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Experimental and Theoretical Investigations in Stimuli Responsive Dendrimer-based Assemblies

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Stimuli-responsive macromolecular assemblies are of great interest in drug delivery applications, as it holds the promise to keep the drug molecules sequestered under one set of conditions and release them under another. The former set of conditions could represent circulation, while the latter could represent a disease location. Over the past two decades, sizeable contributions to this field have come from dendrimers, which along with their monodispersity, provide great scope for structural modifications at the molecular level. In this paper, we briefly discuss the various synthetic strategies that have been developed so far to obtain a range of functional dendrimers. We then discuss the design strategies utilized to introduce stimuli responsive elements within the dendritic architecture. The stimuli itself are broadly classified into two categories, viz. extrinsic and intrinsic. Extrinsic stimuli are externally induced such as temperature and light variations, while intrinsic stimuli involve physiological aberrations such as variations in pH, redox conditions, proteins and enzyme concentrations in pathological tissues. Furthermore, the unique support from molecular dynamics (MD) simulations has been highlighted. MD simulations have helped back many of the observations made from assembly formation properties to rationalized the mechanism of drug release and this has been illustrated with discussions on G4 PPI (Polypropylene imine) dendrimers and biaryl facially amphiphilic dendrimers. The synergy that exists between experimental and theoretical studies open new avenues for the use of dendrimers as versatile drug delivery systems.

1. Introduction

The entry of nanocarriers like polymers, liposomes and dendrimers into pharmacotherapy has revolutionized the field, as these help not only to reduce drug leakage and reduce side effects but also contribute to slow, sustained and targeted drug release. 1-3 These are very important characteristics, especially in cancer therapy, where there is a risk of side effects due to off-target activity of the drugs causing damage to normal cells. Depending on the type of disease, drug, payload capacity, and route of administration, various nano particulate carriers- ranging from inorganic to organic, non-biodegradable to completely biodegradable materials, small molecule to polymeric amphiphiles, dendrimers to quantum dots, and lipids to microemulsions have been developed. These materials, when engineered to be more involved and responsive to external stimuli, become very promising drug delivery vehicles.

Dendrimers are one of the most interesting classes of macromolecules used in the field of drug delivery due to the advantages they hold over the other types of macromolecules. Their ability to form stable assemblies and the capacity to be functionalized at the surface, core, middle or even the branches make them structurally resourceful. They offer the unique advantage of being macromolecular and monodisperse, providing the opportunity to study structure-property relationships at the molecular level. 4,5 Stimuli-responsive assemblies have attracted particular attention due to their interests in a variety of applications, especially in biology and medicine. The targeted stimulus can be an inherent physiological imbalance such as variations in pH, 6-11 redox potential, 12-13 or protein concentrations. 14-20 Alternatively, the environmental change can also be externally stimulated (e.g. temperature and light). 21-28 While a variety of nanoscopic systems are being developed, 5,29-32 this review will focus primarily on stimuli responsive dendrimers.

The incorporation of pH and redox responsive systems into many molecular assemblies, including dendrimers, has attracted significant interest. The interest in pH is mainly driven by the aberrant pH in diseased tissues such as cancer cells and in subcellular compartments such as lysosomes. 33 Various functional groups have been utilized in dendritic assemblies such that a pH-sensitive linker would provide stability to the assembled nanostructure stable at neutral pH 7.4, but would respond to a lower pH. 6,11 Similarly, variations in the redox potential between extracellular space and the cytoplasm has led to explorations in developing redox sensitive molecular assemblies. 12-13 A more recent venture in the area of stimuli-responsive assemblies involves systems that respond to enzymatic and protein activities. 14,20 There is a surge in research involving these.
stimuli, because the imbalances in these bio macromolecules can be considered as the primary reason for physiological imbalances. Therefore, targeting these stimuli might hold significant potential for future therapeutic strategies.

Environmental stimuli can be classified into two main categories: intrinsic or extrinsic. The stimuli outlined above are intrinsic, i.e. the variations are caused by intrinsic changes associated with human pathology. It is also possible that one could use extrinsic stimuli in biological applications, where the key advantage is spatiotemporal control. Among the external stimuli possibilities, temperature variations have attracted significant attention due to the implications in areas such as “thermo-therapy”. Oligo- and poly-ethylene oxide based dendrimers have drawn particular attention in this respect. Similarly features that are responsive to light, magnetic field and ultrasound have been incorporated into molecular assemblies to generate externally-triggerable systems. The idea of using molecular dynamics simulations to gain more insight on stimuli sensitive dendrimers is a rather young field with great potential and has been discussed in this review.

Insights into these experimental findings have been augmented by atomistic modeling, and particularly all-atom molecular dynamics (MD) simulations in solution. The high resolution representation that simulations provide allows to gain unique details of the dendrimer structure, of the interactions with the solvent, and of the structural modifications that follow to changes in the external conditions. For example, effect of pH on structural transitions in dendrimers using MD simulations has not only provided insights into the dendrimers themselves, but also on the interaction of the dendrimers with other molecular targets such as siRNA. In general, molecular modeling has provided complementary and often privileged points of view on the effect of external stimuli on molecular structure and on molecular assemblies, as exemplified by the insights into the effect of salt concentration or temperature on supramolecular assemblies. With these as the prelude, this review is organized in the following manner. First, we briefly outline common synthetic strategies utilized for assembling dendritic macromolecules. Since this component of the dendrimer field is quite extensively reviewed, we will succinctly highlight the key approaches. We will then outline the strategies by which stimuli-responsive functionalities are introduced in dendrimers in general, followed by the characterization of the responsive characteristics in dendritic assemblies. We have divided the stimuli into intrinsic and extrinsic stimuli, while discussing these responsive dendrimers. Finally, we highlight the need for synergy between theoretical modeling and experiments through examples of insights that modelling has provided for interesting experimental observations. This last feature is included with a particular hope that it will stimulate several interactions between computational scientists and experimentalists in order to gain interests into stimuli-responsive nanoassemblies.

Scheme 1. Schematic presentation of various synthetic approaches; Top: Divergent synthetic approach, Middle: Convergent synthetic approach, Bottom: Double exponential method. For all cases repeat of the activation and coupling step will give higher generation of dendrimers.
2. Synthetic Strategies of Dendrimers

Synthetic approaches to dendrimers can be broadly classified into three categories: (i) divergent method, (ii) convergent method, (iii) combination of both. In the divergent approach, the dendrimer structure grows outwards from an initiator core (Scheme 1). The synthesis starts with coupling the reactive periphery of a core moiety with a complimentary reactive functional group of the monomer. After first step, the new latent peripheral functionality is activated. This latent functionality is often similar to the one found in the core moiety, providing for reaction with an additional layer of monomers. This iterative process results in rapid increase in the size of the branched polymer, as illustrated in Scheme 1. While possessing the capability to afford high generations, a drawback of this approach is that the number of reactions that are to be performed on each dendritic molecule increases with generation. This tends to produce defects in these dendrimers at high generations.

