

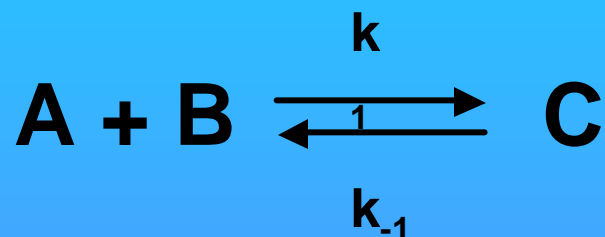
What Are Kinetics Trying to Telling Us?

Sam Dudley, M.D., Ph.D.

404-329-4626

sdudley@emory.edu

The Relationship of Kinetics to Equilibrium



$$V_f = k_1[A][B] \qquad V_r = k_{-1}[C]$$

At equilibrium, $V_f = V_r$

$$k_1[A][B] = k_{-1}[C]$$

$$k_1/k_{-1} = [A][B]/[C] = K_{eq}$$

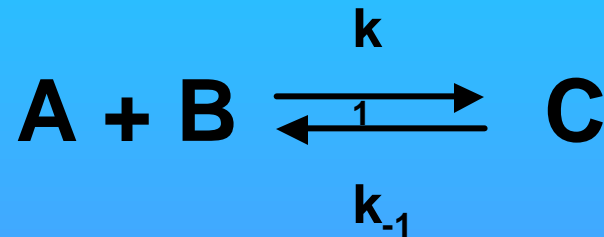
- Kinetics probe the intermediate state
- There are many ways to get the same K_{eq}

Affinity and K_{eq}

A high K_{eq} means:

- A. the ligand has low affinity
- B. the ligand has high affinity
- C. neither

Gibbs Free Energy (ΔG)



- At constant temp and pressure, $\Delta G = (G_A + G_B) - G_C$
- Reactions with a positive and negative ΔG are endergonic and exergonic, respectively
- At equilibrium, $\Delta G = -RT \ln K_d$

ΔG and Reaction Rates

A reaction has a ΔG of -15 kcal/mol.:

A. The reaction will proceed rapidly toward products.

B. The reaction will proceed slowly toward reactants.

C. The reaction may not proceed at all.

What Determines Rates?

$$k = Ae^{-E_a/RT}$$

- The Arrhenius equation (1889)
- The rate is related to some portion of the collisions with the right geometry (A) and with enough energy ($e^{-E_a/RT}$)
- $e^{-E_a/RT}$ looks suspiciously like the Boltzmann distribution and tells you the fraction of molecules with E_a energy at a given T

Reaction Rates

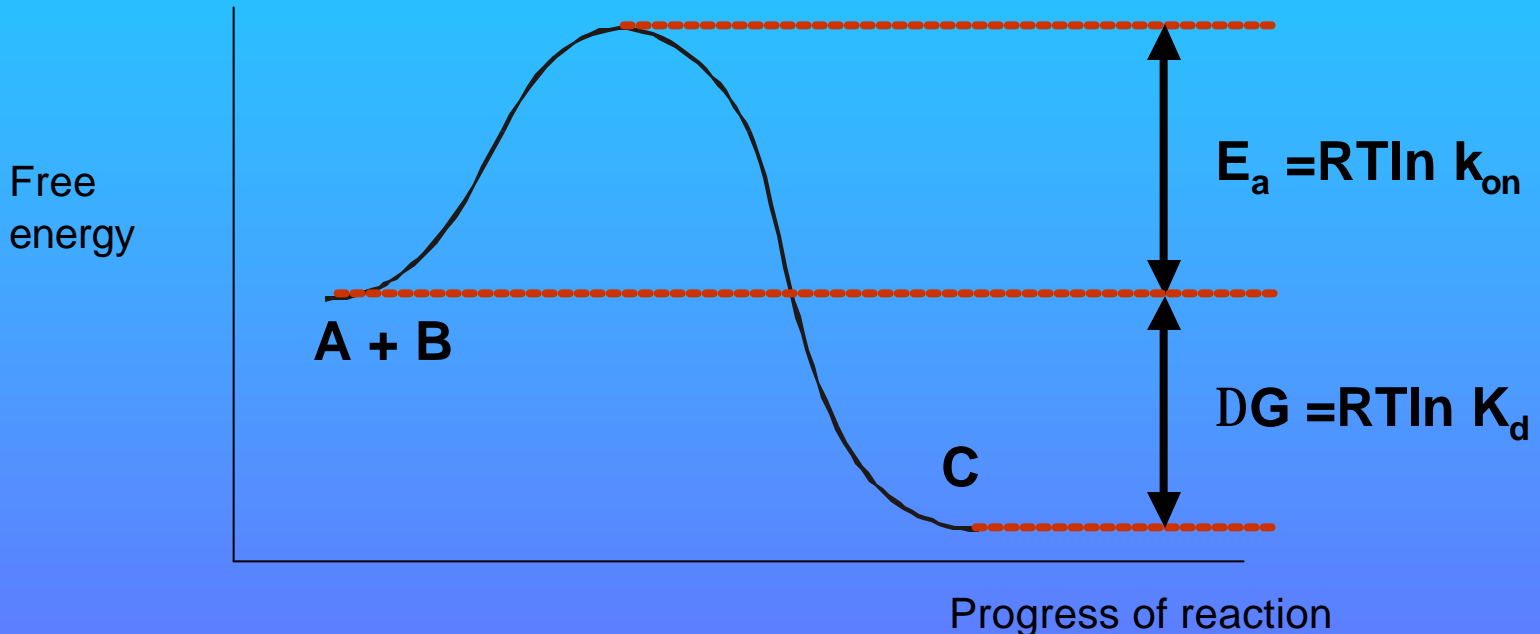
You can raise a reaction rate by all except:

