## Supplementary data

## On-chip Plasmonic Immunoassay Based on Targeted Assembly of

## **Gold Nanoplasmonic Particles**

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Figure S1. Representative dark-field scattering image of intersection areas formed via multiple reactions in the multi-channels on a single glass slide. The dimensions of each square are 50  $\mu$ m x 50  $\mu$ m.



**Figure S2. Identification of the targeted assembly between antibody-conjugated GNPs and Tau.** Changes in scattering color (top) and spectra of GNPs (bottom) due to antibody conjugation and sequential Tau binding to the GNPs. Scale bar represents 2 µm.



Figure S3. Large field of view showing changes in scattering colors of GNPs. (i) Bare GNPs (ii) Antibody-conjuagated GNPs (iii) antibody-conjuagated GNPs which assembled with A $\beta$ . Scale bar represents 10µm.



**Fig. S4. Optimization of the blocking condition for Tau.** (A) Case 1: capture Ab (pAb) and detection Ab (mAb). (B) Case 2: capture Ab (pAb) and detection Ab (pAb). For both cases, 1%, 2%, 3% BSA and 3% BSA/1% PEG were tested as blocking agents. Scale bars represent 20 μm.



**Figure S5. Quantitative detection of Tau based on the targeted assemblies of GNPs.** (A) Concentration dependent dark-field scattering images (top) and their mask images (bottom) obtained with varying the concentration of Tau. Scale bar represents 20 µm. Masks of dark-field scattering images were obtained ImageJ software program. (B) Calibration curves for Tau obtained from the particle coverage ratios.

Table S1. Comparison of working ranges and LOD values in various methods for detecting A $\beta$  and Tau.

Methods	Target	Working range	Limit of detection (LOD)
Our immunoassay system	Aβ	10 fM - 10 nM	170 fM
Commercial ELISA kit	Aβ	1 - 100 pM	1.39 pM
Colorimetric detection with gold nanoparticles <sup>42</sup>	Aβ	10.5 - 313.5 nM	600 pM
Electrochemical immunosensor <sup>43</sup>	Aβ	10 pM - 100 nM	10 pM
Dot-blot immunoassay with gold nanoparticles <sup>44</sup>	Aβ	20 pM - 2 µM	10 pM
Electrochemical detection with gold nanoparticles <sup>45</sup>	Aβ	500 nM - 20 μM	1 µM
Surface plasmon resonance detection <sup>46</sup>	Aβ	20 pM - 5 nM	3.3 pM
Our immunoassay system	Tau	10 fM - 10 nM	190 fM
Commercial ELISA kit	Tau	397 fM – 26 pM	128 fM
Quartz crystal balance (QCM) immunosensor <sup>47</sup>	Tau	0 – 500 nM	42 nM
Fluorescence quenching <sup>48</sup>	Tau	0 – 769 pM	82 pM



**Figure S6. Cross reaction tests of the on-chip plasmonic immunoassay.** (A) Signals of different targets for the A $\beta$  antibody-patterned chip. (B) Signals of different targets for the Tau antibody-patterned chip. (Top) dark-field scattering images and (bottom) particle coverage ratios for different targets. Scale bars represent 20 µm.