Nanomedicine in a Nutshell

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ABSTRACT

Biology is the only known phenomenon which uses molecular nanotechnology – the manipulation of matter locally and deliberately on the atomic or molecular scale. It follows then that biological conditions and diseases must originate from alterations in these nanoscale processes. Mutated genes, misfolded proteins and infections caused by nanoscale viruses and pathogens can lead to cell malfunction or miscommunication, eventually leading to life-threatening diseases. The nascent field of nanomedicine has attempted to treat these systems by interacting with them at their native scale using engineered materials and systems with features in the nanoscale range (0.1-100 nm). In this Review, we highlight the uses of nanomedicine in the diagnosis, treatment and prevention of disease, as well elucidate a common mechanism of action used by many nanomedicine technologies. The incredibly high surface to volume ratio of nanoscale materials allows multivalent presentation of biologically functional molecules. This leads to high local concentration and the enhanced rates of reaction essential for biological processes. Here we show that optimizing this enhancement effect using fundamental principals of statistical mechanics is achievable. The application of this framework to engineering nanomedicine technologies is also presented.

Introduction

Molecular nanotechnology, generally defined as the deliberate manipulation of matter with spatial and temporal precision at the molecular scale, has been long dreamed of by scientists. This field was arguably started by the famous 1959 talk by Richard Feynman entitled "*There is Plenty of Room at the Bottom*" (*1*). In this revolutionary talk, Feynman acknowledged that in pursuing nanotechnology we are indeed only entering a domain with a long-accomplished master; biology. Biology already utilizes molecular nanotechnology to incredible ends; biological nanomachines and processes at the molecular or nanoscale level are able to produce complex products and emergent phenomena, including other nanomachines. Feynman was first inspired to propose the field of nanotechnology by the incredibly dense information storage used in biological systems(*1*). Perhaps motivated by his farseeing vision, the field of nanotechnology began to flourish, with the discovery of molecular beam epitaxy at Bell Labs in 1968 the controlled synthesis of nanoparticles, and the invention of the scanning tunneling microscope for nanoscale manipulation (*2*). Since this time, new phenomena and opportunities have been realized in many branches of technology, including the electronics, chemical and biomedical industries. Fast and energy efficient electronic circuits, biosensors, "smart" polymeric materials and novel surface coatings are some examples of this transdisciplinary field (*3-5*). The field of nanotechnology is still a nascent one, but the rapid emergence of practical applications clearly demonstrates its enormous potential.

The specific application of nanotechnologies to medicine (termed nanomedicine) has been trumped as a marriage of fields yielding an offspring set to bring momentous advances in the fight against a range of diseases (Figure **1)** (*6-8*). The application of nanotechnology to medicine (and biology in general) is a natural one, since biological conditions and diseases originate from alterations in the nanoscale and molecular processes in biology. Mutated genes, misfolded proteins and infections caused by nanoscale viruses and pathogens can lead to cell malfunction or miscommunications, eventually leading to life-threatening diseases. By treating and diagnosing diseases at their native (nano-) scale using engineered materials and systems with features in the 0.1-100 nm range, nanotechnology offers new ways to create better diagnostic cools for non-invasive screening of diseases, safe and effective therapies that are patient-specific and the power to prevent infections directly at the site of action (*9*).

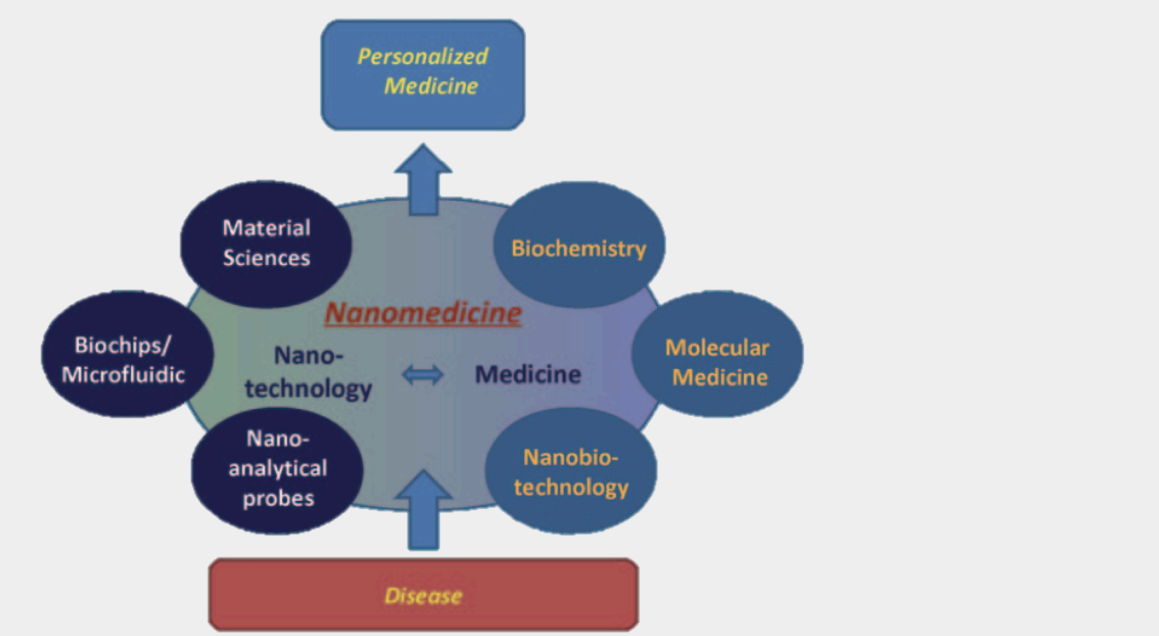

Fig. 1: The general trend of nanotechnology research and development, including the different technologies and fields involved(*9*).

