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Kinematic Design of Functional Nanoscale Mechanisms From Molecular Primitives

Natural nanomechanisms such as capillaries, neurotransmitters, and ion channels play a vital role in the living systems. But the design principles developed by nature through evolution are not well understood and, hence, not applicable to engineered nanomachines. Thus, the design of nanoscale mechanisms with prescribed functions remains a challenge. Here, we present a systematic approach based on established kinematics techniques to designing, analyzing, and controlling manufacturable nanomachines with prescribed mobility and function built from a finite but extendable number of available "molecular primitives." Our framework allows the systematic exploration of the design space of irreducibly simple nanomachines, built with prescribed motion specification by combining available nanocomponents into systems having constrained, and consequently controllable motions. We show that the proposed framework has allowed us to discover and verify a molecule in the form of a seven link, seven revolute (7R) closed-loop spatial linkage with mobility (degree-of-freedom (DOF)) of one. Furthermore, our experiments exhibit the type and range of motion predicted by our simulations. Enhancing such a structure into functional nanomechanisms by exploiting and controlling their motions individually or as part of an ensemble could galvanize development of the multitude of engineering, scientific, medical, and consumer applications that can benefit from engineered nanomachines. [DOI: 10.1115/1.4051472]

Introduction

Having the right set of nanoscale building blocks is perhaps the most critical factor in designing nanomechanisms. In nature, natural building blocks occur in various chemical structures to generate more complex utile molecules. One form of natural building blocks is amino acids, which are organic compounds that combine to form proteins and can play central roles as intermediates in metabolism. Natural translation processes use a set of 20 amino acids in a variety of combinations to create polypeptides and future proteins [1,2], However, there are numerous combinations, and by extension assemblies, of these building components that have never been tried by nature. Therefore, two key motivating questions arise from this understanding. The first, can one use different building blocks to design, build, and control artificial nanomachines that can manipulate matter at nanoscale and function as the "nanoworkers" of the future? If yes, what are the simplest functional nanomechanisms that can be designed systematically, controlled effectively, and fabricated with standard chemical processes?

Fortunately, important insights from across multiple disciplines suggest a practical approach to addressing some of these questions. Molecular biology teaches us that the precise sequence in which amino acids are linked together is often sufficient to determine the three-dimensional conformation(s) the resulting polypeptide folds into or unfolds from. Physicochemcial properties, function, and molecular interactions are all determined by a protein's conformation and therefore predicated by the sequence of amino acids in the polypeptide chain. In addition, a protein molecule's folding into one or more stable conformations and

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transition (motion) between these conformations are determined by the environment in which the protein exists. Therefore, we now have a relationship between environment, molecular design, and function to be explored.

We therefore hypothesize that practical, effective, and controllable nanomechanisms can be systematically developed by exploring the design space of the one degree-of-freedom (1DOF) nanomachines, built from a large but finite number of primitive molecular components. In this paper, we propose the first systematic approach for nanoscale mechanism design. The examples we provide here serve to prove the capabilities of the proposed method and provide insight for improving the current method toward developing more effective molecular design practices.

In what follows, we observe several common assumptions made in ab initio synthesis of protein structures: each primitive in the design space is considered to be rigid; rigid collections of bonded atoms connect through covalent bonds; covalent bonds can exist as revolute joints that allow for rotation of atoms around a spatial axis. Furthermore, the synthesized molecules must each self-assemble into a closed loop that remains closed over its intrinsic range of motion.

Design Space

Our group has been working on the kinematic analysis of biological structures during the last 20 years [3–9]. We presented in an earlier study [10] a novel nanolinkage synthesis method aimed at supporting the synthesis of nanoscale mechanisms that self-assemble from a "link soup" of molecular primitives, with the resulting mechanisms having a prescribed number of DOF. The proposed method, illustrated in Fig. 1, assumes the input as primitives that are either available in nature or can be synthesized in a laboratory setting. It can enumerate all valid topologies that satisfy certain mobility conditions in terms of the desired DOF of the final mechanism. Additionally, it generates all possible linkage arrangements by assigning links from the design space to each topology.

