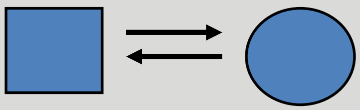
1958: Linked Equilibria

2 or more states are present:



T **R**

L = [T]/[R]

Oxygen has higher affinity for “R”

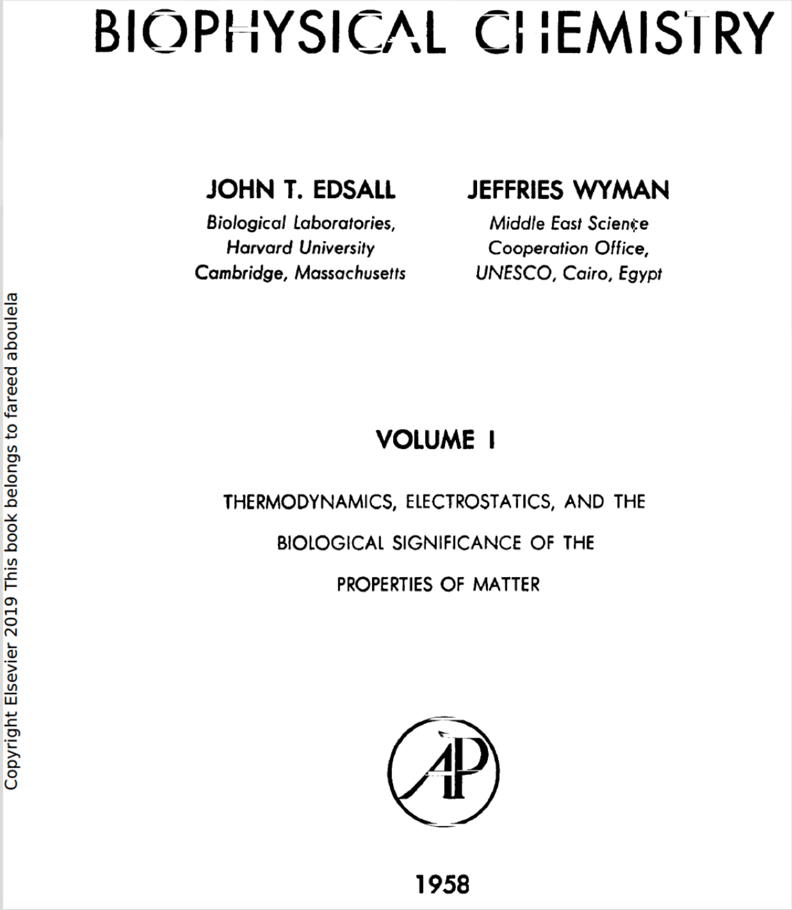
$O_2 + R \overset{\longrightarrow}{\leftarrow} R\cdot O_2$

$$K_R = \frac{[O_2][R]}{[R \bullet O_2]}$$

$O_2 + T \overset{\rightarrow}{\longleftarrow} T\cdot O_2$

$$K_T = \frac{[O_2][T]}{[T \bullet O_2]}$$

$c = K_R/K_T$



BIOPHYSICAL CHEMISTRY

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VOLUME I

THERMODYNAMICS, ELECTROSTATICS, AND THE
BIOLOGICAL SIGNIFICANCE OF THE
PROPERTIES OF MATTER

1958

Linked Equilibria

$L_1K_T = L_0K_R$

$c = K_R/K_T$ $\quad L_1 = L_0\{K_R/K_T\} = L_0c$

$L_2K_T = L_1K_R$

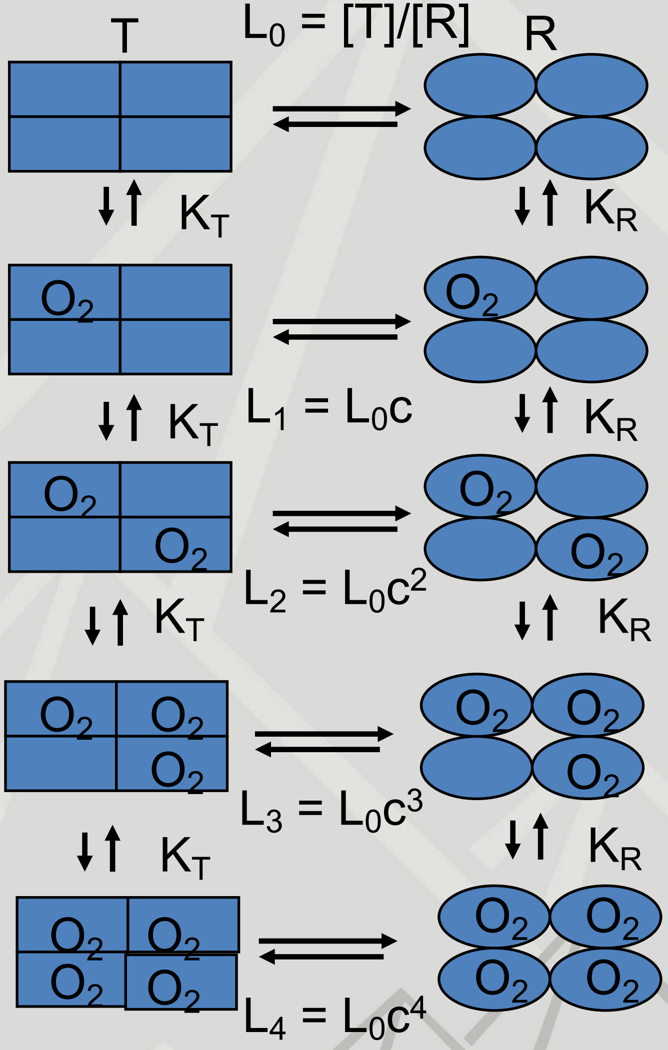
$L_2 = L_1\{K_R/K_T\} =$
$L_1c = L_1c^2$

