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Dendrimers — An Enabling Synthetic Science to Controlled Organic Nanostructures

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20.1 Introduction

20.1.1 Civilizations, Technology Periods, and Historical Revolutions

Throughout the last 120 centuries of human history, a mere handful of civilizations have emerged to dominate the social order and to define the human condition in the world (Figure 20. 1). These unique cultures generally emerged as a result of certain converging societal parameters which included evolving politics, religion, social order, or major military/scientific paradigm shifts. Historical patterns will show, however, that the ultimate magnitude and duration of the civilizations were inextricably connected to their investment in certain key emerging technologies. These broad technologies not only underpinned these cultures but also defined the cutting edge of human knowledge at that time in history. These advancements are referred to as technology ages or periods (Figure 20.1).

The first 118 centuries (10,000 BC–1800 AD) were distinguished by only three major technology periods — the Stone, Bronze, and Iron Ages. Each period was based on materials (building blocks) derived from natural sources and involved empirical knowledge gained through craftsmanship. Developments in these technology periods provided the resources and intellectual forces for dominance by these earlier civilizations. These successful reigns were followed by regression into the dark ages. Emergence of the European influence concurrent with the Renaissance led to an advancement of the Iron Age, to the initiation of the Chemical Age, and ultimately aligned critical forces leading to the great Industrial Revolution. During the past two centuries, there has been a dramatic proliferation of technologies with at least four major technology ages emerging in the last seven decades; namely, nuclear, plastics, materials, and biotechnology ages. These technical advancements have been aligned primarily with Euro-American societies; however, more recently there has been substantial Asian influence. Such technical advancements are very dependent upon certain critical enabling sciences that include *synthesis, engineering,* and, more recently, *combinatorial/simulation* strategies.

Generally speaking, significant new paradigm shifts have initiated each of these technology periods. Typical of each period has been the systematic characterization and exploitation of novel structural and materials properties. Based on property patterns observed within the complexity boundaries of the specific technology, new scientific rules and principles evolve and become defined. Contemporary society has benefited substantially from both the knowledge and the materials created by these technology periods.

The general trend for succession of these technology periods has involved progression from simpler materials to more complex forms. In this fashion, earlier precursor technologies become the platforms upon which subsequent more complex structures (i.e., symmetries) are based. As described by P.W. Anderson,[1] emerging new properties, principles, and rules are exhibited as a function of *symmetry breaking.* This occurs as one advances from more basic structures and progresses to higher orders of complexity. Such a pattern is noted as one follows the hierarchical/development of complexity that has been observed in biological systems over the past several billion years of evolution. Simply stated — "*the whole becomes not only more than, but different from the sum of its parts.*"[1] It is this premise that has driven the human quest for understanding new and higher forms of complexity. It appears that it is from this pattern of inquiry that the industrial revolution was spawned. It may be argued that the convergence of knowledge and new materials created by just five technology areas (Figure 20.1) provided the critical environment and synergy for this historical revolution. Economic benefits and enhancements to the human condition in such areas as transportation, shelter, clothing, energy, and agriculture are now recognized to be immeasurable. It is from this perspective that we now consider the emergence of new technologies such as *genomics, proteomics,* and particularly *nanotechnology* as we examine prospects for the next revolution. The proposed biological revolution, which began with elucidation of the DNA structure by Crick and Watson, is expected to provide viable strategies that sound almost like science fiction at this time. Such predictions include the total elimination of human disease, human life expectancy beyond 100 years, and perhaps extraterrestrial emigration in the next century.

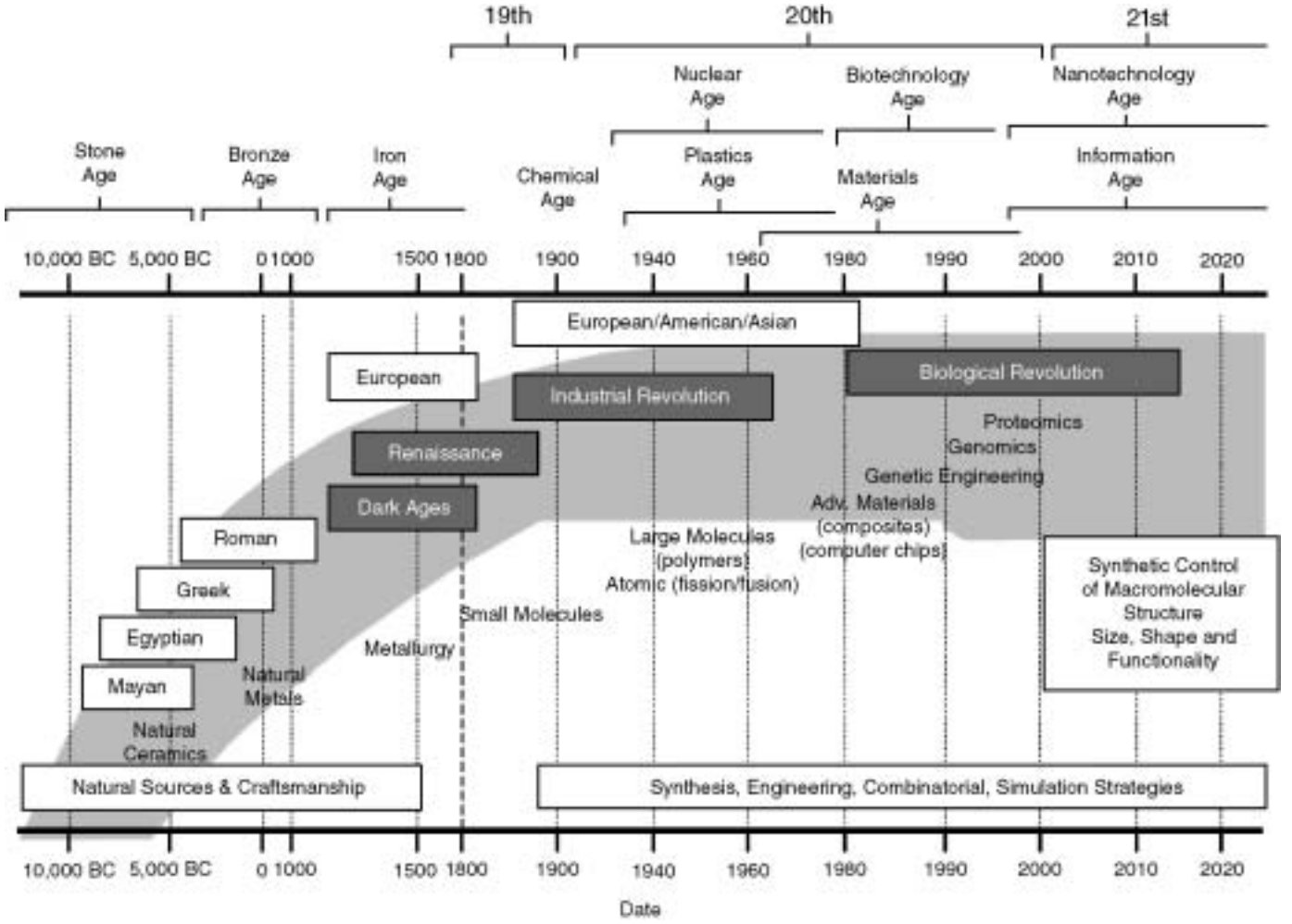


FIGURE 20.1 Civilizations, technology periods (ages), and historical revolutions as a function of time.

20.1.2 Importance of Controlled Organic Nanostructures to Biology

Critical to the successful creation of all biological structures required for life has been the evolutionary development of strategies to produce controlled organic nanostructures. As one reflects on evolutionary progress that defines the dimensional hierarchy of biological matter, it is apparent that it occurred in two significant phases and involved bottom-up synthesis. Clearly, critical parameters such as mass and dimension had to increase in size to define the appropriate building modules. The first phase was abiotic and involved molecular evolution from atoms to small molecules. This began approximately 13 billion years ago and progressed for nearly 8 to 9 billion years. The complexity reached at that point was necessary to set the stage with appropriate building blocks for the subsequent evolution of life, namely macromolecules such as DNA, RNA, and proteins. This latter phase is believed to have begun 3.5 billion years ago. These modules were generally collections of precisely bonded atoms that occupied space with dimensions ranging from 1 to 10^2 nm. Such structures required the controlled assembly of as many as 10^3 to 10^9 atoms and possessed molecular weights ranging from 10^4 to 10^{10} Daltons. These assemblies required the rigorous control of *size, shape, surface chemistries, scaffolding*, and *container properties* as described in Figure 20.2.

20.1.3 The Wet and Dry World of Nanotechnology

According to Nobel laureate R. Smalley,[2] the world of nanotechnology can be subdivided into two major application areas, the *wet* and *dry* sides. The former, of course, includes the biological domain, wherein the water-based science of living entities is dependent upon *hydrophilic* nanostructures and devices that may function within biological cells. Dendritic nanopolymers, especially *dendrimers,* fulfill many applications in the wet world of nanotechnology (Figure 20.3). In contrast, the dry side is expected to include those applications focused on more *hydrophobic* architectures and strategies. Progress in this second area may be expected to enhance the tensile strength of materials, increase the conductivity of electrons, or allow the size reduction of computer chips to levels unattainable with traditional bulk materials.

Although substantial progress has been made concerning the use of fullerenes and carbon nanotubes for dry nanotech applications, their use in biological applications has been hindered by the fact that they are highly hydrophobic and available in only several specific sizes (i.e., usually approximately 1 nm). However, recent advances involving the functionalization of fullerenes may offer future promise for these materials in certain biological applications.[3]

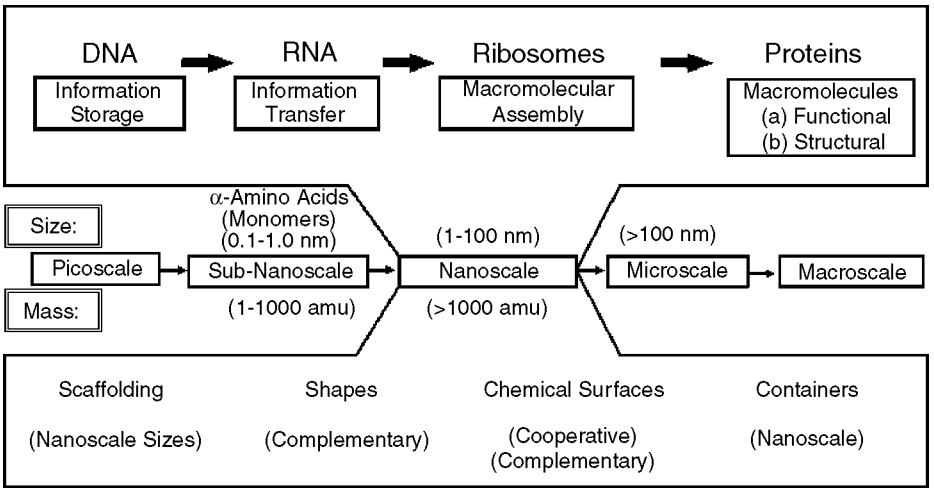


FIGURE 20.2 Biological structure–control strategy leading to nanoscale scaffolding, shapes, chemical surfaces, and containers found in proteins.

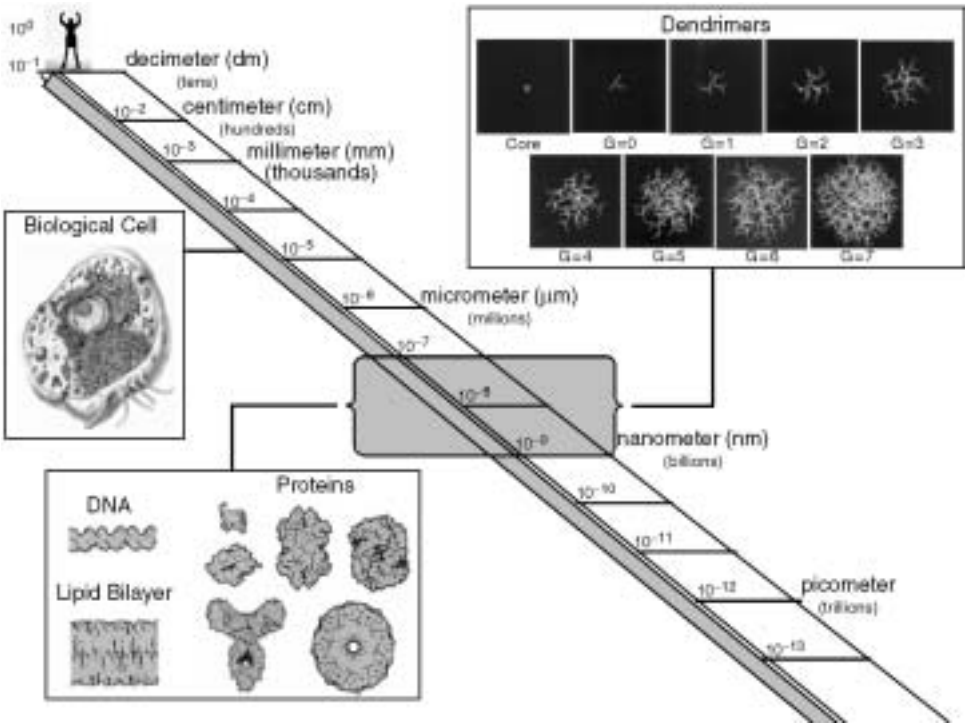


FIGURE 20.3 Comparison of micron-scale biological cells to nanoscale proteins and poly(amidoamine) dendrimers.

