Extreme sensitivity biosensing platform based on hyperbolic metamaterials

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S1. Optical constants of gold and Al₂O₃

Variable angle spectroscopic ellipsometry (J. A. Woollam Co., Inc, V-VASE) has been used to obtain the optical constants of gold and Al₂O₃ thin films. By using thermal evaporation of gold pellets, gold thickness of 20 nm was deposited on a glass substrate and a general oscillator model was used to fit the measured ellipsometry data. For the measurements of Al₂O₃ thin film, a 50 nm Al₂O₃ layer was first deposited on a silicon wafer using electron-beam evaporation and a Cauchy model was used to fit the measured data to obtain the refractive index of Al₂O₃ dielectric layer. The obtained permittivity values of gold and Al₂O₃ thin films are shown in Fig. S1. The real and imaginary permittivity values for gold are represented by the blue and red lines, respectively. Positive dielectric constants of Al₂O₃ are represented by a black line. Due to Drude-type response of free electrons in metals at higher wavelengths the real permittivity values of gold show a well-known decreasing trend towards strongly negative values¹.

![Figure S1. Permittivity values of gold and Al₂O₃ determined using spectroscopic ellipsometry.](image)

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**S2. Time-resolved photoluminescence measurements**

We used time-resolved photoluminescence spectroscopy to confirm the existence of high-k modes in the gold-Al\(_2\)O\(_3\) HMM. The fabricated HMM structure consists of a Coumarin 522 B dye (0.3% by wt. in ethanol solution) dissolved polymer (PMMA) layer of 100 nm thickness on top of a 8 pairs of gold/Al\(_2\)O\(_3\) stack, separated by a Al\(_2\)O\(_3\) spacer layer of 10 nm thickness. A reference sample was used to compare the results, which consists of Coumarin 522 B dye dissolved PMMA layer (100 nm thickness) on a Al\(_2\)O\(_3\)/glass substrate. An ultrafast optical set up has been used to measure the lifetimes of the samples. The optical set up consists of a Ti: Sapphire tunable femtosecond laser (Chameleon Ultra II from Coherent), Pulse Picker (by Coherent), Second Harmonic Generator (Coherent), and a spectrofluorometer for time-correlated single photon counting (TCSPC) instrument (Edinburgh instruments). The time resolution of the TCSPC instrument is \(\leq 5\) ps. In the experiments, the Coumarin 522 B dye was excited by using a pulsed laser at 400 nm with a pulse width of about 120 fs and a repetition rate of 4 MHz. Here the emission wavelengths were varied using a monochromator that belongs to the TCSPC instrument.

The time decay curves of reference and HMM samples are shown in Fig. S2a. It is clear that there is a large variation in time decay for HMM compared to reference sample. In order to obtain the lifetime, the data were fitted using, 

\[ R(t) = B_1 e^{-t/\tau_1} + B_2 e^{-t/\tau_2} + B_3 e^{-t/\tau_3}, \]

where \(\tau_i\) is the lifetime. Since longer times are attributed to uncoupled dye molecules, located above the coupling distance from the HMM, we used shorter decay times (\(\tau_1\)) to predict the existence of high-k modes in HMM, since shorter decay time is related to molecules strongly coupled with HMM\(^2\). The obtained shorter lifetimes (\(\tau_1\)) of reference and HMM samples as a function of emission wavelength are shown in Fig. S2b. In hyperbolic region, a large difference in spontaneous emission lifetime of
dye onto the HMM compared to reference sample is observed. Also, dye on reference sample show an increase in lifetimes with emission wavelengths; however lifetime of dye onto HMM is almost constant in the hyperbolic region of the emission spectra. The observed behaviour of HMM is attributed to the existence of high-k modes as well as non-radiative and SPP modes present in the HMM\(^3\). The observed response of HMM definitely supports the existence of high k-modes in the fabricated gold/Al\(_2\)O\(_3\) HMM. However, the observed results are not in good agreement with previously reported results\(^3\). This could be due to the low internal quantum yield of the Coumarin dye in PMMA, broadband dye emission spectrum (Fig. S3) and higher metallic fill fraction (34% in the present case).

Figure S2. (a) Time-resolved photoluminescence measurements of Coumarin dye on reference and HMM samples. (b) Variation of spontaneous emission lifetimes of the Coumarin dye on reference and HMM samples, as a function of emission wavelength.
Figure S3. Emission spectrum of Coumarin 522 B dye dissolved PMMA layer on HMM and reference samples, excitation at 400 nm wavelength.

