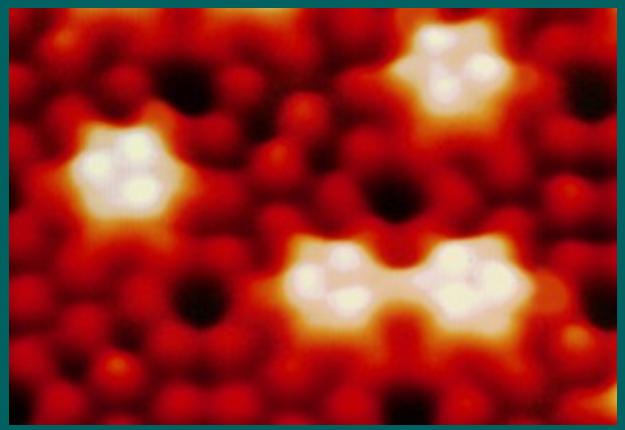
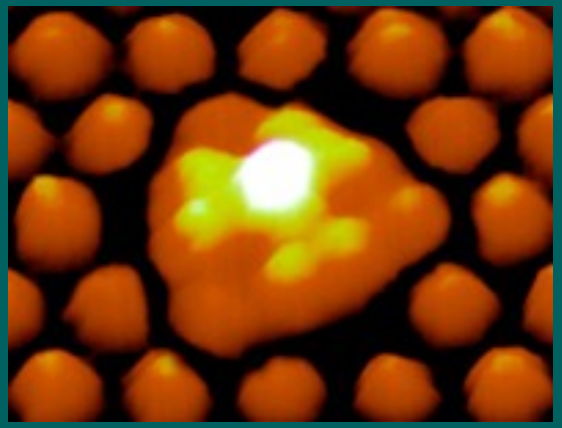
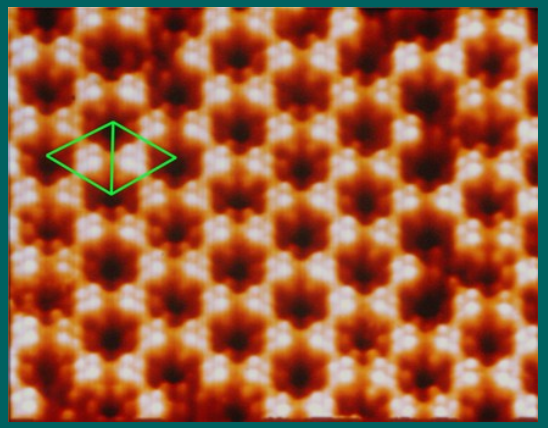
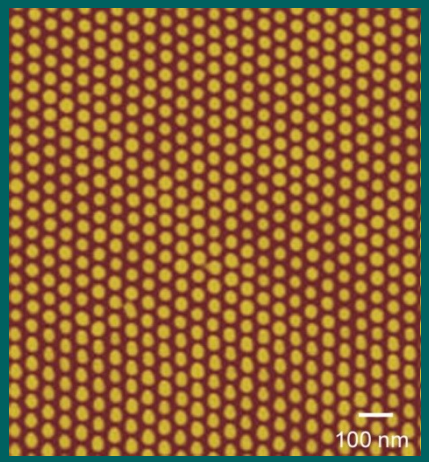
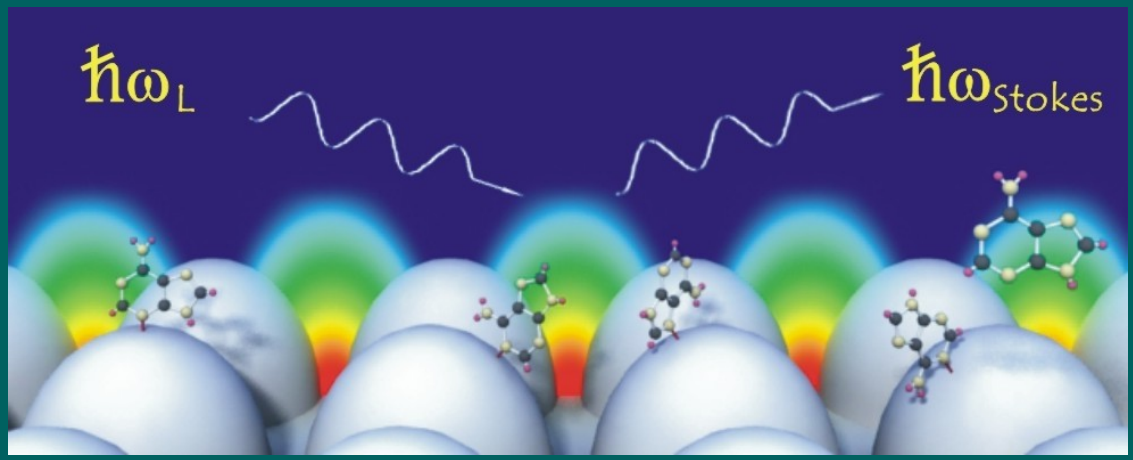
‘Listening’ to the ‘Sound’ of Bacteria Using Raman Scattering Enhanced by Arrays of Nanoparticles











王 玉 麟 (Yuh-Lin Wang)
中研院原分所暨台大物理系
於清大物理系(10/13/2010)

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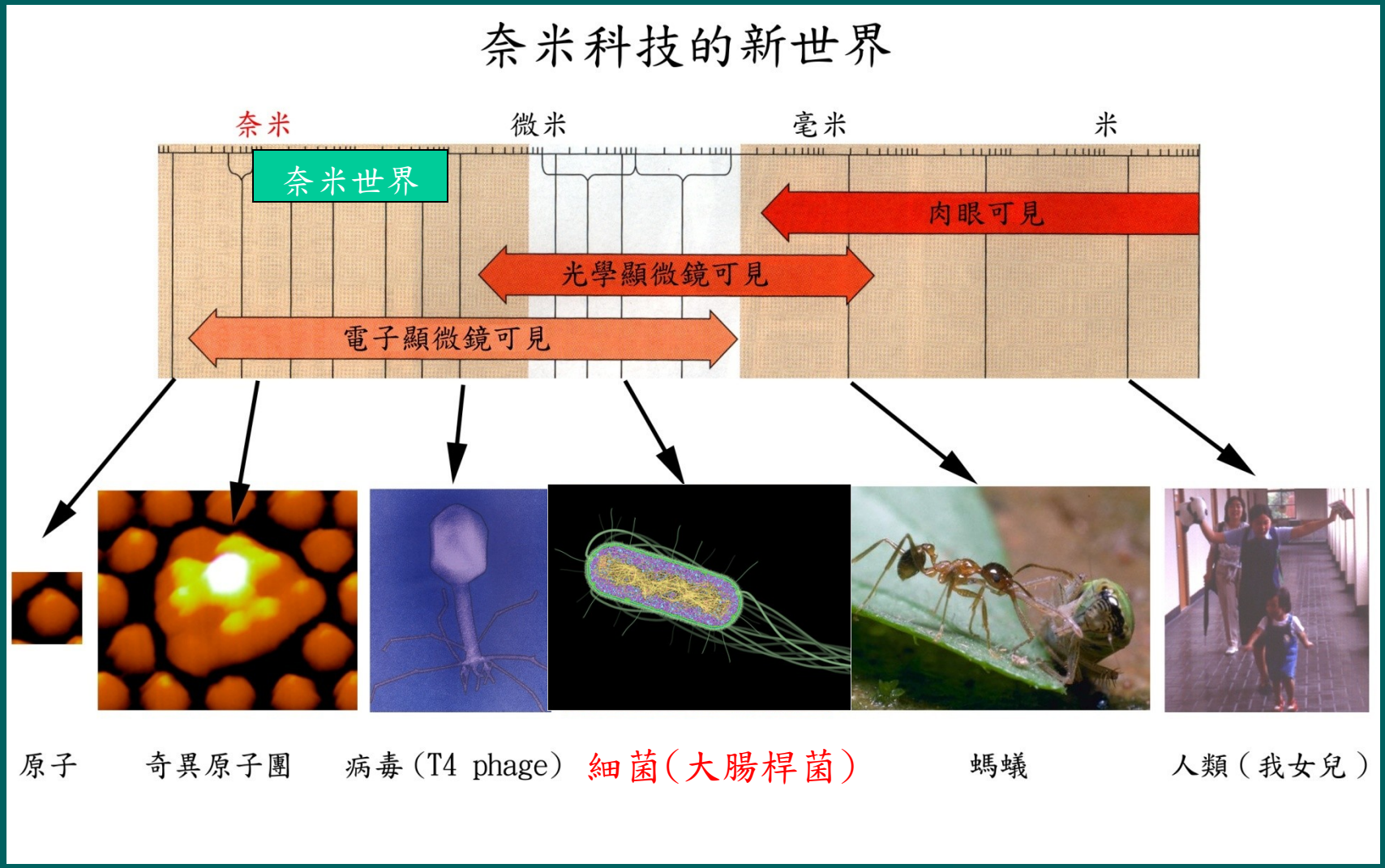
用「奈米電漿子晶體」幫你聽細菌的聲音



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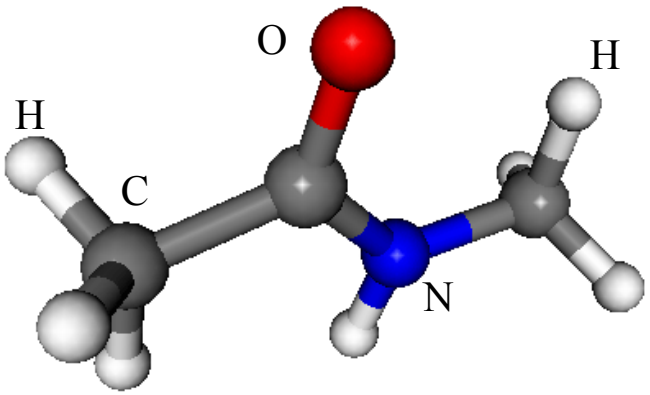
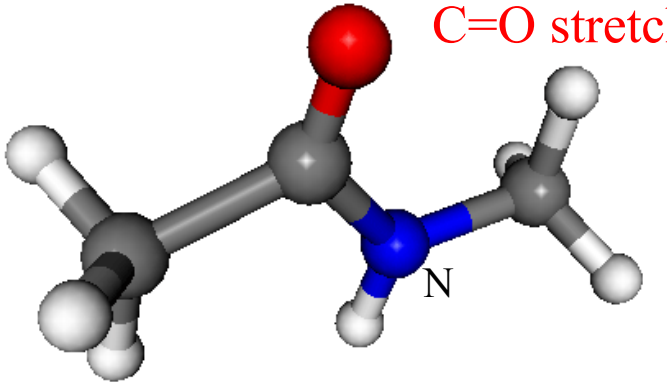
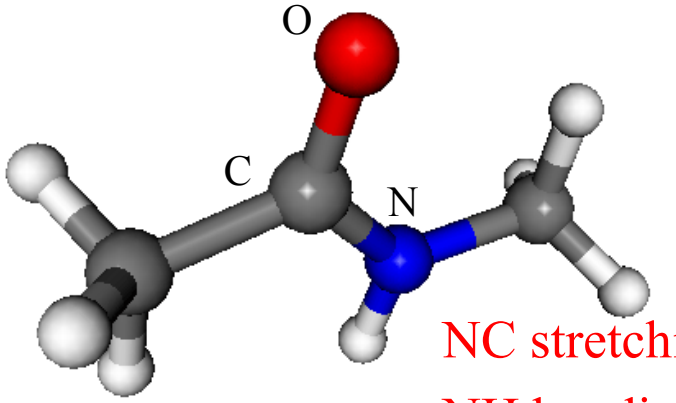
Raman Scattering & Molecular Vibration



CH_3 umbrella

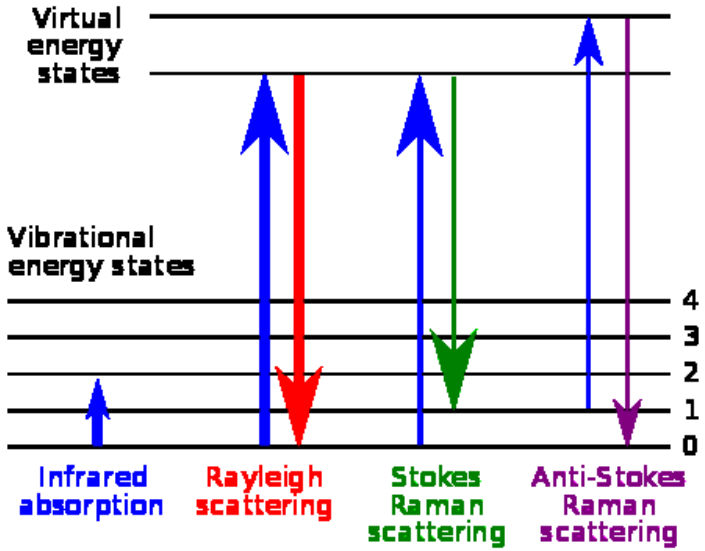


C=O stretching



NC stretching

NH bending



$$\Delta\nu=(1/\lambda_{in})-(1/\lambda_{out})\ (1/cm)$$

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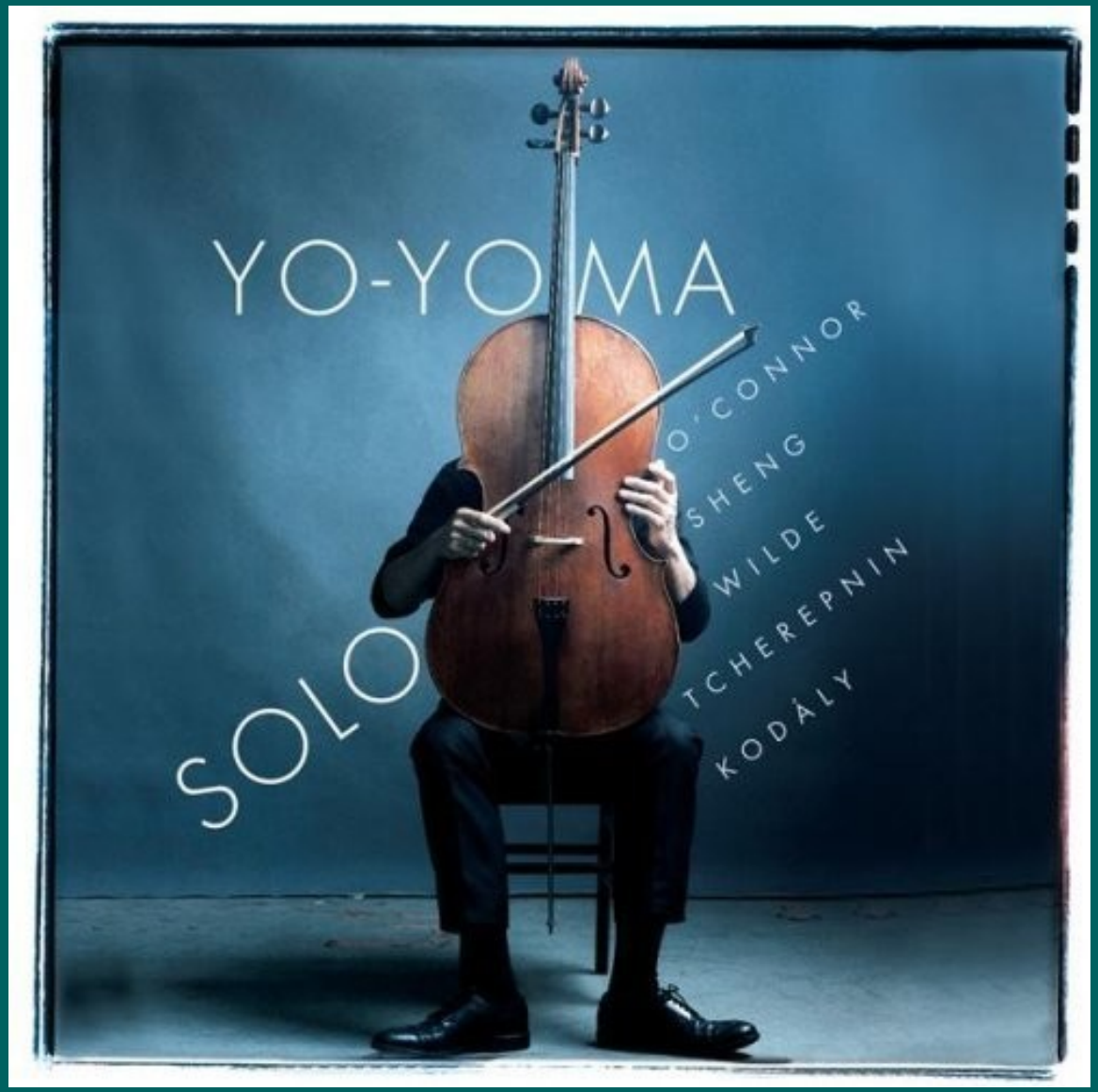
Vibrational Frequency of a Molecular Cello (大提琴)?



