A soft and transparent handleable protein model

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The field of structural biology currently relies on computer-generated graphical representations of three-dimensional (3D) structures to conceptualize biomolecules. As the size and complexity of the molecular structure increases, model generation and peer discussions become more difficult. It is even more problematic when discussing protein–protein interactions wherein large surface area contact is considered. This report demonstrates the viability of a new handleable protein molecular model with a soft and transparent silicone body similar to the molecule's surface. A full-color printed main chain structure embedded in the silicone body enables users to simultaneously feel the molecular surface, view through the main chain structure, and manually simulate molecular docking. The interactive, hands-on experience deepens the user's intuitive understanding of the complicated 3D protein structure and elucidates ligand binding and protein–protein interactions. This model would be an effective discussion tool for the classroom or laboratory that stimulates inspired learning in this study field. [http://dx.doi.org/10.1063/1.4739961]

I. INTRODUCTION

Molecular and structural biology are based on the concept that life phenomena are the result of molecular chemical processes. Of all the biomolecules found in the cell, proteins play the most important roles in nearly every cellular function. In general, each protein has a unique and complex three-dimensional (3D) structure that is specific to its biological function.[1] Therefore, a deep understanding of protein structures is a key to elucidating the molecular mechanism underlying protein functions. In addition, many proteins in nature form oligomeric complexes to achieve more sophisticated functionality, such as allostericity or regulation. Meanwhile, pharmaceutical companies are currently developing engineered next-generation antibody drugs[2] that recognize and bind only to specific target molecules wherein protein–protein interactions are the most important factor. Thus, a deep understanding of the mechanism underlying protein–protein interactions is also of great importance. To elucidate these mechanisms, the shape and physicochemical properties of the molecular surface, especially those for molecular recognition (ligand binding, protein–protein association) sites, are critically important. However, unlike some globular enzymatic proteins, which often have a narrow binding site cleft, protein–protein interaction sites have a large surface area and are complicated in shape, making their 3D conceptualization very difficult, even with the help of graphical or virtual representations.

Currently, the study of protein structures has been dominated by the use of 3D visualization software. Various currently available visualization programs can display a computer-generated 3D protein model. However, with this method, one has to constantly manipulate and rotate the molecular image displayed on the screen to perceive the 3D protein structure. The performance of such software-based modeling depends on user proficiency, which may cause a gap in the individual's understanding of the protein structure possibly leading to a crucial misunderstanding in discussions with others. Although software performance and realistic display of 3D imaging has improved with software advancements, the images are still flat and cannot be touched. Stereo viewing has been routinely used in molecular visualization for decades, and this technique mitigates the need of constant manipulation and rotation of the molecular image. However, stereo viewing uses a static image, and therefore, we can only observe the molecular structure at a fixed angle.

On the other hand, tangible models have an apparent advantage in that any user can manipulate them, explore their 3D structures, and share the structural images with others. Physical molecular models are widely known to be useful tools for teaching basic chemistry and for scientific discussions in the laboratory.[3] Many educators and researchers recognize the importance of hands-on modeling for educational and scientific discussion. A tangible protein model is also an effective tool because it can help users comprehend the spatial aspects of complex protein structures, which is beyond that obtained from a printed 2D computer graphic or a molecular animation displayed on a computer screen. Indeed, in the early study of structural biology, physical models of biomolecules (e.g., DNA[4] and protein[5]) were used to investigate their unknown 3D structures. In this journal, Corey and Pauling reported the space-filling (CPK) model for the study of amino acid and peptide structures.[6] However, protein molecules are usually composed of thousands to tens of thousands of atoms, and as the size and complexity of the molecular structures increase, building the molecular model becomes more difficult if not almost impossible. John Kendrew built a huge brass model of a myoglobin,[7] that was supported by a forest of rods that obscured the view of the model and prevented access to the interior of the model. Today, physical models are rarely seen in educational institutions or laboratories, and they are being

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replaced with cheaper and more adaptable virtual representations.

