ly bound dangling bond sites requires a particular orientation with respect to the Si dimer rows. ESDIAD studies (*18, 19*) did not determine the population of bridging species.
21. Manipulation was achieved by either fixing or scanning the probe tip over the site during the pulse. The latter method was most effective presumably because of a time-dependent variation in the field gradient that promotes diffusion and rebonding. The average conversion efficiency was low (≈5%) and tip-dependent.
22. P. Gupta, P. A. Coon, B. G. Koehler, S. M. George, *Surf. Sci.* **249**, 92 (1991).
23. The interaction strength depends on the orientation of the Si–Cl dipole (Eq. 1) and varies depending on whether Cl adsorbs on the up or down Si dimer atom. Thus, other than molecular precursors, isolated sites may be Cl_2 molecules that decompose diagonally across a pair of dimers forming Si–Cl bonds on the up-atoms. The reason such sites do not switch into type II sites and why the latter are less prone to switching compared with type III sites is not understood.
24. I wish to thank J. T. Yates Jr. for making available reprints (*18, 19*) before publication.

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Attempts to Mimic Docking Processes of the Immune System: Recognition-Induced Formation of Protein Multilayers

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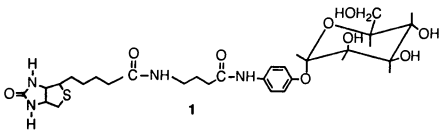
The assemblage of protein multilayers induced by molecular recognition, as seen, for example, in the immune cascade, has been mimicked by using streptavidin as a docking matrix. For these experiments, this protein matrix was organized on liposomes, monolayers at the air-water interface, and self-assembled layers on gold, all three containing biotin lipids. The docking of streptavidin to biotin at liposomal surfaces was confirmed by circular dichroism. Mixed double and triple layers of streptavidin, concanavalin A, antibody Fab fragments, and hormones were prepared at the air-water interface and on gold surfaces and were characterized by fluorescence microscopy and plasmon spectroscopy. With the use of biotin analogs that have lower binding constants it has been possible to achieve multiple formation and competitive replacement of the oriented protein assemblages.

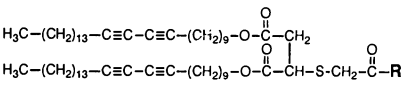
The pathway of the immune cascade (*1*) demonstrates the interplay of molecular recognition and molecular self-organization in the formation of oriented protein assemblages. We have attempted to mimic these protein docking processes and their reversibility. In the system described here, the multiple docking of proteins leads to ordered protein multilayers, which can be disassembled by competitive replacement. It was recently shown that the specific interaction of streptavidin (*2, 3*) with a biotin-containing monolayer at the air-water interface results in two-dimensional (2D) streptavidin crystallization (*4–7*). Each streptavidin in this protein layer has two free binding sites facing the subphase, thus forming a bioreactive docking matrix, shown schematically in Fig. 1 (*8–12*).

We report results on the formation and competitive replacement of mixed protein assemblages using this 2D protein matrix in three different model membrane systems: monolayers at the air-water interface, liposomes, and self-assembled monolayers on gold. We show here how these model systems, each having specific advantages, lead to recognition-induced formation of protein layers and allow their characterization.

Starting with a 2D crystalline streptavidin matrix at the air-water interface, we used bifunctional linking molecules to form well-defined protein triple layers, alternating streptavidin and concanavalin A (Con A). This step-by-step docking process was visualized by fluorescence microscopy. We found that rhodamine-labeled Con A can be docked by means of a biotin-sugar linker **1** to a fluorescein-labeled streptavidin layer.



1



The Con A layer permits perfect imaging of the domain structure of the streptavidin matrix, thus allowing the visualization of the first protein layer even without a fluorescent label attached to streptavidin. An unlabeled primary streptavidin matrix was therefore used for the formation of the alternating protein triple layer. As shown in Fig. 2, the rhodamine-labeled Con A was docked (as a second layer) underneath the crystalline streptavidin matrix with the biotin-sugar linker **1**. Further binding of fluorescein-labeled streptavidin to this second layer (Con A) results in an alternating protein triple layer (see Fig. 2). In addition, fluorescein-labeled streptavidin, even when docked by means of bisbiotin linkers onto a primary amorphous avidin matrix, crystallizes to form needle-like domains, thus offering the possibility of building alternating amorphous-crystalline protein multilayers (*13*).

