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A Review of Surface Functionalized Amine Terminated Dendrimers for Application in Biological and Molecular Sensing

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Dendrimers are three dimensional nanosized synthetic molecules that have internal cavities and numerous surface groups. In recent times they have received increased attention in sensing applications. For dendrimers to be used as sensors, they most commonly require functionalization at their surface. This is because the surface is generally the first point of contact between the dendrimer and the outside world, hence surface functionalization serves to selectively home in on the target analyte. Further, sensor signals may be transmitted through surface functionalities e.g. fluorochromic molecules. It is therefore important to document surface functionalization approaches. Dendrimers with amine surface groups have the advantage of being able to be conjugated to other molecules via an amide linkage, which is one of the most fundamental and widespread chemical bonds in nature. In this paper we demonstrate the properties of dendrimers that make them so applicable to sensing. We review several methods for functionalizing dendrimers via an amide linkage, as well as present a review of surface functionalized polyamidoamine, polyamine, and polypeptide dendrimers that have been employed for biological, chemical and molecular sensing.

Keywords: Amide linkage; Amine; Dendrimers; Ester; Film; Fluorescence; Functionalization; Luminescence; Macromolecules; PAMAM; PPI; Periphery; Sensors; Synthesis

INTRODUCTION

A dendrimer (from the Greek *dendros*, meaning tree, and *meros*, meaning part) is an artificially synthesized nanoscale organic molecule that is built up from branched units called monomers. Dendrimers, like hyperbranched polymers, are built up around a central multifunctional core molecule and have branched structures that grow exponentially in terms of the end-group functionalities and molecular weights. The main difference between a dendrimer and hyperbranched polymers is that dendrimers possess monodispersed properties, which implies that all molecules are alike when it comes to structure and molecular weight. This is largely the result of the synthesis approaches, as hyperbranched polymers are produced by a relatively simplified single step reaction, whereas dendrimers are synthesized iteratively and require stringent control of all synthesis steps.

Despite their first appearance being reported by Vögtle [1], interest in these macromolecules stemmed from reports in the mid 1980s by Tomalia [2], Newkome [3], and Denkewalter [4]. They have attracted immense attention in recent years, as evidenced by the exponential increase in publications and patents concerning them, largely because they may be employed in a broad range of applications ranging from physical, chemical, and biological. Their popularity stems from two facts: (1) their chemical composition and molecular weight can be precisely regulated during their synthesis; and (2) peripheral functionalization can be controlled, which in turn affects numerous properties that include: chemical reactivity, solubility and photo-physical properties. Considering the enormous potential applications of dendrimers, selectively functionalizing their periphery is of high importance. Therefore, finding the appropriate synthetic route for producing these functionalized molecules presents itself as a challenge for researchers to this day, particularly for in vivo applications.

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Among the multitude of dendrimers reported in literature, primary amine ($-NH_2$) polyamidoamine, polyimine, and polyamide dendrimers, also known as PAMAM, PPI and polypeptide dendrimers respectively, have attracted the most attention in recent years, largely as a result of them being commercially available, and that their surface/periphery may be functionalized via amide bonds. In fact, Lysine is the most common amino acid branching unit from which peptide dendrimers are assembled [5]. For sensing applications, functionalizing the surface gives the dendrimers specificity towards a target analyte. Furthermore, transduction of the sensor signal can be tailored through these surface functionalities. For example, by attaching fluorochromic molecules, changes in optical absorption and emission can be monitored though changes in the optical properties of the function groups. In this paper, we first provide an introduction to the applicability and capability of functionalized dendrimers for sensing. We then proceed to review methods for attaching functional groups to primary amine terminated PAMAM, PPI and some polypeptide dendrimers, and present examples of functionalized dendrimers in chemical and biological sensing applications. We have centered the discussion mainly on primary amine dendrimers that have diamino alkane cores (e.g. ethylenediamine, diaminobutane) as a result of them being most readily available commercially. However, many of the principles discussed in this paper are applicable across the range most dendritic molecules.

DENDRIMER PROPERTIES AND THEIR SUITABILITY FOR SENSING

Dendrimers are three-dimensional macromolecules that have a roughly spherical, globular shape in solution. They contain three distinct regions, these being the core, branches and surface/periphery. The central core is covalently linked to layers of repeating units (generations) and a number of terminal groups. A typical dendritic structure can be seen in Fig. 1. As the branches emanate from the central core, the structure becomes more dense and compact, leaving the core relatively bare of molecules. These internal cavities permit the encapsulation of molecules/ions, essentially allowing the dendrimer to perform as a "nanoscopic" container, as exemplified in an early report by Meijer [6]. Such a feature is ideal for sensing applications, as encapsulated "guest" species can alter the electrical and optical properties of the "host" dendrimer.

Another intriguing aspect is that functional units can be placed at specific sites in dendrimers (interior and/or surface) allowing for different functionality of materials. In doing so, dendrimers may provide a new controlled "place of chemical and physical events" [7]. As interactions between analytes and the functionalities can alter dendrimer's chemical, physical and optical properties, controlling the sites at which these interactions occur presents the opportunity for maximizing the number of such interactions

The chemical and physical properties of dendrimers are affected by many parameters, which include the shape and multiplicity of the core/building block, the size and shape of end groups, and of course their chemical composition. The generations are interdependent and create a unique molecular shape, and can lead to intrinsic properties such as high solubility and low viscosity. PAMAM and PPI dendrimers (Fig. 2) typically have a binary structure, whereby two branches emanate from each of the branching points, in which case the number of surface groups for a dendrimer of generation n, is $2^{(n+2)}$. For example PAMAM dendrimers of fourth generation have 64 primary amine groups at their surface, and in solution, the macromolecule is spherical with an approximate diameter of 4.5 nm [8].

For sensing applications, the dendrimer must somehow interact with the analyte molecule/ion, and the interaction must result in a change in quantity that can be monitored, e.g. conductivity, conformation, fluorescence intensity etc. The interaction may result in a non-radiative transfer of energy or electrons, which can occur in a number of ways: If the transfer occurs by a through-bond mechanism, as is the case for most affinity sensors, it is termed the Dexter mechanism [9] and electron

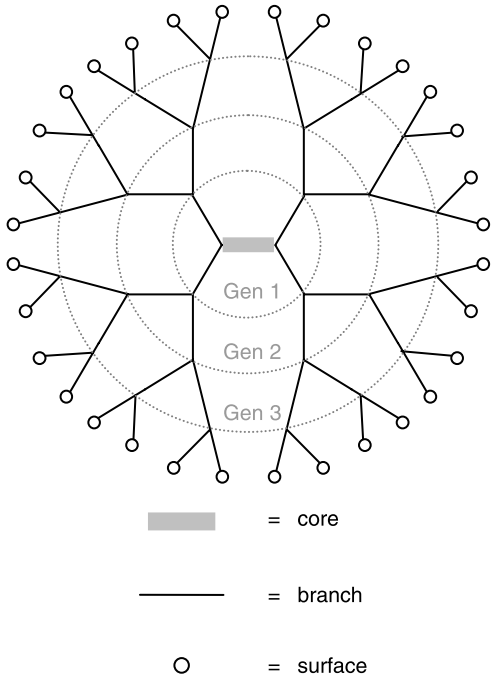

FIGURE 1 3rd generation binary dendrimer.

