Optical spectroscopy and biosensors for investigation of biomolecules and their interactions

Jakub Dostalek

AIT - Austrian Institute of Technology GmbH
Biosensor Technologies Unit
Konrad-Lorenz-Strasse 24 | 3430 Tulln | Austria
T +43(0) 664 2351773

FZU – Institute of Physics of the Czech Academy of Sciences,
Na Slovance 1 | Prague 182 00 | Czech Republic
T+420 776767927

jakub.dostalek@ait.ac.at | http://www.ait.ac.at | http://www.jakubdostalek.cz

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Surface-Enhanced Raman Spectroscopy

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Content

- Raman scattering, Amplification of weak Raman signal by using metallic nanostructures.
- Design rules and sensitivity.
- Fingerprinting of molecular species
- Raman labels serving in assays.
- Optical configurations used for the Raman scattering detection.

Raman @ IR Absoprtion Spectroscopy

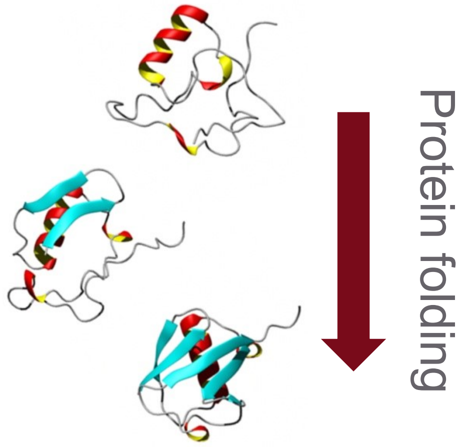
Vibrational spectroscopies - IR and Raman are the most common vibrational spectroscopies for assessing molecular motion and fingerprinting species.

IR and Raman obeys complementary selection rules

- Selection rules dictate, which molecular vibrations are probed.
- Some vibrational modes are both IR and Raman active.

Applications

- Commonly used in chemistry, since vibrational information is specific to the chemical bonds and symmetry of molecules. Therefore, it provides a fingerprint by which the molecule can be identified.
- For larger molecules – information on conformation changes can be obtained rather than identification of a protein itself.



http://web.mit.edu/~tokmakofflab/ResearchProtein.htm

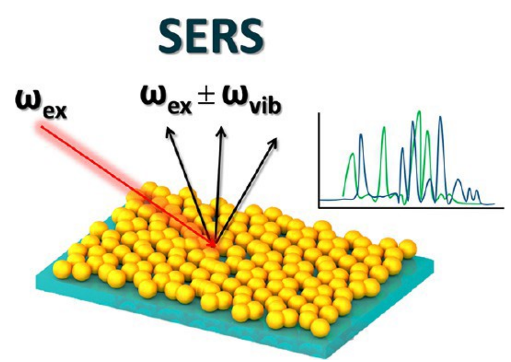
High EM Field Intensity

The description of refractive index independently on the electromagnetic field is valid for weak optical fields. For high field intensities *E* the polarizability can be treated in form of Taylor series:

$$P_i = \varepsilon_0 \left[\sum_j \chi_{ij}^{(1)} E_j + \frac{1}{2} \sum_{j,k} \chi_{ijk}^{(2)} E_j E_k + \frac{1}{6} \sum_{j,k,l} \chi_{ijkl}^{(3)} E_j E_k E_l + \dots \right]$$

The higher order susceptibilities $\chi^{(2),(3)\dots}$ lead to non-linear optical effects such as:

- Second harmonic generation $\chi^{(2)}$
- Raman scattering $\chi^{(3)}$
- ….



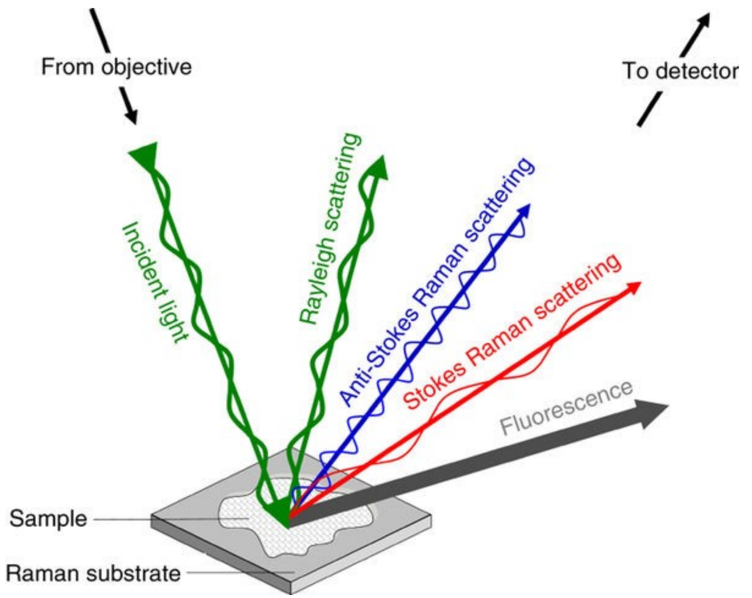
https://doi.org/10.1021/acsnano.9b04224

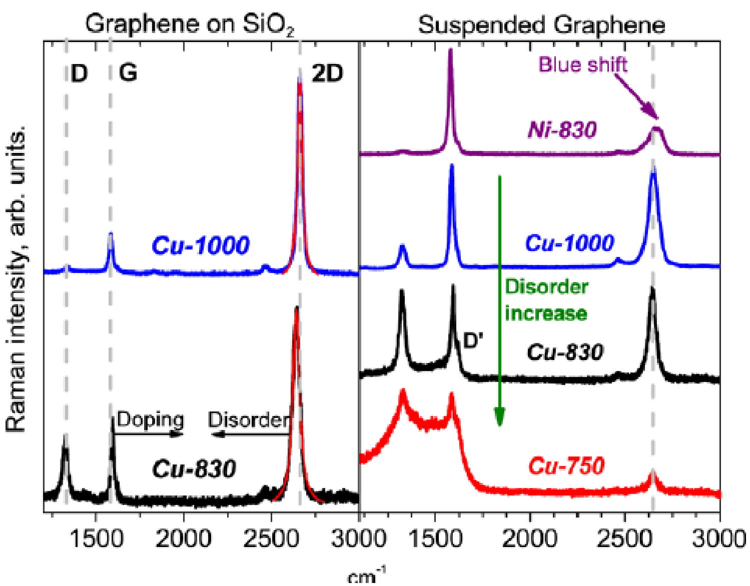
Raman Scattering

Raman scattering probability ~ E^4 ← Field intensity

IR absorption rate ~ E^2

Raman is much weaker effect (often masked by fluorescence) compared to IR.





DOI: 10.1088/0957-4484/22/27/275716

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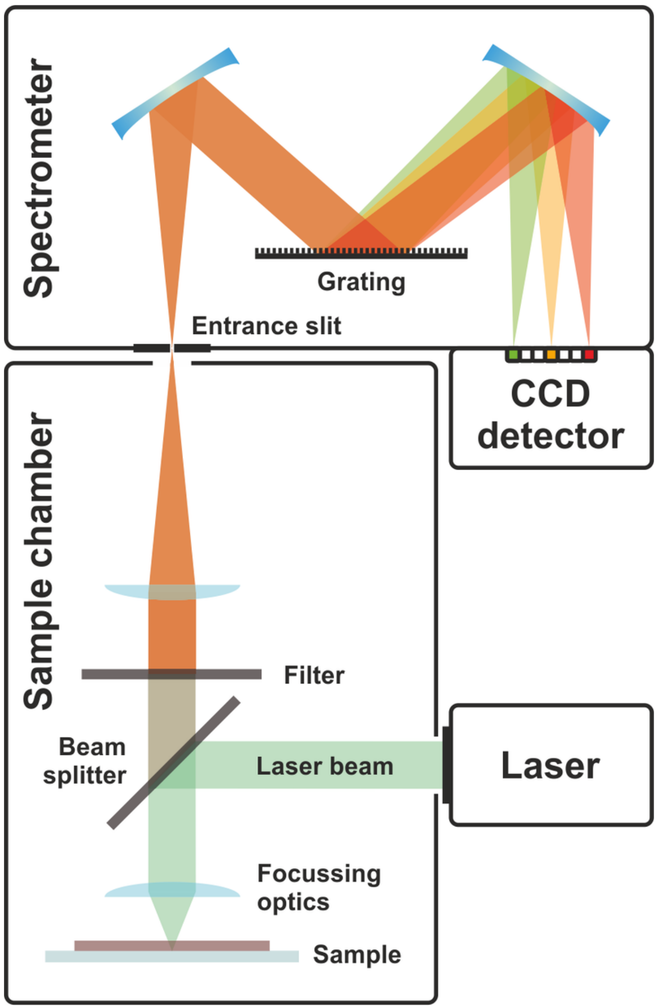
Raman Spectroscopy



