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Alpha-helical protein domains unify strength and robustness through hierarchical nanostructures

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Abstract
Hierarchical nanostructures, ranging through atomistic, molecular and macroscopic scales, represent universal features of biological protein materials. Here we show for the case of alpha-helical (AH) protein domains that this use of molecular hierarchies within the structural arrangement leads to an extended physical dimension in the material design space that resolves the conflict between disparate material properties such as strength and robustness, a limitation faced by many synthetic materials. An optimal combination of redundancies at different hierarchical levels enables superior mechanical performance without additional material use. Our analysis is facilitated by the application of a Hierarchical Bell model (HBM), which explicitly considers the hierarchical architecture of H-bonds within the protein structure, providing a structure–property relationship of strength properties of AH protein nanostructures. The HBM is validated by large-scale molecular dynamics simulations of several model protein structures. Our findings may enable the development of self-assembled *de novo* bioinspired nanomaterials based on peptide and protein building blocks, and could help in elucidating the mechanistic role of AHs in cell signaling and mechanotransduction.

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(Some figures in this article are in colour only in the electronic version)

1. Introduction

The origin of how naturally occurring biological protein materials (e.g. spider silk, bone, tendon, skin) are capable of unifying disparate mechanical properties such as strength (ability to sustain large stresses without fracture) and robustness (ability to undergo deformation without fracture, despite the presence of defects, equivalent to toughness), as well as other physical properties such as self-healing ability, adaptability, changeability, and evolvability into multi-functional materials is of significant interest. However, the molecular basis of these properties remains largely unknown [1–3]. Many synthetic materials are not capable of unifying strength and robustness, being either extremely strong with little ductility (e.g. ceramics, glass, silicon), or weak with extreme ductility (e.g. soft metals like copper) [4]. The combination of these disparate properties into synthetic materials remains an open challenge on the way towards the development of biomimetic structures and material designed from the nanoscale up.

The folded structure of proteins is stabilized by a variety of chemical driving forces including hydrophobic effects, H-bond formation as well as charge interactions [5]. Simultaneously, in a folded state a loss in configurational entropy appears due to the loss of degrees of freedom, as almost all rotomeric angles of the backbone and the side chains are restricted to one position. Thus the enthalpic effects of the folding process need to be higher than the entropic contributions at given conditions

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14. ABSTRACT
Hierarchical nanostructures, ranging through atomistic, molecular and macroscopic scales, represent universal features of biological protein materials. Here we show for the case of alpha-helical (AH) protein domains that this use of molecular hierarchies within the structural arrangement leads to an extended physical dimension in the material design space that resolves the conflict between disparate material properties such as strength and robustness, a limitation faced by many synthetic materials. An optimal combination of redundancies at different

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(e.g. temperature, pH, etc). In alpha-helical (AH) based protein structures (including individual AHs, alpha-helical coiled-coils (CCs), higher order filament assemblies, etc) H-bonds, ionic as well as hydrophobic interactions play an important role in creating and stabilizing the protein structure. Whereas AHs are stabilized primarily by H-bonds, the structure of CCs is related to additional ionic and hydrophobic interactions [6, 7].

An AH is generated when a single polypeptide chain twists around on itself, stabilized by H-bonds made between every fourth residue, linking the O backbone atom (hydrogen acceptor, providing free electrons) of peptide i to the N backbone atom (hydrogen donor) of peptide $i + 4$ in the polypeptide chain. Consequently, at each convolution, 3.5 H-bonds are found in a parallel arrangement that stabilize the helical configuration of AHs [8]. Due to the stabilizing role of H-bonds [5], rupture of these protein structures itself is mainly determined by breaking of these H-bonds. Once these H-bonds are broken, the protein has the degrees of freedom necessary for further protein unfolding. This is in particular the case when the breaking of H-bonds appears in a highly driven system, far away from equilibrium. In contrast, at very small deformation rates near the equilibrium the H-bond rupture needs significantly less force than the protein unfolding itself, which is determined by entropic effects of stretching the protein backbone (and restricting the degrees of freedom again) (see, e.g. the discussion recently reported in [9]). In this paper we focus on protein rupture mechanics in a highly driven system, and therefore consider H-bonded interactions as the main contributor to determine their strength properties.

Although it is recognized that H-bonds play a crucial role in defining the strength properties of fundamental protein constituents in biological protein materials [2, 7, 8, 10], the role of their characteristic nanostructured hierarchical arrangement as well as their influence on the larger-scale mechanical properties of protein filaments (that is, elasticity, fracture/rupture, energy dissipation, etc) remains largely unknown. However, the molecular and supermolecular, mesoscale behavior of basic protein constituents is elementary in order to progress towards an integrated understanding of the mechanical role proteins play in biological systems, for chemomechanical coupling, signaling cascades, and protein–protein interactions [3, 10–12], as well as for the use of protein building blocks and hierarchical structures in the design of new nanomaterials [13].

A promising path to build *de novo* macroscopic structures for technological applications from nanoscopic elementary buildings blocks is to arrange them systematically in a hierarchical order as observed in Nature. Thereby protein domains, macromolecules, nanowires or different types of nanotubes could serve as possible elementary, universal building blocks. However, engineering these structures and controlling their chemomechanical properties across multiple length-scales requires a fundamental understanding of the basic materials science concepts found in these materials. Theoretical efforts to elucidate these concepts are reported in this paper.

Here we focus on an analysis of simple alpha-helical (AH) protein domains as model systems, which are universally found nanostructural components of many biological protein materials. These protein domains play a crucial role in the signaling and deformation behavior of cytoskeletal protein networks in cells (e.g. intermediate filaments vimentin and lamin as well as actin [7, 8, 11]), and in determining the mechanical properties of hair, hoof, feather and many other important structural protein materials [8]. Nanostructured AH-based protein domains universally define the nanoscale architecture of these protein materials. Through the analysis reported here, we illustrate that the nanostructural arrangement of universal protein building blocks plays a crucial role in defining their material performance. Thereby, the occurrence of material hierarchies (that is, the arrangement of subunits to units, which themselves form larger-scale structures, etc) is a particularly important aspect. The analysis reported here is focused on AH-based protein filaments as a model system to illustrate fundamental material concepts that could be translated to the design of other nanostructures such as hierarchically arranged carbon nanotubes or nanowire bundles.

