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Biologically inspired energy: harnessing molecular functionality towards nanosystemic design

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Evaluation of: Ho D, Chu B, Lee H, Montemagno CD: Protein-driven energy transduction across polymeric biomembranes. *Nanotechnology* 15(8), 1084–1094 (2004) [1]. This manuscript highlights the intersection of two rapidly advancing fields: molecular nanotechnology and alternative energy development. The functionalization of biomimetic membranes with the energy-transducing proteins bacteriorhodopsin and cytochrome c oxidase to form biologically active thin films is described. Following the fabrication and characterization of protein-embedded Langmuir–Blodgett films, these materials have been demonstrated to exhibit enhanced mechanical robustness over conventional, lipid-based materials, indicating a potential applicability towards device construction. In addition, these thin films have demonstrated the ability to use green light to generate proton gradients as well as photoelectric currents across these membrane thin films, which indicate their utility in preserving protein activity. We present the results and significance of this paper in its application of nonbiological materials to harness evolutionarily perfected biomolecular energetics.

Keywords: biomolecular energetics, Langmuir–Blodgett method, nanotechnology, polymeric biomembranes

The nanotechnology community has witnessed several recent studies that have used proteins as the underlying technology for energy or medically relevant biofunctionalized devices and beyond. Many of these studies have been based upon the reconstitution or insertion of proteins into biomimetic environments or have draw upon molecular architectures inspired by nature to develop novel classes of materials with mimetic functionality. These materials have included copolymer membranes, which simulate the natural membrane environments found at cell surfaces, and peptide amphiphiles that can preserve or encapsulate proteins for activity harnessing [2–5]. A key motivation for these studies is directed towards the goal of utilizing innate biomolecular activity to elicit desired responses from cells that are interfaced with the material (e.g., directed growth) or embed enhanced amounts of information within the material to deepen its functional complexity (e.g., introducing voltage-gated pore protein activity or energy conversion). As such, the potential applicability of these devices can be directed towards a spectrum of disciplines, from fabricating exciting new classes of nanotechnology-enabled biocompatible materials to acquiring bioinspired energy for medically relevant applications.

Among the advantages possessed by materials that take advantage of the integration of biological with nonbiological components is the ability to activate these materials with functional mechanisms that have been perfected by nature and that govern some of its most intricate processes, such as fluid–analyte balance, homeostasis, energy conversion and cell signaling. At the same time, however, applying engineered materials as matrices for the preservation of these biostructures enables the addition of enhanced robustness and tailorable material architectures and characteristics towards a rationally designed and universally applicable platform for hybrid material fabrication. For example, work performed by Heller and colleagues has used the glucose oxidase redox protein towards the development of electrical outputs with glucose serving as the fuel source for future applications as an energy source for powering implants *in vivo* [6]. Meier and colleagues have utilized a block copolymeric thin film to preserve pore protein (LamB/OmpF) activity for transmembrane gating studies as well as viral receptor-mediated DNA transfer across a biomimetic membrane that also preserved DNA structural integrity [7]. Recently, Stupp and colleagues developed a peptide amphiphile-based nanofiber scaffold that, when introduced into physiological conditions, was able to present an amino acid sequence to induce neural progenitor cells to differentiate into neurons rather than astrocytes that mediate scar tissue formation and paralysis from spinal cord injury [8]. Furthermore, Messersmith and

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colleagues have developed L-3,4-dihydroxyphenylalanine (DOPA)-based mussel adhesive protein mimetics as biomimetic adhesives for medically relevant applications in antifouling, among several others [9]. The encompassing subject of this article as well as the aforementioned work, represents exciting examples of the progression towards the directed embedding of biofunctionality into nonbiological materials.

