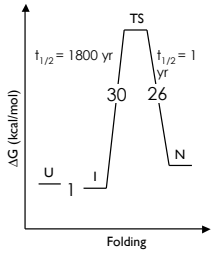
UCSFBIOPHYSICS

ANNUAL STUDENT RETREAT 6 OCTOBER 2006

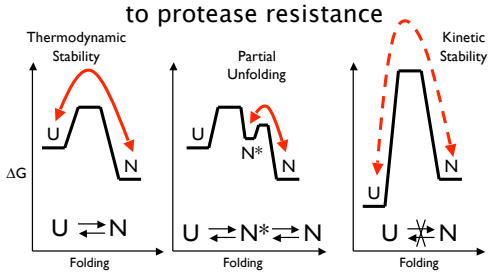
Neema Salimi

αLP is kinetically stable



- Native state not thermodynamically stable
- Native state persists due to high barrier to unfolding
- Why would αLP evolve such an extreme folding landscape?

Kinetic Stability as a path to protease resistance

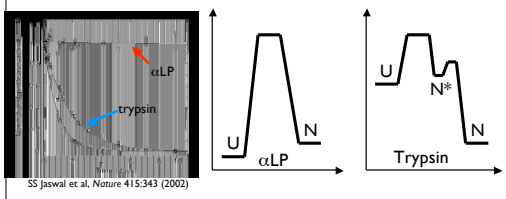


- constantly samples U state
- susceptible to proteolysis

- partial unfolding much faster than global unfolding
- N* can be protease sensitive

- rarely samples U state
- resistant to proteolysis
- longer functional lifetime

αLP unfolding cooperativity much higher than trypsin



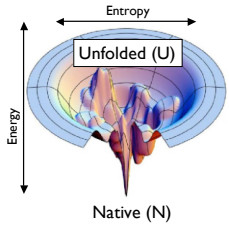
SS Jaswal et al, *Nature* 415:343 (2002)

- Trypsin unfolding rate similar to αLP (Stephanie Truhlar)
- Rapid degradation of trypsin due to partial unfolding
- Trypsin unfolding not as cooperative as αLP

SME Truhlar et al, *Protein Science* 13:381 (2004)

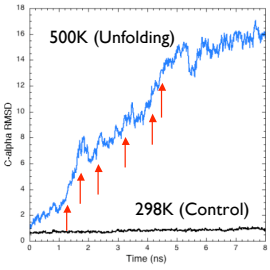
What is the structural basis of αLP's kinetic stability and extreme cooperativity?

- Mapping unfolding landscape requires high structural resolution
- Molecular dynamics (MD) simulations give best structural resolution
- Valerie Daggett's lab (and others) have shown unfolding MD is highly complementary to and consistent with experiment
- Requires high temperature to achieve *in silico* unfolding



Simulation Details:
5 500K sims, 1 298K sim, NVE ensemble
NAMD software, CHARMM forcefield
198 protein residues, ~30,000 total atoms

αLP unfolds rapidly and completely at 500K

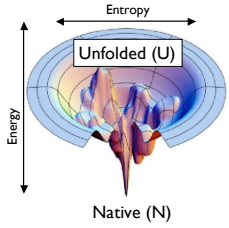


- 298K control averages <1.0 Å RMSD
- 500K simulation shows several large jumps in RMSD
- How do we analyze the trajectories for relevant information?
- How can we find the Transition State Ensemble (TSE)?

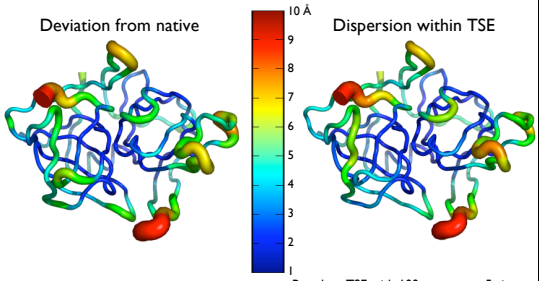
Determining the TSE by MD unfolding

Because the RMSD plot is ambiguous, we need to integrate many parameters in order to find the TSE

- Nativeness
  - RMSD
  - # native contacts
- Structural Properties
  - radius of gyration
  - solvent accessible surface area
- Configurational Entropy
  - Divergence within a simulation
  - Divergence between simulations



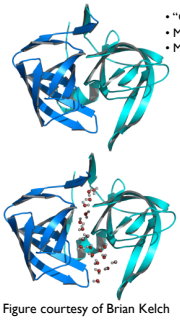
The αLP TSE deviates from native in several key areas



Based on TSE with 100 structures, 5 sims

Unfolding involves splitting the domain interface

- "Cracked egg" model for αLP unfolding was proposed (Sheila Jaswal)
- Model postulates split at domain interface during unfolding
- Model based on thermodynamics and pH dependence of unfolding



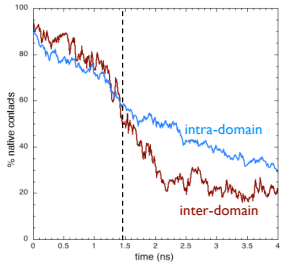
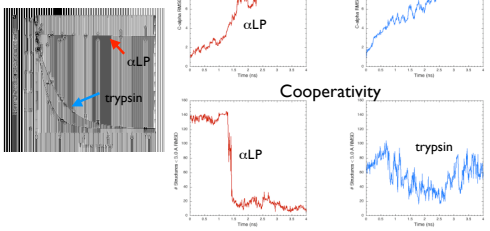


