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Two and Three-Dimensional Pattern Recognition of Organized Surfaces by Specific Antibodies

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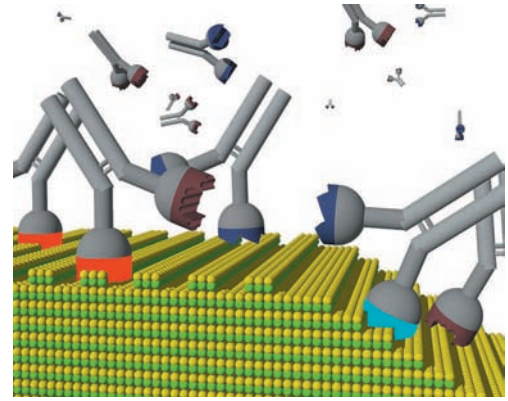

Understanding molecular recognition of supramolecules for solid substrates is essential for designing chemical sensors and molecular devices. The rules of molecular recognition are well established at the level of single molecules. However, during the transition from molecular-scale devices to macroscopic devices, issues concerning control over recognition that are well-established at the molecular level become much more complex. Hopefully, the conceptual and practical considerations reported here will clarify some of these issues.

The immune system uses antibodies to identify molecular surfaces through molecular recognition. Antibodies are thus appropriate tools to study the rules of macromolecule−surface interactions, and this was done using crystal surfaces as substrates. Crystals can be formed or introduced into organisms and should be thus treated by the organism as any other intruder, by eliciting antibodies specific to their surfaces.

A structure-recognizing antibody is defined here as complementary to a certain ordered supramolecular organization. It can be considered as a mold bearing in its binding site memory of the organization against which it was elicited. On the surface of a crystal composed of relatively small organic molecules, an antibody binding site would encompass an array of 10−20 molecular moieties. The antibody binding site would not detect one molecule, but rather a two- or three-dimensional molecular arrangement on the surface, similar to a macromolecular surface. The complementarity between antibody binding site and surface is supported by stereoselective supramolecular interactions to the repetitive structural motifs that are exposed at the surface.

A procedure was developed in order to isolate monoclonal antibodies that specifically recognize a certain crystalline surface. The procedure was applied in particular to crystals of cholesterol monohydrate, of 1,4-dinitrobenzene, and of the tripeptide (*S*)leucine-(*S*)leucine-(*S*)tyrosine (LLY). A series of antibodies were selected and studied, three of which provided reliable specific antibody−antigen structural models. The three docking models show an astounding geometrical and chemical match of the antibody binding sites on the respective crystal surfaces. We also showed that antibodies are intrinsically capable of recognition at the length scale necessary for detection of chirality.

Once the structural parameters determining the antibody specificity to the target surfaces are characterized, the antibodies may be conceivably used as reporters of the existence and location of target domains with similar structure in biological milieus. In this context, we developed and characterized monoclonal antibodies specific to crystalline mixed monolayers of cholesterol and ceramide, fundamental building blocks of lipid microdomains in cellular membranes. When used on cells, one antibody indeed labels cell membrane domains composed of cholesterol and ceramide.

The fundamental contribution of the approach developed here may be in the antibody ability to report on the structural organization of paracrystalline domains that cannot be determined by other means. Alternatively, structure-recognizing antibodies may be conceivably used to carry information or build connections to specific targets, which may offer interesting developments in medicine or electronics.

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Introduction

Molecular recognition between biological macromolecules and their substrates, such as antigen–antibody, DNA–protein, carbohydrate–lectin, is an essential component of the molecular basis of life. Within the framework of surpramolecular chemistry, J. M. Lehn defined molecular recognition as binding and selection of substrate(s) by a given receptor molecule, mediated through a structurally well defined pattern of noncovalent intermolecular interactions.[1]

Supramolecular chemists nowadays try to design complex new molecular functions based on inspiration from biological systems and with applications to medical devices as well as in the fields of electronics and the materials sciences.[2–4] Understanding molecular recognition in supramolecules and recognition of supramolecules for solid substrates is essential for designing chemical sensors and molecular devices. However, during the transition from molecular-scale devices to macroscopic devices, issues concerning control over recognition that are well-established at the molecular level become much more complex. The question of how biological macromolecules recognize organized solid surfaces has not been studied in detail, mainly because it was considered mostly, if not totally, nonspecific. Indeed, hydrophobic or electrostatic interactions are dominating factors that in general result in denaturation of proteins and of macromolecules upon contact with solid surfaces. But is this inevitable?

Researchers have recently been trying to apply the fundamental principles that have been established concerning recognition at the molecular level to larger length scales.[5–7] We wish to understand how molecular recognition is manifested at interfaces between macromolecules and organized surfaces, such as two- or three-dimensional crystals, at length scales ranging from nanometers to micrometers; up to 10 000 times larger than the angstrom scale of molecules. "Crystals are supermolecules par excellence"[8] and thus are especially suitable for the study of molecular recognition at higher length scales.