$L_3K_T = L_2K_R$

$L_3 = L_2\{K_R/K_T\} =$
$L_2c = L_0c^3$

$L_4K_T = L_3K_R$

$L_4 = L_3\{K_R/K_T\} = L_0c^4$



T

$L_0 = [T]/[R]$

R

K_T

K_R

$L_1 = L_0c$

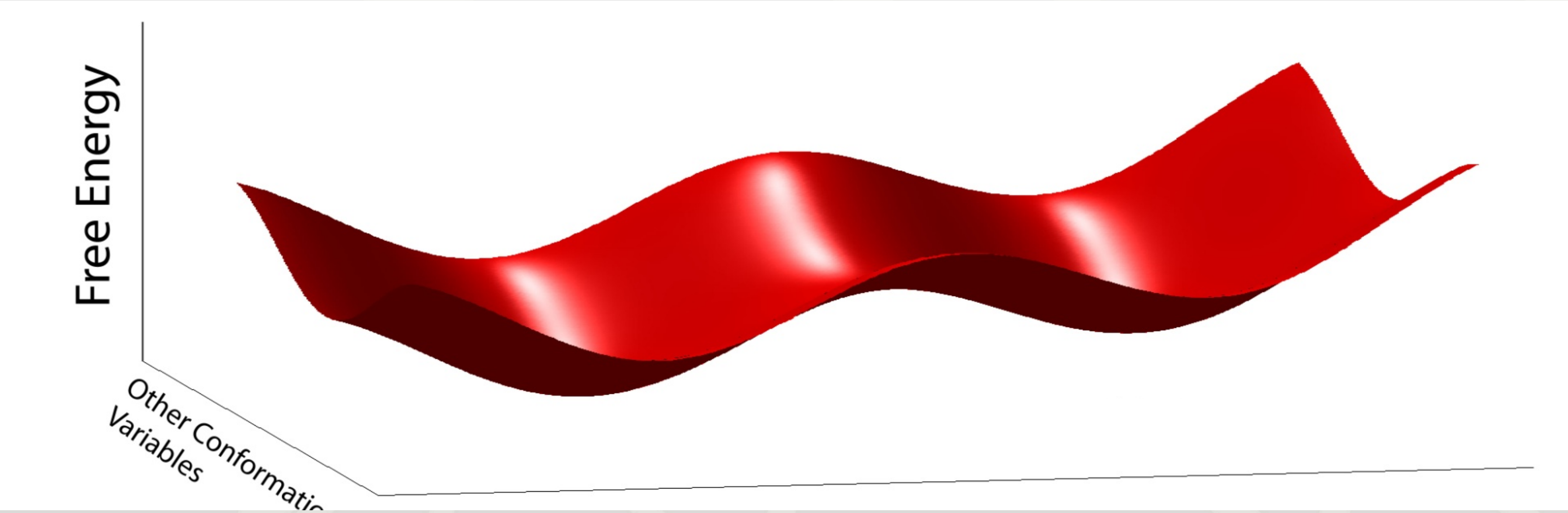
$L_2 = L_0c^2$

$L_3 = L_0c^3$

$L_4 = L_0c^4$

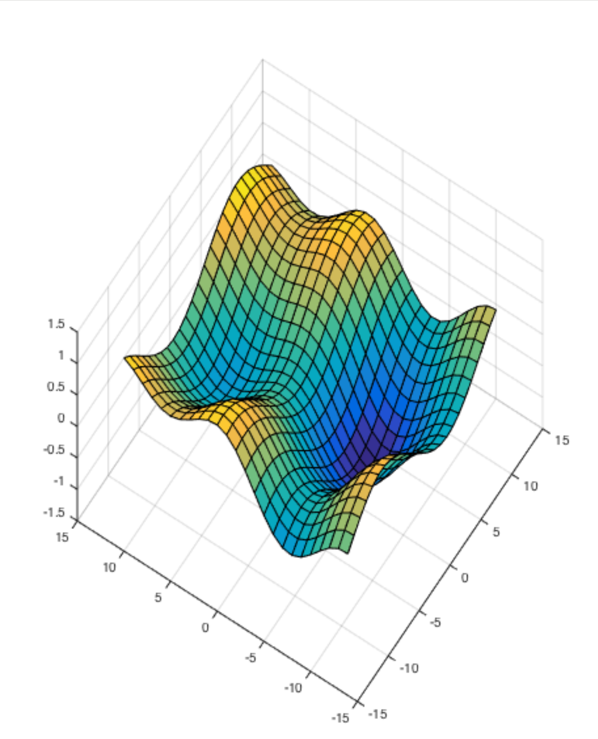
The Free Energy Landscape

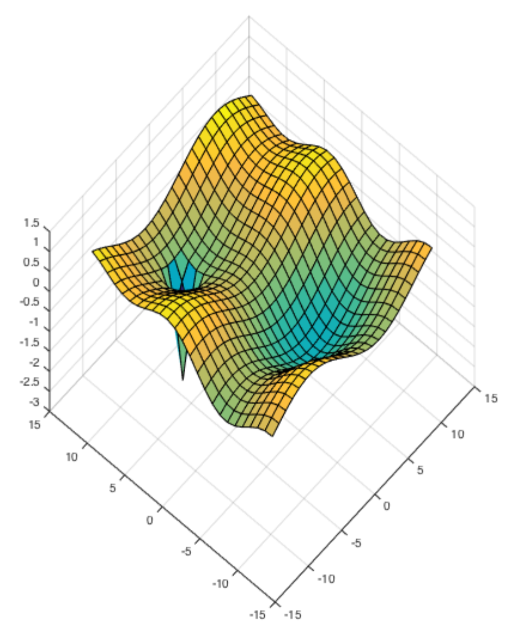
$$P_i \propto e^{\frac{-\Delta G_i}{kT}}$$



"Reaction Coordinate"

