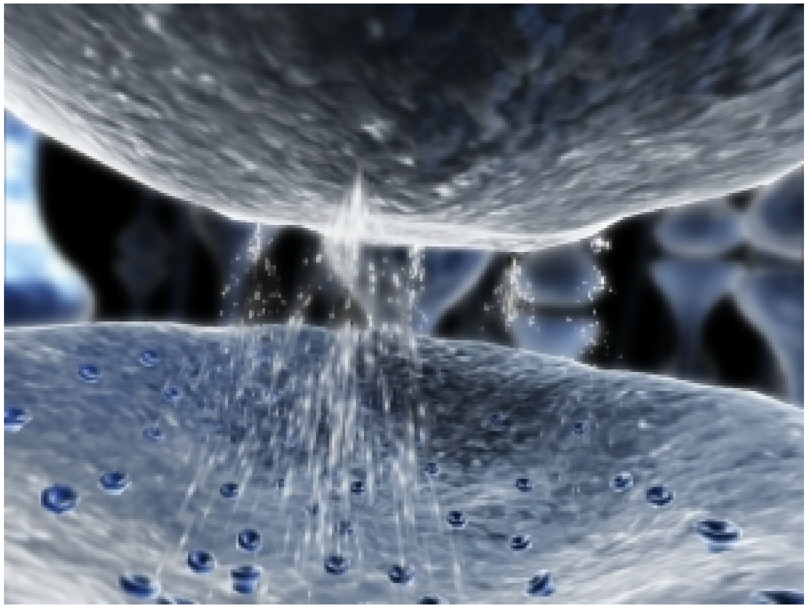
Career of Dennis A. Dougherty: Ion Channels



Alex Warkentin

MacMillan Group Meeting

March 25th, 2010

Dennis A. Dougherty: Affiliations and Awards

- B.S., M.S., Bucknell University, 1974

- Ph.D., Princeton University (Kurt Mislow), 1978
  Computational Chemistry for conformational/stereocehmical analysis
  Strained molecular structural study from force field calculations

- Post-doc, Yale University (Jerome Berson), 1979
  Study of stabilized *m*-quinomethide biradicals

- -

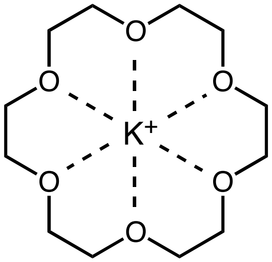
- Assistant Professor, California Institute of Technology, 1979 - 1985
- Associate Professor, California Institute of Technology, 1985 - 1989
- Professor, California Institute of Technology, 1989 - 2001
- Executive Officer for Chemistry, 1994 - 1999
- Hoag Professor, 2002 - present

- -

- National Academy of Sciences (2009); American Academy of Arts and Sciences (1999); many more
- Editorial Board: *Journal of Physical Organic Chemistry, Supramolecular Chemistry, Chemistry and Biology*
- Books: *Modern Physical Organic Chemistry*, with Eric Anslyn
- Henry Lester, Bren Professor of Biology, California Institute of Technology
  Frequent collaborator of 15 years

Dennis A. Dougherty: Early Work

- Not covered here (~1982 - 1996): High spin organic polymers (ferromagnets) *J. Am. Chem. Soc.* **1994**, *116*, 8152 (many pubs.; most recent)
- Non-Kekule Benzenes, *J. Am. Chem. Soc.* **1989**, *111*, 3943 (and refs. therein)
- Other topics: Physical organic probes using matrix isolation, low temp. (~4 K) radical study, polyradicals, radical rearrangements, radical tunneling
- Pedersen launches field of "Host-Guest" chemistry with discovery of 18-crown-6 binding to potassium cation (1967)
- Pedersen shares 1987 Nobel Prize with Cram and Lehn for Host-Guest chemistry



Anslyn, E. V.; Dougherty D. A. *Modern Physical Organic Chemistry* University Science Books (Sausalito, CA) 2005, p 221.

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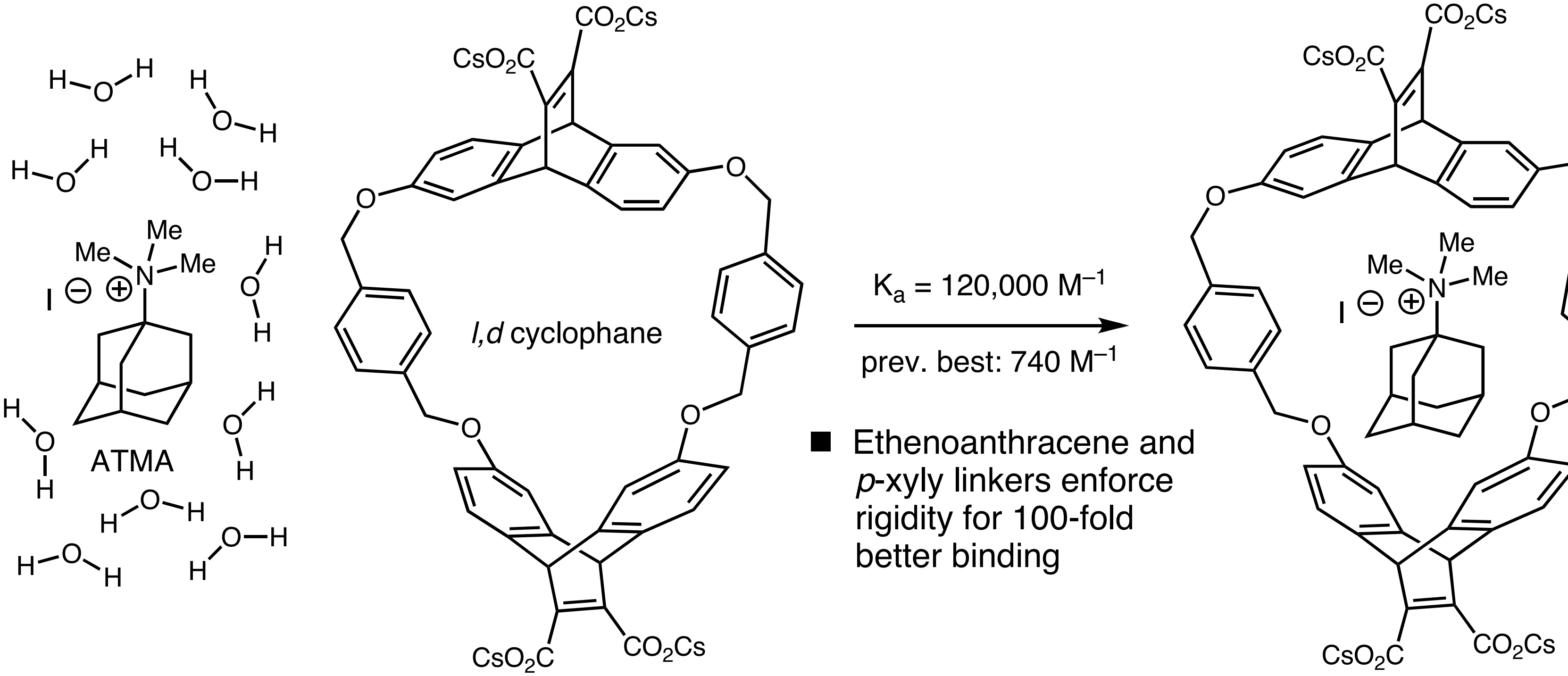
- 1986: First host of aliphatic guest. Δppm over range of concentrations gives binding constant

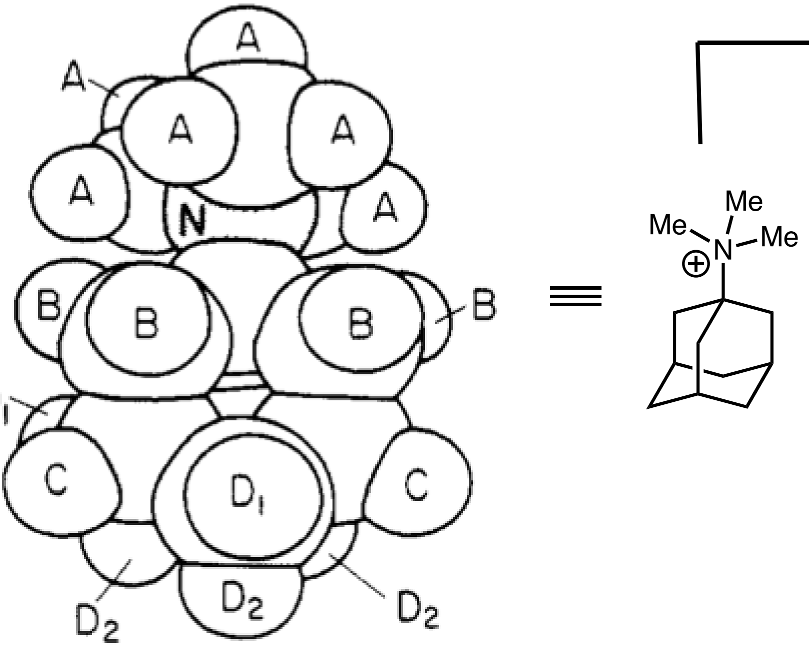


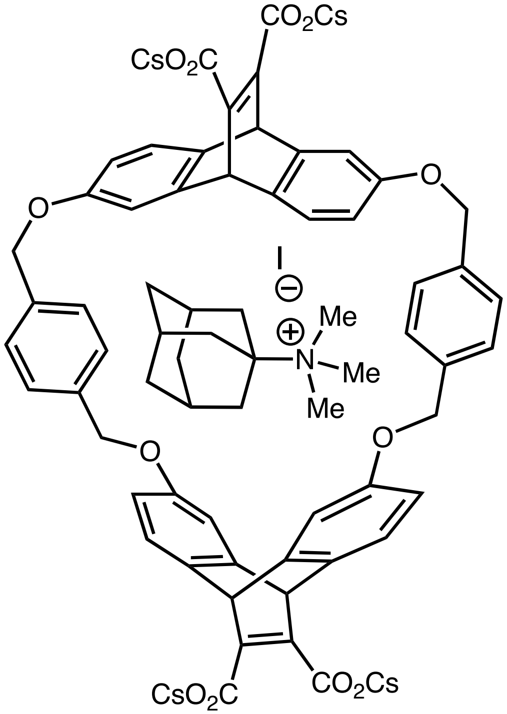
- Ethenoanthracene and *p*-xyly linkers enforce rigidity for 100-fold better binding

Shepodd, T. J.; Petti, M. A.; Dougherty, D. A. *J. Am. Chem. Soc.* **1986**, *108*, 6085.

Dennis A. Dougherty: Early Work

- Chemical shifts for specific protons betray binding orientation of ATMA in *d,l* P
- A-protons bind in cyclophane pocket (A/B most deshielded, D_2 least)
- This would have massive implications for the rest of Dougherty's carreer





ΔA	ΔB	ΔC	ΔD_1	ΔD_2	
1.83	2.90	1.19	1.30	0.76	(ppm)

⇨

$$\Delta\delta = \Delta\delta_{tot}K_a[G]/(1 + K_a[G])$$

G = guest

$K_a = 120{,}000\ M^{-1}$

⇨

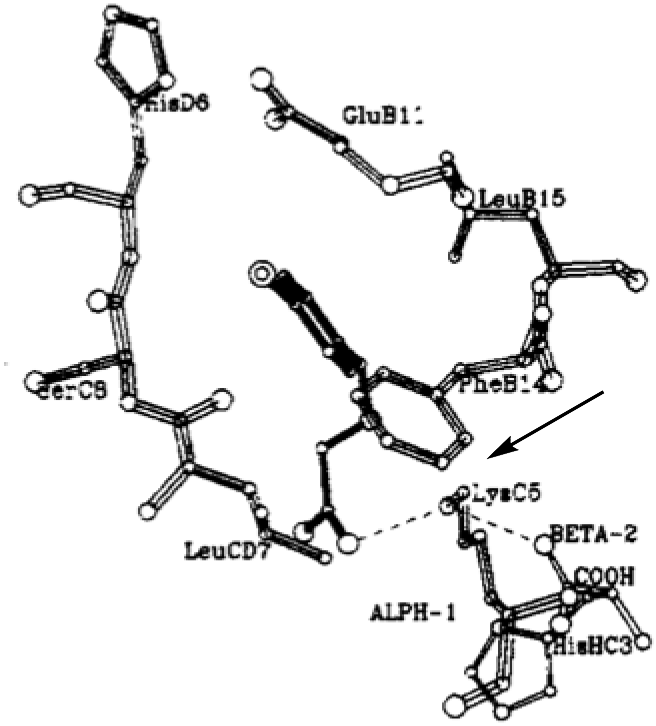
$$-\Delta G^o = RT\ln(K_a)$$

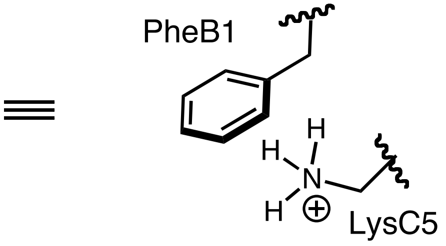
Shepodd, T. J.; Petti, M. A.; Dougherty, D. A. *J. Am. Chem. Soc.* **1986**, *108*, 6085.

Anslyn, E. V.; Dougherty D. A. *Modern Physical Organic Chemistry* University Science Books (Sausalito, CA) 2005, p 221.

Dennis A. Dougherty: Early Influence

- Dougherty influenced by Perutz and others who identify amide H-bonding and other "amino-aromatic" interactions in X-rays of hemoglobin binding to drugs and peptides





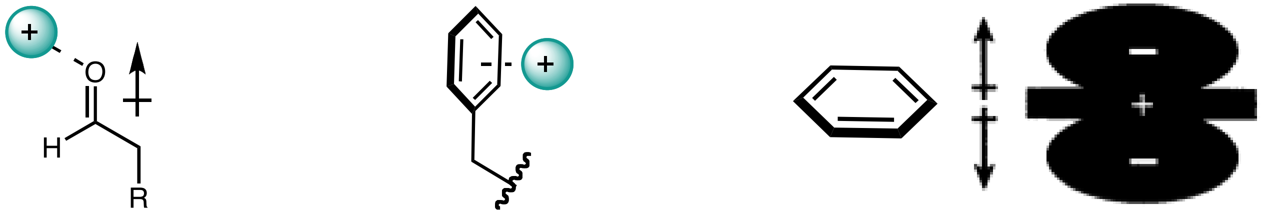
Dubbed "amino-aromatic"
interaction

- What is this interaction composed of?
  How general is it?

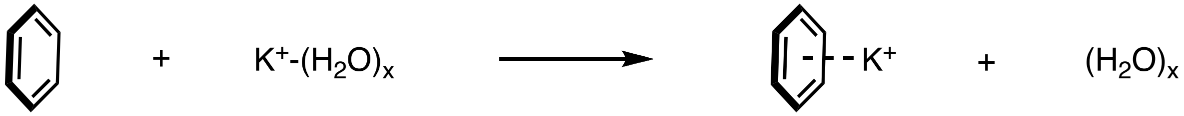
Perutz, M. F.; Fermi, G.; Abraham, D. J.; Poyart, C.; Bursaux, E. *J. Am. Chem. Soc.* **1986**, *108*, 1064.

A Newly Recognized Fundamental Non-Covalent Interaction: Cation-π

- Permanent dipoles attract ions at their poles. How do quadrupoles accomplish this?



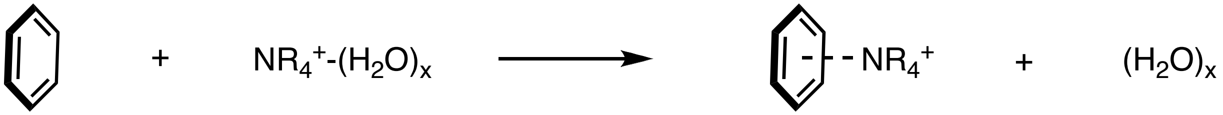
- Gas-phase calculations give trends for binding of cations to aromatic groups



–ΔG = 18 kcal/mol

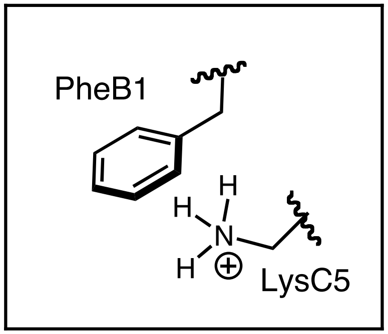
–ΔG = 19 kcal/mol

- More pertinent to biology, NH_4^+ and NMe_4^+ give similar gas phase energies



–ΔG = 18 kcal/mol (R = H)
–ΔG = 9 kcal/mol (R = Me)

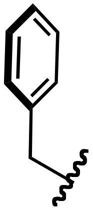
–ΔG = 19 kcal/mol (R = H)
–ΔG = 9 kcal/mol (R = Me)



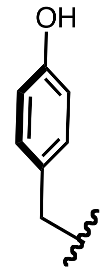
Ma, J. C.; Dougherty, D. A. *Chem. Rev.* **1997**, *97*, 1303.