20.1.4 Potential Bottom-Up Synthesis Strategies for Organic Nanostructures

At least three major strategies are presently available for covalent synthesis of organic nanostructures: (1) *traditional organic chemistry*, (2) *traditional polymer chemistry*, and, more recently, (3) *dendritic macromolecular chemistry* (Figure 20.4).

Broadly speaking, traditional organic chemistry leads to higher complexity by involving the formation of relatively few covalent bonds between small heterogeneous aggregates of atoms or reagents to give

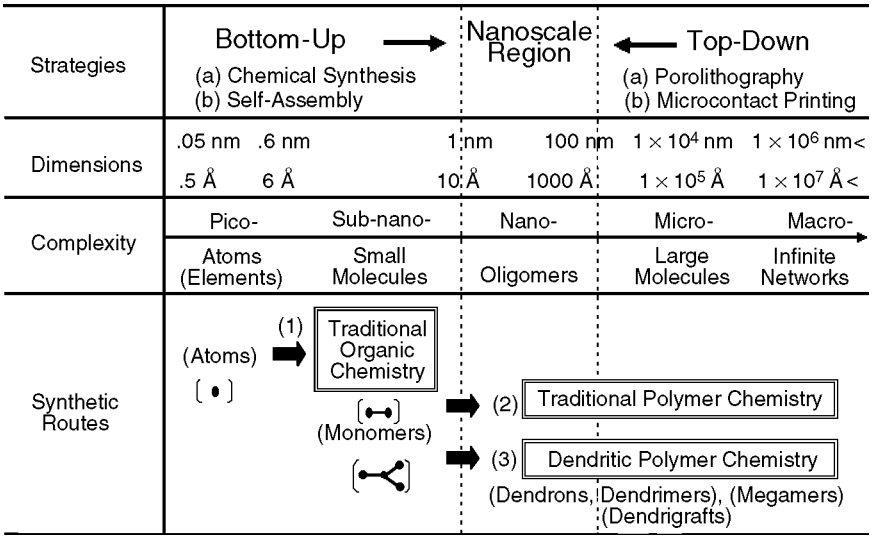


FIGURE 20.4 Molecular complexity as a function of covalent synthesis strategies and molecular dimensions.

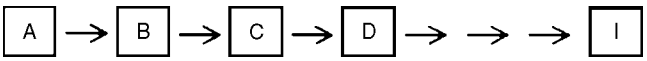
well-defined small molecules. On the other hand, polymerization strategies such as (2) and (3) involve the formation of large multiples of covalent bonds between homogenous monomers to produce large molecules or infinite networks with a broad range of structure control.[4,5]

By comparing these three covalent synthesis strategies, we wish to introduce the third strategy and briefly overview the potential offered by *dendritic macromolecules* for the routine synthesis of controlled organic nanostructures.

20.1.5 Traditional Organic Chemistry

Organic chemistry, originating with Wöhler in 1828, has led to the synthesis of literally millions of small molecules. Organic synthesis involves the use of various hybridization states of carbon combined with specific heteroatoms to produce key hydrocarbon building blocks (modules) or functional groups (connectors). These two construction parameters have been used to assemble literally millions of more complex structures by either *divergent* or *convergent* strategies involving a limited number of step-wise, covalent bond-forming events. Usually product isolation is involved at each stage. Relatively small molecules (i.e., < 1 nm) are produced, allowing the precise control of shape, mass, flexibility, and functional group placement. The divergent and convergent strategies are recognized as the essence of traditional organic synthesis.[6] An example of the divergent strategy may be found in the Merrifield synthesis,[7–10] which involves chronological introduction of precise amino acid sequences to produce structure-controlled, linear architectures.

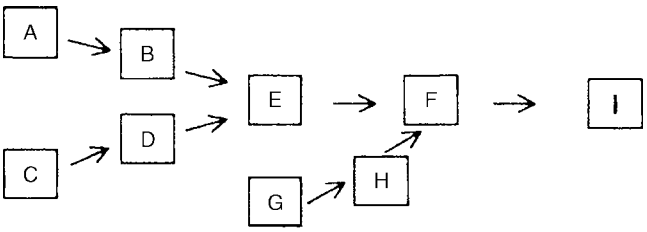
20.1.5.1 Divergent Multistep Synthesis



STRUCTURE (20.1)

Many examples of the convergent strategy can be found in contemporary approaches to natural products synthesis. Usually the routes to target molecules (i.e., I) are derived by retro-synthesis from the final product.[6] This involves transformation of the target molecule to lower molecular weight precursors (i.e., A–F).

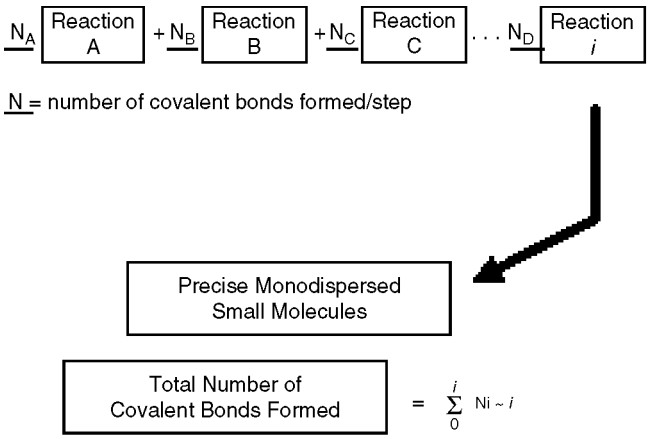
20.1.5.2 Convergent Multistep Synthesis



STRUCTURE (20.2)

Mathematically, at least one covalent bond, or in some cases several bonds, may be formed per reaction step (N_i). Assuming high-yield reaction steps and appropriate isolation stages, one can expect to obtain precise monodispersed products. In either case, the total number of covalent bonds formed can be expressed as shown in Scheme 20.1.

Based on the various hybridization states of carbon (Figure 20.5), at least four major carboskeletal architectures are known.[6,11] They are recognized as (I) *linear*, (II) *bridged* (two-dimensional/three-dimensional), (III) *branched,* and (IV) *dendritic*. It should be noted that buckminsterfullerenes, a subset of Class (II), bridged (three-dimensional) structures, and cascade molecules, a low molecular weight subset of Class (IV) dendritic architectures, are relatively recent examples of organic small molecule topologies. The former,



SCHEME 20.1

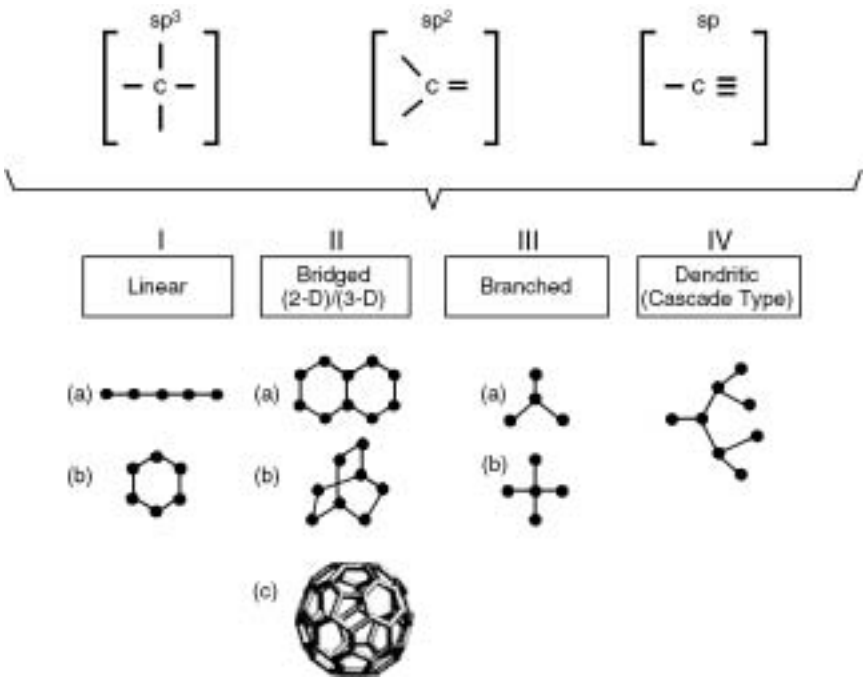


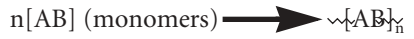
FIGURE 20.5 Four major small molecular architectures derived from the hybridization states of carbon.

recognized as *bucky balls* and *carbon nanotubes*, have enjoyed considerable attention as precise, hydrophobic nanomodules suitable for applications in the dry nanotechnology world. The low molecular weight dendritic architectures have been used as precursors to true dendrimer nanostructures as will be described later.

20.1.6 Traditional Polymer Chemistry

Over the past 70 years, a second covalent synthesis strategy has evolved based on the catenation of reactive small molecular modules or monomers. Broadly speaking, these propagations involve the use of reactive (AB-type monomers) that may be engaged to produce a variety of large, nanoscale molecules with polydispersed masses. Such multiple-bond formation may be driven by a variety of mechanisms including (1) *chain growth*, (2) *ring opening*, (3) *step-growth condensation,* or (4) *enzyme catalyzed*

processes. Staudinger first introduced this paradigm in the 1920s[12–14] by demonstrating that reactive monomers could be used to produce a statistical distribution of one-dimensional (linear) molecules with very high molecular weights (i.e., > 10^6 Daltons). As many as 10,000 or more covalent bonds may be formed in a single chain reaction of monomers. Although macro/megamolecules with nanoscale dimensions may be attained, structure control of critical macromolecular design parameters (CMDPs) such as *size, molecular shape*, *positioning of atoms*, or *covalent connectivity* — other than those affording linear or crosslinked topologies — is difficult. However, recent progress has been made using *living polymerization* techniques that afford better control over molecular weight and some structural elements as described elsewhere.[15]

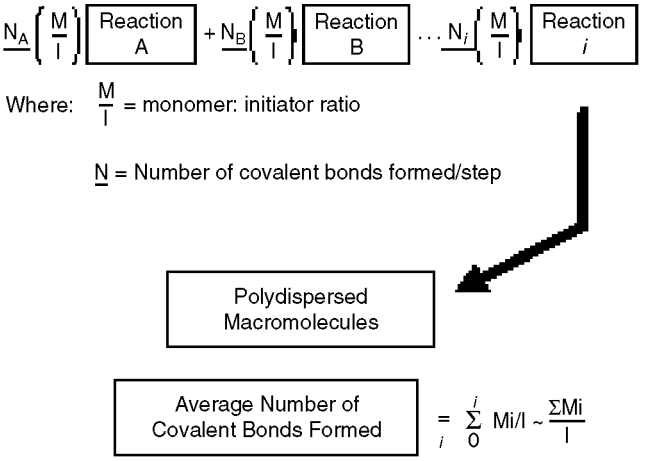


Traditional polymerizations usually involve AB-type monomers based on substituted ethylenes or strained small ring compounds. These monomers may be propagated by using chain reactions initiated by free radical, anionic, or cationic initiators;[16] or, alternatively, AB-type monomers may be used in polycondensation reactions.[5]

Multiple, covalent bonds are formed to produce each macromolecule and, in general, statistical, polydispersed structures are obtained. In the case of controlled vinyl polymerizations, the average length of the macromolecule is determined by monomer-to-initiator ratios. If one views these polymerizations as extraordinarily long sequences of individual reaction steps, the average number of covalent bonds formed may be visualized as shown in Scheme 20.2.

Traditional polymerization strategies generally produce linear architectures; however, branched topologies may be formed either by chain transfer processes or intentionally introduced by grafting techniques. In any case, the linear and branched architectural classes have traditionally defined the broad area of *thermoplastics*. Of equal importance is the major architectural class that is formed by the introduction of covalent bridging bonds between linear or branched polymeric topologies. These cross-linked (bridged) topologies were studied by Flory in the early 1940s and constitute the second major area of traditional polymer chemistry — namely, *thermosets*.

