ACS APPLIED BIO MATERIALS

www.acsabm.org

Review

# Nanoplasmonic Sensor Approaches for Sensitive Detection of Disease-Associated Exosomes

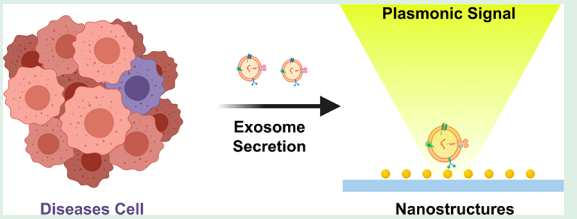
Pouya Amrollahi,[†] Wenshu Zheng,[†] Chandler Monk, Chen-Zhong Li, and Tony Ye Hu*

Cite This: https://doi.org/10.1021/acsabm.1c00113

Read Online

ACCESS | Metrics & More | Article Recommendations

**ABSTRACT:** Exosomes are abundantly secreted by most cells that carry membrane and cytosolic factors that can reflect the physiologic state of their source cells and thus have strong potential to serve as biomarkers for early diagnosis, disease staging, and treatment monitoring. However, traditional diagnostic or prognostic applications that might use exosomes are hindered by the lack of rapid and sensitive assays that can exploit their biological information. An array of assay approaches have been developed to address this deficit, including those that integrate immunoassays with nanoplasmonic sensors to measure changes in optical refractive indexes in response to the binding of low concentrations of their targeted molecules. These sensors take advantage of enhanced and tunable interactions between the electron clouds of nanoplasmonic particles and structures and incident electromagnetic radiation to enable isolation-free and ultrasensitive quantification of disease-associated exosome biomarkers present in complex biological samples. These unique advantages make nanoplasmonic sensing one of the most competitive approaches available for clinical applications and point-of-care tests that evaluate exosome-based biomarkers. This review will briefly summarize the origin and clinical utility of exosomes and the limitations of current isolation and analysis approaches before reviewing the specific advantages and limitations of nanoplasmonic sensing devices and indicating what additional developments are necessary to allow the translation of these approaches into clinical applications.




**KEYWORDS:** *extracellular vesicles, exosomes, nanoplasmonics, metamaterial, biosensor*



## EXOSOMES AS BIOLOGICAL TREASURE

Exosomes are small (30−150 nm) membrane-bound extracellular vesicles (EVs) secreted by all cell types by evolutionarily conserved constitutive and inducible mechanisms.[1,2] Following their secretion, exosomes accumulate in an array of biological fluids (e.g., blood, urine, saliva, breast milk, cerebrospinal fluid, and tears). Exosome secretion was initially considered as a means for cells to discard unwanted material.[3] However, subsequent studies found that the composition of exosomes could differ from that of their parent cells because of selective enrichment effects, which could differ by cell type and other parameters; that exosomes could exhibit enhanced uptake by specific cell types depending upon their cell of origin; and that exosome uptake could alter the phenotype of their recipient cells.[4] Exosomes were also found to differ from other EVs in that they contained selectively packaged material from most cellular compartments and membranes, in part due to their endosomal origin, and to capture a wealth of biological information that is not present in other EV types or membranous particles. This includes information underlying both physiological mechanisms (e.g., intercellular communication[5] and regulation of the immune response and morphogenesis[6,7]) and pathological processes (e.g., cancer,[8] viral and bacterial infection,[9] and neurodegenerative diseases[10]) that may be partially regulated by the transfer of exosome cargoes that exert effector functions. Taken together, these features have led to exosomes being considered an excellent source of potential biomarkers.

Mechanisms governing exosome biogenesis are covered in other reviews, but broadly speaking, the secretion and uptake dynamics of exosomes are largely regulated by their membrane proteins, whereas their biological functions depend upon both their surface and internal composition, and all of these are controlled by mechanisms and stimuli that are active during their biogenesis to control their composition.[11,12] Briefly, exosome biogenesis begins with inward budding of the plasma membrane to form early endosomes that incorporate plasma membrane proteins,[13,14] which can then undergo inward budding events that result in the accumulation of intraluminal vesicles (ILVs) to produce multivesicular bodies (MVBs).[15,16] These MVBs can fuse with lysosomes, resulting in the degradation of their contents,[17] or utilize soluble N-ethylmaleimide-sensitive factor attachment protein receptors

Special Issue: Biospecies Sensors

Received: January 29, 2021
Accepted: July 20, 2021

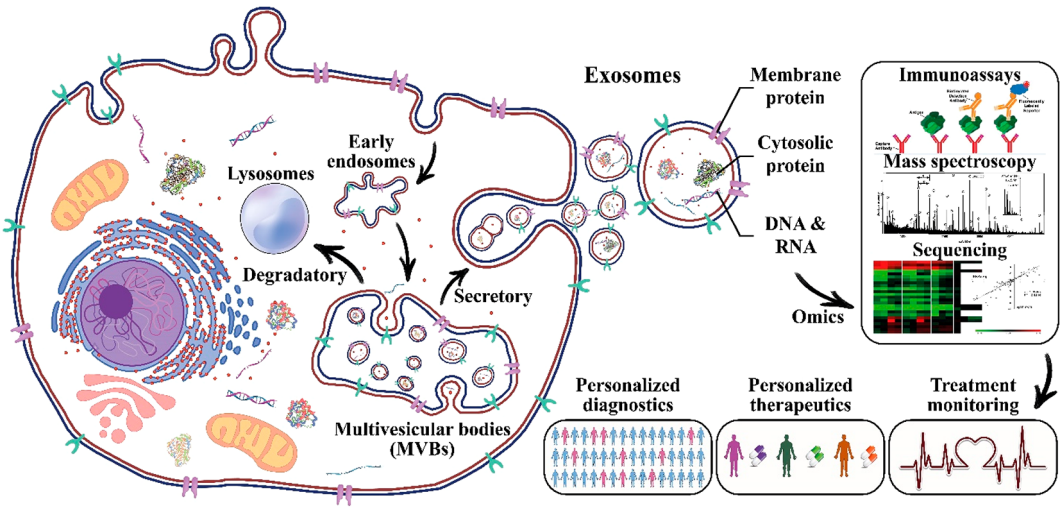



**Figure 1.** Exosome biogenesis and translational applications. (Left) Early endosomes can mature into multivesicular bodies (MVBs) and either fuse with lysosomes and be degraded or migrate to the cell membrane where their intraluminal vesicles (ILVs) can be secreted as exosomes. (Right) Exosomes carry a wealth of material from their cells of origin (e.g., membrane and cytosolic proteins, DNA, and RNA) that can be analyzed via various applications to evaluate the phenotype of these cells. The amount of information carried by exosomes makes them a promising source of candidate biomarkers for translational applications for personalized disease diagnosis, prognostic evaluations, and treatment monitoring.

(SNAREs) to fuse with the plasma membrane[18−20] and release their ILV contents into the extracellular space as exosomes. Cells encapsulate a diverse repertoire of cytosolic and membrane proteins, lipids, and genetic information (double-stranded DNA and various RNA types) into exosomes during their biogenesis.[21] Exosomes, therefore, represent a rich source of biomarkers that may allow greater insight into disease progression, and which can be readily obtained through minimally invasive liquid biopsy procedures, including standard peripheral blood draws (Figure 1).

## EXOSOMES AS POTENTIAL DIAGNOSTIC BIOMARKERS

**Exosomes in Cancer.** Cancer diagnosis is often delayed because of nonspecific symptoms and the lack of cost-effective diagnostic approaches or specific biomarkers.[22] Cancer-derived exosomes have the potential to address this situation because they can exhibit novel or altered expression of cancer-associated factors, including oncoproteins, growth factors, immunomodulatory molecules, RNAs, and genomic DNA fragments containing cancer-associated mutant alleles.[23,24] Furthermore, both cell-intrinsic conditions (e.g., overexpression of endosomal sorting complex required for transport (ESCRT) complex components,[25] activation of oncogenic signaling pathways,[26] and elevated cytosolic DNA concentrations[27]) and microenvironmental conditions (e.g., hypoxia[28]) can promote the enhanced exosome secretion by tumors and therefore increase their utility as biomarkers. Cancer-derived exosomes also play major roles in cell-to-cell communication to regulate tumorigenesis and metastasis. They can alter the tumorigenesis by promoting cancer invasiveness by affecting tumor−stroma interactions, suppressing the immune response,[29] and stimulating tumor angiogenesis.[30] Cancer-derived exosomes can increase directional cell movement and migration speed,[31] degrade the extracellular matrix,[32] and enhance tumor growth by stimulating the differentiation of myofibroblastic cells,[33] and thus specific factors associated with these exosomes have the potential to function as biomarkers for more invasive and aggressive cancer stages. Cancer-derived exosomes released into the circulation can also interact with distant cells to promote their adjacent tissues to adopt traits that lead to the formation of premetastatic niches that promote metastasis,[34,35] and the tissue specificity of this targeting can be regulated by exosome membrane proteins,[36] whereas the transfer of their RNA species to such sites can also increase the aggressiveness of distant tumors.[37] Exosomes can thus serve as biomarkers for metastatic cancer, as well as means to identify anatomical sites most likely to host premetastatic niches to improve tumor surveillance. For example, melanoma-derived exosomes have been shown to increase pro-metastatic behavior by educating bone marrow progenitor cells to develop premetastatic niches,[38] whereas exosomes expressing epidermal growth factor receptor (EGFR) have been reported to educate liver tissue to host premetastatic niches for gastric cancer metastases.[39] In breast cancer studies, it has been shown that miRNAs and miRNA maturation proteins are packaged in cancer-derived exosomes that migrate to distant organs where they can increase vascular leakiness,[40] alter glucose metabolism to enhance tumor growth,[41] increase resistance to chemo therapy,[42] and promote the malignant transformation of nonmalignant cells by altering their transcriptomes.[43] RNA species carried by exosomes can exert effector functions upon their uptake by recipient cells,[44] and have been shown to promote chemo-resistance in triple-negative breast cancer[45] and pancreatic cancer,[46] as well as increasing chemo-sensitivity.[47,48] From a diagnostic/prognostic point-of-view, rapid and ultrasensitive detection of protein and mRNA/miRNAs signatures in cancer-derived exosomes can enhance cancer diagnosis and staging and improve the evaluation of tumor responses to a course of treatment.[49−52]

**Exosomes in Neurodegenerative Diseases.** Conventional techniques for the diagnosis of neurodegenerative diseases primarily rely on positron-emission tomography (PET) imaging studies or require an invasive procedure to obtain cerebrospinal fluid for molecular analyses. Although exosomes secreted by neurons, astrocytes, and microglia can move from the site of their release and cross the blood−brain


https://doi.org/10.1021/acsabm.1c00113
ACS Appl. Bio Mater. XXXX, XXX, XXX−XXX

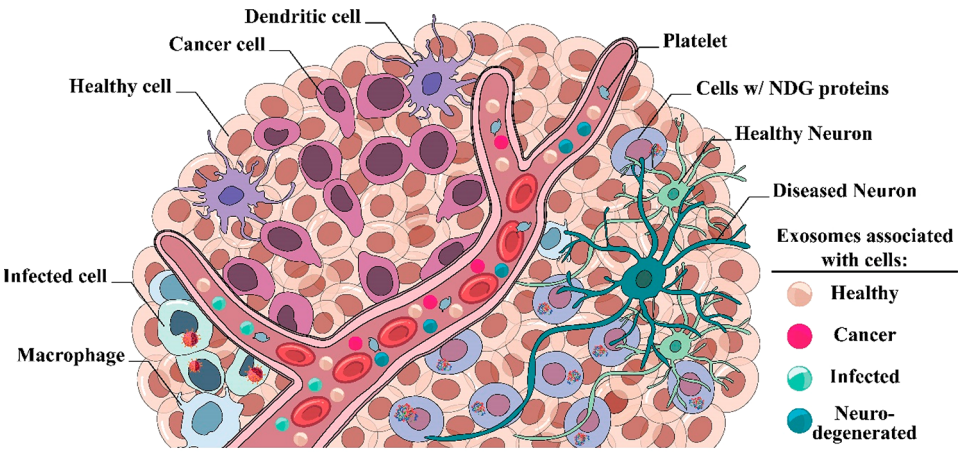



**Figure 2.** Exosomes and disease progression. Cancerous, infected, or damaged cells can secrete exosomes that carry factors (e.g., mutant alleles or cancer-associated factors, viral genomic material or proteins, or factors associated with tissue injury conditions such as neurodegenerative diseases) that can be transferred to adjacent and distant cells to alter the local microenvironment or affect distant tissues and organs.

barrier[53−55] to enter and accumulate in the cerebrospinal fluid,[56] as well as the circulation, and thus have potential utility in the development of minimally invasive assays that can distinguish specific neurological disorders. Exosomes released by neural tissue can carry neuron-specific markers, including transmembrane proteins,[56] and are reported to regulate intercellular communication in the central nervous system to support myelination, plasticity, and antigen presentation.[57] It thus predicted that such exosomes could serve as a source of viable biomarkers for multiple neurodegenerative conditions, including, Alzheimer's[58] and Parkinson's[59] disease and prion-based diseases,[60] as long as the integrity of their surface proteins and cargoes is preserved during their isolation. Indeed, exosomes purified from the central nervous system and brain tissue are reported to contain dysregulated miRNAs[61] and disease-associated proteins and peptides[58,62,63] linked to neurodegenerative diseases. This includes markers such as tau and amyloid-$\beta$ (A$\beta$) that are associated with Alzheimer's disease; Syntenin 1, $\alpha$-synuclein, and amyotrophic lateral sclerosis ($\alpha$-synuclein) associated with Parkinson's disease; and huntingtin that is associated with Huntington's disease.[64−66] Neuronal tissue exosome cargo changes have been investigated in some diseases,[67] although the mechanisms responsible for such changes are not known. The relative scarcity of these biomarker-rich exosomes and the invasive cerebrospinal fluid collection procedure limits their clinical utility when analyzed by current analysis approaches.[10] Novel assay approaches are needed to address these two issues.