Recent advances in computer-aided design (CAD) and rapid prototyping technology have enabled us to directly fabricate a complex 3D object. The technique is known as *additive manufacturing technology* in which an object is created in a layer-by-layer manner. This technique has a great advantage in that nearly any shape, including nesting structures, can be built in one process. This technology was used to build a physical protein model with a complicated structure[8] that was subsequently applied to research and communication in molecular biology with augmented reality[9] and to biological education in a classroom setting.[10,11]

Of the current rapid prototyping techniques, the ZPrinter (3D Systems, Rock Hill) and the selective laser sintering (SLS) technique[12] are most frequently used for the fabrication of physical protein models. The SLS technique uses a nylon powder as a base material, which is sintered by a CO_2 laser to build the final structure in a layer-by-layer manner. The resulting nylon models are flexible and durable, making them well suited for representing ribbon or wire frame models. This fabricating method does not include automatic coloring, and therefore models must be hand-painted to specify structural and physical properties, which is impossible for models with a complicated structure. Meanwhile, the ZPrinter performs 3D printing using inkjets to draw a cross section of the object by depositing pigmented binder on a thinly covered plaster-based powder plane. The drawing process is repeated until every layer is printed and a full-color model is created. This fabricating technique is very useful for constructing the complex topological structure of the protein's main chain, which is represented as a ribbon or wire frame model that is completely colored according to the secondary structure elements or for constructing a molecular surface model colored according to the physical properties of the surface, such as electrostatic potential or hydrophobicity.

However, since educational or discussion tools are supposed to be handled frequently by many users, the above two methods have some critical drawbacks. The SLS model has some durability and flexibility, but it is not sufficiently durable for repeated handling. Moreover, the SLS model sometimes cannot support its own weight and thus requires mechanical supports. Models made using the ZPrinter method are brittle, their surface can be easily stained when handled with bare hands, and it is not waterproof (washable). In addition, these models present an educational drawback because it is desirable that through hands-on experience with a physical model, students/researchers learn biological interactions, such as how a ligand (low molecular weight) is bound to a protein's active site or how protein molecules interact with each other to form a quaternary structure or ligand–receptor complexes.

In this paper, a new concept of physical protein model that circumvents the above problems using a combination of rapid prototyping and resin-casting techniques is presented. The new models are soft, durable, stain-free (waterproof and washable), and above all, they enable the display of the main chain structure, surface shape, and physical properties all in one model. Because the new model is soft, users can deform the model and experience ligand binding/dissociation and protein–protein association, which has never been possible with other physical molecule models. The model presented here would be a useful tool not only for biological education but also for high-level discussion in the laboratory spurring the researcher's inspiration.

II. MATERIALS AND METHODS

The fabrication process for this new molecular model is illustrated in Figure 1. First, the atomic coordination data of a protein molecule were downloaded from the Protein Data Bank (PDB, Figure 1(a)). Protein Viewer software, such as RasMol,[13] PyMol [http://www.pymol.org/], MolMol,[14] or Chimera[15] can export the 3D protein structure data in the Virtual Reality Modeling Language (VRML) format, which can then be edited using CAD software. With this software, users can freely edit protein molecules. The key of this fabrication method is integrating two different sets of structural information. One is the main chain structure data represented in the ribbon or wire frame format and the other is the protein surface data (also known as the solvent-excluded surface[16–18]), as shown in Figure 1(a). When necessary, some side-chain structures of specific amino acid residues (e.g., catalytic active site) may be also drawn, but the presence of excessive side-chain structures complicate handling of the printed object in the post-print processes described below.