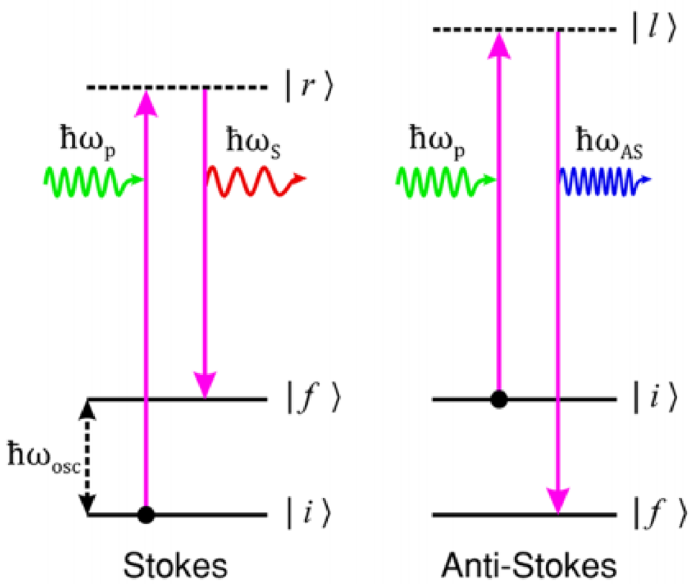
Raman scattering probability ~ λ^4

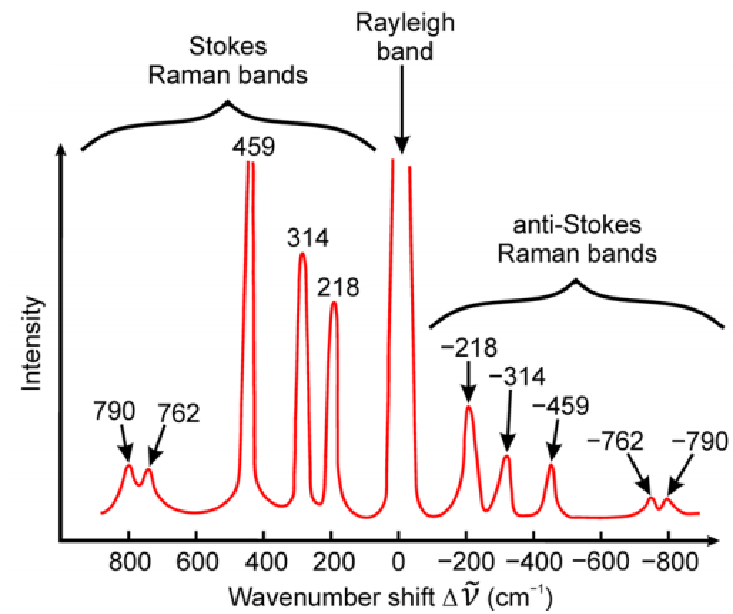
Weak intensity – using of single photon detectors like cooled CCD.

Efficient filtering of the background signal – using of notch filters, confocal configuration.

Typically long integration times are needed to collect a signal in counts per second, operation in a dark room…

Raman Spectra





https://doi.org/10.1186/s11671-019-3039-2

Ratio of Stokes and anti-Stokes band intensity:

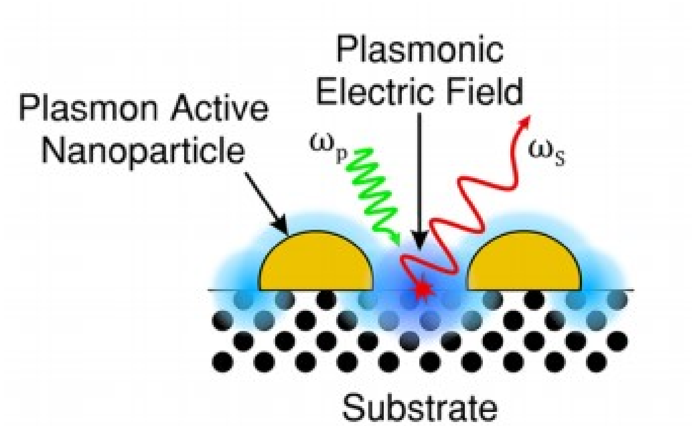
$$\frac{I_{AS}}{I_S} = \left(\frac{\omega_p + \omega_{osc}}{\omega_p - \omega_{osc}}\right)^4 e^{\left(-\frac{\hbar\omega_{osc}}{kT}\right)}$$

The spectra of plotted as depending on energy shift in cm^{-1}:

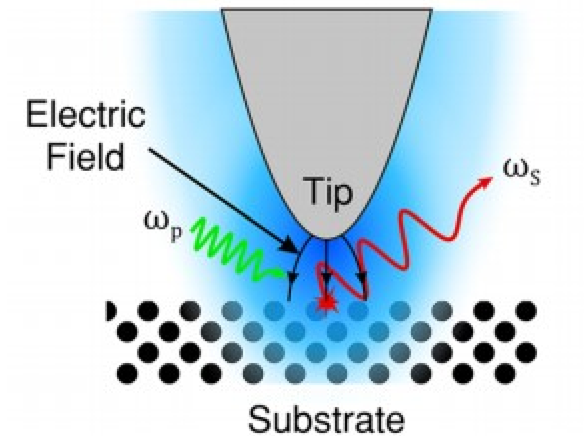
$$E = \hbar \qquad h\frac{c}{\lambda} \qquad \Delta E \sim \frac{1}{\lambda_1} - \frac{1}{\lambda_2}$$

Amplification of Raman Scattering

https://doi.org/10.1186/s11671-019-3039-2



Surface-enhanced Raman spectroscopy (SERS)



Tip-enhanced Raman spectroscopy (TERS)

Analyzed molecules are adsorbed at the surface of plasmonic nanostructures with tuned resonant properties with respect to ω_p and ω_s.

Amplification of Raman Scattering

The discovery of SERS: Fleischmann and co-workers in 1974 during measurements of the Raman scattering of pyridine on rough silver electrodes, and they ascribed the enhancement to a surface-area effect. The phenomenon was identified independently by Jeanmaire and Van Duyne and by Albrecht and Creighton in 1977, both of whom suggested enhancement factors (EFs) 5 – 6 orders of magnitude.

