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Macrocyclic supramolecular biomaterials in anti-cancer therapeutics

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ABSTRACT

Macrocyclic supramolecular complexes demonstrate the dynamic potential to solve global biomedical challenges, a promising cancer treatment modality. The macrocyclic system is an important heterocyclic system widely present in natural products and synthetic molecules. The unique structural feature of macrocyclic supramolecular complexes with desirable donor & acceptor characteristics is beneficial for readily binding with various enzymes and receptors in biological systems through diverse weak interactions, thereby exhibiting broad bioactivities. Macrocyclic-related research and macrocyclic molecules-based medicinal chemistry developments have become rapidly developing areas of study. Numerous macrocyclic-based molecules as clinical drugs have been extensively used in the clinic to treat various diseases with high therapeutic potency. This critically analyzed work systematically reviews current developments of macrocyclic supramolecular complexes-based compounds in the range of medicinal chemistry as anticancer, anti-inflammatory, and other therapeutic agents, together with their potential applications in diagnostics and pathology. This review will be helpful for medicinal chemistry researchers to develop new thoughts in the quest for rational designs of more active and less toxic macrocyclic supramolecular complexes-based medicinal drugs, as well as more effective diagnostic agents and pathologic probes.

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1. Introduction

In all branches of science, covalent bonds have now almost been extended to their conceptual limits. Even current chemists worldwide are trying to develop highly efficient nano-systems equivalent to those encountered in nature. To build such structures, synthetic chemists are employing extensive research in non-covalent bonding systems. These non-covalent interactions include electrostatic interactions, weak intermolecular forces such as H-bonding, stacking interaction, cation-interaction, ionic interaction, hydrophobic interaction, and electrostatic interaction [1]. The energy involved in the non-covalent interactions range of 1–5 kcal/mol, which plays a significant role in the extraordinary properties of various materials [2,3]. These interactions are observed between receptor-ligand like antigen-antibody, DNA-protein, sugar-lectin, RNA-ribosome [4], crystal packing [5], *etc.* Such interactions determine the packing pattern of various molecules in bulk, providing properties like thermal stability, *etc.* [6–10]. These non-covalent interactions are widely recognized with remarkable dis-

coveries like crown ethers [11], spherands [12], and cryptands [13] by Charles Pederson, Jean-Marie Lehn, and Donald J. Cram in 1987. Shepherd and cryptands discoveries make it an innovative research area of supramolecular edifices.

Macrocyclic molecules like cyclodextrins, pillar[n]arenes, crown ethers, cucurbit[n]urils, cyclobis(paraquat-*p*-phenylene) and calix[n]arenes are popular in supramolecular chemistry due to their exceptional host-guest binding abilities assisted by non-covalent interactions. Supramolecular interactions are abundantly found and involved in various metabolic functions in living organisms. Detail study in this area shows that certain structural modifications of macrocycles can induce dynamic changes in many properties like high stability, high binding affinity, biocompatibility, *etc.* These are useful in designing complex supramolecular assemblies for biomedical and material sciences applications [14,15]. Cucurbit[n]urils and calix[n]arenes are two different macrocycles with different architectures. Both are well-known for their ability to form interesting inclusion complexes with an array of biological functions and applications, including self-healing systems, bio-assemblies, drug-delivery, bio-imaging, biosensors, reversible and irreversible enzyme inhibition, antibacterial and chemotherapy. This review gives a brief idea of recognition-based supramolecular assemblies and demonstrates the recent

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biological developments of cucurbit[n]urils and calix[n]arenes in drug-delivery, cell-bioimaging and discussed about their *in-vivo* therapeutic achievements.

2. Intermolecular interactions

The intermolecular interactions are principally linked with comprehensive transdisciplinary research. These interactions led to uncovering the science with the engineering and advancement of the structural complexity of molecules with oligo to polymolecular entities. It is essential to study the nature and the extent of the intermolecular interactions as they govern the molecular arrangements in the crystal lattice and thus provide unique features to crystalline material. Dynamic supramolecular systems can be designed with specific supramolecular interactions, as given in Table 1 [16–25].

3. Supramolecular approach to macrocycles

Macrocyclic host-guest interaction is vital in supramolecular chemistry and has been extensively studied. Macrocycles have versatile synthetic ability and structural functions. They have been considered substrates with high potential for developing and designing simpler, overly complicated supramolecular systems with new and enhanced properties. Macrocyclic supramolecular systems provide applications in molecular recognition, molecular sensing, nanomedicines, catalysis, and polymer chemistry. The macrocyclic compounds commonly contain O, N, S, and P heteroatoms in the ring or functional groups. These heteroatoms enable non-covalent intermolecular interactions in macrocyclic compounds. These compounds become helpful in designing self-assembled supramolecular systems controlled by thermodynamics. In a self-assembly-driven system with discrete entities, supramolecular chemistry is involved in attaining a well-organized architecture with programmed molecular species. These macrocyclic compounds with multiple functional groups may undergo extensive cooperative binding of substrates. Macrocycles are well-known for their physicochemical properties like broad physiological activity [26–29], dynamic self-assembling capacity [30–32] & low toxicity [33,34]. Their structural capabilities bind various inorganic/organic/biological molecules and ions in their cyclic cavity. Chen *et al.* [35] reported a series of mechanically controllable macrocyclic supramolecular systems. All are based on macrocyclic hosts, including crown-ethers, cyclodextrin, calixarene, pillararene, and cucurbituril. These could bind a wide range of neutral to polar substrates in their cavity. Similar macrocycles are commonly used in designing skeletons for host-guest interaction-based polymeric supramolecular complexes [36]. A wide range of advancements in the application of macrocyclic supramolecular systems was reported, including thermal responsive systems [37], multi stimuli-responsive systems [38], drug delivery vehicles [39–41], supramolecular antitoxins [42], supramolecular catalysis [43–45] and medicinal supramolecular systems [46–49]. The magnetic properties emerging from macrocyclic supramolecular systems elevate the interest of future studies.

Cyclodextrin is one of the most important types of host molecules for bio-applications. They have excellent supramolecular properties in the water, which have been known since their discovery by Szejtli in 1891 [50]. Cyclodextrins are cyclic oligosaccharide molecules consisting of glucose units connected by 1,4-glycosidic linkages. Their cone-shaped structure possesses a hydrophilic exterior with an internal hydrophobic cavity formed by the glucose units' inward-directed H3 and H5 atoms. This pocket can easily trap lipophilic molecules or hydrophobic drugs useful for medicinal applications [51,52]. They are utilized in multiple sectors like chemistry, cosmetics, textile industries, and most importantly, pharmaceuticals. Like cyclodextrin, calixarene and cucurbituril are

other macrocyclic hosts that look structurally similar with comparable internal diameters [53–55]. They have emerged significantly till the present day and are being explored to their maximum possibilities. With an inner hydrophobic cavity, their inherent properties and inclusion characteristics differ to a great extent. In the structure of the cucurbituril, a symmetrical plane can be observed with identical cavity portals. The cavity is hydrophobic, and the two portals are polar in nature. It makes them efficient hosts to bind hydrophobic guests, especially those bearing positive charges. Calixarene do not have any symmetry, which is a tub-shaped structure with two openings at the extremities. Calixarenes possess hydrophobic alkyl groups on one side and polar hydroxyl groups on the other. Chemical modifications of the portals of these macrocycles render amphiphilic characteristics of versatile self-assembling features with convincing SARs and nano formulation parameters [56,57]. This property is helpful to design potent macrocyclic substrates for highly efficient materials, probes, drug delivery systems, and bioactive materials.

4. Supramolecular therapeutics

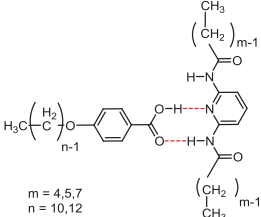
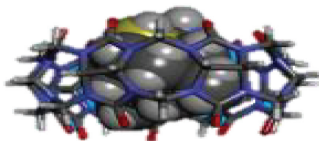
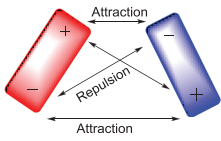
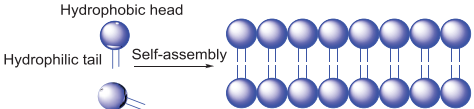
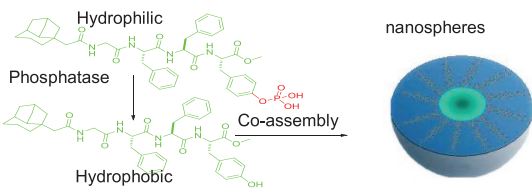
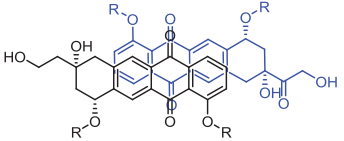
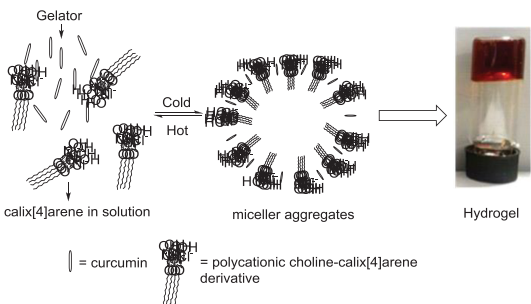
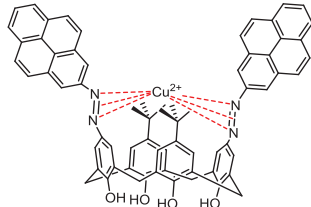
In the last few decades, significant advancements have been achieved in biology and medicinal fields targeting highly efficient nano-drugs with low toxicity effects. Several supramolecular systems, such as nanoparticles, hydrogels, nanofibers, nanovesicles, and nanosponges, have attracted great attention from chemistry, biology, and material chemistry. They have properties like antibacterial [18], anti-inflammatory [58–61], anti-cancer [62–65], photodynamic therapy [66], *etc.* For example, Yan *et al.* [67] reported novel biodegradable polymers, showing effective antibacterial activity towards gram-positive bacteria. It is constructed from derivatives of cationic polyaspartamides and anionic carboxylatopillar[5]arene. This material showed effective membranolysis to Gram-positive methicillin-resistant *Staphylococcus aureus* (MRSA), a vicious bacterial issue in medicinal therapy [68]. Interestingly, the host-guest complexes inhibit the development of anti-microbial resistance, which is usually not found in broad-spectrum antibiotics. *In vivo* analysis in MRSA-infected mice revealed 95% depletion of the Gram-positive bacterial cells. Additionally, from the immunohistochemistry (IHC) study, inhibition of nuclear factor-kappa B (NF- κ B) activation and escalated tumor necrosis factor-alpha (TNF α) levels were also observed in mice.

Calixarenes with good amphiphilicity may serve as valuable nano vehicles. They can co-assemble with various drugs to provide multifunctional drug applications. A unique macrocyclic cavity of calixarenes was utilized to construct a supramolecular drug carrier with clarithromycin. FITC loading potentials were reported by Ali *et al.* [20]. Structurally modified upper and lower rims of amphiphilic sulfonated calix[6]arene formed nanostructures with $57.54\% \pm 1.88\%$ drug encapsulation efficiency against clarithromycin. It is found due to lipophilic modifications in the supramolecular structure.

Moreover, the drug encapsulated supramolecular system resulted in an increased antibacterial activity towards *S. pneumonia* with a minimum inhibitory concentration (MIC) of $11.81 \pm 0.43 \mu\text{g/mL}$ and IC_{50} value of $18.02 \pm 0.91 \mu\text{g/mL}$ compared to standard clarithromycin and unloaded drug carrier. In addition, for *S. pneumonia*, the clarithromycin loaded calix[6]arene derivative displayed biofilm inhibition with a minimum biofilm inhibition concentration (MBIC) of $15.97 \pm 1.68 \mu\text{g/mL}$ and IC_{50} value of $41.55 \pm 0.73 \mu\text{g/mL}$. Interesting drug distribution features were observed using AFM in different strains of *S. pneumonia*. Both standard clarithromycin and its loaded calix[6]arene derivative were incubated with *S. pneumonia* cells, which were entirely ruined after treatment with clarithromycin-loaded calix[6]arene derivative. At the same time, free clarithromycin

Table 1

Different supramolecular assemblies from various non-covalent interactions.

