ABSTRACT

Index No. **159/18/Chem./26**

Thesis Title : **SELF-AGGREGATED ORGANIC MATERIALS IN BIOCATALYSIS AND BIOMEDICINE**

Thesis Submitted by : ANUP KUMAR GHOSH

Supramolecular self-aggregation involves amphiphilic building blocks coming together through various non-covalent interactions, such as hydrogen bonding, π-π stacking, van der Waals forces, electrostatic forces, and hydrophobic forces. These interactions lead to the formation of diverse morphologies, including micelles, reverse micelles, vesicles, microemulsions, and gel fiber. This thesis provides an overview of various self-assembled systems originating from amphiphiles, tailored to specific purposes, highlighting the unique features of soft materials. It encompasses development of fluorescent organic nanoparticles (FONPs) with distinct physicochemical and photoluminescent attributes, utilized in biosensing, bioimaging, and targeted cargo transport within mammalian cells. Furthermore, the thesis showcases fabrication of carbon (CD)-organic nanoparticle (ONP) conjugate with potential implicationsin cancer treatment. Overall, the focus is on "smart" supramolecular systems applicable in bioimaging, sensing biologically significant entities within mammalian cells, and advancing drug delivery.

Chapter 1 focuses on creating a selective and sensitive heparin detection method using a fluorescent organic nanoparticle-protamine (FONP-Pro) conjugate. This innovative approach leverages the aggregation-induced emission (AIE) property of FONPs. Naphthalene diimide-based amphiphilic molecules (NDI-**1**), with naphthyl and 3-aminopyridyl units, form organic nanoparticles in a DMSO-water mixture, exhibiting AIE through excimer formation (orange emission at λ_{em} = 594 nm). Aminopyridine residues on NDI-**1** give NDI-**1** FONPs a negative surface charge, enabling interaction with positively charged protamine to form FONP-Pro conjugates (Figure 1).The FONP-Pro conjugate reduces NDI-**1**'s orange emission. Interestingly, adding heparin turns on FONP fluorescence by displacing protamine due to strong heparin-protamine binding. Sensing heparin involves monitoring NDI-**1** FONP emission changes. This approach is highly selective against other biomolecules. It results in an efficient heparin sensor (FONP-Pro) with a low detection limit of 12 nM, utilizing the fluorescence 'turn-off' and 'turn-on' mechanisms of NDI-**1** FONPs (Figure 1).

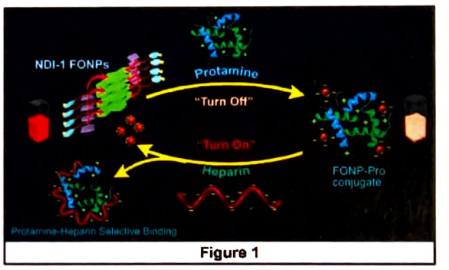

Figure 1

Chapter 2 showcases the precise targeting of specific cellular imaging for cells enriched with GABA (γ-aminobutyric acid) receptors, such as SH-SY5Y and A549, using FONPs derived from naphthalene diimide (NDI). These NDI-based nanoparticles, termed **NDI-nip**, were created through self-assembly in a DMSO-water mixture, driven by *J*-type aggregation, with nipecotic acid tethered L-aspartic acid appended NDI derivatives (Figure 2). **NDI-nip** exhibited aggregation-induced emission (AIE) with orange fluorescence at ≥60% water content in DMSO, emitting at λ_{em} = 579 nm.These orange-emitting **NDI-nip** fluorescent organic nanoparticles, when dispersed in f_w = 99 vol% solution, demonstrated exceptional cell viability and photostability. They were effectively employed for targeted bioimaging and the treatment of cancer cells overexpressing GABA receptors by delivering the anticancer drug curcumin. Notably, the fluorescence of **NDI-nip** FONPs got quenched within $GABA_AR$-enriched neuroblastoma cells (SH-SY5Y) and cancerous cells (A549), but interestingly, the fluorescence was restored in the presence of GABA within these cell lines. This quenching and recovery phenomenon was not observed in non-cancerous cells (NIH3T3).The study revealed that curcumin-loaded **NDI-nip** FONPs exhibited significantly higher efficiency in killing SH-SY5Y (88 ± 3%) and A549 (72 ± 2%) cells compared to NIH3T3 (37 ± 2%). The presence of the nipecotic acid moiety facilitated the selective internalization of **NDI-nip** FONPs into $GABA_AR$-overexpressing cells. Consequently, these orange-emitting **NDI-nip** FONPs hold promise as both targeted diagnostic probes and drug delivery vehicles for $GABA_AR$-enriched cancer cells (Figure 2).

