Opportunities for Theory in Biological Physics.

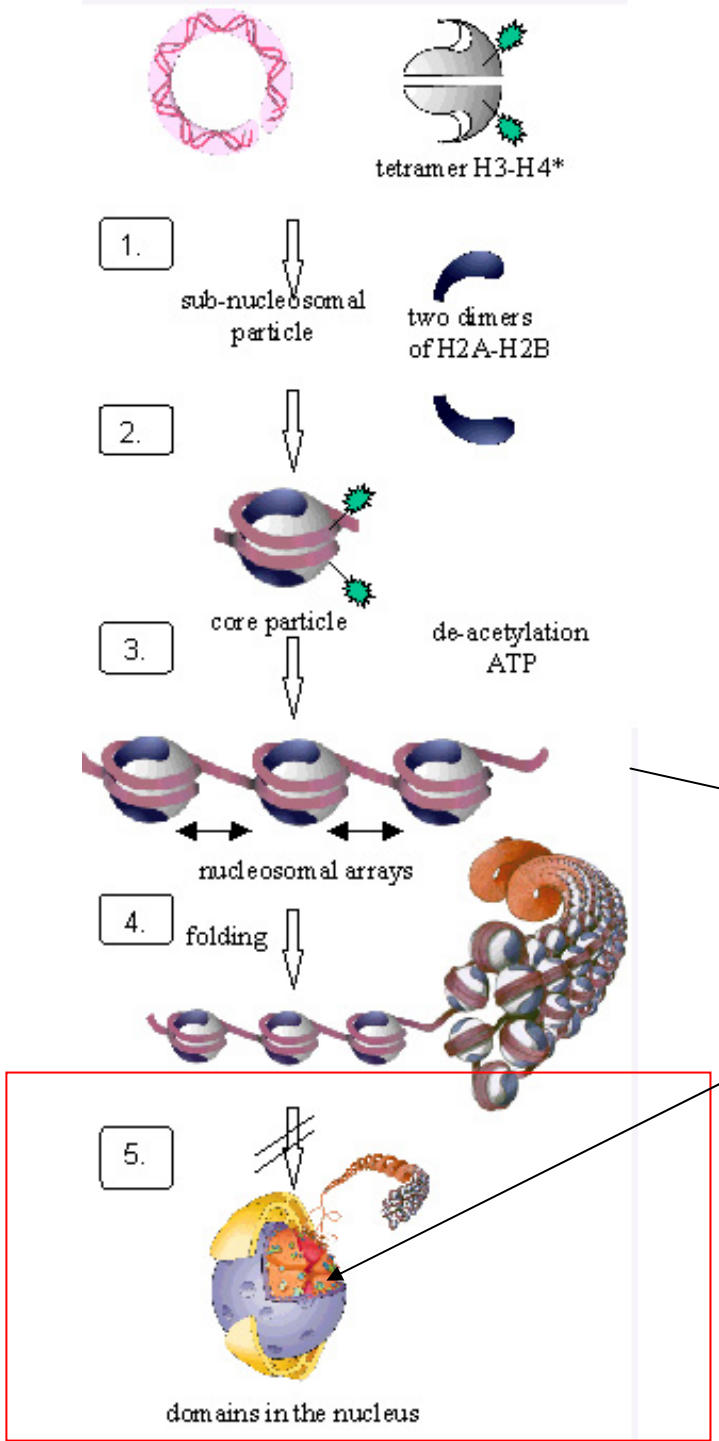
1) Chromosome Control.

2) The Polyglutamine Problem.

3) Transcription Initiation Complex.

4) Ribosomal Proofreading.

5) Focal Adhesion Sites.



*DNA/DNA interaction:

Aqueous electrostatics beyond mean-field theory. (Oosawa)

*DNA/nucleosome interaction: electrostatic attraction versus bending stiffness. (Manning)

*Micromechanics (M.Wang)

Nucleus: 23 chromosomes (1m DNA in micron-sized nucleus)

Gene regulation by compaction.

“Chromosome painting”:

3D-FISH

Statics:
3-D Reconstruction of Nucleus.

DNA-DNA mean spacing:
30-40 Angstrom.
Close-packing is close

(Cremer)

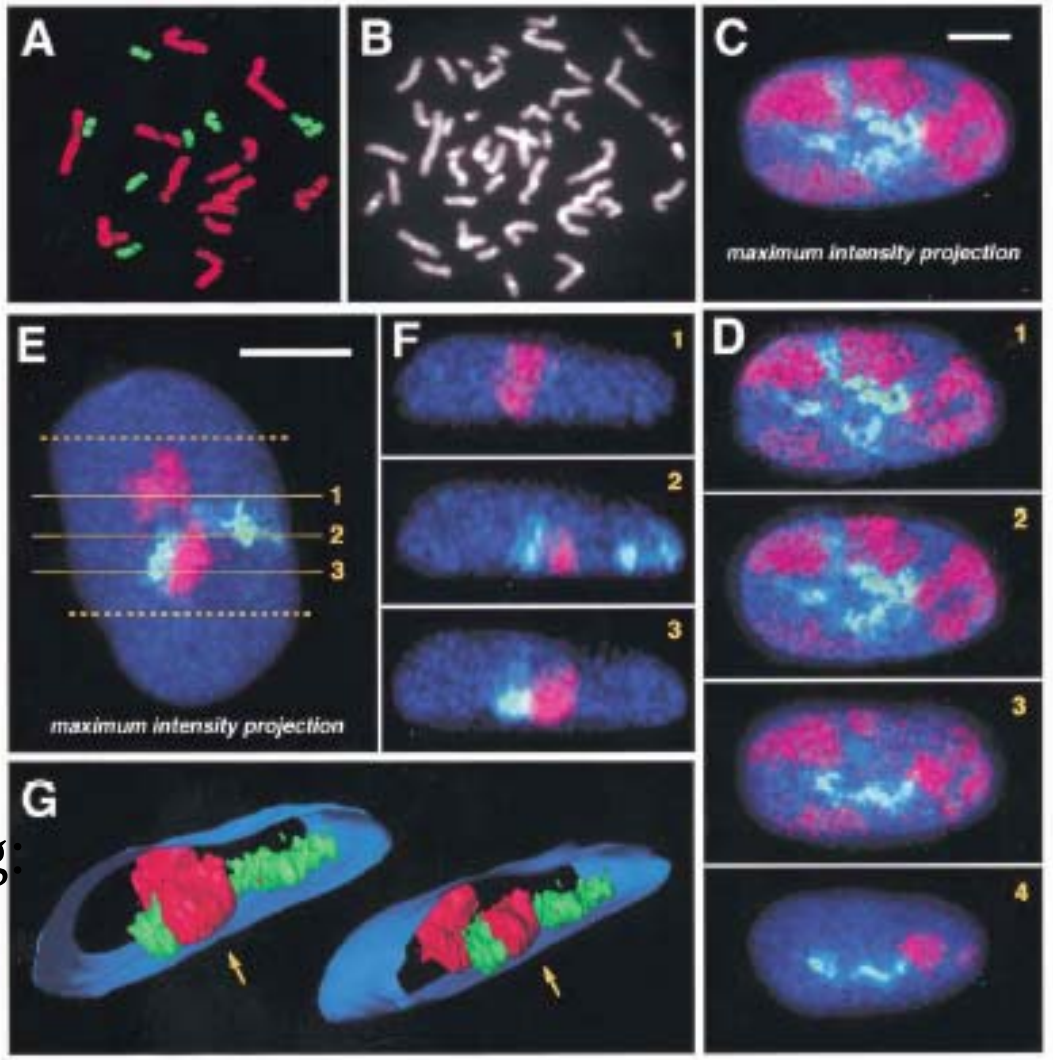
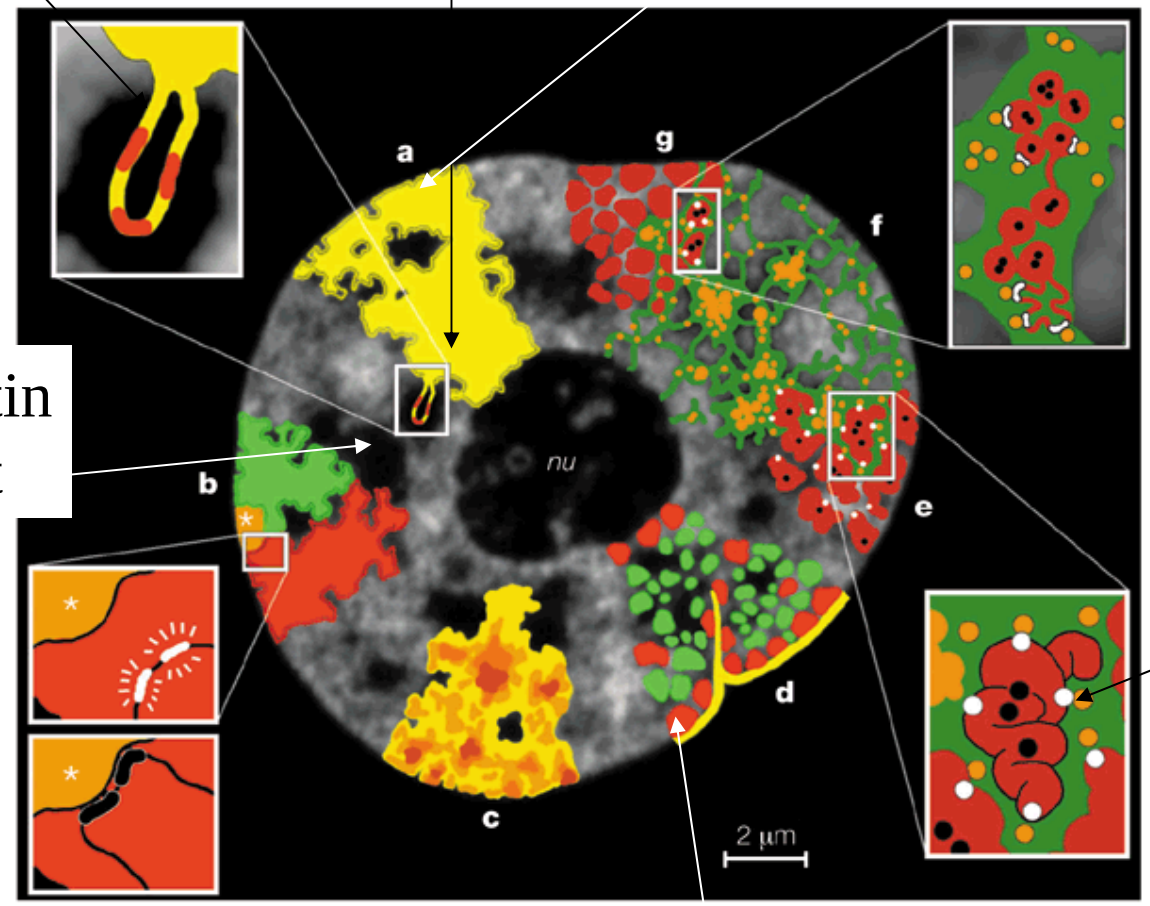


Figure 6. Distribution of small and large CTs in the flat nuclei of primary human fibroblasts. **(A)** FISH on metaphase spread with two probe pools for the large chromosomes (1–5 and X, red) and for the small chromosomes (17–20, green). **(B)** The same metaphase after DAPI staining. **(C)** Maximum intensity projection of a series of confocal sections through a fibroblast nucleus after 3D-FISH with the same two probe pools as shown on A. The nucleus shows the typical distribution pattern of CTs: large CTs (red) occupy peripheral positions, while small CTs (green) are situated more centrally. Counterstaining (PI) is shown in blue. **(D)** Four optical sections, out of a total of 16, from the bottom (section #1) to the top (section #4) of the same nucleus as shown on **(C)**. The distance between optical sections is 0.75 μm. Fibroblasts are strongly flattened – only 3–4 μm thick in the central part – and the majority of painted chromosome territories extend from the bottom to the top of the nucleus. Therefore, projection sufficiently represents the distribution of chromosomes in the whole nucleus (compare the projection on **C** and individual sections on **D**). **(E–G)** Spatial arrangement of CTs

Expanded Chromosome Loop (active genes)