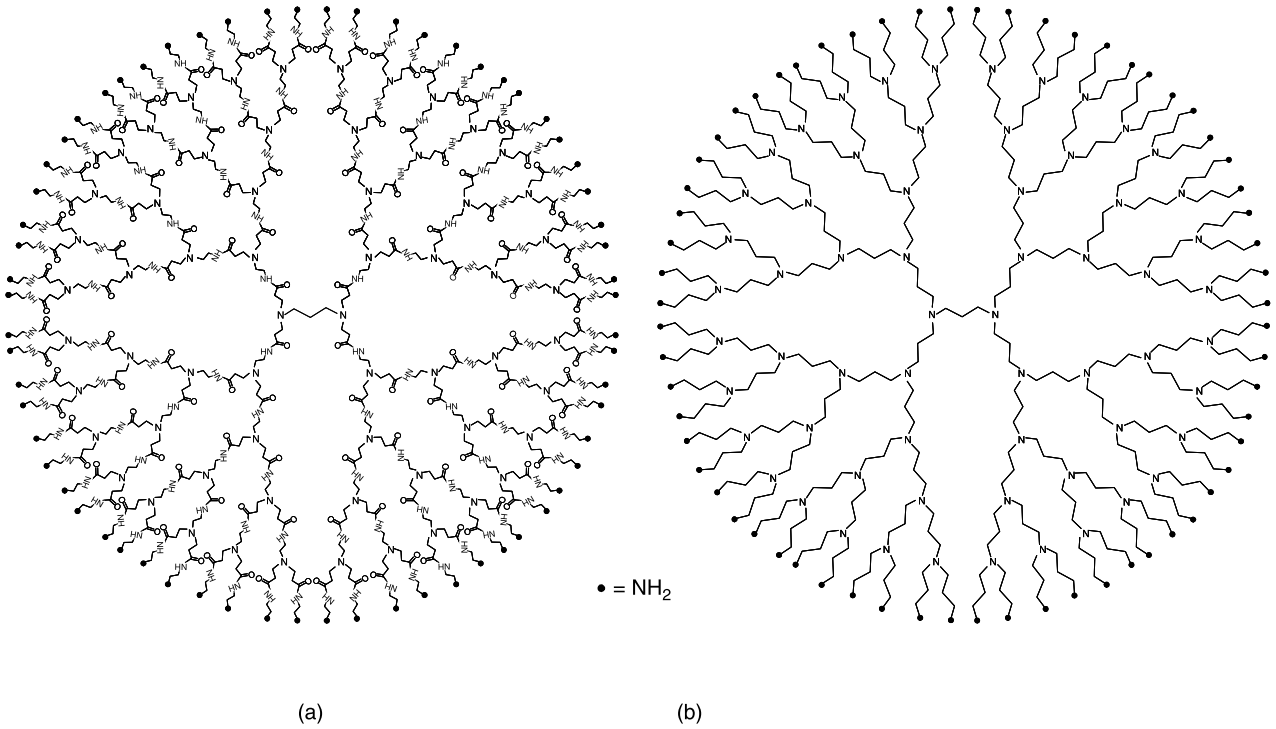

FIGURE 2 4th generation amine terminated (a) PAMAM and (b) PPI dendrimers.

exchange occurs from the first excited state of the donor to the first excited state of the acceptor, with a simultaneous exchange of a ground state electron from the acceptor to the donor (see Fig. 3). This electron exchange requires strong donor-acceptor orbital overlap and is therefore a short-range (<10 Å) interaction that diminishes exponentially with distance. Conversely, transfer can occur between molecules that are separated by distances considerably larger than the sum of their van der Waals radii, and is governed by the Förster mechanism (see Fig. 3) [10]. In such a case no electron exchange is required as the energy transfer is via a through-space dipole–dipole interaction. Donor-acceptor orbital overlap is not required, allowing the molecules to be separated by a relatively large distance (10–100 Å). This mechanism is typically applicable to molecular and ion sensing, in which the analyte is encapsulated within the dendrimer.

In terms of signal transduction for dendrimer based sensing, luminescence (in particular fluorescence) presents itself as an exciting option. Excitation/relaxation results when there is absorption/emission of energy, i.e. when electrons are redistributed into higher/lower energy levels, typically via the absorption/emission of a photon. Here the donor molecule can transfer energy to an acceptor, which is in its ground state and energy transfer can occur such that the donor returns to its ground state simultaneously with the promotion of the acceptor to its excited state. Following excitation from an external source, the dendrimer will absorb and/or emit light, and in the presence of an analyte, its optical absorbance/emittance may change. In such a case the dendrimer would be functionalized with fluorochromic units at the periphery, or it

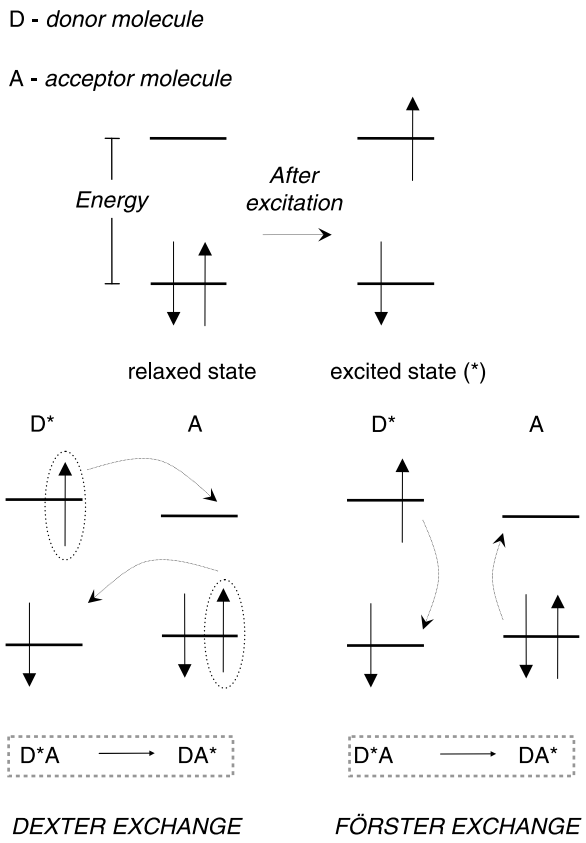

FIGURE 3 Excitation and electron/energy transfer.

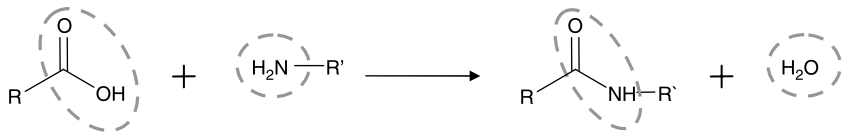

FIGURE 4 Amide bond formation with a carboxylic acid.

may encapsulate molecules in its cavities, or both. Here the interaction between the analyte and the fluorochrome functionalized dendrimer may alter the luminescence properties (i.e. enhances light emission, quenches light emission, changes the wavelength of the emitted light) via the "through bond" or "through space" transfer mechanisms.

What makes dendrimer based sensing far more attractive than those incorporating a single receptor-substrate interaction is that a single analyte can interact with a greater number of fluorescent units and result in signal amplification. Further to this point is perhaps one of the most intriguing, if not the most intriguing aspects of dendrimer based sensing, which was illustrated by Astruc and co-workers [11] They reported the remarkable dendritic effect observed whilst employing amido-ferrocene dendrimers (FcDs) as supramolecular redox sensors for inorganic anions. This dendritic effect is the dendrimer's ability to achieve better sensing and recognition of the analyte as the dendrimer's generation increases.

Another advantage that results from dendrimers tree-like structure is that they can serve as an energy gradient, which allows the funneling of energy from the periphery to the central core [12–14]. Furthermore, dendrimers possess the ability to incorporate several functionalities into the one dendrimer. This presents the possibility of forming multiple sensors into ordered arrays, capable of simultaneously detecting many different analytes. The large number of available receptor sites also lends itself to widespread application in the specific binding of toxins and micro-organisms. All these attributes highlight dendrimers excellent suitability for use in practical applications such as catalysts, electron transfer mediators, energy conversion, ion sensors and in electro-luminescent devices [7].

Although unfunctionalized PAMAM and PPI dendrimers have been shown to be sensitive towards pH, organic vapors, metal ions, organic molecules etc., it is through functionalization that specificity and selectivity towards different analytes can be achieved. Functionalization enables specific binding (as will be demonstrated later) and should result in a stronger sensor signal, higher sensitivity, and lower detection limits. Coupling this with the fact that the highly branched three-dimensional dendrimer structure allows a very high packing density per unit volume, there is no doubt as to the impact dendrimers are having on the field of sensors.

SURFACE/PERIPHERY FUNCTIONALIZATION PROCEDURES

Synthetic approaches offer a myriad of avenues for forming bonds between molecules/compounds etc. As only PAMAM and PPI dendrimers with peripheral amine groups are considered in this review (see Fig. 2), molecules employed to functionalize the surfaces are those that can be connected via an amide linkage, and hence an amide bond. The most obvious approach to functionalizing the dendrimer amine surface is through the reaction between a carboxylic acid and a primary amine (Fig. 4). The carboxylic acid group reacts with the amine group to give an amide, and a water molecule is eliminated (an example of a condensation reaction). Usually the formation of an amide from a carboxylic acid and an amine results in an overall loss of free energy, however there is a high activation energy to be overcome. This energy must be lowered in order to make the synthesis viable.