λ: 1m → 1nm

ν: ? → ?

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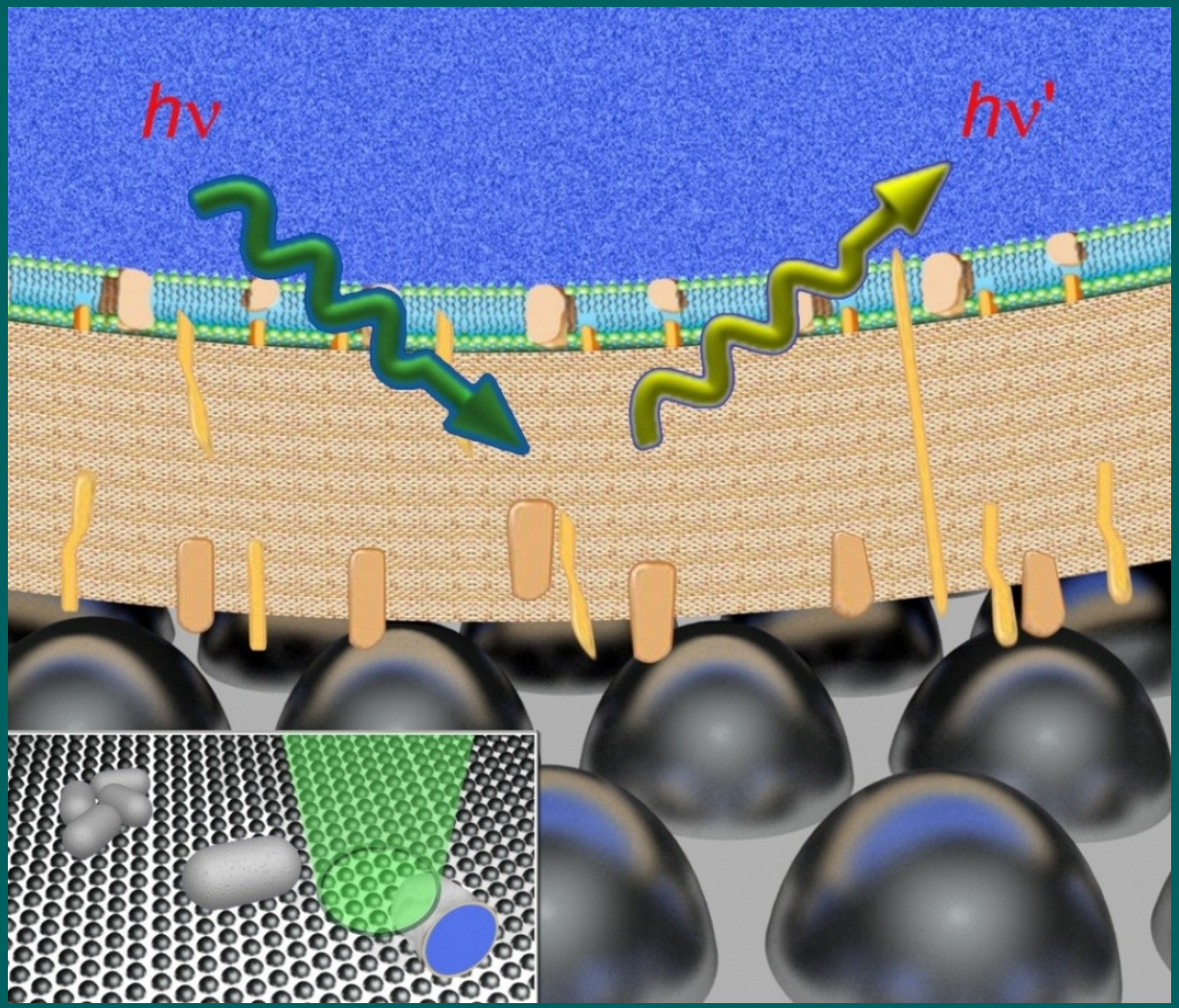
Study of Bacteria by Surface Enhanced Raman Spectroscopy



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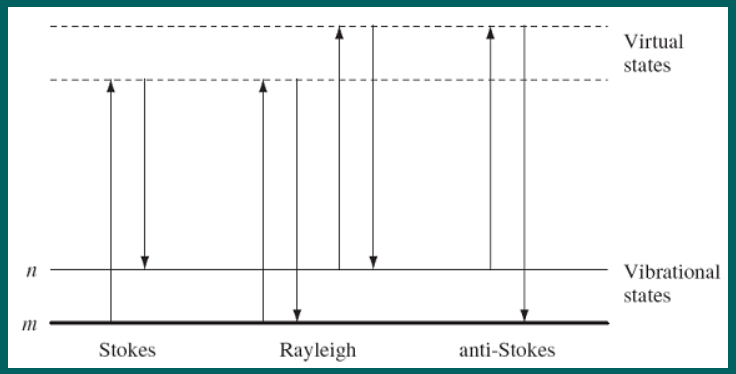
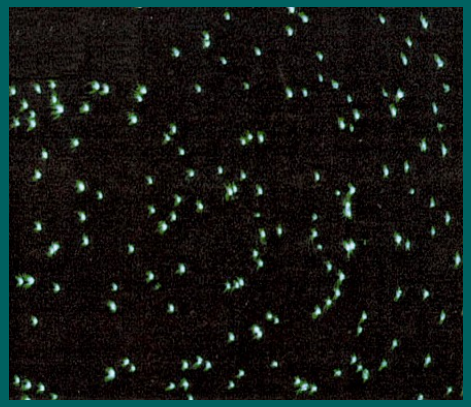
Motivations for Fabricating Substrates for Surface Enhanced Raman Spectroscopy (SERS)

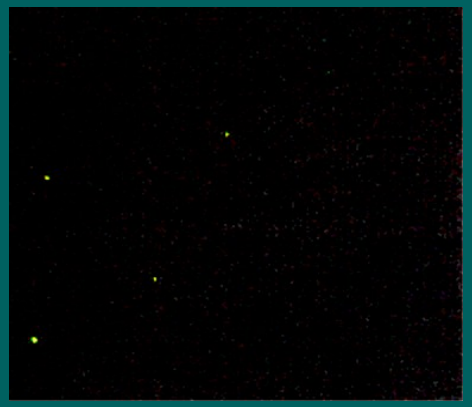
σ (fluorescence) ~ 10^{-16} (cm^2/molecule)

σ (Raman) ~ 10^{-32} (cm^2/molecule)



First Direct Observation of 'Hot-Spots' in SERS





S. Nie and S. R Emory Science 275(1997)1102

First Observation of SERS

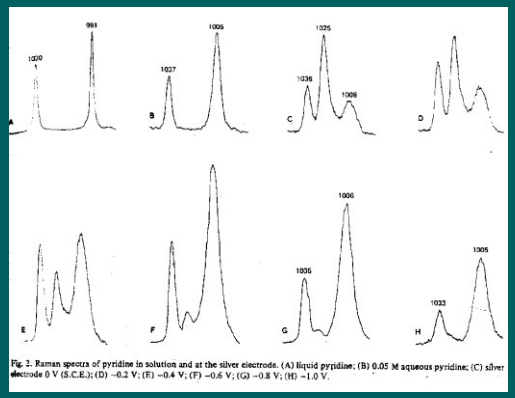


Fig. 2. Raman spectra of pyridine in solution and at the silver electrode. (A) liquid pyridine; (B) 0.05 M aqueous pyridine; (C) silver electrode 0 V (S.C.E.); (D) −0.2 V; (E) −0.4 V; (F) −0.6 V; (G) −0.8 V; (H) −1.0 V.

M. Fleishmann, P. J. Hendra, A. J. MacQuillan, Chem. Phys. Lett. 26 (1974) 163

Exploit field enhancement to increase the Raman signal, facilitating optical spectroscopic detection and study of bio-molecules

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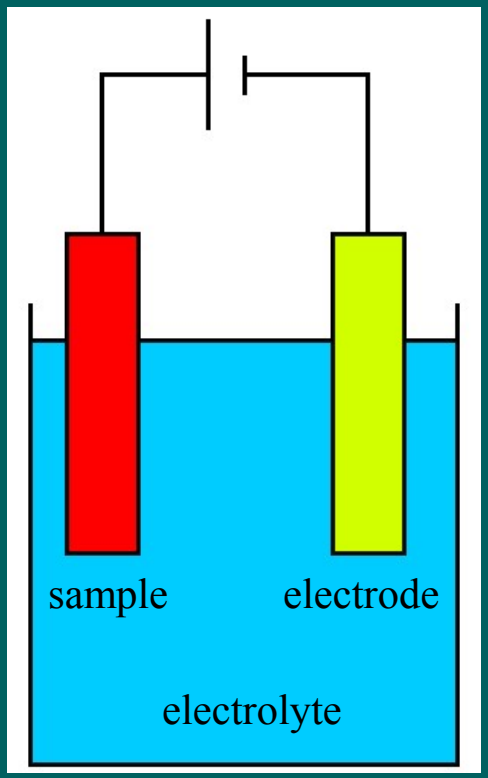
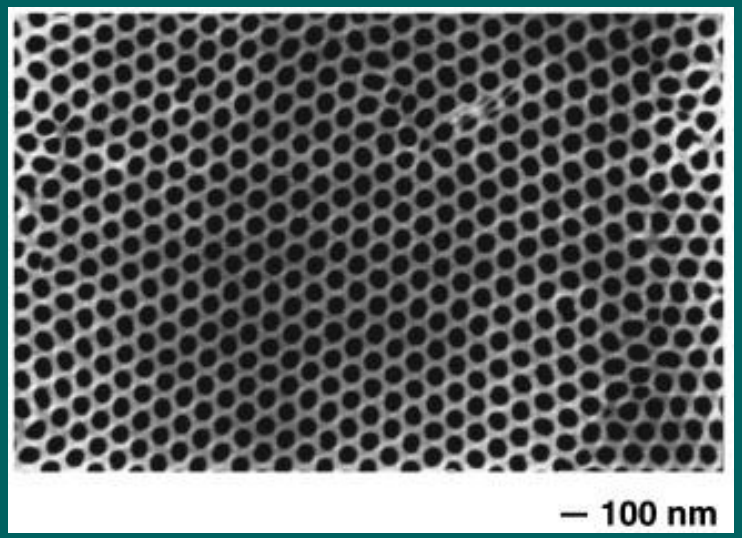
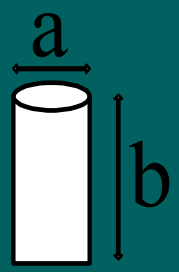
Anodization (陽極處理) of Metal Leading to Porous Oxide: an Example of Material Self-organization in 1~100 nm Scale

example: Al $\longrightarrow$ Al_2O_3 (Anodic Alumina)





O. Jessensky, F. Müller, U. Gösele
Appl. Phys. Lett. **72**, 1173 (1998)



- Range of diameter is between several to a few hundreds of nm
- High aspect ratio: b/a can be larger than 1000

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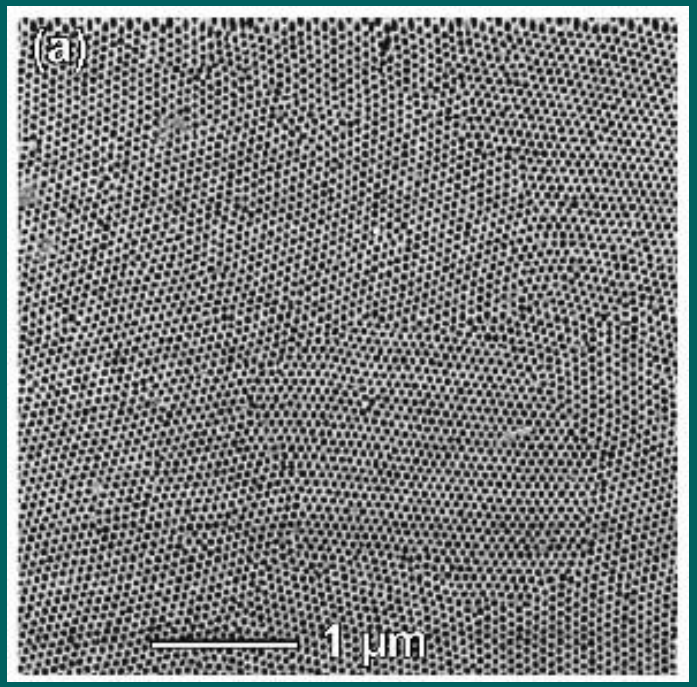
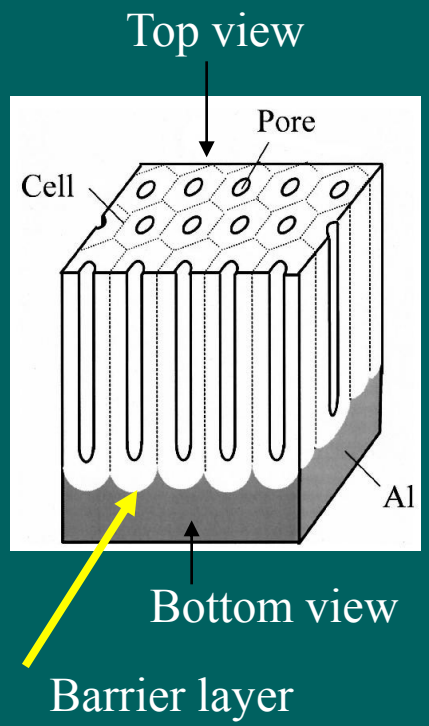
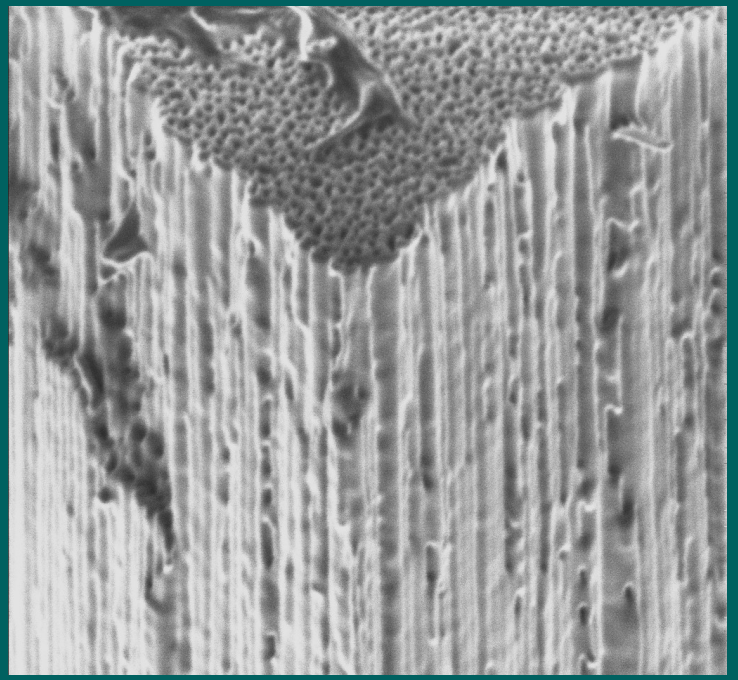
Self-organized ordered and disordered anodic alumina nanochannels (氧化鋁奈米管)