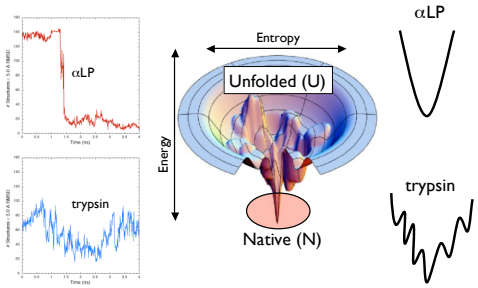
Figure courtesy of Brian Kelch

A new metric for cooperativity



αLP unfolding *in silico* is significantly more cooperative than trypsin

Landscapes for the native states αLP and trypsin



Jerome Nilmeier

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Multiscale Monte Carlo Modeling of Proteins

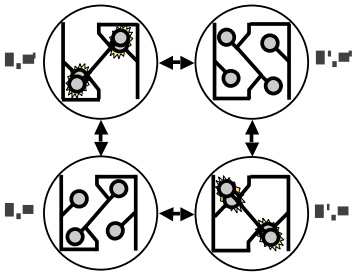
Jerome Nilmeier
Matt Jacobson Group

UCSF
Biophysics Retreat
October 6, 2006

What are we doing?

We are ***using physics based methods*** for ***high resolution structure refinement*** to aid in ***drug design***

A key challenge:
How to identify multiple low free energy structures starting from a close packed protein state?

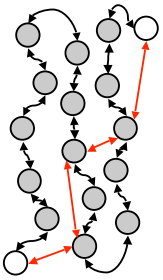


- Large energy and/or dynamical barriers make MD difficult
- Global ptimization methods often neglect entropic components
- Perhaps a ***Monte Carlo*** approach will give the best of both worlds?

Practical Considerations for Monte Carlo

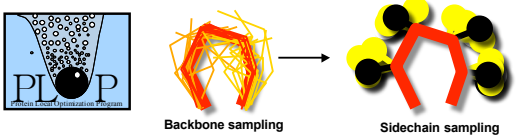
Monte Carlo is a discrete sampling algorithm

- Moves do not need to be connected temporally or spatially.
- The states do need to be ***connected topologically***, however.
- The efficiency of the scheme depends entirely on the choice of move set.



A clever choice of move set will traverse energy barriers efficiently

Protein Local Optimization Program



A key feature of PLOP is the hierarchical decomposition of coordinates.

We can adapt many of the existing routines to a ***Monte Carlo Sampling Strategy***

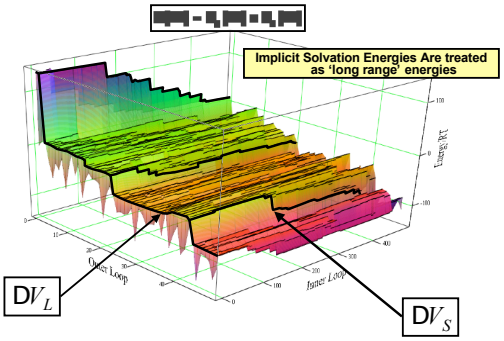
Jacobson, M.P., et al., *A hierarchical approach to all-atom protein loop prediction.* Proteins, 2004. **55**(2): p. 351-67.

Sidechain Monte Carlo

- *Rotamers can be chosen from random for our move set and accepted with the Metropolis Criterion*
- *This corresponds to a random perturbation of the dihedrals of the sidechain*
- *Polar hydrogens are added, with the torsion selected randomly*

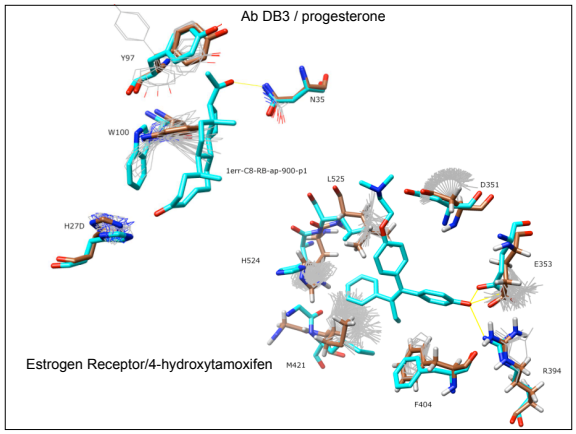
Xiang, Z. and B. Honig, **Extending the accuracy limits of prediction for side-chain conformations**. *J Mol Biol*, **2001**. 311(2): p. 421-30.

