Supporting Information

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Control of Nanoscale Environment to Improve Stability of Immobilized Proteins on Diamond Surfaces

Adarsh D. Radadia, Courtney J. Stavis, Rogan Carr, Hongjun Zeng, William P. King, John A. Carlisle, Aleksei Aksimentiev, Robert J. Hamers, * and Rashid Bashir *

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By *Adarsh D. Radadia*, *Courtney J. Stavis*, *Rogan Carr*, *Hongjun Zeng*, *William P. King*, *John A. Carlisle*, *Aleksei Aksimentiev*, *Robert J. Hamers**, and *Rashid Bashir**

[*] Prof. R. Bashir, Dr. A.D. Radadia
Micro and Nano Technology Laboratory
University of Illinois
208 North Wright Street, Urbana, Illinois 61801 (USA)
E-mail: rbashir@illinois.edu

Prof. W. P. King
Department of Mechanical Science and Engineering, Micro and Nano Technology Laboratory
University of Illinois
208 North Wright Street, Urbana, Illinois 61801 (USA)

Prof. A. Aksimentiev, R. Carr
Department of Physics
University of Illinois
Urbana, IL 61801 (USA)

Prof. R.J. Hamers, C.J. Stavis
Department of Chemistry
University of Wisconsin
Madison, WI 53706 (USA)
E-mail: rjhamers@wisc.edu

Dr. J.A. Carlisle, H. Zeng
Advanced Diamond Technologies, Inc
Romeoville, IL 60446 (USA)

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Experimental:

Check Increase in Non-specific Adsorption During 37 ºC Stability Studies: FITC-labeled goat antibodies were used to quantify increase in non-specific binding on immunosurfaces during the 37 °C stability tests. PBS from wells was removed and surfaces were exposed to FITC-labeled antibodies (5 µg/ml PBS, 30 min, 22 °C). To remove the excess FITC-labeled anti-*O157:H7*, surfaces were further washed with PBS twice. Fluorescence from non-specifically bound antibodies was imaged and fluorescence from the surface was quantified using ImageJ. Surface containing fluorescently labeled bacteria was used as reference.

Generate Calibration for Quantified ELISA: Quantified ELISA was possible due to optimization of HRP-antibody concentration and generation of a calibration curve co-relating antibody mass and optical density readings (see Supplementary Fig. S6). Nunc MaxiSorp 384-well plate was used to adsorb 0.05-0.4 µg anti-*O157:H7* per well by incubation at 4 °C overnight (18-20 hr). Wells were prepared in quadruplicates to test four HRP-antibody concentrations. Next day, MaxiSorp wells were washed with PBS-Tween20 twice and PBS once. Non-specific binding sites in wells were blocked using casein solution (1h, 22 °C) and excess blocking agent was rinsed using the washing routine. HRP-antibody concentrations of 250, 100, 50 and 12.5 ng ml^{-1} were prepared by diluting 0.5 mg ml^{-1} HRP-antibody in BSA diluent. MaxiSorp wells were incubated with HRP-antibody for 30 min at 22 °C. The wells were then rinsed using the washing routine and exposed to HRP substrate, Sure Blue Reserve (KPL) for 5 min. The reacted substrate solution was transferred to a 384 well plate and quenched with equivalent amount of hydrochloric acid (1M). We found that HRP-antibody concentration of 50 ng ml^{-1} gives linear and sensitive optical density reading. Optical density at 450 nm was plot as function of goat antibody concentration to generate the calibration curve.

