How protein materials balance strength, robustness and adaptability

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Abstract: Proteins form the basis of a wide range of biological materials such as hair, skin, bone, spider silk or cells, which play an important role in providing key functions to biological systems. The focus of this article is to discuss how protein materials are capable of balancing multiple, seemingly incompatible properties such as strength, robustness and adaptability. Here we review bottom-up materiomics studies focused on the mechanical behavior of protein materials at multiple scales, from nano to macro. We focus on alpha-helix based intermediate filament proteins as a model system to explain why the utilization of hierarchical structural features is vital to their ability to combine strength, robustness and adaptability. Experimental studies demonstrating the activation of angiogenesis, the growth of new blood vessels, are presented as an example of how adaptability of structure in biological tissue is achieved through changes in gene expression that result in an altered material structure. We analyze the concepts in light of the universality and diversity of the structural makeup of protein materials, and discuss the findings in the context of potential fundamental evolutionary principles that control their nanoscale structure. We conclude with a discussion of multi-scale science in biology and *de novo* materials design.

Strength, robustness and adaptability are properties of fundamental importance to biological materials and structures, and are crucial to providing functional properties to living systems. Strength is defined as the maximum force (or pressure) a material can withstand before breaking. Robustness is defined as the ability of a material to tolerate flaws and defects in its structural makeup while maintaining its ability to provide functionality. Adaptability refers to the ability of a material to cope with changing environmental conditions. These properties are crucial for materials in biology (s.a. skin, bone, spider silk, or cells), which either provide structural support themselves (s.a. the skeleton formed by bone), or need to withstand mechanical deformation under normal physiologic conditions (s.a. cells and tissue associated with blood vessels that are exposed to the pressure of the blood).

In engineering, strength and robustness are disparate properties, as it remains challenging to create materials that combine these two features. Glass or ceramics, for example, are typically very strong materials. However, they are not very robust: Even a small crack in a glass, or an attempt to deform glass considerably will lead to catastrophic failure. In contrast, metals such

as copper are very robust; however, they do typically not resist large forces (Courtney 1990). Yet, these materials allow for large deformation, and even the existence of cracks in the material does not lead to a sudden breakdown. In contrast, many biological materials (s.a. cellular protein filaments, blood vessels, collagenous tissues s.a. tendon, spider silk, bone, tendon, skin) are capable of providing both properties – strength and robustness, very effectively, and also combined with the ability to adapt to changes in the environment (Fratzl and Weinkamer 2007; Meyers, Chen et al. 2008). For example, blood vessel tissues comprised of cells (endothelial and smooth muscle cells) and extracellular matrix protein material secreted by these cells, together from a highly elastic tissue material that is capable of withstanding haemostatic pressure variations, and moreover, is capable of adapting to changes in functional requirements by forming new tissue or removing tissue no longer needed. For example, the activation of angiogenesis (the formation of new blood vessels) occurs in response to physiologic cues where an increase in nutrient and oxygen is required (s.a. development of the embryo, ischemic wound sites, ovulation, etc.) in order to support newly formed tissue or to assist in wound healing (Folkman 2003). Extracellular matrix materials such as collagenous tissues or elastin fibers represent another example of highly adaptive materials that shows great resilience to environmental changes, self-healing ability, as well as deformability and strength (Fratzl 2008). Other examples for biological systems in with hierarchical features include gecko adhesion mechanisms, where extremely strong and robust adhesion is reached through the use of weak van der Waals interactions (Autumn, Sitti et al. 2002; Arzt, Gorb et al. 2003; Gao, Wang et al. 2005).

Most fibers, tissues, organs and organisms found in nature show a highly hierarchical and organized structure, where features are found at all scales, ranging from protein molecules (≈50 Å), protein assemblies (≈1 to 10 nm), fibrils and fibers (≈10 to 100 μm), to cells (≈50 μm), and to tissues and organs (≈1000s and more μm) (Alberts, Johnson et al. 2002; Vollrath and Porter 2006; LeDuc and Schwartz 2007; Rammensee, Slotta et al. 2008). In recent years, the study of the role of these distinct hierarchical structures, how they regulate the growth and function of biological systems, and what the driving forces are for their formation has emerged as an active field of research. Most early studies have focused on investigations at single scales, or treated tissues or the cellular microenvironment as a continuum medium without heterogeneous structures (e.g. studies that examine the isolated effects of material stiffness, or the role of chemical cues alone on cell behavior). However, the cause and effect of biological material mechanics is likely more complex than a singular input at a specific scale, and thus, the examination of how a range of material scales and hierarchies contribute to certain biological function and dysfunction has emerged as a critical aspect in advancing our understanding of the role of materials in biology in both a physiological and pathological context. Specifically, the origin of how naturally occurring biological protein materials are capable of unifying disparate mechanical properties such as strength, robustness and adaptability is of significant interest for both biological and engineering science, and has attracted significant attention. To investigate these issues, this article provides a review of recent work and future challenges in this field. Specifically, we discuss here, the key role that multiscale mechanics plays in defining a material's ultimate response at failure, and how nature's structural design principles define the hierarchical makeup of biological materials. This process, likely evolutionarily driven, enables biological materials to combine disparate properties such as strength, robustness and adaptability, and may explain the existence of universal structural features observed in a variety of biological materials, across species. Figure 1 shows a summary of the structural makeup of three example protein materials: intermediate filaments (an intracellular protein material), collagenous tissue (an extracellular protein material), and amyloids (an ectopic protein material), revealing their hierarchical structures that range from nano to macro. Table 1 provides a summary and definitions of key properties and terms used in this article.

To illustrate the key material concepts in a specific case, much of our discussion is focused on a particular protein material, intermediate filaments - a protein part of the cell's cytoskeleton - and alpha-helical protein structures that form the basic constituent of this class of protein filaments. We begin with a brief review of this class of protein material. The cell's cytoskeleton plays a crucial role in determining the overall cellular mechanical and biological properties. It consists of three major protein networks, actin, microtubules and intermediate filaments (often abbreviated as IFs). Thereby, actin filaments and microtubules, both made up of globular proteins, are responsible for cell dynamics and motility as well as particle transport (Wietz and Janmey 2008). However, these networks are rather brittle and break either at relatively low stress or low strains lower than 50% (Janmey, Euteneuer et al. 1991). The third component of the cell's cytoskeleton are alpha-helix based intermediate filament protein networks. In contrast to actin filaments and microtubules, intermediate filaments withstand much larger strains of up to several hundred percent (Fudge, Russell et al. 2008; Kreplak, Herrmann et al. 2008; Qin, Kreplak et al. 2009). Intermediate filaments also form the structural basis for lamin intermediate filaments, which constitute an important part of the cell's nuclear membrane (Sullivan, Escalante-Alcalde et al. 1999; Dahl, Kahn et al. 2004; Lammerding, Schulze et al. 2004; Houben, Ramaekers et al. 2007; Dahl, Ribeiro et al. 2008). Similar to intermediate filaments in the cell's cytoskeleton, lamin intermediate filaments fulfill the roles of defining the mechanical properties of the nuclear membrane, and also participate in gene regulation (Sullivan, Escalante-Alcalde et al. 1999; Dahl, Kahn et al. 2004; Lammerding, Schulze et al. 2004; Houben, Ramaekers et al. 2007; Dahl, Ribeiro et al. 2008). Their mechanical role has been demonstrated in several studies, which includes analyses of disease mechanisms in the rapid aging disease *progeria* (Dahl, Scaffidi et al. 2006). The hierarchical structure of lamin intermediate filaments features a cascaded hierarchical structure that ranges from the scale of individual H-bonds to the scale of individual cells. Figure 1A shows a schematic representation of the different levels associated with lamin intermediate filaments. The alpha-helix based protein dimer structure is highlighted as well.

Outline of the article

The paper consists of four major sections that cover different scales and aspects related to the issues of strength, robustness, and adaptability of protein materials, here exemplified in studies of alpha-helical protein materials. First, we present a section focused on strength and robustness of individual protein filaments. Second, we present a section dedicated to the analysis of hierarchical protein networks, spanning the scales from individual protein domains to micrometer sized networks. The discussion continues in a third part focused on a review of how adaptability is achieved in biological materials, illustrated based on the example of angiogenesis (blood vessel formation). The paper concludes with a discussion on universality and diversity of the structural makeup of protein materials in part four.

Deformation and failure of protein filaments

This section is focused on strength and robustness of individual protein filaments as they appear in a variety of protein materials (see Figure 1). The ultrastructure of protein materials such as intermediate filaments, spider silk, muscle tissue or amyloid fibers universally consists of alpha-helix and beta-sheet structures as well as other universal structural motifs such as triple helical collagen molecules. These material components are unique in their making as they employ not only covalently bonded polypeptide chains, but also H-bonds that give rise to unique folds and nanostructural arrangements of proteins by forming intramolecular as well as intermolecular contacts. Notably, H-bonds are intermolecular bonds 100 to 1,000 times weaker than those typically found in ceramics or metals. Due to the low bonding energy, individual H-bonds behave like liquids, since their weak interactions can be disrupted even due to moderate thermal fluctuations. This is evident in water, for example,

where a network of H-bonds exists that is established between individual water molecules. Yet, materials such as spider silk, intermediate filaments, and muscles display great mechanical resistance against deformation and failure. The key questions addressed here are: (1) How can mechanically weak structural elements such as proteins stabilized by H-bonds provide the basis to strong materials? (2) What role do hierarchical structures play in providing overall strength and robustness properties of a material?