A. Increasing temperature.

B. Lowering K_{eq}

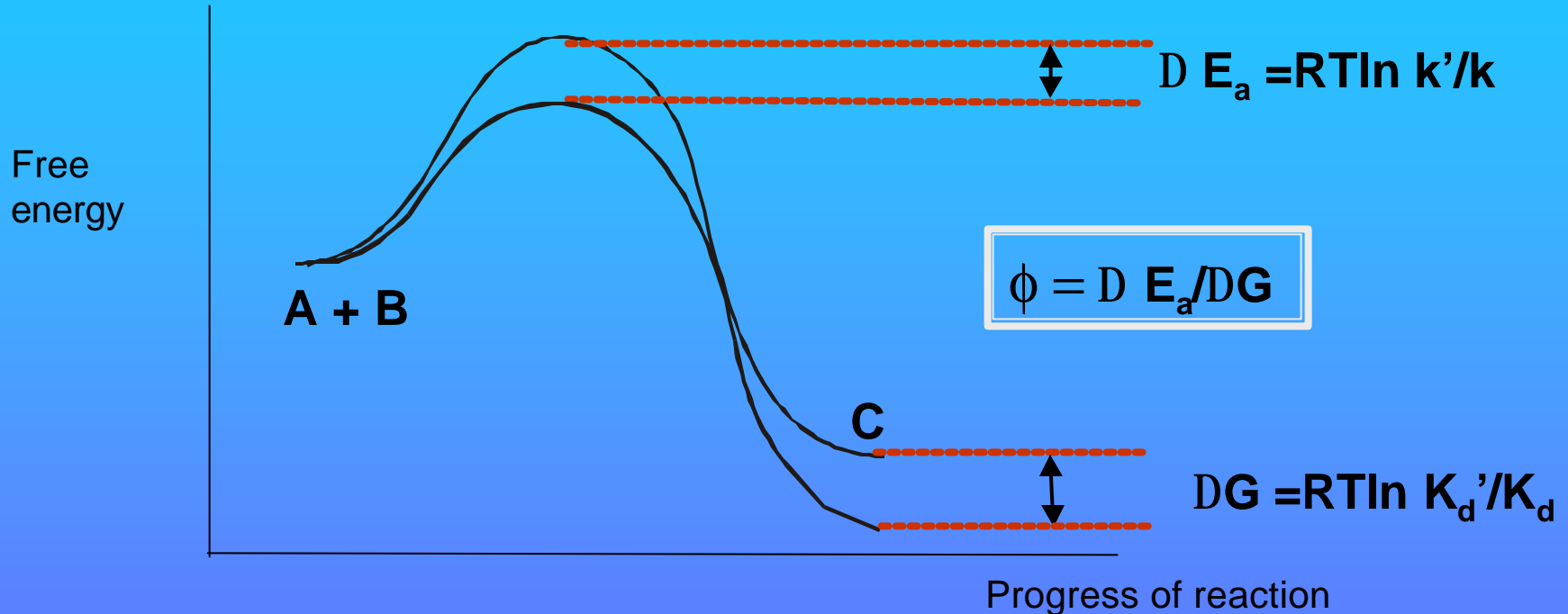
C. Adding an enzyme or catalyst.

