Noble metal nanoparticle biosensors: from fundamental studies towards point-of-care diagnostics

*Hongya Geng, Simon Vilms Pedersen, Yun Ma, Tabasom Haghighi, Hongliang Dai, Philip D. Howes, and Molly M. Stevens**

Corresponding Author

Molly M. Stevens − Department of Materials, Department of Bioengineering and Institute of Biomedical Engineering, Imperial College London, London SW7 2AZ, U.K; Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, 171 77, Sweden. Phone: +44 20 7594 6804;

Email: m.stevens@imperial.ac.uk; orcid.org/0000-0002-7335-266X

Authors

Hongya Geng – Department of Materials, Department of Bioengineering and Institute of Biomedical Engineering, Imperial College London, London SW7 2AZ, U.K.; Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, 171 77, Sweden. orcid.org/0000-0002-4276-352X

Simon Vilms Pedersen – Department of Materials, Department of Materials, Department of Bioengineering and Institute of Biomedical Engineering, Imperial College London, London SW7 2AZ, U.K.; orcid.org/0000-0002-1830-370X

Yun Ma – Department of Materials, Department of Bioengineering and Institute of Biomedical Engineering, Imperial College London, London SW7 2AZ, U.K.; orcid.org/0000-0002-5326-5155

Tabasom Haghighi – Department of Materials, Department of Bioengineering and Institute of Biomedical Engineering, Imperial College London, London SW7 2AZ, U.K.; orcid.org/0000-0002-9570-4048

Hongliang Dai – School of Environmental and Chemical Engineering, Jiangsu University of Science and Technology, Zhenjiang 212003, China; orcid.org/0000-0002-6974-7167

Philip D. Howes – Division of Mechanical Engineering and Design, School of Engineering, London South Bank University, London, SE1 0AA, UK; orcid.org/0000-0002-1862-8395

Conspectus: Noble metal nanoparticles (NMNPs) have become firmly established as effective agents to detect various biomolecules with extremely high sensitivity. This ability stems from the collective oscillation of free electrons and extremely large electric field enhancement under exposure to light, leading to various light-matter interactions such as localized surface plasmon resonance (LSPR) and surface-enhanced Raman scattering. A remarkable feature of NMNPs is their customizability by mechanisms such as particle etching, growth, and aggregation/dispersion, yielding distinct color changes and excellent opportunities for colorimetric biosensing in user-friendly assays and devices. They are readily functionalized with a large variety of capping agents and biomolecules, with resultant bioconjugates often possessing excellent biocompatibility, which can be used to quantitatively detect analytes from physiological fluids. Furthermore, they possess excellent catalytic properties that can achieve significant signal amplification through mechanisms such as the catalytic transformation of colorless substrates to colored reporters. The various excellent attributes of NMNP biosensors have put them in the spotlight for developing high-performance in vitro diagnostic (IVD) devices, that are particularly well-suited to mitigate the societal threat that infectious diseases pose. This threat continues to dominate the global healthcare landscape, claiming millions of lives annually. NMNP IVDs possess the potential to sensitively detect infections even at very early stages with affordable and field-deployable devices, which will be key to strengthening infectious disease management. This has been the major focal point of current research, with a view to new avenues for early multiplexed detection of infectious diseases with portable devices such as smartphones, especially in resource-limited settings.

In this account, we provide an overview of our original inspiration and efforts in NMNP-based assay development, together with some more sophisticated IVD assays by ourselves and many others. Our work in the area has led to our recent efforts in developing IVDs for high-profile infectious diseases, including Ebola and HIV. We emphasize that integration with digital platforms represents an opportunity to establish and efficiently manage widespread testing, tracking, epidemiological intelligence, and data sharing backed by community participation. We highlight how digital technologies can address the limitations of conventional diagnostics technologies at the point of care (POC) and how they may be used to abate and contain the spread of infectious diseases. Finally, we focus on more recent integrations of noble metal nanoparticles with Raman spectroscopy for accurate, non-invasive POC diagnostics with improved sensitivity and specificity.

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1. Introduction

Noble metal nanoparticles (NMNPs) have attracted significant attention for early disease diagnosis through biomarker detection with extremely high sensitivity.[5] In biosensing applications, they are frequently used due to their unique surface plasmon resonance (SPR). SPR is characterized by a local charge oscillation in resonance with incident light when the size of particles is smaller than the light wavelength. The local charge oscillation can confine light at nanoscale dimensions, by which scattering and absorption can be significantly enhanced.[6] Importantly, these NPs exhibit distinct and sensitive changes in optical properties as they aggregate/disaggregate or change shape by etching or growth. Upon functionalization with capping agents, NMNPs provide high flexibility in many biomolecule detection scenarios, for example, by monitoring the refractive index, spectroscopic-based readings, and distinct color changes. Beyond their plasmonic properties, some NMNPs exhibit natural enzyme-like

catalytic properties. Leveraging this, a single NP can produce many reporters and generate colored products from colorless precursors, allowing pronounced signal amplification.[7]

Over recent decades, the frequency and amplitude of infectious disease outbreaks have increased.[8] The death toll of the ongoing severe acute respiratory syndrome coronavirus (COVID-19) pandemic surpassed 4 million during the writing of this paper,[9] and represents the third coronavirus outbreak in less than 20 years after MERS in 2012 and SARS in 2003.[10,11] The Ebola virus outbreak in West Africa in 2013 claimed over 11000 lives,[12] and the human immunodeficiency virus (HIV) and severe Zika virus outbreaks have sparked grave concerns about global health.[13,14] Since infectious diseases can be rapidly transmitted among humans by live pathogens such as airborne or waterborne viruses, parasites and bacteria, early-stage field-deployable in vitro diagnostics (IVD) play a pivotal role in cutting off the transmission routes, helping to protect susceptible people and monitor population recovery.

Despite the high sensitivity and specificity of polymerase-chain-reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) for detection of infections (e.g., through genetic materials, pathogen-related proteins, and human host generated pathogen-specific antibodies), they require skilled professionals, sophisticated infrastructure, and costly reagents, impeding their translation into clinical environments, and more so in low-resource settings.[15] In contrast, Lateral Flow Assays (LFAs) detect biomolecules on a disposable nitrocellulose strip, where a colorimetric response generally allows equipment-free rapid readout, often within five minutes (Figure 1). These advantages have popularized LFAs for the point-of-care (POC) infectious disease diagnosis.

