Electronic Supplementary Information:

A facile and cost-effective TEM grid approach to design gold nano-structured substrates for high throughput plasmonic sensitive detection of biomolecules

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Table S1. LSPR data including resonant wavelength and maximum extinction of gold annealed NPs obtained from three different patterns of 8 independent samples. The data shown represent the average of 5 LSPR measurements inside one pattern. One pattern corresponds to one square of a TEM-grid pattern.

	(resonant wavelength (nm), maximum extinction)			Average wavelength, extinction	RSD	
Sample	Pattern 1	Pattern 2	Pattern 3		Wavelength	Extinction
No 1	566.8, 0.31	567.9, 0.29	567.1, 0.30	567.3, 0.3	0.1%	3.3%
No 2	564.8, 0.26	563.1, 0.25	563.7, 0.26	563.9, 0.26	0.15%	2.25%
No 3	566.2, 0.30	567.8, 0.29	568.5, 0.32	567.5, 0.3	0.21%	5.04%
No 4	566.1, 0.27	564.9, 0.26	565.1, 0.25	565.4, 0.26	0.12%	3.85%
No 5	566.2, 0.28	565.9, 0.29	567.8, 0.31	566.6, 0.29	0.18%	5.21%
No 6	566.8, 0.27	568.4, 0.28	567.9, 0.3	567.7, 0.28	0.14%	5.39%
No 7	565.8, 0.26	566.1, 0.26	567.2, 0.25	566.4, 0.26	0.26%	2.25%
No 8	565.3, 0.31	566.7, 0.3	567.9, 0.32	566.6, 0.31	0.23%	3.23%

In the present article the nanostructures are obtained by metal evaporation followed by high temperature thermal annealing. One major advantage of this protocol is the obtained NPs structures which are stable and optically homogenous according to the authors published results [34,35] and other groups [36, 37]. The reproducibility of optical LSPR measurements is highly improved by using a TEM grid as a marker to guide and position the illumination light beam. Consequently, eight independent nanostructured gold samples are LSPR tested using the same experimental conditions as

reported in the manuscript (Fig. 4 and 5). Moreover, for each sample, three different patterns are selected and for each pattern, five different regions were LSPR tested. The averaged values of wavelength and extinction are summarized in Table S1. For instance, for a sample with 4 nm evporation gold film and annealed at 500 °C for 8 hrs, the averaged plasmonic peak is located at 566.7 ± 1.5 nm with a maximum optical density of 0.27± 0.03. The overall relative standard deviation (RSD) for the 8 independent samples is calculated. Such results indicate that the plasmonic properties of obtained nanostructures are reproducible and homogeneous.

