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Supporting Information

Real-time monitoring of TSP assembly at surfaces with QCM

We observed a typical bi-phase kinetic behavior (Fig. 1b, inset). Nearly 70% of the probes assembled at the Au surface within several minutes, corresponding to the "rapid" phase. The remaining probes assembled within several hours, constituting a "slow" phase. A control experiment incorporating non-thiolated TSP exhibited minimal adsorption at the Au surface, confirming that TSP was anchored at the surface *via* specific Au-S binding.

Additional mechanistic studies using QCM and SPR

Frequency change can be translated into mass change at the surface via the Sauerbrey equation[1]: $\Delta m = C\frac{-\Delta f_n}{n}$, leading to an apparent surface density of 9.0×10^{12} TSP/cm^2. However, we note that QCM mass quantification is overestimated since the QCM frequency change is known to be associated with trapped water[2] (i.e. "wet" mass). An optical method, surface plasmon resonance (SPR), provides a more reliable method to quantify "dry" mass change at the surface, leading to a surface density of 4.8×10^{12} TSP/cm^2 (or 8.0 pmol/cm^2). The two-fold difference in QCM and SPR data reveals that a large amount of water is trapped within the thin TSP film. In addition, the observed large dissipation change in QCM (Fig. S2) also suggests the high capacity of the hollow tetrahedral structures.

Electrochemical characterization of TSP-modified Au surfaces

To evaluate the viability of constructing an electrochemical DNA sensor using TSP-decorated gold electrodes, we first analyzed the electron-transfer reactivity at the TSP-conjugated surface since it has a much thicker layer (the structure of TSP plus a six-carbon spacer theoretically forms a ~6 nm layer) than the conventional ssDNA probe-based ones (a six-carbon spacer forms a layer of ~1 nm). Interestingly, we found that a redox molecule, TMB, exhibited well-defined redox peaks at the TSP-modified surfaces (Fig. S3), which were significantly better that those at the much thinner 11-carbon alkane thiol-modified surfaces (~1.5 nm), suggesting that TMB could easily penetrate the hollow structures comprising the TSP layer.

References

[1] G. Sauerbrey, *Zeitschrift Fur Physik* **1959,** *155,* 206.
[2] M. V. Voinova, M. Rodahl, M. Jonson, B. Kasemo, *Phys. Scr.* **1999,** *59,* 391.

Experiment section

Materials

Tris-(hydroxymethyle)aminomethane was from Cxbio Biotechnology Ltd. Ethylenediaminetetraacetic acid (EDTA), MCH, and tris(2-carboxyehtyl) phosphine hydrochloride (TCEP) were purchased from Sigma-Aldrich (St. Louis, MO). TMB substrate (TMB, 3,3',5,5'-tetramethylbenzidine; Neogen K-blue low activity substrate) was from Neogen (U.S.A.). Avidin-HRP(horseradish peroxidase) was from Roche Diagnostics (Mannheim, Germany). The buffer solutions involved in this study are as follows: the hybridization buffer was 1 M NaCl and 10mM TE buffer (pH 7.4). The buffer for tetrahedral probe assembly was 20 mM Tris (pH 8.0), 50 mM $MgCl_2$. The DNA immobilization buffer was 10mM Tris-HCl, 1 mM EDTA, 10 mM TECP (pH 7.4), and 1 M NaCl. The washing buffer was 0.1 M NaCl and 10 mM PB buffer (pH 7.4). Enzyme diluent was 0.1 M PBS buffer with 0.5 % casein (pH 7.2). All solutions were prepared with Milli-Q water (18MΩ·cm resistivity) from a Millipore system.

All oligonucleotides were synthesized and purified by TaKaRa Inc. (Dalian, China), and the sequences are shown in Table S1.

