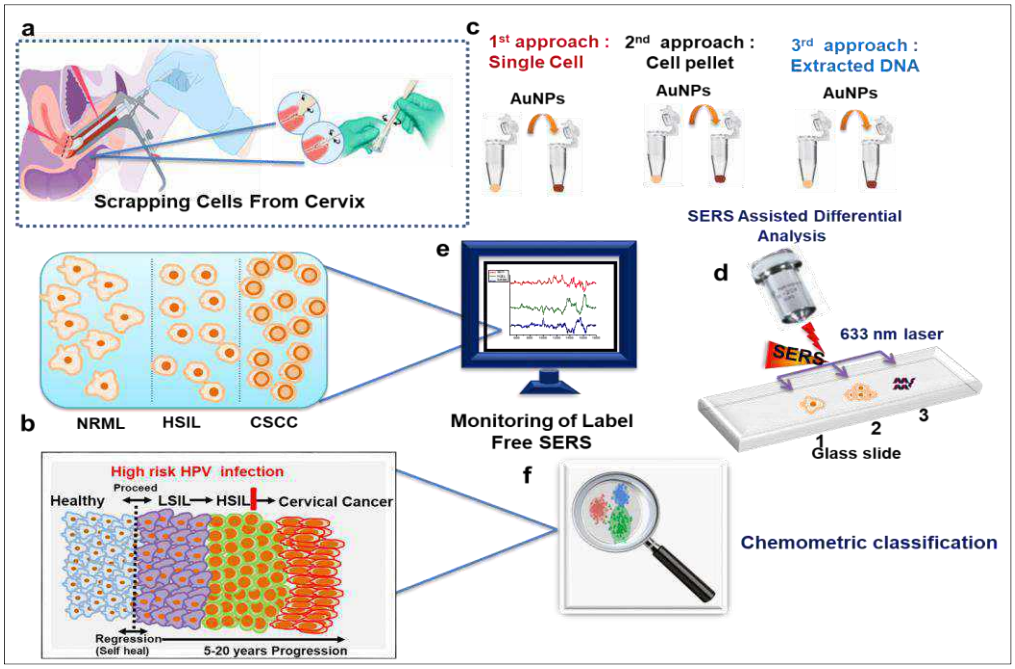
Highlights of ten best publications:

A. Diagnostic Research Based on Raman Spectroscopy:

1. Diagnostic Spectro-cytology revealing differential recognition of **cervical Cancer lesions** by label-free surface enhanced Raman fingerprints and Chemomssetrics; Varsha Karunakaran, Valliamma N. Saritha, Manu M.Joseph, Jyothi B. Nair, Giridharan Saranya, Kozhiparambil G. Raghu, Kunjuraman Sujathan*, Krishnan Nair S. Kumar*, Kaustabh K. Maiti*
   Nanomedicine: Nanotechnology, Biology and Medicine, 2020, 29, 102276 (Impact Factor: 6.45).

Herein, a new spectroscopy based diagnostic modality has been developed by utilizing label free ultrasensitive surface enhanced Raman scattering (SERS) technique to generate a differential spectral fingerprint for the prediction of normal (NRML), high-grade intraepithelial lesion (HSIL) and cervical squamous cell carcinoma (CSCC) from exfoliated cell samples of cervix. Three different approaches i.e., single-cell, cell-pellet and extracted DNA from oncology clinic as confirmed by Pap test and HPV PCR were employed. Gold nanoparticles as the SERS substrate favoured the increment of Raman intensity exhibited signature identity for Amide III/Nucleobases and carotenoid/glycogen respectively from clinical samples for establishing the empirical discrimination. Moreover, all the spectral invention was subjected to Artificial intelligence (AI) tool which includes Support Vector Machine (SVM) and furnished an average diagnostic accuracy of 94%, 74% and 92 % of the three grades. The current discovery with the combination of SERS read-out and AI in field trial promises its potential to reduce the incidence in low resource countries.

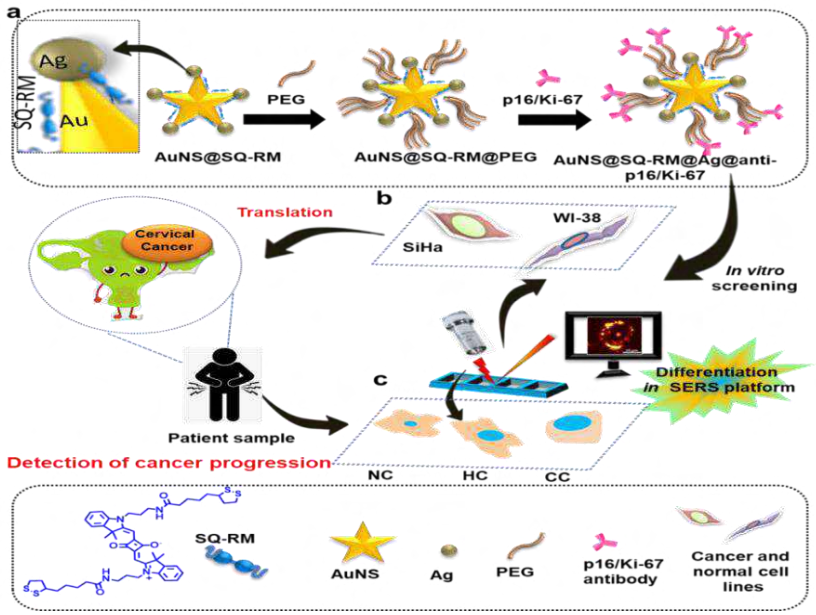


Schematic illustration of experimental design for differentiating three grades viz. normal (NRML), high grade intraepithelial lesion (HSIL), cervical squamous cell carcinoma (CSCC) using SERS., a) Scrapping cells from the cervix using cytobrush, b) progression pattern of cervical cancer c) Set 1: single cell, Set 2: cell pellet, Set 3: extracted DNA (mixed with AuNPs), d) independent SERS analysis

of 1) single cell,2) cell pellet, 3) extracted DNA in glass slide, d) empirical signal monitoring of the three grades f) chemometric analysis.

