JOURNAL OF CHEMICAL EDUCATION

pubs.acs.org/jchemeduc

Demonstration

Using Magnets and Flexible 3D-Printed Structures to Illustrate Protein (Un)folding

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Cite This: https://doi.org/10.1021/acs.jchemed.2c00231

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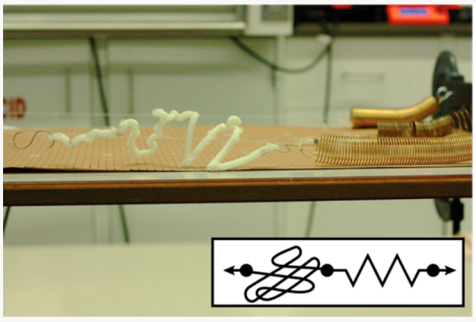

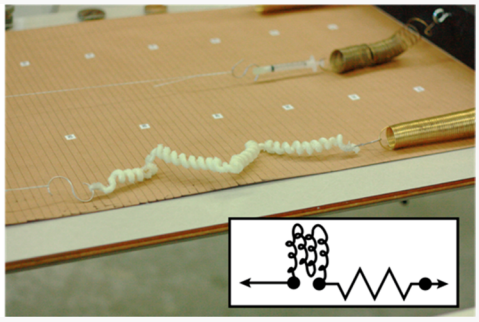

ABSTRACT: Proteins are "magical" workers inside our body, as they accomplish most of the cellular functions. Here we report on a novel approach to teach protein folding and unfolding, using magnets and flexible 3D-printed protein structures. To illustrate this physical process, we used colored circular magnets designed for whiteboards, connected through paper clips. Several protein structures were then 3D-printed, using both standard and flexible materials. Protein unfolding under force was then investigated by adding slotted weights to a setup consisting of three experiments: a simple spring, a spring in series with a sealed syringe (representing a dashpot), and a spring in series with a printed protein structure. All of the experiments shown here were done as part of the event, organized by the University of Wisconsin—Milwaukee. The approach presented here complements the use of other techniques to learn about protein folding and constitutes a novel way to explain how mechanical unfolding *in vivo* relates to a gain-of-function.

KEYWORDS: *Protein Structure, Protein Folding, Mechanical Unfolding of Proteins, 3D-Printing of Flexible Structures*

■ INTRODUCTION

Proteins are molecular machines that carry out the majority of biological functions, from catalyzing reactions and providing structural support to acting as nanometric-sized motors.[1] In order for proteins to perform their function *in vivo*, they first need to acquire a specific 3-dimensional (3D) structure. This process of a polypeptide chain acquiring a specific structure based on a given amino acid sequence is known as protein folding and still represents one of the open questions in science.[2]

Cells represent the smallest structural unit of our body that can self-replicate.[1] Our genetic information is stored inside the nucleus in the form of DNA, which itself is divided into *chromosomes*. Each of the over 20,000 proteins in the human body has a corresponding section in the genome encoding for it, known as a *gene*. The DNA encodes this information via its nucleotide sequence, which has in its structure cytosine [C], guanine [G], adenine [A], or thymine [T] nitrogen-containing bases. Through the process of *transcription*, the sequence encoding for a specific protein is assembled into an mRNA (mRNA) molecule, which has the same C, G, and A nucleotides, while T is replaced by uracil [U]. mRNA may then exit the nucleus to become the template for protein synthesis through *translation*. During translation, the ribosome decodes the mRNA sequence a codon (3 base pairs) at a time to produce a polypeptide with a well-defined sequence, made from a total of 20 amino acids.

There are also several distinct organization levels for protein structure.[1] The first level is the primary structure, which is given by the sequence of amino acids forming the protein backbone. The secondary structure of proteins has two main elements, α-helices and β-strands (or sheets). α-Helices form between adjacent amino acids, while β-strands appear between parts of the protein sequence that can even be at opposite ends. The tertiary structure of a protein, also known as the native state, represents a relatively well-defined stable 3D arrangement of secondary structure elements and unstructured

Received: March 15, 2022
Revised: June 14, 2022

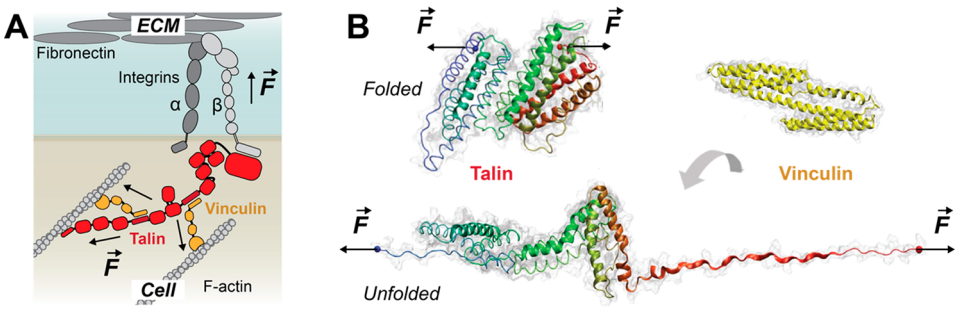

Figure 1. Schematics of how protein unfolding under a force vector represents a mechanically induced signaling mechanism. (A) Schematics of the first steps during the formation of focal adhesions, which are mechanical connections between cells and their extracellular matrix (ECM). Talin unfolding of its tail domains (red) allows for the formation of new connections with actin cytoskeleton via vinculin (orange), as force develops via the integrin-extracellular matrix (fibronectin) pathway. Adapted with permission from ref 7. Copyright 2020 John Wiley and Sons. (B) Molecular representation of the unfolding-induced binding process of vinculin to talin, derived from crystal structures and from molecular dynamics simulations. Top left: Folded talin domains. Bottom: Same domains being mechanically unfolded to expose vinculin binding sites. Adapted with permission from ref 8. Copyright 2009 The American Association for the Advancement of Science.

