The Significance of RNA in Molecular Biology

DNA → **RNA** → Protein

Knowledge of RNA Structure

DNA >> RNA

PROTEIN >> RNA

Causes diseases

(RNA viruses: AIDS, colds hepatitis, polio, etc.)

Catalyzes chemical reactions

(ribozymes)

RNA

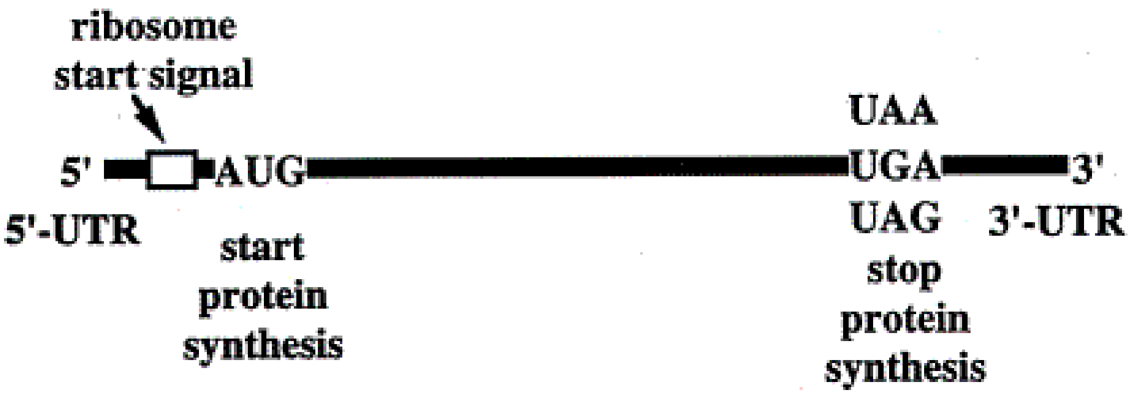
Carries messages for synthesis of all proteins.

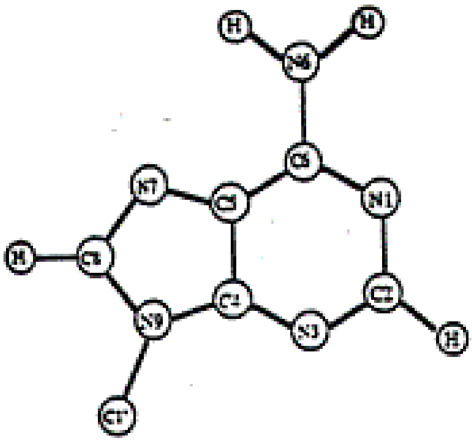
(messenger RNAs)

Translates messages into protein sequences.

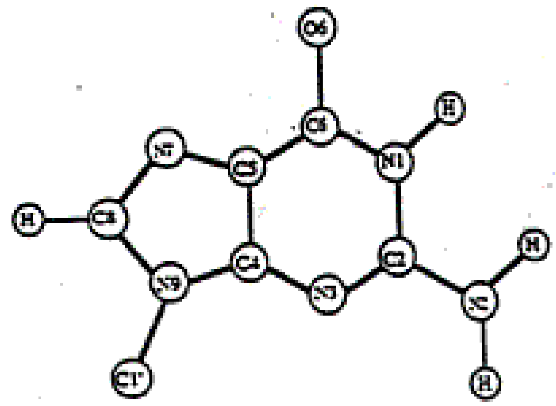
(transfer RNAs, ribosomal RNAs)

Translation of messenger RNA

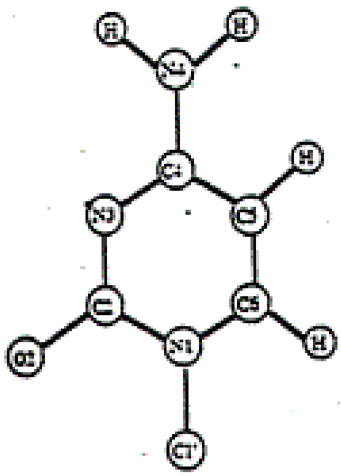




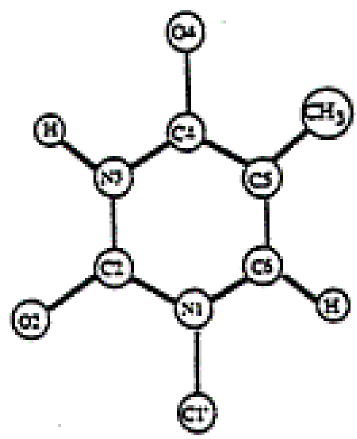
Adenine (A)



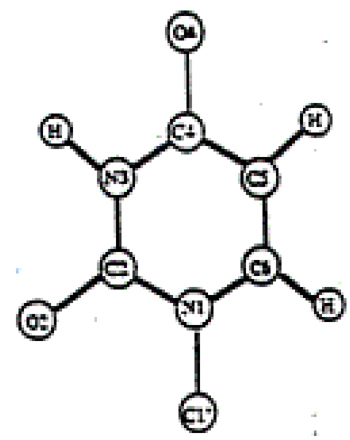
Guanine (G)



Cytosine (C)

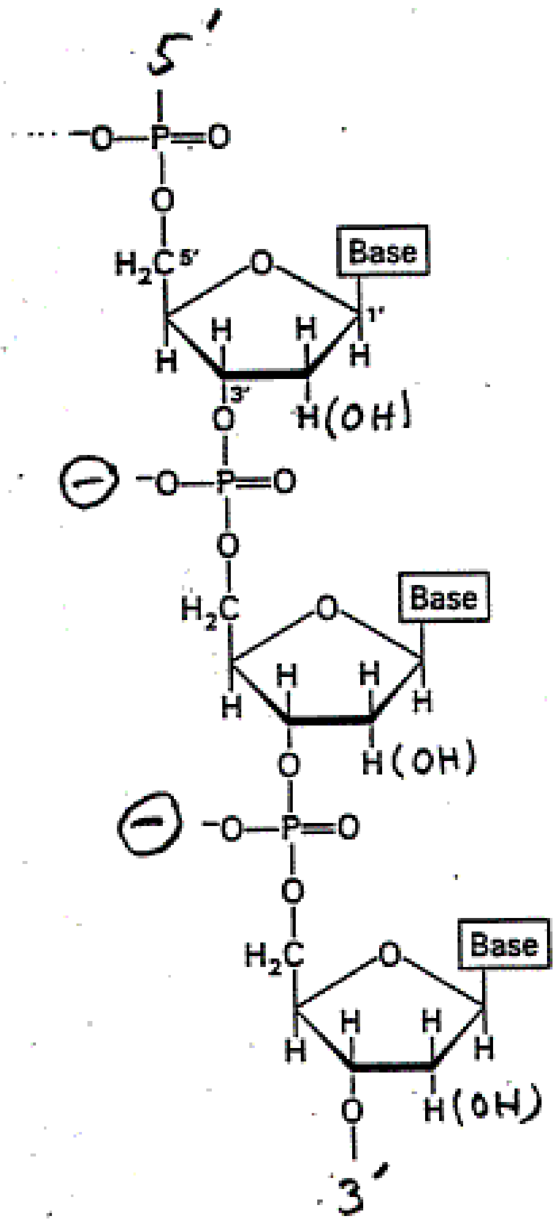


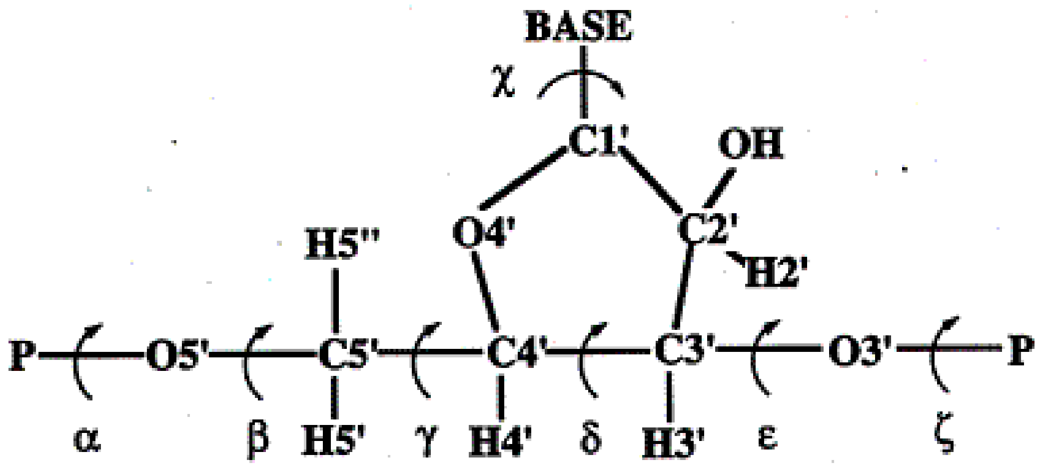
Thymine (T)



Uracil (U)

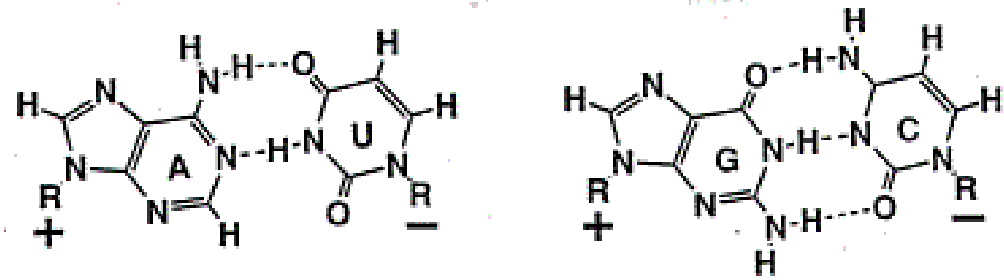
[05] Dr. Ignacio Tinoco, UCB (2/07/01) Unfolding Single RNA Molecules by Force



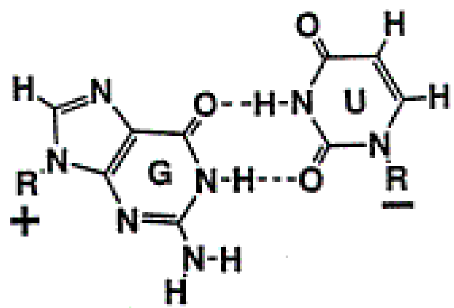

BASE
χ
C1'
OH
O4'
C2'
H5"
H2'
P
O5'
C5'
C4'
C3'
O3'
P
α
β
H5'
γ
H4'
δ
H3'
ε
ζ

Half the RNA folding problem is already mainly solved

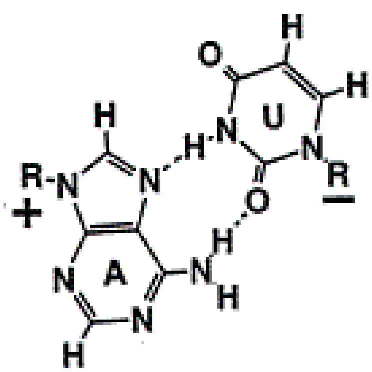
The base pairing—the secondary structure—can be predicted with good accuracy from experimental thermodynamic data on double strands, loops, and bulges.



