Understanding polypharmacology of antibodies: A mathematical approach

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1 Introduction

Antibodies are large Y-shaped proteins that are used by the immune system to identify and neutralize pathogens such as bacteria and viruses. Monoclonal antibodies (mAbs) are a particular type of antibody that are made by identical immune cells that are all clones of a unique parent cell. These mAbs can provide highly-specific high-affinity binding to almost any molecule. Most therapeutic mAbs are monospecific (having affinity only for the same antigen) and these have been found to be highly efficacious for the treatment of diseases ranging from immunoinflammatory [4] to oncology and ophthalmology [5]. In these cases, highly specific and effective disruption of protein-protein interaction is required for the therapeutic effect and the target typically is a membrane-bound receptor for its cognate soluble ligand.

Protein engineering has allowed the creation of bispecific varieties (bs-mAbs) that can bind two different target molecules simultaneously. For example, two recent papers by Mazor et al. [6, 7] describe a novel bs-mAb (DuetMab) that can simultaneously engage with two different antigens on the same cell and form a ternary surface-bound complex. Bispecific mAbs are expected to offer several advantages over traditional antibody technologies by

- Improved targeting of therapeutics to tissues or specific disease processes
- Crosslinking cell-surface receptors to invoke novel biology with powerful therapeutic potential.

To the best of our knowledge, no theoretical description of the action of bs-mAbs exists in the literature, and this was therefore the focus of this study-group problem.

2 Aims

Dr Armin Sepp (GSK) presented the problem. He explained that many alternative formats for bs-mAbs exist including those with one binding site per mAb molecule for either target (two in total, A on Figure 1) and those where there are two for either target (four in total, B on Figure 1). He asked us to focus on the former.

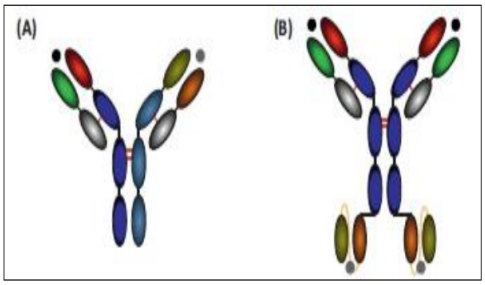

Figure 1: Bispecific antibody formats [1].

Traditional antibodies have 2 binding sites, but typically only bind to a single molecule of a soluble target due to high molar excesses when used as a therapeutic. However, when the target is membrane-bound, bivalent interaction may occur that results in improved binding through the avidity effect [2, 3]. In the case of bispecific mAbs, it is conceivable that two different targets present on the surface of the same cell may be cross-linked in similar manner.

The primary aims were to understand if there were any benefits in using a bispecific molecule over a combination of monospecific molecules when the targets are (i) well-mixed in solution and (ii) anchored on the surface of a cell.

3 Task 1: Targets well-mixed in solution

We consider here two species of target, T_1 and T_2, in solution at a ratio of $1:1$. The targets do not interact with one another and there is no target turnover. Either a combination of monospecific mAbs or bispecific mAbs are to be added to the solution at the same overall concentration. The question to be answered is whether or not this situation would favour using the combination mAb approach over the bispecific mAb approach from the target occupancy point of view.

Combination of monospecific mAbs
In this situation mAb1 binds T_1 with some dissociation constant K_d and mAb2 binds T_2 with the same K_d. We have that mAb1 and mAb2 are co-dosed at a ratio of $1:1$.

A bispecific mAb
In this situation the bs-mAb binds targets T_1 and T_2 with equal K_d

We concluded that, from the target occupancy point of view, both the combination of monospecific mAbs and the bispecific mAb approaches are identical. However, we note that the combination mAb approach is likely to allow greater flexibility in dosing and would therefore have the practical advantage.

4 Task 2: Targets anchored on the surface of a cell

To address this situation, we considered two distinct approaches. In the first, we neglect the spatial distribution of antibodies and targets and devise an Ordinary Differential Equation (ODE) model to describe the range of reactions which may occur. In the second approach, we attempt to account for spatial diffusion of target molecules by introducing a Partial Differential Equation (PDE) model.

