Electronic Supplementary Material (ESI) for Chemical Communications
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Supporting Information for the communication:

Nucleic Acid-Mediated Gold Oxidation: Novel Biolithography for Surface Microfabrication and New Insight on Gold-based Biomaterials

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Materials and Reagents. Glass cover slips (9mm in thickness) and ethanol (anhydrous, 200 proof) were purchased from VWR. Chloroauric acid ($HAuCl_4 \bullet 4H_2O$), sodium borohydride, N-bromosuccinimide (NBS) and pyridine were obtained from Sigma and Wako. PBS buffer was purchased from Invitrogen. ssDNA (single strand DNA, pcr primer with 20-25mer) and dsDNA (double strand DNA, plasmid with 8kb) samples were obtained from IDT (Integrated DNA Technologies). MilliQ water was used in all of the experiments.

Surface Modification Steps to Fabricate Gold-coated Glass and Self-assembled Monolayer (SAM) on Gold Surface. The glass cover slips were first cleaned with piranha solution, and then coated by a 45-nm thick gold layer with a 5-nm thick Cr adhesion layer between the glass and gold. (Caution: Piranha solution reacts violently with organic materials; it must be handled with extreme care, followed by copious rinsing with deionized water.) Gold coating on glass substrate was performed by CHA Solution electron beam (E-Beam) evaporator. The gold-coated cover slips were stored in ethanol at -20 ºC until further use. For formation of alkanethiol self-assembled monolayers (SAMs),[1] ethanol used to make the solutions was degassed for at least 30 min. The gold coated glass was cleaned by a UV-ozone cleaner (UVO cleaner, No. 42, Jellght Company Inc., CA, USA) for 2 min (the shortest distance from light source to glass surface was used during the cleaning process), then directly transferred into the thiol solution (2 mM total thiol concentration). The solution was purged with N_2, sealed, and incubated overnight. The substrate was then rinsed with copious amounts of ethanol and ultrasonicated in ethanol for 2 min. The SAM-modified gold substrate was then directly used in the next step or stored under N_2 at -20 ºC.

Synthesis of Gold Colloidal Aqueous Solution.[2] 500 mL 0.1mM yellow choloauric acid aqueous solution was added by 0.05 g of sodium borohydride under vigorous stirring. Upon adding sodium borohydride, the solution immediately changed to burgundy color. The solution was then kept under ambient condition for 24hr with vigorous stirring to further complete the reaction. The freshly prepared

gold colloidal aqueous solution was immediately used for the experiment.

Synthesis of $HAuBr_4$ solution from $HAuCl_4$.[3] The $HAuCl_4$ aqueous solution was prepared by dissolving 27.7mg (0.067mmol) $HAuCl_4 \bullet 4H_2O$ in 2ml water. The freshly prepared $HAuCl_4$ was then added by excess amount of HBr solution (69.2mg, 47-49% wt in water). Upon adding HBr solution, the $HAuCl_4$ solution changed to dark red from original yellow. The UV-vis spectra demonstrated the characteristic absorption peak red-shifted to 380nm in dark red solution containing $HAuBr_4$ from 290nm in yellow $HAuCl_4$ solution.

Synthesis of [py($AuBr_3$)] (py=pyridine).[4] 1ml freshly prepared $HAuBr_4$ solution (calculated concentration is 0.0335mmol $HAuBr_4$, 17.34mg) was dropwise added by excess amount of pyridine (100 μl) under vigorous stirring. The dark orange-red precipitation was formed immediately. The resulting precipitation without further purification was collected by filtering and drying at vacuum. The final amount of the dried crude product was 25.3mg. The mass spectra gave a peak at 514.8 that was in good agreement with theoretical mass (515.8) of the coordination compound [py($AuBr_3$)].

Gold Peeling from Glass Substrate and Lithography. Gold peeling experiments were performed by immersing gold-coated glass chip (ca. 1×1cm) into the aqueous solution containing certain amount of DNA (ss DNA or ds DNA) and NBS (as called as etching solution). A typical example was that immersing one gold-coated glass chip (ca. 1×1cm) without SAM modification into 500 μl PBS buffer solution containing 100μM DNA and 1mg NBS. If pyridine was used to replace DNA in the case of mechanism investigation, a typical example was that immersing one gold-coated glass chip (ca. 1×1cm) with or without SAM modification into 5ml aqueous solution containing 25μl pyridine and 13mg NBS. After given time, the chip was taken out, washed by copious water and subjected to further characterization. For the lithography at macro-scale, a red rubber pad with regular circular holes (ca. 1cm in diameter) was conformal and tightly applied onto gold-coated glass chip, and then the etching solution was filled in each hole. The open holes were then sealed by coverslips to prevent water

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evaporation. The sealed chamber with filled etching solution was then incubated at ambient condition for given time to finish the etching reaction. For lithography at micro-scale, a PDMS microfluidic channel was conform and tightly applied onto gold-coated glass chip and then the etching solution was allowed to fill into the channel (Figure S1). The solvent used in the etching solution may be changed to ethanol from water if the filling process was hardly preceded due to poor water wettability of hydrophobic gold surface. After finishing the filling, two solution droplets were put on the inlet and outlet position of the microfluidic channels to seal the whole filled channels. Then a 100g load was carefully applied on PDMS stamps to further secure the conformal and tight contact between PDMS channel and gold surface. The whole setup was incubated at ambient condition for determined time to finish the etching reaction.

Gold Nanoparticle Etching by Amino Acids and DNA. For etching by amino acids, 1ml freshly prepared gold colloid aqueous solution (Au NP) was mixed with 1ml 0.1M glutathione (GSH from TCI, reduced form, Mw=307.32) in 10mM Tris-HCl buffer (pH 7.4) and then the mixed solution was incubated at ambient condition for given time. The sample was then subjected to UV-vis scanning in the range of 200-800nm. For etching by the combination of amino acids and DNA, 1ml freshly prepared Au NP was mixed with 1ml 0.1M GSH in 10mM Tris-HCl buffer (pH 7.4), followed by adding 100μl λ-DNA sample (Takara Biomed, 0.345mg/ml in 10mM Tris-HCl with 1mM EDTA added, 48502bp). The mixture solution was incubated at ambient condition for given time. The sample was then subjected to UV-vis scanning in the range of 200-800nm.

Characterization. Optical microscopy was performed by a Nikon Eclipse TE2000-U. X-ray Photoelectron Spectra (XPS) were obtained on an AXIS Ultra (Kratos Analytical, NY) spectrometer with a monochromatic AlKα X-ray source (1.4867 KeV) and a monochromator. UV/vis spectra were acquired by using a Jasco V-670 spectraphotometer. Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectra were performed on an Applied Biosystems Voyager-DE STR

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spectrometer by using 1,8,9-trihydroxyanthracene as matrix. 1H NMR spectra were obtained using a JEOL JNM-LA400 at 400 MHz in $CDCl_3$. Chemical shifts of 1H signal was quoted to internal standard Me_4Si and expressed by chemical shifts in ppm.

