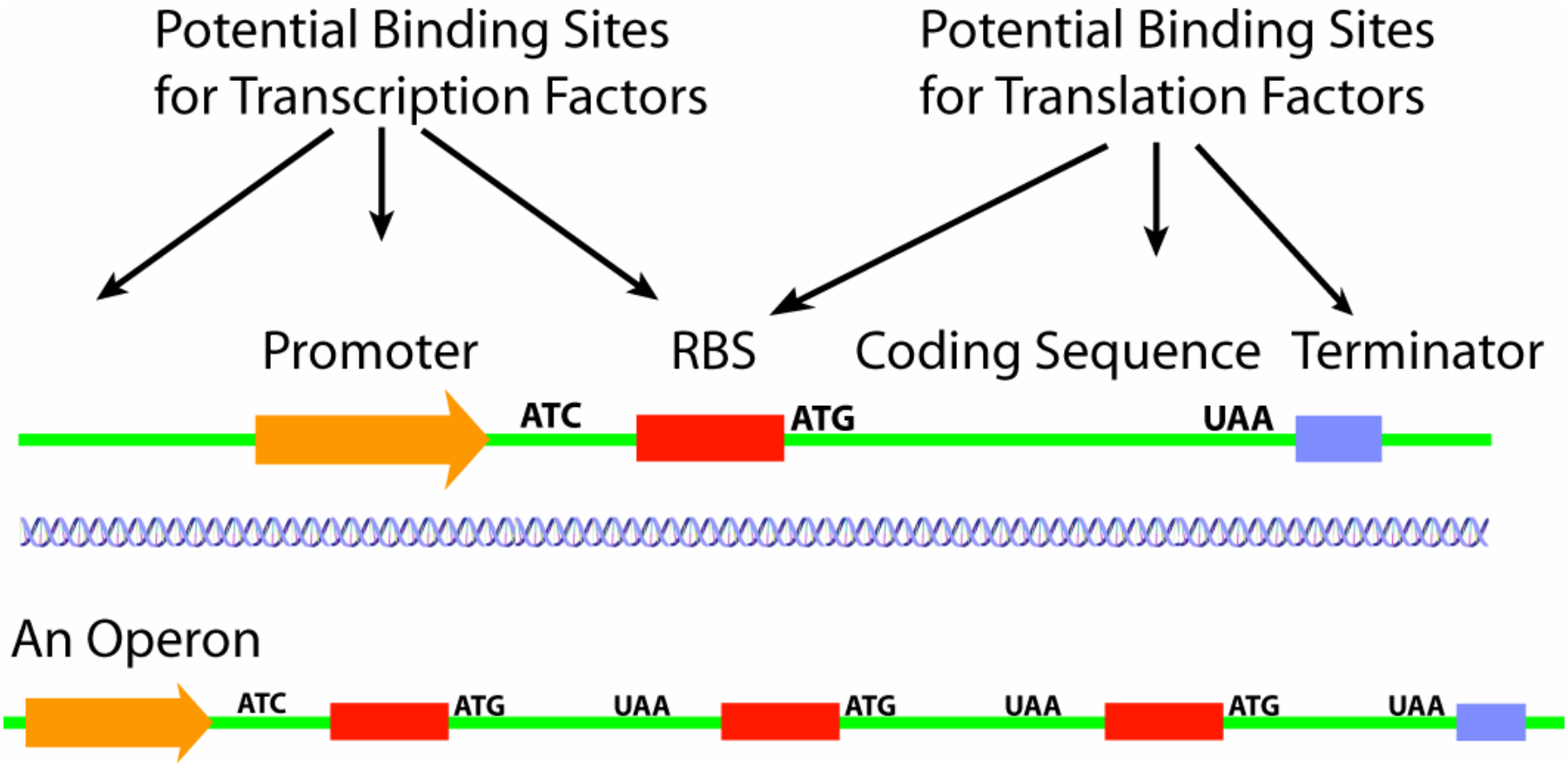
Genetic Parts in Bacterial Gene Expression

The Focus

- Promoters
- Operators
- Transcription Factors
- Transcriptional Terminators
- Ribosome Binding Sites

An Abstract Annotation



We'll Pay Particular Attention To:

- How the DNA sequence of a promoter/operator affects its function
- How transcription factors affect the rate of transcriptional initiation
- How the hybridization & secondary structure of RNA affects its function
- We will try to quantitate differences wherever possible by using thermodynamics

Thermodynamics of binding reactions (macroscopic)

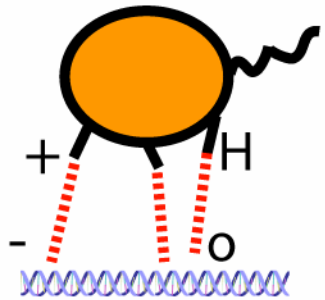
- $A + B \leftrightarrow AB$ complex
- $\Delta G = G_{before} - G_{after}$ at constant P, T
- A more negative ΔG is a stronger bound complex
  - The system wants to <u>minimize</u> its energy
- $K_A = [AB] / [A][B] = \exp(-\Delta G / RT) / (1\ M)$
  - Macroscopically measurable (affinity)

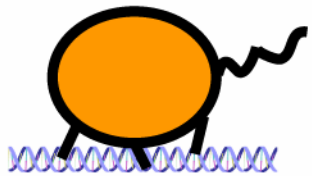
Thermodynamics of binding reactions (microscopic)

- $\Delta G = \Delta H - T\,\Delta S$
- ΔH (change in enthalpy)
  - Negative = stronger attractive interactions
  - Caused by van der Waals, electrostatics, and hydrogen bonding forces between atoms
- ΔS (change in entropy)
  - Entropy wants to be maximized
  - ΔS: the change in the number of the conformations (position + momenta) the atoms may exist in
  - Caused by hydrophobicity, torsional stress, stretching & looping of molecules

