

Sonification-enhanced lattice model animations for teaching the protein folding reaction

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ABSTRACT: The protein folding reaction is one of the most important chemical reactions in the human body. Yet, despite its importance, it is sometimes omitted from undergraduate courses due to the challenging nature of some of the underlying concepts. To help make key concepts of the protein folding reaction accessible to our undergraduate students, we implemented three, simplified 2D lattice models of various amino acid chains, and we used these models to generate sound-enhanced animations that allow students see and hear the dynamics of protein folding in action (for example, [Video S1.6](#)). In spring of 2021, we used these videos in remote-learning biophysics and music courses to introduce four key concepts of the folding reaction: solvation and hydrophobicity; energy and conformational entropy; funneled energy landscape; and frustration and traps. Our lattice model animations and sonifications helped provide insight into protein folding dynamics for undergraduate and graduate biophysical chemistry students, undergraduate musicians, and even for the authors who are experts in this field. We plan to incorporate these and additional animations, along with enhancements to the 2D lattice models in our future courses.

Videos, brief sample lecture material, and sample homework problems are provided in the Supporting Information section

KEYWORDS: Second-year undergraduate; upper-division undergraduate; physical chemistry; multimedia-based learning; proteins/peptides

Introduction

Protein folding is one of the most important chemical reactions of life: it produces the enzymes that run our metabolism, the transcriptases that copy DNA, and the signaling proteins that tell our cells whether everything is functioning normally.¹ Yet folding and its cousin, protein structure prediction,² are difficult subjects at the undergraduate level in a physical chemistry, biochemistry, or computational chemistry class, and often omitted despite their importance for chemistry and biology majors.³ The primary reason is that folded structure does not rely on just one or two strong bonds, as is the case for organic reactions or inorganic catalysis,⁴ but rather on many weak interactions to hold the folded protein together. Conceptually, it has been understood since Anfinsen's work in the 1960s that the folding mechanism is encoded in the amino acid sequence.⁵ Lattice models,⁶ invented in the 1970s and applied more widely starting in the 1980s, supported the idea that proteins have evolved to maximize consistency of many distributed interactions⁷ and minimize frustration among these interactions.¹ Even so, large proteins require unfolding/folding by chaperones because some frustrated interactions remain.⁴

A picture is worth 1000 words, and so visualization plays an important role in educating students about protein dynamics. This includes methods for teaching about energy landscapes using the funnel picture,⁸ combining visualization^{9–11} with a hands-on mechanical model of folding,¹² watching color changes when proteins fold and

unfold reversibly because "seeing is believing,"¹³ interacting with computer games that teach protein folding concepts,¹ and creating physical models to help students visualize proteins in three dimensional space¹⁴ or, if visually impaired, to explore those structures using the hands or mouth.¹⁵

One has only to recall the crackle of a Geiger counter, alerting its users to the presence of alpha particles for over 100 years, to recognize that data sonification, too, is an effective tool for discovering, understanding, and communicating the time-dependent behavior of physical phenomena. We are, at the most fundamental level, multi-modal creatures, having evolved to navigate our 3D world using redundant, synergistic combinations of sensory inputs — vision, audition, proprioception, olfaction, and more — to form an accurate and reliable composite model of our surroundings.¹⁶ We seek to piggyback on this highly-evolved skill for navigating with all one's senses in order to enhance our understanding of an abstract chemical concept: the energy landscape of protein folding.

Whereas sonification has been applied to spectroscopy education,^{17,18} and data sonification of proteins has focused on structure,^{19–22} the goal of our present project was, instead, to convey to our students some of the *dynamics* of protein folding. We apply a real-time lattice model of folding as a new educational tool, and combine this new visualization with sonification to allow students to see and hear changes in the energy and conformation of a protein as it transitions from state to state. Feedback from a diverse set of science and music students indicates that presenting folding as an animated, sonified lattice model increases students' interest in the material and is perceived by them as useful in solving homework problems related to folding.

After introducing key concepts of protein folding and scientific data-sonification, we describe how we constructed the sonified lattice models used to create the videos. We discuss three folding examples using the models, and we include a sample lecture, and sample homework problems with solutions. Finally, we discuss students' responses to these materials and speculate on the outlook for future applications of sonification to dynamics in chemistry.

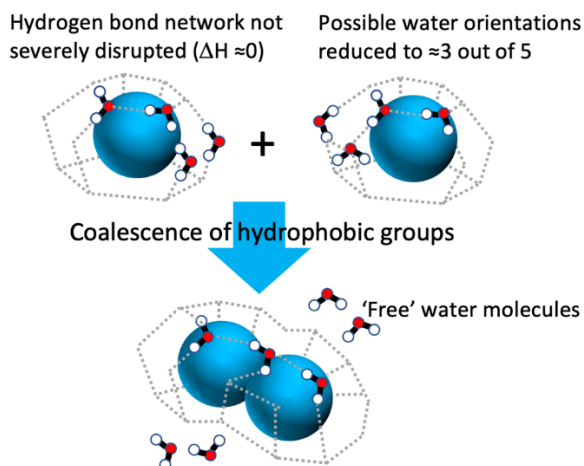


Figure 1. Small hydrophobic chemical groups, such as $-\text{CH}_3$ or phenyl, are solvated by forming less mobile water cages around them, reducing the solvent entropy. When such hydrophobic groups make contact (bottom), water molecules are released, increasing the overall entropy, at least at higher temperatures.²⁰

Protein Folding Concepts

In order to help our students learn to speak the language used by protein scientists, we introduce four key concepts of the folding reaction. These are: solvation and hydrophobicity; energy and conformational entropy; funneled energy landscape; and finally, frustration and traps. For more in-depth study, we reference below several review articles and books with material that would be useful to an instructor or student who is not an expert in the area but who wishes to learn more.