Therefore, approximately 50 years after the introduction of the *macromolecular hypothesis* by Staudinger,[14] the entire field of polymer science could simply be described as consisting of only two major architectural classes: (I) *linear topologies* as found in thermoplastics and (II) *cross-linked architectures* as found in thermosets.[4,16] The major focus of polymer science during the time frame of the 1920s to the 1970s was on the unique architecturally driven properties manifested by either linear or cross-linked topologies. Based on unique properties exhibited by these topologies, many



SCHEME 20.2

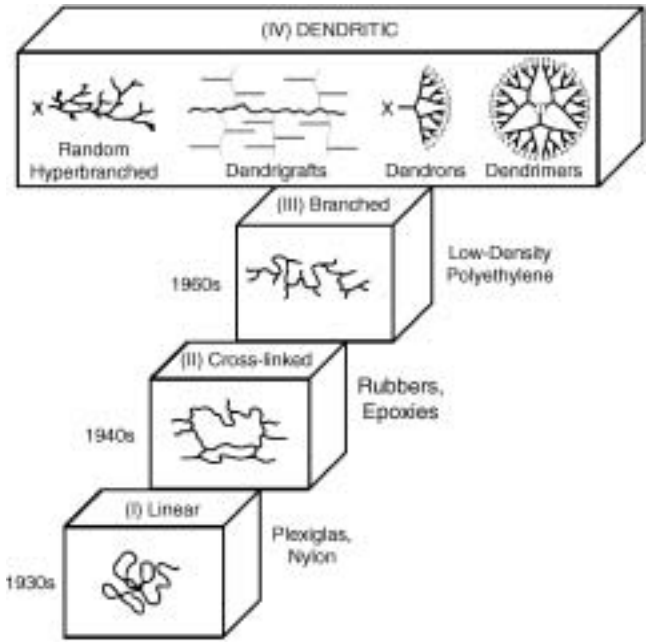


FIGURE 20.6 Three major polymer architectural Classes (I–III) leading to the fourth major class — dendritic (IV) consisting of the subclasses *random hyperbranched, dendrigrafts, dendrons,* and *dendrimers.*

natural polymers critical to success in World War II were replaced with synthetic polymers and were of utmost strategic importance.[4] In the 1960s and 1970s, pioneering investigation into long chain branching (LCB) in polyolefins and other related branching systems began to emerge.[17,18] More recently, intense commercial interest has been focused on new polyolefin architectures based on *random long branched* and *dendritic topologies*.[19] These architectures are produced by *metallocene* and *Brookhart-type* catalysts. In summary, by the end of the 1970s, only three major architectural classes of polymers were recognized and referred to as classical polymers. The fourth major class, dendritic polymers,[166] was only beginning to emerge (Figure 20.6).

20.2 The Dendritic State

Dendritic architecture is one of the most pervasive topologies observed at the micro- and macro-dimensional length scales (i.e., μm to m). At the nanoscale (molecular) level, there are relatively few biological examples of this architecture. Most notable are the glycogen and amylopectin hyperbranched structures that nature uses for energy storage. Presumably, the many chain ends that decorate these macromolecules facilitate enzymatic access to glucose for high-demand bioenergy events.[20] Another nanoscale example of dendritic architecture in biological systems is found in proteoglycans. These macromolecules appear to provide energy-absorbing, cushioning properties and determine the viscoelastic properties of connective tissue (Figure 20.7).

In the past two decades, versatile strategies have been developed that allow the design, synthesis, and functionalization of a wide variety of such dendritic structures.[15]

20.2.1 A Comparison of Organic Chemistry and Polymer Chemistry with Dendritic Macromolecular Chemistry

It is appropriate to compare the well-known concepts of covalent bond formation in traditional organic chemistry with those in classical polymer chemistry and dendritic macromolecular chemistry. This allows

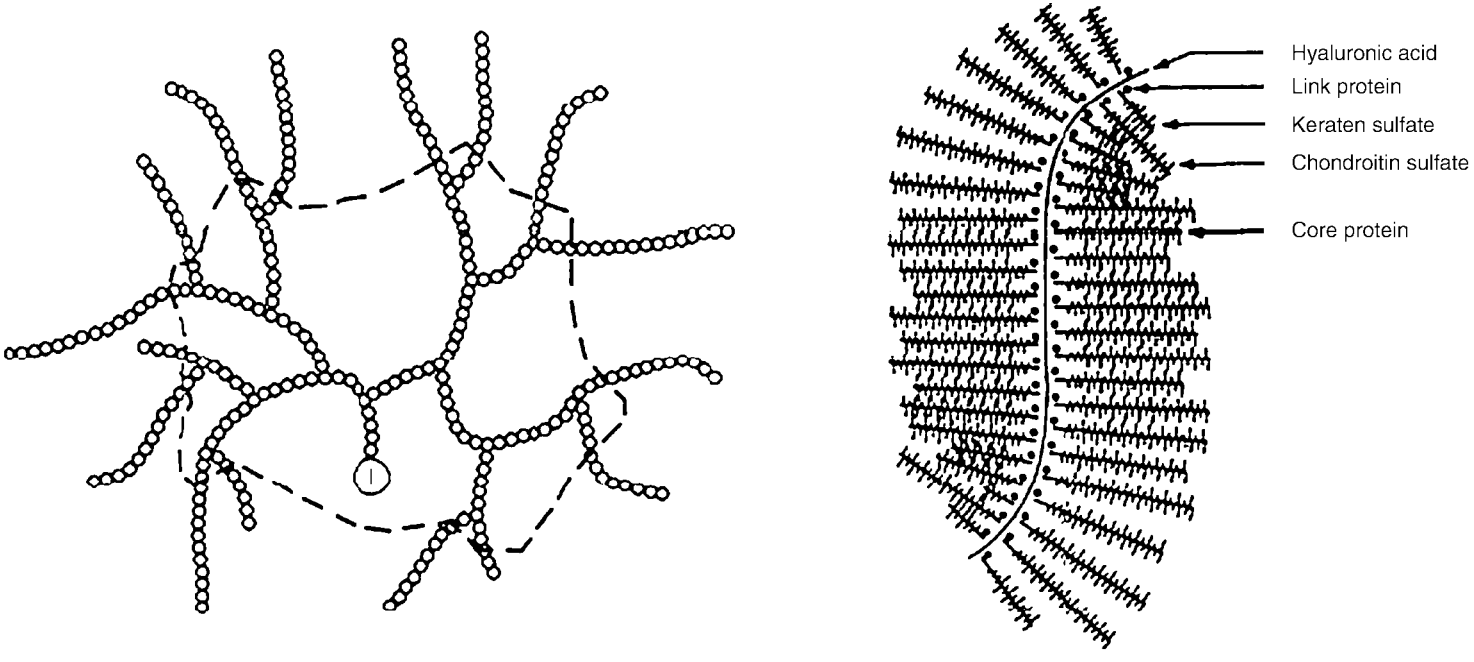


FIGURE 20.7 Topologies for (a) amylopectin and (b) proteoglycans.

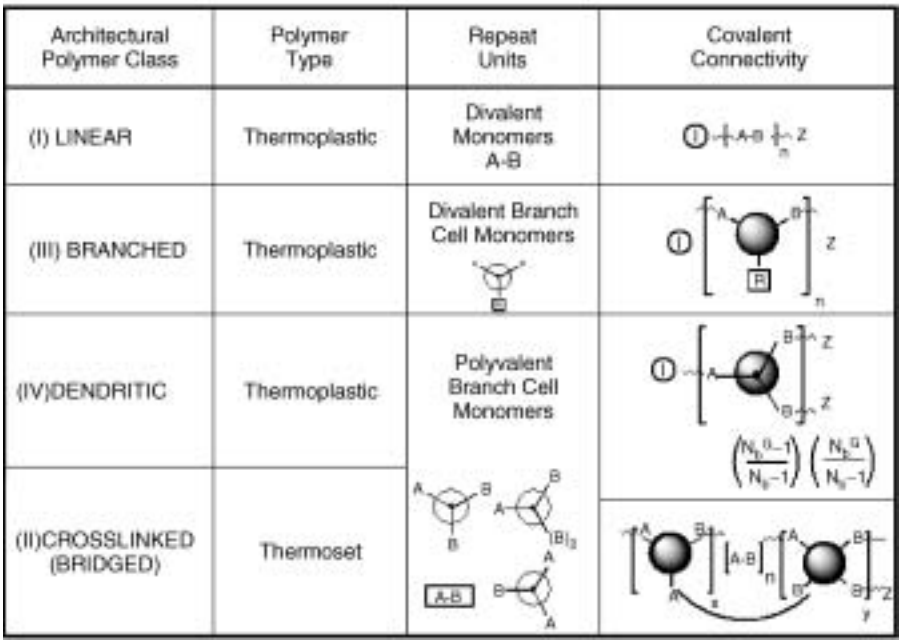


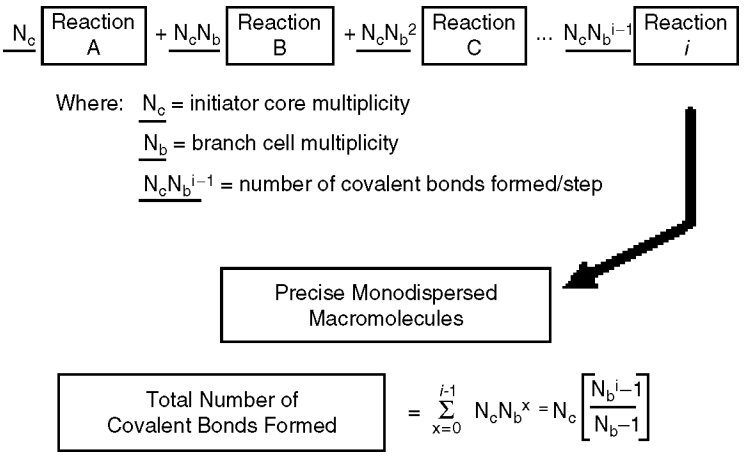
FIGURE 20.8 Examples of architectural polymer classes (I–IV) polymer type, repeat units, and covalent connectivity associated with architectural classes. (From Esfand, R.; Tomalia, D.A. Laboratory synthesis of poly(amidoamine) (PAMAM) dendrimers. In *Dendrimers and Other Dendritic Polymers*; Fréchet, J.M.J. and Tomalia, D.A., Eds.; John Wiley & Sons: West Sussex, 2001. With permission.)

one to fully appreciate the advantages and shortcomings of these three synthetic strategies in the context of bottom-up routes to organic nanostructures. Covalent synthesis in traditional polymer chemistry has evolved around the use of reactive modules (AB-type monomer) or ABR-type branch reagents that may be engaged in multiple covalent bond formation to produce large one-dimensional molecules of various lengths. Such multiple bond formation may be driven either by chain reactions, ring opening reactions, or polycondensation schemes. These propagation schemes lead to products that are recognized as Class I: *linear* or Class III: *branched* architectures. Alternatively, using combinations and permutations of divalent A-B type monomers and/or A-B_n, A_n-B polyvalent, branch cell-type monomers produces Class II, *cross-linked (bridged)* architectures.

A comparison of the covalent connectivity associated with each of these architecture classes (Figure 20.8) reveals that the number of covalent bonds formed per step for linear and branched topology is a multiple (n = degree of polymerization) related to the monomer/initiator ratios. In contrast, ideal *dendritic* (Class IV) propagations involve the formation of an exponential number of covalent bonds per reaction step (all termed G = generation), as well as amplification of both mass (i.e., number of branch cells/G) and terminal groups, (Z) per generation (G).

Mathematically, the number of covalent bonds formed per generation (reaction step) varies in an ideal dendron or dendrimer synthesis, according to a power function of the reaction steps, as illustrated in (Scheme 20.3). It is clear that a dramatic amplification of covalent bond formation occurs in all dendritic synthesis strategies. In addition to new architectural consequences, this feature clearly differentiates dendritic growth processes from covalent bond synthesis found in traditional organic and polymer chemistry.[21]

It should be quite apparent that, although all major architectural polymer classes are derived from common or related repeat units, the covalent connectivity is truly discrete and different. Furthermore, mathematical analyses of the respective propagation strategies clearly illustrate the dramatic differences in structure development as a function of covalent bond formation. It should be noted that linear, branched, and dendritic topologies differ substantially in their covalent connectivity and their terminal



SCHEME 20.3

group-to-initiator site ratios. In spite of these differences, these open, unlooped macromolecular assemblies clearly manifest thermoplastic polymer-type behavior compared with the looped, bridged connectivity associated with cross-linked thermoset systems. In fact, it is now recognized that these three open assembly-topologies (linear, branched, and dendritic) represent a graduated continuum of architectural intermediacy between thermoplastic and thermoset behavior.[22]

In summary, traditional organic chemistry offers exquisite structure control over a wide variety of compositions up to, but not including, *higher nanoscale structural dimensions* (e.g., buckminsterfullerene diameters ≅ 1 nm). Furthermore, such all-carbon nanostructures are limited to only one hydrophobic compositional form and are hampered by the difficulties of functionalizing their surfaces to produce hydrophilic structures.[3]

Classical polymer chemistry offers relatively little structural control, but it facilitates access to statistical distributions of polydispersed nanoscale structures. Living polymerizations provide slightly better, but still imperfect, control over product size and mass distribution or polydispersity. In contrast, as will be seen below, dendritic macromolecular chemistry provides essentially all the features required for unparalleled control over *topology, composition, size, mass, shape,* and *functional group placement.*[15] These are features that truly distinguish many successful nanostructures found in nature.[23]

The quest for nanostructures and devices based on the biomimetic premise of architectural and functional precision is intense and remains an ultimate challenge. One must ask: what new options or unique properties does the dendritic state offer to meet the needs of nanoscale science and technology? The rest of this chapter will attempt to overview key features of the dendritic state that address these and other issues.