Interactions involving	Mechanism directed	Compatible groups	Refs.
H-bonding	 $m = 4, 5, 7$ $n = 10, 12$	Hydroxyl, carboxylic acid, pyridine, DNA base pairing, etc.	[16]
Host-guest		Cyclodextrins, calix[n]arenes, cucurbit[n]urils, pillar[n]arenes, etc.	[17]
Electrostatic interaction		Charged species (adenine, thymine, etc.)	[18,19]
Amphiphilic self-assembly		Groups with hydrophobic head and polar tail (amphiphilic <i>p</i> -sulphonatocalix[6]arene)	[20]
Enzyme assisted	 Hydrophilic Phosphatase Hydrophobic nanospheres	Peptides, enzymes, and similar substrates	[19,21]
Hydrophobic or π - π interaction		Pyrene, tetraphene, phenyl, alkyl chain, cyclohexane, etc.	[22,23]
Ionic interactions	 Gelator calix[4]arene in solution micellar aggregates Hydrogel = curcumin = polycationic choline-calix[4]arene derivative	Natural and synthetic polymers (protein etc.)	[24]
Metal-coordination		Molecules with appropriate coordination sites (azocalix[4]arene etc.)	[25]

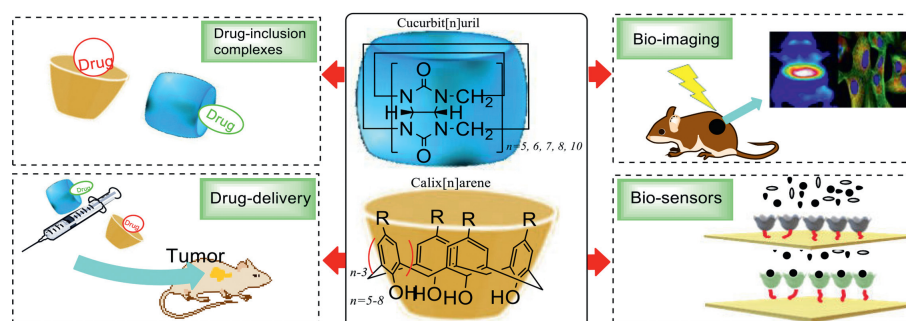


Fig. 1. Bio-applications of calix[n]arenes and cucurbit[n]urils.

could not make significant cell deaths. Thus, the reported calix[6]arene derivative is an excellent biocompatible drug carrier for biomedical applications with the potential to transport clarithromycin without any modulation in its chemical properties securely. Fontana *et al.* reported the application of nanosponges as antibiotics transporter. They designed tetracycline using β -cyclodextrin and a derivative of poly-propargyloxy calix[4]arene using a CuAAC reaction [69]. MIC₉₀ assay was performed for *E. coli*, *P. aeruginosa*, *B. subtilis*, *S. aureus* bacterial strains-displayed bacterial inhibition activities even using minimal amounts ($\sim 0.5 \mu\text{g/mL}$).

When it comes to the leading global health issues, cancer is one of the prominent reasons of death around the world. International Agency for Research on the World Health Organization reported that nearly 10 million people died worldwide due to cancer in 2020 [70]. Although the medicinal industry has significantly developed in cancer diagnosis and therapeutics, but its maximum potential is still not achieved. This fact brings the necessity to explore effective means for treating cancer. Nanomedicines have already entered the field of medical sciences and have shown promising potential to combat global clinical issues. In this regard, nanosystems can provide variable modalities, which is advantageous for enhanced susceptibility and critical analysis of *in vivo* activities. Chemotherapy is a primary method of treating cancer. However, it brings certain difficulties, like toxicity, to healthy cells. It induces side effects that limit the efficacy of the treatment. Other challenges include poor biocompatibility of various anticancer drugs, unstable release, and bad cell-targeted delivery. Concerning this, drug carriers can solve these problems. Drug carriers can be designed for target-specific action with a sustained drug release without interfering with the healthy cells. Recent studies reveal that such nanomaterials increase drug efficiency and biocompatibility. It provides sustained drug release, high cell penetration ability, and amplifies dissolution ability in blood [17].

Supramolecules like calixarene, cucurbituril, can perform host-guest interactions or form complex supramolecular systems through self-assembly. Calix[n]arene and cucurbit[n]urils macrocycles are extensively utilized in supramolecular therapeutic applications (Fig. 1). In a recent article, novel *p*-tertbutylcalix[4]arenes were reported by Mehmet Oguz with the anticancer property. The calixarene derivatives were tailored with potent fluorinated isomers of trifluoromethyl aniline. The compound with CF₃ at the *ortho* position showed high proliferation inhibition in MCF-7 cells up to 25 folds and about 12 folds in Vero cells. The compound can be a potential candidate for human breast cancer cell with an IC₅₀ value of $8.334 \mu\text{mol/L}$ in clinical experiments [71]. The same research group also obtained cancer-selective cationic derivatives of calixarene using 3-bromopropyl-triphenyl-phosphoniumbromide and 5-bromopentyl-trimethyl ammonium bromide as the cationic groups [72].

The phosphonium derivative showed toxicity against A549 cells, and the ammonium derivative against HeLa cell lines without affecting the epithelium cells. Multiple drugs are often combined with increasing therapeutic efficiency, which induces a synergistic action. The ideal drug concentration ratio examined *in vitro* is challenging to maintain after transport to their targets *in vivo* experiment. Drug dissolution differences and varying pharmacokinetics are the main cause of these problems. Today, many nanoscale drug transporters like micelles, liposomes, nanosponges, etc. are used in drug delivery process. Even in these systems, the exact ratio of drug delivered to tumor cells is difficult to estimate, which results in an unsuitable synergistic effect. It is because physical incorporation is still a major technique for loading drugs. GBM or glioblastoma is a well-known primary malignant brain cancer that causes premature death in adults of 50–60 years old and in children at their adolescent age. Commonly for cancer treatment, temozolomide (TMZ) is a famous chemotherapeutic agent used in various therapies for human glioma [73–75]. Its applications have many difficulties, among which low solubility and stability are prominent barrier. However, since its approval by FDA in 2005, in the present-day, temozolomide (TMZ) has been primarily used for the prognosis of glioblastoma patients. It makes the study of TMZ an important topic to explore better treatment and development for clinical applications.

5. Drug delivery in supramolecular systems

At present, nano drug transport systems are developed tremendously for efficient and target-specific delivery of anticancer drugs or biomolecules to tumorous cells. In the tumor, abnormal pathophysiological characteristics are observed. These features allow nano drug-delivery systems to gather and get absorbed in the tumor sites by the enhanced permeation and retention effect, which was first shown by Matsumura and Maeda in 1986 [76]. Although, an unsatisfactory EPR expression is observed in cancer diagnosis. These are primarily due to the heterogeneous behavior of the EPR effect in the tumor microenvironment (TME) because of (1) imbalanced interstitial fluid pressure, (2) irregular bloodstream, (3) hypoxia and necrosis [77]. These defects bring about the attention to design advanced drug-carrying vehicles. The tumor microenvironment's pathophysiological features can be considered to develop drug-delivery systems sensitive to pH, temperature, lights, or enzymes.

It will affect only the tumor cells, reducing side effects and enhancing tumor accumulation with the EPR effect. High GSH concentration is observed in the tumor microenvironment, mainly due to γ -glutamyl-transpeptidase (GGT), γ -glutamylcysteine ligase (GCL), and GSH pumps. Moreover, irregular hemostasis results in amplified levels of reactive oxygen species (ROS). These GSH and ROS concentrations are significantly lower in normal cells as com-

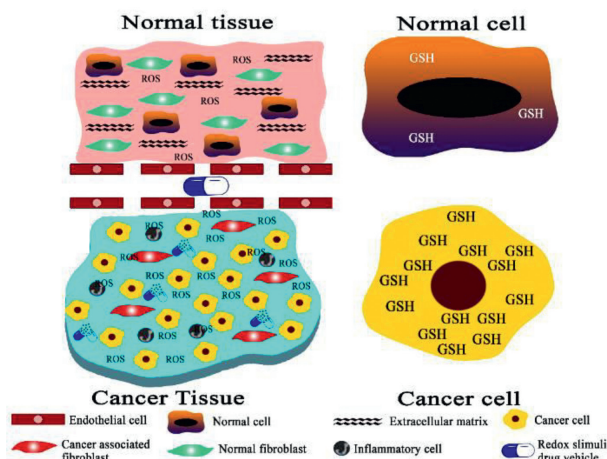


Fig. 2. Schematic diagram showing the difference between GSH and ROS concentrations in tumor and normal cells.

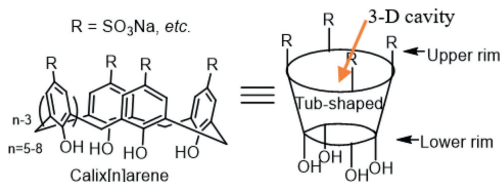


Fig. 3. Structure of calix[n]arene.

pared to cancerous cells (Fig. 2), which enables researchers to construct chemically sensitive nano vehicles that can deliver drugs straight to the TME.

Calix[n]arenes are a class of macrocycles with multiple *para*-phenol groups linked by methylene bridges at *ortho* to hydroxyl groups in the ring (Fig. 3). They form a 3-D hydrophobic cavity that can undergo recognition to capture hydrophobic guests and can be modified by increasing the number of linked groups. Calix[n]arenes possess two characteristic upper and lower rims, which can be designed by introducing different groups to trap guest molecules or ions selectively. Calix[n]arenes have advantageous features like forming ordered aggregates by self-assembly with themselves and other organic molecules, ions, metal oxides, etc. In addition, the *para*-sulphonated calixarenes are also known for their exceptional bio-compatibility function [78]. Owing to their properties, they have wide applications in nanomedicines and pharmaceutical sciences. Numerous drug advancements [79] and remedial genes [80] are known for cancer treatment. However, their fused systems for treating tumor cells are very bounded. Nanoparticles are renowned for their extensive utilization in the target-based therapy of cancer cells [81]. The highly specific drug-delivery and efficient assembly properties of nanoparticles have led to their applications in development of nano-medicine for the treatment of tumor cells [82,83].

The other macrocycle, cucurbit[n]uril (CB[n], $n = 5-10$), is a well-known family of macrocycles having n glycoluril groups. The drum-shaped macrocycle has a non-polar hydrophobic pocket and two polar openings on the two sides of the macrocycle (Fig. 4) [84]. They can form highly stable 1:1 binary and 1:1:1 ternary complex with different guests using non-covalent interactions provided by the hydrophobic cavity with additional binding with ions or polar species assisted by the polar carbonyl head groups. Interesting structural and recognition characteristics make them potent receptors and valuable components for designing supramolecular materials [85,86]. These can be useful for creating nanocar-

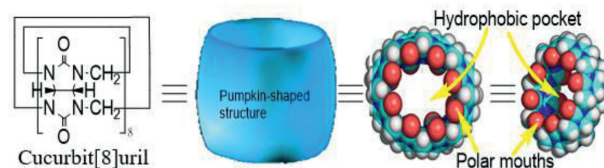


Fig. 4. Structure of cucurbit[8]uril.

riers, ion channels, vesicles, polymers, etc. For example, Liu *et al.* [87] constructed $[\text{ZnCl}_4]^{2-}$ assisted supramolecular organic frameworks (SOFs) by utilizing the positive electrostatic potential of the external face of CB[6] and $[\text{ZnCl}_4]^{2-}$. These were stabilized with added interactions from polar C=O head group of CB[6] and positive electrostatic potential of neighboring CB[6] macrocycles (Fig. 5). CB[6]'s host-guest recognition properties enabled the successful loading of ibuprofen, an NSAID with a stable drug release *in vitro* analysis under physiological temperature and pH.

Despite the enormous medicinal advancements made till now, the design of safe and efficient therapeutics is still challenging. Side effects induced by non-targeted transport or co-morbidity make it difficult to conclude the efficacy of therapeutic entities. In this regard, choosing an appropriate drug delivery pathway can impact on drug's pharmacokinetics and biocompatibility. Stimulus-responsive drug delivery can enhance drug dissolution in a spatiotemporal manner. Several stimuli-sensitive drug delivery systems (Fig. 6) are used based on different supramolecular materials. Preliminary knowledge of the delivery site for therapy can be merged with such supramolecular system, integrating a stimuli-responsive trigger for expanding the therapeutic index ensures a satisfactory amount of targeted drug transport. For example, certain abnormal physiological conditions exist in the tumor microenvironment, like low oxygen concentration, high glutathione (GSH), high reactive organic species (ROS), increased interstitial fluid pressure, and low pH levels. Zheng *et al.* reported nanomaterials constructed from ganoderma lucidum polysaccharides (GLP) with anti-tumor ability. Anti-tumor drugs methotrexate (MTX) and hydroxycamptothecin (HCPT) were loaded together for a combined drug effect studied in a 4T1 mice model for the treatment of breast cancer *in vivo*. The nanoparticles synthesized possessed borate ester bonds which break upon reaching the tumor's acidic environment. This disrupts the nanoparticle and co-deliver MTX and HCPT into the tumor cells [88].