S3. Reflectance spectrum

The reflectance spectrum of sensor device under distilled water environment is shown in Fig. S4. One can see that the reflectance spectrum is modified at longer wavelength compared to the GC-HMM (Fig. 1d), particularly the fourth BPP mode ($\lambda > 2000$ nm) is missing from the spectrum due to the higher absorption of relatively thick polymer channel at longer wavelengths. It is evident that the reflectance minima observed at longer wavelengths are not guided modes because those minima are not blue shifted when the angle of incidence is increased from $20^\circ$ to $50^\circ$. In comparison to reflectance spectra of GC-HMM (Fig. 1d), a large red shift in resonance wavelength is observed in Fig. S4, which is due to the large refractive index contrast between air and distilled water.
S4. Sensor performance evaluation (with functionalization)

In the case of functionalized samples, reflectance minima were obtained at 1300±5 nm for the first mode, 885±4 nm for the second mode, and 685±4 nm for the third mode. Here the angle of incidence was set to 30°. The spectroscopic resolution of 0.2 nm was taken for binding events experiments, which is far above the spectroscopic resolution of the instrument (0.03 nm). A scanning time of around 50 seconds is required to record data points of about 130 for each measurement (binding events experiments). Therefore the time required for recording one data point is around 0.4 seconds, which is higher than the minimum measurement time of the instrument (0.1 seconds). Therefore the noise level is well below the signal amplitude. We have an error bar of +/-0.1 nm since our wavelength step size is 0.2 nm, and +/- 0.2 seconds in time since we have a time step of 0.4 seconds. However, the error bars in time and wavelength are too small that even a dot can cover it.

The reflectance spectra of the functionalized sensor device with different concentrations of biotin in PBS for the third mode are shown in Fig. S5. For the first mode, the maximum with
respect to PBS was 4 nm for 10 pM biotin and 11 nm for 1 µM biotin. A maximum shift of 1.4 nm for 10 pM biotin and 4 nm for 1 µM biotin are obtained for the second mode whereas the third mode provides a shift of 0.8 nm for 10 pM biotin and 2 nm for 1 µM. Note that the molecular binding of lower and higher concentrations of biotin in PBS were performed using different samples of the same batch for different concentrations. The binding of biotin molecules to the sensor surface by recording the wavelength shift for different concentrations (100 pM, 1 nM and 1 µM) over time is shown in Fig. S6. As shown in Fig. S6, obtained step in the wavelength shift is different for lower and higher concentrations. The discrete steps in Fig. S6 are only due to the 0.2 nm discreteness in the wavelength sensitivity, and the variability in the step size is due to statistical fluctuations where larger or smaller number of binding events occur. In all our measurements, there is a tradeoff between scanning time resolution and spectroscopic resolution. In order to get a data point, by scanning a mode, within one minute, we have used 0.2 nm spectroscopic resolution, which means that any mode shift below 0.2 nm is not observed. This is the reason why we did not record considerable shift within 50 sec at given time intervals (Fig. 3c and Fig. S6). The obtained peak shift is due to the change in local refractive index induced by the binding of injected biotin molecules within the illuminated areas of 100 µm × 100 µm. The initial wavelength shift for first mode of four concentrations are shown in Fig. S7. As expected, the initial wavelength shift increases when the concentration of the biotin is increased from 10 pM to 1 µM. Note that no further considerable wavelength shift was observed for higher (10 µM) concentration of biotin in PBS after 1 µM. This is due to the complete coverage of neutravidin sites in the case of functionalized samples.

One of the raw data of 10 pM biotin concentration after rinsing with PBS is shown in Fig. S8. It is clear from the figure that spectral position of the modes are slightly blue shifted when the time
progresses. A considerable blue shift was first obtained by injecting PBS buffer. After that the obtained blue shift was comparable to the red shift obtained while adsorption of molecules. As it is clear from Fig. 3, the wavelength shift of the sensor decreases as the number of adsorbed molecules increases. That means the wavelength shift almost saturates when many molecules are absorbed on the sensor surface. The sensitivity has been found to remarkably decay as the number of molecules on the sensing area increases, producing a relatively small resonance shift per adsorbed molecules. Therefore only a small blue shift is possible after rinsing in PBS due to removal of loosely mound molecules. It is more likely that after injecting PBS the loose molecules are flushed, causing considerable shift, then whatever unbinding happens afterwards should be random and unlikely to happen since it’s not energetically favorable. A blue shift of 1 nm was obtained after rinsing the sample with PBS for 10 min.