**Exosomes in Infectious Disease.** Cells infected with viral and microbial pathogens release exosomes containing factors expressed by these pathogens, which may include immunomodulatory factors, into their microenvironment.[68] Some viral assembly and exosome biogenesis pathways share several components, including ESCRT subcomplexes and tetraspanins, but the knowledge about the mechanisms that govern sorting pathogen-derived components into exosomes is fragmentary. HIV is one of the most widely studied pathogens with regard to its interactions with the exosome secretion machinery. The HIV membrane protein Gag and the HIV virulence factor Nef have been detected on the surface of exosomes secreted by HIV-infected cells, whereas the HIV trans-activation response element RNA has been detected in the exosome cargoes of these cells.[69−71] Similarly, exosomes secreted by Epstein−Barr virus (EBV)-infected cells express EBV latent membrane protein 1, which has immunosuppressive functions. These exosomes also contain numerous EBV-derived miRNAs that can alter the transcriptome of their recipient cells and therefore alter their phenotypes.[72]

Some intracellular pathogens employ their own machinery to incorporate pathogen-derived components into exosomes (HIV),[73] regulate the ESCRT pathway to promote their survival by altering endosome trafficking and antigen presentation (*Mycobacterium tuberculosis*),[74] or can upregulate stress-associated factors (70 kDa heat shock protein) to stimulate exosome production by their host cells (*Mycobacterium avium* and *Mycobacterium smegmatis*).[75] However, exosomes produced by dendritic cells infected with intracellular parasites, such as *Toxoplasma gondii* and *Eimeria* spp., have been shown to stimulate protective immune responses against these species.[76,77] Several studies have also evaluated the potential of exosome biomarkers in the diagnosis, prognosis, and evaluation of treatment response for chronic diseases,[78−83] including inflammatory[84] and cardiovascular diseases[85] and liver pathologies.[86]

Exosomes derived from infected, diseased, or damaged cells therefore appear to have strong potential value as candidates for diagnostic and therapeutic response markers. Figure 2 depicts the diverse range of pathological conditions that have exosome signatures and could be addressed by ultrasensitive exosome assays that detect these biomarkers in clinical samples to improve the diagnosis and treatment evaluation of individuals with these pathologies.

## EXOSOMES DETECTION AND ISOLATION

Lack of unique, exosome-specific markers hinders specific detection of exosomes in complex biological samples, so that isolation steps are often required to allow exosome analyses using both basic and clinical research applications. As summarized in Table 1, most exosome purification methods involve trade-offs between speed, expense, and labor and produce exosome fractions with method-specific differences in exosome yield, purity, and integrity. Common methods are based on differential centrifugation, filtration/size exclusion, and immunoprecipitation approaches, each of which has its own drawbacks. For example, isolation methods based on size exclusion (e.g., volume-excluding polymers precipitation, size


https://doi.org/10.1021/acsabm.1c00113
ACS Appl. Bio Mater. XXXX, XXX, XXX−XXX

**Table 1. Comparison of Several Exosome Detection and Isolation Techniques**

| basis | example(s) | qualitative assessment |
|---|---|---|
| size/density/shape differences | size chromatography | advantages: |
| | microfluidic sorting | exosome-rich samples |
| | differential ultracentrifugation | straightforward protocols |
| | | drawbacks: |
| | | contamination by particles of similar size |
| | | operator-dependent effects on purity/yield |
| | | large volume of clinical samples necessary |
| exosome precipitation | polymer-based precipitation | advantages: |
| | | does not rely on the bulk instrument, short turnaround time, and largely scalable |
| | | drawbacks: |
| | | low purity due to the coprecipitation of other nonexosomal contaminations; multiple washing steps |
| immunoaffinity | ELISA[a] | advantages: |
| | magnetic beads | subjects exosomes to lower shear forces |
| | | marker-specific detection of exosomes |
| | | drawbacks: |
| | | lower yields |
| | | lack of specificity |
| | | isolation step required for scarce markers |
| | | large volumes for scarce markers |
| optical | near- and far-field nPES[a] | advantages |
| | LSPR[a] sensing | isolation-free exosome analysis |
| | | small sample volume requirement |
| | | can employ small volume unprocessed biological samples |
| | | drawbacks: |
| | | lack of specific markers |
| | | complex fabrication steps |

[a]ELISA, enzyme-linked immunosorbent assay; nPES, nanoplasmon-enhanced scattering assay; LSPR, localized surface plasmon resonance.

exclusion chromatography, and microfluidic sorting), tend to produce exosome fractions that are contaminated with small microvesicles of similar size to exosomes, protein aggregates, lipid droplets, lipoproteins, and other plasma membrane-derived vesicles.[87] Differential ultracentrifugation, the gold-standard technique for exosome isolation, not only suffers from these drawbacks but also is highly operator dependent and compromises the morphological integrity of the isolated exosomes as it subjects them to high shear forces. Further, a recent study that employed an asymmetric flow field-flow fractionation approach to isolate vesicles by their relative density and hydrodynamic properties has identified two exosome subpopulations with distinct morphologies and sizes and a new "exomere" category of nonmembranous nanovesicles[88] that can cosegregate to complicate the isolation of pure exosome samples. Segregation of these particles, however, relies on a labor-intensive workflow that requires large samples, expensive equipment, and skilled labor, so that discrimination and independence of these exosome subpopulations are not feasible for clinical applications without substantial methodological improvement.

As a function of their biogenesis, exosomes tend to be enriched in the tetraspanin proteins CD9, CD63, and, CD81, and the ESCRT-associated components TSG101 and Alix.[11] Immunoaffinity precipitation methods using these specific proteins can isolate exosomes without exerting the same degree of physical stress to produce less damaged exosome isolates. However, such methods also tend to produce lower yields and lack specificity, as exosome expression of these markers can demonstrate cell-specific variations and the ESCRT machinery is also involved in microvesicle biogenesis, limiting the utility of these proteins as exosome-specific markers.[89]

Because of the relatively low yield, exosome isolation approaches tend to require large starting sample volumes and are subject to significant variation in their yield and purity. Enzyme-linked immunosorbent assays (ELISAs), which are widely used to quantify the expression of exosome surface biomarkers, do not have high sensitivity and usually require preisolated EV samples to detect biomarkers of interest that are often present at low levels in clinical samples. The volume requirement for exosome purification may thus prevent the analysis of small volume samples, such as those obtained from mouse models of human disease, or samples with low exosome concentrations, although exosome purification approaches are generally not feasible for clinical applications because of their labor and equipment requirements and inherent variability. Several new approaches are therefore under development to address these challenges, including new methods that employ nanoplasmonic structures to improve the direct detection of exosome surface markers from clinical samples for biomarker assay applications. For example, a sandwich immunoassay approach that employs a nanoplasmon-enhanced scattering (nPES) effect to quantify target exosomes has been shown to directly detect small numbers of tumor-derived exosomes from small volumes of serum.[90] Exosomes have also been quantified by small changes in the near-field refractive index generated in proportion to exosome capture on an immuno-functionalized surface of a nanoplasmonic structure. The following section will therefore discuss several recent studies that employ nanoplasmonic signals to detect or quantify exosomes. The methods described in these studies utilize signal enhancement effects from plasmonic or nanoplasmonic materials to specifically analyze low concentration target exosome populations without requiring the preisolation of exosomes from large-volume clinical samples.

## NANOPLASMONIC SENSING OF EXOSOMES

The physical properties of nanomaterials highly depend on their size, shape, and composition. Fabrication of nanostructured metallic materials has led to the development of novel mechanical, chemical, biological, electronic, and optical applications. Nanoplasmonics has emerged as a new field at the intersection of optics and nanoelectronics, two fields that have revolutionized the way and pace at which we can transfer, process, and analyze information from our surroundings[91] through the application of design nanostructures on noble metal substrates. This new field focuses on utilizing unique properties of nanomaterials to manipulate light-matter interactions for new applications. Nanomaterials with plasmonic properties can exhibit different interactions with


https://doi.org/10.1021/acsabm.1c00113
ACS Appl. Bio Mater. XXXX, XXX, XXX−XXX

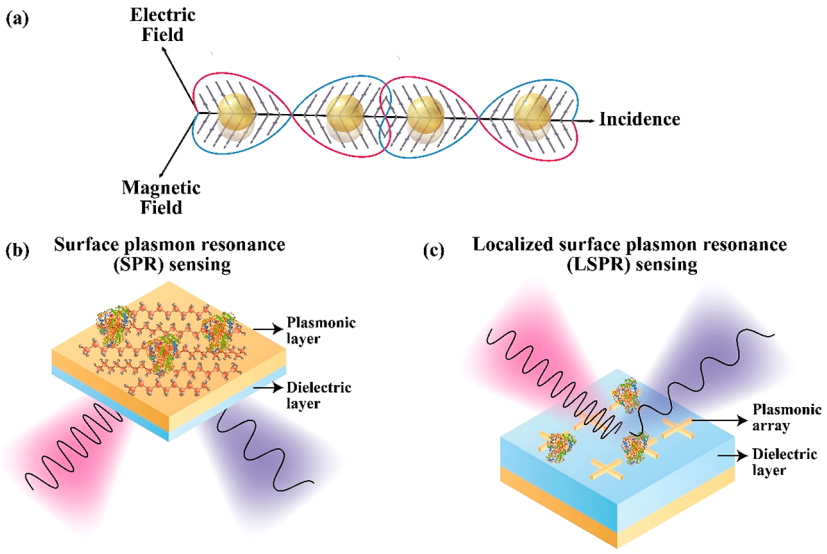



**Figure 3.** Plasmonic effects and their application in sensing applications. (a) Plasmonic effects are caused by the collective oscillation of free electrons in noble metals in response to the electric component of the incident light. (b) Surface plasmon resonance (SPR) and (c) localized surface plasmon resonance (LSPR) is geometry-dependent properties of plasmonic materials that can be used for sensing applications.

light of distinct wavelengths, which can lead to numerous potential applications, such as microscopic imaging beyond the diffraction limit, chemical and biological sensing, invisibility cloaks, optical modulators, photodetectors, and photovoltaic devices. Understanding and translating the plasmonic properties of nanomaterials to real-life applications require not only the utilization of advances in modeling structures with superior functional properties but also highly accurate nanofabrication and synthesis techniques, and characterization tools and optical setups able to make precise measurements. For example, the generation of second-order harmonics from magnetic metamaterials was not observed until after the development of highly accurate nanoscale fabrication techniques,[92] whereas the development of advanced near-field microscopes was required to allow the observation of surface plasmon polariton waves.[93]

**Plasmonic Effect.** From an electromagnetic point of view, metals represent an abundant source of positive ions, free electrons, or plasmons, which occur when metals are exposed to an electromagnetic field (Figure 3). Conductive band electrons collectively oscillate and can be treated as a three-dimensional electron gas, which forms surface plasmon polaritons (SPPs). SPPs are waves that can propagate along the metal−dielectric interface and couple the electric component of light to the surface, confining the light in an area smaller than the diffraction limit.[94] Compared to bulk metals, nanoscale metal structures can also confine light in their near-field and form localized surface plasmon resonances (LSPRs). The absorption cross-section of these nanoscale metals can be maximized at their resonance wavelength, and thus LSPRs can produce much higher signal intensities than SPPs, which are widely applied for catalytic, sensing, and medical applications.[95−97] The resonance wavelength of nanomaterial structures can be adjusted to cover different portions of the ultraviolet, visible, and near-infrared spectrum by tuning the size, shape, and dielectric medium of these nanomaterials for different applications.