A Simplified Example

#1 Before After

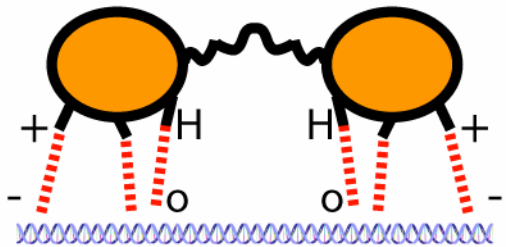


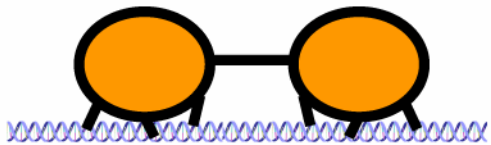


$\Delta H = -3.5$ kcal/mol $\Delta S \sim 0$ cal/mol/K

$\Delta G = -3.5$ kcal/mol

#2 Before After





$\Delta H = 2 \times -3.5 = -7$ kcal/mol $\Delta S = -6.7$ cal/mol/K

$\Delta G = -7 - 298*(-0.0067) = -5$ kcal/mol

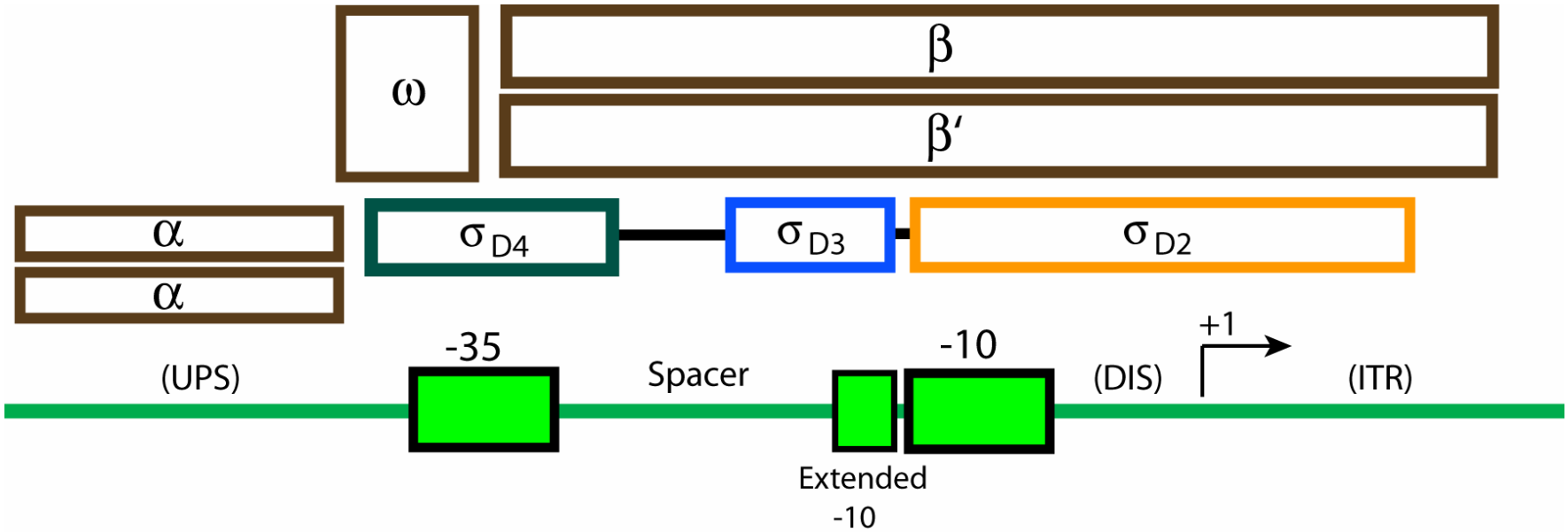
Bacterial Promoters & RNAP/σ

- Transcriptional initiation requires RNA polymerase and a σ factor
  - Steps: recruitment & assembly, closed-to-open conformational change, DNA melting, promoter escape, productive initiation (not abortive)

- The bacterial RNA polymerase
  - contains five subunits (ααββ'ω)
  - makes only weak contacts with promoter DNA

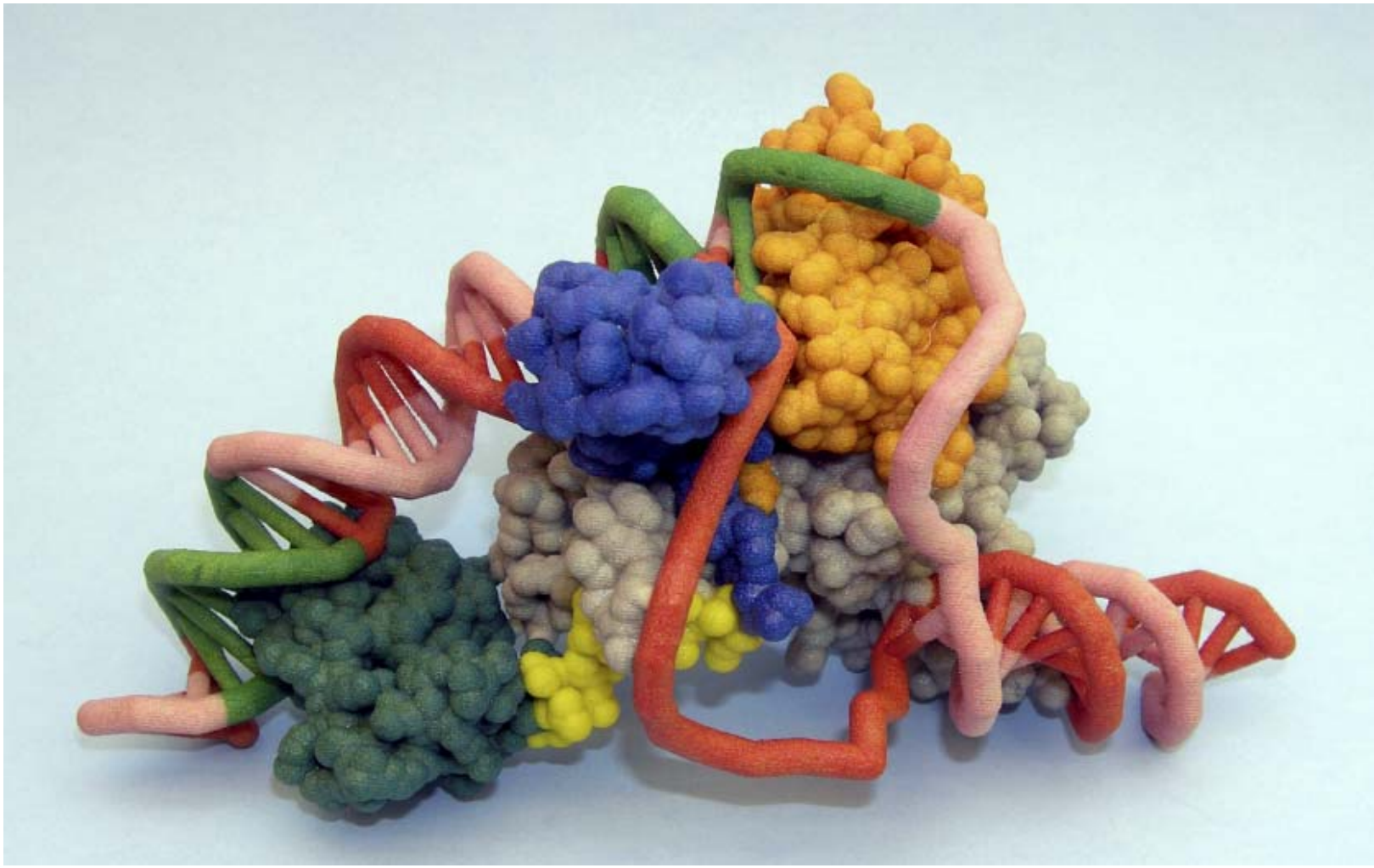
- σ factor
  - In E. coli, there are 7 σ factors
  - σ^{70} (RpoD), σ^{S} (RpoS), σ^{32} (RpoH), σ^{E} (RpoE), σ^{F} (FliA), FecI, σ^{54}
  - Each factor contains 2-4 conserved domains
  - Sigma factor binds to RNAP and mediates promoter specificity
  - σ^{54} requires an ATP-dependent activator to initiate DNA melting

Anatomy & Interactions



σ_{D1} domain is disordered & can self-inhibit DNA binding
σ_{D2} domain contacts the -10 hexamer & helps in DNA melting
σ_{D3} domain contacts the extended -10 sequence
σ_{D4} domain contacts the -35 hexamer
The α subunits of RNAP strongly contacts an AT-rich UPS element
Initial DNA melting occurs in the DIS region
Abortive initiation occurs in the ITR region

RNAP/σ^{70} Assembly



c/o Dr. Tim Herman & the Pingry school

Table of Consensus E. Coli Promoters

Sigma Factor	-35	Spacer Length	-10 / -10 extended
σ^{70}	TTGACA	15-19 (17) bp	TATAAT
σ^{S}	None		CTATACT
σ^{32}	TTGAAA	12-16 (14) bp	CCCCATTT
σ^{F}	TAAA	13-17 (15) bp	GCCGATAA

