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FEATURE ARTICLE

Dendrimers in biosensors: Concept and applications

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The performance of biosensors, *i.e.* the sensitivity, specificity, linearity, reusability, chemical stability, and reproducibility is critically dependent on the biofunctionalization of the sensor platform. The type (s) of linkers used for the immobilization of the capture probes and the exact immobilization protocol play a vital role in the overall performance of sensors. A variety of linker molecules have been used to biofunctionalize technologically important substrates (glass, gold, mica *etc.*). Amongst the different linkers, researchers have paid more attention to two dimensional architectures, *e.g.* silanes, polyaniline (PANI), alkanethiols, poly-L-lysine (PLL), *etc.* Despite extensive research and a large number of reports, researchers still face problems related to limited loading efficiency, limited accessibility of the probes, poor control over uniform spacing among the probes and a loss of functionality due to irregular orientation of the probes, all of which cause variability in the responses. Three dimensional gel based matrices have proved to be a better choice, except for the fact that the leaching of entrapped probe molecules has limited their use in developing sensor platforms. Taking into account the limitations of the two dimensional linker arrays and three dimensional gel matrices, supramolecular dendritic architectures have shown immense potential in designing and developing the sensor platforms. Dendrimers are well-defined, monodispersed, globular macromolecules constructed around a core unit. Different properties of dendrimers, *i.e.* their structural uniformity, globular shape, monodispersity, the existence of dendritic crevices, high functional group density, hydrophilicity, versatility to design

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dendrimer of different composition and their nanometric size can be exploited while developing high sensitivity biosensors. Researchers have demonstrated that these hyperbranched 3D molecules show enhanced sensitivity, reduced nonspecific binding, greater accessibility of the probe for the target analyte, high stability and low variability in their response. Hence, designing a sensor with a dendrimer as a linker is a successful approach to obtain superior sensor performance and minimize the overall cost of a sensor. In this article, we discuss various aspects of dendrimers from the point of view of sensor design, hoping that this review will excite more researchers into exploiting the exceptional properties of dendrimers in biosensor development.

1 Introduction

Development of reliable biosensing devices, especially microarrays and microchips, has been the prime interest of many researchers and commercial agencies ever since biosensors were invented. These devices are of great use not only in molecular medicine for gene expression studies, the detection of nucleotide mutations, and genotyping individuals in forensic applications, but also in bio-analytical chemistry, specifically for food safety and environmental analysis.[1] The performance of these biosensing devices depends on a number of factors including the surface chemistry used for immobilization, micro-/nano-fabrication techniques and the transduction techniques. Among these, substrate functionalization plays a vital role, since the orientation and functionality of the bioreceptor molecules (antibodies, enzymes, DNA, *etc.*) greatly influences the overall performance of a biosensor. Ideally, a biosensor matrix should be a molecularly organized surface that offers a high ligand density, adequate accessibility, low non-specific signals and the stable attachment of ligands throughout the assay and after the regeneration steps. In this respect, much research has been devoted to identify optimal sensor supports for various applications and, as a result, a variety of two-dimensional (2D) matrices, three-dimensional (3D) gels, membrane coatings and supramolecular architectures have been investigated.

1.1 Limitations of conventional functionalization techniques

Two dimensional matrices are primarily built-up with alkanethiol self-assembled monolayers (SAMs) and silane-modified layers on gold and silica substrates respectively. Other examples include chitosan, polyaniline (PANI), poly-L-lysine (PLL), *etc.* These interface layers have limitations in developing a stable, dense and well organized biosensor matrix. For example, silane tends to polymerize upon exposure to air/moisture, and results in a defective and irreproducible adhesion on the glass unless substrates are dehydrated prior to silanization. In addition, silane layers bound or adsorbed to the sensor substrate may not withstand the regeneration steps.[2] PLL polymeric layers tends to lose their adhesive properties due to the damage from the molecular geometry of freely rotating long polypeptide chains after their immobilization onto the glass surface.[3] The loss of the quaternary amine groups from PLL during the covalent immobilization might also contribute to this phenomenon, since their positive charge plays a significant role in the immobilization of biomolecules. Furthermore, some of these linear polymeric linkers form entangled chains, which probably result in a decreased number of available functional groups for subsequent bioconjugation reactions.[4] In cases of short chain linear linkers, the rapid evaporation of the liquid environment and close surface contact of the bioreceptor molecules lead to the denaturation of their native functional structures.[5] Two dimensional linkers have low immobilization efficiencies due to their planar structures.[6] These limitations of the linear linkers are still unresolved and prevent microarray technology from reaching its full potential.[7] The conventional linkers give rise to some level of satisfactory performance only after accounting for the losses arising from their planar surface morphology, such as a reduced number of capture probes, reduced accessibility and the loss of functionality of the bioreceptor molecules.[8]

Use of 3D gels or the membrane-coated surfaces of poly-acrylamide[9] and agarose gel[10] have been suggested to preserve the functionality and affinity of the biomolecules. Although the physical adsorption of proteineous molecules is a viable alternative to preserve the native protein conformation and obtain good binding capacity, the leaching of bioreceptor molecules results in larger variations in signal intensity, thus limiting their use.

Three-dimensional supramolecular architectures, such as 'dendrimeric' linker systems, have been proposed to overcome the above-mentioned limitations. The inherent characteristics of these molecules, such as their structural homogeneity, integrity, controlled composition, and high density multidentate homogeneous ends for consecutive bioconjugation reactions make them unique and stable for versatile applications. Soon after the discovery of dendrimers,[11] these macromolecules received extensive applications in diverse fields; drug and gene delivery, biomedical imaging, microelectronic and biomimetic systems, catalysis, nanocomposite systems, high-capacity chelating agents, detoxication agents for hydrophobic endogenous toxins, and chemical and biochemical sensors.[12] Although dendrimers have been widely employed in various branches of biomedical science, an exponential increase in the number of articles related to its application in sensors and biosensors has been observed in the last

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five years. This review is intended to draw attention of innumerable sensor research groups towards the potential advantages of dendrimers over conventional linkers or functionalization techniques. We have focused on the current surface modification strategies of silica and gold substrates with dendrimers, the influence of dendrimeric properties on sensor performance and the applications of dendrimers in conjunction with different transducers for the detection of a variety of analytes.

1.2 Introduction to dendrimers

Dendrimers are monodispersed, three dimensional, hyperbranched, nanoscale polymeric architectures with a very high density of surface functional groups. These molecules have a definite molecular weight, shape and size, which make them excellent molecules for innumerable applications.[11a] Dendrimer molecules are composed of three distinct domains, the core, the dendron and the terminal functional groups (Fig. 1). The space between the branches of a dendrimer molecule forms cavities which are known as dendrimeric crevices. The properties of the dendrimers are dominated by peripheral functional groups, although the internal functionality of dendrimeric crevices and central core also are of great significance.

1.2.1 Dendrimer synthesis. In the past two decades, various approaches have been developed for dendrimer synthesis. Among these, divergent and convergent growths have emerged as fundamental approaches, in which growth is typically achieved *via* sequential conjugation and/or protection-deprotection reactions. These techniques have been scaled-up and commercialized for a variety of dendrimer families.

Divergent approach. This approach was developed independently by three research groups led by Vögtle,[11b] Newkone[11c] and Tomalia.[11c] In this method, the growth of the dendrimer proceeds from its core to the periphery (Fig. 2).[13] The reaction of the peripheral functionalities of the core with the complementary reactive group of the dendron monomer introduces a new latent branch point at each coupling site and results in an increase in the number of peripheral functionalities.[14] This approach has been successfully used for the synthesis of PAMAM, PPI, Poly-L-Lysine, melamine, citric acid and polyglycerol dendrimers (Table 1).

Convergent approach. This synthetic route was first reported by Hawker and Fréchet.[15] In this method, growth initiates from what will eventually become the exterior of the molecule, and progresses inward by coupling end groups to each branch of the monomer (Fig. 2). This technique has been utilized for polyester, polyether and melamine dendrimer synthesis (Table 1).

In addition to the above-described methods, the double-stage convergent growth approach,[16] double-exponential dendrimer growth approach[17] and orthogonal coupling[18] have also been used for dendrimer synthesis. So far, over one hundred dendrimer families with different internal and external functionalities have been created (Table 1).[19] Common peripheral groups in dendrimers are–NH_2,–COOH,–OH,–CHO, *etc.* which have been exploited to develop more than 1000 types of bioconjugates.

Simple dendrimer synthetic chemistry has led to the creation of dendrimers of different shapes such as star/globular,[11a] cone,[33] bowl,[34] turbine,[35] tadpole,[36] cross,[37] snowflake,[38] dumbbell,[39] *etc.* Globular-shaped PAMAM dendrimers are the first and the most extensively studied family of dendrimers. The shape of the dendrimer molecules depends on the generation, the physicochemical properties of the core material and the dendrons. They undergo conformational change and tend to become compact with an increase in the number of generations. These molecules behave as soft or hard spheres for intermediate (4G to 8G) or higher generations (>8G) respectively.[40]

2 Surface modification using dendrimers

A number of surface modification techniques have been explored for grafting dendrimers on sensor substrates, mainly gold or silica (glass slides, silicon wafers and silica fibers). They include the Langmuir–Blodgett (LB) technique,[41] noncovalent interactions,[42] covalent attachment[43] and the spin casting technique.[44] In this article, we have focused on noncovalent and covalent immobilization techniques of dendrimers

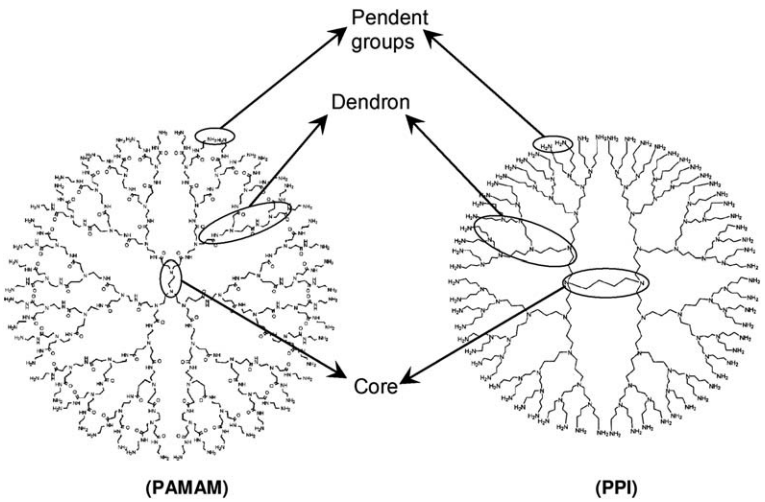


Fig. 1 The typical structure of poly(amido amine) (PAMAM) and poly(propylene imine) (PPI) dendrimer with three distinct domains: core, dendron and pendent groups.

