Nanoplasmonics

DOI: 10.1002/anie.201105121

Catalytic Gold Nanoparticles for Nanoplasmonic Detection of DNA Hybridization**

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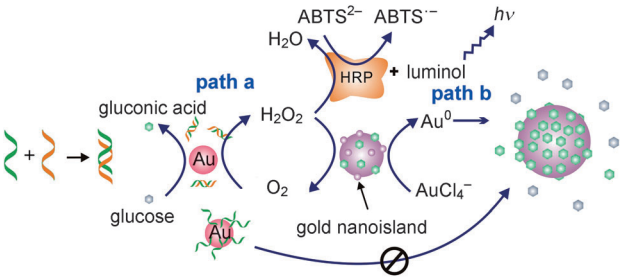
Gold nanoparticles (AuNPs) possess many attractive optical and electronic properties that have proven to be of high utility in biomedical applications.[1] In particular, biomolecular detection methods have been developed by exploiting surface plasmon resonance (SPR),[2] Raman,[3] fluorescence,[4] and conductance[5] properties of AuNPs. However, there is another largely ignored region, that is, the catalytic activity of AuNPs.[6] Although bulk gold is generally considered to be chemically inert, substrate-supported AuNPs have been known to possess surprisingly high catalytic activities toward the oxidation of CO or NO.[7] Recently, colloidal AuNPs were found to exhibit glucose oxidase (GOx)-like activity.[8,9] Our previous work revealed that this enzyme-like activity of AuNPs was extremely sensitive to surface properties, which led to the design of a self-limiting growth system.[9] We speculate that these unprecedented findings open a new avenue toward biological applications with catalytic AuNPs.

AuNPs interact with biomolecules in various ways, based on which hybrid nanobiomaterials with synergetic properties and functions have been developed.[10] DNA–AuNP conjugates that rely on gold–sulfur chemistry is an elegant example,[11] which has become an important building block for a broad spectrum of bioassays,[12] nanostructures, and nanodevices.[13] Alternatively, noncovalent interactions between as-prepared AuNPs and DNA strands have been actively exploited to detect DNA hybridization and aptamer-binding reactions.[14] As unstructured, single-stranded (ss-) DNA noncovalently binds to as-prepared AuNPs much more rapidly and strongly than structured, double-stranded (ds)-DNA,[15] DNA hybridization can effectively tune the stability of AuNPs in a high-concentration salt solution. Herein, we aim to amplify these noncovalent interactions by using catalytic AuNPs, and develop a new nanoplasmonic probe for biomolecular detection.

As-prepared AuNPs can catalytically oxidize glucose in the presence of O_2, producing gluconic acid and H_2O_2 [Scheme 1, Eq. (1)]:[8]

$$\text{glucose} + O_2 \xrightarrow{\text{AuNPs}} \text{gluconic acid} + H_2O_2 \quad (1)$$



Scheme 1. Illustration of the GOx-like catalytic activity of AuNPs regulated by DNA hybridization, which can be either amplified by HRP-cascaded color or chemiluminescence variations (path a) or lead to nanoplasmonic changes owing to size enlargement (path b). Orange strand = target, green strand = adsorption probe.

When this catalytic reaction is coupled with horseradish peroxidase (HRP)-based enzymatic catalysis, this cascade reaction results in a characteristic blue color owing to the oxidation of a cosubstrate of $ABTS^{2-}$ (ABTS = 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) by HRP [Eq. (2); Supporting Information, Figure S1]:

$$H_2O_2 + ABTS^{2-} \xrightarrow{\text{HRP}} H_2O + ABTS^{\bullet-} \quad (2)$$

Given that the GOx-like activity of as-prepared AuNPs is extremely sensitive to surface passivation,[9] we attempted to investigate variation of the catalytic activity of AuNPs upon interaction with ss-DNA (adsorption probe **1**) and ds-DNA (probe **1**/target **3** (Figure 1 A). When **1** was first hybridized with the fully complementary DNA **3** to form a duplex and then subjected to the cascade reaction, we observed the appearance of the characteristic blue color. Interestingly, if AuNPs were first mixed with ss-DNA followed by the HRP cascade reaction, the color change was largely attenuated, suggesting an effective suppression of the cascade reaction by

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[**] This work was supported by the National Basic Research Program of China (973 program, 2012CB932600, 2007CB936000), the National Natural Science Foundation of China (20805055, 20873175, 21105028, 21075128, and 20725516), and 2007AA06A406, the Shanghai Municipal Commission for Science and Technology (10A1408200), and the Chinese Academy of Sciences (KJCX2-EW-N03).

