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Supporting Material

Linking the acetylcholine receptor-channel agonist binding sites with the gate

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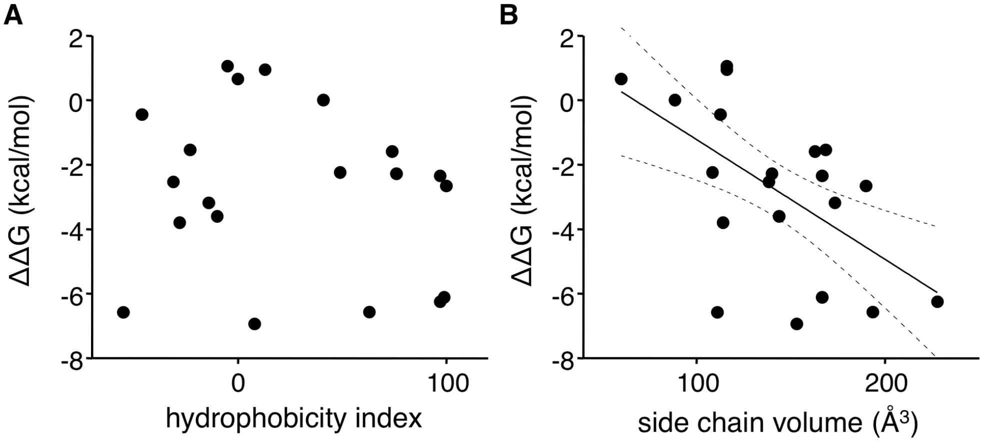

A
B
ΔΔG (kcal/mol)
2
0
-2
-4
-6
-8
0
100
hydrophobicity index
ΔΔG (kcal/mol)
2
0
-2
-4
-6
-8
100
200
side chain volume (Å3)

Figure S1

Figure S1: ΔΔG measurements of αA96 mutations correlate with side chain volume but not hydrophobicity.

A. ΔΔG plotted as a function of side chain hydrophobicity, for all 20 natural amino acid side chains. There is no correlation of this parameter with the equilibrium constant.

B. ΔΔG plotted as a function of side chain volume, for all 20 natural amino acid side chains. There is a tendency for E_0 to increase with increasing side chain volume. The slope of the line is -0.037 kcal mol^{-1} $(\text{Å}^3)^{-1}$ ± 0.012 (SE), $R^2=0.29$. Dotted lines are ±90% confidence limits.

