Supporting Information for

Liquid Crystal Multiplexed Protease Assays Reporting Enzymatic Activities as Optical Bar Charts

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HATR-FTIR

A Fourier transformation infrared (FTIR) spectrophotometer (model: IRPrestige-21) from Shimadzu (Japan) in the Horizontal Attenuated Total Reflectance (HATR) mode was used for all measurements. This spectrophotometer was also equipped with a liquid nitrogen-cooled, mercury-cadmium-telluride (MCT) detector and an infrared polarizer (GPR-8000) to achieve better sensitivity. Prior to each measurement, a silicon trough plate (PIKE Technologies, U.S.A.) was polished with AUTOSOL®, and then rinsed thoroughly with deionized water. Surface modifications of the silicon trough plate are the same as that of glass slides. The resolution was maintained at 4 cm^{-1} and 256 scans were accumulated.

In our experiment, we created an aldehyde-terminated surface by sequential deposition of DMOAP and TEA onto a solid substrate. The resulting surface prepared by this strategy has two main advantages. First, the long hydrocarbon chain of DMOAP helps to orient TEA and the subsequent oligopeptide molecules when they are immobilized on the surface. Second, the orientation of LCs supported on this surface is uniformly homeotropic.[S1,S2] We used HATR-FTIR to examine the immobilization of TEA on the DMOAP-coated surface. As shown in Figure S1, the peak at 1726 cm^{-1} is assigned to the stretching mode of C=O, which confirms that TEA is immobilized on the DMOAP-coated surface.

FITC array

We created an FITC microarray with concentrations ranging from 0.01 μM to 5 μM. After this, we did not rinse the surface to make sure that all of the FITC molecules in solutions deposited to the surface (with a spot size of 0.8 mm in diameter). Therefore, the surface densities of FITC are 0.12, 0.6, 1.2, 2.4, and 6.0 ($\times 10^{10}$ /mm^2), respectively from the top to down in Figure S2a. A linear correlation between the fluorescence intensity and the surface density of FITC can be obtained as shown in Figure S2b. The

correlation coefficient of the fitting line was 0.96. It was noted that the fluorescence intensity did not increase linearly if the surface density of FITC exceeded $2.4 \times 10^{10}/mm^2$ (which was not shown in Figure S2b). This is probably because of the self-quenching at the high surface density of FITC.

Ellipsometry

Ellipsometric thicknesses of oligopeptides coated silicon wafers were measured by using a Stokes Ellipsometer LSE (Gaertner, U.S.A.) at a wavelength of 632 nm and a fixed angle of 70°. Ellipsometric constants, *n* and *k*, of each cleaned silicon wafer were found to be 3.92 and 0.0202, respectively. The ellipsometric thicknesses of the surface organic layers were determined by assuming a single reflective index of 1.46 for a one-layer model. Each thickness represents an average thickness of five different spots.

To confirm that **P1** was immobilized on the aldehyde-terminated surface, we also performed ATR-FTIR on the silicon trough plate. Figure S4a shows that the FTIR spectrum exhibits several peaks after the immobilization of **P1** on the aldehyde-terminated surface. Among them, the peak at 3285 cm^{-1} can be assigned to the stretching mode of primary amine,[S3] suggesting that **P1** was immobilized on the aldehyde-terminated surface. Moreover, the primary amine in lysine residue was free after the immobilization. On the other hand, we also performed the immobilization reaction on DMOAP-coated silicon trough plate; no new peaks were observed (Figure S4b).

References

S1. Kahn, F. J. *Appl. Phys. Lett.* **1973**, *22*, 386.
S2. Kocevar, K.; Musevic, I. *ChemPhysChem* **2003**, *4*, 1049.
S3. Chiang, C.; Irhida, H.; Koenig, J. L. *J. Colloid. Interface Sci.* **1980**, *74*, 396.

