

Viral assembly of oriented quantum dot nanowires

Abstract

The highly organized structure of M13 bacteriophage was used as an evolved biological template for the nucleation and orientation of semiconductor nanowires. To create this organized template, peptides were selected by using a pIII phage display library for their ability to nucleate ZnS or CdS nanocrystals. The successful peptides were expressed as pVIII fusion proteins into the crystalline capsid of the virus. The engineered viruses were exposed to semiconductor precursor solutions, and the resultant nanocrystals that were templated along the viruses to form nanowires were extensively characterized by using high-resolution analytical electron microscopy and photoluminescence. ZnS nanocrystals were well crystallized on the viral capsid in a hexagonal wurtzite or a cubic zinc blende structure, depending on the peptide expressed on the viral capsid. Electron diffraction patterns showed single-crystal type behavior from a polynanocrystalline area of the nanowire formed, suggesting that the nanocrystals on the virus were preferentially oriented with their [001] perpendicular to the viral surface. Peptides that specifically directed CdS nanocrystal growth were also engineered into the viral capsid to create wurtzite CdS virus-based nanowires. Lastly, heterostructured nucleation was achieved with a dual-peptide virus engineered to express two distinct peptides within the same viral capsid. This work represents a genetically controlled biological synthesis route to a semiconductor nanoscale heterostructure.

Exploiting the biologically evolved, self-assembling structures of viral capsids is a possible mechanism in the ordering of technologically important materials. A key characteristic of materials synthesized by natural biological systems is the hierarchical organization of structures on many length scales with controlled size, shape, alignment, and orientation ([1–8](#)). There has been much interest in using biological templates *in vitro* to modulate the growth of inorganic, semiconductor, magnetic, and other technologically important materials ([9–14](#)). Our previous work, along with that of others, has shown that biological combinatorial

approaches can identify amino acid sequences capable of interacting with a variety of materials (11, 15). Here, we present a system that exploits the self-assembling, crystalline structure of the viral coat to nucleate and template II-VI semiconductor nanocrystals into highly oriented quantum dot nanowires. To accomplish this, the pVIII major coat protein was engineered to display evolutionary-selected peptides, enabling directed nanocrystal growth into viral-semiconductor hybrid nanowires that were crystallographically oriented over the micrometer size range.

The helical major coat protein, pVIII, of viruses can be engineered to express fusion proteins that readily self-assemble into a highly oriented viral coat structure. A 5-fold axis of rotation relates symmetric points on the coat (16, 17). Peptide sequences engineered into the pVIII protein for use as nanocrystal templates were selected based on affinity toward either ZnS or CdS, specificity for ZnS over CdS (or vice versa), and the ability to affect nucleation and growth of nanocrystals during synthesis (Fig. 6 and *Supporting Text*, which are published as supporting information on the PNAS web site, www.pnas.org). We have previously identified a conformationally constrained peptide, termed A7 (Cys-Asn-Asn-Pro-Met-His-Gln-Asn-Cys) and an unconstrained peptide, Z8 (Val-Ile-Ser-Asn-His-Ala-Glu-Ser-Ser-Arg-Arg-Leu), both of which specifically recognize and control growth of ZnS. For example, MS data indicate that the A7 peptide, with the disulfide bond formed, bound four Zn(II) ions with at least 3 orders of magnitude more strongly than the S^{2-} (Fig. 7, which is published as supporting information on the PNAS web site; see *Supporting Text*). Further, we have demonstrated that A7-ZnS viral semiconductor complexes can be assembled into self-supporting thin films (12). Similarly, the peptide J140, with a sequence of Ser-Leu-Thr-Pro-Leu-Thr-Thr-Ser-His-Leu-Arg-Ser, was selected for recognition of single-crystal CdS and nucleation control over CdS. Using high-resolution analytical electron microscopy and photoluminescence (PL), we characterized collections of single M13 phage particles engineered to express semiconductor nucleating peptides upon the viral capsid that direct highly oriented, phase-specific nanocrystal growth. This approach combines the use of perfectly ordered and assembled natural viral templates with the flexibility of genetic control over material synthesis to assemble semiconductor wires in supramolecular hybrid structures.

