

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 $\sim 10^5$ to 10^6 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.

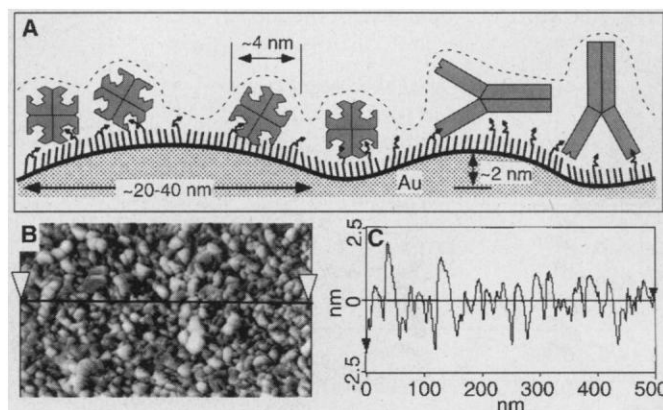
The principles of our approach are based on four properties of LCs (5). First, molecules within LCs (mesogens) can communicate their orientations to regions of the fluid that are up to 100 μm away. This long-range communication between

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-(CH_2)₂[(CH_2)₂O]₂NHCO(CH_2)₁₁SH (BiSH) (8) and $\text{CH}_3(\text{CH}_2)_7\text{SH}$ (C_8SH) by immersion of the anisotropic gold films in ethanolic solutions containing 9.6 μM of BiSH and 70.4 μM of C_8SH for 8 hours (9). The mixed SAMs were estimated to consist of $\sim 27\%$ biotinylated species by linear interpolation of thicknesses (Δ) of single-component SAMs formed from BiSH ($\Delta_{\text{BiSH}} = 3.8$ nm) and C_8SH ($\Delta_{\text{C}_8\text{SH}} = 1$ 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, 0.004 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).



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deionized water for ~ 30 s and dried with a stream of nitrogen for ~ 30 s. LC cells were formed by separating two SAMs with a spacer of thin plastic film (Mylar or Saran Wrap) (Fig. 2). A drop of 4-cyano-4'-pentylbiphenyl (5CB) was drawn in its isotropic phase (isotropic-nematic transition, $\sim 34^\circ\text{C}$) by capillarity into the cavity formed by the two surfaces. The LC was then cooled toward room temperature; a nematic texture was observed to spread across a 1 cm by 1 cm cell in ~ 5 to 10 s. Optical images of the cell were recorded on a polarization microscope using transmission mode.

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 ($\Delta_{\text{Av}} = 2.6$ nm), the polarized light image was highly non-uniform 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) ($\Delta_{\text{blkd Av}} = 0.9$ nm, Fig. 3D) or when SAMs formed from C_8SH were pretreated in PBS containing Av ($\Delta_{\text{Av}} = 0.4$ 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-mm^2 area of the mixed

SAM (an easily visible area), $\sim 10^{10}$ Av molecules (~ 1 ng or ~ 2.6 nm of coverage) control the orientations of $\sim 2 \times 10^{15}$ mesogens (a $2\text{-}\mu\text{m}$ 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 $100\text{ }\mu\text{m}$ 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 $K_d \approx 10^{-15}$ M), we also demonstrated the use of LCs to detect the binding of antibodies to antigens ($K_d \approx 10^{-9}$ 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 C_8SH ($\Delta_{\text{anti-Bi-IgG}} = 5.5$ nm) caused the orientation of a supported LC to become nonuniform (Fig. 3E). In contrast, neither a non-specific antibody such as rabbit polyclonal antibody to fluorescein (anti-fluorescein isothiocyanate IgG, $\Delta_{\text{anti-FITC-IgG}} = 0$ nm) (Fig. 3F) nor bovine serum albumin (BSA, $\Delta_{\text{BSA}} = 1.4$ 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 C_8SH did not significantly bind anti-Bi-IgG ($\Delta_{\text{anti-Bi-IgG}} = 0.1$ 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-

