Tailoring Nanoparticles to Study Interactions in Bio–Systems and Diagnostics Using Raman Spectroscopy

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Light Scattering Laboratory

- Phase transitions in materials – high pressure/temperature studies probed by Raman and Brillouin spectroscopy.
- Elastic properties of nano and bulk materials.
- Surface Enhanced Raman Studies (SERS) for drug-protein interactions, biological systems.
- Raman as diagnostic probe for biological systems.
- Raman Imaging using TERS.
Raman spectra of CCl$_4$
Raman spectra of $\text{CCl}_4$
Raman Spectra of SWNTs

- Milli Dresselhaus

$A_{1g} \ 165 \text{ cm}^{-1}$
Raman Spectra of SWNTs

$E_{lg}$ 1585 cm$^{-1}$
Raman Spectra of SWNTs

$E_{2g}$ 1591 cm$^{-1}$
Energy diagram and Quantum picture

Electronic states

Vibrational states

Virtual states

Raman cross section

If $E_i = E_a$ or $E_s = E_b$
We have Resonance Raman effect
Miniaturization of Raman setup
Miniaturization of Raman
Micro-Raman setup for SERS experiments

- LASER
- Stage
- Objective lens
- Dichroic Mirror
- Optical fiber
- CCD
- Computer
- Mono-chromator
- Camera
- Focusing lens
- Edge filter
- Objective lens
- Microscope

Why Raman Spectroscopy?

- Use photons in the UV, visible and near-infrared - can be used as in situ spectroscopy. Used through a somewhat transparent gas phase, liquid or solid towards the surface under investigation.
- The full spectral range from virtually 0 to more than 4000 wavenumbers is accessible.
- The resolution is a few wavenumbers or better.
- The major drawback is the usually very low Raman cross section. – SERS a saving grace!
Motivation behind Surface Enhanced Raman Spectroscopy (SERS)

Single Molecular detection – $10^{-18}$
Scattering Cross Section for Fluorescence – $10^{-16}$
Scattering Cross Section for Raman – $10^{-32}$
with SERS enhancements of $10^{14} – 10^{-18}$
Challenges in Use of Raman Spectroscopy in Biology

- Normal Raman of Bio-molecules ideal but difficult
- SERS is an alternative – Ag is the best but is toxic to cell – Alkali metals a possibility
- SERS provides limited information – but still has potential
- SERRS a potential tool in diagnostics – a limited number of molecules presently available
Origin of SERS

- Initially – thought to be depended on Surface Area of the rough surface
- It was shown that the scattering cross section far exceeded the number of molecules on the rough surface.
- It was proposed that the origin was due to surface plasmons
- Alkali and Ag the best, Au and Cu the next best, Al, In, Pt followed by transition metals and then bad conductors.
- Exciting wavelength, polarization, exact nature of the nanostructure also effect the SERS
Plasmons in Nanoparticles

**Plasmons**

Jellium model in metals

Discrete positive nuclei

Approximated by continuous, immobile positive charge distribution

Free Electron cloud

Jellium

Displacement → Coulomb Restoring Force

Plasmon Oscillation!

Collective, coherent oscillation of charge
Surface Enhanced Raman Scattering (SERS)

Raman signal intensity gets enhanced when molecules are adsorbed on metal nanoparticles, colloids, island films etc.

Pathways for enhancement:

Electromagnetic enhancement
Enhanced local optical fields of metallic nanostructure

Chemical enhancement
Molecule-nanostructure system provide new energy states
Electromagnetic Effect

- Creation of Surface Plasmon Modes
- Dipole–Dipole interaction to the molecule
- Vibrational Modes created and annihilated
- Molecule to Metal transfer of energy.
- Emission of photon
Chemical Effect

- Excitation of electron from VB to empty CB band
- Charge Transfer process from metal to molecule.
- Vibrational state changes.
- Molecule to metal energy transfer.
- Emission from metal.
- Can be tuned by applying a voltage.

