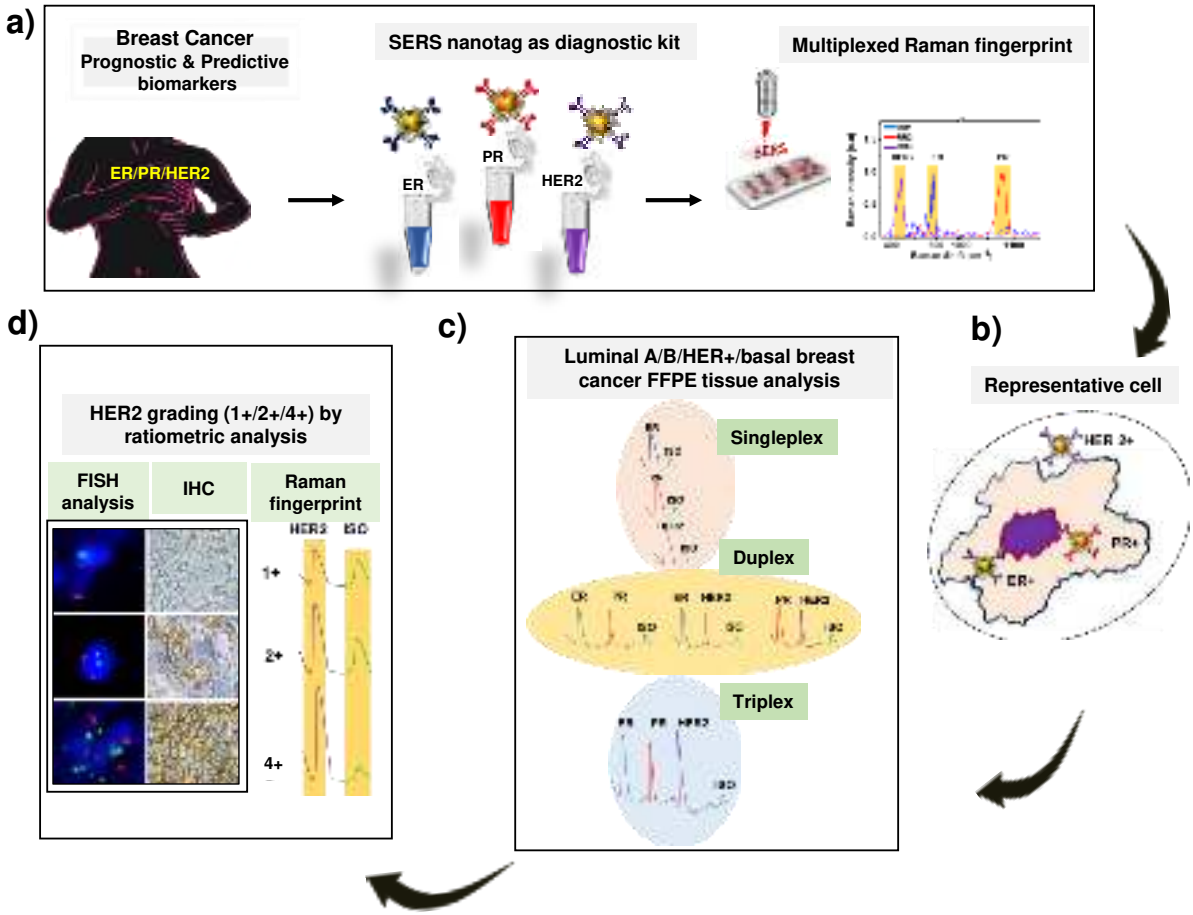
Highlights of ten best publications: Dr. Kaustabh Kumar Maiti

A. Cancer Diagnostic Research Based on Raman Spectroscopy:

1. A clinically feasible diagnostic spectro-histology built on SERS-nanotags for multiplex detection and grading of breast cancer biomarkers
   Vishnu Priya Murali, Varsha Karunakaran, Madhukrishnan Murali, Asha Lekshmi, Shamna Kottarathil, Selvakumar Deepika, Valliamma N. Saritha, Adukkadan N. Ramya, Kozhiparambil G. Raghu, Kunjuraman Sujathan*, Kaustabh Kumar Maiti,*

   Biosensors and Bioelectronics, 227 (2023), 115177 (Impact Factor: 12.54)

