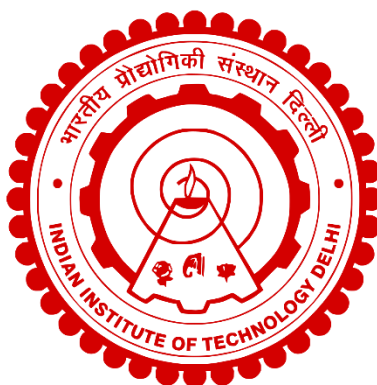


SPECTROSCOPIC STUDIES ON BIO-NANO INTERFACES USING RAMAN AND ENHANCED RAMAN SPECTROSCOPIC TECHNIQUES

Arti Sharma



Department of Chemistry

Indian Institute of Technology Delhi

April 2026

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by

Arti Sharma

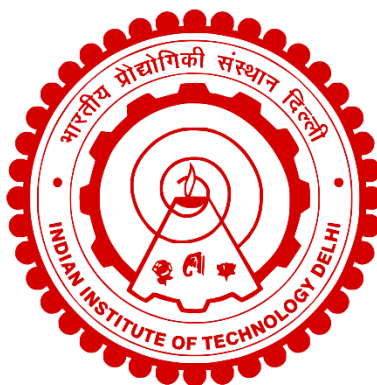
Department of Chemistry

Submitted

In fulfillment of the requirements of the degree of

Doctor of Philosophy

to the



Indian Institute of Technology Delhi

April 2026

Dedicated to my Parents

CERTIFICATE

This is to certify that the thesis entitled “**Spectroscopic Studies on Bio-Nano Interfaces Using Raman and Enhanced Raman Spectroscopic Techniques**” being submitted by **Ms. Arti Sharma** to the **Indian Institute of Technology Delhi** for the award of “**Doctor of Philosophy**” is a record of bonafide research carried out by her. She has worked under my guidance and supervision and has fulfilled the requirements for the submission of the thesis. To the best of my knowledge, the results contained in the thesis have not been submitted, in part or full, to any other university or institute for the award of any degree or diploma.

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Abstract

Proteins are ubiquitous in nature and play a crucial role in every living body, including maintaining cellular processes, controlling metabolism, regulating cell signaling, supporting immune defense, facilitating molecular recognition, and regulating gene expression. To gain more information about the protein's functionality, integrating it with nanoparticles has led to the emergence of a new field called nanotechnology. Nanoparticles have intriguing properties, such as a high surface-to-volume ratio and high electrical and optical properties. However, when these nanoparticles are injected into the body, the protein adsorbs to their surfaces, forming a protein corona that has detrimental effects on both the protein and the nanoparticles, including protein aggregation, amyloid fibril formation, nanoparticle aggregation, and many more.

Given the extensive use of nanoparticles in biomedical applications, people often overlook the adverse effects associated with protein-nanoparticle interactions. This thesis addresses this problem by studying the structural properties of the protein at the bio-nano interface using vibrational Raman spectroscopy. Vibrational Raman spectroscopy is a scattering-based technique that provides molecular-level insights into protein structure, both in the absence and in the presence of a perturbing environment. However, Raman is a weak-scattering phenomenon, and to enhance it, surface-enhanced Raman spectroscopy (SERS) is employed, which involves plasmonic nanoparticles such as gold and silver that use electromagnetic and chemical enhancement mechanisms to achieve high signal intensity at trace-level concentrations.

In this thesis, we investigated protein-nanoparticle interactions at the bio-nano interface. By combining molecular dynamics simulation and SERS, we studied protein-nanoparticle interactions using two bio-ionic liquids: one tends to adsorb to the nanoparticle surface, thereby preventing

direct protein-nanoparticle interaction, whereas the other remains suspended in solution and affects protein structure. Building on this, we studied the effect of iodide ions on two different lectins, wheat germ agglutinin (WGA) and concanavalin A (ConA), with and without their carbohydrate ligands, using SERS and principal component analysis (PCA). This study further unravels the effect of KI, widely used by researchers to obtain stronger SERS signals (for proteins). It discusses the impact of adding KI to protein nanoparticles and protein-ligand complexes.

Since Raman is a weak-scattering phenomenon, to enhance the signal, we tried to increase the chemical enhancement mechanism of SERS by pre-shining the microwave synthesized alumina nanofibers substrate with UV-visible light which leads to the formation of electron hole in the substrate which then subsequently transferred to the silver nanoparticles fermi level and thereby increasing its charge density and then in the presence of laser these electrons gets transferred to the analyte which leads to high signal intensity. This technique is called photoinduced Raman spectroscopy (PIERS). Parallely, we investigated the PIERS effect on the widely used titanium oxide (TiO_2) substrate by synthesizing TiO_2 substrates via different mechanisms, each with distinct oxygen vacancies. This thesis examines how defect engineering in plasmonic-semiconductor hybrids enhances photo-induced Raman responses, offering a route toward ultrasensitive biosensing platforms.