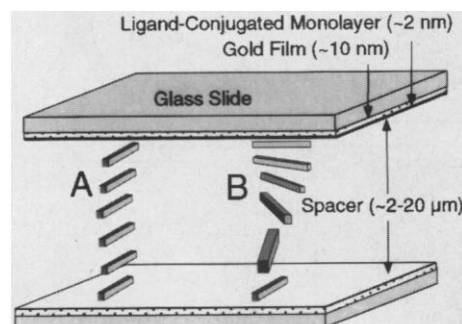


Fig. 2. Schematic illustration of a LC cell assembled using SAMs formed from ligand-conjugated thiols and alkanethiols on semitransparent gold films supported on glass substrates. A thin layer of titanium separates the gold and glass (see Fig. 1). Also shown are uniform (A) and twisted (B) orientations of bulk LC bounded by two surfaces.

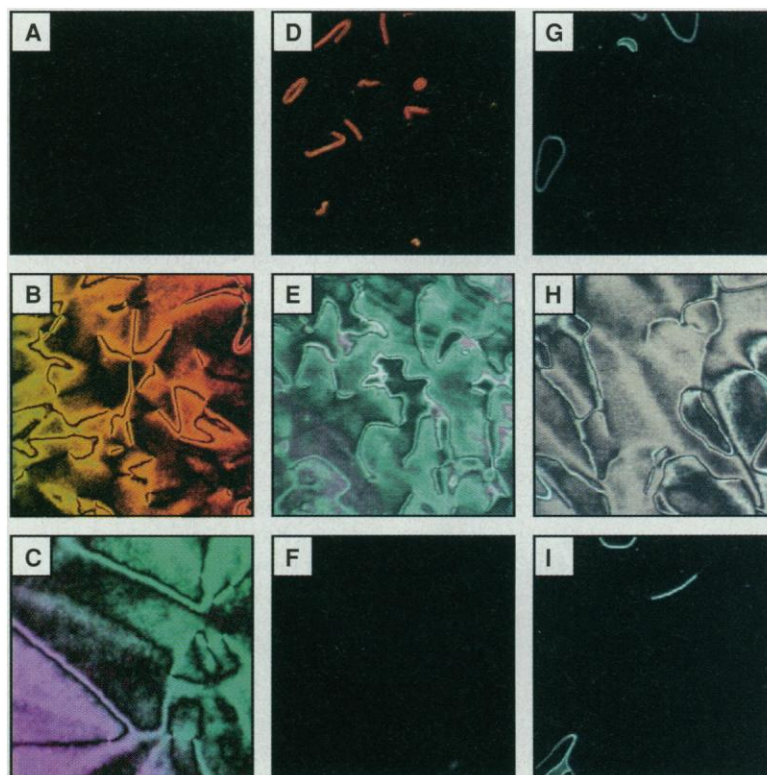


Fig. 3. Optical images (light transmitted through crossed polarizers) of nematic LC sandwiched between mixed SAMs formed from BiSH and C_8SH with or without bound proteins. The thickness of each LC layer was $\sim 2\text{ }\mu\text{m}$. Unless stated otherwise, the mixed SAMs were supported on anisotropic gold films with preferred directions aligned parallel within the LC cell. All images were recorded by aligning one of the polarizers parallel to the preferred direction within the gold films. The horizontal dimension in each image is 1.1 mm. (A) Mixed SAMs before immersion in PBS containing protein molecules. The optical image became uniformly bright when the sample was rotated by 45° between the crossed polarizers. (B) Mixed SAMs pretreated by immersion in PBS containing $0.5\text{ }\mu\text{M}$ Av. (C) Mixed SAMs formed on gold without anisotropic roughness. (D) Mixed SAMs pretreated by immersion in PBS containing $0.5\text{ }\mu\text{M}$ Av blocked with biotin. The bright lines in the image are caused by scattering of light from disclination loops in the LC [similarly for (G) and (I) below]. (E) Mixed SAMs pretreated by immersion for 5 min in PBS containing $0.5\text{ }\mu\text{M}$ anti-Bi-IgG. (F) Mixed SAMs pretreated with a nonspecific antibody by immersion for 15 min in PBS containing $0.5\text{ }\mu\text{M}$ anti-FITC-IgG. (G) Mixed SAMs pretreated by immersion for 10 min in PBS containing $0.5\text{ }\mu\text{M}$ FITC-Av. (H) Mixed SAMs pretreated by immersion for 10 min in PBS containing $0.5\text{ }\mu\text{M}$ FITC-Av, then 15 min in PBS containing $0.5\text{ }\mu\text{M}$ anti-FITC-IgG. (I) Mixed SAMs pretreated by immersion for 10 min in PBS containing $0.5\text{ }\mu\text{M}$ FITC-Av, then 15 min in PBS containing $0.5\text{ }\mu\text{M}$ anti-FITC-IgG blocked with fluorescein.