Simultaneous detection of multiple biomarkers is an obstacle in immunohistochemical (IHC) analysis. A straightforward spectroscopy-driven histopathologic approach has emerged as a paradigm of Raman-label (RL) nanoparticle probes for multiplex recognition of pertinent biomarkers in heterogeneous breast cancer. The nanoprobes are constructed by sequential incorporation of signature RL and target specific antibodies on gold nanoparticles, which are coined as Raman-Label surface-enhanced Raman scattering (RL-SERS)-nanotags to evaluate simultaneous recognition of clinically relevant breast cancer biomarkers i.e., estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor2 (HER2). As a foot-step assessment, breast cancer cell lines having varied expression levels of the triple biomarkers are investigated. Subsequently, the optimized detection strategy using RL-SERS-nanotags is subjected to clinically confirmed, retrospective formalin-fixed paraffin-embedded (FFPE) breast cancer tissue samples to fish out the quick response of singleplex, duplex as well as triplex biomarkers in a single tissue specimen by adopting a ratiometric signature RL-SERS analysis which enabled to minimize the false negative and positive results. Significantly, sensitivity and specificity of 95% and 92% for singleplex, 88% and 85% for duplex, 75% and 67% for triplex biomarker have been achieved by assessing specific Raman fingerprints of the respective SERS-tags. Furthermore, a semi-quantitative evaluation of HER2 grading between 4^+ / 2^+ / 1^+ tissue samples was also achieved by the Raman intensity profiling of SERS-tag, which is fully in agreement with the expensive fluorescent in situ hybridization analysis. Additionally, the practical diagnostic applicability of RL-SERS-tags was acheived by large-area SERS imaging of areas covering 0.5 to 5 mm^2 within 45 minutes. These findings unveil an accurate, inexpensive, and multiplex diagnostics modality envisaging for large-scale multi-centric clinical validation.

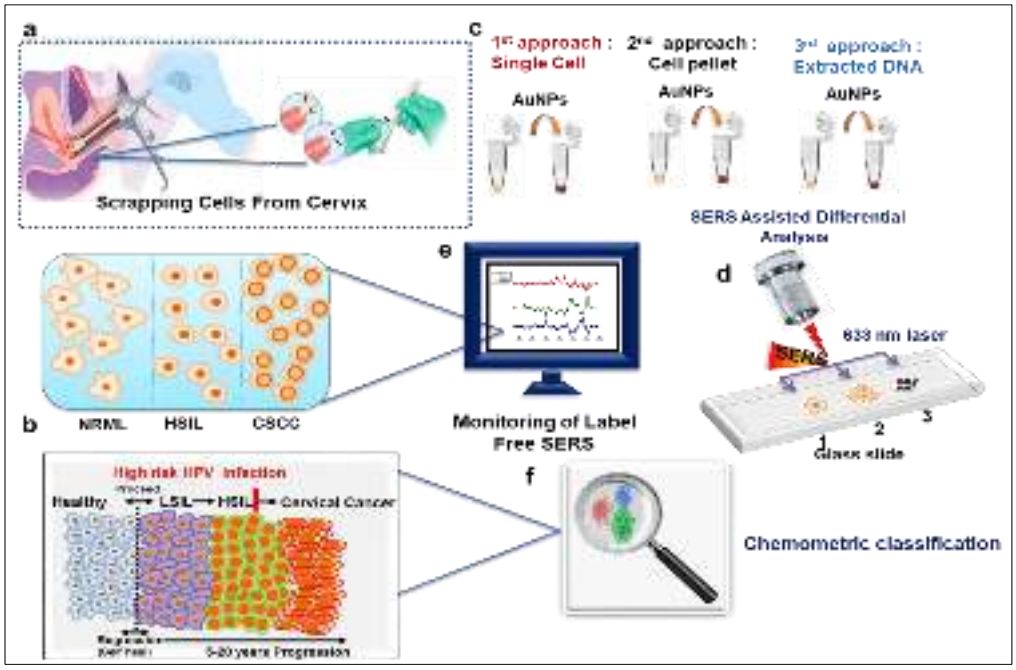


Schematic illustration of the experimental design for differentiating the clinically relevant triple biomarkers, ER, PR and HER2. a) strategy for the multiplexed detection using ER, PR and HER2 conjugated SERS-tags having AuNPs as substrate, b) representative design for detection of biomarkers in cells/paraffin embedded breast tissue samples using SERS mapping, c) Three phases of biomarker detection executed as single, dual and triple biomarker analysis from the breast tissue specimens, d) HER2 grading of IHC and FISH confirmed 1^+, 2^+ and 4^+ tissue sample through ratiometric SERS mapping.

2. 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).

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 the cervix. Three different approaches i.e., single-cell, cell-pellet, and extracted DNA from the oncology clinic as confirmed by Pap test and HPV PCR were employed. Gold nanoparticles as the SERS substrate favored 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.

