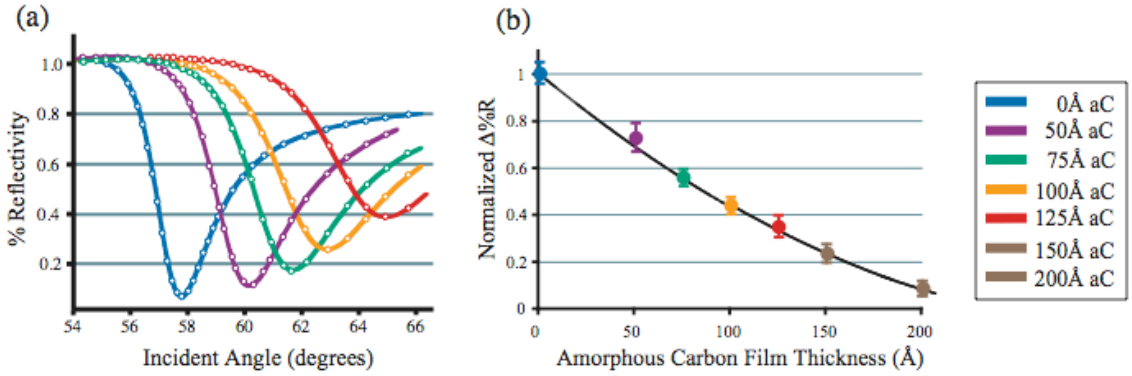

Figure S1:

Varying thicknesses of amorphous carbon were applied to 42.5nm gold thin films, which were then characterized with FTSPR measurements, and monitored with fixed angle SPRi. (a) Scanning angle reflectivity curves (8994 cm^{-1}) of gold substrates containing 5, 7.5, 10, and 12.5nm of amorphous carbon were compared to a bare gold substrate. Spectra were obtained from FTSPR measurements of the individual substrates. Experimentally obtained values (circles) were compared to theoretically calculated reflectivity curves for each substrate (lines). The theoretical values were determined with a *n*-phase Fresnel equation. (b) A series of fixed angle SPRi measurements were made to determine the effect of the amorphous carbon overlayer on binding sensitivity. Each substrate was exposed to two solutions with a 0.03% index of refraction difference and the change in reflectivity measured. The observed reflectivity changes were normalized to the change observed in the bare gold substrate. A second-order polynomial fit has been added to this graph to guide the eye.

Materials and Methods:

(1) Preparation of amorphous carbon SPR surfaces:

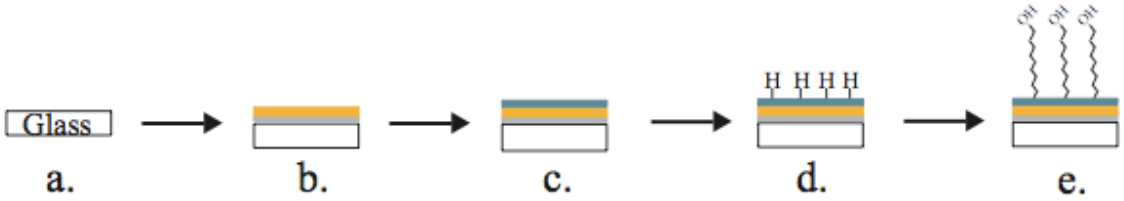

Figure S1: Preparation of amorphous carbon surfaces

The amorphous carbon substrates used throughout this work were prepared on (a) high index glass substrates (SF10 glass, Schott Glass, Duryea, PA). Prior to use, each glass substrate was rinsed with copious amounts of deionized water (DI) and dried under a stream of nitrogen gas. (b) Metal thin films were applied to the substrate using an Angstrom Engineering Åmod metal evaporator (Cambridge, ON). First, a 2.0nm chromium thin film was thermally evaporated onto the substrate, providing increased adhesion of the subsequent layers to the SF10 glass. Next, the SPR-active metal of interest was electron-beam evaporated onto the surface: 42.5nm gold, 40nm silver, or 42.5nm copper. (c) Directly after metal evaporation, amorphous carbon thin films were applied to the surface by DC magnetron sputtering (Denton Vacuum, Moorestown, NJ).

