

Research Highlights

Highlights from the latest articles on molecular imaging with Raman spectroscopy



Gold nanoparticles combined with surface-enhanced Raman spectroscopy

Raman spectroscopy using surface-enhanced Raman scattering (SERS) nanoparticles is an exciting new molecular imaging modality that has emerged over the past decade [1,2]. The approach is based on the inelastic scatter of photons by molecular bonds. Small-molecule dyes adsorbed on a rough metal (usually gold) surface experience a dramatic increase in their Raman scatter intensity due to surface plasmons, which induce strong localized electric fields near the surface [3]. As each bond within a Raman dye has a characteristic vibrational energy, the SERS spectrum is unique for that dye. The nanoparticle for Raman molecular imaging is composed of: a gold core to provide the signal enhancement, an adsorbed dye to provide the SERS spectrum, a biocompatible coating (e.g., polyethylene glycol or silica) and a targeting moiety (e.g., an antibody). By combining unique dye spectra and targeting moieties for different biological targets, high-sensitivity, multiplexed molecular imaging is possible *in vivo* with an environmentally and optically stable contrast agent [4–8]. Summarized below are four exciting recent reports that significantly advance the field of SERS-based molecular imaging.

References

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The origin of surface enhancement of Raman scattering by nanoparticles

Evaluation of: Sun G, Khurgin JB. Origin of giant difference between fluorescence, resonance and nonresonance Raman scattering enhancement by surface plasmons. *Phys. Rev. A* 85, 063410 (2012).

Despite the widespread use of SERS for both *in vitro* diagnostics and *in vivo* molecular imaging, the basic physics underlying the dramatic increase in Raman scattering intensity of over ten orders of magnitude in the vicinity of metal nanoparticles is still relatively poorly understood. In particular,

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the reason why optical processes, such as fluorescence and resonance Raman, do not experience a similar degree of enhancement has been attributed to fluorescence lifetime quenching near the metal surface and a lack of commensurate Raman quenching, without complete explanation. The goal of this work was to establish an analytical model that reveals the physics behind these strikingly different results, explores the limits on the attainable enhancement and outlines a path to optimization of the surface enhancement for all three processes [1].

The authors build on their previous model of enhancement of optical properties by metal nanoparticles, which focused on photoluminescence enhancement by combining models for light absorption and emission in the presence of isolated metal nanoparticles. They have adapted the model by including all competing radiative and nonradiative processes, as well as

higher order surface plasmon modes, and use, as an example, a dye molecule placed at various distances (0–30 nm) from a metal nanosphere that can take a range of diameters (20–100 nm).

The simplified approach used here allowed the authors to draw a number of important conclusions. First, they demonstrate that the attainable enhancement is, above all, dependent on the original efficiency of the given optical process, meaning only weak emitters (Raman) can be strongly enhanced by surface plasmons, while strong emitters may end up quenched by emission into nonradiative higher-order modes. Second, they demonstrate that the separation between the dye molecule and the nanoparticle can be optimized to achieve a maximum enhancement in the case of strong emitters, whereas no such improvement is available for the weaker Raman process, which is always maximally enhanced at the surface. Finally, they demonstrate that the nanosphere size can be tuned to find a compromise between the two competing and mutually exclusive

roles that metal nanoparticles play: that of antenna for efficient in- and out-coupling of energy and that of a nanocavity for energy concentration. An efficient antenna requires a large size particle, while a high concentration of energy requires a small particle size. Finding a compromise between these two demands for a given optical process can maximize surface enhancement.

The conclusions above demonstrate the power of this analytical model, providing an insight into the physical processes behind the SERS phenomenon. Importantly for *in vivo* imaging using SERS-based nanoparticle approaches, the ability to optimize nanoparticle design for a given application using simple *a priori* information will be valuable for future developments in the field.

Reference

- 1 Sun G, Khurgin JB. Origin of giant difference between fluorescence, resonance and nonresonance Raman scattering enhancement by surface plasmons. *Phys. Rev. A* 85, 063410 (2012).

Detection of inflammation via ICAM-1 expression using surface-enhanced Raman scattering

Evaluation of: McQueenie R, Stevenson R, Benson R *et al.* Detection of inflammation *in vivo* by surface-enhanced Raman scattering provides higher sensitivity than conventional fluorescence imaging. *Anal. Chem.* 84, 5968–5975 (2012).

The purpose of this study was to demonstrate the application of SERS molecular imaging for sensitive detection of early-stage inflammation using SERS nanoparticles coded with a unique Raman dye and conjugated to ICAM-1 specific monoclonal antibodies to detect ICAM-1 expression on endothelial cells [1].