A. P. Li *et al.* J. Appl. Phys. **84**, 6023 (1998)





C. Y. Liu, A. Datta, Y. L. Wang, Appl. Phys. Lett. **78**, 120 (2001)

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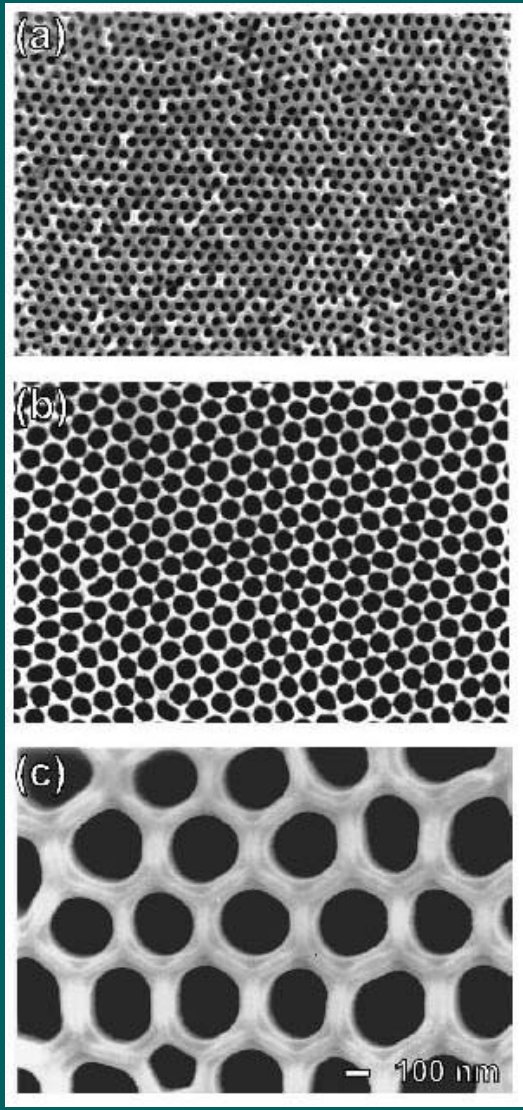
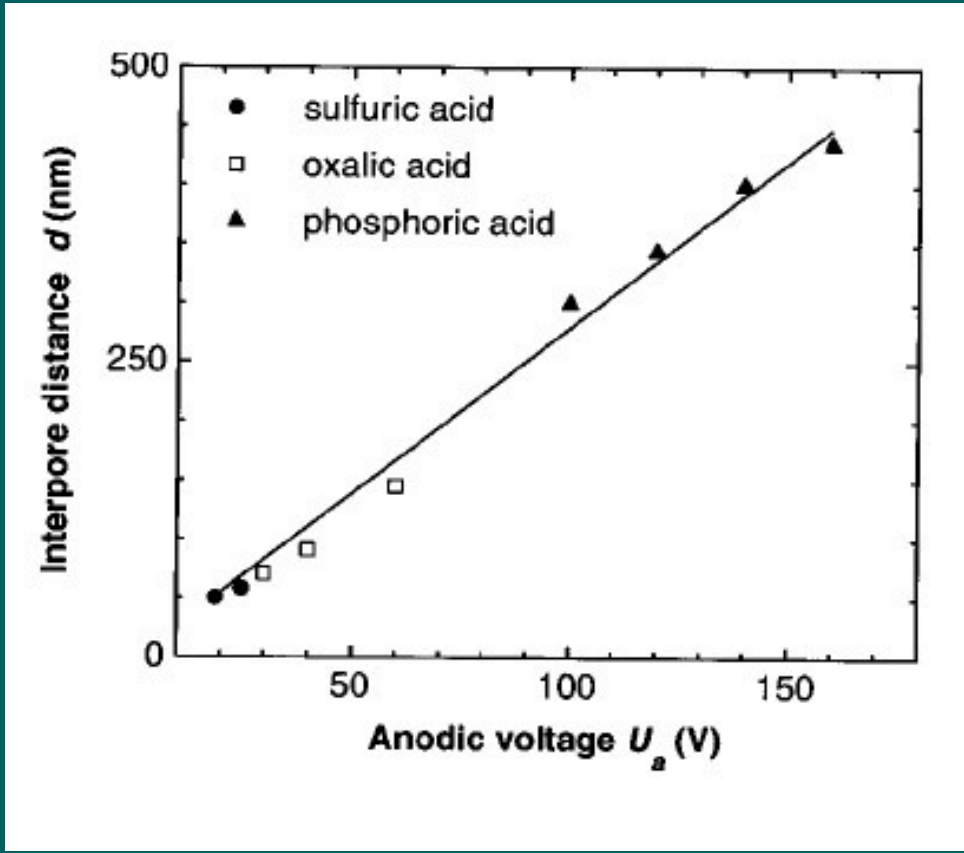
H_2SO_4
25V

$H_2C_2O_4$
40 V

H_3PO_4
160 V



2.5 nm/V



A. P. Li *et al.* J. Appl. Phys. **84**, 6023 (1998)

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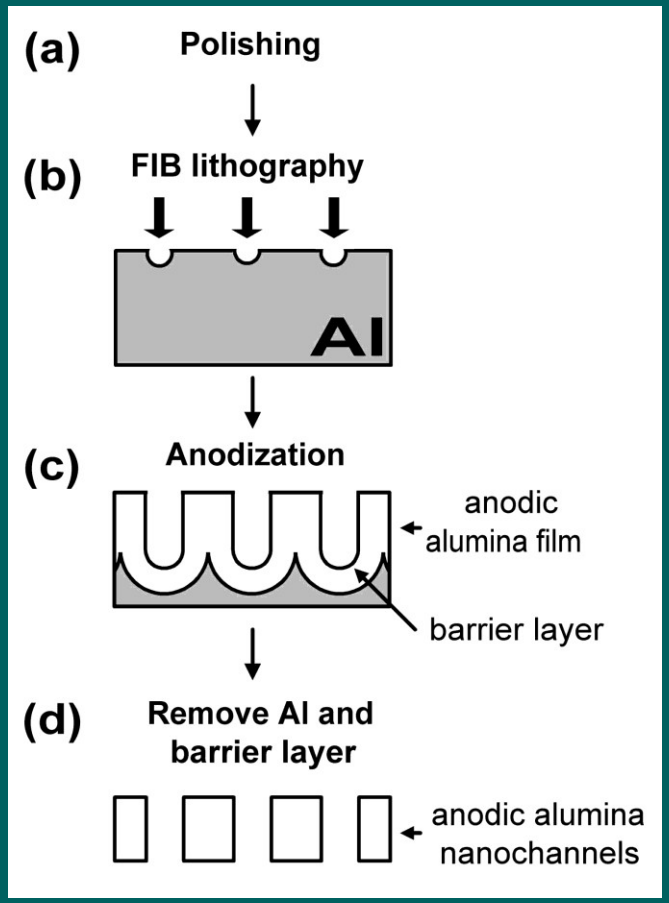
Guiding the growth of anodic alumina nanochannel arrays with focused ion beam (FIB)



- Anodic alumina film with ideally ordered nanochannels are grown on aluminum surfaces that have been pre-patterned by FIB lithography
- The period and arrangement of the lithographic pattern must be similar to that of the self-organized ordered array of nanochannels.

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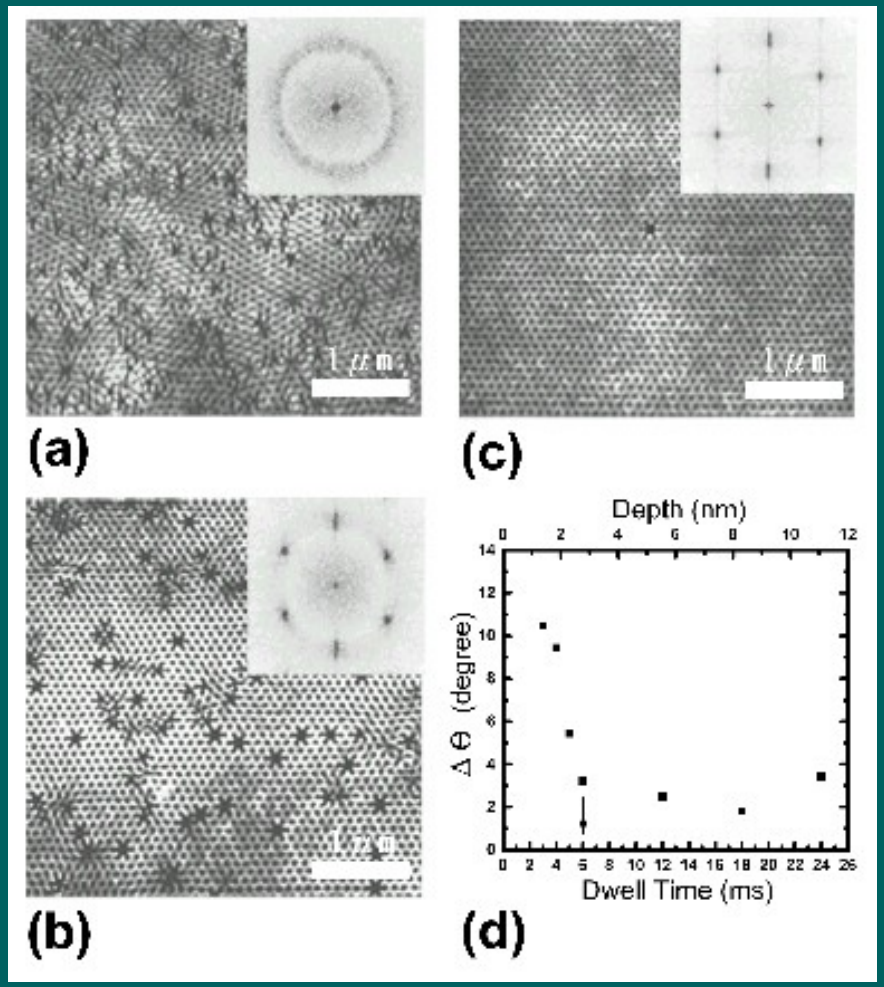
Effect of FIB dose on the order of nanochannels



AFM images of the backside of the nanochannels created by (a)2×10^{15} (1 ms), (b) 9×10^{15} (6 ms), and (c) 2×10^{16} (12 ms) ions/cm^2. The insets show the corresponding Fourier transforms of the images. (d) Angular spread of the intensity peaks ($\Delta\theta$) as a function of FIB dwell time.

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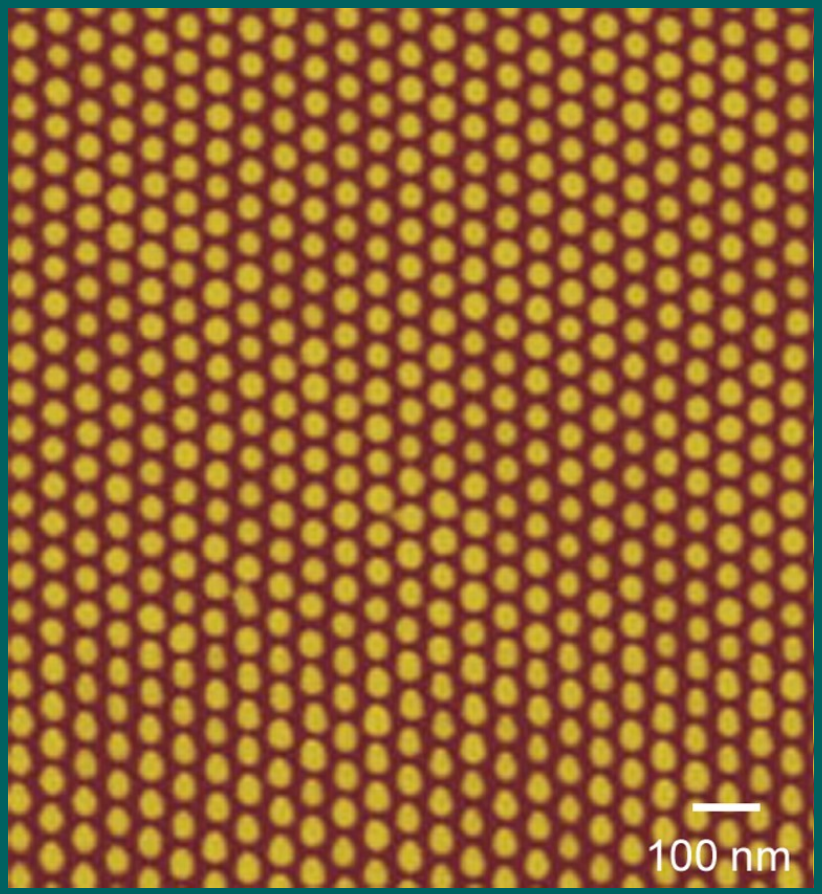

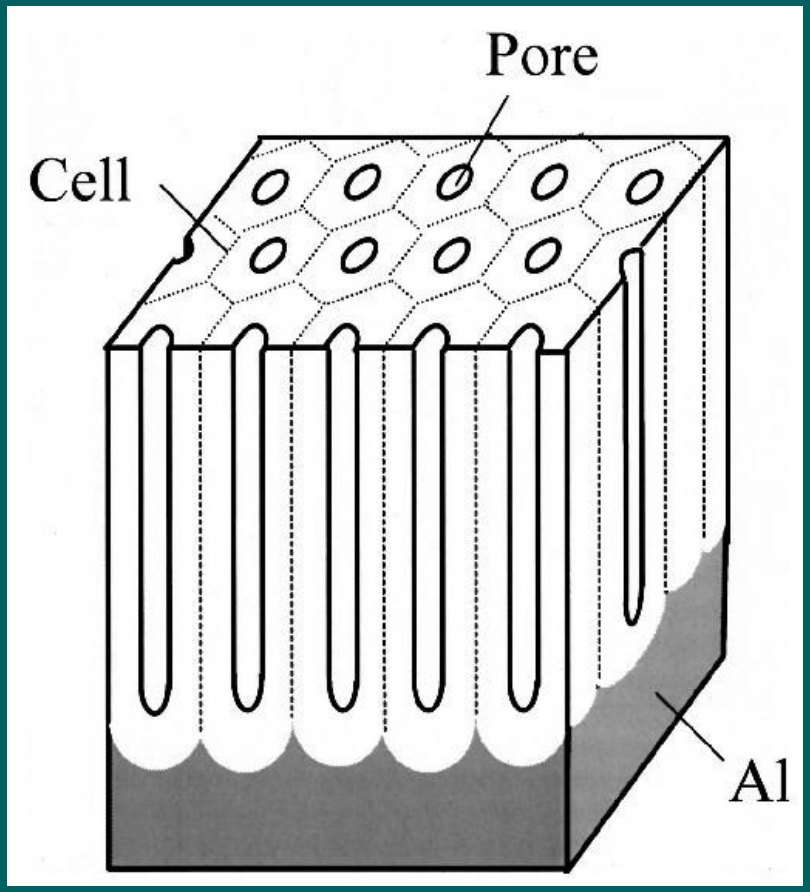

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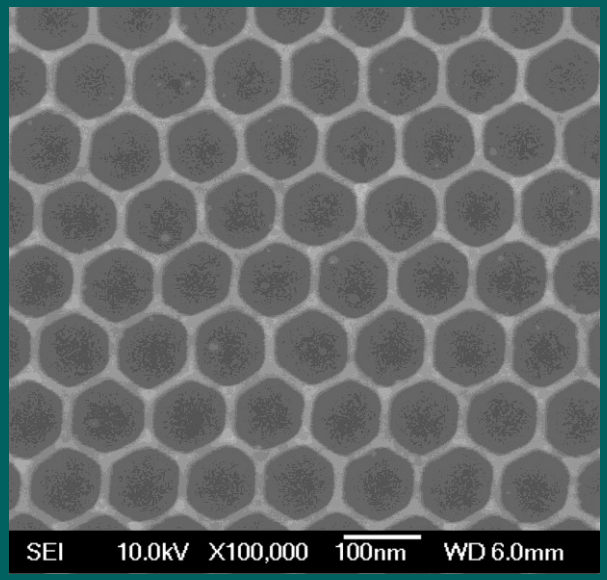
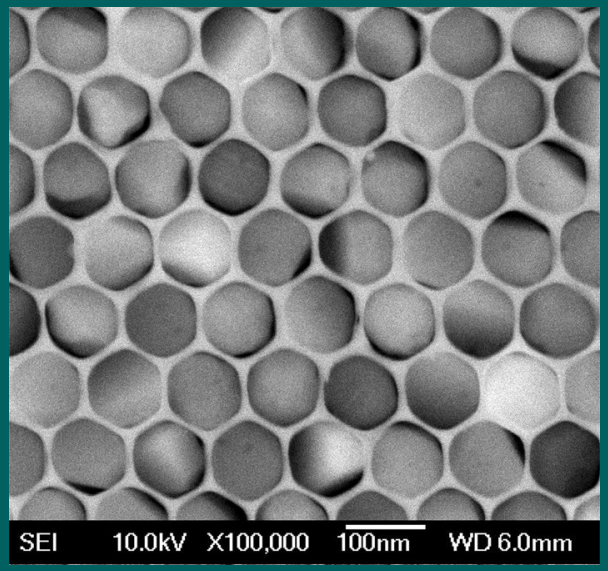
Fabrication of Ordered Array of Ag-nanoparticles

