Archael Transport.

The Archaea are a recent biological discovery. Certainly, the presence of so-called 'extremophiles' - bacteria that are adapted to extraordinarily harsh environments - was known, but the uniqueness of their DNA sequences was not, until Woese proposed that the extremophiles were so different (based on tRNA and rRNA sequences) that they comprised a completely different major clade*: The Archaea. The other major differences are the composition of their membranes and cell walls.

*	
◦ Archaea ◦ Prokarya • Eukarya	Three major clades of life.

Now, it is clear that Archaea are not limited to extreme environments, but are also mesophiles (typically growing between 20 & 40 °C) based on pcr-amplication of Archaea-unique 16S rRNA in water and soil. Even so, our exploration of Archael transport will focus on the extremes.
of which there are many ....

methanogens (strict anaerobes, methane-producers)
halophiles (strict aerobes, high-salt environments)
thermoacidophiles (aerobes in hot acidic environments)
hyperthermophilic (very hot)
psychrophilic (cold)
halophilic (high salt)
alkaliphylic (very alkaline)
} both methanogens & all

etcetera.....

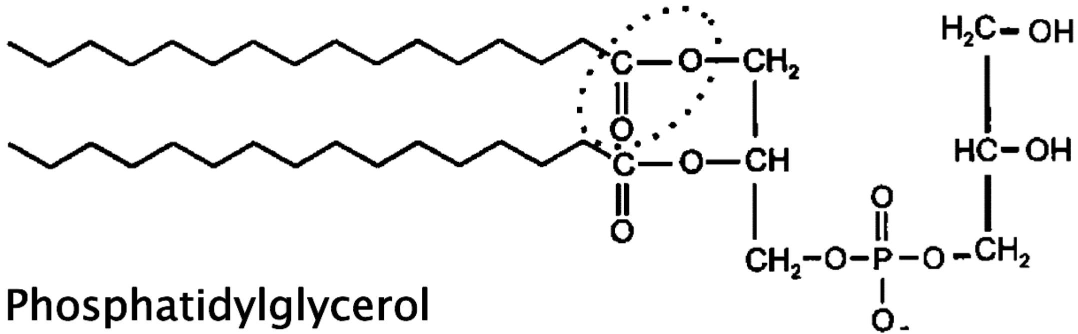
From Forterre P, Brochier C, and Philippe H (2002) Evolution of the Archaea. Theoretical Population Biology 61:409–422.

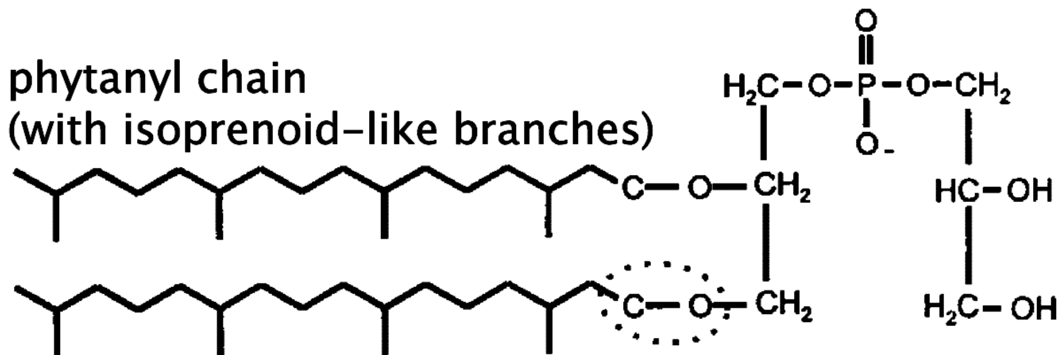
2. BRIEF DESCRIPTION OF THE DOMAIN ARCHAEA

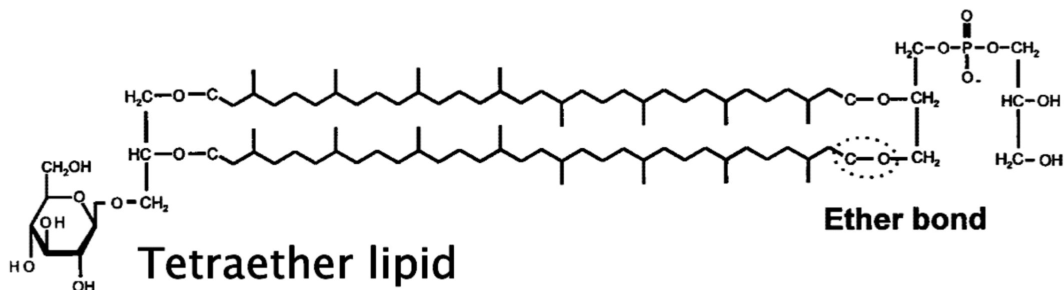
The Archaea are prokaryotes (cell without nucleus) that cannot be easily distinguished from Bacteria by size or shape. However, although most Archaea look like typical Bacteria, some have morphologies that are not found in Bacteria, such as polygonal in halophilic Archaea or very irregular cocci in particular hyperthermophiles (Fig. 1). This could reflect the absence of a rigid cell wall in most Archaea (see below) and/or novel (still unknown) mechanisms for morphogenesis. Archaea exhibit a wide diversity of phenotypes, as is the case for Bacteria. The first three phenotypes to be recognized were the methanogens (strict anaerobes and methane producers), the halophiles (strict aerobes living in high-salt environments) and the thermoacidophiles (aerobes living in hot and acidic environments) (Woese and Fox, 1977). Organisms with such disparate phenotypes were first unified based on the similarities of their rRNA sequences, and later on also by the unique structure of their membrane phospholipids (see below). Many additional phenotypes were discovered among Archaea in the following decades, such as hyperthermophilic or psychrophilic methanogens, halophilic and/or alkaliphilic methanogens, anaerobic, alkaliphilic and neutrophilic hyperthermophiles (Mathrani *et al.*, 1988; Franzmann *et al.*, 1997; Ollivier *et al.*, 1998; Huber *et al.*, 2000). Finally, the explosion of environmental studies based on PCR amplification of 16S rRNA has revealed the widespread occurrence in water and soils of mesophilic Archaea with otherwise unknown phenotypes (Fig. 2).

Nota bene. Psychrophilic (cold temperature), alkaliphilic (alkaline pH), neutrophilic (neutral pH), halophilic (salt tolerant), etc. All extreme environments for a biological organism.

From Konings WN, Albers S-V, Koning S, and Driessen AJM (2002) The cell membrane plays a crucial role in survival of bacteria and archaea in extreme environments. Antonie van Leeuwenhoek 81: 61–72.







The structures of an ester lipid and ether lipids of archaea (and eubacterial thermophiles) are contrasted. Most notable is the ether bond, which is far more resistant to hydrolysis compared to the ester bond (The reason for this is the more –ve dipole of the ester — because of the carbonyl— resulting in easier hydrolysis). The phytanyl, with isoprenoid-like branches, probably creates a more hydrophobic membrane interior with tighter packing.

In many of these extreme environments, a major challenge is to maintain the integrity of the membrane.

For us, a homeotherm adapted to 37°C, a temperature of 80°C would disrupt our cellular integrity (and denature our proteins). Yet Archaea survive - actually grow - at such elevated temperatures.
High salt creates osmo-regulatory challenges
Alkaline & acidic conditions would disrupt normal $\Delta\mu_{H^+}$ required for ATP synthesis. etcetera.
How do Archaea do it?

First, we need to consider the nature of membrane permeability.

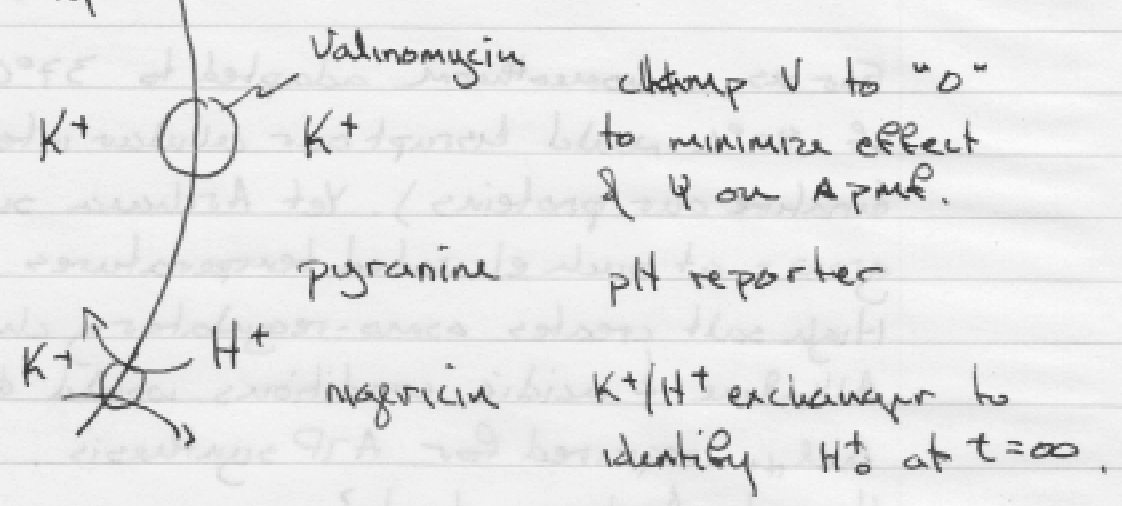
We begin with the measurement of membrane permeability.

