lectively react with fuctionalized disulfides [1]. This method was used to append azide groups on CPMV. The azide groups were then utilized in a 1,3 dipolar cycloaddition reaction [12] with modified alkynes to affix the desired functionality on the virus. Importantly, in this study Finn and coworkers also discovered that it is possible to stitch a virus particle together at adjacent subunits through the reaction of a neighboring pair of tyrosine residues to form dityrosine crosslinks. It is puzzling that despite extensive "darning" of the viral coat with such intersubunit crosslinks, these particles were not observed to be more stable than the unmodified virions. Given the fairly rugged nature of CPMV, this finding is probably more of a curiosity than a concern and can probably be rectified by directing crosslink formation at more labile positions on the capsid. Overall, the studies by the Finn and Francis groups significantly increase the diversity of available reactions that can be utilized to decorate virus particles.

Potential applications of viruses as chemical reagents include the formation of nanometer-scale circuitry upon the appropriate immobilization of metal particles [5, 13], the ability to encapsulate drugs or inorganic species in viral cages [14], the possibility of creating isolated catalytic chambers inside viral capsids, and the use of virions as scaffolds for the polyvalent display of bioactive ligands [15, 16].

What lies beyond modification of virions at lysine, cysteine, aspartic acid, glutamic acid, and now tyrosine? The palette for decorating reactive residues on viral proteins has become quite robust. Further developments may await techniques for the introduction of nonnatural amino acids into viral proteins, which could potentially enable a new suite of synthetic strategies [17]. In addition, there is a wide diversity of nanometer-scaled protein scaffolds other than viruses, such as chaperonins. Now some of these are also being modified [18, 19], along with self-assembled DNA arrays [20].

We can eagerly anticipate that chemists will continue to exploit virus particles for nanotechnology applications. After all the sustenance higher organisms have provided viruses, isn't it about time they did something for us?

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Heparin-Induced Cancer Cell Death

Heparin uptake into cancer cells can be promoted by conjugation to poly (β-amino ester)s. Internalized heparin is cytotoxic, causing cancer cell death by interfering with transcription factor activity and inducing apoptosis, but only certain poly(β-amino ester)s promote this activity.

The heparin polysaccharide is among the most acidic of all natural products with a single molecule carrying from 75 to 100 negative charges [1]. As a result, heparin interacts with a large number of proteins and other basic molecules through ionic and hydrogen bonding interactions [2]. A widely used therapeutic anticoagulant, heparin is biosynthesized and stored intracellularly exclusively in mast cells. Mast-cell-rich animal tissues, such as porcine intestine and bovine lung, are used as commercial sources of heparin. Many of the biological activities ascribed to heparin (i.e., anticoagulation, regulation
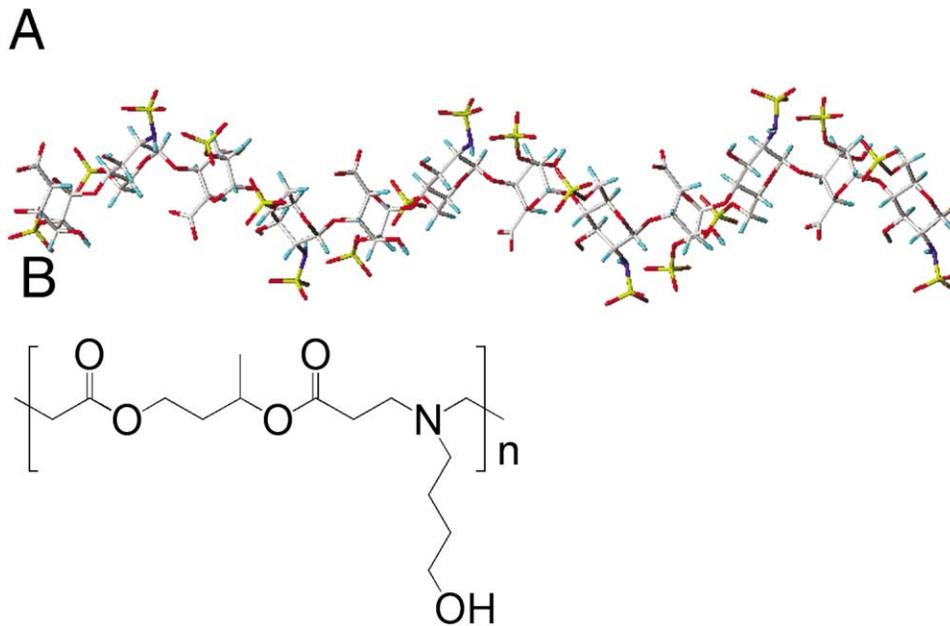
Figure 1. Structure of Heparin and Poly (β-Amino Ester)
Structure of a small heparin chain (A) and that of a poly (β-amino ester) (B) capable of carrying heparin into the cell.

of cell differentiation and cell growth through growth factors, control of chemokine signaling, etc.) are more correctly ascribed to the structurally related intercellular glycosaminoglycan, heparan sulfate [3].

