Single gold-bridged nanoprobes for identification of single point DNA mutations

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Consensus ranking of protein affinity to identify point mutations has not been established. Therefore, analytical techniques that can detect subtle variations without interfering with native biomolecular interactions are required. Here we report a rapid method to identify point mutations by a single nanoparticle sensing system. DNA-directed gold crystallization forms rod-like nanoparticles with bridges based on structural design. The nanoparticles enhance Rayleigh light scattering, achieving high refractive-index sensitivity, and enable the system to monitor even a small number of protein-DNA binding events without interference. Analysis of the binding affinity can compile an atlas to distinguish the potential of various point mutations recognized by MutS protein. We use the atlas to analyze the presence and type of single point mutations in BRCA1 from samples of human breast and ovarian cancer cell lines. The strategy of synthesis-by-design of plasmonic nanoparticles for sensors enables direct identification of subtle biomolecular binding distortions and genetic alterations.

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Many diseases have a genetic component; their detections therefore require a clear understanding of underlying mutations\(^1\). For example, approximately 12% of women will develop breast cancer during their lives, with the highest risk conferred by *BRCA1* mutations (59–87%)\(^2\). Most methods for identifying gene mutations rely on sequencing\(^3\), but a method that can detect the presence and identity of single mutant bases without prior knowledge of the sequence—ideally without artifacts from labels and the in vitro environment—is desired. The specificity of the biological interaction between the post-replicative mismatch repair (MMR) initiation protein MutS and mismatched DNA enables detection of nucleotide polymorphisms by methods such as single-molecule fluorescence resonance energy transfer (smFRET); however, these require labor-intensive steps such as labeling of MutS or fabrication of radioactive probes for DNA\(^4–7\). Visualizing MutS molecules by atomic force microscopy is complicated and difficult to apply to biomedical sensors\(^8\). On the other hand, bulk measurements of point mutations by gel mobility shift and filter/chip binding assays do not output real-time information on molecular interactions\(^10–13\), whereas measurements by surface plasmon resonance (SPR), electrochemical assay, and quartz crystal microbalance (QCM) are time consuming and inefficient (detailed in Supplementary Note 1)\(^14–16\). Accurate methods for quantifying protein binding affinity over arbitrarily full DNA footprints are currently limited to computational approaches\(^17\). An atlas of nucleic acid–MutS binding affinities at single-base resolution that reveals changes in gene regulation in disease states and describes the relationship between point mutation type and repair efficiency of the MMR system would be highly useful, but is currently lacking\(^18\).

For advanced sensor applications, plasmonic nanoparticles (NPs) have attracted interest due to their ability to interact with light and produce localized SPR (LSPR). The collective oscillation of electrons in the nanostructure at a given resonant frequency transduces changes in the local refractive index (RI) into shifts in the plasmonic bands of their absorption and scattering spectra\(^19\). The sensing scale can be reduced to a single NP; such single NP sensing (sNPS) can relay local biological information on a nanometer scale in which the limit of detection (LOD) reaches countable numbers of molecules using a very small sensing volume. For example, the MutS protein is 125 × 90 × 55 Å\(^20\); thus, adsorption of MutS onto a single NP can drastically alter the collective oscillatory behavior of its surface electrons, resulting in wavelength shifts in the NP spectra\(^21\). In contrast, most other sensing techniques using bulk solutions or planar surfaces show a limited ability to localize and separate sensing elements and are limited by slow molecular diffusion, stochastic binding, and frequent dissociation of complexed biomolecules with consequent disequilibrium of reactions, resulting in signal fluctuations with a low signal-to-noise ratio (S/N). An sNPS sensor is a tiny probe capable of high-throughput and parallel readout in which the structure and localized sensing volume/area of the NP are essential for RI sensitivity. Studies have shown that rod-like NPs exhibit the highest sensitivity to changes in RI\(^22\). Nanogap and nanobridge structures are associated with and generate strong optical signals by plasmonic coupling, further enhancing the local field to generate distinct spectral responses\(^23,24\). However, synthesizing colloidal plasmonic NPs with a predefined structure is challenging due to the difficulty in manipulating atoms that are transient in solution\(^25\). Moreover, chemically synthesized nanostructures are restricted to a highly symmetric shape with identical surface facets (e.g., nanospheres (NSphs), nanorods (NRs), nanocubes, nanodisks, and others)\(^26\). Two research groups recently achieved breakthroughs in synthesis-by-design at sub-5 nm precision using a programmable biomolecule—i.e., DNA—to create nanoplasmnic particles either by casting in DNA molds\(^27\) or using DNA frameworks\(^28\). Structurally programmable NPs could overcome sNPS limitations such as low sensitivity and reproducibility.

Here we report the synthesis-by-design of plasmonic nanostructures for detecting a countable number of biomolecule binding events. The nanostructure with a rod shape and one nanobridge (bridged NP) show higher RI sensitivity than similarly sized NSphs, NRs, and NPs with a nanogap (NP-gap). The bridged NPs are synthesized in solution by highly controlled direction-specific crystallization whereby double-stranded DNA (dsDNA) molecules with adjustable lengths and surface charges subtly regulate Au atom crystallization, a process distinct from Au metallization. The Au-bridged NPs utilize low-energy white light as a signal source in an sNPS system, which preserve intrinsic MutS–DNA interactions and enable different point mutations to be recognized by MutS with high speed, resolution, and fidelity. The method generates the atlas of MutS affinities to various synthetic DNA samples, and further applies to analyze genomic DNA extracted from human breast and ovarian cancer cells for directly identifying mutated single bases.