1.1. Hierarchical H-bond structures

In biological tissues, macroscopically applied stress is forwarded to microscopic hierarchical scales, where rupture of H-bonds mediates deformation, thereby controlling the response at the macroscopic protein filament level. Since rupture of individual H-bonds equals a chemical reaction (breaking of the backbone H-bond and building of H-bonds with surrounding water molecules governed by a difference in H-bond building energy, see figure 1(c)), an integrated chemomechanical approach is compulsory for the understanding of protein fracture mechanisms and the development of constitutive mathematical relations.

The lowest hierarchy of AHs (and other protein structures) is typically composed of arrangements of weak H-bond interactions, organizing amino acids in stable elementary building blocks such as AHs, which form hierarchical arrangements such as CCs, supercoils and filamentous structures [7, 8, 11, 12] (see figures 1(a) and (b) and 2(a), exemplified for AH-based structures as they appear in intermediate filaments [7]). The key to enable the development of an accurate bottom-up mechanistic understanding of the strength properties of such AH protein domains and assemblies thereof must therefore include an explicit description of nanopatterned H-bond arrangements, by adapting a system view of materials, in the spirit of a merger of nanostructure and materials. The concept of hierarchical arrangements, where each element consists of several subelements provides a broadly applicable, yet simple model to describe the geometry of AH protein structures [14]. The schematic representation depicted in figures 1(a) and (b) and 2(a) could in principle be used to represent other structural proteins such as beta-sheets, beta-helices or tropocollagen, since the difference between many protein structures is the geometrical arrangement of H-bonds.

H-bond rupture mechanisms can be seen as analogs to the nucleation of dislocations in ductile materials or the rupture of covalent bonds in brittle materials, representing

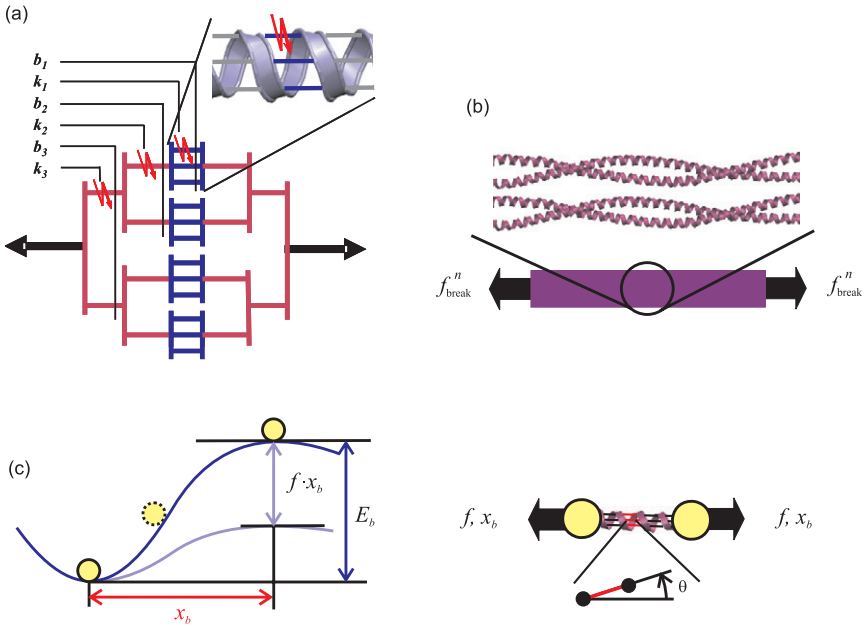

Figure 1. Illustration of structural hierarchies and their representation in the Hierarchical Bell model (subplot (a)), as well as representation of the corresponding physical system (subplot (b)). The inlay in the upper part of subplot (a) shows a single AH structure with ≈3 H-bonds per convolution. The Hierarchical Bell model enables one to predict the strength of different hierarchical bond arrangements as a function of the deformation speed. Subplot (b) shows the physical system that is represented in the hierarchical model in subplot (a). Subplot (c): statistical theory to predict the bond rupture mechanics via the bond energy landscape [15, 17]. The graph depicts the energy as a function of deformation along a deformation variable, along a particular pathway that leads to bond rupture. Here f is the applied force, and x_b is the displacement in the direction of the applied force, corresponding to the lateral displacement that is necessary to overcome the bond breaking distance of a H-bond. Given that x_b^* is the distance to break a single H-bond, the distance $x_b^* = x_b \cos\theta$ denotes the lateral displacement at bond breaking, with the angle θ as the angle between pulling direction and orientation of the H-bond inside the molecule. This fundamental view of single bond behavior is scaled up through several hierarchies in the Hierarchical Bell model.

fundamental unit deformation events [4, 15–19]. Notably, larger-scale, effective properties of these hierarchical H-bond structures can not be calculated by conventional mean-field averaging approaches, not only due to an insufficient number of subelements [14], but also since information may be forfeited that is crucial for the structure's behavior several scales up. Recent results provide strong evidence that the key to understand the mechanical response of AH-based protein structures is to consider the rupture dynamics of H-bonds at mesoscale [17, 18, 20–25].

1.2. Outline of this paper

The central question addressed in this paper is, how does a hierarchical AH protein structure respond to mechanical load and how does this response relate to the protein's structure? Further, how does the hierarchical arrangement control the multi-scale process of fracture and deformation in protein materials? How can AH protein domains overcome the competition between strength and robustness? In this paper we address this question by developing a simple theoretical model coupled with molecular dynamics (MD) simulations. We begin with the presentation of the theoretical model and validation simulations. We then continue with a discussion of several case studies, all focused on illustrating the use of hierarchies in achieving improved material properties that exceed those of the elements alone. We conclude the paper with a discussion of implications for bioinspired approaches in nanotechnology.