Condensed inactive genes

Decondensed, active genes

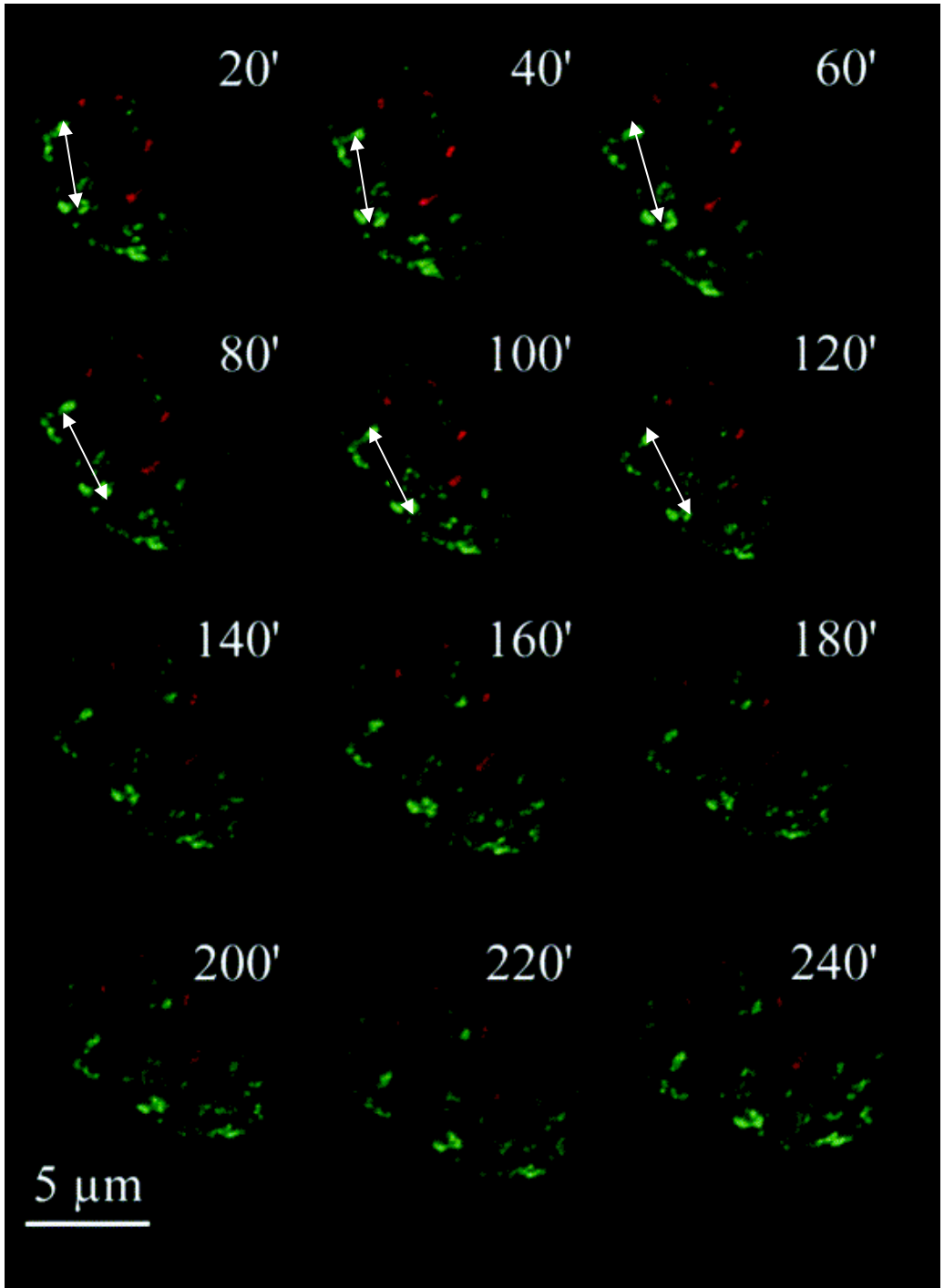


Inter-chromatin Compartment

Active gene: on surface.

Late replicating gene

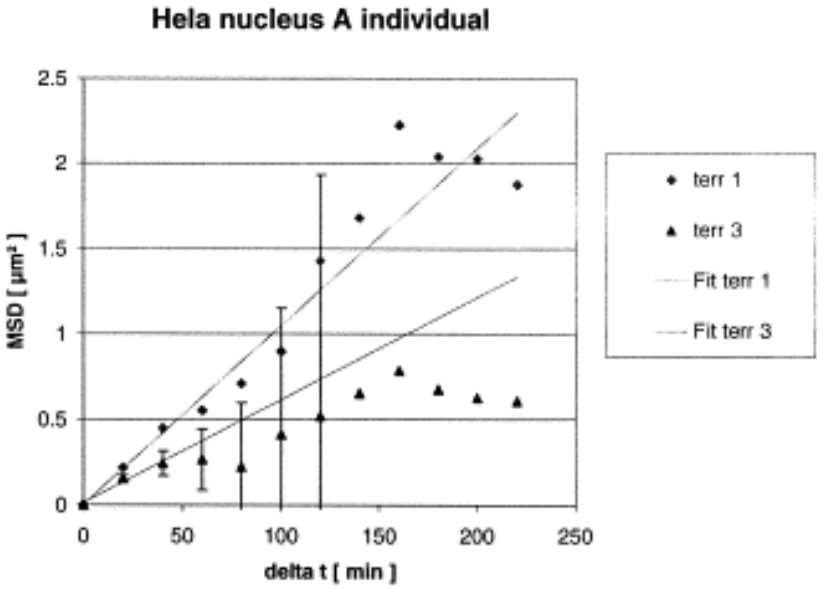
Nucleus is fully accessible to protein transport.

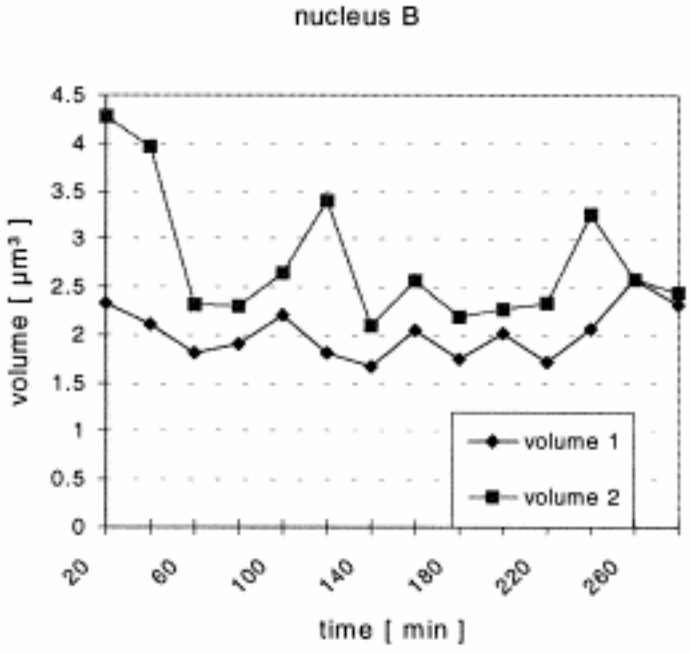


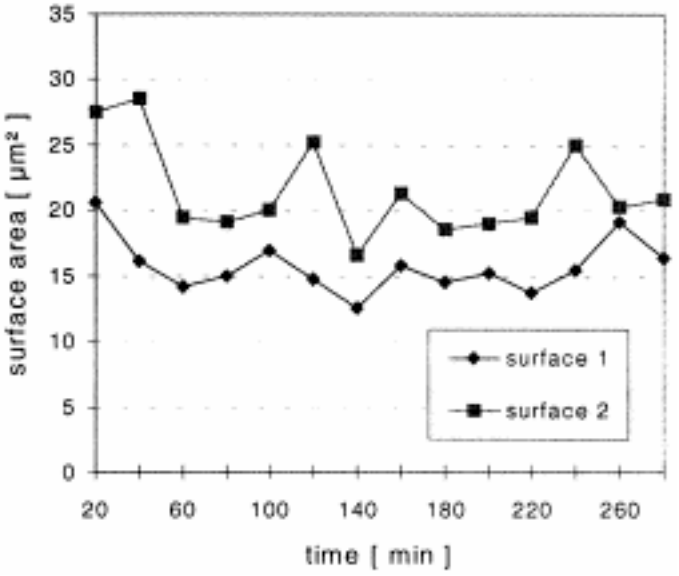
3-D Fish:
Chromosome Dynamics

(20 minute intervals)

Chromosomal “Diffusion”







Chromosomal Volume and Surface Area vs time.

Statics:

How is the “open” architecture of the nucleus maintained and controlled under the osmotic pressure of de-condensed, active DNA sections.
Equation of State of DNA bundles is known.

Dynamics:

Chromosome dynamics driven by DNA condensation/de-condensation events triggered by local gene expression:”gene noise”.

*Can we deduce temporal and spatial correlation functions for gene noise from the motion of the chromosomes by fluctuation analysis and relate it to gene activity?

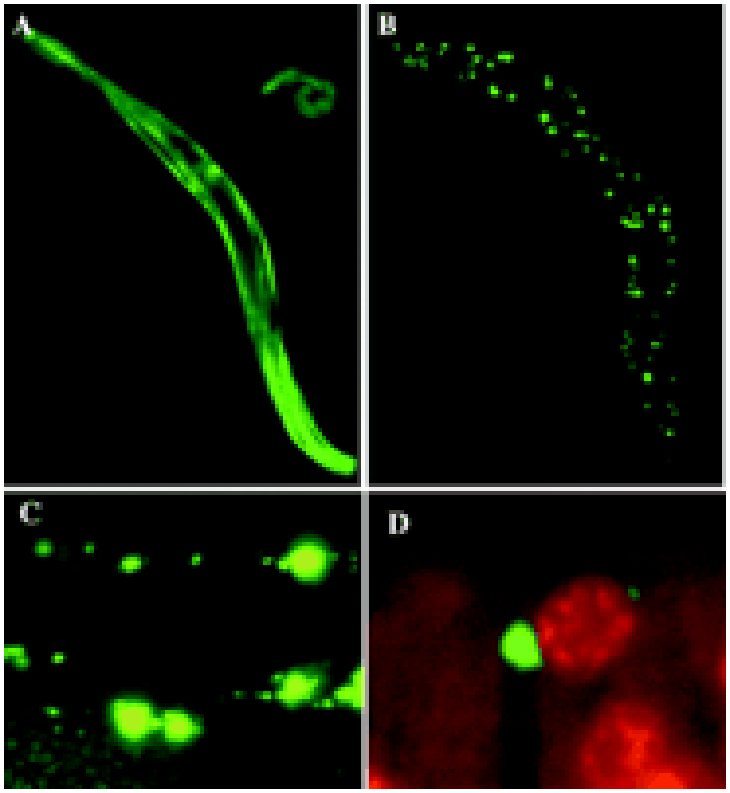
*Chromosome “micro-rheology”?

The Polyglutamine Problem

Nine neuro-degenerative diseases are associated with $(CAG)_N$ triplet repeats: Huntingdon's, spinal dystrophy, ataxia ….
CAG is the code for the amino-acid *glutamine*.

N=19
Homogeneous

N=82 (x 40)
.