regions. Some proteins also have quaternary structures, which are made either through the assembly of several repeats of the same domain (homomers) or assemblies with other proteins (heteromers). The tertiary and quaternary structures play the most important role in the function of proteins. As discussed below, in some cases, the unfolding and refolding of the native structure also influences the function of some proteins *in vivo*.

The process of protein folding entails the transition from the primary to the tertiary structure and is both fascinating and inherently complex. An average polypeptide chain has a staggering number of possible conformations that it could adopt. As proteins typically fold within a few milliseconds, most of these possible conformations are not being sampled, and the folding process is somehow guided.[3] There are several important steps driving protein folding, which can be divided on the basis of their timing and complexity: entropic collapse, hydrophobic collapse, formation of secondary structure elements, and molten globule formation. During entropic collapse, the reduction in dimensionality occurs because it is more energetically favorable for a polymer (or peptide chain) to adopt a collapsed rather than a stretched conformation.[4] Throughout the hydrophobic collapse, amino acids rearrange themselves such that the hydrophilic amino acids form the shell and the hydrophobic amino acids the core of the structure.[5] This process is typically preceded by the formation of α-structure elements and followed by the expulsion of water molecules (known as the "dry" molten globule state[6]). Following the formation of β-strands, the protein acquires its native 3D folded structure. In one of the demonstrations shown in this study, we focus on the hydrophobic collapse step of protein folding.

Recently, experimental evidence has surfaced that suggests that some proteins unfold and refold *in vivo* as a novel and yet poorly understood phenomenon. We refer the reader to a review on this process, where sequential unfolding and refolding of protein domains produces some interesting gain-of-function effects, such as adaptive changes in elasticity, storage, and release of mechanical energy, and exposure of buried binding sites, of disulfate bonds or other amino acids that could be posttranslationally modified.[7] Here we will discuss only one such example.

Cells continuously exchange mechanical cues with themselves and their extracellular matrix (ECM), and this interaction determines if a cell will function normally, or undergo division, or even die by apoptosis.[1,9] When interacting with their ECM, cells form focal adhesions, where their cytoskeleton is rearranged to allow for movement and to match developing extracellular forces. This complex process is controlled by a protein named *talin,* which effectively represents a molecular mechanical computer[9] (Figure 1). Talin can attach between an actin filament and a transmembrane integrin complex. As the integrin complex binds to an extracellular ligand, it can generate more and more pulling force (Figure 1A). So, how do cells match these developing pulling forces? Like in a tug-of-war, talin tail domains unfold and can expose up to 11 previously hidden (cryptic) binding sites for another protein called vinculin (Figure 1B).[8] Vinculin, in turn, can recruit more actin filaments, forming a branched structure and distributing the developing forces. In the second part of this paper, we will focus on how tethered proteins can unfold and refold under a changing force vector. These mechanically controlled processes are currently poorly understood but may play a yet to be fully recognized role on how proteins integrate mechanical signaling.[7]

Hands-on experiments have been shown to improve students' understanding,[10,11] and the use of 3D-printing is becoming an increasingly useful approach toward learning.[12−14] Here we report the use of magnets and 3D-printed structures to illustrate details related to protein (i) folding, (ii) structure, and (iii) unfolding under a force vector. These processes follow the chronological events in the life of a protein inside our cells. All of the experiments shown here were performed as part of an event organized by the author with the help of his host institution. This event aimed to attract a young audience (K−12) to STEM by demonstrating various protein foldings through hands-on experiments. The event was repeated 5 times during the month of January 2022 and had a total audience of 143 people. The approach presented here complements the use of other techniques to learn about protein folding[15−19] and constitutes a novel way to explain how mechanical unfolding *in vivo* relates to a gain-of-function.

Illustrating Protein Folding with Connected Magnets

Here we first aim to illustrate how a polypeptide chain can reduce its number of possible conformations through the segregation of hydrophobic and hydrophilic amino acids, also known as the hydrophobic collapse. Following translation, the emerging polypeptide chain exits the ribosome to encounter water molecules, as well as folding helpers, known as chaperones.