Figure S5. Reflectance spectra of third mode with different concentrations of biotin in PBS.
Figure S6. Binding of biotin molecules on the sensor surface for (a) 100 pM biotin in PBS, (b) 1 nM biotin in PBS, and (c) 1 µM biotin in PBS.

Figure S7. Initial wavelength shift as a function of biotin concentrations.
Then we performed an experiment to study molecular binding events by recording two modes simultaneously. Since the highest sensitive mode (first mode (~1300 nm)) is far away from other modes, we have simultaneously recorded second (~870 nm) and third (~670 nm) modes. Since we needed to scan the reflectance spectra over a broad wavelength range, the wavelength spectroscopic resolution was set to 0.4 nm. The reflectance spectra were recorded every 3 min. Since the lower concentrations do not provide a large wavelength shift for the third mode, we have performed experiments using 1 μM biotin in PBS. The wavelength shifts of the second and third modes with respect to PBS are recorded over time (Fig. S9). One can see that the second and third modes shifts differently over time. The second mode provides larger shift as compared to the third mode due to its higher refractive index sensitivity and FOM.

Also it is clear that there are a significant number of discrete points (red and blue dots) are at basically the exact same level because it was not feasible to record a wavelength shift below 0.4 nm caused by the adsorption of biotin molecules since the wavelength spectroscopic resolution of our measurement was set to 0.4 nm.
Figure S9. Binding of biotin molecules on the sensor surface by simultaneously recording the second and the third modes, using 1 µM biotin in PBS.

**S5. Sensor performance evaluation (without functionalization)**

Relative to the distilled water control, a wavelength shift of 5.2 nm was observed for 10 fM BSA and a maximum shift of 20.2 nm was observed for 100 nM BSA (Fig 4a). Substantial shifts of 1.8, 1.4 and 1.2 nm were observed for the first, second and third modes in the presence of 10 fM biotin. In order to show the binding of BSA molecules on the sensor surface for different concentrations, we have used different samples of the same batch for lower and higher concentrations. Here, we demonstrate the binding of BSA to the sensor surface by recording the wavelength shift of different concentrations (100 fM, 100 pM and 100 nM) of BSA over time (Fig. S10). The reflectance spectra of all samples were recorded at an incident angle of 30 degree. For all samples, the resonance wavelength of first mode after injection of DI water is obtained at 1280±5 nm. It is evident from Fig. S10 that the rate of binding depends on the concentrations and the slope of the initial
wavelength shift increases. An initial wavelength shift of 1.8 nm is obtained after injecting 100 fM BSA whereas 100 pM BSA and 100 nM BSA provided a shift of 3.4 nm and 6.6 nm, respectively (Fig. S11).

Figure S10. Binding of BSA molecules on the sensor surface (a) 100 fM BSA in DI water, (b) 100 pM BSA in DI water, and (c) 100 nM BSA in DI water.

The reflectance spectra of the third mode for BSA, biotin and BSA+biotin solutions with different concentrations are shown in Fig. S12a, Fig. S12b and Fig. S12c, respectively. According to Fig. 4 (in the manuscript), biotin provides comparatively smaller wavelength shifts in comparison with BSA due to the lower molecular weight of biotin. Interestingly, for the third mode, the shift obtained in the case of BSA+biotin is almost similar to the case of biotin alone, which is a clear indication of selective binding of biomolecules. To study this concept, we plotted the difference in wavelength shift between first (NIR) and third (visible) mode of BSA and biotin, which is shown in Fig. S13. We observed that the different modes do not shift in a one to one manner.
Figure S11. Initial wavelength shift as a function of BSA concentrations.

Figure S12. Reflectance spectra (without functionalization) of third mode for different concentrations of (a) BSA in distilled water, (b) Biotin in distilled water, and (c) BSA+biotin in distilled water.
Figure S13. Resonance wavelength difference ($\Delta \lambda$) between the first and the third mode (as a function of concentrations) of BSA and biotin in DI water. Data points represent the error bar.

