

## The Importance of Chemistry in Creating Well-Defined Nanoscopic Embedded Therapeutics: Devices Capable of the Dual Functions of Imaging and Therapy

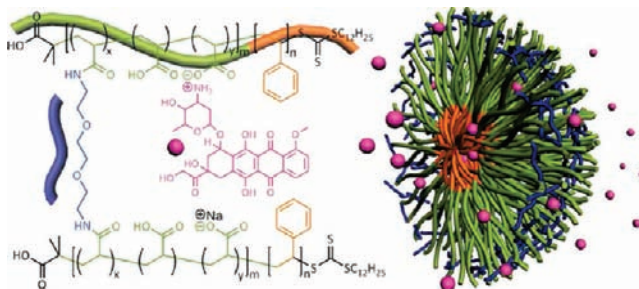
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### CONSPECTUS

Nanomedicine is a rapidly evolving field, for which polymer building blocks are proving useful for the construction of sophisticated devices that provide enhanced diagnostic imaging and treatment of disease, known as theranostics. These well-defined nanoscopic objects have high loading capacities, can protect embedded therapeutic cargo, and offer control over the conditions and rates of release. Theranostics also offer external surface area for the conjugation of ligands to impart stealth characteristics and/or direct their interactions with biological receptors and provide a framework for conjugation of imaging agents to track delivery to diseased site(s). The nanoscopic dimensions allow for extensive biological circulation. The incorporation of such multiple functions is complicated, requiring exquisite chemical control during production and rigorous characterization studies to confirm the compositions, structures, properties, and performance.



We are particularly interested in the study of nanoscopic objects designed for treatment of lung infections and acute lung injury, urinary tract infections, and cancer. This Account highlights our work over several years to tune the assembly of unique nanostructures. We provide examples of how the composition, structure, dimensions, and morphology of theranostic devices can tune their performance as drug delivery agents for the treatment of infectious diseases and cancer.

The evolution of nanostructured materials from relatively simple overall shapes and internal morphologies to those of increasing complexity is driving the development of synthetic methodologies for the preparation of increasingly complex nanomedicine devices. Our nanomedicine devices are derived from macromolecules that have well-defined compositions, structures, and topologies, which provide a framework for their programmed assembly into nanostructures with controlled sizes, shapes, and morphologies. The inclusion of functional units within selective compartments/domains allows us to create (multi)functional materials. We employ combinations of controlled radical and ring-opening polymerizations, chemical transformations, and supramolecular assembly to construct such materials as functional entities. The use of multifunctional monomers with selective polymerization chemistries affords regiochemically functionalized polymers. Further supramolecular assembly processes in water with further chemical transformations provide discrete nanoscopic objects within aqueous solutions. This approach echoes processes in nature, whereby small molecules (amino acids, nucleic acids, saccharides) are linked into polymers (proteins, DNA/RNA, polysaccharides, respectively) and then those polymers fold into three-dimensional conformations that can lead to nanoscopic functional entities.

### Introduction

Well-defined nanoscopic objects that can be embedded with therapeutics have great potential to protect the

therapeutic cargo with high capacity for loading and control over the conditions and rates of release, while also offering external surface area for conjugation of ligands to impart

stealth characteristics and/or direct their interactions with biological receptors. The nanoscopic dimensions allow for extended biological circulation and provide a framework for conjugation of imaging agents to track delivery to diseased site(s). Incorporating such multiple functions is complicated, requiring exquisite chemical control during production and rigorous characterization studies to confirm the compositions, structures, properties, and performance. Direct linkage to a particular disease is necessary to tune the chemical, physical, and morphological features to gain access to the disease site and deliver the cargo appropriately, for example, extravascular, intracellular, and so forth.