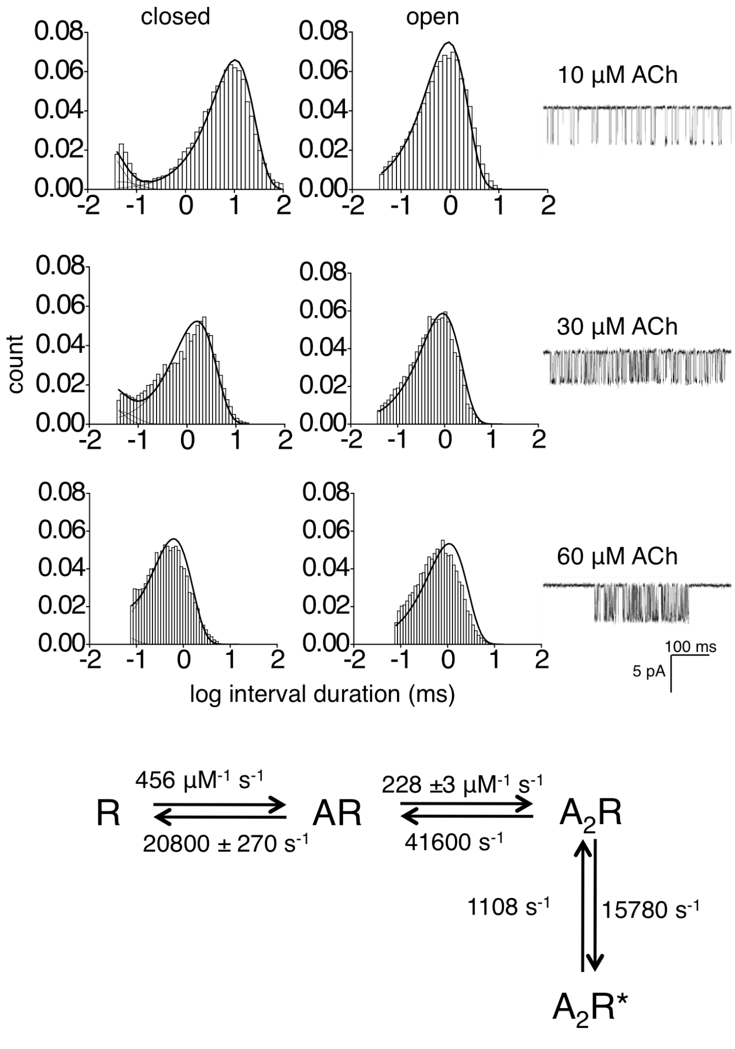
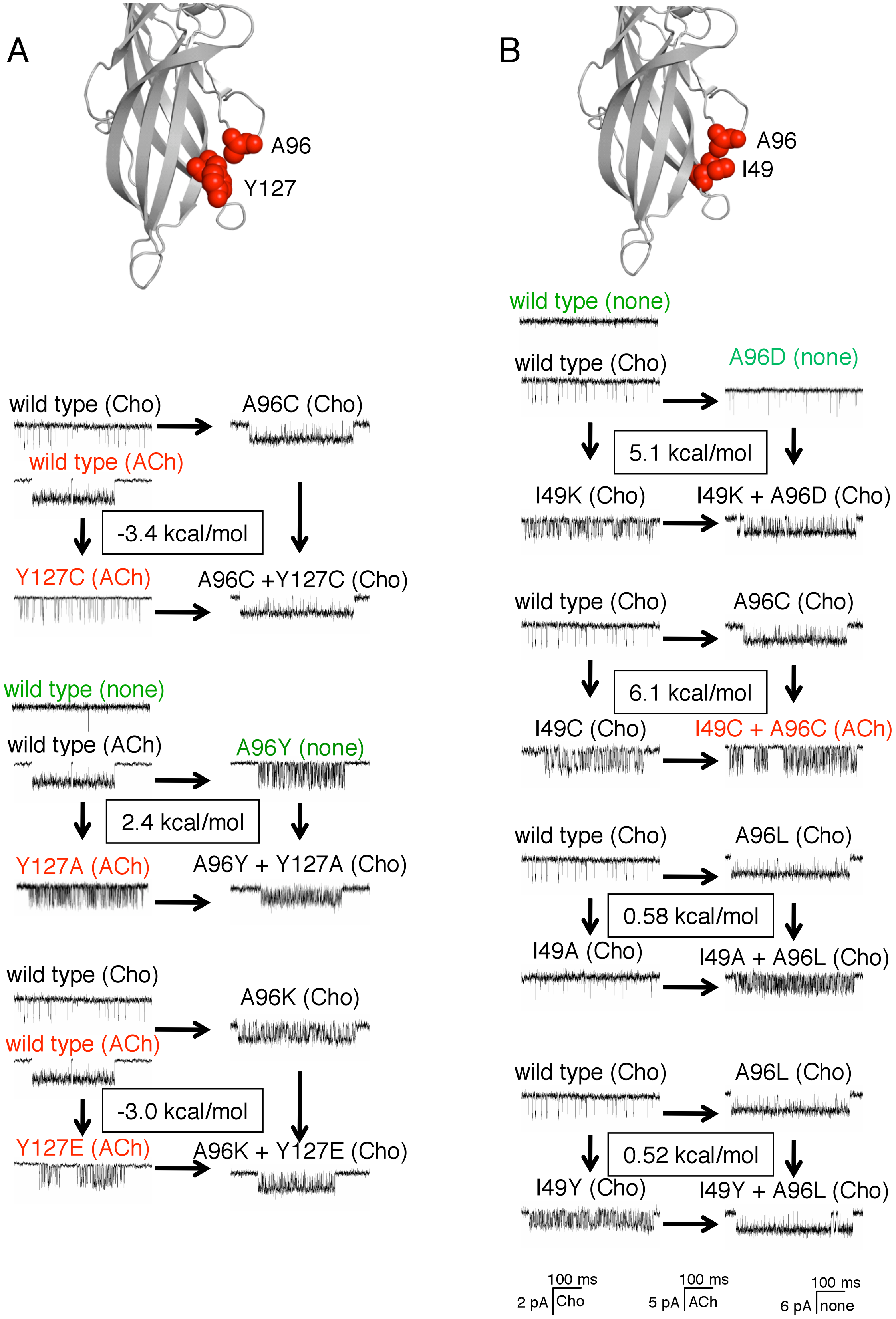

closed
open
0.08
0.06
0.04
0.02
0.00
-2
-1
0
1
2
count
log interval duration (ms)
10 µM ACh
30 µM ACh
60 µM ACh
100 ms
5 pA
R
AR
A_2R
A_2R^*
456 µM^{-1} s^{-1}
20800 ± 270 s^{-1}
228 ±3 µM^{-1} s^{-1}
41600 s^{-1}
1108 s^{-1}
15780 s^{-1}