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 ($\Delta_{\text{FITC-Av}} = 1$ 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 ($\Delta_{\text{anti-FITC-IgG}} = -0.1$ nm), nor did anti-FITC-IgG blocked with fluorescein bind to a surface presenting FITC-Av ($\Delta_{\text{FITC-Av/anti-FITC-IgG}} = 0.7$ nm); both con-

trol 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.

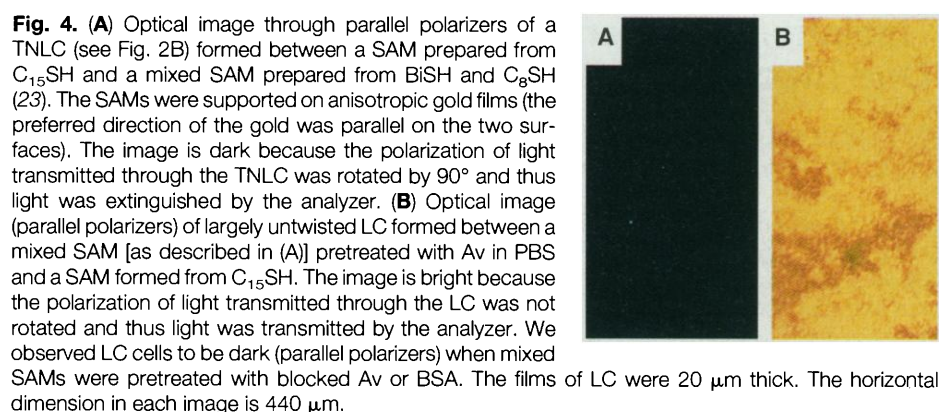
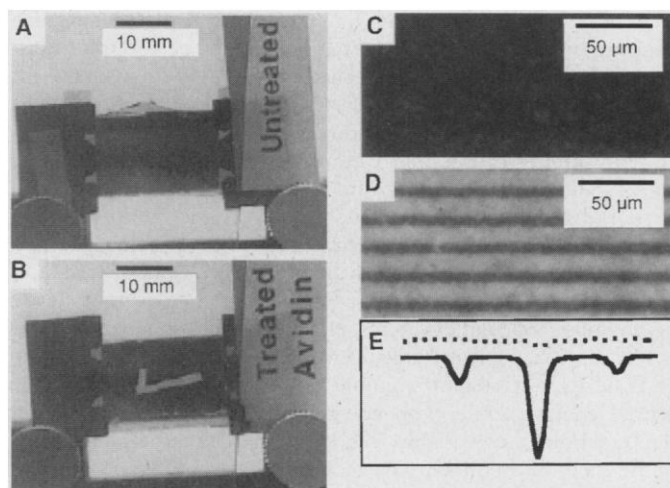


Fig. 5. (A and B) Optical images of LC sandwiched between two gold films, one supporting a SAM formed from C_{15}SH and the other patterned with a mixed SAM formed from C_8SH and BiSH and a SAM formed from C_{16}SH . To prepare the latter surface, we used microcontact printing (27) to pattern a SAM formed from C_{16}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_8SH . 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_{16}SH and a mixture of C_8SH 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.