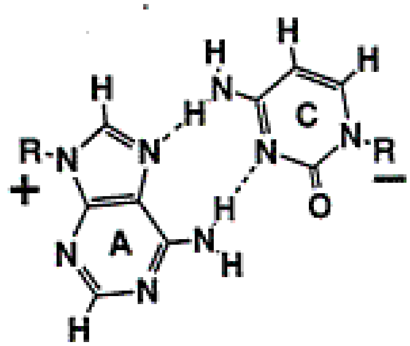
Watson-Crick



G•U Wobble



A•U Reverse Hoogsteen



A•C Reverse Hoogsteen

Fig. 1

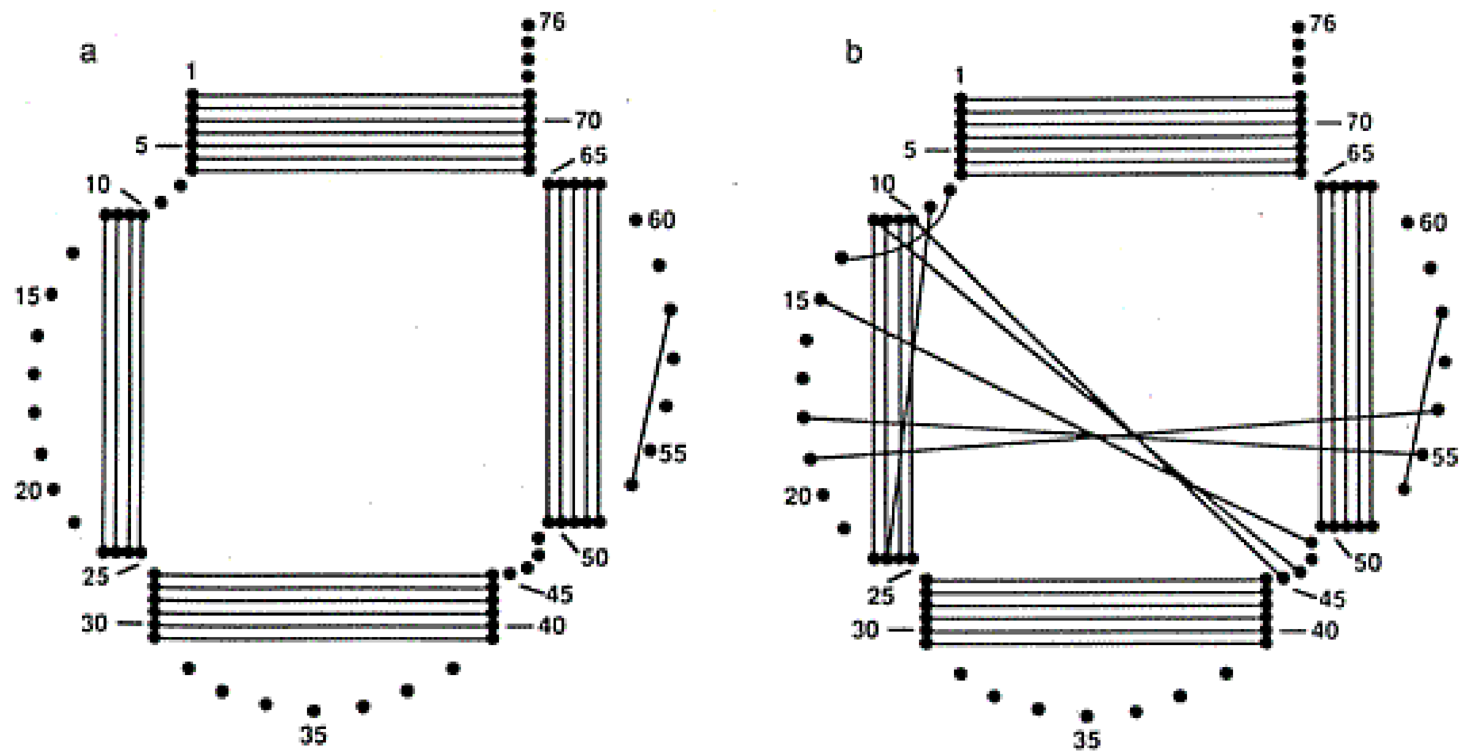
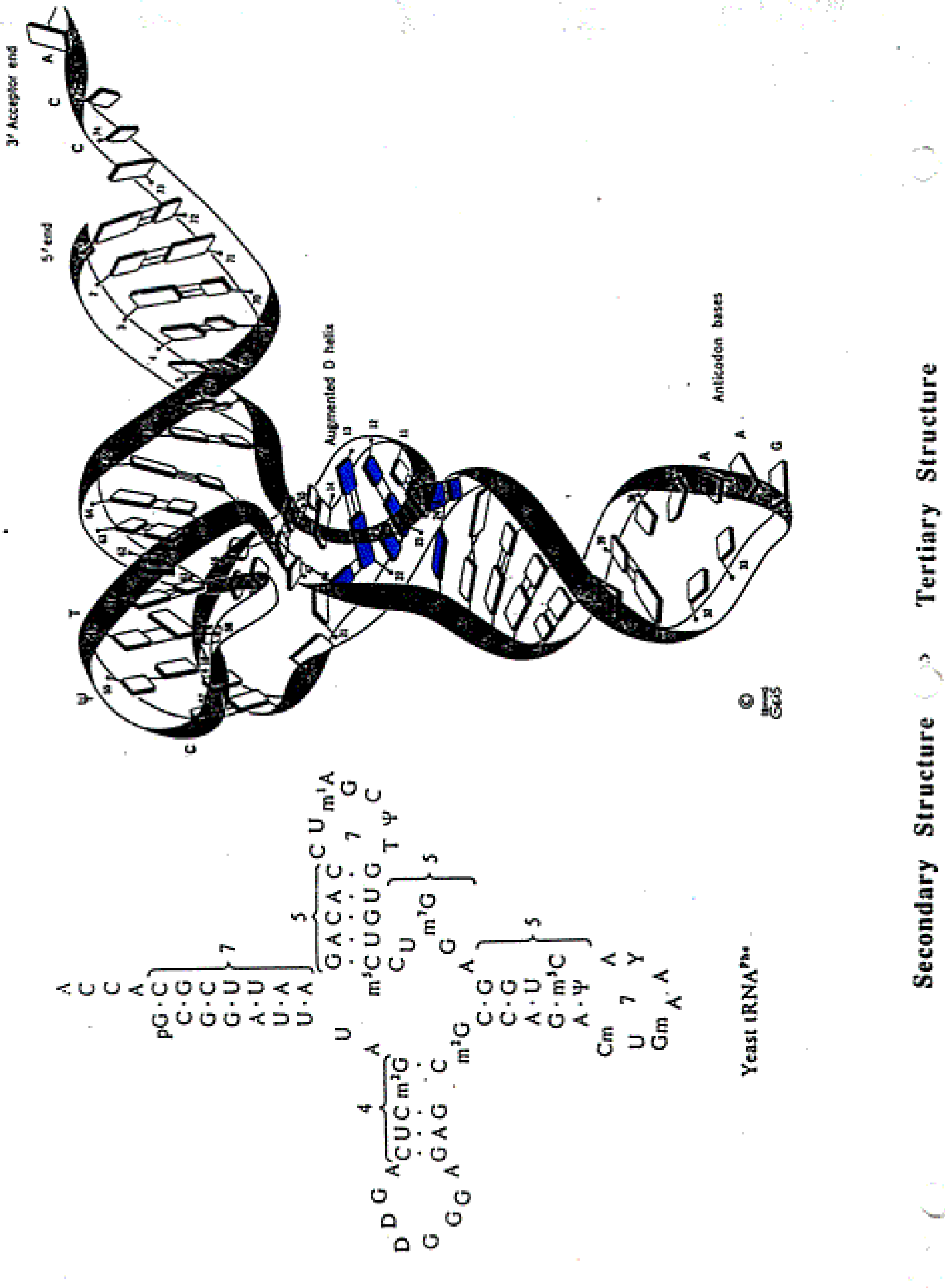


FIG. 1. The definition of secondary structure and tertiary structure in terms of chord crossing. The lines between points represent base-pairs. (a) The secondary structure of $tRNA^{Phe}$. (b) The secondary and tertiary structures of $tRNA^{Phe}$.

[10] Dr. Ignacio Tinoco, UCB (2/07/01) Unfolding Single RNA Molecules by Force

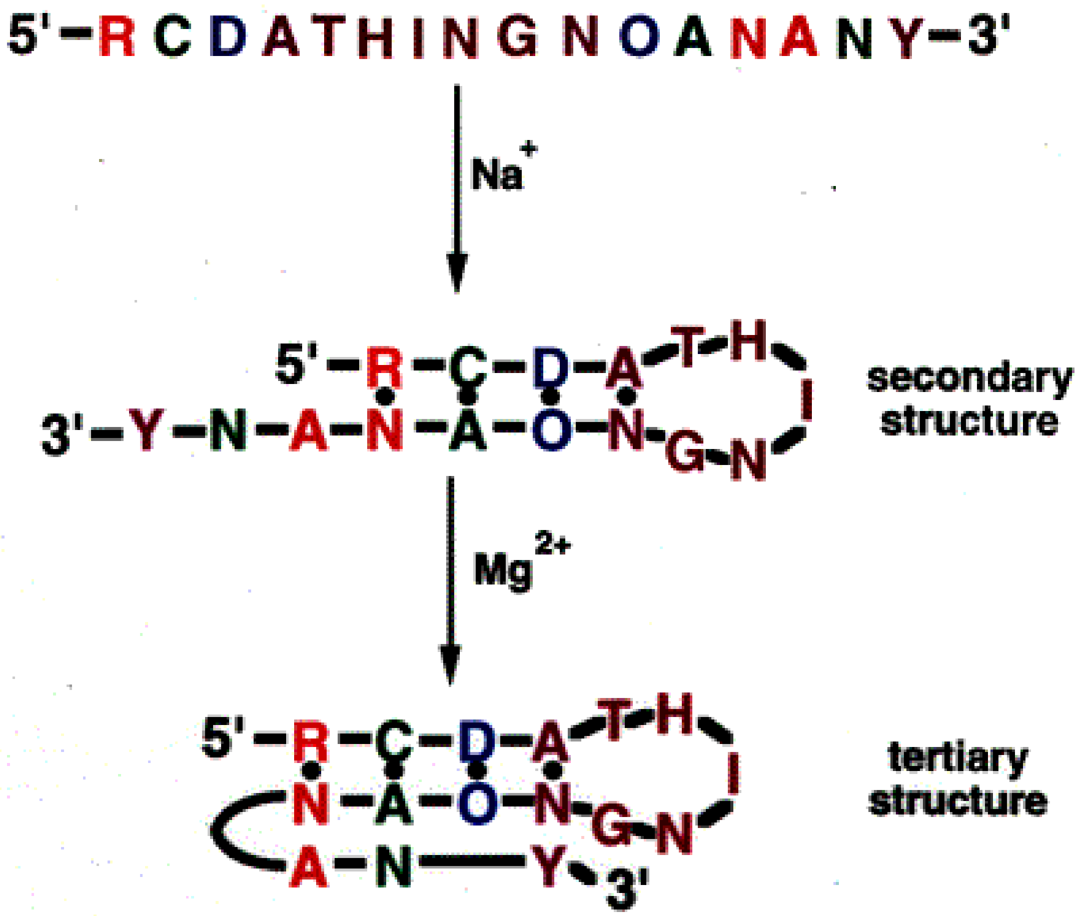


The Gibbs free energy, G, of a closed system at constant temperature, T, and pressure, P, tends toward a minimum.