Multiple "time step" Monte Carlo (MTSMC)

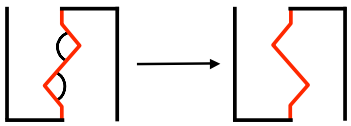


'Multiple "time step" Monte Carlo', B. Hetenyi, K. Bernacki, and B. Berne , JCP 117 8203

Jerome Nilmeier



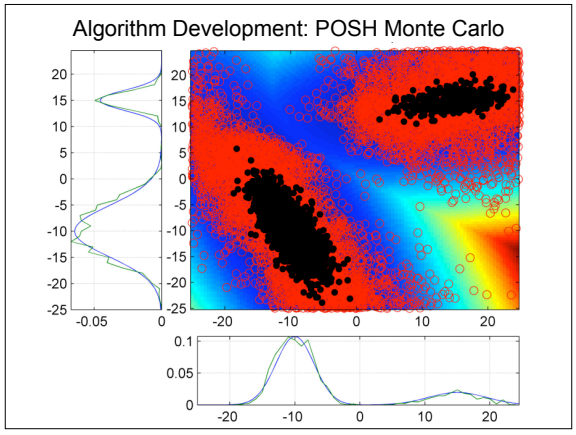
Concerted Rotation:
The basis for protein Monte Carlo

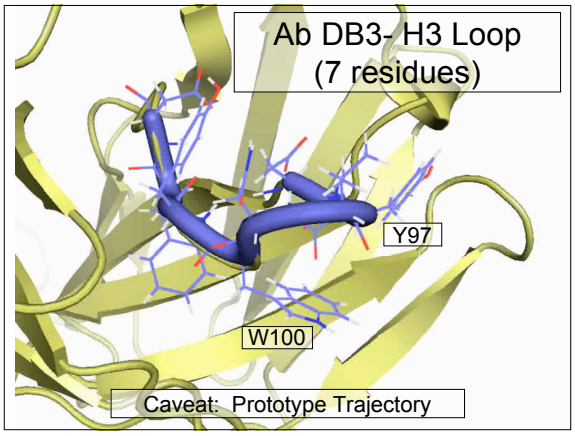


•2 angles must be simultaneously perturbed

The alternative is to completely unfold the structure and repack in the new configuration

Coutsias, E.A., ,Seok, C., Jacobson, M. and Dill, K.A., ***A kinematic view of loop closure***. Journal of Computational Chemistry, **2004**. 25: p. 510-528.





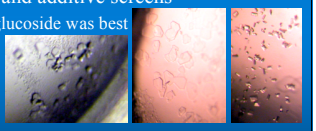
Structural Investigations of the Thyroid Hormone Receptor

Eric Slivka
Biophysics Retreat
October 6, 2006

Nuclear Receptors

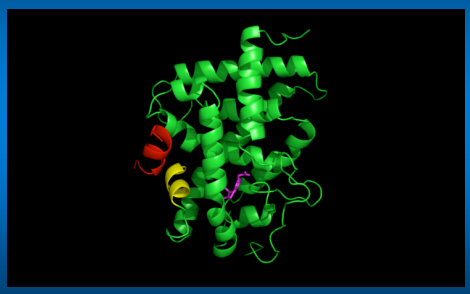
TR/RXR LBD Crystals

- Crystals! (Diffracting to 9Å)
  - Hampton PEG/Ion #25
    - (0.2M MgOAc, 20% w/v PEG 3350)
- Optimization
  - Hampton detergent and additive screens
    - 1-s-nonyl-β-d-thioglucoside was best



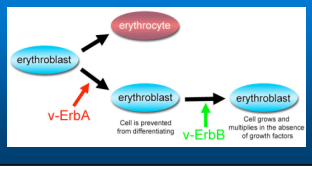
  - Nextal OptiSalts with detergent
    - More crystals
    - Best diffraction ~ 7Å

TR LBD + Peptide Structure



v-erbA

- Avian erythroblastosis virus
  - Retrovirus causing leukemia of red blood cells in chickens
  - Contains two genes (v-erbA and v-erbB) taken from the chicken genome and subsequently mutated
    - v-erbA: mutated thyroid hormone receptor a
    - v-erbB: mutated epidermal growth factor-like receptor

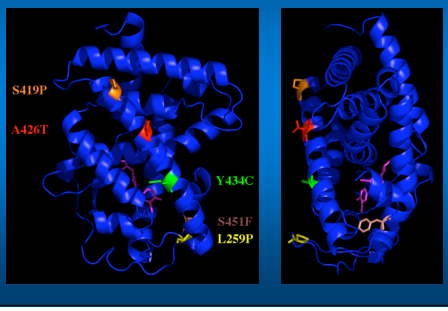


Comparison of v-erbA and TR Ligand Binding Domains

```
                202       210       220       230       240       250       260
v-ErbA LBD      EEMIKSLQHRPSPSAEEWELIHVVTEAHRSTNAQGSHWKQRRKFLEDIGQSPMASMLDG
c-ErbA LBD      EEMIKSLQHRPSPSAEEWELIHVVTEAHRSTNAQGSHWKQRKFLPEDIGQSPMASMPDG

                      270       280       290       300       310       320
v-ErbA LBD      DKVDLEAFSEFTKIITPAITRVVDFAKQLPMFSELPCEDQIILLKGCCMEIMSLRAAVRY
c-ErbA LBD      DKVDLEAFSEFTKIITPAITRVVDFAKKLPMFSELPCEDQIILLKGCCMEIMSLRAAVRY

                      330       340       350       360       370       380
v-ErbA LBD      DPESETLTLSGEMAVKREQLKNGGLGVVSDAIFDLGKSLSAFNLDDTEVALLQAVLLMSS
c-ErbA LBD      DPESETLTLSGEMAVKREQLKNGGLGVVSDAIFDLGKSLSAFNLDDTEVALLQAVLLMSS

                      390       400       410       420       430       440
v-ErbA LBD      DRTGLICVDKIEKCQESYLLAFEHYINYRKHNIPHFWSKLLMKVADLRMIGAYHASRFLH
c-ErbA LBD      DRTGLICVDKIEKCQETYLLAFEHYINYRKHNIPHFWPKLLMKVTDLRMIGACHASRFLH

                      450       460
v-ErbA LBD      MKVECPTELSP---------QEV
c-ErbA LBD      MKVECPTELFPPLFLEVFEDQEV
```