The important lesson that emerged early on from studies of biomineralization is that biological macromolecules may control many aspects of crystal formation.[9] They do so by virtue of matching the macromolecular structure to the crystal structure on one particular plane. Unfortunately, the structure of the proteins involved in direct interactions with the crystals in biomineralization are still largely unknown, such that the three-dimensional complementarity between these macromolecules and the mineral surfaces can only be indirectly deduced from studies on crystal growth, crystal morphology, and X-ray diffraction of the minerals.[10–12] One exception to this general dearth of information is in the area of antifreeze proteins, where the X-ray structure of some proteins was determined. The protein structures were subsequently docked to the ice crystal structure, and models showing good complementarity were proposed (Figure 1).[13–15]

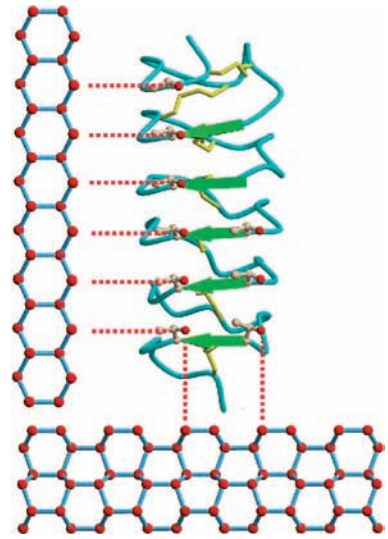


FIGURE 1. Model of an antifreeze protein aligned to the prism face of ice. Red spheres represent oxygen atoms. Dotted red lines highlight the two-dimensional match of threonine residues to the ice planes. Reproduced from ref 13 with permission. Copyright 2002 Elsevier.

An in depth understanding of molecular recognition between macromolecules and organized surfaces would require, however, a systematic study of protein–crystal complexes. This is still inherently difficult because even the most advanced technological tools can barely tackle the formidable task of investigating protein structures at interfaces.[16]

Antibodies are the tools that vertebrates evolved to tag foreign antigens invading the organism. They do so by virtue of molecular recognition. Each antibody tags one or more specific antigens. Antibodies thus appear to be ideal to study the rules of molecular recognition at interfaces with biological environments.

Crystals can be formed or introduced into organisms and should be thus treated by the organism as any other intruder, by eliciting antibodies specific to their surfaces. The question that should be asked is thus: are antibodies capable of intrinsic pattern recognition of two- or three-dimensional repetitive structures? If indeed they are, the next goal should be to understand the rules of this recognition.

Antibodies recognize their targets by virtue of complementarity between their binding site and the target surface over an area of typically 1000 Å^2.[17] On the surface of a crystal composed of, say, relatively small organic molecules, an antibody

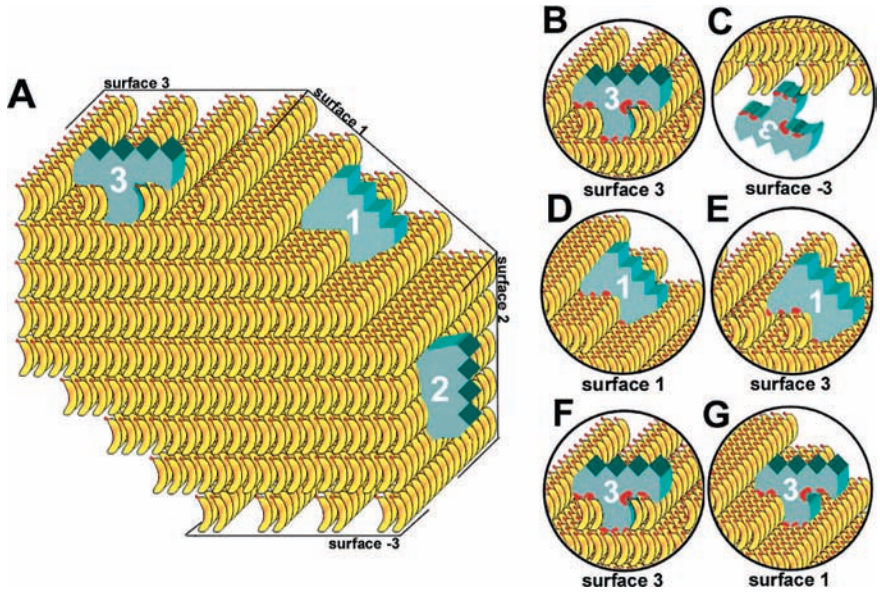


FIGURE 2. (A) Schematic representation of a crystal lattice with three antibody binding sites interacting specifically with three different crystal faces. Color code indicates different chemical character, for example, red denotes polar groups, yellow hydrophobic groups. None of the antibody binding sites could recognize one isolated molecule because its shape and molecular moieties are not reproduced fully in any of the surfaces. Conversely, a binding site encompassing only one to two molecules might easily fit any surface. (B, C) Face 3 is not equivalent to face −3, because different molecular moieties are exposed. Binding of antibody 3 on face −3 is possible geometrically but not chemically. (D−G) The affinity and subsequently the discriminating power of the three antibodies change on the different surfaces. (D, E) Antibody1 binds more strongly to surface 1 than to surface 3 because the contact area is larger; (F, G) antibody 3 binds more strongly to surface 3 than to surface 1 because the contact area is larger.

binding site would encompass an array of 10−20 molecular moieties with a cross section of 50−100 Å^2 (5−10 Å × 5−10 Å). The antibody binding site would thus not detect one molecule but rather a two- or three-dimensional molecular arrangement on the surface, similar to a macromolecular surface. A direct consequence of this pattern recognition is that, at least in principle, one antibody should not recognize different surfaces on the same crystal, if these are not symmetry-related and thus identical (Figure 2).

