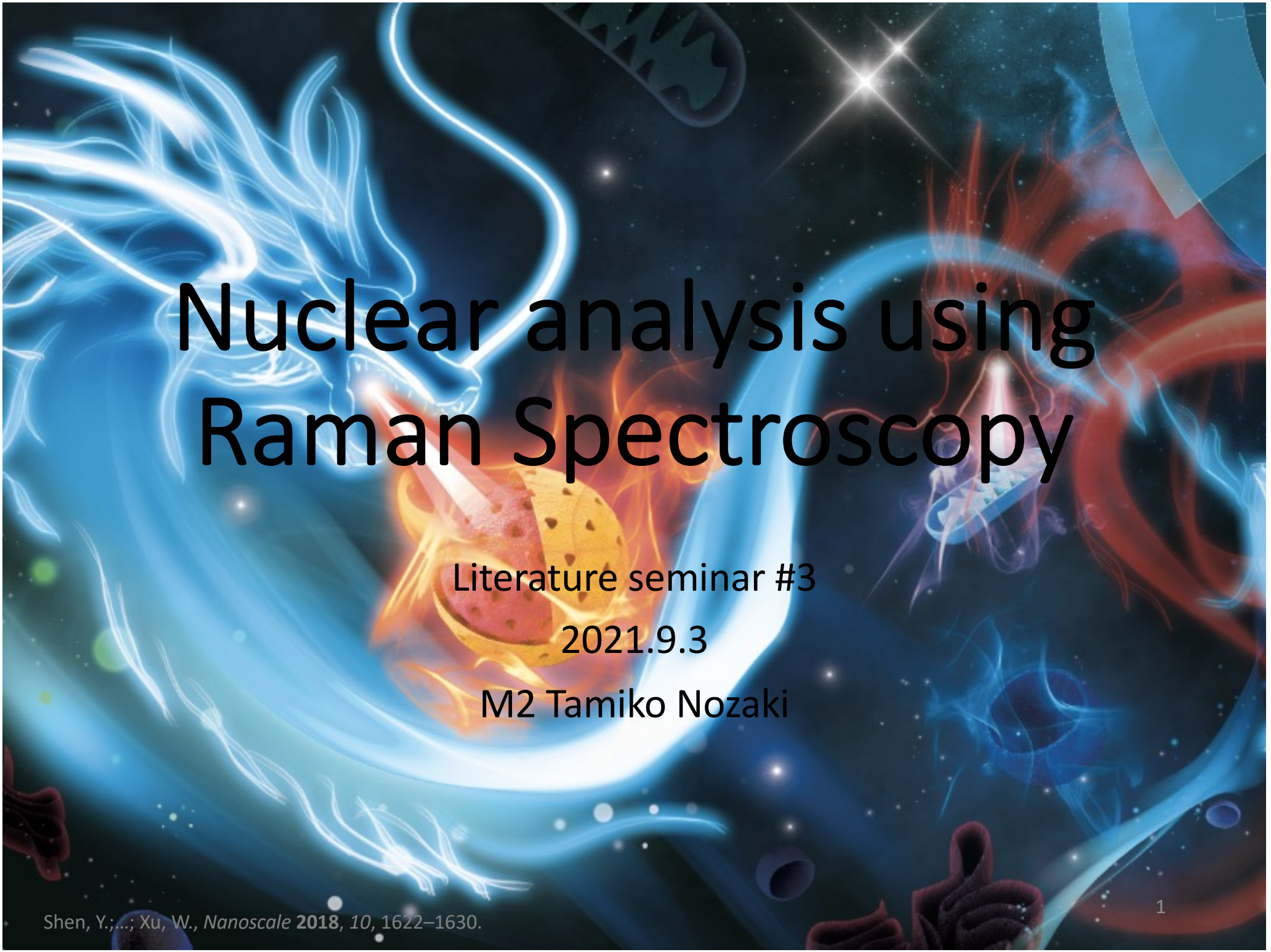


Nuclear analysis using Raman Spectroscopy

Literature seminar #3

2021.9.3

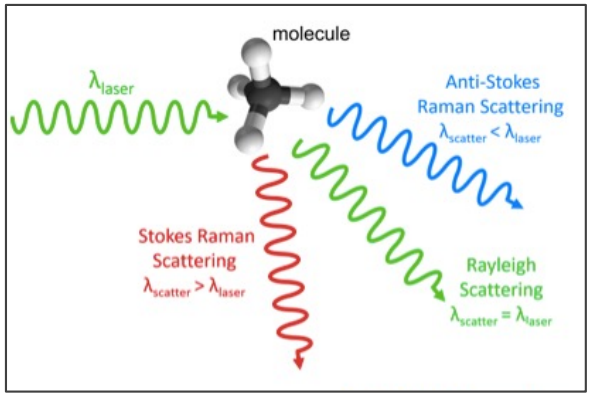
M2 Tamiko Nozaki

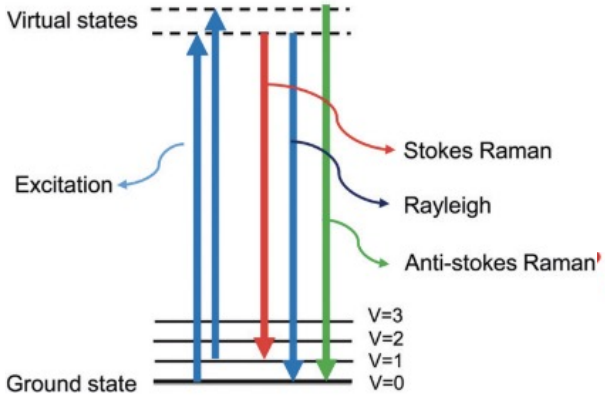
Shen, Y.;…; Xu, W., *Nanoscale* **2018**, *10*, 1622–1630.

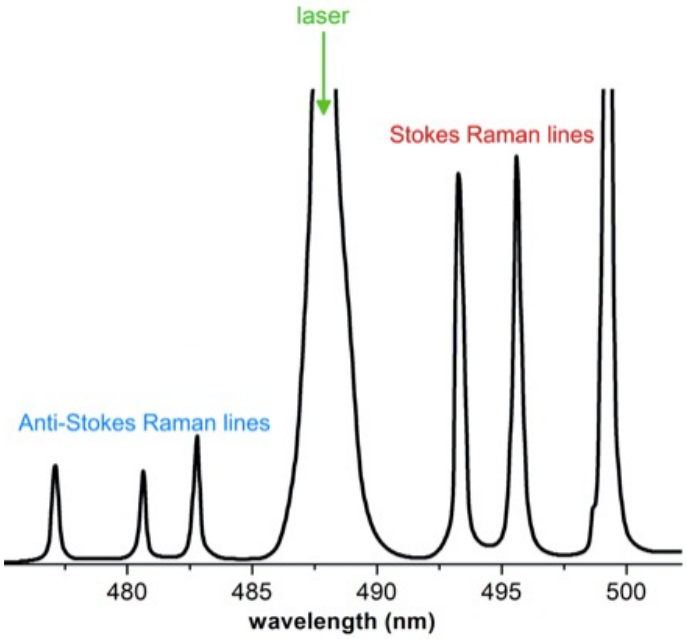
Contents

Contents

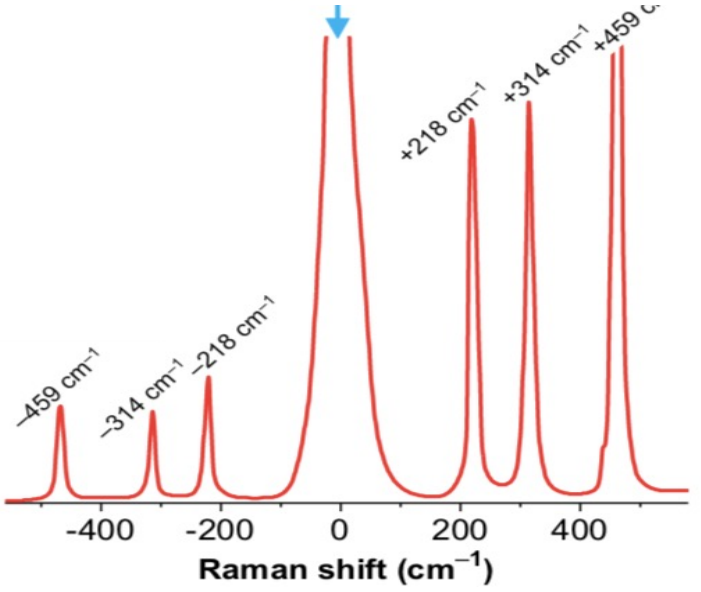
Raman scattering





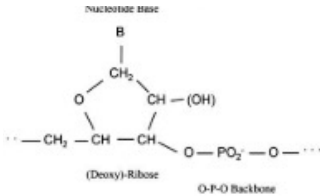


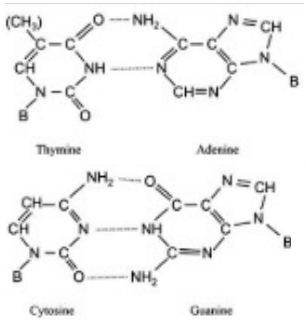
- ✓ Raman scattering is an inelastic process in which the molecule returns to a higher or lower vibrational state.
- ✓ Raman scattering reveals the vibrational, rotational and other low frequency modes of molecules