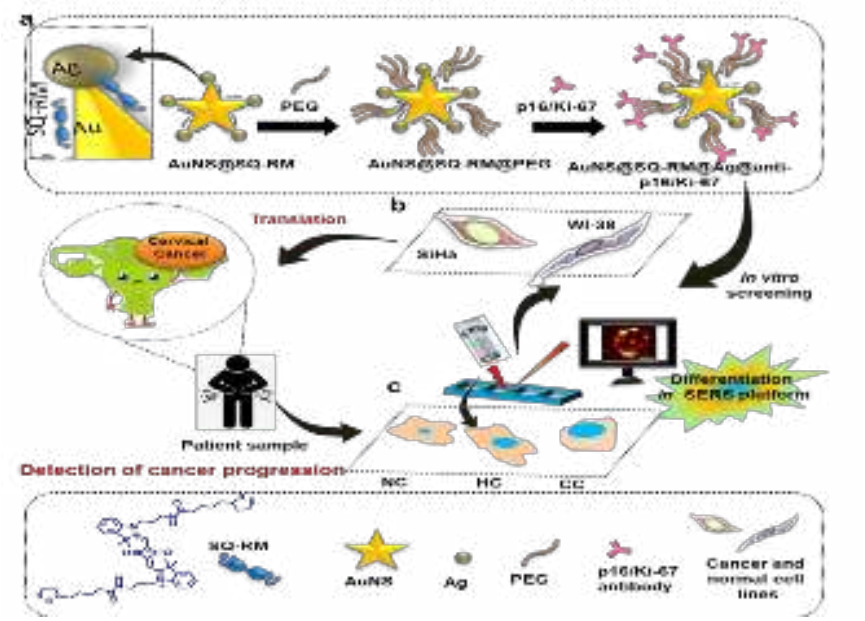


Schematic illustration of experimental design for differentiating three grades viz. normal (NRML), high grade intraepithelial lesion (HSIL), cervical squamous cell carcinoma (CSCC) using SERS., a) Scrapping cells from the cervix using cytobrush, b) progression pattern of cervical cancer c) Set 1: single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

3. 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: 8.00)

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

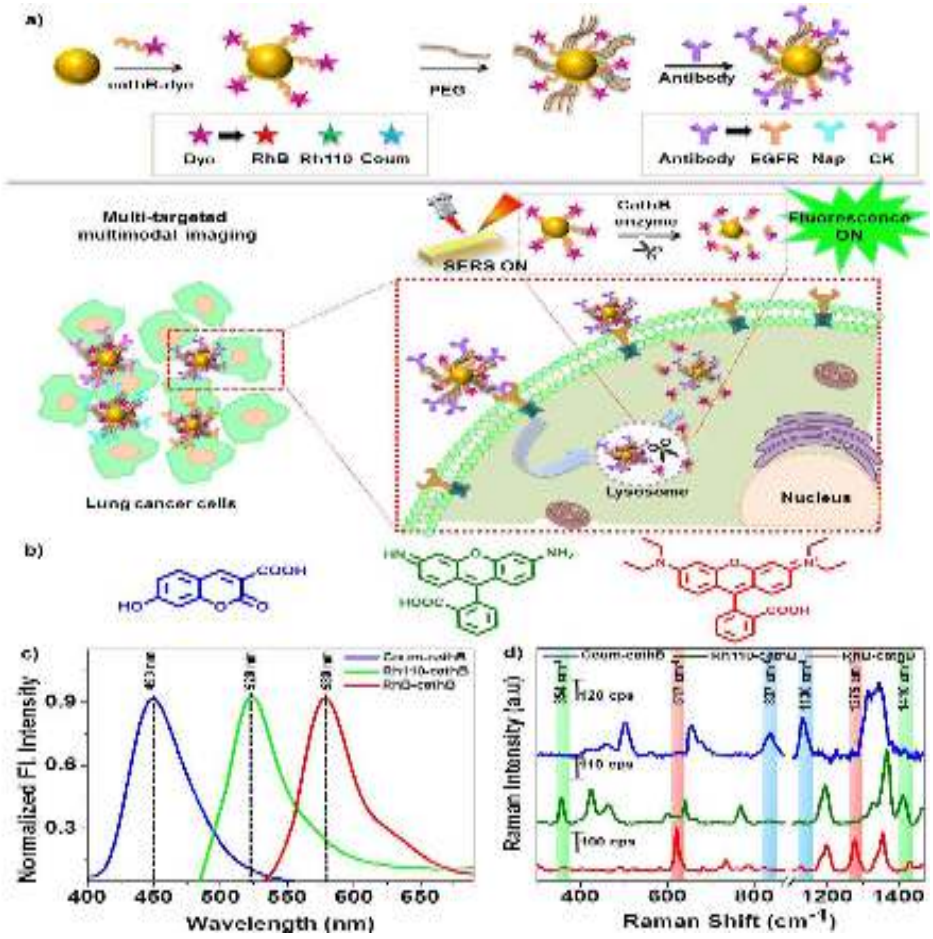
immunocytochemistry technique. Therefore, the newly developed Raman image guided SERS imaging emphasizes the approach of uplifting of SERS in practical utility with further improvement for clinical applications for cervical cancer detection in future.



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.

4. 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: 10.38).