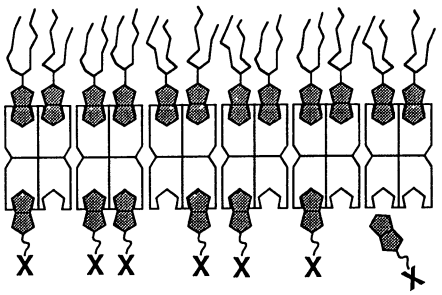
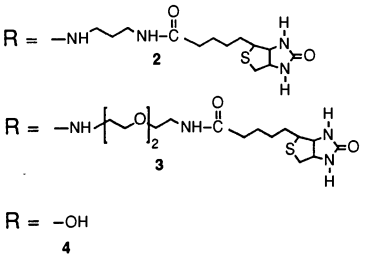


Fig. 1. Schematic representation of the 2D streptavidin crystal, which can serve as a bioreactive matrix. The binding biotin molecules are shown as filled figures, which fit closely into the binding sites in the upper and lower surfaces of the streptavidin matrix. On the lower surface of the figure, they are shown linking to the next layer (**X**), and at the top of the figure, they are shown with free lipid tails.

Liposomes were used as a second model system. They were prepared from various biotin lipids (**2, 3**) containing long hydrophilic spacers, which allowed an optimal protein-ligand interaction, and polymerizable diacetylene moieties which stabilized the liposomes. Upon ultraviolet polymerization of the liposomes, the conjugated backbone of the polydiacetylene lipids becomes a chromophore, which can serve as a sensing unit for studying the docking of streptavidin to the chiral biotin headgroups.



2 **3** **4**

Stable polymerized vesicles were prepared from the amphiphilic diacetylene **4** containing 5 mole percent (mol%) of the biotin lipid **3** (*14*). We found that the

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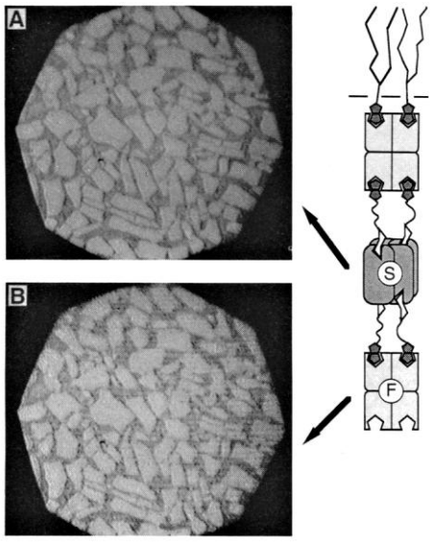


Fig. 2. Fluorescence micrograph of a combined streptavidin–Con A–streptavidin triple layer. (**A**) The first layer of crystalline streptavidin (nonlabled) was imaged and made visible by a docked second layer of Con A (sulforhodamine-labeled). (**B**) The third layer of streptavidin (fluorescein-labeled) was docked onto the second Con A layer, and again the shape of the streptavidin domains in the first layer was imaged.

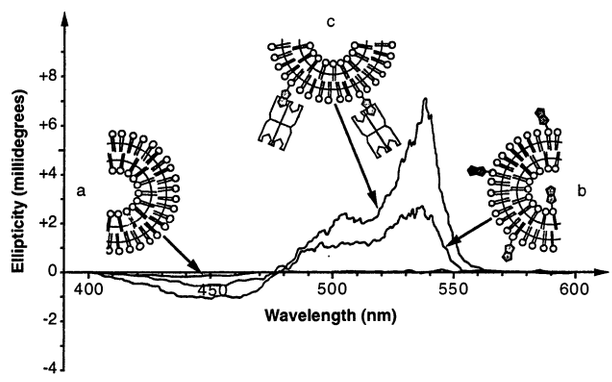


Fig. 3. Specific interaction between streptavidin- and biotin lipid–containing liposomes was studied by circular dichroism: The structure labeled a designates polymerized liposomes without biotin lipid **3**; structure b represents polymerized liposomes with **3**; and structure c shows streptavidin bound to polymerized liposomes by means of biotin.

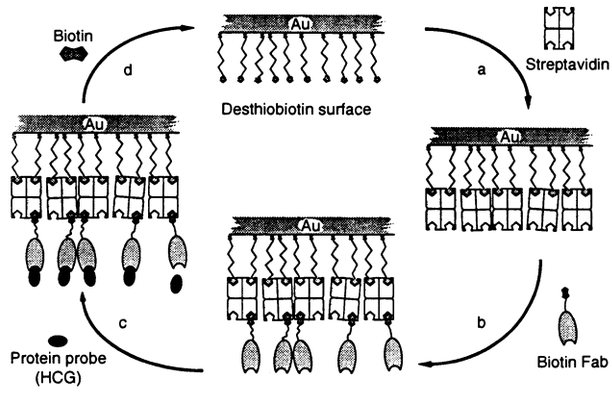
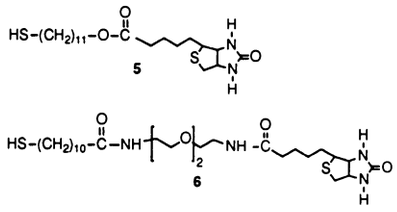