Because of the highly charged nature of heparin and related glycosaminoglycans, it is hard to imagine a mechanism for its uptake by cells through passive diffusion across cell membranes. There is, however, evidence that subcutaneously administered heparin [4] and even orally administered heparin [5] can cross barriers
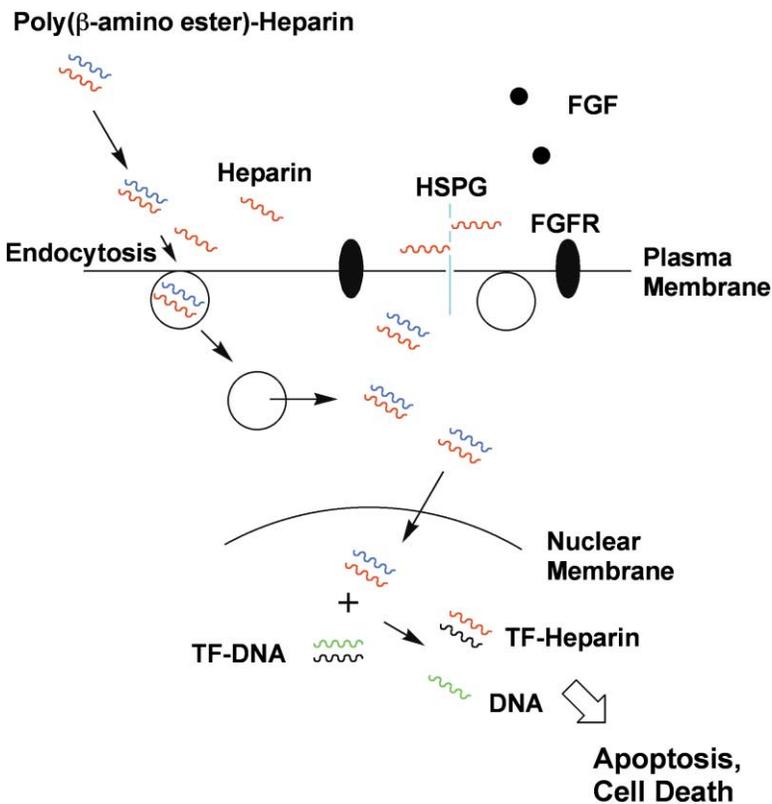

Figure 2. Heparin-Induced Cell Death
Proposed endocytotic mechanism for heparin cellular uptake of heparin (red) poly (β-amino ester) (blue) complex escape, distribution through the cell, displacement of DNA (green), complexation with transcription factor (TF in black), and resulting apoptosis and cell death. Fibroblast growth factor (FGF) and fibroblast growth factor receptor (FGFR) interact with heparan sulfate proteoglycans (HSPG) and form a complex [8] that can be similarly transported into the cell [7].

and enter cells. The bioavailability of glycosaminoglycans can be enhanced through the use of cationic and peptoid-based excipients [6] or by reducing their molecular weight (i.e., low molecular weight heparins [1]). There is also evidence that heparan sulfate and related glycosaminoglycans in the ECM and on the cell surface can be internalized by cells while bound to receptors [7]. For example, the uptake of heparin involves complexation [8] and internalization [7] with fibroblast growth factor and fibroblast growth factor receptor.

Most research on heparin has focused on its anticoagulant activity—this has resulted in the development and successful introduction of low molecular weight heparins into the marketplace [9]. Heparin and related glycosaminoglycans, however, interact with a diverse group of proteins [2] exhibiting a multiplicity of important physiological and pharmacological biological activities [1, 2]. Among the most recently discovered and exciting of these activities is the role of heparin in cancer [10, 11, 12]. While initial discoveries pointed to its important role in regulating growth factor activity [8], recent studies [10] including the one by Berry and coworkers in this issue [13], suggest the anticancer activity ascribed to heparin is considerably more complex.

The work of Berry and coworkers results from a marriage of the polymer technology pioneered in the Langer laboratories and the glycobiology focus of Sasisekharan's laboratory. Poly (β-amino ester)s are one of a number of types of basic polymers that are used for delivering DNA into cells [14]. In pursuing this line of research, these investigators found that heparin could block cellular uptake of the poly (β-amino ester)–DNA complex. Moreover, these poly (β-amino ester)s promoted cellular uptake of heparin, particularly highly sulfated full-length (molecular weight average 12,000) heparin, suggesting that heparin was competing with DNA for poly (β-amino ester)s. A small library of varied poly (β-amino ester)s were next examined for the members' abilities to bind and internalize heparin. Of the 70 poly (β-amino ester)s examined, most bound heparin but only 14 enabled its internalization. This selectivity suggests that an appropriate match between the structure of heparin and the structure of the basic polymer (Figure 1) is essential in promoting cellular uptake. Furthermore, the localization of intracellular heparin was not limited to endosomes and lysosomes. Fluorescently labeled heparin, used to demonstrate internalization, was uniformly distributed throughout the cell. Most intriguingly, the internalized heparin promoted cell death. There are a number of possible mechanisms for the cytotoxicity of internalized heparin. One of the most obvious, interference with the fibroblast growth factor signal transduction pathway, was shown to be unaffected by internalized heparin. Detailed studies by Berry and coworkers [13] suggest that poly (β-amino ester)–heparin complexes affect cellular processes including inducing transcription factor

and caspase activation, ultimately inducing apoptotic cell death. The heparin-poly (β-amino ester) complex appears to be internalized through endocytosis and may enter the cytosol through lysosomal escape mediated by cationic poly (β-amino ester) [15] (Figure 2).

The significance of this study for influencing the design of future cancer-killing compounds is clear. Cancerous cells have faster endocytic rates than normal cells, resulting in enhanced rates of heparin-poly (β-amino ester) uptake and thus, poly (β-amino ester)-mediated internalization of heparin might offer a new, selective approach for inducing cancer cell death. This study should also result in a better understanding of endocytic uptake of both heparin and DNA polyanions. Moreover, since cell surface heparan sulfate is similarly transported, this study may offer an improved understanding of growth factor [7] or even virus uptake [16] by cells through their heparan sulfate receptors [3].

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