

Today's class:

Diffusive transport in the cell - part 2

This lecture follows the Chapter 13 in the book ‘Physical Biology of the Cell’ by Philips et al. and chapter 17 in the book ‘The Molecules of Life’ by Kuriyan et al.

Diffusion constant depends on molecular properties like size and shape

When a particle moves through a fluid it feels a resistance - a frictional force

If the particle moves with a velocity v , the frictional force is proportional to the velocity

$$F_{friction} \propto -v$$

$$\implies F_{friction} = -fv$$

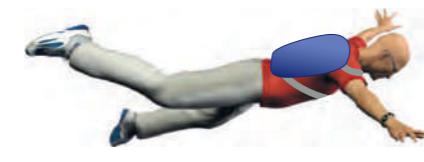
Friction factor

The friction factor is a parameter that relates the size and shape of a molecule to the drag (the resistance to movement) it generates in a fluid.



faster

~ 200 mph



fast

~ 100 mph



~ 10 mph

slow

Here f is called the friction factor

When an object moves under an externally applied force $F_{friction}$ tries to balance it.

The velocity is decided by the balance of forces. When the force is gravity it is called terminal velocity.

This balance strongly depends on the shape of the moving object.

Diffusion constant depends on molecular properties like size and shape...*contd*

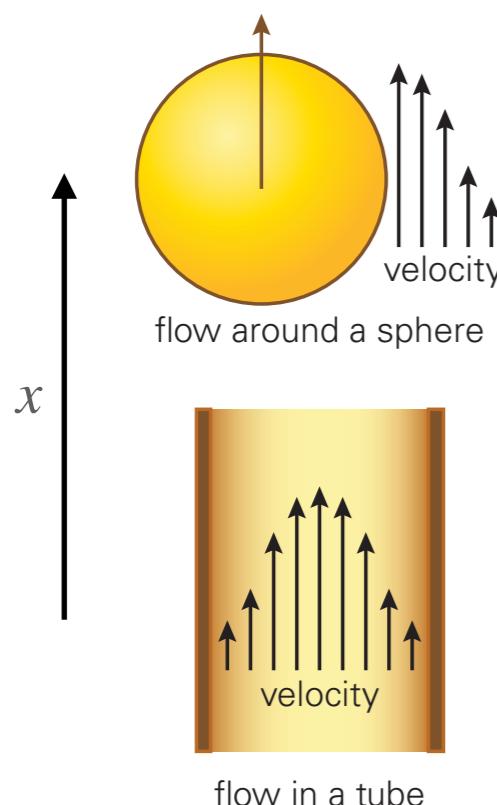
The friction factor is related to the diffusion constant through the Einstein relation

$$D = \frac{k_B T}{f}$$

Where does size and shape enter here?

They enter via viscosity of the fluid.

Gradients of momentum



When an object is moving in a fluid or the fluid is moving against a wall momentum gradients are created away from the object/wall

The momentum flux is proportional to the velocity gradient

$$j_p = -\eta \frac{dv}{dz}$$

Here velocity gradient is created along z or y

η is the viscosity of the fluid

Diffusion constant depends on molecular properties like size and shape...*contd*

$$j_p = -\eta \frac{dv}{dz}$$

For a sticky solvent velocity gradient is shallow to η is high

For a thin solvent velocity gradient is sharp to η is low

η is the viscosity of the fluid

Viscosity also defines the rate of flow of a fluid through a tube

- If η is high, the fluid layers even at the centre of the tube feels the effect of the wall where fluid is slow
- if η is low, the fluid layers at the centre of the tube hardly feels any effect of the wall

Dimension of Viscosity

$$\text{Dimension of } j = \frac{\text{momentum change}}{\text{area} \times \text{time}} = [MLT^{-1} \times L^{-2}T^{-1}] = [ML^{-1}T^{-2}]$$

$$\text{Dimension of } \frac{dv}{dz} = \frac{\text{velocity}}{\text{length}} = [LT^{-1} \times L^{-1}] = [T^{-1}]$$

$$\text{Dimension of } \eta = \frac{[ML^{-1}T^{-2}]}{[T^{-1}]} = [ML^{-1}T^{-1}]$$

Units $\text{g cm}^{-1} \text{ s}^{-1}$ or $\text{Pa} \cdot \text{s}$

Classic unit 1 Poise = $1 \text{ g cm}^{-1} \text{ s}^{-1}$

Viscosity of water at 298K is $\sim 10^{-2}$ Poise

Diffusion constant depends on molecular properties like size and shape...*contd*

Finally, viscosity is related to the friction factor through molecular size

Stokes' law: $f = 6\pi\eta a$ — for a spherical molecule moving in a fluid

Combining this with the Einstein relation: $D = \frac{k_B T}{f}$ we get

$$D = \frac{k_B T}{6\pi\eta a} \quad \text{Stokes-Einstein relation}$$

This relation

- Relates diffusion constant to viscosity
- Relates diffusion constant to molecular size
- Often is used to measure size of molecules

