Probing allostery through DNA: interplay between theory and experiments

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Allosteric regulation

Allostery = “allos” (other) + “stereos” (object)

The binding of a molecule to a macromolecule complex affects the binding affinity or enzymatic activity at a distant site.
Transcriptional factors on DNA: any allosteric effect?
Can DNA-binding proteins “feel” the coming RNAP?

DNA binding proteins including DNA repair protein, DNA modification protein, ...

Similar feature might occur during DNA replication too.
Single molecule measurement of LacR* binding

T7 RNA polymerase (or EcoRV or BamH1)

LacR*
Single molecule measurement of LacR* binding

Histogram of dissociation times of LacR from DNA in the absence of T7 RNAp.

\[ N(t) = A \exp\left(-\frac{t}{\tau}\right) \]

Dissociation assay for protein binding kinetics

Average binding time = \( \tau \)
Standard error = \( \tau / \sqrt{N} \)
\( k_{\text{off}} = 1/ \tau \)
LacR dissociation in the presence of a second binder
LacR dissociation rate as a function of separation

With a periodicity of ~10 base pairs, the helical pitch of B-form DNA, and a decay length of ~15 base pairs
Kinetic mechanism

State 0
- $k_{0 \rightarrow 0}$
- $k_{0 \rightarrow 1}[R]
- $k_{0 \rightarrow 2}[P]

State 1
- $k_{1 \rightarrow 0}$
- $k_{1 \rightarrow 3}[P]$
- $k_{3 \rightarrow 1}$

State 2
- $k_{2 \rightarrow 0}$
- $k_{2 \rightarrow 3}[R]$

State 3
- $L = 5$ or $15$
- $L = 10$ or $20$
- State 3

LacR
- Green

T7 RNAp
- Blue
LacR dissociation time as a function of T7 concentration

\[ k_{\text{off}} = \frac{(k_{3\rightarrow1} + k_{3\rightarrow2})(k_{1\rightarrow0} - k_{3\rightarrow2})}{[P]} \cdot k_{1\rightarrow3} + (k_{3\rightarrow1} + k_{3\rightarrow2}) + k_{3\rightarrow2} \]

Michaelis-Menten type

Reciprocal of the first passage time from state 1 to state 0 or 2

\[ K_M = \frac{k_{3\rightarrow1} + k_{3\rightarrow2}}{k_{1\rightarrow3}} \approx K_d \]

For protein \( P \) in the presence of \( R \)

if \( k_{3\rightarrow2} << k_{3\rightarrow1} \)
Recall the thermodynamic result of bimolecular binding

Consider the case when a ligand molecule $L$ binds to a macromolecule $P$:

$$P + L \rightleftharpoons PL$$

The dissociation constant $K_d$ is defined as

$$K_d = \frac{[L][P]}{[PL]}$$

At equilibrium, the fraction of macromolecules that is bound is given by

$$f = \frac{[PL]}{[P] + [PL]} = \frac{[L][P]}{K_d + [P]} = \frac{[L]}{[L] + K_d}$$
Cooperativity

The two proteins either stabilize or destabilize each other simultaneously!

Through fitting the titration curve
Hence, detailed balance, independent with \([P]\) and \([R]\).

\[ K_i = \frac{k_i}{k_{-i}} \]

\[ \Delta G_i^0 = -kT \log K_i \]

\[ \Delta G_1^0 + \Delta G_2^0 = \Delta G_3^0 + \Delta G_4^0 \]

\[ K_1 K_2 = K_3 K_4 \]

**Detailed balance, independent with** \([P]\) and \([R]\).**

**Hence**

\[ K_3 > K_1 \iff K_2 > K_4 \]

Positive cooperativity

\[ K_3 < K_1 \iff K_2 < K_4 \]

Negative cooperativity
The structure of ternary complex as a function of separation

Structure Origin: Cis vs Trans?
Energy-minimized configurations for two spheres (radius $R=2.5$ nm and fraction wrapping $\gamma=0.50$) bound to a polymer subjected to 4 pN tension with linker lengths of 30 bp (a), 35 bp (b), 61 bp (c), and 66 bp (d).

No bending or twisting, no oscillation!