Maximum mechanical strength of H-bond clusters is reached at a critical length scale

We first address the question of mechanical strength of H-bonds, by combing a chemical and mechanical perspective in the analysis. The key hypothesis considered here is that in order to understand the mechanical strength of H-bonds, it is essential to consider the effect of structural organizing of H-bonds on their effective properties. Indeed, H-bonds in naturally occurring protein motifs often display a high level of structural organization of H-bonds. Based on theoretical and computational molecular dynamics studies (Ackbarow, Chen et al. 2007; Keten and Buehler 2008; Keten and Buehler 2008) and experimental validation (Keten and Buehler 2008), the strength properties of clusters of H-bonds of different size were investigated based on a simple model system in which a single beta-strand with varying number of H-bonds was examined. Figure 2A shows the strength of clusters of H-bonds as a function of the size of the strand, characterized by the number of H-bonds (results obtained under quasistatic deformation at asymptotically vanishing deformation rates). Here the strength is defined as the maximum force required in order to initiate breaking of the cluster, divided by the sheared area.

The results display interesting characteristics. First, as expected based on our knowledge of the weakness of individual H-bonds, the mechanical strength of individual H-bonds is zero. This observation is in agreement with the fact that water is a liquid and not a solid. Second, the analysis reveals that as the number of H-bonds in a cluster is increased, the strength increases as well, reaching a peak at $N_{cr} \approx$ 3-4 H-bonds. Notably, the peak maximum strength is close to 200 MPa, resembling the shear strength of metals (Keten and Buehler 2008). These results show that the maximum mechanical strength is reached at a critical length scale, providing a strategy to overcome the intrinsic limitation of the weakness of H-bonds. It is noted that the H-bond energy itself depends on the solvent environment, which is reflected in changes of the energy barrier associated with breaking H-bonds. This effect is responsible for the variation of H-bond energies from 2-8 kcal/mol in various protein materials and solvent environments. Notably, the effect of these solvent induced variations of the H-bond energy have only a relatively small effect on the scaling behavior of the strength as reviewed above, as discussed in more detail (Keten and Buehler 2008). Specifically, the shape of the scaling as presented here and the fact that the maximum strength is reached at a critical number of H-bonds appears to be a universal feature.

Next we examine the robustness of a cluster of H-bonds, again for different geometries. Robustness is a key measure that reflects the ability of a system to deal with changes in the environment or changes in its structural makeup (e.g. loss of bonding in parts of a protein, crack formation, etc.). The robustness R is defined as the strength F of a filament in which one element (here, one H-bond) is missing, divided by the strength of an intact filament:

$$R(i) = \frac{F(i-1)}{F(i)}$$ (1)

(the number of H-bonds in a cluster refers to the number of H-bonds in a turn as shown in Figure 3A). The results for the robustness as a function of the number of H-bonds are plotted in Figure 2A. The graphs show that the robustness increases continuously with the number of

H-bonds (Ackbarow, Chen et al. 2007). However, the actual increment of robustness due to adding one H-bond decreases with the number of H-bonds. Specifically, it is found that a robustness value close to 100% reached at a size of 3-4 H-bonds, perhaps resembling a balance between optimal material use and strength. The 100% robustness value can be explained by the fact that beyond the critical number of H-bonds N_{cr}, the strength does not change with the number of H-bonds, that is, $F(i) = F_{max}$ for $i \geq N_{cr}$.

This result illustrates that by utilizing a size effect that is rooted in a fundamental scaling of the strength as a function of the geometry, the intrinsic limitation of H-bonds, their mechanical weakness, can be overcome while maintaining a relatively high level of strength *and* robustness. Considering a variety of protein structures found in nature, we find that the size of H-bond clusters in most proteins is close to the critical number N_{cr} associated with maximum mechanical strength, as shown in Figure 2B. Therefore, the occurrence of a strength peak at this characteristic dimension provides a possible explanation for the geometric features of several protein constituents. Possibly, the clustering of H-bonds into small groups could be a universal evolutionary principle, guided by the requirement to present mechanically strong and robust building blocks to form a diverse group of fibers and tissues. This concept may explain the universal nanostructural structural principle found in a diverse set of protein materials. Furthermore, the structure formed by "soft" H-bond clusters, sandwiched between "stiff" polypeptide amino acid chains, resembles a common design principle used in the construction of brick walls used in civil engineering for centuries at the macroscale (see inlay in Figure 2A). Future studies are needed to put these concepts into a more solid footing in the context of evolutionary science.

Strength and robustness of hierarchical H-bond assemblies

We now proceed with a study of strength-robustness properties of filaments composed out of different hierarchical assemblies of alpha-helical protein domains (Keten and Buehler 2008; Ackbarow and Buehler 2009; Qin, Cranford et al. 2009). The basic building block for all filaments considered in this case study is an alpha-helical protein domain as shown in Figure 3A, stabilized by 3-4 H-bonds per turn (an alpha-helical turn has an average of 3.6 H-bonds (Alberts, Johnson et al. 2002)). For this particular geometry the mechanical resistance (both strength and robustness) of the individual protein domain is at its maximum, as shown in Figure 2A. The question examined here is to find out whether or not it is possible to build larger-scale structures out of individual protein domains that maintain high levels of strength and robustness.

In this analysis, the concept of robustness is defined as the strength of an intact filament divided by the strength of a filament in which one element (here, one alpha-helix) is missing at the smallest level (following the definition provided in eq. (1)). To explore the effect of structural variations on the performance in the strength-robustness domain, we consider eight alpha-helices and arrange them in all possible geometries and measure their properties. Figure 3B depicts the geometries and results for eight alpha-helices (the definition of subelements and their arrangement are those shown in the inlay of the figure). The analysis shows that even though no additional material is used, the mechanical performance changes significantly as the hierarchical arrangement of the structure is varied (see caption of Figure 3 for details regarding the nomenclature). The {8} structure provide very high levels of robustness, albeit at low strength. In contrast, the {4,2} structure provide high strength, albeit at low robustness. However, there are some structures that provide an optimal combination of both properties, the {2,2,2} and {2,4} structures. Among these, the {2,4} structure is the best performer as it provides the highest levels of strength and robustness. The {2,4} structure represents a fiber composed of two bundles of four-fold coiled coil alpha-helices (CC4).

The analysis is extended by considering a much larger number of filaments. As in the earlier study with only eight elements, the elements are assembled in all possible hierarchical structures and tested for their strength and robustness. Figure 3C depicts results for 16,384 alpha-helices (Ackbarow and Buehler accepted for publication, in press), where an analysis of the distribution of structures and their performance shows that most structures (> 98%) in Figure 3C fall onto a curve referred to as the banana-curve, where strength and robustness are mutually exclusive properties. Only ≈2 % of all structures lead to high strength and high robustness.

The investigation shows how high-performance materials can be made out of relatively weak constituents such as alpha-helices that are bonded by structurally and mechanically inferior H-bonds, by arranging them into specific hierarchical patterns. The resulting robustness-strength plots suggest a similar behavior as that found in many biological materials, as indicated in Figure 3C, in that they combine disparate properties. The particular distribution of performance characteristics for a large number of elements may explain why most engineered materials (s.a. metals, ceramics, glass, etc.) show a poor performance of strength and robustness. This is because most randomly picked arrangements fall on the banana curve (> 98%). Engineered materials often show this behaviour since hierarchical nanostructural geometries have not yet been utilized engineering materials design. In contrast, biological materials may have achieved the particular high performance structures through the adaptation of hierarchical structures. These observations suggest that the structure of biological materials may have developed under evolutionary pressure to yield materials with multiple objectives, such as high strength and high robustness, a trait that can be achieved by utilization of hierarchical structures. Further exploration of this concept in both experimental and theoretical studies could shed further light into these mechanisms.

Deformation and failure of protein networks: An issue of multiple scales

We now focus on other types of assemblies of proteins, and discuss structures that form hierarchical network structures at levels far beyond a single filament. In the literature, most protein materials have been studied either from a macroscale perspective (e.g. through continuum models) or from a single-molecule level, but not from an intermediate "mesoscale" viewpoint. For example, alpha-helix based intermediate filament networks have been investigated through shear experiments of protein gels (Janmey, Euteneuer et al. 1991) as well as through *in situ* studies with particle tracking rheology (Sivaramakrishnan, DeGiulio et al. 2008), where their material properties have been explored from a macroscopic perspective. On the other hand, the mechanical properties of the elementary nanoscale alpha-helical building blocks were studied extensively, and several publications have reported advances in the understanding of their nanomechanical behavior from both experimental (Lantz, Jarvis et al. 1999; Kageshima, Lantz et al. 2001) and theoretical (Ackbarow and Buehler 2007; Ackbarow, Chen et al. 2007; Buehler and Ackbarow 2007; Buehler, Keten et al. 2008; Ackbarow, Keten et al. 2009; Qin, Kreplak et al. 2009) perspectives. A more complete understanding of properties such as strength, robustness and adaptability, however, requires us to take a mesoscale perspective that considers all scales and the hierarchical structures, from nano to macro.

Here we review studies of a simple model system of a hierarchical protein material, as shown in Figure 4 (Ackbarow, Sen et al. 2009). The model is designed with the objective in mind to devise a simple physics based representation of an intermediate filament network in the nuclear envelope (lamina) (further details on the simulation setup, results and interpretation are included in (Ackbarow, Sen et al. 2009)). The goal is to elucidate the key parameters of interactions between structure and properties at multiple hierarchical levels, without attempting to provide a quantitative model of this particular protein material. A lattice

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structure, resembling the meshwork arrangement of intermediate filaments in the nuclear envelope is subjected to tensile loading as shown in Figure 5A (upper left plot). To model the effect of the existence of structural flaws on the material performance, we insert a crack-like defect in the center of the sample, as highlighted with the white ellipsoid. This setup serves as a simple model to mimic the existence of structural irregularities as shown in Figure 4 (nuclear envelope level *H5*; marked in white color). The protein network itself is modeled based on a coarse-grained bead-spring network as highlighted in Figure 5A (upper inlay), following a multi-scale modeling approach. Each of the one-dimensional chains resembles an alpha-helix based protein filament and serves as a simple model representation of an intermediate filament protein. All parameters in the coarse-grained model are derived from full-atomistic simulations, which have led to the characteristic three-tiered elastic-softening-stiffening response of alpha-helical filaments (see inlay in Figure 5A). The first regime resembles the initial stretching of the filament without rupture of H-bonds, that is, elastic deformation. Regime β resembles the secondary regime of stretching, a very soft plateau, during which the protein filament unravels by unfolding of alpha-helical turns with a slight increase of the force as the strain is increased (which occurs by rupture of individual alpha-helical turns as shown in (Ackbarow, Chen et al. 2007)). Regime γ resembles the stiffening regime, during which the protein filament's stiffness increases manifold due to stretching of strong bonds (covalent bonds).

