UHCL 2019

Physics of Memory and Learning – from the Perspective of Interacting Biomolecules

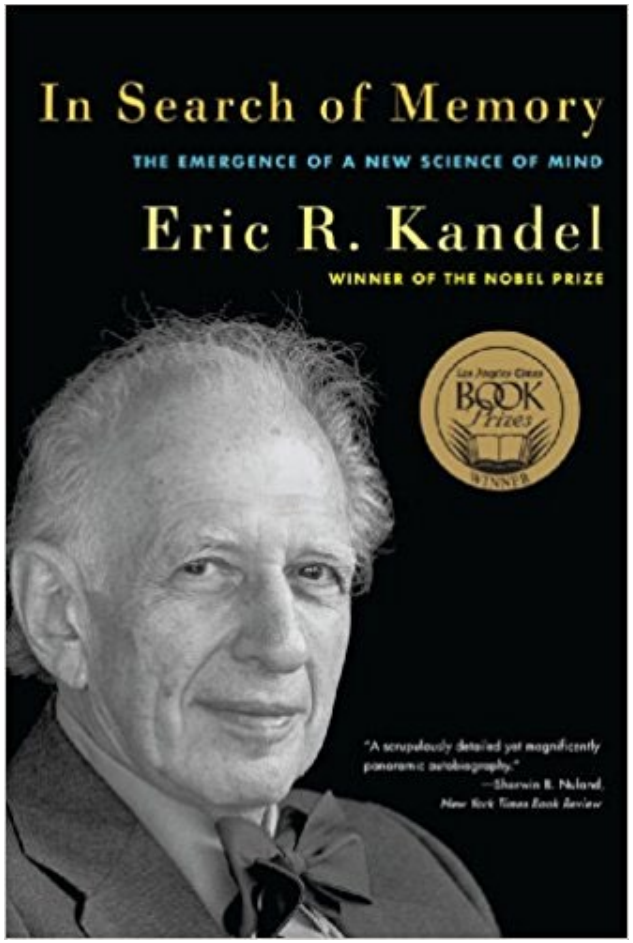
Margaret S. Cheung

Department of Physics
University of Houston
Center for Theoretical Biological Physics
Rice University

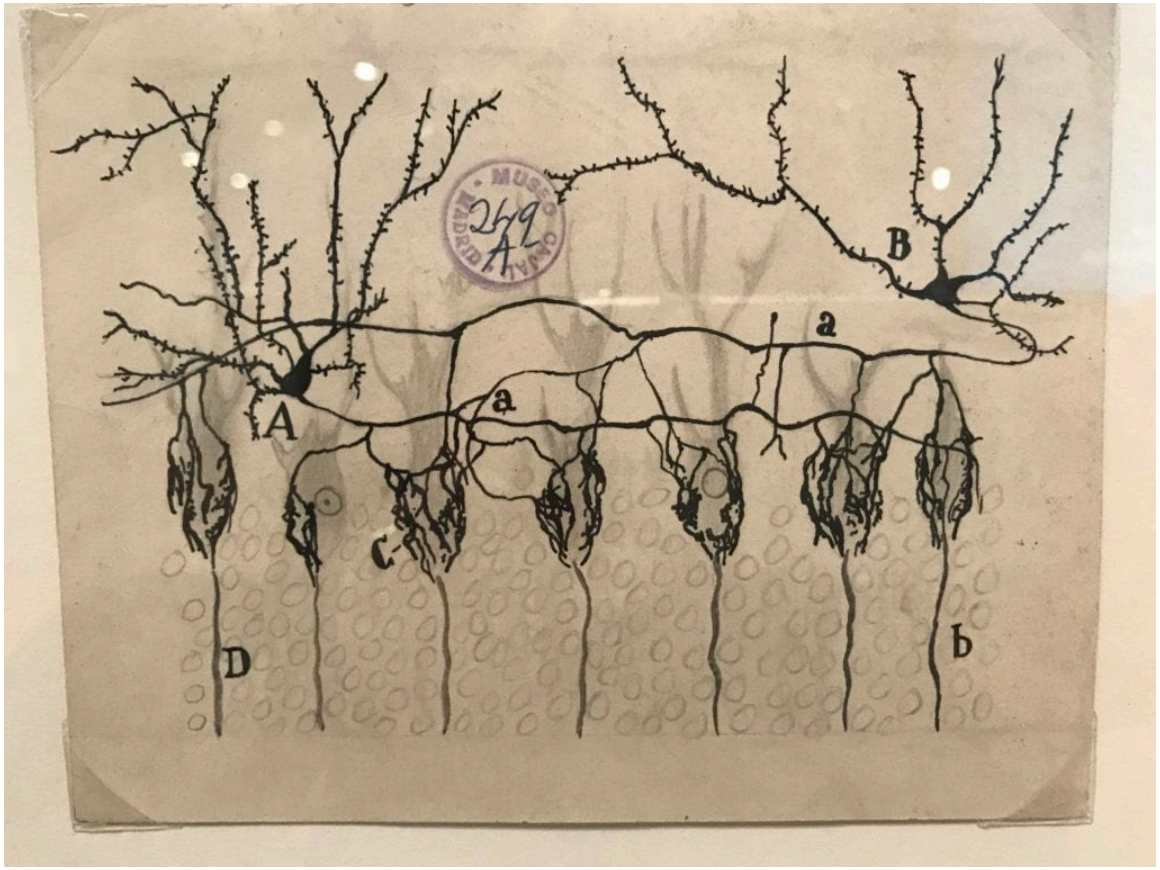
What is the biology of memory and learning?

"The new biology posits that consciousness is a biological process that will eventually be explained in terms of molecular signaling pathways used by interacting populations of nerve cells."

-- Eric R. Kandel,
2000 Nobel laureate

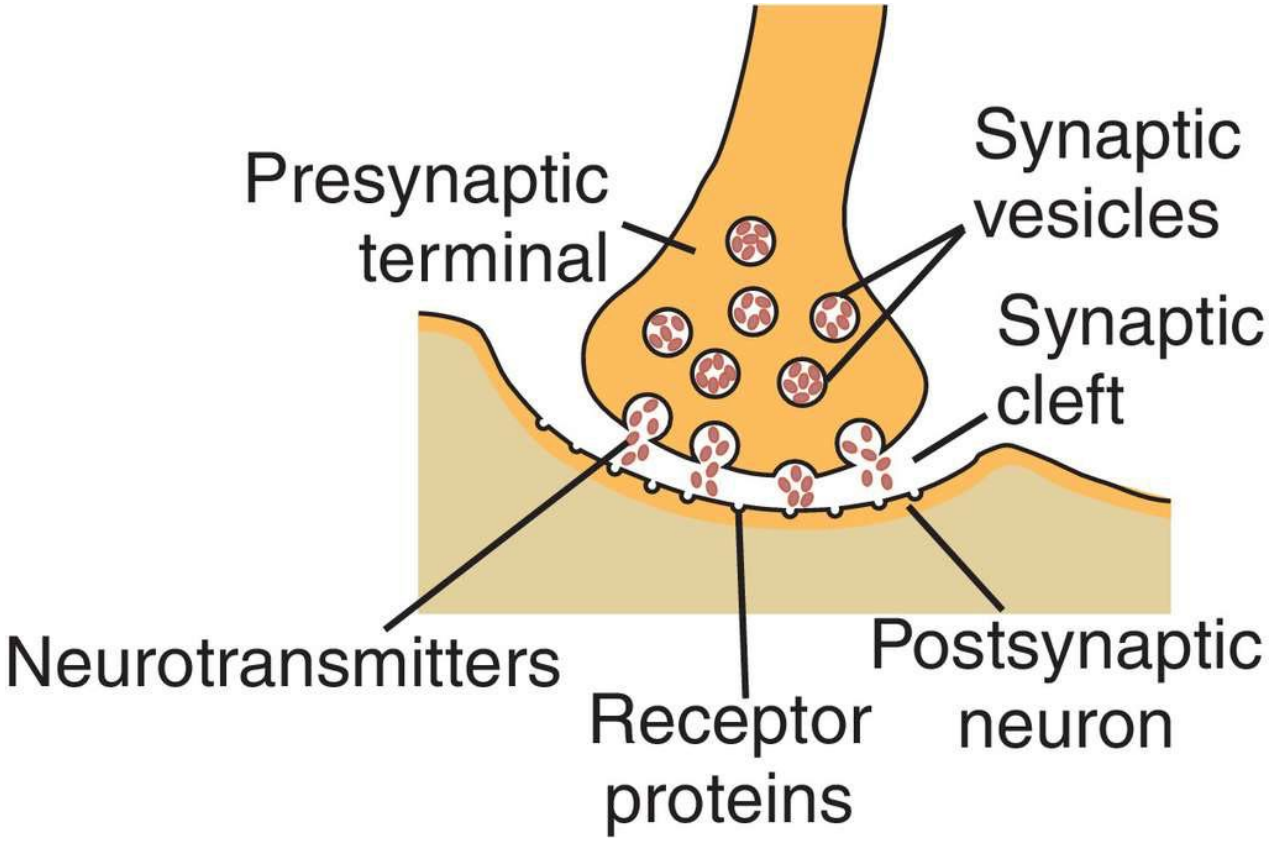


How do neuron cells communicate?
Neurons are in touch, without touching



"**Synaptic contacts** in the cerebellum"
Santiago Ramón y Cajal, Nobel Laureate in 1906

Synaptic plasticity underscores learning -- "Practice makes perfect" makes perfect sense



https://medical-dictionary.thefreedictionary.com/synaptic+transmission

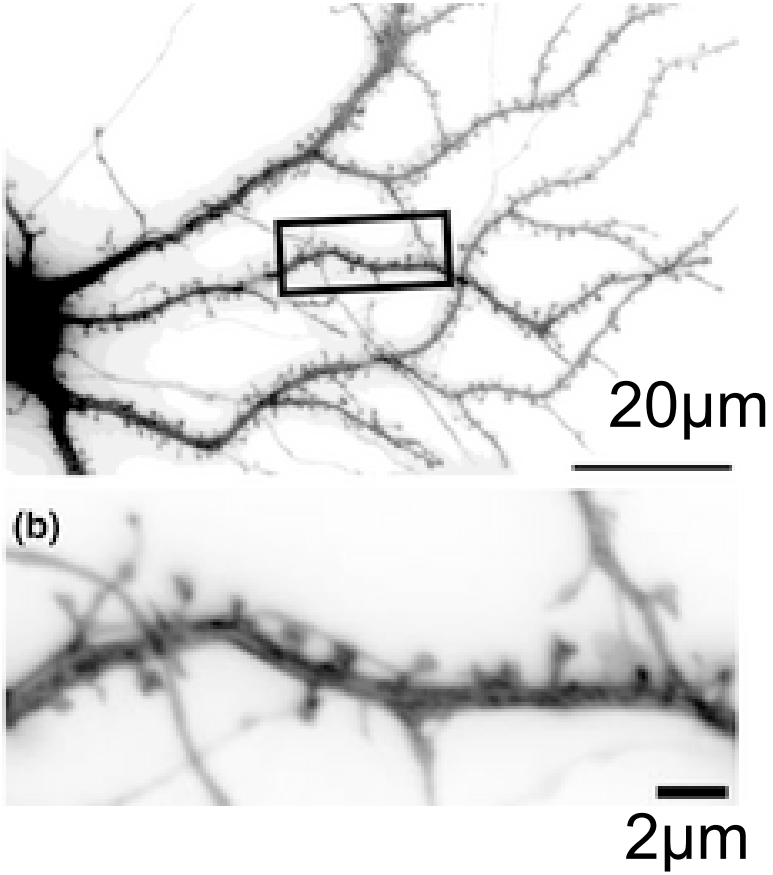
How does a neuron decode extracellular signals?

