Harvard–MIT
Health Sciences & Technology

Department of Physics, MIT

What does a protein need to work?

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What does a protein need to work?

1. Stable structure
2. Specific active/binding sites.

What does a protein need to work?

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2. Specific active/binding sites.

3. Somewhat unstable structure
4. Non-specific binding site

A simple DNA-binding protein

<u>Function</u>

1. Find its site on DNA
2. Bind it tightly
3. IF [ligand]>0
        leave the site
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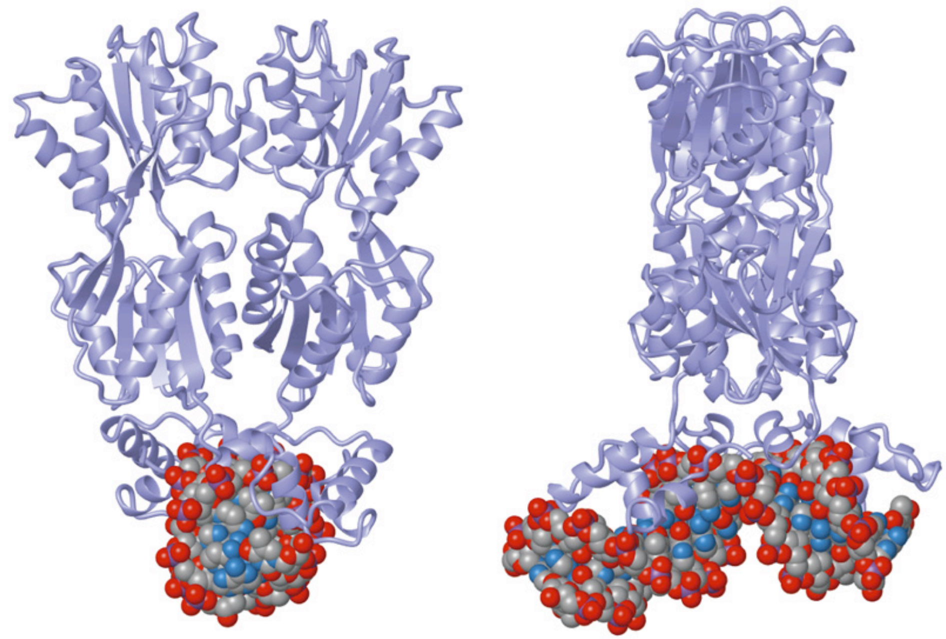

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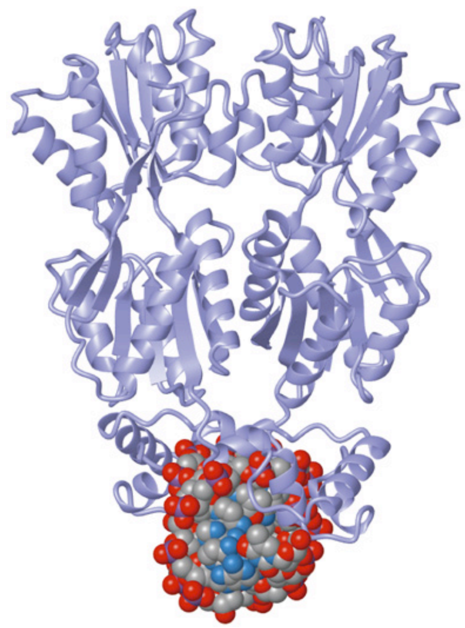
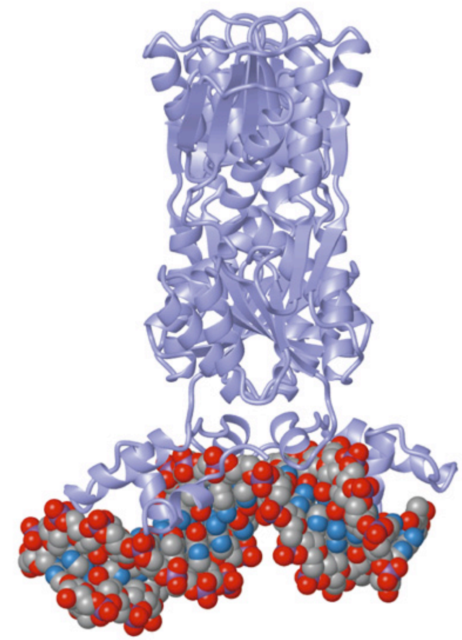
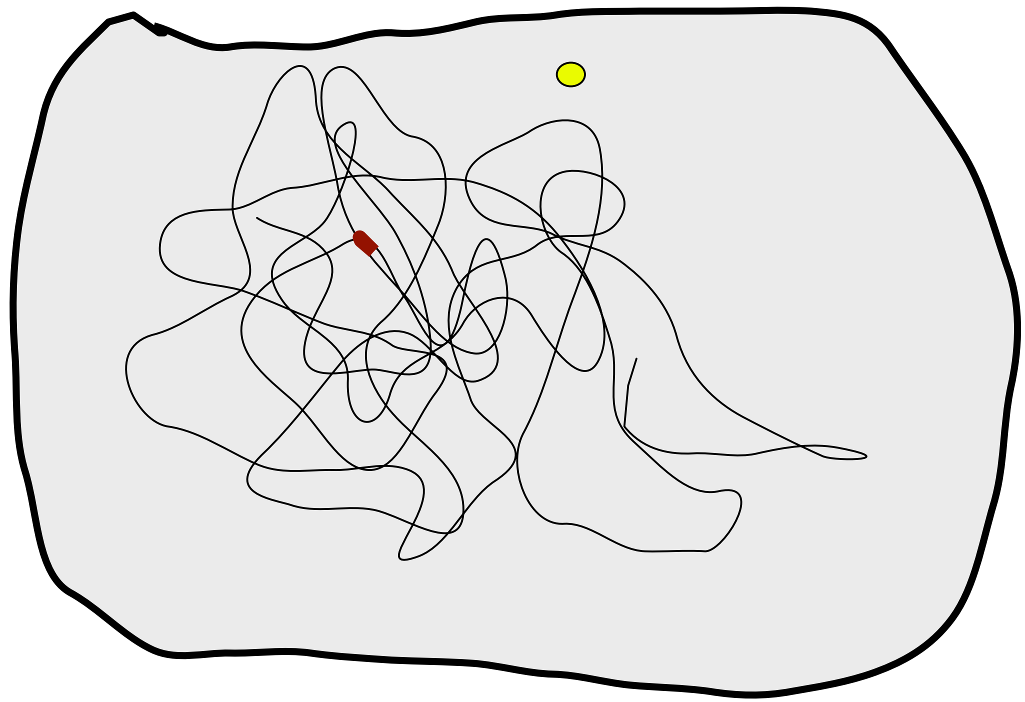




Problem 1:

find cognate site among 10^6-10^9 non-cognate sites



Experiment:

Riggs et al 1970

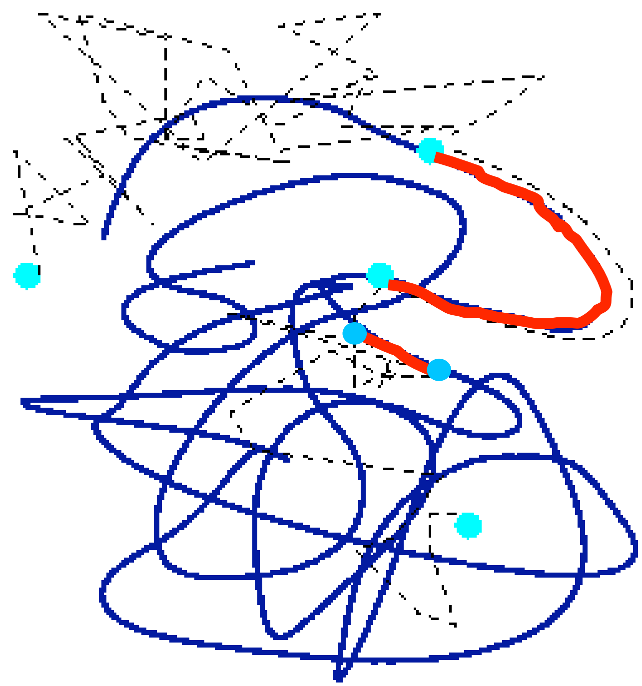
	WATER	CELL
	$k_{on} \approx 10^{10} M^{-1} s^{-1}$	$t_a \sim 1 - 10\ sec$
Diffusion-limited association	$k_{DS} = 4\pi D_{3D} b \sim 10^{8}\ M^{-1}\ s^{-1}$	$t_a \sim 10^2 - 10^3\ sec$

Theory:

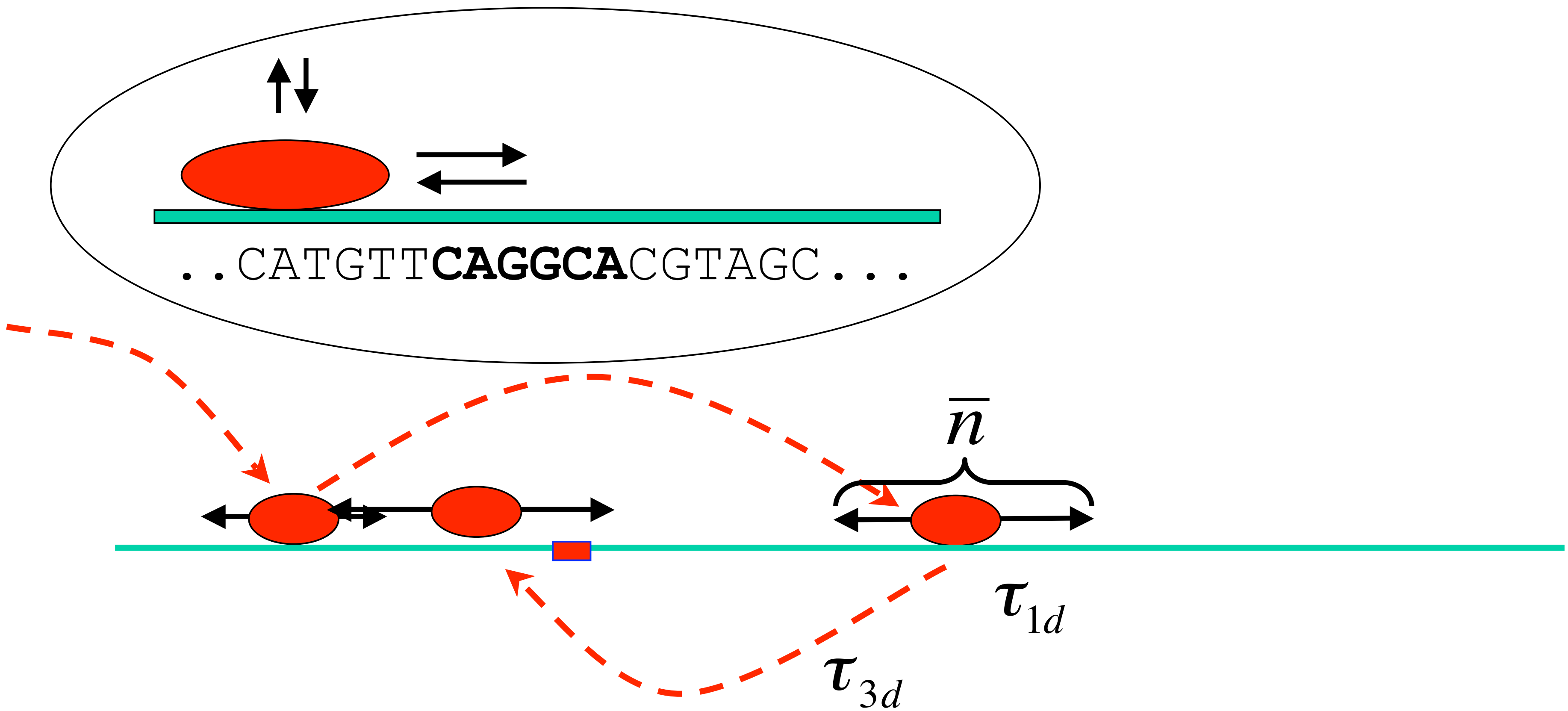
1D diffusion ("sliding") + 3D

Richter and Eigen 1974,

Berg, Winter, von Hippel 1981



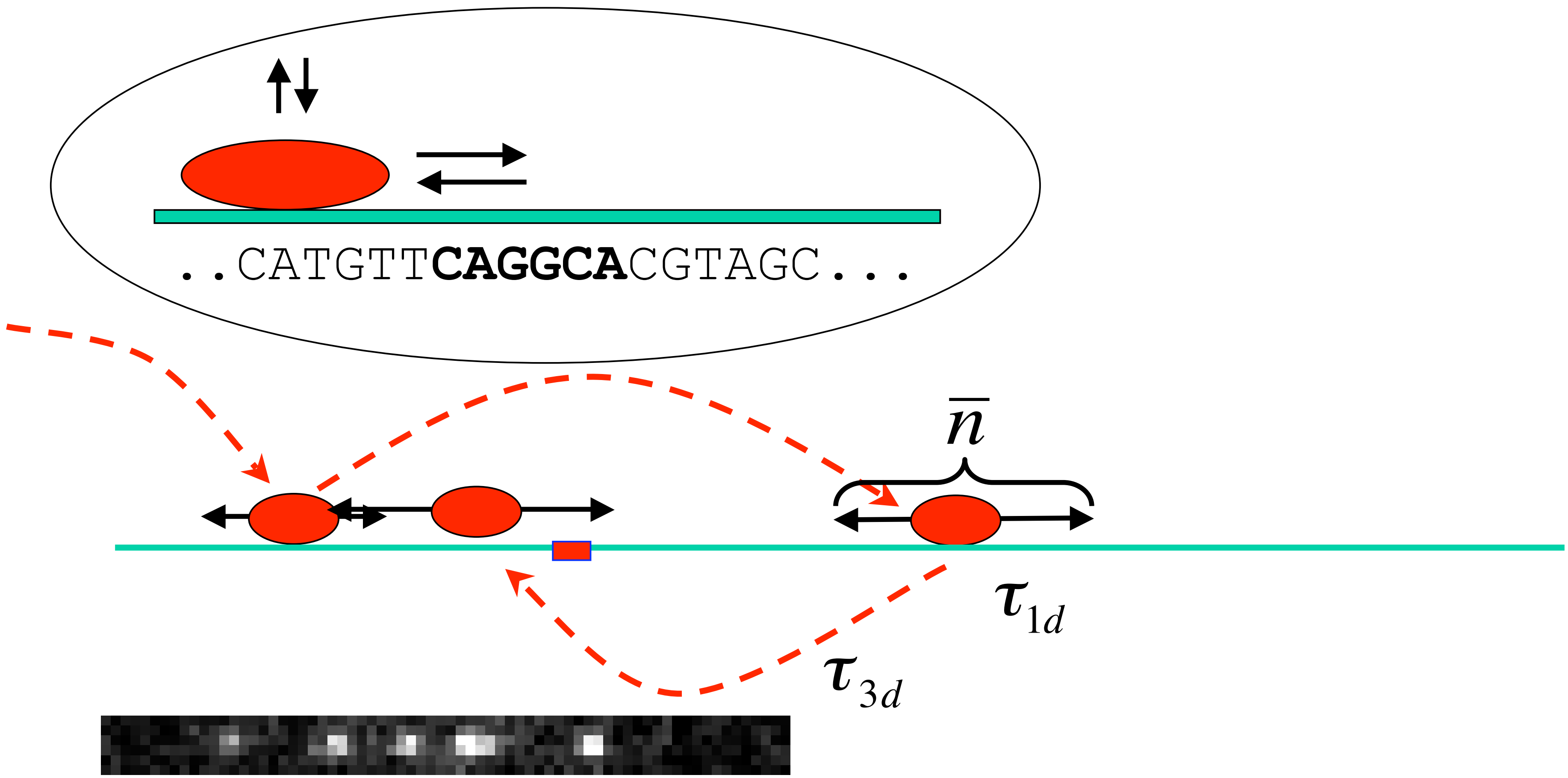
Model: 1D+3D



t_s – search time
M – genome size

$$t_s(\bar{n}, M) = \frac{M}{\bar{n}}\left[\tau_{1d}(\bar{n}) + \bar{\tau}_{3d}\right]$$

Model: 1D+3D



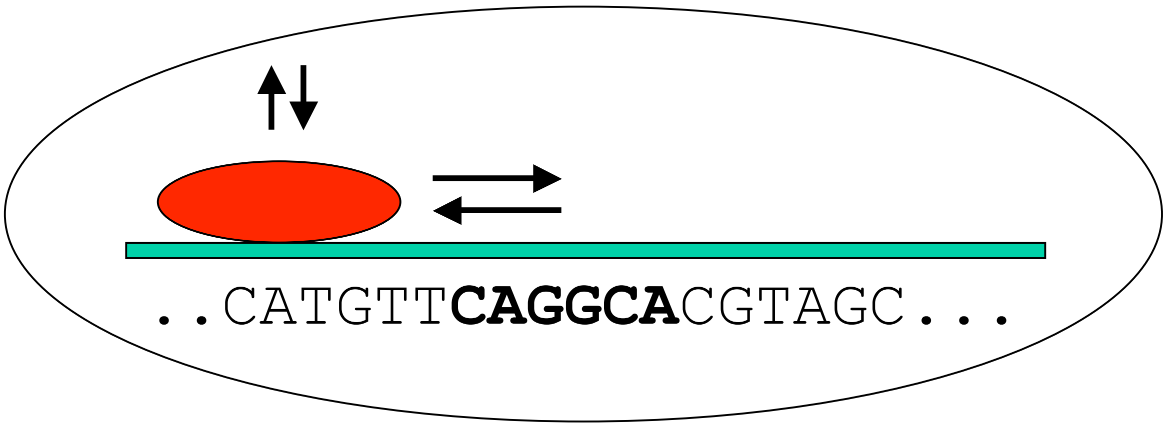
A base-excision DNA-repair protein finds intrahelical lesion bases by fast sliding in contact with DNA

Paul C. Blainey*, Antoine M. van Oijen*†, Anirban Banerjee*, Gregory L. Verdine*§¶, and X. Sunney Xie*¶

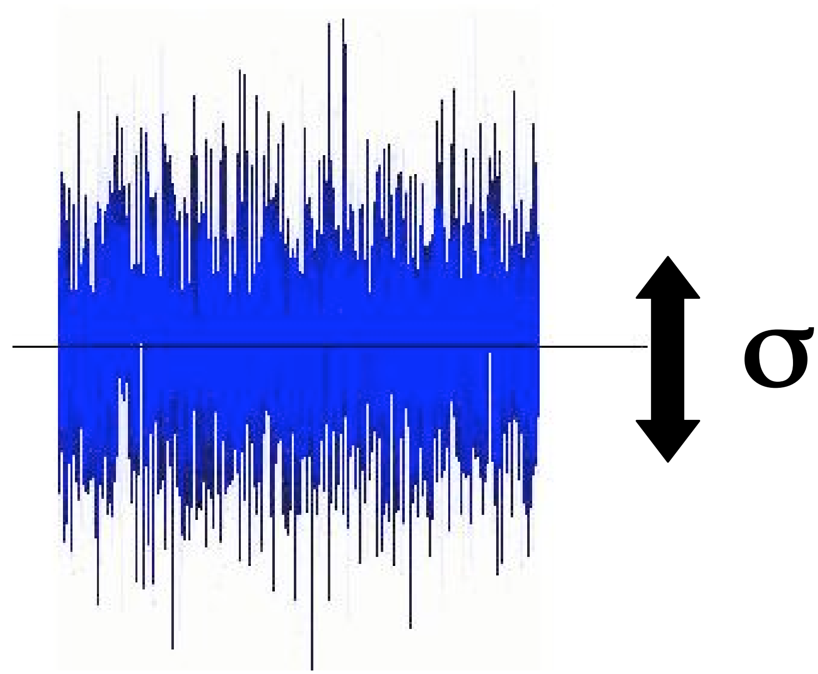
Departments of *Chemistry and Chemical Biology and §Molecular and Cellular Biology, Harvard University, 12 Oxford Street, Cambridge, MA 02138