4.1 The ODE Model

We firstly assume that the two targets expressed on the surface of the same cell are uniformly mixed and are similarly exposed to the antibodies. For the purposes of this model, we assume no spatial gradients of antibody or either target are established. Our model consists of a series of nonlinear ODE's which describe the reversible binding of an antibody A with some target T_1 to form a complex AT_1 or some target T_2 to form a complex AT_2, while either of these can reversibly bind respectively T_2 or T_1 to form a ternary complex AT_1T_2 according to the scheme in Fig. 2.

The scheme is described by a system of differential and algebraic equations with concentrations of species given in square brackets. Concentrations of surface species T_1, T_2, AT_1, AT_2 and AT_1T_2 are in $mole/dm^2$ units and carry subscript s. Free antibody concentration $[A]$ in molar units is assumed constant based on the comparatively large volume of buffer. Association rate constants k_1 and k_2 for reactions between antibody and a surface target molecule are in $(M\ s)^{-1}$ units, while k_3 and k_4 for reactions between two surface molecules are in $dm^2/(mole\ s)$ units. Dissociation rate constants k_{-1}, k_{-2}, k_{-3} and k_{-4} are all in s^{-1} units. The model is

$$
\begin{aligned}
[T_1]_s &= [T_1]_{0,s} - [AT_1]_s - [AT_1T_2]_s, \quad [T_2]_s = [T_2]_{0,s} - [AT_2]_s - [AT_1T_2]_s, && (1)\\
\frac{d[AT_1]_s}{dt} &= k_1[A]_s[T_1]_s - k_{-1}[AT_1]_s - k_3[AT_1]_s[T_2]_s + k_{-3}[AT_1T_2]_s, && (2)\\
\frac{d[AT_2]_s}{dt} &= k_2[A]_s[T_2]_s - k_{-2}[AT_2]_s - k_4[AT_2]_s[T_1]_s + k_{-4}[AT_1T_2]_s, && (3)\\
\frac{d[AT_2T_1]_s}{dt} &= k_3[AT_1]_s[T_2]_s - k_{-3}[AT_1T_2]_s + k_4[AT_2]_s[T_1]_s - k_{-4}[AT_1T_2]_s, && (4)
\end{aligned}
$$

where $[T_1]_{0,s}$ and $[T_2]_{0,s}$ are the initial constant concentrations of targets 1 and 2, respectively. Equation (1) is a statement of conservation of target binding sites. Equations (2-3) describe the rate of change of the concentration of the *one-arm* bound complexes $[AT_1]_s$ and $[AT_2]_s$, whilst

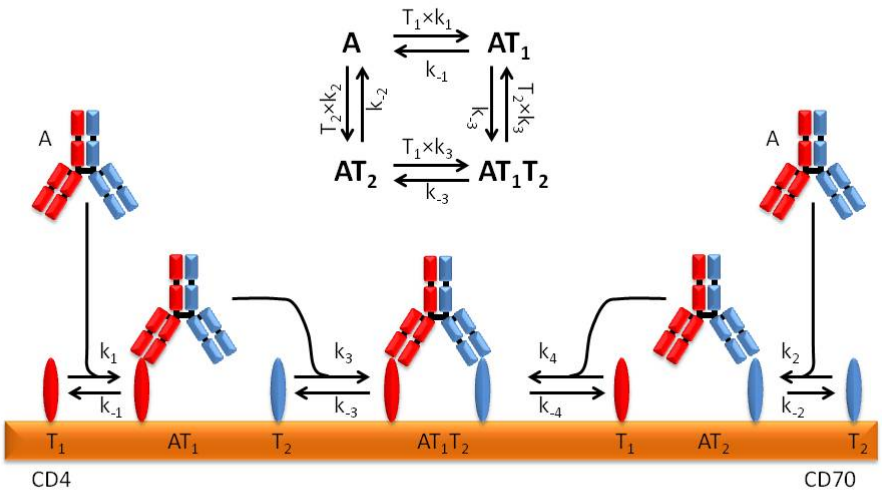

Figure 2: The reaction scheme for DuetMab interaction with two cell membrane targets

equation (4) describes the rate of change of concentration of the triple complex $[AT_1T_2]_s$ (referred to as *two-arm* bound). Initially we assume a constant concentration of antibody ($[A]_{0,s}$), whilst the concentration of the various complexes $[AT_1]_s$, $[AT_2]_s$ and $[AT_3]_s$ are zero:

$$[A]_s = [A]_{0,s}, \quad [T_1]_s = [T_1]_{0,s}, \quad [T_2]_s = [T_2]_{0,s}, \quad [AT_1]_s = [AT_2]_s = [AT_1T_2]_s = 0, \quad at \quad t = 0. \quad (5)$$