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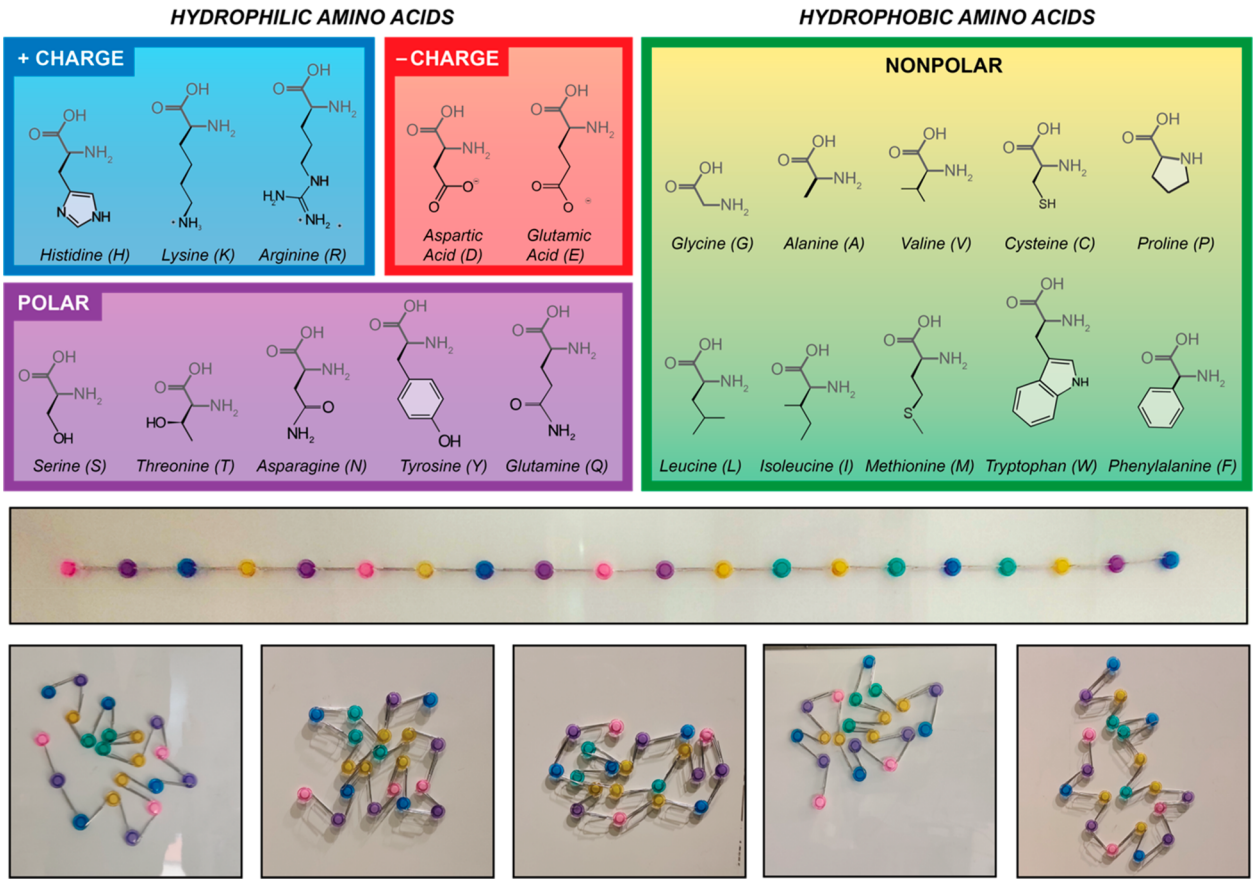

Figure 2. Illustrating hydrophobic−hydrophilic segregation during protein folding. (Top) Structure of the 20 amino acids found in proteins, grouped as positively charged (blue), negatively charged (red), polar (purple), and nonpolar (yellow-green). The charged and polar amino acids are hydrophilic, while the nonpolar amino acids are water hydrophobic. (Middle) Magnets following the same color code arranged in a random sequence and connected with paper clips, representing a stretched amino acid chain. (Bottom) Five arrangements done by participants at the event, who were asked to collapse the stretched sequence such that the hydrophobic amino acids form the core and the hydrophilic amino acids the shell. Note: As the kits come with an equal number of colored magnets, to minimize costs we chose to represent the hydrophobic amino acids with two colors (green and yellow), without making a distinction between these two colors.

There are a total of 20 amino acids that can be viewed on the basis of their interaction with water (Figure 2A, top). Half of these amino acids can be classified as hydrophilic and half as hydrophobic. The hydrophilic amino acids can be divided into three categories: positively charged (marked in blue), negatively charged (marked in red), and polar, having asymmetric charge distribution but no free charge (marked in purple). As the amino acids chain emerges from the ribosome, the hydrophobic ones will minimize their interaction energy by clustering away from the solvent, inside the core of the molecule, while the hydrophilic amino acids will go to form a "protective" shell.[5] Furthermore, amino acids with a similar charge will tend to move away from each other, while oppositely charged amino acids will attract each other. To illustrate this physical process, we used circular magnets designed for whiteboards, which come in sets of 60 pieces and five colors (bought from Toodoo Cat. No. 44111900, via Amazon). Two holes were made at opposite ends of these magnets, which were then connected using paper clips (Figure 2, middle). The magnets were then arranged in random sequences, and 3 chains representing 3 different amino acid sequences were assembled. During the event, the students were asked to collapse the magnet-chains following the rules that yellow and green magnets should not be exposed to the environment, while blue, red, and purple should. Furthermore, red−red and blue−blue magnets should not be in close proximity, while red−blue should. Figure 2, bottom, and Figure S1 show five arrangements done by participants at the event following the instructions above. The students were also asked to have the shortest distance between the magnets larger than one paper clip length. While using the exact same magnet sequence, five different collapsed structures were obtained. We noticed that most of the students had in the beginning a hard time working out a procedure to move around the magnets and match the interactions. However, once the students were told to focus on having the hydrophobic amino acids inside the structure (the yellow and green magnets pointing toward the center), they managed to find functional conformations right away. The organizers also advised the students toward the end of the experiment to try to have only magnets representing charged and polar amino acids exposed to the surroundings, to avoid having positive−positive charges (blue−blue magnets) or negative−negative charges (red−red magnets) adjacent to each other, and to try to have positive−negative charges (blue−red magnets) close together. In some experiments, there were still some yellow/green magnets exposed, and some magnets were closer than a paper clip length. The students learned that a long polypeptide chain becomes a collapsed structure based on the interaction of amino acids with water. This experiment clearly shows how, based on their interaction