C. Elegans worm
GFP $(CAG)_N$

N=82:

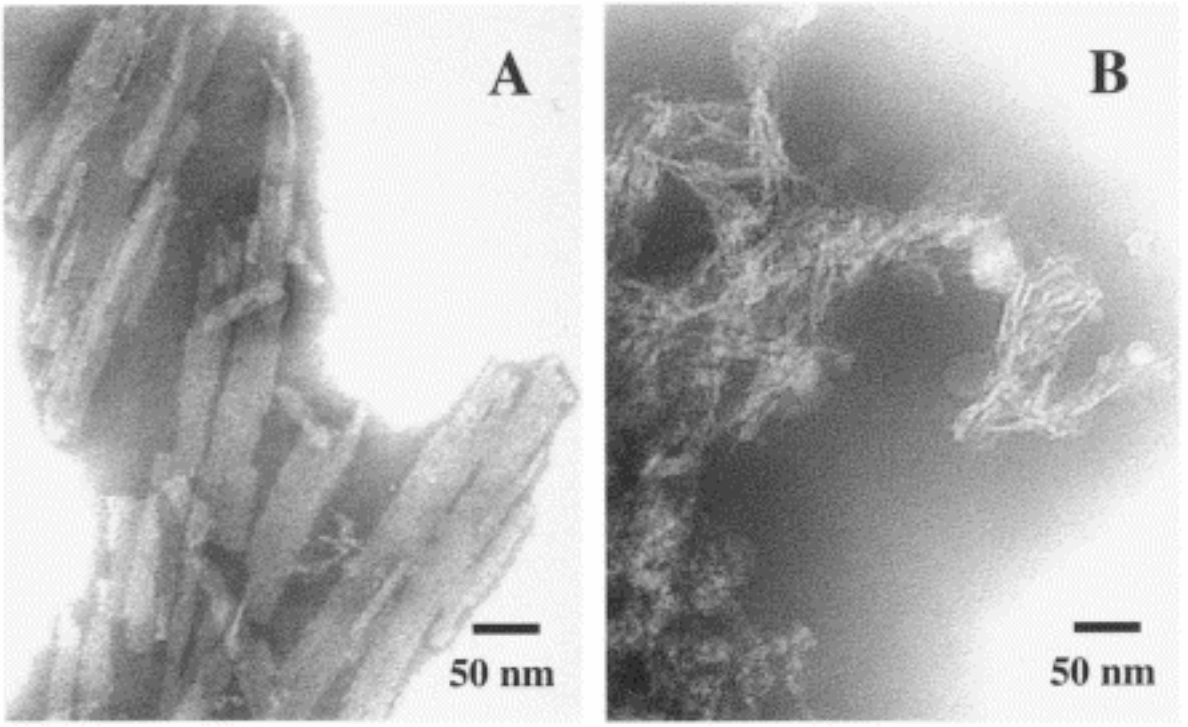
Toxic Aggregates

Impaired motility

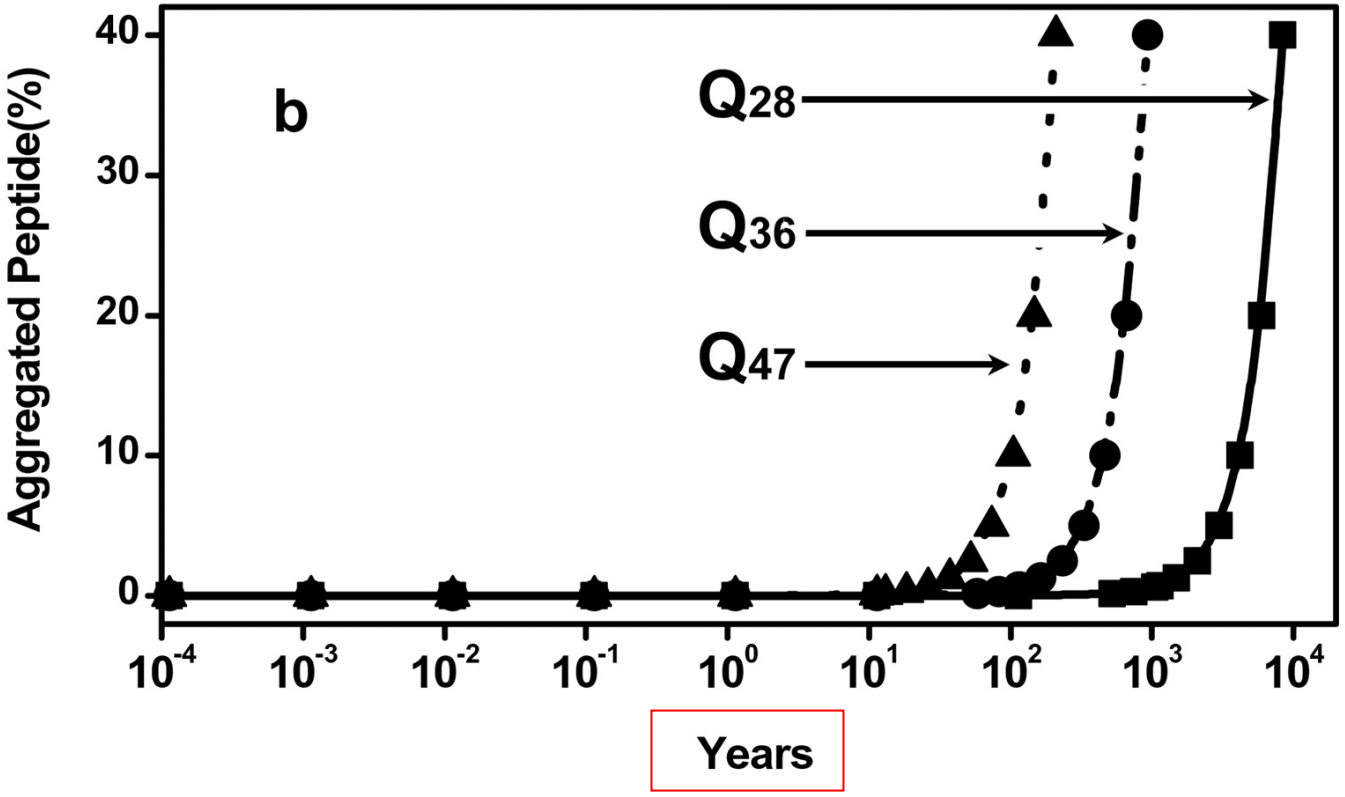
Proteosome action inhibited.

Aggregates: N > 35-40

In vitro polyglutamine homopolymer aggregation (N=37)

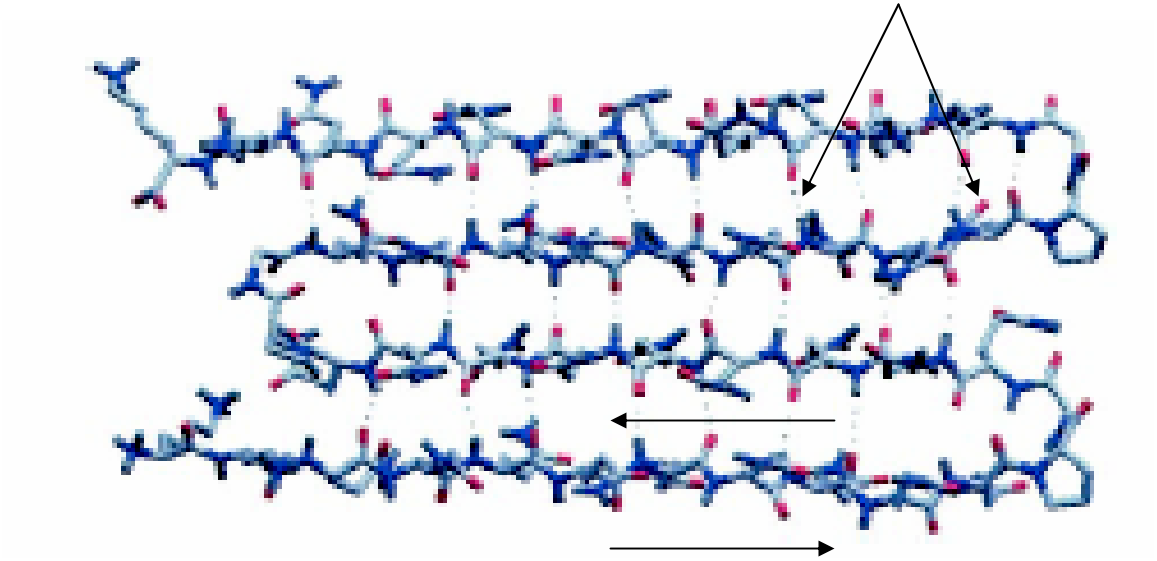


Aggregation Kinetics (Wetzel):



Chen, Songming et al. (2002) Proc. Natl. Acad. Sci. USA 99, 11884-11889

Hydrogen bonds

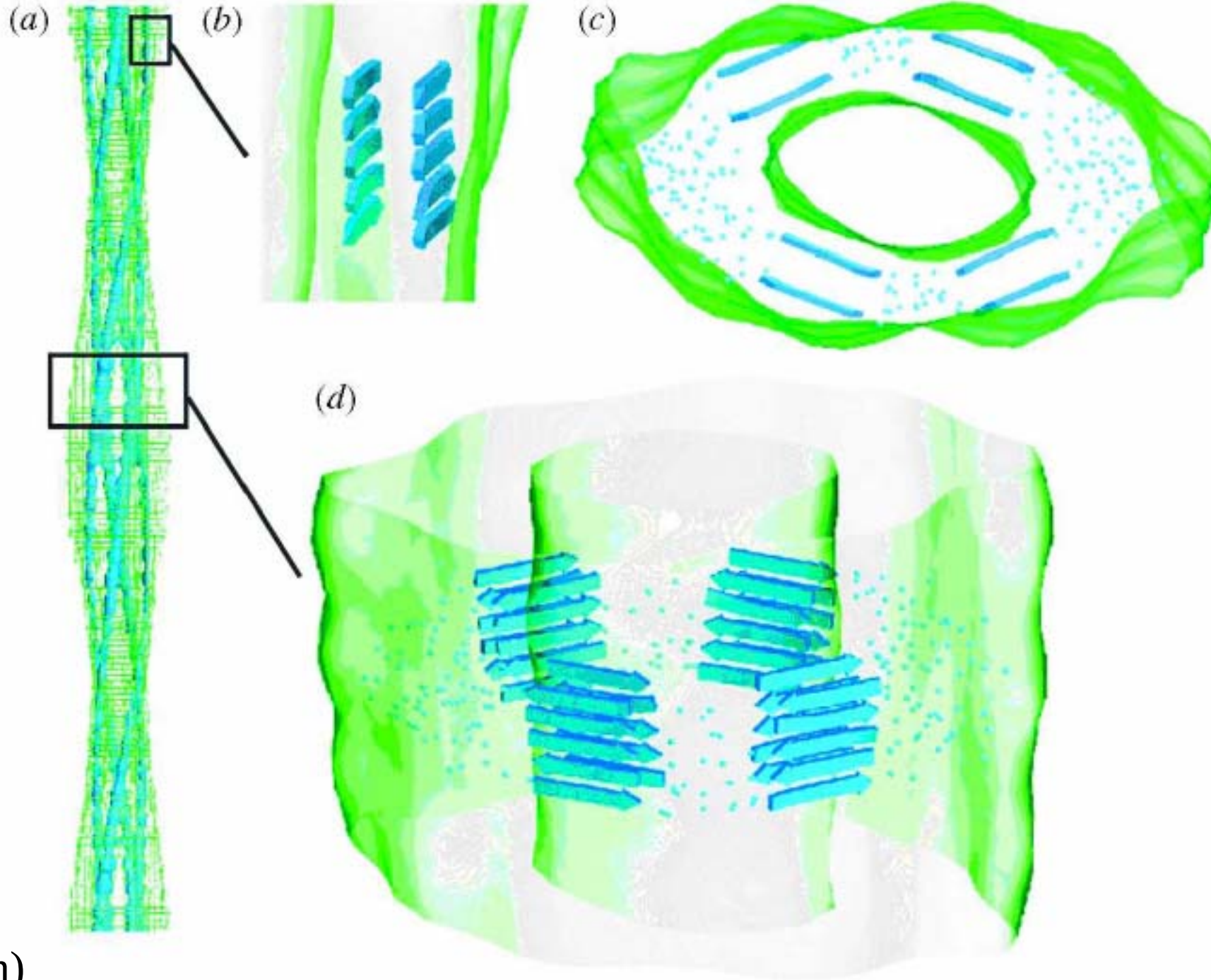


“ Zipper”: Anti-parallel beta sheets.