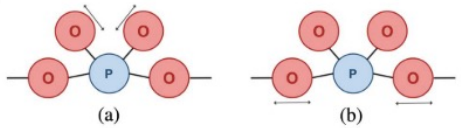


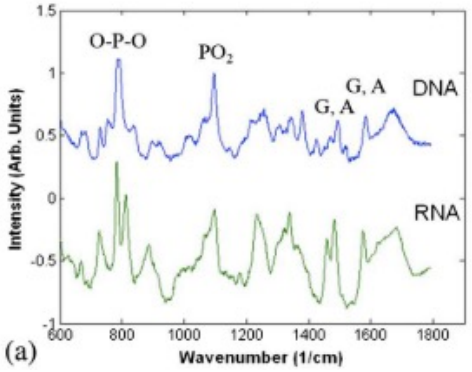
Raman spectra of biological molecules

<Nucleic acid>

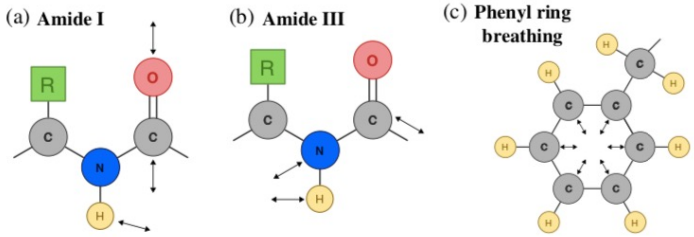


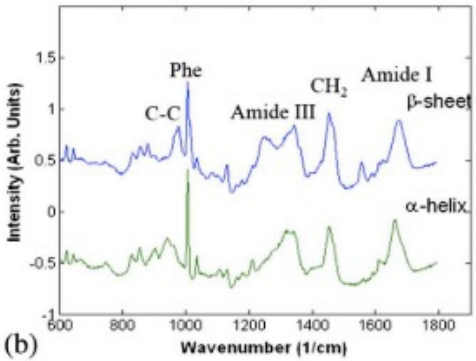




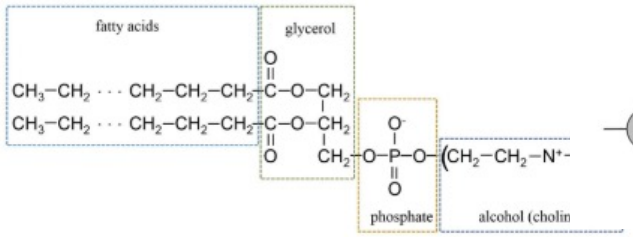


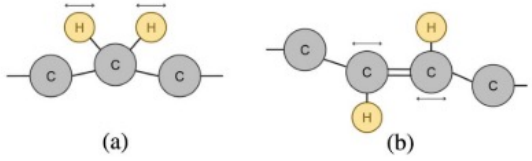
<Protein>

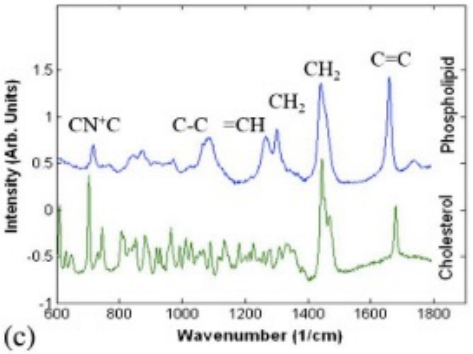




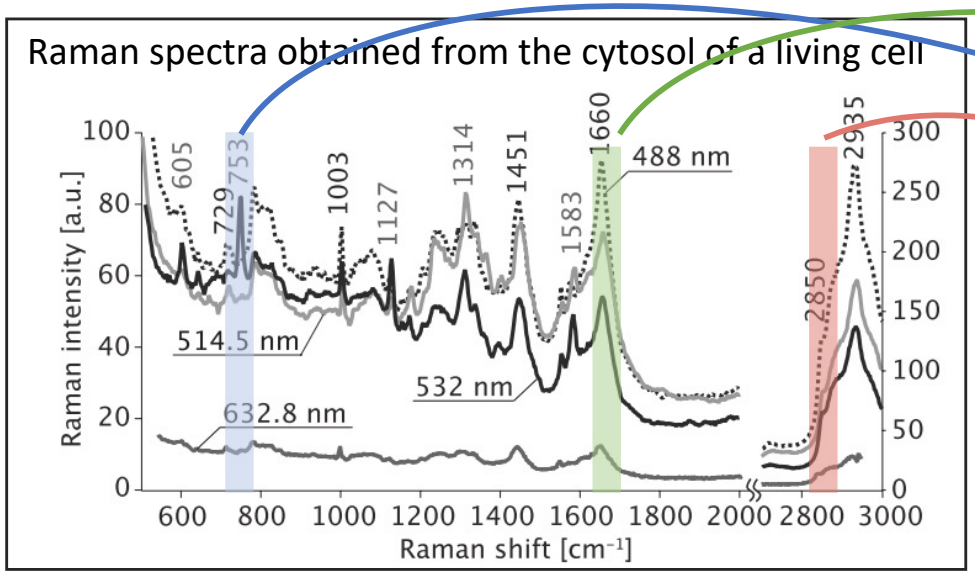
<Lipid>

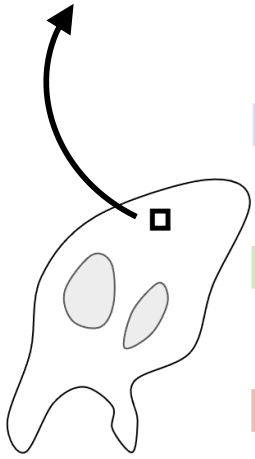




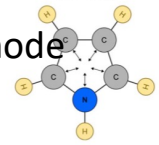


Raman imaging of living cells

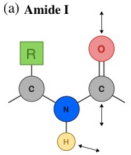




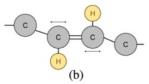
753 cm^{-1} : The pyrrole breathing mode
= cytochrome c

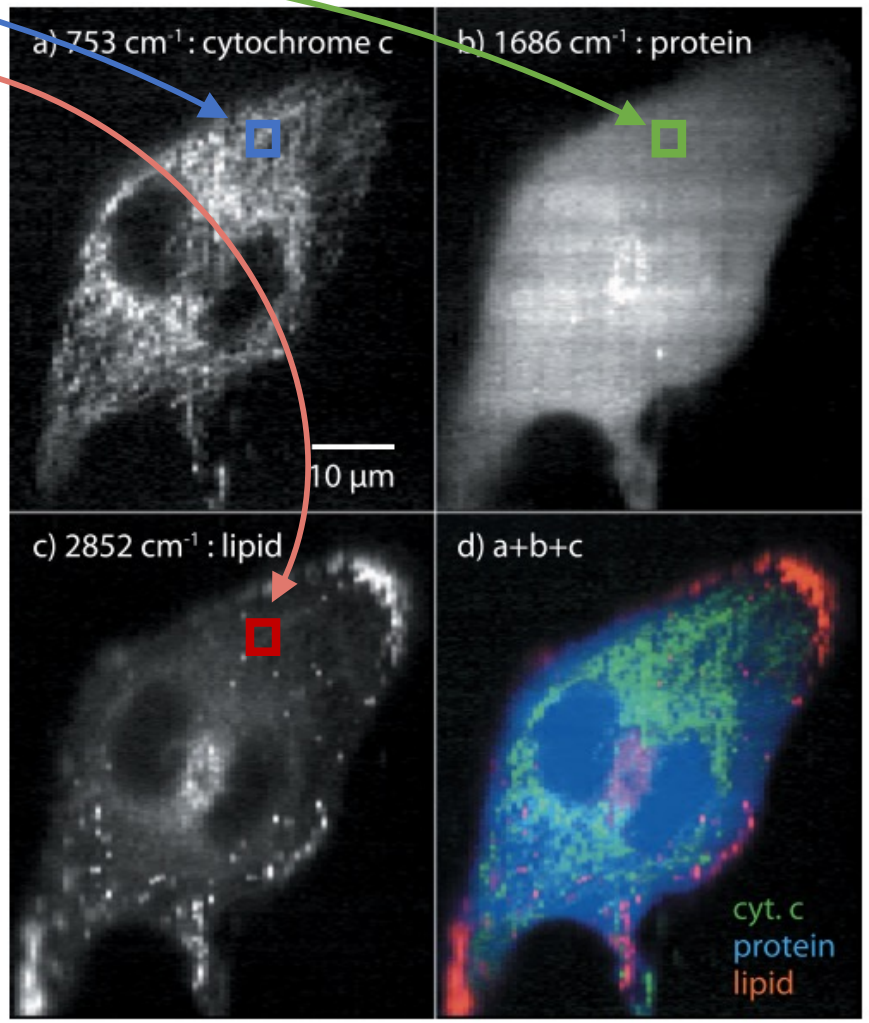


1686 cm^{-1} : Amide-I vibration
= protein β sheet



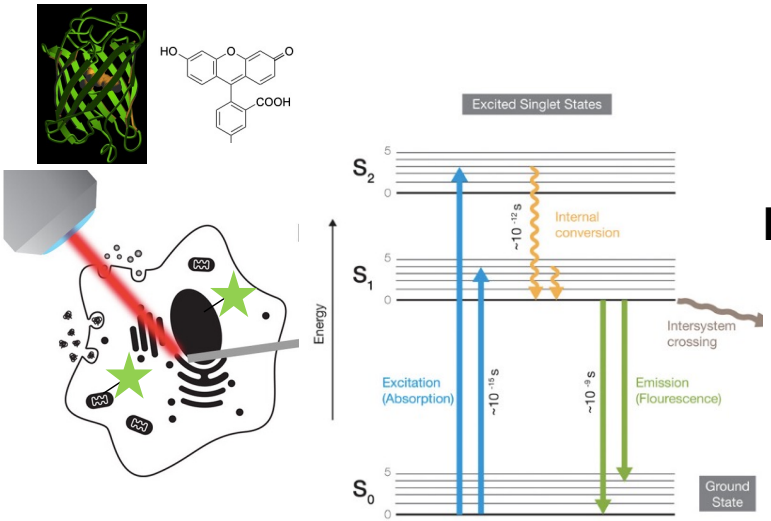
2852 cm^{-1} : C-C stretching vibration modes
= lipid molecules





Fluorescence microscopy vs Raman microscopy

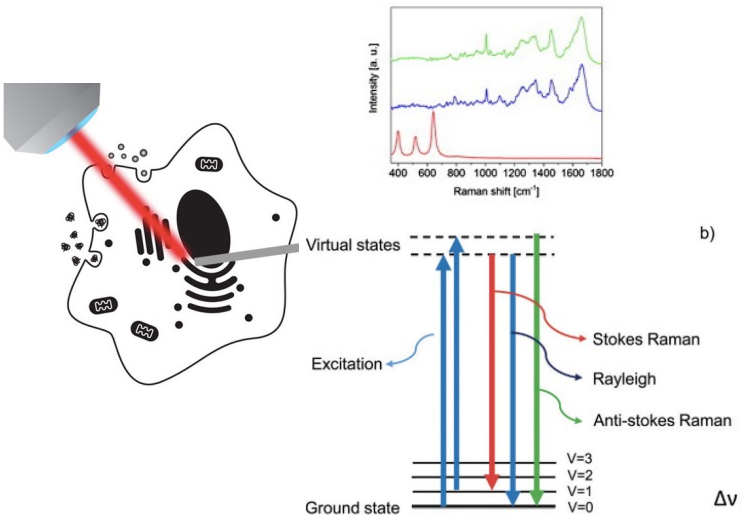
<Fluorescence microscopy >



- ✓ Images with very high spatial resolution.
- ✓ The high temporal resolution.

- ☐ Labeling of the target biomolecules with <u>fluorescent small molecules / genetically encoded fluorescent proteins.</u>
  - Large enough to affect the biochemical/biophysical properties of the target biomolecules.
  - They affect the native cellular environment,

<Raman microscopy>



- ☐ Raman imaging has not yet achieved the spatiotemporal resolution of fluorescence imaging
  = Raman signals are too weak

- ✓ Raman imaging can be performed without labelling or with minimal labelling.
- ✓ The obtained information has molecular level detail.

Kann, B.;… Otto, C. *Adv. Drug Deliv. Rev.* **2015**, *89*, 71.,
Dunst, S.; Tomancak, P. *Genetics* **2019**, *211*, 15.

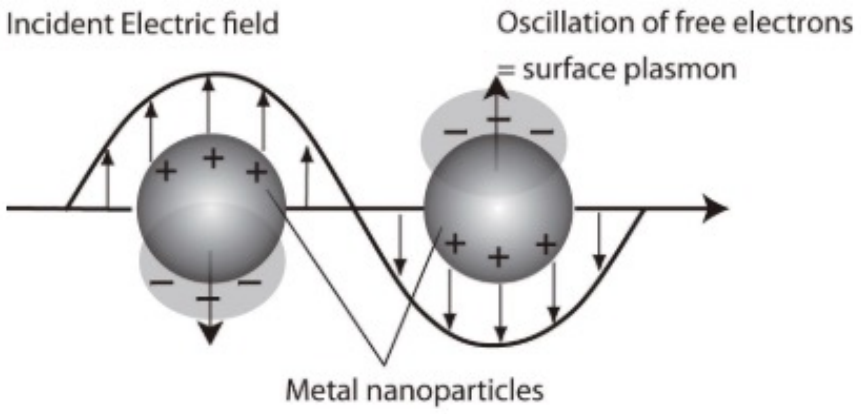
https://www.chem-station.com/molecule/2009/01/green_fluorescent_protein_gfp.html

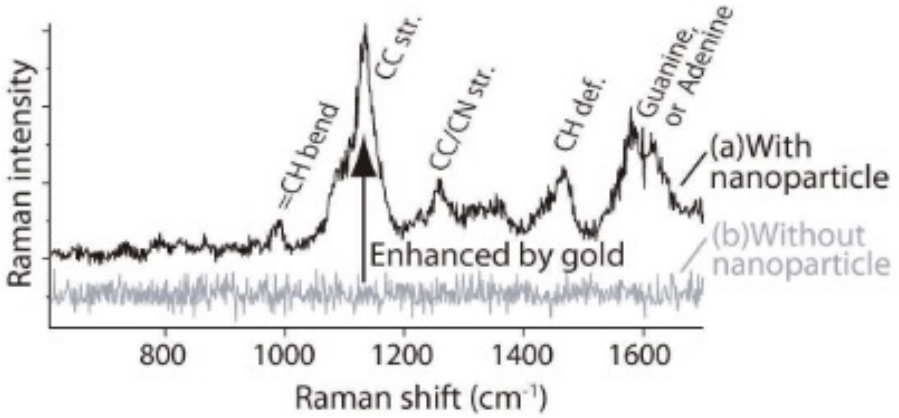
Surface-enhanced Raman spectroscopy (SERS)

- Since typical Raman scattering signals are compared to fluorescence yields.
  ; The measurement of Raman spectra usually requires long exposure times, making observations of living specimen difficult.