2. Theoretical and computational modeling

In this section we summarize theoretical and computational methods used for the analysis reported in this paper.

2.1. Hierarchical Bell model

The Bell theory [26] has been used successfully to describe the rupture mechanics of adhesion bonds, but has also been used to describe the rupture dynamics of H-bonds. The central element of Bell's theory is the off rate, which describes how often a bond dissociates per unit time (this concept is illustrated in figure 1(c)):

$$\chi = \omega_0 \exp\left(-\frac{(E_b - f x_b \cos(\theta))}{k_B T}\right). \quad (1)$$

In order to allow capturing the pulling speed dependence of the strength of H-bonds, here we link the off rate to the bond breaking speed, by multiplying the off rate (reciprocal of the bond breaking time) by the distance x_b, which needs to be overcome in order to break the bond: $v = \chi x_b$. Despite the usefulness of this approach, existing models derived on Bell's concept are only capable of treating conglomerates of bonds, as they only consider an effective energy barrier that corresponds to multiple individual bonds. Moreover, thus far all attempts have failed to predict the strength of hierarchical arrangements of H-bonds from fundamental principles, explicitly considering the

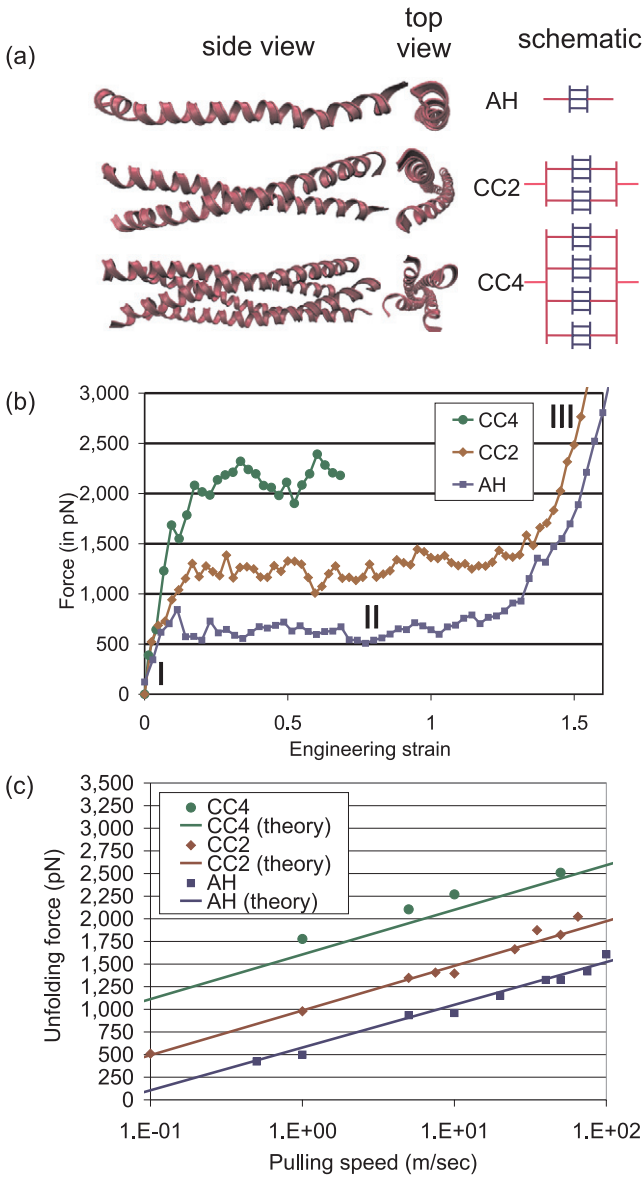

Figure 2. Validation of Hierarchical Bell model via direct atomistic simulation in explicit solvent. Subplot (a): overview of three model systems, including single AH, coiled-coil with two AHs (CC2), and a coiled-coil protein with four AHs (CC4). The schematic on the right shows the hierarchical representation of each physical system (following the approach shown in figure 1). Each blue line represents a single H-bond (in the atomistic simulations, all structures are embedded entirely in a water skin during deformation; however, water molecules are not shown here for clarity). Subplots (b) and (c): force-extension curves and unfolding force as a function of pulling speed. The beginning of the plateau regime (regime II), following the initial elastic stretching (regime I) in subplot (b) defines the unfolding force, the quantity plotted in subplot (c). The continuous lines in subplots (c) are the predictions from the Hierarchical Bell model, equation (2). All predictions have been made based on the same input parameters of H-bond energy and therefore, the differences are solely due to hierarchical effects. It is noted that the force level in the plateau regime is defined by the unfolding force, and therefore, the dissipated energy is proportional to this force value.

geometrical arrangements of the bonds. Here, we introduce the development of the Hierarchical Bell model that overcomes these limitations. A detailed derivation of the mathematical model is included in the supplementary material (available at stacks.iop.org/Nano/20/075103).

As discussed above, the 'original' Bell model (equation (1)) does not distinguish between a single chemical bond and protein architectures that include several fundamental bonds. For instance, whether a single H-bond ruptures or if several H-bonds rupture simultaneously is captured in an effective value of E_b; however, this change in mechanism is not explicitly noted in the model given in equation (1) and subsequent expressions. In order to estimate the strength and the energy landscape of a protein without performing any simulations or experiments and thus to make the model predictive, the model is extended to explicitly consider the structural hierarchies of the protein structure with the only input parameters being the energy of a H-bond and the rupture distance. The H-bond represents a hierarchical structure, reaching from individual H-bonds at the lowest, atomistic level to a collection of H-bonds at the next higher, molecular protein scale.