Estimation of diffusion constant using Stokes-Einstein

$$D = \frac{k_B T}{6\pi\eta a} \quad \text{Stokes-Einstein relation}$$

Estimate the diffusion constants for these species in water

sucrose

$$a \approx 5 \text{ \AA}$$

myoglobin

$$a \approx 20 \text{ \AA}$$

$$D_{\text{sucrose}} \approx 5.5 \times 10^{-6} \text{ cm}^2\text{s}^{-1}$$

$$a \approx 140 \text{ \AA}$$

$$D (\text{myoglobin, 300K}) = 1.1 \times 10^{-6} \text{ cm}^2\text{\bullet sec}^{-1}$$

virus
particle

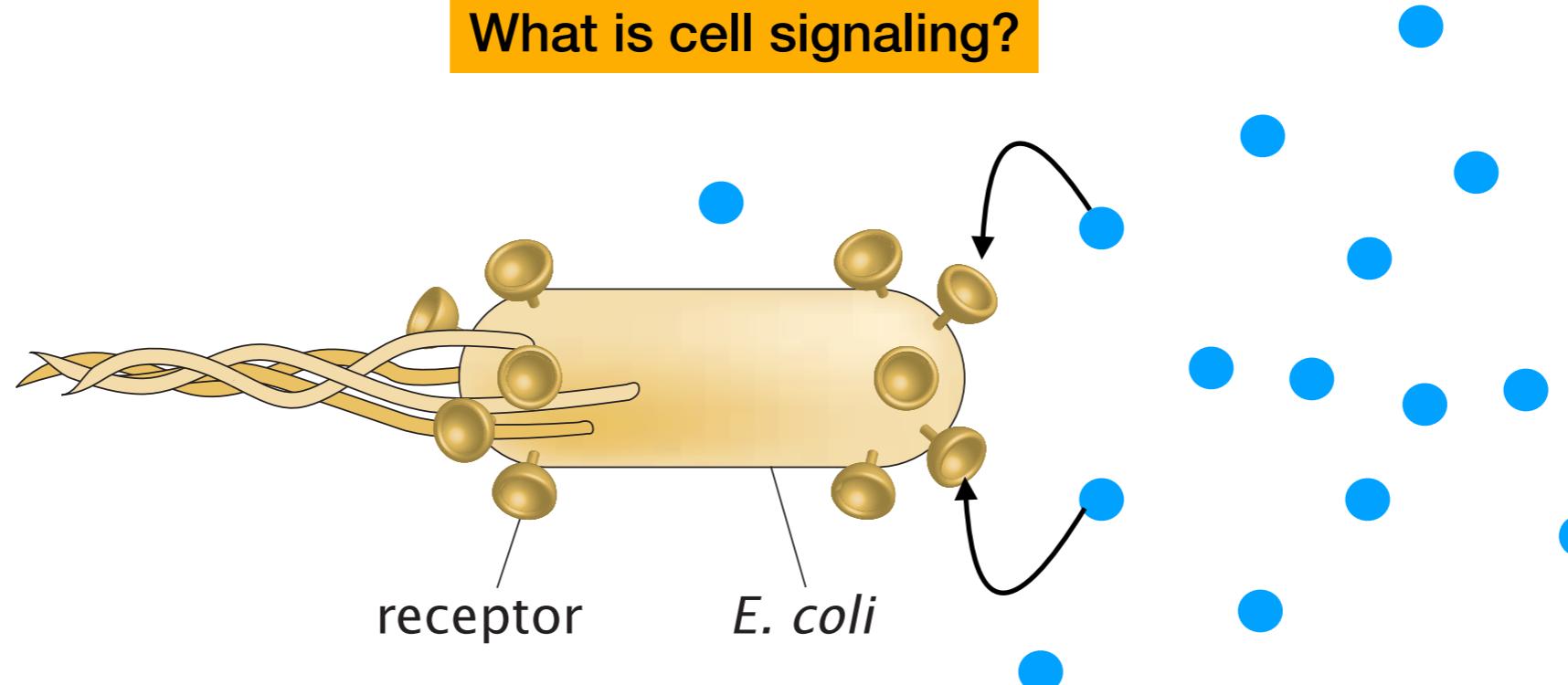
$$D_{\text{virus}} \approx 0.1 \times 10^{-6} \text{ cm}^2\text{s}^{-1}$$