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J. Chem. Educ. XXXX, XXX, XXX−XXX

with water and with themselves, amino acids can collapse to reduce their dimensionality.

Using 3D-Printing to Visualize Folded Structure of Proteins

To illustrate the diversity and beauty of folded proteins, we chose to 3D-print some representative structures. 3D-printing of proteins was reported previously,[20−22] and our protocol was adapted from these studies. Briefly, the steps that were followed were the following:

1. A. pdb file (which is a textual file format describing the three-dimensional coordinates of amino acids for a folded protein, stored in the Protein Data Bank) was downloaded and saved on the desktop. In the case of structures made with several domains or crystal structures with multimeric molecules, only a single domain was kept, and the .pdb file resaved, using molecular visualization software (such as PyMOL available at https://pymol.org/2/).
2. The .pdb file can then be opened in molecular visualization software that is capable of exporting in the .stl format, such as Visual Molecular Dynamics (VMD), available at http://www.ks.uiuc.edu/Research/vmd/, or Chimera, available at https://www.rbvi.ucsf.edu/chimera/. Since the structures generated with VMD produced more cylindrical representations, which printed better, the remainder of this procedure will describe the steps needed to be taken for VMD.
3. Following the loading of a structure in VMD, we chose *Secondary Structure* under *Coloring Method*, and *New Cartoon* under *Drawing Method*. We also changed the *Aspect Ratio* to 1.0, the *Thickness* to 1.65, and the *Resolution* to 50. From the *Display* menu, we then removed the *Axes* (*Off*) from being displayed and changed the perspective to *Orthographic*. The structure was then rendered as an .stl file from the *File* menu.
4. The files were then imported in the *Ultimaker Cura* 3D slicer. Here they were scaled to the desired size. First, the presetting for the used filament was loaded (in our case either *PLA—polylactic acid* or *TPU—thermoplastic polyurethane*). The printing temperature was further adjusted to follow the filament manufacturer specifications. The *Infill Density* was set to 50%, and the layer thickness to 0.3 mm. *Generate Support* was selected having *Tree* as *Support Structure* and the *Support Placement* as *Touching Buildplate*. We recommend Cura as opposed to the Prusa slicer, as it allows generation of tree-supports, that produce prints which are easier to clean.
5. All structures were printed using a Prusa i3MK3S+ 3D-printer (Prusa Research), equipped with a 0.4 mm nozzle. The names of the used filaments are provided in the description of each print. Following the print, the tree-supports were carefully removed with a plier.
6. (Optional) Printed proteins can be painted with acrylic colors to underline various structural elements (see Supporting Information Figure S2).

One print is of the bacterial protein L (pdb code 1hz6; Figure 3A). This simple $\alpha-\beta$ protein is ideal for showing various secondary structure elements. *In vivo*, several domains of protein L are secreted by streptococcus bacteria to target antibodies.[23] We chose this protein, as it has been extensively studied as a model protein for folding[24,25] and is one of the rare cases where the bacterium, which typically is the "prey" for our immune system, becomes the "predator", targeting antibodies in order to disrupt the immune response.[26] We chose to print it using Glow in the Dark PLA Filament (Hatchbox), which glows green in the dark and can also be easily painted on.

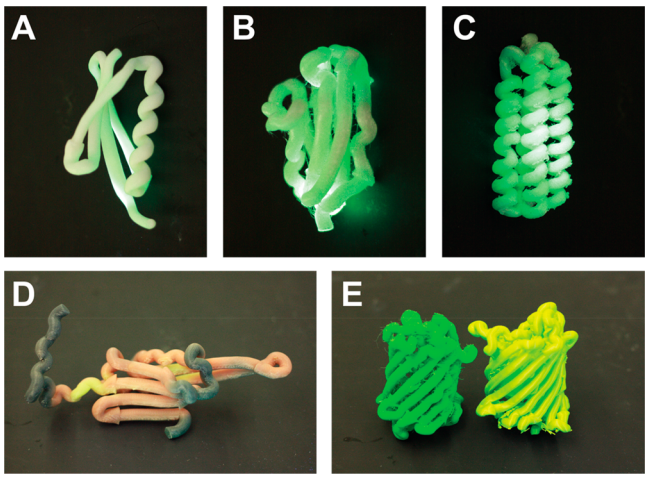

Figure 3. 3D-prints of proteins. (A) Bacterial protein L, printed in glow in the dark PLA. (B) Titin I10, printed in TPU. (C) Talin R9, printed in TPU. (D) Crystallin, printed in Color Changing with Temperature PLA. (E) GFP, printed in Green Flexible PLA Plus and Shiny Lime Green Silk PLA.