Nanoparticles and their Applications in Lipid Signaling

Abstract

This chapter provides an overview of the diverse range of applications associated with nanoparticles. The application of nanoparticles in the medical field has garnered considerable attention due to their unique properties and versatile compositions. They have shown promise in the treatment of cancer, fungal and viral infections, and pain management. These systems provide numerous benefits, such as increased drug stability, improved bioavailability, and targeted delivery to specific tissues or cells. The objective of this chapter is to provide a brief analysis of the differences between nanoparticles and lipid particles, focusing particularly on the importance of nanoparticle size and composition in their interactions with lipids. Additionally, the applications of nanoparticles in lipid signaling will be discussed, considering the vital roles lipids play in cellular signaling pathways. Nanoparticles have shown immense potential in the regulation and control of medical pathways. In this case, we will focus on the manufacture of liposomes, a type of nanoparticle composed of lipids. The reason behind the extensive investigation into liposomes as drug delivery vehicles is their remarkable biocompatibility and adaptability. This section will provide insights into the methods and techniques employed for liposome formulation.

Keywords: Lipid Nanoparticle, Lipid Signaling, drug delivery, solid lipid nanoparticles, liposomes, nanostructured lipid carriers

1. Introduction

1.1. Understanding the Science of Nanoparticles

Nanoparticles (NPs) are extremely small particles having diameters in nanometer scale; smaller than 100 nm. These materials can be 0D, 1D, 2D, or 3D depending on the overall shape. This classification is heavily reliant on electron mobility in

the nanomaterials (NMs) along the dimensions. Electrons in 0D NMs, for example, are entrapped in a dimensionless environment, but electrons in 1D NMs can flow along the x-axis, which is less than 100 nm. Similarly, electrons move along the x-y-axis and the x, y, and z-axes in 2D and 3D NMs, respectively [1]. Nanoparticles are divided into several categories based on their size, shape, and material characteristics. Some classifications divide nanoparticles into organic as dendrimers, liposomes, and polymeric nanoparticles. Or inorganic categories including fullerenes, quantum dots, and gold nanoparticles. Other classifications divide nanoparticles into carbon-based, ceramic, semiconducting, and polymeric nanoparticles. Furthermore, nanoparticles can be hard (such as titania [titanium dioxide], silica [silica dioxide] particles, and fullerenes) or soft (such as liposomes, vesicles, and nanodroplets) [2, 3].

The significance of these materials was apparent when researchers discovered their microscopic size can influence a substance's physiochemical qualities and capabilities [3]. NPs consist of three layers: the surface layer, which can be functionalized with a range of small molecules, metal ions, surfactants, and polymers. The shell layer, which is chemically distinct from the core in all aspects, and the core, which is the NP's central section and commonly refers to the NP itself [4].

1.2. Nanoparticles vs. lipid-particles

Unlike nanoparticles, lipid particles consist of a core of hydrophobic lipids, mainly triglycerides and cholesterol esters. This core is surrounded by a shell of proteins, phospholipids, and free cholesterol. The proteins that make up the shell are known as apolipoproteins which boost lipoprotein synthesis and function, and they are responsible for structural stability as well as interacting with cellular receptors and enzymes [5]. Because lipids are insoluble in water, they must be stored in a soluble form for optimal transit in the bloodstream, Lipid particles function as carriers for carrying lipids throughout the body in combination with proteins (lipoproteins). These lipoproteins are essential for dietary fat absorption and transport.

