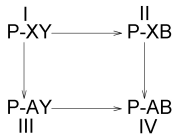
SI Guide for Gadsby ms 25186

1)
File name: GadsbySupplNotes.doc
Title: Supplementary Notes: Thermodynamic double mutant cycles
Derivation of equations used to quantify energetic coupling.
(MS Word document, 80 kB)

2)
File name: GadsbySupplTable.doc
Title: Supplementary Table: Kinetic Parameters of WT and mutant CFTR channels
Part A: kinetic parameters describing single-channel gating, obtained from patches containing a small number of channels (e.g. Figs. 2c, 3b and 4b).
Part B: Current decay time-constants, obtained from patches containing hundreds of channels (e.g. Fig 1b).
(MS Word document, 28 kB)

THERMODYNAMIC DOUBLE-MUTANT CYCLES

We can construct a thermodynamic cycle linking single and double mutations



SCHEME I:

Each corner of the square represents a different construct (I, II, III or IV); X and Y are the target residues mutated to A and B, respectively. Considering changes in an unspecified path-independent state property, if the two mutations are independent their effects will be additive:

$\Delta_{IV-I} = \Delta_{II-I} + \Delta_{III-I}$ (Independence)

where Δ_{IV-I} is the change due to the double mutation and Δ_{II-I} and Δ_{III-I} are the changes due to mutating individually Y to B or X to A, respectively. Discrepancy from additivity gives a measure of the energetic coupling between the two residues in the protein ($\Delta\Delta_{int}$):

$\Delta\Delta_{int} = \Delta_{IV-I} - \Delta_{II-I} - \Delta_{III-I} = (\Delta_{III-I} + \Delta_{IV-III}) - \Delta_{II-I} - \Delta_{III-I} = \Delta_{IV-III} - \Delta_{II-I}$

Thus

$\Delta\Delta_{int} = \Delta_{IV-III} - \Delta_{II-I} = \Delta_{IV-II} - \Delta_{III-I}$ (Coupling) equation [1]

The equations give an intuitive description of coupling, comparing the effects of the mutation in mutant (Δ_{IV-II}, Δ_{IV-III}) and WT (Δ_{III-I}, Δ_{II-I}) background: if the two mutations are not coupled, mutation-linked changes on parallel sides of the mutant cycle are the same. Any non-zero difference in the effects of the mutation in WT and mutant background is a measure of energetic coupling (equation [1]).

We use changes in Gibbs free energy (ΔG) for the process starting in state 1 and ending in state 2 to characterize each construct (each corner in scheme I)

$\Delta G = G_2 - G_1$

From equation [1]:

$\Delta\Delta G_{int} = (\Delta G_{IV} - \Delta G_{II}) - (\Delta G_{III} - \Delta G_{I})$

Substituting $G_2 - G_1$ for ΔG values in this equation yields:

$\Delta\Delta G_{int} = (G_{2\,IV} - G_{1\,IV} - G_{2\,II} + G_{1\,II}) - (G_{2\,III} - G_{1\,III} - G_{2\,I} + G_{1\,I})$

rearranging:

$\Delta\Delta G_{int} = (G_{2\,IV} - G_{2\,II} - G_{2\,III} + G_{2\,I}) - (G_{1\,IV} - G_{1\,II} - G_{1\,III} + G_{1\,I})$

$\Delta\Delta G_{int} = (\Delta G_{2\,IV\text{-}II} - \Delta G_{2\,III\text{-}I}) - (\Delta G_{1\,IV\text{-}II} - \Delta G_{1\,III\text{-}I})$

The quantity within the first bracket, can be thought of as the coupling in state 2, the difference between changes on parallel sides of a cycle resulting from an "alchemical" linking of states 2 for WT, single mutants and double mutant. Similarly, the terms within the second bracket represent coupling in state 1, so that:

$\Delta\Delta G_{int} = \Delta\Delta G_{int\,2} - \Delta\Delta G_{int\,1}$

Therefore, the sign of $\Delta\Delta G_{int}$ will give information on the relative strength of energetic coupling in state 2 and state 1. For example, if the single mutations disrupt a stabilizing interaction in both states, the $\Delta\Delta G_{int\,2}$ and $\Delta\Delta G_{int\,1}$ values are negative, and a negative sign of the global $\Delta\Delta G_{int}$ indicates that such interactions are stronger in state 2 than in state 1 (e.g. **Fig.4a** and **d**). However, a negative overall $\Delta\Delta G_{int}$ will also result if the single mutations disrupt destabilizing interactions ($\Delta\Delta G_{int\,1}$ and $\Delta\Delta G_{int\,2}$ values are positive) but the disrupted interactions are stronger in state 1 than in state 2.

When the kinetic measurement used is the rate of a process (e.g. channel opening rate) the derivation above can be used, substituting the ground state and the transition state for that process as state 1 and state 2, respectively. Transition state theory, developed for bonds obeying quantum theory, relates the rate of a chemical reaction to the reaction's $\Delta G^{\ddagger}$. This theory can be extended to the kinetics of complex conformational changes in proteins as long as the quantities of interest are *changes* in barrier heights, thus eliminating uncertainties regarding the magnitude of the pre-exponential factor (Fersht, 1999).

Coupling energy can be partitioned into several components, direct interactions between the target side-chains being only one of them, and interactions of the targets with the rest of the protein and with solvent and ligand often making important contributions to $\Delta\Delta G$int (Serrano, 1990). The coupling energies measured here are within the very wide range of energy values attributed to hydrogen bonds within proteins (Fersht, 1999), but an increase, upon channel opening, in coupling energy mediated by indirect interactions would be equally consistent with our conclusions.

Kinetic parameters of WT and mutant CFTR channels

A

	WT		**R555K**	
	mean ± SEM	n	mean ± SEM	n
τb	432 ± 20	32	387 ± 39	26
τib	2288 ± 458	16	8531 ± 1227	15
τ_F	16.2 ± 2.3	32	19.9 ± 3.0	26
n_F	0.77 ± 0.10	32	0.95 ± 0.19	26
r_{CO}	0.65 ± 0.09	16	0.16 ± 0.02	15
r_{OC}	2.67 ± 0.22	32	3.22 ± 0.25	26

	T1246N		**R555K T1246N**	
	mean ± SEM	n	mean ± SEM	n
τb	1,196 ± 350	14	2,323 ± 142	16
τib	26,883 ± 5180	7	7,441 ± 1785	7
τ_F	34.5 ± 6.6	14	29.5 ± 3.2	16
n_F	0.95 ± 0.16	14	1.86 ± 0.14	16
r_{CO}	0.06 ± 0.02	7	0.18 ± 0.04	7
r_{OC}	1.30 ± 0.16	14	0.21 ± 0.03	16

Rates (r_{CO}: opening rate; r_{OC}: closing rate) and derived parameters (τb: burst duration; τib: interburst duration; τ_F : duration of flickery closure; n_F: number of flickers per burst) were obtained from maximum likelihood fit of dwell-time histograms at all conductance levels. (Csanády, 2000). Channels were activated to a steady state, by application of 5 mM MgATP + 300 nM PKA. Durations are given in ms and rates in s^{-1}.

B

	K1250R		**R555K K1250R**	
	mean ± SEM	n	mean ± SEM	n
τ	9,323 ± 515	49	27,266 ± 4,439	25

	T1246N K1250R		**R555K T1246N K1250R**	
	mean ± SEM	n	mean ± SEM	n
τ	15,772 ± 2,543	11	55,646 ± 10,782	11

Time constants (in ms) were obtained from least squares fit of macroscopic current decay, after activation with 5 mM MgATP + 300 nM PKA.