Basal Transcription Rate

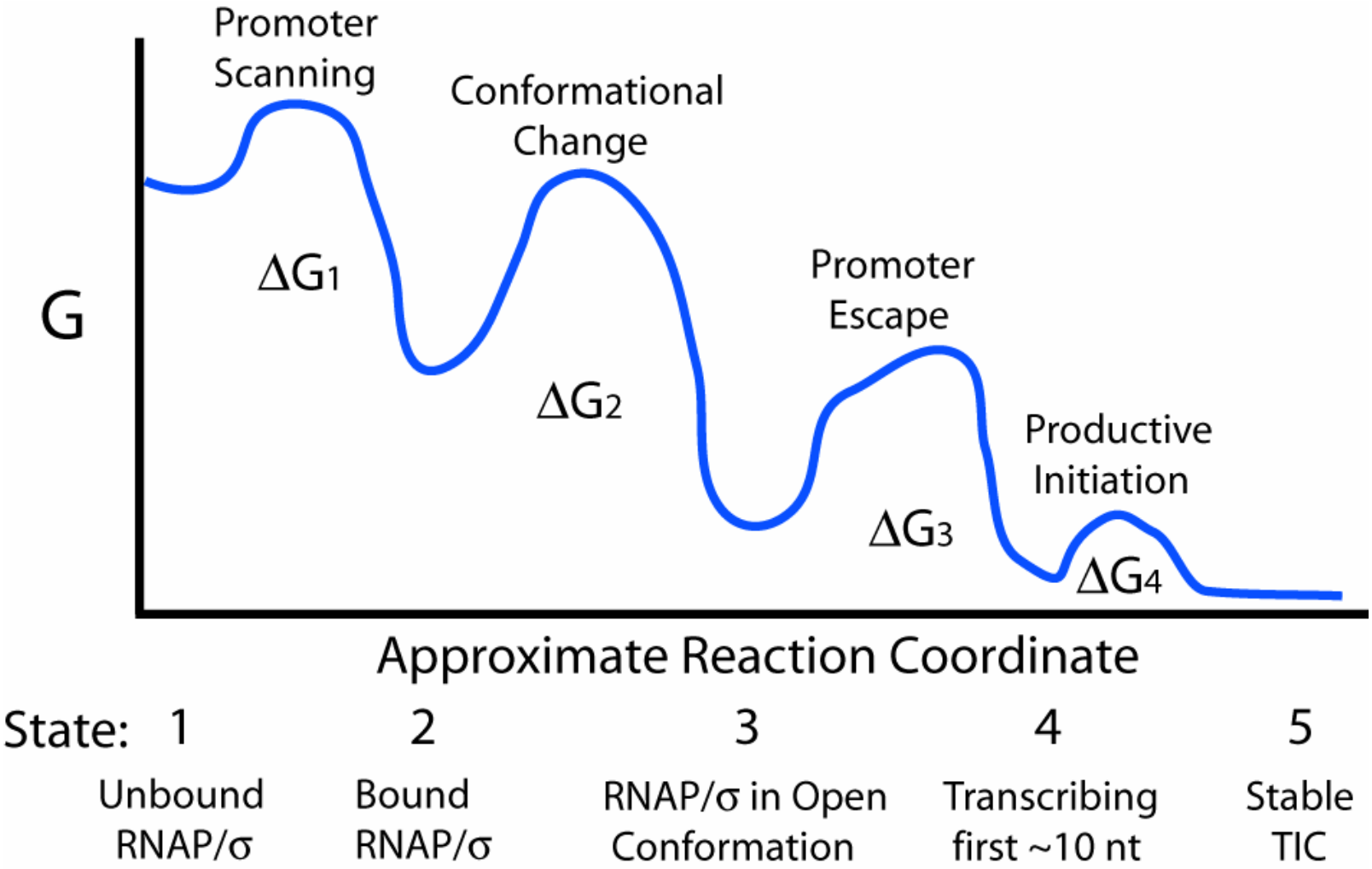
- Depends on the rate-limiting step

- RNAP/σ assembly
  - The available, active amounts of each σ factor
  - The sequences of the UPS / -35 / -10 / extended -10 regions
  - The affinity of each σ factor to the promoter
    - Most promoters can bind to more than one σ factor

- Conformational Change
  - The sequences of the -10 / extended -10 / DIS regions

- Promoter Escape & Productive Initiation
  - Breaking UPS / -35 contacts and forming new ones in the DIS & ITR regions

Thermodynamics of Basal Transcriptional Initiation



To the White Board …

- What happens to the rate of transcript. init. if …
  - The promoter deviates from the consensus sequence of a particular σ factor
  - The spacer region is too large
  - The promoter has the consensus sequence for σ^{70}, the UPS element is AT-rich, and the spacer region is optimal

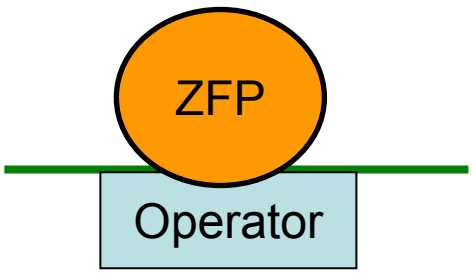
- How about in terms of ΔG's, ΔH's, and ΔS's?

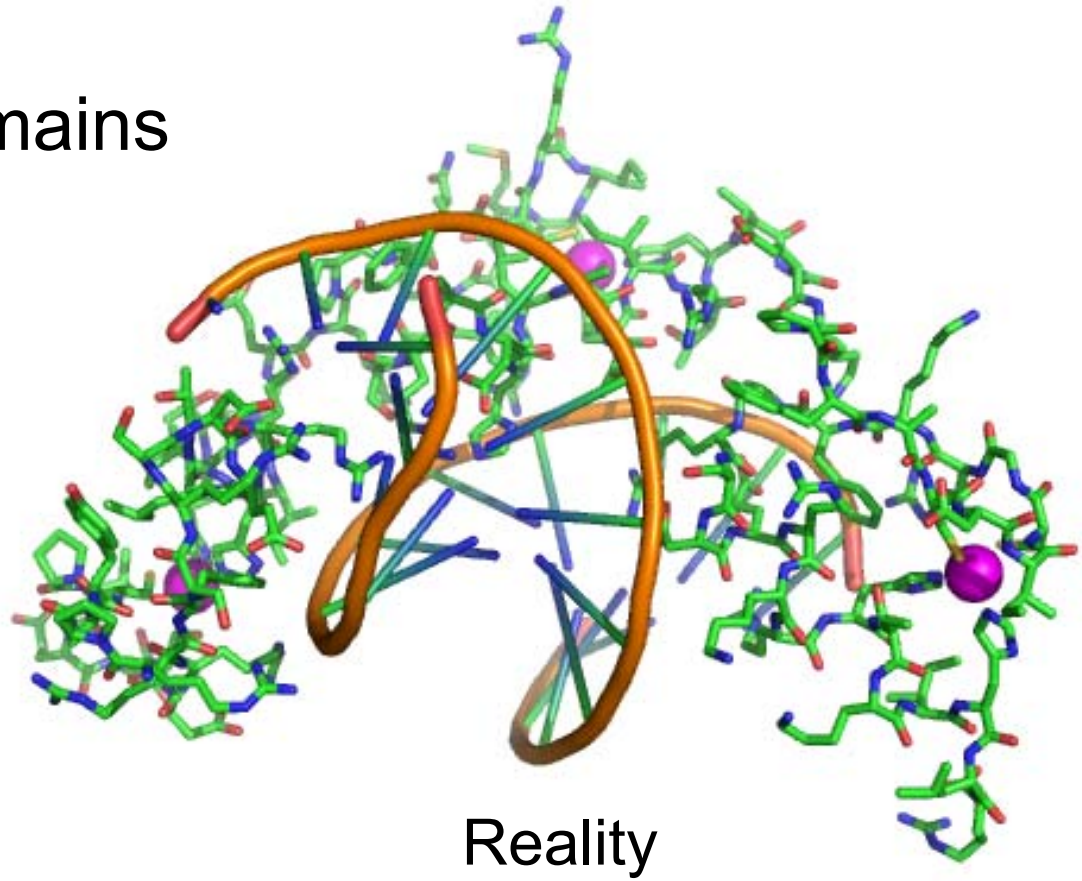
Transcription Factors & Operators

- Operators are DNA sequences that bind to transcription factors (TFs)
  - Affinity of TF depends on operator DNA sequence
- The TF mediates the rate of transcriptional initiation by making contacts with RNAP/σ
- If the contacts increase (decrease) the rate of initiation then the TF is an activator (repressor)
  - Any step in the transcription mechanism may be targeted
- The magnitude of repression / activation depends on the relative spatial position of the TF and RNAP/ σ