Solvation and hydrophobicity While hydrogen bonds between amino acids organize secondary structure (e.g., α -helices and β -sheets),²³ the solvent is critical in bringing amino acids together to form tertiary structure. When water solvates hydrophobic amino acid side chains such as phenylalanine, it forms cages around them with reduced water mobility and entropy. Folding a protein so these hydrophobic side chains release water into the bulk increases the mobility of water and hence overall entropy (Figure 1).²⁴ This hydrophobic driving force is not entirely entropic, especially at low temperature, but increasing solvent entropy does play a major role in folding.

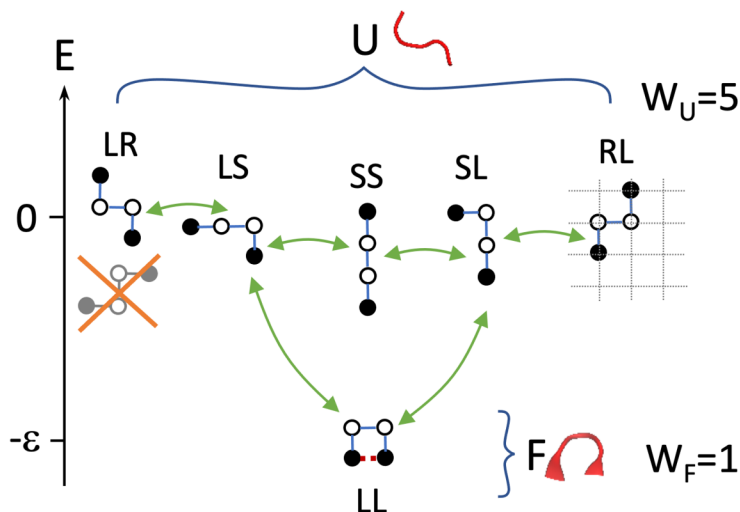


Figure 2. A lattice model with hydrophilic (light) and hydrophobic (dark) beads representing a 4 amino acid tetrapeptide that can form a ‘hairpin.’ The gray, dashed lines represent the square lattice on which beads are allowed to move. The axis on the left shows the energy of each state. The folded ‘state’ F at energy $-\epsilon$ contains only $W=1$ conformation, ‘LL’. In contrast, the unfolded ‘state’ U contains $W=5$ conformations and has conformational entropy $S_c=k_B \ln 5$. Green arrows show the allowed interconversions, by flipping one bond 90° , between conformations. The conformation crossed out in red is not counted because it can be obtained from the one above it by 2D rotation. The ‘LSR’ nomenclature for each conformation is explained in the Methods section, e.g., ‘LL’ refers to the chain making two left turns after the first segment, which is always oriented straight up. The red chain next to U and ribbon next to F show a conventional chain/ribbon representation of a disordered chain or hairpin turn.

Energy and conformational entropy When a protein folds, native contacts are made among side chains, ranging from salt bridges (a positively and a negatively charged amino acid coming together) to the hydrophobic contacts mentioned above. This reduces the energy E (or at constant pressure, the enthalpy H) of the protein as it folds. At the same time, the protein comes to occupy a much smaller number W of conformations, reducing its conformational entropy $S_c=k_B \ln W$ (Figure 2).²⁵

Unlike in many simple chemical reactions, in folding, the solvent plays a key role in mediating contacts. If these critical solvent coordinates are included in the analysis, then they are part of the energy E shown in Figure 2. Otherwise, the vertical axis in Figure 2 becomes a free energy with respect to the solvent. We consider the critical solvent coordinates included here to distinguish the ‘energy’ of the energy landscape from the overall ‘free energy’ of the chemical reaction.

Funneled energy landscape Large molecules have an energy landscape, a generalization of the ‘potential surface’ for small molecules.²⁶ The full energy landscape depends on many coordinates and can include relevant solvent degrees of freedom that are part of the folding process, such as water molecules excluded from amino acid interfaces due to hydrophobicity. A minimal plot of the energy landscape features E as the vertical axis, and S_c as the horizontal axis, indicating how many conformations are possible at each energy. The energy landscapes of unevolved macromolecules have many local minima with many conformations at each energy (Figure 3, top).

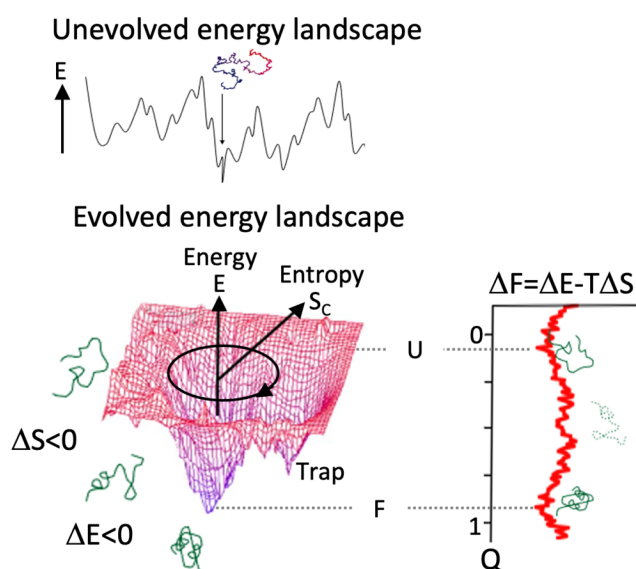


Figure 3. Top left: an unevolved energy landscape. Random contacts can lower the energy somewhat, but because the amino acid sequence does not facilitate all such contacts being made simultaneously, the energy landscape is ‘frustrated.’ Bottom left: an evolved energy landscape has a sequence that facilitates multiple simultaneous contacts, offsetting the loss of conformational entropy. It ‘minimizes frustration.’⁶ Bottom right: energy-entropy compensation means that the folding free energy $FE=E-TS$ ($G=H-TS$ at constant pressure) is small and free energy barriers are small, making folding a very fast (milliseconds to hours at room temperature) reaction between states (here U and F) that have similar free energy under physiological conditions. Representative structures of the polypeptide chain are shown.