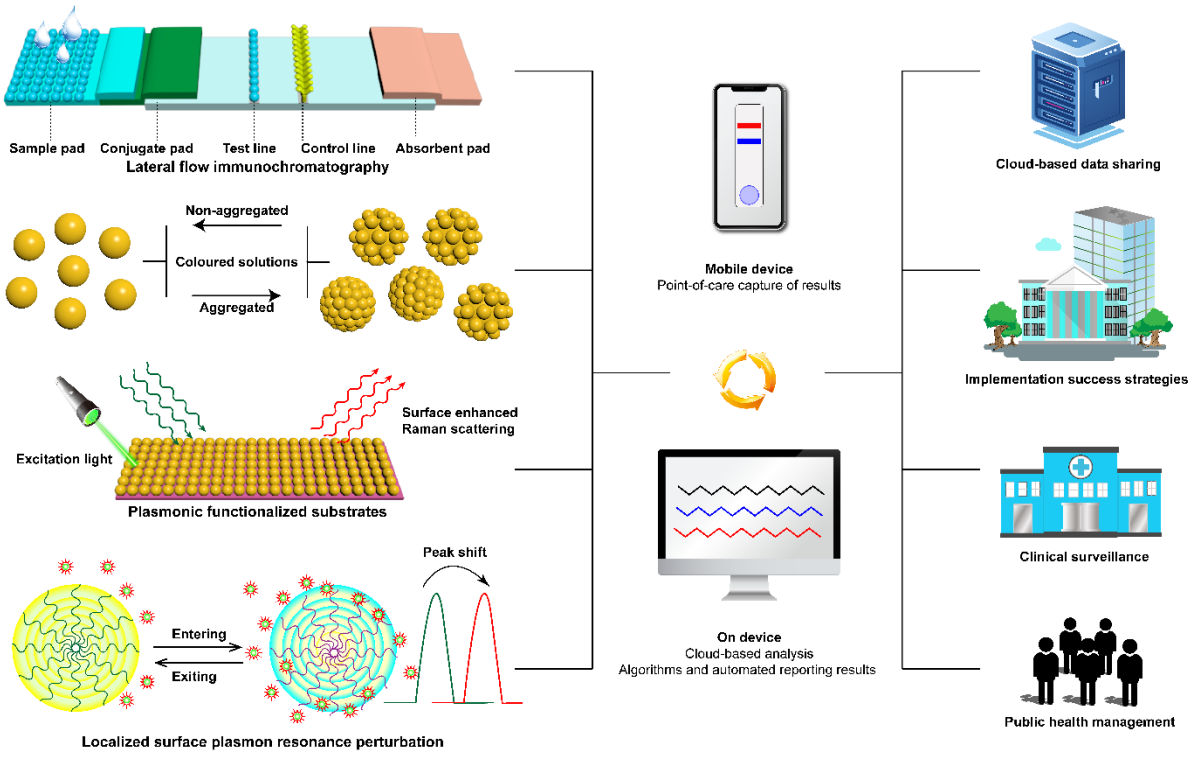

Figure 1. Deploying noble metal nanoparticles with integrated digital technologies drives early infectious disease detection and diagnosis.

This account provides an overview of our work and experience in developing NMNP biosensors, and also discusses some of the excellent work from other groups. We emphasize advanced diagnostic strategies based on aggregation/disaggregation, anisotropic growth, liposome-mediated aggregation, and signal amplification of NMNPs that arise from their plasmonic and catalytic activity, respectively. Further, we provide suggestions and perspectives on integrating NMNPs with portable devices, such as smartphones and LFAs, to improve POC testing. The potential in exploiting surface-enhanced Raman scattering using NMNPs for diagnostics is also proposed.

2. Fundamental mechanism of noble metal nanoparticles as biosensors

NMNPs can detect analytes through three main mechanisms (Figure 1): (1) Aggregation/disaggregation, surface etching, and anisotropic growth-induced visible color change in the presence of analytes, arising due to position and/or shape changes of the localized surface plasmon resonance (LSPR) peak(s) in the visible range. This occurs as the plasmonic character of nanostructures is strongly dependent on particle shape, size, interparticle distance, environment, etc.;[16] (2) Shifting of the LSPR peak due to the differences in refractive index between the binding/deposited molecules and the bulk solution;[17] (3) Raman intensities can be modulated by binding or removing Raman-active probes from NPs. Small variations in either the number density of molecules being probed, or the arrangement of metal atoms within the nanostructures, have a significant effect on Raman intensities.[18] In principle, the sensitivity of such methods is increased if a single analyte molecule leads to a cascade of chemical events amplifying response signals due to the NMNP-catalyzed reactions.[19] The surface composition of nanoparticles is key in controlling their behaviour in all analyte detection scenarios, most often through bioconjugation of recognition elements such as antibodies, nucleic acids, aptamers, proteins and peptides (Figure 2a). These often confer the extra benefit of enhacing colloidal stability, especially when paired with advanced ligand chemistries, for example employing zwitterionic or polymeric capping layers.[5]

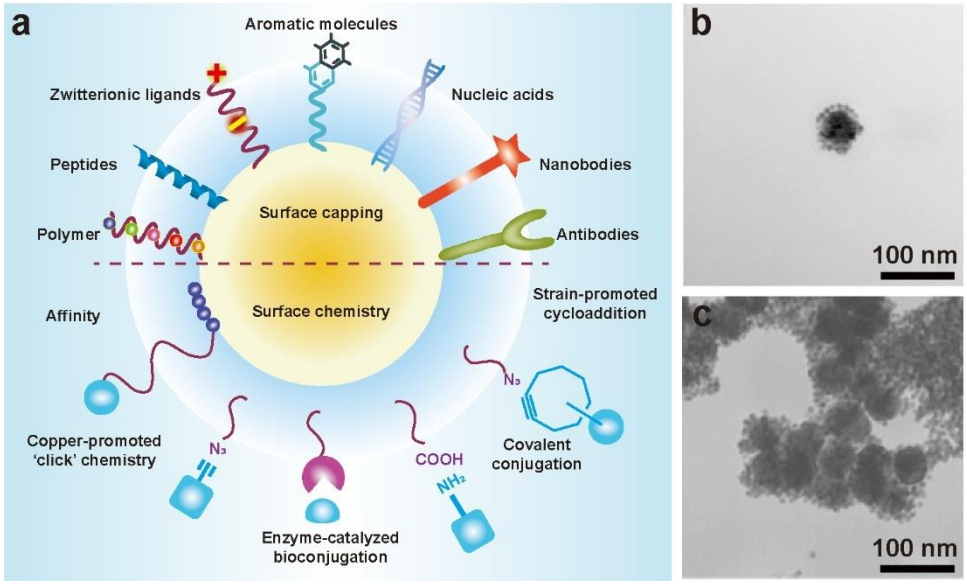

Figure 2. (a) Bioconjugation of a layer of ligands providing colloidal stability and functional groups targeting biomolecules. Adapted with permission from Ref.[5] Copyright 2014 American Association for the Advancement of Science. (b) and (c) Typical TEM images showing the self-assembly of peptide coated AuNPs. Reproduced with permission from Ref. [20] Copyright 2004 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