In this Review, we discuss the field of nanomedicine in its broad strokes, and summarize specific examples of applications in the diagnosis, treatment and prevention of disease. The complexity of biological systems and the historical lack of detailed molecular mechanisms of many diseases have resulted in the nanomedicine field being a largely empirical enterprise. In the process of examining this field, we will present a common mechanism of action shared by many nanomedicine implementations. By staging the mechanism of nanomedicine action in a statistical mechanical framework, a rational method of engineering and optimization of the effectiveness of a nanomedicine diagnosis or treatment is presented. Specific examples given are selected to demonstrate the phenomenon of engineered high local concentration and its applications in nanomedicine. To begin, it is instructive to give an overview of concepts and principals of nanomedicine.

Principals and Concepts

While nanotechnology can be relatively easily and generally defined as the deliberate control of nanoscale (0.1–100 nm) features or materials, and nanomedicine is essentially applying this to medicine, it is useful to differentiate nanomedicine from the fields of molecular medicine and nanobiotechnology. The differentiation between these fields emerges from their focus, with nanomedicine focusing on the applications of nanotechnology directly to medical applications, while nanobiotechnology broadly covers all basic research in nanoscience on biological systems, including those which have little relation to medicine such as nanoengineered pesticides or fungicides for use on plants (*9*). Molecular medicine meanwhile focuses on a more conventional biochemical approach of drug discovery (*10*).

Just as medicine focuses on the diagnosis, treatment and prevention of disease, nanomedicine can also be broadly classified into these three areas. While traditionally, medical diagnosis is the process of linking observable bodily symptoms to a condition or disease, nanomedicine takes a different tact, and instead diagnoses diseases or conditions through analysis and quantification of an appropriately chosen biological marker. For treatments and therapies, in contrast to conventional surgery, chemical or radiation approaches which basically attempt to remove diseased cells faster than

healthy cells, nanomedicine uses a more sophisticated and targeted approach to either kill specific cells or repair them one by one. In order to prevent infection, nanomedicine uses specific knowledge of the molecular mechanism of the pathogenic or infectious agent to prevent harmful interactions with cells and tissues.

Diagnosis

Diagnostic technologies play a very important role in medicine to enable the successful prevention and effective treatment of diseases. Cancer is the canonical example which exemplifies this role, and it remains the leading cause of death in industrial countries. The WHO has reported that nearly one third of the cancer burden could be avoided if efficient early-detection diagnostic techniques could become widely available (*11*). Nanomedical diagnostics hold promise to fill this need due to their potentially higher sensitivity and selectivity compared to classical methods (*9*).

As with most nanomedicine technologies, the power of nanostructure-based diagnostics comes with interacting with the body (and the cause or mechanism of disease) at the molecular scale. To perform this task however requires knowledge of the molecular mechanisms of the disease or condition to be diagnosed. Nanomedical diagnostics depend on the knowledge of a biological marker (or biomarker) which can be used as a proxy for the presence or absence of a disease. An easy example is the heavily researched field of HIV diagnosis (*6*). Upon infection with HIV, the body's cells act as factories for the HIV virus particle and rapidly over a period of weeks create many trillions of copies, resulting in a high blood concentration of virus which can be detected. This concentration does not persist however. After some months, the disease enters a different state, and not wishing to overwhelm its host by producing too many viruses, begins to lower production until it reaches a steady low virus concentration which persists for the rest of the patient's life. In this case, the cause of the disease itself (the HIV viral particle) would be a poor biomarker, since a diagnosis based on this molecule would only be feasible in the first few months following infection. However often the alternative to detecting the instigator of the disease itself is to detect the body's response to the disease. Whenever the body's immune system detects foreign invaders, it develops antibodies to attempt to prevent infection. The general idea is that these antibodies will find and either inhibit the invaders mechanism of action, or label them for later destruction and disposal by other bodily systems. In the case of HIV these antibodies are not very effective, but their presence is a characteristic indicator that an HIV infection exists. By understanding the mechanism in which anti-HIV antibodies are developed by the body, they can be identified in blood samples and the magnitude of their presence can be linked to the presence and progress of AIDS.

In order to sensitively and specifically detect a biomarker of interest, a biosensor must perform two actions; first a biochemical recognition event must occur, in which a target biomarker is recognized by the device through a binding or unbinding event, and secondly this event must cause a measurable change in the device which can be transduced to a signal readable by a human user or another secondary device such as a change in color, fluorescence, or bulk electronic properties. Nanostructure-based diagnostics are capable of increasing the efficiency of both of these steps.

