Supporting Information for:

Monolayer Sensitivity Enables a 2D IR Immuno-biosensor for Studying Protein Structures: Application to Amyloid Polymorphs

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2D IR Spectroscopy

The pulse-shaping based 2D IR spectrometer has been described in detail previously,[1] with a few minor modifications. A Ti:Sapphire regenerative amplifier (Libra, Coherent) generates 50 fs pulses centered at 800 nm. The average power is about 3.5 W with about 3.5 mJ per pulse. This is used to pump a commercial optical parametric amplifier (OPA, Topas, Light Conversion) to generate a combined 850 mW of signal and idler pulses. A home-build difference frequency generation (DFG) apparatus based on $AgGaS_2$ is used to generate mid-IR light centered at about 6050 nm with a pulse energy of about 15 μJ. The FWHM of the mid-IR spectrum is typically around 250 cm^{-1} and the pulse duration <100 fs measured with a home-built FROG set up. The mid-IR light is split into separate pump and probe lines with a 90/10 wedged CaF_2 beam splitter. The pump light is directed into a home-build mid-IR 4*f* pulser shaper that utilizes a Ge acousto optic modulator (AOM). The shaper allows for amplitude and phase modulation in the frequency domain on a shot-to-shot basis. The pump pulse is shaped in order to generate a pump pulse pair with computer-controlled time delays and relative phases. The delays are scanned on a shot-to-shot basis out to 3.1 ps. We perform measurements in the rotating frame by changing the phase of the moving pulse as a function of the pump pulse time delay to allow unambiguous undersampling of the experimental interferograms.[2] The measurements reported are collected with a rotating frame of 1500 cm^{-1} and 40 fs time steps. The pump frequency axis is corrected with the rotating frame frequency during data processing. This significantly reduces the amount of time needed to collect a single 2D spectrum. The only pre-processing used in the experiments was a Tukey window function applied to the time-domain data and the data zero-padded by a factor of two.[3] Diagonal slices are interpolated by a factor of two.

The pump and probe are spatially overlapped at the sample with gold parabolic mirrors. The spot size is about 100 μm at the sample. The relative timing between the pump and probe pulses is controlled with a motorized translation stage. For the experiments reported in this paper, the waiting time is set to maximize the transient absorption signal, which corresponds to a waiting time of about 150 fs for the pulse durations used here. The signal is self-heterodyned with the probe pulse and dispersed on a 32x1 MCT array detector.[3] The spectra in the main text were averaged for 1 hour. The probe frequency axis is calibrated with the water vapor lines. All spectra are collected with *s* polarized

pump and probe pulses relative to the substrate. The spectrometer is purged with dry air to remove contributions from water vapor before experiments.

Preparation of Gold Coated CaF_2 Substrates

3 nm thick-gold films were deposited on 25 mm diameter x 2 mm thick CaF_2 (Crystran) substrates by thermal evaporation of gold pellets purchased from Kurt Lesker (>99.999% purity). The base pressure prior to deposition was 4e-7 torr. A deposition rate of 0.1 Å/s was maintained and the thickness was monitored using a quartz crystal microbalance. The rotational speed of the stage was 60 rpm.

Synthesis of hIAPP

Both the isotope and native hIAPP was synthesized with FMOC solid-phase peptide synthesis using an automated microwave assisted peptide synthesizer (Liberty Blue, CEM) according to previous reports.[2,4] The isotope-labeled amino acids were synthesized from ^{13}C C1-labeled FMOC protected amino acids (Cambridge Isotope Laboratories). The ^{18}O was exchanged using ^{18}O water. In contrast to previous methods, we used 1,4 dioxanes containing 4 M HCl for anhydrous acidification. Synthesized peptides were cleaved from the resin and side-chain protecting groups removed using an acidic cleavage cocktail comprised of trifluoroaceticacid, thioanisole, 1,2-ethanedithiol, and anisole in a 90:5:4:1 ratio by volume, respectively, at 37^{o}C for 30 minutes in a microwave-assisted cleavage system (Accent, CEM). The synthesized peptide was incubated in a 1:1 ratio of dimethylsulfoxide (DMSO) and 20% acidic acid for 12 hours to ensure proper formation of the disulfide bond. Peptides were purified using reverse-phase HPLC using a C18 preparative column (XSelect, CSH, Waters). Two rounds of purification were employed to guaranteed purity and the masses of the peptides were confirmed with MALDI-TOF-MS.