Various functional groups (Table 2) are being incorporated into drug delivery systems to impart sensitivity to the tumor microenvironment [90–98]. Functional groups that are redox-responsive moieties are critical regarding their reactivity. It is crucial to understand how they interact or react to target cells. Drug-delivery systems sensitive to tumor physiological conditions carry great potential for cancer therapy. More effective stimuli targeted delivery to the intracellular and extracellular tumor matrix can be explored using multiple stimuli combinations. For example, acidity regulations in the intracellular organelles with the redox reactions inside the cell can cause drug release into the ground plasma matrix from drug delivery systems in response to redox reactions and acidity [89].

6. Inclusion complexes

Hu *et al.* [99] reported pegylated guanidinium-modified calix[5]arene pentadecyl ether (GC5A-12C) as an advanced ATP-responsive nanocarrier for the treatment of cancer. This treatment is based on biomarker displacement activation (BDA). The PEG group prevents interaction with the extracellular matrix, which in-

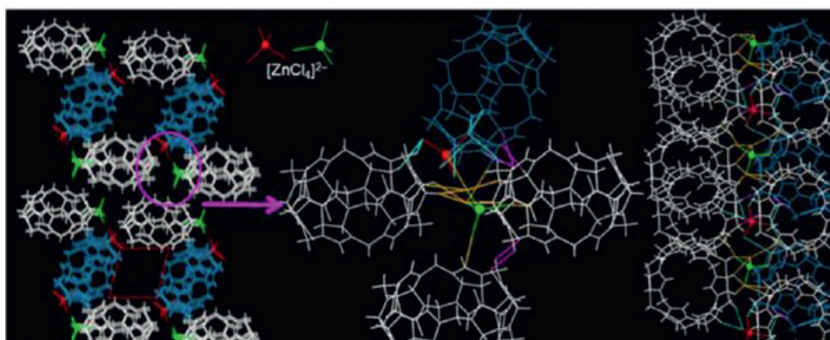


Fig. 5. Interactions between cucurbit[6]uril and $[ZnCl_4]^{2-}$ (C-H...Cl, green and orange color and C-H...O, purple color). Reprinted with permission [87]. Copyright 2022, Elsevier.

Table 2
Stimuli-responsive functional groups for drug cargo release.

Drug carrier (based on calixarene/cucurbituril)	Functional group	Sensitivity towards	Refs.
Liposomes	Polyethylene glycol	Tumor pH	[90]
Polymeric nanoparticles	Amine group	Tumor pH	[91]
	Poly(ethylene glycol)- <i>b</i> -polylysine	Tumor pH	[62]
	Azo group	Bio-reductive enzymes	[92]
Micelles	Amide and ester bonds	Tumor pH	[93]
	Phosphonate group	Tumor pH	[56]
Nanocapsules	Azo group	Hypoxia	[94]
	Disulfide bond	Tumor GSH	[95]
Inclusion complexes	Hexanoate group	Tumor pH	[96]
	$-H_2N^+(CH_3)_2$	$h\nu$	[97]
Vesicles	3,4,5-Tris(<i>n</i> -dodecyloxy)benzoylamide (TBA-Azo)	UV radiation	[98]

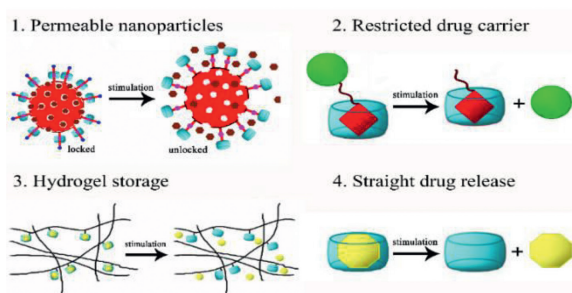


Fig. 6. Different types of the stimuli-responsive drug discharge.

increases its stability during blood circulation. Recognition properties of the macrocycle with guanidium groups at the upper rim of the calix enabled strong binding with anticancer drugs like OX, MTX, and chl. The nanocarrier GC5A-12C showed no cytotoxicity against HeLa, HepG2, and MCF-7 cells up to 20 $\mu\text{mol/L}$ concentration. Nanomaterial (AlPcS₄+GC5A-12C) was established from sulfonated aluminum phthalocyanine (AlPcS₄) and GC5A-12C to understand the drug binding and release capability. AlPcS₄ was used as a photosensitizer loaded into the pocket of the calix so that as it reaches the tumor site, it readily induces the photo reaction. AlPcS₄+GC5A-12C showed excellent cellular internalization and high drug-delivering ability from flow cytometry in HeLa cells with intense fluorescence, whereas in free AlPcS₄, mild fluorescence signals were observed from CLSM studies. These pieces of evidence indicate the ATP-sensitive release ability of GC5A-12C. The three drugs, OX, MTX, and chl, were easily added to the GC5A-12C nanocarrier and showed lesser IC₅₀ values than the free drugs in HeLa, HepG2, and MCF-7. The nanomaterial's smaller size and additional positive charge make easy passage for them across the cell membrane. This passage got the assistive endocytosis support by salt bridges of guanidium groups having high pKa of 13.65.

Zn and Cd coordinated calixarene-based nanocages with potential binding properties obtained from terpyridine-modified calix[4]arene macrocycle (Fig. 7) [100]. D4h symmetrical structures demonstrated the host-guest binding between the host and anticancer drug mercaptopurine through S- π and π - π interactions. The nanocapsules have shown ordered drug accumulation and remarkably interesting fluorescence suppression upon binding with host, which switched on again after the liberation of the drug. Nanocapsules are found to have low toxicity. Slow diffusion of mercaptopurine was seen in PBS experiments which presents its importance for exploration in medicinal applications like drug delivery and imaging.

Renziehausen *et al.* [78] encapsulated TMZ in a *p*-sulphonatocalix[4]arene with enhanced stability, which gets hydrolyzed very quickly to 5-(3-methyltriazene-1-yl)imidazole-4-carboxamide (MTIC) at pH > 7 that further degenerates to methyl diazonium cation and metabolite 5-aminoimidazole-4-carboxamide. The MTIC derived from TMZ cannot easily surpass the blood-brain barrier (BBB) and does not satisfy cellular dissolution. The hydrophobic nature of the calix cavity assists the methyl of the imidazotetrazine ring of TMZ, which prevents it from getting hydrolyzed rapidly and inhibits DNA replication. The therapeutic efficacy of MTIC received to the tumor location severely depends on the intact existence and ability of TMZ to transcend the BBB because of its high degradation and short half-life of 1.8 h. *In vivo* experiments revealed TMZ+calix complex achieved slow hydrolysis of TMZ with the additional enhancement of half-life (>12 h) observed from LC-MS/MS plasma stability assays. *In vitro* cytotoxicity experiments showed high inhibition of tumor cell growth (up to 81%) in GBM cell cultures.

Zhang *et al.* [94] designed a cancer-targeted drug carrier using the calixarene macrocycle. It can bind with different chemotherapeutic drugs like doxorubicin (DOX), epirubicin (EPI), pirarubicin (THP), daunorubicin (DNR), idarubicin (IDA), etc. Among these

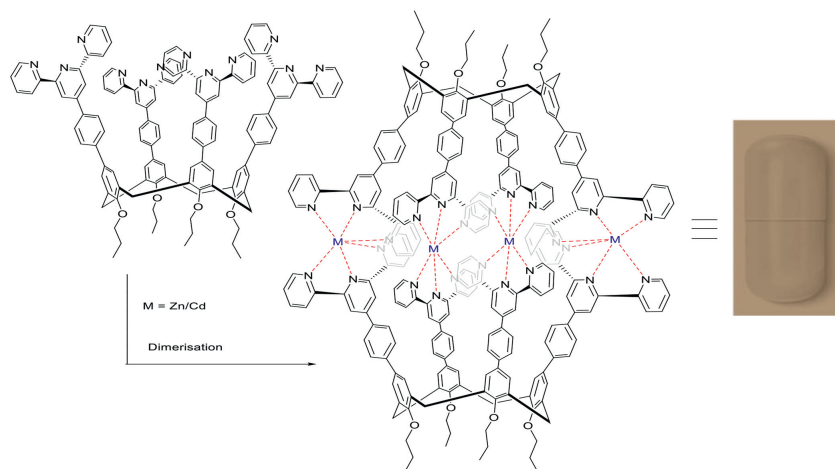


Fig. 7. Formation of metal coordinated nanocapsule.

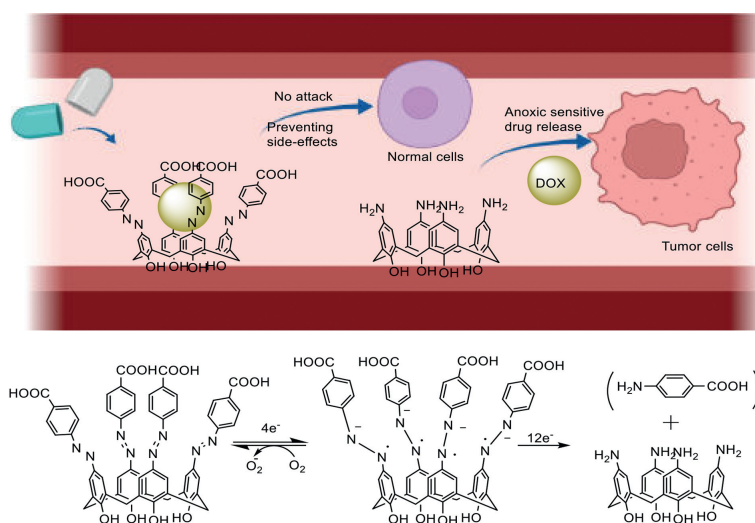


Fig. 8. Cancer selective drug-delivery.

drugs, DOX was predominantly studied. The host-guest complexation between the macrocycle and the drugs enhances their dispersibility property. They can break down under hypoxic conditions. Carboxylated azocalix[4]arene (CAC4A) macrocycle consists of COOH groups at the upper rim providing water solubility. At the same time, it supplements binding affinity to positively charged guests. CAC4A can be reduced to aminocalix[4]arene (NH₂C4A) by nicotinamide adenine dinucleotide phosphate (NADPH) in the presence of DT-diaphorase (tumor-activated reductase) (Fig. 8).

Low binding constant ($(1.0 \pm 0.4) \times 10^4$ L/mol) of reduced NH₂C4A and DOX was observed compared to CAC4A and DOX (binding constant = $(1.6 \pm 0.5) \times 10^8$ L/mol) which helps to understand the release sensitivity to hypoxia. It was also studied using mimicking hypoxia conditions with sodium dithionite utilizing the fluorescence property of DOX. In addition, DOX, a fluorescent compound, remains unaffected in encounters with NH₂C4A. At the same time, CAC4A suppresses the fluorescent property of DOX. The MTT assay found low cytotoxicity for CAC4A in 4T1 cells and CAC4A.

Carbon allotrope fullerene has gained a significant surge in biomedical applications (antioxidants, antiviral/antibacterial agents, drug delivery, and MRI contrast agents) due to its electronic properties [101–109]. Their unique structural features have abetted their use in designing multifunctional nanomaterials, and their hy-

drophobic nature makes them quite challenging for designing biomaterials.

Zhang *et al.* reported a modified derivative of SC4A-sulfonated azocalix[4]arene (SAC4A) with a deep hydrophobic pocket that can efficiently encapsulate fullerenes (C₆₀ and C₇₀) [110] obtained by simple grinding. The fullerene solubilizing property of SAC4A was found more significant than simple SCnAs. From dynamic light scattering analysis (DLS), the C₆₀ appended SAC4A was found to be more stable. C₆₀+SAC4A assembly caused the formation of ROS species, which can be incredibly useful for applications in photodynamic therapy.

Recently, Chen *et al.* [96] developed a highly pH-sensitive drug-delivery system from sodium hexanoate-CB[7], with high aqueous solubility of more than 600 mg/mL. In acidic conditions, the alkyl chain of the hexanoate group gets encapsulated in CB[7], resulting in the subsequent pH-induced release of encapsulated drugs inside the CB[7] cavity. They have achieved a competitive binding effect from the hexanoate group. *In vitro* activity analysis of irinotecan loading using A549 cells revealed anticancer ability (binding affinity = 10^6 L/mol at pH 7.4) with a cytotoxicity effect at pH 5.4.

