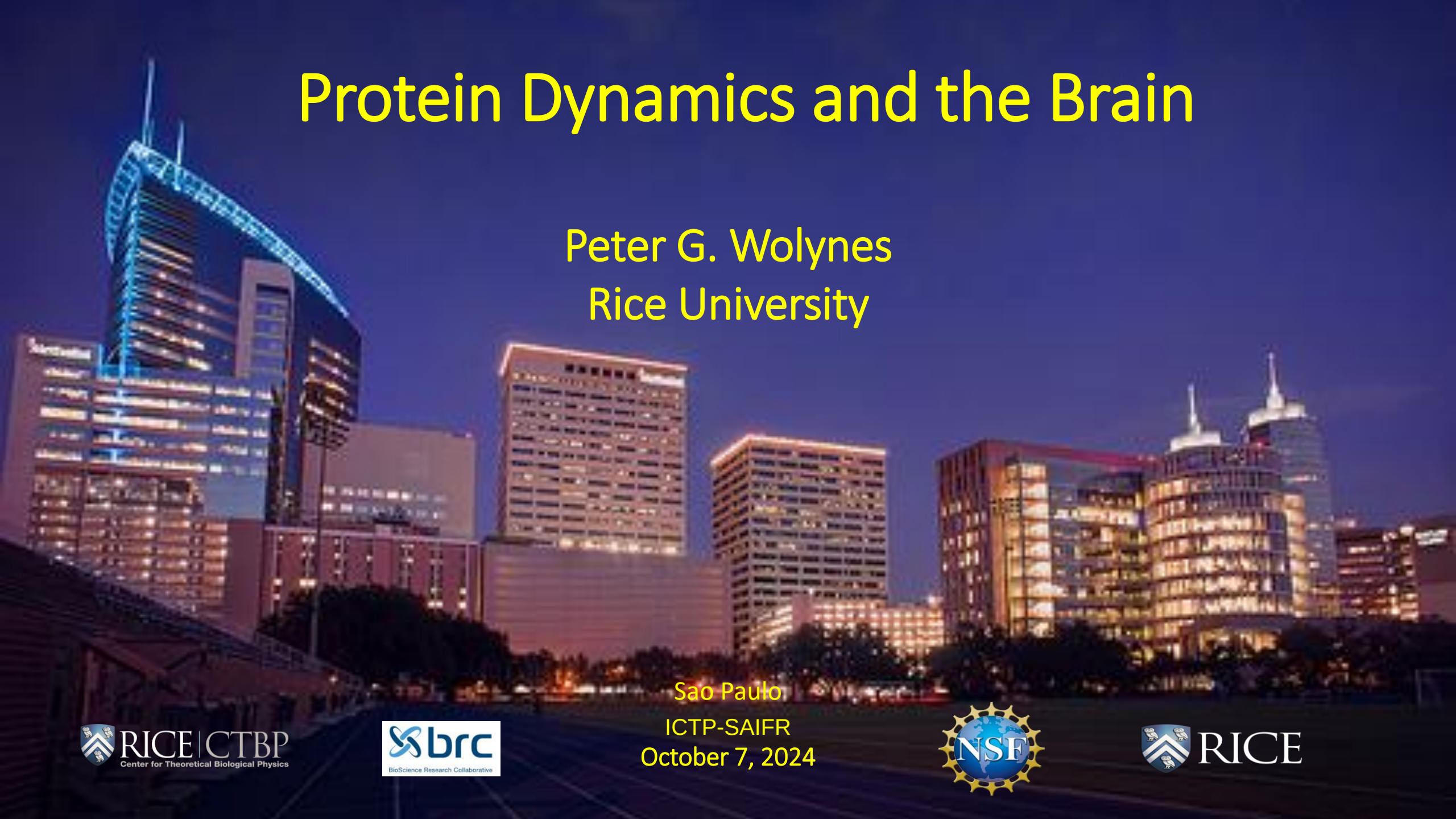
Protein Dynamics and the Brain

Peter G. Wolynes
Rice University



Sao Paulo
ICTP-SAIFR
October 7, 2024

RICE | CTBP Center for Theoretical Biological Physics

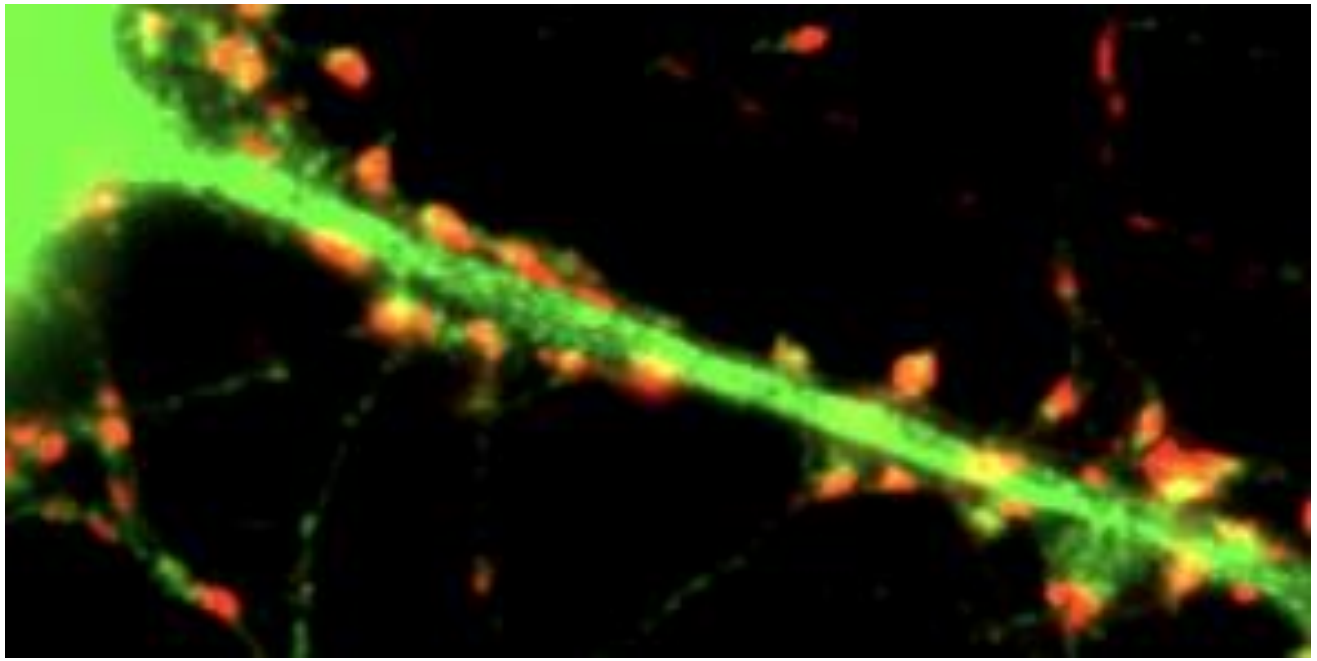
brc BioScience Research Collaborative

NSF

RICE

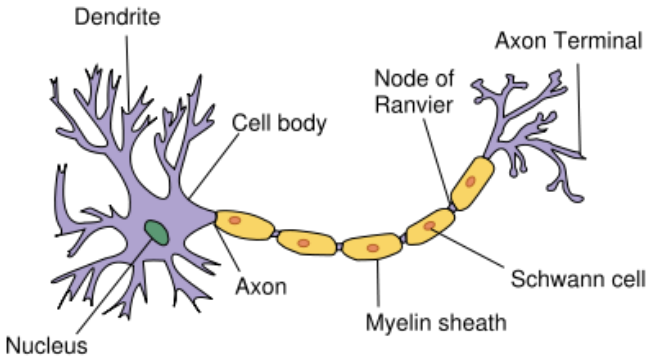
“No sooner had the warm liquid mixed with the crumbs touched my palate than a shudder ran through me and I stopped, intent upon the extraordinary thing that was happening to me. An exquisite pleasure had invaded my senses, something isolated, detached, with no suggestion of its origin. And at once the vicissitudes of life had become indifferent to me, its disasters innocuous, its brevity illusory—this new sensation having had on me the effect which love has of filling me with a precious essence; or rather this essence was not in me it was me. ... Whence did it come? What did it mean? How could I seize and apprehend it? ... And suddenly the memory revealed itself. The taste was that of the little piece of madeleine which on Sunday mornings at Combray (because on those mornings I did not go out before mass), when I went to say good morning to her in her bedroom, my aunt Léonie used to give me, dipping it first in her own cup of tea or tisane. The sight of the little madeleine had recalled nothing to my mind before I tasted it. And all from my cup of tea.”

In Search of Lost Time,
Marcel Proust, 1913 - 1927

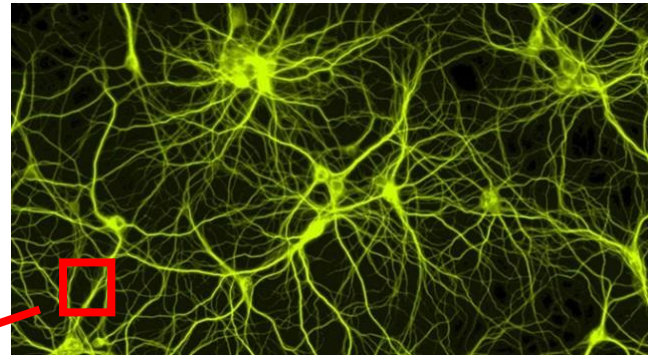


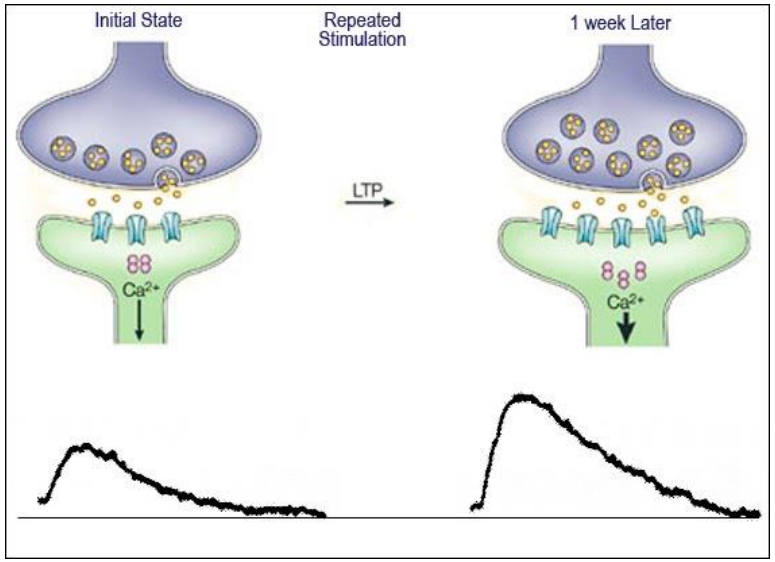
How Are Memories Formed? Hebbian Learning!

Neural cell

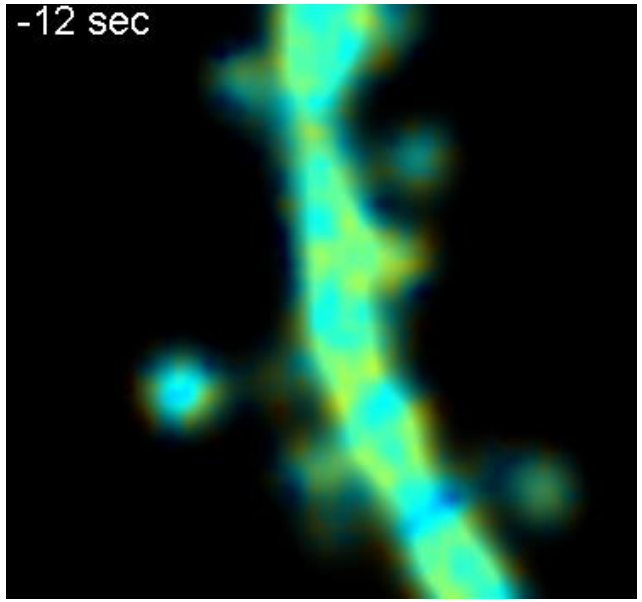


Neural network





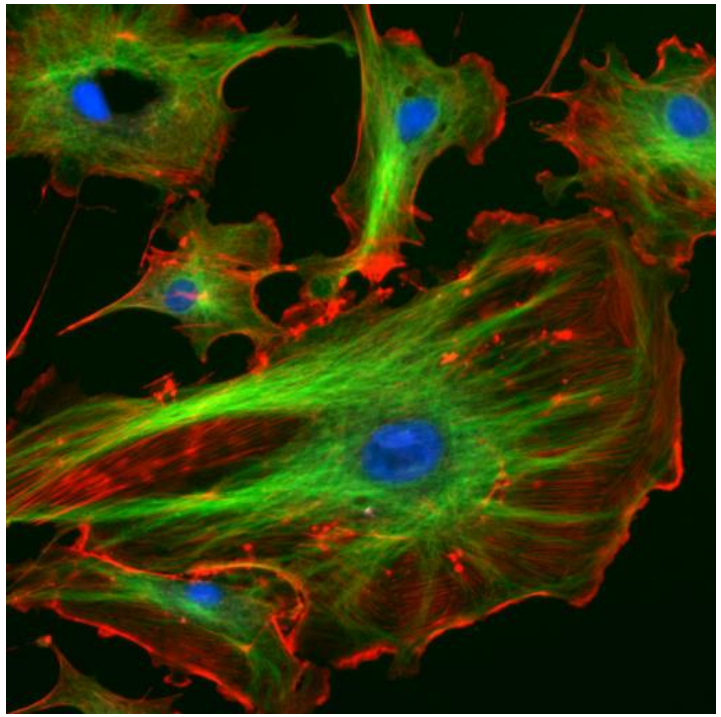
Repetition of electrical signals leads to long-term strengthening of dendritic spines. This is called **long term potentiation** (LTP).



Lee et al., Nature, 2009.

https://sites.google.com/site/mcauliffeneur493/home/synaptic-plasticity

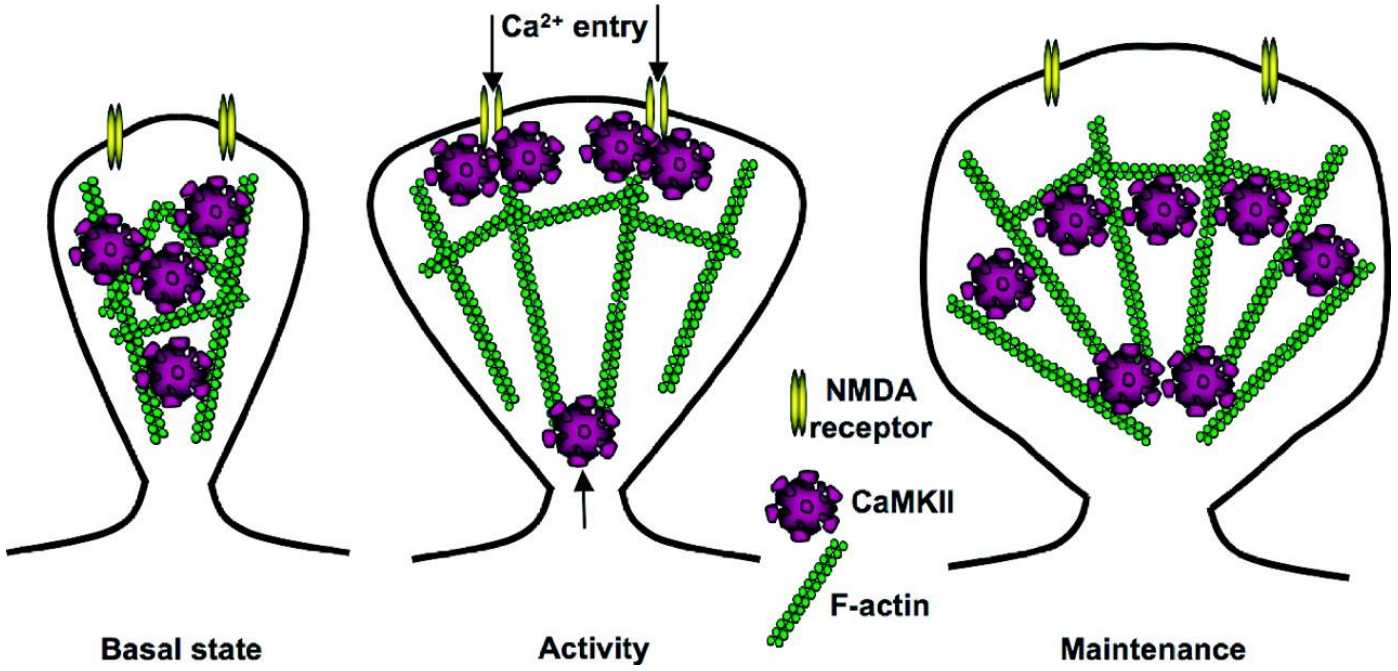
Calcium Influx Initiates the Remodeling of the Actin Cytoskeleton with Calcium Calmodulin Dependent Kinase (CaMKII)



http://rsb.info.nih.gov/ij/images/

The shape of a cell depends on a complex, dynamic network of interlinking protein filaments.

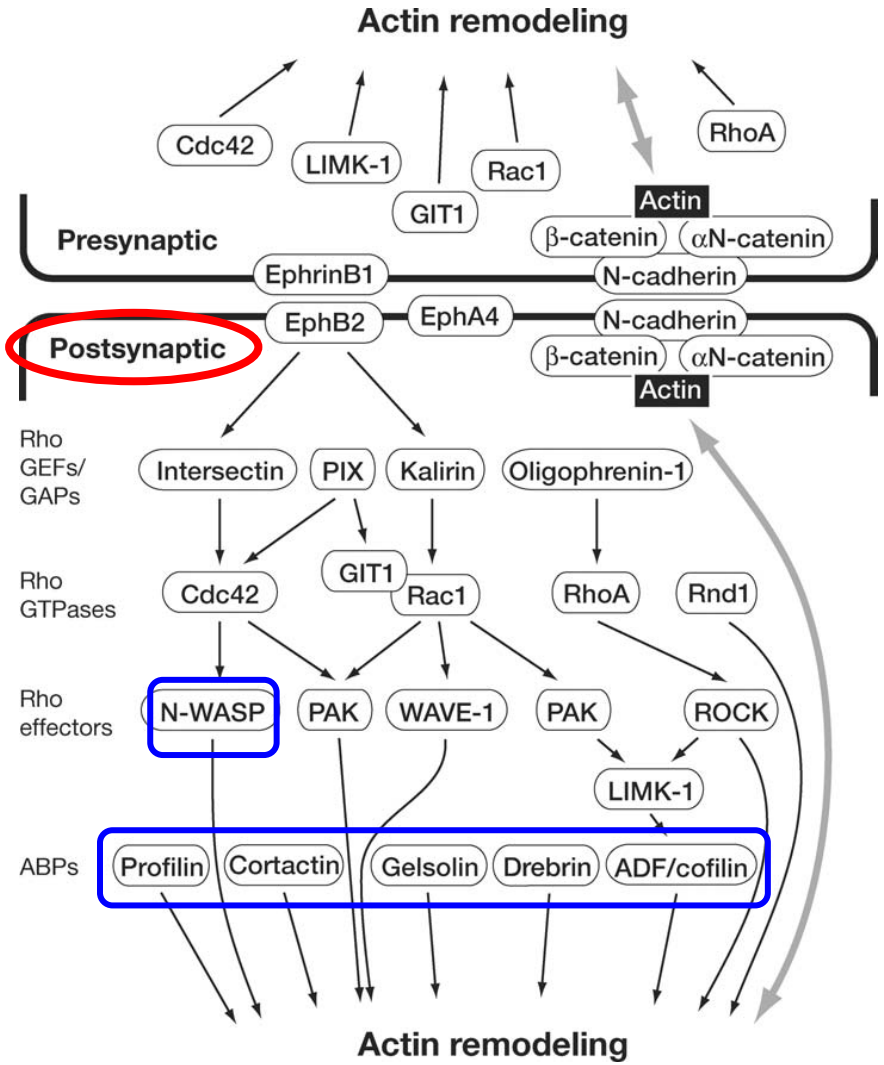
Key protein: CaMKII

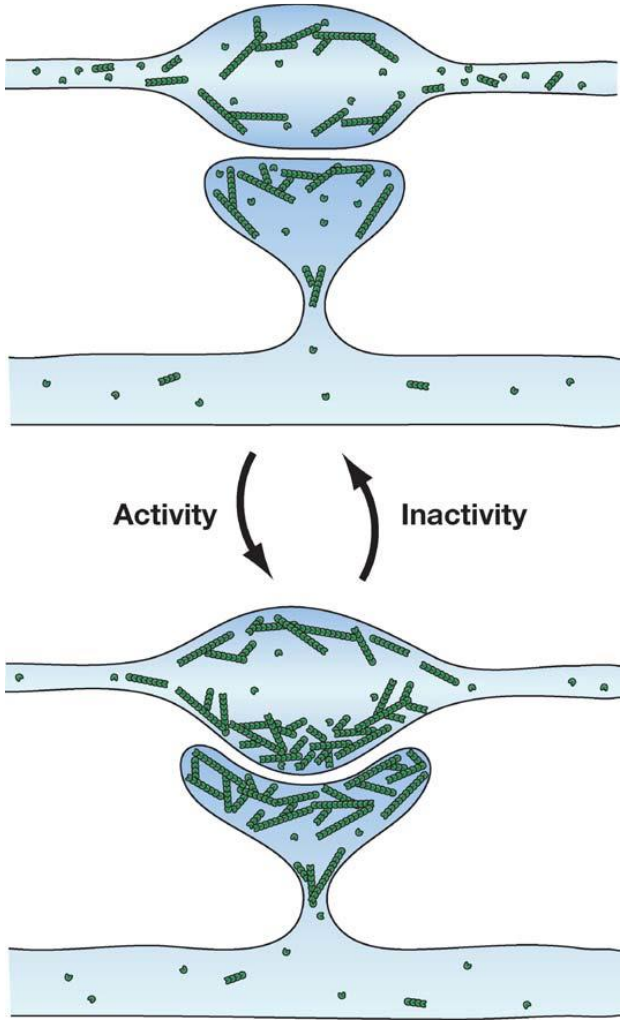


Yasunori Hayashi Group, *PNAS*, 2007.