Our initial development of NMNP biosensors grew out of historical interest in studying functional peptides for biomedical applications.[21] Artificial bioactive peptides exhibit versatile and programmable protein-like functionalities while possessing a high degree of flexibility in molecular design and manufacturing.[22,23] The screening of peptide libraries facilitates the discovery of effective binders against a large variety of analytes with high specificity, including cells, RNAs, DNAs, proteins, and other peptides.[24] Ran et al. enabled the simultaneous detection of multiple inflammatory biomarkers over a broad range of concentrations, from pg/mL to μg/mL, using peptide-mediated aggregation of AuNPs.[25] Three phosphorylated peptides possessing different responses to alkaline phosphatase (ALP) were used. These influenced the degree of AuNP aggregation differently, due to the difference of the ALP hydrolysis efficiency and the electrostatic/spatial interactions between the peptides and the AuNPs. More sensitive peptides could induce aggregation of AuNPs in solutions with lower ALP, and vice versa. Therefore, biomarkers that present at a broad range of concentrations could be detected in the presence of a suitable peptide and ALP, linked to the detection antibody of the corresponding biomarker, by monitoring the aggregation of AuNPs. Additionally, we established an early platform for biomolecular recognition based on the functionality of coiled-coil peptides conjugated to gold NPs (AuNPs).[20] An acidic leucine zipper-like peptide and a basic leucine zipper-like peptide were separately conjugated onto AuNPs via the terminal cysteine residues. Hydrophobic packing between these peptides drove the formation of a left-handed coiled-coil structure between the leucine zipper-like domains. The oppositely charged lysine-glutamate ion pairs in the glutamic acid on the coiled-coil

structure were able to dynamically assemble and disassemble the AuNPs. For example, a low pH value (4.5) could increase the helicity of the peptide, leading to the destabilization of the coiled-coil structure and thus the disassembly of the AuNPs (Figure 2b and 2c). The proposed approach was of great versatility for biosensing of target analytes, by dynamically controlling the assembly of AuNPs and screening peptide libraries. From this fundamental study, we developed a novel strategy for real-time monitoring of protease activity with a single population of protease-responsive AuNPs.[26] A tripeptide was functionalized with AuNPs, whereby i) N-(fluorenyl-9-methoxycarbonyl) assembled AuNPs through π-stacking interaction, ii) a scissible bond at the amine side of Gly-Phe served as a protease-cleavable unit, iii) cysteine-containing thiol groups anchored onto AuNPs (Fmoc-Gly↓Phe-Cys-NH_2). Upon enzymatic hydrolysis by thermolysin, disassembly occurred due to the electrostatic repulsion of NH_3^+ groups (noted by the arrow), leading to a visible color change from blue to red.

These early studies in NMNP bioconjugate design and fabrication enabled our colorimetric kinase detection by incorporating them in a particle aggregation mechanism, which could be carried out in two different assay formats, sensor chip and in-solution.[27] This system consisted of two populations of AuNPs coated with protein kinase peptide and complementary antiphosphotyrosine antibodies. The addition of kinase caused enzymatic phosphorylation of the protein kinase peptide-capped AuNPs, followed by interparticle assembly with the antibody-capped particles due to recognition of the antibody with high specificity. In the sensor chip case, aggregates were captured by antibodies on the chip, giving changes of SPR at the test line. Aggregation induced remarkable visible color changes and a redshift in the UV-vis spectra.

3. Advanced strategies that govern the detection of infectious diseases

3.1 Anisotropic growth and etching of the NMNPs

Since our early assays of peptide-mediated AuNPs assembly, we have started exploiting shifts in plasmonic peaks (and therefore colorimetric response) resulting from structural changes of NMNPs (size, shape, interparticle spacing and particle composition), giving rise to extremely sensitive diagnostics assays.

A fundamental mechanism utilized in NMNPs biosensors is the colorimetric response arising from a shift of plasmonic peaks. This was harnessed in an early assay of ours, where we lowered the limit of detection (LOD) of a cancer biomarker (prostate specific antigen, PSA) via the controlled overgrowth of Ag on Au nanostars.[28] The controllable crystal growth of Ag on Au nanostars, and nucleation in solution, generated plasmon-derived color changes. A lower concentration of glucose oxidase (GOx) slowed down Ag ion nucleation, favoring the formation of a Ag coating layer on Au nanostars, generating a blueshift in the localized SPR (LSPR). Correspondingly, more GOx accelerated nucleation of Ag ions in the solutions, leading to less of a blueshift of Au nanostars in the LSPR. We then bound

GOx to polyclonal antibodies against PSA. The concentration of PSA was thus directly inversely proportional to the concentration of GOx between 10^{-18} g mL^{-1} and 10^{-13} g mL^{-1}. Next, we developed a plasmonic ELISA, where enzyme-mediated growth of AuNPs allowed extremely sensitive detection of HIV-1 capsid antigen p24 (Figure 3).[29] Similar to conventional ELISA, p24 was first captured on the plates using capture antibodies (human monoclonal anti-p24), then primary and secondary antibodies were added to link p24 to streptavidin-catalase conjugates selectively. Catalase consumed H_2O_2, slowing down the AuNP growth resulting in a blue color. Non-aggregated quasi-spherical AuNPs formed in the absence of p24 due to decreased consumption of H_2O_2 and a fast rate reduction of Au ions, giving a red color.[30] Naked-eye detection of HIV-1 capsid antigen p24 (from 30 HIV-infected patients) as low as 1×10^{-18} g mL^{-1} was realized.

We then took advantage of anisotropic growth of Au nanorods (AuNRs) to detect analytes.[31] The LSPR modes along the longitudinal and transverse axes of the nanorods cover the visible and near-infrared regions, allowing ready and broad spectral adjustment through aspect ratio tuning.[32] Our results showed that high concentrations of reducing molecules such as 4-aminophenol (AP) and 4-aminophenyl phosphate (APP), resulted in a higher number of seeds, limiting the extent of AuNRs during the growth process, thus decreasing the redshift of the produced nanorods. We exploited this concept to similarly detect PSA in a sandwich ELISA, where a higher concentration of PSA increased the conversion of APP to AP leading to the growth of AuNRs with a higher aspect ratio. A redshift in longtitudinal LSPR during the growth of AuNRs yielded an LOD of PSA at 0.16 ng mL^{-1}.

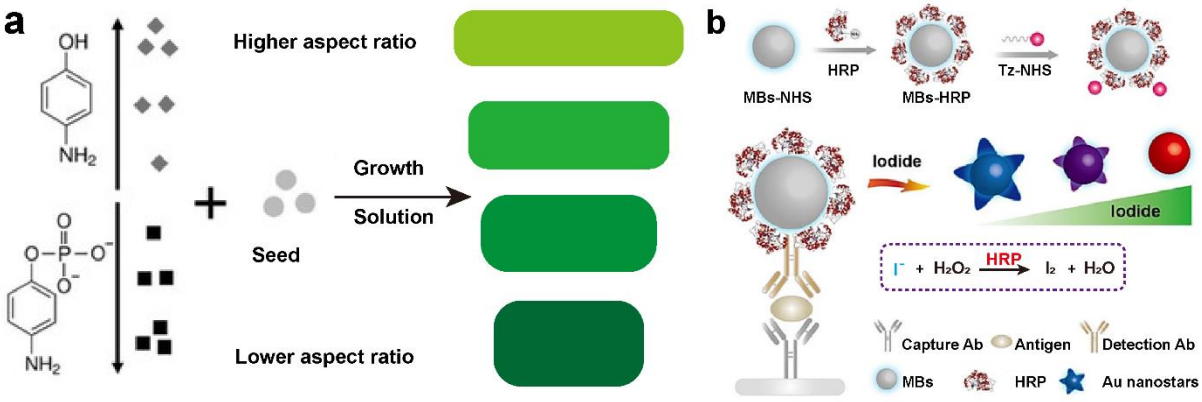

Figure 3. (a) Schematic illustration of the concentration-dependent effects of AP and APP on the aspect ratio of AuNRs. Adapted with permission from Ref.[31] Copyright 2017 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. (b) Illustration of a sandwich immunoassay based on the surface etching of the Au nanostars. Reproduced with permission from Ref.[33] Copyright 2021 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