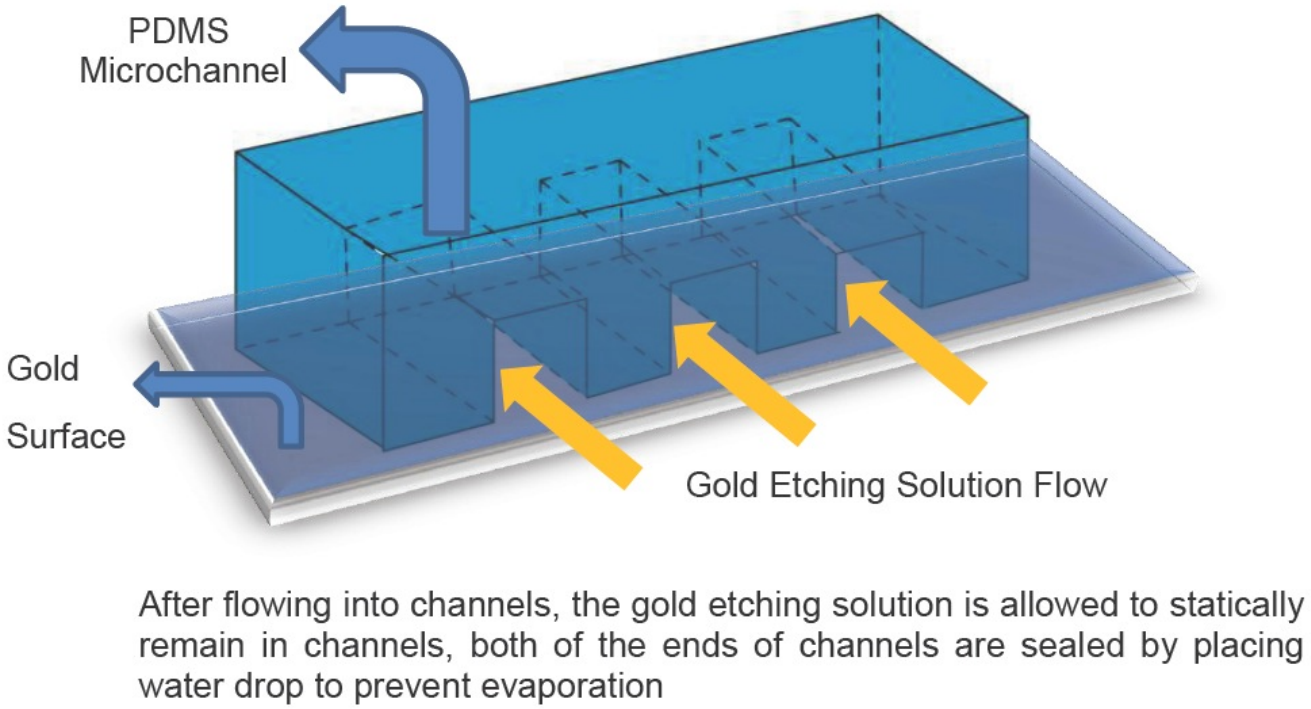

Figure S1. The schematic process for patterned lithography at micro-scale by the combinational use of gold etching solution and microfluidic channels.

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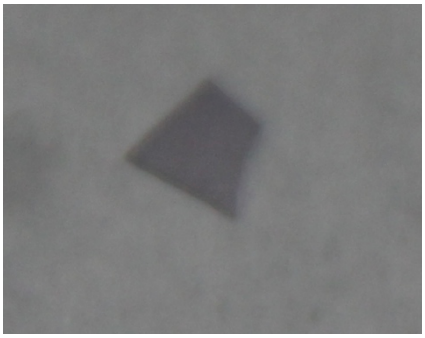
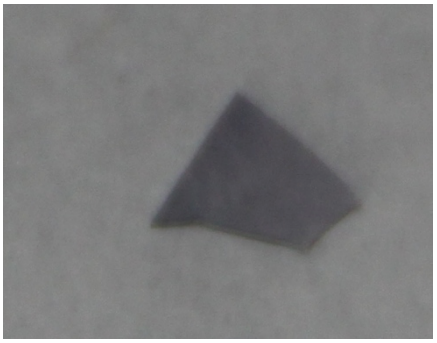
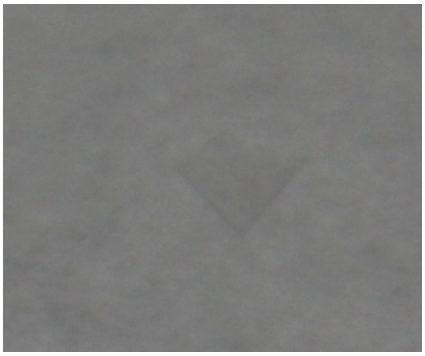
	Coating for 5 s (c.a. 5 nm)	Coating for 10 s (c.a. 10 nm)
The As-deposited		
After etching by GSH/DNA for 8hr		
After etching by GSH/DNA for 32 hr		

Figure S2. The etching of gold film coated on glass substrate with different thickness (5 nm and 10 nm in thickness respectively) by immersing the samples in GSH/DNA (λ-DNA) solution for different time.

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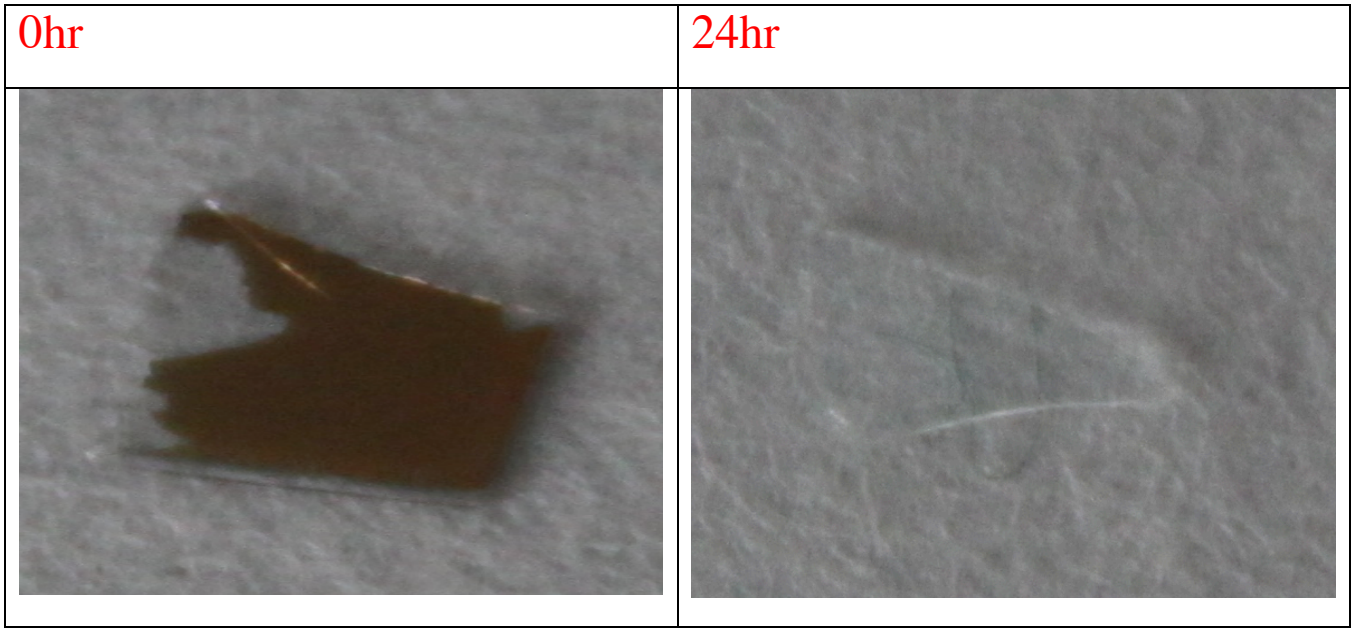