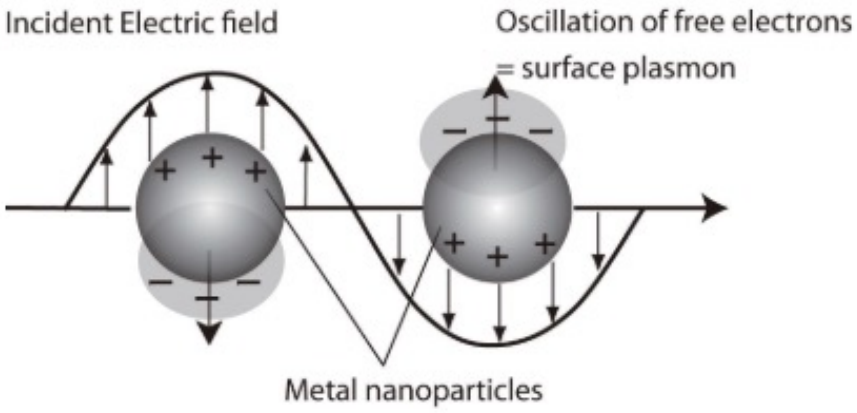
Surface-enhanced Raman spectroscopy (SERS)

The Raman signal of the target molecule is enhanced when it is close to the surface of metals such as silver or gold.





Surface-enhanced Raman spectroscopy (SERS)



$$P = \frac{3}{4\pi}\left[\frac{\varepsilon(\omega) - \varepsilon_m}{\varepsilon(\omega) + 2\varepsilon_m}\right] E_0$$

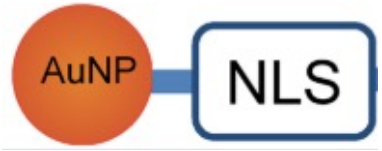
(P : polarization, $\varepsilon(\omega)$: dielectric function
ε_m : dielectric constant, E_0 : external electric field)

$\mathrm{Re}[\varepsilon(\omega)] = -2\varepsilon_m$
: Localized Surface Plasmon Resonance

- ✓ Localized surface plasmon polaritons are generated due to oscillation of free electrons in the metal.
- ✓ SERS can enhance the Raman signal on average by 10^4 to 10^8 times, and up to 10^{14}-10^{15} times.
- ✓ Optical detection and spectroscopy of single molecules have been achieved.

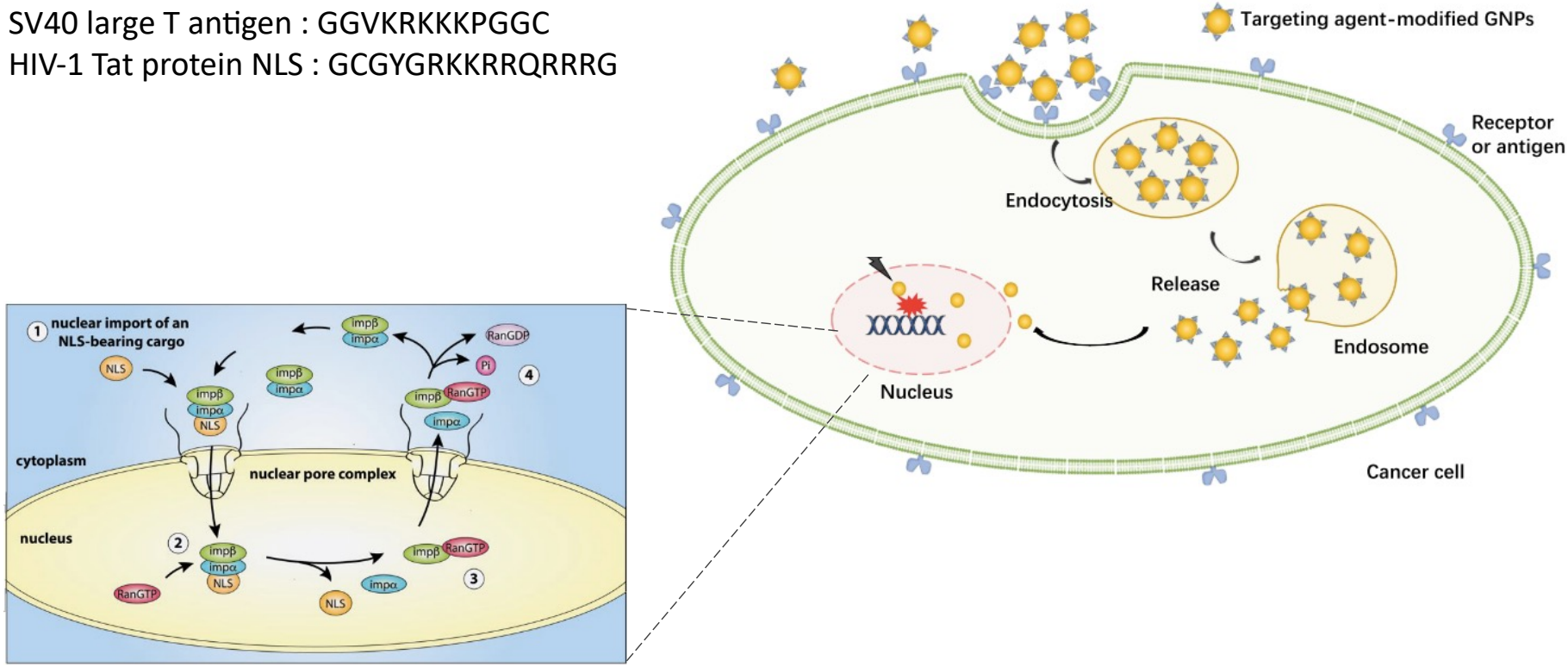
Contents

SERS for nuclear analysis



Nuclear localization signal peptides (NLS)

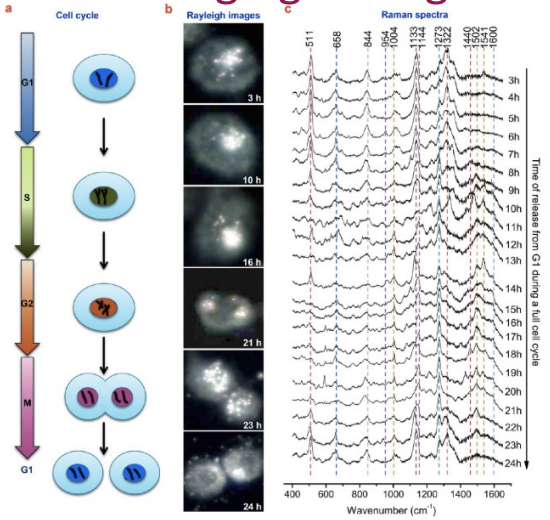
SV40 large T antigen : GGVKRKKKPGGC
HIV-1 Tat protein NLS : GCGYGRKKRRQRRRG



✓ Conjugation with NLS allows the targeting of NPs to the cell nucleus.

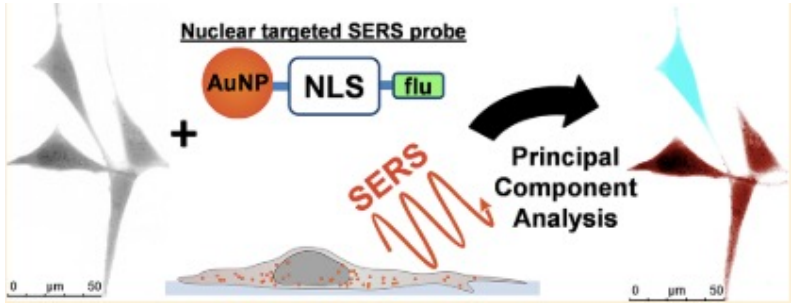
SERS for label free Nuclear analysis

Cell nucleus imaging during the cell cycle



Identifying cell types

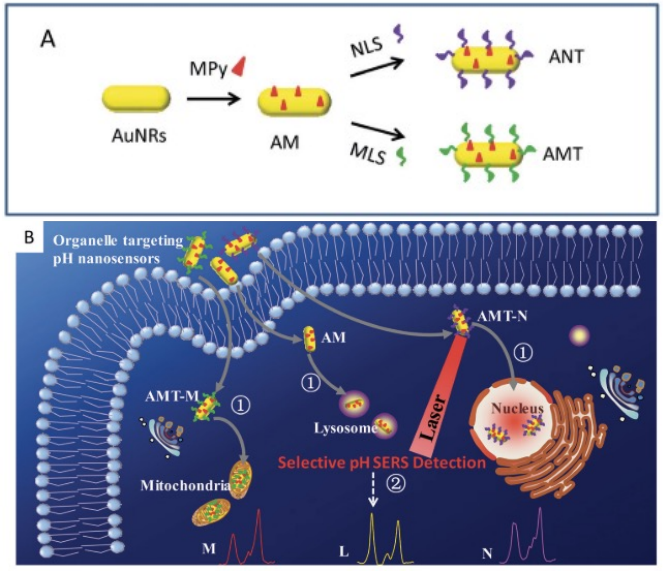
Undifferentiated cell Differentiated cell



