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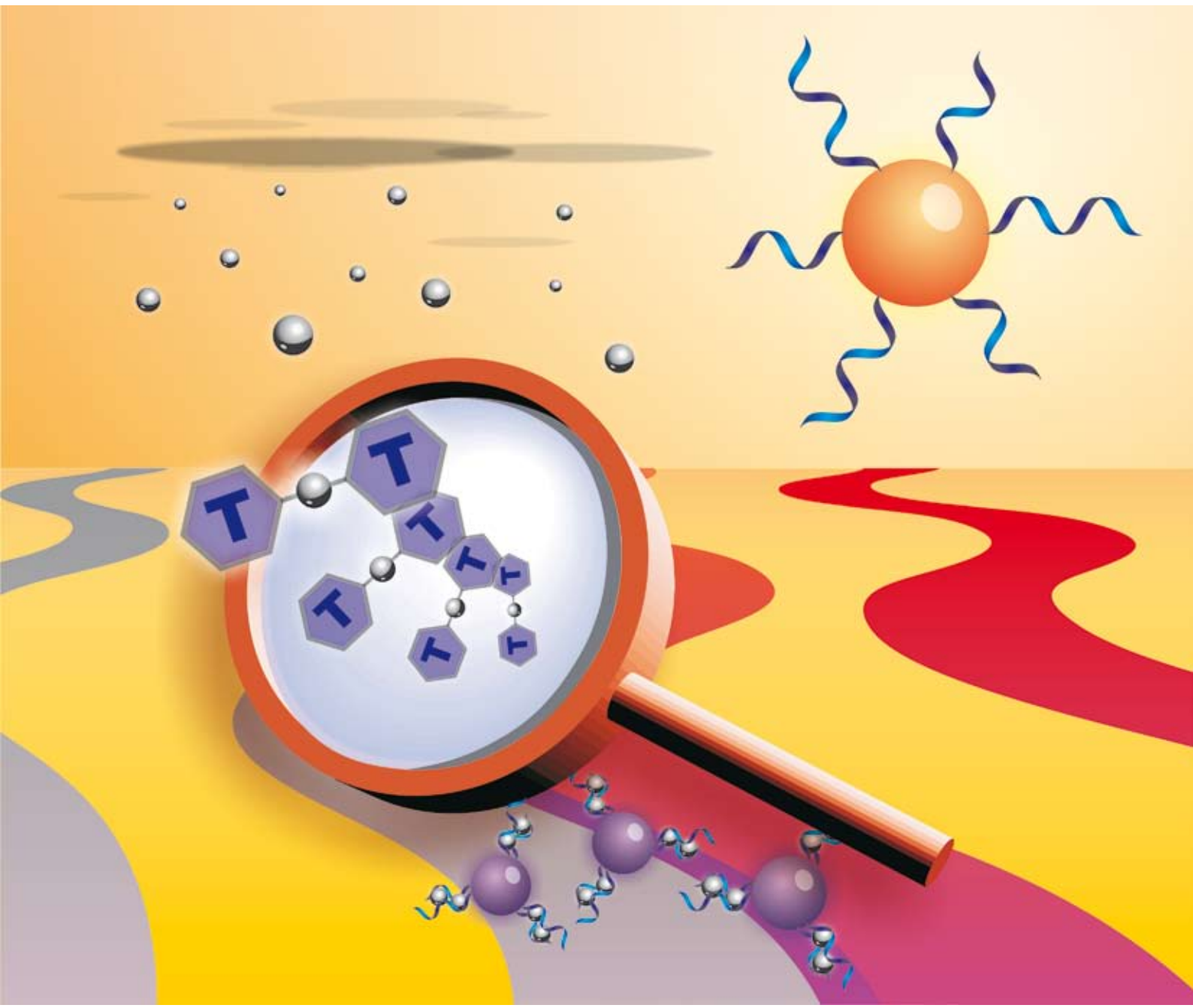

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Design of a gold nanoprobe for rapid and portable mercury detection with the naked eye†

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A gold nanoprobe that can respond colorimetrically to Hg^{2+} is designed and coupled with a power-free PDMS device; the system can be used for rapid and visual detection of low micromolar Hg^{2+} in real environmental samples.