Functionalizing the Gold Surface with Anti-amylin Antibodies

All chemistry to link the antibodies to the surface was completed in a purchased flow cell (Harrick). The total system volume of the manual flow cell was ~100 µl. The anti-amylin antibodies were used as received without further purification. A 300 µl solution of antibodies in deuterated PBS buffer was used for each experiment. 285 µl of PBS buffer was mixed with 10 µl of the antibody stock solution (1 mg/ml) and 5 µl of CS_2. Approximately 100 µl of the mixture was then introduced through the flow cell to completely cover the substrate surface. Antibody solution was used within 24 hours of preparation. Figure S1 shows the 2D IR spectrum of the antibodies before addition of the blocking agents.

To prevent non-specific binding of hIAPP, we incubated the antibody-coated substrate with 100 µl of 1% casein in deuterated PBS buffer. Before experiments the surface is washed with 100 µl of PBS buffer three times. To confirm that the bind sites are saturated, we repeated this step multiple times and recorded the subsequent 2D IR spectrum. We only observed an increase in the 2D IR signal after the first wash, indicating that the casein blocking agents cover the surface. Experiments were completed in triplicate.

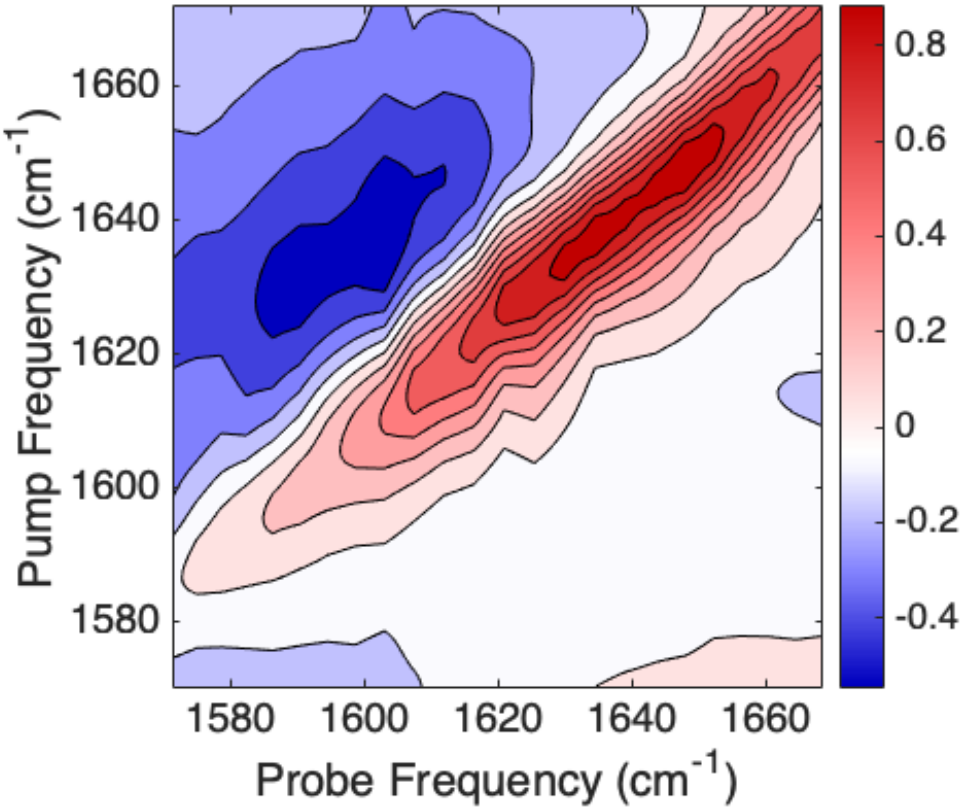

Figure S1. 2D IR Spectrum of the monolayer of antibodies on the gold surface before addition of the casein.

Control Experiments with Hen Egg White Lysozyme

To test the specificity of the purchased anti-amylin antibodies, we completed the experiments reported in the main text, but instead of using the hIAPP, we used Hen Egg White Lysozyme (HEWL). This experiment tests the ability of the antibodies to specifically extract hIAPP from a complex solution. We purchased the HEWL from Sigma Aldrich and used without further purification. The sensor was prepared as stated in the preceding section, and then a 5 mM solution of HEWL was incubated on the antibody-functionalized sensor for about 2 hours. The solution was then rinsed with PBS buffer and spectrum collected. Figure S2 shows the difference spectrum obtained after soaking the antibodies on the

same colormap as those presented in the main text. We observed no 2D IR signal from the HEWL, indicating that the antibody sensor is specific to hIAPP. We note that the anti-amylin will not likely distinguish between amylin of different species because of similarities in the N-terminal sequence. We also zoom in on the noise by a factor of 33 in Figure S3 to highlight the complete lack of signal, thus showing that within our signal-to-noise there is no non-specific binding.