A Newly Recognized Fundamental Non-Covalent Interaction: Cation-π

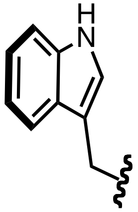
- Aromatic amino acids now seen in a new light: "Polar, yet hydrophobic residues"



Phenylalanine

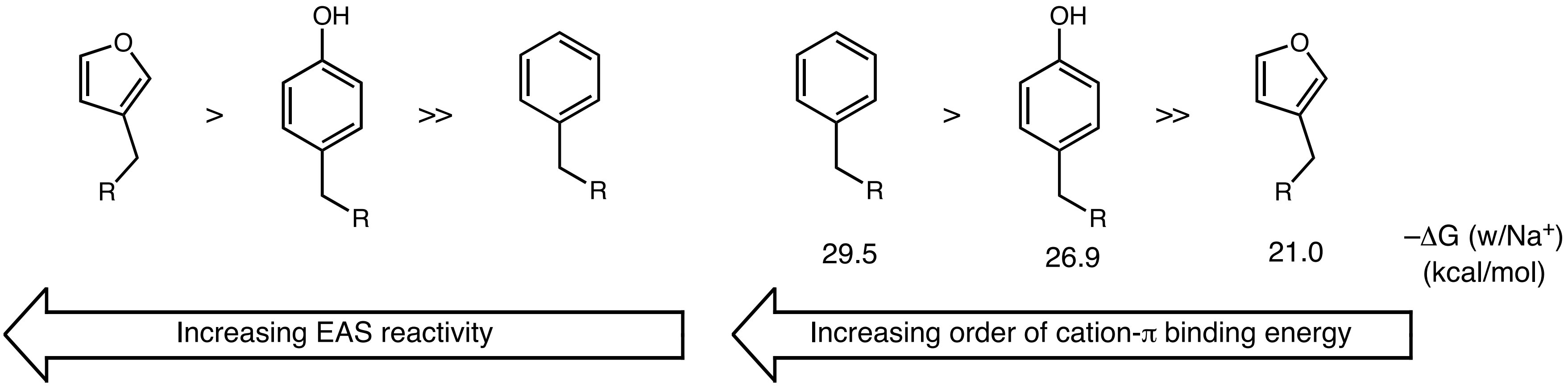


Tyrosine



Tryptophan

- Given an aromatic interacting with an electronphile, we would expect a Friedel-Crafts order of reactivity



29.5 26.9 21.0 –ΔG (w/Na$^+$) (kcal/mol)

Increasing EAS reactivity

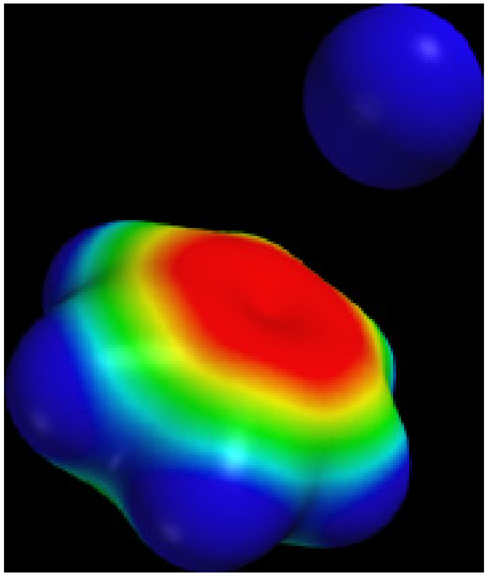
Increasing order of cation-π binding energy

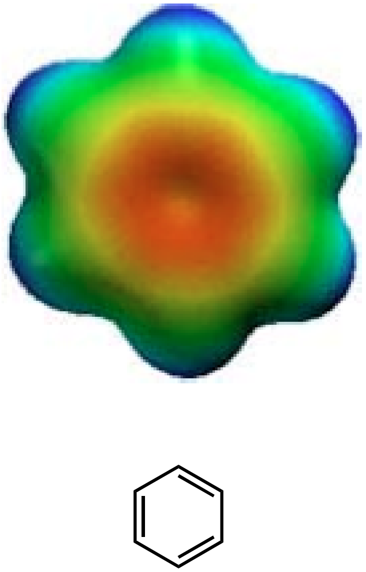
- Predictable trends occur for metal cations: Li$^+$ (38), Na$^+$ (27), K$^+$ (19), Rb$^+$ (16) with benzene (kcal/mol)
  This mirrors charge density: 76, 108, 138, 152 ionic radius (pm) respectively

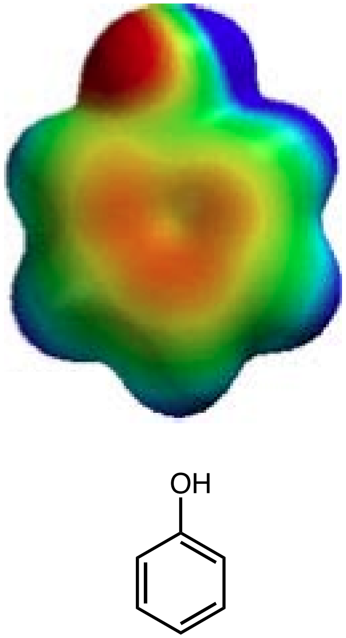
Ma, J. C.; Dougherty, D. A. *Chem. Rev.* **1997**, *97*, 1303.

A Newly Recognized Fundamental Non-Covalent Interaction: Cation-π

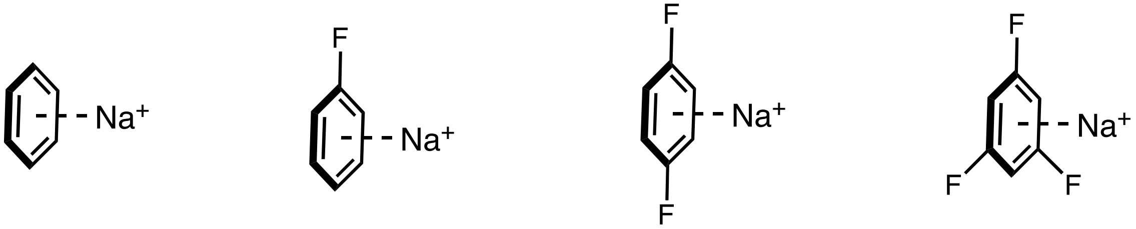
- Model(s) for cation-π interactions should be based on electron-density maps, not electro-statics or polarizability alone.
- Reversed Friedel-Crafts-type reactivity explained by distribution of density relative to center







- Fluorine substitution does have an intuitive and additive influence on cation-π interactions

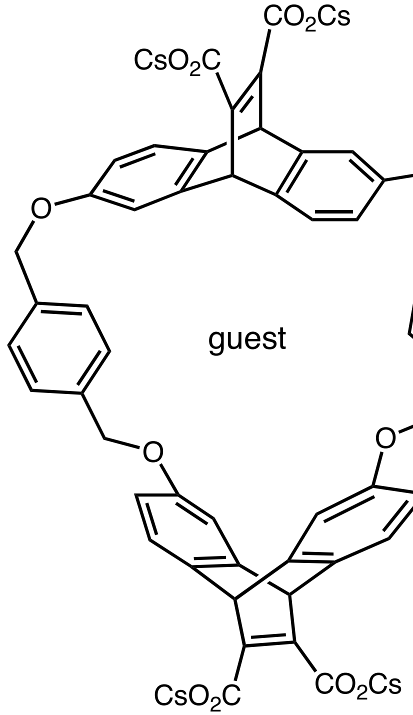


27.1	20.0	16.8	12.4	kcal/mol –ΔG binding energy

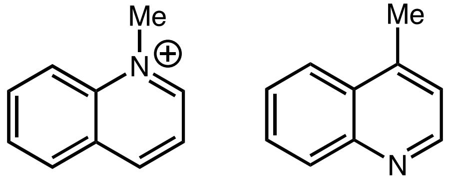
Ma, J. C.; Dougherty, D. A. *Chem. Rev.* **1997**, *97*, 1303.

A Newly Recognized Fundamental Non-Covalent Interaction: Cation-π

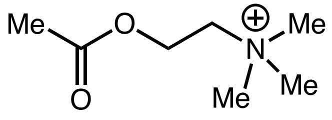
- Highlights of large study of guests of physical organic and biological import



Cyclophane host

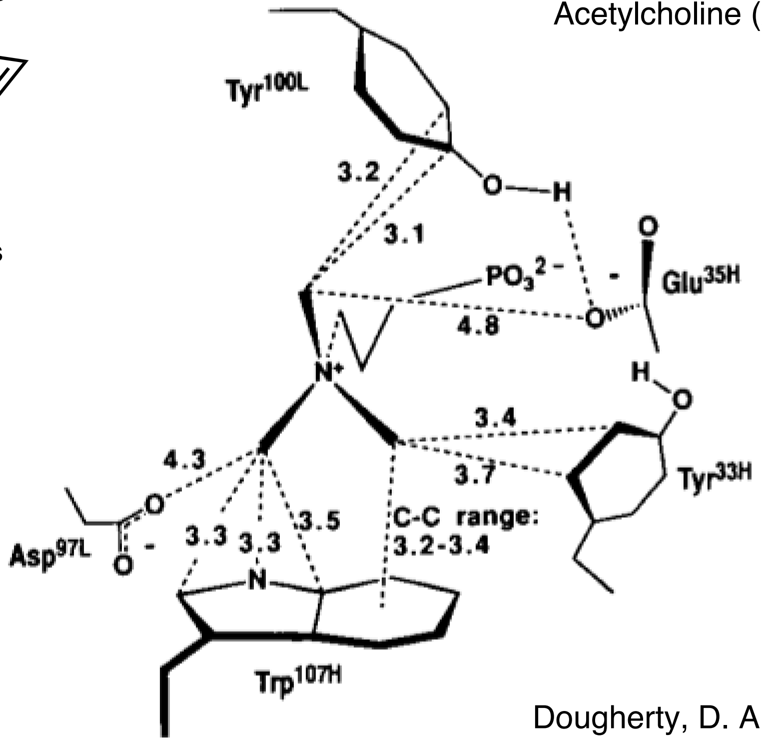


Cation bound 2.5 kcal/mol over neutral even though cation is solvated 46.5 kcal/mol over neutral

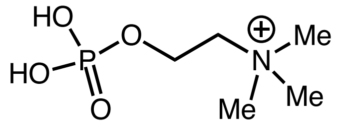


Acetylcholine (ACh)

cyclophane binds ACh similarly to nAChR (K_d = 50 μM)



- Cation-π existence requires more than just proximity

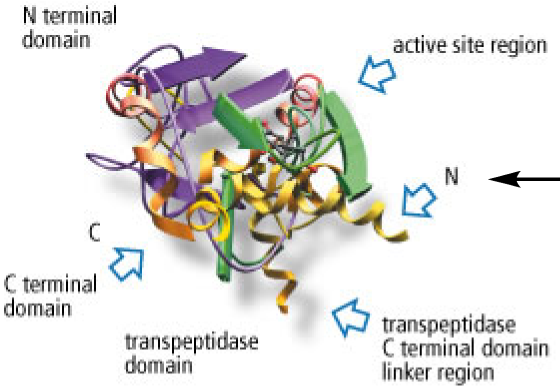


Phosphocholine

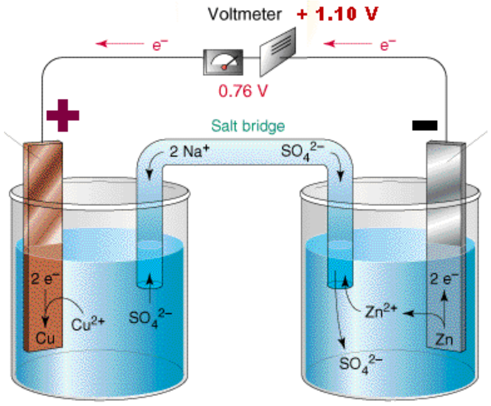
Dougherty, D. A.; Stauffer, D. A. *Science* **1990**, *250,* 1558.

The Platform Potency of Cation-π Cenceptualization

- The Dougherty group has extended cation-π to a wealth of diverse fields of study (leading refs. shown)



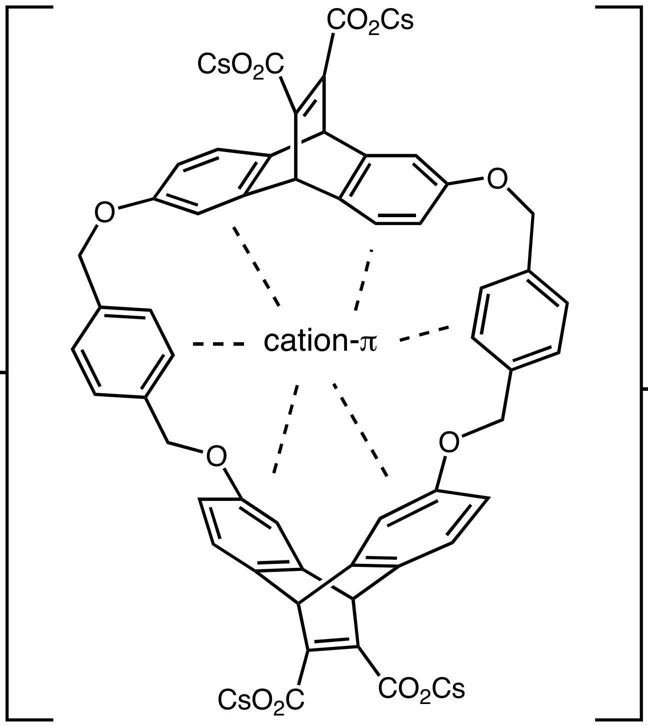
Protein engineering
J. Am. Chem. Soc. **2000**, *122*, 870.

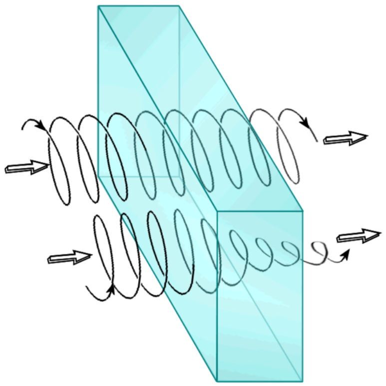


Salt bridge study
J. Am. Chem. Soc. **1999**, *121*, 1192.

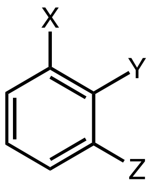
Biomimetic S_N2 catalysis
J. Am. Chem. Soc.
1992, *114*, 10314.

nAChR mimic + cationic guest



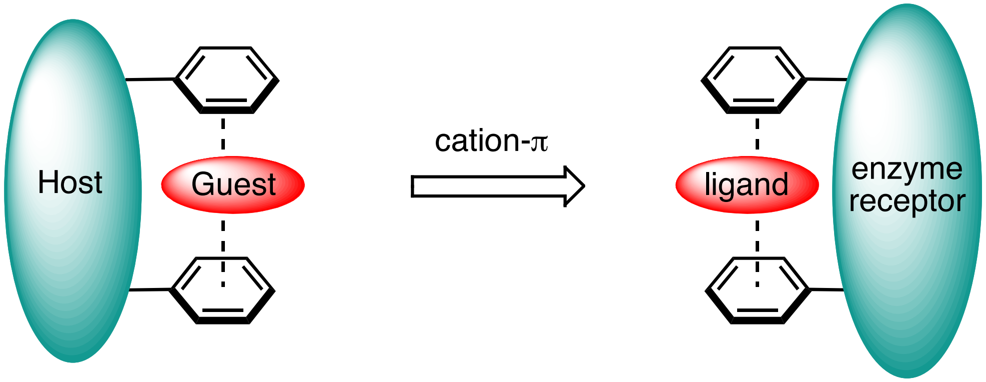


Circular dichroism
J. Am. Chem. Soc.
1995, *117*, 9213.



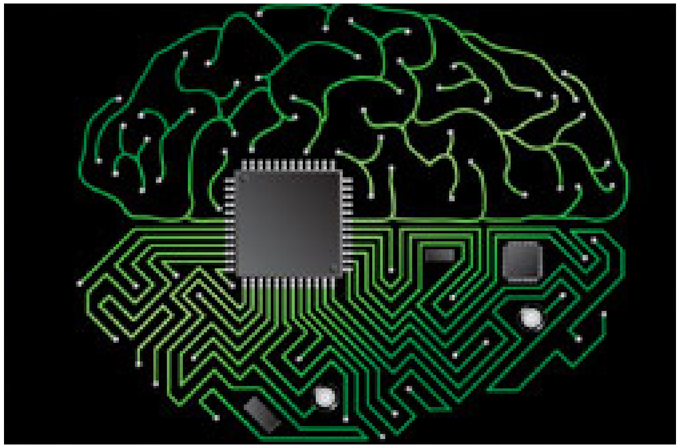
SAR-binding studies
J. Am. Chem. Soc.
1993, *115*, 9907.

Ion channels


Host
Guest
cation-π
ligand
enzyme
receptor

Basics of Neuroscience and Ion Channels

- 10^{12} Neurons in human; 1,000 different types; each connects to 10^4 others --> 10^{16} synapses (3 x 10^9 bp genome)
- 30% proteins are ion channels; 60% of pharmaceutical targets; <0.1% PDB (even that: prokaryote/fragment)



Hubner, C. A.; Jentsch, T. J. *Hum. Mol. Gen.* **2002**, *11*, 2435.

