Concept Paper

The Binding Landscape of Serum Antibodies: How Physical and Mathematical Concepts can Advance Systems Immunology

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Abstract: Antibodies constitute a major component of serum on protein mass basis. We also know that the structural diversity of these antibodies exceeds that of all other proteins in the body and they react with an immense number of molecular targets. What we still cannot quantitatively describe is, how antibody abundance is related to affinity, specificity and cross reactivity. This ignorance has important practical consequences: we also do not have proper biochemical units for characterizing polyclonal serum antibody binding. The solution requires both a theoretical foundation, a physical model of the system, and technology for the experimental confirmation of theory. Here we argue that the quantitative characterization of interactions between serum antibodies and their targets requires systems-level physical chemistry approach and generates results that should help create maps of antibody binding landscape.

Keywords: antibody; binding energy; binding landscape; logistic curve; network

1. Introduction

Our immune system is responsible for more than protecting us from pathogens. It also regulates the removal of our own molecules and cells, once these are losing function due to ageing, attrition, infection. It maintains a healthy balance with the myriad of microbes and viruses present in our bodies[1]. To carry out these functions, the system utilizes an intricate regulatory mechanism that tunes its potential for destruction over a very wide range[2][3]. The humoral adaptive immune system consists of cells (B cells) and soluble molecules (antibodies) and has the remarkable ability to generate an immensely diverse repertoire of its element by adjusting, amongst others, one critical factor of molecular interactions: affinity[4,5]. Together with albumin and other macromolecules antibodies (Ab) create a molecularly crowded environment in blood, where molecules are in constant interaction with each other. Because of the huge structural diversity of antibody binding sites these interactions in the blood and with all molecules and cells contacted by blood, the strength of binding interactions also spans a huge range. In this article we expand a conceptual framework, based on physics and B-cell differentiation, for the distribution and organization of antibody interactions and argue that a recently developed quantitative serology technology is suitable for characterizing the proposed model.

2. Immunological and Physical Rules of the System: B Cells as Sensors and Effectors

Antibodies are present in three main forms in blood: as part of a receptor complex, the B-cell antigen receptor (BCR) or membrane immunoglobulin (mIg), with cellular signaling capacity[6–8]; in secreted, freely circulating form (this is what we usually refer to as serum antibodies)[9][10], and in receptor-bound form, attached to the immunoglobulin Fc receptors of cells[11–13]. The last form is responsible for effector functions and is not dealt with in this article, but is also important in quantitative modeling of antibody homeostasis.

Based on the form of antibody they express there are three categories of B cells: 1) resting naïve B2 lymphocytes and memory B cells (MBC) display BCR but do not secrete Abs; 2) activated B1 cells, pre-plasmablasts, lymphoblasts express both surface and secreted Ab; 3) plasma cells, such as short-lived plasma cells (SLPC) and long-lived plasma cells (LLPC) only secrete antibodies. The second and third group together is also called antibody secreting cells (ASC). In accord with these categories, these cell types function as antigen (Ag) sensors, as both sensors and effectors and as effectors only (Figure 1). A feedback mechanism based on antigen concentration and antibody engagement operates to generate sensors and effectors against all potential targets[8]. In short, the extent of antigen binding to BCR determines cell survival via signals delivered through the BCR. Too much or too little BCR engagement leads to cell death, while the proper extent of BCR engagement initiates cell activation or cell survival. Activated B cells become lymphoblasts, with the ability to secrete antibody and yet depend on BCR signals for survival. Terminally differentiated antigen secreting cells, plasma cells, do not depend on BCR signals but produce secreted antibodies, which in turn reduce the concentration of target antigen. As antigen is cleared the immune response retracts, short-lived effector cells (SLPC) die and new steady state equilibrium is established. Affinity maturation of antibodies changes the concentration of antigen required for a given extent of antibody engagement, therefore results in memory cells capable of more sensitive detection (sensor MBC) or more effective removal (LLPC) of antigen. The new equilibrium allows MBC with increased sensitivity to survive, backing up the front line of secreted antibodies. Cycles of these events shape the lymphocyte repertoire and the theoretical space of all antibody interactions.

