Supporting Information for:

Using Measurements of Anchoring Energies of Liquid Crystals on Surfaces to Quantify Proteins Captured by Immobilized Ligands

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Below we provide additional details regarding the preparation and analysis of samples used to determine azimuthal anchoring energies with the torque balance method.

Infrared analysis. Infrared spectra of SAMs supported on gold films (thickness of 2000Å) were obtained using a Nicolet Magna-IR 860 FT-IR spectrometer with photoelastic modulator (PEM-90, Hinds Instruments, Hillsboro, OR), synchronous sampling demodulator (SSD-100, GWC Technologies, Madison, WI) and a liquid N_2-cooled mercury cadmium telluride (MCT) detector. All spectra were taken at an incident angle of 83° with the modulation centered at 1800 cm^{-1}. For each sample, 500 scans were taken at a resolution of 4 cm^{-1}. Data was collected as differential reflectance vs. wavenumber and spectra were normalized and converted to absorbance units (Figure S1).

Sample assembly. Patterned SAMs were formed on gold films deposited at an angle of θi = 49°, as described in the main text of the manuscript. An optical cell was fabricated using the patterned substrate and a reference plate comprised of a pentadecanethiol SAM supported on a gold substrate deposited at an angle of θ_i = 64° (Figure S2). The orientations of nematic liquid crystals on obliquely deposited gold films depend on the molecular structures of SAMs formed on the films.[1] Nematic 5CB orients on **EG4** SAMs with an easy axis $\boldsymbol{\eta_0}$ that is perpendicular to the azimuthal direction of gold deposition.[2] In contrast, 5CB in contact with contact printed-hexadecanethiol (C16) SAMs orients such that $\boldsymbol{\eta_0}$ is parallel to the direction of gold deposition. Of relevance to the reference surface, 5CB in contact with the pentadecanethiol (C15) SAM will orient such that $\boldsymbol{\eta_0}$ is perpendicular to the direction of gold deposition.[1] These boundary conditions give rise to the patterned orientations depicted in Figure S2C. The boundary conditions defined by the C15 and C16 SAMs give rise to uniform planar orientations of the LC.

Determination of 5CB film thickness (d). We used the optical properties of the LC in the hybrid regions to determine the thickness of the film of LC at a given position in the wedge-shaped optical cell (Figure S2B). The LC cell was positioned on the stage of a polarizing light microscope with source polarizer and analyzer set at 90° (Figure S3A). We note here that the region of twisted LC appears bright when viewed under crossed polarizers as it rotates plane polarized light. The optical appearance of the LC confined

in the hybrid region is dark, when the orientation of the LC is parallel to either polarizer or analyzer. Rotation of both the analyzer and the polarizer 45° (Figure S3B) caused the hybrid regions to create brightly colored interference bands. The bands are caused by the wavelength-specific retardation of light passing through the film of liquid crystal as a function of thickness.[3] By using the Michel-Levy interference chart,[4] one can relate the observed interference color to the thickness of the LC film, when the birefringence of the material is known.[4] To determine the thickness of the LC film in regions 1-7 of the twisted nematic LC, we noted the color of the interference band in the hybrid region immediately adjacent to the region of interest. In the example shown in Figure S3B, all measurements were taken along the dotted line, where the optical appearance of the LC in the hybrid region was green and corresponded to a thickness of 6.0 ± 0.5 μm.

Measurement of angles for calculation of anchoring energies. The angle diagram used to identify Ψ and φ from the experimentally measured parameters δ and γ is shown in Figure 1 in the manuscript.[2,5] The angle γ, measured experimentally, is orthogonal the equilibrium position of the director $\boldsymbol{\eta}_d$-top. Therefore the angle formed between the source polarizer P and η_d-top, corresponding to Ψ, is (γ - 90°). Similarly, we experimentally determined δ; we calculated φ from $\varphi = \delta - \Psi$ (Figure S4).

Quantification of experimental error. To quantify the experimental error involved in measurements of the anchoring energy, we treated each of the Regions 3-7 of a sample with 0.1 nM anti-phosphotyrosine antibody (Table S1). From these measurements a standard error of the mean (*n*=5) was calculated to be ± 0.5° (as indicated by the error bars in Figure 7 of the manuscript). The corresponding standard error in calculated values of anchoring energies was 0.3 $\mu J/m^2$ (Table S1, error bars in Figure 8).

References

(1) Skaife, J. J.; Abbott, N. L.; *Chem. Mater*. **1999**, *11*, 612-623.

(2) Clare, B. H.; Guzman, O.; de Pablo, J. J.; Abbott, N. L. *Langmuir* **2006**, *22*, 4654-4659.

(3) Hartshorne, N. H.; Stuart, A. *Crystals and the Polarizing Microscope*, 4th ed. ed.; Edward Arnold Publishers, Ltd.: London, 1970.

(4) More recent versions of the Michel-Levy can be found at the Olympus website: www.olympusmicro.com.