Specificity of Antibodies Recognizing Surfaces: General Considerations

Specificity, when discussing antibody recognition, is a misleading term. At the beginning of the investigation of antibodies, the classical lock-and-key concept was applied to antibody recognition, in analogy to enzyme−substrate recognition. A strict interpretation of this concept would imply that every potential target has a complementary antibody binding site that binds to it, which binds to nothing else but that specific target. Subsequent studies on antibody binding have shown that this is far from true.

A well-studied case is that of an antibody raised and selected to recognize a dinitrophenyl target (hapten). This antibody was found to bind to vitamin K and other haptens that do not have any molecular similarity to dinitrophenyl.[18,19] There is thus no predetermination for a specific target in antibody recognition, and this may be expected to hold true also for antigens such as the repetitive organized surfaces of crystals. A consequence of this conclusion is that all the antibodies to any given surface could be ideally placed on a relative binding scale, defining which antibody has a stronger affinity for a certain surface in a certain environment. Some ground rules of interaction may however be predicted to a certain extent, as discussed below and illustrated in Figure 2.

The binding affinity and the selectivity of structure-recognizing antibodies will be determined by the balance of interactions and their correct organization and orientation, as in any other antibody−antigen complex.[20,21] Crystal−antibody interaction will, in addition, be regulated by the crystal structure and symmetry, which establishes which functional groups are exposed at which surface and how they are organized (Figure 2B,C). Because all interactions occur in an aqueous environment, water is expected to play a crucial role in both the enthalpy and entropy balance of the process and, in particular, in surface desolvation, water ordering, and establishment of hydrophobic interactions.

Binding stability and selectivity will depend, in addition, on the contact area of the binding site, on the cooperativity of the interactions, and on the conformational freedom of the binding site. Across a large interface area, multiple interactions will

be established, thus increasing the chances of good discrimination (Figure 2D–G). Multivalency, such as is found in antibodies of the IgM isotype with 10 binding sites, will cooperatively increase both affinity and discriminating power. The ability to discriminate between different surfaces depends also on the conformational freedom of the ligand, insofar as this allows it to adapt to different patterns. Antibodies have rather large binding sites (~1000 Å^2) and, due to strong β-sheet backbone interactions, have strict limitations on the conformation of the binding site loops.[22] This notwithstanding, there is still a certain conformational flexibility in antibody binding sites, which may reduce discrimination between different surfaces.

In contrast to antibodies and for these same reasons, small molecules such as peptides would be expected to perform less well in discrimination of similar surfaces, because of their small contact area and large conformational freedom.

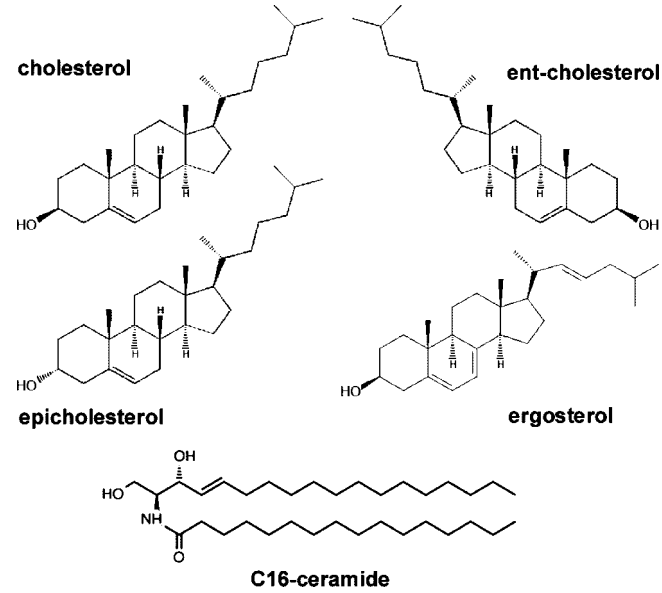
When dealing with crystals as antigens, it must also be taken into account that steps, kinks, or imperfections, such as dislocations, have a tendency to adsorb nonspecifically any additive or impurity present in the medium. These high-energy sites must be neutralized by a competitor to allow the detection of specific interactions. If this is not done, nonspecific adsorption will overwhelm the specific interactions, preventing their detection. It is therefore important in general to perform binding experiments in the presence of a competitor in concentrations higher than that of the antibody of interest by an order of magnitude to avoid nonspecific binding.

Monoclonal Antibodies That Specifically Recognize Structured Surfaces: Examples

Based on the considerations above, a procedure was developed in order to isolate monoclonal antibodies that specifically recognize a certain surface. The procedure was applied to a number of crystals, in particular to crystals of cholesterol monohydrate (Scheme 1),[23] of 1,4-dinitrobenzene,[24] and of the tripeptide (*S*)leucine-(*S*)leucine-(*S*)tyrosine (LLY).[25] Figure 3 summarizes the sequence of steps involved, together with some examples of the specific systems. A series of antibodies were selected and studied, three of which provided reliable specific antibody–antigen structural models (Figure 4).[26,27]

The three docking models show an astounding geometrical and chemical match of the antibody binding sites on the respective crystal surfaces. The flat crystal surface in panel A, the stepped crystal surface in panel B, and the ridge and groove motif in panel C are matched by the antibody binding sites, which are flat or stepped or penetrate the groove and hug the ridge structure of the crystal surface, respectively. The interactions also appear to be well-matched and complementary (Figure 4).

SCHEME 1



None of the antibodies has many charged residues in the binding site. On the contrary, it was observed for antibodies selected against different crystals that the presence of several charged residues leads to the absence of binding specificity.[26,27] This can be rationalized when considering that electrostatic interactions are strong and isotropic, and crystal surfaces can easily be locally polarized. A charged antibody can also easily overwhelm a competitor, even when present in greater concentration, and bind to imperfections and high-energy sites nonspecifically.