The Perturbed Free Energy Landscape





$$P_{ifree} \propto e^{\frac{-\Delta G_{ifree}}{kT}}$$

$$P_{ibound} \propto e^{\frac{-(\Delta G_{ifree}+\Delta G_{binding})}{kT}}$$

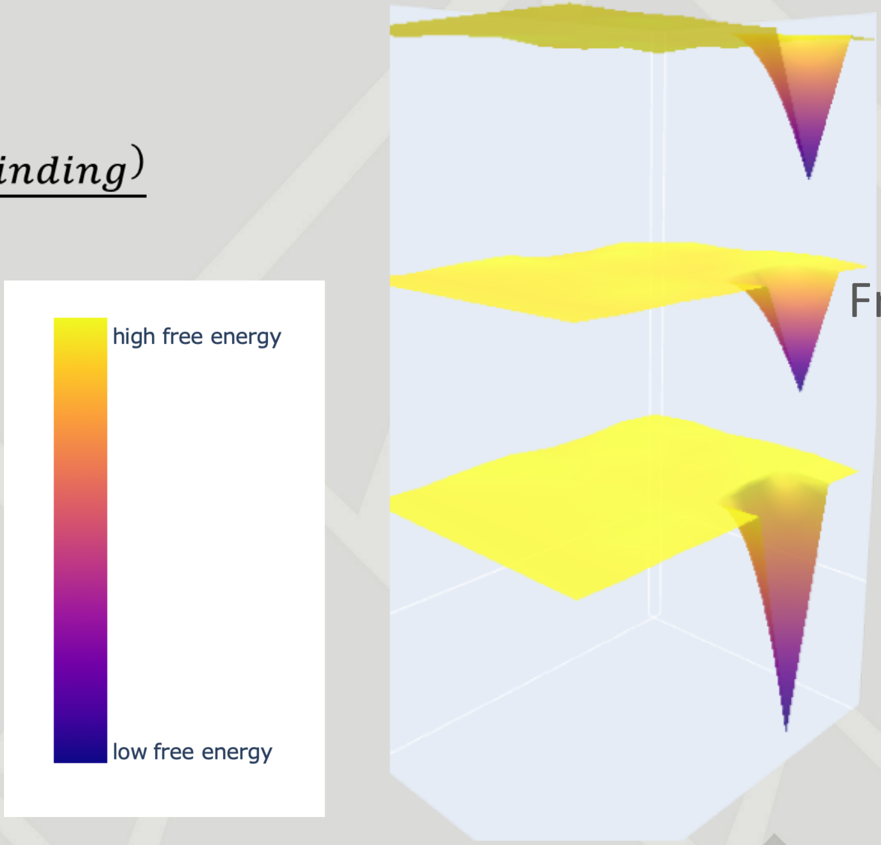
n. b. The Protein is Divided into Free and Bound fractions

The Binding Affinity Landscape

$$P_{ibound} \propto e^{\frac{-(\Delta G_{ifree}+\Delta G_{binding})}{kT}}$$

“Lock and Key” requires a very narrow and deep minimum

The “fit” must be between the FEL and the BAL



FEL

+

Binding Free Energy

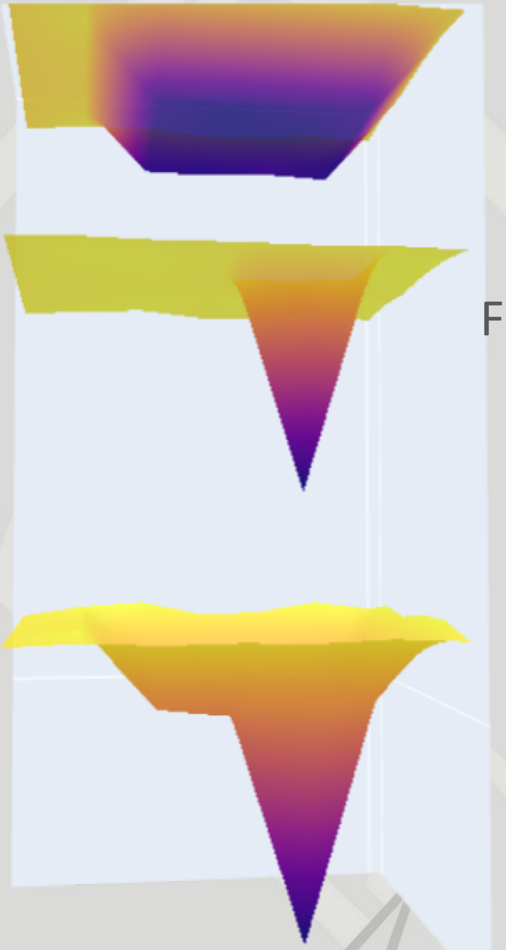
↓

Perturbed FEL

The Binding Affinity Landscape

$$P_{ibound} \propto e^{\frac{-(\Delta G_{ifree}+\Delta G_{binding})}{kT}}$$

"Conformational Capture" results from a deep, narrow min in the BAL and a broad min in the FEL



FEL

+

Binding Free Energy

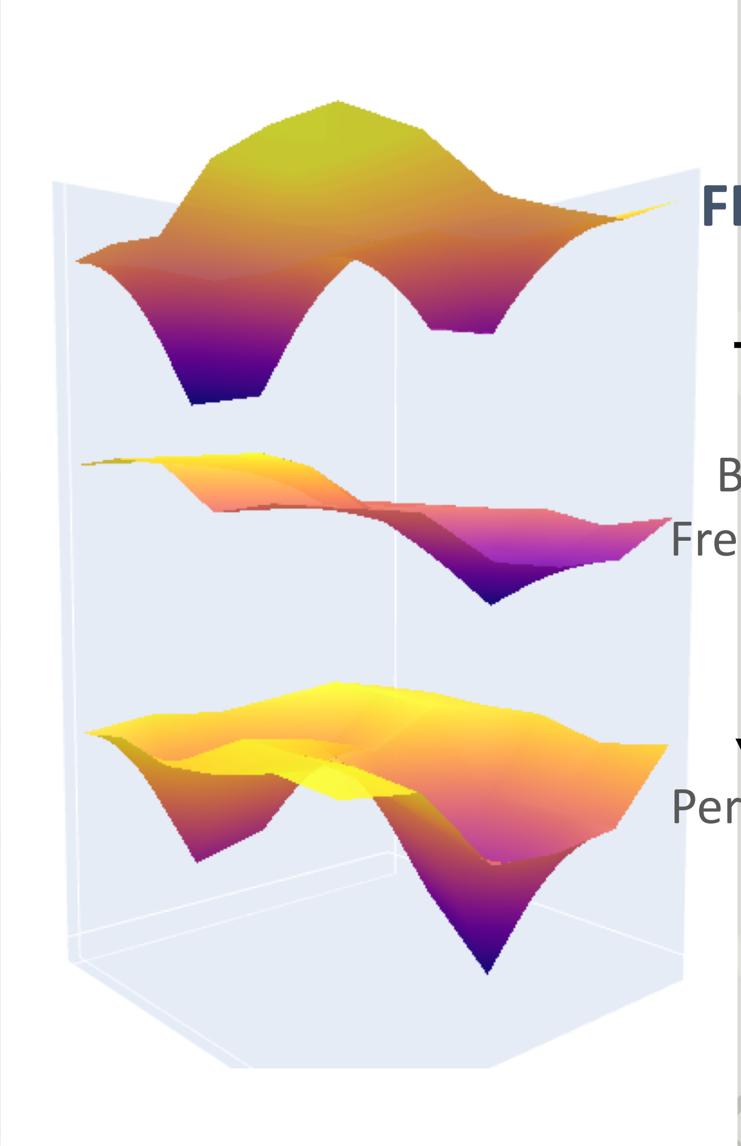
↓

Perturbed FEL

The Binding Affinity Landscape (BAL)

$$P_{ibound} \propto e^{\frac{-(\Delta G_{ifree}+\Delta G_{binding})}{kT}}$$

“Induced Fit” results from overlap of the BAL with the FEL, but with a min in a different position