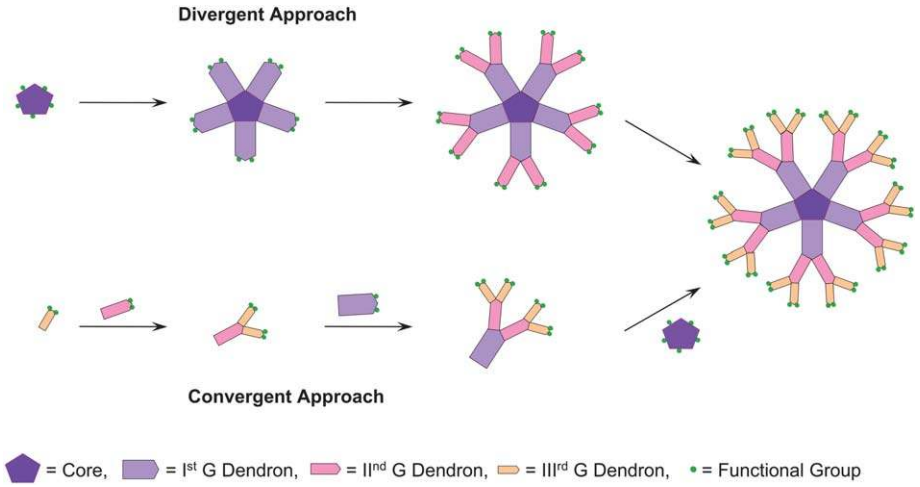


Fig. 2 A scheme illustrating the convergent and divergent growth approach for dendrimer synthesis.

2.1 Non-covalent immobilization of dendrimers

Non-covalent immobilization involves either physical or chemical adsorption, which are caused by hydrogen bonding, van der Waal's forces and electrostatic interactions. Either cationic or anionic dendrimeric architectures may be bound to bare and functionalized substrates.[45] For example, cationic dendrimers are adsorbed on silica substrates through ionic interactions with negatively charged silanol sites and, to some extent by hydrogen bonding. Binding is stronger for higher generations due to increased electrostatic adsorption caused by the high degree of multidentate interactions.[42a] In a similar fashion, amine terminated dendrimers are chemisorbed on different gold substrates including micropatterned surfaces,[42b] electrodes,[46] and nanoparticles.[6]

Multilayer dendrimer structures have been obtained by the layer-by-layer (LBL) assembly of oppositely charged species, of which at least one is a dendrimer. Examples include (i) a multilayered assembly of PAMAM dendrimer and K_2PtCl_4 on silicon wafers,[47] (ii) polyoxometalates (Keggin-structure $PMo_{12}O_{40}^{3-}$ or Dawson-structure $P_2W_{18}O_{62}^{6-}$) and a PAMAM dendrimer on gold or quartz surfaces,[48] (iii) a multilayered matrix composed of a cationic *n*.0G PAMAM-NH_2 and a *n*.5G anionic PAMAM-COOH dendrimer[49] and (iv) a carboxyl-terminated poly-ether dendrimer playing the dual role of hydrogen donor as well as acceptor.[50] These multilayered matrices have been used to embed nanoparticles to obtain highly active surface enhanced Raman scattering (SERS) substrates.[51]

Although non-covalent binding is a simple technique, it is not a preferred protocol due to its poor reproducibility and the possible delamination of the dendrimer layer. In particular, the lower generation dendrimeric layers are less stable and more readily desorb upon exposure to water (pH 5.5), chloroform or ethanol, compared to the higher generations.[45a]

2.2 Covalent immobilization

Covalent immobilization is an attractive choice for surface functionalization since it is stable, durable, reproducible, and its assembly can be controlled to a greater degree. Typically, covalent immobilization involves the introduction of functional groups by means of conventional surface modification techniques such as silanization or alkanethiol self assembled monolayers for silica or gold surfaces, respectively. Most of the studies have utilized dendrimers more like a linker layer between a functionalized substrate and bioreceptor molecules, barring a few reports where modified dendrimers were used directly with substrates. Some other studies involved the *in situ* fabrication of a pseudodendritic linker by a four step chemical reaction on a glass and polypropylene surface[52] or the assembly of dendrimers on flat surfaces by means of "click" chemistry, using homo or hetero bifunctional coupling agents.[53] Covalent techniques for the immobilization of dendrimers on silica and gold substrates are mainly discussed here (Fig. 3 and Table 2).

2.2.1 Coupling to silica. A variety of linker molecules have been used to facilitate dendrimer coupling to silica surfaces. Among these, silane molecules have been commonly used

Silane method. Silanization is the most widely adapted technique for introducing various functional groups to a silica surface. A variety of silane molecules including aminosilane, cyanosilane, epoxysilane, *etc.* have been utilized to immobilize dendrimers on glass/quartz surfaces through suitable homo-or hetero-bifunctional cross linkers.[2,56,65] For example, a surface is aminosilanized to bind an amine terminated dendrimer using glutaraldehyde as a cross linker[66] or alternatively, an aldehyde/carboxy terminated dendrimer *via* Schiff base formation.[5,55]

Multilayer dendrimeric films have been assembled using supercritical carbon dioxide ($SCCO_2$) on a silica surface.[56] Cyano functional groups of 3-cyanopropyltrichlorosilane coated silica substrates were hydrolyzed to carboxylic acid terminals by treating them with sulfuric acid, and subsequently converted into anhydride groups using trifluoroacetic anhydride in $SCCO_2$. PAMAM dendrimers were then covalently attached to the reactive anhydride groups in $SCCO_2$. In the same study, the authors also reported the assembly of a PAMAM dendrimer on p-aminophenyl-trimethoxysilane coated silicon substrates using pyromellitic dianhydride as a coupling agent.

Table 1 Structural composition of some important families of dendrimer

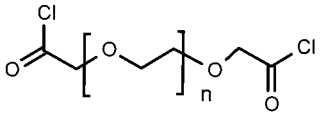
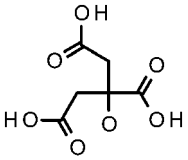
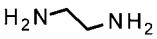
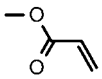
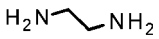
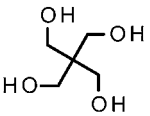
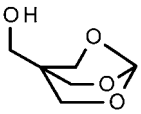
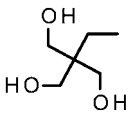
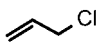
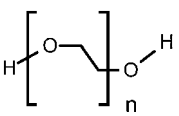
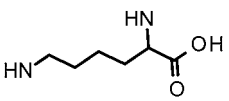
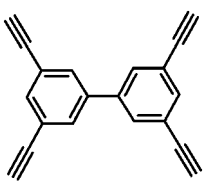
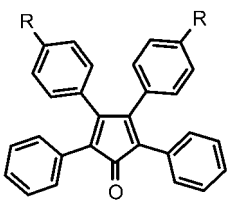
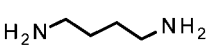
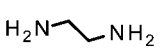
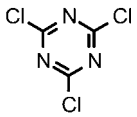
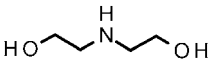
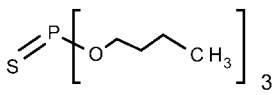
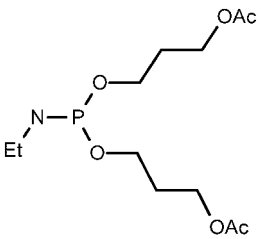
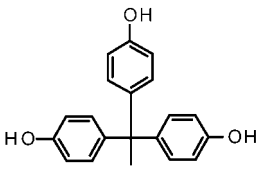
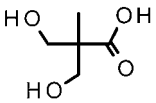
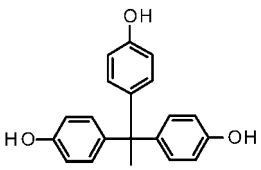
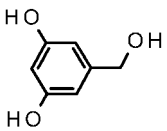
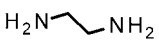
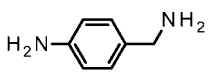
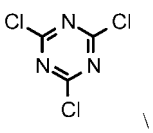
Dendrimer	Core	Dendron/monomer	Pendent group (P)	nG-mP[a]	References
Divergent Approach					
Citric Acid	 diacyl chloride poly (ethyleneglycol)	 citric acid	–COOH	1G–6 2G–18 3G–54	Namazi and Adeli[20]
Poly(amido amine)	 ethylene diamine	 (i) methyl acrylate	$-NH_2$	1G–4 4G–32 5G–64	
		 (ii) ethylene diamine	–COOH	0.5G–4 3.5G–32 4.5G–64	Tomalia *et al.*[11a]
Polyether	 pentaerythritol	 4-(hydroxymethyl)-2,6,7-trioxabicyclo[2.2.2]octa	–OH	1G-12 2G–36 3G–108	Padias *et al.*[21]
Polyglycerol	 1,1,1-trimethylolpropane	 Allyl chloride	–OH	3G-24 4G–48 5G–96	Haag *et al.*;[22a] Ooya *et al.*[22b]
Poly-L-lysine	 polyethyleneglycol	 L –lysine	$-NH_2$	3G-16 4G–32 5G–64	Kaminskas *et al.*;[23] Agrawal *et al.*[24]
Polyphenylene	 tetraethynylbiphenyl	 tetraphenylcyclopentadienone	–R	1G-8 2G–16 3G–32	Morgenroth *et al.*[25]
Poly(propylene imine)	 1,4-diaminobutane	 acrylonitrile	$-NH_2$	1G-4 4G–32 5G–64	de Brabander-van den Berg and Meijer[26]
	 ethylene diamine	 acrylonitrile	–CN	0.5G-4 3.5G–32 4.5G–64	Kumar *et al.*[27]

Table 1 (*Contd.*)

Dendrimer	Core	Dendron/monomer	Pendent group (P)	nG-mP[a]	References
Triazine	 triazine trichloride	 diethanolamine	–OH	1G-6 2G–24 3G–96	Bansal *et al.*[28]
Thiophosphate	 tri(3-hydroxy)propyl thionophosphate	 di(3-acetoxypropoxy)-N,N-diethylaminophosphine	–OH	1G-6 2G–12 5G–96	Salamończyk *et al.*;[29] Domański *et al.*[30]
Convergent Approach					
Polyester	 1,1,1-tris(4-hydroxyphenyl)ethane	 2,2-bis(hydroxymethyl) propanoic acid	–OH	2G-8 3G–16 4G–32	Ihre *et al.*[31]
Polyether	 1,1,1-tris(4-hydroxyphenyl)ethane	 3,5-di-hydroxybenzyl alcohol	–OH	1G-4 2G–8 3G–16	Hawker and Fréchet,[15]
Both Approach					
Melamine	 ethylene diamine	 (i) p-aminobenzylamine  (ii) cyanuric chloride	$-NH_2$	1G-12 2G–24 3G–48	Zhang and Simanek.;[32a] Chen *et al.*[32b]

[a] nG and mP represent the dendrimer generation and the number of pendent groups, respectively.