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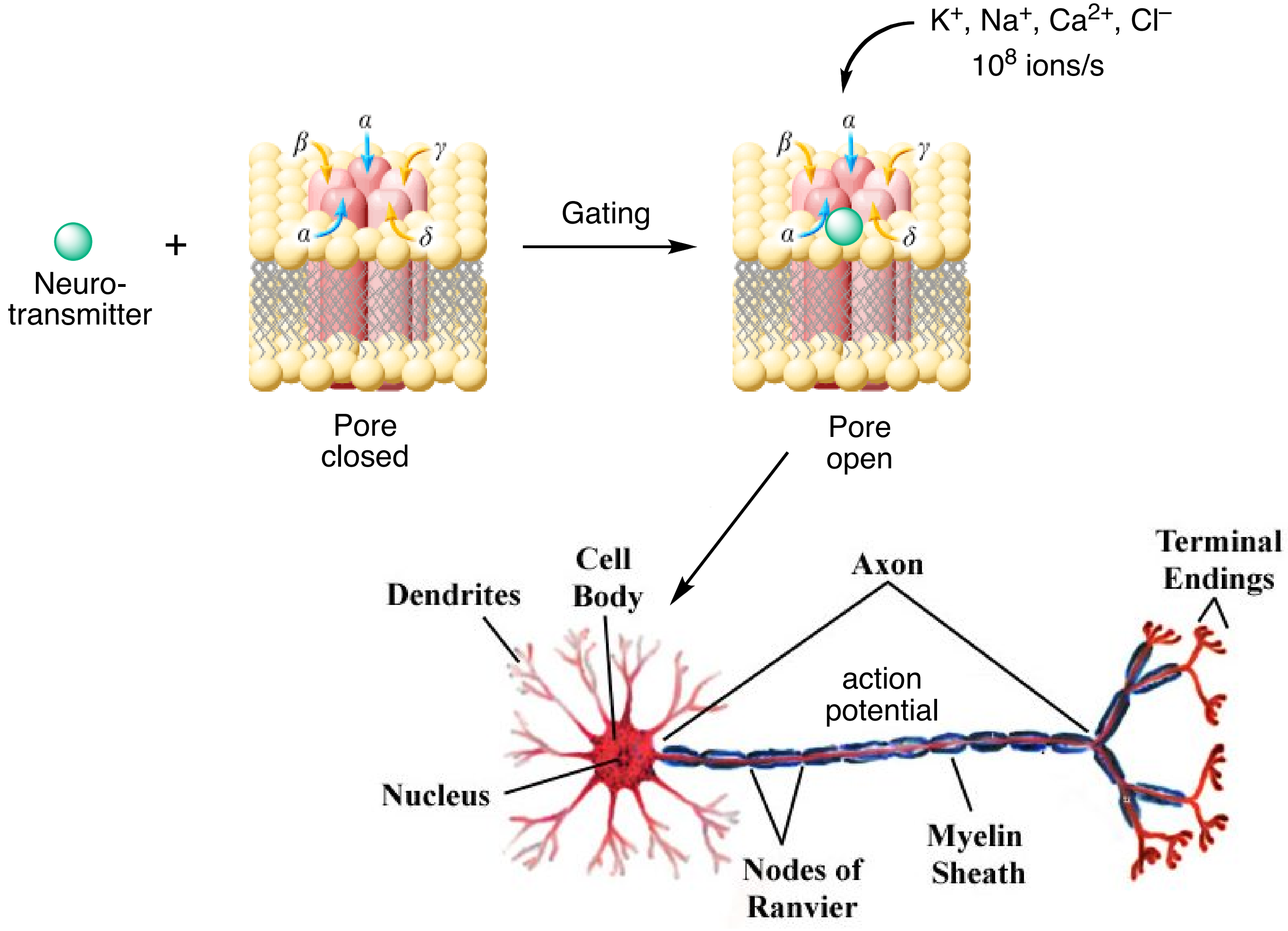

K^+, Na^+, Ca^{2+}, Cl^-

10^8 ions/s

Neuro-transmitter +

Gating

Pore closed

Pore open

action potential

Hubner, C. A.; Jentsch, T. J. *Hum. Mol. Gen.* **2002**, *11*, 2435.

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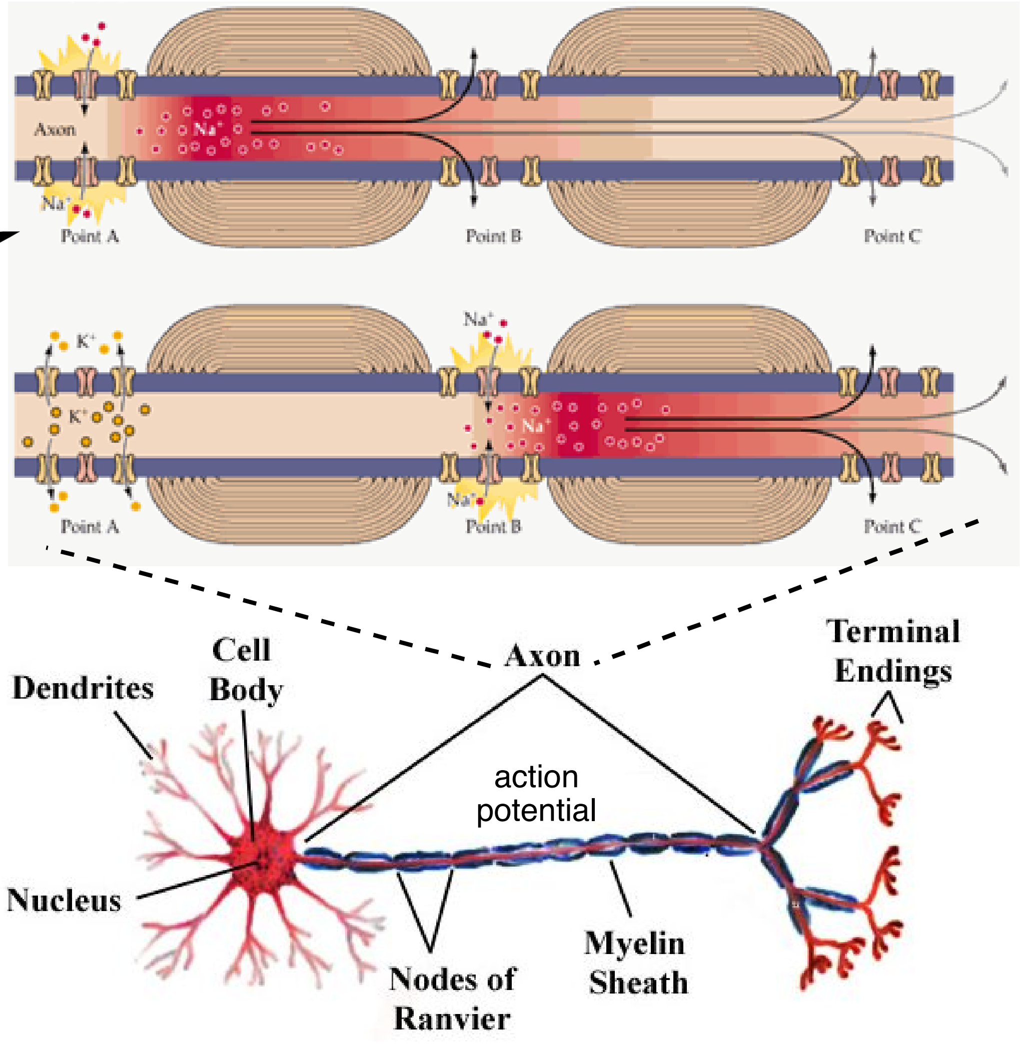

voltage gated ion channels

action potential

Hubner, C. A.; Jentsch, T. J. *Hum. Mol. Gen.* **2002**, *11*, 2435.

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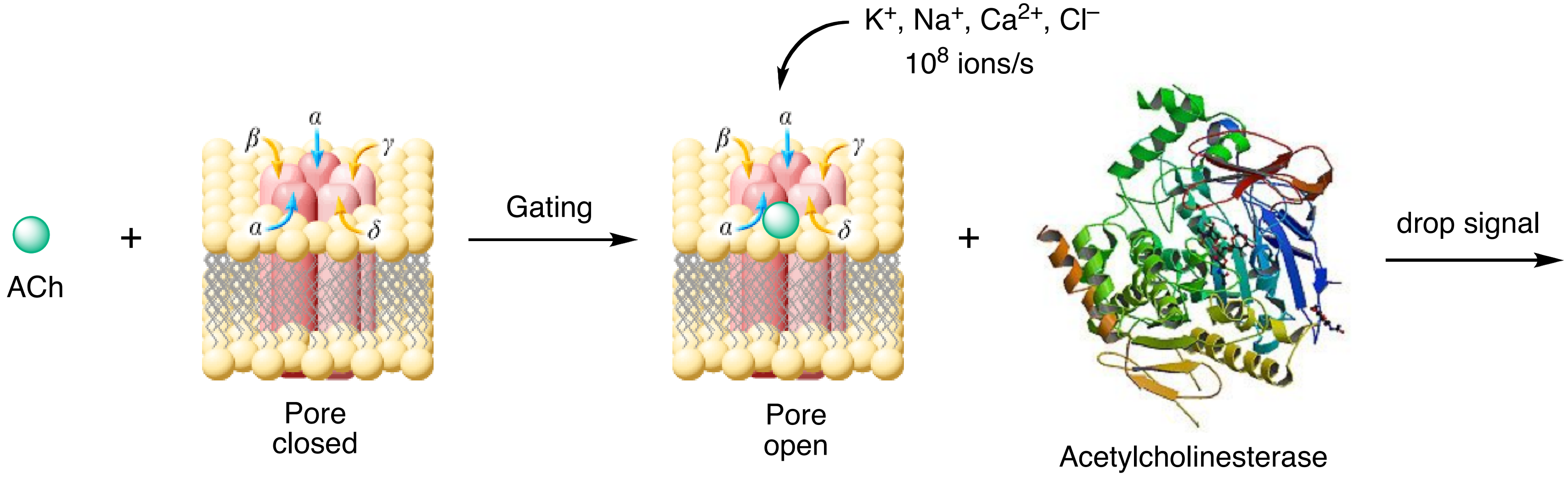
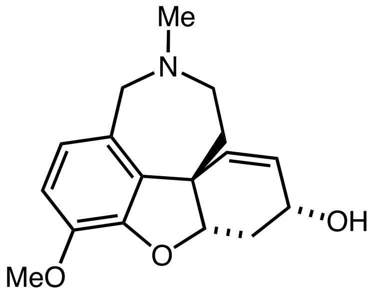
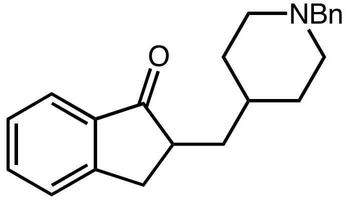
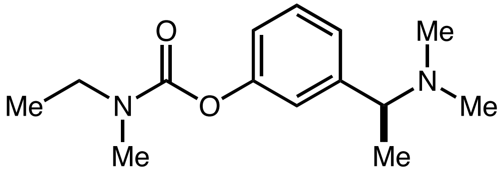




Reminyl (Memeron)



Aricept (Pfizer)



Exelon (Novartis)

Hubner, C. A.; Jentsch, T. J. *Hum. Mol. Gen.* **2002**, *11*, 2435.

Basics of Neuroscience and Ion Channels

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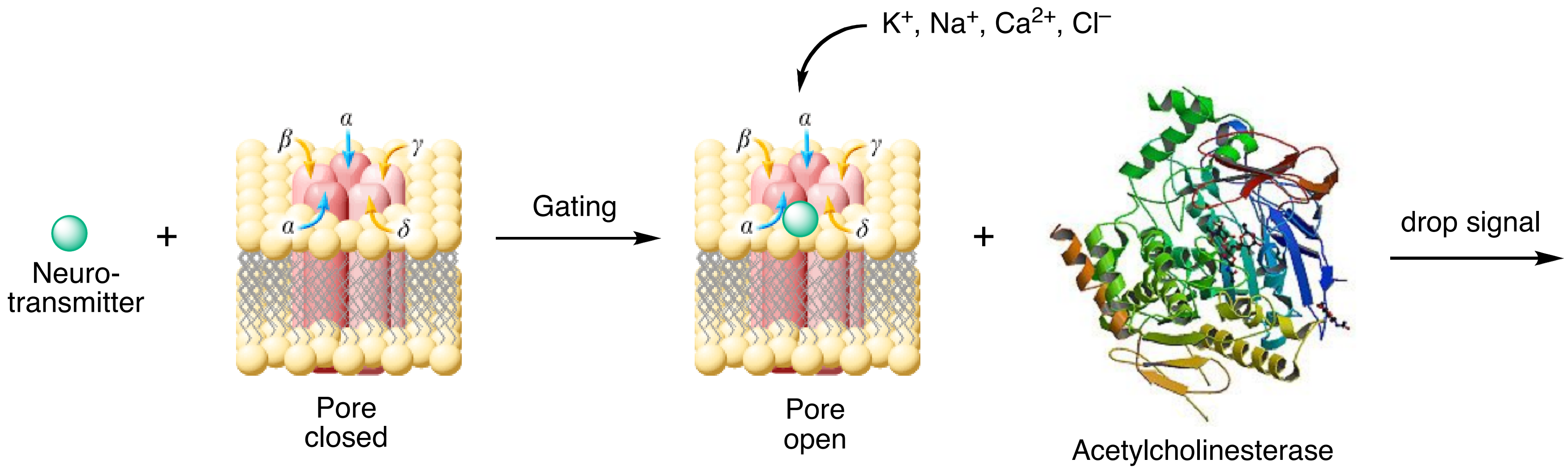


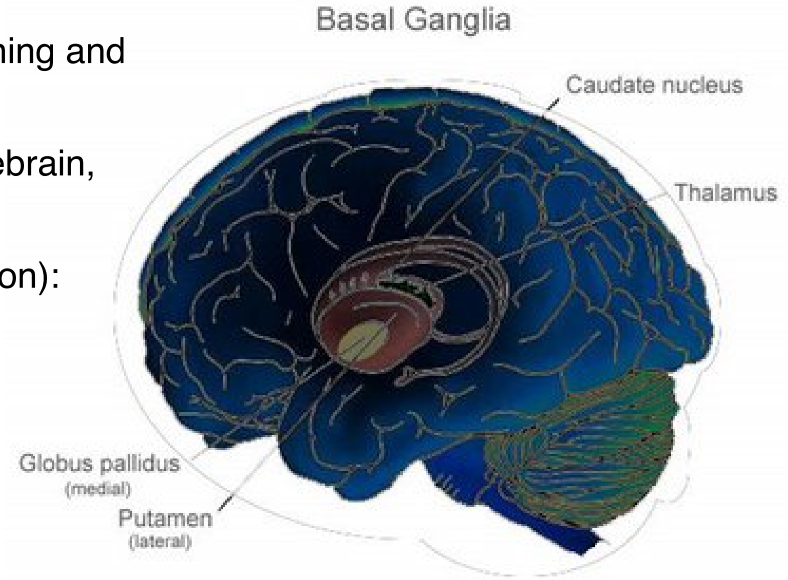
- Complex: regulated by active pumps, cotransporters, Ca^{2+}, phosphorylation, lipids
- Widespread: all membranes have a potential and use ion channels; also organelles (mitochondria, ER)

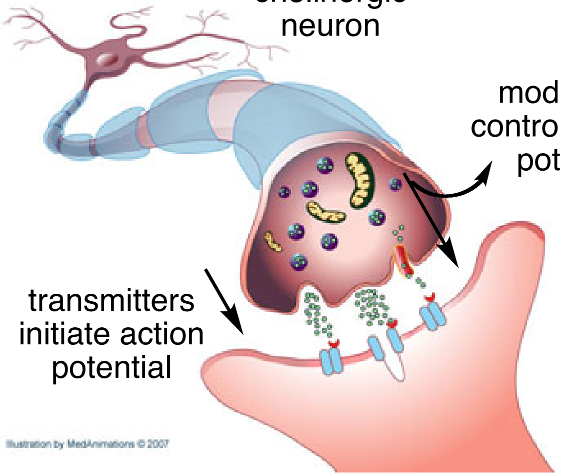
- Disease: Epilepsy, ataxia, cardiac arrhythmia, cistic fribrosis, insulin secretion, Alzheimer's, Parkinson's, pain, schizophrenia, ADHD

Hubner, C. A.; Jentsch, T. J. *Hum. Mol. Gen.* **2002**, *11*, 2435.

History and Basics of Acetylcholine

- Acetylcholine first neuromodulator discovered (1914), thought to be oldest in evolutionary sense
- Associated with excitatory responces, arousal, reward, learning and short-term memory
- CNS locations: brain stem, cerebellum, thalamus, basal forebrain, basal ganglia (many more)
- PNS locations: musculo-skeletal junctions (muscle contraction):
- Neuromodulative enhancement of action potential = synaptic plasticity = learning

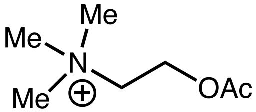


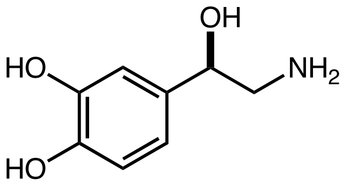

"cholinergic" neuron

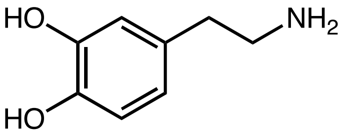
modulators control existing potential

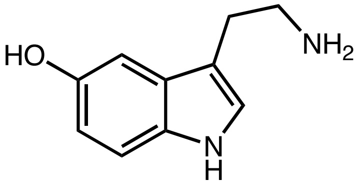
transmitters initiate action potential

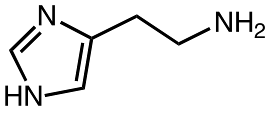
Illustration by MedAnimations © 2007


acetylcholine (ACh)


norepinephrine

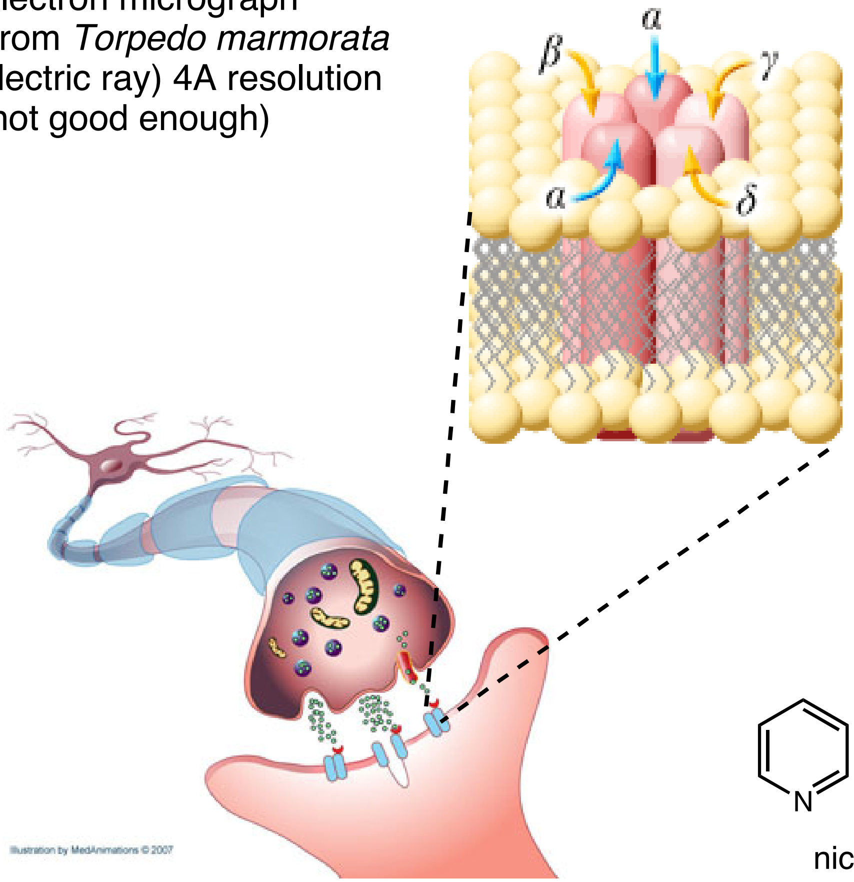

dopamine


seratonine (5-HT)


histamine

The Nicotinic Acetylcholine Receptor (nAChR)