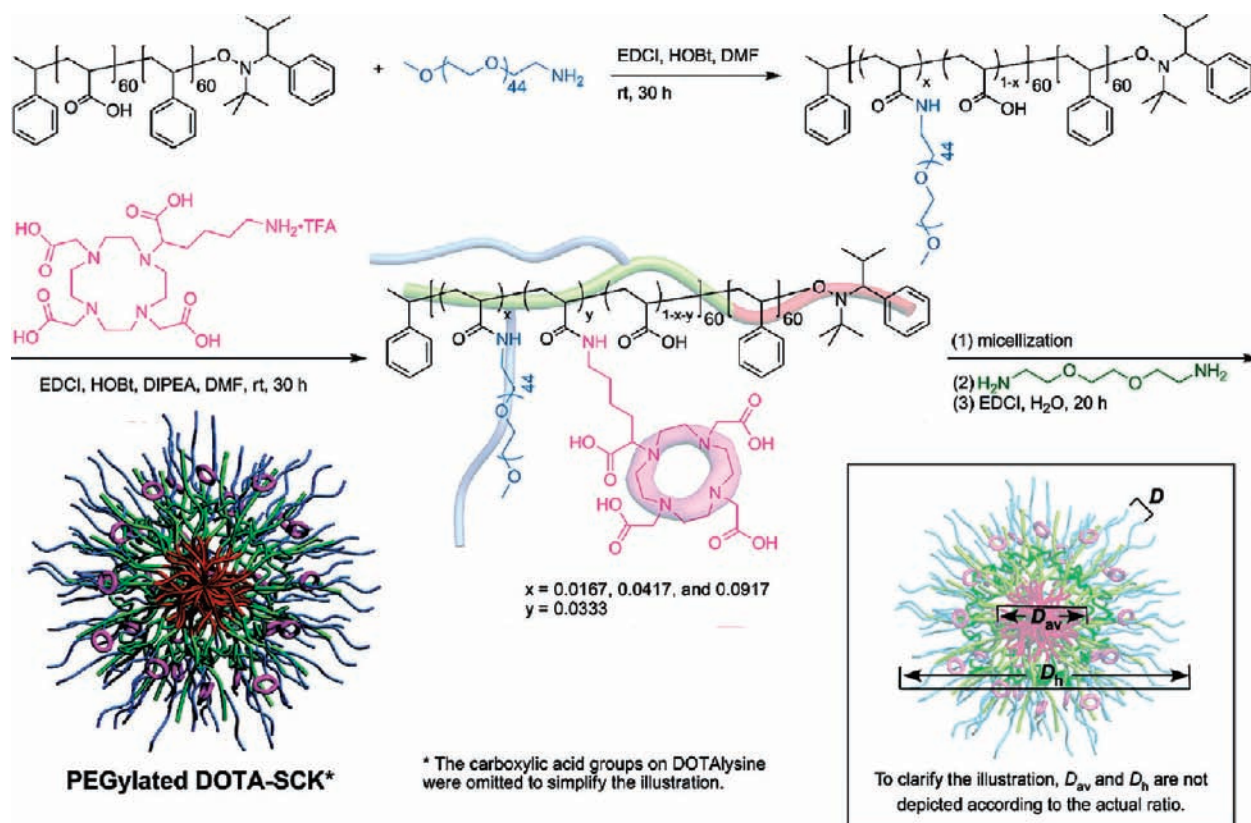
Nanomedicine is a rapidly evolving field, for which polymer building blocks are proving useful for the construction of sophisticated devices, theranostics, that provide enhanced diagnostic imaging and treatment of disease. We have special interests in the study of nanoscopic objects that are designed for treatment of lung infectious diseases, urinary tract infections, acute lung injury, and cancer. Our nanomedicine devices are derived from macromolecules that have well-defined compositions, structures, and topologies, allowing for programmed assembly into nanostructures having controlled sizes, shapes, and morphologies. The evolution of nanostructured materials from relatively simple overall shapes and internal morphologies to those of increasing complexity is driving the development of synthetic methodologies that allow for the preparation of increasingly complex nanomedicine devices. Moreover, the inclusion of functional units within selective compartments/domains is of great importance to create (multi)functional materials. Combinations of controlled radical and ring-opening polymerizations, chemical transformations, and supramolecular assembly are employed to construct such materials as functional entities. We employ a variety of multifunctional monomers, together with selective polymerization chemistries, to afford regiochemically functionalized polymers, and then further conduct supramolecular assembly processes in water, often together with chemical transformations, to afford discrete nanoscopic objects within aqueous solutions. This approach has similarities to the processes of nature, whereby small molecules (amino acids, nucleic acids, saccharides) are linked into polymers (proteins, DNA/RNA, polysaccharides, respectively) and then those polymers fold into three-dimensional conformations that can lead to nanoscopic functional entities. This Account will highlight our work over several years to tune the assembly of unique nanostructures, with examples of how their compositions, structures, dimensions, and morphologies can

tune their performance as theranostic agents for the treatment of infectious diseases and cancer.

## Tuning the Biological Interactions and Biodistribution

As indicated by their name, theranostic nanoparticle systems (TNPs) are designed to fulfill two objectives: therapeutic delivery and diagnostic imaging. Parameters such as the biodistribution (BioD), pharmacokinetics (PK), and pharmacodynamics (PD) are inherently different between drug delivery systems (DDS) and imaging systems. Nanoparticles for drug delivery typically exhibit extended blood circulation times in order to increase plasma concentration of the pharmacologically active substance, and also to exploit fully the enhanced permeation and retention (EPR) effect or ligand-mediated targeting, to reach further increases in local drug concentrations at disease sites. On the other hand, nanoparticulate imaging agents often require a different set of BioD, PK, and PD properties to operate with optimized signal to background. Nanoscopic imaging agents are administered to increase the signal-to-noise ratio in a specific tissue and allow for a diagnostic image to be obtained. In the case where the nanoparticle imaging agent is combined with an "active" targeting ligand, the circulation half-life of the construct becomes a critical parameter. In this setting, a longer circulation will allow for accumulation (via more circulation passes) at the target, but will require that the nanoparticle ultimately clears in order to reduce background (concentration) in the circulating blood to provide contrast. For successful realization of functional TNPs, this means that these parameters of the device must compromise between the optimum for drug delivery and that for diagnostic imaging, which in turn requires flexible chemical synthesis to systematically tailor the size, shape, charge, placement of targeting ligands, degradation, drug loading and release kinetics, and so forth.