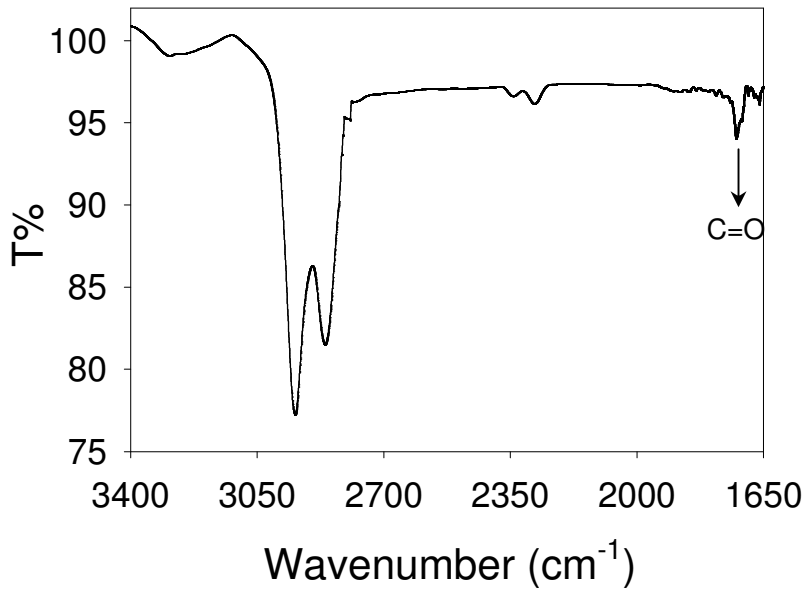

Figure S1. HATR-FTIR spectrum of silicon trough plate after it was modified with DMOAP for 1 min and then 2% TEA for 2 h. The unmodified silicon trough plate was used as a reference.

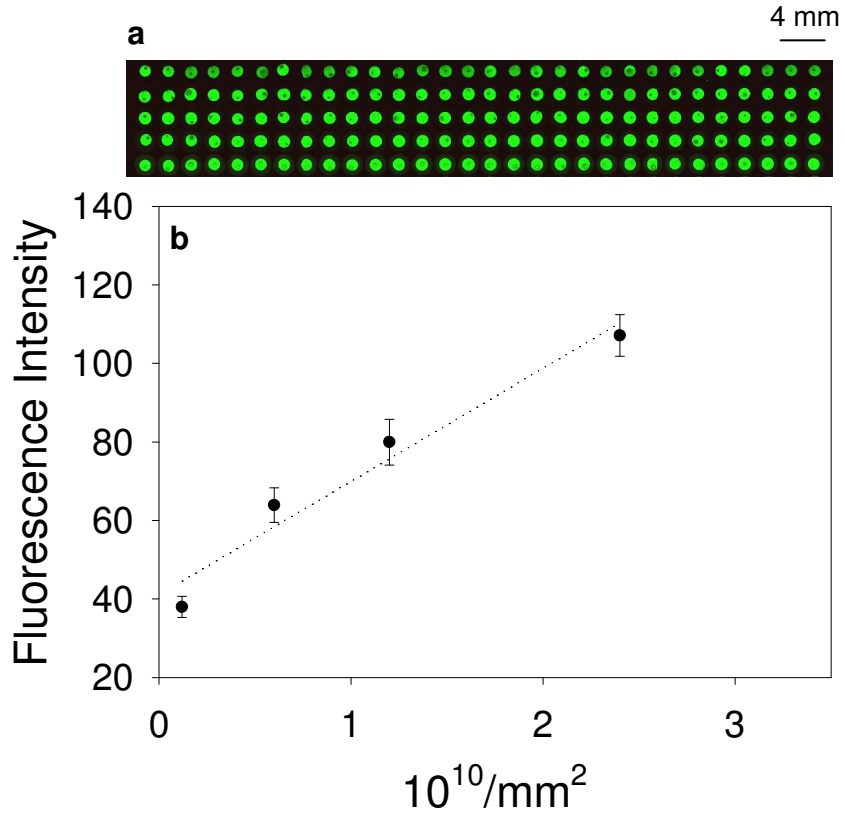

Figure S2. (a) Fluorescence images of different concentrations of FITC deposited on aldehyde-terminated surface. (b) Average fluorescence intensity obtained at different surface densities of FTIC.

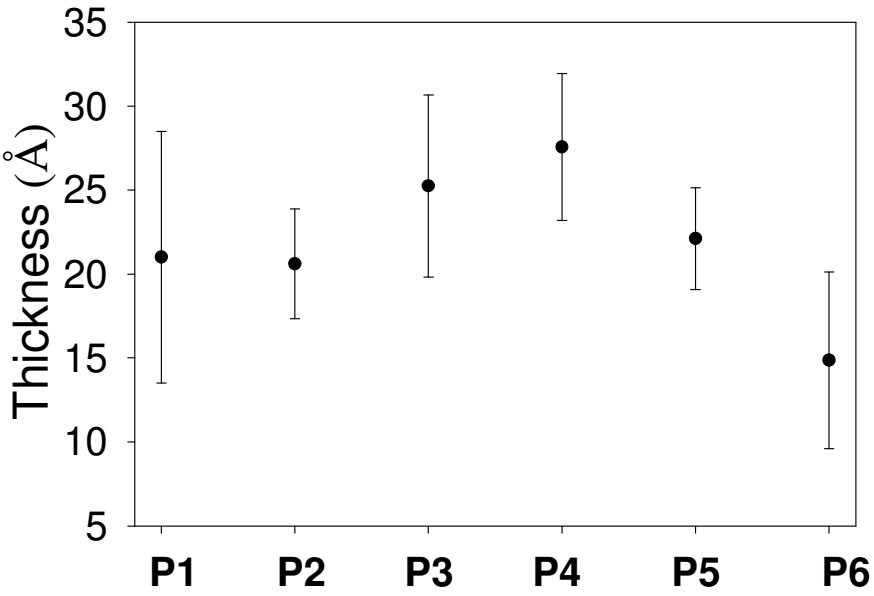

Figure S3. Ellipsometric thicknesses of aldehyde-terminated silicon wafers after they were modified with **P1**-**P6**, respectively.