4.1.1 Numerical Solution

We proceeded to solve the algebraic and differential equations (1-4) subject to (5) numerically using ODE45 in Matlab, which is based on the explicit Runge-Kutta method. For the purposes of this task we considered three cases: cells expressing a single target T_1; cells expressing a single target T_2; cells expressing both targets T_1 and T_2. Simulations were performed both for the original bispecific antibody and those for a modified antibody in which the binding affinity to one of the targets was reduced, as specified in [7]. The parameter values used in the simulations, similar to those used by Mazor et al. [6, 7], are summarised in Tables 1 and 2, with $[T_1]_{0,s}$ corresponding to CD4 and $[T_2]_{0,s}$ to CD70. The number of targets per cell was set at 30000 of either T_1 or T_2 for cells expressing a single target, or 30000 of each for cells expressing both targets. The target surface density was then calculated based on a spherical cell with a diameter of 10 μm. Parameters k_3 and k_4 were assumed to be dominated by surface diffusion, with equal values for both targets based on the interactions between similar membrane embedded proteins [8].

The results for the simulation of antibody binding as a function of time are displayed in Fig. 3 for a cell expressing both T_1 and T_2. It can be observed in Fig. 3 (upper) that for low concentrations of antibody the level of total bound antibody increases linearly as a function of time and no equilibrium

Parameter	Value	unit
$[T_1]_{0,s}$	1.6×10^{-12}	mol/dm^2
$[T_2]_{0,s}$	1.6×10^{-12}	mol/dm^2
k_1	2.8×10^{5}	$(M\ s)^{-1}$
k_{-1}	2.6×10^{-4}	s^{-1}
k_2	2.0×10^{5}	$(M\ s)^{-1}$
k_{-2}	4.9×10^{-3}	s^{-1}
k_3	4.8×10^{13}	$dm^2/(mol\ s)$
k_{-3}	4.9×10^{-3}	s^{-1}
k_4	4.8×10^{13}	$dm^2/(mol\ s)$
k_{-4}	2.6×10^{-4}	s^{-1}

Table 1: Parameter values used in the simulations for the original bispecific antibody [7].

Parameter	Value	unit
k_1	5.2×10^{5}	$(M\ s)^{-1}$
k_{-1}	3.6×10^{-2}	s^{-1}
k_{-4}	3.6×10^{-2}	s^{-1}

Table 2: Parameter values used in the simulations for the modified bispecific antibody [7]. All other parameters as reported in Table 1.

is reached within the 1 hour experiment. In contrast, for high antibody concentration, equilibrium is reached well within 10 minutes as shown in Fig. 3 (lower). For both low and high antibody concentrations it is clear that the largest fraction of antibody bound to the cell is bound to both T_1 and T_2 in the *two-arm* binding configuration, with only a small fraction bound with only one arm to either T_1 or T_2.

To enable a direct comparison with experimental results, the total bound concentration at the end of the simulated experiment (t=3600 s) was plotted for a wide range of antibody concentrations in Fig. 4. Overall, the simulated results for antibody binding to cells expressing either T_1 or T_2, or both, corresponded remarkably well to the experiments, without any parameter tuning (see Fig. 2A in Mazor et al. [7]). As in the experiments, the strongest binding was observed for cells expressing both T_1 and T_2, followed by those expressing only T_1, while much lower levels of binding were observed for cells expressing only T_2. However, for high antibody concentrations in solution, the model predictions were higher than observed experimentally for the cells expressing only a single target (T_1 or T_2). This is likely due to the dissociation of antibody during the measurement procedure after the experiment has been stopped, which was not taken into account in the model.

