Optical Amplification of Ligand-Receptor Binding Using Liquid Crystals

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Liquid crystals (LCs) were used to amplify and transduce receptor-mediated binding of proteins at surfaces into optical outputs. Spontaneously organized surfaces were designed so that protein molecules, upon binding to ligands hosted on these surfaces, triggered changes in the orientations of 1- to 20-micrometer-thick films of supported LCs, thus corresponding to a reorientation of ~10° to ~10° mesogens per protein. Binding-induced changes in the intensity of light transmitted through the LC were easily seen with the naked eye and could be further amplified by using surfaces designed so that protein-ligand recognition causes twisted nematic LCs to untwist. This approach to the detection of ligand-receptor binding does not require labeling of the analyte, does not require the use of electroanalytical apparatus, provides a spatial resolution of micrometers, and is sufficiently simple that it may find use in biochemical assays and imaging of spatially resolved chemical libraries.

Detection of the binding of ligands to receptors forms the basis of methods to screen for the presence of narcotics in blood and hair (1), biomarkers in food products (2), and immunological species that indicate disease (3). Current methods used to detect the binding of biomolecules and ligands (such as antibodies and antigens) generally require laboratory-based analytical apparatus or procedures in bulk solution that involve species labeled with latex beads, enzymes, radioactive isotopes, or fluorophores (3). Here, we describe the use of thermotropic LCs to directly amplify and transduce the binding of analytes at surfaces into optical outputs that can be easily read with the naked eye (4). Detection of the ligand-receptor interaction can be performed in ambient lighting without the need for electrical power, can be localized to specific regions of a surface with micrometer resolution, requires nanogram quantities of materials, and is sufficiently simple that it may find use in diagnostic (yes/no) assays performed in locations remote from central laboratories, as well as in primary screening assays of patterned arrays of chemical species.

The principles of our approach are based on four properties of LCs (5). First, molecules within LCs (mesogens) can communicate their orientations to regions of the fluid that are up to 100 µm away. This long-range communication between mesogens permits ligand-mediated binding of proteins to surfaces to be amplified into changes in the orientations of 1- to 20-µm-thick films of supported LCs. Second, because mesogens within LCs have mobilities that are characteristic of liquids, information about the binding of ligands and receptors at surfaces propagates rapidly from the surface into the bulk of the LC (amplification and transduction can occur in a few seconds) (6). Third, optical anisotropy caused by the preferred orientations of mesogens within LCs provides a straightforward way to transduce changes in the orientations of bulk LCs into optical signals that are easily read using ambient light and the naked eye. Fourth, because the orientations assumed by LCs near surfaces reflect the molecular-level or mesoscale structure of a surface, surfaces can be designed such that the binding of both macromolecules and small molecules is amplified and transduced into optical signals.

We designed surfaces with nanometer-scale topographies that could be erased by the specific binding of proteins to surface-immobilized ligands (Fig. 1A), thus leading to macroscopic changes in the orientations of LCs supported on these surfaces. First, we prepared thin films of polycrystalline gold (Fig. 1B) with roughnesses characterized by a maximum amplitude of ~2 nm and a maximum wavelength of ~50 nm (Fig. 1C). The deposition of the gold films was controlled so as to introduce an anisotropic roughness within the films (hereafter called "anisotropic gold films") (7). Although subtle, the anisotropic roughness was easily detected by observing the orientations of supported LCs (7). Second, we formed mixed, self-assembled monolayers (SAMs) from biotin-(CH₂)₉[(CH₂)₂O]₂NHCO(CH₂)₁₁SH (BiSH) (8) and CH₃(CH₂)₁₁SH (C₈SH) (8) by immersion of the anisotropic gold films in ethanolic solutions containing 9.6 µM of BiSH and 70.4 µM of C₈SH for 8 hours (9). The mixed SAMs were estimated to consist of ~27% biotinylated species by linear interpolation of thicknesses (Δ) of single-component SAMs formed from BiSH (ΔBiSH = 3.8 nm) and C₈SH (ΔC₈SH = 1 nm) (10). Binding of the protein avidin (Av, 4.2 nm by 4.2 nm by 5.6 nm) (11) to biotin hosted within these SAMs was achieved by incubating the SAMs for 10 to 15 min in phosphate-buffered saline (PBS) (pH 7.4, 100 mM NaCl, 0.024 volume % Triton X-100) containing 0.5 µM Av (12). The surfaces were then rinsed in...