In this context, shell cross-linked knedel-like nanoparticles (SCKs) are versatile constructs for which a substantial body of work has been performed to establish protocols for controlling such parameters that are fundamental for nanomedical research, in general, and theranostic applications, in particular. In 2008, the Wooley lab developed a protocol for designing SCK nanoparticles that exhibited tailored PK and BioD.<sup>1</sup> The foundation for preparing these cross-linked NPs was to utilize an amphiphilic block copolymer scaffold with specific hydrophilic/hydrophobic ratios to render SCKs with controlled core and shell dimensions (Figure 1). To tailor the BioD and PK characteristics, these block copolymers were



**FIGURE 1.** SCKs having tailored in vivo behavior involve coupling onto polymer precursors to provide for chemical control and detailed characterization. Reprinted with permission from ref 1. Copyright 2008 American Chemical Society.

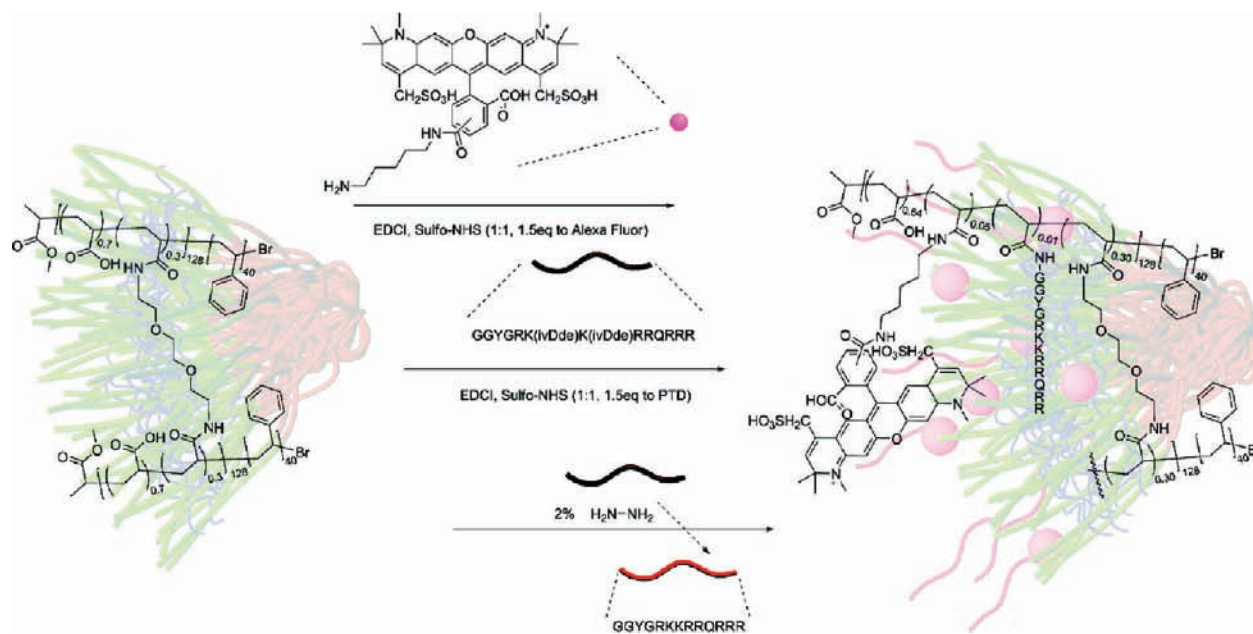
equipped with poly(ethylene glycol) (PEG) grafts of different molecular weight and grafting density, combined with  $^{64}\text{Cu}$  radiolabels for diagnostic imaging and full BioD assessment. Importantly, the grafting of PEG chains was performed on the block copolymers prior to formation of the SCKs, to achieve accurate determination and control over the grafting density on the final nanostructures. It was observed that five PEG grafts per block copolymer gave a PEG spacing,  $D$ , of ca. 1 nm, capable of resisting adsorption of large and small proteins<sup>2,3</sup> and resulting in extended blood circulation times. This approach proved to be a much more efficient route to obtaining NPs with high surface density of PEG and improved stealth properties, compared to a triblock copolymer of PEG-*b*-PAA-*b*-PS, even with longer PEG chain length, which is a traditional way of obtaining stealthy micellar nanostructures.

The concept of targeting TNPs to specific tissue or disease of choice is the fundamental premise of creating nanomedicines with high efficacy. The concept is not new; Paul Ehrlich postulated the magic bullet concept in the early 1900s, and it is still a highly appealing idea.<sup>4</sup> The rationale for creating such systems is simple: targeting drugs or contrast can substantially increase the efficacy of treatment,

minimize the injected dose, overcome toxicity issues, provide superior contrast (or superior local therapeutic concentrations), and allow for the intelligent design of personalized medicines specifically designed for each patient subtype. However, there are several conflicts that result from the competing effects from the use of ligands for active targeting by binding to biological receptors, including alteration of the BioD<sup>5</sup> by hitting other targets and immunogenic responses.<sup>6</sup>