Mercury ions (Hg^{2+}), the most stable form of inorganic mercury, are highly toxic environmental pollutants and known to have serious effects on human beings.[1] For example, microbial biomethylation of Hg^{2+} yields methyl mercury that accumulates in the body through the food chain, causing brain damage as well as other chronic diseases.[2] Therefore, there is an ever-growing demand for rapid and field detection of mercury contaminants in drinking water, food and soil. Various electrochemical and optical methods have been developed for the analysis of Hg^{2+}.[3–11] Recently, Hg^{2+}–bis-thymine (T–Hg^{2+}–T) complexes have been well studied,[12,13] which motivated the design of a variety of novel Hg^{2+} detection assays.[14–21] However, most of these reported methods are instrument-based, which makes them inconvenient for field detection. In this work, we developed a novel oligo-T-based gold nanoprobe for rapid and portable detection of Hg^{2+} with the help of a power-free poly(dimethylsiloxane) (PDMS) microfluidic device.

Gold nanoparticles (AuNPs) have long been used as biological labels because of their attractive optical and electronic properties.[22–24] Significant research interest has been directed toward AuNPs-based colorimetric bioassays for nucleic acids, proteins as well as small molecules, which rely on the plasmonic properties of AuNPs, *i.e.*, AuNPs have very high extinction coefficients and show size-dependent brilliant colors due to the light-induced plasmons.[25–28] Very recently, several groups including ours proposed that AuNPs modified T-containing oligonucleotides could serve as visual probes for Hg^{2+} detection.[14,17–20,29] However, while these AuNPs-based nanoprobes worked very well in test tubes, we found that they were not compatible with Hg^{2+} detection in a simple microfluidic device.

In this work, we designed a new oligo-T/AuNPs-based gold nanoprobe that could rapidly respond to Hg^{2+} within microfluidic channels and at room temperature (Fig. 1). We prepared the Au nanoprobe through modification of AuNPs with thiolated thymine oligonucleotides (a mixture of T6/T10). Because of the strong Coulombic repulsion between highly negatively charged DNA strands located at the AuNP surface, these nanoprobes were highly stable and remained dispersed (with a characteristic red color) even at high ionic strength. In the presence of Hg^{2+}, adjacent thymine probes at the surface of each AuNP formed the T–Hg^{2+}–T complex, which changed the charge distribution at the surface and destabilized AuNPs against aggregation at high ionic strength. As a result, the color of the gold nanoprobes turned purple as purple-colored aggregates formed, an effect originating from the interparticle coupled plasmon absorbance of aggregated AuNPs.[25,27,28] This simple strategy forms the basis of our visual approach for Hg^{2+} detection.

As shown in Fig. 2, the gold nanoprobes retained their red color in the presence of salt of high concentration (2.25 M $NaClO_4$, tube 1). We then challenged the nanoprobes with Hg^{2+} of 0.1 mM (tube 7). After 5 min, we added highly concentrated salt, then a red-to-purple color change was almost instantly observed. We also tested a series of non-specific heavy metal ions (all 0.1 mM with 5-min incubation, tubes 2–6), *i.e.*, Pb^{2+}, Cd^{2+}, Cu^{2+}, Mn^{2+} and Zn^{2+}, which did not lead to an obvious color change. These results suggested that the Au nanoprobes were highly selective for Hg^{2+} detection due to the highly specific T–Hg^{2+}–T bonding. Tubes 8–11 compare the color change of nanoprobes at a series of concentrations of Hg^{2+}, ranging from 5 to 100 μM. Obviously, the color of the nanoprobes became more purple-like along with the increase of the concentration of Hg^{2+}, suggesting a higher degree of aggregation. It is worthwhile to point out that the ratio of two different oligo-Ts at the surface of the AuNPs is critical for the performance of the gold nanoprobes. We found experimentally that gold nanoprobes prepared from thiolated oligonucleotide solution with a molar ratio of 2 : 3 (T6 : T10) possessed the highest sensitivity for Hg^{2+} detection, which was ascribed to the optimum spacing of probes that allows efficient T–Hg^{2+}–T formation at the surface.