Another exciting drug carrier was constructed by Wu *et al.* [111], utilizing host-guest recognition properties of pegylated CB[7] and oxaliplatin (OxPt, a chemotherapy drug). The drug release ability was achieved by manipulating the thermodynamic features of the carrier in water. When CB[7]+OxPt complex of 1:1 ratio was

studied in 1640 cell culture, a significant decrease in binding constant and enthalpy with a strict increase in entropy was observed. Phenylalanine (Phe) amino acid in 1640 medium has a high binding affinity towards CB[7], gets encapsulated in CB[7] pocket, and liberates water. Moreover, the inorganic salts of the medium also interact with the polar carbonyl groups at the openings of CB[7], which affects the host-guest binding interactions. Though different pegylated CB[7]s were synthesized and tested to analyze their host-guest binding abilities, there is no change in binding. Enhanced toxicity in cancer cells was found with CB[8] macrocycles using combined loading of OxPt and acridine orange (AO) [112]. A high number of dead HeLa cells were observed from the cooperative toxicity of AO and OxPt, achieved within a short incubation time.

Light-sensitive drug release advancement was demonstrated by Škalamera *et al.* [97], in which two host-guest complexes were constructed from two positively charged cresol prodrugs and CB[7]. The inclusion complexes showed stable dissolution and drug carriage properties for an efficient drug delivery system. Photo-induced deamination of the encapsulated prodrugs leads to conversion into their biologically active form, quinone methides (QMs). QMs are neutral zwitterionic intermediates that can cause DNA alkylation and cross-link, a popular approach for anticancer chemotherapy [113]. QMs were not found to form a sturdy host-guest complex with CB[7], which resulted in their dissociation from the supramolecular hosts. MTT assay on H460 and MCF-7 cancer cell lines revealed increased proliferation inhibition from the inclusion complexes under UV light than QMs alone.

Co-ordination assisted supramolecular self-assembly was developed by Datta *et al.* [114] from an organoplatinum(II) with methyl viologen (MV) and CB[8]. It can trap curcumin in aqueous media and transport to tumor cells. Interesting curcumin concentration-dependent structural modulations were seen. Curcumin sustained release in pH 6.8 to 7.4, while a significant increase of curcumin release was observed under acidic conditions. *In vitro* study results observed negligible cytotoxicity, excellent suppression of cancer cells in C32, B16F10, MCF-7, and MDA-MB231 cells with low IC₅₀ values.

6.1. Hybrid nanoparticles

Nanomaterials specifically responsive to cancer-selective enzymes are in high demand to achieve efficient, targeted delivery materials for cancer treatment. Indoleamine 2,3-dioxygenase 1 (IDO1), a metabolic enzyme observed in various cancer cells, converts the amino acid tryptophan (Trp) to kynurenines through catabolism. It is a key step to unlock the drug complex for the liberation of drugs. Such nanocarriers sensitive to IDO1 were reported by Qiao *et al.* [115], in which they have developed hybrid raspberry-like nanoparticles (HRNs) from a homoternary complex CB[8]+[Trp, Trp], a nanoporous silica cavity, and Trp-modified Fe₃O₄ nanoparticles (Fig. 9). Nanocarriers with 100 nm diameter (confirmed from TEM images) easily pass-through cell membranes. DOX was encapsulated in the silica core and locked by Fe₃O₄ nanoparticles. They are released after the breakage of the supramolecular cover in the presence of the IDO1 enzyme. High cellular uptake and biodistribution were observed *in vitro* and *in vivo* investigations. Considering this tumor-induced cargo release effect of the nanocarriers within a cell makes them promising candidates as intelligent drug carriers.

6.2. Micelles and hydrogels

Hydrogels are extensively used for clinical purposes, especially their drug conveying capability. Their cross-linked structures with

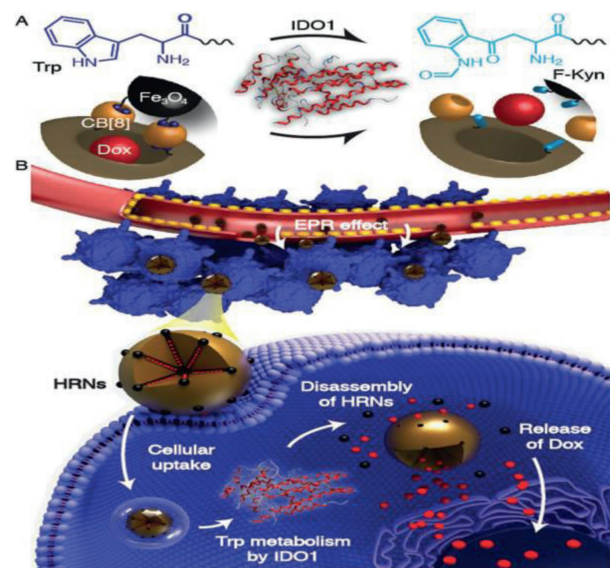


Fig. 9. IDO1 sensitive drug-delivery of CB[8] based self-assembled supramolecular coronas. Reprinted with permission [115]. Copyright 2019, John Wiley and Sons.

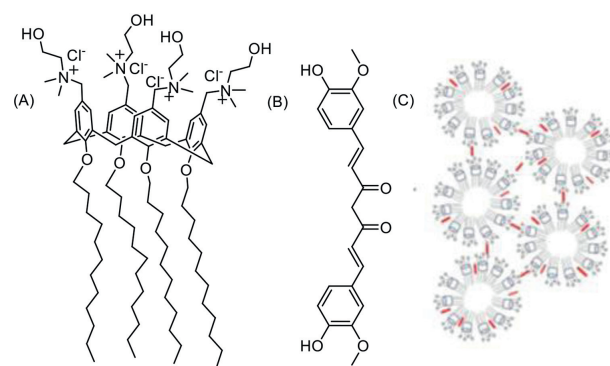


Fig. 10. Structures of choline calix[4]arene derivative (A), curcumin (B), curcumin (red bars) (C) in sandwich and bridge model. Reprinted with permission [24]. Copyright 2020, Elsevier B.V.

impressive elasticity and viscid property with high hydration abilities make them highly bio-compatible for applications in drug development. Granata *et al.* [24] designed self-assembly assisted nano hydrogel micellar polycationic choline-calix[4]arene derivative (Fig. 10) with curcumin for their multi-target pharmacological applications.

Curcumin was efficiently released in PBS solution with increased time at human body temperature. The micelle-based hydrogel effectively protected the curcumin. Its photostability was evaluated from steady-state photolysis tests. The hydrogel displayed properties like that of non-Newtonian pseudoplastic fluid. It showed a decreased viscosity at high shear strain revealed from rheological studies. The hydrogel's fast self-healing properties were observed. The micellar hydrogel appears to be a potential media for drug delivery of multi-target drugs like curcumin, which was also confirmed from anti-inflammatory studies as eye drops in the eyes of rats with LPS-induced uveitis [116].

An *et al.* [117] constructed doxorubicin (DOX) loaded micelles via self-assembly between DSPE-PEG2000-FA folate, DOX, and PEG550 grafted calix[4]arene. The micelles showed more significant inhibition of anti-tumor HepG2 cells than free DOX. Two bioactive amide groups were attached at the lower rim for the bio-

compatibility of the DOX. Hydrophobic alkyl chains protected both groups at the lower rim of the calix. Hydrophilic PEG550 chains at the upper rim of the calix inside the micelle structure prevent interaction with other cells in the blood for safe carriage. *In vitro*, drug release experiments revealed that DOX release is pH-sensitive and at slight acidic pH than physiological pH, DOX released for 10 h which became very stable at around 24–48 h due to the pH-sensitive PEG550 chains at the upper rim. The reported micelles thus can be especially useful for biomedical applications as it is water-soluble and can deliver DOX to tumor cells specifically for minimized side effects and enhanced efficiency. In addition to providing hydrophilicity, the PEG550 chains also prevent intervention from serum proteins and other micelles.

Dual drug-loaded nanocarriers for various therapeutic applications [118–120] have gained massive attention in the present time, as they were found to impact therapeutic efficacy by providing target-specific interaction with drugs significantly. Such nanomaterials are mostly seen in anticancer treatment, where they strictly affect cell cycle arrest leading to inhibition of cancer growth [121–123]. Micellar nano-container was constructed with *p*-sulfonato-calix[4]arene macrocycle to co-encapsulate temozolomide (TMZ) and curcumin (CUR). A highly significant effect was seen on glioblastoma cells, as reported by Rossella Migliore *et al.* [124]. The significant stability and solubility enhancement of TMZ and CUR in the TMZ+CUR+calix[4]arene was observed compare to the free drug. The nanomaterials can be potent nano-vehicles for dual-drug responsive cancer treatment, which supports the anti-tumor effect of TMZ bound in sulfonated calix[4]arenes. They have negligible cytotoxicity, and their hydrodynamic diameter is 122 nm.

6.3. Amphiphilic aggregates

Liposomes can encapsulate hydrophilic, lipophilic, and amphiphilic molecules with unique biocompatibility. Enabling it as a highly functional drug delivery system in medicinal applications [125]. Hu *et al.* [98] reported photo responsive drug-delivering nano-vesicle constructed from CB[8], maleimide modified methyl viologen (MMV), and 3,4,5-tris(*n*-dodecyloxy)benzoylamide (TBA-Azo) in 1:1:1 ratio. MMV gives hydrophilicity to the complex. The TBA-Azo group provides hydrophobicity, which isomerizes to *cis* form (Fig. 11) under UV beams and induces photosensitive disruption of the supramolecular construction to achieve a sustained drug release. Dox encapsulated vesicles showed high efficiency, low cytotoxicity, and enhanced cell death in A549 cells by >31.5 fold compared to free vesicles under UV light irradiation. It shows the integrity of the nanovesicles in photo-operated drug delivery for tumor medications.

Lebron *et al.* [126] synthesized liposomes from different calix[4]arene derivatives with modified polar moieties at the upper rim and hydrophobic tails at the lower rim. Dioleoyl-*sn*-glycerol-3-phosphoethanolamine (DOPE) phospholipid used in liposome form spherical shaped liposome bilayers observed from TEM images. Compared to their micellar and vesicular form, lipoplexes displayed enhanced drug transportability with sufficiently positive charges for easy passage through cell membrane. Lipoplexes showed easy encapsulation against antineoplastic drug doxorubicin and were found functional till six days. A sustained *pseudo*-first-order kinetics of doxorubicin release was observed *in vitro* analysis at physiological temperature with suppressed side effects.

Similar liposomes were synthesized by hydration of lipid films with 1,2-dioleoyl-*sn*-glycerol-3-phosphocholine (DOPC), 1,2-dioleoyl-*sn*-glycerol-3-phosphoethanolamine (DOPE) and cholesterol in water with a Cy5 fluorescent dye [127]. Further addition of guanidium appended calix[4]arenes by post-insertion technique resulted an enhanced cell-penetrating characteristics of liposomes. Positive zeta-potential values and high cellular accumulation was

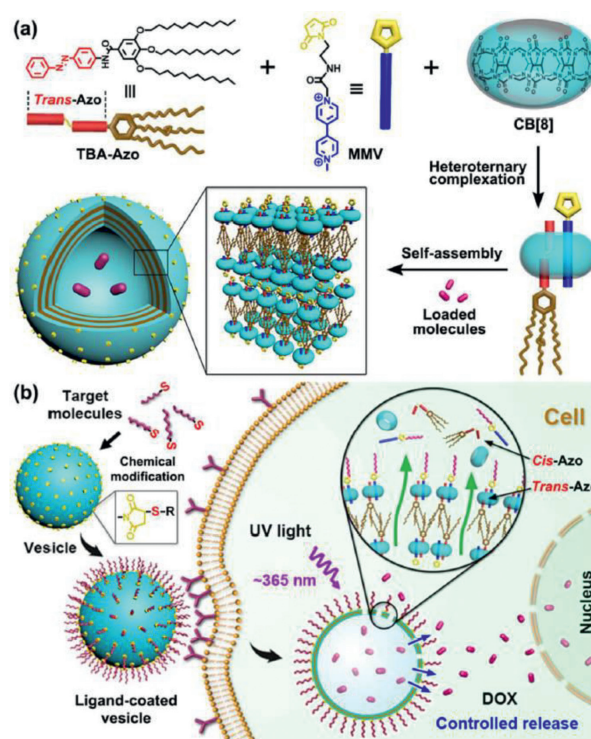


Fig. 11. Schematic illustration of supramolecular vesicle formation and photodegradation. Adapted with permission [98]. Copyright 2018, American Chemical Society.

observed for the liposomes in CHO-K1 cells, demonstrating their good cellular uptake capacities compared to the low uptake of unmodified liposomes. Moreover, in pgsA-745 cells (mutant CHO-K1 cell-line that does not express heparan sulfate proteoglycans (HSPGs)), cell uptake of modified liposomes was not satisfactory. These cellular internalization features of the guanidium-modified calixarene-based liposomes can help to design a drug delivery media.