Ligand-mediated targeting of nanoparticles, or “active” targeting, utilizes biomacromolecules, such as antibodies, proteins, peptides, aptamers, or small molecular entities attached to the surface of the nanoconstruct.<sup>7</sup> These ligands are expected to bind overexpressed or specific receptors in the tissue selected, followed often by receptor-mediated endocytosis for transport into cells. Whether the ligand targeting affects the PK or only the BioD is under debate. Studies have shown that ligand-targeted liposomes do not show a difference in the PK characteristics, but in the tumor localization (BioD). Similarly, studies have indicated that targeting NPs to a tumor has a more profound effect on the local tumor distribution, where the targeting ligand acts to distribute the NPs from the tumor stroma to an intracellular localization.<sup>8</sup> Such effects are suggested to be an effect





**FIGURE 2.** SCKs having fluorescent labels and Tat peptides were prepared by conjugation of the active units onto pre-established SCKs. Reprinted with permission from ref 11. Copyright 2008 American Chemical Society.

of the PEGylation of these nanocarriers, prohibiting effective targeting due to interference with receptor binding.<sup>8</sup> In most cases, the PEG shell is critical and cannot be excluded, as the NP construct would otherwise rapidly activate mononuclear phagocyte system (MPS) clearance mechanisms. However, other important factors have not been studied in great detail in vivo with respect to targeting, such as how particle shape<sup>9,10</sup> and flexibility affect targeting with the same ligand.<sup>11,12</sup> In such studies, nonbinding ligands of similar shape, size, and charge, for example, scrambled peptides for peptide-based targeting ligands, must be used as controls.

In the case of SCKs of varying shapes, the inherent carboxylic acid functionality of the hydrophilic poly(acrylic acid) shell layer has been utilized via the formation of amide bonds for cross-linking and the attachment of imaging agents, PEGs for circulation enhancement, and targeting components. Given that the SCK nanostructure contains thousands of carboxylic acids or activated esters,<sup>13</sup> there are many sites available, but the chemistry must be conducted with care to avoid loss of ligand binding function. For example, with peptides, this requires that the N-terminus is the only amino group reacted, in order to avoid intra- and intermolecular cross-linking. Conjugation of the protein transduction domain of human immunodeficiency virus type 1 Tat protein, a commonly employed cell penetrating peptide sequence having the sequence GYGRKRRQRRR, onto spherical and cylindrical SCKs was accomplished by using protected Tat peptides that could then be deprotected

(Figure 2).<sup>11</sup> It was found that smaller spherical Tat-SCKs (ca. 10 nm) were internalized by cells more rapidly than were longer cylindrical (ca. 10–30 nm diameter but lengths of  $\geq 200$  nm) nanostructures of the same composition. Differing degrees of Tat functionalization were also studied, for which it was observed that the highest Tat peptide concentration resulted in a 5-fold increase in cellular uptake, as compared to the naked SCK. In contrast, the opposite behavior was observed for spherical versus cylindrical SCKs labeled with folate targeting ligands.

The folate receptor (FR), or membrane-associated folate-binding protein, is one of the cellular transport systems that binds folates with high affinity in the nanomolar range and undergoes receptor-mediated endocytosis. Because the folate receptor is well characterized and is overexpressed in several cancer types with limited expression in normal tissue, the FR is an attractive target for nanoparticles to treat tumors.<sup>14</sup> Three studies have been reported for folate-labeled SCKs: one that involved synthetic methodology development,<sup>15</sup> another primarily in vivo oriented,<sup>16</sup> and one in vitro focused.<sup>12</sup> In the first studies, folate was placed on the terminus of a 1.6 kDa PEG spacer that was conjugated to the surface of pre-established SCKs, using their surface available COOH groups. These particles were further labeled with fluorophores and <sup>64</sup>Cu radioisotopes for in vitro and in vivo studies.<sup>16</sup> The in vitro experiments indicated rapid uptake of the folate-functionalized SCKs within 4 h at 37 °C and only a surface bound fraction at 4 °C, each of

which could be blocked with the addition of free folic acid. The BioD data *in vivo* indicated rapid and high MPS uptake (53% in liver and spleen in 10 min), suggesting that the folate-PEG did not provide a sufficient stealth layer and should be complemented with unfunctionalized PEG chains at a higher surface density. The BioD data showed no evidence of the benefit of folate conjugation on *in vivo* accumulation of nanoparticles in FR-overexpressing large tumors, for which the EPR effect appeared to dominate SCK uptake. However, there was some indication that smaller, less developed tumors may have been targeted by folate SCK-to-folate receptor interactions. In the most recent study, the focus was on investigation of how the *in vitro* cellular internalization was affected by the nanomorphology for spherical versus cylindrical SCKs.<sup>12</sup> Contrary to the results observed for the Tat-modified cylinders, it was found that folate-functionalized cylinders were better candidates for cell internalization than were folate-functionalized spheres. This effect may be a result of receptor clustering, where the cylinders are more capable of multivalent interactions due to their ("longer") size, and thereby triggering of multiple receptor-mediated endocytotic processes.