Overall, this thesis explains the complex interplay between proteins and nanoparticles at the bio-nano interface and demonstrates how interfacial engineering can control protein behavior. The findings in this thesis are very crucial in developing safer, more efficient nanomaterial-based platforms for biosensing, diagnostics, and biomedical applications. Moreover, the efforts involved in studying the PIERS mechanism with different substrates are useful for trace-level molecule detection.

सार

प्रोटीन प्रकृति में हर जगह पाए जाते हैं और हर जीवित शरीर में महत्वपूर्ण भूमिका निभाते हैं, जिसमें सेलुलर प्रक्रियाओं को बनाए रखना, मेटाबॉलिज्म को नियंत्रित करना, सेल सिग्नलिंग को रेगुलेट करना, इम्यून डिफेंस को सपोर्ट करना, मॉलिक्यूलर पहचान को आसान बनाना और जीन एक्सप्रेशन को रेगुलेट करना शामिल है। प्रोटीन की कार्यप्रणाली के बारे में अधिक जानकारी हासिल करने के लिए, इसे नैनोपार्टिकल्स के साथ इंटीग्रेट करने से नैनोटेक्नोलॉजी नामक एक नए क्षेत्र का उदय हुआ है। नैनोपार्टिकल्स में दिलचस्प गुण होते हैं, जैसे कि उच्च सतह-से-आयतन अनुपात और उच्च विद्युत और ऑप्टिकल गुण। हालांकि, जब इन नैनोपार्टिकल्स को शरीर में इंजेक्ट किया जाता है, तो प्रोटीन उनकी सतहों पर एड्सॉर्ब हो जाता है, जिससे एक प्रोटीन कोरोना बनता है जिसका प्रोटीन और नैनोपार्टिकल्स दोनों पर हानिकारक प्रभाव पड़ता है, जिसमें प्रोटीन एग्रीगेशन, एमाइलॉइड फाइब्रिल निर्माण, नैनोपार्टिकल एग्रीगेशन, और भी बहुत कुछ शामिल है।

बायोमेडिकल अनुप्रयोगों में नैनोपार्टिकल्स के व्यापक उपयोग को देखते हुए, लोग अक्सर प्रोटीन-नैनोपार्टिकल इंटरैक्शन से जुड़े प्रतिकूल प्रभावों को नज़रअंदाज़ कर देते हैं। यह थीसिस वाइब्रेशनल रमन स्पेक्ट्रोस्कोपी का उपयोग करके बायो-नैनो इंटरफ़ेस पर प्रोटीन के संरचनात्मक गुणों का अध्ययन करके इस समस्या का समाधान करती है। वाइब्रेशनल रमन स्पेक्ट्रोस्कोपी एक स्कैटरिंग-आधारित तकनीक है जो किसी भी परेशान करने वाले वातावरण के साथ या उसके बिना प्रोटीन संरचना में आणविक-स्तर की जानकारी प्रदान करती है। हालांकि, रमन एक कमजोर-स्कैटरिंग घटना है, और इसे बढ़ाने के लिए, सतह-संवर्धित रमन स्पेक्ट्रोस्कोपी (SERS) का उपयोग किया जाता है, जिसमें गोल्ड और सिल्वर जैसे प्लास्मोनिक नैनोपार्टिकल्स शामिल होते हैं जो ट्रेस-स्तर की सांद्रता पर उच्च सिग्नल तीव्रता प्राप्त करने के लिए इलेक्ट्रोमैग्नेटिक और रासायनिक वृद्धि तंत्र का उपयोग करते हैं।

