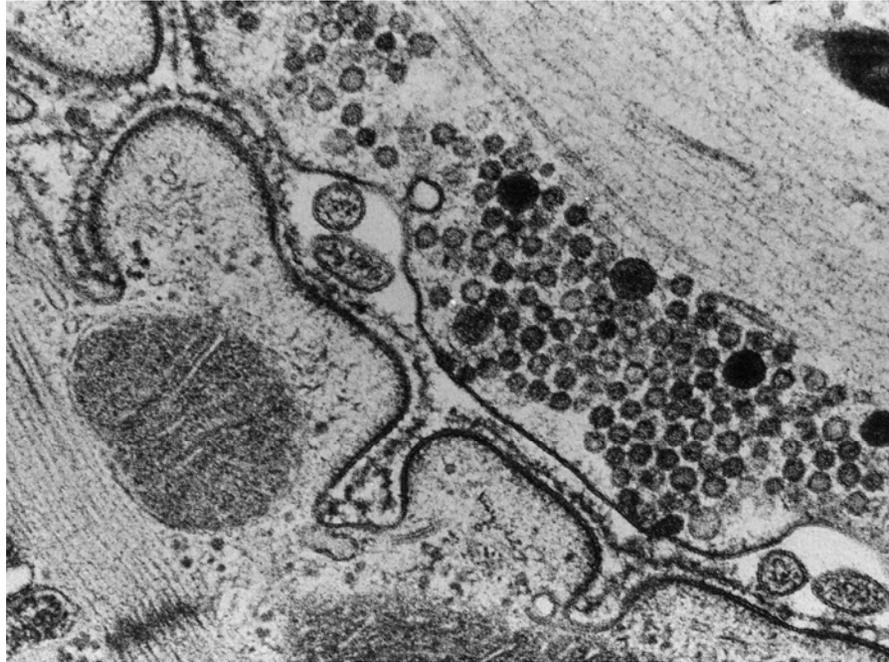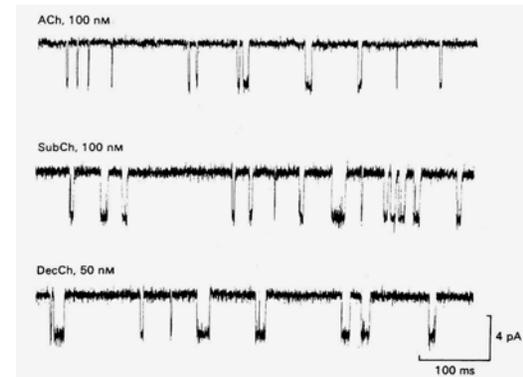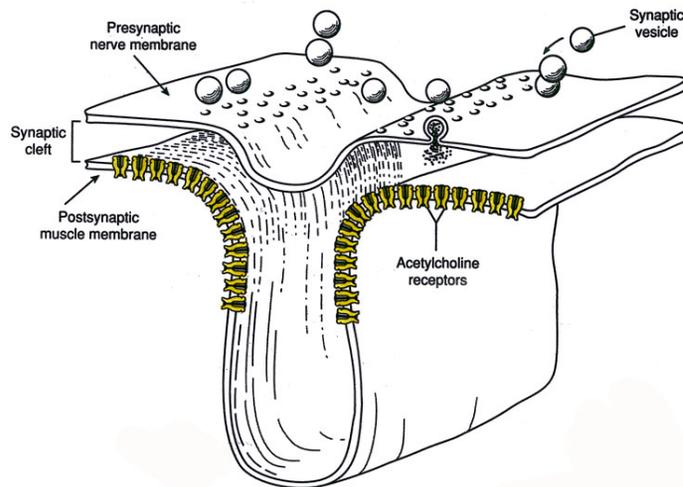# Electron crystallography of tubes: nicotinic acetylcholine receptor

Nigel Unwin,
MRC Lab of Molecular
Biology,
Cambridge, UK

The nerve-muscle synapse

John Heuser, 1975

Colquhoun and Sakmann, 1985

Fundamental questions:

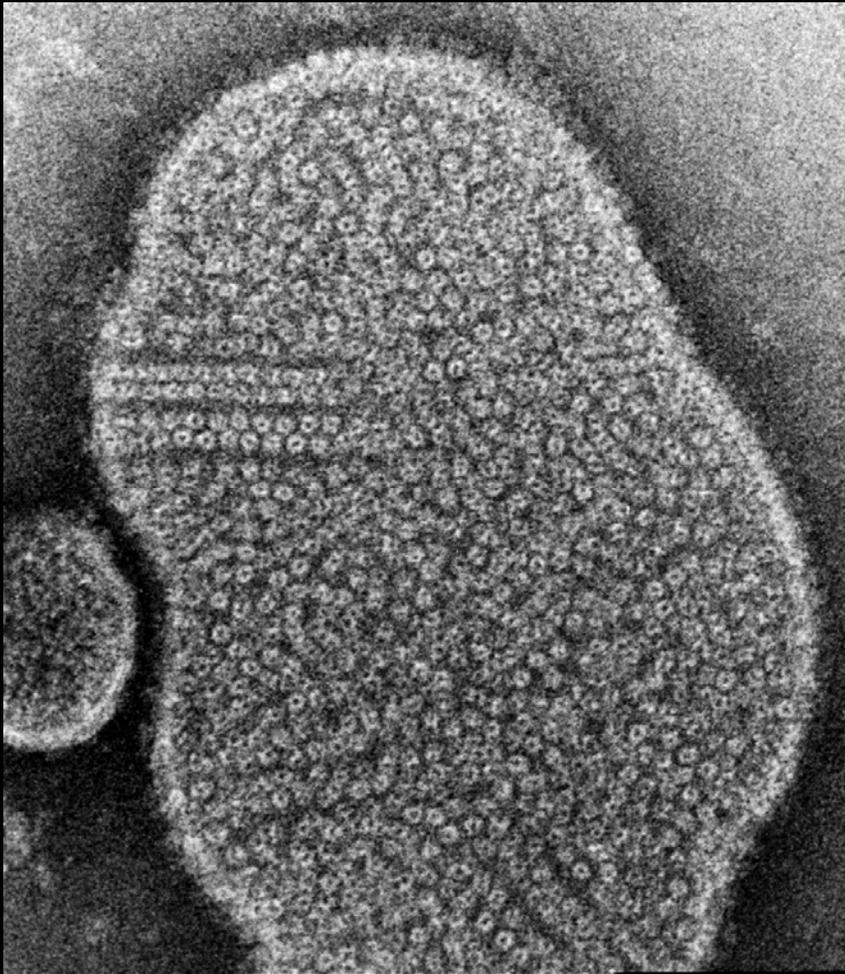How does the transmitter initiate the movements which open the channel?

How does the structure change between closed and open states?
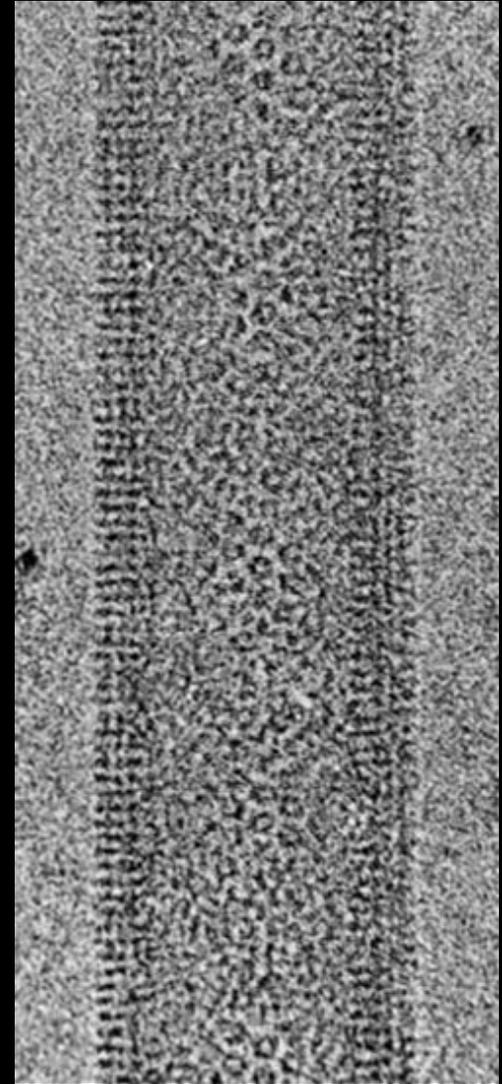
How is ion selectivity achieved?