FEL

+

Binding Free Energy

↓

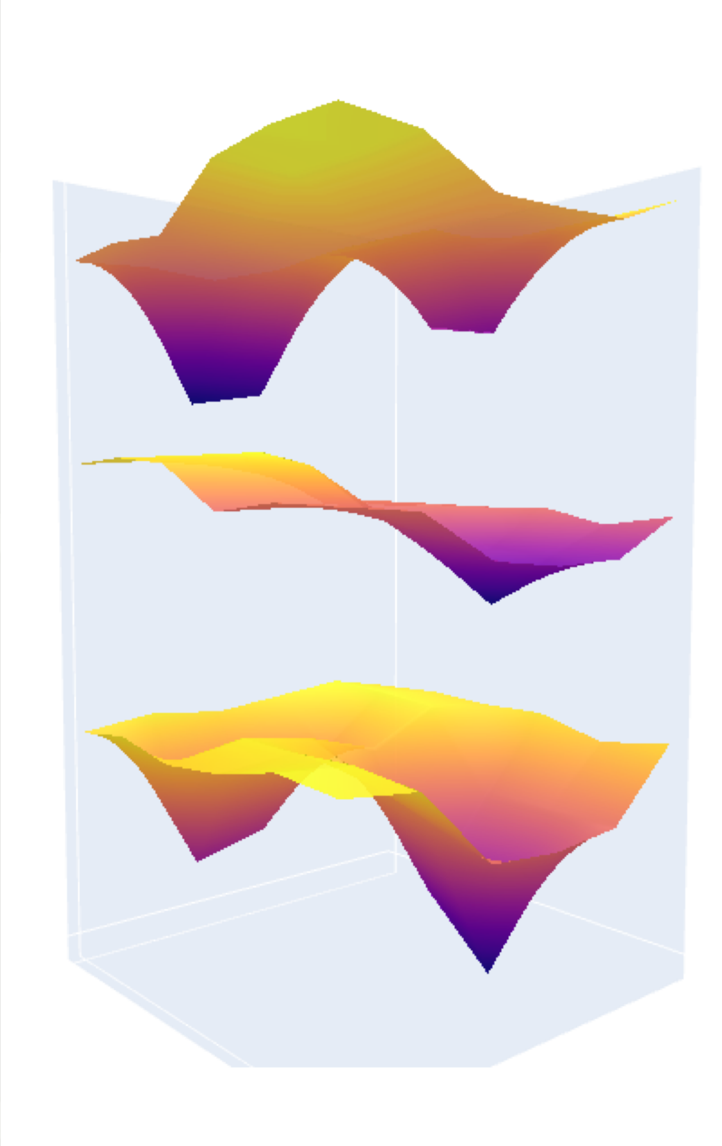
Perturbed FEL

The Perturbed FEL

$$P_{ibound} \propto e^{\frac{-(\Delta G_{ifree}+\Delta G_{binding})}{kT}}$$

We could predict/engineer biomolecular conformation from the BAL/perturbed FEL

BUT, to do so requires isolating (fixing) the representative conformers



The Perturbed FEL

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We could predict/engineer biomolecular conformation from the BAL/perturbed FEL

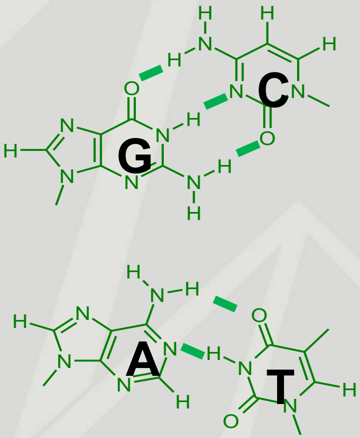
BUT, to do so requires isolating (fixing) the representative conformers

Fixing/predicting protein secondary or tertiary structure is challenging

Fixing/predicting RNA secondary structure is (nearly) tractable

The Perturbed FEL

$$P_{ibound} \propto e^{\frac{-(\Delta G_{ifree}+\Delta G_{binding})}{kT}}$$



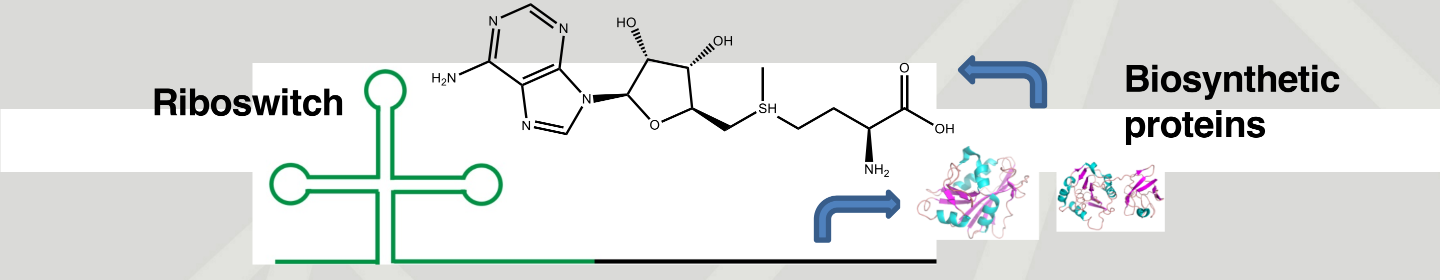
Fixing/predicting protein secondary or tertiary structure is challenging