Not specific for glutamine

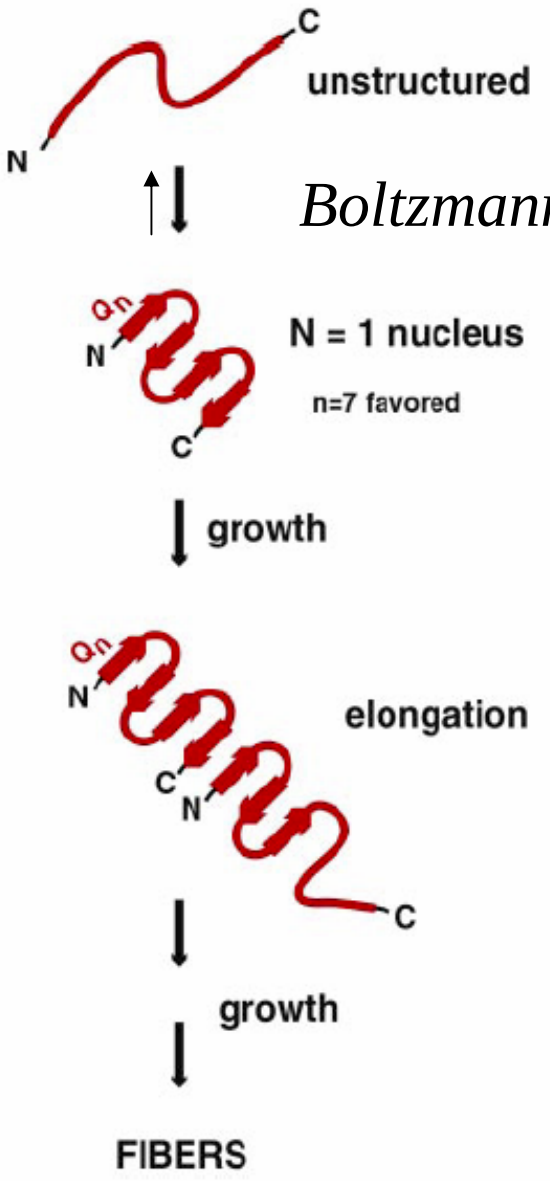
“Polymers physics” of alpha-helix forming homopolymers is well understood (Bruno Zimm). Ising model.

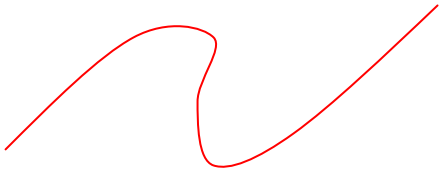
Beta-sheet homopolymers: first-order phase transition (Finkelstein)



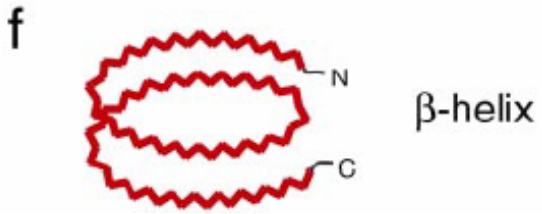
(Dobson)

sheet nuclei: can “infect” unstructured peptide sequences.

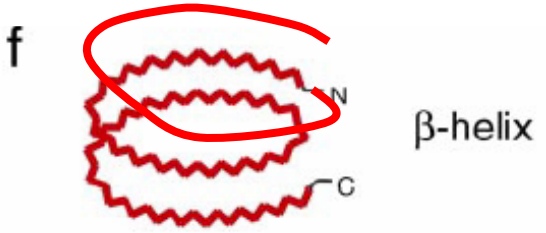




unstructured



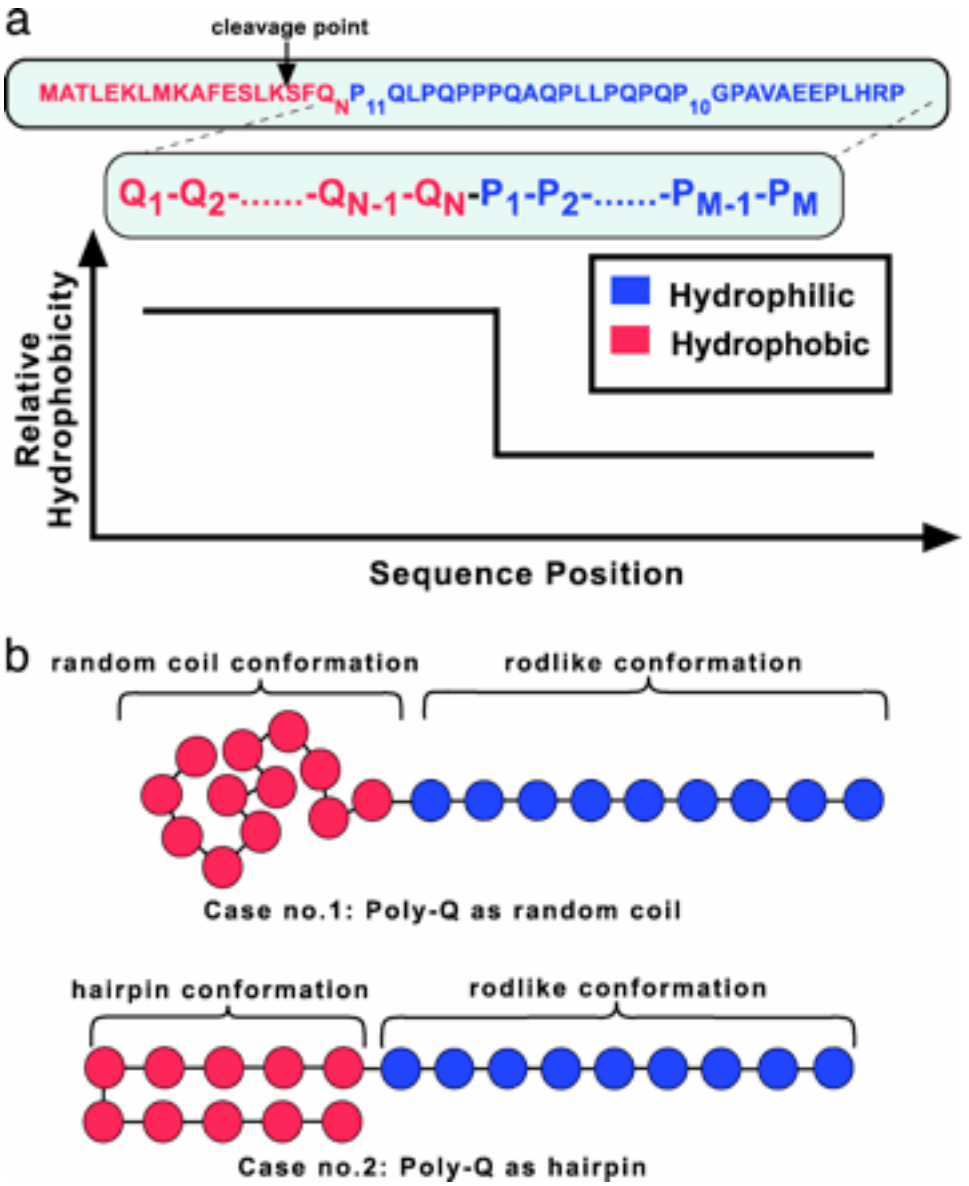
Critical nucleus



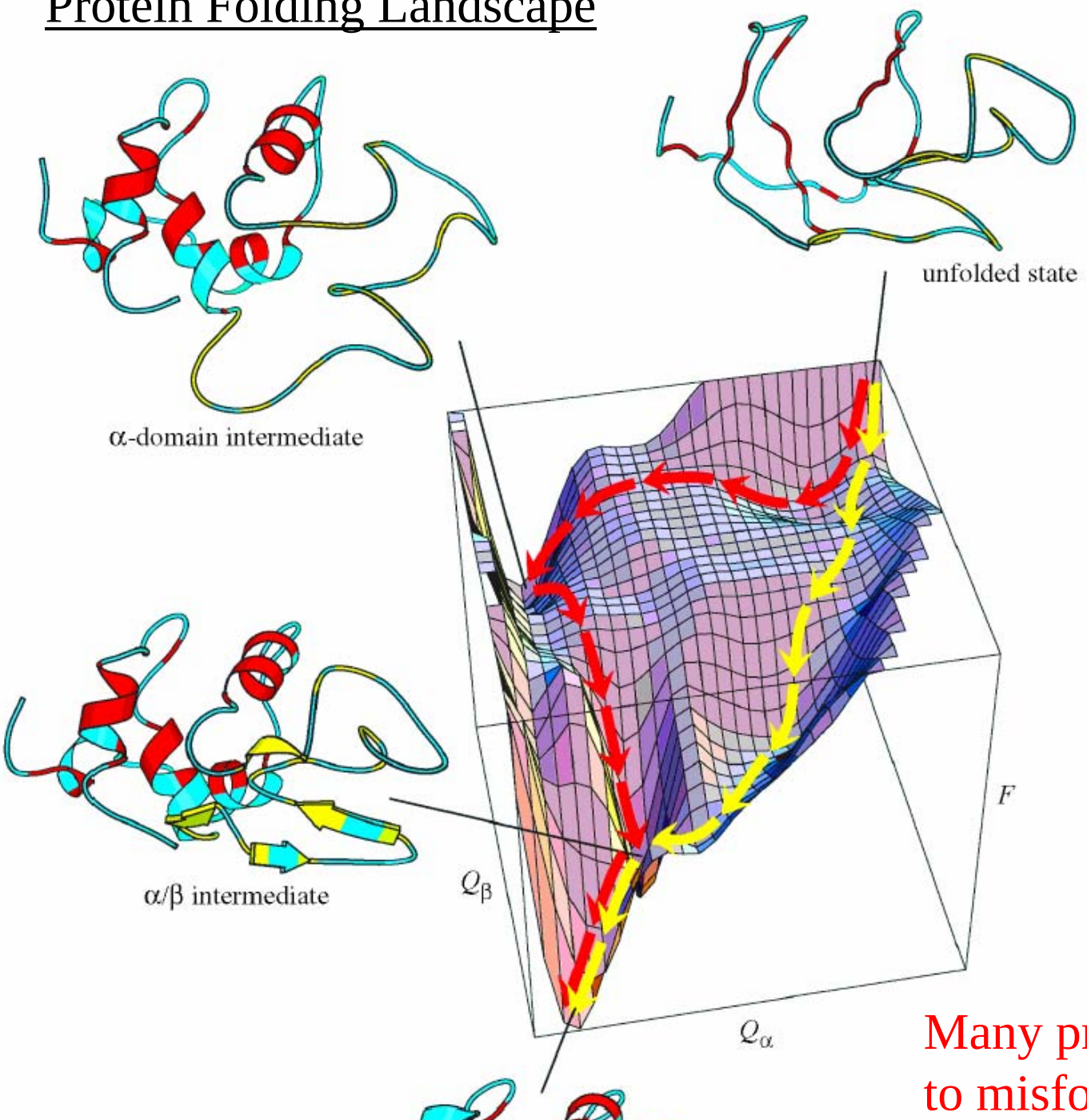
elongation

but….

Huntingtin exon 1 *actually* produces a PolyQ/PolyP block copolymer!

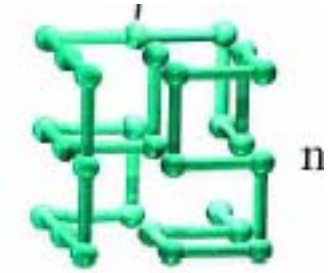


Protein Folding Landscape

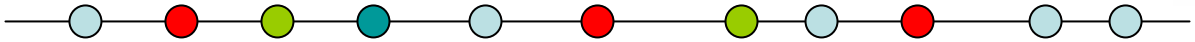


Many proteins can be made to misfold into beta-sheet

Gõ model.



i=1, 2, …. N



$M_{i\ j}$ (*non specific*) *

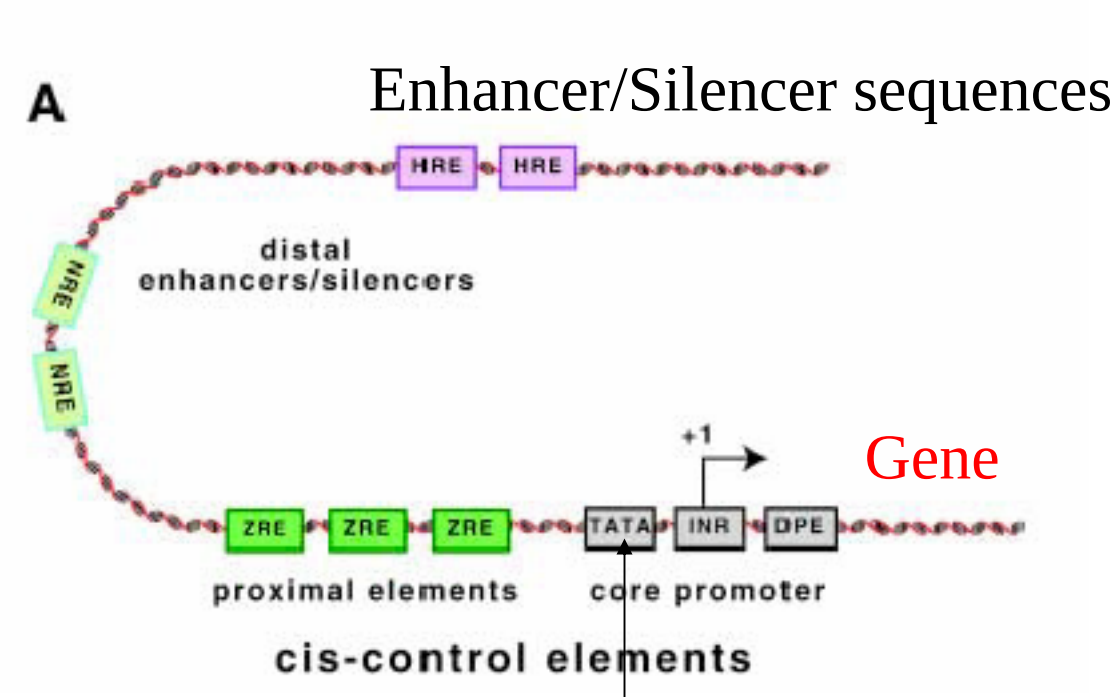
.	0	1	0
0	.	0	1
1	0	.	1
0	1	1	.