2. Elucidating Raman Image-Guided Differential Recognition of **Clinically Confirmed Grades of Cervical Exfoliated Cells** by Dual Biomarker-Appended SERS-Tag; Varsha Karunakaran, Valliamma N. Saritha, Adukkadan N. Ramya, Vishnu Priya Murali, Kozhiparambil G. Raghu, Kunjuraman Sujathan,* and Kaustabh Kumar Maiti*, ***Analytical Chemistry,*** 2021, 93, 32, 11140–11150, (Impact Factor: 6.98)

Ultrasensitive detection of cancer biomarkers via single cell analysis through Raman imaging is an impending approach which modulates the possibility of early diagnosis. Cervical cancer is one such type which can be monitored for a sufficiently long period towards invasive cancer phenotype. Herein, a surface enhanced Raman scattering (SERS) nanotag (SERS-tag) has been successfully implemented for the simultaneous detection of p16/K-i67, a dual biomarker persisting in the progression of squamous cell carcinoma of human cervix. The recognition by the SERS-tag was first validated in cervical squamous cell carcinoma cell line, SiHa as a foot-step study and subsequently implemented to different grades of clinically confirmed exfoliated cells including normal cell (NC), high-grade intraepithelial lesion (HC) and squamous cell carcinoma (CC) samples of the cervix. We observed a distinct intensity hike of around ten-fold in the single dysplastic HC and CC samples in comparison to NC specimen which clearly justify the prevalence of p16/Ki-67. Amidst the challenges in Raman image guided modality, the technique was further complemented with the gold standard immunocytochemistry dual staining analysis. The synthesized probe is able to map the abnormal cells within 20 min with high reproducibility and stability after antigen retrieval step for 1mm x 1mm mapping area with good contrast. The tedious time-consuming steps can be avoided and real time read out can be achieved using the SERS mapping unlike immunocytochemistry technique. Therefore, the newly developed Raman image guided SERS imaging emphasizes the approach of uplifting of SERS in practical utility with further improvement for clinical applications for cervical cancer detection in future.

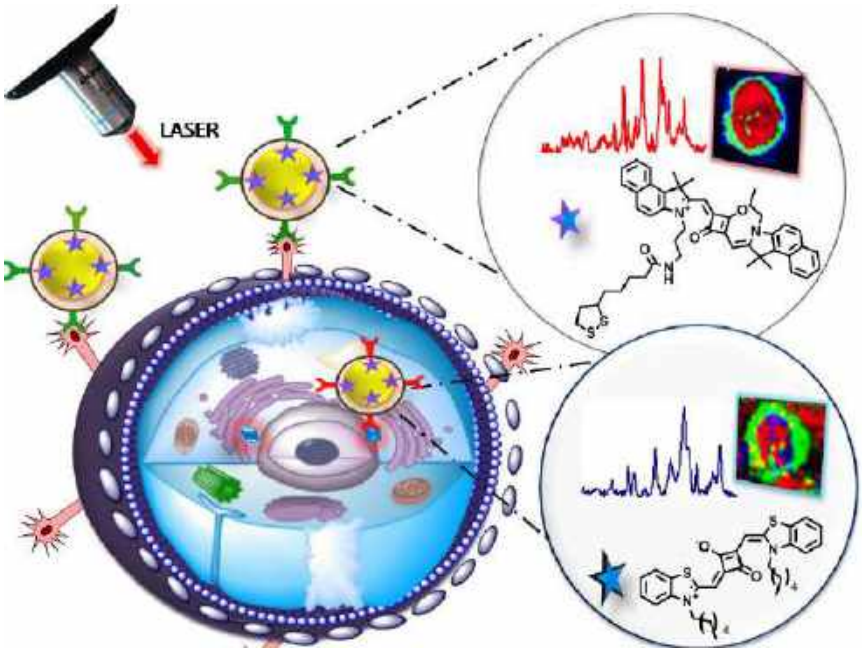


Schematic illustration for experimental design for differentiating three grades viz. normal cell (NC), high grade intraepithelial lesion (HC), cervical squamous cell carcinoma (CC) using SERS nanotags,

a) synthetic scheme of AuNS@SQ-RM@Ag@PEG@anti-p16/Ki-67, b) SERS mapping in cell lines, SiHa and WI-38, c) SERS mapping in clinical samples, Normal cell (NC), b) High grade squamous intra epithelial lesion cell (HC) and c) cervical squamous cell carcinoma (CC) samples.