The electric ray: Torpedo marmorata
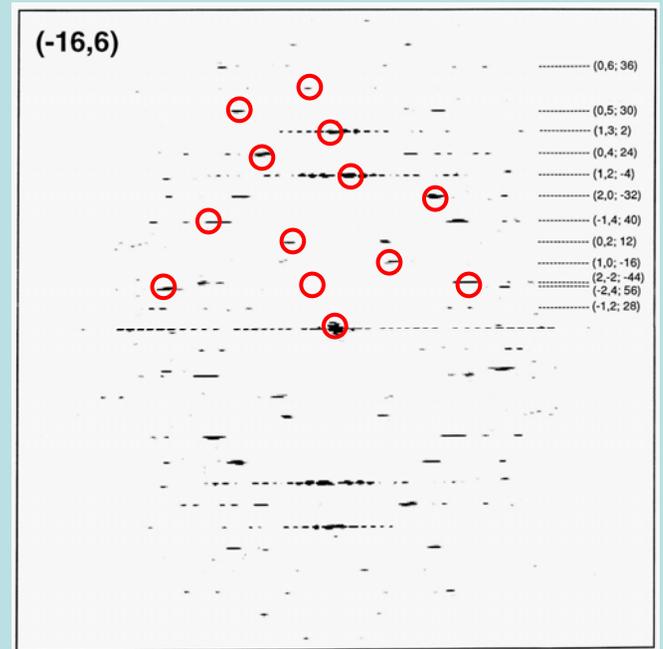
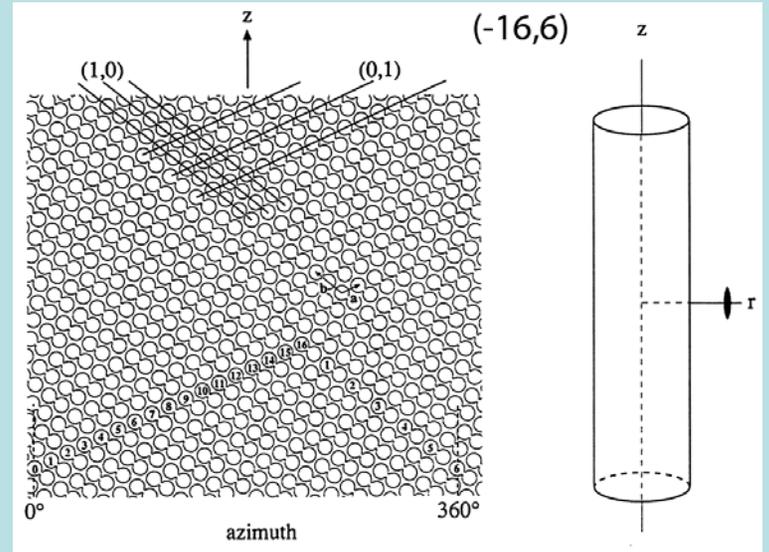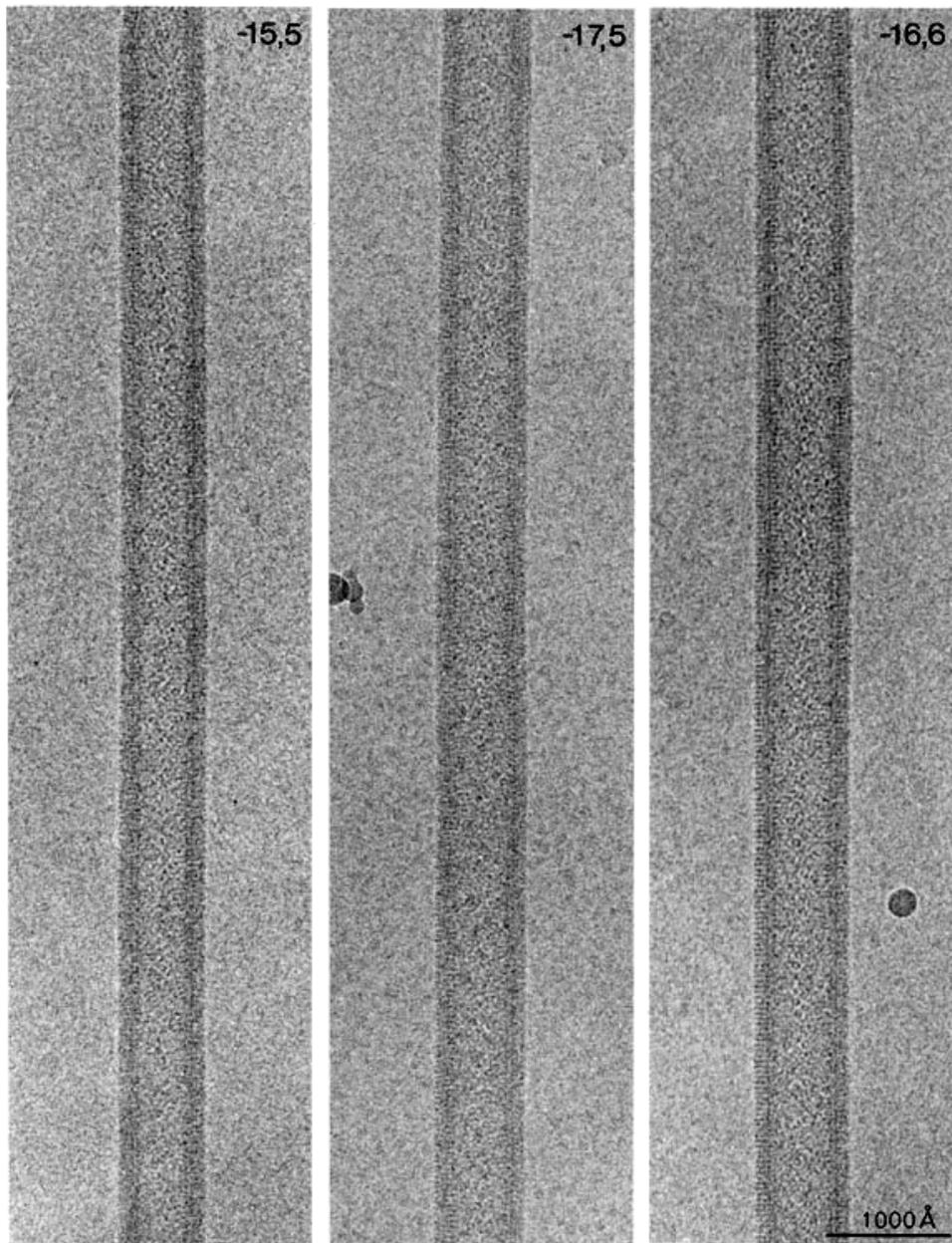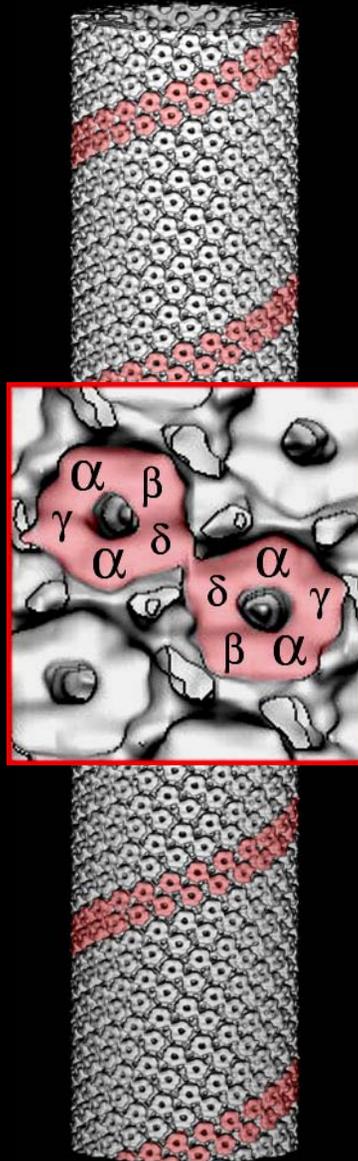# Postsynaptic membranes from the Torpedo ray

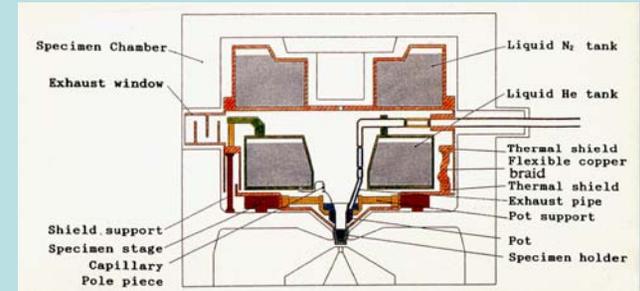Vesicle (neg. stain)

Tube (ice)

Different helical families

Reconstruction of a (-16,6) tube

Important Techniques

Electron microscopy at liquid helium temperatures
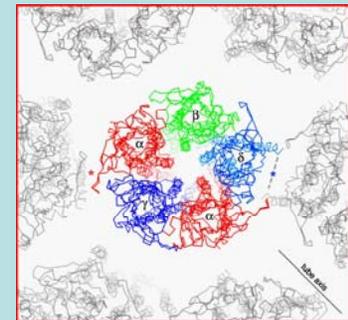
(Fujiyoshi et al., Ultramicroscopy, **38**, 241-251;1991)

Undistorting tube images by alignment of short segments to a reference structure
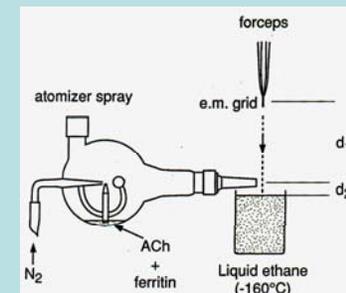
(Beroukhim & Unwin, Ultramicroscopy, **70**, 57-81;1997)