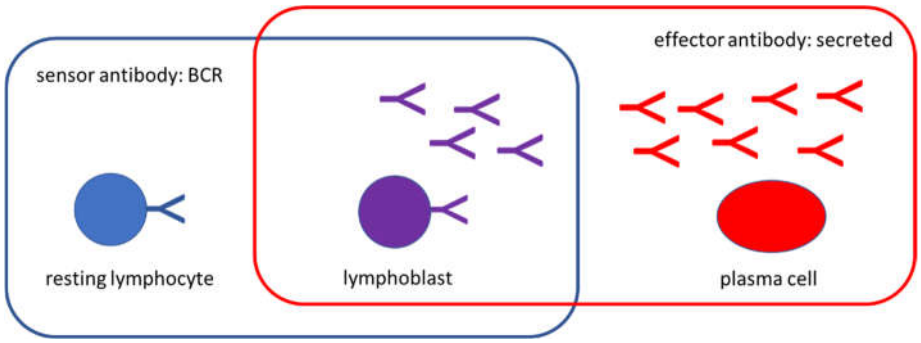

Figure 1. Categorization of antibodies based on sensor and effector function. The two functional types of antibodies, two corresponding cell types and the mixed type are shown. BCR, B-cell receptor.

3. The Configuration Space of Serum Antibodies

From the medical and biological perspective, the humoral immune response takes place in various anatomical locations of the host: lymph nodes, spleen, bone marrow, blood or periphery. The specialized structure of these tissues contributes to the development, differentiation and activation of B-cells and antibody secreting cells. Nevertheless, all these tissues are physically connected, and while cell trafficking is regulated and cells are not allowed to go anywhere, secreted circulating antibodies do reach most tissues. Naïve and memory B cells are recruited into the pool of ASC by antigenic stimulation and co-stimulation by other cells. There is a continuous supply of antibodies from ASC into the circulation, along with a continuous removal via immunoglobulin Fc receptors on immune effector cells. This flow of antibodies maintains target antigen concentrations at levels defined by the immune system. Where antibodies are present, they continuously search for their highest affinity binding partner – in other words for their lowest energy bound state. For a physical interpretation of the whole system of antibody interactions, it is reasonable to simplify the system, neglect anatomy and introduce an abstract space instead: the antibody interaction space.

This interaction space can be thought of as a coordinate system of chemical potentials. Chemical potential here refers to the ability of the system to contribute to the generation of Ab-Ag complexes. An Ab with given specificity can be identified by a vector pointing towards a given direction in the landscape of molecular targets (Figure 2). The chemical potential of the antibody is determined by the affinity (standard chemical potential), the concentration and its thermodynamic activity coefficient (see later). In the center of the system is the generation of lymphocyte precursors, which develop into antibody secreting cells as they mature[14]. Within the boundaries of the system cells generate a diverse repertoire of surface antibodies (B-cell receptors, BCR) that allows them to probe the complete antigen landscape or antigenome. In fact, BCRs are probing not whole molecules but rather patches of molecular surfaces called epitopes. We can think of the horizon of interaction space as the continuity of epitopes forming a canvas around the interaction space, as the landscape of target molecular surface patterns. Once a B-cell starts secreting an antibody, it will push the boundary of the system towards the recognized epitope to an extent determined by its chemical potential (Figure 2).

