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Nucleic acid-mediated gold oxidation: novel biolithography for surface microfabrication and new insight into gold-based biomaterials†

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In Nature, certain organisms can perform microbial corrosion on base metals by oxidation of neutral metallic atoms (H. L. Ehrlich, *Appl. Microbiol. Biotechnol.*, 1997). Herein we describe the first discovery of biological nucleic acids able to catalyze and mediate gold oxidation from neutral Au^0 to trivalent Au(III) under certain oxidative environments provided by mild oxidizing reagent *N*-bromosuccinimide or amino acids. A new biolithography technique for gold patterning is further developed.

The development of novel applications based on nucleic acids such as nucleic acid nanotechnology,[2] self-assembly and templating synthesis of nanoparticles, biosensors, bioactive coating, and antennas[3] is gaining increasing importance. In particular, the marriage between nucleic acids and gold has shown promising applications in a variety of fields such as biosensoring and gene delivery.[4] Two aspects that have been specifically noted in gold-based biotechnology are surface patterning of gold on the (sub)micron scale and understanding the interaction between gold and biomolecules. The former is important for the microfabrication of sensor arrays and electronics, and the latter could provide insight into sensing and biological activity evaluation of gold-based biomaterials.

For the first aspect, selective etching methods for gold have been developed.[5] The two fundamental components of a gold etchant include an oxidant to convert Au^0 atoms to Au ions and a ligand that binds the oxidized Au ions to decrease its redox potential.[6] Iodine,[5] oxygen or hydrogen peroxide,[7,8] Au^{3+}-surfactant (sodium dodecylbenzenesulfonate (SDBS)[9] or cetyl-trimethylammonium bromide (CTAB)),[10] and Aqua Regia[11] are typical oxidants, and cyanide, thiourea, thiosulfate as well as thiosulfonate are frequently used ligands.[6,12] These selective etching solutions open up a new path for the micro/nano-fabrication of gold 1D–3D structures *e.g.* patterned gold layers,[6,12] nanoparticles,[10] nanotubes,[13] nanorods,[14] and nanoplates.[9] A number of important drawbacks exist in these conventional chemical etching baths, which mainly include toxicity (*e.g.* thiourea and cyanide) and stability (*e.g.* thiosulfate).[12] For this reason, a new gold etchant system with extremely low toxicity and high durability is promising for practical industrial applications. Different from the conventional systems based on synthetical chemicals, this communication is the first report on biomolecules-based gold etching by the combinational use of nucleic acids and amino acids, providing a new bio-mimetic gold etching system to convert neutral Au^0 to Au(III) ions. Recently, Liu *et al.*[15] found that surface-adsorbed DNA regulated the etching of SiO_2 through tuning the concentration of surface-adsorbed water. The use of aqua-based biological molecules makes our method stand out as it is environmentally benign and highly durable.

For the second aspect, conventional opinions consider gold to have a superior chemical inertness, and to only show minute bio-dissolution when forming Au(I) that is partially oxidized to Au(III) in the presence of macrophages or amino acids of sweat and saliva.[16] It has thus never been recognized that nucleic acids could be involved in the oxidation process from Au^0 atoms to Au(III). The present results suggest that, under certain oxidative environments provided by synthetic or biological substances, the coordination of nucleic acids with Au(III)[17] has the potential to enhance a continuous dissolution of Au^0.

The gold-coated substrate consisted of a top gold layer, 45 nm thick, and a middle Cr adhesion layer, 5 nm thick, on a 1 mm glass coverslip. After incubating this substrate in the aqueous solution containing specific amounts of DNA (dsDNA or ssDNA) and *N*-bromosuccinimide (NBS) at room temperature overnight, the gold layer was found to peel off from the glass and to be subsequently diminished, thus forming a yellow dispersion (Fig. 1B and C). Finally, this process resulted in the complete peeling-off of the gold layer, leaving only the underlying Cr adhesion layer on the glass (*vide infra*). Patterned gold macro- and micro-structures such as macroporous gold films and gold micro-lines (Fig. 1D and E) could be obtained with the assistance of a porous rubber pad or a microfluidic technique (Fig. S1, ESI†). Compared with the conventional gold etching system, the present DNA–NBS system had a lower etching speed, probably due to the diluted concentration of the reactants, and thus a slower interaction time of nucleic acids with Au(III).[17] Further optimization with regard to reaction conditions such as the concentration, DNA structures, nucleic acid sequence could be applicable to enhance the etching speed.