Alignment of v-erbA LBD Homology Model and TRa

Interesting v-erbA LBD Mutations

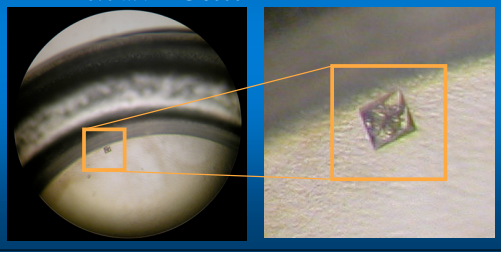


TR DBD/LBD Homodimer with DNA and Corepressor Peptide

- Aim: Determine the crystal structure of a homodimer of a TR DBD/LBD construct bound to DNA and a corepressor peptide
- Strategies:
  - DNA: F2 everted palindrome best for TR homodimer binding
  - Peptide from NCoR to stabilize unliganded state

TR DBD/LBD Crystals

- Two crystals in Classics Lite #69
  - 0.05 potassium phosphate monobasic
  - 10% w/v PEG 8000



Brendan Murphy

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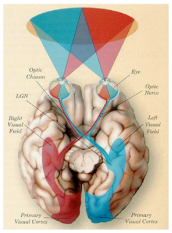
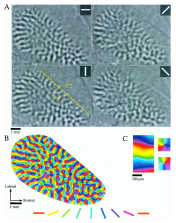
What is primary visual cortex (V1)?



- The first area of cortex to receive visual information
- Neurons respond selectively to oriented visual stimuli

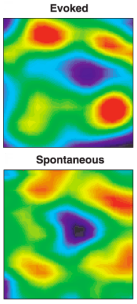
A map of orientation preference



Bosking et al, 1997

- Prefered orientation is mapped across the surface of V1
- Nearby neurons prefer similar orientations

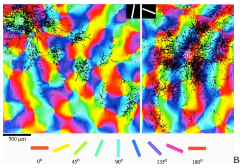
Structure of spontaneous activity in visual cortex



Tsodyks et al 1999

- Presenting an oriented stimulus causes areas of cortex that prefer that orientation light up
- Surprisingly similar patterns of activity occur in the absence of a visual stimulus

V1 physiology

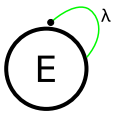


Bosking et al, 1997

- Each neuron in V1 is recurrently connected to thousands of other neurons
- Long range synaptic connections are made preferentially between neurons with similar orientation preferences

Recurrent connectivity

A single neuron recurrently exciting itself



$$\tau \frac{dr}{dt} = -r + \lambda r + h$$

$$\tau \frac{dr}{dt} = -(1-\lambda) r + h$$

$$r(t) = r(0) e^{-t/\tau'} + h'(1 - e^{-t/\tau'})$$

$$\tau' = \frac{\tau}{1-\lambda} \qquad h' = \frac{h}{1-\lambda}$$

Patterns of activity: eigenvectors and eigenvalues

Similar equation for a network of neurons with arbitrary connectivity

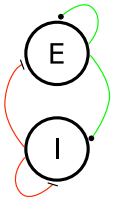
$$\tau \frac{d\mathbf{r}}{dt} = -\mathbf{r} + \mathbf{W}\mathbf{r} + \mathbf{h}$$

$$\mathbf{r} = \begin{pmatrix} r_1 \\ r_2 \\ \vdots \end{pmatrix} \mathbf{W} = \begin{pmatrix} w_{11} & w_{12} & \cdots \\ w_{21} & w_{22} & \cdots \\ \vdots & \vdots & \ddots \end{pmatrix}$$

Eigenvectors of $\mathbf{W}$ are patterns that grow or shrink independently

$$\mathbf{W}\mathbf{e_i} = \lambda_i \mathbf{e_i}$$

Separate excitatory and inhibitory neurons



$$\mathbf{r} = \begin{pmatrix} e \\ i \end{pmatrix}$$

$$\mathbf{W} = \begin{pmatrix} a & -a \\ a & -a \end{pmatrix}$$

Properties of the weight matrix

Eigenvector

$$\begin{pmatrix} a & -a \\ a & -a \end{pmatrix}\begin{pmatrix} 1 \\ 1 \end{pmatrix} = \begin{pmatrix} 0 \\ 0 \end{pmatrix}$$

Generalized eigenvector

$$\begin{pmatrix} a & -a \\ a & -a \end{pmatrix}\begin{pmatrix} 1 \\ -1 \end{pmatrix} = 2a\begin{pmatrix} 1 \\ 1 \end{pmatrix}$$

$$\begin{pmatrix} a & -a \\ a & -a \end{pmatrix}^2\begin{pmatrix} e \\ i \end{pmatrix} = \begin{pmatrix} a & -a \\ a & -a \end{pmatrix}\begin{pmatrix} a(e-i) \\ a(e-i) \end{pmatrix} = \begin{pmatrix} 0 \\ 0 \end{pmatrix}$$