This may be achieved either by catalysis or by forming carboxylic acid derivatives (RCOX, Fig. 5). It is imperative to select the correct leaving group 'X', as it determines the value of the activation energy. It would thus seem desirable to form amino acid derivatives with a strongly electron withdrawing 'X',

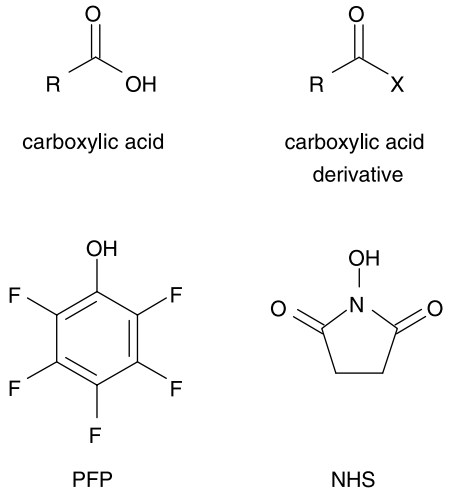

FIGURE 5 Carboxylic acid terminated functional group and substituents for activation.

making the carbonyl carbon more prone to nucleophilic attack and thereby achieving high reaction rates at ambient temperatures.

Compounds other than carboxylic acids may be utilized for amide linkage formation, for example: sulfonyl chlorides and those having azide, fluoride and chloride as leaving groups. In the case of acid chlorides, the chloride ion is easily eliminated which renders the carbonyl prone to attack, even from weak nucleophiles, making them highly susceptible to hydrolysis. Alternatively, polyamine dendrimers can be readily functionalized with various functional groups attached as carboxamides by standard peptide chemistry procedures, with some of the more common procedures detailed below.

Surfaces Functionalized with Carboxylic Acid Terminated Molecules

In general, the direct reaction of a carboxylic acid or an acid chloride group with an amine may be synthetically unfavorable or prohibitive, depending on the ease with which the intermediate acyl-amine complex can be deprotonated. Failure to deprotonate causes the ammonium ion to be the preferred leaving group and hence the starting materials will be reformed. Therefore, the end group requires activation so that it is capable of accepting a proton. Quite commonly in such a case, a carboxylic acid end group is transformed into an ester, to form what is termed an "activated ester". In particular, pentafluorophenol (PFP) and *N*-hydroxysuccinimide (NHS) are used as they form excellent leaving groups (Fig. 5), and these esters are well-known in peptide chemistry for their high stability towards hydrolysis and their great selectivity for amide formation.

Carbodiimides, such as dicyclohexylcarbodiimide (DCC) are commonly utilized as coupling mediators for esterificating carboxylic acids, and have been employed for functionalizing PAMAM and PPI dendrimers [15–19]. They have a downside however, as in these reactions they precipitate a dicyclohexyl urea (DCU) byproduct, which although does not participate in subsequent reactions with amines, can be cumbersome to remove. Further purification, by column chromatography or HPLC for example, may be required. A general procedure for the formation of an NHS ester is illustrated by Telser and co-workers [20], in which 1 equivalent of the monoacid and 1 equivalent of NHS were dissolved in dry *N*,*N*-dimethylformamide (DMF). To this mixture, 1 equivalent DCC dissolved in DMF was added dropwise, and after stirring for 12 hours, the solution was purified to yield the activated ester.

Balzani and Vögtle [21] prepared 3-(phenylazo)-benzoic acid NHS esters which were attached to amine terminated PPI dendrimers with a yield of 80–90%. They illustrated the importance of esterification, as they found that prior attempts to prepare azobenzene-substituted cascade molecules by the reaction of the PPI dendrimers with the corresponding acid chlorides in the presence of a base led to incomplete conversion. This they presumed was because quaternary ammonium chloride salts were formed, aside from the desired amides.

PFP has been utilized by Meijer and co-workers [22] to activate π-conjugated oligo(p-phenylene vinylene), or OPV's. In their procedure the OPV-containing dendrimers were synthesized from a tri(p-phenylene vinylene) derivative, which was converted to a carboxylic acid. This carboxylic acid and DCC (1.1 equiv) were added to a tetrahydrofuran (THF) solution at 0°C. PFP (1.1 equiv) was added after the reaction mixture was allowed to come to room temperature. After stirring for 14 h, THF was evaporated and the desired compound was purified with flash chromatography, yielding the activated ester (70%) as a yellow solid. The peripheral moieties of PPI dendrimers were then functionalized with the activated esters. The appropriate amounts of the ester and dendrimer, dissolved in dichloromethane (DCM), were added and stirred, followed by filtering, washing, drying and purification, giving a yield of 59%. Meijer and co-workers have also reported several other dendrimers functionalized with different NHS activated esters [6,23].

Furthermore, the rapid increase in the preparation of dendrimer derivatives containing reactive nucleophilic and electrophilic groups for coupling proteins in sequence has been a result of their use in DNA and drug delivery applications. For example, Singh [24] functionalized PAMAM dendrimers with various active esters which were utilized to couple two proteins in sequence. Examples of ester activated peptide functionalized dendrimers may be found elsewhere [25].

Dendrimer functionalization with activated esters can be summarized in Fig. 6, in which to an amine terminated dendrimer of a given concentration, having *n* peripheral amines, *n* equivalents of the activated molecule, which are to functionalize the dendrimer, are slowly added, with the choice of solvent depending on the polarity of the dendrimer and the functional groups. The amide forming reaction is carried out in the presence of a base, typically triethylamine (Et_3N), and left to stir for periods ranging between 12 hours to 7 days. The reaction mixture is then extensively washed, commonly with a saturated sodium carbonate solution and water, then dried over sodium sulfate and filtered. Solvents are then removed *in vacuo* to yield the final product, although additional purification may be required.

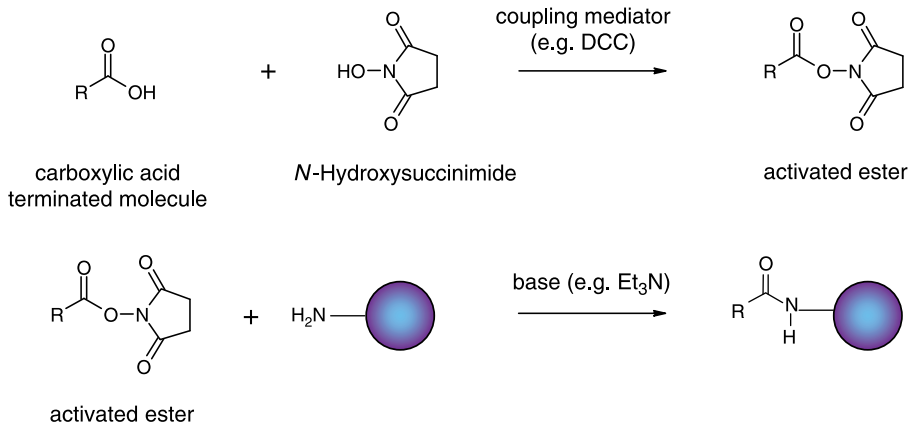

FIGURE 6 A general procedure for functionalizing amine terminated dendrimers with molecules having carboxylic acid end groups.

Surfaces Functionalized with Acid Chloride Terminated Molecules

Crooks *et al.* [26] reported the functionalization of amine terminated PPI dendrimers with different acid chlorides in a one step reaction. These acid chlorides were added dropwise to dry DCM solutions containing the dendrimer and dry Et_3N under an N_2 atmosphere at room temperature. After stirring overnight, the solvent was removed and the residue dissolved in $CHCl_3$, then washed with saturated aqueous solutions of Na_2CO_3 and NaCl. Drying with Na_2SO_4 was followed by evaporation of the solvent. The product was confirmed by NMR and found that 90–98% of the amine groups of dendrimers were functionalized by this approach. Similarly Put *et al.* [27] produced different generations of PPI dendrimers with 4-dimethylaminophenylcarboxamide end groups, from the corresponding acid chloride, with yields ranging between 70–80%.

Vögtle [28,29] demonstrated the surface functionalization of amine terminated dendrimers with sulphonic acid chlorides through a sulphonamide linkage. Reactions were carried out in boiling DCM or at a temperature of 25°C, under an argon atmosphere, with triethylamine being employed as a base. Reaction times of up to 5 days were reported, depending on dendrimer generation, and the mixtures were washed in aqueous $NaHCO_3$, then purified via column chromatography.