To a large extent, the richness of the output of the nanomechanism synthesis method depends on the composition of the input primitives, as well as on the proper selection of the synthesis criteria. Natural and synthesized polymer molecules are reliable resources, both for extracting building blocks and for defining the synthesis rules. The formation of protein chains and disulfide crosslinks, for instance, suggests a set of building blocks with one, two, or three connections (joints), as illustrated in Fig. 2.

One-Degree-of-Freedom Nanomechanisms

In this section, we report our initial efforts, which led to the discovery of two structures theoretically belonging to the class of simplest nontrivial spatial 1DOF nanomachines that can be fabricated using the kinematic design methodology. This design framework can be used to search systematically the design space for all 1DOF machines that can be assembled from the molecular primitives. Figure 1 demonstrates the synthesis hierarchy we developed and used to search the design space for molecules having prescribed mobility for different topologies and assortments of links.

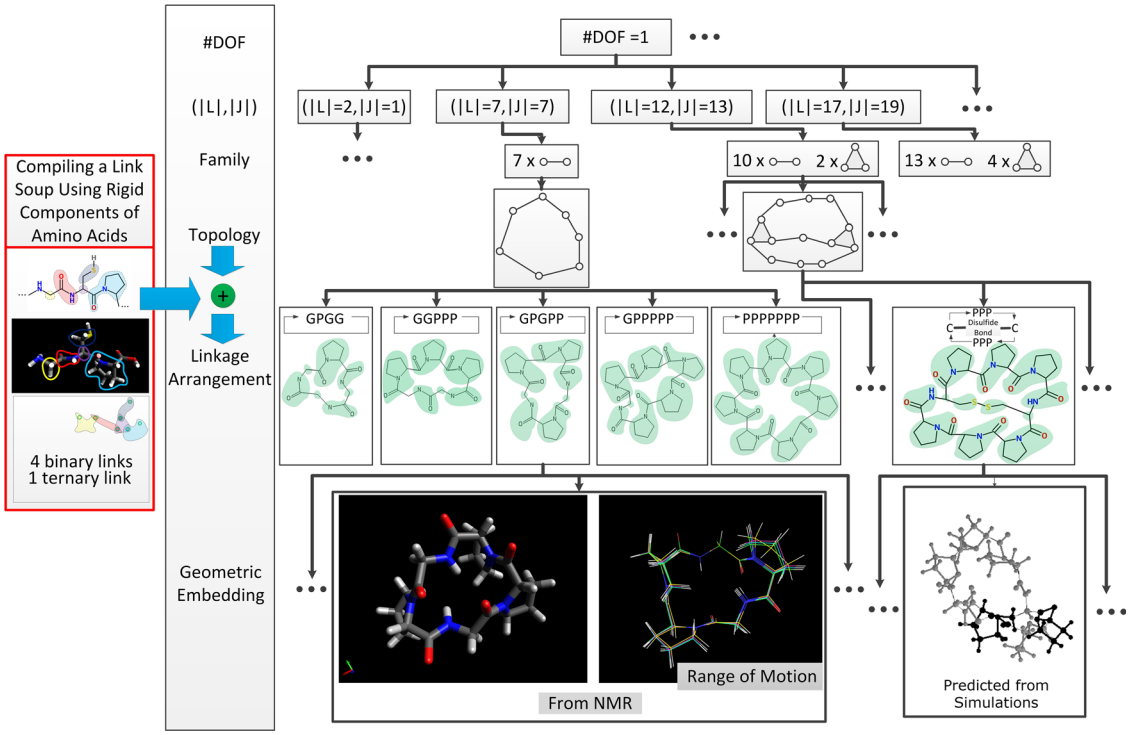

Fig. 1 The synthesis hierarchy developed and implemented to search the design space of nanomachines having prescribed mobility (number of DOFs). Given the prescribed DOF, first, all possible link families are enumerated. Next, for each link family, all the possible topologies are generated. Here, two topologies of seven-bar and twelve-bar are shown. Geometric links from the link soups are then assigned to the vertices of the generated topologies, in different combinations, to produce all the possible different linkage arrangements. The figure shows all of the five linkage arrangements of the seven-bar topology and one of the linkage arrangements of the twelve-bar typology that can be obtained using the link soup shown on the left. Note that the rigid links of these linkage arrangements are highlighted in gray. Finally, geometric embeddings, which fulfill the kinematic loop closure criteria, are obtained for the generated linkage arrangements, and the range of motion is calculated for each geometric solution. The bottom section shows the experimentally verified geometry of the seven-bar typology (consisting of five peptides) and the simulated geometry of the twelve-bar typology. A more elaborate description of the synthesis process can be found at Ref. [10].