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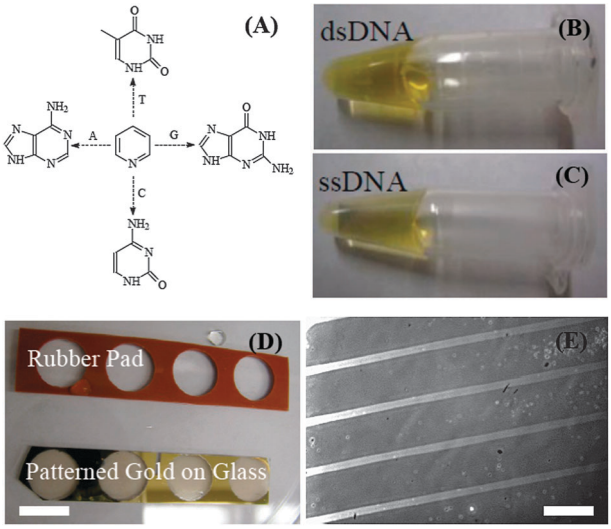


Fig. 1 The structures of four kinds of bases in DNA (A) having pyridine-like derivatives for NBS oxidation on a gold layer. As a result, a yellow solution was obtained after incubating gold-coated glass substrates overnight at room temperature in the solution containing 100 μM DNA (either dsDNA, B or ssDNA, C) and 14.6 mM NBS. This method is also applicable for the patterning of gold layers on glass substrates with macro/micro-resolutions by filling the etching solution into a rubber pad (D) or microfluidic channels (E) on a gold surface. The scale bars in (D) and (E) are 1 cm and 10 μm, respectively.

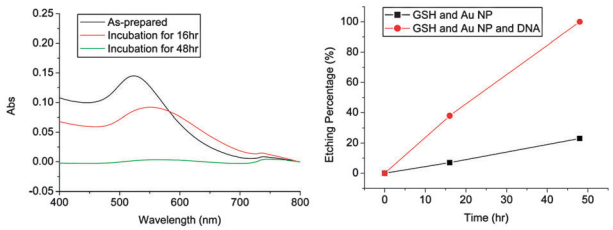


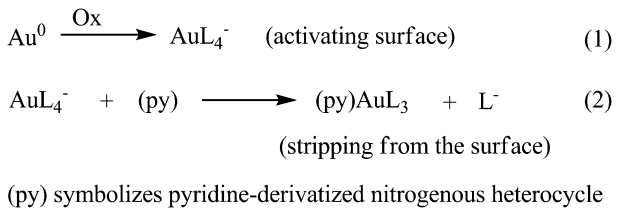
Fig. 2 The UV-vis spectra of gold colloidal solution containing GSH and DNA. The equivalent Au atom concentration in the solution is 0.1 mM, the GSH concentration is 100 mM, and the λ-DNA (48 502 bp) concentration is 5.93×10^{-4} μM. From the left figure, it is concluded that the characteristic peak for gold nanoparticles decreases gradually with incubation time prolonging. Defining the absorption peak value of the as-prepared solution, the solution after incubation for time t (h) as Abs(as-prepared) and Abs(t), respectively, the etching percentage is then calculated as [Abs(as-prepared) − Abs(t)]/Abs(as-prepared), and plotted in the right figure. It is found that compared with the slow etching process (black curve) from GSH on gold nanoparticles (Au NPs), the addition of DNA would accelerate largely the etching rate of Au NP (*ca.* five-fold increases, red curve).