The 3D data converted for the main chain and surface structures are edited using conventional Magics CAD software (Figure 1(b); Materialise, Leuven). Magics CAD is equipped with a "hollow" function that detects a thin-walled object and thickens it to provide a shell structure with a user-defined thickness. Usually, this function is used to mechanically strengthen printed parts and thereby avoid deformations that may result from internal stresses from its own weight and to save printing materials (binder ink and plaster powder). Note that in this data processing, the protein surface data are "mounded" to have a shell structure whose inner surface is exactly the same as the original surface structure. In other words, data for a silicone resin casting mold is created using protein surface data. Typically, the shell thickness is 1 mm–2 mm, which is sufficiently thick for mechanical durability during the casting, degassing, and curing of the silicone resin and also sufficiently thin for easy peeling by hand after the resin has cured. The main chain structure data (usually drawn as a ribbon or wire frame model) and protein surface mold data are then superimposed (Figure 1(b), center). In CAD (Rhinoceros 3D, Robert McNeel & Associates, Seattle), the main chain structure object is fixed to the surface shell by adding mechanical support shafts, such as a roller coaster track (Figure 1(b), right). As the surface structure is like an eggshell, there is no access to the interior of the molecule, which is necessary for the removal of unused powder (depowdering via compressed air) after printing and impregnation for hardening. The shell structure data are cut into two or more pieces to obtain segmented datasets.

The modified VRML data for the protein structure are then printed out using the full-color ZPrinter 450 (Figure 1(c)), and the printed objects are depowdered and hardened using an impregnation process. The impregnation