Stable **Regulatable**

The Role of Actin Remodeling in Synaptic Memory Formation

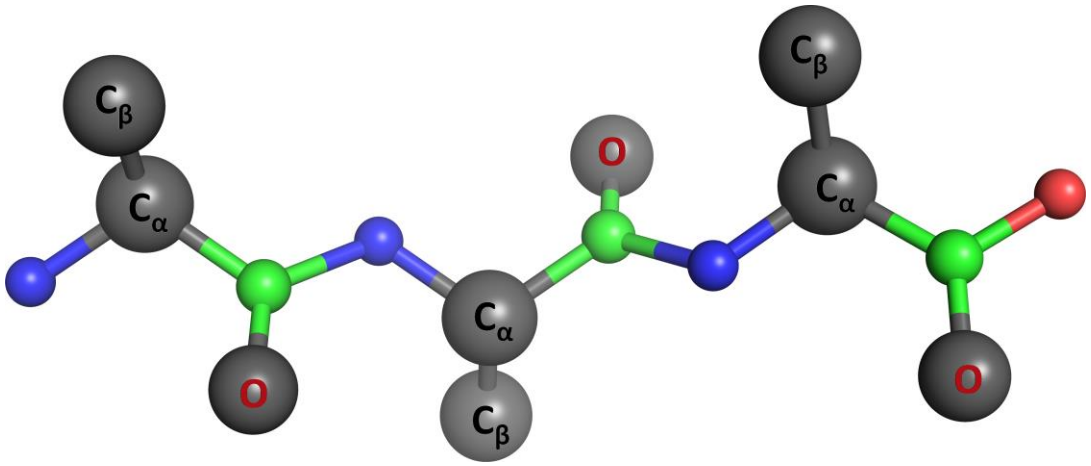




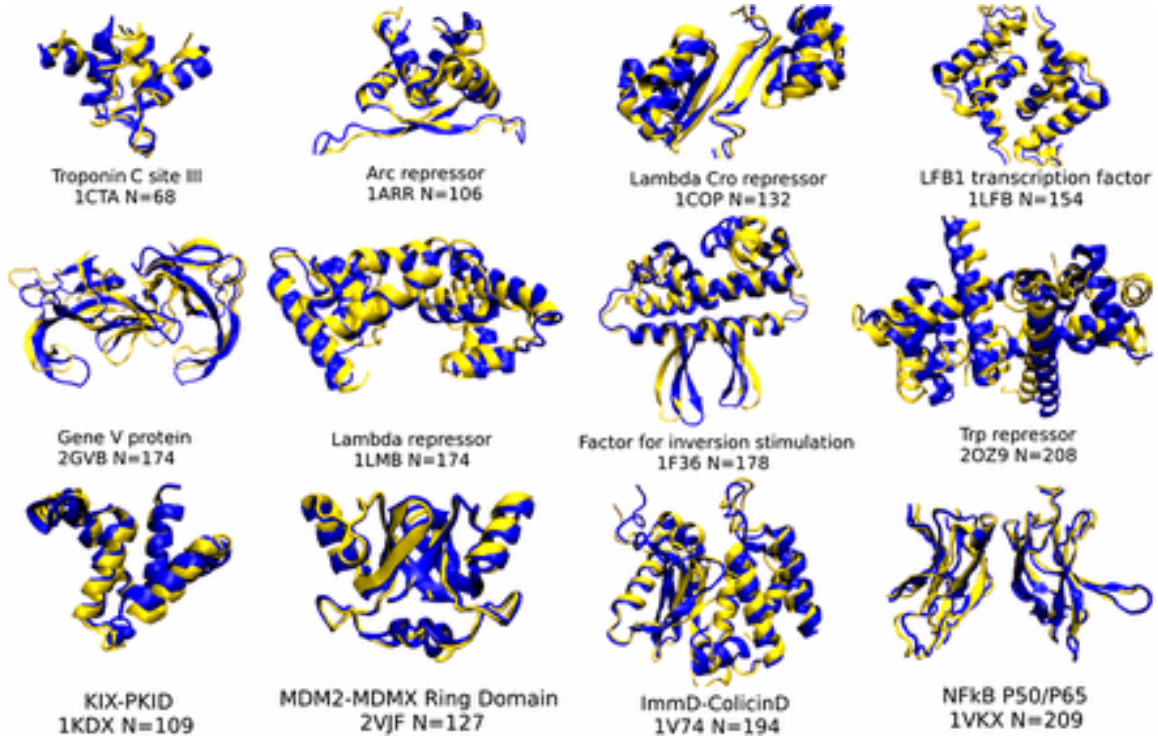
Dillon .. Goda et al., 2005, *Annual Review.*

AWSEM
(Associative Memory, Water Mediated, Structure and Energy Model)
"Machine Learning Since 1989"

Coarse-grained representation

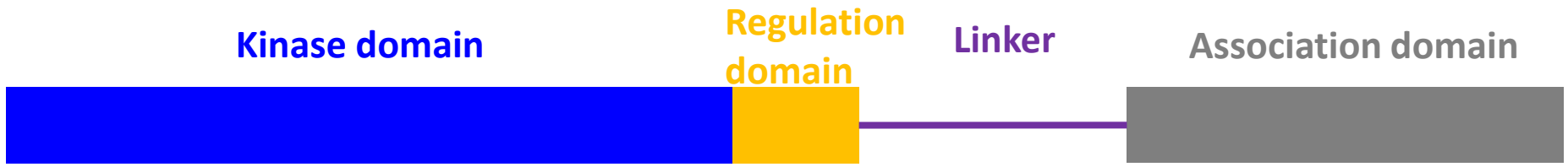


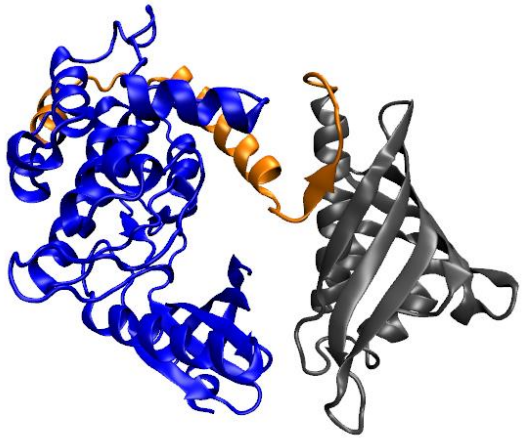
Friedrichs, M. S.; Wolynes, P. G.; "Toward Protein Tertiary Structure Recognition by Means of Associative Memory Hamiltonians" *Science,* **1989**, *246,* 371-373.



Aram Davtyan, Nicholas P. Schafer, Weihua Zheng, Cecilia Clementi, Peter G. Wolynes, and Garegin A. Papoian; "AWSEM-MD: Protein Structure Prediction Using Coarse-Grained Physical Potentials and Bioinformatically Based Local Structure Biasing" *J. Phys. Chem. B,* **2012**, 116 (29), 8494-8503.

Structure of CaMKII

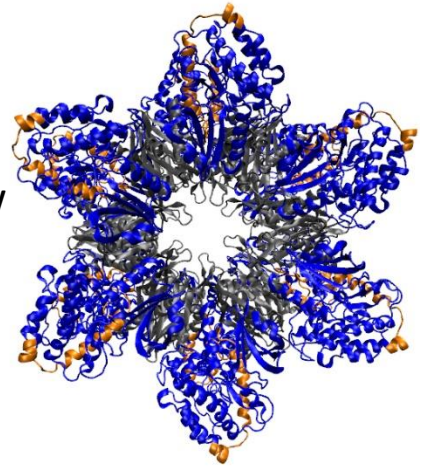




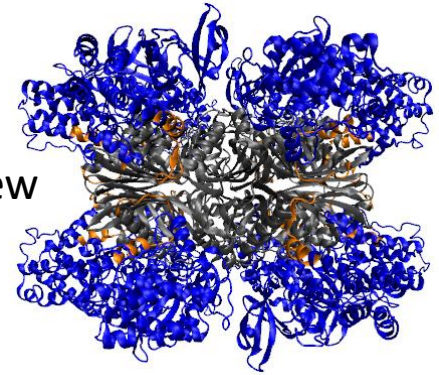
CaMKII monomoer
PDB ID: 3SOA

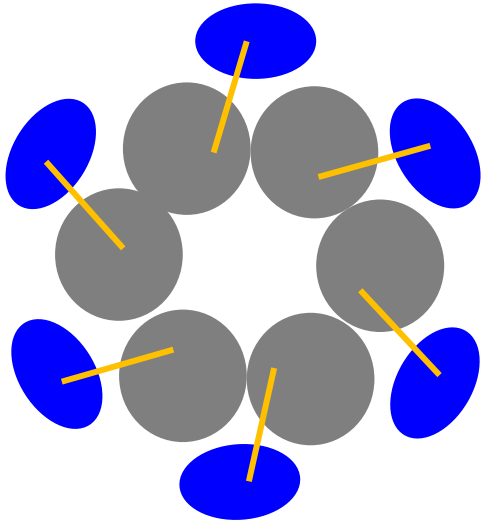
oligomer

Top view



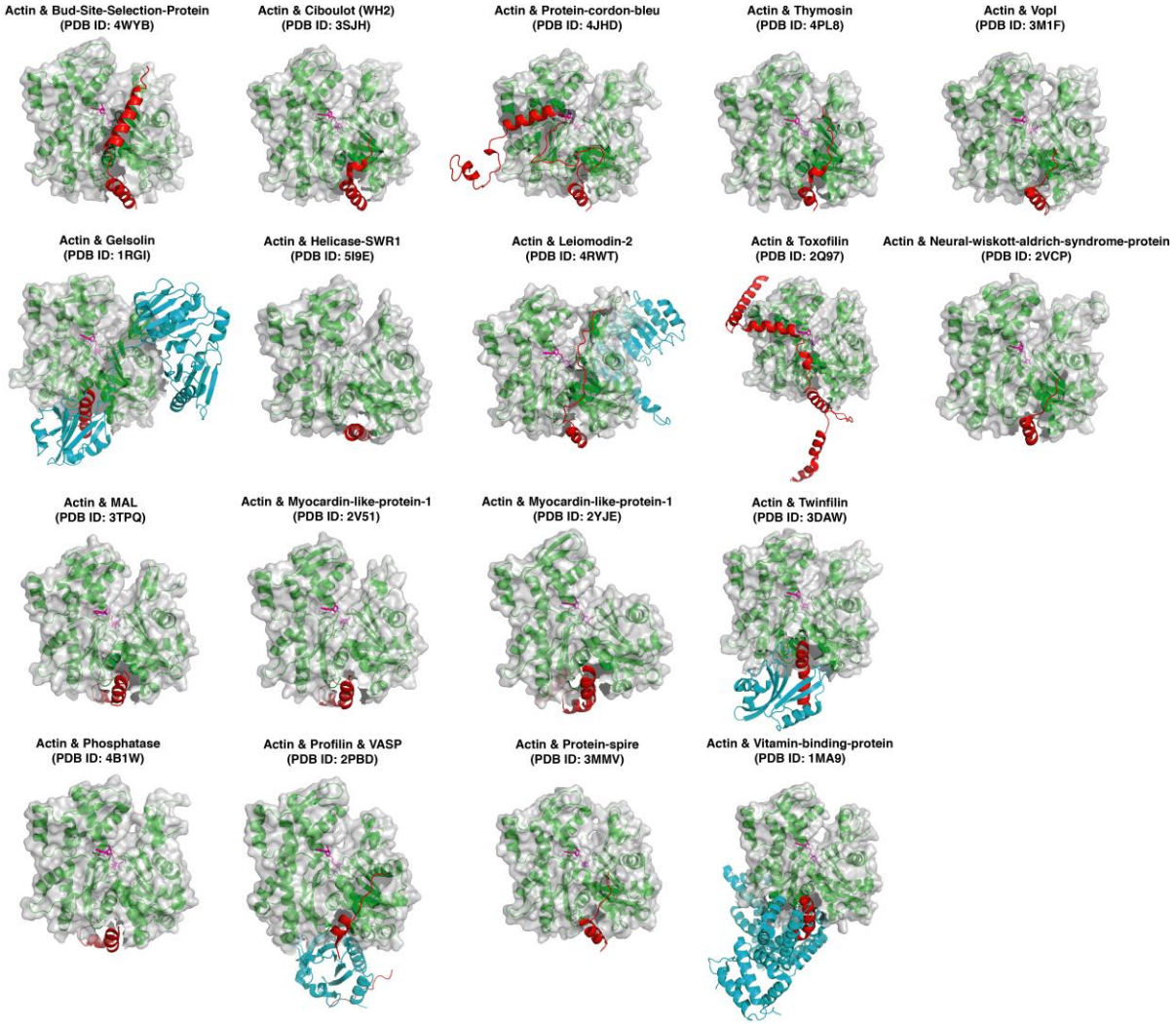
Side view



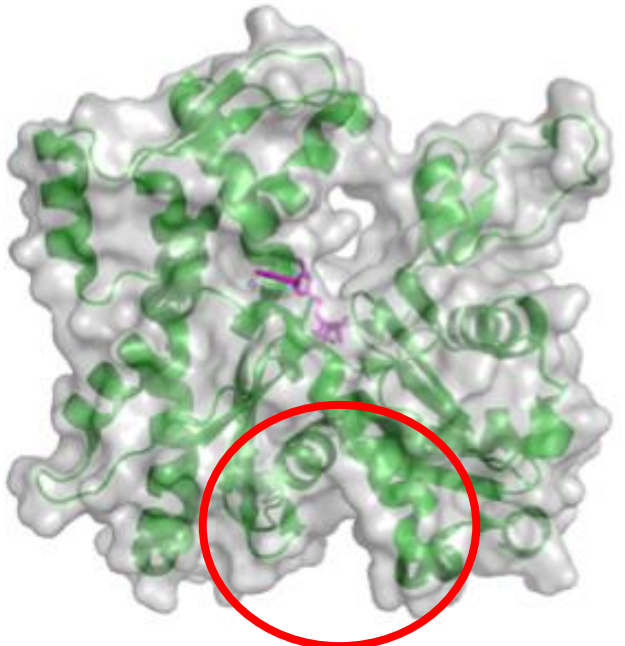


Kuriyan group, Cell, 2011.

Remodeling Proteins for Actin Display a Common Binding Pocket

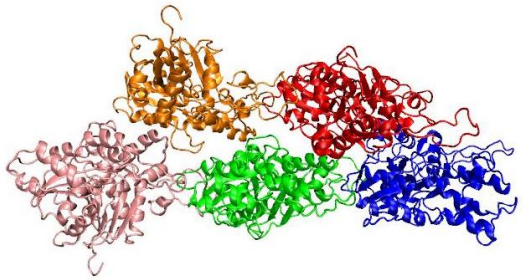


80% of the proteins that remodel Actin bind to a common hydrophobic cleft, but other regions bind too.

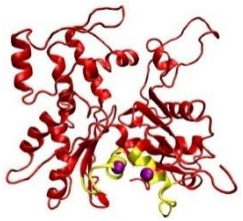


Predicting the CaMKII-Actin Binding Complex Using a Minimal Construct

F-actin



CaMKII monomer

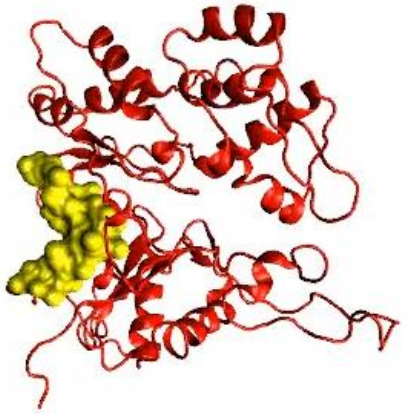


AWSEM-MD simulation

Red: actin

Yellow: conserved binding pocket for many actin-binding proteins

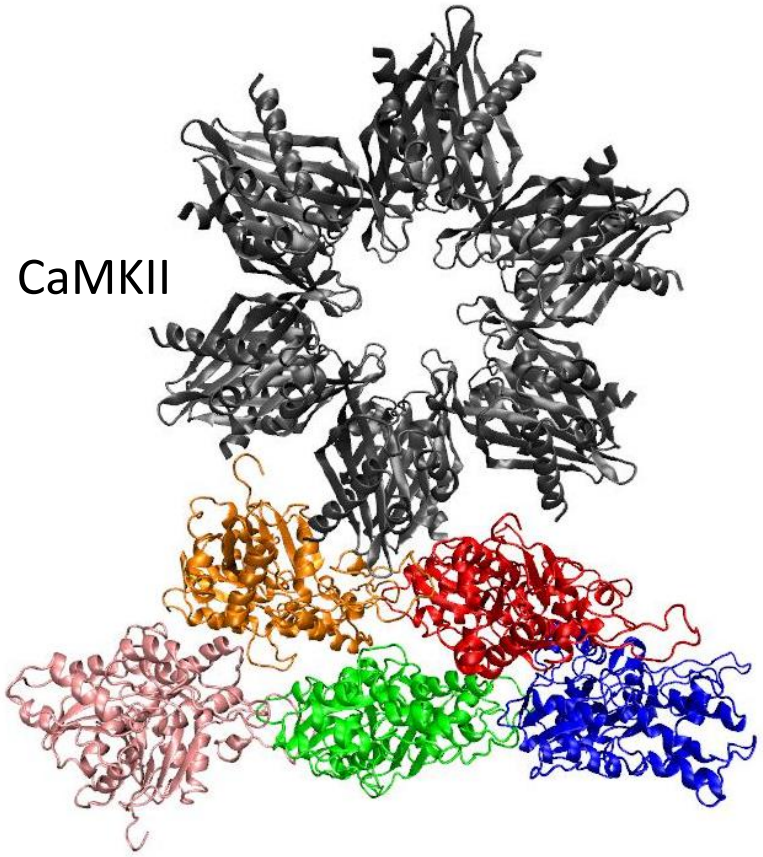
Gray: CaMKII



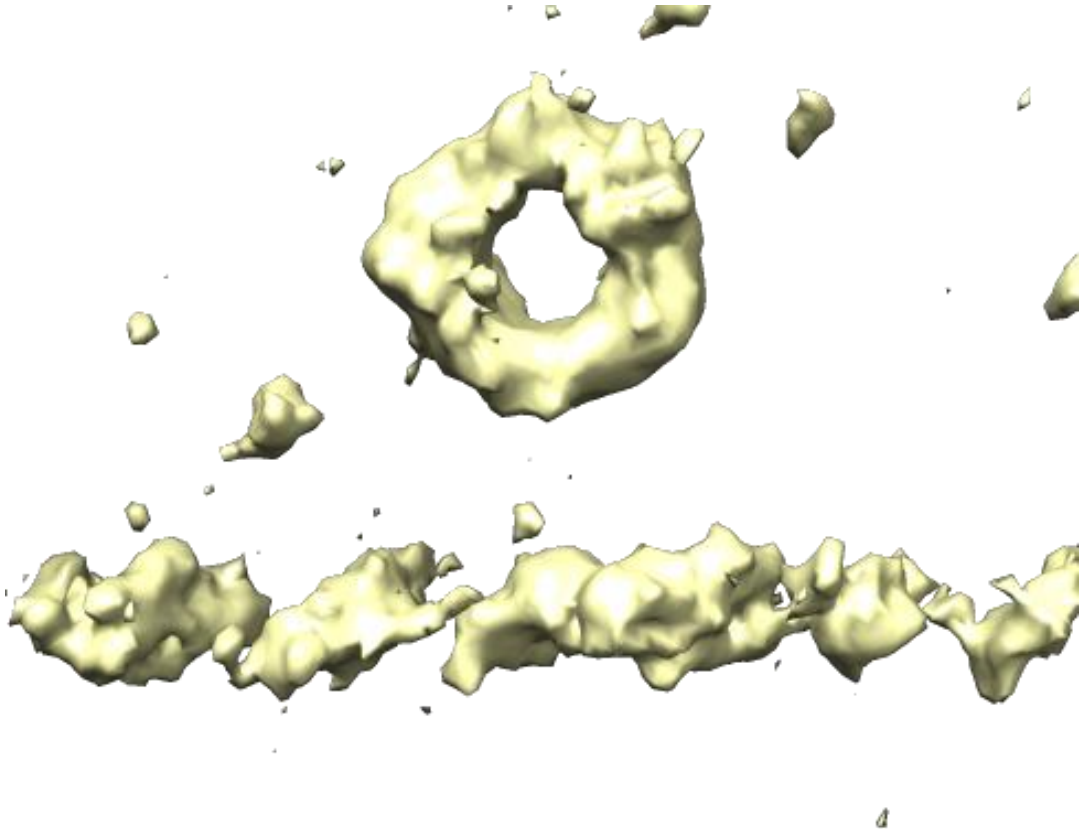
Margaret Cheung, Qian Wang, Mingchen Chen, PGW.

Structure of the CaMKII-Actin Binding Complex Predicted By AWSEM

Computational prediction:



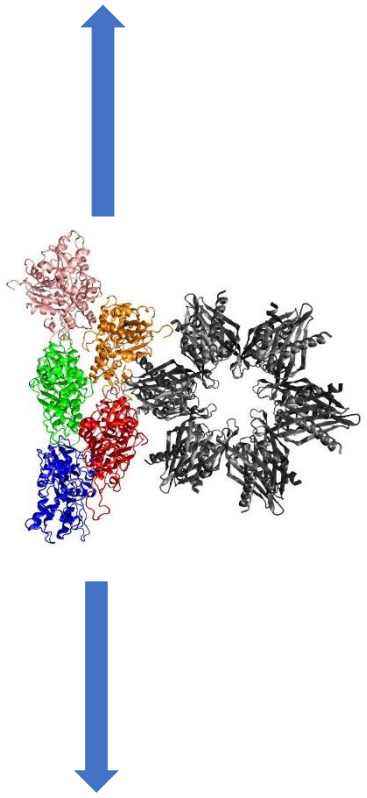
Cryo-EM image from Neal Waxham lab:

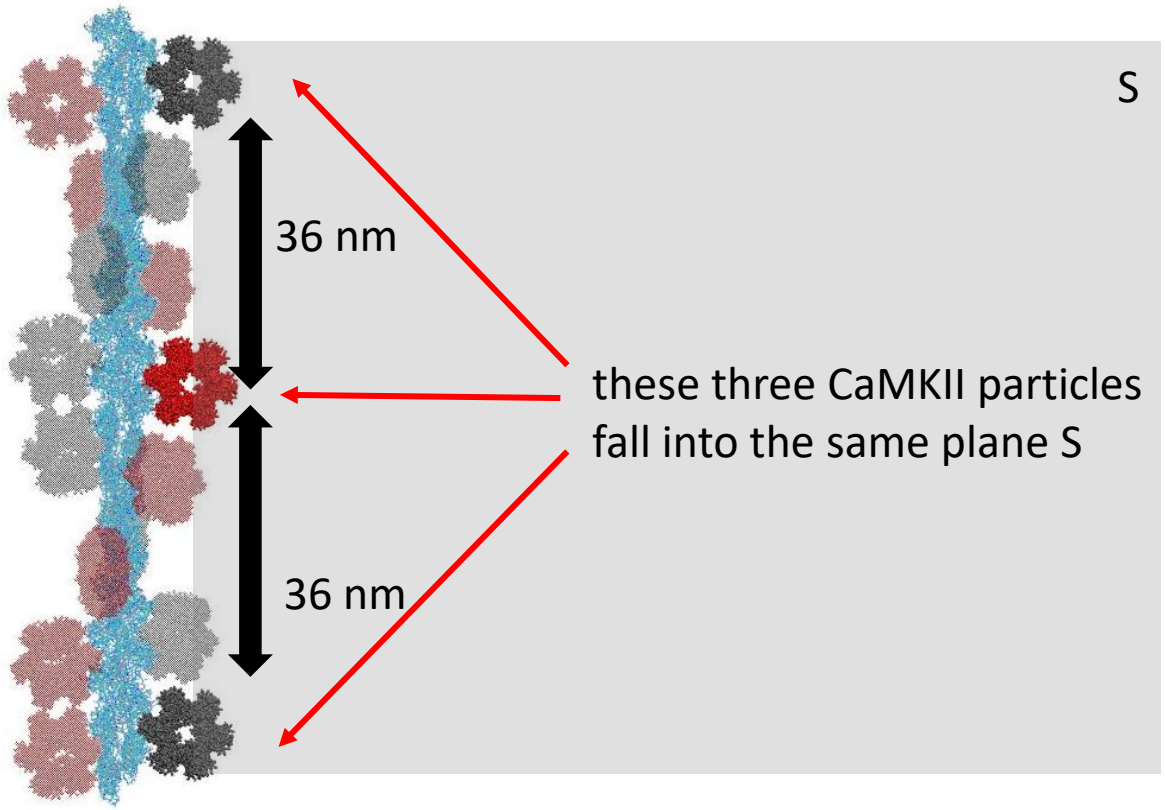


Predicted structure matches the cryo-EM!

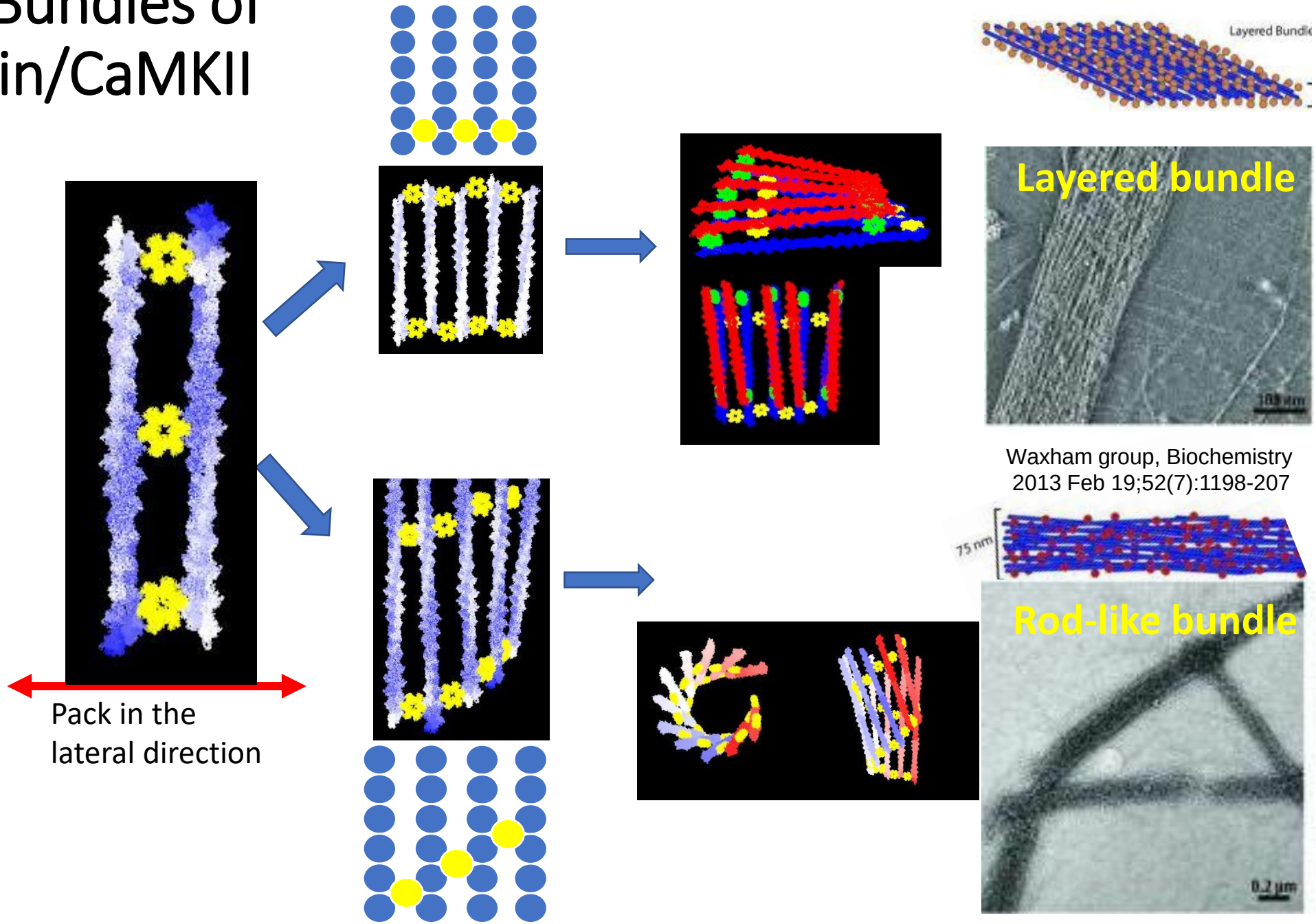
Building a CaMKII-Actin Bundle Using the Predicted CaMKII-Actin Binding Complex Structure

- CaMKII particles form two right-handed helical structures (gray, red)
- The predicted CaMKII spiral has a periodicity of 36 nm – matching the spacing in the Cryo-EM image!