Typically, the structure consists of a glycerol backbone, two hydrophobic fatty acid tails, and a hydrophilic phosphate group. Phospholipids are hence amphipathic. The bilayer arrangement of phospholipids in the cell membrane protects the cell and acts as a barrier to some chemicals. The hydrophobic portion is facing in,

while the hydrophilic portion is facing out. This configuration aids in controlling which molecules can enter and leave the cell. Small polar and nonpolar molecules, like oxygen and water, can easily diffuse into and out of the cell, for instance. Large, polar molecules like glucose cannot move about freely and so need the assistance of transport proteins [6].

Based on their density, which is determined by the quantities of lipids and proteins they contain, lipid particles are categorized. Higher lipid content results in decreased density, which is inversely correlated with density. Chylomicrons have the lowest density; they are large triglyceride-rich particles that are formed in the small intestine. Followed by increasing densities of Very-low-density lipoproteins (VLDL), Low-density lipoproteins (LDL), and High-density lipoproteins (HDL). The liver produces VLDL which are triglyceride-rich particles. While LDL is rich in cholesterol and formed from the combination of VLDL and Intermediate-density lipoproteins IDL. The last one is HDL particles are rich in cholesterol and phospholipid [5, 6].

1.3. Interaction between nanoparticles and lipids

Lipid particles can interact with nanoparticles through different contexts. For instance, adsorption on surface and surface interactions allows nanoparticles to interact with lipid particles. Charge, lipophilicity, and functional groups on nanoparticle surfaces can all influence how they interact with lipids. Hydrophobic bonds are possible between lipid particles, notably lipoproteins, and hydrophobic nanoparticles. Electrostatic forces can also occur between charged nanoparticles and the charged areas of lipids [7]. The second context would involve mimic the structure of the cell membrane in which nanoparticles will be coated with lipids (phospholipids) forming a lipid bilayer around the nanoparticle thus increasing their stability, biocompatibility, and effectiveness. Lipid coatings can provide a protective layer around the nanoparticle core, avoiding aggregation and increasing the time it spends in circulation in the body [8].

Moreover, nanoparticles can be modified using ligands or antibodies that bind to receptors on lipid particles specifically, LDL lipid particles are the most notably used for this interaction. This permits the nanoparticles to be taken up by cells that express these receptors, potentially improving therapeutic or imaging agent delivery that is best used in targeting drug delivery [9–11]. It is possible for lipid

transfer to occur between lipid particles and nanoparticles as another type of lipid-particle and nanoparticle interaction. Lipids from lipoproteins, for example, can be transferred to lipid-based nanoparticles, causing changes in the composition and properties of the nanoparticles. Enzymes, transporters, or other systems may mediate this transfer [12]. Interaction between lipid particles and nanoparticles can result in the formulation of lipid-based nanoparticles (LNPs). Liposomes, micelles, solid lipid nanoparticles (SLN), and nanostructured lipid carriers (NLC) are all examples of nanoparticle formulations that contain lipids. These formulations can ensure the stability and controlled release of the delivered drugs. LNPs can interact with lipid particles, particularly lipoproteins [13, 14].

1.4. Lipid nanocarrier (LNPs) for drug delivery

LNPs are well-positioned in the advancement of genomic therapies because they can encapsulate a wide range of pharmaceutical loads, including small molecules, nucleic acids, monoclonal antibodies, imaging agents, and peptides [15]. LNP-based medicines have also been approved by the FDA for treating additional diseases, including COVID-19 vaccination (Spikevax, Comirnaty) and Amyloidosis (Onpattro), by delivering mRNA and siRNA, respectively [16, 17]. Additionally, different types of LNPs have been designed to deliver different types of drugs, for example, immunosuppressant cancer drugs, HAV viral vaccine, anesthetic drugs, and antiviral HIV-1 drugs [18]. LNPs have various advantages, including protecting drugs from in vivo degradation, increasing solubility and efficacy, allowing targeted drug delivery, modulating drug release, and changing drug biodistribution. They offer sheltering of the API from damaging enzymes and allowing transit across biological membranes. Thus, nanocarriers have the potential to overcome fundamental constraints of existing medicinal agents, such as ineffectiveness, enzymatic degradation, limited bioavailability, and off-target adverse effects.