Figure S3. The etching of gold film coated on glass substrate (c.a. 50 nm in thickness) by NBS/DNA solution in the absence of oxygen. In another experiment, the experiment on gold nanoparticle etching by GSH/DNA under oxygen-free condition was repeated. The results showed the etching percentage as 45% at 16hr and 97% at 48hr that were comparable with 40% at 16hr and 100% at 48hr from the same experiment but under normal condition (Figure 2). This result indicated that the contribution from oxygen to this etching process was low.

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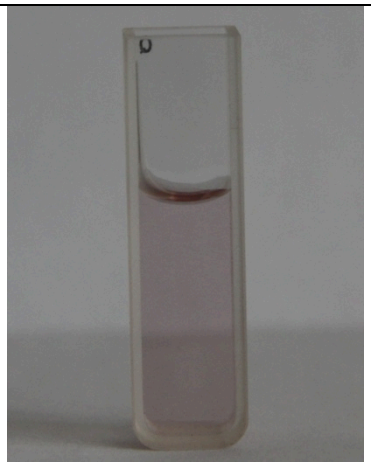
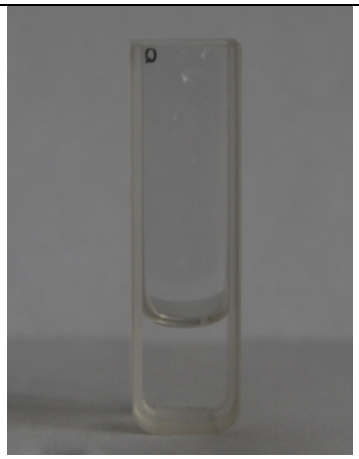
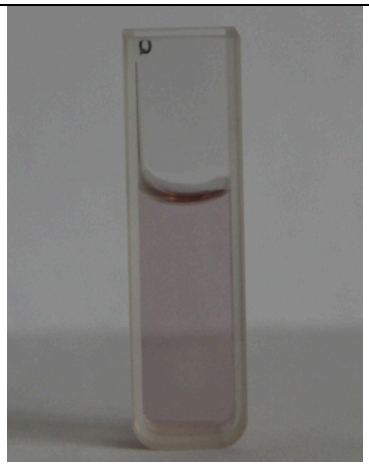
	Before mixing with GSH/DNA	After mixing with GSH/DNA
pH 3.0		
pH 7.4		

Figure S4. The etching of gold colloidal solution by GSH/DNA solution for 48 hr at different initial pH values: a). pH 3.0; b) pH 7.4. The initial gold colloidal solution before mixing with GSH/DNA buffer presented burgundy color, and after mixing with GSH/DNA buffer at different pH for 48 hr, the burgundy color disappeared, indicating the almost complete oxidation of gold colloids in the solution. We also measured the pH during the experiment (3.0 for GSH/DNA solution and 7.4 for NBS/DNA solution) by using pH test paper, and found that pH did not have a discernable change during the experimental period (0 to 48 hr).

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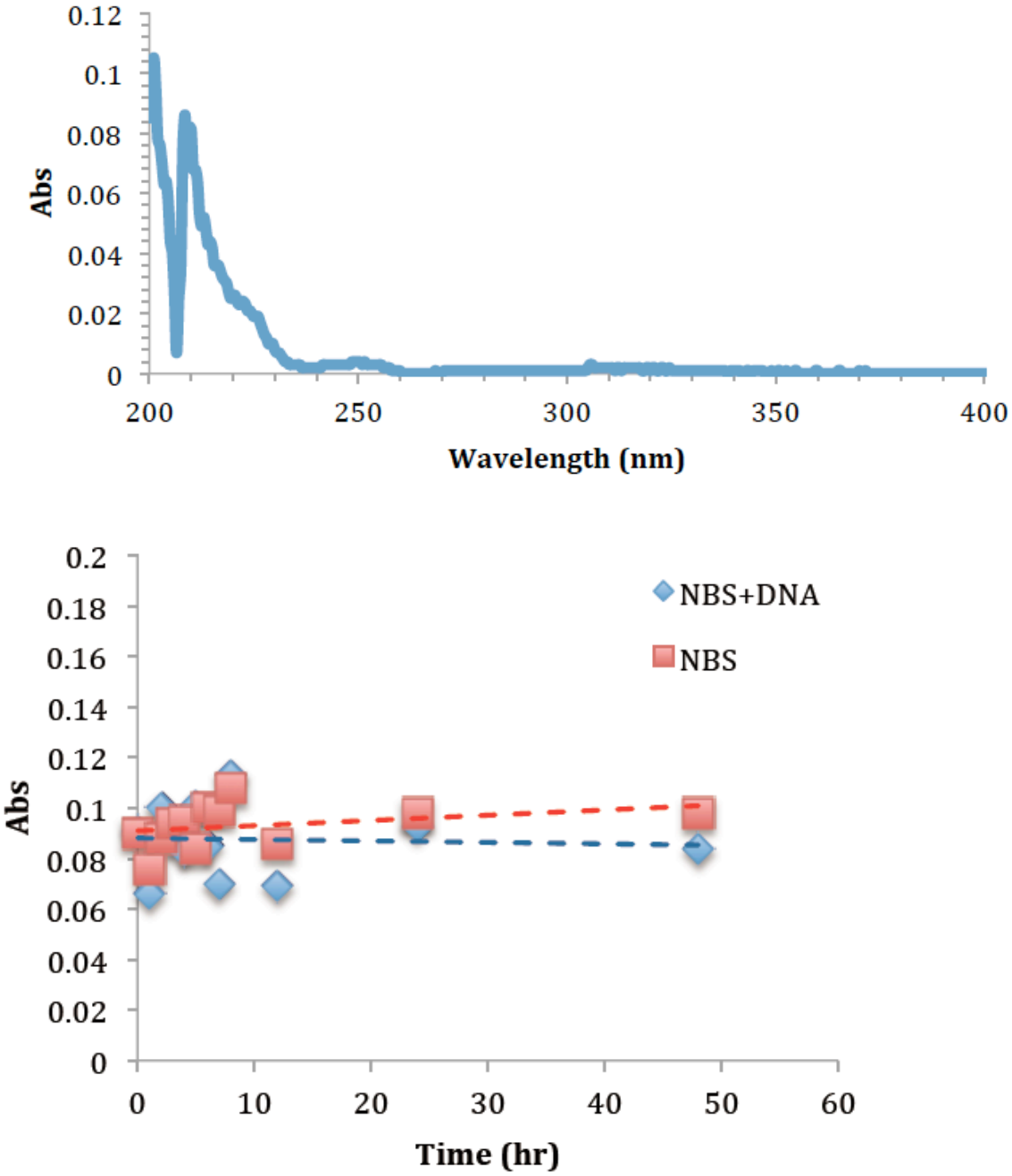