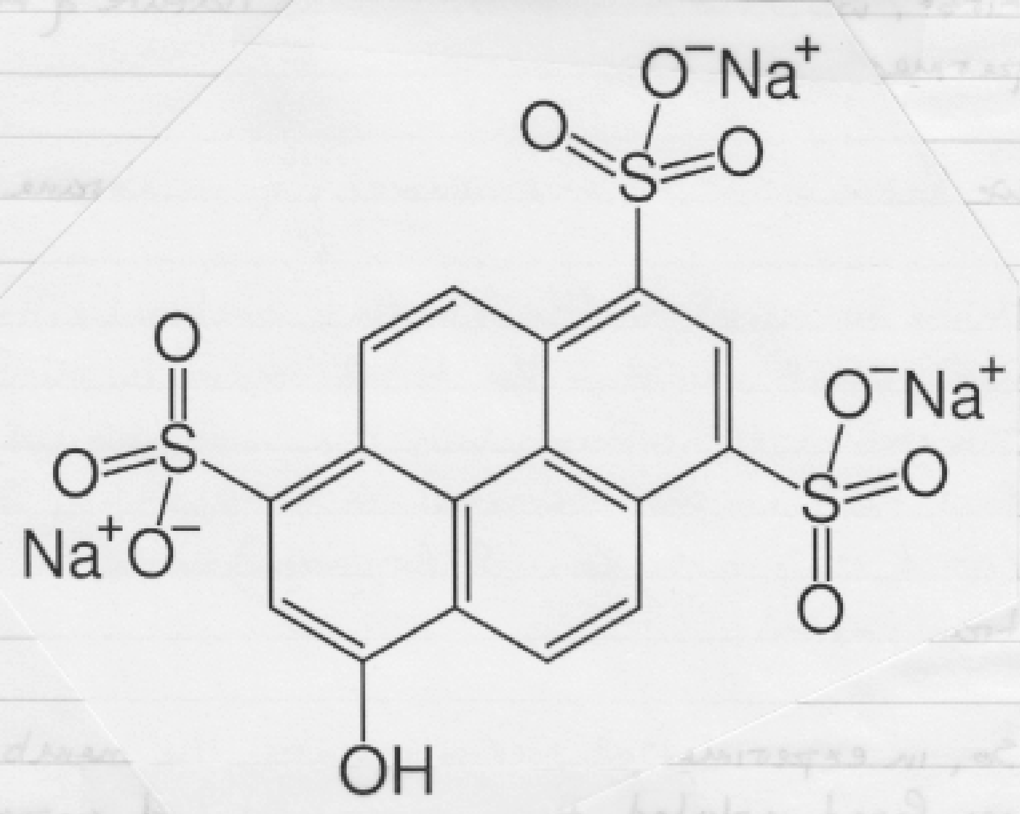
Unlike the situation with Chara, archael cells are very small, so that the total volume is minute.
This creates difficulties which are compounded by the fact that we are interested in permeability of solutes (H^+ & Na^+ in the case of Archaea) which are actively transported.

So, in experimental measurements, the membrane lipids are first isolated, then re-constituted separate from other constituents of the cell.
[overhead from van den Vossenberg et al. 1995]

high buffer capacity

low buffer capacity (plus HCl → pH changes a lot)

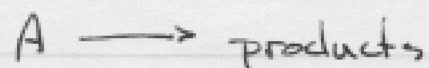




From van de Vossenberg JLCM, Ubbink-Kok T, Elferink MGL, Driessen AJM and Konings WN (1995) Ion permeability of the cytoplasmic membrane limits the maximum growth temperature of bacteria and archaea. Molecular Microbiology 18:925–932 .

Lipids were isolated from *Sulfolobus acidocaldarius* grown at 80ºC (whose membrane is comprised predominantly of membrane-spanning tetraether lipids). The lipids were formed into liposomes and their H^+ and Na^+ permeabilities measured, as indicated by the diagram above. A pH jump was used, followed by tracking the rate ΔpH dissipation (a first order kinetic reaction). Or, the liposomes were pre-loaded with $^{22}Na^+$, and efflux of $^{22}Na^+$ monitored by measuring loss of the radioisotope when the liposomes were placed in a solution in which $^{22}Na^+$ was replaced with $^{23}Na^+$ (the exchange would be a first order kinetic reaction).

First order reaction:



If c is the concentration of A, $-\frac{dc}{dt} = k_1 c$
(1st order)

Re-arranging, $\frac{1}{c}\,dc = -k_1\,dt$

Integrating

$$\ln c = -kt + C$$

where C is the constant of integration

If c is initially a (at time "0") the $\ln a = C$

and the equation becomes:

$$\ln c = -kt + \ln a$$

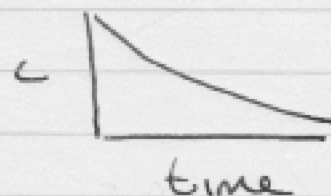
or $(\ln c - \ln a) = -kt$

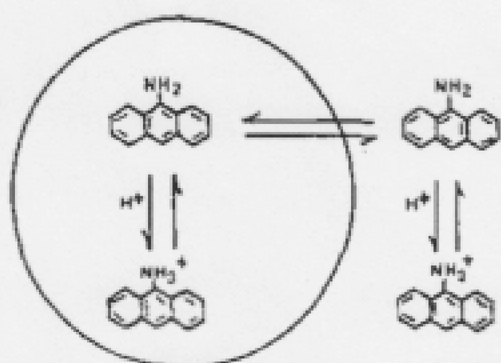
or $\ln \frac{c}{a} = -kt$

exponentiating, $\frac{c}{a} = e^{-kt}$

or $c(t) = a e^{-kt}$

an exponential decay

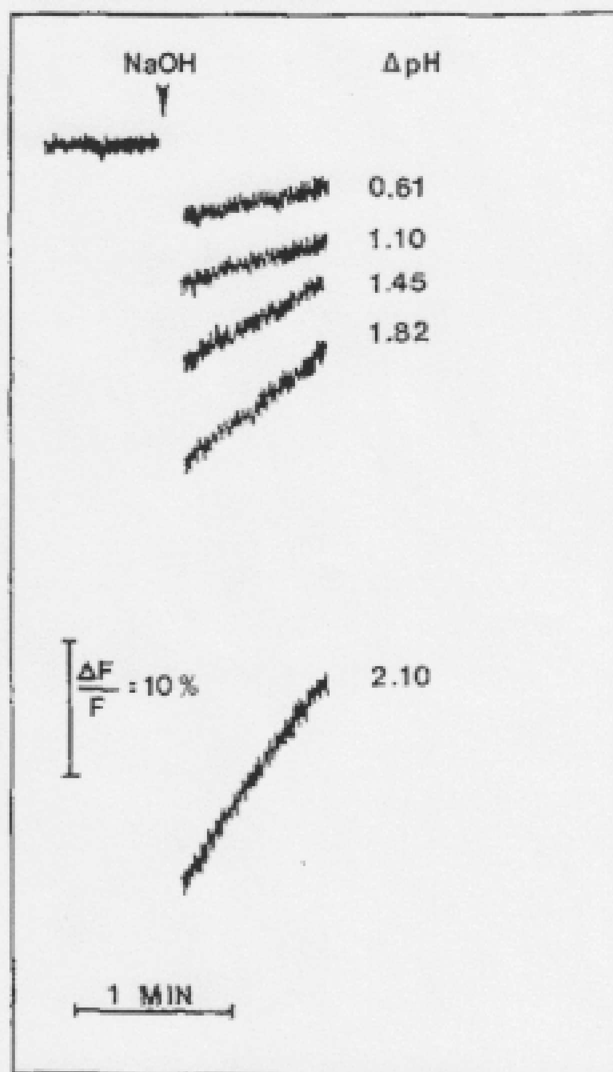




$$\Delta pH = \log \frac{[AH]_i}{[AH]_o} \qquad (1)$$

$$\Delta pH = \log \frac{\%Q}{100 - \%Q} + \log \frac{V_o}{V_i} \qquad (2)$$

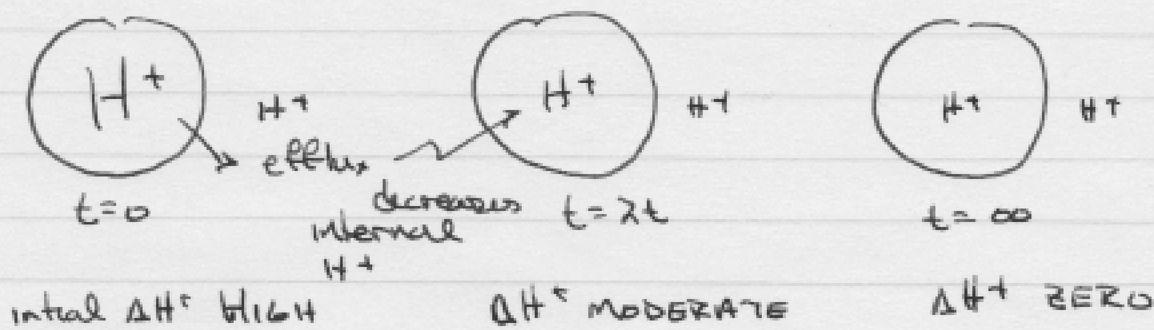
$$\log \frac{\%Q}{100 - \%Q} = \Delta pH + \log \frac{V_i}{V_o} \qquad (3)$$



9-aminoacridine as a pH gradient probe. The fluorochrome freely passes through the membrane in its unprotonated state if the vesicular pH is acid, it will accumulate in its protonated state. The elevated concentration 'quenches' fluorescence due to self-absorption.

