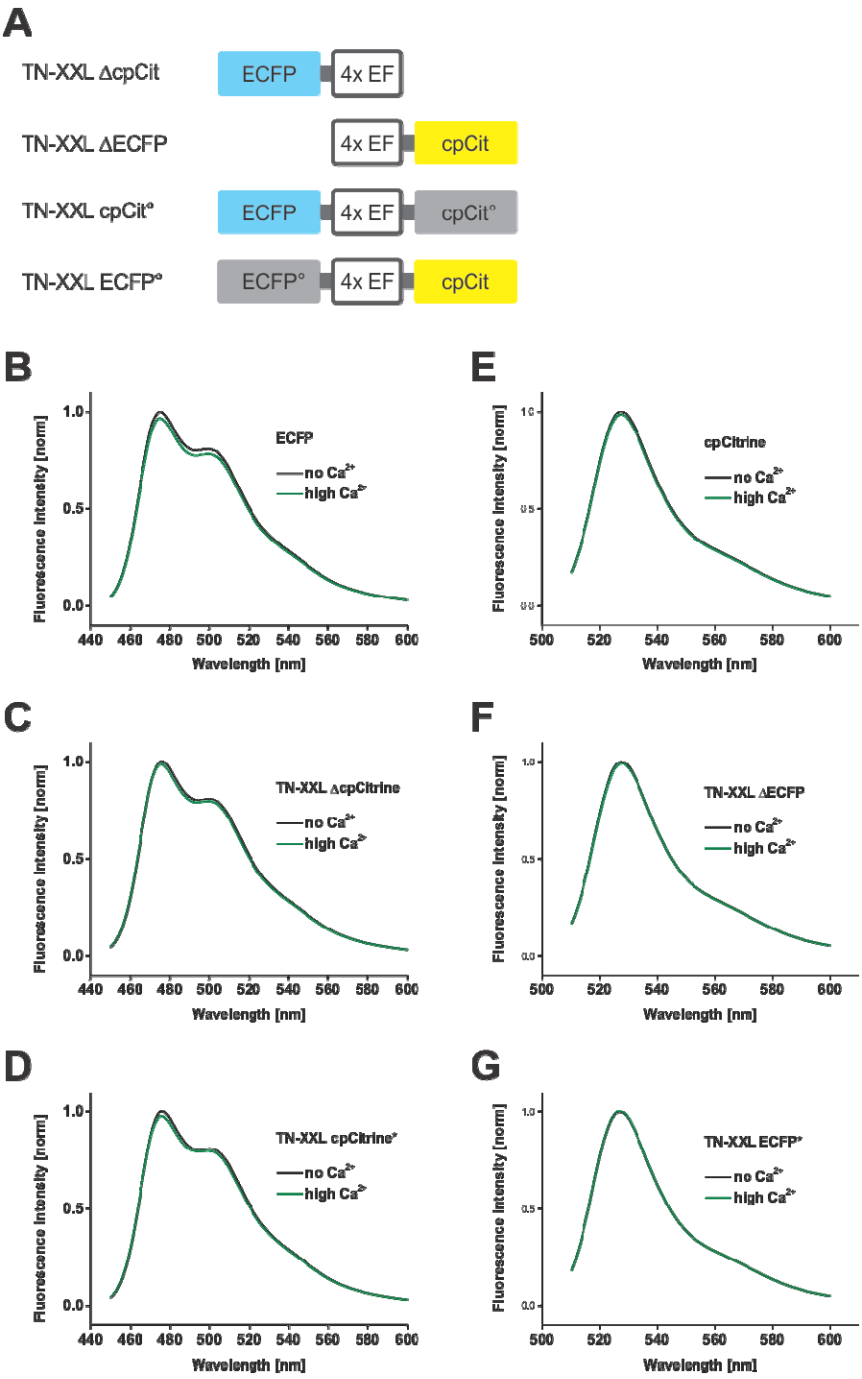
These parameters represent the averages of the values obtained from three independent experiments. The τ_2 value fixed in the tri-exponential fits was obtained from averaging the τ_2 values from three Ca^{2+} titrations of TN-XXL. Subsequently this value was kept fixed throughout further tri-exponential fits. The tri-exponential fits highlighted in bold letters were used further on in discussion and figures.