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201105121.

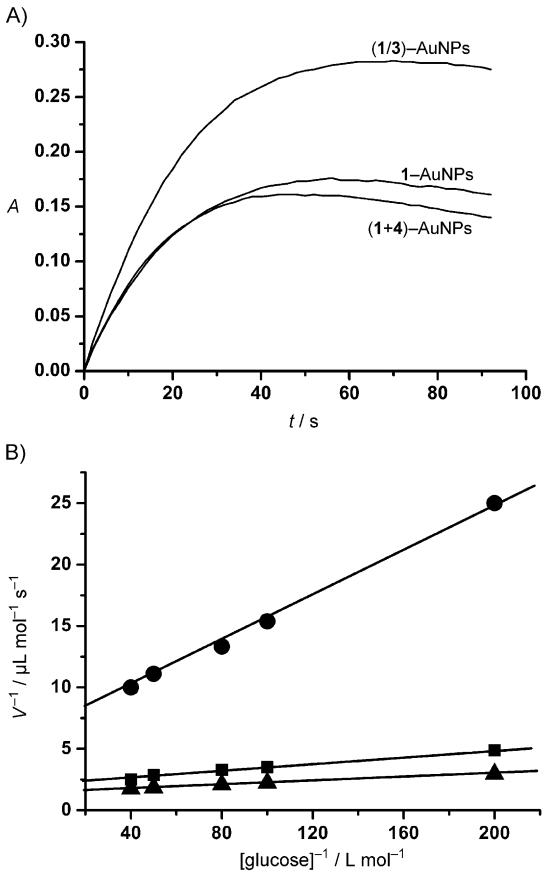

Figure 1. A) Time-dependent changes to absorbance of $ABTS^{\bullet-}$ upon treating bare AuNPs (5 nM) with ds-DNA (**1**/**3**, 1 μM each), or ss-DNA alone (**1**, 1 μM), or mixture of ss-DNA (**1** and **4**, 1 μM each) for 5 min. B) Kinetics study of bare AuNPs (▲), ss-DNA–AuNPs (●), and ds-DNA–AuNPs (■). The reaction rate *V* is defined as the amount of generated of gluconic acid in a fixed reaction time of 30 min. The concentration of AuNPs was 15 nM and the concentrations of ss- and ds-DNA were 0.75 μM.

ss-DNA. As AuNPs were removed by ultracentrifugation before the addition of HRP, this suppression should solely arise from the binding of ss-DNA to the surface of AuNPs. This remarkable difference reflects the different adsorption ability of ss- and ds-DNA to as-prepared AuNPs, a phenomenon consistent with previous observations in salt-stability-based colorimetric assays.[15] In a control experiment, we challenged AuNPs with a mixture of probe **1** and a non-cognate DNA strand **4**, the solution was also less intensively blue as in the case of probe **1** alone, suggesting that the sequence-specific formation of DNA duplex is critical for regulating the catalysis of AuNPs. In this control, the absorption intensity was even lower than that of probe **1** alone. This is because the additional non-cognate ss-DNA **4** further increased the coverage of ss-DNA on the surface of AuNPs. Therefore, ss-DNA can adsorb strongly to the surface of AuNPs, resulting in deactivated catalysis, while ds-DNA binds weakly to AuNPs and only slightly perturbs the catalytic activity of AuNPs.

We further performed kinetic studies by using the Michaelis–Menten model to provide a quantitative measurement of this hybridization-regulated catalytic reaction. Figure 1B illustrates the double-reciprocal plots for the as-prepared AuNPs, ss-DNA-AuNPs, and ds-DNA-AuNPs. AuNPs were centrifuged to prevent possible influence of the color of AuNPs to the colorimetric reaction. Significantly, the values for the Michaelis–Menten constant (K_m) and the catalytic constant (k_{cat}) of ss-DNA–AuNPs was about three times larger and about four times lower than that of as-prepared AuNPs, respectively (Table 1). The decrease in both thermodynamic affinity (increased K_m) and kinetics (decreased k_{cat}) implies that the catalysis of AuNPs toward glucose is suppressed by binding with ss-DNA. In contrast, the K_m of ds-DNA–AuNPs was only slightly increased as compared to as-prepared AuNPs, and the corresponding k_{cat} was decreased by about 25%, suggesting that the activity of as-prepared AuNPs was only slightly perturbed by ds-DNA.