A remarkable feature of many macromolecular biological reactions is energy-entropy compensation. For example, during folding of an evolved sequence, the reduction of conformational entropy of the peptide chain (Figure 3) is accompanied by simultaneous reduction in energy (or enthalpy at constant pressure) through contacts between amino acids: The evolved energy landscape has a funnel shape, with states of lower S_c also having lower E .²⁷ The sequence of amino acids is organized in a way that allows each residue to lower its energy on the order of RT while losing conformational entropy on the order of R ($R = 0.00831 \text{ kJ/[K}\cdot\text{mol]}$ is the universal gas constant). Thus, the free energy $FE=E-TS$ upon folding changes much less than its constituents E (energy) and TS (product of temperature and entropy) (Figure 3, bottom right), resulting in low protein stability and small free energy

barriers for folding; low stability is important for function by allowing fluctuations, and small barriers are important for getting to the folded state quickly.

Frustration and traps In large molecules such as proteins, not all contacts that lower the energy can be made simultaneously, a concept known as frustration. Evolved sequences position amino acids in such a way that a specific 3D protein structure allows a maximum number of native contacts.⁷ This structure is thus minimally frustrated.²⁸ However, protein function, and the finite number (~ 20) of amino acids dictate that frustration cannot be eliminated completely, creating frustrated structures that are local minima on the energy landscape. Thus, even evolved landscapes retain some roughness, and local minima can act as traps that delay the folding process (Figure 3, bottom left): in order to fold, the protein must first unfold in order to try a different path down the funnel. However, when a protein can escape a local minimum directly towards the folded state, we call it an ‘intermediate,’ and it could even accelerate the folding process.

Sonification Concepts

Data sonification is a mapping from data (whether generated by a model, captured in an experiment, or gathered through observation) to one or more parameters of an audio signal or sound synthesis model for the purpose of better understanding, communicating or reasoning about the original model, experiment or underlying phenomenon.²⁹ For example, you could map the value of a measurement, such as the radius of gyration, to the pitch of an audio signal (Figure 4).

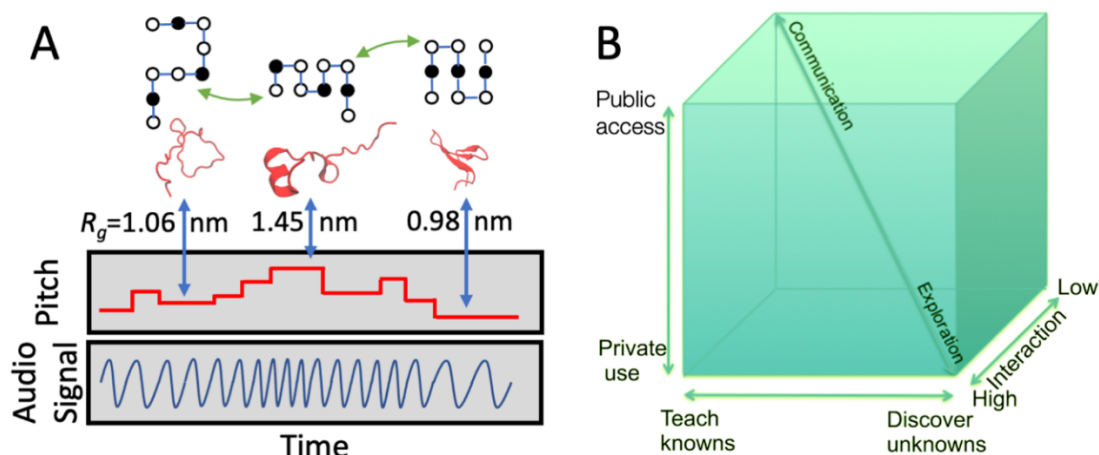


Figure 4. (A) A sonification mapping the radius of gyration (size) of a WW domain protein model to pitch of an audio signal. The lattice model as well as three ribbon structures of an atomic model of WW domain are shown: unfolded (left), misfolded (middle, formed a non-native helix) and folded (right, all beta sheet). (B) The space of data sonification has three dimensions²⁹: intended use (vertical axis), instruct vs. discover (front axis) and interactivity. Our application is a public presentation for teaching known concepts with a moderate degree of interactivity.

A well-designed data sonification (or visualization) mapping should be inference-preserving; in other words, any observations you make or conclusions you can draw in the target (sound) domain should also hold true in the source (simulation/measurement/observation) domain. This is the primary design goal for any data sonification — to preserve the critical features of and interrelationships within the original phenomenon.

Data sonification can lie anywhere along a continuum from exploration and discovery of previously unknown patterns to presentation of known information; it can range from highly interactive within a tight feedback loop to passive listening, and it can be carried out privately by a single individual, by a small research group, or in the public sphere. A particular instance of data sonification can exist at any point in this continuum of passive-interactive, public-private, known-unknown space shown in Figure 4B.

Any data visualization, even one as simple as a 2D graph, is the outcome of multiple design decisions; the choice of which variables to represent, whether to use linear or log scales, how to normalize the data, whether to use dotted or solid lines, which colors to use, and so on, can all impact the clarity and communicative power of a visual graph. In data sonification, too, multiple design decisions — for example, the choice of synthesis model, deciding which data variables to map to which sound parameters, whether to use discrete or continuous values, and the scaling and timing of parameter changes — can all contribute to the intelligibility and clarity of the result.²⁷

Methods

A sonified lattice model for protein folding To help explain the four key protein folding concepts to students having a minimum of thermodynamics and kinetics knowledge, we implemented a protein “lattice model”,^{30–32} a model not previously widely used in chemical education, and we used this model to generate data-visualization animations with data-sonification sound tracks. We briefly describe our simple model and its implementation in software as a state machine, with more details provided in the Supporting Information.