Methods & results

Utilizing the native expression system of both proteins, which were *Halobacterium Halobium* (bacteriorhodopsin [BR]) and *Rhodobacter Sphaeroides* (cytochrome c oxidase [COX]), Ho and colleagues isolated both proteins for resultant integration into the polymeric thin film systems for hybrid membrane fabrication. The protein-embedded polymer films were fabricated using the Langmuir–Blodgett (LB) method. The amphiphilic triblock copolymer solubilized in chloroform was deposited at the air–water interface, whereby the hydrophobic 'B' block of the membrane (polydimethylsiloxane [PDMS]) was capable of suspending the copolymer molecules on the surface of the water. Ho and colleagues used this method, owing to its ability to transfer the free-floating Langmuir films onto a solid support, thereby enabling the films to possess larger areas compared with those formed by the traditional 'black lipid membrane' suspended film technique. Furthermore, the proteins could also be introduced in high quantity to be embedded into the film by injecting BR or COX into the subphase of the Langmuir film for diffusion-mediated insertion at the air–water interface.

Following film compression and substrate deposition, Ho and colleagues provide a very thorough series of characterization trials, including membrane surface tension evaluation, to determine the the architectural outcome of the membrane integrated with the protein (e.g., copolymer block orientation), photoelectric measurement, impedance spectroscopy and ultraviolet (UV)-vis spectrophotometry. They also used transmembrane pH change analysis by coating the Nafion® fluoropolymer, which is used commonly as a fuel cell membrane, as a proton-transporting substrate to allow for large-area BR-embedded film support while simultaneously enabling film activity tests. This series of experiments indicated that the compressed LB film was able to achieve a vertical 'ABA' orientation utilizing contact angle measurements. This orientation was further confirmed by acquiring photoelectric transport plots that were indicative of embedded BR activity and demonstration of hybrid protein–polymer transmembrane energy conversion. Impedance spectroscopy was conducted whereby protein-mediated resistance was observed as a parallel resistance to that generated by the membrane itself. Ho and colleagues were able to observe light-dependent BR activity as well as cytochrome *c*-mediated activity of COX as indicated by light and redox mediator-induced decreases in total membrane resistance due to protein activity of BR and COX, respectively. Furthermore, extensive pH experimentation was performed using purple membrane with site-specific labeled Biotin XX-SSE and BR/polymer-functionalized Nafion. Changes in pH were measured over time by examining BR-mediated proton transport across the fluoropolymer as well as the ability of BR to preserve ionic gradients.

In summary, these hybrid membranes were able to form very stable Langmuir-films for subsequent substrate deposition with surface pressures as high as approximately 50 mN/m and were capable of generating a current density of approximately 100 nA/cm^2, with measured pH level changes of 0.5–1.75, utilizing LB-coated large-area substrates of 0.5 × 0.5 inches in dimension.

Discussion & significance

The article by Ho and colleagues represents an approach to utilizing LB fabrication to generate energy-transducing/block copolymer hybrid films that are, in turn, interfaced with a proton-conducting fluoropolymer that is extremely robust, easily coated with surface-activating materials (e.g., gold, chromium adhesion layers) and highly flexible for increased versatility in handling. As such, the fluoropolymeric substrate was more than capable of serving as a solid support for the deposition of the biomolecular hybrids. Instead, it served as an active component of the composite device. Paired with the robustness of the fluoropolymer was the enhanced durability of the block copolymer that has been demonstrated previously to possess enhanced mechanical robustness over lipid-based membranes [2,3]. In addition, BR is known to be among the most robust membrane proteins in existence, with reported stability within temperature ranges of 0–140°C and pH levels of 0–12 [1]. The interface of these robust components then

represented a logistically feasible approach towards rational, active material fabrication based upon protein activity harnessing.

The extensive protein–polymer characterization conducted provided a well-rounded view of the film capabilities. The authors were able to clearly examine LB film fabrication parameters with respect to phase transition properties and, using contact angle measurements, were able to examine the surface properties and resultant architectural characteristics. UV-vis spectrophotometry was able to confirm the presence of BR in the films, based upon its spectral properties and key absorbance peaks at 278 and 563 nm. Confirmation of vertical film deposition at a pressure suitable for acquisition of protein activity was then proven via photoelectric measurement, impedance spectroscopy and transmembrane pH assessment.