A number of intravital microscopy approaches have previously been applied to study immune responses in living subjects; two-photon fluorescence

microscopy (TPM) is usually the method of choice as near-infrared light is able to excite fluorophores at depth in tissue. Fluorescence approaches, however, suffer from background autofluorescence, broad emission spectra and photobleaching. To demonstrate the potential of SERS molecular imaging compared with equivalent fluorescent approaches, the authors quantitatively compared their results to flow cytometry *in vitro*, immunofluorescence *ex vivo* and TPM *in vivo*.

Antibody-conjugated SERS nanoparticles (~100 nm diameter) were shown to be stable for at least 6 months. Measurements were first made in microvascular endothelial cells in culture that were stimulated with the proinflammatory cytokine TNF- α for 18 h prior to experiments. The relative sensitivities of SERS and flow cytometry

were compared using the ratios of mean fluorescence intensity and the highest intensity peak in the SERS spectrum, to their isotype control contrast agents (IgG2b-conjugated). These data show that SERS could detect ICAM-1 upregulation under stimulation by lower concentrations of TNF- α (1 ng/ml) than flow cytometry (10 ng/ml). Next, the expression of ICAM-1 by aortic endothelial cells in the apoE-deficient mouse model of atherosclerosis was explored. Scanning of tissue sections demonstrated colocalization of SERS signals with the endothelial cells inside the luminal wall, at signal-to-noise ratios nearly threefold greater than immunofluorescence.

To study the sensitivity of their ICAM-1-targeted SERS nanoparticles compared with anti-ICAM-1-FITC for fluorescence imaging using TPM in small living subjects, the authors induced



inflammation in the mouse ear pinna using an intradermal lipopolysaccharide challenge. They intravenously administered the molecular imaging agents (20- μ g anti-ICAM-1-FITC and 0.82-nM anti-ICAM-1-nanotags) in separate animal cohorts 24 h after the lipopolysaccharide challenge, and normalized the data to isotype antibody control. Signal-to-noise ratios for SERS imaging were over twofold greater than in TPM and were more sensitive in the detection of ICAM-1 in this model of inflammation. Importantly, they also demonstrated detection of their

targeted nanoparticles at depths of up to 1 cm in tissue, which significantly exceeded the depth available using TPM ($\sim 100 \mu\text{m}$).

The authors conclude that targeted SERS nanoparticles produce higher sensitivity detection of ICAM-1 expression than conventional fluorophore-antibody conjugates and offer improvements in spectral definition, signal specificity, depth resolution and the achievable signal-to-noise ratio. This study is significant as it demonstrates for the first time that SERS molecular imaging can

be used to detect inflammation in small living subjects.

Reference

- 1 McQueenie R, Stevenson R, Benson R *et al.* Detection of inflammation *in vivo* by surface-enhanced Raman scattering provides higher sensitivity than conventional fluorescence imaging. *Anal. Chem.* 84, 5968–5975 (2012).

Porphyrin-lipid stabilized gold nanoparticles for Raman spectroscopic imaging

Evaluation of: Tam NCM, McVeigh PZ, MacDonald TD, Farhadi A, Wilson BC, Zheng G. Porphyrin-lipid stabilized gold nanoparticles for surface enhanced Raman scattering based imaging. *Bioconjug. Chem.* 23, 1726–1730 (2012).

Currently, one of the major limitations in the synthesis of SERS-active gold nanoparticles is the selection of dyes that contain strong Raman-active modes and functional groups (e.g., thiol and -SH) that allow for chemi- or physi-adsorption to metallic surfaces. Since these dyes often have altered affinities in the presence of a biological matrix, nanoparticles are often coated with poly(ethylene glycol) or silica (as described in the ‘Molecular imaging using gold nanoparticles combined with surface enhanced Raman spectroscopy’ section) to produce stable probes for *in vivo* imaging. The initial step of loading dyes onto gold nanoparticles can give rise to concentration- and dye-dependent inconsistencies, and uncontrolled aggregation.

To overcome this limitation, the authors of this study have developed a porphyrin-phospholipid conjugate with quenched fluorescence to serve as both a Raman dye and a stabilizing biocompatible surface coating [1]. Porphyrins exhibit strong Raman scattering but this is usually

overwhelmed by endogenous fluorescence; incorporation of metal ions (Mn^{3+}) quenches this fluorescence. The conjugate was able to self assemble into a 4–7 nm thick bilayer around 60-nm gold nanoparticles suspended in water. SERS enhancement of the Mn^{3+} loaded pyropheophorbide-a (porphyrin) situated within the lipid coating was detectable by Raman spectroscopy; no Raman signal could be detected from the conjugate in solution at over a 1000-fold concentration in the absence of gold nanoparticles, confirming the surface-enhancement effect. Importantly, the enhancement was still observed without the Raman dye being directly adsorbed onto the metal surface (see evaluation of Sun and Khurgin [2] for more details).