- The nAChR is a target of Alzheimer's and nicotine dependence drugs (and other neuromusculature disorders)
- The nAChR is membrane bound. ACh binds to two receptors (star) trigering pore opening (top) and $K^+/Na^+/Ca^{2+}$ ions flowing through causing an action potential
- Not crystal structure
  Electron micrograph
  (from *Torpedo marmorata*
  electric ray) 4A resolution
  (not good enough)



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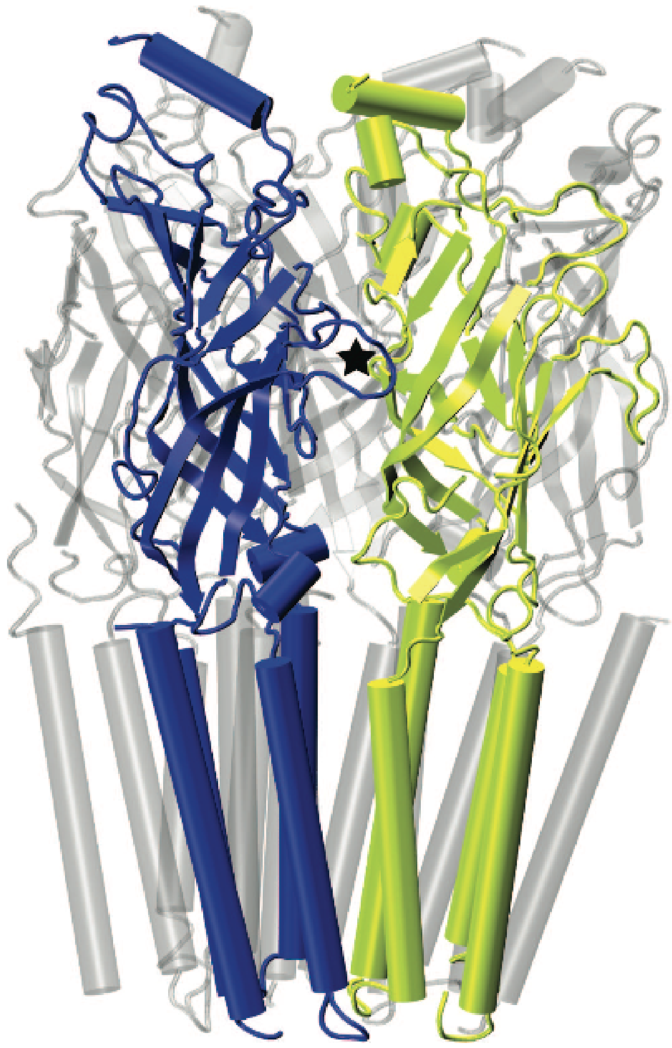
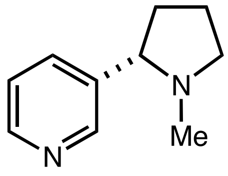




nicotine

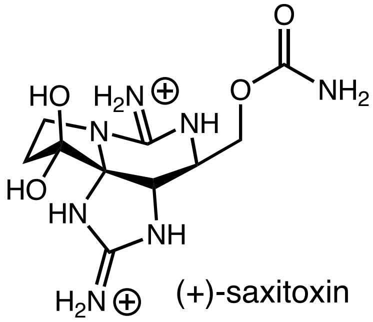
Dougherty, D. A. *Chem. Rev.* **2008**, *108*, 1642.
Unwin, N. *J. Mol. Biol.* **2005**, *346*, 967.

Pharmacology of the nAChR and other Ion Channels

- Neurotoxins like α–Latrotoxin from the black widow block ACh from nAChR, sending a massive and sustained action potential which locks muscles and can stop the heart

- Botulin toxin (Botox) also activates the nAChR

- Saxitoxin acts differently by blocking the pore of voltage gated ion channels



(+)-saxitoxin

Andresen, B. M.; J. Du Bois
J. Am. Chem. Soc. **2009**, *131*, 12524

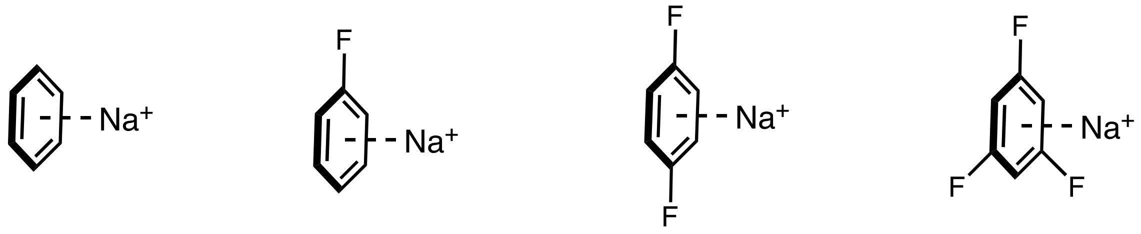
- Alzheimers drugs target ACh esterase since the disease is associated with a low concentration of ACh
Others target the nAChR as ACh mimics for a similar effect

- ACh transferase catalyzes the production of ACh from choline and acetate. The cysteine in it's active site is the the toxic target of organo Hg^{2+} poisoning

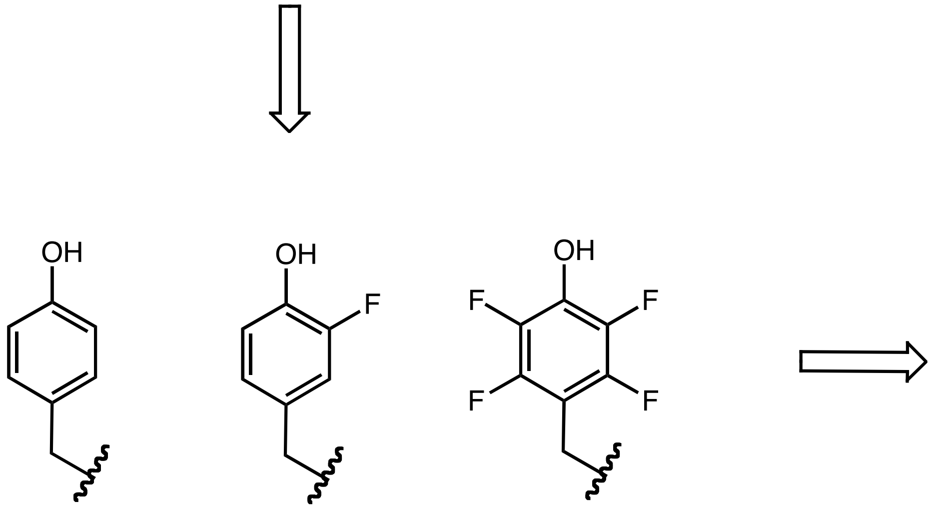
T. C. Sudhof *Annu. Rev. Neurosci.* **2001**, *24*, 933.

Example One: Use Cation-π Fluorination to Probe Mechanism of ACh Binding

- Fluorine substitution does have an intuitive and additive influence on cation-π interactions



27.1	20.0	16.8	12.4	kcal/mol $-\Delta G$ binding energy



Tyr

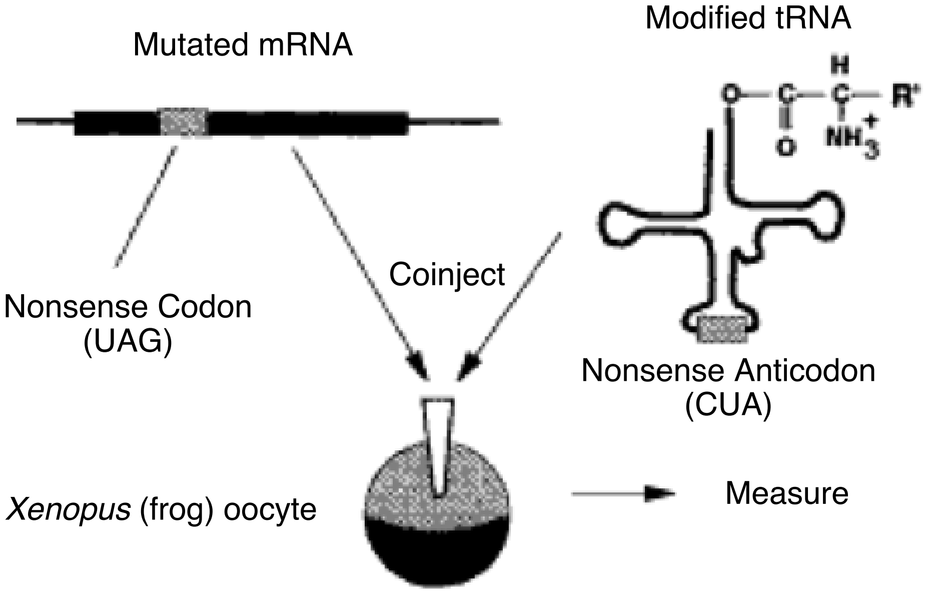
3F-Tyr

F_4-Tyr

Correlation with binding afinity (EC_{50})?

Initial experiments to probe nAChR with Physical Organic Chemistry

- 3F-Tyr mutation, first use of unnatural amino acid incorporation in living cell
- Controls: heterologous coinjection/expression of WT mRNA and tRNA give WT functional ion channels
- Controls: Injection of mRNA (with nonsense (stop) codon) with no tRNA gives no signal



Mutated mRNA

Nonsense Codon (UAG)

Coinject

Modified tRNA

Nonsense Anticodon (CUA)

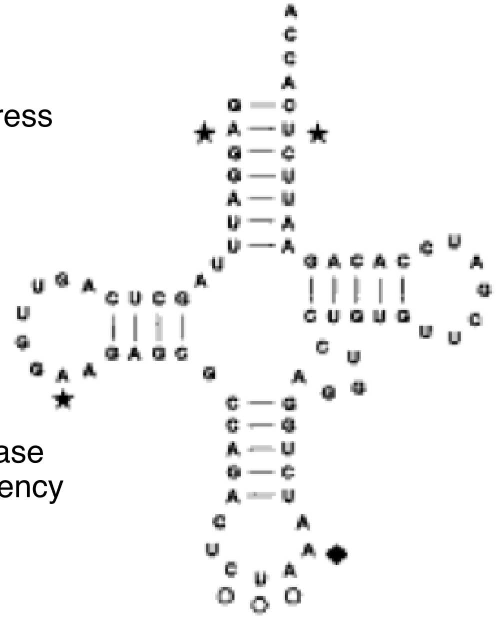
Xenopus (frog) oocyte

Measure

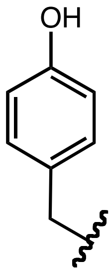
★ Changed to suppress reacylation

◆ Changed to increase suppression efficiency

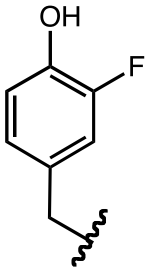
○ Anticodon



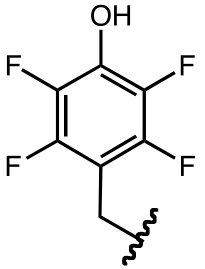
- First use of unnatural AA in vivo



Tyr



3F-Tyr



F_4-Tyr

Schultz, P. G.; Dougherty, D. A.; Lester, H. A.;
et al. Science **1995**, *268*, 439.

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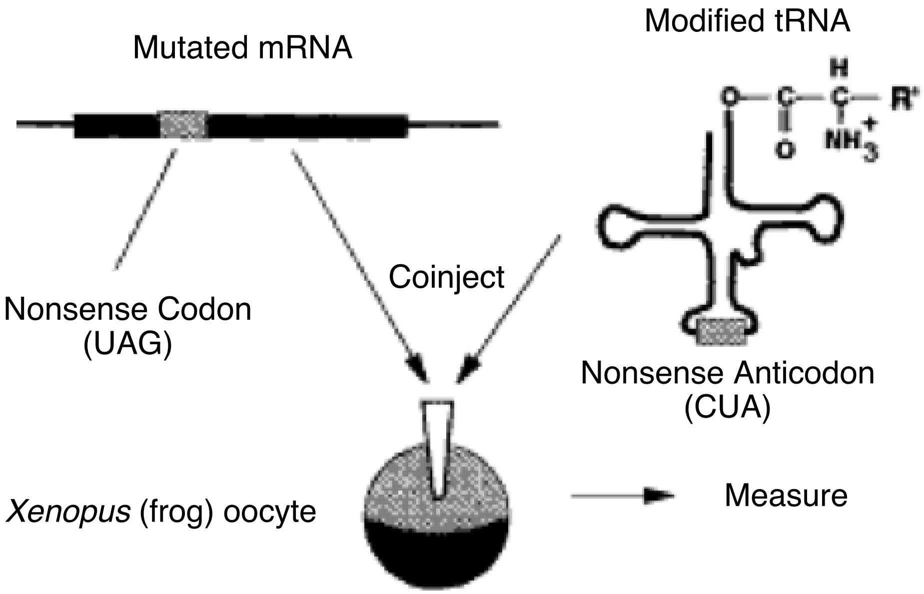
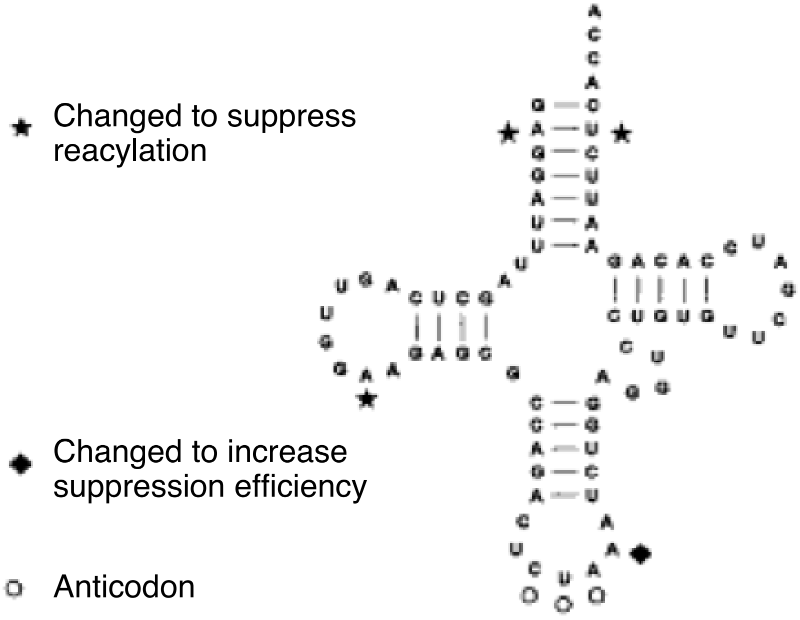
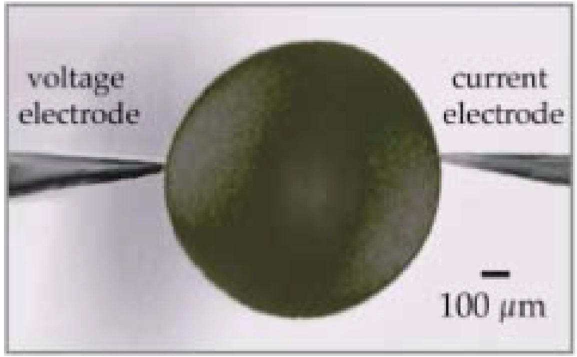
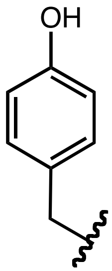
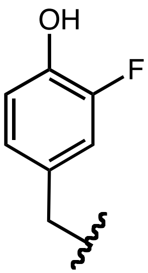