The electrical polarization of a material is a frequency-dependent complex function (eq 1) that indicates the way a material interacts with the electrical component of light. A material's polarizability, also known as electric permittivity ($\varepsilon(\omega)$), comprises a real component ($\varepsilon(\omega)'$) and an imaginary component ($\varepsilon(\omega)''$) that respectively describe its inducible polarization strength and its associated losses. Plasmonic materials require negative real permittivity, provided by their free electrons, and low loss. For noble metals, electrons are free to move and accelerate in an electric field in the visible and near-infrared region of the spectrum and $\varepsilon(\omega)$ is explained by the Drude theory (eq 2). This theory can thus explain experimentally collected data for $\varepsilon(\omega)'$; however, at shorter wavelengths, when the energy of the incoming photon is larger than the bandgap of the plasmonic material (e.g., plasmonic semiconductors), the contribution of the interband transitions to the permittivity is better described by the Lorentz model (eq 3) with one or multiple terms ($\Delta\varepsilon$). Both models demonstrate how plasma frequency ($\omega_p$ and $\Omega_P$ terms) and damping frequency ($\gamma$ and T terms) influence the electrical polarization of a material. For gold, at frequencies lower than 0.5 THz, the balance is tipped in favor of the large negative value of $\varepsilon(\omega)'$, whereas at higher frequencies, the energy dissipation within the metal increases and $\varepsilon(\omega)''$ takes over.

$$\varepsilon(\omega) = \varepsilon(\omega)' + i\varepsilon(\omega)'' \tag{1}$$

$$\varepsilon_{\text{Drude}}(\omega) = \varepsilon_\infty - \frac{\omega_p^{\,2}}{\omega(\omega + i\gamma)} \tag{2}$$

$$\varepsilon_{\text{Lorentz}}(\omega) = \frac{\Delta\varepsilon\Omega_P^2}{\Omega_P^2 - \omega(\omega + i\text{T})} \tag{3}$$

Electron−electron and electron−phonon interactions of conduction band electrons and frequent interband electronic transitions plague most metals with large detrimental losses.[98] One strategy to reduce the contribution from interband transitions is to dope noble metals lightly with transition metals, such as cadmium and zinc,[99] or alkali metals, such as potassium.[100] Grain boundary scatterings and lattice defects also contribute to the overall losses of a material.[101] Among highly conductive metals, silver and gold have low losses and are the materials of choice at optical and near-infrared frequencies, respectively. The optical properties of silver are


https://doi.org/10.1021/acsabm.1c00113
ACS Appl. Bio Mater. XXXX, XXX, XXX−XXX

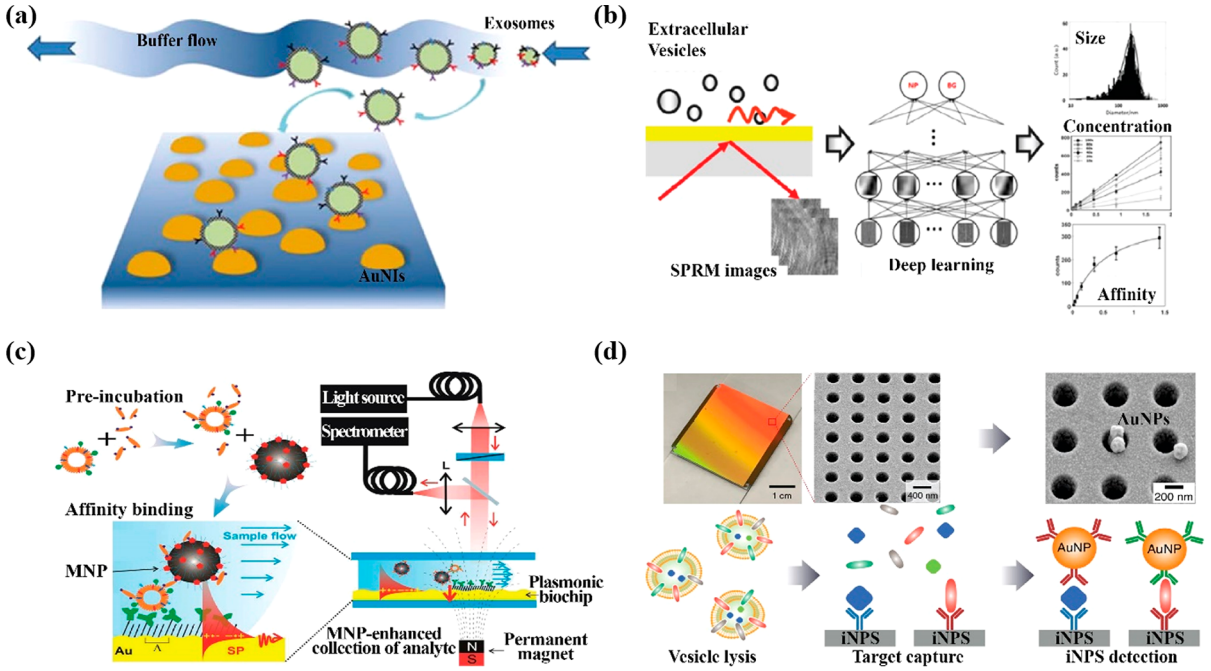



**Figure 4.** Current LSPR-based exosome sensor platforms. Schematic representations of LSPR platforms employed for exosome analyses. (a) Self-assembled gold nanoislands functioning as LISPR interferometers to distinguish exosomes from microvesicles without antibody functionalization. Reproduced with permission from ref 141. Copyright 2017 Elsevier. (b) Antibody-modified SPR sensor approach that employs deep learning approach employed to characterize the physical and chemical properties of interacting EVs. Reproduced with permission from ref 146. Copyright 2020 American Chemical Society. (c) Grating-coupled SPR biosensor approach that employs a magnetic field, an antibody-conjugated sensor, and antibody-labeled magnetic particles to enhance the detection of a specific exosome population. Reproduced with permission from ref 145. Copyright 2017 Royal Society of Chemistry. (d) Nanohole-based SPR approach to detect specific EV transmembrane proteins and cargoes in a sandwich immunoassay approach where target concentration its measured by the immobilization of an antibody-conjugated gold nanoparticle probe adjacent to the SPR surface. Reproduced with permission from ref 148. Copyright 2018 American Chemical Society.

highly susceptible to sulfidation, however, and the formation of a thin layer of silver sulfide can significantly increase the loss component of its permittivity.[102] The remarkable chemical stability of gold in various solutions and the diverse surface chemistry modifications possible with gold make it an ideal candidate for many applications, even though it suffers from higher losses than silver. The use of noble metals as plasmonic materials is limited by fact that their optical properties are tunable only to the degree that it is feasible to change the size, shape, and composition of the final material. For example, noble metals are not good candidates for applications that require modulation or switching because, unlike semiconductors, it is difficult to adjust the electron carrier concentration of noble metals. Many novel applications require much lower losses at or close to their optical frequencies than can be achieved with noble metals.[103] Plasmonic effects with low losses have been shown in copper,[104] aluminum,[105] nickel,[106] palladium,[107] and platinum[108] structures and have potential utility. Alkali metals have relatively low losses,[109] but their high reactivity with air and water renders them impractical for use in plasmonic applications.[110] Finally, although semiconductors are dielectrics and not traditional conductors, their optical properties can be tuned to perform as low-loss plasmonic materials under heavy doping concentrations.[111]

**Nanoplasmonic Metamaterials.** Natural plasmonic materials do not offer sufficient ability to achieve controllable electrical and magnetic properties. To overcome this obstacle, micro/nanofabrication approaches that produce structures comprising carefully designed layers of metals, semiconductors, and dielectrics have been used to achieve superior light−matter interaction at specific wavelengths.[112] Over the past decades, there have been major advancements[113] in both bottom-up approaches (e.g., chemical and photochemical processes[114,115] and colloidal lithography[116]) and top-down nanofabrication processes (e.g., deep ultraviolet projection lithography,[117] electron beam lithography,[118] focused ion beam lithography,[119] and nanoimprint lithography[120]) that enable such fabrication approaches. Different layers of materials with specific morphologies, thicknesses, and geometries are carefully arranged in plasmonic metamaterials to optimize the coupling of the energy and momentum of photons and electrons at specific frequencies. These structures can absorb and scatter light more effectively at their resonance wavelength, and anisotropic plasmonic material can support resonant modes at multiple spectral locations when the incident light is aligned with their axes of symmetry.[121] An efficient plasmonic metamaterial should also allow robust confinement of the incident light for strong field enhancement in its near field.[122] One strategy to increase field enhancement is to include gaps at the center of the long axis of the plasmonic structure.[123] The strong shape dependency of these metallic elements on metamaterials (e.g., networks of metallic wires,[124] split-ring resonators,[125] rings,[126] H-shaped resonators,[127] rods,[128] cross-bars,[129] triangles,[130] bowties,[131] stars,[132] discs,[133] chiral structures,[134] flowers,[135] and cavities[136]) has been the subject of extensive study. Morphological characterization of such plasmonic structures, also referred to as nanoantennas, can be performed using field-mission electron scanning microscopy (FESEM) and atomic force microscopy (AFM). Measurement


https://doi.org/10.1021/acsabm.1c00113
ACS Appl. Bio Mater. XXXX, XXX, XXX−XXX

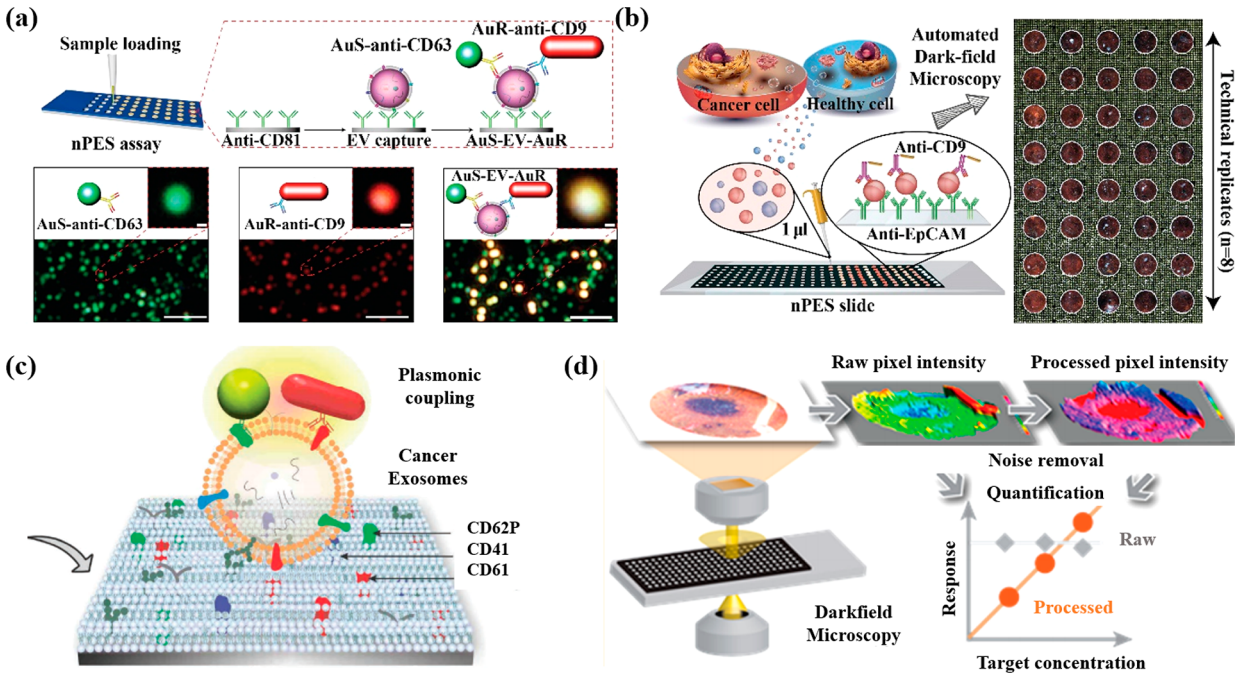



**Figure 5.** Nanoplasmon-enhanced scattering assays for exosome analysis. (a) Near-field nanoplasmon-enhanced scattering (nPES) assay approach for specific EV detection where exosomes captured by a general exosome marker (e.g., anti-CD81 antibody) are incubated with morphologically distinct gold nanoparticles conjugated with antibody probes to general exosome (e.g., CD9 and CD63) and disease-specific surface markers. A strong plasmonic enhancement effect (intensity increase and resonance shift) is observed when both nanoparticle probes bind the same exosome and are constrained within the plasmon interaction limit. Reproduced with permission from ref 90. Copyright 2017 Springer Nature. (b) Far-field nPES assay approach four automated dark-field imaging of plasmon-enhanced light scattering from gold nanoparticles conjugated to a general exosome marker (e.g., CD9) that are bound to target EVs captured by a disease-associated exosome surface marker (e.g., EpCAM). Reproduced with permission from ref 49. Copyright 2019 Frontiers. (c) Biomimetic coating approach allows selective capture of cancer cell-derived exosomes and reduces signal artifacts when plasmonic signal from captured nanoprobes is detected by dark field microscope image analysis. Reproduced with permission from ref 150. Copyright 2019 John Wiley and Sons. (d) Noise reduction approach to resolve nPES signal from nanoparticle probes from scattering artifacts. Reproduced with permission from ref 151. Copyright 2016 American Chemical Society.

of their optical properties, such as field enhancement and plasmon excitations, can be performed using scanning transmission electron microscope electron energy-loss spectroscopy (STEM-EELS),[137] photon-induced near-field electron microscopy (PINEM),[138] and Fourier-transform infrared spectroscopy (FTIR).[129] Advanced fabrication and characterization tools are required to produce nanoantennas with desired characteristics due to the extreme shape and size dependency of their plasmonic behavior. After considering the shape and size of the nanoantenna that best suits the intended application, it is important to determine if periodic arrays of these nanostructures behave differently than single instances or small clusters of these elements. Incident light interacts with different elements on two-dimensional arrays of plasmonic nanoantennas, and the reflected beam can travel as a wave throughout the structure for many periods before being radiated back. This aids in further tuning the optical properties of nanoantennas.[139] Choosing the best nanofabrication process is always a trade-off between accuracy, availability of source materials, cost, and the throughput of the suitable fabrication techniques.