Structural refinement by R-factor minimisation and comparison of calculated with experimental phases
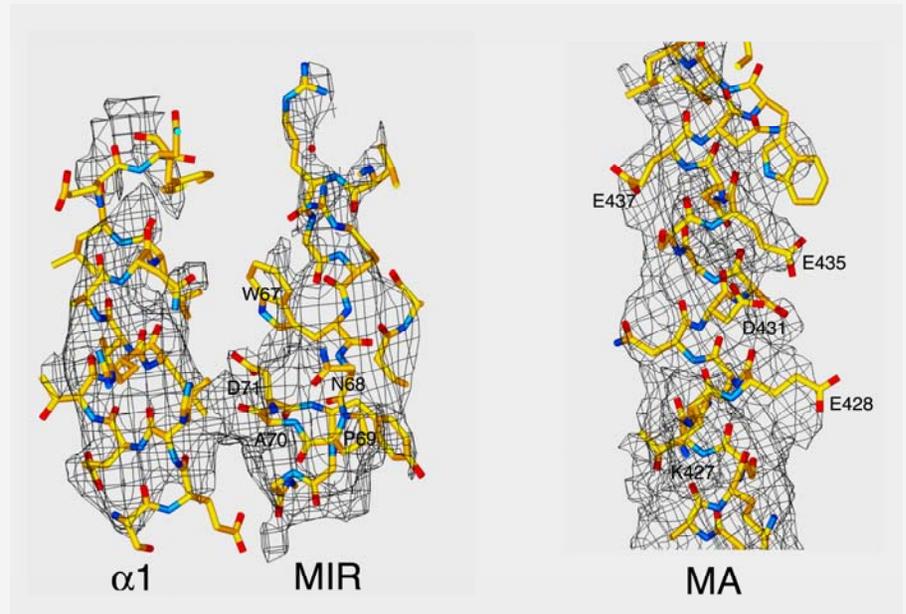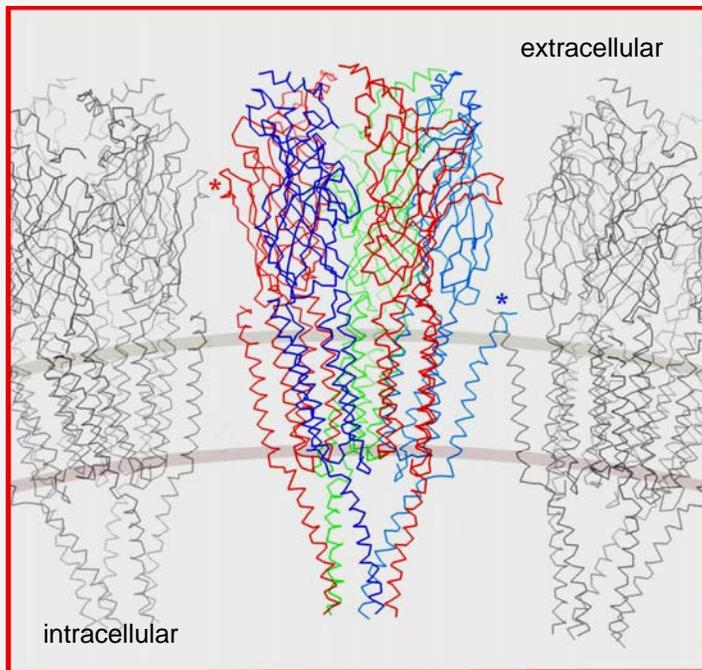
(Unwin, J. Mol.Biol., **346**, 967-989; 2005)

Freeze-trapping to image gating movements

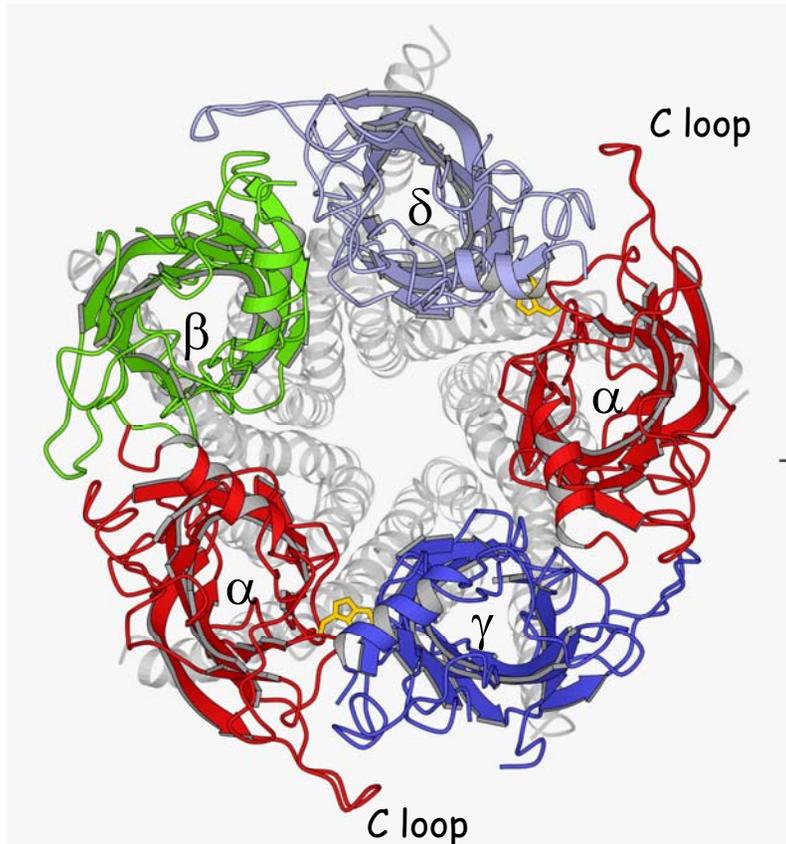(Berriman & Unwin, Ultramicroscopy, **56**, 241-252; 1994)

3D map at 4Å resolution

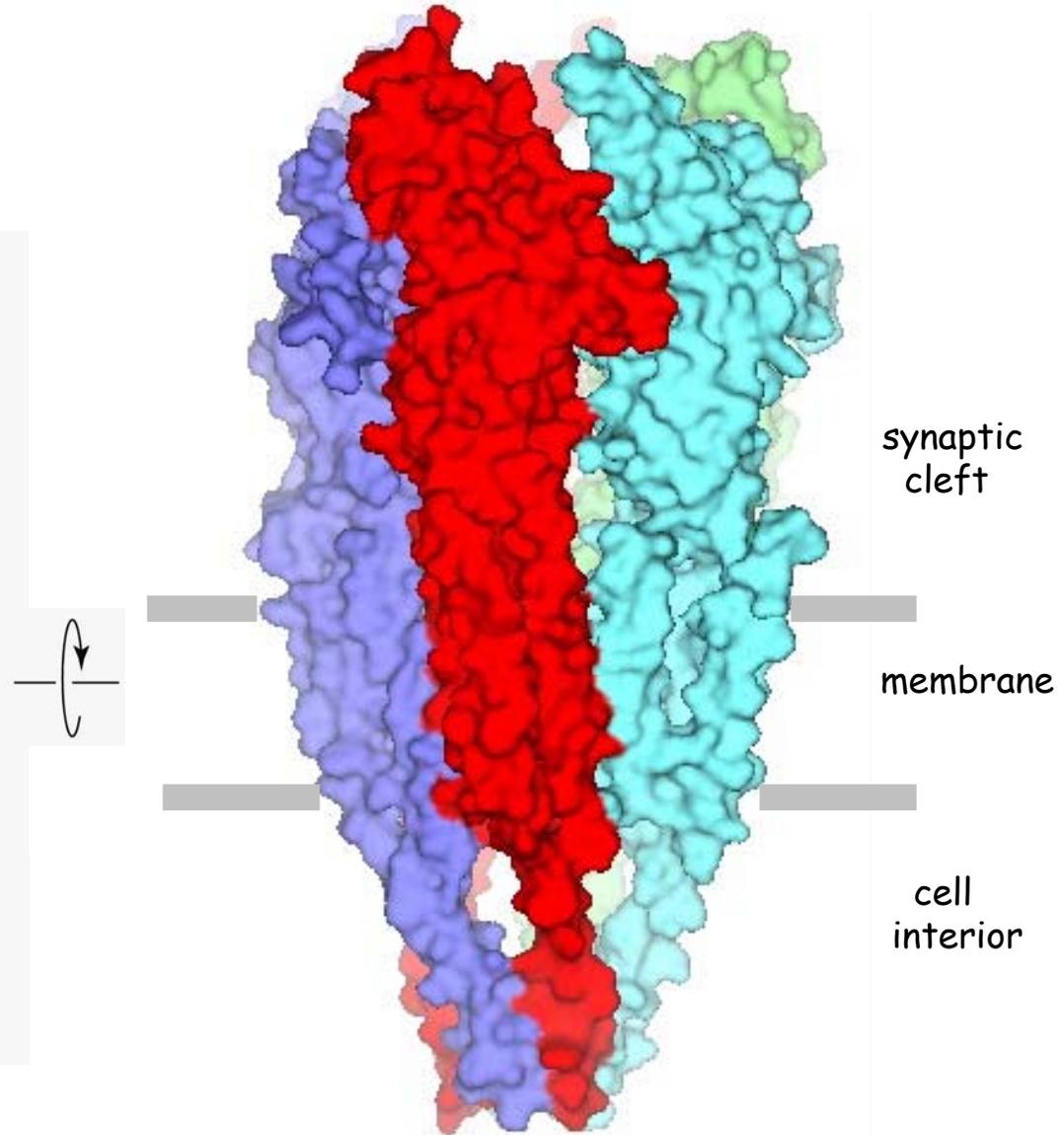|	
Number of images	342
Number of receptors	$\sim 10^6$
No. Fourier terms	$\sim 10^5$
Amp. wted phase error	51°
R-factor	36.7%
(R_{free}	37.9%)