Other methods. Fail *et al.*[57] immobilized PAMAM-NH_2 dendrimers on pulse polymerized maleic anhydride surfaces *via* amide linkage. This matrix was used for a variety of surface related phenomena including fluorination and imidization. In another strategy, a homogeneous film of PPI dendrimers was generated on a silica substrate using 1,1′-carbonyldiimidazole as a coupling agent.[43b] Recently, dendrimer conjugation has been reported by exploiting the avidin-biotin specific interactions.[67] In this approach, pre-produced avidin-biotin-dendrimers composites were specifically bound on biotin functionalized glass substrates using selective avidin-biotin interactions.

Multilayer films of a carboxyl terminated PAMAM dendrimer and nitro-containing diazoresin on a silica surface were formed *via* the LBL technique. The diazonium groups were decomposed

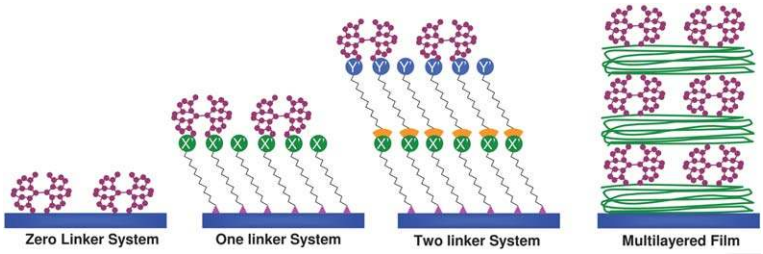
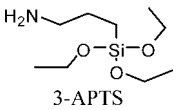
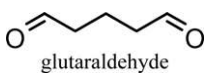
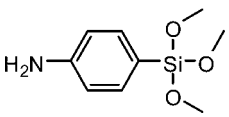
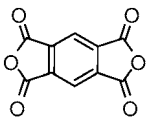
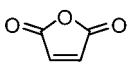
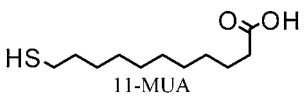
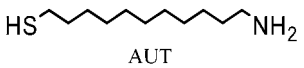
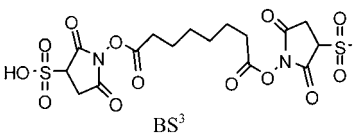
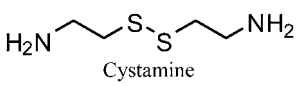
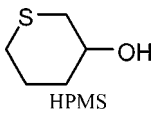
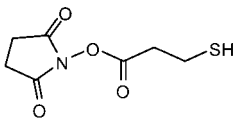
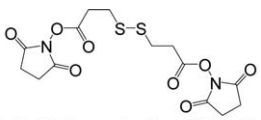


Fig. 3 Schematic representation of dendrimer immobilization strategies on solid substrates using zero, one or two linker system and multilayered matrix fabrication.

into phenyl cations by UV-irradiation and allowed to bind with the carboxyl groups to produce a covalent ester interlayer linkage.[68]

2.2.2 Coupling to gold. Dendrimers have been grafted onto gold substrates using SAMs of heterobifunctional coupling agents with a thiol group at one of the ends. Thiol groups of these

Table 2 Different strategies for dendrimer immobilization on silica and gold substrates[a]

Substrate	X′	Y′	References
Silica	 3-APTS	 glutaraldehyde or OHC-Dend-CHO	Benters *et al.*;[54] Ji *et al.*[3] Slomkowski *et al.*[55]
	 p-APhTMS	 PMDA H_2SO_4, CF_3COOH	Puniredd and Srinivasan[56]
	 maleic anhydride	NU	Fail *et al.*[57]
Gold	 11-MUA	DCC-NHS or PF_5-EDAC	Yam *et al.*[58] Hong *et al.*[59]
	 AUT	 BS^3	Mark *et al.*;[60] Singh *et al.*[61]
	 Cystamine		Liu *et al.*[6]
	 HPMS	NU	Liu and Amiridis[62]
	 NHS-3-mercaptopropanoate	NU	Chechik and Crooks[63]
	 3,3-dithiopropionic acid bis-N-hydroxysuccinimide ester	NU	Yoon *et al.*[64]

[a] NHS: N-hydroxysuccinimide; PMDA: pyromellitic dianhydride; 3-APTS: 3-aminopropyltriethoxysilane; BS^3: bis(sulfo succinimidyl) suberate; 11-MUA: 11-mercapto undecanoic acid; p-APhTMS: p-aminophenyltrimethoxysilane; 3-CPTCS: 3-cyanopropyltrichlorosilane; AUT: amino undecanethiol; HPMS: 2-hydroxypentamethylene sulphide; PF_5-EDAC: pentafluorophenol, 1-ethyl-3-(3-dimethyl aminopropyl)carbodiimide hydrochloride; CF_3COOH: trifluoroacetic acid; DCC: 1,3-dicyclohexylcarbodiimide; NU: not used.

linkers are known to chemisorb on gold surfaces while the other functional group binds to dendrimer molecules.

Mercaptoundecanoic acid (MUA) and analogs. Different generations of PAMAM dendrimers have been immobilized on MUA coated gold surfaces using EDC-NHS mediated coupling chemistry.[43a,58,69] Covalent attachment is accomplished by forming amide bonds between the peripheral amine groups of the PAMAM dendrimer and the carboxylic acid groups of MUA-SAMs on gold substrates. Surface-confined dendrimer interfaces have been prepared on mixed SAM (*e.g.* MUA + mercaptopentane (MP)) coated gold substrates in a similar way.[70] This chemistry has been exploited in fabricating biochips for protein–ligand interactions to bind PAMAM dendrimers by microcontact printing.[59,71] Yoon *et al.*[72] used pentafluorophenyl-activated ester groups for grafting amine terminated dendrimers onto a MUA treated gold surface and claimed 10 times faster reaction rates than for other reactive esters. The self assembled monolayers of 3-mercaptopropionic acid have also been used to anchor PAMAM dendrimers on gold electrodes to develop electrochemical biosensors.[73]

A durable, polyfunctional and highly impermeable thin film has been prepared using amine-/hydroxyl-terminated PAMAM dendrimers, first as building blocks and then as *in situ* thermosetting agents.[74] Amine terminated PAMAM dendrimers were covalently linked to chloroformate-activated MUA-SAMs *via* amide bond formation. Further, poly(maleic anhydride)-c-poly(methyl vinyl ether) was covalently linked to the amine functionalized substrate *via* amic acid bonds. Imidization and decomposition of PAMAM dendrimers in the film were accomplished by thermal treatment to realize the highly impermeable thin films.

Amino alkane thiol SAMs. The formation of SAMs using cystamine, a short chain amino alkane thiol molecule, is a simple and inexpensive approach to obtain amine groups on a gold surface. Dendrimers can be covalently bound to the free amines *via* coupling agents, such as glutaraldehyde, bis(sulfo succinimidyl) suberate (BS^3), *etc.*[6,61] For example, amine functional SAMs have been utilized to obtain multilayered glucose biosensing films on gold disk electrodes by LBL depositions of alternating layers of 4G PAMAM dendrimers and –CHO terminated periodate-oxidized glucose oxidase (GOx).[75] Alternatively, dendrimers are bound to gold using 11-amino 1-undecanethiol hydrochloride (AUT), a long chain amino alkane thiol molecule that aids the retention of bioreceptor activity to a greater degree than cystamine. Following this dendrimer conjugation chemistry, DNA[60,76] and explosive[61] sensor chips have been fabricated.

Miscellaneous. An amine-reactive SAM has been constructed by chemisorption of 3,3-dithiopropionic acid bis-*N*-hydroxysuccinimide ester on gold surfaces and utilized to bind ferrocenyl-tethered dendrimers.[64] PAMAM dendrimers have also been immobilized in a similar fashion using 11,11′-dithiobis(*N*-hydroxy succinimidylundecanoate) as a coupling agent.[77] Micro-/nano-fabricated robust reactive arrays were developed *via* solution phase chemistry, microcontact printing (μCP), and atomic force microscopy (AFM) tip-mediated transfer (dip pen nanolithography). In another approach, aldehyde functionalities were generated on gold substrate using 2-hydroxypentamethylene sulfide (HPMS) to bind an amine terminated dendrimer layer to the sensor surface.[62] Crooks and coworkers bypassed the necessity of alkyl thiol SAMs with the help of thiol terminated dendrimers obtained by treating a PAMAM dendrimer with N-hydroxy succinimidyl 3-mercaptopropanoate.[63]

3 Influence of dendrimer properties on sensor performance

The exciting properties of dendrimers, such as globular geometry, their controllable size, high surface functionality, hydrophilicity and high mechanical and chemical stability make them an ideal matrix for the immobilization of biomolecules. These properties result in the enhanced target capturing ability, sensitivity, specificity, stability and reusability of the biosensors. The typical structural features of dendrimers and their influence on sensor performance are explained in this section.

3.1 The size and shape of the dendrimer

Nanoscopic supramolecular architectures of dendrimers spread three dimensionally and attain globular geometry with each subsequent higher generation. Their tiny size and quasi three-dimensional structure provide better control over the thickness of the sensor matrix and the spacing among the immobilized bioreceptor molecules, thereby preserving the functionality of immobilized ligand groups and reducing nonspecific binding (NSB).

3.1.1 The thickness of the dendrimeric interface. The sensitivity of a sensor is greatly influenced by the distance between the solid substrate and the biorecognition element. For affinity based biosensors, biomolecular interactions must be within close vicinity of the transducer surface or field of influence, as seen in many label-free biosensors, *e.g.* surface plasmon resonance (SPR) and SERS. However, very close proximity of the bioreceptor molecules to solid substrates leads to steric hindrance and a loss of functionality due to the less hydrophilic nature of unmodified surface. Thus, an intermediate layer is necessary to act as a spacer to keep bioreceptors away from the substrate. Ideally, this makes the surface hydrophilic. The monolayer thickness of 2D linkers on silica and gold substrates is 20 Å or less.[8d,58] The incorporation of a spacer, typically >28 Å thick, is desirable in order to preserve the functionality of the probe biomolecules.[78] Dendrimers are close to ideal candidates for providing an additional grafting layer.

The necessary thickness of the grafting layer can be tuned by suitable generation and composition of the dendrimers. For instance, the hydrodynamic diameter of PAMAM dendrimers in solution is in the range of 4.5 nm (4G) to 13.5 nm (10G).[44a] However, the thickness of dendrimeric architectures grafted on a solid surface significantly reduces to only 1–5 nm due to substantial flattening (Fig. 4, Table 3). The collapse of a dendrimer occurs due to the multiplicity of the interaction sites to form a densely-packed film.[45e,80] Thus, the grafting of dendrimeric spacer in a sensor matrix might help in alleviating problems such as steric hindrance of the recognition sites, possible denaturation of bioreceptors and quenching of the luminescence based signalling molecules close to the sensor substrate.

