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Structural and physical basis for the elasticity of elastin

Camille Depenveiller[1,2], Stéphanie Baud[1], Nicolas Belloy[1], Brigida Bochicchio[3], Jany Dandurand[4], Manuel Dauchez[1], Antonietta Pepe[3], Régis Pomès[5,6], Valérie Samouillan[4] and Laurent Debelle[1]

[1]UMR URCA/CNRS 7369, Matrice Extracellulaire et Dynamique Cellulaire (MEDyC), UFR Sciences Exactes et Naturelles, SFR CAP Santé, Université de Reims Champagne-Ardenne, Reims, France; [2]Unité de Génie Enzymatique et Cellulaire UMR 7025 CNRS, Université de Picardie Jules Verne, Amiens, France; [3]Laboratory of Bioinspired Materials, Department of Science, University of Basilicata, Potenza, Italy; [4]CIRIMAT UMR 5085, Université Paul Sabatier, Université de Toulouse, Toulouse, France; [5]Molecular Medicine, Hospital for Sick Children, Toronto, ON, Canada and [6]Department of Biochemistry, University of Toronto, Toronto, ON, Canada

Review

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Corresponding author:
Laurent Debelle;
Email: laurent.debelle@univ-reims.fr

Abstract

Elastin function is to endow vertebrate tissues with elasticity so that they can adapt to local mechanical constraints. The hydrophobicity and insolubility of the mature elastin polymer have hampered studies of its molecular organisation and structure-elasticity relationships. Nevertheless, a growing number of studies from a broad range of disciplines have provided invaluable insights, and several structural models of elastin have been proposed. However, many questions remain regarding how the primary sequence of elastin (and the soluble precursor tropoelastin) governs the molecular structure, its organisation into a polymeric network, and the mechanical properties of the resulting material. The elasticity of elastin is known to be largely entropic in origin, a property that is understood to arise from both its disordered molecular structure and its hydrophobic character. Despite a high degree of hydrophobicity, elastin does not form compact, water-excluding domains and remains highly disordered. However, elastin contains both stable and labile secondary structure elements. Current models of elastin structure and function are drawn from data collected on tropoelastin and on elastin-like peptides (ELPs) but at the tissue level, elasticity is only achieved after polymerisation of the mature elastin. In tissues, the reticulation of tropoelastin chains in water defines the polymer elastin that bears elasticity. Similarly, ELPs require polymerisation to become elastic. There is considerable interest in elastin especially in the biomaterials and cosmetic fields where ELPs are widely used. This review aims to provide an up-to-date survey of/perspective on current knowledge about the interplay between elastin structure, solvation, and entropic elasticity.

Foreword

Since its first traceable report in the literature in the early twentieth century (Engeland, 1925), elastin has attracted much attention, especially in recent decades.

In the 1950s, the first purification methods were devised to extract elastin from tissues (Lansing *et al.,* 1952; Partridge *et al.,* 1955). Partridge *et al.* (1955) observe that elastin absorbs water and swells in a hydrated medium. At that time, despite a relative lack of information about its structure, the first theory for elastin elasticity was proposed (Hoeve and Flory, 1958). However, until 1960, the number of articles containing the term 'elastin' as referenced in PubMed (Figure 1) remained low. From the 1960s through the late 1970s, elastin started to attract more and more attention. This period, marked by the discovery of elastin cross-links by Partridge *et al.* (1963), was dominated by publications on the chemical and biochemical aspects of elastin. Purification methods were devised to extract and isolate elastin from tissues (reviewed in Mecham, 2008) and the amino-acid composition of elastin was established (Gotte *et al.,* 1963). Thanks to the efforts of numerous groups in the chemical and biochemical community, fragments from elastin, mostly from bovine *ligamentum nuchae*, were identified and characterised, as was their ability to coacervate (Partridge *et al.,* 1955; Ledvina and Bartoš, 1968; Urry *et al.,* 1969). In parallel, in the biomedical field, the evidence for a link between structural degradation of elastin and pathological conditions was increasingly documented, and elastin emerged as a critical compound involved in arterial calcification (Urry, 1974; Sandberg, 1976).

During the 1980s, considerable efforts were also made to understand elastin genetics and gene regulation. For an excellent overview of elastin knowledge at that time, see (Cunningham, 1987).

In August 1987, the sequence of human tropoelastin was published (Indik *et al.,* 1987). Although the global organisation of elastin domains was revealed, it was also clear that elastin's

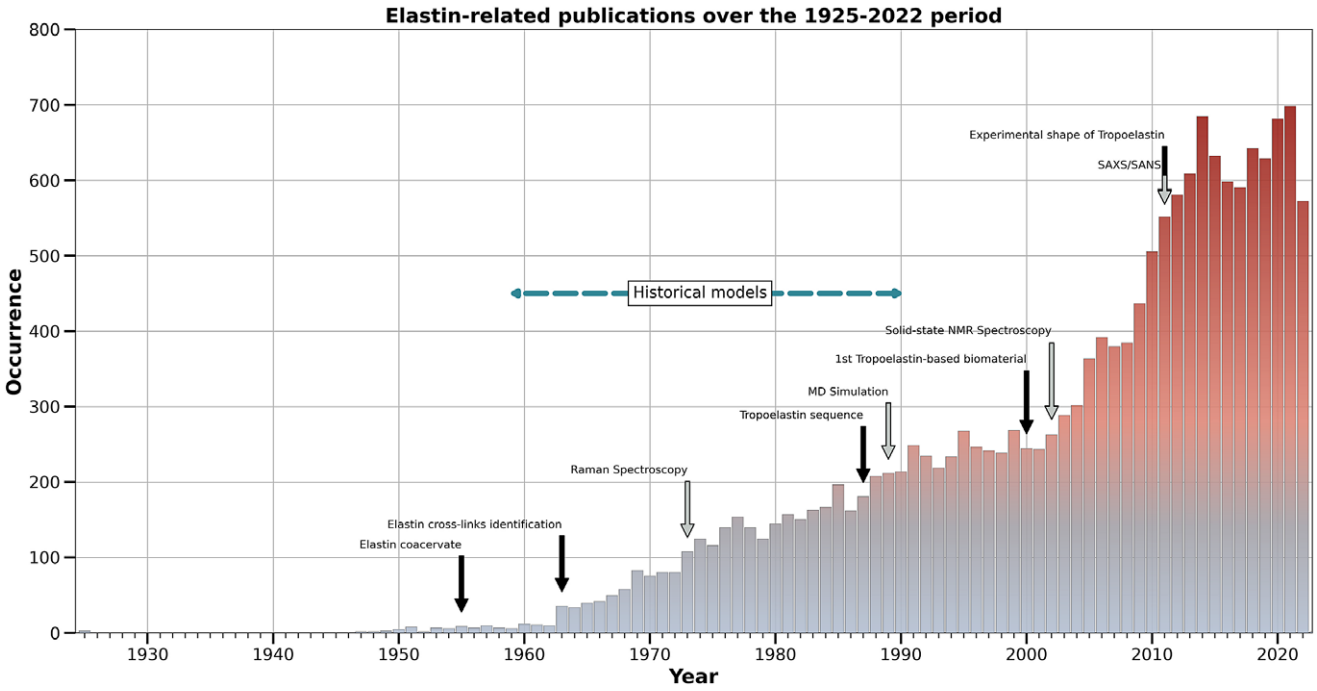

Figure 1. Time evolution of the number of elastin-related articles published since 1925. The literature search was performed in PubMed. The term 'elastin' was searched in the title, abstract and keywords. Black vertical arrows delineate important milestones in the field of elastin biology. The grey arrows point out important dates where biophysical methods were successfully applied to elastin material.

amino acid sequence and organisation were very different from that of known globular proteins. The structure of elastin remained a puzzle!

Since that time, the number of publications on elastin has grown rapidly and elastin biology has become a central aspect of matrix biology, thanks notably to advances made in molecular biology and matrix genetics. Renewing elastin and promoting elastogenesis has become a major challenge. Therefore, elastin-inspired materials have been developed as they offer promising alternative solutions to replacing native elastin.

Since 2000, the literature on elastin has considerably widened and evolved. In particular, important data have been gathered on elastin and tropoelastin. Elastin-mimics have tackled new problems and continue to develop in various fields.

Nevertheless, despite significant advances, many questions about elastin remain. Of central importance to the function of elastin is the relationship between structure and elasticity. Can the structure of elastin be captured by a unique or small number of representative conformations, or is it best described as a large ensemble of highly degenerate conformations with vanishing propensities? How do these structural properties change under stress, how do they evolve in time upon extension, recoil, or stress relaxation, and how do they give rise to elasticity?

This review aims to provide a state-of-the-art survey of current knowledge and emerging paradigms regarding the structural and physical basis for the elasticity/mechanical properties of elastin, what is known and what is predicted.

Introduction

The extracellular matrix is a three-dimensional macromolecular network offering adhesion sites, mechanical protection, and regulatory cues to cells (Kyriakopoulou *et al.*, 2023). The reversible extensibility of tissues is mostly due to the presence of elastic fibres in their extracellular matrix (Gosline *et al.*, 2002). These fibrillar structures are particularly abundant in tissues where resilience is required. Consequently, elastic fibres are found in mechanically active organs such as skin, lung, ligaments, and large arteries. Their presence fulfils a physiological need as they ensure the proper and continuous functioning of these tissues. Depending on their location, elastic fibres can be present in various architectures nevertheless, their composition and molecular structure are remarkably invariant between organs (Heinz, 2021; Schmelzer and Duca, 2022).

In mature tissues, elastic fibres are formed by a central component, elastin, surrounded by a mantle of microfibrils, mostly composed of fibrillin-1 and other glycoproteins (Singh *et al.*, 2021). While microfibrils appear to act as a protective scaffold, the central elastin core, which represents about 90% of the fibre, is responsible for resilience as well as elastic recoil. The formation of elastic fibres, which includes the deposition of elastin, is a process referred to as elastogenesis. This complex process involves the timely expression of various genes during foetal stages and early infancy (Wagenseil and Mecham, 2007). As describing this process is beyond the scope of this review, interested readers should consider the following recent reviews (Heinz, 2021; Keeley, 2021; Schmelzer and Duca, 2022). In this review, we focus on elastin, an insoluble and hydrophobic protein formed by cross-linking of its soluble monomeric precursor, tropoelastin.