In this model, the system breaking force f_{hn} and the energy barrier E_b^{hn} of a system consisting of n hierarchies (the mathematical symbols are explained in table 1) is given by

$$f_{hn} = \frac{k_B T}{x_b \cos\theta}\left[\ln\left(\frac{v}{x_b \omega_0}\right) + \ln\left(\frac{b_n}{k_n}\right) + \sum_{i=2}^{n}\left(\frac{b_i}{k_i}\right) \times \ln\left(\frac{b_{i-1}}{k_{i-1}}\right)\right] + \frac{\prod_{i=1}^{n} k_i E_b^0}{x_b \cos\theta} = f_v + \sum_{i=1}^{n} f_{hi} + f_{h0} \quad (2)$$

and

$$E_b^{hn} = \prod_{i=1}^{n} k_i E_b^0 + k_B T \times \left[\sum_{i=2}^{n}\left(\frac{b_i}{k_i}\right)\ln\left(\frac{b_{i-1}}{k_{i-1}}\right) + \ln\left(\frac{b_n}{k_n}\right)\right]. \quad (3)$$

These equations now enable us to predict the unfolding force at any pulling speed, once the structural geometry and the energy landscape of a single H-bond is known.

We note that this model only considers H-bonds as structural elements in the definition of AH-based protein structures, representing a limitation of this model. Thereby it does not consider hydrophobic effects and other chemical interactions between molecules, which may play a role in defining the strength properties. This is a limitation of the model; however, there is currently no method to explicitly include these effects in the model and thus this task is left to future work. We expect, however, that the effect of intermolecular adhesion is limited with respect to the prediction of the initial strength values. This is based on our observation that in AH-based coiled-coil protein structures, failure initiates first in the individual AHs (thus defining its strength properties) and is later followed by uncoiling of the overall coiled-coil structure [27]. This suggests that the approach taken here by focusing solely on H-bonds is a good approximation for the strength properties of AH-based protein domains.

2.2. Molecular dynamics simulation procedure and comparison with HBM

Atomistic MD with explicit solvent is used to simulate the atomistic mechanisms that control the deformation and

Table 1. Overview of important variables and symbols used in the manuscript.

Mathematical symbol	Unit	Description
E_b	kcal mol^{-1}	Height of the energy barrier at the transition state
E_b^0	kcal mol^{-1}	Energy barrier of a single H-bond
x_b	Å	Location of the energy barrier
f	pN	Applied force at molecule
θ	° (degrees)	Angle between pulling direction and reaction coordinate of breaking H-bond
k_B	J K^{-1}	Boltzmann constant
T	K	Absolute temperature
ω_0	s^{-1}	Natural bond vibration frequency (1×10^{13} s^{-1})
χ	s^{-1}	Off rate: bond dissociation per second
v	m s^{-1}	Macroscopically: pulling speed, microscopically bond breaking speed
v_0	m s^{-1}	Natural bond breaking speed, when no load is applied
ε	%	Engineering/molecular strain: displacement of the pulled atom normalized by the length of the molecule
b_i	—	Number of parallel elements at hierarchy level i
k_i	—	Number of elements at hierarchy level i that rupture simultaneously
f_v	pN	Force contribution as a consequence of pulling speed
f_{hi}	pN	Force contribution as a consequence of hierarchy level i
f_{h0}	pN	Force contribution as a consequence of the basic hierarchy (individual H-bond)
r	%	Robustness, values ranging from 0% to 100%

rupture of bonds at nanoscale, and are used here to validate the predictions put forward by the Hierarchical Bell model developed above.

MD calculations are carried out using NAMD [28] with a CHARMM [29] force field with explicit water. Each molecular assembly is embedded in a skin of explicit TIP3 water at pH 7. The single AH and the two-stranded coiled-coils (CC2) are taken from the 2B segment of vimentin IF (PDB ID 1GK6, residues 355–406). The four-stranded CC (CC4) is taken from the early endosomal SNARE complex (PDB ID 2NPS, residues A: 60–112, B: 196–248, C: 138–190, D: 180–232). Energy minimization is carried out for 100 000 steps, which allows for a favorable conformation to be achieved. This is followed by an equilibration procedure, during which the molecular assembly is heated up to 300 K with a rate of 25 K every 25 steps. An NVT ensemble is employed to hold the temperature constant at the final temperature of 300 K. Using a time step of 1 fs, each molecular is subjected to equilibration for 1 ns. To simulate forced rupture of H-bonds between the strands, we use the steered molecular dynamics procedure (SMD) with a constant velocity pulling scheme. A spring constant of 10 kcal mol^{-1} Å^{-2} and a pulling rate ranging form 1 to 100 m s^{-1} are used for this purpose. In interatomic potentials (such as the CHARMM force field as used for the studies here), H-bonds are modeled based on empirical energy expressions. Such expressions are fitted to first principles or experimental measurements of H-bond geometries and energies. In CHARMM, Coulomb and Lennard-Jones terms between the donor and acceptor accurately represent the H-bond interaction, making an explicit H-bond interaction formulation unnecessary [29]. Thereby, H-bond models in such force fields describe the ability of donor–acceptor pairs of H-bond formation to interact and form intermolecular or intramolecular bonding. H-bonds in CHARMM force fields are 'reactive', that is, they can break and reform with any donor–acceptor pair in a simulation model. This resembles the conventional way of thinking of H-bonds as weak interactions formed within and between protein domains.

The analysis with the Hierarchical Bell model shown in figure 2(c) is carried out using $k_1 = 1$ according to the elementary rupture mode under the loading conditions (see reference [17] for details). The results shown in this article are visualized using visual molecular dynamics (VMD) [30].

2.3. Strength analysis

In all examples, we plot the force per AH, that is, we normalize the strength to enable a suitable comparison between different hierarchical structures. We focus on the hierarchical contribution to the strength and robustness of the protein structure (the contribution to strength and robustness from the pulling velocity has the same absolute value at a fixed deformation velocity, for different structures).