Another pH-responsive micellar drug carrier was reported previously by Chen *et al.* [56] as an efficient tumor-targeted micelle container for curcumin encapsulated in calix[4]arene supramolecule. Curcumin has some demerits like poor pharmacokinetics, pharmacodynamics, and low efficiency in some diseases. Many pathways were approached by utilizing nanomaterials to enhance efficiency for various biomedical applications of nanomaterials, which showed promising cancer-targeted capabilities [124]. Curcumin has loaded with a phosphorylated calix[4]arene with high amphiphilicity. That forms micelles and releases curcumin at different pH (slow release at neutral pH and fast release at pH < 7.2). Because of uncharged phosphonate groups of the calix upper rim at acidic pH, dissolution of micelles increases, making it more unstable, leading to rapid release of confined curcumin. The curcumin-loaded micelles showed high inhibition of triple-negative breast cancer (TNBC) and lowered levels of nuclear β -catenin and androgen receptors, significantly increasing breast tumor suppression. The encapsulated micelles were also found highly effective against CD44⁺ and CD133⁺ breast cancer stem cells, which signifies the therapeutic ability of the micelles for the treatment of breast cancer.

The use of cyclodextrin as dynamic drug-delivery media is ubiquitously found in biomedical research. Approved by the US FDA and considered harmless for medical applications. The pruned cone shape of cyclodextrins possesses a hydrophobic inner pocket that can encapsulate hydrophobic drugs [128,129]. Amphiphilic cyclodextrins can form nano-vesicles or nanocapsules. That is utilized

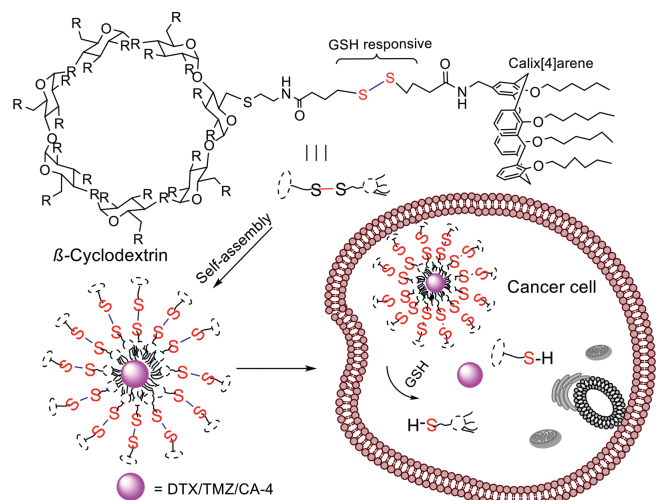


Fig. 12. De-assembly of nanosphere via disruption of disulfide bond in the tumor environment.

for designing sustainable drug loading and releasing systems for targeted pharmaceutical functions [130,131]. However, only a few studies have been done on nanocapsule combinations with other biocompatible macrocyclic systems in the context of biomedical applications.

Gallego-Yerga *et al.* [95] reported disulfide bridge grafted amphiphiles constructed from modified β -cyclodextrin (β CD) and calix[4]arene that assemble themselves to provide redox stimulated drug delivery characteristics. Nanospheres and nanocapsules were constructed to capture anticancer drugs like docetaxel (DTX), temozolomide (TMZ), or combretastatin (CA-4) with high drug capturing efficiencies (>81%). They disrupt and efficiently liberate encapsulated drugs in high glutathione (GSH) environments similar to those found in cancerous cells (Fig. 12).

Water-soluble obtained nanosystems were amicable for accumulation in the blood (estimated using a critical micellar concentration study). Using these nanosystems effective inhibition of various cancer cell lines (PC3 prostate cells, LnCap cells, MCF-7 cells, glioblastoma U87 cells, cervical and colon cancer cells) and significantly enhanced cytotoxicity were observed. It decreased IC_{50} values compared to individual reference drugs. Additionally, high apoptosis of cells was found with Annexin V/propidium iodide (PI) double staining technique in NP-treated cells, demonstrating the effect of GSH-responsive drug release. Further group study has displayed β -cyclodextrin and calixarene-based nanoscale amphiphilic hosts effectively binds with docetaxel (a chemotherapy drug) [132]. They can cause significant cell death in human LnCap, PC3, U87, and rat C6 cells with slow diffusion of the loaded docetaxel. The β -CD affords undercover against serum proteins yet gives access for nano surface modification that can modulate the specificity and effectiveness of the designed nanomaterial.

Colon cancer or colorectal cancer (CRC) is third among all common types of cancer. It is considered the second major cause of cancer-associated mortality rates throughout the globe. Even though present-day research sheds light on the pathogenesis of CRC, its treatment, understanding the genomic and epigenomic instability, and providing enhanced screening strategies, the prevalence of CRC is progressing [133–136].

Li *et al.* reported that cup-like nanomaterial established from phosphonate calix[4]arene have effective inhibition against HT-29 adenocarcinoma cells. These are prominent among colon cancer types [137]. They have phosphonic groups at the upper rim and alkyl chains at the lower rims of the calix[4]arene. They are assembled into the liposomal structure (Fig. 13). It was utilized to cap-

ture two chemotherapeutic drugs, paclitaxel (PTX) and carboplatin (CPT). CPT occupies an external bowl-shaped cavity, while PTX is solubilized between the bilayers of liposomal structure. They have low polydispersity and hydrodynamic diameter that can overcome renal excretion. The nanomaterials were intact for more than 72 h and were experimented with at different pH values. The dual-loaded nanomaterials showed more significant cytotoxicity towards HT-29 cells and had higher cell apoptosis than the free drugs and empty nanomaterials. This shows the potential of PTX+CPT loaded nanoparticles to develop effective nanomedicine for CRC.

6.4. Biomolecular nanoassemblies

Carboxylated azocalix[4]arene-embedded nanoparticles (CENP) modified with phenylboronic acid (PBA) & polyethyleneimine (PEI) were reported by Liu *et al.* [62]. It can efficiently co-deliver molecular drug-DOX and therapeutic gene-plasmid DNA (pDNA) for an interference-free gene-drug combination cancer therapy system. A polymeric shell was constructed from *cis*-aconitic anhydride (CA)-modified poly(ethylene glycol)-*b*-polylysine (mPEG113-*b*-PLys110/CA) with acidic and hypoxic degradation features (Fig. 14). In an acidic environment (pH 6.5–6.8), the release of therapeutic genes with stable blood circulation and high cellular internalization of CENP was observed. At the same time, the hypoxia-responsive calixarene derivative prevented the interaction between the genes and drugs. It is done by separating the drugs encapsulated into the pocket of the calixarene. Doxorubicin (DOX) appended CENP suppressed tumor growth was obtained in mice by miR-21 inhibition. Along with a plasma DNA clustered regularly interspaced short palindromic repeats interference (CRISPRi).

Supramolecular biomaterials are of enormous interest in novel drug-delivery systems in terms of efficient target delivery, enhanced biocompatibility, stability, and protection of drugs. Bovine serum albumin (BSA) is a protein consisting of tryptophan (Trp), tyrosine (Tyr), and phenylalanine (Phy).

It provides beneficial physiological activities because of its high presence in the circulatory system. Its high amino acid composition contributes to many interaction sites to design stable carriage systems of bioactive molecules [138–140]. Barooah *et al.* [141] reported a BSA-based supramolecular drug carrier with CB[7]. DOX (a fluorescent anticancer drug) was loaded into BSA+CB[7] supramolecular assembly, significantly reducing fluorescence emission. DOX can be encapsulated easily at biocompatible pH. Thus, it showed a trigger inducible release at pH 5, or adamantylamine (AD) can be used as a competitive binder at pH 7.4 (Fig. 15). The quenched fluorescence of DOX after encapsulation was recovered when pH was decreased and was similar to the emission of free DOX, which indicates the damaged structure of the serum protein-based supramolecular assembly. MTT experiments revealed no cytotoxicity in MCF-7 and CHO cells with a promising anticancer effect in MCF-7 cells.

One more supramolecular medicinal cargo carrier with a biopolymer consisting of switchable peptides was synthesized [142]. This therapeutic cargo aims to develop an efficient and highly targeted drug delivery system. BSA+CB[7] supramolecular complex was synthesized and grafted with peptide using succinimidyl-3-(2-pyridyldithio)propionate (SDDP). SDDP is a cross-linking reagent linked through disulfide linkage. The peptide was constructed with three important constituents: a CPP motif, FAM-E9 (PLGLAGR9GGC), and cPep (GLAGR9GGC). CPP motif induces cell internalization, FAM-E9 prevents CPP cell penetration, and cPep links to BSA and is cut by MMP2 to give tumor-sensitive action by the system (Fig. 16). DOX encapsulation was attained satisfactorily, and no toxicity was observed in MCF-7 and MCF-10A cells with high cell uptake. GSH broke disulfide bonds of the peptide to release DOX under acidic pH. These features demonstrate

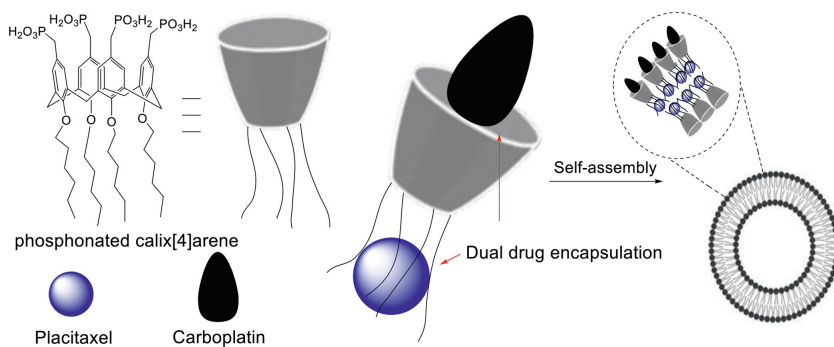


Fig. 13. Liposome formation from self-assembled phosphonate calix[4]arene.

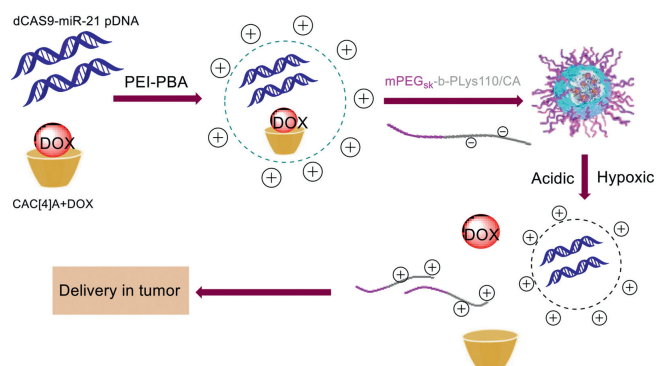


Fig. 14. Acidic and hypoxic sensitive gene & drug delivery in tumors.

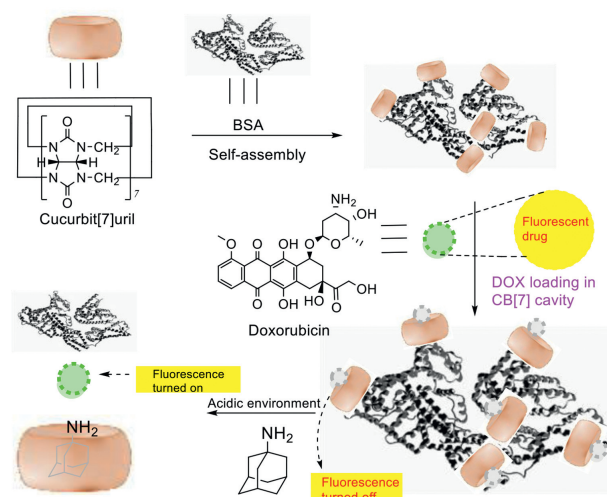


Fig. 15. Adamantylamine and pH-induced drug release from BSA+CB[7] aggregates.

the cancer-selective property of the supramolecular drug carrier. It prevents interactions with normal cells that can be further explored to treat tumors using supramolecular vehicles.