We begin our analysis with carrying out a tensile deformation test of the protein network. We carry out a detailed analysis of the deformation mechanism, as shown in Figure 5A, where the color of the alpha-helical filaments indicates how much it has been deformed (identifying the three regimes: α, β and γ, as described above). At small deformation, the protein filaments start to unfold as H-bonds begin to rupture and the alpha-helical proteins uncoil, turn by turn (Figure 5A, snapshot II). At small to moderate deformation, the deformation mechanism of the network is characterized by molecular unfolding of the alpha-helical protein domains, leading to the formation of very large yield regions. This is shown in Figure 5A (snapshots III-IV) where the yield regions appear first in yellow and then in red color. These yield regions represent an energy dissipation mechanism to resist catastrophic failure of the system (referred to as "dissipative yield regions"). Rather than dissipating mechanical energy stored in the material due to the external strain by breaking of strong molecular bonds as it would happen in a brittle material like glass or a ceramic, the particular structure of alpha-helical proteins makes it possible that mechanical energy is dissipated via a benign and reversible mechanism, the breaking of H-bonds. Catastrophic failure of the structure does not occur until a very large region of the structure has been stretched so significantly that the strong bonds within and between alpha-helical protein filaments begin to fail. As shown in Figure 5A through the highlighted crack shape, we observe that the formation of yield regions enables a significant change of the shape of the crack, from an initial ellipsoidal shape where the longest axis points in the *x*-direction (horizontal orientation) to an ellipsoidal shape where the longest axis points in the *y*-direction (vertical orientation).

This microscopic change of the crack shape induced by the macroscopic applied load has important implications on the failure behavior of the system, and provides an intrinsic mechanism to mitigate the adverse effects of the flaw. A simple approximation of stress fields at a crack tip can be obtained using the Inglis solution for elliptical cracks (Lawn 1993) (see schematic in Figure 5B with explanation of variables), where the crack tip stress is given by

$$\sigma_{tip} = \sigma_0 \left(1 + 2 \frac{\xi'}{\delta'} \right). \tag{2}$$

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In eq. (2), σ_{tip} (= σ_{yy} at the crack tip) and σ_0 are the stresses at the crack tip and the far-field respectively, and ξ' and δ' are the x and y-axes lengths of the elliptical crack shape before failure. Specifically, the parameters ξ' and δ' describe the transformed crack geometry after blunting has occurred through formation of large yield regions mediated by protein filament stretching, but before the final stage of deformation has begun. We note that the parameters ξ and δ describe the initial crack geometry at the beginning of the simulation, before the transformation has occurred. Equation (2) can be used to make a few interesting predictions. The equation provides a simple model for the reduction of stress magnification at corners due to structural transformation as discussed above. For an ellipsoidal crack shape where the longest axis points in the x-direction, the ratio $\xi'/\delta' \gg 1$ (Figure 5B, left), the stress at the tip is much larger ($\sigma_{tip} \gg \sigma_0$) than for an ellipsoidal crack shape where the longest axis points in the x-direction, the ratio $\xi'/\delta' < 1$ (Figure 5B, right), where σ_{tip} is only slightly larger than σ_0. For example, for the geometry discussed here the initial ratio $\xi/\delta \approx 5$, leading to $\sigma_{tip} = 11\sigma_0$. After the crack shape transformation has occurred, $\xi'/\delta' \approx 0.3$, leading to $\sigma_{tip} = 1.9\sigma_0$, reduced by a factor of ≈ 6.

The analysis of the protein network reviewed here shows that the cascaded activation of deformation mechanisms at multiple scales enables the material to tolerate structural flaws (cracks) of virtually any size. This unique behavior is in stark contrast to engineered materials (e.g. metals or ceramics; materials constructed with no hierarchies), where the presence of cracks leads to a severe reduction of strength and is the most common cause for catastrophic materials failure (Broberg 1990). Materials failure typically initiates at locations of peak internal material stress at the corners of cracks), where atomic bonds are likely to break, leading to the propagation of fractures. Table 2 provides a summary of the roles and mechanisms of individual levels of structural hierarchies shown in Figure 5 for the overall system behavior, illustrating that each hierarchical level plays a key role in achieving the overall system performance. The detailed deformation and failure mechanism is summarized as follows:

- Initially, the system is loaded in Mode I (tensile load), with the load applied vertically to the long axis of the crack. In solids, this represents the most critical mode of loading with respect to inducing high local stresses in the vicinity of the crack tip.

- As load is applied, the protein filaments start to unfold, as H-bonds begin to rupture and the alpha-helical proteins uncoil (see blowups in Figure 5A).

- The system elongates in the loading direction, and the shape (morphology) of the crack undergoes a dramatic transformation from mode I, to a circular hole, to finally an elongated crack aligned with the direction of loading (see Figure 5A). This transformation is caused by the continuous unfolding of the individual proteins around the crack, which can proceed largely independently from their neighbors.

- As discussed based on the simple analysis derived from Inglis' solution, the elongated crack features very small stresses in the vicinity of the crack. The transformation of the crack shape is thus reminiscent of an intrinsic ability of this material to provide self-protection against the adverse effects of the existence of cracks.

- The almost identical strain at fracture regardless of crack size is due to the similar stretching mechanism and unfolding of the proteins at the initial stages of loading. Due to the self-protection mechanism and the related change of the crack shape (that

is, the alignment along the stress direction) the crack becomes almost invisible, even if dominating large parts of the cross-sectional area, and has little adverse effect on the overall system performance.

These investigations provide insight into the fundamental deformation and failure mechanisms of an abundant class of biological materials that feature networks of similar protein filaments. Specifically, the results may explain the ability of cells to undergo very large deformation despite irregularities in the structural makeup of the protein network. More generally, the concepts identified here may also apply to many other protein materials, and suggest that the controlled structure formation at multiple levels could be the key to obtain an integrated performance that combines disparate properties. Overall, intrinsic mechanisms such as the flaw-tolerance mechanisms revealed in the protein network present an intriguing ability of this class of materials to self-protect themselves against adverse effects of structural irregularities and other defects. Avoiding such structural irregularities in the material makeup would require a high energetic cost (e.g. through the need for strong bonding as it appears in crystalline solids). Biological materials appear to solve this challenge by adapting a structure that is intrinsically capable of mitigating structural irregularities or flaws while maintaining high performance, presenting a built-in capability to tolerate defects. These properties effectively result in self-protecting and flaw-tolerant materials.

The ability of the material to change its structural makeup, as demonstrated here by changing the crack orientation, reflects a level of responsiveness that transcends the concept of "static" structural optimization of hierarchical structures as described in the previous section. It mirrors an innate ability of biological materials to adapt to the environment by mutating their structural makeup at multiple scales and as such demonstrates that cross-scale interactions are crucial elements in understanding the mechanical performance of these materials.

Adaptive material properties

The adaptability of biological materials goes far beyond intrinsic mechanisms of crack shape change or flaw tolerance that are built in biological materials and structures. A greated level of adaptability can also involve cascades of signalling that link mechanical or other material cues to biochemical signals, resulting in the alteration of structure or the formation of new tissue. In this section we provide a brief review of how an important biological process, angiogenesis (the process of new blood vessel formation from existing vessels) respond and adapt to environmental cues via signalling cascades. The mechanism that regulates angiogenesis is complex and has been demonstrated to occur through the coupling of mechanical strain signals to biochemical factors, where the secretion of endogenous angiogenic factors was shown to be regulated by strain at multiple scales (Yung, Chae et al. 2009). This example illustrates how biological systems are capable of adapting to different boundary conditions by forming new tissue via the coupling of material synthesis and structure formation with physiological cues. The significance of this aspect of protein materials in the context of the focus of this paper is that it shows that the study of biological systems with material concepts alone is insufficient. Rather, biological materials must be understood as complex hierarchical signalling cascades that are interrelated and that involve intervention mechanisms that are rooted in changing the structure of the most fundamental constituents, through altering gene expression.

Angiogenesis requires an orchestrated series of cell activities in a specific spatial and temporal sequence. Figure 6 summarizes the key results and a potential mechanobiological mechanism of angiogenesis. These nascent vessels feature a characteristic bilayer makeup of endothelial cells, which serve as the blood barrier, and surrounded by a supportive elastic layer of smooth muscle cells. Human umbilical vein endothelial cells (HUVECs), and human

aortic smooth muscle cells (HASMCs) were used in these studies as model cell types to study the angiogenic process. A schematic illustration of a microdevice used to apply cyclic strain at 1 Hz to the cell cultures, cultured in PDMS wells, is shown in Figure 6A (Yung, Vandenburgh et al. 2009). The application of mechanical strain was used to replicate the physiologic environment, where endothelial and smooth muscle cells are exposed to cyclical pulsations due to change in haemostatic pressures. A model system used to examine sprouting, a process identified to represent angiogenesis, is shown in Figure 6B where confocal images show HUVECs seeded onto microcarriers, embedded into fibrin gels, and forming tube-like extensions in response to specific cues. HUVECs cultured under static (no strain) conditions as shown in the left, form minimal sprouts, whereas those subject to cyclic strain, image to the right, form an enhanced quantity of sprouts. These images qualitatively demonstrate how cyclic strain significantly enhances sprout formation, suggesting that mechanical cues alone are capable of triggering the formation of nascent blood vessels. The mechanism that regulates this angiogenic process, here represented by sprout formations, is analyzed through examination of angiogenic biochemical factors regulated by strain. Figure 6C displays the temporal pattern of angiogenic factors secreted by HUVECs (PDGF and Ang-2) and HSMCs (VEGF and Ang-1) in response to cyclic strain. The results show that Ang-2 and PDGF are both upregulated in a temporal fashion relevant to their role in the angiogenic process, where the Ang-2 peak secretion occurred approximately at day 1, and the PDGF peak at day 2.