Another protein, crystallin (pdb code 3l1e), was printed using Tri Color Changing with Temperature PLA Filament (Amolen Tech). Crystallin is part of the cornea and the lens of our eyes.[27] Rather ironically, when present in low concentrations, as is the case in most of our cells, this protein prevents other proteins from forming crystals and aggregates. In this capacity, it is known as a heat-shock protein, which is expressed when the body temperature rises. To illustrate the role of crystallin to mitigate cellular stress, as it performs chaperone functions and helps other proteins fold correctly, as the body temperature rises, we chose a PLA that changes color with temperature. Using a heat-blower, the printed structure can be exposed to temperatures equal to or higher than the body temperature, turning its color from black to red or yellow, respectively (Figure 3B).

The proteins in Figure 3C,D represent the I10 domain of titin (from PDB code 5jde), and the R9 domain of talin (pdb code 2kbb), respectively. They were printed using Glow in The Dark Luminous Green TPU (from SainSmart). As mentioned in the Introduction, titin, the largest protein in the human body, is made of up to 100 folded domains and two spring-like unstructured regions.[28] Titin acts as both a biological spring and an active participant in muscle contraction by continuously unfolding and refolding some of its Immunoglobulin (Ig)-like domains, such as I10 (Figure 1A). Talin, as well, operates under a force vector and unfolds its rode domains to expose binding sites and drive focal adhesion formation (Figure 1B). While both proteins operate in a similar way, they have vastly different structures, with titin domains being mostly β-strands, and talin domain having only α-helices. The proteins printed in TPU can be unfolded and extended and were further used to demonstrate protein unfolding.

Another protein that we chose to print is green fluorescence protein (GFP, Figure 3E; pdb code 4kw4). This protein

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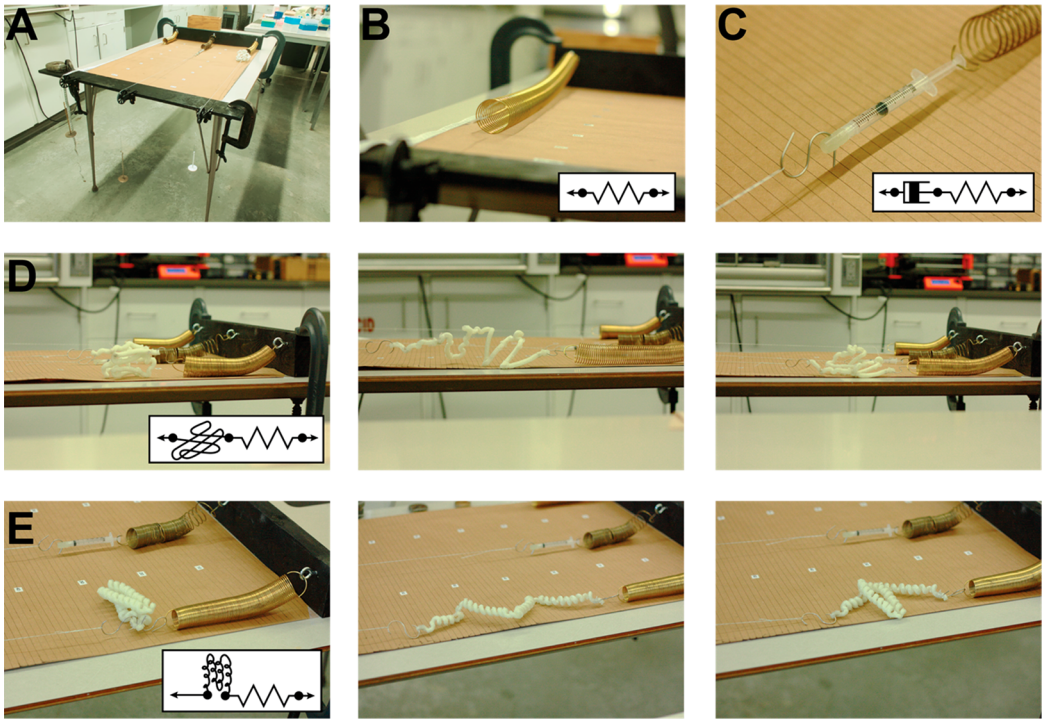

Figure 4. Simple setup to demonstrate how spring, dashpots, and proteins respond to mechanical forces. (A) Picture of the overall setup, reassembled in our lab, showing the three main components, as well as the general assembly. (B) Picture of a spring under force, extending proportionally. (C) Picture of a spring in series with a syringe. Over a certain range of forces, the syringe plunger slowly moves, representing the viscoelastic response of a dashpot. (D) Pictures of a muscle titin I10 domain in series with a spring. (E) Pictures of a talin R9 domain in series with a spring. For both panels D and E, left, snapshot is at a low force (before the protein domain unfolds), middle, at a higher force (following unfolding) and, right, back at a lower force (where both the spring and protein contract, but before domain refolding).

discovered in jellyfish is extensively used by scientists as a molecular reporter.[29] When exposed to the blue or ultraviolet (UV) light, GFP glows bright green. Many other variants were developed that produce other colors (which have different emission spectra). GFP has an interesting β-barrel structure and was printed in a Shiny Lime Green Silk PLA filament (Polymaker). As this filament was quite brittle and GFP has a very complex structure that is hard to remove supports, we also printed it using Green Flexible PLA Plus (Ataraxia Art). When talking about GFP, purified solutions of various variants, from blue to red, were shown under a UV light, to demonstrate their fluorescence.