Figure S2

Figure S2: The mutation αA96G has little effect on ACh binding

Intracluster interval durations were fitted globally using a linear binding-gating kinetic scheme (below) at three different concentrations of ACh. The models assumed two equal and independent binding steps followed by a single isomerization step. Left, observed interval duration histograms (bars) and probability density functions (solid lines) calculated from the globally-fitted rate constant estimates. Right, example clusters. The optimal binding rate constants were: association, $k_+ = 228 \pm 3\ \mu M^{-1}s^{-1}$; dissociation, $k_- = 20{,}800 \pm 270\ s^{-1}$. $K_d = k_-/k_+ = 91\ \mu M$. These values are similar to the wt values: $k_+=140\ \mu M^{-1}s^{-1}$, $k_- = 20{,}000\ s^{-1}$. $K_d = 140\ \mu M$.


A
A96
Y127
wild type (Cho)
A96C (Cho)
wild type (ACh)
-3.4 kcal/mol
Y127C (ACh)
A96C +Y127C (Cho)
wild type (none)
wild type (ACh)
A96Y (none)
2.4 kcal/mol
Y127A (ACh)
A96Y + Y127A (Cho)
wild type (Cho)
A96K (Cho)
wild type (ACh)
-3.0 kcal/mol
Y127E (ACh)
A96K + Y127E (Cho)
B
A96
I49
wild type (none)
wild type (Cho)
A96D (none)
5.1 kcal/mol
I49K (Cho)
I49K + A96D (Cho)
wild type (Cho)
A96C (Cho)
6.1 kcal/mol
I49C (Cho)
I49C + A96C (ACh)
wild type (Cho)
A96L (Cho)
0.58 kcal/mol
I49A (Cho)
I49A + A96L (Cho)
wild type (Cho)
A96L (Cho)
0.52 kcal/mol
I49Y (Cho)
I49Y + A96L (Cho)
100 ms
2 pA
Cho
100 ms
5 pA
ACh
100 ms
6 pA
none