Electromagnetic field mechanism:

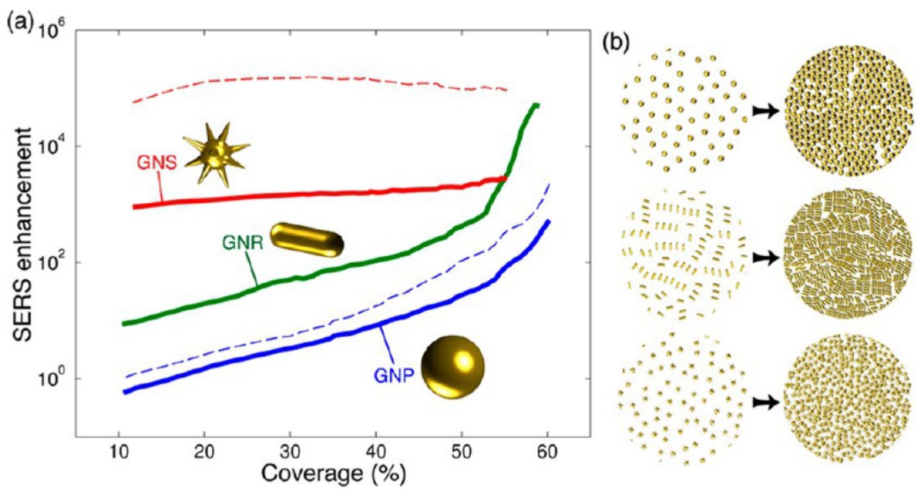
Universal, associated to the field intensity strength, reaching up to EF=10^9

$$EF \sim \frac{\left|E(\omega_P)\right|^2 \left|E(\omega_S)\right|^2}{\left|E_0\right|^4}$$

Chemical mechanism:

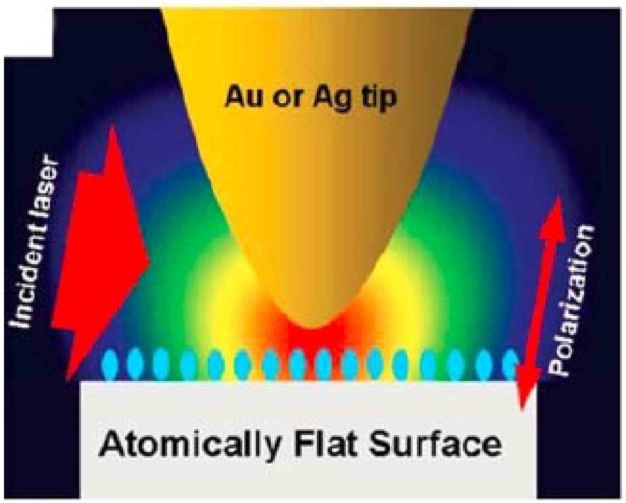
Ascribed to transfer of electrons between analyte and the metal, yields EF=10^2-10^3.

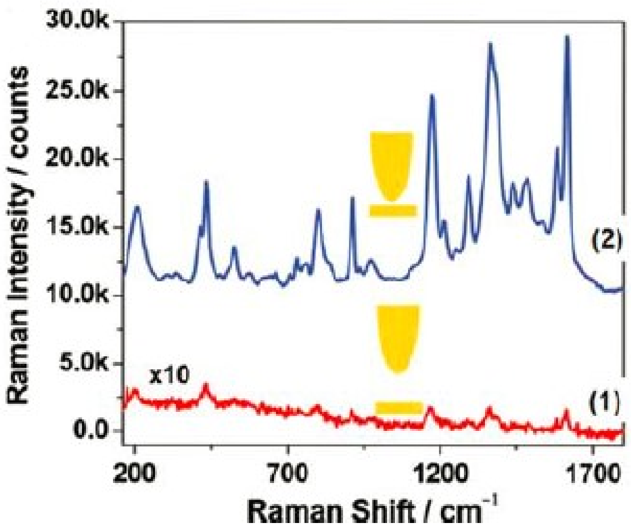
Solis, S. M.; Taboada, J. M.; Obelleiro, F.; Liz-Marzán, L. M.; García de Abajo, F. J. Optimization of Nanoparticle-Based SERS Substrates through Large-Scale Realistic Simulations. ACS Photonics, 2017, 4, 329–337.



Surface Enhanced Raman Spectroscopy (SERS)

Raman scattering is extremely weak phenomenon, however since introduction of surface-enhanced Raman spectroscopy (SERS) it become important tool in analysis of biomolecules and for detection of chemical and biological species.

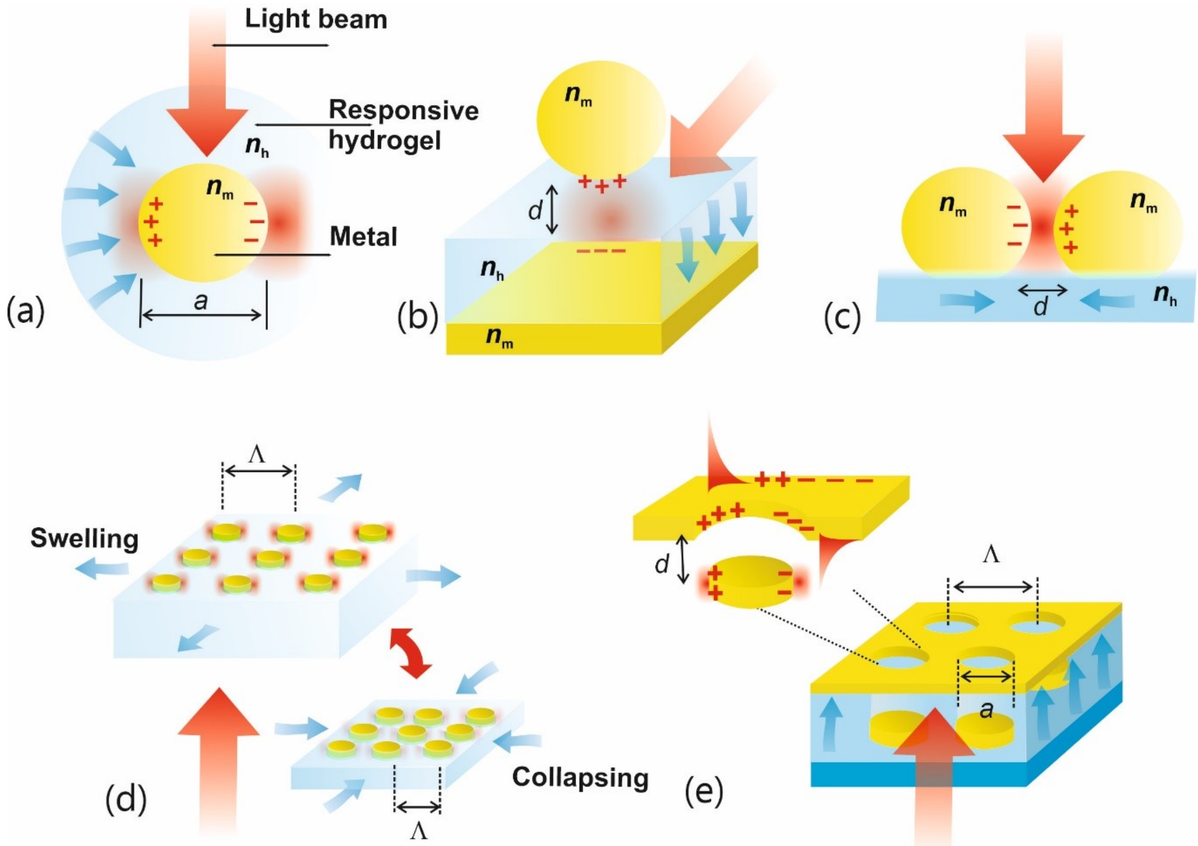




Tian Z-Q, Ren B, Li J-F, Yang Z-L (2007) Expanding generality of surface-enhanced Raman spectroscopy with borrowing SERS activity strategy. Chem Commn (34): 3514–3534

Blackie, Evan J.; Le Ru, Eric C.; Etchegoin, Pablo G. (2009). "Single-Molecule Surface-Enhanced Raman Spectroscopy of Nonresonant Molecules". *J. Am. Chem. Soc.* **131** (40): 14466–14472. doi:10.1021/ja905319w.PMID 19807188.