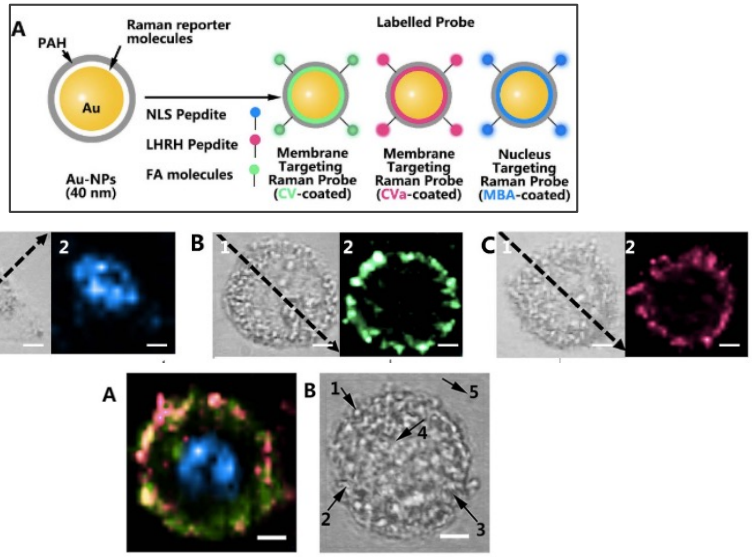
RNA peaks
; looser packaging

DNA/histone peaks
; DNA packaged with chromosomal proteins in heterochromatin

Intracellular monitoring of pH



Multi-targeting SERS imaging



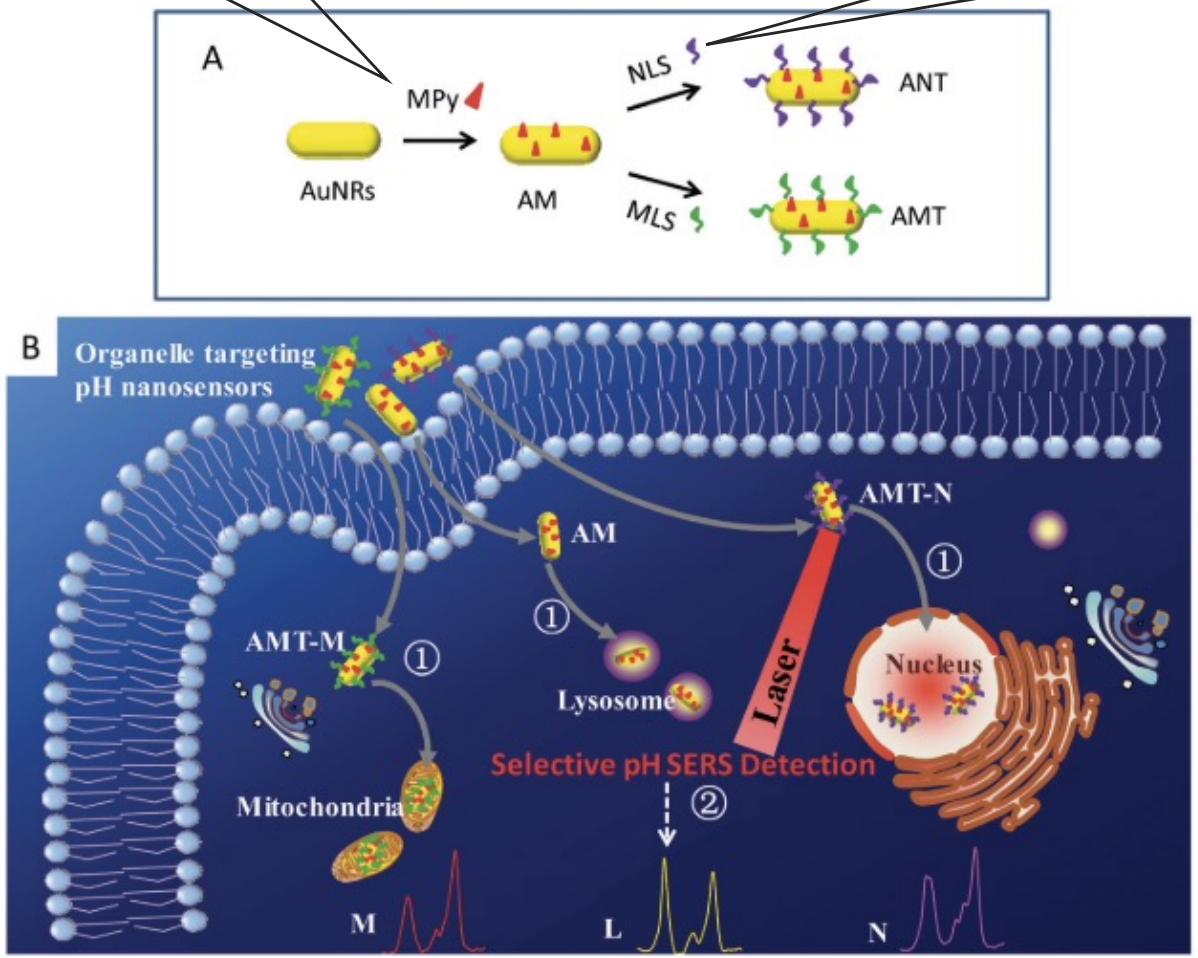
Kang, B.; …El-Sayed, M. A. *Nano Lett.* **2012**, *12*, 5369.
Shen, Y.;…; Xu, W., *Nanoscale* **2018**, *10*, 1622–1630.
Huefner, A.;… Mahajan, S. *Nano Lett.* **2013**, *13*, 2463.
Chen, Y.; …; Hu, J.. *Sci. Rep.* **2016**, *6*, 1

AuNR-MPy-targeting (AMT) pH nano-sensors

<AuNR-MPy-targeting (AMT) pH nano-sensors>

pH-sensitive molecule

The organelle-targeting peptides
(nucleus targeting peptide (NLS))

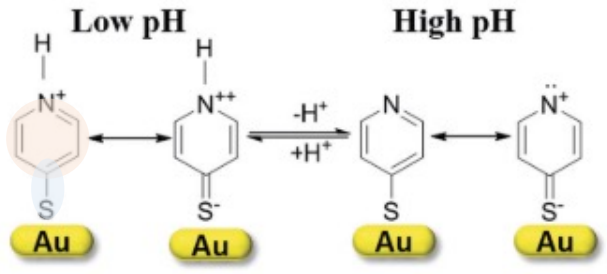


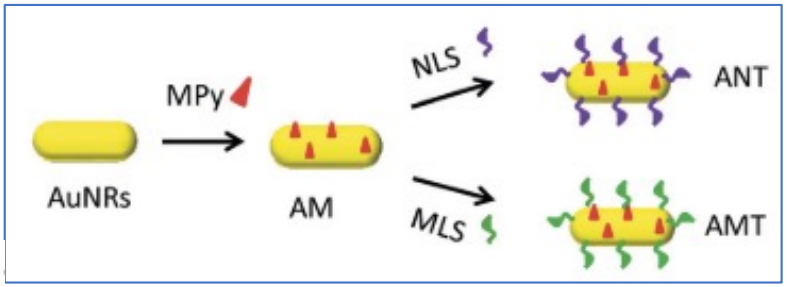
AuNR-MPy-targeting (AMT) pH nano-sensors

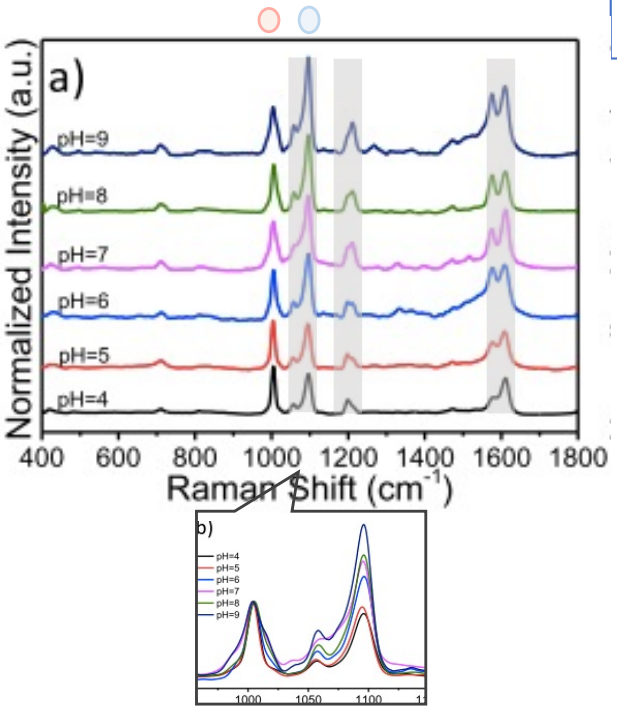
<AuNR-MPy-targeting (AMT) pH nano-sensors>

pH-sensitive molecule

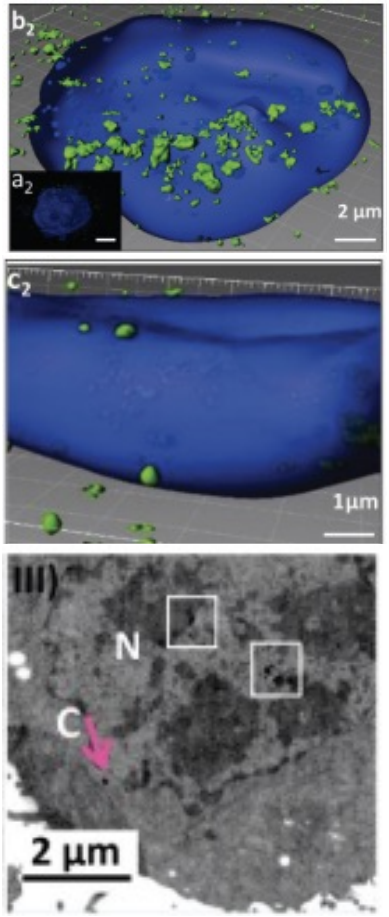
The organelle-targeting peptides
(nucleus targeting peptide (NLS))



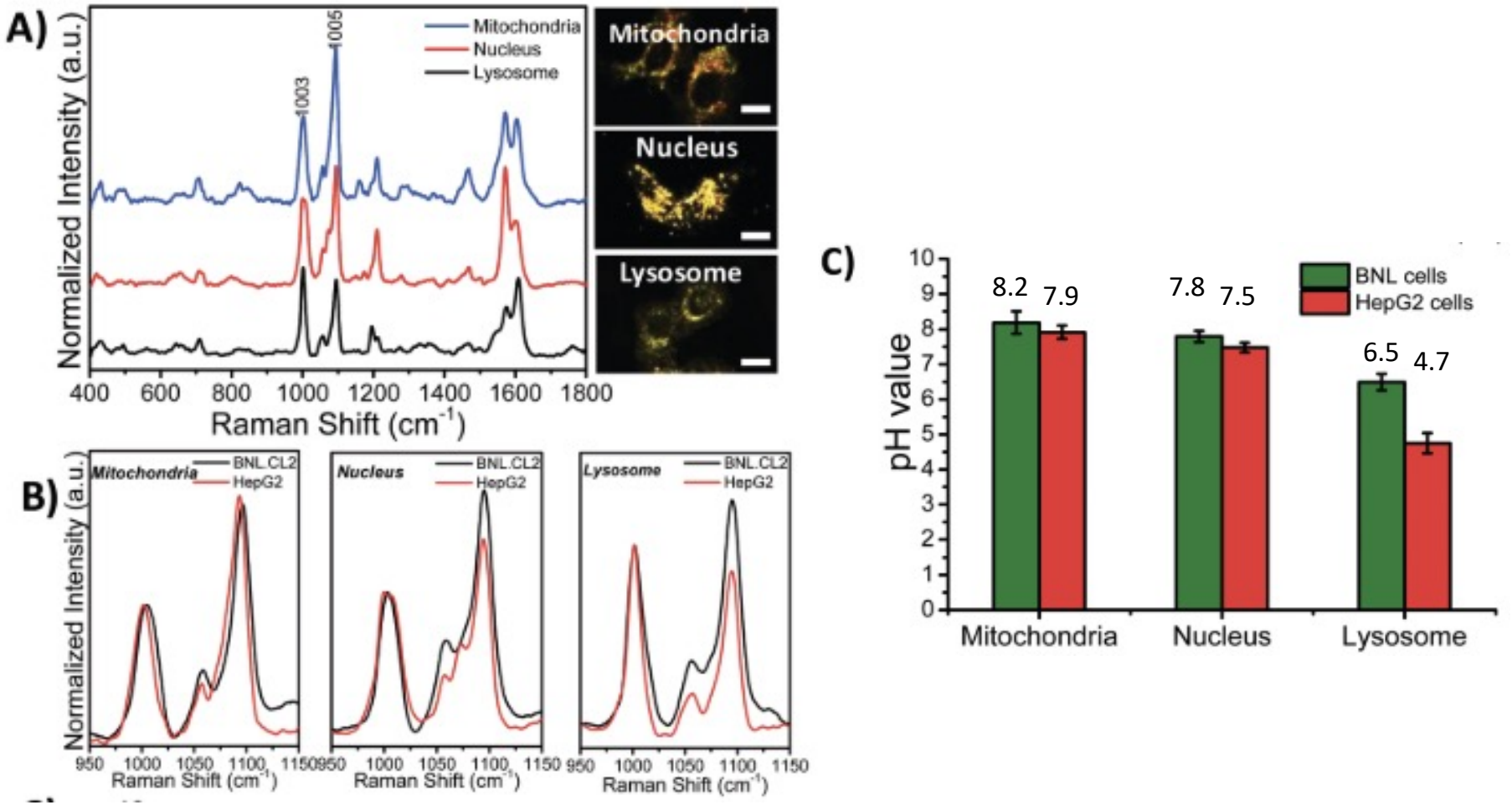




- ✓ AM-pH nano-sensor has a good response for pH
- ✓ Most of ANT can locate in the nucleus or its adjacent area.