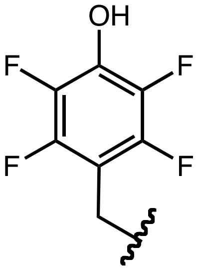




Tyr



3F-Tyr



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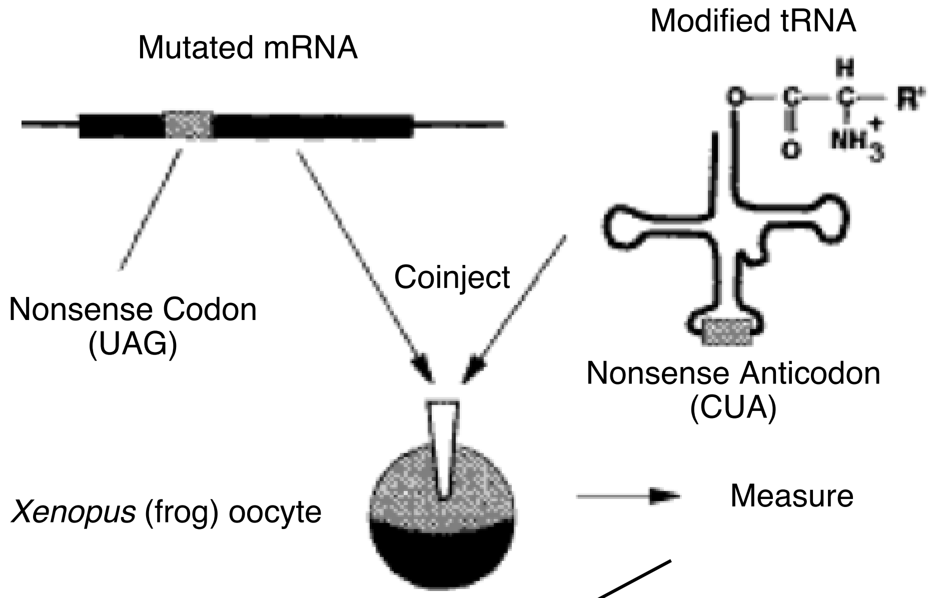
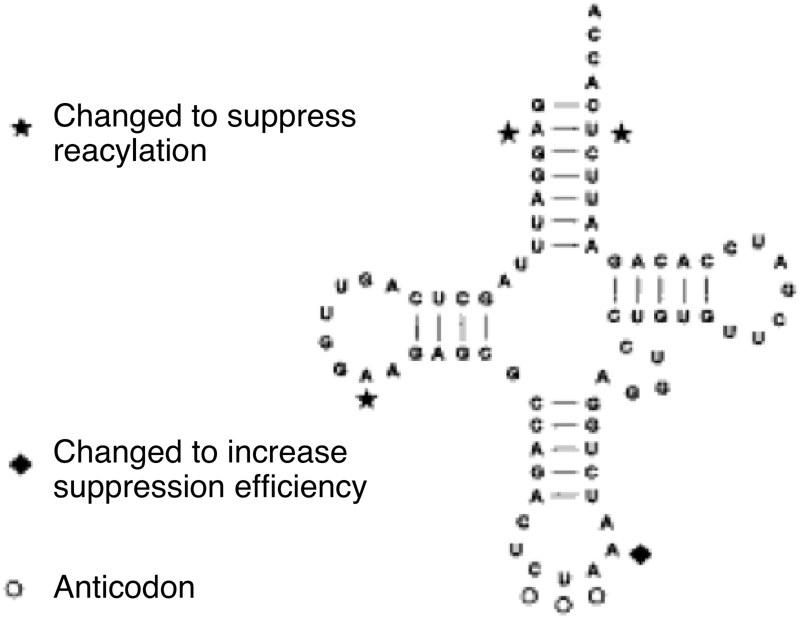
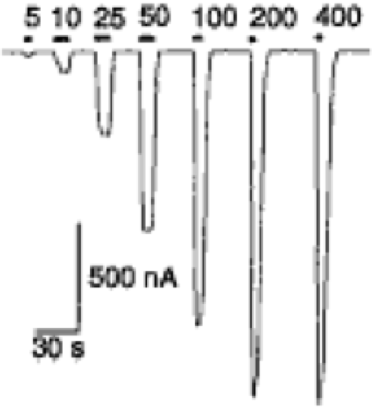


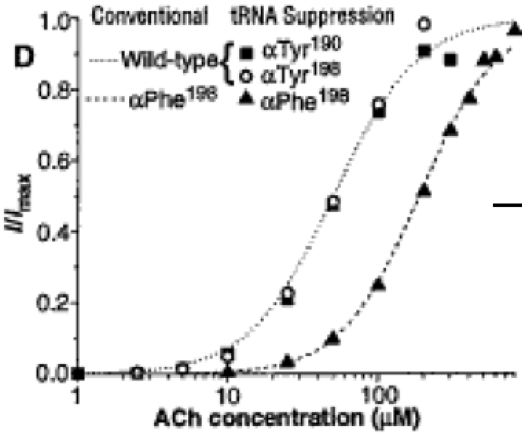


★ Changed to suppress reacylation

◆ Changed to increase suppression efficiency

○ Anticodon



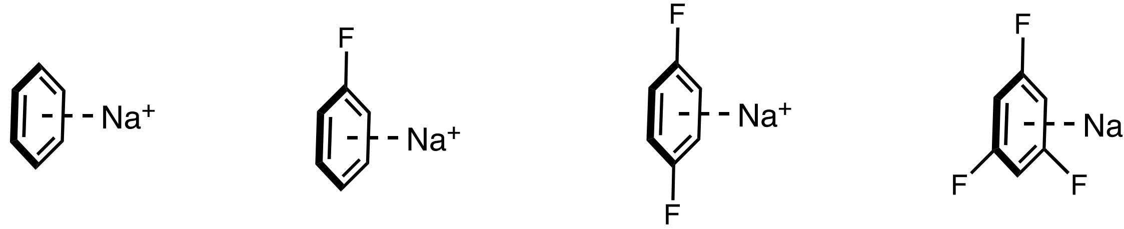


EC_{50}, effector conc. at 1/2 max (vs. IC_{50}, LD_{50})

Schultz, P. G.; Dougherty, D. A.; Lester, H. A.; *et al. Science* **1995**, *268*, 439.

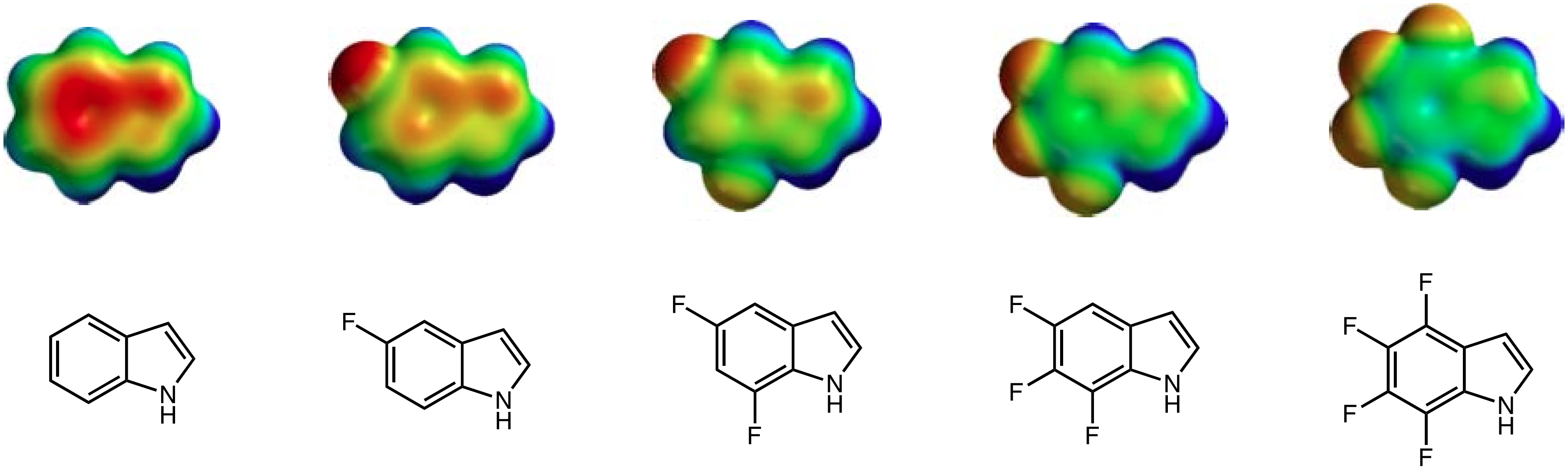
Unnatural Amino Acid Incorporation: Perturb, do not Disrupt

- Dougherty, Lester and coworkers incorporate serial fluorine substitutions in αTrp-149 of the muscle type nAChR
- *Xenopus leavis* (frog) oocytes used; as a vertebrate: close homology to human nAChR
  Frog oocytes very robust to patch-clamp single channel electrophysiology measurements



27.1 20.0 16.8 12.4

kcal/mol –ΔG
binding energy



32.6 27.5 23.2 18.9 14.4

Zhong, W.; Gallivan, J. P.; Zhang, Y.; Li, L.; Lester, H. A.; Dougherty, D. A. *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95,* 12088.

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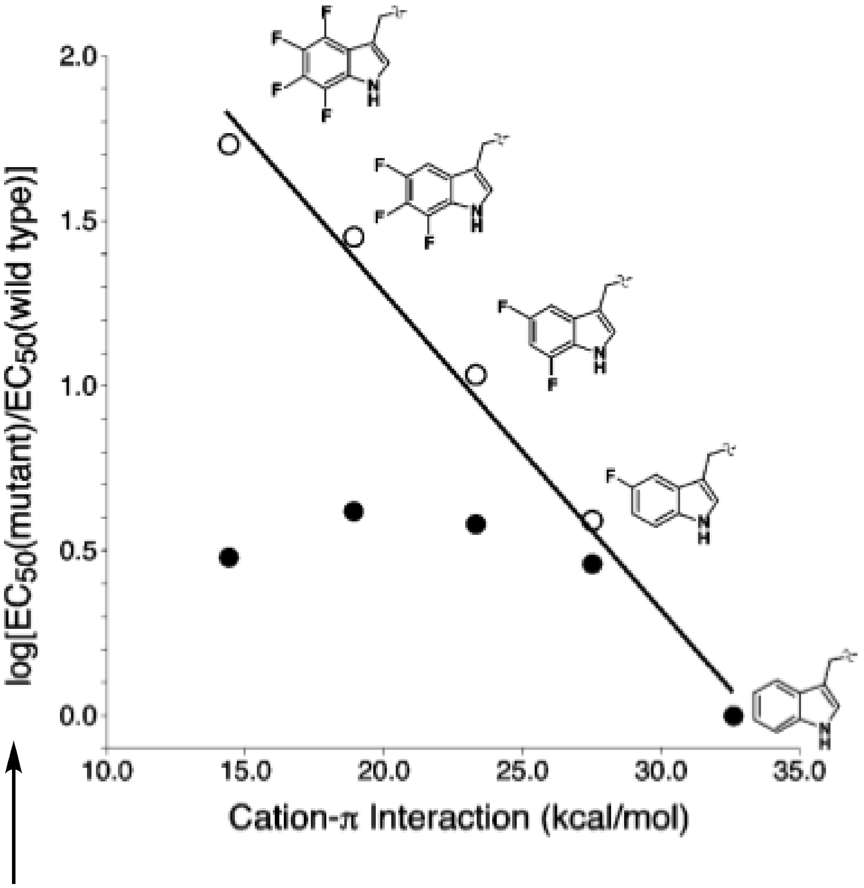
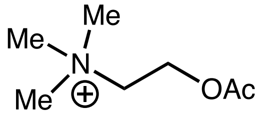
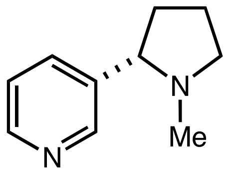


$-\Delta G^o = RT\ln(K_a)$

- Sequential $F_x\alpha$Trp-149 mutagenesis probes function of an aromatic active site residue without disrupting the function of the channel
- Conclusions (of cation-π role) vindicated three years later in study of nAChR "aromatic box" Sixma, T. K.; Smit, A. B. *Ann. Rev. Biophys. Biomol. Struct.* **2003**, *32*, 311
- Nicotine shows no clear trend with cation-π relationship: nicotine does not bind like ACh (Implications for nicotine drug discoverty)

○


acetylcholine (ACh)

●


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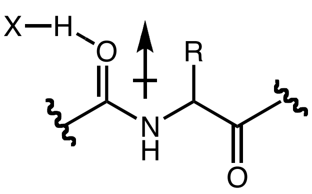
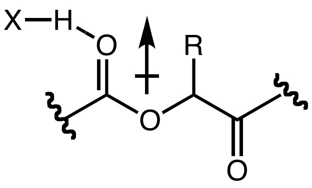


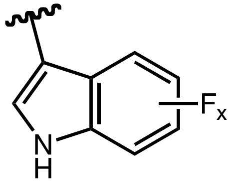
3.5 Debye

Better H-bond acceptor



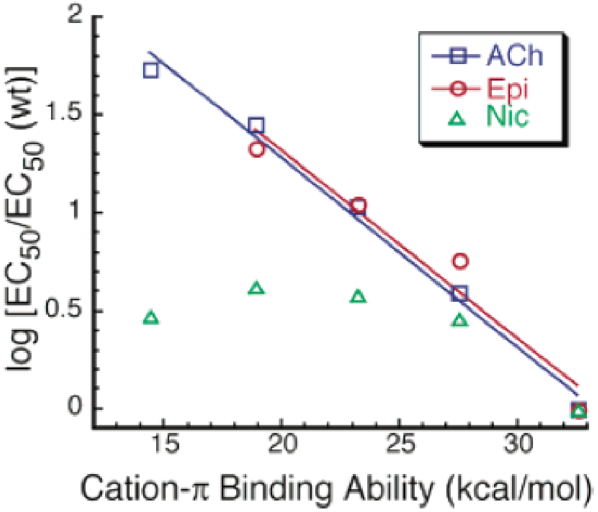
1.7 Debye

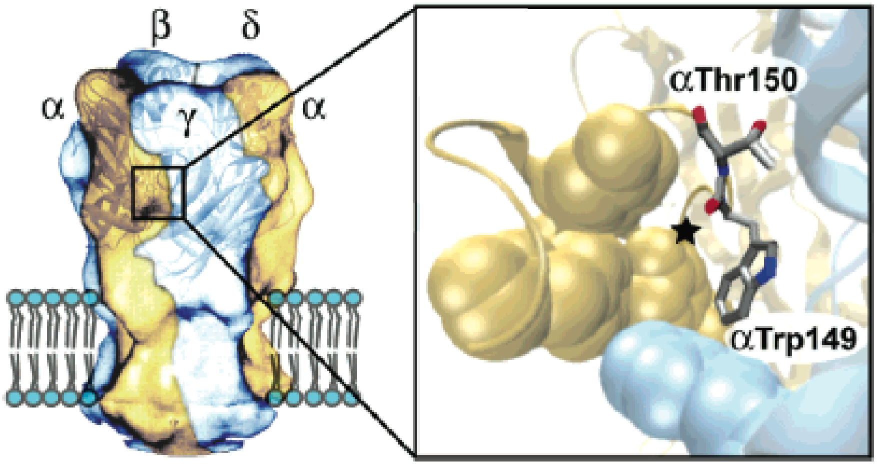
Worse H-bond acceptor
Not a donor

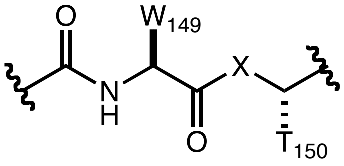


0.6 - 0.9 kcal/mol
for α-helices/β-sheets

(1D = 3.33564 x 10^{-30} C·m)







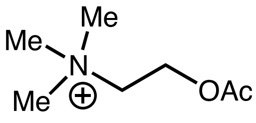
W = Tryptophan

T = Threonine

Mutations Testing H-Bond Interactions at α150[a]

agonist	Thr[b]	Tah	Tah/Thr
ACh	0.83 ± 0.04	0.25 ± 0.01	0.30
nicotine	57 ± 2	92 ± 4	1.6
epibatidine	0.60 ± 0.04	2.2 ± 0.2	3.7

[a] EC_{50} (μM) ± standard error of the mean. The receptor has a Leu9′Ser mutation in M2 of the β subunit. [b] Rescue of wild type by nonsense suppression.



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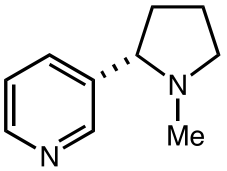
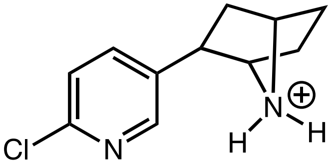


nicotine



(+)-epibatidine

Cashin, A. L.; Petersson, E. J.; Lester, H. A.;
Dougherty, D. A *J. Am. Chem. Soc.* **2004**, *127*, 350.

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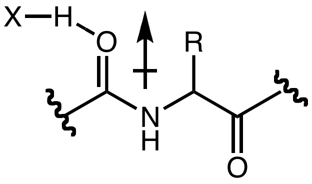

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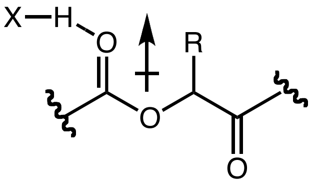