Reaction Coordinates



- DG and K_d are functions of state and independent of the reaction path
- E_a is path dependent
- Enzymes affect lower E_a without affecting DG or K_d

ϕ kinetic analysis



- $\phi_{\text{on}} = 1 - \phi_{\text{off}}$
- $\phi_{\text{on}} = 1$, interaction forms before the transition state
- $\phi_{\text{on}} = 0$, interaction forms after the transition state
- $0 < \phi < 1$ is difficult to interpret

Kinetic Analysis Assumptions

- there are no intermediate states of lower energy than the starting state
- mutations only alter the energetics of transition and bound states not the reaction coordinate

Interpreting ϕ

A mutation gave a ϕ_{off} of 0.05. This amino acid is likely to affect:

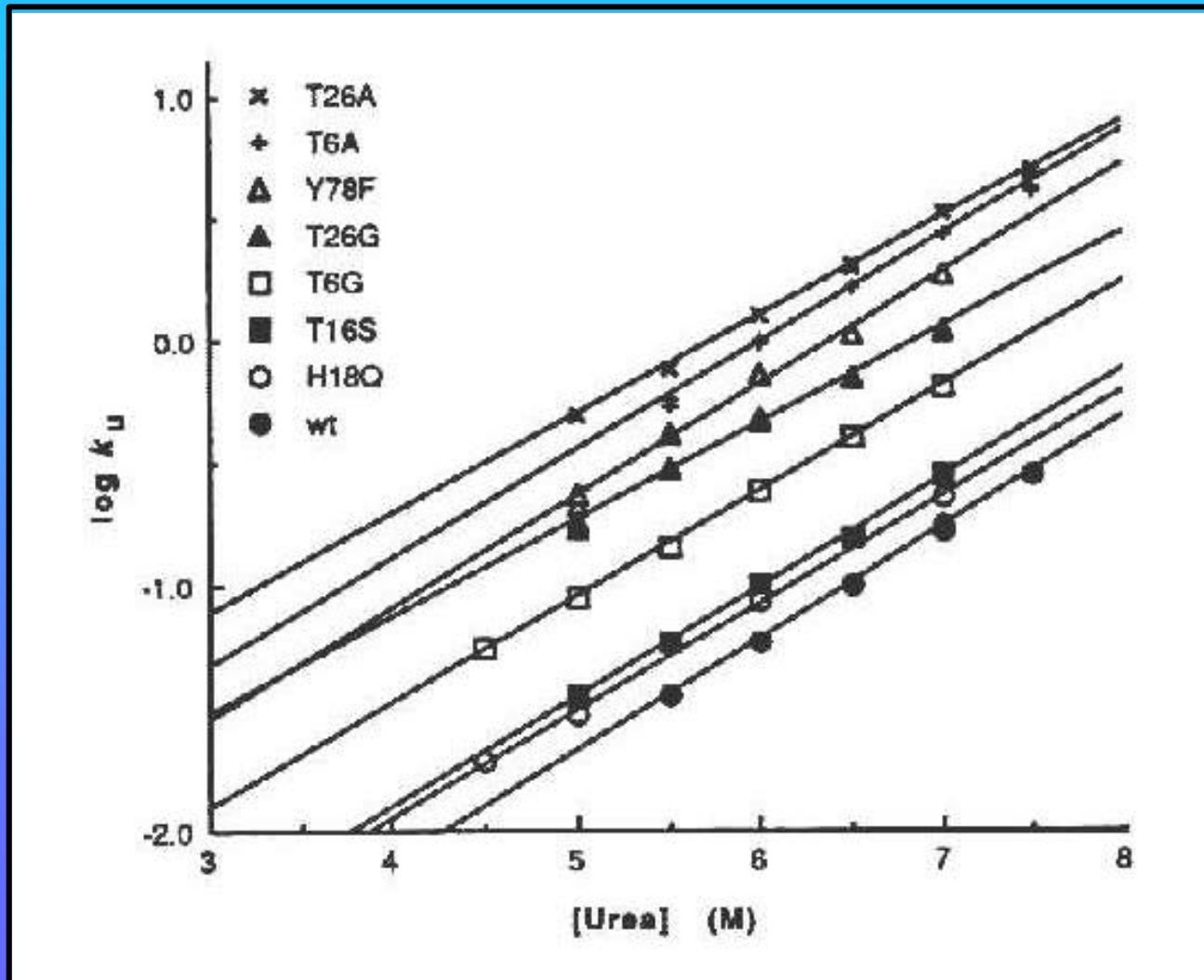
A. the on rate.