इस थीसिस में, हमने बायो-नैनो इंटरफ़ेस पर प्रोटीन-नैनोपार्टिकल इंटरैक्शन की जांच की। मॉलिक्यूलर डायनामिक्स सिमुलेशन और SERS को मिलाकर, हमने दो बायो-आयनिक तरल पदार्थों का उपयोग करके प्रोटीन-नैनोपार्टिकल इंटरैक्शन का अध्ययन किया: एक नैनोपार्टिकल सतह पर एड्सॉर्ब होने की प्रवृत्ति रखता है, जिससे प्रत्यक्ष प्रोटीन-नैनोपार्टिकल इंटरैक्शन को रोका जा सके, जबकि दूसरा घोल में निलंबित रहता है और प्रोटीन संरचना को प्रभावित करता है। इसी आधार पर, हमने SERS और प्रिंसिपल कंपोनेंट एनालिसिस (PCA) का उपयोग करके, दो अलग-अलग लेक्टिन, व्हीट जर्म एग्लूटिनिन (WGA) और कॉनकैनावेलिन A (ConA) पर आयोडाइड आयनों के प्रभाव का अध्ययन किया, जिसमें उनके कार्बोहाइड्रेट लिगेंड के साथ और बिना शामिल थे। यह अध्ययन KI के प्रभाव को और उजागर करता है, जिसका उपयोग शोधकर्ताओं द्वारा मजबूत SERS सिग्नल (प्रोटीन के लिए) प्राप्त करने के लिए व्यापक रूप से किया जाता है। यह प्रोटीन नैनोपार्टिकल्स और प्रोटीन-लिगेंड कॉम्प्लेक्स में KI जोड़ने के प्रभाव पर चर्चा करता है। क्योंकि रमन एक कमजोर स्कैटरिंग घटना है, इसलिए सिग्नल को बढ़ाने के लिए, हमने सबस्ट्रेट को UV-विजिबल लाइट से पहले से चमकाकर SERS के केमिकल एन्हांसमेंट मैकेनिज्म को बढ़ाने की कोशिश की। इससे सबस्ट्रेट में इलेक्ट्रॉन होल बनते हैं जो बाद में सिल्वर नैनोपार्टिकल्स के फर्मी लेवल में ट्रांसफर हो जाते हैं, जिससे उनकी चार्ज डेंसिटी बढ़ जाती है और फिर लेजर की मौजूदगी में ये इलेक्ट्रॉन एनालाइट में ट्रांसफर हो जाते हैं, जिससे हाई सिग्नल इंटेन्सिटी मिलती है। इस तकनीक को फोटोइंड्यूस्ड रमन स्पेक्ट्रोस्कोपी (PIERS) कहा जाता है। इसके साथ ही, हमने अलग-अलग मैकेनिज्म से TiO₂ सबस्ट्रेट्स को सिंथेसाइज करके, हर एक में अलग-अलग ऑक्सीजन वैकेंसी के साथ, व्यापक रूप से इस्तेमाल होने वाले टाइटेनियम ऑक्साइड (TiO₂) सबस्ट्रेट पर PIERS प्रभाव की जांच की। यह काम जांचता है कि प्लास्मोनिक-सेमीकंडक्टर हाइब्रिड में डिफेक्ट इंजीनियरिंग फोटो-इंड्यूस्ड रमन रिस्पॉन्स को कैसे बढ़ाती है, जो अल्ट्रासेंसिटिव बायोसेंसिंग प्लेटफॉर्म की दिशा में एक रास्ता दिखाती है।

कुल मिलाकर, यह अध्ययन बायो-नैनो इंटरफ़ेस पर प्रोटीन और नैनोपार्टिकल्स के बीच जटिल तालमेल को समझाता है और दिखाता है कि इंटरफेशियल इंजीनियरिंग प्रोटीन के व्यवहार को कैसे नियंत्रित कर सकती है। ये निष्कर्ष बायोसेसिंग, डायग्नोस्टिक्स और बायोमेडिकल एप्लीकेशन के लिए सुरक्षित, अधिक कुशल नैनोमटेरियल-आधारित प्लेटफॉर्म विकसित करने में बहुत महत्वपूर्ण हैं। इसके अलावा, अलग-अलग सबस्ट्रेट के साथ PIERS मैकेनिज्म का अध्ययन करने में किए गए प्रयास ट्रेस-लेवल मॉलिक्यूल डिटेक्शन के लिए उपयोगी हैं।

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List of abbreviations

UV Ultraviolet

FTIR Fourier Transform Infrared

IR Infrared

SERS Surface-enhanced Raman Spectroscopy

DFT Density Functional Theory

UTI Urinary tract infection

STD Sexually transmitted diseases

XPS X-ray Photoelectron spectroscopy

CIDs Charge-Injection Devices

CCD's Charged Coupled Devices

PMT Photomultiplier Tube

CHA Concentric Hemispherical Analyzer

GERS Graphene-Enhanced Raman Scattering

PIERS Photoinduced-Enhanced Raman spectroscopy

WGA Wheat Germ Agglutinin

ConA Concanavalin

NAG N-acetylglucosamine

DLS Dynamic light scattering

PCA Principal Component analysis

TEM Transmission Electron Microscopy

AlNFs Alumina Nanofibers

USP Ultrasonic spray pyrolysis

AgNPs Silver Nanoparticles

CD Circular Dichroism
COM Center of mass
DCCM Dynamic Cross-correlation maps
[Ch][DHC] Choline Dihydrogen Citrate
[Ch][Cl] Choline Chloride
LSPR Localized Surface Plasmonic Resonance
Lyz Lysozyme
MRE Mean Residual Ellipticity
MD molecular Dynamics
PBC Periodic boundary Condition
RMSD Root Mean Square Deviation
RMSF Root Mean Square Fluctuations
PME Particle Mesh Ewald
PDB Protein Data Bank
LINCS Linear Constraint Solver
NP nanoparticle
PRET Plasmon Resonant Energy Transfer
PICS Plasmon-induced charge separation