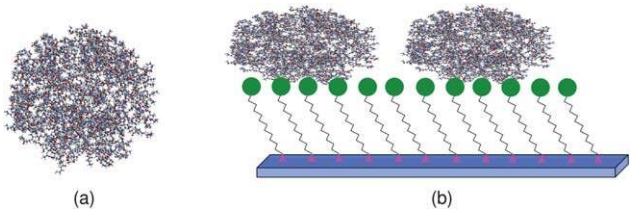


Fig. 4 Schematic presentation of globular shaped dendrimer structure (a) in solution phase (Çağın *et al.*[79]) and (b) flattened or collapsed structure after immobilization on solid substrate.

3.1.2 Shape and nonspecific binding. The functional specificity of a ligand is another key factor for the success of a biosensor. Many proteins are known to adsorb non-specifically at biointerface layers and pose a constant problem by generating false positive signals. This nonselective binding is likely to be related to free active functionalities present on the sensor surface and the cumulative effect of the number of biological samples. Non-relevant adsorption is driven by the net influence of various interdependent interactions, such as van der Waals forces, dipolar or hydrogen bonds, electrostatic forces and hydrophobic effects.[82] Therefore, great care must be taken while designing sensor surface functionalization and immobilization protocols. Conventional linkers are more prone to NSB because of their planar structure. In contrast, dendrimeric matrices have shown reduced NSB due to their intrinsic globular shape, which prevents access to its free terminal groups due to the steric constraint imposed by immobilized bioreceptor molecules. In addition, 3D architectures of dendrimers also covers some surface defects on substrates (*e.g.* free –COOH groups of MUA-SAMs on gold substrates), which remain exposed in 2D matrices.

Mark *et al.*[60] found an insignificant amount of nonspecific adsorption of bovine serum albumin (BSA) on a streptavidin derivatized PAMAM dendrimer modified sensor surface. When the nonrelevant interactions of avidin on amine terminated-dendrimer were compared with 2,2′-(ethylenedioxy)bis(ethylenediamine) (DADOO) and 3-aminopropyltrimethoxysilane (AMPTS) coated sensor matrices, the dendrimeric film exhibited approximately 10% NSB of the maximum specific binding capacity as opposed to 27% and 76% for the DADOO and AMPTS surfaces, respectively.[66]

Mesospacing. The controlled lateral spacing among biomolecules on a sensor surface, *i.e.* mesospacing, is another prerequisite for unhindered recognition, especially for single nucleotide polymorphism (SNP) detection. In addition, it lessens the nonspecific adsorption of biomolecules on the surface.[83] Previously, mesospaced biosensor matrices have been developed using mixed SAMs of linear linkers. However, a lower degree of analyte binding was observed due to steric constraints.[83b] Instead of 2D linkers, dendrimers have shown outstanding performance in the design of a mesospaced bioplatform, purely due to their shape. For example, globular shaped PAMAM dendrimers have provided interspacing of the order of size of the bioaffinity molecules (~18 Å). In contrast, a nondendrimeric matrix manifested interspacing of ~10 Å which may be due to steric hindrance of the analyte binding sites. Cone shaped dendrons also offered greater control over the mesospacing by choosing the type and generation of the dendrons.[84]

The detection ability of mesospaced dendrimeric bioplatforms has been shown to be two to three times higher than aminosilanized glass slides for target oligonucleotides.[85] The space between immobilized oligonucleotides on the dendrimeric solid support is expected to be wide enough to accommodate more target oligonucleotides, resulting in signal enhancement. These matrices showed a good discrimination efficiency for single base mismatched DNA detection.[33] A similar trend was found for an avidin-biotin assay.[71b] The avidin surface density approached 5.0 ng mm^{-2} for the dendrimeric sensor matrix, which was 50% higher than that for the PLL layers (3.2 ng mm^{-2}). The inaccessibility of the biotin molecules caused by their entrapment within the entangled layers of PLL was cited as a reason for the lower surface density of avidin.

3.2 Composition and high functional group density

In the past two decades, dendrimers of various compositions and of different generations have been developed (Table 1). Also, it is well known that their end groups increase in number with each

Table 3 Thickness and contact angle of different dendrimerized substrates

Substrate	Dendrimer generation-pendent group type	Analytical methods-Thickness (nm)	Contact angle	References
Silica	5G PPI-NH_2	E–2.3	12°	Pathak *et al.*[43b]
	4.5G PAMAM-COOH	AFM–20	48°	Ajikumar *et al.*[5]
	[a]PAMAM-NH_2	E–2.9	NR	Puniredd and Srinivasan[56]
	10G PAMAM	SPM–5.3	NR	Tsukruk *et al.*[80]
Mica	4.5G PAMAM-COOH	XRD–4.6	NR	Wang *et al.*[81]
Gold	4G PAMAM-SH (100%)	E–1.1	26°	Chechik and Crooks[63]
	4G PAMAM-NH_2	E–1.6	48°	Yam *et al.*[58]
	6G PAMAM-EG	E–2.5	45°	Yam *et al.*[58]
	4G PAMAM-NH_2	E–2.8	32°	Tokuhisa *et al.*[42b]
	4G PAMAM-OH	E–2.3	23°	Tokuhisa *et al.*[42b]
	4G PAMAM-NH_2	E–2.8	31°	Hong *et al.*[71a]
	4G PAMAM-NH_2	E–2.6	NR	Yoon and Kim[75]
	4G PAMAM-NH_2	E–1.2	NR	Mark *et al.*[60]
	5G PAMAM-NH_2	NR	14°	Goddard and Erickson[8c]
	4.5G PAMAM-COOH	NR	14°	Goddard and Erickson[8c]

[a] generation not reported; NR: not reported; E: ellipsometry; AFM:atomic force microscopy; SPM:scanning probe microscopy; XRD:X-ray diffraction.

generation. For instance, a 1G EDA-PAMAM dendrimer has 4 amine groups, whereas 5G has 64 groups. The versatility in designing dendrimers of any composition and/or functional groups has been exploited for biosensor applications.

3.2.1 High ligand immobilization. Dendrimer molecules form a densely packed film on a flat surface in order to maintain a lower surface tension, which helps these molecules bind strongly through a higher number of end groups.[73a] The remaining free pendent groups help in the formation of a highly dense layer of bioreceptors (Fig. 5), which improves signal to noise ratio (SNR) and minimum detection limit.[61] However, there exists an optimum surface density beyond which steric hindrance at binding sites limits the overall efficiency of the analyte binding and thus limiting the maximum achievable signal intensity.

At least a two-fold increase in the loading efficiency of single stranded DNA (ssDNA) on a dendrimeric surface was confirmed by fluorescence, as well as radio-label based assays.[2] Approximately 50 fmol mm^{-2} of Cy5-labelled oligonucleotides were bound to dendrichip, which was three orders of magnitude greater than commercial aldehyde functionalized slides.[86] In another study, the immobilization efficiency of DNA on a dendrimer derived matrix was measured to be twenty five times better than the aminoethanethiol (AET) based linear film using electrochemical impedance spectroscopy.[76] Similar observations were made in the case of protein immobilization as well. The surface density of antibodies immobilized on a 4G PAMAM-AUT modified gold substrate was enhanced by 1.6- to 2.5- times compared to the unmodified, AUT-only surface.[60,61]

Obviously, the number of pendent groups (*i.e.* the generation) on a dendrimer is expected to influence the sensitivity. A higher generation dendrimeric matrix, 4.5G PAMAM-COOH, was found to be 1000-fold more sensitive compared to linear linkers (succinamic acid) and lower generation (1.5G) dendrimer modified surfaces.[5]

Nucleic acid dendrimer. In order to amplify the sensitivity of nucleic acid based sensors, DNA dendrimers have been used. These are complex, highly branched molecules possessing multiple single-stranded arms capable of hybridizing with a complementary nucleic acid sequence.[87,88]

Nucleic acid dendrimer derived matrices have shown greater hybridization capacity, enhanced sensitivity and an extended linear response towards DNA detection. The sensitivity and detection limit of these sensors was at least eight times better than the conventional oligonucleotide based biosensor.[89] The superior performance of nucleic acid dendrimer based biosensors is due to the substantially large number of ssDNA arms available for hybridization. These bioplatforms have also shown a negligible response to various noncomplementary oligomers.

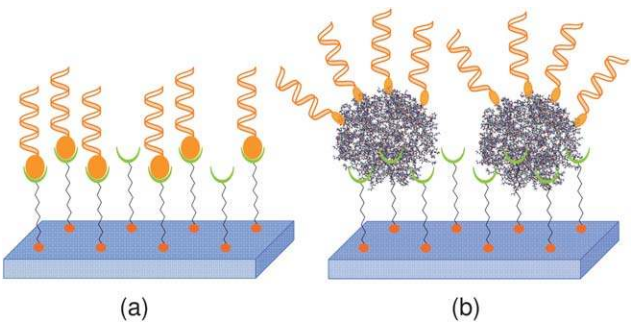


Fig. 5 A cartoon representing the immobilization efficiency of ssDNA on (a) linear linker and (b) on dendrimer derived substrate.

3.2.2 Stability and regeneration of biosensor matrix. Ideal characteristics of a biosensor matrix include (i) resistance to changes in wide range of physiological environments; such as the pH, temperature, ionic strength and chemical composition of the sample matrix, (ii) long-term stability, durability *etc.* Given the complex and time consuming protocols for the immobilization of bioaffinity ligands on sensor surfaces and the cost, the regeneration of immobilized ligands after analyte detection has been of interest to researchers. Regeneration involves the treatment of the sensor matrix in harsh conditions such as washing with highly acidic or basic solution, which may sometimes lead to the complete desorption of the recognition matrix. Hence, the regeneration ability and stability of the biosensor matrix depends on the method of immobilization and the type of linkers on which affinity ligands have been attached.