**Results**

**NP design with numerical simulations.** Since each NP functions as a signal transducer in the sNPS platform, NP structure and shape should be homogeneous and controllable\(^19\). This excludes irregularly shaped nanocrystals (e.g., branched nanostars), since their formation is empirical rather than based on the principles of synthesis\(^26–28\). Furthermore, the controllability of polyhedral nanostructures is limited by the lack of chemicals that can specifically tune targeted crystal facets and thus produce NPs with a relatively high yield\(^29\). We therefore selected nanostructures in the shape of spheres and rods as substrates for sNPS, since both can be synthesized in a uniform and scalable manner. We also introduced structures consisting of nanogaps and nanobridges that induce distinct spectral responses and influence the magnitude of plasmonic coupling, polarization direction, signal intensity, and RI sensitivity (Fig. 1a)\(^23,24\). Instead of using complex biomarkers to quantify RI sensitivity, we performed optical simulations of single NPs with predesigned structures in which the RI of the surrounding medium was set to change (Supplementary Fig. 1). RI sensitivity was quantified by analyzing changes in LSPR wavelength (λ\(_{\text{max}}\)) of single Au-NPs induced by different RI solutions\(^22\). Changes in λ\(_{\text{max}}\) corresponding to each change in RI were expressed as a linear fit (Fig. 1b) in which the slope of the line represents RI sensitivity of the NPs. Interestingly, NP-gap and bridged NP showed better performance than the NR, which was previously thought to be the optimal structure\(^22\). This is a direct consequence of the high field concentration provided by nanogap and nanobridge structures. In particular, the Au-bridged NP showed twofold higher sensitivity than did the AuNR, making it an ultrasensitive candidate material for sNPS fabrication.

**Synthesis-by-design of NPs.** We explored the feasibility of direction-specific crystallization by which one dsDNA anchored between two Au nanoseeds (AuNSs; 5 nm in diameter) served as a directional guide for the crystallization of Au atoms into nanobridges (Supplementary Fig. 2). Interestingly, altering the surface charge of dsDNA by adjusting the pH also generated nanogaps smaller than 1 nm (Fig. 1c). At pH 5, dsDNA is slightly negatively charged and electrostatically concentrates NH\(_3\)OH\(^+\). The reaction between NH\(_3\)OH\(^+\) and AuCl\(_4^-\) induced Au crystallization along the DNA. Consequently, Au-bridged NPs with a length of 31.15 ± 1.00 nm and a diameter of 14.38 ± 0.58 nm for the two spherical ends and 8.79 ± 0.96 nm for the bridge were...
formed (Supplementary Table 1). The yield of the desired morphology was 87%, and the nanostructures were in a relatively high monodispersity (Supplementary Fig. 3). In contrast, at pH 4, dsDNA is positively charged owing to its isoelectric point (pI) of 4–4.5.\(^{-1}\) The DNA repelled NH\(_3\)OH\(^+\) by an electrostatic repulsive force, and therefore, the reaction between NH\(_3\)OH\(^+\) and AuCl\(_4\)^{−}\) occurred mostly near the AuNS, which further autocatalyzed the crystallization of Au atoms surrounding its surface. The reaction ended with the complete oxidation of Au ions into atoms, leaving a 0.44 nm gap between the two nanospheres (17.01 ± 1.07 nm in diameter).

Notably, crystallization occurred in specific directions from the AuNS-dsDNA boundaries to the mid-point of the dsDNA strand, with nanoscale controllability defined by the length of dsDNA. This method differs fundamentally from conventional approaches involving metallizing DNA or DNA origami, in which either sequential necklaces or continuous bulges are formed with poorly controlled structural precision (>100 nm).\(^{31}\) The directional effect of DNA in the synthesis of Au-bridged NPs was evaluated by X-ray diffraction and high-resolution transmission electron microscopy (HR-TEM) (Fig. 1d, e). The AuNSs exhibited peaks of an fcc structure of Au (JCPDS No. 03-0921) at 38° (111) and 44.1° (200).
44° (200). The peak positions showed clear shifts after DNA-directed crystallization, indicating that the DNA-induced significant lattice strain in the Au-bridged nanostructure26. The narrower linewidth of the peaks indirectly reflected enlarged particle sizes. Furthermore, HR-TEM images of the nanoscale bridge regime revealed crystal planes with a spacing of 0.208 ± 0.004 nm, corresponding to (200) lattice fringes in the <100> crystallization direction28.

sNPS with Au-bridged NPs. We investigated resonant Rayleigh light scattering (RLS) responses of a single Au-bridged NP by sNPS with a white light source (Supplementary Fig. 4). The light generated LSPR with NPs that sufficiently enhanced light scattering to allow for direct observation of individual NPs; on the other hand, the white light illumination avoided high energy and heat that could denature target biomolecules or block molecular interactions in the microfluidic reaction chamber19 (Fig. 2a). The RLS spectrum of a single Au-bridged NP had two surface plasmon peaks (Fig. 2b): one was related to electron oscillation in the transverse direction, resulting in a relatively weak resonance band, whereas the other was stronger and was related to electron oscillation in the longitudinal direction. We focused on longitudinal surface plasmon peaks since the longitudinal mode is more sensitive to changes in the dielectric constant of the medium than the transverse one22. The adsorbates of the medium were single-stranded DNA (ssDNA) and MutS protein. The probe ssDNA was anchored on Au-bridged NPs by thiol modification and then hybridized with a target ssDNA through hydrogen (H)-bonding and π–π stacking, during which process the $\lambda_{\text{max}}$ red shifted from 561.1 ± 0.5 nm to 570.7 ± 0.5 nm (steps 1 and 2; Fig. 2c). MutS with a positively charged surface sequence independently contacted and caused clenching of the negatively charged DNA backbone at mismatched points with the conserved Phe-Xaa-Glu motif39, resulting in a red shift of up to 24 nm relative to the $\lambda_{\text{max}}$ of bare NPs to 585.3 ± 0.5 nm (steps 2 and 3; Fig. 2c). According to the Mic theory, the LSPR of metallic NPs depends on the shape, size, and RI of the local dielectric environment. Using an individual NP eliminated differences in shape and size; thus, the LSPR $\lambda_{\text{max}}$ shifts were attributed to changes in the NP interface upon DNA hybridization and subsequent MutS binding (Fig. 2d). To verify the specificity of the peak shifts that occurred with the recognition of mutations by MutS, we tested the DNA target in human serum without MutS as well as DNA without mutations (i.e., a perfectly matched target). As expected, few spectral changes were observed in either experiment (Supplementary Fig. 5). After treating the microfluidic chambers at 95 °C, we detected the mutant target in serum solution containing MutS and observed identical red shifts, confirming that the fabricated single NP sensor preserved the specificity of MutS for DNA mutations.