B. the off rate.

C. both.

D. neither.

Urea Accelerates Unfolding

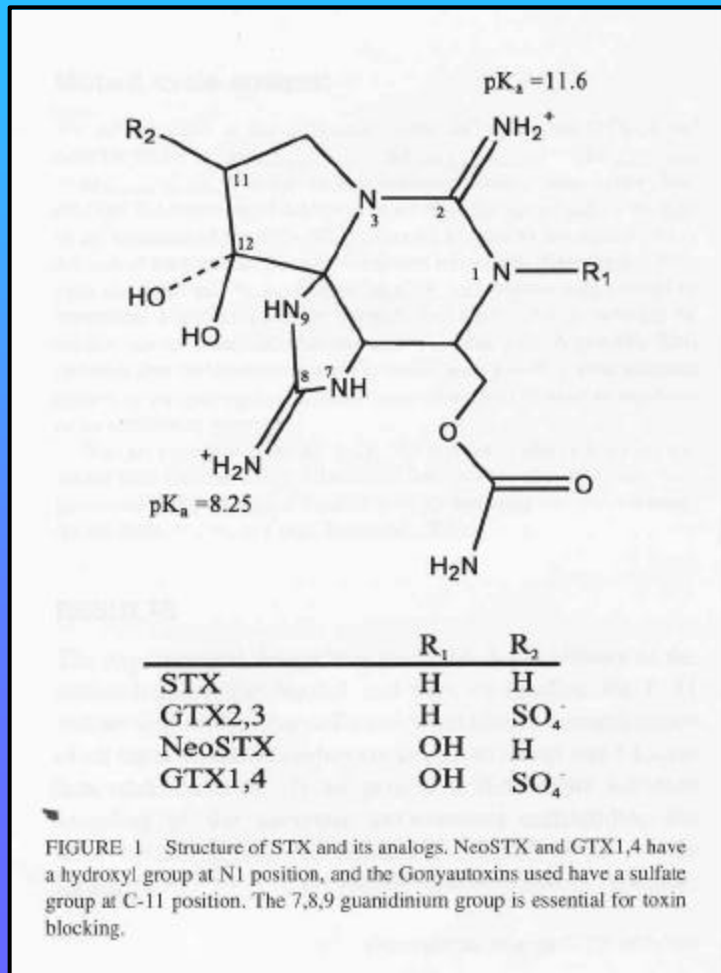


Kinetics Suggest Significant Secondary Structure at the Transition State

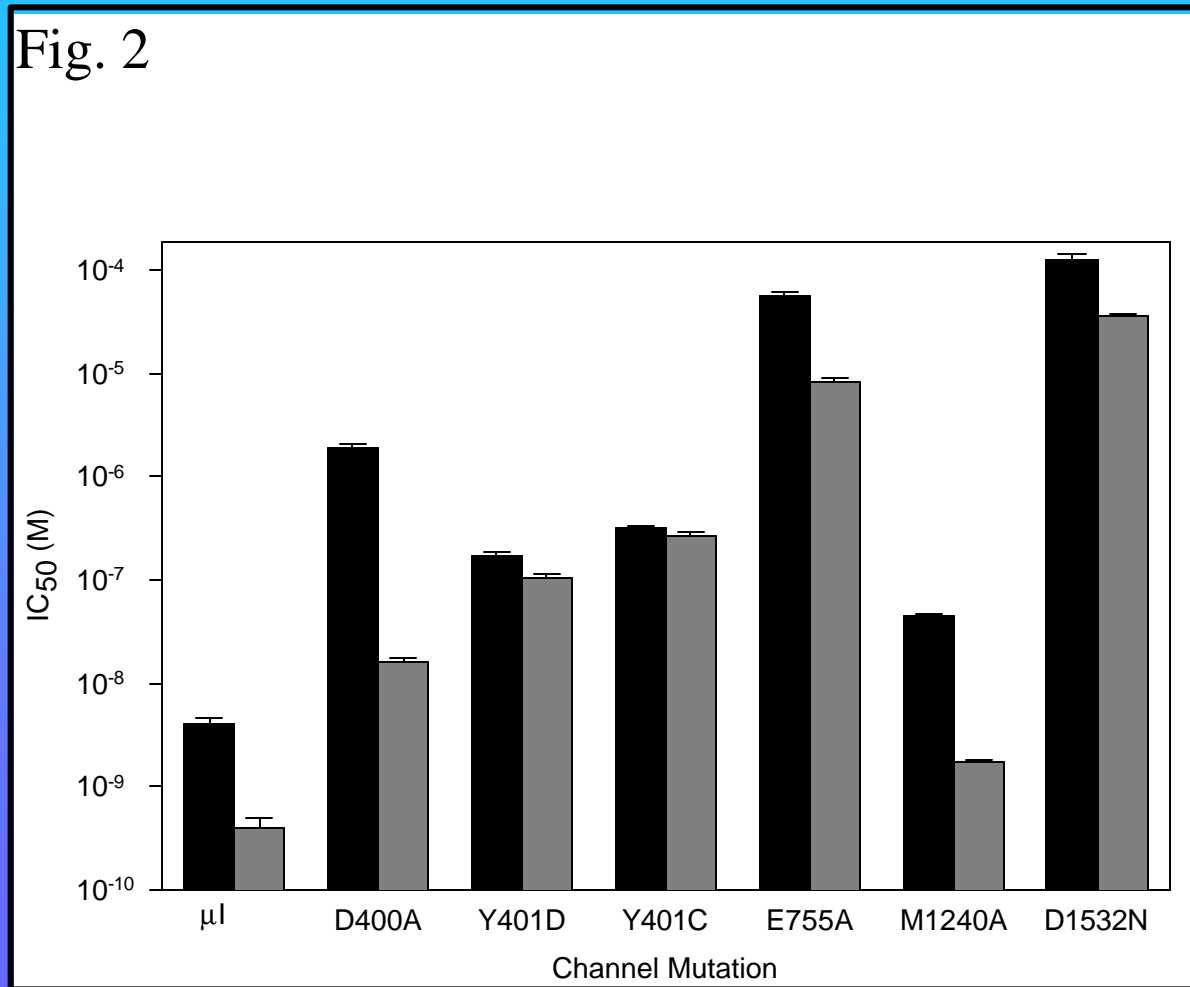
TABLE 1 Changes in activation ($\Delta\Delta G_u^\ddagger$) and free energies of unfolding ($\Delta\Delta G_u$) on mutation of barnase