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3. C. P. Price and D. J. Newman, *Principles and Practice of Immunoassays* (Macmillan, New York, 1991); D. Wild, *The Immunoassay Handbook* (Macmillan, New York, 1994).
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 [B. A. Cornell *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. Spevak, M. D. Bednarski, *Science* **261**, 585 (1993); D. Charych *et al.*, *Chem. Biol.* **3**, 113 (1996); J. Pan and D. Charych, *Langmuir* **13**, 1365 (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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6. Indirect amplification of surface-bound analytes can be achieved with the use of enzyme-linked IgGs that bind to antigenic regions on analytes (3). In contrast to our approach, these methods require the use of IgGs labeled with enzymes and incubation of the surface-bound analytes with IgGs for tens of minutes, during which time the enzyme-linked IgGs bind to the analytes. A second incubation step, typically taking several minutes, is then required to enzymatically transform substrates into colored products.
7. We prepared gold films on a stationary glass substrate with a fixed angle of incidence of gold during deposition. Analysis of atomic force microscopy images of these films reveals their roughness to be greatest in a direction parallel to the direction of deposition of the gold. Using ellipsometry, we have also measured anisotropy in the amplitude and phase of reflected light (J. J. Skaife, V. K. Gupta, N. L. Abbott, unpublished data). On these gold films, nematic LCs orient uniformly in a direction perpendicular to the direction of incidence of the gold and parallel to the plane of the surface [V. K. Gupta and N. L. Abbott, *Langmuir* **12**, 2587 (1996)]. A nematic LC has no preferred orientation in the plane of a gold film that is prepared by rotating the glass substrate during deposition of the gold.
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9. Mixed SAMs were also formed in a few minutes by using solutions that contained millimolar concentrations of the organothiols.
10. All thickness measurements were performed using a Rudolph Auto ellipsometer with light (633 nm) incident at an angle of 70°. A refractive index of 1.45 was used to estimate the thickness of bound layers of thiols and proteins. Standard deviations of the reported ellipsometric thicknesses (Δ) are ± 0.2 nm.
11. We used a form of Av modified to reduce nonspecific adsorption at surfaces (Neutravidin, Pierce). The molecular weight of Av is ~ 60 kD and its dissociation constant with biotin is $K_d = 10^{-15}$ M (3, 8) [P. C. Weber *et al.*, *Science* **243**, 85 (1989)].
12. Triton X-100 (Sigma) is a nonionic detergent that was added to the PBS to minimize nonspecific adsorption of proteins. Use of Triton X-100 reduced the nonspecific adsorption of Av on SAMs formed from C_6SH to $\Delta_{av} = 0.2$ to 0.6 nm. Nonspecific adsorption of proteins on SAMs can also be minimized by using alkane-thiols end-functionalized with oligoethylene glycol or hydroxyl groups [K. L. Prime and G. M. Whitesides, *J. Am. Chem. Soc.* **115**, 10714 (1993)].
13. When rotated between crossed polarizers, the light transmitted through the sample did not show a large modulation in intensity. This result indicates the absence of a preferred orientation of the LC within the cell. The general features of the optical textures were not influenced by variations in rates of cooling of the LC to the ambient temperature.
14. We have shown that specific binding of Av can cause reorientation of films of LCs as thick as 20 μm ($\sim 2 \times 10^6$ mesogens per protein). We did not investigate LC films thicker than 20 μm because the optical effects of birefringence within a 20- μm -thick film of LC are easily seen with the naked eye. The threshold surface mass of avidin that causes reorientation of the LC is a strong function of the manner of deposition of the gold, the composition of the mixed SAM, and the type of LC (J. J. Skaife and N. L. Abbott, unpublished data).
15. Values of K_d reported here correspond to values measured in bulk solution [see, for example, H. Bagci *et al.*, *FEBS Lett.* **322**, 47 (1993)].
16. Anti-Bi-IgG was purchased from Sigma BioScience and anti-FITC-IgG was purchased from Molecular Probes. All measurements were performed in PBS containing 0.5 μM IgG and 0.004% Triton X-100. After binding of the IgG in PBS, the samples were rinsed with deionized water and dried under a stream of nitrogen.
17. Uniform anchoring of nematic 5CB was measured on mixed SAMs formed on anisotropic gold films by coadsorption from an ethanolic solution of 9 mM

$C_{11}SH$ and 1 mM $HS(CH_2)_{11}-Ala-Ala-Pro-Phe-p$ -nitroanilide.