Sensitivity of sensing. We further investigated the sensitivity of the sNPS sensing method according to two parameters: the lowest concentration of MutS protein enabling an LSPR $\lambda_{\text{max}}$ shift ($\Delta \lambda_{\text{max}}$) to be effective within a certain detection time, and the LOD for analytes. After the MutS solution had arrived at the DNA-modified Au-bridged NPs in the microfluidic chamber, we allowed the reaction to continue for 1 min before obtaining RLS spectra for 10 s. An excess of DNA target was added to ensure complete hybridization with the probes. The effective concentration of MutS protein for the LSPR readout was 6.17 nM, corresponding to a 3.40 nm red shift in $\lambda_{\text{max}}$ in the linear range of 10–25 nM MutS (Fig. 2e). We performed measurements using a blank sample and DNA targets at different concentrations to determine the LOD (Fig. 2f). The analytical range of 5–150 nM, where a plot of concentrations versus responses went linearly with an $R^2$ of 0.9954, was observed, beyond which the linearity was inconsistent. The S/N was 9.86 while monitoring the 5 nM target. The LOD was calculated as 8.63 nM, which is comparable to that obtained with the label-free QCM method, and tens of fold lower than the value determined by label-free SPR bulk detection (Supplementary Note 1, Supplementary Fig. 14 and Supplementary Table 4)14,16. Most importantly, this high sensitivity was achieved at a flow rate of 1 μl min$^{-1}$, and the total sample volume required for each detection was 30 μl with trace levels of sample. Excess and nonspecific materials were washed out of the microfluidic chamber and did not interfere with particle sensing. In contrast, fluorescence sensing methods require large amounts of reagent and many processing steps (e.g., MutS requires fluorophore modification at a pre-concentration >3 µM in buffers to achieve a labeling efficiency of <55%)6,33. A gel mobility shift assay requires loading of only a small volume, but samples must be highly concentrated for visualization10,34. Notably, the MutS footprint is 24 base pairs (bp), whereas interactions between protein and DNA are distributed over a large surface area (1250 Å$^2$ or approximately 50 bp$^2$). The 51 bp ssDNA probe used in this study minimized signal loss due to nonspecific target binding and ensured clear differentiation between signal changes induced by different point mutations.

Identification of single point mutations. Point mutations are the most difficult to detect of all genetic alterations due to their subtle nature. We speculated that sequence-nonspecific binding of MutS to point mutations alter LSPR signals; to test this hypothesis, we examined the relative activity of MutS towards different nucleotide variants. We reviewed and selected eight most frequent polymorphisms from the spectrum of BRCA1 mutations35–39, including six single-nucleotide substitutions (GT, GG, AC, TC, AA, and GA), an insertion (+C), and a deletion (−C). The DNA sequences, mutant names, genomic locations, functional consequences, and target populations are summarized in Supplementary Table 2. Upon injection into the sNPS chamber, MutS was allowed to bind to DNA-conjugated Au-bridged NPs for 150 s, and the changes in the optical response of a single NP were monitored every 1 s (Fig. 3a). Notably, MutS was loaded on homoduplex (perfectly matched) DNA for approximately 15 s according to real-time signal responses. This was consistent with a previous report that MutS forms a short-lived clamp and moves along homoduplex DNA by one-dimensional diffusional sliding, presumably in search of mismatched bases4. Mismatch identification resulted in a MutS binding time 10-fold longer than that of the homoduplex and induced serial $\Delta \lambda_{\text{max}}$. Since this was caused by RI changes upon MutS binding to a DNA-conjugated NP, the time course clearly reflects the distinct activities of MutS in recognizing different point mutations, which presumably altered the contact between MutS and DNA, thus producing variable reaction constants in kinetic assays of MutS–DNA interactions.

We defined the relative activity of MutS to mutant DNA ($R_{\text{act}}$) as the efficiency with which MutS binds to mutant DNA, expressed as $R_{\text{act}} = K \times k_{\text{reaction}}$ where $K$ is an occupancy constant and $k_{\text{reaction}}$ is the rate constant of the protein–DNA interaction40. This is a simple approximation of a stochastic binding event in which DNA on the Au-bridged NP is equally available for MutS; therefore, the same detection conditions allow the same event in which DNA on the Au-bridged NP is equally available for MutS; therefore, the same detection conditions allow the same $K$ and $R_{\text{act}}$ to be evaluated according to $k_{\text{reaction}}$. The DNA probe length in this study (51 bp) implied 1:1 binding stoichiometry with MutS; thus, the time course of binding and dissociation can be described as a single exponential process. By fitting to the exponential equation, the $k_{\text{reaction}}$ (10$^{-2}$ s$^{-1}$) values of MutS binding to different DNA targets were 9.95 ± 0.420, 6.15 ± 0.208,
5.80 ± 0.189, 4.92 ± 0.214, 3.82 ± 0.212, 3.60 ± 0.243, 3.25 ± 0.184, and 2.82 ± 0.197 for the point mutations GT, GG, +C, AA, TC, −C, AC, and GA, respectively. By replotting the reaction values as a function of each target DNA, the order of relative activity of MutS towards the mutations was determined as GT>GG>−C>AA>TC>AC>GA (Fig. 3b), which is consistent with previous gel mobility shift assay data10,34.

Reliability of sensing. The crystal structure and interactions of MutS binding to a GT mismatch have been previously described in detail20,32,41,42. We therefore evaluated the reliability of the sNPS platform based on the reaction of MutS and GT-mutant DNA interaction. The kinetics of MutS binding to and dissociation from DNA can be described as

\[ k_{\text{reaction}} = k_{\text{binding}} [\text{MutS}] + k_{\text{dissociation}} \]

where \( k_{\text{binding}} \) and \( k_{\text{dissociation}} \) are the binding and dissociation rate constants, respectively, and [MutS] represents the free molar concentration of MutS. [MutS] clearly affected reaction kinetics and corresponded to the amplitude of variation in \( \Delta \lambda_{\text{max}} \) (Fig. 4a). Higher [MutS] induced greater increases in \( \lambda_{\text{max}} \) prior to equilibrium, ultimately leading to longer shifts at the end of the interaction, which started at the maximum rate since no MutS had been consumed before the reaction slowed in a predictable manner to an equilibrium distribution of MutS. The rate constant \( k_{\text{reaction}} \) was quantitatively estimated by exponential fitting.