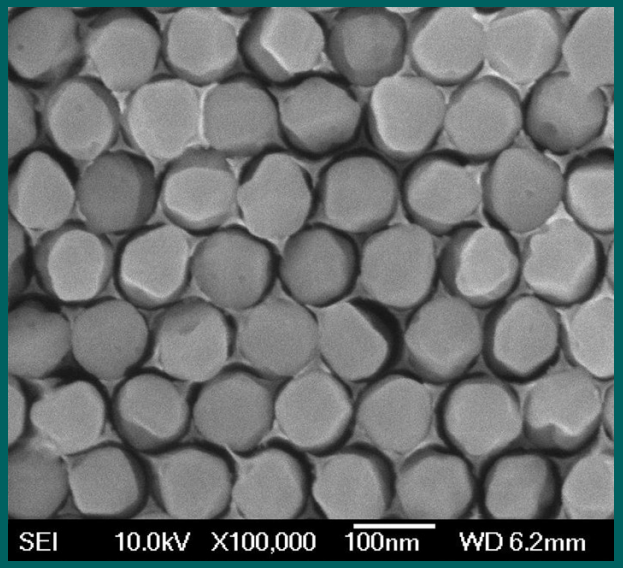
AAO nanochannel array with 10 nm channel wall



AAO nanochannel array partially filled with Ag



AAO nanochannel array filled with Ag nanocrystals



- USA Patent granted, 2008
- SERS substrate fabrication nanotechnology transferred to MA-tek, 2009

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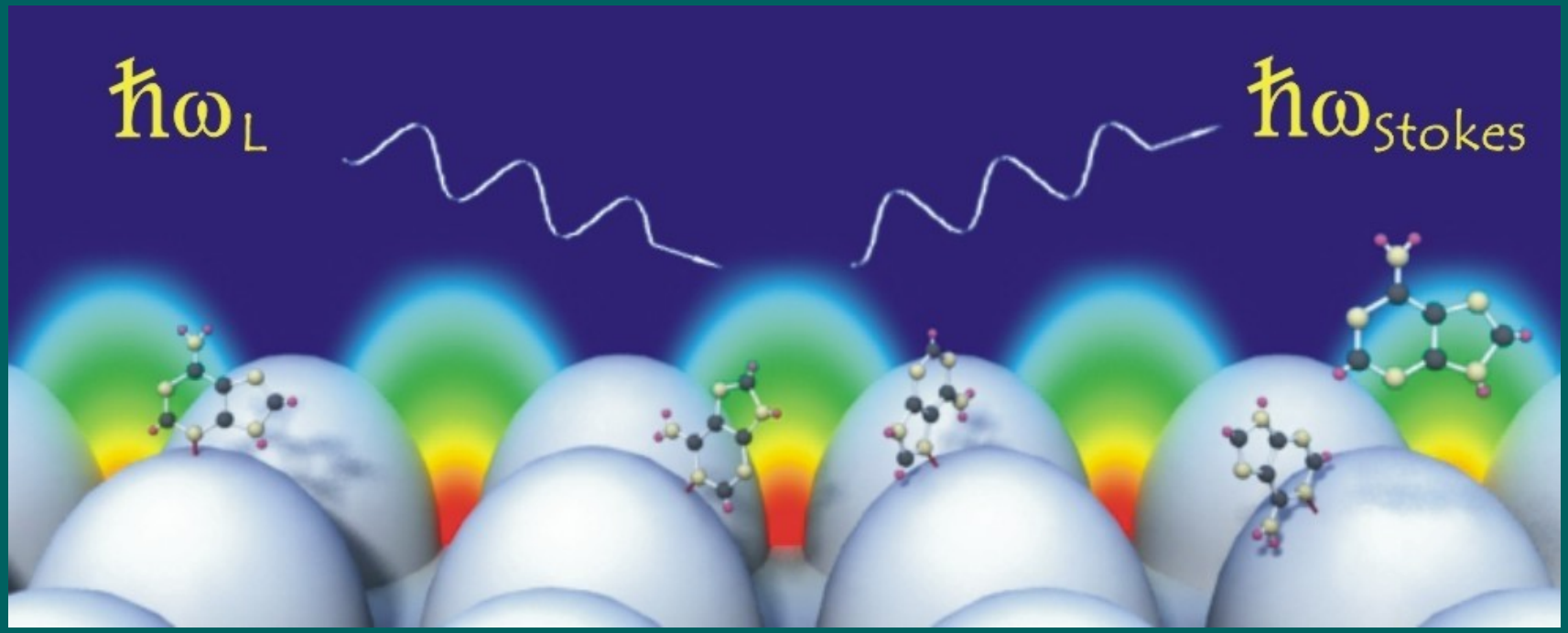
Nanoparticle-Array-based Surface Enhanced Raman Scattering (NPA-based SERS)



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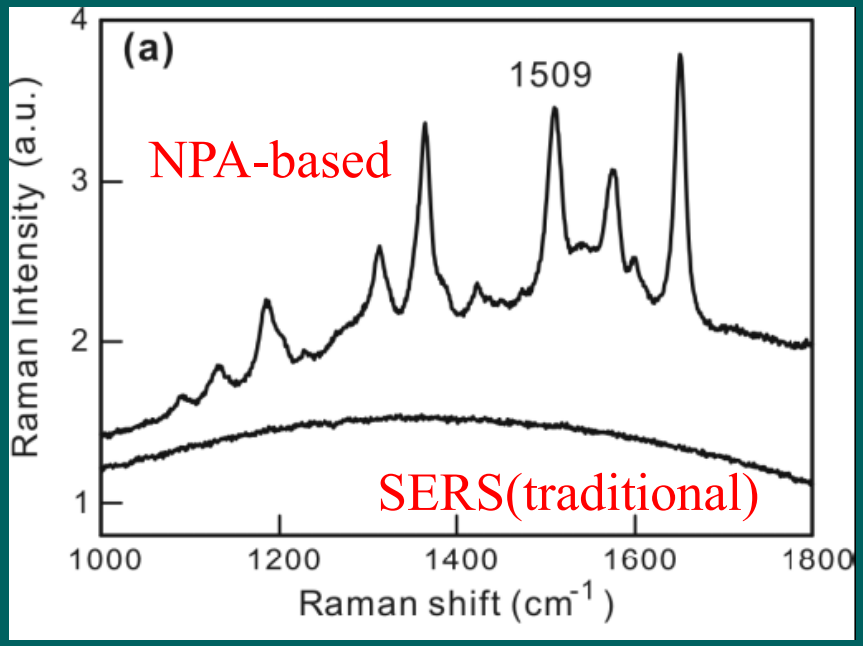
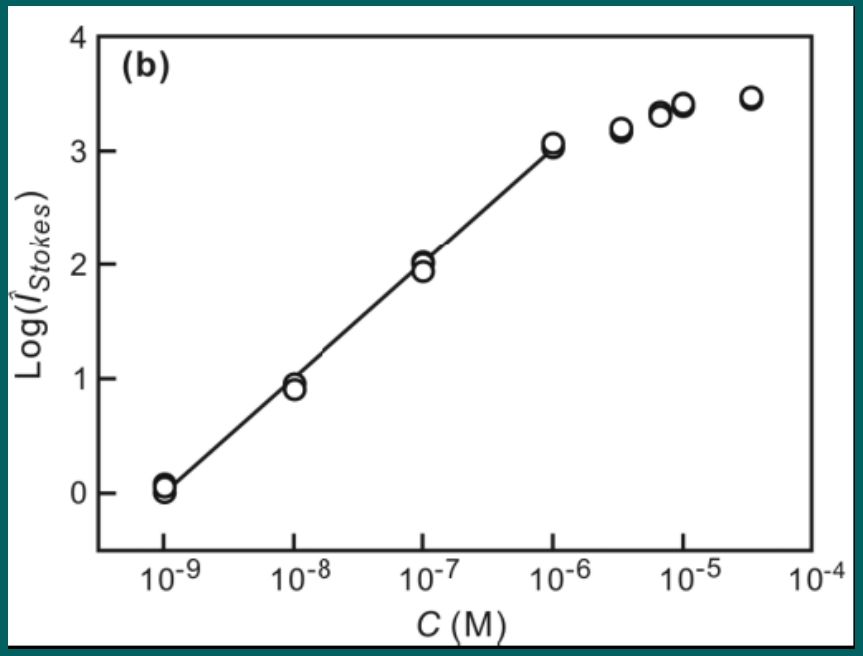
Comparison between NPA-based and Traditional SERS





a) NPA-based SERS of 10–6 M R6G solution (top) and on a typical SERS substrate prepared by depositing ~ 30 nm Ag on Si (bottom); b) NPA-based SERS at 1509 cm^{-1} as a function of the molecular concentration on a logarithmic scale.

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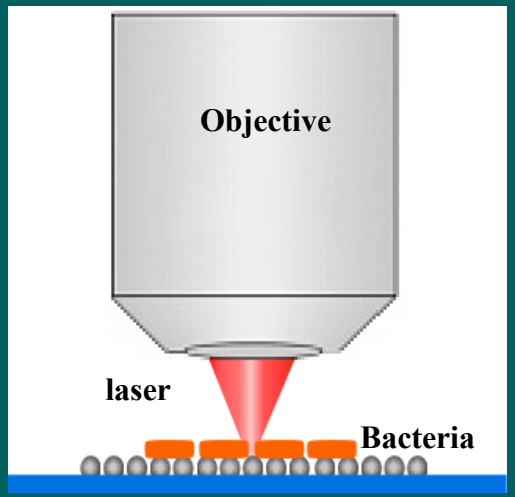
A High Platform for Bacteria Detection and Monitoring

NPA-based SERS

- *Microbial biology*
  To detect the changes of bacterial cell wall in the distinct growth phases
- *Antibiotic susceptibility*
  To delineate bacteria's response to therapeutic treatments
- *Discrimination of bacteria*



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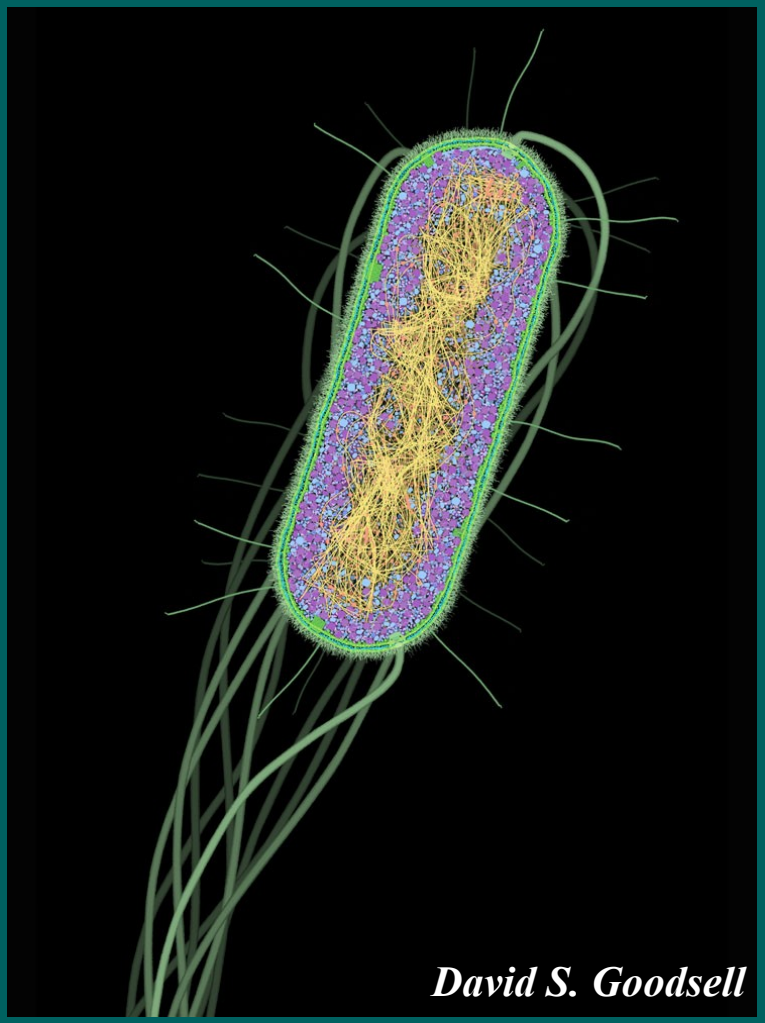
Escherichia coli (*E. Coli*, 大腸桿菌)



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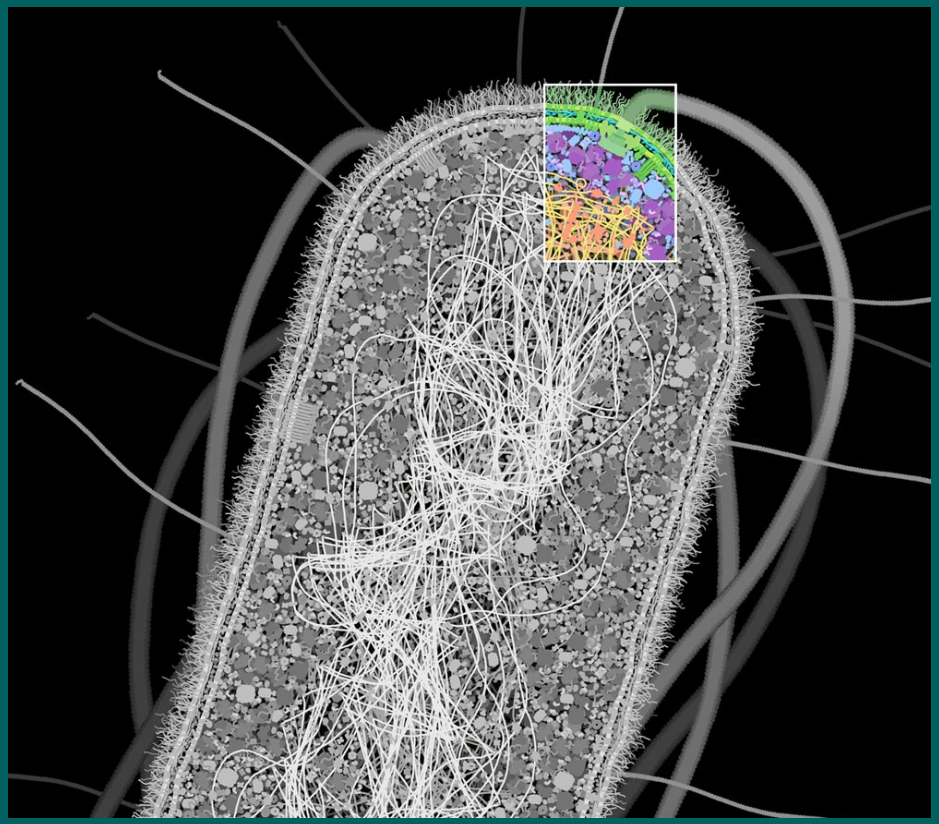
Escherichia coli (*E. Coli*, 大腸桿菌)