The use of acid chlorides removes the need for the coupling mediator and allows the dendrimer to be functionalized in a one step reaction. Hence using them for forming amides has an advantage over carboxylic acids, as well as requiring one less purification step. There is a downside however, as acid chlorides are highly reactive and hence are not as stable as the activated esters. An alternative approach is to form a PFP ester from the acid chloride, without the need for a coupling mediator, in a method described by Babu *et al.* [30]. They utilized a biphasic system that employed $NaHCO_3$ in the aqueous layer, to form the PFP ester. Reaction products were highly pure and produced in yields of around 75%.

SENSING APPLICATIONS OF FUNCTIONALIZED DENDRIMERS

Biological Sensors

Dendritic biosensors typically have surface functional units which display a high affinity towards a particular analyte (enzymes, proteins, peptide, antigen etc). For biosensing, in particular affinity-biosensing, interfaces that are highly selective and dense are required to achieve high sensitivity and selectivity. The inherent properties that dendrimers possess make them well suited to such applications. For example, biomolecules that have specific affinity for other molecules (such as white blood cells, proteins etc.) can partially functionalize the dendrimer surface, while the remaining surface can be functionalized with fluorescent material that will change its fluorescent properties upon interaction with the biomolecules. What makes PPI and PAMAM dendrimers particularly appealing for biological applications is their three-dimensional structure, which mimics those of biomacromolecules, in addition to their biocompatibility. An example of a functionalized dendrimer monolayer for affinity sensing is seen in Fig. 7.

One of the first examples of dendrimer based biosensors has been provided by Shinkai *et al.* [31]. In their first report, they demonstrated water soluble dendrimers functionalized with boronic acids and fluorescent nathyl moeties for the detection of saccharides. Here the napthyl groups' fluorescence intensity changes in relation to the covalent interactions between the boronic acids and the sugars. They also functionalized second generation

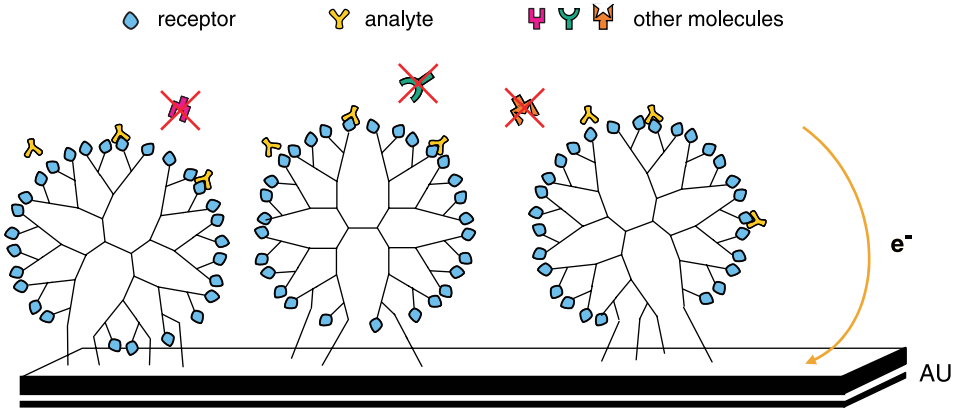

FIGURE 7 Affinity sensing with dendrimer monolayers.

PAMAM dendrimers with eight boronic acids and eight anthracene units at the periphery (see Fig. 8) [32]. When in solution, the fluorescence intensity of the macromolecules were monitored prior to and subsequently after the addition of D-galactose and D-fructose, and were found to be sensitive to concentrations as low as 10^{-4} M, highlighting there suitability for biosensing.

A key step in fabricating biosensors is the immobilization of proteins or enzymes on surfaces. Kim *et al.* [33] demonstrated the use of dendrimers as bioconjugating reagents for constructing multilayered enzyme nanostructures. They utilized this concept to investigate a new type of biosensor (avidin), incorporating a fourth generation PAMAM dendrimer monolayer [15]. The dendrimers surface primary amines were partially modified with ferrocenyl groups, and then self assembled onto a gold surface. The remaining peripheral amines were functionalized with biotin analogues. An electrochemical signal from

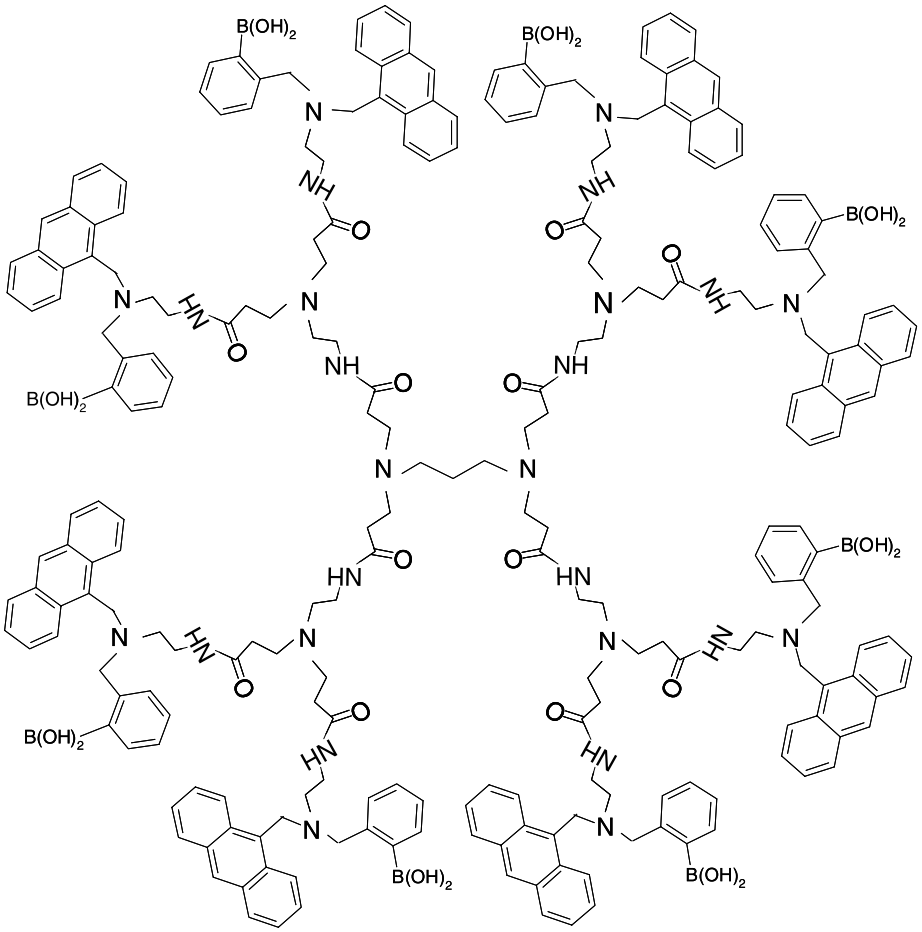

FIGURE 8 Second generation PAMAM dendrimer functionalized with anthracene and boronic acids.

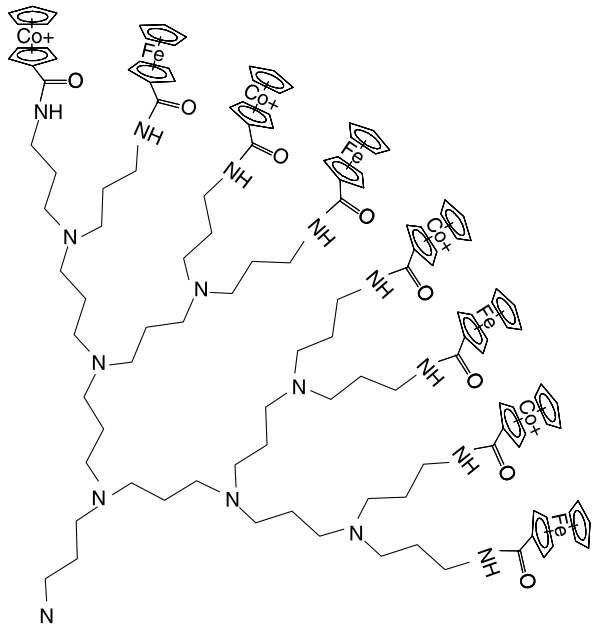

FIGURE 9 3rd generation mixed ferrocene–cobaltocenium terminated PPI dendron.

the affinity biosensor was generated by free glucose oxidase in electrolyte, which depends on the degree of coverage of the sensing surface with avidin. The ferrocene was employed as an electron-transferring mediator between the glucose oxidase and the PAMAM. As the concentration of avidin in the electrolyte solution is increased, the electrochemical signal is decreased because of the steric blockage by the avidin adlayer formed on the modified electrode. They took this research further by demonstrating that monolayers of PAMAM dendrimers, functionalized with biotin analogues, could be repeatedly regenerated as an affinity bio-sensing surface by a displacement reaction with biotin [17].