Among all feasible spatial 1DOF mechanisms, the simplest nontrivial topology is a closed-loop, seven-bar linkage [11].

Our design space comprises five lumped groups of atoms that appear repeatedly in the structure of different protein molecules and are observed to remain mostly rigid during protein conformational changes. This set of five links basically covers all the binary (with two connection points) and ternary (with three connection points) links that are naturally available and can be used to form closed-loop linkages without further modifications. Specifically,

- Link 1 comprises one alpha carbon atom, with two hydrogen atoms attached to it and comes from glycine. The link can either be connected to two links of type 2 or to one link of type 2 and one link of type 5.
- Link 2 is a planar link—that is, a peptide plane comprising four atoms: C, O, N, and H. The link can make two connections in several different forms namely, two links of type 1, two links of type 3, two links of type 1 and 3, two links of type 1 and 5, and Finally, two links of type 3 and 5.
- Link 3 comprises one alpha carbon atom, with one hydrogen atom attached to it and comes from cysteine. Link 3 acts similarly to link 1, except it forms an extra connection with a side chain link namely, link 4.
- Link 4 is a side chain link comprising a beta carbon with two hydrogens attached to it and a sulfur atom that enables formation of disulfide bond in the molecule. The link connects from one end to a type 3 link and from the other to another type 4 link.
- Link 5 comprises a ring structure and a carboxyl group, which manifests the extra rigidity of the proline amino acid and is taken advantage of to assemble linkage loops with odd numbers of links. Link 5 connects to two other links. One end connects to a type 2 link and the other end connects to a type 1 or type 3 link.

Selection of these five links has been in line with our objective of designing irreducibly simple machines. That is, among all the amino acids that could contribute two links and two backbone hinges to the designed mechanisms, we have chosen only glycine, as this amino acid does not have a conformable side chain. Amino acids with conformable side chains add flexibility to the mechanism irrelevant to the constrained motion produced from the remainder of the structure. The additional structural complexity also makes its performance analysis more challenging. In fact, if such a feature is incorporated into a molecular machine, the machine can no longer be considered a one-degree-of-freedom mechanism. This, in turn, makes motion control more difficult. Although amino acids other than glycine have immobile side chains and can add links to the side design space, we chose glycine for preliminary stages of design to minimize steric clash among these small-size molecules and to observe the largest possible range of motion. Furthermore, we used proline to handle our odd number link, simplest topology consisting of seven links and seven hinges. Given that ω is always locked around 0 deg or 180 deg, each amino acid contributes two hinges to a mechanism. However, proline has a ring structure formed between its side and

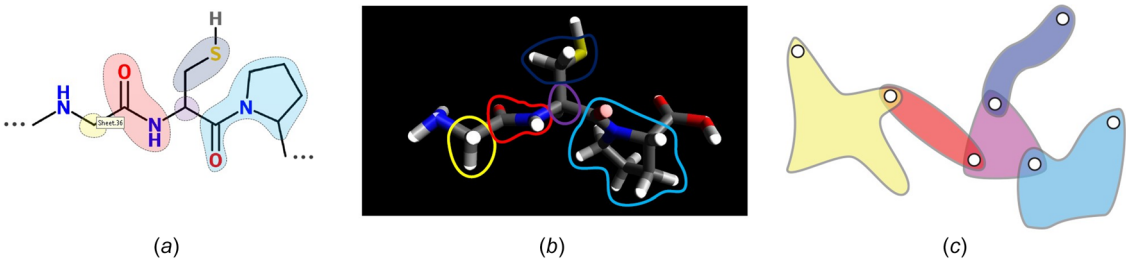

Fig. 2 Exemplifying the identification of potential molecular primitives comprising the link soup. The structural formula, skeletal model, and schematic representation of the five links that comprise the link soup used for synthesizing the reported molecular machines in this paper are reflected by (*a*), (*b*), and (*c*), respectively. Note that these rigid links are extracted from a larger structure.