Use of Chirality and Stereospecificity as a Tool for the Study of Antibody Recognition

The driving force for the selectivity of the antibodies to different crystal faces might conceivably be due to the general chemical character or the general topography of the surfaces, while their exact spatial organization and interactions are not important for binding specificity. Thus antibody 36A1 might bind to the (301) face of cholesterol because it has a mixed hydrophobic–hydrophilic character (Figure 4A), and antibody 122B1 might bind to the $(10\bar{1})$ face of 1,4-dinitrobenzene because it is flat relative to the other faces (Figure 4B).

To better understand the interplay of the surface geometrical and chemical parameters on antibody recognition, chemically equivalent systems differing only in their geometry were studied, using chirality and stereoisomerism as a tool. In par-

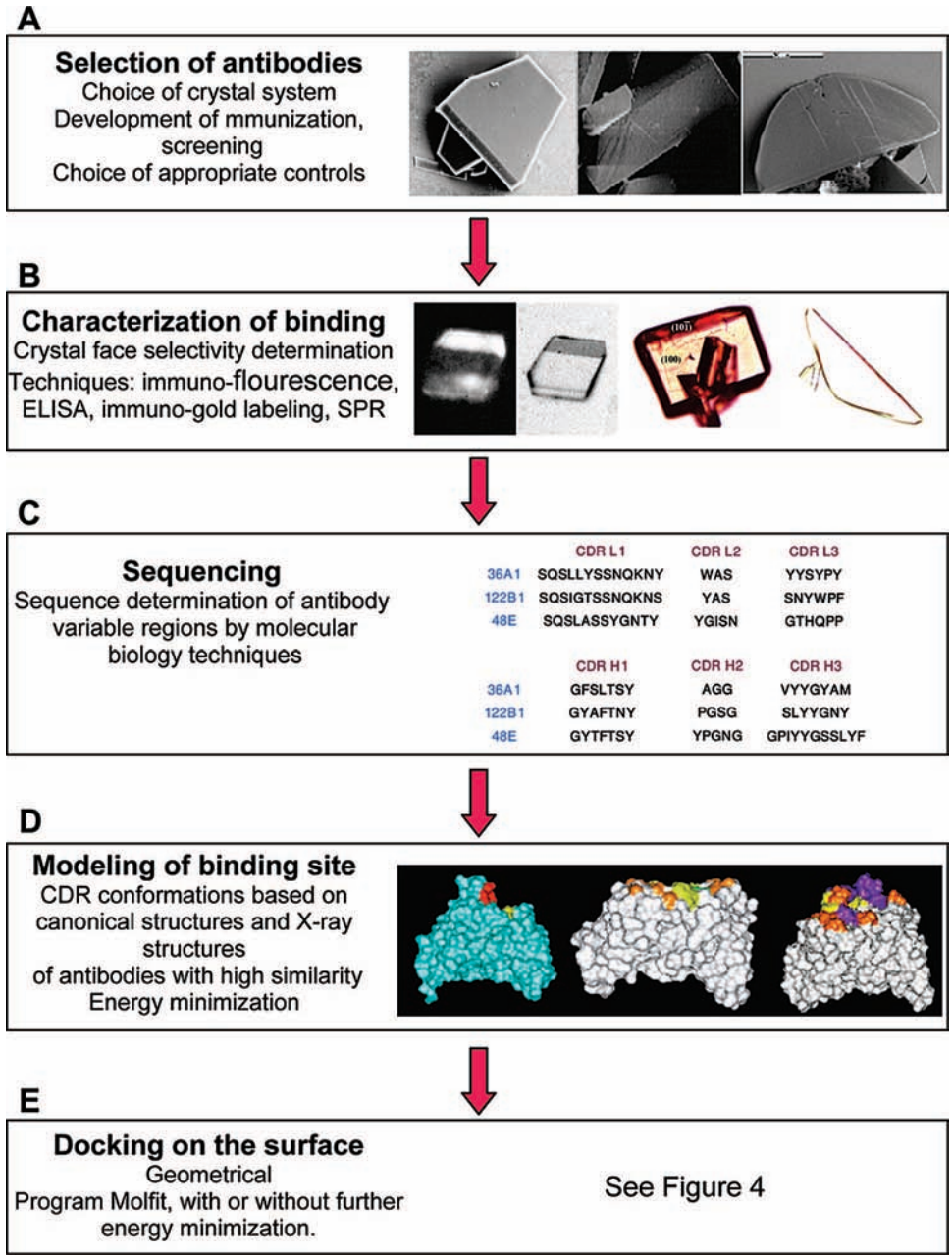


FIGURE 3. Steps A–E in the procedure involved in isolating and characterizing a structure-recognizing antibody, with illustrative examples for the antibody–crystal systems in Figure 4: (A) crystals of cholesterol monohydrate (left),[23] 1,4-dinitrobenzene (center),[24] and LLY (right);[25] (B) left, immunofluorescence labeling of a cholesterol monohydrate crystal with antibody 36A1;[23] center, immunocolor labeling of a 1,4-dinitrobenzene crystal with antibody 122B1;[26] right, immunocolor labeling of a LLY crystal with antibody 48E;[25] (C) sequences of the complementarity-determining regions (CDR) in antibodies 36A1,[26] 122B1,[26] and 48E;[27] (D) modeling of the binding sites of antibodies 36A1 (left),[26] 122B1 (center),[26] and 48E (right).[27]

ticular, when enantiomorphous crystals are used as a characterization tool, any differences in chemical specificity are eliminated because the surfaces expose the same chemical moieties. The effect of the geometrical–stereochemical match can thus be isolated.