Figures:

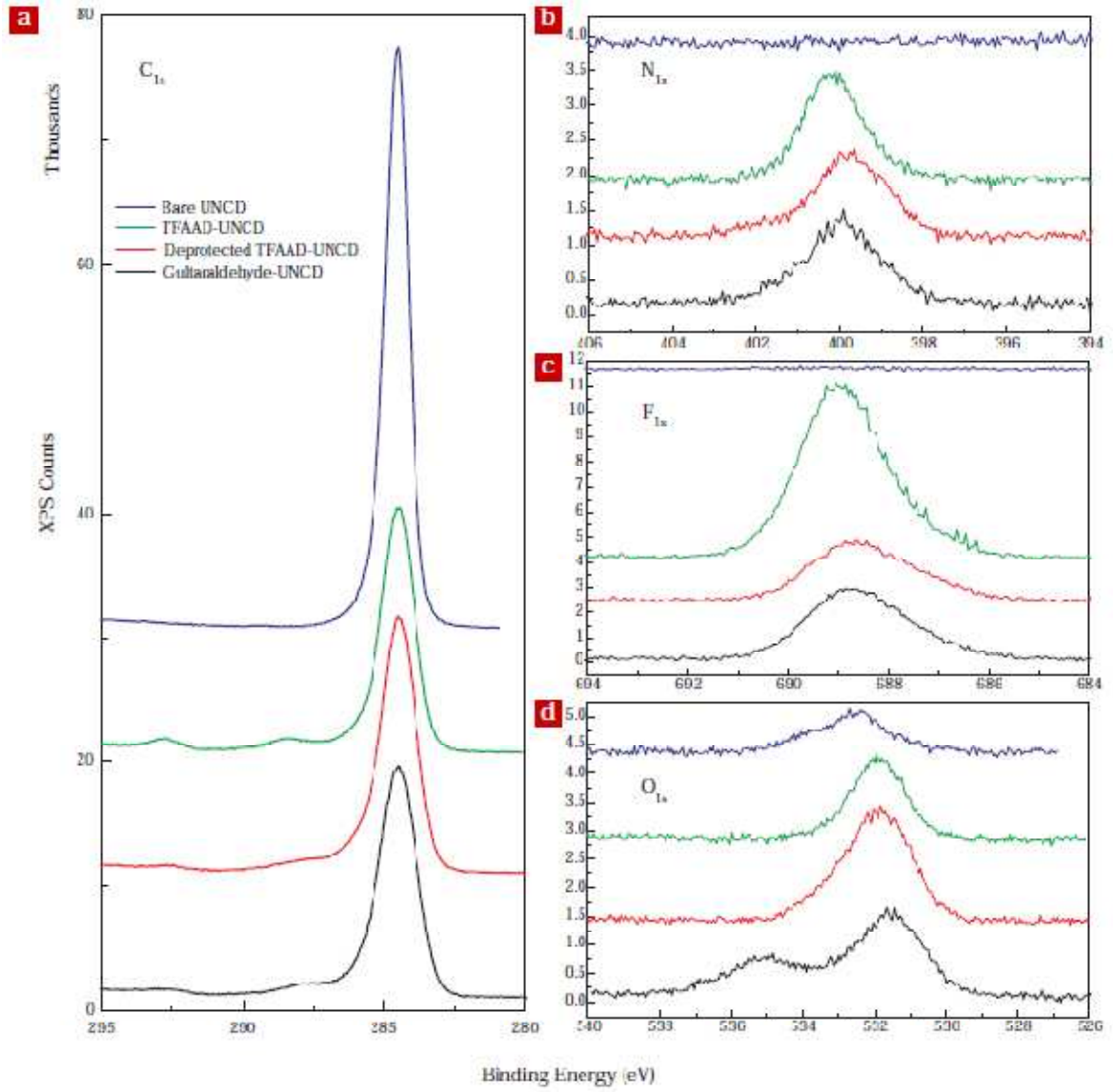

Figure S1. XPS analysis of UNCD-surface functionalization. C_{1s} (a), N_{1s} (b), F_{1s} (c), and O_{1s} (d) regions of XPS spectra taken after UNCD deposition, TFAAD/dodecane grafting, TFAAD deprotection, and glutaraldehyde attachment (top to bottom). XPS data were obtained using an ultrahigh vacuum (P < 7 x 10-10 Torr) XPS system with a monochromatized Mg Kα (1253.6 eV) source (350 W, 14.0 kV) and a hemispherical analyzer equipped with a multichannel detector. The entrance to the detector was aligned 90 degrees from the incident x-ray beam and 45 degrees from the sample surface. The hydrogen-terminated bare UNCD surface has a sharp C_{1s} peak set to 284.5 eV, no detectable N or F, and only a small amount of oxygen (d). Photochemical grafting of a TFAAD/dodecene mixture leads to new peaks in the F_{1s}, N_{1s}, and C_{1s} regions. In the C_{1s} spectrum, peaks at 292.7 eV and 288.4 eV arise from the CF_3 and CO functionalities in the TFA protecting group, and a small shoulder at ~285.9 eV arises from the NH-CH_2 group. The N_{1s}, F_{1s}, and O_{1s} all show single peaks consistent with the covalent grafting of TFAAD to the

