Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Photothermal palladium nanoballs coupled with a smartphone-thermal reader for photothermal lateral flow immunoassay of Aβ 1-40

Xiaoli Cai,[a, †, *] Yangxing Luo,[c, †] Yang Song [b, *]

[a] Academy of Nutrition and Health, Hubei Province Key Laboratory of Occupational Hazard Identification and Control, School of Medicine, School of Public Health, Wuhan University of Science and Technology, Wuhan 430065, China

[b] NANOGENE LLC, Gainesville, Florida 32611, United States

[c] Department of Orthopedics, Renmin Hospital of Wuhan University, Wuhan 430060, China

Corresponding Author

*E-mail: xlcai@wust.edu.cn; yang.song@wsu.edu

† X. Cai and Y. Luo contributed equally to this manuscript.

Materials and experimental section

Materials. Palladium (II) acetylacetonate (99%), polyvinylpyrrolidone (Mw=30,000), sodium bromide (99%), N, N-dimethylpropionamide, Bovine serum albumin (BSA), N-(3-dimethylamino propyl)-N′-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were purchased from Sigma. Poly(D,L-lactide-co-glycolide) (PLGA-acid or PLGA-COOH, D, L-LA/GA=50:50, Mn 24000) was purchased from Nanosoftpolymers, USA. Other chemical reagents were analytical grade and used without further purification. Backing cards (HF000MC100) and absorbent pads (Surewick) were purchased from Millipore (Bedford, MA, USA). Glass fiber sample pads (HV Plus 1668) and conjugation pads (8964) were purchased from Ahlstrom-munksjo (Helsinki, Finland). Nitrocellulose membranes (CN140) were obtained from Sartorius Stedim Biotech (Göttingen, Germany). Double distilled water was used in all experiments. Anti-Amyloid beta 1-40 monoclonal antibody (Target the residue 40 on the C-terminus), anti-Amyloid beta 1-40 polyclonal antibodies (Target N-terminus region) and recombinant amyloid beta 1-40 were obtained from Sigma-Aldrich.

Characterizations. TEM samples were prepared by pipetting one drop of water-diluted sample suspensions onto a carbon-coated electron microscopy grid. After 8 min, the liquid was removed by slowly sucking using filter paper. The grids were dried at room temperature for overnight. TEM measurements were conducted on a 200 kV FEI Tecnai TEM microscope. X-ray diffraction (XRD) trials were carried out using a Rigaku Miniflex 600 diffractometer. UV-vis spectra were obtained using a UV-2450 spectrometer. The particle size distributions and zeta potentials were measured with a Zetasizer Nano ZS system (Malvern) with a 633 nm laser. The amount of Pd incorporated into the nanoball was determined by using a microwave plasma-

atomic emission spectrometer (Agilent 4100 MP-AES). Samples were previously digested, with a mixture of aqua regia and distilled water (1:9) and filtered using a 0.2 μm PTFE filter.

Synthesis of Pd nanocrystal (Pd NC). Pd NC was synthesized following previous reports.[43] Pd(II) acetylacetonate ($Pd(acac)_2$, 10.0 mg), poly(vinylpyrrolidone) (PVP, MW=30,000, 32.0 mg) and NaBr (30.6 mg) were mixed with N, N-dimethylpropionamide (2 mL) and water (4 mL). After standing for 10 hours, the resulting homogeneous yellow solution was transferred to a glass pressure vessel. The vessel was then charged with CO to 1 bar and heated at 100 °C for 2 h before it was cooled to room temperature. The dark blue products were precipitated by acetone, separated via centrifugation and further purified with an ethanol-acetone mixture.

Modification of PNB with mAbs. Firstly, 10 μL of PNB (1 mg/mL) and 990 μL of MES (50 mM, pH 6.1) were mixed in a 2 mL microcentrifuge tube. Then, 25 μL NHS (5 mg/mL) and 4.8 μL EDC (5 mg/mL) were added and shocked for 30 min. After centrifugation (18,000 rpm, 15 min), the resulting carboxyl-activated PNB was resuspended in 1 mL MES (50 mM, pH 6.1). Afterward, different volumes of mouse anti-Aβ 1-40 monoclonal antibodies (final concentration range from 2 to 14 μg/mL) were added and incubated for 1 h on a rocking shaker to conjugate with PNB. Besides, BSA (100 μL, 10%) was added for an additional 1 h incubation to block the excess binding sites. Then, the materials were centrifugated and washed with PBS (1% w/v of BSA). Finally, the prepared PNB-Abs conjugates (~100 μg/mL) were collected and suspended in a resuspension buffer (0.05 M Tris-HCl, pH 8.0, 0.05% Tween-20 and 10% FBS).