(5) Fonseca, J. G.; Galerne, Y. *Appl. Phys. Lett.* **2001**, *79*, 2910.

Table S1. Anchoring energies (**W**) of LC measured upon exposure of **Y1173-** and **pY1173-** decorated surfaces to 100 pM anti-phosphotyrosine antibody.

Region	**Peptide** (0.01 μ M) + **antibody** (100 pM)	γ (degrees)	δ (degrees)	φ (degrees)	ψ (degrees)	**W** (**μJ/m^2**)
1	EG4	165.09	84.95	9.86	75.09	5.44
2	Y1173	162.2	83	10.8	72.2	4.83
3	pY1173	156.56	81.41	14.85	66.56	3.30
4	pY1173	156.95	79.35	12.4	66.95	3.91
5	pY1173	156.24	79.9	13.66	66.24	3.55
6	pY1173	156.52	78.73	12.21	66.52	3.95
7	pY1173	159.35	82.16	12.81	69.35	3.93

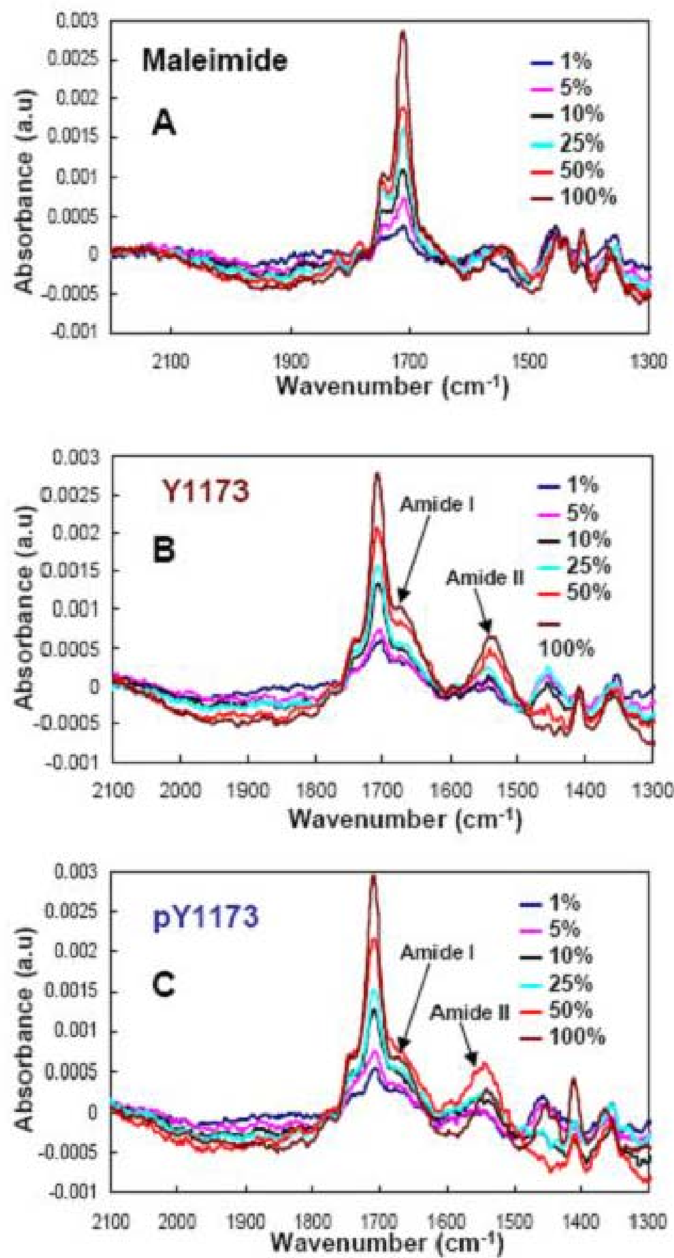

Figure S1. Baseline-corrected PM-IRRAS spectra of maleimide modified SAMs (**A**), and EFGR peptide (Y1173 and pY1173) modified SAMs (**B** and **C** respectively).