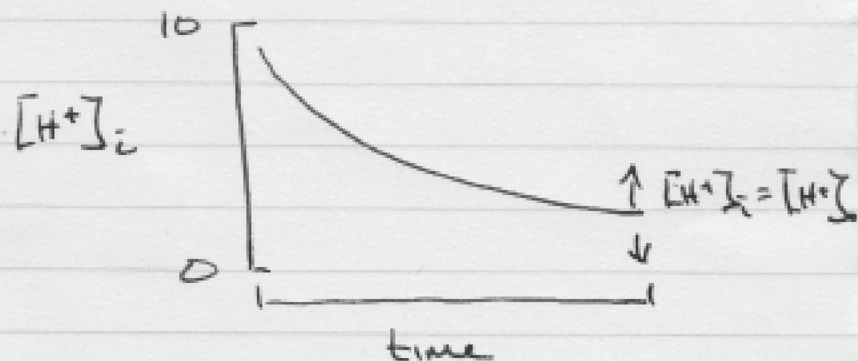
Bennett & Spanswick 1983 J. Membr. Biol. 71:95-107.

In the case of ion gradients across a vesicle:



The initial gradient dissipates over time. The dissipation is exponential (thus, first order kinetics)

Note that external volume is much greater than internal volume, so $[H^+]_o$ is unchanging.



↳ This leads to a re-statement of the flux equ: $J_{net} = P_{H+}([H^+]_o - [H^+]_i)$

$$\text{to } J_{net} = \frac{V}{A}\frac{dH^+_i}{dt} = P_{H+}(H^+_o - H^+_i)$$

For the appropriate boundary conditions

$$H^+_i \propto \underbrace{\exp\left[-\frac{A}{V}P_H t\right]}_{\text{an exponential change}}$$

From Lew RR and Spanswick RM (1985) Characterization of anion effects on the nitrate-sensitive ATP-dependent proton pumping activity of soybean (*Glycine max* L.) seedling root microsomes. Plant Physiology 77:352–357.

The assumption that the equilibration of the pH gradient is described by an exponential function is based on the following:

We assume that $\Delta\Psi$ is constant (KCl is present); thus, we can use the equation for the flux of a neutral solute:

$$J_{net} = P_H\,(H_o^+ - H_i^+) \tag{1a}$$

where H_i^+ and H_o^+ are the internal and external concentrations, respectively, and P_H is the permeability of the membranes to H^+. J_{net} is also described by:

$$J_{net} = \frac{V}{A}\frac{dH_i^+}{dt} = P_H\,(H_o^+ - H_i^+) \tag{2a}$$

Since the external volume is very large relative to the internal volume of the vesicles and is buffered, we can assume that H_o^+ will remain constant. If $H_i^+ = [H_i^+]_o$ at $t = 0$ and H_i^+ at $t = t$, we can integrate to give the solution:

$$H_i^+ = H_o^+ + [\Delta H^+]_0 \exp\,[-\frac{A}{V} P_H\, t] \tag{3a}$$

where $[\Delta H^+]_0 = [H_i^+]_0 - H_o^+$.

The halftime is given by:

$$t_{1/2} = 0.693\,\frac{V}{A}\frac{1}{P_H} \tag{4a}$$

If we examine the effect of volume change on the $t_{1/2}$, we find that it is proportional to the inverse of the cube root of the osmoticum concentration, assuming the vesicles act as perfect osmometers. Since $V \propto 1/[\text{osmotic concentration}]$, and the term V/A in Eq. 4a is equal to $r/3$ for spherical vesicles, then $4/3\ r^3 \propto 1/[\text{osmotic concentration}]$ so that

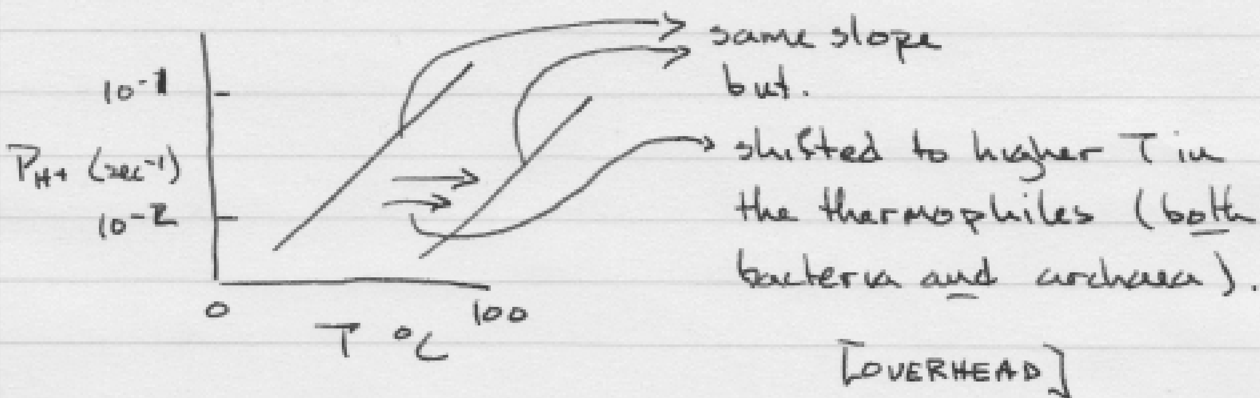
$$t_{1/2} \propto 1/[\text{osmotic concentration}]^{1/3} \tag{5a}$$

Equation 5a suggests that increasing osmoticum concentration will cause a decrease in the $t_{1/2}$ if P_H is constant. In fact it increases (Table I), implying that P_H itself is decreasing.

We are not measuring H^+ directly, but the $\%Q$. For this reason, it is the plot of $\%Q/(100 - \%Q)$ *versus* time which should be an exponential function. We tested this for some of the data shown in Figure 2. The r^2 values for an exponential fit were greater than 0.9, and greater than r^2 values for plots of $\%Q$ *versus* time.

So, with techniques available to measure the H^+ & Na^+ permeabilities of the membrane lipids, what is the temperature-dependence of permeability? And, how does it relate to the normal growth temperature of the organism?

It turns out that all org. lipids exhibit a similar T-dependence



[OVERHEAD]

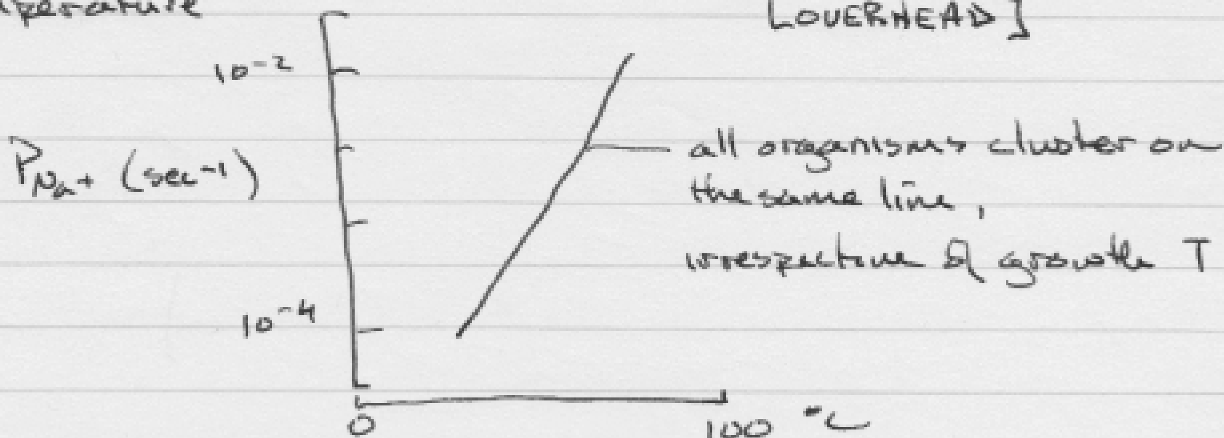
In fact, there appears to be an optimal P_{H^+} (ca 2×10^{-1} sec⁻¹) for all organisms, irrespective of growth temperature.

The thermophiles do adapt their lipid composition at elevated temperature. In Sulfobolus solfataricus, this is done by increasing the cyclic structures in the 'hydrophobic core' of the tetra-ether lipids.

