Commercialization and Future Developments in Bionanotechnology

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The perfection of nanotechnology has long been achieved by biological systems. An enzyme represents a near perfect robot, stamping out molecular patterns from unique templates, each designed to execute its task with near perfect efficiency. Evolution has driven this design, driven the efficiency higher, as needed for life in harsh environments. This improvement process has run for at least 3.5 billion years, and the results are quite impressive. The fact that we are here at all is a vast testament to the power and potential of nanotechnology. Recently, however, we have begun with our first blunt-fingered attempts to extend the capabilities of biological systems. Our first attempts have harnessed innovations in materials chemistry and electronics, and coupled these to biologically defined specificity for both magnetic and fluorescent probes. In these cases, we have introduced relatively limited functionality to the existing biological systems, but the potential exists for far greater engineering into these systems and the applications from here will undoubtedly expand dramatically. However, at the present time, we are guided by empirical observations, not by a detailed understanding of the interactions of the biological systems with the materials and devices we are preparing. As such, we are not only blunt-fingered, but we are also nearly blind. We will have to expand our efforts dramatically in the development of new characterization methods, and the basic specifications and predictors of biological performance before substantial commercial rewards and benefits in health and medicine can be realized from the emerging bionanotechnology products.

What is Bionanotechnology?

At the present time, there is no consensus definition of bionanotechnology. Funding agency enthusiasm has led a number of old (and important) areas to be relabeled "nanotechnology" including colloid science, molecular biology, and implantable materials surface science. Coupled with biological systems, all of these fields should be considered to be bionanotechnology. In general, however, the idea of engineering the interface between molecules or materials and biological systems can be taken broadly to encompass bionanotechnology. Looking forward, however, bringing engineered systems into biological contact and biological function is a key area where commercialization will continue.

The general idea of bionanotechnology, however, that has been popularized by the lay-media is largely oversold. This idea, popular 20 years ago as the "magic bullet" theory of biotechnology, and now adopted as the bionanotechnology target, can be described as the "dump-truck" model of technology. In this model, the technology components consist of a targeting moiety, which could be biological or nanotechnological, and one or more cargos, which are envisioned as small machines, capable of specific destructive or corrective action. Design of targeting molecules,

selective for diseased tissues and capable of targeting cargo larger than the typical antibody has proven extraordinarily difficult, and molecular targeting of nanoscale devices greater than 5 nm outside of the vascular space may prove to be prohibitively difficult. However, the lack of guiding principles for effective biological direction of non-biological molecules leaves this as a currently open question.

Here, I will discuss three recent examples of commercialized bionanotechnology, in order in decreasing characterization of the final nanotechnology system—the Antibody Directed Enzyme Prodrug Therapy (ADEPT), SuperParamagnetic Iron Oxide particles for MRI contrast enhancement, and the application of quantum dot technology in biological detection. Each of these examples shows the potential power and some of the challenges of integrating technology at the molecular level.

Examples of commercial bionanotechnology

Perhaps the most salient and relevant example of a bionanotechnology undergoing commercialization is the ADEPT methods currently being explored by Genencor and Seattle Genetics. In this method, an antibody-enzyme fusion is prepared and isolated. This molecule can be designed with precise chemical (biological) composition, precise linkage geometry, and complete definition and characterization using standard molecular biology techniques and biochemical methods. The modification of the enzyme with the antibody fragment can be used to target the particular antibody-enzyme conjugate to the site of interest. In this way, a small antibody fragment is used to target a molecular machine (an enzyme) to a particular site of interest, and then the machine is used to generate a specific molecule at that site. In the clinic, a pro-drug (a drug molecule modified to an inactive state that can be converted to active drug "in-situ") is administered to the patient. At the site targeted by this particular antibody-enzyme construct, the pro-drug is converted to an active drug. The local concentration of the active drug can be driven very high, while maintaining very low overall concentration, improving the safety and efficacy of the therapy. This is a highly characterized, and highly effective example of bionanotechnology in action. However, in spite of 15 years of active research, these drug strategies are still in the research and early clinical trials, and not in general practice. This is a testament to the complexity of developing biospecific performing technologies, and is likely to be a general problem for the development of nanotechnologies with high clinical impact.