Supplementary Table 3: Temperature and pH dependency of TN-XXL

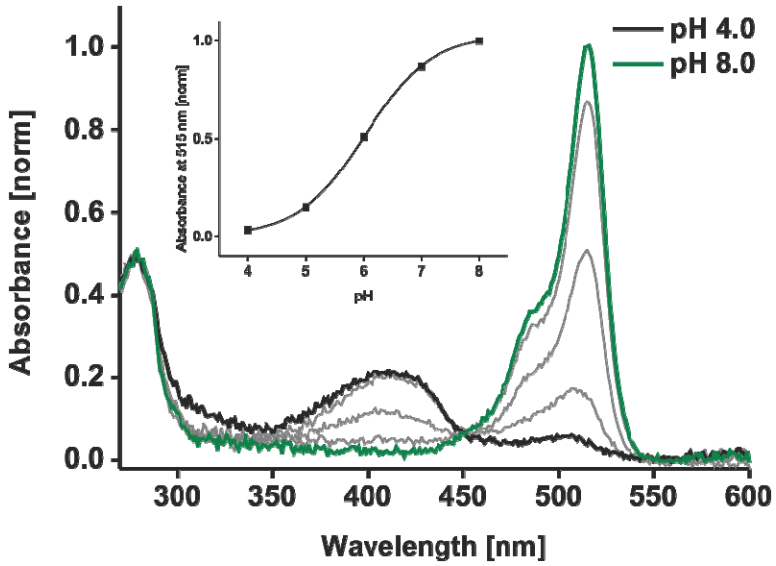
Condition		Affinity K_d [nM]	Off-Kinetics t_{decay} [ms]
23°C	pH = 6.5	1030	620
	pH = 7.2	830	--
	pH = 7.5	--	522
	pH = 8.0	451	425
30°C	pH = 7.2	946	--
	pH = 7.5	--	264
37°C	pH = 7.2	1210	--
	pH = 7.5	--	129



Supplementary Figure S1 Tyrosine substitutions in TN-XXL.
(*A* and *B*) Tyrosine substitutions in all four EF-hands (TN-XXL 4xTyr) do not alter calcium binding properties of TN-XXL as monitored by FRET. Calcium affinity titrations (*A*) and calcium dissociation kinetics (*B*) of TN-XXL 4xTyr were indistinguishable from those of TN-XXL. Excitation was at 432 nm and emission recorded at 475/527 nm. (*C* and *D*) Emission spectra of calcium titrations with single tyrosine substitutions within TN-XXL. EF3-1 (*C*) and EF4-1 (*D*) showed comparable fluorescence modulation to EF3-2 and EF4-2, respectively (excitation at 275 nm with emission spectra recorded from 285-400 nm).



Supplementary Figure S2 Truncation and Amber Substitutions in TN-XXL.
(*A*) TN-XXL truncation and Amber substitution constructs used to test the structure effects on the fluorescence signal of each FP variant. For ECFP emission (excitation: 432 nm) ECFP alone (B), TN-XXL ΔcpCit (C) and TN-XXL cpCit° including the Amber mutation Y67C in cpCitrine (D) were tested and only a minor drop in fluorescence of ~2% upon addition of 40µM Ca^{2+} was detected. cpCitrine emission (excitation: 500 nm) was tested with cpCitrine alone (E), TN-XXL ΔECFP (F) and TN-XXL ECFP° including the Amber mutation W67C in ECFP (G) without any fluorescence change upon addition of 40 µM Ca^{2+}.



Supplementary Figure S3 pH effect on cpCitrine chromophore.
UV Absorption spectra of cpCitrine recorded at different pH values of 4.0 (cyan), 5.0, 6.0, 7.0 and 8.0 (black). The inset shows the normalized absorption readout at 515 nm indicating ~88% absorption at pH 7.2 (i.e., 88% of the cpCitrine chromophores deprotonated).