In order to explore the use of diverse and biologically complex targeting ligands, new conjugation methodologies have been developed for SCKs. One such example is the utilization of protected thiols, placed on the termini of PEG grafts presented topologically on the outer surface of the SCK nanoparticle, that can be deprotected/activated for further conjugation with thiol- or maleimide-containing targeting groups to form reversible disulfide or thioether linkages, respectively.<sup>17</sup> These SCKs were shown to have high thiol reactivity and could be used to attach the model protein albumin with good coupling efficacy. The thiol–maleimide Michael addition or furan–maleimide Diels–Alder conjugation are highly attractive conjugation strategies for the attachment of antibodies, since site-specific Fc-introduction of maleimide groups on antibodies is well established and demonstrated on nanoparticles to maintain binding efficacy *in vitro*.<sup>18</sup> Although not tested yet on the SCK scaffold, thiol groups can also be utilized in high yielding thiol–ene couplings.<sup>19</sup>

In our experience with SCK nanoparticles, it has been clear that achieving efficient and quantifiable targeting (including results not published) is indeed difficult. Our hypothesis is that the ligand presentation is not optimum in many targeted NPs and that the chemical design of the carriers must be more sophisticated. If the ligand is placed on a spacer unit such as PEG, the remaining PEG (necessary for

circulation enhancement) must be of sufficiently shorter length in order not to interfere with binding to the receptor. In addition, covalent attachment of the ligand to the spacer molecule must be such that the binding characteristics are not hampered. In the antibody, antibody fragment or protein case, this requires site specific modification reactions, and the employment of rapid, efficient and orthogonal (REO) ligation methods for covalent attachment to NPs.<sup>20</sup> With small molecule ligands, control is achieved more easily. Similarly, the nature of the spacer is worthwhile to consider, as one can imagine that a more rigid spacer with longer persistence length may well present the targeting ligand in a better conformation for receptor interaction. Jiang and Cao have demonstrated that zwitterionic polymers offer advantages over PEG for inhibition of protein adsorption,<sup>21</sup> and these polymers are quite attractive for SCKs because they also offer functionalizability. Strategies that employ cleavable or other linkages to bury the ligands until the target site (e.g., tumor) is reached may offer advantages for cell uptake and so on, but they fail to address the challenges associated with getting to that site.

Control over the route of administration of the nanoconstruct is a highly attractive means by which to exploit the advantage that the specific organ provides combined with selected targeting approaches. For example, localized therapy of urinary tract or lung infections may be achieved via catheter delivery to the bladder or inhalation delivery of aerosols to the lung, respectively. We are currently devoting significant efforts toward the treatment of infectious diseases by the direct administration of antimicrobial-loaded SCKs<sup>22</sup> to the lung and bladder. Preliminary *in vivo* tests have shown outstanding performance. Nasal delivery has been suggested as another direct administration method for achieving delivery in the brain, utilizing the olfactory pathway where the nervous system is in contact with the surrounding environment.<sup>23</sup>

A directed approach to actively target tissues *in vivo*, without the use of targeting ligands, utilizes superparamagnetic nanoparticles together with an external magnetic field to localize the drug carrier to the tissue of choice.<sup>24</sup> This method requires that the diseased tissue is known and can be visualized for guidance procedures. The concept is indeed interesting for some clinical applications, but several problems still exist, such as scaling up from the animal models, embolization of blood vessels in the area, controlling the magnetic field geometry, and dealing with biological clearance issues for nondegradable inorganic nanoparticles.

## Imaging Modalities

Positron emission tomography is a highly sensitive imaging modality, with which we have had great success in tracking SCKs in vivo. As with the PEGylation of SCKs, it was found that conjugation of chelators for the PET-active radionuclides was best performed onto the polymer precursors to allow for efficient coupling and accurate quantification and characterization studies to be performed.<sup>25</sup> Earlier attempts to couple chelators and perform radiolabeling onto pre-established SCKs experienced difficulties.<sup>26</sup> The advantages of nanoparticles for PET imaging applications has already been reviewed.<sup>27</sup> We have also begun developments of SCKs toward optical imaging modalities.<sup>28,29</sup>

Magnetic resonance imaging (MRI) is powerful in its ability to visualize soft tissues. Nanoparticles for MRI include both  $T_1$  (positive contrast) based agents, such as NPs containing rare earth metals,  $Gd^{3+}$ , and others, as relaxation aids, or superparamagnetic NPs, such as SPIONs for  $T_2$  (negative contrast) weighted imaging. Apart from these standard MRI applications, NPs for  $T_1$  imaging of other natural isotopes such as  $^{19}F$ ,  $^{31}P$ ,  $^{13}C$ ,  $^{14}N$ , and others have been developed. Of these isotopes,  $^{19}F$  nuclei are attractive due to the lack of background in normal tissue (only low levels in teeth and bone), good biocompatibility, sensitive chemical shift, high gyromagnetic ratio, and high natural abundance. We have explored the use of nanoscale  $^{19}F$ -MRI agents capable of both chemotherapeutic delivery and  $^{19}F$ -MRI either in the form of hyperbranched polymer micelles<sup>30,31</sup> or SCKs.<sup>32</sup> Fluorinated hyperbranched polymer micelles were initially investigated, since it was hypothesized that the multicompartamental amphiphilic structure of the polymer scaffold would allow sufficient mobility for the fluorinated component to achieve beneficial relaxation kinetics for imaging while also providing nanoscopic compartments for chemotherapeutic loading via hydrophobic interactions. We found that this design strategy indeed produced nanoparticles with one narrow  $^{19}F$  signal and much better  $T_1$  and slightly better  $T_2$  relaxation times for imaging applications compared to perfluorocrown ether (PFCE).<sup>31</sup> In vitro evaluation indicated low toxicity and good biocompatibility of the NP itself, but the absolute fluorine concentration was too low for in vivo imaging.<sup>30</sup> To overcome the limitations in fluorine concentration that the hyperbranched fluoropolymer system experienced, we also explored fluorinated SCKs, as these structures have shown high loading capacities for hydrophobic chemotherapeutics.<sup>33,34</sup> SCKs with fluorinated hydrophobic cores were further loaded with PFCEs having 15 chemically equivalent fluorine atoms. Despite the high fluorine