The identification of the molecule or structure used for the recognition of the biomarker is a biochemical problem, and the nature of this molecule is not essential to the discussion of the intrinsic advantages of nanomedical diagnostics. This recognition molecule is likely a biomolecule itself, either specifically coded to interact with the biomarker of interest (perhaps through complementary DNA/RNA basepairs), or through some more exotic selection method such as the development of a monoclonal antibody, or the evolution of a DNA aptamer designed to bind the biomarker of interest (*12*). Some examples of recognition molecules used with nanoparticle-based diagnostics are shown in Figure 2 below. However this recognition event occurs, its effect can be amplified by a common quality of nanostructured systems; their extremely high surface area to volume ratio which may be orders of magnitude greater than that of macroscopic

materials. Cutting a 1-cm cube into 10^{21} cubes each 1nm on a side will result in the same overall volume and mass, but the surface area will be increased by a factor of 10 million. Thus it can be readily seen that the advantages of using nanostructured materials as biosensors is that their surface can be coated with many times more recognition molecules than a material with macroscopic dimensions. For this reason, current nanomedical diagnostics generally utilize functionalized nanoparticles – tiny particles with diameters in the range of 1-100nm which are coated with a recognition molecule of choice. These types of systems are able to present very high effective concentrations of recognition molecules, which directly results in increased sensitivity of detection due to the simple fact that a stray biomarker is much more likely to encounter a recognition molecule in a high surface area nanostructured system. Furthermore, once the recognition event has occurred, the multivalent presentation of many recognition molecules on a nanostructure surface makes it very unlikely that a biomarker will escape. While a single recognition molecule might have a certain probability of losing hold of a biomarker, a particle completely covered with recognition molecules creates a very high local concentration in the vicinity of the biomarker, drastically increasing the effective rate of binding and decreasing the rate of escape. It is worth noting that not only can the sensitivity of a biosensor be increased through nanomaterials, results from the Mirkin group have shown that the geometry of densely packed DNA recognition molecules on a nanoparticle surface makes the sensor much more picky (or selective) in which complementary DNA biomarker the particle will bind to (*13*). While DNA recognition molecules layed out on a flat surface might be able to discriminate between a 90% and a 100% DNA sequence match, Mirkin's spherical nucleic acid nanoparticles can easily detect a single basepair mismatch. This increase in sensitivity and selectivity associated with nanostructure-based biosensors depends heavily on the physical parameters of the system, including the nature of the biomarker (monovalent or polyvalent, membrane-bound or free in solution), the density of recognition molecule on the nanoparticle, as well as the nature of the linker between the nanoparticle and the recognition molecule.

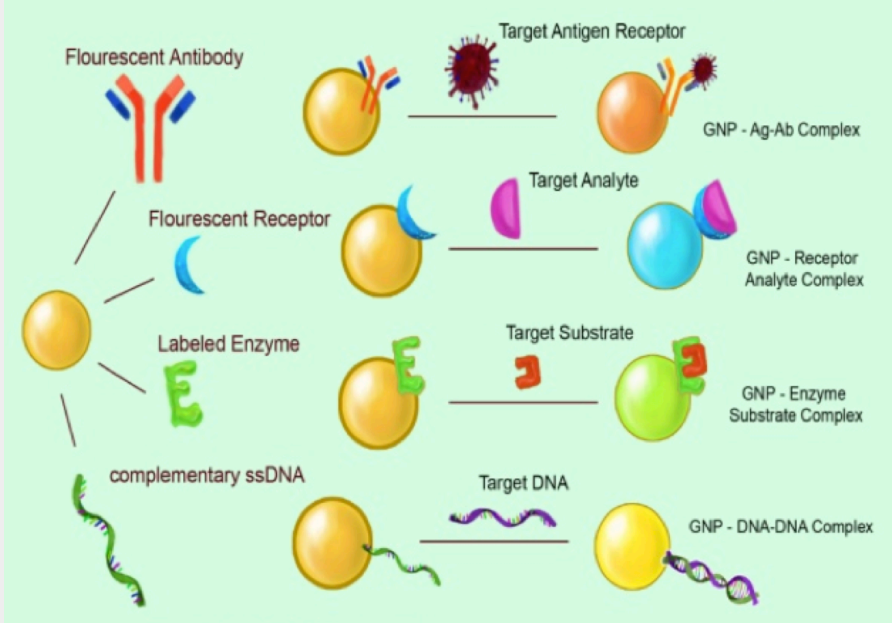

Fig. 2: Some examples of different types of recognition molecules and their complementary ligands used in nanoparticle-based diagnostic biosensors (*14*).

Nanostructured materials can also bring to bear their unique electronic, optical and surface effects to facilitate novel signal transduction methods for reading out a biochemical recognition event at the macro scale (*9, 15*). Nanoparticles again are the optimal structures to enable these distinctive effects. Silver and gold nanoparticles for example have exceedingly high absorbance cross section, meaning immobilization of a single nanoparticle due to a biochemical recognition event would create about one million fold more change in color than a binding event with a traditional organic dye. Shrinking a bulk semiconductor material below 10 nanometers in size creates a particle known as a quantum dot. These nanoparticles have a very strong and persistent fluorescence which can be used to signal when a recognition binding or unbinding effect has occurred. This fluorescence is resistant to many environments which would quickly eclipse a traditional organic fluorophore, and even more fascinatingly, the color of the fluorescence can be tuned by controlling the size of the nanoparticle, enabling multiplexed diagnoses of multiple biomarkers simultaneously. This list of effects is not exhaustive, and shrinking a material down to the nanoscale nearly always produces some interesting effect which has remained unseen in macroscopic materials (*15*).