2.4. Definition of robustness in biological protein constituents

We calculate robustness as the ratio of strength of a defected system and an intact system, by following Kitano's definition of robustness as fault insensitivity [31]. For the calculations reported below, the intact system is defined as a system in which all AHs contribute to strength, whereas in the defected system all except one AHs (fault on second hierarchy, variation of b_2 [=number of AHs in a bundle], resulting in b_2^*) contribute to the strength. The robustness is defined as the ratio of the strength of the flawed system divided by the strength of the intact system (see equation (2) for the definition of f_{hi}),

$$r(b_i) = \frac{f(b_i, k_i, b_2^* = b_2 - 1)}{f(b_i, k_i)}$$
$$= \left\{ f_{h0} + f_{h1}(k_1 = 3, b_1 = 3) + f_{h2}(k_2 = 1, b_2^* = b_2 - 1) + \sum_{i=3}^{N} f_{hi}(k_i = 1, b_i) \right\} \left\{ f_{h0} + f_{h1}(k_1 = 3, b_1 = 3) + \sum_{i=2}^{N} f_{hi}(k_i = 1, b_i) \right\}^{-1}. \quad (4)$$

The robustness converges towards complete fault tolerance when $b_i \to \infty$, as shown in figure 4. We note that other

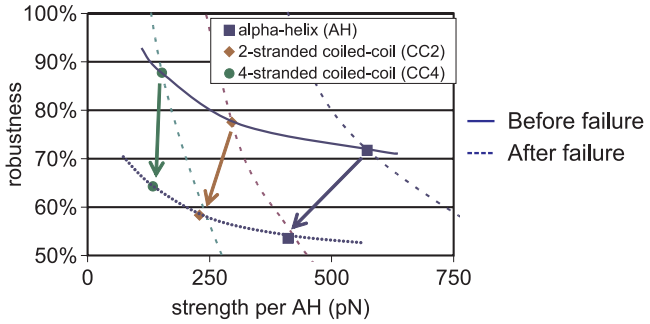

Figure 3. Performance of the analyzed structures in the strength per AH-robustness space, with and without a defect. The coarsely dashed lines represent levels of equal strength (s)–robustness (r) potential (that is, the product of both values is equal on these lines, $r \cdot s = \text{const.}$). Robustness and strength compete on these lines. The first data point for each structure represents the intact system, whereas the second data point shows the system after failure. CC2s have a robustness degree of 80% (robustness equals to the force from hierarchical strengthening of a defect system, two instead of three H-bonds rupture simultaneously, divided by the force of an intact system, when all three H-bonds rupture at once). The presence of a defect moves the system to another potential line. For example, due to the high level of robustness, the CC4 structure hardly changes its strength, whereas the strength of a single AH is significantly reduced. This illustrates how robustness and strength are combined in dependence of functional requirements.

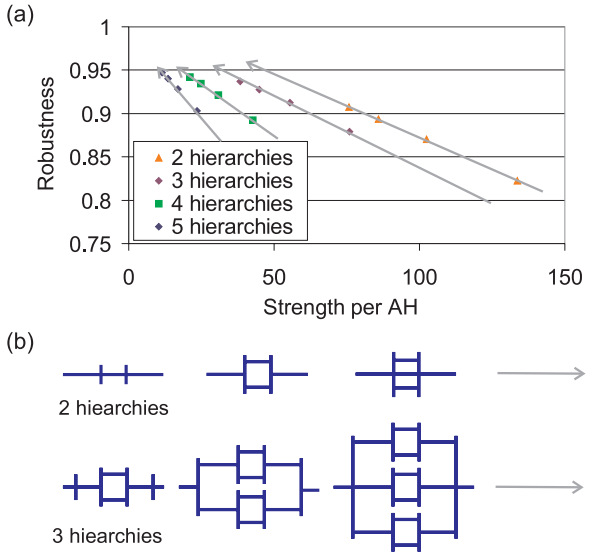

Figure 4. Robustness–strength domain for different hierarchical arrangements, considering the effect of increasing the number of subelements on the particular hierarchy (arrow points in direction of increasing number of hierarchies). Subplot (b) depicts examples for 2-hierarchy and 3-hierarchy systems, each with one, two and three elements. These results show that in general, the more elements, the more robust but the less strong (per AH) is a system. This also shows the tradeoff between robustness and strength. The strength per AH is plotted, to enable a better comparison of different structures, which feature different amounts of material (that is, number of AHs) per cross-sectional area. This equals to a normalization of force by the cross-sectional area, which leads to the strength of a material.

robustness perspectives in biological materials include adaptation to environmental changes, as well as graceful degradation [31]. This definition of robustness is related to a structure's ability to tolerate structural defects; it is therefore related to toughness. Tough materials show high robustness against catastrophic failure despite the presence of a defect.

3. Computational and theoretical results

3.1. Validation of the Hierarchical Bell model via direct MD simulation

We first validate the Hierarchical Bell model by carrying out full atomistic simulation studies in explicit water of the rupture force of three different hierarchical AH protein motifs (see figure 2(a) for the geometries). Force-extension histories from the MD simulations are shown in figure 2(b). Figure 2(c) shows theoretical predictions of the strength of the structures according to equation (2) (see continuous lines), and the direct comparison with results of MD simulations. The predictions based on the model require only two fundamental input parameters, the H-bond energy E_b^0 and the bond breaking distance x_b. Notably, these values are the same for all structures shown in figure 2. Thus, the analysis shows that the different strength values for the different geometries are a result solely of hierarchical effects resulting from different geometries. These simulations confirm that the formulation of the Bell model for hierarchical systems given in equations (2) and (3) provides an accurate prediction of the rupture forces or strength values based on the particular geometry of the protein structure.

3.2. Applications and case studies

The Hierarchical Bell model is now used in a series of theoretical analyses of three case studies, addressing the question, how are AH-based protein domains capable of unifying strength and robustness? The direct atomistic simulation of the systems considered here is not possible due to computational limitations, so that the analysis is limited to theoretical considerations.