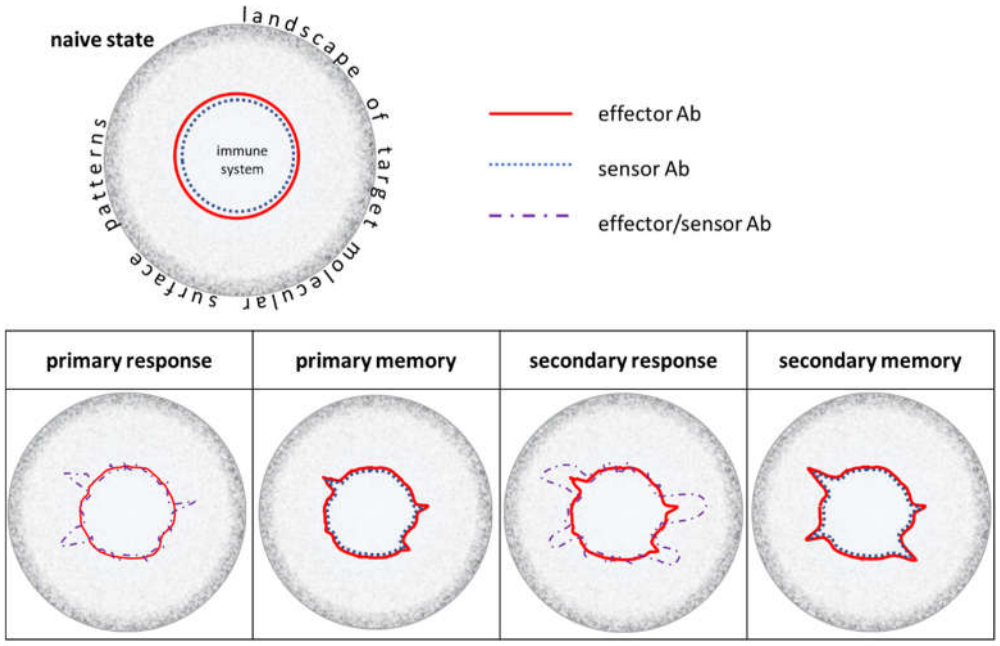

Figure 2. Immune responses displayed in configuration space of antibody interactions. Distance of the lines representing immune system boundary from the center corresponds to chemical potential.

During an immune response, naïve and memory cells of the adaptive immune system are activated, expanded and differentiated. B cells in germinal centers undergo affinity maturation: somatic hypermutations introduce changes into antibody structure, followed by the selection of structural variants with higher affinity[4,5,15,16]. The process gives rise to genetically different new clones carrying antibodies with higher chemical potential. As long as the stimulus persists, germinal centers generate these new clones by cycles of random somatic hypermutation and selection. The result is the expansion of the system in the configuration space (Figures 2 and 3): sensor-effector lymphoblasts start secreting antibodies and also increase their antibodies' affinity by mutations.

As the stimulus is cleared by the immune response, most effector cells die and only memory cells remain. This corresponds to a retraction and reorganization in configuration space. It is important to note that the new boundary of the system is established by the negotiation between the host and the intruder: very harmful intruders will tend to leave a long-lasting and high affinity imprint, while softer attacks will have weaker effects. Regulatory mechanisms in the host also cut back clones with potentially harmful autoimmune effects. This negotiation results in a steady state, which entails the formation of networks that insert newly generated clones into a previously established architecture. The system

of interactions optimizes itself: randomness is finally replaced by hierarchy and optimized antibody cross-reactivity networks. Activated cells will disappear, with resting lymphocytes and LLPC with adjusted affinity surviving (Figure 3).

The immune system is never totally at rest. It is the dynamism, the constant restructuring of this landscape by antigenic stimuli that maintains system architecture and adjusts the configuration space to the molecular environment. Therefore, whilst the overall hierarchy is expected to be governed by the laws of physics, shifting and changing locally active sites respond to the biological needs of the system.