Treatment

When considering the application of nanomedicine to treatments and therapeutics, we must consider the problems that exist with currently used techniques. We are not lacking in drugs and agents which are capable of killing, inhibiting or removing diseased or cancerous tissue, but instead we are unable to apply these drugs selectively to affected cells and tissues (*9*). Applied indiscriminately, anti-cancer drugs for example are more than capable of killing quickly replicating but non-cancerous cells such as those in the hair and stomach lining, creating serious and sometimes even life-threatening side effects to chemotherapy. Nanomedicine addresses this problem through the use of targeted delivery systems which can efficiently deliver a payload preferentially to affected cells and tissues.

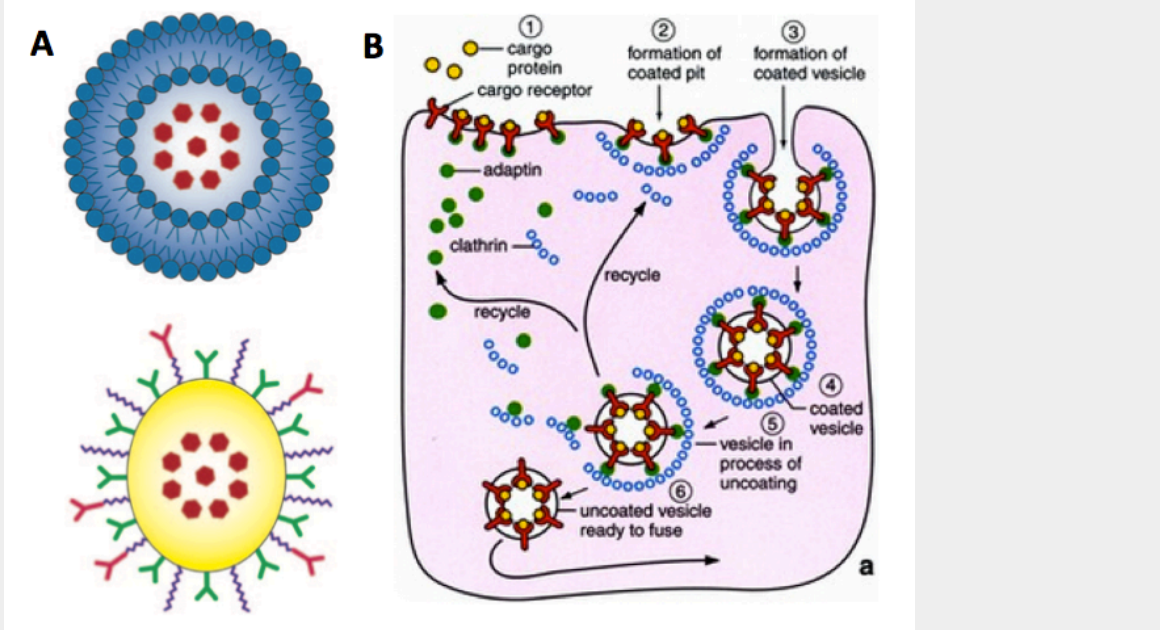

Fig. 3: A: A cartoon representation of passively targeted generation 1 delivery vehicles (top) and actively targeted generation 2 delivery vehicles (bottom). B: The mechanism of receptor mediated endocytosis. (*9*).

One advantage of these delivery vehicles – which are normally composed of nanoparticles capable of transporting and delivering one of more bioactive molecules, including therapeutic agents and imaging agents – for biomedical applications is their ability to bypass many barriers used by the body to prevent invasion by foreign agents and to localize into the target tissue. These types of delivery vehicles can be broadly split into two different main groups of "generations" (*11*). The first generation uses a passive delivery system that localizes into the targeted tissue type, and is limited in which types of tissues it can target and in its delivery efficiency. The most common system of this type is the use of liposomes (membrane bound particles) to deliver a drug payload to tumors. In this case, the mechanism of action is the so-called "enhanced permeation and retention effect" (EPR), which drives the delivery vehicle to selectively leave the vasculature and enter tumor tissue through characteristic openings in the hastily developed vasculature surrounding tumors (*16*). The cutoff size of these openings through the blood-tumor barrier depends on the location and type of tumor, but is generally in the range of 300-800 nm (*9*). Another barrier that must be considered however is the activation of phagocytotic (or cell-eating) cells, which generally consume and evacuate vehicles that are larger than 200nm. This leads to an ideal size on the order of 100nm for drug delivery vehicles if they wish to persist for long periods of time in the body, and selectively permeate from the blood into the tumor tissues (*9, 12*). To compound this effect, tumors are generally masses of undifferentiated cells, and have very poor lymphatic drainage to remove contaminants and foreign bodies. This helps to keep the drug-loaded delivery vehicles in the tumors for a longer time and enhance their effects.