20.2.2 Dendritic Polymers — A Fourth Major New Architectural Class

Dendritic topologies are recognized as a fourth major class of macromolecular architecture.[24–27,166] The signature for such a distinction is the unique repertoire of new properties exhibited by this class of polymers. Many new synthetic strategies have been reported for the preparation of these materials, thus providing access to a broad range of dendritic structures. Presently, this architectural class consists of three dendritic subclasses as shown in Figure 20.9: (IVa) *random hyperbranched polymers*, (IVb) *dendrigraft polymers,* and (IVc) *dendrimers*. This subset order (IVa–c) reflects the relative degree of structural control present in each of these dendritic architectures.

All dendritic polymers are open, covalent nanoassemblies of branch cells. They may be organized as very symmetrical, monodispersed arrays, as is the case for dendrimers, or as irregular, polydispersed assemblies that typically define random hyperbranched polymers. As such, the respective subclasses and the level of structure control are defined by the propagation methodology as well as by the branch cell (BC) construction

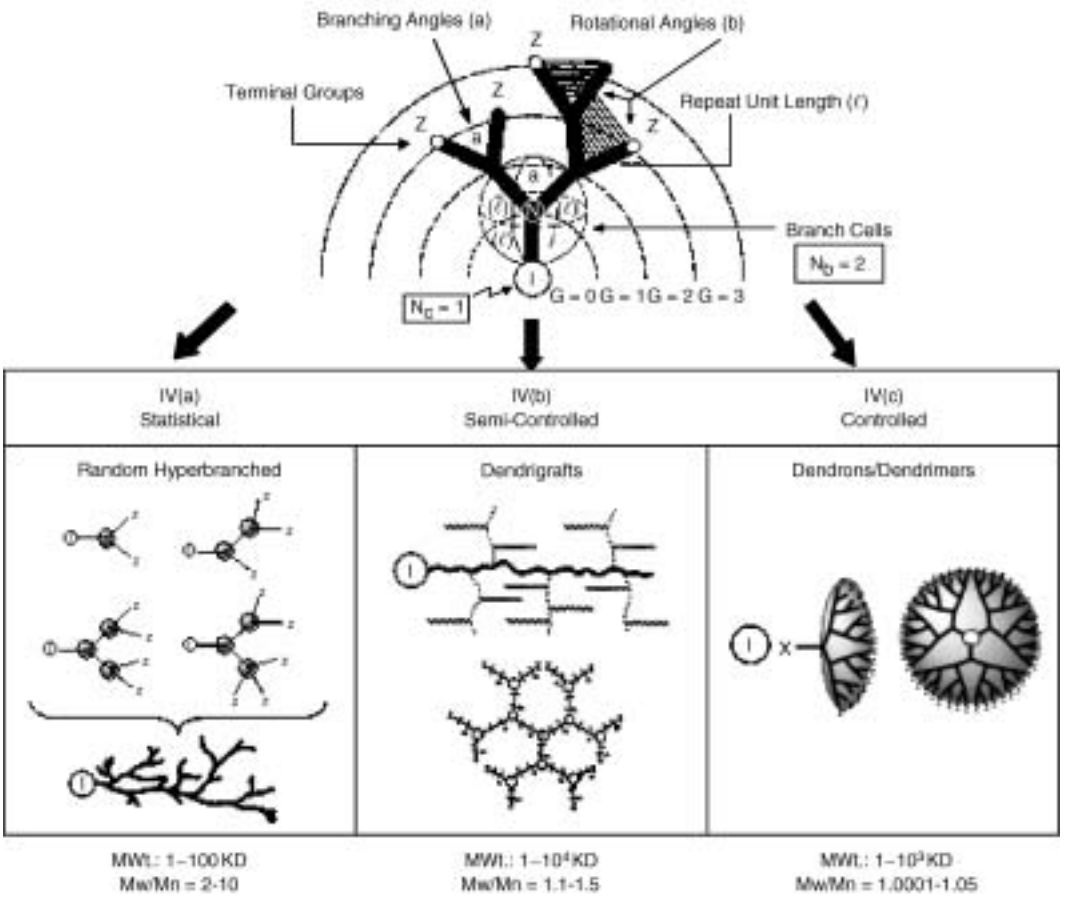


FIGURE 20.9 Branch cell structural parameters: (a) branching angles, (b) rotational angles, (*l*) repeat units lengths, (Z) terminal groups and dendritic subclasses derived from branches, (IVa) random hyperbranched, (IVb) dendrigrafts, and (IVc) dendrons/dendrimers.

parameters used to produce these assemblies. These BC parameters are determined by the composition of the BC monomers as well as the nature of the *excluded volume* defined by the BC. The excluded volume of the BC is determined by the length of the arms, the symmetry, rigidity/flexibility, the branching, and rotation angles involved within each of the branch cell domains.[28] As shown in Figure 20.9, these dendritic arrays of branch cells usually manifest covalent connectivity relative to some molecular reference marker (I) or core. As such, these branch cell arrays may be very non-ideal/polydispersed (e.g., Mw/Mn ≅ 2–10), as observed for random hyperbranched polymers (IVa), or very ideally organized into highly controlled, core–shell-type structures as noted for dendrons/dendrimers (IVc): Mw/Mn ≅ 1.01–1.0001. Dendrigraft (arborescent) polymers reside between these two extremes of structure control, frequently manifesting rather narrow polydispersities of Mw/Mn 1.1–1.5 depending on their mode of preparation.

20.2.3 Dendrons and Dendrimers

Dendrons and dendrimers are the most intensely investigated subset of dendritic polymers. In the past decade over 5000 literature references have appeared on this unique class of structure-controlled polymers. The word *dendrimer* is derived from the Greek words *dendri* (branch, tree-like) and *meros* (part of). The term was coined by Tomalia et al. over 15 years ago in the first full paper on poly(amidoamine)

(PAMAM) dendrimers.[29,30,166] Poly(amidoamine) dendrimers constitute the first dendrimer family to be commercialized and undoubtedly represent the most extensively characterized and best understood series at this time. In view of the vast amount of literature in this field, the remaining overview will focus on PAMAM dendrimers and limit the scope to critical property features and unique biomedical applications offered by these fascinating nanostructures.

20.2.3.1 Synthesis — Divergent and Convergent Methods

In contrast to traditional polymers, dendrimers are unique core–shell structures possessing three basic architectural components: (1) *a core*, (2) *an interior of shells (generation)* consisting of repetitive branch cell units, and (3) *terminal functional groups* (i.e., the outer shell or periphery) as illustrated in Figures 20.10 and 20.11.

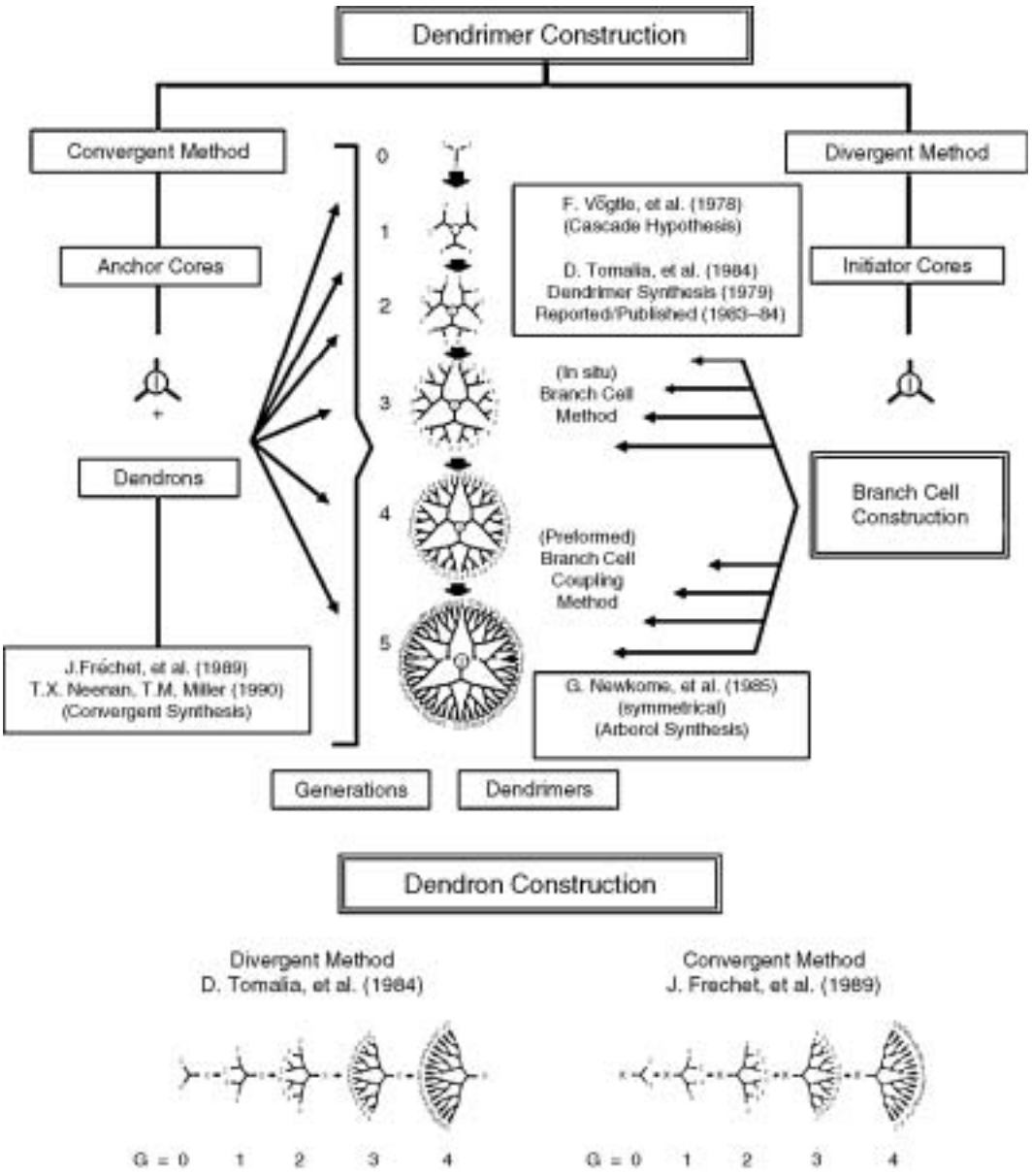


FIGURE 20.10 Overview of synthetic strategies for branch cell construction, dendron construction, and dendrimer construction annotated with discovery scientists.

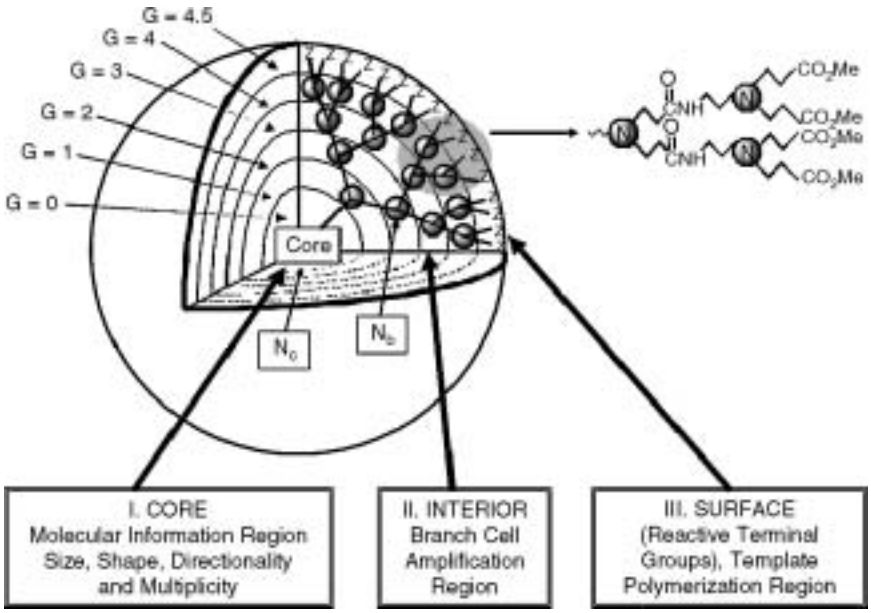


FIGURE 20.11 Three-dimensional projection of dendrimer core-shell architecture for G = 4.5 poly(amidoamine) (PAMAM) dendrimer with principal architectural components (I) core, (II) interior, and (III) surface. (From Esfand, R.; Tomalia, D.A. Laboratory synthesis of poly(amidoamine) (PAMAM) dendrimers. In *Dendrimers and Other Dendritic Polymers*; Fréchet, J.M.J. and Tomalia, D.A., Eds.; John Wiley & Sons: West Sussex, 2001. With permission.)

In general, dendrimer synthesis involves hierarchical assembly strategies that require the construction components shown in Structure 20.3.

Cores : Monomers ➡ Branch Cells ➡ Dendrons ➡ Dendrimers

STRUCTURE (20.3)

Many methods for assembling these components have been reported; however, they can be broadly categorized as either *divergent* or *convergent* strategies. Within each of these major approaches there may be variations in methodology for branch cell construction (i.e., *in situ* vs. *preformed*) or dendron construction (i.e., *divergent* vs. *convergent*), as overviewed in (Figure 20.10).