Elastin appeared late in vertebrates evolution (Keeley, 2013). This macromolecule is characterised by exceptional mechanical properties (Gosline *et al.*, 2002) and longevity. With a half-life of 70 years in lung (Shapiro *et al.*, 1991), elastin is one of the most stable and durable proteins known in humans. It is extremely resistant to mechanical deformations and is chemically stable. Indeed, few enzymes can degrade mature elastin (Heinz, 2020). The exceptional stability of elastin is mostly due to the extreme hydrophobicity of its chains and its numerous cross-links.

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The function of elastin is to endow biological tissues with elasticity. In contrast with Hookean linear elasticity, which is a property shared by all solids subjected to small deformations (<1%), elastin is an elastomeric polymer supporting large deformations (~150%) and characterised by rubber-like elasticity of entropic origin (Urry *et al.,* 2002). These properties are imparted by the cross-linked structure of the polymer and modulated by solvent conditions: the elasticity of elastin is achieved only when tropoelastin chains are polymerised and swollen in water. In fact, dry elastin is brittle.

Despite over 80 years of study (Meyer and Ferri, 1937), neither the molecular basis for the self-assembly of elastin nor the structure of the functional, polymerised state are fully understood. Competing structural models of elastin have been proposed, which span a range from fully ordered (Venkatachalam and Urry, 1981) to fully disordered (Hoeve and Flory, 1958, 1974) and emphasise either the hydrophobic effect (Weis-Fogh and Andersen, 1970; Gray *et al.,* 1973; Venkatachalam and Urry, 1981; Li *et al.,* 2001a) or conformational entropy (Hoeve and Flory, 1958, 1974; Dorrington *et al.,* 1975) as the dominant contribution to the elastic recoil force.

High conformational entropy is the key feature of the random network model of elastin (Hoeve and Flory, 1958, 1974). Based on thermo-elasticity measurements indicating that elastin's recoil force is 85% entropic, Hoeve and Flory postulated that elastin's structure is an isotropic, rubber-like polymer network consisting of cross-linked, random chains. In this model, elastic recoil is driven by the difference in conformational entropy (i.e. disorder) of the polypeptide chains, which increases upon collapse, relative to the stretched state, in which conformational entropy approaches zero – much as the structural disorder of cooked spaghetti differs from that of its uncooked counterpart.

In contrast to the random coil model, specific secondary structure preferences and significant burial of non-polar groups are common features of several other structural models of elastin (Weis-Fogh and Andersen, 1970; Gray *et al.,* 1973; Venkatachalam and Urry, 1981; Li *et al.,* 2001a). Elastin's highly hydrophobic sequence led several groups to propose that the hydrophobic effect, rather than conformational entropy, is the major entropic driving force for elasticity (Weis-Fogh and Andersen, 1970; Gray *et al.,* 1973; Gosline, 1978). In these models, non-polar side chains are successively exposed to water upon extension of the polypeptide and buried upon recoil. Several models were proposed in which non-polar side chains are arranged to exclude water molecules; the most ordered of these models is the β-spiral, which consists of repeated β-turns (Venkatachalam and Urry, 1981).

To go beyond these idealised, qualitative, and seemingly contradictory models, it is essential to gain insights into the molecular basis of elasticity as grounded in high-resolution structural information, in which conformational disorder is quantified statistically, in terms of structural propensities.

Definitions

In the literature, the term 'elastin' is sometimes misused. To clarify the vocabulary devoted to elastin, we provide here a glossary of the main terms.

Elastic fibres– These elastic structures are large fibres located in the extracellular matrix of tissues requiring extensibility with resiliency (Kozel *et al.,* 2011). They can have various 3D organisations depending on the tissue and its local mechanical constraints. For instance, they form a loose fibrillar network in skin and nested tubular structures in the arterial wall.

Elastin– This macromolecule is made of assembled tropoelastin molecules bound covalently together upon the formation of specific cross-links (Partridge *et al.,* 1963). The molecular weight of elastin can be considered as infinite as it forms a very large and extensive network. For instance, arterial elastin forms a continuous network encompassing the elastic lamellae of all arterial conducts (as they are interconnected). The term 'elastic network' is sometimes used for elastin.

Tropoelastin– This molecule is the expression product of the *eln* gene. It is the monomer that will give rise to elastin once polymerised. The *eln* gene can encode various tropoelastin isoforms due to alternative splicing (Indik *et al.,* 1987). This process is regulated in time and space (Boyd *et al.,* 1993). Therefore, elastin is made of various tropoelastin building blocks. Full-length human tropoelastin (UniProt accession number P15502–3) comprises 786 residues and has an apparent molecular weight of 70 kDa. Post-translational modifications of tropoelastin include the cleavage of its signal peptide and the hydroxylation of selected prolyl sites (Schmelzer *et al.,* 2016). Importantly, these modifications are absent in tropoelastins recombinantly expressed in bacteria (Jensen *et al.,* 2000). The overall organisation of full-length human tropoelastin is depicted in Figure 2.

Cross-links– This term refers to the covalent cross-links formed between tropoelastin molecules during elastogenesis. Those cross-links are formed following the action of lysyl oxidases on exposed lysyl residues to form allysyl moieties that spontaneously condensate with modified or un-modified lysyl residues resulting in covalent bi-, tri- or tetrafunctional cross-links. The typical cross-links of elastin are desmosine and isodesmosine. Allysine aldol and lysonorleucine are also found. Importantly, recent experimental findings establish that elastin cross-links are formed randomly both inside and between tropoelastin chains (Schräder *et al.,* 2018; Hedtke *et al.,* 2019).

In the biomaterial field, numerous studies report the presence of cross-linked elastin chains. In these cases, cross-linking is artificial and can be achieved by chemical, physical, thermal, or enzymatic processes.

Assembly– The assembly of elastin designates both the process occurring during elastogenesis when tropoelastin molecules organise in 3D before being cross-linked to form elastin, and the final 3D organisation of tropoelastin molecules.

Elastin domains– These domains are numbered. They are not domains (in the 3D acceptance of the term) like those found in globular or modular proteins. They correspond to the sequences encoded by the exon with matching number in the *eln* gene. For instance, domain 24 is the domain encoded by exon 24. This denomination is made possible because of the cassette-like organisation of the gene, where each exon encodes for a hydrophobic or hydrophilic domain. It is important to note here that this numbering refers to the structure of bovine *eln* gene (36 exons), since exons 34 and 35 are absent from the genomic sequence of human tropoelastin.

Hydrophobic/hydrophilic domains– These domains, that alternate in the sequence of tropoelastin, correspond to tropoelastin sequence domains mostly made of G, P, A, V, and L residues for the first one, while the second are characterised by the presence of the lysyl residues that are used for cross-linking. The latter can also be termed cross-linking domains (see below). The hydrophilic qualification of these domains must be explained. In general, whilst the entire sequence of tropoelastin is extremely hydrophobic, several segments appear less hydrophobic due to the local presence of

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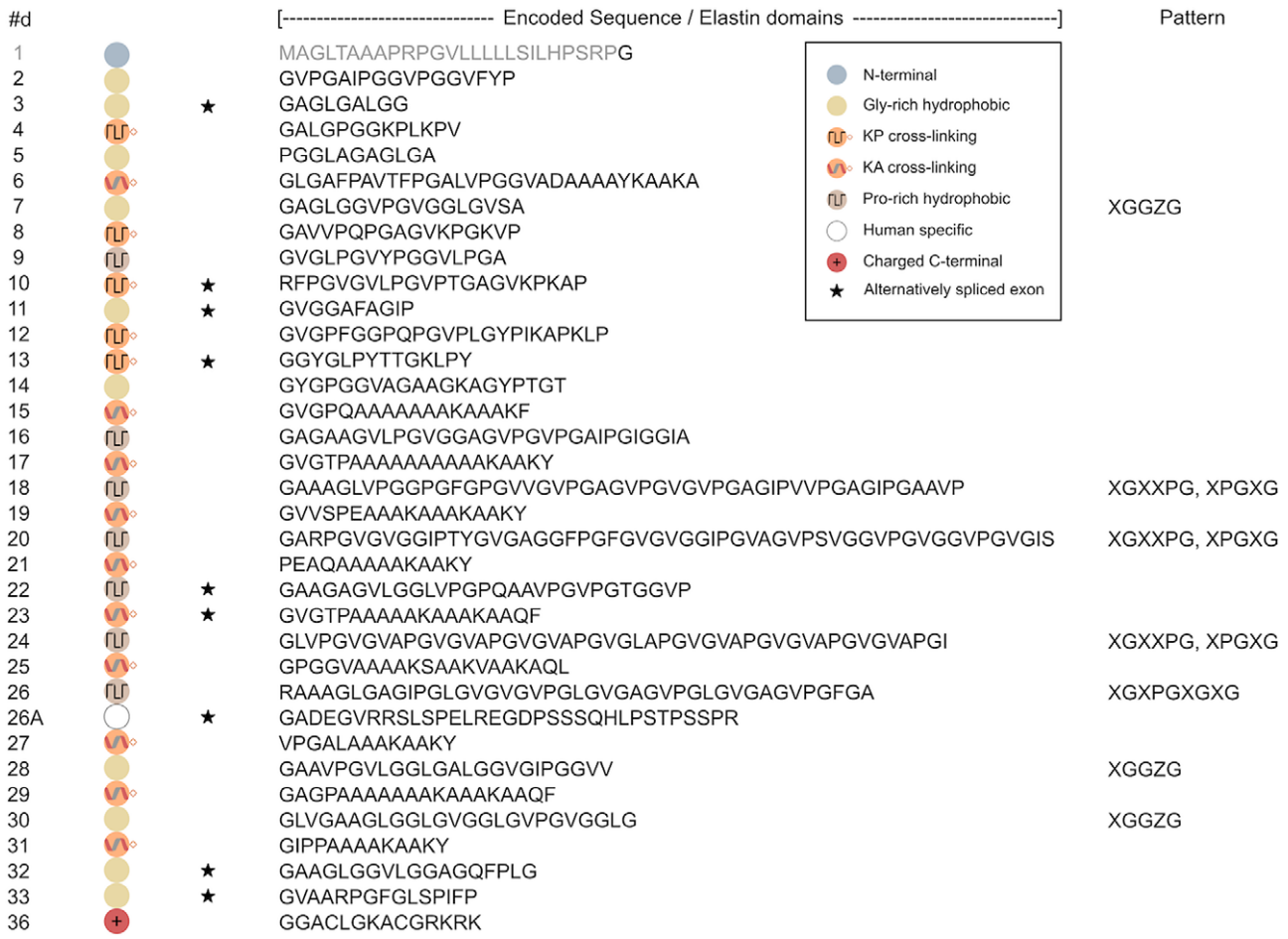

Figure 2. Overall organisation of full-length human tropoelastin. #d, number of the domain. This number is the number of the exon encoding the sequence. Exon numbering follows the structure of the bovine *eln* gene. The symbols on the left of this number indicate the type of domain (see inset). The sequence of each domain is reported as well as the typical patterns found inside. The sequence in grey in domain 1 corresponds to the signal peptide. The protein sequence of the human-specific domain 26A is highly uncharacteristic of elastins. It is rarely expressed, and may be artefactual (Bisaccia *et al.,* 1998).

charged residues, that is, lysyl residues. These regions are considered as hydrophilic as compared to the rest of the sequence. In elastin, lysyl residues engage in cross-links and lose their charges making of these initially 'hydrophilic' cross-linking domains more hydrophobic ones in the mature elastin molecule.