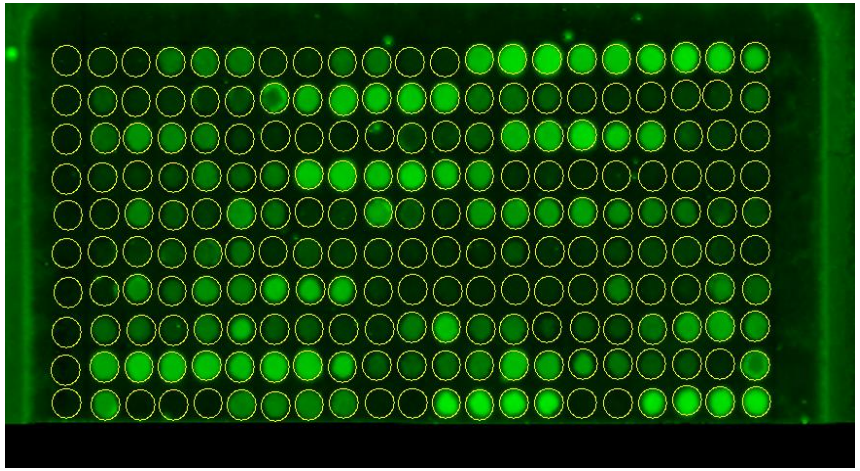




3D Bundles of Actin/CaMKII

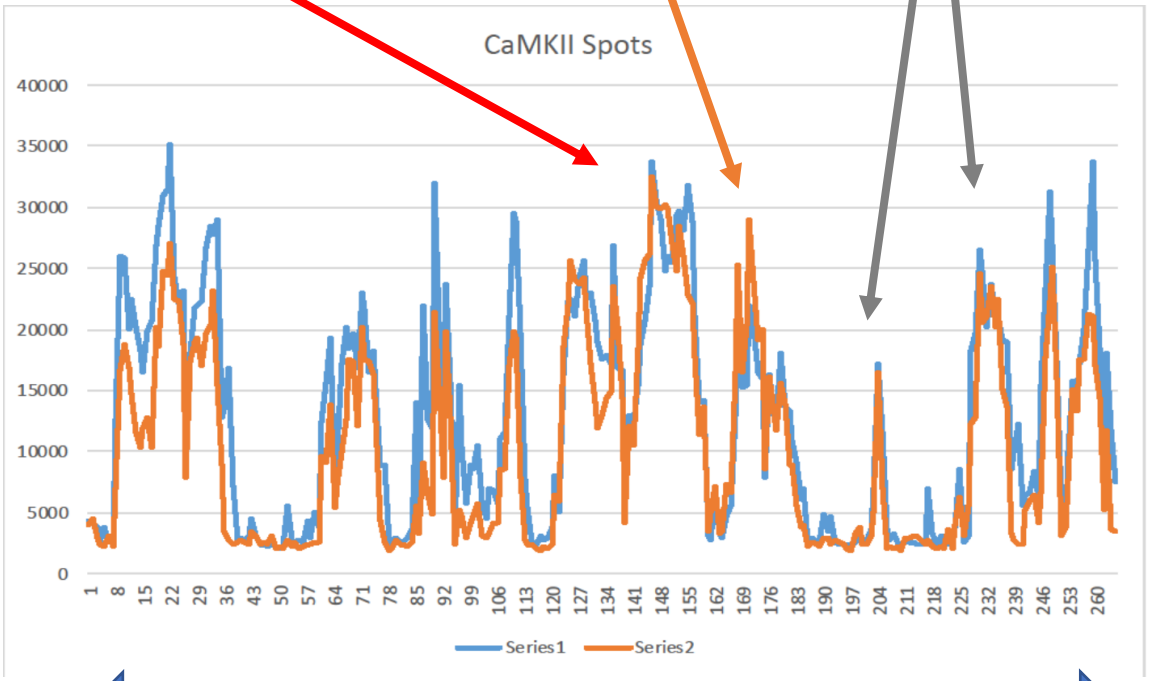


The Actin Binding Interface Involves Multiple Domains of CaMKII



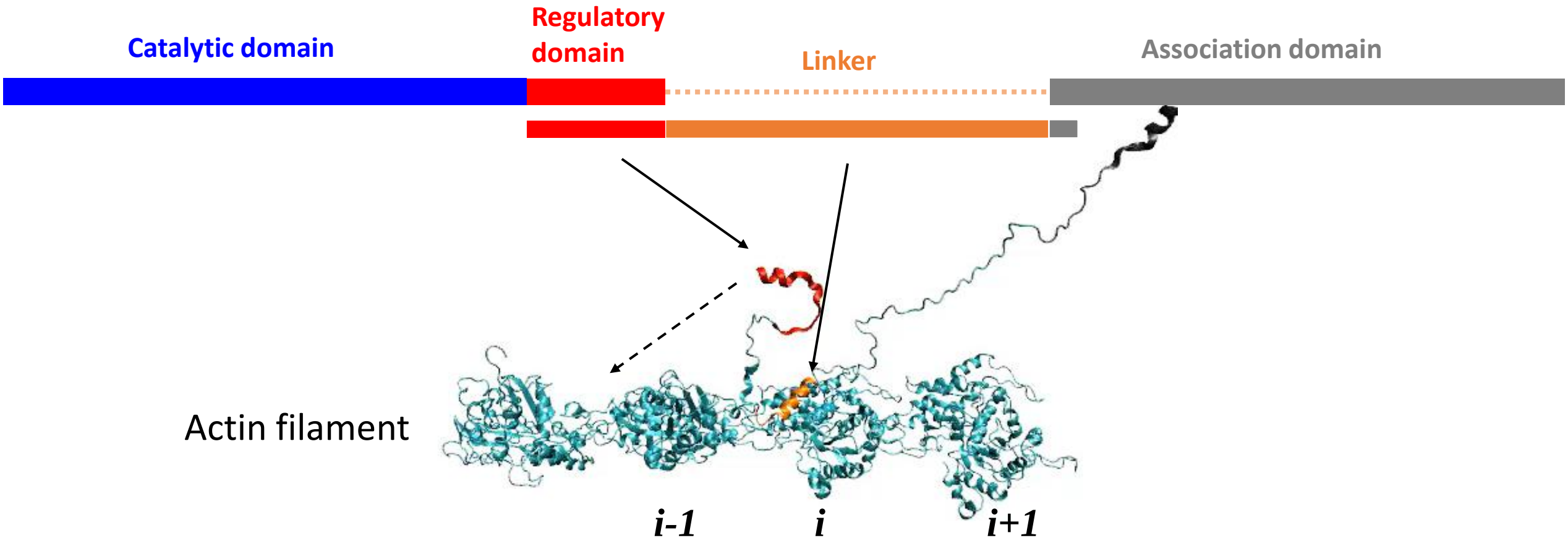
Data from the Waxham Lab

Region I:
Proposed binding domain in current literature



CaMKII full sequence

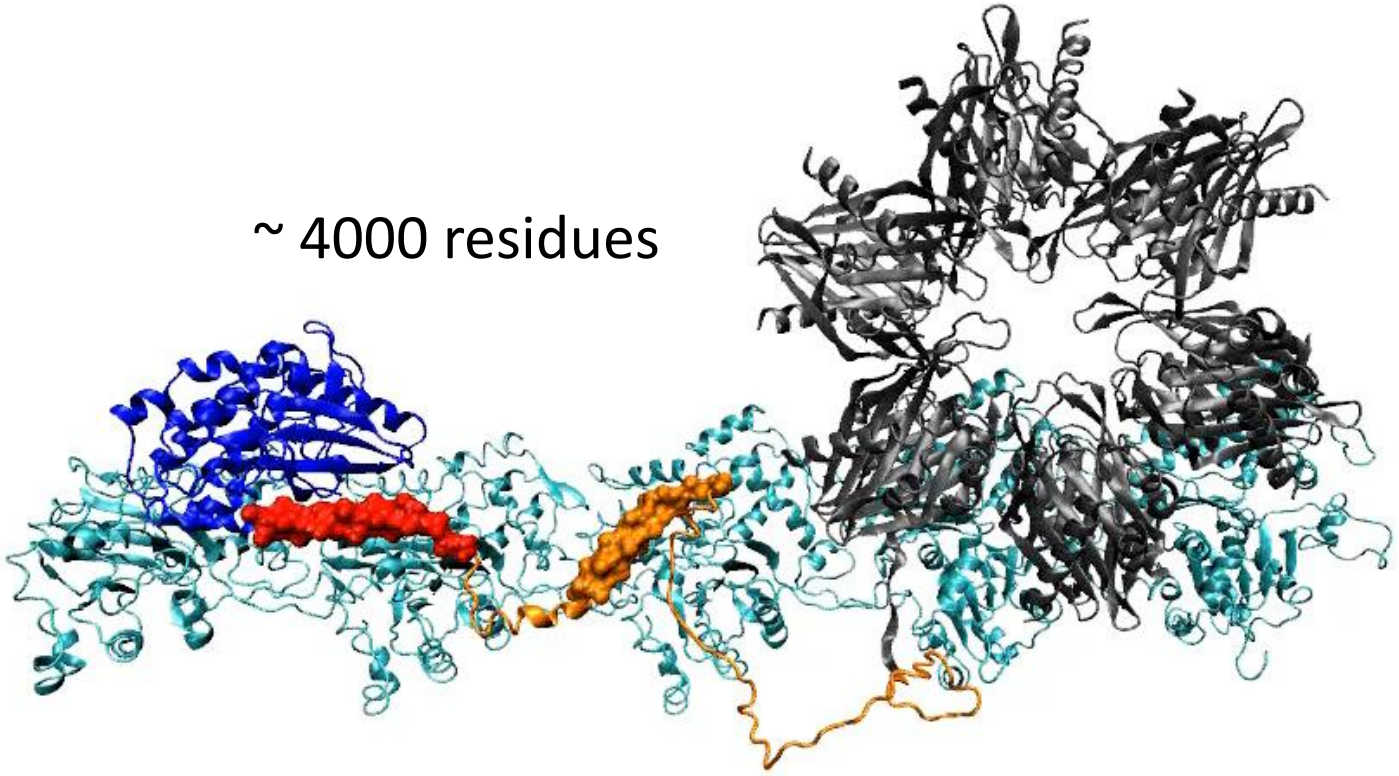
Binding Between the Regulatory Domain of CaMKII and F-Actin



ab initio AWSEM-MD with
no bias to specific region

The First Atomistic Structural Model for CaMKII – Actin Complex

~ 4000 residues



What can we learn from the structure?

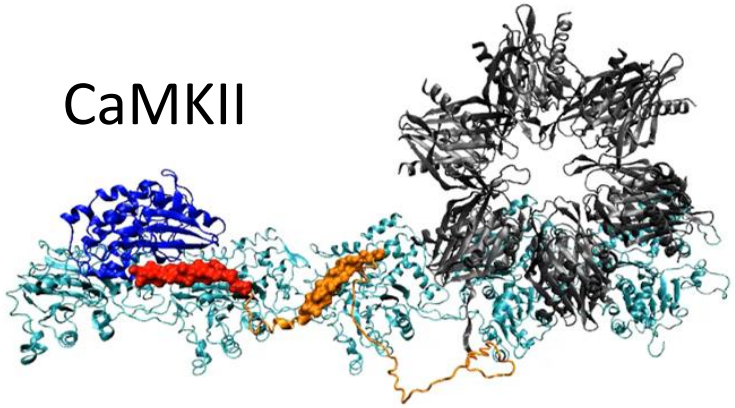
CaMKII utilizes three different domains to bind five adjacent actin subunits

"Stable and regulatable" binding is achieved using distinct domains.

Qian Wang, Mingchen Chen, Nicholas P. Schafer, Carlos Bueno, Sarah S. Song, Andy Hudmon, Peter G. Wolynes*, M. Neal Waxham*, Margaret S. Cheung*, *PNAS*, 2019.

Regulation Mechanism: Low Ca^{2+} Concentration

CaMKII

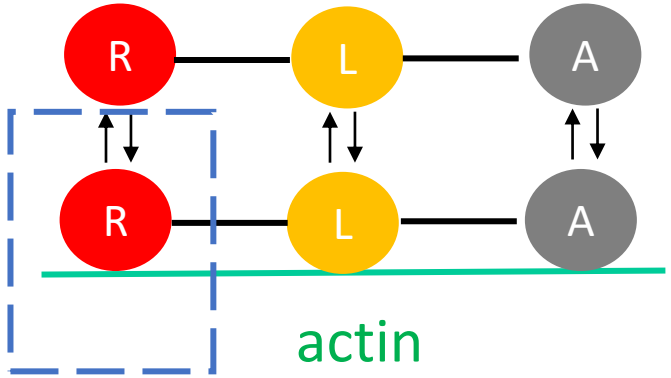


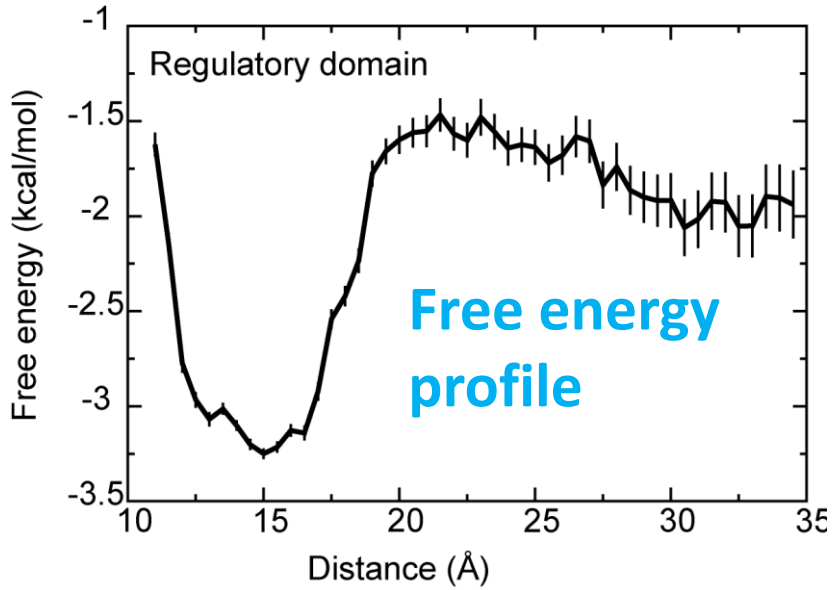
1.8 kcal/mol low

3.8 kcal/mol low

3.7 kcal/mol low

9.3 kcal/mol high

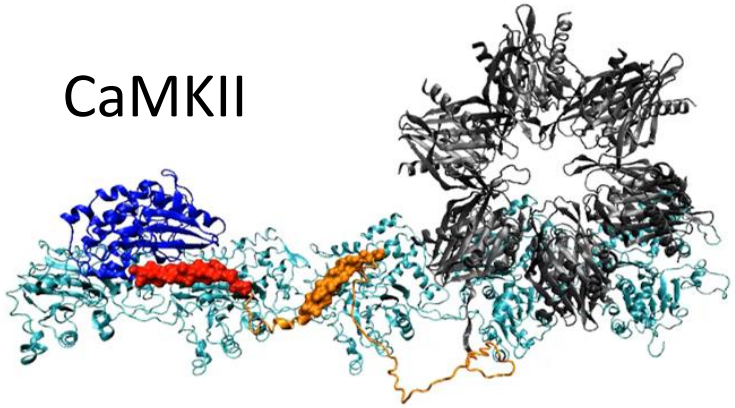




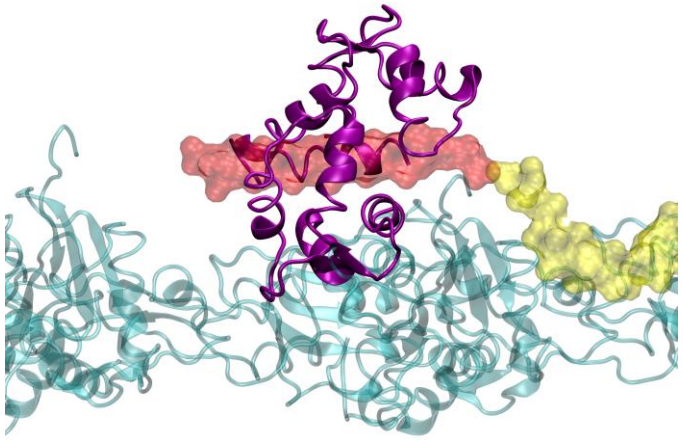
Regulation Mechanism: High Ca^{2+} Concentration

stimulus → Ca^{2+} concentration rises

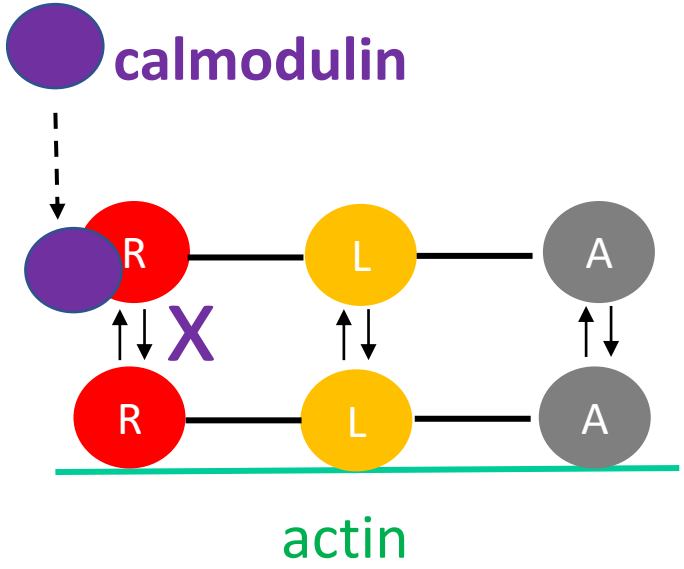
CaMKII



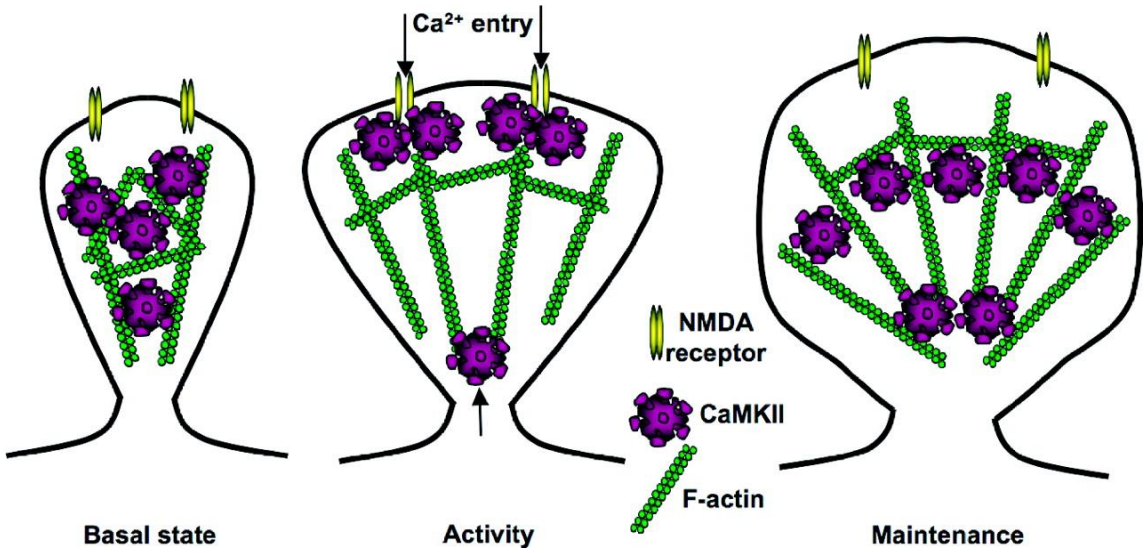
Calmodulin activated



Calmodulin and actin compete for the same binding interface:
Molecular stripping!

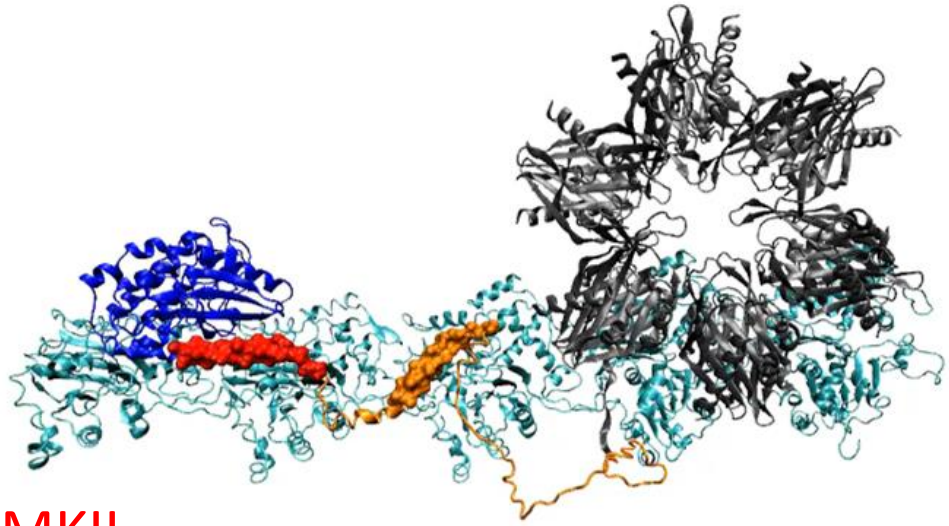


Specificity vs. Adaptability



Yasunori Hayashi group, *PNAS*, 2007.

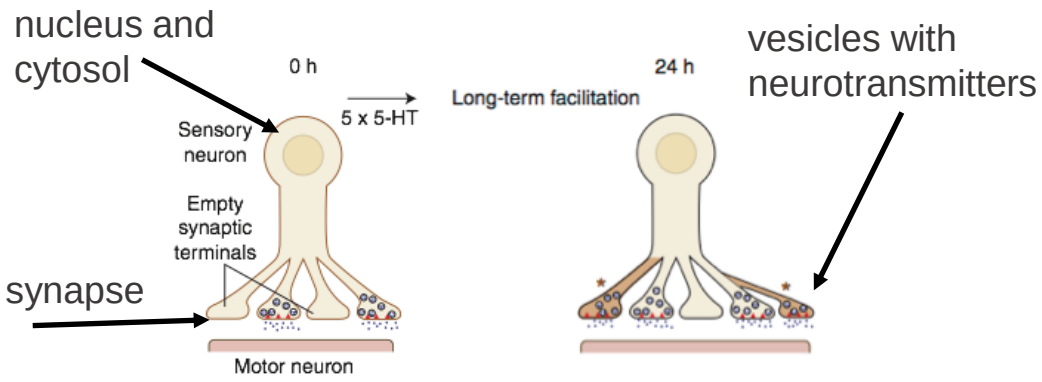
The association domain of CaMKII serves as a specific anchor to bundle actin filaments and stabilize the cytoskeleton.



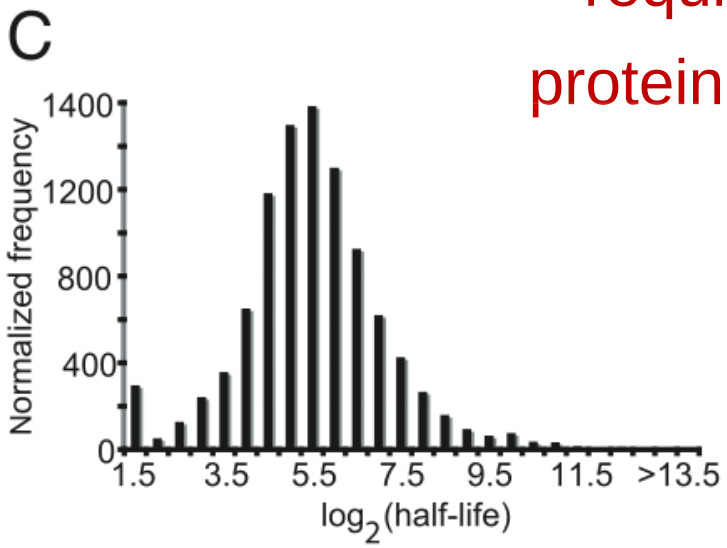
The regulatory domain of CaMKII regulates the binding between CaMKII and actin by responding to the cellular Ca^{2+} level

The Memory Timescale Puzzle

Long-term memory requires stable protein production.



Bailey et al., 2015, CSHL.



Belle et al. 2006, PNAS.