Delivery vehicles of this first type generally fall apart and release their drug into the fluid surrounding the cells once they have entered the tumor target. The last step of targeting is to promote delivery vehicles to enter tumor cells and release their life-saving cargo there, rather than just outside. This would reduce the toxic effects of released drug leaving the tumor, as well as increase the delivery efficiency (*9*). The second generation of nanomedical targeted delivery vehicles are defined as having specific additional functionalities which allow for molecular recognition of the target tissue, or for active (event triggered) release of the drug payload at the targeted site. Though this generation of delivery mechanisms includes functional nanoparticles such as gold shells or magnetic liposomes which can be "activated" to release their contents or treatment by incident light or magnetic field, the most common type of second generation delivery vehicles uses receptor-mediated endocytosis to actively penetrate into diseased cells(*9*). These second generation delivery vehicles are generally formulated in much the same way (and the same size) as the first generation, in order to use the EPR effect to passively target the tumorous tissues. In addition, the outside of the delivery vehicles are coated with a high density of ligands chosen to initiate the process of receptor mediated endocytosis (*17, 18*). The complementary receptors for these ligands are overexpressed (more prevalent) in the membranes of cancerous cells, and once a recognition event has occurred with the correct ligand, a pathway leading to swallowing up a small volume outside of the cell membrane begins. As shown in Figure **3B**, each ligand-receptor binding event outside of the cell membrane recruits a protein known as a clathyrin, which coats the opposite side of the membrane. Once enough clathyrins have been recruited in a specific area, an invagination occurs in the membrane which absorbs the bound ligand-receptor complexes along with any attached nanoparticle or drug (*17, 18*). The initiation of this pathway is a non-linear process, and depends heavily on the density of recognition events on the outside of the cell membrane. Functional nanoparticles with a high concentration of presented complementary ligands are ideal for the purposes of activating the endocytosis process. When a nanoparticle approaches a cell, the local concentration of ligand is very high, drastically increasing the rate of recognition reaction with the membrane-bound receptor proteins. This in turn accelerates the recruitment of clathyrins and the initiation of endocytosis. Parameters such as the geometry of the nanoparticle delivery

vehicle, the density of exposed ligand, and the nature of the connective linkers from nanoparticle to ligand all have an effect on the efficiency of endocytosis and eventual drug delivery. It should be noted that a small molecule (such as a drug-ligand conjugate) is not able to initiate the endocytosis cascade without a very high extracellular concentration – a very difficult task to accomplish if the drug molecule of interest is toxic to surrounding healthy tissues (*17*).

Prevention

The majority of recent advances in the development of nanomedicine have focused on advanced diagnosis and treatment methods, however there is no area of nanomedicine in which the unique abilities of nanoengineered systems have been exploited more than the recent advancements in the development of preventative nanomedicines. As mentioned previously, it is through interfacing with biology at its native scale that nanomedicine is available to accomplish some of its most useful applications. Preventative nanotechnologies are designed to inhibit the processes which allow pathogenic and infectious agents such as bacteria and viruses to attach cells and tissues at the molecular level. In order to accomplish this through clever nanoengineering, scientists have been able to create competitive biomimics able to fool infectious agents enough to prevent their ability to infect human cells (*19–21*). As with both biosensors and actively targeted delivery vehicles, knowledge of the molecular mechanism is required for this task to be possible. A canonical example is the reasonably well-understood infectious mechanism is shared by a large variety of mammalian viruses, including HIV and influenza A.

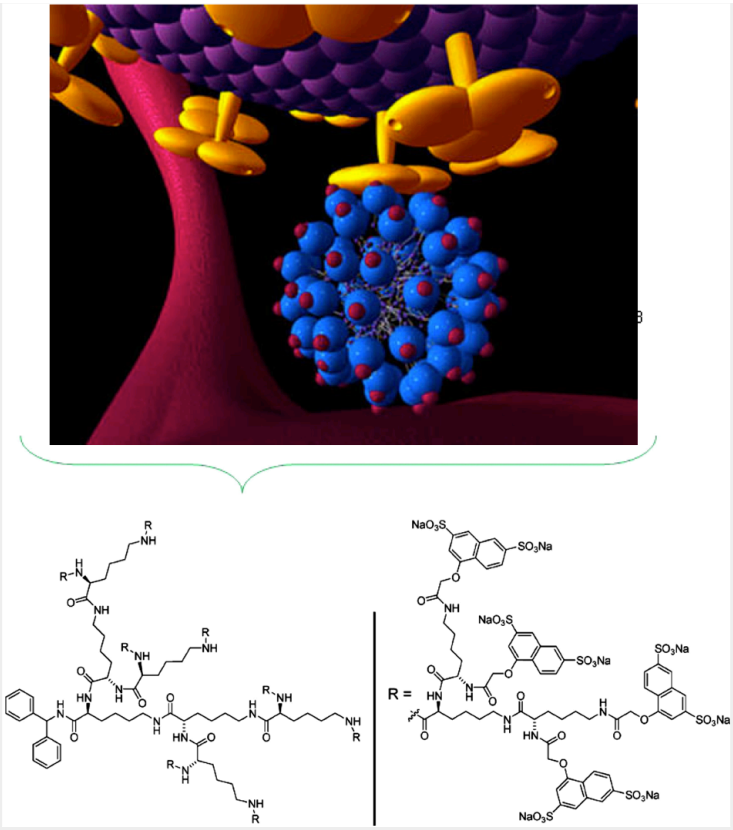

Fig. 4: An artists' rendition of a mammalian cell membrane mimicking dendrimer blocking the viral spike of the HIV viral capsid (top), and the chemical functionalities used for HIV and influenza targeting (sialic acid and sulfonate) (*22*).