Representation of proteins on a lattice The protein is represented on a 2D square lattice as in Figure 2: dark beads correspond to hydrophobic amino acids that interact with an energy $-\epsilon$ when they are adjacent; light beads correspond to hydrophilic amino acids that interact with energy $-\epsilon'$ when they are adjacent (see Figure 6 for such hydrophilic pairs). The total energy E of the system is the sum of all the pair energies $-\epsilon$ and $-\epsilon'$. We specifically used $\epsilon = 1$ kJ/mole and $\epsilon' = 0.25$ kJ/mole in our implementation of the problem for lecture demonstrations and homework assignments.

The configuration with the lowest energy is considered the ‘folded state’ (F in Figure 2); all others are lumped together as the ‘unfolded state’ (U in Figure 2), although one may further differentiate long-lived traps (defined below) within U. F and U are the macroscopically distinguished states in the model, which may contain one or more conformations. Thus, the entropy of state F is $S_F = k_B \ln(W_F) = 0$ as it corresponds to a single folded conformation, and the entropy of the state U is $S_U = k_B \ln(W_U) = k_B \ln 5$ in Figure 2 because U consists of 5

macroscopically indistinguishable conformations. Of course, the folded state of a real protein has several slightly different conformations, and better experiments could distinguish some of the conformations in the unfolded state from one another.

The protein folds by moving from one configuration to the next. In real-life, this motion is driven by thermal excitation (e.g. water molecules bumping into the protein) and forces between amino acids (e.g. attraction of two hydrophobic amino acids). Folding kinetics of the polypeptide chain in this model follows three simple rules: (1) A single randomly selected bond per time step can be flipped by 90° to make a transition between two conformations, as indicated by the green arrows in Figure 2. (2) Beads cannot be superimposed on the same lattice point, they avoid each other due to steric hindrance. (3) To satisfy thermodynamic equilibrium, flips are chosen by Metropolis sampling:³³ if a randomly chosen flip lowers the total energy E , it is automatically accepted. If the flip would raise the energy, it is only accepted if the Boltzmann factor $\exp[-\Delta E/k_B T]$ is greater than a number randomly chosen between 0 and 1. This procedure produces the correct thermodynamic equilibrium. Thus, the model will tend to fold into the lowest energy state at low temperature, and it will tend to unfold to the state U at high temperature because that state contains more conformations that can be visited at high temperature. A ‘trap’ is a conformation or a group of conformations, of energy higher than the folded state F, from which all allowed transitions lead upward in energy.

In this model, each move corresponds to a time step Δt from time t to $t+\Delta t$; the real-world characteristic duration for such steps is the time required for the diffusion of an amino acid residue over a distance comparable to its size, which is about 10 ns.³⁴ The ‘flipping’ dynamics of the model thus mimic the real dynamics of a polypeptide chain moving continuously in space.

Implementing the lattice model of protein dynamics as a state machine We used the sound design language Kyma³⁵ to implement the lattice model as a state machine and to map observables (variables) from each conformation to sound parameters and images. Here we give a brief overview of the implementation, with a more detailed description provided in SI Sections 2 and 3. Given a lattice model, defined in terms of the number of elements in the chain, the positions of hydrophobic elements, and the energy associated with hydrophobic and hydrophilic bonds, Kyma generates, in real time, a sequence of valid conformations, each of which has associated observables such as the size or total energy of that conformation (see below).

A conformation is represented in Kyma as a string of directions, starting with the first bond pointing ‘up’, and successively labeling each joint (angle between two bonds) as 90° to the left (L), 90° to the right (R), or straight ahead (S). For the 4-bead hairpin in Figure 2, this representation yields $3^2 = 9$ shapes, identified by their joint directions (starting from the first bead): {LS, SL, SR, RS, RL, SS, LR, RR, LL}. If the first and last beads are considered equivalent and conformations that interconvert by rotation in the 2D plane are considered to be identical, then the simple model {LS=SR, SL=RS, RL, SS, LR, LL} in Figure 2 is obtained.

Calculating chemically interesting observables Observables analogous to those computed in all-atom molecular dynamics (MD) simulations (e.g., Q) or measured in folding experiments (e.g., R_g) can introduce a conceptual link between these 2D lattice models and the kinds of behaviors students might see reflected in data found in the literature.

The observables, $\mathbf{O} = (E, R_g, d_{ee}, Q)$, computed for each conformation, are defined as:

- The total energy E of the conformation, which depends on the number and type of bonds present
- The radius of gyration R_g , which measures the diameter of the protein (see SI for full definition)
- The end-to-end distance d_{ee} , also labeled ‘E2E’ in some videos, from the first bead to the last bead³⁶
- The fraction of native contacts Q , ranging from 0 (unfolded) to 1 (folded) (see SI for full definition)

To generate a characteristic sound for each conformation, Kyma maps the observable values associated with that time step to sound synthesis or processing parameters and displays an image associated with that conformation.

[Video S1.1](#) is a sonification/visualization of the 4-bead lattice model shown in Figure 2. The graph (G) of the funneled energy landscape is displayed on the left (with the current state highlighted in yellow and edges out of that state highlighted in purple); the current conformation (the current state) is displayed on the right, and the changing values of the observables are displayed just below the conformation. Each conformation is associated with a unique percussive sound, and each time a new state is entered (each time the conformation changes), that conformation’s sound is triggered. The pitch of the recording (low to high) and its pan position (left to right) reflect the energy of the protein conformation.

