Physical Biomodeling: a new field enabled by 3-D printing in biomodeling

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Abstract

3D printing technology opens the door for a new field at the intersection of experimental data, computational biology and physical modeling for study of biological systems, such as protein folding at nano-scale.

Key insights

• 3D-printing enabled new possibilities for scientific modeling: We explore a new domain of precision physical modeling and correlate it with existing visualization and computational systems.
• Dynamic physical models of biomolecular models can be designed to-scale that can serve as research tools along with existing biocomputational tools and databases.
• Accurate physical modeling with 3D-printing techniques will lead to new approaches to study dynamics of biological systems complementing computational methods.

Introduction

Biological structures, from the nano-scale to macro-scale are known to flex, fold, grow or shrink, and are constantly evolving from one form to another. The dynamics of these structures further add to the complexity. A remarkable example of complex dynamic structures is the protein folding problem. At present we have sophisticated computational models to study the folding process. But is that enough? In 2011 in an article, TIME Magazine reported the amazing success of online FoldIt gamers in solving enzyme structure that baffled scientists for decades [1, 2]. These gamers solved the problem, not as a scientific undertaking, but as a 3D puzzle. It highlights the findings that human beings are naturally good at spatial reasoning, methods in which computers struggle for a foothold. So the question is: Is it going to be any better to have scaled, self-folding physical models sitting at your desk computing the details and dynamics of biological systems? And more importantly: Is it feasible?

Simulations provide a successful means to study complex biosystems, but they still lack a platform that will facilitate an intuitive understanding of the underlying complexity. For example in protein folding, biocomputational models have limited themselves to modeling in terms of force fields, energy and entropy. The dynamics is thought of as entropy minimization problem. Even though these are precise and successful methodologies for studying shapes and dynamics in the protein folding process, the embodiment of properties within a physical model –
the tangibility-factor – is still hard to capture given the complexity of biological systems. There is a void, a need felt for direct representation and manipulation of these dynamic shapes that can have a digital presence as well as physical.

In recent years, extensive studies have been made in CAD methodologies, solid modeling geometry and 3D printing techniques. But the biology research community is yet to gain from the advancements. A marriage of 3D CAD and biocomputation is in sight with cutting-edge technologies like 3D bioprinting, CAD-Nano and DNA scaffolds. These are transformative and disruptive technologies that society will greatly benefit from in future. Specifically, the advent of 3D-printing adds a new player to the game in physical modeling with the Design ➔ Print ➔ Play paradigm. The questions that come to mind is: What happens if we want to build macro-scale, precise physical models of any of these? Can these models actually serve as instruments for future research – and if this is indeed possible, can we imbibe digital knowledge into them so that they can “compute” by virtue of the shape and dynamics? The Peppytide project (example below) is one of the initial efforts to establish the feasibility of such physical models for protein folding [3-6].

Form-specific physical objects are easy to manipulate by hand, and can be designed to emulate the shape and mechanics of bio-systems. The interplay between the physical and computational models can be analyzed, evaluated and designed to contribute in a complementary manner, so that the two can work together to provide a more effective tool, e.g. mimicking the process of protein folding with your hand and parallely generating its digital shadow.

A New Field

Physical Biomodeling is a new area of exploration at the interface of computer science and the biological systems. While tremendous advances have been made in computational biology, the cutting-edge 3D printing provides unprecedented opportunities for a third angle into the landscape, thus uncovering this new computational space for modeling that has remained unexplored so far (Figure 1). In this paper, we tie together these concepts of form-specific physical-digital interfaces. With these principles, a new computational paradigm emerges for physical-digital interfaces for studying of biological phenomenon (e.g. protein folding timesteps) that focuses on shape and dynamics.

