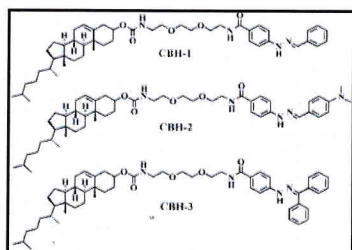


Thesis Title : **SYNTHESIS AND DEVELOPMENT OF STIMULI RESPONSIVE SELF-AGGREGATES FOR TASK SPECIFIC APPLICATIONS**

Thesis Submitted by : **DEBLINA SARKAR**

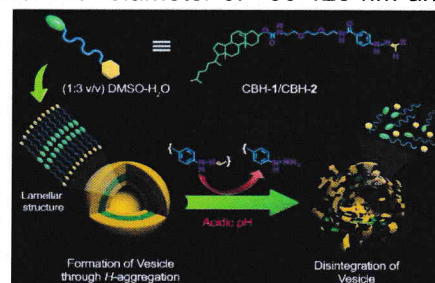
In supramolecular self-aggregation, amphiphilic building blocks get together by several non-covalent interactions (e.g. hydrogen bonding,  $\pi$ - $\pi$  stacking, van der Waal's interactions, electrostatic forces, hydrophobic forces e.t.c.) and give rise to the different morphologies (micelles, reverse micelles, vesicles, microemulsions, gel fibres e.t.c.). Self-assembly or disassembly can be judiciously controlled by tuning the amphiphilic back bone. Stimuli responsive destruction of self-aggregates have huge application in the domain of "smart" Drug Delivery Systems. Also, organic nanoparticles with unique luminogenic properties have revolutionized the bio-medicinal arena. To this end, the present thesis gives an overview on fabrication of supramolecular vesicles with stimuli responsive junction that leads to pH sensitive and hypoxia responsive drug release; development of fluorescent organic nanoparticles (FONPs) based  $\text{Fe}^{3+}$  sensor with ability to differentiate between +2 and +3 state of iron and fabrication of FONPs with tunable emissions by altering the functionalities of the amphiphiles.

**Chapter 1** describes development of cholesterol-based pH-responsive hydrazone appended amphiphiles (**CBH-1-3**) varying the carbonyl moieties from aldehyde (benzaldehyde (**CBH-1**), *p*-dimethylaminobenzaldehyde (**CBH-2**)) to ketone (benzophenone (**CBH-3**)) (Figure 1). Among them, **CBH-1** and **CBH-2** formed vesicles via *H*-aggregation in 1:3 v/v, DMSO-water with diameter of ~50-120 nm and ~150-250 nm for **CBH-1** and **CBH-2** respectively.



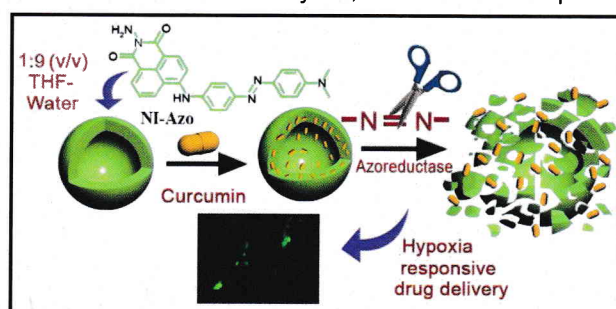
**Figure 1**

Furthermore, the formation of bilayer vesicles possibly through highly ordered lamellar like structure was validated by low angle X-ray diffraction (XRD) patterns. These vesicles were stable at neutral environment but got disassembled at acidic pH (pH < 5.0) (Figure 2). Cleavage of the hydrazone bond in acidic environment, that lead to destruction of self-assembly, was investigated by UV-visible, FTIR and mass spectrometric studies. In the transmission electron microscopic (TEM) images of the acid treated vesicles, absence of spherical structures established its disintegration. In addition, drug loading inside the vesicles and its pH-sensitive release from the **CBH-1** vesicles were confirmed using doxorubicin.



**Figure 2**

**Chapter 2** illustrates construction of self-aggregated vesicle in 1:9 (v/v), tetrahydrofuran (THF)-water from naphthalimide-based azo moiety containing amphiphile (**NI-Azo**) through *H*-aggregation having diameter of 200-250 nm (Figure 3). Azo ( $-\text{N}=\text{N}-$ ) bond is subject to be reduced in the presence of the azoreductase enzyme, which is overexpressed in the hypoxic microenvironment. The optical



**Figure 3**

density of this characteristic azo ( $-\text{N}=\text{N}-$ ) moiety of **NI-Azo** vesicles at 458 nm got weakened in the presence of both bacterial azoreductase (extracted from *Escherichia coli*) and sodium dithionite (chemical mimic of azoreductase). This signifies that they induce azo bond cleavage in **NI-Azo**. Formation of aniline derivatives through the cleavage of the azo bond by azoreductase and sodium dithionite was confirmed by the UV spectroscopic study of **NI-Azo** vesicles and MALDI-TOF spectrometric study of fragments obtained after sodium dithionite treatment. Anticancer drug curcumin was encapsulated within **NI-Azo** vesicles and that killed cancerous B16F10 cells in  $\text{CoCl}_2$ -induced hypoxic environment because of the azoreductase-sensitive drug release through the early apoptotic pathway,

where the cell killing was 2.15-fold higher than that of the normoxic condition and 2.4-fold higher compared to that of native curcumin in the hypoxic environment (Figure 3).