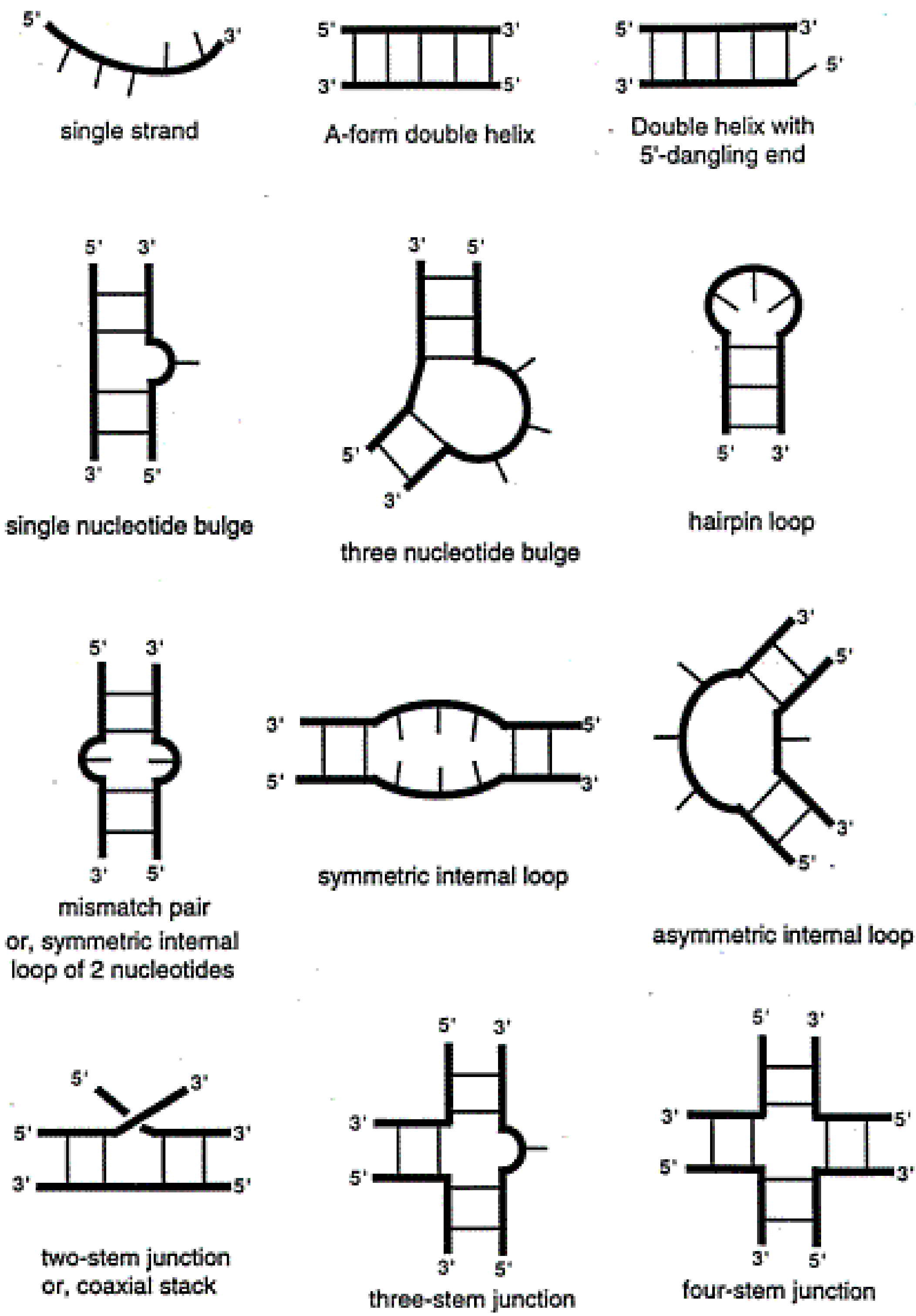
$$G = H - TS = E + PV - TS$$

H = enthalpy
S = entropy
E = energy
V = volume

[12] Dr. Ignacio Tinoco, UCB (2/07/01) Unfolding Single RNA Molecules by Force



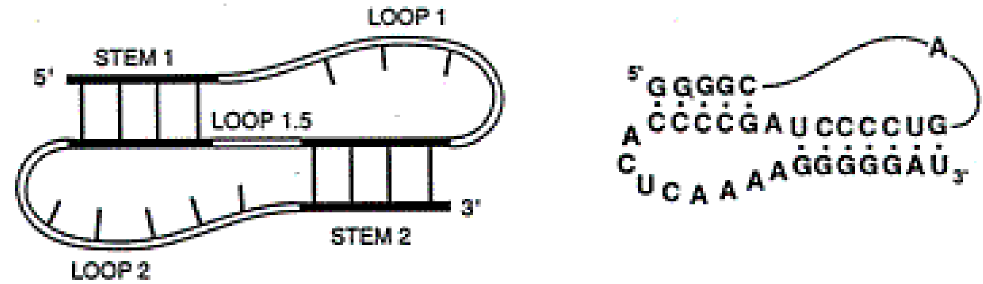
[13] Dr. Ignacio Tinoco, UCB (2/07/01) Unfolding Single RNA Molecules by Force



Appendix 2, Fig 1 corrected

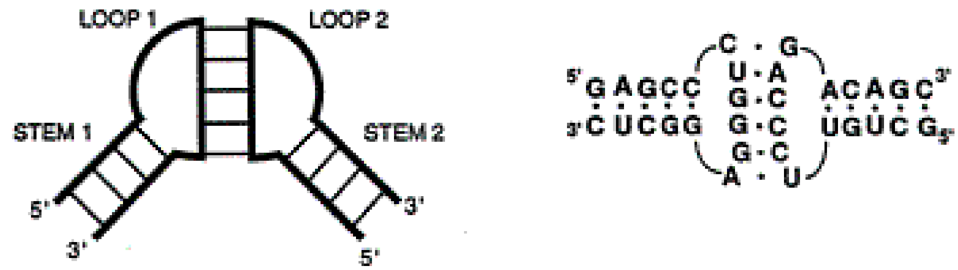
[14] Dr. Ignacio Tinoco, UCB (2/07/01) Unfolding Single RNA Molecules by Force

a)



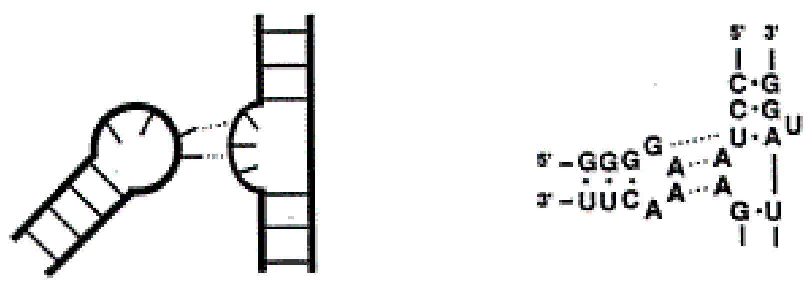
Pseudoknot

b)



Kissing hairpins

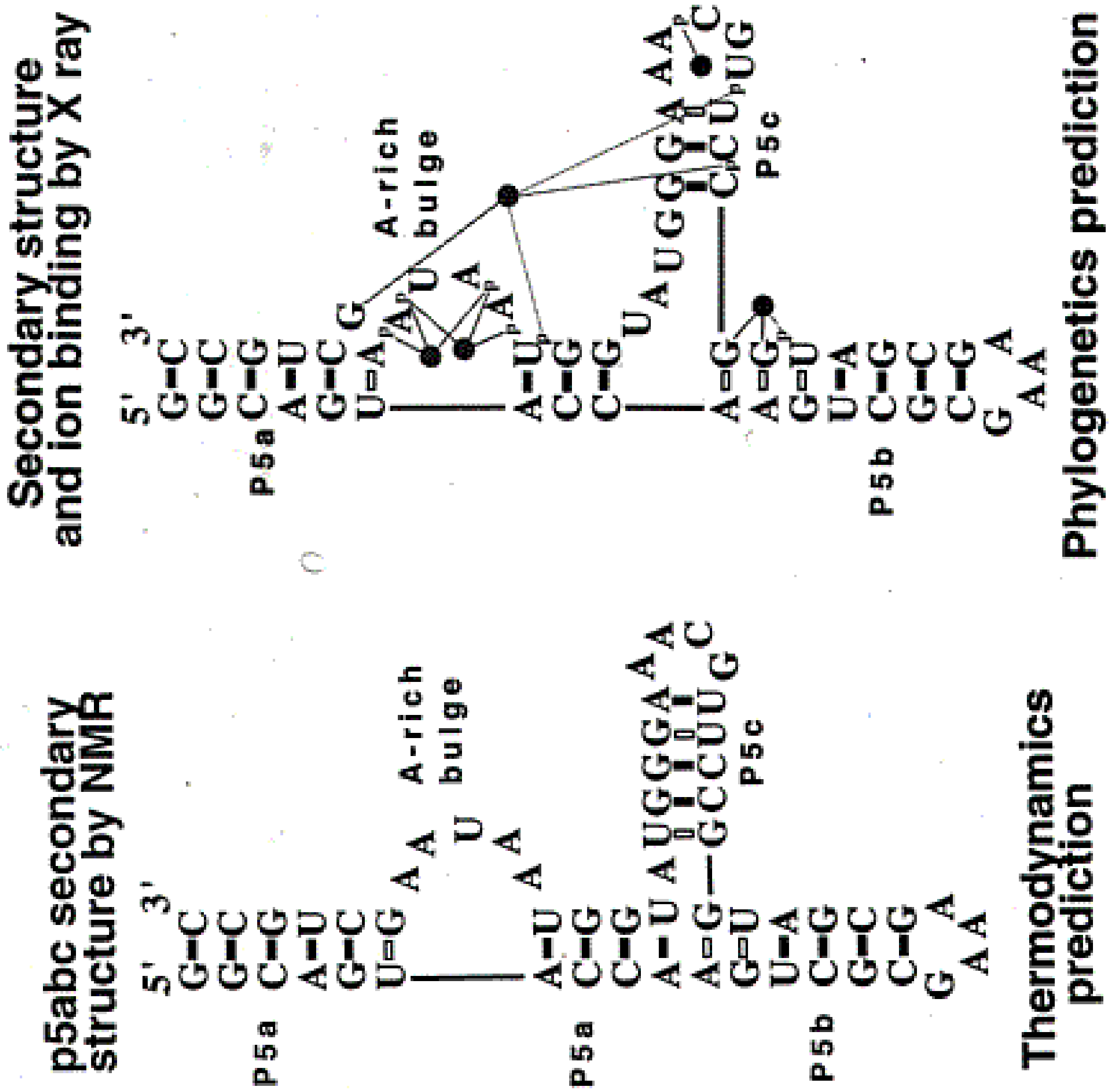
c)