It is further found that this newly found DNA-mediated etching could be similarly applicable in the oxidation environment different from that provided by NBS. Inspired by the *in vivo* oxidation of gold materials based on biothiols such as thiol-based amino acids, we infer that instead of NBS, the combinational use of amino acids and DNA would also produce notably oxidation on gold materials. Previous studies have shown that thiol-containing amino acids such as glutathione (GSH) only oxidize neutral gold colloids in a very slow speed (over a month).[18] In contrast, when we used the mixed solution of GSH and DNA to oxidize gold colloids, the oxidation precipitations typically found by mere use of GSH oxidation for a long period could be observed in a substantially shortened time (16–48 h). Microscopic UV spectra further revealed that the oxidation rate of GSH–DNA was accelerated largely (*ca.* 5-fold higher) due to the catalysis and mediation ability of DNA (Fig. 2). Similarly, the oxidation of the gold film coating on the glass substrate by GSH–DNA was also successfully conducted (Fig. S2, ESI†).

Control experiments involving the separate use of NBS or DNA did not give rise to etching on the gold surface. Oxygen did not play a key role in this process since similar experimental results for gold etching by NBS–DNA or GSH–DNA were also obtained under oxygen-free conditions (Fig. S3, ESI†). The pH value also did not affect the etching process substantially, because the initial pH values did not change obviously with the etching process proceeding, and the etching on gold nanoparticles by GSH–DNA at different initial pH values showed similar results (Fig. S4, ESI†). The catalytic role of a pyridine unit in DNA as a kind of potential *tert*-amine to accelerate the release of Br^- from NBS was not obvious in the present experimental window (0–48 h), showing insufficient changes in the UV-vis spectra of NBS dissolved in the buffer with or without DNA (Fig. S5, ESI†).

We considered that there was a cooperative role of these two compounds in the etching. We correlated the role of nucleic acids to that of its four kinds of nucleobases A, C, G, T (Fig. 1A). Actually, it has been shown that nitrogen atoms in A, C, G, T nucleobases can bind with metal ions such as trivalent Au(III) for templating synthesis, self-assembly of metallic nanoparticles and photosensitization.[17,19] The initial oxidation of neutral gold atoms to Au(III) was conducted by NBS[20] or biothiols.[18] For example, in the NBS oxidative process, bromine molecules released from NBS could attack the gold atoms to form $[AuBr_4]^-$. We therefore postulate that in our system, Au(III) produced by NBS or biothiol oxidation could bind with DNA to decrease the redox potential, as a result, continuous dissolution of gold atoms is facilitated.

In order to support the above hypothesis, we replaced DNA with a model compound, pyridine, to simplify the DNA–NBS oxidation system for the mechanism investigation, since nucleic acids could be considered as chains carrying pyridine-derivatized nitrogenous heterocycles. As expected, when immersing a gold-coated glass into an aqueous solution of pyridine and NBS, a similar oxidative etching was observed but with a much faster etching speed. X-ray Photoelectron Spectroscopy (XPS) (Fig. S6–S9, ESI†) demonstrated that the Au4d and Au4f binding energy peaks at 335 and 546 eV completely disappeared after incubating the substrate in the etching solution for 10 min. Upon addition of pyridine to a freshly prepared gold colloidal solution, the UV absorption peak red-shifted to 570 nm from initial 520 nm due to a pyridine-induced aggregation,[21] accompanied with the color change from burgundy[20] to blue (Fig. S10, ESI†). When NBS was further sequentially added to this solution, the solution became colorless quickly and the corresponding electronic spectra showed zero absorption until 300 nm, reflecting that the reaction finished with formation of a poorly water-soluble coordination complex [py(AuX_3)] between pyridine (py) and AuX_4 (X = Cl, Br).[22] This judgement was confirmed by a



Scheme 1 The plausible reaction mechanism for the etching reaction on a gold surface mediated by pyridine-like units.