100 \AA

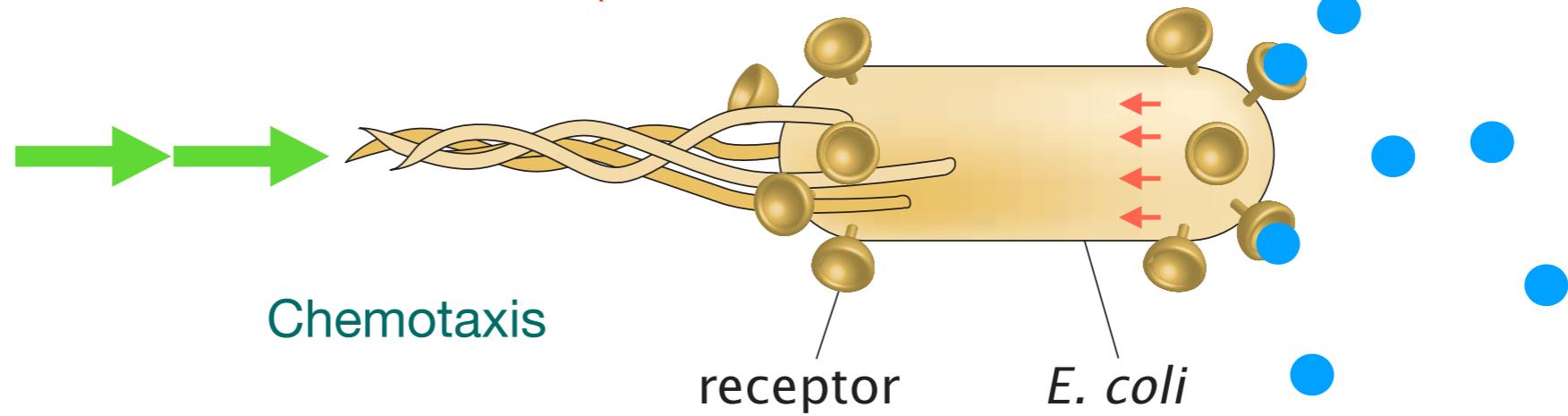


Cell signaling as a diffusive process

What is cell signaling?



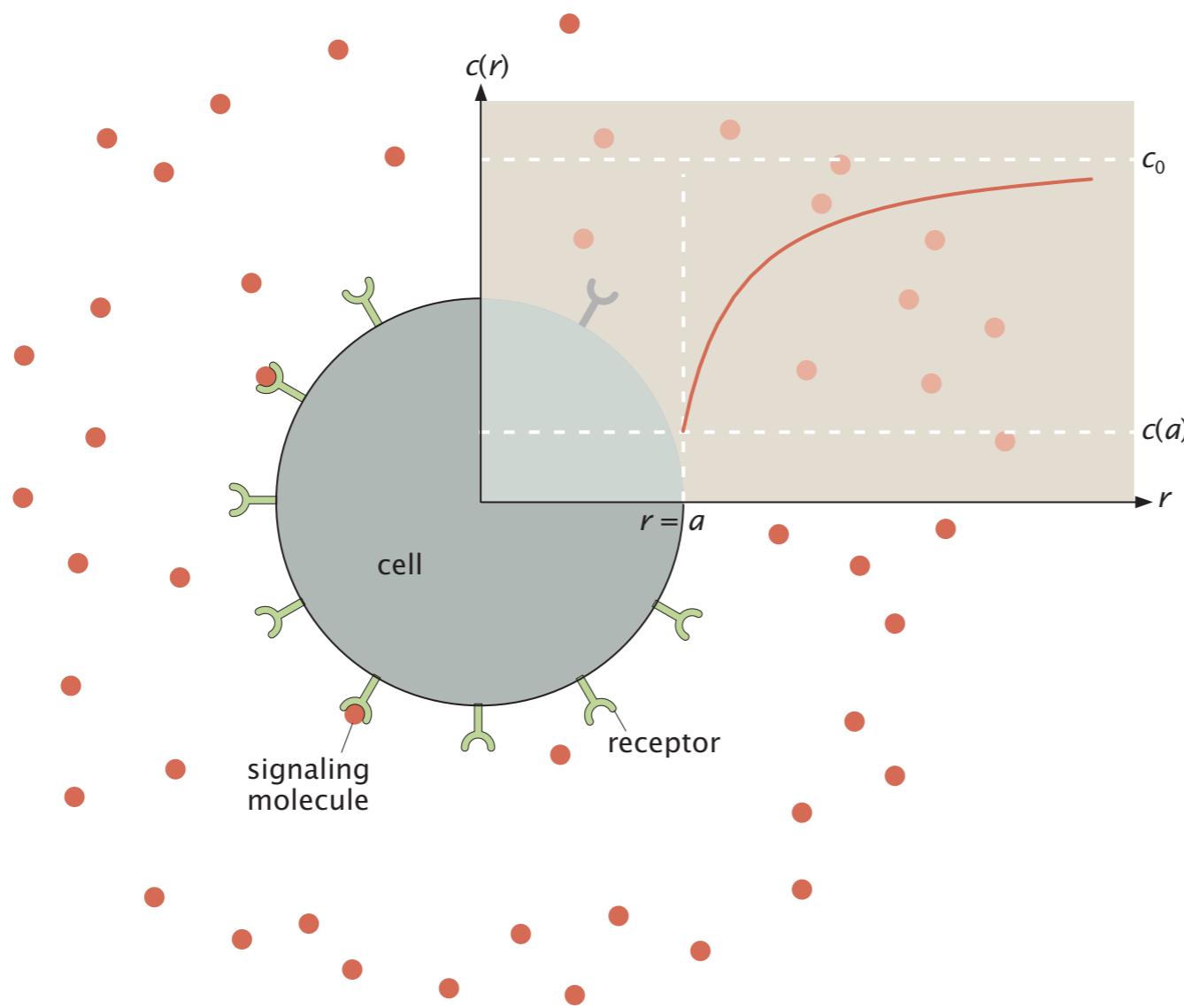
Downstream processes are activated in the cell



Can we understand this process using diffusion?