surface. After deprotecting the TFAAD by methods previously described, the N_{1s} peak undergoes a slight broadening and shift from 400.3 eV to 399.7 eV that is consistent with transformation from an amide-type linkage to a primary amine . The F_{1s} peak and also the C_{1s} peaks at 292.7 eV and 288.4 eV are all reduced in intensity to 41%, 15% and 42% of their respective initial values. However, the peak most clearly indicative of the TFA protecting group is the isolated CF_3 peak at 292.7 eV. Changes in this peak demonstrate that ~85% of the TFAAD groups are deprotected to form the primary amine, which is consistent with previous studies[23]. After reductive amination of the primary amine with glutaraldehyde to form secondary amine, the N(1s) peak shifts slightly to 399.9 eV, and a new O(1s) peak at 535.1 eV appears. These changes are consistent with the grafting of glutaraldehyde to the surface.

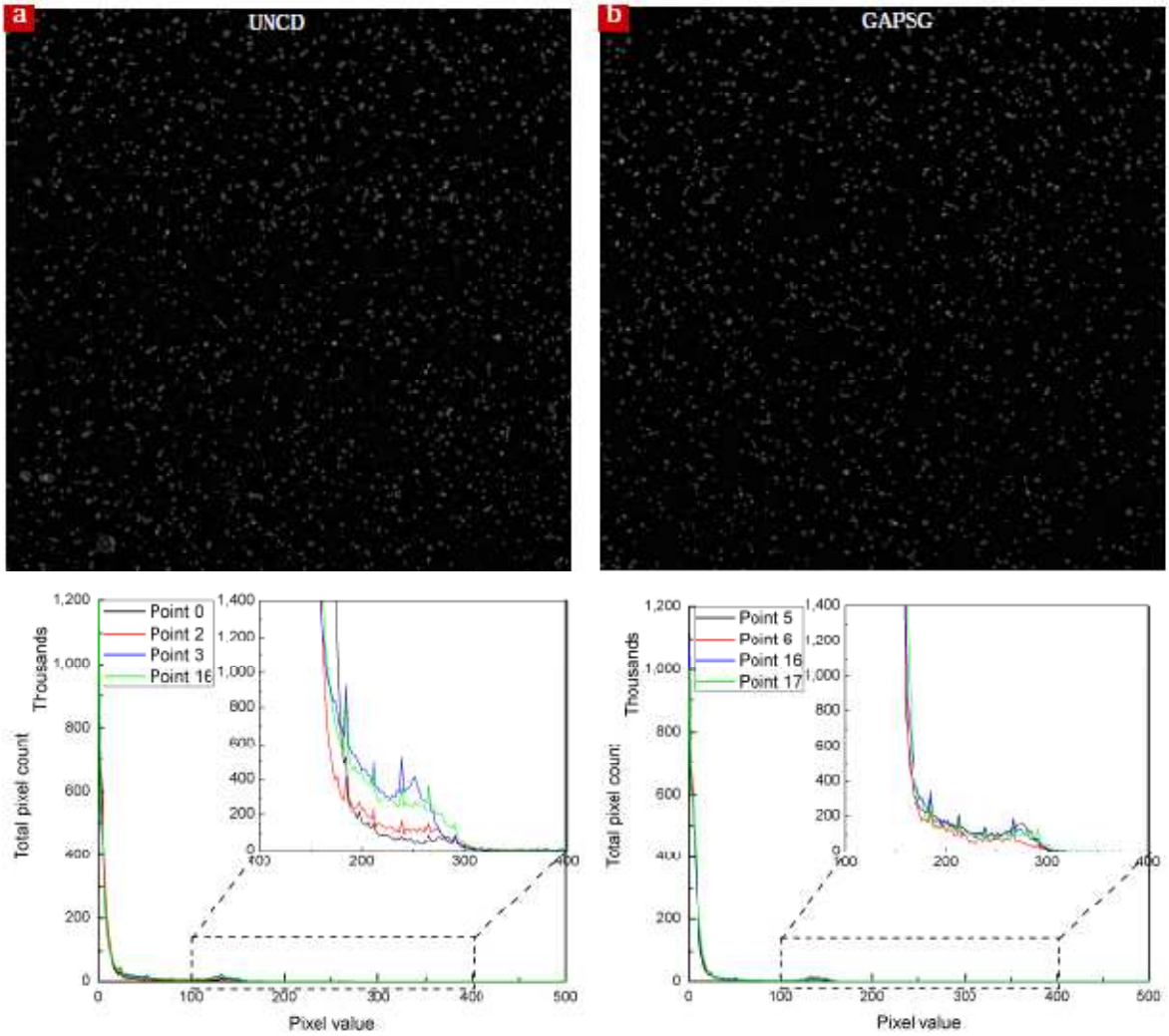

Figure S2. Histograms of z-projections (Fig. 3) showing randomness in bacteria capture events between five consecutive capture and regeneration cycles. (a) On GAPSG, we found pixels showing capture multiple times during the five capture-regeneration cycles, accounted for <1% of the total area under the histogram. (b) On UNCD, pixels showing capture multiple times accounted for <1.5% of the total area under the histogram. The histograms show the probability of capturing a bacterium at the same exact location is low and implies that the surface is homogenous in surface capture capability.