\[ h\omega = E_{ct} - \left[ E_r(0) + eV \right] \]
Tip Enhanced Raman Spectroscopy (TERS)

- Coupling Scanning Microscope or Atomic Microscope to Raman spectroscopy.
- Huge electromagnetic enhancement takes place at the tip with dimensions less than wavelength of light.
- Small fraction of the molecules close to the tip feel the electromagnetic enhancement.
- Has a potential as single molecular spectroscopy.
TERS of Cresyl Blue dye

Brilliant Cresyl Blue (BCB) Dye on Gold

BCB on smooth gold
integration time: 100 s
accumulations: 2
objective: 100X
laser power: 1mW

tip tunneling
tip retracted
Cellulose - Ag and Ag@Fe$_3$O$_4$ Composites
Time-dependent cytotoxicity of Ag@Fe$_3$O$_4$ nanoparticles
Ag Core – Au Shell Nanoparticles with hot spots

SERS from Hot Spots

Ad – Adenine, TP – Thiophenol, Imd – Imidazole, ATP – adenosine Triphosphate, Hb - Hemoglobin

Tailoring Gold’s Plasmonic properties
Tailoring Gold’s Plasmonic properties

Crystal Violet

Raman Intensity

Wavenumber / cm⁻¹
Drug Protein Interaction studies using SERS

With Tapas Kundu, JNCASR
The Dynamic Chromatin

9.5 The solenoid model of condensed chromatin

Hizume et al, Biochemistry, 2005, 44, p12978
Kundu and Takeyasu, 2005
Factors Influencing Transcriptional Competence

- **Histone Acetyltransferases**: p300, CBP, GCN5
- **Histone Methyltransferases**: Ash1, CARM1, PRMT1
- **ATP Dependent Remodeling Complexes**: SWI/SNF, Rsc, NURF
- **Non-Histone Chromatin Proteins**: HMG’s

**OPEN**

- **Histone Chaperones**: Asf1, CAF1, Spt16, NPM1

**Transcriptionally Active**

**CLOSED**

- **Histone Deacetylase**: HDAC1, HDAC4, SIRT1
- **Histone Methyltransferases**: SuV39A, Dot1, G9a
- **ATP Dependent Remodeling Complexes**: NuRD
- **Non-Histone Chromatin Proteins**: HP1, MeCP2, PARP1, PC4
SERS of p300

at 10 nM concentration

coactivator-associated arginine methyltransferase 1 (CARM1)

Autoacetylation of HAT domain

<table>
<thead>
<tr>
<th>S.No</th>
<th>HAT modulator</th>
<th>Reference</th>
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<tbody>
<tr>
<td>1</td>
<td>Lysyl-CoA</td>
<td>Lau et al., 2000</td>
</tr>
<tr>
<td>2</td>
<td>H3-CoA-20</td>
<td>Lau et al., 2000</td>
</tr>
<tr>
<td>3</td>
<td>Anacardic acid</td>
<td>Balasubramanyam et al., 2003</td>
</tr>
<tr>
<td>4</td>
<td>CTPB (activator)</td>
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<tr>
<td>5</td>
<td>Garcinol</td>
<td>Balasubramanyam et al., 2004</td>
</tr>
<tr>
<td>6</td>
<td>Curcumin</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>γ-butyrolactones</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Isothiazolones</td>
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</tbody>
</table>
Activity

CTPB

Interaction studies

Synthetic HAT Domain of p300

Effect of hydrophobic functional group

Conformation Changes in Transactivator Protein C by Mg$^{2+}$ Viewed by SERS

With V. Nagaraja, IISc
C- protein transactivator of Bacteriophage Mu

- Mg$^{2+}$ is an important ion in DNA replication, transcription, repair, restriction, transposition.
- C-protein is transcription activator for RNA polymerase.
- It binds to DNA at a specific recognition sequence only in presence of Mg$^{2+}$.
- Recent CD spectra suggests conformational changes – changes in $\alpha$ – helix
C-Protein SERS – effect of Mg$^{2+}$