Antibody 36A1 was raised and selected against cholesterol monohydrate crystals. The antibody recognizes specifically also monolayers of cholesterol at the air–water interface.[28] Although exact binding constants cannot be derived for these systems, the binding affinity is extremely high, judging by the fact that the first detectable binding occurs at an antibody concentration in solution of 10^{-11} M and saturation is reached at 10^{-9} M. [29]

The same antibody (36A1) does not bind, however, to epicholesterol monolayers, where the hydroxyl group is in the 3α-position (axial) instead of the 3β-position (equa-

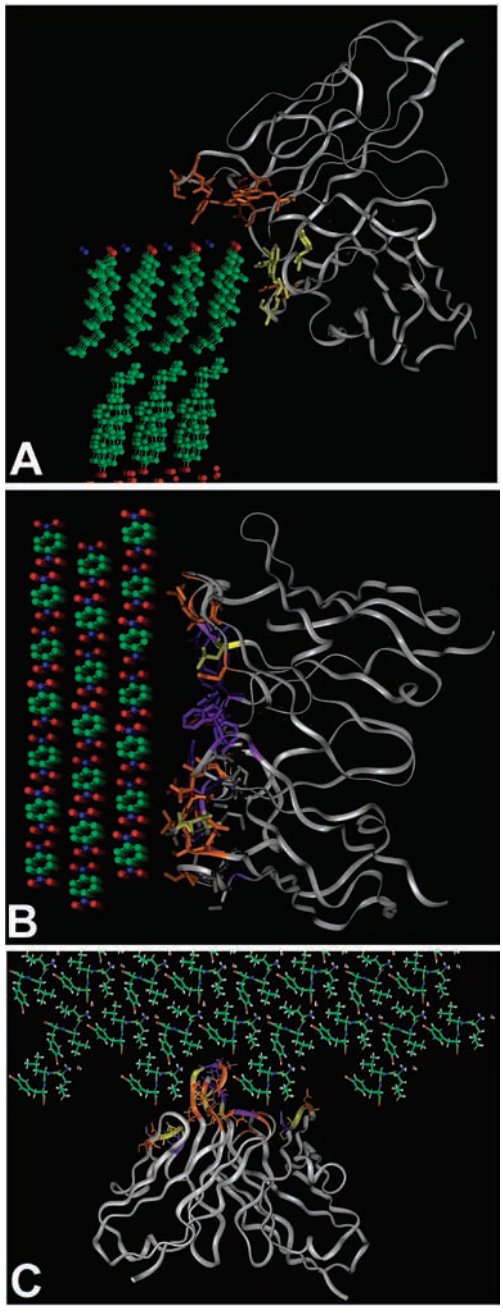


FIGURE 4. Docking model of three antibody binding sites (polar residues are orange, hydrophobic are yellow, aromatic are purple, and backbone is gray) on the surfaces of the respective crystals (green represents C, red O, blue N, and cyan water): (A) 36A1 on the (301) face of cholesterol monohydrate; the molecular step on the crystal has a hydrophilic and a hydrophobic side, matched by the antibody binding site with hydrophilic and hydrophobic groups;[26] (B) 122B1 on the $(10\bar{1})$ face of dinitrobenzene; the aromatic groups, exposed edge-on to the flat crystal face in a stacked herringbone motif are well-matched by five aromatic side chains and various polar groups of the antibody;[26] (C) 48E1 on the $(0\bar{1}1)$ face of LLY; the hydrophobic and hydrophilic groups exposed on the crystal surface along the groove walls and the ridge surface are matched one-to-one by the antibody.[27]

torial) [30](Scheme 1). The change in the angle between the headgroup and the rigid cholesterol backbone in epicholesterol must impose different packing of the molecules in the monolayer, eliminating antibody recognition. This is made even more evident by the lack of recognition of the antibody for ergosterol (Scheme 1), where the stereochemistry of the hydroxyl functional group is 3β as in cholesterol.[31] The reason in this case can be attributed only to the molecular organization of ergosterol, rather than to the stereochemistry of the single molecule.

Antibody 36A1, however, binds to monolayers of entcholesterol, the enantiomer of cholesterol (Scheme 1) with the same affinity as to cholesterol monolayers.[29] In this case, the monolayer has by definition the same structural organization as that of cholesterol, but the molecules and consequently the surface lattice patterns are enantiomeric.

The recognition of antibody 36A1 on cholesterol monolayers is thus stereoselective but not enantioselective. This may be explained when considering that the antibody binding site interacts with an array of hydroxyl groups arranged at defined distances on one side of the steps and with a hydrophobic domain on the other side of the step (Figure 4A), identical to the interaction with the enantiomeric structure. It does not recognize or interact directly with chiral centers that are entirely exposed at the surface.

