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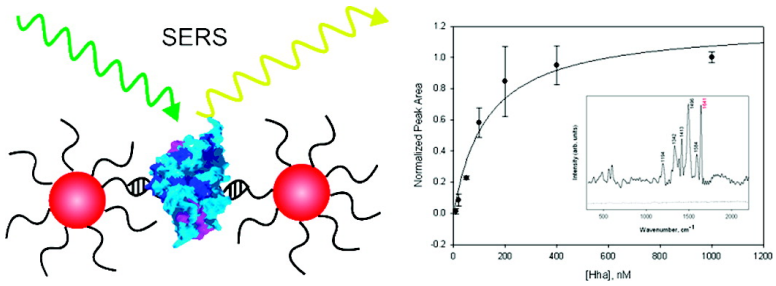

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Detection of Sequence-Specific Protein-DNA Interactions via Surface Enhanced Resonance Raman Scattering

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Practical and high-throughput assays for probing protein−ligand interactions are essential for proteomics and drug development.[1] For example, the analysis of multiprotein complexes involved in gene regulation is a combinatorial challenge with applications in medical diagnostics.[2] Here we describe an approach using surface-enhanced resonance Raman scattering (SERRS) for protein sensing in a tightly controlled assembly of gold nanoparticles and DNA, which has great potential for high sensitivity with high-throughput multiplexing capacity.[3]

SERRS techniques greatly enhance signal strength and sensitivity in many applications, with demonstrations of detection limits at the single-molecule level,[4,5] while offering other important benefits over fluorescent detection methods, including resistance to photobleaching and narrow emission peaks for spectral multiplexing.[6] However, the enhancement possible from SERRS is very dependent on the distance between, the surface morphology of, and the optical resonance of closely associated metal nanoparticles, making the design of controlled assemblies paramount to correctly position analytes for optimal detection.[7]

We describe an effective architecture of DNA-bridged nanoparticle assemblies for binding and detecting sequence and concentration dependent protein-DNA interactions. Each short stretch of duplex DNA, which is to be bound by the analyte protein, is prepared with overhangs that hybridize and cross-link a generic set of gold nanoparticles (NPs) functionalized with complementary DNA.[8] This self-assembling scaffold allows control of the positioning of metallic NPs to directly surround a DNA sequence recognized by an analyte protein (tagged with a resonance Raman molecule). These NPs are subsequently grown using a silver plating step to decrease the distance between the surfaces and the analyte causing a large increase in SERRS signal, detected by a confocal Raman microprobe.[5,9]

The assembly consists of a three-part oligonucleotide scaffolding tethering NPs as shown in Figure 1A. Double-stranded oligonucleotides C (oligo-C) of lengths 15 to 39 base pairs containing the protein binding site of interest were designed to generate appropriate spacing for protein access into the final assemblies, with 12 base pair single-stranded overhangs on each end that are complementary to surface-bound 22 base pair oligonucleotides A or B (oligo-A or oligo-B, DNA sequences in Supporting Information). Gold NPs diameter of ∼13 nm were prepared by citrate reduction of gold aurate,[11] and the resultant citrate shell was displaced by thiol-modified oligo-A or oligo-B[10]. Conjugates were determined to have 183 ± 20 oligonucleotides per particle (Supporting Information). Nanoparticles of this size have previously been used as seeds for silver plating and multiplexed detection by SERRS.[11]

Upon annealing of the single-stranded overhangs, the three components condense into assemblies.[10] Variations of assemblies were formed with oligo-**C** containing the GCGC recognition site of M.HhaI,[12] the TATA-box recognition site of TATA-binding protein (TBP),[13] or with a central thymine base coupled to a biotin for streptavidin.[14] Noncognate sequences were used as controls to test for nonspecific protein-DNA binding. TEM imaging was used to analyze particle size and dispersity for oligo-NPs and assemblies (Supporting Information).

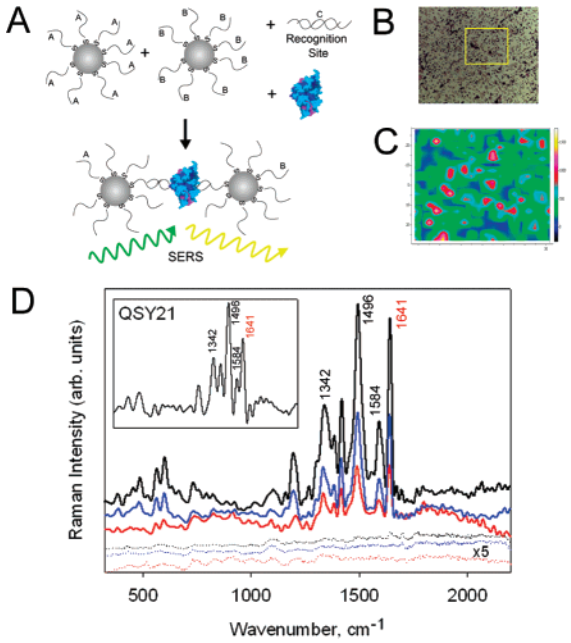

Figure 1. Detection of protein binding to ABC assemblies via SERRS: (A) schema of the formation of assemblies; (B) optical image of ABC assemblies deposited on glass and silver plated. Yellow rectangle indicates size of area probed by Raman mapping; (C) Raman point map of a 50 μm × 50 μm region, with position intensity corresponding to a peak at 1641 cm^{-1} of the Raman label; (**D**) averaged spectra of labeled proteins: streptavidin (black), TBP (blue), and M.HhaI (red) with cognate-DNA (solid lines) and noncognate-DNA (×5 signal, dotted lines). Background correction has been applied. Inset shows spectrum of QSY21 in silver colloid solution as a reference. The peak at 1641 cm^{-1} (red) was used as the reference for comparison of signal intensity across trials.