top, α subunit   bottom, γ subunit

Structure of the closed channel

Viewed from synaptic cleft

Viewed from the side

Fit of mouse α subunit ligand-binding domain to Torpedo ACh receptor

β-sheet core
r.m.s deviations (Å):
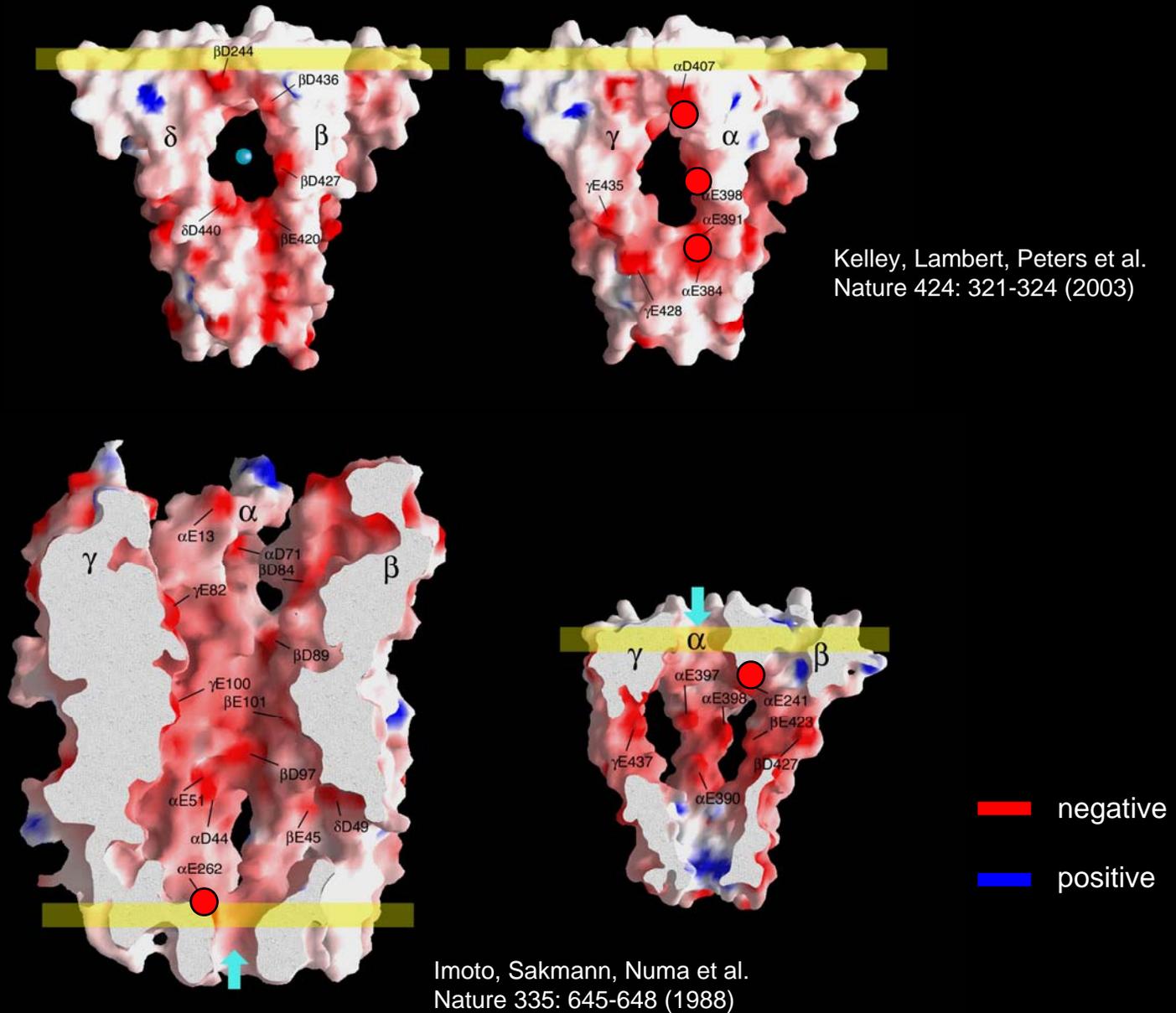
$\alpha_m/\alpha_\gamma = 2.16$
$\alpha_m/\alpha_\delta = 2.10$
$\alpha_m/\beta = 2.17$
$\alpha_m/\gamma = 1.81$
$\alpha_m/\delta = 1.86$
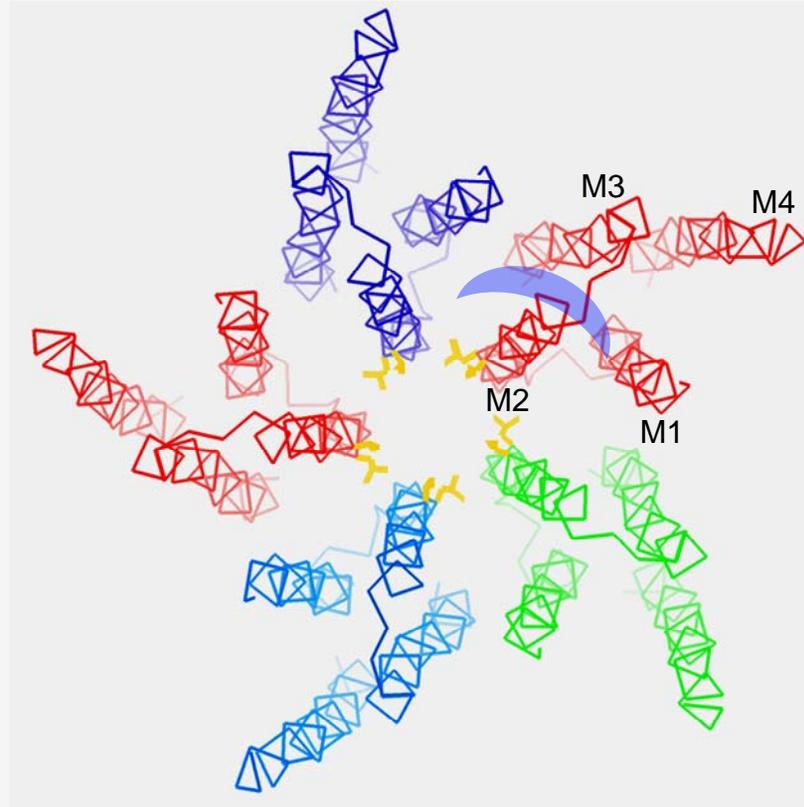
(AChBP/$\alpha_\gamma = 2.43$)

Vestibules are negatively charged

Kelley, Lambert, Peters et al.
Nature 424: 321-324 (2003)

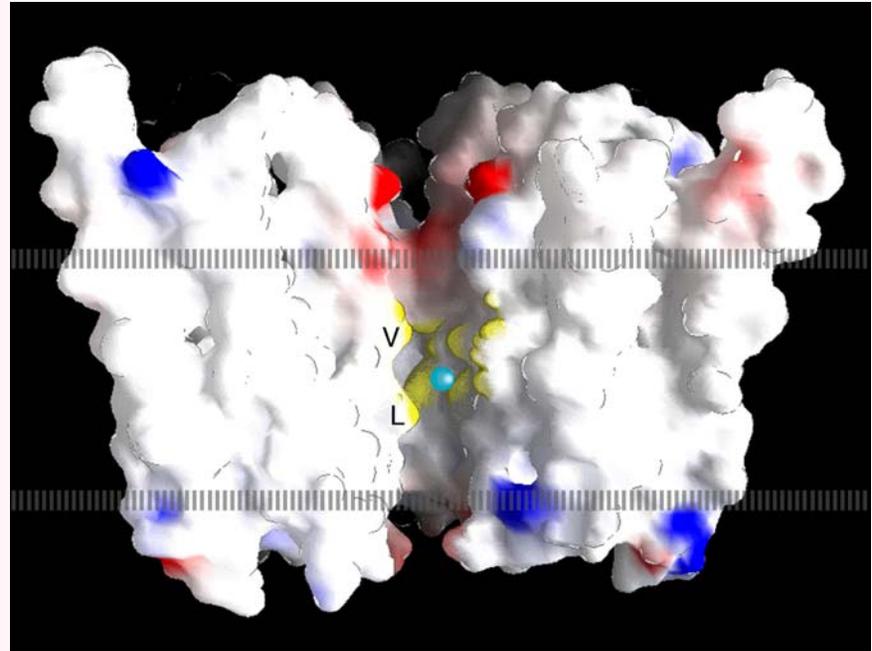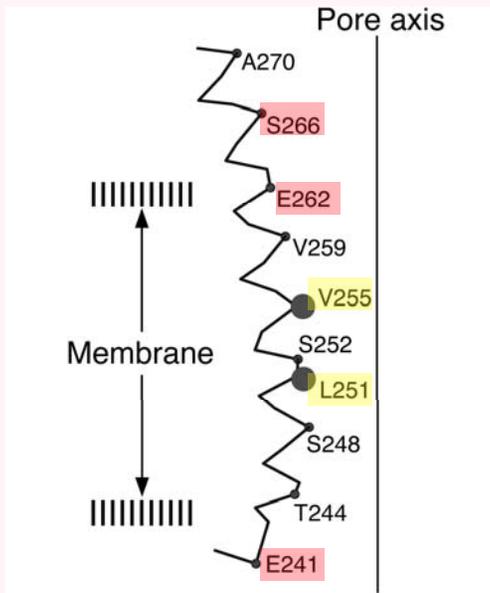Imoto, Sakmann, Numa et al.
Nature 335: 645-648 (1988)