3. Aggregation induced Raman scattering of squaraine dye: Implementation in diagnosis of **cervical cancer dysplasia** by SERS imaging ; Nisha Narayanan, Varsha Karunakaran, Willi Paul, Karunakaran Venugopal, K. Sujathan, Kaustabh Kumar Maiti*, ***Biosensors and Bioelectronics.,*** 2015, 70, 145-152 (Impact Factor: 10.25)

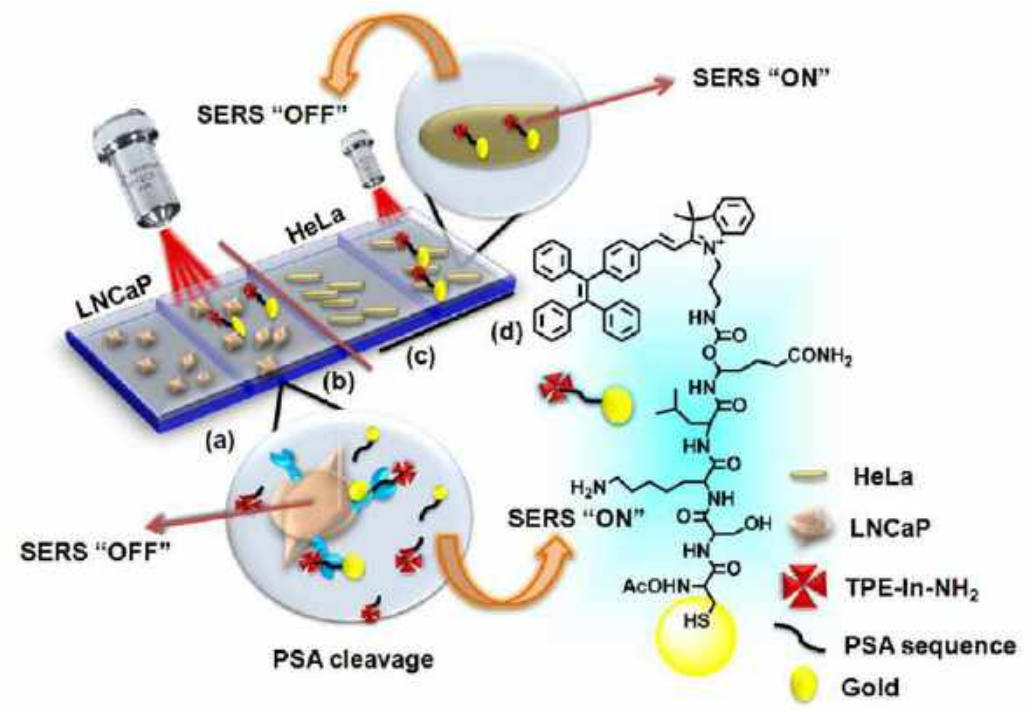
The extent of Raman reporter (RM) molecule aggregation that reflects on surface enhanced Raman signal scattering (SERS) intensity upon adsorption on nano-roughened gold surface has been discovered. Herein, a serious of six squaraine based RM designated as SQ1, SQ2, SQ3, SQ4, SQ5 and SQ6 has been synthesized. Interestingly, SQ2 (mono lipoic acid appended), SQ5 and SQ6 (conjugated with hexyl and dodecyl side chain) derivatives having more tendency of aggregation in DMSO-water mixed solvent showed significant increase of Raman scattering in the fingerprint region when chemisorbed on spherical gold nanoparticles. Two sets of SERS nanotags were prepared with colloidal gold nanoparticle (Au-NPs size: 40nm) by incorporating Raman reporters SQ2 and SQ5 followed by thiolated PEG encapsulation (SH-PEG, SH-PEG-COOH) denoted as AuNPs-SQ2-PEG and AuNPs- SQ5- PEG. Further conjugation of these nanotag with monoclonal antibodies specific to over expressed receptors, EGFR and p16/Ki-67 in cervical cancer cell, HeLa showed prominent SERS mapping intensity and selectivity towards cell surface and nucleus. The fast and accurate recognition obtained by antibody triggered SERS-nanotag has been compared with conventional time-consuming immunocytochemistry technique which prompted us to extend further investigation using real patient cervical smear sample for a non-invasive, ultrafast and accurate diagnosis.



Schematic illustration of cancer cell recognition by SERS by nanotags

4. New Insight of Tetraphenylethylene-based Raman Signatures for Targeted SERS Nanoprobe Construction Toward **Prostate Cancer Cell** Detection;Adukkadan N. Ramya, Manu M. Joseph, Jyothi B. Nair, Varsha Karunakaran,Nisha Narayanan, and Kaustabh Kumar Maiti*, ***ACS Appl. Mater. Interfaces***., 2016, 8, 10220-10225 (Impact Factor: 9.22).

Newly designed and synthesized tetraphenylethylene (TPE) appended molecular probe unfold their unique Raman fingerprints reflected by surface enhanced Raman scattering (SERS) upon adsorption on nano-roughened gold surface. A series of five TPE analogues has been synthesized and interestingly, all the five TPE analogues produced multiplexing Raman signal pattern, out of which TPE-In-Boc showed significant increase in signal intensity in the fingerprint region. An efficient SERS nanoprobe has been constructed using gold nanoparticles as SERS substrate, and the TPE-In as the Raman reporter, which conjugated with a specific peptide substrate, Cys-Ser-Lys-Leu-Gln-OH, well known for the recognition of prostate specific antigen (PSA). The designated nanoprobe TPE-In-PSA@Au acted as SERS "ON/OFF" probe in peace with the vicinity of PSA protease which distinctly recognizes PSA expression with a limit of detection (LOD) of 0.5 ng in SERS platform. Furthermore, TPE-In-PSA@Au nanoprobe was efficiently recognized the over-expressed PSA in human LNCaP cell which can be visualized through SERS spectral analysis and SERS mapping.