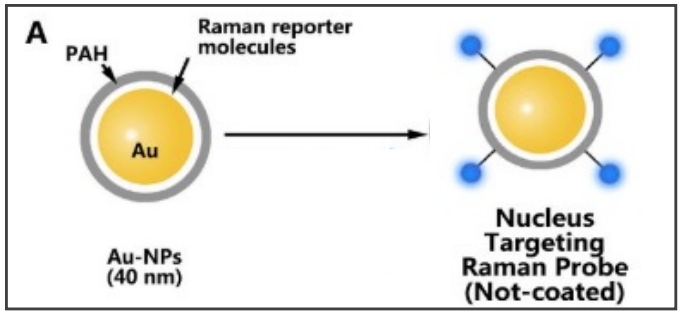
Intracellular monitoring of pH fluctuations in organelles of cancer and normal cells

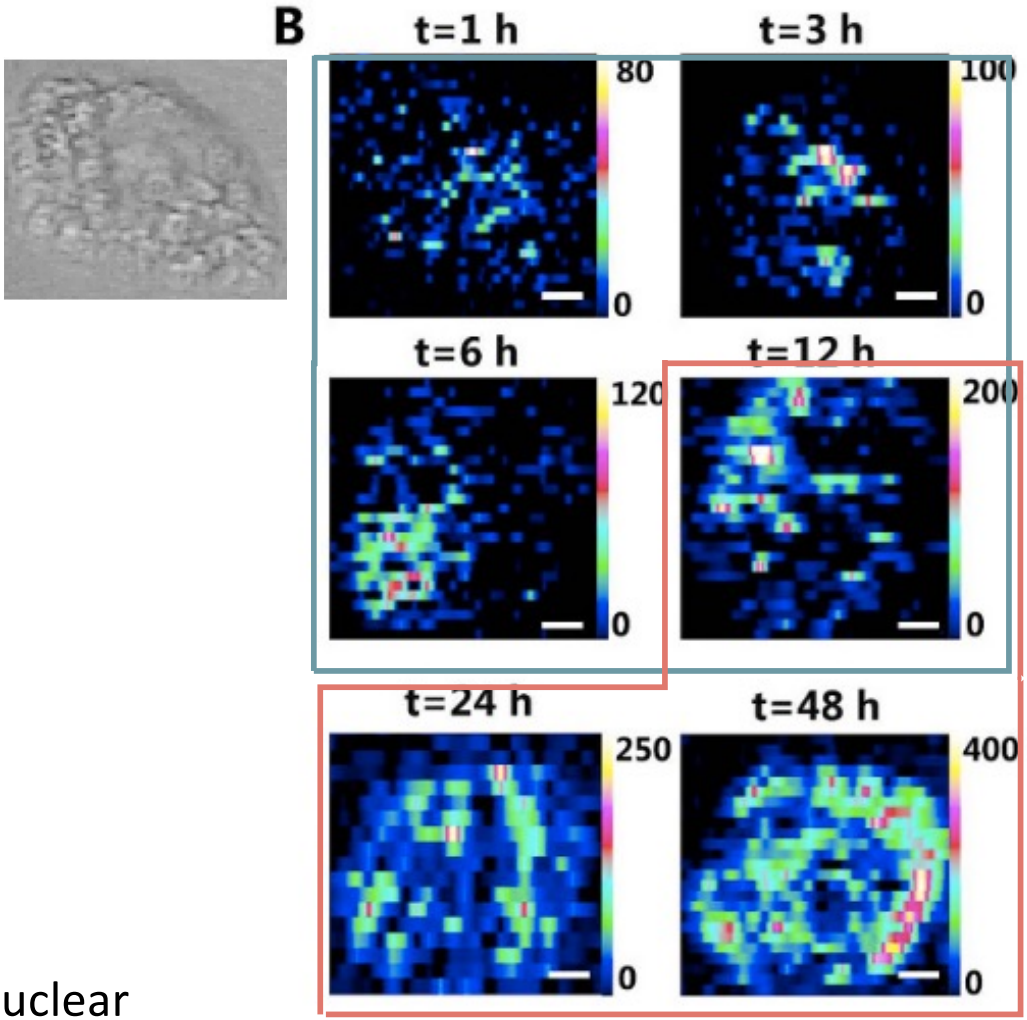


- ✓ The lysosome is acidic, and nucleus and mitochondria are neutral or slightly alkaline.
- ✓ The pH of the subcellular organelles in the cancer cells is more acidic as compared to that in the normal cells.

Contents

SERS imaging of the time-dependent changes of cell nuclei during apoptosis





Early apoptosis stage

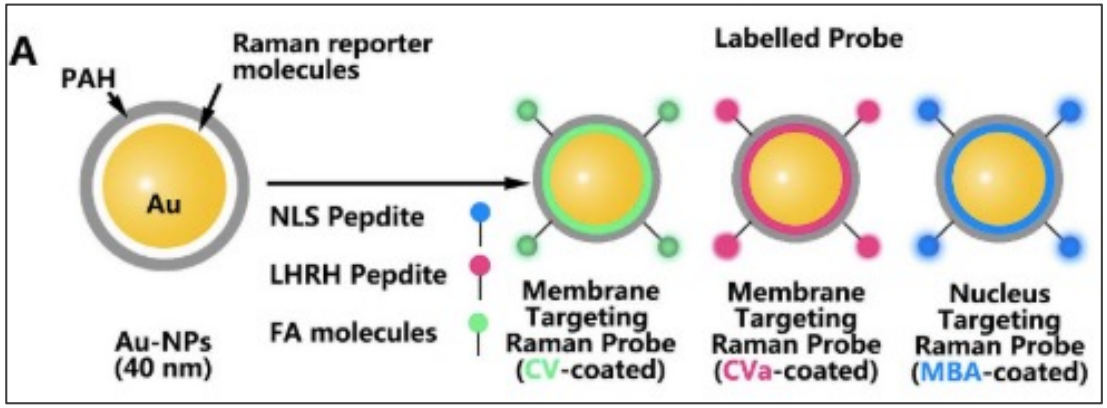
: diminishing nuclei and slight condensation of chromatin

Late apoptosis stage :

: chromatin decomposition and nuclear membrane rupture

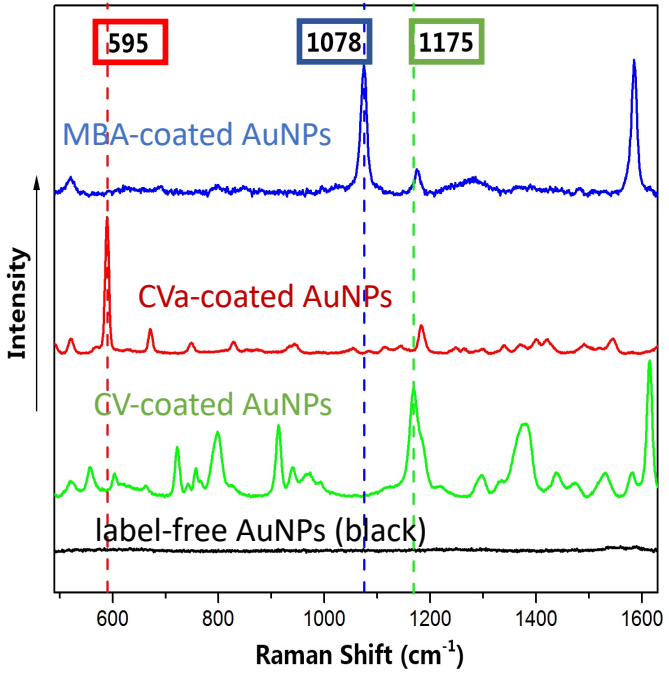
The high-resolution SERS imaging of HeLa was constructed from the total SERS intensity between 490 and 1630 cm^{-1}.

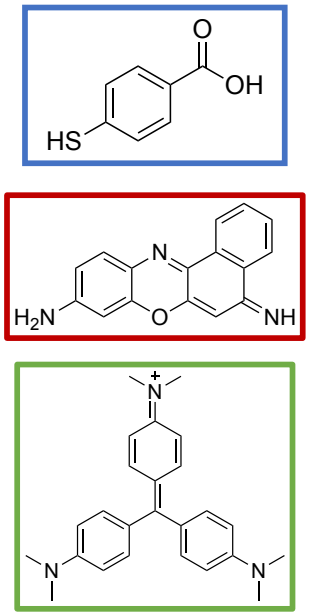
Multi-targeting SERS imaging of cell



Three different Raman-active molecules was inserted into the gap of the Au@Au core-shell structure.