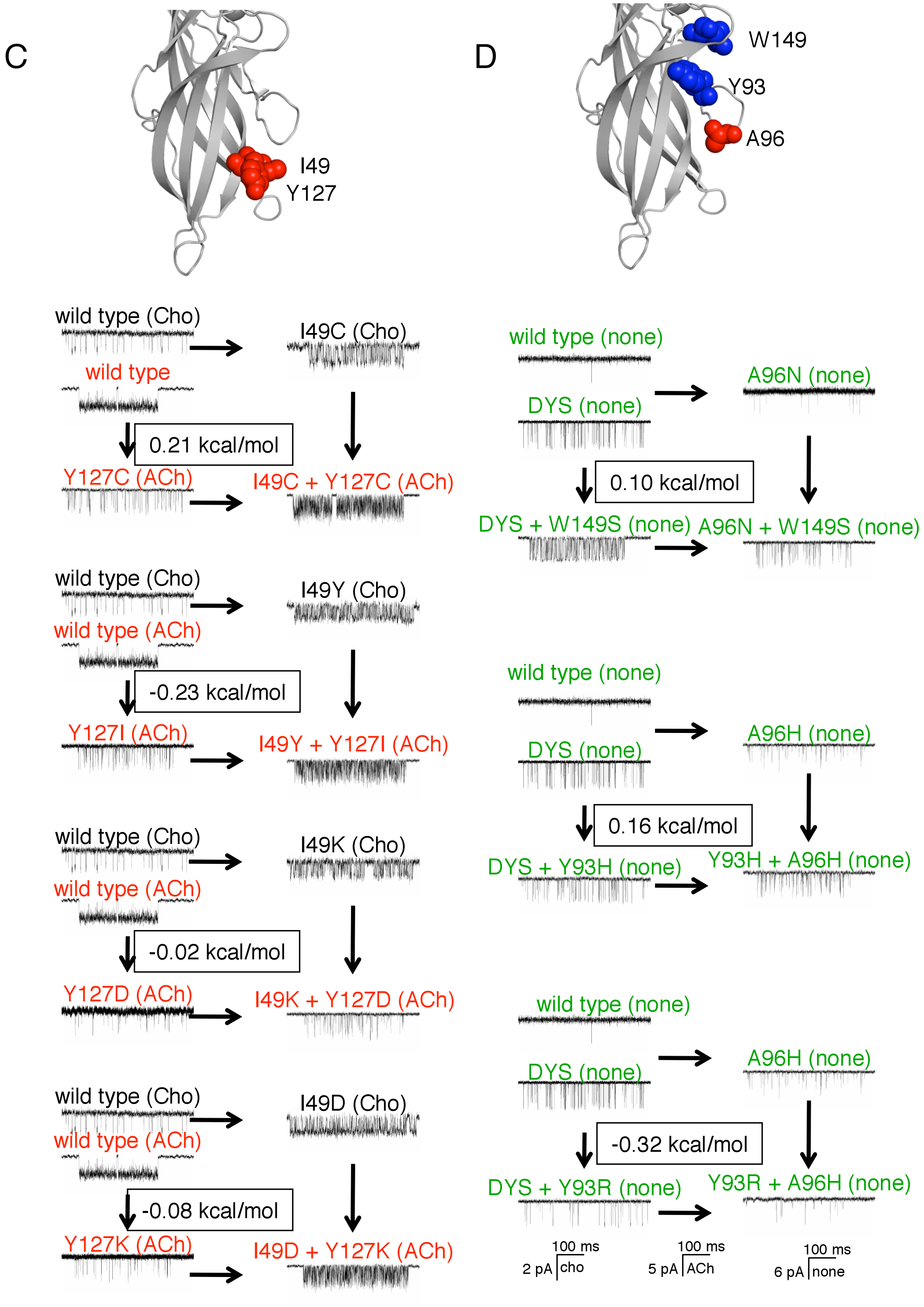

C
I49
Y127
wild type (Cho)
I49C (Cho)
wild type
0.21 kcal/mol
Y127C (ACh)
I49C + Y127C (ACh)
wild type (Cho)
I49Y (Cho)
wild type (ACh)
-0.23 kcal/mol
Y127I (ACh)
I49Y + Y127I (ACh)
wild type (Cho)
I49K (Cho)
wild type (ACh)
-0.02 kcal/mol
Y127D (ACh)
I49K + Y127D (ACh)
wild type (Cho)
I49D (Cho)
wild type (ACh)
-0.08 kcal/mol
Y127K (ACh)
I49D + Y127K (ACh)
D
W149
Y93
A96
wild type (none)
A96N (none)
DYS (none)
0.10 kcal/mol
DYS + W149S (none)
A96N + W149S (none)
wild type (none)
A96H (none)
DYS (none)
0.16 kcal/mol
DYS + Y93H (none)
Y93H + A96H (none)
wild type (none)
A96H (none)
DYS (none)
-0.32 kcal/mol
DYS + Y93R (none)
Y93R + A96H (none)
100 ms
2 pA
cho
100 ms
5 pA
ACh
100 ms
6 pA
none

Figure S3: Mutant cycle analysis

Coupling energies (boxed; Table 2) were calculated for each pair of side chain substitution: (kcal/mol)=-0.59ln[(E$^{\text{double mutant}}$)(E$^{\text{wild type}}$)/(E$^{\text{mutant 1}}$)/(E$^{\text{mutant 2}}$)]. An example cluster for each construct is shown. For each cycle, upper left is wild type and lower right is double mutant. Red letters, ACh-activated; black letters, choline-activated; green letters, spontaneously active. A. αA96-αY127 show a coupling energy that ranges between -3.4 kcal/mol (Cys-Cys) to +2.4 kcal/mol (Tyr-Ala). B. αA96-αI49 show a coupling energy that ranges between +0.5 kcal/mol (Tyr-Leu) to +5.1 kcal/mol (Lys-Asp). C. αI49-αY127 show little interaction, as the coupling energy ranges between -0.2 kcal/mol (Tyr-Ile) to +2 kcal/mol (Cys-Cys). D. αIA96 is not coupled significantly to either αW149 or αY93.