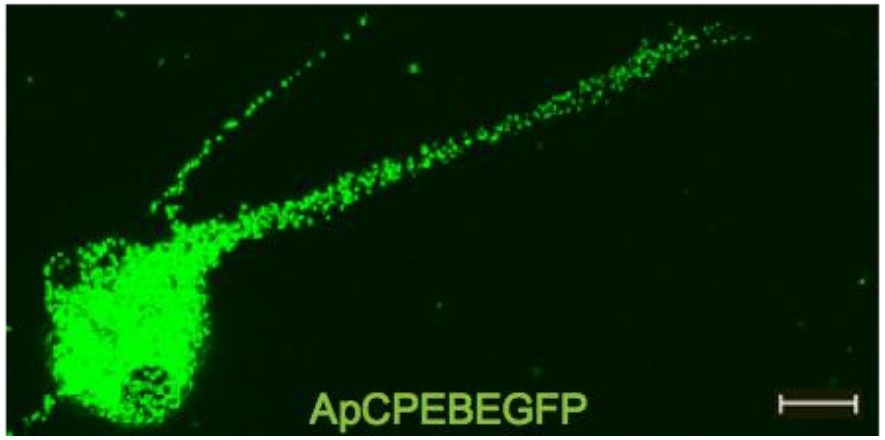
Time scale of >> 24h

Mean and median half-life ~43 min for yeast proteins.

F. Crick, "Memory and Molecular Turnover," *Nature,* 1984.

Functional Prions May Resolve the Timescale Puzzle

Crick, Tompas, Lindquist, Kandel and their collaborators.

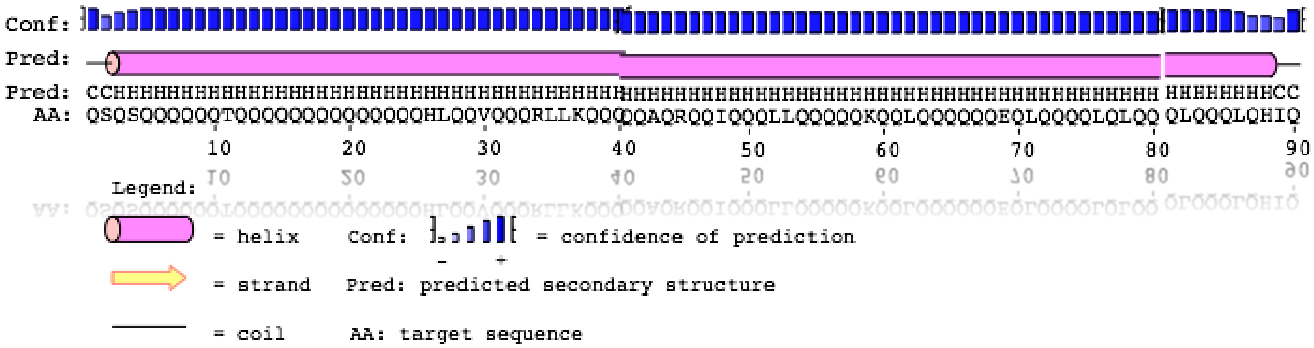


Aplysia CPEB (translational regulator) forms aggregates through its Q-rich domain in vivo in sensory neurons.

Protein-only mechanism induces self-perpetuating changes in the activity of neuronal *Aplysia* cytoplasmic polyadenylation element binding protein (CPEB)

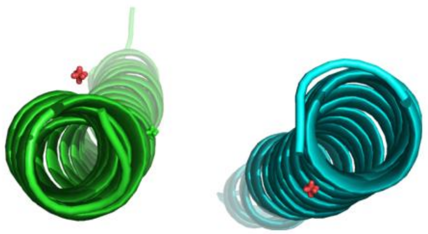
Sven U. Heinrich[a] and Susan Lindquist[a,b,1]

Predicted Structures of the Q-Rich Region of CPEB Using AWSEM

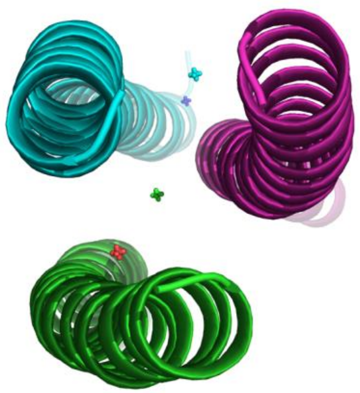


Secondary structure prediction using PsiPred.

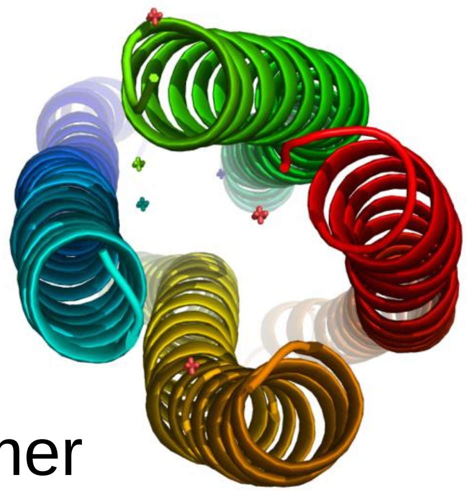
AWSEM prediction mode (fragment memory).



Dimer



Trimer

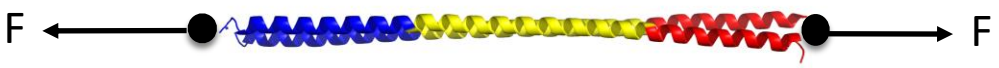


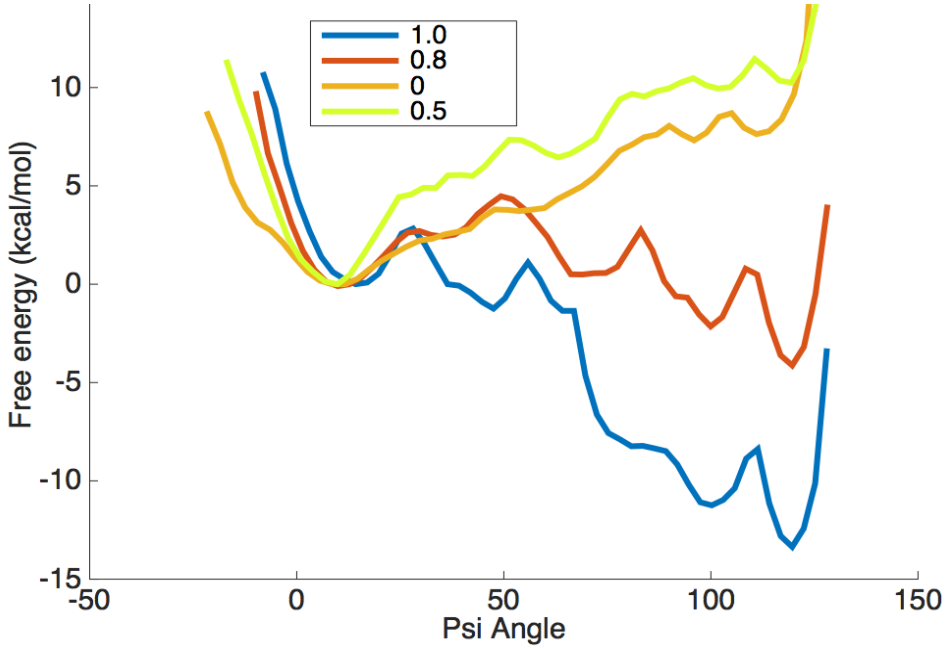
Tetramer

Mingchen Chen, Weihua Zheng and PGW, *PNAS,* 2016.

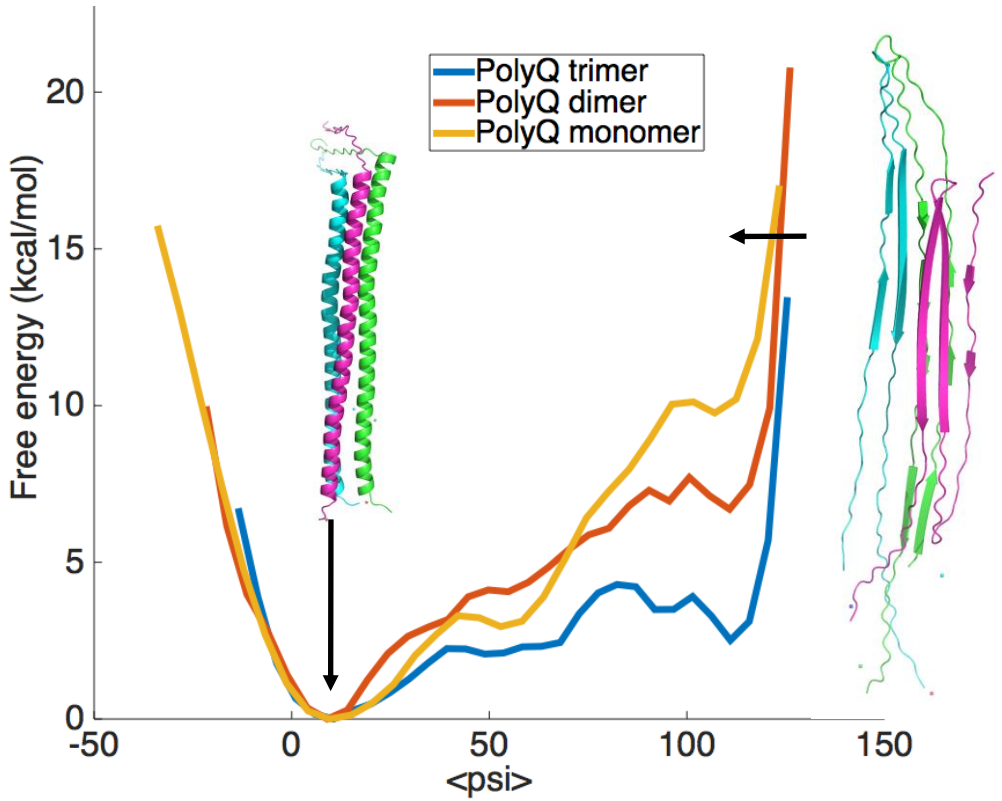
Mechanical Pulling Facilitates the Coiled-Coil to β-Strand Transition

CPEB is hard to aggregate on its own (as a β sheet)!





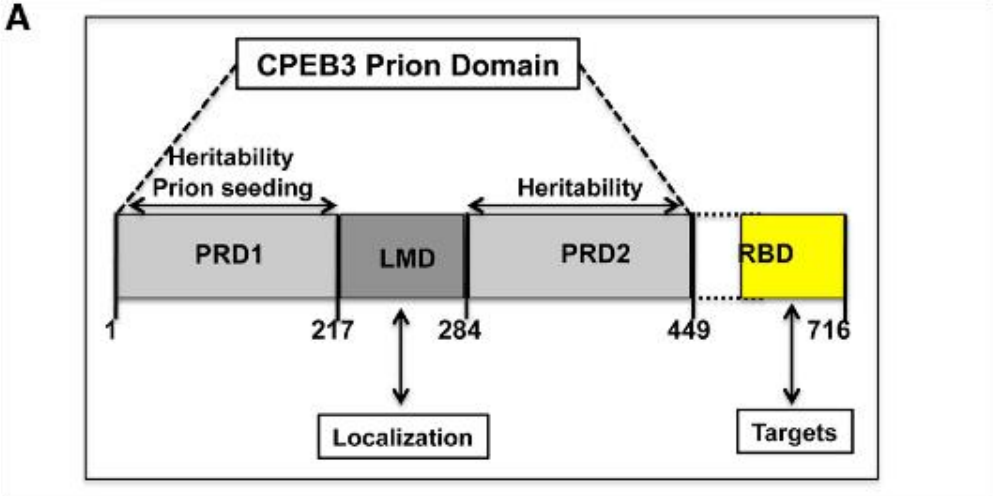
<psi>: average psi angle



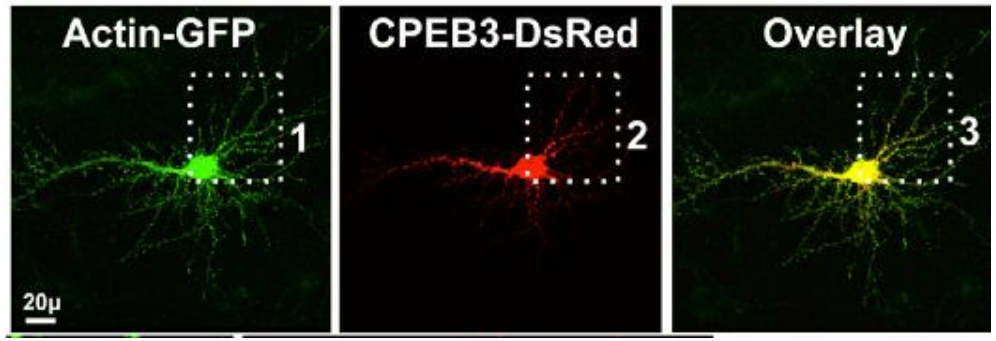
<psi>: average psi angle

The Cytoskeleton Can Provide the Needed Force!

CPEB3 Interacts with Actin (fluorescence and co-IP); and CPEB3 Aggregates Colocalize with the Actin Cytoskeleton.

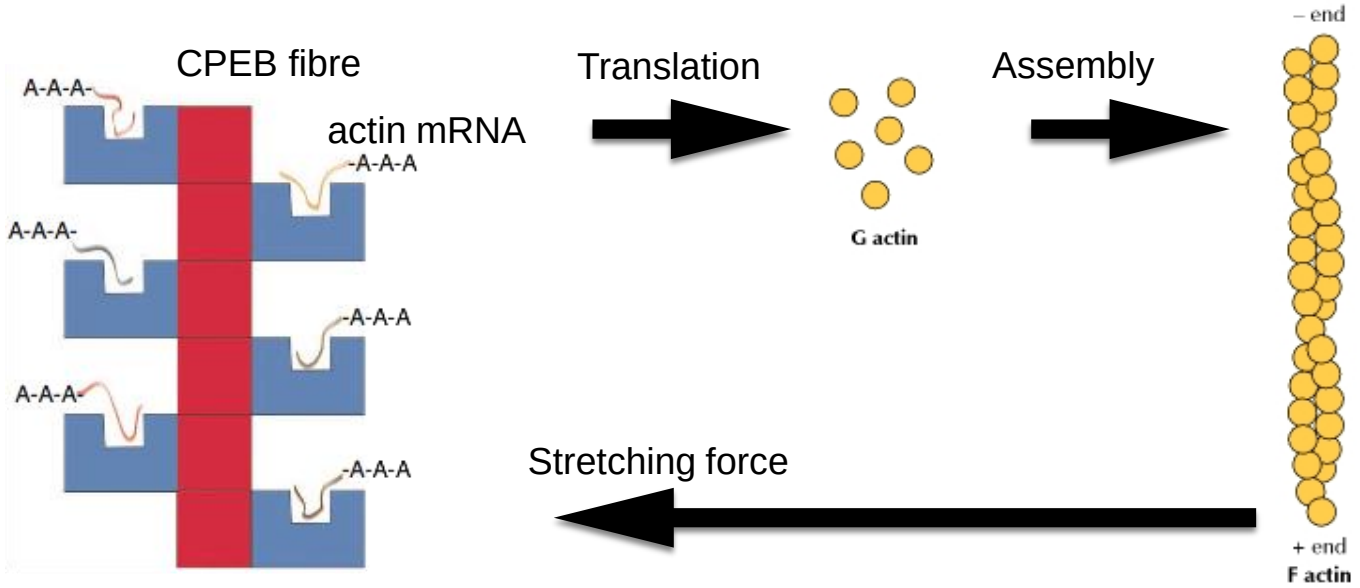


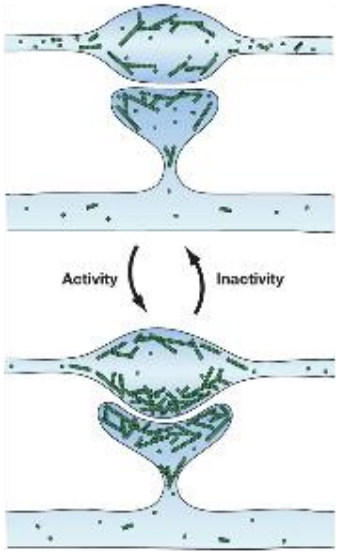
CPEB3 Aggregation Requires an Intact Actin Cytoskeleton



Stephan et al., 2015, Cell Reports.

Cytoskeletal Growth and CPEB Prion Formation Provide a Stable Positive Feedback Loop for Preserving Long-lived Local Structures





Positive feedback results in continuous formation of actin filaments and CPEB fibres localized in the synapse area thus marking the synapse.

Crick, F. (1984). Neurobiology: Memory and Molecular Turnover, Nature.

Aggregation Landscapes

PNAS

Exploring the aggregation free energy landscape of the amyloid-β protein (1–40)

Weihua Zheng[a,b], Min-Yeh Tsai[a,b], Mingchen Chen[a,c], and Peter G. Wolynes[a,b,1]

[a]Center for Theoretical Biological Physics, Rice University, Houston, TX 77005; [b]Department of Chemistry, Rice University, Houston, TX 77005; and [c]Department of Bioengineering, Rice University, Houston, TX 77005

Contributed by Peter G. Wolynes, August 17, 2016 (sent for review July 28, 2016; reviewed by William A. Eaton and Angel E. Garcia)

A predictive coarse-grained protein force field [associative memory, water-mediated, structure, and energy model for molecular structurally characterizing monomeric protein folding, dimer binding, and misfolding of multidomain proteins (9–12). The

THE JOURNAL OF PHYSICAL CHEMISTRY B

Article

Cite This: *J. Phys. Chem. B* 2018, 122, 11414–11430

pubs.acs.org/JPCB

Surveying the Energy Landscapes of Aβ Fibril Polymorphism

Mingchen Chen,[†,‡,¶] Nicholas P. Schafer,[†,§,¶] and Peter G. Wolynes*[,†,§]

[†]Center for Theoretical Biological Physics, Rice University, Houston, Texas 77005, United States

[‡]Department of Bioengineering, Rice University, Houston, Texas 77005, United States

[§]Department of Chemistry, Rice University, Houston, Texas 77005, United States

PNAS

Exploring the Interplay Between Fibrillization and Amorphous Aggregation Channels on the Energy Landscapes of Tau Repeat Isoforms

Xun Chen[a,b,1], Mingchen Chen[a,c,1], Nicholas P. Schafer[a], and Peter G. Wolynes[a,b,d]

[a]Center for Theoretical Biological Physics; [b]Department of Cahemistry, Rice University; [c]Department of Bioengineering, Rice University; [d]Department of Biosciences, Rice University

J|A|C|S JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Article

Cite This: *J. Am. Chem. Soc.* 2017, 139, 16666-16676

pubs.acs.org/JACS

Comparing the Aggregation Free Energy Landscapes of Amyloid Beta(1–42) and Amyloid Beta(1–40)

Weihua Zheng, Min-Yeh Tsai, and Peter G. Wolynes*

Department of Chemistry, and Center for Theoretical Biological Physics, Rice University, Houston, Texas 77005, United States

Cell, Vol. 72, 971–983, March 26, 1993, Copyright © 1993 by Cell Press

A Novel Gene Containing a Trinucleotide Repeat That Is Expanded and Unstable on Huntington's Disease Chromosomes

The Huntington's Disease Collaborative Research Group*

Nancy Wexler

Summary

The Huntington's disease (HD) gene has been mapped in 4p16.3 but has eluded identification. We have used haplotype analysis of linkage disequilibrium to spotlight a small segment of 4p16.3 as the likely location of the defect. A new gene, IT15, isolated using cloned trapped exons from the target area contains a polymorphic trinucleotide repeat that is expanded and unstable on *HD* chromosomes. A $(CAG)_n$ repeat longer than the normal range was observed on *HD* chromosomes from all 75 disease families examined, comprising a variety of ethnic backgrounds and 4p16.3 haplotypes. The $(CAG)_n$ repeat appears to be located within the coding sequence of a predicted ~348 kd protein that is widely expressed but unrelated to any known gene. Thus, the *HD* mutation involves an unstable DNA segment, similar to those described in fragile X syndrome, spino-bulbar muscular atrophy, and myotonic dystrophy, acting in the context of a novel 4p16.3 gene to produce a dominant phenotype.

*The Huntington's Disease Collaborative Research Group comprises:

Group 1:

Marcy E. MacDonald,[1] Christine M. Ambrose,[1] Mabel P. Duyao,[1] Richard H. Myers,[2] Carol Lin,[1] Lakshmi Srinidhi,[1] Glenn Barnes,[1] Sherryl A. Taylor,[1] Marianne James,[1] Nicolet Groot,[1] Heather MacFarlane, Barbara Jenkins,[1] Mary Anne Anderson,[1] Nancy S. Wexler,[3] and James F. Gusella[1†]

[1]Molecular Neurogenetics Unit
Massachusetts General Hospital
and Department of Genetics
Harvard Medical School
Boston, Massachusetts 02114
[2]Department of Neurology
Boston University Medical School
Boston, Massachusetts 02118
[3]Hereditary Disease Foundation
1427 7th Street, Suite 2
Santa Monica, California 90401

Group 2:

Gillian P. Bates, Sarah Baxendale, Holger Hummerich, Susan Kirby, Mike North, Sandra Youngman, Richard Mott, Gunther Zehetner, Zdenek Sedlacek, Annemarie Poustka, Anna-Maria Frischauf, and Hans Lehrach

Genome Analysis Laboratory
Imperial Cancer Research Fund
Lincoln's Inn Fields
London, WC2A 3PX, England

Group 3:

Alan J. Buckler,[1] Deanna Church,[1] Lynn Doucette-Stamm,[1] Michael C. O'Donovan,[1]

*The Huntington's Disease Collaborative Research Group comprises:

Group 1:

Marcy E. MacDonald,[1] Christine M. Ambrose,[1] Mabel P. Duyao,[1] Richard H. Myers,[2] Carol Lin,[1] Lakshmi Srinidhi,[1] Glenn Barnes,[1] Sherryl A. Taylor,[1] Marianne James,[1] Nicolet Groot,[1] Heather MacFarlane, Barbara Jenkins,[1] Mary Anne Anderson,[1] Nancy S. Wexler,[3] and James F. Gusella[1†]

[1]Molecular Neurogenetics Unit
Massachusetts General Hospital
and Department of Genetics
Harvard Medical School
Boston, Massachusetts 02114
[2]Department of Neurology
Boston University Medical School
Boston, Massachusetts 02118
[3]Hereditary Disease Foundation
1427 7th Street, Suite 2
Santa Monica, California 90401

Group 2:

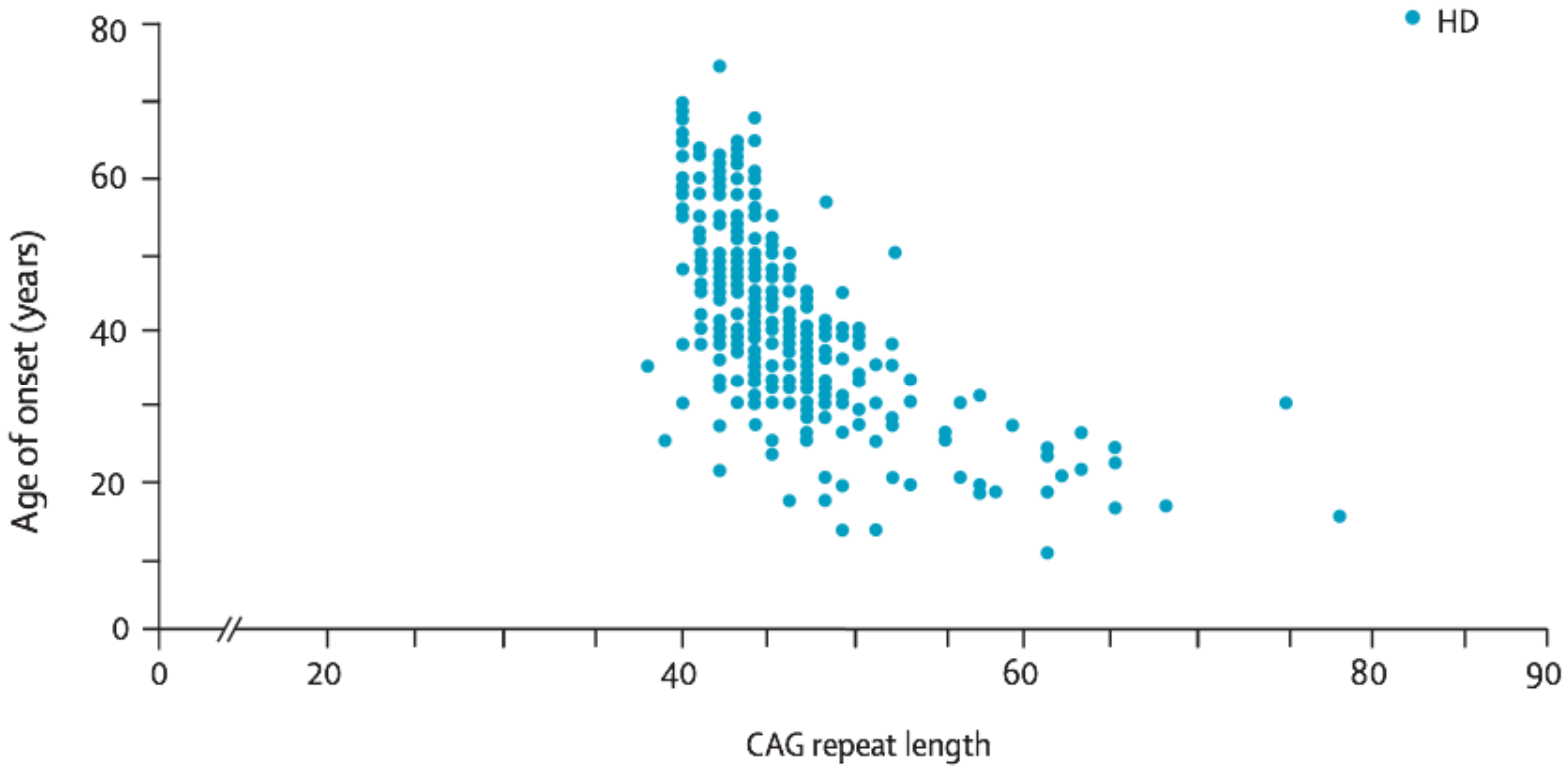
Gillian P. Bates, Sarah Baxendale, Holger Hummerich, Susan Kirby, Mike North, Sandra Youngman, Richard Mott, Gunther Zehetner, Zdenek Sedlacek, Annemarie Poustka, Anna-Maria Frischauf, and Hans Lehrach

