Nature versus Nurture in Cellular Behavior and Disease

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Abstract

This project pursued two complementary, interrelated goals: 1) the incorporation of individual or groups of bacterial, fungal, or mammalian cells within novel three-dimensional (3D) cell-built or lithographically defined matrices that provide an engineered chemical and physical background to inform cells and direct their behavior; and 2) the development of two classes of targeted nanoparticle delivery platforms, protocells (porous nanoparticle supported lipid bilayers) and virus-like particles (VLPs), which could be selected against dormant/drug resistant/metastatic cells and selectively deliver multicomponent cargos (cocktails) to this recalcitrant population. This project provided a unique means to understand environmental influences on cellular behavior, in particular, dormancy, drug resistance, metastasis and
nanoparticle toxicology. It enabled the development of new targeting and drug delivery strategies
designed to selectively attack and kill dormant and drug resistant cells, thereby reducing a
significant reservoir of human disease. It established the scientific basis for creating new classes
of cell based sensors, and it will provide a platform in which to understand nanoparticle
toxicology in 3D environments, which better represent in vivo conditions.

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1. EXECUTIVE SUMMARY

There is now overwhelming evidence of environmental influences on cellular behavior. For example, many drugs screened/developed against 2D cell cultures in vitro are often ineffective against the same cells contained within 3D environments in vivo. Environmental effects are crucial to understanding a diverse spectrum of problems including cancer metastasis, drug resistance, TB dormancy, and nanoparticle toxicology. For example, it is proposed that cancer cells may use a quorum sensing mechanism similar to bacteria to regulate multicellular functions and control steps in metastatic colonization. Progress on addressing these problems is currently hindered by: 1) an inability to incorporate cells into three-dimensional architectures that better represent the nanostructured, 3-D extracellular matrix (ECM), tissues, or niches, where cells may reside in vivo and 2) an inability to selectively target and deliver effective therapeutics to dormant/drug resistant cell populations. This project vigorously pursued two complementary, interrelated goals: 1) the incorporation of individual or groups of bacterial, fungal, or mammalian cells within novel three-dimensional (3D) cell-built or lithographically defined matrices that provide an engineered chemical and physical background to inform cells and direct their behavior; and 2) the development of two classes of targeted nanoparticle delivery platforms, protocells (porous nanoparticle supported lipid bilayers) and virus-like particles (VLPs), which could be selected against arbitrary dormant/drug resistant/metastatic cells and selectively deliver multicomponent cargos (cocktails) to this recalcitrant population. This project provided a unique means to understand environmental influences on cellular behavior, in particular, dormancy, drug resistance, metastasis and nanoparticle toxicology. It enabled the development of new targeting and drug delivery strategies designed to selectively attack and kill dormant and drug resistant cells, thereby reducing a significant reservoir of human disease. It will establish the scientific basis for creating new classes of cell based sensors, and it will provide a platform in which to understand nanoparticle toxicology in 3D environments, which better represent in vivo conditions.
2. EXPERIMENTS AND RESULTS

2.1 Confinement-Induced Quorum Sensing of Individual *Staphylococcus Aureus* Bacteria

It is postulated that in addition to cell density, other factors such as the dimensions and diffusional characteristics of the environment could influence quorum sensing (QS) and induction of genetic reprogramming. Modeling studies predict that QS may operate at the level of a single cell, but, owing to experimental challenges, the potential benefits of QS by individual cells remain virtually unexplored. Here we report a physical system that mimics isolation of a bacterium, such as within an endosome or phagosome during infection, and maintains cell viability under conditions of complete chemical and physical isolation. For *Staphylococcus aureus*, we show that quorum sensing and genetic reprogramming can occur in a single isolated organism. Quorum sensing allows *S. aureus* to sense confinement and to activate virulence and metabolic pathways needed for survival. To demonstrate the benefit of confinement-induced quorum sensing to individuals, we showed that quorum-sensing bacteria have significantly greater viability over non-QS bacteria.