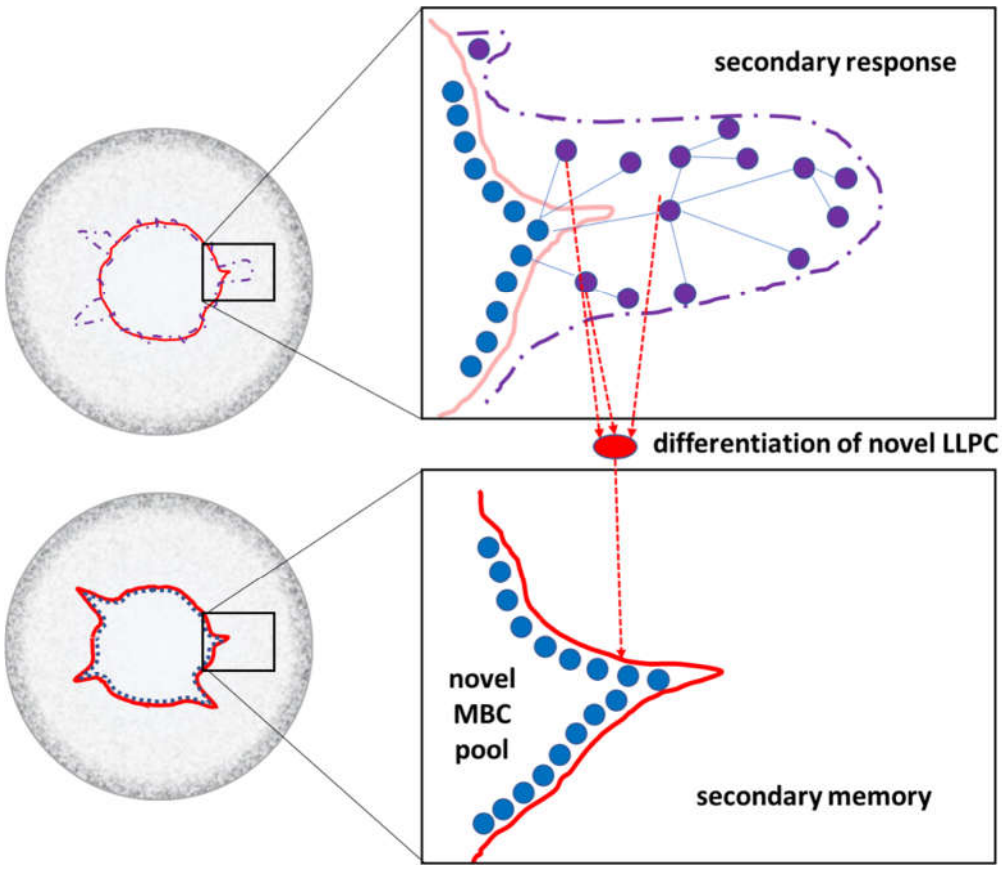

Figure 3. Expansion and affinity maturation of germinal center B cells displayed in configuration space. Naive and memory sensor B cells seed an active response, being activated via BCR. Somatic hypermutations generate random shifts in configuration space (blue lines between purple nodes), selection of higher affinity mutants produces lymphoblasts. Secretion of antibodies with higher affinity appears as a protrusion of the interaction space towards the targeted antigen. Following an active immune response, the system retracts leading to a steady state with new borders, corresponding to LLPC and MBC with an affinity higher than original.

4. Probing Serum Antibody Configuration Space: Quantitative Systems Serology

Understanding the underlying hierarchy and architecture of the network of antibodies has an immediate practical use: the design of serological assays with results that characterize this network. Current serological assays are standardized according to medical purposes, with the aim of establishing optimal cut-off values for diagnostic accuracy. The units obtained this way do not allow any kind of comparison of results even for the identical antigen when different platforms are used or different antibody isotypes are measured. The units are standardized but arbitrary with no biochemical meaning [17].

By using the configuration space model, we can identify the parameters that are required to describe such a system. Considering that serum antibodies are mixtures of molecules with a wide range of affinities against antigenic targets and wide range of concentrations of each molecule, it is reasonable to assume that these parameters need to be esti-

mated. We can probe this space by measuring the formation of antigen-antibody complexes in immunoassays and map the space by applying mathematical functions that model physical properties of the system.