A potential mechanism of angiogenesis, as regulated by cyclic strain, is shown in Figure 6D demonstrating a coupled mechanical-biochemical process. Availability of Ang-2, an early angiogenic factor, in the microenvironment (via upregulated secretion in response to strain) resulted in the increased formation of HUVEC sprouts. Whereas the offset increased secretion of PDGF, a late stage cytokine, and chemotactant for HASMCs resulted in the recruitment of HASMCs, to likely stabilize the nascent blood vessels (sprouts) in order to form the characteristic bilayer geometry of HUVECs and HASMCs. Under a lack of cyclic strain, both the Ang-2 secretion and PDGF secretion are reduced, resulting in reduced angiogenic activity. The mechanistic role of Ang-2 in strain regulated angiogenesis was examined using molecular biology knockdown techniques (RNAi), where the endogenous production of Ang-2 was suppressed. The angiogenic activity was reduced, both under static and more clearly, under stained conditions, thus verifying Ang-2's causal role in strain regulated angiogenic activation. The study reviewed here showed that autocrine signaling via activation of Ang-2 may be the mechanistic pathway by which HUVECs transduce mechanical signals to process angiogenic responses. Furthermore, cyclic strain modulated the intercellular communication between endothelial cells and smooth muscle cells by upregulating chemotactic paracrine factors secreted by HUVECs to recruit HASMCs. The study reviewed in Figure 6 shows for the first time, that a single mechanical input can regulate intercellular biochemical communication between vascular cells to activate angiogenesis.

Universality-diversity paradigm of biological protein materials

The evolution of protein materials through genetic selection and structural alterations has resulted in a specific set of protein building blocks that define their structural makeup. As outlined throughout this article, protein materials exist in an abundant variety, and the need exists to formulate a widely applicable model to systematically categorize all such materials, in order to establish a fundamental understanding, and to exploit the use of hierarchical structural building blocks to develop a new generation of advanced nanomaterials (Csete and Doyle 2002; Alon 2007; Buehler and Yung 2009). A *protocol* is defined here as a term that encompasses a general analysis of protein materials that describes the *use of structural building blocks* (e.g. alpha-helices, beta-sheets, random coils) during their formation and

function, and the *process or mechanism of use* of this material (e.g. synthesis, breakdown, self assembly). The phenomenon of *universality* exists ubiquitously in biology, where certain protocols are commonly found in all protein materials (s.a. the use of hierarchical levels of building blocks: DNA nucleotides, DNA double helical structure, alpha-helices, beta-sheets), and the process of transcription/translation, protein synthesis etc.). However, other protocols are highly specialized (s.a. the use of specific DNA sequences for a particular protein structure, the resultant protein motifs of tendon fascicles, lattice-like lamin structure, etc.), thus representing *diversity*. Therefore, protocols can be classified as either universal or diverse.

Universal and diverse protocols are distributed heterogeneously across different hierarchical levels, as shown in Figure 7. The four DNA nucleotides (ACGT) represent a universal protocol common to all protein materials, where their arrangements in diverse patterns form the immense variety of genetic sequences found in biology. Genetic sequences are universally encoded in the double-helical DNA structure, regardless of the specific nucleotide sequence. Through the universal process of transcription and translation, protein molecules are synthesized into a one-dimensional sequence of the universal 20 amino acid building blocks, which fold into 3D protein structures. Virtually all protein structures contain one or more of the universally found motifs: alpha-helices, beta-sheets and random coils. These universal motifs arrange into unique, diverse larger-scale protein structures (e.g. enzymes, fibres, filaments). Generally, a greater diversity of protocols is found at higher hierarchical levels, suggesting that biological functionality is associated with structural diversity. Universality is generally associated with protocols that can be used to derive diverse functionality at larger hierarchical levels. A fundamental difference between engineered materials and naturally formed biological materials is that functionality in biology can be created by arranging universal building blocks in different patterns, rather than by inventing new types of building blocks, as in many engineered materials. The formation of hierarchical arrangements provides the structural basis to enable the existence of universality and diversity within a single material. This combination of dissimilar concepts may explain how protein materials are capable of combining disparate material properties, such as high strength and high robustness, together with multi-functionality.

Biological functionality must be understood at varying scales. Biochemistry focuses on biological functionality at the molecular scales. The mesoscale that encompasses length-scales that range from nanometers to micrometers and time-scales of nanoseconds to microseconds is a particularly important level necessary to understand how specific protein materials derive their unique properties and what role they play in biological systems. Many material properties and mechanisms associated with physiologic and pathologic phenomena originate at this scale. The mesoscale science of protein materials, through the linking of molecular properties to properties of protein materials at the microscale, thus represents an important frontier of materials science with high potential for fundamental contributions to biology, medicine as well as for the *de novo* synthesis of engineered materials such as polymer nanocomposites and other hierarchical materials derived from self-assembly mechanisms (Glotzer and Solomon 2007).

The approach of utilizing universal building blocks to create diverse multifunctional hierarchical structures has been successfully applied in current macroscale engineering paradigms. For example, in the design of structures such as buildings or bridges, universal constituents (bricks, cement, steel trusses, glass) are combined to create multifunctionality (structural support, living space, thermal properties, light harvesting) at larger length-scales. The challenge of utilizing similar concepts that span to the nanoscale, as exemplified in biological protein materials, through the integration of structure and material, could enable the

emergence of novel technological concepts. A key obstacle in the development of new materials lies in our inability to directly control the structure formation at multiple hierarchical levels, an area of research that should be actively pursued from both an experimental and theoretical angle. The concept of universality and diversity and the knowledge gained from how to characterize these materials at different hierarchical levels can hopefully contribute to addressing these challenges.

As discussed in (Buehler and Yung 2009), nature's utilization of a limited number of universal building blocks, arranged diversely in a variety of ways, is a limitation as well as a strength of biological systems that could be exploited for materials design. For example, although the performance of structural tissues in our body is poor compared with most engineered materials (e.g. steel, ceramics, composites), their performance is remarkably good considering the inferior building blocks they are made out of. Understanding these material concepts and the translation to the design of synthetic materials, perhaps based on new nanostructured building blocks such as carbon nanotubes or graphene platelets, could thus provide us with new ideas for materials design based on inexpensive, abundant constituents.

Conclusion

Biology utilizes hierarchical structures in an intriguing way to create multifunctional materials. This explains the formation of hierarchical structures with defined length-scales for key protein domains that are, as a consequence, found as universal features. We have further observed that the cascaded activation of deformation mechanisms at multiple scales enables the material to tolerate structural flaws (cracks) of virtually any size, representing an innate mechanism of structural transformation that enables protein materials to mutate their structure to cope with the adverse effects of a structural flaw. Complex biological feedback loops explain additional mechanisms of adaptation of biological to changes of the environment or to deal with new physiological requests (s.a. blood vessel formation). As shown in the example discussed here, mechanical strain signals at the scale of 10-100 cells ($\approx$2000 μm) induce the secretion of signaling proteins at the molecular level ($\approx$10-100 Å). Diffusive processes in the tissue transport these signaling proteins that activate cell-cell interactions at the level of several cells ($\approx$100 μm). This example shows a complex interplay of biochemistry, mechanics, and material properties. Table 3 summarizes the key mechanisms by which protein materials balance strength, robustness and adaptability.

A materials science approach is a powerful approach to investigate biological systems from this perspective, a field of study referred to as materiomics (Buehler and Keten 2008; Buehler, Keten et al. 2008). Figure 8A shows the conventional materials science triangle that links structure, process and property. Figure 8B displays the materials science paradigm applied to the hierarchical structure of protein materials (*Hi* refers to hierarchy levels *i*=0..*N,* and *Ri* refers to material property requirements at hierarchy levels *i*=0..*N*). The expanded triangle shown in Figure 8B specifically includes the link of material properties and genetic processes (s.a. gene activation), which play a key role in understanding adaptability of biological materials. Thereby, biochemical processes facilitated by sensing of the environment of cells (as illustrated here for the example of angiogenesis) may change gene expression or activation, which results in inducing a change in cell behavior. This type of research, understanding the role of materials at the interface of physics, chemistry and biology, could have great impact in various areas of biological and biomedical research. For example, atherosclerosis (hardening of blood vessels due to plaque formation), or blood clots in large vessels (e.g. carotid artery), and other blood vessel diseased states are related to a complex interplay of materials-cell interactions at multiple scales.

The ability to adapt to changes in the environment, and to provide simultaneously strength and robustness is a behavior that is in stark contrast to engineered materials (e.g. metals or ceramics; materials constructed with no hierarchies), where the presence of cracks leads to a severe reduction of strength and is the most common cause for catastrophic materials failure (Broberg 1990). Materials failure typically initiates at locations of peak internal material stress at the corners of cracks), where atomic bonds are likely to break, leading to the propagation of fractures. Unfortunately, flaws and cracks in materials can not be avoided. The current engineering paradigm to address this issue is to over-dimension materials, which has resulted in heavyweight structures where most of the excess material is never needed during regular operation. Biological materials, however, have an intrinsic ability to mitigate the adverse effects of material flaws (cracks) and are capable to render them innocuous, even to very large cracks. It was demonstrated (Ackbarow, Sen et al. 2009) that the hierarchical makeup facilitated the dissipation of the local stress in the material by re-orientating a crack in an alpha-helical protein network under tension from a horizontal to a vertical orientation, leading to a marginal increase in the stresses at the corners of the crack (Figure 5). This change in the crack orientation provides a mechanism for the flawed material to deform several hundred per cent and still avoid catastrophic failure, despite the presence of large flaws. New computational strategies must be developed that are capable of incorporating changes at the genetic level into structural alterations at the level of proteins and protein assemblies. Such approaches could combine protein structure prediction methods with an analysis of the material performance, and reveal interesting insight into the details of structure-process-property relationships.