Alonso [34] also showed that thin films of mixed ferrocene–cobaltocenium terminated PPI dendrimers (Fig. 9) with glucose oxidase immobilized electrostatically on the surface, and immersed in solutions containing different glucose concentrations, produced an output signal directly proportional the glucose concentration. Cyclic voltammograms revealed that under anaerobic conditions the ferrocene units acted as mediators in enzymatic processes, while in the presence of oxygen the cobaltocenium moieties took part in electrocatalytic processes.

Ferritin functionalized PAMAM dendrimers that were deposited onto micropatterned gold coated silicon wafers have been employed as antiferritin immunosensors by Yoon and co-workers [35]. Here the sensor signal is obtained by cyclic voltammetry, and is the electrochemical current generated through antibody-antigen binding interactions. Ferritin is a globular protein that is well known for storing Fe^{3+} ions, as well as being marker for tumors. Upon the addition of different concentrations of the target protein in antiferritin antiserum, a distinct change in current was observed, whose value was inversely proportional to the target protein concentration. In this research, two electrode geometries were investigated, rectangular and circular, the latter proving to be preferred for sensing as the output current was less susceptible to drift and fluctuations in the voltage.

Examining the fluorescence properties of molecules in the presence of an analyte can provide valuable information regarding analyte concentration. The fluorescence of tryptophans (which are essential amino acids) is one of the most frequently used methods for studying protein structure and function, especially for membrane-active proteins and peptides [36,37]. Klajnert [38] showed that interactions between both amine and carboxylic acid terminated PAMAM dendrimers with bovine serum albumin (BSA), which contained two tryptophan residues (Trp-213 and Trp-134), resulted in fluorescence quenching of the tryptophan. The fluorescence quenching was attributed to electrostatic dendrimer–protein interactions. The quenching was accompanied by a slight hypsochromic shift in the emission maxima, which was attributed to changes in conformation resulting from PAMAM–BSA interactions. Stronger fluorescence quenching was observed for amine terminated dendrimers. More recently, Ibey *et al.* [39] created a new fluorescent glucose assay based on Alexa Fluor 647-labeled concanavalin A (Con A) and a fourth-generation PAMAM Alexa Fluor 594-labeled glycodendrimer. The glucose response was based on a single fluorophore quenching reaction that occurred upon binding between the dendrimer and glucose with Con A. According to their results, glucose was monitored across the biological range with a dynamic range even broader than that seen in previous assays. The larger dynamic range occurs since the assay is based upon a single fluorophore phenomenon rather than a FRET-based phenomenon.

An exciting application for dendrimers has been highlighted by Baker *et al.* [40], in which fifth generation PAMAM dendrimers, functionalized with 6-carboxytetramethylrhodamine fluorescent probes and targeted with folic acid, were used to label human epidermoid carcinoma (also known as KB) cell tumors *in vivo*. The high affinity folic acid receptor (FAR) is known to be over-expressed in several human carcinomas, and hence acts as a site for folic acid to home in on the tumor. Using a two-photon optical fiber fluorescence probe, they were able to achieve a fourfold increase in tumor fluorescence in animals that received the targeted dendrimer. Such results highlight the viability of utilizing biocompatible materials for drug distribution in tumors, and in particular for identifying cancer signatures that can be tagged fluorescently via ligand binding. Such results may be a stepping stone

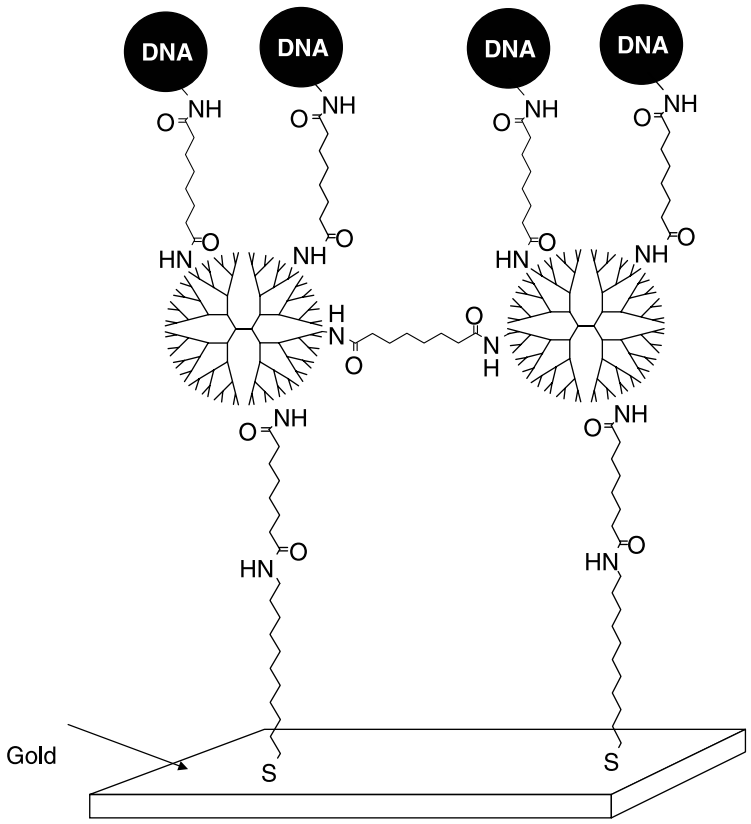

FIGURE 10 Example of DNA functionalized PAMAM dendrimers anchored to SAMs on gold.

required for moving away from harmful techniques such as radiation and chemical analysis.

DNA and protein molecules can also be attached to a dendrimer's surface for use in sensing applications, as reported by Mark [41]. Amine terminated PAMAM dendrimers, anchored to self-assembled monolayers (SAMs) of amino undecanethiol on gold, were functionalized with model proteins, such streptavidin and rabbit immunoglobulin proteins, and DNA molecules (Fig. 10). The sensing of these bioactive thin films was achieved through surface plasmon resonance (SPR) after covalent attachment of the proteins and DNA. SPR measurements revealed that with dendrimer-based films, it was possible to detect DNA–DNA interactions with a high degree of selectivity and specificity. The responses of these multicomponent films exhibited stable and repeatable regeneration and hybridization cycles, and surface mass transport limiting effects did not appear to affect the kinetics of the DNA–DNA hybridization process.

Molecular/Ion Sensors

As mentioned earlier, Astruc and co-workers [11] demonstrated that ferrocene-modified PAMAM dendrimers with 1, 3, 9, 18 and 36 terminal functional groups could serve as supramolecular redox sensors for small inorganic anions. They achieved this by monitoring how titrations of the fictionalized dendrimers were effected by n-Bu4N$^+$ salts of $H_2PO_4^-$, HSO_4^-, Cl^-, Br^- and NO_3^-, and monitoring them by cyclic voltammetry (CV) and nuclear magnetic resonance (NMR) spectroscopy. From the CV experiments on these dendrimers, they found that a progressive shift of the wave was observed for the titration of the dendrimers by the addition of inorganic salts, and the standard redox potential changed significantly by the addition of one equivalent of salt per ferrocene unit. This made it possible to distinguish between the different inorganic anions. They observed that not only did ΔE° values increase with each generation, indicating that the redox centers at the Fe^{II}/Fe^{III} are equivalent, also they behaved independently. By far the most outstanding result observed was that as the number of equivalents of inorganic salts was added per branch of dendrimer, the E^o values increased with increasing dendrimer generation. This *dendritic effect* demonstrates that better sensing of anions by cyclic voltammetry can be achieved as the dendrimer generation increases! They also found that highest overall binding affinity was observed in the case of the dendrimer with 18 ferrocene surface groups, as steric surface saturation was observed for higher generation dendrimers, which limited the penetration of the anions through the ferrocene surface. In addition to this research, there is work carried out by

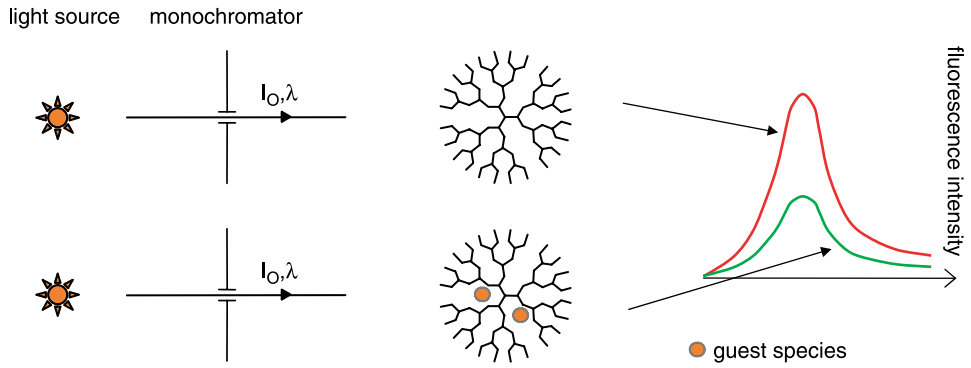

FIGURE 11 Changing fluorescence intensity upon guest encapsulation.