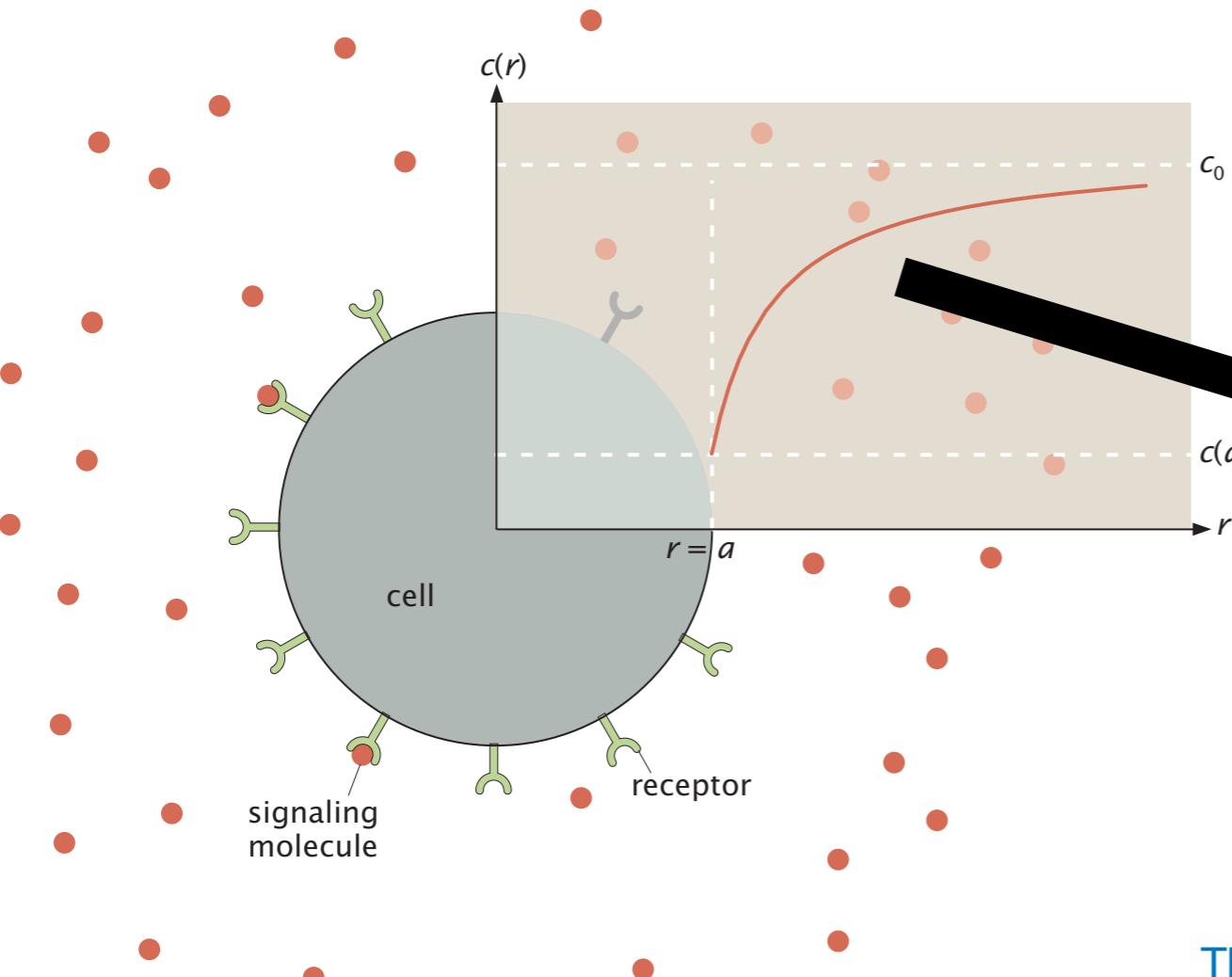
Main questions

- What is the number of ligand molecules that arrive on cell surface purely by diffusion?
- What is the resultant distribution of receptors on the cell surface?