Edited by Peter H. von Hippel, University of Oregon, Eugene, OR, and approved February 14, 2006 (received for review November 8, 2005)

Our model: 1D+3D

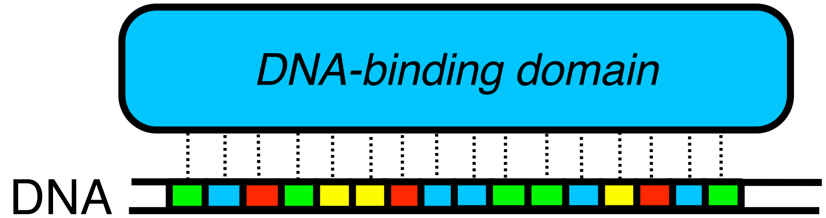


Energy landscape of 1D sliding



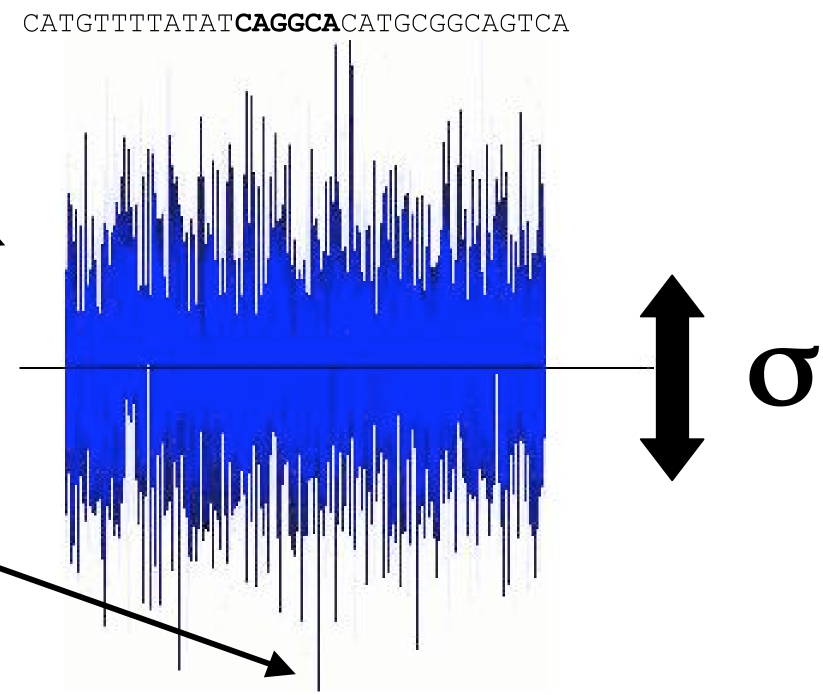
Protein-DNA interaction energy

$$E = \sum_{i=1}^{l} e(i, b_i)$$



Energy is strongly sequence dependent

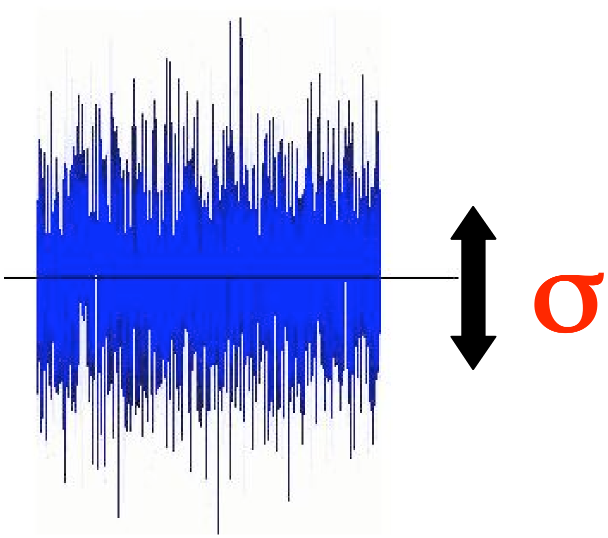
Energy landscape

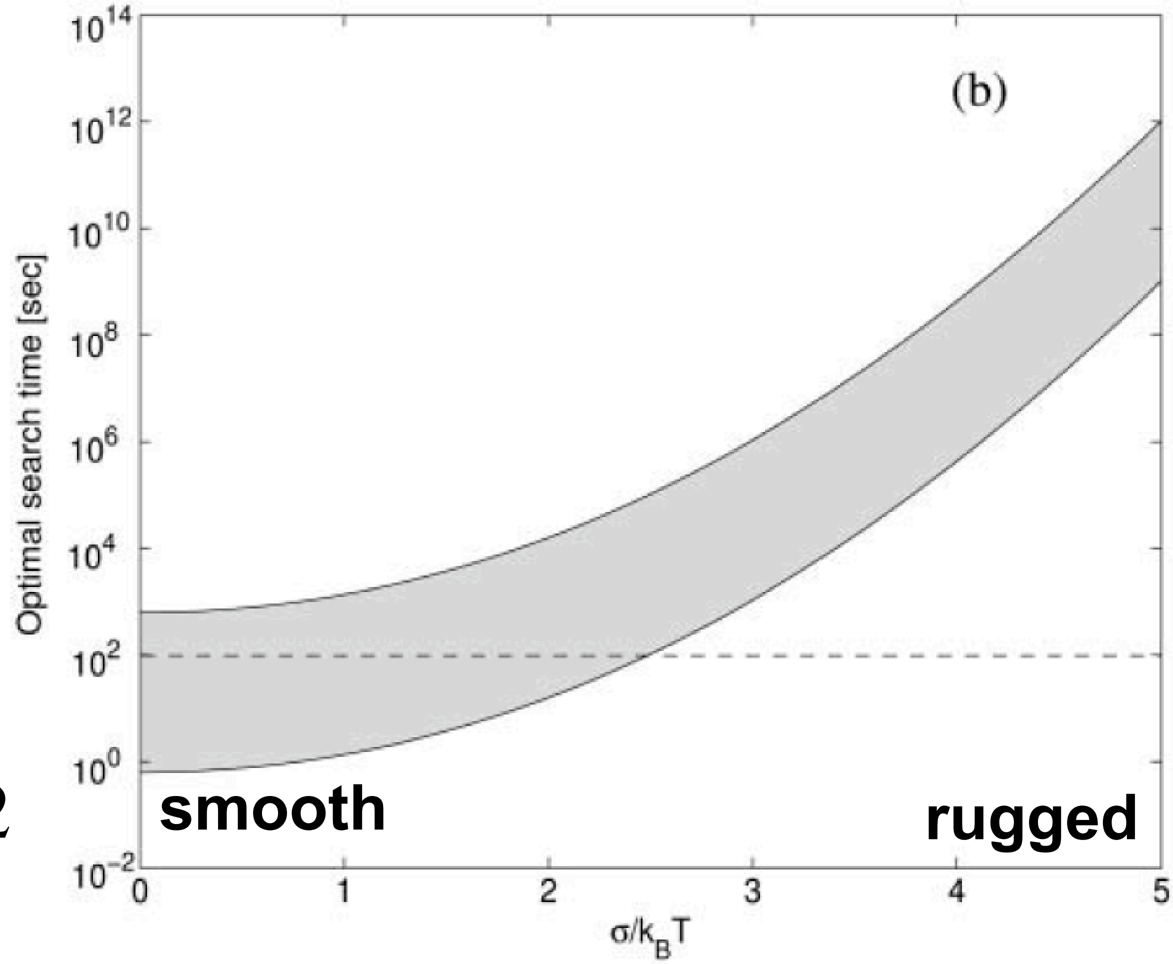


NO ENERGY GAP between cognate and random sites

Results