![Figure 1](image)

**Figure 1. Isolation of individual *S. aureus* within a nanostructured droplet.** (a) Schematic of physical system (not to scale) showing a cell incorporated in an endosome-like lipid vesicle within a surrounding nanostructured lipid/silica droplet deposited on glass substrate. (b) Scanning electron microscopy image of physical system. The nanostructure maintains cell viability under dry external conditions and allows complete chemical and physical isolation of one cell from all others. (c,d) Plain-view optical microscope images of individual cells in droplets (large outer circular areas). Magnified areas show differential interference contrast image and red fluorescence image of individual stained, isolated cells (both c and d) and green fluorescence image of NBD-labeled lipid localization at cell surface (c) or localized pH (d, using Oregon Green pH-sensitive dye). We find that, within the droplet, the cells become enveloped in an endosome-like lipid vesicle (c) and establish a localized pH consistent with physiological early endosomal conditions (~5.5).
Summary: Our results clearly illustrate that under certain conditions induction can be independent of both cellular density and spatial distribution. Thus the term ‘quorum sensing’ (and its implicit definition of ‘sensing a quorum’) is a misnomer, especially when applied to isolated, individual cells. Furthermore, our results confirm one experimental prediction of the diffusion-sensing hypothesis: “that isolated cells should be able to produce enough autoinducer for self-induction under plausible natural conditions”. But regarding whether autoinducer peptide–controlled genetic reprogramming should be classified as quorum sensing, diffusion sensing or efficiency sensing, we advocate a systems biology perspective where the underlying two-component regulatory system is inherently sensitive to the combined factors that control the concentration of extracellular autoinducer peptides proximate to the cell surface. This view readily extends the QS concept and attendant benefits to the individual cell level, where it is unnecessary to invoke complex social interactions for its evolution and maintenance. Importantly, it emphasizes that for medically important pathogens such as *S. aureus*, QS can contribute significantly to the survival of the isolated individual, as we showed in our reduced physical system.

Reference

2.2 Cell-Directed Integration into Three-Dimensional Lipid-Silica Nanostructured Matrices

Recently, we reported a unique approach in which living cells direct their integration into 3D solid-state nanostructures. Yeast cells deposited on a weakly condensed lipid/silica thin film mesophase actively reconstruct the surface to create a fully 3D bio/nano interface, composed of localized lipid bilayers enveloped by a lipid/silica mesophase, through a self-catalyzed silica condensation process. Remarkably, this integration process selects exclusively for living cells over the corresponding apoptotic cells (those undergoing programmed cell death), via the development of a pH gradient, which catalyzes silica deposition and the formation of a coherent interface between the cell and surrounding silica matrix. Added long-chain lipids or auxiliary nanocomponents are localized within the pH gradient, allowing the development of complex active and accessible bio/nano interfaces not achievable by other synthetic methods. Overall, this approach provides the first demonstration of active cell directed integration into a nominally solid-state three-dimensional architecture. It promises a new means to integrate “bio” with “nano” into platforms useful to study and manipulate cellular behavior at the individual cell level and to interface living organisms with electronics, photonics, and fluidics.

We attribute this cell-directed integration to the ability of cells to actively develop a pH gradient that catalyzes silica condensation within a thin three-dimensional volume surrounding the cells. Unlike *S. cerevisiae*, no pH gradient was observed near latex beads introduced to lipid-
silica films. As the rate of silica condensation is a strong function of pH and increases significantly between pH 3 and pH 6, it is likely that the development of the localized pH gradient governs microorganism self-encapsulation within silica. The capability of viable cells to actively facilitate their integration was demonstrated by depositing an aqueous mixture of yeast cells and neutrally charged latex beads, serving as yeast cell surrogates (equivalent vol. fraction), onto a weakly condensed lipid-templated silica film (30 min aging from film formation).