There is however an example in which the antibody recognition is both stereoselective and enantioselective. Antibody 48E recognizes the $\{0\bar{1}1\}$ faces of the tripeptide (*S*)leucine-(*S*)leucine-(*S*)tyrosine crystals (Figures 4C and 5B). The antibody however, does not recognize the $\{01\bar{1}\}$ crystal faces of the tripeptide (*R*)leucine-(*R*)leucine-(*R*)tyrosine (Figure 5A). In this case, the recognized face must explicitly express the chirality of the molecules resulting in a distinctly asymmetric surface, although this is far from obvious from visual inspection. Interestingly, the geometrical docking program MolFit did not distinguish between antibody docking on the two enantiomorphs.[27] The antibody discrimination is thus higher than what the modeling may predict.

When an antibody distinguishes between chemically equivalent enantiomorphous surfaces, this directly implies that there are surface geometrical differences at the length scale at which chirality is manifested at the surface. When it does not, however, it cannot be concluded that the chirality is not expressed or that the geometrical differences are minute. Antibody binding sites are flexible and may withstand some steric modification if energetically compensated. Moreover, intrinsic specificity varies from one antibody to another in their interactions with the same crystal face. Thus, antibody 602E,

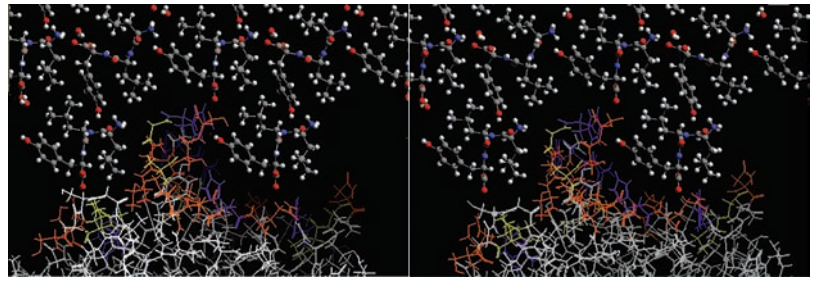


FIGURE 5. Docking of antibody 48E on the (01$\bar{1}$) face of (*R*,*R*,*R*)-LLY (left) and on the (0$\bar{1}$1) face of (*S*,*S*,*S*)-LLY (right). Color code is the same as in Figure 4, except carbon atoms are dark gray. The antibody–antigen complexes are very similar, yet the antibody is enantiospecific.[27]

also specific to LLY crystals, is not enantiospecific and only mildly stereoselective in its interactions with the various crystal surfaces.[25,27]

We conclude from all the above that antibodies are intrinsically capable of recognition at the length scale necessary for detection of chirality. The interaction is not determined, however, by the surface properties exclusively, and the appropriate antibody must be selected with care. The screening process may help eliminate nonspecific antibodies, but an extensive characterization of the antibody prior to its use as reporter at the level of stereochemistry and chirality is needed.

Use of Antibodies As Reporters of Structural Organization in Biological Systems

The remarkable complementarity and specificity observed between the antibodies and the recognized surfaces hints at the possibility of obtaining structural information on a biological surface using the antibody as sensor of a specific and known molecular organization.

Kruth et al. provided a proof-of-concept using antibodies as reporters of cell membrane domain structure in cholesterol-enriched membranes of macrophages and fibroblasts.[32] Antibody 58B1, which was selected for its recognition of cholesterol monohydrate crystals,[23] does not interact with the surface of these cultured cells under normal conditions. Only when the plasma membrane is enriched with cholesterol is binding to the cells observed.[32] The antibody specifically recognizes cholesterol when it is organized in the crystalline form: it follows from the sensitivity of the antibody binding to modulation of cholesterol levels in the cell that the recognized cellular target consists of paracrystalline cholesterol domains rather than of single molecules of cholesterol dispersed in the cell membrane. The potential thus exists for the development of monoclonal antibodies that detect structured domains within the plasma membrane.

Ordered microdomains in eukaryotic cell membranes are only one example of biological structures that cannot be unraveled with conventional biophysical or biological techniques.[33–35] The existence of the so-called "lipid rafts" in cell membranes is well established and accepted. Very little information is however available on the molecular structure and organization of these domains. The idea is to develop tailor-made antibodies that recognize known molecular organizations of lipids relevant to membrane domain composition and to subsequently test them in biological membranes.[36]

The concept involves creating a lipid mixture relevant to lipid domain organization in cells, determining its structure in the form of monolayer, raising and selecting a specific antibody that selectively recognizes this structural organization, characterizing the antibody binding on the artificial monolayers, and finally using the antibody as reporter of the presence of the characterized epitope in cell membranes (Figure 6).

In this context, we developed monoclonal antibodies specific to mixed monolayers of cholesterol and ceramide. One of the developed antibodies was shown to be selective toward crystalline cholesterol/ceramide mixed monolayers of known structural organization[37] but does not bind to separate cholesterol or ceramide monolayers nor to phosphatidylcholine monolayers (Figure 6A). It also does not bind to ergosterol/ceramide monolayers.[31] On cells, the antibody labels cell membrane domains.[38] The antibody labeling is sensitive to changes in cholesterol and ceramide levels, as well as to the selective modulation of the levels of ceramides with different acyl chain lengths.

The potential of these types of structure-recognizing antibodies for providing information on biologically relevant systems such as cellular membranes is still to be demonstrated. It is however important to stress that the fundamental contribution of the approach developed here will be not in producing antibodies as tagging tools for single molecules but rather in the antibody ability to report on the structural organization of paracrystalline domains that cannot be determined by other means.