We define 3 categories, and 6 processes connecting these 3 categories (Figure 1). These categories and processes together form the basis of the philosophy behind the field of Physical Biomodeling. Through exploring Processes 1-6, we see that a relationship-triangle exists between the experimental data from natural systems (N), the computational models for biosystems (C), and scaled, accurate physical models of biosystems (P). Processes 1 and 2 have already existed in the literature for a long time. Physical Biomodeling brings forth Processes 3-6 that will provide a new way to look at the old problems in biology. We arrive at a
computational space at the intersection of N, C and P that has so far remained unexplored because of the difficulty in designing and fabricating accurate, scaled physical models of biosystems. Further, as we discuss in detail later, no such bio-cum-CAD platform exists yet to facilitate this union.

Biological systems involve complex phenomena, and encapsulating these characteristics within a physical body is thought to be difficult. For example, representing the polypeptide chain, a generalized protein chain, along with its complex degrees-of-freedom, by a physical scaled model that will fold dimensionally-accurately, was thought to be quite difficult before the Peppyptide project proved otherwise [3,4]. Now with 3D-printing technologies, and possibilities for CAD-cum-biocomputation platforms, we are poised to explore this new domain of study.

We define 3 sets as:

- N: experimental data from natural systems
- C: existing computational models
- P: form-specific, dynamic physical models
Fig 1: A new field of study at the intersection of N, C and P: The Entity-Relationship diagram in the physical-digital computing space.

We explore below the relationship between these sets to identify their current scopes and to pinpoint the unexplored areas that might complement the existing computing frameworks. Peppytide, a scaled physical model of the polypeptides with flexible/foldable backbone, is explained as a proof-of-concept of the Physical Biomodeling principle.

A new paradigm emerges from this analysis wherefrom the form and flexibility of the real-world object can contribute to the better parameterization of the computer models, and to a deeper intuitive understanding of the biological phenomena. Thus this paradigm sets forth new ways to think about bio-systems, that complement the existing methods. We illustrate the six processes and their utility, with the Peppytide model as a case study.
Peppytide: A case study for the Physical Biomodeling paradigm

Processes 1 and 2. These are the two traditional processes involved in simulation, modeling and prediction. Process 1 involves fitting experimental data to mathematical and computational models, while Process 2 entails using the model to predict system behavior. For example, numerous studies have been done in attaining precise computational models and folding principles using template matching, force field calculations and other methods [7-9] (Process 1), as it is an extremely important topic that has implications for medicine and drug design in addition to understanding the fundamental rules of protein folding. Extensive studies have also been made within the last two decades in designing small de novo proteins with less than 40% homology to known sequences to achieve a preferred structure [10,11] (Process 2). This body of work has firmly established the relationship between N and C (Figure 2) with the protein folding data available from X-ray crystallography and NMR structures of native states, and the de novo protein folds.

Fig 2: Processes 1 & 2: A look at computational modeling based on existing observations and current concepts.

Process 3. This process, from N to P, explores the design cycle for focusing on the meticulous design of exact-scale physical models that address open problems in biology, bringing with it a new way to look at the same problems. The spatial, tangible, geometric approach to looking at a problem that has been extensively explored computationally and experimentally, would bring in fresh aspects – the third angle – to our knowledge and would feed-forward into the computational space. Even though there has been extensive developments in 3D-printers and CAD modeling techniques within the last 10 years, the idea of designing physical models for biosystems that demand precision, exactness and dynamics, is still daunting. Moreover, prototyping such models are themselves hard problems. But recently the feasibility of this concept has been established. For example, Peppytide which is one of the first such models that focus on the protein folding problem, demonstrates the exact-scaling, accuracy in degrees-of-freedom and the ability of the model to form various folded secondary and tertiary structure motifs found in proteins at their native states [3,4] (Figure 3, 4). This is a very exciting development.
**Fig 3:** The Peppytide model. (a) A scaled, dynamic physical model of the polypeptide chain folded into various secondary structures found in proteins, (b) a comparison of the model folded into $3_{10}$ helix, looser alpha helix and loosest pi helix, demonstrating the realistic flexibility of model backbone, (c) making antiparallel beta sheet, (d) demonstrating folding of secondary structures in Summer NanoCamp at Foothill College, 2014, (e) demonstrating folding at Maker Faire 2014, (f) demonstrating folding at Lawrence Hall of Science, UC Berkeley, 2013, (g) protein folding with participants from Johns Hopkins Center for Talented Youth at Lawrence Berkeley National Laboratory, 2014 (photos with permissions).