Given that DNA hybridization can specifically switch on the catalytic activity of AuNPs, we were motivated to design a catalytic AuNP-based strategy for DNA analysis (Scheme 1, path a). We reason that the catalytic activity of AuNPs, as well as its coupled cascade amplification, can be finely regulated by DNA hybridization, providing a quantitative measurement for target DNA. The HRP-based colorimetric or chemiluminescent (CL) detection led to limit of detection (LOD) of 14 nM and 0.75 nM, respectively (Supporting Information, Figure S2,S3). This catalytic AuNP-based strategy can be employed to detect microRNAs (miRNAs; Supporting Information, Figure S4), a class of short (19–24 bases), endogenous non-coding RNAs with promising applications in clinical diagnostics.[16,17] By using probe DNA **2** that was complementary to hsa-let-7e, which is a target belonging to the let-7 miRNA family that has been known to be associated with tumors,[18,19] we found that hsa-let-7e could be quantified by using the catalytic AuNP-based colorimetric measurements (Supporting Information, Figure S4A), leading to a LOD of 8 nM ($>3\sigma$). Significantly, we found that the specific hsa-let-7e exhibited significantly higher color intensity than all other miRNAs (Supporting Information, Figure S4B).

The same strategy was adaptable to aptamer-based assays, as aptamers often undergo significant structural variations from unstructured random coils to rigid structures upon target

Table 1: Kinetic parameters of as-prepared 13 nm AuNPs, ss-DNA–AuNPs, and ds-DNA–AuNPs with glucose substrate.[a]

	[AuNP] [nmol L^{-1}]	K_m [mmol L^{-1}]	V_{max} [μL mol s^{-1}]	k_{cat} [s^{-1}]	k_{cat}/K_m [mL mol^{-1} s^{-1}]
bare AuNPs	15	4.73 ± 0.37	0.68 ± 0.03	47.33 ± 2.00	9.99
ss-DNA–AuNPs	15	17.67 ± 2.07	0.18 ± 0.04	12.00 ± 2.00	0.68
ds-DNA–AuNPs	15	6.98 ± 0.69	0.53 ± 0.04	35.33 ± 2.67	5.06

[a] K_m = Michaelis–Menten constant, k_{cat} = catalytic constant, V_{max} = maximum reaction rate.

binding.[14e,20,21] A K^+-specific G quartet **7** was employed to demonstrate the possibility of using catalytic AuNPs in aptamer-based assays (Supporting Information, Figure S5). Similar to DNA hybridization, we found that this binding process recovered the catalytic activity of AuNPs, as manifested by the intensified color change in the presence of K^+. This assay could detect as low as 0.5 mM of K^+ with high selectivity over other monovalent cations (Li^+, Na^+, Rb^+, NH_4^+).

Apart from the cascade reaction with HRP, the catalytic AuNPs can be coupled with the AuNP-mediated seed growth in the presence of $HAuCl_4$ (Scheme 1, path b). The H_2O_2 generated in situ reduces $HAuCl_4$ to Au^0, which is deposited on the surface of AuNP seed, resulting in gradual size enlargement of AuNPs.[9] AuNP-seeded growth significantly alters the localized SPR (or nanoplasmonic) properties of AuNPs, which has been exploited to develop high-sensitivity bioassays and cellular/tissue imaging.[22] AuNPs possess intense SPR-enhanced light scattering,[23] and the true color of the light scattered from a single AuNP can be visually detected by using dark-field illumination. Dark-field microscopy (DFM) provides a powerful means to directly image size and shape variations of AuNPs in real time and at the single-nanoparticle level.[24] The advantage of DFM to track single plasmonic NPs facilitates its use as labels in bioassays.[25] Particularly, multiplex targets detection and in vivo imaging using plasmonic NPs of different colors are of great promise for applications.[26] Simultaneously recorded Rayleigh scattering spectra also provides a mechanistic understanding of the AuNP growth system. In our DFM setup, the optical resolution allows the tracking of single AuNPs with a minimum size of 50 nm in diameter.