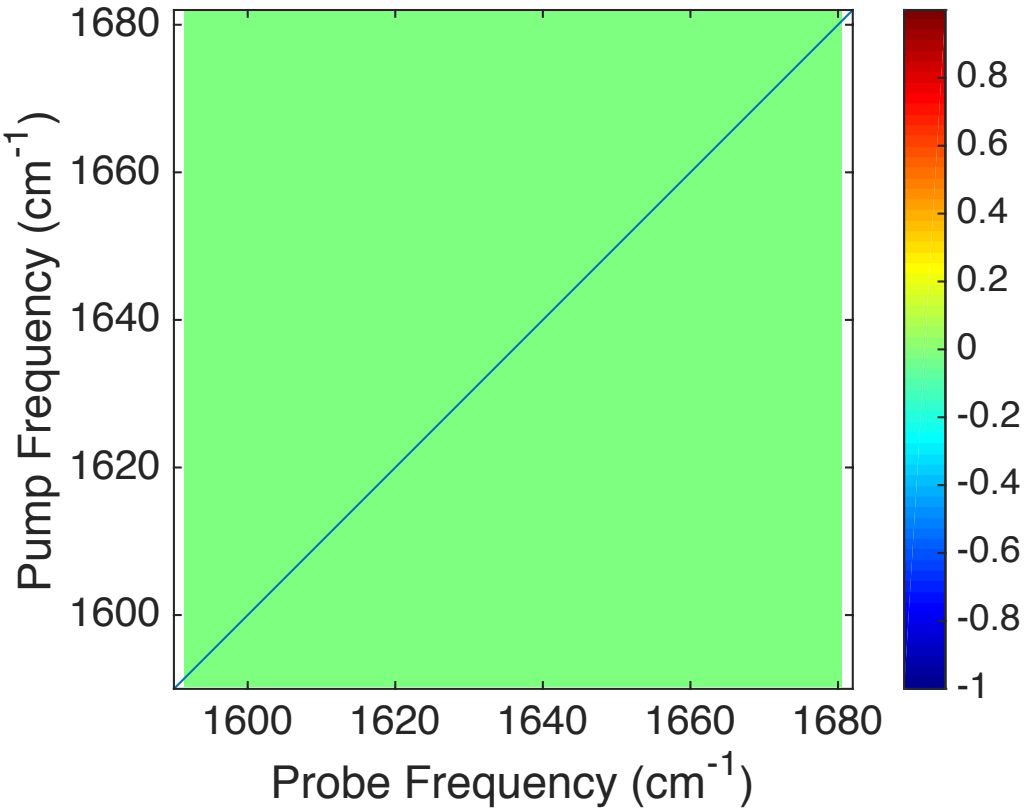

Figure S2. 2D IR difference spectrum upon binding of the HEWL. No non-specific binding is observed. The color map is the same as that of Figure S1, and those in the main text.