Alving et al. were the first to suggest that the state of molecular aggregation of cholesterol together with other mol-

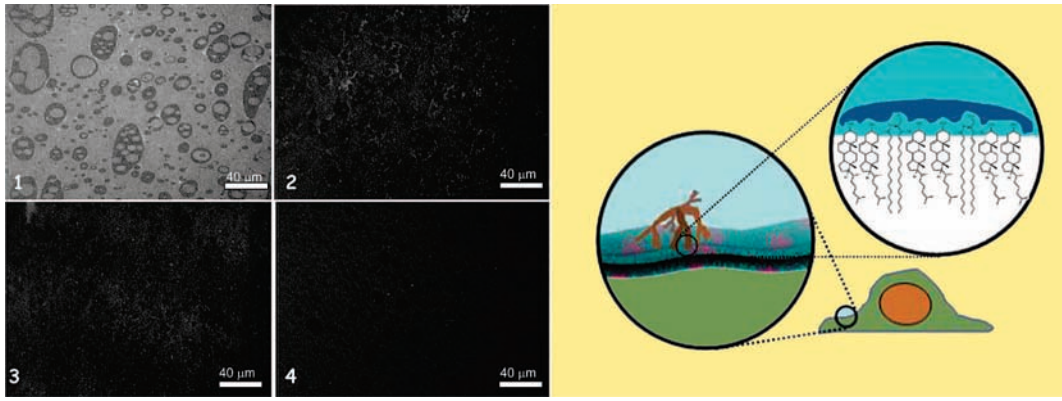


FIGURE 6. Immunofluorescence labeling (left) with antibody 405F, specific to a 60:40% cholesterol/C16-ceramide mixture of monolayers (1, 60:40% cholesterol/C16-ceramide mixture; 2, C16-ceramide; 3, phosphatidyl-choline; 4, cholesterol) where the antibody binds only to the mixed phase of cholesterol and ceramide, which has a known crystalline structure[31] and schematic representation (right) of the application of the antibody to cells displaying the specific microdomain structure on the cell membrane.

ecules, rather than its molecular structure, may be recognized by specific antibodies. They produced monoclonal antibodies against liposomes composed of cholesterol and lipid A.[39,40] However, considerable cross-reactivity with related lipids was observed due to the presence in the binding site of a subsite that recognized phosphate ions.[41] Alving's antibodies may be able to recognize organized molecular microdomains on biological surfaces. Because the structure of the liposome model system has not been determined, it is difficult to define, however, whether these antibodies pertain to the same group of structure-recognizing antibodies discussed here.

One consequence of the observations above is that antibodies that have been raised and selected to identify single lipid molecules must be used with caution in labeling cells and tissues, because the state of aggregation and the environment of the molecule determine whether it may be detected. Cholesterol is a physiological component of cell membranes, where it is dispersed at the molecular level among other lipids. Only when its aggregation state is abnormal, such as in cholesterol crystals or liposomes containing excess cholesterol, may antibodies be raised against it. By the same token, antibodies raised against other lipids may recognize not the molecule but the molecular arrangement in which it appears. Examples are three commercial anticeramide antibodies, generated for establishing the topology of ceramide generation or subcellular localization.[42,43] As long as nothing is known about the structure of the target that is recognized or on the characteristics of the antibody–antigen complex, caution needs to be exercised in the interpretation of antibody labeling or lack thereof.

Structure-Recognizing Antibodies in Medical Research

A structure-recognizing antibody is defined here as complementary to a certain ordered supramolecular organization. It can thus be considered as a mold bearing in its binding site memory of the organization against which it was elicited. This concept may have both interesting and disturbing consequences in pathological conditions.

The positive side of structural antibodies may be their potential use in tagging pathological crystalline aggregates and reporting on their presence and location. Thus, antibodies against cholesterol monohydrate crystals may conceivably be used to report on the existence, location, and amount of crystals in advanced atherosclerotic plaques, which contain crystals of cholesterol monohydrate.[44]

The potentially negative consequence of structure-recognizing antibodies is that such antibodies, preserving the memory of the crystal structure to which they bind, may reduce the activation barrier to crystallization and stabilize crystal nuclei by providing a template of the crystal surface in the correct organization. Indeed, antibodies isolated from synovial fluids of patients suffering from gout, and thus associated with the pathological presence of monosodium urate monohydrate crystals, were shown to accelerate in vitro the formation of the respective crystals in new nucleation events.[10,45] Analogously, antibodies raised and selected against cholesterol monohydrate crystals accelerate the crystallization of cholesterol in vitro.[10] A consequence of such a process in living organisms may be the amplification of crystal deposition in diseases associated with pathological crystal formation, such as gout[46,47] (monosodium urate monohydrate), pseudogout (cal-

cium pyrophosphate), osteoarthritis (apatite), kidney stones (calcium oxalate and others), gall stones[48] (cholesterol), and others. Such amplification may be triggered by antibodies elicited in the body by the crystals in a previous event. The same mechanism may be operative in northern ocean fish harboring ice crystals in their blood.[49]

Other pathological conditions are associated with deposition of physiological peptides and proteins in ordered molecular organizations different from their native structure, such as amyloid-related diseases. Amyloids are fibrillar aggregates caused by pathological protein misfolding in a characteristic cross-β conformation.[50,51] More than 20 diseases have been identified as amyloid-related, among them Alzheimer's, Parkinson's, diabetes type II, and the well-known prion diseases. Each disease is associated with the deposition of a specific polypeptide in a specific body organ.