Mechanical Unfolding Experiment Using Springs and 3D-Printed Flexible Structures

There is a profound, yet poorly understood, physics behind how protein unfolding under a force vector affects the overall extension and elasticity of cells and tissues. Unfolding of a protein domain results in a sudden increase in the overall contour length, and it looks like a spring being instantaneously added or subtracted over/under a certain force. To make things even more complicated, at the single molecule level, the process is probabilistic (and not deterministic), meaning that the same protein domain at the same pulling force will take some finite amount of time to unfold.[30] This time will be slightly different if the experiment is repeated over and over again and depends on the local interactions between the domain and the solvent molecules, as well as on the continuous formation and breaking of hydrogen bonds inside the protein structure. This addition and subtraction of contour length as the force changes, in the context of tens-to-thousands of domains in series, increases the extension range and response time of a molecule without significant variations on the force-per-molecule. This process provides, for example, a large range of operating conditions for our muscles,[28,31] which can be stiff while we stand, as most domains can be folded, and elastic when we exercise, as some domains may unfold. To illustrate this process, we designed a setup that shows how a spring, a dashpot, and a protein domain respond to changing forces. A dashpot is a damper used to describe time-dependent motions. Our setup consisted of a ledge clamped on a table, with three components (Figure 4A). A first component had a spring attached to the ledge in series with a string that was passed over a wheel and connected to a weight hanger (parts bought from Pasco) (Figure 4B and Movie S1). The second component had a spring in series with a 3 mL syringe (Teleflex), followed by the same wheel-weight hanger setup. The syringe had a 3D-printed plunger at its end, to seal it (printed in TPU, see design file in Supporting Information) (Figure 4C and Movie S2). A larger syringe (20 mL), with its end sealed with a finger, was shown to demonstrate that when the force is released, the plunger slowly moves back due to the vacuum force. The third component had a spring in series with a 3D-printed protein structure (in TPU) (Figure 4D,E, and Movie S3 and Movie S4). The protein had at its mechanical clamp, which for both titin I10 and talin R9 is between the first and last structural elements, glued magnets (N52, K&J Magnetics). These magnets were inserted in holes predrilled at the opposite sides and glued with epoxy glue. Magnets are ideal to represent the hydrogen bonds that hold together the protein structure, as they have a strong attractive interaction, which is relatively short-ranged. For the protein structure, the metal weight hanger (which weight ~50g) was replaced with a

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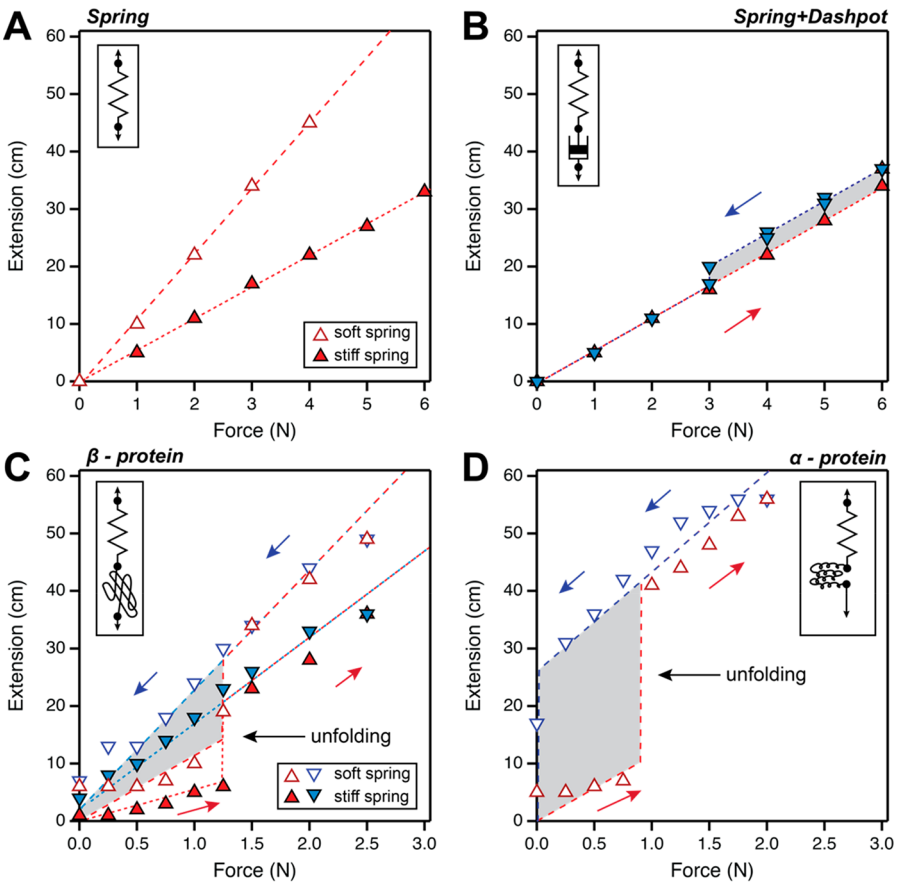

Figure 5. Data recorded for the extension as a function of force. Measured length change as a function of force for (A) two springs with different elasticities; (B) a sealed syringe in series with stiff spring; (C) β-protein I10 in series with a stiff and a soft spring; and (D) α-protein R9 in series with a soft spring. Filled symbols were used for experiments with a stiff spring, while empty ones were used for the experiments with a soft spring. Upward pointing triangles refer to the experiments when force was increased (also denoted by the red arrows), while downward pointing triangles refer to those where force was decreased (also denoted by the blue arrows). As with real proteins, our structures also show hysteresis, with a higher unfolding than refolding force, which allows for a storage/release mechanism for mechanical energy. This hysteresis is marked with a gray area. Data were recorded from a total of $N = 3$ experiments and represent real values expressed in Newtons and centimeters. Error bars are smaller than the markers used in the diagram.