- Localized nature of calcium signals.
- Encoding the time-varying calcium signal by tuning the affinity of calcium-binding proteins for calcium ions.
- Protein-mediated calcium signaling pathways.
- Protein-mediated calcium signaling pathways.

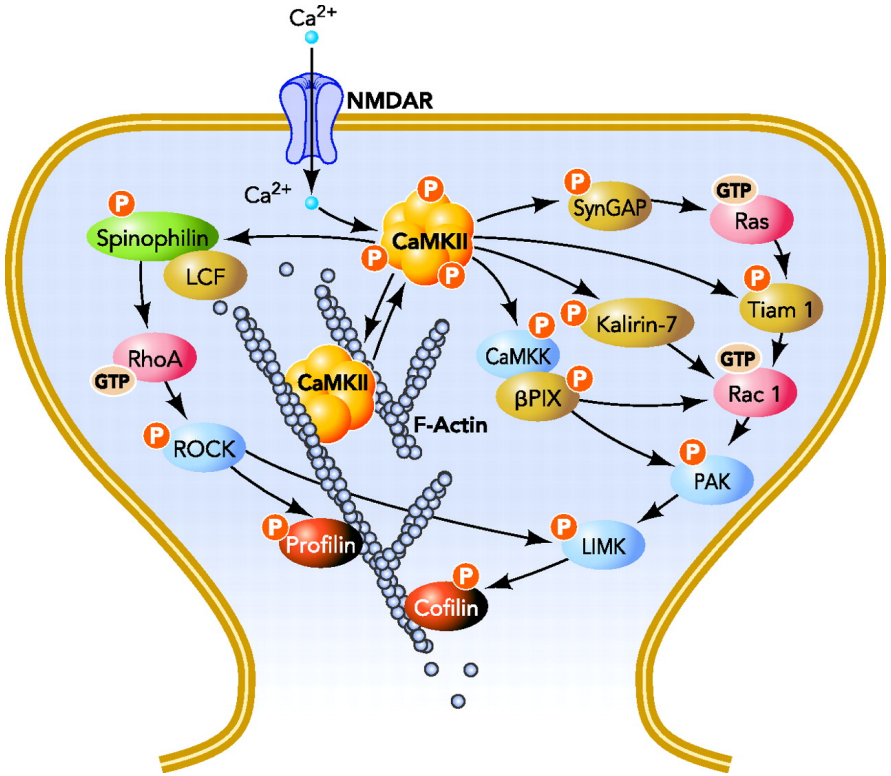
Kubota Y, Putkey JA, Shouval HZ, Waxham MN. 2008, J. Neurophysiology

Clapham. 2007. Cell

Calcium influx activates calcium signaling pathways in a dendritic spine

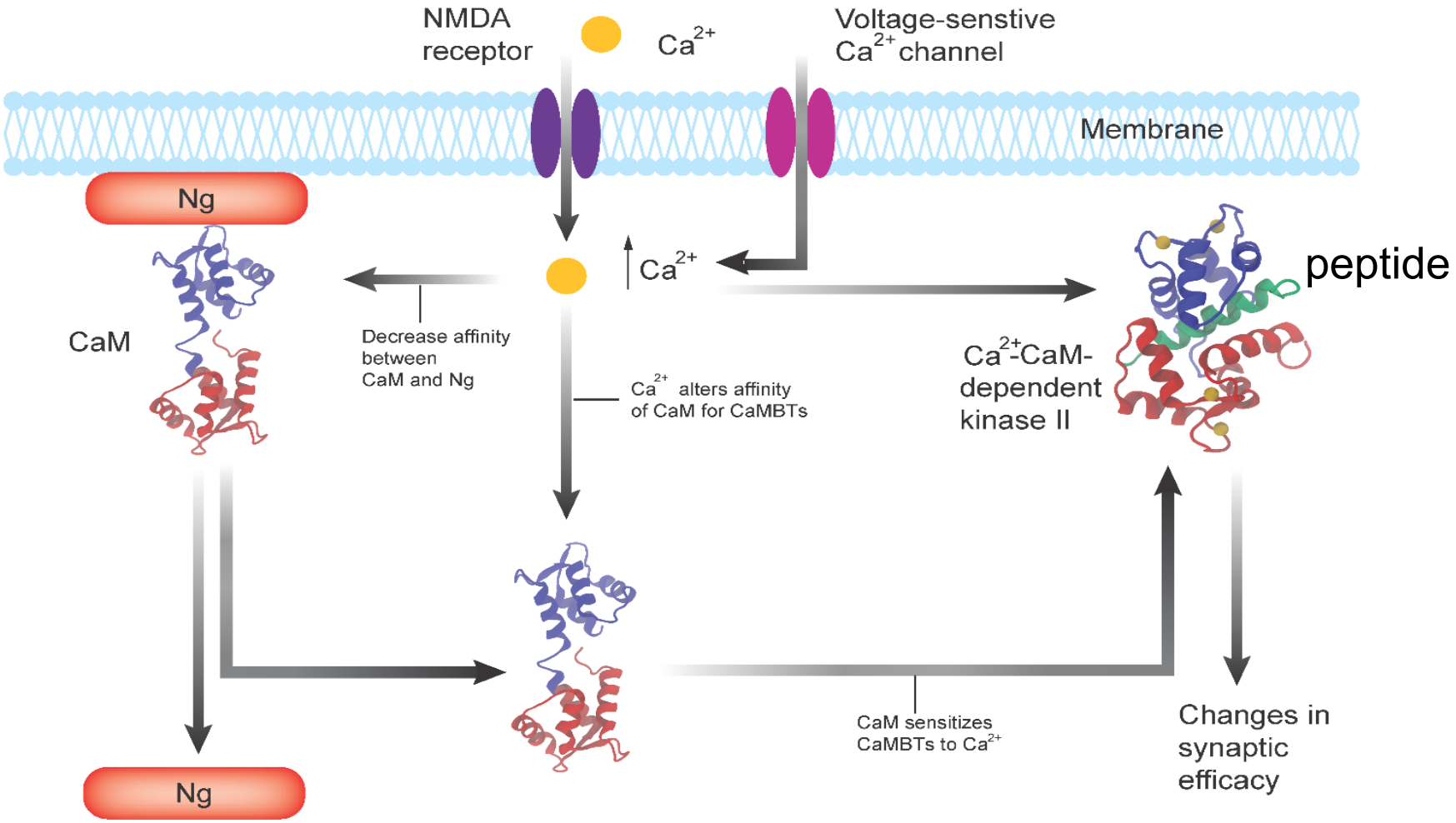


Shirao, J. Neurochemistry (2013)

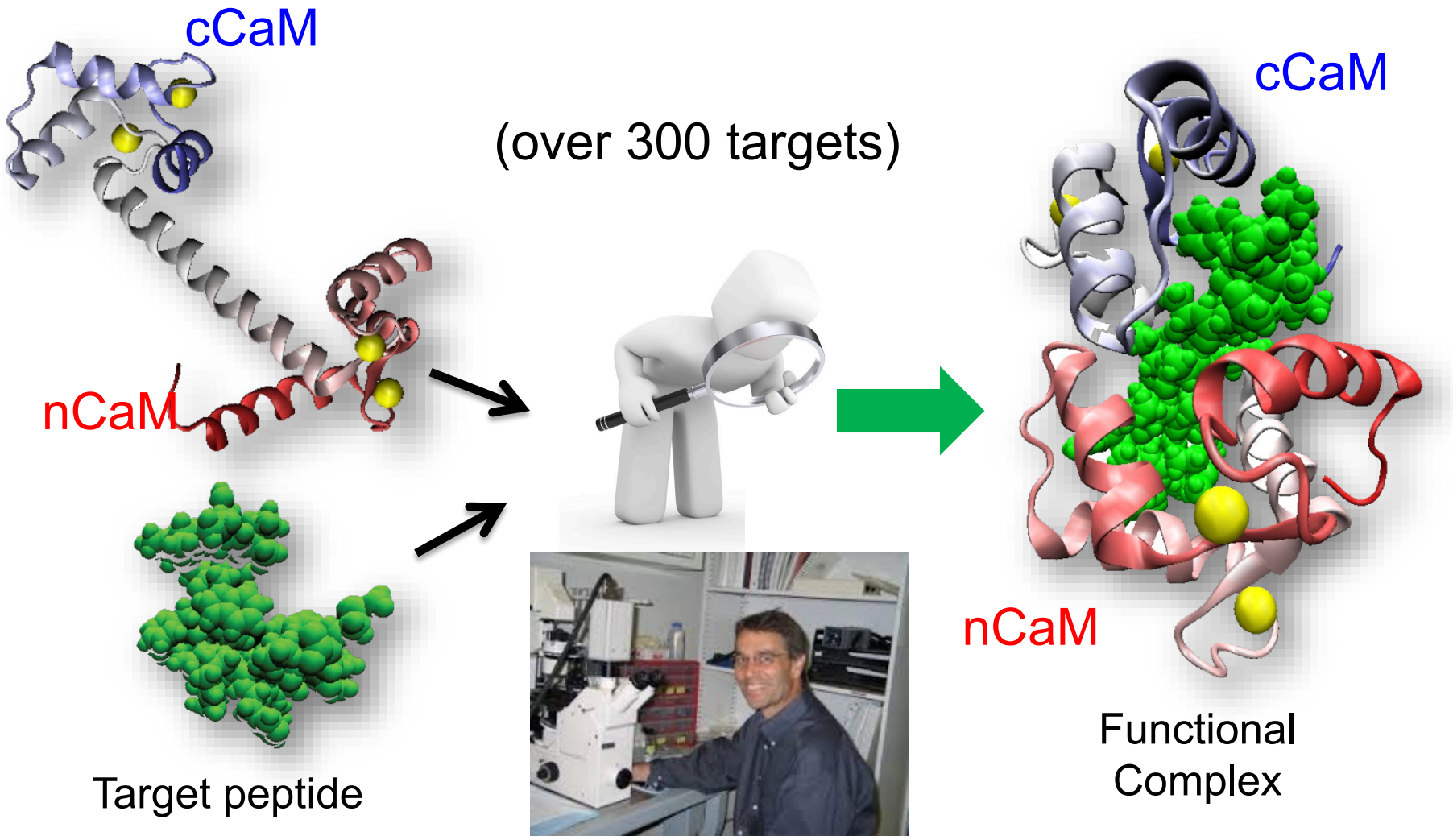


Okamoto, Physiology (2009)

The protein calmodulin is crucial in the first second upon stimulation by neurotransmitters



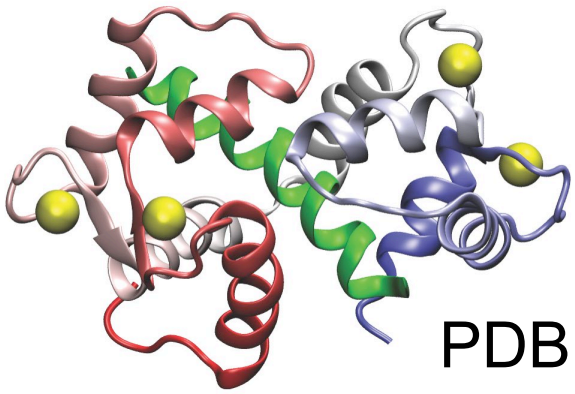
CaM is structurally flexible and adopts distinct conformations when bound to different protein targets



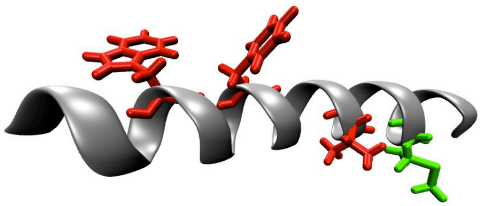
M. Neal Waxham (UTH)