Historically, early developments in the field were based on divergent methods. Vögtle et al. (University of Bonn) first reported the synthesis of several low molecular weight (<900 Daltons; G = 0–2) cascade structures[31] using the divergent, *in situ* branch cell method. This synthesis was based on a combination of acrylonitrile and reduction chemistry. As Vögtle reported later,[32] higher generation cascade structures and, indeed, dendrimers could not be obtained by this process due to synthetic and analytical difficulties. Nearly simultaneously, a completely characterized series of high molecular weight (i.e., > 58000 Daltons; G = 0–7), poly(amidoamine) (PAMAM) dendrimers was synthesized by Tomalia et al.[29,30,166] Success with his approach was based on a two-step reaction sequence involving acrylate Michael addition and amidation chemistry.[30,33,34] Historically, this methodology provided the first commercial route to dendrimers as well as the first opportunity to observe unique dendrimer property development that occurs *only* at higher generations (i.e., G = 4 or higher). Many of these observations were described in a publication that appeared in 1985[30] and later reviewed extensively in 1990.[35,36]

The first published use of preformed branch cell methodology was reported in a communication by Newkome et al.[37] This approach involved the coupling of preformed branch cell reagents around a core to produce low molecular weight (i.e., > 2000 Daltons, G = 3) *arborol structures*. This approach has been used to synthesize many other dendrimer families including *dendri-poly(ethers)*,[38] *dendri-*

poly(thioethers),[21] and others.[15,39] Each of these methods involved the systematic divergent growth of branch cells around a core that defined shells within the dendrons. The multiplicity and directionality of the initiator sites (N_c) on the core determine the number of dendrons and the ultimate shape of the dendrimer. In essence, dendrimers propagated by this method constitute groups of molecular trees (i.e., two or more dendrons) that are propagated outwardly from their roots (cores). This occurs in stages (generations), wherein the functional leaves of these trees become reactive precursor templates (scaffolding) upon which to assemble the next generation of branches. This methodology can be used to produce multiples of trees — (dendrimers) — or single trees — (dendrons) as shown in Figure 20.10.

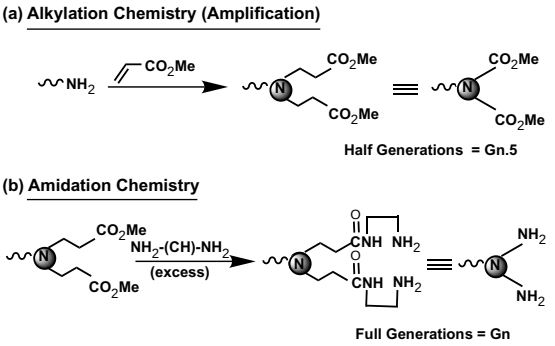
Using a totally novel approach, Hawker and Fréchet[40,41] followed by Neenan and Miller[42] reported the convergent construction of such molecular trees by first starting with the leaves or surface branch cell reagents. By amplifying with these reagents in stages (generations), one produces a dendron possessing a single reactive group at the root or focal point of the structure. Subsequent coupling of these reactive dendrons through their focal points to a common *anchoring core* yields the corresponding dendrimers. Because of the availability of orthogonal functional groups at the focal point and periphery of the dendrons, the convergent synthesis is particularly useful for the preparation of more complex macromolecular architectures[43] such as linear dendritic hybrids, block copolymeric dendrimers, or dendronized polymers. Another significant difference is that the divergent approach requires an exponential increase in the number of coupling steps for generation growth, whereas the convergent method involves only a constant number of reactions (typically two to three) at each stage of the synthesis. Today, several hundred reports utilizing the original poly(ether), Fréchet-type dendron[44] method make this the best understood and structurally most precise family of convergent dendrimers.

Overall, each of these dendrimer construction strategies offers respective advantages and disadvantages. Some of these issues, together with experimental laboratory procedures, are reviewed elsewhere.[15]

20.2.3.2 Dendrimer Features of Interest to Nanoscientists

Dendrimers may be viewed as unique, information processing, nanoscale devices. Each architectural component manifests a specific function while also defining properties for these nanostructures as they are grown generation by generation. For example, the *core* may be thought of as the molecular information center from which *size*, *shape*, *directionality,* and *multiplicity* are expressed *via* the covalent connectivity to the outer shells. Within the *interior*, one finds the *branch cell amplification region,* which defines the type and amount of interior void space that may be enclosed by the terminal groups as the dendrimer is grown. Branch cell multiplicity (N_b) determines the density and degree of amplification as an exponential function of generation (G). The interior composition and amount of solvent-filled void space determines the extent and nature of guest–host (endo-receptor) properties that are possible within a particular dendrimer family and generation. Finally, the surface consists of reactive or passive terminal groups that may perform several functions. With appropriate function, they serve as a *template polymerization region* as each generation is amplified and covalently attached to the precursor generation. Secondly, the surface groups may function as passive or reactive gates controlling entry or departure of guest molecules from the dendrimer interior. These three architectural components essentially determine the physical/chemical properties as well as the overall sizes, shapes, and flexibility of dendrimers. It is important to note that dendrimer diameters increase linearly as a function of shells or generations added, whereas the terminal functional groups increase exponentially as a function of generation. This dilemma enhances *tethered congestion* of the anchored dendrons as a function of generation, due to the steric crowding of the end groups. As a consequence, lower generations are generally open, floppy structures — whereas higher generations become robust, less deformable spheroids, ellipsoids, or cylinders, depending on the shape and directionality of the core.

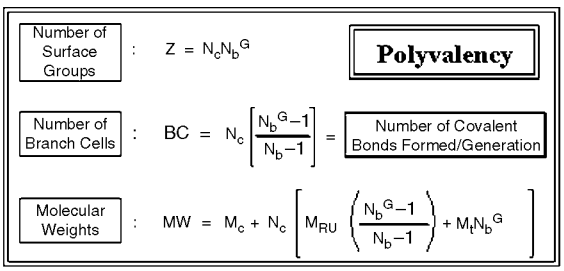
PAMAM dendrimers are synthesized by the divergent approach. This methodology involves *in situ* branch cell construction in step-wise, iterative stages (i.e., generation = 1,2,3…) around a desired core to produce mathematically defined *core-shell* structures. Typically, ethylenediamine ($N_c = 4$) or ammonia ($N_c = 3$) are used as cores and allowed to undergo reiterative two-step reaction sequences involving (1) exhaustive alkylation of primary amines (Michael addition) with methyl acrylate and (2) amidation



SCHEME 20.4 (From Esfand, R.; Tomalia, D.A. Laboratory synthesis of poly(amidoamine) (PAMAM) dendrimers. In *Dendrimers and Other Dendritic Polymers*; Fréchet, J.M.J. and Tomalia, D.A., Eds.; John Wiley & Sons: West Sussex, 2001. With permission.)

of amplified ester groups with a large excess of ethylenediamine to produce primary amine terminal groups as illustrated in Scheme 20.4.

This first reaction sequence on the exposed dendron (Figure 20.11) creates G = 0 (i.e., the core branch cell), wherein the number of arms (i.e., dendrons) anchored to the core is determined by N_c. Iteration of the alkylation/amidation sequence produces an amplification of terminal groups from 1 to 2 with the *in situ* creation of a branch cell at the anchoring site of the dendron that constitutes G = 1. Repeating these iterative sequences (Scheme 20.4) produces additional shells (generations) of branch cells that amplify mass and terminal groups according to the mathematical expressions described in Figure 20.12.



Generation	Surface Groups (Z)	Molecular Formula	MW	Diameter (nm)
0	4	$C_{22}H_{48}N_{10}O_4$	517	1.4
1	8	$C_{62}H_{128}N_{26}O_{12}$	1,430	1.9
2	16	$C_{142}H_{288}N_{58}O_{28}$	3,256	2.6
3	32	$C_{302}H_{608}N_{122}O_{60}$	6,909	3.6
4	64	$C_{622}H_{1248}N_{250}O_{124}$	14,215	4.4
5	128	$C_{1262}H_{2528}N_{506}O_{252}$	28,826	5.7
6	256	$C_{2542}H_{5088}N_{1018}O_{508}$	58,048	7.2
7	512	$C_{5102}H_{10208}N_{2042}O_{1020}$	116,493	8.8
8	1,024	$C_{10222}H_{20448}N_{4090}O_{2044}$	233,383	9.8
9	2,048	$C_{20462}H_{40928}N_{8186}O_{4092}$	467,162	11.4
10	4,096	$C_{40942}H_{81888}N_{16378}O_{8188}$	934,720	~13.0

FIGURE 20.12 Dendritic branching mathematics for predicting number of dendrimer surface groups, number of branch cells, and molecular weights. Calculated values for [ethylenediamine core]; *dendri*-poly(amidoamine) series with nanoscale diameters (nm).

It is apparent that both the core multiplicity (N_c) and branch cell multiplicity (N_b) determine the precise number of terminal groups (Z) and mass amplification as a function of generation (G). One may view those generation sequences as quantized polymerization events. The assembly of reactive monomers,[33,35] branch cells,[15,35,39] or dendrons[15,40,45] around atomic or molecular cores to produce dendrimers according to divergent/convergent dendritic branching principles, has been well demonstrated. Such systematic filling of space around cores with branch cells, as a function of generational growth stages (branch cell shells), to give discrete, quantized bundles of mass has been shown to be mathematically predictable.[21,46] Predicted molecular weights have been confirmed by mass spectroscopy[47–49] and other analytical methods.[35,40,50–52] Predicted numbers of branch cells, terminal groups (Z), and molecular weights as a function of generation for an ethylenediamine core ($N_c = 4$) PAMAM dendrimer are shown in Figure 20.12. It should be noted that the molecular weights approximately double as one progresses from one generation to the next. The surface groups (Z) and branch cells (BC) amplify mathematically according to a power function, thus producing discrete, monodispersed structures with precise molecular weights and nanoscale diameter enhancement as described in Figure 20.12. These predicted values are routinely verified by mass spectroscopy for the earlier generations (i.e., G = 4–5); however, with divergent dendrimers, minor mass defects are often observed for higher generations as congestion-induced *de Gennes dense packing* begins to take effect (Figure 20.13).

20.2.3.3. Dendrimer Shape Changes — A Nanoscale Molecular Morphogenesis

As illustrated in Figure 20.13, dendrimers undergo *congestion-induced* molecular shape changes from flat, floppy conformations to robust spheroids as first predicted by Goddard et al.[53] Shape change transitions were subsequently confirmed by extensive photophysical measurements, pioneered by Turro et al.,[54–57] and solvatochromic measurements by Hawker et al.[58] Depending upon the accumulative core and branch cell multiplicities of the dendrimer family under consideration, these transitions were found to occur between G = 3 and G = 5. Ammonia core, PAMAM dendrimers ($N_c = 3$, $N_b = 2$) exhibited a molecular morphogenesis break at G = 4.5, whereas the ethylenediamine (EDA) PAMAM dendrimer family ($N_c = 4$; $N_b = 2$) manifested a shape change break around G = 3–4,[53] and the Fréchet-type convergent dendrons ($N_b = 2$) around G = 4.[58] It is readily apparent that increasing the core multiplicity from $N_c = 3$ to $N_c = 4$ accelerates congestion and forces a shape change at least one generation earlier. Beyond these generational transitions, one can visualize these dendrimeric shapes as nearly spheroidal or slightly ellipsoidal *core-shell type architectures.*

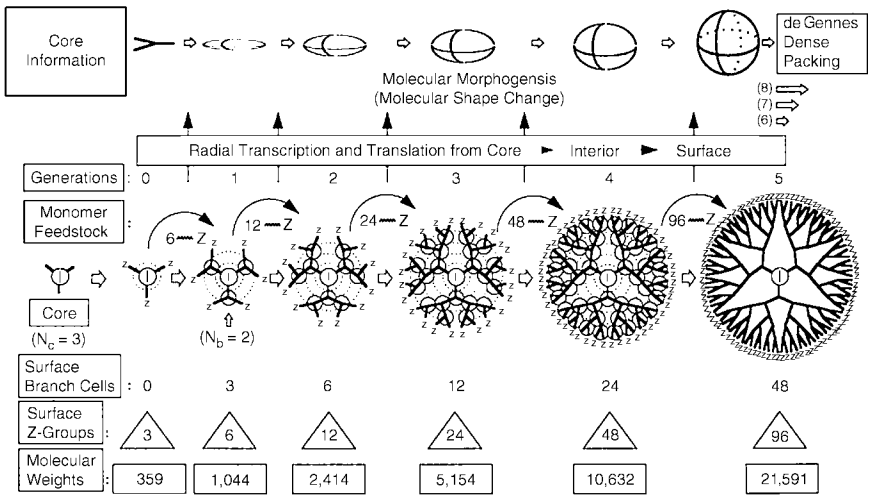


FIGURE 20.13 Comparison of molecular shape change, two-dimensional branch cell amplification, surface branch cells, surface groups (Z), and molecular weights as function of generation: G = 0–5.

20.2.3.4 de Gennes Dense Packing — A Nanoscale Steric Phenomenon

As a consequence of the excluded volume associated with the core, interior, and surface branch cells, steric congestion is expected to occur due to tethered connectivity to the core. Furthermore, the number of dendrimer surface groups, Z, amplifies with each subsequent generation (G). This occurs according to geometric *branching laws*, which are related to core multiplicity, (N_c), and branch cell multiplicity, (N_b).[21,30] These values are defined by the following equation:

$$Z = N_c N_b{}^G$$

Because the radii of the dendrimers increase in a linear manner as a function of G, whereas the surface cells amplify according to $N_c N_b{}^G$, it is implicit from this equation that generational reiteration of branch cells ultimately will lead to a so-called *dense-packed state*.

