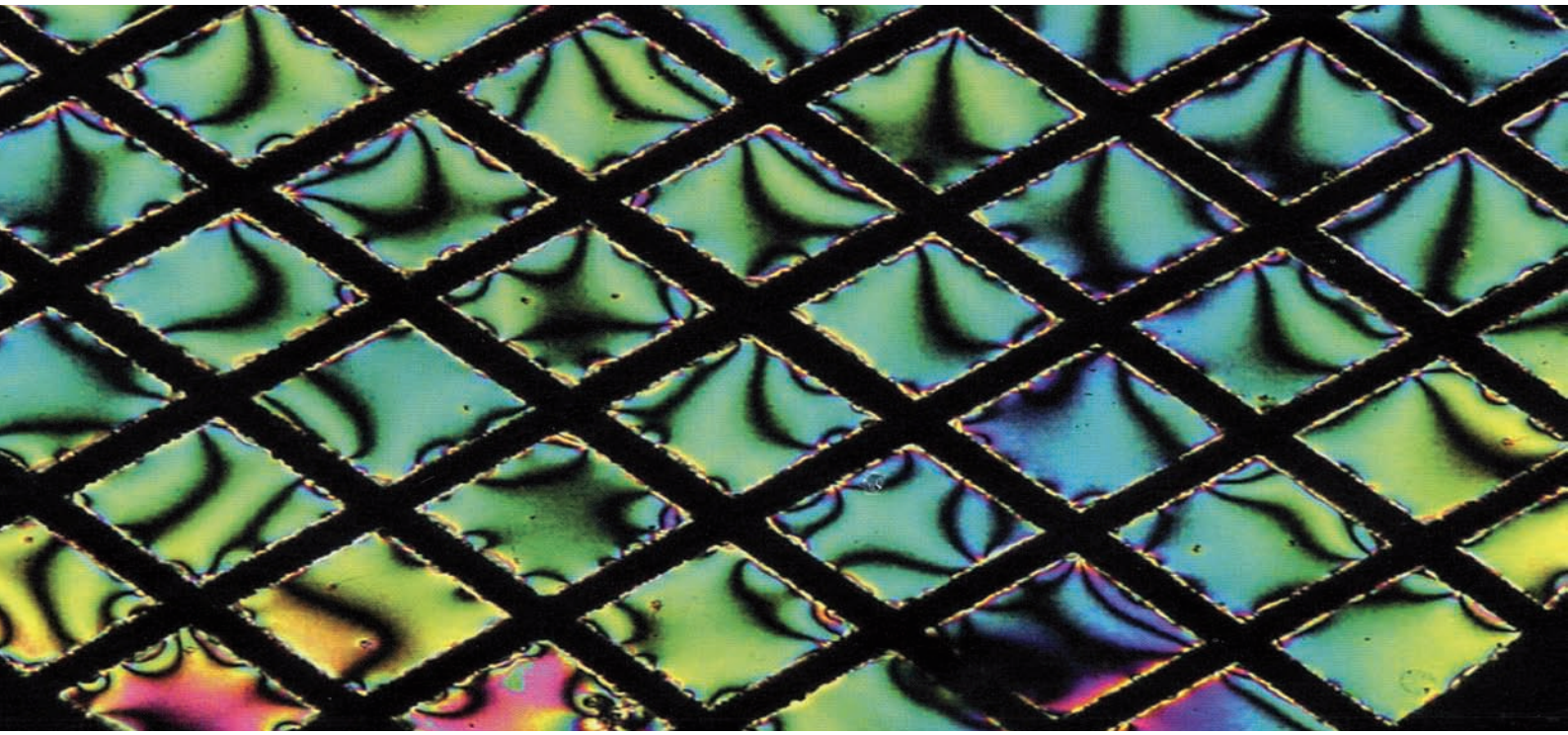
Liquid Crystal-based Analytic Technology

Enabling a Molecular View of Cancer



Liquid crystals (LCs) have wide application in devices such as computer displays, wherein thin films of LCs are sandwiched between two glass surfaces that have electrodes printed on their surfaces. Traditional LC devices employ applied electric fields to control LC ordering and thus optical appearance, whereas here we describe a new class of highly sensitive devices that use LC reordering to report molecular interactions at the surface, which in turn may have application in cancer diagnostics.