Elastic domains– In contrast with the *cross-linking domains*, the elastic domains are thought to confer elasticity. It is generally accepted that these domains are hydrophobic (Rauscher *et al.,* 2006). Nevertheless, it must be stressed that elasticity is only achieved after proper cross-linking of tropoelastin chains. Therefore, sequences in the cross-linking domains must also somehow affect elastic properties.

Mini-elastin– A mini-elastin is a protein model of elastin created by genetic engineering. The building block of a mini-elastin is a protein sequence containing alternating hydrophobic and cross-linking domains of elastin, thereby mimicking the sequence organisation of tropoelastin. These building blocks are polymerised to form a 3D network. The domains within mini-elastin are often simplified versions of the natural domains, that is, they are regular repeats of a given sequence motif. Despite being a synthetic and simplified versions of elastin, the mini-elastins are excellent models for studying elastin-related processes (Keeley *et al.,* 2002; Bellingham *et al.,* 2003; Vidal Ceballos *et al.,* 2022).

Soluble elastin– Purified elastin is totally insoluble in water. Consequently, methods were devised to make it soluble. The term 'soluble elastin' designates the product resulting from the solubilisation of purified elastin. Depending on the hydrolysis agent, usually oxalic acid or KOH, the resulting soluble elastin is named α-elastin (Partridge *et al.,* 1955) or κ-elastin (Jacob and Hornebeck, 1985), respectively. Soluble elastins are mixtures of cleaved elastin peptides or chains of various lengths.

Elastin peptides– These peptides are the degradation/fragmentation products of mature elastin. They are sometimes called *elastin-derived peptides* (EDP) because they truly originate from elastin. These peptides can have various lengths and some of them exhibit biological activities. For a review of these aspects, see (Schmelzer and Duca, 2022). The term 'elastin peptides' also designates synthetic peptides exhibiting typical elastin sequences. In this case, those are commonly produced by organic synthesis. By extension, any peptide harbouring a sequence resembling an actual elastin sequence, or repeats of such sequence, is termed *elastin-like peptide* (ELP). The development of ELP has led to elastin-inspired technologies.

Coacervation– The coacervation process is a reversible phase separation in which the peptides in solution form 'liquid droplets' that eventually merge to form a hydrogel, the coacervate (Partridge

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et al., 1955). Tropoelastin, soluble elastin, elastin peptides, mini-elastin and ELP exhibit this behaviour. The coacervation process occurs at a fixed temperature, which depends on the nature of the solute. When this critical temperature is reached, coacervation occurs if the solute is sufficiently concentrated. Coacervation is influenced by the pH and ionic strength of the medium and is one of the key processes driving elastin assembly (Yeo *et al.,* 2011). The hydrophobic domains of elastin are responsible for this self-assembly (Rauscher *et al.,* 2006).

Aggregation– This term designates the formation of irreversible interactions between peptide chains. This behaviour is typical of amyloidogenic sequences present in elastin, notably in domain 30 (Kozel *et al.,* 2003; Tamburro *et al.,* 2005). In older papers, when the amyloidogenic behaviour of elastin was unknown, the term 'aggregation' was used instead of 'coacervation' (Bressan *et al.,* 1986).

Elastin structure

Since elastin elasticity has an entropic origin, it was initially thought that, as for typical elastomers, elastin must be mostly disordered (Hoeve and Flory, 1974). However, elastin elasticity requires that the hydrophobic chains are cross-linked and hydrated. Thus, even if conformational entropy is a key feature contributing largely to elastin entropy (and function), it is not sufficient to explain it. As a disordered protein, tropoelastin/elastin challenges Anfinsen's dogma of protein structure (Anfinsen, 1972), the so-called 'thermodynamic hypothesis'. In this postulate, Anfinsen states that a protein folds in a unique configuration, corresponding to its local free energy minimum, that is solely defined by the sequence of the protein itself. This could be summarised by the following logical chain of events:

sequence → folding → set of stable conformations → function.

As we will underline, elastin follows another path:

sequence → partial folding → no stable conformation → function.

In fact, elastin is characterised by its inability to pack its secondary structure elements into a stable tertiary structure, making of it a prototypical member of the intrinsically disorder proteins group (Muiznieks *et al.,* 2018).

Elastin organisation

The elastic network is a 3D object endowing tissues with rubber-like elasticity. In this network, the 3D arrangement of polymerised tropoelastin explains and sustains this function. Consequently, most structural models of elastin have been devised to explain both its assembly and elastic behaviour in keeping with what was known at the time.

Microscopic observations

The first insight into elastin assembly came from histological observations. As elastin possesses few charged residues, its affinity for common dyes was low and specific dyes were developed to detect it in tissues (Dempsey and Lansing, 1954). Unfortunately, their use did not permit to assess the fine structures of the imaged elastic fibres. However, they underlined the regular and cylindrical shape of elastic fibres, and the possibility of fibrillar elements, at least in *ligamentum nuchae.* Further, histological analysis clearly demonstrated that the 3D organisation of the elastic network was tissue-dependent (Partridge, 1963).

The application of electron microscopy to the study of elastic fibres was not possible until thin section preparation was possible. In 1986, the group of Dino Volpin in Padova (Italy) demonstrated that, above 40 °C, tropoelastin assembled into thin 5 nm-thick filaments that could, in turn, merge to form an elastin fibre adopting a rope-like appearance (Bressan *et al.,* 1986). Elastin was conventionally described as unstructured and amorphous based on transmission electronic microscopy observations where the elastic fibres appeared devoid of any internal order/organisation. This discrepancy is explained by the fact that most reports used conventional sample preparation techniques. However, a possible internal organisation of elastin had been proposed earlier using quick freeze deep etch methods to observe elastic fibres ultrastructure (Pasquali Ronchetti *et al.,* 1979). The fact that elastin sample preparation greatly influences its appearance is now accepted (Kozel and Mecham, 2019), as is the rope-like organisation of elastin within the fibre.

Historical models

The *random chain network* (Hoeve and Flory, 1958) was the first model proposed for elastin. It describes elastin as an ideal rubber and explains its mechanical behaviour with reference to the classical theory of rubber elasticity. In this model, elastin is described as a collection of long, unordered, cross-linked hydrophobic chains swollen in water.

In 1970, an alternative model was proposed to explain elastin structure and elasticity (Weis-Fogh and Andersen, 1970). In the *liquid drop model*, tropoelastin molecules are assembled in a regular 3D arrangement creating intramolecular spaces filled by bulk water. According to this model, upon extension, the individual tropoelastin units change shape and expose their hydrophobic side chains to bulk water resulting in the formation of low-entropy water of hydrophobic hydration. This model therefore proposes that hydrophobic hydration would be the driving mechanism for entropic elasticity and hence that water is a critical actor in mediating elastin elasticity. This model was supported by the experimental finding that elastin contains 'water spaces' (Partridge, 1967). However, Hoeve and Flory (1974) contested this view by stating that 'polymer backbone, not solvent, must bear the force'.

The *oiled coil model* proposed in 1973 (Gray *et al.,* 1973) was devised following extensive analysis of partial sequences derived from elastin. This model describes elastin as a network where the cross-linking domains are rigid α-helices while the hydrophobic domains are organised in a spring-like structure explaining elasticity. In this coil structure, glycyl residues are in external positions where they can interact with the solvent, while the more hydrophobic residues are buried. In this configuration, the backbone is fully hydrated. This model suggests that in a static state, elastin chains are organised in a particular way that would be lost upon extension but that this organisation could be fully recovered after stretching. In this context, both the chains and water seem to be important for elasticity.

After considerable work performed on the VPGVG sequence, which is tandemly repeated in bovine elastin, Urry proposed the *β-spiral model* in 1977 (Urry and Long, 1977). In this model, repeated motifs form consecutive *β*-turns that organise in a helix-like structure, which would be expected to manifest as a filamentous organisation at the supramolecular level. In the folded *β*-spiral, water surrounds *β*-turn chains that are free to rotate about their backbone bonds. These moieties are expected to rock or 'librate'. According to Urry's model, the entropic nature of elastin originates

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from these librational motions that decrease when the polymer is stretched.

When the full sequence of tropoelastin became available, it became clear that the sequences that gave rise to the oiled coil and β-spiral model only represented a small portion of the sequence and could not fully explain elastin 3D structure. While the oiled drop model was rapidly abandoned by the community, the β-spiral was still discussed because numerous experimental data suggested that the β-turn would be an important secondary structure in elastin.