CaM-target binding kinetics varies by the sequence of its target

CaM-CaMKI peptide



PDB: 2L7L

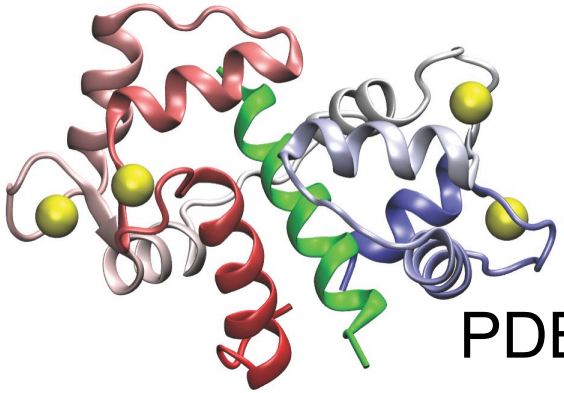


AKSKWKQAFNATAVVRHMRKLQ

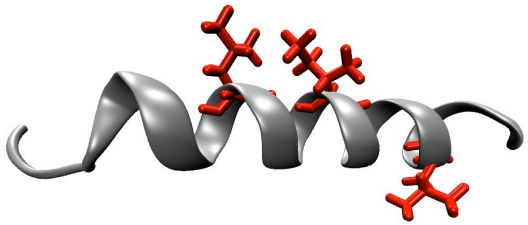
1 5 10 14

CaMKI peptide

CaM-CaMKII peptide



PDB: 1CDM

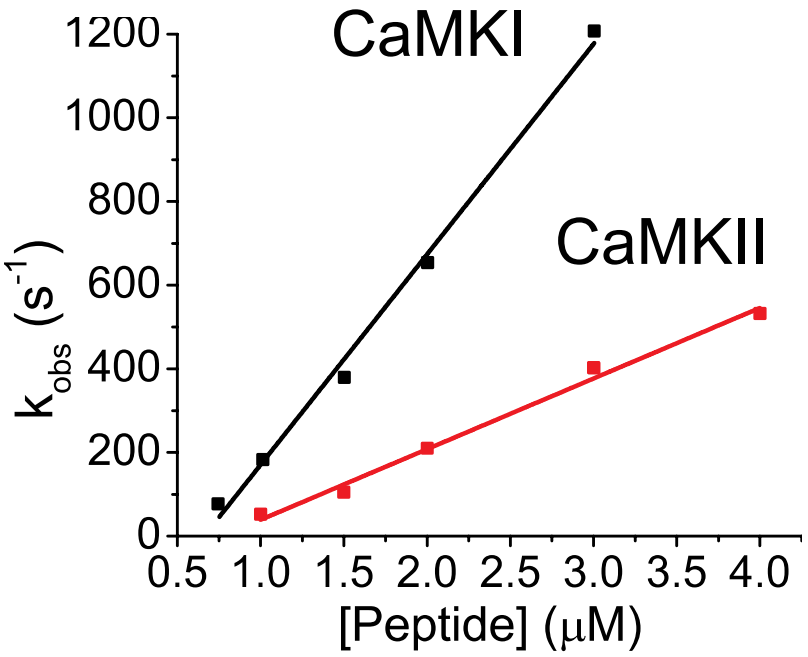


KFNARRKLKGAILTTMLATRN

1 5 10

CaMKII peptide

A factor of two in binding rates can be significant in CaM's target selection and recognition.



Wang, Zhang.... Cheung, Waxham (PNAS 2013)

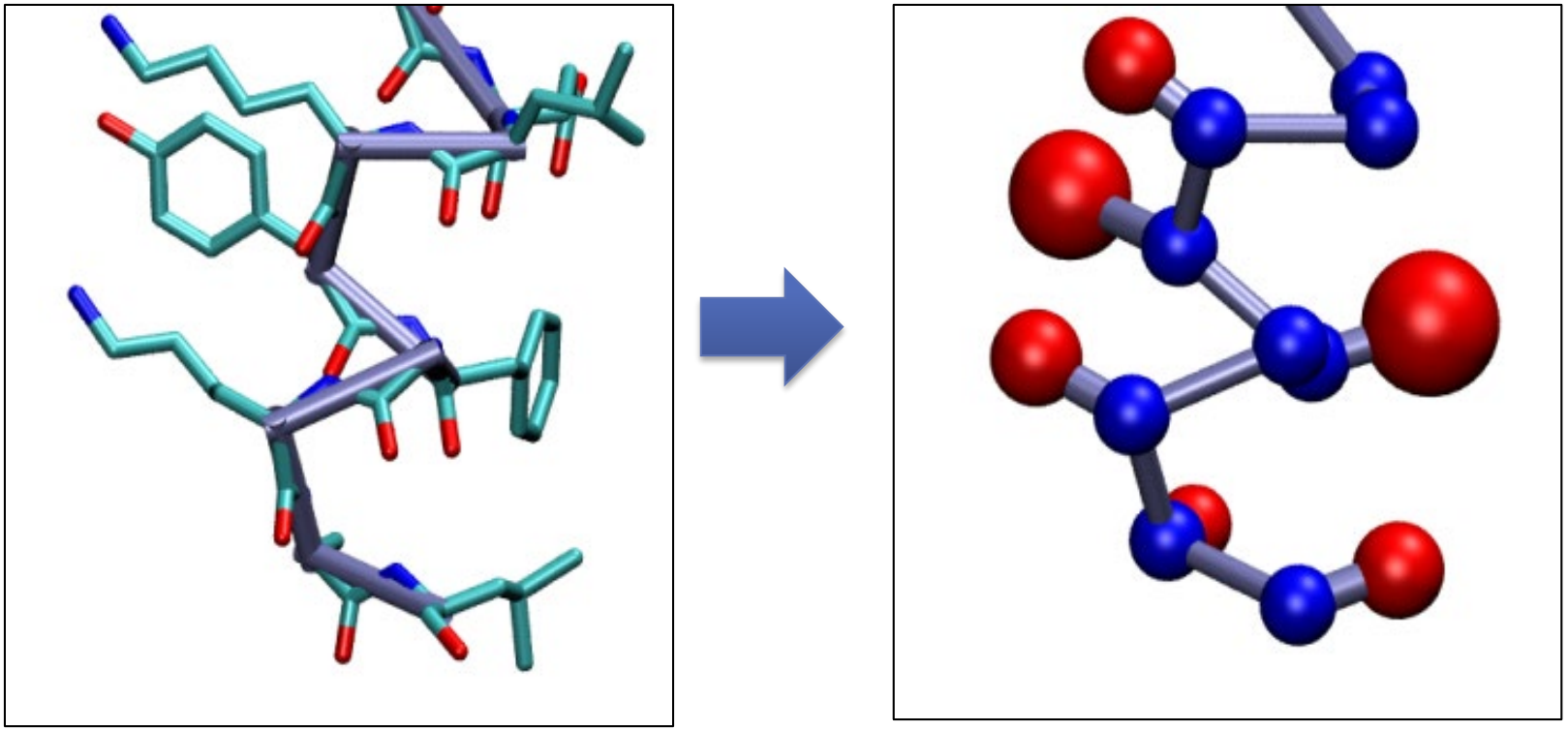
At 4 °C, experimental rates:

CaM-CaMKI k_{on} (10^8 $M^{-1}s^{-1}$)	CaM-CaMKII k_{on} (10^8 $M^{-1}s^{-1}$)
3.79	1.54

- A factor of 2 on-rates cannot be explained by solely a diffusion-controlled mechanism
- The differences in on-rates must involve post-contact events.

Need computations and theories!

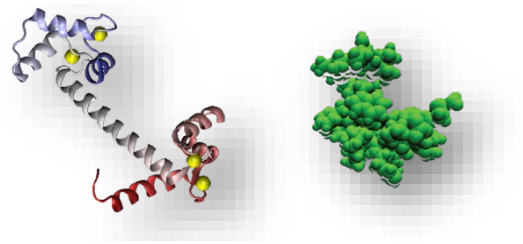
A coarse-grained side-chain Cα protein model for both CaM and target efficiently samples a broad conformational ensemble



Cheung, M.S., Finke, J.M., Callahan, B. & Onuchic, J.N. JPCB (2003)

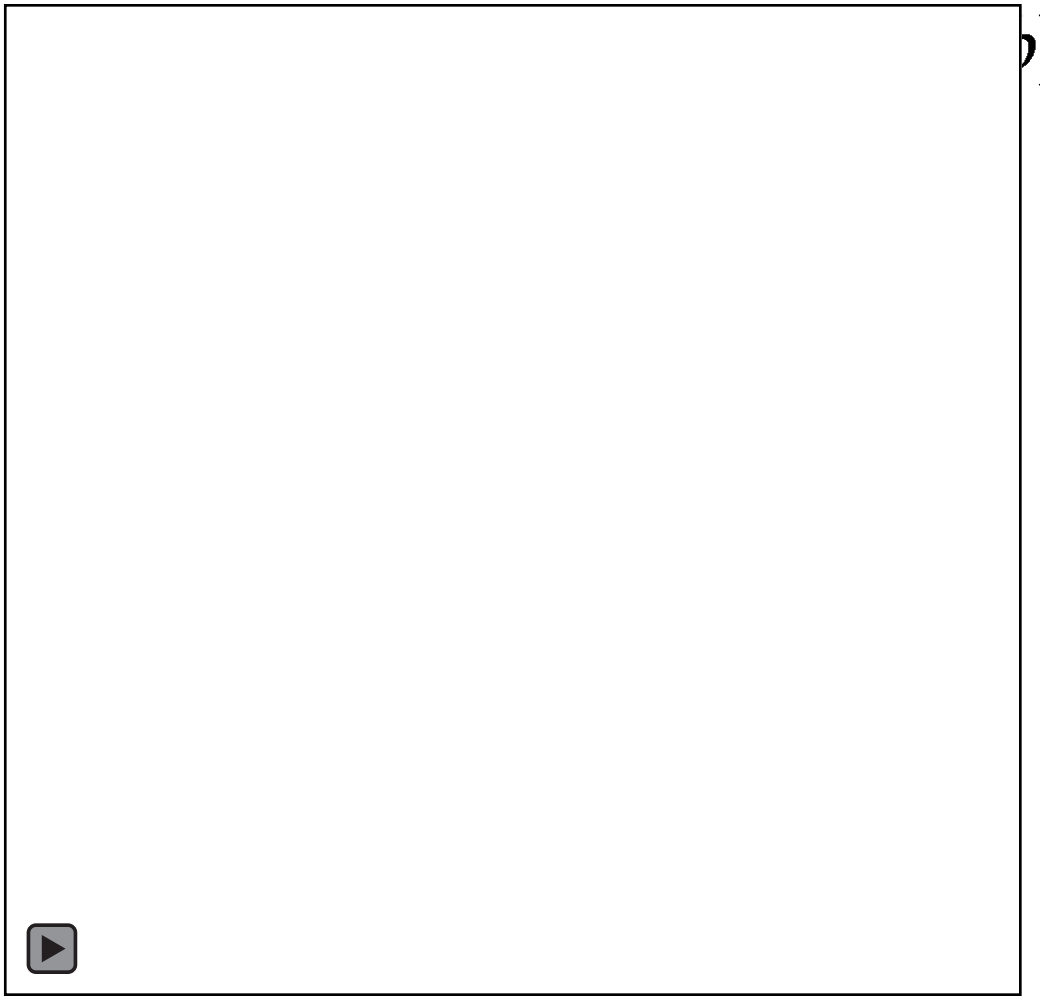
The Hamiltonian for the CaM-target complex is not biased toward a specific complex structure