The amorphous carbon substrates were chemically functionalized using a modified procedure for the functionalization of diamond substrates, developed in our laboratory.(*1*) (d) First, each amorphous carbon thin film was hydrogen-terminated in a 13.56MHz inductively coupled hydrogen plasma for 12 minutes (30Torr H_2, room temperature). (e) Next, 30mL of 9-decene-1-ol (Sigma Aldrich, St. Louis, MO) was placed directly onto the newly hydrogen-terminated surface and covered with a quartz coverslip. The substrates were then irradiated for 8 hours with a low-pressure mercury grid lamp (λ_{max} = 254nm, 0.35mW/cm^2), under nitrogen purge. After the photoreaction the substrates were rinsed with copious amounts of ethanol and DI, dried under a nitrogen stream, and stored in a desiccator until needed.

(2) *In-situ* oligonucleotide array synthesis on amorphous carbon substrates:

All of the oligonucleotide arrays used in this work were fabricated using a previously described light-directed photolithographic synthesis method,(*2*) allowing the oligonucleotide sequences to be synthesized in a base-by-base manner. Syntheses were performed with oligonucleotide bases modified with a photolabile 3'-nitrophenylpropyloxycarbonyl- (NPPOC-) protecting group and a digital micromirror-based Biological Exposure and Synthesis System (BESS) connected to a Perspective Biosystems Expedite Nucleic Acid Synthesis System (Farmingham, MA).

Array synthesis proceeded as follows: (a) after condensation of the previous NPPOC-protected base to the growing DNA strand, the synthesis flow cell (volume ~100µl) was flushed with 500µl of exposure solvent; (b) a digital image (mask) representing the locations for the next base addition illuminated the surface with 3.95J/cm^2 of 365nm light (200W Hg/Xe arc lamp, Newport, Stratford, CT). Exposure solvent was constantly flowed through the flow cell at a rate of 100µl / 0.5J/cm^2 during illumination, sufficiently maintaining the basic conditions needed to drive the photo-catalyzed elimination reaction. (c) Following irradiation, the array was washed with acetonitrile (~400µl) to remove residual exposure solvent, dry wash (~300µl) to remove trace water, and activator solution (~100µl). (d) Coupling of the next base was achieved by filling the flow cell with a 1:1 solution of the desired phosphoramidite and activator. All 5'-NPPOC-protected amidites underwent a single 40s coupling step. (e) After amidite coupling, the array was washed with acetonitrile (~100µl) and either oxidized by flushing the cell with oxidizer solution (THF, pyridine, iodine, and water; ~500µl) or subjected to the next phosphoramidite addition. The non-acidic conditions of deprotection allow for oxidation of the backbone phosphite groups after every 4th coupling step and at the end of the synthesis, rather than at every coupling step. (f) After synthesis is completed, the nucleoside bases are deprotected in 1:1 ethylenediamine:absolute ethanol solution at room temperature for 2-4 hr.

Oligonucleotide synthesis reagents (DCI activator, acetonitrile (dry wash and amidite diluent), and oxidizer solution) were purchased from Sigma-Proligo, exposure solvent was purchased from Nimblegen Systems Inc. (Madison, WI). All anhydrous reagents were kept over molecular sieves (Trap Packs, Aldrich). All NPPOC-protected

phosphoramidites [5'-NPPOC-dAdenosine(tac) 3'-β-cyanoethylphosphoramidite (dA), 5'-NPPOC-dThymidine 3'-β-Cyanoethylphosphoramidite (dT), 5'-NPPOC-dCytidine(ib) 3'-β-cyanoethylphosphoramidite (dC), 5'-NPPOC-dGuanosine(ipac) 3'-β-cyanoethylphosphoramidite (dG)] were manufactured by Proligo Biochemie GmbH (Hamburg, Germany) and purchased from Nimblegen Systems Inc.; NPPOC-Phosphoramidites were diluted (1g in 60mL) with dry acetonitrile (amidite diluent).

(3) FTSPR experimental conditions:

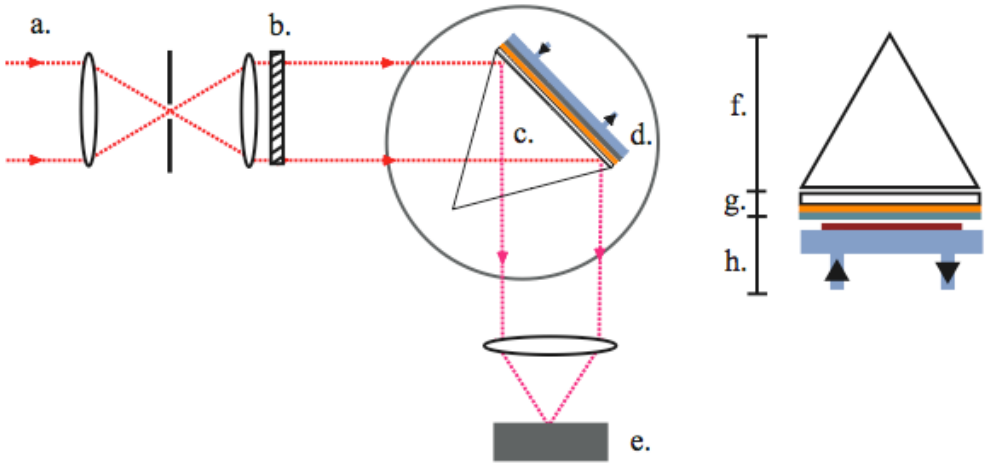

Figure S2: FTSPR instrument configuration and experimental setup

A FT-SPR 100 instrument (GWC Technologies, Madison, WI) was used to characterize the angle- and wavelength-dependent effects of the amorphous carbon thin films on gold and silver thin film substrates. (a) A light beam from the external port of a Fourier transform near infrared (FT-NIR) spectrometer is focused onto an aperture and recollimated with a second lens. (b) The light beam is then polarized with an NIR film polarizer, efficient over the 6000-12000cm^{-1} range. In a typical experiment, a reference spectrum is acquired with light perpendicular (s-polarized) to the incident plane. Subsequent spectra are acquired with light parallel (p-polarized) to the incident plane. Each spectrum is corrected by ratioing the p- to s-polarized light intensities. (c) Once polarized the light beam is directed onto the substrate, mounted in a Kretschmann configuration. The substrate is attached to a (d) rotating stage with an angular precision of 0.001^{o} with a wavelength precision of 0.01cm^{-1}. (e) The light reflected from the substrate is focused onto an InGaAs photodiode detector.

To achieve the proper configuration for photon-plasmon coupling, a Kretschmann configuration is used. This configuration consists of (f) a prism mounted to the (g) substrate with a layer of index matching fluid (n=1.721, Cargille, Cedar Grove, NJ). The Kretschmann configuration is then mounted to a (h) flow cell, via a Teflon o-ring, connected to a peristaltic pump. Spectra were recorded after the flow cell was filled with deionized water.

(4) SPR imaging experimental conditions:

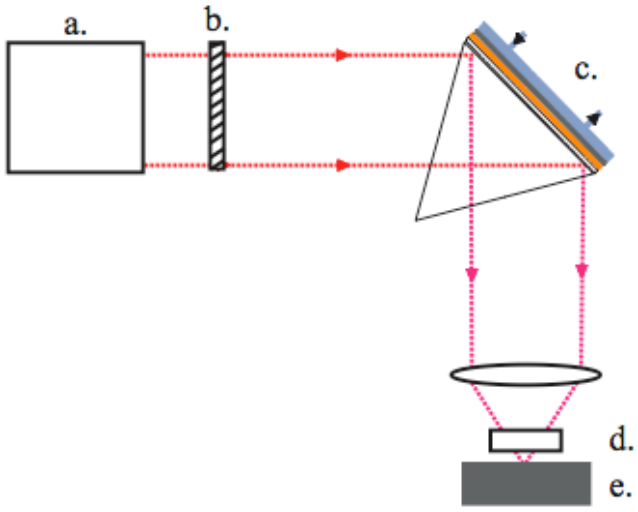

Figure S3: SPRi instrument configuration and experimental conditions

A SPR Imager II instrument (GWC Technologies) was used for all SPRi binding experiments. (a) A collimated white light source is (b) polarized with a visible film polarizer. In a typical experiment a reference image is collected with s-polarized light. Subsequent images are acquired with p-polarized light and corrected by ratioing each image with the reference. (c) The polarized light beam is directed onto the substrate, mounted in a Kretschmann configuration. The configuration is set to a fixed angle throughout the experiment. The optimal angle is substrate dependent and is determined experimentally as approximately 60% of the attainable reflectance. The light reflected from the substrate is refocused and passed through a (d) narrow band pass filter onto a (e) CCD camera.

The substrate configuration (prism, substrate, flow cell) used in SPRi experiments is described in FigureS3.