Figure S5. The effect of DNA on the decomposition of NBS in a buffer without reductants, thermo and light stimuli. The UV absorption peak at 208 nm was found in the UV-vis spectra of NBS dissolved in a buffer with or without DNA added (the upper panel), and then the intensity of this peak at different incubation time was plotted as Y-axis with the incubation time (hr) being as X-axis (the lower panel). The scatter points in both of conditions (NBS or NBS+DNA) were then linearly fitted to depict a tendency line. Such tendency lines at both of conditions revealed that the addition of DNA would not affect the decomposition of NBS during the experimental time window (0-48 hr). The characteristic peaks for the decomposed products of NBS i.e. a wide absorption band for succinimide in 200-260 nm and three absorption peaks at 214, 262 and 393 nm for bromine in water were not observed in the buffer containing NBS or the mixture of NBS and DNA.

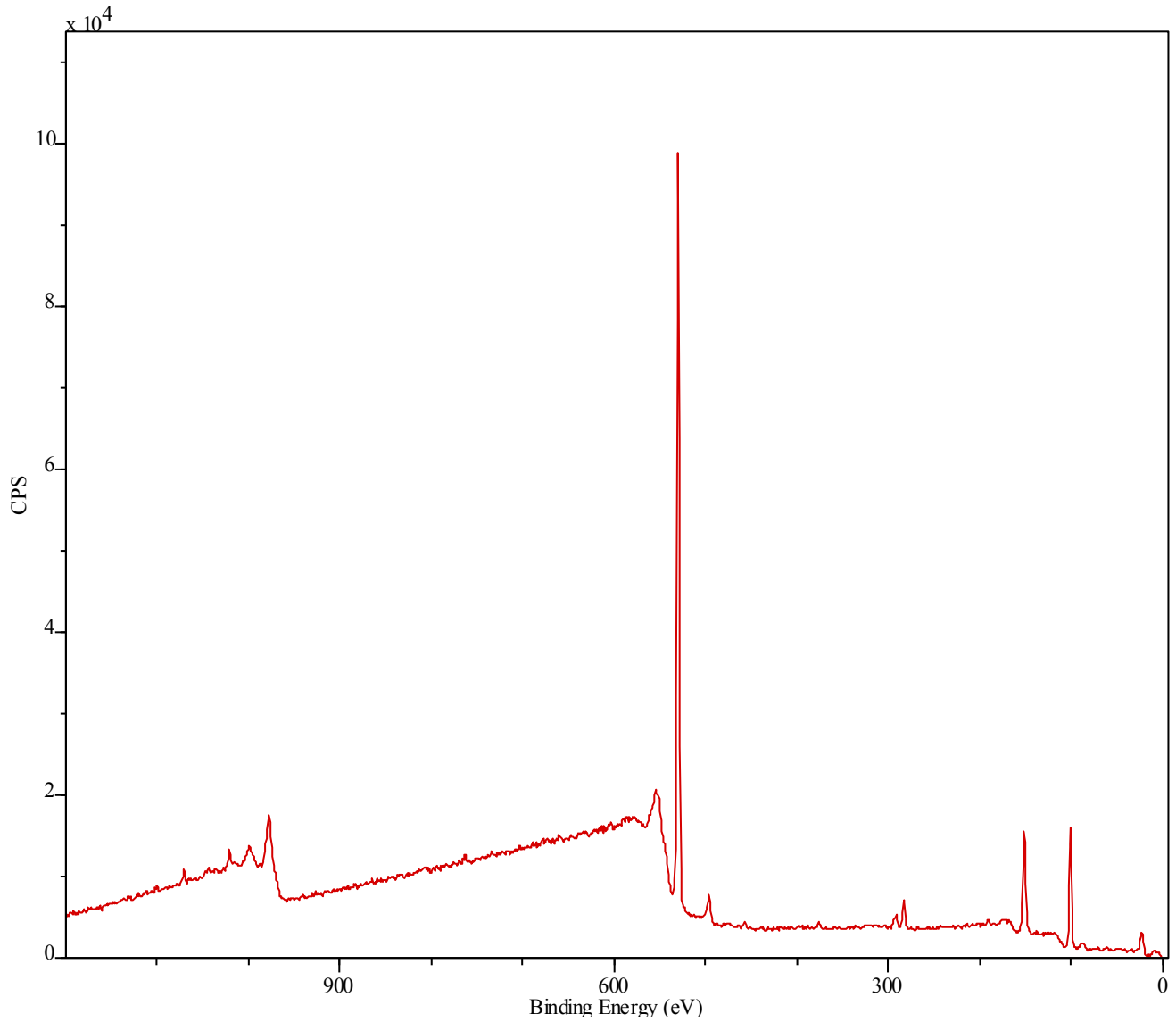

Figure S6. XPS survey spectrum for bare pristine glass surface without any Cr and Au coatings. The characteristic binding energy for Cr and Au is not detected.