S6. Adsorbed molecule sensitivity analysis

In this section, we estimate the sensitivity of the resonant wavelength shift to the number of molecules adsorbed on the sensor surface. We focus on the experiments without functionalization, where the smallest concentration values of BSA and biotin were probed, and the resulting response of the mode at 1280 nm in DI water at 30° angle of incidence. For each concentration $c$ of the biomolecule in our device, there will be a corresponding saturating wavelength shift $\Delta \lambda(c)$ of this mode in the long-time limit (after approximately 45 minutes). This shift is due to the presence of an average equilibrium population $N(c)$ of adsorbed particles on the sensor surface, a number which we cannot directly measure. The sensitivity can be defined as $\Delta \lambda(c)/ N(c)$, or mean wavelength shift per adsorbed particle. Since the minimum shift resolvable in our apparatus is $A_{\text{min}} = 0.2$ nm, a sensitivity of $\Delta \lambda(c)/ N(c) \geq A_{\text{min}}$ would be required to resolve individual molecules binding to the sensor surface. How close is our system to this single-molecule detection threshold?
To answer this question, let us analyze the adsorption process. Though $N(c)$ is inaccessible, we can determine a reliable upper bound on this number, which we will call $N_{\text{max}}(c)$, the maximum number of molecules on average that can be adsorbed on the sensor surface. Since $N(c) \leq N_{\text{max}}(c)$, we know $\Delta \lambda(c)/N_{\text{max}}(c)$ will be a lower bound on the true sensitivity $\Delta \lambda(c)/N(c)$. To derive $N_{\text{max}}(c)$, we consider several factors that contribute to $N(c)$, and how they can be manipulated to yield a bound on the adsorbed population:

- **Illuminated sensor area**: Only molecules that bind to the 100 $\mu$m X 100 $\mu$m grating regions (shaded squares in Fig. S14a) illuminated by the beam can couple to the plasmonic system and hence affect the wavelength shift. The illuminated area is an ellipse with major radius $R_{\text{sec}} \theta$ and minor radius $R$, where $R = 1$ mm is the radius of the beam, and $\theta=30^\circ$ is the angle of incidence. The grating squares form a 4 x 15 array on the bottom surface of the microfluidic device, spaced out by 200 $\mu$m intervals of no grating. Depending on the orientation and position of the elliptical illuminated area with respect to the grating array, the amount of illuminated sensing surface can vary. Figure S14a shows the case where the largest number of grating squares lie inside the beam boundary, which we will use to calculate $N_{\text{max}}$. This corresponds to approximately 28 squares, or a total effective sensor area of 0.28 mm$^2$.

- **Adsorption on boundaries of microfluidic volume**: Only a small fraction of the total population of BSA or biotin molecules will end up adsorbed on the illuminated sensor area. Adsorption can occur anywhere along the bottom gold surface, not just the sensor, and also on the PMMA top and side surfaces of the microfluidic device. For $N_{\text{max}}$, we will assume only the bottom surface is adsorbing, since any competition from the PMMA surfaces will always lead to fewer molecules on the sensor. We will also assume the adsorbed molecules
are equally distributed across the entire bottom surface, which has dimensions of 2 mm x 14 mm, an area of 28 mm$^2$. Hence, given a certain maximum possible adsorbed population on the bottom, only a fraction $0.28 \text{ mm}^2 / 28 \text{ mm}^2 = 0.01$ will be in the sensing region and relevant to the wavelength shift.

- **Irreversibility of adsorption:** In principle the equilibrium adsorbed population $N$ reflects the net flux of molecules binding to the sensor areas minus the flux of molecules unbinding. To obtain $N_{\text{max}}$ we will assume that binding is irreversible, since any unbinding events will always lower than adsorbed number. Based on earlier work looking at BSA adsorption on a gold surface, this irreversibility is likely to be approximately true on the time scales of the experiment (~ 1 hr).

Putting these considerations together, we can now calculate $N_{\text{max}}$. Since the microfluidic channel has a height of 0.05 mm, initially there are $c (28 \text{ mm} \times 0.05 \text{ mm/1L}) \text{ M}^{-1} = 8.4c \times 10^{17} \text{ M}^{-1}$ biomolecules in solution inside the device. In the long time limit, if all of these were to be adsorbed irreversibly on the bottom gold surface, on average 1% of the total would be in the sensor areas. Thus:

$$N_{\text{max}}(c) = 8.4c \times 10^{15} \text{ M}^{-1}$$

In Fig. S14b, this number is plotted versus $\Delta\lambda(c)$ for BSA, with eight different values of $c$ between 10 fM to 100 nM, corresponding to $N_{\text{max}}$ ranging from 84 to $8.4 \times 10^8$ molecules. The relationship between $N_{\text{max}}$ and $\Delta\lambda$ is highly nonlinear, and is consistent with the following phenomenological fitting function (drawn as a blue curve):

$$N_{\text{max}} = A_1(e^{\Delta\lambda/A_1} - 1) + A_2(e^{\Delta\lambda/A_2} - 1)$$