Amplified patterns do not persist - no network time constant

A network of excitatory and inhibitory pairs

For N excitatory/inhibitory pairs: **e** and **i** are now N dimensional vectors and **A** is an NxN matrix representing the pattern of connectivity

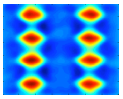
$$\mathbf{W} = \begin{pmatrix} e2e & i2e \\ e2i & i2i \end{pmatrix} = \begin{pmatrix} \mathbf{A} & -\mathbf{A} \\ \mathbf{A} & -\mathbf{A} \end{pmatrix}$$

Patterns that are amplified most are the eigenvectors of the sub-matrix **A** with the largest eigenvalues

$$\begin{pmatrix} \mathbf{A} & -\mathbf{A} \\ \mathbf{A} & -\mathbf{A} \end{pmatrix}\begin{pmatrix} \mathbf{f_i} \\ -\mathbf{f_i} \end{pmatrix} = \begin{pmatrix} 2\mathbf{A}\mathbf{f_i} \\ 2\mathbf{A}\mathbf{f_i} \end{pmatrix} = \begin{pmatrix} 2\lambda_i\mathbf{f_i} \\ 2\lambda_i\mathbf{f_i} \end{pmatrix}$$

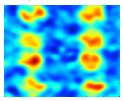
Simulation results

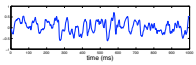
Evoked



⇐ Correlation Coefficient ⇒

Spontaneous





Both the linear and a more realistic biophysical model with spiking neurons display strong patterns

Time constant of the activity is the longer of the input correlation time and the cellular time constant

Conclusion

Spatial patterns can arise without a positive eigenvalue and without a network time constant

Detailed biophysical simulations match other aspects of cortical activity like membrane potential noise and spike statistics

The Grand Goal

Knowledge from protein folding experiments

+

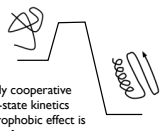
Physics-based theory

↓

Efficient physics-based computer algorithms for protein folding

What We Know

macroscopic cooperativity



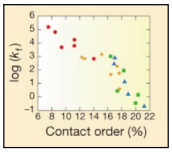
- highly cooperative
- two-state kinetics
- hydrophobic effect is driving force

microscopic cooperativity

- protein folding is greedy: only a small fraction conformational space is searched

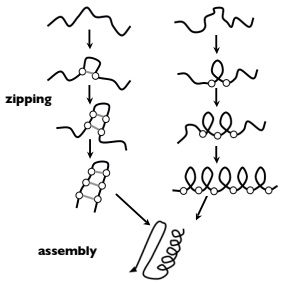
topology-dependent folding rates

- topological "frustration": rate-limiting step is finding the right topology
- loop-closure entropy

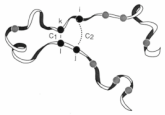


Baker D. *Nature* (2000).

Zipping and Assembly (Z&A)

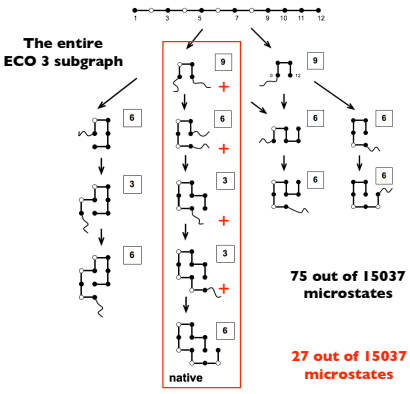


The ZIPSEARCH algorithm searches along topologically local contacts



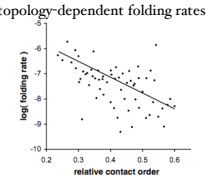
Effective Contact Order (ECO)

- measures topological localness
- is related to the entropy of loop closure

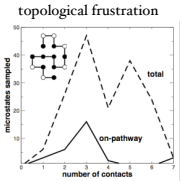


Z&A recapitulates known folding behavior

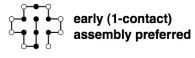
topology-dependent folding rates



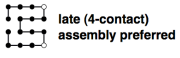
topological frustration



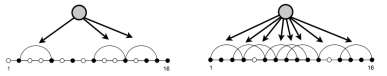
Hierarchical assembly is a result of optimal search efficiency



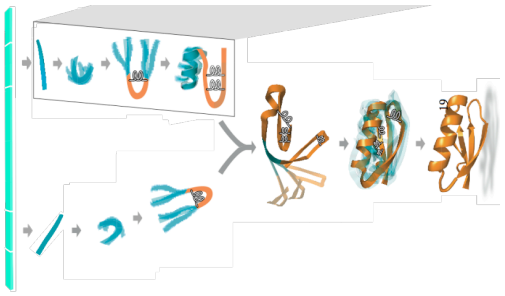
early (1-contact) assembly preferred



late (4-contact) assembly preferred

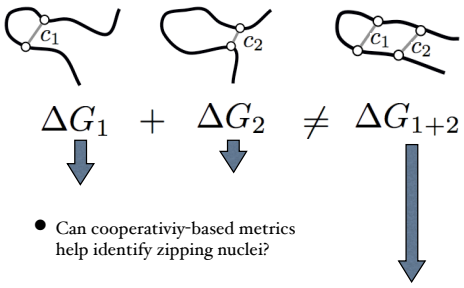


Proof of principle: Can a Z&A strategy be used to fold more realistic all-atom models?