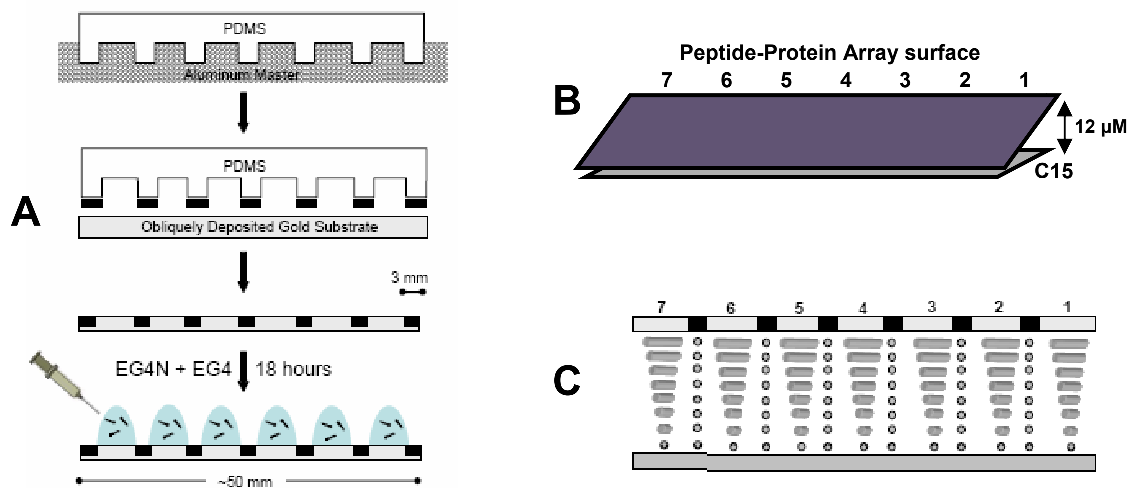

Figure S2. **A**: Procedure used to pattern SAMs onto obliquely deposited gold substrates. **B**: Pictorial representation of optical cell prepared from peptide-protein array (top) and reference surface (C15, bottom). **C**: Schematic illustration of orientation of LC between surfaces indicated in **B**.

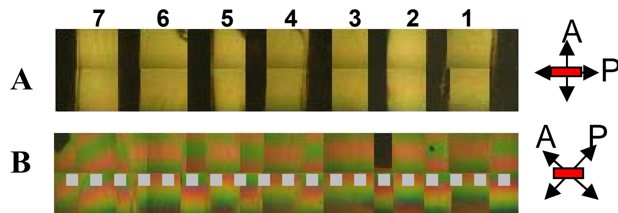

Figure S3. Optical images of control experiment used to determine standard error with 0.1 nM antibody (IgG) and 0.01 µM pY1173. **A and B**: Polarized light micrographs of the peptide-antibody array viewed with the orientation of the polarizer and analyzer as shown to the right of each image. A: Analyzer, P: Polarizer. The dotted line in B indicates the position (constant thickness d = 6.0 ± 0.5 µm, as determined from Michel-Levy chart) at which all measurements were made.

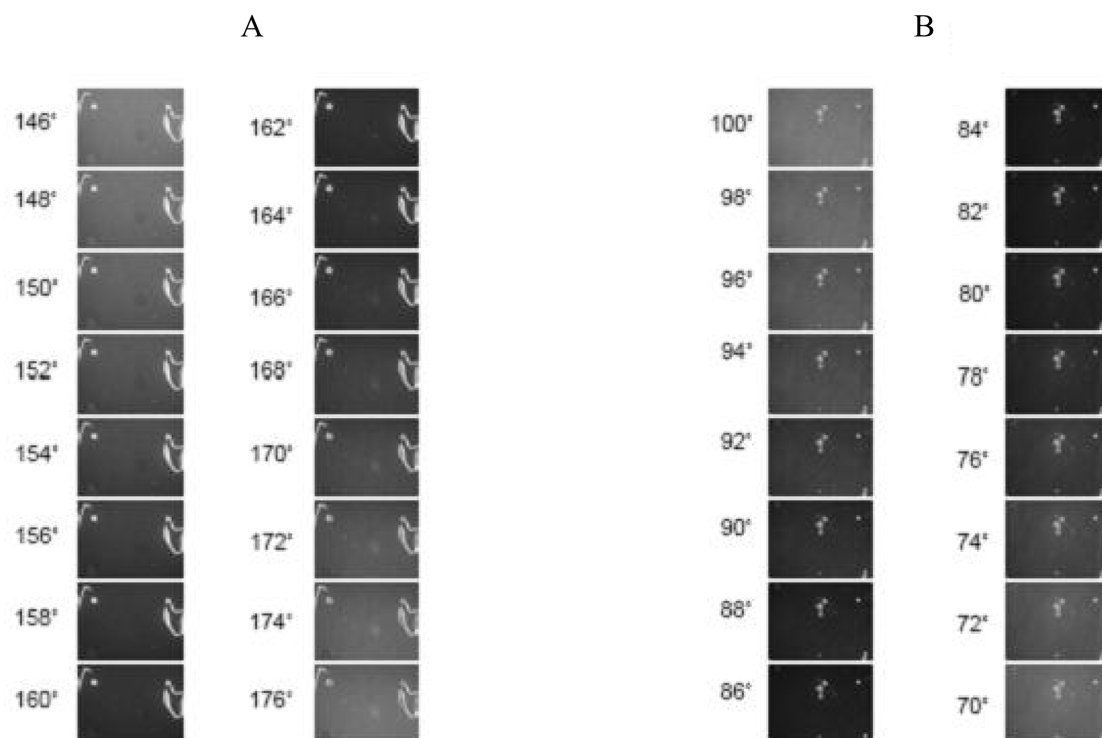

Figure S4: Optical images used to determine angles **γ** and **δ** from region 3 of the array. **A** and **B**: Gray-scale polarized light images captured as a function of analyzer position for twisted (to determine **γ)** and hybrid regions (to determine **δ)** respectively.