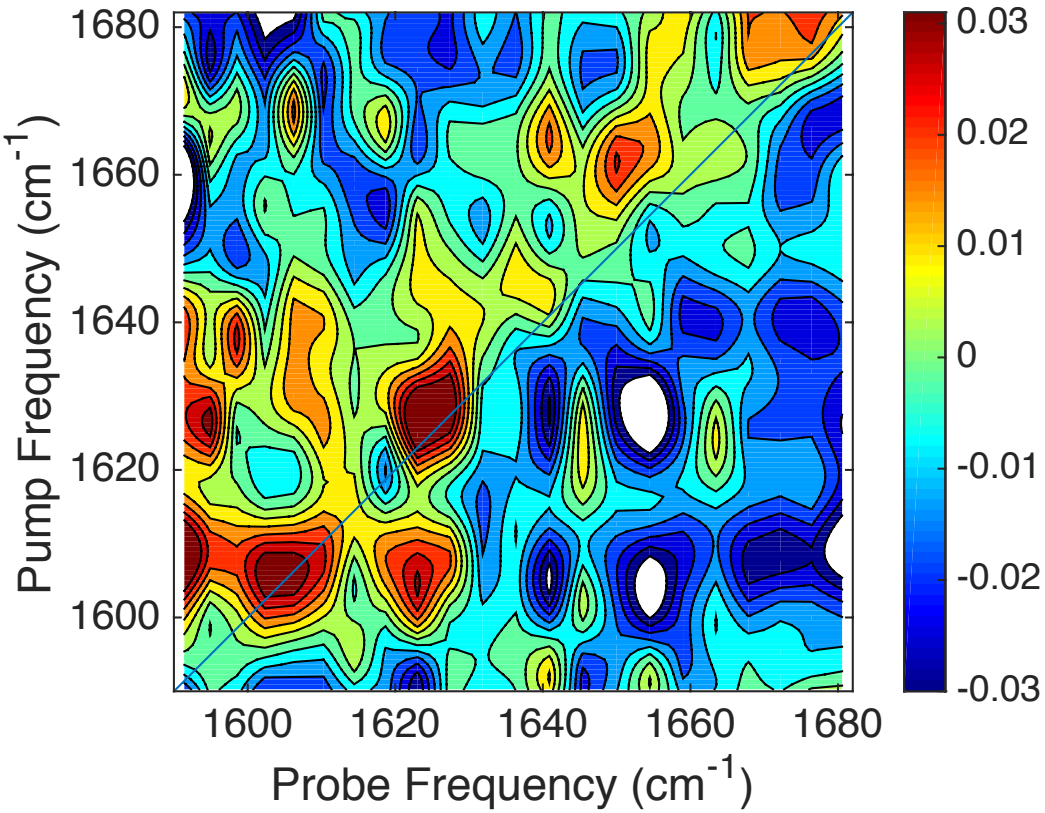

Figure S3. 2D IR difference spectrum of HEWL added to to the hIAPP immuno-sensor plotted zoomed in by a factor of 33 compared to the difference spectra presented in the main text.

Control Experiments with hIAPP and No Anti-Amylin

To confirm that the hIAPP cannot bind to the casein or residual free binding sites on the gold, we processed the antibody sensor as in the main text, but instead of introducing the anti-amylin, we introduced casein to be chemically linked to the gold surface. Although the exact concentration of the antibody in buffer is not known and cannot be determined, we approximated the same conditions by assuming the antibody has the same molecular weight as rabbit IgG. The sensor was processed with the casein to block free binding sites identically to the main text. hIAPP was added at 5 mM concentration to maximize the likelihood of binding. Figure S4 shows the residual 2D IR difference spectrum. We observe no signal and thus conclude that the hIAPP does not bind to the gold or casein.