separate experiment where a diluted $HAuX_4$ (X = Cl, Br) solution was added to an excess amount of pyridines. The characteristic peaks of $HAuX_4$ were gradually quenched (Fig. S11 and S12, ESI†). When an excess amount of pyridine was mixed with a concentrated $HAuBr_4$ solution, a precipitate was visually collected that was quantitatively consistent with the molecular formula [py($AuBr_3$)] based on yield calculation, ^{1}HNMR (Fig. S13, ESI†) and molecular mass measurement. These findings indicated a general mechanism for pyridine-derivatives mediated gold oxidation as depicted in Scheme 1. The standard oxidation potential for gold oxidation by NBS ($Au^0 + 4Br^- - 3e^- \rightarrow AuBr_4^-$) is −0.854 V.[20] This redox tendency is favourable for a spontaneous reaction between Br_2 and Au^0 to form AuL_4^-. By this reaction, the surface gold atom is firstly activated by the oxidizer (NBS or biothiol), and the resulting AuL_4^- effectively coordinates with the surrounding pyridine-derivatized unit (py) to form Au(py)L_3 and releases a free L anion. The binding constant of AuL_4^- with nucleobases is estimated to be 10^5–10^6 M.[23] This energy-favourable reaction facilitates the existing redox equilibrium to shift more to the right side, catalysing and accelerating the oxidation reaction. Consequently, a continuous stripping of $[AuL_4]^-$ (L is a ligand) enriched on the oxidized gold surface and the subsequent exposure of a fresh surface for further oxidation is created.

In conclusion, the present research involves an important and interesting fundamental finding: nucleic acids could mediate and catalyze the oxidative etching process of gold materials. Surface patternable gold macro–micro-structures can be obtained with the assistance of a porous rubber pad or a microfluidic technique. Based on this newly found phenomenon, an all-biomolecule-based etching system is developed by the combinational use of DNA and amino acids. This biomimetic finding differs significantly from conventional gold etching systems where strong and toxic oxidizing as well as ligand reagents or harsh conditions are required. It is the first controlled biolithography system to use nucleic acids to catalyze and mediate the oxidative etching of metals in mild aqueous solutions at room temperature.

This research is expected to find novel and significant fundamental meaning in single-molecular lithography, DNA metallization, DNA-directed templating synthesis and self-assembly of nanoparticles, bioelectronics, and biosensors. Since it is found that the gold oxidation by thiol-based amino acids is a main route for bio-dissolution of gold in biological bodies, our findings on these new functions of nucleic acids should also shed important light on *in vivo* or *in vitro* biomimetic metal material synthesis and the biological activity of gold materials when they are used as drug delivery carriers in biological bodies.