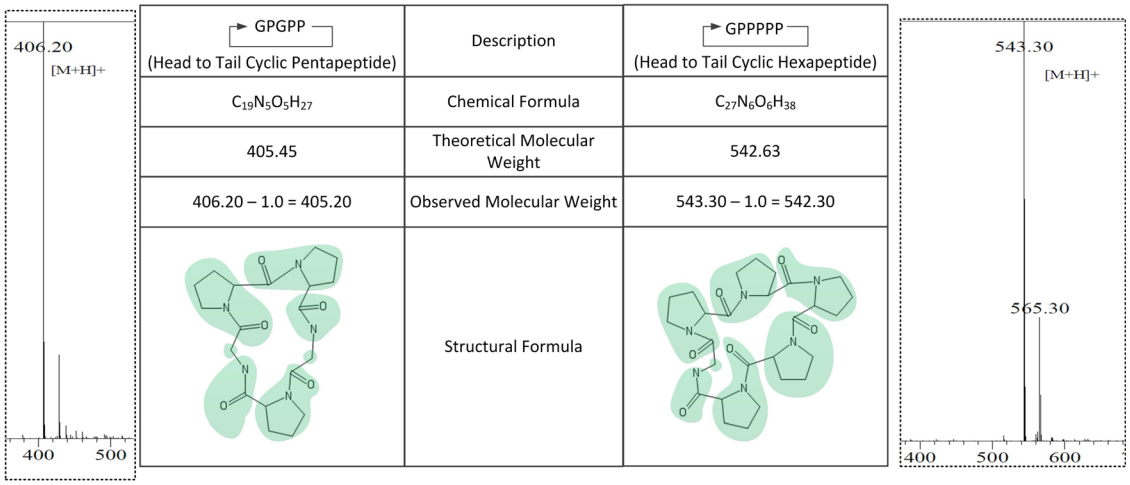

Fig. 3 A description of the structure, the structural formula, the chemical formula, and the theoretical molecular weight, as well as the mass spectrometry measurements for the two chemically synthesized molecules, are shown. The measured and the theoretical molecular weights are in close agreement for both molecules, which supports the chemical formation of these cyclic molecules.

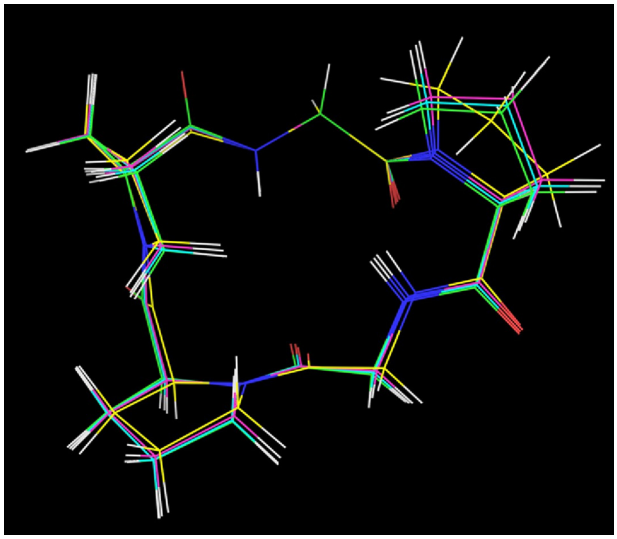

Fig. 4 Alignment of some of the 20 candidate structures. Structural differences can be clearly seen among these candidates.

main chain locking a generally mobile amino acid dihedral angle. As a result, proline contributes only one hinge and one link component to the mechanism. This makes proline suitable to fulfill the odd number link and hinge requirement.