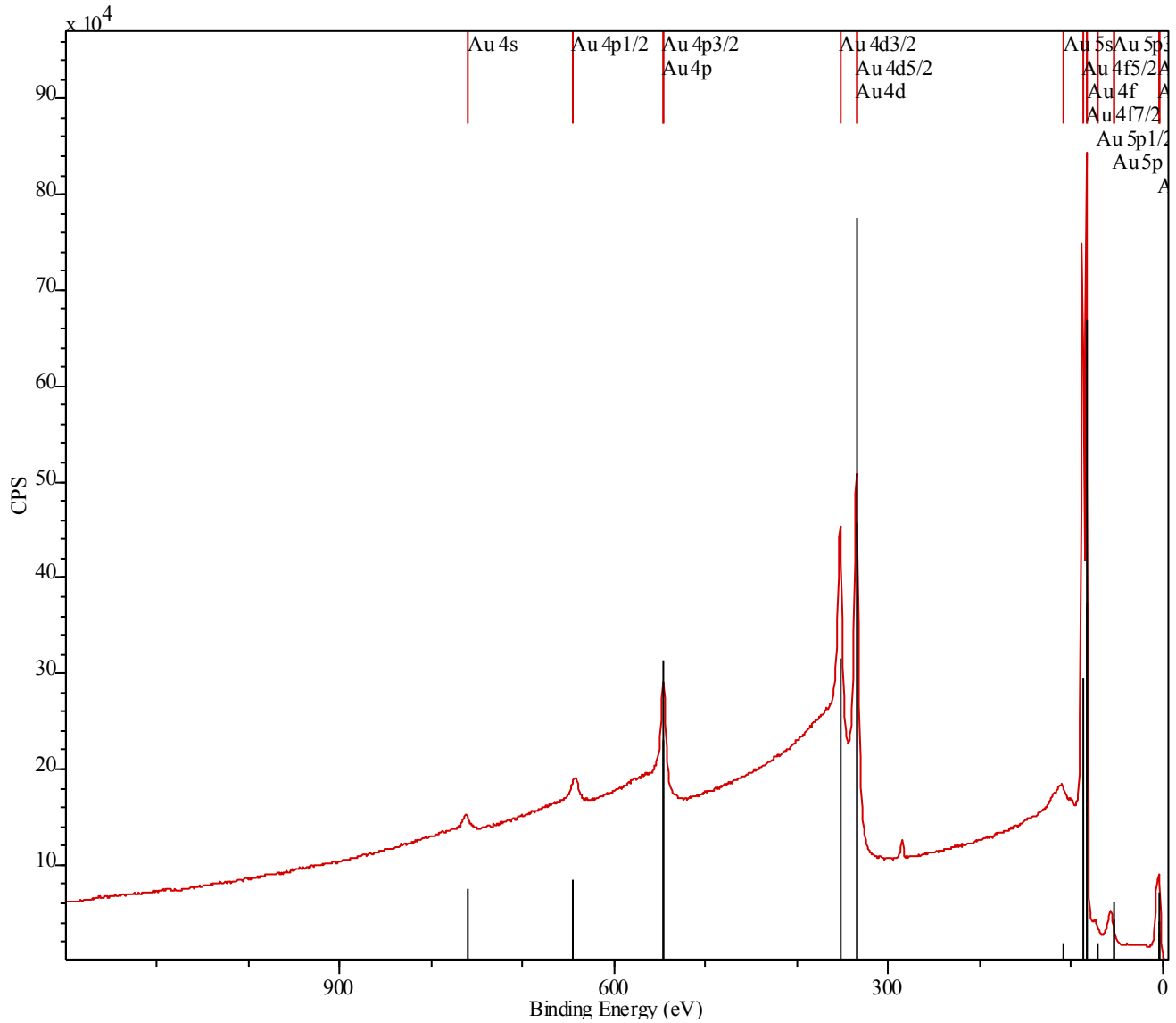

Figure S7. XPS survey spectrum for bare pristine gold surface before incubating in the etching solution containing certain amount of pyridine and NBS. The binding energy for Au is clearly observed which mainly locates at 334.91 and 545.91eV from Au4d and Au4f levels respectively. The binding energy from Cr layer that is beneath gold layer is not observable because 45nm-thick gold layer would prevent x-ray photoelectron emission from underlying Cr layer.

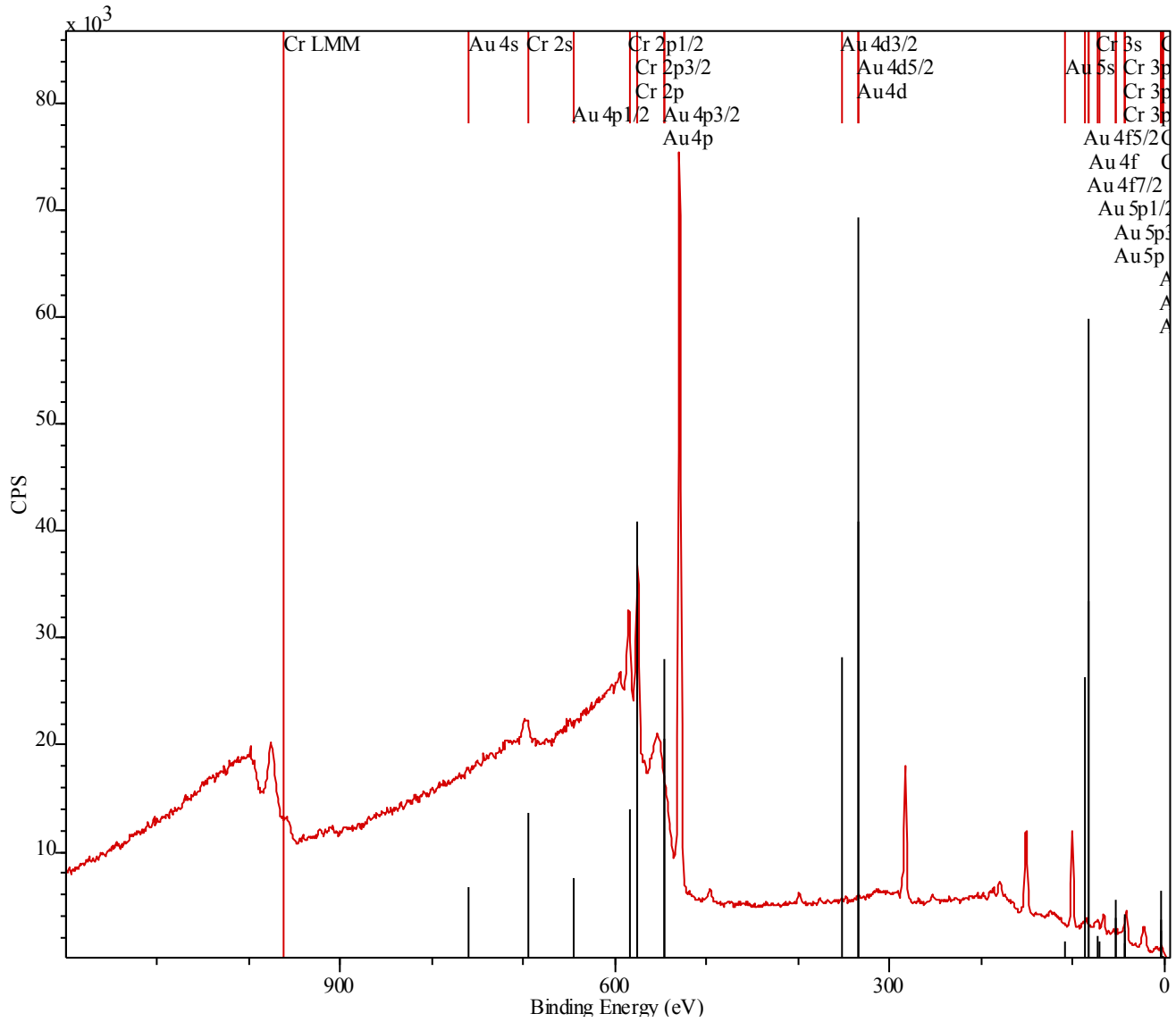

Figure S8. XPS survey spectrum for bare pristine gold surface after contacting with the etching solution containing certain amount of pyridine and NBS for 10min at room temperature. The binding energy for Au4d and Au4f levels at 334.91 and 545.91eV are diminished, reflecting the gold layer could be completely peeled off within 10min. As a result, because of the disappearance of gold shell layer on 5nm-thick Cr coating, the binding energy for Cr is observed mainly at 574.91 and 698.91eV from Cr2p and Cr2s respectively.