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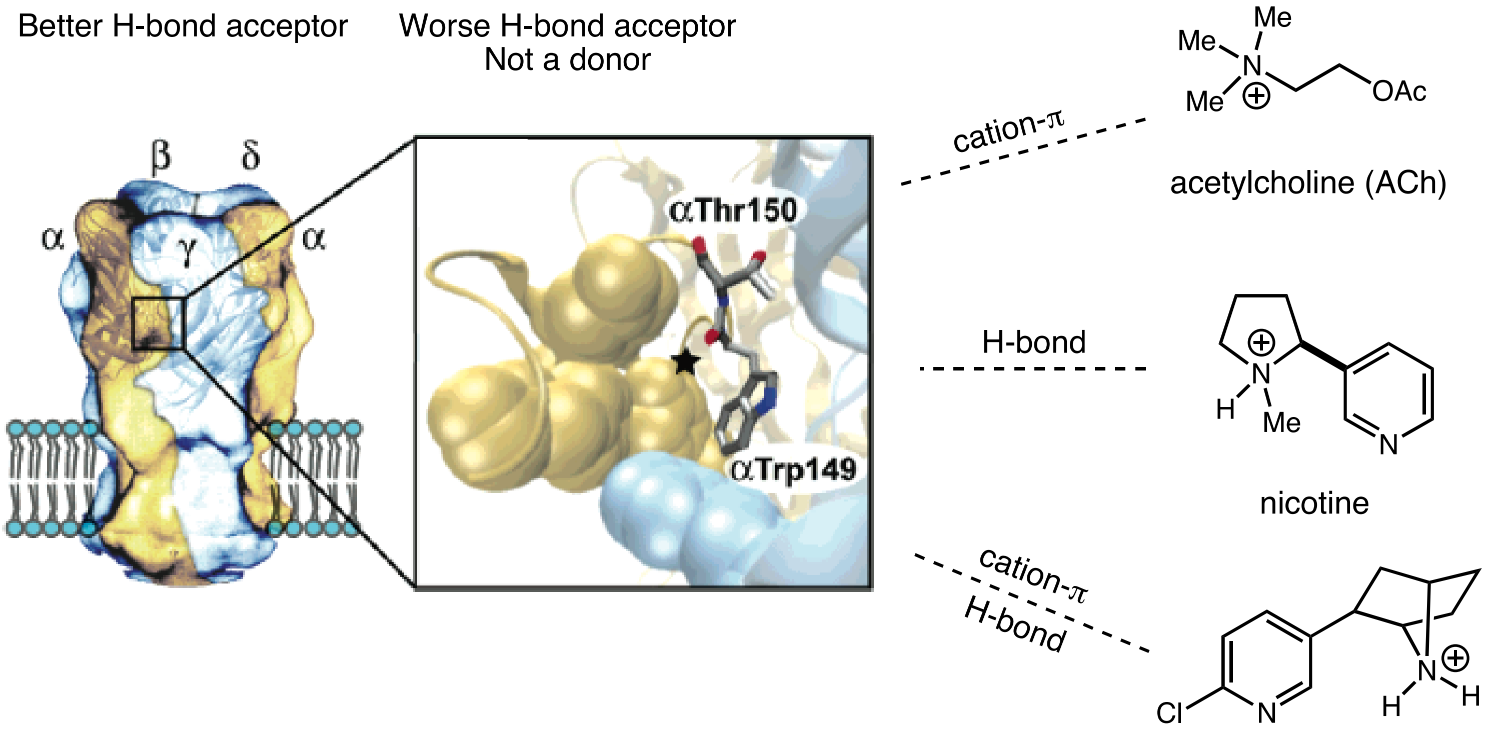


acetylcholine (ACh)

nicotine

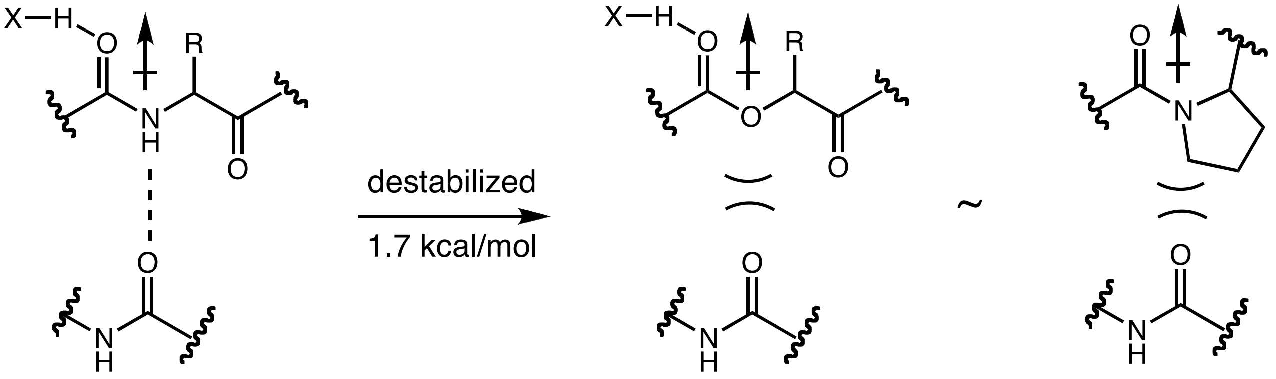
(+)-epibatidine

- Implications for medicinal drug discovery

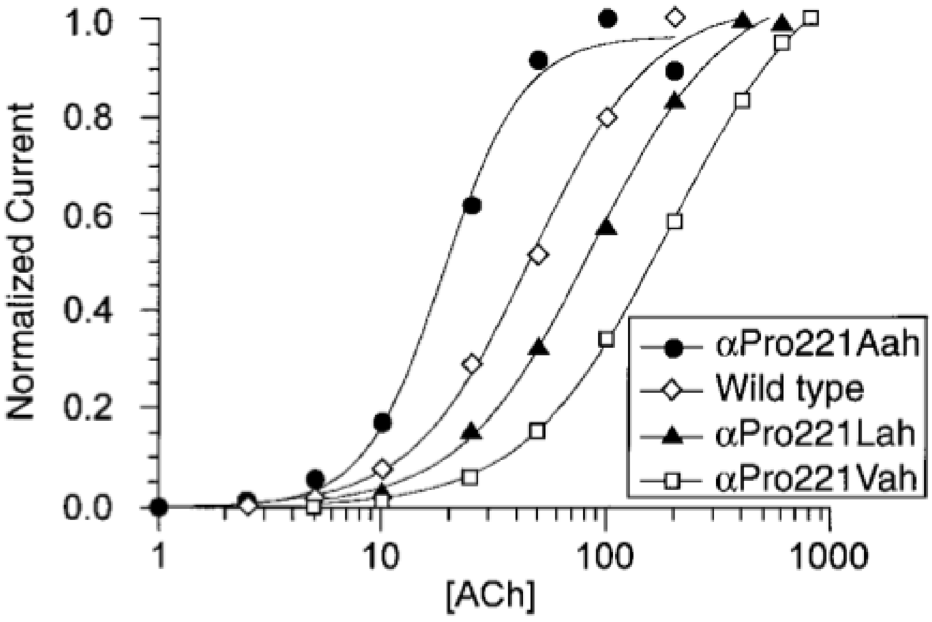
Cashin, A. L.; Petersson, E. J.; Lester, H. A.;
Dougherty, D. A *J. Am. Chem. Soc.* **2004**, *127*, 350.

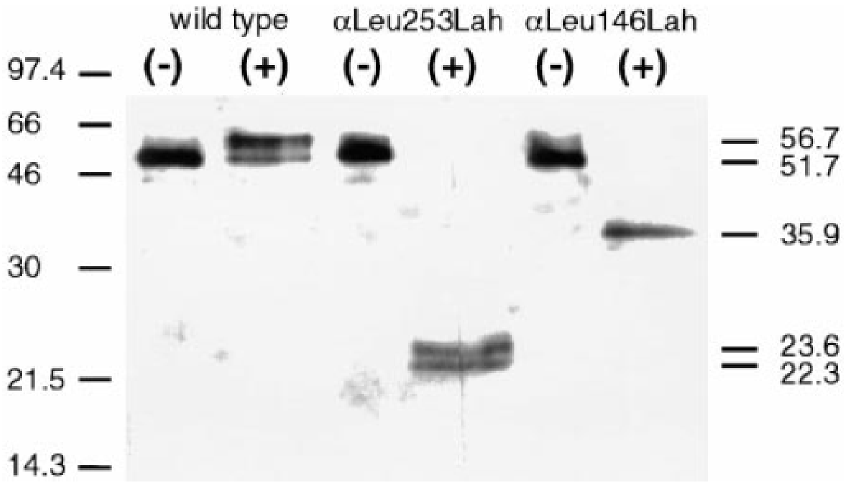
Unnatural Amino Acid Incorporation: Perturb, do not Disrupt

- Ester incorporation can also be used to probe amide H-bond *donation* in nAChR (first example of ester *in vivo*)
- αPro-221 conserved among the Cys-loop family of ion channel receptors (includes nAChR)



- What is the gating mechanism based on the large distance between receptor and channel pore (~50 A)?



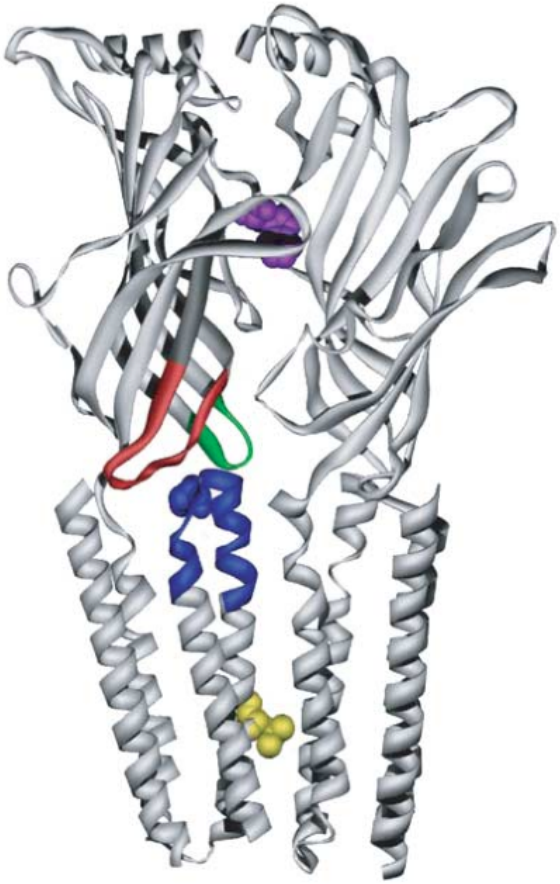


England, P. M.; Zhang, Y,; Dougherty, D. A.; Lester, H. A. *Cell* **1999**, *96*, 89.

Importance of Proline flexibility in other Cys Loop Ion Channels

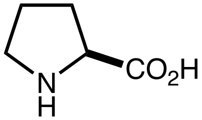
- Conserved Pro 8* among Cys loop receptors which sits at site thought to pivot open pore (gating) upon binding
- Could serial mutation of Pro 8* lead to understanding of this binding-to-gating mechanism?

- At first, no trend emerged between functional and non-functional mutants

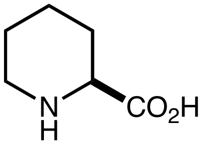


5-HT_3 ion channel

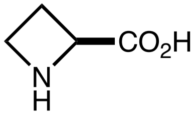
Functional channels



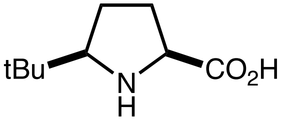
Pro



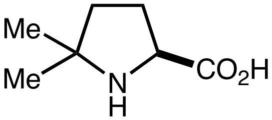
Pip



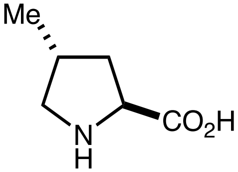
Aze



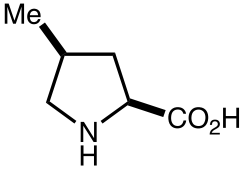
Tbp



Dmp

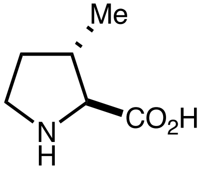


t-4F-Pro

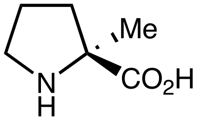


c-4F-Pro

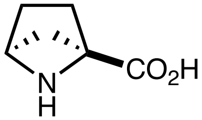
Non-functional channels



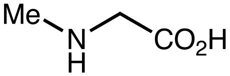
3-Me-Pro



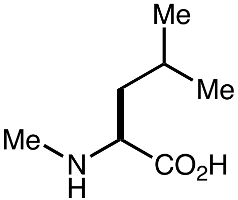
2-Me-Pro



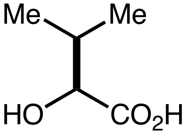
2,4-CH_2-Pro



Sar



N-Me-Leu



Vah

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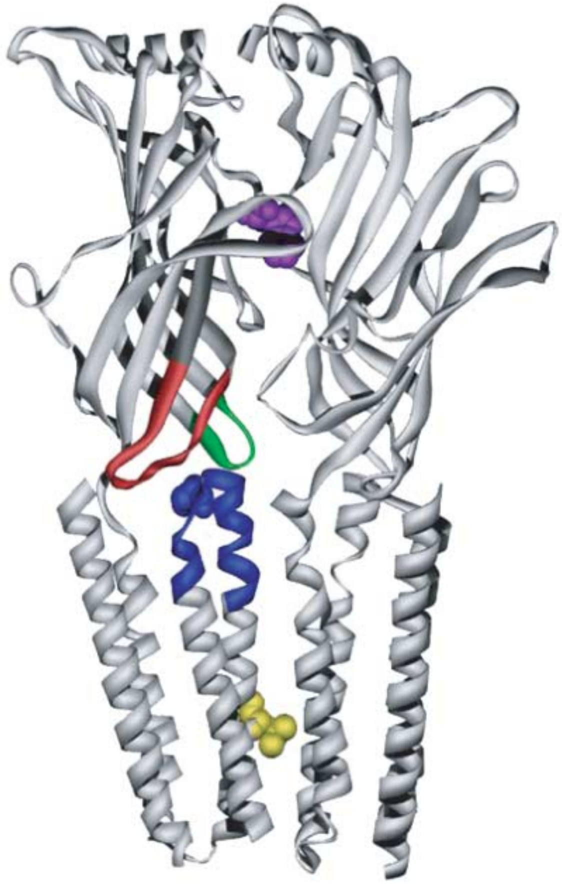
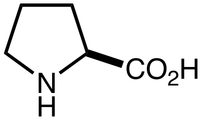
- Conserved Pro 8* among Cys loop receptors which sits at site thought to pivot open pore (gating) upon binding
- Could serial mutation of Pro 8* lead to understanding of this binding-to-gating mechanism?
- At first, no trend emerged between functional and non-functional mutants

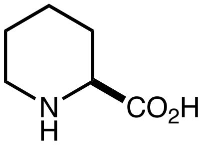


5-HT_3 ion channel

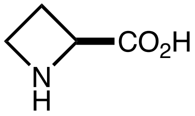
Functional channels



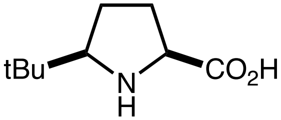
Pro



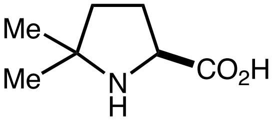
Pip



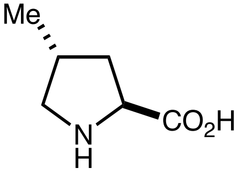
Aze



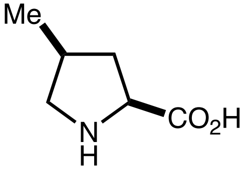
Tbp



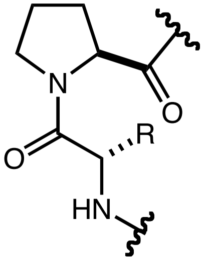
Dmp

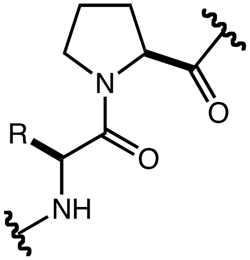


t-4F-Pro



c-4F-Pro





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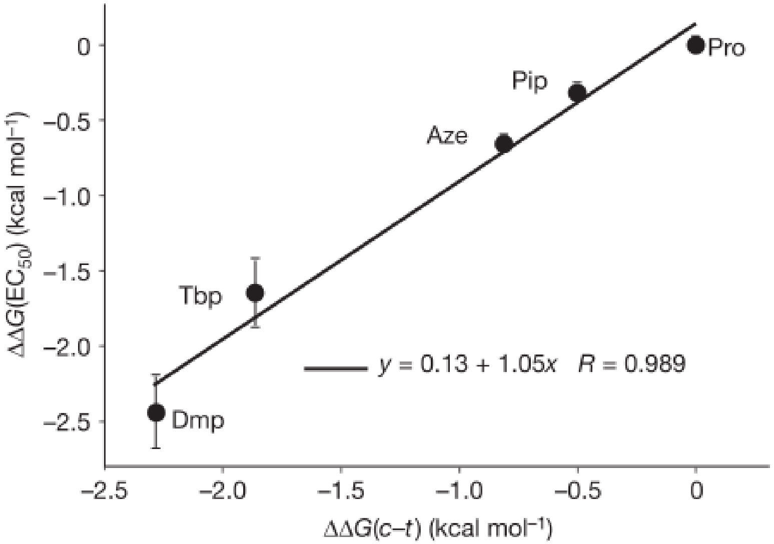
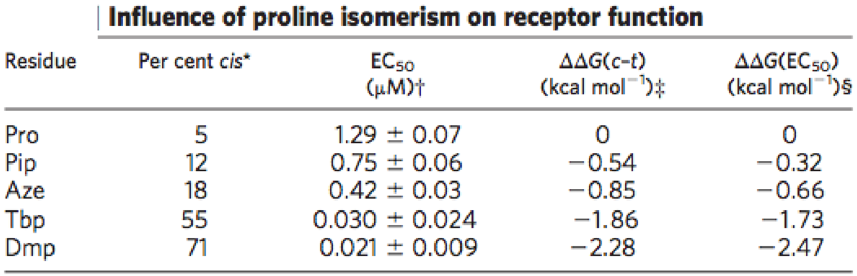
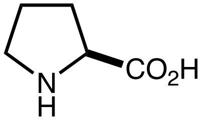




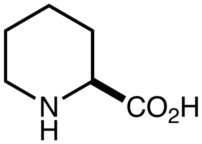
Influence of proline isomerism on receptor function

Residue	Per cent *cis**	EC$_{50}$ (μM)†	ΔΔG(*c*–*t*) (kcal mol^{-1})‡	ΔΔG(EC$_{50}$) (kcal mol^{-1})§
Pro	5	1.29 ± 0.07	0	0
Pip	12	0.75 ± 0.06	−0.54	−0.32
Aze	18	0.42 ± 0.03	−0.85	−0.66
Tbp	55	0.030 ± 0.024	−1.86	−1.73
Dmp	71	0.021 ± 0.009	−2.28	−2.47

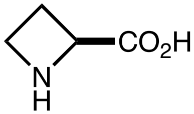
Functional channels



Pro



Pip



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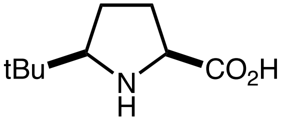

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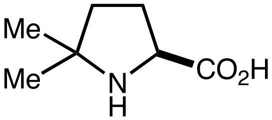

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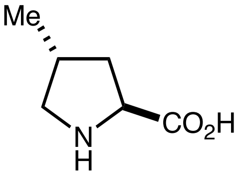
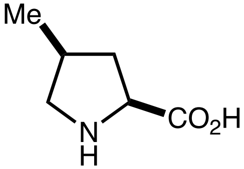


t-4F-Pro



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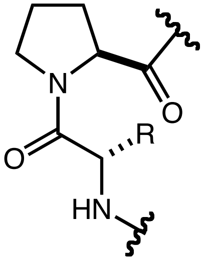

