Phagocytosis: Biology and Mathematical Model (CW1)

Question 1

Phagocytosis is a vital process that occurs in many types of cells, including white blood cells, and uni-cellular organisms. It is the way in which cells engulf and digest foreign materials such as bacteria, apoptotic cells, and other large particles >0.5µm in size that would be too large to be taken in by diffusion. This process is crucial for the immune system to protect the body from harmful substances, but it is also used by other cells such as single-celled organisms as a way of obtaining nutrients.

The process of phagocytosis begins when a phagocyte comes into contact with a particle, such as a bacterium. It recognises the foreign particle by use of specialised proteins (receptors) that are present across the entirety of the cell's surface; this occurs either directly by recognition of molecular groups on the target, or indirectly via extracellular proteins called opsonins [1]. Opsonins are present in the circulating blood and bind to the target particles, which can in turn be recognised by the phagocyte's opsonic receptors [5,6]. An example of opsonic receptors are Fcγ receptors, which are glycoproteins that specifically bind to part of IgG molecules, which are antibodies produced by the adaptive immune response [10]. These receptors bind to ligands on the target particle and cause a signalling cascade which instructs the reshaping of the cell membrane to extend around the particle [7]. Part of this reshaping happens through the rearrangement of the cytoskeleton. The cytoskeleton is a network of filaments within the cell which act to give the cell structure, as well as mediating intracellular movement of materials. Forming a cup-like structure around it, the cell engulfs the foreign particle, while receptors diffuse across the surface towards and into the cup to increase density of receptors and allow for more attachment sites. This is especially important as binding of the receptors to the ligands is not an easy task because many of the cell's receptors are very short in comparison to longer, rigid, glycoproteins that are present across the cell [1]. As more receptors move towards the target particle and the cup closes around it, the particle is trapped and formed into a phagosome.

The phagosome is its own vesicle within the host cell. Maturation of the phagosome occurs for it to become a phagolysosome. Lysosomes fuse with the trapped particle — lysosomes are small vesicles that contain enzymes that can digest the particle. The phagolysosome has a different membrane composition to the original cell in order to allow for a highly acidic environment for the enzymes to degrade the particle in [8, 9]. Once the phagosome has fused with the lysosomes, the enzymes in the lysosomes break down the particle into smaller molecules in a process called intracellular digestion. The smaller molecules are then transported out of the phagosome and into the cytoplasm of the phagocyte. This allows the phagocyte to use the nutrients from the particle for energy.

In addition to providing the cell with nutrients, the process of phagocytosis also helps to remove harmful particles from the body when used in an immune response. Once the particle has been completely broken down and absorbed, the phagosome is degraded, and the phagocyte is ready to move on to the next particle. Some phagocytes, such as macrophages, will also process the pathogen's antigens in order to present its fragments on its own surface for T cells to recognise a current infection. This is done using MHC molecules which bind to the fragments and display them on the cell's surface [2-4].

The most efficient phagocyte is the neutrophil, which is capable of engulfing up to 50 bacteria at a time, and phagocytosing in only a few seconds [13]. They are able to detect and bind to a wide range of ligands including ones on fungi, bacteria, and even on cancer cells [14].

Another important use of phagocytosis is for removing apoptotic cells; ones which have undergone a programmed cell death [9]. Removal of apoptotic cells is fundamental for tissue homeostasis as the human body can turn over more than 200 billion cells per day. Apoptosis helps to eliminate cells that are no longer needed without causing damage to their surroundings [11]. The dying cell releases signals to attract the phagocytes, which then recognise these signals and can proceed to consume them. When degrading the apoptotic cells its extremely important for DNA to be removed, as rogue bits of DNA can lead to autoimmune diseases [12].

In conclusion, phagocytosis is a vital process that is used by many different types of cells. It's an essential process in the immune system where it is used to remove harmful pathogens from the host, and to clear up dead cells in order to aid in tissue homeostasis. Phagocytosis relies on the recognition of target particles by use of receptors on the cell's surface.