Implementing a lattice model as a nondeterministic finite state machine — the basis of formal languages and the theory of computation — invites analogies between chemical reactions and information processing. For example, in the lectures (listed in SI section 6 as separate SI file and discussed in Results), biochemical processes are described in terms of information theory, noting that chiral synthesis of a protein chain that encodes its own folded structure through the amino acid sequence (the information) relies on information that was pre-stored at great ATP expense over billions of years of evolution.

Results

In the classroom and homework, we focused on the folding dynamics of four simple lattice proteins: an unevolved polypeptide, a beta hairpin, an alpha helix,²³ and the WW domain, a small protein that helps prevent cell apoptosis (death) among its other functions.³⁷

Unevolved sequence An arbitrary chain of amino acids generally does not fold into a unique shape: it has many low-energy states where the peptide can get trapped (Figure 3, top). Similarly, a lattice model with an arbitrary string of hydrophobic and hydrophilic beads does not necessarily result in a well-defined folding funnel with a single folded state.

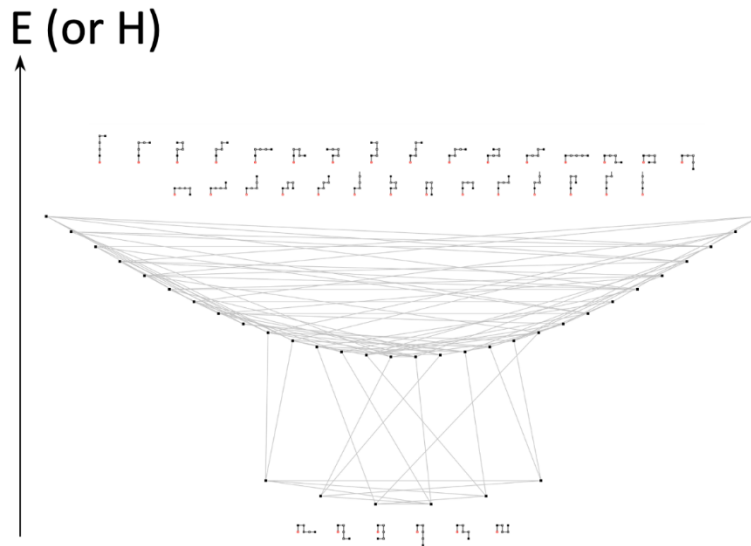


Figure 5. Energy landscape for a random (unevolved) sequence of 6 beads. There is no well-defined native state, and there are six very different structures at the same energy, analogous to Figure 3. The low and high energy structures are shown in no particular order. Sonification at [Video S1.2](#).

For example, the 6-bead chain ①②③④⑤⑥ (where beads 2 and 6 are hydrophobic) does not yield a graph with a single minimum energy state; instead, it has 6 lower energy conformations connected to 30 conformations at higher energy (Figure 5 and [Video S1.2](#)). A chain with this kind of energy landscape cannot fold to a unique native state.

Beta hairpin To see an example of how the process of evolution can store information to generate spatial structure, try changing the position of one of the hydrophobic beads from position 6 to 5 to make the sequence ①②③④⑤⑥ (so beads 2 and 5 are hydrophobic). The state space for this 6-bead beta hairpin model, like the 4-bead example, is generated by taking the space of all $3^4 = 81$ possible conformations, removing any conformations that overlay beads, that are rotations of another conformation in the 2D plane, or that are equivalent under order-reversal. One can also enforce a preference for chirality (e.g., every chain begins as either a straight or as a right turn), resulting in a state space of 22 shapes and a 4-level energy landscape (left example in Figure 6A and [Video S1.3](#)).

Alpha helix The alpha helix lattice model is based on a chain of 8 beads ①②③④⑤⑥⑦⑧ with hydrophobic elements in positions 2 and 7. As with the 4-bead and beta hairpin models, we start by taking the space of all $3^6 = 729$ possible combinations of left, right, or straight angles at each joint, remove any conformations that cross themselves or that are rotations of another conformation in the 2D plane. To mimic chirality in actual proteins, we allow only the right-handed variant of each shape. To mimic the N- and C- termini of actual proteins, in this model, we do not consider the first and last beads to be equivalent, so we do not remove shapes that would be equivalent under order-reversal of the beads. After this pruning, there are 272 shapes left. Since alpha-helical peptides form near-optimal linear hydrogen bonds, we give only the helical hydrogen bonds $\epsilon=1$ kJ/mole (Figure 6B and [Video S1.4](#)).

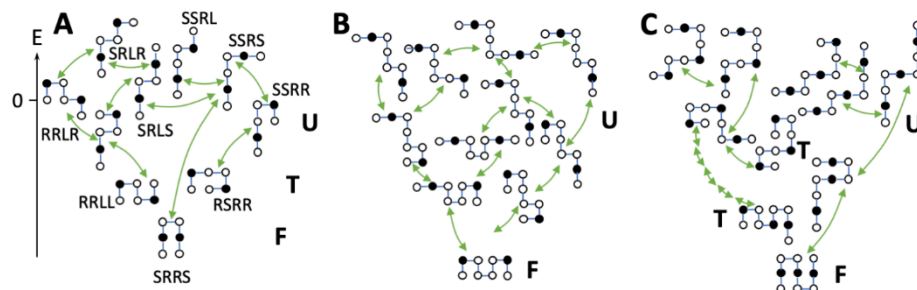


Figure 6. Summary of (A) the beta hairpin ([Video S1.3](#)), (B) alpha helix ([Video S1.4](#)) and (C) WW domain ([Video S1.5](#)) lattice models, showing a small selection of the conformations for each, with allowed transitions as green arrows. Each protein has a unique lowest energy conformation, the folded state 'F', unfolded conformations that make up the unfolded state U, and in some cases traps T which have no path downhill in energy towards the folded state. The energy landscapes are funnel-shaped. The hairpin landscape in (A) also highlights the 'RLS' nomenclature discussed in the text.