**Current Platforms for Nanoplasmonic Exosome Sensing.** The confinement of an electromagnetic field within a few nanometers around these plasmonic nanomaterials/nanostructures makes them exceptionally sensitive to minute changes in the refractive index of the proximal medium. Therefore, nanomaterials/nanostructures functionalized with capture elements (e.g., antibodies or aptamers against specific biomolecules) can sensitively detect and quantify the presence of small biomolecular analytes by changes in the refractive index that occurs when these analytes bind to their surfaces. The quality of such plasmonic nanosensors can be evaluated by the degree of spectral shift that they experience for one unit of change in the refractive index, and the lowest concentration of their specific analyte they can detect. Unlike surface plasmon resonance (SPR)-based biosensing that requires bulky expensive tools for characterization, LSPR-based biosensors can be realized in miniaturized setups that have the potential for routine clinical applications.[140] In this section, we cover several recent representative studies that focus on the use of plasmonic metamaterials for sensitive EV detection.

**Nanoplasmon-Enhanced LSPR Assays for Exosome Detection.** Self-assembling gold nanoislands (Figure 4a) have been shown to function as LSPR-based sensors that can detect low concentrations (194 ng $mL^{-1}$) of exosomes purified from the urine of a mouse lung cancer model and an adenocarcinomic human alveolar basal epithelial cell line by ultracentrifugation.[141] This analysis demonstrated that the electrokinetic potential of the immobilized particles distinguished exosomes from larger EVs. Another study employed the same concept to analyze exosomes purified from a breast cancer cell line[142] and performed simulations indicating that each square micrometer of the gold nanoisland structure of the sensor platform could accommodate 27 exosomes, three times more than reported in the literature. However, the reliance of this approach on isolated exosomes renders it unsuitable for


https://doi.org/10.1021/acsabm.1c00113
ACS Appl. Bio Mater. XXXX, XXX, XXX−XXX

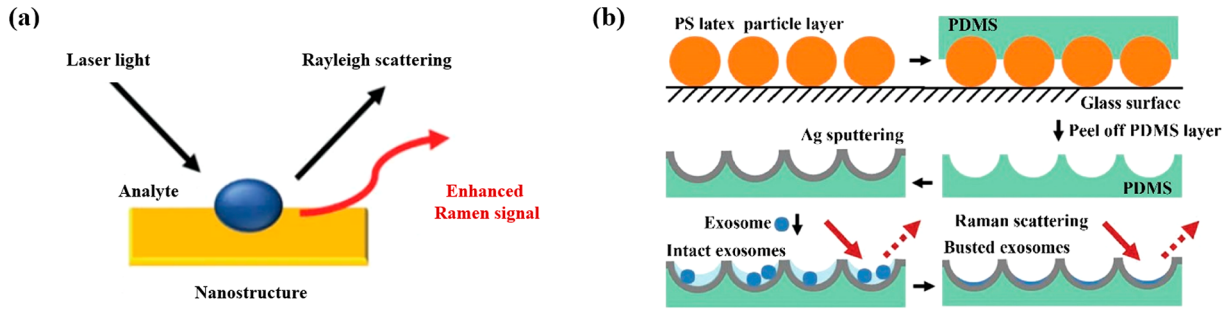



**Figure 6.** Nanoplasmonic exosome sensing by SERS. (a) Schematic representation of the SERS principle for nanoplasmonic detection. Reproduced with permission from ref 152. Copyright 2020 Springer Nature. (b) Silver film-coated nanobowl structures preparation peeling a PDMS layer off an array of polystyrene particles and its use in SERS detection of intact and ruptured exosomes. Reproduced with permission from ref 153. Copyright 2015 Royal Society of Chemistry.

clinical translation. Another group reported using an LSPR-based sensor that employs a gold nanoellipsoid array integrated on a microfluidic chip, which too could detect approximately 1 ng $mL^{-1}$ of isolated exosomes using a general exosome marker (CD63).[143] An antibody-functionalized plasmonic biosensor, based on titanium nitride as an alternative to gold and silver, was also reported to detect low concentrations (4.29 ng $mL^{-1}$) of glioma-derived exosomes captured by CD63-specific antibodies conjugated on the sensor. However, although this sensor platform could be tuned to function at different visible and near-infrared wavelengths and detect target exosomes from dilute samples of serum-derived exosomes, the proof-of-concept study required an exosome purification step, and further studies evaluating its ability to directly analyze clinical samples are required to judge its potential clinical utility.[144] Nevertheless, this approach was noteworthy as it characterized a new plasmonic material with a potential application as an exosome biosensor. Several other studies have also shown affinity capture and SPR-based sensing of exosomes (Figure 4b, c) from breast cancer cell lines, lung cancer cell lines, and mesenchymal stem cells,[145,146] further demonstrating the potential of this sensor approach.

Several plasmonic nanopore structures have been reported in the literature, but thus far only one has demonstrated label-free quantification of low concentration exosomes (approximately 670 aM). In this study, exosomes purified from a primary ovarian cancer cell line (CaOV3) were captured on a nanohole array coated with cancer-specific antibodies to produce transmission surface plasmon resonance through the periodic nanohole array and induce a spectral shift in the transmission resonance (Figure 4d). This approach was used to distinguish ovarian cancer patients from healthy controls in a small cohort.[147] Limited data from processing clinical samples still leaves an opportunity for additional improvements on this device. In another study, the same nanohole array was used to detect both transmembrane and intravesicular proteins of lysed exosomes after their purification from several ovarian cancer cell lines (OVCAR3, OV420, and CaOV3). This approach exhibited a detection limit of approximately $1 \times 10^4$ EVs through affinity capture of the lysed EV cargo and labeling with gold nanoparticles.[148] Because of their technology relying on lysed purified EV samples, its performance is contingent on finding two specific markers for capture and signal enhancement, which limits the potential to be used in the clinic.

**Nanoplasmon-Enhanced Scattering Assays.** Nanoplasmonic effects can also enhance the light scattering detected by dark-field microscopy when nanomaterials are illuminated with ultraviolet or visible light. On the basis of this principle, our team developed a nanoplasmon-enhanced scattering (nPES) assay approach that can detect 200 ng $mL^{-1}$ tumor-associated exosomes directly from as little as 1 $\mu$L of unprocessed serum (Figure 5a). In this assay, serum exosomes are captured directly from serum samples using a ubiquitously expressed exosome surface marker (e.g., CD81) immobilized on a slide. The exosomes are then hybridized with two antibody-conjugated gold nanoparticles that have different morphologies, and thus distinct resonances that recognize two separate EV surface biomarkers, such as a second ubiquitous exosome surface protein (CD63) and a target cancer-associated EV surface biomarker. Exosomes secreted by nonmalignant cells only bind the first of these two nanoparticle probes, but tumor-derived exosomes can bind both probes. This brings them within the minimum distance required to enhance the intensity and cause a resonance shift in their combined light scattering when analyzed under near-field microscopy.[90] This near-field nPES assay approach exhibits high sensitivity for target EVs using a standard user-friendly sandwich immunoassay approach frequently employed in clinical settings. Clinical application is limited because manual focusing may be required to maintain its near-field effect. To address this issue, we developed a far-field variant of this nPES assay (Figure 5b), that detects light scattering from a single target nanoparticle.[49,149] This far-field approach has less sensitivity and higher background than near-field nPES assays but does not require manual focusing for accurate sample analyses, rendering it more suitable for use in clinical settings. Notably, the small sample quantity required for both assays proves useful for EV analyses performed using small animal models, which are normally restricted by the large sample volumes required in conventional EV assays. This is particularly important for studies that require longitudinal analyses, such as cancer models or the evaluation of drug responses.

Assays that employ nanoplasmon-enhanced scattering of visible light allow ultrasensitive detection of EVs that can be captured directly from small volumes of complex biological samples. However, scattering artifacts arising due to non-specific binding of particulates and other factors present in complex samples to the surface of assays wells can decrease the analytical performance of such assays. One group has reported that a biomimetic platelet membrane coating step greatly reduced biofouling on assay well surfaces in addition to its ability to promote selective capture of EVs from cancer versus nonmalignant cell lines due to the inherent properties of


https://doi.org/10.1021/acsabm.1c00113
ACS Appl. Bio Mater. XXXX, XXX, XXX−XXX

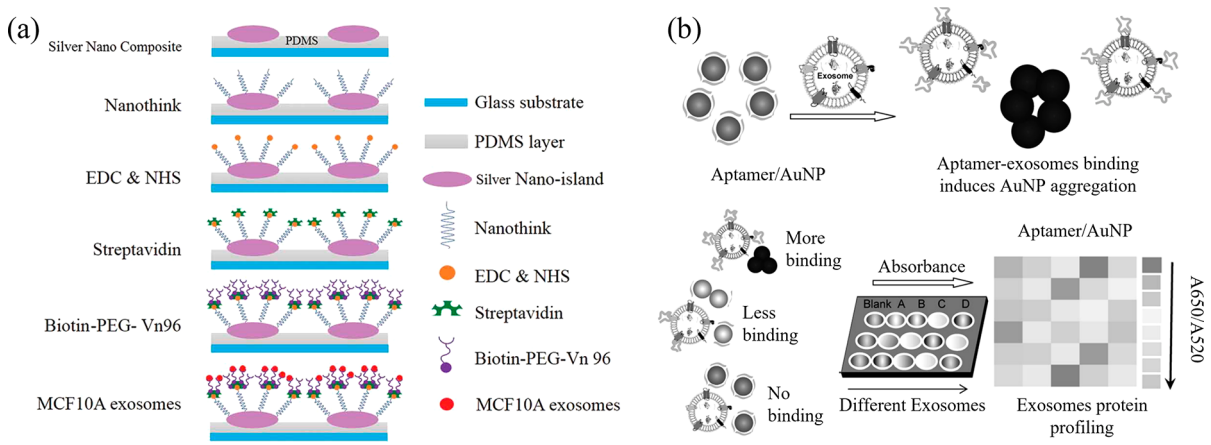



**Figure 7.** Nanoplasmon-enhanced adsorption spectrometry assays for exosomes detection. (a) Schematic of the fabrication and surface functionalization of silver nanocomposite for the exosomes sensing. Reproduced with permission from ref 155. Copyright 2020 Taylor & Francis. (b) Schematic of a nanoparticle aggregation assay where binding of aptamers complexed to the surface of gold nanoparticles causes these nanoparticles to rapidly aggregate to produce a colorimetric signal in proportion to EV concentration. Reproduced with permission from ref 156. Copyright 2017 John Wiley and Sons.

platelet membranes to interact with cancer cells (Figure 5c),[150] suggesting that similar membrane conjugation steps may be useful to reduce signal artifacts when analyzing EVs captured from other complex biological samples. Our group has also recently developed a noise reduction approach to distinguish plasmonic signals produced from nanoprobe binding and scattering artifacts produced by other interfering factors (Figure 5d)[151] to increase assay performance when analyzing complex biological samples.

Raman signals from biomolecules can also be significantly enhanced when target analytes bind to the surface of metallic nanostructures due to an LSPR effect (Figure 6a).[152] This principle has promoted the development of a variety of assays for biological analytes, including exosomes, that exhibit high sensitivity and signal-to-background ratios. For example, one group detected purified exosomes from the SKOV-3 ovarian cancer cell line after their affinity capture on nanobowl structures coated with a thin silver film (Figure 6b) using surface-enhanced Raman spectroscopy (SERS).[153] The limit of detection reported for this device (580 fM) was about 10-fold lower than reported for a high-sensitivity chemiluminescence ELISA, although its translational value is not clear because this study used dilutions of purified exosomes rather than analyzing exosomes directly captured from clinical samples. Interpretation of these results is further complicated by the fact that the morphological integrity of the analyzed exosomes was likely compromised by both the isolation procedure and the drying during the analysis procedure.