The logistic function (also referred to as logistic equation, logistic growth curve or Verhulst model) describes population growth with an exponential growth limited by maximum capacity of the system. While originally it was introduced for modeling growth in time, it is also used for modeling chemical reactions and antibody-antigen binding reactions. In immunoassays we follow the increase of concentration of reaction products (Ab-Ag complexes) as a function of the logarithm of increasing concentrations of a reactant. Thus, the growth in this case is not in time but along an experimentally created concentration series. Time factor in these reactions can be omitted if the reaction is allowed to reach a point where concentrations of reaction components do not change any more: equilibrium is reached.

For a reaction where we increase Ag concentration and follow the concentration of bound Ab (equivalent to measuring Ab-Ag complexes), we can rewrite the differential equation

$$\frac{dN}{dt} = r * N * (1 - \frac{N}{K}) \tag{1}$$

where N is the number of entities and K is the capacity of the system for such entities, and r is the rate of exponential growth, as

$$\frac{dAb_b}{dlog(Ag)} = r * Ab_b * (1 - \frac{Ab_b}{Ab_t}) \tag{2}$$

where Ab_b is bound antibody concentration, Ab_t is total antibody and Ag is total antigen concentration.

This means that the rate of change of generation of bound antibody while increasing Ag is determined by the actual bound antibody concentration, its relationship to the total antibody concentration and the rate parameter. Because of the use of the logarithm of Ag concentration the increase of bound antibody concentration is exponential, limited by the availability of antibody, as expressed in the second part of the function.

We can simplify this expression by using the relative proportion of concentration of antibody bound under equilibrium conditions to total antibody concentration. This is in fact characterized by the thermodynamic activity coefficient of serum antibodies γ_{Ab}, a coefficient that adjusts concentrations to relative thermodynamic activity a

$$a = \gamma_{Ab} * Ab_t \tag{3}$$

Using $\gamma_{Ab} = a/Ab_t$ and $a = Ab_b$, equation (2) becomes

$$\frac{d\gamma_{Ab}}{dlog(Ag)} = r * \gamma_{Ab} * (1 - \gamma_{Ab}) \tag{4}$$

The explicit solution of this differential equation is the function known to immunologists as the four-parameter logistic function or 4PL (see Appendix A) with a lower limit of zero. The 4PL can be used to estimate the affinity of a monoclonal antibody with known concentration [18]. However, the 4PL models an ideal binding curve, which may not reflect real binding that is modified by other interactions. To allow for an asymmetry in the binding curve and thereby take into account such intricate events, the five-parameter model, 5PL was introduced (see Appendix A) [19].