After extensive conformational analysis of synthetic elastin-derived sequences, Tamburro's *sliding turn model* was proposed (Tamburro, 1990). As Urry's, this model mainly focuses on the conformational behaviour of the hydrophobic domains. But while Urry proposed regular arrangement of turns, the sliding turn model states that these regions are characterised by the presence of labile and rapidly exchanging secondary structure features, including turns, distorted β-stands or poly-proline II elements (Tamburro, 2009). This dynamical interchange is amplified by hydration and makes turns 'slide' along the chains in a perpetual movement (Tamburro *et al.,* 1990; Lelj *et al.,* 1992). For Tamburro, while the cross-linking domains are rather stable and rigid, the elastic domains of elastin are dominated by these sliding structures, which explains the high conformational entropy required for elasticity.

Based on small-angle X-ray and neutron scattering experiments, Weiss and colleagues (Baldock *et al.,* 2011) described the shape of hydrated tropoelastin. Further, they analysed the elastic behaviour of the isolated monomer using the worm-like chain model of polymer elasticity (Lapidus *et al.,* 2002) and concluded that elastin elasticity is explained by the mechanical properties of its monomer. In this model, elastin is described as an 'oiled coiled coil' in which tropoelastin molecules are arranged head to tail to form intertwined microfibers. Different from other models, this model is not directly linked to a specific explanation for the elastin entropic behaviour as it does not provide data about the conformational behaviour of structural ensembles found within the tropoelastin shape. Because this model consists of a single conformation that resembles the shape derived from SAXS data, it is not readily reconcilable with the concept of structural disorder and statistical mechanics prevailing for elastin structure and function. Consequently, *Weiss model* for elastin should be better described as a model for tropoelastin assembly.

Cross-links formation

Since their first identification (Partridge *et al.,* 1963), considerable effort has been made to understand the processes leading to the formation of mature elastin via cross-linking of tropoelastin. To date, this process is not fully understood but important advances have been made. The identification of cross-links joining domains 10, 19, and 25 was a key step (Brown-Augsburger *et al.,* 1995). Indeed, this finding suggested that, at least in the initial steps of polymerisation, a regular organisation of tropoelastin molecules would be required to assemble elastin. However, more recently, extensive mass spectrometry analyses performed by Schmelzer's group convincingly established that the formation of elastin cross-links was mainly a random process (Schräder *et al.,* 2018; Hedtke *et al.,* 2019). Therefore, it seems reasonable to propose that, despite some level of ordered structures, cross-link formation is a random process that would enhance the overall entropic nature of the polymer.

Fractality

The supramolecular organisation of bovine *ligamentum nuchae* elastin and α-elastin either as coacervate or as a lyophilised powder has been studied by scanning and transmission microscopy (Tamburro *et al.,* 1995). Importantly, these experiments evidenced filaments, fibrils, fibres, networks and dendritic, leaf-like forms, that could be rationalised in terms of fractal geometry.

Fractal geometry describes natural occurring objects with non-integer dimensions, including complex chemical systems (Borman, 1991) and large aggregates (Family, 1993). The most important property of a fractal is its self-similarity. A piece of a fractal object looks like the whole, namely it has no characteristic length scale. When a fractal is observed at increasing resolution, it shows increasingly higher level of detail and the following power law (Sander *et al.,* 1994) holds: $N = cs^{-D}$, where N is the smallest number of boxes of linear dimension s that can cover the object. D is the fractal dimension and c is a constant. For elastin, the fractal dimension D was determined by using the box-counting method and was in the range between 1.4 and 1.9 (Tamburro *et al.,* 1995). Comparable fractal characteristics were calculated for some aggregates from recombinant elastin-inspired partially ordered polypeptides. A two-stage aggregation process was suggested and predicted by coarse-grained simulations for these peptides depending on temperature: when it is raised beyond the transition temperature (T_t) the mesoscopic nuclei rapidly link, forming fractal networks (Roberts *et al.,* 2018). This type of coacervation is mirrored in tropoelastin which undergoes a multistage process (Yeo *et al.,* 2011).

Structural insights

Mature, cross-linked elastin does not crystallise thereby preventing structural analysis by X-ray diffraction. In 2002, elastin extracted from bovine nuchal ligament was characterised in the hydrated and lyophilised form by solid-state nuclear magnetic resonance (NMR) (Perry *et al.,* 2002; Kumashiro *et al.,* 2003). Given the difficulty in crystallising elastin and elastin-derived sequences, hydrolysis products, such as α- and κ-elastin, have been used for spectroscopic studies in solution (Tamburro *et al.,* 1978; Debelle *et al.,* 1995). Later, the availability of soluble human recombinant tropoelastin, allowed researchers to study the protein with a high purification degree and to use it for potential applications as a biomaterial (Vrhovski *et al.,* 1997). Whilst recombinant tropoelastin represents a breakthrough in biomaterials applied science, the use of tropoelastin in conformational studies presents some drawbacks. First, the repetition of identical amino acid residues such as glycine, alanine, and valine along the primary structure impedes the conformational investigation of the protein in solution by NMR given the severe degeneracy of signals. Consequently, the data gathered experimentally for elastin and tropoelastin structures are limited. However, circular dichroism (CD) (Vrhovski *et al.,* 1997) and Raman (Debelle *et al.,* 1995) spectra underline the presence of α-helical conformations, β-turns and β-strands in both elastin and tropoelastin. Therefore, the intrinsically disordered elastin polymer possesses at least some degree of ordered structures. As we will illustrate below, this apparent discrepancy is explained by the fact that these spectroscopic techniques provide an average static description of elastin structures at a given observation time. A key feature of elastin structures is their dynamic behaviour: they are mostly transient, labile, and rapidly changing between states.

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The sequence of tropoelastin displays a low complexity, notably in its hydrophobic domains (Figure 2). About 80% of the residues found in tropoelastin sequence are P, V, G, L, I, or A. The hydrophobic domains are rich in G, V, P, and L, while K and A are mainly found in the hydrophilic cross-linking domains. In the hydrophobic domains, non-polar residues are often found in tandem repeat and pseudo-repeat sequences (Keeley, 2013). In these regions, characteristic 2-mer and 3-mer motifs can be found, such as GX, PX, GGX, and PGX, where X corresponds to a G, A, V, L, or I (Debelle and Tamburro, 1999; Shewry *et al.,* 2003). The GV, GGV, or VPG motifs are common in these domains (Muiznieks and Keeley, 2017).

The study of these typical elastin repeats is of particular interest when it comes to understanding tropoelastin through its characteristic elements. For instance, VPG motifs have been suggested to form transient β-turn structures which are necessary to maintain conformational flexibility (Reichheld *et al.,* 2021).

Reductionist approach

The fractal nature of assembled elastin and hence self-similarity means that even very short elastin sequences should show molecular and supramolecular features which are similar to those of the whole elastomer. As a consequence, past studies performed on short synthetic peptides corresponding to repeating sequences (Long *et al.,* 1980; Tamburro *et al.,* 1990, 1992) or to recombinant polypeptides spanning regions encoded by exons 20 to 24 (Miao *et al.,* 2005; Reichheld *et al.,* 2014) may be highly informative regarding larger-scale elastin assembly. However, the choice of specific sequences within elastin chains may mean that such studies are not representative of all exons within the monomer.

To fill this gap, Tamburro and colleagues took advantage of the cassette-like organisation of the *eln* gene, where exons encoding hydrophobic sequences alternate regularly with lysine-rich domains containing of those rich in alanine (KA domains) and those containing proline (KP domains), and undertook the exon-by-exon synthesis of human tropoelastin domains (Tamburro *et al.,* 2003, 2006; Pepe *et al.,* 2008). The working hypothesis of their 'reductionist approach' (Salvi *et al.,* 2013) was that each domain would adopt a specific and independent conformation in the polymer, and possibly play a specific function.

CD is a useful and quick tool for assessing the secondary structure of polypeptides in solution, which, in the context of the reductionist approach, depends on temperature and solvent. In particular, temperature is a critical parameter for extended conformations such as poly-L-proline II (PPII) and folded structures like turns (Tamburro *et al.,* 2005; Bochicchio and Pepe, 2011). Therefore, studies as a function of temperature were carried out both in aqueous solution and 2,2,2-trifluoroethanol (TFE). TFE is a solvent which is less polar than water and which favours regular secondary structures such as helices and turns stabilised by intramolecular hydrogen bonds. The rationale behind the use of TFE is to assess the possible conformations adopted by the polypeptides in a more shielded environment as compared to the real local conditions they experience in hydrated elastin. These *in situ* conditions are unknown and more likely intermediate between the two solvent extremes, water and TFE. Of particular interest, is the finding that in an aqueous solution the dominant conformation for hydrophobic domain peptides is apparently the PPII often in equilibrium with the so-called 'unordered' state. The results are summarised in Table 1.

The reductionist approach underlined that PPII is a significant contributor to the conformational ensemble in flexible, unordered polypeptides (Bochicchio and Pepe, 2011). In fact, recently a new picture of unordered conformations emerged from the analysis of unfolded proteins and unordered peptides that points to more restricted conformational sampling in the unordered state with a limited range of accessible conformations.

Many spectroscopic studies and molecular dynamics simulations propose that short stretches of 2–5 residues in PPII are the most favoured conformations in solution for unordered and unfolded peptides (Bochicchio *et al.,* 2015). Furthermore, the unordered form is constituted not only by PPII-helical stretches with three or more residues but also by conformers in which individual residues are in PPII, α-, or β-regions of the Ramachandran map, rapidly interconverting. In TFE, in contrast, folded conformations, such as type I and type II β-turns are found together with α-helices in polyalanine sequences and with unordered conformations in hydrophobic and KP domains (Tamburro *et al.,* 2006).

In conclusion, because the polarity of the microenvironment might be intermediate between water and TFE, the reductionist approach led to the proposal of a conformational equilibrium for elastin, comprising PPII, β-turns, and unordered conformations, as illustrated in Figure 3. Each of the three terms at the equilibrium should comprise, on their own, additional internal fluctuations. Such is, for example, the case of PPII, where most probably the trans-conformational transitions involve the entire upper left region of the Ramachandran map, that is, the region of the (quasi) extended conformation, including also β-strands. Similarly, the term 'β-turns' refers to fluctuations around the energy minimum of isolated β-turns and also equilibria such as type I ⇄ type II, which have been proposed as a common structure in elastin, and are central to the 'sliding β-turns' model proposed by Tamburro as an explanation for elastin elasticity (Tamburro *et al.,* 1990; Lelj *et al.,* 1992).