Fig. 2 Fabrication of single nanoparticle (NP) biosensor of high sensitivity and fidelity. a Schematic illustration of single NP sensing (sNPS) for identifying single point DNA mutations. b Representative Rayleigh light scattering (RLS) spectra and in-situ dark-field microscopy image of an Au-bridged NP. Scale bar, 1 μm. c Localized surface plasmon resonance (LSPR) \( \lambda_{\text{max}} \) shifts upon binding of DNA and MutS. Same line legend for (b, c). Each step of molecular binding (1, 2, and 3) illustrated in (d). Insets: real-time images of a single NP obtained with charge-coupled device (CCD) camera. e Concentration of MutS for adequate signal response. Calibration curve showing the linear relationship between the \( \Delta \lambda_{\text{max}} \) and various concentrations of MutS. f Limit of detection (LOD) of the mutant DNA target. Calibration curve showing the linear relationship between the \( \Delta \lambda_{\text{max}} \) and various concentrations of target DNA. For all panels, error bars represent mean ± S.E.M. All experiments were performed in triplicate. Source data of Fig. 2b, c, e, and f are provided as a Source Data file.
Replotting $k_{\text{reaction}}$ as a function of [MutS] yielded a linear equation (Fig. 4b) with $k_{\text{dissociation}}$ as the y intercept and $k_{\text{binding}}$ as the slope. The $k_{\text{binding}}$ of $2.97 \times 10^6 \, \text{M}^{-1} \, \text{s}^{-1}$ was close to previously reported bulk kinetic measurements of $3\text{–}6 \times 10^6 \, \text{M}^{-1} \, \text{s}^{-1}$.

Kinetic studies of the ratio between $k_{\text{dissociation}}$ and $k_{\text{binding}}$ revealed the dissociation equilibrium constant of MutS to DNA—i.e., $K_D$, a fundamental parameter of ligand affinity. The $K_D$ of MutS was found to be 4.46 nM, which was in agreement with reported smFRET and bulk measurements of 2–20 nM.$^{4,43}$ This is the validation by kinetic studies of the equilibrium constant of MutS on a precise scale around a single NP, and supports the utility of the sNPS assay for biological applications.

**Atlas of MutS affinities to point mutations.** We further established an atlas of protein binding affinities to DNA with four types of point mutation (Fig. 5): highly identifiable ($k_{\text{reaction}} > 0.07$), identifiable ($k_{\text{reaction}} = 0.05\text{–}0.07$), highly detectable ($k_{\text{reaction}} = 0.03\text{–}0.05$), and detectable ($k_{\text{reaction}} < 0.03$). The atlas shows comprehensive information obtained by low-input, high-fidelity sNPS of the relative activity and reaction half-time for each target, mutation type, and detection signal. Specifically, each circle represents a single point mutation, with the diameter and color reflecting the signal response for quantifying the LSPR peak shift and mutation category, respectively. For example, the target DNA with GT mutation generated a peak shift of 14.2 nm, the value of which is affected by and is in proportion to the concentration of targets (100 nM). The mutation category is biologically divided into three types, colored in blue, green, and red, of which the blue one indicates a transition mutation (replacement of a pyrimidine with a pyrimidine or vice versa), the green one indicates a transversion mutation (replacement of a pyrimidine with a purine or vice versa), and the red one indicates a bulging mutation (a base insertion or deletion). The $y$ and $x$ coordinates of the center of each circle represent the relative activity and half-time of the reaction, respectively. The relative activity predicted by $k_{\text{reaction}}$ is dependent on the concentration of MutS used in the detections and the $K_D$ of MutS to the target DNA. Therefore,
single point mutations can be identified using MutS of the same quality and quantity by this sNPS method. The half-time of the reaction gives knowledge of the time it takes for MutS binding to the individual NPs to reach half of the maximum LSPR shift. Such knowledge can help to explain why, for instance, purine–purine mutations show better repair rates than pyrimidine–pyrimidine mutations in cells, as evidenced by our observation that MutS bound more strongly to the purine–purine mutation (e.g., identifiable GG and AA) than to the pyrimidine–pyrimidine mutation (e.g., highly detectable TC). These results also indicate that repair of −C, AC, and GA mutations will be less effective since MutS has lower relative activity towards these than towards TC.

As a proof-of-principle demonstration of clinical applications of the atlas, we prepared biological DNA samples from the human breast cancer cell lines, HCC1937 and MCF7, as an analyte and a control, respectively, and detected the presence and type of a potential point mutation among the eight mutations shown in the atlas (Supplementary Figs. 6–8). Conspicuously, the glass chip designed for the detection of 5382insC (Supplementary Table 2) exhibited a series of peak shifts, while the other chips did not show significant signal variations (Fig. 6). Kinetic studies on the peak shifts in response to the detection time yielded a $k_{\text{reaction}}$ of $5.73 \pm 0.071 \times 10^{-2} \text{s}^{-1}$, which was in close proximity to the position of +C in the atlas (Fig. 7a, c), demonstrating that BRCA1 of HCC1937 contains a single cytosine duplication. This result was confirmed by DNA sequencing (Supplementary Fig. 9).

Additionally, the circle diameter of the target outputted the information of its concentration; for example, an 8.25 nm diameter indicated a 68.8 nM target according to the circle diameter (12 nm) of the +C in the atlas, which was obtained with a standard concentration of 100 nM.

Finally, we applied this sNPS system to detect potential point mutations in a user-assigned genomic region. A potential BRCA1 point mutation located at 43047665 on region 2 band 1 of the long arm of chromosome 17 was assigned to test an ovarian cancer cell line, SNU251. We fabricated the chip with the same Au-bridged NP but with a new 64 bp probe. Interestingly, continuous shifts of the spectral peaks were observed, validating the effectiveness of the sNPS with the new probe to monitor a specific interval in the gene (Fig. 7b and Supplementary Fig. 10). Further, input of the detection results into the atlas indicated that the type of mutation was highly similar to AC point mutation (Fig. 7c). It is noteworthy that the $k_{\text{reaction}}$ (10$^{-2}$ s$^{-1}$) yielded the similar values (3.20 in the detection versus 3.24 in the atlas), although the flanking sequences of the newly designed probe and the previously used AC probe were different. The small diameter of the purple circle indicated a low concentration of the target. The prediction of the AC mutation was confirmed to be accurate by DNA sequencing (Supplementary Fig. 11).