Dendrimer modified sensor surfaces have revealed remarkable stability, even after numerous cycles of regeneration for DNA hybridization and antigen-antibody assays. These matrices have been found stable in different types of regeneration systems, which include alkaline stripping,[2] urea stripping,[60] glycine-HCl stripping[61] and thermal stripping.[58] The high stability of the dendrimer film is attributed to the attachment of each bioreceptor molecule through multiple bonds with several functional groups present on the sensor substrates, as well as the higher holding capacity of the bioaffinity groups. Benters *et al.*[2] observed an interesting trend during the regeneration of a dendrimer based sensor matrix. The dendrimeric biomolecular film showed increased signal intensity after the first cycle of alkaline stripping. In subsequent regeneration and hybridization experiments, the signal intensity decreased slightly and reached a plateau, which remained constant for more than 100 simulated regeneration cycles. There are some other reports suggesting the rejuvenation of dendrimer derived matrices at least 10 times without any significant loss of the signal intensity.[60,85]

3.3 Homogeneous end group distribution

The uniform orientation of probe molecules on sensor surfaces is essential to achieve greater analyte binding capacity, as well as homogeneous signals. For example, in fluorescence based protein microarrays, target protein molecules have the tendency to form ring like structures.[90] The attachment of the probes through linear linkers often causes a loss of activity and heterogeneous binding affinities caused by (i) the direct chemical modification of the target-binding site of the probes, (ii) steric hindrance by the surface and/or by adjacently immobilized biorecognition moieties, (iii) the denaturation of immobilized biomolecules, *etc.*[91,92] Conversely, dendrimeric structures have regularly spaced terminal groups, which provide homogeneous orientation and spatial distribution of bioreceptor molecules in the sensor matrix. Consequently, these structures are capable of displaying

homogeneous spots in fluorescence based assays which make quantification much easier.

Benters *et al.*[2] compared the spot homogeneity of DNA hybridization assays for three different arrays spotted on epoxysilane-activated slides, nitrocellulose covered slides and PAMAM dendrimer slides. Amongst these, the dendrislides displayed sharp spots of Cy5-labeled oligomer with the best homogeneity of signal distribution. Similar findings were reported when comparing the surface homogeneity obtained from a 4.5G PAMAM-COOH dendrimer with that of 2D surfaces of succinamic acid.[5] Higher generation dendrimers exhibit sharper spots and homogeneous signal distribution due to larger number of binding sites.

3.4 Hydrophilicity and pendent group type

Globular proteins in their native state usually display a hydrophilic exterior and a hydrophobic interior. Immobilization on a hydrophobic surface may destabilize the structure and turn it inside out, thereby rendering the protein inactive. Thus, a hydrophilic surface is needed to conserve the functionality of immobilized bioreceptor molecules. In addition, increased hydrophilicity contributes towards the reduction of analyte-wall interactions, thereby increasing the efficiency of separation and improving resolution. However, too great a hydrophilicity might affect the stability of the coating.[93] Similar to 2D linkers, dendrimers have shown their efficacy in reducing the hydrophobic nature of solid substrates. Contact angle studies have revealed that dendrimers and linear linkers have an approximately similar ability to reduce the contact angle of a sensor surface (Table 3). For example, a 4.5G PAMAM-COOH dendrimer derived surface showed contact angles similar to the linear linker succinamic acid. This hydrophilic nature can be attributed to large number of end groups in the dendrimers.[5] Amongst different generations of dendrimers, lower generation dendrimers have shown less potential compared to higher generation dendrimers in imparting a hydrophilic nature to the surface. The high hydrophilicity of higher generation dendrimers was associated with a higher number of functional groups on the surface.[5]

3.4.1 Type of pendent group and nonspecific binding. The degree of NSB on the dendrimeric matrix is influenced by the type and generation of a dendrimer as well as its characteristic pendent group. For example, amine terminated dendrimers have shown relatively high nonspecific interactions due to their cationic nature and highly reactive amine functionalities. At pH 7.5, a PAMAM-NH_2 dendrimer remains positively charged because the pK_a values of the primary and tertiary amines of the dendrimer are 9 and 6.[94] However, this cationic nature of the dendrimer has also been exploited in reducing the NSB of avidin (pI value of ~10) due to repulsive interactions between the dendrimer (pK_a value of ~9.5) and the avidin molecules at physiological pH.[71b] The use of passivating agents, such as Tris, ethanolamine, oligoethylene glycols, BSA and nonionic detergents have rendered dendrimer matrices more effective at reducing NSB compared to the naked dendrimer.[5,77] Hong *et al.*[71a] demonstrated that tri(ethylene oxide) (EO_3) conjugated PAMAM dendrimers reduce the nonspecific adsorption of proteins compared to PAMAM dendrimers alone. The extent of the nonselective adsorption of BSA and the serum proteins on an EO_3 conjugated dendrimeric matrix was 11.4% and 29.5% of those on the bare PAMAM dendrimer layers, respectively.

The types of pendent groups on the dendrimer molecules play a significant role in controlling NSB and SNR. Carboxyl and hydroxyl pendent functionalities have shown a lesser degree of NSB compared to cationic amine groups. Ajikumar *et al.*[5] reported 100-fold lower NSB and improved SNR for a PAMAM-COOH dendrimer compared to a PAMAM-NH_2 dendrimer. Also, compared to lower generation dendrimers, higher generation PAMAM-COOH (4.5G) were shown to reduce NSB due to the presence of an increased number of carboxyl groups. Advances in dendrimer synthesis chemistry have introduced hydroxyl terminated polyglycerol (PG) dendrimers. These dendrimers possess characteristic structural features of highly protein-resistant surfaces such as PEG SAMs.[95] Also, these dendritic PG monolayers are significantly more active than dextran-coated surfaces. In addition, they also have a higher thermal and oxidative stability compared to PEG. Recently, bifunctional (hydroxyl and thiol-functionalized) dendrimeric nanocomposites have also been found to be resistant to the nonspecific adsorption of proteins with a wide range of molecular weight and isoelectric points.[96]

3.4.2 Ease of bioconjugation. The availability of dendrimers with different types of terminal functionalities has made bioconjugation chemistry much easier. There are a number of reported bioconjugation protocols to bind DNA, antibodies, enzymes or other biomolecules to dendrimeric surfaces.[2,12d,64,97] The ease in carrying out any bioconjugation protocol on dendrimeric surfaces has opened the path to detect any chemical or biological substance using any sensing mechanisms. As a result, a variety of sensors have been developed for the detection of DNA, protein, pathogens, glucose, TNT, *etc.*

3.4.3 Multilayering. The end groups of dendrimers, especially cationic amine groups, have been used to fabricate multilayer films of dendrimers and metallic nanoparticles (silver/gold colloids) using LBL techniques for SERS and electrochemical based biosensor applications.[51,75,98,99] The multilayer film characteristics depend on the chemistry, size and concentration of the species involved, as well as the generation of the dendrimer and the number of deposition steps. The roughness of multilayered surface increases with the increasing number of bilayers and reaches a saturation value after a certain number of bilayers. For example, the film surface roughness rose from 5.1 nm to 14 nm as the number of bilayers increased from 1 to 5, after which it remained practically constant for 7 and 9-bilayer films.[99] In another study comparing different generations of a dendrimer, the adsorption of silver nanoparticles (AgNPs) was higher for the 5G dendrimer than for the 1G dendrimer due to the larger number of functional groups available to bind the AgNPs.[98]

3.5 Miscellaneous

3.5.1 Dendrimeric crevices. Dendrimeric macromolecules contain unique tunable inner cavities which enable covalent and

non-covalent host–guest interactions. Among the different types of dendrimers, polyphenylene dendrimers (PDs) have rigid frameworks with aromatic groups in internal voids. These dendrimers are most suitable for hosting volatile organic compounds because of the stable cavities in their interior.[100] These crevices have also been exploited to help in sensor applications, *e.g.* pathogen detection.[3,101] Ji *et al.*[3] introduced a membrane-reactive fluorophore, FAST DiA, into dendrimeric crevices through host–guest interactions and demonstrated the real time detection of bacterial contamination in dynamic aqueous environments.

3.5.2 Flexibility of dendritic chains. Depending on the chain length of the dendrons, dendrimer architectures can be flexible in nature. Flexible arms provide a "cushioning effect" that helps preserve the native conformation of immobilized proteins and their accessibility to the analyte.[45e] Intuitively, higher generation dendrimers possess better flexibility. In the case of ferrocenyl dendrimers, which are typically used in electrochemical sensors, higher generation dendrimers provide more flexibility and shorter separation between ferrocenyl neighbours, resulting in enhanced electron transfer between the immobilized enzymes and substrates, and therefore, a greater biocatalytic response.[102]

4 Application of dendrimers in different sensors

Dendrimeric bioplatforms have been used successfully for the detection of proteins, DNA, pathogens, chemicals, *etc.* using different sensor mechanisms, *e.g.* electrochemical, fluorescence, SPR, SERS, gravimetric, *etc.* A nanomolar to attomolar detection limit has been achieved for a variety of analyte(s) (Table 4).

4.1 Electrochemical

Ideally, a conductive surface is needed for electrochemical detection. Although dendrimers are not known to be good conductors, metallic compounds or colloids can easily be coupled to their abundant functional groups to improve their conductivity. Partial ferrocenyl tethered dendrimers (Fc-D) and dendrimer-AuNPs nanocomposites are examples of conductive dendrimers that have been frequently used in electrochemical sensing (Fig. 6). Sensing in such a type of matrix is strongly dependent on the type of supramolecular interaction localized near the redox center, *i.e.* ferrocenyl or AuNP.[103]

High sensitivity and specificity have been achieved by exploiting the properties of conducting dendrimers in electrochemical sensors. A partial-ferrocenyl tethered PAMAM dendrimer based electrochemical affinity biosensor has been reported to detect 4.5 pM of avidin with a linear response of up to 10 nM.[64] A DNA biosensor based on electrocatalysis measurements has been fabricated using Fc-D support.[70b] The functionalized dendrimer layer acted as the building block to immobilize capture probes as well as acting as an electrocatalyst to enhance the electrochemical signals due to DNA hybridization.

Exploiting the conductive properties of metallodendrimers, a hydrogen peroxide (H_2O_2) electrochemical sensor has been developed using a HRP/nano-Au/PAMAM/cystamine modified gold electrode. This sensor was sensitive to H_2O_2 concentrations between 10 μM and 2.5 mM with a detection limit of 2.0 μM.[6] The apparent Michaelis–Menten constant (K_m^{app}) of the biosensor was evaluated to be 0.52 mM. In another approach, DNA analysis was carried out using probe DNA immobilized on a PAMAM coated gold electrode with the help of daunomycin as an electroactive hybridization indicator.[108] For a complementary sequence, the average currents of daunomycin were found to be linear for a concentration range of 11 pM to 110 pM.

Shim and co-workers[110,128] developed sandwich and competitive type electrocatalytic bioassays for protein and DNA detection based on H_2O_2 reduction catalysed by a hydrazine label available upon target analyte detection (Fig. 7). The biosensor matrix was fabricated on a conducting polymer layer, poly-5, 2′:5′,2″-terthiophene-3′-carboxylic acid (pTTCA). A monolayer of a 3G PAMAM dendrimer was covalently linked to the polymer followed by the chemisorption of AuNPs/CdS NPs. Bioreceptor molecules were immobilized on AuNPs. This method allowed for the detection of DNA and protein down to 450 aM and 4 fg mL^{-1}, respectively. Over 70-fold sensitivity enhancement was observed using a pTTCA/DEN/AuNP assembly instead of a plain pTTCA layer. The enhancement of the signal was due to the high AuNP loading provided by the pTTCA/DEN layer and thus the larger number of protein/hydrazine/avidin complexes associated with AuNPs. Recently this sensor has been employed to detect biomarkers and antibacterial agents using competitive immuno-interactions. This immunosensor exhibited a detection limit of 51 pg mL^{-1} for Annexin II[113] and 45 pg mL^{-1} for chloramphenicol.[118] In a similar fashion, glassy carbon electrodes modified with composites of –OH terminated PAMAM dendrimers containing rhodium nanoparticles (RhNPs) have been developed for the amperometric detection of dopamine in urine.[109] The Rh nanoparticles served as electrocatalysts for the electro-oxidation of dopamine. The authors claim that this electroanalytical approach suffers minimally from the matrix effects caused by the adsorption of interferents on the electrode surface when the direct analysis of dopamine from urine samples is taking place.