(1)
where $A_i$, $A_i$, $i=1,2$, are parameters. The best-fit values are shown in the figure. At large $\Delta \lambda$, this function captures the exponential changes in $N_{\text{max}}$ associated with small increases of $\Delta \lambda$. In fact there are clearly two exponential regimes in the data, necessitating a biexponential fitting form.

With increasing $c$, there is a crossover around $\Delta \lambda = 13$ nm from the regime with exponential constant $A_1 = 2.1$ nm to a faster growing one with $A_1 = 0.56$ nm. The fitting function is not just a sum of two exponentials, but includes a constant term - $(A_1+A_2)$ so that $N_{\text{max}}=0$ when $\Delta \lambda = 0$. In the limit $\Delta \lambda \to 0$ (or equivalently $c \to 0$) we know that the number of adsorbed particles on the sensor areas becomes tiny. If $N_{\text{max}} \ll 28$ ($c \ll 3 \text{ fM}$), then typically there will be one or zero adsorbed molecules in each grating square. Since each square contributes independently to the wavelength shift, we know that $\Delta \lambda$ must be proportional to $N$ (and by extension $N_{\text{max}}$) in this limit.

Eq. (1) does indeed exhibit this property, with $N_{\text{max}} = (A_1 / \Lambda_1 + A_2 / \Lambda_2) \Delta \lambda$ for $\Delta \lambda \to 0$. As $c$ increases, the possibility of multiple adsorbed molecules per square leads to interference effects and hence nonlinearity, with each additional molecule having a decreasing impact on the wavelength shift.

Figure S14c shows the ratio $\Delta \lambda / N_{\text{max}}$ versus $N_{\text{max}}$. Since this ratio is a lower bound on the true sensitivity, it is instructive to compare it to the threshold $A_{\text{min}}$ required to detect single molecule adsorption. The smallest experimental concentration, $c= 10 \text{ fM}$, has the largest observed ratio, $\Delta \lambda / N_{\text{max}} = 0.06$ nm. This is nearly at the single molecule threshold: no more than 3 molecules adsorb on average for every 0.2 nm shift in wavelength. Consistent with the discussion above, the extrapolated $\Delta \lambda / N_{\text{max}}$ from the fitting function (blue curve) plateaus for $c < 3 \text{ fM}$, in the linear regime. If the $c \to 0$ extrapolation is valid, a single molecule adsorbed on one of the sensor areas gives a wavelength shift of ~0.26 nm, above the resolution threshold. For BSA, constraints on the range of experimentally accessible $c$ do not allow us to directly test this prediction. For larger $c$, © 2016 Macmillan Publishers Limited. All rights reserved.
and hence larger $N_{\text{max}}$, the interference effects of many adsorbed particles in every square lead to drastically reduced sensitivity. The $\Delta \lambda / N_{\text{max}}$ curve decays roughly like $\sim 1 / N_{\text{max}}$ with increasing $N_{\text{max}}$.

Figure S14. (a): Shaded squares show the array of 100 µm X 100 µm sensor regions, while the red ellipse indicates the illuminated area for a beam radius of 1 mm and a 30° angle of incidence.
This diagram shows the arrangement with the maximum sensor area inside the illuminated boundary. (b): The maximum number of BSA molecules, $N_{\text{max}}$, adsorbed in the illuminated sensor areas in the long-time limit, versus the corresponding wavelength shift $\Delta \lambda$ for the mode located at 1280 nm in DI water without BSA. Each circle represents a different initial concentration of BSA, as indicated by the labels. The curve is a best-fit function, described in the text and (c): The same data and curve as in b, but plotted in terms of the sensitivity $\Delta \lambda / N_{\text{max}}$ (a lower bound on the true sensitivity) versus $N_{\text{max}}$. The dotted line shows the threshold $A_{\text{min}} = 0.2$ nm required to resolve individual molecular adsorption events. Figures d and e: analogous to b and c, but with biotin instead of BSA.