**Chapter 3** deals with green emitting Fluorescent Organic Nanoparticles (FONPs) formed by naphthalimide based L-histidine appended amphiphile (**NID**) in 99% water-DMSO binary solvent system via *J*-type aggregation (Figure 4). Aggregation-induced emission (AIE) was observed with excimer formation at 470 nm. These FONPs were employed in selective sensing of  $\text{Fe}^{3+}$  and bioimaging of  $\text{Fe}^{3+}$  inside cells due to their emissive properties and low toxicity (Figure 4). Among the various tested metal ions, presence of  $\text{Fe}^{3+}$  led to the selective quenching of the emission intensity of **NID** FONPs due to 1:1 stoichiometric complex formation with the histidine residue. The limit of detection (LOD) of this turn-off sensor was calculated to be  $12.5 \pm 1.2 \mu\text{M}$  having high selectivity over other metal ions. Concurrently, **NID** FONP was successfully employed for bioimaging of  $\text{Fe}^{3+}$  ions via fluorescence quenching within living cells as well as detecting  $\text{Fe}^{2+}/\text{Fe}^{3+}$  transition selectively inside cancer cell due to its high  $\text{H}_2\text{O}_2$  content. Co-culture experiment and flow cytometry further confirmed selective sensing of cancer cells. Hence, **NID** FONPs can be a selective diagnostic tool for cancerous cells on account of their higher  $\text{H}_2\text{O}_2$  amount.

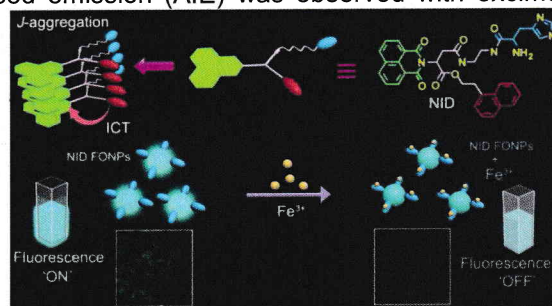


Figure 4

**Chapter 4** Chapter 4 demonstrates development of naphthalenediimide (NDI) based amphiphiles with varying amino acid substitution from L-alanine to L-phenyl alanine to 3-(2-naphthyl)-L-alanine (increasing  $\pi$ -electrons) with or without alkyl spacer (C-6, C-11). Construction of self-aggregated organic nanoparticles of  $\sim 50$  nm occurred at 50 vol% water or methyl cyclohexane (MCH) onwards in DMSO/DMF or  $\text{CHCl}_3$  via *J*-aggregation. Amphiphiles, where amino acids are directly connected to the NDI, self-assembled into FONPs (MCH in  $\text{CHCl}_3$ ) and displayed AIE through excimer formation at 484 nm to 495 nm, and 590 where red shift was observed with increasing  $\pi$ -electrons donor side. FONPs of 6-amino caproic acid spacer containing NDI derivatives showed AIE at 505 nm and 545 nm (water in DMF/DMSO) and 11-amino undecanoic acid containing analogues showed AIE at 480 nm and 585 nm in  $\text{CHCl}_3$ -MCH and DMF/DMSO-water. The multi colour emission from blue-green to yellow-orange with enhancement of  $\pi$ -electron cloud in the side chain of NDI derivatives was detected probably due to the facilitated electron transfer at a lower energy to the electron deficient NDI core that caused red shifted emission maxima. This was again clarified by controlling the availability of  $\pi$ -electrons of phenyl ring by including electron withdrawing  $-\text{NO}_2$  and donating  $-\text{OMe}$  groups at the molecular structure of other two amphiphiles that exhibited blue shifted (475 nm) and red shifted (570 nm) emission maxima compared to the unsubstituted phenyl alanine appended one (495 nm) (Figure 6). Nevertheless, increased alkyl spacer length produced relatively lower quantum yield apparently due to the impeded electron transfer.

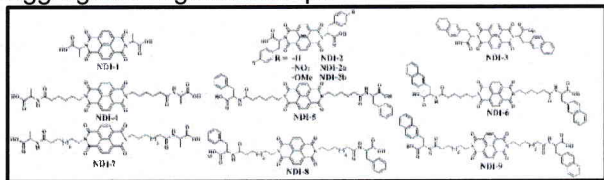


Figure 5

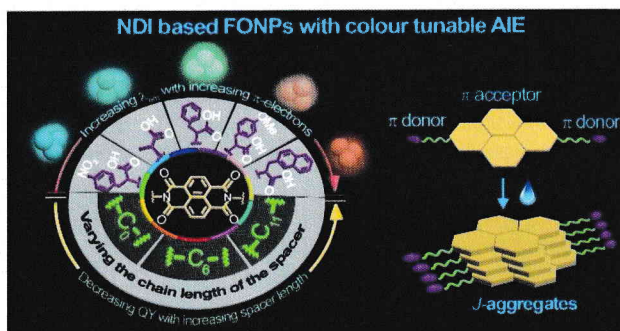


Figure 6

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