Liquid Crystals as Amplifiers of Biomolecular Interactions

Many molecular species presented at surfaces can be reported by LCs, including small organic molecules and large biomolecules such as proteins [1, 2]. By combining the use of LCs with surfaces engineered to have nanoscopic topography and well-defined surface chemistry, we have demonstrated the detection of unlabeled proteins at sensitivity limits ≤ 1 ng/cm^2. Such high sensitivity, without the modification of the target molecule or the use of a secondary antibody, is possible because LCs, by virtue of their molecular properties, provide amplification and transduction of sub-nm-scale processes associated with molecular recognition (fig. 1). The LC output can be an optical signal that is measured with common instrumentation (e.g., a light microscope, optical scanner or plate reader). The combination of speed, sensitivity, spatial resolution, and its label-free nature suggests that LC-based assays possess distinct advantages over conventional molecular detection techniques (e.g., ELISAs) and positions them to advance technologies for the measurement of biomolecular recognition, including clinical applications such as the detection of molecular markers of cancer.

The use of LCs as amplifiers and transducers of molecular events at surfaces is

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based on four fundamental properties (table 1): 1) Molecules forming LCs (called mesogens) can communicate their orientations to each other over distances as large as 100 µm (>10^5 molecular lengths). Thus nanoscopic events of molecular recognition can lead to ordering transitions in LCs that occur on the µm-scale, thereby enabling optical detection. 2) Because mesogens within the LCs possess mobilities that are characteristic of liquids, information about molecular binding events propagates rapidly (within sec) from the surface into the bulk of the LC. 3) Optical anisotropy caused by preferred orientations of mesogens within LCs provides a way to transduce changes in the orientations of bulk LCs into optical signals that are easily read using ambient light. 4) Because the orientational order of an LC near a surface can reflect the molecular-level and/or mesoscopic structure of the surface, surfaces can be tailored such that the presence of proteins on surfaces is amplified and transduced into optical signals.

Measuring LC Output

The output of an LC-based assay results from the interaction of light with the LC, and can be measured as the transmission of polarised light through the LC that contacts the bioanalytic surface. In this format, the film of LC is squeezed between two surfaces, much like an LC display: one surface is the bioanalytic surface, and the two surfaces define a LC cell. When the molecules comprising the LCs are oriented perpendicular to both surfaces, the LC will appear black when viewed in transmission-mode between crossed-polarisers (fig. 1). If a protein is captured onto the analytic surface of the LC cell, the perpendicular alignment of the LC is disturbed and the LC will adopt an orientation that is tilted towards one surface. This change in orientational order, when viewed through crossed polarisers, leads to a dramatically different optical appearance (dark to bright colours) and is the basis for the use of LCs in the detection of biomolecules.

Many variations of the aforementioned device are possible. One approach that leads to enhanced sensitivity, quantitative utility, and a large dynamic range involves the use of "twisted LC cells". This format is related to the "twisted" LC displays that represent the high end of the LC display market. In this approach, the LC is held in the plane of two surfaces that are structured to cause the LC to adopt orientations that are mutually orthogonal (fig. 1). This design leads to a twisting of the LC. The precise degree of twist of the LC is determined by a competition between the mechanical properties of the LC (twist elastic modulus) and the strength of interaction of the LC with the surfaces (anchoring energy). If a targeted analyte, such as a protein, is captured onto the analytic surface, the surface anchoring energy will change, and the torque caused by the bulk elastic properties of the LC will drive a change in the twist angle of the LC. Using optical techniques, the torque (or twist)