Fixing/predicting RNA secondary structure is (nearly) tractable

Model System Needed for the Binding Energy Landscape

Riboswitches

S-Adenosyl Methionine (SAM) Riboswitch: Feedback Regulation

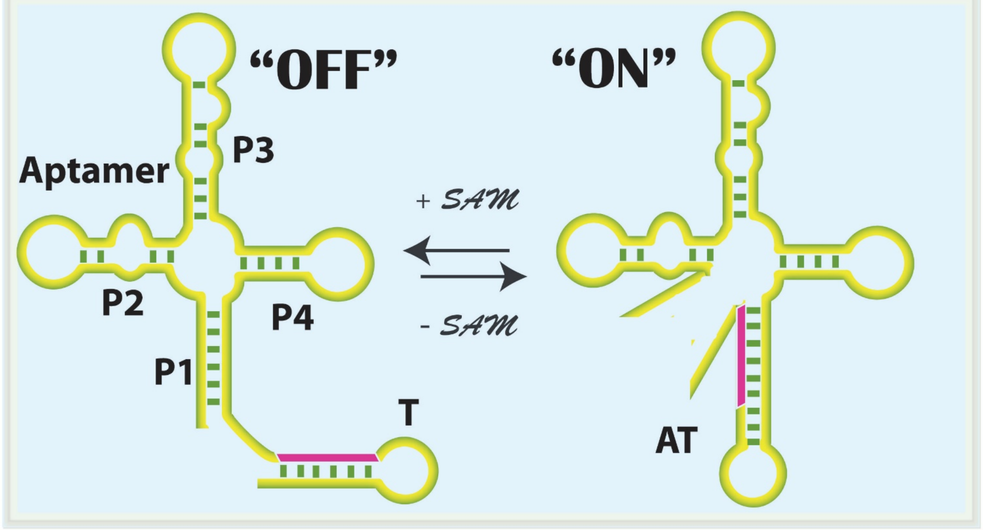


Riboswitch

Biosynthetic proteins

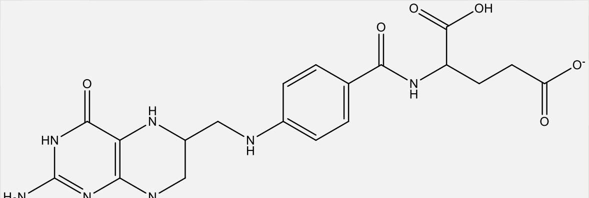
5’ UTR

Biosynthesis genes

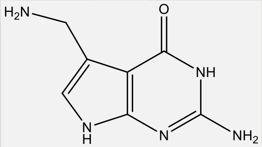


Significance of “Riboswitches”

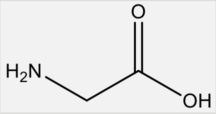
- Riboswitches are Bio-Sensors AND Gene Regulators
- Tuning of Metabolism
- (Mostly) in Bacteria
- Nanoengineering/Synthetic Biology



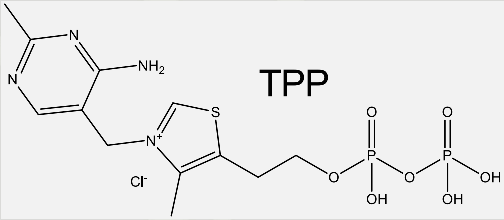
THF



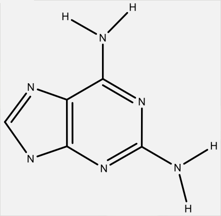
Pre-Quenosine



Glycine



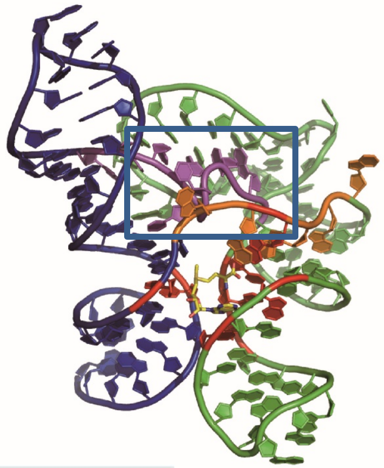
TPP

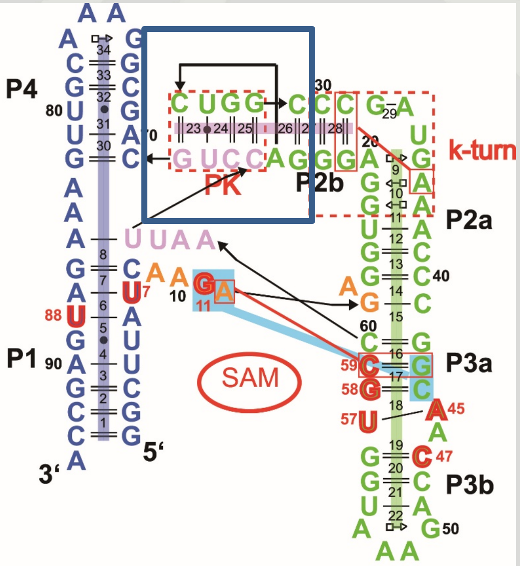


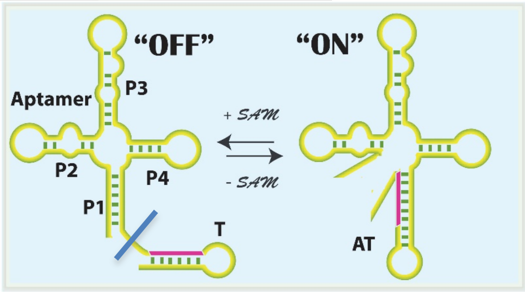
2-Aminopurine

Structure of Isolated Riboswitch Aptamer

Montange & Batey, Nature Structural Biology, 2006



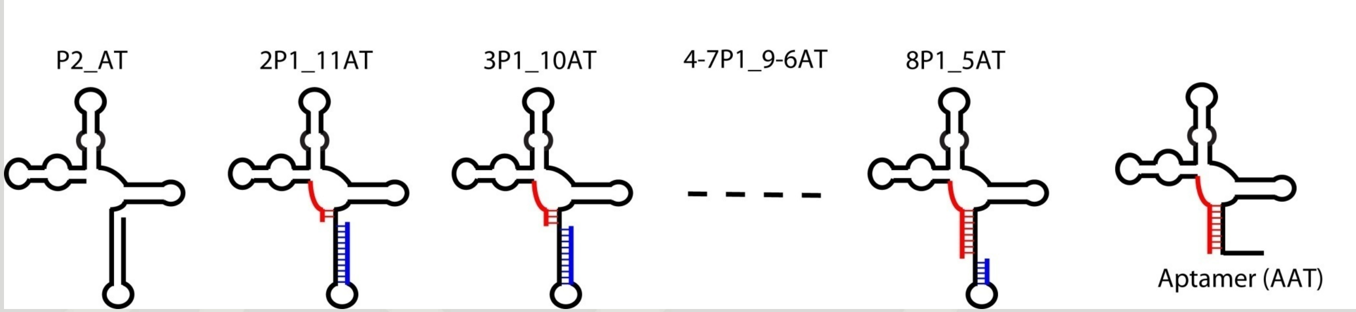


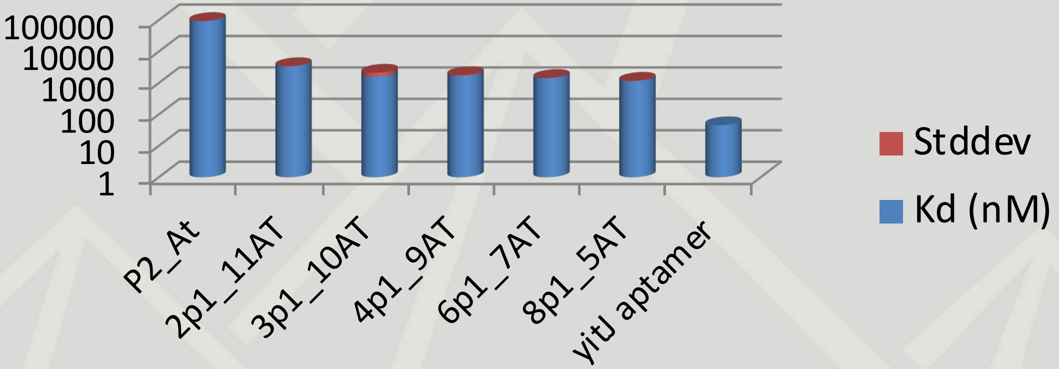


Thermoanaerobacter tencongensis

WC GC pairs ═
WC AU pairs —
GU wobble pairs ●
10th pair family ⊲–□
A-minor motif ☐
Ribose zipper ▬