WW domain The WW domain (illustrated in the sample lectures) is our most complex lattice model, suitable for introduction after students have first worked with the simpler examples in homework. This model is based on a 9-bead string where the first and last beads are not equivalent, and with hydrophobic residues in positions 2, 5, and 8 (Figure 6C, Figure 7, and [Video S1.5](#)). There are 740 shapes in the state space and 10 discrete energy levels in the energy landscape. In this model, the traps correspond to incorrect secondary structure, such as a helix or mis-registered hairpins.

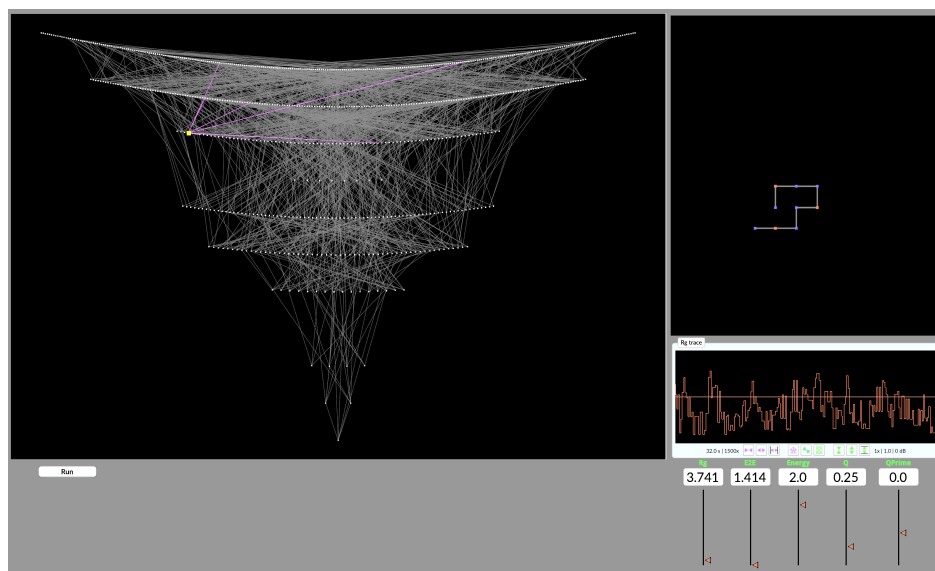


Figure 7. Screenshot from an animation of a state machine that models WW domain folding. The funnel is shown on the left, with the current state highlighted in yellow and all possible transitions to the next conformation highlighted in purple; other allowed transitions are shown as a white web connecting conformations. The eight traps, which can be escaped only by going to higher energy, are in the fourth row from the top. The currently occupied conformation (whose shape is shown on the right) is a “degenerate trap” which can be escaped only by going to a conformation at the same energy (degeneracy = equal energy). The “oscilloscope display” on the right can highlight variables such as fraction of native contacts Q or (in this case) radius of gyration R_g , and the observables are displayed on meters below the oscilloscope. In [Video S1.6](#), you hear the model at slightly above its folding temperature T_m , where it explores both the folded state and unfolded conformations.

Visualizing and Sonifying Lattice Models For each state, we map one or more features of the current conformation to one or more parameters of a sound synthesis algorithm; simultaneously we display an image of the conformation (see Figure 7) alongside an image of the energy funnel highlighting the current state (as a yellow square) and the potential transitions from that state to the next state (as purple edges). As discussed earlier, a single time-step (one Monte Carlo move of the chain) corresponds to about 10 nanoseconds for a natural amino acid chain.

In the lecture ([Playlist S1.7](#)) and homework ([Playlist S1.8](#)) videos, we used a variety of sound mappings, each one designed to bring out a particular aspect of the model in order to help the students make a comparison or answer a question. In SI section 3 “Step-by-step sound-mapping examples” we describe one such mapping in detail and provide brief descriptions of some of the alternative mappings.

Teaching the Four Key Concepts of Protein Folding A course instructor could utilize a combination of the slides, figures, and videos provided in the SI to introduce the four key concepts of protein folding, for example:

- **Solvation and hydrophobicity:** The WW model forms the folded state when all three hydrophobic beads are lined up, excluding the solvent as much as possible from their surfaces (Figure 6C and [Video S1.9](#)). At high temperature, solvation “wins” over hydrophobicity, even though hydrophobicity increases with temperature,²⁴ because the solvated chain has more conformational entropy. ([Video S1.10](#))
- **Energy and conformational entropy:** In the unevolved peptide (Figure 5 and [Video S1.2](#)), the entropy of the lowest energy state is $S_c = k_B \ln(6) \neq 0$. For the evolved proteins in Figure 6, $S_c = k_B \ln(1) = 0$ ([Video S1.3](#)). The energy of the native state of the WW domain lattice model is -2.5 kJ/mole, due to two hydrophobic interactions ($\epsilon = 1$ kJ/mole each) and two hydrophilic interactions (pairs of light beads, $\epsilon' = 0.25$ kJ/mole each) ([Video S1.5](#)).
- **Funneled energy landscape:** Figure 7 shows the funneled landscape for WW domain, and all the videos except for [Video S1.2](#) show the protein hopping between conformations, occasionally folding into the unique folded conformation.
- **Frustration and Traps:** In the WW domain ([Video S1.11](#)), and hairpin ([Video S1.12](#)) models the model protein gets stuck in traps that have a small radius of gyration but are not the folded state. In **Sample homework problem e** (below), students are encouraged to find these traps by listening to the value of R_g mapped to the frequency of the audio signal (Figure 4A and [Video S1.6](#)).