Fast sliding requires smooth landscape



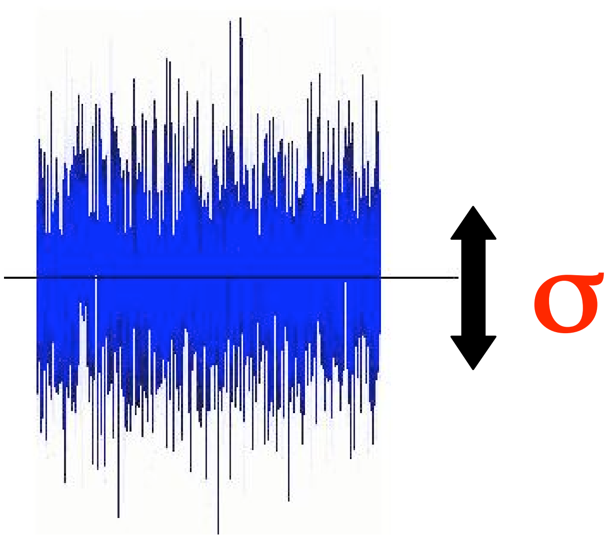


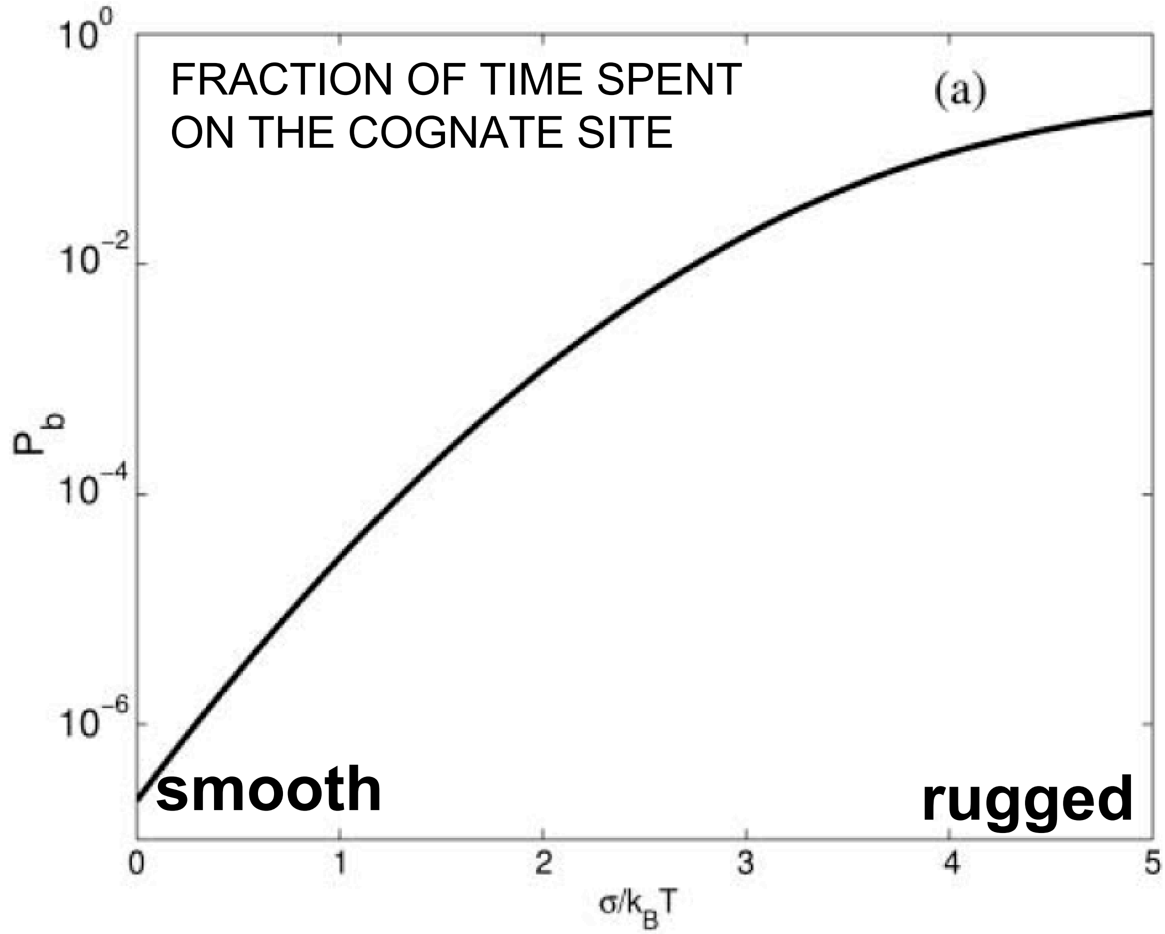
$$D_{1d} \sim e^{-\frac{7}{4}(\sigma / k_B T)^2}$$

Roughness of the energy landscape

Results

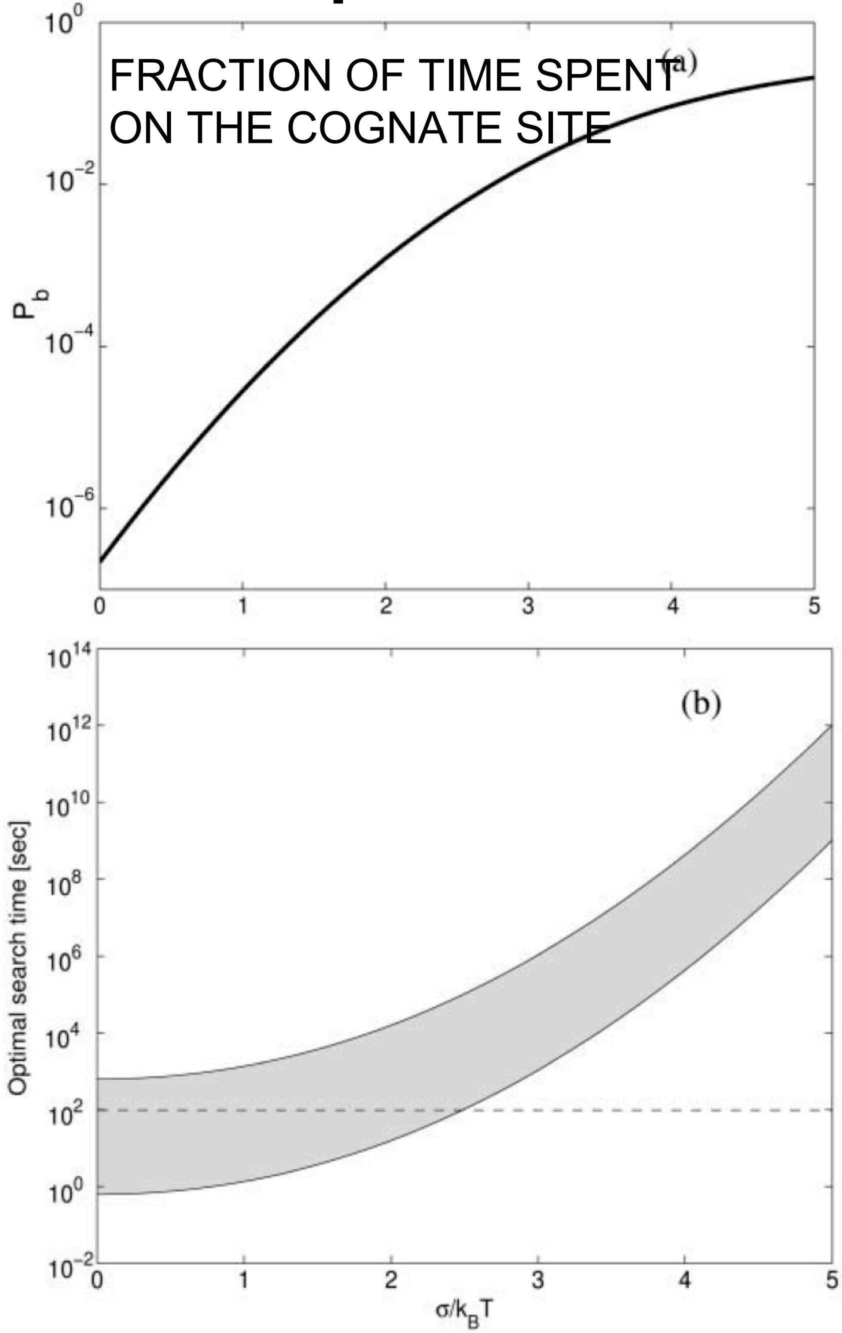
Specific recognition requires rough landscape





Roughness of the energy landscape

Speed-stability paradox



STABILITY:

$\sigma > 5kT$

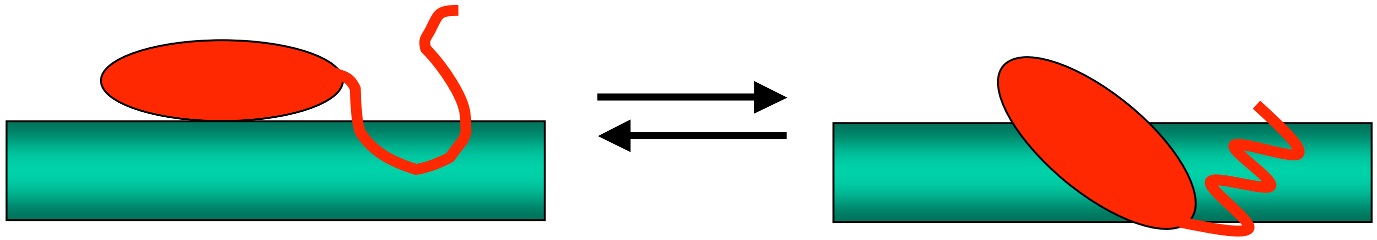
SPEED

$\sigma < 2kT$

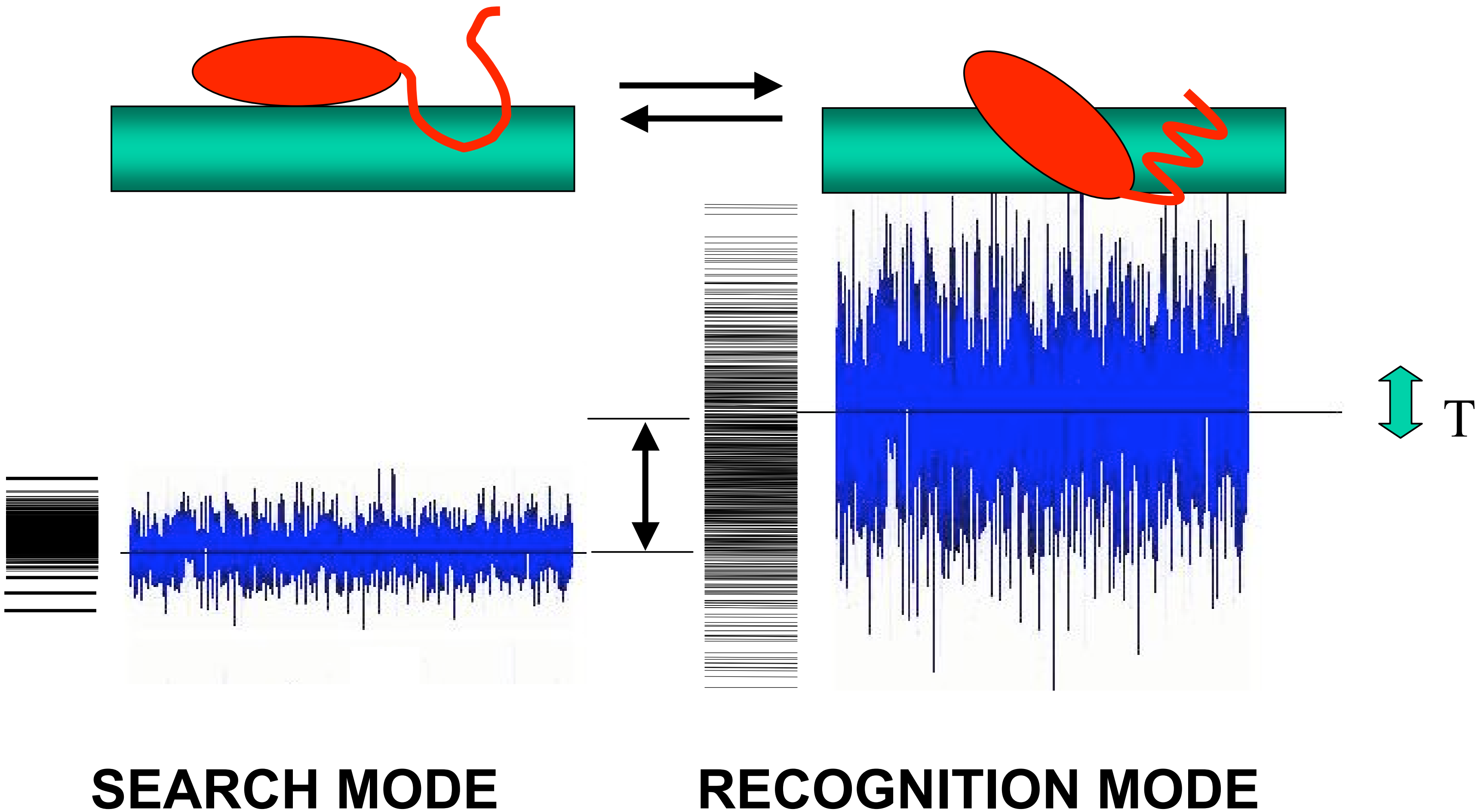
Either speed or stability
but not both !