David S. Goodsell

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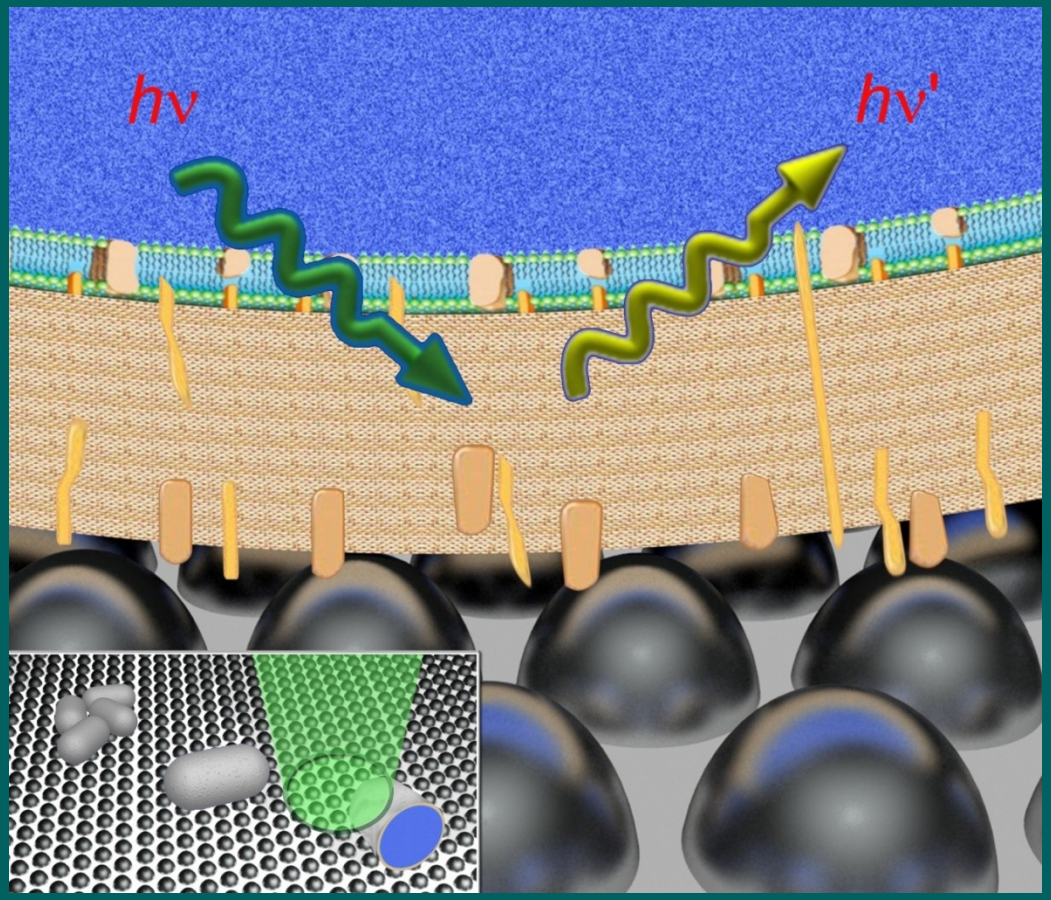
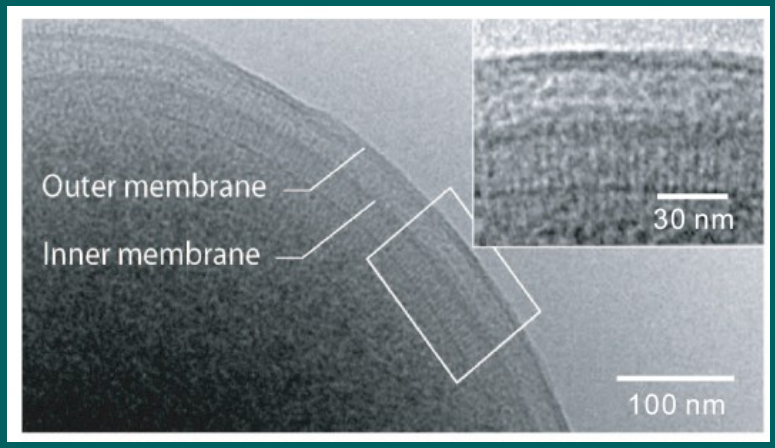
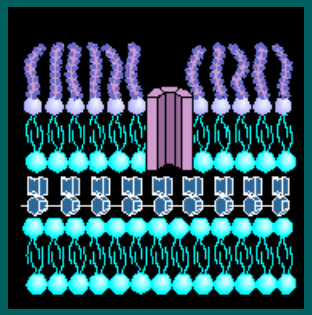
SERS of Bacteria Cell Wall

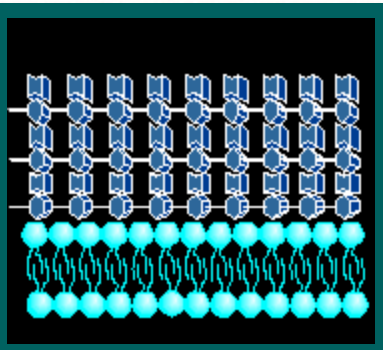


Cryo-TEM image of *E. coli*





Gram negative



Gram positive

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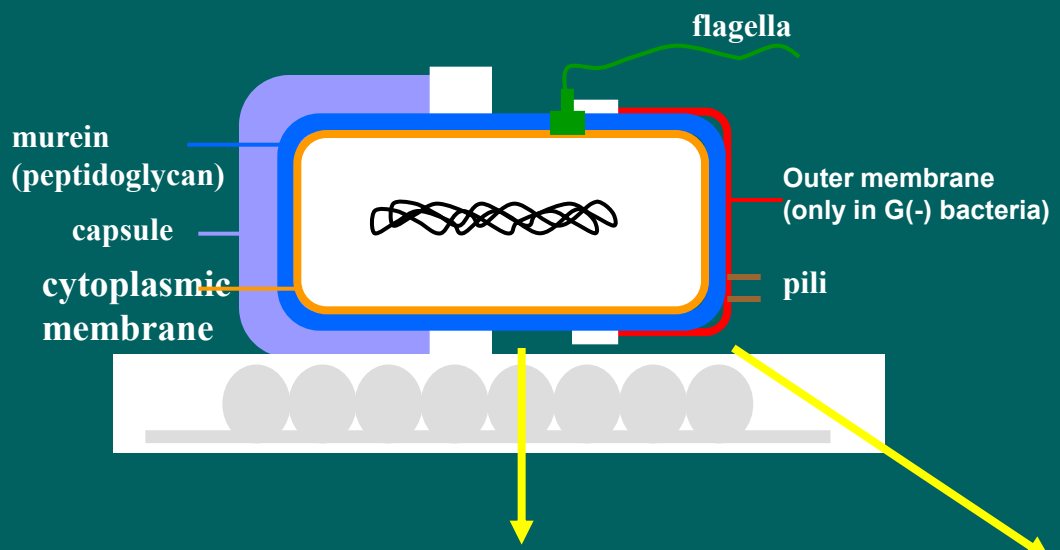
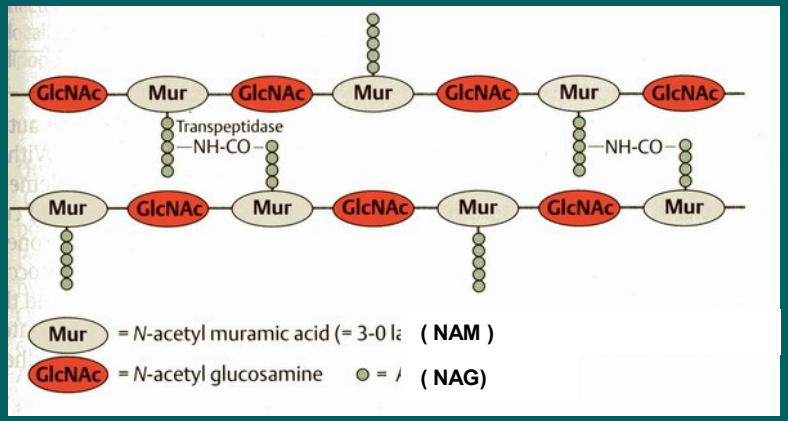
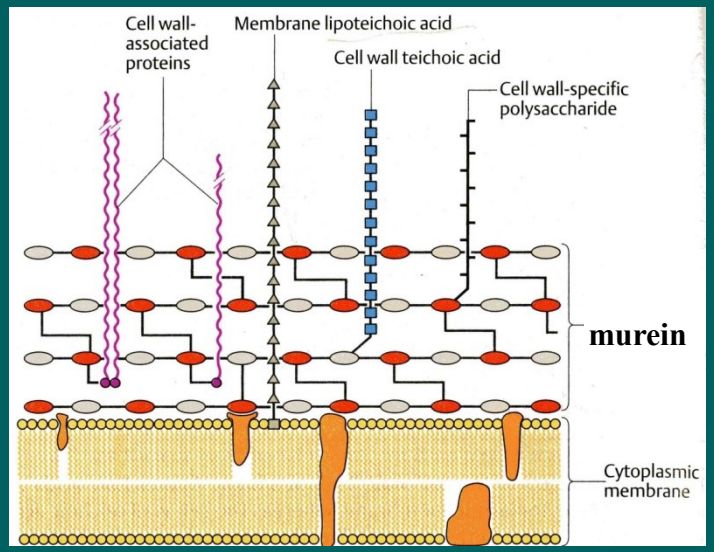
Basic bacterial cell structure



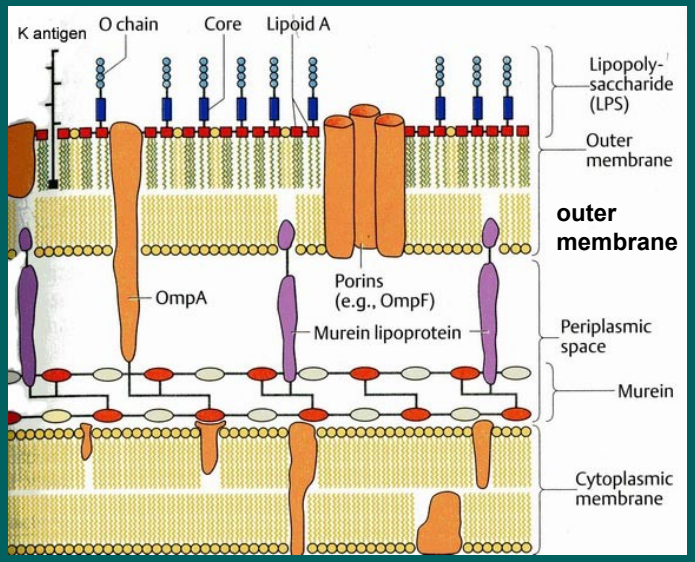
The structure of murein (peptidoglycan)



The cell wall of Gram-positive bacteria



The cell wall of Gram-negative bacteria



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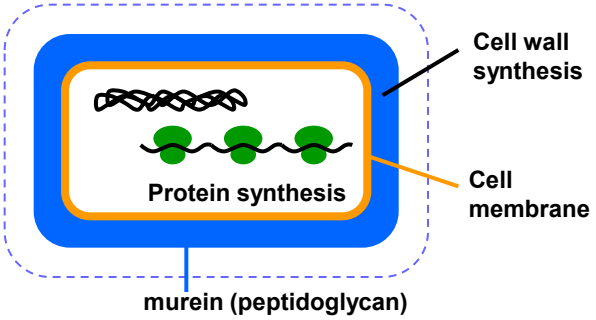
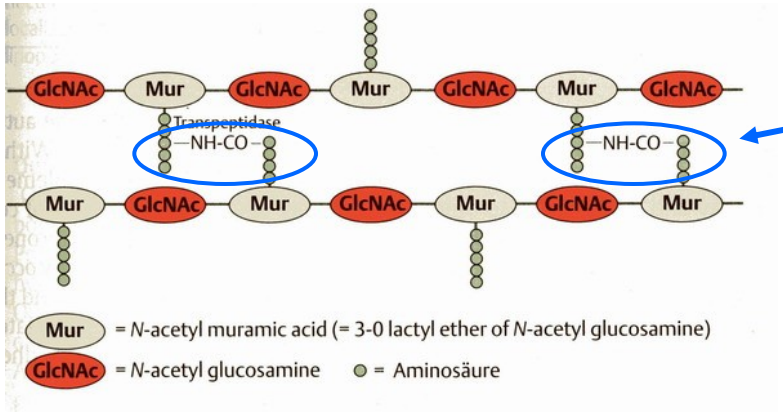
Two types of Change of Bacterial Cell Wall Resulting from Antibiotic Treatment

Basic Mechanisms of Antibiotic Action against Bacterial Cells



1. Inhibition of Cell Wall Synthesis (most common mechanism)
   Vancomycin / Ampicillin
2. Inhibition of Protein Synthesis (second largest class)
   Tetracycline / Gentamicin
3. Alteration of Cell Membranes
4. Inhibition of Nucleic Acid Synthesis

The structure of murein (peptidoglycan) and the action site of vancomycin



Vancomycin disrupts peptidoglycan cross-linkage and inhibits steps in murein (peptidoglycan) biosynthesis.