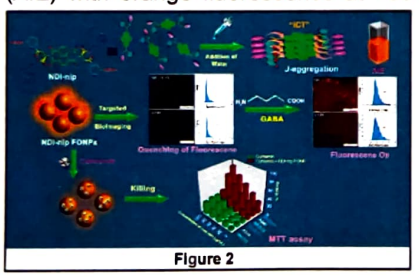

Figure 2

Chapter 3 delineates detection and analyzing the redox behavior of protein disulfide isomerase (PDI) enzyme by exploitation of AIE of novel naphthalimide (NI) derivatives having thiol (-SH) and disulfide moiety (-S-S-). Self-aggregated spherical shaped organic nanoparticles were prepared by synthesized NI-based amphiphiles (**NISH**, **NISS**, **NINSS** and **TNINSH**) through *J*-type aggregation in DMSO-water (f_w = 99 vol%) (Figure 3). Naphthyl residue containing NI-derived amphiphiles (**NINSS** and **TNINSH**) exhibited AIE (blue and yellow) at 470 and 550 nm, respectively in DMSO-water (f_w = 99 vol%). **NINSS** and **TNINSH** FONPs were suitably utilized in sensing PDI through its redox nature of thiol-disulfide exchange. Fluorescence quenching of **NINSS** FONPs was observed due to reduction of disulfide to thiol by PDI whereas emission intensity got progressively red shifted and enhanced ("Dual-AIE") for **TNINSH** (containing ER targeting N-tosylethylenediamine) owing to oxidation of thiol to disulfide by PDI (Figure 3). **NINSS** and **TNINSH** FONPs were found to be highly efficient in sensing PDI through AIE-based "fluorescence off/on" mechanism having limit of detection of ~12.6-17.7 ng/mL and ~11.7-16.5 ng/mL, respectively. In vitro cell imaging for NIH3T3 (non-cancer) and B16F10 (melanoma) cells with **NINSS** and **TNINSH** FONPs displayed excellent diagnosis of eukaryotic cells upon interaction with indigenous PDI. Notably, detection of cancer cells was more sensitive over the non-cancerous cells by these FONPs due to overexpression of PDI within cancer cells (Figure 3).

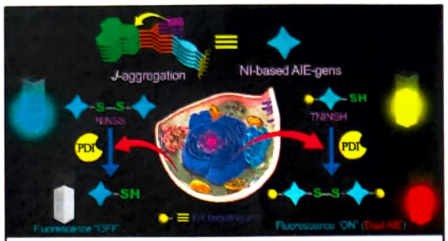

Figure 3

Chapter 4 describes the design and fabrication of **NIONP-CaCD** conjugate through boronic acid-diol covalent linkage between a self-assembled organic nanoparticle (**NIONP**) and Ca^{2+}-doped carbon dots (**CaCD**) (Figure 4). Naphthalimide based lysosome targeting unit appended boronic acid tailed amphiphile (**NIONP**) was synthesized that formed organic nanoparticle (ONP) through *J*-aggregation in DMSO-water system. Ca^{2+}-doped carbon dot (**CaCD**) was synthesized via hydrothermal method. These two nanomaterials were covalently linked by using a boronic acid-diol interaction between phenylboronic acid based **NIONP** and hydroxyl group functionalized **CaCD** to develop **NIONP-CaCD** conjugate facilitated by Lewis acid-base chemistry. The formation of **NIONP**, **CaCD**, and the **NIONP-CaCD** conjugate was characterized by microscopic and spectroscopic techniques. This newly construct blue emitting **NIONP-CaCD** conjugate was use in bioimaging well as in pro-drug-free drug combination therapy for cancer treatment. Hydroxychloroquine (HCQ) was loaded within this **NIONP-CaCD** conjugate with a higher loading capacity compared to the individual cargo carrier (**NIONP** or **CaCD**). Notably, the disintegration of the **NIONP-CaCD** conjugate into its separate constituents took place through the cleavage of the boronate-diol bond at the lysosomal pH range (4.5-5.0) resulting in the liberation of HCQ and **CaCD**(Figure 4). This cytocompatible pro-drug–free drug formulation **NIONP-CaCD-HCQ** resulted in a ~1.5-fold increase in cancer cell death with compared to standalone therapy with individually loaded cargo on the native compounds. This anticancer potential of **NIONP-CaCD-HCQ** could be attributed to Ca^{2+} overload induced apoptosis in conjunction with lysosomal cell death by HCQ resulting in the efficient killing of cancer cells. Hence, owing to its ability to induce Ca^{2+} overload-based oncotherapy along with HCQ-induced lysosomal cell death, the **NIONP-CaCD**conjugate holds promise as a potential candidate for cancer theranostics (Figure 4).

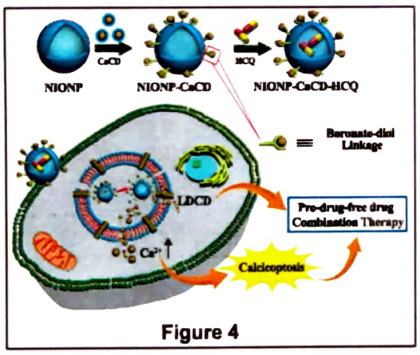

Figure 4

Prasanta Kr. Das 07/09/23
(PRASANTA KUMAR DAS)
Signature of the supervisor
and date

Dr. Prasanta K. Das
Senior Professor
Chair-School of Biological Sciences
Indian Association for the Cultivation of Science
Jadavpur, Kolkata - 700032

Anup Kumar Ghosh
07/09/2023
(ANUP KUMAR GHOSH)
Signature of the candidate
and date