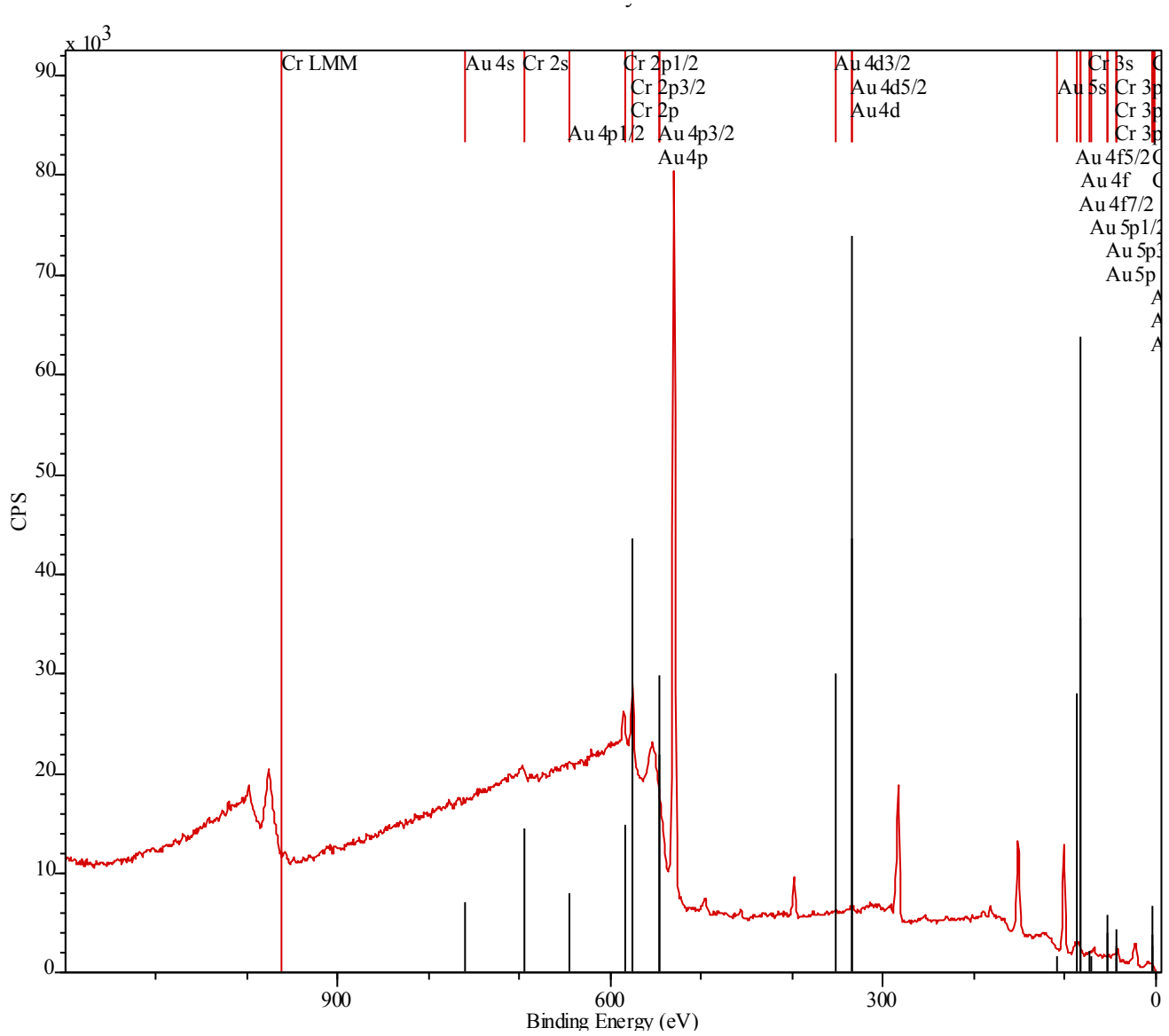

Figure S9. XPS survey spectrum for bare pristine gold surface after contacting with the etching solution containing certain amount of pyridine and NBS for 16hr at room temperature. The concentration of the etching solution used here is same as that used in Figure S3. It is found that under such condition, gold layer would be completely peeled off in 10min. Therefore, the present Figure shows that when the gold etching is complete, the further incubating in the etching solution for a relative long time (16hr) still results in the observable binding energy peaks from 5nm-thick Cr coating with slightly decreased intensity when comparing them with the binding energy signals from Cr layer after incubating in the etching solution for a short time (10min), indicating the etching from the etching solution would be largely suppressed on Cr layer.