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Notes and references

1 H. L. Ehrlich, *Appl. Microbiol. Biotechnol.*, 1997, **48**, 687.
2 N. C. Seeman, *J. Theor. Biol.*, 1982, **99**, 237; N. C. Seeman, *Annu. Rev. Biophys. Biomol. Struct.*, 1998, **27**, 225; J. Nangreave, D. Han, Y. Liu and H. Yan, *Curr. Opin. Chem. Biol.*, 2010, **14**, 608.
3 G. Tikhomirov, S. Hoogland, P. E. Lee, A. Fischer, E.-H. Sargent and S. O. Kelley, *Nat. Nanotechnol.*, 2011, **6**, 485; D. Nykypanchuk, M. M. Maye, D. van der Lelie and O. Gang, *Nature*, 2008, **451**, 549; S. Y. Park, A. K. R. Lytton-Jean, B. Lee, S. Weigand, G. C. Schatz and C. A. Mirkin, *Nature*, 2008, **451**, 553; Y. Hatakeyama, M. Umetsu, S. Ohara, F. Kawadai, S. Takami, T. Naka and T. Adschiri, *Adv. Mater.*, 2008, **20**, 1122; A. Rotaru, S. Dutta, E. Jentzsch, K. Gothelf and A. Mokhir, *Angew. Chem., Int. Ed.*, 2010, **49**, 5665; K. Suzuki, K. Hosokawa and M. Maeda, *J. Am. Chem. Soc.*, 2009, **131**, 7518.
4 G. J. Hutchings, M. Brust and H. Schmidbaur, *Chem. Soc. Rev.*, 2008, **37**, 1759; L. Xu and D. Liu, *Chem. Soc. Rev.*, 2010, **39**, 150; A. E. Nel, L. Mädler, D. Velego, T. Xia, E. M. V. Hoek, P. Somasundaran, F. Klasessig and M. Thompson, *Nat. Mater.*, 2009, **8**, 543.
5 K. R. Williams, K. Gupta and M. Wasilik, *J. Microelectromech. Syst.*, 2003, **12**, 761.
6 D. Burdinski and M. H. Blees, *Chem. Mater.*, 2007, **19**, 3933.
7 N. R. Jana, L. Gearheart, S. O. Obare and C. J. Murphy, *Langmuir*, 2002, **18**, 922.
8 X. Kou, W. Ni, C.-K. Tsung, K. Chan, H.-Q. Lin, G. D. Stucky and J. Wang, *Small*, 2007, **3**, 2103.
9 Y. Cheng, C. Qiu, H. Ma, X. Zhang and X. Gu, *Nanoscale*, 2010, **2**, 685.
10 J. Rodríguez-Fernández, J. Pérez-Juste, P. Mulvaney and L. M. Liz-Marzán, *J. Phys. Chem. B*, 2005, **109**, 14257.
11 R. W. Stark, M. S. Stalder and A. Stemmer, *Microelectron. Eng.*, 2003, **67–68**, 229.
12 Y. Xia, X. M. Zhao, E. Kim and G. M. Whitesides, *Chem. Mater.*, 1995, **7**, 2332; A. Kumar, H. A. Biebuyck and G. M. Whitesides, *Langmuir*, 1994, **10**, 1498.
13 S. O. Obare, N. R. Jana and C. J. Murphy, *Nano Lett.*, 2001, **1**, 601.
14 C.-K. Tsung, X. Kou, Q. Shi, J. Zhang, M. H. Yeung, J. Wang and G. D. Stucky, *J. Am. Chem. Soc.*, 2006, **128**, 5352.
15 S. P. Surwade, S. Zhao and H. Liu, *J. Am. Chem. Soc.*, 2011, **133**, 11868.
16 A. Larsen, K. Kolind, D. S. Pedersen, P. Doering, M. Ø. Pedersen, G. Danscher, M. Penkowa and M. Stoltenberg, *Histochem. Cell Biol.*, 2008, **130**, 681; A. S. Thakor, J. Jokerst, C. Zavaleta, T. F. Massoud and S. S. Gambhir, *Nano Lett.*, 2011, **11**, 4029.
17 C. K. S. Pillai and U. S. Nandi, *Biopolymers*, 1973, **12**, 143l; D. W. Gibson, M. Beer and R. J. Barrnett, *Biochemistry*, 1971, **10**, 3669; N. Sundaresan, C. H. Suresh, T. Thomas, T. J. Thomas and C. K. S. Pillai, *Biomacromolecules*, 2008, **9**, 1860; R. J. Wilkins, *Nucleic Acids Res.*, 1978, **5**, 3731.
18 W. S. Rapson, *Contact Dermatitis*, 1985, **13**, 56.
19 L. Berti and G. A. Burley, *Nat. Nanotechnol.*, 2008, **3**, 81.
20 S. Singh and B. L. V. Prasad, *J. Phys. Chem. C*, 2007, **111**, 14348.
21 C. G. Blatchford, J. R. Campbell and J. A. Creighton, *Surf. Sci.*, 1982, **120**, 435; P. Galletto, P. F. Brevet, H. H. Girault, R. Antoine and M. Broyer, *J. Phys. Chem. B*, 1999, **103**, 8706; T. J. Norman Jr, C. P. Grant, D. Magana, J. Z. Zhang, J. Liu, D. Cao, F. Bridges and A. Van Buuren, *J. Phys. Chem. B*, 2002, **106**, 7005.
22 L. Cattxlini, M. Sicolisi and A. Orio, *Inorg. Chem.*, 1966, **5**, 1674; L. Cattalini and M. L. Tobe, *Inorg. Chem.*, 1966, **5**, 1145; L. Cattalini, V. Ricevuto, A. Orio and M. L. Tobe, *Inorg. Chem.*, 1968, **7**, 51.
23 C. Mandal and U. S. Nandi, *Proc.- Indian Acad. Sci., Sect. A*, 1979, **88**, 263.