Genome Analysis Laboratory
Imperial Cancer Research Fund
Lincoln's Inn Fields
London, WC2A 3PX, England

Group 3:

Alan J. Buckler,[1] Deanna Church,[1] Lynn Doucette-Stamm,[1] Michael C. O'Donovan,[1]

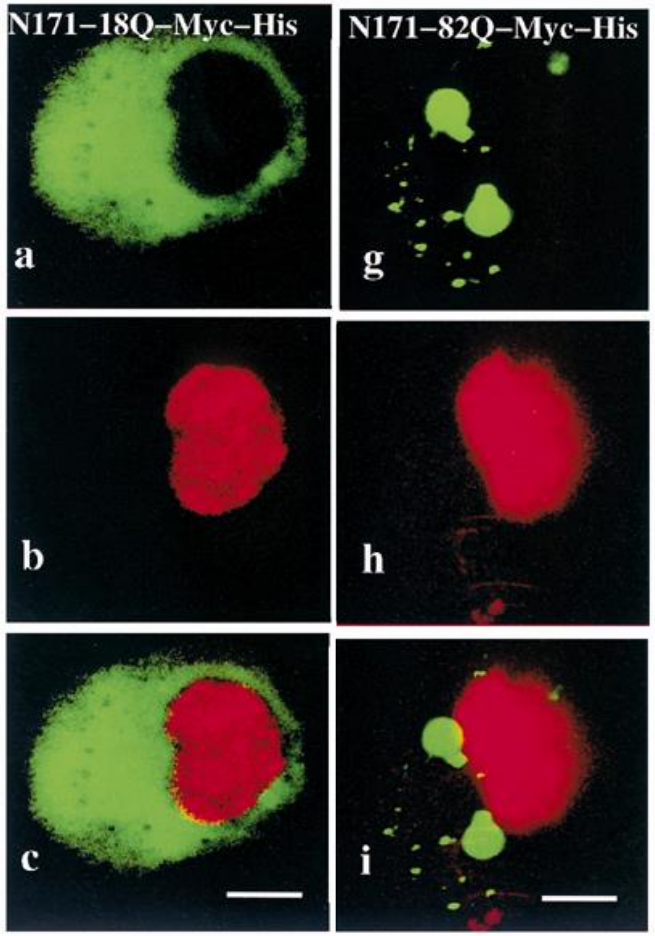
Age of Onset for Huntington's Disease



Repeat count	Classification	Disease status
<28	Normal	Unaffected
28–35	Intermediate	Unaffected
36–40	Reduced penetrance	+/- Affected
>40	Full penetrance	Affected

Walker FO (2007). "Huntington's Disease", *Lancet.*

Formation of Inclusion Bodies as Signature of the Disease



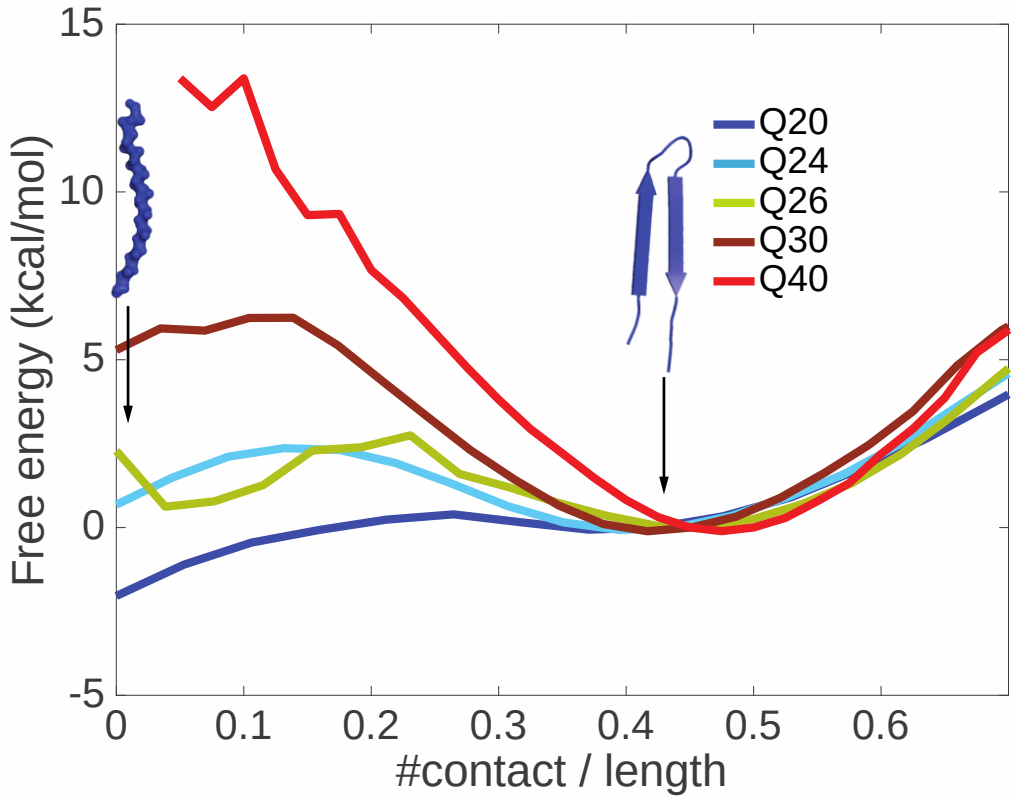
Green: HTT exon1 encoded protein fragments

Red: Nucleus

Inclusion Bodies Enriched with HTT appear in the Cytosol!

Cooper .. Christopher Ross,. 1998 Human Mol Genetics.

The Monomer Structure of PolyQ Depends on Repeat Length



- 1: A **extended form** is favored in shorter repeats (20); while a **beta-hairpin** in longer repeats (30,40)
- 2: For **Q24 and Q26**, both states are equally likely!!!

Length Dependence of the Critical Nucleus Size

Nucleation kinetics analysis on various polyQ peptides[a]

Peptide	Concentrations examined	Slope	R^2	Slope variation[b]	n*
$AT7^{NT}Q_{30}K_2$	6	2.7	0.9806	2.61 – 3.23	0.7
$SFQ_{37}P_{10}K_2$	10	3.0	0.9852	2.92 – 3.08	1.0
$K_2Q_{37}K_2$	9	2.7	0.9632	2.52 – 2.85	0.7
$K_2Q_{27}K_2$	5	2.9	0.9264	2.88 – 3.11	0.9
$K_2Q_{26}K_2$	6	2.9	0.9850	2.77 – 3.17	0.9
$K_2Q_{25}K_2$	7	4.0	0.9571	3.86 – 4.31	2.0
$K_2Q_{24}K_2$	6	5.3	0.968	5.03 – 5.54	3.3
$K_2Q_{23}K_2$	8	5.9	0.9760	5.73 – 6.31	3.9
$K_2Q_{18}K_2$	7	5.7	0.9793	5.44 – 6.04	3.7

Q26

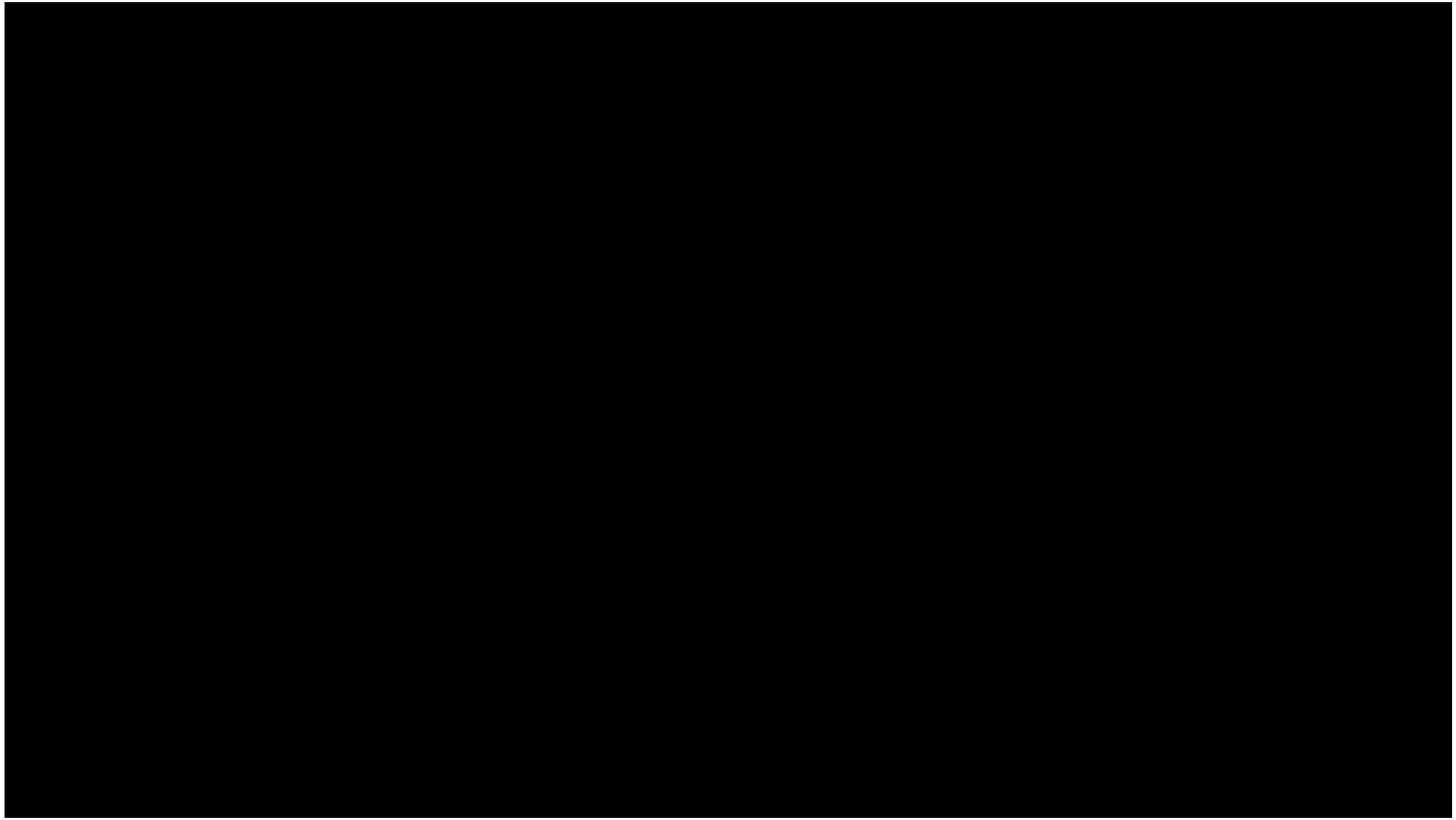
Q23

1

4

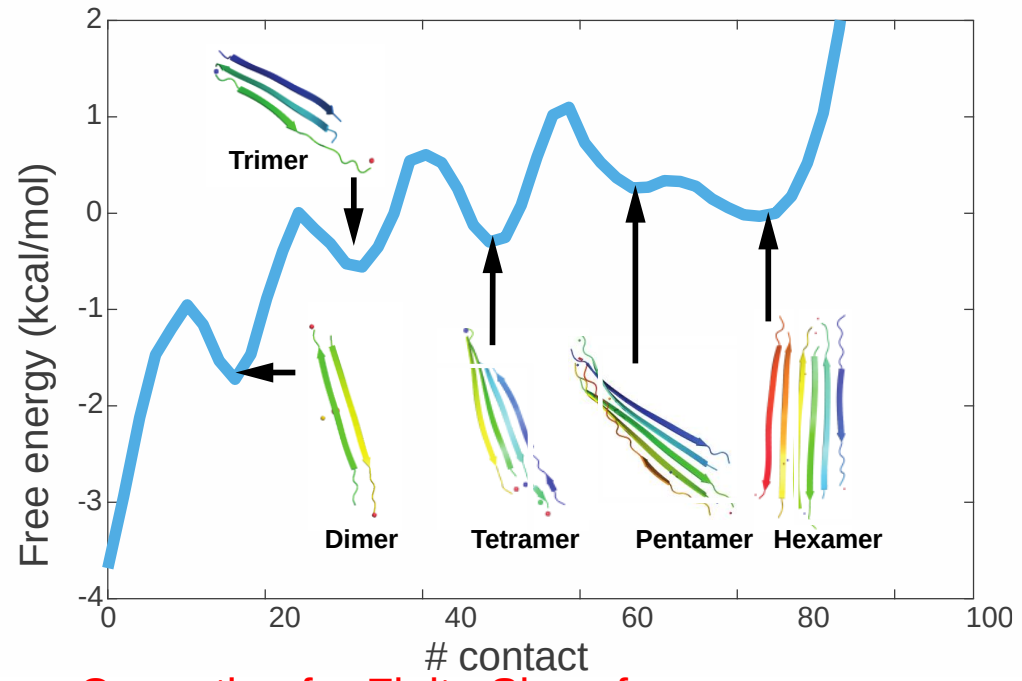
Kar .. Ronald Wetzel et al, (2011) NSMB.

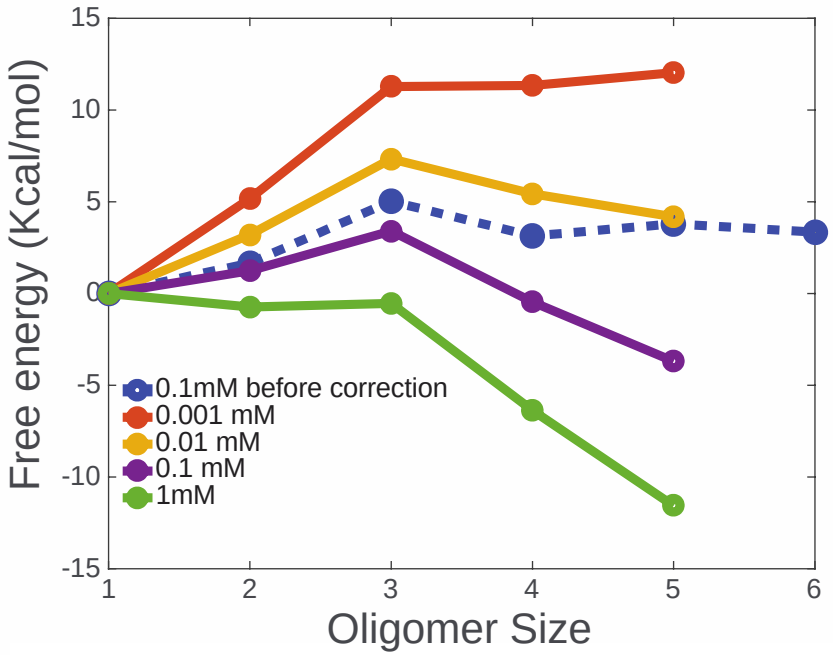
AWSEM Simulation of Q20 Aggregation



Aggregation Free Energy Profile for Q20

Nucleus Size: n*=3





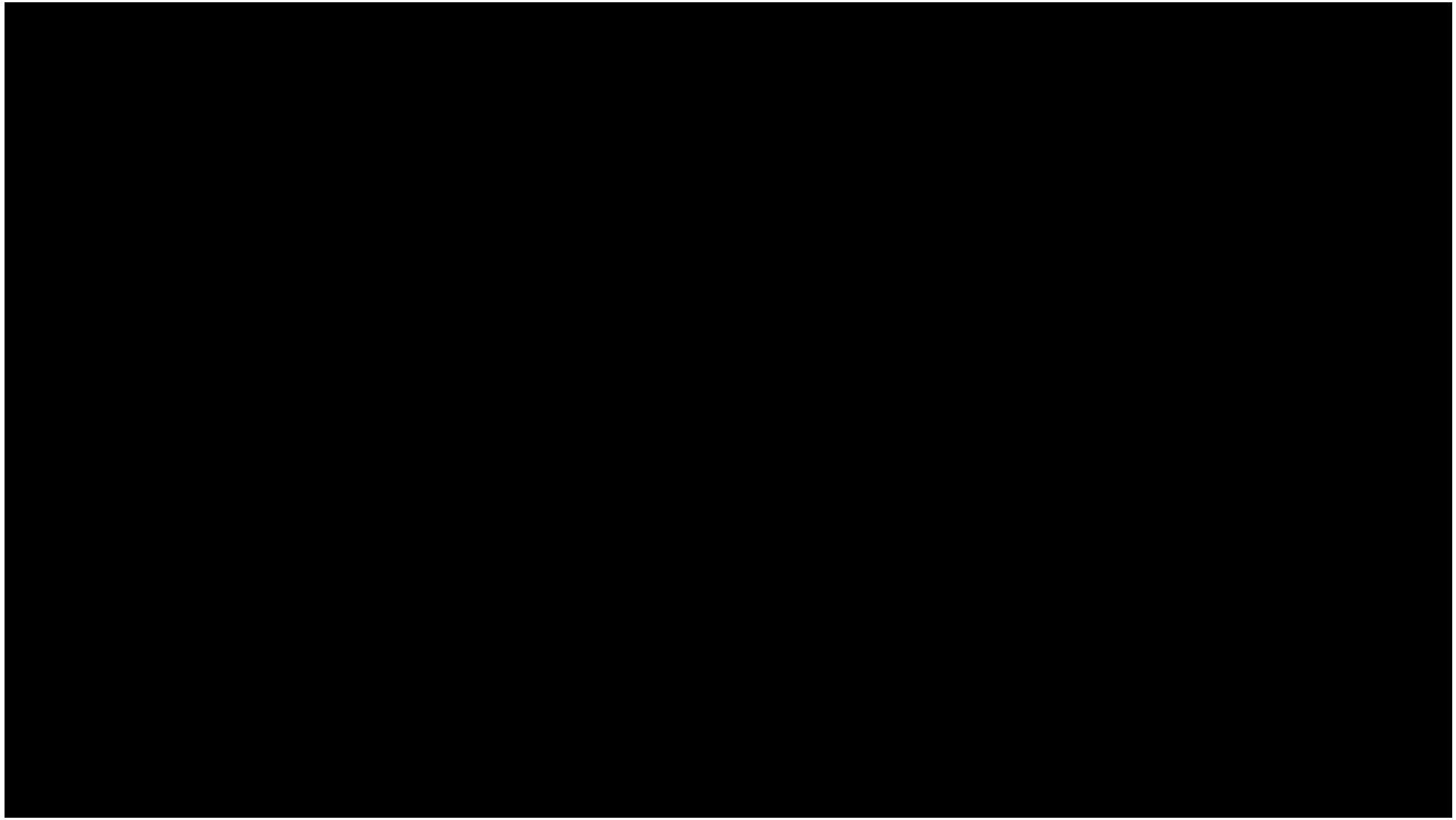
Correction for Finite Size of Simulation Box:

$$F_n = -k_B T \ln P_n = -k_B T \ln q_n - n\mu.$$

Extrapolated Profiles at Other Concentrations:

$$F_n(C_1) = F_n - n\mu_1 = F_n(C_0) - n(\mu_1 - \mu_0) = F_n(C_0) - nkTln\frac{C_1}{C_0}$$

AWSEM Simulation of Q30 Aggregation



The Sequence Structure of Huntingtin (htt)

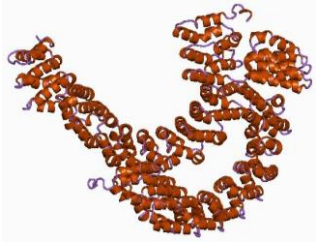
3144 Residues in total!

NT17: structure has been solved with alpha-helix secondary structure.

PolyQ: secondary structure not certain. Fibre form has been validated to be beta (FTIR, ssNMR, TF-dye, x-ray diffraction).

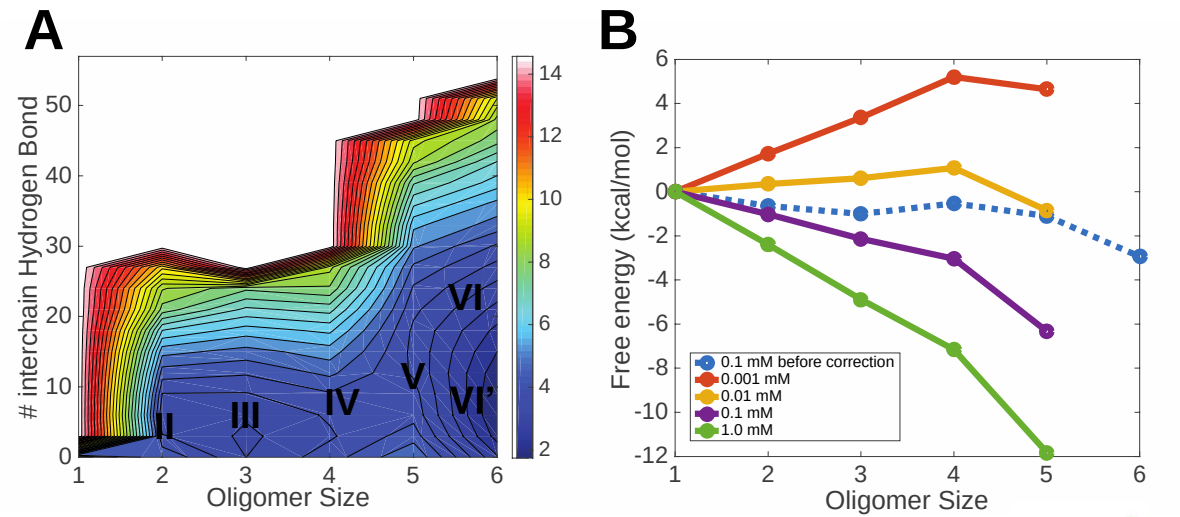
PolyP: Reported to prevent helix formation in PolyQ segment (*Anusri et al,. 2006 JMB*).