For the following analysis we use the E_b^0 from the single AH ($E_b^0 = 4.21$ kcal mol^{-1} and $x_b = 1.21$ Å) as extracted from full atomistic simulations of AH structures [17]. These values are close to results of experimental and theoretical investigations of the rupture mechanism of AH protein domains [17, 32]. Earlier full atomistic studies in explicit water solvent by the same authors have shown that the fundamental fracture mechanism of a single AH element is the simultaneous rupture of approximately 3 H-bonds [17]. This mode represents the basic unit mechanism of rupture *in vivo*, at pulling rates below 0.1 m s^{-1} [17]. This mechanism is considered in the case studies reported here (figures 3–5), represented by $k_1 = 3$, thereby providing a realistic description of the behavior closer to experimentally accessible pulling rates (it was shown in an earlier analysis [17] that this mode of deformation, $k_1 = 3$, dominates at low deformation rates, instead of $k_1 = 1$, which is due to the high pulling rates in MD simulations).

We now apply the Hierarchical Bell model to three AH-based structures (these are the same structures as discussed in

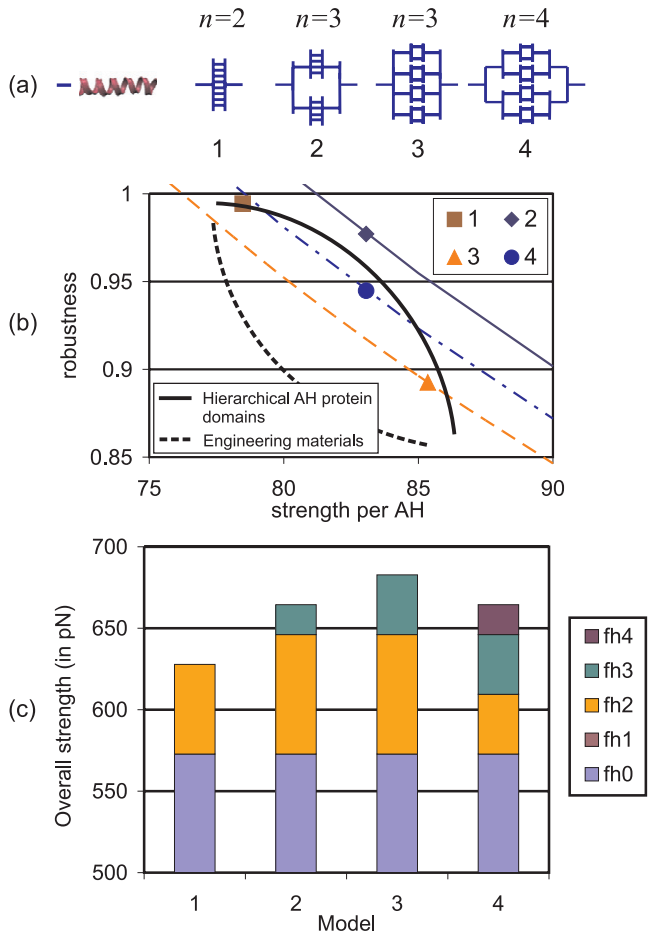

Figure 5. This figure gives an example of four different structures with the same number of subelements (that is, eight AHs) but in different hierarchical arrangements. Subplot (a) shows the four different architectures. For simplicity, individual H-bonds on the lowest hierarchical scale are not shown; instead one line represents three H-bonds as one AH. Subplot (b) shows the concurrence between strength and robustness, which depends on the degree of redundancies on the different hierarchical levels. The level of robustness increases with increasing redundancies on a particular level. Dependent on the hierarchical arrangement of the elements, different potentials of strength and robustness can be reached. Subplot (c) shows the contributions of each hierarchy to the overall strength (not strength per AH, as shown in subplot (b)). As we assume that in each AH three H-bonds break simultaneously, each structure (featuring an AH as the smallest subelement) has the same contribution from hierarchy 0. This is also the highest amount of strength contribution and shows the significance of the strength of H-bonds, which depends on the solvent and the environment. The other contributions are of hierarchical origin. The force contribution from hierarchy 1 is zero, since 3 out of 3 H-bonds break, which lowers the logarithmic multiplicator to zero.

the validation of the model, shown in figure 2(a)). Figure 3 shows the performance of the analyzed structures in the strength per AH-robustness space with and without a defect of one basic element (see section 2.4 for the definition of robustness; typical strength values under slow deformation conditions [17] range between 100 and several hundred pN, at deformation speeds below 0.1 m s^{-1}). A system with a high level of robustness and thus with a high level of redundancies hardly changes the strength even when a defect appears (as seen in the 4-stranded CC, CC4). In contrast, a system with a low level of robustness and thus less redundancies (as seen in the single AH) has a significantly reduced strength when a defect appears. However, increasing robustness goes along with a loss of strength per element (that is, per AH), since the overall strength of a structure is not directly proportional to the number of parallel elements. Theoretically, a robustness value of 100%—leading to the highest strength of a defected system—would appear for an infinite number of elements at each hierarchical scale.

Figure 4 depicts a systematic investigation of the robustness–strength behavior under an increasing number of elements at each hierarchy, illustrating this effect. Each line represents one level of hierarchies (e.g. 2 hierarchies equal to the AH level, 3 hierarchies equal to the CC level), where the number of elements on this particular hierarchies is varied (e.g. one AH, two AHs, for 2 hierarchies system and one CC, two CCs, and others, for a three-hierarchy system). We find that at each equihierarchical line, with an increasing number of elements, the strength per AH decreases, while the robustness increases. However, increasing the number of elements at a specific hierarchical scale is inefficient, as it leads to extensive material use and a decrease in the overall strength. Therefore, the introduction of hierarchies, enabling the optimization of strength and robustness under limited material use becomes significant.