Table S1. oligonucleotides used in this work

Oligo DNA	sequence
A	5’-ACA TTC CTA AGT CTG AAA CAT TAC AGC TTG CTA CAC GAG AAG AGC CGC CAT AGT A-3’
B	5’-TAT CAC CAG GCA GTT GAC AGT GTA GCA AGC TGT AAT AGA TGC GAG GGT CCA ATA C-3’
C	5’-TCA ACT GCC TGG TGA TAA AAC GAC ACT ACG TGG GAA TCT ACT ATG GCG GCT CTT C-3’
D	5’-TTC AGA CTT AGG AAT GTG CTT CCC ACG TAG TGT CGT TTG TAT TGG ACC CTC GCA T-3’
Tetra-A	**5’-GTATC CAGTG GCTCA** TTTTTTTTTT ACA TTC CTA AGT CTG AAA CAT TAC AGC TTG CTA CAC GAG AAG AGC CGC CAT AGT A-3’
Tetra-B	5'-HS-C6- TAT CAC CAG GCA GTT GAC AGT GTA GCA AGC TGT AAT AGA TGC GAG GGT CCA ATA C -3'
Tetra-C	5'-HS-C6- TCA ACT GCC TGG TGA TAA AAC GAC ACT ACG TGG GAA TCT ACT ATG GCG GCT CTT C -3'
Tetra-D	5'-HS-C6- TTC AGA CTT AGG AAT GTG CTT CCC ACG TAG TGT CGT TTG TAT TGG ACC CTC GCA T -3'
Dye-A	**5’-TAMRA**-ACA TTC CTA AGT CTG AAA CAT TAC AGC TTG CTA CAC GAG AAG AGC CGC CAT AGT A -3
Dye-D	5’-HS-C6- TTC AGA CTT AGG AAT GTG CTT CCC ACG TAG TGT CGT TTG TAT TGG ACC CTC GCA T -**TAMRA**-3’
Tetra-Thr	5’-GGTTGGTGTGGTTGG TTTTTTTTTT ACA TTC CTA AGT CTG AAA CAT TAC AGC TTG CTA CAC GAG AAG AGC CGC CAT AGT A -3’
Thr-RP	5’-Biotin-TTTTTTTTAGTCCGTGGTAGGGCAGGTTGGGGTGACT-3’
SSP	5’-GTATC CAGTG GCTCATTTTT-SH-3’
T3	5’-TGA GCC TCT GGA TAC-biotin-3’
T4	5’-TGA GCC GCT GGA TAC-biotin-3’
T5	5’-ACG AAT TCC GAG ATG-biotin-3’
RP	5’-biotin-GCATGCTAGTAATGCTCTTG-3’

Formation of DNA Tetrahedra probe

We mixed the four oligonucleotides (Tetra-A, Tetra-B, Tetra-C and Tetra-D) in equimolar quantities in TM buffer (20 mM Tris, 50 mM $MgCl_2$, pH 8.0), heated the mixture to 95°C for 2 min and then cooled to 4°C in 30 s. For DNA assays: TSP was synthesized with Tetra-A, Tetra-B, Tetra-C and Tetra-D. The DNA Tetrahedra probes were analyzed using polyacryamide gel electrophoresis (PAGE, 12.5%) in TBE buffer at a constant current of 5 mA at 4°C.

Electrophoretic analysis demonstrated that the yield for tetrahedron synthesis was of ~85%. However, unpurified tetrahedron could be directly employed for electrode modification. In our design, only the intact tetrahedron carries both the thiol group and the capture probe sequence, and can serve as the recognition part at the surface of gold electrodes. A small amount of thiolated ssDNA might remain in the system and possibly attached to the

surface. However, since tetrahedron carries three thiol moieties, the binding of which to gold surfaces is kinetically faster and thermodynamically more stable than single-thiolated DNA. Therefore, the tetrahedron should occupy the majority of the surface. Indeed, we have compared the sensor performance by using either purified or unpurified tetrahedron, and find no significant difference.

For fluorescence experiment: Thiol-free, TAMRA-tagged DNA tetrahedron (top) was synthesized with Dye-A, B, C and D. Thiol-modified TAMRA-tagged DNA tetrahedron (top) was synthesized with Dye-A, Tetra-B, Tetra-C and Tetra-D. Thiol-modified TAMRA-tagged DNA tetrahedron (bottom) was synthesized with A, Tetra-B, Dye-C and Tetra-D.

QCM

Q-Sense D300 (Q-Sense, Gothenburg, Sweden) was used here. A QCM chip was first loaded onto a QCM sensor. Then, TM (pH 8.0) solution was pumped into the QCM chamber. After a stable baseline was established, a solution of 2 μM tetrahedron DNA in TM solution was introduced. The immobilization of DNA on the chip was monitored online. The DNA solution was then replaced by TM, and incubated for 30 min. Finally, the target DNA was introduced.