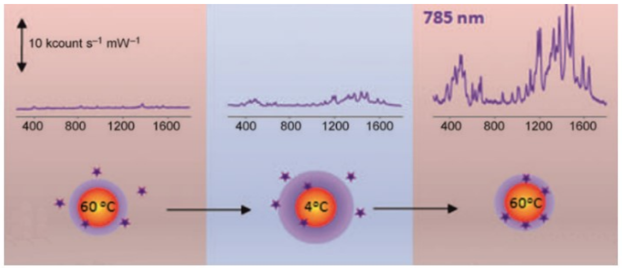
Plasmonic Structures



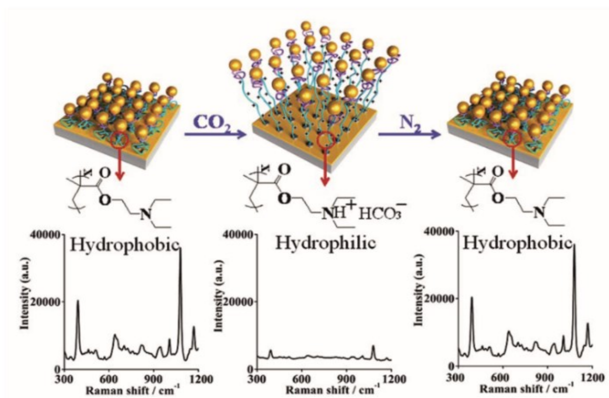
- Examples of plasmonic architectures assembled from individual building blocks.

SERS with Dynamically Formed Hotspot

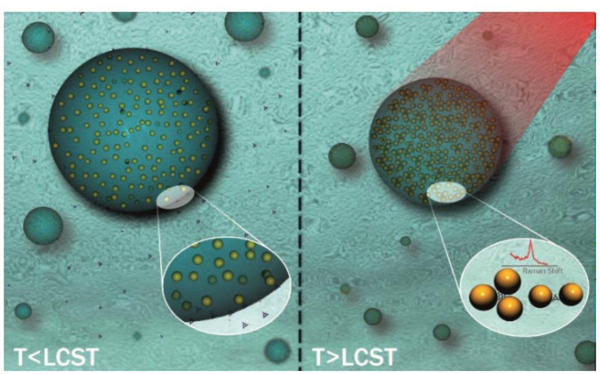
(A)



(B)



(C)



Embedding of metallic nanoparticles to architectures with responsive hydrogel moieties:

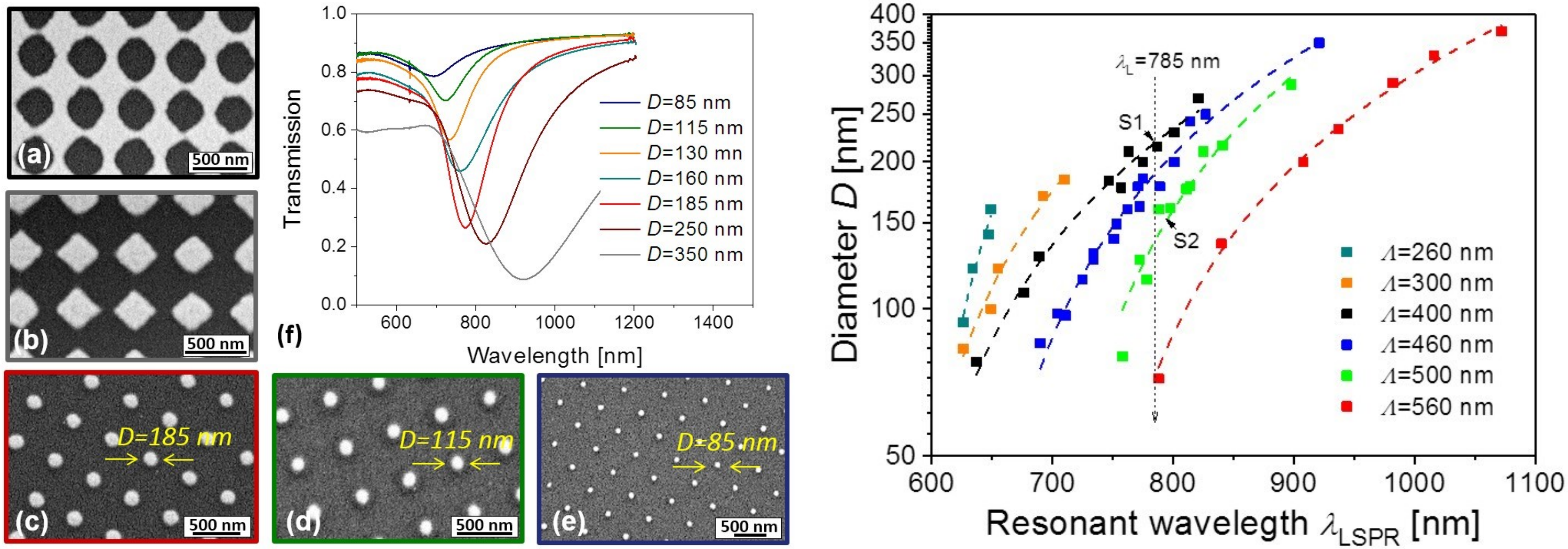
- On demand tuning of resonant wavelength and forming tight hotspots when closing gaps
- Collecting of analyzed species in the gaps where the sensitivity is highest

H. Chen, Y. Wang, X. Li, B. Liang, S. Dong, T. You and P. Yin, *RSC Adv.*, 2018, **8**, 22177–22181

R. A. Álvarez-Puebla, R. Contreras-Cáceres, I. Pastoriza-Santos, J. Pérez-Juste and L. M. Liz-Marzán, *Angew. Chemie - Int. Ed.*, 2009, **48**, 138–143.