In another study, we demonstrated an enzyme biosensor by reshaping Au nanostars using iodide etching.[33] The higher binding energies of I^- to Au surfaces compared to other halide ions, enabled iodide-specific surface etching due to an electrochemical reaction.[34] Gold nanostars could be etched to

form rounded particles (low iodide concentration) and spherical structures (high iodide concentration). In the presence of H_2O_2, horseradish peroxidase (HRP) catalyzed the oxidation of iodide into iodine that cannot etch Au nanostars. A versatile plasmonic ELISA was developed based on this HRP-modulated etching, to detect human IgG as a protein model. Anti-human IgG (detection antibody), goat anti-human IgG (capture antibody), and HRP were first co-functionalized with magnetic beads (MBs, ~26530 HRP per MB) to catalyze iodide consumption. As human IgG increased, more antibody functionalized HRP-MBs were captured by the plate to consume iodide. By monitoring changes in transverse and longitudinal bands at 550 nm and 780 nm, we achieved a LOD of 0.2 ng mL^{-1}.

Similarly, Suea-Ngam et al. detected Methicillin-resistant staphylococcus aureus (MRSA) based on selective etching of Ag nanoplates.[35] Loop-mediated isothermal amplification (LAMP) was used to amplifiy the target MRSA gene (*mecA*) from a test sample on a paper-based analytical device. The long LAMP DNA amplicons bind to Ag nanoplates on the strip, and stop etching by NaBr. In the absence of the target gene, the silver nanoplates are not protected and get rapidly etched by the salt, leading to a dramatic shift in the plasmonic peak and colour change from blue to yellow. Gu et al. reported the assay of microRNA-141 in serum (hose serum was used to mimic human blood serum) based on the SPR change of silver-coated gold nanorods (Au@Ag NRs).[36] In response to microRNA-141, hydroxyl radicals generated by H_2O_2 due to the catalyzed hairpin assembly and hybridization chain reactions etched Ag shell of the NRs. As low as 1.0×10^{-14} M microRNA can be quantified via the notable red SPR shifts.

3.2 Aggregation assays

Reversible biological assembly (aggregation) of NMNPs using highly specific biomolecular recognition systems yields distinct shifts in LSPR, valuable for sensitive biosensing.[20,37] As a means of targeting specific analytes, the interactions between NMNPs and analytes can be driven by various mechanisms, including (1) highly specific antigen-antibody interactions,[4] (2) enzyme substrates containing proteins or peptides that can be removed by protease cleavage,[38] (3) charge-charge and hydrogen bonding interactions between nanoparticles and polymers,[39] (4) various types of aptamers that target ions, biotoxins and biomarkers,[40] and (5) primers that can guide the amplification of DNA when polymerase and nucleotides are present.[41]

Using highly selective antigen-antibody interactions to trigger aggregation of AuNPs and detection of antibodies/antigens is of particular importance for infectious disease management. Here, the antibody response can be used to detect later-stage active infections, a past infection, ongoing immunity status, or response to vaccination. Accordingly, we developed a rapid and sensitive assay for the antiviral epitope Haemophilus influenzae (HA) antibodies by employing aggregation of peptide epitope-capped AuNPs.[42] Additions of 1–50 nM of antiviral epitope antibodies with monoclonal specificity (mAbs) into AuNP dispersions caused a drop in plasmon peak intensity and an immediate red-shift, while 100–

300 nM of mAbs resulted in extensive red-shift and broadening of the plasmon peak. This assay enabled the detection of disease-specific antibody levels of 0.01–10,000 μg mL^{-1} in serum.

A conceptually related mechanism was reported recently in application to the diagnosis of acute severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) virus. Prikshit et al. designed AuNPs capped with thiol-modified antisense oligonucleotides that aggregate in the presence of the N-gene of target RNA of SARS-CoV-2.[43] The selective agglomeration of AuNPs occurred upon the addition of thermostable RNaseH that specifically cleave the phosphodiester bonds of the N-gene strand (Figure 4a). The visible precipitate in solution, and the absorbance at 660 nm of AuNPs treated with SARS-CoV-2, gave a LOD down to 1 ng mL^{-1}. Many other bioconjugate formats can be explored to have similar effects. For example, Zheng et al.[44] monitored the color change of aggregating AuNPs to indicate different concentrations of *Escherichia coli*. Aggregation was triggered by mixing AuNPs with cross-linking agents (phenolic hydroxyl moieties in tyramine) in the presence of capture antibody-capped magnetic NPs, and detection antibody-modified polystyrene microspheres. Here the color change could be monitored in a microfluidic chamber with catalase and *Escherichia coli* cells at concentrations as low as 50 CFU/mL within 1 hour.

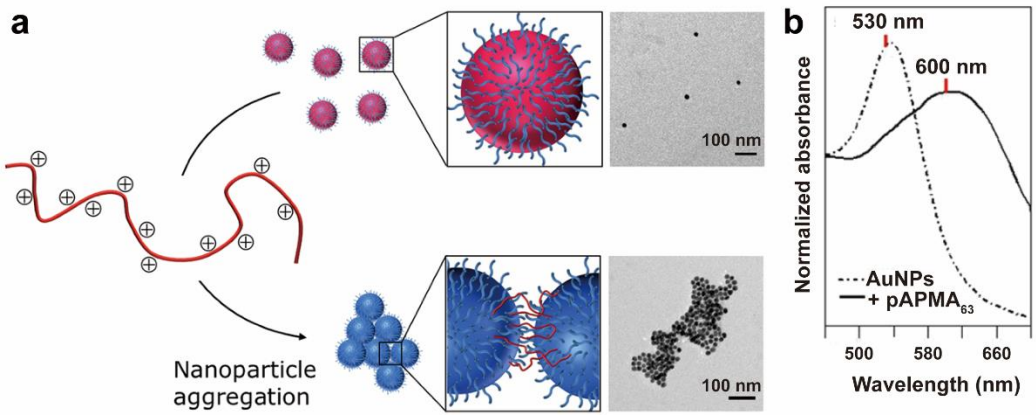

Figure 4. (a) Schematic illustration of an assay design that utilizes polymerization-based signal amplification. (b) Absorbance spectra of dispersed and aggregated AuNPs due to the presence of pAPMA. Reproduced with permission from Ref. [45] Copyright 2015 American Chemical Society.

Building on previous aggregation-based assays, we later designed platforms aimed towards enzymes (e.g., HRP and catalase) and ions (e.g., iron and copper) as they can generate radicals to form polymers that entangle AuNPs into aggregates (Figure 4b).[45] This polymer-mediated assay offers a promising signal amplification strategy. A cascade initiated in the presence of a very low concentration of free radicals can trigger AuNPs aggregation by formation of large polymer chains, resulting in a remarkable visible color change. For example, 1 μg mL^{-1} of HRP could be detected as it triggers the polymerization of 3-aminopropyl methacrylamide (> 10^{-1} mg mL^{-1}), enabling LOD of catalase down to 0.7 ng mL^{-1}

using an inverse assay format.