Without Mg ion

With Mg ion

Region of $\alpha$ and $\beta$ – helix
In vivo tumor targeting and spectroscopic detection with surface-enhanced Raman nanoparticle tags
Design of pegylated SERS nanotags

- The basic functional and structural properties of nanometer-sized particles are different from either discrete molecules or bulk materials
- Pegylated gold particle exhibit excellent in vivo biodistribution and pharmacokinetic properties
- Non toxic & enhances the Raman signal
- Doesn’t replace the Raman reporters & hence SERS signal remain the same
- PEG coating layer allows efficient conjugation
Preparation of targeted SERS nanoparticles using mixture of SH-PEG & a heterofunctional PEG (SH-PEG-COOH)

The human head & neck carcinoma cells (Tu 686)
(a,b) SERS spectra obtained from the tumor and the liver locations by using targeted (a) and nontargeted (b) nanoparticles. (c) Photographs showing a laser beam focusing on the tumor site or on the anatomical location of Liver.
Non-PCR based detection of HIV-1 subtypes and recombinants using SERRS

With Udaykumar Ranga, JNCASR
HIV STRAINS

HIV-1

M
N
O

Viral subtypes

A B C D J

---to---

HIV-2

A B C D E

99% (India)
13% 56% (Global)
Two types of HIV-1 Recombinants

- 32 circulating recombinant forms (CRF) are known today
  - Molecularly identical viruses isolated from at least from 3 subjects who are not related to each other
  - Molecular clones are isolated
- Several hundreds of unique recombinant forms (URF)
  - The JNC lab detected two B/C recombinant viruses in India for the first time
    - Siddappa NB et al, AIDS, 2005
Pathogenic significance of the recombinant viruses

- Recombinants could have survival advantage over the parental strains
  - B/C of China
  - A/E of Thailand
  - A/G of Africa

- Intervention strategies must be reevaluated
  - Vaccine design
  - Medicines
Strategies to detect recombinant viruses

• **Sequencing the whole virus**
  - Golden standard
  - Universal application: useful for all the known and emerging viruses
  - Technically demanding
  - Expensive
  - Doesn’t lend for automation

• **Multi-probe hybridization assay**
  - A real-time PCR based technique
  - Expensive: several TaqMan probes required
  - Limited application: Useful for detecting 2 or 3 subtypes
  - False positive results (especially in sub-optimal conditions)
Schematic representation of the genetic characterization of the HIV-1 subtypes

- Silane-modified glass surface
- Amino-modified Capture probe (Subtype-specific)
- Raman probe Modified Detector Probe (Common to all viral subtypes)
- RNA (Template)
- Glass slide

A microarray-based detection strategy on a glass slide

- **Capture probes**: Multiple window- and subtype-specific probes
- **Direct capture** of the viral RNA (no PCR)
- **Detection probes**: 2 or 3 highly conserved probes detect all subtypes and conjugated to a Raman reporter
- **SERS-mediated probe detection**

- To identify capture probes in the backdrop of the viral genomic diversity
- To ensure the integrity of the viral RNA

- **No amplification required**
- A single or few detector probes

- **False negative results**
HIV-1 subtype-C capture probe detects only C, not A and B strains

- Window-1 (LTR)
- Subtype-C capture probe (N 730c)
- Acquisition time 1 sec
- Assay sensitivity approx. $10^4$ molecules

Template molecules (ss DNA)

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<tr>
<th></th>
<th>$10^{13}$</th>
<th>$10^{12}$</th>
<th>$10^{11}$</th>
<th>$10^{10}$</th>
</tr>
</thead>
</table>

SERRS intensity

Raman shift

Viral subtype

- A
- C
- B

20 Oct 2006
Checking for B Virus

Capture probe

B-virus

Summary

• SERS shows a great potential in study of Biological systems
• New nanostructures and new materials to be found
• Coupling of SERS with other techniques like CD and Optical Activity would increase the reach
• New techniques for high sensitivity needs to be devised
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