Matching cellular dimensions with molecular sizes

Michael Reth

This Commentary discusses the spatial perception of receptors and their nanoscale organization at the surface of the lymphocyte membrane.

The topic of immunoreceptors and their organization in the plasma membrane seems to represent a confusing area to most immunology students and even to more experienced researchers. Most students think of the plasma membrane as a lipid bilayer in which the membrane proteins float around chaotically until they are induced to cluster by contact with other cells or after soluble ligands bind. As most immunology professors have probably discovered, many students cannot approximate the number of various receptor proteins present on the cell surface, nor do they know the real size of those proteins relative to the size of the cell surface. A possible reason for this lack of clear information or even misunderstanding about the number, size or density of membrane receptors could be the widespread trend for most immunology textbooks, together with many immunology review articles, to generally ignore many quantitative aspects of biological receptors.

Too big to be true

In addition to loving sugar, the human brain loves pictures, and therefore most science and immunology textbooks abound with drawings. The problem is, however, that none of these textbooks include an introductory chapter or clear specifications in figure leg-

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ends about molecular sizes and the correct way to interpret such schematic drawings. For example, the ratio of the size of a B cell to that of an antigen receptor (depicted as a membrane-bound antibody molecule) in a typical figure in a popular immunology textbook (**Fig. 1a**) would suggest that the receptor depicted would measure about 3 μm in length, compared with a resting B lymphocyte, with an average diameter of about 7 μm. Even though it is likely that no one would accept such a drawing at face value, such schematic representations stick in the mind. The real size of an antibody molecule is about 10 nm, and thus the antibody depicted would not be visible on the surface of the B cells if drawn to scale, but this is not clearly specified in the figure legend.

The relationship between cellular dimensions and the size of molecules is not naturally understood, and thus students are not alone in being uncertain of the sizes and organizational principles of membrane proteins. Nanometer distances are not intuitive for human brains, which have been evolutionarily optimized to judge distances in the range of 1 m to 100 m. Within milliseconds, the brain can calculate the distance between an approaching lion, the closest life-saving tree and the owner of that brain. However, viewing the moon and the stars in the night sky does not provide a good estimation of the size of such cosmic objects and the immense distances between them. Humans do not have a natural 'feeling' for the difference between 1 light year and 100 light years. The same holds true for objects smaller than 1 mm. How small is 'small', and what is the difference between 1 nm and 100 nm? Despite all these limitations on spatial perception, the

human brain can rely on imagination—and according to Albert Einstein, imagination is more important than knowledge. To aid in the understanding of molecular sizes in relation to cellular dimensions, I propose the following *Gedankenexperiment* (thought experiment).

A walk in the park

A useful way of understanding such relationships is to envision a membrane-bound antibody molecule that is the size of the human body. In fact, an antibody shares with the human body such common design principles as a bilateral symmetry and two freely moving 'arms' ready to grab things, such as antigen (**Fig. 1b,c**). If an antibody were the size of an adult human, how big would the surface of a lymphocyte be? If a lymphocyte were assumed to have a somewhat folded plasma membrane, the available area for the human-sized antibody to move around would be 9.9 km^2. That area happens to be roughly the size of Freiburg (the old town and suburbs), or three times larger than Central Park in New York City (3.4 km^2). Thus, the total surface of the lymphocyte would cover the entirety of Central Park and large parts of the adjacent Upper West Side and Upper East Side of Manhattan (**Fig. 2**).

Strolling through Central Park, humans the size of membrane-bound antibody molecules could probably walk for several days without completely covering this enormous 'membrane' surface. On a walk starting at 72nd Street and Central Park West, in Strawberry Fields on the 'Imagine' mosaic dedicated to the memory of John Lennon, and then heading south toward Sheep Meadow, it would become clear that such human antibodies are not equally distributed but instead tend to cluster
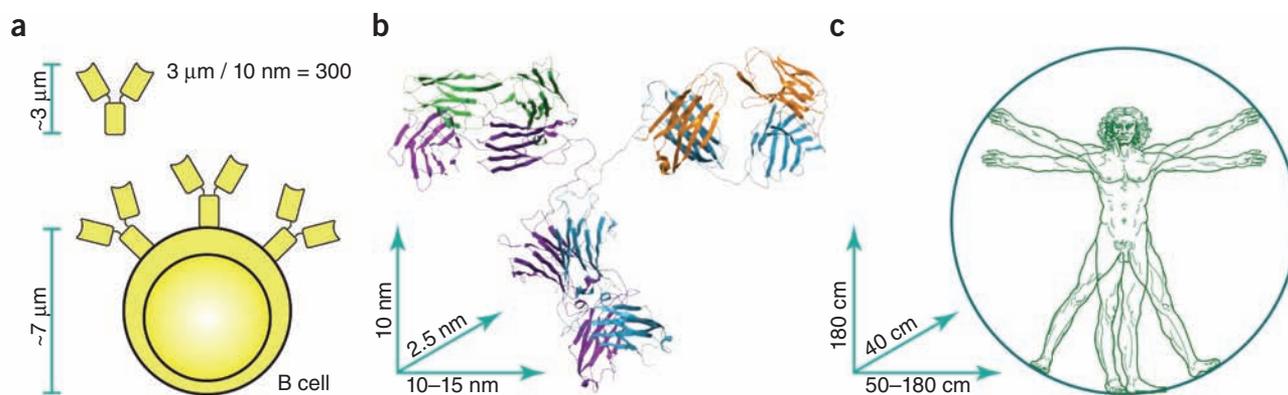
Figure 1 Size comparison of a textbook model of a B cell, an antibody molecule and the human body. (**a**) B cell with attached membrane-bound antibody molecules[26], and their apparent sizes. Copyright 2011, *Janeway's Immunobiology*, 8th Edition, by K.M. Murphy. Reproduced with permission from Garland Science/Taylor & Francis LLC. (**b**) Structure and size of an IgG2 antibody molecule (Protein Data Bank accession code, 1IGT). (**c**) An adult man and his average size.