To illustrate this point, we arrange eight single AHs in different hierarchical structures—asking the question: how can one arrange eight AHs to obtain different levels of robustness and strength? As shown in figure 5, the systems (schematics in figure 5(a)) consist of two, three and four hierarchies. The differences in robustness and strength calculated with the Hierarchical Bell model are not achieved through additional use of materials, but purely through different hierarchical arrangements (figures 5(b) and (c)). To the best of our knowledge, this tuning of properties in the strength–robustness domain as illustrated here has been shown theoretically here for the first time. In the robustness–strength map, the 'best' material behavior is the one in which high robustness is achieved at large strength—referred to as a 'high potential'. It can be seen that system 2 has the highest potential. Notably, it is not the system with the highest hierarchical level (system 4), nor the system with the highest level of redundancies (system 1). In other words, system 2 features the best combination of redundancies at different hierarchical levels, and features superior mechanical performance without additional material use.

The analysis reported here shows that with different arrangements (by changing the number of subelements as shown in figure 4, and by changing the hierarchical geometries as shown in figure 5), almost any point in the strength–robustness space can be achieved. These results illustrate how AH protein domains solve the conflict between strength and robustness, by introducing hierarchies as an additional design variable. This finding is the most important result of the study reported in this paper. The results shown in figure 5 results suggest that the level of hierarchical depth

and strength may be balanced in biological protein materials, allowing biological materials to maximize the mechanical performance while minimizing the use of materials. In agreement with this notion, proteomics analysis reveals that CC2 structures (see figure 2(a)) are most common in biology (due their dominance in intermediate filaments, feather, hair, hoof and other materials [7, 8, 11]), maybe since they provide a compromise between strength and robustness (having two AHs instead of one is the minimum level of possible redundancies). Further, the energy dissipation per AH in the plateau regime II (see, e.g. figure 2(b)) depends directly on the force level and might be another reason for the dominance of CC2 structures, as they feature a minimum level of redundancies at high levels of energy dissipation density.

4. Discussion and conclusions

We have shown here that with different structural arrangements, different combinations of strength and robustness can be achieved. This finding is the most important result of the case studies put forth in this article: it illustrates that the conflict between strength and robustness can be resolved by introducing hierarchies as an additional design variable. This provides important insight into structure–property relationships in protein materials, contributing to on-going efforts at the interface of materials science and biology [33]. These results further suggest that the level of hierarchical depth and strength may be balanced in biological protein materials, since the robustness and strength are not completely inversely proportional, allowing biological materials to maximize the mechanical performance while minimizing the use of materials. Overall our analysis illustrates that the introduction of hierarchies is the key to unify disparate material properties. Applying this insight to the design of materials will allow an extended use of hierarchies in bioinspired or biomimetic synthetic materials at nanoscale, such as hierarchically organized CNT-bundles, nanowires, CNT–protein or polymer–protein composites [34–36]. The combination of synthetic and natural constituting elements (e.g. proteins) could be a particularly promising strategy.

The increasing use of protein building blocks in the development of novel nanomaterials, such as nanowires, nanotubes, and others [13] requires the development of new engineering models that enable the systematic design of the use of nanoscale constituents in the makeup of larger-scale materials. Our model provides such insight and design rules, here shown for the example of AH structures, which facilitate the development of novel nanostructures based on proteins. Most importantly, our model explains a fundamentally new concept, that is, by simply rearranging the same number of nanoscale elements into hierarchies, one can change the performance of the material in the strength–robustness space (see figure 5). This makes the continuous invention of new basic building blocks unnecessary. Thus the broad application of universal building blocks in highly diverse architectures might be a biological strategy that enables adaptation to changes in the environment directly by adopting the structural arrangement of the same basic building blocks. This concept also appears at the level of primary structure of proteins. Instead of inventing new amino acids, a limited number of universal 20 amino acids are combined in different ways to create functional complexity, accounting for the great variety of biological protein materials. These types of concepts represent an opportunity for further studies that could investigate this hypothesis in greater detail. In additional to the biological context, detailed studies of this concept as a novel 'engineering paradigm' are crucial to advance this field. This might result in synthetic materials as mechanomutable, smart structures, which continuously and independently adapt to environmental changes at each length- and timescale, consisting of a handful of 'universal' building blocks at the nanoscale.

Even though our model is focused on AH protein structures, the main results of the work should be generally valid for other protein structures that are primarily stabilized by H-bonds, in particular the finding that hierarchical arrangements of H-bonds are crucial for the ability of protein materials to combine strength and robustness. This property of biological protein materials has often been pointed out in the literature [2, 37], but has not yet been explained based on a fundamental physics model as accomplished here with the Hierarchical Bell model (equations (2) and (3)). The observed behavior provides further evidence that this behavior may have its origin at the molecular scale. This insight could be crucial to translate biological material concepts towards nanotechnology applications. However, caution must be taken since other types of chemical bonding as well as the environmental conditions such as temperature or pH (possibly strengthening and weakening different types of bonds) may also contribute to a protein's mechanical stability (see also discussion in the introduction), and that these models should be considered appropriately. Further studies, perhaps using full atomistic MD with different model protein structures in explicit solvent, could provide crucial insight into these aspects.

Figure 6 shows how our theory can be applied in an analysis of the mechanical performance of a different protein nanostructure, a triple beta-helix amyloid protein nanotube that is part of the T4 bacteriophage virus (structure shown in figure 6(a)). This amyloid structure is able to withstanding large pulling and compression forces in the order of several nN [38], and might be of great interest for future mechanical and nanoelectronic applications [13, 39]. Each convolution in this structure consists of three beta-sheets arranged in an equilateral triangle, where each beta-sheet consists of a cluster of several H-bonds. The structure therefore consists of two hierarchies per convolution. Figure 6(b) depicts the representation of this protein structure in the Hierarchical Bell model. How would the performance of this structure change if the structure is altered? Figure 6(c) depicts a representation of a system that would contain all H-bonds per convolution in one large cluster, that is, this structure has lost the arrangement of H-bonds in clusters. Applying equation (3), and $E_b^0 = 5$ kcal mol^{-1}, and assuming that the bonds rupture one by one ($k_1 = 1$ as expected from MD simulations of similar structures), the Hierarchical Bell model predicts that the introduction of the hierarchical level by clustering H-bonds increases the effective