*Monte-Carlo.

- Beta-sheet: off-diagonal entries.
- Competing energy minimum versus folding pathway

Transcription Inititiation Complex

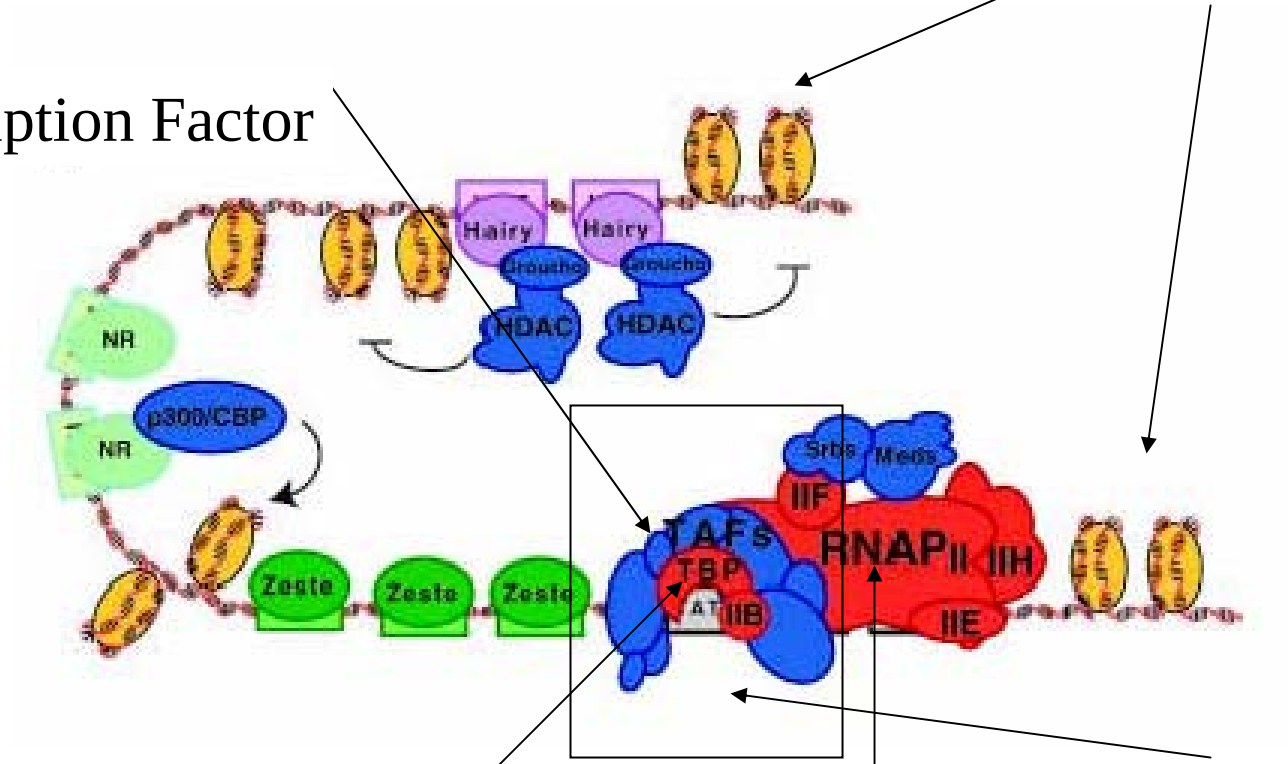
1) Eukaryotic Transcription Complex: “Structural Calculator”



Silencers/enhanhers modulate gene expression.

Nucleosomes

Transcription Factor



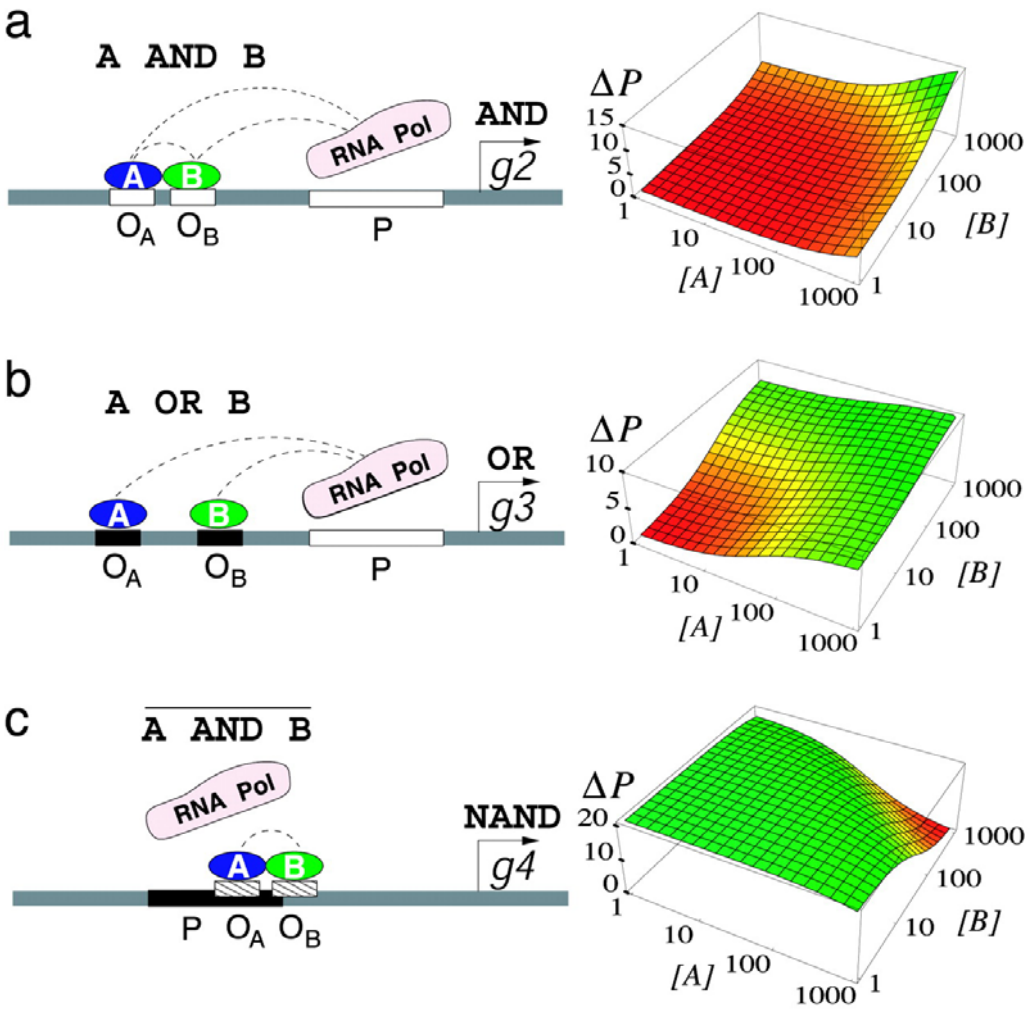
Basal Complex

RNA Polymerase

TATA box binding protein

Universal Molecular Computer

“Boolean logic” (T.Hwa)



Complex controls statistically the rate of gene expression by altering the RNA Polymerase binding energy.

RNA Polymerase

*How do large protein complexes “grow” and form well-defined, unique structures ?

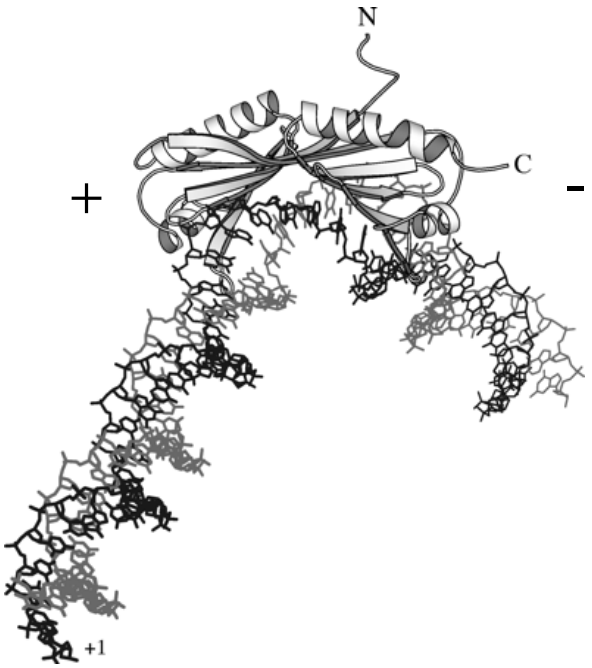
*How is the “signal” communicated from silencer/enhancer to the RNA Pol binding site? (super-allostery?)

*Is the DNA bending stress relevant? (Austin)

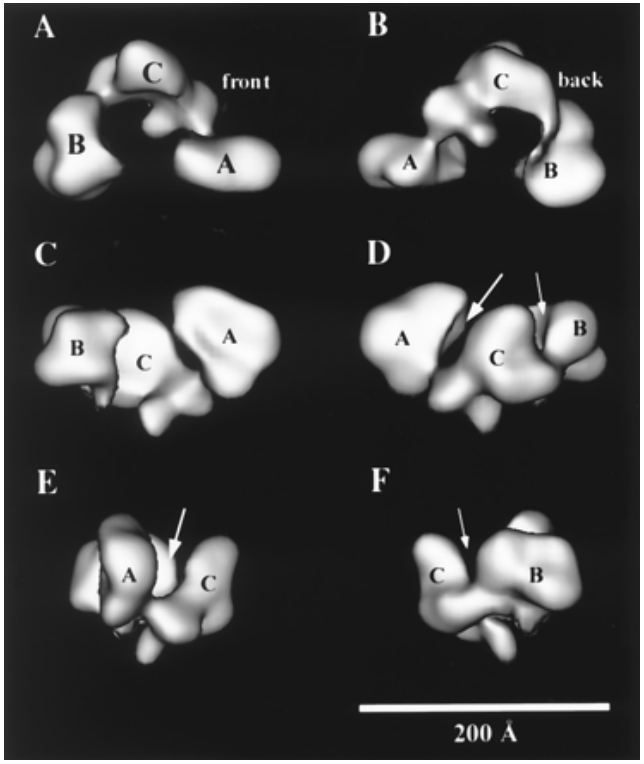
Thermal fluctuations play a key role:

Basal Complex

TATA box binding protein:
near-symmetric dimer



Electron Micrograph
(TFIIA, TFIIB)



TATA box:
Thermal sliding fluctuations.
Thermal orientational fluctuations.