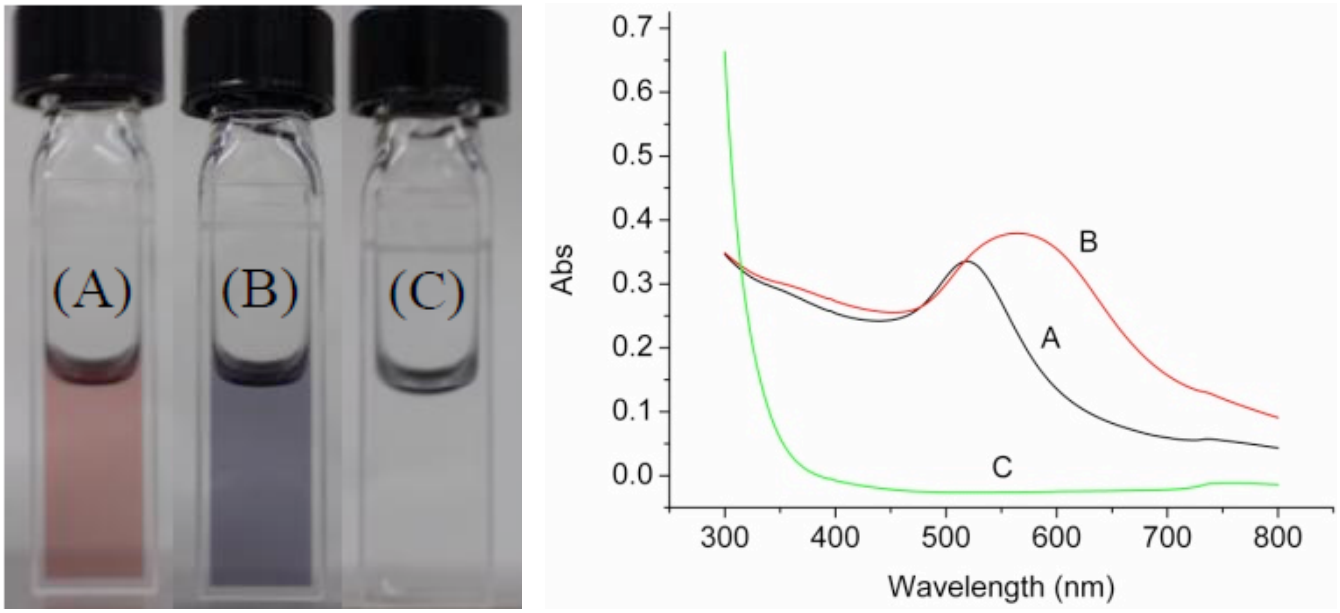

Figure S10. The optical images and UV-vis absorption spectra of gold colloidal solutions before (the equivalent Au atom concentration is 0.1 mM, A) and after sequentially adding pyridine (155 mM, B) and NBS (64.6 mM, C).

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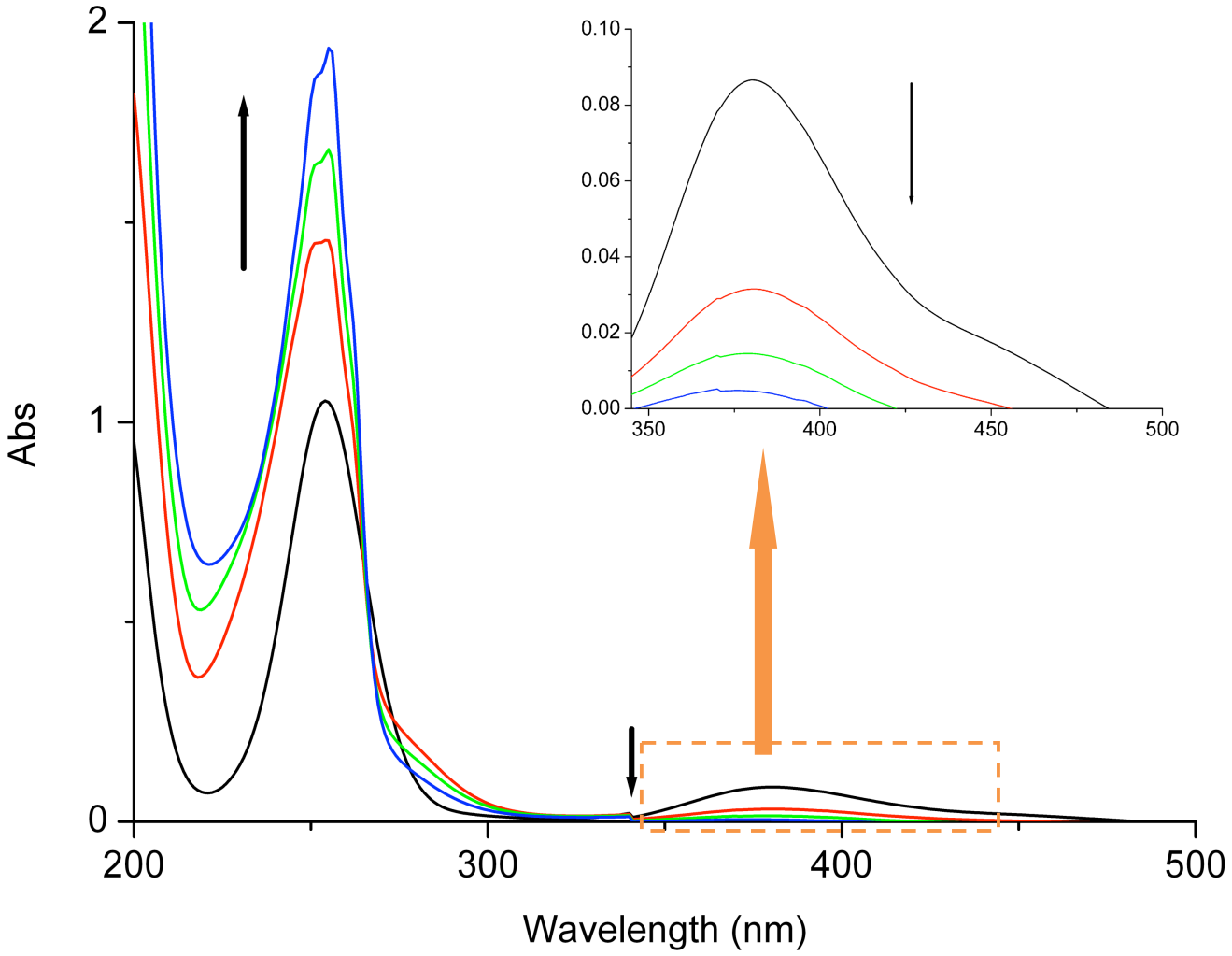

Figure S11. The UV-vis spectra of $HAuBr_4$ solution (0.025mM) before (black) and after adding different amount of pyridine: 12mM (red); 18mM (green) and 24mM (blue).