As a native, relaxed state at high entropy is essential for conferring elasticity according to the classical theory of rubber elasticity, a pivotal role in this context is played by the PPII flexibility. In fact, the main peculiarity of PPII lies in its high flexibility undoubtedly due to the absence of intra-chain hydrogen bonds. Accordingly, the possibility that PPII is involved, as suggested above, in multi-conformational equilibria such as PPII/unordered/β-turns, should conceivably increase the entropy of the relaxed state, and therefore allow the expression of elasticity. In this sense, the reductionist approach strongly supports the general equilibrium extended ⇄ folded, proposed by Tamburro model as the source of elastin high entropy in the relaxed state.

Numerical simulations

Molecular dynamics (MD) simulation is a powerful computational technique for studying the properties of biological molecules at the atomic level. A protein is represented as a set of atoms that interact according to the laws of classical mechanics. By simulating the motion of these atoms, the conformational changes and dynamic properties of the protein can be sampled. The result is a trajectory that describes the positions and velocities of all the atoms in the molecule over time. Simulations have been used to study a wide range of biological processes, including protein folding/unfolding, ligand binding and enzyme catalysis, and have provided valuable insights into the molecular basis of these phenomena. Despite its computational cost, MD simulation has become an essential tool for studying globular proteins, filling the gap between experiment and theory and shedding new light on the fundamental principles governing protein structure and function (Bottaro and Lindorff-Larsen, 2018).

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Table 1. Summary of secondary structure elements presence in elastin domains as evidenced by CD and NMR following the reductionist approach

#d	Type	Secondary structures					#d	Type	Secondary structures					
2	h		PPII	β1	β2	U	19	KA	α		β1			U
3	h		PPII		β2	U	20	h		PPII		β2		U
4	KP			β1		U	21	KA	α	PPII				U
5	h		PPII	β1	β2	U	22	h				β2		U
6	KA	α	PPII			U	23	KA	α	PPII			T	U
7	h				β2	U	24	h		PPII		β2		U
8	KP		PPII	β1	β2	U	25	KA	α	PPII			T	U
9	h		PPII	β1	β2	U	26	h		PPII		β2		U
10	KP		PPII	β1		U	26A[†]					β2		U
11	h					U	27	KA	α	PPII	β1			U
12	KP		PPII	β1	β2	U	28	h		PPII	β1			U
13	KP			β1	β2	U	29	KA	α	PPII				U
14	h				β2	U	30	h		PPII		β2		U
15	KA	α	PPII			U	31	KA	α	PPII				U
16	h		PPII		β2	U	32	h		PPII	β1	β2		U
17	KA	α		β1		U	33	h		PPII	β1	β2		U
18	h		PPII		β2	U	36	CT			β1			U

#d, domain number. Domain types: h, hydrophobic domain; KP, KP cross-linking domain; KA, KA cross-linking domain; CT, C-terminal. Secondary structures: α, α-helix; PPII, poly-proline II helix; β1, type I β-turn; β2, type II β-turn; T, turn; U, unordered. [†]Domain 26A is not a distinct domain but rather an extension of domain 26 generated by read-through beyond the normal donor splice site in the genomic sequence. The protein sequence is highly uncharacteristic of elastins, rarely expressed, and may be artefactual (Bisaccia *et al.,* 1998).

MD simulation is an essential tool for studying poorly structured or intrinsically disordered proteins (IDPs), that is proteins lacking a well-defined 3D structure under physiological conditions. When studying IDP by MD, simulation allows the exploration of the IDP conformational space and dynamics, generating an ensemble of conformations which can be compared to experimental observables. Although the conformational flexibility of IDPs makes their simulation particularly challenging, MD simulations constitute a valuable complement to experimental techniques, notably in the study of IDPs (Hernández *et al.,* 2020; Salvi *et al.,* 2022).

MD relies on a force field, a mathematical model describing the interactions between atoms in a molecular system. It consists of a set of equations that computes the forces acting on each atom based on its position and the positions of neighbouring atoms. These forces, typically derived from experimental or theoretical data, determine the motion of the atoms and therefore the behaviour of the whole system. A force field typically includes terms for bond stretching, angle bending, torsional rotation, non-bonded interactions (such as van der Waals forces and electrostatic interactions), and other contributions such as solvent effects. Different force fields can have different levels of accuracy and computational efficiency, and their selection depends on the specific system being studied and the research question of interest. The choice of a force field can have a significant impact on the simulation results, and it is important to validate the model against experimental data whenever possible.

Several force fields have been developed for several decades, mainly for globular proteins. These force fields have shown their efficiency for simulating such systems, but have limitations for simulating IDPs because of their inability to reproduce the conformational ensembles explored by disordered systems (Rauscher *et al.,* 2015). Consequently, new sets of parameters have been developed or adjusted from existing force fields for these systems. For instance, the CHARMM36m force field (Huang *et al.,* 2017), developed from CHARMM36, allows to generate conformational ensembles for IDPs, the sampling method being based on the search for interactions between protein and water, to explore more specifically extended conformations. Currently, convenient simulation protocols are still under development.

In the field of elastin research, molecular modelling was used to investigate the structural organisation, elasticity, or phase transition of tropoelastin-inspired peptides. Pioneering computations were initially performed *in vacuo* (Chang *et al.,* 1989; Lelj *et al.,* 1992; Villani and Tamburro, 1995), then with one added shell of water molecules around $(VPGVG)_n$ (Wasserman and Salemme, 1990), and later on, on a fully hydrated system with explicit water (Li *et al.,* 2001a).

Whereas all-atom simulations can be performed on characteristic peptide motifs of tropoelastin, more recent approaches like coarse-grained modelling do not consider each atom individually, but rather one particle as a group of several atoms. This approach is difficult to use with molecules such as tropoelastin and its motifs (Depenveiller *et al.,* 2023). Coarse-grained simulation approaches have been applied to ELP, but they require a systematic re-parameterisation of the potentials of the dihedral angles to prevent the secondary structures from 'freezing' during the simulation.

The dynamical behaviour *in vacuo* of an elastin-derived peptide, GLGG, was modelled (Villani *et al.,* 1997) and small amplitude motions named *solitons* were observed. In this picture, the molecular motion is essentially quasiperiodic and of rather lower entropy. In

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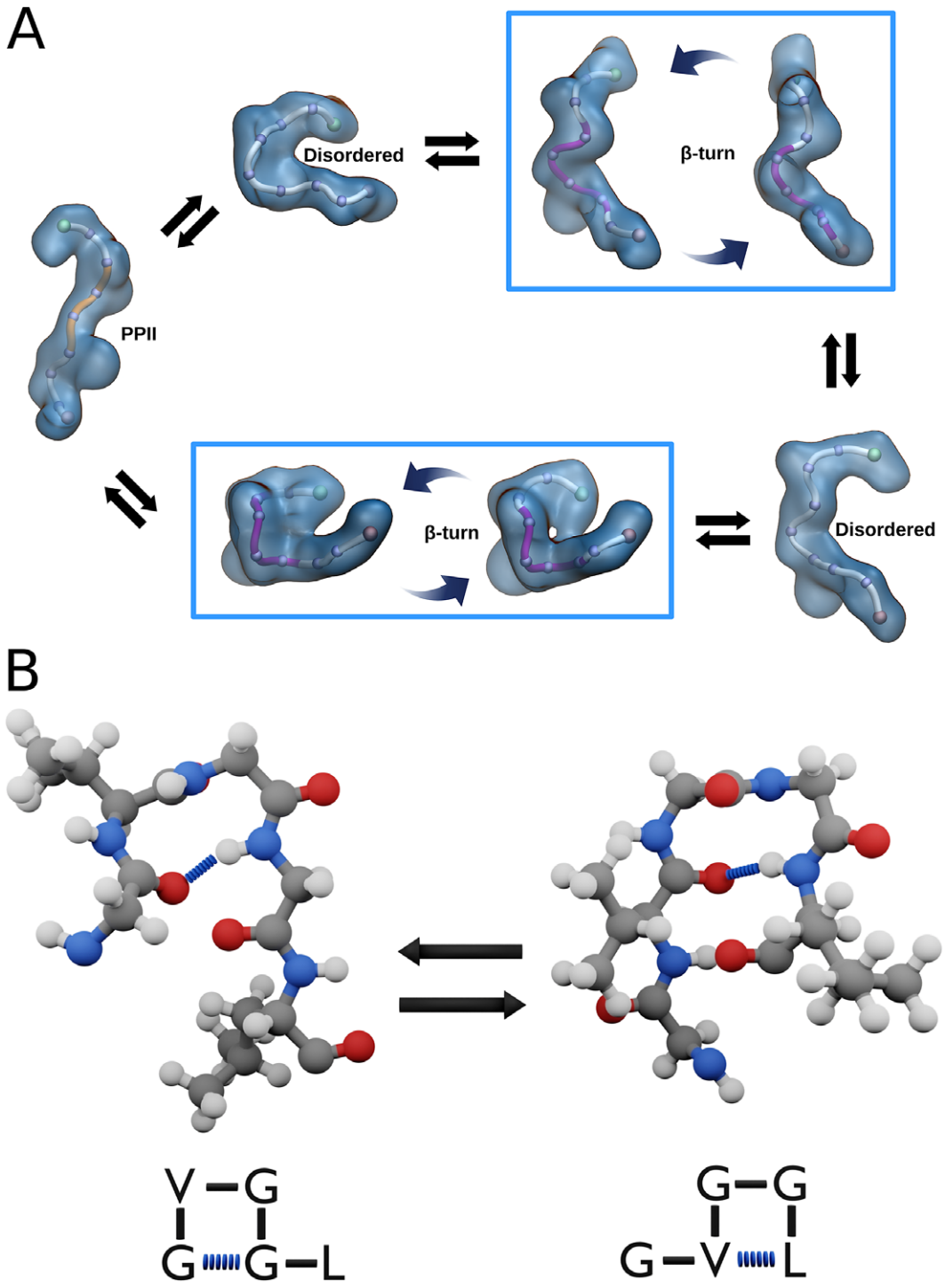