Slutsky.M, Mirny,LA, *Biophys J (*2004)

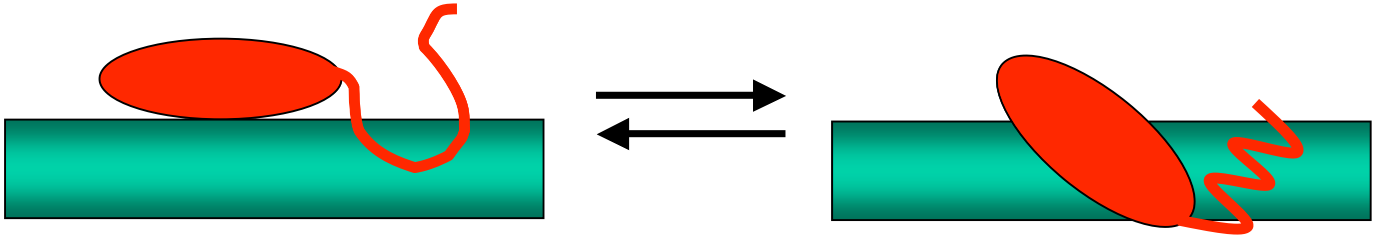
Proposed Mechanism



Proposed Mechanism



Experiments



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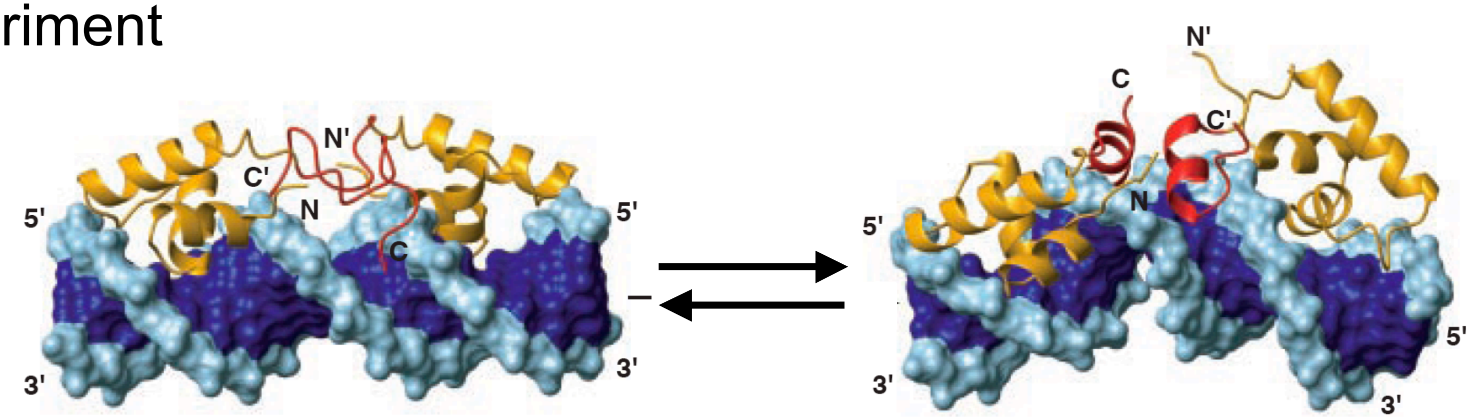
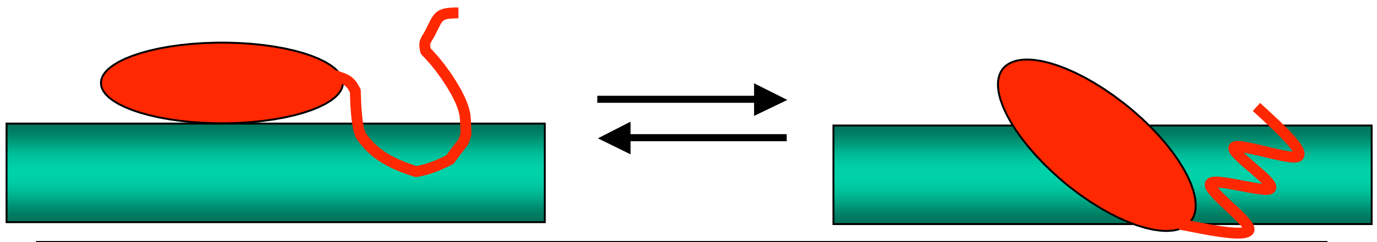
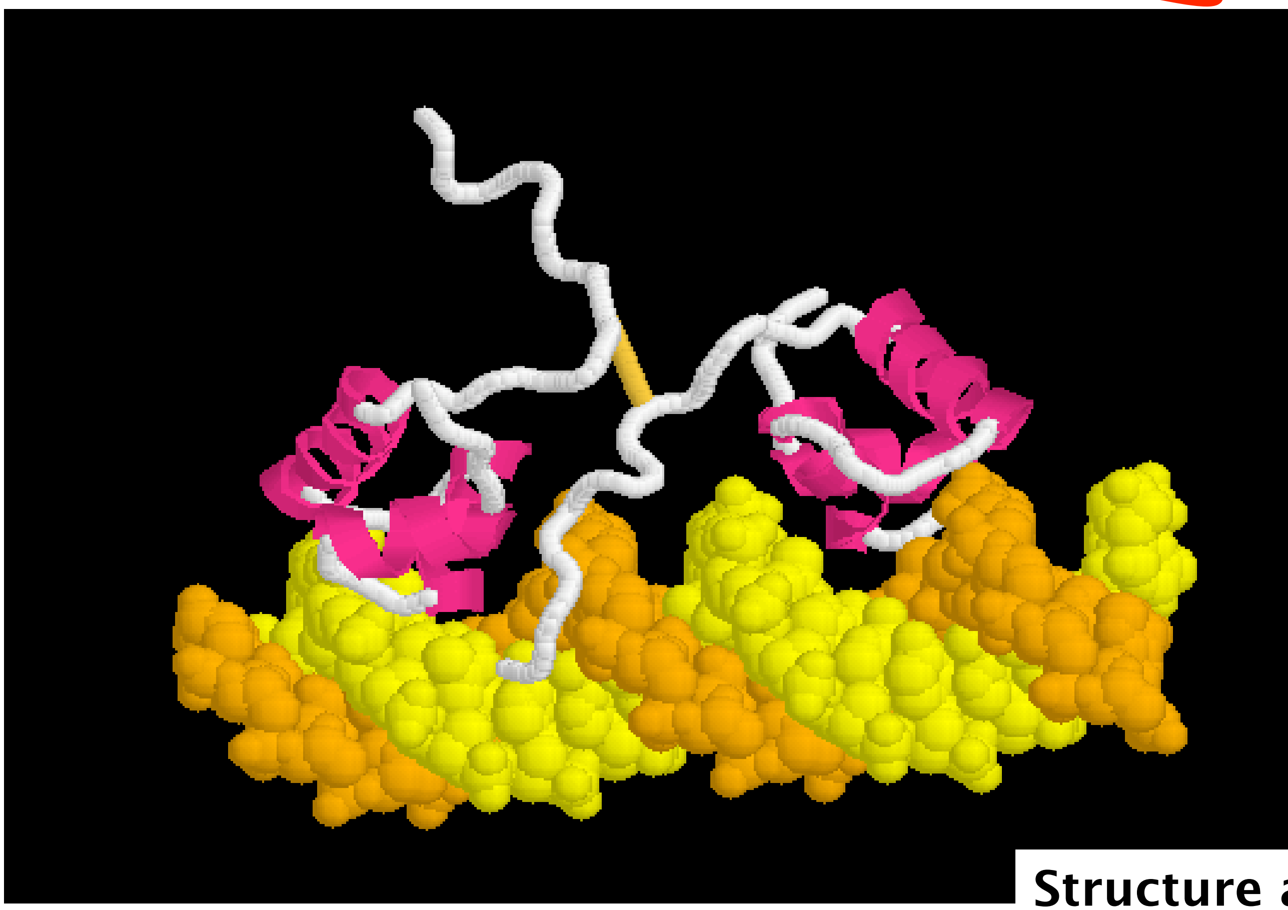