Alternatively, a convergent synthetic approach to dendrimers was developed to overcome the defect problems. In the convergent synthesis, each dendron is coupled through the focal point to produce various generations of the dendrimer, i.e. the dendrimer grows from outside-in (Scheme 1). Contrary to the divergent approach where the number of reactions on the dendron increases with generation, the number of reactions required on a single molecule in each step of the dendrimer growth is constant in the convergent approach. A key disadvantage here is that the process suffers from poor yields at higher generations, attributed to increased steric crowding at higher generations.

To overcome the limitations of the individual synthetic approaches and to accelerate the dendrimer synthesis, a new combination approach, called the double exponential growth approach, was developed. In this strategy, an AB₃ monomer with protected peripheral and focal point functional groups can be activated selectively at either of the locations (Scheme 1). Repetition of these two selective activation reactions, including the intermediate dendrons, can more rapidly generate high generation dendrimers.


In principle, one would want to design the dendrimer to be responsive to an environmental stimulus. At a molecular level, this involves strategic placement of the functional groups that are known to endow that response to stimulus change. For example, when one requires a material to be sensitive to ionic strength, a key interaction incorporated into the molecule could be based on electrostatics. Similarly, secondary interactions such as hydrogen bonding or π-π interactions can be used in dendrimers. Interestingly, there are several commonly used functional groups that are generally employed to elicit responses from specific stimuli. For example, to make a material thermo-responsive, polyethylene glycol (PEG) and poly-N-isopropylacrylamide (PNIPAM) are commonly used. Each of these functional groups is responsive to temperature for the same reason with only subtle differences. They can both be hydrated due to hydrogen bonding with water. However, these rather weak interactions are broken at higher temperatures causing them to lose their hydrophilicity. When engineering for biological applications, ideal thermo-responsive delivery systems should be rendered stable at physiological temperature, but predictably transformed due to a temperature change, for example in locally heated area.

The non-invasiveness and the opportunity of remote and spatiotemporal control makes light a vital stimulus for on demand drug release. Azobenzene and α-nitrobenzyl ether (or ester) derivates are commonly used as light sensitive functional groups. The former moiety undergoes a reversible trans-to-cis isomerization, while the latter one undergoes an irreversible cleavage reaction upon photoirradiation. In many cases, hydrophilic or hydrophobic counterparts have been attached using α-nitro benzyl ester derivatives. Photo regulated cleavage of the α-nitro benzyl group then leads to transformation of the dendrimer assembly. Reversible and irreversible transformations are possible in pH-sensitive moieties as well. Ionizable functional groups, such as amines or carboxylates, are reversible as their transformation depends on their inherent pKₐ and the solution pH. On the other hand, functionalities such as acetals/ketals, imines, ester, hydrazone and hydrazide can be irreversibly cleaved in response to pH changes. Many anticancer drug delivery systems have been developed so far by taking advantage of the small difference in pH between healthy tissue (~7.4) and extracellular environment of tumor (6.5-7.2). Redox sensitive functional groups that simply undergo electron transfer and change its extent of charge can be reversible (e.g. oxidation of neutral ferrocene to charged ferrocenium units), while redox sensitive cleavage of a bond is irreversible (e.g. cleavage of a disulfide functionality by thiol-based molecules such as glutathione (GSH)). In case of biological stimuli such as enzymatic and non-enzymatic proteins, the former one is often irreversible and the latter is often reversible. In these cases, protein specific functional groups need to be incorporated on the scaffolds to achieve stimulus-responsive materials.

Disassembly of the self-assembled systems in response to biological stimuli can be classified into two broad categories: first category involves covalent modification of a dendrimer-based assembly to cause release of a molecule either due to disassembly of a self-assembled structure or due to cleavage of an appended drug molecule. In the second category, the dendrimer is modified due to a non-covalent interaction with a protein that causes release of the bound molecules. Examples of both these categories have been introduced in the literature recently and these are described below.

To gain insights into the driving forces involved in the self-organization of the dendrimers, self-assembly of the dendrimers has been investigated. In most of the cases dendrimer self-assembly occurs by utilizing noncovalent interactions such as hydrogen bonding, π-π stacking and hydrophobic interactions. In some cases metal-complexation mediated self-assembly of dendrimer molecules have also been observed. Hydrogen bonding interactions allow directionality, specificity and cooperativity in the self-assembly process. Inspired by self-assembly in biology, scientists have used hydrogen-bonding interactions to achieve stable aggregation of the synthesized...
molecules. For example it has been reported in the literature\textsuperscript{77} that first generation poly (triazole-phenylene) dendrimers self-assembled into specific 2D nanostructures utilizing van der Waals as well as hydrogen bonding interactions. In case of highly branched polyester molecules, multiple hydrogen bonding interactions expedited their self-assembly into remarkably well-ordered, 1D supramolecular structures including long micro and nanofibers.\textsuperscript{78} Like hydrogen bonding, hydrophobic interaction also plays a crucial role in self-assembly processes in nature. The stability and nature of the aggregates are dependent on relative ratio of the hydrophilic segment to the hydrophobic segment, along with external conditions such as concentration and temperature. For example, a bolaamphiphile made of water soluble dendritic polyls linked with hydrophobic spacer such as simple aliphatic chains or biphenyl or spirane units were found to form rod shaped aggregates with uniform widths, but variable lengths.\textsuperscript{79} Similarly, a dendritic bolaamphiphile with a central triple bond was shown to form a helical superstructure.\textsuperscript{80} Dendritic bolaamphiphile with tetrathiafulvalene (TTF) spacer was found to form large band-like structure which might potentially act as a “molecular wire”.\textsuperscript{81} A novel class of amphiphilic star shape dendrimer that exhibits interesting self-assembly properties has been investigated. These star shaped dendrimers formed different micellar structures depending on the environment, which made this system a potential candidate for solvent specific encapsulation.\textsuperscript{82} Fourth generation amine terminated poly(propylene imine) (PPI) forms diverse supramolecular structures. Similarly, poly(amido amine) (PAMAM) dendrimers have also been used to prepare various types of nanostructures. It has been reported that fractal like aggregates formed via electrostatic interaction between carboxylate and amine terminated PAMAM dendrimers in aqueous medium as well as silicon surface.\textsuperscript{83} More recently, self-assembly of Janus dendrimers have been sound to afford new morphologies.\textsuperscript{84}

4. Stimuli Responsive Dendrimers

Recently, enormous attention has been focused on stimuli responsive macromolecular aggregates, which undergo significant physical or chemical changes in response to a stimulus. Stimuli have been categorized in to two parts, viz. exogenous and endogenous stimuli.\textsuperscript{85} Exogenous stimuli include temperature, light, magnetic field, ultrasound, and electric field, while endogenous stimuli include pH, redox potential and enzymes. We have organized this sub-section under these two categories.