**Nanoplasmon-Enabled Exosome Detection via Absorption Spectroscopy.** Exosome binding to metallic nanomaterials or nanostructures can alter the refractive indexes to produce a resonance wavelength shift that can be monitored by absorption spectroscopy.[154] In a recent example of this detection approach, silver nanoislands formed on a PDMS membrane were functionalized with a synthetic peptide (Vn96) to allow specific capture of exosomes derived from MCF10A cells, and exosome binding was detected by a peak shift detectable by absorption spectroscopy (Figure 7a).[155] Nanoprobes can be also designed to aggregate in the presence of the exosome target to produce a colorimetric signal that can be detected by UV−vis spectroscopy or direct observation. For example, one study employed a sensor platform in which gold nanoparticles were solubilized by aptamers noncovalently bound to their surface so that specific binding of these aptamers to their EV target causes the rapid aggregation of these gold nanoparticles to produce a concentration-dependent colorimetric signal (Figure 7b).[156] Several amplification strategies have been used to improve the sensitivity of nanoparticles-based colorimetric assays, including magnetic isolation/separation and enzyme-controlled silver deposition on captured gold nanoparticles.[157,158] However, these approaches introduce several additional steps to improve the limit of detection, but increase assay labor and complexity, and potentially variability, which may render such assays less suitable for clinical applications. Nanoplasmon-enhanced absorption is also employed in lateral flow immunoassays that detect antibody-captured exosomes through specific binding of gold nanoparticle detection probes that are conjugated with target-specific antibodies. However, although the simple workflow of lateral flow assays is suitable for point-of-care tests, it is not clear if exosome assays that use this approach will exhibit sufficient sensitivity and diagnostic performance.[159]

**Nanoplasmon-Enhanced Fluorescence for Exosome Detection.** Fluorescence-based methods are widely used to detect a variety of biological targets due to the simplicity and versatility of this detection approach, and its suitability for high-throughput detection approaches.[160,161] However, fluorescence-based assays that target exosome biomarkers are likely to exhibit very poor analytical sensitivity due to the limited surface area and volume of exosomes, which limits the abundance of target biomarkers on or within these vesicles and thus the amount of fluorescent signal that can be generated per each target exosome.

Multiple studies have shown that fluorescence emission can be significantly enhanced when fluorescent molecules come within a specific distance of a nanoparticle or nanostructure surface,[162] and this principle has been employed to more sensitively detect a variety of targets, including exosomes. One recent study has employed a periodic nanohole structure to amplify exosome fluorescent signal using the SPR effect to amplify the fluorescent signal overlapping the plasmonic resonance of this nanostructure.[163] In another approach, LSPR and an SPR-mediated fluorescence amplification of the


https://doi.org/10.1021/acsabm.1c00113
ACS Appl. Bio Mater. XXXX, XXX, XXX−XXX

target signal were combined to enhance the signal-to-noise ratio of exosome detection, which resulted in a 140-fold signal-to-noise increase when fluorescently labeled target proteins were captured on a plasmonic sensor grating.[164] However, this approach employed purified exosome samples, and it is not clear if this method can be utilized to directly capture and analyze exosomes from complex biological specimens for clinical translation. Considering the plasmonic resonance characteristic of most nanostructures can enhance red and near-infrared fluorescent signals,[165] combining plasmon-enhanced fluorescence amplification approaches with other plasmon enhancement approaches (e.g., LSPR) should be considered to permit multiplex detection of different exosome markers.

## CONCLUSIONS AND FUTURE CHALLENGES

As one of the major subtypes of EVs, exosomes are unique, encapsulated biomolecules that are abundantly secreted by all cells and carry crucial biological information from their cell of origin. These nanometric particles can be detected in different biofluids (e.g., blood, cerebrospinal fluid, urine, saliva) via procedures with close-to-no invasiveness. However, exosome analysis is not yet clinically translatable because of the requirement for a purification step and the lack of a standard technique for reproducible, high yield, pure exosomes. The explosive rise in studying exosomes as promising biomarkers for disease diagnosis, prognosis, and treatment monitoring (in maladies such as cancer, infectious diseases, and neuro-degenerative diseases), now more than ever, underscores the necessity of developing highly sensitive, reproducible, and scalable techniques that can examine and implement clinically relevant applications of exosomes. Because all the currently known size-based and affinity-based assays have inherent drawbacks for clinical applicability, researchers have turned to nanoplasmonic structures and explored them as novel compact devices with great potential to make to the clinics. Therefore, many distinct types of plasmonic metamaterials are readily functionalized with molecules that exhibit affinity to target biomarkers and thus be employed as biosensors in multiple different applications. The specificity of such biosensors can be facilely modified by altering the specificity of their conjugated affinity molecules, such that specific antibodies can be employed to recognize EV biomarkers associated with particular diseases or disease stages to permit disease diagnosis, staging, and treatment monitoring. However, for such plasmonic biosensors to have clinical utility, they must satisfy several essential requirements. These include the ability to (1) rapidly analyze unprocessed clinical specimens, such as serum or plasma, in a high-throughput manner; (2) allow more sensitive and specific detection of existing biomarkers, or novel biomarkers not evaluable by other methods; and (3) utilize simple, efficient, high-yield, and cost-effective fabrication and characterization procedures. Most current nanoplasmonic assays do not yet meet one or more of these requirements, and future studies are necessary to address these issues and ultimately demonstrate the utility of a refined assay platform in a clinical validation study. However, nanoplasmonic sensing and detection approaches remain a strong potential approach for future clinical applications due to (1) the versatility of plasmonic materials that can be designed, fabricated, and tailored to work at a range of biological wavelengths; (2) the promise they show for reaching ultralow limits of detection; (3) their potential for developing compact, portable, silicon-based devices; and (4) their potential in developing purification-free, automated exosome assays that can reduce variability and operator bias.

## AUTHOR INFORMATION


### Corresponding Author

**Tony Ye Hu** − *Center of Cellular and Molecular Diagnosis, Tulane University, New Orleans, Louisiana 70118, United States;* orcid.org/0000-0002-5166-4937; Email: tonyhu@tulane.edu

### Authors

**Pouya Amrollahi** − *Center of Cellular and Molecular Diagnosis, Tulane University, New Orleans, Louisiana 70118, United States; School of Biological and Health Systems Engineering, Arizona State University, Tempe, Arizona 85281, United States*

**Wenshu Zheng** − *Center of Cellular and Molecular Diagnosis, Tulane University, New Orleans, Louisiana 70118, United States*

**Chandler Monk** − *Center of Cellular and Molecular Diagnosis, Tulane University, New Orleans, Louisiana 70118, United States*

**Chen-Zhong Li** − *Center of Cellular and Molecular Diagnosis, Tulane University, New Orleans, Louisiana 70118, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsabm.1c00113

### Author Contributions

†P.A. and W.Z. contributed equally to this work.


### Notes

The authors declare no competing financial interest.

## REFERENCES


(1) Dreyer, F.; Baur, A. *Biogenesis and Functions of Exosomes and Extracellular Vesicles*; Springer, 2016; pp 201−216.

(2) Raposo, G.; Stoorvogel, W. Extracellular vesicles: exosomes, microvesicles, and friends. *J. Cell Biol.* **2013**, *200* (4), 373−383.

(3) Johnstone, R. M.; Adam, M.; Hammond, J.; Orr, L.; Turbide, C. Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). *J. Biol. Chem.* **1987**, *262* (19), 9412−9420.

(4) Horibe, S.; Tanahashi, T.; Kawauchi, S.; Murakami, Y.; Rikitake, Y. Mechanism of recipient cell-dependent differences in exosome uptake. *BMC Cancer* **2018**, *18* (1), 1−9.

(5) Colombo, M.; Raposo, G.; Théry, C. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. *Annu. Rev. Cell Dev. Biol.* **2014**, *30*, 255−289.

(6) Fuccillo, M.; Joyner, A. L.; Fishell, G. Morphogen to mitogen: the multiple roles of hedgehog signalling in vertebrate neural development. *Nat. Rev. Neurosci.* **2006**, *7* (10), 772−783.

(7) van den Brink, G. R. Hedgehog signaling in development and homeostasis of the gastrointestinal tract. *Physiol. Rev.* **2007**, *87* (4), 1343−1375.

(8) Minciacchi, V. R.; Freeman, M. R.; Di Vizio, D. Extracellular vesicles in cancer: exosomes, microvesicles and the emerging role of large oncosomes. *Semin. Cell Dev. Biol.* **2015**, *40*, 41−51.

(9) Rodrigues, M.; Fan, J.; Lyon, C.; Wan, M.; Hu, Y. Role of extracellular vesicles in viral and bacterial infections: pathogenesis, diagnostics, and therapeutics. *Theranostics* **2018**, *8* (10), 2709.

(10) Thompson, A. G.; Gray, E.; Heman-Ackah, S. M.; Mäger, I.; Talbot, K.; El Andaloussi, S.; Wood, M. J.; Turner, M. R. Extracellular vesicles in neurodegenerative disease—pathogenesis to biomarkers. *Nat. Rev. Neurol.* **2016**, *12* (6), 346.

