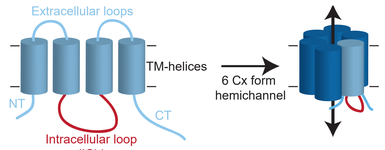
Calmodulin interaction with gap junction intracellular loop peptides

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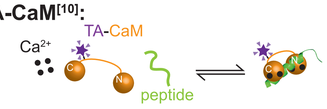
Connexins and calmodulin (CaM)

- CaM binding sites have been identified on N- and C-terminus of connexins (Cx): 26, 32, 34.7, 35 and 36[1-4]
- CaM binding to intracellular loop (ILC) of Cx43, 44, 45 and 46 was shown[5-8]
- CaM binding sites are predicted in the ICL of most Cx, thus CaM may modulate gap junction permeablity
- A representative ICL peptide from each α-, β- and γ-type Cx was selected and investigated for interaction with CaM

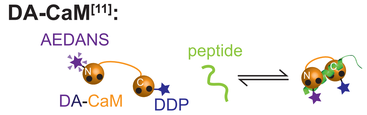


β Cx32 ICL KRHKVISGTLWWTYVISVVFRLLFE
γ Cx35 ICL TKSKMRRQEGISRFYIIQVVFRNALE
α CX45 ICL DGRRRIREDGLMKIYVLQLLARTVFE
α Cx57 ICL KIHKVPLKGCLLRTYVLHILTRSVLE
mTRP[9] [myr]-RRKWQKTGHAVRAIGRL

TA-CaM[10]:

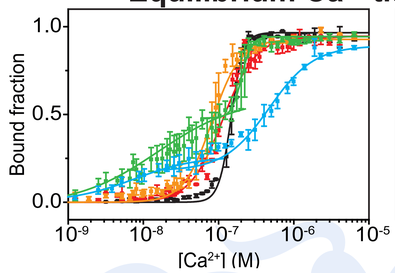


DA-CaM[11]:



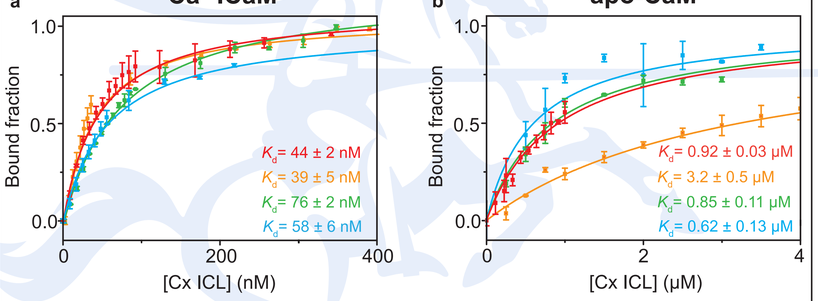
Binding to Ca^{2+}.CaM and apo-CaM

Equilibrium Ca^{2+} titration with TA-CaM



	$K_d(Ca^{2+})$ (nM)	n	
TA-CaM	147 ± 2	5.3 ± 0.3	
Cx32 ICL	82 ± 3	2.3 ± 0.2	left shift
Cx35 ICL	114 ± 3	2.3 ± 0.1	
Cx45 ICL	16 ± 4 202 ± 4	0.8 ± 0.2 6.6 ± 0.7	two-site binding
Cx57 ICL	7.7 ± 0.9 471 ± 33	1.1 ± 0.2 1.4 ± 0.1	

Cx ICL peptide affinities for CaM

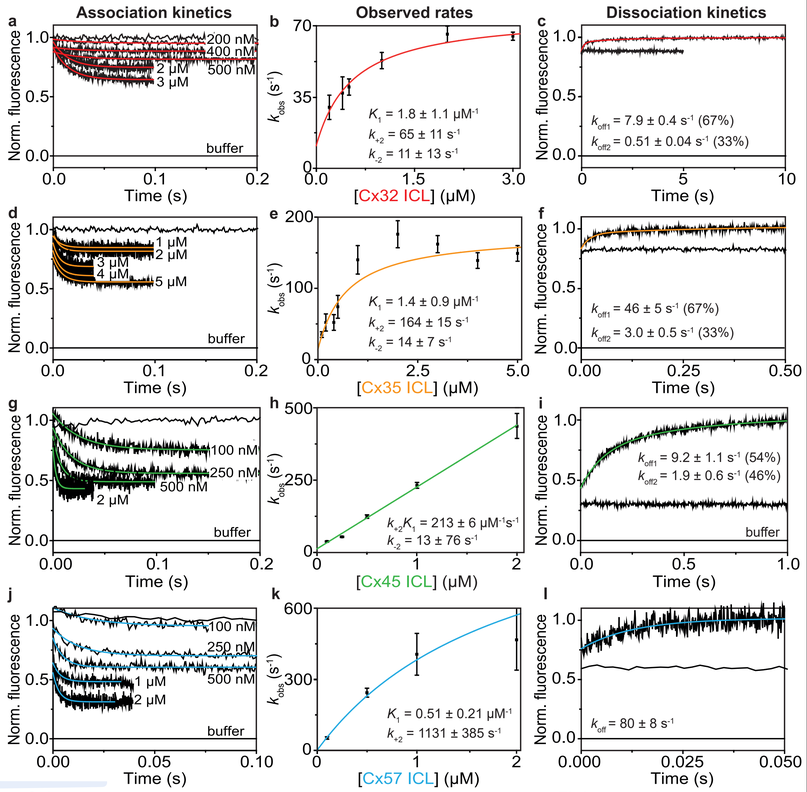


15 nM TA-CaM + 100 μM $CaCl_2$
500 nM DA-CaM + 4 mM EGTA
Assay buffer: 50 mM K^+-HEPES pH 7.5, 100 mM KCl, 2 mM $MgCl_2$ at 20 °C.