We first assume an idealized situation with a spherical cell and perfectly adsorbing receptors

Diffusion limited number of ligand arriving on cell surface



Let the no of receptors be M

Conc of ligands far away from cell surface = c_0

Conc of ligands at the cell surface = $c(a)$

$$c(a) \approx 0$$

The diffusion equation for the ligands in 1D is

$$\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial x^2}$$

The diffusion equation for the ligands in 3D is then

$$\frac{\partial c}{\partial t} = D \nabla^2 c$$

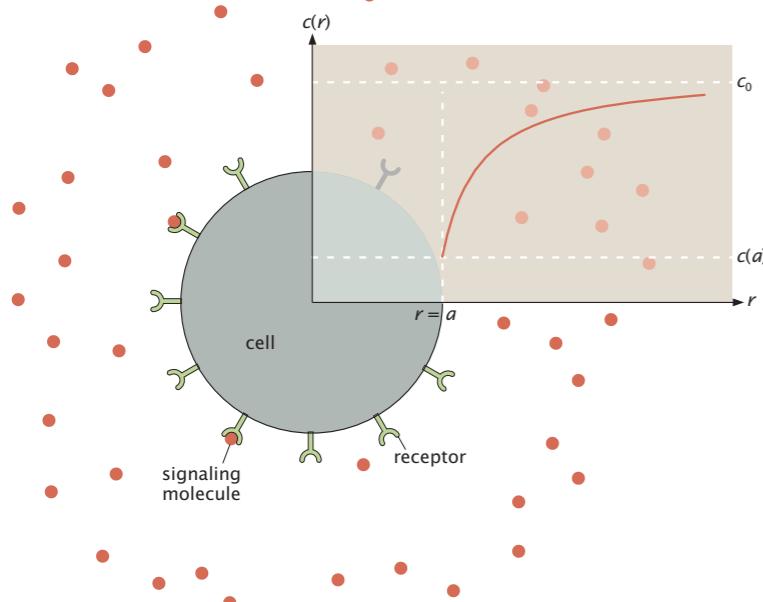
$$\text{Where } \nabla = \frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} + \frac{\partial^2}{\partial z^2}$$

Idea of partial derivative

$$\text{If } f = f(x, y)$$

Then, $\frac{\partial f}{\partial x}$ = derivative of f with respect to x keeping y fixed

Diffusion limited number of ligand arriving on cell surface ...*contd*



Let's assume

$$r^2 \frac{\partial c}{\partial r} = A$$

$$\Rightarrow \frac{\partial c}{\partial r} = \frac{A}{r^2}$$

At steady state $\frac{\partial c}{\partial t} = 0 = D \nabla^2 c$

For spherical geometry $\nabla^2 c = \frac{1}{r^2} \frac{\partial}{\partial r} \left(r^2 \frac{\partial c}{\partial r} \right)$

So, $\frac{1}{r^2} \frac{\partial}{\partial r} \left(r^2 \frac{\partial c}{\partial r} \right) = 0$

$$\Rightarrow r^2 \frac{\partial c}{\partial r} = \text{constant}$$

Solving the differential eq $\Rightarrow c = -\frac{A}{r} + B$

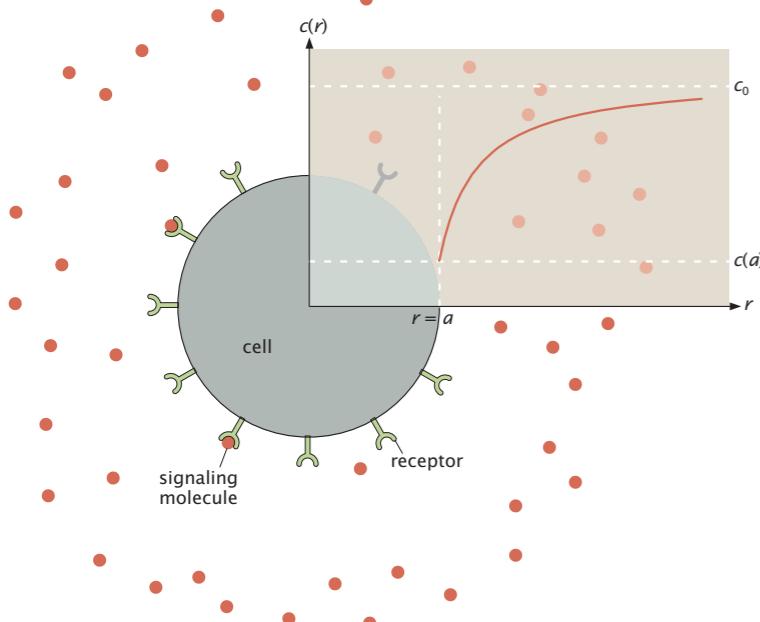
Now imposing boundary conditions we can get A and B

$$c(a) = 0 \text{ & } c(\infty) = c_0$$

Finally,

$$c = c_0 \left(1 - \frac{a}{r} \right)$$

Diffusion limited number of ligand arriving on cell surface ...*contd*



Conc profile of the ligand

$$c = c_0 \left(1 - \frac{a}{r} \right)$$

Molecular flux at the cell surface

$$j(a) = -D \left[\frac{\partial c}{\partial r} \right]_{r=a}$$

From the profile:

$$\frac{\partial c}{\partial r} = \frac{ac_0}{r^2} \implies \left[\frac{\partial c}{\partial r} \right]_{r=a} = \frac{c_0}{a}$$