These viruses identify potential mammalian hosts through glycoproteins (sugar-containing proteins) present on the cell membrane. These types of proteins are commonly used in intercellular signaling (and in fact are used to distinguish blood type in humans), and their presence is a good indicator for the virus that the cell is a potential host. Glycoproteins are generally negatively charged due to the preponderance of negatively charged building blocks (such as sulfonate and sialic acid), and as a result, the area of the virus used to identify these proteins is generally positively charged, and uses display peptides with a very high affinity for these types of building blocks. Once the virus finds itself bound to its preferred type of membrane-bound glycoproteins, a conformational change occurs which either initiates the penetration of the cell membrane by a viral "spike" (as is the case with influenza), or the fusion of the viral and cell membranes (as is the case with HIV). However, the virus has such high affinity for the identifying glycoproteins on the cell surface that the binding is effectively irreversible. Viruses do not generally detach once bound, and it is this knowledge of the molecular mechanism with enables the development of a preventative nanomedicine(*19-21*).

Dendrimers (from the Greek *dendros* for tree) are a class of polymers with a star-like structure as shown in Figure **4.** Dendrimers are grown by ligating divalent building blocks in successive generations, with each generation doubling the number of addressable functional groups. After four or five generations, the number of addressable groups becomes very high, and steric interactions lead to a globular structure. If the efficiency of each generation's addition is high, the polydispersity of the resulting polymeric structures is very close to 1, earning dendrimers the label of "artificial proteins". In later generations, the density of addressable functional groups is very high, with effective local concentrations in the order of 10M. Furthermore, the choice of the chemical nature of the building block allows the bulk chemical characteristics of the dendrimer to be tuned. By choosing a positively or negatively charged building block, the entire dendrimer can be tuned to have a positive or negative zeta potential. The terminal function groups can also be chemically modified following growth of the dendrimer to display a variety of chemical moieties. It follows then, that by tuning the zeta potential and the displayed surface groups, a dendrimer can be designed to mimic an arbitrary chemical surface such as a mammalian cell membrane(*23, 24*).

Indeed scientists have shown that by using a negatively charged building block for the dendrimer, and by functionalizing the surface with sulfonate and sialic acid residues, dendrimers can act as competitive biomimics to inhibit viral infection. The local concentration of functional groups on the dendrimer far exceeds that of the identifiable functional groups on a cell membrane, which accelerates virus-dendrimer binding compared to virus-cell binding. Since the viral binding is effectively irreversible, dendrimers act to inhibit the "teeth" of the virus, creating an effectively inert capsid. With detailed knowledge of the molecular mechanism of viral binding, the parameters of the dendrimer such as generation, zeta potential and surface group density can be optimized for a given inhibition purpose.

Applications

In examining the current implementations of nanomedicine for the diagnosis, treatment and prevention of disease, some common mechanisms of action have become apparent. In each of these three cases, it is the geometry of the nanostructured system – specifically the extremely high surface to volume ratio – which enables and enhances nanomedicine's benevolent and effective interface with biology. The key element appears to be the high local concentration which occurs during reactions between nanoparticles and biological systems, a result of the inherent multivalent nature of both sides. If the nature of these multivalent interactions could be better understood and quantified, it would be possible to design a nanomedical system for diagnosis,

treatment or preventative use given detailed information about the molecular mechanism of the targeted disease or condition.

Empirical evidence from biophysical studies has shown that multivalent ligand-receptor interactions can form complexes which are orders of magnitude more stable than their monovalent counterparts. As exemplified in the previous section, these highly specific multivalent interactions play a crucial role in interactions between nanomedicine and biology, including cell adhesion, receptor mediated endocytosis, and recognition events in biosensors. Though the use of nanoparticles is recognized to facilitate these multivalent interactions, the design and optimization of the nanostructures used has largely remained an empirical process without a significant amount of *ab initio* rational design. Recently researchers have conducted biophysical studies on the multivalent enhancement observed in the inhibition of proteasomes and cholera toxin, concluding that it is the enhanced stability of multivalent complexes over their monovalent correlates which allows a desired effect (such as initiation of an endocytosis cascade) to be accomplished at concentrations of a multivalent construct (such as a functionalized nanoparticle) much lower than those required for monovalent ligands(*25, 26*). This conclusion has obvious implications for the design of therapeutic and diagnostic agents if the multivalent effect can be optimized for a given system.

A Statistical Mechanics View of Multivalent Interactions

In an attempt to generate a simple framework for understanding the mechanism of an amplifying effect of multivalent interactions, a recent letter by Diestler and Knapp has looked at the problem of multivalent binding from the first principals of statistical thermodynamics (*27*). By taking a simplified view of a divalent ligand binding to a divalent receptor, they have been able to derive an explicit relation between monovalent and multivalent reaction constants in terms of molecular properties which clearly reveal how enhancement occurs. In their treatment, the specific chemical and physical nature of the ligands is ignored, as are all the internal degrees of freedom of the ligands and receptor. In attempting to make a generalized framework, it was recognized that the specific nature of the receptor and ligands would be shared between the multivalent and monovalent cases, and in determining the reaction rate amplification factor, their effect would mostly cancel out. In ignoring these contributions, the only difference between the multivalent and monovalent systems is the linker molecule, and the effect that chemically linking two or more ligands has on the available conformations and energetics of the complex. Using only the requirements of chemical equilibrium and taking into account the total potential and kinetic energies of each player in the system, a very simple expression was found which relates the enhanced multivalent rate constant to the monovalent correlate (*27*):

$$K_{di} = \frac{e^{-\beta F(\rho)}}{\int_{V_{link}} dr e^{-\beta F(r)}} K_{mono}^2$$