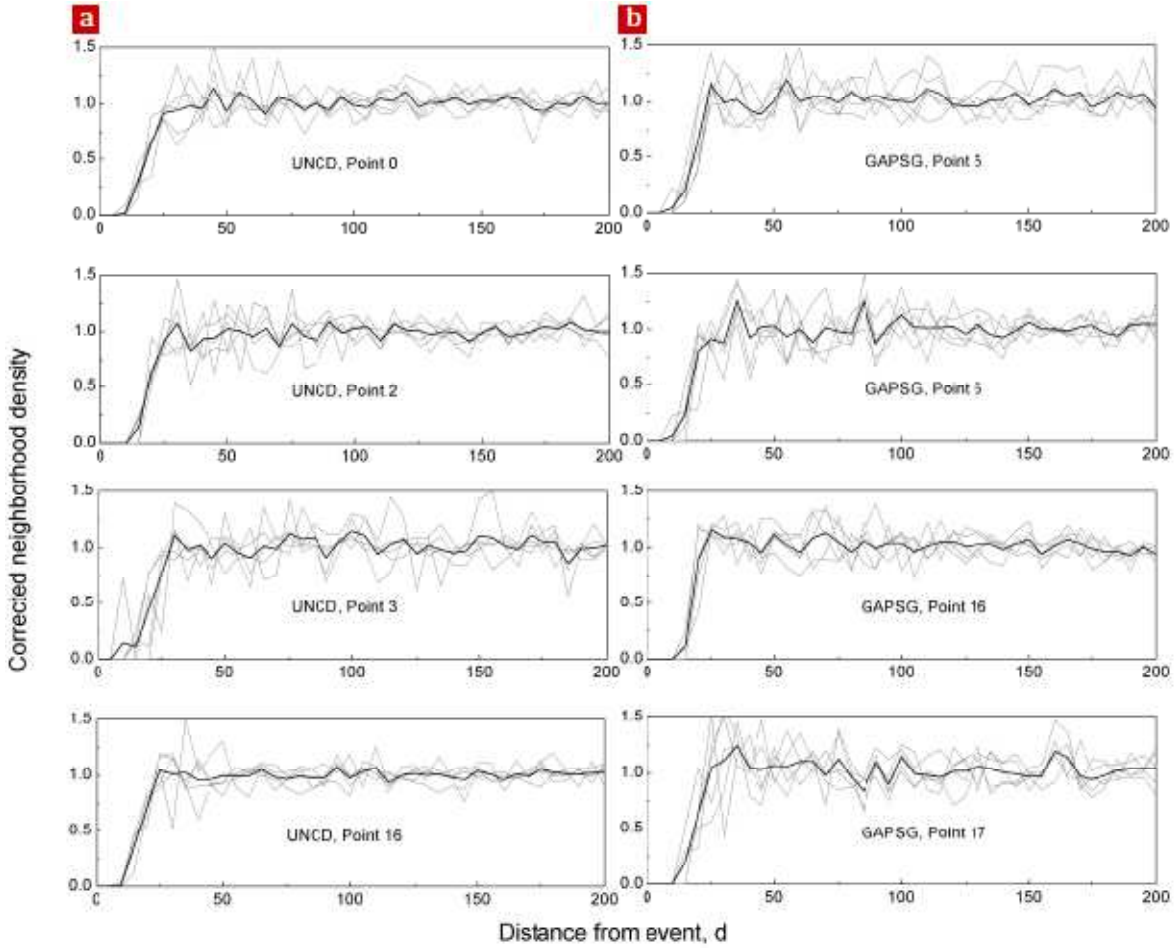

Figure S3. Neighborhood density function calculated for captures at more locations on the UNCD (a) and GAPSG (b) surfaces. The light grey lines indicate the NDF calculated for individual capture steps during the consecutive five capture-regeneration cycling and the black line indicated the average of the grey lines. The average NDF value converges to ~1 at ~25 pixel distance in most cases, which reflects randomness in the process of bacteria capture process on antibody functionalized UNCD (a) and GAPSG (b).