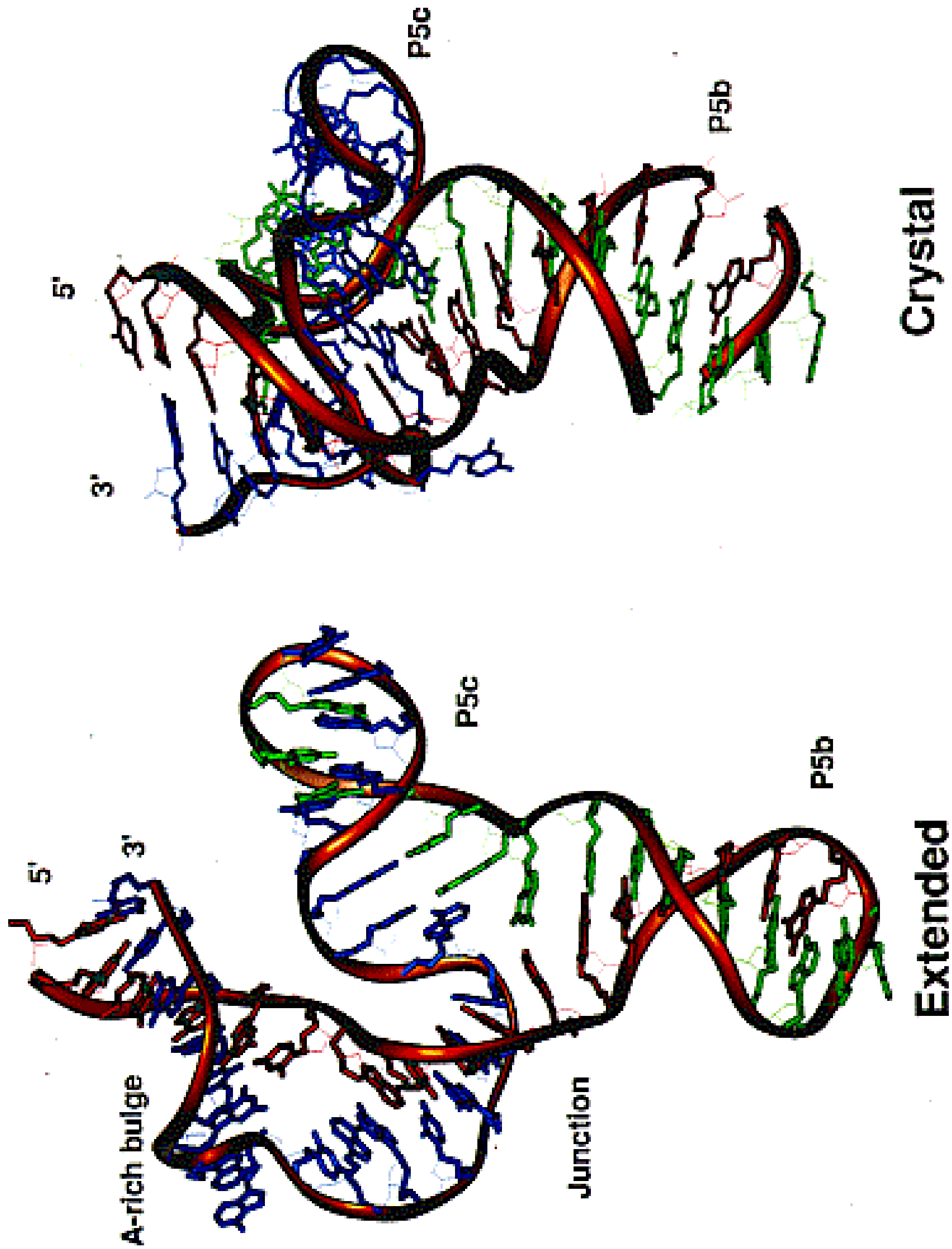
Hairpin loop - bulge contact

Appendix J, Fig 2

[15] Dr. Ignacio Tinoco, UCB (2/07/01) Unfolding Single RNA Molecules by Force



[16] Dr. Ignacio Tinoco, UCB (2/07/01) Unfolding Single RNA Molecules by Force



Coworkers

Professor Carlos Bustamante, Departments of Chemistry, Molecular and Cell Biology, and Physics, and Howard Hughes Medical Institute, UCB

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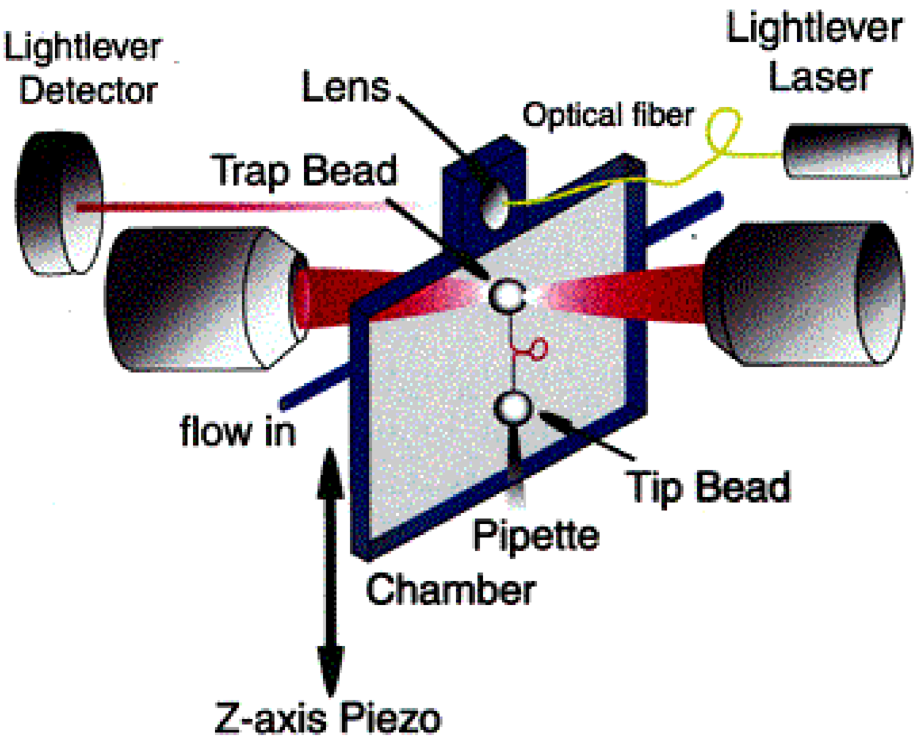
Dr. Jan Liphardt, Department of Chemistry, UCB

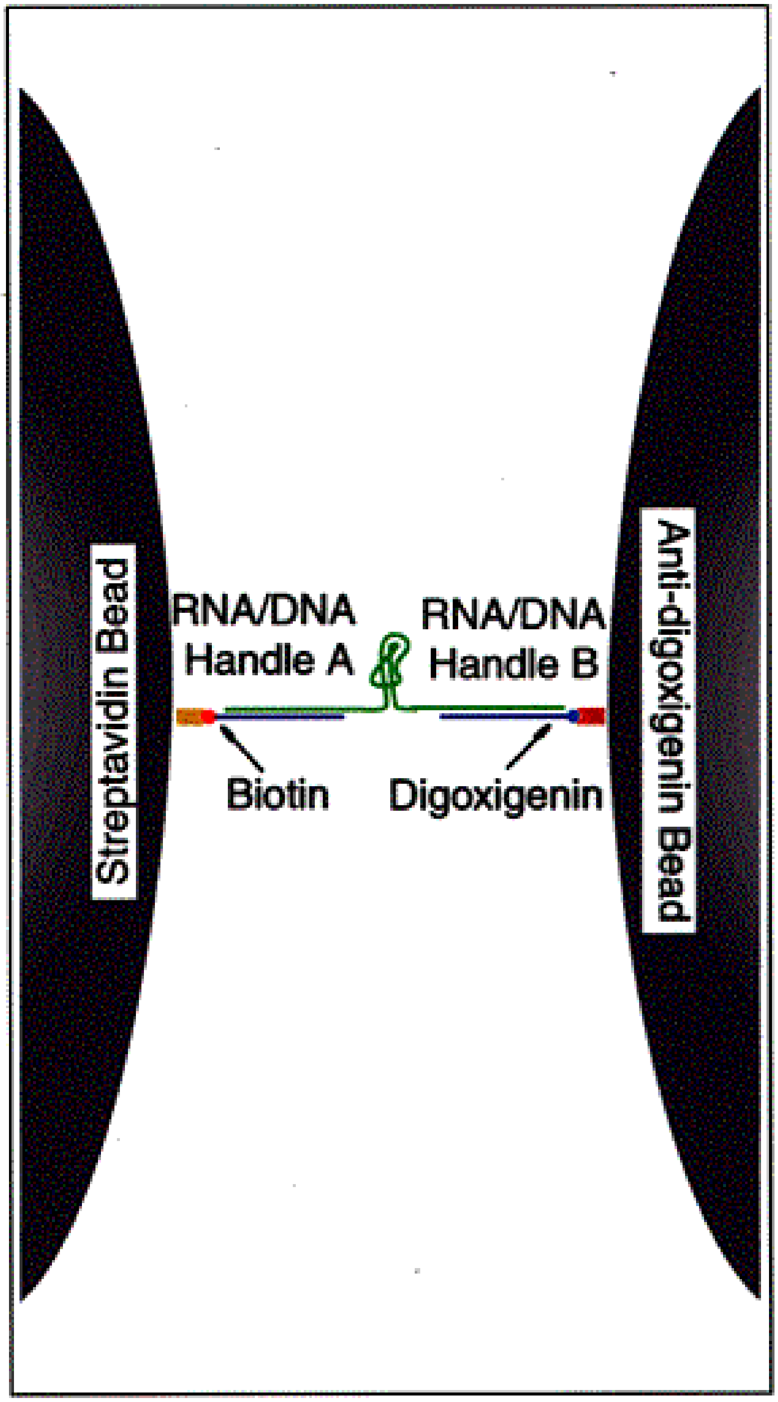
Dr. Bibiana Onoa, Department of Chemistry, UCB