The binding of DNA cytosine-C5 methyltransferase M.HhaI and yeast TBP to DNA were investigated by collecting SERRS from the ABC assemblies. The interaction of streptavidin with a biotinylated DNA target served as both proof of concept for small ligand binding interactions in the assemblies and as a positive binding control.[15] All proteins used in this study were labeled with an amine-reactive, fluorescence quencher molecule QSY21 (Invitrogen), chosen because of its strong resonance at the laser line used (633 nm) and low fluorescence emission to minimize any background.[16] Extent of protein-labeling was determined by UV−vis absorbance to be 1.8 ± 0.9 labels/protein, and electrophoretic binding measurements were used to confirm that the dye-modified proteins display the expected affinity toward DNA (Supporting

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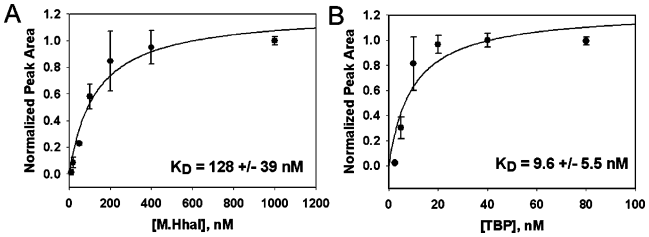

Figure 2. Raman mapping measurement of protein-DNA complex formation using normalized peak areas from ABC assemblies. (A) QSY-21 labeled M.HhaI was incubated with ABC assemblies with M.HhaI recognition sequence on C duplex. (B) QSY-21 labeled TBP was incubated with ABC assemblies with TATA recognition sequence on C duplex. Each data point was derived from multiple Raman maps, $n = 3-5$.

Information). This confirmation is necessary as many DNA-binding proteins interact with DNA through binding-site lysine residues which could be disrupted by amine-reactive labels.

An important feature of protein-DNA assays is the ability to robustly distinguish between specific and nonspecific interactions. To test this, proteins were incubated with cognate and noncognate assemblies at concentrations in excess of their dissociation constants for DNA, then spotted and silver plated. Clusters of assemblies are clearly visible at 50× magnification (Figure 1B) and spectra were collected over a period of 2 min from 400 points across a 50 μm × 50 μm grid in line scanning mode to detect the presence of the Raman label QSY21. The spectra display distinct peaks at 1342, 1584, and 1641 cm^{-1} which are strong in all QSY21 spectra collected and are characteristic of the xanthene ring stretching vibrations, observed in structurally similar dyes such as rhodamine 6G and fluorescein.[17] For quantitation we chose to integrate the 1641 cm^{-1} band and generate Raman maps (Figure 1C). The signals were averaged from a set of ABC assemblies to correct for the variability in signal present due to differences in the dispersion deposited on the glass substrate and the occupation of labeled-protein present in the hot spots.

Figure 1D shows that spectra obtained from each labeled protein (streptavidin, M.HhaI, and TBP) with their respective cognate assemblies (solid lines) display strong peaks not found in the noncognate samples (dotted lines). Noncognate DNA signal intensity is at near baseline levels, displaying only weak Raman signals from the (unlabeled) DNA scaffold and glass substrate, indicating little if any nonspecific protein binding. When one NP was not included in the self-assembly reaction, signal was not distinguishable from background until a concentration of 1 uM M.HhaI was present, showing the necessity of the full assembly for efficient hot spot formation. Furthermore, QSY21 signals were not observed in free NP-**A** or NP-**B** incubated with QSY21 molecules or QSY21-labeled protein (centrifuged and washed as described, data not shown), indicating that label-induced binding to the NPs does not occur.

Another important feature to those studying protein−nucleic acid interactions is the affinity of a particular protein for its recognition sequence.[18] The Raman intensity correlates with the concentrations of QSY21-labeled M.HhaI (5 nM−1 μM) and TBP (1−80 nM) in a sequence-specific manner (Figure 2). The binding isotherms generated from this data were used to calculate apparent dissociation constants which correlate well with literature values obtained by conventional gel-based methods.[12,13] Using this method, TBP has an apparent K_D of 9.6 ± 5.5 nM, which falls within the range error limits of the literature value 5 nM.[13] M.HhaI in the absence of its cofactor, *S*-adenosyl-methionine, (not used in this study) has an apparent K_D of 128 ± 39 nM, comparable to the literature value of 100 ± 30 nM.[12]

The SERRS approach described here provides a new strategy to investigate protein−DNA interactions which is applicable to a range of proteins, either by direct recognition of the DNA by DNA-binding proteins or by attachment of ligand moieties to the central DNA strand (or an alternative chemical linker). Raman bands have very narrow widths in comparison to traditional detection labels such as fluorophores and quantum dots[6] and so could, in principle, be chosen to identify over a dozen unique and assignable peaks simultaneously. Spectral deconvolution of features from multiple labels across an array of assemblies would allow multiplex probing of a series of NP-DNA sites for the various proteins, using a single laser excitation line.

An in-depth understanding of transcriptional regulation specificity and dynamics will require techniques to probe this large interaction-space of possible transient and stable complexes.[19] The programmability of DNA-NP assemblies allows for systematic studies in sequence space, scaffold size, architecture, attachment chemistry, and DNA/NP stoichiometry. We are currently exploring mismatch recognition and methods to modify assembly size, including single protein analysis through isolated NP−NP dimers or through scanning a monolayer of NPs bound to metal films through DNA.[20]

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Supporting Information Available: Details of experimental procedures, DNA sequences used, NP data, and TEM and Raman conditions. This material is available free of charge via the Internet at http://pubs.acs.org.

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