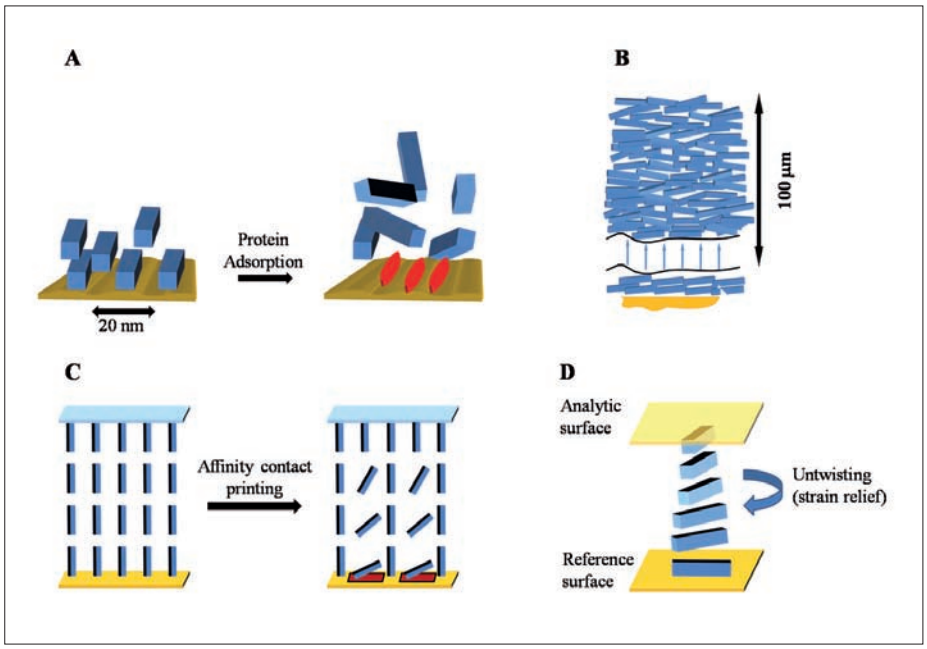

Fig. 1: Previously demonstrated LC-based detection schemes. A) LC mesogens aligned by nanostructured gold become perturbed when biomolecules adsorb to the gold surface. B) The orientation adopted by surface-bound mesogens is communicated to other mesogens within the LC up to 100 µm away. C) After affinity contact printing, the homeotropic alignment of LC mesogens is disrupted by the presence of adsorbed proteins. D) Surface-displayed biomolecules cause a twisted LC to relax proportionately to the amount of bound protein. The exact degree of LC twist is used to calculate surface anchoring energy.

can be precisely quantified and related to the amount of protein present on the surface. Compared to simple LC cells, the twisted LC cells allow for more precise measurements of captured biomolecules with at least an order of magnitude greater sensitivity.

Cancer and the Epidermal Growth Factor Receptor (EGFR)

Cancer is a leading cause of mortality, and promising applications of LC-based analytics lie in the detection and characterisation of biomarkers for various cancers and in the analysis of the effectiveness of chemotherapeutics. Certain cancers develop slowly, and the advent of routine screening procedures such as Pap smears, prostate examinations, and colonoscopies have helped to curtail several cancer-related mortalities. With many other cancers, however, the disease course is often too rapid or silent to benefit from these types of physical examinations. Also, many screening procedures suffer from poor detection sensitivity and yield little data on the manner by which the cancer has developed; in this vein, more effective assays are needed.

Our understanding of cancer development has benefited from recent advances in cell and molecular biology as we are beginning to define the changes in gene expression and protein production/activity that lead to malignancies. These cancer biomarkers lend insight into the course of the disease, and may serve as early indicators of transformation, therapeutic targets and a way to monitor treatment efficacy. Such a biomarker is the EGFR [3], which is a tyrosine kinase that regulates cell growth, migration, and angiogenesis in many tissues [4]. EGFR and related proteins are often overexpressed or mutated in cancers, including lung cancer, which is responsible for the most cancer mortalities in both Europe and North America. Thus, there is a clear need for developing rapid, sensitive, and affordable techniques by which to detect EGFR expression, activity and mutation in tissue samples and biopsies.