(5) Experiment 1: DNA-DNA binding

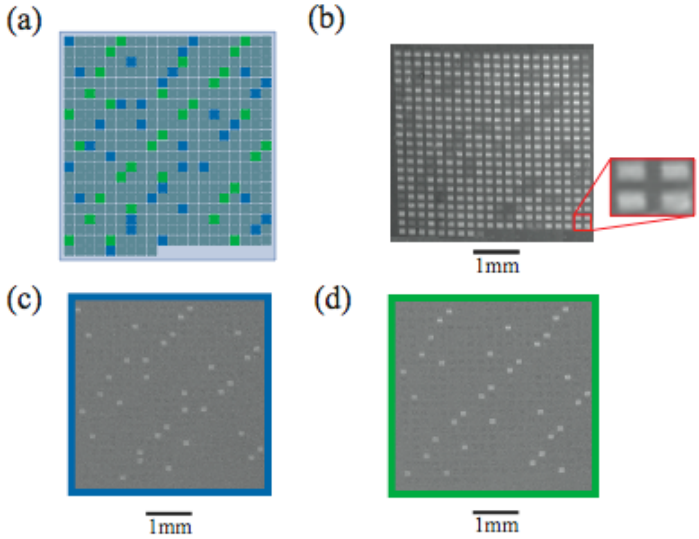

Figure 3 from the manuscript is provided for reference.

Array design: A 420-oligonucleotide feature array (128μm x 128μm per feature, with a 96μm spacing between each feature) was fabricated on a substrate containing 7.5nm of amorphous carbon sputtered onto a 42.5nm gold thin film. The array contained 58 reference features, 17 features of Probe 1 (a, blue), 17 features of Probe 2 (a, green), and 328 oligonucleotide features that were randomly generated. (b) The reference features within the array are less intense, due to the fact they are 8 nucleotides shorter in length than the other features (18 nucleotides). Each oligonucleotide sequence contained a 10 dT spacer, providing the necessary distance from the surface for efficient oligonucleotide hybridization.

Name	**Sequence (5' → 3')**
Reference	TTTTTTTTTT
Probe 1	CCACTGTTGCAAAGTTAT
Probe 2	CGCTTCTGTATATTCATC
Complement 1	ATAACTTTGCAACAGTGG
Complement 2	GATGAATATACAGAAGCG

Experimental procedure: The array was mounted into an SPR Imager II flow cell (described above) and placed in the imager. The flow cell was filled with 1xSSPE buffer (10mM NaH_2PO_4, 150mM NaCl, 1mM EDTA, pH 7.4), the angle was set to 60^o, a reference image (using s-polarized light) taken, and the experiment started. First, a 100nM solution of Complement 1 (1xSSPE) was introduced into the flow cell and binding monitored. Image (c) is the difference image obtained 2 minutes after Complement 1 was introduced. Next, a 100nM solution of Complement 2 (1xSSPE) was introduced and images collected. Difference image (c) obtained by subtracting image t=120s from t=0s. Difference image (d) obtained by subtracting image t=240s from t=120s. Complementary oligonucleotides were synthesized using standard phosphoramidite chemistries (Integrated DNA Technologies, Coralville, IA).