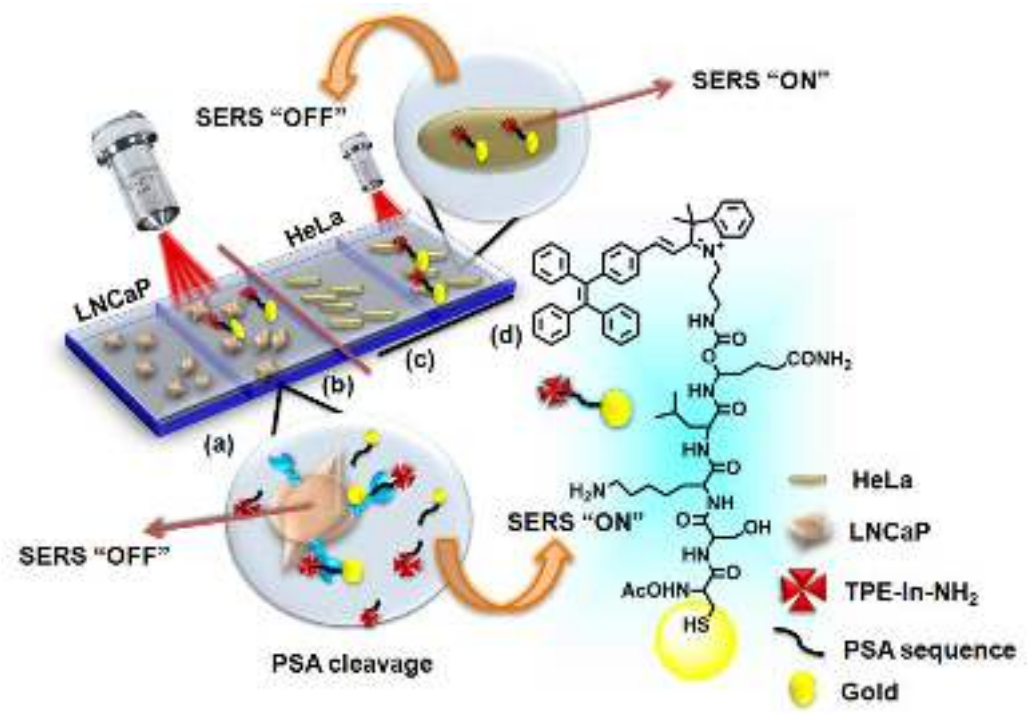
Comprehensive profiling of multiple protein targets plays a critical role in the deeper understanding of specific disease conditions associated with high heterogeneity and complexity. Herein, a modular fabrication of smart programmable nanoarchitectures, which could integrate clinically relevant diagnostic 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

5. 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: 10.38).

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.



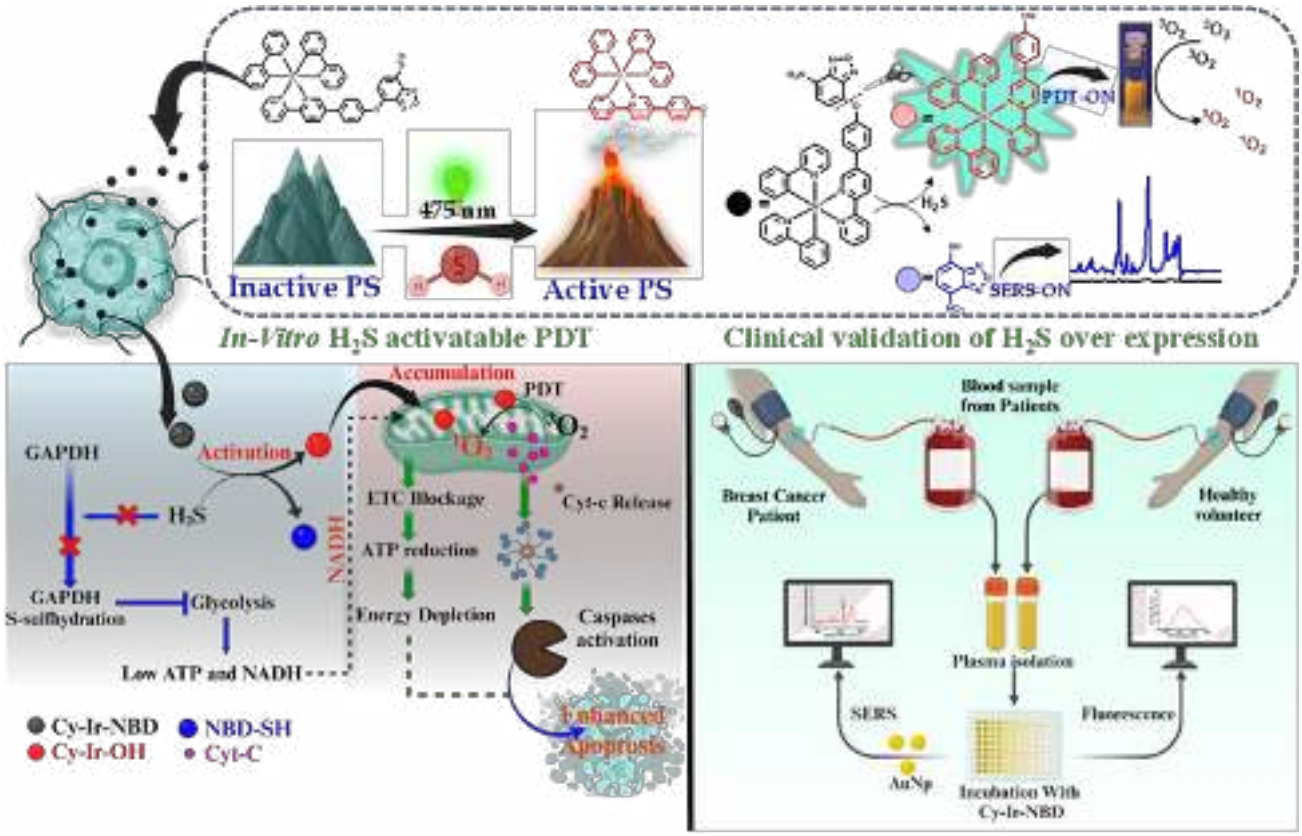
Thematic representation of PSA recognition by TPE-In-PSA@Au nanoprobe by SERS; (a) LNCaP cells without nanoprobe (b) with nanoprobe, HeLa cells (c) without nanoprobe (d) with nanoprobe.