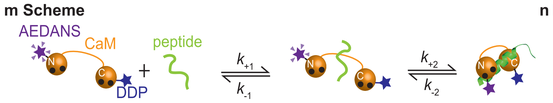
Kinetics of Ca^{2+}.CaM interactions

Measured by stopped-flow fluorimetry in assay buffer with 100 μM $CaCl_2$, 20 °C.

- Association kinetics: 500 nM DA-CaM mixed with different concentrations of Cx ICL peptides
- Dissociation kinetics: 500 nM DA-CaM with saturating Cx ICL peptide concentration mixed with excess of CaM (2.5 μM)



m Scheme



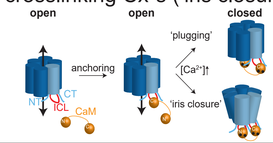
n Fitting equation

$$k_{obs} = \frac{k_{+2}K_1[\text{Cx ICL}]}{1 + K_1[\text{Cx ICL}]} + k_{-2}$$

- Association: 2-step process with an equilibrium of free and loosely bound Cx ICL peptide followed by reversible conformation changes (Scheme) were fitted with the equation shown above
- Disssociation: were fitted to a single or biexponential decay

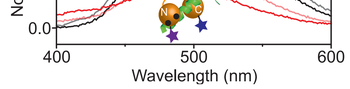
Conclusion

- Ca^{2+}.CaM binds α, β or γ Cx ICL peptides with high affinity (K_d 40-80 nM)
- Ca^{2+}-independent CaM binding to Cx ICL peptides is demonstrated for the first time (K_d 0.5-3 μM)
- Ca^{2+}.CaM is partially compacted on binding Cx ICL peptides with fast rates of 70 to 500 s^{-1} (20 °C)
- CaM may be anchored to gap junctions in resting cells
- Increase in intracellular [Ca^{2+}] may lead to CaM modulating gap juction permeability by
  a) A CaM lobe acting as a 'plug'
  b) crosslinking Cx-s ('iris closure')



Structural insight by FRET

Quenching of DA-CaM by Cx ICL peptides



peptides	Quencing (%)	
	+ Ca^{2+}	- Ca^{2+}
mTRP	80 ± 3	45 ± 5
Cx32 ICL	64 ± 3	34 ± 9
Cx35 ICL	62 ± 6	45 ± 6
Cx45 ICL	70 ± 5	33 ± 5
Cx57 ICL	54 ± 4	42 ± 4

Fluorescence lifetime measurements

- Lifetime measurements were performed of 500 nM DA-CaM in assay buffer with 100 μM $CaCl_2$ or 4 mM EGTA under Cx ICL saturating conditions using TCSPC
- Lifetime data was fitted to one or two exponentials
- Förster radius is determined by: $R_0 = 0.2844\sqrt[6]{\frac{\Phi_D \kappa^2 J_{DA}}{n^4 N_A}} = 24 \pm 2$ Å

Φ_D = 0.24 ± 0.02, κ^2 = 2/3, the overlap integral J_{DA} = 4.86/5.19 · 10^{13} $nm^4M^{-1}cm^{-1}$, in Ca^{2+}/EGTA and n = 1.4

- Distance R was calculated by $R = R_0\sqrt[6]{\frac{1-E}{E}}$ with $E = 1 - \frac{\tau_{DA}}{\tau_D}$

	condition	$\tau_{(d)}$ (ns)	$\tau_{1(da)}$ (ns)	A_1	distance (da_1) (Å)	$\tau_{1(da)}$ (ns)	A_1	distance (da_1) (Å)
DA-CaM	+ Ca^{2+}	13.14 ± 0.09	12.0 ± 0.1	1	**36 ± 3**			
mTRP		14.8 ± 0.01	12.2 ± 1.1	0. 53 ± 0.02	**32 ± 4**	2.9 ± 0.2	0.47 ± 0.02	**19 ± 2**
Cx32 ICL		13.4 ± 0.1	12.4 ± 0.3	0.85 ± 0.01	**37 ± 3**	2.7 ± 0.4	0.15 ± 0.01	**19 ± 4**
Cx35 ICL		13.3 ± 0.1	12.4 ± 0.5	0.85 ± 0.01	**38 ± 3**	3.1 ± 0.3	0.15 ± 0.01	**20 ± 3**
Cx45 ICL		13.7 ± 0.1	12.6 ± 0.4	0.66 ± 0.02	**37 ± 3**	3.2 ± 0.4	0.34 ± 0.01	**20 ± 4**
Cx57 ICL		13.9 ± 0.1	10.7 ± 0.2	0.85 ± 0.01	**30 ± 2**	2.3 ± 0.3	0.15 ± 0.01	**20 ± 3**
DA-CaM	- Ca^{2+}	13.15 ± 0.06	12.3 ± 0.1	1	**39 ± 3**			
mTRP		17.9 ± 0.1	11.7 ± 0.2	1	**28 ± 2**			
Cx32 ICL		13.2 ± 0.1	10.8 ± 0.2	1	**32 ± 2**			
Cx35 ICL		13.1 ± 0.1	10.3 ± 0.3	1	**31 ± 3**			
Cx45 ICL		13.9 ± 0.1	10.6 ± 0.2	1	**30 ± 2**			
Cx57 ICL		13.7 ± 0.1	10.2 ± 0.2	1	**30 ± 2**			

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