negative
positive

Membrane-spanning portion
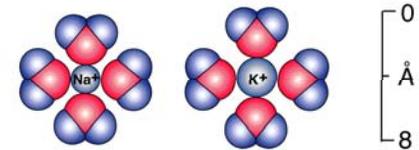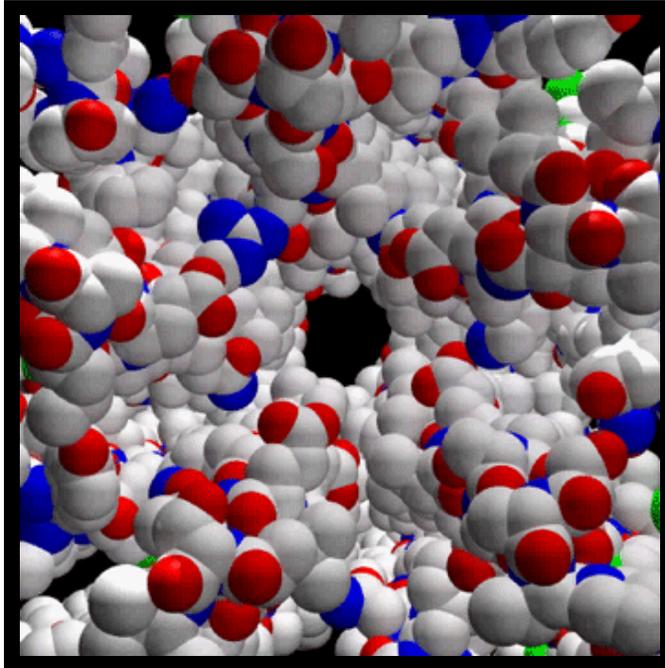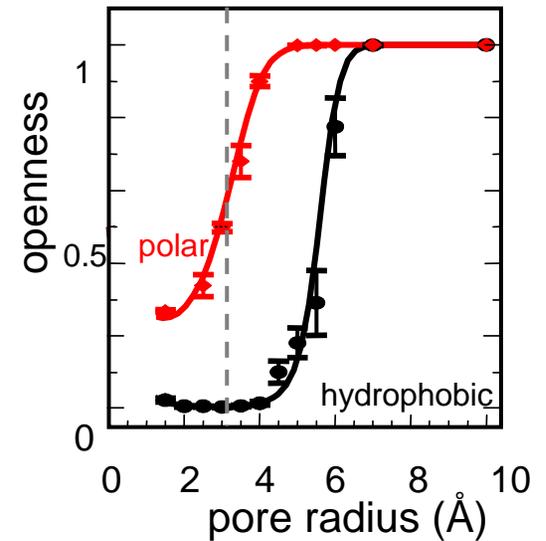
Membrane-spanning portion
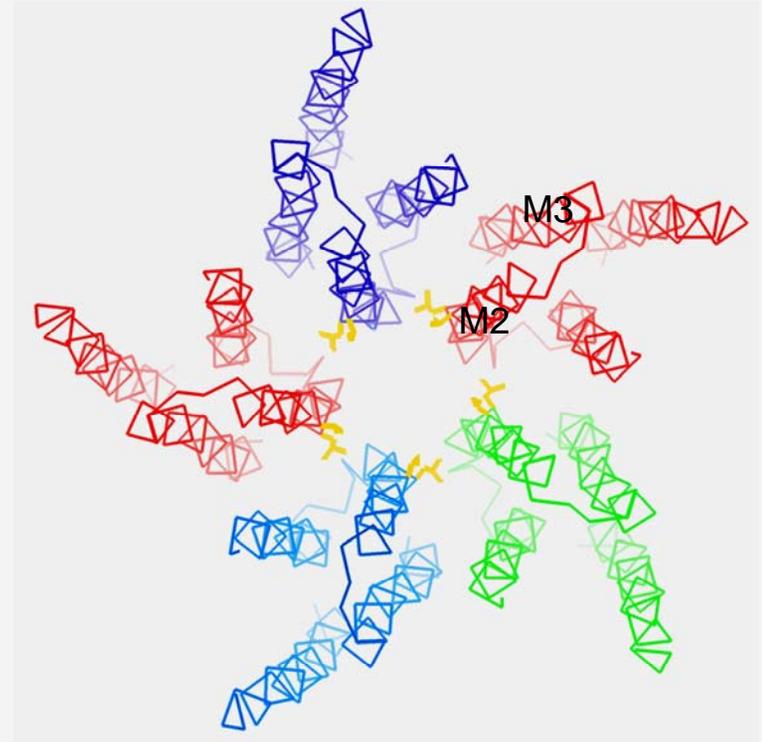
M2 (α subunit)

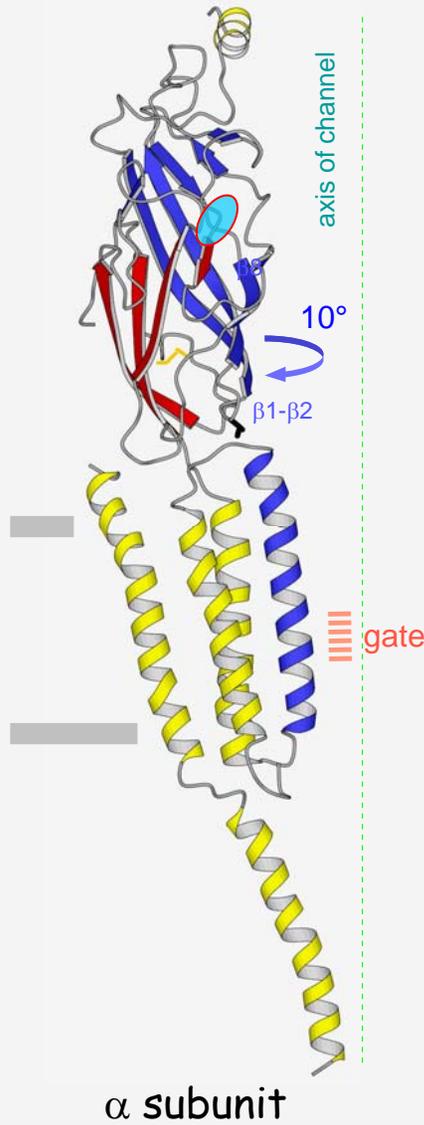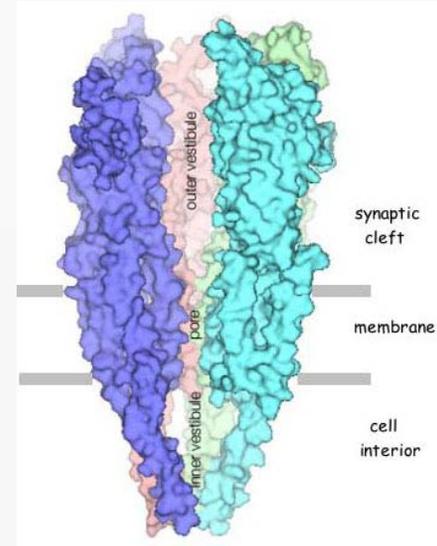# Hydrophobic girdle at middle of membrane

hydrophobic gating
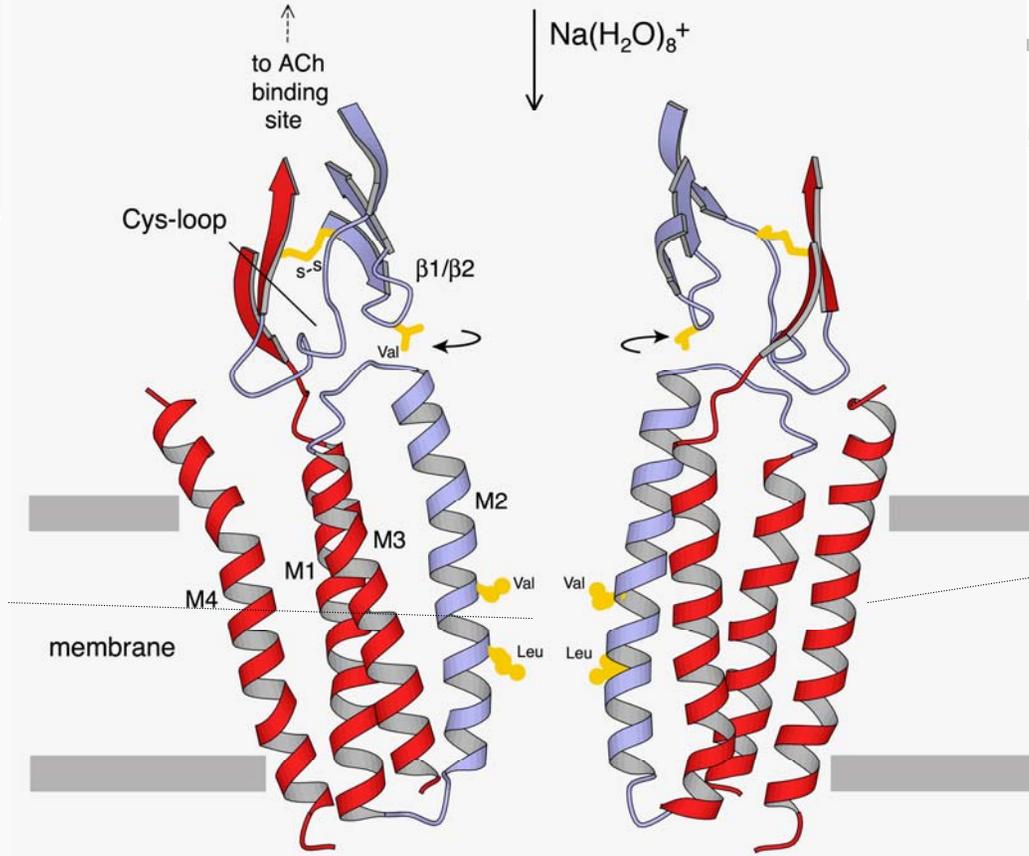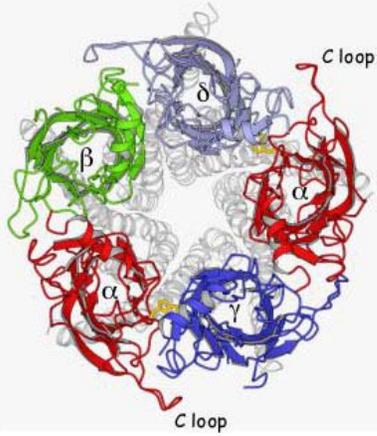
Beckstein, Biggin & Sansom (2001)
J. Phys. Chem. B 105:12902