For a variety of applications, cross-scale multiscale effects will be very important as we push the limits of what we can see and how small and how effective we can design, for example in the development of new types of composites that could be inspired from the structural features found in bone or nacre that could utilize fundamental scaling laws for strength and plasticity (Gao, Ji et al. 2003; Gao 2006; Katz, Misra et al. 2007). For efficiency and conservation of finite resources, novel multi-scale modeling methods will be required that enable us to explore the full design space, from nano to macro in a realization of a merger of structure and material. New interatomic force fields and potentials that can accurately describe the formation and breaking of diverse types of chemical bonds (H-bonds, covalent bonds, different solvent environments, etc.) in a seamless multi-scale scheme are needed to include the full complexity of chemical bonding in a numerically efficient description. New types of models and approaches that bridge the knowledge between disparate engineering and scientific disciplines are necessary, and may lead to emerging fields with huge potential impact for society and technological advancement as synergies between research fields are identified. The concept of designing materials with hierarchical structures, by deliberately determining a cascade of multi-scale mechanisms is a largely unexplored aspect in materials science that could lead to advances in *de novo* materials design. By utilizing self-assembly processes from nano to macro (Reches and Gazit 2007), hierarchical structures may be the key that can enable us to take advantage of properties at all scales, and to exploit superior nanoscale properties. Such work has the potential to extend the current state of the art towards developing a new generation of intelligent biomaterials that integrates structure and function, from the nano to macro scales.

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Tables and table legends

Table 1: Definition of major terms used in this article

Term	Definition
Strength	The maximum applied force or stress (pressure) at which failure of a system occurs (e.g. through fracture, tissue break down, etc.).
Robustness	Measures the ability of a system to tolerate flaws and defects, that is, still being capable of providing the required function under the presence of flaws and defects. A definition of robustness related to strength properties is the ratio of strength of a flawed structure divided by the strength of a perfect structure.
Adaptability	Ability of system to respond to changes in the environment (s.a. formation of defects due to injuries, or changes in physiological requirements, or due to the formation of fractures, etc.).
Flaw (defect)	Deviation of the structure of a system from its perfect, ideal or reference configuration. Examples for defects include cracks, inclusions, protein misfolds, or mutations in the amino acid sequence.
Failure	Sudden, typically uncontrolled and irreversible loss of the functional properties of a system. An example is the breakdown of tissue due to injuries under very large applied forces.
Self-healing ability	Ability of a system to reform from a perturbed structure to its reference configuration (reassemble). May involve for example the curing of flaws and defects such as cracks or voids, or the replacement or the addition of tissue.
Changeability and mutability	Formation of distinct (sometimes preprogrammed) structures with different properties, which can be controlled by external cues. Examples include the existence of multiple conformations of proteins based on pH, or applied forces.
Multifunctionality	Ability of a system to provide multiple properties to satisfy a set of target properties. An example is the combination of strength and robustness.
Evolvability	Ability of a system to evolve over generations of synthesis. In contrast to adaptability, evolvability reflects a change of structural makeup and/or properties over generations of synthesis.

Table 2: Role of hierarchical levels in the deformation and failure behavior of alpha-helical protein network (see Figure 4 for schematic of the structure considered here) (Ackbarow, Sen et al. 2009)

Hierarchy level Hn	Description	Key mechanism(s)
H0	Level of chemistry; Intrabackbone H-bond; Basic chemical bonding, enabled by particular polypeptide structure	H-bonds form at moderate temperatures; Drive self-assembly of alpha-helices.
H1	Alpha-helix turn defined by cluster of 3-4 H-bonds; Basic building block of alpha-helix filament	Clusters of 3-4 H-bonds provide optimal resistance against mechanical failure (Keten and Buehler 2008) (3-4 H-bonds break concurrently, providing maximum possible mechanical strength at minimal material cost).
H2	Alpha-helix filament; Basic building block of square lattice	Particular geometry with linear array of turns provides structural basis for large extensibility of >150% strain via repeated rupture of turns.
H3	Square lattice unit cell; Microstructural geometry of network level	Distance between filaments provides structural basis to independently stretch without affecting neighboring bonds, since there are no immediate interactions between individual filaments in the network that prevent microscopic rotations and shear; Facilitates extreme strain gradients at low energy cost ($\approx 2 \times 10^{11}$ %/Å).
H4	Network; Macroscopic functional scale (e.g. nuclear envelope for mechanical integrity)	Structural transformation of crack-like defects to mitigate stress concentrations.

Table 3: How protein materials balance strength, robustness and adaptability

Property	Mechanism(s)	Example(s)
Strength	Size effects (scaling of strength with respect to structure size provides basis for peak at critical scale)	Clusters of 3-4 H-bonds maximizes strength of H-bonded protein domains (Figure 2A)
Robustness	Formation of hierarchical structures (small subelements combine to form larger-scale structure)	Coiled-coil proteins or other bundled filaments that provide high strength at high robustness (Figure 3B,C)
Adaptability	Change of structural makeup of material (microstructure)	Change of crack shape from horizontal to vertical to mitigate stress concentrations (Figure 6)
	Change of gene expression (biochemical signaling) in response to mechanical cues	Angiogenesis (blood vessel formation) (Figure 8)