Results for the modified antibody are shown in Fig. 5. It can be seen that reducing the binding affinity of the antibody for T_1 dramatically reduced binding to cells expressing only T_1, as could be expected. Because only the antibody binding to T_1 was modified, logically, binding to cells expressing only T_2 was not affected and remained low. Binding to cells expressing both T_1 and T_2 was largely unaffected and even improved slightly for low concentrations of antibody in solution. Overall, these model predictions corresponded closely to the previous experimental results (see Fig. 3 in Mazor et al. [7]). Importantly, by modulating binding affinity the modelling results demonstrate how bispecific antibodies can be used to specifically target only those cells that express two different targets and not those that express only a single target, which is highly valuable from

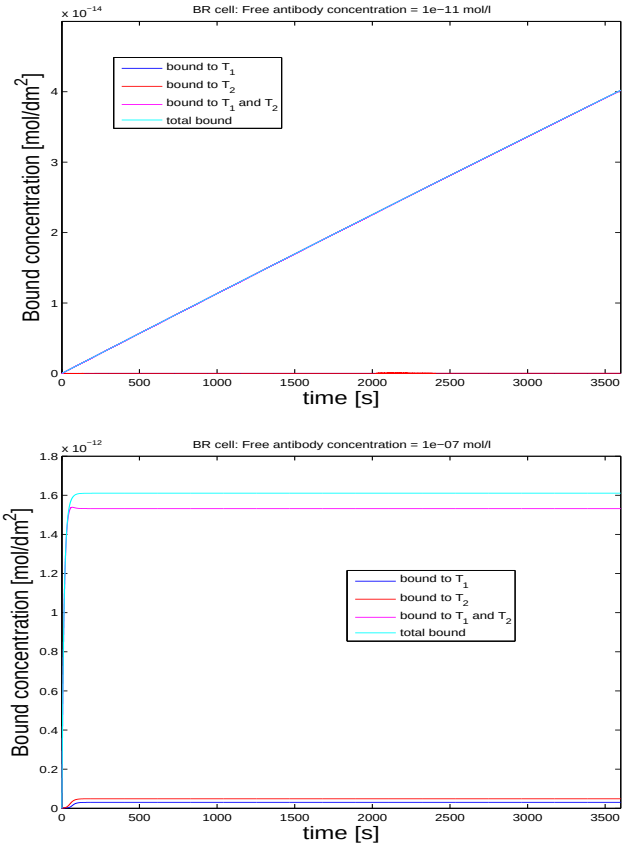

Figure 3: Time series results simulating antibody binding to a cell expressing both T_1 and T_2 during the 1 h experiment. Upper: Low antibody concentration $[A] = 1 \times 10^{-11} M$. Lower: High antibody concentration $[A] = 1 \times 10^{-7} M$. Note that for low antibody concentration (upper) the profiles for "total bound" and "bound to T_1 and T_2" overlap, while "bound to T_1" and "bound to T_2" are both close to zero.

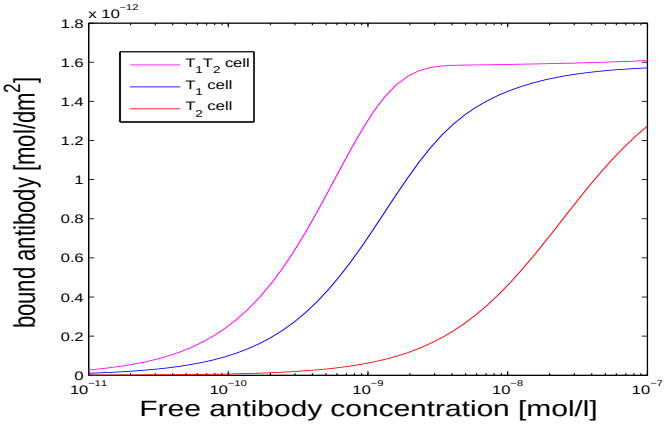

Figure 4: Results for the original antibody binding to dual-positive CD4+/CD70+ cells: Total bound antibody concentration as a function of free antibody concentration in solution.

a pharmaceutical perspective. In addition the model can predict optimal concentration ranges based on binding data for single antibodies, thus reducing costly experimentation and aiding the development of novel bispecific antibodies.