Recently O'Nuallain et al. raised structure-recognizing antibodies against the mature amyloid fibril state of the Alzheimer's peptide ($A\beta_{1-40}$).[52] The antibodies distinguish between the amyloid structure and the soluble structure of the peptide, and also bind to other amyloid fibers, albeit with lower affinity. This led to the suggestion that the antibodies are specific for a generic epitope that is characteristic to the amyloid structure.

Kayed et al. produced structure-recognizing antibodies that bind to soluble amyloid oligomers of the Alzheimer's peptide.[53] The resulting antibodies are specific to a common generic target present in the oligomer structure but not in the native or mature amyloid fiber structure. In addition to the information that can be derived from these experiments on the structure of amyloids and of their intermediates on the pathway to mature fibers, there could be medical implications to these experiments.[54,55] Kayed et al. showed that the antibodies reduce the toxicity of the different amyloidogenic protein oligomers, suggesting that they could be used as passive vaccines. In the absence of precise knowledge on the structure of the antibody–antigen complex, these concepts must however be considered with great caution.

Antibodies as Sensors in Electronics

A different approach in the use of molecular recognition of surfaces emerged in nanotechnology. Achieving discrimination of semiconductor surfaces by specific ligands, such as antibodies, could provide a powerful tool for the assembly of devices at molecular length scale. The approach is fundamentally different from that discussed above, insofar as the discrimination is planned to occur between a defined number of predetermined surfaces rather than among a large number of unknown substrates with unknown organization, such as in biological systems.

Barbas et al.[56] used phage-display techniques to select semisynthetic antibodies interacting with magnetite, establishing the potential of this technique for production of surface-selective ligands. The procedure involves the presentation of large peptide and protein libraries on the surface of filamentous phages and allows the selection of peptides and proteins, including antibodies, with high affinity and specificity for almost any target.[57,58] Phage-display libraries were subsequently used for the selection of ligands binding to inorganic surfaces such as semiconductors, silica, and metals.[59–61]

Whaley et al.[59] claimed that combinatorial phage-display libraries can be used to develop short peptides (12 amino acids) that bind to a range of semiconductor surfaces with high specificity, depending on the crystallographic orientation of the semiconductor. It was however subsequently shown that even the phage itself, without the peptide, binds through its coat protein to the same facet of GaAs as observed by Whaley et al.[60] The mechanism underlying the process of molecular recognition between the semiconductors and the peptides thus still has to be demonstrated. In addition, even assuming that the short peptides are specific when bound to the phage, when unbound in solution they may lose their specificity because of the large conformational freedom.

By the use of phage-display technology, antibody fragments that selectively bind to GaAs facets were recently produced.[60] In this case, the antibody fragments were selective to the specific crystal faces even when separated from the phage and in the presence of a competitor. Although the recognition mechanism is still uncertain, this study establishes the basis of future use of antibodies binding to inorganic surfaces in nanotechnology.

Concluding Remarks

The questions that were asked at the outset can now be answered with more assurance: interactions between proteins and crystal surfaces may be specific, and antibodies are intrinsically capable of recognizing two- and three-dimensional repetitive structures. The rules of the game are however not straightforward, and caution must be exercised in the choice of the substrate and in the characterization of the antibody–antigen interactions. In molecular crystals, specific antibodies have good geometric and chemical complementarity to their targets. Possible applications of such structure-recognizing antibodies are conceivable in biology, medicine, and electronics, in order to obtain information that cannot be directly achieved by other means or to transfer information from one system to another. A

particularly careful approach should be taken when switching from in vitro systems to biological systems, due to the enormous variety and complexity of the substrates involved.

BIOGRAPHICAL INFORMATION

Born in Padova, Italy, **Lia Addadi** obtained her B.Sc. and M.Sc. degrees in Organic Chemistry at the Università degli Studi di Padova and a Ph.D. in Structural Chemistry from the Weizmann Institute of Science in 1979. Her postdoctoral studies were carried out at Harvard University. In 1982, Lia Addadi returned to the Weizmann Institute, where she was appointed Associate Professor in 1988 and Full Professor in 1993. Lia Addadi pursues a range of research interests centered on stereochemistry and molecular recognition at biological interfaces. She studies mineralization in biological systems, antibody recognition of organized molecular assemblies, and cell adhesion.

Noa Rubin was born in Tel Aviv, Israel. She obtained her B.Sc. degree in Material Engineering in 2001 at Ben Gurion University of the Negev and M.Sc. degree in Chemistry in 2003 at the Weizmann Institute of Science. She is currently pursuing her Ph.D. under the supervision of Prof. Addadi. Her scientific interests are focused on organized molecular assemblies, in particular amyloid structure, using antibody molecular recognition and chirality.

Luana Scheffer was born in Bucarest, Romania, where she obtained her M.Sc. degree in Physics in 1990. She obtained a first doctoral degree from Tel Aviv University, Israel, in 2002, and a second Ph.D. from the Weizmann Institute of Science in 2007, under the supervision of Prof. Addadi. Dr. Scheffer is presently pursuing postdoctoral studies at the Mayo Clinic in Rochester, MN. Her scientific interests range from Physics to Chemistry and Biology, centering on cell membrane structure and organization.

Roy Ziblat was born in Rehovot, Israel. He obtained a B.Sc. degree in Physics and Mathematics in 2003 and M.Sc. degree in Physics in 2005, both at the Hebrew University of Jerusalem. His work focused on photonic crystals and implementation of surface plasmons on cells. He is currently pursuing his Ph.D. under the supervision of Prof. Addadi. His scientific interests lay both in physics and structural biology, specifically on the structure and organization of cell membranes.

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