18. Fluorescein-labeled streptavidin (FITC-Av) was purchased from Pierce. All measurements were performed in PBS containing 0.5 μM FITC-Av and 0.004% Triton X-100. After binding of the FITC-Av in PBS, the samples were rinsed with deionized water.
19. Studies based on stress-induced chromatic transitions in polymer films have reported limits of detection for specific binding of pentavalent cholera toxin to ganglioside G_{M1} (molecular weight ~ 100 kD, $K_d \approx 10^{-10}$ M) to be 100 ppm ($\sim 1 \mu M$) when using liposomes in solution and 20 ppm ($\sim 0.2 \mu M$) when using supported films of the polymer (4). The use of this method to detect the binding of antibodies to antigens has not been reported.
20. The surfaces of a TNLC cell are designed such that the region of LC in contact with one surface is oriented at right angles to the region of LC in contact with the opposing surface (Fig. 2B). The LC sandwiched between the two surface regions of the cell undergoes a 90° twist-type deformation, and the polarization of linearly polarized light transmitted through

such a cell is rotated by 90°. A twisted LC cell, when viewed between two parallel polarizers, appears dark. In contrast, a cell containing LC that is not twisted appears bright between parallel polars [B. Bahadur, Ed., *Liquid Crystals: Applications and Uses* (World Scientific, Singapore, 1990)].

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23. The preferred orientation of nematic 5CB in the plane of the SAM is perpendicular to the direction of deposition of gold on SAMs formed from $C_{15}SH$ and parallel to the direction of deposition of gold on SAMs formed from C_6SH [V. K. Gupta and N. L. Abbott, *Phys. Rev. E* **54**, 4540 (1996)].
24. Supported in part by NSF grants CTS-9502263 and DMR-9400354 and by the Office of Naval Research Presidential Early Career Award for Scientists and Engineers (N.L.A.). We thank Boehringer Mannheim and W. Knoll for their gift of BISH, and G. Lopez and K. Oppermann for helpful suggestions.

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Isolation of a Benzene Valence Isomer with One-Electron Phosphorus-Phosphorus Bonds

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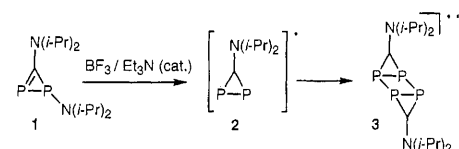
A tetraphosphabenzene analog of the postulated *anti*-tricyclohexylene, a singlet biradical valence isomer of benzene, has been isolated. The tricyclic derivative features one-electron phosphorus-phosphorus bonds, which result from the $\pi^*-\pi^*$ interaction between two diphosphirennyl radicals. Such one-electron bonds may play a wider role in phosphorus chemistry.

Despite the long history associated with the chemistry of benzene (C_6H_6), new and fascinating benzene isomers are still being discovered (1). In contrast to the large number of possible C_6H_6 isomers [217 generated by a computer-aided 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, benzmobiusstripane **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*-tricyclohexylene **H** has been postulated to be the transition state in the Cope rearrangement of the bicycloprop-2-enyl derivatives **D'** into **D''** (6).

Heterobenzene chemistry, especially that involving the heavier main group elements, is comparatively poorly developed (7). For the phosphorus-containing series (P is isolobal to the CH fragment), several phosphazene (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 diphosphirennyl radical **2** and the ensuing stable dimer **3** (Scheme 1). The latter



Scheme 1. Synthesis of diphosphirennyl radical **2** and of its stable dimer **3**. N(*i*-Pr)₂, diisopropylamino.

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