B. Theranostics and Nanomedicine: Nano Delivery System (NDS) with Raman Spectroscopy as a diagnostic modality

6. Hydrogen Sulfide-induced activatable photodynamic therapy adjunct to disruption of subcellular glycolysis in cancer cells by a fluorescence-SERS bimodal Iridium metal-organic hybrid; Shanmughan Shamjith, Vishnu Priya Murali, Manu M. Joseph, Fathima T S, Reghukumar Chandana, Roopasree O. Jayarajan, Kaustabh Kumar Maiti*, **ACS Applied Materials and Interfaces, (2024), https://doi.org/10.1021/acsami.4c02761 , (Impact Factor: 9.5)**

The practical application of photodynamic therapy (PDT) demands targeted and activatable photosensitizers to mitigate off-target phototoxicity common in "always on" photosensitizers during light exposure. Herein, a cyclometalated iridium complex-based activatable photodynamic molecular hybrid (APMH), Cy-Ir-NBD, is demonstrated as a biomedicine for molecular precision. This design integrates a hydrogen sulfide (H_2S)-responsive 7-nitrobenzofurazan (NBD) unit with a hydroxy-appended iridium complex, Cy-Ir-OH. In normal physiological conditions, the electron-rich Ir metal center exerts electron transfer to the NBD unit, quenches the excited state dynamics, and establishes a PDT-off state. Upon exposure to H_2S activates Cy-Ir-NBD into the potent photosensitizer Cy-Ir-OH through nucleophilic substitution. This mechanism ensures exceptional specificity, enabling targeted phototherapy in H_2S-rich cancer cells. Additionally, we observed that Cy-Ir-NBD-induced H_2S depletion disrupts S-sulfhydration

of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) enzyme, impairing glycolysis and ATP production in the cellular milieu. This sequential therapeutic process of Cy-Ir-NBD is governed by the positively charged central iridium ion that ensures mitochondria-mediated apoptosis in cancer cells. Dual-modality SERS and fluorescence imaging validate apoptotic events, highlighting Cy-Ir-NBD as an advanced theranostic molecular entity for activatable PDT. Finally, as a proof-of-concept clinical assessment is evaluated with the blood samples of breast cancer patients, and healthy volunteers, based on their H_2S overexpression capability through SERS and fluorescence, revealing Cy-Ir-NBD, a promising predictor for PDT activation in advanced cancer phototherapy.