Figures and figure captions
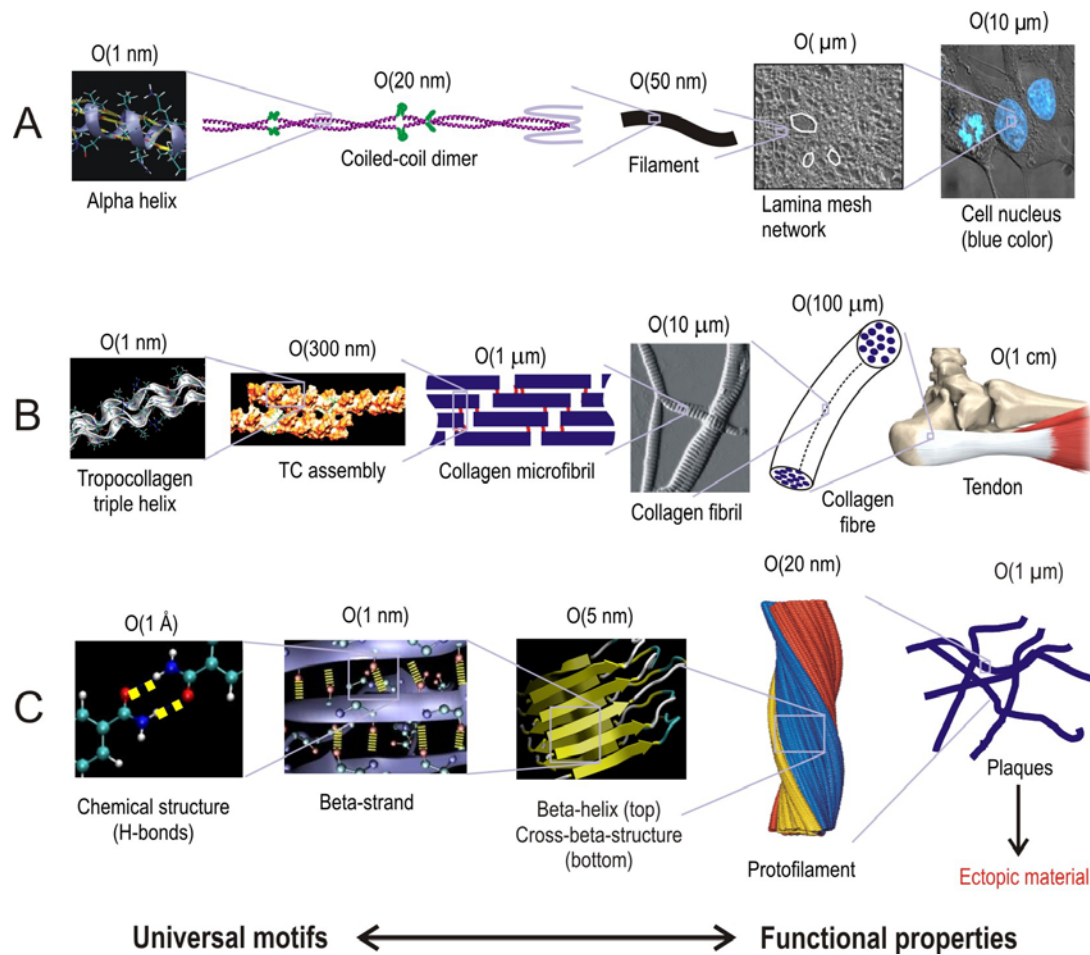
Figure 1: Hierarchical structure of three example biological protein materials, intermediate filaments (panel A), collagenous tissues (panel B), and amyloids (panel C). Figure adapted from (Buehler and Yung 2009).
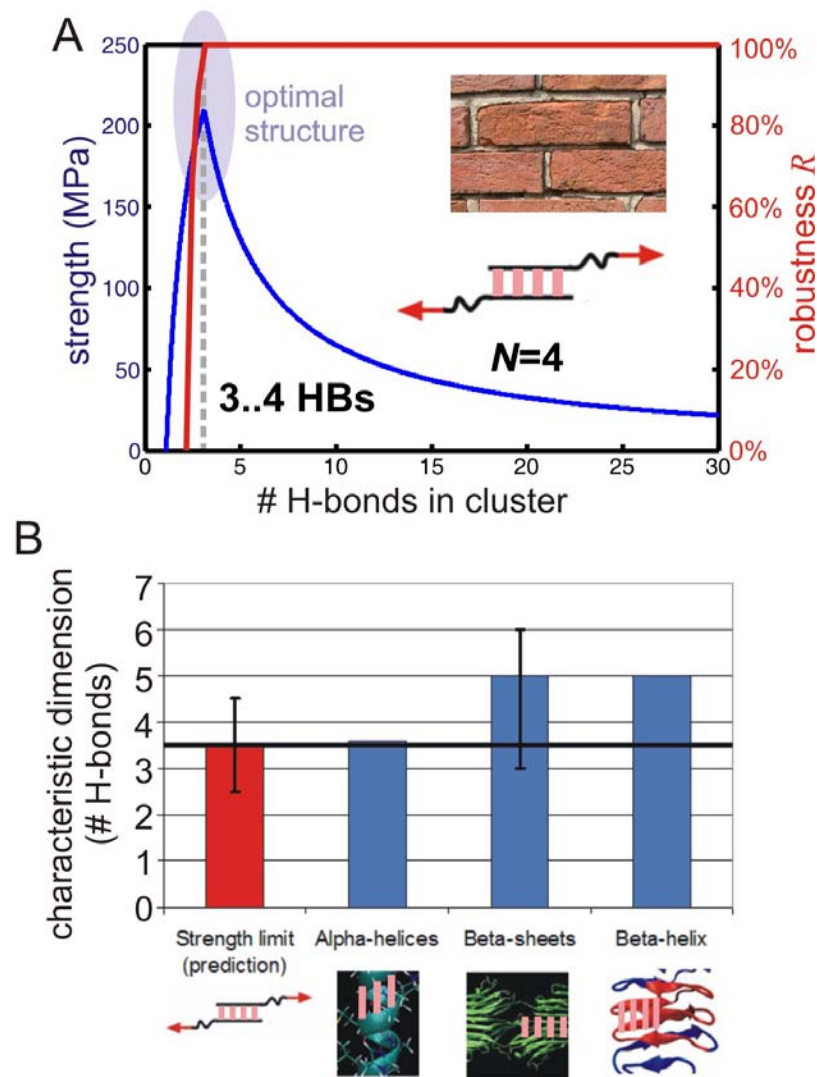
Figure 2: Size effect associated with clusters of H-bonds (Ackbarow, Chen et al. 2007; Keten and Buehler 2008; Keten and Buehler 2008). Panel A: Shear strength and robustness of clusters of H-bonds as a function of the size of the strand, showing a peak maximum strength of ≈200 MPa at a critical cluster size of 3-4 H-bonds. By utilizing this size effect, the fundamental limitation of H-bonds, being mechanically weak, can be overcome (Keten and Buehler 2008; Buehler, Keten et al. 2008). The analysis further shows that the robustness increases continuously with the number of H-bonds. Panel B: Number of H-bonds in common protein motifs. The comparison with the number of 3-4 H-bonds for optimal mechanical performance suggests that most natural protein motifs fall into this range.
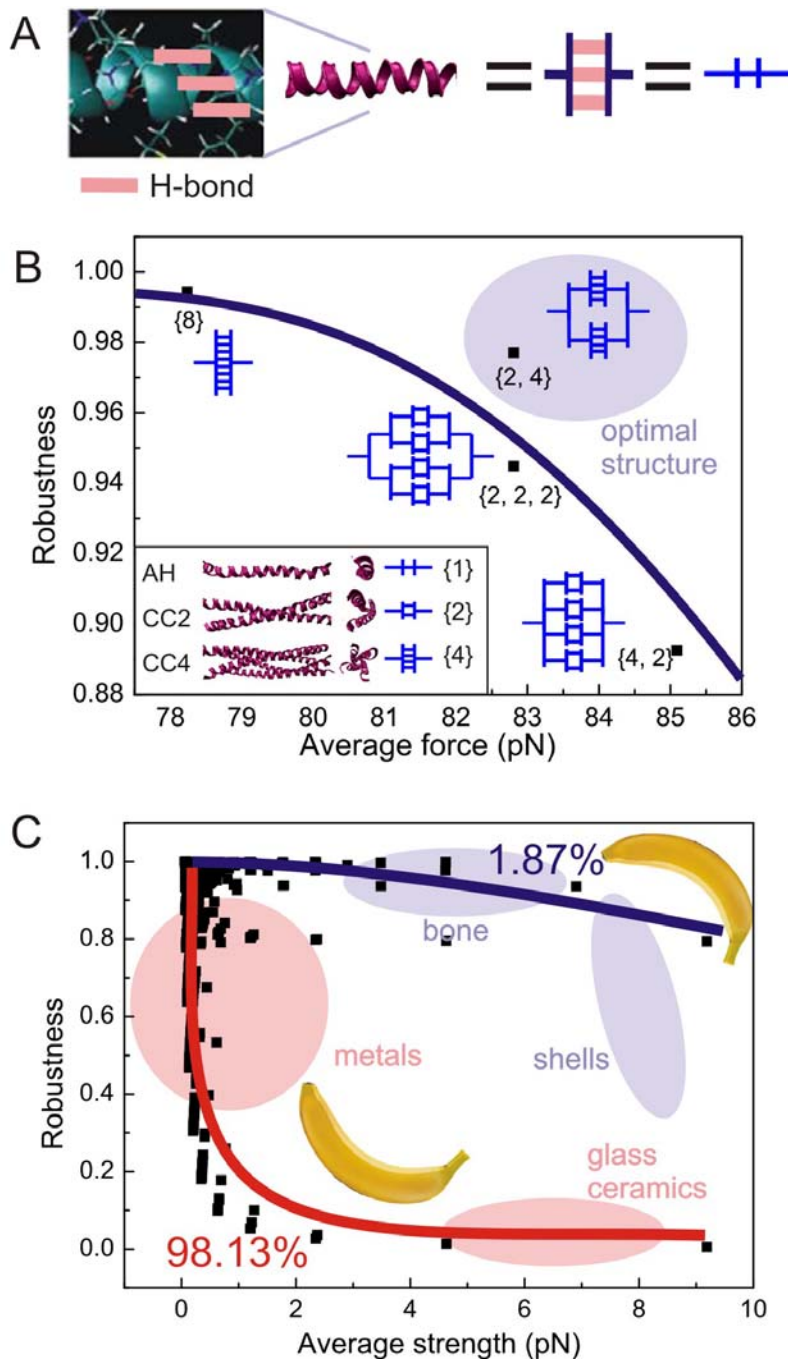
Figure 3: Strength-robustness relation for alpha-helical protein filaments (results adapted from (Keten and Buehler 2008; Ackbarow and Buehler 2009; Qin, Cranford et al. 2009)). Panel A shows the geometry of a single alpha-helix, composed of 3-4 H-bonds per turn. We study how the performance in the strength-robustness domain changes if several alpha-helices are assembled in different hierarchical patterns, as shown schematically in the plot (for 8 alpha-helices). Panel B shows the results for eight subelements in the protein filament arranged in all possible hierarchical patterns. The definition of subelements and their arrangement are those shown in panel A. To present the results, we use the following nomenclature $\{b_N, b_{N-1},..,b_2, b_1\}$ to uniquely describe the various hierarchical structures. The values of b_i in this expression thereby define the number of elements that are found in parallel with each other at a particular hierarchical level, from the largest to the smallest elements. A single alpha-helix is characterized by $\{1\}$, a bundle of two alpha-helices $\{2\}$