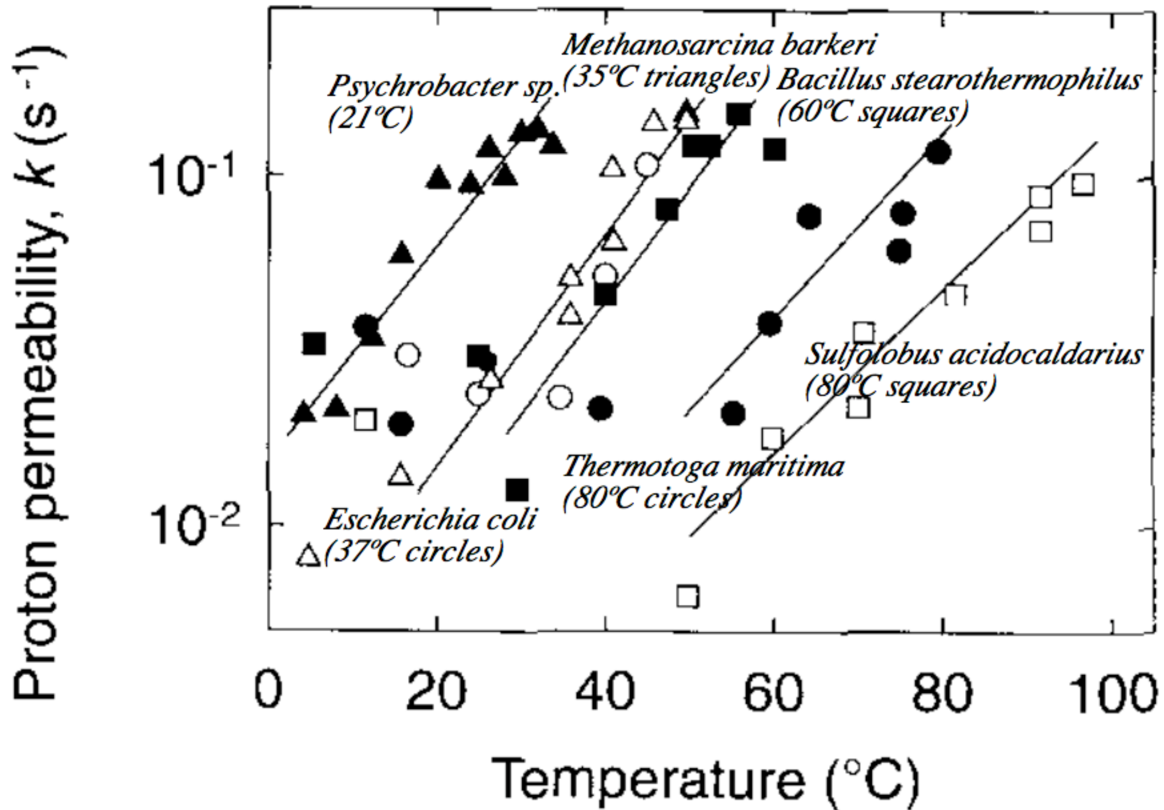
[OVERHEAD]

Notably, Na^+ permeability does not shift with growth temperature

[OVERHEAD]



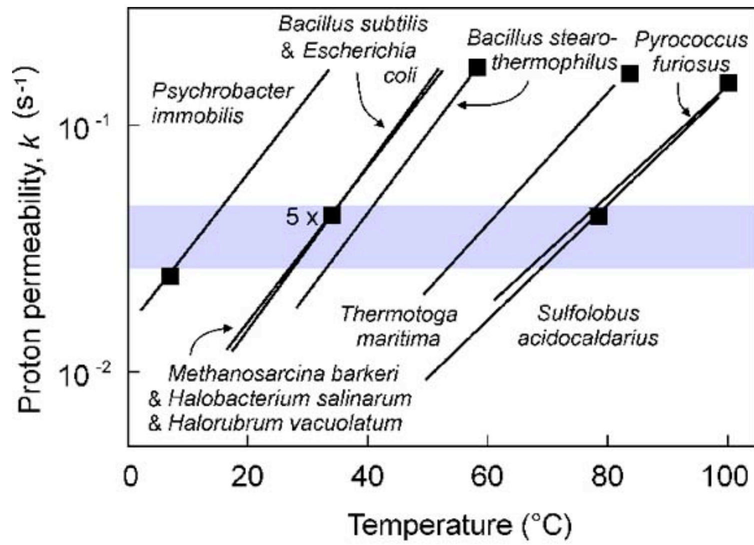
From van de Vossenberg JLCM, Ubbink-Kok T, Elferink MGL, Driessen AJM and Konings WN (1995) Ion permeability of the cytoplasmic membrane limits the maximum growth temperature of bacteria and archaea. Molecular Microbiology 18:925–932.



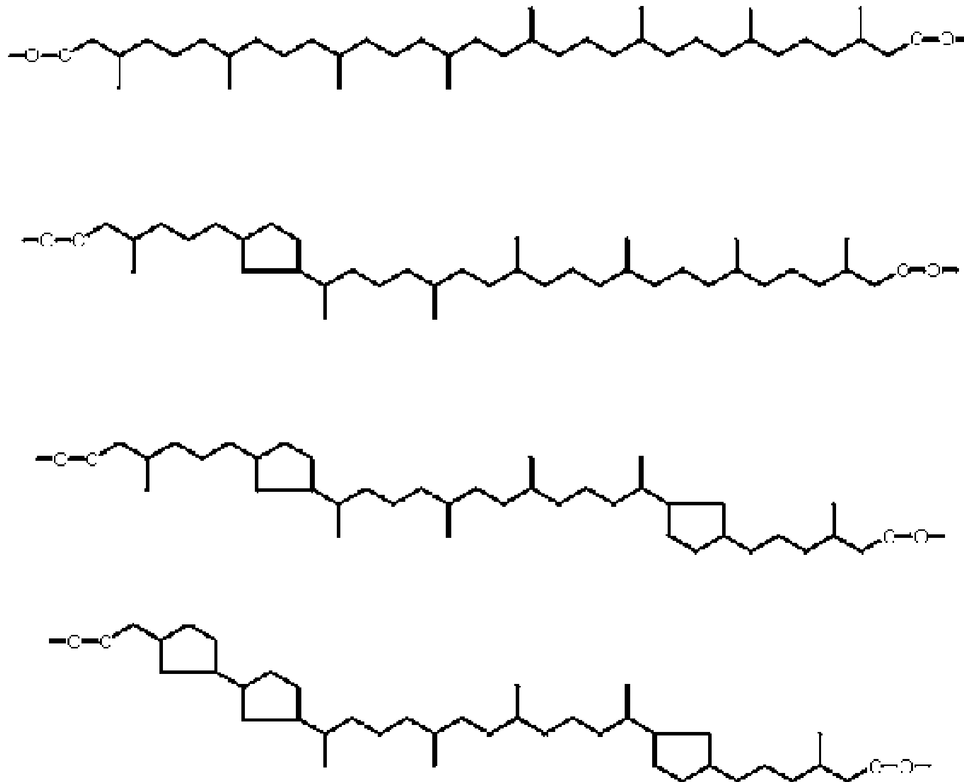
The proton permeabilities of lipids isolated from a diverse range of prokaryotes and Archaea are shown. Note that the slopes of proton permeabilities *versus* temperature are very similar, increasing with elevated temperature. But, the all the thermophiles are shifted to the right: They exhibit lower permeabilities at any given temperature.

	Temperature (°C)		
Species	Growth	Maximum	Acyl chain composition
Bacteria			
Psychrobacter sp	21	29	93% mono-unsaturated; shorter chains
Escherichia coli	37	42	32% mono-unsaturated; shorter chains
Bacillus stearothermophilus	60	70	Saturated, 80% branched acyl chains
Thermotoga maritima	80	90	Saturated, 7% membrane-spanning lipid esters
Archaea			
Methanosarcina barkeri	35	42	Diether lipids, isoprenoid and hydroxy-acyl chains
Sulfolobus acidocaldarius	80	83	99% membrane-spanning tetraether lipids; cyclopentane rings

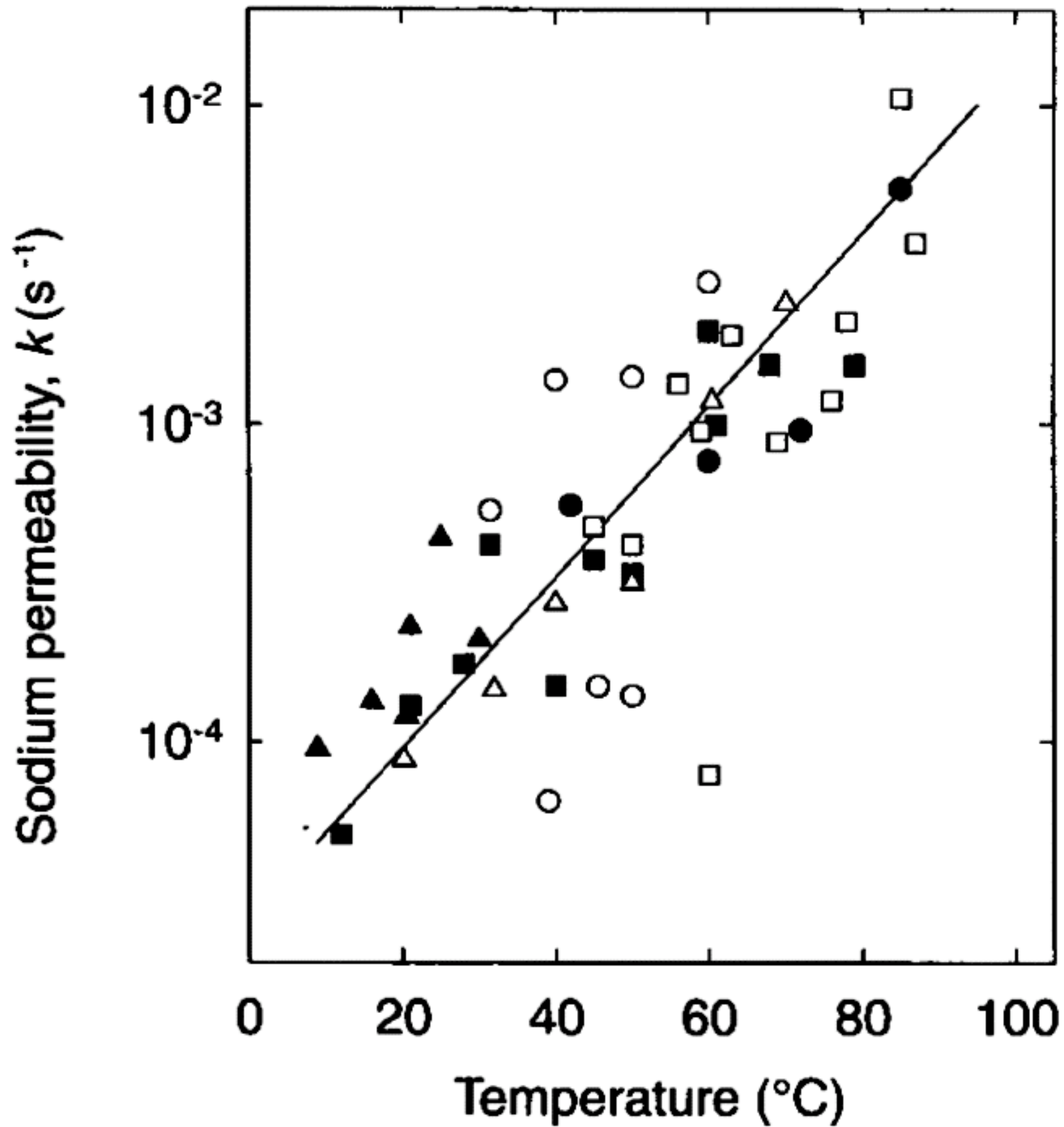
From Albers S-V, van de Vossenberg JLCM, Driessen AJM and Konings WN (2005) Adaptations of the archaeal cell membrane to heat stress. Frontiers in Bioscience 5:d796–803.