The size of the nanoparticles influences their stability, biodistribution, cellular uptake, and overall therapeutic efficacy. Smaller LNPs (size 100 nm) have higher cellular uptake and a longer circulation period, whereas bigger LNPs (size >100 nm) have a higher drug-loading capacity but lower cellular uptake [19]. Furthermore, LNP surface charge is a vital metric indicating LNP stability in suspension. A significant surface charge would result in electrostatic repulsion between particles and increase their stability in the dispersion. While, a surface

charge value close to zero, indicates a low surface charge and a stronger tendency for particle aggregation or coalescence, which could contribute to the destabilization of the LNP suspension [20]. Surface morphology analysis of LNPs can provide useful insights into the physical structure and properties of the LNP's outer surface. A well-defined and smooth surface morphology is extremely beneficial for LNPs in biological contexts because it leads to increased stability and reduces the risk of opsonization, which occurs when serum proteins attach to the surface and stimulate immune system clearance. This flat surface reduces undesired interactions with biological components, allowing optimal LNP biodistribution and therapeutic efficacy [21]. LNPs with rough or uneven surface morphology may interact with biological entities more frequently, thereby affecting LNP biodistribution patterns and impacting therapeutic effectiveness [21].

1.5. Examples of nanoparticles

The first example of LNPs known as liposomes, these spherical vesicles are made up of lipid bilayers. Their capacity to contain hydrophilic and hydrophobic medical entities within their water core or fatty lipid membrane makes them one of the most extensively utilized drug delivery methods. Drug delivery can be more precise, stable, and solubilized with the help of liposomes[22]. SLNs as another example are nanoparticles formed from waxes or triglycerides, which are solid lipids. They are used to encapsulate pharmaceuticals and for sustained drug delivery. SLNs have benefits such increased medication absorption, improved drug stability, and defense against degradation [23]. While NLCs combine liquid and solid lipids. Compared to SLNs, this special structure offers a higher drug-loading capacity and better drug release kinetics. NLCs are utilized to deliver medical drugs, including targeted therapies and pharmaceuticals with poor solubility[24, 25].

Hybrid of Lipids and Polymers Nanoparticles are nanomaterials which mix lipids and polymers to take advantage of the benefits of both components. The lipid part allows for biological compatibility and drug encapsulation, while the polymer part provides stability and sustained release. They are used in medical treatments delivery, imaging agents, and gene therapy [26]. Another reported example is Ethosomes that are lipid-based nanoparticles with significant ethanol concentration. Ethosomes are advantageous for transdermal medication delivery

applications because the incorporation of ethanol improves the penetration of pharmaceuticals that are encapsulated through the skin. They play a key role in topical preparations, which enhance medication absorption[[27](#)]. The latter example is transferosomes that are lipid vesicles with high flexibility and have the capacity to pass through pores in the skin and other small openings. They help medications get through biological barriers better, such as the skin or mucosal surfaces. Gene therapy, vaccine, and transdermal delivery are all possible uses for transferosomes [[28](#)].

1.6. Applications of nanoparticles in lipid signaling

Nanoparticle technology is recognized as a promising tool for various biomedical applications in biology. Many innovative techniques are being developed for the detection of analytes, pathogens, and cells using nanoparticles. Furthermore, the utilization of nanoparticles has proven to be effective in the fields of biomedical imaging, biosensing, and as carriers for drug delivery [[29](#)].