Dr. Steven B. Smith, Department of Physics, UCB

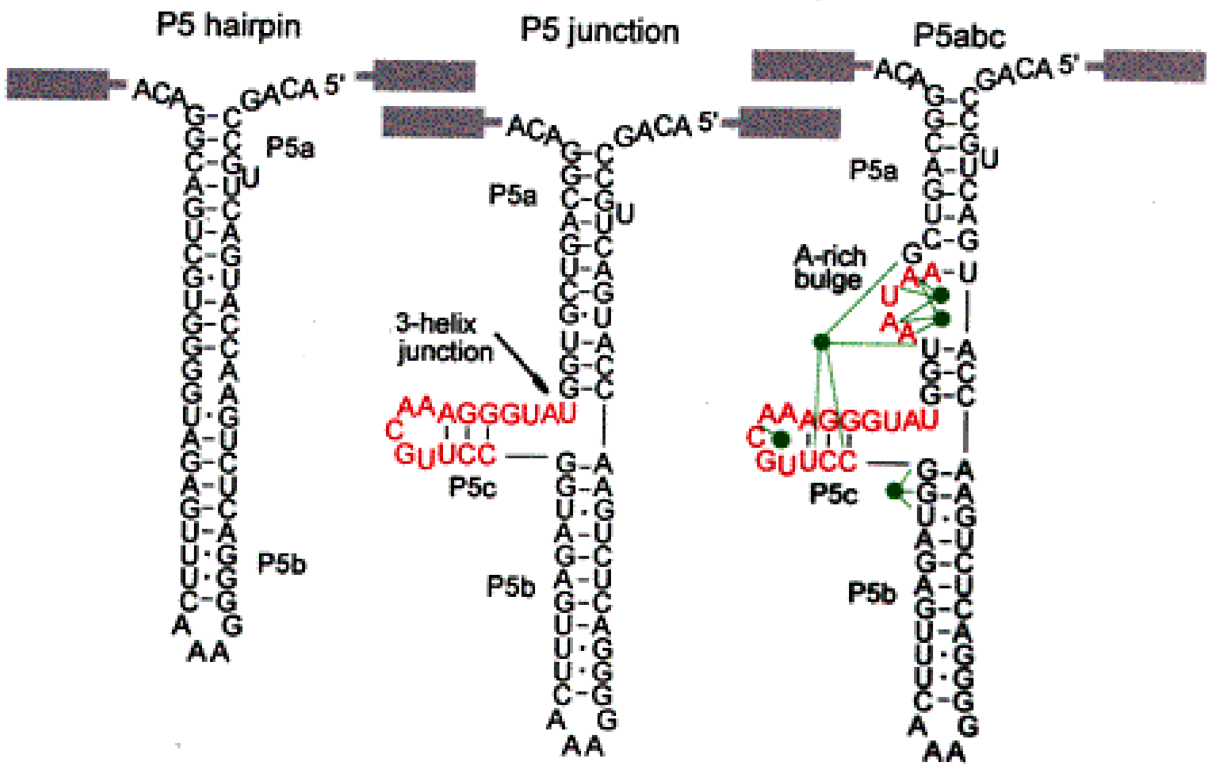
[18] Dr. Ignacio Tinoco, UCB (2/07/01) Unfolding Single RNA Molecules by Force