Mutation	Function of position	$\Delta\Delta G_u$ kcal mol ⁻¹	$\Delta\Delta G_u^\ddagger$ (at 4 M urea) kcal mol ⁻¹	$\Delta\Delta G_u^\ddagger$ (in H ₂ O) kcal mol ⁻¹	$\Delta\Delta G_u^\ddagger/\Delta\Delta G_u$ (at 4 M urea)	$\Delta\Delta G_u^\ddagger/\Delta\Delta G_u$ (in H ₂ O)
Thr → Gly 6	N cap*	1.34	0.86	1.01	0.64	0.76
Thr → Ala 6	N cap*	2.23	1.66	1.76	0.74	0.79
Thr → Gly 26	N cap*	1.58	1.33	1.67	0.84	1.06
Thr → Ala 26	N cap*	2.14	1.91	2.19	0.89	1.02
His → Gln 18	C cap—charge/helix dipole	1.60	0.23	0.36	0.14	0.22
Thr → Ser 16	Hydrophobic on helix surface	1.87	0.28	0.37	0.15	0.20
Tyr → Phe 78	Bridges loop	1.50	1.39	1.41	0.92	0.94
Leu → Ala 14	Hydrophobic core	4.80	1.91	1.86	0.40	0.39
Ile → Val 88	Hydrophobic core	1.49	0.30	0.28	0.20	0.19
Ile → Ala 88	Hydrophobic core	4.46	0.68	0.33	0.15	0.07
Ile → Val 96	Hydrophobic core	0.98	0.48	0.55	0.49	0.56
Ile → Ala 96	Hydrophobic core	3.52	0.74	0.60	0.21	0.17

Structure of STX and its mutation, neoSTX



IC₅₀ Values of STX and NeoSTX for the Na⁺ Channel and Mutations

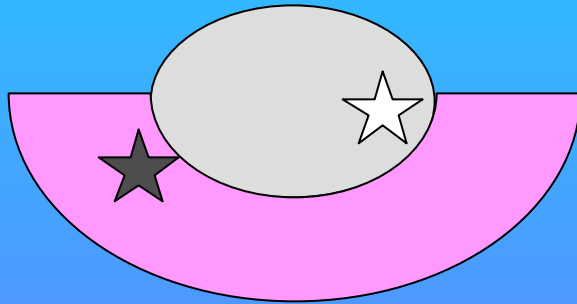


Mutations Affect Binding Kinetics

TABLE 1 Comparison of the effects of channel mutations on neoSTX and STX blocking efficacy

