Proximity hybridization-triggered cascade amplification for label-free SERS detection of Alzheimer's amyloid-β oligomer

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2.1. Chemicals and materials

Hydrazine monohydrate (85%, AR), silver nitrate ($AgNO_3$), sodium dodecyl sulfate (SDS, 99%), and l-cysteine (99%) were provided by Sinopharm Chemical Reagent Co., Ltd. Oligonucleotides were purified using high performance liquid chromatography (Sangong Bioengineering Technology Co., Ltd. (Shanghai, China)).

DNA oligonucleotides used in this work were synthesized and purified by Takara Biotechnology Co., Ltd. (Dalian, China).

DNA1: 5'-SH-TTTTTTTTTTTTTTTTGTGAGGTTACTG ATGTTG A-3'

DNA2: 5'-TAG CTT ATCAGAACCTCACTTTTTTTTTTTTTTT-SH-3'

MB1: 5'-TCAACATCAGTCTGATAAGCTACCATGTGTAGATAGCTTATCAGA CTTTTTTTTTTT CGACATCTAACCTAGCTCACTGAC-3'

MB2: 5'-TAAGCTATCTACACATGGTAGCTTATCAGACTCCATGTGTAGATT TTTTTTTT CGACATCTAACCTAGCTCACTGAC-3'

H1:5'-SH-GTCAGTGAGCTAGGTTAGATGTCGCCATGTGTAGACGACATC TAACCTAGC-3'

H2: 5'-AGATGTCGTCTACACATGGCGACATCTAACCTAGCCCATG TGTAGA-3'

2.2 Probes of Ab-DNA1 and Ab-DNA2.

Prior research was followed in synthesizing the DNA-labeled antibodies (Ab-DNA1 or Ab-DNA2). In PBS with a 20-fold molar excess of SMCC, anti-amyloid-β oligomer antibody (2 mg mL^{-1}) was initially incubated for 2 h at room temperature. Parallel reduction of 3 ml 100 mM thiolated DNA1 with 4 μL 100 mM dithiothreitol (DTT) in PBS was carried out for 1 h at 37 °C. Both products underwent ultrafiltration using a

Millipore membrane with a 10, 000 MW cutoff, and the buffer was altered to PBE (55 mM phosphate, pH 7.4, 150 mM NaCl, 5 mM EDTA). The Ab-DNA1 was produced after the products were combined and incubated overnight at 4 °C with the unreacted DNA being filtered out using ultrafiltration.

2.3. Detection of AβO in serum samples.

The human serum samples employed in this research were provided by Xuzhou medical university-affiliated hospital, and our study received the approval of the ethics committee at Xuzhou Medical University. The obtained blood from six people was centrifuged after the natural coagulation, and we collected the supernatant for further use. After that, all the experimental conditions were similar to the targeted detection described above.

2.4. Raman analysis.

SERS were carried out using the Renishaw inVia Reflex Raman microscopy equipment (Renishaw, UK). A continuous He-Ne laser with a 514 nm wavelength and a 10 mW power output was employed for the excitation, the spectra across 400-1700 cm^{-1} were collected using 10 X objective lenses. Each sample has its spectra obtained at various random locations. Utilizing a commercially accessible spectroscopic analysis software program, spectra are examined after averaging and normalizing.