Example #1: Zinc Finger TFs

3 Zinc-finger Domains



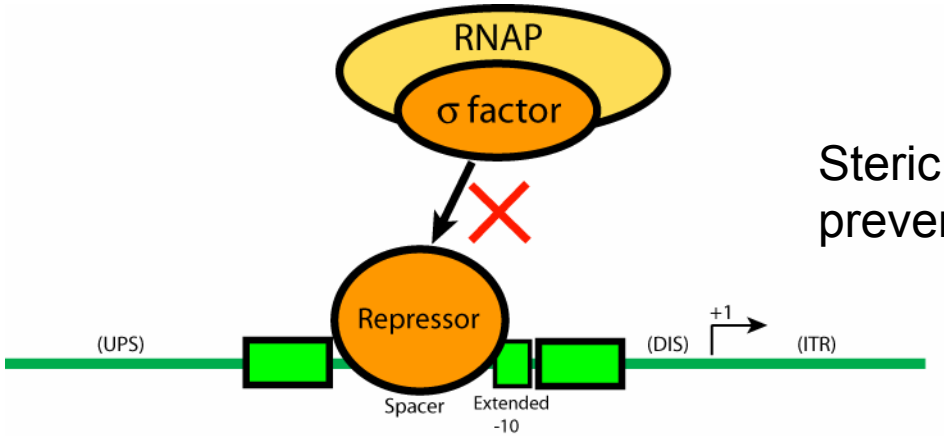


Abstraction

Reality

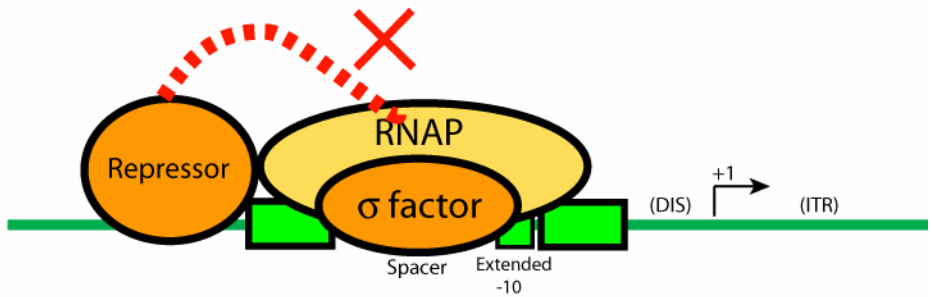
Small number of residues contact specific nucleotides while others contact the phosphate backbone (non-specific DNA-binding)