nonspecific complex specific complex

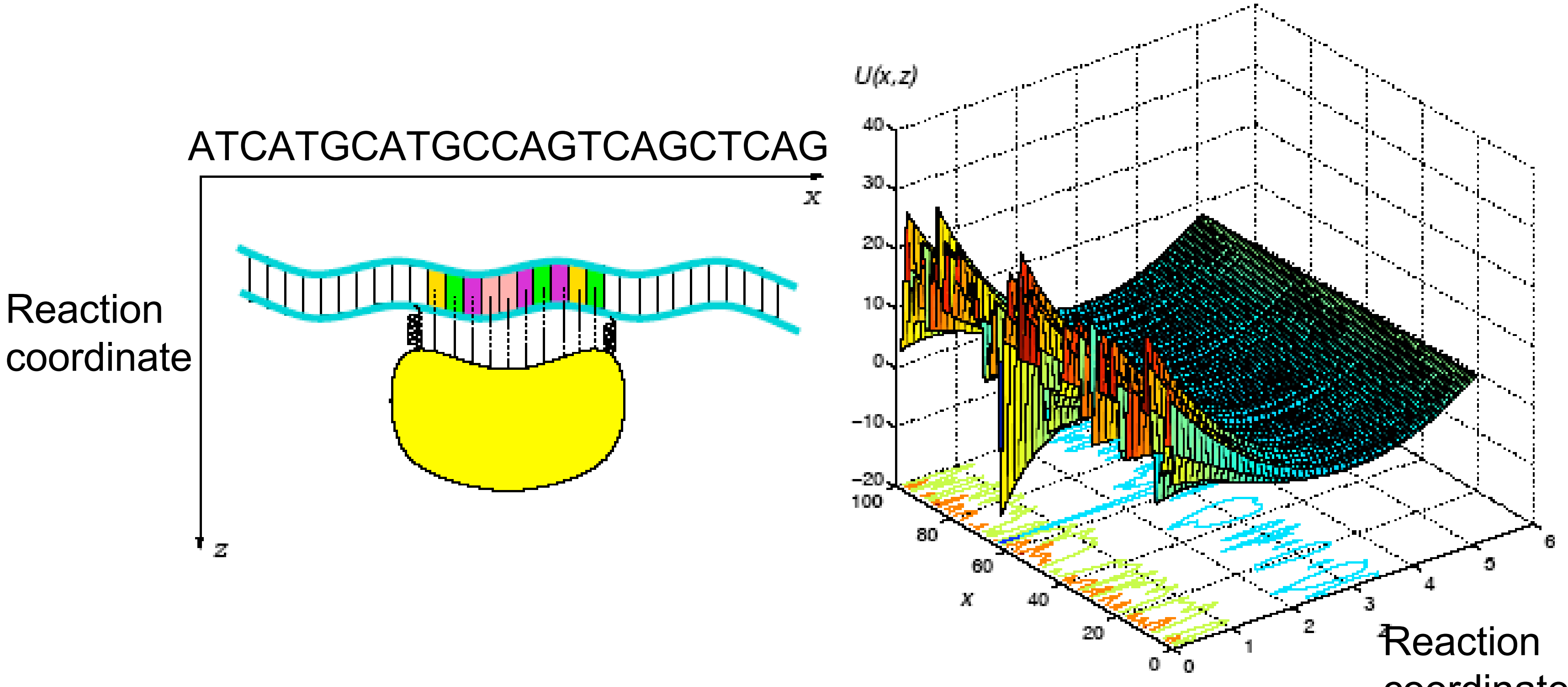
Kalodimos et.al *Science.2004*





Structure and animation
by Babis Kalodimos et al

Landscape model

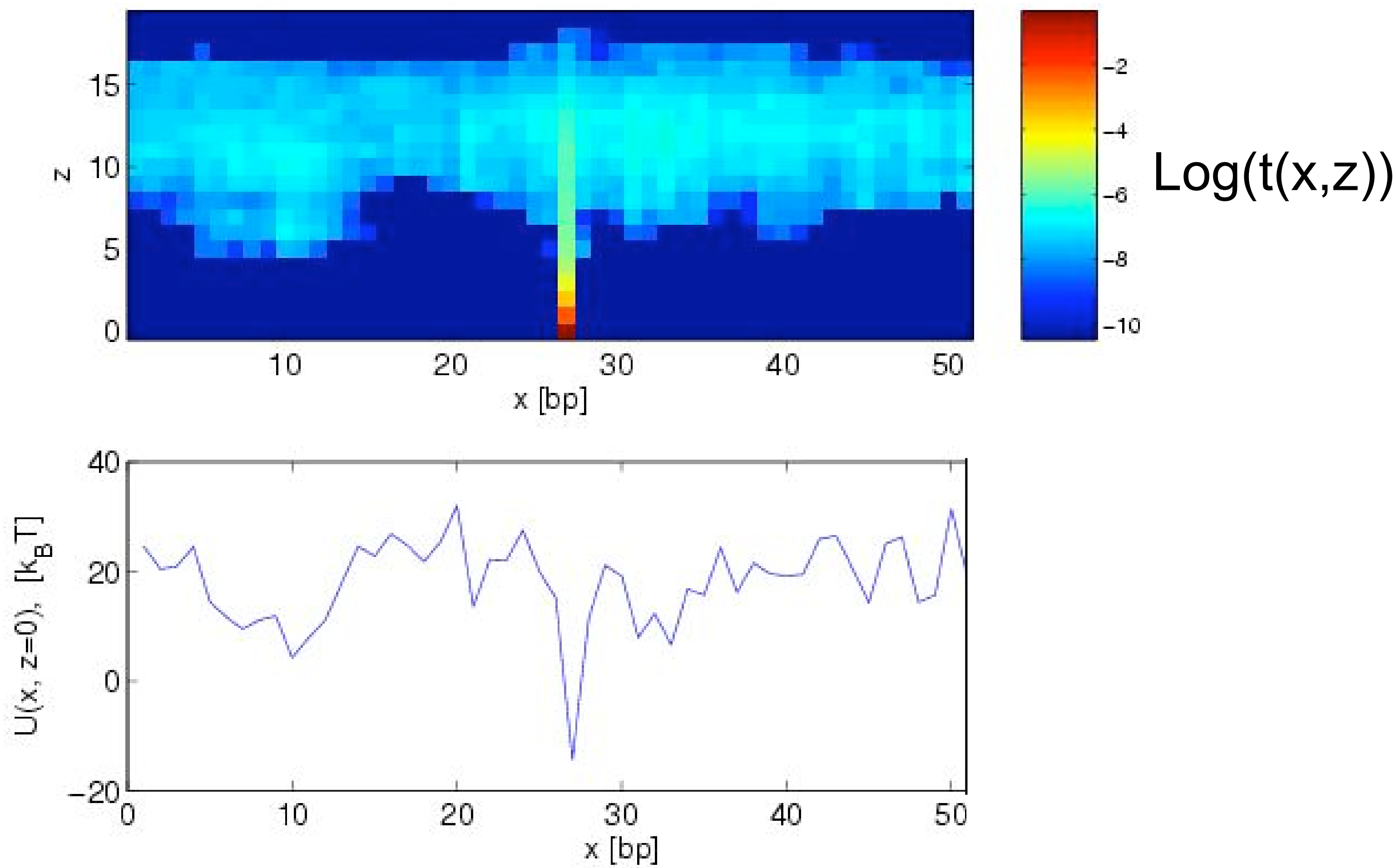


(a) (b)

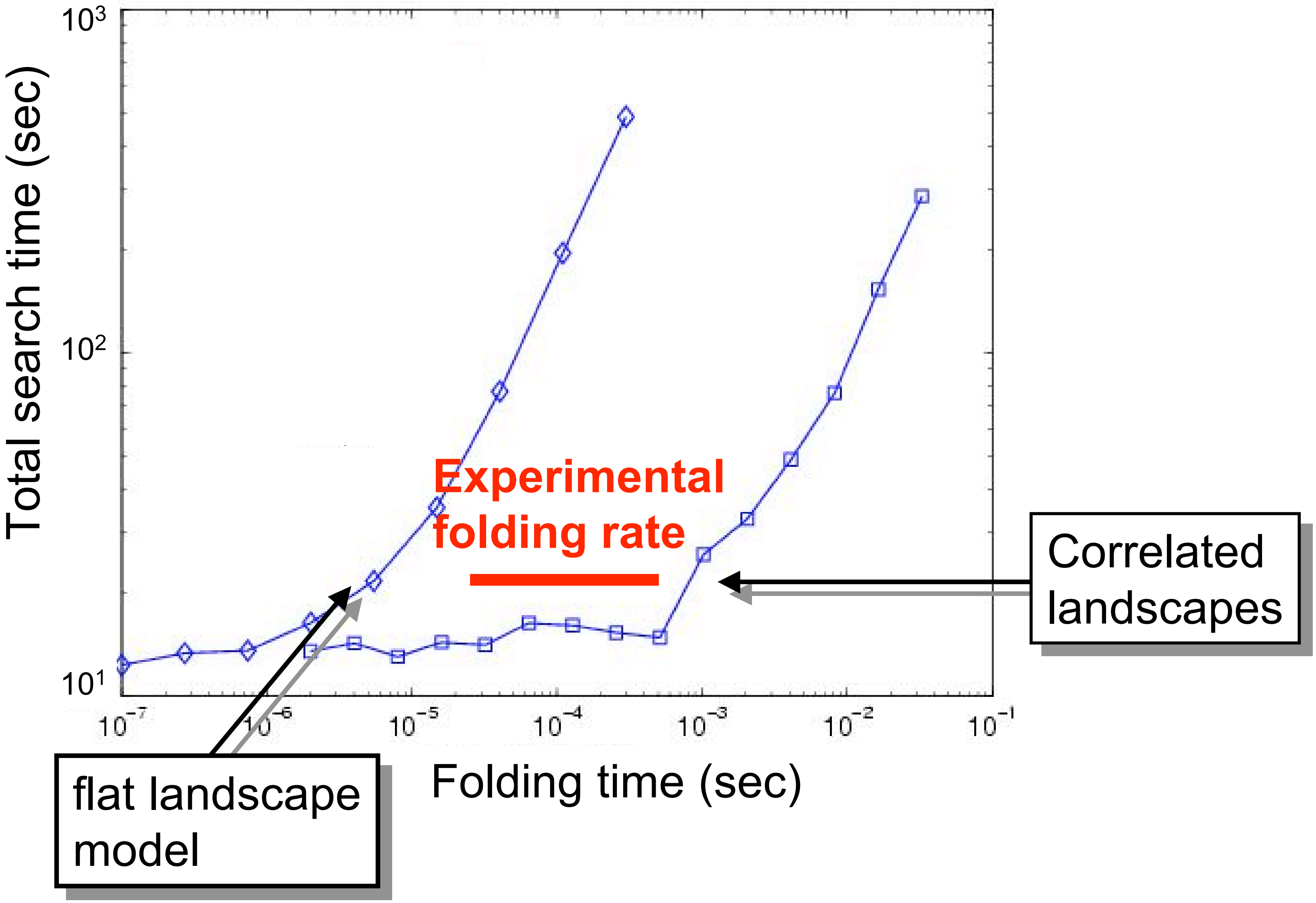
$$U(x, z) = U_{\rm spec}(x, z) + U_{\rm non-spec}(z)$$