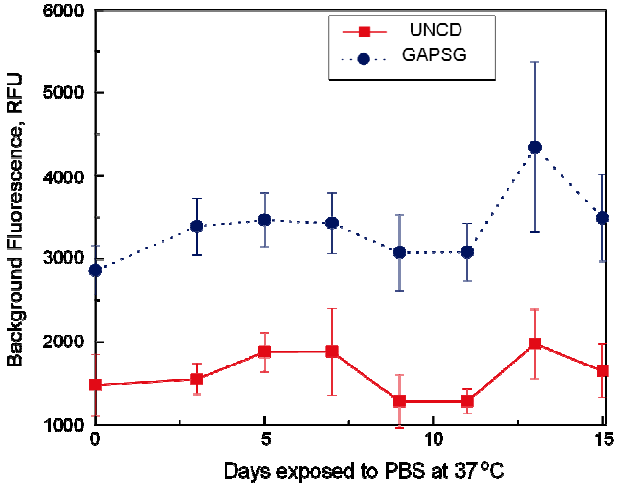

Figure S4. Non-specific binding and antibody loss justified using fluorescence spectroscopy and XPS. a, Fluorescence measured from non-specifically bound FTIC-labeled goat antibody on functionalized substrates exposed to PBS at 37 °C for up to 14 days. The fluorescence values directly correlate to creation of non-specific binding sites when the substrates are exposed to PBS for prolonged duration.