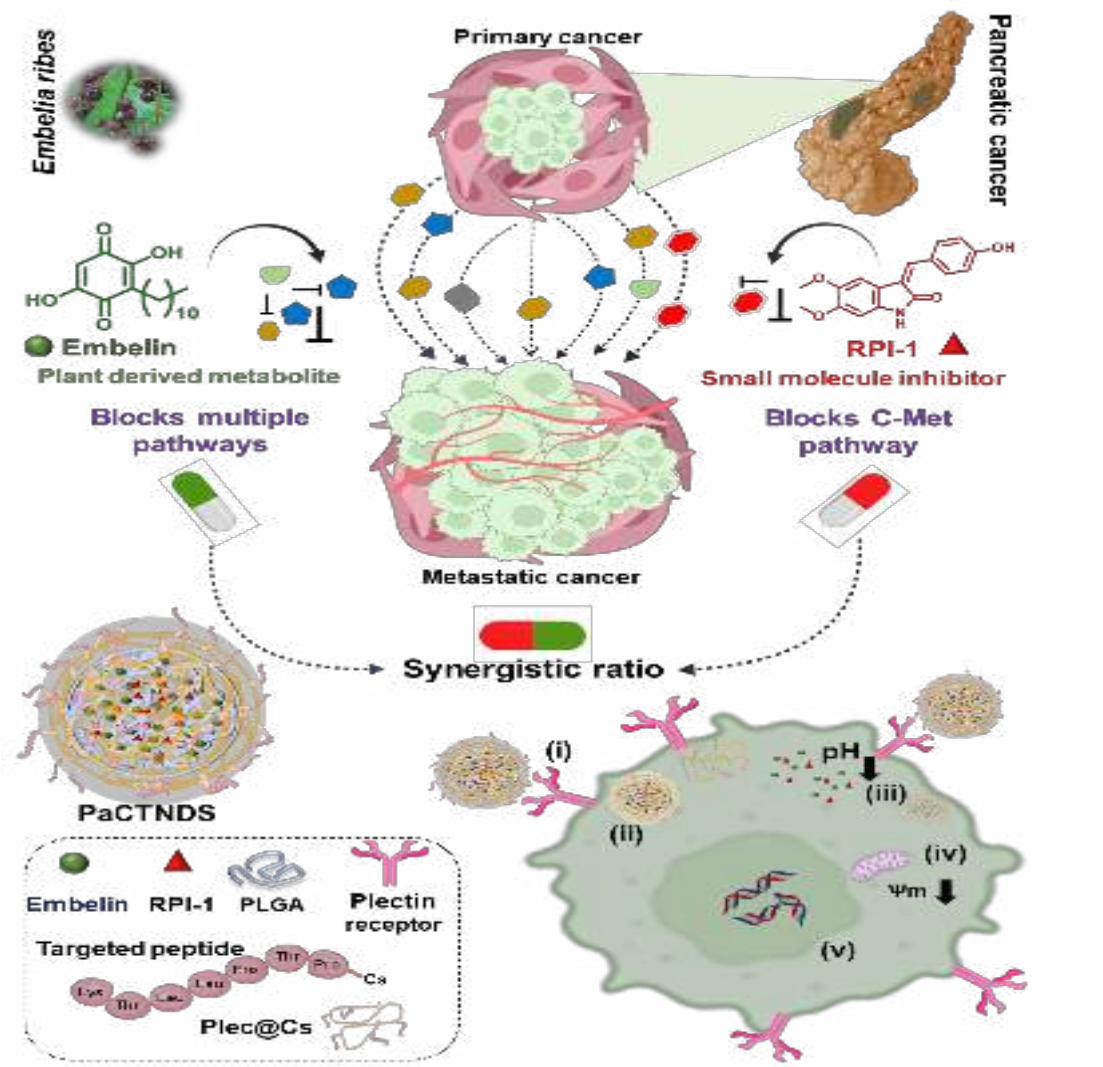
Schematic depiction elucidating the cascade of processes elicited by the activatable photodynamic therapy (PDT) agent Cy-Ir-NBD within the cellular milieu and the validation of H_2S expression in clinical sam

7. Targeted Delivery Polymeric Nanosystem Reinforced by Synergism of Embilin and RPI-1 for Therapeutics of Pancreatic Cancer

   Jayadev S. Arya, Manu M. Joseph,* Vishnu Priya Murali, Murukan S. Vidyalekshmi, and Kaustabh Kumar Maiti*

   ACS Appl. Nano Mater., (2022); 2022, 5, 12, 18622–18636 (Impact Factor: 6.14)

   Pancreatic cancer (PC) is an aggressive form of malignancy with poor prognosis and feeble survival benefits. A personalized nanomedicine constituted of the synergistic therapeutic benefit of two agents, namely, embelin and RPI-1, coloaded in a biodegradable nano-delivery system which has been tethered with a targeting peptide substrate for plectin-1, a surface biomarker of PC. The phytomolecule embelin, an alkyl substituted hydroxyl benzoquinone isolated from the seeds of Embelia ribes, is introduced in this combination therapy and is known as a natural inhibitor of X-linked inhibitor of apoptosis protein and executes anticancer activity via the NF-kB pathway. On the other hand, tyrosine-protein kinase Met (c-MET) is a biomarker of PC wherein the molecule RPI-1, an indolinone derivative, is selective to the c-MET inhibitor. After conducting a series of systematic cytotoxicity evaluations followed by enumeration of combination index, a standalone synergic ratio of embelin and RPI-1 (1: 4.7) was evolved that executed a benchmarked PC-selective toxicity profile. This composition was precisely incorporated within a PC-targeted PLGA−chitosan core− shell nanoparticle delivery system for avoiding collateral damages. Appealing features of the nanoconstruct including biocompatibility, PC-targeted uptake, and subsequent execution of cytotoxicity and antimetastatic properties have been systematically evaluated on the PC cell line PANC-1 and later on the xenograft PC model of zebra fish. Finally, the unique metabolic changes associated with the therapeutic action of embelin and RPI-1 was scrutinized by surface-enhanced Raman spectroscopy and liquid chromatography−mass spectrometry analysis wherein almost 25 metabolites associated with the cell death pathway deciphered a significant variation. Therefore, this proof-of-concept personalized nano-delivery warrants further preclinical and clinical evaluations for the management of PC