concentration, unfortunately, it was found that the hydrophobic core restricted the mobility of the PFCE to such an extent that the signal was not detectable without plasticizing the core.

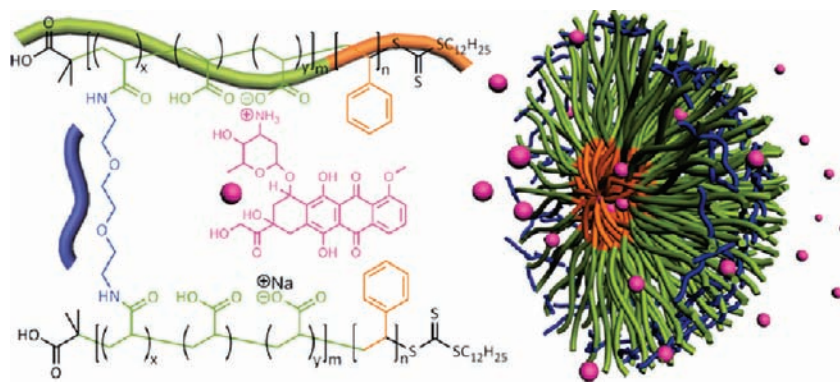
## Therapeutic Cargo

SCKs have been investigated as carriers of therapeutics directed against three main applications: cancer,<sup>33–35</sup> infectious diseases,<sup>22</sup> and acute lung injury. In drug delivery applications, SCKs are attractive candidates due to their stability, high loading capacity, and the broad tunability of composition, structure, dimension and morphology. Our interest has been to craft the SCK to optimize drug loading capacity and control the release kinetics. The amphiphilic core–shell morphology is taken advantage of, and we have performed studies that have involved packaging of therapeutics in the core, at the core–shell interface and in the shell, while tuning the compositions, properties, and dimensions of each.

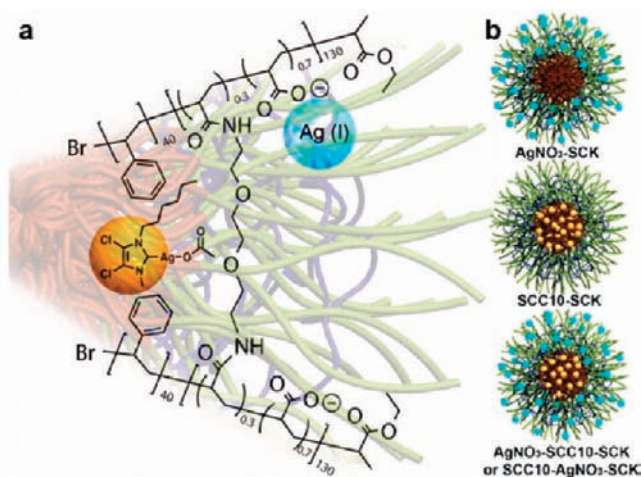
In one case, we probed effects of amorphous versus semicrystalline core domains, with the intention being to trap doxorubicin, an anthracycline antibiotic and a very common chemotherapeutic agent used in the treatment of many cancer types, within the semicrystalline core at room temperature and promote their release at physiological temperature.<sup>33,35</sup> We found that high loading concentrations (20% w/w) of doxorubicin in SCKs could be achieved with both amorphous and crystalline nanoparticle cores, due to the combined favorable hydrophobic and  $\pi$ – $\pi$  interactions for doxorubicin with the cores and ionic interactions with the SCK shells. The drug-loaded SCKs were found to kill U87 cancer cells via apoptosis in vitro, while no negative effect on cell viability was found from the SCKs themselves.

We also conducted an in-depth study of the effects of the particle dimensions, including overall diameter, core diameter, and shell thickness, on the drug release kinetics. Effects of the nanoparticle composition were held constant, by employing SCKs constructed from a single type of amphiphilic diblock copolymer (Figure 3). A series of four SCK nanoparticle samples, ranging in number-average hydrodynamic diameter from 14 to 30 nm, was prepared from four block copolymers having different relative block lengths and absolute degrees of polymerization. Their shell-to-core volume ratio showed impact on the rates and extents of release of DOX, with the volume occupied by the poly(acrylic acid) shell relative to the volume occupied by the polystyrene core correlating inversely with the diffusion-based release of DOX.





**FIGURE 3.** SCKs as carriers of doxorubicin, with the ability to tune the compositions and dimensions of the core and shell domains independently. Reprinted with permission from ref 34. Copyright Elsevier 2011.