Question 2

The pure diffusion model of phagocytosis from [A] models the density of Fcγ receptors across the cell membrane over time and so, by proxy, models their movement. The model assumes a spherical bead to be engulfed and so, taking the origin to be the initial point of contact, the model is circularly symmetric. This means that the problem can be reduced to a semi-infinite 1-dimensional problem in the range $[0,\infty)$. In this model we assume that the receptors passively diffuse across the membrane with a constant diffusion factor, rather than an active movement of receptors via retrograde actin flow for example.

The line upon which the diffusion is modelled is parameterised by the distance from the origin, r, and the receptor density is described by $\rho(r,t)$ for some time t. At time t=0, before first contact with the bead, the receptor density across the cell's surface is constant, given by ρ_0. After engulfment begins the receptor density is not constant but is assumed to remain at ρ_0 at infinity. In addition, the cup-size is described by $a(t)$. Within the cup, i.e. where $r<a$, the receptors are attached to the ligands of the bead and we assume that in this region the receptor density remains constant and is denoted ρ_L, where $\rho_L > \rho_0$. This constant value is due to the assumption that, within the engulfment region, ρ is related directly to the ligand density on the bead. Finally we denote the receptor density at the cup edge, $\rho_+ = \rho(a(t),t)$.

The diffusion of the receptors is described by the partial differential equation:

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

With diffusion constant D. This equation, given that we model on a spherical bead, uses the radial Laplacian operator and so can be expanded:

$$\frac{\partial \rho}{\partial t} = \frac{D}{r}\frac{\partial}{\partial r}\left(r\frac{\partial \rho}{\partial r}\right)$$

Additionally, $a(t)$ is described by:

$$\frac{da}{dt} = \frac{D\rho'_+}{\rho_L - \rho_+}$$

Where ρ'_+ denotes $\partial\rho/\partial t\big|_{r=a}$. The equation for a relies on the conservation of receptors, using the receptor flux $(-)D\rho'_+$.

The initial conditions of this set of equations are $\rho(r,0) = \rho_0$ and $a(0) = 0$.

To find a solution the system of equations, an extra condition is needed: there must be no free-energy change as receptors diffuse to the engulfed region and the cup is widened. This means that the receptor-ligand binding energy, membrane curvature, and receptor entropy must be taken into account. From this requirement, one can construct an equation for the free energy which can be reduced down to an equation numerically solvable to find ρ_+.

The system can then be solved analytically, or numerically by the Euler method, for example.

Question 3

Parameters:

D = 1 $\mu m^2 s^{-1}$ – diffusion constant

ρ_0 = 50 μm^{-2} – typical receptor density

ρ_L = 50 μm^{-2} – typical ligand density

ε = 15 – free binding energy

β = 20 – bending modulus

R = 2 µm – target radius

L = 50 µm – domain size (size of cell)