We reason that the aggregation of AuNPs mainly arises from the T–Hg^{2+}–T formation at the surface of individual particles. Hg^{2+} glues adjacent oligo-T probes on a single particle, leading to an uneven charge distribution at the surface of the nanoparticles. That is, some sites at the surface are opened for the ion permeation, resulting in the instability of

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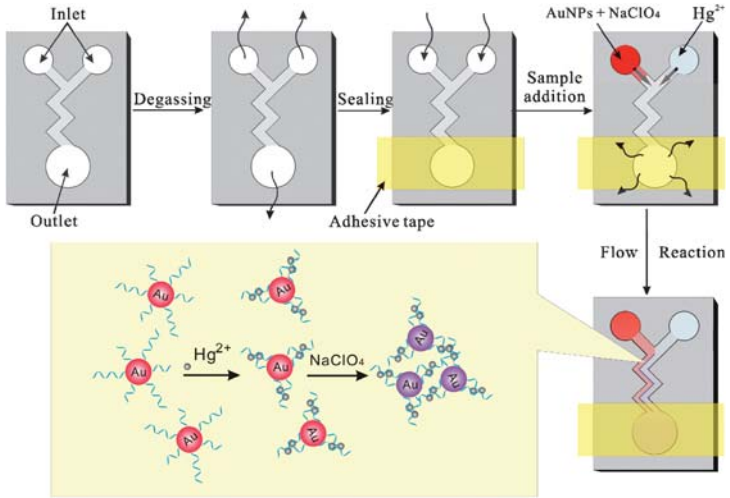

Fig. 1 Scheme for power-free Hg^{2+} sensing strategy. The black arrows in each step indicate the directions of air transfer. Also shown is the scheme for optical detection of Hg^{2+} using thymine-modified Au nanoparticles.

AuNPs at high ionic strength. One might argue the possibility of Hg^{2+}-mediated inter-particle aggregation. However, we did not find any evidence of aggregation formation before the salt addition in our UV–Vis and light scattering studies (data not shown). Therefore, while we could not completely exclude the inter-particle aggregation mechanism due to the limited sensitivity available in spectroscopic studies, we believe that the inner-particle mechanism plays a major role. Due to this new mechanism, our nanoprobes can work reliably and rapidly at room temperature, avoiding either precise temperature control[17] or elaborate sequence design[29] as described in previous reports.

PDMS is an optically transparent, soft elastomer suitable for the fabrication of microfluidic devices.[30–32] PDMS is known to possess high gas solubility,[31] and the amount of dissolved gas in PDMS is directly proportional to the partial pressure of the gas in equilibrium with the PMDS. This important property forms the basis for the fabrication of PDMS devices with the power-free pumping feature.[31] We fabricated a PDMS device with two Y-shaped zigzag microchannels (Fig. 1). Of note, the zigzag design facilitates mixing in the channel. The device was first degassed in vacuum and then the outlet reservoir was sealed with a piece of adhesive tape. A mixture of gold nanoprobes and $NaClO_4$ (0.6 M) was added to one inlet. Interestingly, while the Hg^{2+} sample was added to the other inlet, both droplets instantly flew into the channel. As described above, the Au nanoprobes readily formed purple-colored aggregates as they met Hg^{2+} at high ionic strength, which resulted in a clearly visible deposition line in the zigzag-shaped microchannel.

Fig. 2 Color change of gold nanoprobes upon being treated with pure water (1) and a series of metal ions of 100 μM, *i.e.*, Pb^{2+}, Cd^{2+}, Cu^{2+}, Mn^{2+} and Zn^{2+} (2–6). Tubes 7–11 show the concentration profile for the Hg^{2+}-induced color change of gold nanoprobes (from left to right: 100 μM, 50 μM, 25 μM, 10 μM and 5 μM). In all these experiments, $NaClO_4$ of 2.25 M was added to tubes after 5-min incubation.

This power-free pumping arises from the energy stored in the degassed PDMS.[31] When the degassed PDMS device was brought back to the air, both the inlets and the outlet were filled with air instantly, while the concentration of dissolved gas in PDMS remained at a low level due to the relatively slow diffusion in the bulk polymer. After the sealing of the outlet, the air filling the channel and the outlet would diffuse into PDMS, leading to a pressure difference with the inlets that were open in air. As a result, when and only when both inlets were filled with the liquid samples, the pressure difference "pumped" the samples into the channel.