ACh-induced rotations in the ligand-binding domain break open the gate

axis of channel

10°

β1-β2
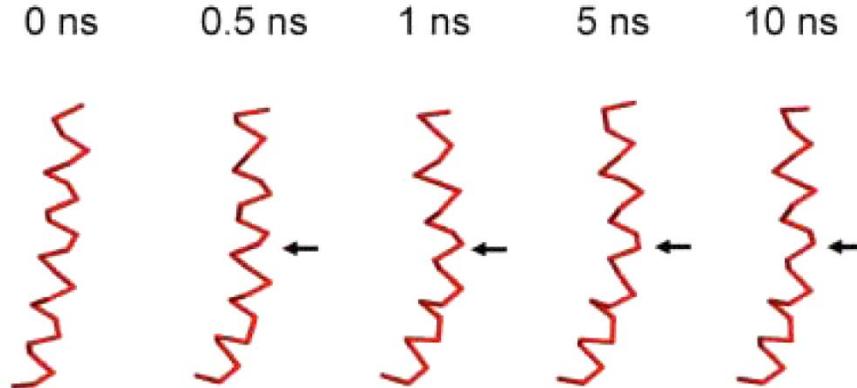
gate

α subunit

M3

M2

Summary of proposed gating mechanism

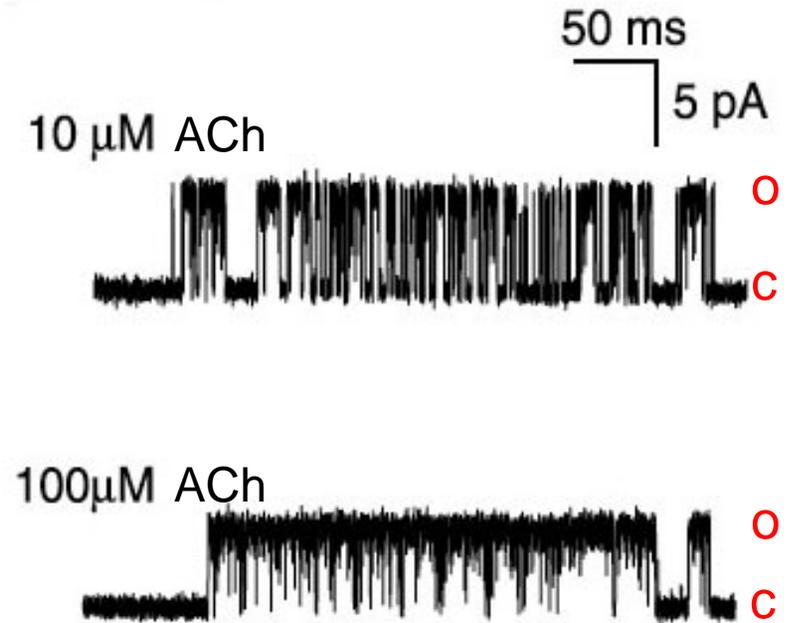Hydrophobic girdle: energetic barrier to ion permeation when channel is closed

Protein scaffold (M1, M3, M4) shielding gating motions from lipids

Do gating movements involve helix bending?

0 ns   0.5 ns   1 ns   5 ns   10 ns

MD simulations on membrane-spanning
portion of ACh receptor

(Hung, Sansom et al., Biophys. J. 88: 3321-3333 (2005))

50 ms

5 pA

10 µM ACh

O

C

100µM ACh

O

C
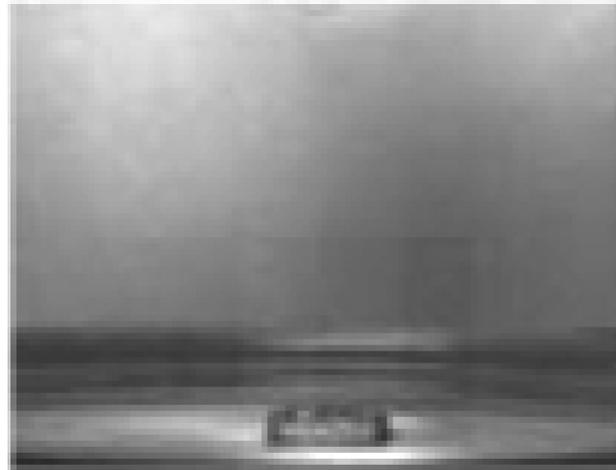
Electrophysiological recordings

Wang, Sine et al., Nat. Neurosc., 2: 226-233 (1999)

Catching the gating movement by plunge-freezing

forceps

atomizer spray

e.m. grid

d_1

d_2 | ~10ms

ferritin

ACh
+
ferritin

N$_2$

Liquid ethane
(-160°C)
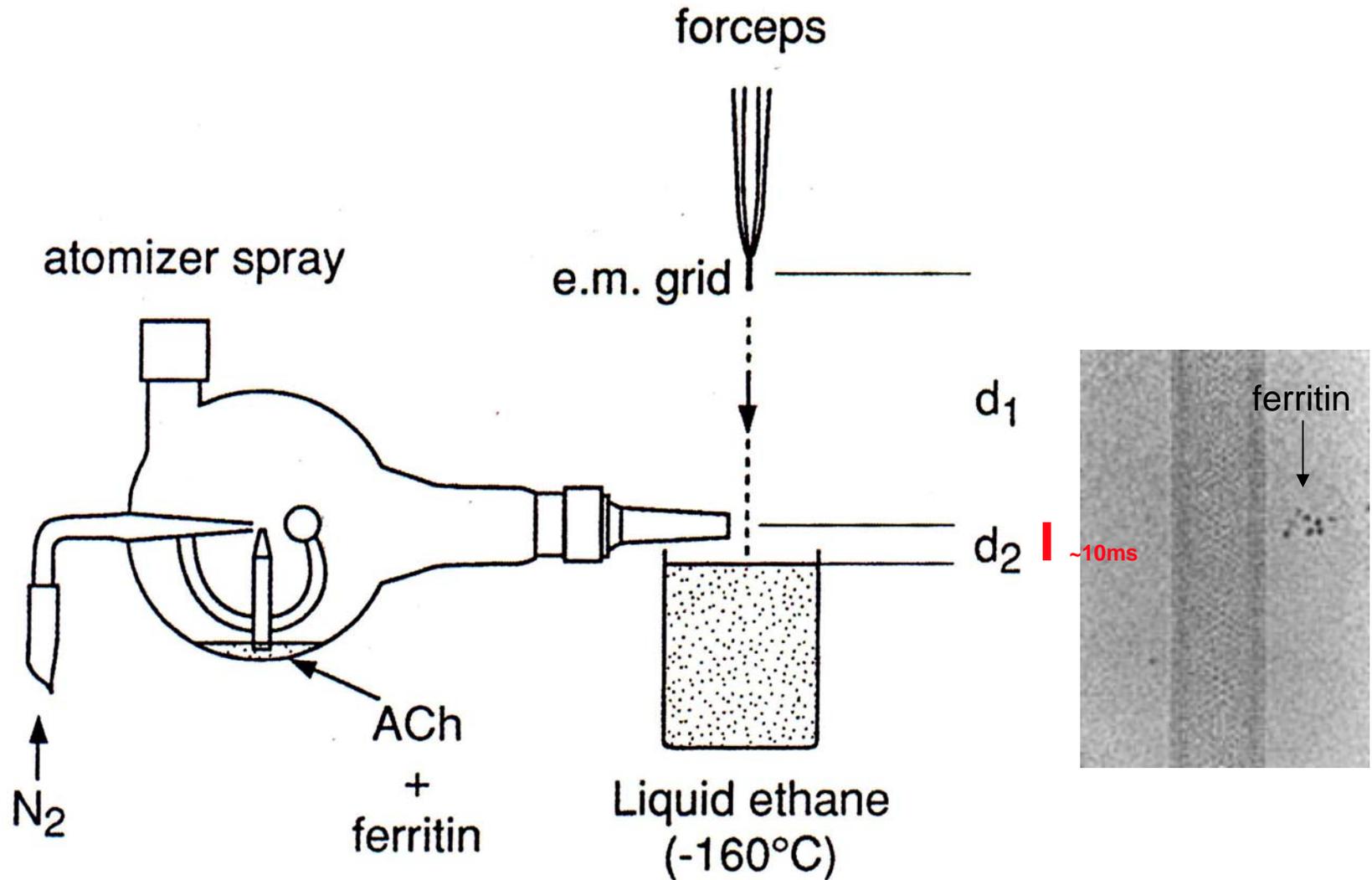
Spread of droplet over a thin aqueous film