Figure 3. Conformational equilibrium characterising the elastic domains of elastin. (*a*) Elastic domains do not present stable conformations but rather rapidly and constantly interchanging turns, PPII and unordered states engaged in a conformational equilibrium sustained by the presence of surrounding water molecules. This process explains elastin exceptional conformational entropy. Boxes point at turns sliding along the sequence. (*b*) Illustration of a turn sliding along the sequence. The example sequence is GVGGL. The H-bond defining the turn shifts from positions $G_1 \rightarrow G_4$ to $V_2 \rightarrow L_5$.

dynamical system theory it is well established that, increasing the amplitude fluctuations of *solitons*, a transition to chaos of high entropy is expected (Walker and Ford, 1969). Then, peptide chaotic motion is suggested to take place in solution or at higher temperature where large motions should occur. Moreover, considering that the common characteristic of the proposed elastin elasticity mechanism is a higher molecular freedom inside either the peptide chains or solvent molecules for the relaxed state with respect to the extended one, a *transition to chaos* has been proposed as a source of entropy differences and the driving force for the refolding of stretched elastin when the external force is removed. This was the starting point for a theoretical study based on non-linear systems theory (Villani *et al.*, 1997; Villani and Tamburro, 1999). One of the global variables considered was the trajectory of the end-to-end vector R_{ee} defined

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as the difference of position components of the Cartesian coordinate of the end carbon atoms of acetyl and *N*-methyl-amide groups. The perfectly irregular trajectory $R_{ee}(t)$ found is considered typical of fractional Brownian motion (chaos). A typical Brownian motion is that resulting from the random walk assumed in the classical theory of elasticity (Flory, 1953) and according to the fractal nature suggested for elastin. The large conformational freedom in solution of the free chains, either folded or extended, is apparent from the structural parameter trajectories. The motions are, in all cases, larger than the corresponding ones *in vacuo*. Moreover, this picture is in agreement with proposed plasticiser role of water (Megret *et al.,* 1993). In fact, the flexibility of the peptide backbone is increased by the interaction with the solvent molecules and by efficient solvation of peptide units that reduces the intramolecular hydrogen-bonding interactions.

With increases in computational power, elaborate elastin-related systems have been simulated in explicit water. The analysis of the corresponding trajectories evidenced essential features of hydrated elastin chains dynamics. Based on the reductionist approach, numerical studies have been performed on peptides bearing the characteristic sequence patterns of tropoelastin, namely XPGXG, XGGZG, XGXXPG, XGXPGXGXG (Figure 2).

Domain 18 of bovine elastin contains 11 VPGVG tandem repeats (Raju and Anwar, 1987). Similar VPGXG motifs are used to design intrinsically disordered biomaterials (Roberts *et al.,* 2015). The XPGXG motifs originating from tropoelastin sequence have been extensively studied, both experimentally by different methodologies, and in simulations, VPGVG being the consensus motif classically described (Muiznieks and Keeley, 2017). The VPGEG and VPGKG forms are also found and widely used in biomedical applications and biomaterials (Rodríguez-Cabello *et al.,* 2016; Wang *et al.,* 2017; Rodriguez-Cabello *et al.,* 2021).

Most simulations performed on these peptides have been run on structural models of elastin. From such data, Urry proposed a model where the repeating $(VPGVG)_n$ sequence would form a β-spiral (Venkatachalam and Urry, 1981). However, such an organisation implies restricted conformations, which is incompatible with the high degree of conformational flexibility of elastin (Rauscher and Pomès, 2012). Further, this β-spiral structure has been shown to be unstable during simulations (Li and Daggett, 2002).

The XGGZG type motifs, with X and Z being mainly permutations of L or V residues, are also important motifs of tropoelastin. They are found in domains 7, 28, and 30 (Figure 2). These sequences play a major role in aggregation and elastic fibre formation (Bochicchio *et al.,* 2004). A variant of a natural sequence, VGGVG, is often used in biomaterial studies. Numerical simulations performed on these patterns (Bochicchio *et al.,* 2015) show that, in solution, these peptides mostly adopt a type I β-turn rapidly exchanging with type II β-turns and irregular β-stands.

The VGVAPG is the archetypical XGXXPG-type motif. It is found six times in domain 24 of human tropoelastin (Indik *et al.,* 1987) where it is tandemly repeated three times, followed by VGLAPG and then again tandemly repeated three times. The VGVAPG motif has deserved considerable attention because it presents numerous biological activities (Le Page *et al.,* 2019). As such, VGVAPG belongs to the group of matrikines and more specifically to the group of elastokines, bioactive peptides derived from elastin sequences (Maurice *et al.,* 2013). Numerical simulations performed on XGXXPG peptides show that this pattern adopts a mixture of extended PPII and folded turn-like conformations in equilibrium (Floquet *et al.,* 2004; Rauscher *et al.,* 2006). This description supports the sliding turns model (Tamburro *et al.,* 2003, 2005). The canonical β-turn classification defines 8 types of β-turns (Wilmot and Thornton, 1988). Strikingly, within the VGVAPG peptide, the type VIII β-turn is very frequently encountered. This propensity of VGVAPG (and XGXXPG peptides) to adopt a type VIII β-turn conformation correlates with their biological activity (Floquet *et al.,* 2004; Moroy *et al.,* 2005; Fuchs *et al.,* 2006; Blanchevoye *et al.,* 2013).

The XGXPGXGXG sequence motif found in domain 26 of human tropoelastin defines a class of elastokines distinct from the XGXXPG (Long *et al.,* 1988; Maeda *et al.,* 2007). Numerical and experimental analyses have been used to address their conformational behaviour (Hernández *et al.,* 2020). As with other elastin-related sequences, these peptides are extremely dynamic when hydrated. Nevertheless, it appears that their intrinsic conformational changes favour type II β-turn conformation suggesting a possible role in their biological activities.

Overall, independently of the considered sequence motif, numerical simulations strongly support a conformational equilibrium in hydrated elastin peptides (Figure 3) as suggested by the reductionist approach. In agreement with spectroscopic studies, the structure of the hydrophobic domains of elastin therefore consists of sparse, transient, and local secondary structure elements which can be described in a statistical way.

Following the description of the molecular shape of tropoelastin derived from the SAXS/SANS envelope of a recombinant tropoelastin and some of its fragments, namely domains 2–18 and 2–25 (Baldock *et al.,* 2011), the first molecular model of a 698-residue long tropoelastin was obtained by folding an extended monomer combining implicit and explicit replica exchange molecular dynamics simulations (Tarakanova *et al.,* 2018). Although these results are remarkable, the proposed model only represents one possible conformation. Further, given the demonstrated highly flexible and mobile nature of elastin chains, one can wonder about the generation of a unique model, lacking publicly available coordinates.

The tropoelastin model presents several convincing features, but it is incompatible with the established concept of structural disorder and the notion of conformational equilibrium that rule elastin function. Therefore, we feel that this model should be cautiously considered as it is misleading to represent a highly disordered protein by a single conformation.

The elastin/water system

Native elastin, which reaches the top of hydrophobic proteins, also has a hydrophilic character when classified as a polymer. Water molecules play a considerable role in governing the structure, stability, dynamics, and function of biomolecules in general and notably of elastin. Changes in the thermodynamic and motional properties of water at different hydration levels of elastin and associated polypeptides indicate the existence of different fractions of water which vary in structures and properties and presumably play different biological roles. Moreover, the insolubility of elastin forbids the use of proven techniques dedicated to the determination of the structure and dynamics of globular proteins in solution. Consequently, techniques from material science have been adapted to elastin characterisation and shed new light on the peculiar role of water on elastin structure and dynamics.

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The different types of water in the elastin-water system

The functional properties of natural polymers can be defined when they coexist with water molecules (Kaatze, 1997; Sun and Boutis, 2010; Cametti *et al.,* 2011). Biopolymers in their native, *in vivo* state, typically contain a considerable amount of water, and it is necessary to evaluate the water combined with the polymers to elucidate characteristic features of polymer-water systems. Different types of techniques have been used to investigate the structure and properties of ab/adsorbed water on hydrophilic polymers (Hatakeyama *et al.,* 1988; Kaatze *et al.,* 2002; Sun and Boutis, 2010; Cametti *et al.,* 2011): sorption isotherm, thermal analysis, nuclear magnetic resonance spectroscopy, dielectric measurements, viscoelastic measurements, revealing the great structural complexity of water in biological systems.

The elastin-water system has been analysed via these different techniques, giving both qualitative and quantitative information of these different types of water. In the following section, hydration h is defined as the weight of water per weight of dry protein while the water content x_w is defined as the weight of water per weight of hydrated protein.

Monolayer, multilayer, and bulk water

Water equilibrium sorption–desorption isotherms of initially dry elastin revealed critical water contents for the gradual formation and completion of the protein hydration (Panagopoulou *et al.,* 2013). The S-shaped sorption isotherm satisfactory fits the well-known Guggenheim-Anderson-de Boer equation (Timmermann, 1989), allowing to define monolayer, multilayer, and bulk water. A first critical value $x_w = 6.5\%$ ($h = 0.07$) is found for the filling of the first sorption layer, corresponding to 0.3 molecules of water by residue of elastin. A second critical value $x_w = 11.5\%$ ($h = 0.13$) is found as the onset of water clustering (filling of multilayers); a third critical value of $x_w = 18\%$ ($h = 0.15$) must be reached for the organisation of water in the protein hydration shell, which roughly corresponds to 0.7 molecules of water per residue and could be related to the beginning of swelling. Finally, a maximum water uptake of 23% ($h = 0.3$) is determined from vapour sorption experiments leading to significant bulk water, which roughly corresponds to 1.4 molecules of water per residue. It must be noted that usually about 0.3–0.7 g of water remains associated per gram of 'dry' protein (Nandi *et al.,* 2000). In the case of elastin, the low value of 0.3 ranks elastin in the category of natural hydrophobic proteins, but in comparison with synthetic polymers it also allows to classify elastin as a hydrophilic polymer.

