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Raman Dye-Labeled Nanoparticle Probes for Proteins

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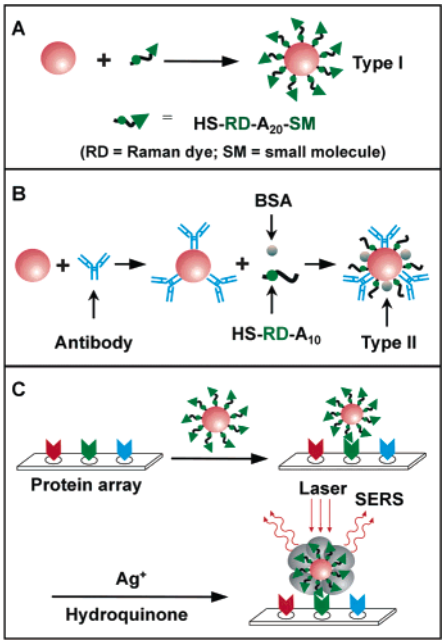
The detection of proteins and the subsequent understanding of their functions can provide direct answers as to how genetic alteration, drug exposure, or changes in the environment affect cellular physiology and pathology.[1] In principle, protein microarrays allow one to simultaneously map thousands of proteins in a high-throughput fashion, holding promise for the acceleration and development of molecular biology research, clinical diagnosis, and drug discovery.[2,3] Currently, microarray techniques mainly rely on the use of fluorescent molecular dye labels.[2] However, because of the broad emission spectra and narrow excitation spectra displayed by molecular fluorophores, this approach has several potential complications with respect to the parallel multiplexing and ratioing of analytes.[4−12] Recently, we developed a nanoparticle-based Raman spectroscopic encoding approach for DNA and RNA detection, which can overcome many of the drawbacks inherent to molecular fluorophores.[13] Herein, we demonstrate how one can chemically design Raman dye-functionalized nanoparticle probes with specific protein-binding affinities and use these probes, coupled with surface-enhanced Raman scattering (SERS) spectroscopy, to perform multiplexed screening of protein−small molecule interactions and protein−protein interactions in a microarray format.

Two types of nanoparticle probes have been designed for the studies reported herein. Type I probes are for screening protein−small molecule interactions, and Type II probes are for protein−protein interactions. All of the probes, regardless of their intended use, were designed to be water soluble, provide unique Raman spectra, exhibit specific target recognition properties, and be catalytically active with respect to silver plating in the presence of Ag(I) and hydroquinone.

For the Type I probes, to attain water-soluble structures, we have chosen nanoparticles coated with hydrophilic oligonucleotides rather than hydrophobic alkanethiols as our starting point in probe design. One advantage of this strategy is that very stable, water-soluble particles can be produced that withstand the chemical complexity of assay mixtures. Moreover, additional chemical functionality can be introduced to the particle via: (1) coupling chemistry during the synthesis of the oligonucleotides and (2) direct binding of molecules to the gold surface (Scheme 1A). A typical nanoparticle probe prepared via this strategy consists of a 13-nm diameter gold particle modified with alkylthiol-capped oligoadenotides (A_{20}), each terminally capped with a small molecule probe recognition element (e.g., biotin), and further modified at the opposite end with a Raman dye (Scheme 1A; see the protocol in Supporting Information). The resulting gold particle probes exhibit extraordinary stability to high salt concentrations (e.g., no change after 5 days under 1 M NaCl) and very specific target recognition properties (vide infra).

Type II particle probes, used for screening protein−protein interactions, were prepared via a modification of the strategy used to make Type I probes (Scheme 1B). Gold nanoparticles (13 nm) were first functionalized with antibodies by adding the antibody (10 μg, 100 μL, pH = 9.2) to a solution of the dispersed particles (10 nM, 1 mL, pH = 9.2). The solution was shaken for 20 min,

Scheme 1



and then a Raman dye-modified alkylthiol-capped oligoadenotide (HS-RD-A_{10}, 0.2 OD at 260 nm) was added to the solution. After 12 h, a 10% BSA solution (0.3 mL) was added to the solution to further passivate the surface of the gold particles. The solution was allowed to stand for 10 min, and then, the Raman dye-functionalized gold particle−antibody conjugates were purified by centrifugation (13 000g, 30 min) and separated from the supernatant. The particle probes were then redispersed in a PBS buffer solution (0.3 M NaCl, 10 mM phosphate, pH = 7.4).

In a typical assay (Scheme 1C), a protein chip was made by spotting the protein solution (200 μg/mL, 5% glycerol) onto aldehyde-functionalized glass slides with a commercial arrayer (GMS 417 Array, Genetic MicroSystem, Inc.).[14] The protein chip was exposed to a solution containing Raman-labeled nanoparticle probes (Type I or II, 2 nM, Scheme 1A,B) for 2 h at 4 °C. After being rinsed with 0.2 M $NaNO_3$ PBS buffer solution, the chip was treated with silver enhancement solution (Ted Pella, Inc.) for 8 min, subsequently washed with Nanopure water (18.2 MΩ·cm), and dried with a microarray centrifuge (2000g, 2 min). After being silver stained, the chip exhibited gray spots visible to the naked eye. The spots were studied using a Raman spectrometer coupled to a fiber-optic probe with a 0.65 N.A. microscope objective (25 μm laser beam diameter) in a 0.3 M NaCl PBS buffer solution (Solution Raman 633 spectrometer from Concurrent Analytical, Inc., 30 mW He−Ne laser).