$$U(x, z) = U_{\rm s}(x)e^{-z} + \frac{\alpha}{2}(z - z_0)^2$$

Meso-scale dynamics of landscape model

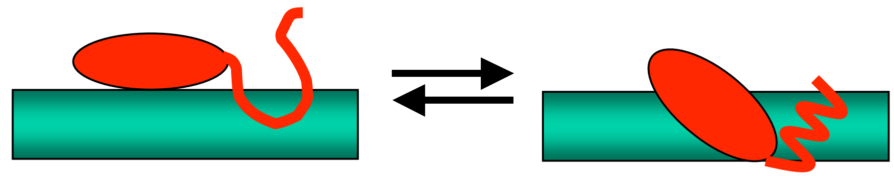


Macroscale dynamics



Summary

1. 1D+3D search is fast if the protein-DNA complex is <u>FLEXIBLE.</u>



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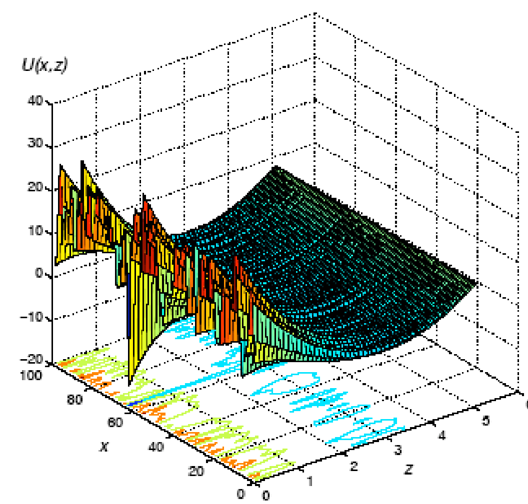


(b)

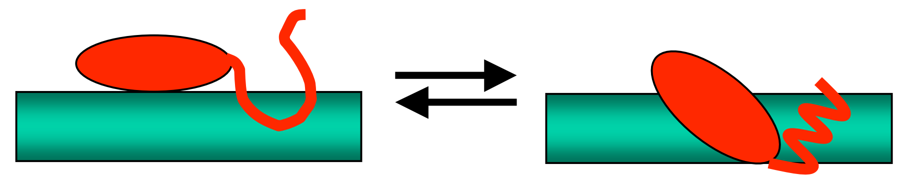
Somewhat unstable structure is needed for …

<u>Function</u>

✓1. Find its site on DNA

✓2. Bind it tightly

3. IF [ligand]>0
   leave the site
  ELSE
   goto step 1.
  END



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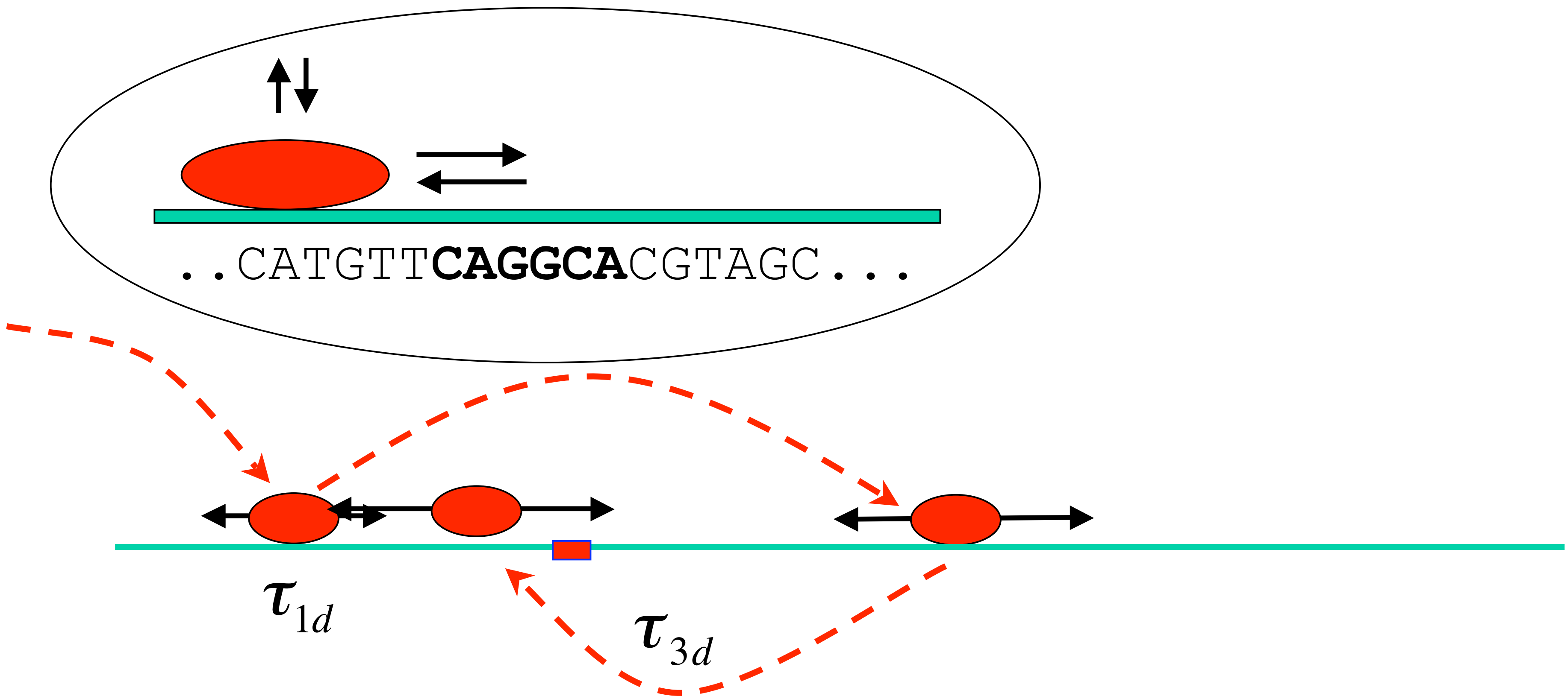

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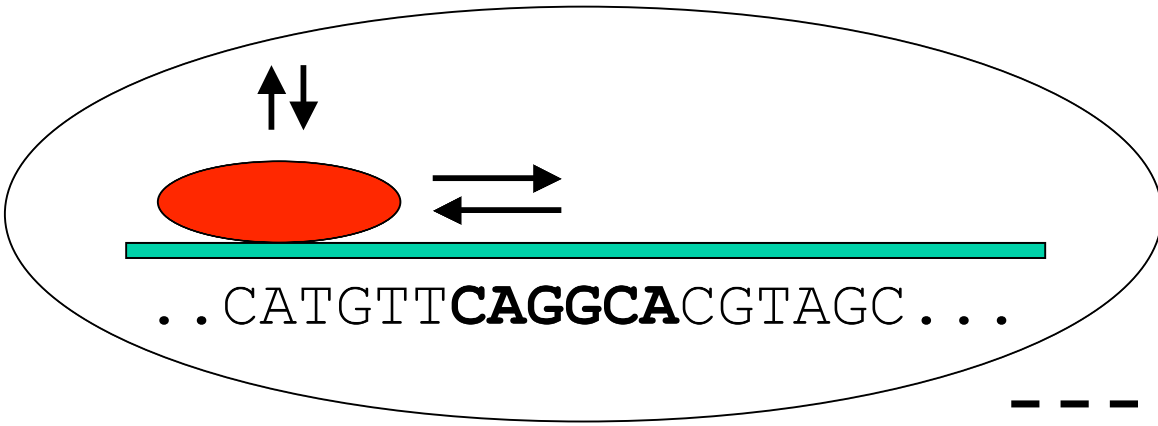
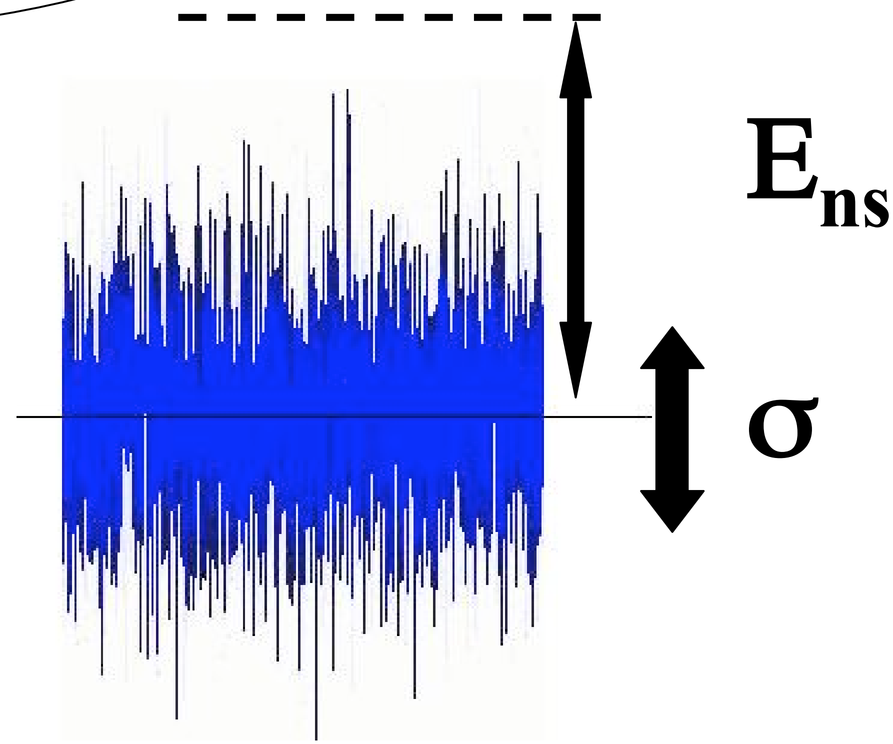
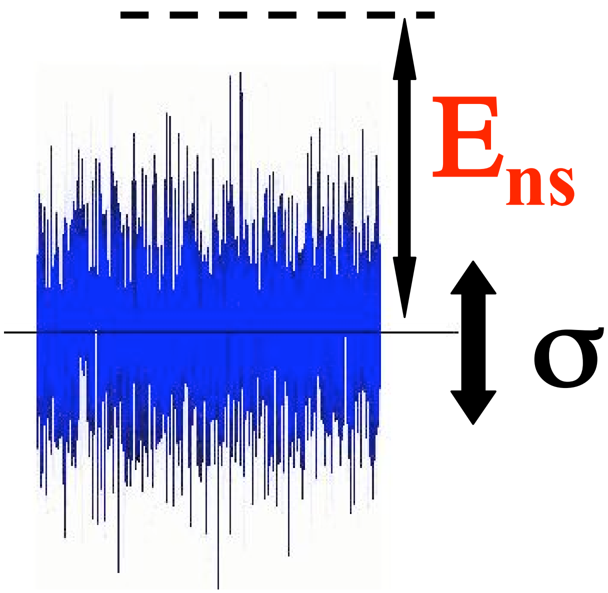


