

AMPLIFICATION OF BIOMOLECULAR INTERACTIONS USING LIQUID CRYSTALLINE MATERIALS

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INTRODUCTION: The immobilization of oriented and functional proteins as well as detection of specific binding events to immobilized proteins are two key challenges (amongst many) that must be addressed if surface-based proteomics tools are to be successfully developed. These tools possess the potential to substantially accelerate investigations aimed at understanding issues such as the roles of populations of regulatory proteins in cell signaling processes. This paper reports a strategy for the oriented immobilization of protein receptors on gold films possessing nanometer-scale topographies, and detection of protein binding events to these receptors by using the surface-driven orientational behavior of liquid crystals.^{1,2}

METHODS: The approach reported in this paper revolves around the use of self-assembled monolayers (SAMs) formed from nitrilotriacetic acid (NTA)-terminated alkanethiols, **1**, and tri(ethylene glycol)-terminated alkanethiols, **2**. The SAMs are formed on ultrathin gold films that are deposited from a vapor onto silica substrates oriented at an oblique angle of incidence. These polycrystalline gold films possess both in-plane and out-of-plane crystallographic textures as well as an anisotropic topography that can be idealized as a corrugation with an amplitude of 1-2 nm and a wavelength of 10-40 nm. Because the sizes of many proteins are comparable to the spatial scale of the topography of the surface, proteins bound to these SAMs can mask or erase the topography of the surface. The uniform alignment of liquid crystal (e.g., 4-cyano-4'-pentylbiphenyl (5CB) or 4-methoxybenzylidene-4'-butylaniline (MBBA)), which is observed on these surfaces not supporting bound protein, is disrupted by the presence of bound protein. The change in orientation of the liquid crystal is observed by the transmission of polarized light through the liquid crystal.

RESULTS: We have found that single component SAMs formed from **2** on these gold films resist non-specific protein adsorption (using cell lysates) and promote uniform planar anchoring of the nematic liquid crystal, 4-cyano-4'-pentylbiphenyl (5CB). Surprisingly, the azimuthal orientation of nematic 5CB is parallel to the direction of maximum roughness within the gold film when using SAMs formed from **2**, but perpendicular to the direction of maximum roughness when tetra(ethylene glycol)-terminated SAMs are formed on the gold films. Mixed SAMs formed from **1** and **2** bind the hexahistidine-tagged protein MEK via specific complexation of the hexahistidine tags of MEK to the Ni(II)-NTA complexes on the surface.

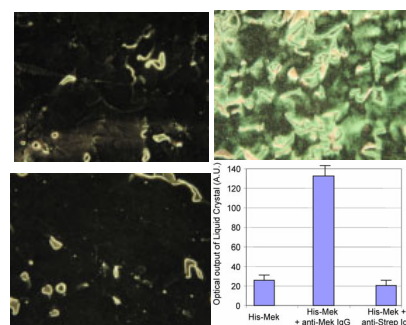


Fig. 1: Optical images (crossed polarizers) of nematic 5CB supported on surfaces that present: (A) His-tag Mek, (B) His-tag Mek to which anti-Mek IgG was bound, (C) His-tag Mek exposed to a solution of anti-streptavidin IgG (control experiment). Binding of anti-Mek IgG to the his-tag Mek gives rise to an easily distinguished (and quantifiable) change in light transmission through the LC (error bars indicate standard error of mean luminosity of liquid crystal).

When gold films are prepared by oblique deposition at an angle of 30° from normal, we measured bound MEK to disrupt the uniform orientation of 5CB, thus leading to an easily visualized change in the optical appearance of the liquid crystal. However, by using gold films deposited at an angle of 40° from normal, we report that bound MEK does not disrupt the alignment of the liquid crystal whereas anti-MEK IgG bound to the MEK does lead to a non-uniform alignment of liquid crystal (Fig 1).

DISCUSSION & CONCLUSIONS: These results, when combined with appropriate control experiments (see Figure 1), suggest that nanostructured surfaces presenting NTA and ethylene glycol-terminated SAMs form a useful interface for liquid-crystal-based reporting of specific binding events between proteins.

REFERENCES: ¹V.K. Gupta, J.J. Skaife, T.B. Dubrovsky, and N.L. Abbott (1998) *Science*, **279**: 2077-2080. ²Y.Y. Luk, M.L. Tingey, D.J. Hall, B.A. Israel, C.J. Murphy, P.J. Bertics, and N.L. Abbott (2003) *Langmuir*, **19**: 1671-1680

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