Sample lectures We developed two sample lectures containing no previously copyrighted material that are suitable at various levels from sophomore (very little background in thermodynamics or partition functions) to seniors (some background in thermodynamics and partition functions). The presentation in PowerPoint format contains only 10 slides per lecture, so the material can be covered in two lectures at a pace allowing the instructor to interact with the students and show sonifications, rather than just lecture. Material that is optional for an upper division or graduate course has been minimized and is in an Appendix, so the lectures can be given at a wide range

of levels. Talking points for each lecture, referring to the four key concepts introduced in the Protein Folding Concepts sub-section of this paper, are summarized in the Comment area of each slide.

Sample homework problems The problem set and solutions are presented in the SI (section 4) and the solution to each problem relies on the information presented in the associated animation / sonification video.

Problems a and b: To introduce students to the experience of analytical listening and give them some practice extracting information from sound, we started with a simple coin-toss model ([Video S1.13](#)). Each virtual coin toss triggers a sound event: a high frequency tone corresponds to a result of Heads and a lower frequency tone corresponds to Tails. Students are asked to identify whether a coin is “fair” or biased towards heads or tails ([Video S1.14](#)), and then to quantitatively establish their reasoning (the sound-mapping is described in SI section 3).

Problems c and d: The homework progresses to more difficult questions involving protein folding models of a 6-bead hairpin such as the one in Figure 5B. After listening to examples of the hairpin model at temperatures above ([Video S1.15](#)), below ([Video S1.16](#)), and at the ‘folding temperature’ ([Video S1.17](#)), students are asked to determine whether the video of the protein at an unknown temperature is at a temperature low enough to be mostly in the folded state ($T < T_m$) ([Video S1.18](#)). The sound-mapping is similar to the one used in the coin toss so as to facilitate comparisons between the simple coin-toss model and the 6-bead lattice model.

Problem e: These problems focus on the WW domain model (Figures 6C and 7) and ask more subtle questions about identifying traps by ear (this can be solved by noticing that a smaller radius of gyration is mapped to a lower pitch) ([Video S1.6](#)).

Problem f: In a final, open-ended homework question, the students are invited to view a visualization / sonification of an all-atom molecular dynamics simulation the CHARMM22* force field³⁸ and then to speculate about how one could enhance the lattice model simulations to make them closer-to-life. ([Video S1.19](#))

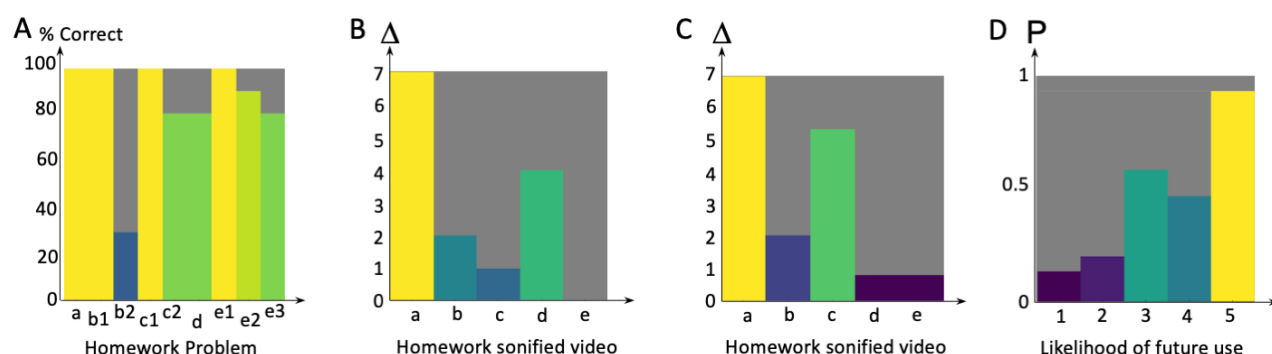


Figure 8. Assessment (A) Fraction of correct answers to questions in the sample homework assignment. Only the numerical calculation problem ‘b’ was an outlier; the sonification problems were solved correctly in 80%+ of cases. (B & C) Clarity of the videos as rated by science students and music students, respectively. The videos are: a Coin-toss, b Coin-toss unknown, c Hairpin high & low temperatures, d Hairpin unknown temperature, and e WW Lattice R_g . Colors range from purple (low value) to yellow (high value). (D) Correlation of prior exposure to sonification ($P=0$ to 1) vs. likelihood of future use (1=not, 5=definitely) among MUS 208 students.

Assessment of the course materials We presented the lecture and homework assignment to interested participants in the remote learning course PHYS 498 at the University of Illinois at Urbana-Champaign (38% undergraduates, 62% graduates), as well as to music majors in the course MUS 208. PHYS 498 was taught partly asynchronously, with students reading literature, and ~50% of class time spent observing and discussing sonification videos. Post-class, we performed an assessment of the material via a survey questionnaire with qualitative and quantitative content (SI section 5). Students in PHYS 498 were exposed to the two lectures (SI section , separate SI files) and homework (SI section 4) including the example sonification videos (SI section 1). Students in MUS 208 received a single qualitative lecture and were exposed to the sonification videos before their questionnaire (SI section 5).

As expected, simple sonification questions about the coin toss (a) were mostly answered correctly (Figure 8A), whereas the one question that involved calculation (b), even though it was also about the coin toss, proved the most difficult. Even the most difficult sonification question (e), asking for identification of traps in WW domain, was answered correctly at least 75% of the time. This trend also matches subjective assessment of the clarity of the videos (Figure 8B) based on questions 8 and 9 in the PHYS 498 survey. The following week we presented the same sonifications to music majors. We presented a simplified version of the lecture, aimed toward non-specialists. Although most of them knew very little about protein folding, their assessment of sonification clarity (Figure 8C) largely tracks with the scientists'; we did not have them work on the computational problem.