Channel Mutation	IC ₅₀ ± SEM (nM)	n	IC ₅₀ Ratio	k _{on} ± SEM (nM ⁻¹ s ⁻¹)	n	k _{off} ± SEM (s ⁻¹)	n	K _d ± SEM (nM)	n
neoSTX									
μI	0.4 ± 0.1	9	1	8.4 × 10 ⁻³ ± 8.5 × 10 ⁻⁴	7	3.8 × 10 ⁻³ ± 2.3 × 10 ⁻⁴	8	0.5 ± 0.1	7
D400A	16.3 ± 1.1	8	41	4.0 × 10 ⁻⁴ ± 1.0 × 10 ⁻⁴	4	8.2 × 10 ⁻³ ± 1.1 × 10 ⁻³	4	24.3 ± 6.3	4
Y401D	106.8 ± 6.2	8	267	2.4 × 10 ⁻⁵ ± 4.4 × 10 ⁻⁶	7	5.2 × 10 ⁻³ ± 1.0 × 10 ⁻³	7	231.9 ± 30.7	7
Y401C	263.4 ± 25.3	8	659	5.2 × 10 ⁻⁵ ± 1.0 × 10 ⁻⁵	6	1.4 × 10 ⁻² ± 2.5 × 10 ⁻³	6	306.9 ± 64.4	6
E755A	8199.5 ± 989.7	4	20499						
M1240A	1.7 ± 0.1	10	4	5.5 × 10 ⁻³ ± 1.2 × 10 ⁻³	10	1.0 × 10 ⁻² ± 8.1 × 10 ⁻⁴	10	2.4 ± 0.3	10
D1532N	35575.7 ± 2263.6	4	88939						
STX									
μI	4.1 ± 0.5	13	1	4.3 × 10 ⁻³ ± 1.5 × 10 ⁻³	8	1.3 × 10 ⁻² ± 7.4 × 10 ⁻⁴	9	6.3 ± 1.8	8
D400A	1924.9 ± 155.0	10	469	1.8 × 10 ⁻⁶ ± 3.3 × 10 ⁻⁷	6	1.0 × 10 ⁻² ± 1.7 × 10 ⁻³	6	6524.8 ± 1461.5	6
Y401D	169.3 ± 13.7	10	41	2.4 × 10 ⁻⁵ ± 6.2 × 10 ⁻⁶	6	7.1 × 10 ⁻³ ± 5.1 × 10 ⁻⁴	6	373.6 ± 73.9	6
Y401C	314.4 ± 12.5	5	77	6.5 × 10 ⁻⁵ ± 1.4 × 10 ⁻⁵	4	2.2 × 10 ⁻² ± 3.4 × 10 ⁻³	4	376.7 ± 70.6	4
E755A	55035.8 ± 5277.7	8	13423						
M1240A	44.7 ± 3.0	12	11	5.9 × 10 ⁻⁴ ± 1.6 × 10 ⁻⁴	10	1.3 × 10 ⁻² ± 1.6 × 10 ⁻³	10	29.9 ± 5.6	10
D1532N	127487.2 ± 13078.5	8	31094						