Photodynamic therapy (PDT) is a considerably safe cancer treatment without prolonged side effects, which mainly involves photoresponsive medicines to cause cancer cell death. Combining photoactive medicine with a suitable macrocycle can be developed as potent PDT therapeutics. Robinson-Duggon *et al.* [66] investigated

the delivery and phototoxicity of different photosensitizers (toluidine blue (TBO), TBOC6 and TBOC14 fatty acids), appended with CB[7] and HSA macrocyclic host. Characteristic binding was ob-

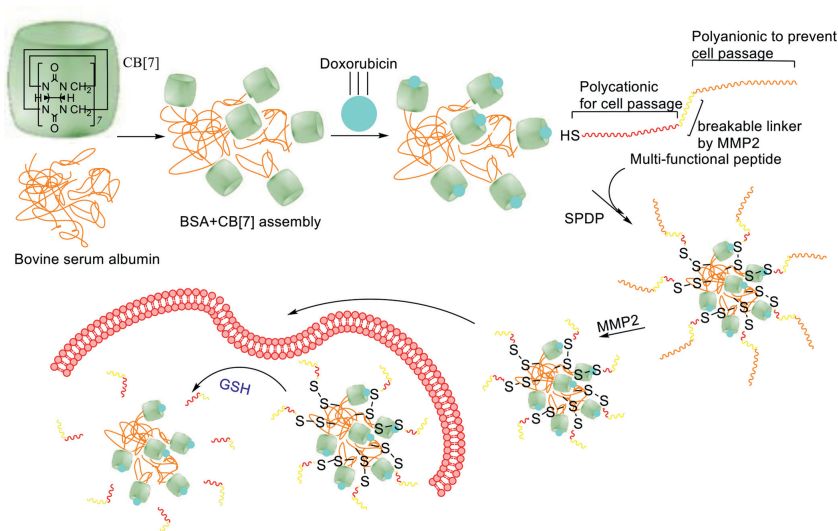


Fig. 16. Peptide-based drug release of BSA+CB[7] assembly.

served for TBO and TBOC14 with CB[7], while low affinity towards TBOC6. It showed high binding constants in the case of HSA, TBO and TBOC14. High cell internalization was observed when the photosensitizers were grafted in the HSA of the CB[7]+HSA assembly. Under UV radiation, the supramolecular system-induced remarkable toxicity with all photosensitizers. It was attained from an indirect ROS production from oxidative photodegradation of HSA.

6.5. Nanofiber and nanosponges

pH-sensitive nanofiber mats were constructed by Ozcan *et al.* [143] as a tumor-targeted drug delivery system using human serum albumin (HSA) grafted calix[4]arene. The nanofiber mats were added with DOX in various buffers solutions with varying pH, and the addition was studied using FT-IR, TEM, SEM, and EDX methods. *In vitro* drug discharge analysis was performed for the DOX-added nanofiber mats at different pH and time intervals, revealing the pH-sensitive drug discharge characteristics at 90 min. Adding HSA, FA, and GSH enhances the nanofiber's biocompatibility. Nanofibers can assist in tumor therapeutic applications with the presence of DOX.

Electrospun nanofibers have recently gained interest in material science and bio-medical fields due to their high specific surface area, porosity, biocompatibility, and small-fiber diameter. These make them ideal for numerous industrial and medicinal applications [144]. Cagil *et al.* [145] reported calixarene-based nanofibers with potent drug loading and releasing features. The nanofiber was found pH sensitive. They can efficiently release loaded drugs like thiabendazole (Tbz) (anthelmintic) and donepezil (Dnp) (acetylcholinesterase inhibitor) using different buffers. Drug accumulation and release were studied directly from the nanofibers. The number of unaccumulated drugs was analyzed through regression equations of fluorescence intensity calibration graphs at their respective emission intensities. Even though the drugs Tbz and Dnp are hydrophobic, their loading capacities into the nanofibers were different at different pH (maximum drug loading efficacy for Tbz and Dnp are 1.688 μg at pH 7.4 and 30.529 μg at pH 7.4, respectively). From a comparative drug release study at pH 7.4, 6, 4, and 2.2 the maximum release efficiency of the nanofibers for Tbz was observed to be 0.243 μg at pH 7.4. While in the case of Dnp, it is much higher $\sim 9.72 \mu\text{g}$ at pH 2.2, which can be due to the lower order of intermolecular interactions between Dnp and calixarene-based nanofibers as compared to Tbz. These newly designed calixarene-based nanofibers with pore size 140–170 nm and diameter of 600 nm prepared by a simple electrospinning method can thus be an efficient pH stimuli drug carrier for bio-active applications.

7. Bioimaging

Biological imaging is a dynamic tool that enables us to track and study cellular and molecular imaging of biological processes and investigate metabolites for biomarker identification and treatment of lethal diseases. Bioimaging tools were significantly developed for studying cellular processes linked to diseases like cancer, wilson, Alzheimer's, utilizing various fluorescent organic dyes [146–148]. Generally, systems with high water dispersibility, good fluorescent properties, high biocompatibility, and low toxicity are potential candidates for bioimaging studies. Numerous examples show how bioimaging is evolving from qualitative visual analysis to quantitative measurements of biological imaging [149–153]. Supramolecular fluorescent probes like hydrogels with three-dimensional cross-linking structures have high biocompatibility and promising bio-imaging applications. Highly efficient and sensitive gelators can be constructed by incorporating fluorescent

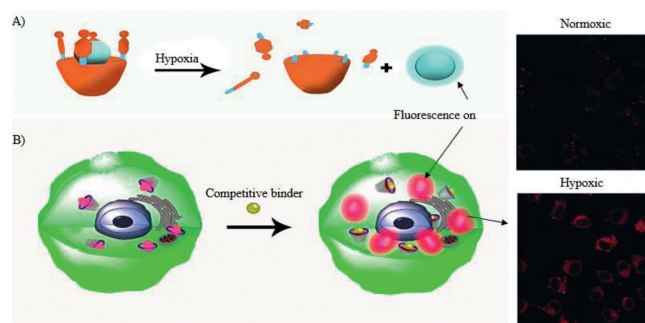


Fig. 17. (A) Hypoxia responsive cargo release and (B) hypoxia responsive cargo release in the presence of a competitive and their utilization in bioimaging.

materials or dyes that provide less interference and very high sensitivity with detection limits of 10^{-8} – 10^{-10} s. Supramolecular fluorescent hydrogels are highly advantageous to other bio-imaging probes regarding biocompatibility and stability. The cell imaging resolution can also be significantly modulated with varying fluorescent probes, which highly demand bio-marking advancements. Nowadays, the probes used in supramolecular chemistry are widely studied to ameliorate imaging potentiality [154–156], such as hypoxia imaging (Fig. 17). Macrocyclic host-guest complexation critical in supramolecular chemistry with its effective animated features and has brought up a broad dynamic scope of study related to its interactions and structural properties.

p-Sulfonatocalix[*n*]arenes (SCnAs) are well-known water-soluble and biocompatible calixarene derivatives. They have a three-dimensional cavity with interesting binding properties, especially to organic cations [157]. Their host-guest complexation properties make them ideal macrocyclic hosts for supramolecular architectures. There has been an increasing demand to explore the organic molecular framework with ion sensing capabilities that can even function in biological cells. Recently, an article was published by Noruzi *et al.*, where a water-soluble supramolecular system based on *p*-sulfonatocalix[4]arene derivative was reported with a high binding affinity towards Hg^{2+} [157]. The supramolecular materials showed characteristic fluorescence when studied in SW-620 cell lines. The material was potent for imaging studies in living cells with Low toxicity for SW-620 cells.

Bhatti *et al.* [158] reported Hg^{2+} detection with the supramolecular assembly of *p*-sulfonatocalix[4]arene and rhodamine triamine. It was used to design a chemosensor (fluorescence off-on) system to detect Hg^{2+} ions. Upon complexing with the Hg^{2+} ion, the synthesized water-soluble compound showed apparent accretion in fluorescence intensity at nearly 573 nm, attributed to the chelation-enhanced fluorescence (CHEF) effect. However, no bio-imaging studies were performed for the reported material.

Other host-guest complexes were exploited based on biologically important acridine and *p*-sulfonatocalix[4]arene. They have dynamic photophysical characteristics upon complexation [159]. Prototropic forms of acridine form complex with *p*-sulfonatocalix[4]arene were studied at different pH. It resulted in fascinating optical properties and pH-sensitive variations upon acridine's encapsulation in the calixarene derivatives. The modulations can be regulated using acetylcholine as a stimulant familiar with its neuro-transmitting properties [160,161]. The acridine-calixarene complex's fluorescence properties were sensitive to acetylcholine, which was studied using absorption, steady-state, and time-resolved fluorescence. In short, a systematic off-on supramolecular sensitive system can efficiently release encapsulated acridine upon adding acetylcholine. It can be advantageous for the development of bio-imaging probes and biosensors.

Yilmaz *et al.* constructed Hg^{2+} sensitive supramolecular systems using modified upper rim calixarene derivatives containing rhodamine [162]. The Hg^{2+} selective binding affinities were explored using fluorescence titrations spectral analysis with various metal ion solutions. Upon successfully binding Hg^{2+} ions, the synthesized material showed distinct fluorescent emission even with highly diluted Hg^{2+} solutions. The binding interaction was also studied from UV-vis spectroscopy, in which the solutions of free reported compounds did not show any emission characteristics. While, after the addition of Hg^{2+} , the colorless solution turned pink. The reported rhodamine-based calixarene derivatives showed low cytotoxicity ($77.42\% \pm 1.323\%$ to $86.18\% \pm 0.654\%$ viability in HEK 293 cells) and efficient inhibition of MCF-7 studied in MIA PaCa-2 cancer cell lines. Live-cell imaging of MCF-7 and MIA PaCa-2 cell lines, the Hg^{2+} appended compounds displayed red fluorescence, which can apply Hg^{2+} ion sensing in bio-imaging studies.

Cu^{2+} selective chemosensing supramolecular complexes with azocalix[4]arene derivatives based on azocalix[4]arene were synthesized by Serkan Elcin and his co-workers [25]. They reported two compounds: 25,26,27,28-tetra-hydroxy-11,23-di-(*tert*-butyl)-5,17-di-(2-anthracenyl)azocalix[4]arene (X) and 25,26,27,28-tetra-hydroxy-11,23-di-(*tert*-butyl)-5,17-di-(1-pyrenyl)azocalix[4]arene (Y) with chelation-enhanced fluorescence (CHEF) properties forming 1:1 metal-ligand complexes. Live cell imaging was performed using ZOETM fluorescent cell imager from which the compound X was found efficient towards human colon adenocarcinoma cell line: SW-620 and showed fluorescence when appended to Cu^{2+} ions. Nag *et al.* reported triazole-linked spiroindoline calix[4]arene derivative with selective ion binding affinity towards Cu^{2+} ion [163].

Indoline calix[4]arene derivative is a closed form with triazole groups. When this derivative irradiated with light or added to trifluoroacetic acid (TFA), it gets converted to an open form, thus, making space for Cu^{2+} binding. Fluorescence was observed from cell imaging studies for both the spiroderivative and its Cu^{2+} bound complex. Additionally, anticancer properties with MDA-MB-231 cells were observed from the MTT assay, presenting very compelling cancer cell death ($\text{IC}_{50} = 165 \text{ nmol/L}$) from the calix- Cu^{2+} complex. Colocalization analysis of these complexes against Mito Tracker Green overlap integral of 0.9 was observed, which explains the intake of the derivative into mitochondria that make the derivative potent for cancer cell imaging.

Self-assembled fluorescent supramolecular nanoparticles were developed using quaternary ammonium-modified tetraphenylethene (QA-TPE) with SC4As and bis-SC4As controlled by multiple catalytic features. Thus, developing a novel supramolecular system with aggregation induced emission (AIE) based fluorescence properties [164]. Upon complexation between calixarene derivatives and QATPE, fluorescence was observed but similar experimentation for β -cyclodextrin and cucurbit[7]uril performed and no fluorescence was detected. Photoreactivity of TPE derivatives changes fluorescence properties (fluorescent to non-fluorescent) in the supramolecular material upon UV light irradiation. This property can be helpful for imaging applications.

Chen *et al.* [165] designed a supramolecular system in a dual-step process with near-infrared emission (NIR). At first step, Anthracyl pyridinium derivative, 4,4'-anthracene-9,10-diylbis(ethene-2,1-diyl)bis(1-ethylpyridin-1-ium)bromide (ENDT) aggregation with cucurbit[8]uril (CB[8]) forming nanorods that displayed NIR fluorescence at 625 nm. The fluorescence was further mediated in the next step using calixarene-induced aggregation advantages of water-soluble *p*-sulfonatocalix[4]arene with augmentation of the NIR fluorescence. Interestingly, when ENDT was directly subjected to complex with *p*-sulfonatocalix[4]arene, blue shift fluorescence at 600 nm was observed. The structural anatomy of the fluorescent supramolecular materials was studied using scanning electron mi-

croscopy (SEM), XRD, and Transmission electron microscopy (TEM). They confirmed that nanorods formed from ENDT+CB[8] and supramolecular nanoparticles made-up of ENDT+CB[8]+SC4AD aggregates. The supramolecular complex ENDT+CB[8]+SC4AD was a potential candidate against human lung adenocarcinoma cells (A549 cells). Imaging studies of this complex showed bright red fluorescence in the cells. Cell viability studies from the MTT assay indicated negligible toxicity against A549 cells. Co-stained experiments using ENDT+CB[8]+SC4AD and commercially available lysosome staining dye LysoTracker Blue against A549 cells were carried out. Satisfactory overlapping stain positions were observed in co-stained experiments that revealed an efficient lysosome-based cell imaging method.