The permeability of the membrane appears to be optimized to the growth temperature. That is, at the growth temperature, the permeability to protons is about $2 \bullet 10^{-2}$ sec^{-1} for any of the bacteria examined. With increased temperature, one adaptation is to increase the extent of cyclization of the 'hydrophobic core' of the phytanyl chains of the tetraether lipids (in *Sulfolobus solfataricus*) (from top to bottom in the figure below).

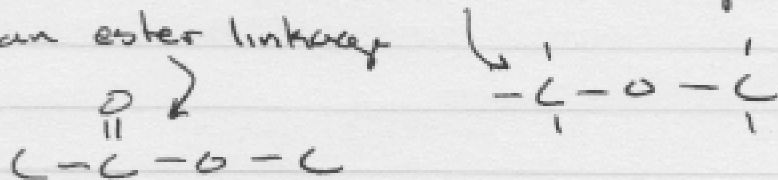


From van de Vossenberg JLCM, Ubbink-Kok T, Elferink MGL, Driessen AJM and Konings WN (1995) Ion permeability of the cytoplasmic membrane limits the maximum growth temperature of bacteria and archaea. Molecular Microbiology 18:925–932.

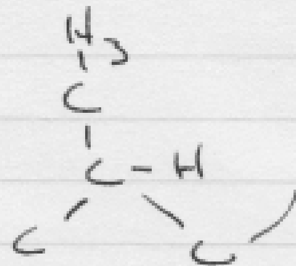


The sodium permeabilities of lipids isolated from the same diverse range of prokaryotes and Archaea are all similar (unlike proton permeabilities). **Bacteria** (*Psychrobacter* sp., *Escherichia coli, Bacillus stearothermophilus, Thermotoga maritima*); **Archaea** (*Methanosarcina barkeri* and *Sulfolobus acidocaldarius*).

So, that's the lipid connection.
In a nutshell, Archaea (and eubacteria of extreme environments) tend to use an ether linkage rather than an ester linkage



which is less sensitive to hydrolysis.



And phytanyl hydrophobic cores with isoprenoid branching.

And, finally – "double-headed" lipids to create a monolayer

rather than a bilayer

All of these factors (plus cyclopentane rings in the hydrophobic core) will "protect" the membrane in extreme environments, as demonstrated directly by their effect on H^+ permeability (leakage).

Now, onto transport processes in the Archaea.

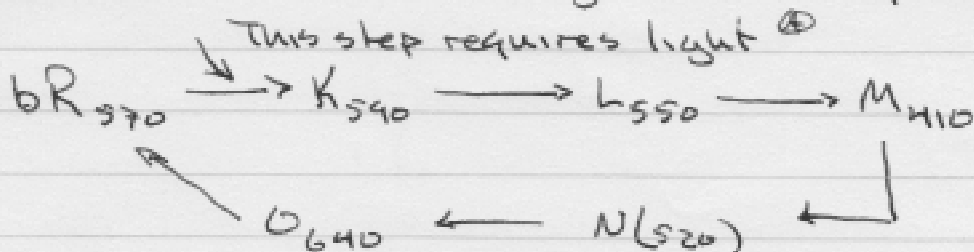
Amongst the Archaea, there is one (or two) extraordinarily well-known transporter(s). These are found in Halobacteria halobium, now
(salt) (salt)

described as Halobacteria salinarum
(from the latin for 'salt-works')

It is red to purple in color, and grows at high salinity. It is found in man-made evaporative ponds used to extract salt from ocean waters.

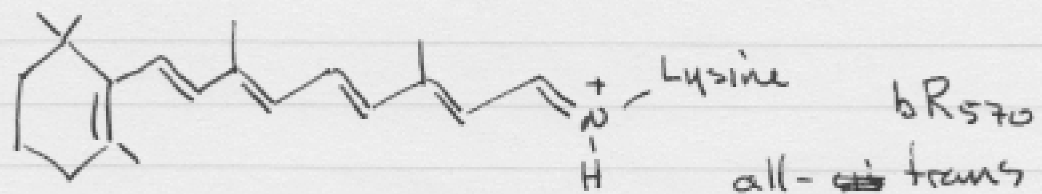
The color is due to a rhodopsin-protein/pigment - quite similar to the retinal used in human vision. The purple coloration forms patches on the bacteria (known as purple membranes). These could be isolated relatively easily.

The rhodopsin is a 25 kDa protein that spans the membrane via seven α-helical segments. The photopigment undergoes a well-defined cycle of intermediates - all defined by their absorption maxima.



*light absorp

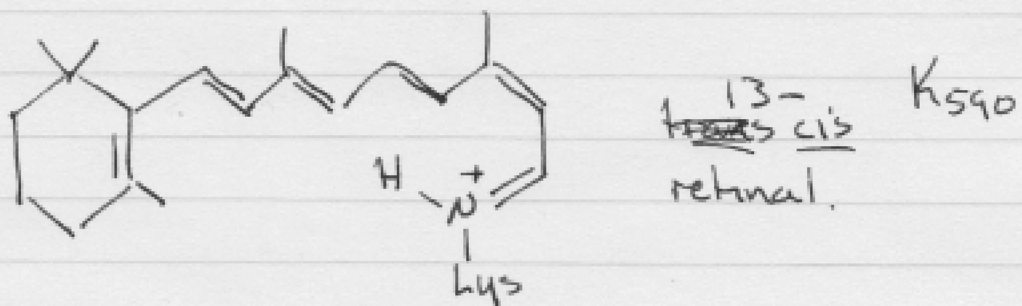
During this reaction photocycle, 1 H^+ is transported from the inside of the bacterium to the outside. It occurred in concert with a "Schiff-base" transition



bR_{570}

all-~~cis~~ trans

↓ $h\nu$ (light.

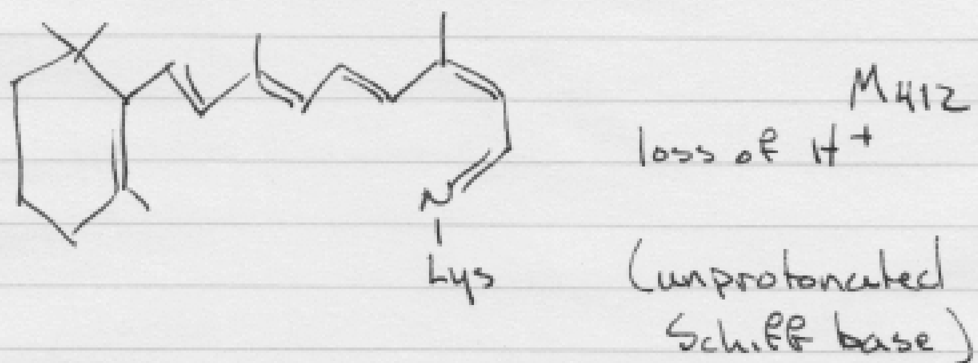


K_{590}

13-~~trans~~ cis

retinal.