(11) Baietti, M. F.; Zhang, Z.; Mortier, E.; Melchior, A.; Degeest, G.; Geeraerts, A.; Ivarsson, Y.; Depoortere, F.; Coomans, C.; Vermeiren, E.; et al. Syndecan−syntenin−ALIX regulates the biogenesis of exosomes. *Nat. Cell Biol.* **2012**, *14* (7), 677−685.
(12) Mazurov, D.; Barbashova, L.; Filatov, A. Tetraspanin protein CD 9 interacts with metalloprotease CD 10 and enhances its release via exosomes. *FEBS J.* **2013**, *280* (5), 1200−1213.
(13) Harding, C.; Heuser, J.; Stahl, P. Receptor-mediated endocytosis of transferrin and recycling of the transferrin receptor in rat reticulocytes. *J. Cell Biol.* **1983**, *97* (2), 329−339.
(14) Pan, B.-T.; Teng, K.; Wu, C.; Adam, M.; Johnstone, R. M. Electron microscopic evidence for externalization of the transferrin receptor in vesicular form in sheep reticulocytes. *J. Cell Biol.* **1985**, *101* (3), 942−948.
(15) Poteryaev, D.; Datta, S.; Ackema, K.; Zerial, M.; Spang, A. Identification of the switch in early-to-late endosome transition. *Cell* **2010**, *141* (3), 497−508.
(16) Théry, C.; Zitvogel, L.; Amigorena, S. Exosomes: composition, biogenesis and function. *Nat. Rev. Immunol.* **2002**, *2* (8), 569−579.
(17) Robbins, P. D.; Morelli, A. E. Regulation of immune responses by extracellular vesicles. *Nat. Rev. Immunol.* **2014**, *14* (3), 195−208.
(18) Ostrowski, M.; Carmo, N. B.; Krumeich, S.; Fanget, I.; Raposo, G.; Savina, A.; Moita, C. F.; Schauer, K.; Hume, A. N.; Freitas, R. P.; et al. Rab27a and Rab27b control different steps of the exosome secretion pathway. *Nat. Cell Biol.* **2010**, *12* (1), 19−30.
(19) Hsu, C.; Morohashi, Y.; Yoshimura, S.-i.; Manrique-Hoyos, N.; Jung, S.; Lauterbach, M. A.; Bakhti, M.; Grønborg, M.; Möbius, W.; Rhee, J.; et al. Regulation of exosome secretion by Rab35 and its GTPase-activating proteins TBC1D10A−C. *J. Cell Biol.* **2010**, *189* (2), 223−232.
(20) Fader, C. M.; Sánchez, D. G.; Mestre, M. B.; Colombo, M. I. TI-VAMP/VAMP7 and VAMP3/cellubrevin: two v-SNARE proteins involved in specific steps of the autophagy/multivesicular body pathways. *Biochim. Biophys. Acta, Mol. Cell Res.* **2009**, *1793* (12), 1901−1916.
(21) Burgoyne, T.; Jolly, R.; Martin-Martin, B.; Seabra, M. C.; Piccirillo, R.; Schiaffino, M. V.; Futter, C. E. Expression of OA1 limits the fusion of a subset of MVBs with lysosomes−a mechanism potentially involved in the initial biogenesis of melanosomes. *J. Cell Sci.* **2013**, *126* (22), 5143−5152.
(22) Sharifi, M.; Avadi, M. R.; Attar, F.; Dashtestani, F.; Ghorchian, H.; Rezayat, S. M.; Saboury, A. A.; Falahati, M. Cancer diagnosis using nanomaterials based electrochemical nanobiosensors. *Biosens. Bioelectron.* **2019**, *126*, 773−784.
(23) Griffiths, S. G.; Cormier, M. T.; Clayton, A.; Doucette, A. A. Differential proteome analysis of extracellular vesicles from breast cancer cell lines by chaperone affinity enrichment. *Proteomes* **2017**, *5* (4), 25.
(24) Chalmin, F.; Ladoire, S.; Mignot, G.; Vincent, J.; Bruchard, M.; Remy-Martin, J.-P.; Boireau, W.; Rouleau, A.; Simon, B.; Lanneau, D.; et al. Membrane-associated Hsp72 from tumor-derived exosomes mediates STAT3-dependent immunosuppressive function of mouse and human myeloid-derived suppressor cells. *J. Clin. Invest.* **2010**, *120* (2), 457−471.
(25) Liu, R.-T.; Huang, C.-C.; You, H.-L.; Chou, F.-F.; Hu, C.-C. A.; Chao, F.-P.; Chen, C.-M.; Cheng, J.-T. Overexpression of tumor susceptibility gene TSG101 in human papillary thyroid carcinomas. *Oncogene* **2002**, *21* (31), 4830−4837.
(26) Takasugi, M.; Okada, R.; Takahashi, A.; Virya Chen, D.; Watanabe, S.; Hara, E. Small extracellular vesicles secreted from senescent cells promote cancer cell proliferation through EphA2. *Nat. Commun.* **2017**, *8* (1), 1−11.
(27) Bebelman, M. P.; Smit, M. J.; Pegtel, D. M.; Baglio, S. R. Biogenesis and function of extracellular vesicles in cancer. *Pharmacol. Ther.* **2018**, *188*, 1−11.
(28) King, H. W.; Michael, M. Z.; Gleadle, J. M. Hypoxic enhancement of exosome release by breast cancer cells. *BMC Cancer* **2012**, *12* (1), 1−10.
(29) Clayton, A.; Mitchell, J. P.; Court, J.; Mason, M. D.; Tabi, Z. Human tumor-derived exosomes selectively impair lymphocyte responses to interleukin-2. *Cancer Res.* **2007**, *67* (15), 7458−7466.
(30) Kucharzewska, P.; Christianson, H. C.; Welch, J. E.; Svensson, K. J.; Fredlund, E.; Ringnér, M.; Mörgelin, M.; Bourseau-Guilmain, E.; Bengzon, J.; Belting, M. Exosomes reflect the hypoxic status of glioma cells and mediate hypoxia-dependent activation of vascular cells during tumor development. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110* (18), 7312−7317.
(31) Sung, B. H.; Ketova, T.; Hoshino, D.; Zijlstra, A.; Weaver, A. M. Directional cell movement through tissues is controlled by exosome secretion. *Nat. Commun.* **2015**, *6* (1), 1−14.
(32) Hoshino, D.; Kirkbride, K. C.; Costello, K.; Clark, E. S.; Sinha, S.; Grega-Larson, N.; Tyska, M. J.; Weaver, A. M. Exosome secretion is enhanced by invadopodia and drives invasive behavior. *Cell Rep.* **2013**, *5* (5), 1159−1168.
(33) Webber, J.; Steadman, R.; Mason, M. D.; Tabi, Z.; Clayton, A. Cancer exosomes trigger fibroblast to myofibroblast differentiation. *Cancer Res.* **2010**, *70* (23), 9621−9630.
(34) Peinado, H.; Zhang, H.; Matei, I. R.; Costa-Silva, B.; Hoshino, A.; Rodrigues, G.; Psaila, B.; Kaplan, R. N.; Bromberg, J. F.; Kang, Y.; et al. Pre-metastatic niches: organ-specific homes for metastases. *Nat. Rev. Cancer* **2017**, *17* (5), 302−317.
(35) Liu, Y.; Cao, X. Organotropic metastasis: role of tumor exosomes. *Cell Res.* **2016**, *26* (2), 149−150.
(36) Morton, D. J.; Kuiper, E. G.; Jones, S. K.; Leung, S. W.; Corbett, A. H.; Fasken, M. B. The RNA exosome and RNA exosome-linked disease. *RNA* **2018**, *24* (2), 127−142.
(37) Zomer, A.; Maynard, C.; Verweij, F. J.; Kamermans, A.; Schäfer, R.; Beerling, E.; Schiffelers, R. M.; de Wit, E.; Berenguer, J.; Ellenbroek, S. I. J.; et al. In vivo imaging reveals extracellular vesicle-mediated phenocopying of metastatic behavior. *Cell* **2015**, *161* (5), 1046−1057.
(38) Peinado, H.; Alečković, M.; Lavotshkin, S.; Matei, I.; Costa-Silva, B.; Moreno-Bueno, G.; Hergueta-Redondo, M.; Williams, C.; García-Santos, G.; Ghajar, C. M.; et al. Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. *Nat. Med.* **2012**, *18* (6), 883−891.
(39) Zhang, H.; Deng, T.; Liu, R.; Bai, M.; Zhou, L.; Wang, X.; Li, S.; Wang, X.; Yang, H.; Li, J.; et al. Exosome-delivered EGFR regulates liver microenvironment to promote gastric cancer liver metastasis. *Nat. Commun.* **2017**, *8* (1), 1−11.
(40) Zhou, W.; Fong, M. Y.; Min, Y.; Somlo, G.; Liu, L.; Palomares, M. R.; Yu, Y.; Chow, A.; O'Connor, S. T. F.; Chin, A. R.; et al. Cancer-secreted miR-105 destroys vascular endothelial barriers to promote metastasis. *Cancer Cell* **2014**, *25* (4), 501−515.
(41) Fong, M. Y.; Zhou, W.; Liu, L.; Alontaga, A. Y.; Chandra, M.; Ashby, J.; Chow, A.; O'Connor, S. T. F.; Li, S.; Chin, A. R.; et al. Breast-cancer-secreted miR-122 reprograms glucose metabolism in premetastatic niche to promote metastasis. *Nat. Cell Biol.* **2015**, *17* (2), 183−194.
(42) Santos, J. C.; da Silva Lima, N.; Sarian, L. O.; Matheu, A.; Ribeiro, M. L.; Derchain, S. F. M. Exosome-mediated breast cancer chemoresistance via miR-155 transfer. *Sci. Rep.* **2018**, *8* (1), 1−11.
(43) Melo, S. A.; Sugimoto, H.; O'Connell, J. T.; Kato, N.; Villanueva, A.; Vidal, A.; Qiu, L.; Vitkin, E.; Perelman, L. T.; Melo, C. A.; et al. Cancer exosomes perform cell-independent microRNA biogenesis and promote tumorigenesis. *Cancer Cell* **2014**, *26* (5), 707−721.
(44) Valadi, H.; Ekström, K.; Bossios, A.; Sjöstrand, M.; Lee, J. J.; Lötvall, J. O. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat. Cell Biol.* **2007**, *9* (6), 654−659.
(45) Boelens, M. C.; Wu, T. J.; Nabet, B. Y.; Xu, B.; Qiu, Y.; Yoon, T.; Azzam, D. J.; Twyman-Saint Victor, C.; Wiemann, B. Z.; Ishwaran, H.; et al. Exosome transfer from stromal to breast cancer cells regulates therapy resistance pathways. *Cell* **2014**, *159* (3), 499−513.
(46) Fan, J.; Wei, Q.; Koay, E. J; Liu, Y.; Ning, B.; Bernard, P. W; Zhang, N.; Han, H.; Katz, M. H; Zhao, Z.; Hu, Y. Chemoresistance


https://doi.org/10.1021/acsabm.1c00113
ACS Appl. Bio Mater. XXXX, XXX, XXX−XXX

transmission via exosome-mediated EphA2 transfer in pancreatic cancer. *Theranostics* **2018**, *8* (21), 5986.
(47) Han, M.; Hu, J.; Lu, P.; Cao, H.; Yu, C.; Li, X.; Qian, X.; Yang, X.; Yang, Y.; Han, N.; et al. Exosome-transmitted miR-567 reverses trastuzumab resistance by inhibiting ATG5 in breast cancer. *Cell Death Dis.* **2020**, *11* (1), 1−15.
(48) Liu, T.; Zhang, X.; Du, L.; Wang, Y.; Liu, X.; Tian, H.; Wang, L.; Li, P.; Zhao, Y.; Duan, W.; et al. Exosome-transmitted miR-128−3p increase chemosensitivity of oxaliplatin-resistant colorectal cancer. *Mol. Cancer* **2019**, *18* (1), 43.
(49) Amrollahi, P.; Rodrigues, M.; Lyon, C. J.; Goel, A.; Han, H.; Hu, T. Y. Ultra-sensitive automated profiling of EpCAM expression on tumor-derived extracellular vesicles. *Front. Genet.* **2019**, *10*, 1273.
(50) Rabinowits, G.; Gerçel-Taylor, C.; Day, J. M.; Taylor, D. D.; Kloecker, G. H. Exosomal microRNA: a diagnostic marker for lung cancer. *Clin. Lung Cancer* **2009**, *10* (1), 42−46.
(51) Whiteside, T. L. The potential of tumor-derived exosomes for noninvasive cancer monitoring. *Expert Rev. Mol. Diagn.* **2015**, *15* (10), 1293−1310.
(52) Panigrahi, G. K.; Praharaj, P. P.; Kittaka, H.; Mridha, A. R.; Black, O. M.; Singh, R.; Mercer, R.; Van Bokhoven, A.; Torkko, K. C.; Agarwal, C.; et al. Exosome proteomic analyses identify inflammatory phenotype and novel biomarkers in African American prostate cancer patients. *Cancer Med.* **2019**, *8* (3), 1110−1123.
(53) Yang, T.; Martin, P.; Fogarty, B.; Brown, A.; Schurman, K.; Phipps, R.; Yin, V. P.; Lockman, P.; Bai, S. Exosome delivered anticancer drugs across the blood-brain barrier for brain cancer therapy in Danio rerio. *Pharm. Res.* **2015**, *32* (6), 2003−2014.
(54) Chen, C. C.; Liu, L.; Ma, F.; Wong, C. W.; Guo, X. E.; Chacko, J. V.; Farhoodi, H. P.; Zhang, S. X.; Zimak, J.; Ségaliny, A.; et al. Elucidation of exosome migration across the blood−brain barrier model in vitro. *Cell. Mol. Bioeng.* **2016**, *9* (4), 509−529.
(55) Wood, M. J.; O'Loughlin, A. J.; Lakhal, S. Exosomes and the blood−brain barrier: implications for neurological diseases. *Ther. Delivery* **2011**, *2* (9), 1095−1099.
(56) Chiasserini, D.; van Weering, J. R.; Piersma, S. R.; Pham, T. V.; Malekzadeh, A.; Teunissen, C. E.; de Wit, H.; Jiménez, C. R. Proteomic analysis of cerebrospinal fluid extracellular vesicles: a comprehensive dataset. *J. Proteomics* **2014**, *106*, 191−204.
(57) Krämer-Albers, E. M.; Bretz, N.; Tenzer, S.; Winterstein, C.; Möbius, W.; Berger, H.; Nave, K. A.; Schild, H.; Trotter, J. Oligodendrocytes secrete exosomes containing major myelin and stress-protective proteins: Trophic support for axons? *Proteomics: Clin. Appl.* **2007**, *1* (11), 1446−1461.
(58) Rajendran, L.; Honsho, M.; Zahn, T. R.; Keller, P.; Geiger, K. D.; Verkade, P.; Simons, K. Alzheimer's disease $\beta$-amyloid peptides are released in association with exosomes. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103* (30), 11172−11177.
(59) Stuendl, A.; Kunadt, M.; Kruse, N.; Bartels, C.; Moebius, W.; Danzer, K. M.; Mollenhauer, B.; Schneider, A. Induction of $\alpha$-synuclein aggregate formation by CSF exosomes from patients with Parkinson's disease and dementia with Lewy bodies. *Brain* **2016**, *139* (2), 481−494.
(60) Fevrier, B.; Vilette, D.; Archer, F.; Loew, D.; Faigle, W.; Vidal, M.; Laude, H.; Raposo, G. Cells release prions in association with exosomes. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101* (26), 9683−9688.
(61) Serafin, A.; Foco, L.; Zanigni, S.; Blankenburg, H.; Picard, A.; Zanon, A.; Giannini, G.; Pichler, I.; Facheris, M. F.; Cortelli, P.; et al. Overexpression of blood microRNAs 103a, 30b, and 29a in l-dopa−treated patients with PD. *Neurology* **2015**, *84* (7), 645−653.
(62) McCann, H.; Stevens, C. H.; Cartwright, H.; Halliday, G. M. $\alpha$-Synucleinopathy phenotypes. *Parkinsonism Relat. Disord.* **2014**, *20*, S62−S67.
(63) Fraser, K. B.; Moehle, M. S.; Daher, J. P.; Webber, P. J.; Williams, J. Y.; Stewart, C. A.; Yacoubian, T. A.; Cowell, R. M.; Dokland, T.; Ye, T.; et al. LRRK2 secretion in exosomes is regulated by 14−3-3. *Hum. Mol. Genet.* **2013**, *22* (24), 4988−5000.
(64) Blennow, K.; Zetterberg, H. Understanding biomarkers of neurodegeneration: ultrasensitive detection techniques pave the way for mechanistic understanding. *Nat. Med.* **2015**, *21* (3), 217−219.
(65) Vingtdeux, V.; Sergeant, N.; Buée, L. Potential contribution of exosomes to the prion-like propagation of lesions in Alzheimer's disease. *Front. Physiol.* **2012**, *3*, 229.
(66) Schneider, A.; Simons, M. Exosomes: vesicular carriers for intercellular communication in neurodegenerative disorders. *Cell Tissue Res.* **2013**, *352* (1), 33−47.
(67) Guescini, M.; Genedani, S.; Stocchi, V.; Agnati, L. F. Astrocytes and Glioblastoma cells release exosomes carrying mtDNA. *J. Neural Transm.* **2010**, *117* (1), 1.
(68) Schorey, J. S.; Cheng, Y.; Singh, P. P.; Smith, V. L. Exosomes and other extracellular vesicles in host−pathogen interactions. *EMBO Rep.* **2015**, *16* (1), 24−43.
(69) Fang, Y.; Wu, N.; Gan, X.; Yan, W.; Morrell, J. C.; Gould, S. J. Higher-order oligomerization targets plasma membrane proteins and HIV gag to exosomes. *PLoS Biol.* **2007**, *5* (6), e158.
(70) Narayanan, A.; Iordanskiy, S.; Das, R.; Van Duyne, R.; Santos, S.; Jaworski, E.; Guendel, I.; Sampey, G.; Dalby, E.; Iglesias-Ussel, M.; et al. Exosomes derived from HIV-1-infected cells contain trans-activation response element RNA. *J. Biol. Chem.* **2013**, *288* (27), 20014−20033.
(71) Campbell, T. D.; Khan, M.; Huang, M.-B.; Bond, V. C.; Powell, M. D. HIV-1 Nef protein is secreted into vesicles that can fuse with target cells and virions. *Ethn. Dis.* **2008**, *18* (2), S2-14-9.
(72) Yang, H.-J.; Huang, T.-J.; Yang, C.-F.; Peng, L.-X.; Liu, R.-Y.; Yang, G.-D.; Chu, Q.-Q.; Huang, J.-L.; Liu, N.; Huang, H.-B.; et al. Comprehensive profiling of Epstein-Barr virus-encoded miRNA species associated with specific latency types in tumor cells. *Virol. J.* **2013**, *10* (1), 314.
(73) Lenassi, M.; Cagney, G.; Liao, M.; Vaupotič, T.; Bartholomeeusen, K.; Cheng, Y.; Krogan, N. J.; Plemenitaš, A.; Peterlin, B. M. HIV Nef is secreted in exosomes and triggers apoptosis in bystander CD4+ T cells. *Traffic* **2010**, *11* (1), 110−122.
(74) Mehra, A.; Zahra, A.; Thompson, V.; Sirisaengtaksin, N.; Wells, A.; Porto, M.; Köster, S.; Penberthy, K.; Kubota, Y.; Dricot, A.; et al. Mycobacterium tuberculosis type VII secreted effector EsxH targets host ESCRT to impair trafficking. *PLoS Pathog.* **2013**, *9* (10), e1003734.
(75) Anand, P. K.; Anand, E.; Bleck, C. K.; Anes, E.; Griffiths, G. Exosomal Hsp70 induces a pro-inflammatory response to foreign particles including mycobacteria. *PLoS One* **2010**, *5* (4), e10136.
(76) Aline, F.; Bout, D.; Amigorena, S.; Roingeard, P.; Dimier-Poisson, I. Toxoplasma gondii antigen-pulsed-dendritic cell-derived exosomes induce a protective immune response against T. gondii infection. *Infect. Immun.* **2004**, *72* (7), 4127−4137.
(77) del Cacho, E.; Gallego, M.; Lee, S. H.; Lillehoj, H. S.; Quilez, J.; Lillehoj, E. P.; Sánchez-Acedo, C. Induction of protective immunity against Eimeria tenella infection using antigen-loaded dendritic cells (DC) and DC-derived exosomes. *Vaccine* **2011**, *29* (21), 3818−3825.
(78) Gurunathan, S.; Kang, M.-H.; Jeyaraj, M.; Qasim, M.; Kim, J.-H. Review of the isolation, characterization, biological function, and multifarious therapeutic approaches of exosomes. *Cells* **2019**, *8* (4), 307.
(79) Zhang, H.; Wang, L.; Li, C.; Yu, Y.; Yi, Y.; Wang, J.; Chen, D. Exosome-induced regulation in inflammatory bowel disease. *Front. Immunol.* **2019**, *10*, 1464.
(80) Azmi, A. S.; Bao, B.; Sarkar, F. H. Exosomes in cancer development, metastasis, and drug resistance: a comprehensive review. *Cancer Metastasis Rev.* **2013**, *32* (3), 623−642.
(81) Alipoor, S. D.; Mortaz, E.; Garssen, J.; Movassaghi, M.; Mirsaeidi, M.; Adcock, I. M. Exosomes and exosomal miRNA in respiratory diseases. *Mediators Inflammation* **2016**, *2016*, 5628404.
(82) Schorey, J. S.; Bhatnagar, S. Exosome function: from tumor immunology to pathogen biology. *Traffic* **2008**, *9* (6), 871−881.
(83) Zhu, T.; Wang, Y.; Jin, H.; Li, L. The role of exosome in autoimmune connective tissue disease. *Ann. Med.* **2019**, *51* (2), 101−108.