Freezable and unfreezable water

Differential scanning calorimetry (DSC), that measures the variation of the heat capacity during a temperature program, is a well-suited technique to determine phase transitions in any kind of sample. In calorimetric experiments of polymers, water molecules are categorised into two types according to their behaviour during freezing and thawing. Freezable water gives crystallisation endotherms during cooling and melting endotherms during heating. In contrast, unfreezable water cannot freeze and does not generate transitions.

The term 'freezable water' should be preferred to the term 'free' water, reserved to vibrational or relaxational techniques (Rault *et al.,* 1995; Tang *et al.,* 2017). In the same way, care should be taken when associating unfreezable water with 'bound' water, since hydrogen bonding is not the 'exclusive' factor influencing water crystallisation. Indeed, nanocavities in the polymer can be also an important reason for the formation of unfrozen water (Liu and Yao, 2001).

The first DSC experiments performed on native, purified and soluble elastin (Ceccorulli *et al.,* 1977) evidenced a maximal amount of unfreezable water at $x_w = 25\%$ ($h = 0.33$) in the elastin-water system, leading to a molar ratio of unfreezable water per residue close to 1.5. This value was consistent with the hypothesis of a direct solvation of the main chain and interpreted according to a model previously proposed for polyamides, with three water molecules for two residues: the first strongly bound to two carbonyl groups of elastin, the others weakly bound, and inserted between pre-existing hydrogen bonds between carbonyl and amine groups.

As shown in Figure 4, calorimetric experiments on hydrated elastin evidenced the appearance of freezable water for water amounts above $x_w = 22\%$, that is, $h = 0.3$ (Samouillan *et al.,* 2004, 2011; Panagopoulou *et al.,* 2013), in accordance with equilibrium sorption–desorption isotherm experiments. Cross-links and conformational changes can explain the slight decrease of the maximal molar ratio of unfreezable water per elastin residue (1.4) when compared to soluble elastin (1.5).

Interestingly, solid-state ^{13}C NMR spectra on differentially hydrated elastins exhibit a clear evolution above $h = 0.3$, supporting the existence of a localised water phase under this peculiar hydration (Perry *et al.,* 2002). As water is added, one plausible model might include an extensive hydrogen-bonding network from peptide backbone to water molecules in the first, second and subsequent hydration layers.

The endothermic peak corresponding to the melting of freezable water is widely used to quantify freezable water in proteins and tissues, by dividing the area of the peak by 0.334 $J.kg^{-1}$, corresponding to the melting enthalpy of pure ice at 0 °C (Heys *et al.,* 2008; Panagopoulou *et al.,* 2013). Total hydration can be reached by thermogravimetric analysis and the amount of unfreezable water by a simple difference (Tang *et al.,* 2017). The broadness of the endotherm or presence of shoulders can be due to the subdivision of freezable water into different categories as reported for various biopolymers/water systems: (1) bulk water in excess, which can form its classical four H-bonds with neighbouring water molecules and freezes/melts at the usual freezing point of water, (2) intermediate water (Liu and Yao, 2001), which freezes/melts at a lower temperature and can be associated with water in mesopores (Hay and Laity, 2000; Tang *et al.,* 2017), (3) freezable 'bound' water, less closely bound and immobilised (Hatakeyama *et al.,* 1988). Another kind of water, often named 'hydrophobic hydration', corresponds to clathrate-like structures of water around hydrophobic solutes (Teeter, 1984). In water/hydrophobic compounds mixtures, clathrate-like structures of water generally dissociate in the 273 K region (Nguyen *et al.,* 2021) or at slightly higher temperature (López-Bueno *et al.,* 2021) due to the specific arrangement of water around the hydrophobic solute, arising from a steric effect (Rezus and Bakker, 2007).

Even if hydrophobic hydration is hardly detectable by calorimetric measurements in hydrated cross-linked elastin because it overlaps with frozen water melting, this type of hydration is crucial to explain the thermal behaviour of aqueous solutions of elastin polypeptide analogues (Luan *et al.,* 1992; Urry, 2004; Ribeiro *et al.,* 2009). It was shown to be intricately linked with the hydrophobic effect, that plays a major role in the coacervation phenomenon leading to fibrillogenesis of elastin. The hydrophobic effect can be described as the tendency of apolar groups to associate in an aqueous solution, minimising the total hydrophobic surface exposed to water (Urry, 2004; Rezus and Bakker, 2007) – and thereby the hydrophobic

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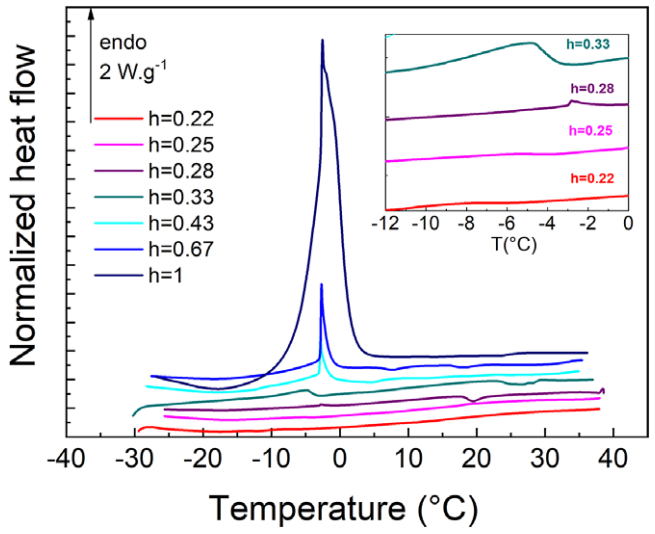

Figure 4. Freezable and unfreezable water in elastin. DSC heating thermograms (10 °C/min) of elastin-water systems evidencing the detection of endothermic melting of ice (i.e. detection of freezable water) for h = 0.28 (x_w = 22%) and intensification of the endotherm for higher hydration. Reprinted (adapted) with permission from Samouillan *et al.* (2011). © 2011 Elsevier.

hydration – when the temperature is raised, leading to a phase separation. During this phase separation, the decrease in order of structured water surrounding hydrophobic groups that becomes bulk water exceeds the ordering of the protein or polypeptide, implying that the entropy of the whole system (water and protein) increases. This transition referred as an inverse temperature transition (ITT) in proteins or lower critical solution temperature (LCST) in polymers, has been evidenced by classic calorimetric measurements to be dominated by the endothermic process of the clathrate-like water melting occurring in the 20–40 °C range (Urry *et al.,* 1991; Rodríguez-Cabello *et al.,* 2004; Ribeiro *et al.,* 2009).

Modulated differential scanning calorimetry (M-DSC) is required to detect the minor exothermic part due to Van der Waals cohesive interactions of the polypeptide chains accompanying the association (Rodriguez-Cabello *et al.,* 1999; Urry, 2004). Calorimetric experiments on specific domains from elastin have shown that the temperature for the formation of clathrate-like water, which depends on sequence, was closely connected to the coacervation temperature (Dandurand *et al.,* 2015) and could be used to tune the coacervation of polypeptides. The dependence of ITT/LCST on the amino acid sequence points out that the water structures in elastin present a heterogeneous distribution (Ribeiro *et al.,* 2009).

Free, bound, and hydrophobic water

Interfacial water is considered as a part of the structures of macromolecules. The mobility of these water molecules is very different from that of pure bulk or 'free' water. Dynamically oriented and exhibiting restricted motions, these 'bound' water molecules are identified by a slowing down of their dynamics as shown by NMR spectroscopy, quasi-elastic neutron-scattering spectroscopy and dielectric relaxation techniques (Gniadecka and Jemec, 1998; Kaatze *et al.,* 2002; Sun and Boutis, 2010; Cametti *et al.,* 2011; Reuhl and Vogel, 2022).

With the emergence of 2D NMR spectroscopies, magnetic relaxation methods have played a leading role in the study of protein hydration dynamics (Halle, 2004). The dynamics properties of water are probed by the correlation time between the proton spin–lattice (longitudinal) relaxation time T1 and the proton spin–spin (transverse) relaxation time T2. Complementing the earlier work of Ellis and Packer (1976), four reservoirs of water could be discriminated by their mobilities in the elastin-water system (for an hydration h = 1) at 25 °C using deuterium 2D T1-T2 NMR (Sun *et al.,* 2011; Sun and Boutis, 2011; Silverstein *et al.,* 2015). Correlation times ranged from 5 ps, for the free water outside the fibres, to 112 ns for the more restrained water, closer to the protein (Figure 5). Interestingly, with increasing strain on the polymer, experimental ^{13}C NMR data evidenced an increase of the frequency of the protein backbone motion associated with an entropy decrease, as commonly described for the rubber elasticity of elastomers.

Surprisingly, an increase of the random tumbling of localised water is also observed in this water-elastin system. This fact can be interpreted by an increase of the orientational entropy of water molecules. This could appear in contradiction with the short-time dynamics simulations performed on $(VPGVG)_{18}$ (Li and Daggett, 2002) suggesting a decrease of water orientational entropy near hydrophobic groups during the elongation process. Nevertheless, it agrees with the simulations of $(VPGVG)_3$ which show that the lifetime of peptide-water H-bonds decreases with increasing strain, while the peptide entropy decreases (1.63–1.46 kJ/mol.K).

Dielectric relaxational spectroscopy (DRS), which consists of measuring the complex dielectric permittivity of a sample as a function of frequency and temperature, has been widely used to investigate the arrangement of water in hydrated biopolymers or aqueous solutions of proteins (Bone and Pethig, 1985; Nandi *et al.,* 2000). Indeed this technique provides information on the orientational dynamics of molecular dipoles and covers all kinds of polarisation fluctuations in the ms to ps time scales (Cametti *et al.,* 2011). To extract reliable information about protein hydration dynamics from DRS, which gives average information, relatively high protein concentrations are required since only water molecules in the vicinity of protein surface are perturbed (Halle, 2004).