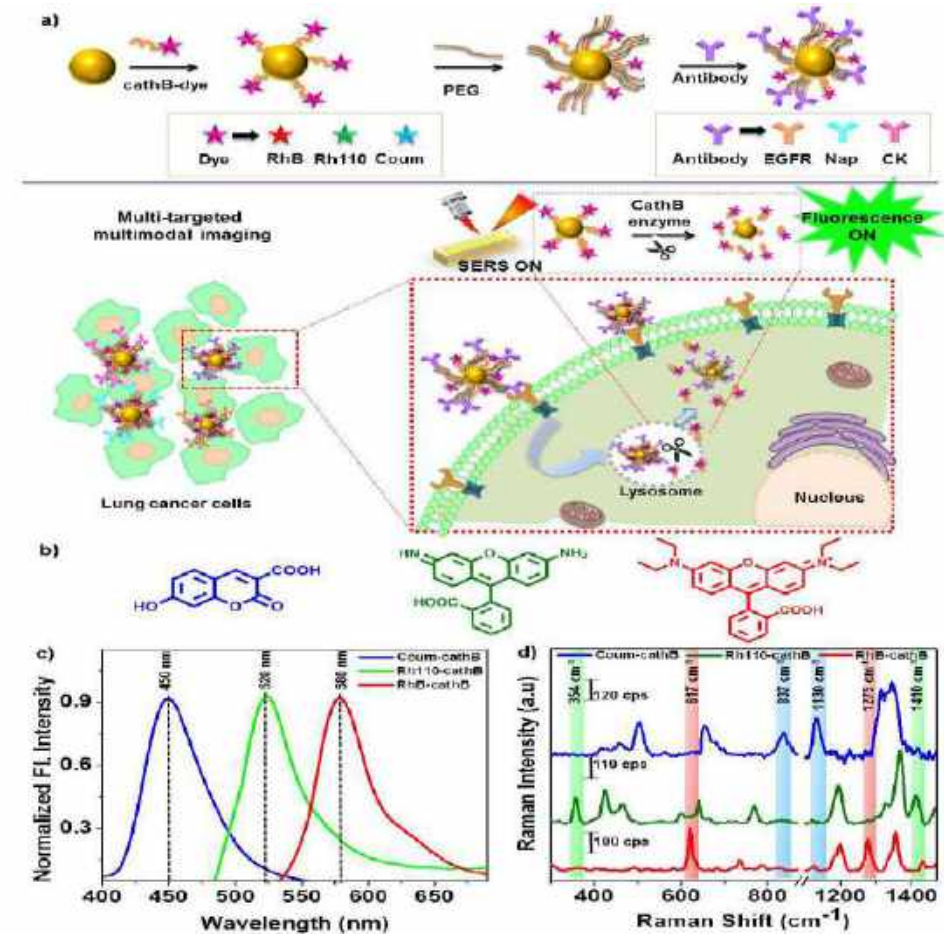


Thematic representation of PSA recognition by TPE-In-PSA@Au nanoprobe by SERS; (a) LNCaP cells without nanoprobe (b) with nanoprobe, HeLa cells (c) without nanoprobe (d) with nanoprobe.

5. Enzyme-Driven Switchable Fluorescence-SERS Diagnostic Nanococktail for the Multiplex Detection of **Lung Cancer Biomarkers**; Giridharan Saranya,Manu M. Joseph, Varsha Karunakaran, Jyothi B. Nair, Valliamma N. Saritha, Vamadevan S. Veena, Kunjuraman Sujathan, Ayyappanpillai Ajayaghosh*, and Kaustabh K.

Maiti*, ***ACS Applied Materials and Interfaces,*** 2018, 10 (45), pp 38807–38818 (Impact Factor: 9.22).