Summary: We have described a unique method in which cells introduced onto a solid state lipid-silica mesophase film actively reconstruct the surface to create a fully 3D bio/nano interface composed of a lipid-rich silica shell that coherently interfaces the cells with the nanostructured inorganic matrix. Auxiliary nanocomponents added to the lipid/silica mesophase film, or introduced with the cells, were localized at the cellular surface providing a means to introduce foreign functionalities, which remained accessible to and interacted with the cellular surface. This process is simple and does not expose cells to toxic solvents, chemical byproducts or drying stresses, preserving the original cell morphology. We expect this method to be general and adaptable to other bacterial and fungal cells and even mammalian cells. It promises to be useful as a new technique for integrating bio with nano, impacting fundamental studies in nanoparticle toxicology, cell-cell signaling, and development of bioelectronics and cell-based biosensors.
Reference

2.3 Protein-Directed Assembly of Arbitrary Three-Dimensional Nanoporous Silica Architectures

Through precise control of nanoscale building blocks such as proteins and polyamines, silica condensing microorganisms are able to create intricate mineral structures displaying hierarchical features from nano- to millimeter length scales. The creation of artificial structures of similar characteristics is facilitated through biomimetic approaches, for instance, by first creating a bio-scaffold comprised of silica condensing moieties which, in turn, govern silica deposition into 3D structures. In this work, we demonstrate a protein-directed approach to template silica into true arbitrary 3D architectures by employing cross-linked protein hydrogels to controllably direct silica condensation. Protein hydrogels are fabricated using multiphoton lithography which enables user-defined control over template features in three dimensions. Silica deposition, under acidic conditions, proceeds throughout protein hydrogel templates via flocculation.

Figure 3 Microfabrication of artificial diatom and radiolarian frustules. a) Images of diatom frustules (upper left panels) direct the fabrication of BSA protein microstructures (DIC micrograph, upper right panel; scale bar, 20 μm) using MDML. (lower panel; scale bar, 20 μm) Phase micrograph of diatom-like silica microstructures. b) CAD designed microfabrication of artificial radiolarian frustules using MDML (scale bars, 10 μm). Upper inset, top view; lower inset shows 3D rendering generated from the image sequence used to direct multiphoton fabrication. c) Characterization of hierarchical features displayed by microfabricated diatom structures using SEM and AFM. The smallest constituent particles, on the order of ~16 nm in size, are visible in the AFM phase image, bottom right panel (scale bars clockwise from top left, 20 μm, 2 μm, 200 nm, 50 nm).
of silica nano-particles by protein molecules as indicated by dynamic light scattering (DLS) and time-dependent measurements of elastic modulus. Following silica deposition, the protein template can be removed using mild thermal processing yielding high surface area (625 m²/g) porous silica replicas that do not undergo significant volume change compared to the starting template. We demonstrate the capabilities of this approach to create bio-inspired silica microstructures displaying hierarchical features over broad length scales, and demonstrate the infiltration/functionality capabilities of the nanoporous silica matrix by laser printing a 3D gold image within a 3D silica matrix. This work provides a foundation to potentially understand and mimic biogenic silica condensation under the constraints of user-defined bio-templates, and further should enable a wide range of complex inorganic architectures to be explored using silica transformational chemistries, for instance silica to silicon, as demonstrated herein.

**Summary:** We have described a strategy to generate arbitrary 3D silica architectures using protein-directed condensation of silica. The key method underpinning template fabrication, multiphoton lithography, can be readily extended to mm length scales, offering control over hierarchical features across ~7 orders of magnitude. Importantly, protein-directed assembly of silica is amenable to single photon patterning of the protein template, facilitating translation of this approach to standard 2D photo-lithographic practices. In contrast to nearly all photolithographic processes that involve chemical templating, development and/or thermal processing procedures (e.g., shrinkage of SU846 and direct ink writing scaffolds), the use of protein hydrogel scaffolds permits high fidelity replication of the template without significant volume changes in the resulting replica. We further demonstrated both replication (Figure 3a,c) and design (Figure 3b) of complex diatom/radiolarian morphologies comprised of silica. The ability to design/prototype silica architectures in 3D followed by transformational chemistries, for instance silica to silicon as demonstrated here, should enable a wide range of complex inorganic architectures to be explored, in particular those that require precise 3D geometries (e.g., photonics, bio-implants), hierarchical, high surface area features with robust mechanical properties and that benefit from high density functionalization (e.g., sensors, catalysts).