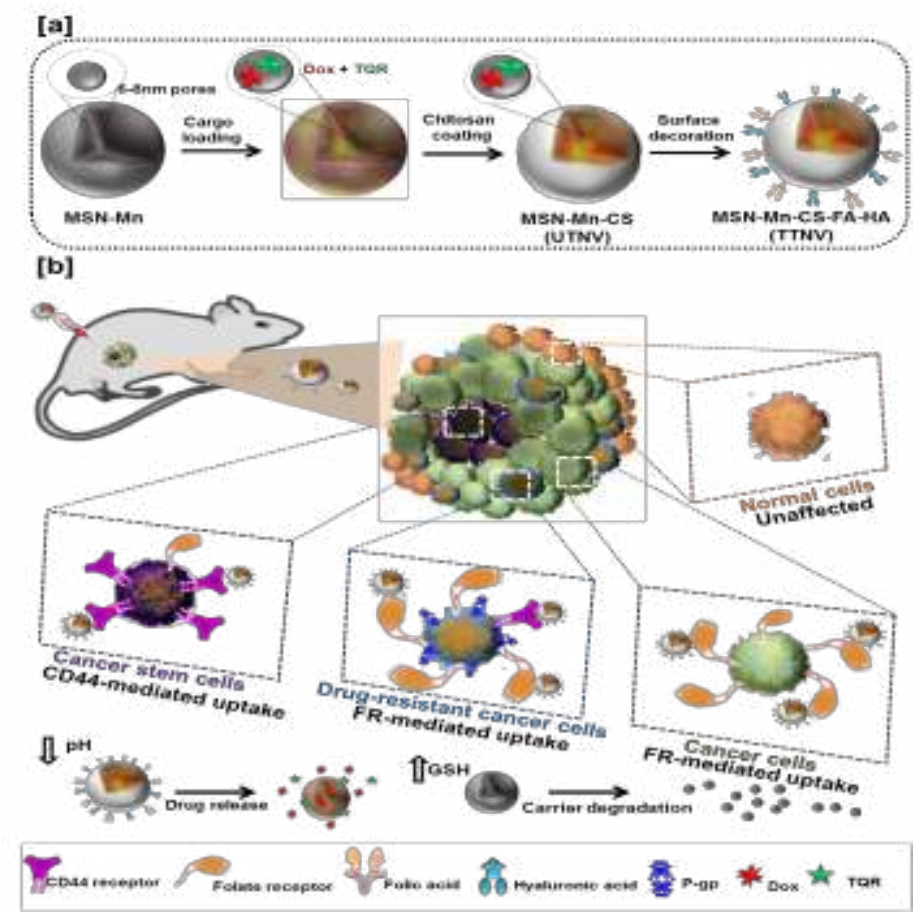


Schematic representation of the fabrication and mechanistic action of a pancreatic cancer targeted nano-delivery system (PaCTNDS, (i) PaCTNDS will get selectively recognized by the Plectin receptor of pancreatic cancer cells and will undergo (ii) targeted endocytosis to gain rapid cell entry. Later, (iii) the intracellular acidic pH causes the chitosan layer to swell, facilitating the release of trapped cargo and biodegradation of the carrier. Subsequently a cascade of biochemical and metabolic changes including (iv) reduction in ΔΨm, release of cyt C and (v) execution of programmed cell death

8. 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: 15.15)