$$E = E_{CaM} + E_{target} + E_{CaM\text{-}target}$$



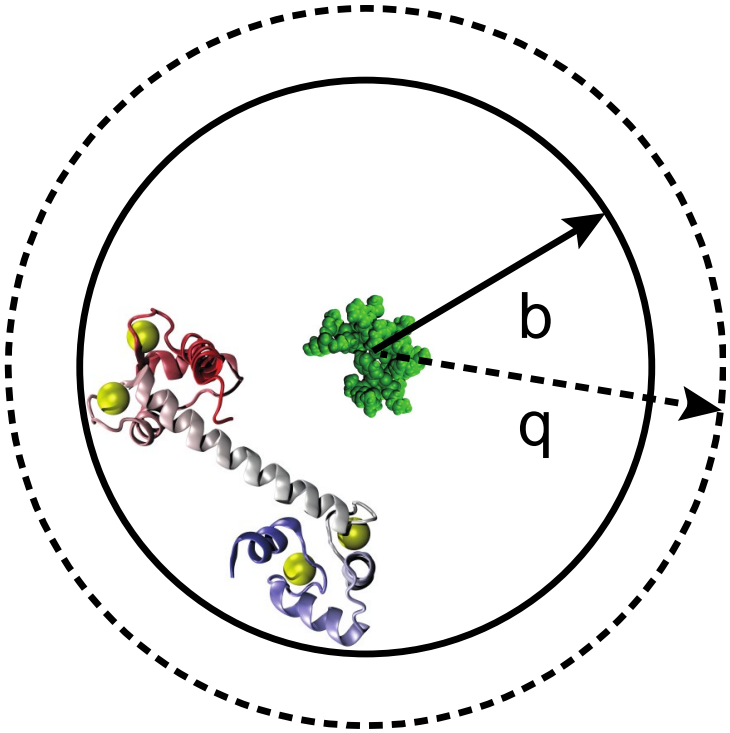
Compute association rates (k_a) by running tens of thousands of Brownian dynamics simulations

$$\left[\frac{\beta}{1-(1-\beta)\Omega}\right]$$



(Cont.d) Compute association rates (k_a) by running tens of thousands of Brownian dynamics simulations

$$k_a = k_D(b)\left[\frac{\beta}{1-(1-\beta)\Omega}\right]$$



β: the probability of successful events

Ω = b/q =0.20: the probability that a target at $r=q$ will eventually return to $r=b$

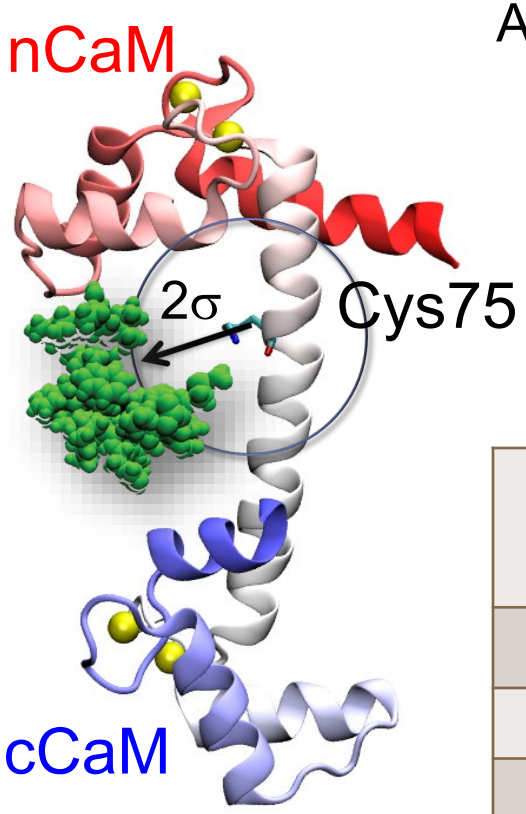
k_D (b) = 4πDb, the rate that a target achieve at b; D is diffusion coefficient.

Northrup, Allison, McCammon, JCP 1984

How to define a successful event?

What is an encounter complex?

Experiments guide the calculation of K_a from computer simulations by setting up a proper order parameter



At 4 °C, experimental measured association rates

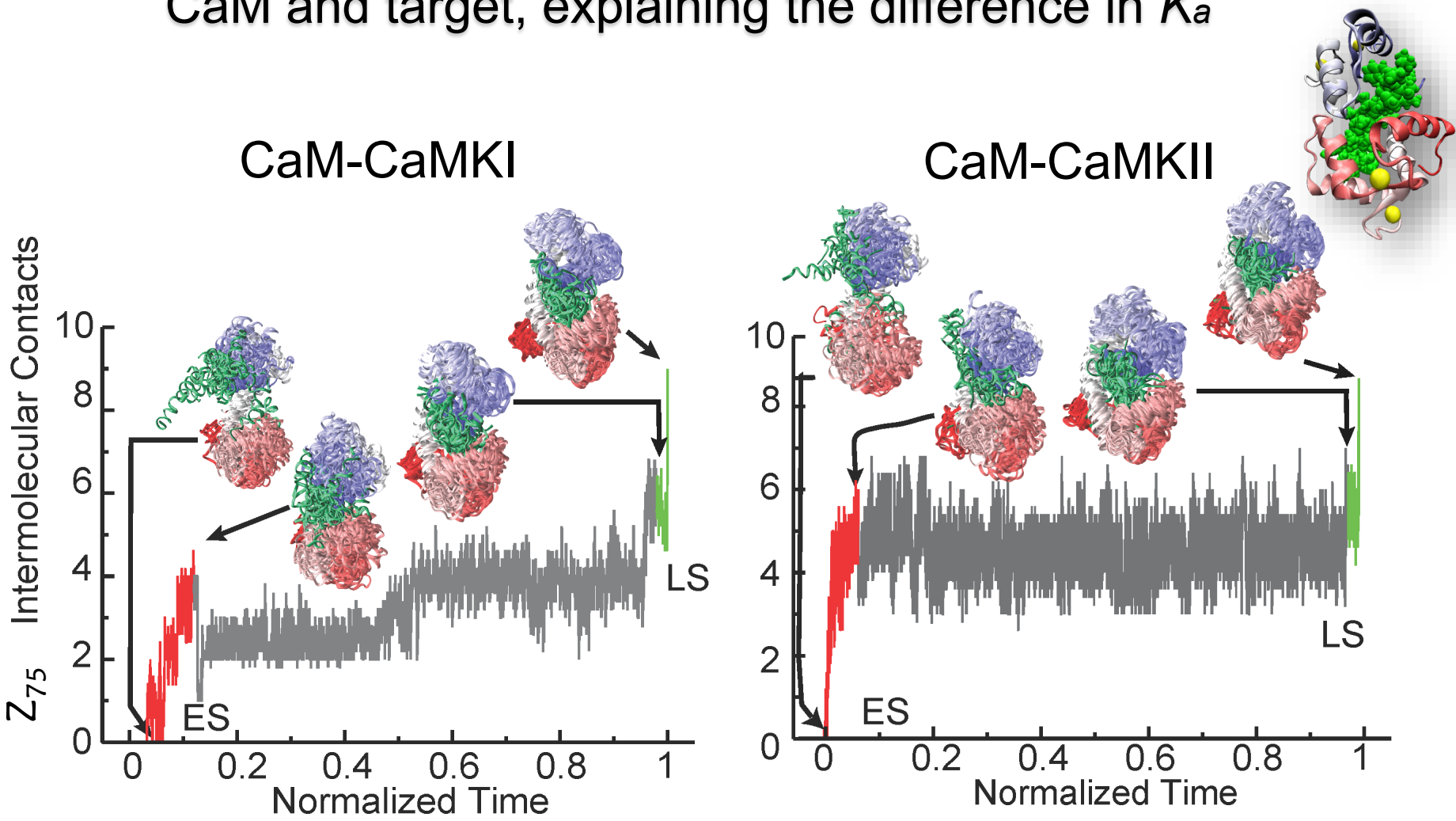
CaM-CaMKI (10^8 $M^{-1}s^{-1}$)	CaM-CaMKII (10^8 $M^{-1}s^{-1}$)
3.79	1.54

Threshold Z_{75}	CaM-CaMKI (10^8 $M^{-1}s^{-1}$)	CaM-CaMKII (10^8 $M^{-1}s^{-1}$)
5	57.305	59.084
6	41.591	47.339
7	28.248	27.882
8	18.018	14.560
9	5.618	2.669
10	0.252	0.126

Wang, MSC... PNAS (2013)

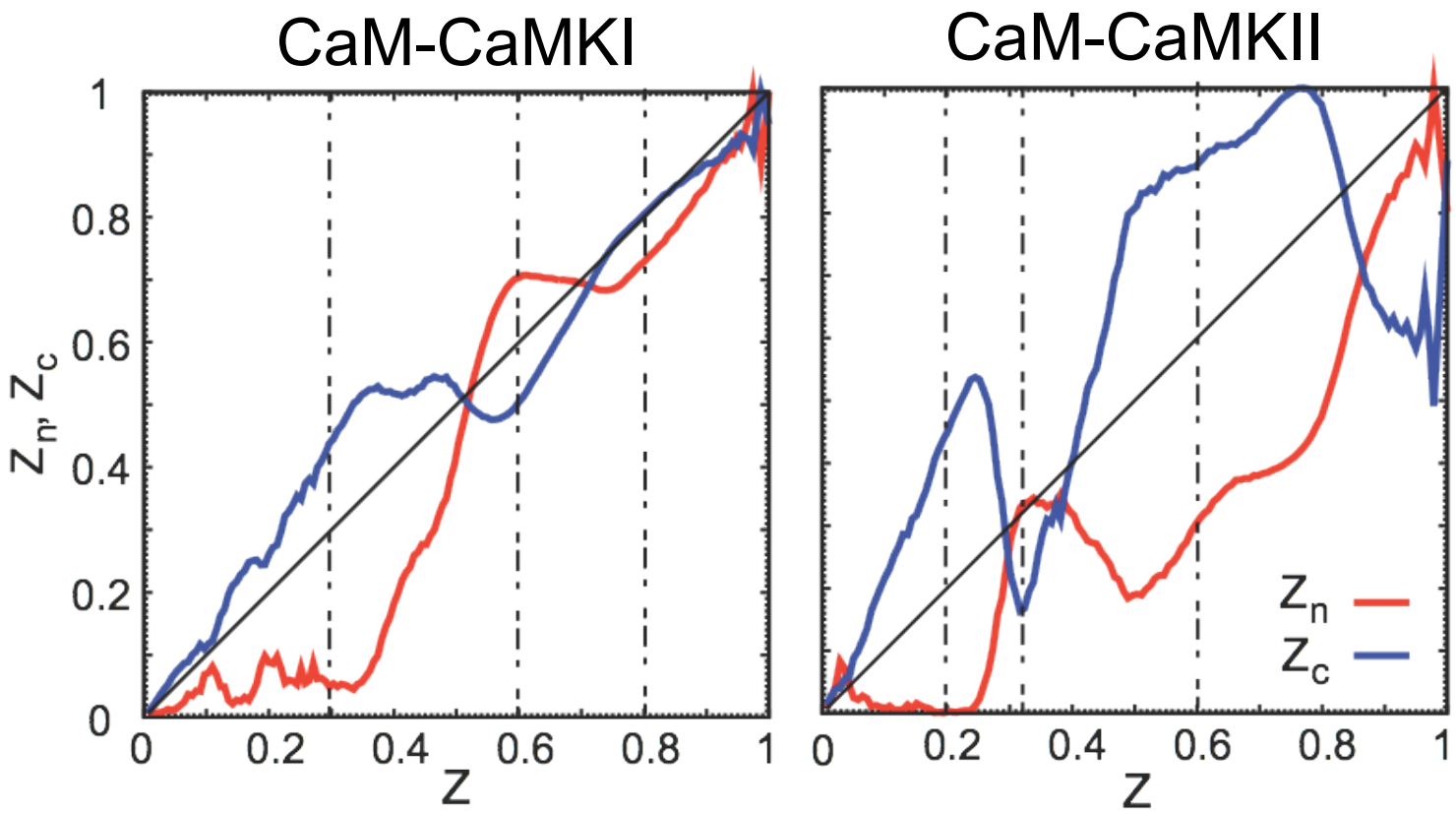
Intermolecular contacts: Z_{75}

The post-collisional events involve structural arrangement of both CaM and target, explaining the difference in K_a