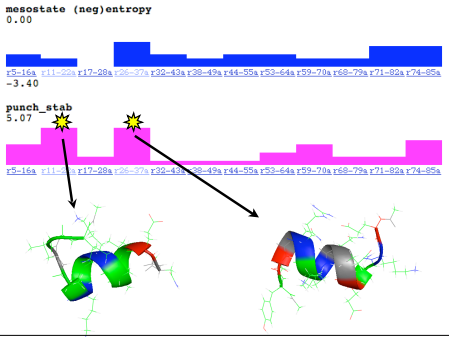
Ozkan et al. *PNAS, in press* (2006)

Cooperativity is useful to quantify

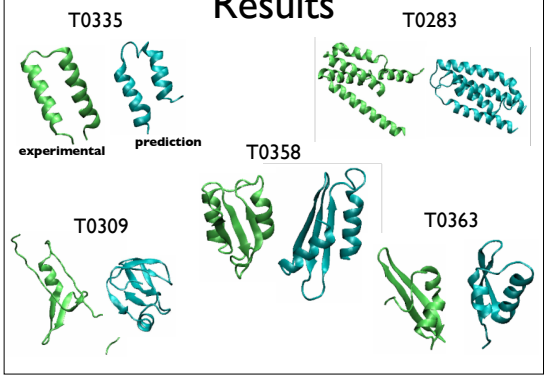


- Can cooperativiy-based metrics help identify zipping nuclei?

T0335 12-mers



Results



Conclusions

- Zipping and Assembly is a viable Folding Principle
  - fast and efficient search strategy
  - explains observed protein folding behavior
- Quantifying cooperativity can be useful for all-atom protein folding

Quincey Justman

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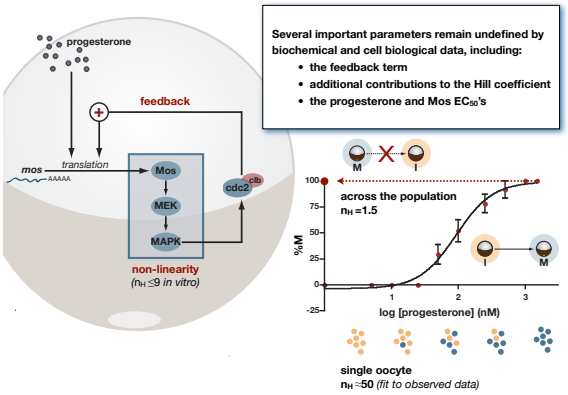
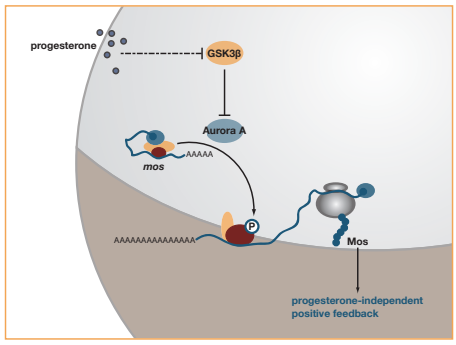
Xenopus oocyte maturation is bistable and irreversible

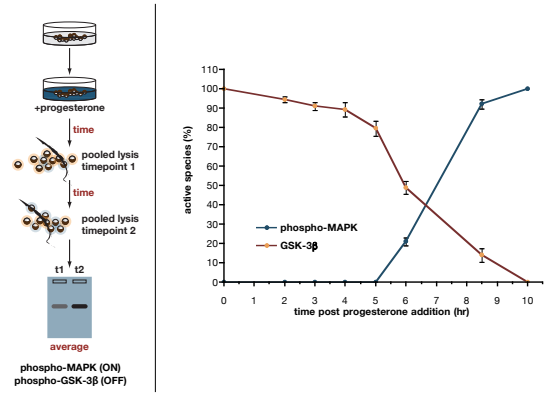


GSK-3β regulates Mos translation

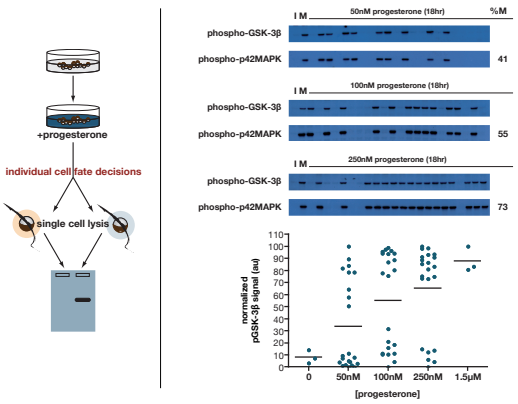


Starkissian *et al.*, Genes Dev 18(1): 48-61.