https://doi.org/10.1021/acsabm.1c00113
*ACS Appl. Bio Mater.* XXXX, XXX, XXX−XXX

(84) Buzas, E. I.; György, B.; Nagy, G.; Falus, A.; Gay, S. Emerging role of extracellular vesicles in inflammatory diseases. *Nat. Rev. Rheumatol.* **2014**, *10* (6), 356−364.
(85) Fleury, A.; Martinez, M. C.; Le Lay, S. Extracellular vesicles as therapeutic tools in cardiovascular diseases. *Front. Immunol.* **2014**, *5*, 370.
(86) Sehrawat, T. S.; Arab, J. P.; Liu, M.; Amrollahi, P.; Wan, M.; Fan, J.; Nakao, Y.; Pose, E.; Navarro-Corcuera, A.; Dasgupta, D. Circulating extracellular vesicles carrying sphingolipid cargo for the diagnosis and dynamic risk profiling of alcoholic hepatitis. *Hepatology* **2021**, *73*, 571−585.
(87) Hessvik, N. P.; Llorente, A. Current knowledge on exosome biogenesis and release. *Cell. Mol. Life Sci.* **2018**, *75* (2), 193−208.
(88) Zhang, H.; Freitas, D.; Kim, H. S.; Fabijanic, K.; Li, Z.; Chen, H.; Mark, M. T.; Molina, H.; Martin, A. B.; Bojmar, L. Identification of distinct nanoparticles and subsets of extracellular vesicles by asymmetric flow field-flow fractionation. *Nat. Cell Biol.* **2018**, *20* (3), 332−343.
(89) Hurley, J. H. ESCRT s are everywhere. *EMBO J.* **2015**, *34* (19), 2398−2407.
(90) Liang, K.; Liu, F.; Fan, J.; Sun, D.; Liu, C.; Lyon, C. J.; Bernard, D. W.; Li, Y.; Yokoi, K.; Katz, M. H.; et al. Nanoplasmonic quantification of tumour-derived extracellular vesicles in plasma microsamples for diagnosis and treatment monitoring. *Nat. Biomed. Eng.* **2017**, *1* (4), 1−11.
(91) Moore, G. E. *Cramming More Components onto Integrated Circuits*; McGraw-Hill, 1998; Vol. *38*, 114−117.
(92) Klein, M. W.; Enkrich, C.; Wegener, M.; Linden, S. Second-harmonic generation from magnetic metamaterials. *Science* **2006**, *313* (5786), 502−504.
(93) Kawata, S.; Ohtsu, M.; Irie, M. *near-Field Optics and Surface Plasmon Polaritons*; Springer Science & Business Media, 2001; Vol. *81*.
(94) Naik, G. V.; Shalaev, V. M.; Boltasseva, A. Alternative plasmonic materials: beyond gold and silver. *Adv. Mater.* **2013**, *25* (24), 3264−3294.
(95) Atwater, H. A. The promise of plasmonics. *Sci. Am.* **2007**, *296* (4), 56−63.
(96) Lal, S.; Link, S.; Halas, N. J. Nano-optics from sensing to waveguiding. *Nat. Photonics* **2007**, *1* (11), 641−648.
(97) Stewart, M. E.; Mack, N. H.; Malyarchuk, V.; Soares, J. A.; Lee, T.-W.; Gray, S. K.; Nuzzo, R. G.; Rogers, J. A. Quantitative multispectral biosensing and 1D imaging using quasi-3D plasmonic crystals. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103* (46), 17143−17148.
(98) Johnson, P. B.; Christy, R.-W. Optical constants of the noble metals. *Phys. Rev. B* **1972**, *6* (12), 4370.
(99) Bobb, D.; Zhu, G.; Mayy, M.; Gavrilenko, A.; Mead, P.; Gavrilenko, V.; Noginov, M. Engineering of low-loss metal for nanoplasmonic and metamaterials applications. *Appl. Phys. Lett.* **2009**, *95* (15), 151102.
(100) Blaber, M.; Arnold, M.; Ford, M. Designing materials for plasmonic systems: the alkali−noble intermetallics. *J. Phys.: Condens. Matter* **2010**, *22* (9), 095501.
(101) Khurgin, J. B.; Boltasseva, A. Reflecting upon the losses in plasmonics and metamaterials. *MRS Bull.* **2012**, *37* (8), 768.
(102) Bennett, H.; Peck, R.; Burge, D.; Bennett, J. Formation and growth of tarnish on evaporated silver films. *J. Appl. Phys.* **1969**, *40* (8), 3351−3360.
(103) West, P. R.; Ishii, S.; Naik, G. V.; Emani, N. K.; Shalaev, V. M.; Boltasseva, A. Searching for better plasmonic materials. *Laser Photonics Rev.* **2010**, *4* (6), 795−808.
(104) Chan, G. H.; Zhao, J.; Hicks, E. M.; Schatz, G. C.; Van Duyne, R. P. Plasmonic properties of copper nanoparticles fabricated by nanosphere lithography. *Nano Lett.* **2007**, *7* (7), 1947−1952.
(105) Langhammer, C.; Schwind, M.; Kasemo, B.; Zoric, I. Localized surface plasmon resonances in aluminum nanodisks. *Nano Lett.* **2008**, *8* (5), 1461−1471.
(106) Krutyanskiy, V.; Kolmychek, I.; Gan'shina, E.; Murzina, T.; Evans, P.; Pollard, R.; Stashkevich, A.; Wurtz, G.; Zayats, A. Plasmonic enhancement of nonlinear magneto-optical response in nickel nanorod metamaterials. *Phys. Rev. B: Condens. Matter Mater. Phys.* **2013**, *87* (3), 035116.
(107) Tobiška, P.; Hugon, O.; Trouillet, A.; Gagnaire, H. An integrated optic hydrogen sensor based on SPR on palladium. *Sens. Actuators, B* **2001**, *74* (1−3), 168−172.
(108) Baldelli, S.; Eppler, A. S.; Anderson, E.; Shen, Y.-R.; Somorjai, G. A. Surface enhanced sum frequency generation of carbon monoxide adsorbed on platinum nanoparticle arrays. *J. Chem. Phys.* **2000**, *113* (13), 5432−5438.
(109) Palik, E. D. *Handbook of Optical Constants of Solids*; Academic press, 1998; Vol. *3*.
(110) Blaber, M.; Arnold, M.; Harris, N.; Ford, M.; Cortie, M. Plasmon absorption in nanospheres: A comparison of sodium, potassium, aluminium, silver and gold. *Phys. B* **2007**, *394* (2), 184−187.
(111) Hoffman, A. J.; Alekseyev, L.; Howard, S. S.; Franz, K. J.; Wasserman, D.; Podolskiy, V. A.; Narimanov, E. E.; Sivco, D. L.; Gmachl, C. Negative refraction in semiconductor metamaterials. *Nat. Mater.* **2007**, *6* (12), 946−950.
(112) Sihvola, A. Metamaterials in electromagnetics. *Metamaterials* **2007**, *1* (1), 2−11.
(113) Pelton, M.; Aizpurua, J.; Bryant, G. Metal-nanoparticle plasmonics. *Laser Photonics Rev.* **2008**, *2* (3), 136−159.
(114) Washio, I.; Xiong, Y.; Yin, Y.; Xia, Y. Reduction by the end groups of poly (vinyl pyrrolidone): a new and versatile route to the kinetically controlled synthesis of Ag triangular nanoplates. *Adv. Mater.* **2006**, *18* (13), 1745−1749.
(115) Carbó-Argibay, E.; Rodríguez-González, B.; Pacifico, J.; Pastoriza-Santos, I.; Pérez-Juste, J.; Liz-Marzán, L. M. Chemical sharpening of gold nanorods: the rod-to-octahedron transition. *Angew. Chem., Int. Ed.* **2007**, *46* (47), 8983−8987.
(116) Yang, S. M.; Jang, S. G.; Choi, D. G.; Kim, S.; Yu, H. K. Nanomachining by colloidal lithography. *Small* **2006**, *2* (4), 458−475.
(117) Boltasseva, A.; Nikolajsen, T.; Leosson, K.; Kjaer, K.; Larsen, M. S.; Bozhevolnyi, S. I. Integrated optical components utilizing long-range surface plasmon polaritons. *J. Lightwave Technol.* **2005**, *23* (1), 413.
(118) Abargues, R.; Marqués-Hueso, J.; Canet-Ferrer, J.; Pedrueza, E.; Valdés, J.; Jiménez, E.; Martínez-Pastor, J. High-resolution electron-beam patternable nanocomposite containing metal nanoparticles for plasmonics. *Nanotechnology* **2008**, *19* (35), 355308.
(119) De Angelis, F.; Liberale, C.; Coluccio, M.; Cojoc, G.; Di Fabrizio, E. Emerging fabrication techniques for 3D nano-structuring in plasmonics and single molecule studies. *Nanoscale* **2011**, *3* (7), 2689−2696.
(120) Boltasseva, A. Plasmonic components fabrication via nanoimprint. *J. Opt. A: Pure Appl. Opt.* **2009**, *11* (11), 114001.
(121) Payne, E. K.; Shuford, K. L.; Park, S.; Schatz, G. C.; Mirkin, C. A. Multipole plasmon resonances in gold nanorods. *J. Phys. Chem. B* **2006**, *110* (5), 2150−2154.
(122) Bharadwaj, P.; Deutsch, B.; Novotny, L. Optical antennas. *Adv. Opt. Photonics* **2009**, *1* (3), 438−483.
(123) Ghenuche, P.; Cherukulappurath, S.; Taminiau, T. H.; van Hulst, N. F.; Quidant, R. Spectroscopic mode mapping of resonant plasmon nanoantennas. *Phys. Rev. Lett.* **2008**, *101* (11), 116805.
(124) Pendry, J. B.; Holden, A.; Stewart, W.; Youngs, I. Extremely low frequency plasmons in metallic mesostructures. *Phys. Rev. Lett.* **1996**, *76* (25), 4773.
(125) Shelby, R. A.; Smith, D. R.; Schultz, S. Experimental verification of a negative index of refraction. *Science* **2001**, *292* (5514), 77−79.
(126) Aizpurua, J.; Hanarp, P.; Sutherland, D. S.; Kall, M.; Bryant, G. W.; Garcia de Abajo, F. J. Optical properties of gold nanorings. *Phys. Rev. Lett.* **2003**, *90* (5), 057401.
(127) Zhou, J.; Koschny, T.; Zhang, L.; Tuttle, G.; Soukoulis, C. M. Experimental demonstration of negative index of refraction. *Appl. Phys. Lett.* **2006**, *88* (22), 221103.