As early as 1983, de Gennes and Hervet[59,166] proposed a simple equation derived from fundamental principles to predict the dense-packed generation for PAMAM dendrimers. It was predicted that at this generation, ideal branching can no longer occur as available surface space becomes too limited for the mathematically predicted number of surface cells to occupy. This produces a *closed geometric structure*. The surface is *crowded* with exterior groups, which, although potentially chemically reactive, are sterically prohibited from participating in ideal dendrimer growth.

This *critical packing state* does not preclude further dendrimer growth beyond this point in the genealogical history of the dendrimer preparation. On the contrary, although continuation of dendrimer step-growth beyond the dense-packed state cannot yield structurally ideal, next-generation dendrimers, it can nevertheless occur as indicated by further increases in the molecular weight of the resulting products. Predictions by de Gennes[59] suggested that the PAMAM dendrimer series should reach a critical packing state at generations 9–10. Experimentally, we observed a moderate molecular weight deviation from predicted ideal values beginning at generation 4–7 (Figure 20.15). This digression became very significant at generations 7–8 as dendrimer growth was continued to generations 12.[24] The products thus obtained are of imperfect structure because of the inability of all surface groups to undergo further reaction. Presumably a fraction of these surface groups remains trapped or is sterically encumbered under the surface of the newly formed dendrimer shell, yielding a unique architecture possessing two types of terminal groups. This new surface group population will consist of both those groups that are accessible to subsequent reiteration reagents and those that will be sterically screened. The total number of these groups will not, however, correspond to the predictions of the mathematical branching law, but will fall between that value mathematically predicted for the next generations (i.e., G + 1) and that expected for the precursor generation (G). Thus, a mass defective dendrimer generation is formed.

Dendrimer surface congestion can be appraised mathematically as a function of generation, from the following simple relationship:

$$A_z = \frac{A_D}{N_Z} \alpha \frac{r^2}{N_c N_b{}^G}$$

where A_z is the surface area per terminal group Z, A_D the dendrimer surface area, and N_z the number of surface groups Z per generation. This relationship predicts that at higher generations G, the surface area per Z group becomes increasingly smaller and experimentally approaches the cross-sectional area or van der Waals dimension of the surface groups Z. The generation G thus reached is referred to as the *de Gennes dense-packed generation*.[15,21,35] Ideal dendritic growth without branch defects is possible only for those generations preceding this dense-packed state. This critical dendrimer property gives rise to self-limiting dendrimer dimensions, which are a function of the branch cell segment length (l), the core multiplicity N_c, the branch cell juncture multiplicity N_b, and the steric dimensions of the terminal group Z (Figure 20.9). Whereas the dendrimer radius r in the above expression is dependent on the branch cell segment lengths l, large l values delay this congestion. On the other hand, larger N_c, N_b values and larger Z dimensions dramatically hasten it.

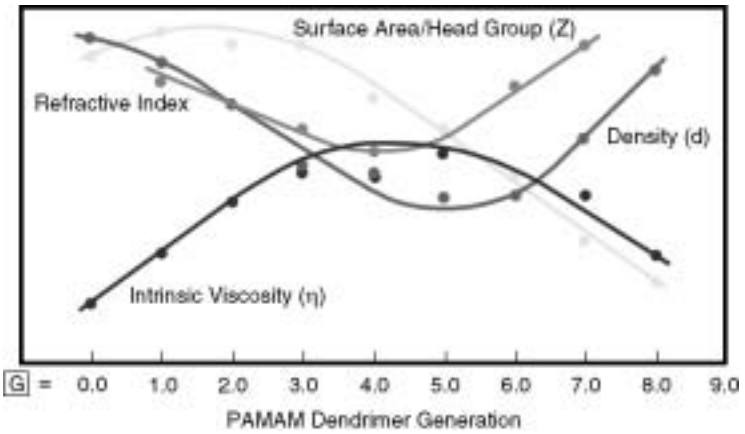


FIGURE 20.14 Comparison of surface area/head group (Z), refractive index, density (d), and intrinsic viscosity (η) as a function of generation: G = 1–9. (From Esfand, R.; Tomalia, D.A. Laboratory synthesis of poly(amidoamine) (PAMAM) dendrimers. In *Dendrimers and Other Dendritic Polymers*; Fréchet, J.M.J. and Tomalia, D.A., Eds.; John Wiley & Sons: West Sussex, 2001. With permission.)

Additional physical evidence supporting the anticipated development of congestion as a function of generation is shown in the composite comparison of property changes in Figure 20.14. Plots of intrinsic viscosity [η],[35,60] density z, surface area per Z group (A_z), and refractive index (η) as a function of generation clearly show maxima or minima at generations = 3–5, paralleling computer-assisted molecular-simulation predictions[53] as well as extensive photochemical probe experiments reported by Turro et al.[54–57]

The intrinsic viscosities [η] increase in a very classical fashion as a function of molar mass (generation) but decline beyond a certain generation because of change from an extended to a globular shape.[35,53] In effect, once this critical generation is reached, the dendrimer begins to act more like an Einstein spheroid. The intrinsic viscosity is a physical property that is expressed in dL/g (i.e., the ratio of volume to mass). As the generation number increases and transition to a spherical shape takes place, the volume of the spherical dendrimer roughly increases in cubic fashion while its mass increases exponentially; hence, the value of [η] must decrease once a certain generation is reached. This prediction has now been confirmed experimentally.[35,60]

The dendrimer density (d) (atomic mass units per unit volume) clearly minimizes between generations 4 and 5. It then begins to increase as a function of generation due to the increasingly larger, exponential accumulation of surface groups. Because refractive indices are directly related to density parameters, their values minimize and parallel the above density relationship.

Clearly, this de Gennes dense-packed congestion would be expected to contribute to sterically inhibited reaction rates and sterically induced stoichiometry.[35] Each of these effects was observed experimentally at higher generations.[34] The latter would be expected to induce dendrimer mass defects at higher generations which we have used as a diagnostic signature for appraising the de Gennes dense-packing effect.

Theoretical dendrimer mass values were compared with experimental values by performing electrospray and MALDI-TOF mass spectral analysis on the respective PAMAM families (i.e., N_c = 3 and 4).[48] Note there is essentially complete shell filling for the first five generations of the (NH_3) core (N_c = 3; N_b = 2) poly(amidoamine) (PAMAM) series (Figure 20.15b). A gradual digression from theoretical masses occurs for G = 5–8, followed by a substantial break (i.e., Δ = 23%) between G = 8 and 9. *This discontinuity in shell saturation is interpreted as a signature for de Gennes dense packing*. It should be noted that shell saturation values continue to decline monotonically beyond this breakpoint to a value of 35.7% of theoretical at G = 12. A similar trend is noted for the EDA core, PAMAM series (N_c = 4; N_b = 2); however, the shell saturation inflection point occurs at least one generation earlier (i.e., G = 4–7, see Figure 20.15a). This suggests that the onset of de Gennes dense packing may be occurring between G = 7 and 8.

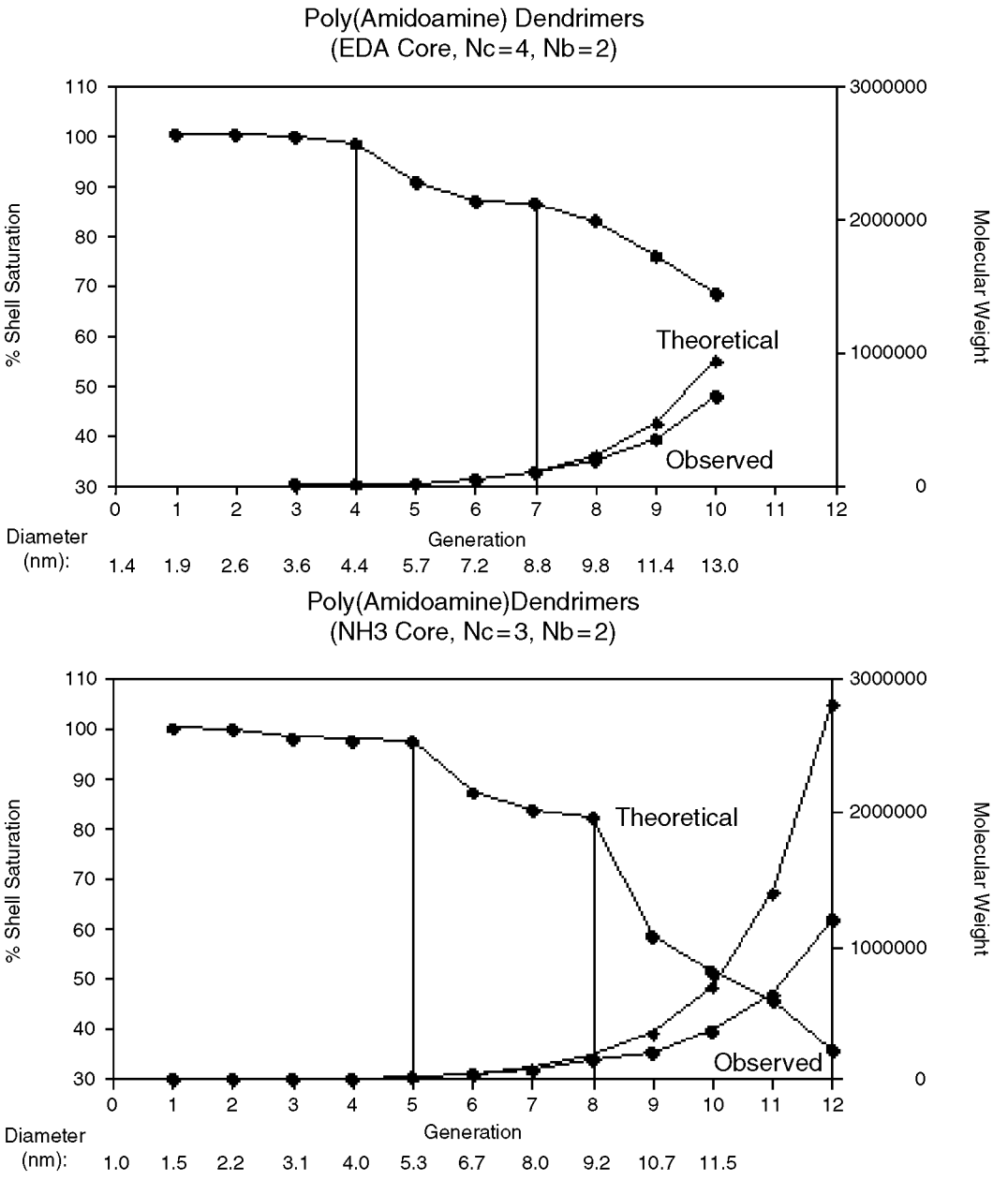


FIGURE 20.15 Top: Comparison of theoretical/observed molecular weights and percentage shell filling for EDA core PAMAM dendrimers as a function of generation: G = 1–10. Bottom: Comparison of theoretical/observed molecular weights and percentage shell filling for NH_3 core PAMAM dendrimers as a function of generation: G = 1–12. (From Esfand, R.; Tomalia, D.A. Laboratory synthesis of poly(amidoamine) (PAMAM) dendrimers. In *Dendrimers and Other Dendritic Polymers*; Fréchet, J.M.J. and Tomalia, D.A., Eds.; John Wiley & Sons: West Sussex, 2001. With permission.)

Unique features offered by the dendritic state that have no equivalency in classical polymer topologies are found almost exclusively in the dendron/dendrimer subset or, to a slightly lesser degree, in the dendrigrafts. They include:

1. Nearly complete nanoscale monodispersity
2. The ability to control nanoscale container/scaffolding properties
3. Exponential amplification and functionalization of dendrimer surfaces
4. Nanoscale dimension and shape mimicry of proteins

These features are captured to some degree with *dendrigraft* polymers, but are either absent or present to a vanishing small extent for random *hyperbranched* polymers.

20.3 Unique Dendrimer Properties

20.3.1 Nanoscale Monodispersity

The monodispersed nature of dendrimers has been verified extensively by mass spectroscopy,[155] size exclusion chromatography, gel electrophoresis,[52] electron microscopy (TEM),[15,167] and atomic force microscopy (AFM).[152] As is always the case, the level of monodispersity is determined by the skill of the synthetic chemist as well as the isolation/purification methods utilized.

In general, convergent methods produce the most nearly isomolecular dendrimers. This is because the convergent growth process allows purification at each step of the synthesis and eliminates cumulative effects due to failed couplings.[40,43] Appropriately purified, convergent dendrimers are probably the most precise synthetic macromolecules that exist today.

As discussed earlier, mass spectroscopy has shown that PAMAM dendrimers (Figure 20.15) produced by the divergent method are very monodisperse and have masses consistent with predicted values for the earlier generations (G = 0–5). Even at higher generations, as one enters the de Gennes dense-packed region, the molecular weight distributions remain very narrow (i.e., 1.05) and consistent, in spite of the fact that experimental masses deviate substantially from predicted theoretical values. Presumably, de Gennes dense packing produces a very regular and dependable effect that is manifested in the narrow molecular weight distribution.