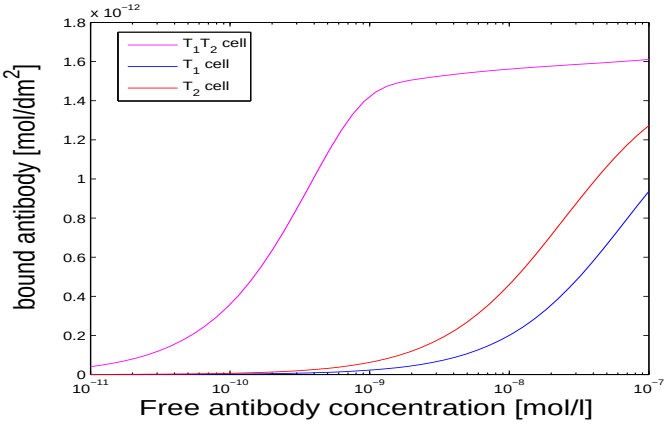

Figure 5: Results for the modified antibody: Total bound antibody concentration as a function of free antibody concentration in solution.

4.2 The PDE Model

It is known that the possible role of diffusion in this antibody-antigen interaction is not explicitly defined in the ODE model presented. Under certain circumstances binding may be diffusion limited. For example, after one antibody arm has bound the first target on cell membrane, there may be a delay before the other arm binds due to the time it takes for a nearby target to diffuse to within reach of the second arm. Likewise, if the well-stirred assumption does not hold in the volume compartment, antibody concentration gradients can develop near the cell surface. Therefore, in this section we attempt to account for diffusion by devising a PDE model.

Assumptions

1. We simplify the full geometry and consider only a single cell membrane surface in contact with a large volume of solution containing mAbs (Fig. 6).
2. The mAbs diffuse through the solution with isotropic diffusion coefficient D_A and are initially distributed evenly through the solution at concentration $[A]_0$.
3. At the cell surface, the unoccupied mAbs $[A]$ come into contact with target molecules $[T_1]$ and $[T_2]$ and form *one-arm* complexes (either $[AT_1]$ or $[AT_2]$). We describe this with a flux condition at the boundary between the solution and the cell surface.
4. After binding *one-arm* to a target molecule on the surface, there is likely a delay in binding with the other arm (to produce the *two-arm* complex $[AT_1T_2]$). We assume that target molecules diffuse across the cell surface with some diffusion coefficient D_S.

Let us define the following regions of the computational domain Γ:

$$\text{Solution containing mAbs} \quad \Gamma_1 := \{L_s < x < L_x, 0 < y < L_y\}$$

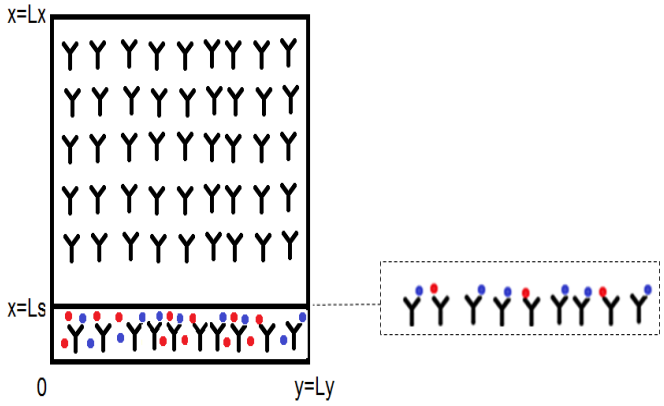

Figure 6: Schematic of the geometry of the simplified PDE model. A solution of evenly distributed mAbs is in contact with a single cell membrane of breadth L_y and thickness L_S. One-arm binding between mAbs and single targets occurs at the surface located at $x = L_S$ (shown in insert). Target molecules diffuse across the cell surface and two-arm binding may occur.

$$\text{Cell membrane layer} \quad \Gamma_2 := \{0 < x < L_s, 0 < y < L_y\}$$
$$\text{Cell membrane surface} \quad \Gamma_3 := \{x = L_s, 0 < y < L_y\}$$

In the solution (step 2) we then have:

$$\frac{\partial [A]}{\partial t} = D_A \left(\frac{\partial^2 [A]}{\partial x^2} + \frac{\partial^2 [A]}{\partial y^2} \right), \quad \text{on } \Gamma_1, \quad \text{subject to} \quad [A(x, y, 0)] = [A]_0. \tag{6}$$