Figure S14d shows $N_{\text{max}}$ versus $\Delta \lambda(c)$ for the case of biotin. As with BSA, the model in Eq. (1) describes the data well. The overall magnitudes of $\Delta \lambda(c)$ are smaller for biotin than for BSA at the same concentrations, which makes sense since biotin is a much smaller molecule (244 Da for biotin compared to 66,430 Da for BSA). Figure S14e shows the sensitivity ratio $\Delta \lambda / N_{\text{max}}$ for biotin. Despite the two orders of magnitude difference in molecular weights, the sensitivity at the smallest concentrations of biotin is about one-third that of BSA in Fig. S14c. Hence the system maintains its remarkably high level of responsiveness even for low weight molecules.

S7. Numerical simulations

Numerical simulations have been performed to justify the physical reasons behind the extreme sensitivity of our device. Scattering matrix method$^{5-7}$ was used to obtain the reflectivity of the GC-HMM and finite-difference time domain (FDTD) (using commercially available lumerical FDTD software) method has been used to show the transversal confinement of the different modes and their mode profile. For a comparison purpose, a reference sample has been designed and fabricated, which consists of 2D gold hole array on a Al$_2$O$_3$ spacer layer (10 nm) deposited glass substrate.
The grating parameters are same as that of GC-HMM. A schematic representation of fabricated reference samples is shown in Fig. S16c.

The reflectivity plot of GC-HMM under air environment was first simulated and validated with experimental results. In our simulations, the optical constants of gold are calculated based on Drude free-electron theory, 

$$\varepsilon_m = 1 - \left( \frac{\omega_p^2}{\omega(\omega + i/\tau)} \right)$$

where $\omega_p$ is the plasma frequency of gold and $\tau$ is the relaxation time. As evidenced from Fig. S15, the simulated reflectance spectrum is well correlated with experimentally obtained results (Fig. 1d in the manuscript). Importantly, the higher wavelength BPP mode around 2000 nm is clearly visible in the simulated spectrum. However, all the BPP modes are slightly red shifted as compared to experimental reflectance spectra.

![Simulation](image1.png) ![Experiment](image2.png)

Figure S15. Simulated and experimental reflectance spectrum of GC-HMM in the absence of microfluidic channel (under air environment). Angle of incidence was set to 30°.

The reflectance spectrum (simulation and experiment) of both samples when DI water as the superstrate is shown in Fig. S16. A microfluidic channel was fabricated on top of the sample to flow the DI water. Here the incident angle was set to 30°. It is evident from the figures that
reference sample shows only two low optical quality factor modes at shorter wavelengths (below 500 nm) and those two modes are corresponding to the surface plasmon polaritons (SPP) of metallic diffraction grating. However, GC-HMM supports four extra high quality factor modes above 500 nm and one low quality factor mode below 500 nm. It shows that the four modes obtained above 500 nm are the bulk plasmon polariton modes of HMM and one mode obtained below 500 nm is the SPP mode of diffraction grating. However, the SPP mode of GC-HMM provides comparatively higher quality factor than that of reference sample. This is because GC-HMM supports long and short range surface plasmon polaritons (LRSP and SRSP) in addition to SPP and BPP. It shows that simulation results are in good agreement with experimental results. However, the spectral positions of the modes of the GC-HMM (Fig. S16a) are red shifted as compared to experimental results (Fig. S16b). Nevertheless, the purpose these simulations is to show the supporting SPP and BPP modes of GC-HMM and their response to the external bulk refractive index changes.

Since the reference sample supports only SPP modes, we have considered that sample as the standard SPP-based plasmonic sensor. To provide further evidence for the existence of SPP modes in the reference sample, the reflectance spectra of two modes were recorded for different angles of incidence (Fig. S17a). A blue shift in resonance wavelength with increasing angle of incidence predicts that those modes are SPP. The refractive index sensitivity of reference sample was studied by injecting DI water and 0.5% glycerol in DI water in the sensor microfluidic channel (Fig. S17b). A wavelength red shift of 1.2 nm was obtained for the mode at 450 nm and no considerable shift was obtained for the mode at 375 nm. The calculated refractive index sensitivity of SPP mode at 450 nm is around 2000 nm/RIU. However, GC-HMM provides a very high sensitivity of 10,000 nm/RIU for that particular SPP mode (see Fig. S17c). This is due to the high quality factor of the...
SPP mode of GC-HMM. It shows that RI sensitivity of our device based on SPP mode is even better than that of the standard SPP-based plasmonic sensors (reference sample).