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resembles a coiled-coil structure (CC2), and a bundle of four alpha-helices {4} resembles a four-fold coiled coil structure (CC4; see inset in plot). The {8} structure represents a single bundle of eight alpha-helices in parallel; the {2,4} structure represents a fiber composed of two bundles of four alpha-helices; the {2,2,2} structure represents a fiber composed of two bundles of two bundles of two alpha-helices each; the {4,2} structure represents a fiber composed of four bundles of two alpha-helices. Panel C shows results for 16,384 subelements in the protein filament. An analysis of the distribution of structures and their performance shows that most data points (>98 %) in panel d fall onto the banana-curve. Only less than 2% of all structures lead to high strength and high robustness. This analysis shows how high-performance materials can be made out of relatively weak constituents such as alpha-helices that are bonded by mechanically inferior H-bonds (Keten and Buehler 2008; Qin, Cranford et al. 2009).
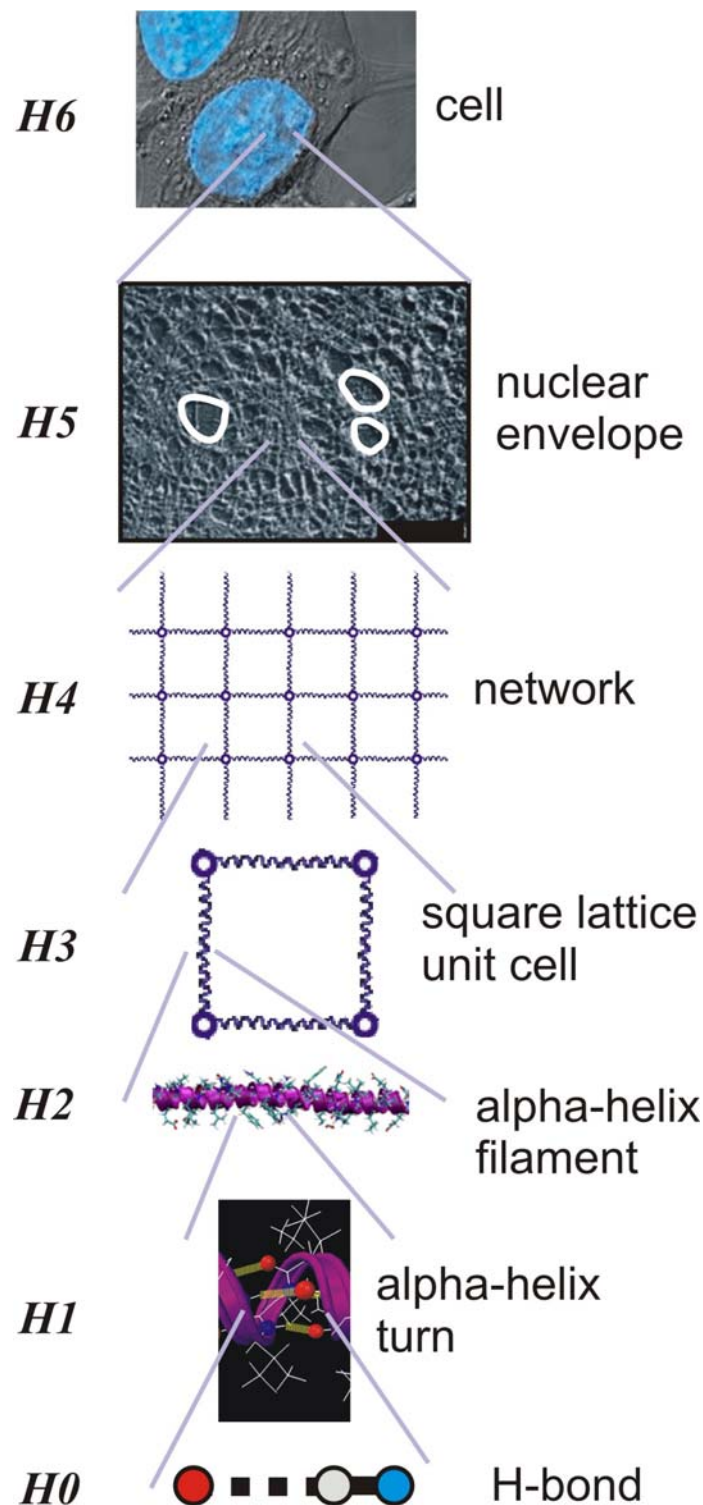
Figure 4: Hierarchical structure of a simplistic model of the intermediate filament protein network in cells (figure adapted from (Ackbarow, Sen et al. 2009)). Seven levels of hierarchies are considered, from intrabackbone hydrogen bond (*H0*), alpha-helical turns (*H1*), filaments of alpha-helices (*H2*), to the representative unit cell (*H3*) of protein networks (H4) that form the cell nucleus (defects in the network highlighted) (*H5*) of eukaryotic cells (*H6*). Even though this is a simple model system, it enables us to illustrate the major points associated with the deformation mechanics of hierarchical biological materials throughout multiple scales. The structure at each level is adapted to provide a suitable mechanical

response and plays a key role in defining the overall mechanical behavior. Unfolding of alpha-helix turns (*H1*) proceeds via breaking of strong clusters of 3-4 H-bonds (*H0*). The large deformation of alpha-helix filaments (with maximum strains of 100-200 %) (*H2*) is enabled by the serial arrangements of many alpha-helical turns (*H1*). The severe stiffening of the filaments is enabled by alpha-to-beta-sheet transitions and backbone stretching, followed by interprotein sliding at the filament level (*H2*), is a direct consequence of the structure of coiled alpha-helical proteins (it is noted, that in the simple model system and case study reviewed here interprotein beta-sheet formation and sliding is not considered, as only a single alpha-helical protein filament is modeled; however, recent studies of realistic intermediate filaments showed that the above mentioned mechanisms indeed occur (Qin, Kreplak et al. 2009)). The lattice structure (*H3*) is the key to facilitate large strain gradients in the protein network, enabling gigantic strain gradients at virtually no energetic cost at the network level (*H4*). This behavior is crucial for the flaw-tolerant behavior of the nuclear envelope level (*H5*), which is relevant to provide robust structural support to cells under large deformation (*H6*).
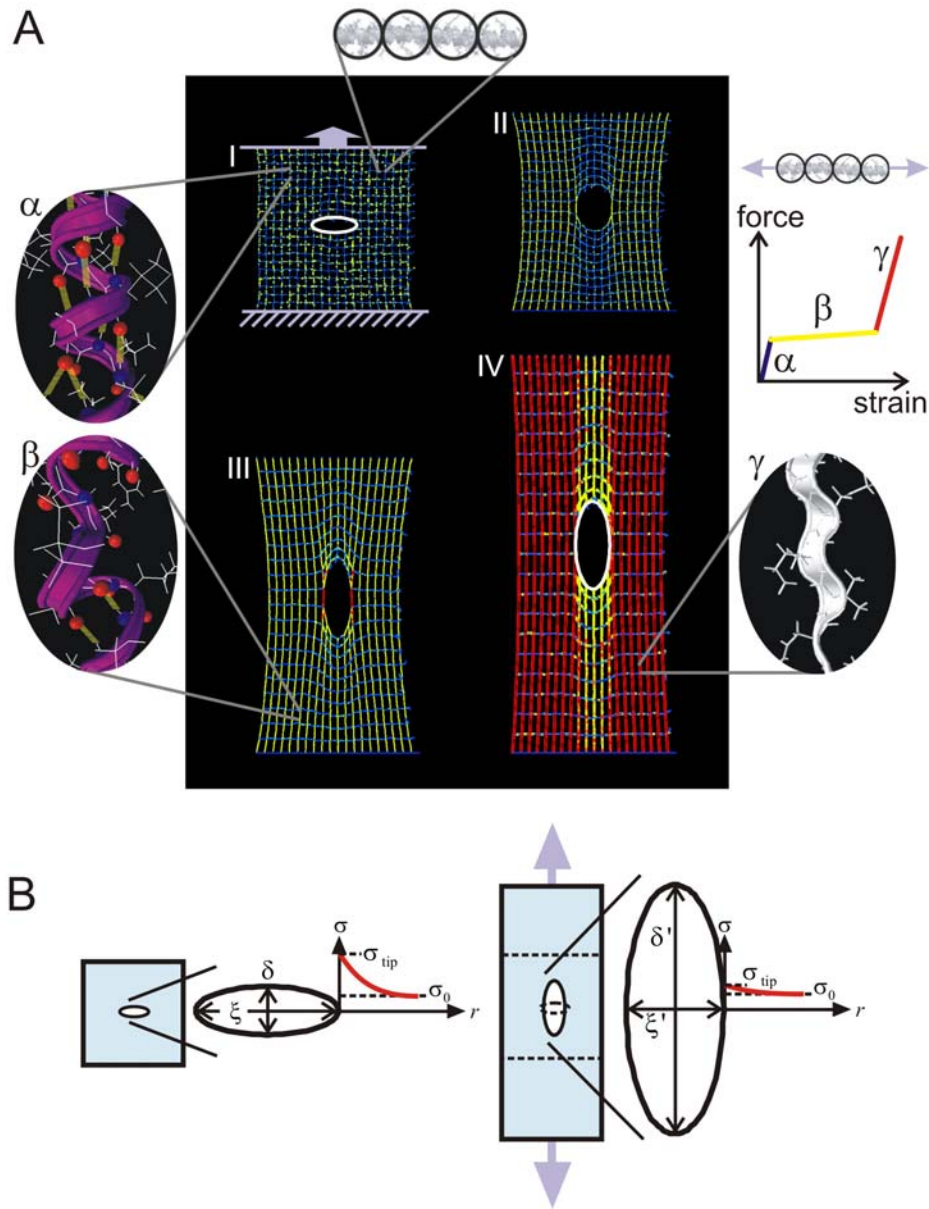
Figure 5: Deformation field of the protein network (plot adapted from (Ackbarow, Sen et al. 2009)). The transformation of the crack shape can be recognized from the plots. Panel A shows an overview over different deformation stages. Panel B shows the stress field close to the crack tip, illustrating how the transformation from a horizontal crack to a vertical crack reduces the concentration of stresses at the tip of the crack. The cascaded activation of mechanisms at multiple levels is a remarkable behavior ubiquitously found in biological materials that renders them capable to withstanding extreme deformation and large loads.
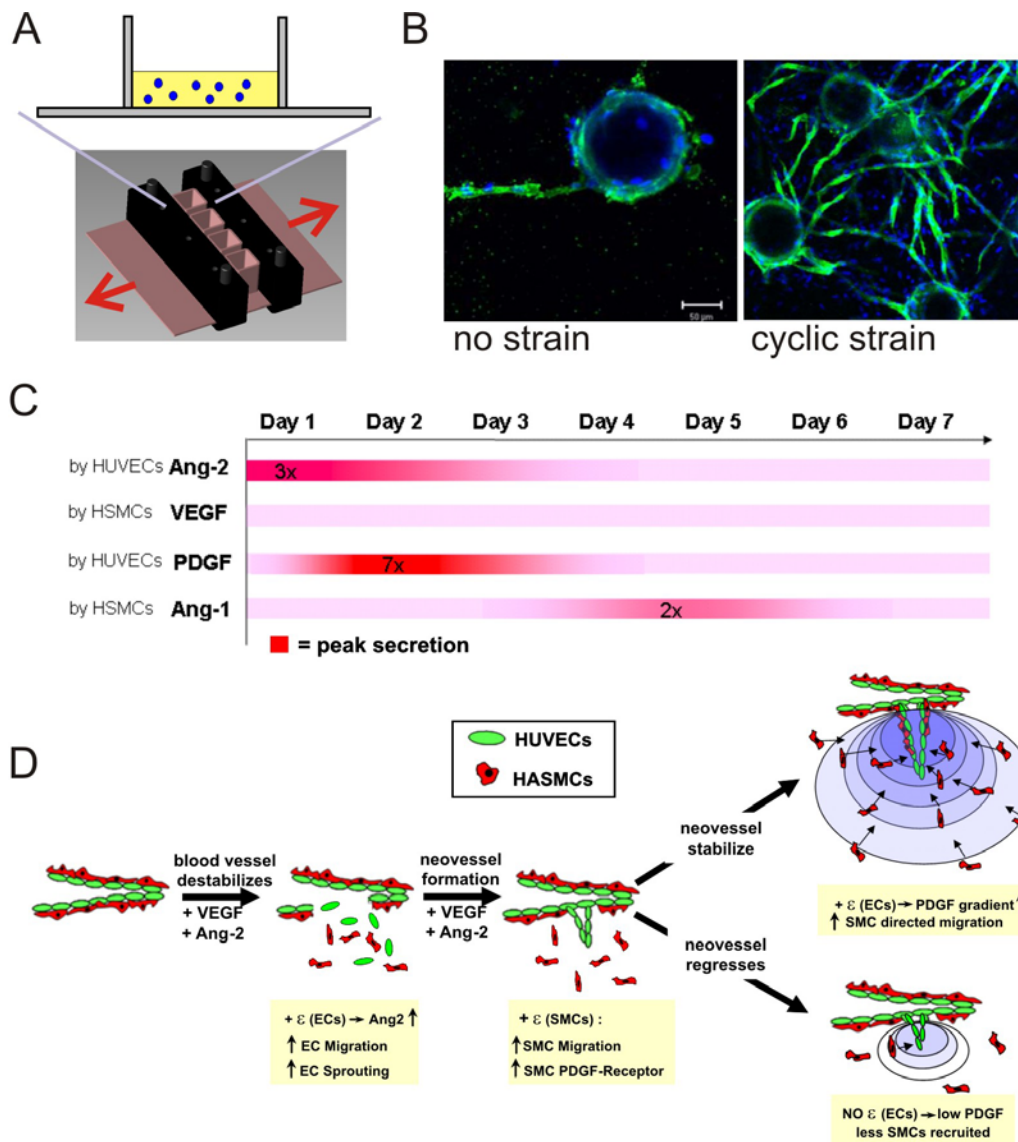
Figure 6: Examining the mechanobiological mechanism of angiogenesis (Yung, Chae et al. 2009). Human umbilical vein endothelial cells (HUVECs) and human aortic smooth muscle cells (HASMCs) were used as model cell types to study the angiogenic process. Panel A: Strain microdevice used to apply cyclic strain to cell cultures (conferred via straining PDMS wells, the culture substrate, as shown in the inlay) (Yung, Vandenburgh et al. 2009). Panel B: A model system to investigate angiogenic sprouting, demonstrated using HUVECs seeded onto microcarriers and embedded into fibrin gels, is induced to form tube-like extensions in response to microenvironmental cues. Images show cultures both under static conditions (left, no strain) and under application of cyclic strain (right). Image documentation of sprouts are observed after 5 days of culture, using confocal microscopy, where endothelial CD31 membrane receptors are immunostained with FITC (green) and the nucleus with DAPI (blue). The images qualitatively show that application of cyclic strain significantly enhances sprout formation, and suggests that mechanical cues alone are capable of triggering the formation of nascent blood vessels. Panel C: Temporal secretion profile of angiogenic factors secreted by HUVECs and HASMCs. In response to cyclic strain, Ang-2 and PDGF are both upregulated, where the Ang-2 peak occurs at day 1, and the PDGF peak at day 2, in a temporal fashion relevant to their physiologic role in angiogenesis Panel D: Potential mechanism of strain regulated angiogenesis, representing a coupled mechanical-biochemical process. The unregulated secretion of Ang-2 results in the formation of HUVEC sprouts. The subsequent