**Discussion**

Our sNPS system enables low-input profiling of single point mutations in DNA by taking advantage of redesigned Au-bridged NPs. Plasmonics combined with scattering spectroscopy has contributed to advances in single-particle investigations by allowing light to be concentrated in plasmonic hotspots, while different nanostructures with bridges and gaps have been used in Raman and Rayleigh spectroscopy. Despite significant advances, these technologies have two critical challenges: (1) artificial signal reporters and high-energy laser concentration on NPs for Raman sensing hinder original probe–target interactions; and (2) pre-designed plasmonic nanostructures are difficult to synthesize in solution. We used white light for label-free RLS from single particles, thereby preserving the physiological and biochemical conditions of the molecules. NPs with nanoscale bridges and gaps were obtained by direction-specific synthesis; this synthesis strategy controls the crystallization of Au atoms along the DNA framework from the nanoseed surface. In contrast to DNA or DNA origami-mediated metallization of polyploid structures, the products are colloidal crystalline particles comprising either nanobridges or nanogaps, which are adjustable by altering the

**Fig. 5** Atlas of MutS affinities to different point mutations. The atlas was established by single Au-bridged nanoparticle (NP) sensing a countable number of binding events between MutS and eight different mutations. Source data are provided as a Source Data file.
ionic environment of the DNA interface. The nanobridge effectively increased refractometric plasmonic sensitivity for RLS, making the Au-bridged NP more sensitive than an AuNR of the same size. This demonstrates the DNA-directed synthesis-by-design of colloidal plasmonic nanostructures; the synthesized NPs can potentially be used for sensing and imaging biological interactions at high speed, resolution, and fidelity.

The implementation of the sNPS platform relies on microfluidic systems. On the scale of a few tens of nanometers, uidic systems can be manufactured by advanced nanofabrication techniques. To count the number of biomolecules in the sNPS, we investigated the effective space of plasmonic sensitivity to refractive index changes surrounding an individual nanoparticle, termed as plasmonic field of interest (FOI). The volume of the FOI ($V$) was calculated to be 0.953 $\mu$m$^3$ (Supplementary Fig. 12). Here, the $N$ can be obtained from the molar concentration of biomolecules ($C$) by $N = C \times V \times N_A$, where $N_A$ is the Avogadro’s constant. The lowest concentration of 6.17 nM protein in the $\Delta \lambda_{\text{max}}$ confidence assays indicated that at least 3.5 MutS proteins in average must concurrently bind to the mutant DNA for a 3.40 nm red shift in $\lambda_{\text{max}}$. The LOD value of 8.63 nM indicated that the detection was reliable after recognition of five strands of mutant DNA. The average number of probes ($N'$) loaded on an Au-bridged NP was estimated to be 207 by modeling of the nanoparticle and DNA footprint (Supplementary Fig. 13). The proportion of the probes completely capturing target DNA was hypothesized as 59.4%, corresponding to 215 nM of the saturation concentration for target detections. In consistence, the sensor signal was saturated at 200 nM (Fig. 2f). The 200 nM target binding is equal to 115 MutS molecules on the Au-bridged NP–DNA surface. In principle, loading all 115 molecules requires a minimum package volume of $7.12 \times 10^{-5} \mu$m$^3$, which fits the available space of $9.91 \times 10^{-5} \mu$m$^3$ when the straight bar-like dsDNA is uniformly anchored on the NP as illustrated in Supplementary Fig. 12a. Thus, quantitative analysis confirmed that sNPS is useful for detecting small numbers of molecular binding events.

We predicted that the type of DNA point mutation was related to adaptations in base geometry and dynamics (Supplementary Note 2 and Supplementary Fig. 15). Mutations alter DNA geometry, with compensatory changes depending on the nature of the mismatched base and flexibility of the environment. These

**Fig. 6** Detections of BRCA1 point mutation with the eight single nanoparticle sensing (sNPS) chips developed in this study. **a–h** The sample from human breast cancer cell line, HCC1937, was detected as the analyte. None of the chips yielded an effective $k_{\text{reaction}}$ except the 5382insC, indicating that the analyte contains a single cytosine duplication. **i** The sample from MCF7 was detected as the control. Source data are provided as a Source Data file.
perturbations induce significant alterations in the ionic milieu of the DNA, which in turn influence DNA recognition by external factors such as the positively charged clamp domain of MutS. Additionally, the dynamic nature of mismatched bases affects the availability of DNA to proteins arising from the equilibrium between several different states of the bases. However, the correlation between the flanking sequence and the tautomeric or ionization state of DNA also affects its binding with MutS and potentially alter the kinetic parameters (e.g., $\Delta \lambda_{\text{max}}$, LOD)\(^\text{26}\). In our analysis of the eight mutations, surrounding sequences were originally predetermined according to \textit{BRCA1} and distinct from one another. To eliminate their influence, we used DNA probes that matched the MutS footprint. The design of probes should follow these principles: (1) the probe must guarantee one and only one MutS binding to potentially mutated targets by controlling its length to be shorter than 100 bp; (2) the probe must guarantee MutS binding near to the nanoparticle surface to sensitively generate peak shifts; and (3) the sequence of the probe needs to refer to the computed digestion maps of a target gene based on the available restriction enzymes. Given that MutS is in continuous rotational contact with duplex DNA followed by one-dimensional diffusion at the very high rate of approximately 700 bp s\(^{-1}\) to detect mutations\(^\text{4}\), the $k_{\text{reaction}}$ of MutS binding to mutations of target DNA ($\sim$700 bp) is expected to be specifically dominated by the following conformational dynamics: (1) base pair geometry, which induces changes in the tautomeric or ionization state of the bases; and (2) intermolecular interactions that undergo dynamic changes to modulate the orientations of nucleic and amino acids in DNA–MutS to achieve a mostly stable conformation. Therefore, the relative activity quantified by $k_{\text{reaction}}$ in the atlas is more promising than an end-point result of peak shift for identifying a point mutation\(^\text{21}\). Long-term adaptions from a single mutation to surrounding bases and then to the active sites of a protein also suggest a mechanism by which MutS retains a memory of certain mutant conformations after binding\(^\text{48}\). Given that structural biological studies examining DNA–protein interactions provide insufficient information\(^\text{49}\) and that indirect reading mechanisms may play a role in defining MutS preferences\(^\text{32}\), it is difficult to unambiguously relate the distortions induced by mutations to the recognition ability of MutS. However, our data using synthetic DNA samples reveal the susceptibility of different mutations to recognition by MutS.