4.1 Exogenous Stimuli (Temperature and light) Responsive Dendrimers

Thermo-responsive materials for drug delivery and tissue engineering have been widely explored. In drug delivery, often the strategy is to utilize an external heating mechanism to locally heat an area, such as a malignant tumor, which then causes the release of an encapsulated drug, selectively in the tumor tissue.\textsuperscript{86} A number of reports on modification of dendrimer surfaces to endow them with temperature-sensitive characteristics exist as a result of this motivation, among others. Periphery of PAMAM dendrimers has been modified to present temperature-sensitive functional groups on their surfaces. Incorporation of PIPAAm functional groups imparts temperature-sensitive features, which has been used to modify the activity of an encapsulated catalyst (Fig.1).\textsuperscript{87} In this report, catalytically active water-soluble guest molecules were non-covalently bound to the dendrimer. The authors observed that the temperature-dependence of the catalytic activity was induced by the change in structure of the dendritic host.

![Fig.1 Schematic presentation PAMAM G4 dendrimer decorated with temperature sensitive functionality](image1)

![Fig.2 Schematic presentation of synthesis of PAMAM-g-PDMA dendrimer](image2)

A pH and temperature sensitive polymer, poly (N, N-dimethylaminoethyl methacrylate) (PDMA), was attached to the surface of a dendrimer.\textsuperscript{87} This PAMAM-g-PDMA exhibited lower critical solution temperature (LCST), which is often used as a marker for temperature-sensitive characteristics of molecule (Fig.2). The LCST itself was found to be dependent on the graft chain length, which is understandable as the overall hydrophobicity of the dendrimer increases with graft length. Since the PDMA functional groups can also be protonated at lower pH and since this protonation event causes a change in the hydrophilicity of the dendrimer, the LCST of the dendrimer was found to vary with pH. To investigate the utility of such a system in drug delivery, the authors encapsulated chlorambucil (CLB) as an anticancer model drug in this dendritic scaffold. Release rate of the encapsulated CLB molecule was found to be indeed faster at lower pH. This result has been attributed to the conformational change in the PDMA from a coil to an expanded shape due to the protonation of the tertiary amine moieties in PDMA.
In an effort to mimic collagen with improved drug encapsulation and release properties, dendrimers have been modified with a collagen model peptide, (Pro-Pro-Gly)$_5$. The peptide chains in this dendrimer formed a triple helix, which showed thermal reversibility and endowed the dendrimer with drug carrier characteristics (Fig. 3). This dendrimer exhibited thermosensitive molecular release, although it did not show LCST transitions. It was found that the release rate of the encapsulated rose Bengal (RB) at 4 °C was slower than at 37 °C. This was attributed to the temperature responsive change in the extent of triple helix character in the dendrimer, which was found to be 58% and 0% at 4 and 35 °C respectively. It is noteworthy that unlike PNIPAM-based systems, these collagen-mimic dendrimers did not exhibit a phase transition, but simply caused a change in the helix formation.

Considering that both PEG and PNIPAM units have temperature-sensitive features, both of these functionalities have been incorporated onto surfaces of dendrimers (Fig. 4). The authors synthesized two dendrimers, viz. PAMAM–g–PNIPAAm and PAMAM–g–PNIPAAm–co–PEG. Interestingly, the PAMAM-g–PNIPAAm dendrimer exhibited LCST at 32 °C, while the PEG co-grafting causes the LCST decrease of about 3 °C. This relatively small difference was attributed to the loose packing of PNIPAAm, which decreases their interaction and dehydration of the moiety. These dendrimers were then utilized to encapsulate indomethacin as a model drug, which was shown to exhibit a
temperature-dependent release profile. The unmodified PAMAM dendrimer itself does not exhibit any temperature-dependent guest encapsulation and release characteristics.

Our group has developed a new class of bi-aryl based amphiphilic dendrons with polyethylene glycol (PEG) as the hydrophilic segment and decyl chain as the hydrophobic segment (Fig. 5). We examined temperature dependent characteristics of these dendrimers and found generation dependent temperature sensitivity. More specifically, higher generation dendrimers showed lower LCST. Interestingly, these dendrimer scaffolds were found to form micellar aggregates in water. Considering these aggregating features and yet very different LCST for different generations, we envisaged that there must be a cooperativity in temperature-sensitive transitions, when these PEG moieties are tethered together. This hypothesis was later confirmed by systematically synthesizing amphiphilic oligomers. More recently, we found that this G1 dendron exhibits an interesting size transition at a temperature (17.5°C) lower than the LCST, and this was designated as a sub-LCST behavior. At this sub-LCST, the size of the aggregates changes from ~160 nm to ~30 nm as measured by DLS. Interestingly, this behaviour was not observed for G2 and G3 dendrons, presumably because there is a larger energetic barrier for reorganization of the assembly in higher generation. Since these molecules form micellar aggregates, we examined both hydrophobic guest encapsulation ability and stability of the guest encapsulation at various temperatures, using the fluorescence resonance energy transfer (FRET) technique. It was found that guest molecules were stably encapsulated at ambient temperatures, whereas dynamic exchange was observed at lower temperatures, due to the greater hydration of the PEG units (Fig. 5).

Photoresponsive materials have drawn significant attention because of their non-invasiveness and the possibility of remote spatiotemporal control in causing a change in a material. Such a feature will be useful in applications such as on-demand drug release. Self-immolative dendrimers, where a single photochemical reaction can cause a cascade of reactions to disassemble a dendrimer, have been developed (Fig. 6). Here, one adapter molecule was linked to two reporter molecules and one photolabile trigger in a G1 dendron. The cleavage of the trigger molecule initiates a sequence of self-immolative reactions which eventually leads to the release of the two reporter molecules. Upon shining of UV light, cleavage of the trigger molecule occurs, followed by self-immolative release of aminomethylpyrene which was completed within 21 h (Fig. 7).