**Reference**

**2.4 The Targeted Delivery of Multicomponent Cargos to Cancer Cells by Nanoporous Particle-Supported Lipid Bilayers**

Encapsulation of drugs within nanocarriers that selectively target malignant cells promises to mitigate side effects of conventional chemotherapy and to enable delivery of the unique drug combinations needed for personalized medicine. To realize this potential, however, targeted nanocarriers must simultaneously overcome multiple challenges, including specificity, stability and a high capacity for disparate cargos. Here we report porous nanoparticle-supported
lipid bilayers (protocells) that synergistically combine properties of liposomes and nanoporous particles. Protocells modified with a targeting peptide that binds to human hepatocellular carcinoma exhibit a 10,000-fold greater affinity for human hepatocellular carcinoma than for hepatocytes, endothelial cells or immune cells. Furthermore, protocells can be loaded with combinations of therapeutic (drugs, small interfering RNA and toxins) and diagnostic (quantum dots) agents and modified to promote endosomal escape and nuclear accumulation of selected cargos. The enormous capacity of the high-surface-area nanoporous core combined with the enhanced targeting efficacy enabled by the fluid supported lipid bilayer enable a single protocell loaded with a drug cocktail to kill a drug-resistant human hepatocellular carcinoma cell, representing a 106-fold improvement over comparable liposomes.

Summary: This nanoparticle-based delivery vehicle is the first example of a nanocarrier that simultaneously addresses the complex requirements of targeted, multicomponent delivery. Perhaps the most striking feature of protocells is their ability to deliver high concentrations of

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Figure 4. Schematic depicting the successive steps of multivalent binding and internalization of targeted protocells, followed by endosomal escape and nuclear localization of protocell-encapsulated cargo. DOPC protocells [1] bind to HCC with high affinity due to recruitment of SP94 targeting peptides (magenta) to the cell surface, [2] become internalized via receptor-mediated endocytosis, and [3] release their cargo into the cytosol upon endosome acidification and protonation of the H5WYG fusogenic peptide (blue). Cargos modified with a NLS are transported through the nuclear pore complex and become concentrated in the nucleus [4].
diverse cargos and 'cocktails' of chemically disparate components. Protocells encapsulate 1,000 times more siRNA than similarly sized liposomes with the same bilayer composition and, when targeted with the SP94 peptide, induce apoptosis in 50% of Hep3B within 36 h without affecting the viability of hepatocytes. Another distinctive characteristic of protocells is that the enhanced fluidity and stability of the SLB support multivalent peptide recruitment to surface receptors expressed by the target cell, which suggests that displaying two or more types of ligand on the protocell surface might enable complex binding interactions. We expect, therefore, that modifying the protocell SLB with ligand(s) that bind to surface receptor(s) uniquely or overexpressed by the target cell along with a ligand that promotes internalization (for example the octaarginine peptide, which stimulates macropinocytosis would enable both selective targeting and intracellular delivery for cancers where cell-specific receptors are not normally endocytosed.

**News & Views Commentary on this work:** Irvine, D. J.: News & Views: One nanoparticle, one kill. *Nature Materials* 2011, 10, 342. “Ashley et al. describe in Nature Materials a new type of composite nanoparticle — a hybrid between liposomes and nanoporous silica nanoparticles. The properties engineered into this system elegantly synergize to approach the goal of an ideal targeted-delivery agent. They combine unprecedented specificity in binding to cancer cells with the combinatorial delivery of drug cocktails. (Darrell J. Irvine, the Koch Institute for Integrative Cancer Research, MIT, the Ragon Institute of Massachusetts General Hospital, MIT and Harvard University, and Howard Hughes Medical Institute.)