Gene mutations are associated with 10–30% of spontaneous cancers in a variety of tissues\(^\text{50}\). The sNPS technology described in this study provides a theoretical and experimental basis for analyzing interactions between mutant DNA and MutS for elucidating subtle variations in genes. Since the consistency of point mutation identifications between sensing and sequencing has been firmly established with cell lines, we believe that this sNPS system and the typical atlas will be useful for quick and reliable point mutation analysis in clinical applications. Our next step is to update the microfluidic units to fully integrate clinical sample processing\(^\text{51}\), nucleic acid extraction\(^\text{52}\), fragments of interest cutting\(^\text{53}\), and detection for rapid and sensitive sample-to-result genetic testing of clinical samples\(^\text{54}\). The introduction of another MMR protein, MutL, which can trap MutS at a DNA mismatch, will generate larger $\Delta \lambda_{\text{max}}$ values for higher detection sensitivity; this could improve our understanding of the mechanisms by which MutS and MutL assemble on mismatched DNA for subsequent activation of MutH\(^\text{46}\). The fidelity to biomolecular interactions of this sensitive platform is also useful for investigating the bending kinetics of mutant DNA that control the activation of downstream signaling in the MMR system\(^\text{33}\).

**Methods**

**Material information.** Au nanoseed (AuNS; 5 nm) solution was obtained from Ocean NanoTech (10 mM phosphate-buffered saline with 0.02% NaN\(_3\); 0.01% Tween 20, 0.1% bovine serum albumin, pH 7.4; catalog no.: WB-100, San Diego, CA, USA). Dithiothreitol (DTT) and restriction enzyme Sty I (#R648A) were from British BioCell International (Crumlin, UK). Wash/storage buffers were from Ocean NanoTech (10 mM phosphate-buffered saline with 0.02% NaN\(_3\); 0.01% Tween 20, 0.1% bovine serum albumin, pH 7.4; catalog no.: WB-100, San Diego, CA, USA).
Promega (Madison, WI, USA). Microseep and Nanosep centrifuge tubes were from Pall Life Sciences (Ann Arbor, MI, USA). 2- (2-(2- (2-(1-Mercaptoundec-11-yloxy)ethyldithiolato)-10-phenanthroline)gold(III) chloride) (AuCl3) was purchased from Alfa Aesar (Haverhill, MA, USA). Au nanoparticles (AuNPs) were synthesized from sodium citrate and silver nitrate following the method described by Faraday (1995). In brief, the seed solution was prepared by mixing 0.5 M NaCl, 10 mM DTT, 0.5 μM labeled DNA, and 14 μM AuNPs. The seed solution was gently stirred for 10 min at 100 °C. The seed solution was then mixed with gold precursor for 10 min at a rate of 10 ml/min before use. All oligonucleotides used in this study were from Integrated DNA Technologies (Corvalde, IA, USA). The sequences of the DNA targets and probes are shown in Table 1. The names and information of single-stranded DNA (ssDNA) are shown in Supplementary Table 3.

NP modeling and numerical simulation. We performed modeling and optical simulations of nanostructures with spherical, rod, NP-gap, and bridged NP shapes using the commercial software COMSOL. NPs were composed of Au; particle sizes were set as uniform to facilitate comparisons. Optical simulations were performed in the local dielectric environment where water–gycerol mixtures of varying weight ratios were prepared so that the RI of the surrounding medium ranged from 1.333 to 1.443 (Supplementary Fig. 1).

Conjugation of AuNPs with ssDNA. All 5′-thiol-modified oligonucleotides were incubated with DTT solution in a 1:100 ratio for 15 min and purified three times with ethyl acetate before use. The disulfide bond of the 5′-thiol was cleaved into an active sulfhydryl group and immediately conjugated with the Au surface. Before conjugation with DNA in solution, AuNPs were coated with bis(p-sulfonatophenyl)phosphine hydroxide diatopium (BSPP; 100 μM AuNPs solution mixed with 100 mg BSPP for 10 h) to improve the tolerance of AuNPs to the highly ionic environment. The AuNPs solution was then mixed with NaCl, which resulted in a color change from dark red to light violet. The solution was centrifuged for 30 min at 500 g and the precipitate was resuspended in 1 ml of 0.5 mM BSPP. The solution was adjusted to the desired concentration, and the concentration of AuNPs was measured by ultraviolet–visible light–near-infrared spectrophotometer (UV–VIS: Shimadzu, Kyoto, Japan) 1 OD of 5 μM AuNPs is equal to 5.0 × 10^13 particles per microliter according to the manufacturer’s instructions. The AuNPs were incubated overnight at room temperature with ssDNA-1 in a stoichiometric ratio of 1:1 in 0.5× TBE buffer containing 50 mM NaCl. The following day, 60% glycerol was added to the solution to obtain a final mixture of 10% glycerol to prevent AuNPs-ssDNA from spreading in the buffer upon centrifugation. AuNPs-ssDNA were then centrifuged at 5000 g for 10 min. The ssDNA separated into different bands on a 3% agarose gel in 0.5× TBE buffer at 10 V cm^-1 for 1 h (Supplementary Fig. 2a). The gel containing AuNPs conjugated with one strand of ssDNA (AuNPs–1ssDNA-1) was incubated in 0.5× TBE buffer for further use. The procedures were carried out to conjugate AuNPs and ssDNA 2 to obtain AuNPs–1ssDNA-2.

Synthesis-by-design of NPs. Gold precursor (HauCl₄, 0.03%) and reductant (NH₄OH·HCl, 1 mM) were separately dissolved in water and the pH of each solution was adjusted to 5 or 4 (±0.1) by gradually adding NaOH under a nitrogen atmosphere. The seed for DNA-directed synthesis was produced by hybridization of AuNPs–1ssDNA-1 with AuNPs–1ssDNA-2 in the form of AuNPs-dsDNA-AuNPs. To increase hybridization efficiency, equal volumes of the two conjugates in 0.5× TBE buffer were added to a 10 mM NaCl buffer for 5 h at 25 °C. The resulting mixture was then diluted 10× by centrifugation (5000 g for 10 min) and the mixture was shaken overnight at 37 °C and the AuNPs-dsDNA-AuNPs was separated by gel electrophoresis with the same procedure of AuNPs–1ssDNA-1 separation (Supplementary Fig. 2b). The gel containing AuNPs-dsDNA-AuNPs was soaked in 50 ml wash/storage buffer with a final PEG/seed molar ratio of 100:1. The solution was poured into a centrifuge tube (molecular weight cut-off 30 K, 3000 x g), and then the seeds were protected by the neutral PEG layer to improve stability and reduce the nonspecific absorption of charged molecules onto the AuNPs surface. The seeds were gently stirred with gold precursor for 10 min at a final concentration of 2 mM; 10 μl of the solution was mixed with 17.54 μl reduc- tant, and a color change from colorless to light-red was observed within 1 min. After 15 min, the synthesized NPs were washed by repeated resuspension in water and centrifugation. TEM and HR-TEM images of the NPs were obtained (HD2300; Hitachi, Tokyo, Japan) in z-contrast and secondary electron modes at an accel- erating voltage of 300 kV. Samples were prepared for TEM using a staining plate (Electron Microscopy Sciences, Hatfield, PA, USA) and 400-mesh copper grids with carbon film (Ted Pella, Redding, CA, USA). The lengths and diameters of the nanostructures in the plane of TEM were measured using the software ImageJ.