A second, and more recognizable, example of bionanotechnology in the clinic is the Ferridex and Combidex particles which are commercialized for MRI (magnetic resonance imaging) contrast enhancement in medical imaging by Advanced Magnetics. These particles are nanoscale magnetic particles, that cause MRI signal enhancement because they are superparamagnetic. Modification of these particles with dextran (a polymerized sugar molecule) makes a biocompatible coating which dramatically reduces the nonspecific interactions in the body. These particles, when administered intraveneously, can easily be measured in a standard clinical MRI imaging instrument, enhancing the sensitivity of the instrument wherever they are present. These materials are currently approved for liver and spleen imaging of cancers. Recently, however, the Combidex agent was rejected by the FDA due to safety concerns and a lack of efficacy data that arose when at least one patient died in a clinical trial investigating the use of this agent for sentinel node detection (finding the near-nodes that are most likely to contain cancerous cells—critical to grading, staging, and treating cancers). In this case,

however, the FDA did recommend that with further appropriately designed trials, the compound may be approvable for specific indications.

The final example is one that I have been extensively involved in: the use of fluorescent quantum dots for biological detection in research and ultimately clinical applications. In this application, semiconductor nanocrystals (Quantum Dots—QDs), specifically designed to have intense monochromatic emission spectra, can be coupled to biological targeting molecules, such as antibodies and nucleic acids, and can then be used to detect the presence of particular analytes in biological samples. While these particles afford dramatic improvement in experimental complexity and sensitivity, they have been slowly adopted, due to subtle protocol differences between these materials and the typical fluorescence dyes used in detection. Many of these protocol differences are thought to arise from distinct differences in the size of typical probes and the nanotechnology based probes, and such idiosyncracies are likely to be ubiquitous in nanotechnology enabled product commercialization.

Outlook

Where then, will bionanotechnology take us. These examples have progressed from a completely characterized system with defined molecular structure (ADEPT), still encountering difficulty in effecting a clinical improvement, to a system where each of the components is well characterized (Combidex), to a system which is still lacking in fundamental characterization capability (QDs). Chemists have tools like mass spectrometry and NMR to guide them. Engineers have test and measurement systems to validate systems as small as a few hundred nanometers. In the middle, though, the nanoengineer (or the nanochemist) is still lacking in the fundamental tools to determine how his job has been done, or in what directions to look for improvements. Devices are synthesized on molar ($\sim 10^{26}$) scales, but the characterization tools designed for molecules do not work effectively. Clearly, the device characterization methods (typically single "device" characterization on enough devices to ensure a reliable measurement of the production run statistics) are inappropriate, especially when a dose is 10^{13} devices and a minor population component can dominate the bad effects (for instance pore-clogging).

There is currently an acute and growing need for specifiability in the design of bionanotechnology tools. To achieve this hallmark of engineerable systems, a concerted effort should bring basic scientific investigation of the impact of materials properties on the biological behavior together with physical scientific investigation of new methods to characterize the detailed physical and population properties of nanometer scale materials and components. Such work will yield predictability, falsifiability, and rapid progress in commercial bionanotechnology.

Further Reading:

ADEPT Technology:

Alderson, R. F., Toki, B. E., Roberge, M., Geng, W., Basler, J., Chin, R., et al. (2006). Characterization of a CC49-based single-chain fragment-beta-lactamase fusion protein for antibody-directed enzyme prodrug therapy (ADEPT). Bioconjugate Chemistry, 17(2), 410-418.

Bagshawe, K. D., Sharma, S. K., & Begent, R. H. J. (2004). Antibody-directed enzyme prodrug therapy (ADEPT) for cancer. Expert Opinion On Biological Therapy, 4(11), 1777-1789.

Wu, A. M., & Senter, P. D. (2005). Arming antibodies: prospects and challenges for immunoconjugates. Nature Biotechnology, 23(9), 1137-1146.

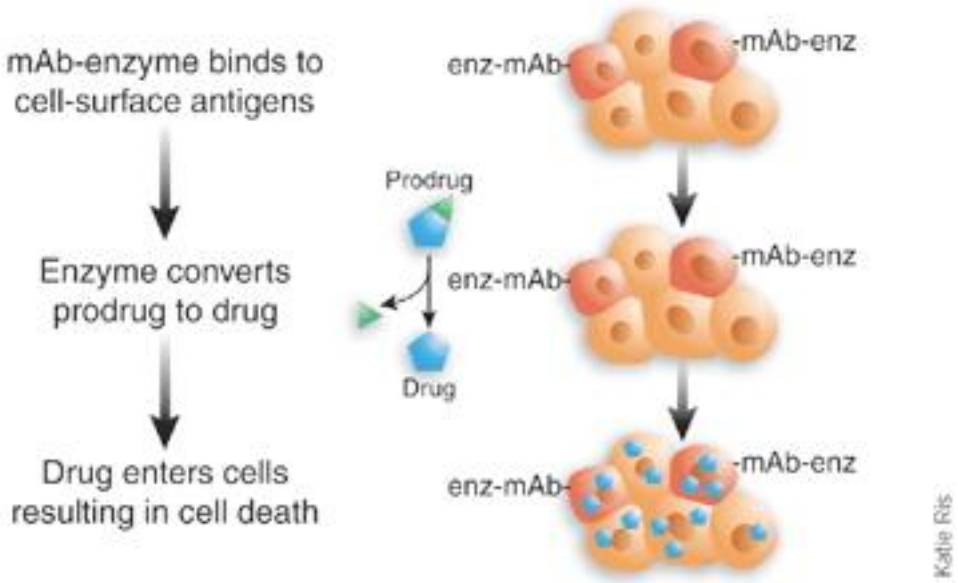
Combidex Technology:
Harisinghani, M. G., Barentsz, J., Hahn, P. F., Deserno, W. M., Tabatabaei, S., van de Kaa, C. H., et al. (2003). Noninvasive detection of clinically occult lymph-node metastases in prostate cancer. New England Journal Of Medicine, 348(25), 2491-U2495.