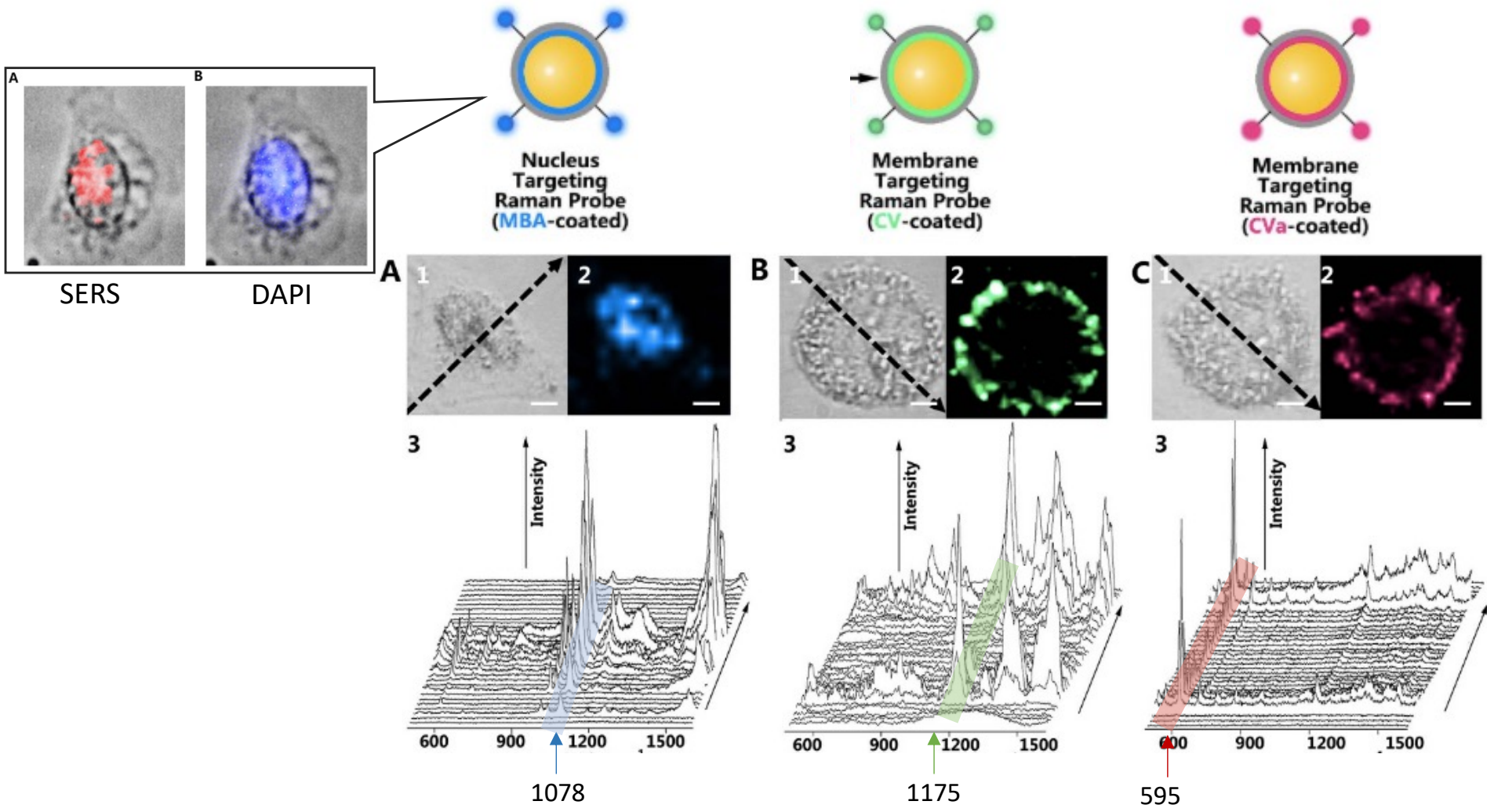
<Raman-dyes as SERS reporters>





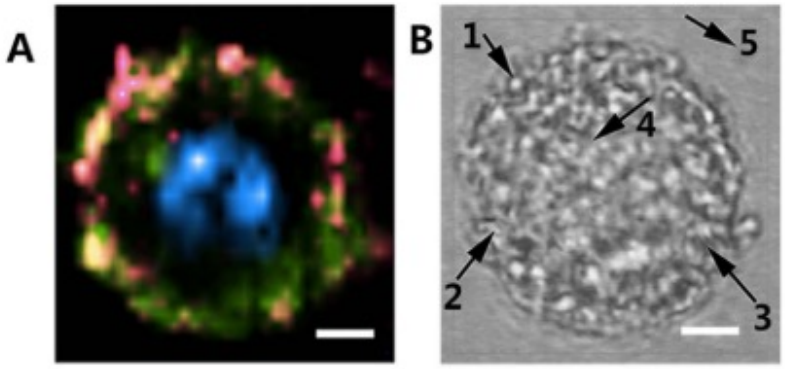
Chen, Y.; …Hu, J.. *Sci. Rep.* **2016**, *6*, 1. Liu, X.; …Wang, Z.. *Nanoscale* **2017**, *9*, 15390.
Caires, A. J.;… Ladeira, L. O. *J. Colloid Interface Sci.* **2015**, *455*, 78.

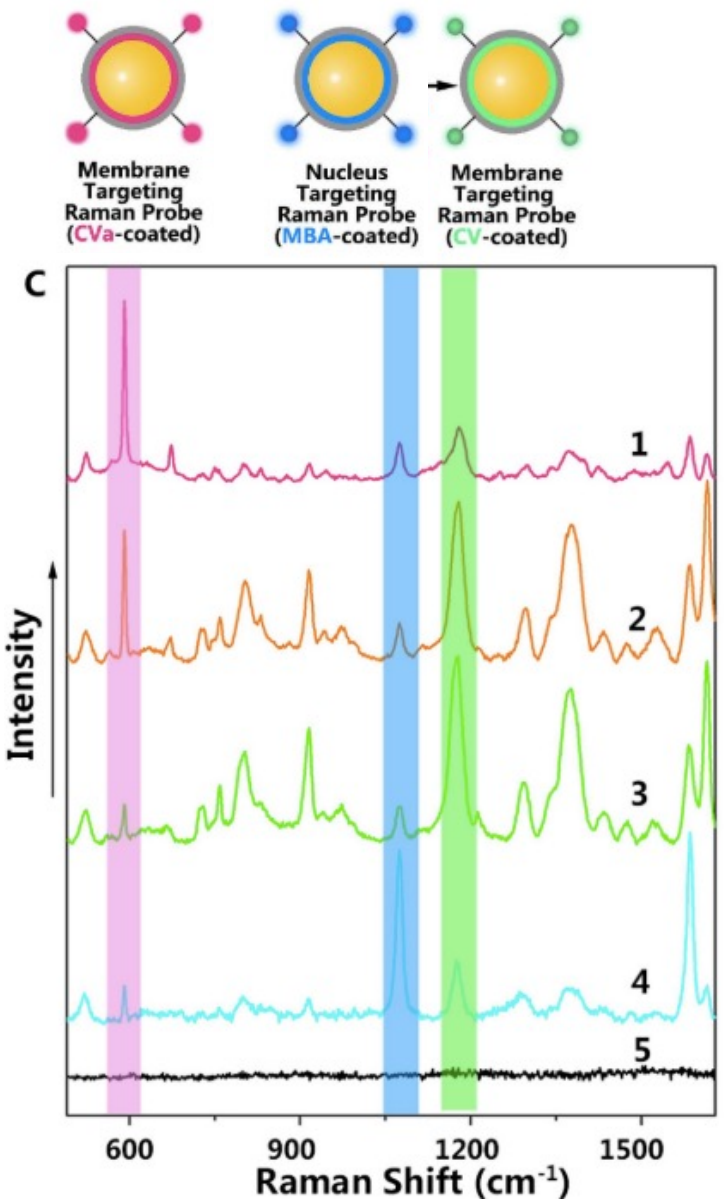
SERS imaging of a cell treated with Raman-dye coated AuNP



✓ The membrane- or nuclear-targeting AuNPs were found inside the nucleus and surrounding the cells, respectively.

Multi-targeting SERS imaging using coated AuNPs in the same single cell

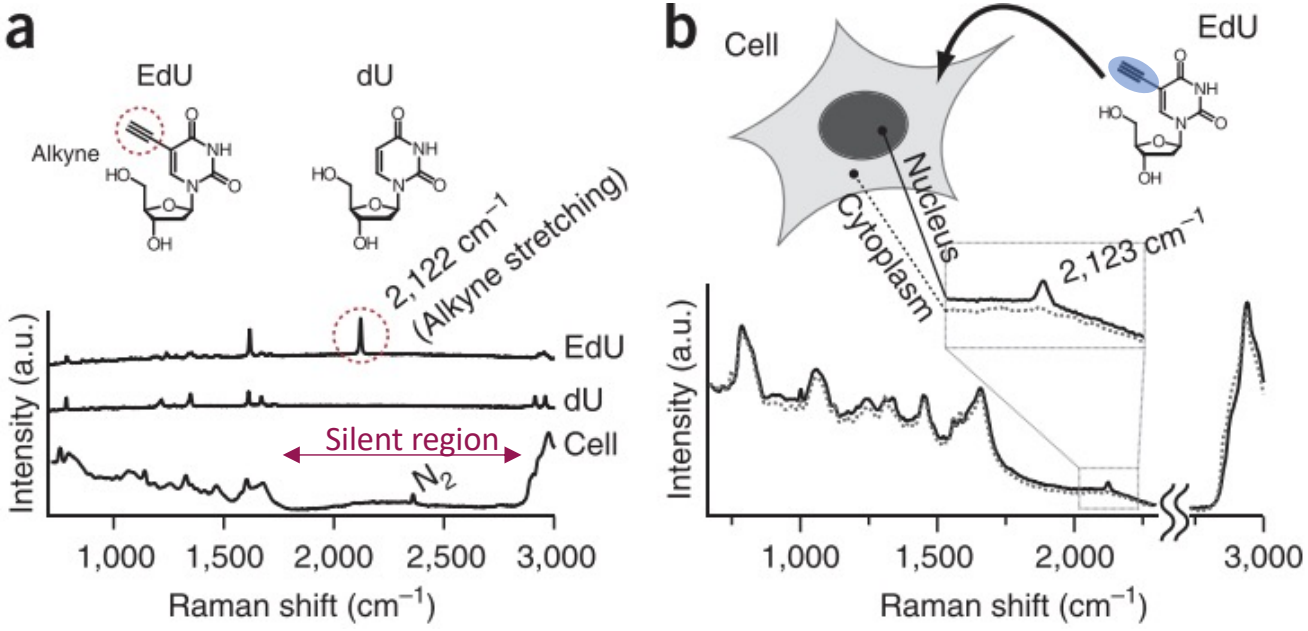


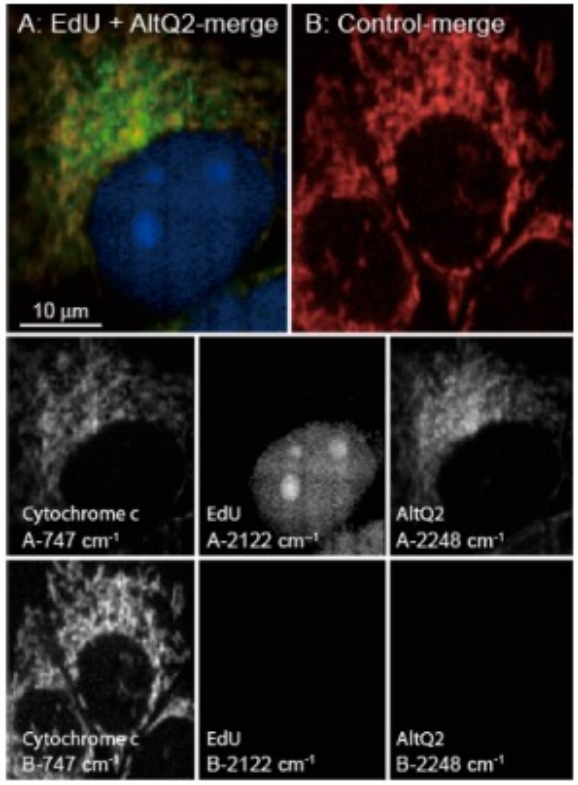


- ✓ The positions of the nucleus and membrane can be clearly distinguished.
- ✓ The multi-targeting SERS imaging of various dye-AuNPs in the same cell.