In order to realize multiplex detection with a single device, we designed a multi-channel PDMS device. As shown in Fig. 3A, Hg^{2+} of 100 μM induced the formation of a clearly visible deposition line in the microchannel, while the other metal ions did not have this effect, suggesting that the gold nanoprobes were highly compatible with the PDMS device, and retained high selectivity in the microchannel-based detection. We also evaluated the sensitivity of the device in samples mimicking real-world applications (Hg^{2+} spiked in river water). Fig. 3B compares the microscopic images of the microfluidic channels for a series of Hg^{2+} concentrations ranging from 5 μM to 100 μM, with higher Hg^{2+} concentration leading to denser deposition lines. Of note, as little as 5 μM Hg^{2+} still led to the formation of a detectable deposition line. This micromolar sensitivity is comparable to that of state-of-the-art colorimetric Hg^{2+} sensors.[16,17,29] We expect that the use of larger AuNPs or the employment of amplification methods may further increase the sensitivity to meet the requirements of real-world applications.

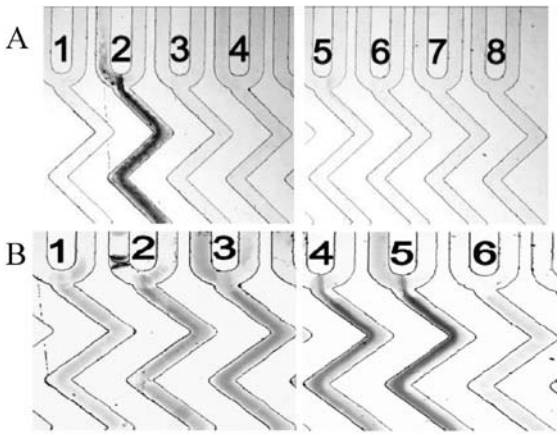

Fig. 3 (A) Microscopic images of the PDMS channels for gold nanoprobes treated with pure water (1) and a series of metal ions of 100 μM, *i.e.*, Hg^{2+}, Cd^{2+}, Mn^{2+}, Cu^{2+}, Zn^{2+}, $Ca^{2+}$and Mg^{2+} (2–8). In all these experiments, 0.6 M $NaClO_4$ was mixed with the gold nanoprobes and added to the left inlet while metal ions were added to the right inlet. (B) Microscopic images of the PDMS channels for gold nanoprobes treated with a series of Hg^{2+} concentrations, *i.e.*, 5 μM (1), 10 μM (2), 25 μM (3), 50 μM (4), 100 μM (5), 0 M (6, blank river water). In all these experiments, Hg^{2+} was dissolved in real river water samples.

The PDMS device should be equipped with a microscope for visual detection since the channels are only of micrometre sizes. Interestingly, we found that when a droplet of water was cast on the microchannels, it served as a "magnifying glass", and the deposition lines were clearly visible even with the naked eye (Fig. S2 of the Supporting Information†). It is worth noting that this Hg^{2+} sensor is a fully portable device. That is, we can simply store a degassed PDMS device in a vacuum bag, which can be carried to any location and be ready for Hg^{2+} detection with the naked eye. Therefore, in view of its remarkable simplicity, our device could be useful for rapid screening in field detection.

In summary, we have demonstrated a gold nanoprobe that can rapidly, sensitively and selectively detect mercury ions with the naked eye. This method has several important advantages. First, we have developed a microfluidic device that is fully portable, power-free and cost-effective. Since the detection does not rely on an expensive instrument, this device is highly suitable for field detection. Second, the gold nanoprobes are highly soluble in aqueous solution, which is different from most small-molecule probes that only work in organic media. Third, the gold nanoprobes that we have designed rely on the inner-particle interaction mechanism,[17,29] therefore the reaction is rapid (the assay time is in minutes) and insensitive to the environmental temperature. We thus believe that this contribution opens a new opportunity for rapid, field detection of mercury ions as well as a range of clinically, environmentally or security related targets.

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