Cardona and Kaifer [42] who fabricated polyamide dendrons with ferrocene moieties at the core/focal position. They also found the redox behavior to be dependant on the generation, as were the electron transfer rate and the diffusion coefficient.

More recently, Astruc and co-workers reported the surface functionalization of amino dendrimers with the tetrairon cluster $[CpFe(\mu_3\text{-}CO)]_4$, showing their application as selective sensors for oxo anions $H_2PO_4^-$, HSO_4^- and adenosine-5′-triphosphate (ATP^{2-}) [43]. Sensing and anion recognition was performed by cyclic voltammetry, which revealed a single reversible wave for the redox change $Fe_4 \rightarrow Fe_4^+$. Furthermore, in the presence the other oxo anions, they were able to distinguish ATP^{2-} by the replacement of the initial wave observed in the voltammogram by a new wave at a less positive potential, with the shape of the new wave being a fingerprint of the oxo anion. Dendritic and structural effects were also found to play a role on anion recognition.

Dendritic fluorescent sensors can be synthesized by linking a photoactive (fluorochrome) molecule to a receptor subunit displaying affinity toward the envisaged substrate. As we know, one of the most publicized uses of dendrimers is for hosting encapsulated guest molecules [6,44], and encapsulated guests can alter the dendrimer's properties. Moreover, molecules that are encapsulated within dendrimer cavities can alter the photoluminescence absorption and/or emission properties, and hence the sensor output signal is the change in fluorescence. This concept is illustrated in Fig. 11. Host-guest systems, as opposed to dendrimers containing a luminescent core, have the advantage of being able to tune the emission wavelength. This is a result of the sensitized emission from the macromolecule's dependence on the guest that is hosted in the very same dendrimer [45–47].

One family of compounds which has gained much attention in the world of dendrimers are naphthyl groups, in particular the 5-(dimethylamino)-1-naphthalenesulfonyl (or dansyl) groups (Fig. 12). They show intense absorption bands in the near UV and strong fluorescence in the visible region. Their absorption and emission properties are very sensitive to the addition of acids because of the protonation of the dansylamine subunit. It is this property that has seen them grow ever more popular in sensing and labeling applications, and they have been employed to functionalize a variety dendrimers [48–51].

The preparation, absorption spectra and photophysical properties of solutions of PPI dendrimers functionalized with dansyl groups at the periphery (Fig. 13) have been reported by Vögtle, Balzani and co-workers [29]. In their investigation, the properties of these dendrimers were compared to those of a monodansyl model compound when different acid concentrations were added. Interestingly, it was found that the functionalized dendrimers required greater acid concentrations to quench the fluorescence in comparison to the monodansyl compound. This was due to the peripheral dansylamine sites being weaker bases than the tertiary amines of the interior dendrimer branches, where the added ion of the acid first causes the protonation of inner tertiary amines of the dendrimer. Furthermore, in cases where only a fraction of the peripheral dansyl units were protonated, they observed that electronic energy transfer is directed from the fluorescent excited state of the protonated units to the ground state of the unprotonated units. Such a situation caused increased changes in the spectroscopic signals that accompany the protonation/deprotonation of separated dansyl units.

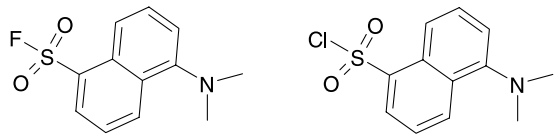

FIGURE 12 Dansyl compounds.

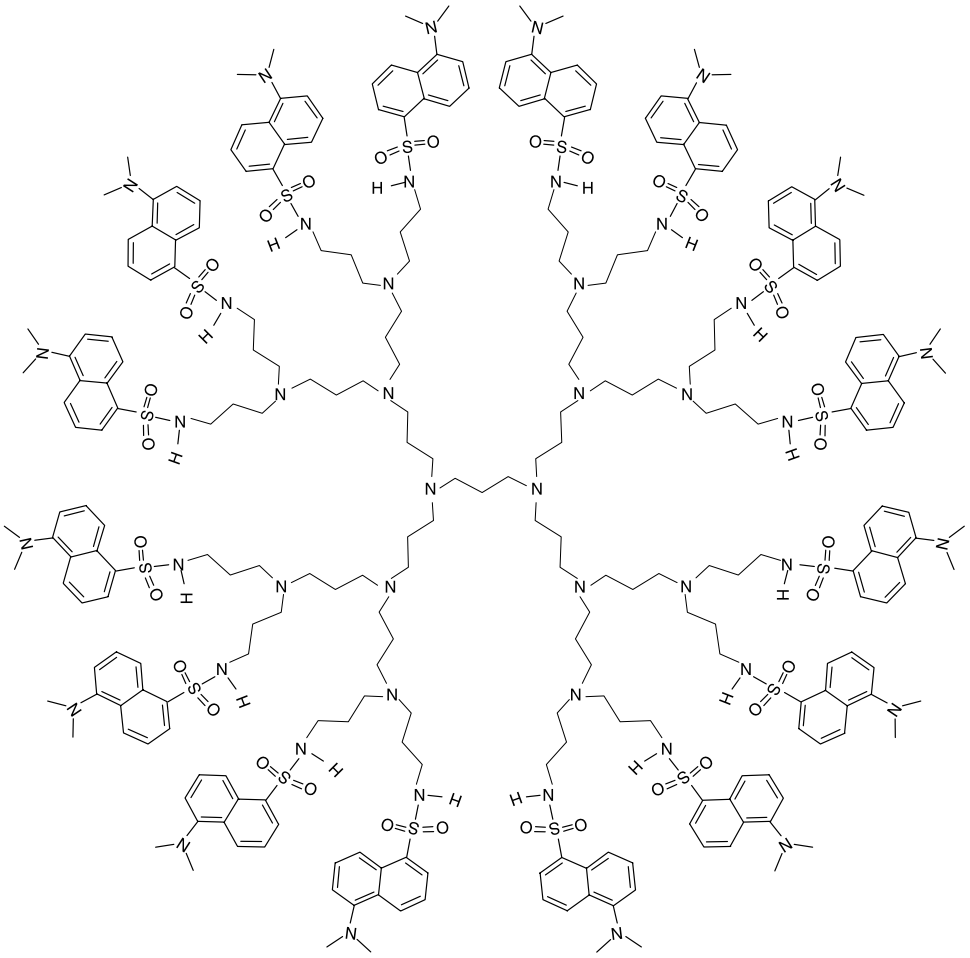

FIGURE 13 2nd generation dansyl functionalized PPI dendrimer.

Vögtle and co-workers went on to demonstrate that metal ions (e.g. Co^{2+}) could be coordinated in the aliphatic amine branches of dansyl functionalized PPI dendrimers, and in doing so can quench the fluorescence of the peripheral dansyl groups [52]. The interactions between the Co^{2+} ions and the monodansyl compound were shown not to cause any change in the monodansyl fluorescence properties, hence the quenching of the dendrimers was not attributed to dynamic processes. The efficiency of fluorescence quenching was found to increase with increasing dendrimer generation. A most interesting result was that signal amplification was observed, as the co-ordination of a single metal ion was capable of quenching the fluorescence of all the peripheral dansyl groups. Furthermore, the metal ion coordination was a fully reversible process, for when excess triflic acid was added to the metal ion loaded dendrimer, full recovery of the fluorescence properties was seen. Other ions such as Cu^{2+} and Zn^{2+} were found to either not affect the fluorescence intensity, or to form irreversible complexes.