TEM images with scale bars of 20 nm and 50 nm showed nanostructures large enough for precise measurements. Particle yield was calculated as the ratio of Au- bridged NPs. The unbound particles were easily distinguished as oversized or undersized bridged-nanoparticles and as nanospheres grown from AuNPs-ssDNA that were denatured from AuNPs-dsDNA-AuNPs during the synthetic reaction. Fast Fourier transform patterns of HR-TEM images were analyzed with Digital Micrograph software (Gatan, Pleasanton, CA, USA) to confirm the crys- talline structure and growth orientation.

sNPS platform settings. The overall configuration of the sNPS system is shown in Supplementary Fig. 4a. To construct the detection chamber, microscope glass slides (22 × 40 × 0.1 mm) (Warner Instruments, Hamden, CT, USA) were coated with First Contact cleaning polymer (Photonic Cleaning Technologies, Plateville, WI, USA), which was immediately peeled off after curing for 15 min. The Au slide was then rinsed overnight with freshly prepared aqueous rega solution; after rinsing with ultrapure water, the slide was immersed in 5% (v/v) 3-aminopropyltriethoxysilane in absolute ethanol for 15 min followed by sonication in ultrapure water for 5 min. This process was repeated three times. A 3 μl volume of diluted Au-bridged NP solution (30 μM Au and 10% (±0.5%) was added drop by drop into the chamber followed by incubation for 1 min at room temperature. The slide was then washed with ultrapure water and ethanol in a sterile fume hood to prevent contamination with airborne debris before air drying with nitrogen gas. The slide was placed in a closed-bath imaging microfluidic chamber (RC-30; Warner Instruments) that was washed with a nitrogen flow and a stage controller (Marsharau Sensotech, Wetzlar, Germany) and connected to a flow device for solution mixing and a flow rate control system (PHD 2000; Harvard Apparatus, Holliston, MA, USA) (Supplementary Fig. 4b). Images of the field of view of the inverted microscope (Euclipse TE2000-U; Nikon, Tokyo, Japan) equipped with a 100 W halogen source (Type 7724, Philips), a dark-field dry objective (5×, Nikon) with a NA of 0.80–0.85; Nikon, and 100 x oil objective (CFI Plan Fluor ELWD, NA = 0.6; Nikon) were acquired with a color camera (DS5; Nikon), and only individual nanoparticles with inter-particle spacing ~5-fold greater than the diameter of shining dots were analyzed to minimize the effects of inter-particle resonance coupling (Supplementary Fig. 4c). Images from the chamber were acquired using a charge-coupled device (CCD) camera (Polaris Instruments, Trenton, NJ, USA) at ~70 °C with a 100 ms frame integration time. A beam splitter at the output port of the microscope and long-pass filter were placed before the CCD. The platform allowed the determination of RLS properties of each NP in the chamber using an RLS spectrograph (Microspec 2300; Roper Scientific, Lincoln, Nebraska) in a dark room. A Drop Spec (Microspec Ltd, Dinton, UK) was used to record acquisition time of 1 s. The spectral data were fitted with the Lorentzian algorithm to eliminate noise, and an accurate λ_m was determined using Origin2018 software (OriginLab, Northampton, MA, USA). Application of this method to analyze 10 spectra acquired once per minute from the same nanoparticle yielded a fitting-limited peak measurement precision of 0.188 nm (Supplementary Fig. 4e–f). The fluctuation in peak positions is attributed to instrumental factors including spectrometer resolution, physical uniformity in chambers, transient variations in temperature, and flow rate of liquid, and analytical factors such as microscope focus control, spectral source correction, exposed pixel selection, and detector gain (0.188 nm). Besides the 0.29 nm peak uncertainty measured at random readings of 168 nanoparticles is 0.487 nm, where the 0.29 nm difference resulted from nanoparticle factors of size, shape, and orientation.

Detection of point mutations. After mounting the glass slide in the sNPS system, the chamber was rinsed by injecting 75% ethanol for 5 min followed by rinsing with wash/storage buffer for 20 min to remove contaminant’s and unused AuNPs. The positions of Au-bridged NPs were recorded after photographing the chamber. One NP was representative to one detection set and its optical properties were determined for each step of molecule binding. The chamber was filled with 100 nM probe DNA (e.g., Probe-GT) for 8 h at room temperature and rinsed with wash/storage buffer for 20 min to remove probes from the chamber. The chamber was washed with nanospheres pendant and then the spectrum in a range of 300–900 nm was acquired after washing with ultrapure water for 5 min. The same procedure used 20 mM MutS protein to injected into the chamber after capturing the same target, and then the spectra were recorded again for further analysis (Supplementary Fig. 5).