To test the selectivity and suitability of this approach for the screening of protein−small molecule interactions, we selected three unrelated small molecules for which specific protein receptors are available: biotin (BTN) and its mouse monoclonal antibody,

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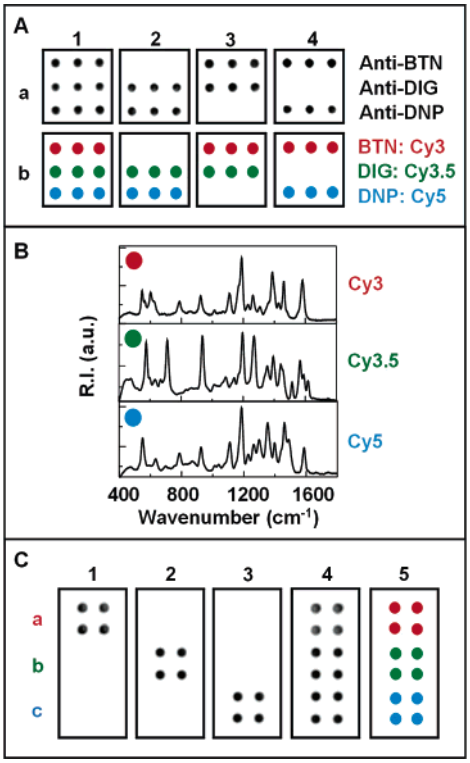

Figure 1. (A) Flatbed scanner images of silver-stained microarrays for protein−small molecule screening experiments (a1−4) and color code for the Raman spectra for the probes in the silver-stained spots (b1−4). (B) Typical Raman spectra (after background subtraction) corresponding to the colored dots in A (b1−4) and C5. (C) Flatbed scanner images of silver-stained microarrays for the protein−protein screening experiments (1−4) and color code for the Raman identification of the probes in the silver stained spots (5). For (C), (a) anti-mouse IgG was labeled with Cy3-modified alkylthiol-capped A10, (b) anti-ubiquitin by Cy3.5-modified alkylthiol-capped A10, and (c) anti-human protein C by Cy5-modified alkylthiol-capped A10. The A10 oligonucleotide spacer was used to enhance the stability of the particle probes. DNA spot diameter = 175 μm, distance = 375μm).

digoxigenin (DIG) and its monclonal antibody, and dinitrophenyl (DNP) and its mouse monoclonal antibody. Each of the three small molecules (BTN, DIG, or DNP) was labeled with Raman dye (Cy3, Cy3.5, or Cy5)-functionalized and alkylthiol-capped oligoadenotides (Scheme 1A). These oligonucleotides were subsequently used to functionalize 13-nm gold particles, respectively, using literature protocols.[15]

To evaluate the effectiveness of the Type I probes for detecting protein−small molecule interactions, a chip was spotted in triplicate with each monoclonal antibody and then exposed to a solution containing all three Raman-labeled Type I probes. They were subsequently rinsed with 0.2 M $NaNO_3$ PBS buffer and treated with silver enhancement solution for 8 min, which resulted in silver plating where the nanoparticle probes were bound to the substrate. After silver enhancement, the triplet dot array was clearly visible, even to the naked eye (Figure 1A, a1). When measuring the Raman spectra from each dot, we obtained the correct probe spectra with no evidence of cross-reactivity (Cy3 for biotin, Cy3.5 for DIG, and Cy5 for DNP) (Figure 1A, b1, and B). In a second test, we studied the detection selectivity using the same type of chip but in the presence of only two probes. One probe for the array was intentionally absent, serving as a control for screening the other interaction pairs. When DIG and DNP probes were present, the expected results were obtained (Figure 1A, a2 and b2). This type of experiment was repeated for all two-probe combinations, and again the expected results were obtained, thus demonstrating the high selectivity of the system (Figure 1A, a3, b3, a4, and b4).

This novel Raman dye labeling approach works equally well for screening protein−protein interactions. Conventionally, gold nanoparticles functionalized with proteins have been widely used in immunohistochemistry, primarily for mapping out protein−protein interactions.[15] However, a drawback of this single-color detection method is that it limits target multiplexing capabilities. Herein, we demonstrate that one can overcome this disadvantage using Raman-labeled gold probes (Type II, Scheme 1B). To test the multiplexing capabilities of this Raman approach for screening protein−protein interactions, we chose three pairs of proteins to study: mouse immunoglobulin G (IgG), ubiquitin, and human protein C, and their respective antibodies. Mouse IgG, ubiquitin, and human protein C were spotted in quadruplicate on aldehyde modified slides.[14] The probes (2 nM for gold nanoparticles and about 2 μg/mL for the antibodies) were then used to develop the protein chips. The protocol for screening the protein−protein interactions was similar to that for protein−small molecule interactions. First, the chip was probed with all three Raman-labeled antibody gold probes simultaneously (Figure 1C, 4). After silver enhancement, all three 2 × 2 dot arrays were clearly visible. Raman analysis showed no detectable cross-reactivity, and all of the dyes were on the correct spots. Next we studied the same type of chip but in the presence of each antibody probe, in turn, to demonstrate the high selectivity of the system (Figure 1C, 1−3). This Raman-based detection format displays all of the advantages of conventional gold particle labeling but allows for the multiplexing capabilities gained from the Raman spectroscopic fingerprints of different Raman dyes on the surface of gold nanoparticles. All of the Raman detection experiments above were carried out on the surface of glass chips. However, we found that the selection of substrates is also very flexible. Polymer (e.g., nitrocellulose, PVDF) substrates also work well for Raman detection experiments, exhibiting no substantial background from the polymer substrates that are typically used for Western blotting experiments (Supporting Information). Therefore, the Raman labeling technique described herein can also be applied to Western blotting experiments.

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Supporting Information Available: Details of the oligonucleotide synthesis and protein chip passivation procedures are available (PDF). This material is available free of charge via the Internet at http://pubs.acs. org

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