↓ → H^+



M_{412}

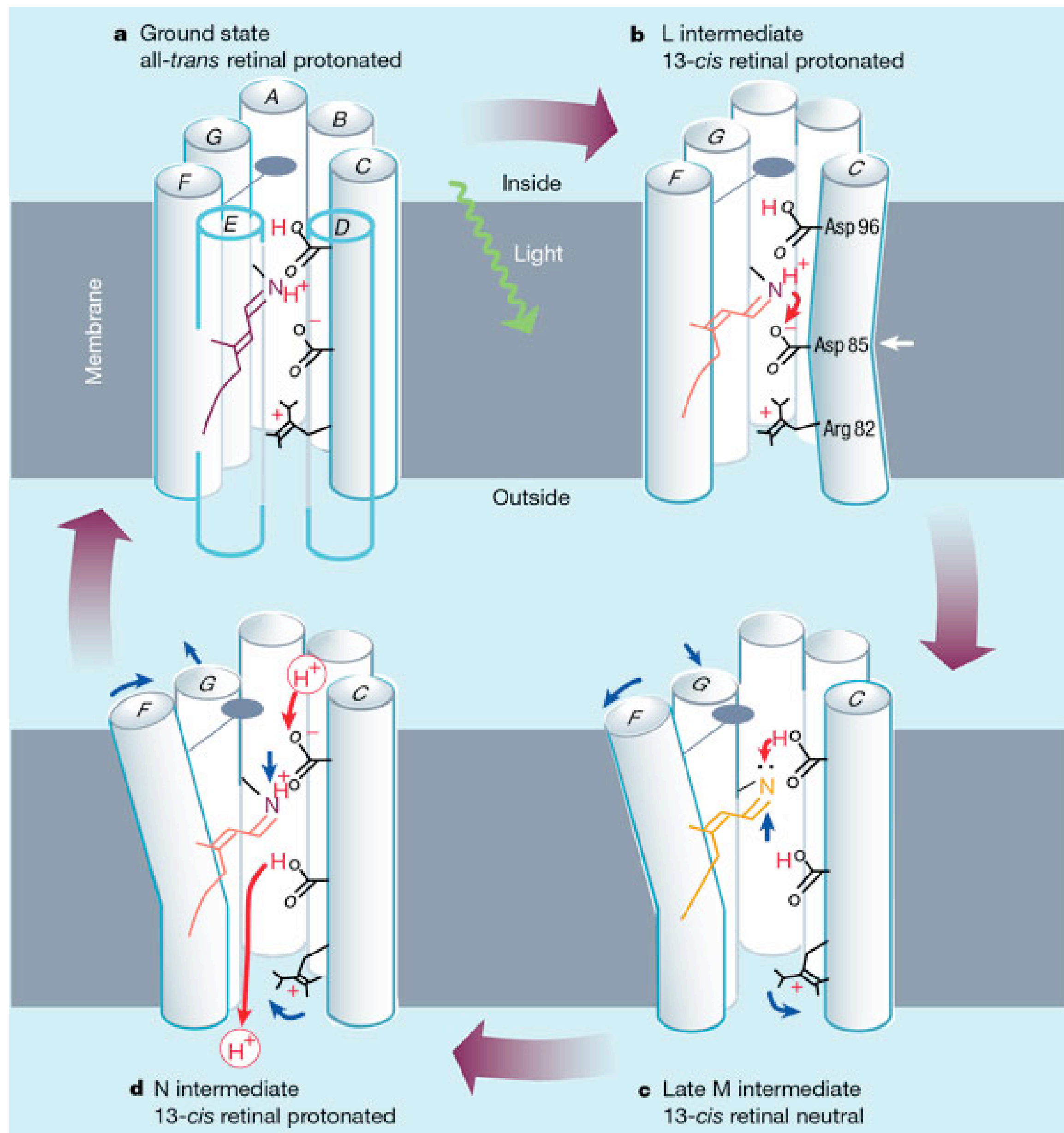
loss of H^+

(unprotonated Schiff base)

↓ (re-protonation & re-cycle back to bR_{570}).

Source: Stoeckenius W 1999 Bacterial rhodopsins: Evolution of a mechanistic model for the ion pumps. Protein Science 8: 447-459.

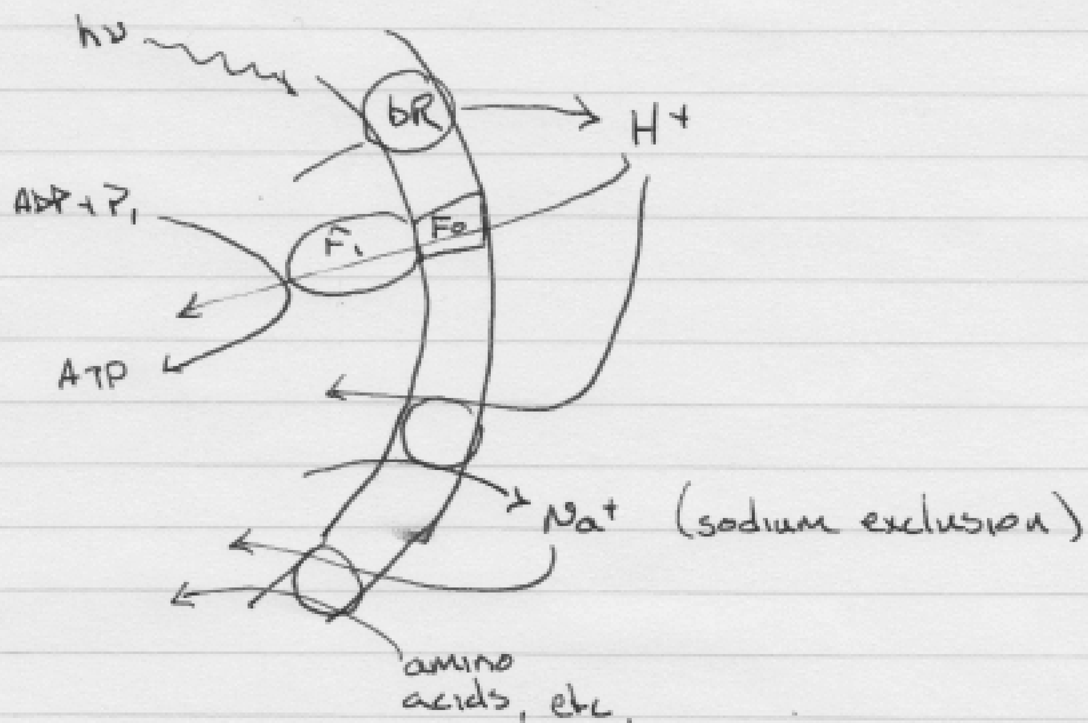
From Kuhlbrandt W (2000) Bacteriorhodopsin — the movie. Nature 406:569–570.



Light-induced isomerization of the protonated retinal from all- trans (purple—**a**) to 13-cis (pink—**b**) triggers the transfer of the proton to aspartate 85, aided by a slight movement of this residue in the L intermediate (**b**) towards the nitrogen atom. The deprotonated retinal (yellow—**c**) straightens, pushing against helix F and causing it to tilt. This opens a channel on the inner, cytoplasmic side of the membrane through which aspartate 96 is reprotonated (**d**), having given up its proton to the nitrogen on the retinal. Aspartate 85 transfers its proton through a network of hydrogen bonds and water molecules to the outside medium, past arginine 82, which has moved slightly.

There is a lot of history associated with Bacteriorhodopsin. In the heyday of bioenergetics, it was reconstituted with the F_1/F_0 ATP synthetase to demonstrate that the F_1/F_0 ATP synthetase uses the proton motive force (a vectorial reaction) to synthesize ATP (a chemical reaction).

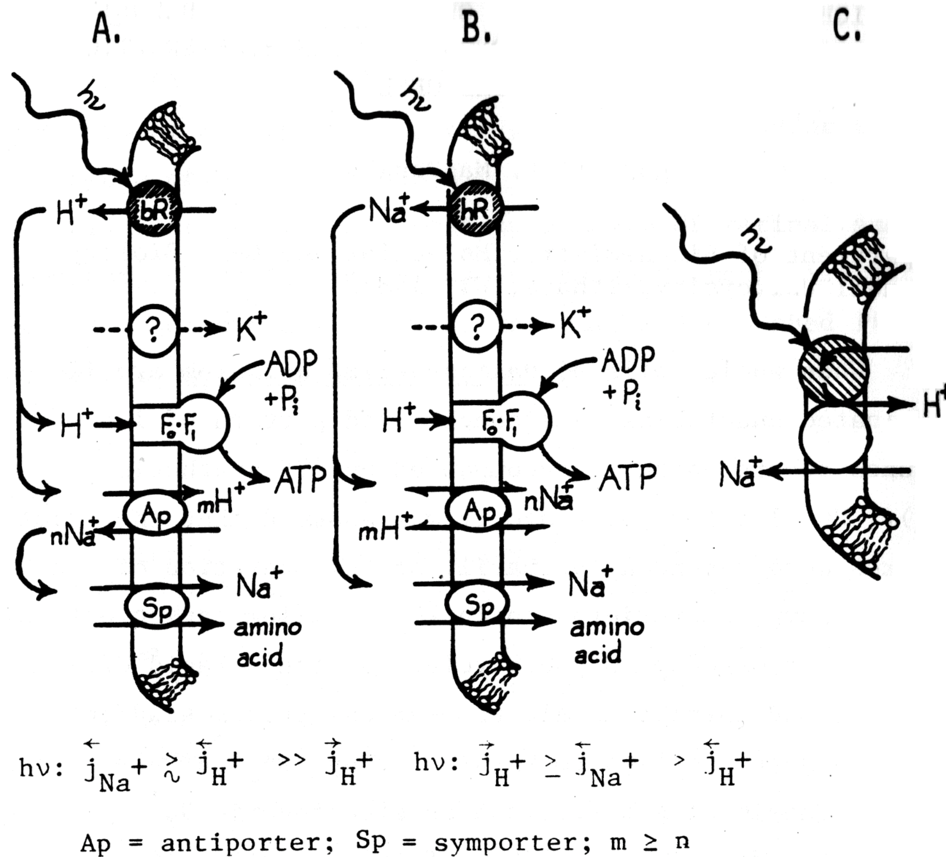
In the Halobacterium, the general transport scheme:



Historically, researchers identified two types of vesicles from the bacterium. In one type, light caused acidification of the extracellular medium. In the other type, alkalinization was observed. Initially, it was proposed to be a Na^+ influx pump [OVERHEAD], but soon it was identified as a Cl^--influx pump.⊕

⊕ discussed by Stoeckenius, W (1999) Bacterial rhodopsins: Evolution of a mechanistic model for the ion pumps. Protein Science 8: 447-459.