Fabrication of the PNB-strip (PNB-based LFS). The PNB-strip consisted of a sample pad (Ahlstrom, HV Plus 1668), a conjugate pad (Ahlstrom, 8964), a nitrocellulose membrane (UniSart® CN140, 25 mm × 30 cm), an absorbent pad (Millipore, SureWick) and an interoperable backing. The sample pad was constructed from glass fiber and saturated with

0.05M Tris-HCl, pH 8.0 containing 1 wt% BSA and 0.5 wt% Tween-20 and dried overnight at room temperature. Test line (1 μL/cm) was prepared by dispensing anti-Aβ 1-40 pAbs (1 mg/mL) on the nitrocellulose membrane using BioDot BioJet BJQ 3000 dispenser (Irvine, CA, USA). The distance between each line was approximately 5 mm. The nitrocellulose membranes were then dried in a dry room overnight.

The conjugation pad (8 mm × 30 cm) was made from glass fiber. The eluent solution was dispensed onto the conjugated pad using a BioDot AirJet BJQ 3000 dispenser (Irvine, CA, USA). To achieve optimum performance and good repeatability of the conjugate pad, the airjet dispenser should be set at the dispense speed of 15 mm/s with a dispenser rate of 3 μL/cm. The dispenser pressure is set to 10 psi. After dispensing the conjugate, the conjugate pad was then dried at 37°C for 30 min and stored in a dry room for further use.

The different pads were assembled on backing (60 mm × 30 cm) with an overlap between them of approximately 1 mm to ensure that the solution could migrate through the lateral flow strips. The lateral flow strip was cut at a width of 3.5 mm using BioDot Paper Cutter module CM4000 (Irvine, CA, USA).

Developing Pd-based LFS

(1) Pd NC conjugation

Carboxylic functionalized Pd NCs were prepared according to the literature.[1] Firstly, 10 μL of carboxylic functionalized Pd NCs (1 mg/mL) and 990 μL of MES (50 mM, pH 6.1) were mixed in a 2 mL microcentrifuge tube. Then, 25 μL NHS (5 mg/mL) and 4.8 μL EDC (5 mg/mL) were added and shocked for 30 min. After centrifugation (18,000 rpm, 15 min), the resulting carboxyl-activated Pd NCs were resuspended in 1 mL MES (50 mM, pH 6.1). Afterward, mouse anti-Aβ 1-40 monoclonal antibodies (final concentration 12 μg/mL) were added and incubated for 1 h on a rocking shaker to conjugate with Pd NCs. Besides, BSA (100 μL, 10%) was added for an

additional 1 h incubation to block the excess binding sites. Then, the materials were centrifuged and washed with PBS (1% w/v of BSA). Finally, the prepared Pd NC-Abs conjugates (~100 μg/mL) were collected and suspended in a resuspension buffer (0.05 M Tris-HCl, pH 8.0, 0.05% Tween-20 and 10% FBS).

(2) Fabrication of Pd-based LFS

The fabrication steps of Pd-based LFS are consistent with the synthesis of PNB-based LFS.

Fabrication of the smartphone-thermal reader. The smartphone-thermal reader consisted of four main components namely a 3D printed accessory, a laser, a thermal detector, and a smartphone with a preloaded app. The smartphone accessory was composed of computer-aided design software SolidWorks (Dassault System., MA, USA) using an Apple iPhone 5s (Apple Inc., CA, USA). A commercial fused filament deposition (FFD) 3D printer (Einstart S, Shining 3D, Hangzhou, Zhejiang, China) was used for the rapid prototyping of the smartphone accessory. A portable laser (Century Star Technology Co., Ltd., Wuhan, China) with a 705 nm wavelength emitter was inserted into the 3D printed accessory. A thermal detector was purchased from Seek Thermal, Inc. and inserted into the 3D-printed accessory. The "Seek Thermal" app, obtained from the Apple Store, can convert a thermal signal into a visible image and real-time data.

Preparation of real samples. Each 50 μL of the serum sample, purchased from BioreclamationIVT, was added to 50 μL of running buffer (2 x PBS containing 2 wt% BSA, 0.1 wt% Tween-20) and loaded onto the sample pad. In the recovery experiment, a series of Aβ 1-40 were spiked into the supernatant to achieve the final Aβ 1-40 concentration of 50 pg/mL, 100 pg/mL, and 1000 pg/mL, which were used to simulate overexpression samples.