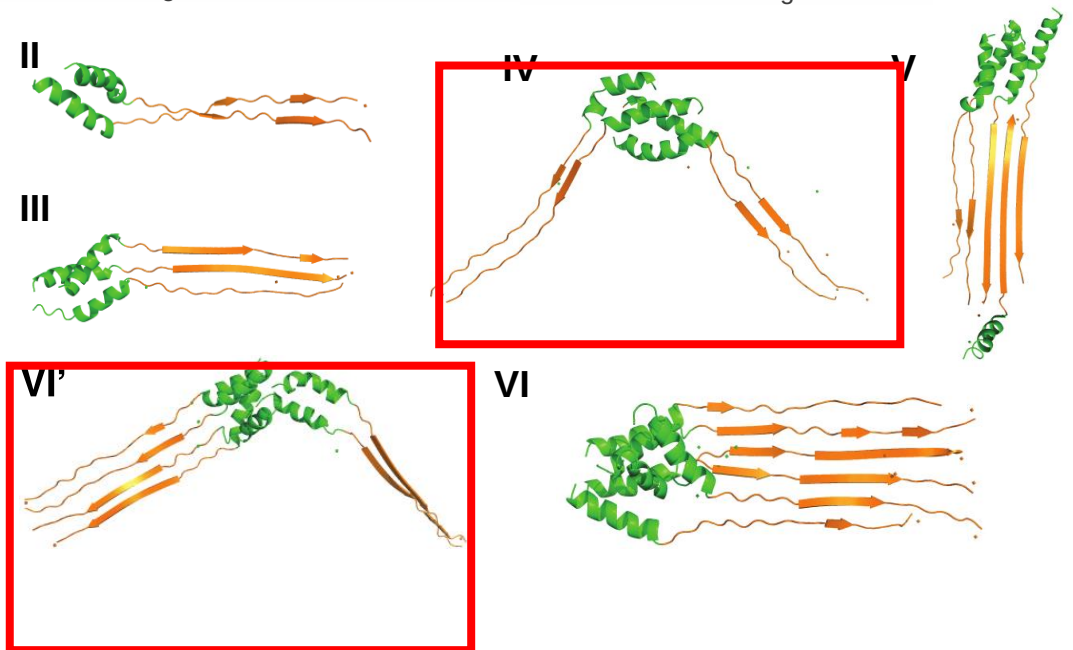
Heat-repeat domain: structure solved



PDB ID: 1B3U

The N-Terminal Region Facilitates Aggregation By Forming Pre-Fibrillar Oligomers (Q20)



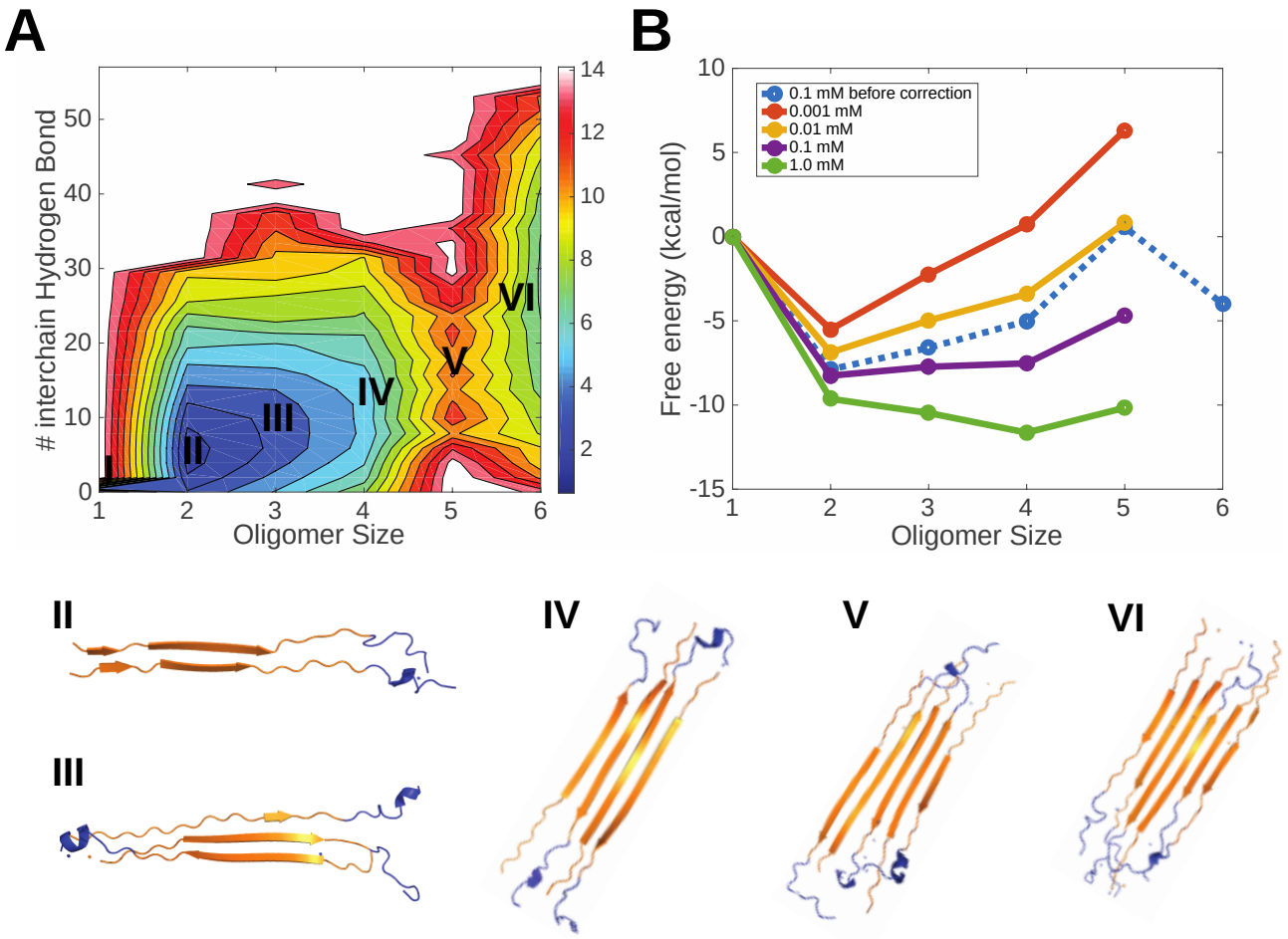


The free energy profile is more downhill compared to pure Q20!

Formation of pre-fibrillar oligomers eliminates the free energy barrier.

Similar facilitation is observed for NT17-Q30 and NT17-Q40!!

The C-Terminal PolyProline Inhibits Aggregation



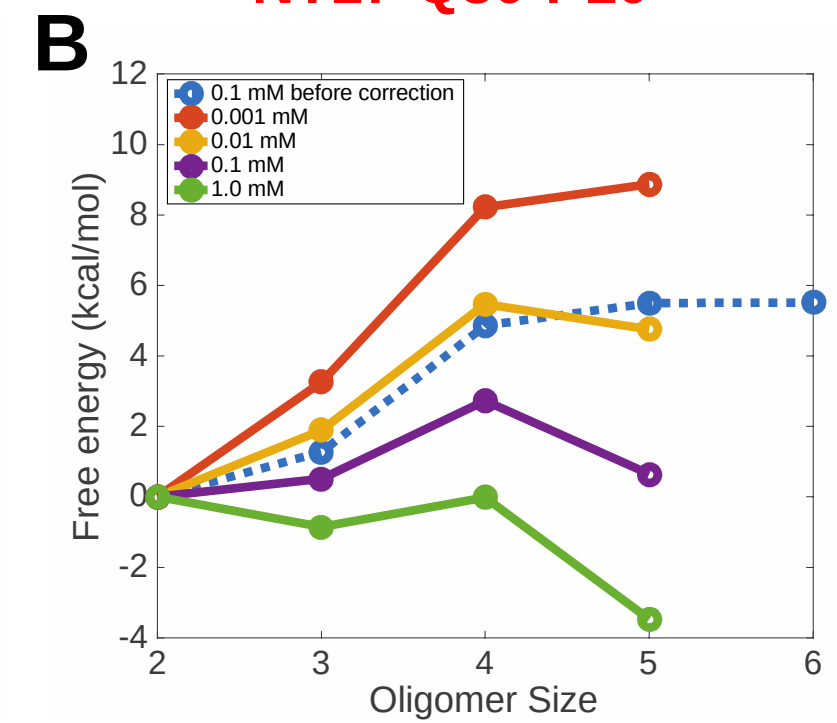
The free energy profile is more uphill compared to pure Q20!

Similar inhibition is observed for Q30-P10 and Q40-P10!!

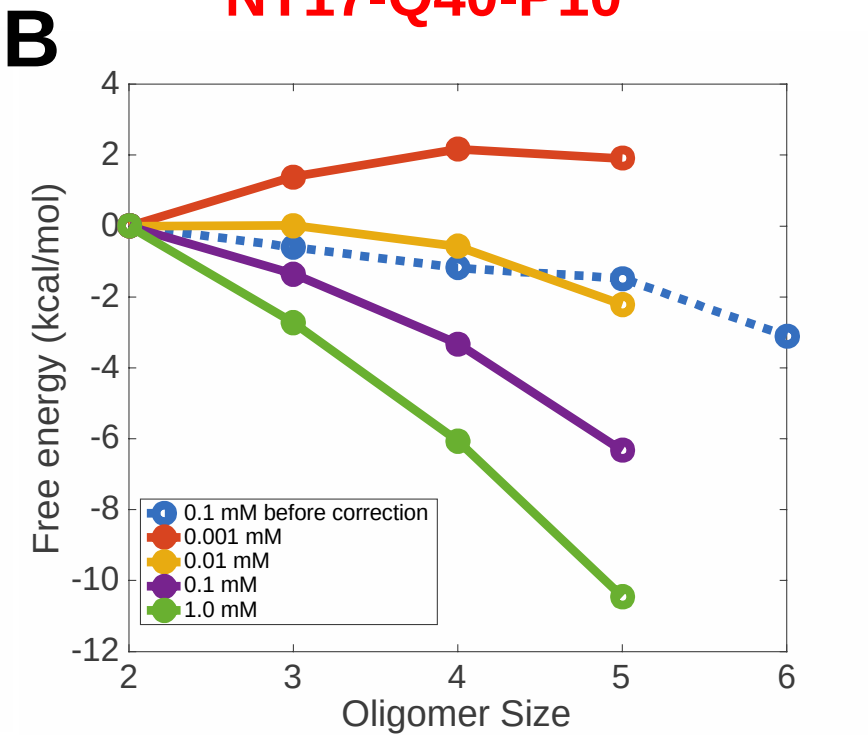
The aggregation mechanism is not altered!

Aggregation Behavior at the Estimated Concentration in the Inclusion Bodies

NT17-Q30-P10



NT17-Q40-P10



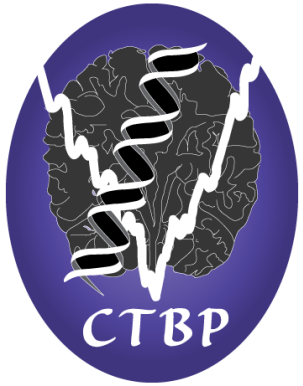
Below the critical length, the aggregation is uphill.

The CTBP Memory Group

Mingchen Chen
Qian Wang
Nick Schafer
Carlos Bueno

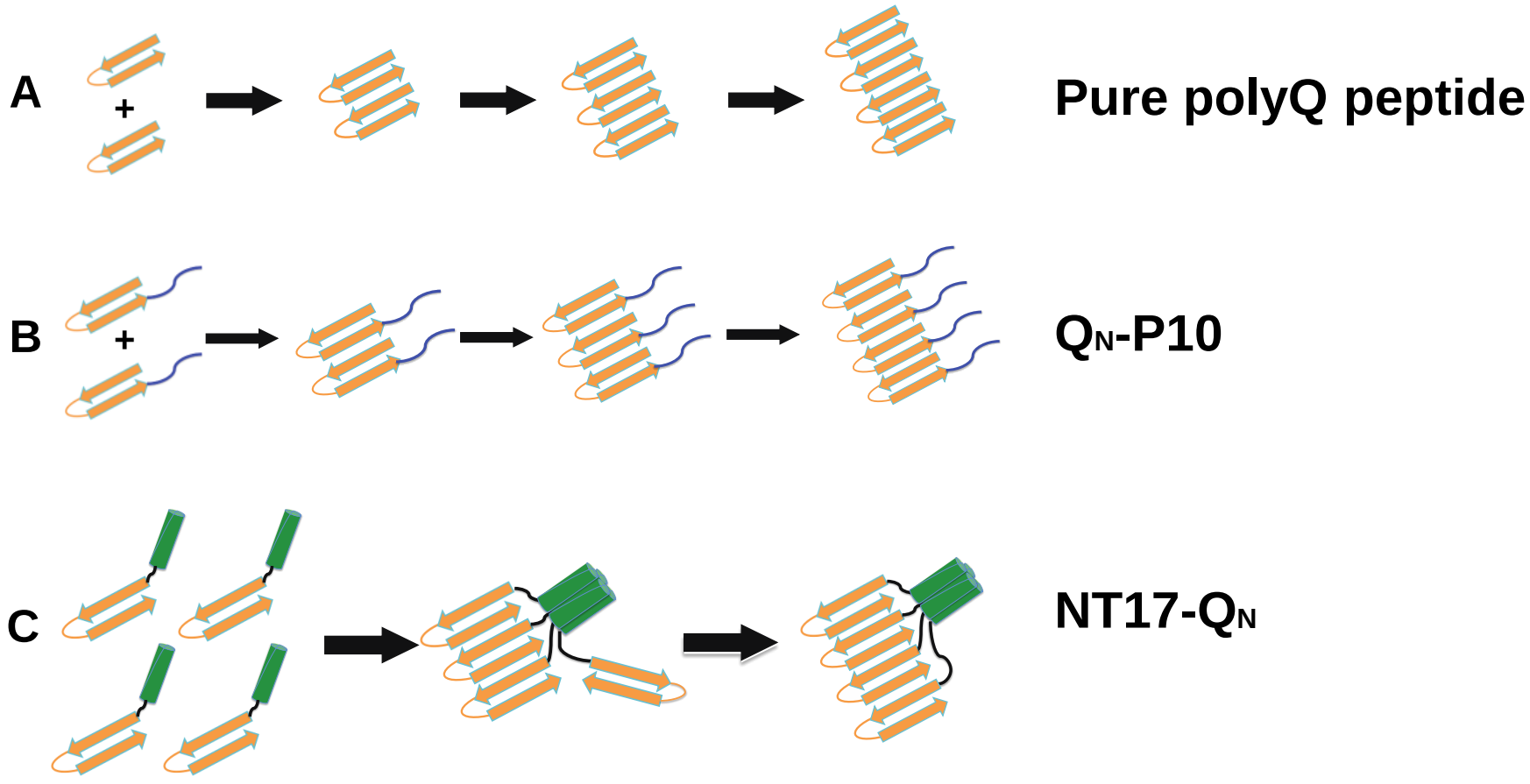
Margaret Cheung, University of Houston
Neal Waxham, UT Health Science Center
Herb Levine, Northeastern University
PGW

CHE 1743392
NSF RAISE: Dendritic Spine Mechano-biology and the Process of Memory Formation



Back-up Slides

Summary of the Terminal Effects



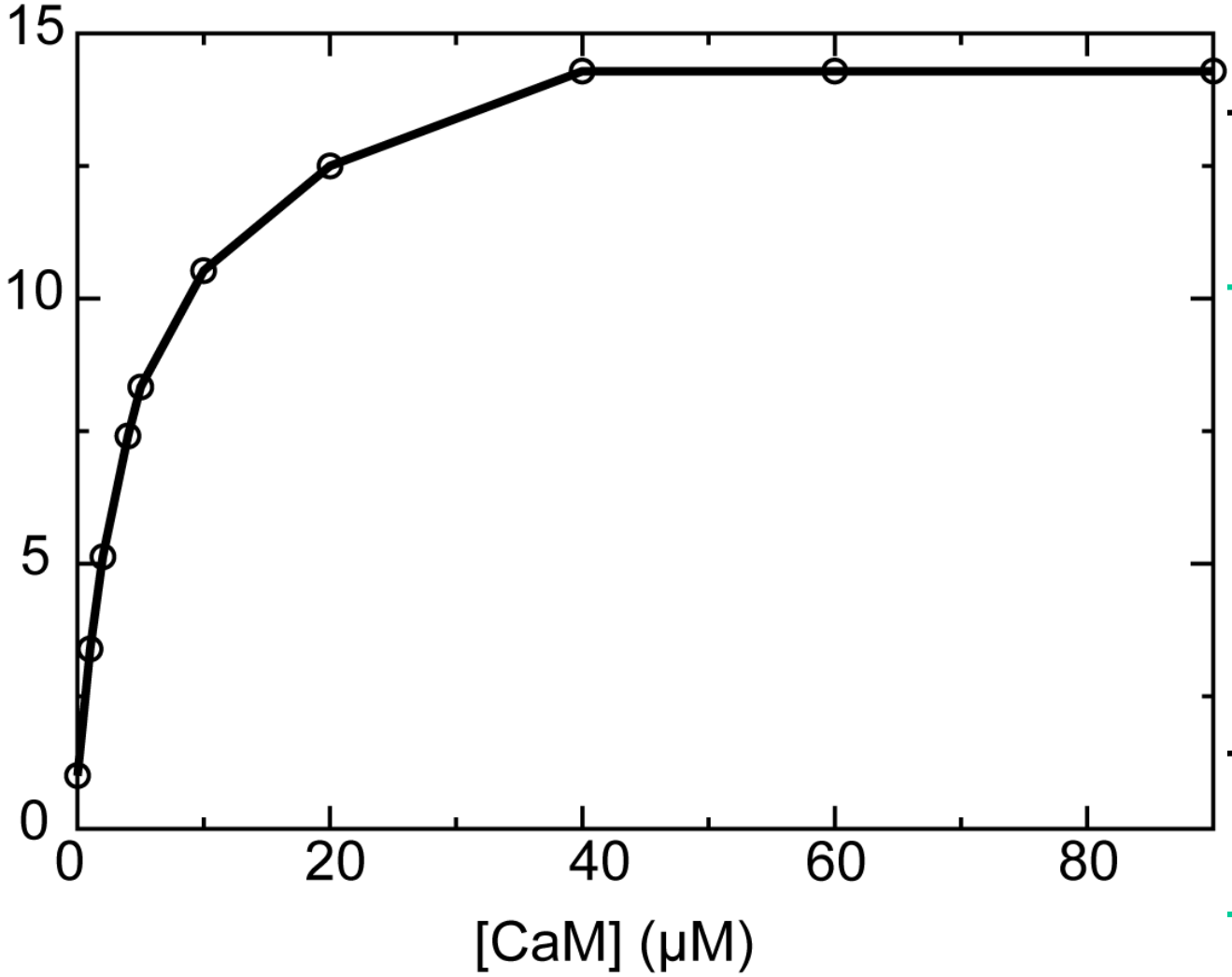
The arrows represent relative kinetic rates.

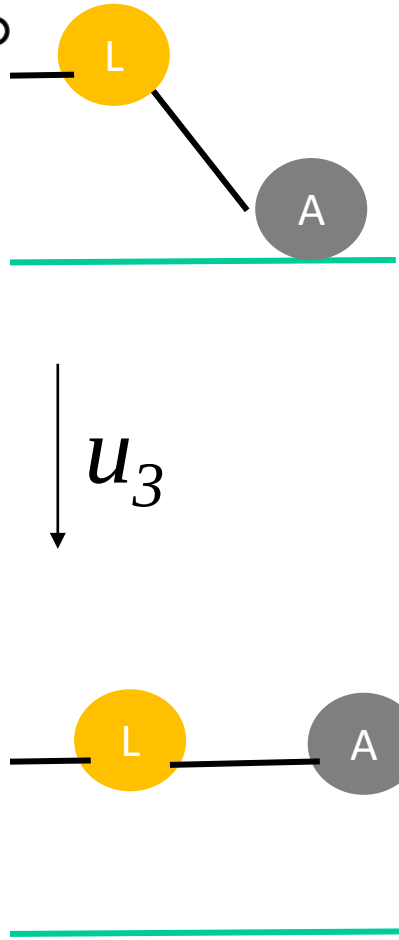
Kinetic model

u

$$\frac{k_{off}\ (CaM)}{k_{off}\ (no\ CaM)}$$

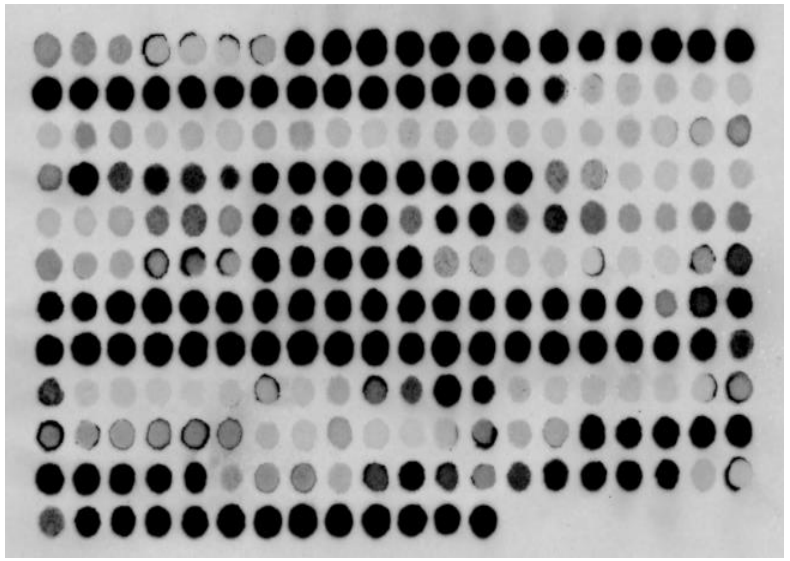
calmodulin





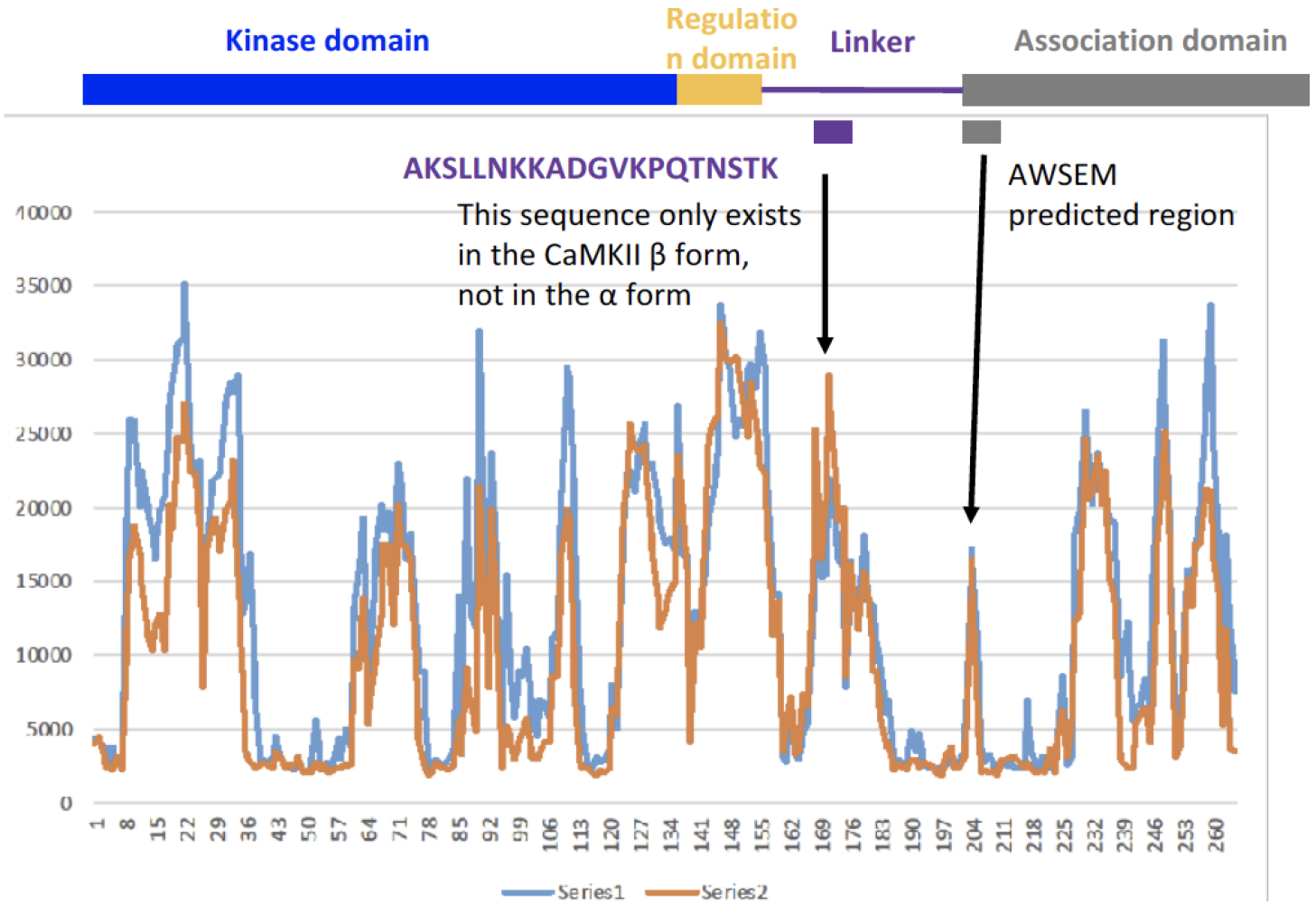
Peptide Arrays Provide Clues on Additional Binding Regions