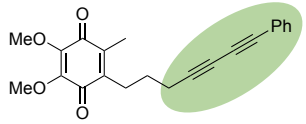
Contents

Alkyne-tag Raman imaging (ATRI) for nuclear imaging



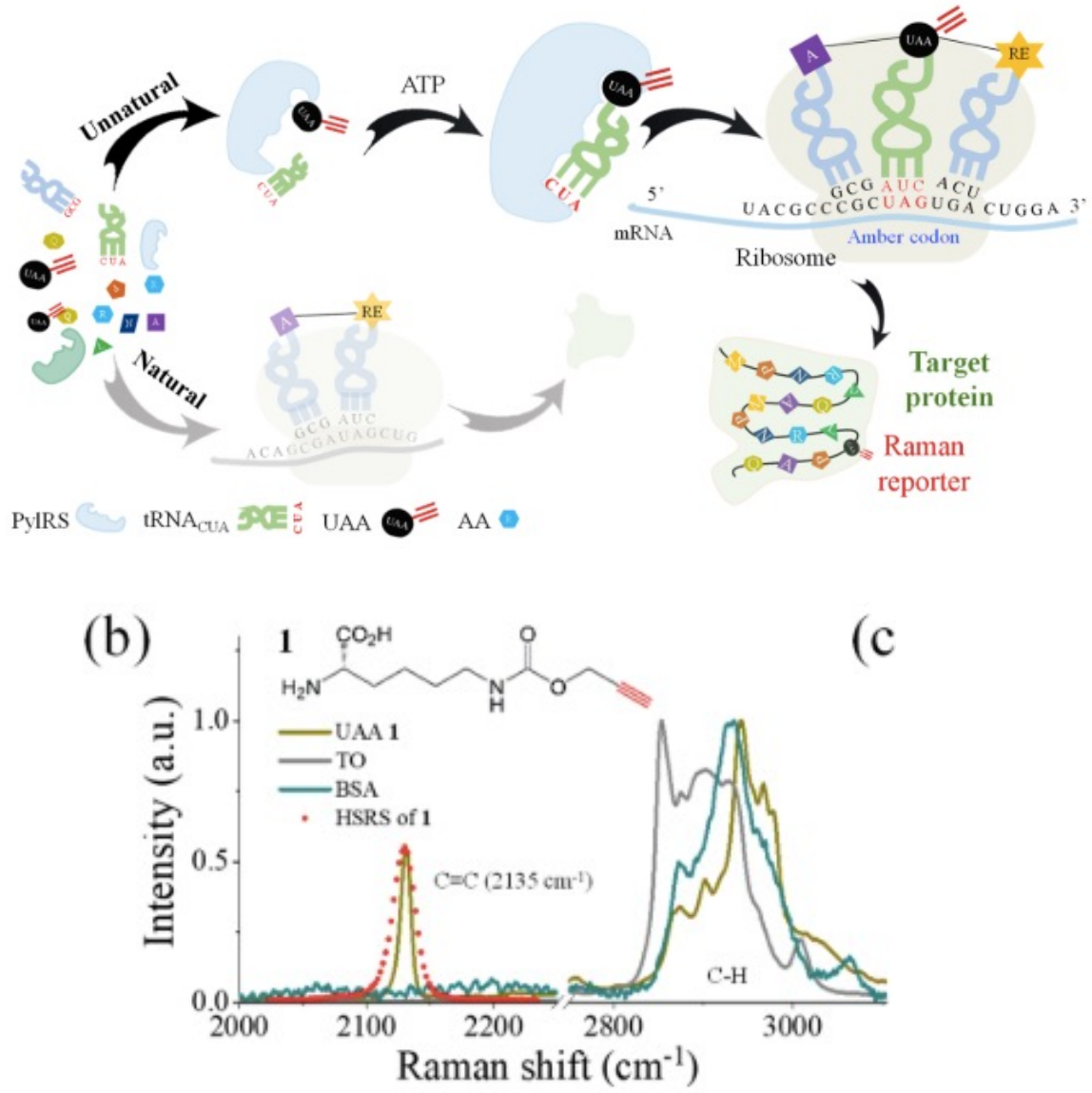


- ✓ Alkynes have strong Raman signals in the cellular silent region and can be excellent tags.
- ✓ Multi-color imaging with ATRI was achieved due to the narrow line width of alkyne peak.



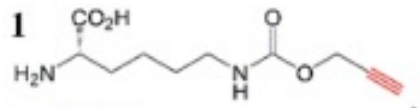
AltQ2

Histone imaging with Raman tag incorporated via genetic code expansion

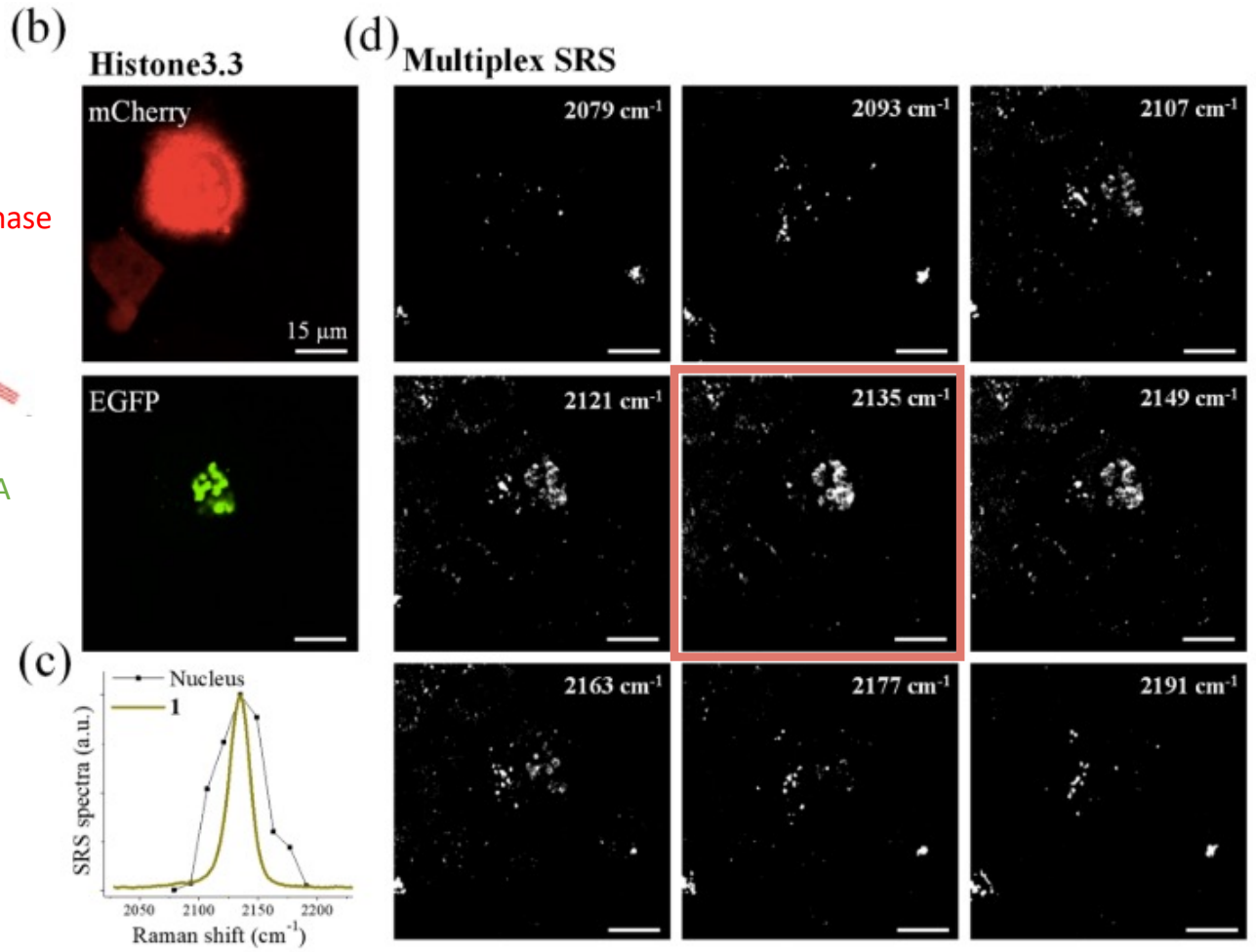


Incorporation of a Raman tag via genetic code expansion

○ Expression of tRNA synthase



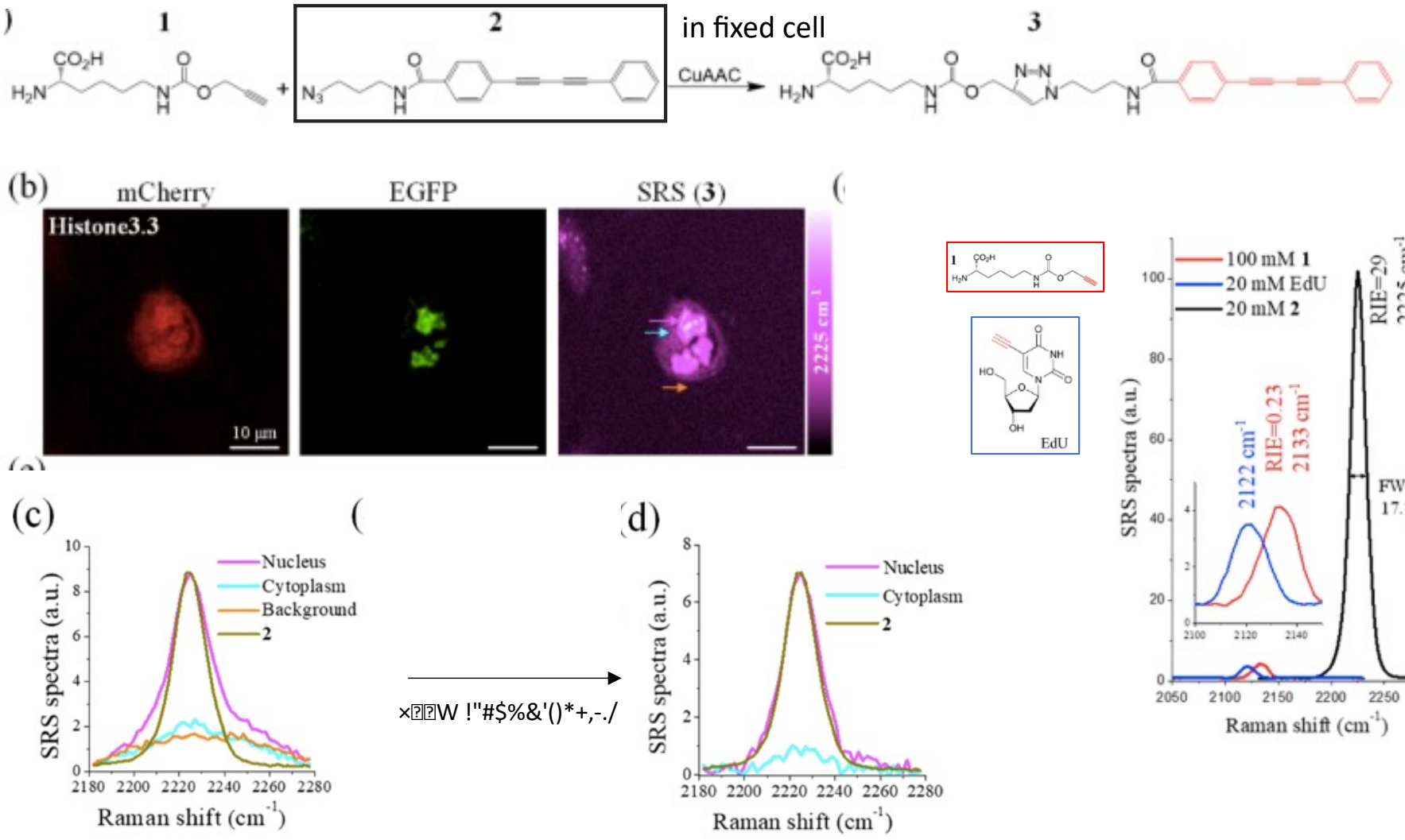
○ The incorporation of UAA



✓ A clear image of the Histone3.3 protein in the nucleus was observed @2135 cm^{-1}.

✓ The vibrational signal of alkyne was very weak.