Similarly, multivalent dendrons that are capable of binding DNA and then releasing it upon exposure to UV light have been designed and synthesized (Fig. 8). Here, dendron surfaces were decorated with cationic spermine groups through an o-nitrobenzyl linker. Since spermine groups are cationic, the DNA could bind to the dendritic surface. However, irradiation of the dendrimer at 350 nm caused photolytic degradation of the o-nitrobenzyl linker, followed by release of the non-covalently bound DNA. This was attributed to the degradation of the o-nitrobenzyl group, which caused the spermine groups to be cleaved from the surface of the dendrons depleting the cationic multivalency, and providing very weak affinity towards DNA. Polyamide dendrons containing azobenzene or o-nitrobenzyl ether functional groups have been found to self-assemble into vesicle type structure, which can encapsulate both hydrophilic and hydrophobic guest molecules. Both these guest molecules were released in response to light, where irradiation of the dendron caused a morphological change in the self-assembled structure from a vesicular assembly to a nanofibrous structure due...
acid, on the surface of the dendrimer along with the drug molecules. The surface moieties of the dendrimer have been additionally used to incorporate a fluorophore, which has been used to monitor the cellular uptake of these drug molecules. The authors found that the active form of the caged molecule is irreversibly released upon photoirradiation. The dendrimer-drug conjugates containing a folic acid ligand showed significant cellular uptake with KB cells that overexpress folate receptor. However, the conjugates without the folic acid ligand did not show any significant level of cellular association. It is also important to note that dendrimer-drug conjugate was cytotoxic, only upon UV irradiation indicating the possibility of light activated therapy.

More recently, our group has designed and synthesized photodegradable facially amphiphilic dendrimers composed of hydrophilic PEG chain and hydrophobic alkyl chain (Fig. 11a). The hydrophobic chain in this molecule has been linked to the dendritic backbone through the photocleavable ortho-nitrobenzyl group. This dendrimer was shown to form micellar aggregates in aqueous medium. Photochemical cleavage of the ortho-nitrobenzyl group destroys the hydrophilic-lipophilic balance of the dendron and consequently disassembles the micellar aggregates (Fig. 11b). To test molecular encapsulation and photo-responsive release possibilities, Nile red was used as a model drug. Upon irradiation at 365 nm, a systematic decrease in the emission intensity of Nile red was observed over time, indicating disassembly of the micellar aggregates and the simultaneous release of Nile red from dendritic scaffold. It was found that after 200 seconds the % of Nile red release from the G1 micelle was ~88%, whereas for the G2 micelle it was 72%. To test whether the release of Nile red was indeed due to cleavage of ortho-nitrobenzyl group, a control G1 dendron that lacks photo-cleavable functionalities was synthesized and tested for photo-release characteristics. This dendritic assembly did not exhibit any molecular release due to light irradiation, suggesting that the

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Fig. 8 Structural presentation of photocleavable spermine dendrimer to the cleavage of the ortho-nitrobenzyl ether moiety. This morphological change was also accompanied by a light-induced molecular release, where hydrophilic calcein was released in response to photoirradiation. In the azobenzene case, photoirradiation presumably caused a trans to cis isomerization of the focal azobenzene unit, but the vesicular structure remains unchanged. Interestingly, the irradiated vesicles were found to be more permeable for the release of the encapsulated molecule, which was attributed to the repulsive interaction between the geometrically distorted amphiphiles when the azobenzene moiety is in its cis form (Fig. 9).

The ortho-nitrobenzyl ether moiety has also been used to directly cage drug molecules, such as doxorubicin and methotrexate using the dendritic scaffold (Fig. 10). The key advantage here of the dendritic scaffold is that the multiple functional groups also allows for the incorporation of targeting moieties, such as folic acid, on the surface of the dendrimer along with the drug molecules. The surface moieties of the dendrimer have been additionally used to incorporate a fluorophore, which has been used to monitor the cellular uptake of these drug molecules. The authors found that the active form of the caged molecule is irreversibly released upon photoirradiation. The dendrimer-drug conjugates containing a folic acid ligand showed significant cellular uptake with KB cells that overexpress folate receptor. However, the conjugates without the folic acid ligand did not show any significant level of cellular association. It is also important to note that dendrimer-drug conjugate was cytotoxic, only upon UV irradiation indicating the possibility of light activated therapy.

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Fig. 10 Top: Structure of fifth generation PAAM dendrimer-DOX conjugates; DOX is caged with a photocleavable ortho-nitrobenzyl group. Bottom: Structure of fifth generation PAAM dendrimer-MTX conjugate.
cleavage of the α-nitrobenzyl group is indeed responsible for the light-induced guest release.

### 4.2 Endogenous Stimuli (pH, Redox and Enzyme) Responsive Dendrimers

Lower pH at the extracellular space of solid tumours and also in sub-cellular compartments such as the endosome and the lysosome have generated significant interests in pH-sensitive supramolecular nanoassemblies. A pH-sensitive polymer, poly(2-(N, N-diethylamino) ethyl methacrylate) (PDEA), has been grafted to the surface of a PAMAM dendrimer along with mPEG chains (Fig. 12). The resultant nanocarrier has a core-shell structure, where the PAMAM dendrimer forms the core and the pH-sensitive PDEA forms the shell along with the hydrophilic and charge-neutral PEG. Significant change in the size of the dendrimer was observed in response to pH changes, which has been attributed to the pH-responsive chain elongation and contraction of the PDEA units. It was found that the release rate of the entrapped 5-fluorouracil (5-FU) molecules from the nanocarrier at pH 7.4 was slower than at pH 6.5. The effect of 5-FU-loaded nanocarrier was also evaluated in mice and it was observed that the nanocarrier had a long half-life and showed good tumor targeting capabilities. Polyether dendrimers based on 2, 2-bis(hydroxymethyl) propanoic acid have also been used to covalently conjugate anti-cancer drug molecules such as doxorubicin (Fig. 13). Hydrazine functionality, which is known to be acid labile, has been used as the linker for conjugating the drug molecule to the dendrimer. The dendrimer was shown to have reduced accumulation in liver in their biodistribution experiments. The drug molecule was also shown to release from the dendrimer at a much faster rate at lower pH, which is attributed to the pH-sensitive hydrazine linker.

Similarly, a pH-responsive nanoassembly based on a linear-dendrimer hybrid has been achieved, where the linear polymer is based on PEG and the dendrimer is based on a polylysine or polyether dendron. Here, the hydrophobic segments were attached through acid labile linker, cyclic acetal (Fig. 14). Therefore, the micellar assembly, formed from the dendritic copolymer, was stable at pH 7.4, but disintegrated at pH 5 attributed to the hydrolysis of the acetal group. The degradation of the assembly was monitored using techniques such as dynamic light scattering (DLS). The encapsulation and pH-sensitive release potential of this assembly was demonstrated using Nile red as the fluorescent probe.