Wang, Zhang, MSC.. PNAS (2013)

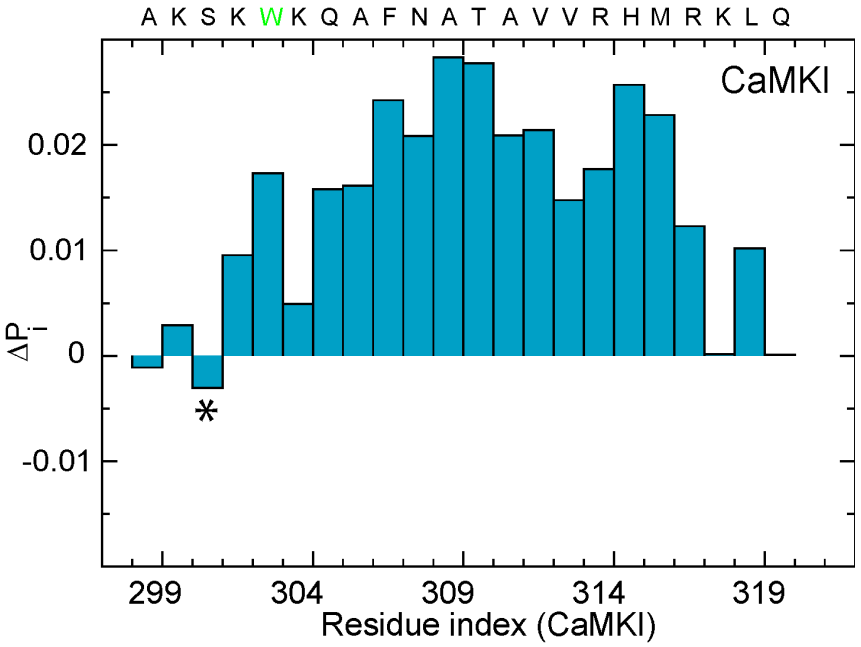
CaM-CaMKI is less frustrated than CaM-CaMKII

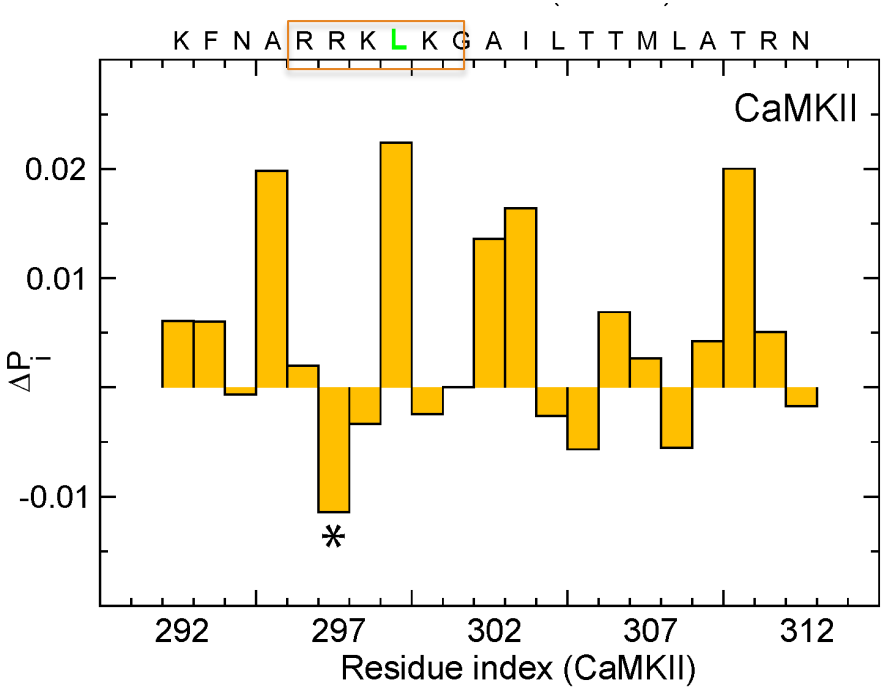


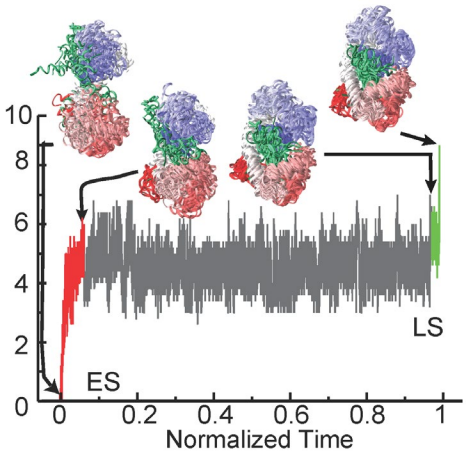
Z=Zn+Zc is the total no. of (normalized) side-chain contacts between CaM and targets

Tripathi, MSC et al J. Mol Reg. (2015)

Distinctive charge distributions from the target peptides contribute to CaM's binding frustration

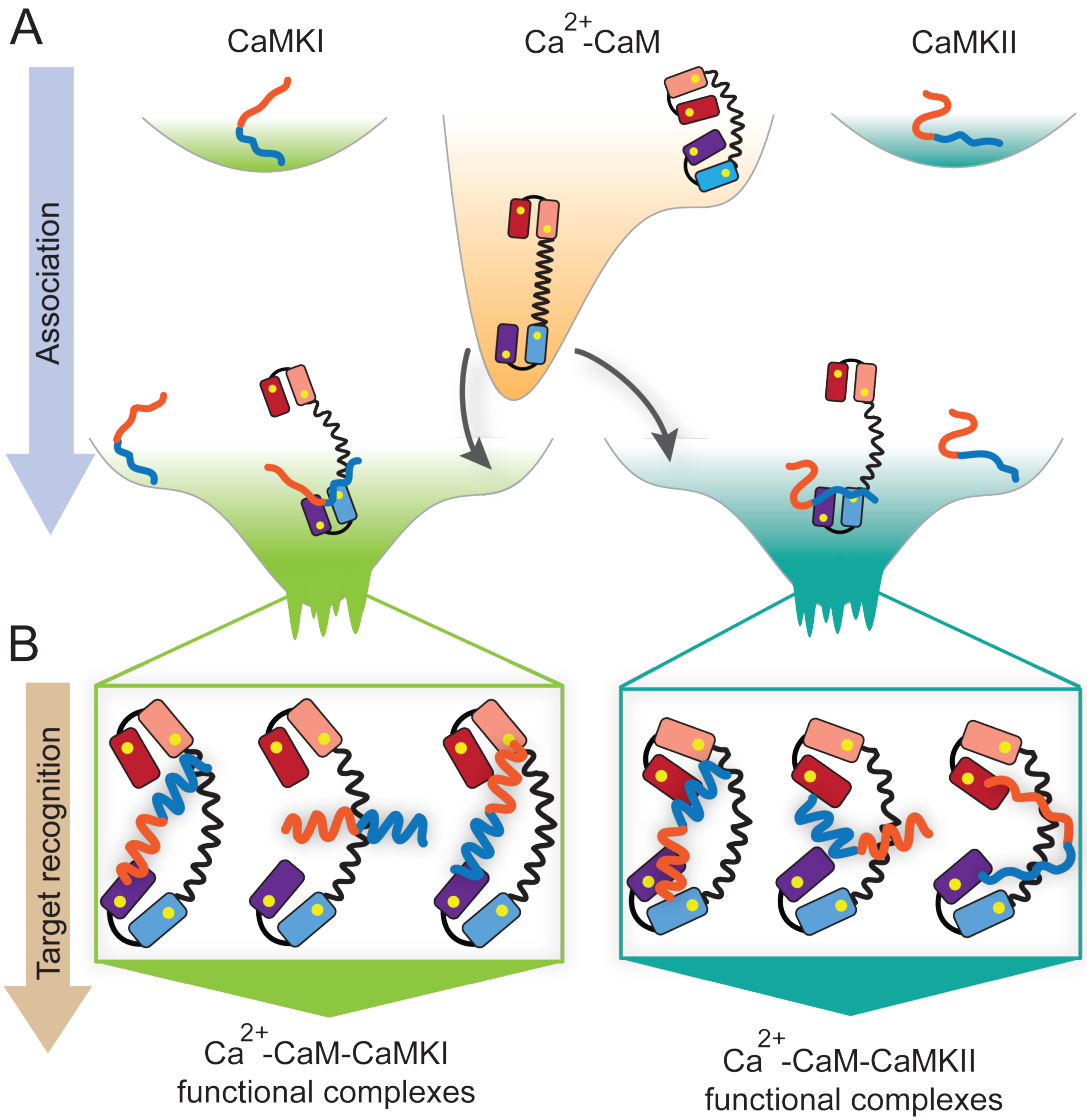




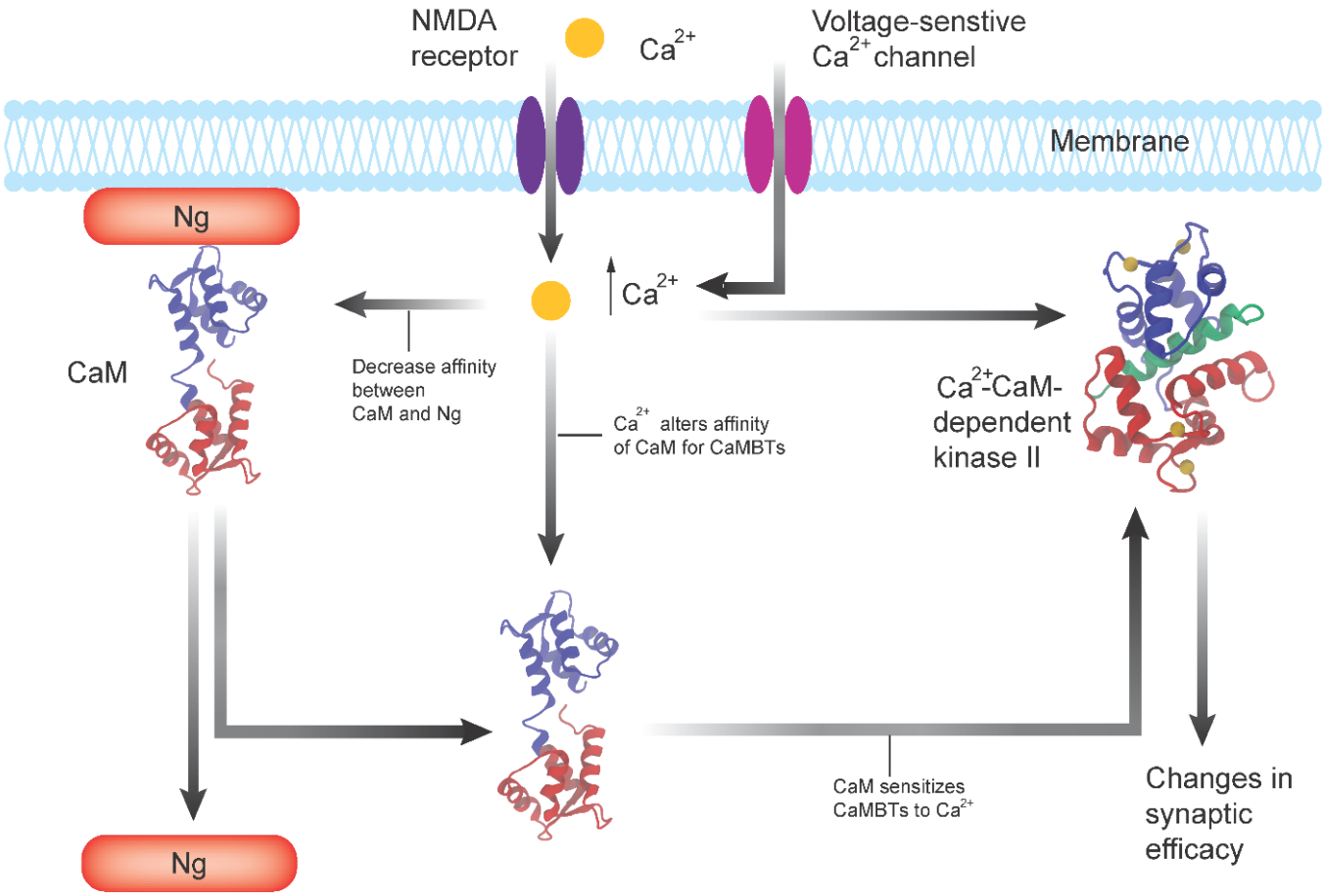


Tripathi, MSC J. Mol Reg. (2015)