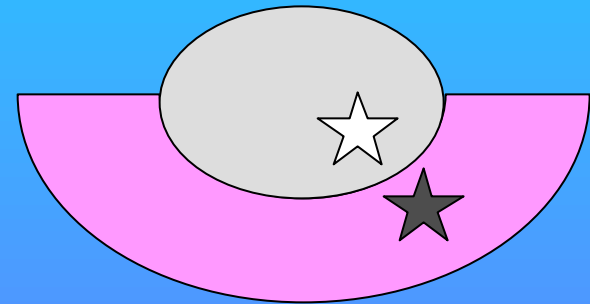
Mutant Cycles: The Basics



1 nM \longrightarrow 10 nM

5 nM \longrightarrow 50 nM

$$DDG = RT \ln (1/10)/(5/50) \\ = 0 \text{ kcal/mol}$$

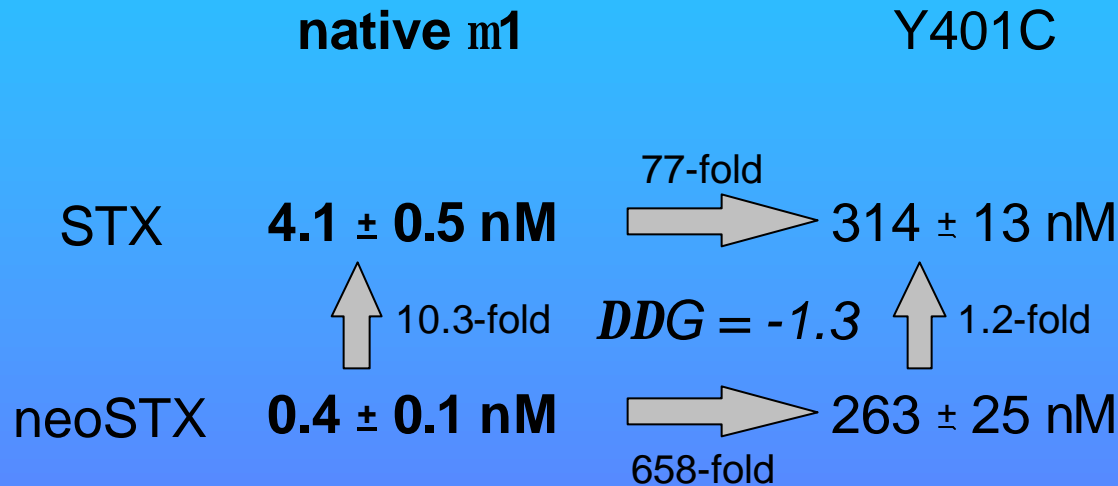


1 nM \longrightarrow 10 nM

10 nM \longrightarrow 10 nM

$$DDG = RT \ln \\ (1/10)/(10/10) = 1.3 \\ \text{kcal/mol}$$

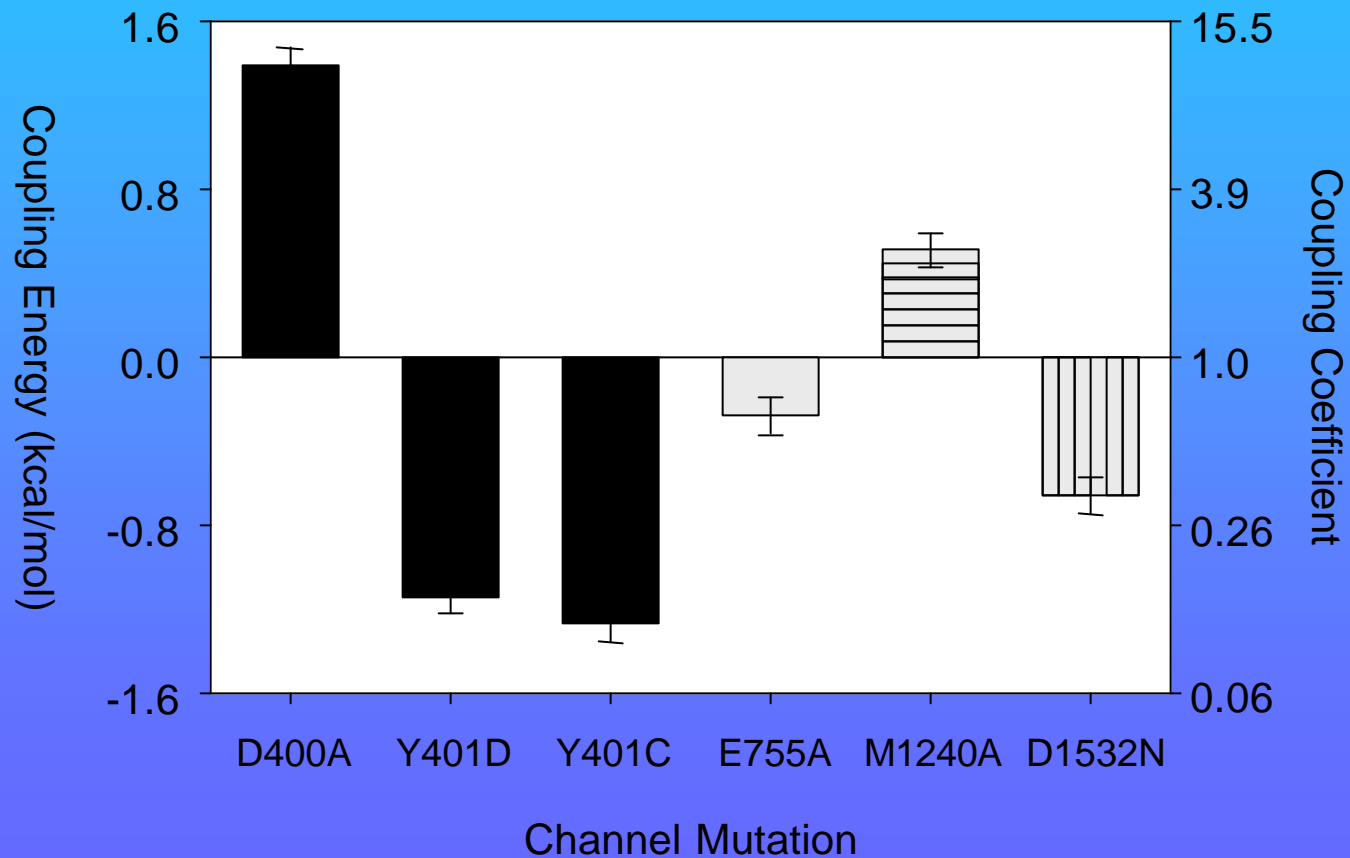
Mutant Cycles - Isolating Interactions Between Two Residues