Fig. 4. Schematic representation of the reversible formation of a protein triple layer based on a streptavidin matrix: step a, formation of the streptavidin matrix on a self-assembled monolayer on gold (10% mercaptodesthiobiotin **5** and 90% 11-hydroxyundecylmercaptane); step b, docking of the biotinylated Fab-fragment; step c, binding of the hormone HCG to the Fab fragment; step d, competitive replacement of the triple layer after addition of an excess of biotin.

chiral biotin headgroups produced circular dichroism, even though the biotin moiety is decoupled from the chromophore (polymer backbone) by a long spacer (Fig. 3, structure b). This effect was used to study the docking process of streptavidin to the biotin headgroup of the polymerized diacetylene lipids. After the binding of the protein to the biotin headgroups, the circular dichroism is increased by a factor of 2.5, as shown in Fig. 3, structure c. Separate reference measurements were made with both inactivated (biotin-saturated) streptavidin and liposomes without biotin lipids, and neither showed enhancement of the circular dichroism. This result confirms the specific interaction of streptavidin with biotin at liposomal surfaces.

Using the docking concept to fabricate layered protein assemblages on solid supports opens up the possibility for various applications, especially in the field of biosensors. These supported protein surfaces have numerous advantages, such as ease of handling, stability of the layers, and the availability of characterization methods for thin films (for example, atomic force microscopy or surface plasmon spectroscopy). Self-assembled monolayers of thiol-functionalized biotins on gold were used to prepare streptavidin matrices on solid supports (*15*). Previous studies with various mercaptobiotins (**5, 6**) revealed that recognition-induced protein docking to such self-assembled monolayers could only be achieved if a hydrophilic spacer of sufficient length (see **6**) decouples the motion of the



ligand headgroups from the rigid supported membrane (*16*).

Applying the spacer concept, we were able to form protein triple layers on solid supports based on self-assembled monolayers on gold. In these experiments, an anti–HCG-Fab fragment (HCG is human chorionic gonadotropin) was used as the second layer coupled to the primary streptavidin matrix. For this purpose, the hinge, or linkage, region of the Fab fragment was specifically functionalized with biotin. Surface plasmon spectroscopy studies revealed (Figs. 4 and 5) that, in addition to the double layer formation (streptavidin, Fab fragment), the antigen HCG could dock to the Fab fragment, forming a third layer, and this process could be quantified.

Considering the importance of competitive replacement of docked proteins in naturally occurring systems, we attempted to

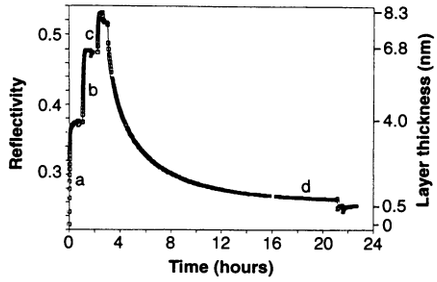
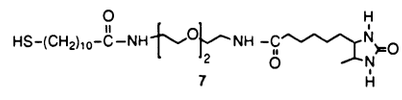


Fig. 5. Stepwise formation and competitive replacement of a protein triple layer of streptavidin–Fab fragment–HCG was studied in situ with the use of surface plasmon spectroscopy. Portions of the curve labeled a, b, c, and d refer to steps diagramed in Fig. 4.

transfer this concept to the streptavidin–Fab fragment–HCG triple layer, as shown in Fig. 4. Because of the extremely strong binding between biotin and streptavidin (association constant $K_a = 10^{15}\ M^{-1}$), this recognition process was essentially irreversible. Competitive replacement was achieved with the use of biotin analogs that exhibited lower affinities to streptavidin, for example, desthiobiotin ($K_a = 5 \times 10^{13}\ M^{-1}$) (*3*). Therefore, a series of mercaptanes with biotin analogs as headgroups were synthesized (**7**). A triple



layer of streptavidin–Fab fragment–HCG was constructed on a desthiobiotin surface, as shown in Fig. 4, steps a through c (10 mol% **7** and 90 mol% 11-hydroxyundecylmercaptane). The addition of an excess of free biotin resulted in the competitive replacement of the entire multilayer as a result of the higher affinity of biotin to streptavidin. The restored desthiobiotin surface was used again for the reassembly of the streptavidin–Fab fragment–HCG triple layer, and the layer thickness of each of the proteins was as determined for the first triple layer. Moreover, using a single streptavidin-desthiobiotin layer, we repeated this cycle of docking and competitive replacement of streptavidin several times on the same desthiobiotin functionalized surface (*17*).

In conclusion, streptavidin matrices are versatile model systems for the tailoring of bioreactive surfaces and for the cyclic formation of protein multilayer structures. On the basis of this concept, questions concerning naturally occurring recognition processes, as well as the development of diagnostic tools such as biosensors, can be addressed. Furthermore, recognition-induced formation of organized protein–inorganic multilayers will become possible.