CaM-target recognition is mediated through conformational and mutually induced fit



CaM needs another CaM-binding protein to tune its affinity for calcium



Kubota Y, Putkey JA, Shouval HZ, Waxham MN 2008, J. Neurophysiology

THE JOURNAL OF BIOLOGICAL CHEMISTRY

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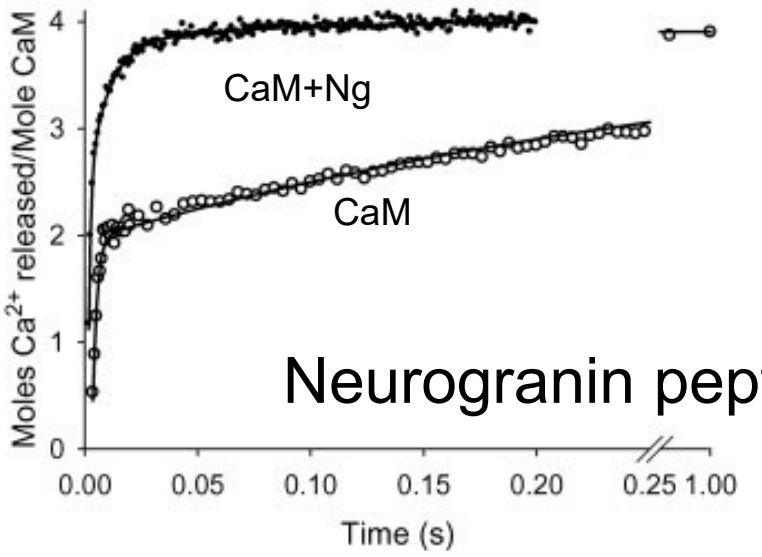
Printed in U.S.A.

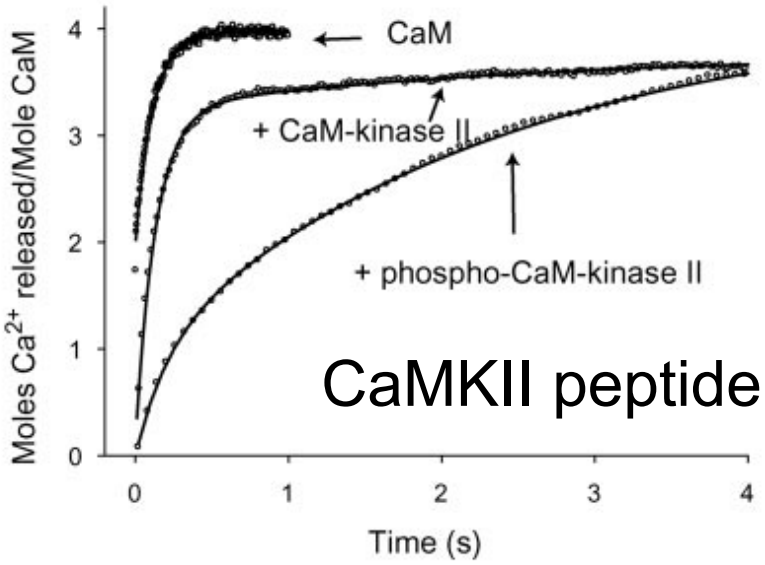
RC3/Neurogranin and Ca^{2+}/Calmodulin-dependent Protein Kinase II Produce Opposing Effects on the Affinity of Calmodulin for Calcium*

Received for publication, May 13, 2004, and in revised form, June 23, 2004
Published, JBC Papers in Press, July 15, 2004, DOI 10.1074/jbc.M405352200

Tara R. Gaertner‡§, John A. Putkey¶, and M. Neal Waxham‡‖

From the Departments of ‡Neurobiology and Anatomy and ¶Biochemistry and Molecular Biology, University of Texas Medical School, Houston, Texas 77030





Neurogranin (Ng) is abundant in neurons

1. Ng knock-out mice exhibited deficits in spatial learning (Pak, PNAS, 2000)

2. Ng has a slightly higher binding affinity for apoCaM than holoCaM by a factor of 2 (Kd~nM, Waxham, JBC, 2014)

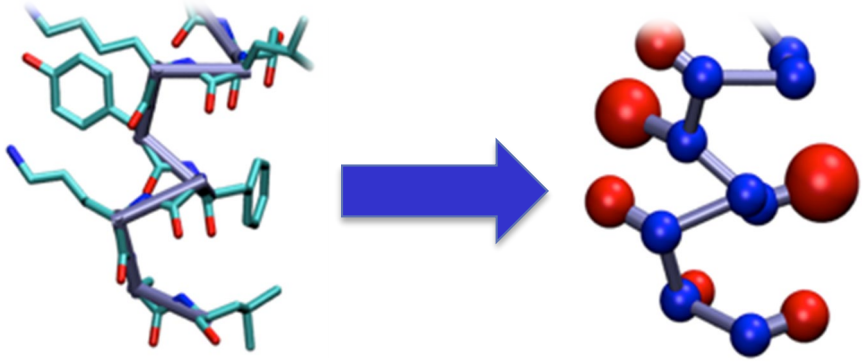
3. The **acidic region** and **IQ domains** (Ng_{13-49}) are essential for function
   DDDILDIPLDDPGANAAAAKIQASFRGHMARKKIKSGECG
   IQ motif: IQXXXRXXXXR (Waxham, JBC, 2014)

4. There is no structure of a CaM-Ng bound complex except with a tethered Ng

5. We modeled the bound CaM-Ng using additional information from NMR

Hamiltonian of coarse-grained molecular simulations for CaM-Ng

Structural information from the target and the bound complex is absent



$$E = E_{CaM} + E_{CaM\text{-}target} + E_{target}$$

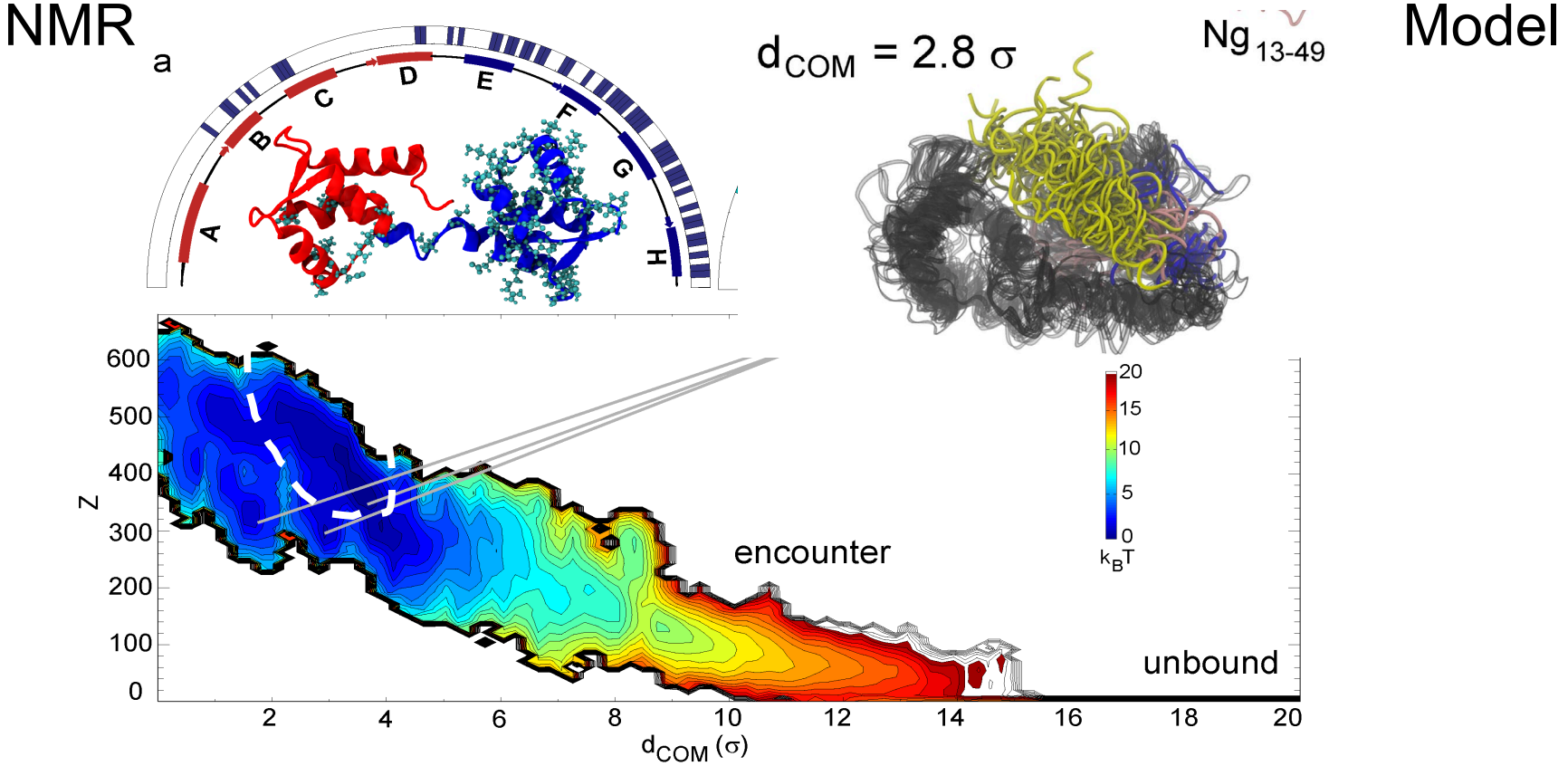
Sequence dependent

$$E_{target} = E_{structural} + E_{vdW/HB} + E_{Debye\text{-}Hückel}$$

$$\text{Target: } E_{structural} = E_{bond} + E_{angle} + E_{dihedral} + E_{chiral}$$

$$E_{CaM\text{-}target} = E_{vdW/HB} + E_{Debye\text{-}Hückel}$$

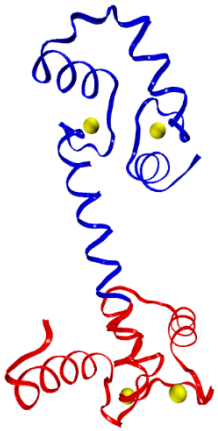
The distribution of bound CaM-Ng conformations is broad (I=0.1M, pH = 6.3)



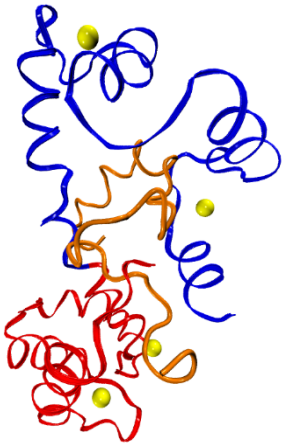
CaM is still structurally extended upon Ng binding, not wrapping around a kinked Ng