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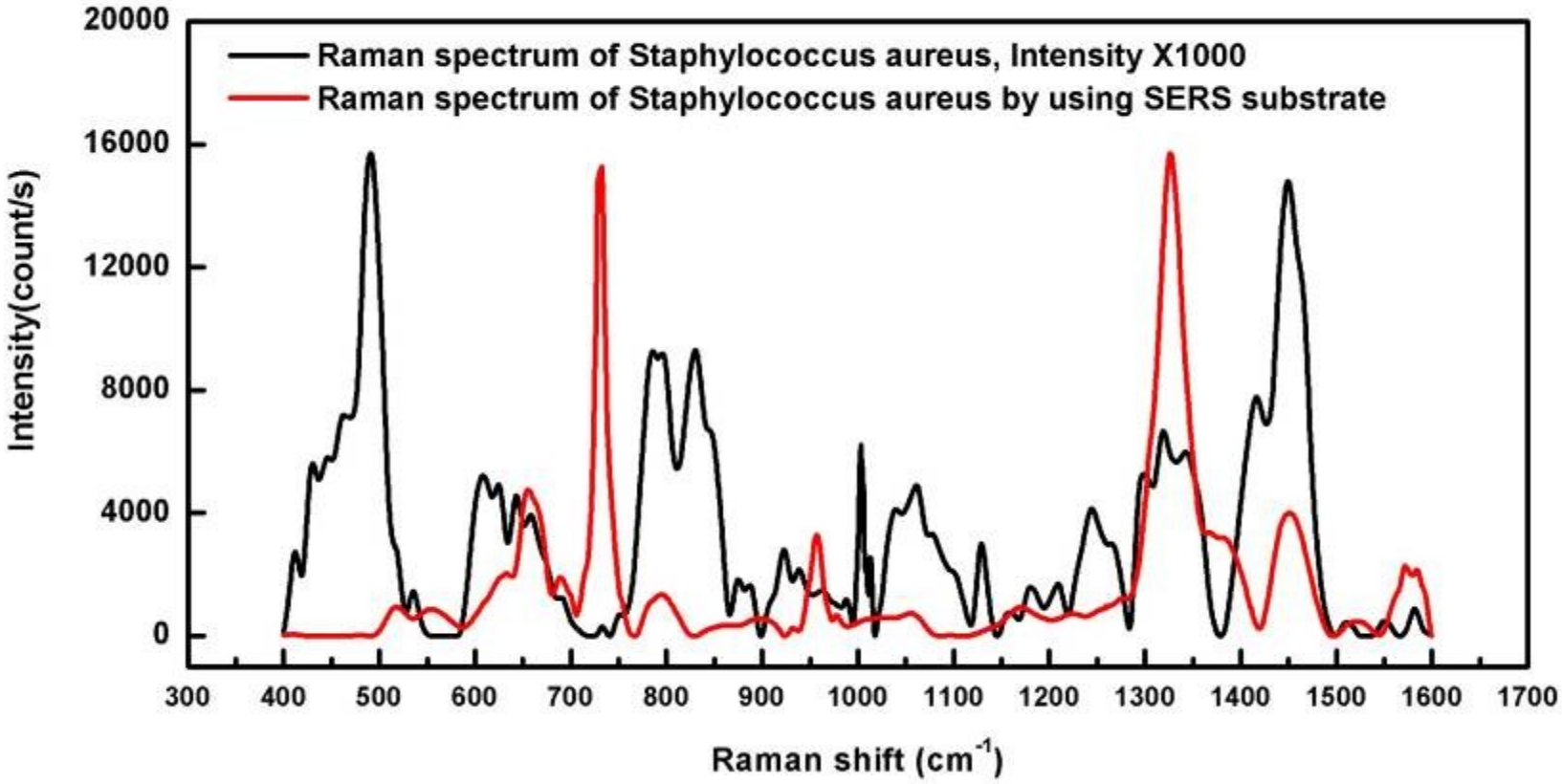
Comparison between Raman and SERS of Bacteria



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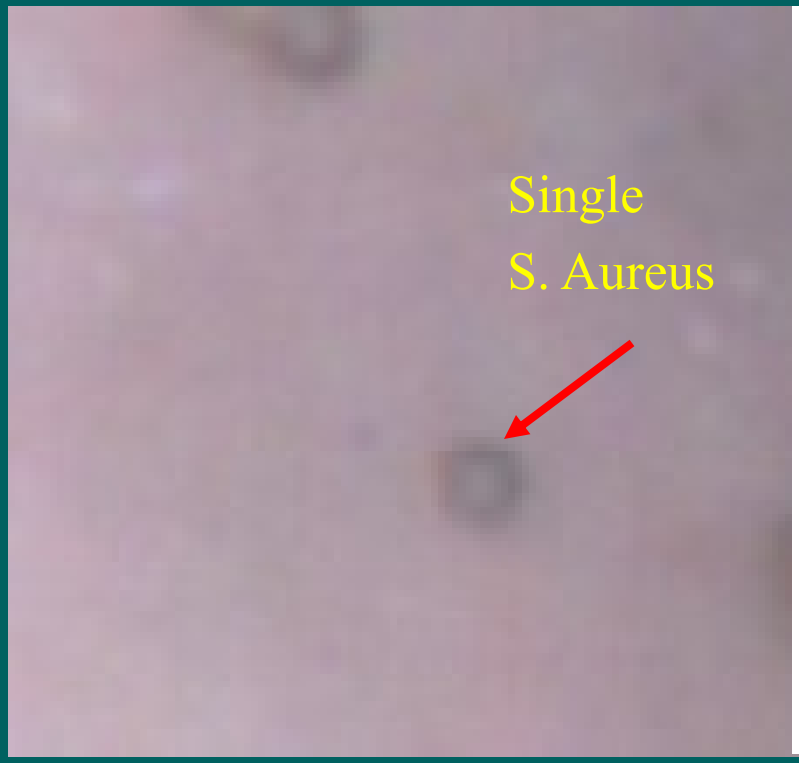
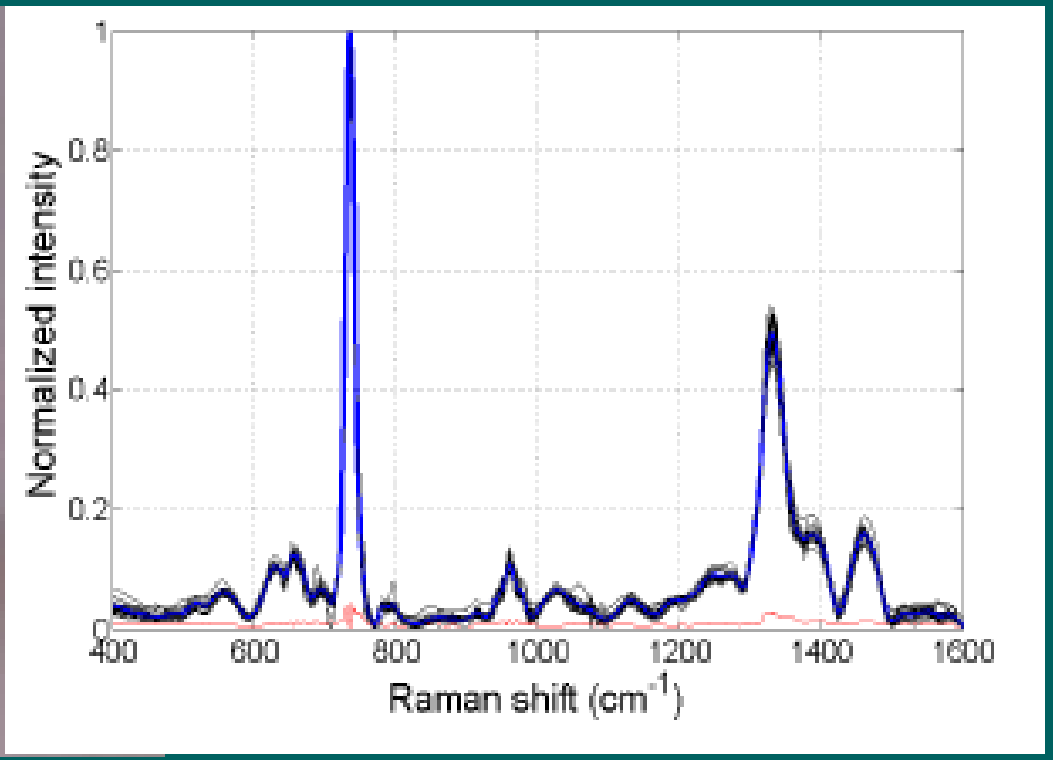
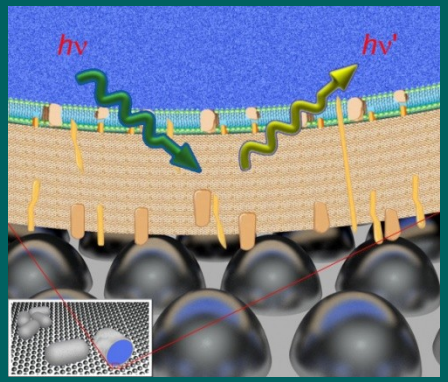
SERS of Single Bacterium







Nine spectra of *Staphylococcus aureus,* taken every 10 min, are overlapped to demonstrate the reproducibility and stability of SERS signal

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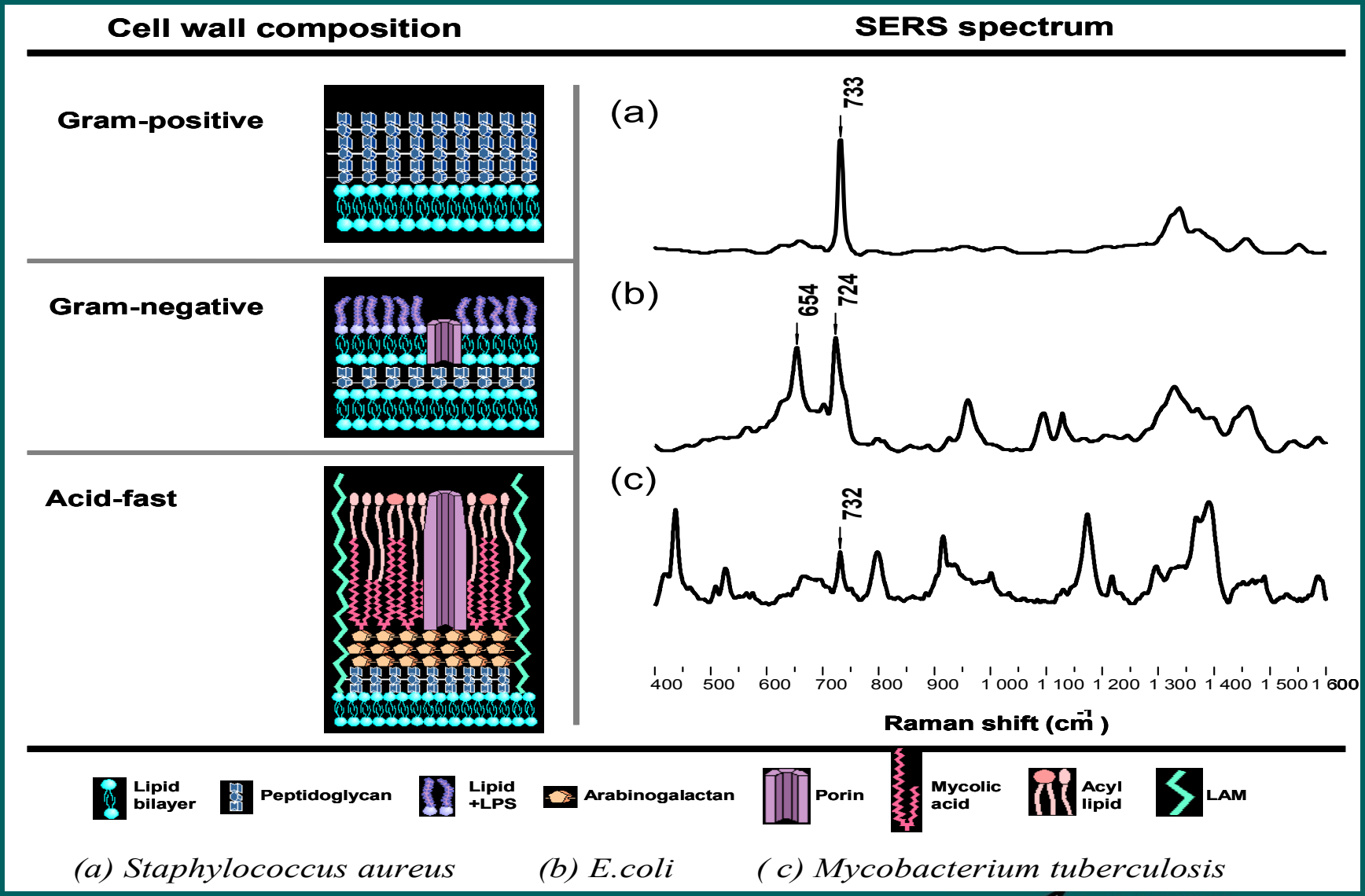
Typical SERS of Three Types of Bacteria



(a) Staphylococcus aureus *(b) E.coli* *(c) Mycobacterium tuberculosis*

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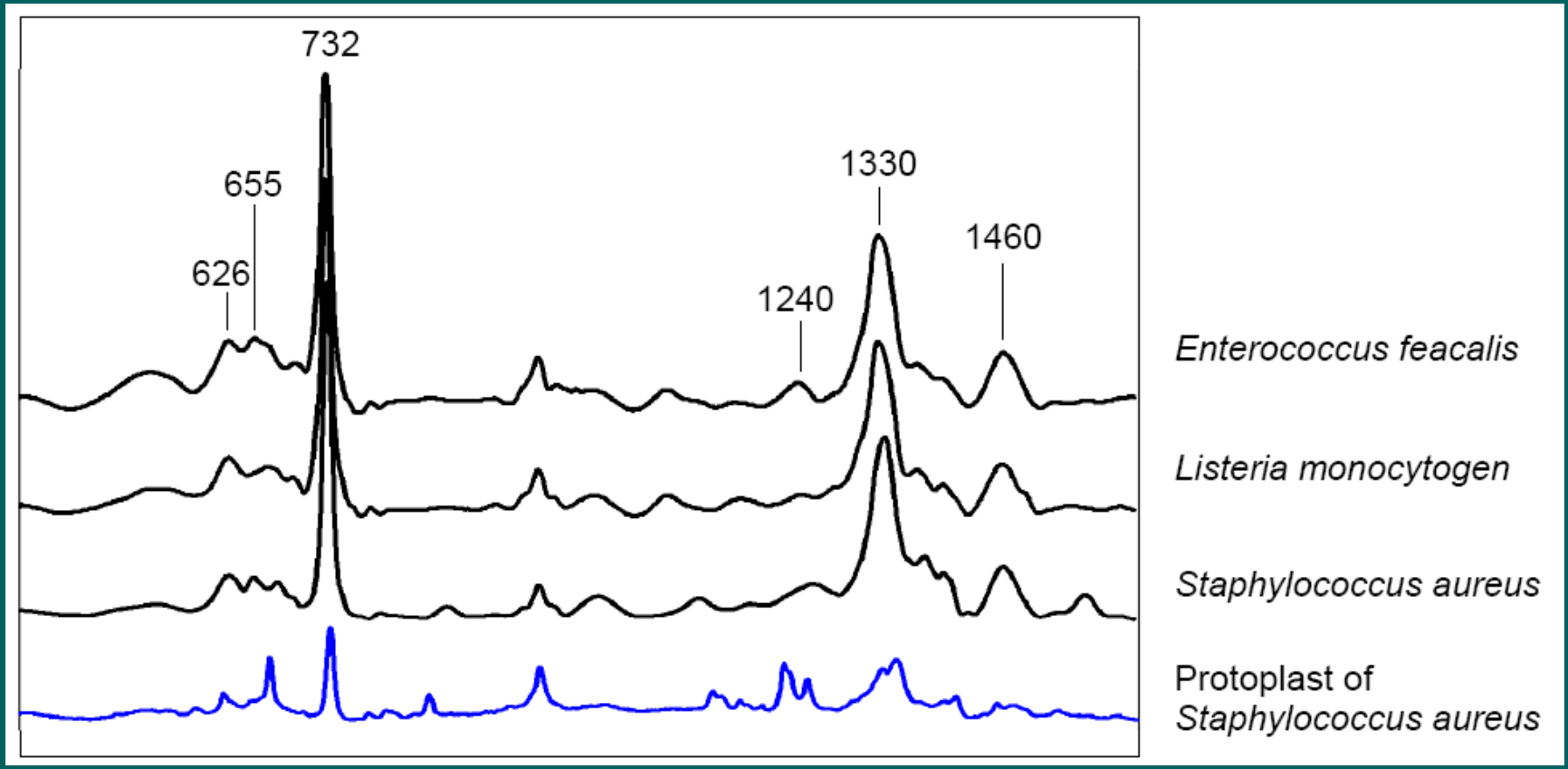
NPA-based SERS of Gram Positive Bacteria and Protoplast of S. Aureus