While relatively few science students had heard of sonification before (27%), enough music students had prior familiarity with the term that we could correlate 'likelihood of using sonification' vs. 'having heard of sonification before'. Fig. 8D shows a rather strong correlation, supporting the idea that increasing familiarity with sonification will make it more likely that this modality will be accepted by students.

The overall feedback in PHYS 498 was positive, with 100% respondents rating 'difficulty' 'just right', and 82% of respondents rating 'lecture speed' 'just right', the remainder rating it 'too fast,' indicating that the students were not overwhelmed by seeing and discussing sonifications in lecture, even though few had prior familiarity with that format. Question 7 on the survey was answered correctly by 100% of survey participants: this question tested whether the key message of the sonified lattice model of folding had come across. On a scale of 1 (very likely) to 5 (very unlikely) in question 10 'use sonification yourself,' the average response was 2.2 ± 1.0 . This indicates a generally favorable attitude towards sonification after the class, even though the majority of students (73%) had not heard of the concept before.

The qualitative feedback was positive, with listeners generally noting that watching the lattice movies and hearing various observables **O** of the protein sonified gave them a much more dynamic picture of protein folding, as opposed to a reaction that simply jumps directly from reactant to product in a single step. Comments included *'I don't know how I can incorporate sonification in my research but I'll definitely learn more about it,'* and *'lectures are very intuitive and easy to understand even if the subjects are complicated.'*

Discussion

In the real world, human beings acquire information via combinations of multiple sensory input channels. Even in machine learning, performance on information retrieval and classification tasks is more accurate and robust when two sensory modalities are combined.³⁹ In this project, we tried to engage our students' multi-modal perceptual abilities by using both visualization and sonification of a lattice model to help them understand the dynamics of protein folding.

Sonification can reinforce visualization. For example, high/low-pitched sound conveys high/low energy levels of a conformation (e.g. Figures 6 and 7) as intuitively as its visual location along the energy axis, while increasing accessibility. While it is known that adding speech audio to written text to increase redundancy can hinder learning,⁴⁰ when non-speech audio and imagery channels are redundant (and not conflicting), memory for information increases with increasing redundancy.⁴¹

Perhaps more importantly, sonification can complement visualization, that is, it can present aspects of the data that are difficult to visualize. In agreement with earlier experiments,⁴² we also saw evidence that data-driven sound tracks can increase the bandwidth of scientific visualization, and that sound and animation lend themselves particularly well to the presentation of time-dependent phenomena and models. When the image and sound channels are complementary, the sound is not just reinforcing the image; it is crucial to conveying the desired information. The authors have found that with minimal practice, they could watch protein visualizations while listening to a sonification to monitor changes in a reaction coordinate that was not explicitly present in the visualization, such as R_g (size) of the conformation. Indeed, we found it easier to identify compact states based on sound than it was to identify them in the visual oscilloscope trace in Figure 7. Also, use of sonification allowed us to identify compact states while running the simulation at a faster pace, making it more engaging for the students (who were under time pressure to finish their homework quickly). This phenomenon can enable students to process other variables that are difficult to visualize quickly in real-time, such as protein size (often distorted by the orientation of the protein in a simulation on a 2D computer screen) or solvation (where the huge number of water molecules becomes impossible to count or visually assess).

Scientists as well as musicians overall found sonification videos easy to follow, although musicians rated the videos easier (greater Δ in Figures 8B and 8C) than the scientists. A reasonable (but untested) hypothesis based on these results is that sonification builds listener intuition, and that the combination of visualization, sonification and calculation is a good way for students to internalize what is taught by appealing to multiple senses. It remains to be seen whether perception of sonification problems as easier also translates into better retention of the most important concepts.

Outlook

With the ongoing shift to electronic presentation of educational materials, there are now fewer barriers to including information-bearing sound to accompany other modalities (graphics, animation, and interactive problem-solving

discussions) in science classes. By increasing the sensory channels of educational materials to include data sonification (non-speech audio), educators can increase the bandwidth of these materials and engage the multiple learning styles of students.

Several future improvements could make these materials even more useful. For instance, adding interactive controls to the lattice models could allow students to formulate hypotheses (“the average radius of gyration of a protein increases with temperature”) and perform computer-experiments to test these hypotheses in an interactive way. Thus, the use of the sonified lattice models could expand to virtual labs. Sonification could also be applied to more realistic models, such as solvation of a protein visualized by an all-atom molecular dynamics simulation, where plotting the water molecules can obscure the protein, and solvation can be difficult to judge visually.

Data-driven sound has been applied as an educational tool in the past to understand structure¹⁹ and spectra,¹⁸ and here we see that dynamics, or other data that are easily represented by time series, are also excellent candidates for sonification.

Our initial foray into using data-driven sound and animation as an adjunct to lectures, discussion sections and homework for undergraduate and graduate students has been encouraging. The listeners found that sonification was not only engaging but was a useful tool in problem-solving; most importantly, even specialist co-authors in the protein folding field of research found that it helped increase their intuition for how proteins fold and misfold over time. This encourages us to continue to explore other opportunities for enhancing our lectures and homework with data-driven sound and animations, as well as future assessment with control groups.

Supporting Information

1. **Videos: lattice model animations with data-driven sound**
2. **Method for implementing the lattice model in software**
3. **Step-by-step sound-mapping examples**
4. **Example homework and solutions**
5. **Survey instruments used in the PHY 498 and MUS 208 courses to assess student response**
6. **Sample lecture slides with comments (separate Power Point and PDF files, *SI6_SampleLectures.pptx* and *SI6_SampleLectures.pdf*)**

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Acknowledgements

This work was supported by the James R. Eiszner Chair in Chemistry and NSF grant MCB 1803786 (M.G.) and Symbolic Sound Corporation (C.S. and K.H.). We also would like to thank Franz Danksagmüller for many enlightening discussions during this project. The assessment survey was reviewed by the Illinois IRB under protocol # 22360.

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