SPR

All surface plasmon resonance (SPR) experiments were performed at 25℃ on Biacore 3000 instrument (Biacore Inc.). For preparation of the biosensor surface with TSP. 1μM TSP in running buffer was automatically injected onto an untreated gold surface sensor chips (Sensor Chip Au, GE Healthcare Inc.) to the saturated density in different flow cell. Proteins and target DNA were diluted in running buffer.

Microcantilever

Cantilever measurements were performed in real time at 25 °C using Cantisens CSR-801(Concentris GmbH, Basel, Switzerland). We employed arrays of eight gold-coated cantilevers (CLA-750-010-08, Concentris, Basel, Switzerland), with a dimension of 750 μm in length, 100 μm in width and 1 μm in thickness. Reference cantilevers were first blocked by immersing them in capillaries filled with 2 mM MCH for 2 h, and then the array was mounted. After a stable baseline was obtained, 180 μL TSP immobilization solution was introduced at a speed of 0.42 μL/s. When the DNA solution reached the measurement chamber, the signal started to increase and became stable after a certain period of time. After being washed with buffer, DNA hybridization solution was pumped into the chamber.

Electrochemical measurements.

Electrochemical measurements were performed with a CHI 660 electrochemical workstation (CH Instruments Inc., Ausin, TX). A conventional three-electrode configuration was employed all through the experiment, involving a gold working electrode, an Ag/AgCl reference electrode, and a platinum counter electrode. A glass cell with 3 mL of electrolytic buffer was placed on a cell stand. All potentials were referred to the Ag/AgCl (3 M KCl) electrode, and all measurements were carried out at a scan rate of 100 mV/s. Amperometric detection was performed with a fixed potential of 150mV, and steady state was usually reached and recorded within 100 s.

Fabrication of electrochemical DNA sensors

Gold electrodes were cleaned following the reported protocol. The cleaned electrodes were incubated with 1 μM TSP or ssDNA probe in the immobilization buffer for 3 h at room temperature. After that, probe-modified electrodes were treated with 2 mM MCH, or not. A 3 μL droplet of target DNA was pipetted onto the electrode surface, and incubated for 30 min at

37 °C. In the direct detection mode, biotinylated target DNA was employed, while label-free target was first mixed with a biotinylated reporter probe (100 nM) in the sandwich detection mode. Electrodes were then rinsed with washing buffer and then incubated with 2 μL of avidin-HRP (0.5 U/mL) for 15 min at room temperature. Finally, the sensor was extensively rinsed and subjected to electrochemical measurements.

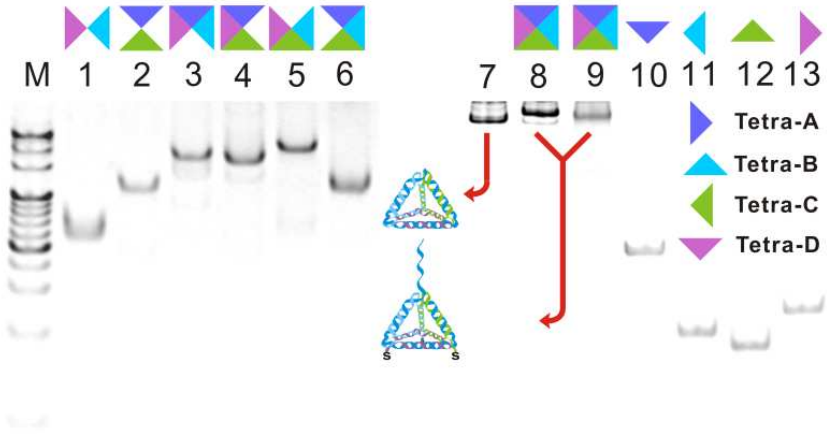


Figure S1. Gel electrophoretic analysis of the formation of TSP. Lane 7 stands for a non-modified tetrahedron, lane 8 for TSP and lane 9 for TSP incubated in 50 % serum. Control experiment for single-stranded (ss-) DNA (lane 10, 11, 12, 13) or any other combinations lacking one (lane 3, 4, 5, 6) or two strands(lane 1, 2). Lane M, 20 bp ladder.