3.3 Liposome-triggered aggregation assay

Although the colorimetric response of NMNPs has been demonstrated in many systems, a compromise is often made between non-specific interaction with the substrates and particle stability. Liposome provides a multifunctional platform that can be activated to release cargos such as DNA, polypeptides, and proteins to guide the assembly/disassembly process of NPs.

Degradation of liposomes can be triggered by phospholipases, an enzyme involved in various physiological processes such as inflammatory responses and intercellular signalling. Phospholipases act particularly on lipid bilayers and micelles rather than free lipid monomers, giving a high selectivity. One class of phospholipases, Phospholipase A_2 (PLA_2), have been extensively demonstrated as biomarkers of many infectious conditions such as bacterial sepsis,[46] which is why we developed a liposome-triggered NMNP aggregation assay for PLA_2.[47] Our system comprised polypeptide-functionalized AuNPs (JR2EC) and complementary polypeptide ($JR2KC_2$) capped AuNPs. The 42-residue JR2EC could form bridges with nanomolar amounts of $JR2KC_2$, causing rapid assembly of AuNPs (Figure 5a). When exposed to PLA_2, the liposomes were degraded, releasing the pre-encapsulated $JR2KC_2$ functionalized AuNPs, enabling aggregation of AuNPs and real-time monitoring of the significant redshift. In a rapid testing format, monitoring aggregation of AuNPs allowed us to detect PLA_2 at concentrations down to 700 pM. We then undertook a detailed characterization of this highly controllable assembly of AuNPs using UV-vis spectroscopy and small-angle X-ray scattering.[48] Our results showed that JR2EC monomer was immobilized on AuNPs through a cysteine residue folded into a four-helix bundle, which triggered the assembly of NPs via a folding-dependent bridge. Specifically, two JR2EC monomers on AuNPs heteroassociate with one $JR2KC_2$ and fold into two disulfide-linked four-helix bundles (Figure 5b), driving the assembly of AuNPs.

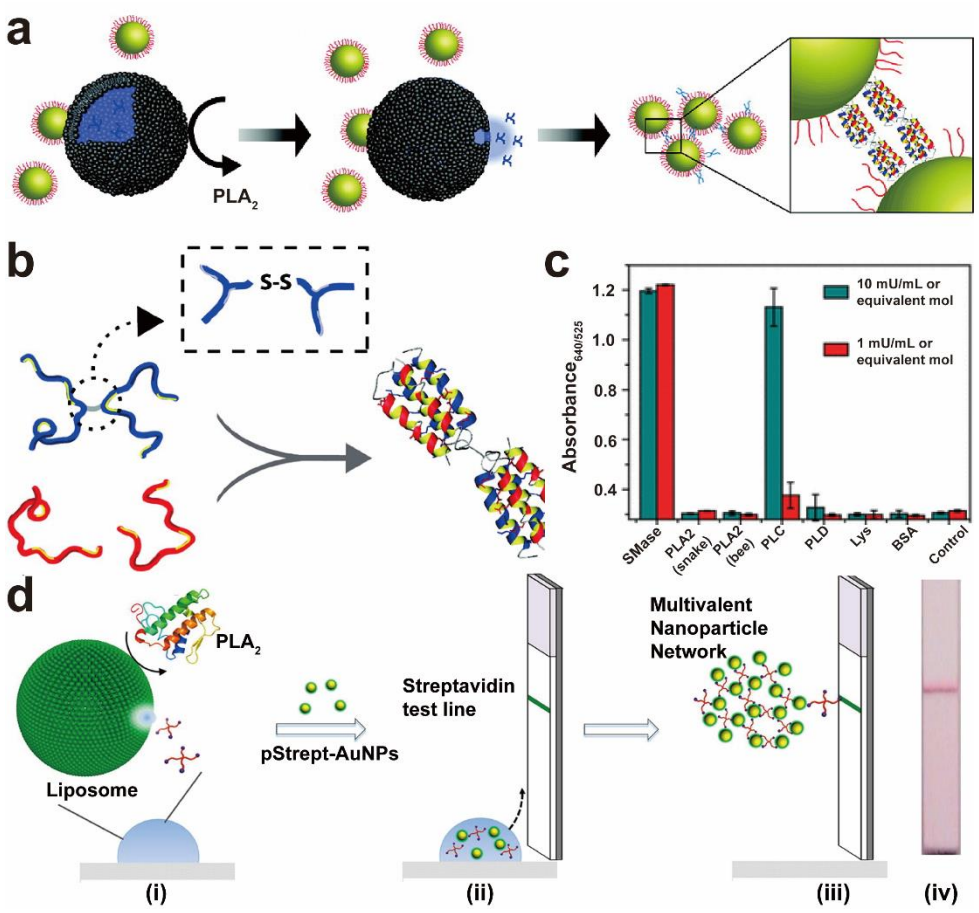

Figure 5. (a) Mechanism of PLA_2 detection based on the release of $JR2KC_2$ from a liposome. Reproduced with permission from Ref.[47] Copyright 2011 American Chemical Society. (b) The formation of AuNPs assemblies based on heteroassociation between JR2KC and glutamate-rich helix-loop-helix polypeptide. Reproduced with permission from Ref.[48] Copyright 2011 American Chemical Society. (c) Sensitivity assay in reaction buffer containing SMase, denatured SMase, Triton X100, PLA_2, phospholipase D (PLD), phospholipase C (PLC), lysozyme (Lys), and bovine serum albumin (BSA). Reproduced with permission from Ref.[49] Copyright 2018 American Chemical Society. (d) LFA device schematic based on the liposome-triggered assay. Reproduced with permission from Ref.[50] Copyright 2015 American Chemical Society.

In a related study, we demonstrated cysteine release from sphingomyelinase-based liposomes, driving AuNPs aggregation to detect the sphingomyelinase enzyme (SMase), a biomarker of several infectious diseases including HIV.[49] Here, a red AuNP dispersion was first added to the solution of cysteine pre-loaded liposomes, as cysteine is a small zwitterionic molecule with a thiol moiety that strongly binds gold.[51] In the presence of SMase, cysteine was released as the liposome was enzymatically hydrolyzed into ceramide and phosphocholine. The released cysteine caused the aggregation of AuNPs through zwitterionic electrostatic interactions and hydrogen bonding between cysteines as they bound onto AuNPs. The color change from red to blue after aggregation was evident by peak broadening at 525 nm

due to the quadrupole plasmon excitation, and a shoulder peak at 640 nm due to the dipole plasmon resonance. We used the ratio between absorbance at 525 nm and 640 nm to determine the concentration of cysteine. The LOD determined using the absorbance ratio was 0.02 mU mL^{-1}, which is equal to an enzyme concentration of 1.4 pM. Compared to other common phospholipases, the liposome-triggered aggregation exhibited higher selectivity (Figure 5c).