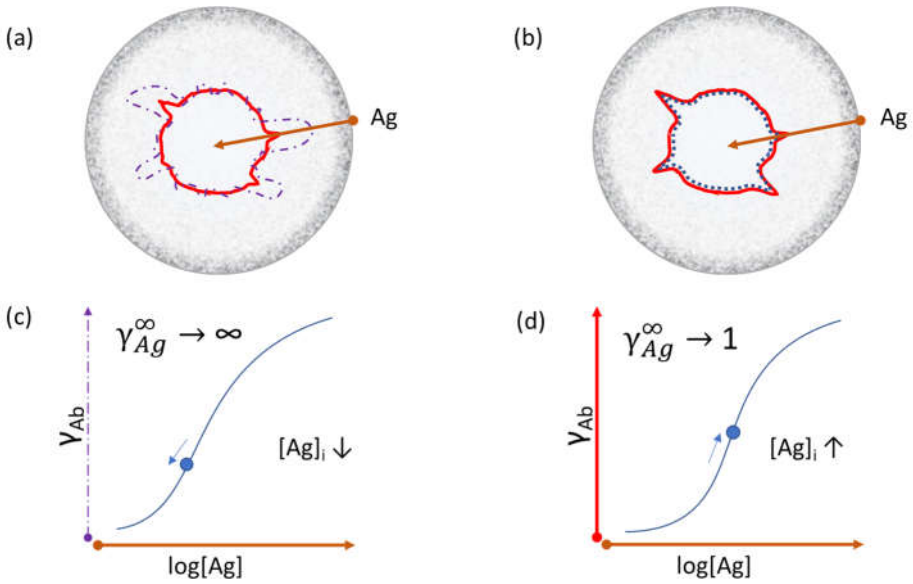

Figure 4. Probing the configuration space with antigen. Configuration space (a and b) can be probed (brown arrow) by measuring the changes of chemical potential of serum antibodies with the antigen of interest, using an immunoassay. Using antigen microspot titration key parameters of interaction, such as standard chemical potential and limiting activity coefficient can be modeled by the Richards curve (c and d), including the determination of inflection point (blue circle) position. During an active immune response (a and c), the apparent affinity increases, as reflected by a decreased average standard chemical potential, and changes in clonal composition alter the limiting coefficient. A memory response (b and d) is characterized by optimized affinity and clonal heterogeneity. $[Ag]_i$, antigen concentration at point of inflection.

However, while the 5PL is the solution of a modified differential equation, it is less suitable for the description of a system's behavior because of its parametrization. First order differential equations define relationships between functions representing physical quantities and their derivatives, the latter representing rates of change of the physical quantity. In an immunoassay the physical quantity is the amount of antigen-antibody complex (or bound antibody) formed during the assay, once equilibrium is reached. The rate of change in this quantity, while changing reaction conditions by titrating antibody or antigen, is the derivative of the function that relates the amount of complex formed to the logarithm of the titrated component. We proposed the use of the generalized logistic model or Richards growth model[20,21] instead of 5PL[22], because the Richards growth function, like the 5PL, is the solution of the differential equation

$$\frac{d\gamma_{Ab}}{dlog(Ag)} = \frac{r}{\nu} * \gamma_{Ab} * (1 - \{\gamma_{Ab}\}^{\nu}) \quad (5)$$

but is parameterized in a way that $[Ag]_i$ is the point of inflection of the curve. This differential function implies that besides the activity coefficient of antibody and the rate parameter, the rate of generation of bound antibody while increasing Ag, is determined by a power of the activity coefficient. The exponent in the power expression ν is a parameter that modifies the influence of the ratio $\frac{Ab_b}{Ab_t}$ on the rate of growth of bound antibody. The reason for this modification is the changing behavior of antibodies at different antigen concentrations. This parameter introduces asymmetry into the sigmoid binding curve, in a way that is more suitable for the description of the system. We propose that ν is related to a special activity coefficient, $\gamma_{Ag}^{\infty} = 1/\nu$, which defines Ag thermodynamic activity at infinite Ab dilution, and is determined by the composition of antibodies in the total pool (Figure 4 and 5).

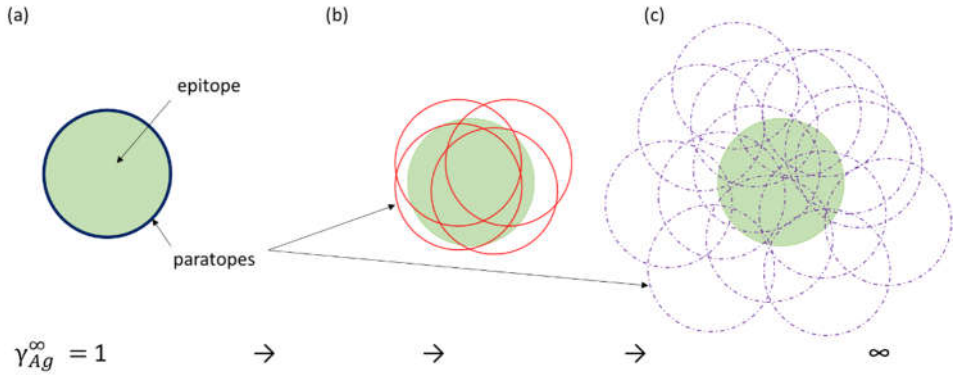

Figure 5. Interpretation of the limiting activity coefficient of antigen. The limiting thermodynamic activity coefficient reflects the contribution of epitope to binding by all antibody structures or the epitope-paratope fit in other words. Only the outlines of the paratope surfaces are shown (circles) to allow the visualization of overlaps. (a) A monoclonal antibody paratope-epitope fit is shown for comparison. Memory formation (b) reduces surviving clones to minimal optimal binders, while during an active immune response (c) several structurally distinct antibodies co-exist and compete for binding.