(6) Experiment 2: DNA-RNA binding

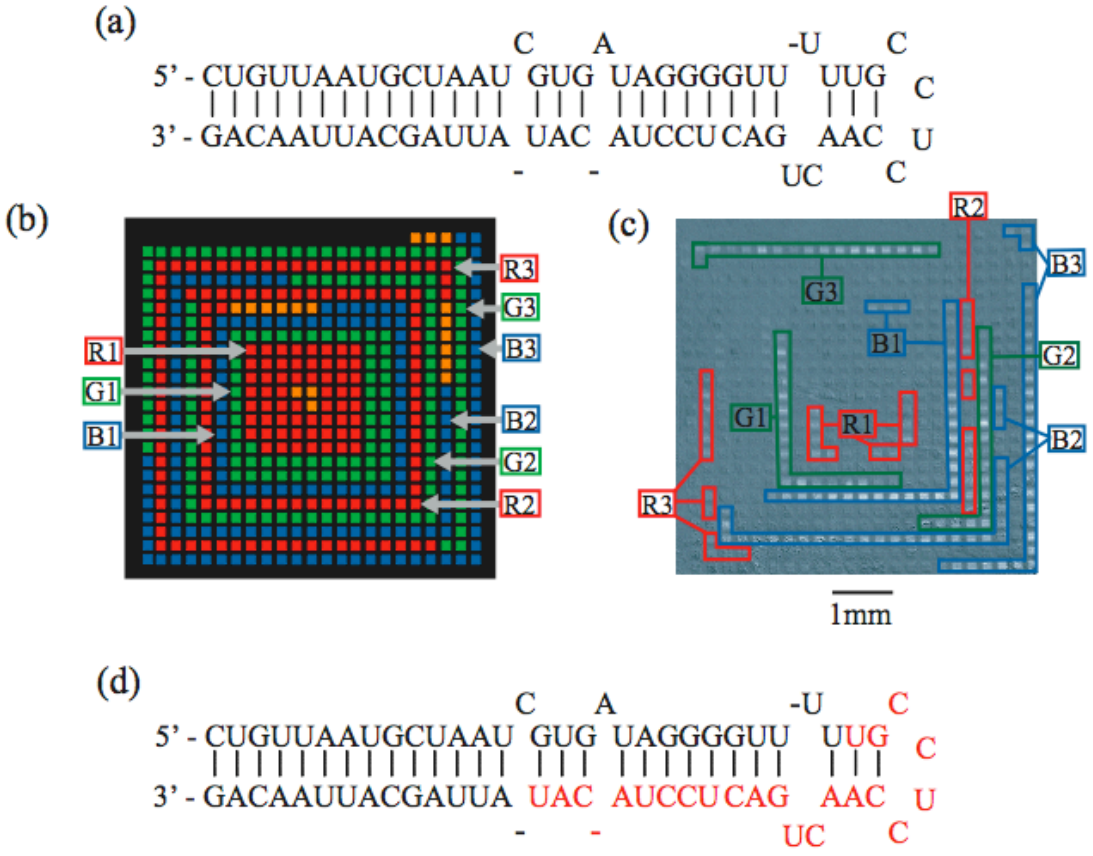

Figure 4 from the manuscript is provided for reference.

Array design: A 534-oligonucleotide feature tiling array (192μm x 192μm per feature, with a 128μm spacing between each feature) was fabricated on a substrate containing 7.5nm of amorphous carbon sputtered onto a 42.5nm gold thin film. A tiling array of complementary oligonucleotides of the has-mir-155 RNA molecule,(*3*) stepping through the entire RNA molecule one base at a time in a 5' → 3' direction, was designed and fabricated. The array contained three separate sub-tiling arrays of 6-, 8-, and 12-mer complementary sequences of the RNA molecule, each in triplicate. These regions are labeled by a color corresponding to the oligonucleotide length (R=red, 6mers; G=green, 8mers; B=blue, 12mers), starting in the center of the array and spiraling outward in a counter clockwise pattern with the designations 1, 2, 3.

The tiling portion of the array consists of 516 features. The remaining features are three different control oligonucleotide sequences (each on 6 features) to test the fidelity and specificity of the array. The fidelity was determined by checking the fluorescence of Cy3-terminated features (Control 3), while the specificity was determined using fluorescently labeled complement hybridized to two orthogonal sequences (Control 1 and Control 2). Each oligonucleotide sequence contained a 10 dT spacer, providing the necessary distance from the surface for efficient oligonucleotide hybridization.

Name	**Sequence (5' → 3')**
hsa-miR-155	CUGUUAAUGCUAAUCGUGAUAGGGGUUUUUGCCUCCAACUGACUCCUACAUAUUAGCAUUAACAG
Control 1	TCACTGTTGCAAAGTTATTG
Control 2	ACTCTTGCAGGTCATCGG
Control 3	Cy3-TTTTTTTTTT
Complement 1	CAATAACTTTGCAACAGTGA-FAM
Complement 2	CCGATGACCTGCAAGAGT-FAM

Experimental procedure: The array was mounted into an SPR Imager II flow cell (described above) and placed in the imager. The flow cell was filled with buffer (7.5mM $MgCl_2$, 10mM MOPS, pH 7.4), the angle set to 60^o, a reference (s-polarized light) image was taken, and the experiment started. A 1μM solution of RNA (7.5mM $MgCl_2$, 10mM MOPS, pH 7.5) was introduced into the flow cell and binding monitored. In less than one minute, binding was observed. The hsa-miR-155 RNA molecule was synthesized using

standard phosphoramidite chemistries (University of Wisconsin Biotechnology Center, Madison, WI). Complement 1 and 2 were synthesized by Integrated DNA Technologies (Coralville, IA) with 3' fluorescein dye (3' 6-FAM) modifications.