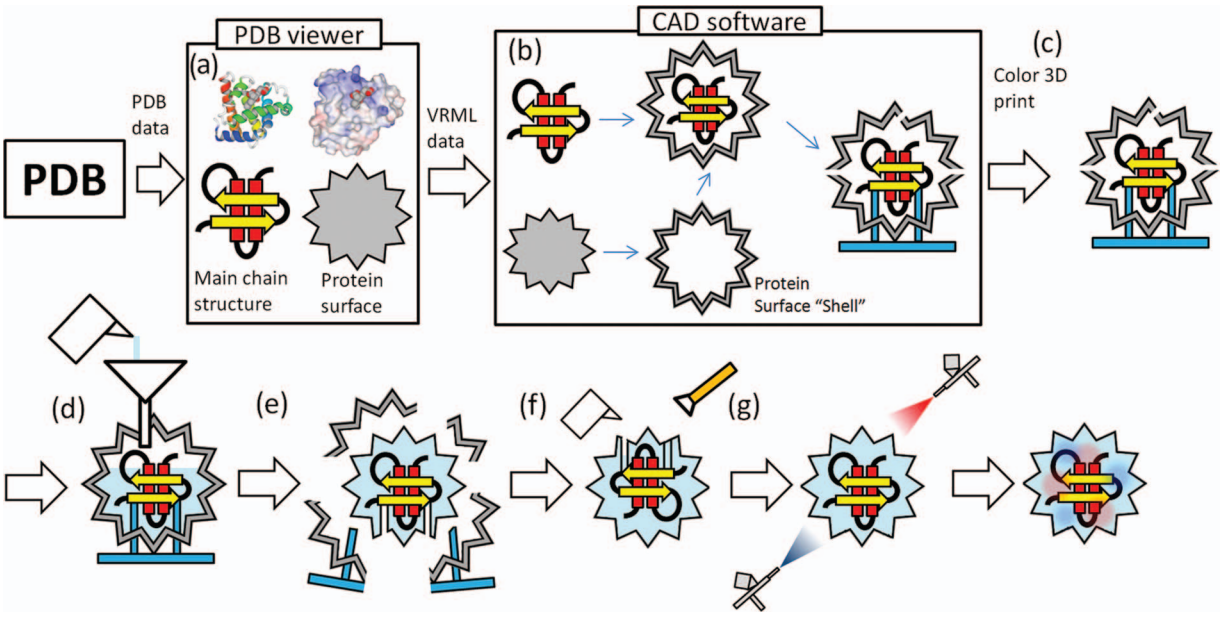

FIG. 1. A schematic diagram of the fabrication of a soft and transparent physical protein model. (a) 3D data from a main chain (left) and surface (right) of a protein molecule are edited using PDB Viewer software and exported as a VRML file. (b) The structural data are edited using CAD software. The main chain structure is fixed to the surface structure with a mechanical support, and the surface structure is formed like an eggshell. The surface and main chain data are superimposed, and the surface shell is cut into a few pieces, depending on the complexity of the molecular shape. (c) The superimposed data are then printed out using ZPrinter, and each part is hardened by impregnation with super glue or silicone resin. (d) The shell is closed and sealed with a silicone RTV sealant and a transparent silicone resin is then poured into the cavity and cured at 80 °C for 2 h. (e) The shell and mechanical supports are released. (f) The silicone resin is thinly applied to the model surface and poured into the holes, which were from the mechanical supports. (g) If necessary, the surface of the model is thinly painted using an airbrush according to physicochemical properties.

liquid is typically a cyanoacrylate-based glue (Aron Alpha 201, Toagosei, Tokyo) for mechanical durability or thinned silicone rubber for pliability. The printed parts are put together, and a mixture of catalyst and transparent silicone monomer liquid (Silpot 184®, Toray-Dow Corning, Tokyo) is poured into the casting mold, as shown in Figure 1(d).

Because the 3D-printed model is made of a glue and plaster-based powder, it is porous and produces a lot of small bubbles in the poured silicone resin that spoils the transparency and appearance of the model. To avoid bubbles, the model should be extensively degassed in a vacuum chamber before the silicone resin is cured. After degassing, the model is incubated at a high temperature to cure the silicone rubber. The shell and mechanical supports are then peeled off (Figure 1(e)), resulting in large holes that are backfilled with silicone resin (Figure 1(f)). The resulting surface of the cast is not smooth and has a lusterless and hazy surface, such as ground glass. A thin coating of the silicone resin makes the model surface smooth and glossy (Figure 1(f)). If necessary, the model can be colored to represent physical properties of the surface, such as electrostatic potential or hydrophobicity[19] (Figure 1(g)).

The resulting molecular model has a soft transparent silicone body in which a full-colored main chain (and a part of the side-chain structure) structure is embedded at the exact correct position. Users can handle the surface model and view through the inner main chain structure at the same time. Although the inner structure is transparent, it does not resemble what we see using PDB Viewer software. This is because the transparent body is made of silicone resin that has a refraction index ($\eta = 1.41$) much higher than that of air ($\eta = 1.00$), with refraction occurring on the uneven surface of the model. This optical distortion can be improved when the model is soaked in water, which has a refraction index ($\eta = 1.33$) close to that of silicone resin. The tangible, malleable, and transparent characteristics of the protein model will aid one's understanding of a protein's 3D structure, its function, and dynamics. In addition, this model is useful for understanding how protein molecules associate to form a quaternary structure or protein–protein complex, which has been difficult to represent with computer graphical representations.

III. RESULT AND DISCUSSION

A. A single domain model and its ligands: Myoglobin and the heme molecule

In 1959, the Kendrew group completed the first protein structure elucidation using sperm whale myoglobin.[20] Myoglobin is a protein that can bind oxygen reversibly and whose structure, dynamics, and function have been most intensively studied using experimental and computational methods. The myoglobin backbone forms eight α-helices in a globin fold, encapsulating a heme group as a cofactor in its hydrophobic cleft. Figure 2 shows a new physical model of myoglobin (PDB ID: 1mbn). With this model, the main chain structure is computer generated, with the eight α-helices drawn as thick colored ribbons. The loop structures are represented as white tubes. The main chain structure is covered with silicone resin whose outer shape models the molecular surface, resulting in a tangible structure that gives users a hands-on understanding of the surface structure. It is of great advantage, especially in