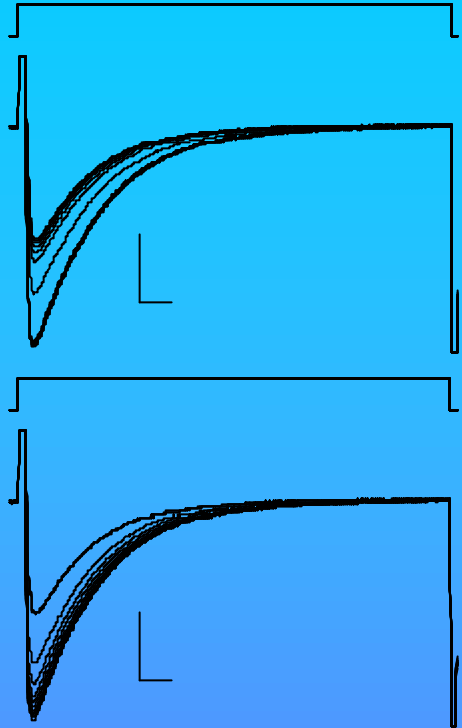
$$DDG = RT \ln W = RT \ln (IC50'/IC50'')/(IC50'''/IC50''''')$$

- $\Omega = 1$ means there is no coupling.
- The larger the deviation of Ω from 1, the more energy of interaction.

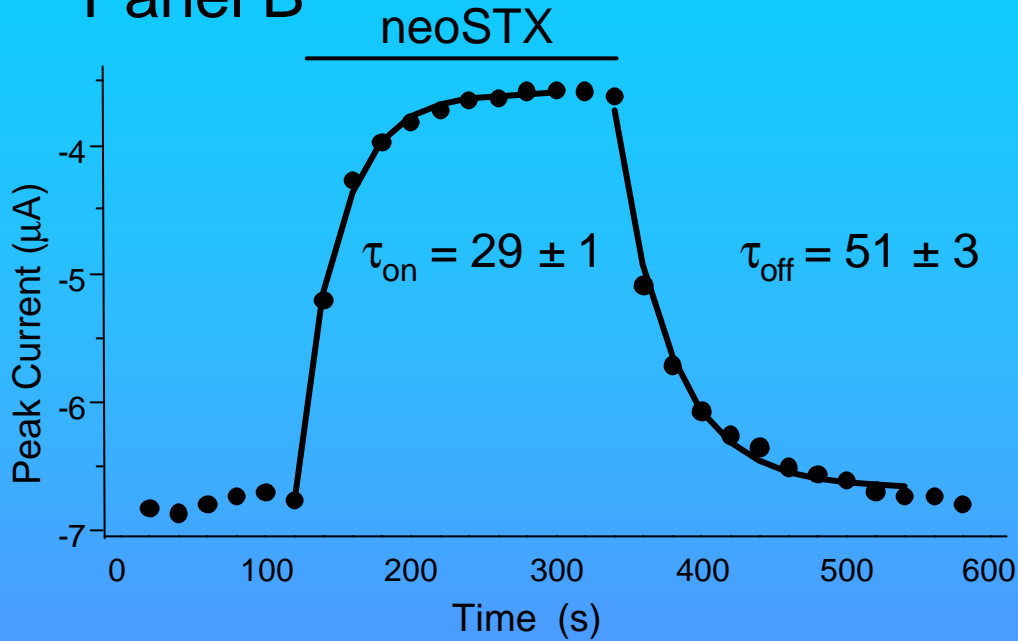
Coupling Energies of N1-OH with Various Channel Residues



Panel A



Panel B



Panel C

Y401C

native m1

D400A

STX

6.5×10^{-5}

4.3×10^{-3}

1.8×10^{-6}

0.8-fold

$\Omega = 0.4$

2.0-fold

$\Omega = 111$

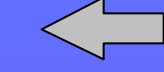
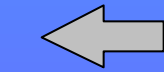
222-fold

neoSTX

5.2×10^{-5}

8.4×10^{-3}

4.0×10^{-4}



NeoSTX in a Model of the Na⁺ Channel Outer Vestibule