20.3.2 Nanoscale Container/Scaffolding Properties

Unimolecular container/scaffolding behavior appears to be a periodic property that is specific to each dendrimer family or series. These properties are determined by the size, shape, and multiplicity of the construction components that are used for the core, interior, and surface of the dendrimer (Figure 20.9). Higher multiplicity components and those that contribute to tethered congestion will hasten the development of container properties or rigid surface scaffolding as a function of generation. Within the PAMAM dendrimer family, these periodic properties are generally manifested in three phases as shown in Figure 20.16.

The earlier generations (i.e., G = 0–3) exhibit no well-defined interior characteristics, whereas interior development related to geometric closure is observed for the intermediate generations (i.e., G = 4–6/7). Accessibility and departure from the interior is determined by the size and gating properties of the surface groups. At higher generations (i.e., G ≥ 7), where de Gennes dense packing is severe, rigid scaffolding properties are observed, allowing relatively little access to the interior except for very small guest molecules. The site isolation and encapsulation properties of dendrimers have been reviewed recently by Tomalia et al.,[61] Hecht and Fréchet,[62] and Meijer et al.[63]

20.3.3 Amplification and Functionalization of Dendrimer Surface Groups

Dendrimers within a generational series can be expected to present their terminal groups in at least three different modes: *flexible, semi-flexible,* or *rigid functionalized* scaffolding. Based on mathematically defined dendritic branching rules (i.e., $Z = N_c N_b^G$), the various surface presentations become more congested and rigid as a function of increasing generation levels. It is implicit that this surface amplification may be designed to control gating properties associated with unimolecular container development.

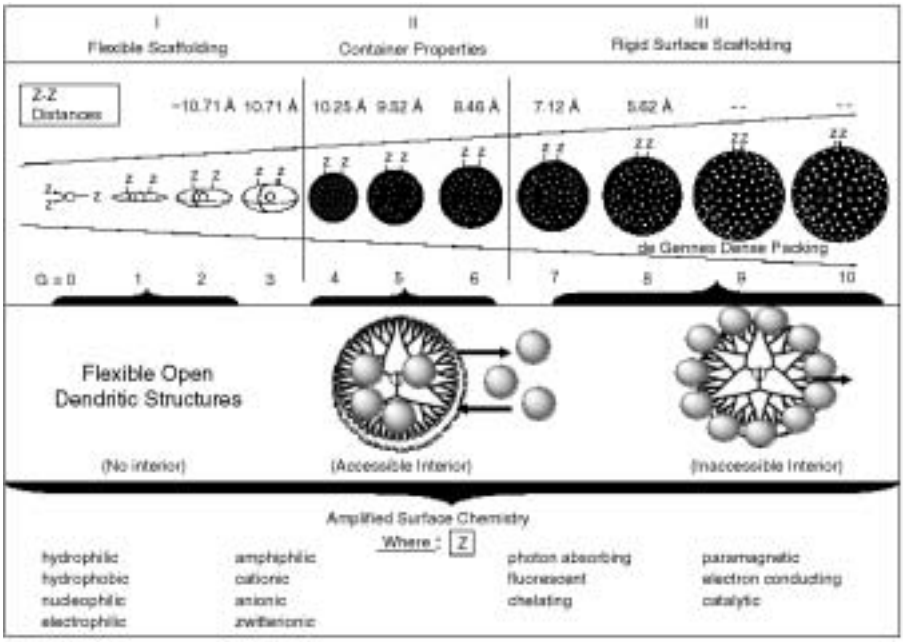


FIGURE 20.16 Periodic properties for PAMAM dendrimers as a function of generation G = 0–10: (I) flexible scaffolding (G = 0–3); (II) container properties (G = 4–6); and (III) rigid surface scaffolding (G = 7–10). Various chemo/physical dendrimer surfaces amplified according to: Z – $N_cN_b^G$ where: N_c = core multiplicity, N_b = branch cell multiplicity, G = generation. (From Esfand, R.; Tomalia, D.A. Laboratory synthesis of poly(amidoamine) (PAMAM) dendrimers. In *Dendrimers and Other Dendritic Polymers*; Fréchet, J.M.J. and Tomalia, D.A., Eds.; John Wiley & Sons: West Sussex, 2001. With permission.)

Furthermore, dendrimers may be viewed as versatile, nanosized objects that can be surface functionalized with a vast array of chemical and application features (Figure 20.16). The ability to control and engineer these parameters provides an endless list of possibilities for utilizing dendrimers as modules for nanodevice design.[24,46,64,65] Recent reviews have begun to focus on this area.[35,62,65–67]

20.3.4 Nanoscale Dimensions and Shapes Mimicking Proteins

In view of the extraordinary structure control and nanoscale dimensions observed for dendrimers, it is not surprising to find extensive interest in their use as globular protein mimics.[24] Based on their systematic, dimensional length scaling properties (Figure 20.17) and electrophoretic/hydrodynamic[50,52] behavior, they are referred to as *artificial proteins*.[24,61] Substantial effort has been focused recently on the use of dendrimers for "site isolation" mimicry of proteins[35] and enzyme-like catalysis,[68] as well as for other biomimetic applications,[24,69] drug delivery,[61] surface engineering,[70] and light harvesting.[71,72] These fundamental properties have in fact led to their commercial use as globular protein replacements for gene therapy, immunodiagnostics,[73,74] and a variety of other biological applications described below.

20.4 Dendrimers as Nanopharmaceuticals and Nanomedical Devices

Many promising biomedical applications for these compounds have been discovered and several are currently under development.[69] A current overview of dendrimer applications in the wet nanotechnology area is described below.

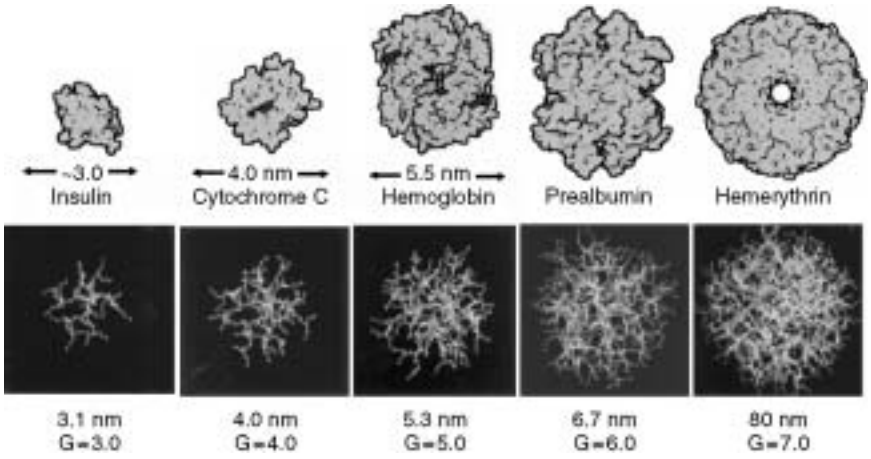


FIGURE 20.17 Comparison of selected proteins showing the close dimensional size/scaling (nm) to respective generations [-ammonia core]; *dendri*-poly(amidoamine) dendrimer.

20.4.1 Dendrimers as Genetic Material Transfer Agents

Antisense oligonucleotides have been shown to down-regulate the expression of specific genes in both *in vivo* and *in vitro* systems.[75,76] Gene regulation has many potential applications in treating disease states such as cancer, tissue graft survival, multiple drug resistance, and other conditions where it would be beneficial to reduce or eliminate messages produced from genes causing the adverse effect. The main drawback with antisense treatment is the delivery of "naked" nucleic material to the cell and, in particular, the cell nucleus. Technologies using viral[77,78] and synthetic cationic lipid preparations[79] have been shown to improve antisense delivery but demonstrate severe limiting side effects *in vivo*. Viral vectors have immunogenicity and cell targeting problems,[80] whereas the cationic lipids cause inflammatory reactions and cytotoxicity.[81,82]

The application of PAMAM dendrimers as transfection agents for antisense oligonucleotides and plasmids was described by Tomalia and co-workers in 1996.[83,84] They found that stable DNA–dendrimer complexes formed and could successfully suppress luciferase expression in an *in vitro* cell culture system.[83] Further work by Kukowska–Latallo et al.,[85] Baker et al.,[86] and DeLong et al.[87] provided additional evidence that the stability of these DNA–dendrimer complexes was dependent on electrostatic charge of the PAMAM dendrimer and that the complexes formed were stable at a range of pH values. These complexes were also stable to restriction endonucleases.

Thus, PAMAM and, to some extent the poly(lysine) dendrimers,[88] overcame some of the problems associated with delivery of nucleic acids intracellularly. In preliminary studies, Roberts et al.[89] showed that several generations of the PAMAM dendrimers elicited no immunogenicity and possessed very low *in vivo* toxicity. The bioavailability results were somewhat unusual, with all methylated dendrimers showing high pancreatic uptake. However, more recent evaluation of the toxicity and bioavailability of PAMAM dendrimers has been reported.[90]

20.4.2 Dendrimer–Carbohydrate Conjugates for Polyvalent Recognition

Carbohydrate–protein interactions play a vital role in many biological functions such as cell adhesion, receptor-mediated events, cellular recognition processes, and microbial–host cell interactions. These individual sugar–receptor complexes are often weak and nonselective.[91] Pioneering work by Roy et al., Stoddart et al., Lindhorst et al., and Okada et al. led to the development of a special class of dendrimers known as carbohydrate dendrimers (see review by Jayaraman et al.[92]). These carbohydrate-linked dendrimers had several sugar groups attached to the outside of the dendrimer shell, which led to a greatly enhanced affinity and selectivity of the sugar–receptor reaction, believed to be mediated via polyvalent

(multivalent) interactions (for a review of polyvalent interactions, see Mammen et al.[93]). This enhanced affinity has been demonstrated for carbohydrate-linked poly(lysine),[94] PAMAM,[95] and poly(propyleneimine) (PPI)[96] dendrimers. Many potential biomedical applications exist for the concentration of glycodendrimers as anti-viral,[97] bacterial species,[98] and anti-cell adhesion agents in general.[99]

20.4.3 Dendrimers as Targeted Drug Delivery Agents

Dendrimers may be used as hosts or *molecular* or *dendritic boxes* to encapsulate guest molecules and transport them to biological targets.[105] The use of a PPI dendrimer as a host for the dye Bengal Rose was first described by Meijer and co-workers.[100–102] Under certain conditions, the guest molecules can slowly diffuse out of the dendritic host, although large molecules such as Bengal Rose require quite aggressive denaturing of the dendrimer before release occurs. There are many potential applications of dendrimers as drug delivery vehicles, and several of these have been described by Tomalia and co-workers[61,69] and Behr.[103] In a broad patent issued in 1996, Tomalia and colleagues[104] claimed dendrimer polymers as complexing and carrier materials for biological agents, including genes, DNA for transfection, IgG and such agents as the herbicides 2,4-dichlorophenoacetic acid, and abscissic acid.[104]

Additionally, the surface of the dendrimer may possess recognition functionality covalently attached suitable for targeting the dendrimer–guest complexes to a specific biological destination.[105] An example of this application was shown by Moroder and co-workers[106] where antibody-labeled dendrimers containing radioactive boron isotopes were targeted to tumor cells. Barth et al.[107] had previously shown that incurable forms of glioblastoma cancer could be treated with ^{10}B isotope containing dendrimers, which concentrated high levels of neutron-capture agents within the tumor and reduced the tumor size. Duncan and co-workers[108] demonstrated that a PAMAM dendrimer conjugated to cisplatin (producing a dendrimer–platinate) was highly water soluble and released platinum slowly *in vitro*. When tested *in vivo*, the dendrimer–platinate displayed greater anti-tumor activity than naked cisplatin and exhibited lower toxicity. Jansen et al.[109] conjugated a PPI dendrimer with platinum in order to overcome cisplatin resistance in cancer cells. In preliminary *in vitro* tests, this PPI–platinum complex showed low cytotoxicity in a range of human tumor cell lines.

20.4.4 Dendrimers as Magnetic Resonance Imaging Contrast Agents

Certainly dendrimers can play an important role as both tumor diagnosis and therapeutic agents because they are capable of incorporating large amounts of paramagnetic or radioactive metal ions into their interiors. The use of dendrimers to treat tumors as neutron capture agents has been described above; however, a similar principle can be used to image various tumors and cancers.

An example of such a use is that offered by folate-conjugated PAMAM dendrimer chelates containing gadolinium that were used to target tumor cells.[110] These folate-conjugated chelates targeted the complex to folate binding proteins on the tumor cell surfaces. Such high-affinity folate binding sites are upregulated on the cell surface of many human cancers of epithelial origin.[79]

Margerum et al. have also shown that PAMAM–gadolinium complexes have a reduced liver uptake and increased bioavailability when grafted with polyethylene glycol (PEG) side chains.[111]

20.4.5 Dendrimers as Antiviral Agents

Currently there have been few reports of the use of PAMAM or poly(lysine) dendrimers as potential antiviral or antimicrobial therapeutics. Reuter et al.[97] reported the use of PAMAM dendrimers with sialic acid functional groups as effective inhibitors of influenza virus binding to host cell receptors and noted the potential for these compounds to be used therapeutically. Whitesides and co-workers[112] had previously shown that sialic acid containing polyacrylamide inhibitors prevented the attachment of influenza virus to mammalian erythrocytes. They proposed that the underlying mechanism for this inhibition was due to polyvalent interactions coupled with steric stabilization by the dendrimers to prevent the interaction between the virus and the erythrocyte.