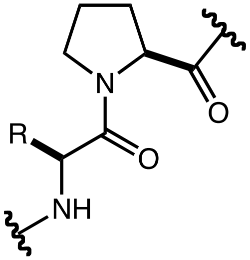

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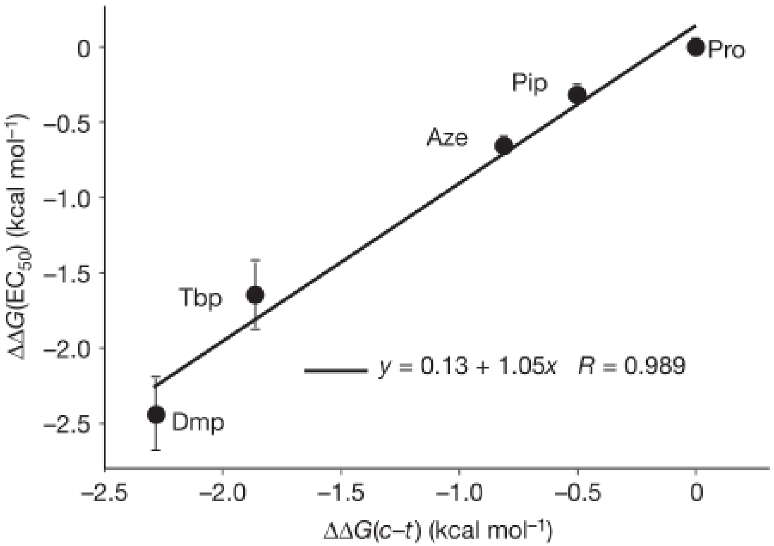

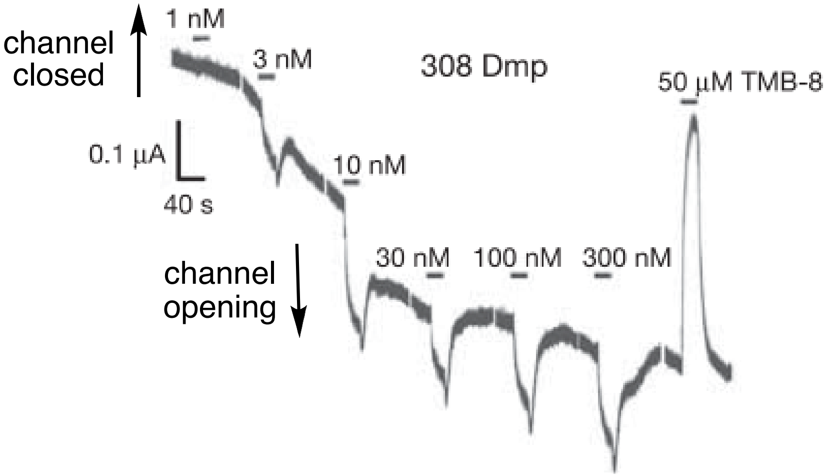

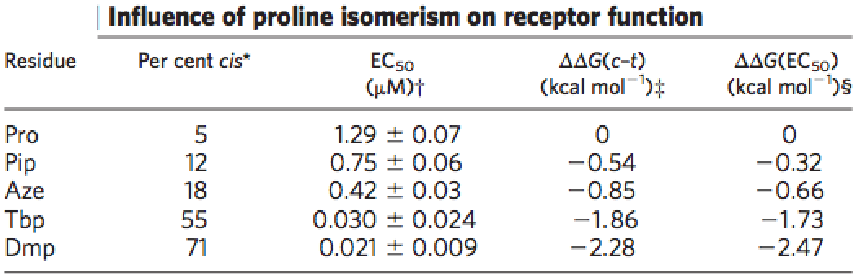

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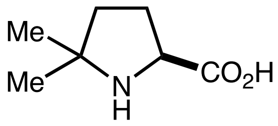

Dmp

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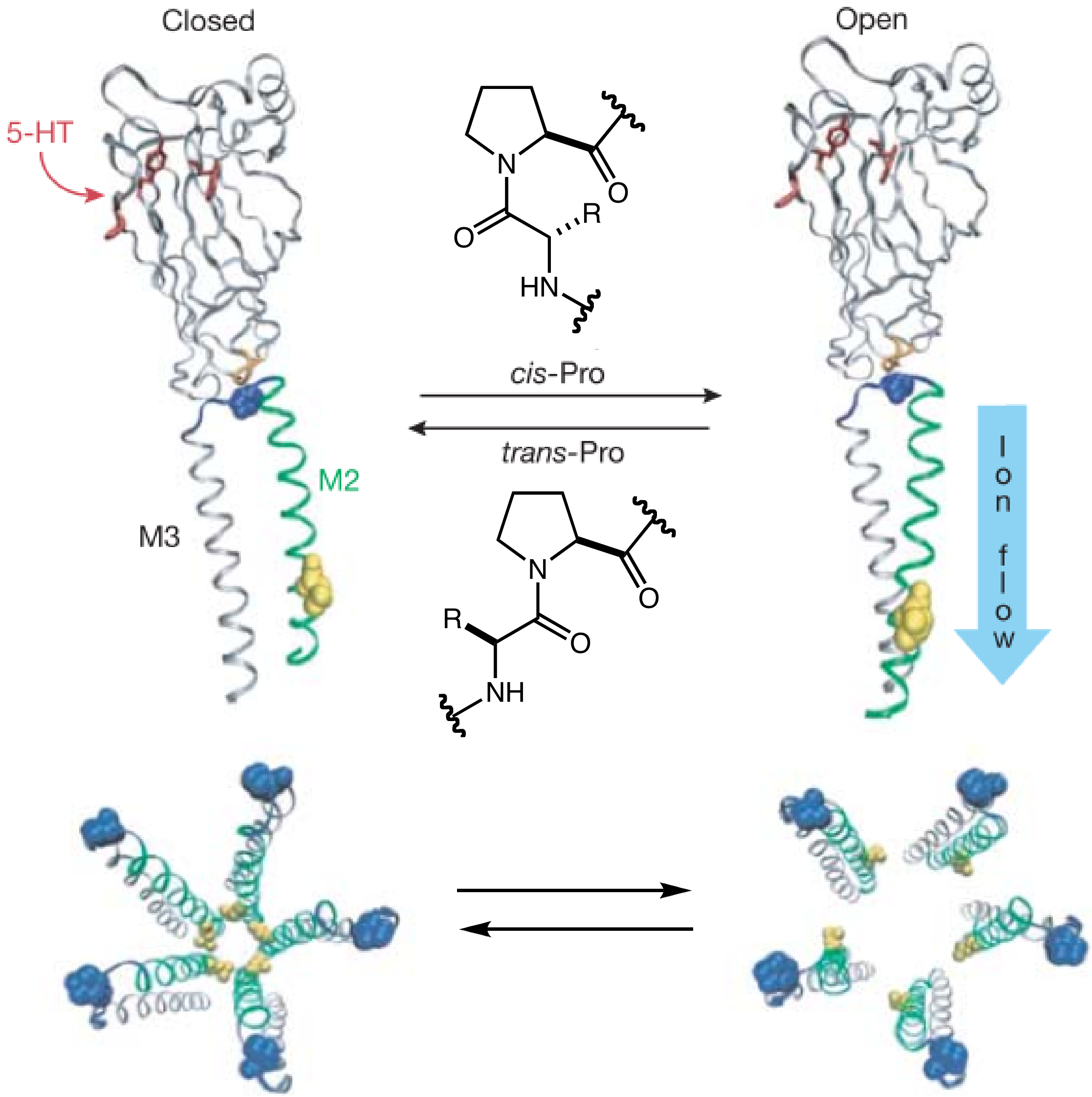

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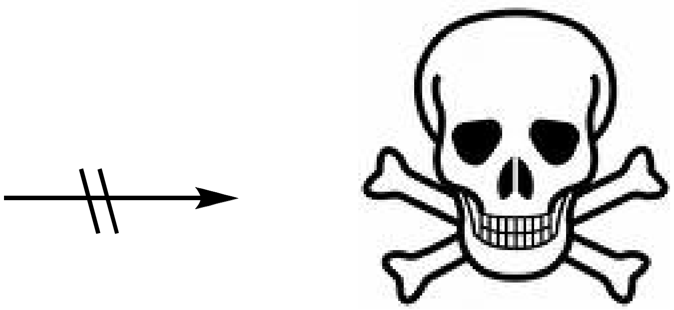
Differential Effect of Nicotine to CNS and Neuromuscular nAChRs



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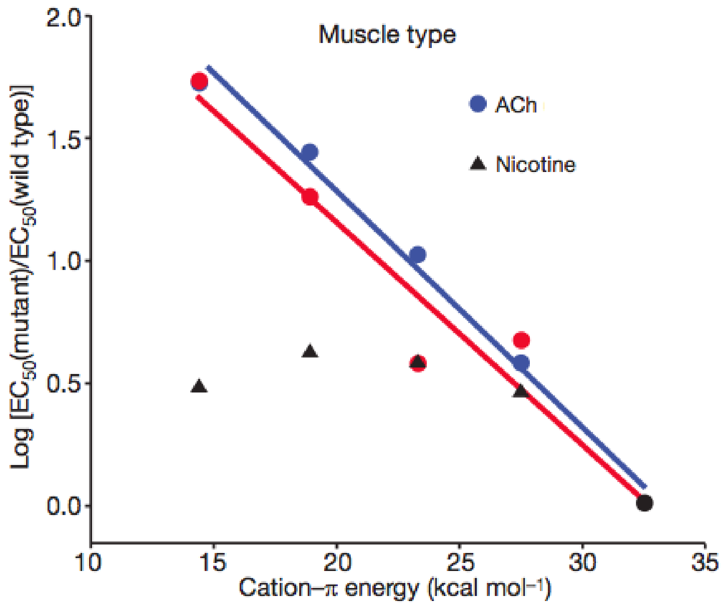
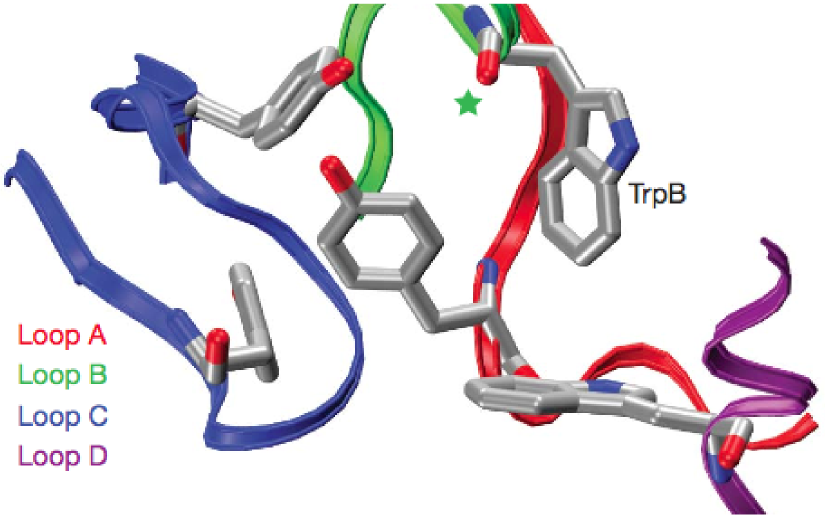
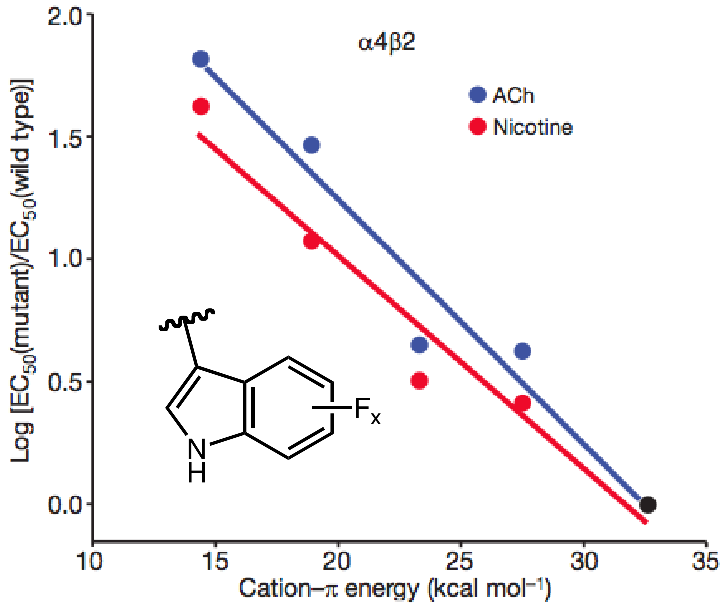
Differential Effect of Nicotine to CNS and Neuromuscular nAChRs

- nAChRs in CNS ($\alpha 4 \beta 2$) and neuromuscular junction: Why do smokers not have potentially fatal muscle contractions?
- "Aromatic box"-active site conserved between both subclasses (CNS vs. PNS)
- Cation-π interaction probed as before with serial fluorination of TrpB: as before, no Cation-π with muscle jct. nAChR and nicotine but now see one with CNS ($\alpha 4 \beta 2$) receptor
- Is this defference in effect due to binding or gating? (EC_{50} = composite of both)







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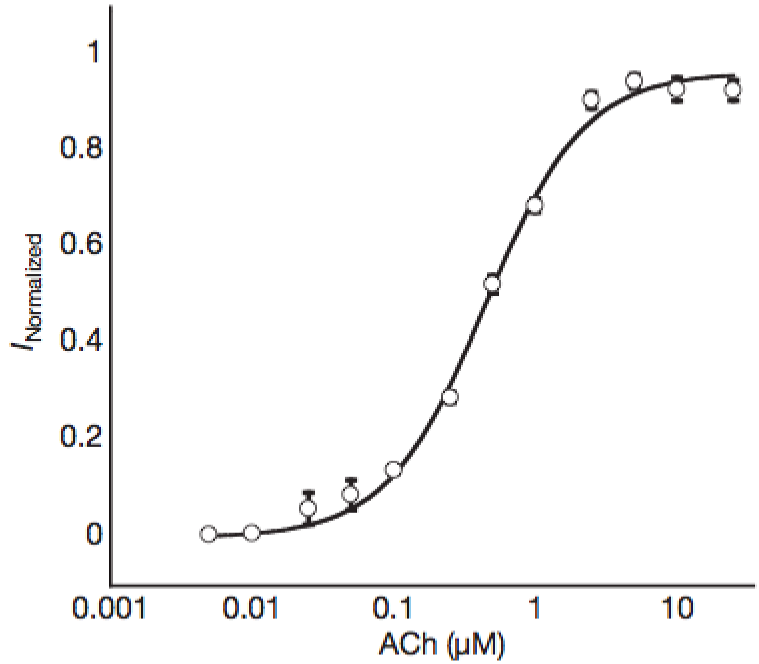
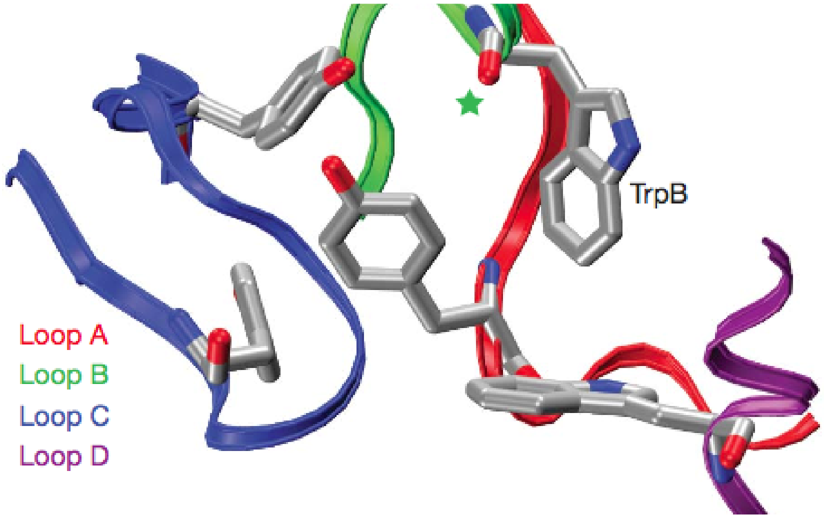
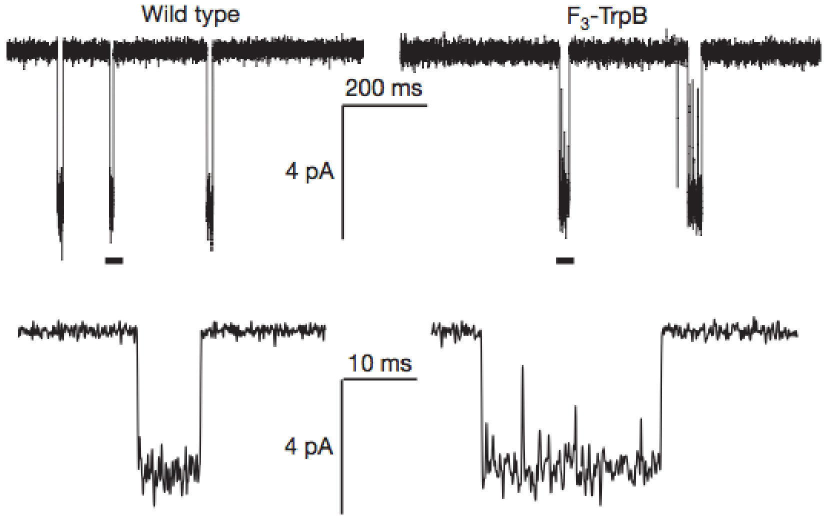
- Is this difference in effect due to binding or gating? (EC_{50} composite of both)
- Single channel recordings show "nearly indistinguishable" gating between WT and F_3-Trp (Population open) indicating that EC_{50} result of change in agonist binding
- TrpB amide to ester mutation showed little change in efficacy (ratio: 1.6) in neuromuscular nAChR but a 19-fold increase in EC_{50} for $\alpha4\beta2$