Affinity Contact Printing (αCP) and LCs in Biomolecular Analysis

We have recently shown that molecular reporting can be enhanced by combining the use of LCs with αCP, which facilitates the separation and detection of specific proteins from complex mixtures (cell lysates) via the use of surface-immobilised antibodies (fig. 2). Once the antibody is covalently linked to an elastomeric stamp, it can capture and, through physical contact, deliver the specific target molecule to another surface designed for LC detection (the analytic surface). Because the antibody is not transferred to the analytic surface, but rather is linked to a reusable stamp, one can optimise the analytic surface to exhibit maximum sensitivity to the transferred protein. With the wide availability of monoclonal antibodies possessing very high affinities (Kd ≤1 nM), αCP is an attractive technique to use in combination with LC detection.

An example of EGFR detection using αCP and LCs [5] involves EGFR capture from cell membrane preparations of murine fibroblasts transfected with the human EGFR (fig. 2). As a negative control, preparations from non-transfected cells that do not express EGFR were used. The density of EGFR transferred from the elastomer stamp to the analytic surface was estimated at 1,000 molecules/µm^2. The observation that EGFR can be captured and detected from cell membrane preparations illustrates the potential clinical applications of an LC-based assay for rapid biopsy characterisation.

A challenge in using antibodies for affinity-based detection platforms lies in the immobilisation strategies used to obtain a functional surface orientation. This issue is key given that the efficacy and sensitivity of LC-based assays hinge directly upon the design of a capture surface, abundantly decorated with an affinity reagent that can be finely tuned with regard to its functional availability and specificity. Although we have successfully used passive adsorption of antibodies and covalent crosslinking to the assay surface using non-specific sites such as primary amines, we are developing site-specific antibody functionalisation [6, 7] methods and EGFR-directed single chain antibodies that should allow oriented presentation of these affinity reagents on the assay surface.

Besides the use of immobilised antibodies, experiments using twisted LC cells have also employed peptide substrates of EGFR immobilised on a surface in a controlled density [8]. The peptide-laden surface was incubated with an antibody specific for the phosphorylated form of the peptide. By measuring the change in twist of the LC, the decrease in surface anchoring energy of the LC was determined as a function of phosphospecific antibody concentration. This study established a correlation between antibody concentration and the surface anchoring energy (fig. 2) of the LC, and demonstrated the capacity to detect very low levels (10 pM) of bound antibody. Strikingly, the anchoring energy measurements remained linear over four orders of magnitude of antibody concentration.