2 ms

Manzello & Yang,
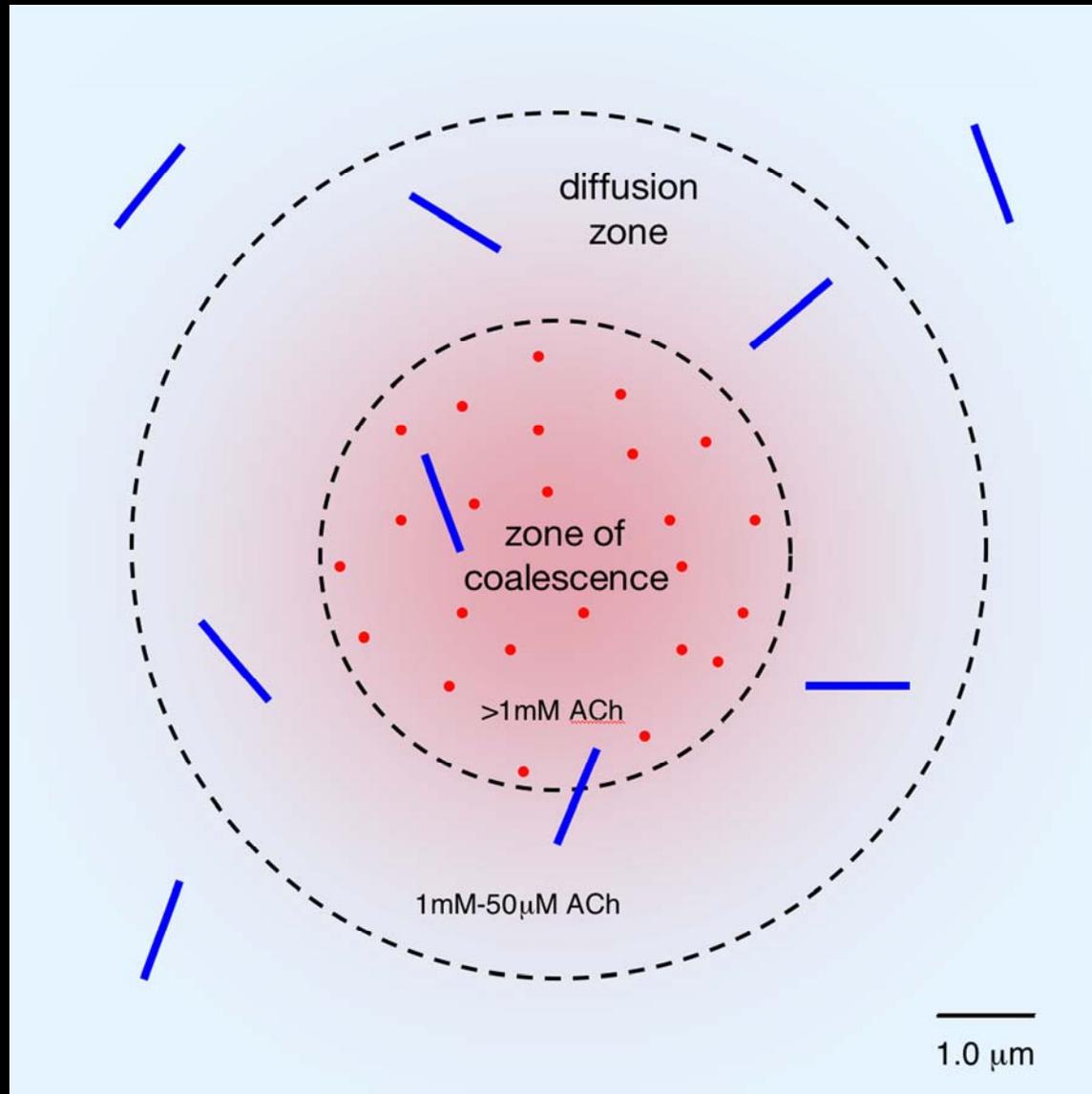Exps. in Fluids, 32: 580-589 (2002)

Measurements from 1μm droplet, after 10 ms:

(Berriman & Unwin, Ultramicroscopy 56: 241-252 (1994))

Zone of coalescence extends to radius of ~3 μm
Diffusing ions extend to radius of ~7 μm

Estimated diffusion distance for ions $(2(Dt)^{1/2})$: 9.0 μm

1µm droplet after 10 ms

Data collection

$$P = RISHFP$$

R = sample from a suitable *Torpedo* Ray (~1 in 50)
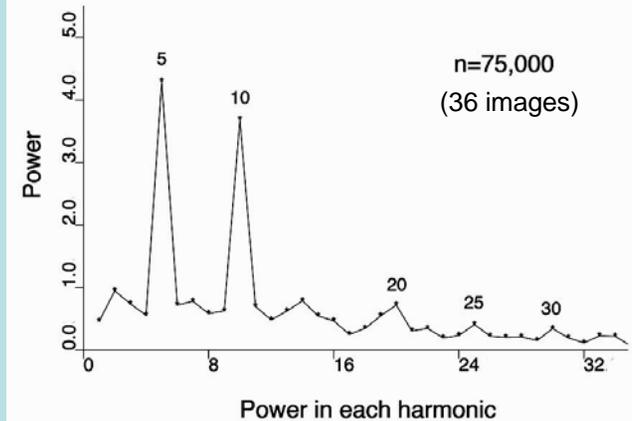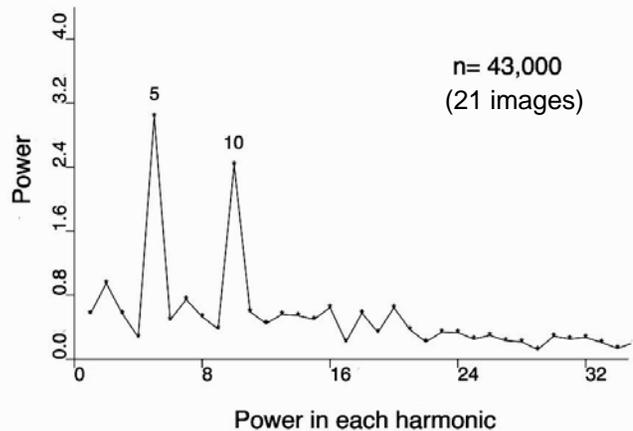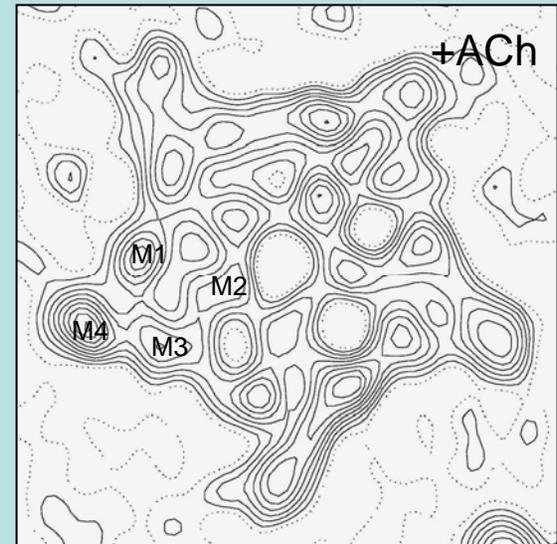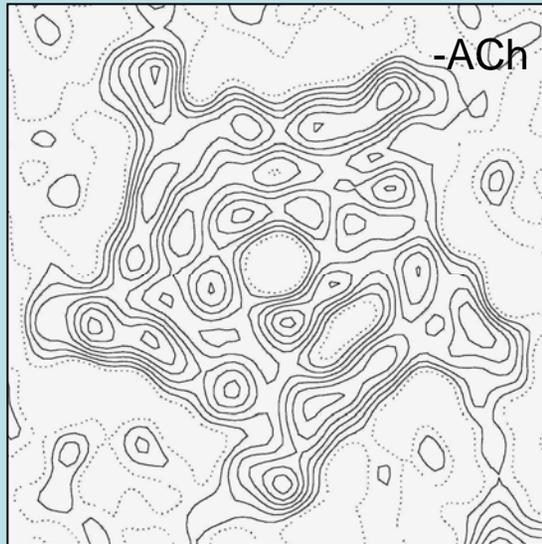 I = good thickness of Ice on em grid (± 200Å)
S = Spray droplet lands appropriate distance from tube