Harisinghani, M. G., & Weissleder, R. (2004). Sensitive, noninvasive detection of lymph node metastases. Plos Medicine, 1(3), 202-209.

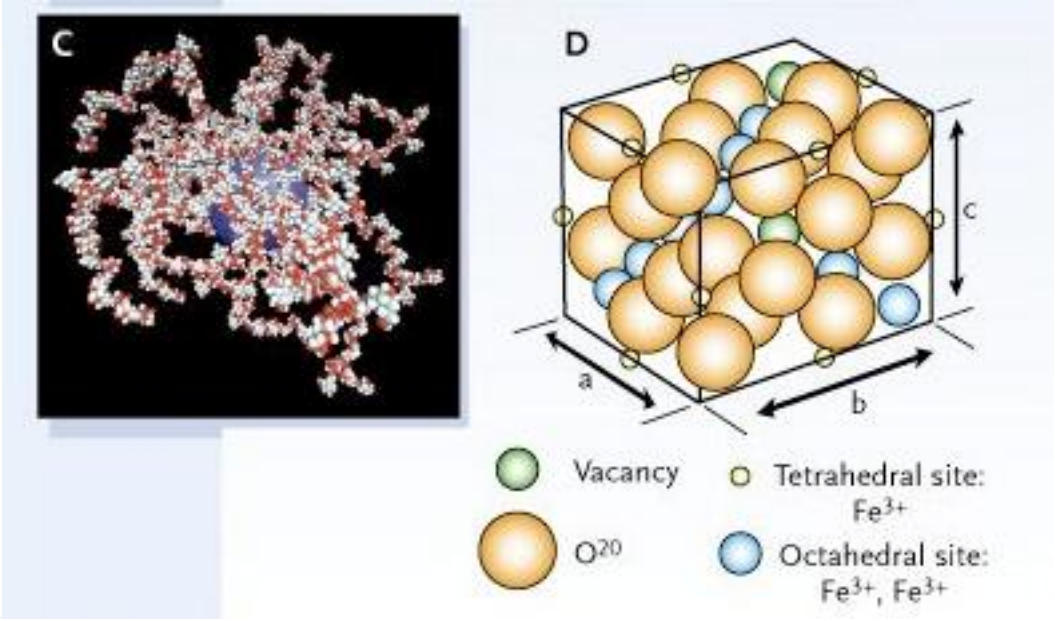
Quantum Dot Technology:
Alivisatos, P. (2004). The use of nanocrystals in biological detection. Nature Biotechnology, 22(1), 47-52.

Michalet, X., Pinaud, F. F., Bentolila, L. A., Tsay, J. M., Doose, S., Li, J. J., et al. (2005). Quantum dots for live cells, in vivo imaging, and diagnostics. Science, 307(5709), 538-544.

Figure 1. Three examples of commercialized bionanotechnologies. A. The Antibody Directed Enzyme Prodrug Therapy (ADEPT) uses antibodies to target particular cells with an enzyme which then converts prodrug molecules to active drug at the localized site. B. Combidex particles for MRI contrast enhancement rely on superparamagnetic iron oxide nanoparticles covered with dextran. These particles are excluded from tumor bearing regions of lymph nodes, helping to identify the tumor bearing nodes non-invasively. C. Qdot conjugates have bright emission and multicolor capability, allowing researchers to view more targets in a single sample, and simplifying detection strategies.


mAb-enzyme binds to cell-surface antigens
Enzyme converts prodrug to drug
Drug enters cells resulting in cell death
Prodrug
Drug
enz-mAb-
-mAb-enz
Katie Ris

A.


C
D
c
a
b
Vacancy
Tetrahedral site: Fe^{3+}
O^{20}
Octahedral site: Fe^{3+}, Fe^{3+}

B.

C.