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

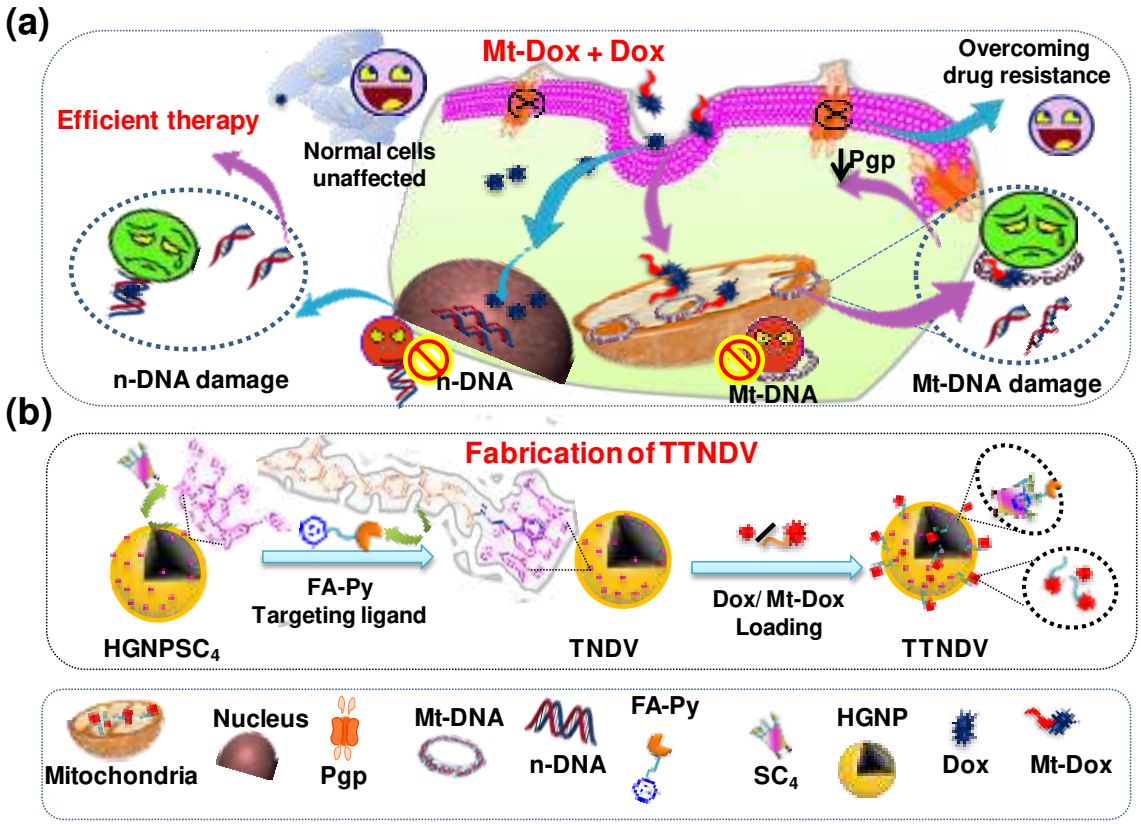
framed TTNV was chemically conjugated with folic acid and hyaluronic acid as a dual-targeting entity to promote folate receptor (FR) mediated cancer cell and CD44 mediated CSC uptake, respectively. Interestingly, surface-enhanced Raman spectroscopy (SERS) was exploited to monitor drug release kinetics, 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.

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: 10.383).

In an attempt to circumvent the major pitfalls associated with conventional chemotherapy including drug resistance and off-target toxicity, a strategy have been adopted 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 intervention 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. Moreover, the cavity of SC_4 facilitated the formation of an inclusion complex with folic acid to target folate receptor expressing cancer cells and imparted enhanced biocompatibility. 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.



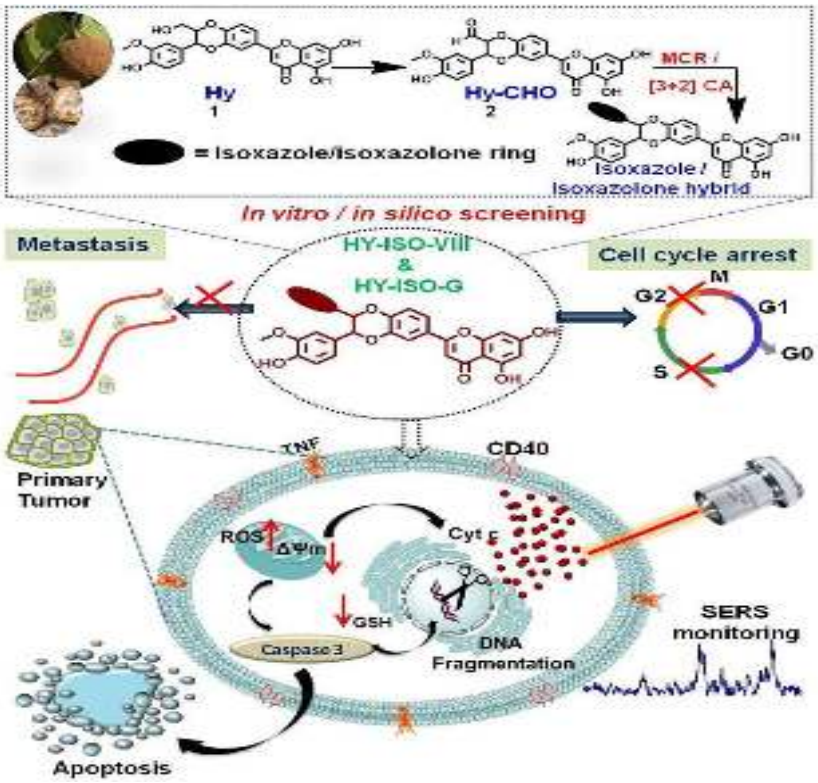
Schematic illustration with the mechanistic action of a combination of Dox and Mt-Dox on cancer cells. Mt-Dox facilitates the damage of mitochondrial DNA thereby helping to overcome the troubles associated with Mt-DNA escape viz drug resistance, metastatis etc, Dox will execute the damage of nuclear DNA for an efficint chemotherapy. (b) Various synthetic steps involved in the fabrication of the TTNDV, viz, tethering of folic acid as a targeting ligand for selective recognition of folate receptors

on $HGNPSC_4$, loading of pretuned ratio of Dox: Mt-Dox (1:100) in to the targeted nanodelivery vector to yield TTNDV

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