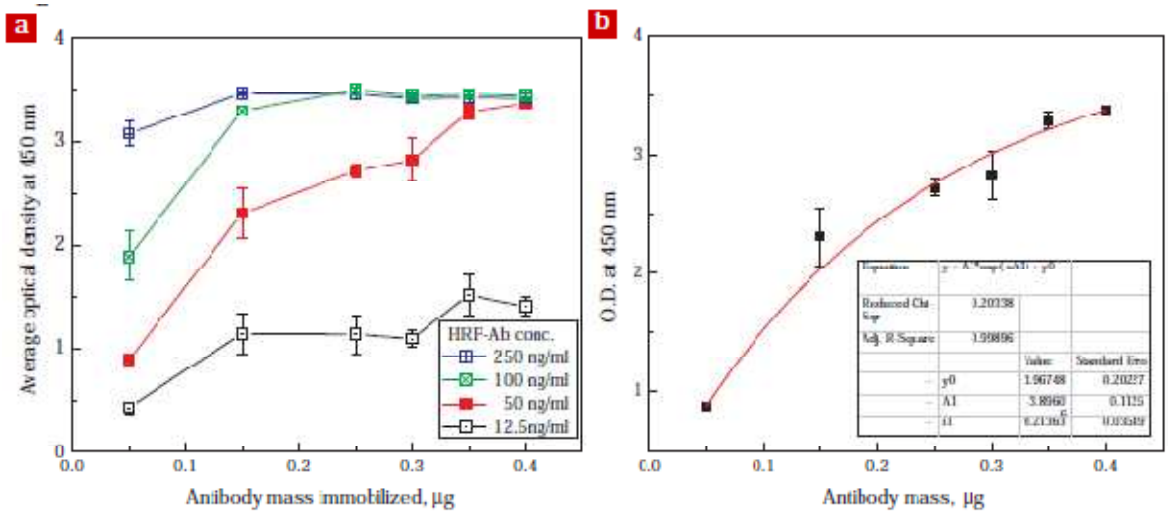

Figure S5. Quantified-ELISA parameter optimization. (a) Plot of optical density reading in ELISA versus antibody (goat anti-*E.coli O157:H7*) mass adsorbed on Nunc Maxisorp 384-wellplate for various detector antibody concentrations (HRP conjugated rabbit anti-goat). We find that a concentration of 50 ng HRP-antibody per ml covers a wide range of antigen (goat antibody) concentration. (b) Calibration curve relating the mass of goat anti-E.coli O157:H7 adsorbed on Maxisorp wells to the optical density readings obtained during ELISA.

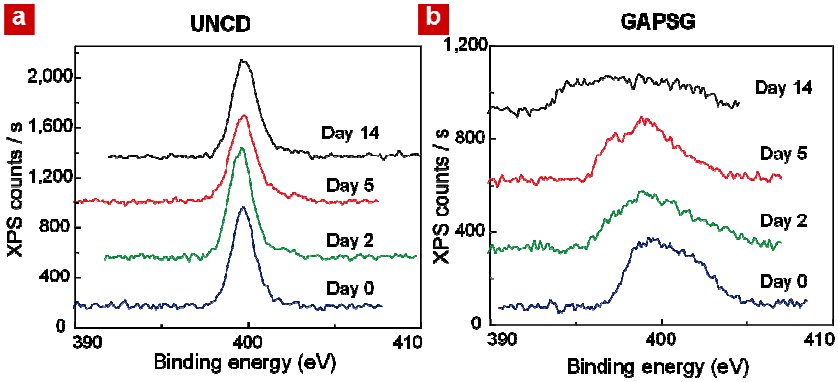

Figure S6. N_{1s} peak obtained from UNCD (a) and GAPSG (b) immunosurfaces during 37 °C stability tests.