For the design space illustrated in Fig. 2 and detailed above, there are five linkage arrangements, but only four of these have a geometric solution—that is, geometric embedding as a closed kinematic chain. Two of these linkages, cyclic GPGPP and cyclic GPPPPP, were chemically synthesized by an established biotech company specializing in fundamental life sciences research and early-phase drug discovery services. We performed experimental investigations to see whether the synthesized chains exhibit the conformational changes predicted by the 1DOF model or not.

Experimental Observations

Figure 3 provides a description of the structure, the structural formula, the chemical formula, and the theoretical molecular weight, as well as the mass spectrometry measurements carried out by the biotech company that synthesized the two chemical compounds mentioned previously.

In order to investigate the conformational changes predicted by the one degree-of-freedom model, we performed nuclear magnetic resonance (NMR) spectroscopy of one of the peptides, GPGPP [12]. The compound was dissolved in dimethylsulfoxide solvent and NMR experiments produced (using ARIA software package [13]) a list of up to 20 candidate structures from with the lowest energy levels. Some of these structures are overlapped in Fig. 4 with small structural differences evident among the candidates.

Each of these penta-peptides has 15 dihedral angles (three for each of the five amino acids). Our 1DOF linkage model of cyclic GPGPP predicts that among these 15 angles, eight are fixed and only the remaining seven are variable. The left-hand section of Fig. 5 illustrates the cyclic chain with links and hinges labeled and detailed as locked or variable hinges (as shown by the symbols). The right-hand section shows the measured values of these 15 angles for each of the 20 candidate structures and displays a strong correlation between the model prediction and the measured variation of the 15 angles even in the absence of additional physical stimuli. More specifically, the large torsion angle variations (extracted from experimental data) were associated with the joints assumed to be variable and small torsion angle variations were associated with the joints assumed to be locked.

By aligning the 20 candidate structures output by ARIA from the NMR data, we see structural differences, illustrated in Fig. 4, which can be attributed to either (1) expected angle variations in

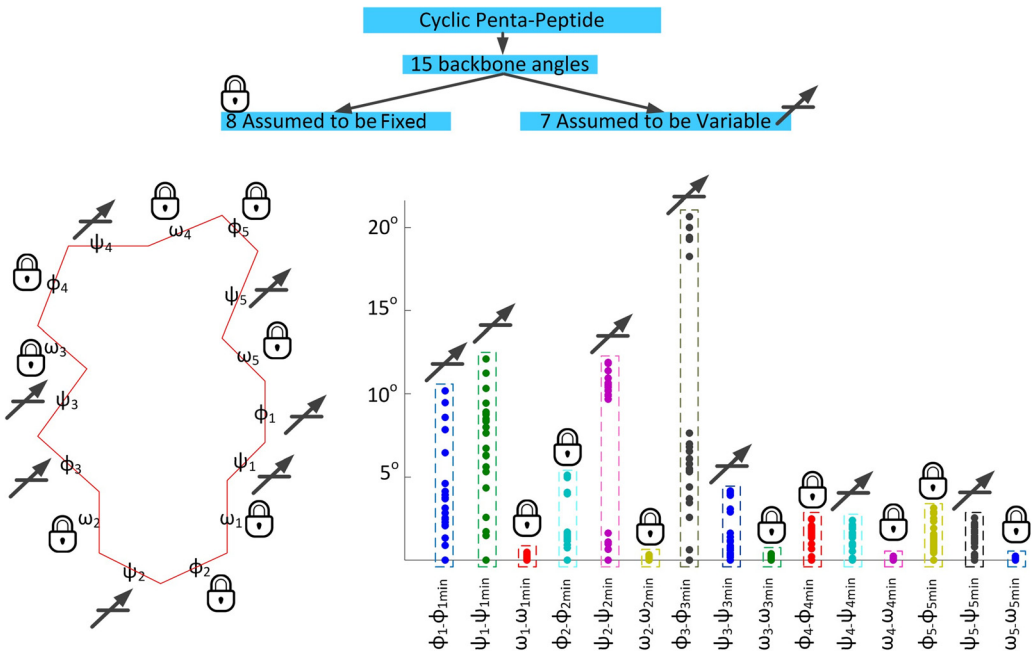

Fig. 5 Left: A cyclic chain of 15 link components connected via 15 hinges, among which eight are locked and seven are variable (as shown by the symbols). Right: Measured values of these 15 angles for each of the 20 candidate structures. A strong correlation between the model prediction and measured variation of the 15 angles is observed, even in the absence of additional physical stimuli. Large torsion angle variations (extracted from experimental data) are associated with the joints that are predicted to be variable and the small torsion angle variations with the joints that are predicted to be locked.