The usage of liposomes that take advantage of simultaneous binding to multiple ligands on NPs has also been applied to amplify signals of LFAs. We developed a rapid lateral flow assay for PLA_2 detection in serum using adhesion of polystreptavidin-coated AuNPs (Figure 5d).[50] PLA_2 triggered the release of a biotinylated four-armed poly(ethylene glycol) (PEG) from liposomes due to the cleavage of phospholipids. Imaging devices including cameras, commercially available readers, and smartphones could be used to capture the colorimetric signal enabling in-field diagnosis. Our design demonstrated detection of 1–10 nM of PLA_2 in sera samples of acute pancreatitis, which was lower than the commercial kits (50 nM). This design also offers high flexibility, as changing the liposome components could tune it towards various natural enzyme activities. For example, we utilized a secretory phospholipase A2 group IIA ($sPLA_2$-IIA)-based liposome substrate to release the four-armed PEG in LFA devices for POC measurement of $sPLA_2$-IIA, which is associated with rheumatoid arthritis.[52] The concentration of $sPLA_2$-IIA in healthy serum measured using our LFA was 21.9 ± 23.9 ng mL^{-1}, in line with ELISA rheumatoid analysis. However, our simple LFA-based method should be an attractive alternative for rapid testing outside clinical settings, given that there is no need for laboratory facilities.

3.4 Signal amplification based on catalytic NMNPs

All of the assays discussed so far have utilized the plasmonic properties of NMNPs to generate colorimetric signals. However, NMNPs also possess powerful enzyme-like catalytic properties, including peroxidase-, oxidase-, hydrolase-, esterase-, phosphatase-, and superoxide-like enzymatic activities, which can increase sensitivity by orders of magnitude.[53-55] Bare NMNPs exhibited the highest activity, which could be inhibited after covering with a wide range of ligands such as phosphate and citric acid. On the other hand, metal ions, including Cr^{3+} and Fe^{2+}, could activate redox cycle reactions to improve catalytic activity. A colorimetric sensor is thus available by monitoring the change of chromogenic substrates such as 3,3′,5,5′-tetramethylbenzidine (TMB) in the presence of hydrogen peroxide (H_2O_2).[56] Another recent example of using NMNP catalytic properties came from Mohamed et al.,[57], who labeled PtNPs with a monoclonal antibody against the envelope protein of ZIKV virus to detect the virus on-chip (Figure 6a). On-chip stable gas bubble formation in the presence of H_2O_2 was detectable by a smartphone. Sensitivity and specificity of 98.97% and 91.89%, respectively, were achieved under different temperature and humidity conditions with a virus concentration of 250 copies/mL for ZIKV samples, HBV, and HCV patient plasma/serum samples.

Our interest in the catalytic properties of NMNPs has allowed us to develop multiplexed protease nanosensors for *in vivo* early detection of both infectious and non-communicable diseases.[2] Molecular-like ultrasmall gold nanoclusters (AuNCs) can function as peroxidase-like enzymes to oxidize chromogenic substrates (e.g., TMB) for signal generation and amplification. This is of particular interest, as they exhibit promising renal clearance properties. Based on our previously reported protease responsive NMNP systems consisting of bioactive peptides,[26,27,29,30,45] catalytic AuNCs were capped with thiol-terminated peptides that could be selectively cleaved by the serine protease thrombin or the zinc-dependent matrix metalloproteinase 9 (MMP9), which have been used as biomarkers for cardiovascular diseases and cancer, respectively. The functionalized AuNCs were intravenously administered and disassembled at the site of diseases. After being proteolytically liberated from the MMP9-responsive or thrombin-responsive peptides, urine samples containing catalytic AuNCs were collected and generated an observable blue color after adding TMB (Figure 6b). Compared to healthy mice, the urinary signal increased by 13-fold as measured by the colorimetric readout.

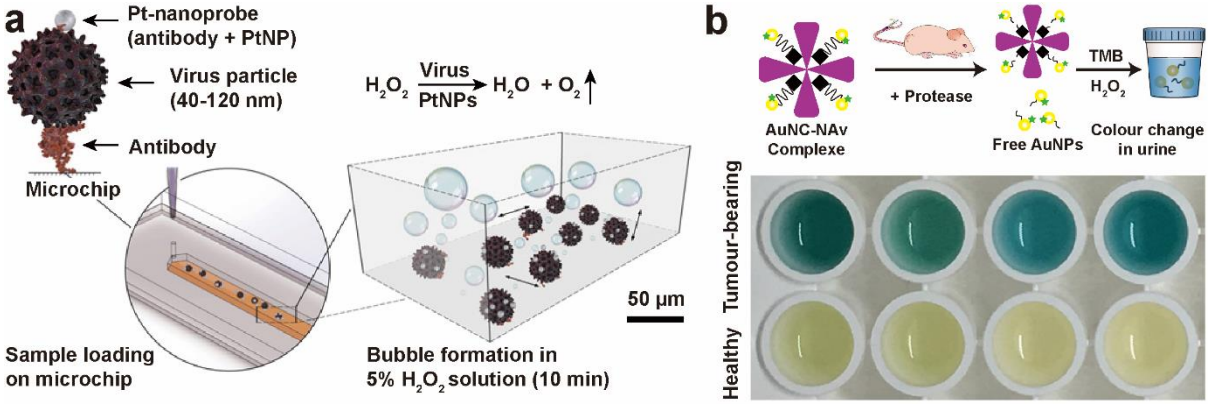

Figure 6. (a) Detection of virus particles on a microchip by bubble formation in the presence of PtNPs and H_2O_2. Reproduced from Ref. [57] Copyright The Authors. (b) Design of a renally-clearable sensing system based on the nanocatalyst signal amplification. Photograph shows the colorimetric assay of urine from tumour-bearing (top) and healthy mice (bottom). Reproduced from Ref. [2] Copyright The Authors.

4. Towards infectious disease diagnostics at the point-of-care using noble metal nanoparticles

Our development of NP-based biosensors has yielded assays for a variety of non-infectious disease targets over the years, including cancer,[26] pancreatitis,[38,58] arthritis,[52] blood coagulation,[59] prostate-related diseases,[29] and cardiovascular disease.[2] During this time, our attention began to turn to the threat of infectious diseases, as it was apparent that issues such as the increasing prevalence of antimicrobial resistance and risk of zoonotic or mutant-driven pandemic were not being matched by an increase in invention and translation of viable and field-deployable IVD devices. In 2013, we became founding partners of an Interdisciplinary Research Collaboration (IRC) in the UK, called *i-sense*, funded by the

Engineering and Physical Sciences Research Council (EPSRC) to develop digital health systems to test, track and treat infectious diseases.[60] Subsequently, we have developed assays targetting various infectious diseases, including influenza,[61-63] HIV,[3,49,64] tuberculosis,[65] and Ebola.[4]

In the context of infectious disease, actionable information obtained at or near the location of the patient, be it at home, in the field or in a hospital bed, is the key to better management, early containment and treatment, and improved patient outcomes.[66] The current state-of-the-art technology is LFAs, which has become a hallmark in many national testing strategies during the COVID-19 pandemic. Particularly, the strong visible color changes due to the SPR variations of NMNPs triggered by analytes offer an elegant solution to enhance the diagnostic readout. Our group developed an immunochromatographic POC test comprising antihuman IgG antibody-capped AuNPs (40 nm) and a mobile phone for semiquantitative multiplexed antibody detection.[4] As shown in Figure 7a, our test strip design is universal by using recombinant viral protein to detect IgG antibodies against several viral subtypes, including Ebola, Bundibugyo, and Sudan virus. The LOD of the platform is 200 ng mL^{-1} with an assay time of 15 min for 15 µL of serum. Combined with in-app analysis, semiquantitative results could be achieved by capturing images and analyzing color intensities of the test strip (Figure 7b). With geographic tagging, secure data storage and management, our platform showed great potential as a POC technology to manage recovered patients and improve epidemic control.