[19] Dr. Ignacio Tinoco, UCB (2/07/01) Unfolding Single RNA Molecules by Force

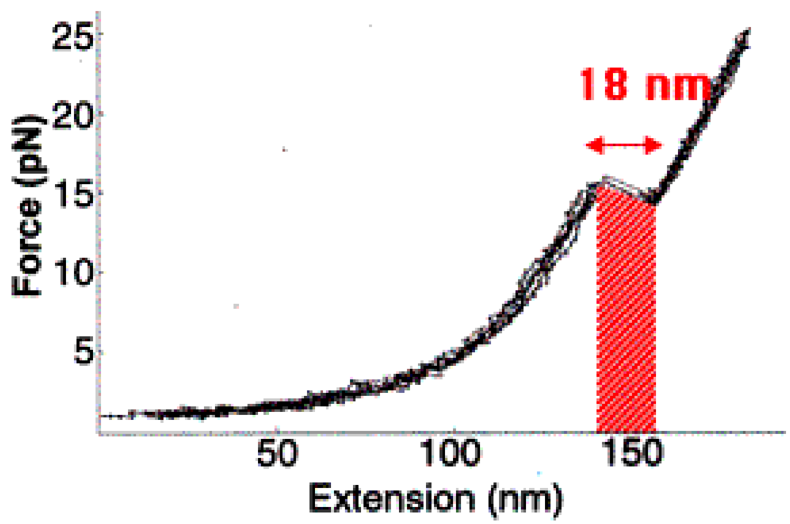


[20] Dr. Ignacio Tinoco, UCB (2/07/01) Unfolding Single RNA Molecules by Force

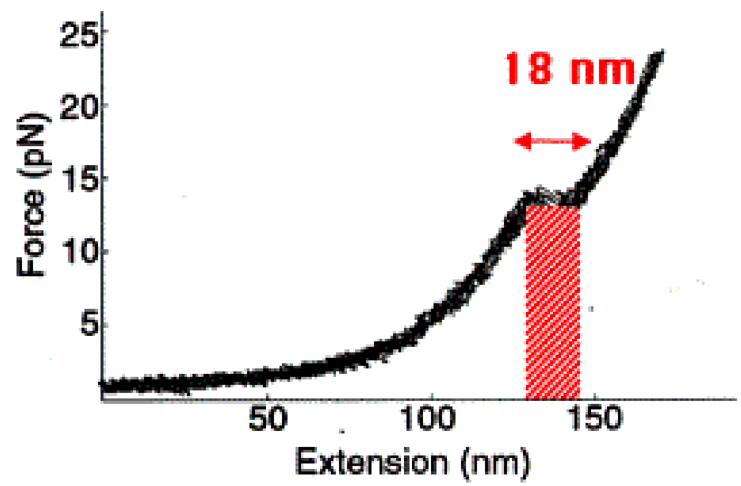


Mechanical unfolding of RNA

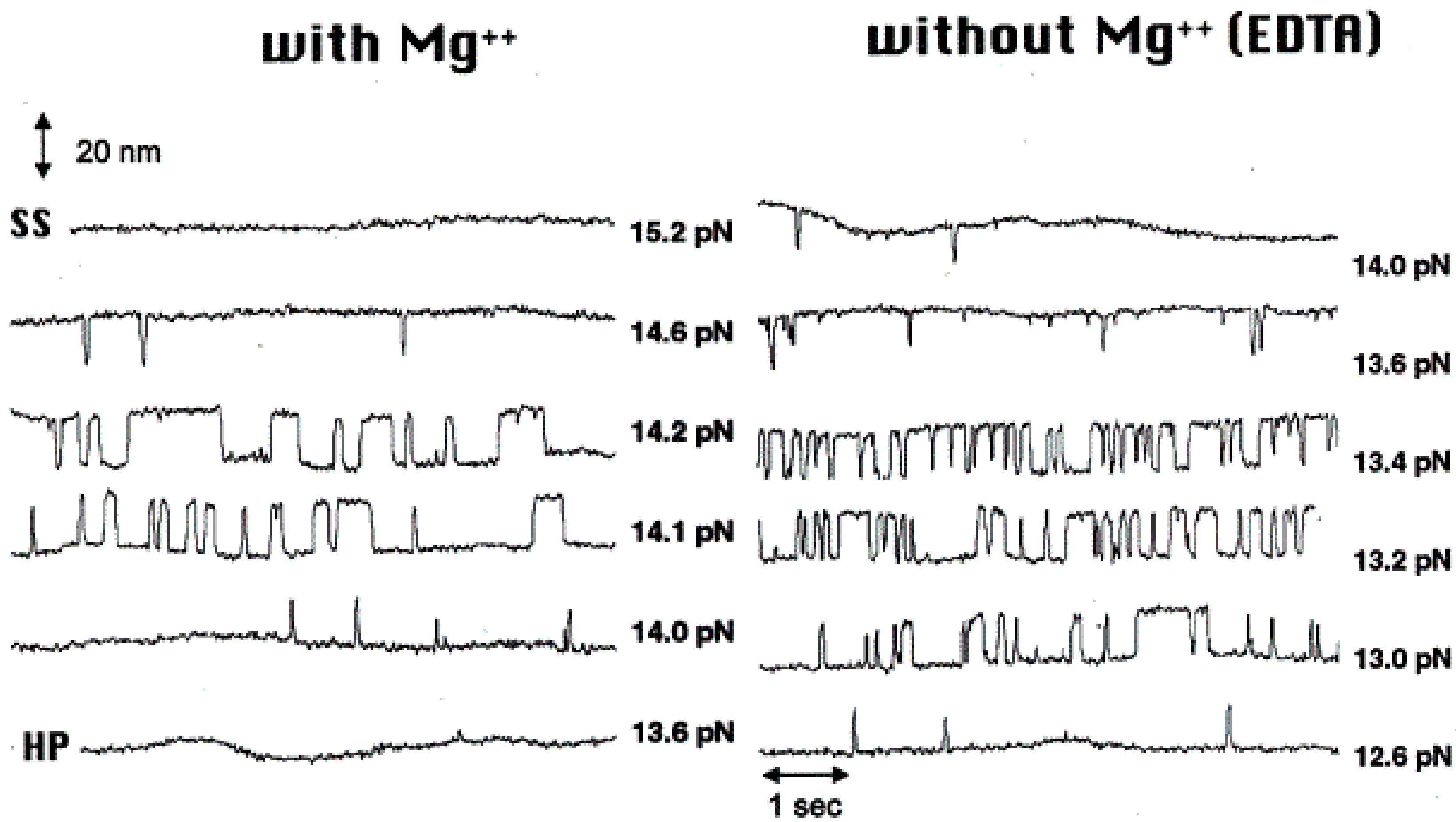
P5 hairpin in Mg^{++}



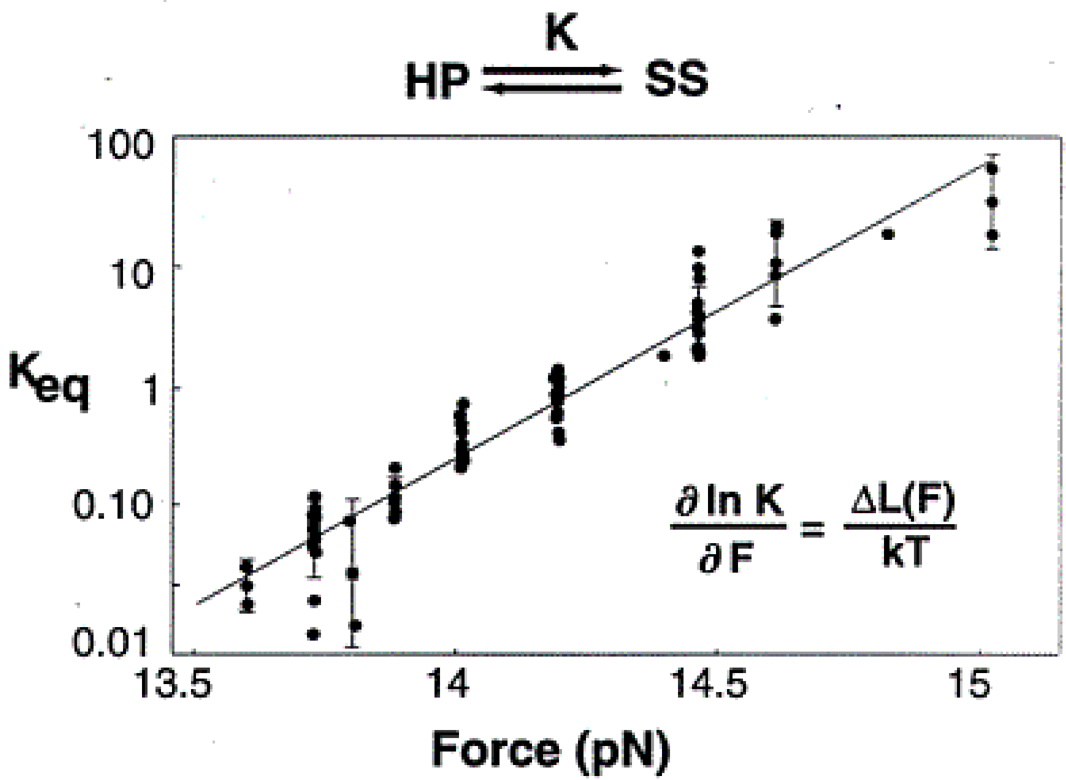
P5 hairpin in EDTA



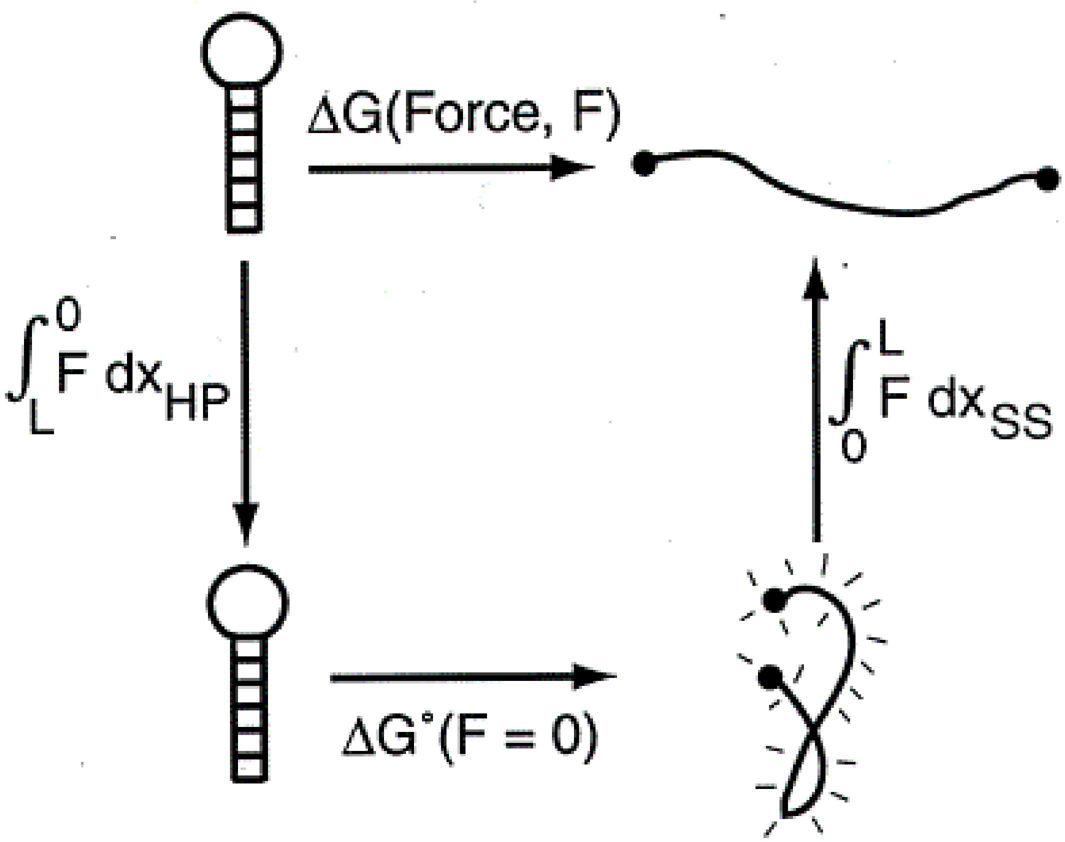
P5 hairpin constant force experiments



[23] Dr. Ignacio Tinoco, UCB (2/07/01) Unfolding Single RNA Molecules by Force



[24] Dr. Ignacio Tinoco, UCB (2/07/01) Unfolding Single RNA Molecules by Force

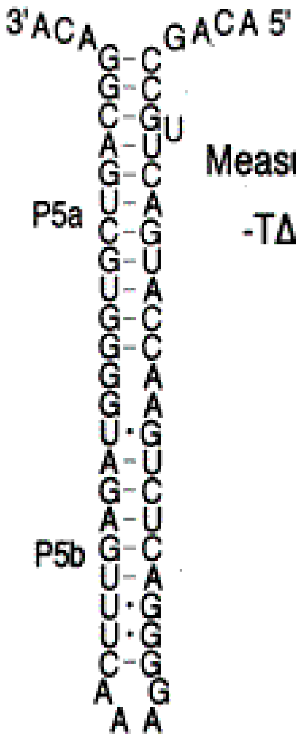


$$\Delta G(\text{Force, F}) = \Delta G^\circ(F = 0) + \int_0^L F\, dx_{SS} - \int_0^L F\, dx_{HP}$$

$$\Delta G(\text{Force, F}) = \Delta G^\circ(F = 0) + \Delta G(0 \text{ -> } F)_{\text{stretching}}$$

[25] Dr. Ignacio Tinoco, UCB (2/07/01) Unfolding Single RNA Molecules by Force

$$\Delta G^{\circ}(F = 0) = F_{1/2}\,\Delta L - \Delta G(0 \rightarrow F_{1/2})_{stretch}$$



P5 hairpin

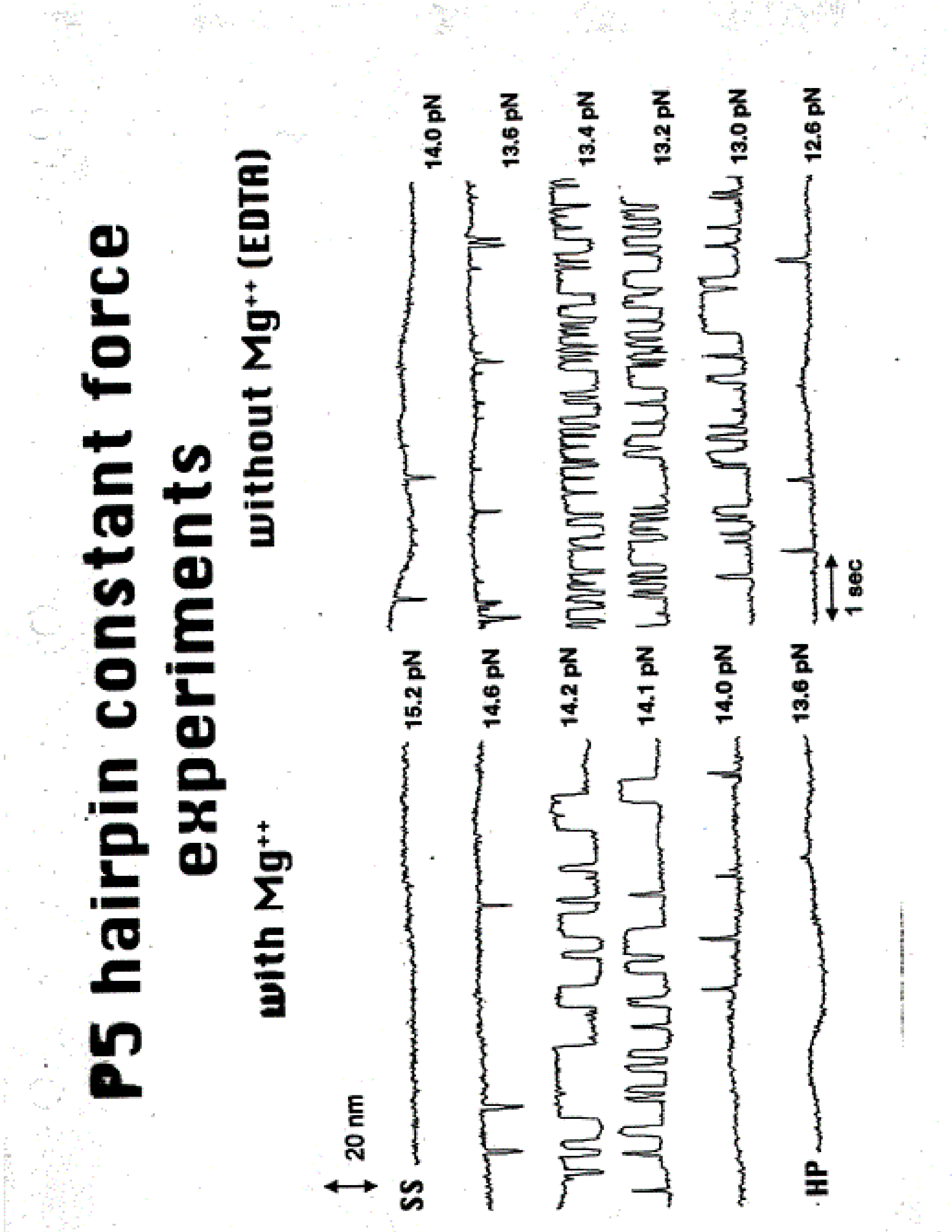
Measured $\Delta G(298)$ at 14.5 pN = 157 ± 20 kJ/mol = 37.6 kcal/mol

$-T\Delta S(298)$ for tethered ends = - 40 ± 10 kJ/mol = - 9.6 kcal/mol

$\Delta G^{\circ}(298)$ free in solution = 117 ± 30 kJ/mol = 28.0 kcal/mol

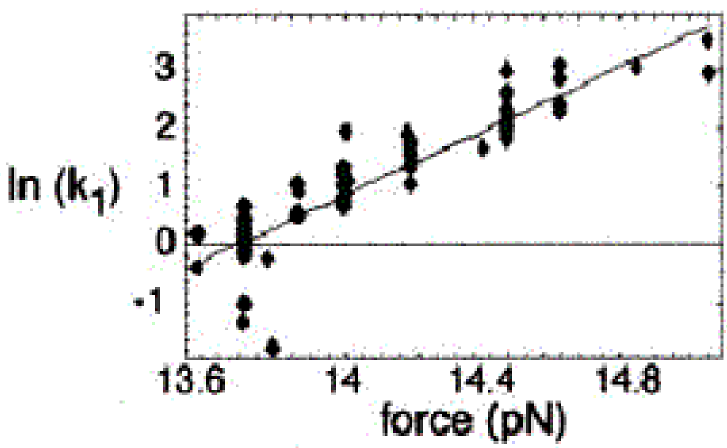
$\Delta G^{\circ}(298)$ Zuker's mfold = 141 ± 10 kJ/mol = 33.7 kcal/mol