3D-printed weight hanger, weighing less than 2 g (printed using Tough PLA Plus from Duramic, design file available as part of Supporting Information). The reason for using a lighter weight hanger is that the metal weight hanger applies too much force and does not allow the protein to refold back. The setup also had a brown paper with markings every centimeter and numbers every 10 cm, for easy reading of the extension.

Our experiment consisted of having members of the audience add slotted weights and record both the extension and the force. To simplify the force readout, every 100 g of added weight was approximated to 1 N. For the first setup, as weights were added in increments of 100 g, the extension was recorded for both a soft and a stiff spring (Figure 5A). When weights were removed, the extension was recorded as well. The springs behaved the same when weights where added or subtracted, and only the points where weights were added are shown in Figure 5A. The stiff spring in our case had a spring constant of 0.18 N/cm (filled triangles), while the soft spring showed 0.09 N/cm (empty triangles). The second experiment, consisting of a spring in series with a dashpot was done only with the stiff spring, as the soft spring would reach the maximum extension in our setup (which was ∼83 cm in total) before the syringe would start to extend. As force is being applied, the extension follows the same behavior as measured for the spring alone, until a loading of 600 g. At this force, the syringe plunger starts to slowly move, and after ∼5 s, it reaches an equilibrium point (Figure 5B). As adding more weights would break the seal between the plunger and the barrel, no other weights were added. When weights were subtracted, the plunger showed some time delay in its response, and at 300 g is fully closed. This experiment aims to explain the concept of viscoelasticity. The hysteresis seen is related to the friction forces developed between the rubber end of the plunger and the syringe barrel. In the last experiment, a printed protein was put in series with a spring (Figure 5C,D, and Movie S1 and Movie S2). Smaller weights were added (25 g) to first capture the extension of the spring, while the protein remained folded. At a given force (125 g for I10 and 90 g for R9), the protein suddenly unfolded, and after that, both the spring and the unfolded structure extended together. When removing weights, the extension kept decreasing without refolding of the protein structure, well beyond the unfolding force. The reason here is related to the fact that the magnets, while very strong, have a small attractive range. In fact, for the α helix protein, the magnets do not come close enough to re-form the original structure. There is an obvious hysteresis in our measurements, as the proteins unfold at a significantly higher force than when they refold. As we already know the spring constants from the

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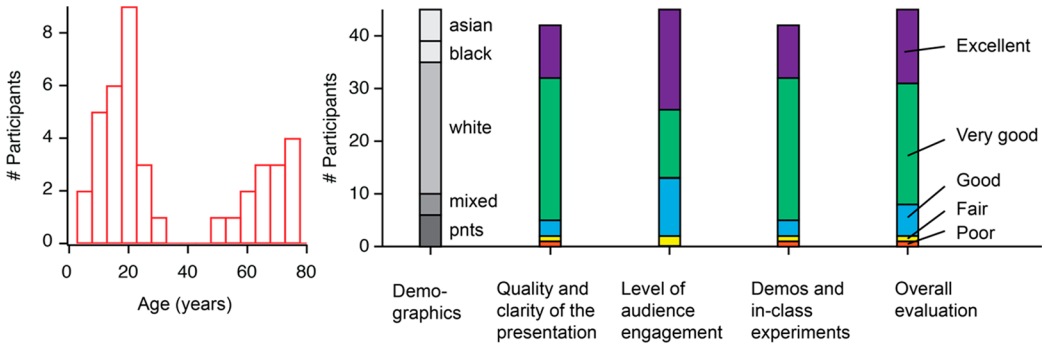

Figure 6. Summary of evaluations. (Left) Age distribution (pnts = prefer not to say). (Middle) Demographics. (Right) Program evaluations from a total of 45 evaluation forms.