From MacDonald RE (1981) The light-driven sodium pump of *Halobacterium halobium*: Its discovery and speculations about its bioenergetic role in the cell. *In* Skulachev VP and Hinkle PC (ed.) Chemisomotic Proton Circuits in Biological Membranes. In honor of Peter Mitchell. Addison-Wesley. pp. 321–335.



The above diagram is an historic snapshot of proposed transport processes in the halophilic purple membrane bacteria. In **A**, the 'classic' transport model is shown: Generation of a proton motive force by bacterioRhodopsin (bR) is used to synthesize ATP and 'drive' the transport of other solutes either into or out of the cell. In **B**, a model is shown to explain some odd results (that were initially dismissed as artifacts of the biochemical techniques) that suggested light-driven Na^+ transport (haloRhodopsin [hR]). ***Nota bene*** **The concept of a Na^+ efflux pump was soon discounted in the scientific community: Instead, haloRhodopsin is a Cl^- influx pump.**

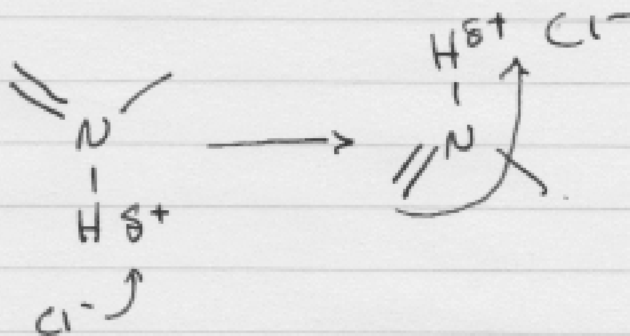
The sequence & structural homology of bR and hR are very high. Yet, the nature of the transported molecule is very different: a H^+ & a Cl^-?

It is known that bR will transport Cl^- at an acid pH and that hR transports H^+ in the presence of azide (N_3^-)

The x-ray crystallographic solution of halorhodopsin at 1.8 Å resolution shed light on the mechanism, since a Cl^- could be resolved next to the Schiff base nitrogen. Thus, Cl^- transport must involve the isomerization reactions at the retinal.

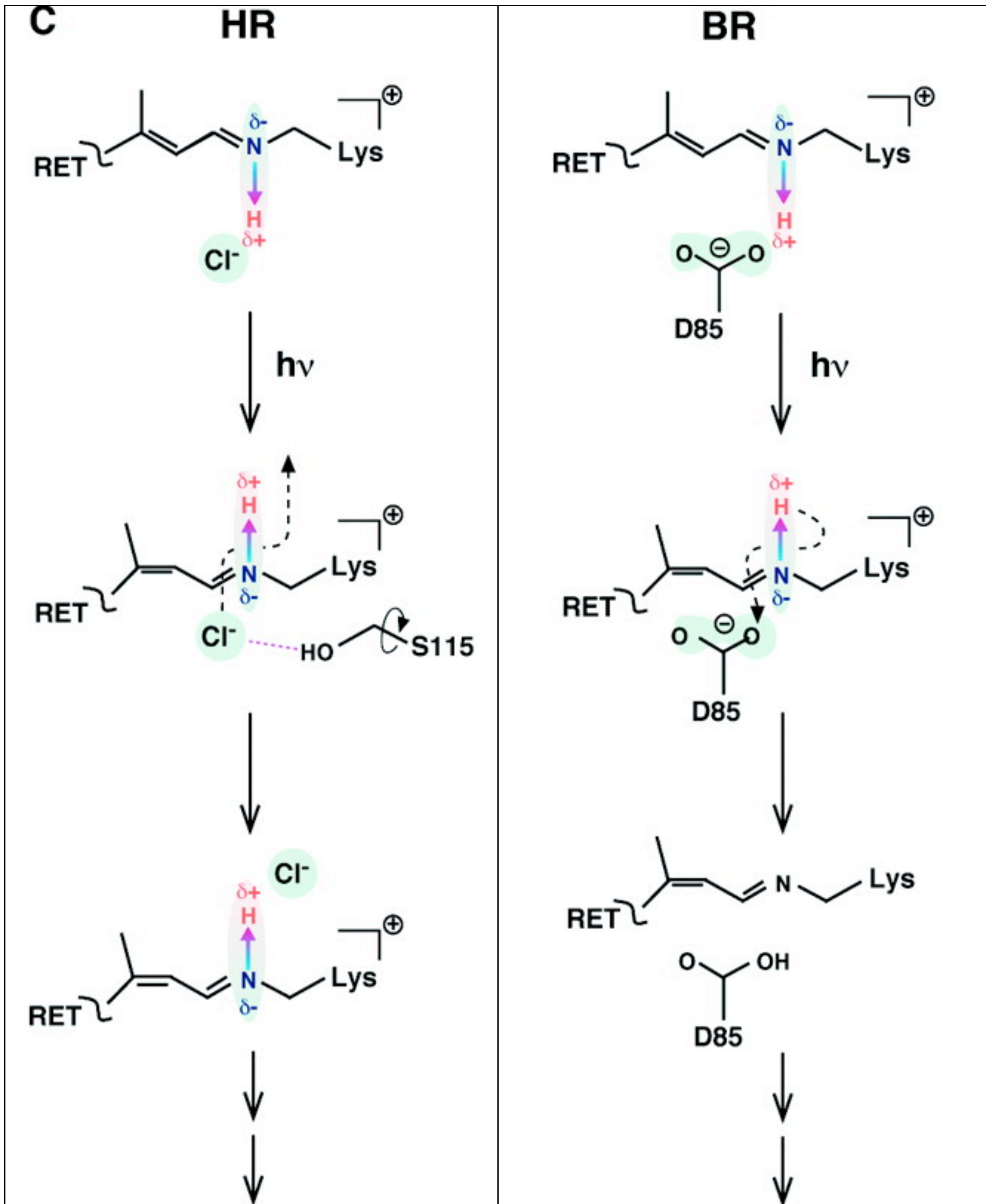
[OVERHEAD]

The proposed mechanism is "ion-dragging". Wherein the re-location of the +ve charged hydrogen on the amide



would "drag" the Cl^- anion from one side to the other - effecting Cl^- transport across the membrane.

From Kolbe M, Besir H, Essen L-O, Oesterhelt D (2000) Structure of the light-driven chloride pump halorhodopsin at 1.8 Å resolution. Science 288:1390–1396.



The transport mechanisms for Cl^- (**HR**) are contrasted to that of H^+ (**BR**). The authors describe the proposed mechanism as 'ion-dragging' due to ion-dipole interactions caused by the shift in the location of the +ve charge on the lysine.

There is a fascinating sequel to this story.
Now that we understand these light-driven pumps,
can they be harnessed for a biotech application?
Apparently, yes.

Chow et al. created mammalian codon-usage
optimized constructs and inserted into expression vectors

Expression was confirmed with GFP-fusion proteins

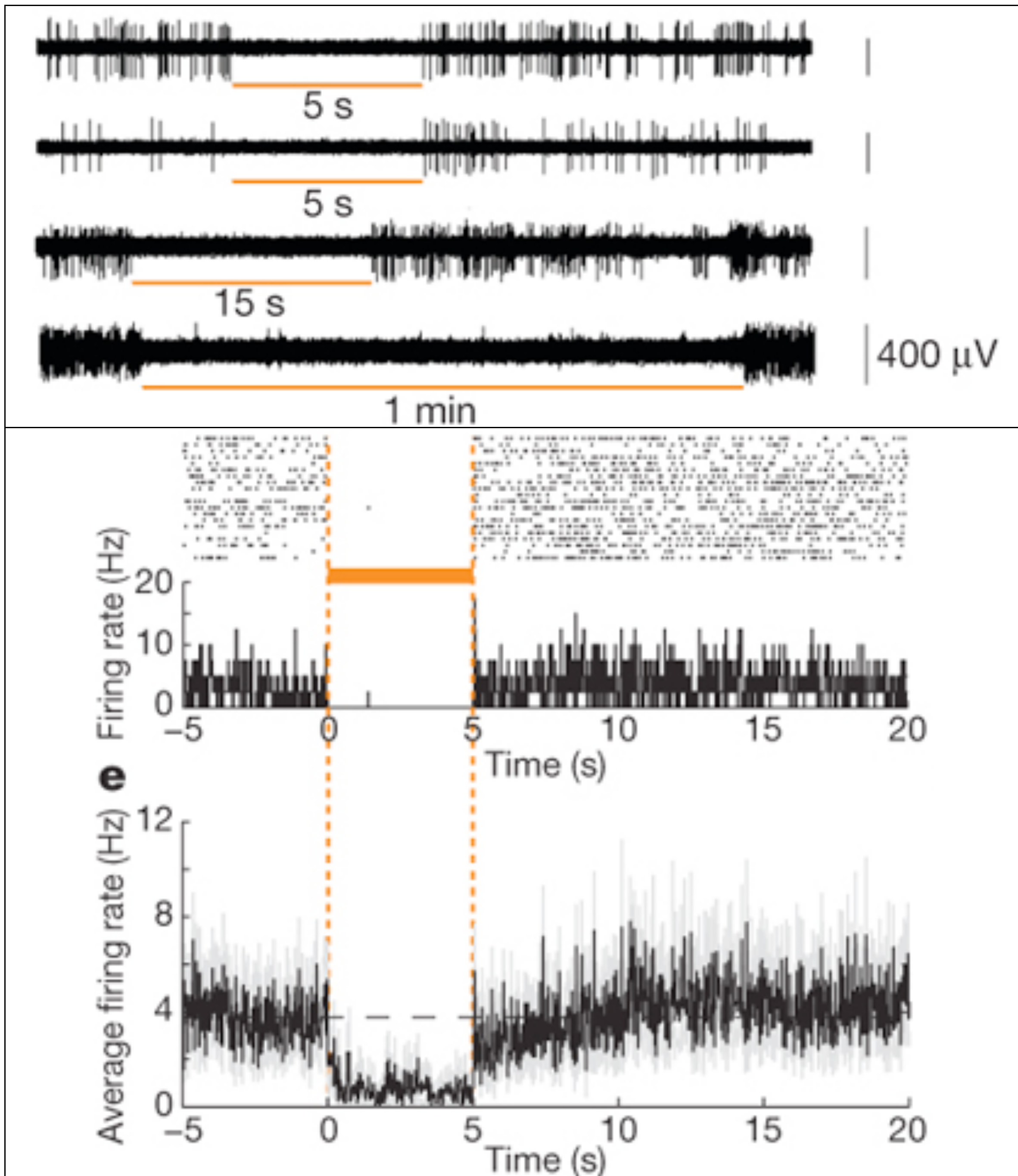
When irradiated with 593 nm light, photocurrents
were observed and caused AP-silencing.
[OVERHEAD]

Thus light-mediated control of neuronal activity
is possible.