[26] Dr. Ignacio Tinoco, UCB (2/07/01) Unfolding Single RNA Molecules by Force



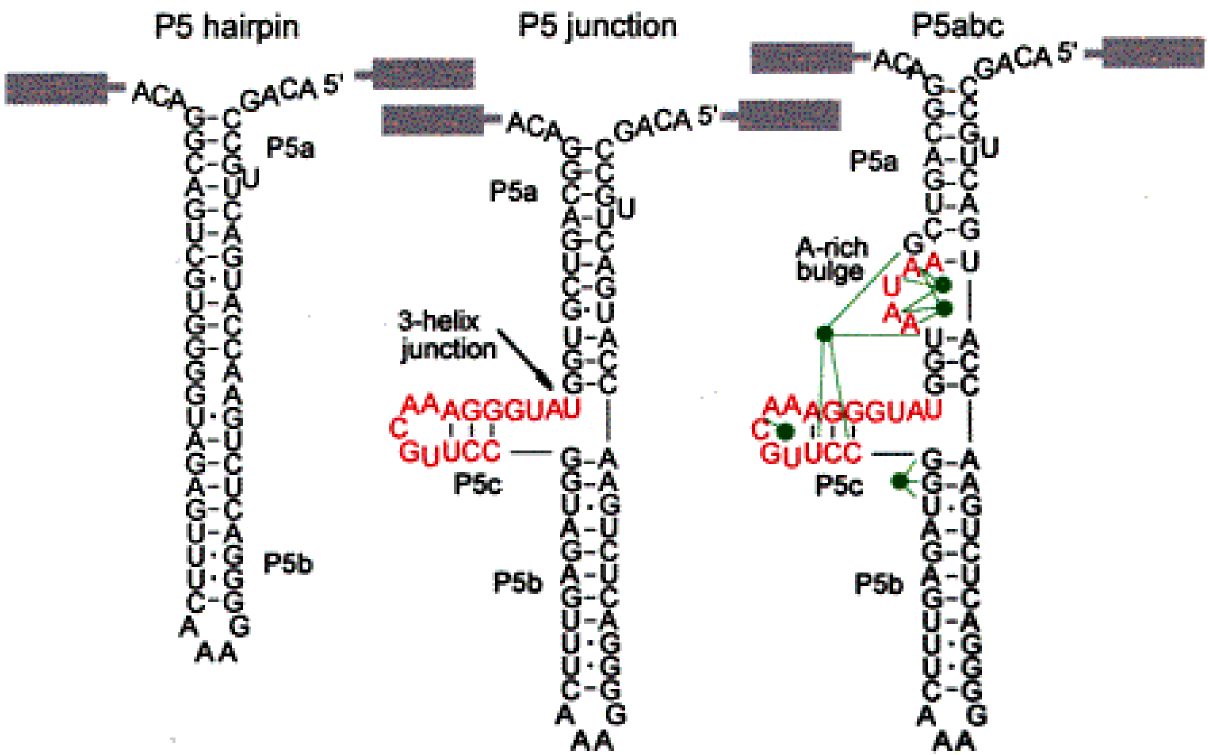
Effect of force on the rate of unfolding P5 HP

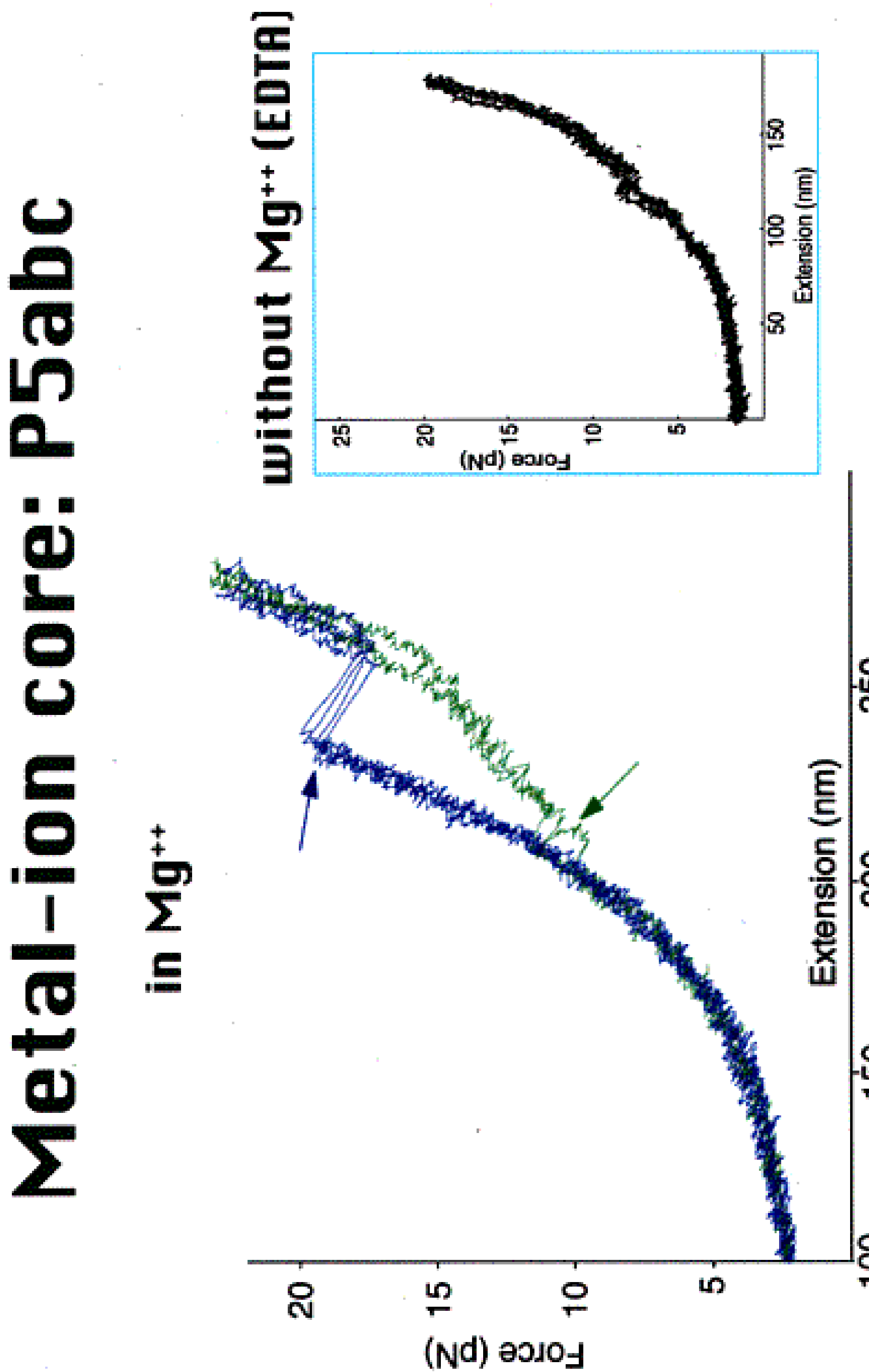
$$\text{Hairpin} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{Single strand}$$



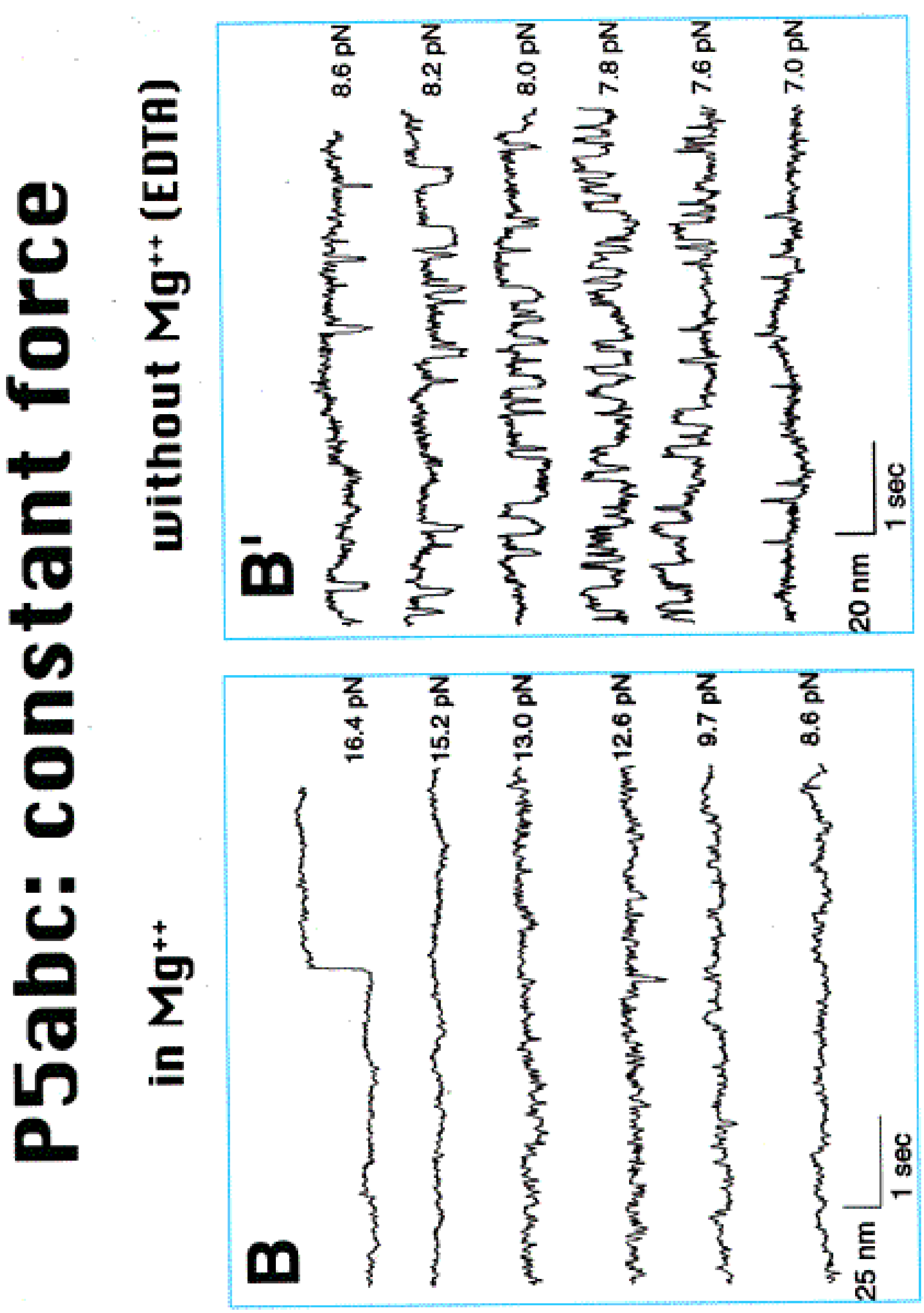
$$\frac{\partial \ln k_1}{\partial F} = \frac{\Delta l^*(F)}{kT} \qquad \Delta l^* = 12 \text{ nm}$$