All-atom simulations: bound complexes determine affinity for Ca^{2+}

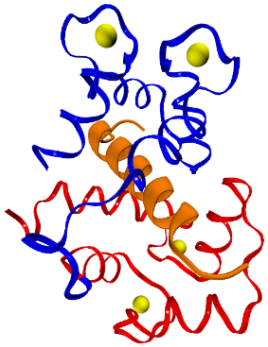
holoCaM
PDB: 1CLL

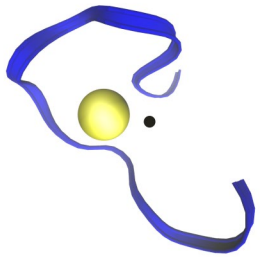


holoCaM-Ng
(reconstructed)



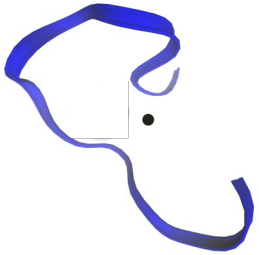
holoCaM-CaMKII
PDB: 1CDM





Jarzynski equality

nonequilibrium work (*w*)



$$\exp(\beta\Delta G) = \langle \exp(-w) \rangle_{paths}$$

$$\Delta G = G_B - G_U$$

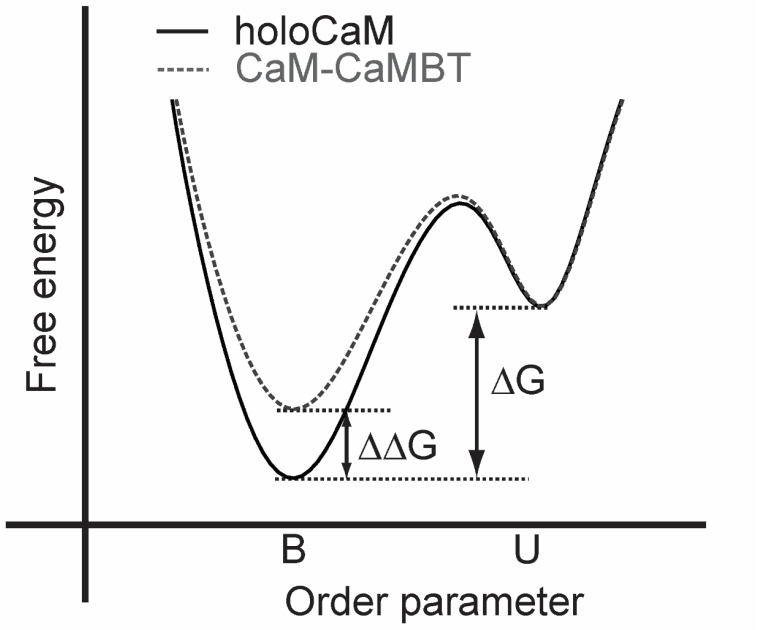
CaM-CaMKII complex retains Ca^{2+}; CaM-Ng did not

$\Delta G = G_B - G_U$

$\Delta\Delta G$ = ΔG(holoCaM-CaMBT) - ΔG(holoCaM)

$\Delta\Delta G$ of CaM with CaMKII < 0; Ca^{2+} affinity increases

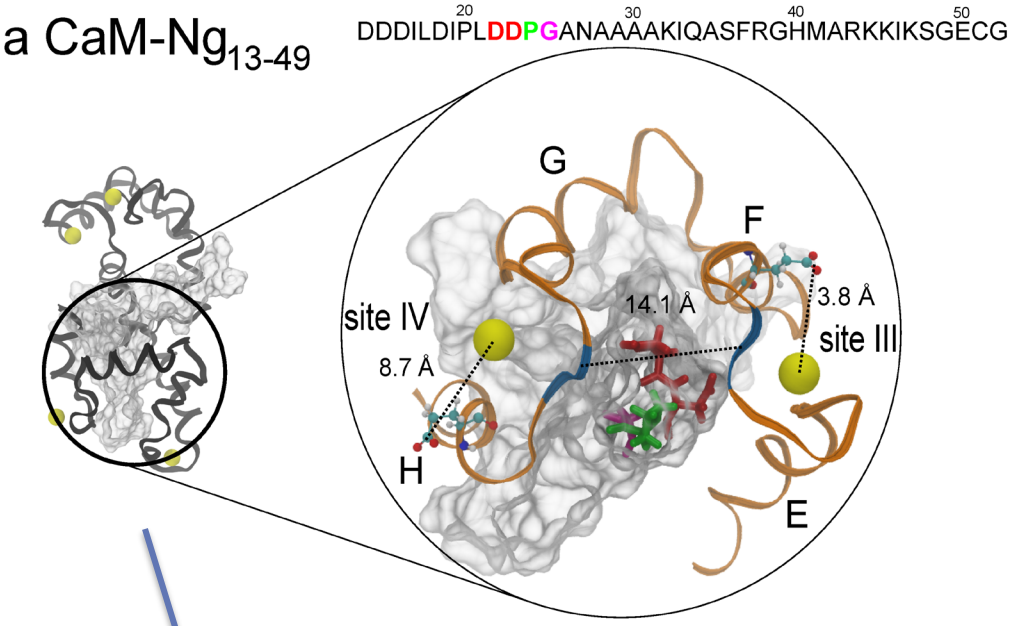
$\Delta\Delta G$ of CaM with Ng > 0; Ca^{2+} affinity decreases

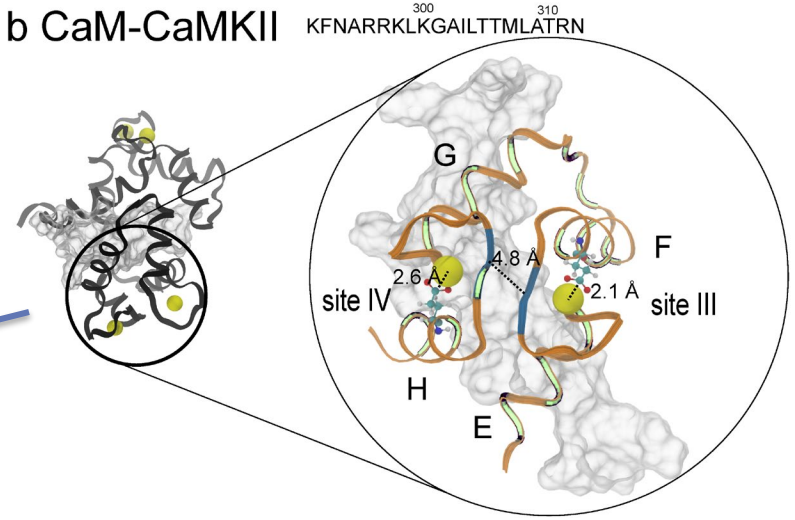


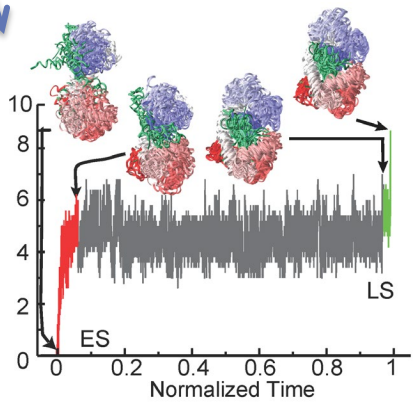
pH=7.4 I=0.1M		holoCaM-Ng_{13-49} (average)	holoCaM-CaMKII
ΔΔG (kcal/mol) CI extrapolation	Site III	9.2±2.2	-2.5
	Site IV	22.4±0.9	-1.7
ΔΔG (kcal/mol) from experimental measurements	Site III/IV	2.5	-3.3

Zhang, Tripathi, Trinh, Cheung.
Biophysical Journal 2017

Distinctive bound complexes delineate the importance of CaM's progressive mechanism of target binding on its Ca^{2+} binding affinities





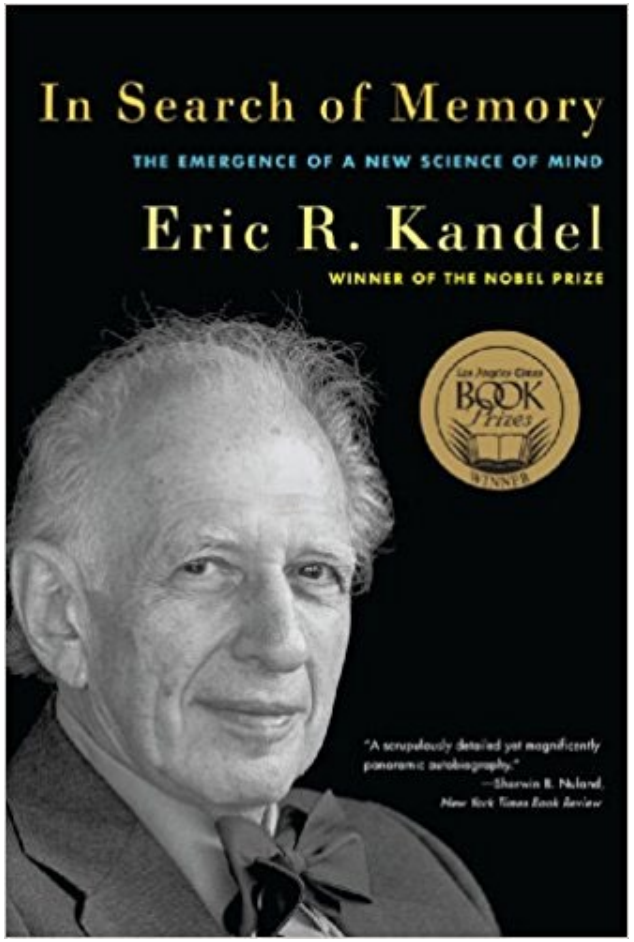


Zhang, Tripathi, Trinh, Cheung.
Biophysical Journal 2017

What is the biology of mind?

"The new biology of mind is potentially more disturbing because it suggests that not only the body, but also mind and the specific molecules that underlie our highest mental processes – consciousness of self and of others, consciousness of the past and the future – have evolved from our animal ancestors."