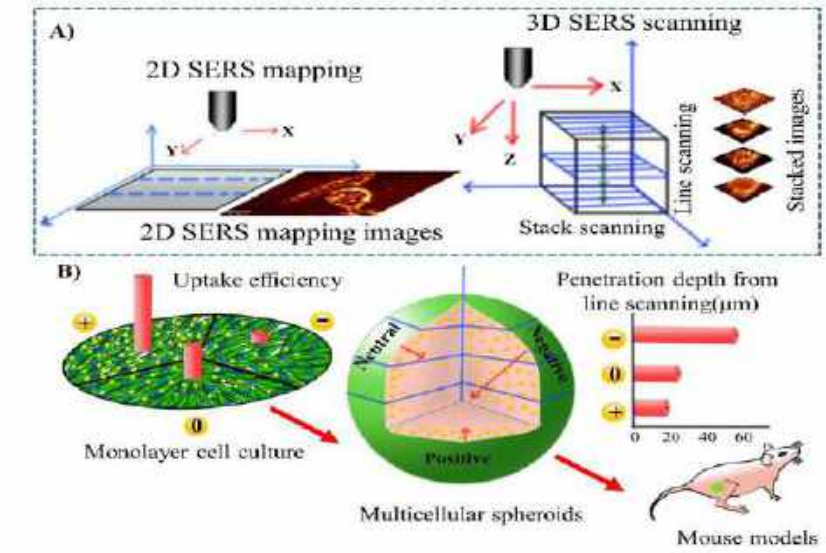
Comprehensive profiling of multiple protein targets plays a critical role in the deeper understanding of specific disease conditions associated with high heterogeneity and complexity. Herein, a modular fabrication of smart programmable nanoarchitectures, which could integrate clinically relevant diagnostic modalities for the multiplexed detection of most prevalent panel of disease biomarkers present in lung cancer. The multiplex nanoprobes were prepared by attaching dual-functional Raman active fluorogens onto spherical gold nanoparticles through a peptide linker, Phe-Lys-Cys (FKC) which is engineered with a cathepsin B (cathB) enzyme cleavage site. Presence of the cathB induces the scission of FKC upon homing into the cancer cells, resulting in the release of the initially latent fluorophores with a concomitant quenching of the surface enhanced Raman signal intensity, thereby realizing an on-off switching between the fluorescence and Raman modalities. The enzyme triggered switchable nanoprobes were utilized for the simultaneous detection of pathologically relevant lung cancer targets by tethering with specific antibody units. The multiplex-targeted multi-color-coded detection capability of the antitags was successfully developed as a valid protein screening methodology which can address the unmet challenges in the conventional clinical scenario for the precise and early diagnosis of lung cancer.



Schematic illustration of enzyme triggered switching of antibody functionalized FSENPs for the multiplexed detection of lung cancer biomarkers. b) Chemical structures of 7-hydroxy-3-carboxycoumarin (blue), rhodamine 110 (green) and rhodamine B (red). c) Fluorescence and d) SERS spectral analysis

6. Surface charge modulates the internalization vs penetration of gold nanoparticles: comprehensive scrutiny on **monolayer cancer cells, multicellular spheroids and solid tumors by SERS modality**; Palasseri T. Sujai, Manu M. Joseph,* Giridharan Saranya, Jyothi B. Nair, Vishnu Priya Murali and Kaustabh Kumar Maiti*; ***Nanoscale***, 2020, 12, 6971–6975 (Impact Factor: 7.79).

Precise control over the dynamics of nanoparticles (NPs) in a tumor microenvironment is highly warranted for the development of an efficient nanotheranostic agent. Even though inductively coupled plasma mass spectrometry can provide a quantitative assessment regarding the uptake efficiency of metal NPs, enumeration of deep tissue penetration capacity remains as a challenge. Herein, an accurate tracking of the uptake efficiency and penetration phenomenon of gold nanoparticles (AuNPs: 40–50 nm) has been evaluated with respect to three different surface charges in monolayer (2D) cells, multicellular spheroids (3D) and in vivo tumors by surface-enhanced Raman spectroscopy (SERS). While positively charged AuNPs showed around two-fold increased internalization in monolayer cells, SERS-tag-based line scanning on multi-layered tumor spheroids illustrated almost nine-fold superior penetration capability with negatively charged AuNPs. Further, the enhanced solid tumor distribution contributed by the negatively charged AuNPs could appreciably escalate its clinical utility in cancer management.



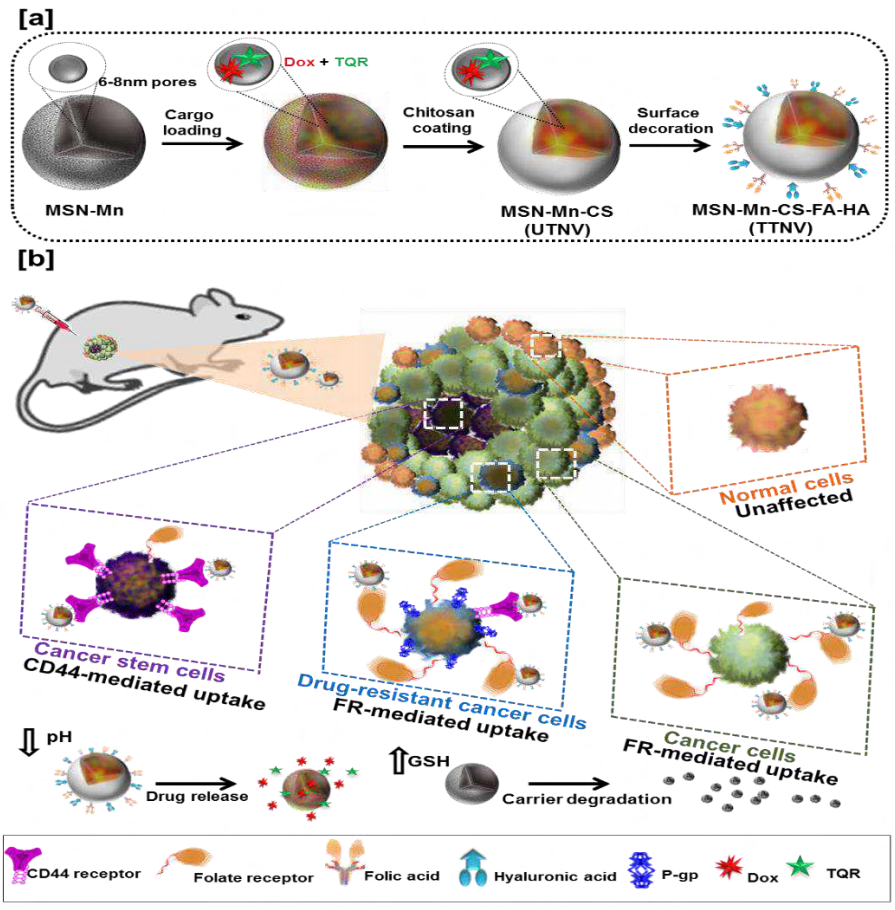
Different scanning modes on confocal Raman microscope used for SERS studies. (B) Schematic representation of differential distribution of AuNPs with respect to surface charges on monolayer cell culture, multicellular spheroids and in mouse models.