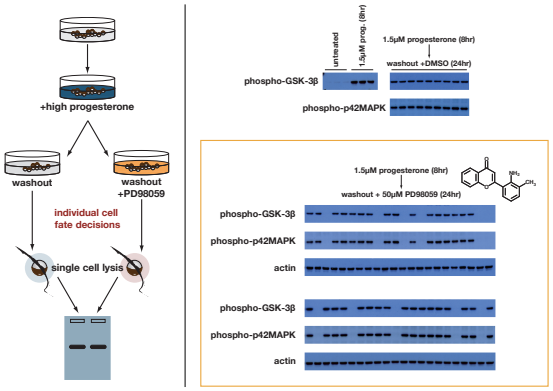
GSK-3β inactivation and MAPK activation are concomitant



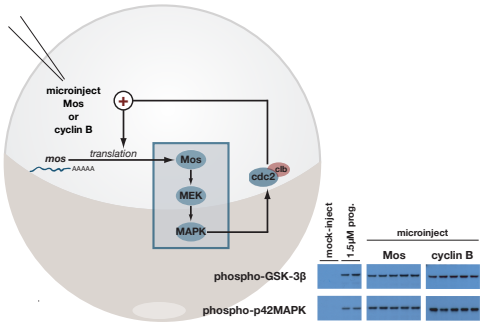
GSK-3β inactivation is non-linear and correlates with cell-fate decision



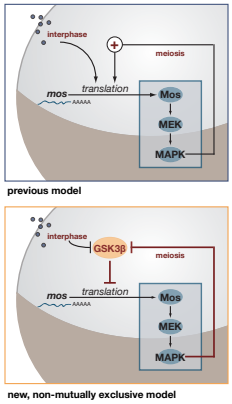
M-phase GSK-3β inactivation is MEK-dependent



Mos or cyclin B is sufficient to induce GSK-3β inactivation



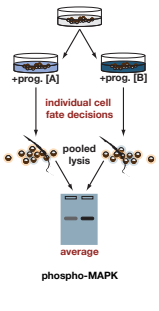
Quincey Justman

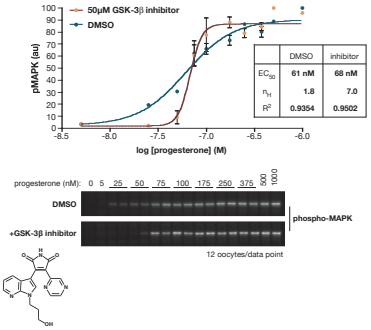


Summary

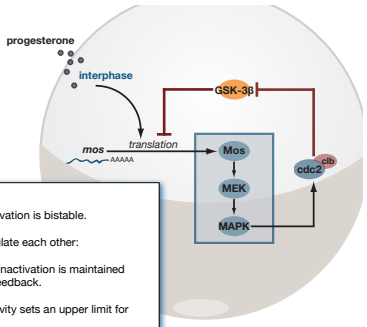
- GSK-3β inactivation correlates with MAPK activation, demonstrating that it is an M-phase event.
- On the single oocyte level, GSK-3β phosphorylation is non-linear with respect to [progesterone]. It is also irreversible.
- The feedback that stabilizes the OFF state of GSK-3β during M-phase is MEK dependent.
- Microinjection of Mos or cyclin B is sufficient to elicit full GSK-3β inactivation.

GSK-3β regulates the M-phase feedback loop.





Conclusions: GSK-3β

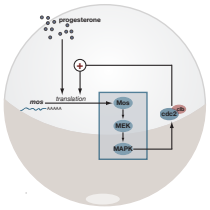


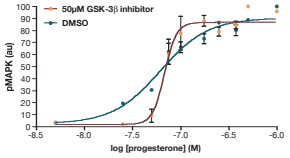
- M-phase GSK-3β inactivation is bistable.
- GSK-3β and MAPK regulate each other:

  In M-phase, GSK-3β inactivation is maintained by MEK-dependent feedback.

  Residual GSK-3β activity sets an upper limit for MAPK's non-linearity.
- GSK-3β regulates oocyte-to-oocyte variability.

A general approach to uncover non-essential but biologically relevant functions and relationships

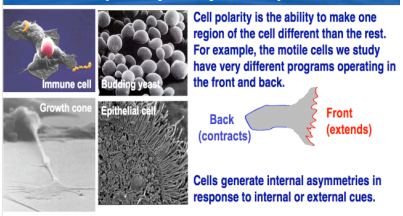




	perturbation/target	phenotype	conclusions
genetics	knockdown/out library crosses	changes in growth rate	epistasis, identification of unknown functions
chemical genetics	"drug-like" kinase inhibitors (with cognate *in vivo* targets) inhibitor combinations	changes in a quantifyable, system-level property	quantified contribution of target kinase to that property chemical"epistasis" (network topology)

Orion Weiner

What is cell polarity? Why is it important?



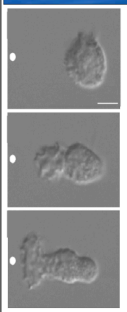
Cell polarity is the ability to make one region of the cell different than the rest. For example, the motile cells we study have very different programs operating in the front and back.

Cells generate internal asymmetries in response to internal or external cues.

Directed polarity is essential for single-celled organisms to hunt and mate, axons to find their way in the developing nervous system, and cells in the innate immune system to find and kill invading pathogens.

Linked positive and negative feedback loops have been suggested to represent core elements of cell polarity in many systems such as yeast budding and eukaryotic chemotaxis. Although we can capture elements of polarity in these descriptions, there is still an incomplete understanding of how polarity is generated and signaling is linked to morphological change.

Our model system: human neutrophils



These cells from your immune system hunt and kill bacteria

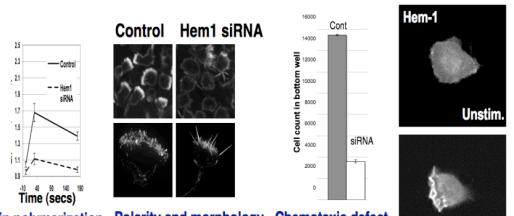
They will orient and migrate towards a single bacterium

So-- one cell, no brain, but is able to spatially interpret its surroundings-- how?