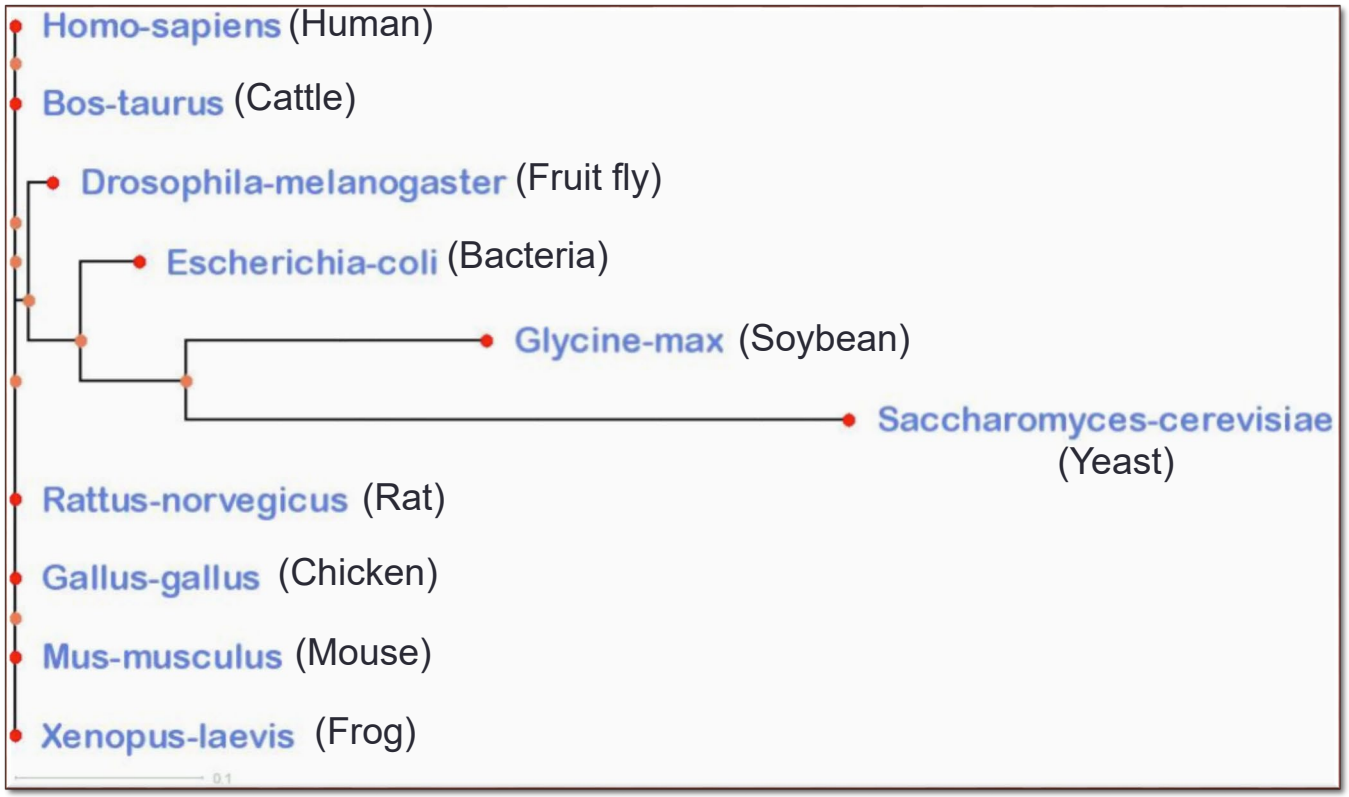
-- Eric R. Kandel,
2000 Nobel laureate



CaM is found in eukaryotes and its primary amino acid sequence is highly conserved among eukaryotes
(In fact, all 148 of the a.a. are conserved for vertebrates.....)

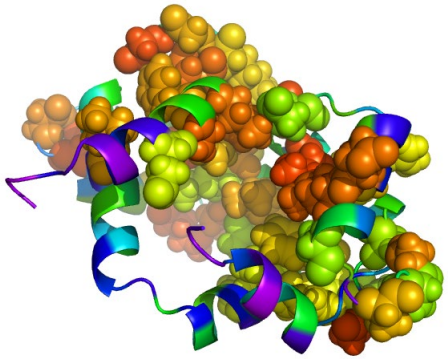
The function of CaM is essential for various pathways in almost all eukaryotes
(*e.g.* calcium binding signal transducers is consistent throughout all eukaryotes)

Evolutionary tree
(CaM sequence)

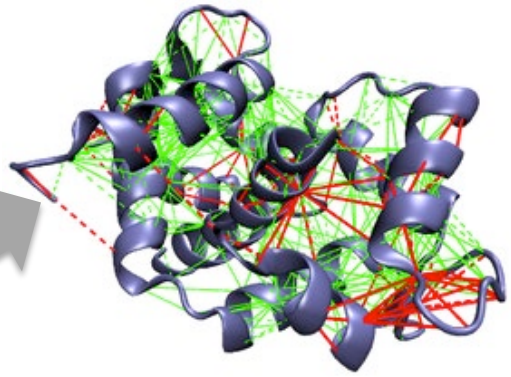


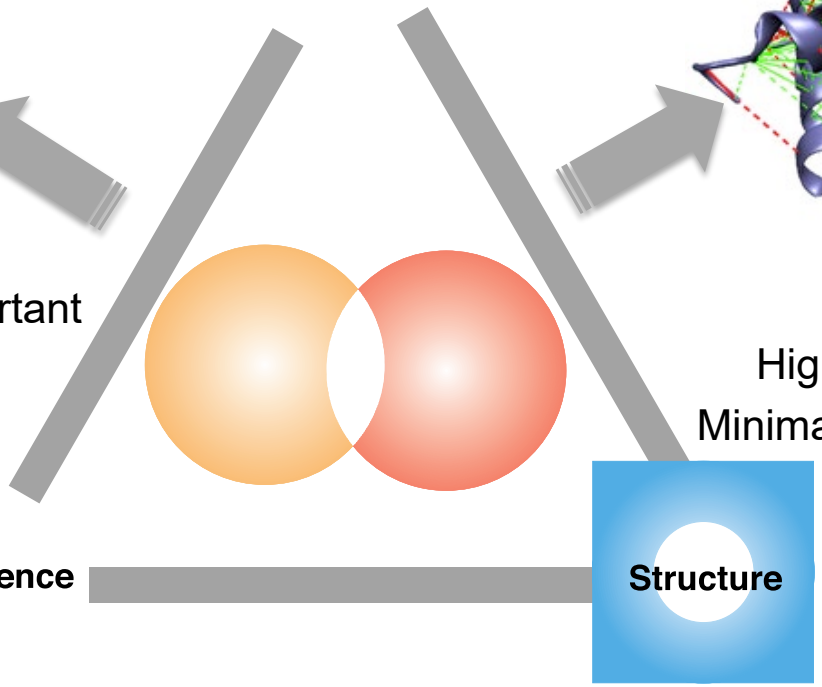
Can dynamics (physics) be an evolutionary constraint?

Evolutionary Trace



Frustratometer



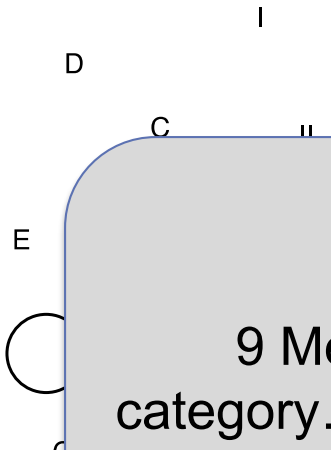


Lichtarge JMB 2004
http://mammoth.bcm.tmc.edu/

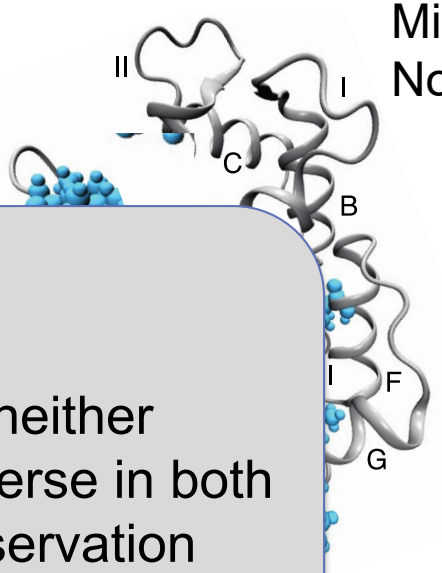
Wolynes PNAS 2010
http://www.frustratometer.tk/

Highly frustrated
Non-conserved

Post Translational Modification



Minimally frustrated
Non-conserved

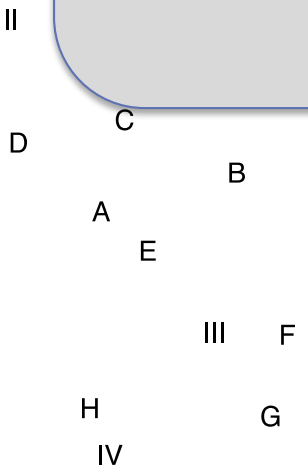


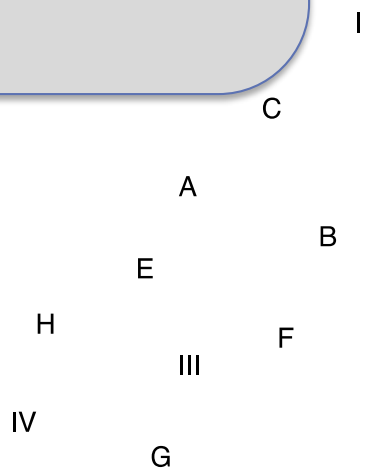
Modular

9 Mets belong to neither category…they are diverse in both frustration and conservation

Functional

Highly frustrated
Conserved



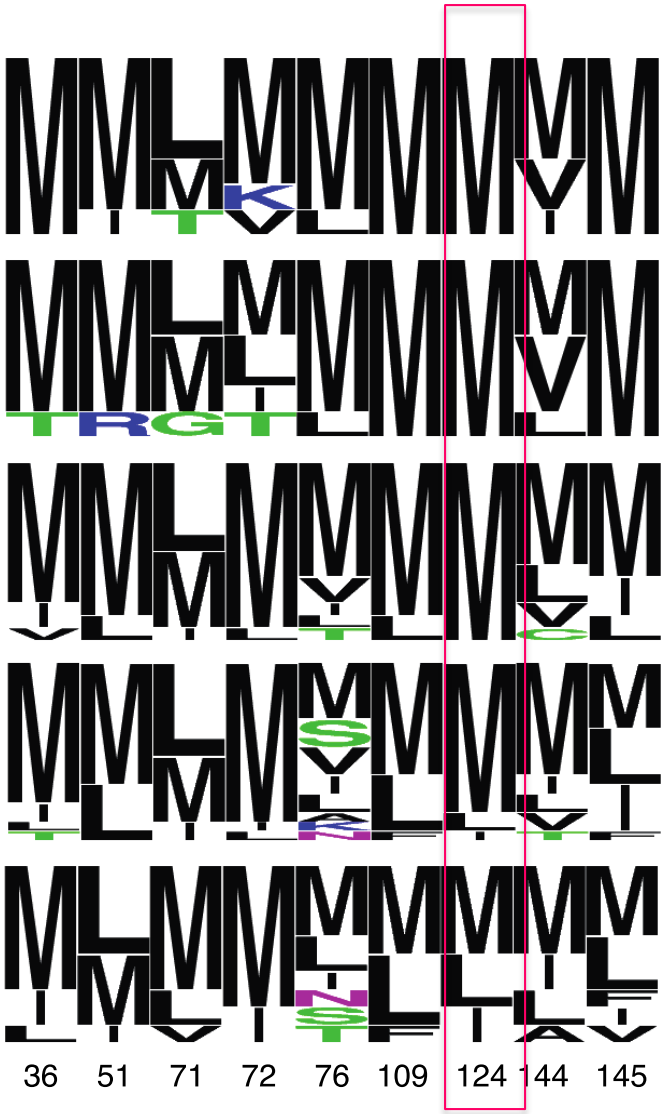


Folding

Minimally frustrated
Conserved

Tripathi, Waxham, Cheung, Liu, Scientific Report 2015

CaM becomes less hydrophobic throughout evolutionary history



Now

Ancient

Met124 has evolved against a singular notion of stability. Instead, its conformational dynamics is a tradeoff for binding promiscuity to diversify

Tripathi, Waxham, Cheung, Liu, Scientific Report 2015

Conclusions and outlook

- A “conformationally and mutually induced fit” as a mechanism for CaM to recognize targets that lack distinct structures
- CaM’s progressive mechanism of target binding regulates its Ca^{2+} binding affinities
- Acidic region of Ng is key to lessen binding affinity of CaM for calcium. Bidirectional binding of CaM-target is critical to the reciprocal relation to calcium affinity.
- Dynamics is an evolutionary driving force for promiscuous proteins to achieve their binding multi-specificity and diverse biological functions.

- Need novel computational tools to simulate and characterize IDPs that explain the observations from experiments.

- Need to move beyond the peptide models for CaM-binding targets.

- Need novel models and force fields for Ca^{2+}-binding proteins.

- Need novel theoretical approaches to connect time-varying calcium signals to the molecular mechanism of CaM binding for target selection.

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