(7) Experiment 3: DNA-protein binding

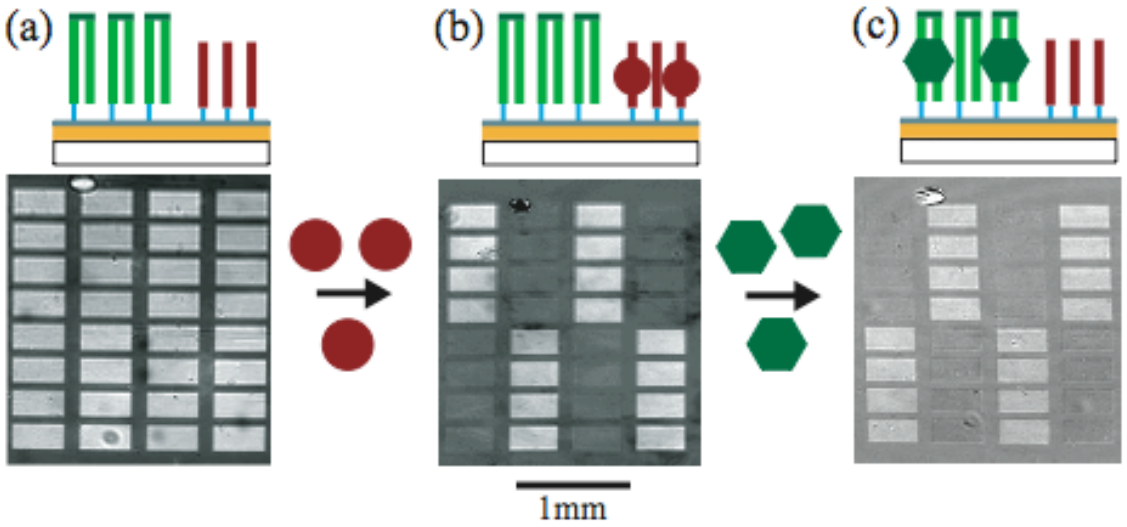

Figure 5 from the manuscript is provided for reference.

Array design: A 32-oligonucleotide feature array (256μm x 256μm per feature, with a 192μm spacing between each feature) was fabricated on a substrate containing 7.5nm of amorphous carbon sputtered onto a 42.5nm gold thin film. The array consisted of two oligonucleotide sequences, a 19nt oligonucleotide with known thrombin binding (Probe Thr) and a 52nt self-complementary oligonucleotide with known virulence factor regulator protein binding (Probe VFR). Probe VFR is self-complementary with a 4nt (TCCT) loop. A hairpin structure was formed by incubating the surface at 65°C followed by a slow cooling process. Each oligonucleotide sequence contains a 15 dT spacer, providing a spacer from the surface.

Name	**Sequence (5' → 3')**
Probe Thr	TTGGTTGGTGTGGTTGGTT
Probe VFR	AGGACGGGTATCGTACTAGGTGCATCCTTGCACCTAGTACGATACCCGTCCT

Experimental procedure: the array was mounted into an SPR Imager II flow cell (described above), filled with thrombin binding buffer (20mM HEPES, 150mM NaCl,

2mM $CaCl_2$, pH 7.4), incubated at 60ºC for 10 minutes, and then slowly cooled to room temperature. This process allows the probe VFR hairpins to form. Next, the flow cell was mounted into the SPR imaging instrument, the angle set to 60^o, a reference (s-polarized light) image taken, and the experiment started. First, a 10μg/mL solution of thrombin (Sigma Aldrich, St. Louis, MO) in thrombin binding buffer was introduced into the flow cell and binding monitored. The difference image was obtained 10 minutes after the thrombin was introduced. Next, the flow cell was filled with VFR binding buffer (100mM HEPES buffer, 1mM DTT, and 1mM EDTA; at pH 7.0) and a reference image taken. A 10μg/mL solution of VFR (Aseem Ansari laboratory, University of Wisconsin Department of Biochemistry) in VFR binding buffer was introduced into the flow cell and binding monitored. The difference image was obtained 10 minutes after VFR was introduced.

1. T. Strother *et al.*, *Langmuir* **18**, 968 (2002).
2. S. Singh-Gasson *et al.*, *Nature Biotechnology* **17**, 974 (1999).
3. J. Krol *et al.*, *Journal of Biological Chemistry* **279**, 42230.