Therefore, surface flux

$$j(a) = - \frac{Dc_0}{a}$$

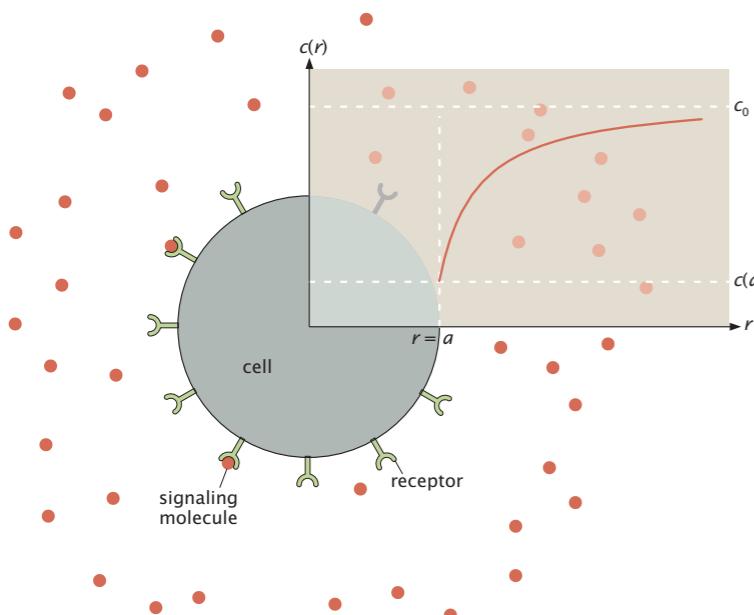
Therefore, no. of ligand molecules arriving at the cell surface per unit time

$$\implies \frac{dn}{dt} = |j(a)| \times A_{sphere} = \frac{Dc_0}{a} \times 4\pi a^2 = 4\pi Dc_0 a$$

This result assumes perfect adsorbing receptors but real one's not perfect, so?

Number of ligand adsorbed on cell surface for finite binding rate

If there is a finite binding rate of the receptors k_{on} then no of molecules adsorbed per unit time



Let the no of receptors be M

$$\frac{dn_1}{dt} = M k_{on} c(a)$$

We can write LHS in terms of molecular flux through any sphere of radius r

$$\frac{dn_1}{dt} = |j(r)| 4\pi r^2$$

$$\Rightarrow \frac{dn_1}{dt} = D \frac{\partial c}{\partial r} 4\pi r^2 = M k_{on} c(a)$$

$$\Rightarrow \frac{\partial c}{\partial r} = \frac{M k_{on} c(a)}{4D\pi r^2}$$

Separation of variable gives

$$\Rightarrow dc = \frac{M k_{on} c(a)}{4D\pi r^2} dr$$

$$\Rightarrow \int_{c(a)}^{c(r)} dc = \int_a^r \frac{M k_{on} c(a)}{4D\pi r^2} dr$$

$$\Rightarrow c(r) - c(a) = \frac{M k_{on} c(a)}{4\pi D} \left(\frac{1}{a} - \frac{1}{r} \right)$$

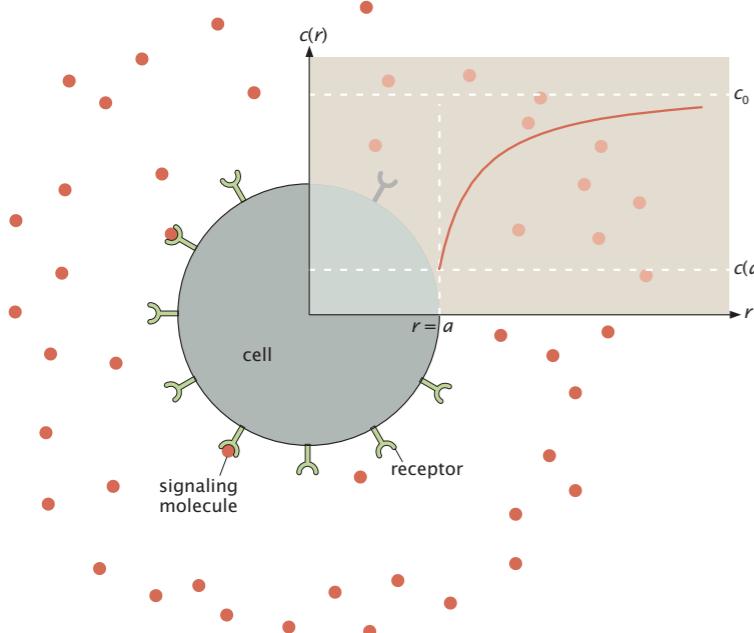
Now if we use limit $r \rightarrow \infty$

$$c(a) = \frac{c_0}{1 + \frac{M k_{on} c(a)}{4\pi D a}}$$

This is the conc of ligand molecules at the cell surface if receptors are not perfectly adsorbing