where K_{di} is the multivalent reaction constant (higher is faster), and K_{mono} is the monovalent reaction constant. The monovalent constant raised to the power of 2 (the number of ligands in the multivalent complex) to show the number of sequential monovalent reactions required to equal a single multivalent reaction. This number however is modulated by a Boltzmann-weighted fraction term which is a function of $F(\mathbf{r})$, the pair potential free energy between any two ligands in the multivalent complex (which is dependent only on the nature of the linker molecule). In physical terms, this fraction has units of inverse volume (or number concentration), and shows the amount of conformations in which any two ligands in the multivalent complex will be ρ units of distance apart, which is the distance between receptor binding sites. This amount is normalized by a Boltzmann-weighted sum of all possible separations and orientations of the two ligands allowed by the geometry of the linker. In their study, this modulating term was defined as C_{eff}, the effective concentration of ligand present at the correct

orientation with respect to another binding site, and used as a quantity of merit for the amplification effect of the system.

It can be seen that at high effective concentrations of ligand, the rate will be accelerated, meaning that the linker is designed such that ligand is correctly placed to bind with subsequent receptor sites after an initial binding event. At low C_{eff}, the probability of the linker bringing a subsequent ligand in the correct orientation to bind will be low, which will produce very little acceleration of the rate constant.

A Simplified Real-world Demonstration

In an attempt to apply this simple model to a real world system, the authors had to consider a form of the free energy expression dictated by a typical linker. As an initial proof of concept which could be related to a simple real-world demonstration, the model of an entropic chain (a 3-dimensional random walk) polymeric linker was used. This is a simple but often physically insightful model of polymers in which interactions between constituent building blocks are ignored, and each building block is considered as a rigid rod of a fixed length. From a random walk distribution, the free energy landscape between each ligand-functionalized end of the polymer linker can be easily analytically calculated as shown in many standard statistical mechanics textbooks. This free energy as a function of end-separation distance is dependent only on the number of building blocks in the polymer chain, and the length of each of these blocks (*27*).

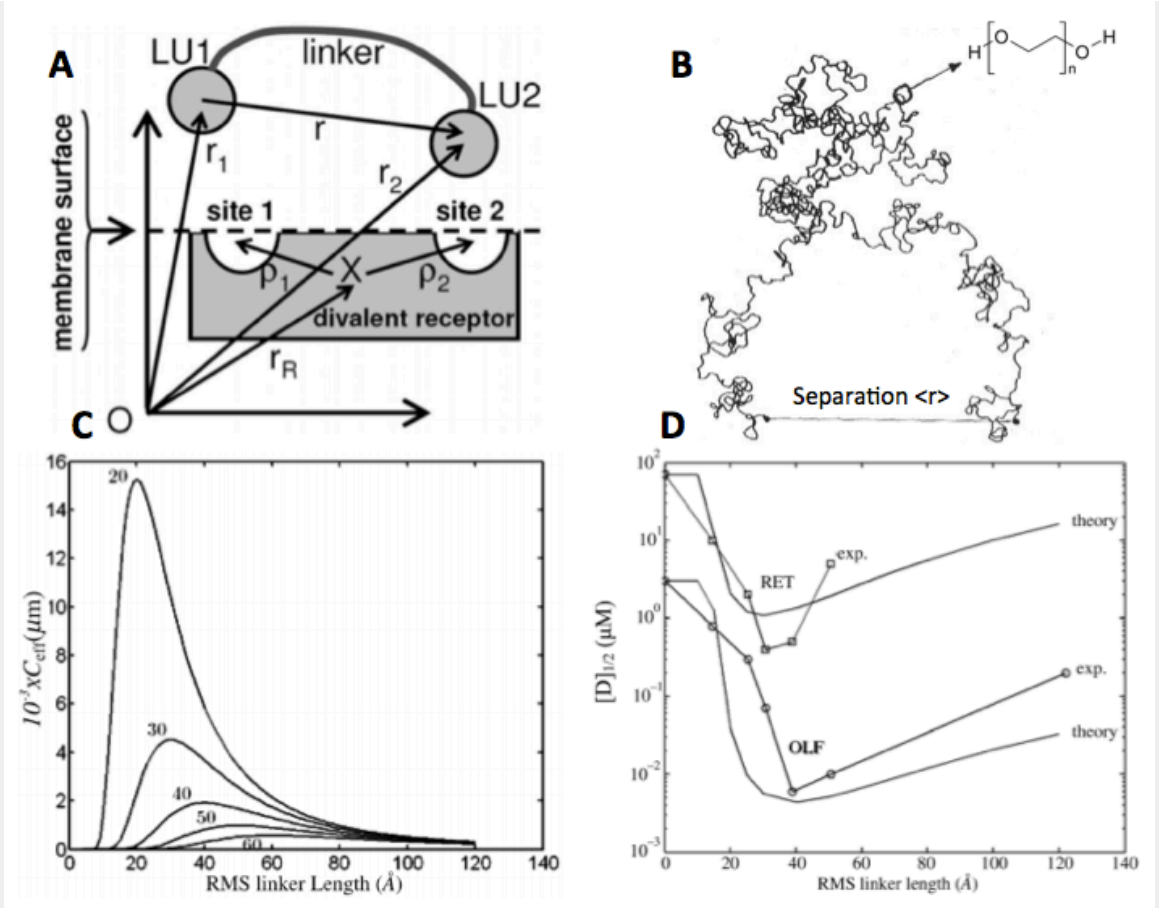

Fig. 5 A: Schematic side view of system showing divalent ligand in solution in contact with cell membrane containing divalent receptor referenced in text. B: Typical "random walk" structure of a flexible polymer backbone showing end-to-end distance <r>. C: Effective concentration C_{eff} as a function of root mean squared separation of polymer linker. D: Plot showing predicted 50% binding concentrations of dimer and

experimentally determined values for two membrane-bound receptors at a range of polymer linker lengths. **(*27*).**