To investigate the reproducibility and biocompatibility of their nanoparticles, the authors incubated them in either phosphate-buffered saline or 50% fetal bovine serum for up to 24 h. The lack of any red shift observed in the nanoparticle absorption peak demonstrates that the porphyrin-lipid coating is sufficient to prevent aggregation from serum proteins and at physiological ion concentrations. Furthermore, they incubated cells from four different cancer cell lines with concentrations of nanoparticles between 0.28 fM and 2.8 nM for 2, 8 and 24 h in four different cancer cell lines; no cytotoxicity was observed in any of these cases. Imaging with a confocal Raman

microscope demonstrated localization of the nanoparticles on the periphery and inside the cells, taken up by nonspecific endocytosis. The limitations of this work were that the authors did not demonstrate the conjugation of a targeting moiety to the porphyrin-phospholipid nanoparticles and did not perform any imaging of small living subjects or pilot toxicity studies.

The novel strategy the authors describe for creating SERS nanoparticles by combining a phospholipid surface coating with a chromophore will expand the selection of dyes that can be used for Raman multiplexed detection, since it is no longer necessary to employ specific thiol groups for adsorption to the gold nanoparticle surface. This approach streamlines the synthesis of nanoparticles, provides stability to the resulting contrast agent and reduces variability. All of these factors will be key to the future clinical development of SERS molecular imaging.

References

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- 2 Sun G, Khurgin JB. Origin of giant difference between fluorescence, resonance and nonresonance Raman scattering enhancement by surface plasmons. *Phys. Rev. A* 85, 063410 (2012).



Plasmonic vesicles for Raman traceable intracellular drug delivery

Evaluation of: Song J, Zhao J, Duan H. Self-assembled plasmonic vesicles of SERS-encoded amphiphilic gold nanoparticles for cancer cell targeting and traceable intracellular drug delivery. *J. Am. Chem. Soc.* 134, 13458–13469 (2012).

All prior reports of SERS nanoparticle targeting involve the conjugation of antibodies for a particular cell surface receptor to the individual nanoparticles. While this technique provides a sensitive approach for localizing SERS nanoparticles to a particular cell type or condition (as demonstrated above in McQueenie *et al.* [1]), it does not report specifically on the extra or intracellular location of the particle, or allow for delivery of additional payloads. In this study, the authors demonstrate the use of a pH-responsive SERS nanoparticle assembly for triggered intracellular drug release and noninvasive readout of the event.

The use of nanoparticle assemblies in the biological environment remains largely unexplored, and this study is one of the first to demonstrate that plasmonic assemblies with a hollow cavity can play multifunctional roles as delivery carriers for anticancer drugs as well as plasmonic imaging probes to specifically report on drug release [2]. Stimuli triggered payload release and feedback mechanisms are key

to the field of ‘theranostic nanomedicine’, but current readouts rely on changes in fluorescence, for example with fluorescence resonance energy transfer.

In this study, an assembly was prepared using amphiphilic gold nanoparticles coated with a Raman dye and mixed polymer brushes of hydrophilic poly(ethylene glycol) and a pH-sensitive, hydrophobic copolymer. The resulting plasmonic vesicle was approximately 200 nm in diameter, with the shell thickness of approximately 20 nm corresponding to a monolayer of densely packed amphiphilic nanoparticles around a hollow central cavity. The dense packing of gold nanoparticles in the shell led to a strong interparticle plasmonic coupling that yields increased SERS enhancement, evidenced by a 25-nm red-shift of the absorption peak relative to that of individual nanoparticles. The plasmonic vesicles showed excellent colloidal stability, a stable hydrodynamic size for at least 3 months and were dissociated upon lowering of the solution pH from 7.4 to 5.0.

To test these vesicles as cancer cell targeting drug delivery agents, the vesicles were conjugated with a monoclonal antibody to HER2, loaded with doxorubicin and incubated with HER2⁺ SKBR-3 breast cancer cells. Dark field and fluorescence microscopy indicated efficient receptor-mediated cellular uptake of the vesicles. Although the targeted plasmonic vesicles had no direct effect on cell

viability, when loaded with doxorubicin, they became highly toxic to the cells, with an estimated IC₅₀ of 0.3 µg/ml. Raman spectroscopy readouts demonstrated gradually reduced SERS intensity over time, which gave a noninvasive readout of the dissociation of the internalized vesicles over time within endosomes/lysosomes. A limitation of this study is that the authors only performed dark field and fluorescence microscopy imaging but did not include images from Raman microscopy, only point measurements. Furthermore, the targeting and activation of the plasmonic vesicles was demonstrated solely in cells in culture.

In summary, this study is valuable as it demonstrates that SERS molecular imaging can be used to give independent spectroscopic feedback on the cargo release by a novel theranostic platform, cancer drug-loaded plasmonic assemblies.

References

- 1 McQueenie R, Stevenson R, Benson R *et al.* Detection of inflammation *in vivo* by surface-enhanced Raman scattering provides higher sensitivity than conventional fluorescence imaging. *Anal. Chem.* 84, 5968–5975 (2012).
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