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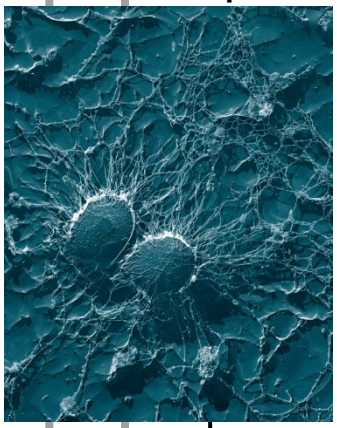
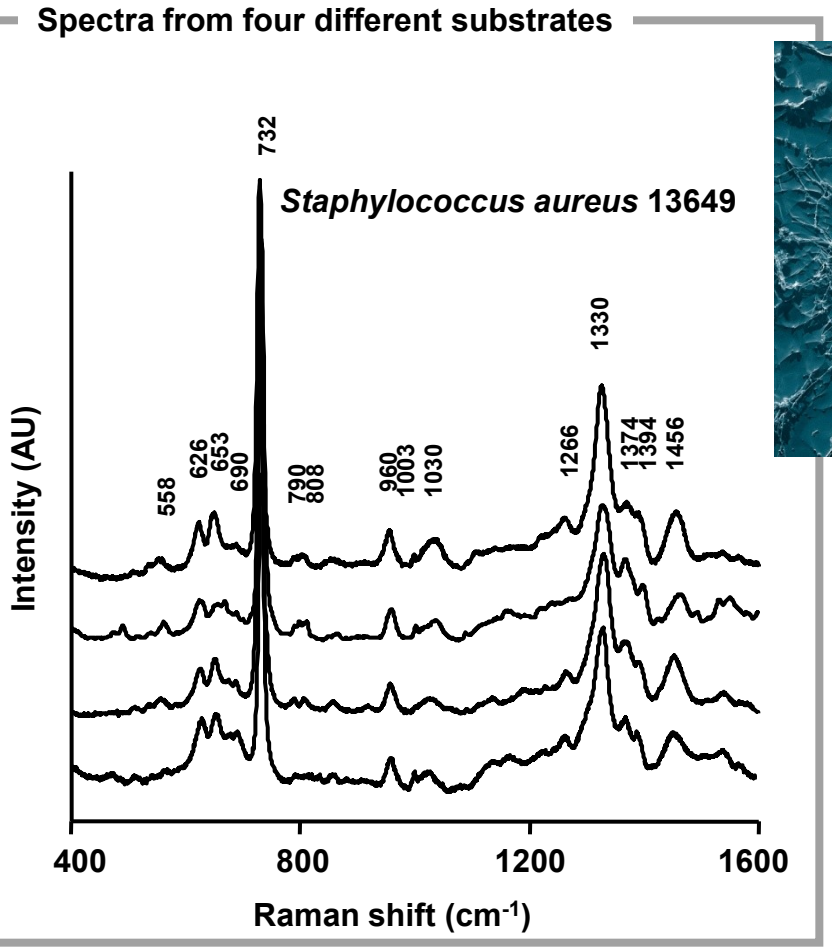
Stability and Reproducibility of NPA-Based SERS





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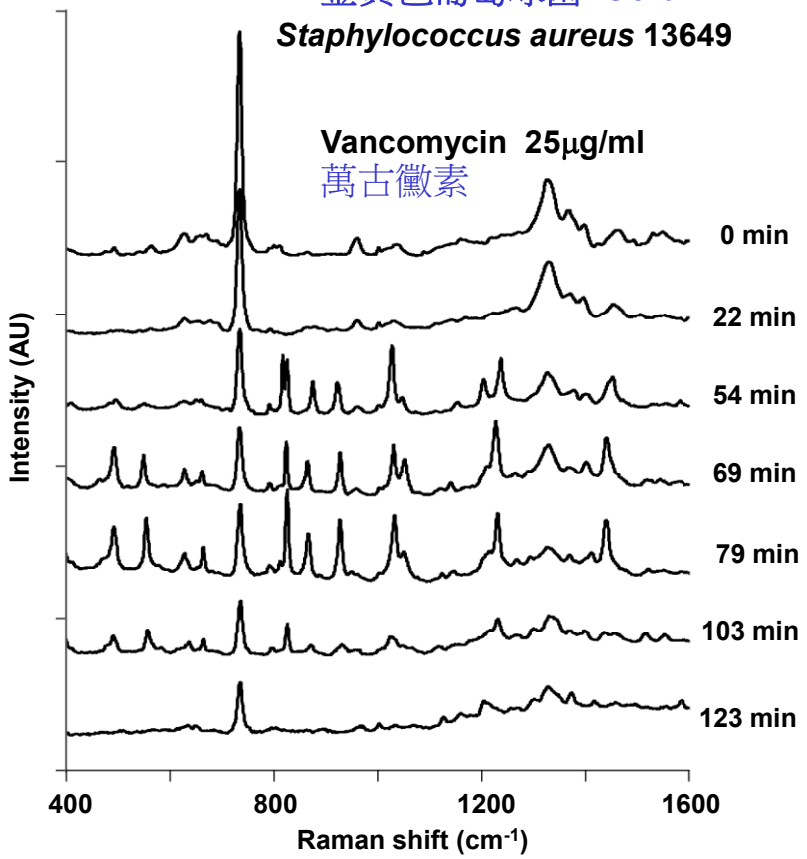
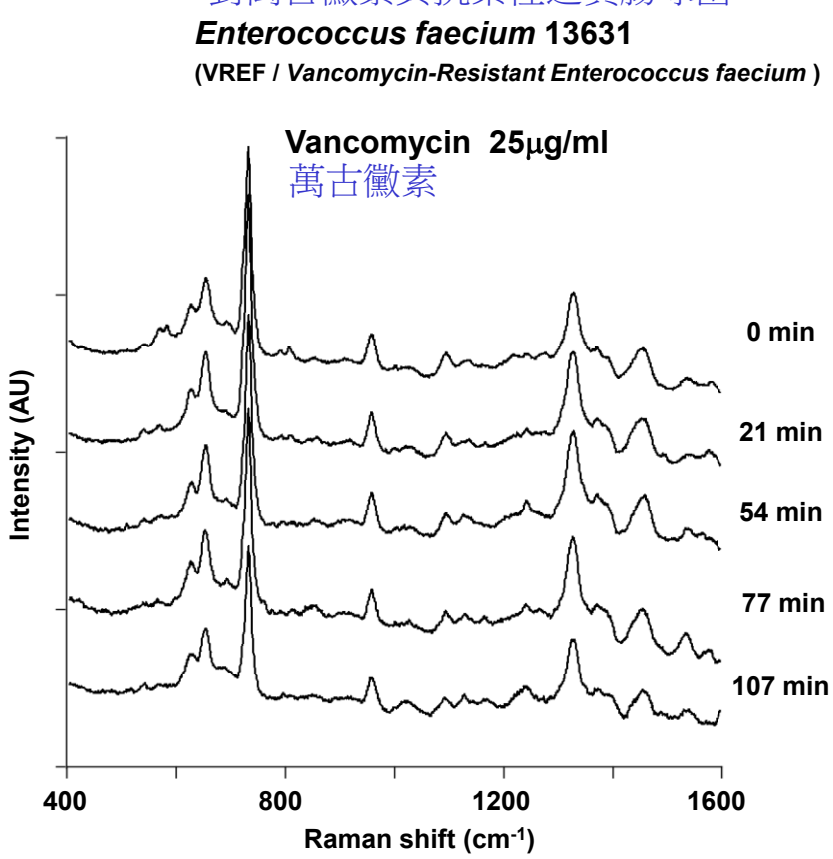
Changes of Bacterial Cell Wall Chemistry During Antibiotic Treatment





Upon the treatment of vancomycin, the spectra of *Staphylococcus aureus* 13649 (vancomycin-susceptible) was dramatically shifted. But the spectra of *Enterococcus faecium* (vancomycin-resistant) 13631 had no changes.

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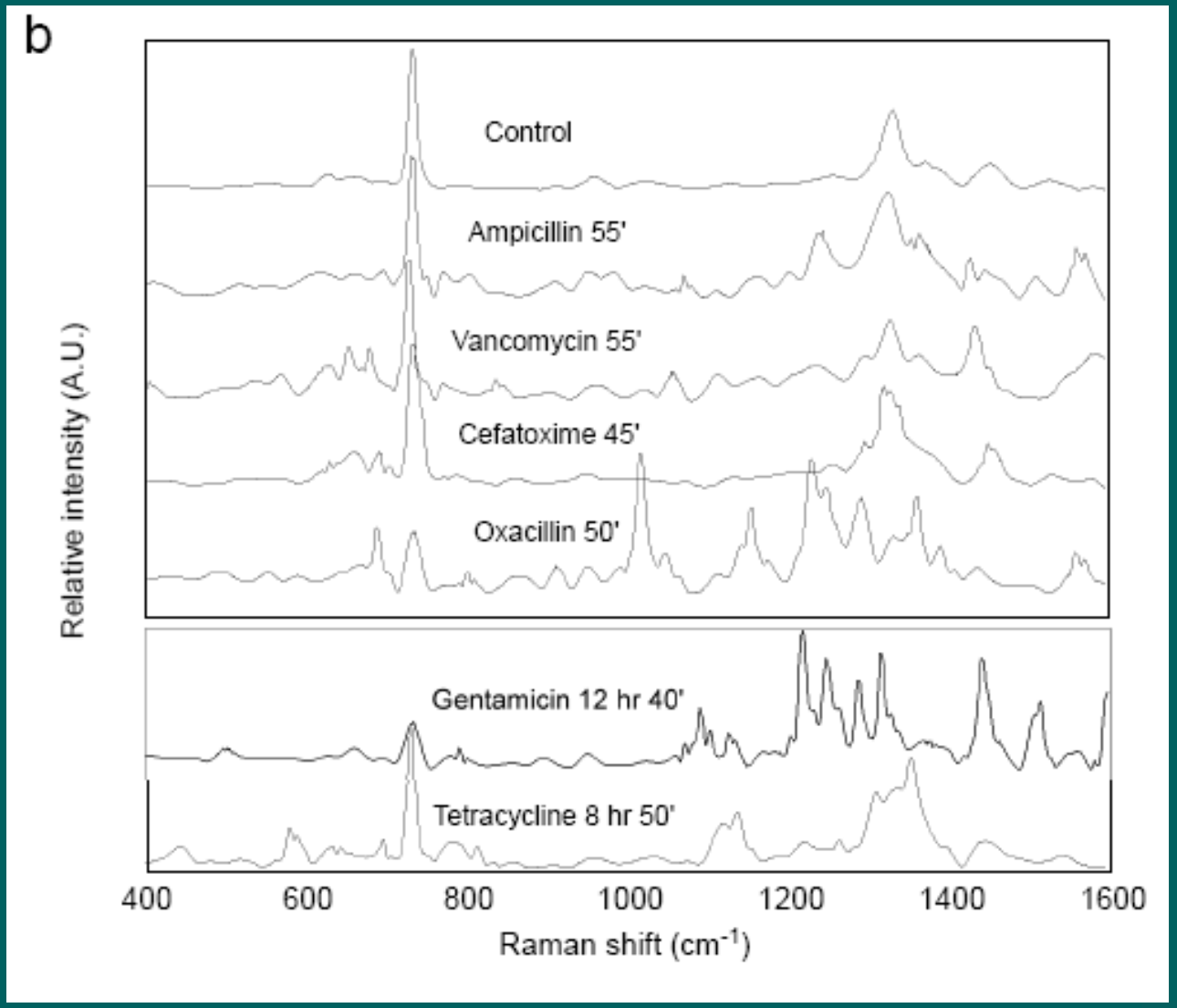
Response of S. Aureus to Various Antibiotics



Inhibition of Cell Wall Synthesis (most common mechanism)

Inhibition of Protein Synthesis (second largest class)

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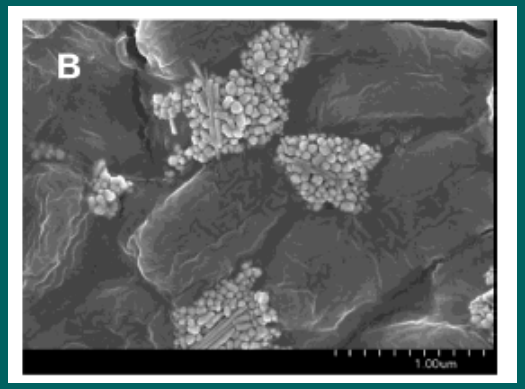
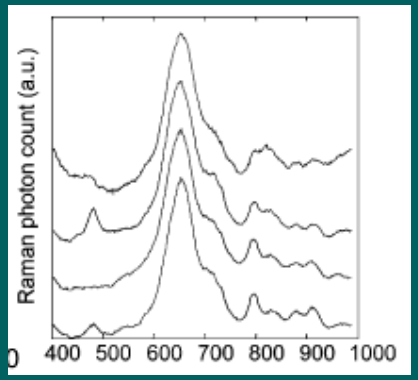
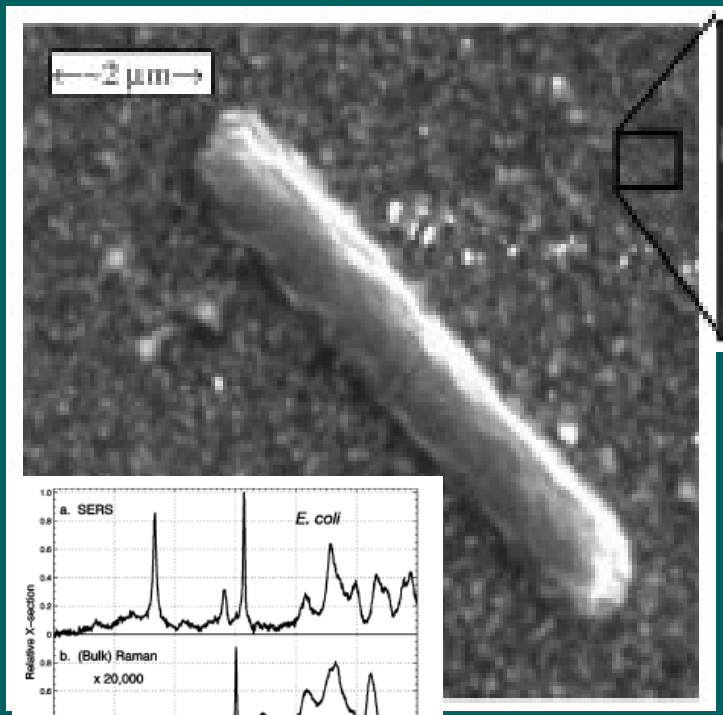
Comparison between previous and our ONPA-SERS substrates for bacteria studies

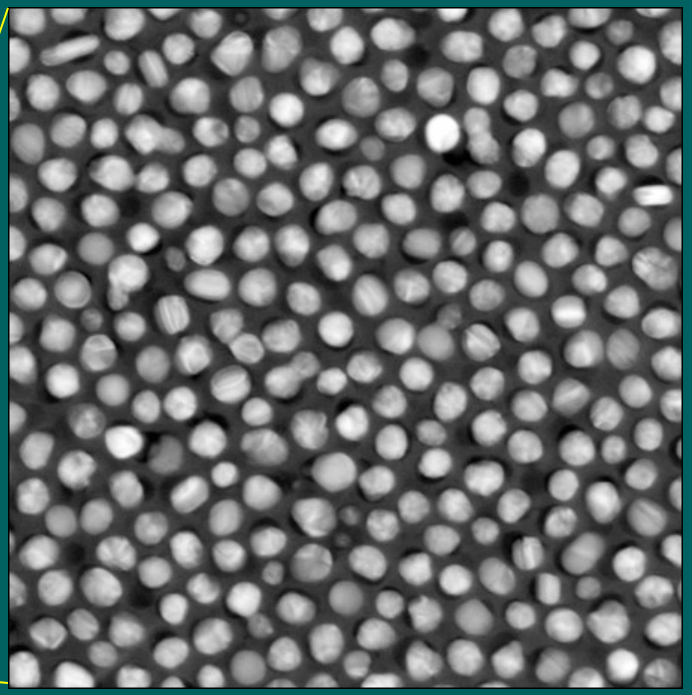




SERS achieved by mixing Au-nanoparticles with bacteria
Anal. Chem. 2004, 76, 40-47