Overlapping peptides of CaMKII are spotted on an array

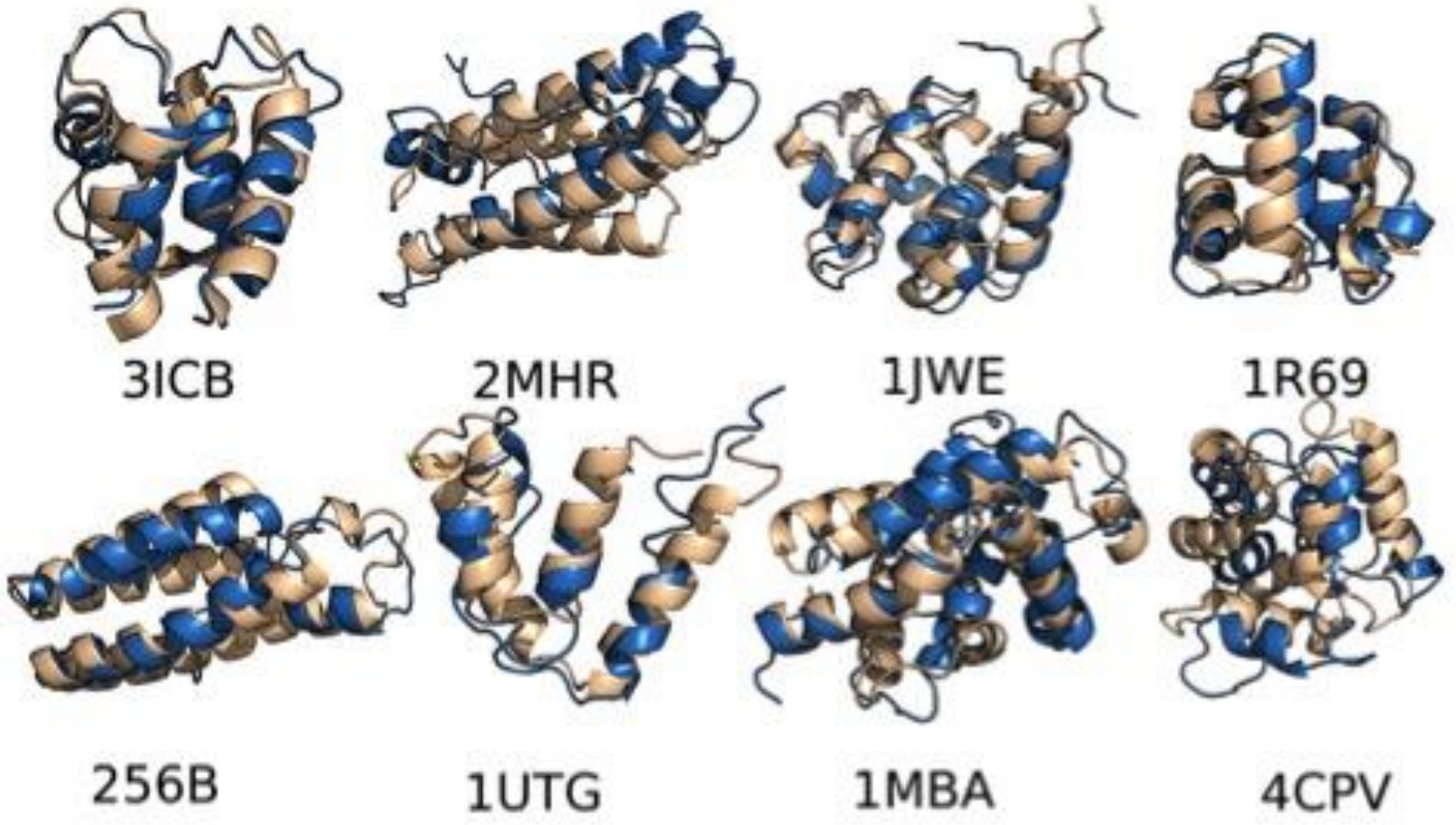


Actin fluorescence signals

Neal Waxham

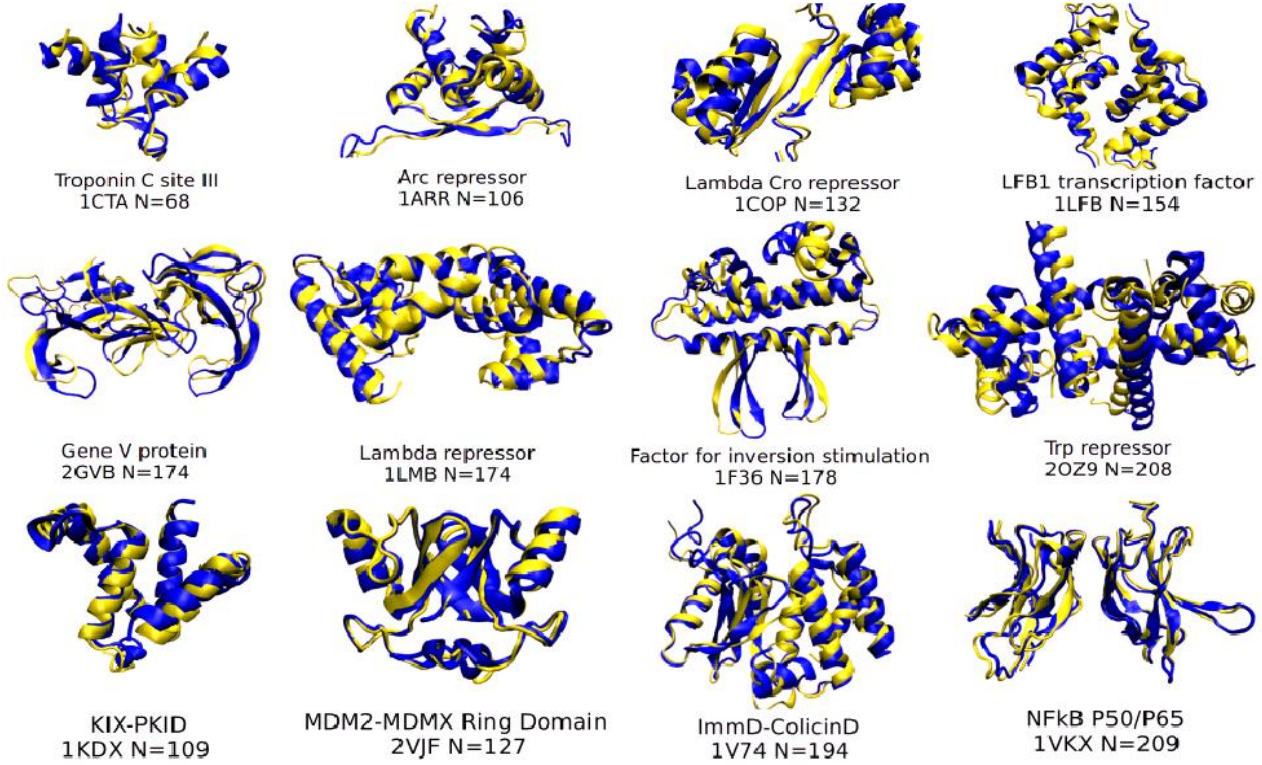


AWSEM predicts Tertiary Structure of Globular Proteins from Their Sequence Alone

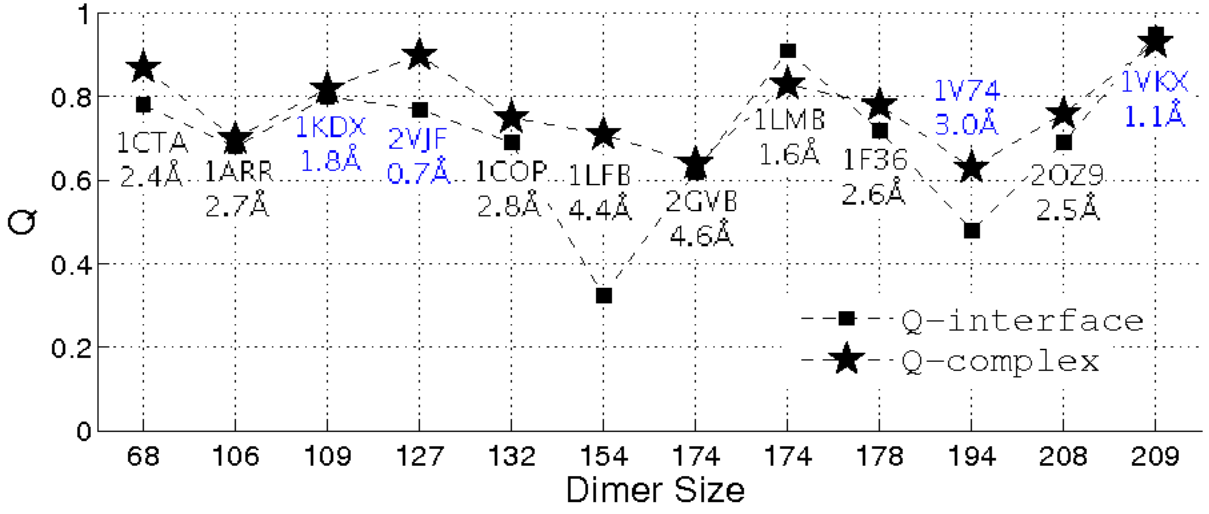


Davtyan, A.; Schafer, N.; Zheng, W.; Clementi, C;. Wolynes, P. G.; Papoian, G. A. *J. Phys. Chem.,* **2012**, *116*, 8494-8503.

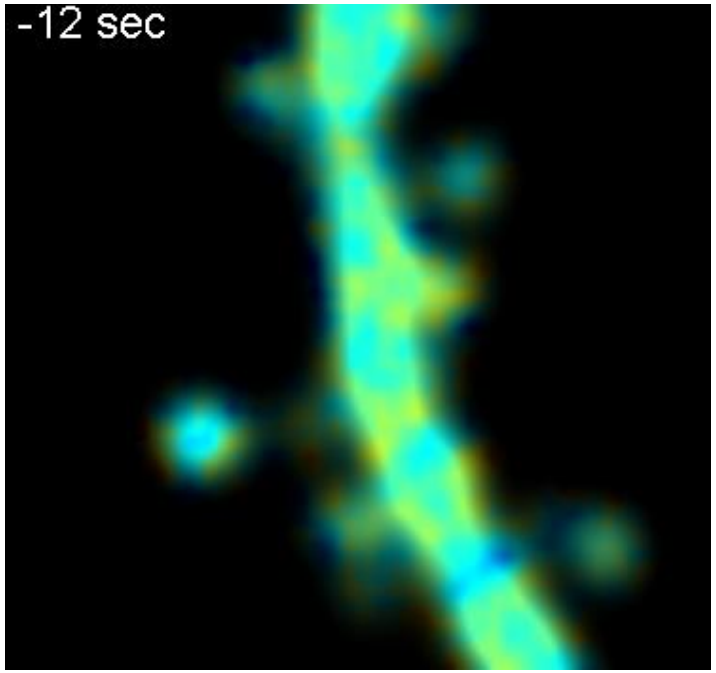
AWSEM Predicted structures of dimers



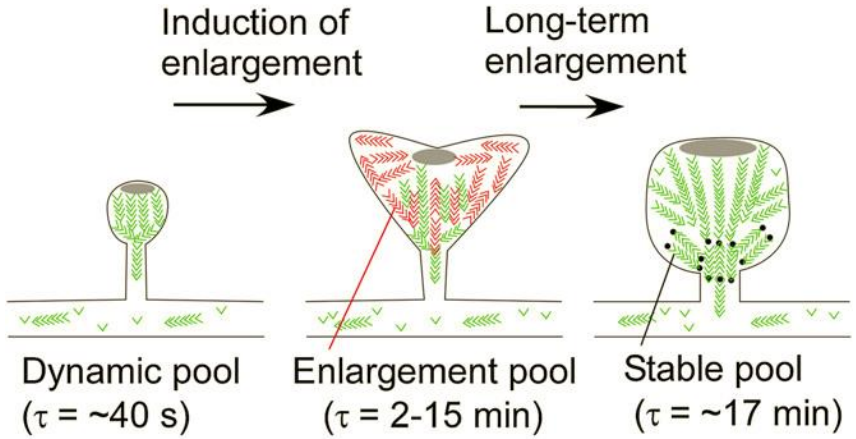
Zheng W, Schafer N, Davtyan A, Papoian G, Wolynes PG, "Predictive Energy Landscapes for Protein-Protein Association" *PNAS* **109**, 19244, 2012.



Memories Seem to Be Initiated By Calcium Influx Key Players: Actin and CamKinase II



From Lee et al., *Nature* 2009



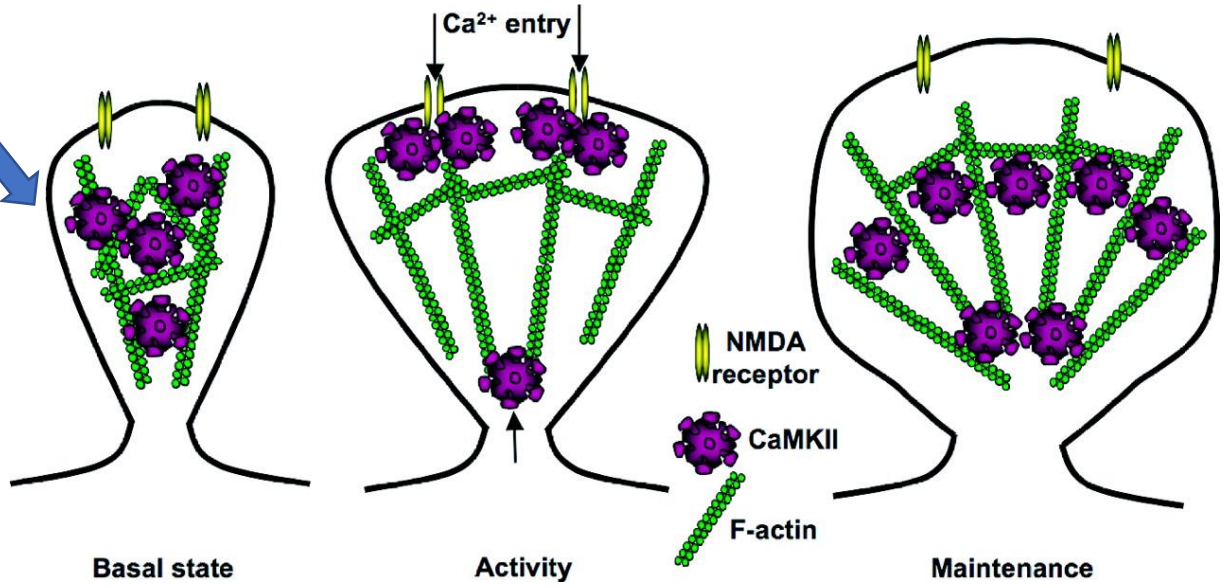
From Honkura et al., Neuron, 2008

Spine enlargement correlates with long-term plasticity and requires calmodulin and CaMKII

CaMKII remodels the actin cytoskeleton

Enlarged, mushroom-like dendritic spine

dendritic spine



Yasunori Hayashi group, PNAS, 2007

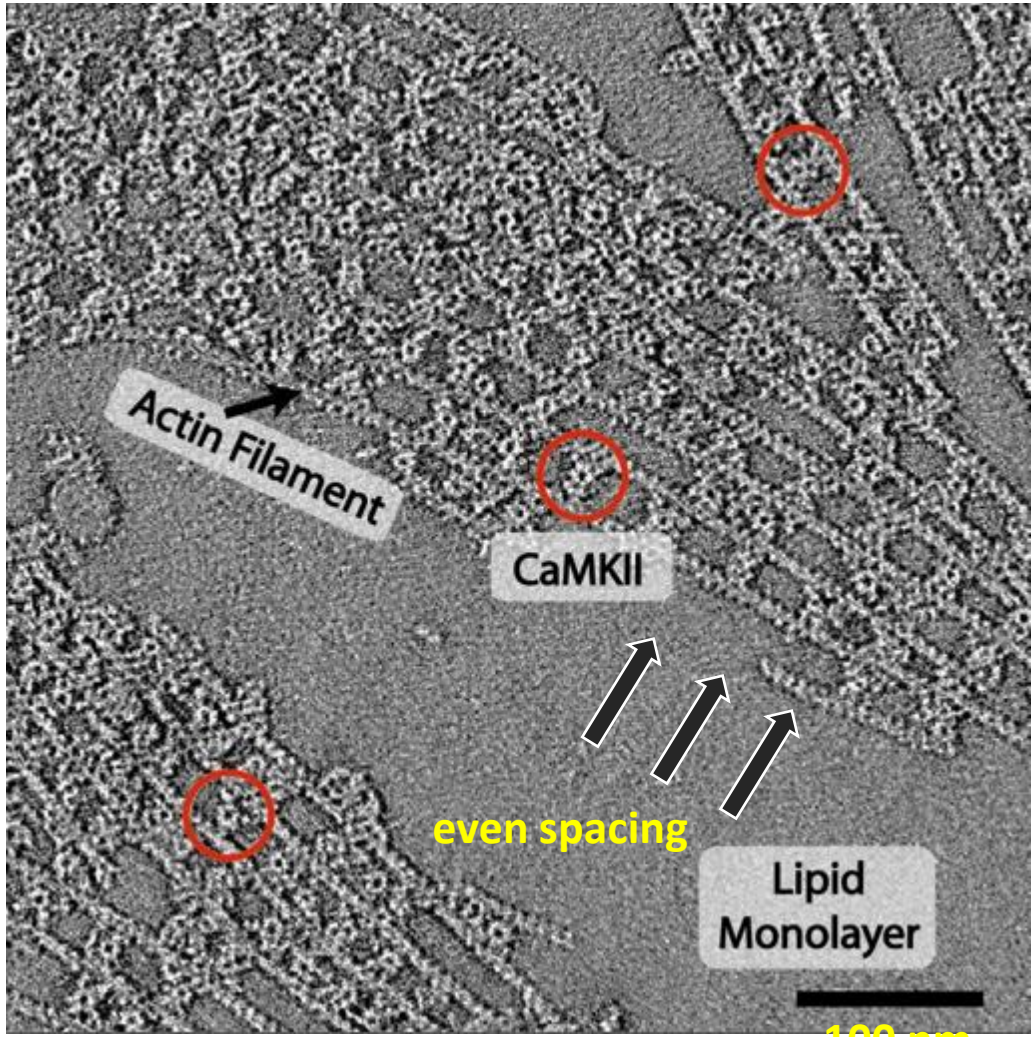
CaMKII binds and bundles F-actin

CaMKII dissociates from, thus unbundles F-actin

CaMKII re-binds and bundles F-actin

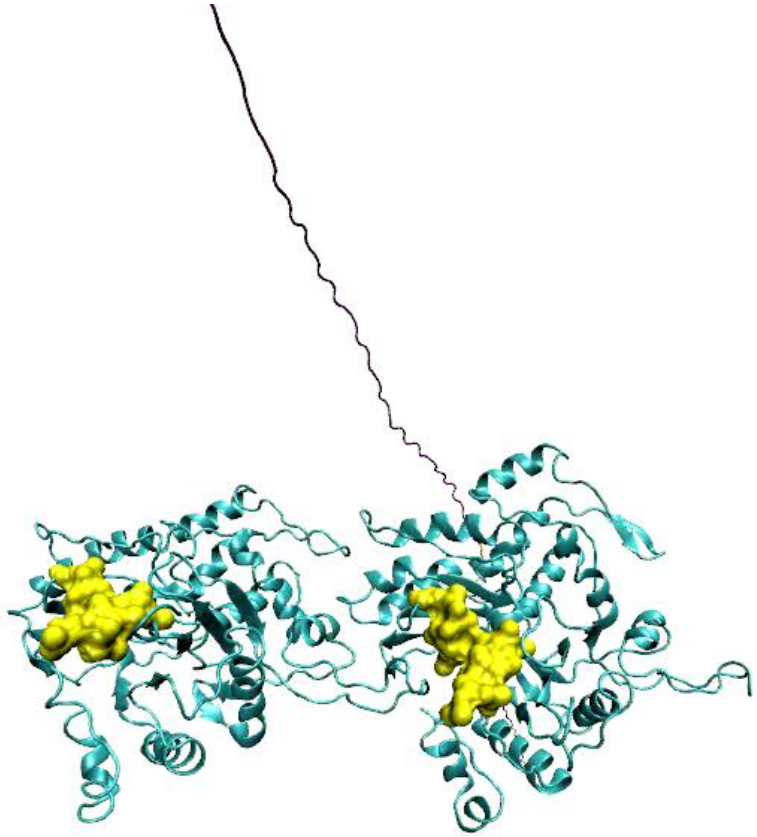
What are the molecular details?

Cryo-EM image from Neal Waxham lab



The actin filaments bundle in parallel with a periodic spacing of the CaMKII molecules

Multivalent binding of CamKII beta form to actin



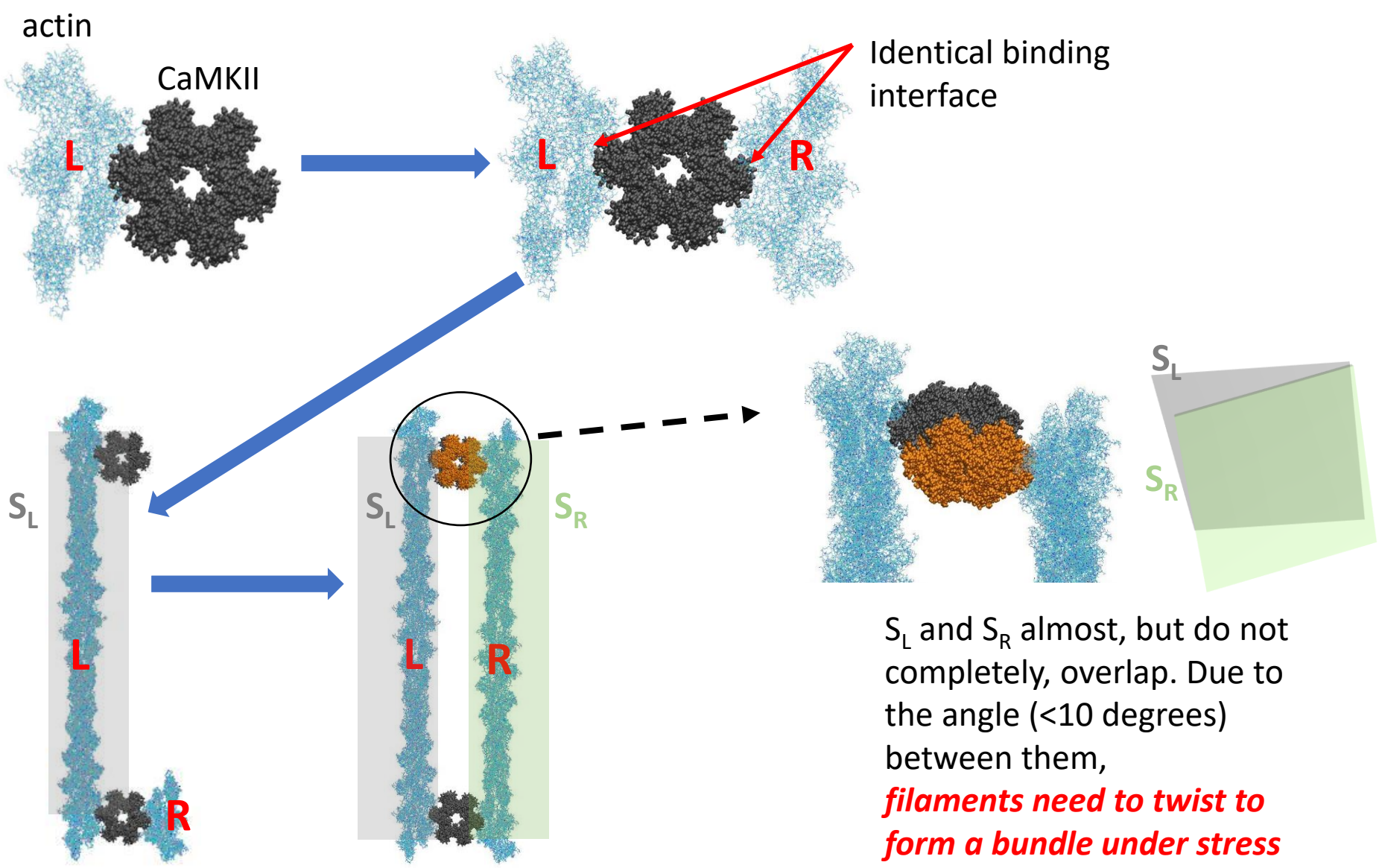
Grey: N-terminus of the association domain

Orange: Linker

Cyan: actin

Yellow: conserved binding pocket

Qian Wang, Mingchen Chen, Margaret Cheung, Neal Waxham, PGW

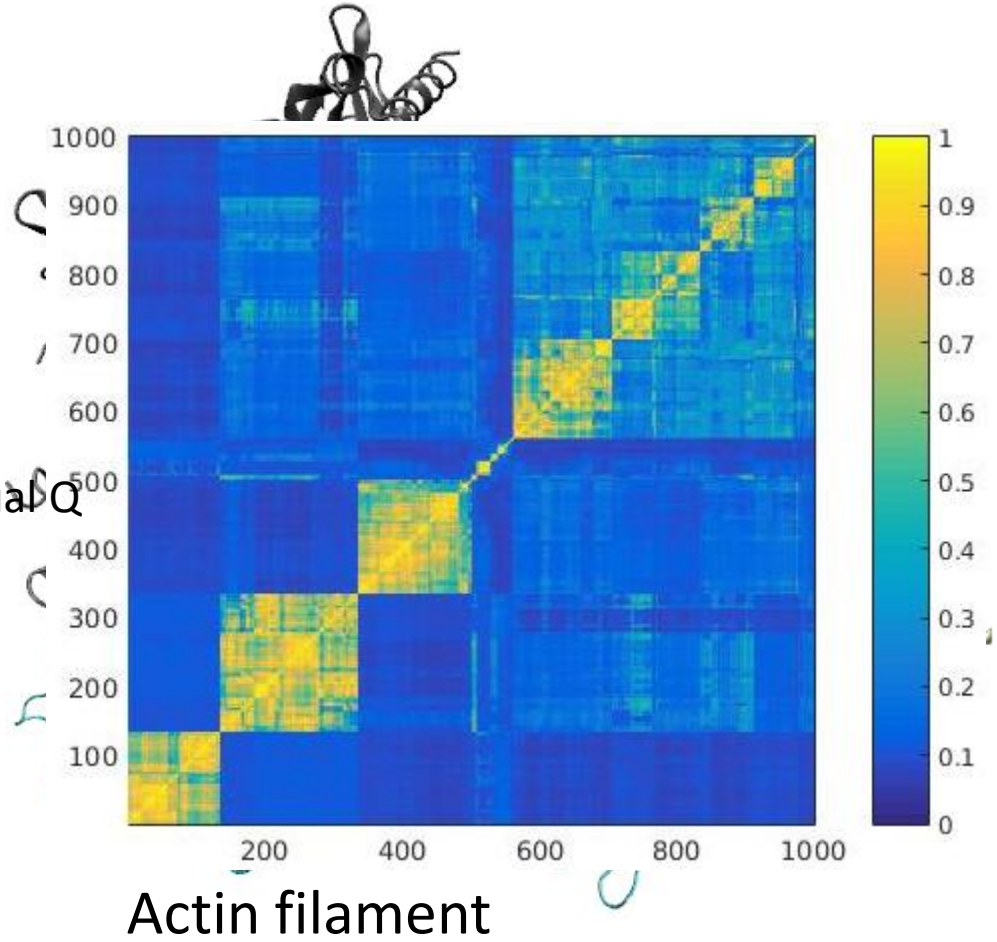


Predicted relative orientation between CaMKII and actin strongly favors the formation of parallel aligned filaments geometrically