first experiment, we can use those values to determine the spring constant of the unfolded structure. To model such a behavior, a second spring was added or subtracted as the force increased or decreased, at the values measured experimentally for (un)folding (see dotted lines in Figure 5C,D). The titin I10 β-strand structure had an apparent spring constant of 0.11 N/cm in both experiments (with soft and stiff spring), while for the all-α talin R9 structure we measured an apparent spring constant of 0.17 N/cm. Several things are worth mentioning here. First, the same hysteresis originating in our experiment from the fact that the magnets exert a strong, but short-range force, is seen for proteins, where the hydrogen bonds holding the 3D structure also have a strong, but short-range, attraction effect.[30] By having this hysteresis, proteins can effectively store and dissipate important amounts of energy. This behavior is also measured in tissue-like materials, where biomaterials made from globular proteins show a different strain response upon increasing stress and decreasing it.[32,33] However, unlike the third experiment, which sees the unfolding taking place every time at the same force, proteins *in vivo* have a probabilistic behavior, and a molecule made from repeats of the same protein domain will display under a constant force, with unfolding steps of different time intervals.[30] This time-dependent behavior can be better related to the second experiment, where viscoelastic effects introduce a time-dependent variable. Finally, force is a vector, and not only its magnitude but also its direction are important. It is well-known that the same protein pulled from different directions will show vastly different unfolding kinetics.[34,35] Typically, when hydrogen bonds are perpendicular to the pulling direction (known as sheering geometry, which is the case for titin I10 domain), the force required to unfold is higher than when hydrogen bonds are parallel to the force (known as unzipping geometry, as is the case for talin R9 domain). Interestingly, our experiment follows this behavior, with the all-α structure unfolding at a lower force than the all-β structure. We note that the mechanical pulling apart demonstration that we show here with flexible 3D-printed structures and springs under a given force actually happens at the molecular level *in vivo*, as detailed above for talin. Obviously, the forces are much smaller (picoNewtons when scaled to single proteins vs Newtons for printed structures), and the extensions are proportional to the protein structure (nanometers for the unfolding of a protein *in vivo* vs millimeter extensions measured in our demonstration). We also note a limitation of the 3D-printed structures having α-helices, which in the demonstration retain the spring structure after unfolding, no matter the applied force. Unlike the macroscopic demonstration, the secondary structure elements of proteins also denature at high forces, transforming the protein chain into a simple polymer.[25]

■ EVALUATIONS

The event was held 5 times during the month of January 2022 (which unfortunately coincided with one of the peaks of the COVID-19 pandemic and a cold wave in Milwaukee). The program was advertised through Milwaukee and Shorewood public schools and recreation departments, as well as through social media such as Twitter, Facebook, etc. The event was attended by a total of 143 people. Members of the audience were actively involved in the experiments described above and were rewarded with 3D-prints of the proteins from Figure 3. Evaluation forms were designed following standard templates from surveymonkey.com and personalized for this event. Evaluation forms were distributed one-per-group (family) and left at end of the event in an unattended box. The answers to the questions are summarized in Figure 6. We saw a bimodal distribution of age, with 55% of the self-reporting members of the audience being of K−12 age. This bimodal distribution reflects that smaller children were typically accompanied by their parents or grandparents. The demographics from those who reported on the question about race show an attendance of 44% from minority (26% from underrepresented minorities in science), above the Wisconsin demographics (which is 18% for minorities and 15% for under-represented minorities, according to census data from 2021 https://www.census.gov/quickfacts/WI). All the questions on the evaluation of the quality of the event were over 50% rated as "Very good" or "Excellent", with the assessment on the level of engagement having the highest proportion of "Excellent" rankings.

■ CONCLUSIONS

We have described a set of experiments that have been successfully implemented in an open-public event and can constitute an improved learning experience for students being taught protein folding. The experiments shown use inexpensive magnets and take advantage of the large availability and increased affordability of 3D-printing technology. Several simple structures printed from materials that reflect the *in vivo* properties of proteins are demonstrated in this study. Furthermore, structures printed in a flexible material were used to demonstrate a profound behavior of mechanical unfolding of proteins and relate it to macroscopic changes on how cells and tissues respond. During this event, we learned that direct involvement of the audience increases both their attention and their understanding. A challenge came from the time needed to

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print the 3D structures. While only costing less than a quarter in material, the time needed for printing and cleaning of each structure was ∼3.5 h. Furthermore, generating removable tree-supports allowed us to print protein structures without any artificial linking parts. While the demonstrations presented here were done as part of an outreach event, they could easily be incorporated into a course, as most high schools and universities have access to 3D-printers. Another demonstration could also come from having students identify a protein structure and follow the instructions in the manuscript to 3D-print that structure. The approach presented here will help visual learners by allowing students to physically interact with protein structures that are large enough to be held in their hands. Furthermore, by combining experiments on spring elasticity and dashpot response, that are typically encountered in mechanics laboratories, with unfolding of flexible protein structures, students can better understand how proteins operate *in vivo* under a force to change their extension. Obviously, the demonstrations provided here have the potential to improve engagement and interest in a classroom setting. We envision the use of hands-on demonstrations as a successful approach to teach students about the wonders of science.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available at https://pubs.acs.org/doi/10.1021/acs.jchemed.2c00231.

- File to use as weight hangar .stl upon changing the extension from .txt to .stl (TXT)
- Additional figures showing hydrophobic collapse during protein folding and various representations of proteins (PDF)
- File to use as syringe plunger .stl upon changing the extension from .txt to .stl (TXT)
- Movie S1 showing extension and contraction of a spring (MP4)
- Movie S2 showing extension and contraction of a spring in series with a dashpot (MP4)
- Movie S3 showing unfolding and refolding of a β-protein (MP4)
- Movie S4 showing unfolding and refolding of an α-protein (MP4)
- Weight hanger and syringe plunger .stl files (ZIP)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We acknowledge Michael Gerard Condon from the UWM Physics Machine Shop for helping us to assemble the demonstration setups and Naomi Raicu, Suha Malik, Christine Wiley, Norman P. Lasca, and L&S UWM, for organizing the event. This study was funded by National Science Foundation (MCB-1846143 and DBI-1919670).

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