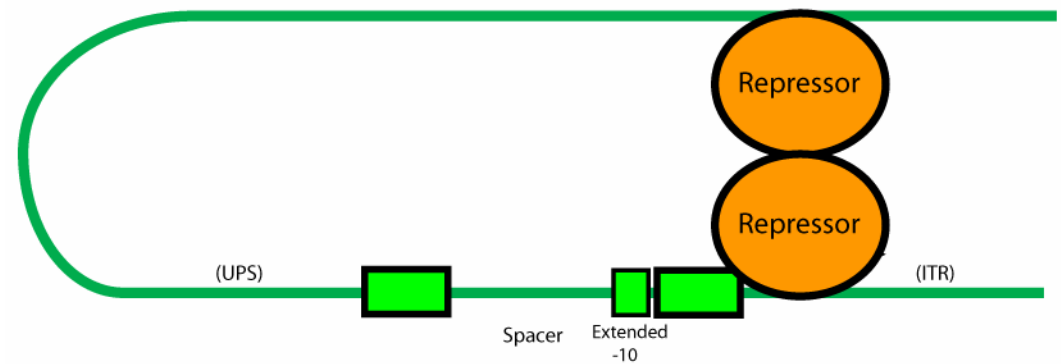
Transcriptional Repressors



Steric Interactions (van der Waals) prevent RNAP from assembling

Inhibiting the conformational change and/or promoter escape

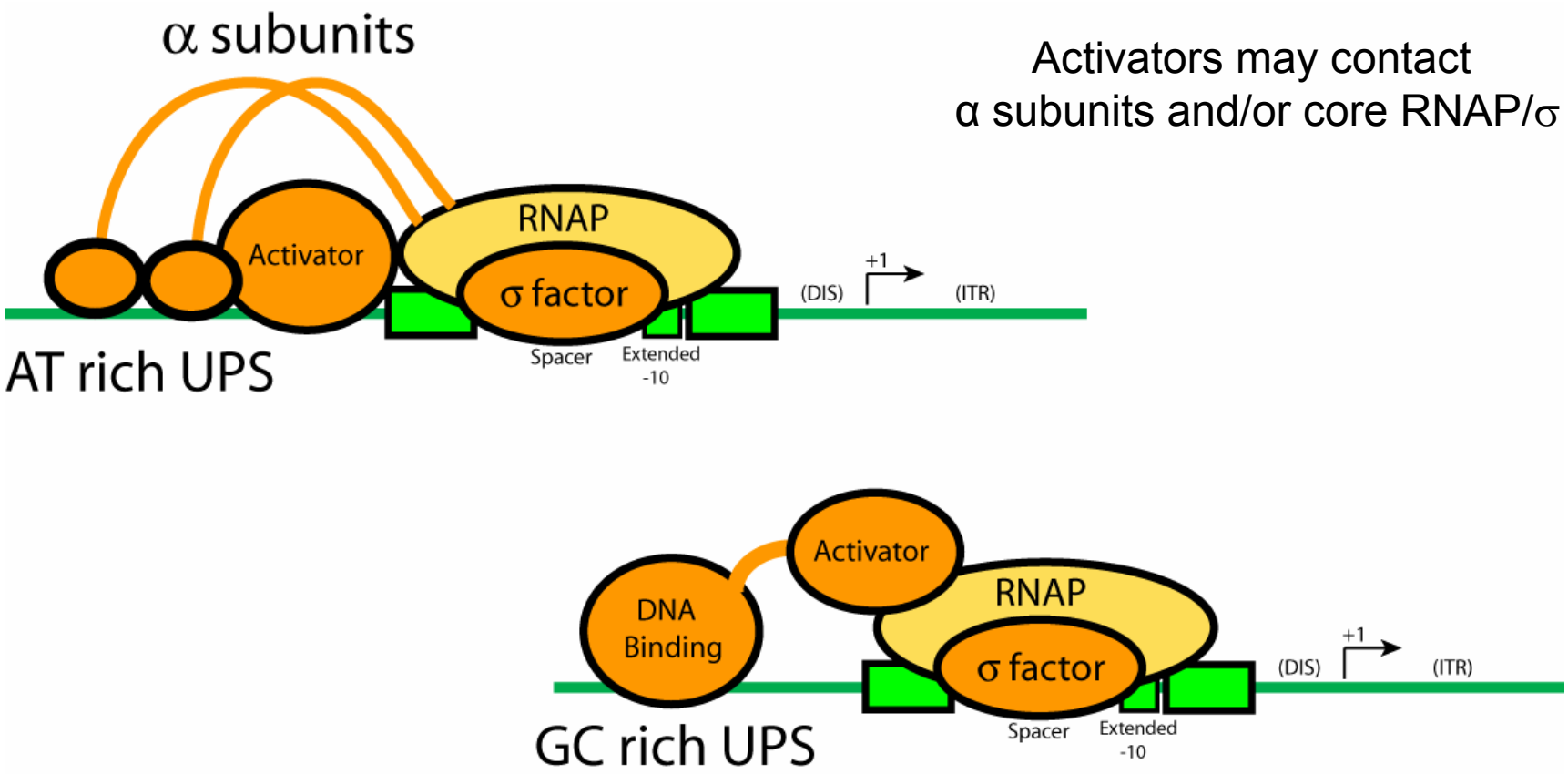




DNA-looping allows distant operators to participate in repression

H-NS protein also grabs and bends DNA at kinks

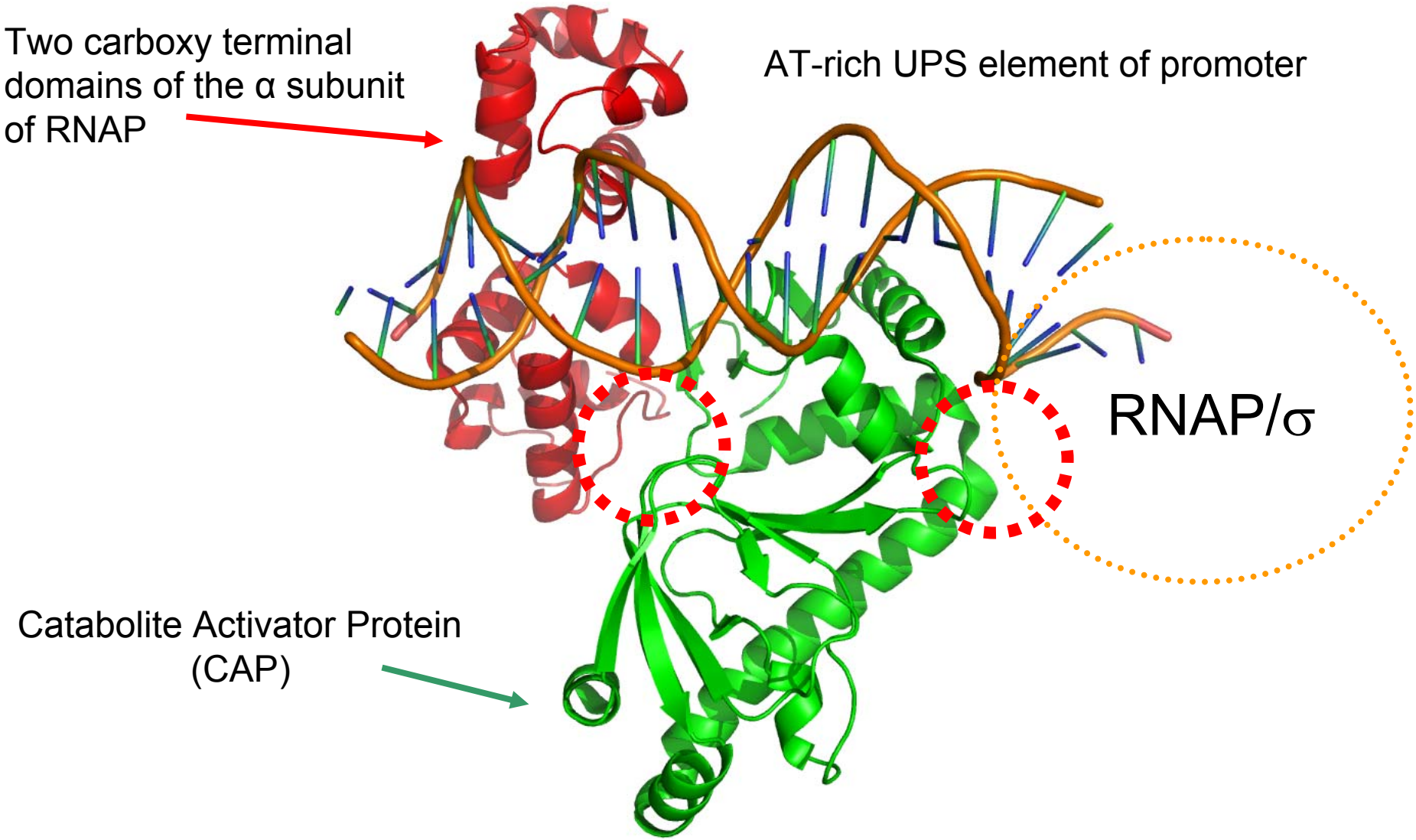
Transcriptional Activators



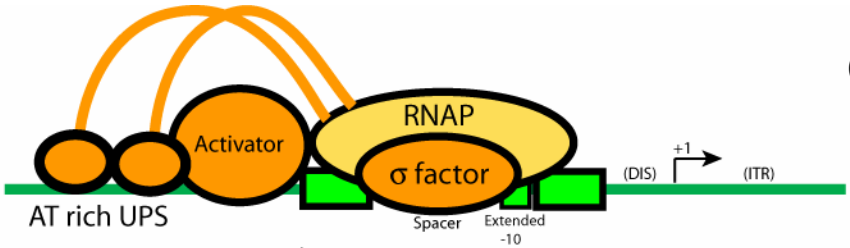
Activators may contact
α subunits and/or core RNAP/σ

Activators may have separate domains
for DNA-binding and transactivation,
connected by a flexible linker