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13. The streptavidin matrix was prepared at the air-water interface as described by Blankenburg *et al.* (*4*). We then removed the excess of streptavidin by exchanging the subphase. Sulforhodamine-labeled Con A, saturated with four equivalents of biotin-sugar linker **1**, was injected underneath the streptavidin matrix and incubated for 30 min. The subphase was exchanged again to remove the excess of Con A. The third protein layer was prepared by injection of fluorescein-labeled streptavidin underneath the streptavidin–Con A double layer.
14. The liposomes were prepared by sonification of a mixed lipid film (5 mol% **3**, 95 mol% **4**) in phosphate buffer (50 mM, pH 8.5) at 60°C. We performed the ultraviolet polymerization of the diacetylene units by irradiating the suspension at 254 nm.
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17. Self-assembled biotin-containing monolayers were formed on clean, vacuum-evaporated gold films. The adsorptions of the thiols were performed in ethanolic solutions with a total thiol concentration of 5×10^{-4} M (10 mol% **7**, 90 mol% 11-undecylhydroxymercaptane). All proteins were adsorbed from solutions whose concentrations were 5×10^{-7} M with respect to the proteins and 0.5 M with respect to NaCl. The adsorption processes were followed in real time by surface plasmon spectroscopy. First, streptavidin was adsorbed onto the functionalized gold, and then after the adsorption process was finished, the solution was exchanged and the biotinylated Fab fragment was adsorbed (5×10^{-7} M Fab fragment, 0.5 M NaCl) up to saturation. The solution was exchanged once again and HCG was then added. Once the triple layer formation was completed and the solution had been exchanged, biotin was added in a concentration of 2×10^{-4} M.

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Inner Core Anisotropy Due to the Magnetic Field–Induced Preferred Orientation of Iron

Shun-ichiro Karato

Anisotropy of the inner core of the Earth is proposed to result from the lattice preferred orientation of anisotropic iron crystals during their solidification in the presence of a magnetic field. The resultant seismic anisotropy is related to the geometry of the magnetic field in the core. This hypothesis implies that the observed anisotropy (fast velocity along the rotation axis) indicates a strong toroidal field in the core, which supports a strong field model for the geodynamo if the inner core is made of hexagonal close-packed iron.

The Earth's inner core is considered to be made of solid iron (or iron-nickel alloy) (*1*). Like the silicate upper mantle, the presence of anisotropic structure has been inferred by recent seismological studies (*2*). Anisotropy appears to have axial symmetry, the fast direction being parallel to the rotation axis with an amplitude of ~3% (*2*). Understanding the origin of this anisotropic structure will effect a better understanding of the dynamics of the core.

Jeanloz and Wenk (*3*) proposed that this anisotropy might be caused by the lattice preferred orientation (LPO) of iron with hexagonal close-packed (hcp) structure (ε-Fe) due to plastic deformation. Seismic anisotropy due to deformation-induced preferred orientation is well documented in the upper mantle of the Earth (*4*), but there are a number of problems in applying such a model to the inner core. First, the differential stresses in the inner core are expected to be low ($\sim 10^{-3}$ to 10^{-1} MPa) (*3*) because of the small thermal expansion, small gravity, and high thermal conductivity (Table 1). Small stresses favor diffusion or superplastic creep rather than dislocation creep for a reasonable range of grain size (*5*). Diffusion or superplastic creep produces no preferred orientation and even destroys any preexisting preferred orientation, and hence no anisotropy is expected in materials deformed by diffusion or superplastic creep (*6*). Second, and more fundamentally, even the presence of convection in the inner core can be questioned for the following reasons: (i) Heat generation due to radioactive elements in the core is likely to be low (*7*); (ii) a likely presence of gradient of oxygen content in the inner core will have a stabilizing effect against convection (*8*); and (iii) the presence of a strong magnetic field will tend to stabilize against convective instability (*9*). Third, it is not straightforward to explain the observed axial symmetry of seismic anisotropy that is based on Jeanloz and Wenk's model because there is no obvious relation between convective pattern and rotation axis. Fourth, the strength of anisotropy predicted by Jeanloz and Wenk's model is much weaker (~0.4%) than that observed (~3%) (*10*).

In this report, I propose an alternative model. This model invokes an interaction of the magnetic field with growing magnetically anisotropic iron crystals. As such, the model implies that the seismic anisotropy carries information about the geometry (and strength) of the magnetic field in the core that is not directly observable. It is shown that the observed seismic anisotropy is consistent with the strong toroidal field, but not with the weak field model of the geodynamo.

There has been a considerable debate about the likely crystal structure of iron in the inner core. Following Jephcoat and Olson (*1*) and Jeanloz (*1*), I will assume that the Earth's inner core is predominantly

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