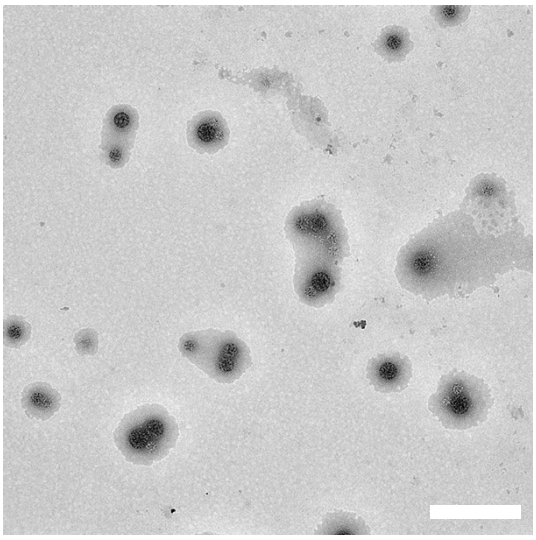

Figure S1. TEM images of PNB. Scale bar: 500 nm.

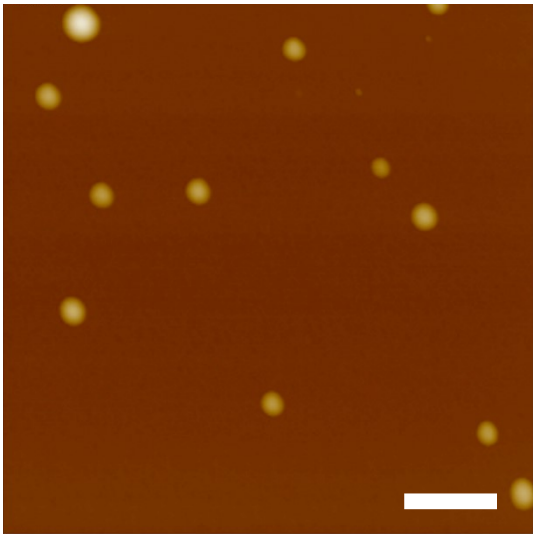

Figure S2. AFM images of PNB. Scale bar: 500 nm.