Number of ligand adsorbed on cell surface for finite binding rate

If there is a finite binding rate of the receptors k_{on} then no of molecules adsorbed per unit time



Let the no of receptors be M

$$\frac{dn_1}{dt} = M k_{on} c(a)$$

$$c(a) = \frac{c_0}{1 + \frac{M k_{on} c(a)}{4\pi D a}}$$

conc of ligand molecules at
the cell surface

Therefore,

$$\frac{dn_1}{dt} = \frac{M k_{on} c_0}{1 + \frac{M k_{on} c(a)}{4\pi D a}}$$

How do we get M using this result for a given ligand concentration?

Number of receptors for a given surface concentration of ligands?

For imperfect adsorbing case

$$\frac{dn_1}{dt} = \frac{Mk_{on}c_0}{1 + \frac{Mk_{on}c(a)}{4\pi Da}}$$

For perfect adsorbing case we had earlier

$$\frac{dn}{dt} = 4\pi Dc_0 a$$

This is the upper limit

So no of receptors needed for half the diffusive limit

$$\frac{dn_1}{dt} = \frac{4\pi Dc_0 a}{2}$$
 can be obtained by

$$\frac{Mk_{on}c_0}{1 + \frac{Mk_{on}c(a)}{4\pi Da}} = \frac{4\pi Dc_0 a}{2}$$

This is equivalent to solving

$$\frac{\beta}{1 + \beta} = \frac{1}{2} \quad \text{Where} \quad \beta = \frac{Mk_{on}}{4\pi Da}$$

Solution to this is

$$\beta = \frac{Mk_{on}}{4\pi Da} = 1 \implies M = \frac{4\pi Da}{k_{on}}$$

What this relation means physically?

Estimation of actual no of ligands for a typical cell

If cell diameter is $10 \mu\text{m}$

D for ligand is $100 \mu\text{m}^2\text{s}^{-1}$

$$M = \frac{4\pi Da}{k_{on}} = ?$$

Rate of binding, $k_{on} \approx 10 \mu\text{M}^{-1}\text{s}^{-1}$

First we simplify k_{on} in to suitable units

$$k_{on} = \frac{10}{10^{-6}} \text{ M}^{-1}\text{s}^{-1} = \frac{10^7}{6 \times 10^{23}} \times 1000 \text{ cm}^3\text{s}^{-1} = \frac{10^{10}}{6 \times 10^{23}} \times 10^{-6} \text{ m}^3\text{s}^{-1}$$

$$\Rightarrow k_{on} = \frac{10^4}{6 \times 10^5} \mu\text{m}^3\text{s}^{-1} = \frac{1}{60} \mu\text{m}^3\text{s}^{-1}$$

$$M = 4 \times 3.14 \times 100 \times 10 \times 60$$

$$M \approx 7 \times 10^5$$

What is the covered membrane fraction?

$$\nu = \frac{MA_{receptor}}{A_{membrane}} = \frac{7 \times 10^5 \times 10 \text{ nm}^2}{4\pi \times 10^2 \text{ } \mu\text{m}^2} = \frac{7 \times 10^6 \times 10^{-18} \text{ m}^2}{4\pi \times 10^2 \times 10^{-12} \text{ m}^2} \approx 10^{-3}$$

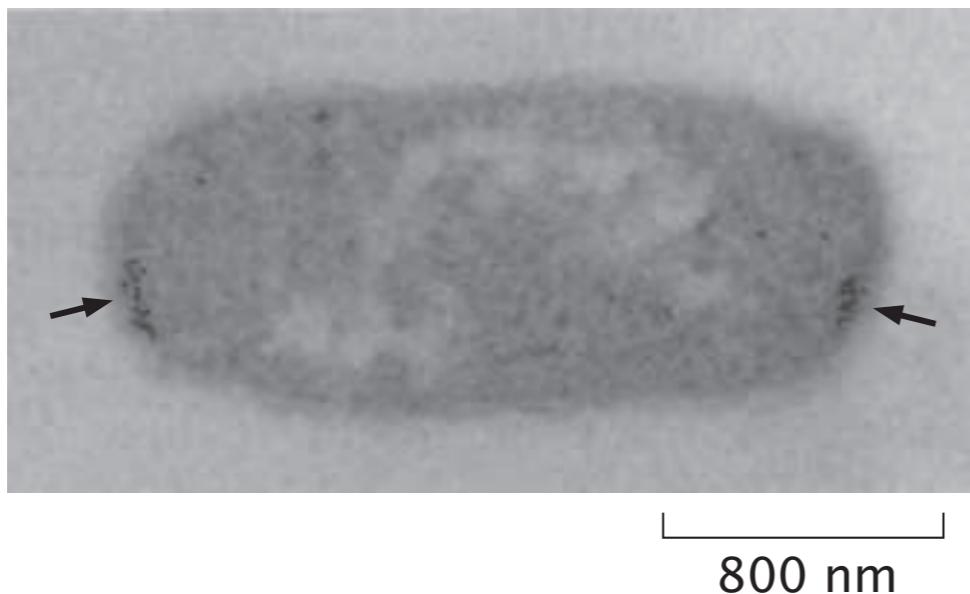
Assume $A_{receptor} \approx 10 \text{ nm}^2$