B. Nano-drug Delivery System (NDDS) with Raman Spectroscopy as a diagnostic modality

7. Targeted Theranostic **Nano Vehicle** Endorsed with Self-Destruction and Immunostimulatory Features to Circumvent Drug Resistance and Wipe-Out Tumor Reinitiating Cancer Stem Cells; Manu M. Joseph,* Adukkadan N. Ramya, Vineeth M.

Vijayan, Jyothi B. Nair, Blossom T. Bastian, Raveendran K. Pillai, Sreelekha T. Therakathinal,* and Kaustabh K. Maiti* ***Small***, 2020, 16, 2003309 (Impact Factor: 13.28)

The downsides of conventional cancer monotherapies are profound and enormously consequential, as drug-resistant cancer cells and cancer stem cells (CSC) are typically not eliminated. Here, a targeted theranostic nano vehicle (TTNV) using manganese-doped mesoporous silica nanoparticle (5 wt% of Mn in MSN) has been constructed with an ideal surface area and pore volume (389 m2/g and 6-8 nm) for co-loading an optimized ratio of antineoplastic doxorubicin and a drug efflux inhibitor tariquidar (TQR). This strategically framed TTNV was chemically conjugated with folic acid and hyaluronic acid as a dual-targeting entity to promote folate receptor (FR) mediated cancer cell and CD44 mediated CSC uptake, respectively. Interestingly, surface-enhanced Raman spectroscopy (SERS) was exploited to monitor drug release kinetics, differentiate drug resistance and also to evaluate the molecular changes associated with therapeutic progression. The superior antitumor response in FR-positive syngeneic and CSC-rich human xenograft murine models was associated with a tumor-targeted biodistribution, favorable pharmacokinetics, and an appealing bioelimination pattern of the TTNV with no palpable signs of toxicity. This dual drug-loaded, metal ion-doped nano vehicle, offers a feasible approach for efficient cancer therapy by on demand cargo release in order to execute complete wipe-out of tumor reinitiating cancer stem cells.

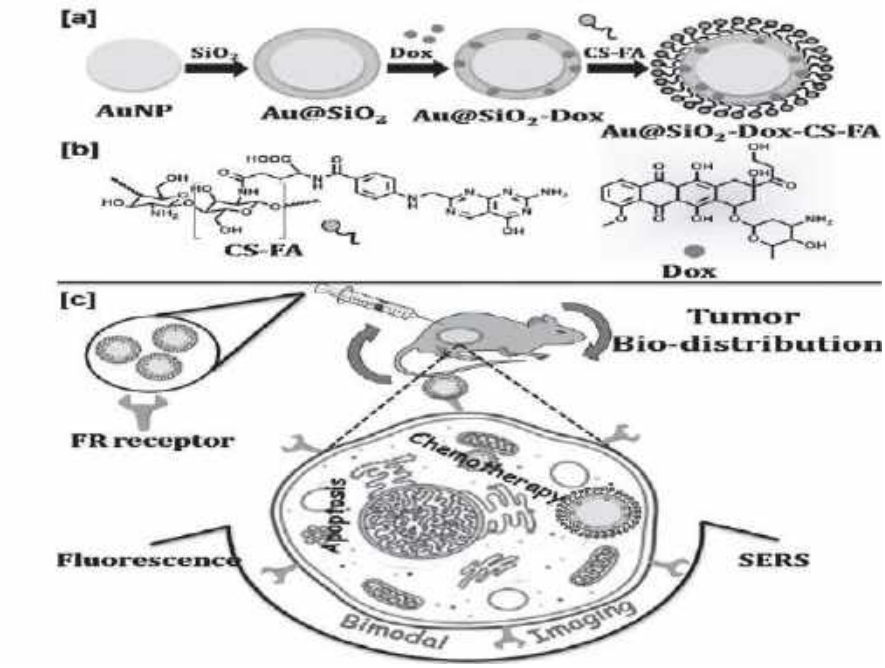


Various steps involved in the fabrication of TTNV. [b] Biological fate of TTNV on tumor-bearing mice. Heterogeneous tumor mass comprises normal cells, drug-resistant and drug-sensitive cancer cells and

CSCs. TTNV undergoes FR-mediated selective up take by cancer cells and CD44-mediated uptake by CSCs.

8. Emergence of Gold-Mesoporous Silica Hybrid **Nano-Theranostic: Dox-Encoded, Folate Targeted Chemotherapy** with Modulation of **SERS Fingerprinting** for Apoptosis Toward Tumor Eradication;Adukkadan N. Ramya, Manu M. Joseph,* Santhi Maniganda, Varsha Karunakaran, Sreelekha T.T,* and Kaustabh Kumar Maiti*,***Small,** 2017, 13, 1700819 (Impact Factor: 13.28).