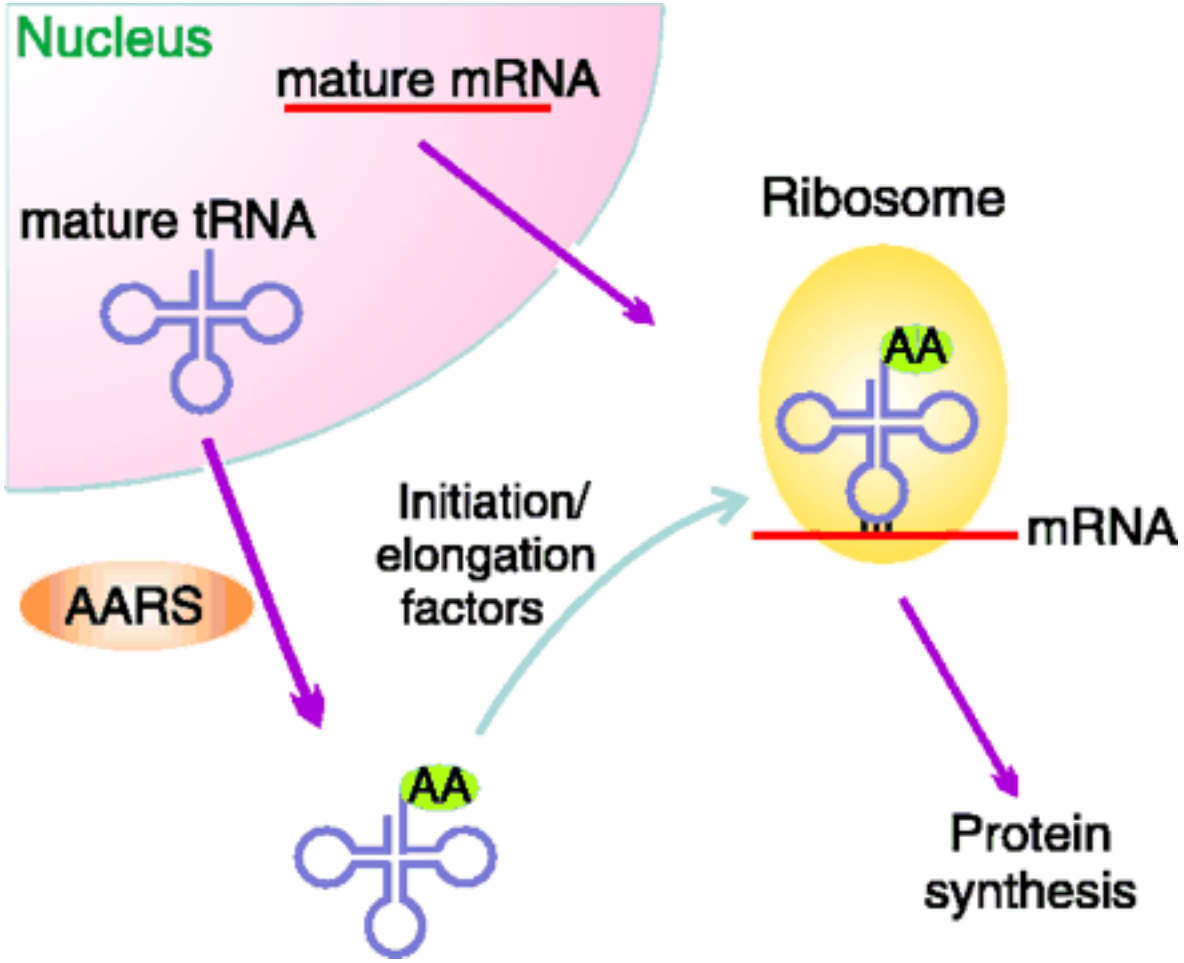
Disaster ?? No, apparently
F of order few kT

QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

Positional and orientational order:
improve when TFIIA&B are added.
Statistical building scheme?

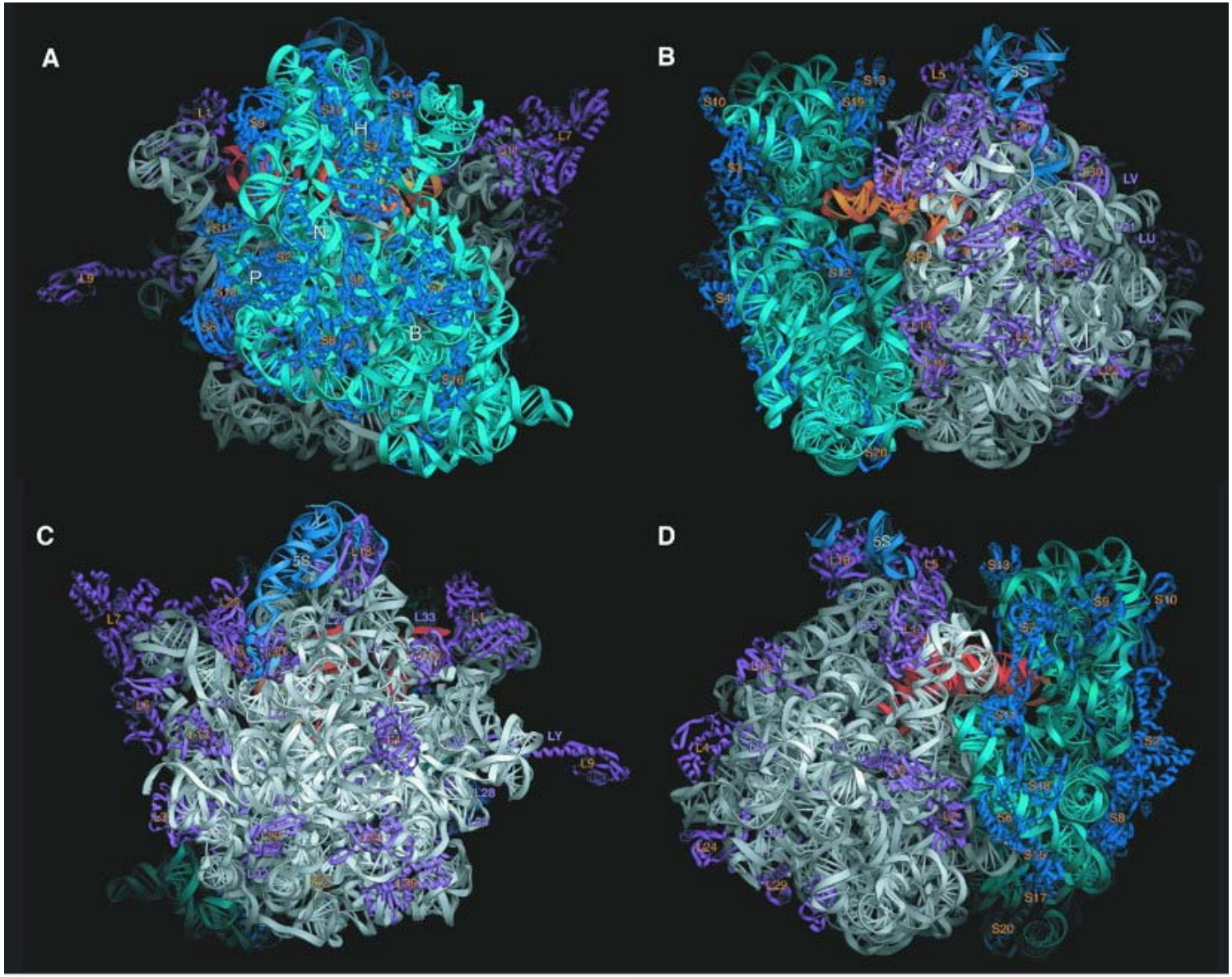
Crystal Structure of:
TFIIA, TFIIB, TBP
complex known.

Ribosomes.



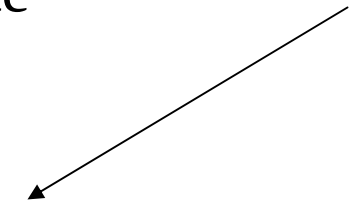
Ribosome must match right Amino Acid (20) to given RNA codon.

Ribosome crystal structure (2.4 Angstrom):



Thermodynamic error rate

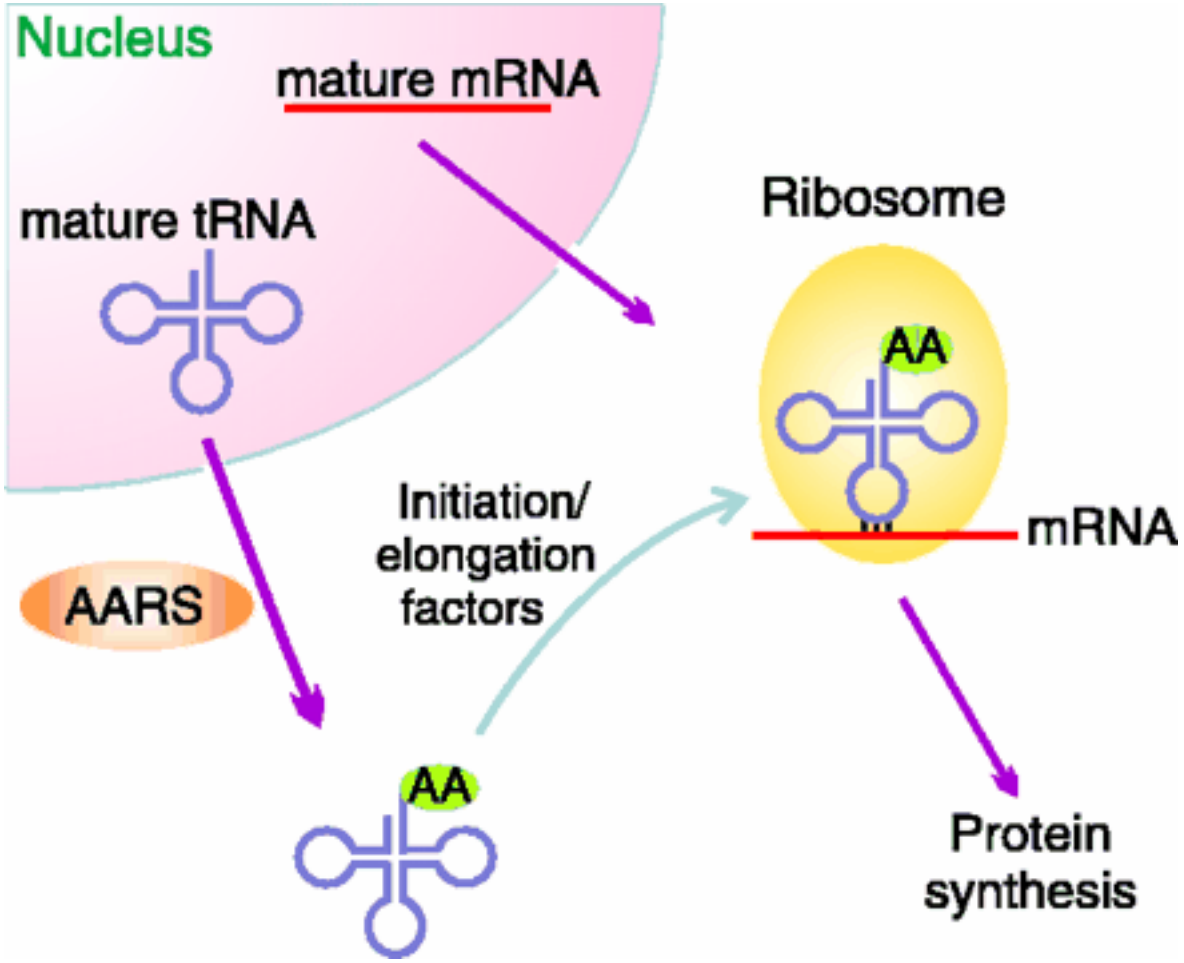
Chemical Thermodynamics.