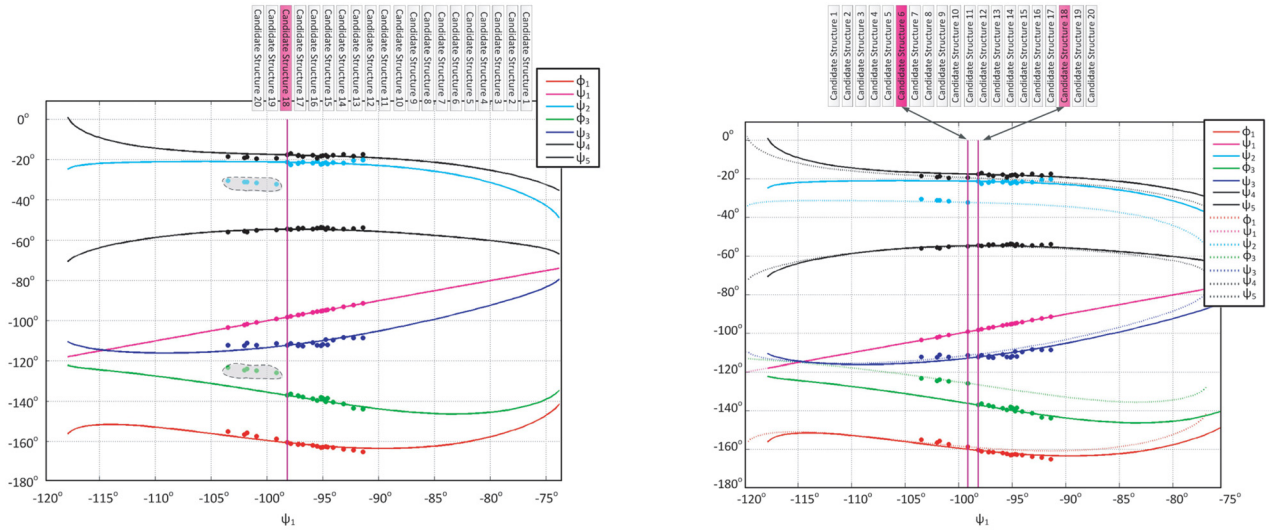

Fig. 6 Changes in variable dihedral angles as functions of change in control variable (predicted versus actual). Through simulation, seven curves were produced that reflected the predicted range of conformational changes across which the structural integrity of ring molecule was preserved and only the value of seven dihedral angles associated with the variable joints was altered. Dots, on the other hand, correspond to the dihedral angle values obtained from NMR data. Correlation between the predicted and measured variations is observed, supporting the hypothesis of one degree-of-freedom motion for the synthesized machine. The five off-the-curve dots correspond to a structural difference in one of the prolines comprising the molecular candidates, dividing the 20 into groups of 5 and 15. Slightly different models must be used for the two groups. All 20 candidate structures relative to the prediction of a single model are shown in (*a*), while (*b*) shows the 20 candidate structures versus two different models matching the distinct structures of the proline ring.

the seven variable angles, or (2) changes in either the other eight "fixed" angles or in the distances between atoms that are assumed to be constant.

To investigate further the dominant factor in the observed conformational differences illustrated in Fig. 4, we selected one of the 20 candidate structures at random (here structure 18) and investigated the range of motion with long-established kinematic simulations. In our simulation, we obtained the range of conformational changes across which the structural integrity of the ring molecule was preserved and only the value of seven dihedral angles associated with the variable joints was altered. Since the designed mechanism, by definition, is a one degree-of-freedom mechanism, its motion could be described using only one variable—that is, with the value of one of the joint angles set, only one value would be possible for each of the other six angles. We then randomly selected one of the seven angles considered to be variable as a control angle (ψ_1), and we plotted in Fig. 6 the variation of the other six variable angles against the control angle. Observe from Fig. 5 that ψ_1 has a large range of variation compared to the other six variable angles. The seven curves in Fig. 6 (one of which, corresponding to ψ_1 versus ψ_1, will clearly be a line segment) represent the theoretical predictions of these variations based on the kinematic model, while the dots correspond to the dihedral angle values obtained from NMR data for the seven variable joints of the 20 candidate structures.