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Preparation and detection of samples from cell lines. We prepared biological DNA samples from the human cancer cell lines HCC1937 (American Type Culture Collection (ATCC), CRL-2336TM), SKNCC2 (Korean Cell Line Bank, 00251)38, and MCF7 (ATCC, HTB-22TM). MCF7 is an authenticated cell line used as control for BACR1 analysis. All cell lines had no mycoplasma contamination. The genomic DNA was extracted using G-spinTM Total DNA Extraction Kit and treated with 200 ng ml−1 proteinase K and 10 ng ml−1 RNase A at 55 °C for 30 min before purification and further restriction digestion. The digestion was performed with restriction enzymes Mbol, Alul, and StyI to generate 50–60 bp nucleotides. In detail, digestion by Mbol and Alul yielded fragments of 100–500 bp. Since there are StyI sites in BACR1, the fragments were further digested by StyI to the target sample of 50–50 bp in length. The specific sites of the enzymes and the computed fragmentation maps can be found in Supplementary Figs. 6 and 7. To efficiently collect the DNA, glycerol was added during the ethanol precipitation following by centrifugation for 15 min at 10,400 × g. The concentration and purity of the DNA were assessed using a Nano-200 Micro-Spectrophotometer DC24V (#AS-11030-00; Allsheng Instruments, Hangzhou, China). The integrity of the DNA was evaluated by gel electrophoresis where 300 ng DNA samples were loaded onto 7.0% agarose gels at 2.5 V cm−1 at 4 °C, stained with 0.5% ethidium bromide, and detected by UV illumination with a Davinch-GelTM Gel Imaging System (Young Wha Scientific, Seoul, Korea) (Supplementary Fig. 8). 30 μl of the nucleotides was melted into single-stranded targets at 90 °C for 1 min and injected into the SNUS251 cell line, a new 64 bp probe was designed. Real-time RLS spectra were recorded and the peak positions were analyzed in the wavelength range of 500–650 nm with Lorentzian fitting.

Demonstration of FOI of an individual NP. The plasmonic FOI of an individual nanoparticle is defined as the effective space of plasmonic sensitivity to refractive index changes, where Eq. 1 is applicable to calculate the molecular concentration in direct proportion to the amplitude of red shifts in IFOI. The FOI was supposed to be cuboid (Supplementary Fig. 12a). The two-dimensional area of the FOI was directly delineated with 8 pixels in CCD images by the WinSpec software of the sensing system (Fig. 2d), the length and width of which were measured to be 3.36 μm based on the scale calibration (Supplementary Fig. 12b). We notified that the length and width of this two-dimensional area were set before the spectral monitoring and were maintained for all detections. As shown in the conformational scheme (Supplementary Fig. 12c), the height (h) of the FOI is the sum of the diameter (Dsphere) and the t of the nanostructure, where t is defined as the threshold thickness of the region that can induce peak red shifts. In detail, LSPR peak shifts exhibit an oscillatory behavior with a periodicity close to the p = hmn/2m, where n is the refractive index of the coating layer on the surface (Supplementary Fig. 12d)37. In the first half of the cycle, spectra exhibit red shifts with increasing thickness of less than p/2. Here, the thickness threshold of p/2 is t. Beyond the threshold thickness, the LSPR peak shifts begin to blue shift and then exhibit a periodic oscillation behavior. Some studies have demonstrated that the refractive index of the DNA layer (δsphere/δnuclea) is 2 and that the proteins and DNA behave similarly with respect to the refractive index change they induce38–40. Therefore, the r of Au-bridged NPs with hmax of 561 nm was calculated to be 70.1 nm. Finally, the volume of the FOI (V = πw h = Vsphere) was calculated as 3.36 μm × 3.36 μm × 0.00844 μm = 993.53 μm3.

Estimation of Nper NP. The N per NP is quantitatively predicted based on the modeling of the nanoparticle and DNA footprint (Supplementary Fig. 13). In detail, N was calculated by dividing the surface area of a particle by the area of effective footprint of a probe. The footprint is defined as the average area each probe occupies on the nanoparticle surface. Several assumptions were made for the calculation of the nanoparticle surface area: models perfect spheres with a cylinder; the footprints with the closest distance from each other were modeled as a circular area on the spheres and an ellipse on the cylinder; the contact-points of the two spheres on glass were not considered; and the probes were assumed to be evenly distributed on the particle surface.

The footprint area on the spheres (Nsphere) is indexed to be 6πm2 according to the diameter of the sphere37. The area of the two spheres (Arhersphere) was calculated by

\[ \text{Arhersphere} = \pi \times \text{diameter of sphere}^2 \]

where the diameter of the sphere is 1.6nm. The footprint is the contact area between the spheres and the cylinder; consequently, the area of the footprint on the spheres was

\[ \text{Arhersphere} = \pi \times \text{diameter of sphere}^2 \times \text{number of probes} \]

where the number of probes is calculated as 2 × 4(\times D_sphere/2) + 2 × \pi (Dbridge/2)2 = 1178 nm2, and thus the number of probes that can be placed on the spheres was \( N_{\text{sphere}} = \frac{\text{Arhersphere}}{\text{diameter of sphere}^2} = 196 \). The footprint area on the outer wall of the cylinder was calculated by the equation:

\[ N_{\text{cylinder}} = \text{n short-axis} \times \text{n long-axis} \times \pi \times \text{diameter of cylinder} \]

where the number of probes along the short axis of the cylinder is specified as the number of probes that can be placed on the cylinder; therefore, the number of probes that can be placed on the cylinder is calculated as \( N_{\text{cylinder}} = \frac{\text{Arhersphere}}{\text{diameter of cylinder}} = 196 \times \frac{1}{2} \), and thus the number of probes that can be placed on the cylinder is calculated as \( N_{\text{cylinder}} = 196 \times 2 = 392 \). Therefore, \( N_{\text{total}} = \text{n short-axis} = 1 \times \text{n long-axis} = 1 \times n_{\text{cylinder}} \).
Further transformation of the Eqs. (9) and (10) can get the equation:

$$r_{1/2} = \ln 2 \cdot \frac{1}{k_{\text{reaction}}}$$

where $k_{\text{reaction}}$ is independent of concentration and indicates the probability that the complex will spontaneously fall apart in a unit of time.

Based on time courses of the $\Delta_{\text{max}}$ change, the time for bindings to reach half of the maximum $\Delta_{\text{max}}$ was evaluated by the half-time of the reaction ($r_{1/2}$):

$$r_{1/2} = \ln 2 \cdot \frac{1}{k_{\text{reaction}}}$$

## Reporting summary

Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.

## Data availability

The data supporting the findings of this study are available within the article and its Supplementary Information. The source data for Figs. 1a, b, 2b, c, e, f and 3–7, and Supplementary Figs. 4e, 5 and 10 are provided as Source Data files. All data are available from the corresponding author upon reasonable request.

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Author contributions

S.J.S. supervised the whole work and revised the manuscript. X.M. conducted the experiments and wrote the manuscript. S.S. participated in detections with clinical samples. S.K. carried out nanoparticle synthesis. M.K. and H.L. cultivated cell lines and prepared DNA samples. W.P. performed optical simulations. All authors proofread and provided comments on the manuscript.

Additional information

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