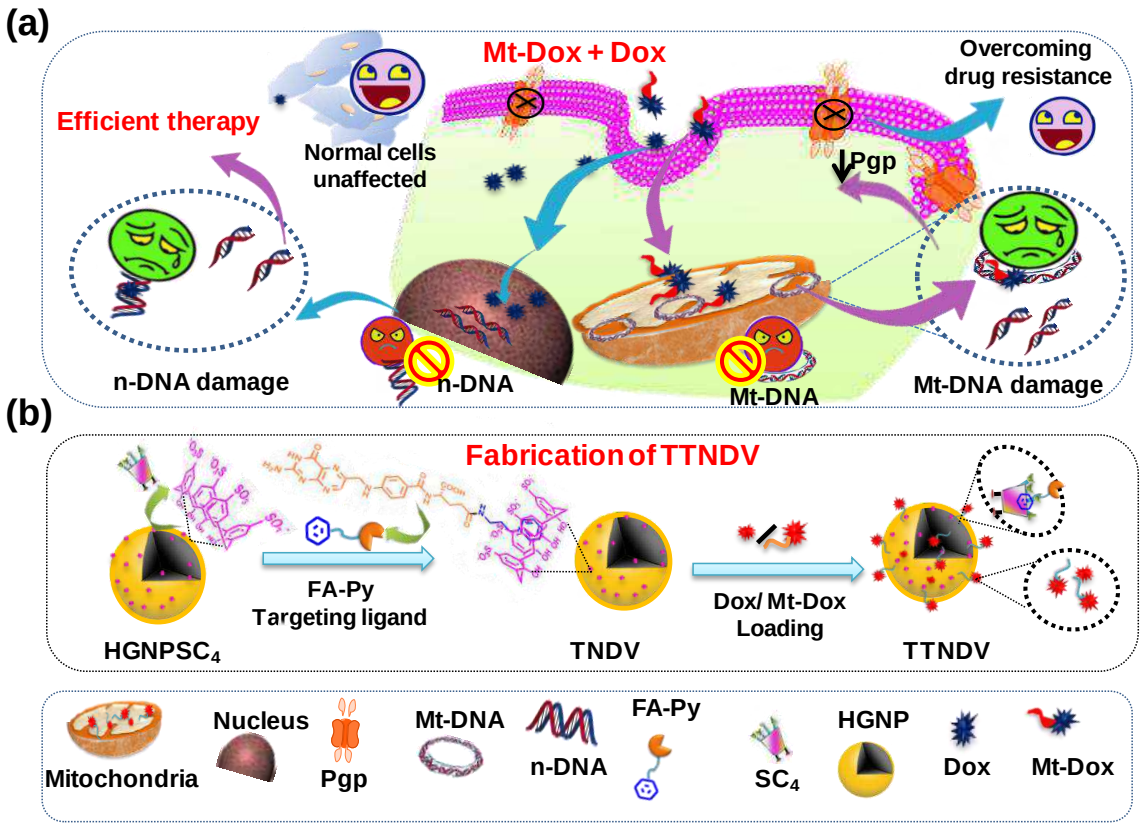
Strategically fabricated theranostic nanocarrier delivery system is an unmet need in personalized medicine. Herein, this study reports a versatile folate receptor (FR) targeted nanoenvelope delivery system (TNEDS) fabricated with gold core silica shell followed by chitosan–folic acid conjugate surface functionalization by for precise loading of doxorubicin (Dox), resembled as Au@SiO2-Dox-CS-FA. TNEDS possesses up to 90% Dox loading efficiency and internalized through endocytosis pathway leading to pH and redox-sensitive release kinetics. The superior FR-targeted cytotoxicity is evaluated by the nanocarrier in comparison with US Food and Drug Administration (FDA)-approved liposomal Dox conjugate, Lipodox. Moreover, TNEDS exhibits theranostic features through caspase-mediated apoptosis and envisages high surface plasmon resonance enabling the nanoconstruct as a promising surface enhanced Raman scattering (SERS) nanotag. Minuscule changes in the biochemical components inside cells exerted by the TNEDS along with the Dox release are evaluated explicitly in a time-dependent fashion using bimodal SERS/ fluorescence nanoprobe. Finally, TNEDS displays superior antitumor response in FR-positive ascites as well as solid tumor syngraft mouse models. Therefore, this futuristic TNEDS is expected to be a potential alternative as a clinically relevant theranostic nanomedicine to effectively combat neoplasia.



Various steps involved in the fabrication of Au@SiO2-Dox-CS-FA starting from AuNPs. b) Chemical structure of chitosan–folic acid(CS-FA) and Dox. c) Biological evaluation after i.p. administration of TNEDS on tumor-bearing mice.

9. Elucidating a Thermo-responsive Multimodal Photo-Chemotherapeutic **Nano-delivery Vehicle to Overcome the Barriers of Doxorubicin Therapy** ; Jyothi B Nair, Manu M Joseph*, Jayadev S Arya, Padincharapad Sreedevi, Palasseri T Sujai, and Kaustabh Kumar Maiti*; ***ACS Applied Materials and Interfaces***, 2020, 12, 39, 43365–43379 (Impact Factor: 9.22).