A. C. Manikas, A. Aliberti, F. Causa, E. Battista and P. A. Netti, *J. Mater. Chem. B*, 2015, **3**, 53–58.

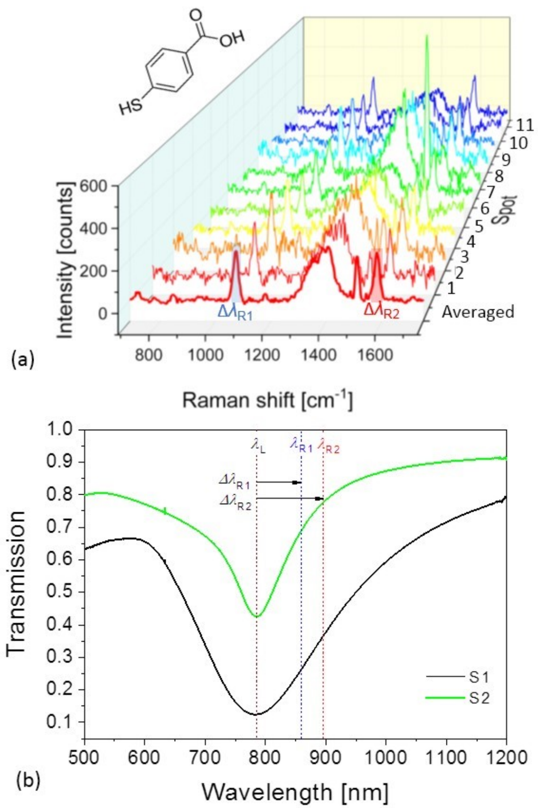
SERS on Arrays of Au Nanoparticles

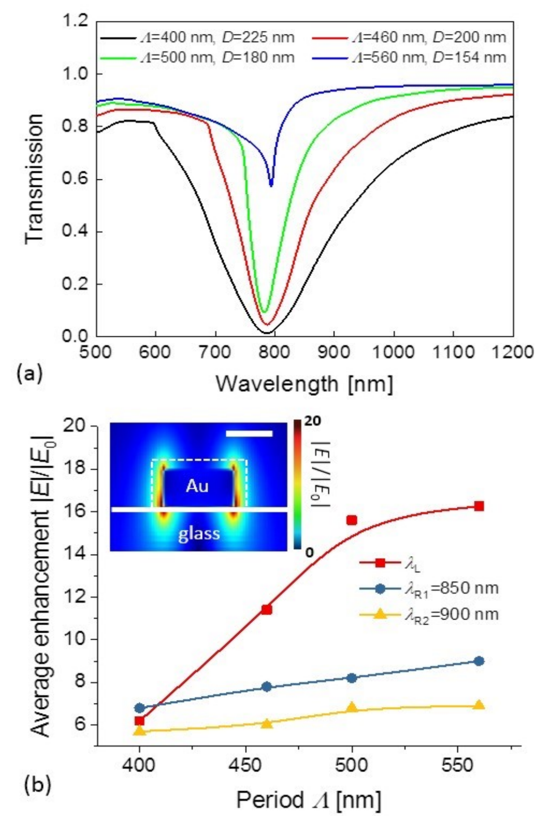


Nestor G. Quilis, Mederic Lequeux, Pryiamvada Venugopalan, Imran Khan, Souhir Boujday, Wolfgang Knoll, Marc Lamy de la Chapelle, Jakub Dostalek, Tunable laser interference lithography preparation of plasmonic nanoparticle arrays tailored for SERS, Nanoscale, 2018, 10, 10268-10276.

- Example of tuning of LSPR wavelength by period and diameter of gold disk-shaped nanoparticles.

SERS on Arrays of Au Nanoparticles

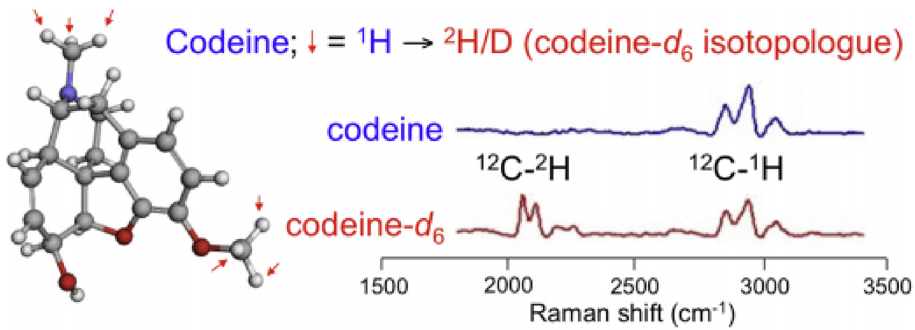




- Example of SERS spectra of MBA and tuning of the spectral width and field intensity enhancement affecting the Raman-scattered light intensity.

Nestor G. Quilis, Mederic Lequeux, Pryiamvada Venugopalan, Imran Khan, Souhir Boujday, Wolfgang Knoll, Marc Lamy de la Chapelle, Jakub Dostalek, Tunable laser interference lithography preparation of plasmonic nanoparticle arrays tailored for SERS, Nanoscale, 2018, 10, 10268-10276.

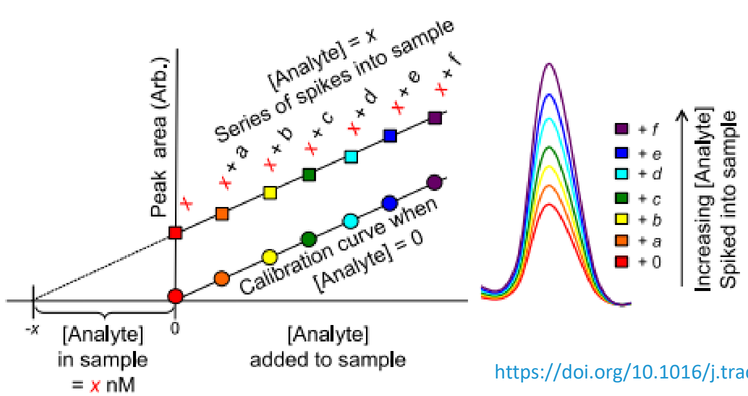
SERS Chemosensors



$$v = \frac{1}{2\pi}\sqrt{\frac{k}{\mu}} \quad \dashrightarrow \quad \mu = \frac{m_1 m_2}{m_1 + m_2}$$

Where: v = vibrational frequency
k = force constant
μ = reduced mass

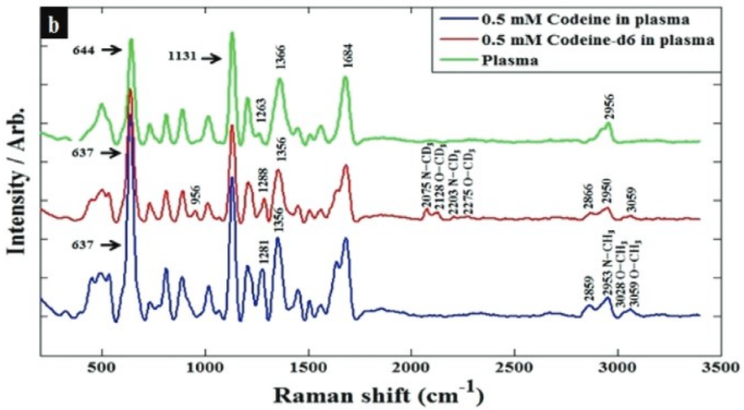
$\therefore$ ^{12}C-^{1}H, $\mu = 12/13 = 0.92$
^{13}C-^{1}H, $\mu = 13/14 = 0.93$
^{12}C-^{2}H, $\mu = 24/14 = 1.71$

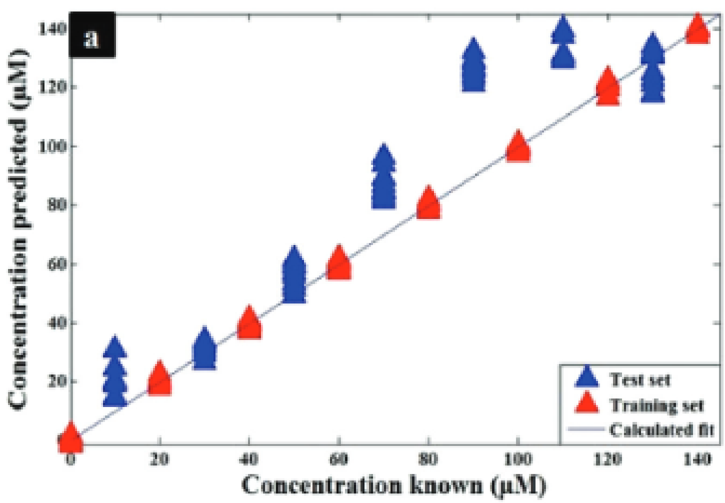


https://doi.org/10.1016/j.trac.2018.03.005

- Direct finger-printing approach for detection of target analytes suitable for low molecular weight analytes.
- Quantitative SERS is possible to construct by appropriate calibration, e.g. by using the isotopologues.

SERS Chemosensors - Codein



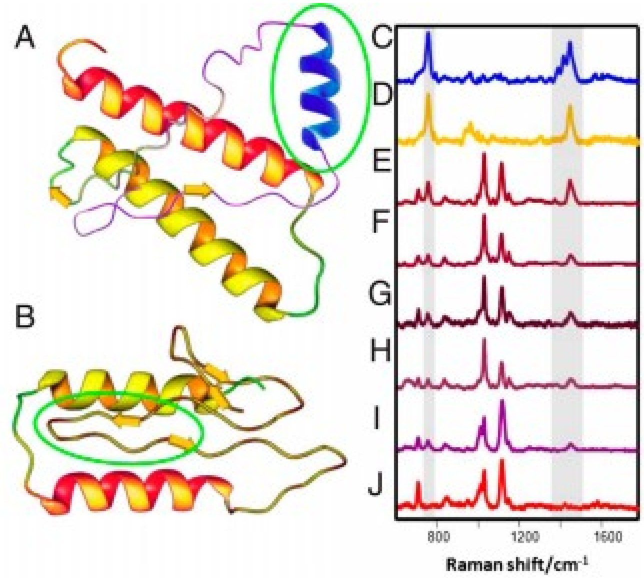


- Implementation by using synthetic Ag nanoparticles mixed with plasma for detection of codeine.
- Using of miniature portable Raman spectrometers possible.