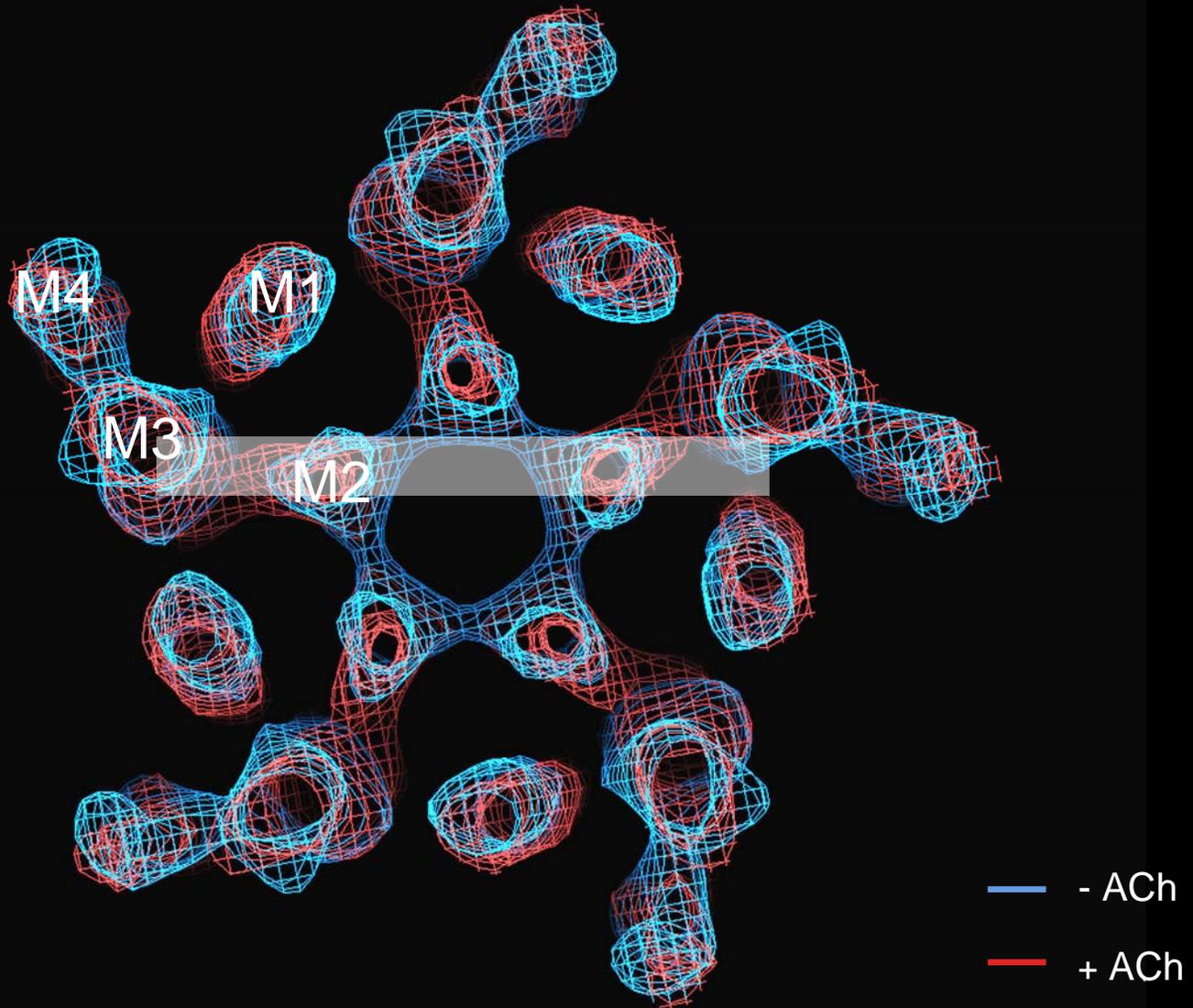H = tube is straight, over a Hole in the support film
 F = tube belongs to a suitable helical Family
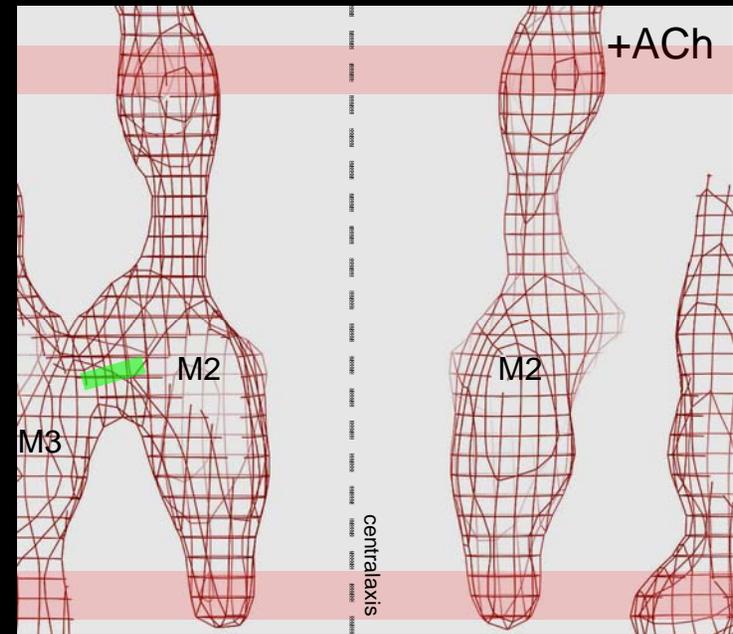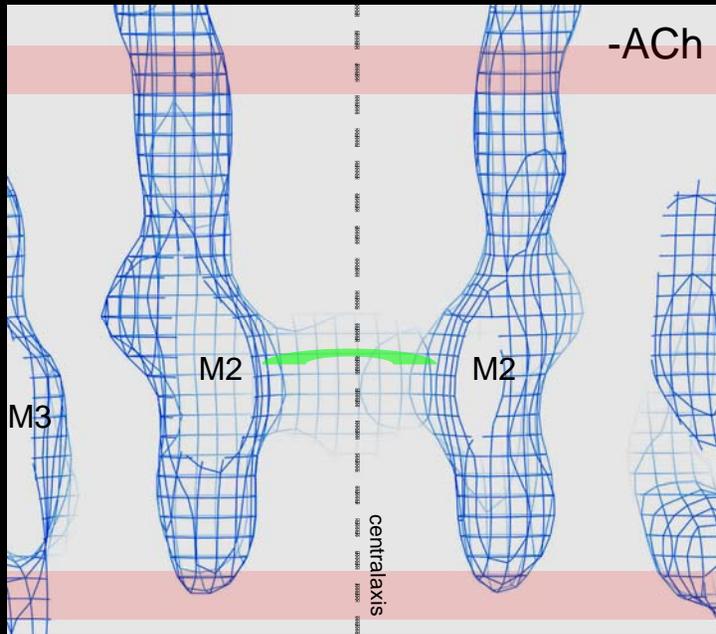 P = microscope records a perfect Picture

Comparison of +ACh with -ACh images (so far)
(-15,5) family; ~6Å resolution

-ACh

+ACh

M1
M2
M4
M3

n= 43,000
(21 images)

5
10

Power

Power in each harmonic

n=75,000
(36 images)
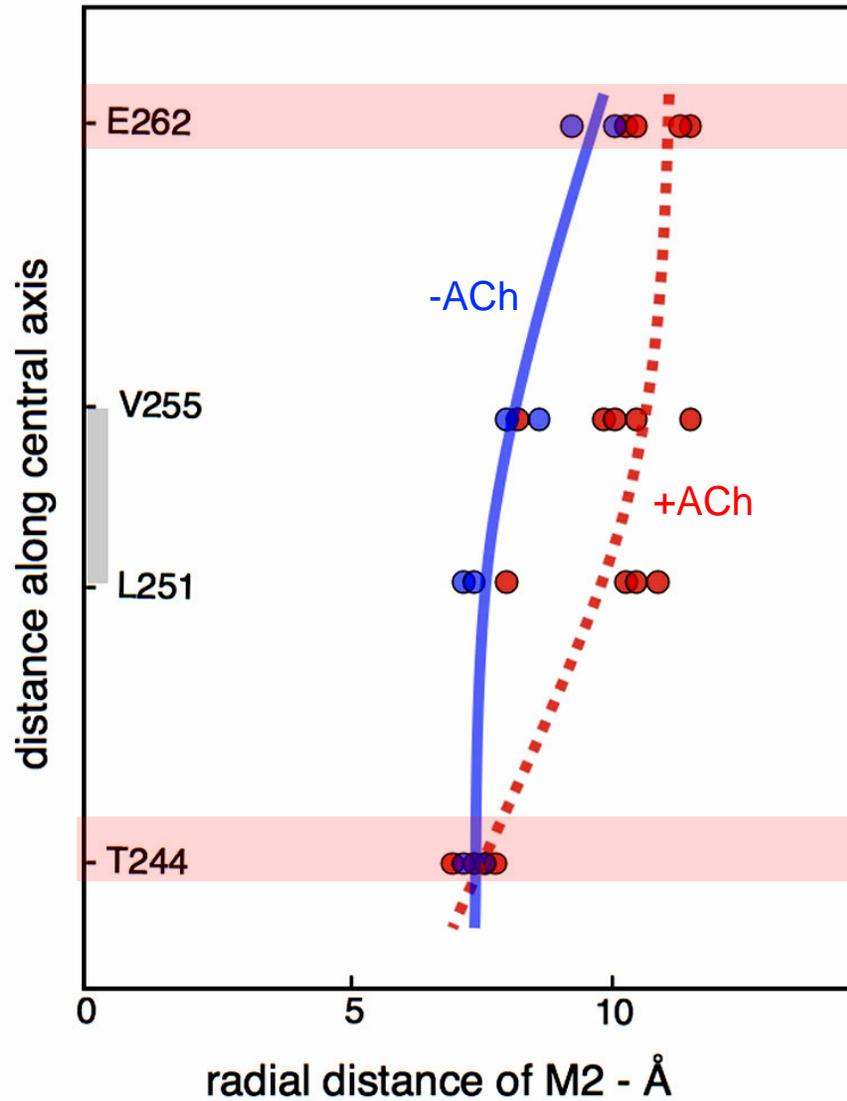
5
10
20
25
30

Power

Power in each harmonic

Slab through upper leaflet of lipid bilayer
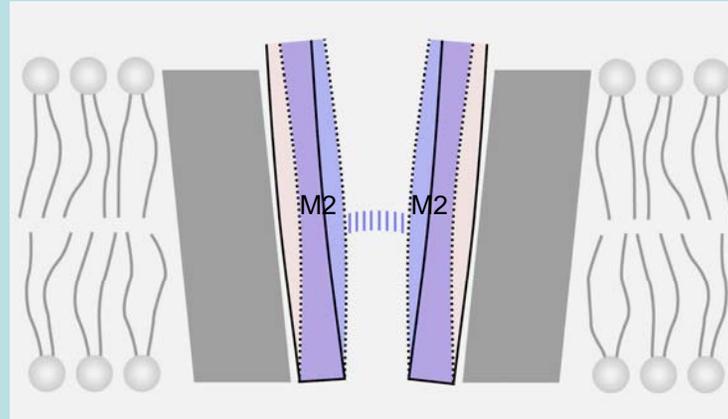
Central sections normal to plane of lipid bilayer

Radial distance of M2 from central axis measured from averages of nine images (n=~18,000)
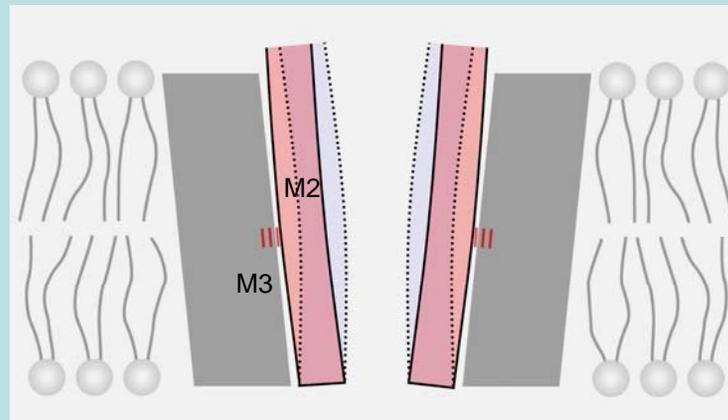
Tentative Conclusion

Closed channel: stabilised by interactions between
inner helices (and by ligand-binding domain)

Open channel: stabilised by interactions between
inner helices and outer wall

Rameen
Beroukhim

Yoshi

Atsuo
Miyazawa