ONPA-SERS substrate by IAMS/MA-tek





F. S.= 650 nm

SERS from a substrate with random Au-NP
Chem. Soc. Rev., 2008, 37, 883-884

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A high speed detection platform based on surface-enhanced Raman scattering for monitoring antibiotic-induced chemical changes in bacteria cell wall

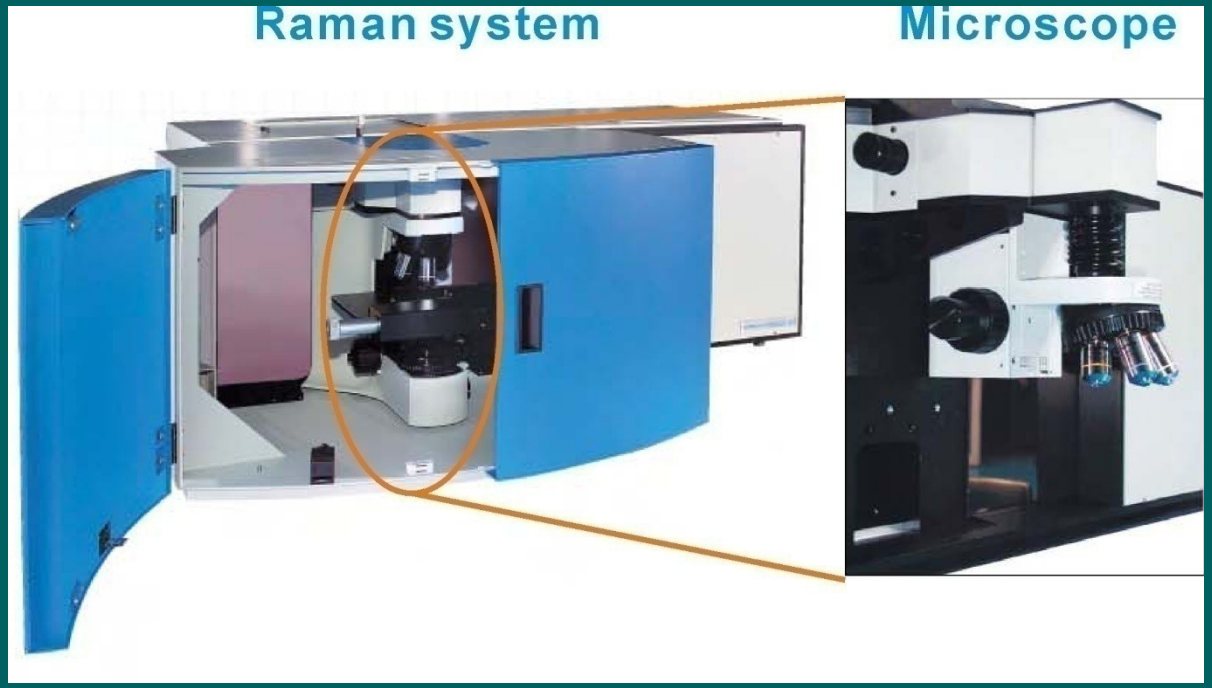
PLoS ONE 4(5), e5470 (2009)

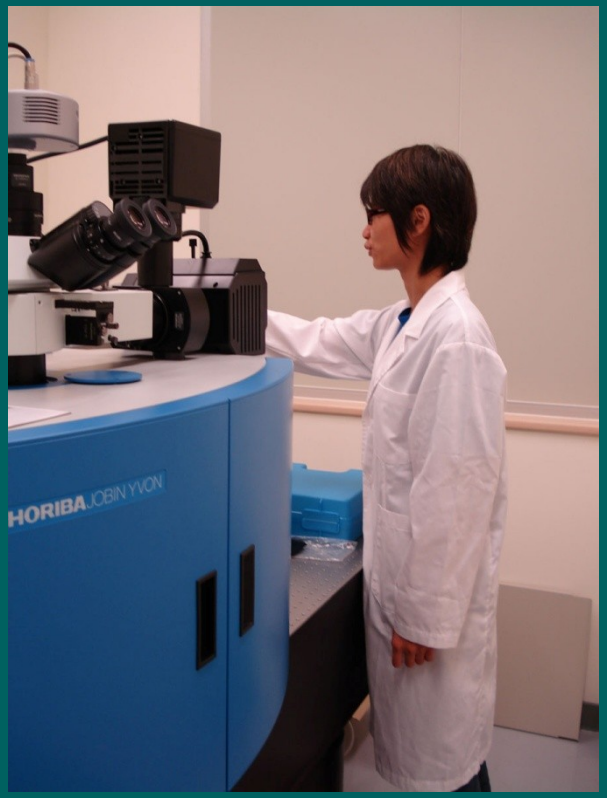
Ting-Ting Liu1, You-Hsuan Lin2, Chia-Sui Hung3, Tian-Jiun Liu2, Yu Chen3, Yung-Ching, Huang2, Tsung-Heng Tsai4, Huai-Hsien Wang5,6, Da-Wei Wang4, Juen-Kai Wang5,7, Yuh-Lin Wang5,6 & Chi-Hung Lin2,3,8,9

1 Division of Research and Development, National Yang-Ming University, 2 Institute of Biophotonics, School of Biomedical Science and Engineering, National Yang-Ming University, 3 Institute of Microbiology and Immunology, School of Life Science, National Yang-Ming University, Taipei, Taiwan, 4 Institute of Information Science, Academia Sinica, Taipei, Taiwan, 5 Institute of Atomic and Molecular Sciences, Academia Sinica, Taipei, Taiwan, 6 Department of Physics, National Taiwan University, 7 Center for Condensed Matter Sciences, National Taiwan University, Taipei, Taiwan, 8 Taipei City Hospital, 9 Taipei Veteran General Hospital.

Correspondence should be J. K. W. (jkwang@ntu.edu.tw), Y. L. W. (ylwnag@pub.iams.sinica.edu.tw), or C.-H. L. (linch@ym.edu.tw)

High resolution Raman analyzer





- 632.8 and 785 nm excitation
- Spectral coverage: 800 cm^{-1}
- Spectral resolution: 2 cm^{-1}
- Fast multichannel detection
- Combined with microscope

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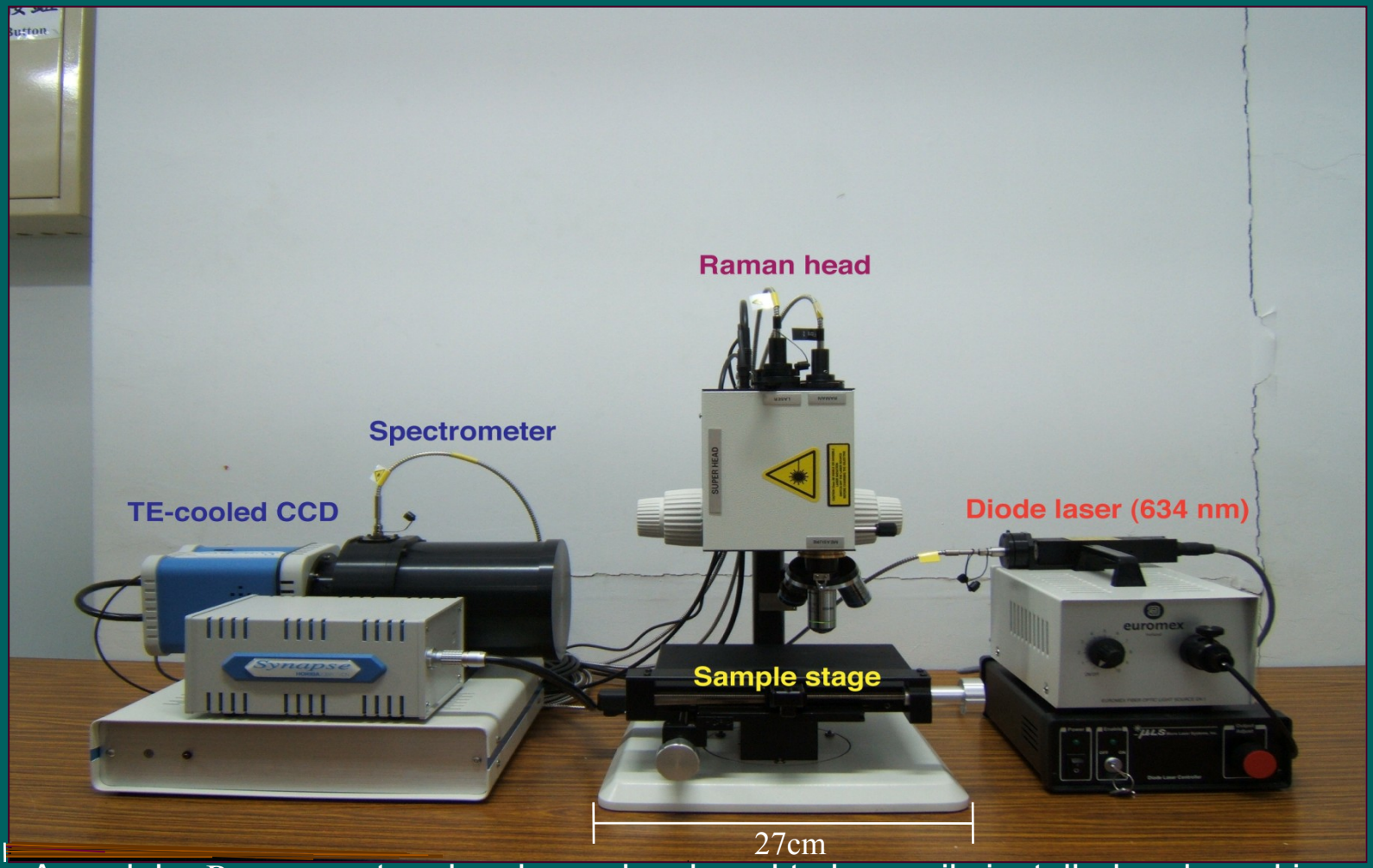
Modular Raman System



- A modular Raman system has been developed to be easily installed and used in biosafety labs.

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Custom-Designed Desktop SERS System

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我們的工作伙伴

王玉麟(1)謝詠芬(2)王俊凱(3)林奇宏(4)王大為(5)
林俊成(6) 薛博仁(7)周如文(8)

(1)中央研究院 原子與分子研究所
(2)閎康科技股份有限公司
(3)台灣大學 凝態科學研究中心
(4)陽明大學 微生物及免疫學研究所
(5)中研究院 資訊科學研究所
(6)清華大學化學系
(7)臺大檢驗醫學科
(8)衛生署疾管局分枝桿菌實驗室

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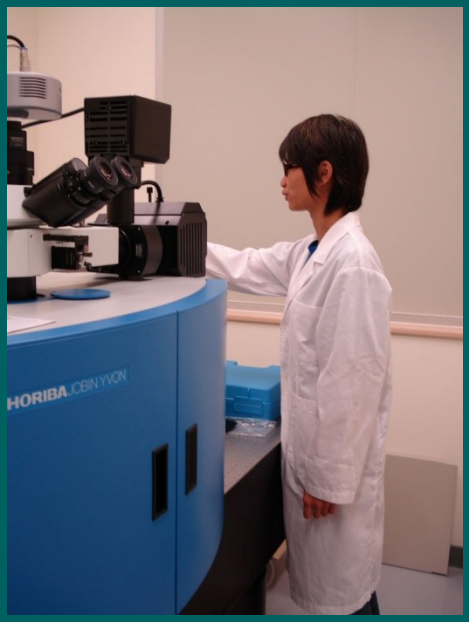
我們的工作伙伴



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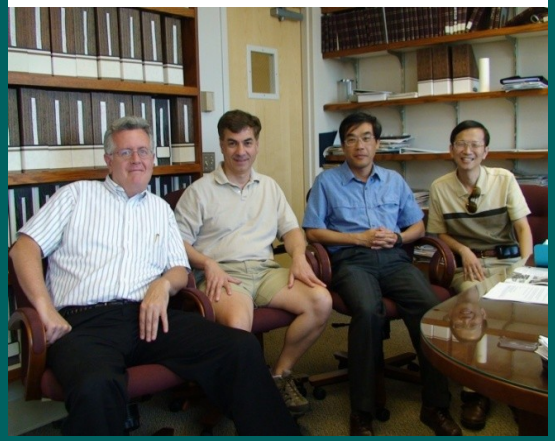
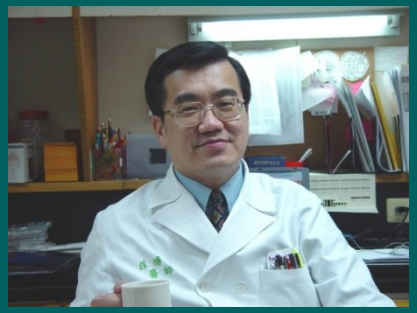
我們的工作伙伴

台美檢驗科技公司



Prof. Shahriar Mobashery
University of Notre Dame



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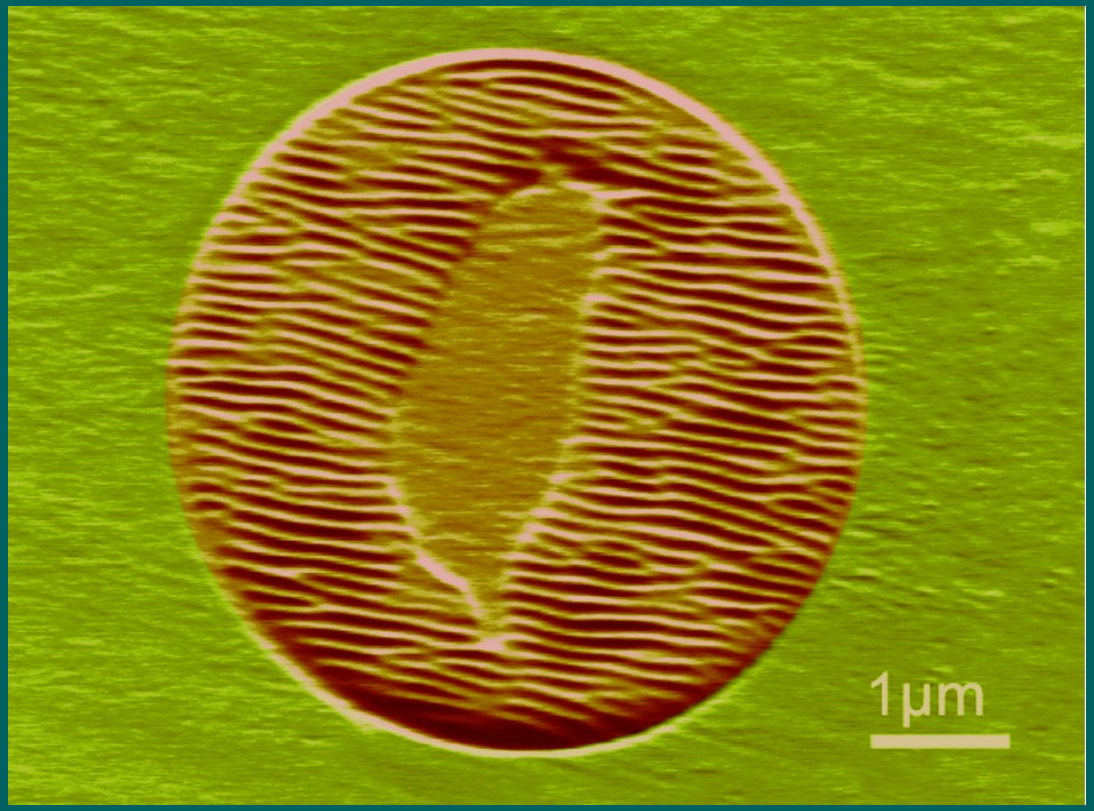
Thank you for your attention



'Certification of diamond using an irreproducible nano-textured pattern fabricated by ion-beam bombardment induced self-organization (Taiwan, R.O.C. Patent Number I 203406, April 24, 2006)

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