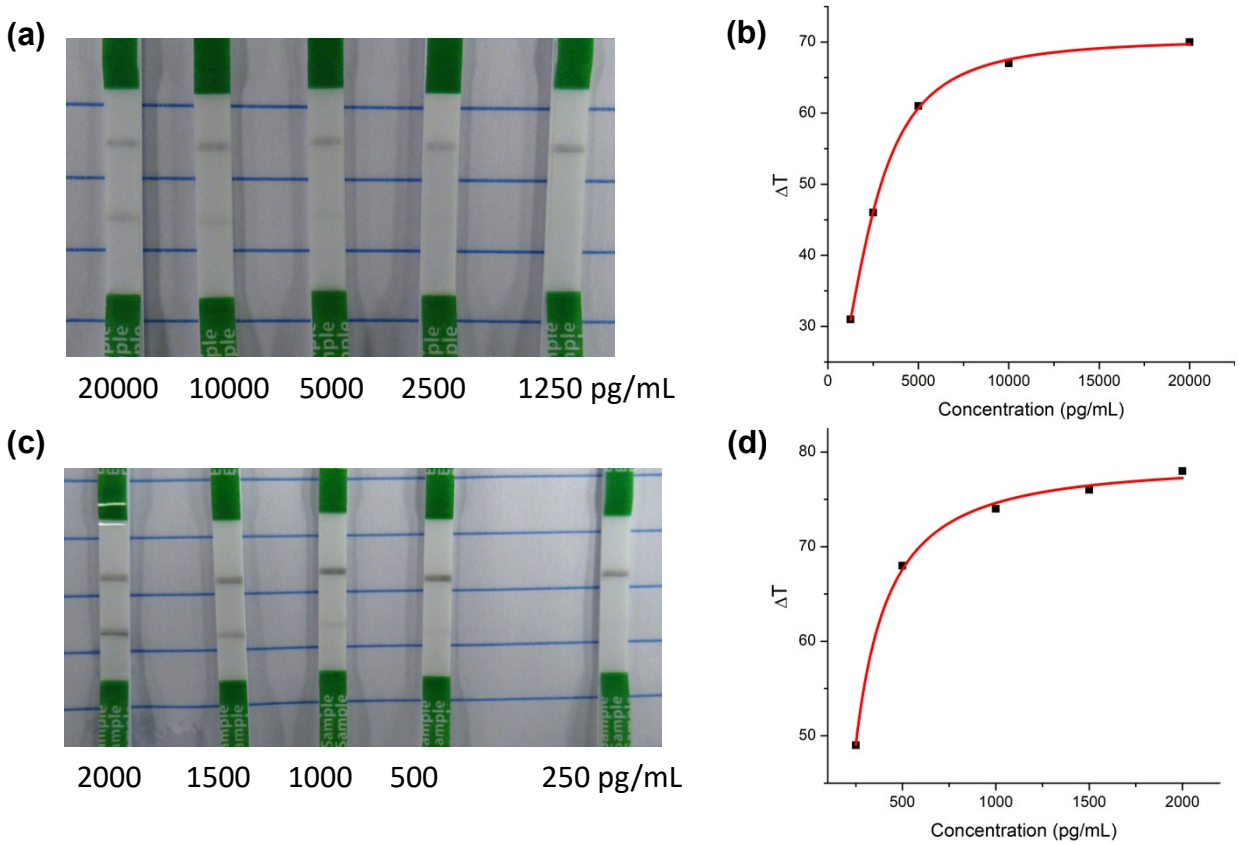

Figure S3. Representative photographs (a) and the relationship of the temperature signal (b) of Pd-based LFS. Representative photographs (c) and the relationship of the temperature signal (d) of PNB-based LFS.

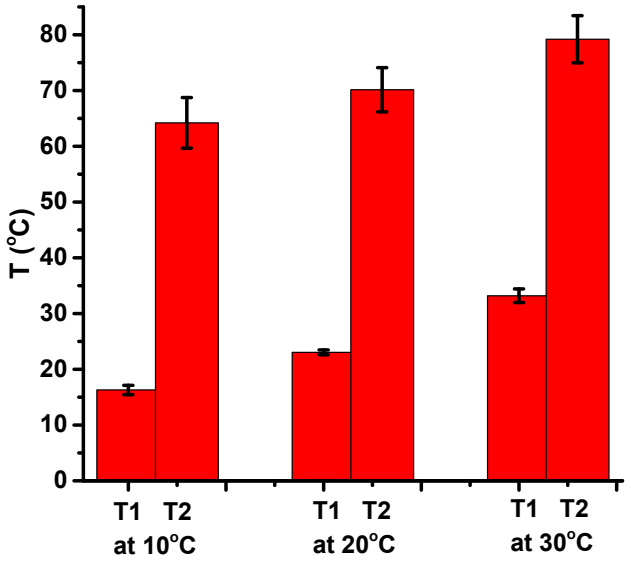

Figure S4. Thermal signals of PNB-based LFS at different room temperatures (10 ℃, 20 ℃ and 30 ℃).

Table S1. Comparison of the limit of detection (LOD) and linear range of this method and other reported methods for the detection of Aβ 1-40.

Techniques	LOD	Linear range	Reference
electrochemical method	77936.4 $pg \cdot mL^{-1}$	0.4-21.6 $\mu g \cdot mL^{-1}$	[12]
AuNP-Aβ-nickel (Ni)-horseradish peroxidase	995.8 $pg \cdot mL^{-1}$	0-43298 $pg \cdot mL^{-1}$	[16]
AuNP-based colorimetric method	43298.0 $pg \cdot mL^{-1}$	151.5-3030.9 $ng \cdot mL^{-1}$	[18]
gold nanoparticle-based aptasensor	2424.7 $pg \cdot mL^{-1}$	4.3-2597.9 $ng \cdot mL^{-1}$	[19]
Guanine-rich DNA quadruplexes based colorimetric technique	21.2 $pg \cdot mL^{-1}$	108.2 $pg \cdot mL^{-1}$-1082.5 $ng \cdot mL^{-1}$	[21]
Photothermal lateral flow immunoassay	21.0 $pg \cdot mL^{-1}$	0-500 $pg \cdot mL^{-1}$	This work

AuNP: Gold-nanoparticle.

1. H. O. Qu, D. Caruntu, H. X. Liu and C. J. O'Connor, *Langmuir*, 2011, **27**, 2271-2278.