Example #2: CAP & CTD of α RNAP subunit

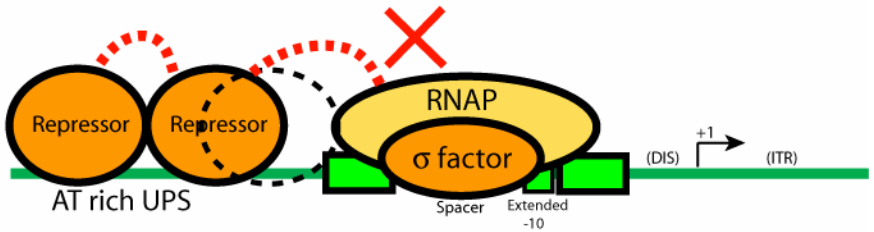


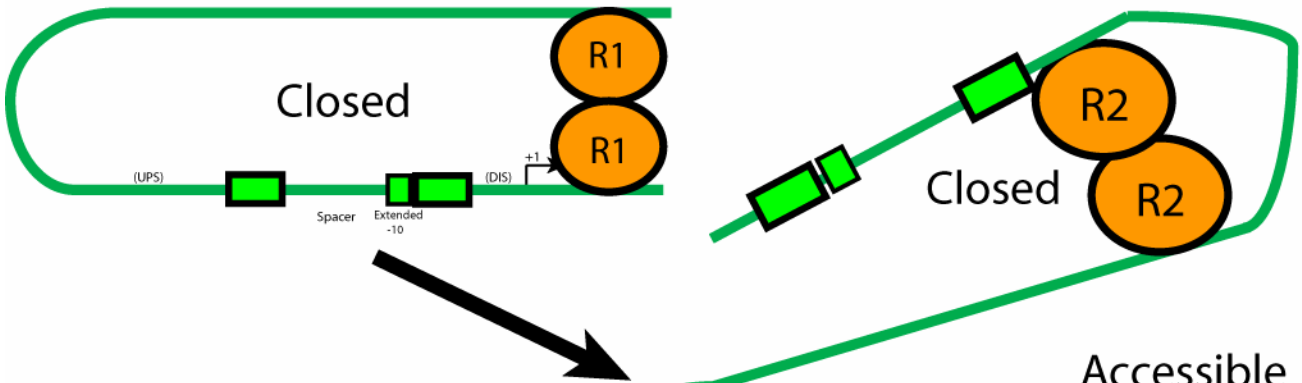
Multiple Transcription Factors



Competition between multiple TFs at overlapping operator sites

Cooperative binding via protein-protein interactions



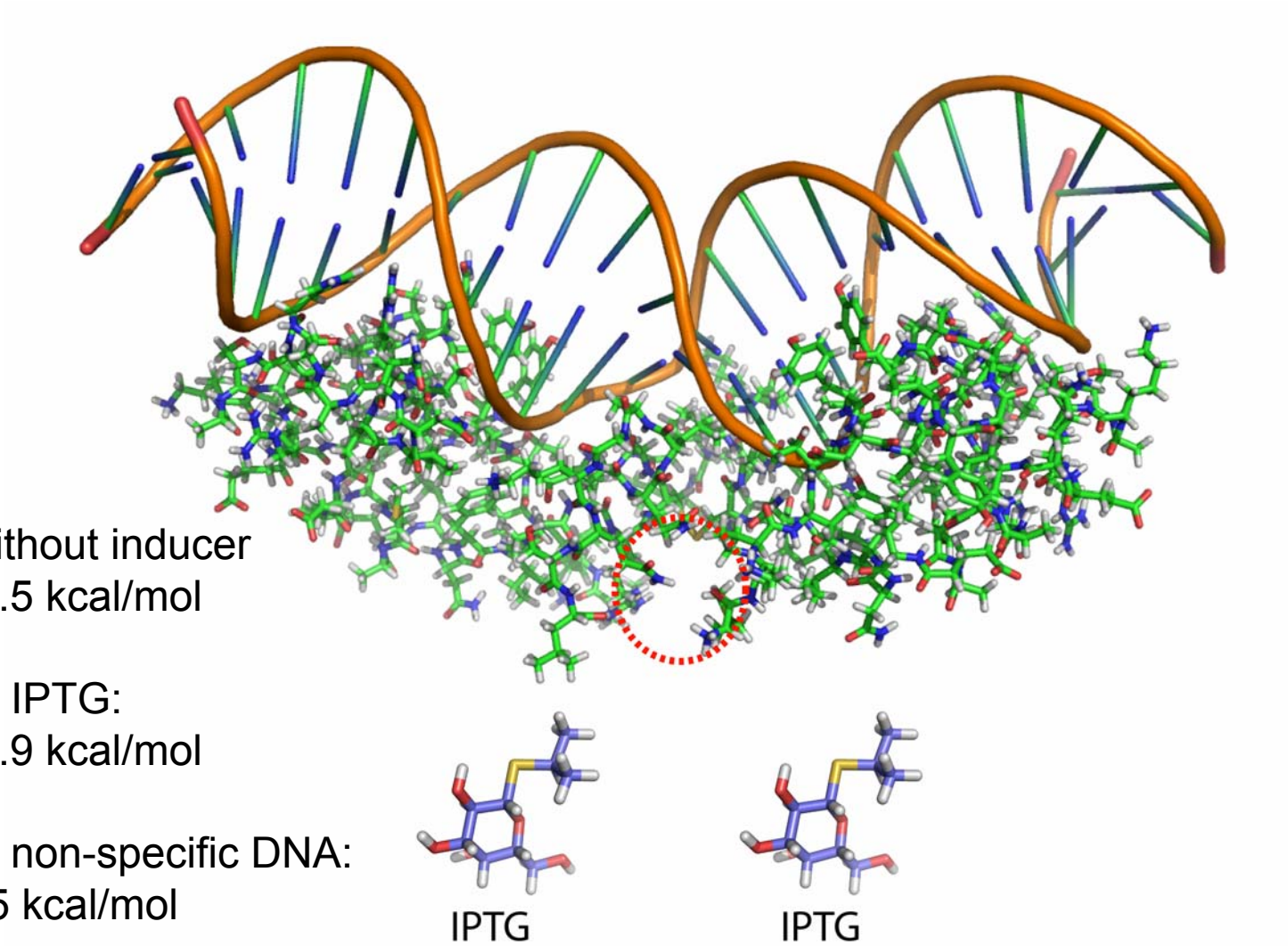


Competition between different DNA loop formations

Inducible Transcription Factors

- Small molecules can bind to TFs and alter their DNA-binding affinities and/or their structural rigidity
  - Ligand binding typically alters ΔS
- Examples
  - LacI repressor & lactose / IPTG
  - TetR repressor & tetracycline / aTC
  - AraC repressor/activator & arabinose

Example #3: Lac repressor



Bound without inducer
ΔG = -14.5 kcal/mol

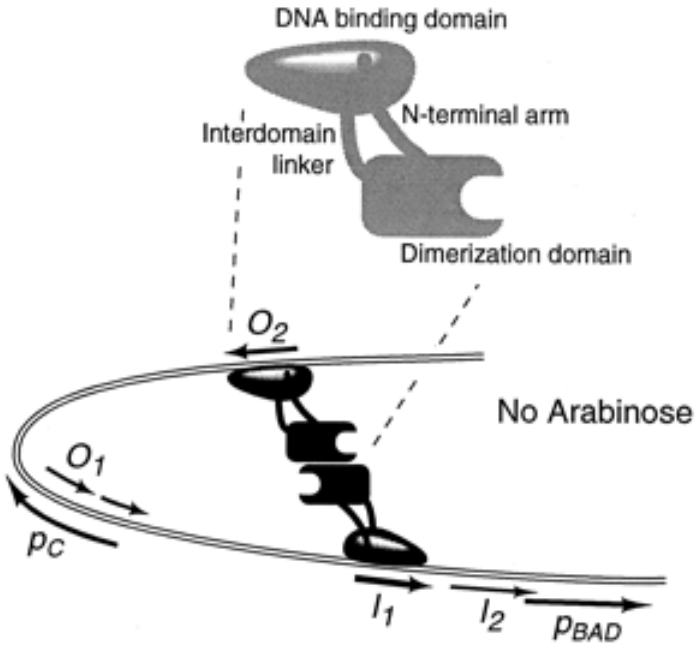
Bound to IPTG:
ΔG = -10.9 kcal/mol

Bound to non-specific DNA:
ΔG ~ -7.5 kcal/mol

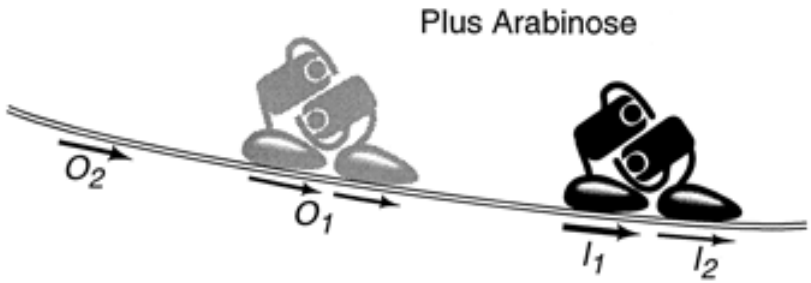
Lac dimer bound to wild-type O1 operator

Notice the DNA torsion/kinking

Example #4: AraC Activator & Repressor



DNA loop prevents RNAP/σ assembly



AraC dimer helps recruit RNAP/σ

Table of Classic TFs & Operators

- LacI tetramer (ΔG [kcal/mol])
  - O1: AATTGTGAGCGGATAACAATT (-14.5)
  - O2: AAATGTGAGCGAGTAACAACC (-13.22)
  - O3: GGCAGTGAGCGCAACGCAATT (-12)
  - Bound to O1 & 2-4 IPTG (-10.9)
  - NS: ~-7 kcal/mol
- TetR dimer
  - O1: ACTCTATCAATGATAGAGTC (-15)
  - O2: TCCCTATCAGTGATAGAGA (?)
  - Bound to O1 & 2 aTC (-11)
- AraC dimer
  - I1: CATAGCATTTTTATCCATAA (-10.7)
  - I2: AGCGGATCCTA (-8.9)
  - I1 + 4 bp spacer + I2 + arabinose (-16)