They continued their investigation, this time demonstrating the dansyl functionalized PPI dendrimers could form host-guest complexes with

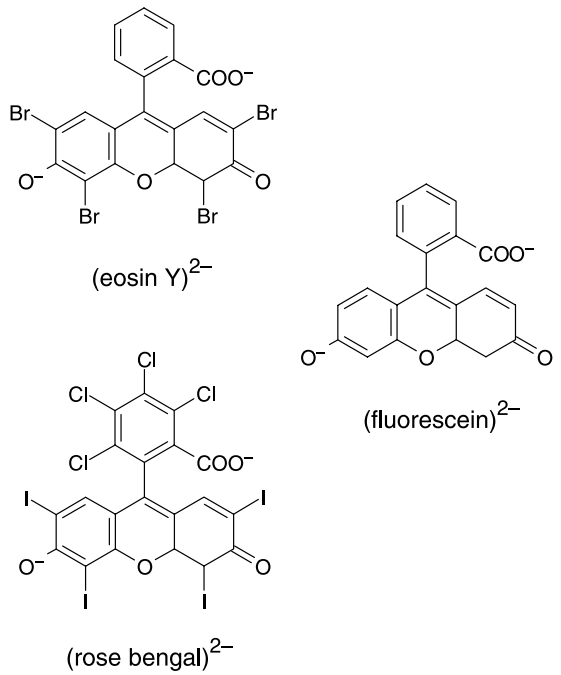

FIGURE 14 Fluorescent molecules encapsulated within dendrimers.

several fluorescent dye molecules (Fig. 14) [53]. The dyes were water soluble, whereas the dendrimers were soluble in DCM. When a colored aqueous solution of the (eosin Y)$^{2+}$ was shaken in DCM, no color changes in the two phases were observed. However, when dansyl functionalized dendrimers were present, the color of the aqueous solution faded and the DCM solution containing the dansylated dendrimer became colored. Hence eosin molecules were extracted from the aqueous phase in their diacid form (eosin Y)H_2 and were shown to be hosted in the interior of the dendrimers. They also observed the dendritic effect in the formation of host-guest species. The host dye molecules quench the peripheral dansyl units fluorescence via energy transfer (Förster mechanism), and in turn their fluorescence is sensitized. These results are consistent with their research into photo-physical effects of dendrimers functionalized with other naphthyl units [54].

The discussion is by no means limited to PAMAM and PPI dendrimers, as it has also been shown that polylisine dendrimers can be employed as sensors for lanthanide ions (Fig. 15) [48,55]. These dendrimers containing aliphatic amide units at their interior can reversibly bind metal ions and protons. Coordination of metals was found to take place by a cooperative action of deprotonated (in basic solution) aliphatic amide units, whereas protonation takes place on the amine moiety of the dansyl

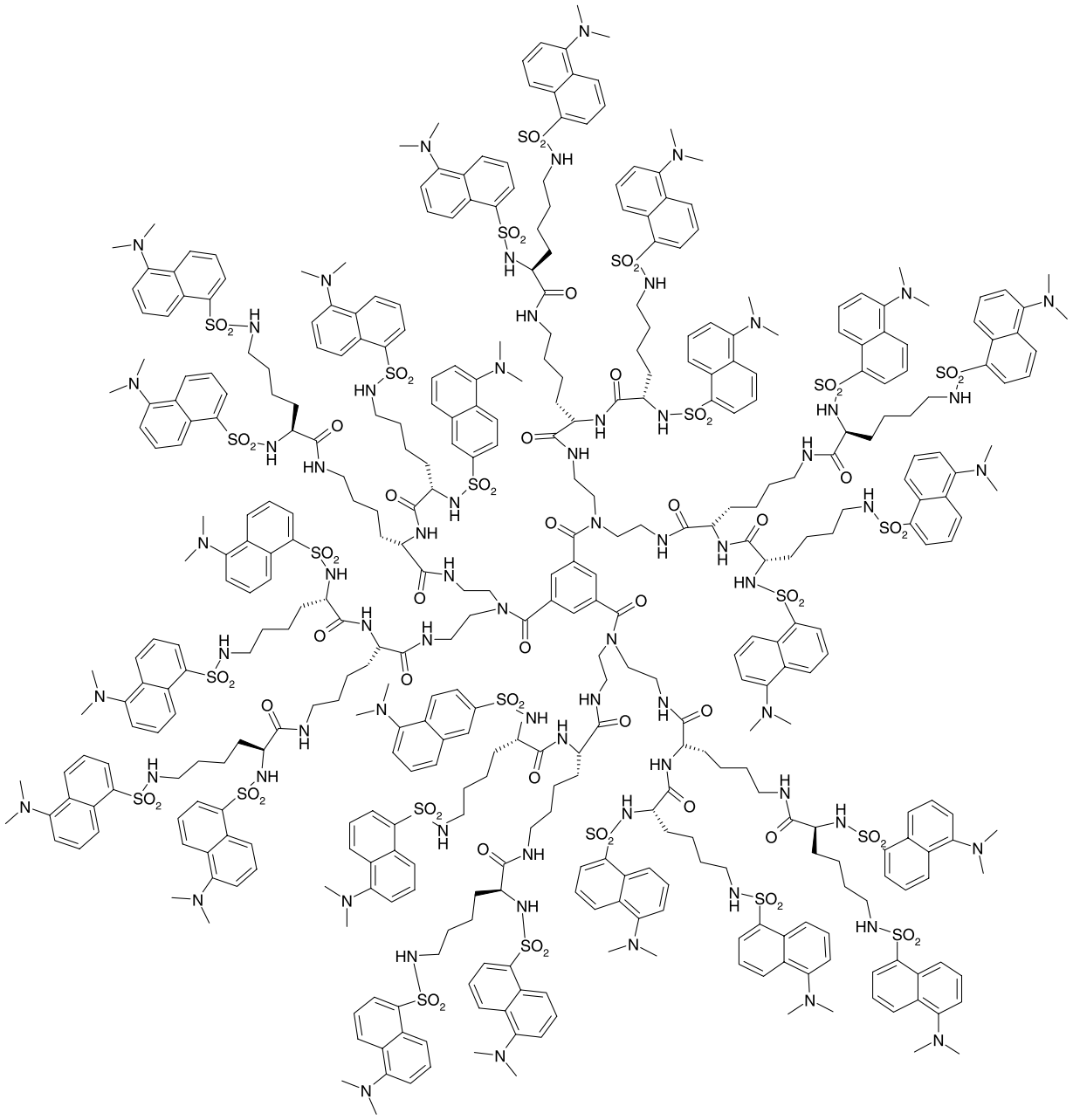

FIGURE 15 Functionalized polylysine dendrimer for sensing lanthanide ions.