Glucose sensing is one of the important clinical analyses in the healthcare sector. Many products for glucose sensing already exists in the market and new designs for robust and reliable sensors are in progress. In addition, many researchers choose to evaluate their new sensor designs using the simple and standard glucose sensing assay. Svobodova *et al.*[97] developed an amperometric electrochemical biosensor for glucose detection using a GOx enzyme coated dendrimeric matrix. The authors reported a 25 μM LOD for glucose and the apparent Michaelis–Menten constant was found to be relatively low in comparison to GOx in solution. A novel amperometric glucose biosensor based on the nanocomposites of PANI coated multi-wall carbon nanotubes (MWNT) and dendrimer-encapsulated Pt nanoparticles (Pt-DENs) has been prepared.[116] Pt-DENs were absorbed on *in situ* synthesized PANI/CNT composite surface by a self-assembly method followed by the crosslinking of GOx on it. The sensor response was found to be excellent, with a low detection limit (0.5 μM), a wide linear range (1 μM–12 mM), a short response time (about 5 s) and high sensitivity (42.0 μA $mM^{-1}cm^{-2}$) and stability (83% of GOx remains functional after 3 weeks). In a recent study, a glassy carbon electrode coated with a nanohybrid sensing material has been reported for glucose detection. This nanohybrid material consisted of clay clusters incorporated

Table 4 Ligand–receptor interactions studied on dendrimeric bioplatforms using variety of measurement techniques

Dendrimer	Ligand	Analyte	Limit of detection	Sensor type	References
Electrochemical biosensor applications					
4G PAMAM	biotin	avidin	4.5 pM	voltammetry	Yoon *et al.*[64]
4G PAMAM	GOx	glucose	1 μM	voltammetry	Yoon *et al.*[104]
1G PAMAM	GOx	glucose	25 μM	amperometry	Svobodova *et al.*[97]
4G PAMAM	pDNA	cDNA	0.1 nM	electrocatalysis	Kim *et al.*[70b]
4G PAMAM	acetylcholinesterase	DDVP	1.3×10^{-3} ppb		
		carbofuran	0.01 ppb	amperometery	Snejdarkova *et al.*[105]
		eserine	0.03 ppb		
4G PAMAM	acetylcholinesterase	trichlorfon	100 nM		
		carbofuran	6 nM	potentiometry	Snejdarkova *et al.*[46b]
		eserine	700 nM		
4G PAMAM	HRP	H_2O_2	2.0 μM	amperometry	Liu *et al.*,[6]
4G PAMAM	pDNP	anti-DNP	2×10^{-5} gL^{-1}	electrochemical	Won *et al.*[106]
3G PPI	GOx	Glucose	1.2 μM	amperometery	Armada *et al.*[107]
4G PAMAM	biotin	anti-biotin	0.1 μg mL^{-1}	voltammetry	Kwon *et al.*[70c]
2G PPI	GOx	glucose	25 μM	amperometery	Losada *et al.*[102]
4G PAMAM	pDNA	cDNA	8.0 pM	differential pulse voltammetry	Zhu *et al.*[108]
4G PAMAM-OH	Rh nanoparticles	dopamine	0.15 μM	amperometry	Bustos *et al.*[109]
4G PAMAM	tyrosinase	penicillamine	54 nM	square-wave voltammetry	Li and Kwak[73a]
3G PAMAM	pDNA	cDNA	450 aM	amperometry	Shiddiky *et al.*[110]
	Anti-IgG	IgG	4 fg mL^{-1}		
3G PAMAM	laccase	catechin	0.05 μM	chronoamperometry	Rahman *et al.*[111]
4G PAMAM	GOx	glucose	0.15 mM	enzyme-linked field-effect transistors	Yao *et al.*[112]
3G PAMAM	anti-annexin II)	annexin II	0.051 ng mL^{-1}	amperometry	Kim *et al.*[113]
4G PAMAM	GOx	glucose	1 μM	amperometery	Sun *et al.*[114]
	human IL-8 MAb	IL-8 protein	200 fg mL^{-1}		
DNA Dendrimer	human IL-1β MAb	IL-1β protein	100 fg mL^{-1}	amperometery	Wei *et al.*[115]
	pDNA	IL-8 RNA	10 aM		
4G PAMAM	GOx	glucose	0.5 μM	amperometery	Xu *et al.*[116]
4G PAMAM	pDNA	cDNA (avian influenza virus)	1 pg mL^{-1}	differential pulse voltammetry	Zhu *et al.*[117]
4G PAMAM	anti-chloramphenicol acetyltransferase antibody	chloramphenicol	45 pg mL^{-1}	amperometery	Kim *et al.*[118]
4G PAMAM	acetylcholinesterase	carbofuran	4.0 nM	amperometery	Qu *et al.*[119]
Fluorescence based biosensor applications					
4G PAMAM	pDNA	cDNA	1 nM	fluorescence microscopy	Benters *et al.*[54]
Dendrimer slide[a]	anti-HAS	HAS	63 aM/spot		Angenendt *et al.*[120]
4G Phsophorous	pDNA	cDNA	0.1 pM		Le Berre *et al.*[85]
4G Phsophorous	pDNA	cDNA	1 pM		Trévisiol *et al.*[86]
4.5G PAMAM	mouse monoclonal anti-GFP antibody	GFP	1 pM		Ajikumar *et al.*[5]
4.5G PAMAM	anti-rabbit IgG	rabbit IgG	1 pM		Ng *et al.*[92]
Porphyrin	GOx	glucose	4 mM		Lee *et al.*[45e]
4G Phsophorous	pDNA	cDNA	30 pM		Yu *et al.*[121]
SPR based biosensor applications					
4G PAMAM	pDNA	cDNA	3.9 nM	SPR	Mark *et al.*[60]
4G PAMAM	anti-BSA dinitro phenylated-keyhole	BSA	0.1 ng mL^{-1}		Seok *et al.*[69]
4G PAMAM	limpet hemocyanin (DNP-KLH) protein	TNT	110 ppt		Singh *et al.*[61]
Impedimetric biosensor applications					
4G PAMAM	Cl-catechol 1,2-dioxygenase	catechol	10^{-10} M	impedance spectroscopy	Zucolotto *et al.*[122]
4G PAMAM	pDNA	cDNA	3.8 pM	impedance spectroscopy	Li *et al.*[76]
4G PAMAM	alcohol dehydrogenase	ethanol	1 ppm	impedance spectroscopy	Perinotto *et al.*[123]
4G PAMAM	aptamer	thrombin	0.01 nM	electrochemical impedance spectroscopy	Zhang *et al.*[124]
4G PAMAM	pDNA	cDNA	14 pM	impedance spectroscopy	Li *et al.*[73b]
salicylaldiimine	pDNA	cDNA	0.34 pM	impedance spectroscopy	Martinovic *et al.*[125]
2G PAMAM	pDNA	cDNA	0.1 pM	electrochemical impedance spectroscopy	Zhu *et al.*[126]

Table 4 (*Contd.*)

Dendrimer	Ligand	Analyte	Limit of detection	Sensor type	References
Other biosensor applications					
4G PAMAM	biotin	avidin	0.04 nM	quantitative FT-IRRAS spectroscopy	Liu and Amiridis[66]
5G PPI	Ag Nanowires	n-pentyl-5-salicylimidoperylene	1 aM	SERS	Aroca *et al.*[51]
4G PAMAM	HIgG	anti IgG	7 nM	QCM	Svobodova *et al.*[127]

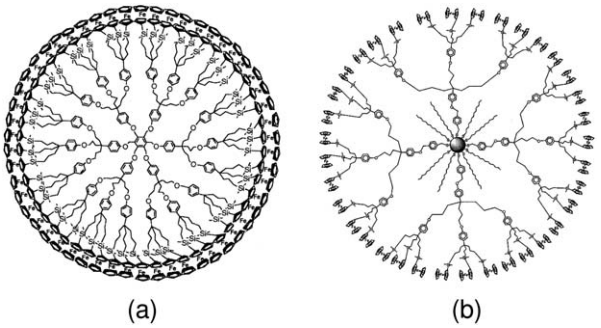


Fig. 6 Structure of (a) ferrocenyl dendrimer (reproduced with permission from Astruc and Ruiz[103b]) (b) dendronized gold colloid containing the ferrocenyl thiol dendron (reproduced with permission from Daniel *et al.*[103c])

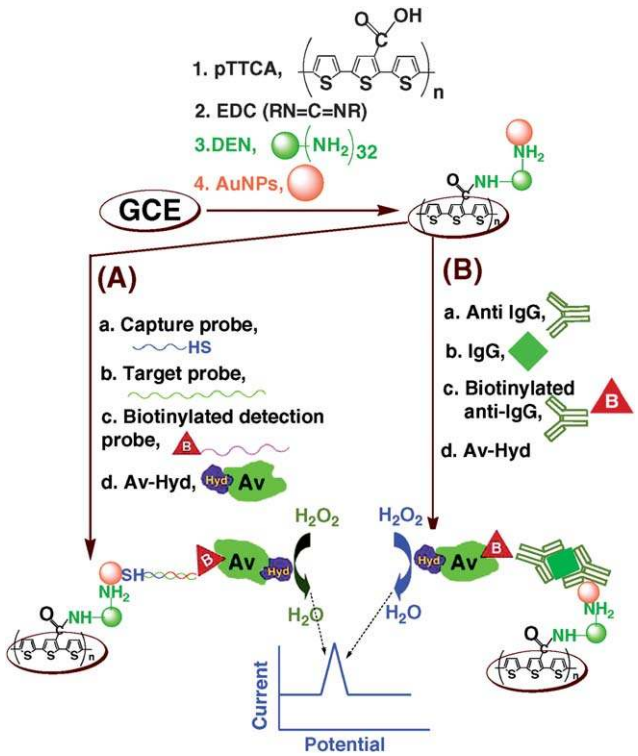


Fig. 7 Schematic presentation of the pTTCA/DEN/AuNP/biomolecules-linked avidin-hydrazine assembly for (A) DNA and (B) protein sensors, which are based on the electrocatalytic activity of hydrazine. Reproduced with permission from Shiddiky *et al.*[110]

with composites of dendrimer-platinum nanoparticles in solution phase and GOx immobilized in the hybrid. This material was used to modify the electrode and obtain the sensor in a single step. The hybrid sensor matrix showed a response time of <3 s and a dynamic range of 0.01 to 16 mM.[129]

By adopting a LBL approach, bilayers of GOx/dendrimer were formed to increase the GOx loading density and therefore significantly amplify the signal intensity. The addition of each bilayer almost doubled the sensitivity, from 3.2 to 14.7 μA mM^{-1} glucose cm^{-2} for one to three bilayers respectively.[75] Glucose sensing based on enhanced photochemical reactions induced by quantum dot-PAMAM nanocomposites has also been explored.[114] The CdS-PAMAM nanocomposites and GOx were immobilized on a Pt electrode using LBL technique to fabricate a multilayered bio–inorganic hybrid system. This sensor showed a sensitivity of 1.83μA mM^{-1} cm^{-2} and a 1 μM lower detection limit for glucose with acceptable reproducibility and stability.