By fitting the Richards curve to experimental binding data from Ag and Ab titration experiments we can obtain the parameters that are suitable for the quantitative characterization of serum Ab. One of these is the antigen concentration at the point of inflection $[Ag]_i$ and the other is the limiting activity coefficient (Figure 4.) [22]. The first is an estimate of the apparent equilibrium dissociation constant (determined by average standard chemical potential) of the antibodies bound to the Ag. The second characterizes the hierarchy of antibodies bound to the Ag. The mapping of these values to collections of structurally related epitopes could serve as a starting point for describing the serum antibody binding landscape.

5. Steps towards Systems Serological Mapping of Immunity

The key message of our article is that the binding landscape of serum antibodies cannot be approached as the simple sum of individual, independent interactions. The word binding is rather meaningless unless we identify conditions and quantify interaction energy. Blood plasma is crowded with macromolecules, with a significant contribution from circulating antibodies. The conditions are therefore defined by the composition of antibodies, by the intricate cross-reactivity network of antibodies, their structures and concentrations. Most of the immunological studies have been directed towards defining how an active immune response happens. Molecular biology helped us clone, sequence and recombinantly express monoclonal antibodies. Structural biology allowed the characterization of antibody structures in detail. Now it is time to organize this information into a complex biological system. We propose that instead of examining the active phase of an immune response, the characterization of landscape of serum antibody binding in steady state is a better goal from the point of view of physics.

The ability to quantitatively characterize and map serum antibody binding to vast collections of antigens can open several possibilities. Via the standardization of simplex measurements, we could generate comparable binding data from quantitative immunoassays and integrate that into epitope databases. Epitope databases would develop into quantitative databases in terms of incorporating binding strength data. By generating antigen arrays with whole molecule antigens, peptides, and modified random peptides suitable for binding strength quantitation we can attempt to create complete maps of serum antibody binding landscape. By the selective detection of isoforms: IgG, IgA, IgM, IgE a further dimension, related to biological effects, can be introduced into the database. On the long term such quantitative maps of individual's Ab interaction spaces should become the foundations for immunodiagnostics and therapeutics as well.

Supplementary Materials: Figure S1: title; Table S1: title; Video S1: title.

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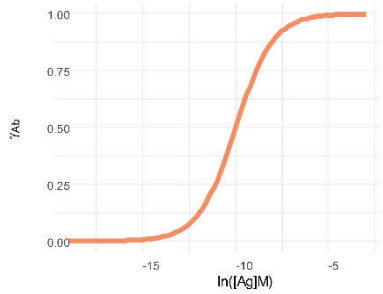
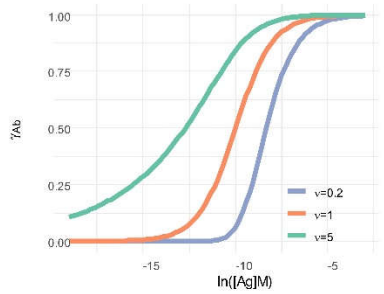
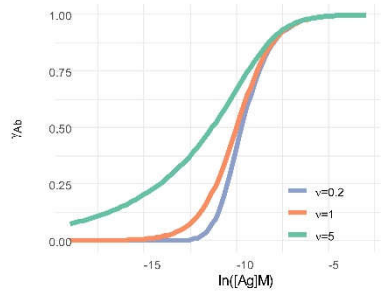
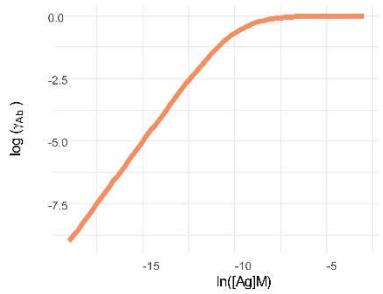
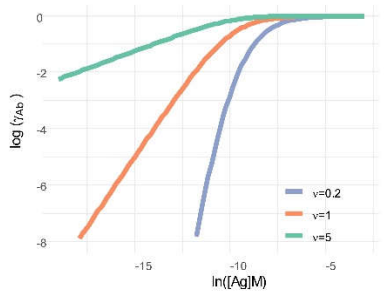
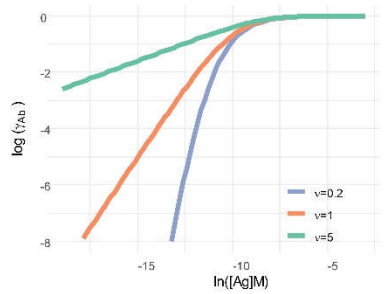
Appendix A