Supramolecular assembly of modified calix[5]arenepentadodecyl ether (CC5A-12C) and aggregation-induced emission luminogens (AIEgens) was utilized in the synthesis of highly bright supramolecular AIE dots [166]. The AIE supramolecular dots in water have high integrity for biomedical applications (fluorescence imaging). A high PL quantum yield of 0.72 was observed for the supramolecular dots with low cytotoxicity. A peritoneal carcinomatosis-affected mouse model was used for *in vivo* analysis. The supramolecular AIE dots were observed to nicely access and brighten the tumor nodules observed from IVIS® *in vivo* fluorescence imaging during the image-guided removal of the tumor. Such interesting fluorescence properties of the supramolecular AIE dots make them promising bioprobes for application in fluorescence image-guided cancer surgery (FIGCS) and an efficient tool for designing bio-imaging systems.

Consoli *et al.* reported several calixarene-based micelles as bio-imaging probes [167]. Calixarene micelles of 7 nm diameter (AFM, TEM) were designed with PEGylated cyanine 3 and 5 dyes with two azide groups to enable micelle shell cross-linking Cy3L and Cy5L cross-linkers to form a cyanine corona [168]. Micelle NPs showed fluorescent red shift emission, with an interesting *circa* 2-fold fluorescence enhancement obtained for Cy3L-micelle NPs in aqueous media compared to quantum dots (QD-585) (Wide-field fluorescence microscopy). NPs displayed fluorescent dots upon incubation with HeLa cells, thus revealing the NPs as potent fluorescent probes for bio-imaging.

Similar micelles-based amphiphilic derivatives of *p*-sulfonatocalix[4]arene appended with indocyanine green (ICG) were reported to increase the solubility and fluorescence emission properties of NIR fluorescent dye. ICG dominates its aqueous instability and photodegradation characteristics making the system efficient for bio-imaging [169]. The ICG incorporated micelles were initially tested against bovine serum albumin (BSA) protein for *in-vivo* analysis. Fluorescence modulations were observed with enhanced fluorescence lifetime and stability of the BSA appended micelle complex. Using ICG incorporated micelles; fluorescence imaging of mouse liver and lymph nodes, intense NIR fluorescence emission was observed. The ICG-micelle complex showed negligible cytotoxicity and was easily eliminated from the body by renal clearance. Additionally, breast tumor imaging using KPL-4107 cells in mice with ICG conjugated antibodies efficiently demonstrated the excellent integrity of the reported material for *in vivo* and *in vitro* imaging.

Uttam *et al.* [170] reported an article that demonstrates a derivative of calix[4]arene that can efficiently sense fluoride ion in solution and biological cells with fluorescent tag 7-chloro-4-nitrobenzofuran (NBD) embedded at the lower rim of calix[4]arene. Designed compounds exhibited low detection values of 100 nm in THF, which were analyzed using UV-vis and fluorescence emission spectroscopy. Biocompatibility of derivative of calix[4]arene using MTT assays was performed on HeLa cells with various concentrations and showed 90% viable cells. Confocal microscopy studies revealed green fluorescence for the treated cells. Decreased fluores-

cence intensity was observed with increased F^- concentration and quenched after adding 50 $\mu\text{mol/L}$ of F^- to the cells.

Endogenous polyamine spermine ubiquitously found in all eukaryotic cells is involved in various metabolic processes. Recently, many research have been reported to detect spermine in the early stages of cancer. However, their detection using time-efficient fluorescence imaging techniques was not studied extensively.

Supramolecular systems based on cucurbit[7]uril (CB[7]) and aggregation-induced emission luminogen (AIEgen) was designed by Jiang *et al.* [171] that can selectively detect spermine. Tetraphenylethylene (TPE) AIEgen, when appended into the cavity of CB[7], showed a fluorescence turn-on feature analyzed from fluorescent imaging studies. Competitive spermine binding upon addition to the supramolecular system AIE+CB[7] abetted the release of the trapped AIEgen. Its emissive characteristics were observed as very weak, as in free AIEgen due to switching off the fluorescence of the system. PL spectral studies of the complex concerning spermine revealed the detection limit of 1.0 $\mu\text{mol/L}$, which can be utilized for spermine detection in the early stages of cancer (1–10 $\mu\text{mol/L}$ for spermine in urine).

Li *et al.* [172] studied endocytosis and excretion mechanisms of cyanine 3-conjugated CB[7] (Cy3+CB[7]) and rhodamine X-conjugated CB[7] (ROX+CB[7]) with human breast carcinoma cells (MCF-7). Live MCF-7 cultured cell medium bearing the Cy3-CB[7] and ROX-CB[7] derivatives showed fluorescent emission up to a sufficiently long time (from CLSM studies), which revealed the accumulation of the derivatives within the cells. However, a distinct decrease in the fluorescence was observed as the derivatives were excreted from the MCF-7 cells. Different inhibitors like chlorpromazine (CPZ), genistein, and sodium azide (NaN_3) were treated to study the intake mechanism of the derivatives, from which different uptake routes were obtained, predominantly the clathrin-mediated endocytosis. Cellular excretion pathways were explored using nocodazole inhibitors, which explained the lysosome-associated exocytosis. It demonstrates the dynamic potential of synthesized compounds for analyzing cellular processes for application in bioimaging diagnosis.

Bockus *et al.* [173] reported indicator displacement assay (IDA) based supramolecular conjugate (Q7R). Cucurbit[7]uril was covalently linked to tetramethylrhodamine (TMR) with fluorescent properties, which were used for cell imaging. Guests bind with supramolecular materials, resulting in the fluorescence of the combined system being diminished. The decrease in fluorescence is attributed to the supplanting of the TMR group from the supramolecular complex. Also, confocal fluorescence microscopy was used for cell imaging to study HT22 neurons in living cells appended with Q7R, which revealed its accumulation in the cytoplasm. However, it is observed that no significant cell growth modulation characteristics in the addition of Q7R up-to 2.2 $\mu\text{mol/L}$ concentrations.

Aggregation-induced emission (AIE) based cucurbit[7]uril supramolecular aggregates (AIECB[7]) were constructed in aqueous media with pH sensitivity leading to singlet oxygen ($^1\text{O}_2$) production [174]. It was promising for cell imaging and photodynamic applications. Cellular intake of AIECB[7] was studied using live A549 cells, and an increased fluorescence in the lysosome (pH 4.5–5.5) was observed compared to the intrinsic fluorescence of AIECB[7]. $^1\text{O}_2$ abetted PDT-induced cell apoptosis upon irradiation of light in AIECB[7] treated A549 cells. *In vitro*, MTT assay with incubated A549 cells and AIECB[7] under different light conditions showed low cytotoxicity without light irradiation, even at high AIECB[7] concentrations. Additionally, several potential chemotherapeutic pieces of evidence were also explored using oxaliplatin and banoxantrone (AQ4N).

Zhou *et al.* [175] reported biocompatible supramolecular complexes (BPV2²⁺+CB[7] & BPV2²⁺+2CB[7]) of bis-viologen biphenyl

molecule (BPV2²⁺) appended to CB[7]. Both of them have high fluorescence emission and low cytotoxicity characteristics. MTT assay using MCF-7 cells were studied in different concentrations of BPV2²⁺ and CB[7] over varying periods, which revealed no cell death till 125 $\mu\text{mol/L}$ concentration for BPV2²⁺+CB[7] & BPV2²⁺+2CB[7] system. Cell imaging in MCF-7 cells was performed using confocal laser microscopy with BPV2²⁺, BPV2²⁺+CB[7], and BPV2²⁺+2CB[7], which showed bright blue light emission in cells for BPV2²⁺+CB[7] and a comparatively lower blue light emission in BPV2²⁺+2CB[7] images. Also, using WGA stain in cellular membranes, the accumulated BPV2²⁺ was imaged in CLSM with effective internalization of BPV2²⁺ in the cells.

Wang *et al.* reported dynamic supramolecular assembly of coumarin-modified tetraphenylethylene derivatives (TPEC) and CB[8]. These assemblies have structurally tunable fluorescent characteristics [176]. Under light irradiation, the compounds undergo dimerization with reduced fluorescence intensity. Initially, TPEC+CB[8] complex showed yellow fluorescence after light irradiation showed blue fluorescence. Such interesting fluorescent properties of the supramolecular complex accentuate its application in designing photosensitive systems.

Ma *et al.* [177] synthesized water-soluble supramolecular material (DA-PY+CB[8]) based on twin axial pseudorotaxane with cucurbit[8]uril and phenyl pyridine derivative (DA-PY) phosphorescence material. They inspected its application in A549 cells. Confocal laser scanning microscopy revealed efficient internalization of DA-PY+CB[8] in the A549 cells with green phosphorescence stain. Colocalizing stains using mitochondria marker Mitotracker RED were obtained, indicating its potential for utilization in mitochondria imaging in cells. At lower concentrations, DA-PY+CB[8] showed no cytotoxicity against A549 cancer cells.

Supramolecular nanoparticles TPE-2SP+CB[7] and TPE-2SP+CB[8]+HA-CD with near-infrared emission were reported by Shen *et al.* [178] that were synthesized using tetraphenylethylene derivative (TPE-2SP), cucurbit[8]uril (CB[8]), and β -cyclodextrin modified hyaluronic acid (HA-CD). TPE-2SP+CB[8] showed red-shifted fluorescence emission in water, amplified after treating tumor-targeting agent HA-CD to TPE-2SP+CB[8]. The supramolecular system TPE-2SP+CB[8]+HA-CD was used to image human lung adenocarcinoma cells (A549 cells). Mito-Tracker Green dye stained TPE-2SP+CB[8]+HA-CD complex treated A549 cells and accumulated complex in the cells displayed red luminescence and overlapped with Mito-Tracker Green dye stains were observed from CLSM studies. In CCK-8 assays of these materials, cell viability in A549 cells was analyzed, and low toxicity towards A549 cells was observed for the synthesized supramolecular materials.

Liu *et al.* [179] reported a dual-light responsive supramolecular complex (DTE-MPBT+CB[8]) with enhanced fluorescence and high water dispersibility. Complex obtained from cucurbit[8]uril and dithienylethylene derivative dithienylethylene-bridged-3-methyl-2-phenylbenzo[d]thiazol-3-ium used to inspect for targeted lysosome imaging. DTE-MPBT+CB[8] treated A549 cells were stained with lysosome staining LysoBlue dye, combining green stains of DTE-MPBT+CB[8] and blue color of LysoBlue. That explained the internalization of DTE-MPBT+CB[8] in the cells, and the MTT assay obtained very low toxicity towards A549 and 293T cells in concentrations less than 20 $\mu\text{mol/L}$. Photo-controlled lysosome imaging was investigated utilizing the dual-visible-light sensitive fluorescence of DTE-MPBT+CB[8]. It showed stable fluorescence intensity effective for light-responsive lysosome imaging.

Phosphorescent supramolecular materials based on molecular folding interactions between cucurbit[8]uril and alkyl-bridged phenylpyridinium salts were reported for mitochondrial imaging [180]. These supramolecular complexes exhibit high phosphorescence quantum yields of about 99.38% and display unique photoluminescence upon light irradiation. Red phosphorescence

was observed from the reported supramolecular complex through an interesting inter-system crossing process and applied in cellular imaging using confocal laser scanning microscopy. The supramolecular material and mitochondrial staining MitoTracker Green dye was appended to A549 living cells, which showed an efficient exhibition inside the cells.

Supramolecular nanomaterials for drug transmission in tumor cells were reported by Yue *et al.* [181] using MRI (*in vivo*) imaging techniques. MRI gives distinct MR indications in tumor cells. The nanomaterials were constructed from cucurbit[7]uril (CB[7]) and Fe₃O₄ nanoparticles that exhibit efficient host-guest binding and delivery of adamantanamine and oxaliplatin for tumor-targeted studies. The CB[7]+Fe₃O₄ nanomaterial was studied with MCF-7 cells, 4T1 cells & L02 cells for toxicity evaluation using MTT assays, which revealed very low toxicity till 250 µg/mL of CB[7]+Fe₃O₄. Hysteresis and T₂ transverse relaxation studies revealed the magnetic properties of the nanomaterial. Benefiting from magnetic properties of complex, it was used in MRI imaging of 4T1 cancerous mice that showed distinct signals for internalization in cancer cells.

Ozkan *et al.* synthesized supramolecular nanomaterials based on gold nanoparticles capped with cucurbit[7]uril. Upon photo excitation, they show unique fluorescence [182]. Their intrinsic fluorescent properties were used to study the imaging-assisted treatment of tumor cells. The nanomaterials were treated in MCF-7 living cells with DMEM medium stained with DAPI dye. Intracellular accumulation of the nanoparticles was confirmed by overlapped stains obtained from Confocal Laser Scanning Microscopy. With low toxicity (*in vitro* MTT assay) toward MCF-7 breast cancer tumor cells, the cytotoxicity can be controlled by adding CB[7].