Incorporation of a Raman tag via genetic code expansion

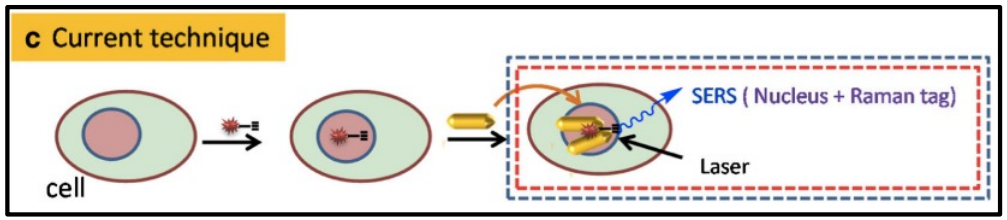


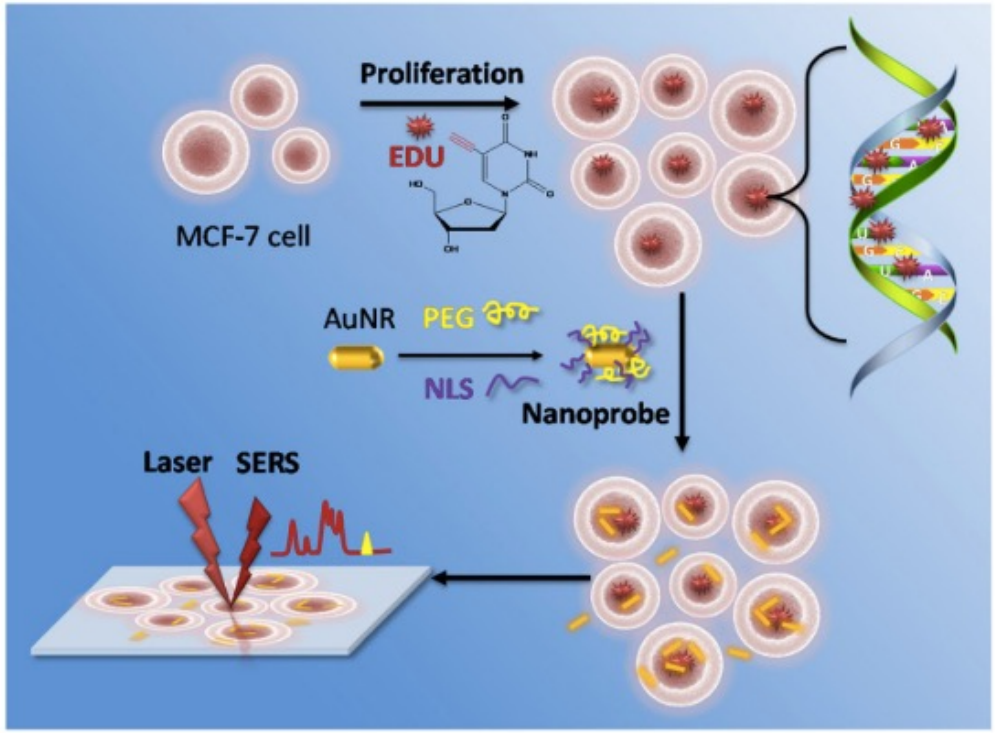
✓ In the nucleus a strong Raman signal was observed @2225 cm^{-1}.

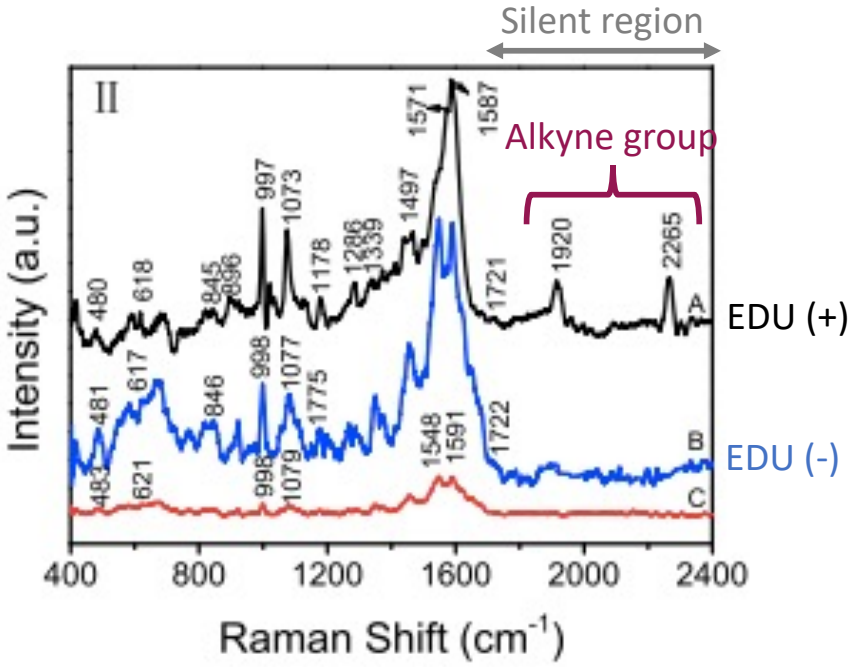
Contents

EdU as a nucleus localization interior label for SERS detection

EdU (ATRI-tag) was adopted as an internal label to locate the nuclear region accurately.

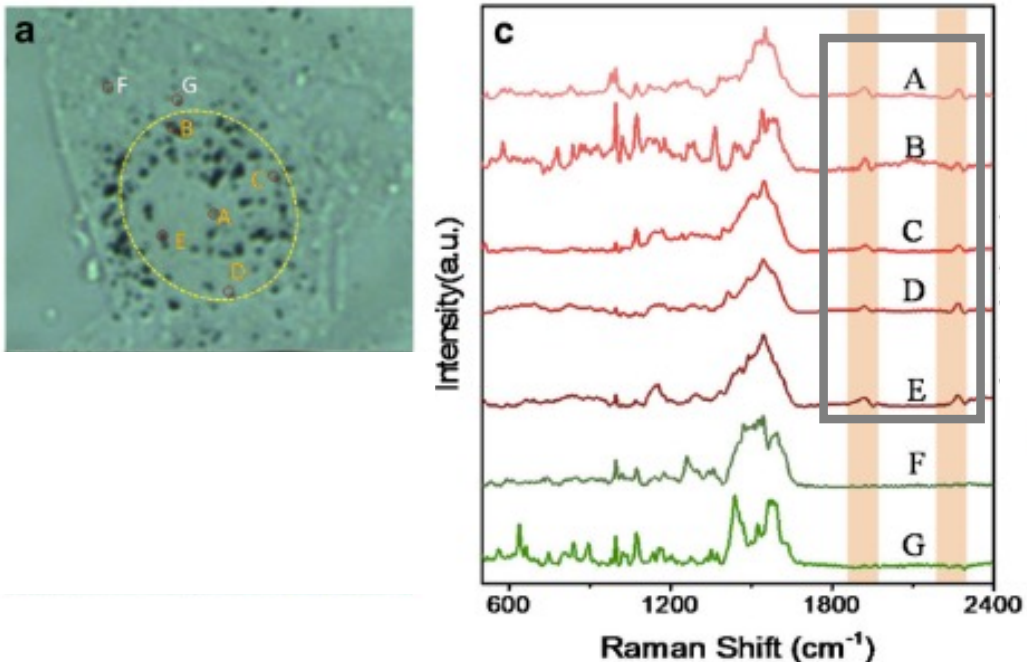






two accumulations. (II) Spontaneous Raman (C) and SERS spectra (A and B) of nucleus of MCF-7 cells without and with AuNRs-PEG-NLS (0.05 nM). SERS spectra (A) of nucleus of MCF-7 cells with 200 μM EDU, with the laser power of 4.3 mW, a collection time of 40 s, and two accumulations

EdU as a nucleus localization interior label for SERS detection



SERS spectra of presenting SERS signals at 1920 and 2265 cm^{-1} are from the nuclear region.

Raman shift (cm^{-1})	DNA	Assignments of protein/lipid
506		S-S
577/1360/1553		Trp
663		ν(C-S)
741		C-S
780/837	O-P-O	
840/1177		Tyr
904/944/1145/1448	Dr-P	
980	Dr	
997		Phe
1021/1039	Dr ν(C-O)	
1072	Dr ν(C-O)	C-N ν(C-O),ν(C-O-C)
1238	U,C	
1261/1286	T	AmideIII
1365/1438		$\gamma(CH_2/CH_3)$
1386		$\gamma(CH_3)$
1485/1492/1570	G,A	
1505/1519	A	
1534/1540	A,C,G	
1580	G,A	
1585		$-N_7-H_7$
1621	U	

✓ EdU as a nucleus localization interior label can allow us to realize accurate location of nuclei.

→ Obtaining precise SERS spectra of nucleus to investigation the biomolecules from the cell nucleus by SERS.

Contents

Summary

- ✓ Raman imaging can be performed without labelling or with minimal labelling and obtain information in molecular detail level.
- ✓ SERS enhance the Raman signal of the target molecule when it is close to the surface of metals such as silver or gold.
- ✓ SERS can be applied for nuclear analysis and the dynamics tracking of physiological processes.
- ✓ ATRI realized the multi-color imaging of biological molecules using alkyne functional group.
- ✓ Combination of ATRI and SERS realized the detection of precise nuclear signal.

Appendix

SRES

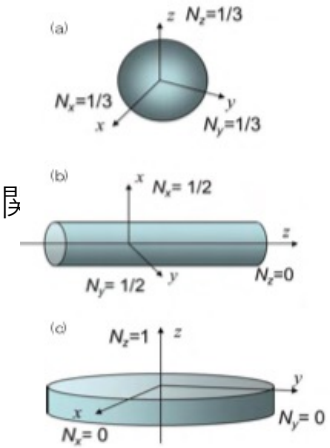
×□□Wについては、金属微小球の 直径が光の波長よりも十分小さい場合に、静電気学的に取り扱う（静電近似）ことができる

誘電率 ε_mの媒質中に誘電関数 $\varepsilon(\omega)$をもった球形の金属微粒子が存在し（ω は光電場の振動の角周波数）、そこに外部電場$\boldsymbol{E_0}$が加えられている場合、

金属微粒子内部の電 子が力を受けて変位し、分極 $\boldsymbol{P}$が誘起される。
同時に、微粒子表面には外部電 場と逆方向の電場を発生させるような表面電荷が誘起される。

反分極場($\boldsymbol{E_d}$)　$E_d = -4\pi L\boldsymbol{P}$　（媒質が真空のとき）

L : 反分極係数, 微粒子の形状に依存、球形粒子の場合 L=1/3）



誘電体中では、分極電荷がつくる電場も考慮する必要があり、ガウスの法則から、

$$\boldsymbol{D} = \varepsilon(\omega)\boldsymbol{E} = \boldsymbol{E} + 4\pi\boldsymbol{P}$$

$\boldsymbol{E}$: 物質中の電場, $\boldsymbol{D}$: 電束密度, $\boldsymbol{P}$: 分極, $\varepsilon(\omega)$: 誘電関

したがって、　$\boldsymbol{P} = \frac{\varepsilon(\omega)-1}{4\pi}$　(1.1)

微粒子内部の電場$\boldsymbol{E}$は、外部電場$\boldsymbol{E_0}$と反分極電場$\boldsymbol{E_d}$から、

$$\boldsymbol{E} = \boldsymbol{E_0} + \boldsymbol{E_d} \qquad (1.2)$$

(1.1), (1.2)より、誘電率 ε_mの媒質中における分極は、$\boldsymbol{P} = \frac{1}{4\pi}\left[\frac{\frac{\varepsilon(\omega)}{\varepsilon_m}-1}{1+\left[\frac{\varepsilon(\omega)}{\varepsilon_m}-1\right]}\right]\boldsymbol{E_0}$　L=1/3として、

$$\boldsymbol{P} = \frac{3}{4\pi}\left[\frac{\varepsilon(\omega)-\varepsilon_m}{\varepsilon(\omega)+2\varepsilon_m}\right]\boldsymbol{E_0}$$

http://home.sato-gallery.com/research/JEMEA_databaseWG_metal_yudenritsu.pdf

https://ir.library.osaka-u.ac.jp/repo/ouka/all/34490/26976_論文.pdf