SAM Binds to Riboswitch with Hybrid ON/OFF Conformation





Vamsi Boyapati

Boyapati et al, RNA 2012

Thermodynamic Cycle for Binding and Conformation

Osama Alaidi

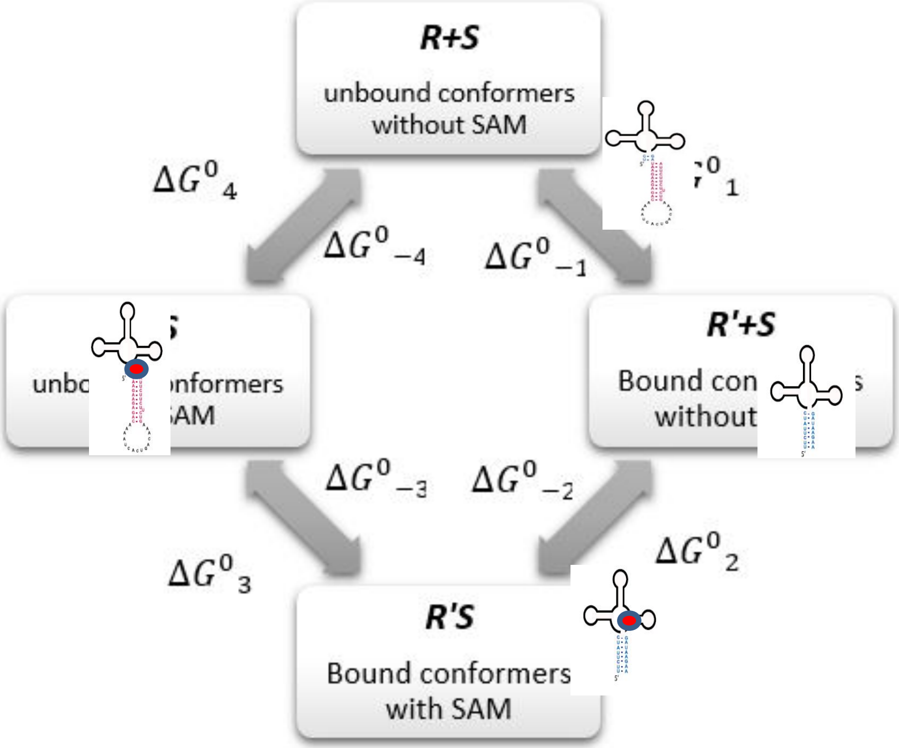
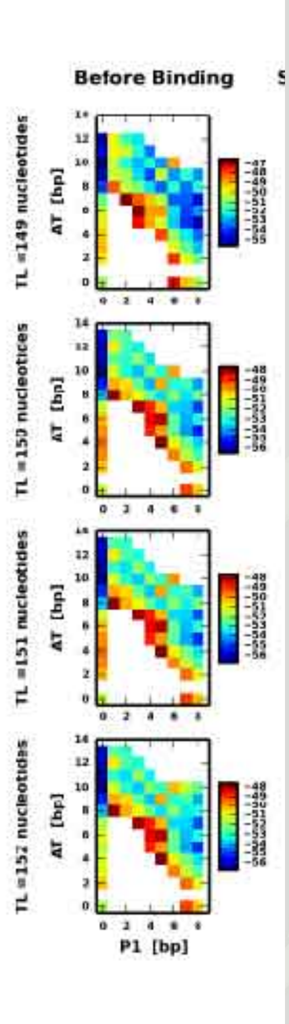
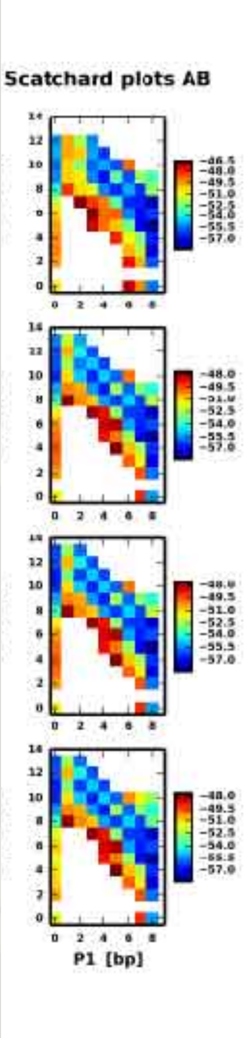


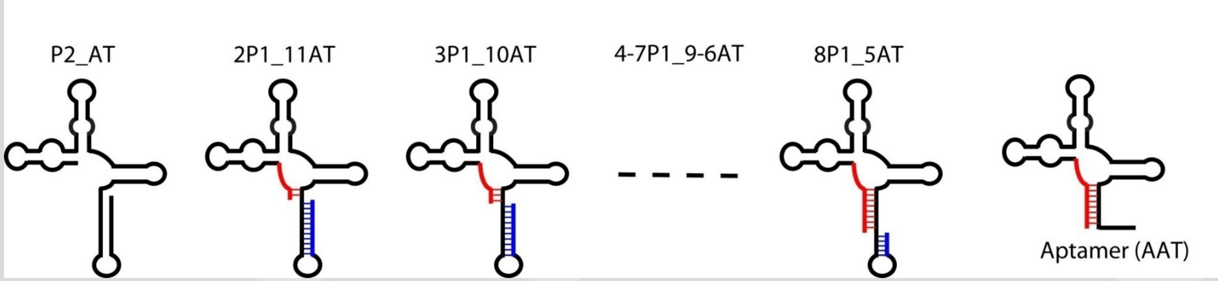
Figure 1. The figure shows the thermodynamic cycle used to calculate the change of the free energy of the population in the presence of SAM. The measured values used for the calculations are obtained in the presence of Mg^{++} ions.