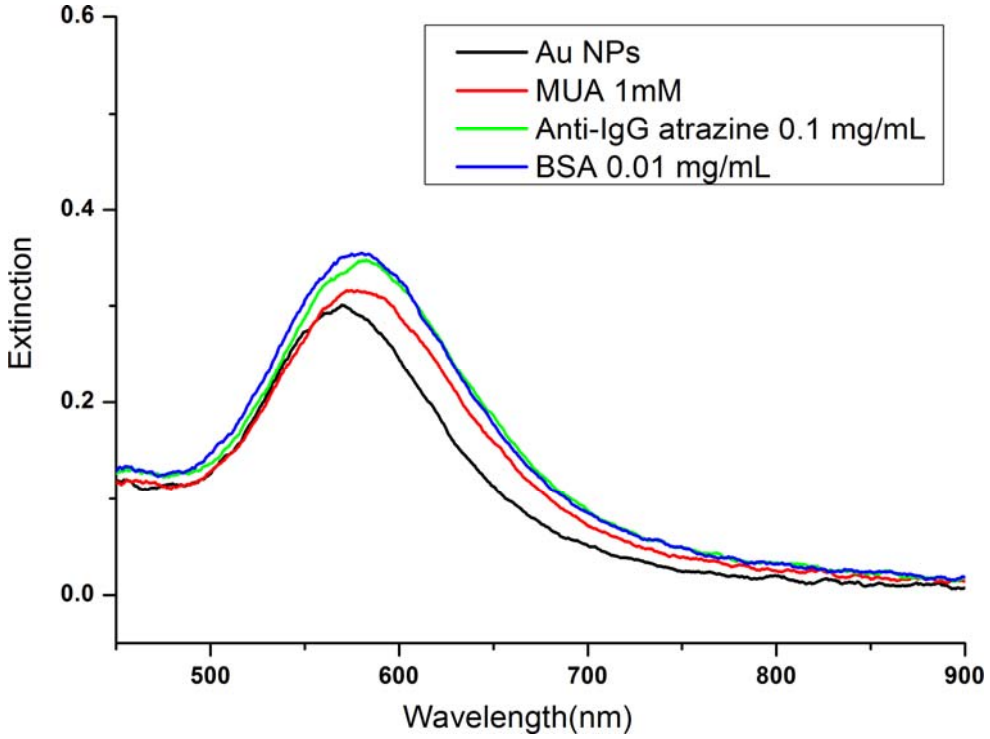

Fig.S1 LSPR spectra recorded for control experiments using the same gold nanostructures area for different biofunctionalization steps such as non-specific antibody (anti-atrazine IgG antibody) and antigen (BSA) for bio recognition.

Since the size of the collected zone is much smaller than the size of one pattern, 5 different zones or regions inside one pattern are detected. Moreover, the LSPR spectra of three different patterns of a given sample are monitored. The spectra shown in Fig. 4 represent the average of 15 independent LSPR measurements for each step of (bio)functionalization.

In the first sets of control experiments, there are observed well-defined plasmonic wavelength shifts and increases of optical densities as the nanostructures are modified by the thiol and anti-atrazine IgG antibody. These results confirm the robustness of the surface biomodification based on high temperature annealed gold substrates. However, when the high concentration of BSA (0.01 mg/mL) is deposited on the non-specific antibody modified substrate, no wavelength shift combined with a slightly variance of the maximum optical density are observed. These experiments strongly confirm the non-specific interaction of BSA with the anti-atrazine IgG antibody modified surfaces.