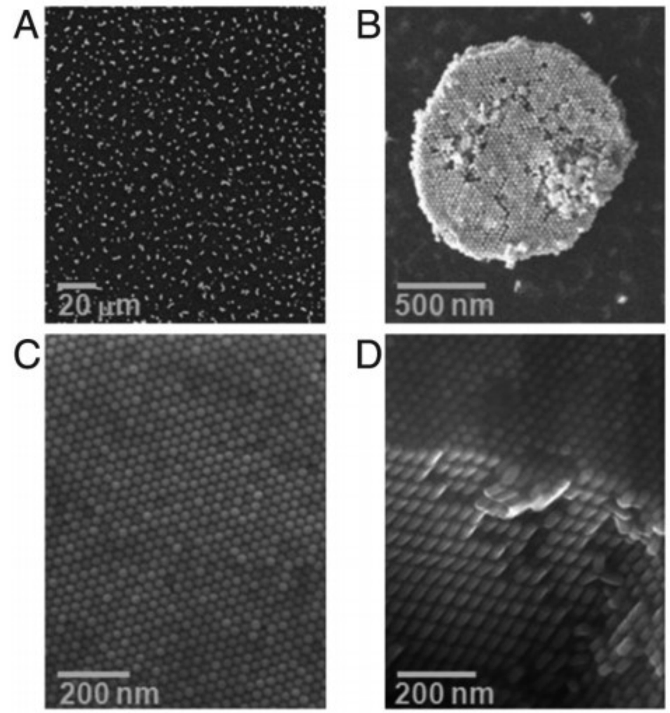
Ahura Scientific

10.1039/c7an0019

SERS Chemosensors - Prion



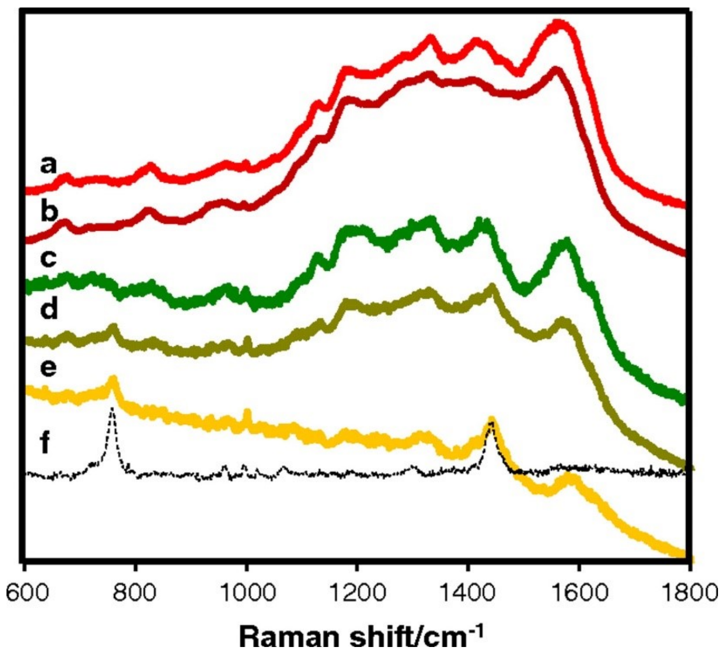
Scheme showing the prion mutation and detection limits for the scrambled version in bovine serum. (A) Biologically active (PrPC α-helix) and (B) scrambled (PrPSC β-sheet) prions; the fragment corresponding to 106–126 peptide is highlighted in green. SERS spectra of (C) PrPC α -helix and (D) PrPSC(scrambled). (E–I) Detection limits of PrPSC:PrPC (1:99) in bovine serum at 10−6, 10−7, 10−8, 10−9, and 10−10 M in total prion, respectively; and (J) bovine serum.



https://doi.org/10.1073/pnas.1016530108

- Target analyte – scrambled sequence of a prion (related to neurodegenerative diseases)
- Plasmonic substrate – super-crystal of gold nanorod particles

SERS Chemosensors - Prions



Prion ultradetection in human blood, SERS spectra of:

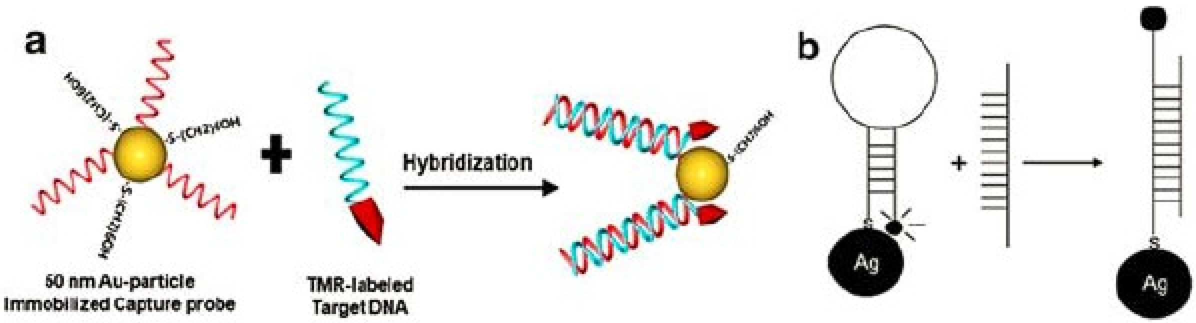
(A) natural human blood
(B) spiked human blood
(C) natural human plasma
(D) spiked human plasma.
(E) SERS spectra spiked human plasma after spectral subtraction of the matrix (human plasma)
(F) SERS spectra of the scrambled prion.

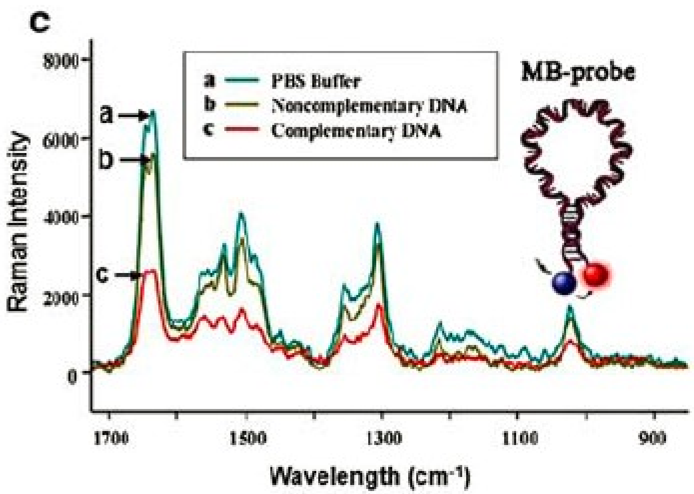
https://doi.org/10.1073/pnas.1016530108

➡ **Direct SERS monitoring of target analyte bands.**

SERS Assay – Analogy to FRET

Microfluid Nanofluid (2009) 6:285–297, 123



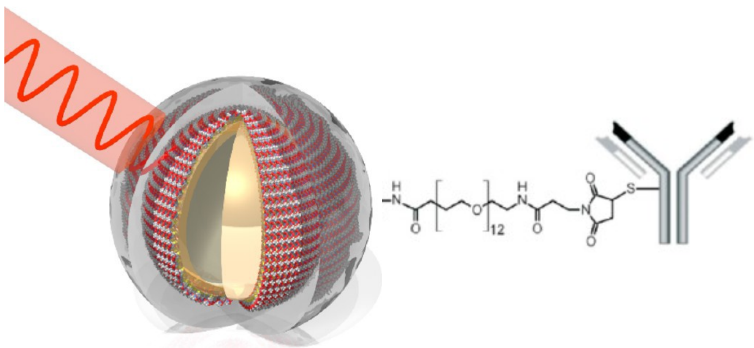


a) Hybridization accompanied with a close contact of a dye and metallic NP – increase of SERS signal