Hybrid Promoters

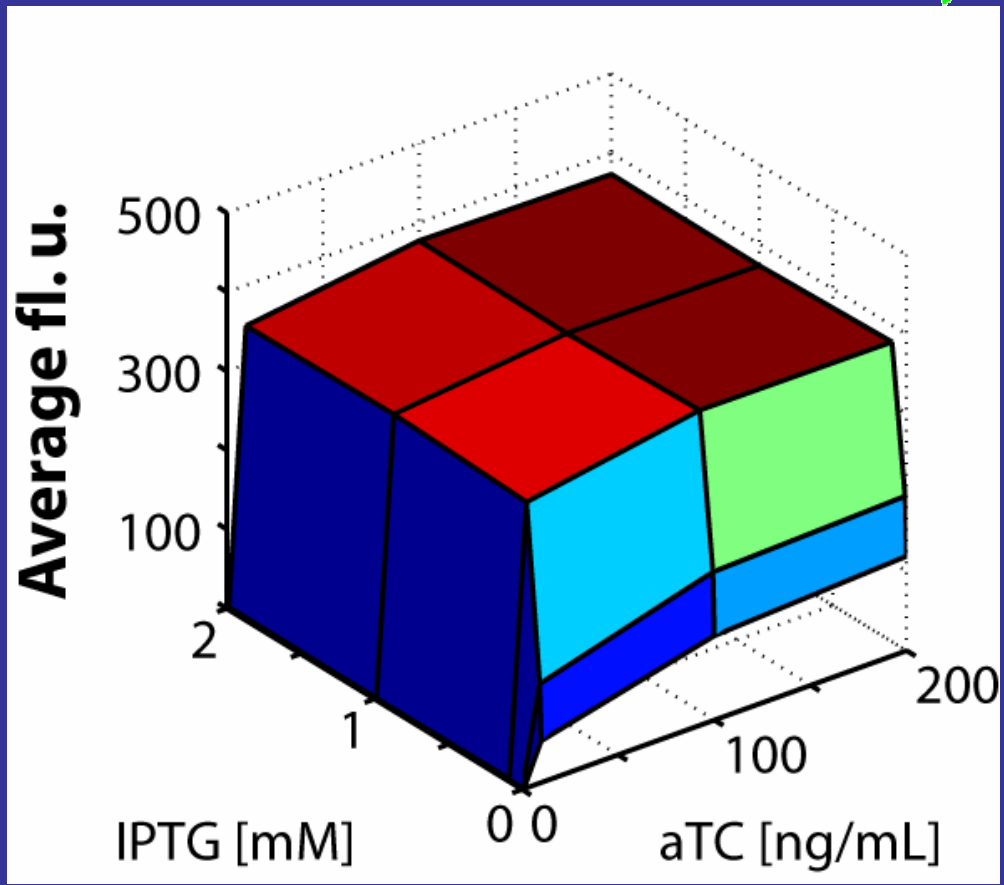
- Mix and match activator- & repressor-binding operators in a promoter
  - Each TF, bound to its operator, must individually be capable of physically contacting RNAP/σ or another TF/operator

- With inducible transcription factors, the promoter can respond to two or more signals

A Lac/Tet Hybrid Promoter

An “AND” Response

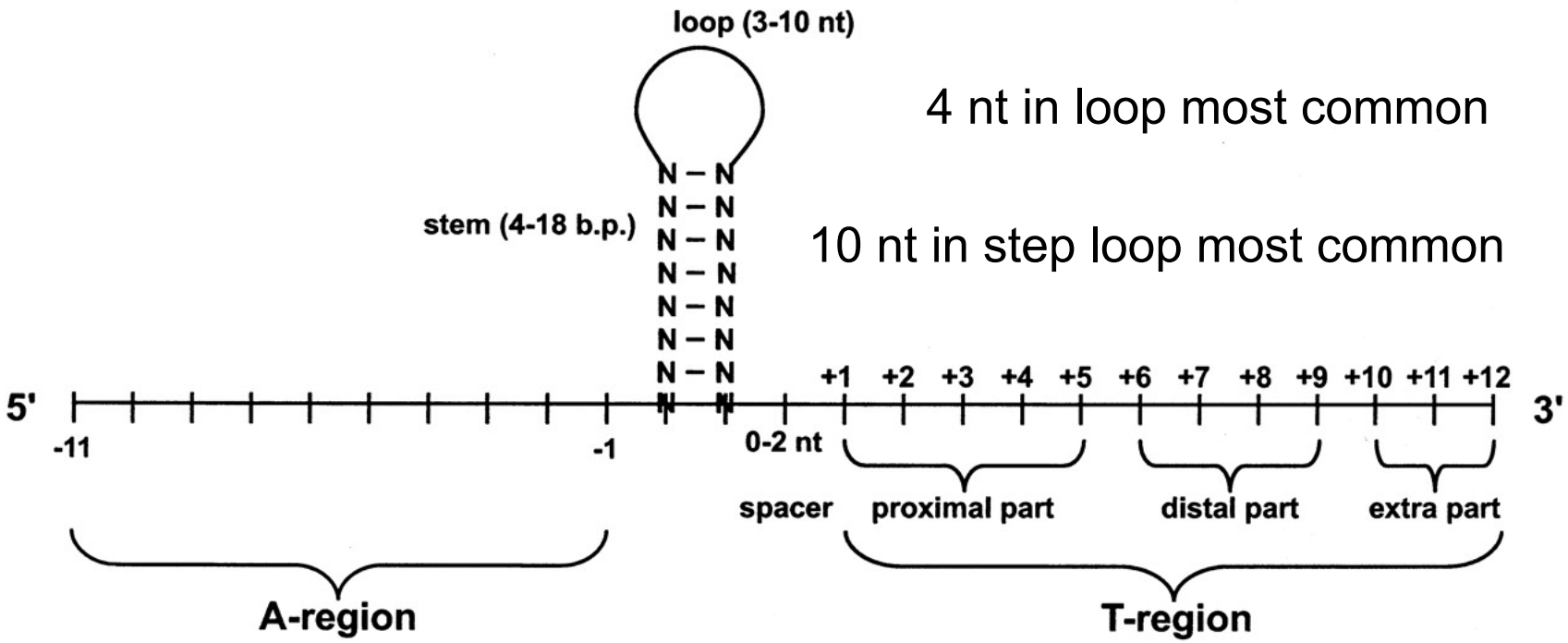
High GFP only when both IPTG and aTC are sufficiently present



RNA secondary structures

- Single stranded RNA can hybridize with other ssRNA to form secondary structures
- A single molecule of RNA can also hybridize with itself to form hairpins, clover leafs, and other structures
- These secondary structures are important to codon recognition (tRNAs), ribosome binding (rRNA:mRNA), transcriptional termination (mRNA), and translation factors (microRNAs, snRNAs, etc)

Transcriptional Terminators (Unidirectional, rho-independent)