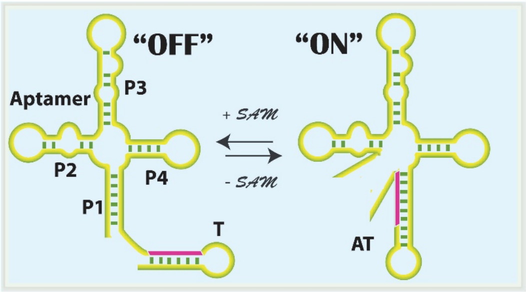
Alaidi & Aboul-ela, JCC 2020

Perturbed FEL for SAM-I Riboswitch RNA

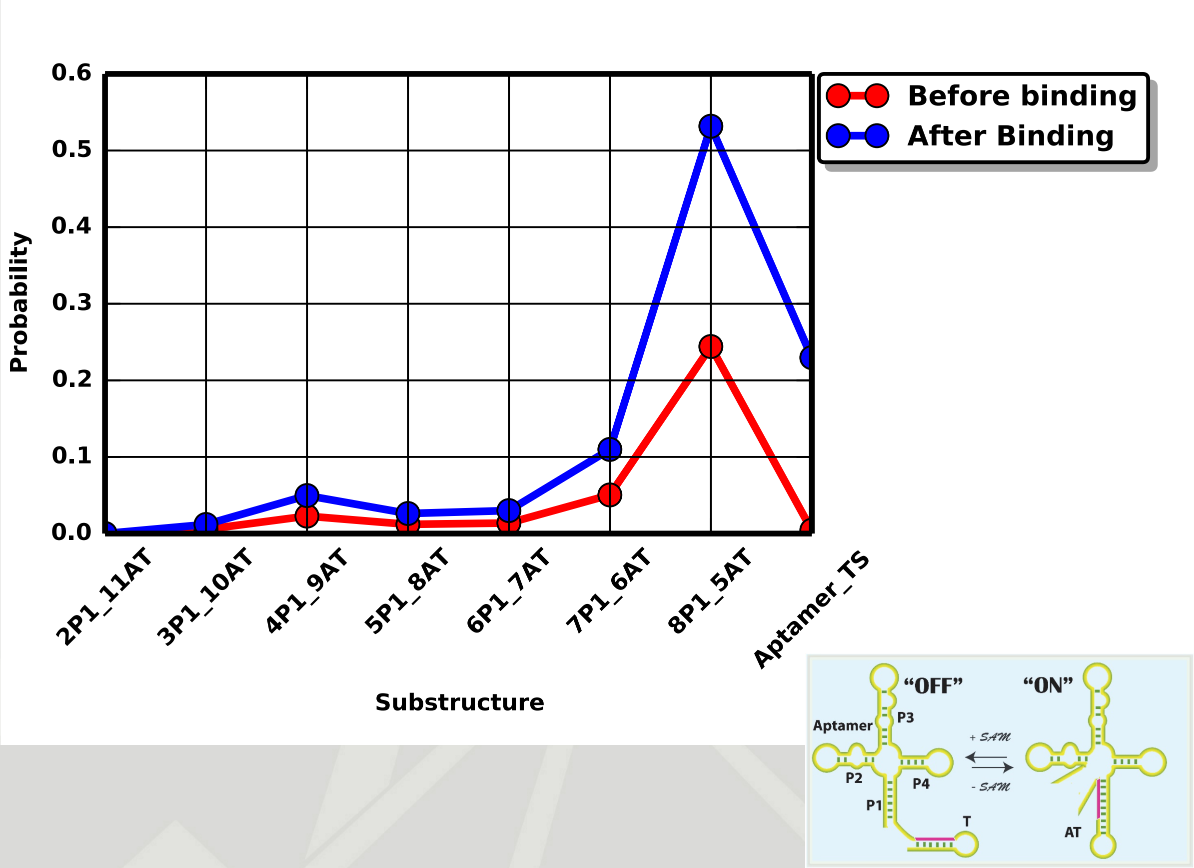




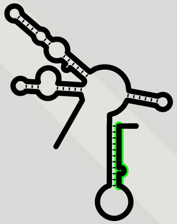




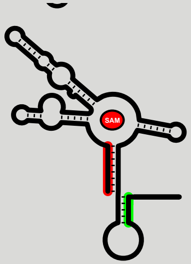
SAM Binding Facilitates Formation of RNA Transition State



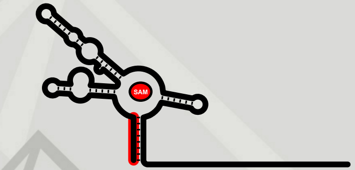
0P1_11AT



8P1_5AT



Aptamer_TS



Summary

- Binding Energy Landscape has been measured
- “Tuned” riboswitch response can be calculated/predicted/engineered
- Experimental evidence?
- Prediction of BAL/pFEL and ligand-induced allostery?

Acknowledgements

ZC Center for X-Ray Determination of the Structure of Matter

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Omar Taha
Dahlia Awwad
Fayrouz Mohamed