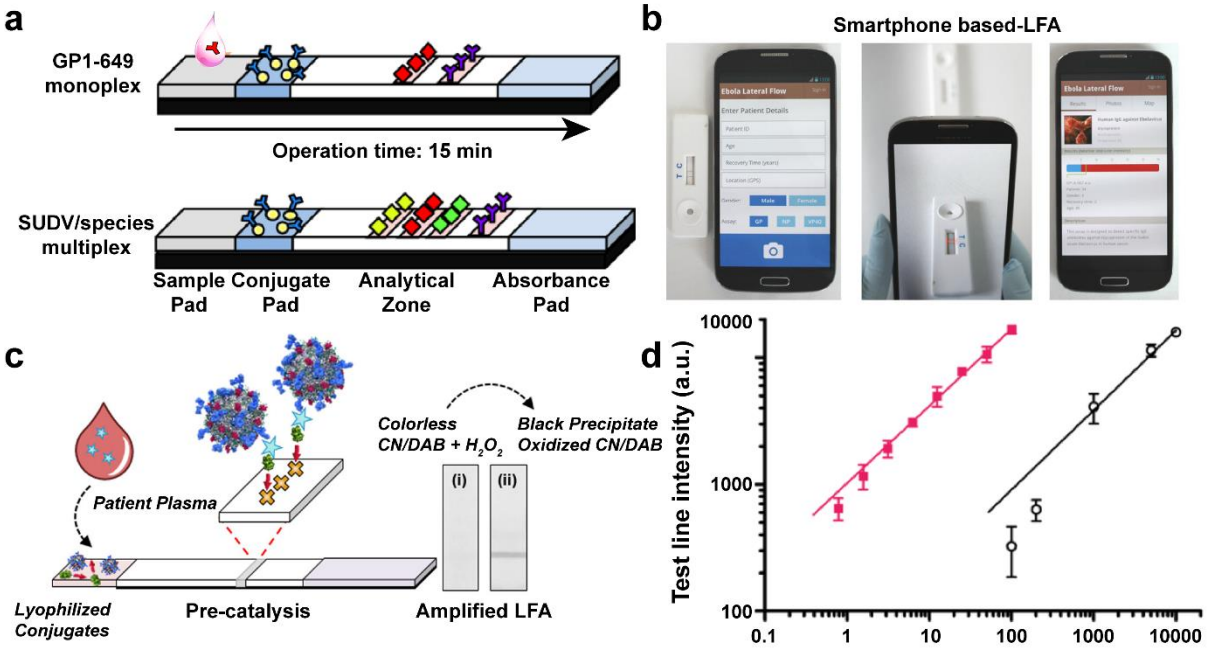

Figure 7. (a) Lateral flow test for Ebola virus IgG detection. (b) Integration of a smartphone application for recording patient details. Reproduced from Ref. [4] Copyright 2018 American Chemical Society. (c) Schematic illustration of catalytic NMNP amplified LFA. (d) Test line intensity illustrating the broad linear dynamic range in spiked sera across four orders of magnitude (from < 1 pg mL^{-1} to 10^4 pg mL^{-1}) of the protein target before and after amplification. R^2 = 0.9813. Reproduced from Ref. [3] Copyright 2018 American Chemical Society.

The ability of NMNPs to oxidize chromogenic substrates provides a powerful method to improve LFA sensitivity. Inspired by this, we incorporated NMNPs into an LFA test for HIV, targeting p24, which allows disease diagnosis within two weeks of infection.[3] The monoclonal antibody (IgG antibody) was bound onto platinum nanocatalysts (PtNCs) to target p24. An anti-p24 nanobody against various subtypes of HIV was used to biotinylate the PtNCs to a streptavidin test line. Nanobodies can be easily produced by bacteria and show high site-selectivity to the target epitope. Then, p24 in human plasma samples could be sandwiched between this anti-p24 nanobody and PtNCs, followed by capture at the streptavidin modified test line (Figure 7c). A clear black line could be observed by the naked eye or smartphone imaging when the concentration of analytes ranged from 100 to 10000 pg mL^{-1}. PtNCs captured on the test line disproportionated H_2O_2 to oxidize a chromogenic substrate (e.g., TMB), generating an unambiguous colored product. Consequently, lower concentrations (1–100 pg mL^{-1}) could be detected (Figure 7d). The dual range diagnostic regime empowers smartphones or other image analysis systems to quantitatively or semi-quantitatively evaluate the antigen level. Human plasma samples from ZeptoMetrix with low levels of p24 (10.8 pg mL^{-1}) and HIV-1 RNA (4.6 pg mL^{-1}) were also successfully analyzed. This LOD covers the concentration window from initial infection to seroconversion (a period over which antibodies develop), which important for early-stage detection.

5. Summary and outlook

Although much research of NMNPs has been conducted, there is still much to learn and much potential to be unlocked, particularly concerning improving signal transduction and protection against non-specific binding and aggregation. Surface modification of NMNPs with molecules including protein, nucleic acid, drugs, polymers, and peptides is of significant importance to solve these problems and are widely demonstrated in *in vitro* and *in vivo* applications.[67] Other nanomaterials will be useful for ultra-high sensitivity including fluorescent materials (quantum dots, upconverting nanoparticles, fluorescent dyes), photothermal/photoacoustic AuNPs, magnetic NPs, electrochemical labels etc.[68] Deployment in the field necessitates minimal manual operation. A trade-off must be weighed up, given the usage of those materials will complicate testing. To ensure the performance of LFAs, pads (detection, conjugate, absorbent), buffers (lysis and immobilization), membranes, and bioreceptors requires optimization to achieve sufficient shelf life, further necessitating research into stabilizers like sugars, agars, and gelatins.[69] Barcode-style assays with each line representing different targets could prove to be the solution towards challenges with multiplexed detection, which is highly desirable for POC testing. The increasing computational power of smartphones facilitate data acquisition, analysis and processing, which will revolutionize the use of field-deployable IVDs.[1,70,71]

Surface-enhanced Raman spectroscopy (SERS) has proven LODs down to the single-molecule level by taking advantage of electromagnetic properties of confined NMNP surfaces to amplify light scattering

up to 10^{13}-fold compared to conventional Raman spectroscopy.[72] Our group has previously exploited SERS and confocal Raman spectroscopy to interrogate and visualize 3D biomolecule distributions in disease progression of infections such as tuberculosis in 3D cell cultures and zebrafish.[73-75] While reaping the benefits of SERS might be considered a straightforward idea of POC, the introduction and manufacturing of nanostructures, surface modifications, and reader instrumentation significantly complicate SERS-based diagnostics. The need for instrumentation increasingly requires our careful reconsideration of not just biosensors and diagnostics, but of the entire POC testing continuum as well, from research through translation, production, and ultimately reading. Increased focus will be put on the challenge of condensing the physical footprint of test and reader devices, driven by test specifications and rational device design choices, in an effort to make the ultrasensitive NMNP-based tests more economically viable and widely distributable. While a failure to do so cannot limit the value of engineering novel biosensor technologies or their applications in established laboratory environments, it does hinder their clinical and commercial translation.