Similar tumor-targeted cell imaging was achieved by Zhou *et al.* [183] using different cucurbit[n]urils ($n = 7, 8$) complexes with hyaluronic acid (HA), 4-(4-bromophenyl)-pyridine-1-ium (BrBP) organic phosphor. These complexes give phosphorescent biaxial pseudorotaxane materials in an aqueous solution. CB[8]+HA+BrBP showed intense phosphorescence with a long phosphorescence lifetime than CB[7]+HA+BrBP at room temperature. Phosphorescence lifetime for both the synthesized materials was further found to increase upon the decrease in temperature. To study live cell imaging, A549, HeLa, KYSE-150, and 293T cells were treated with CB[8]+HA+BrBP. They displayed distinct green phosphorescence for A549, HeLa, and KYSE-150 cells except for 293T cells, in which no phosphorescence was seen (Fig. 18). Green phosphorescence of A549 cells was found to merge nicely with the MitoTracker Red mitochondrion marker, which revealed their efficient potential to function on cancer cells. The CCK-8 assay studied the low cytotoxicity of CB[8]+HA+BrBP towards A549 cells.

Xuan Wu presented a supramolecular complex of carbazole derivative and curcubit[8]uril (CB[8]) for lysosomal imaging. The complex has ROS generating ability for photodynamic therapy [184]. 1:1 molar complexation resulted in an oligomer formation with an apparent red shift in the near-infrared region with the fluorescence of 4.21 ns. Biocompatibility was investigated using live-cell imaging in A549 cells incubated with C+CB[8]. The CLSM study of C+CB[8] revealed efficient internalization of nanomaterial in the cells. The MTT assay showed its anticancer activity and low cytotoxicity. However, the viable cells treated with C+CB[8] under white light irradiation were found to damage all cells using 4 µmol/L of C+CB[8] complex. Further, HA-CD appended to C+CB[8] displayed specifically targeted functions by reducing phototoxicity towards 293T cells but still damaging A549 cancer cells.

8. Therapeutic assessment *in vivo*

Many *in vitro* investigations have been reported on calix[n]arenes and cucurbiturils. However, a few drugs were

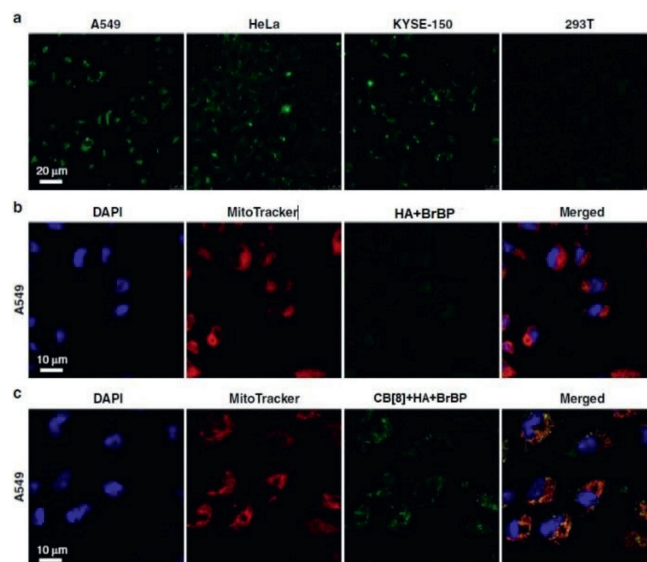


Fig. 18. (a) Cell imaging in A549, HeLa, KYSE-150 and 293T cells with CB[8]+HA+BrBP. (b) A549 cells incubated with HA+BrBP. (c) A549 cells incubated with CB[8]+HA+BrBP. DAPI blue and MitoTracker red was used to stain the nuclei and mitochondria respectively. Reproduced with permission [183]. Copyright 2020, Springer Nature Ltd.

used *in vivo* for various clinical evaluations. In recent studies, CA and CB were mainly involved in anticancer and antibacterial investigations. Carboplatin, paclitaxel, temozolomide, docetaxel, *etc.*, are commonly used medications against different types of cancer such as colon, glioblastoma, ovarian, lung cancer, anaplastic astrocytoma, and many more. Research related to cancer has developed enormously since the last century. However, using particular drugs brings certain clinical limitations, from low biocompatibility and cell toxicity. Research should focus more on these drugs to bridge the gap in drug development to overcome the low pharmacokinetic profiles. These will overcome the therapeutic limitations through designing analogs or using biocompatible supramolecular assistance.

Alkylating agent TMZ is a chemotherapeutic drug approved by the FDA and used as a primary treatment of glioblastoma (GBM). TMZ can pass the blood-brain barrier (BBB) and does not cause direct DNA damage. Renziehausen *et al.* [78] constructed a nanocapsule from *p*-sulphonatocalix[4]arene, loaded with TMZ, to obtain an enhanced bio-compatible feature. Increased stability and slow degradation were observed for TMZ+calix in mice studied under physiological conditions under LC-MS/MS plasma. *In vitro* bioactivity tests were performed with TMZ+calix in GBM cell lines and patient-derived primary cells with known O6-methylguanine-DNA methyltransferase (MGMT) to study the expression status of complexes. TMZ+calix was more effective than free TMZ in suppressing tumor growth in GBM cell lines and patient-derived MGMT. The stability of TMZ+calix was 4-fold the half-life, while independent TMZ has easily deteriorated in mouse plasma.

Meiying Li *et al.* [137] encapsulated carboplatin and paclitaxel in phosphonate calix[4]arene and synthesized nanoparticles. It enhanced structural stability and sustained zeta potential over a wide range of time (0–78 h) and pH (2–10). Effective antitumor potency was observed on HT-29 human breast tumor-bearing nude mice after treatment of oral doses. The nanoparticles caused high tumor inhibition with an increased apoptosis rate observed from the TUNEL Staining technique.

Zhang *et al.* [94] constructed a supramolecular drug container from carboxylated azocalix[4]arene and DOX, an FDA-approved an-

ticancer drug. They observed a hypoxia-sensitive drug carrier with high biocompatibility with no toxicity. Highly effective antitumor ability was found for CAC4A+DOX after injection in T1-bearing BALB/c mice. At the same time, no tumor inhibition was seen in mice treated with DOX alone at the same chemical conditions. The supramolecular combination enhances the anticancer effect and reduces the drug dose.

Curcumin is a polyphenolic compound found in turmeric and known for its versatile bioactivity, including anticancer, antioxidant, neuroprotective, and anti-inflammatory activities. However, their clinical use brings limitations like low bioavailability, absorption, aqueous solubility, and tissue-targeted drug transport.

Platinum(II) containing cisplatin, carboplatin, nedaplatin, lobaplatin, and oxaliplatin are commonly used for chemotherapeutic treatments. However, their applications have several side effects like hepatotoxicity, cardiotoxicity, anaphylaxis, ototoxicity, etc. Cucurbit[n]urils are well-known for their intrinsic host binding ability and biocompatibility, which have been utilized to enhance drug stability, efficacy and reduce toxicity levels. Several inclusion complexes of cucurbiturils have been reported with different platinum-based drugs till now [48].

Two platinum-based supramolecular complexes, CB[7] with oxaliplatin and CB[7] with carboplatin, were synthesized, and it shows a negligible change in cytotoxicity of the drug in peripheral blood mononuclear cells [185]. Compared to CB[7]+carboplatin complex, CB[7]+oxaliplatin revealed enhanced toxicity effects in B16 and K562 cells *in vitro*. An *in vivo* study of murine B16 cell line-based melanoma mice experiments observed a synergic toxic effect. This treatment was done either with carboplatin or CB[7] caused by the degradation of carboplatin to *cis*-PtL₂(NH₃)₂ (L = H₂O or OH⁻) and 1,1-cyclobutane carboxylic acid in the aqueous medium. Further use of CB[7]+carboplatin complex showed acute toxicity in the mice with an enhanced antitumor effect. On the contrary, CB[7]+Oxaliplatin complex did not display any impressive tumor inhibition, although reduced toxicity and reduced weight loss were observed.

Granata *et al.* [116] reported the inclusion of curcumin in polycationic calix[4]arene-based nano-assembly, in which calix[4]arene remarkably enhanced the solubility and stability of curcumin. The anti-inflammatory property was observed relative to unbound curcumin *in vivo* and *in vitro* study. Further experiments using LPS-induced uveitis mice treated with curcumin+calix in the eyes revealed a significant decrease in inflammation and protein levels in aqueous humor. Also, healed indications of uveitis were observed in the iris-ciliary body, such as cytoplasm retrieval and nucleus visibility.

The host-guest complexation between a pegylated CB[7] polymeric assembly and drug oxaliplatin used in chemotherapy increased solubility and induced prolonged drug circulation in the blood [186]. The supramolecular complex was nontoxic to normal cells but highly toxic against cancer cells. The inclusion complex was compared with free oxaliplatin in an *in vivo* xenograft HCT116 tumor mice model, which revealed a significantly higher tumor suppression compared to CB[7]+oxaliplatin and oxaliplatin, which demonstrates the interesting antitumor behavior of the supramolecular polymer. In addition, a reduced side-effect nature was also observed from relative weight comparison in the mice group with polyCB[7]+oxaliplatin to that of the controlled group.

Another biocompatible supramolecular organic framework (MOFs) based on CB[8] was constructed to encapsulate DOX for the treatment of MDR MCF-7/ADR tumors [187]. It reduced IC₅₀ values of 13–19-fold for DOX+SOF relative to free DOX. Tumor-bearing mice were treated with DOX with SOF. It showed high cell apoptosis from hematoxylin and eosin (H&E). Terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL)-stain imaging shows the antitumor potential of the DOX-loaded SOF organic framework.

Similar SOFs combined with pemetrexed disodium (PMX) were reported antitumor properties [84].

These findings are very interesting and crucial for calix[n]arenes and cucurbit[n]uril-based supramolecular medicinal applications. However, combining these macrocycles with commercial drugs has significantly amplified bioactivity [20,94,98,111,112,188]. Only a few *in vivo* experiments related to them have been done. More research is needed to obtain effective clinical potential from these supramolecular systems to understand their physiological effects with optimum doses, which should be corroborated *in vivo*.

9. Conclusion

In summary, we have briefly demonstrated how non-covalent interactions participate in supramolecular self-assemblies. Inspired by these associations, they are ubiquitously applied in various medicinal and material sciences pioneered in recent decades. We reviewed the current drug delivery and bio-imaging advances of calix[n]arene and cucurbit[n]uril based supramolecular systems. The structural rigidity, biocompatibility, and high affinities towards various guests have gained significant interest due to their promising potential to design novel and multifunctional systems. Ascribable to synergic multiple non-covalent interactions, calix[n]arenes and cucurbit[n]urils developed their strand to construct simple to complex self-assemblies. Hybrid nanomaterials and therapeutic host-guest complexes designed with enhanced biocompatibility, stability, and controllable functionality are commendable for different bio-applications. These macrocycles carriers can be modified to deliver a wide range of hydrophobic or hydrophilic drugs with increased aqueous dispersibility, stability, and toxicity. Similarly, photosensitizers can be trapped in the cavities or encapsulated by supramolecular aggregates formed by their self-assembly to obtain effective PDT-based treatments. Stimuli-responsive supramolecular systems can be designed for pH-sensitive drugs. They released drugs in the cancer microenvironment with acidic pH, preventing interaction with other cells for reduced side effects. Although many quality reports have been published on calix[n]arenes and cucurbituril[n]urils based on supramolecular systems with high therapeutic efficiencies, their exploration is still difficult for practical and clinical applications. From several perspectives, attention is needed to obtain more clinical advancements like experiments of higher calix[n]arenes ($n > 6$) and cucurbit[n]urils ($n > 8$) as these macrocycles have more oversized pockets. Utilizing a more significant number of non-bonding interactions may lead to self-assemblies of high structural rigidity with controllable shape, size, and charge with prolonged blood circulation for target selective cargo discharge, minimizing toxicity to healthy cells. Calixarenes and cucurbiturils have many bio-compatible applications due to non-covalent interactions with different bio-interfaces that can be tested with *in vivo* studies for various applications. Minimum usage of drug concentrations with high efficiency should be investigated for different types of diseases using different supramolecular assemblies. Chemiluminescent imaging probes with supramolecular systems are advantageous over fluorescence to make an early diagnosis. It provides safe usage of *in-vivo* testing for clinical experiments. Nowadays, Multidisciplinary research is going on in chemistry, biology, and medicinal fields on supramolecular systems. We believe a new era of supramolecular systems is quickly evolving to tackle global healthcare objectives.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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