Koslover and Spakowitz: *PRL* (2009)
Phase shift for reversed compliment of LacR template

T7 promoter

TAATACGACTCACTATAGXXXXAATTGTGACGGATACAAATT

forward lacO1

TAATACGACTCACTATAGXXXXAATTGTGTTATCCGCTCAAAATT

reverse lacO1
DNA allostery still exists in the presence of straight binders.
The binding affinity of a protein near a DNA hairpin
Allostery through linker DNA structure

Nicked DNA

Mismatched DNA

GC rich v.s. AT rich
DNA structure

Worm-like chain: bending and twisting

- DNA is relatively rigid.
- Bending persistence length: \(~50\) nm or \(150\) bp
- Twisting persistence length: \(~100\) nm or \(300\) bp

Figure 2. Elastic Rod Model of DNA

Structural Basis for Cooperative DNA Binding by CAP and Lac Repressor, Balaeff, Mahadevan, and Schulten, Structure, 12:123-132, 2004

A hypothetic model

Protein A
specific binding

Chemical bonds

hydrogen bonds

Protein A on forward sequence

Protein B

more stable

less stable

GRDBD

GRDBD

R(n)
The hypothetic model is correct or not? From Molecular dynamics

Interhelical distances

Decay extremely rapid
Allostery through DNA induced by distortion of the major groove

Spatial correlation of major groove distances in free DNA

Major groove distances after a base pair being pulled

Allostery in nucleosomal DNA
Allostery in living cells
A course-grained model

\[ U_{\text{tot}} = U_{\text{backbone}} + U_{\text{hydrogen}} + U_{\text{stacking}} \]

\[ U_{\text{stacking}} = U_r \left( 1 - \omega_1 \theta_n^2 \right) \left( 1 - \omega_1 \theta_{n+1}^2 \right) \left( 1 - \omega_2 \Delta \theta_n^2 \right) \]
Analytical solution

\[ U_{\text{stacking}} = U_r \left(1 - \omega_1 \theta_n^2\right) \left(1 - \omega_1 \theta_{n+1}^2\right) \left(1 - \omega_2 \Delta \theta_n^2\right) \]

\[ \omega_1 \ll \omega_2 \]

\[ \theta_n \sim \left(1 - e^{-n/l_1}\right) e^{-n/l_D} \]

\( l_1 \) shows the relaxation length scale of interhelical distance changes and is estimated to be on the order of one base-pair step

\[ l_D \sim \left(\frac{\omega_2}{\omega_1}\right)^{1/2} \sim 10 \text{bp} \]
Simulation result

The position of phosphate group $(px, py, pz)$

$$\cos \theta = \frac{px}{\sqrt{(px)^2 + (py)^2}}$$

$$z = pz$$
Compared with experimental data
Future work

Structure and functions of short-length DNA

- Bioinformatics: more physiological or evolutionary significance embedded in genome;
- Detailed Structure model: Molecular dynamics (collaborated with Yiqin Gao);
- Coarse grained model: Go-type model, energy minimization;
- From two body problem to n body problem (Phase transition);
Summary

- DNA-binding affinities of two proteins exhibit either positive or negative cooperativity, dependent on the separation between the proteins on DNA;
- This through-DNA allosteric effect is ubiquitous among many different pairs of DNA-binding proteins that we examined, suggesting that gene expression and regulation are more complex than previously understood;
- Such an allosteric phenomenon has explained by molecular dynamics and a coarse-grained model.

Mathematical/physical modeling is not only just involved in the project, but is also crucial!
References

REPORTS

Probing Allostery Through DNA

Sangjin Kim,† Erik Broström,‡ Dong Xing,† Jianshi Jin,*,‡ Shasha Chong,† Hao Ge,‡ Siyuan Wang,† Chan Gu,‡ Lijiang Yang,‡ Yi Qin Gao,‡ Xiao-dong Su,‡ Yujie Sun,‡ X. Sunney Xie*,‡

Science 339, 816-819 (2013)

Modeling Spatial Correlation of DNA Deformation: DNA Allostery in Protein Binding

Xinliang Xu,†‡§ Hao Ge,‡¶§ Chan Gu,‡¶§ Yi Qin Gao,‡¶§ Siyuan S. Wang,‡ Beng Joo Reginald Thio,‡ James T. Hynes,*,∥∥∥ X. Sunney Xie,*,∥∥∥ and Jianshu Cao*,‡∥∥∥

21st Century Systems Biology


Adam P. Arkin and David V. Schaffer: Cell 144, 844 2011

Clustering of landmark papers in systems biology

Statistical models

Mechanical models
Single-cell sequencing

Single-Cell Sequencing

With the rapid progress in sequencing technologies, single-cell sequencing is now possible, promising insight into how cell-to-cell heterogeneity affects biological behavior. Achieving adequate genome coverage remains a challenge because single-cell sequencing relies on genome amplification that is prone to sequence bias. Zong et al. (p. 1622) report a new amplification method: multiple annealing and looping-based amplification cycles that allowed 93% genome coverage for a human cell. This coverage facilitated accurate detection of point mutations and copy number variations. Lu et al. (p. 1627) used the method to sequence 99 sperm cells from a single individual. Mapping the meiotic crossovers revealed a nonrandom distribution with a reduced recombination rate near transcription start sites.

Single molecule/cell meets systems biology
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Allostery in nucleosomal DNA
Allostery through linker DNA structure

If DNA is nicked
If DNA has mismatched bases