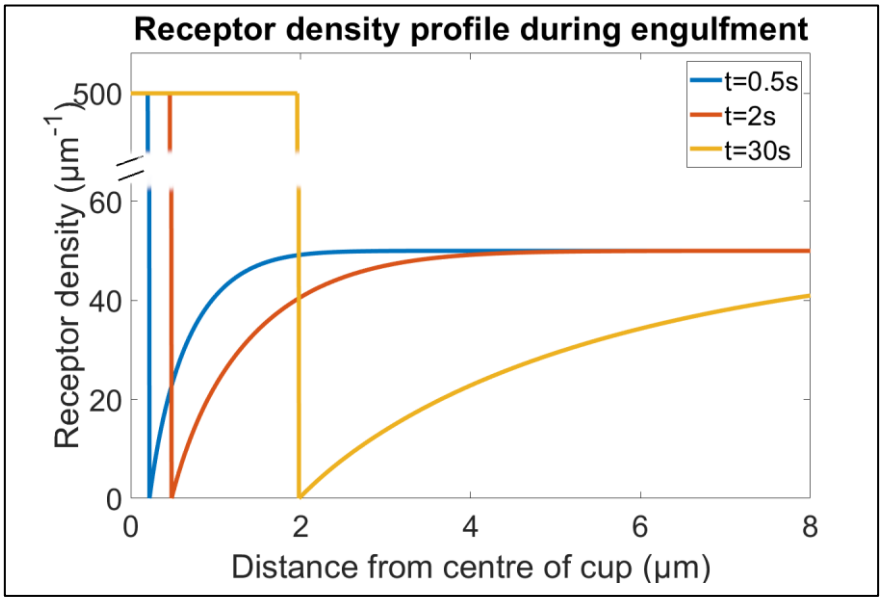

Figure 1. Receptor density profile of the pure diffusion model of phagocytosis (numerically solved), showing the density at three different times, t=0.5s, t=2s, and t=30s.

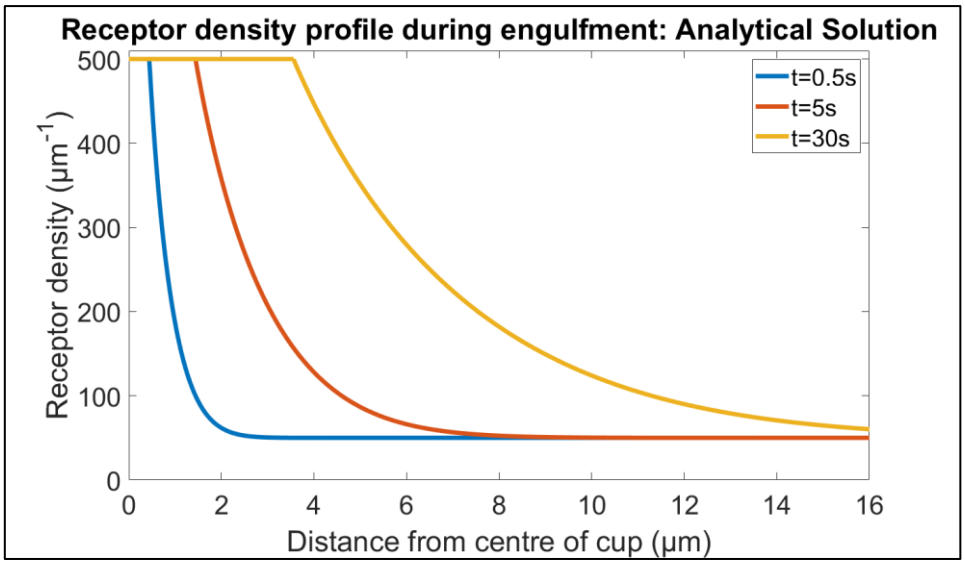

Figure 2. Receptor density profile of the pure diffusion model, analytically solved, showing the density at three different times, t=0.5s, t=2s, and t=30s.