Zero flux conditions are imposed on all external boundaries ($\Gamma \setminus (\Gamma_1 \cup \Gamma_2 \cup \Gamma_3)$). At the boundary between the solution and the cell membrane we impose a flux condition which accounts for the initial one-arm complex production (step 3):

$$-D_A \frac{\partial [A]}{\partial x} = k_1 [T_1][A] - k_{-1}[AT_1] + k_2 [T_2][A] - k_{-2}[AT_2], \quad \text{on } \Gamma_3. \tag{7}$$

Finally, within the cell membrane we have five reaction diffusion equations (step 4):

$$\frac{\partial [T_1]}{\partial t} = D_S \frac{\partial^2 [T_1]}{\partial x^2} - k_1 [T_1][A] + k_{-1}[AT_1], \quad \text{on } \Gamma_2, \tag{8}$$

$$\frac{\partial [T_2]}{\partial t} = D_S \frac{\partial^2 [T_2]}{\partial x^2} - k_2 [T_2][A] + k_{-2}[AT_2], \quad \text{on } \Gamma_2, \tag{9}$$

$$\frac{\partial [AT_1]}{\partial t} = D_S \frac{\partial^2 [AT_1]}{\partial x^2} - k_3 [T_2][AT_1] + k_{-3}[AT_1T_2], \quad \text{on } \Gamma_2, \tag{10}$$

$$\frac{\partial [AT_2]}{\partial t} = D_S \frac{\partial^2 [AT_2]}{\partial x^2} - k_4 [T_1][AT_2] + k_{-4}[AT_1T_2], \quad \text{on } \Gamma_2, \tag{11}$$

$$\frac{\partial [AT_1T_2]}{\partial t} = D_S \frac{\partial^2 [AT_1T_2]}{\partial x^2} + k_3 [T_2][AT_1] - k_{-3}[AT_1T_2] + k_4 [T_1][AT_2] - k_{-4}[AT_1T_2], \quad \text{on } \Gamma_2, \tag{12}$$

subject to the initial conditions:

$$[T_1(x,0)] = [T_1]_0, \;\; [T_2(x,0)] = [T_2]_0, \;\; [AT_1(x,0)] = [AT_2(x,0)] = [AT_1T_2(x,0)] = 0. \tag{13}$$

where $[T_1]_0$ and $[T_2]_0$ are the initial (uniform) densities of target molecules on the cell membrane surface. Note that, as with the ODE model (1), we must have conservation of target binding sites.

4.2.1 Numerical solution

The PDE model requires to be solved numerically. One such approach would be to devise a finite element scheme by considering the corresponding variational formulation of the model. The results of such a model will yield important information regarding the spatial gradients as well as the effect of diffusion on the binding processes.

5 Conclusions and Future Work

In this report we have attempted to use mathematical modelling to better understand the situations in which bispecific mAbs are preferential over a combination of monospecific mAbs. We started by considering the case of bispecific mAbs and an even distribution of two targets in solution versus the same overall concentration of two monospecific mAbs and two targets in solution. We concluded that from the target occupancy point of view, both the combination of monospecific mAbs and the bispecific mAb approaches are identical. However, we noted that the combination mAb approach is likely to allow greater flexibility in dosing and would therefore have the practical advantage. We then considered the more relevant case of targets anchored to the surface of a cell and developed an ODE model to describe the various binding mechanisms. The ODE model has produced very encouraging results which broadly agree with published experimental data. Discrepancies between the simulations and experimental results may well be attributed to rapid dissociation of monovalently bound antibodies of lower affinities from single-positive CD4+/CD70- and CD4-/CD70+ cells or the need to consider more explicitly the surface target diffusion in the model. We hypothesised that a PDE model which accounts for target diffusion may yield better agreement with the experiments and we have written down a preliminary PDE model. Further development of this PDE model is expected to provide a useful comparison for the ODE model and experimental results. Future work should also explore the possibility of deriving modified binding rates to account for the diffusive processes. This would represent something of a compromise between solving the full PDE model and qualitatively capturing the delayed binding effect as a result of diffusion. These modified rates should then be incorporated within the ODE model, and compared with the original ODE model, the experimental results, and the PDE model.

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