This result illustrates two things

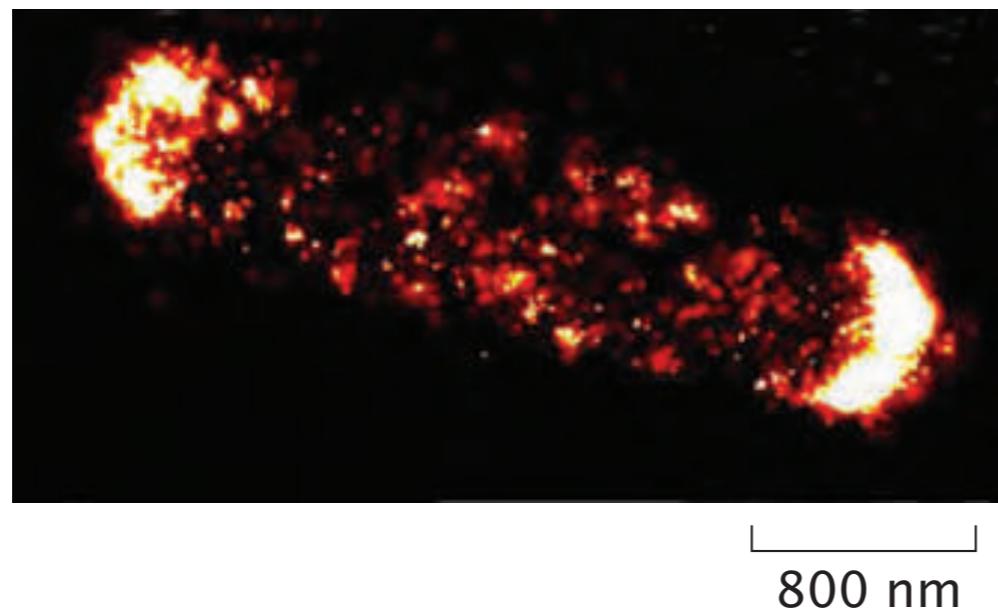
- these simple estimates demonstrate that even a relatively sparse distribution of membrane-bound receptors can rival a perfectly absorbing sphere of receptors.
- Further, this estimate also reveals that many different species of receptor can decorate the cell surface simultaneously while leaving room for the others and with all receptors operating nearly as perfect absorbers.

Real Receptors Are Not Always Uniformly Distributed

Localization of chemotaxis receptors in *E. coli* as shown by immunogold labeling in a thin-section electron micrograph.



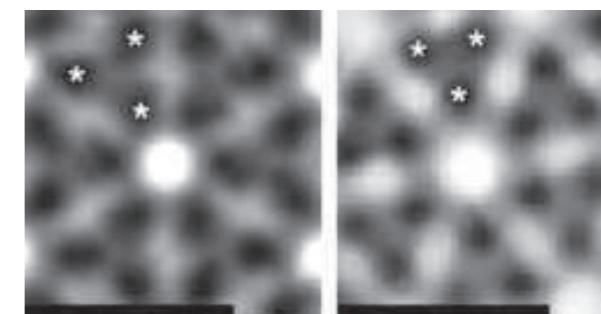
High-resolution fluorescence image of *E. coli* chemotaxis receptors



This reveals non-uniform distribution of receptors on the bacterial cell surface which was not expected from the diffusive assumption

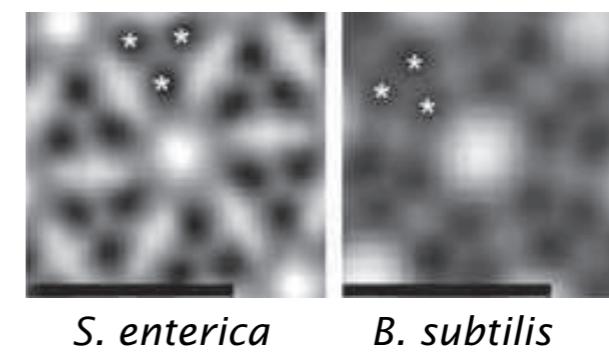
In fact different bacteria organize receptors in hexagonal arrays which is striking! So, the bacteria must care for such a specific arrangement!

(D)



E. coli

H. hepaticus



S. enterica

B. subtilis