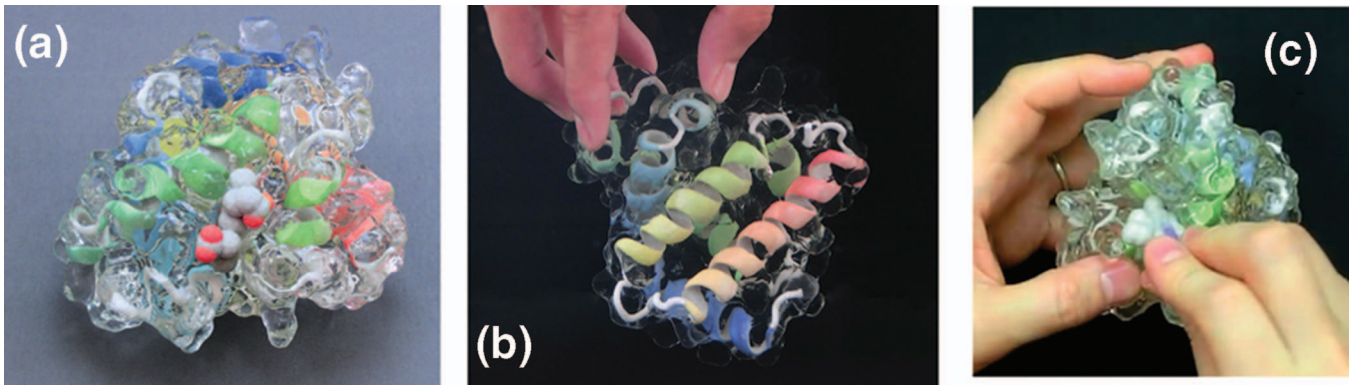

FIG. 2. Demonstration of a new molecular model of sperm whale myoglobin (PDB ID: 1mbn) fabricated using the process illustrated in Figure 1. (a) A photograph of the myoglobin model. The rugged molecular surface and the inner main chain structure can be viewed through the silicone resin body. (b) The myoglobin model soaked in water. (c) "Hands-on" simulation of heme binding to myoglobin[28] (enhanced online) [URL: http://dx.doi.org/10.1063/1.4739961.1].

the case of myoglobin, that users can manually explore the shape details of the intricate heme cleft.

One remarkable characteristic of this new model is the transparency of the body, which enables users to view through the inner main chain structure, providing a deeper understanding of the complicated topological structure of the molecules. As mentioned, because of differences between the refraction index of silicone and air, the inner structure is observed with some distortion. Nevertheless, even with the distortion, users can continue to grasp the topology and how each secondary element is placed, relative to the surface structure. Furthermore, the distorted appearance can be greatly reduced when the model is soaked in water and observed through the flat side of a water tank, as shown in Figure 2(b). Extensive degassing and careful removal of mechanical supports ensure that no bubbles or holes remain in the resin body (Figure 2(b)), further improving visual accuracy. The silicone resin body is water resistant and washable, which is advantageous for a model that is handled often and requires high mechanical and chemical endurance.

Another remarkable characteristic of this model is its resilient and malleable body, which enables various hands-on simulations that are impossible with conventional models. Although plaster-based substrate is used for the ribbon "core" of the models, impregnation with thinned silicon resin reduces the brittleness and gives a significant plasticity and durability to the ribbon structure. For example, heme binding can be examined when the heme is maneuvered in and out of the myoglobin cleft. Because the mouth of the cleft is very narrow, a hard plaster heme CPK model will undergo steric collision that prevents docking. In contrast, with this new soft model, the mouth of the cleft can be easily opened and the heme CPK model can be manipulated in and out of the cleft (Figure 2(c)).[28] Users can then view and identify the main chain helices (E and F) that are moved for opening the cleft mouth to allow the heme to dock. Once the heme is docked, users can feel how the heme is firmly fixed and see that there is no passageway for oxygen molecules. Users may remember that this structure is taken from a crystal form, and under its actual physiological condition, myoglobin requires further conformational fluctuations to take in and release an oxygen molecule.[21]