**Reference**

### 2.5 Cell-Specific Delivery of Diverse Cargos by Bacteriophage MS2 Virus-like Particles

Virus-like particles (VLPs) of bacteriophage MS2 possess numerous features that make them well-suited for use in targeted delivery of therapeutic and imaging agents. MS2 VLPs can be rapidly produced in large quantities using in vivo or in vitro synthesis techniques. Their capsids can be modified in precise locations via genetic insertion or chemical conjugation, facilitating the multivalent display of targeting ligands. MS2 VLPs also self-assemble in the presence of nucleic acids to specifically encapsidate siRNA and RNA-modified cargos. Here we report the use of MS2 VLPs to selectively deliver nanoparticles, chemotherapeutic drugs, siRNA cocktails, and protein toxins to human hepatocellular carcinoma (HCC). MS2 VLPs modified with a peptide (SP94) that binds HCC exhibit a 104-fold higher avidity for HCC than for hepatocytes, endothelial cells, monocytes, or lymphocytes and can deliver high concentrations of
Figure 5. Schematic depicting the process used to synthesize HCC-specific MS2 VLPs that encapsidate chemically disparate therapeutic and imaging agents. Nanoparticles (e.g., quantum dots), protein toxins (e.g., ricin toxin A-chain), and drugs (e.g., doxorubicin) are first conjugated to the pac site using an appropriate crosslinker; for example, quantum dots encapsulated within an amine-terminated PEG layer are linked to a derivative of the pac site that contains a 3’ uracil spacer and sulfhydryl group using the amine-to-sulfhydryl crosslinker, LC-SPDP. Ninety coat protein dimers then self-assemble around RNA-modified cargo to form the 27.5-nm capsid. siRNA molecules drive capsid re-assembly in the absence of the pac site and become incorporated within VLPs at an average concentration of 90 siRNAs per particle; the yield of fully re-assembled, siRNA-loaded capsids is depicted in the TEM image (scale bar = 50 nm). Cargo-loaded VLPs can be further modified with targeting peptides to promote selective internalization by cancer cells, with fusogenic peptides to promote endosomal escape of internalized VLPs, and with PEG to reduce non-specific interactions and mitigate the humoral immune response against coat protein. Peptides synthesized with a C-terminal cysteine residue are linked to lysine residues (red) on the exterior capsid surface (yellow) via a heterobifunctional crosslinker with a PEG spacer arm.

encapsidated cargo to the cytosol of HCC cells. SP94-targeted VLPs loaded with doxorubicin, cisplatin, and 5-fluorouracil selectively kill the HCC cell line, Hep3B, at drug concentrations <1
nM, while SP94-targeted VLPs that encapsidate a siRNA cocktail, which silences expression of cyclin family members, induce growth arrest and apoptosis of Hep3B at siRNA concentrations <150 pM. Impressively, MS2 VLPs, when loaded with ricin toxin A-chain (RTA) and modified to codisplay the SP94 targeting peptide and a histidine-rich fusogenic peptide (H5WYG) that promotes endosomal escape, kill virtually the entire population of Hep3B cells at an RTA concentration of 100 fM without affecting the viability of control cells. Our results demonstrate that MS2 VLPs, because of their tolerance of multivalent peptide display and their ability to specifically encapsidate a variety of chemically disparate cargos, induce selective cytotoxicity of cancer in vitro and represent a significant improvement in the characteristics of VLP-based delivery systems.

Summary: MS2 VLPs can be readily adapted for specific delivery of a variety of molecular cargos to diverse cell types. Because of their natural ability to encapsidate nucleic acids, they are especially well-suited for delivering RNA- and DNA-based drugs, but they can also encapsulate diverse, non-nucleic acid cargos (e.g., quantum dots, chemotherapy drugs, and protein toxins) when the cargo molecules are linked to the MS2 pac site. In the experiments described here, we decorated MS2 VLPs with SP94, an HCC-specific peptide, but other targeting strategies are possible. Indeed, prior work reported the use of transferrin, of a DNA aptamer that binds to a tyrosine kinase receptor, and of folic acid for this purpose. The MS2 VLP platform has a special advantage when peptides are used for targeting, however. We previously showed that peptides can be displayed on the VLP surface by genetic fusion to coat protein and that these VLPs encapsidate the mRNA that encodes the fusion protein. On this basis, we created a system for VLP-based peptide display analogous to conventional filamentous phage display, which allows for affinity selection of arbitrary binding activities from complex random sequence peptide libraries. Selected sequences are then recovered by reverse transcription and polymerase chain reaction and recloned for synthesis of the selected VLPs in bacteria. The existence of this affinity selection capability means that a single particle can serve both for identification of cell-specific targeting ligands and as a drug delivery vehicle.