With respect to biomedical applications that this reported biomolecular hybrid may find, the versatility and robustness of the device contribute to the range of possibilities available. In the medically relevant community, photoactive proteins would potentially generate significant interest in the ophthalmology community as engineered replacements for damaged photoreceptors in the context of vision research. As the copolymer membranes and the proteins themselves would appear to possess a high degree of biocompatibility given the protein reconstitution trials and seeing as they can be further functionalized for potential *in vitro* or *in vivo* targeting applications, they would seem to be ideal candidates as biomimetic photoactive device components.

Outside of using these materials as potential functional tissue replacements, the novel energy-related aspects of the BR protein and the demonstrated integration of the composite membrane with the Nafion fluoropolymer form a strong partnership towards potential applications as biocompatible energy sources or proton leakage reversal materials for the aforementioned envisioned implantable fuel cells or for powering implants directly. Nafion is characterized as a perfluorosulfonic acid polymer, where it is comprised of a perfluorinated carbon backbone with a structure similar to Teflon®, which contains sulfonic acid sidechains that serve as proton donors that enable the transmembrane transport mechanism. However, these acidic sidechains are embedded within the fluoropolymer matrix and are essentially immobile, rendering the Nafion capable of nonharmful contact with the skin. Furthermore, as the fluoropolymer itself is coated completely by the protein-functionalized film, it is further shielded from the external environment.

In evaluating the reported technology, the findings of the article by Ho and colleagues report the development of a versatile approach towards facilitating one of nature's most elegant photo-active processes towards applications that are medically relevant and significant to other fields, such as biocompatible energy development, both of which would benefit from the nonpathogenic aspects of the protein and membrane material utilized. Continued work that would benefit the maturation of this technology towards it fruition as a translational technology would include exploration of packaging solutions to preserve protein functionality in the ambient environment. Furthermore, the development of solutions to enhance thin film to substrate adsorption, should it be required, would further enhance systemic robustness, especially if it will find *in vivo* applicability. The innate properties of the device components render it amenable to coalescence with these optimization parameters. Therefore, the device reported represents potentially very beneficial outcomes at the intersection of molecular nanotechnology and energy development.

Conclusion & future perspective

The introduction of biologically inspired technologies, such as those mentioned previously as well as the technology developed by the Ho and colleagues paper, has illuminated a field that is capable of methodically engineering desired functionality into robust, tailorable materials for therapeutic applications and beyond. A spectrum of potential applications of this exciting class of materials has been illustrated, from generating extremely strong adhesives to inducing cellular differentiation for spinal cord injury therapeutics and harvesting bioenergy to drive implants.

The manuscript by Ho and colleagues represents an exciting advance towards device-scale fabrication of active materials with specific emphasis on biologically inspired energy. It provides a methodical approach towards systemic characterization as well as fabrication, which truly exemplifies and illustrates the versatility of generating functional architectures from molecular buildings blocks comprised of

membrane proteins, biomimetic thin films and active substrates. Coupled with the aforementioned advancements in the field, the utilization of optimized biological mechanisms and phenomena as foundations for the development of nanotechnology are expected to generate exciting breakthroughs for quite some time.

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Executive summary

- Bacteriorhodopsin represents a robust energy-transducing biomolecule with a mechanism that can be harnessed for potential bioenergy device construction. It remains functional in extreme pH as well as extreme temperature conditions.
- Block copolymers mimic the alternating hydrophilic and hydrophobic (amphiphilic) properties found in biological membranes.
- The amphiphilic characteristics of copolymers enable the deposition of large-area Langmuir–Blodgett membranes with embedded proteins introduced into the film subphase. Observed surface pressures were in excess of 50 mN/m, indicating highly stable film formation.
- Copolymer membranes support the photoelectric activity of bacteriorhodopsin upon hybrid film exposure to light. Optimal retinal chromophore photoisomerization occurs at approximately 560 nm (green light).
- Protein-driven energy transduction was characterized thoroughly using a suite of modalities, including transmembrane electrical measurement and pH change, as well as impedance spectroscopy.
- Biomolecule–copolymer hybrids were transferred to Nafion® proton exchange membrane substrates and protein activity was shown to be preserved, indicating the potential translational application of these films as biofuel cell components.
- Fruition of bioinspired energy devices will be empowered by strategies to package the proteins to preclude denaturation, as well as functionalization of the membrane to preclude delamination of the films from the substrates.

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