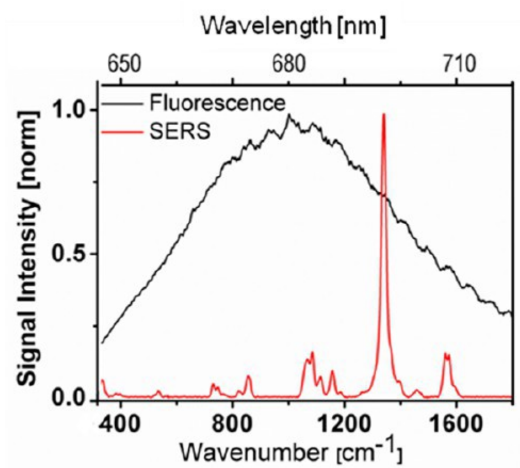
b+c) Hybridization opens the hairpin and leads to a decrease in SERS signal

➡ **Indirect SERS monitoring of target analyte-induced changes of other molecule.**

SERS Tags



Schlücker, S. Surface Enhanced Raman Spectroscopy: Analytical, Biophysical and Life Science Applications; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2011.



Salehi, M.; Schneider, L.; Ströbel, P.; Marx, A.; Packeisen, J.; Schlücker, S. Two-Color SERS Microscopy for Protein Co-Localization in Prostate Tissue with Primary Antibody–Protein A/G–Gold Nanocluster Conjugates. Nanoscale 2014, 6, 2361–2367.

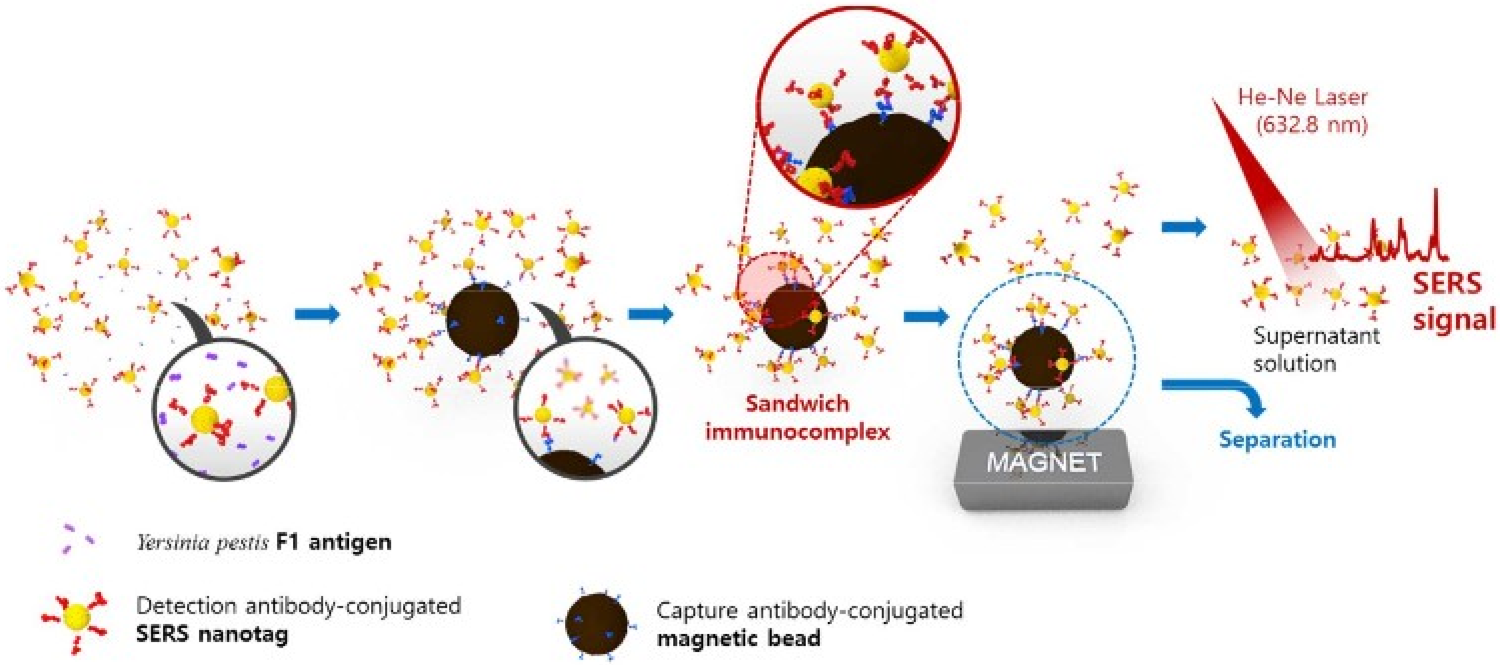
Assays employing labels (e.g. dyes) with SERS readout were pursued for the detection of protein and DNA biomolecules. The advantage over traditional fluorescence is narrow Raman spectra which are better suited for multiplexing and thus enabling detection of multiple reactions in parallel.

- Fluorescence emission band width for dyes >50 nm
- Fluorescence emission band width for quantum dots ~30 nm
- Raman peaks ~ 1 nm

Potential advantages:

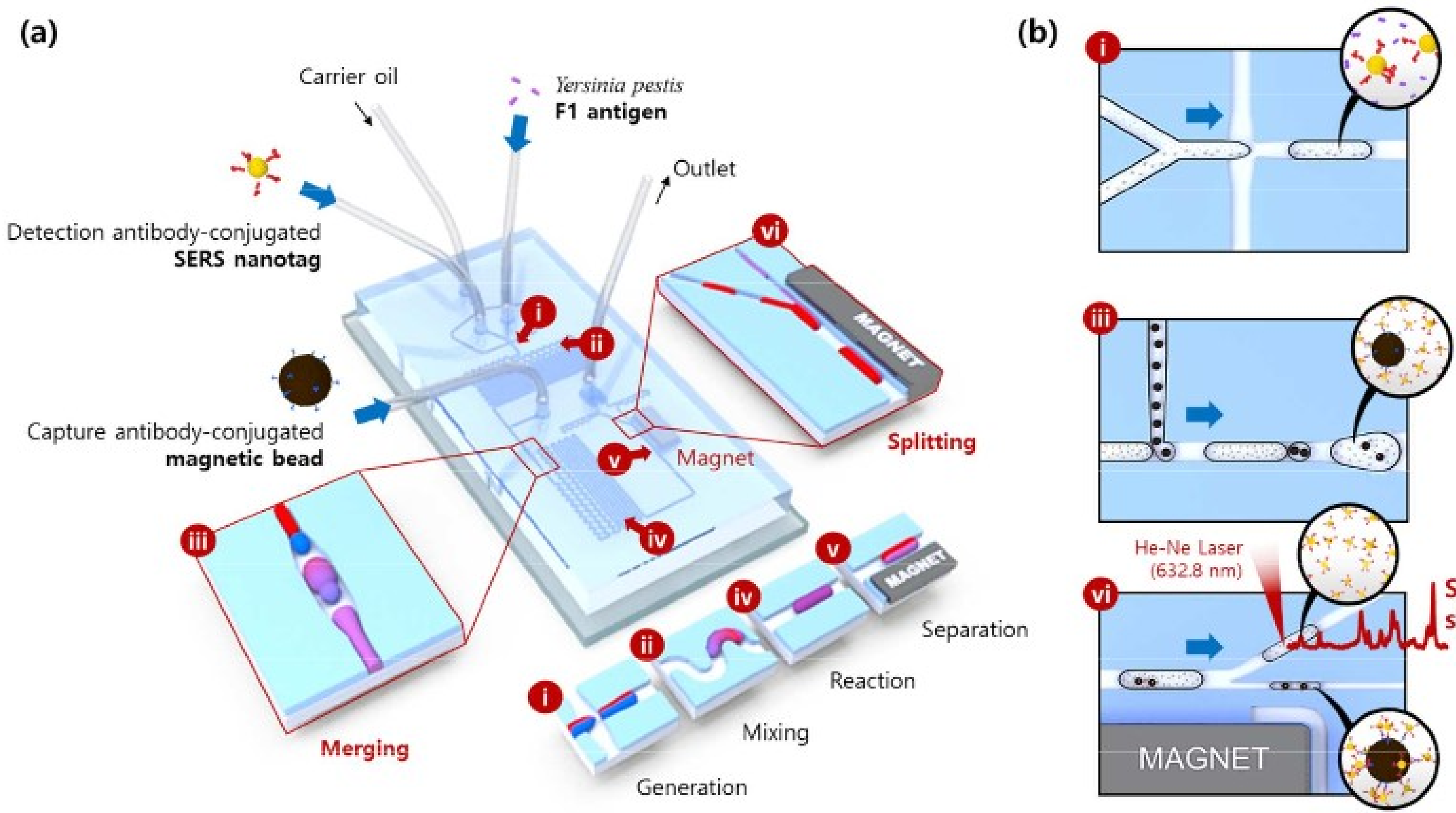
- Multiplexing
- No photobleaching
- Single wavelength excitation
- Suppression of autofluorescence
- SERS can provide similar brightness to fluorescence tags