**Process 4.** Once a physical model is prototyped, it is crucial to test the performance of the model through rigorous testing and by comparison with established data. This validation establishes the relationship from P to N forming the process 4 testing and design cycle. As a use case of the process, the polypeptide chain model Peppytide was tested by folding it manually into a variety of secondary structures, like the most frequently found $\alpha$-helix, $\beta$-sheets
and β-turns, and the less frequent 3_10 helix and π-helix (Figure 3). A few tertiary structure motifs (ββα, Osteocalcin) were also made. The successful testing implies that the structures that can be made by hand with the model are possible structure-candidates for native-state protein conformations and *vice versa* (Figure 4). Further, the scaled model lets anyone get an accurate idea of dimensions of these structures at nano-scale, just by measuring the model with a ruler and using the conversion-factor.

Processes 3 and 4 demonstrate that a bijective mapping can exist between N and P for biosystems. It is a very crucial relationship in physical modeling of biological systems. For successfully modeling future physical biosystems, it is the necessary and sufficient condition to satisfy this relationship.

**Fig 4:** Processes 3 & 4: Designing and validating physical models of polypeptide chain.

**Processes 5 & 6.** Tying together precision physical models with computational models is another hand problem. Firstly, no bio-CAD modeling platform currently exists that can easily map the digital representation of such a complicated physical model with the existing biocomputational platforms. Conversely, tracking a dynamic physical model with large degrees-of-freedom through cameras real-time is a challenge. There is no framework that can make a smooth transition between computational and physical models back and forth. Thus, we have immense potential and lots of scope here to develop CAD-cum-biocomputational models to initiate the dialog between these two types of modeling. Marrying CAD-based platforms and 3D-printing with biocomputational simulations opens up new possibilities for research in biology, and would eventually establish new grounds with Physical Biomodeling techniques. New standards for file conversions between CAD and computational representations need to be established as a first step towards designing such platforms.

We have initiated a new exploration to establish the ground rules for designing such a platform for polypeptides. In this preliminary plan, we aimed to convert the elements of the physical model design into a digital-representation, for 3D-
printing as a first step (Figure 5). We used Cyborg Project, the forthcoming CAD-enabled generic biocomputation framework beta-released by Autodesk Inc. [12], with its software features and modeling languages as a platform to program the digital-representation for polypeptides that would serve as the underlying knowledge base. Eventually, we envision an easy conversion of the design principles between CAD-oriented modeling for biological systems.

![Diagram](image)

**Fig 5: Processes 5 & 6: The Digital-physical Interfacing.**

A natural next-step would be to capture the structure of the physical models which can then be shadowed digitally in real-time (Process 6). Similar kinds of object tracking experiments have been made with simpler objects like lego blocks where a Microsoft Kinect camera with depth-sensing feature. The camera tracks the assembly, break-down and change-of-form of an object from its constituent lego parts [13,14]. It is still a long way before we can achieve such operations with biomodels that have many degrees-of-freedom.

**Possibilities for exploration in this computational space**

The physical modeling technique along with advances in 3D-printing, opens up new possibilities and challenges. Here I outline a few possibilities that can be built upon the foundation that we have laid down in this paper.

(a) A viable input device for biocomputing. Manipulating models with hand making desired shapes and then conveying that information to the
computer, can give us a powerful tool for initial setup of conformational details for algorithmic analysis. It can also be used to study step-by-step change in conformations.