as they walk slowly along the paths or lie on the grass. This is a good way of appreciating the nanocluster organization of proteins in the membrane[1,2]. On closer inspection, it becomes apparent that 'human membrane proteins' of the same family prefer to stick together. That observation, first made for the Lat family of membrane adaptors by electron microscopy[3], despite being not universally accepted or completely understood, has been confirmed for several other proteins[4–6] and represents one of the most exciting discoveries of membrane research in the past 10 years.

South of Sheep Meadow, the Chess & Checkers House is situated on the former *Kinderberg* (children's mountain). The pieces on the chess tables correspond roughly to the size of an amino acid backbone. Viewed south from the Chess & Checkers House, the Empire State building is visible in the far distance. Its height (443.2 m) corresponds roughly to the size used in most immunological textbooks for an immunoglobulin molecule relative to the surface of an Upper Manhattan–sized lymphocyte surface. This comparison can help in visualizing the magnitude of misrepresentation in most schematic drawings in textbooks. North toward the Reservoir, ball games are played at the many baseball fields on the Great Lawn. The infield's dimensions correspond to less than half the area of the 250-nanometer diffraction limit spot of a light microscope (**Fig. 3**). Thus, live-imaging techniques would allow visualization of the movements of the players but never directly of the players themselves in the infield[7]. During concerts in the park on the Great Lawn, thousands of human-sized antibody molecules assemble in this section of the park, leading to a very crowded 'membrane'. For example, 500,000 humans assembled on 19 September 1981 for the historic Simon and Garfunkel concert on the Great Lawn.

Similarly, the whole lymphocyte surface in a crowded state can carry several million receptor molecules. On a random October night, however, a 'human antibody' would be quite alone in Central Park.

If an actual visit to Central Park is not possible, the Google Earth view of it provides an alternative (**Fig. 2**). This aerial view is similar to the view of a cellular membrane through a confocal light microscope. The human antibodies are not visible, but the structures they have built are. For example, the orange baseball fields poking out of the green meadows are easily recognizable. Each baseball field has roughly the size of a receptor microcluster on the surface of activated lymphocytes

detectable by confocal light microscopy[8–10]. These structures can contain several hundred receptor molecules coupled to green fluorescent protein. Thus, these enormous receptor assemblies should not be mistaken for nanoclusters or protein islands on resting lymphocytes, which are organizational structures that contain only 10–50 molecules[4].

A walk in the park as a human antibody can thus provide better understanding of the dimensions and structural variability of the plasma membrane of lymphocytes and may amend some of the misperceptions that immunology textbooks and review journals impose on their readers when they do not properly explain the use of different size