When conjugating a drug molecule to the surface of a dendrimer using a pH-sensitive linker, a problem involving the hydrophobization of the dendrimer is experienced, as most of the drug molecules are hydrophobic. This feature limits the loading of the drug molecule on the dendritic surface. To circumvent this issue, PAMAM dendrimer-drug conjugates have been further modified with PEG chains. Here, the anti-cancer drug

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**Fig. 11** (a) Structure of photolabile G1 dendron; (b) Structural presentation of light induced cleavage of dendron

**Fig. 12** (a) Structural representation of PAMAM-PDEA dendrimer; (b) Schematic representation of pH effect on the dendrimer; at low pH, PDEA chains were hydrophilic and at neutral or slightly basic pH it becomes hydrophobic and contracted so drug molecules were tightly locked within the hydrophobic environment

**Fig. 13** Structure of doxorubicin (DOX) conjugated dendrimer. DOX was conjugated via acid labile linker hydrazone
adriamycin has been conjugated to a PAMAM dendrimer through a hydrazone functionality to generate a pH-sensitive anticancer drug delivery system (Fig.15). Recently Jiang and co-workers investigated how PEGylation degree and style of drug conjugation affects the drug delivery behaviour of PAMAM based dendrimers. They synthesised two different categories of PAMAM dendrimer-doxorubicin conjugates (Fig.16). Similarly, doxorubicin (DOX) was conjugated to pegylated PAMAM dendrimers through an acid-sensitive linkage, $\text{cis}$-acetonityl (PPCD) or an acid-insensitive succinic linkage (PPSD). Cytotoxicity against ovarian cancer cells was found to be higher for PPCD conjugates compared to PPSD conjugates, presumably because of the presence of acid sensitive linkage. It is interesting that PPCD conjugates with highest PEGylation degree showed the highest tumour accumulation in mice.

Similar to the pH-sensitive materials, redox-responsive nanoassemblies are also valuable, given their implications in drug delivery. Specifically, redox-responsive materials are useful in cytosolic delivery of drug molecules, while the extracellular concentration of glutathione (GSH) is micromolar, its cytosolic concentration is much higher (millimolar). This feature has been used to deliver $N$-acetyl-$L$-cysteine (NAC), an antioxidant and anti-inflammatory drug with clinical use in the treatment of neuroinflammation, stroke and cerebral palsy. High plasma binding of NAC requires it to be administered in high doses and causes many side effects. To address this challenge, NAC was conjugated to PAMAM dendrimers via a disulfide linkage, which can cleave in presence of GSH and release the NAC drug molecule (Fig.17). NAC release was studied by reverse phase HPLC and it has been reported that ~70% of NAC payload was released within one hour at intracellular GSH concentrations (~10 mM), whereas insignificant amount of NAC release was found at extracellular GSH concentrations (2 μM). To monitor efficacy of the dendrimer-NAC conjugates they studied FITC-labeled conjugates and found a significantly improved efficacy for both the conjugates. The authors also showed that the disulfide linkage was stable in the presence of serum proteins and lysosomal pH.

Since aberrant expression of proteins is often a specific indicator of human pathology, it is interesting to be able to design systems that respond to a particular protein or enzyme. There are various reports of enzyme-sensitive drug delivery systems based on liposomes and polymers but there are a relatively few reports based on dendrimers. It was conceived that self-immolative dendrimers can facilitate the conversion of multiple prodrugs into drugs through one enzymatic reaction. Using the catalytic antibody 38C2 as the model enzyme, a dendrimer was designed to be able to release DOX, camptothecin (CPT) or both. The molecular design is shown in Fig. 18. Despite the clever design, a pending challenge in this design is the incorporation of hydrophobic drugs on the dendrimer surface and while maintaining the overall aqueous solubility of the dendrimer-drug conjugates.

Recently, we reported a water-soluble assembly that can non-covalently sequester hydrophobic guest molecules. We have rendered this facially amphiphilic dendrimer based assembly sensitive to specific enzymes by incorporating enzyme-cleavable functionalities within the hydrophobic part of the dendrimer. We envisioned that the enzymatic cleavage that converts the hydrophobic moiety in the
amphiphilic dendrimer to a hydrophilic one would cause a disassembly, since the basis for the formation of the assembly is the hydrophilic-lipophilic balance in the molecule. When this balance is disturbed, there should be disassembly. Structures of the dendrons used for this purpose and the supramolecular disassembly concept are illustrated in Fig. 19. We have shown that this disassembly is accompanied by a guest molecule release, the kinetics of which is dependent on the generation of the dendron; the rate of guest release decreases with increase in dendrimer generation.

To stabilize these micelle-like aggregates, we have also partially crosslinked amphiphilic assemblies. In this case, we monitored enzymatic cleavage reaction using the release of a covalently conjugated fluorophore, 4-methylumbelliferone (MUF) (Fig. 20). Concurrent monitoring of MUF fluorescence and that of a non-covalently encapsulated fluorophore suggested that there is a clear correlation between the kinetics of the enzymatic reaction and that of the guest molecule release.

While stimuli induced disassembly is achievable through engineering the enzyme-cleavable function group, it is far more cumbersome to design an assembly that would be responsive to a non-enzymatic protein. To address this challenge, we hypothesized that an amphiphilic dendron would have a very different HLB compared to the corresponding dendron-protein complex. If this difference is sufficiently large such that the former provides a micelle-like assembly, while the latter does not, we could introduce a novel approach for binding-induced disassembly. To test this idea, we designed and synthesized dendrons that contain a single biotin ligand at the focal point on its hydrophilic face (Fig. 21). To examine whether binding-induced disassembly and the corresponding guest release phenomenon can be achieved, we monitored the release of non-covalently encapsulated pyrene in the presence of the complementary protein extravidin. Indeed, addition of extravidin caused release of the pyrene molecules, whereas no such protein-sensitive molecular release was observed in case of a control.
dendr
on that lacked the biotion moiety (G1-control). More recently, we have developed a deeper insight into the mechanism of the binding induced disassembly process using a combination of experiment and theory (vide infra).

In the above design, the ligand moiety is presented at the hydrophobic face of the dendritic assembly (Fig.22). We were interested in identifying whether the presentation of a significantly hydrophobic ligand that is likely to be buried in the pockets of the micellar assembly would still be available for protein binding and supramolecular disassembly. To test this possibility, we also designed a dendrimer system using dinitrophenyl (DNP) moiety as the hydrophobic ligand functionality, which is complementary to anti-DNP immunoglobulin G (IgG). Indeed, we were able to show that the binding induced disassembly possibility does exist and is likely due to the equilibrium between the unimeric and the aggregated states of the amphiphilic dendrimer assembly. Even though such equilibrium should heavily favour the aggregated state of the amphiphile, a Le Chatelier type effect should be sufficient to funnel the bound dendrons towards the disassembled state.