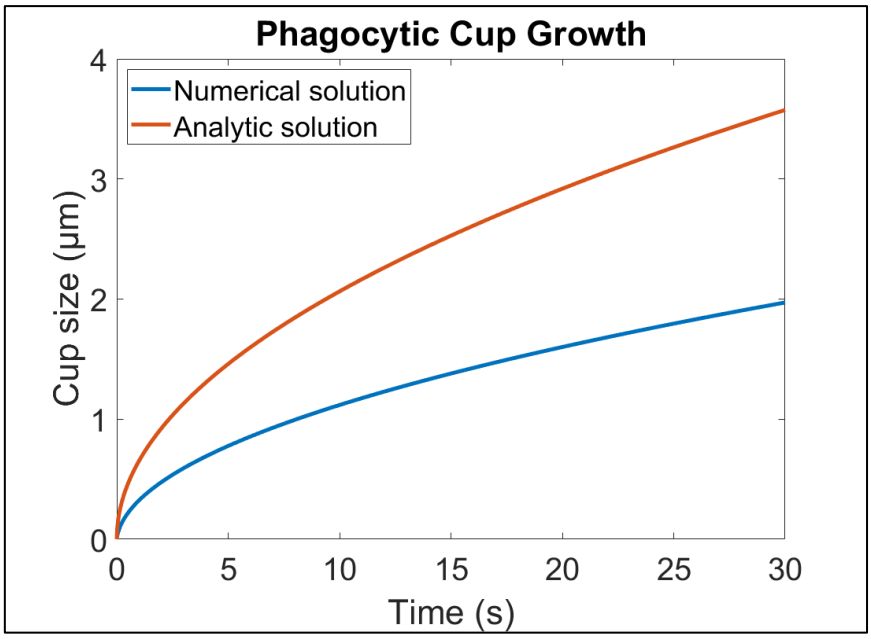

Figure 3. Particle engulfment plotted against time, showing the numerical solution and the analytical solution up to time t=30s.

There is a clear difference in the numerical and analytical solutions. The numerical solution has a distinct curve with a sharp drop in density at the cup's edge giving $\rho_+ < \rho_0$ and steadily increasing back to ρ_0 as distance from the cup increases. As time progresses, the plateau in the region [0,a] steadily increases and the curve stretches with it to be a more gradual gradient towards the cup. The cup size increases with the square root of time. In the analytical solution, the same plateau is present at r<a but the drop in density is then gradual down to ρ_0. In this solution, $\rho_0 < \rho_+$ and so the density steadily

decreases away from the cup; the opposite to that of the numerical solution. Cup size (or equivalently, engulfment) also increases with the square root of time but at a higher rate than the numerical solution.

A big difference between the two is the processing time: the numerical model completed processing in less than two minutes, whereas the analytical model took over an hour to complete its calculations. A drawback however is that the numerical solution doesn't appear to accurately model the speed at which phagocytosis occurs, with the analytical solution predicting a much faster engulfment of the particle: reaching a cup size of 2 μm in about 10 seconds, where the numerical solution took about 30 seconds to reach the same size.

Question 4

Altering the particle radius had no discernible effect on the total engulfment time. This can be understood by realising that a large particle will have many more attachment sites at once versus a smaller particle. As a result, the cup size will increase at a proportional speed and engulfment will take a very similar time. Another way of viewing this is that, assuming a spherical particle, the circumference is directly proportional to the radius ($C = 2\pi R$) and so, equivalently, the radius is directly proportional to the attachment site area.

References

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[A] Richards, D. M., & Endres, R. G. (2014). The mechanism of phagocytosis: two stages of engulfment. *Biophysical journal*, *107*(7), 1542-1553.