This new model's characteristics that allow users to manipulate a ligand model and perform a hands-on docking simulation are quite useful for understanding how the ligand is bound and how steric collision occurs. Of course, a computational approach is the most accurate method of performing a docking simulation, and virtual haptics software might simulate steric collisions. Information obtained through such indirect methods; however, is no match for that obtained from simultaneously seeing, touching, and manipulating both the physical protein and ligand models as presented here. Other examples in the supplementary material show physical protein models of the checkpoint 1 kinase (PDB ID: 2cgu) and hen egg white lysozyme (PDB ID: 1hew) (see Figure S1 in the supplementary material[28]).

B. Demonstration of quaternary structure formation: Hemoglobin model

As demonstrated above, the model presented here facilitates the user's understanding of a protein's 3D surface structure and the topology of its main chain. This model is also very useful when used for the study of protein–protein interactions, such as the formation of quaternary structures and molecular recognition.

Hemoglobin is a tetramer of two alpha chains and two beta chains associating into a quaternary structure. The structure of each subunit is in a globin fold, which is similar to that of myoglobin. The formation of the quaternary structure provides physiologically important homotropic allosteric interactions between the subunits.[22] Consequently, understanding how the four subunits are interacting with each other in a 3D manner is critical in grasping the function of this protein complex. However, the mechanism for the association of the four subunits is like a parquetry, and it is very difficult to conceptualize the image. Moreover, it is extremely difficult to share information regarding the quaternary structure with others and discuss it without any tools. Figure 3(a) shows a new molecular model of human hemoglobin (PDB ID: 4hhb) soaked in water. The surface of the model is thinly painted according to the electric potential. The inner structure, surface shape, and electrostatic potential can be observed at the same time. Similar to the myoglobin model, a main

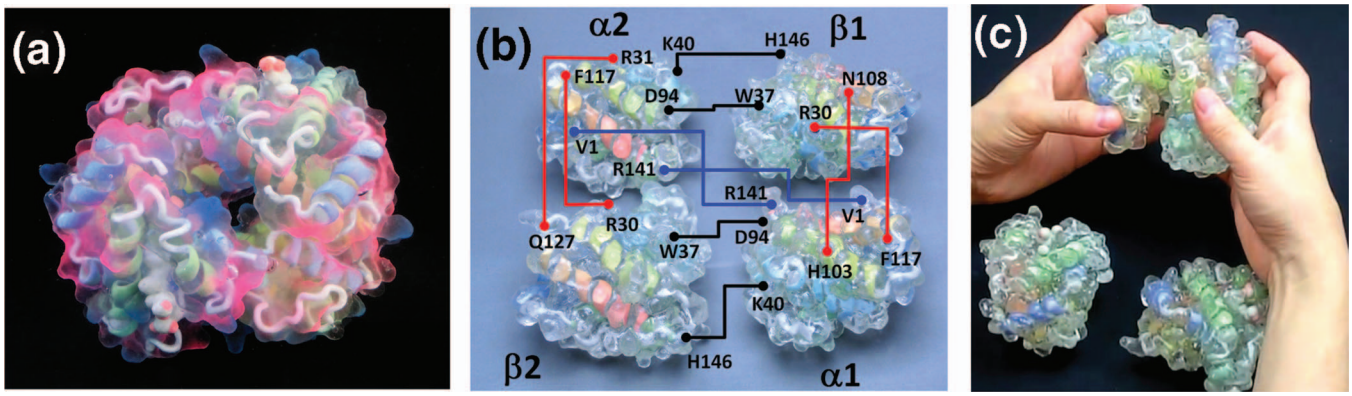

FIG. 3. Tetrameric complex models of human hemoglobin (PDB ID: 4hhb). (a) Photograph of a colored hemoglobin tetramer model soaked in water. (b) The dissected four subdomain models. The positions of embedded magnets are indicated and connecting lines for the magnet pairs are colored with respect of subunit interactions (α1-α2: blue, α1-β1 or α2-β2: red, α1-β2 or α2-β1: black). (c) The embedded magnets guide users how to assemble the subunit models into a tetrameric complex[28] (enhanced online) [URL: http://dx.doi.org/10.1063/1.4739961.2].

chain structure of each hemoglobin subunit is graphically represented and the subunit models are soft and users can simulate the heme binding.