Reference
2.6 Delivery of Small Interfering RNA by Peptide-Targeted Mesoporous Silica Nanoparticle-Supported Lipid Bilayers

The discovery of RNA interference (RNAi) as a robust modulator of eukaryotic gene expression has provided unique insights into cellular pathways that regulate a number of fundamental biological processes. In addition, it has allowed the development of a new class of reagents with powerful therapeutic potential. Under physiological conditions, double-stranded RNAs are digested into 21-23 base pair fragments. The resulting cleavage product binds to an RNA-induced silencing complex (RISC) where the sense strand (relative to an endogenous mRNA) is discarded. RISC loaded with single-stranded RNA binds corresponding mRNAs in the cytoplasm and mediates either a translational repression or an enzymatic cleavage depending on the nature of the base pairing. Based on remarkable progress in identifying critical aspects of this pathway, it has become possible to envision utilizing the features of RNAi to treat any of a variety of diseases whose pathology can be modulated by a decrease in the expression of a specific gene product. Small-interfering RNA (siRNA) is a double-stranded RNA sequence with perfect homology to a region of a cellular message that can be either ectopically introduced into cells or generated from a precursor RNA expressed from a transfected plasmid or transduced virus. siRNAs enter the RISC pathway and mediate cleavage of the targeted message, providing a mechanism whereby, in theory, any cellular mRNA can be inactivated in a precise and controlled manner. siRNAs are especially attractive as anti-cancer therapies, since profound changes in the behavior or survival of neoplastic cells are induced by decreases in the expression of activated oncogenes, cell cycle regulators, or pro-apoptotic genes. The expression of transcripts whose products are involved in the induction of drug resistance can also be targeted by siRNAs. The cytotoxic activity of siRNAs has been clearly demonstrated in a number of in vitro and in vivo model systems. Other researchers (Davis et al. Nature Materials 264, 1067-1070, 2010) recently extended these studies by reporting that the systemic administration of siRNA encapsulated in targeted nanoparticles repressed gene expression in the tumor cells of human patients. Thus, targeted delivery of RNAi agents promises to effectively treat a variety of cancers.

Despite this promise, however, it is clear that a number of significant barriers must be overcome before the widespread clinical use of siRNA technology becomes feasible. Several issues, including ensuring specificity for the target gene, prolonging the duration of siRNA activity, and preventing the induction of an innate immune response have been addressed, at least to some extent, by a careful consideration of siRNA sequences and chemical modifications of the ribose backbone structure. The major obstacle remaining for the development of successful siRNA therapeutics is an optimization of the multiple components of an efficient delivery system. In the previous sections of this report, we described a novel and remarkably versatile nanoparticle, termed a protocell, which synergistically combines features of both mesoporous silica particles and liposomes to exhibit many features of an ideal targeted therapeutic delivery platform. Here we describe the ability of protocells to serve as a delivery platform for siRNAs.
The unique characteristics of targeted protocells address many of the deficiencies that currently limit the clinical use of these macromolecular agents.

**Summary:** The therapeutic potential of small interfering RNAs (siRNAs) is severely limited by the availability of delivery platforms that protect siRNA from degradation, deliver it to the target cell with high specificity and efficiency, and promote its endosomal escape and cytosolic dispersion. We have shown that mesoporous silica nanoparticle-supported lipid bilayers (or “protocells”) exhibit multiple properties that overcome many of the limitations of existing delivery platforms. Protocells have a 10- to 100-fold greater capacity for siRNA than corresponding lipid nanoparticles and are markedly more stable when incubated under physiological conditions. Protocells loaded with a cocktail of siRNAs bind to cells in a manner dependent on the presence of an appropriate targeting peptide and, through an endocytic pathway.