Code

```
% NSCM005 CA1
% modelling a pure diffusion model of phagocytosis for a spherical particle
% clear all variables
clear

% Define parameters
DT = 0.0001; % time step, [s]
DR = 0.02; % lattice step, [um]
T_MAX = 30; % max time, [s]
L = 50; % domain length, [um]
D = 1; % diffusion constant, [um^2/s]
p0 = 50; % initial receptor density, [um^-2]
pL = 500; % bound receptor density, [um^-2]
E = 15; % binding energy per receptor-ligand bond
B = 20; % bending modulus
R = 16; % target radius (radius of bead), [um]

% Declare/allocate variables
num_steps = round(T_MAX/DT); % number of time steps [-]
num_latt_pts = round(L/DR); % number of lattice points [-]

p = zeros(num_steps,num_latt_pts); % initialise receptor density array
a = zeros(num_steps, 1); % cup size

p_plus = fzero(@(p_plus)p_plus/pL - log(p_plus/pL)-E+2*B/(pL*R^2)-1,...
    [1e-6 5]); % calculate p_plus from Eq 4

% Initial conditions
for i = 1:num_latt_pts
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```
    p(1,i) = p0; % initialise receptor densities to p0
end
a(1) = 0; % initialise cup size to 0

% Main loop
for i = 2:num_steps
    %cup size in lattice steps
    a_latt_pt = round( a(i-1)/DR + 1 );

    %set p to pL at r<a
    for j=1:a_latt_pt-1
        p(i,j) = pL;
    end

    % set p to p_plus at r=a
    p(i, a_latt_pt) = p_plus;

    %calculate p for r>a (derived using the Euler method on Eq 2a)
    for j = a_latt_pt+1:num_latt_pts-1
        p(i,j) = p(i-1,j) + D*DT/DR * ( 1/(j*DR)*(p(i-1,j+1)-p(i-1,j)) + ...
            1/DR*(p(i-1,j-1)-2*p(i-1,j)+p(i-1,j+1)));
    end

    %no flux boundary condition at x=L
    p(i, num_latt_pts) = p(i-1,num_latt_pts) + D*DT/DR^2 * (p(i-1,num_latt_pts-1)-
p(i-1,num_latt_pts));

    %calculate p'_plus (derivative dp/dr evaluated at r=a)
    p_dash_plus = (p(i,a_latt_pt+1)-p(i,a_latt_pt)) /DR;

    %calculate da/dt from Eq 2b
    a_dot = D*p_dash_plus/(pL-p_plus);

    %recalculate cup size using da/dt
    a(i) = a(i-1) + a_dot*DT;
end



%neither fzero nor fsolve could solve for alpha due to the expint function,
%so I used desmos instead. Nonetheless I keep the code commented below to
%show the equation that was solved to find alpha.

%alpha = fsolve(@(alpha)alpha^2*exp(alpha^2)*expint(alpha^2)-(p0-p_plus)/(pL-
p_plus),[0 5]);
alpha = 0.326338;

% Analytic Solution NOTE: This took over an hour to run on my machine
A = (p0-pL)/expint(alpha^2);
p_an = zeros(num_steps,num_latt_pts);
for i = 1:num_steps
    a_an = 2*alpha*sqrt(D*i*DT); % a = 2*a*sqrt(Dt)
    a_an_latt = round( a_an/DR + 1 ); % find cup size in lattice points
    for j = 1:a_an_latt
        p_an(i,j) = pL; % density at r<a is equal to pL
    end
    for j = a_an_latt:num_latt_pts % density at r>a is equal to p0 - AE1(r^2/4Dt)
        p_an(i,j) = p0-A*expint((j*DR)^2/(4*D*i*DT));
    end
```

```
end


%Plot receptor density and cup size (change p to p_an for plots of
%analytical solution)

time_int = [0.5 5 T_MAX];
time_int_steps = round(time_int/DT);

clf;
%subplot(1,2,1);
x = 0:DR:(num_latt_pts-1)*DR;
plot(x,p(time_int_steps(1),:), ...
    x,p(time_int_steps(2),:), ...
    x,p(time_int_steps(3),:),'LineWidth',4);
set(gca,'FontSize',24);
legend(['t=' num2str(time_int(1)) 's'], ...
        ['t=' num2str(time_int(2)) 's'], ...
        ['t=' num2str(time_int(3)) 's']);
xlim([0 16]);
ylim([0 510]);
%xticks([0 2 4 6 8 10 12 14 16])
xlabel('Distance from centre of cup (μm)');
ylabel('Receptor density (μm^{-1})');
title('Receptor density profile during engulfment');

subplot(1,2,2);
t = 0:DT:(num_steps-1)*DT;
plot(t,a,t,2*alpha*sqrt(D*t),'LineWidth',3);
set(gca,'FontSize',24);
xlabel('Time (s)');
ylabel('Cup size (μm)');
legend('Numerical solution','Analytic solution','Location','NorthWest');
title('Phagocytic Cup Growth');
```