Recently, a field-effect-based sensor was developed using a light-addressable potentiometric sensor (LAPS) platform modified with LBL films of single-walled carbon nanotubes (SWNT) and PAMAM dendrimers.[130] The biosensing ability of the devices was tested for penicillin-G *via* adsorptive immobilization of the enzyme penicillinase atop the LBL film. LAPS architectures modified with the LBL film exhibited higher sensitivity, *ca.* 100 mV $decade^{-1}$, in comparison to *ca.* 79 mV $decade^{-1}$ for an unmodified LAPS.

Dendrimer based electrochemical DNA biosensors show pronounced discrimination efficiency between different types of target DNA (fully complementary, three-base mismatched sequences and non-complementary).[70b] Zhu *et al.*[108] designed probe DNA/PAMAM/Au based matrix and reported a five-fold increase in a differential pulse voltammogram (DPV) signal for complementary DNA over that of noncomplementary DNA. Recently, a dendrimer-modified nanopipette has been developed to detect the hybridization of a specific DNA sequence.[131] The extent of the rectification of the ionic current observed in the measured current–voltage response indicates DNA hybridization. The response for the single-base mismatches was 33% of the perfectly matched sequences.

Recently, an amperometric alcohol biosensor has been developed by immobilizing alcohol oxidase (AOx) through PAMAM dendrimers on a cysteamine-modified gold electrode surface.[132] Ethanol determination was based on the consumption of the dissolved oxygen content due to the enzymatic reaction. The decrease in the oxygen level was monitored at −0.7 V *vs.* Ag/AgCl and correlated with the ethanol concentration. The

optimized ethanol biosensor showed a wide linearity from 0.025 to 1.0 mM with a 100 s response time and a detection limit of 0.016 mM. Stability studies showed a good preservation of the bioanalytical properties of the sensor, 67% of its initial activity was retained after 1 month storage at 4 °C.

4.2 Fluorescence

Fluorescence detection on dendrimer surfaces has resulted in improved detection limits, down to 1 pM of target analytes. Superior fluorescence assays are obtained due to high loading efficiency, greater accessibility and the uniform distribution of the bioreceptor molecules on dendrimeric matrices. In addition, dendrimeric layers minimize fluorescence quenching by providing sufficient spacing between the sensor substrate and the fluorophores. Due to these merits, dendrimers have been utilized in surface functionalization of micro and nanostructures meant for protein or DNA/RNA microarrays. Dendrimers were immobilized on sensor substrates prepared by electron beam lithography[133] or into well-defined patterns by microcontact patterning using a "dendri-stamp" through "click" chemistry (Fig. 8).[53,134] These microarrays, meant for the fluorescence based detection of protein and DNA, have shown highly uniform and regular spot morphology upon receptor-ligand interactions, indicating the homogeneous density and intact functionality of the probes.

DNA hybridization has been detected at picomolar analyte concentrations using a Cy5-labelled fluorescence assay on phosphorous dendrimer derived DNA microarrays.[86] A five-fold improvement in the detection limit was observed for the dendrimer modified surface in comparison to silanized slides.[135] When probe DNA was bound to PAMAM dendrimers through an avidin-biotin bridge, a four-fold improvement in sensitivity and a significant extension of the dynamic range were observed.[67] Recently, a multilayered dendrimer structure has been found beneficial over a single layer. The DNA detection limits for the sensor substrates consisting of 1 or 4 bilayers of polycationic and polyanionic 4G phosphorous dendrimers measured by surface plasmon field-enhanced fluorescence spectroscopy (SPFS) were 50 pM and 30 pM for 1 and 4 bilayers respectively.[121]

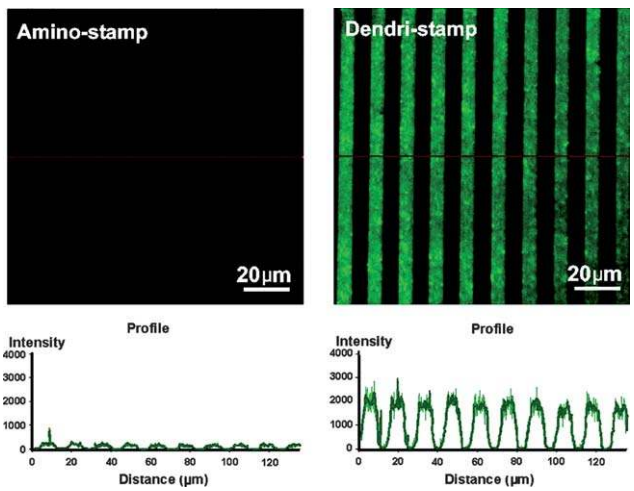


Fig. 8 Patterns of fluorescein-labeled DNA patterns obtained by microcontact printing using an APTES-modified PDMS stamp (left) and transfer printing with a dendri-stamp (right). Reproduced with permission from Rozkiewicz *et al.*[53]

An immunoassay study carried out on a carboxyl terminated PAMAM dendrimer matrix using a DNA directed approach demonstrated the ability to detect 1 pM of Cy5-labeled rabbit IgG antigen.[92] Investigating the properties of seven different bioplatforms in the context of both protein and antibody microarray technologies, Angenendt *et al.*[120] observed relatively low detection limits of about 63 aM spot^{-1} on a dendrimerized matrix, compared to 190 aM spot^{-1} on polystyrene slides for human serum albumin. All other nondendrimeric matrices exhibited detection limits of about 94 aM spot^{-1}.

Discrimination efficiency *i.e.*, the ability of surface immobilized probe DNA to distinguish between matched and mismatched DNA, has also proved to be excellent for dendrimer coated DNA biosensors. Hong *et al.*[83c] demonstrated that DNA microarrays incorporated with cone shaped dendrimers could not only provide each probe DNA with ample space for hybridization with the target DNA, but also show a <1% (0.01) discrimination ratio for single base pair mismatched DNA. These microarrays could detect target DNA in nanomolar concentrations with high discrimination in the broad range of the target DNA concentration.[84] The normalized fluorescence signal ratio, *i.e.* the intensity for single base mismatched pairs to that for the perfectly matched pairs, was found in the range of 0.007 to 0.16. In a similar study, authors found that the dendron-modified surface was capable of reliably detecting heterozygous mutations. Similar to cone shaped dendrimers, DNA microarrays derived on globular shaped phosphorous dendrimers could differentiate single or double base mismatches efficiently.[136]

4.3 Quantum dots

Of late, a growing interest in use of dendrimers coupled with quantum dots has developed. A sensitive fluorescence resonance energy transfer (FRET) based DNA sensor was constructed by forming a stack of suitable quantum dots and a superficial probe DNA layer on the surface of a nanotube with cascade energy band-gap architecture.[137] Different types of QDs were assembled using LBL assembly with dendrimers as the interfacial layers. This allowed the formation of FRET structures in the nanotubes (Fig. 9). These dendrimeric bioplatforms resulted in enhanced fluorescence emission upon binding of the dye labelled target oligonucleotide to probe DNA.

Recently, controlled patterns of quantum dots functionalized with β-cyclodextrin were obtained on admantyl terminated PPI dendrimers coated glass substrate.[138] In this matrix, dendrimers acted as a "supramolecular glue". The interaction of QDs with molecules such as ferrocene functionalized analytes in the unoccupied binding cavities led to the quenching of the fluorescence from the QDs, enabling their use for sensor applications.

4.4 Gravimetric

Gravimetric sensing is a technique by which the interaction of an analyte with recognition moieties immobilized on the surface of a quartz crystal results in the increase of the mass of the crystal and thereby a decrease in its resonant frequency. The presence of

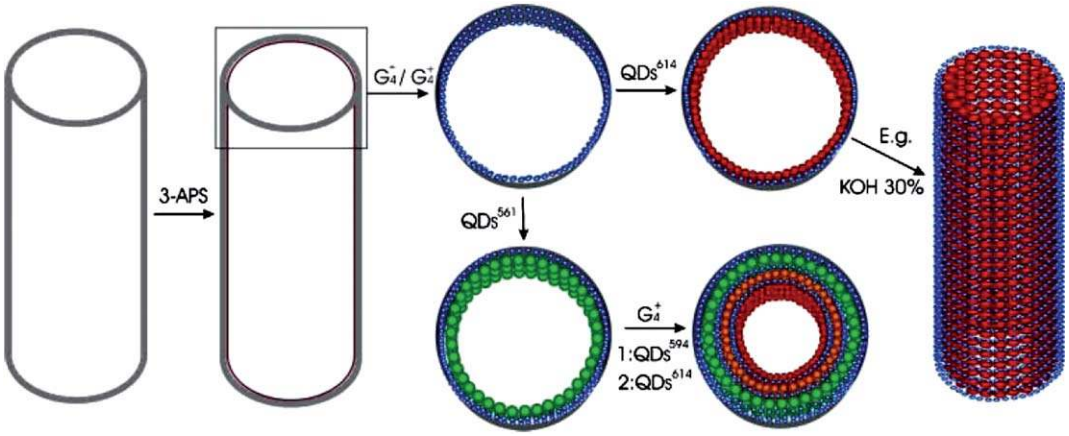


Fig. 9 Schematic diagram of the preparation of QD/dendrimer composite NTs. Reproduced with permission from Feng *et al.*,[137] Copyright Wiley-VCH Verlag GmbH & Co. KGaA.

a large number of analyte molecules on the sensor surface is necessary to achieve high sensitivity.