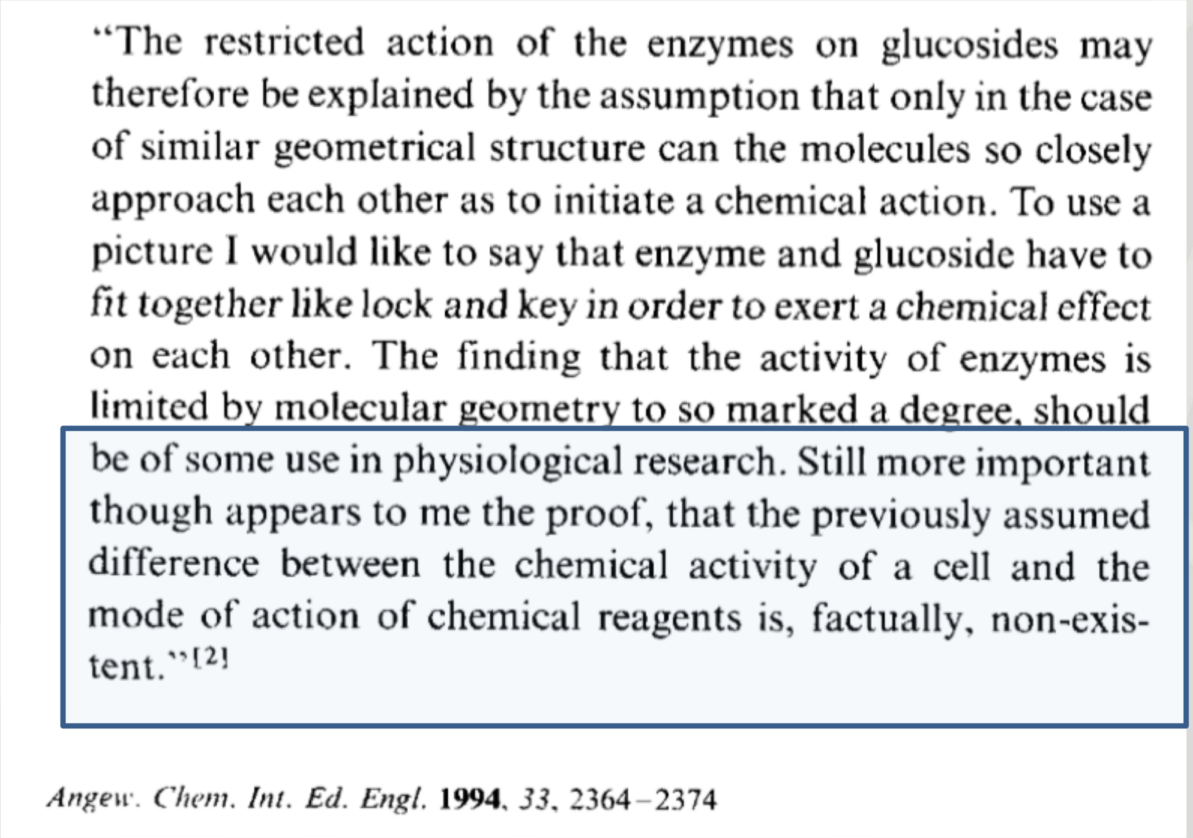
Biocomplexity for Research

Osama Alaidi

LSU

Vamsi Boyapati
Wei Huang
Joohyun Kim
Shantenu Jha

Emil Fischer: The Lock and Key "Model" (1894)



"The restricted action of the enzymes on glucosides may therefore be explained by the assumption that only in the case of similar geometrical structure can the molecules so closely approach each other as to initiate a chemical action. To use a picture I would like to say that enzyme and glucoside have to fit together like lock and key in order to exert a chemical effect on each other. The finding that the activity of enzymes is limited by molecular geometry to so marked a degree, should be of some use in physiological research. Still more important though appears to me the proof, that the previously assumed difference between the chemical activity of a cell and the mode of action of chemical reagents is, factually, non-existent."[2]

Angew. Chem. Int. Ed. Engl. **1994**, *33*, 2364–2374

Louis Pasteur
Biography.com

smFRET evidence for SAM-I riboswitch conformers

Manz et al Nature Chem Biol 2017

Manz et al J. Chem Phys 2018

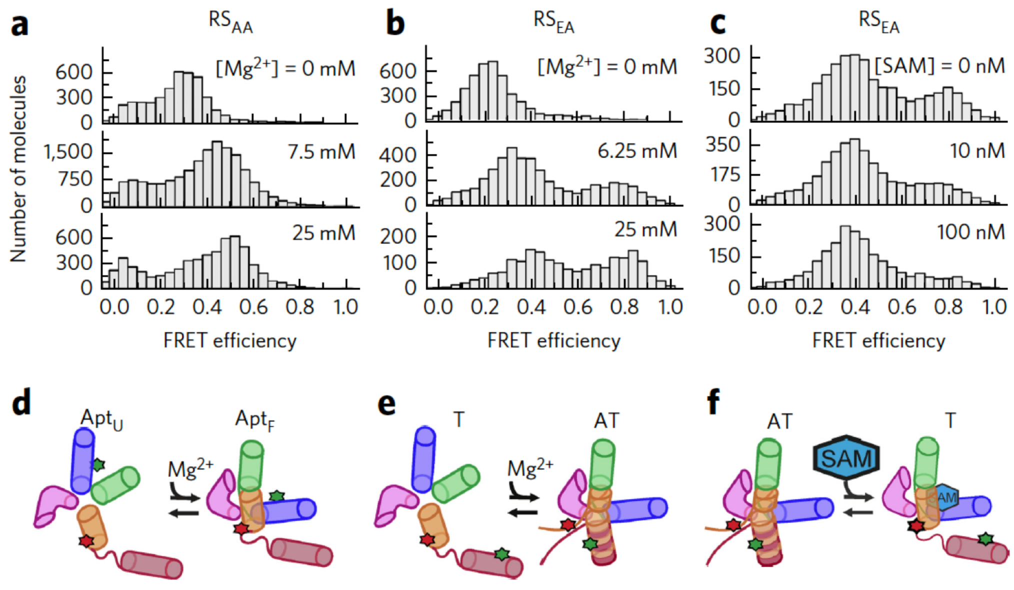
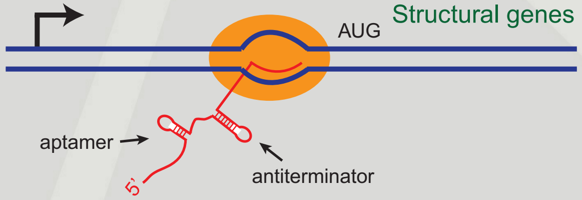


Figure 2 | Mg^{2+}- and ligand-dependent folding of SAM-I riboswitch. (**a**–**c**) Histograms of smFRET efficiency values of riboswitch constructs exposed to different buffer solutions: RS_{AA} at 0, 7.5 and 25 mM Mg^{2+} (**a**); RS_{EA} at 0, 6.25 and 25 mM Mg^{2+} (**b**) and RS_{EA} at 0, 10 and 100 nM SAM in the presence of 20 mM Mg^{2+} (**c**). (**d**–**f**) Schematic depictions of structural changes revealed by the FRET histograms: Mg^{2+}-induced unfolded (U) to folded (F) state transition of the aptamer (Apt) domain that causes the shift of the main peak (**d**); Mg^{2+}-induced transition from the T to the AT structure (**e**) and SAM-induced transition from the AT to the T conformation (**f**).

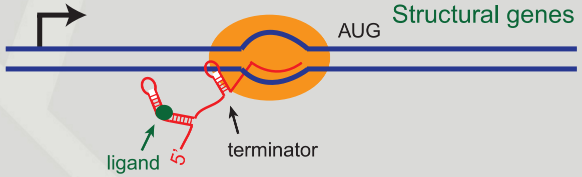
Riboswitch RNA Function Depends on Kinetics of Folding

(a) First steps in transcription of leader RNA

(b) In the absence of ligand



(c) In excess of ligand



(d) When RNAP proceeds faster than folding