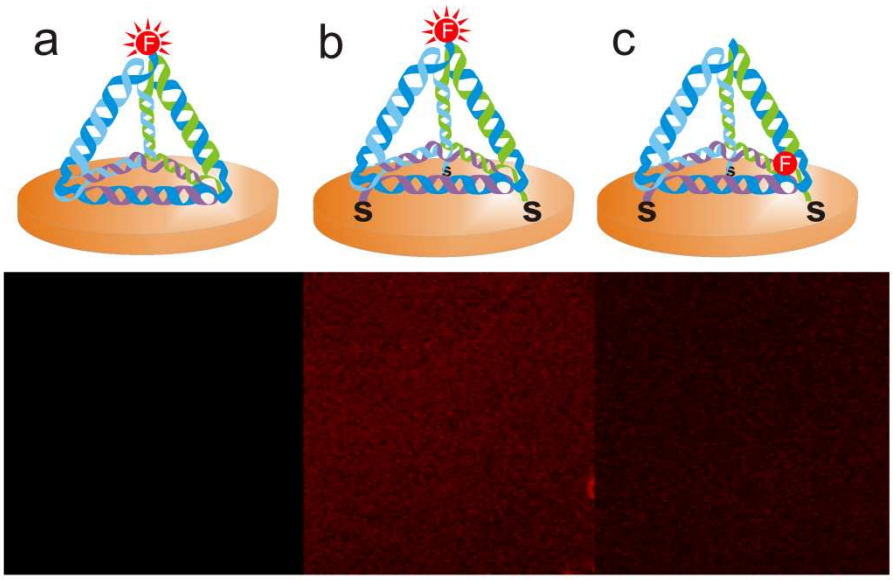


Figure S2. Fluorescence studies on TSP-modified Au surfaces. a) Au surface assembled with non-thiolated tetrahedra; b) TSP labeled with TAMRA at the top vertex; and c) TSP labeled with TAMRA at one of the bottom vertices.

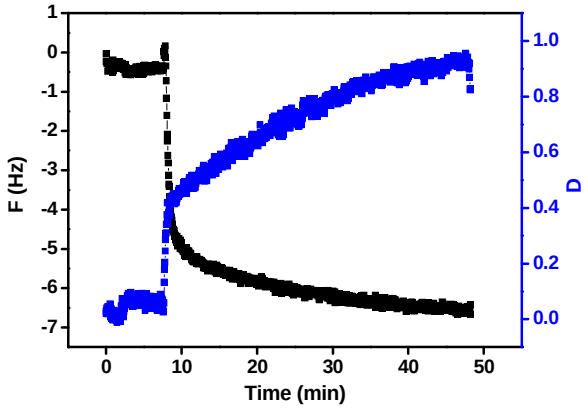


Figure S3. QCM-D sensorgrams for hybridization of a surface-attached tetrahedron-shaped DNA probe with 1 μM target DNA in hybridization buffer.

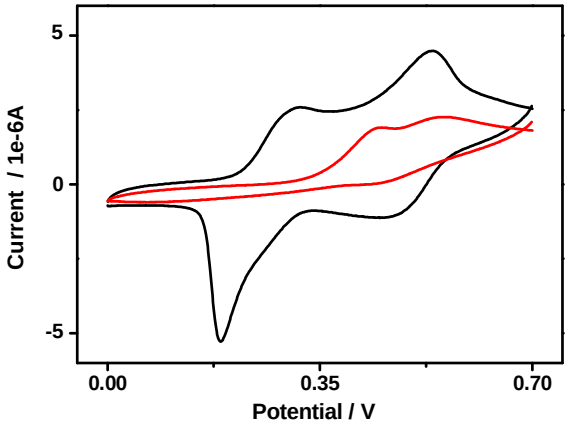


Figure S4. Cyclic voltammograms for the redox reaction of the TMB substrate at gold electrode modified with TSP (black), thiolated 11-MUA/11-MU (red). Scan rate :100 mV/s