Fig. 1. (A) Schematic illustration of the change in surface roughness caused by the binding of molecules of Av (left) or IgG (right) to ligands hosted within a SAM of molecules supported on a gold film. The approximate roughness of the surface after binding of Av and IgG is shown by a dashed line. The presence of the SAM on the gold renormalizes the position of the surface and does not erase the roughness of the gold. (B) Scanning tunneling microscopy image of the surface of a thin (~10 nm) semitransparent, obliquely deposited (50° from normal) gold film prepared by electron beam evaporation onto a glass substrate at 0.02 nm s⁻¹. A layer of titanium (~2 nm, also deposited obliquely) was used to promote adhesion between the gold and the glass. The vertical and horizontal dimensions of the image are 300 nm and 500 nm, respectively. The gray scale of contrast represents a height range of 0 to 5 nm. (C) Profile of the surface of the gold along the black line shown in (B).
pentylbiphenyl (5CB) was drawn in its acrylate monomer cell in nematic texture was observed to spread then cooled toward room temperature; a film formed by the two surfaces. The LC was polarized, and optical images of the cell were recorded on a polarization microscope using transmitted light. Optical images of the cell were recorded from C8SH were pretreated in PBS containing 0.9 nm, Fig. 3D) or when SAMs formed 2078 titanium separates the gold and glass (see Fig. 1).

When two mixed SAMs supported on anisotropic gold films were paired to form a LC cell, the polarized-light image of the LC cell was uniform and featureless (Fig. 3A). Therefore, the LC within the cell was uniformly oriented (Fig. 2A). When a second LC cell was prepared using mixed SAMs that were pretreated with PBS containing Av before filling with LC (ΔAV = 2.6 nm), the polarized light image was highly nonuniform and colored (Fig. 3B). The orientation of the LC showed no memory of the anisotropic roughness of the gold film (13) and resembled optical images of LC supported on mixed SAMs formed on gold films with no anisotropic roughness (Fig. 3C). In contrast, the LC remained uniformly oriented when mixed SAMs were pretreated with PBS containing Av blocked with biotin (100-fold excess) (ΔAV,ΔAv = 0.9 nm, Fig. 3D) or when SAMs formed from C6SH were pretreated in PBS containing Av (ΔAV = 0.4 nm) (12). We conclude, therefore, that specific binding of Av to mixed SAMs erases the effect of the nanometer-scale, anisotropic roughness of the gold on the orientation of the bulk LC and thus leads to a readily visualized change in the optical texture of the LC cell. We estimate that within a 1-mm² area of the mixed SAM (an easily visible area), ~10^10 Av molecules (~1 ng or ~2.6 nm of coverage) control the orientations of ~2 × 10^15 mesogens (a 2-μm film of LC). The binding of each Av molecule to the surface is, therefore, amplified into a reorientation of >10^5 mesogens. Because less than half a monolayer of Av can change the orientation of the LC, and because LC films as thick as 100 μm can be oriented by surfaces, higher levels of amplification are likely to be possible (14).

Because the binding of Av to biotin is unusually strong for a protein-ligand interaction (dissociation constant Kd ≈ 10^-15 M), we also demonstrated the use of LCs to detect the binding of antibodies to antigens (Kd ≈ 10^-9 M) (15). For example, the binding of affinity-isolated goat anti-biotin immunoglobulin G (anti–Bi-IgG) (16) to a mixed SAM formed from BiSH and C6SH (Δanti-Bi-IgG = 5.5 nm) caused the orientation of a supported LC to become nonuniform (Fig. 3E). In contrast, neither a nonspecific antibody such as rabbit polyclonal antibody to fluorescein (anti–fluorescein isothiocyanate IgG, Δanti–FITC-IgG = 0 nm) (Fig. 3F) nor bovine serum albumin (BSA, ΔBSA = 1.4 nm) (the optical texture was the same as Fig. 3F) caused a change in the orientation of the LC. In addition, a SAM formed from C6SH did not significantly bind anti–Bi-IgG (Δanti–Bi-IgG = 0.1 nm) and thus oriented 5CB uniformly (the optical texture was the same as Fig. 3F).