[28] Dr. Ignacio Tinoco, UCB (2/07/01) Unfolding Single RNA Molecules by Force



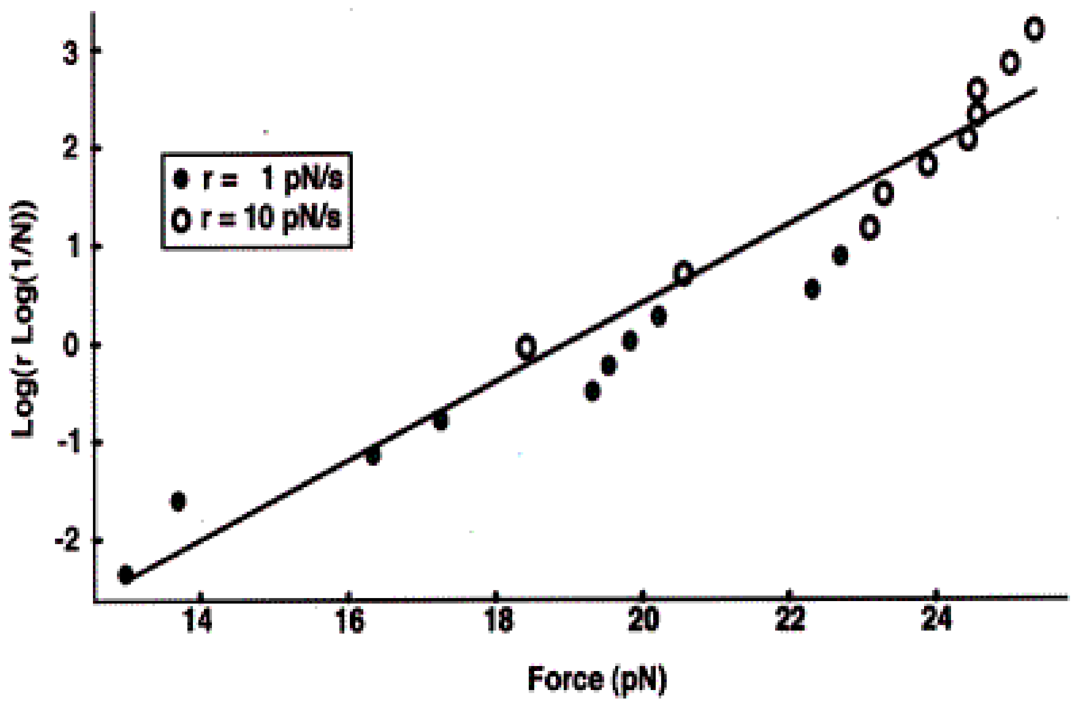

Metal-ion core: P5abc
in Mg++
without Mg++ (EDTA)
Force (pN)
Extension (nm)
100
150
200
250
5
10
15
20
25
50

[30] Dr. Ignacio Tinoco, UCB (2/07/01) Unfolding Single RNA Molecules by Force



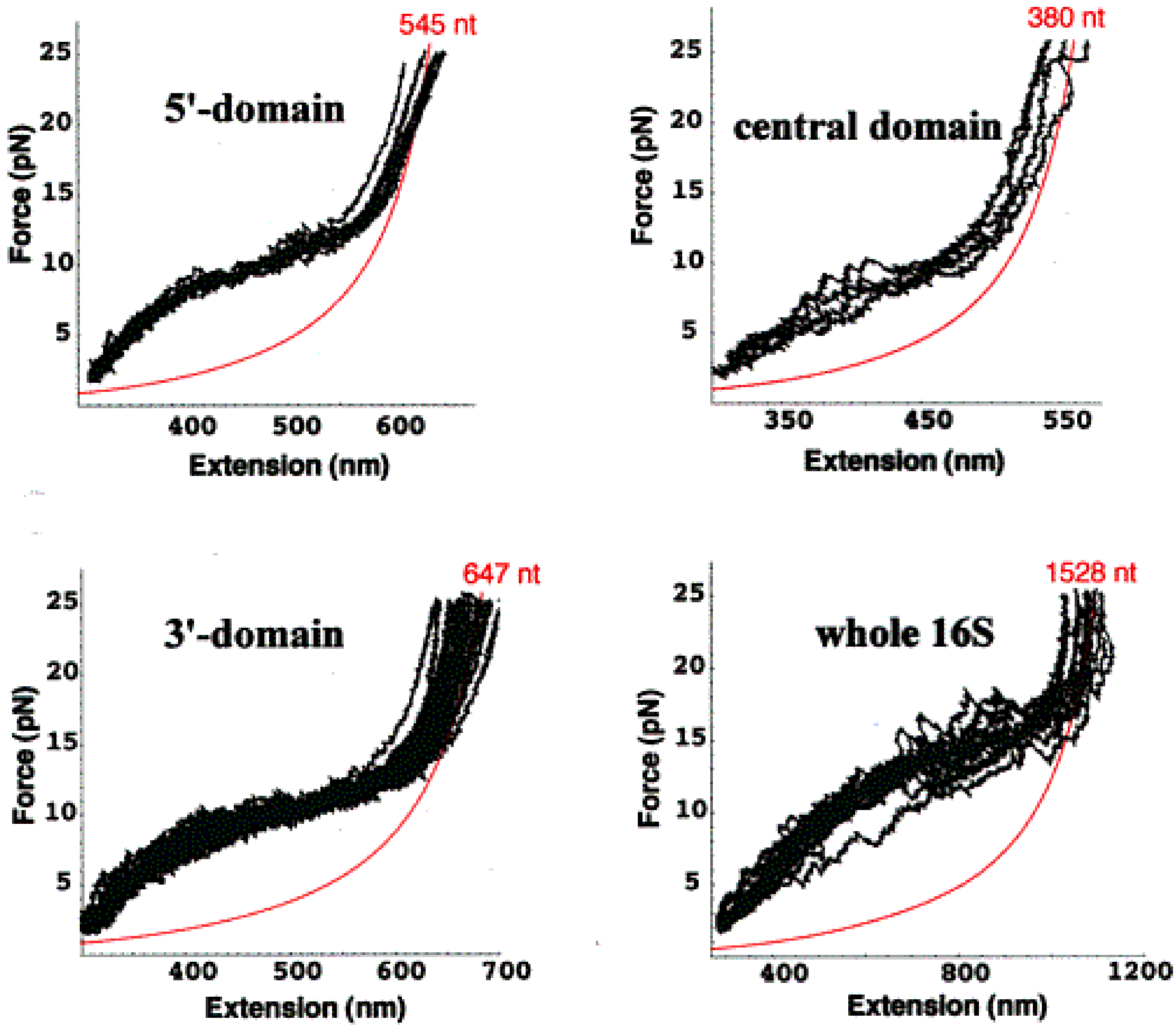
Non-equilibrium Unfolding

$$N(F,r) = e^{-\frac{k_0}{br}(e^{bF}-1)} \quad \textbf{where} \quad b = \frac{\Delta l^*}{k_B T}$$



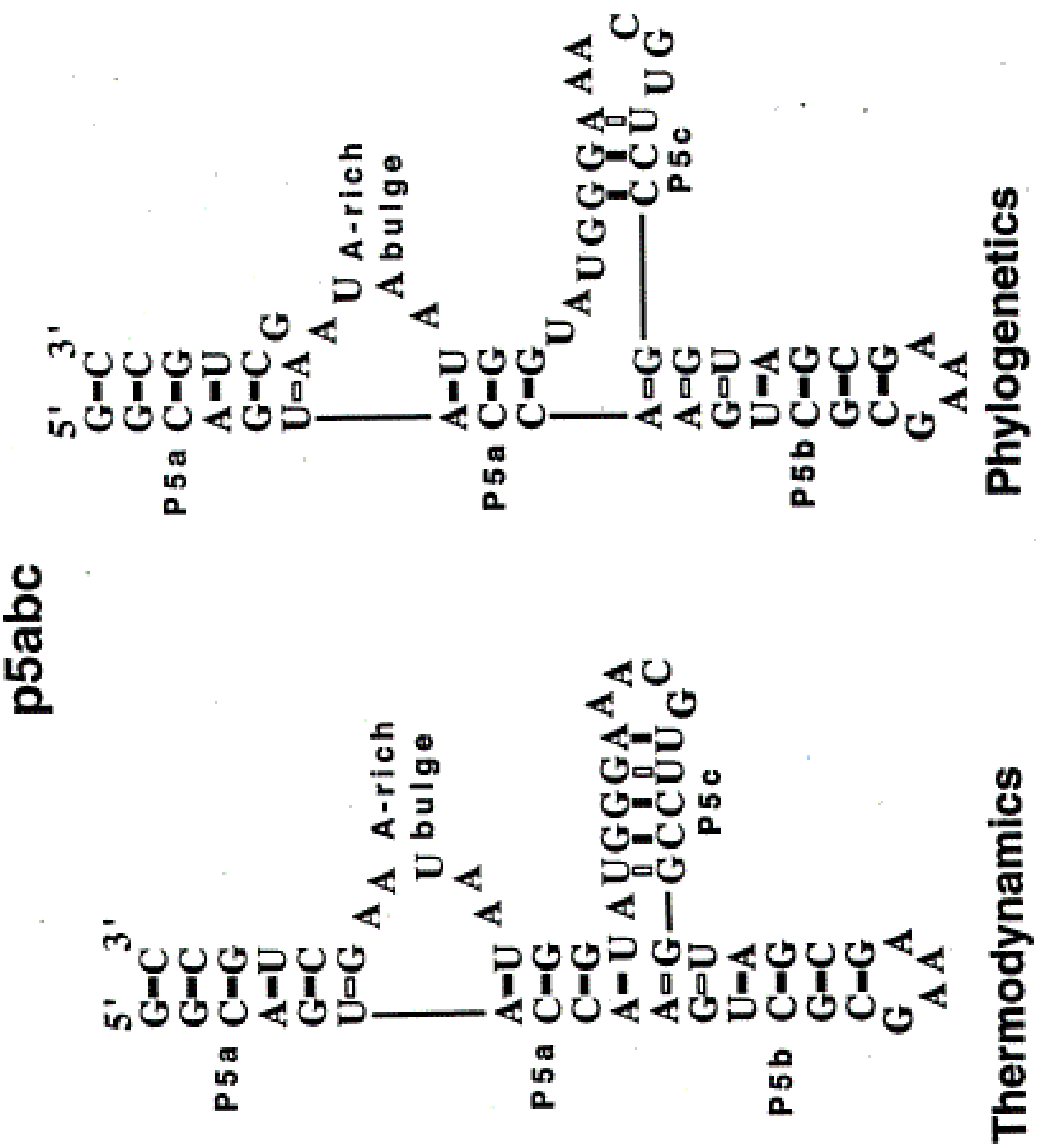
$$\Delta l^* = 1.6\, nm$$

16S Ribosomal RNA in reconstitution buffer minus magnesium



Summary

1. You can measure the work of unfolding a molecule from the product of force times extension; if the process is reversible the work is equal to the free energy change.
2. You can change equilibrium constants and rate constants by applying force.
   a. The difference in end-to-end distance between initial and final states determines effect of force on equilibrium.
   b. The difference in length between initial state and transition state determines effect of force on kinetics.
3. Mg^{2+} markedly decreases the kinetics of unfolding RNA molecules with tertiary interactions.
4. Size is not important.



p5abc
Phylogenetics
Thermodynamics
P5a
P5b
P5c
A-rich
A bulge
U bulge
5'
3'