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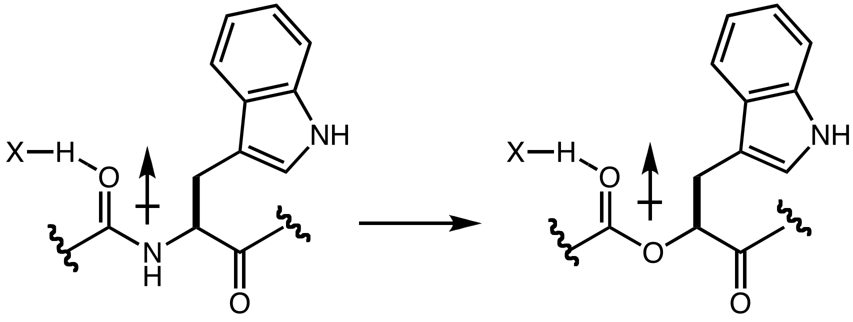
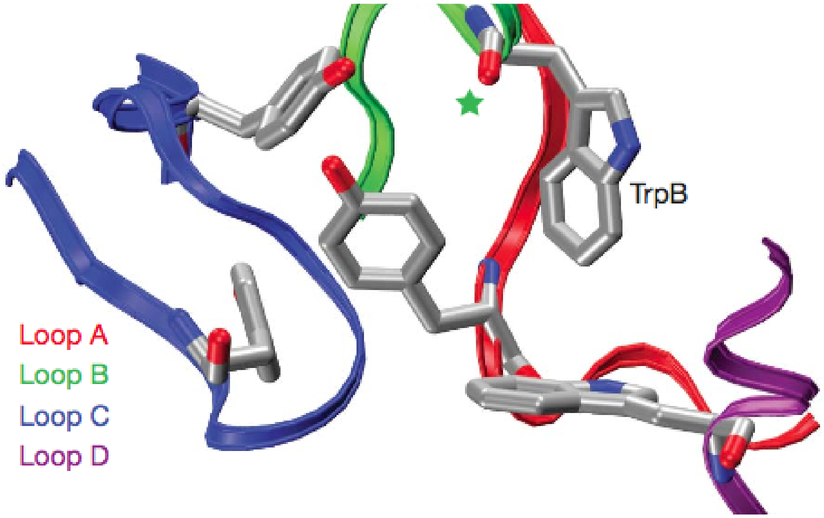
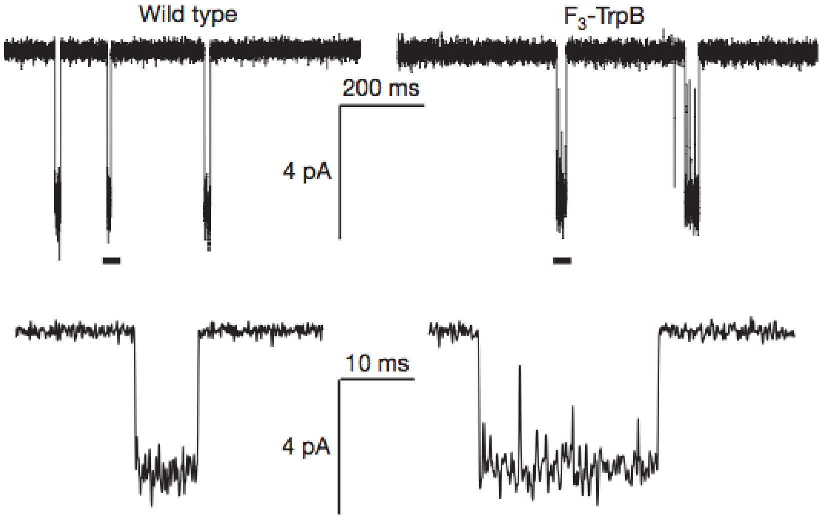


region	change in EC_{50}
neuro-muscular	1.6 fold
CNS ($\alpha4\beta2$)	19 fold





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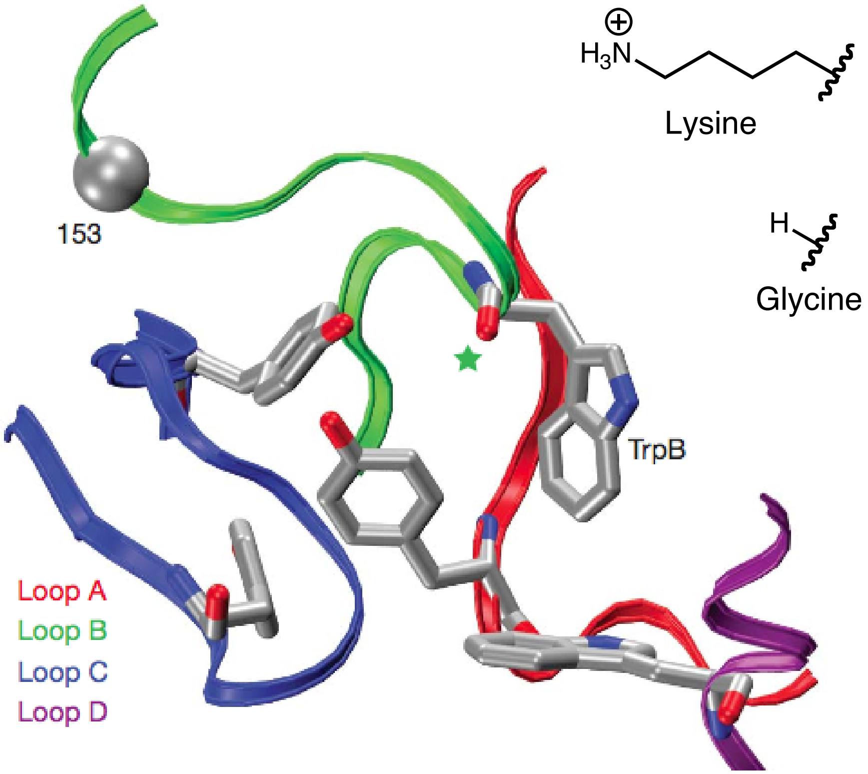
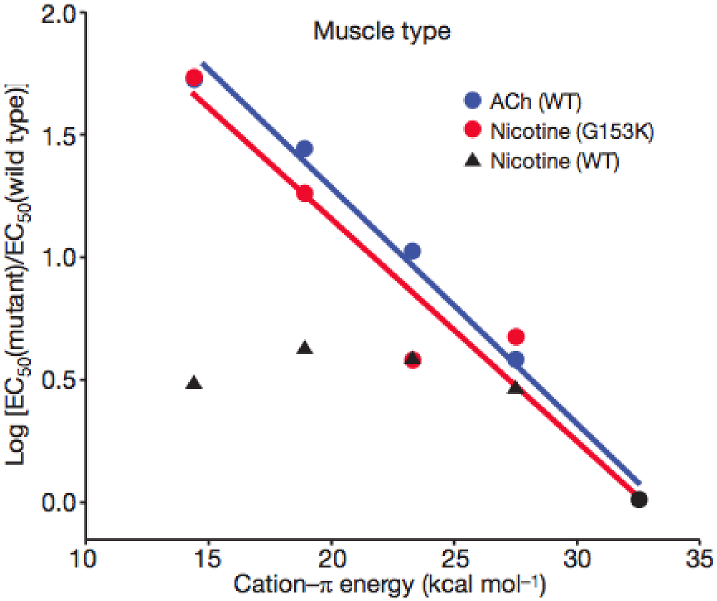
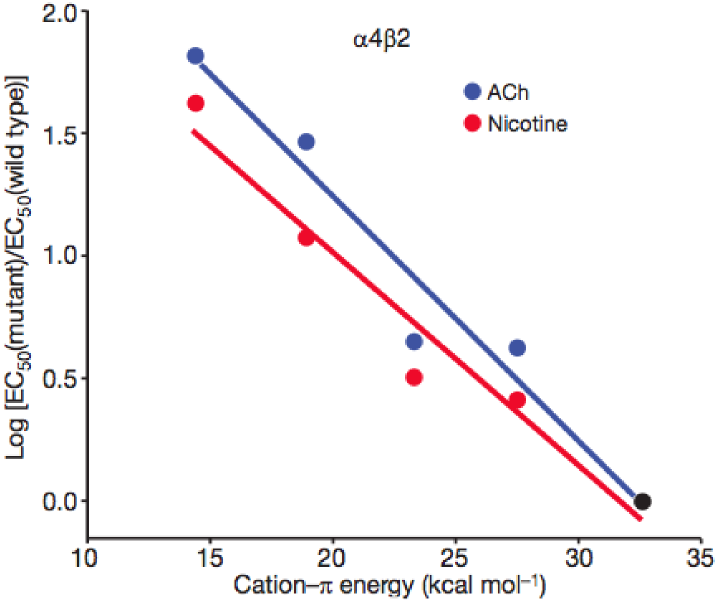
Differential Effect of Nicotine to CNS and Neuromuscular nAChRs

- Differences in nicotine binding bewteen CNS and PNS found to occur becuase of a non-active site lysine which reorients "aromatic box" in CNS (Gly in PNS)
- Mutation of muscle-type nAChR glycine to lysine (G153K) affords functional rescue in cation-π serial fluorination plot







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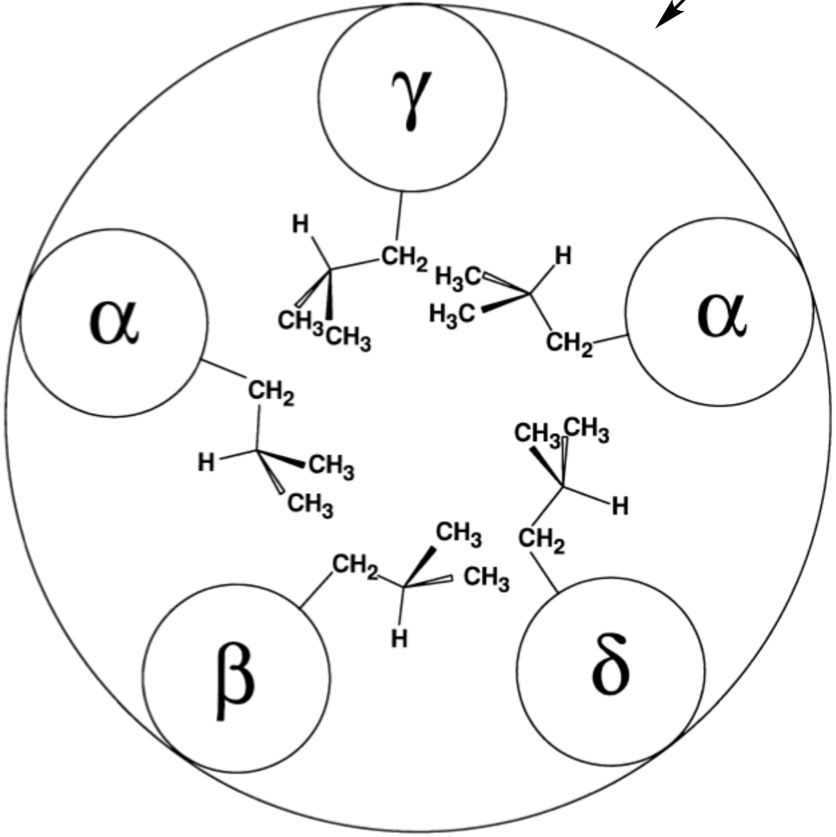
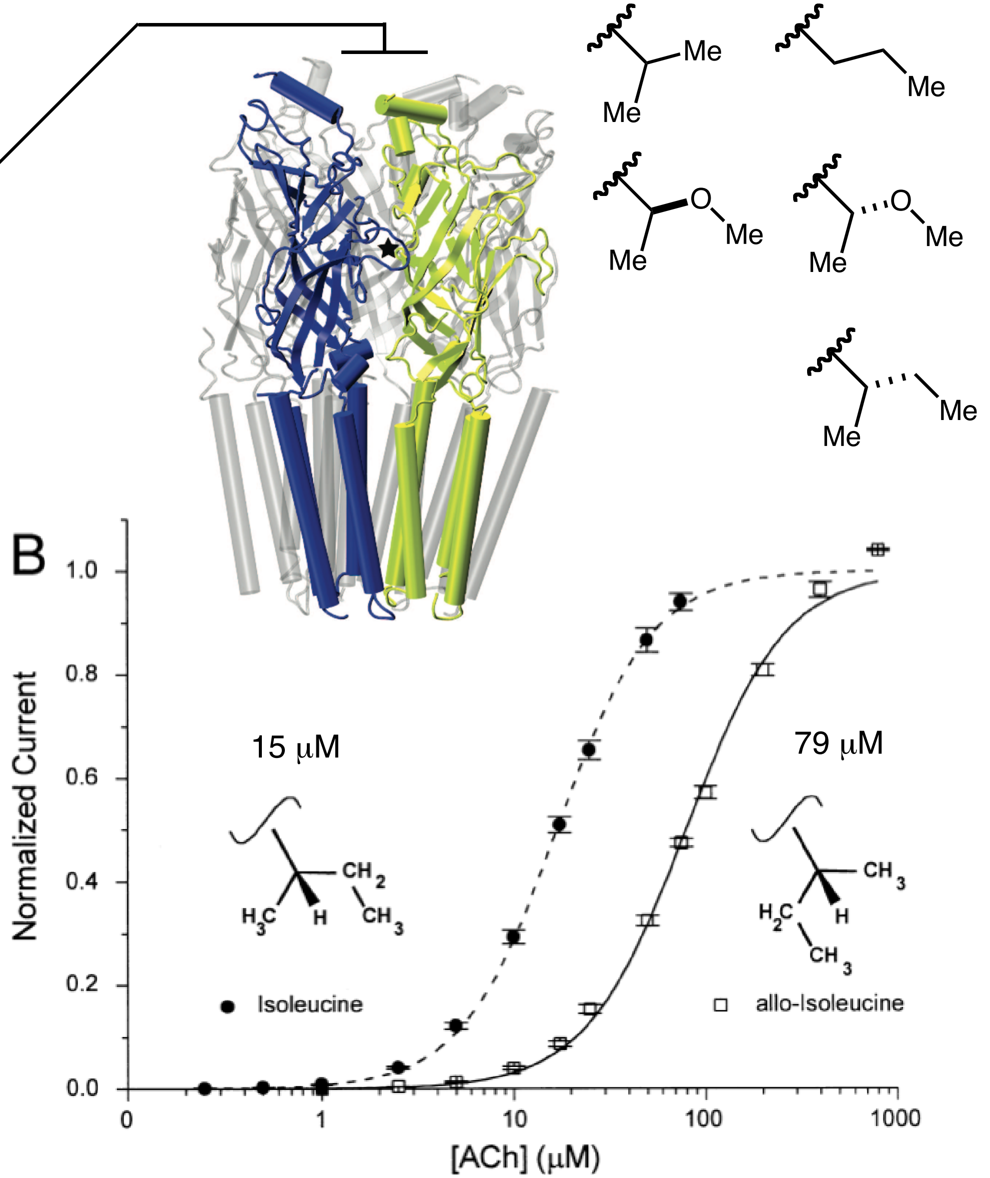
Initial experiments to probe nAChR with Physical Organic Chemistry

- Several Leu9'AA mutations in channel pore aim to probe "hydrophobic plug" model of gating
- Model holds with change to polar UAA residues (OMe) but see biggest shift with *allo*-leu: more subtle model needed

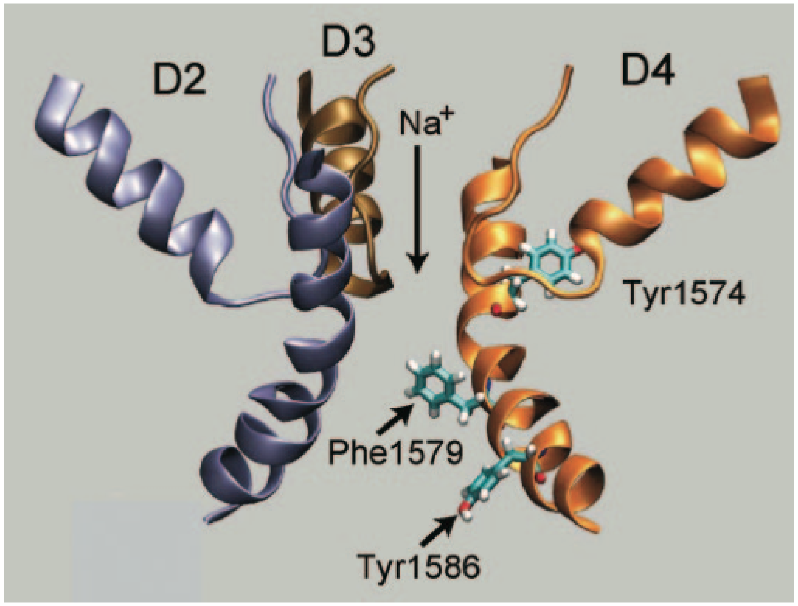


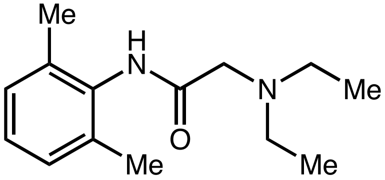


Kearney, P. C.; Zhang, H.; Zhong, W.; Dougherty, D. A.; Lester, H. A. *Neuron* **1996**, *17*, 1221.

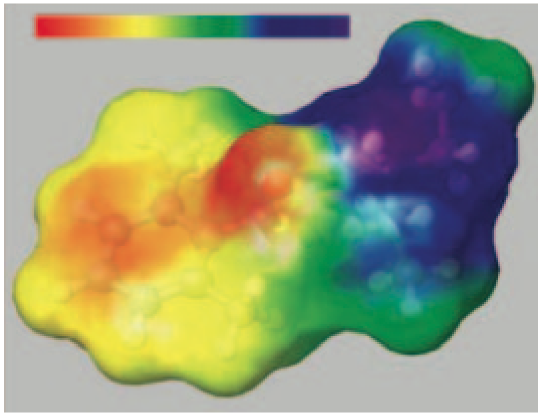
Cation-p Mutagenesis Studies Amenable to Voltage-Gated Ions Channels

- Dougherty settles dispute over which residues in the ion channel pore contribute to lidocaine binding (Phe1579, not Tyr1574 or Tyr1586)
- Cation π interactions probed using similar serial fluorine substitution as with tryptophan





lidocaine



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Early quad. codon: double unnatural amino acid incorp.
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