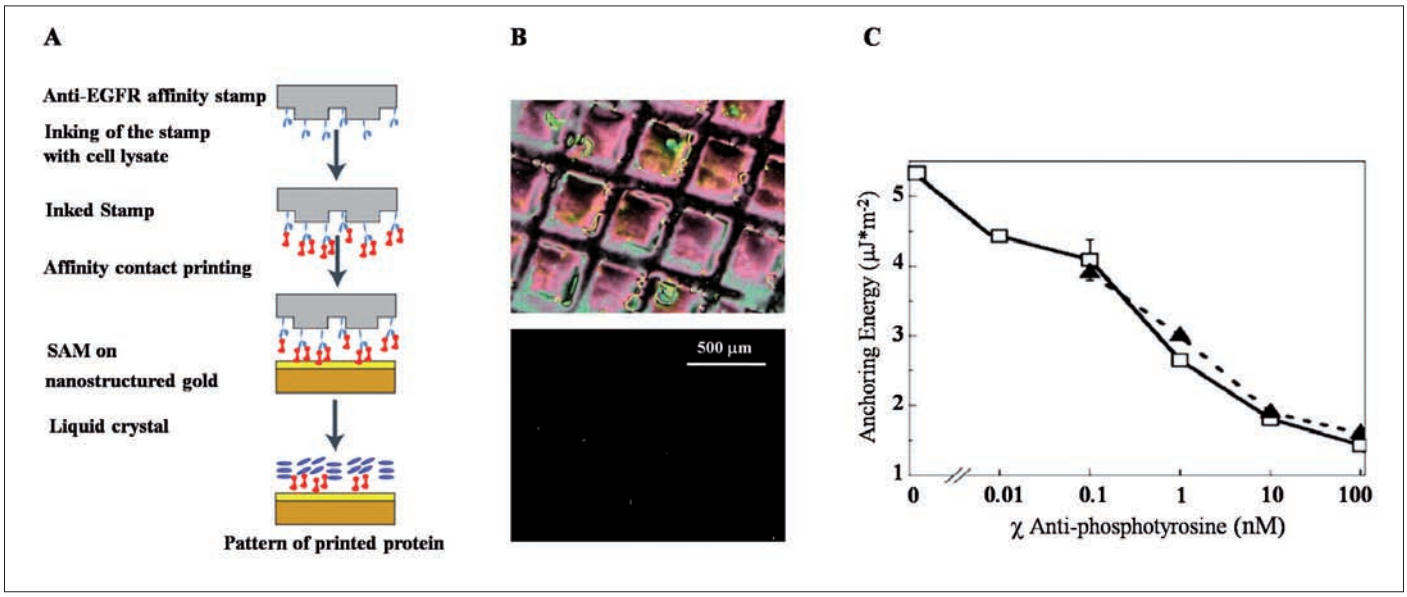

Fig. 2: A) Schematic illustration of αCP, where an EGFR specific antibody that is covalently attached to an elastomeric substrate (PDMS) captures EGFR from a cell lysate. The captured EGFR is printed onto a nanostructured gold surface for LC imaging. B) Polarised light micrograph of affinity captured and printed EGFR. Regions with printed protein are visible as pink squares. The bottom image is a negative control from a cell line that does not express EGFR. Figure reproduced with permission from J Am Chem Soc 2005, 127:8912. Copyright 2005 American Chemical Society C) Anchoring energy measurements from a twisted LC cell. An oligopeptide substrate of EGFR was immobilised onto a nanostructured gold surface and incubated with varying concentrations (χ) of an antibody specific for the oligopeptide sequence. Two independent data sets are shown, with error bars calculated from 5 repeat measurements at 100 pM antibody incubation. The peptide was immobilised at ~0.001% of full monolayer coverage. Figure reproduced with permission from J Am Chem Soc 2007, 129:11223. Copyright 2007 American Chemical Society

In sum, the use of the torque balance technique (twisted LC cells) to detect and quantify biomolecules is a significant development in LC based assays. The sensitive and quantitative nature of the technique suggests that these assays possess the potential for clinical utility.

The Future of Rapid and Facile Biomolecular Analyses

LC-based detection schemes using αCP are a promising development in the engineering of rapid biomolecular and chemical assays. Furthermore, the broad applicability of this technology will likely be enhanced by employing vast antibody libraries that can be easily modified for effective surface functionalisation. A technology that will lead to sensitive, rapid, and affordable assays for detecting a panel of toxins and chemical agents, a set of cancer biomarkers, activated signaling molecules, or even portions of an entire proteome is almost within our reach.

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Table: 1: Advantages of Liquid Crystals as Amplifiers and Transducers of Molecular Events at Surfaces

Liquid Crystal Attributes	Advantages in the Application for Protein Detection
Sensitivity	Detection limits lower than 10^4 copies of a protein; 3–4 orders of magnitude more sensitive than standard methods (gel electrophoresis, ELISA)
Resolution	Detection can occur in localised regions on the micrometer scale; permits the imaging of binding events at the surface in multiplexed analyses
Output	Generation of optical signal in the visual range that can be easily detected and quantified; does not require labeling, fluorescent or redox tags, nor complex instrumentation
Speed	Output (via liquid crystal ordering) occurs in < 1 second; does not require lengthy incubations or precise timings as with other methods (e.g. ELISAs)

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