These results demonstrate two further principles. First, it is possible to control the anisotropy within gold films so that the immobilization of ligands (such as BiSH) on these surfaces does not disturb the uni-

![Fig. 2. Schematic illustration of a LC cell assembled using SAMs formed from ligand-conjugated thiols and alkanethiols on semitransparent gold films supported on glass substrates. A thin layer of titanium separates the gold and glass (see Fig. 1). Also shown are uniform (A) and twisted (B) orientations of bulk LC bounded by two surfaces.](image-url)
form orientation of the LC before the proteins are bound. We have also successfully introduced oligopeptides (for example, Ala-Ala-Pro-Phe) into SAMs without disturbing the uniform orientation of LCs (17). Second, the roughness of the gold film used in our experiments was such that the threshold surface concentration of Av or anti–Bi-IgG needed to change the orientation of 5CB was greater than the nonspecific adsorption but less than the specific adsorption. This characteristic makes possible a sandwich-type assay in which a capture protein (macromolecular ligand) is supported on a surface, and the binding of a second protein (for example, detecting antibody) to the capture protein is detected by a change in the orientation of the LC.

To demonstrate this principle, we first treated a mixed SAM with fluorescein-labeled streptavidin (FITC-Av) for 10 min (18). The bound FITC-Av (ΔF_{FITC-Av} = 1 nm) was below the threshold required to trigger a change in the orientation of 5CB (Fig. 3G). We then immersed the SAM supporting the bound FITC-Av into a solution of 0.5 µM anti–FITC-IgG in PBS. The ellipsometric thickness of the bound protein after the second step was 3.5 nm and thus was sufficient to trigger a change in the orientation of the LC (Fig. 3H). Anti–FITC-IgG did not bind to a mixed SAM in the absence of bound FITC-Av (ΔF_{anti–FITC-IgG} = -0.1 nm), nor did anti–FITC-IgG blocked with fluorescein bind to a surface presenting FITC-Av (ΔF_{FITC-Av/anti–FITC-IgG} = 0.7 nm); both control experiments produced uniformly oriented LCs (Fig. 3, F and I). By repeating the above experiments using Av, and by binding anti–Av-IgG to an antigenic epitope on Av, we have detected concentrations of anti–Av-IgG in solution as low as 2.3 nM (19).

Twisted nematic LCs (TNLCs) are widely used in flat panel displays because reorientation of the twisted LC by an electric field provides high optical contrast ratios (20). We used TNLCs to enhance the optical transduction of biotin-mediated binding of avidin to surfaces (Fig. 4). Optical readout of the binding of proteins and ligands at surfaces can be further facilitated by using patterned SAMs (21, 22). We designed surfaces such that binding of Av to biotin-derivatized regions of a patterned SAM caused area-specific untwisting of a TNLC cell (Fig. 5). Patterns so formed with sizes of a few centimeters provide an easily read indicator of the presence of a biomolecule in solution (Fig. 5, A and B). By using micrometer-sized patterns, we also demonstrated that the binding of biomolecules at surfaces can be detected optically by the diffraction of light from periodic LC structures that form only when the biomolecules are bound to the surfaces (Fig. 5, C to E).

Our results demonstrate that the binding of proteins to ligands on surfaces can be amplified and transduced into optical signals by using LCs. Because the orientations of LCs are sensitive to a wide variety of physicochemical properties of surfaces, we believe that this approach can be extended to detect the binding of potential-determining ions, small-molecule ligands for proteins, and aggregates of molecules (such as virus particles) to surfaces.

**REFERENCES AND NOTES**


4. Several recent studies have detected the binding of proteins and ligands at surfaces by means of electroanalytical methods in combination with sliding ion channels (8, A. Cornet et al., Nature 387, 580 (1997)) or interferometric methods based on porous silicon [V. S.-Y. Lin et al., Science 278, 840 (1997)]. Stress-induced chromatic transitions caused by multivalent attachment of proteins and viruses to liposomes in solution or to polymer films prepared at the surface of water and transferred to a solid substrate have also been reported [D. H. Charych, J. O. Nagy, W. Spevák, M. D. Bednar- ski, Science 261, 585 (1993); D. Charych et al., Chem. Biol. 3, 113 (1996); J. Pan and D. Charych, Langmuir 13, 1385 (1997)]. Our approach, in contrast, is not restricted to multivalent binding events, does not require the use of complex instrumentation, and uses surfaces that can be formed spontaneously from solution and readily patterned.