The developments in the systems above are uniquely positioned to achieve dendrimer-based assemblies that are sensitive to multiple stimuli. Recently, a dual protein stimuli responsive drug delivery system based on an ‘AND’ logic gate was designed by our group, the ambition being a structure that would respond to the simultaneous presence of two proteins. The design resulted in a dendron that contained an enzyme-cleavable coumarin ester in the hydrophobic face and a protein specific ligand on the hydrophilic face of the dendron (Fig.23). It was shown that the system disassembles and provides a fluorescence signal, only in the presence of both the complementary protein and the complementary enzyme. This is because, when the protein was bound to the ligands on the surface of the assembly, the aggregate-unimer equilibrium was shifted towards the unimer form due to the disruption of the HLB, exposing the previously buried coumarin ester. This coumarin ester was cleaved by the enzyme to produce a fluorescent product. These aggregates did not produce the fluorescent product in the presence of the protein or the enzyme alone. We have also combined the photocrosslinking motif into this dendrimer to generate a system that is sensitive only to the concurrent presence of three different stimuli.
studies.

5. Difficulties in Connecting Length Scales in Self-Assembling Dendrimers

The experimental characterization of supramolecular assemblies is a challenging issue, especially due to the fact that properties are controlled at the molecular level. In cases where these molecules aggregate, the assembly and disassembly processes are typically characterized using DLS to monitor the temporal evolution of the aggregate size in solution. This allows for study of the molecules in an unconstrained regime. However, typically the size distribution reported by DLS can be very broad, and for small particles (e.g., below ≈10 nm of diameter) the errors become too large compared to the measured size and the characterization becomes very rough. In this case, it is very difficult to obtain characterization of what is present in solution after disassembly, as it is almost impossible to discriminate, for example, between monomers, dimers, etc., since these all fit in the size distributions reported by DLS. For what pertains to the study of the interactions leading to self-assembly (dendrimer-dendrimer) or to disassembly in presence of an external stimulus (e.g., the interaction of the dendrimer aggregate with a protein), the issue is even more difficult. In fact, experimental techniques such as isothermal titration calorimetry (ITC) can provide good overall estimation of the energies involved in self-assembly, but fail in the detailed description of the interactions in the different steps of the disassembly process. For example, in the case of a dendrons decorated with one biotin ligand that are responsive to the binding with the complementary protein - i.e., extravidin. Our DLS measurements indicate that the dendron aggregates present in solution undergo disassembly in presence of a specific interaction with the complementary protein. However, data on the release of the guests are difficult to rationalize, as they show that release is not uniform among the dendrons, and can be even negligible depending on structural parameters such as the ligand location within the dendrons structure. Similarly, diffusion ordered nuclear magnetic resonance spectroscopy (NMR) also can provide information about the hydrodynamic radii of the aggregates and monomers. Unlike DLS, diffusion NMR provides more reliable value regarding the size of the small particles (below 10 nm), but it fails to endow any information about the different interactions associated with self-assembly and disassembly processes. Another technique, vapour pressure osmometry (VPO) can be useful to know the molecularity of self-assembled dendrimers. VPO measurement provides molecular weight of the assemblies and from that result molecularity of the assemblies can be calculated provided that molecular weight of the single dendron is known. Although VPO technique is not as accurate as mass spectrometry, it gives the opportunity to perform the measurement under specific conditions like temperature and concentration. One will be able to get information about the self-assembled state but will be unable to get the insight about the driving force involved in the stimuli responsive disassembly process. Similarly viscosity measurements can give an idea about the molecular weight or branching in the dendrimers, but again it fails to provide insight about the self-assembly and disassembly process. All the aforementioned techniques are not enough to get the detail account of the interaction and driving force associated with the self-assembly and stimuli responsive disassembly process followed by guest release. Indeed, deeper molecular level understanding is needed to obtain clear perception of the behaviour of these supramolecular systems and the real effect of external stimuli on the structure.

The development of ad hoc molecular models and the use of molecular simulation can complement the experiments, and allow for obtaining high resolution details that are not accessible by experiments. In the further sections we will discuss the remarkable advantage that can be gained by using simulation and assisting the experiments with models. This combined theoretical-experimental approach aims at using molecular modeling to target the blind spots of the experiments, and as a useful framework to rationalize their results.

6. Models Supporting Experiments

Molecular modeling has the potential to address the above mentioned challenges, providing a high-level description and characterization of the dendritic constructs in the solvent and a real support to the experiments. Insight into the interaction between the different dendrimers during self-assembly, and thus on the perturbation induced by the external stimulus can also be extracted.

6.1 Computational Characterization of Dendrimers in Solution: The Effect of Shape on Self-Assembly
The shape and configuration assumed by dendrimers in the solvent is of key importance to understand how they interact with each other during self-assembly or with other molecules. Molecular simulation has a successful history in the molecular-level characterization of dendritic molecules in solution. In particular, all-atom molecular dynamics (MD) of the dendrimers immerged in a periodic simulation box containing explicit solvent molecules (e.g., water) can provide a detailed picture of what the dendrimers look like in solution at the equilibrium, accounting correctly for the interaction with the external solution. This is particularly important, as presence of charges, of hydrophobic patches, etc., at the surface of the dendrimer can be triggers for controlling interactions, aggregation and self-assembly.125-127

Recently, for example, it was demonstrated that the shape assumed by G4 PPI dendrimers in a solution containing water and cadmium acetate salt controls the self-assembly of dendrimers into supramolecular fibers.45 In this case, the process is controlled by ionic effects, and by formation of hydrophobic patches at the surface of the dendrimers (Figure 24a).

Similarly, the facially amphiphilic dendrons (G2) synthesized by our group undergo folding in solution assuming a globular shape in the solvated state, where the hydrophobic chains collapse in the interior and are surrounded by the hydrophilic PEG chains (Figure 24b). MD simulations show that the hydrophilic chains of the dendrimers are not long enough to screen the hydrophobic part of the dendrons completely from interacting with the solvent, and that, hydrophobic patches are present at the surface of the dendrons. This lead to self-assembly of dendrons in the solution.71 Interestingly, modelling can capture the structural rearrangement of the dendron aggregate during the self-assembly process. During the equilibration of the aggregate, the hydrophobic tails of all dendrons converge in the interior of the micelle and are surrounded by the hydrophilic PEG chains (see the radius of gyration plot of the different groups as a function of simulation time).14

These are structure/shape molecular effects on the higher scale that can be successfully captured by the MD simulations. Furthermore, modelling also allows to obtain deeper details such as how much of the self-assembly interaction is due to hydrophobicity, rather than to ionic effects and electrostatic interactions, or to hydrogen bonding, etc., and can be used as a reference to study the effect of external stimuli on the molecular structure.