Comparison of logistic functions

Basic function: $y = \frac{1}{1+(\frac{x}{x_i})^{-r}}$

	4PL	5PL	GL
x=[Ag] x_i=[Ag]$_i$	$\gamma_{Ab} = \frac{1}{1+(\frac{[Ag]}{[Ag]_i})^{-r}}$	$\gamma_{Ab} = \frac{1}{\left\{1+(\frac{[Ag]}{[Ag]_i})^{-r}\right\}^{\frac{1}{\nu}}}$	$\gamma_{Ab} = \frac{1}{\left\{1+\nu(\frac{[Ag]}{[Ag]_i})^{-r}\right\}^{\frac{1}{\nu}}}$
x=log[Ag] x_i=log[Ag]$_i$	$\gamma_{Ab} = \frac{1}{1+e^{-r(\log[Ag]-\log[Ag]_i)}}$	$\gamma_{Ab} = \frac{1}{\{1+e^{-r(\log[Ag]-\log[Ag]_i)}\}^{\frac{1}{\nu}}}$	$\gamma_{Ab} = \frac{1}{\{1+\nu e^{-r(\log[Ag]-\log[Ag]_i)}\}^{\frac{1}{\nu}}}$
diff. eq.	$\frac{d\gamma_{Ab}}{dlog[Ag]} = r\gamma_{Ab}(1-\gamma_{Ab})$	$\frac{d\gamma_{Ab}}{dlog[Ag]} = \frac{r}{\nu}\gamma_{Ab}(1-\{\gamma_{Ab}\}^{\nu})$	
f(x)			
y = $log\gamma_{Ab}$	$log\gamma_{Ab} = -log\,(1+e^{-r(log[Ag]-log[Ag]_i)})$	$log\gamma_{Ab} = -\frac{1}{\nu}\log\,(1+e^{-r(\log[Ag]-log[Ag]_i)})$	$log\gamma_{Ab} = -\frac{1}{\nu}\log\,(1+\nu e^{-r(\log[Ag]-log[Ag]_i)})$
log f(x)			

Representative curves in the diagrams use identical [Ag]$_i$ and the indicated ν values. diff.eq., differential equation

To help the comparison of the three logistic functions the minimum and maximum values are set to 0 and 1, respectively. This simplifies the functions, though the number of parameters is less than suggested by the name. This normalization also allows to treat y as the thermodynamic activity coefficient of Ab, instead of the concentration of bound antibodies. The basic function is modified as implicated in the table to obtain the respective functions: four-parameter logistic (4PL), five-parameter logistic (5PL) and generalized logistic (GL) or Richards function. As the functions show, compared to the 4PL, the 5PL function introduces parameter ν, as the exponent 1/ν of the denominator. The generalized form, in addition to this change, also introduces ν as multiplying factor of the ratio of antigen concentration [Ag] and antigen concentration at inflection point [Ag]$_i$ with power -r.