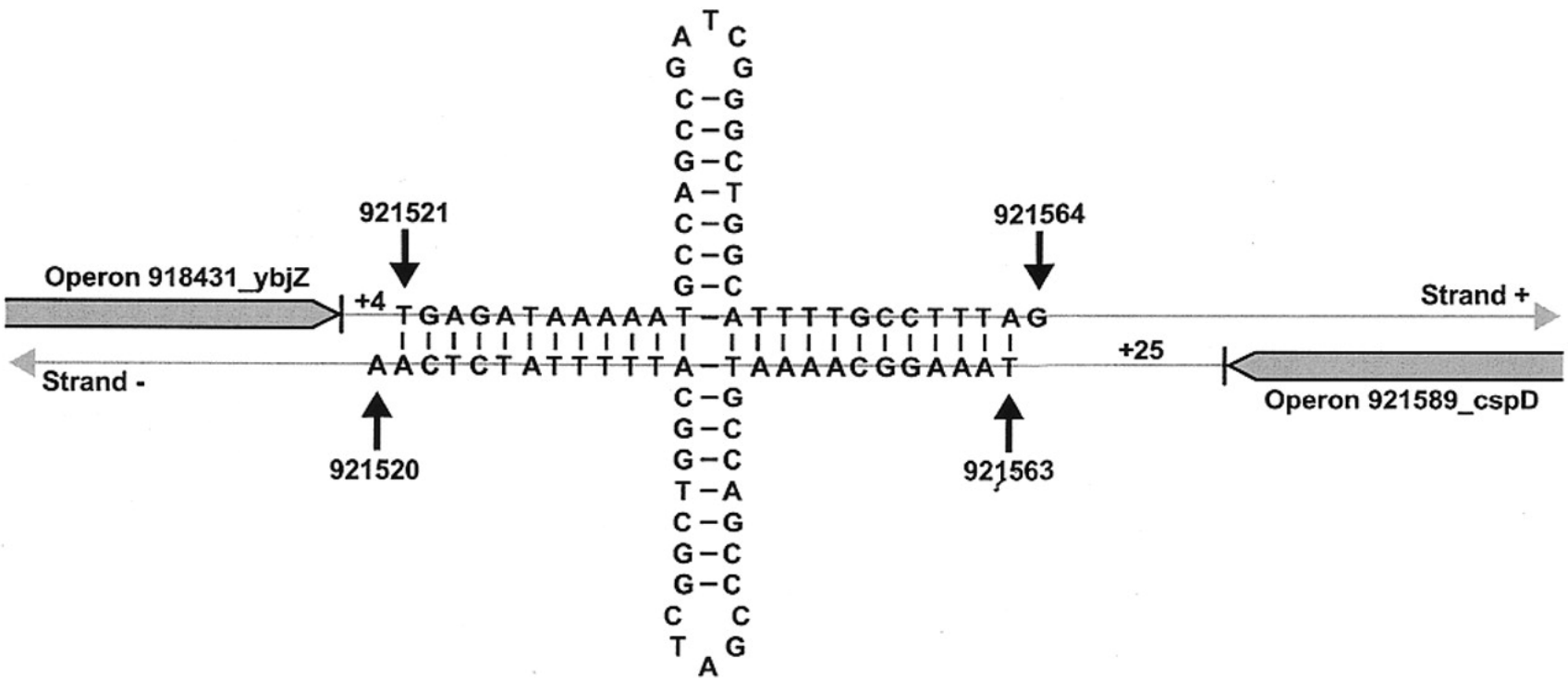
4 nt in loop most common

10 nt in step loop most common

An A-rich region, an ultra-stable stem loop, and a T (U) rich region

The stem loop forms inside the RNA polymerase, causing it to pause. The U-rich RNA forms weak RNA:DNA contacts and RNAP dissociates.

Transcriptional Terminators (Bi-directional, rho-independent)

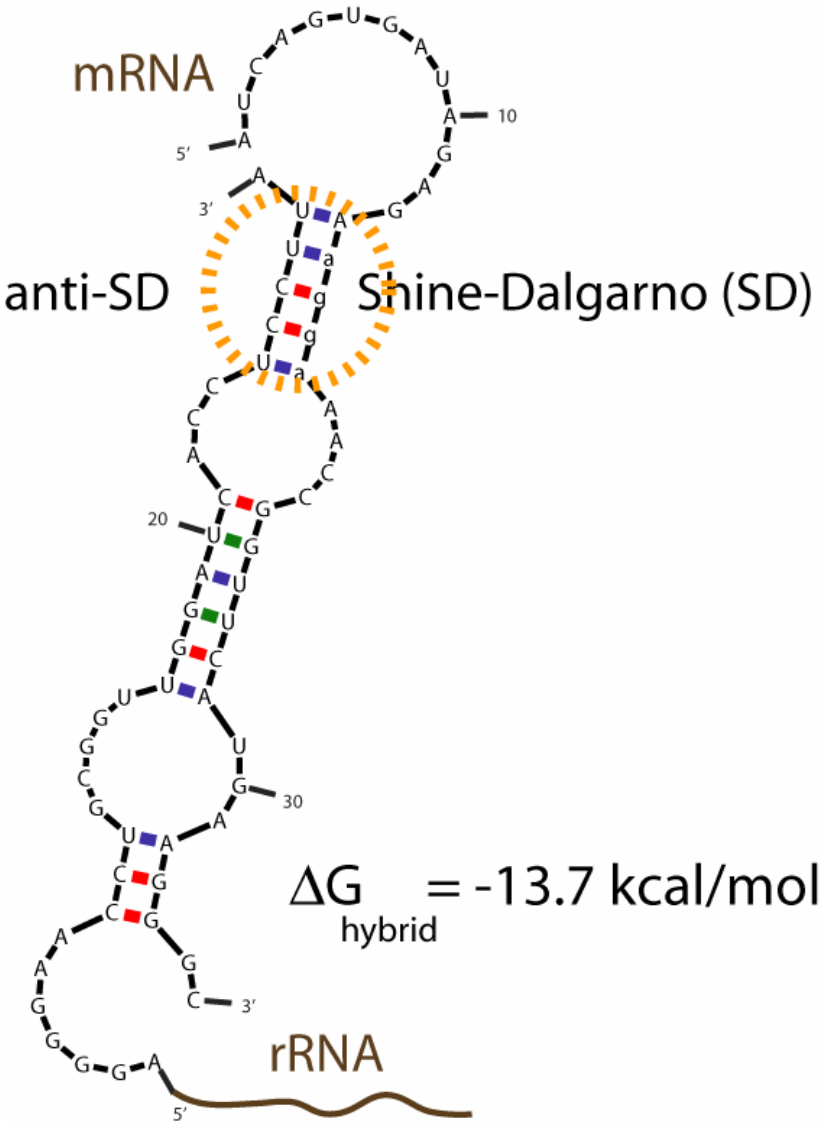


Located between the ybj and csp operons of E. coli

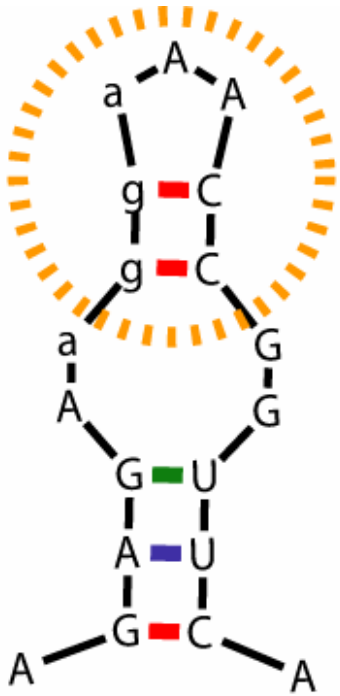
The ribosome & mRNA

- The 30S subunit of ribosome makes two key contacts with 5' UTR mRNA
  - 3' end of 16S rRNA binds to a Shine-Dalgarno sequence in mRNA
  - Ribosomal S1 protein binds to AU rich sequences in mRNA
- These contacts physically align the 30S subunit and the charged Met-tRNA with the AUG start codon
- Afterwards, the 50S subunit quickly assembles and translation initiates
- The rate of translation initiation (and protein production) is proportional to the rate of 30S subunit assembly

rRNA:mRNA Hybridization



mRNA secondary structures can inhibit translation initiation



Shine-Dalgarno (SD)

$$[\mathrm{mRNA}_{\mathrm{unfolded}}] = \frac{[\mathrm{mRNA}]_{\mathrm{tot}}}{1 + \exp\left(-\frac{\Delta G_{\mathrm{folding}}}{\mathrm{RT}}\right)}$$

$\Delta G_{\mathrm{folding}} = -2.5$ kcal/mol

$$[\mathrm{mRNA}_{\mathrm{unfolded}}] = \frac{[\mathrm{mRNA}]_{\mathrm{tot}}}{69.175}$$

Thermodynamics of some RBSs

RBS	$\Delta G_{folding}$	ΔG_{hybrid}
AGGAGGAAAAAA ATG	> 1.5	-14.6 kcal/mol
AGGAATTTAA ATG	0.1	-10.3
AGGAAACAGACC ATG	-0.2	-12.6
AGGAAACCGGTTCG ATG	-2.8	-16
AGGAAACCGGTTC ATG	-2.5	-13.7
AGGAAACCGGTT ATG	-0.7	-10.7
AGGACGGTTCG ATG	-1.3	-16.1
AGGAAAGGCCTCG ATG	-1.8	-12.6
AGGACGGCCGG ATG	-3.1	-11.2

Basal Translation Initiation

- The competition between 30S subunit assembly and mRNA secondary structure formation
- But what about other RNAs in the system?
- Translation factors ….