6.2 Modeling The Effect of External Stimuli

Atomistic simulation can capture the transformations of the dendrimer scaffold following to variations in the external conditions. For example, molecular dynamics (MD) simulations of cationic G4 PAMAM dendrimers in water allowed to study the remarkable structural transition of the dendrimer changing the pH from neutral to low – decreasing the pH from 7.4 to 4.0 the dendrimer undergoes reconfiguration from a dense core conformation to a dense surface one due to change in the protonation state.41,42 Another case where MD calculations enabled to capture the effect of an intrinsic stimulus on the molecular structure is related to catalytic chameleon dendrimers. In particular, it was shown that changes in the surface groups...
obtained through reversible covalent chemistry transform the folded state of the dendrimers in solution, preventing or allowing access to the dendrimer’s interior of substrate molecules, and controlling the on/off functionality of these macromolecular catalytic switches. Molecular simulations can be also used to study the effect of external stimuli on self-assembly; i.e., it is possible to estimate the variation in the self-assembly stability provoked by a change in the external conditions (e.g., temperature, pH, ionic strength, etc.) or by the interaction with other molecules (e.g., proteins, etc.). Such an evidence links the external stimulus to transitions in the molecular structures, and the latter to supramolecular interactions, providing unique details on the behaviour of stimuli responsive supramolecular assemblies.

For example, MD simulations of a portion of PPI dendrimer fibers (Figure 24a) composed of two bound dendrimers pre-equilibrated in a solution of water and Cd(OAc)₂ demonstrate that even low concentrations of NaCl in solution can destabilize the fiber and trigger disassembly. In fact, Cl⁻ ions have higher affinity for cadmium than acetate (AcO⁻) ions. In the ionic competition and substitution, the Cl⁻ ions replace the AcO⁻ ions that are essential for the stability of the fiber. The destabilizing effect of increasing NaCl salt concentration on self-assembly was also proven in the case of the dendron-triggered aggregation of supramolecular virus capsids, and it was demonstrated to vary significantly depending on whether the self-assembly is controlled by electrostatic or hydrophobic forces. One of the main advantages of molecular modeling is that it is possible to construct ad hoc molecular models to study complex phenomena, such as self-assembly or self-assembly destabilization from a privileged point of view. This represents a unique opportunity exploited for the combined experimental-computational characterization of stimuli responsive amphiphilic dendron aggregates synthesized in our group.

7. Practical Examples of Synergy Between Theory and Experiments

One good example of synergy between experimental observations and MD (Molecular Dynamics) simulations is a recently published study of amphiphilic dendron aggregates undergoing disassembly in response to the specific interaction between a ligand and protein (stimulus). Protein-induced disassembly of dendron assemblies in solution, due to a specific binding between complementary ligand-protein pairs such as biotin-avidin and 2, 4-DNP- Anti DNP IgG is well established in our research groups. The change in the hydrophilic lipophilic balance (HLB) upon protein binding was hypothesized to be the driving force for disassembly. The ligand placement had a significant role in encapsulation efficiency, but its role in disassembly needed to be understood. The support of MD simulations was sought for this purpose. MD simulations helped account for every observation, from the facial amphiphilicity of the biaryl dendrimers, to the rate, extent and mechanism of disassembly upon binding of the protein with the ligand.

Generations G1 and G2 of the facially amphiphilic biaryl based dendrimers were chosen for this study, since these provided scope to vary ligand positions. The focal point (F), middle layer (M) and periphery (P) were the three positions targeted in the G2 dendrimer (G2-F, G2-M and G2-P) whereas G1-F and G1-P were the two positions in the G1 dendrimer (Fig. 25).

Upon monitoring the sizes of these assemblies via dynamic light scattering (DLS) before and after the addition of protein, the event of disassembly was established since the size became smaller post protein-addition in each of the five dendrimer assemblies. However, upon probing the efficiency of encapsulated hydrophobic dye (Nile red) release, there was a large discrepancy between the different systems. In the case of the G2-P and G1-P, a total of 65% (in 1 hour) and 77% dye release (over 6 hours) could be achieved whereas only 25%, 13% and 35% was achieved with G2-F, G2-M and G1-F respectively. These results lead to the hypothesis that the accessibility and surface availability of the ligand varied greatly between the constructs, controlling the protein-binding event, and thus the release of the encapsulated guests. Moreover, G1-F and G2-F dendron assemblies exhibited a static fluorescence quenching, when encapsulated with Nile red and exposed to a non-specific protein, Myoglobin. This made way for a presumption that a higher percentage of PEG groups on the periphery of the assemblies may increase the non-specific interactions with the metalloprotein, Myo.

7.1 Structure-Property Relationships

In this framework, MD was first used to characterize the dendrons in solution, focusing at this stage on G2 dendrons which offer the complete series of ligand grafting positions, and the more net discrepancy in the guest release properties. G2-P, G2-M and G2-F dendrons were simulated as surrounded by explicit water molecules. At the equilibrium, the three dendrons look almost identical, having similar size in solution (radius of gyration – Rg=1 nm). However, deeper analysis of the equilibrated dendron structures reveals differences between the three dendrons. The radial distribution functions (g(r)) of the
biotin ligand for G2-P, G2-M and G2-F extracted from the MD simulations showed different levels of biotin ligand exposure to the external solution. If the dendrons are treated as spherical molecules of radius equal to \( R_p \), the most probable position where a biotin could be found is identified by the \( g(r) \) peak position (\( \approx R_q \) for G2-P and \( \approx 0.5 \) \( R_q \) for G2-M and G2-F). Thus, while the biotin ligand is well exposed toward the external solution in the case of G2-P, it is backfolded in G2-M and G2-F. Based on ligand availability, the specific binding between the dendron and the complementary extravidin will be thus highly probable for G2-P, and more hindered for G2-M and G2-F.

According to a simple model, the protein-ligand interaction can be perceived to be occurring in two concomitant steps (i) unfolding of the biotin ligand to become accessible for interacting with the protein (ii) specific binding between the ligand and the protein-extravidin. In this way it is possible to extract the overall dendron-extravidin affinity as the free energy of binding (\( \Delta_{\text{bind}} \)) necessary to complete the specific interaction between the biotin ligand and the complementary protein: \( \Delta_{\text{bind}} = \Delta_{\text{specific}} + \Delta_{\text{ unfolding}} \). Where \( \Delta_{\text{specific}} \) is the free energy of binding for the biotin ligand interactions, which is known constant (-20.4 kcal/mol) based on previously experimental studied and constant for the three dendrons according to this scheme. The unfolding is the free energy required to unfold the biotin ligand and to make it available for the binding event. This unfavourable term depends on the extent of backfolding. In particular, \( \Delta_{\text{unfolding}} \) is negligible for G2-P, as in this case the ligand is well available at the surface, and has a positive (unfavourable) value for G2-M and G2-F. The overall dendron affinity for avidin is thus higher in the case of G2-P than for G2-M and G2-F (Fig. 25), which, in terms of probability, indicates that the relative probability of having a specific avidin-dendron interaction in the case of G2-P is more than ten times higher than for G2-M and G2-F.