We helped to uncover linked positive and negative feedback circuits used in a self-organizing system for polarity

Hem-1, an essential component of cell polarity

RNAi knockdown of a Hem-1 (component of leukocyte WAVE2 complex and other polarity complexes) gives profound cytoskeletal, polarity, and signaling defects in neutrophils.



Actin polymerization defect

Polarity and morphology defect

Chemotaxis defect

Front localization

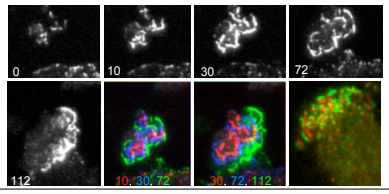
In collaboration w/Maike Rentel & Henry Bourne (UCSF)

We recently discovered a self-organizing pattern formation system that generates polarity in motile cells.

In previous work we identified a set of protein complexes that are essential for organizing cell polarity. We now find that these complexes generate multiple propagating waves of actin polymerization that collectively organize the front of migrating cells. Similar to action potentials, these polarity waves are self-renewing and generate their own inhibitors to produce directional movement.

This simple wave-generating circuit could account for several previously inexplicable behaviors of motile cells including coordinated behavior of the leading edge, cells that flow around boundaries, and dynamic polarity. Waves represent a new framework for understanding cell movement.

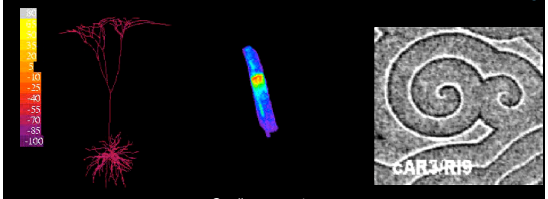
We are developing tools to test how the waves are born, how they move and die, and how they talk to one another in our quest to understand the basic building blocks of cell motility.



Biological waves have similar properties

Intracellular ion waves

Multicellular patterning



Action potential
Destexhe lab

Cardiac myocyte calcium waves
Ernst Niggli lab

Dictyostelium spirals
Cornelis Weijer lab

What mechanistic insights can we gain from other biological waves?

1. Waves move activity, not individual molecules
2. Similar wiring
3. Refractory period, destructive interference

General wave: Activator (delayed) Inhibitor

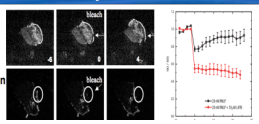
Action potential: Na channels open; Depolarization (delayed) Potassium channels open

Hem-1 wave: Hem-1 (delayed) Actin polymerization

Hem-1 waves move activity, not individual molecules

Control photobleach

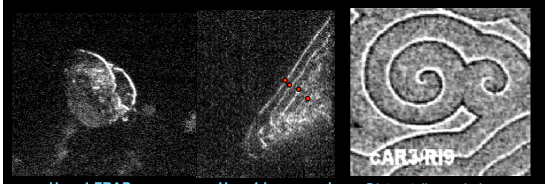
Depolymerize actin photobleach



Recruited Hem-1 rapidly exchanges with the cytosolic pool in an actin polymer-dependent fashion

Evidence that Hem-1 waves are similar to other biological waves

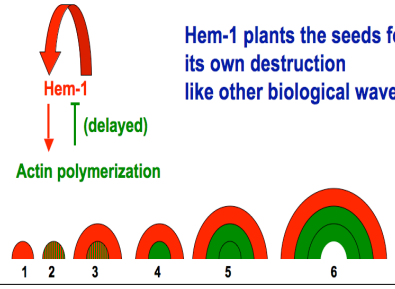
1. Activity, not protein, propagates
2. Refractory period between waves



Hem-1 FRAP | Hem-1 kymograph | *Dictyostelium* spiral waves

3. One of outputs of system (actin polymers) inhibits the activator (removes Hem-1 from membranes)

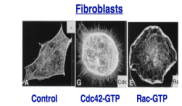
Hem-1 wave generator working model

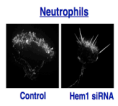


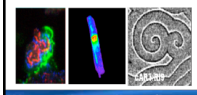
Hem-1 plants the seeds for its own destruction like other biological waves.

Waves could explain cell shape and movement

Hem-1 waves could explain characteristic morphology of motile cells and Rac-GTP



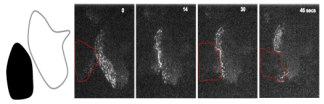




Motility is a direct consequence of waves

Allows positive feedback without locking

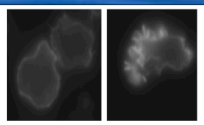
Waves allow cells to flow around boundaries



Reciprocal interaction between signaling and the cytoskeleton is essential for this behavior.

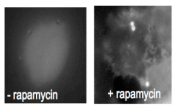
Future Directions

Identify key missing components
Using permeabilized cell system and biochemical reconstitutions

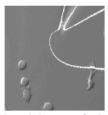


Polarity in Permeabilized cells

Spatially manipulating activators and inhibitors to determine spatial logic of polarity.



Chemically-controlled recruitment
Nanolithography (Desai lab)



Local drug perfusion
Microfluidics