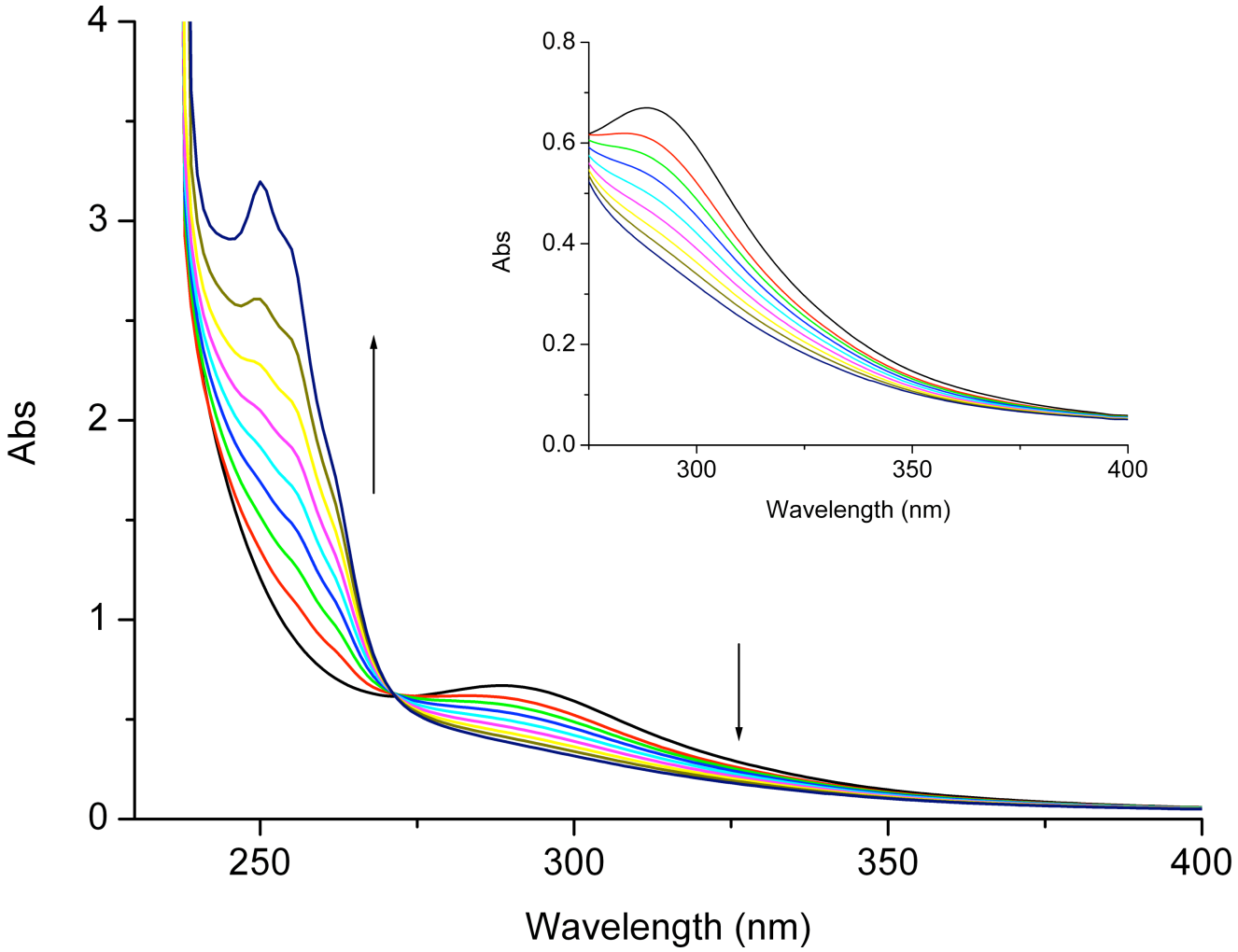

Figure S12. The UV-vis spectra of $HAuCl_4$ solution after adding different amount of pyridine: 0mM (black); 3mM (red); 6mM (green); 9mM (blue); 12mM (cyan); 15mM (magenta); 18mM (yellow); 21mM (dark yellow); 24mM (navy). The characteristic peak for $[AuCl_4]^-$ around 290nm is continuously decreased when pyridine concentration increases gradually. In contrast, the characteristic peak for pyridine around 250nm is enhanced gradually with pyridine concentration increasing.

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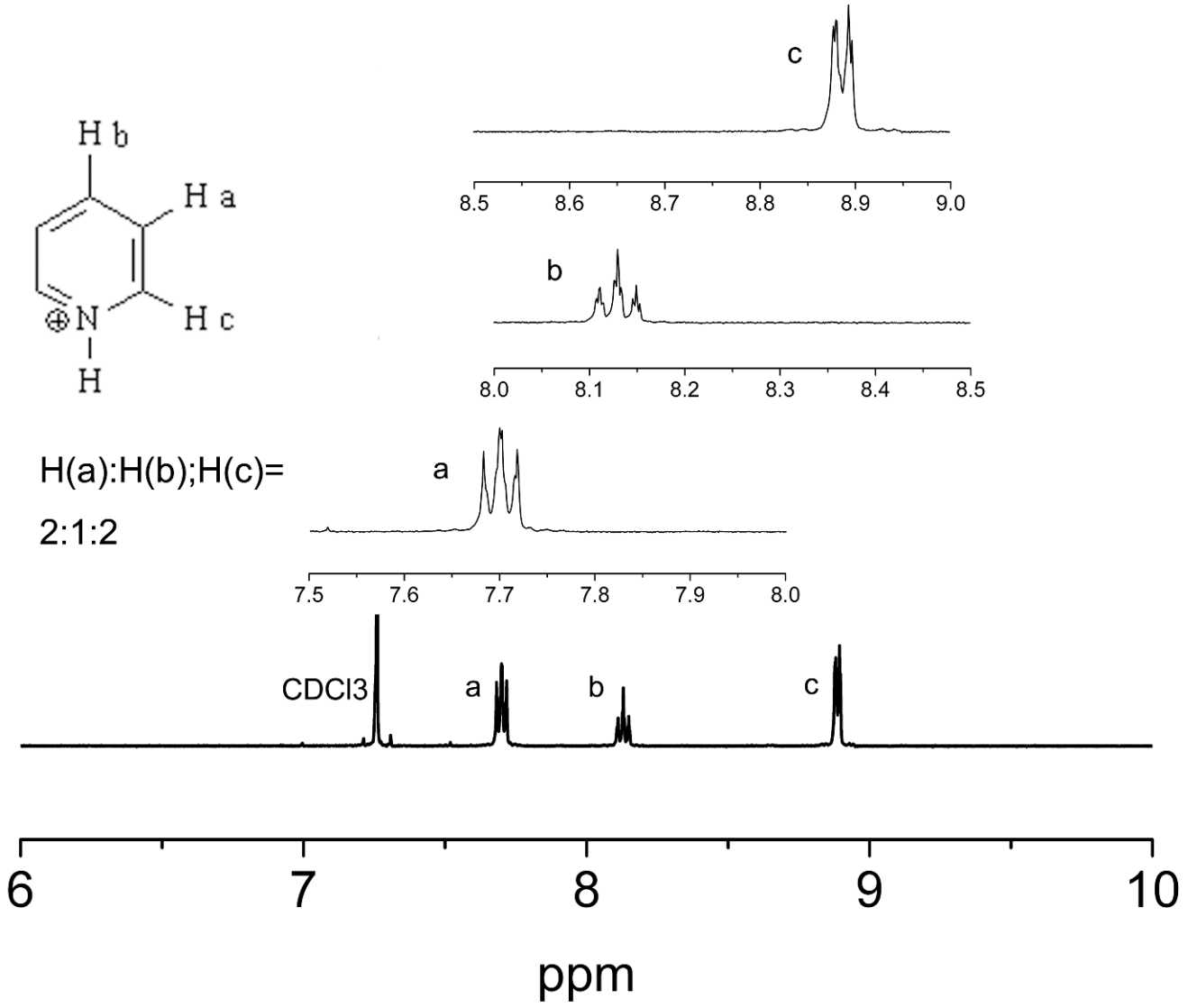

Figure S13. ^{1}HNMR spectra of the coordination complex [py($AuBr_3$)] formed between pyridine and $[AuBr_4]^-$. The solvent used in NMR is CDCl3.

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References

1. The detailed procedures for the preparation and characterization on these functionalized SAM-gold surface could be found in recent paper: P. Yang, S. Marinakos, A. Chilkoti, *Langmuir*, 2011, **27**, 1463.

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