In this case, MD simulations highlighted a real structure-property relationship for these dendrons – a slight difference in the molecular structure can indeed impact the functionality of the dendrons. In fact, if one considers that the specific biotin-avidin binding is a key trigger for disassembly, this result correlates with the experimental evidence that disassembly is faster in the case of G2-P than for G2-M and G2-F.

### 7.2 Molecular Insight Into Protein-Binding Induced Destabilization of The Aggregates

It is possible to use molecular simulation to study both dendrons self-assembly and self-assembly destabilization following to protein specific binding by creating ad hoc molecular systems capable of capturing the stimuli responsive phenomenon. For example, in this case nine unbound G2-P dendrons (pre-equilibrated in solution) were immerged in a water simulation box. Another molecular system was created from the same initial dendrons configuration, with the exception of having the biotin ligand of the central dendron specifically bound to extravidin. The MD simulations of both systems in explicit water demonstrated that the dendrons undergo self-assembly in solution. Moreover, the energy of self-assembly of the dendrons (\( \Delta_{\text{E_{assembly}}} \)) extracted from both MD runs shows that the aggregation of dendrons is strongly destabilized in presence of the external stimulus (specific interaction with extravidin) – \( \Delta_{\text{E_{assembly}}} \) is as high as \( \approx 33 \) kcal/mol in the native aggregate, while it is decreased to \( \approx 17 \) kcal/mol in presence of the specific biotin-avidin binding. This result indicates that even a single specific interaction at the surface of the aggregate can impair the self-assembly of the dendrons by one half of its stability (Figure 26).

This outcome captures the effect of the external stimulus on the self-assembled system. Other interesting insights can be obtained by dissecting the models. For example, the interaction energies calculated from the simulations show that the non-specific interactions between the dendrons in the aggregate with avidin (\( \Delta_{\text{E_{non-specific}}} \approx 9 \) kcal/mol) are relatively weaker than the stability

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**Fig. 26** Modelling binding, self-assembly and stimuli responsive disassembly. (a) Radial distribution functions \( g(r) \) of the biotin ligands in G2-F (black), G2-M (blue) and G2-P (red) dendrons. (b) Simplified model for the dendron-avidin affinity. (c,d) G2-P dendrons self-assembly in absence (c) and in presence (d) of specific binding with avidin (dark ribbon). (e) Self-assembly (\( \Delta_{\text{E_{assembly}}} \)) and binding (\( \Delta_{\text{E_{specific}}} \)) energies calculated from the MD simulations.
of self-assembly ($\Delta E_{\text{ass}} \approx -33$ kcal/mol). On the other hand, the specific interaction with avidin is relatively stronger, being as high as $\Delta E_{\text{bound}} \approx -92$ kcal/mol, and is thus capable of destabilizing the dendrons from self-assembly. This rationalizes why non-specific interactions with non-complementary proteins do not produce any disassembly – they are not strong enough to perturb self-assembly –, and why a specific interaction is needed to cause disassembly.

The theoretical insight produced by the simulations also allowed for another realization in the characterization of these complex systems. Multivalent binding of avidin with biotin is well known being a cooperative effect favouring the binding of multiple ligands to the same target. MD simulation of four G2-P dendrons specifically bound to the four binding sites of the avidin tetramer reported an interaction energy between the four dendrons and the protein ($\Delta E_{\text{bound}}$) of $\approx -510$ kcal/mol, well above the value of one single specifically-bound dendron multiplied per four ($\Delta E_{\text{bound}} \approx -370$ kcal/mol), reinforcing this concept.

According to our scheme, if high ligand availability favours the probability of specific events, then in the case of G2-P dendrons, where multiple biotin ligands are available at the surface of the aggregate, multivalent specific interactions will also be highly probable. The contemporary interaction of one avidin protein with multiple dendrons at the surface of the same aggregate, or from different aggregates, can have important impact, resulting in overall speedup of the disassembly process according, for example, to different schemes of exfoliation, or of protein bridging between different aggregates respectively. The chance of similar events in the cases of G2-M and G2-F are much lower due to avidin backfolding and reduced ligand availability, which reduces the probability of multivalent interactions in these cases. This provides a scenario where for G2-M and G2-F disassembly occurs due to a destabilization imparted upon avidin binding, proceeding slowly and producing larger aggregates than those obtained for G2-P. During this process, the byproducts of disassembly are probably capable of structural rearrangements allowing retention of the guests, consistent with the lack of guest release as evidenced from the experiments in the cases of G2-M and G2-F. These results show how MD simulations can bridge the gap between hypotheses (theory) and proof (practice), and in this case, supporting the experiments in the characterization of the mechanism of protein binding-induced disassembly.


In general, the use of simulations to complement experiments represents a new venue in the characterization of stimuli responsive supramolecular systems. Nevertheless, this combination of theory and experiments enriches the tools that experimentalists have, in the form of a high-resolution "virtual microscope" which allows to study complex phenomena from a privileged (and simpler) point of view.

All-atom MD simulations have already proved useful by capturing the effect of variations of pH, temperature, and ionic strength in solution on molecular structures, molecular interactions, and complex supramolecular properties. As a result of this successful study, our groups are moving forward to study various other stimuli (temperature, pH etc) responsive systems, by the same approach. The goal is to obtain a molecular rationale explaining the supramolecular properties useful for understanding and for the rational design of novel structures with desired and controllable properties.

9. Conclusions

This review discusses recent advances in the field of stimuli responsive supramolecular dendritic systems, with a particular focus on the potential impacts on drug delivery. Considerable efforts have been devoted to the synthesis of molecular candidates capable of forming aggregates in solution with controllable properties. In particular, supramolecular aggregates that are stable in biological conditions, and capable of hosting hydrophobic guests, which then undergo disassembly releasing the guests upon presence of an external stimulus are attractive systems for the development of smart and efficient drug delivery systems. The task of designing such systems can be simplified, if one could develop the connections between the molecular scale (molecular level change in the presence of a specific stimulus) and the nanoscale (assembly and disassembly in the absence and presence of the stimulus).

Herein, particular emphasis is put on the recent addition of molecular modeling and simulations to support experimental results and aid in the characterization of the stimuli responsive properties of dendrimer-based complex systems. The high-resolution details that can be obtained from atomistic simulations constitute a unique added value for the experimentalists, increasing the understanding of the complex behaviour of these systems through different scales, and providing useful indications for the rational design of efficient molecules. Indeed, the synergy between modeling and experiments represents a new frontier in the field aiding the design of stimuli responsive supramolecular systems with controlled functionality.

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Notes and references

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Entry for TOC only:

Experimental and Theoretical Investigations in Stimuli Responsive Dendrimer-based Assemblies

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Diagram of self-assembly and drug release mechanism.
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