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secretion of PDGF resulted in a chemotactic recruitment effect on HASMCs, necessary to stabilize nascent blood vessels in order to form the characteristic double layer comprised of HUVECs and HASMCs in small blood vessel tissues. Under a lack of cyclic strain, both the initial Ang-2 secretion and PDGF secretion are reduced, resulting in reduced angiogenic activity. This study showed that autocrine signaling via activation of Ang-2 may be the mechanistic pathway by which ECs transduce mechanical signals to process angiogenic responses. Furthremore, cyclic strain modulates the intercellular communication between EC and SMC by upregulating chemotactic paracrine factors secreted by ECs to recruit SMCs.
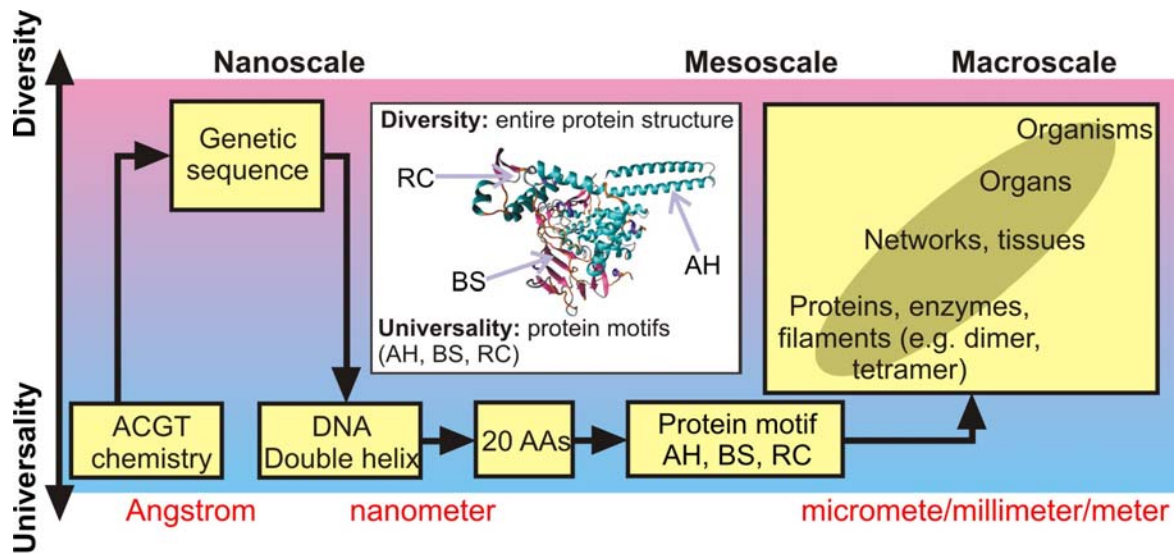
Figure 7: Universality and diversity of the structural makeup of biological protein materials, as discussed in (Buehler and Yung 2009). The illustration shows that universal and diverse protocols are distributed heterogeneously across different hierarchical levels in the material. The inlay shows a visualization of the topoisomerase protein, whose biological role is to cut strands of the DNA double helix. This example illustrates how universal motifs define the overall functional properties of this protein, while the entire protein structure represents diversity.
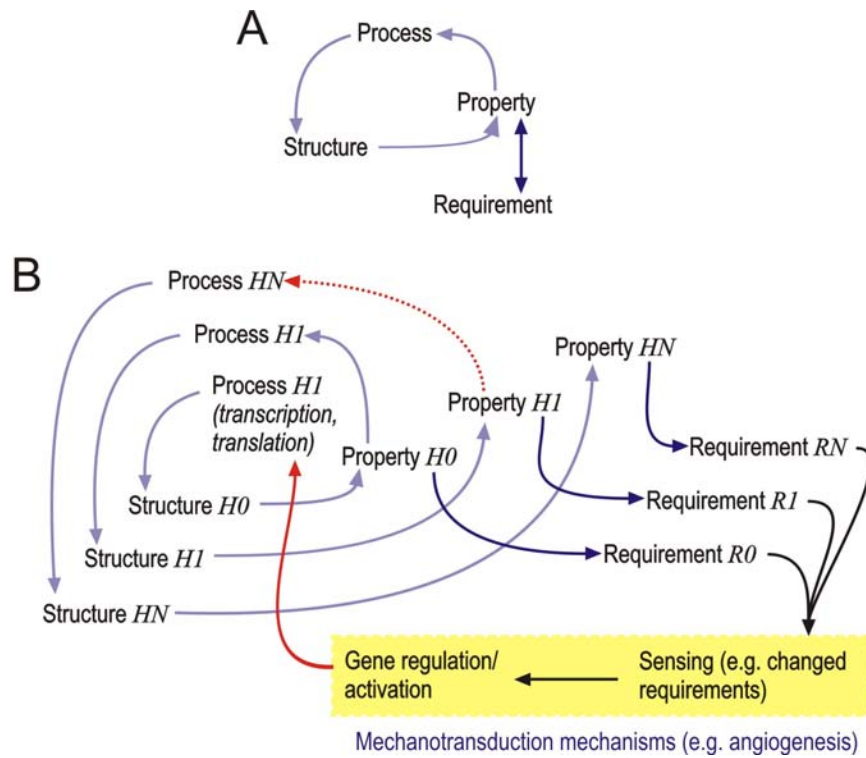
Figure 8: Conventional materials science paradigm (panel A) and hierarchical materials science paradigm applied to biological systems, referred to as materiomics (panel B). The variables *Hi* refer to hierarchy levels *i*=0..*N,* and *Ri* refer to material property requirements at hierarchy levels *i*=0..*N* (see Figure 4 for an example of a hierarchical structure with labels for different levels). Figure adapted from (Buehler and Yung 2009). A crucial issue in studying the material properties of biological systems is the existence of feedback loops, for example realized through mechanotransduction mechanisms. As illustrated in the example of angiogenesis (see Figure 6), environmental cues (in our study, mechanical strain) are sensed and result in changes to gene regulation (realized through a cascade of biochemical signals), which in turn change the structural makeup of tissues at multiple levels (in our study, the formation of new blood vessels through sprouting).