The key observation is the strong correlation between the predicted and measured variations (Fig. 6), which further supports the conjecture that the dominant factor for the structural differences displayed in Fig. 4 are the variations in the seven variable angles predicted by our model.

Designing Larger Molecules

Although we report in this paper the chemical synthesis of seven-bar molecular machines, the size of the molecules that can be designed using this approach is unlimited. An example of the results from our continuing explorations is the in silico design of

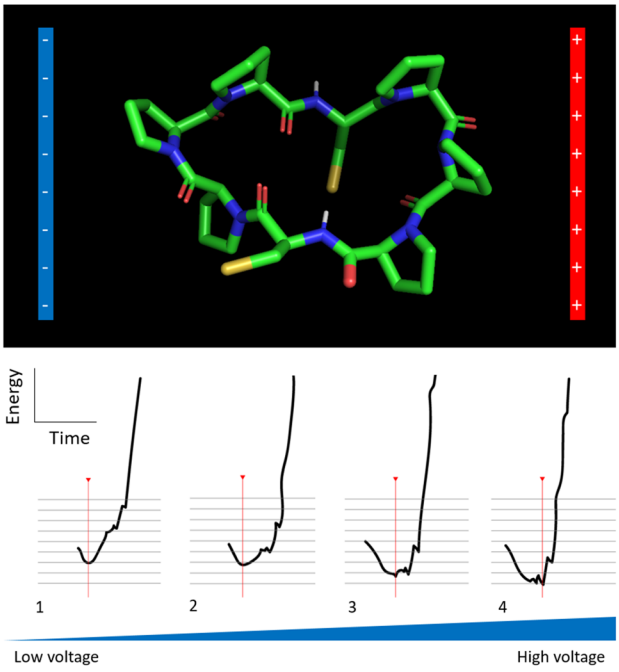

Fig. 7 An electric field is applied via two oppositely charged (virtual) plates, where red indicates positive and blue negative. The charges of the plates have been gradually altered to make changes to the electric field. The effect of such a change on the stable conformation of the CPPPCPPP cyclic molecule is shown in (1) through (4). The curve in each frame represents the energy profile associated with a conformation range of the molecule under a certain electric field condition, and the red arrow indicates the location of minimum energy and, thus, the favorable conformation. Changes in the electric field cause changes in the energy profile and, consequently, in the location of the global minimum. The changes in the conformation are subtle in this case.

the CPPPCPPP cyclic molecule, which acts as a twelve-bar mechanism with two closed loops. Figure 7 shows the simulated conformational change of the CPPPCPPP cyclic molecule under the effect of a variable electric field applied by two oppositely charged plates. The curve in each frame of the figure represents the energy profile associated with a conformation range of the molecule under a certain electric field condition, and the red arrow indicates the location of minimum energy and, thus, the favorable conformation. Changes in the electric field cause changes in the energy profile and, consequently, in the location of the global minimum. The changes in the conformation are subtle in this case.

Conclusion

We have presented an overview of a novel method capable of synthesizing nanomachines from existing or manufacturable nanoprimitives and discussed our linkage design process. Two theoretical structures were designed among the simplest 1DOF seven-bar linkage nanomachines that can be fabricated and NMR experiments were carried out to validate our method. Results of the preliminary tests carried out on one of these two structures showed good comparison between our predictions and the exhibited type and range of motion of the molecule. These results validate our predictions of these 1DOF systems as potential functionalized nanomachines. Our results here only scratch the surface of possible linkage designs and paves way for more complex mechanism design, functionality, and application.

This work explores methods to control the motion of 1DOF structures. If the control of such structures is confirmed through physical experiments, this discovery alone could represent a major breakthrough in biotechnology in general, and in the design of nanomachines in particular.

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