**FIGURE 4.** Loading of silver-based antimicrobial agents within the shell or core-shell interface of SCK nanoparticles. Reprinted with permission from ref 22. Copyright Royal Society of Chemistry 2010.

We have also investigated the SCK scaffold for the delivery of silver-based antimicrobial agents, which are of interest for treating bacterial infections in the lung and bladder. Silver-based agents are interesting as antibacterial therapeutics because toxicity to human tissues is low and bacteria rarely develop resistance against silver-based agents. The SCKs were either loaded with  $\text{Ag}^+$  within the framework of the poly(acrylic acid) shell or silver carbene complexes that were expected to reside near the core-shell interface (Figure 4). The SCKs were found to have high loading capability and sustained release of the silver agents over the course of several days. These silver-containing SCKs were found to be effective inhibitors of infection in vitro against urinary isolates of *Escherichia coli* and respiratory isolates of *Pseudomonas aeruginosa*.

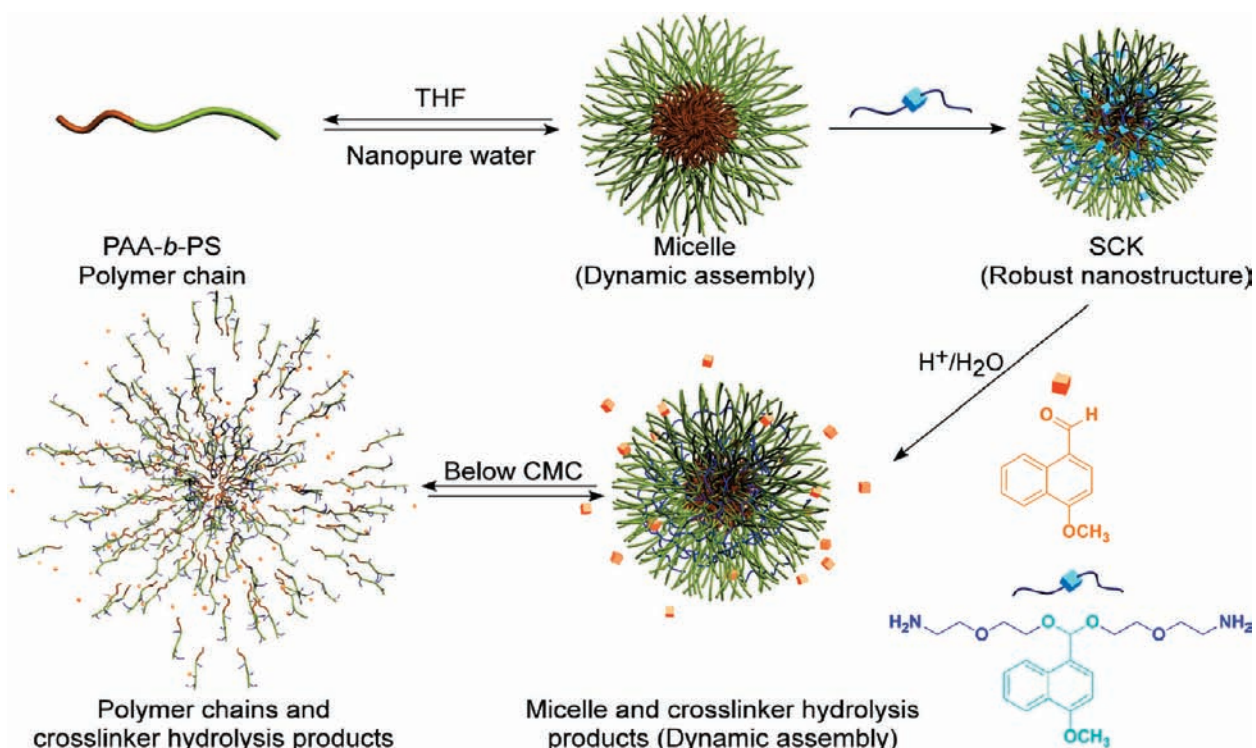
Using nature as a guide, SCKs have been prepared as synthetic analogs of histones,<sup>36</sup> of ca. 10 nm diameter and

carrying positive surface charge character, and studied for their ability to package nucleic acid-based materials (plasmid DNA, oligoDNA, peptide nucleic acids, siRNA).<sup>36–39</sup> Ultimately, the interest is in the delivery of nucleic acids for molecular recognition of mRNA overexpressed in sites of acute lung injury, to allow for diagnostic imaging and/or therapy. A current target is the mRNA of inducible nitric oxide synthase; however, much work remains to advance from promising in vitro cell transfection results to a working system in vivo.

## Degradability

For SCKs described so far, degradability has not been regarded as the primary objective, mainly because the physicochemical, in vitro, and in vivo studies have been time-consuming, and a rapidly degradable NP system would pose challenges with study prior to the disintegration of the controlled morphology. However, to successfully utilize nanoparticle constructs for therapeutic delivery in the clinic, clearance of the NP or its building blocks must be assured, while also avoiding toxic effects from the particles and degradation products. The metabolic fate of the nanoparticle and the single and multiple dose toxicity must be investigated, along with many other factors such as clearance routes.