By inputting this free energy into the expression for C_{eff}, above, an analytical form of this quantity of merit can be derived as a function of the geometry of the linker molecule. As shown in Figure **5**, this function is dependent on the average separation distance ρ between receptor sites, as well as the length and number of building blocks in the linker. Given a random walk, the characteristic linear dimension usually considered is the mean squared chain length, given by $< r^2 >= Na^2$, where *N* is the number of building blocks, and *a* is their length. The value $< r^2 >^{1/2}$ then is the average separation distance between the two ends. As would likely be expected, the C_{eff} reaches a maximum when the average separation between the chain ends is equal to the average separation between receptor binding sites. In addition, the maximum value of the effective concentration lowers as the distance between receptors increases. This seemingly obvious result demonstrates the validity of this model, at least for this simple cartoon system. To further test this, the the authors conducted an experiment to quantify binding between a divalent inhibitor and a tetravalent membrane-bound protein to get as close an approximation to their simple system as possible. They found that their model very accurately predicted the kinetics of the binding when using different lengths of polyethylene glycol linker molecules and membrane-bound proteins with different inter-receptor distances (*27*).

Further Applications:

The approach taken by Diestler and Knapp in attempting to analytically quantify the enhancement effect of multivalent interactions is a very powerful one (*27*). In addition, their method is quite general, and it can be seen how to readily apply it to systems with higher valency, and with a geometry much more complicated than a simple random walk polymer chain linker. An arbitrarily complex multivalent system, such as that of a rigid nanoparticle with a certain surface density and arrangement of ligands or a dendrimer with a certain generation number and electrostatic potential can theoretically be modeled with appropriate parameters to produce a pair potential-like free energy of interaction between any two ligands in the multivalent system.

The previous section of this review has examined the application of nanoparticles and dendrimeric molecules to nanomedicine. When applied today, the geometrical and chemical parameters of these nanostructures are generally empirically optimized to produce a useful effect or product. In general, studies have found for example that very flexible hydrophilic polymer linkers of intermediate length (1-5k molecular weight) produce the highest surface reaction rates for immobilized polymers or ligands on a nanoparticle surface. This has become the prevailing wisdom in the field, and now polyethylene glycol (PEG) is nearly universally used as a linking molecule due to its flexibility and water solubility (*3*). While superficially the choice of a flexible polymer linker makes practical sense due to its range of conformational variance and low enthalpy of deformation, for nanoparticle-nanoparticle interactions and nanoparticle-cell interactions with known parameters for the average separation of receptors or binding geometry, this may not be the case. Similarly for a dendrimer system, studies generally develop different generations of dendrimer with different surface charge distribution and zeta potential, and empirically determine the most effective candidate. By using a statistical thermodynamic framework with ligand pair potentials dependent on the physical parameters of the system, the optimization of these types of nanostructures is possible.

The molecular dynamics of functionalized nanoparticles (*28*) and dendrimers (*29*) have both been the subject of significant research in recent years, and well-characterized expressions for pair correlation and pair potential functions are known. It remains only to apply our knowledge of these types of systems with a molecular

mechanism of interest for a biosensor, therapeutic or preventative nanomedicine of interest to see whether rational design can be of some use.

Conclusions and Outlook:

The development of a man-made system capable of truly interfacing with biology at the molecular scale is one of the grand challenges of the next century that can only be accomplished through molecular nanotechnology and nanomedicine. The potential applications of nanomedicine for the diagnosis, treatment and prevention of disease are currently very broad. Advancement and practical application of nanomedicine requires, therefore, besides creativity and visionary power, simple approaches and rational design.

Simplifying the massive field of endeavor into the most potent features and characteristics of nanomedicine technologies, such as those of controlled high surface area and volume confinement effects may prove useful in achieving these goals. This Review we have provided an overview of what we consider the main ideas and approaches used in nanomedicine today, and through this examination extracted a common mechanism of action of nanomedicine's interactions with biological systems. The high effective concentration of functional groups on engineered high surface area nanostructures is integral to achieving the accelerated rate of reaction with biological molecules and the very "sharp" effects in concentration that cannot be achieved with traditional approaches. With knowledge of the molecular mechanisms at work and through powerful synthetic techniques, we are at a point where we can design a nanosystem from the ground up to perfectly fit a medical need. Simple thermodynamic descriptions of the pair-wise interaction between ligands on the structure can act as useful quantities of merit to use in optimization of these systems.

We are in the midst of a shift for nanomedicine and nanotechnology engineering from an empirical mindset which tests the properties and usefulness of a given structure towards a rational design mindset in which a structure is designed with a specific purpose in mind. By taking pointers from biology when needed and exploiting our increased knowledge of molecular mechanisms of disease, the future of nanomedicine will surely be bright.

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