It should be noted that other light-gated channels
have also been used, to activate neurons. Now,
it is possible to silence them.

The possibilities are more than a little spooky...

From Chow BY, Han X, Dobry AS, Qian X, Chuong AS, Li M, Henninger MA, Belfort GM, Lin Y, Monahan PE, Boyden ES (2010) High-performance genetically targetable optical neural silencing by light-driven proton pumps. Nature 463:98–102.



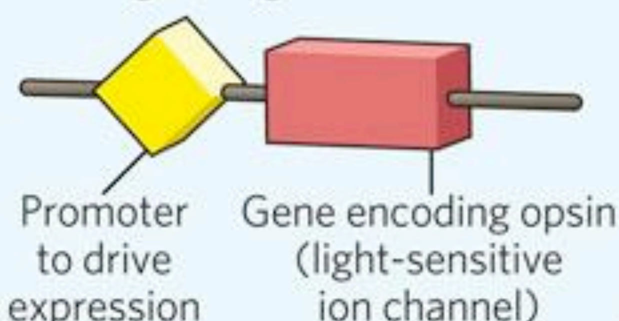
The above show extracellular electrical recordings from mouse cortex expressing ‘Arch’ (a proton pumping bacteriorhodopsin). At the horizontal bars, light is shone on the cortex, causing pumping. This should hyperpolarize the membrane potential, causing silencing of neuronal activity in the illuminated cells (which indeed does occur).

SIX STEPS TO OPTOGENETICS

With optogenetic techniques, researchers can modulate the activity of targeted neurons using light.

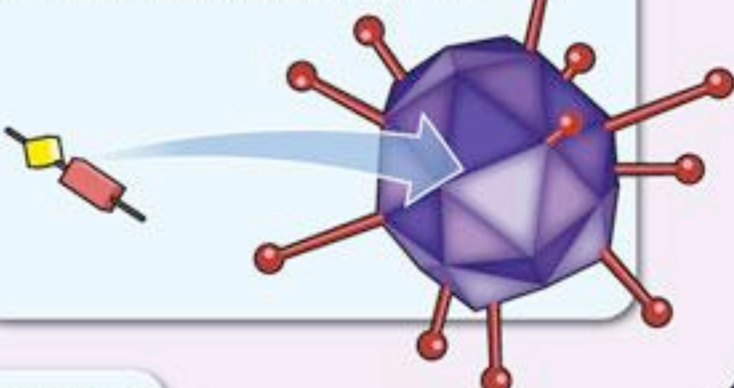
STEP 1

Piece together genetic construct.



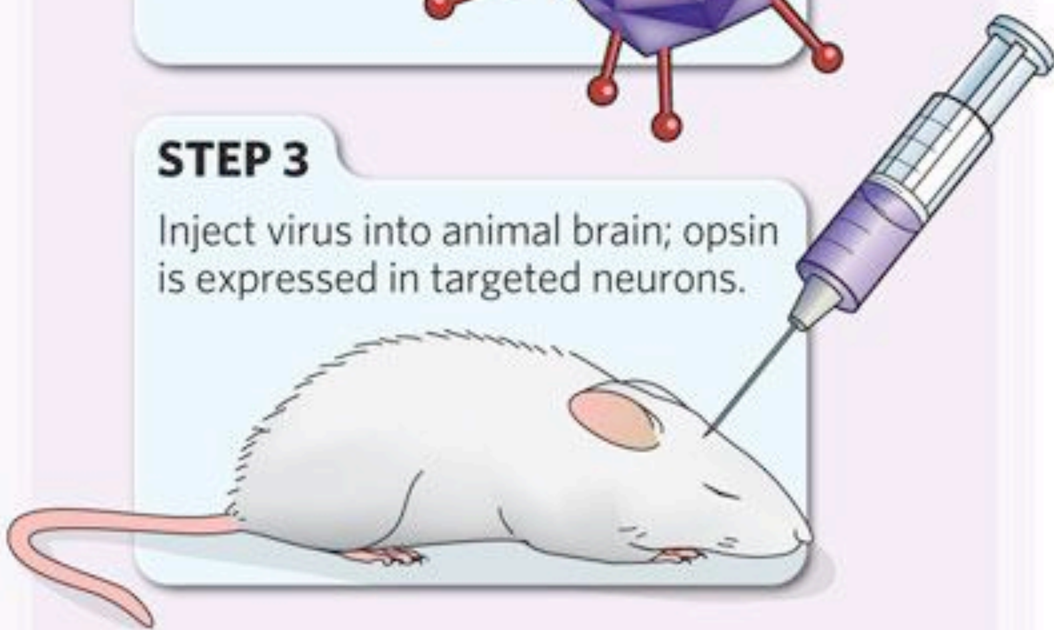
STEP 2

Insert construct into virus.



STEP 3

Inject virus into animal brain; opsin is expressed in targeted neurons.

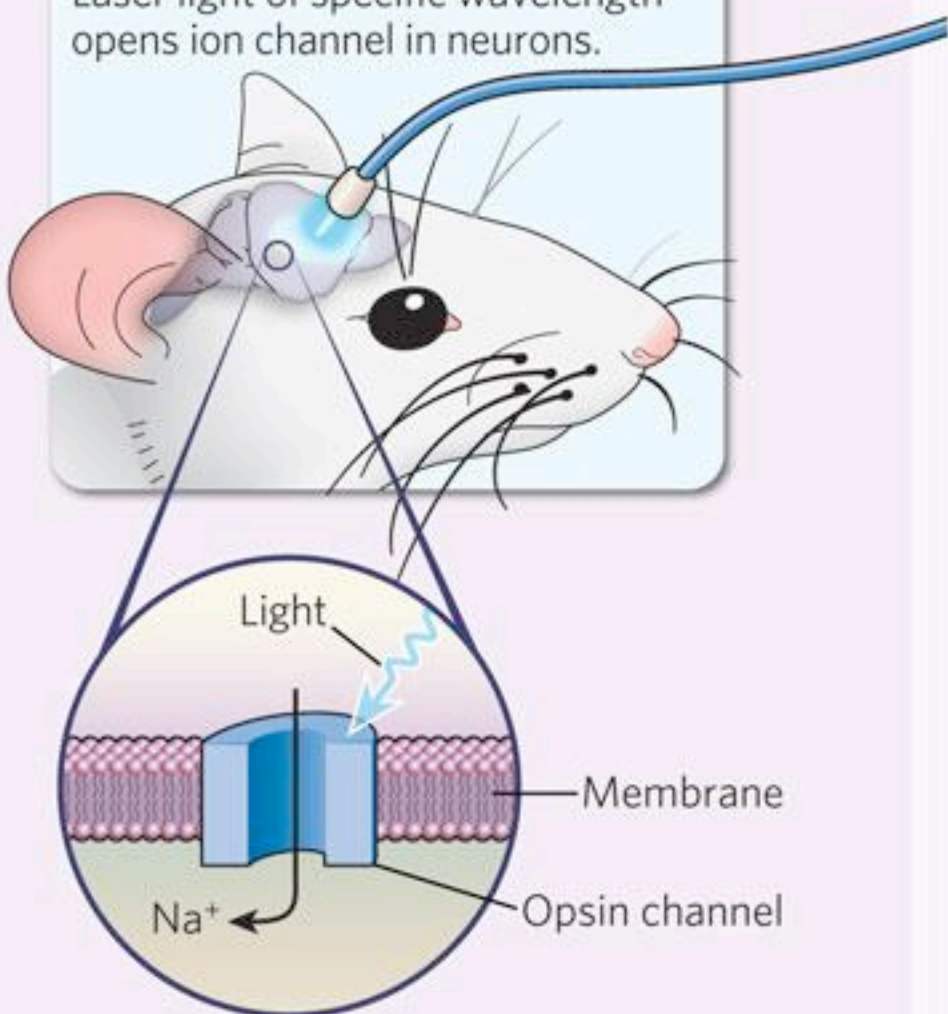


STEP 4

Insert 'optrode', fibre-optic cable plus electrode.

STEP 5

Laser light of specific wavelength opens ion channel in neurons.



STEP 6

Record electrophysiological and behavioural results.