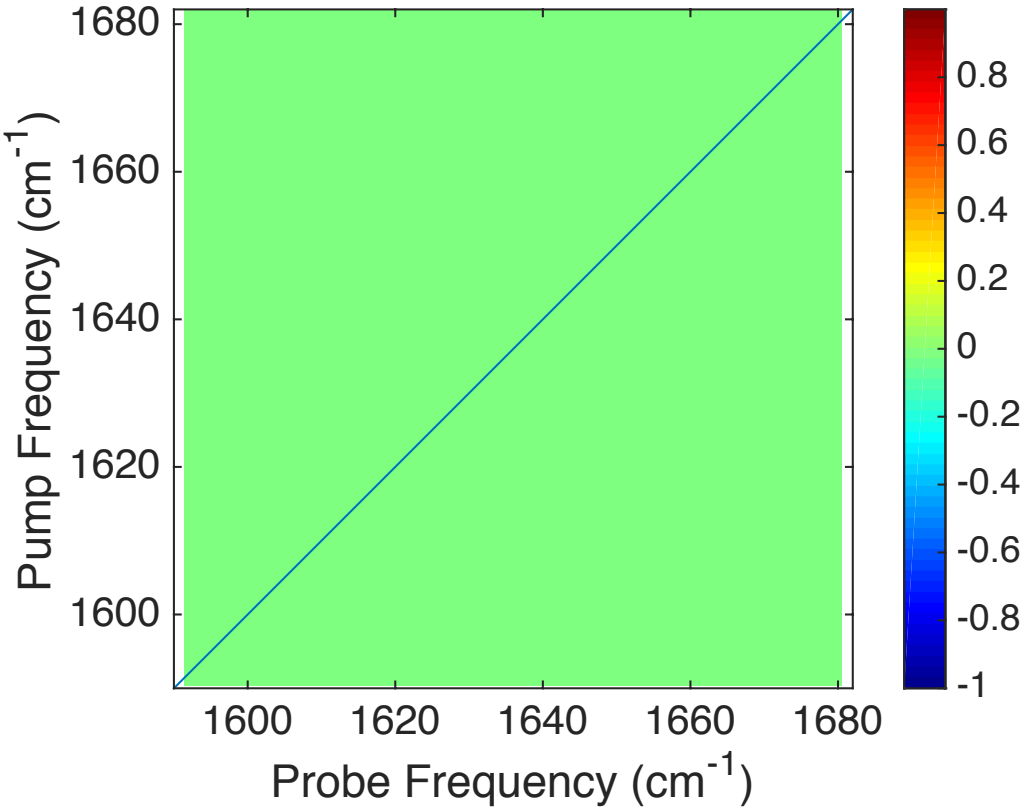

Figure S4. 2D IR difference spectrum upon addition of 5 mM hIAPP with no antibody on the gold surface. We oberserve no binding of the hIAPP to the gold surface or to the casein. Color map is the same as that used in the figures in the SI and main text.

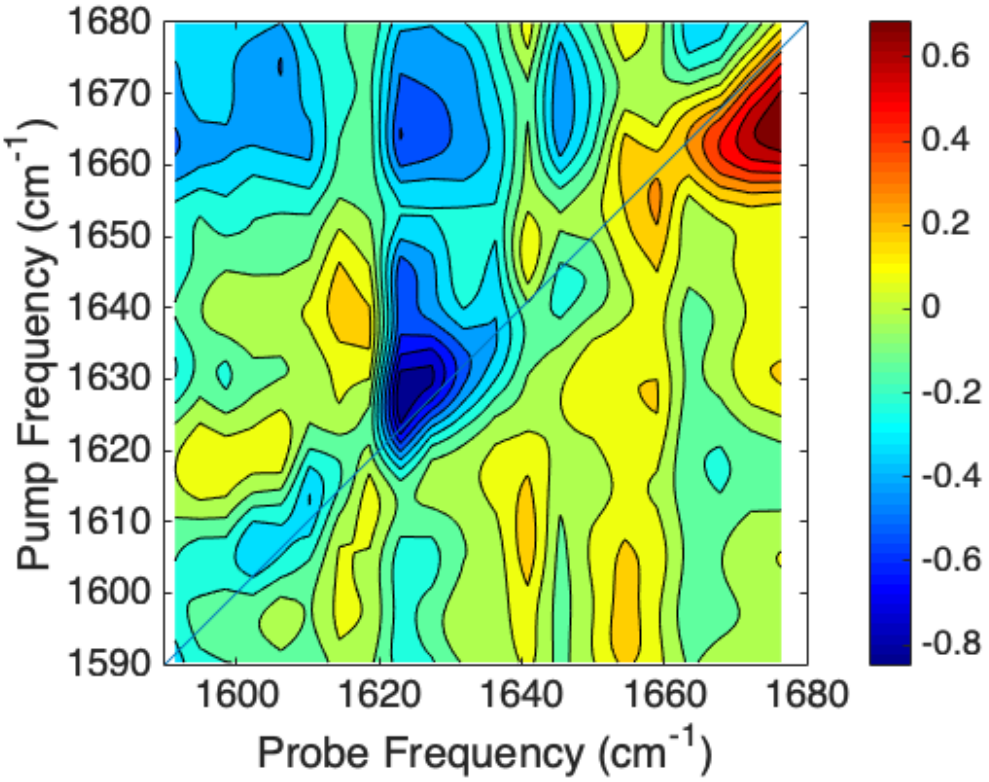

Figure S5. 2D IR differences spectrum when adding hIAPP to casein coated substrate of gold without antibodies. Color map is multiplied by a factor of 33 as in Figure S3.

References

(1) Ghosh, A.; Serrano, A. L.; Oudenhoven, T. A.; Ostrander, J. S.; Eklund, E. C.; Blair, A. F.; Zanni, M. T. Experimental Implementations of 2D IR Spectroscopy through a Horizontal Pulse Shaper Design and a Focal Plane Array Detector. *Opt. Lett.* **2016**, *41*, 524.

(2) Middleton, C. T.; Woys, A. M.; Mukherjee, S. S.; Zanni, M. T. Residue-Specific Structural Kinetics of Proteins through the Union of Isotope Labeling, Mid-IR Pulse Shaping, and Coherent 2D IR Spectroscopy. *Methods* **2010**, *52*, 12–22.

(3) Hamm, P.; Zanni, M. T. *Concepts and Methods of 2D Infrared Spectroscopy*, 1st ed.; Cambridge University Press: New York, 2011.

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