For instance, SLNs have the potential to disrupt inflammatory pathways. An instance of cationic lipid-based nanoparticles was found to activate the toll-like receptor 4 (TLR4) pathway. Moreover, it was observed that in RAW262.7 macrophages, various solid lipid nanoparticles (SLN), predominantly composed of glyceroltrimyristate and cetylpyridinium chloride, elicited cytotoxic effects and resulted in reduced levels of interleukin 6 and TNF- α expression [[30](#)]. Toll-like receptors (TLRs) like TLR4 are found in immune cells like macrophages. These receptors help the innate immune system produce cytokines and chemokines in response to infection and tissue damage. TLRs are also found in adipocytes, hepatocytes, and skeletal muscle cells [[31–33](#)]. These non-immune cells can boost immunity by secreting chemokines and cytokines [[34](#)]. Recent studies have examined human, mice, and skeletal muscle cell cultures. Many methods, including gain and loss of function, were used in these studies. These studies found that TLR4 signaling reduces fatty acid oxidation. Thus, this muscle activity may raise blood triglycerides [[35](#)]. Fasting and TLR4 loss increased skeletal muscle oxidative capacity and decrease triglycerides and non-esterified free fatty acids [[35](#)].

Lipid sensors are used to detect changes in lipid composition and concentration, which are important in lipid signaling pathways. For example, the detection of

CVD biomarkers. The cardiac troponin complex is comprised of three regulatory proteins, namely troponin I (cTnI) and troponin T (cTnT). These proteins are released into the blood flow as a result of cardiomyocyte injury. The ongoing research of nanoparticle-based diagnostics is focused on the detection of troponins and other biomarkers associated with cardiovascular disease (CVD) [36]. For example, a research investigation utilized electrochemical impedance spectroscopy in conjunction with a peptide sequence (CFYSHSFHENWPS) that was deliberately engineered to have a strong binding affinity to cardiac troponin I (cTnI). This peptide sequence was immobilized onto gold nanoparticles, which were in turn coated onto a glassy carbon electrode [37]. The charge transfer resistance that was discovered had a clear positive relationship with the quantity of cTnI, hence facilitating the detection and quantification of the analyte in actual blood samples without the need for labeling. Potentiometric sensors, which utilize the differences in electrical potential between a working electrode and a reference electrode, have also been employed in the diagnosis of (CVD) to quantify the quantity of analytes [38].

Gold nanoparticles are frequently employed in photoacoustic imaging within the field of bioimaging to enhance the visualization of atherosclerotic plaque [39]. This methodology utilizes gold nanoparticles as contrast agents, specifically engineered to selectively bind to vascular cell adhesion molecule 1. This targeting mechanism organizes the accumulation of these nanoparticles in portions of the vasculature that are susceptible to illness. The nanoparticles absorb pulsed laser beams, resulting in thermal expansion and the production of sonic sounds that are detected by an ultrasound transducer [40].

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Cyborgs and Enhancement Technology

Abstract

As we move deeper into the twenty-first century there is a major trend to enhance the body with “cyborg technology”. In fact, due to medical necessity, there are currently millions of people worldwide equipped with prosthetic devices to restore lost functions, and there is a growing DIY movement to self-enhance the body to create new senses or to enhance current senses to “beyond normal” levels of performance. From prosthetic limbs, artificial heart pacers and defibrillators, implants creating brain–computer interfaces, cochlear implants, retinal prosthesis, magnets as implants, exoskeletons, and a host of other enhancement technologies, the human body is becoming more mechanical and computational and thus less biological. This trend will continue to accelerate as the body becomes transformed into an information processing technology, which ultimately will challenge one’s sense of identity and what it means to be human. This paper reviews “cyborg enhancement technologies”, with an emphasis placed on technological enhancements to the brain and the creation of new senses—the benefits of which may allow information to be directly implanted into the brain, memories to be edited, wireless brain-to-brain (i.e., thought-to-thought) communication, and a broad range of sensory information to be explored and experienced. The paper concludes with musings on the future direction of cyborgs and the meaning and implications of becoming more cyborg and less human in an age of rapid advances in the design and use of computing technologies.