**Figure 6.** Schematic depicting the process used to synthesize siRNA-loaded nanoporous particle-supported lipid bilayers (protocells). To form protocells loaded with therapeutic RNA and targeted to hepatocellular carcinoma (HCC), nanoporous silica cores modified with an amine-containing silane (AEPTMS) were first soaked in a solution of small interfering RNA (siRNA). Liposomes composed of DOPC, DOPE, cholesterol, and 18:1 PEG-2000 PE (55:5:30:10 mass ratio) were then fused to siRNA-loaded cores. The resulting supported lipid bilayer (SLB) was modified with a targeting peptide (SP94) that binds to HCC and an endosomolytic peptide (H5WYG) that promotes endosomal/lysosomal escape of internalized protocells. Peptides, modified with glycine-glycine (GG) spacers and C-terminal cysteine residues, were conjugated to primary amines present in DOPE moieties via a heterobifunctional crosslinker (SM(PEG)_24) with a 9.5-nm polyethylene glycol (PEG) spacer. The SP94 and H5WYG sequences are given in red.
followed by endosomal disruption, promote delivery of the silencing nucleotides to the cytoplasm. The expression of each of the genes targeted by the siRNAs was shown to be repressed at the protein level, resulting in a potent induction of growth arrest and apoptosis. Incubation of control cells that lack expression of the antigen recognized by the targeting peptide with siRNA-loaded protocells induced neither repression of protein expression nor apoptosis, indicating the precise specificity of cytotoxic activity. In terms of loading capacity, targeting capabilities, and potency of action, protocells provide unique attributes as a delivery platform for therapeutic oligonucleotides.

Reference

3. SUMMARY

1) We adapted our tailorable hybrid bio/inorganic 3D matrices (CJB et al. *Science* 2006; *Nature Chem Biol.* 2010) to cancer hepatocarcinoma cells. Integrated cells are non-replicative and drug resistant but maintain biofunctions like protein production, pinocytosis and receptor mediated endocytosis. These dormant cell states are resistant to external threats and could serve as reporters of environmental/battlefield conditions or as autonomous factories for enzyme production.

2) Using multiphoton protein lithography, we fabricated arbitrary 3D biomolecular architectures with natural or non-natural structures but retained biomolecular activity and biocompatibility. Through gray scale control, the properties, e.g. modulus and diffusivity, and activity of the resulting protein architecture were optically defined. Such structures serve as 3D micro-environmental chambers in which to study and direct cellular behavior at the individual cell and multi-cellular levels.

3) We showed the direct conversion of lithographically-defined 3D protein scaffolds to silicon dioxide and their further transformation to silicon. This biomineralization process mimics features of diatom formation and results in a new class of rugged biocomposites with retained biofunctionality.

4) We discovered a generalized process, Silica Cell Replication, wherein mammalian cells direct their exact replication in silico. The silica cell replicas preserve nm- to macro-scale cellular features and dimensions after drying at room temperature. Re-exposure to water provides access
to intracellular components, where preliminary experiments show retention of enzymatic activity.

5) We showed that the combined stability, cargo capacity, and targeting selectivity of protocells results in a million times improvement in selective cytotoxicity to drug resistant hepatocarcinoma cancer cell lines compared to the corresponding FDA-approved liposomal delivery agent (Nature Materials, feature cover article, May 2011).

6) We demonstrated selective delivery of nanoparticles, chemotherapeutic drugs, siRNA cocktails, and protein toxins to human hepatocellular carcinoma with MS2 virus-like particles (ACS Nano, feature cover article July 2011).
4. APPENDIX

PUBLICATIONS 2009-2012


## 5. DISTRIBUTION

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