Polyanionic compounds such as dextran sulfate, heparin, and suramin inhibit HIV viral attachment by blocking the binding of the viral envelope glycoprotein gp120 to the cellular CD4 receptor of T lymphocytes. Thus, these polyanionic compounds can inhibit virus binding to cells, virus-induced syncytium formation, and, consequently, viral transmission.[113,114]

Heparin and dextran sulfate, which are both sulfated polysaccharides, have been shown to inhibit the replication of several viruses including HIV, HSV, and cytomegalovirus (CMV), as well as several strains of bacteria.[115–119] The sulfated carbohydrates are believed to block the adhesion to cell surfaces by the pathogens. Several studies suggest that a common cell surface molecule, heparin sulfate (HS), a glycosaminoglycan (GAG), is involved.[119–121]

In recent work a series of PAMAM and poly(lysine) dendrimers were developed and tested as antiviral agents.[122] This dendrimer series contained aryl-sulphonic acid salts on the outer surface and exhibited antiviral/antimicrobial activity to a broad range of viruses and other micro-organisms. It has been postulated that these dendrimers mimic several of the biological activities of heparin[129] but do not possess some of the attributes such as anticoagulant activity, which limits the usefulness of heparin as an anti-infective agent.[130] Wivrouw et al.[122,123] utilized *time of addition* studies to determine the mode of action of these dendrimers. They found that specific PAMAM dendrimers inhibit viral attachment to cells as well as the action of HIV viral reverse transcriptase and virally encoded integrase, which occur intracellularly during the virus replicative cycle. It is interesting to note, however, that some dendrimers showed inhibition of viral attachment only.

In addition to inhibition of HIV viral attachment, the dendrimers also inhibit adsorption of certain enveloped viruses such as herpes simplex virus 2 (HSV)[124] and respiratory syncytial virus (RSV)[125,126] in cell culture at very low concentrations (0.1–1 μg/ml). In recent work, PAMAM and poly(lysine) dendrimers were also shown to inhibit human cytomegalovirus (HCMV), Ebola virus, Hepatitis B virus (HBV), Influenza A and B, Epstein–Barr virus (EbV), and Adeno- and Rhino-viruses.[127]

20.4.6 Dendrimers as Angiogenesis Inhibitors

Angiogenesis, the formation of new blood vessels, is a tightly controlled process that occurs rarely in adult tissue and is limited to wound healing and the female reproductive cycle. Uncontrolled angiogenesis plays a role in the pathogenesis of a number of major diseases including cancer, arthritis, psoriasis, and ocular diseases.

Polyanionic dendrimers are claimed as angiogenesis inhibitors in a patent by Matthews and Holan.[128] It was postulated that these dendrimers mimic heparin or heparin sulfate, sequestering fibroblast growth factor (FGF) and possibly other growth factors, thereby disrupting the formation of new blood vessels.[129]

While having heparin-like properties, polyanionic dendrimers do not possess the anticoagulant activity associated with heparin or other antiangiogenic compounds used as heparin mimetics.[130] These large amino acid-containing structures are nonantigenic when compared with other peptide-based macromolecules.

The results of chorioalantoic membrane (CAM), rat aorta, human umbilical vein endothelial cells (HUVEC), and blood vessel growth inhibition assays demonstrate the potential of these polyanionic dendrimers as angiogenesis inhibitors.[129]

20.4.7 Dendrimers as Antitoxin Agents

Thompson and Schengrund[131,132] reported the use of oligosaccharide-derivatized dendrimers as bacterial enterotoxin inhibitors. The authors have also patented several oligosaccharide-linked PAMAM dendrimers as potential antibacterial and antiviral compounds.[133] They attributed the antitoxin effects of the dendrimers to multivalent (or polyvalent) interactions leading to neutralization of the bacterial toxins. Roy[134] also described the potential use of multivalent glycoconjugates as bacterial toxin inhibitors. Pieters et al.[135] demonstrated that lactose-containing dendrimers bound, in a multivalent fashion, to the cholera toxin B subunit. There have been many recent publications that describe the use of carbohydrate ligands (often toxin receptor analogues) linked to a polymer/dendrimer backbone, which enables a multivalent inhibition of the toxin–cell interaction.[136–139]

In another approach, the use of PAMAM and poly(lysine) dendrimers functionalized with polyanionic groups has been claimed to inhibit a range of viral and bacterial toxins as well as a number of crude snake venoms.[140]

20.4.8 Dendrimers as Antiprion Agents

Recently, Prusiner and co-workers[141] demonstrated the ability of PAMAM and PPI dendrimers to reduce PrP^{Sc}, the protease-resistant isoform of the prion protein, from cultured scrapie-infected neuroblastoma cells to undetectable levels as shown by Western blot analysis.[141] Structure–activity analysis showed that in acidic environments, the dendrimers altered the structure of the PrP^{Sc} and rendered it susceptible to protease degradation.

Further work by Prusiner[142] demonstrated the ability of PAMAM and PPI dendrimers not only to reduce the PrP^{Sc} to undetectable levels but to also eradicate prion infectivity as shown by a bioassay in mice. Additionally, PPI dendrimers denatured the PrP^{Sc} from bovine spongiform encephalopathy-infected brain homogenates; however, PrP^{Sc} from sheep scrapie-infected brain homogenates were resistant. Thus, the dendrimer-induced denaturation of prion protein may be strain-dependent.

This pioneering work by Prusiner and colleagues showed dendrimers to be the first class of compounds that could cure prion infection in living cells. This work has implications not only for the treatment of prion-based diseases but also for important applications in the sterilization of equipment and in diagnostic techniques.

20.4.9 Other Potential Biomedical Applications of Dendrimers

Tam et al.[143,144] demonstrated that peptide sequences that are too light to cause the production of antibodies within a host organism can be functionalized onto a dendrimer backbone to produce a highly antigenic compound called a *multiple antigen peptide* (MAP) dendrimer. This unique use of the dendrimer structure has vast implications for the production of vaccines from important peptide sequences (i.e., haptens from viruses) previously thought incapable of producing immunogenicity.

Dendrimers that act as catalysts, named *dendrizymes*, have been described by Brunner.[145] These dendritic catalysts were made to model enzymes and contained an organometallic active site buried in the branches of the dendritic structures. Initial work carried out by Diedrich and co-workers[146–148] and later by Fréchet and co-workers[149,150] utilized dendrimeric porphyrins as models for enzyme systems such as cytochrome *c*. Jiang and Aida[151] described the use of dendrimeric porphyrins as hemoglobin mimics. Recent advances in the synthesis of peptide dendrimers hold promise that dendrimers could be tailored to act as artificial enzymes in biological systems.

20.5 Dendrimers as Reactive Modules for the Synthesis of More Complex Nanoscale Architectures

20.5.1 Megamers, Saturated Shell, and Partial Shell-Filled Core-Shell Tecto(dendrimers)

In the first full paper published on dendritic polymers,[30] dendrimers were defined as "reactive, structure-controlled macromolecular building blocks." It was proposed that they could be used as repeat units for the construction of a new class of topological macromolecules now referred to as *megamers.*[24,152] Although there is intense activity in the field of dendrimer science, there have been relatively few references focused on this specific concept.[33,46,152–154] The generic term *megamers* has been proposed to describe those new architectures derived from the combination of two or more dendrimer molecules (Figure 20.18).[24,152,153]

Examples of both statistical[152] and structure-controlled[24,153,155] megamer assemblies have been reported and reviewed recently. Covalent oligomeric assemblies of dendrimers (i.e., dimers, trimers, etc.) are well-

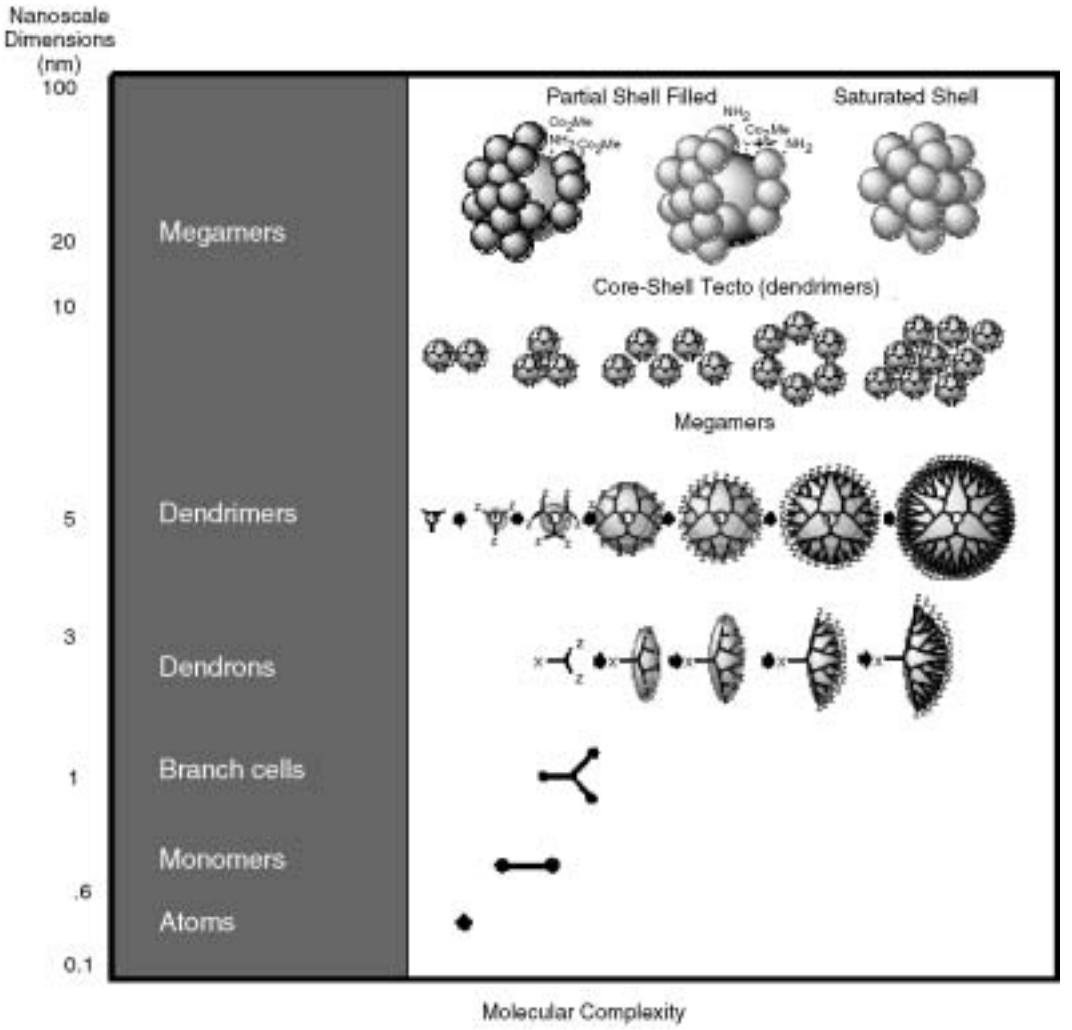


FIGURE 20.18 Approximate nanoscale dimensions as a function of atoms, monomers, branch cells, dendrimers, and megamers.

documented examples of low molecular weight megamers. Statistical megamer assemblies have been reported as both *supramacromolecular*[65,152] and *supermacromolecular*[36] (covalent) topologies.

Recently, mathematically defined, structure-controlled, covalent megamers have been reported. They are a major subclass of megamers referred to as *core–shell tecto(dendrimers)*.[24,155–157] Synthetic methodologies for these new architectures have been reported to produce precise megameric structures that adhere to mathematically defined bonding rules.[24,46,155,158] It appears that *structure-controlled nanoscale complexity beyond dendrimers* is now possible. The demonstrated ability to mathematically predict and synthesize structure-controlled dendrimer assemblies provides a broad concept for the systematic construction of nanostructures with dimensions that could span the entire nanoscale region (Figure 20.18).

20.6 Conclusions

In summary, using strictly abiotic methods, it has been widely demonstrated over the past decade that dendrimers[15] can be routinely constructed with control that rivals the structural regulation found in biological systems. The close scaling of size,[61,159] shape, and quasi-equivalency of surfaces[160–162] observed

between nanoscale biostructures and various dendrimer families/generational levels are both striking and provocative.[61,62,159–165] These remarkable similarities suggest a broad strategy based on rational biomimicry as a means for creating a repertoire of structure-controlled, size- and shape-variable dendrimer assemblies. Successful demonstrations of such a biomimetic approach have proven to be a versatile and powerful synthetic strategy for systematically accessing virtually any desired combination of size, shape, and surface in the nanoscale region. Future extensions will involve combinational variation of dendrimer module parameters such as (1) families (interior compositions), (2) surfaces, (3) generational levels, or (4) architectural shapes (i.e., spheroids, rods, etc.).

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