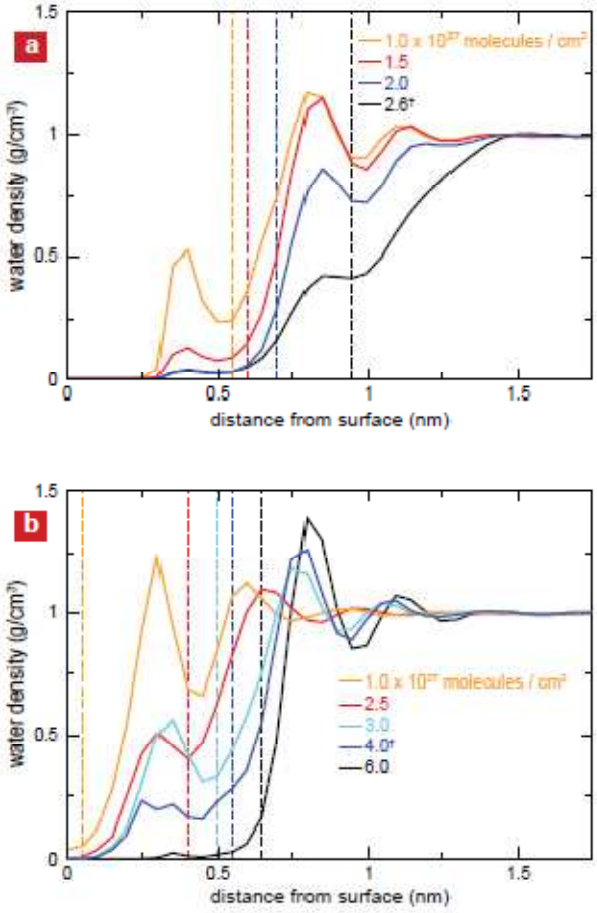

Figure S7. Simulated density of water near functionalized UNCD and silica substrates. (a) The average density of water versus the distance from the UNCD substrate coated with aminodecane to different surface density. Dashed lines indicate the distance at which the density of aminodecane becomes equal to the density of water. (b) The average density of water versus the distance from the silica substrate coated with aminopropylsilane to different surface density. Dashed lines indicate the distance at which the density of aminopropylsilane becomes equal to the density of water. †Corresponds to the expected experimental density of the functional groups.

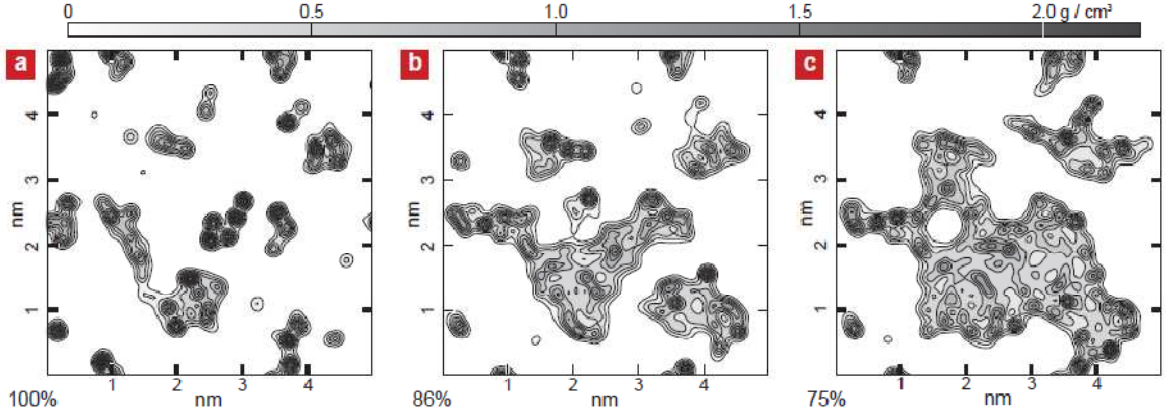

Figure S8. Growth of water pockets associated with degradation of GAPSG functionalization layer. (a) Contour plot of the water density within 0.5 nm of the silica substrate coated with 4.0 x 10^{14} molecules/cm^2 of aminopropylsilane, averaged over a representative 1 ns period of the 40 ns MD trajectory. Starting from this structure, functional groups were removed using a procedure described in the Supporting Methods. (b) The water density on the silica substrate with 86% of the functionalization groups remaining. (c) The water density on the silica substrate with 75% of the functionalization groups remaining.

Movie 1. The dynamics of water pockets on H-terminated UNCD immunosurfaces revealed by MD simulations. Water molecules within 0.5 nm of the UNCD surface (gray molecular surface) are shown as blue vdW spheres. The aminodecane coating is not shown. Water molecules can be seen to spontaneously approach the surface during this representative 3-ns fragment of the 40-ns MD trajectory.

Movie 2. The dynamics of water pockets on silica immunosurfaces revealed by MD simulations. Water molecules within 0.5 nm of the silica surface (gray molecular surface) are shown as blue vdW spheres. The aminopropylsilane coating is not shown. The silica surface can be seen to have constant pockets of water in addition to those spontaneously forming during this representative 3-ns fragment of the 40-ns MD trajectory.