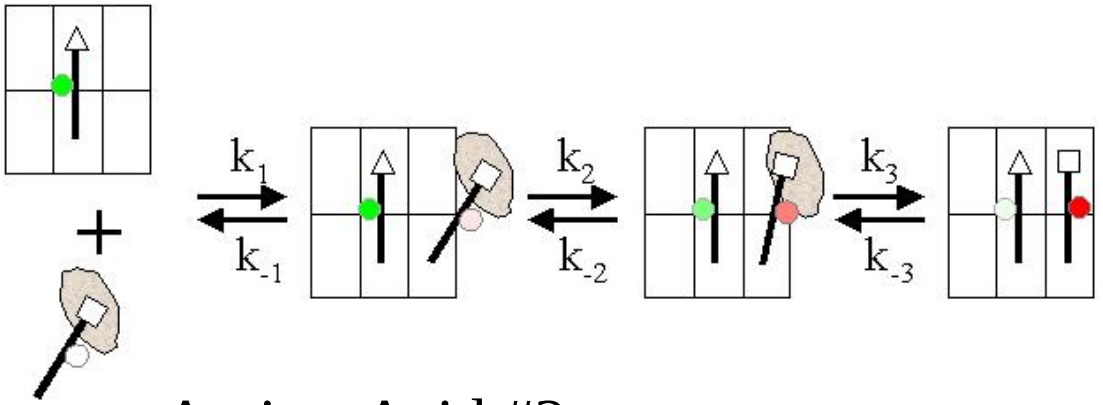
$$P(wrong) \quad \exp\left(\quad F_{right/wrong} / kT \right)$$

Thermodynamics error rate for insertion of wrong amino-acids is much too high!

Attach fluorescent donors and acceptors to amino acids, ribosome. (S.Chu)



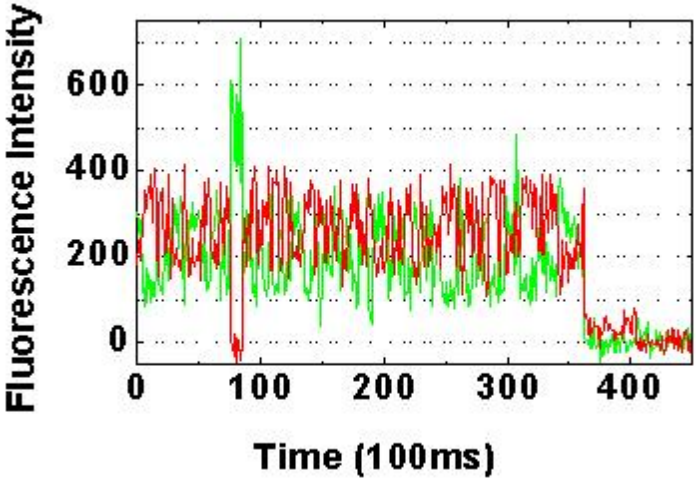
Donor: Amino-Acid#1

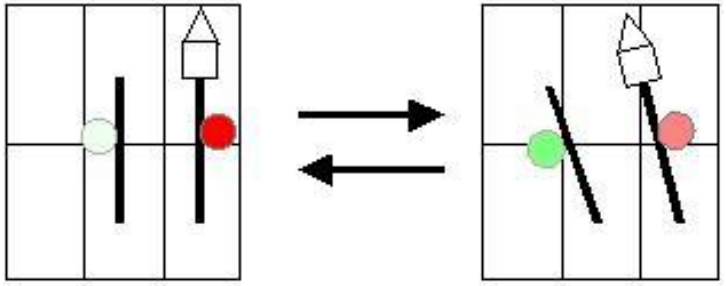


Acceptor: Amino-Acid #2

Hydrolysis : TU/GTP

Time record:

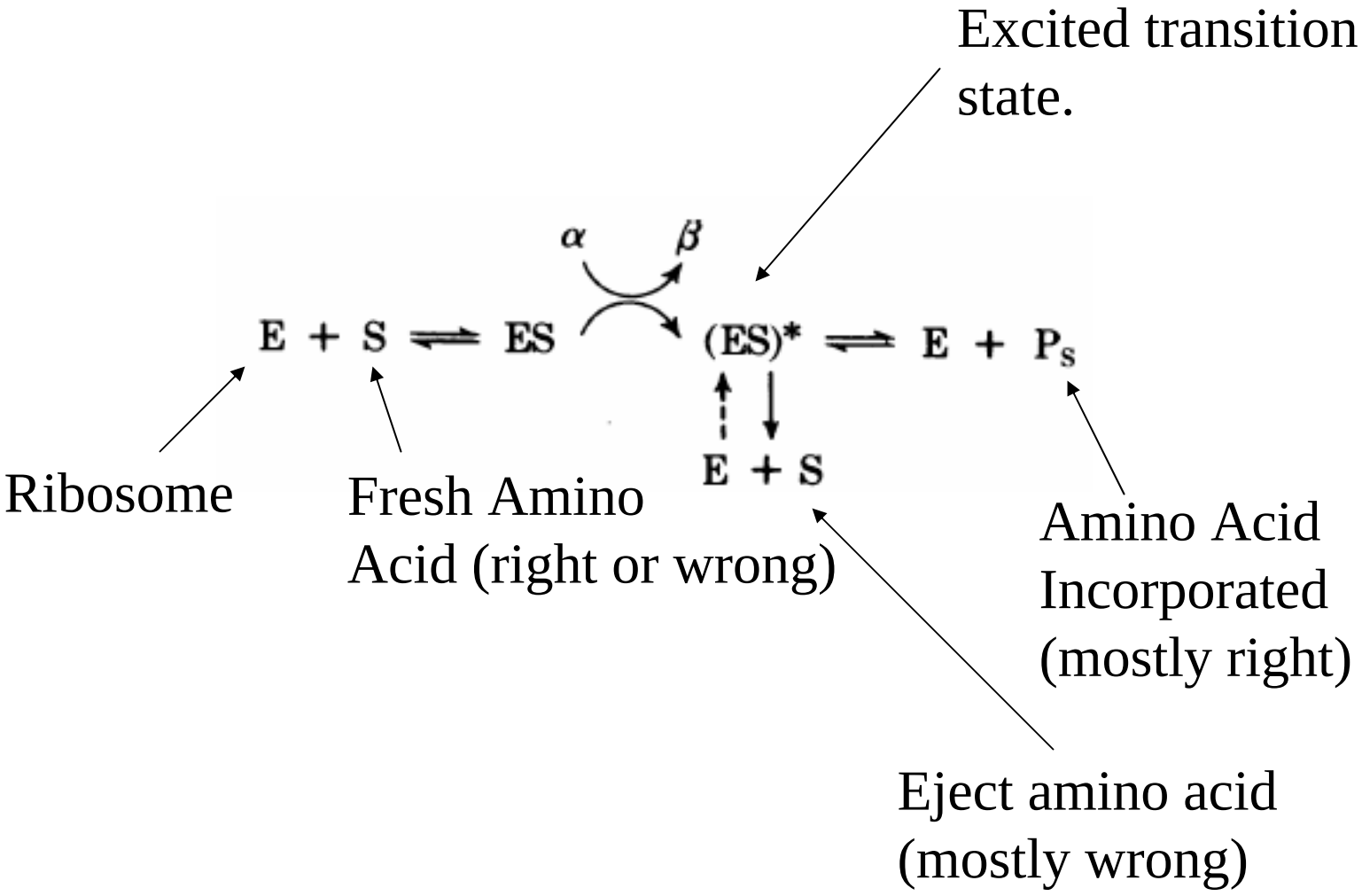




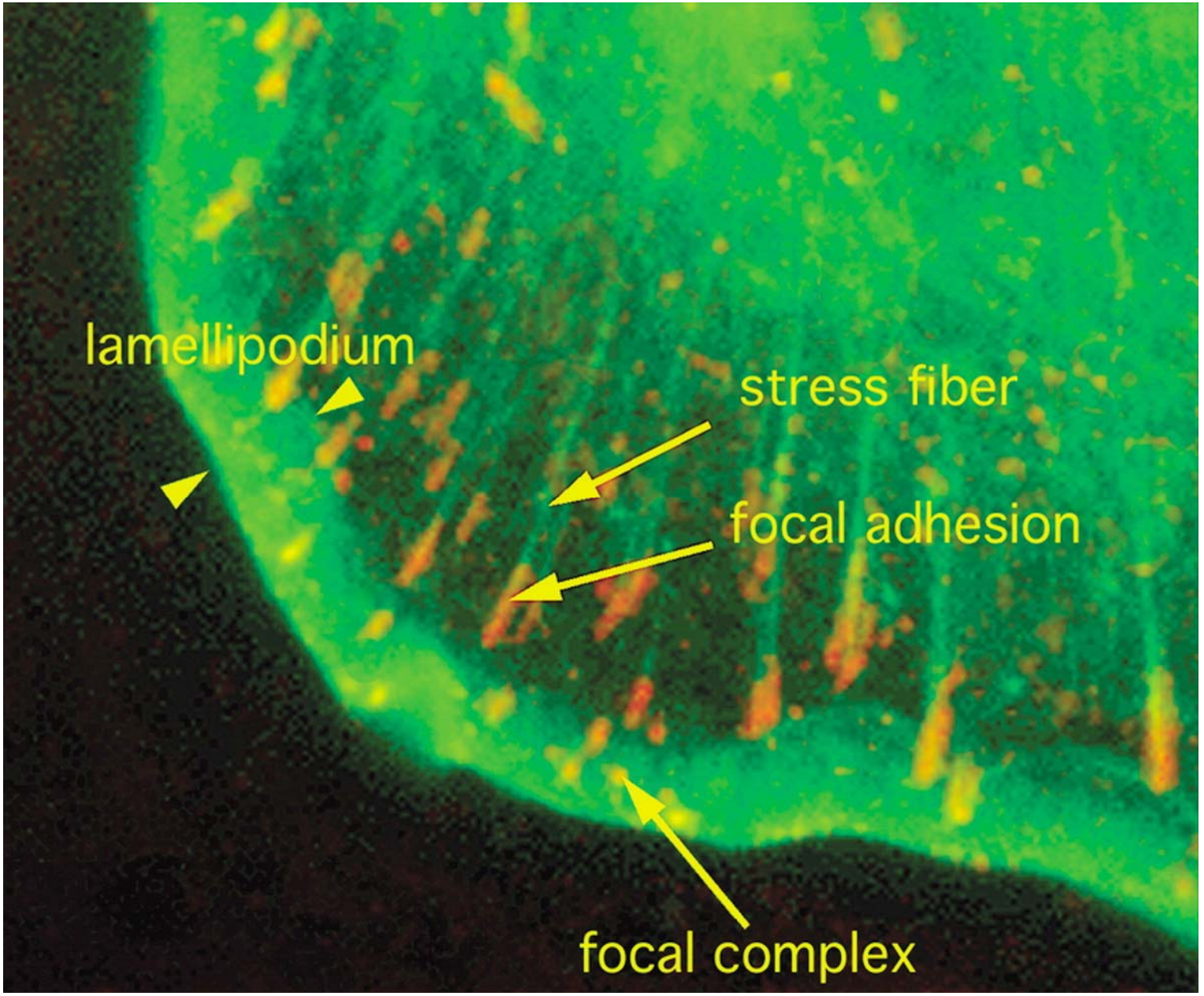
Proof-reading step.

Finds *two* “proofreading” check-points.