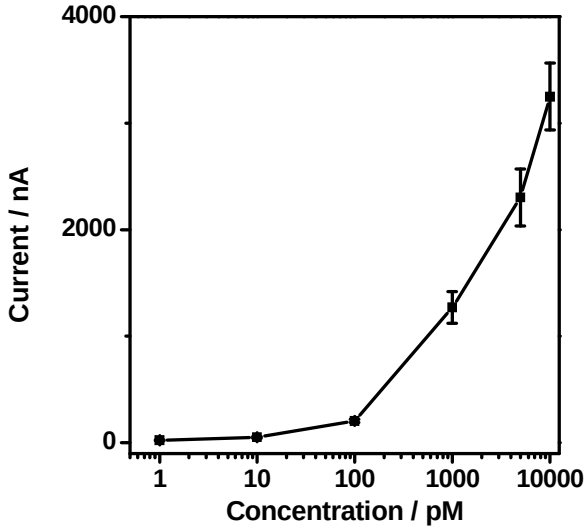
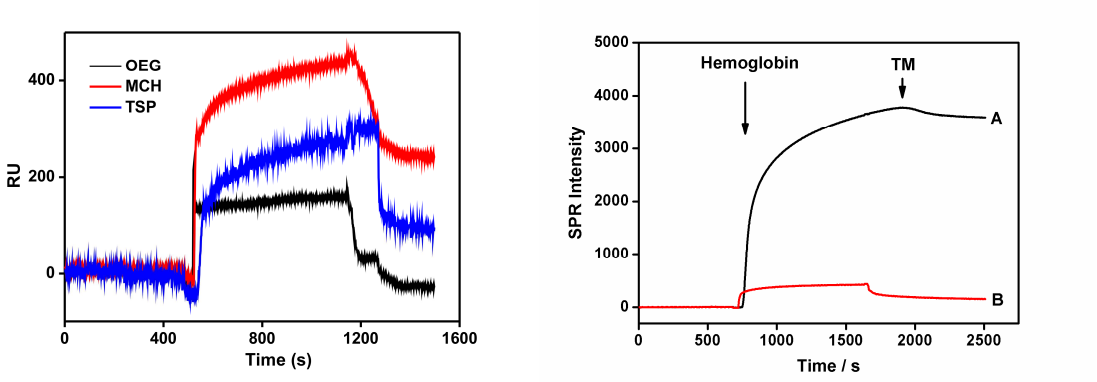


Figure S5. Amperometric signal versus concentration of target DNA on TSP-based sensor in the direct detection mode.



(a) (b)

Figure S6. a) Investigation of protein-resistant ability of Au surfaces modified with MCH, TSP and a well known protein-resistant OEG by SPR. b) SPR sensorgram of protein adsorbed onto bare gold surface (black) and DNA tetrahedron-shaped-probe-modified surface (red). The concentration of hemoglobin is 1 mg/mL.

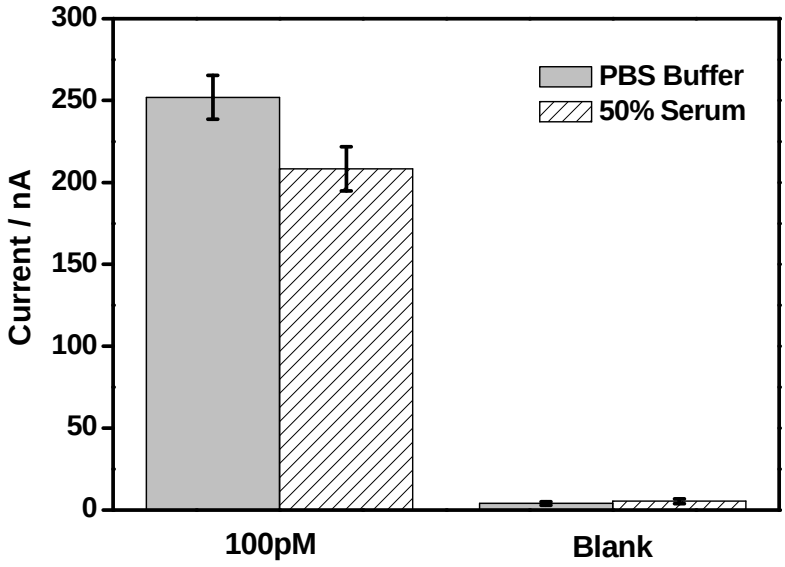


Figure S7. Detection of synthetic target DNA in PBS buffer and 50% serum.