As shown in Figure 2 A1, bare AuNPs of 50 nm that were deposited on silanized glass slides exhibited green scattering light with the maximum wavelength at about 570 nm. It is worth noting that, while the majority of AuNPs on a slide were of a green color, there were also AuNPs of other colors, reflecting the heterogeneous size distribution of chemically synthesized AuNPs. Importantly, since the positions of AuNPs were fixed on silanized slides, it is possible to track single AuNP with DFM and obtain reliable imaging information. Upon the addition of glucose and $HAuCl_4$, which activates the growth process, a gradual color change that resulted in red scattering light was observed after 25 min, with the scattering peak intensity enhanced and red-shifted to 675 nm (Figure 2 A2). This result suggests that bare AuNPs are enlarged during the coupled reaction (Scheme 1, path b), which coincides well with our observations in solution-phase studies.[9] The presence of ss-DNA (probe **1**) largely suppressed the growth of AuNPs (Figure 2 B1,B2), with minimum change in both DFM imaging and scattering spectra, which further confirms that ss-DNA adsorbs on AuNPs and blocks its catalytic activity. Significantly, hybridization of the probe **1** with target **3** could switch on the growth of AuNPs. DFM clearly showed that the color and brightness change of ds-DNA–AuNPs after the coupled growth reaction (Figure 2 C1,C2). While the peak shift and intensity are smaller than that of as-prepared bare AuNPs, they are significantly larger than that of ss-DNA-AuNPs, confirming that ds-DNA

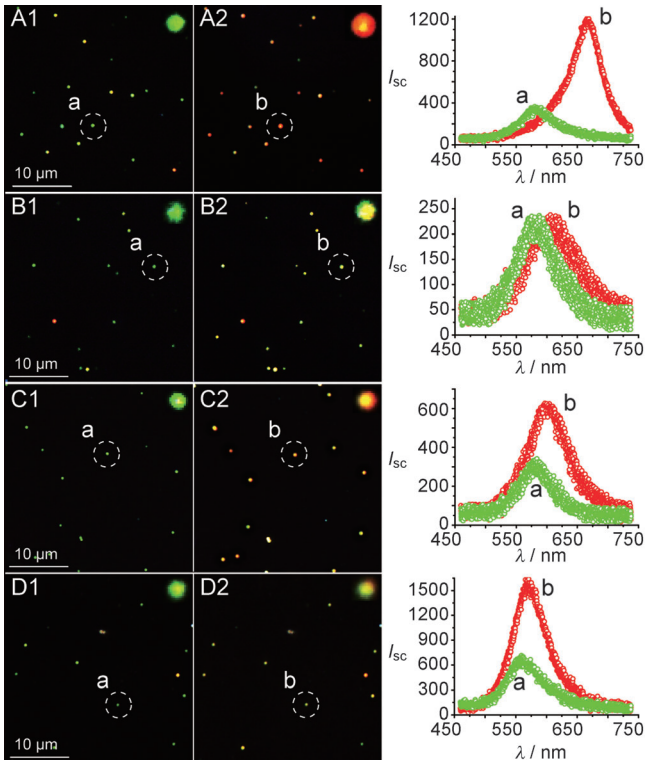

Figure 2. Left: Dark-field microscopy (DFM) images of large AuNPs (50 nm in diameter) before (1) and after (2) the 25 min glucose-induced enlargement process. A) Bare AuNPs, B) ss-DNA–AuNPs, C) ds-DNA–AuNPs, D) ds–DNA with 1-base mismatches interacting with AuNPs. Right: The light scattering spectra corresponding to individual nanoparticle marked with the circle in the DFM images. I_{sc} = scattering intensity. See the text for further details.

adsorbs on AuNPs with much weaker affinity than ss-DNA. Meanwhile, DFM images of probe **1** with single-base mismatched DNA samples **5** exhibited a slight color variation and moderate brightness change after the enlargement reaction (Figure 2 D1,D2), implying that the catalysis-based nanoplasmonic Au nanoprobe can identify DNA targets with high specificity. The DNA regulated growth of AuNPs was also monitored by SEM characterizations with similar conclusion (Supporting Information, Figure S6).

We found that the growth rate of AuNPs with different sizes was regulated differentially with ss-DNA. When ss-DNA was present, larger AuNPs (orange-colored dots) typically grew faster than smaller ones (green-colored dots; Supporting Information, Figure S7). Since our previous solution-phase study has revealed that AuNPs with smaller size possessed higher catalytic activity,[9] this difference in growth rates should arise from the surface coverage of ss-DNA on AuNPs. Under the same conditions, larger AuNPs has larger surface areas and are less covered by ss-DNA; thereby, more unoccupied surface could proceed to catalyze glucose to produce larger NPs.