https://doi.org/10.1021/acsabm.1c00113
*ACS Appl. Bio Mater.* XXXX, XXX, XXX−XXX

(128) Podolskiy, V. A.; Sarychev, A. K.; Narimanov, E. E.; Shalaev, V. M. Resonant light interaction with plasmonic nanowire systems. *J. Opt. A: Pure Appl. Opt.* **2005**, *7* (2), S32.
(129) Bai, J.; Wang, C.; Chen, X.; Basiri, A.; Wang, C.; Yao, Y. Chip-integrated plasmonic flat optics for mid-infrared full-Stokes polarization detection. *Photonics Res.* **2019**, *7* (9), 1051−1060.
(130) Nelayah, J.; Kociak, M.; Stephan, O.; Garcia de Abajo, F. J.; Tence, M.; Henrard, L.; Taverna, D.; Pastoriza-Santos, I.; Liz-Marzan, L. M.; Colliex, C. Mapping surface plasmons on a single metallic nanoparticle. *Nat. Phys.* **2007**, *3* (5), 348−353.
(131) Schuck, P.; Fromm, D.; Sundaramurthy, A.; Kino, G.; Moerner, W. Improving the mismatch between light and nanoscale objects with gold bowtie nanoantennas. *Phys. Rev. Lett.* **2005**, *94* (1), 017402.
(132) Hao, F.; Nehl, C. L.; Hafner, J. H.; Nordlander, P. Plasmon resonances of a gold nanostar. *Nano Lett.* **2007**, *7* (3), 729−732.
(133) Langhammer, C.; Kasemo, B.; Zorić, I. Absorption and scattering of light by Pt, Pd, Ag, and Au nanodisks: absolute cross sections and branching ratios. *J. Chem. Phys.* **2007**, *126* (19), 194702.
(134) Pendry, J. A chiral route to negative refraction. *Science* **2004**, *306* (5700), 1353−1355.
(135) Giannini, V.; Rodríguez-Oliveros, R.; Sánchez-Gil, J. A. Surface plasmon resonances of metallic nanostars/nanoflowers for surface-enhanced Raman scattering. *Plasmonics* **2010**, *5* (1), 99−104.
(136) Kim, J.; Liu, G. L.; Lu, Y.; Lee, L. P. Intra-particle plasmonic coupling of tip and cavity resonance modes in metallic apertured nanocavities. *Opt. Express* **2005**, *13* (21), 8332−8338.
(137) Hohenester, U.; Ditlbacher, H.; Krenn, J. R. Electron-energy-loss spectra of plasmonic nanoparticles. *Phys. Rev. Lett.* **2009**, *103* (10), 106801.
(138) Barwick, B.; Flannigan, D. J.; Zewail, A. H. Photon-induced near-field electron microscopy. *Nature* **2009**, *462* (7275), 902−906.
(139) Auguié, B.; Barnes, W. L. Collective resonances in gold nanoparticle arrays. *Phys. Rev. Lett.* **2008**, *101* (14), 143902.
(140) Chen, S.; Svedendahl, M.; Käll, M.; Gunnarsson, L.; Dmitriev, A. Ultrahigh sensitivity made simple: nanoplasmonic label-free biosensing with an extremely low limit-of-detection for bacterial and cancer diagnostics. *Nanotechnology* **2009**, *20* (43), 434015.
(141) Thakur, A.; Qiu, G.; Ng, S.-P.; Guan, J.; Yue, J.; Lee, Y.; Wu, C.-M. L. Direct detection of two different tumor-derived extracellular vesicles by SAM-AuNIs LSPR biosensor. *Biosens. Bioelectron.* **2017**, *94*, 400−407.
(142) Bathini, S.; Raju, D.; Badilescu, S.; Kumar, A.; Ouellette, R.; Ghosh, A.; Packirisamy, M. Nano−Bio Interactions of Extracellular Vesicles with Gold Nanoislands for Early Cancer Diagnosis. *Research* **2018**, *2018*, 3917986.
(143) Lv, X.; Geng, Z.; Su, Y.; Fan, Z.; Wang, S.; Fang, W.; Chen, H. Label-Free Exosome Detection Based on a Low-Cost Plasmonic Biosensor Array Integrated with Microfluidics. *Langmuir* **2019**, *35* (30), 9816−9824.
(144) Qiu, G.; Thakur, A.; Xu, C.; Ng, S. P.; Lee, Y.; Wu, C. M. L. Detection of Glioma-Derived Exosomes with the Biotinylated Antibody-Functionalized Titanium Nitride Plasmonic Biosensor. *Adv. Funct. Mater.* **2019**, *29* (9), 1806761.
(145) Reiner, A. T.; Ferrer, N.-G.; Venugopalan, P.; Lai, R. C.; Lim, S. K.; Dostálek, J. Magnetic nanoparticle-enhanced surface plasmon resonance biosensor for extracellular vesicle analysis. *Analyst* **2017**, *142* (20), 3913−3921.
(146) Yang, Y.; Zhai, C.; Zeng, Q.; Khan, A. L.; Yu, H. Multifunctional Detection of Extracellular Vesicles with Surface Plasmon Resonance Microscopy. *Anal. Chem.* **2020**, *92* (7), 4884−4890.
(147) Im, H.; Shao, H.; Park, Y. I.; Peterson, V. M.; Castro, C. M.; Weissleder, R.; Lee, H. Label-free detection and molecular profiling of exosomes with a nano-plasmonic sensor. *Nat. Biotechnol.* **2014**, *32* (5), 490−495.
(148) Park, J.; Im, H.; Hong, S.; Castro, C. M.; Weissleder, R.; Lee, H. Analyses of intravesicular exosomal proteins using a nano-plasmonic system. *ACS Photonics* **2018**, *5* (2), 487−494.
(149) Wan, M.; Amrollahi, P.; Sun, D.; Lyon, C.; Hu, T. Y. Using nanoplasmon-enhanced scattering and low-magnification microscope imaging to quantify tumor-derived exosomes. *J. Visualized Exp.* **2019**, No. 147, e59177.
(150) Kumar, S.; Han, J. A.; Michael, I. J.; Ki, D.; Sunkara, V.; Park, J.; Gautam, S.; Ha, H. K.; Zhang, L.; Cho, Y. K. Human Platelet Membrane Functionalized Microchips with Plasmonic Codes for Cancer Detection. *Adv. Funct. Mater.* **2019**, *29* (30), 1902669.
(151) Sun, D.; Fan, J.; Liu, C.; Liu, Y.; Bu, Y.; Lyon, C. J.; Hu, Y. Noise reduction method for quantifying nanoparticle light scattering in low magnification dark-field microscope far-field images. *Anal. Chem.* **2016**, *88* (24), 12001−12005.
(152) Lee, J. U.; Kim, S.; Sim, S. J. SERS-based Nanoplasmonic Exosome Analysis: Enabling Liquid Biopsy for Cancer Diagnosis and Monitoring Progression. *BioChip J.* **2020**, *14*, 231.
(153) Lee, C.; Carney, R. P.; Hazari, S.; Smith, Z. J.; Knudson, A.; Robertson, C. S.; Lam, K. S.; Wachsmann-Hogiu, S. 3D plasmonic nanobowl platform for the study of exosomes in solution. *Nanoscale* **2015**, *7* (20), 9290−9297.
(154) Chin, L. K.; Son, T.; Hong, J.-S.; Liu, A.-Q.; Skog, J.; Castro, C. M.; Weissleder, R.; Lee, H.; Im, H. Plasmonic Sensors for Extracellular Vesicle Analysis: From Scientific Development to Translational Research. *ACS Nano* **2020**, *14* (11), 14528−14548.
(155) Raju, D.; Bathini, S.; Badilescu, S.; Ouellette, R. J.; Ghosh, A.; Packirisamy, M. LSPR detection of extracellular vesicles using a silver-PDMS nano-composite platform suitable for sensor networks. *Enterp. Inf. Syst.* **2020**, *14* (4), 532−541.
(156) Jiang, Y.; Shi, M.; Liu, Y.; Wan, S.; Cui, C.; Zhang, L.; Tan, W. Aptamer/AuNP biosensor for colorimetric profiling of exosomal proteins. *Angew. Chem., Int. Ed.* **2017**, *56* (39), 11916−11920.
(157) Liu, W.; Li, J.; Wu, Y.; Xing, S.; Lai, Y.; Zhang, G. Target-induced proximity ligation triggers recombinase polymerase amplification and transcription-mediated amplification to detect tumor-derived exosomes in nasopharyngeal carcinoma with high sensitivity. *Biosens. Bioelectron.* **2018**, *102*, 204−210.
(158) Zhang, Y.; Wang, D.; Yue, S.; Lu, Y.; Yang, C.; Fang, J.; Xu, Z. Sensitive multicolor visual detection of exosomes via dual signal amplification strategy of enzyme-catalyzed metallization of Au nanorods and hybridization chain reaction. *ACS Sensors* **2019**, *4* (12), 3210−3218.
(159) Oliveira-Rodríguez, M.; Serrano-Pertierra, E.; García, A. C.; López-Martín, S.; Yañez-Mo, M.; Cernuda-Morollón, E.; Blanco-López, M. d. C. Point-of-care detection of extracellular vesicles: sensitivity optimization and multiple-target detection. *Biosens. Bioelectron.* **2017**, *87*, 38−45.
(160) Bauch, M.; Toma, K.; Toma, M.; Zhang, Q.; Dostalek, J. Plasmon-enhanced fluorescence biosensors: a review. *Plasmonics* **2014**, *9* (4), 781−799.
(161) Lee, K.; Fraser, K.; Ghaddar, B.; Yang, K.; Kim, E.; Balaj, L.; Chiocca, E. A.; Breakefield, X. O.; Lee, H.; Weissleder, R. Multiplexed profiling of single extracellular vesicles. *ACS Nano* **2018**, *12* (1), 494−503.
(162) Knoblauch, R.; Geddes, C. D. Review of Advances in Metal-Enhanced Fluorescence. In *Reviews in Plasmonics 2017*; Springer, 2019; pp 253−283.
(163) Min, J.; Son, T.; Hong, J. S.; Cheah, P. S.; Wegemann, A.; Murlidharan, K.; Weissleder, R.; Lee, H.; Im, H. Plasmon-Enhanced Biosensing for Multiplexed Profiling of Extracellular Vesicles. *Adv. Biosyst.* **2020**, *4* (12), 2000003.
(164) Reiner, A. T.; Fossati, S.; Dostalek, J. Biosensor platform for parallel surface plasmon-enhanced epifluorescence and surface plasmon resonance detection. *Sens. Actuators, B* **2018**, *257*, 594−601.
(165) Li, J.-F.; Li, C.-Y.; Aroca, R. F. Plasmon-enhanced fluorescence spectroscopy. *Chem. Soc. Rev.* **2017**, *46* (13), 3962−3979.


https://doi.org/10.1021/acsabm.1c00113
ACS Appl. Bio Mater. XXXX, XXX, XXX−XXX