Figure 2 Aerial view of Central Park in New York City (from http://www.alamy.com) and its correspondence to the size of the surface of a B cell. Size calculations are as follows: the surface of a sphere with a diameter of 7 μm is $4\pi \times (3.5)^2$, or 154 μm^2. The surface of a B cell could reach 300 μm^2 (given that the membrane is not flat). If a membrane-bound antibody were the size of a man, 10 nm would become 1.8 m, 7 μm would become 1.26 km, and 300 μm^2 would become 9.9 km^2. The area of Central Park is 3.4 km^2.
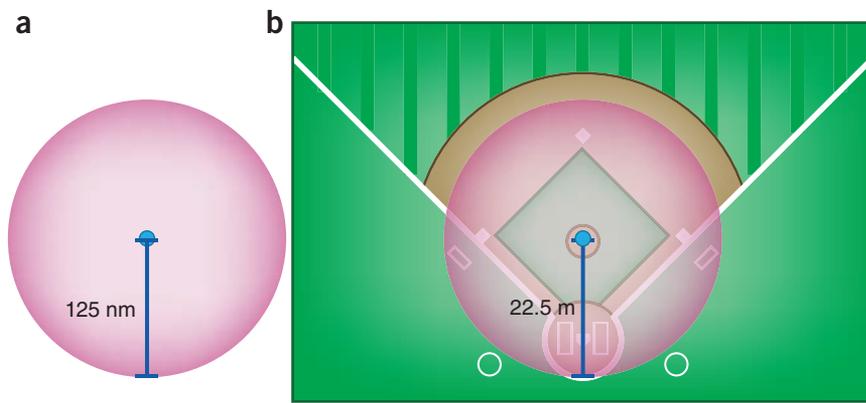
Figure 3 Comparison of a membrane-bound antibody in one diffraction-limited spot and a person on the infield of a baseball field. (**a**) Membrane-bound antibody (blue dot) in a diffraction-limited spot of a modern scanning confocal microscope with a full width at a half maximum of 250 nm. (**b**) A baseball field (from Wikipedia). If the membrane-bound antibody were the size of a human adult, one diffraction-limited spot would cover more than twice the area of the infield diamond.

scales in the same figure. It also reflects the emerging view that the plasma membrane of the resting lymphocyte is not a chaotic assembly of freely moving molecules but is a more organized space with lateral segregation of proteins and lipids in structures the size of nanometers. Such highly organized membrane domains have been found even on the surface of yeast and thus seem to be an evolutionarily highly conserved feature of cellular life[11,12].

Health and therapy implications

Understanding the biology and spatial distribution of membrane receptors represents an important task for future research. With an increasing number of biological reagents entering clinical use, membrane proteins are major targets for the treatment of human diseases. More profound knowledge of the nanoscale organization of the plasma membrane can improve the efficiency of such treatments. For example, the monoclonal antibody rituximab, which binds to the membrane tetraspanner CD20, has been used successfully for the treatment of B cell lymphomas and autoimmune diseases[13]. However, the exact location of CD20 on the surface of B cells and the nanoscale membrane neighborhood of CD20 are not well studied. Better understanding of these aspects of CD20 biology could explain why only a few antibodies to B cell–surface proteins are successful in treating disease.

Membrane proteins can also cause disease. This is true for tyrosine kinase receptors such as the EGF receptor[14]. The B cell antigen receptor (BCR) has also been found to carry alterations in the cytoplasmic tail of the immunoglobulin signaling subunits CD79a (immunoglobulin α-chain) and

CD79b (immunoglobulin β-chain) in up to 20% of diffuse large B cell lymphomas of the activated B cell type[15]. Furthermore, the most prevalent human B cell tumor disease, B cell chronic lymphocytic leukemia, is driven by an autonomous, aggregated and signaling BCR[16]. Better understanding of the nanoscale environment of the BCR in the resting state and its transformation during B cell activation might allow the identification of new treatments for such BCR-driven tumors. The crosslinking hypothesis, which proposes that crosslinking of the BCR takes place after activation, has been the prevailing BCR-activation model for the past 20 years[17]. The problem with the crosslinking hypothesis is that it assumes that in its resting state, the BCR is a freely diffusing monomer. The crosslinking hypothesis has many flaws, and it is somewhat surprising that it has prevailed for so long[18,19].

Future endeavors

Evidence suggests that dissociation of a highly regulated oligomeric BCR precedes receptor crosslinkage[20,21], and the race is on to learn more about the nanoscale organization of the BCR and other receptors on the resting B cell surface. This, however, is not an easy stroll but is a technically demanding hike. Membranes are still among the most difficult biological objects to study. Biochemical methods are limited by the problem that the lysis of cells with detergents destroys the membrane organization; light microscopy studies, by the diffraction barrier of visible light; and electron microscopy, by artifacts generated through the fixation and staining protocols associated with this technique. In the end, it may be a combination of improvements in all these techniques that will help to resolve the secrets of the nanoscale organization of biological

membranes. For example, super-resolution microscopy is a rapidly advancing new field that allows the resolution of structures below the diffraction limit in the 20- to 80-nm range[22,23]. High-pressure freezing techniques may allow the study of more naturally preserved membrane environments by electron microscopy[24]. The proximity-ligation assay is a promising new technique with which to study the relative location of two membrane proteins at distances on a nanoscale[25]. Thus, the next 10 years should provide better insight into the rules of membranes organized on a nanoscale and perhaps also new versions of textbooks that better explain the correct relative molecular sizes and their importance for the understanding of immune reactions at different molecular dimensions.

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