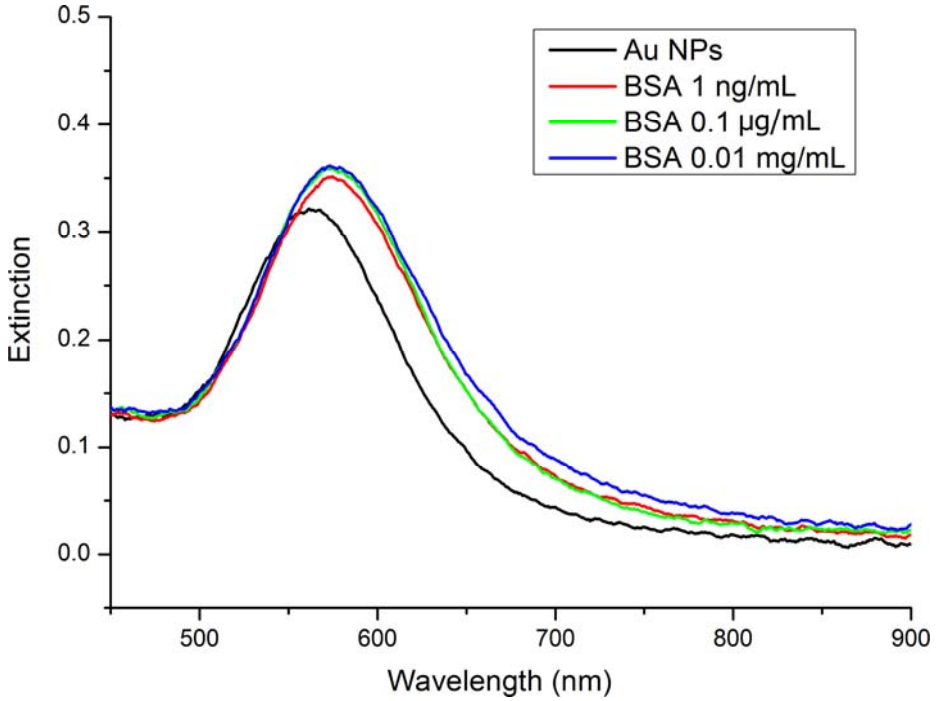

Fig. S2 LSPR spectra of nanostructures modified with three different BSA concentrations deposited onto a non-specific anti-atrazine IgG antibody substrate.

In the second sets of control experiments, one unique area of gold nanostructures was biofunctionalized following the same protocol reported in Fig. 5 except that the specific anti-BSA IgG antibody was replaced with a non-specific anti-atrazine IgG antibody. Thus, it can be seen in Fig.S2, the LSPR spectra of three different albumin modified substrates cannot be distinguished, due to the random non-specific interaction between BSA molecule and NPs modified with atrazine antibody.

Table S2. Plasmonic properties (resonant wavelength and maximum extinction) of stepwise preparation of LSPR biosensors (sample 1) and high throughput specific detection of three different concentrations of BSA in a single experiment (sample 2).

Sample	(resonant wavelength (nm), maximum extinction)				Average wavelength, extinction	RSD	
		Pattern1	Pattern2	Pattern3		Wavelength	Extinction
No 1	NPs	566.8, 0.31	567.9, 0.29	566.2, 0.30	566.9, 0.3	0.15%	3.3%
	MUA 1mM	571.2, 0.32	570.1, 0.33	572.1, 0.34	571.1, 0.33	0.18%	3.03%
	Antibody 0.1 mg/mL	579.5, 0.36	576.8, 0.35	576.8, 0.38	577.7, 0.36	0.27%	4.2%
	BSA 0.01 mg/mL	584.9, 0.38	582.6, 0.42	582.8, 0.39	583.4, 0.4	0.33%	5.25%
No 2	NPs	564.8, 0.26	563.1, 0.25	563.7, 0.26	563.9, 0.26	0.15%	2.25%
	BSA 1 ng/mL	567.8, 0.33	566.2, 0.33	568.9, 0.35	567.6, 0.34	0.24%	3.43%
	BSA 0.1 µg/mL	571.5, 0.34	574.1, 0.36	572.8, 0.35	572.8, 0.35	0.23%	2.86%
	BSA 0.01 mg/mL	578.5, 0.39	581.8, 0.38	582.1, 0.41	580.8, 0.39	0.34%	5.13%
	Inter-assay RSD of BSA 0.01 mg/mL between sample1 and sample 2: For wavelength: 0.36% ; for extinction: 4.16%						

In order to evaluate the reproducibility of the surface biofunctionalization, two nanostructured samples are modified in the same manner as discussed in the manuscript. As mentioned before, the LSPR measurements are recorded for the same patterns (means 3 independent patterns for one sample) during all the biomodification steps. The results (an average of 5 different LSPR optical measurements) are obtained thanks to the TEM-grid patterned substrates that allows a precise control of the collected zone at any time of the experiments. It is found that for these two samples, the maximum RSD between different positions inside one sample is around 0.34 % for

the resonant wavelength and 5.25 % for the optical density. In addition, when compared with the LSPR results of spots modifed by the same concentration of BSA (0.01 mg/mL) from these two different samples, a small inter-assay RSD is obtained as 0.36 % and 4.16% for wavelength and optical density, respectively, which confirms the reproducibility of surface biomodifications.

In conclusion, the results obtained from the two sets of control experiments, concerning the logical plasmonic peak evolutions shown in Fig.4 and Fig.5 are really attributed to the specific interaction between BSA antibody and its antigen.