Going forward, as researchers, we need to balance the need for realistic, commercially viable IVDs against the need for fundamental discovery involving potentially exotic and expensive new materials and biomolecules. On the one hand, we must consider from the outset whether it is even feasible for an idea to one day reach a deployable product, for we cannot afford to waste time on dead ends. On the other hand, we cannot let this pressure stifle the exploration and innovation that happens in fundamental research. There is no easy answer to this, but it is critical that we find a balance. A key recent example of this is the discovery of the CRISPR-Cas enzyme family, which was born out of observations of anomalies in genetic code,[76] and has gone on to revolutionize various areas of application-oriented research, including many high profile examples in IVDs.[77]

The Covid-19 pandemic has brought to attention the drastic need for infectious disease management and pandemic preparedness, and the vital importance of effective IVD devices in these processes. We must build on the hard lessons learned in managing COVID-19 to get ready for other emerging and potentially hard-hitting infectious disease threats. Further, we need to be prepared for the imminent threat of antimicrobial resistance-associated infectious diseases. These are not currently an everyday problem for most people, but they likely soon will be.

We see plenty of opportunities to apply versatile NMNP-based diagnostic technologies integrated with LFAs, SERS, and smartphones, and we are excited to see how the exceptional proliferation of IVDs becomes a widely accessible reality in the coming years. NMNPs-based diagnostic systems and their integration with self-testing devices are increasingly likely to generate breakthroughs to improve healthcare systems by driving more sensitive detection and more efficient care that overcome the current limitations of detection and enhance the rapid response to both established and emergent infectious diseases.

AUTHOR INFORMATION

Author Contributions

The manuscript was written through the contributions of all authors.

Notes

The authors declare the following competing financial interest: MMS is co-inventor on a patent application related to this work filed by the Imperial College London (no. PCT/EP201 1/061695, filed 8 July 201; no. PCT/EP20 19/066 106, filed 18 June 2019; no. PCT/US2019/056454, filed 16 October 2019; no. 2110729.7, filed 26 July 2021). The remaining authors declare no competing interests.

Biographies

Hongya Geng

Hongya Geng received his PhD from the Chinese Academy of Science in 2017 and now serve as a postdoctoral research associate in the Department of Materials, Imperial College London. His works have been published in Nat. Commun., J. Am. Chem. Soc., Angew. Chem., Adv. Mater., and ACS Nano etc. During the years, his research interest centres on nanoparticles systems, hydrogels, conductive polymers and biomaterials for regenerative medicine and diagnosis.

Simon Vilms Pedersen

Simon Vilms Pedersen holds a PhD degree in engineering from the University of Southern Denmark (2020). He is currently a postdoctoral researcher at the Department of Materials, Imperial College London, funded by Independent Research Fund Denmark. His research focuses on the development and application of Raman devices for ultrasensitive biosensing and diagnostics at the point-of-need.

Yun Ma

Mr. Yun Ma received a master’s degree from The University of Sheffield in 2019 for work with Dr. Xiangbing Zeng on Optical Properties and Structure of Liquid Crystal Compounds. Since he joined Prof. Molly Stevens’s Group, Imperial College, as a Ph.D. student, his work has been focused on polypyrrole-coated gold nanoparticles for 3D-cell imaging and biomarker analysis

Tabasom Haghighi

Tabasom Haghighi received her master degree in Chemistry with Biomedicine at King's College London in 2018. She is currently a PhD student in the group of Professor Molly Stevens at Imperial College London. Her research focuses on developing ultrasensitive diagnostic devices based on

nanoparticles for early detection of diseases.

Hongliang Dai

Hongliang Dai, is currently an associate professor in the School of Environmental and Chemical Engineering, Jiangsu University of Science and Technology. He is mainly engaged in the research of pollute detection in water using Raman spectroscopy. His works have been published in Environ. Sci. Technol., Chem. Eng. J., Bioresource Technol., Environ. Pollut., Sci. Total Environ., etc.

Philip Howes

Philip Howes is a lecturer in Mechanical Engineering and Design at London South Bank University, UK. His research focuses on the development of nanomaterials for application in energy, environment and healthcare technologies, which includes the use of microfluidic engineering to expedite the translation of nanomaterials from fundamental study through to advanced applications.

Molly M. Stevens

Molly Stevens is a Professor of Biomedical Materials and Regenerative Medicine at Imperial College London and the Director of the UK Regenerative Medicine Platform Hub for Smart Materials. She is Foreign Member of the US National Academy of Engineering and a Fellow of the Royal Society and the Royal Academy of Engineering. Her research focuses on material-based approaches for applications in advanced therapeutics, regenerative medicine and biosensing.

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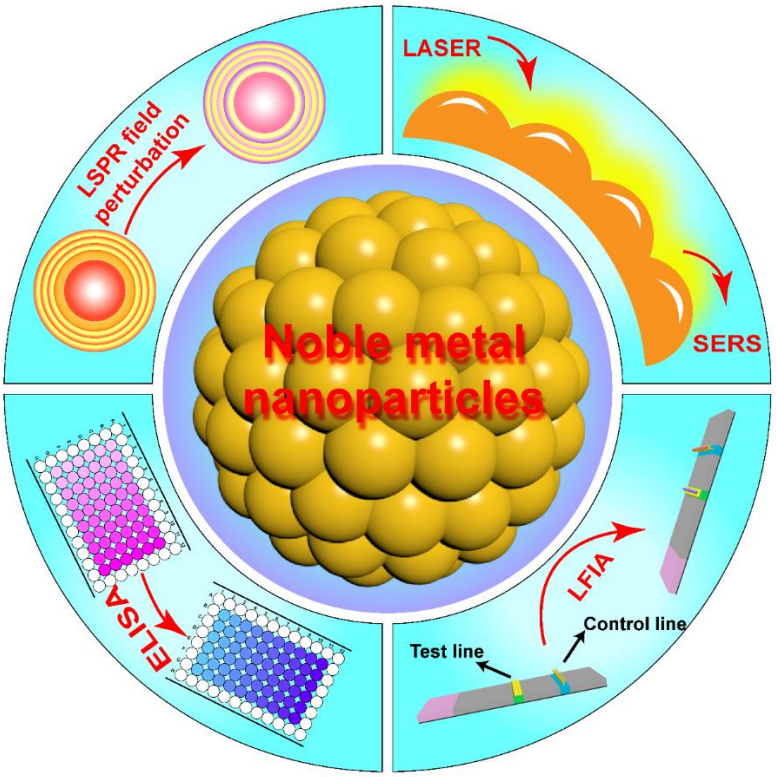
TOC


LSPR field perturbation
LASER
SERS
Noble metal nanoparticles
ELISA
LFIA
Control line
Test line