Furthermore, we have calculated the optical quality factor of GC-HMM and the reference sample using experimentally obtained reflectivity data. Here the optical quality factor is considered as the ratio between resonant wavelength and FWHM (full width at half maximum) of the mode. Note that the direct comparison between the simulated and experimental optical quality factor of GC-HMM is not possible because the resonant wavelength of all modes are different in both cases. The optical quality factor of GC-HMM for NIR wavelength modes to visible wavelength modes are 25.5, 22, 17.7, 8.4 and 5, respectively. As expected, maximum quality factor is obtained for the first BPP mode (1300 nm) and quality factor of modes decrease with decreasing mode wavelength. However, the quality factor of the SPP mode (at 450 nm) of reference sample is less than that of the same mode of GC-HMM (at 450 nm). This is the reason why GC-HMM provides higher refractive index sensitivity compared to the reference sample for that wavelength.
Figure S16. Reflectance spectra obtained at 30° angle of incidence. DI water was considered as the superstrate. (a) Simulation for GC-HMM, (b) experiment for GC-HMM, (c) simulation for the reference sample, and (d) experiment for the reference sample.

Figure S17. (a) Experimental reflectance spectra of reference sample for different angles of incidence. Refractive index sensing using SPP modes: (b) for the reference sample, and (c) for GC-HMM.
Figure S18. Simulated reflectance spectra when distilled water and 0.5% glycerol in distilled water as the superstrate. (i–iv) Enlarged plot of the reflectance spectra of four BPP modes.

Then, we have performed numerical simulations to demonstrate the different refractive index sensitivity of different modes for the bulk refractive index changes. The reflectance spectrum of GC-HMM under DI water and 0.5% glycerol in DI water environment is shown in Fig. S18. It is evident that different modes behave differently for the bulk refractive index change. As shown in Fig. S18 (i–iv), the maximum wavelength shift is possible for the first BPP mode and wavelength shift obtained for third and fourth mode is almost the same. The simulated behavior of the modes exactly matches the experimentally realized results (Fig. 2).

In addition, we have simulated the mode profile of all modes using FDTD method. In order to show the extraordinary properties of the modes supported by GC-HMM we compared the results of GC-HMM with the reference sample. In the FDTD numerical simulations, the Bloch boundary condition with smallest spatial grid size of 1 nm was used for the iteration to maintain the accuracy and stability. In our simulation models, we have considered a sensor grating geometry (Fig. S16c) in which the gold layer only covers the top and bottom of the PMMA grating (not the side walls). The optical constants of gold and Al₂O₃ were taken from Palik⁹ and a constant refractive index of
1.49 was used for PMMA. The electric field (EF) cross-sectional maps (xz-plane) of both GC-HMM and reference samples for different mode wavelengths are shown in Fig. S19. It is visible from the maps that GC-HMMs show enhanced electric field distribution on the superstrate as well as near to the hole array for all modes as compared to the reference sample. This indicates that BPP modes are present at those wavelengths, but those modes are absent in the case of reference sample. However, both GC-HMM and reference samples provide almost same electric field distributions on the superstrate at 450 nm wavelength. It confirms that both samples support SPP mode at 450 nm. In addition, the transversal confinement of last three BPP modes (used for biosensing) on the superstrate obtained at a same point in the x-direction (Fig. S19 (i-iii)) is shown in Fig. S20. It is evident that transversal confinement is higher for longer wavelength modes as compared to shorter wavelength modes. One can see that the transverse decay of the field strongly varies (slope of the intensity vs transverse distance curve) from one mode to another. It turns out that different modes behave differently for the size of the biomolecules to be sensed. Since the first BPP mode at 1300 nm shows maximum transversal confinement on the superstrate, it provides maximum RI sensitivity as confirmed in both experiment and simulation. The sensitivity also decreases with decreasing mode wavelength due to the reduction of transversal confinement on the superstrate. Since the enhanced electromagnetic field is strongly confined near the 2D hole array (in the order of few nanometers), the size of the captured biomolecules (even small molecular weight) overlaps with this field and enhance the sensitivity.
Figure S19. FDTD numerical simulation results of mode profile and transversal confinement on the superstrate. The electric field cross-sectional map (xz-plane) of different modes (a) for GC-HMM and (b) for reference sample. The superstrate was considered as DI water with refractive index value 1.3330.