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Fig. 4. (A) Optical image through parallel polarizers of a TNLC (see Fig. 2B) formed between a SAM prepared from C_{15}SH and a mixed SAM prepared from BISH and C_8SH (23). The SAMs were supported on anisotropic gold films (the preferred direction of the gold was parallel on the two surfaces). The image is dark because the polarization of light transmitted through the TNLC was rotated by 90° and thus light was extinguished by the analyzer. (B) Optical image (parallel polarizers) of a twisted LC formed between a mixed SAM [as described in (A)] pretreated with Av in PBS and a SAM formed from C_{15}SH. The image is bright because the polarization of light transmitted through the LC was not rotated and thus light was transmitted by the analyzer. We observed LC cells to be dark (parallel polarizers) when mixed SAMs were pretreated with blocked Av or BSA. The films of LC were 20 µm thick. The horizontal dimension in each image is 440 µm.

Fig. 5. (A and B) Optical images of LC sandwiched between two gold films, one supporting a SAM formed from C_{15}SH and the other patterned with a mixed SAM formed from C_{15}SH and BISH and a SAM formed from C_{15}SH. To prepare the latter surface, we used microcontact printing (21) to pattern a SAM formed from C_{15}SH on the surface of a gold film such that an area of gold in the shape of a check mark was left unreacted. The unreacted area of gold was then derivatized with a mixed SAM formed by coadsorption of BISH and C_{15}SH. When viewed by eye through parallel polarizers (plastic sheet, Polaroid) in ambient light, the optical texture of the uniformly twisted LC was featureless and dark (A). When the patterned SAM was immersed in PBS containing Av before contact with the LC, Av bound to the mixed SAM prevented the formation of twisted LC in localized regions of the cell, and thus caused the appearance of an easily visible check mark (parallel polarizers) (B). (C and D) Optical images (parallel polarizers) of LC sandwiched between two gold films, one supporting a SAM formed from C_{15}SH and the other supporting a grating-like pattern of SAMs formed from C_{15}SH and a mixture of C_{8}SH and BISH. Patterned SAMs are shown without bound Av (C) and pretreated with Av in PBS (D). (E) Diffraction pattern formed by laser light incident on the LC cell shown in (C) (dashed line) and (D) (solid line). The cells were held between parallel polarizers, and the spatial variation of the intensity of light in the diffraction pattern was obtained from a digital image of the pattern. The LC layers were 20 µm thick.
Isolation of a Benzene Valve Isomer with One-Electron Phosphorus-Phosphorus Bonds

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A tetraphosphabenzene analog of the postulated anti-tricyclohexyliene, a single biradical valve isomer of benzene, has been isolated. The tricyclic derivative features one-electron phosphorus-phosphorus bonds, which result from the π•−π• interaction between two diphenylerylen radicals. Such one-electron bonds may play a wider role in phosphorus chemistry.

Despite the long history associated with the chemistry of benzene (C6H6), new and fascinating benzene isomers are still being discovered (1). In contrast to the large number of accessible C2H4 isomers [21], generated by a concatenated procedure (2)], the number of possible valence isomers of benzene [(CH)6] is quite small (Fig. 1). To date, only four such compounds have been observed experimentally (3): cis-Dewar benzene A, benzvalene B, prismane C, and bicycloprop-2-enyl D. Recent ab initio calculations have predicted that Mobius benzene E and trans-Dewar benzene F lie in very shallow minima on the potential energy profile, with energies 418 and 660 kJ/mol, respectively, higher than benzene (4); furthermore, benzmobiusstriene G has been proposed by Balaban (5). In addition to these valence isomers, which obey the octet rule, one can imagine a number of biradical structures. So far, none of these species have been isolated, although anti-tricyclohexyliene H has been postulated to be the transition state in the Cope rearrangement of the bicycloprop-2-enyl derivatives D into Dv (6).

Heterobenzene chemistry, especially that involving the heavier main group elements, is comparatively poorly developed (7). For the phosphorus-containing series (P is isologal to the CH fragment), several phospa- (8) and diphosphabenzene (9) valence isomer derivatives, a few 1,3,5-triphosphabenzene derivatives (10), and hexaphosphabenzene in the coordination sphere of transition metals (11) are known. No tetra- and pentaphosphabenzene species have been described.

We report here the synthesis of the transient diphenylerylen radical 2 and the ensuing stable dimer 3 (Scheme 1). The latter

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