Polyphenylene dendrimers (PDs) have been employed as sensor layers for monitoring volatile organic compounds in the gaseous phase using a quartz microbalance.[100] The authors observed remarkably high sensitivity (around 5 ppm) for aniline and acetophenone. Recently, a modified phenylene dendrimer coated quartz crystal was used for the detection of triacetone triperoxide (TATP) with good discrimination efficiency.[139] The reversible binding of TATP to dendrimers permits its applicability as an online detection device with a potential to monitor airborne TATP. In another study, a MEMS (microelectromechanical systems) based resonating piezoelectric membrane has been designed for the detection of DNA through a sandwich assay.[140] This biosensor matrix consisted of aminosilane/phosphorous dendrimer-CHO/probe DNA on piezoelectric membranes. Target DNA was detected with the help of biotinylated DNA to which streptavidin coated gold nanoparticles bind and give rise to amplified response. The mass sensitivity of the device was estimated to be 3.6 Hz per pg of DNA, which is several hundred times better than conventional piezoelectric mass-sensing techniques.

4.5 SERS

Dendrimers have been exploited for the deposition of gold and silver colloidal monolayers and multilayers on glass, silicon and indium-tin oxide surfaces to obtain highly active surface enhanced Raman scattering (SERS) substrates. Starburst dendrimers (PAMAM) or DAB-Am dendrimers (PPI) with amino surface groups have been adsorbed directly onto glass substrates to obtain dense functional surfaces. Dendrimers of higher generations result in a greater surface coverage and thereby greater immobilization of nanoparticles. Monolayers of metal nanoparticles may be adsorbed onto dendrimer coated glass substrates *via* electrostatic means. It is possible to control the interparticle spacing and surface coverage, known to greatly influence SERS enhancement, by adjusting the incubation time and dendrimer size. These films have been reported to be stable for several weeks, even after repeated rinsing with water or ethanol.[141] Multilayers of colloids and silver nanowires obtained by the LBL technique using 5G dendrimers were shown to have better enhancement factors (by a factor of 1.33) compared to that of 1G dendrimers. A limit to the enhancement was observed after 4 bilayers.[98] A multilayer composite of Ag nanowires and dendrimers as SERS substrates were shown to detect 1 aM concentration of n-pentyl-5-salicylimidoperylene molecules.[51]

4.6 SPR

Typical SPR sensors rely on the evanescent field created at the interface of the total internal reflection of light. The penetration of the evanescent field is of the order of 100–150 nm.[142] Researchers have used many immobilization techniques like thiol chemistry, CM dextran matrices, *etc.* to confine the biomolecule interaction to this depth in order to exploit the SPR phenomenon for sensing. The thickness of the dendrimeric layer on the sensor matrix is around 2–3 nm, which suits such uses and is likely to have great benefit in SPR sensors. A typical SPR sensor matrix may consist of layers of an alkane thiol SAM on a gold surface, an intermediate dendrimer (*e.g.* PAMAM) layer and a biomolecular recognition layer. A dendrimer as an intermediate layer improves the ligand immobilization efficiency by overcoming mass transport-limiting effects, as in the case of a simple alkane thiol SAM. An ultrathin film SPR matrix made of AUT SAM/PAMAM/pDNA has been shown to detect 3.9 nM of target DNA.[60] In addition, dendrimer-based affinity sensor matrices were found to exhibit greater stability and regeneration of bioreceptor layer. Recently, biofunctionalized PAMAM dendrimers have been utilized for SPR based explosive detection.[61] A competitive inhibition assay for TNT detection using dinitro phenylated-keyhole limpet hemocyanin (DNP-KLH) conjugated dendrimeric chips exhibited a detection limit of 100 ppt as compared to 150 ppt without the dendrimer layer. Bifunctional metallodendrimers (hydroxyl/thiol-functionalized PAMAM-AuNP) have been explored for SPR based insulin detection. Significantly enhanced sensitivity along with reduced NSB and detection limits as low as 0.5 pM were achieved.[96]

4.7 Impedimetry

Electrochemical impedance spectroscopy (EIS) based sensors have also shown an improved response with the use of

dendrimers as intermediate layers. Zucolotto *et al.*[122] used electrical capacitance measurements as the detection method for enzyme-based LBL biosensors. This sensor allowed the detection of 100 pM catechol using Cl-catechol 1,2-dioxygenase/dendrimer bilayers. A similar sensor configuration was used to detect ethanol, down to 1ppm by volume, using multilayers of alcohol dehydrogenase and 4G PAMAM dendrimer.[123] Recently, a label-free impedimetric sensor for thrombin has been developed with the help of aptamer probes immobilized on PAMAM dendrimer coated gold electrodes. Improved aptamer density and an amplified response due to dendrimer involvement resulted in detectable impedance changes for concentrations as low as 10 pM thrombin in the presence of a reversible $[Fe(CN)_6]^{3-/4-}$ redox couple.[124]

A metallodendrimer-based impedimetric DNA biosensor has been constructed by a LBL assembly of cobalt(II) salicylaldiimine metallodendrimer (SDD–Co(II)) and a 21 base oligonucleotide (pDNA) on a gold electrode.[125] The hybridisation of the pDNA with tDNA resulted in an increase in the charge-transfer resistance (R_{ct}) value from 6.52 to 12.85 kΩ. The sensitivity and limit of detection of the sensor were 1.29 kΩ nM^{-1} and 0.34 pM, respectively. In another recent study, an impedimetric DNA biosensor was demonstrated using a composite of CNTs and dendrimers.[126] This composite (2G-PAMAM/MWNT) serves as a support to confine the ssDNA probe, as well as the electronic transducer. The hybridization of tDNA to pDNA gives rise to an additional negative charge in the electrode/electrolyte interface and thereby enhances the interfacial charge-transfer resistance of the electrodes towards the $Fe(CN)_6^{3-/4-}$ redox couple. The sensor response was logarithmically proportional to the tDNA concentration within a range of 0.5 to 500 pM with a detection limit of 0.1 pM.

4.8 Miscellaneous

The advantages of dendrimers have been well utilized for environmental monitoring applications by complexing them with different fluorescent compounds sensitive to bacteria and metal ion pollutants. In an interesting study, *Pseudomonas aeruginosa* has been detected by the dendrimer mediated transport of the nucleic acid stain across the bacterial cell membrane.[101] The sensing film was constructed on disposable plastic coupons or optical fibers with a 4G hydroxy-terminated PAMAM dendrimer and SYTOX Green dye. Due to the presence of the dendrimer, the bacterial cells become permeable to the SYTOX dye and gave rise to enhanced fluorescence. The fluorescence intensity increased with the bacterial concentration and the intensity at 5.4×10^7 cells mL^{-1} was 350% higher than the liquid control without any dendrimer. In a similar approach, FAST DiA, a membrane-receptor fluorophore that exhibits an enhanced quantum yield in lipid environments, was used to capture and detect *E.coli* cells. *E.coli* concentrations of 10^4 cells mL^{-1} a minute and 1.7×10^3 cells mL^{-1} in 2 h were detected by imaging the fluorescence from the FAST DiA-PAMAM dendrimer complex bound to glass slides.[3] In another study, dendrimers tagged with metal ion sensitive fluorescent compounds (*e.g.* 1,8-naphthalimide units) have been demonstrated to detect pollutants such as Ag^+, Cu^{2+}, Co^{2+}, Ni^{2+}, Fe^{3+} and Zn^{2+} with high sensitivity.[143]

Recently, dendrimeric matrices have been investigated for the detection of IL-6 and IL-1β, important biomarkers for the early stage diagnosis of chorioamnionitis, using an enzyme-linked immunosorbent assay (ELISA).[144] A hydroxyl-/thiol-4G PAMAM dendrimer modified plate provided assays with significantly enhanced sensitivity, lower nonspecific adsorption and a detection limit of 0.13 pg mL^{-1} for IL-6 luminol detection and 1.15 pg mL^{-1} for IL-1β TMB detection, which are significantly better than those for the traditional ELISA.

5 Current trends and future scope

The examples given in previous sections illustrate the potential of dendrimers in different types of sensors. Some of the latest research in this field, such as new types of dendrimers and dendrimeric nanocomposites and easier and faster dendrimer immobilization techniques, *etc.* have paved the way for the further exploitation of these molecules for sensing. Researchers, especially from chemistry and polymer sciences domains, are trying to develop new dendrimeric architectures with some beneficial properties suitable for such applications. Recently, a novel antibody-like nanostructure has been developed utilizing two nucleic acid aptamers and a dendrimer which is nearly identical in size to that of a bivalent antibody.[145] This nanostructure has the ability to carry fluorophores at a defined conjugation site. Also, a new dendrimeric nanocomposite film, composed of a PPI dendrimer and nickel tetrasulfonated phthalocyanine (NiTsPc) has been created to serve as chemically sensitive membranes in separative extended-gate field effect transistor (SEGFET) based pH sensors.[146] These PPI/NiTsPc films in SEGFETs represent an alternative in the development of nanostructured, electroactive and stable gate materials to be used in enzymatic biosensors.

Faster immobilization techniques will bypass the current complex immobilization chemistries and will be readily adopted for commercialization. Recently, a simple one step approach has been developed for preparing a redox active dendrimer-modified glassy carbon nanoelectrode by electrodeposition.[147] Advancement in technology and chemosynthesis has also been benefited by the *in situ* synthesis of dendrimer films on solid surfaces with greater control of the dendrimeric architecture. As a result, recently, a PAMAM dendrimeric film has been created on a poly ethylene terephthalate surface by combining plasma technology and chemical reactions.[148] The *in situ* fabrication of dendrimeric sensor matrices has also been demonstrated by electrochemical cross-linking and electronanopatterning using a carbazole peripheral poly(benzyl ether) dendrimer.[149] Similar techniques may be developed and adopted for other dendrimeric architectures to fabricate sensor matrices with good reproducibility.

The unique and exciting properties of dendrimeric nanocomposites have attracted researchers to a wide range of dendrimeric sensor applications. Exploiting these dendrimeric features, some novel applications of dendrimeric sensor matrices have been demonstrated. For example, DNA damage evaluation and the antioxidant capacity measurement of sericin have been performed by a DNA electrochemical biosensor using a dendrimer-encapsulated Au-Pd/chitosan composite.[150] Recently, dendrimeric matrices have been used to develop an online immobilized capillary enzyme microreactor.[151] Advances

in dendrimer synthesis chemistry have also expanded their applicability in nanodevice development by allowing a tighter control on the number and position of different functional groups.[152]

The future of dendrimers as sensor matrices relies on fine tuning three components of dendrimer architecture *i.e.* core, dendrons and surface groups which makes them inimitable macromolecules. It is anticipated that the development of multifunctional dendrimeric architecture may endow in sensors with multiplexing properties.[144] Furthermore, several novel dendrimeric structures such as core-shell "tecto(dendrimers)"[153] and looped dendrimers[154] are being investigated as linkers. There have been only a few reports in which dendrimers of different shapes have been used for sensor applications, but more focused studies are expected in the near future in this area. It is further expected that sensors developed earlier using various immobilization strategies and matrices[8b,155,156] would see dendrimers as possible matrices to enhance their sensitivity and a large number of research products will result from such investigations.

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