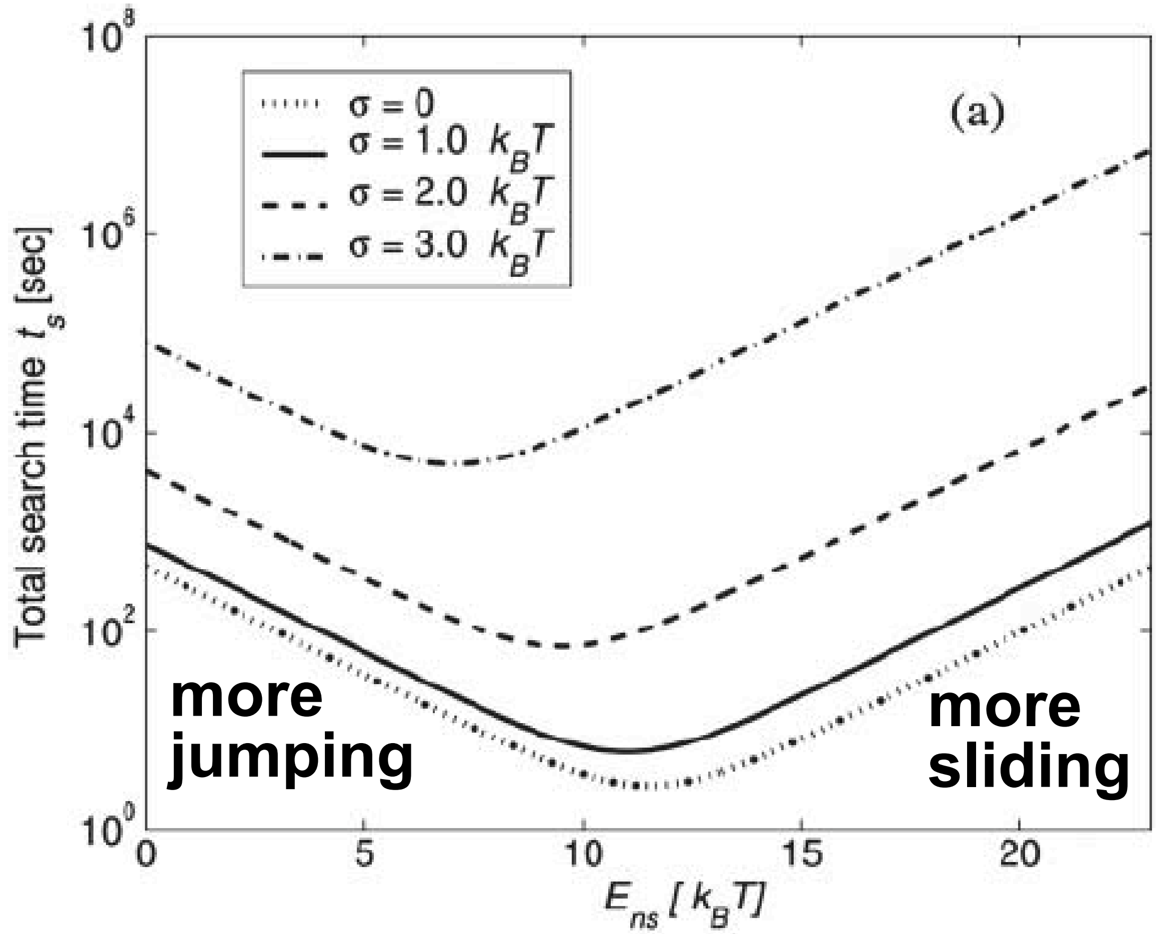


Energy landscape of 1D sliding

Results

Fast sliding requires optimal non-specific binding



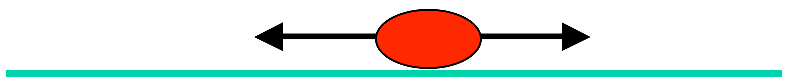


Free energy of non-specific binding

Non-specific binding is needed for …

<u>Function</u>

✓1. Find its site on DNA



2. Bind it tightly

3. IF [ligand]>0

        leave the site

    ELSE

        goto step 1.

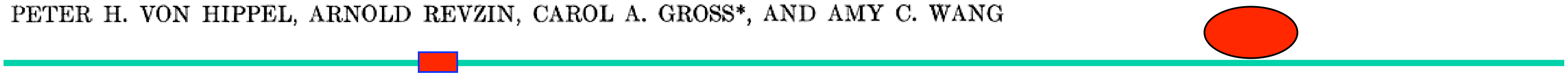
    END

Proc. Nat. Acad. Sci. USA
Vol. 71, No. 12, pp. 4808–4812, December 1974

Non-specific DNA Binding of Genome Regulating Proteins as a Biological Control Mechanism: I. The *lac* Operon: Equilibrium Aspects

(*lac* repressor/DNA-protein interactions/RNA polymerase/repressor–inducer complexes)

PETER H. VON HIPPEL, ARNOLD REVZIN, CAROL A. GROSS*, AND AMY C. WANG



$M \sim 10^6$ - non-specific sites
$m \sim 10$ - number of LacI proteins per cell

$$P = \frac{1}{1 + \frac{M}{m}\frac{K_d^s}{K_d^{ns}}}$$

$$K_d^{ns} \approx 10^{-6}\,M$$
$$K_d^{s,NO-LIGAND} \approx 10^{-12}\,M$$
$$K_d^{s,LIGAND} \approx 10^{-9}\,M$$

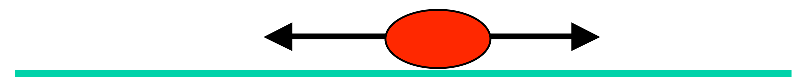
Fraction of time the site is bound

$$P^{NO-LIGAND} = \frac{1}{1+0.1} = 0.9$$
$$P^{LIGAND} = \frac{1}{1+100} = 0.01$$

Non-specific binding is needed for …

Function

✓1. Find its site on DNA



2. Bind it tightly

✓3. IF [ligand]>0

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END

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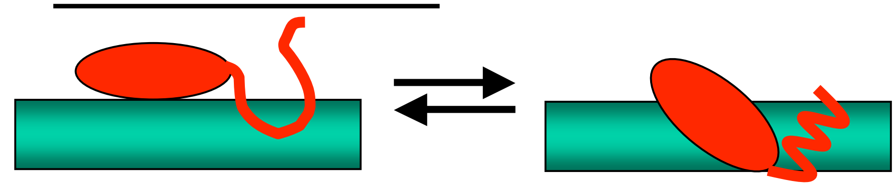

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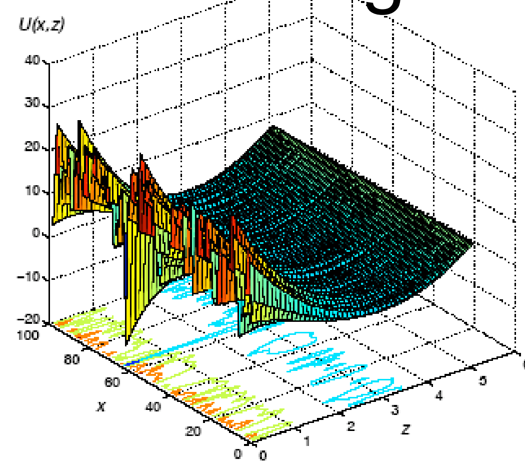


(b)

3. Non-specific binding is essential for protein function.

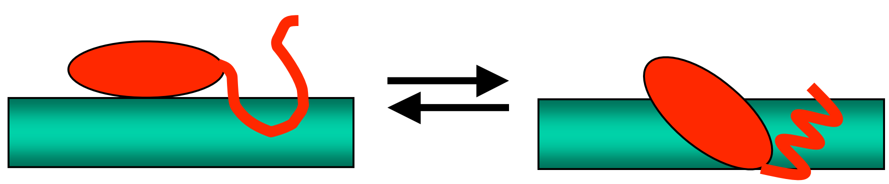
What does a protein need to work?

1. Stable structure
2. Specific active/binding sites.

3. Somewhat unstable structure
4. Non-specific binding site

Testable predictions

1. Diffusion of a protein on DNA is sequence-dependent.
2. DNA sequences can influence
   - folded/unfolded equilibrium
   - rate of conf.transition in the protein (nucleate folding on the target site)
3. Mutations that change the stability and rate can have affect on the total search time and timing of gene expression.



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Acknowledgements

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