SERS Tags – Immunoassay Readout



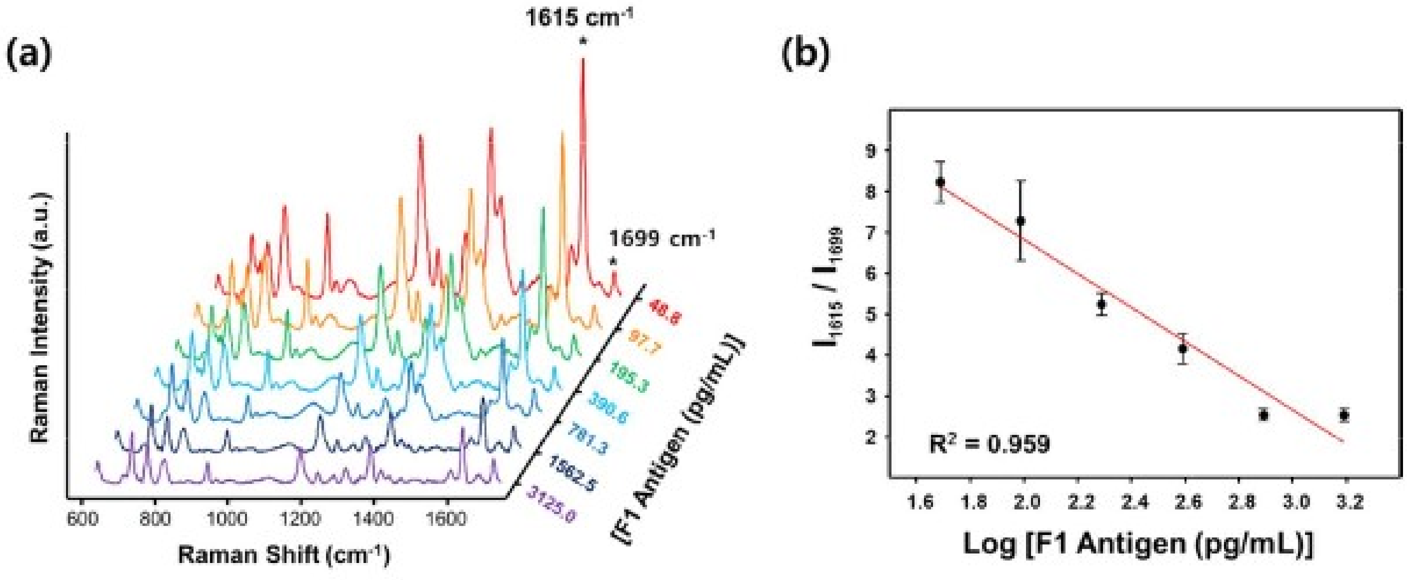
- Sandwich immunoassay for detection of F1 antigen.

SERS Tags – Immunoassay Readout



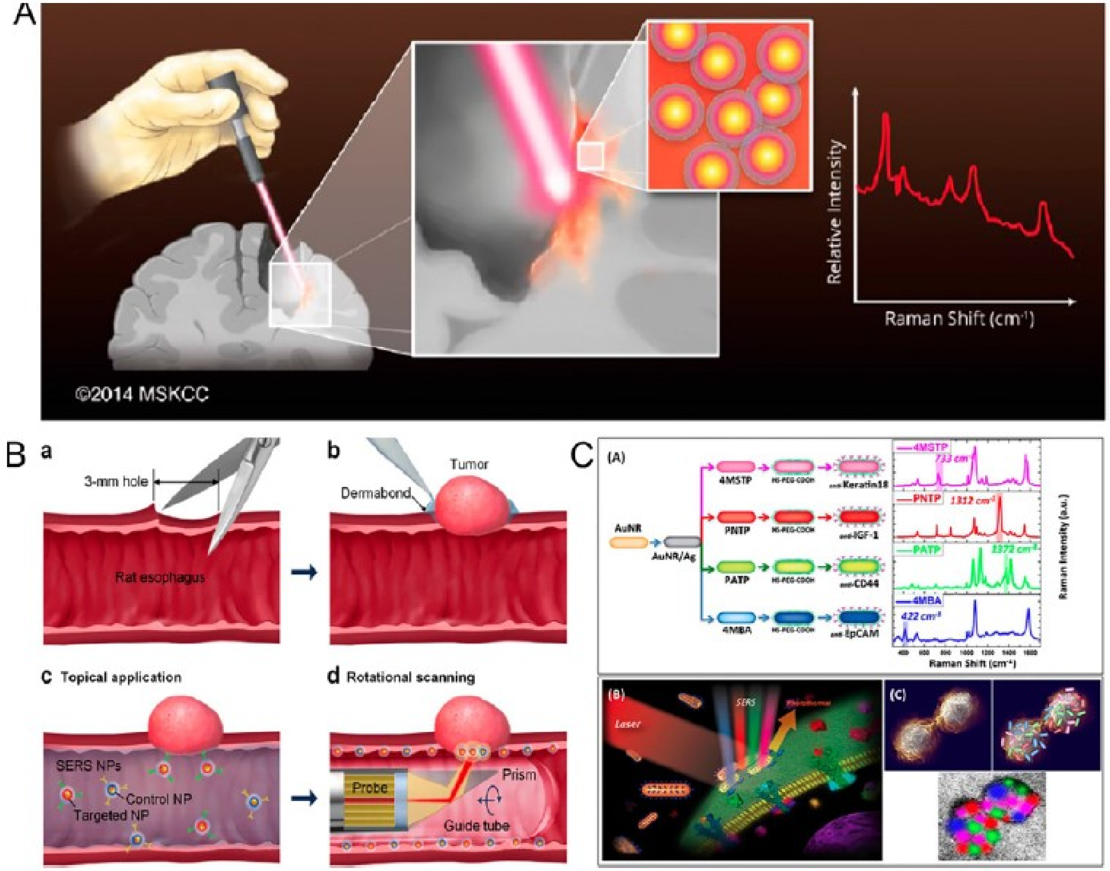
- Microfluidic-based separation of the reacted conjugates of SERS-tag-antibody and F1 antigen.

SERS Tags – Immunoassay Readout



- Readout of the amount of unreacted SERS-tag-antibody, calibration curve.

SERS Tags – Immunoassay Readout



- SERS-based imaging of cancer tissue with the use of SERS-tags conjugated with antibodies specific to a panel of biomarkers.