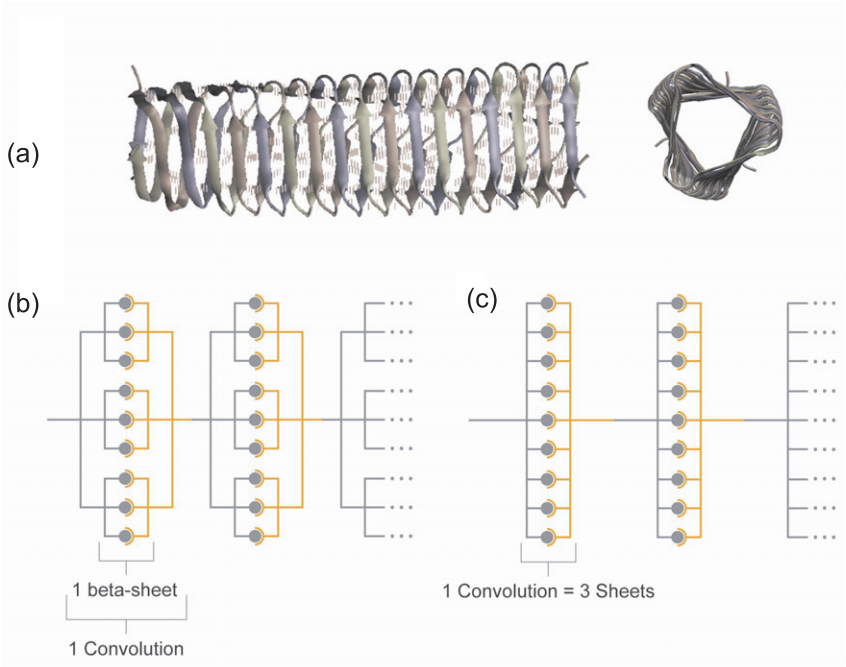

Figure 6. The applicability of our model to a different protein nanostructure, a triple beta-helix, as it could be used for mechanical and electronic nanodevices. The analysis shown here is for the needle of a T4 bacteriophage. Subplot (a) depicts the protein's ribbon structure from two different views. Each convolution consists of three beta-sheets arranged in an equilateral triangle, where each beta-sheet consists of a cluster of several H-bonds. Subplot (b) depicts the representation of this protein structure. The structure consists of two hierarchies per convolution, where H-bonds are arranged in three clusters in each convolution. Subplot (c) depicts the representation of a system that would contain nine parallel bonds per convolution, that is, all available H-bonds per convolution are arranged in one large cluster. Our model predicts that the height of the effective energy barrier E_b of system (b) is 28% higher than the one of system (c), illustrating the effect of using hierarchies to improve the performance of nanostructures.

energy barrier E_b by 28% compared to the arrangement with only one hierarchy, leading to greater chemomechanical stability. This illustrates the effect of using hierarchies to improve the performance of nanostructures. This simple analysis illustrates for a specific example how our model could be used in the design of novel protein-based nanomaterials.

The failure of engineering materials and structures has been studied extensively and has impacted our world by enabling the design of complex structures such as buildings, airplanes, cars and devices. However, the mechanisms of failure in biological materials and how it leads to the breakdown of components in our body is not well understood. Thus, characterizing how protein materials fail has significant implications that may eventually lead to an improved understanding of diseases and injuries. The link between molecular structure and material properties in the strength–robustness domain may lead to a paradigm shift in the understanding of which physical mechanisms govern the behavior of biological systems. Understanding the fundamental physical laws that control the properties of hierarchical protein materials enables us also to link the structural protein organization to the appropriate biological functions. Engineering materials using a bottom-up approach that begins at the atomistic level, inspired by biological protein material concepts, may transcend the borders that currently lie between life sciences and engineering. The transfer towards the design of novel nanostructures facilitates the development of de novo multi-functional and mechanically active, tunable and changeable materials [1, 40–44], for example new organic and organic–inorganic composites that primarily consist of chemical elements that appear in our environment in practically unlimited amount (chemical elements such as C, H, N, O, S).

This may lay the foundation for a new engineering paradigm that includes the design of structures and materials starting at the molecular level, from bottom-up, to the macroscale, to create new materials and structures that mimic and exceed the properties found in biological analogs. The development of a fundamental science driven framework that involves a solid understanding of fundamental concepts is crucial for studies of biological systems, disease diagnosis and treatment, as well as the design of novel biomaterials. It is the key to reverse-engineer the human body, the key to understand diseases at multi-scale levels (cancer, genetic diseases, infectious diseases), to enable advanced treatments (intervention at level of relevant proteins, nanomedicine, protein hierarchies, biomaterials) and diagnostics (mechanical disease signature, protein misregulation).

Recently it was reported that the first bundles of coiled-coil proteins were generated synthetically [40], and many other peptide synthesis techniques are progressing rapidly [1, 40, 42–44], allowing to create self-assembled nanostructures of amyloid fibers [13] or collagen fibrils [45]. The model reported in this paper, combined with these new manufacturing techniques, may be the first step towards a *de novo* bottom-up structural design of protein-based materials. In

addition to protein materials this theory could be applicable to other nanoscale devices, which exhibit a hierarchical structure and are governed by stochastic processes of failure, such as polymer brushes or multi-layer films. A detailed analysis of the applicability of this theory to these structures is left to future work.

The field of genomics is concerned with the study of genes and their effects on macroscopic functions, and has led to considerable medical advances. Genomics, however, does not elucidate material properties, nor the mechanistic relation of hierarchical multi-scale structures and their resulting properties. The multi-scale behavior of protein assemblies with the goal of elucidating the relation between structure and material properties represents a grand challenge at the interface of materials science and biology. This gap in understanding could be closed by systematically studying the material properties of hierarchical protein structures, their effect on the macroscopic properties (through development of structure–property relationships), and the role of material properties in their biological context, an effort defined as materiomics [19].

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