When editing the molecular surface data for the four hemoglobin subunits in CAD, some overlapping molecular surface areas at the original complex position of the subunits were found. Removal of the overlapping area is necessary to enable the subunits to join without physical collision. As expected, the overlapping areas reflected inter-subunit interactions such as hydrogen bonding, hydrophobic packing, or ionic interactions. After the removal of the overlapping area, holes for embedding a magnet were created on both models. This drilling should not be performed manually after the models are fabricated, but during data processing in CAD, otherwise the models may not face each other at right position and angle.

Of note, the position of the embedded magnets indicates the contact points where major inter-subunit interactions exist, as illustrated in Figure 3(b). The embedded magnet positions are included on the inter-subdomain surfaces as suggested in previous works.[23] The magnets guide users and show how the subunit models form a complex (Figure 3(c)).[28] Remarkably, it was found that each subunit model had to be slightly deformed before fitting into their respective positions in the tetrameric complex, indicating that conventional models without a pliable body cannot undergo similar manipulations.

The supplementary material also includes other demonstrations of protein binding or quaternary structure formation; these processes can only be easily explained using soft models (see Figure S2 and movie 4 in the supplementary material[28]).

C. Other prototype models and applications of the new protein model

1. G-protein coupled receptor (GPCR) model

As mentioned above, to view through the inner main chain structure with no distortion, the model should be soaked in water, which might be troublesome for discussion in the laboratory or classroom. Although the advantage of tangibility would be lost, a model could be buried in silicone resin with a flat exterior wall, allowing users to view through the inner main chain structure of the model. In Figure 4(a), a hybrid molecule model of a transmembrane photo-sensing protein rhodopsin from bovine (PDB ID: 1u19) is shown. Rhodopsin belongs to the GPCR family, which is the most utilized group of molecules in the pharmaceutical industry. Adequate modeling of the rhodopsin structure is important because its x-ray crystallographic data are often used as a template for