In short, the extreme sensitivity of our sensing platform for the detection of lower molecular weight biomolecules in a highly diluted solution is due to the highly confined field distributions on the superstrate at hyperbolic dispersion. In particular, high-k modes (BPP) of HMM are responsible for this enhanced field distribution. Therefore, the different sensitivity of the modes with respect to the size/molecular weight of the molecules is due to the fact that the transverse decay of the field strongly varies from one mode to another.
Figure S20. Simulated transversal confinement of three modes (1300, 880 and 680 nm) on the superstrate. Data are taken by selecting a point (x=200 nm) in figures S19a (i to iii) for three wavelengths.

In order to validate the enhanced measured shift of lower molecular weight biomolecules, we have performed numerical simulations to calculate the refractive index sensitivity of different modes for the shallow (thin dielectric layer) refractive index (RI) changes. To mimic the resonance shift induced by the binding of small molecules at very low concentrations (10 pM), a very thin dielectric layer of thickness 10 nm was used as superstrate. The refractive index of the thin dielectric layer was varied from 1.333 (DI water) to 1.3353 (2% glycerol in DI water). The reflectance spectra of the three modes of GC-HMM for shallow RI changes are shown in Fig. S21a. By comparing Fig. 2 and Fig. S18, it is clear that the sensor provides higher sensitivity for bulk RI changes as compared to shallow changes. This is a well-known behavior of plasmonic sensors\textsuperscript{10}. The wavelength shift calculated with respect to DI water for shallow RI changes of the three modes of GC-HMM is shown in Fig. S21b. As expected, the wavelength shift of the modes vary from first to third in which the first mode provides maximum wavelength shift and third mode
provides minimum shift due to different transverse decay of the different modes. The same behavior was experimentally confirmed for biomolecules (Fig. 3 & Fig. 4).

However, the direct comparison between the enhanced measured shift and calculated shift is not straightforward because the calculation of the RI changes induced by the binding of small biomolecules on the sensor surface is not feasible and the spectral positions of the three modes are different in both experiment and simulation. Also, it is well known that surface label free biosensors behave differently for homogenous RI changes compared to RI changes due to biomolecules adhesion. Therefore, we put forward an alternative approach to validate the enhanced measured shift with calculated shifts. Here, the wavelength shift ratio between the three modes of GC-HMM are compared. Specifically, the wavelength shift ratio between the first and the second modes, the first and the third modes, and the second and the third modes are compared. The corresponding values of the three modes for experiment and simulations are shown in Table 1. For three modes, the wavelength shift obtained for the different concentrations of biotin (Fig. 3d in the manuscript text) is considered for the experimental values whereas the shift calculated with respect to DI water (1.3330) when the refractive index values of 10 nm dielectric layer varied from 1.3336 (0.5 % glycerol in DI water) to 1.3353 (2 % glycerol in DI water) are considered for the simulation (Fig.S21). It is clear from the table that the average wavelength shift ratio obtained for the three modes in the experiments are in good agreement with the average values of shift ratio calculated using numerical simulations. These observations support the experimentally obtained sensitivity enhancement of small biomolecules.
Figure S21. Simulated refractive index sensitivity of three modes for shallow (thin dielectric layer of thickness 10 nm) refractive index changes. (a) Reflectivity plot for DI water and 2% glycerol in DI water and (b) Wavelength shift with respect to DI water as a function of different weight ratios of glycerol in DI water for three modes.
<table>
<thead>
<tr>
<th>Experiment (Biotin Concentrations)</th>
<th>Wavelength shift (nm)</th>
<th>Wavelength shift ratio</th>
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</thead>
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<tr>
<td></td>
<td>1(^{\text{st}}) mode</td>
<td>2(^{\text{nd}}) mode</td>
</tr>
<tr>
<td>10 pM</td>
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<td>1.4</td>
</tr>
<tr>
<td>100 pM</td>
<td>5.4</td>
<td>2</td>
</tr>
<tr>
<td>10 nM</td>
<td>7</td>
<td>2.4</td>
</tr>
<tr>
<td>100 nM</td>
<td>10</td>
<td>3.4</td>
</tr>
<tr>
<td>1 (\mu)M</td>
<td>11</td>
<td>4</td>
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<tr>
<td>Average values (experiment)</td>
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<td><strong>2.83</strong></td>
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<tr>
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</tr>
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<tr>
<td>Average values (simulation)</td>
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<td><strong>2.6</strong></td>
</tr>
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</table>

Table 1: Wavelength shift ratio of different modes in the experiment (using biotin) and simulation.
References