Hopfield proofreading:



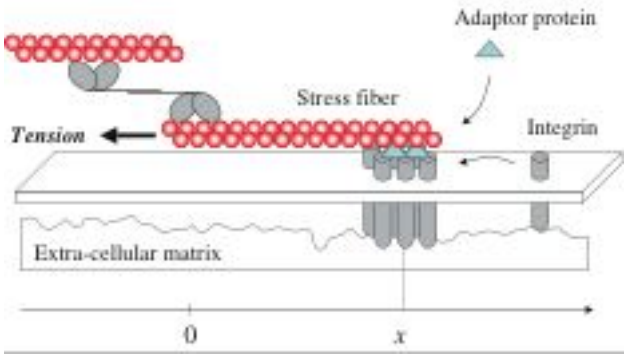
Focal Adhesion Sites: Motor protein regulation

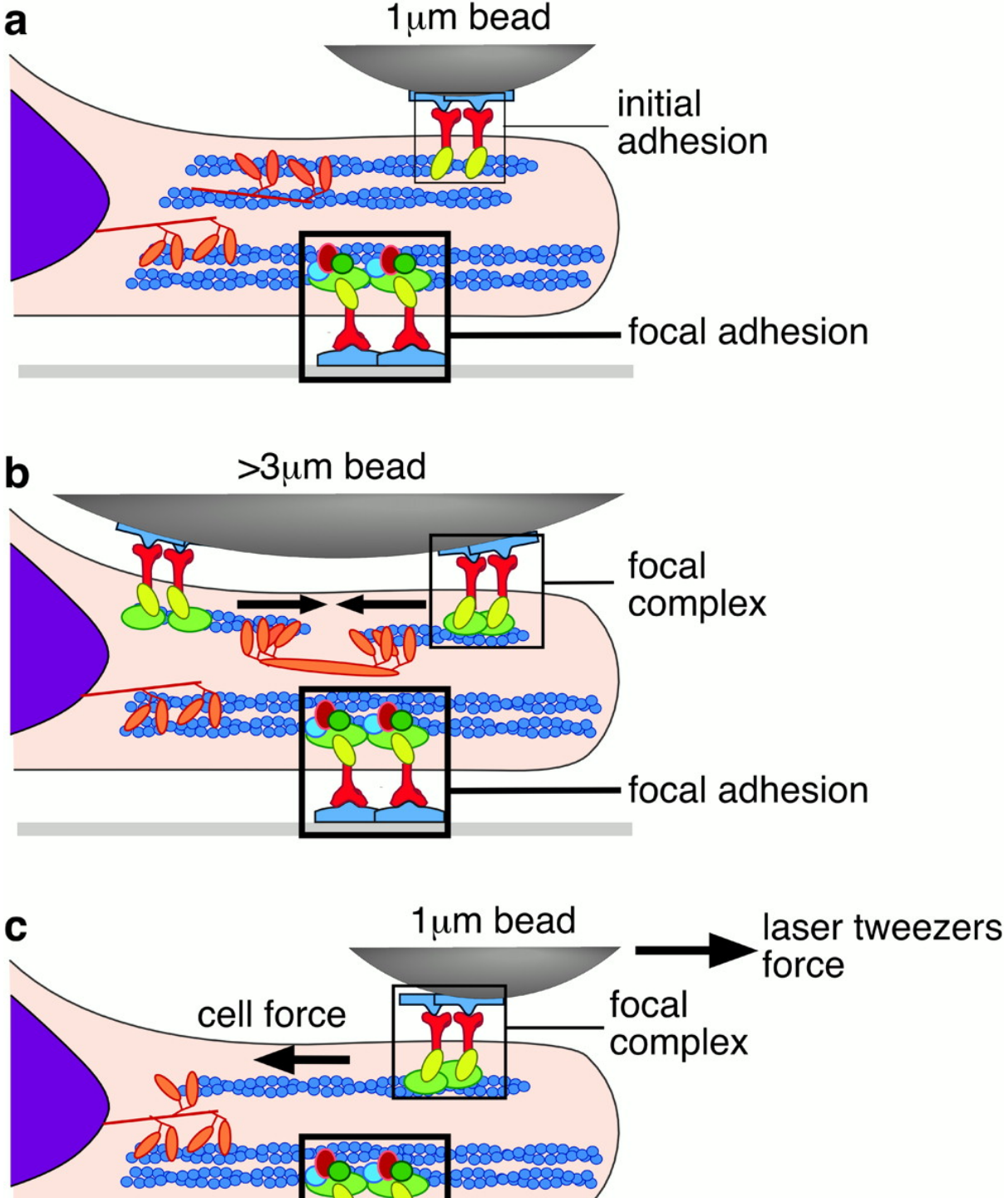


Rigidity Sensing

QuickTime™ and a
Video decompressor
are needed to see this picture.

- Soft substrate: slipping motion, tension in the pN range.
- Rigid substrate: stationary, tension in the nN range.
- External tension stimulates reinforcement.
- Integrin proteins linked to Actin filaments by Adaptor proteins.

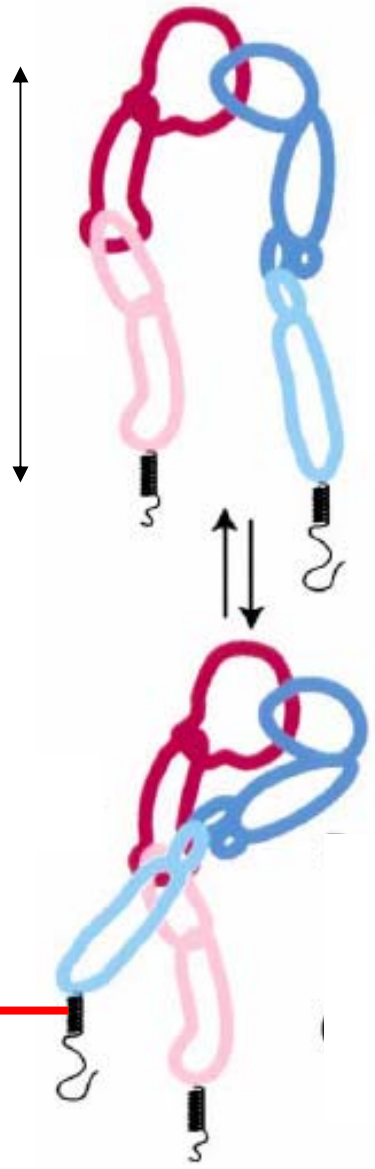



a
1μm bead
initial
adhesion
focal adhesion
b
>3μm bead
focal
complex
focal adhesion
c
1μm bead
laser tweezers
force
cell force
focal
complex

(Sheetz)

Mechanical Activation?

Lever arm L:
(30 nm)



Traction F:

Mechanical Work:

$$W \quad F\ L \sin \qquad G$$

$$10\ k_B T$$

Mechanical Activation Integrin.

Tyrosine Phosphatase Activation.
(RPTP)

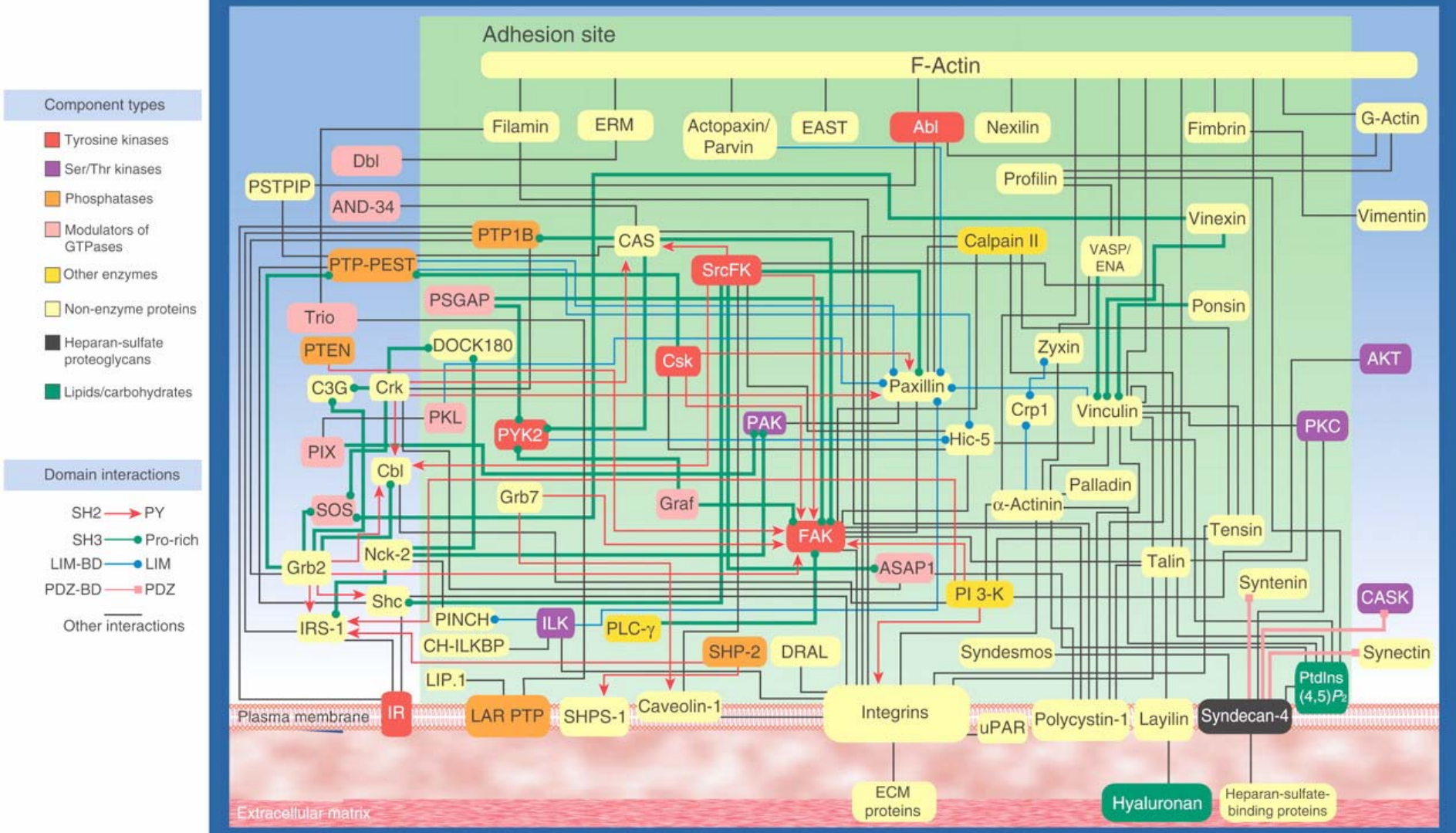
Src kinase pathway.

Adhesion-site Reinforcement.

Journal of Cell Science

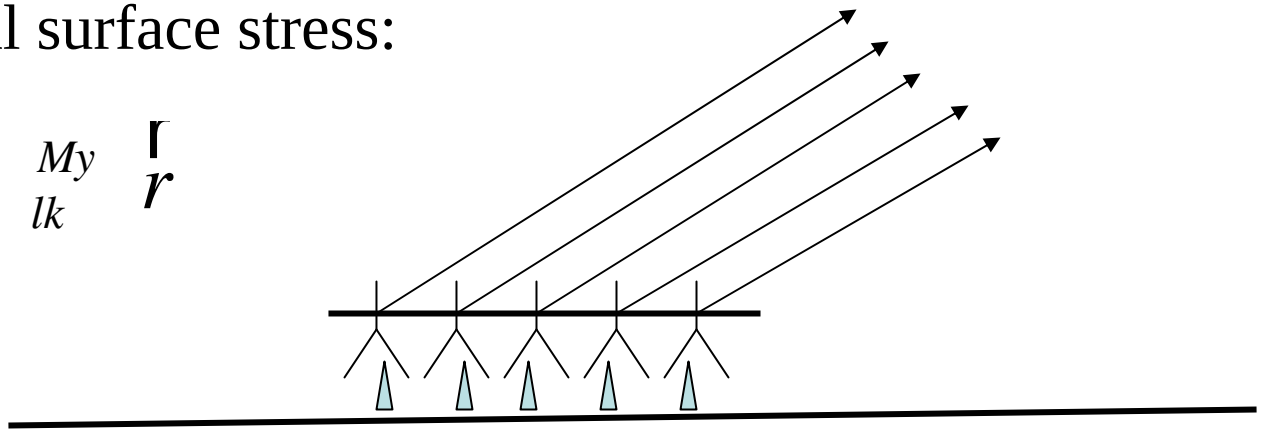
Components of Cell-Matrix Adhesions

Eli Zamir and Benjamin Geiger



Linear Elasticity

External surface stress:



$$Sub_{ij}(\vec{r}) = \int G_{ijkl}(\vec{r},\vec{r}\,')\, My_{kl}(\vec{r}\,')\, d^2\vec{r}\,'$$

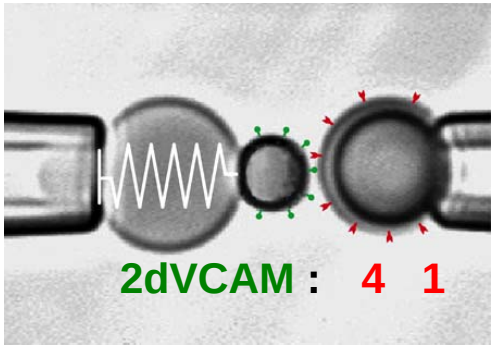
Substrate

Dimensionless

- Does not depend on Young's Modulus!
- "Dynamic" sensing?

Integrin-Ligand Rupture

(K.Kinoshita & E.Evans, 2003)



$$\langle F_{RUP} \rangle \quad f \ \ln \frac{Loading\ Rate}{k_{off}\ f}$$

15 pN

Hz (varies)

$$k_{off} \quad \exp \quad U / k_B T$$

Activation Energy