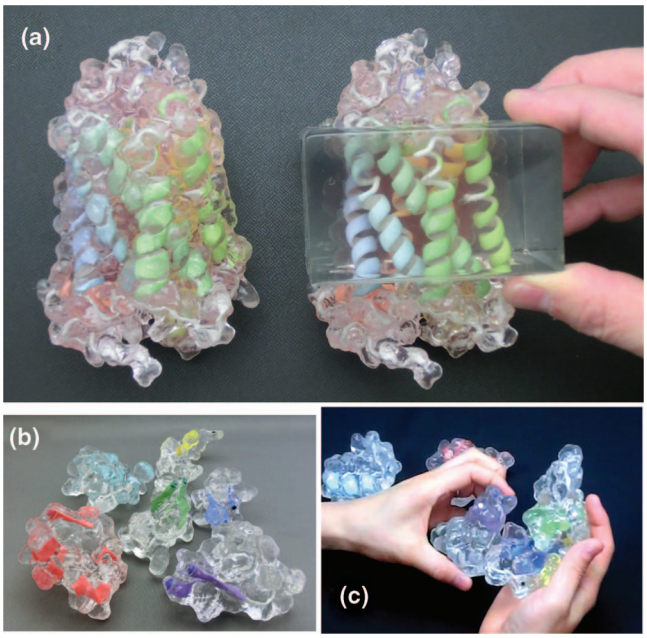

FIG. 4. Demonstration of new molecular models. (a) A photograph of two bovine rhodopsin models. The right model is a hybrid whose transmembrane domain is buried in a flat silicone block to help users view through the domain without distortion and without soaking the model in liquid. (b) Protein module model in which models of modules M1–M6 are independently fabricated. The secondary elements in the modules are colored according to the modules order (M1: cyan, M2: red, M3: purple, M4: green, M5: blue, M6: yellow). (c) Assembly process of the six module models. It is troublesome when assembling the six module models in ascending order (M1–M6). Meanwhile, users can feel that it is much easier to assemble the modules in descending order (M6–M1)[28] (enhanced online) [URL: http://dx.doi.org/10.1063/1.4739961.3].

modeling unknown GPCR molecules. As shown in Figure 4(a) (right), the transmembrane area of the rhodopsin model is buried in a silicone resin block. Users can intuitively understand how the seven helices run through the membrane with the transparent model.

2. Barnase module joint model

Finally, a prototype model that is practical for discussion in a laboratory or classroom was introduced. In Figure 4(b), a protein model of barnase (PDB ID: 1rnb) is shown. This model is composed of six pieces, each representing a module, small folding elements. Module boundaries are closely correlated with the intron positions of genes that encode proteins in eukaryotes.[24,25] At first glance, users can see that each model has a small globular shape indicating its compactness as module.[25] The color-coded secondary structures are observed through the transparent body and magnets are embedded in the C and N termini of each module, mimicking the peptide bond formation and allowing the segmented models to join together. These features enable easy identification of each module, guiding users in the assembly of the six modules into a complete barnase molecule (i.e., which side of a model should be attached to which side of another model). In the assembly process, users can feel how each module interacts with the other modules. Indeed, users will find that it is highly troublesome if the modules are put together starting from M1 to M6 (Figure 4(c)).[28] This is simply because there are few interactions (contacting surfaces) between M1, M2, and M3, and even the M4 model that interacts with M1, M2, and M3 remain unstable. On the other hand, assembly can be easily performed if users start from M6 to M1 (Figure 4(c)).[28] This is explained by the fact that M6, M5, and M4 have large contacting areas forming a tightly packed building block that facilitates joining of the other modules. Of course, this may not reflect the right folding order or thermodynamically stable block structure, but users can roughly conceptualize how the modules interact with each other.

IV. CONCLUSION

A transparent, colorful, soft, easily manipulated, and durable protein molecule model has been demonstrated. The goal of this effort is to provide a new physical model to intuitively convey 3D and inter-molecular information to users. The new model can represent various structural and physical property information inside and on the surface of the model, and above all, its malleable form facilitates learning about various molecular processes, which has never been possible with other methods or tools. Physical models, however, are inferior to computer graphical presentation in terms of flexibility and costs. The fabrication process shown here requires an expensive full-color 3D printer and knowledge of commercial CAD software, which is a barrier to widespread use of these models in research and educational settings. Development of personal 3D printer technology, such as MakerBot™ at http://www.makerbot.com/ or Cube™ at http://cubify.com/, and handy free CAD software may lower the fabrication cost and accelerate the distribution of such a hands-on research or education tools. A combined use of physical models and computer graphical presentation would work synergistically to deepen the user's understanding of protein structures.

Finally, humans are talented to intuitively conceptualize a protein's 3D structure, and such ability has been displayed recently in protein structure refinement using the multiplayer gaming methodology.[26,27] Similarly, each researcher's intuitive concepts affect research results in structural biology, molecular biology, and the pharmaceutical industry. In fact, the manner in which 3D information is fed to users still depends on conceptual predictions using 2D graphics. Improvements on this input method might achieve a breakthrough in this research field and the use of physical models will facilitate that advancement. Imagine the positive impact that a viable physical protein model, which is readily available and continuously used by researchers, would have on the progress and outcome of his/her research.

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