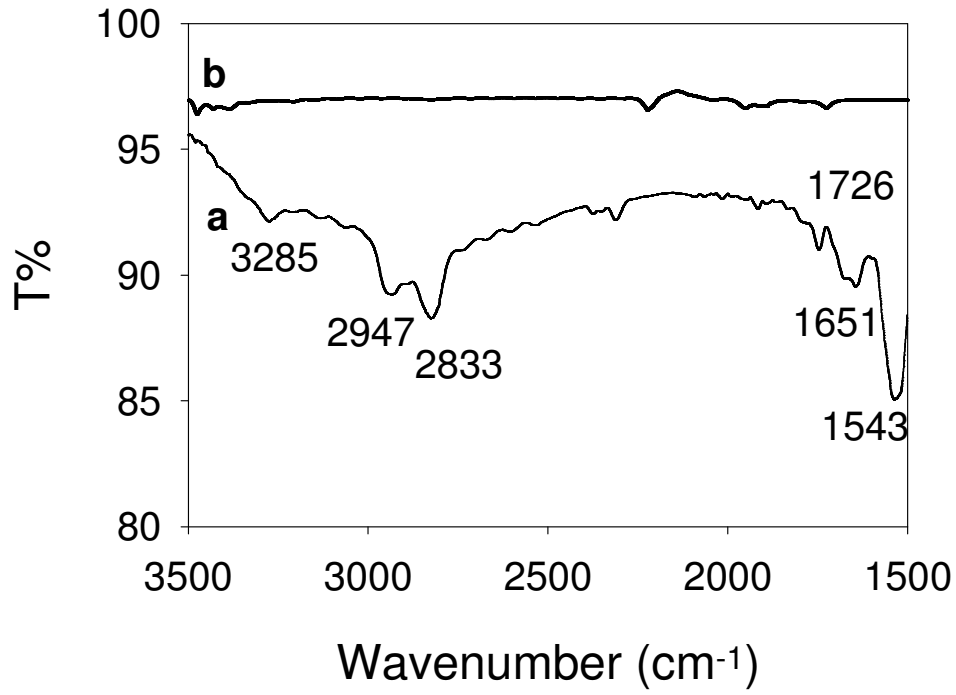

Figure S4. HATR-FTIR spectra of silicon trough plates after they were modified with (a) DMOAP for 1 min and then 2% TEA for 2 h and (b) DMOAP for 1 min and then incubated in 50 μM **P1** for 12 h. The aldehyde-terminated and DMOAP-coated silicon trough plates were used as references for (a) and (b), respectively.

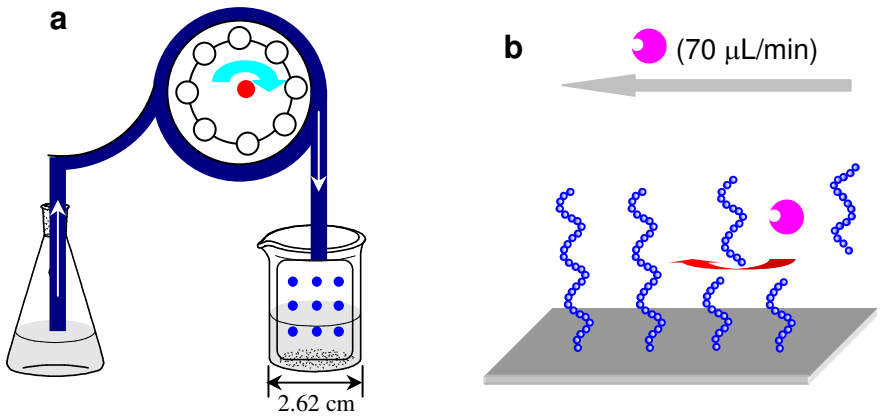

Figure S5. (a) Experimental set-up for the gradient immersion time mode (delivery of trypsin solution to **P1** microarray by using the peristaltic pump). (b) Schematic illustrations of the trypsin cleavage on the oligopeptide substrates.