For SCKs, both degradability of the cross-links stabilizing the micelles<sup>40</sup> and the polymer scaffold used for construction have been studied.<sup>41</sup> Creating pH-degradable crosslinks in the nanostructure is an interesting design feature that would allow degradability to be easily incorporated in any SCK construct, and result in the rapid disassembly of the nanoparticle. Provided that the constituent building blocks are small enough to allow for renal clearance, accumulation of the nanoparticle or its components can be avoided. To



**FIGURE 5.** SCKs containing degradable cross-links. Reprinted with permission from ref 40. Copyright 2008 American Chemical Society.

demonstrate hydrolytic cleavage of degradable cross-links, a self-reporting cross-linking system was utilized, having a UV–vis reporter unit that was released upon hydrolysis of acetal linkages (Figure 5). This system allowed for real-time monitoring of the degradation process *in vitro* via quantification of the released 4-methoxy-1-naphthaldehyde. It was found that the half-life of the neat cross-linker at pH 7.4 was 29 h and that incorporation of the cross-linker in the SCK nanostructure reduced the half-life to ca. 1.5 h; at pH 5.0, the half-life dropped to 13 min. The rapid acceleration of hydrolysis in the SCK shell suggests use *in vivo* for both therapeutic delivery and diagnostic imaging, as localization in diseased tissue should occur within a few circulation passes (seconds to minutes), followed by degradation of cross-links to release therapeutics and clear the background NP in circulation. Furthermore, the decreased half-life under acidic conditions will promote accelerated degradation and release in cells or tumor environment. Further, degradability of the polymer building blocks can be advantageous for nanoparticles that are composed of larger polymer chains, or for applications where complete degradation of the NP systems is desired.<sup>41–43</sup>

## Conclusions/Outlook

The primary advantage of theranostics is that they are not limited to therapy or imaging, but allow for combinations, to

give coincident diagnostic information plus delivery of therapeutics. Nanoscopic objects serve as scaffolds that have the optimum, intermediate size between the molecular level and microscopic materials, to provide domains for high capacities of therapeutic loading and sites for labeling, while also having high surface areas for the presentation of targeting ligands. Although imaging of the nanoscopic device and delivery of a molecular therapeutic does not offer dual tracking of each independently, having information about the conditions under which the therapeutic is released and the ability to monitor the nanostructure gives some indication of the theranostic benefit. However, if each is labeled separately or if the nanoscopic diagnostic agent is the therapeutic, then dual tracking is built in. This result occurs with labeled protein or antibody therapeutics, but could also be made to be true, for instance, if synthetic nanomaterials could be advanced to the point of offering therapeutic benefit directly, by triggering biological responses without the need for release of small molecule therapeutics. We are particularly interested in studying hierarchically assembled nanomaterials that have complex structures and functions,<sup>44,45</sup> and can trigger unique biological responses or carry multiple diagnostic and therapeutic payloads.

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**Andreas M. Nyström** received his M.Sc. in Chemical Engineering and Polymer Technology in 2002 from the Royal Institute of Technology (KTH) in Stockholm, Sweden, after which he continued with his Ph.D. (engineering) in dendrimer chemistry under the guidance of Prof. Anders Hult at KTH. In 2006, Andreas joined Prof. Karen L. Wooley at Washington University in Saint Louis for a 2 year postdoctoral position, financed by a distinguished assistant professor and postdoctoral grant from Knut and Alice Wallenberg foundation. In 2009, Andreas joined the faculty at Karolinska Institute (KI) of Medicine as an assistant professor working with polymeric nanosystems for cancer therapy and imaging, and in 2010 joined the Swedish Medical Nanoscience Center at KI as an Associate Professor of Nanomedicine. Andreas serves as international reviewer for the Czech National Science Foundation and is a part-owner and member of the management of Polymer Factory AB, a company that is commercializing polyester based dendrimers.

**Karen L. Wooley** holds the W. T. Doherty-Welch Chair in the Department of Chemistry at Texas A&M University, with a joint appointment in the Department of Chemical Engineering. She received a B.S. in chemistry from Oregon State University in 1988 and then studied under the direction of Professor Jean M. J. Fréchet at Cornell University, obtaining a Ph.D. in polymer/organic chemistry in 1993. She began an academic career as an assistant professor of chemistry at Washington University in St. Louis, Missouri, was promoted in 1999 to full professor with tenure, and was installed as a James S. McDonnell Distinguished University Professor in Arts & Sciences in 2006. In 2009, she relocated to Texas A&M University. Research areas include the synthesis and characterization of degradable polymers, unique macromolecular architectures and complex polymer assemblies, and the design and development of well-defined nanostructured materials, for which she has received several awards, including an Arthur C. Cope Scholar Award, a Herman F. Mark Scholar Award, and awards from the National Science Foundation, the Office of Naval Research, and the Army Research Office. Karen serves as an Editor for the *Journal of Polymer Science, Part A: Polymer Chemistry*. She directs a NHLBI-supported Program of Excellence in Nanotechnology, serves on the National Institutes of Health NANO study section, and also serves on the Scientific Advisory Panel for the NIH

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#### FOOTNOTES

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