S_L and S_R almost, but do not completely, overlap. Due to the angle (<10 degrees) between them, ***filaments need to twist to form a bundle under stress – what is the consequence?***

Double layer screening process –
the structure which we seek should simultaneously satisfy two conditions:

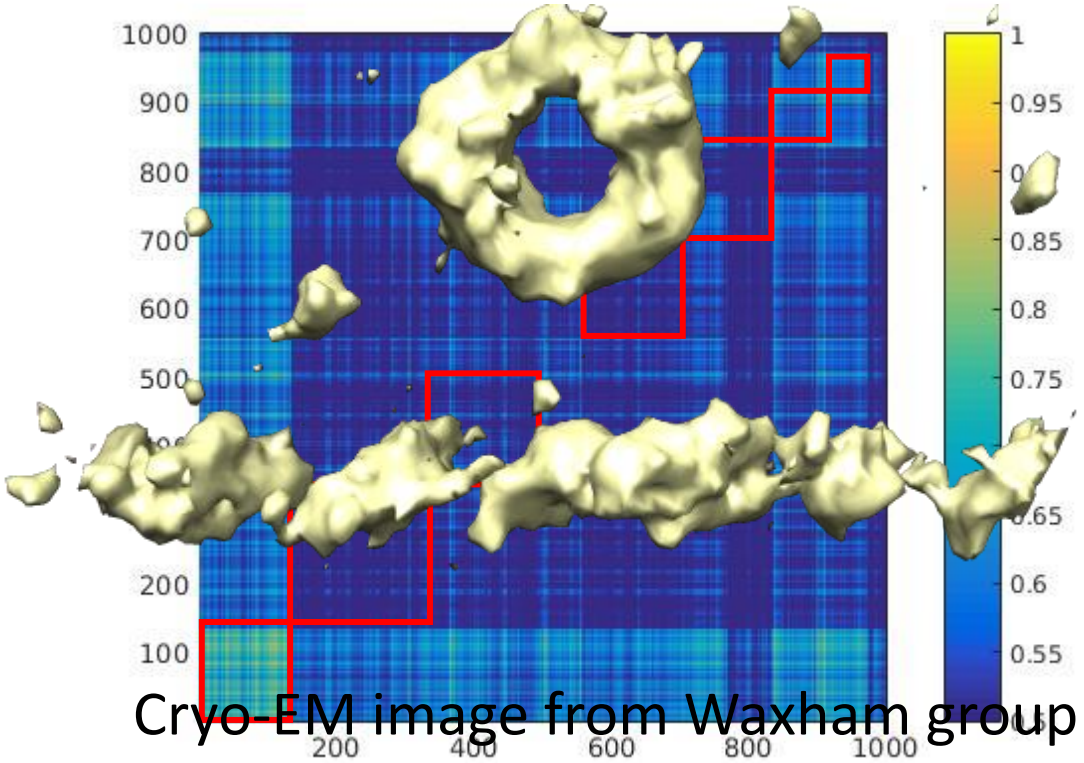
- Locating in a big cluster (low free energy)
- Having a high peptide score (to match with the experimental data)



Mutual Q

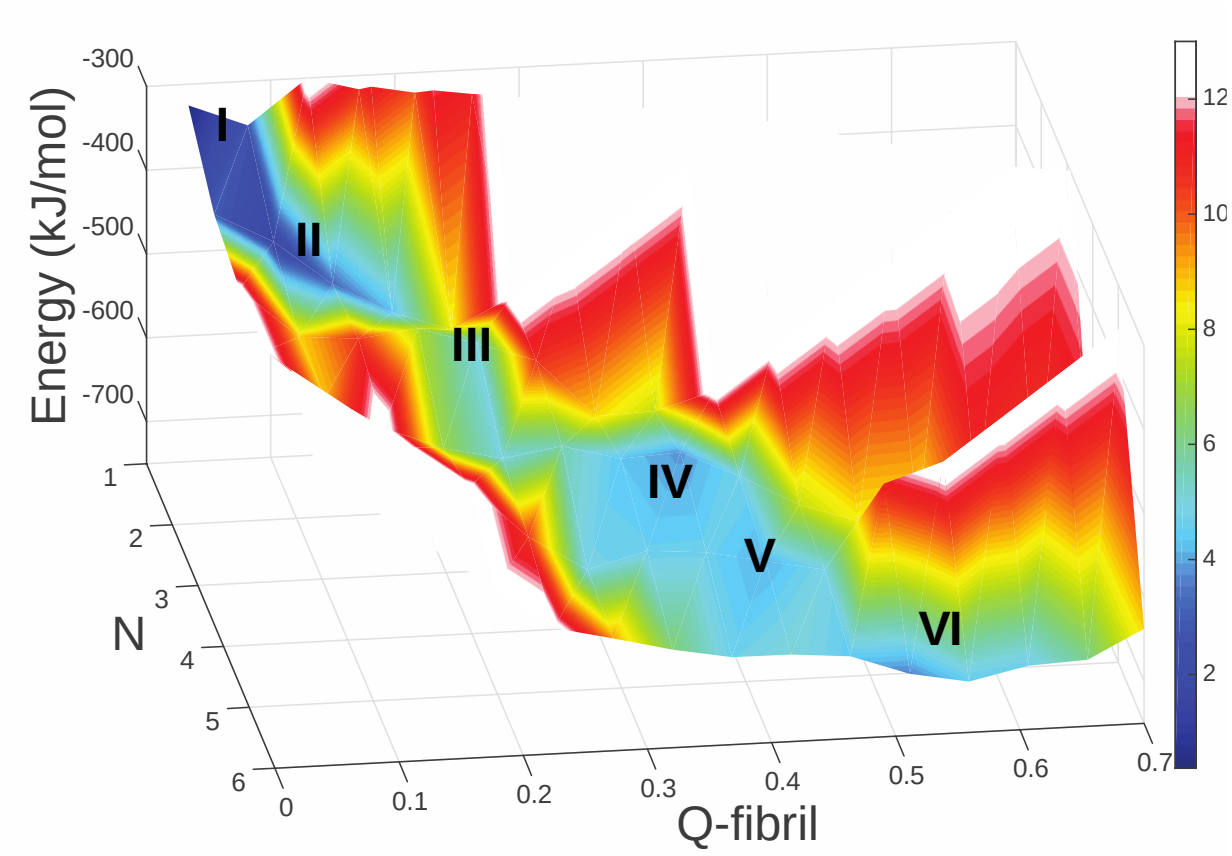
Actin filament

Peptide score matrix



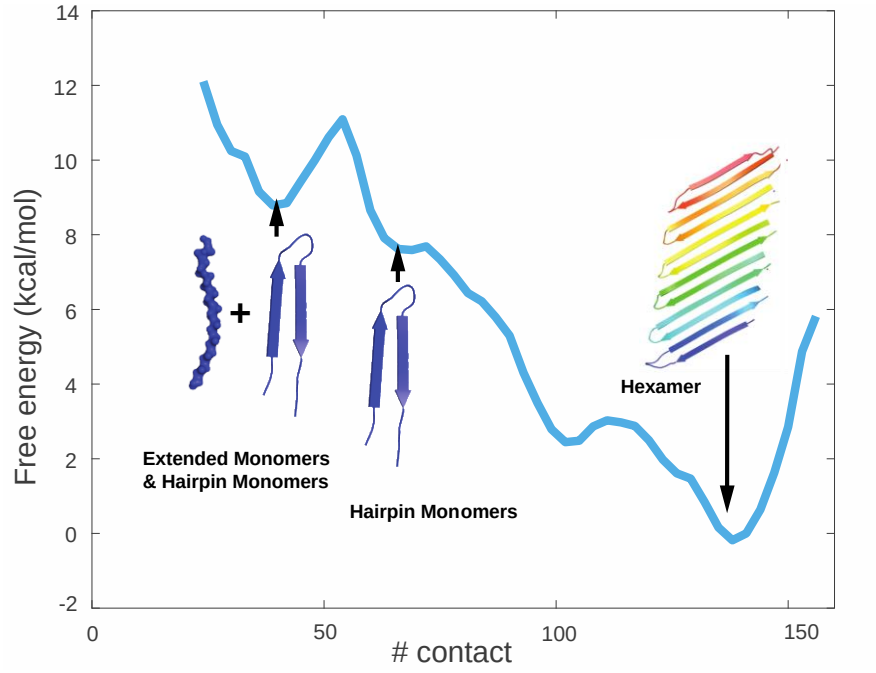
Cryo-EM image from Waxham group

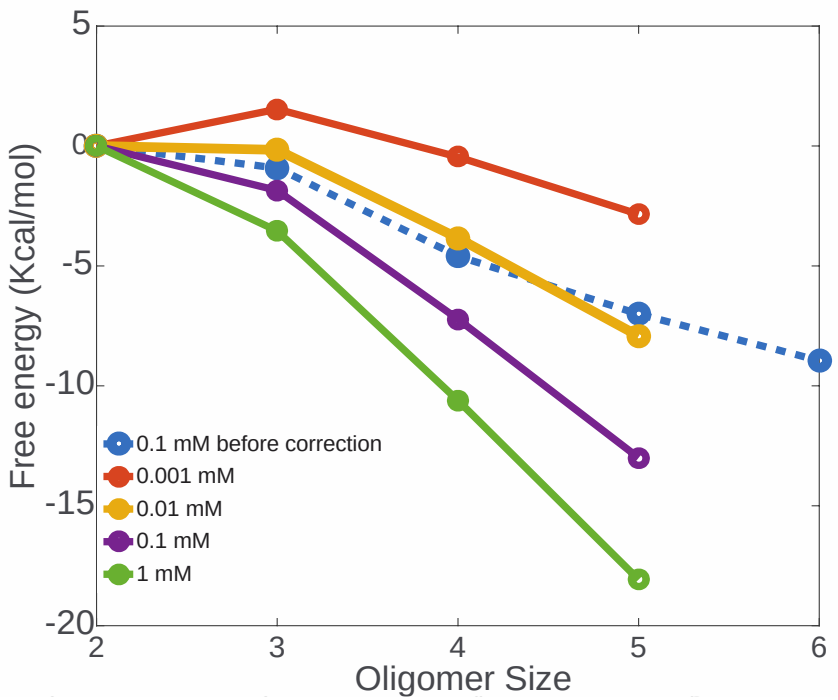
The Aggregation Landscape of Q20 is Funneled



Aggregation Free Energy Profile for Q30

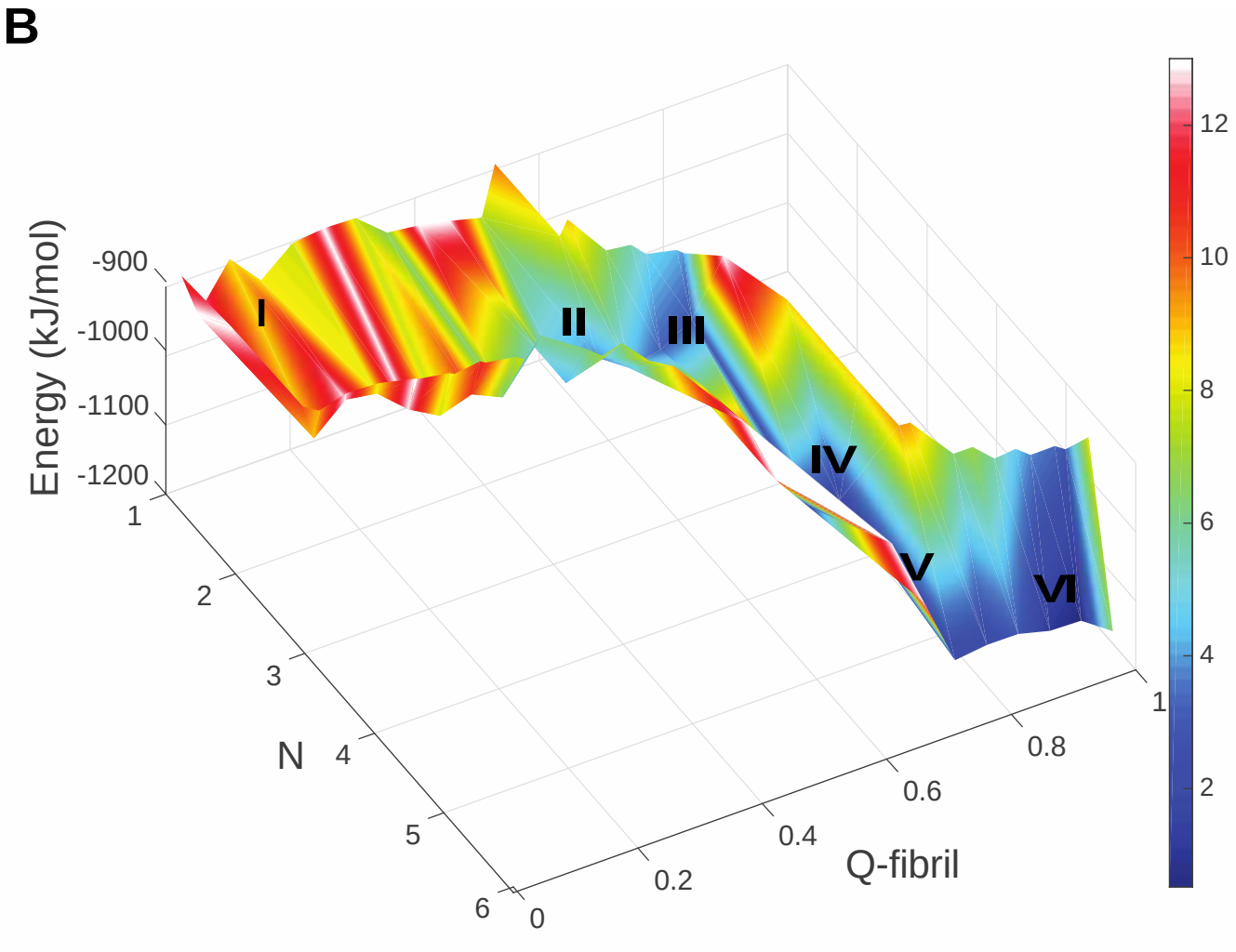
Nucleus Size: n*=1



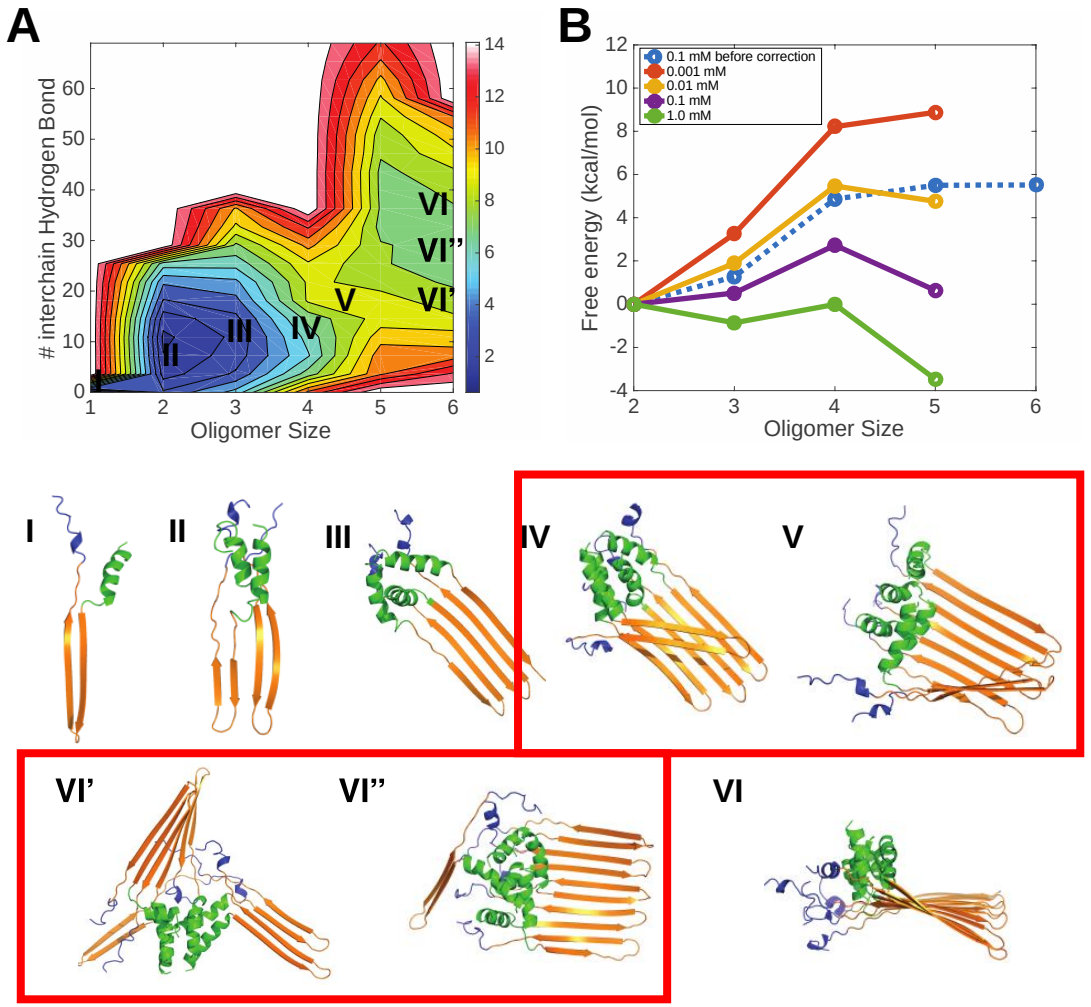


The critical concentration (solubility limit ~1 uM) agrees well with experiments that the solubility limit for Q30K2 is around 5 uM (Crick et al., 2013).

The aggregation free energy landscape for Q30 is funneled



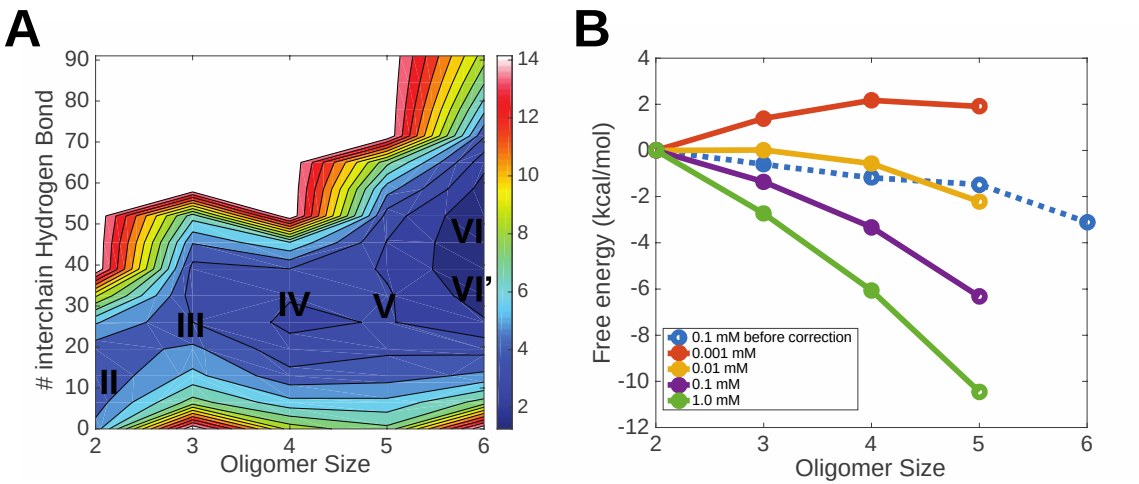
The Aggregation Free Energy Landscape of NT17-Q30-P10

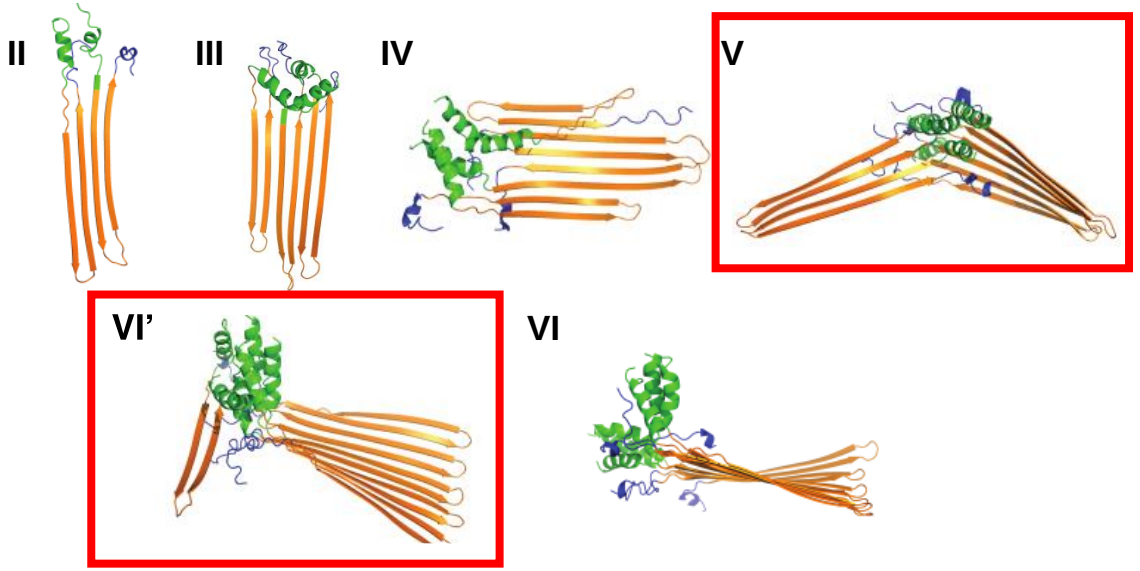


Pre-fibrillar species mediated by N-terminus are observed.

The aggregation is not favored even at high concentration (100 uM).

Aggregation Free Energy Landscapes of NT17-Q40-P10





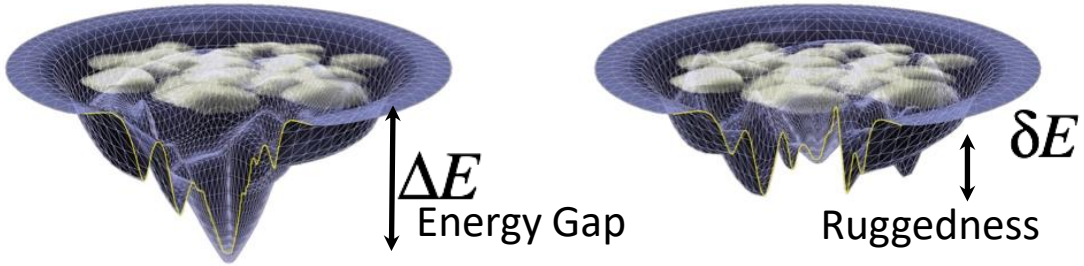
Pre-fibrillar species mediated by N-terminus are observed.

The aggregation becomes favored even at physiological concentration inside the inclusion bodies (~10 uM).

Energy Landscape Theory of Protein Folding

Natural Protein Random Sequence

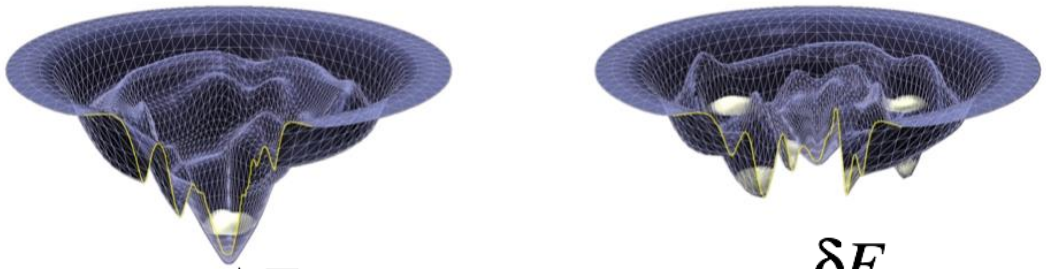
High Temperature



Denatured Ensemble:

- Large Structural Entropy
- Energetic Ruggedness
- Low Stability

Below T_F At T_G

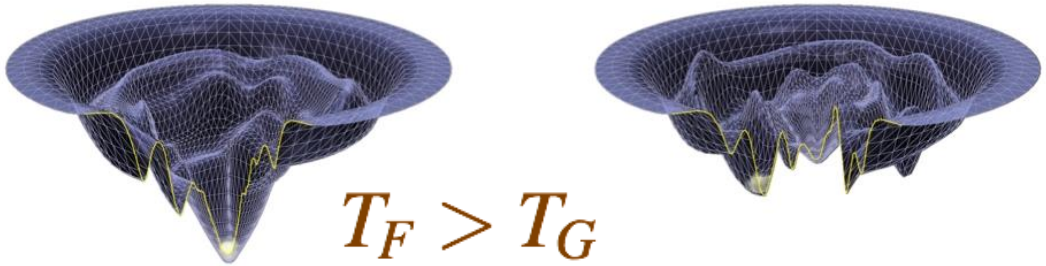


$$T_F \approx \frac{\Delta E}{S_C} \qquad T_G \approx \frac{\delta E}{\sqrt{S_C}}$$

Native Ensemble:

- Energetically Stable

Low Temperature



$$T_F > T_G$$

Principle of Minimal Frustration

JD Bryngelson & PG Wolynes, *PNAS*, 1987