Therefore, we could in principle employ smaller AuNPs to increase the difference in nanoplasmonic signals in response to ss- and ds-DNA. In principle, smaller AuNPs possess higher catalytic activity and can be more easily covered by ss-DNA than larger ones. To test this hypothesis, we further

employed AuNPs of 12 nm in diameter (Supporting Information, Figure S8). These small AuNPs were barely visible under DFM owing to the limited optical resolution of our system. The growth processes were recorded in real time (see the video in the Supporting Information). Interestingly, colored dots rapidly appeared within only 10 minutes upon addition of glucose and $HAuCl_4$. After 30 minutes of enlargement, hundreds of red dots with high brightness levels appeared in the view (Supporting Information, Figure S8A). This result suggests that small AuNPs can rapidly grow into larger AuNPs with sizes exceeding 50 nm. Obviously, this signal-on process with nearly zero background is more sensitive than that with 50 nm AuNPs, which was then employed to differentiate ss-and ds-DNA. Indeed, the critical time for the appearance of colored dots (t_{50}, defined by a greater than 50% increase in the number of dots) was delayed from 10 min for the as-prepared AuNPs to 30 min for ss-DNA–AuNPs (Supporting Information, Figure S8B). The t_{50} value for ds-DNA–AuNPs was 18 minutes, although the color change from green-to-orange was much slower than for as-prepared AuNPs.

We have demonstrated that the GOx-like catalytic activity of AuNPs can be finely regulated by DNA hybridization, and that these catalytic AuNPs can be employed as a nanoprobe for a variety of biomolecular detections. This new strategy has several unprecedented advantages. First, it exploits the catalytic rather than optical or electric properties of AuNPs. Therefore, it could be easily coupled with various enzymatic cascade reactions to amply hybridization signals. Furthermore, the use of more active Au nanomaterials, for example, hollow Au nanocages with more active atoms at the surface,[27] is expected to improve the sensitivity of this system. Second, this assay method avoids labeled DNA probes, which simplifies the system and lowers the assay cost. Third, this strategy could potentially be used in any systems that could incorporate catalytic AuNPs. As we have demonstrated its applicability in aptamer-based assays, it should in principle be a generic platform for the detection of any small molecules, proteins or even cell targets that have specific aptamers.

The mechanism for the observed remarkable difference in adsorption ability of DNA to AuNPs arises primarily from different accessibility of AuNPs to nitrogen-containting nucleotides in ss- and ds-DNA. Moreover, higher surface charge density and rigidity of ds-DNA (or aptamers) also make it more difficult to bind to AuNPs than ss-DNA. Significantly, the adsorption of nucleotides on Au atoms inhibits their catalytic activity, resulting in the DNA hybridization-regulated catalysis of AuNPs. Our previous study has revealed that the size, shape, and catalytic activity of AuNPs could be simultaneously controlled to result in a self-limiting system.[9] This implies that there is plenty of room to further optimize the system by finely tuning DNA–AuNPs interactions to minimize the background and amply the signal.

It is worthwhile to point out that, as AuNP-based catalysis is a complicated reaction with multiple active sites for glucose oxidation and possible substrate suppression, the classic Michaelis–Menten equation cannot precisely describe this reaction. Furthermore, the rate of catalysis would decrease with the adsorption of glucose and its product gluconic acid owing to reduced access to the NP surface, which also does not fit the Michaelis–Menten equation. This substrate suppression is, nevertheless, at a much smaller level than DNA-adsorption based suppression. However, this equation is still employed as it provides a simple measurement for comparison of kinetics.

Our DFM study opens a new avenue for the use of catalytic AuNPs as a nanoplasmonic probe for various types of biomolecular recognition. While our present system is limited by heterogeneous size distribution of chemically synthesized AuNPs, the recently developed methods for the synthesis of monodispersed AuNPs provide a route to increase the detection sensitivity.[28] Also interestingly, this catalysis-based nanoplasmonic Au probe can be coupled with DNA nanotechnology-based solution-phase DNA chips[29] to amplify the hybridization signal. We also note that citrate-capped AuNPs may not be directly (that is, without prior sample separation) applied in biological fluids owing to strong protein adsorption. However, it is possible to use AuNPs with stronger ligands that are not so surface sensitive (for example hydroxylamine-capped AuNPs) to realize bioanalysis in biological fluids. Furthermore, AuNPs are known to possess high cellular uptake ability in various cell types without apparent cell toxicity. Therefore, it is possible to design a system for tracking cellular events in real time by using this catalytic nanoplasmonic probe.[26b] The single NP tracking ability of DFM could provide invaluable information occurring on the NP surface.[30]

Received: July 21, 2011
Revised: August 16, 2011
Published online: October 13, 2011

Keywords: catalysis · DNA · gold nanoparticles · microscopy · nanoplasmonics

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