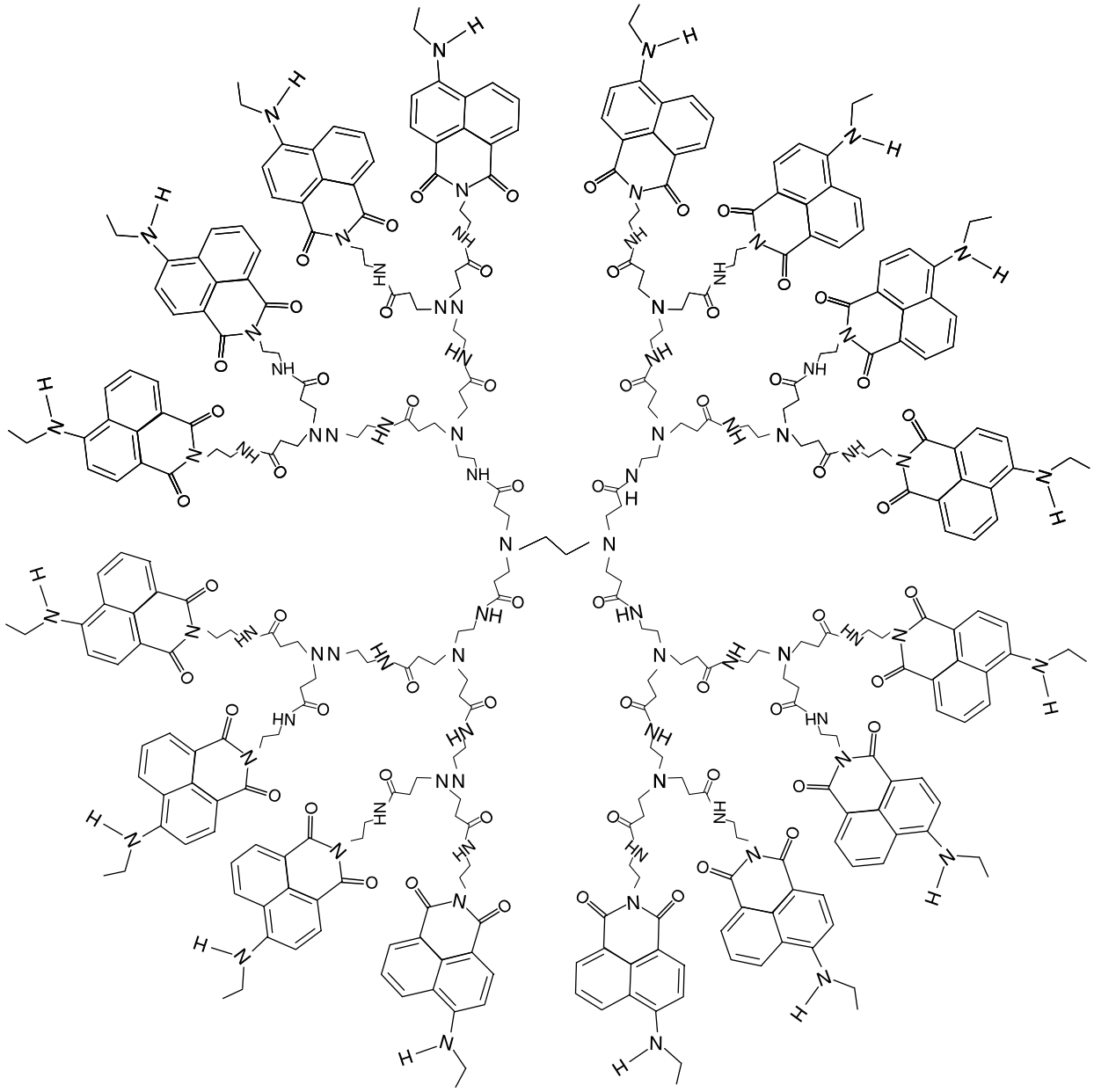

FIGURE 16 Second generation PAMAM dendrimer functionalized with 4-ethylamino-1,8-naphthalimide.

groups. Also, the fluorescence quenching of the dansyl groups was accompanied by a sensitized near-infrared emission of the lanthanide ion.

Naphthalimide functionalized PAMAM dendrimers have been shown for sensing transition metal ions, protons [56–63], and even for rare earth cations [64] in a number of solvents. In a typical example, the effect of the coordination of 4-ethylamino-1,8-naphthalimide functionalized PAMAM dendrimers, shown in Fig. 16, with transition metal cations in DMF solution has been discussed [56]. The fluorescence quenching arising from the addition of the cations was shown not only to depend on the cation concentration, but also on the cation itself. Furthermore, the wavelength at which maximum absorption appears is not altered by the presence of the metal cations, indicating the ligand sites responsible for metal-ion coordination are located within the dendrimer at the aliphatic amine branches.

By contrast, luminescence quenching of the photo-excited sensitizer $[Ru(phen)3]^{2+}$ by $[Co(phen)3]^{3+}$, was shown to occur when the quencher molecules were non-covalently associated on the surface of PAMAM dendrimers [65]. This research was carried out to statistically analyze luminescence quenching resulting from electron transfer between dendrimer surface and the electron acceptor. However, it demonstrated that increased clustering of the sensitizer and acceptor molecules resulted in an increase of luminescence quenching.

PPI dendrimers functionalized with methyl orange at the periphery have revealed a strong sensitivity

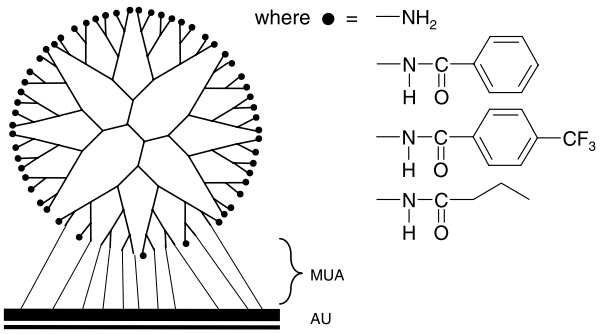

FIGURE 17 Dendrimer SAMs as functionalized by Crooks *et al.*

not only to light, but also towards pH [66]. Methyl orange is a well known pH indicator which is also light sensitive, undergoing isomerization, and the response of the sensors was measured using UV-vis spectroscopy. Upon protonation it was found that the lower generations (GO and G1) behaved in a similar manner to methyl orange itself. However, for higher generations, changes in the isobestic points were observed and the absorption band of the protonated molecules became broader and less bathochromically shifted. Consequently the responses of the methyl orange groups toward changes in pH were dependant on the generation.

A down side in many of these molecular sensing experiments is that they are mostly carried out in solutions, which often makes them impractical for real world sensing applications. Limited work has been conducted that examines the optical and electrical properties of dendrimer thin film sensors. Crooks [26,67] reported the first account of covalent surface confinement of dendrimers, linking PAMAM dendrimers to a mercaptoundecanoic acid (MUA) self-assembled monolayer (SAM). The dendrimer monolayer was formed by covalent attachment via amide bond formation in chloroformate. Dendrimers with different functionalities were employed for the SAMs (Fig. 17) and were deposited onto surface acoustic wave (SAW) transducers, which were shown to respond selectively and rapidly to several volatile organic compounds (VCOs). They were found to depend not only on the chemical nature of the interaction between the analyte, but also on the dendrimer generation.

CONCLUSIONS

Although still in their infancy, functionalized dendrimers have demonstrated there applicability in sensor technology, and many discoveries still lie ahead. Their robustness is highly appealing, and considering the possibilities for surface functionalization, they can be tailored towards specific analytes. All sensing materials are application specific and hence the appropriate dendrimer surface functionality must be selected for the particular application.

Commercially available PAMAM and PPI dendrimers with amine surface groups are widely reported and have been demonstrated in many sensing applications. Their appeal is attributed to the numerous structural variations that can be implemented during their preparation, and their terminal primary amines allows functionalization via reaction with carboxylic acids and acid chlorides. However, whilst the synthesis route required to form amide linkages are often molecule dependent (its terminal groups, solubility, chemical reactivity, etc.) then there will be also be subtle obstacles to be overcome in each of these routes. For instance, the direct reactions of carboxylic acids with amines are not always favorable and further steps are generally required for synthesis, such as activation with NHS or PFP. Also, acid chloride reagents used to provide a direct route for amide bond synthesis are not stable in air and must either be used immediately, or activated as for carboxylic acids.

Dendrimers show great potential in biological and chemical sensing applications. They possess several advantages over biosensors, small molecule sensors and polymer based sensors. Firstly, their generation is pertinent to their sensitivity. In affinity sensing applications, this is due to the exponential increase in binding sites with increasing generation. For example when functionalized with fluorescence molecules, the dynamic quenching of fluorochromic functionalities, the Stern–Volmer quenching constants increase with increasing dendrimer generation, so that higher generation dendrimers can be more sensitive fluorescent sensors. Furthermore, it is seen from the reports in literature that dendrimers contain many potential ligand sites that possess extraordinary ability for coordination as exemplified by reversible binding and the macrocylic effect, and coordination cages for encapsulating guest species. Other advantages include signal amplification resulting from the dendritic structure, low cytoxicity (for application in *in vivo* sensing), ability to tailor any part of the dendrimer for the sensing application, and the fact that they can be made soluble in a variety of solvents. All of these properties have shown them be extremely suited for sensing applications. Yet by far their most attractive property for sensing the dendritic effect.

In many of the sensing examples provided, in particular ion sensing, it was seen that the dendrimers were constrained to the laboratory, and only serve to demonstrate the possibility for utilizing such materials/structures for sensing applications. One of the aims of current research is to discover new methods of implementing such sensors for real-world applications. This will most likely be in the form of thin film monolayers. This has already been

observed in dendrimer based biosensing films, in which the macromolecules are immobilized on a metallic electrode. However, for sensing based on encapsulated species, there is still much work to be done to regenerate the surface and remove the encapsulated species from thin films.

Although many advantages have been presented in this document, to this day, cost remains a significant limiting factor in dendrimers becoming widely adopted. The cost of fabricating dendrimers still remains relatively high, despite them having been in existence for over two decades. This problem could be overcome if the multistep synthesis route can be ultimately reduced to a single step, without compromising the monodisperse properties that make dendrimers so unique. By and large it is hoped that considering the recent attention that is being paid to them, a significant break through in the synthesis cost will allow research into dendritic sensors to flourish and result in their widespread usage.

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