(b) A viable output device for biocomputing. Incorporating rotational degrees-of-freedom in physical models is still cumbersome with even the lightest of servos, but alternative technologies like shape-memory alloys and pneumatic devices might help. One application of having such an output device in Peppyptide project might be to make the model “display” the folding pathway as a function of time, given the ability to self-fold through actuators. Another use-case is to have two remotely located models interacting with each other, with one model’s configuration transmitted to another which can then fold accordingly.

Moreover, a few exciting and important possibilities for particularly research in the protein folding problem is as follows.

(a) Exploring de novo protein structures. Designing de novo structures of proteins is a time-consuming process with computational predictions followed by experimental verifications. A scaled model can act as an in-between to speed up the cycle and might help in quicker rejections of false positives.

(b) Study of misfolded proteins and aggregates. Proteins often misfold and form aggregates, but have specific structural traits. An important problem would be to explore the behavior of insoluble proteins using physical models, to pinpoint the idiosyncrasies of misfolded ones like β-amyloids. By easily changing side chains, the models could provide a quick, initial study on the effects of mutation in protein folding without extensive calculations. For such studies to be most effective, we need to provide a means for the physical model to send its conformational information to the computer for further processing.

(c) Electrostatic and hydrophobic interactions. These forces are critical in protein folding. Implementing electrostatic effects and hydrophobicity in a scaled, realistic way with respect to other interactions, is the key to making stable tertiary structures using a physical model. The concepts in simulation platform sketched as example Process 5 above, might be eventually extended to develop simulations of physical models to computationally plan out a hydrophobic design scheme for the physical models. These interactions need to be orthogonal to the hydrogen bonding forces.

(d) Self-folding of protein models. Currently there is a growing interest in developing self-folding modular robots that can perform a particular task. The intersection of microelectronics, pervasive computing, and growing interests in biolocomotion have paved the path for self-folding 2D and 3D objects. Posey [15, 16], Moteins [17], Programmable matter [18] are a few examples from this class of objects. It is time to borrow these concepts to develop self-folding biopolymers.
(e) Exploring other types of polymers. The concepts of models of polypeptides can be extended to other polymer chains like poly-nucleotides, β−peptides, biomimetic peptoids, and industrially important polymers like Kevlar and polystyrene. Other options for synthetic polymers are conducting polymers and polyethyleneoxide. With exciting developments in material science and new materials, the possibility is endless here.

In a nutshell

Over the last 50 years, bioinformatics and computational biology has revolutionized the way we do research in biology. Since the mid-twentieth century, increasing amount of data from experiments, biological sequencing, structure determination and other studies eventually fueled and outlined the developments in these two disciplines. Addressing these problems have in turn fed forward to developments in various branches within computer science including algorithms, graph theory, computational modeling, parallel computation methods, pattern recognition and visualization. The prevalence of visualizing tools repeatedly highlights the eternal human need for spatial understanding, to orient their insights in terms of geometry. However, the current biocomputational approaches leave tangibility and geometry at the periphery. With the latest developments in 3D-printing and CAD technologies it will now be possible to explore the convergence of these domains with biocomputation.

Moreover, it is possible to extract the guiding rules, as we learned from the Peppytide project, and use them in other problems in biology that focus on shapes and dynamics. These investigations can eventually define a new field of exploration at the physical-digital interface for biology, where the physical models and computational ones complement each other to enable better insights into the fundamental principles of biological systems.

The idea of embodying the physical and mechanical information of molecules directly into the artifact itself and to let it move at its own accord, was the underlying motivation behind the work with Peppytides, and is exportable to other complex systems. This point-of-view of looking at the problem is a substantial shift from having a representative wood block or other manipulative as a tactile handle to direct the digital environment with augmented reality. Next, a way to successfully gather these physical quantities, like position and orientation of different subparts, directly from the artifact would pave the way for innovative ways of biomodeling in future. Such futuristic tools have the potential to change user behavior within the structural biology community the same way as mouse and new visualizing tools disrupted and changed design of biocomputation tools a few decades ago.
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