In an attempt to circumvent the major pitfalls associated with conventional chemotherapy including drug resistance and off-target toxicity, a strategic design has been evolved to simultaneously target both mitochondrial DNA (Mt-DNA) and nuclear DNA (n-DNA) with the aid of a targeted theranostic nano-delivery vehicle (TTNDV). Herein, a folic acid anchored *p*-sulfo-calix[4]arene (SC_4) capped hollow gold nanoparticles (HGNP) was meticulously loaded with a pre-tuned ratio (1:100) of antineoplastic doxorubicin (Dox) and its mitochondria targeted analogue, Mt-Dox for sustained thermo-responsive release of cargo. This therapeutic strategy was enabled to eradicate both n-DNA and Mt-DNA leaving no space to develop drug resistance. The SC_4 capped HGNPs ($HGNPSC_4$) was experimented for the first time as a photothermal (PTT) agent with 61.6% photothermal conversion efficiency, to generate tunable localized heat more efficiently than bare HGNPs. The as-synthesized TTNDV demonstrated to be an ideal substrate for surface-enhanced Raman scattering (SERS) to monitor the molecular level therapeutic progression in cells and spheroidal model. A significant reduction in the tumor mass with a marked survival benefits was archived in syngraft murine models through this synergistic photo-chemotherapy. Collectively, this multifunctional nanoplatform offers a robust approach to treat cancer without any scope of generating Dox resistance and off-target toxicity.



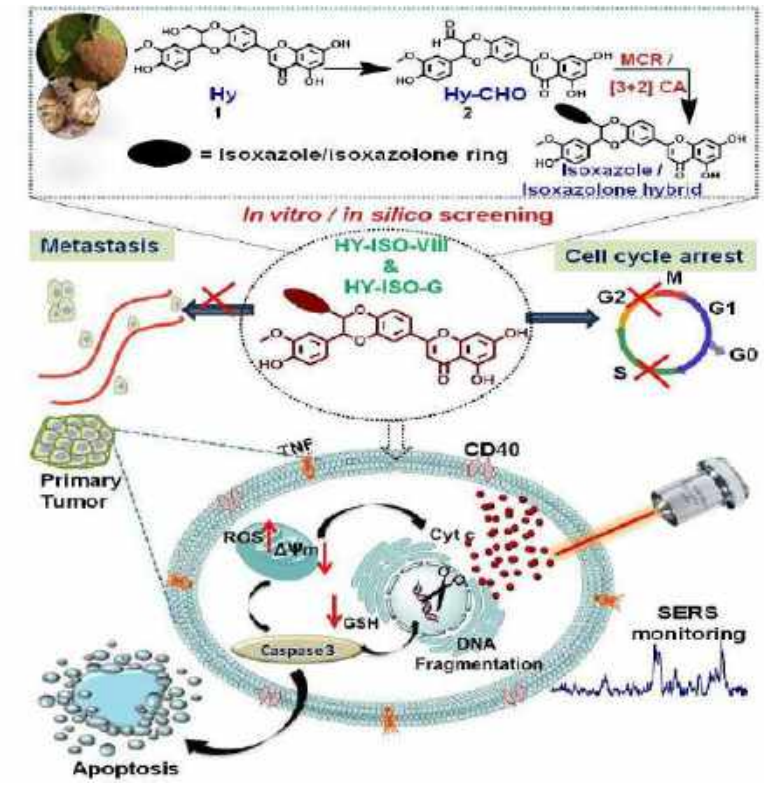
A schematic illustration with the mechanistic action of a combination of Dox and Mt-Dox on cancer cells. Mt-Dox facilitates the damage of mitochondrial DNA thereby helping to overcome the troubles associated with Mt-DNA escape *viz* drug resistance, metastatis etc, Dox will execute the damage of nuclear DNA for an efficint chemotherapy.

C. New Phytochemical Entities (NPCEs) as Anti-cancer Hits: Evaluation by Raman Spectroscopy

10. Exploring Mitochondria Mediated Intrinsic Apoptosis by **New Phytochemical Entities: An Explicit Observation of Cytochrome c Dynamics on Lung and Melanoma Cancer Cells**; Jayadev S Arya, Manu M Joseph*, Daisy Sherin, Jyothi B Nair, Thanathu Krishnan Manojkumar*, and Kaustabh Kumar Maiti* ***J. Med. Chem.,*** 2019, 62, 8311-8329 (Impact Factor: 7.44)

Hydnocarpin (Hy) is a flavonoid isolated and purified from the seeds of *Hydnocarpus wightiana* Blume. Herein, a built-in semi-synthetic modification has been adopted on Hy by one pot multi-component reaction (MCR) and [3+2] cycloaddition strategy to append five membered isoxazole and isoxazolone as new phytochemical entities (NPCEs). Two selected NPCEs *viz* Hy-ISO-VIII and Hy-ISO-G from the library of 20 newly synthesized derivatives after *in vitro* screening unveiled promising cytotoxicity and induced caspase mediated apoptosis against human lung and melanoma cancer cells which was well supported by the virtual screening based on ligand binding affinity and molecular dynamic simulations. As a new insight, we introduced surface-enhanced Raman spectroscopy to identify the chemo-marker molecular fingerprint to confirm the cellular uptake, cytochrome c release and DNA

fragmentation in label-free manner. The present findings throw up a surfeit of seminal reasons behind the semi-synthetic modification of Hy, stepping forward to cancer chemotherapy.



Hy-isoxazole/isoxazolone derivatives promoting apoptosis and inhibiting metastasis