At temperatures above 0 °C, several relaxation processes are detected in the dielectric spectra of hydrated biomacromolecules or aqueous protein solutions. They are assigned to molecular mechanisms involving motion of the protein backbone, of side groups in the protein conformation, and of various layers of strongly and/or weakly bound water and free water. In order of increasing relaxation frequency, the three main relaxations are generally associated with protein tumbling (frequency $f < 10$ MHz, relaxation time $\tau > 15$ ns), to hydration water in the interfacial region surrounding the biomolecule (τ in the 20 ps–10 ns range) and to the orientational polarisation of the free water molecules ($f > 10$ GHz, $\tau < 15$ ps), respectively) (Kaatze, 1997; Cametti *et al.,* 2011). Dielectric studies on concentrated solutions of polypentapeptide analogues of elastin (Buchet *et al.,* 1988; Urry *et al.,* 1997), an elastin coacervate (Urry *et al.,* 1985), or fibrous elastin (Bone and Pethig, 1985; Urry *et al.,* 2002) highlighted both the low-frequency dielectric dispersion due to a librational mode of the protein/polypeptide and the high-frequency dispersion due to water. This latter dispersion was found to be a composite dispersion (Urry *et al.,* 1997) due to at least three kinds of water: free water, water hydrogen bonded to the polar groups of the polypeptide backbone and clathrate-like water associated with the hydrophobic side chains. In the case of elastin-like polypeptides, the magnitude of water relaxation, depending on the hydrophobicity, decreased with increasing temperature between 0 and 60 °C, while the magnitude of the librational mode increased showing an ITT to a more ordered structure. These results corroborate calorimetric

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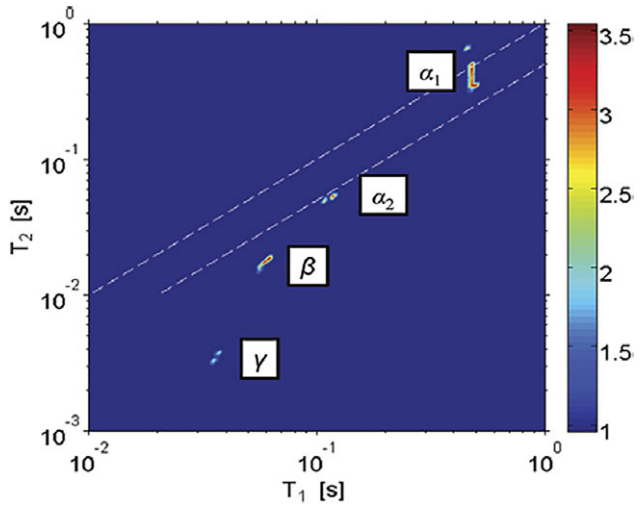

Figure 5. Four types of water in hydrated elastin are identified by their distinct dynamics. ^{2}H T1–T2 NMR relaxation times of water in hydrated elastin from bovine ligament (h = 1) at 25 °C. Component α_1 corresponds to free water external to elastin fibres, component α_2 to free water between the elastin fibres, component β to mobile water within the elastin fibres moving along tortuous channels and component γ to more restricted water. Reprinted (adapted) with permission from Sun *et al.* (2011). © 2011 American Chemical Society.

studies and demonstrate the temperature dependence of the water-polypeptide system, highlighting the role of hydrophobicity in the structure formation of these bioelastomers.

Complementary dielectric measurements on hydrated elastin were also performed at low temperature, in a zone where the relaxation time of the hydration water orientation drastically slows down and can be examined in detail. These experiments evidenced three relaxation modes for the complex formed by elastin and its hydration shell (Figure 6). This first one is a rapid process, known as the universal relaxation in deeply super-cooled confined water due to the reorientation of water molecules in the external layer of the hydration shell of elastin (secondary hydration shell), with an associated energy corresponding to the breaking of two hydrogen bonds (Gainaru *et al.*, 2009; Sudo and Yagihara, 2009). This process is also detected by ^{2}H NMR spin–lattice relaxation (Lusceac *et al.*, 2010). The second is an intermediate process associated with the dynamics of the internal layer of the hydration shell directly linked to the protein (primary hydration shell). This third is a slower, highly cooperative process, involving large motions and mainly due to protein dynamics.

The direct power law found between the relaxation time of the two fastest processes demonstrates that the fast dynamics of water in the secondary hydration shell drive the slower dynamics of the complex protein-strongly confined/bound water. As shown for hydrated globular proteins (Fenimore *et al.*, 2004; Frauenfelder *et al.*, 2009), internal proteins motions are conditioned by the fluctuations of their hydration shells.

It must be pointed out that even for a low hydration level (h = 0.1), the dynamics of the complex protein/primary hydration shell are detectable, and like those observed for higher hydration levels. As formerly demonstrated for hydrated elastin (Bone and Pethig, 1985), in contrast to what is observed for globular proteins, bound water exhibits some freedom of motion, even at very low hydration (Samouillan *et al.*, 2011), correlated to the onset of flexibility of the protein (Nandi *et al.*, 2000).

It was recently shown by DRS/NMR/DSC that the dynamics of hydrated elastin are exactly the same as those of highly asymmetric mixtures of glass formers (Reuhl and Vogel, 2022). This finding strongly supports the fact that water is a crucial and indissociable partner of elastin. Water does play an essential role in the structure and dynamics of hydrated elastin, such as in an extensive hydrogen-bonding network, where water molecules cannot be considered just as a discrete phase (Perry *et al.*, 2002).

Evolution of the physical properties of elastin with hydration

The first DSC measurements performed on purified elastin (Kakivaya and Hoeve, 1975) evidenced no intrinsic first-order phase transition, indicating an absence of long-range order for this protein. The only intrinsic transition detected for both dry and hydrated elastin is the classical step of the specific heat, characteristic of a glass transition phenomenon as widely observed in

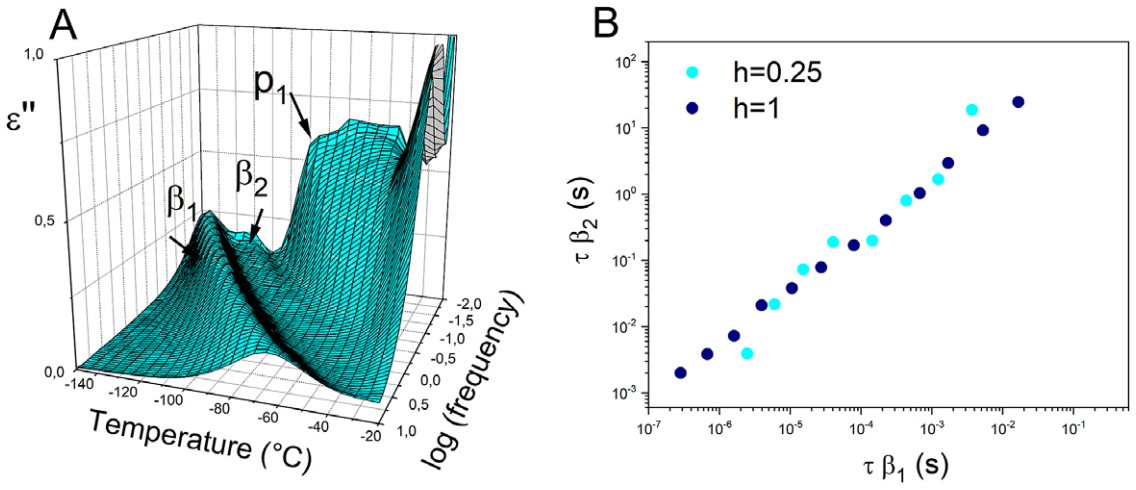

Figure 6. Dynamics of elastin and its hydration shell. A. Dielectric relaxation map of hydrated elastin (h = 0.25) as a function of frequency and temperature, showing the fastest (β_1), intermediate (β_2) and slowest (p1) relaxation modes. Reprinted (adapted) with permission from Samouillan *et al.* (2011). © 2011 Elsevier. B. Relation between the relaxation time of the two fastest β of hydrated elastin indicating that the motions of the protein/water complex are controlled by the fast motions of water in the secondary hydration shell.

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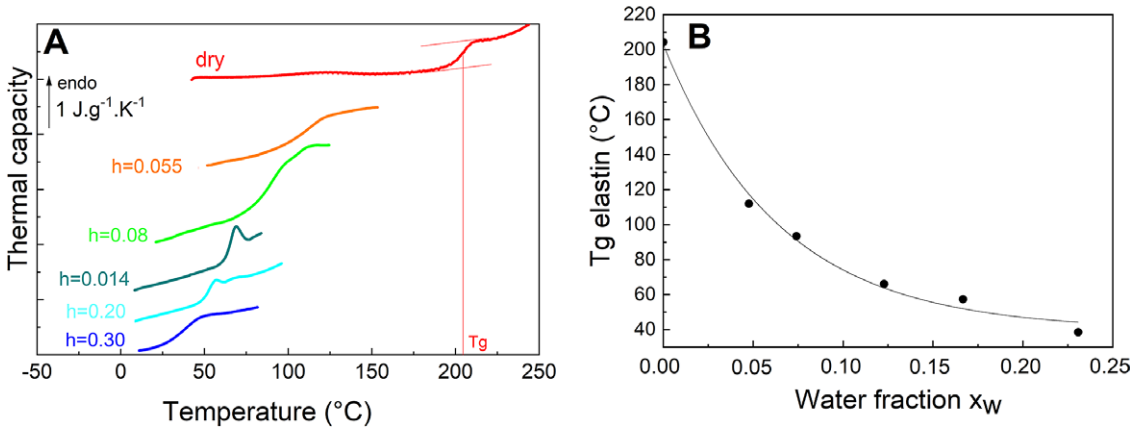

Figure 7. Glass to rubber transition of elastin. (*a*) DSC thermograms of purified elastin at various hydrations; the glass transition corresponds to the step of the specific heat. (*b*) Evolution of the glass transition temperature of elastin with the water content.

amorphous polymers. Corresponding to the reversible transition from a glassy to a rubbery state as the temperature increases, this transition dependent on hydration is accompanied by deep changes in the mechanical properties of elastin (Lillie and Gosline, 1990, 2002; Gosline *et al.,* 2002).

Further, DSC and M-DSC measurements in a wider hydration range confirm the drastic evolution of the glass transition of elastin with hydration (Samouillan *et al.,* 2004) (Figure 7). The glass transition temperature (T_g), which is at about 200 °C in the freeze-dried state, falls to room temperature at 24% hydration, demonstrating that the water-elastin system works as a rubber at body temperature and physiological conditions of hydration.

Strain–stress curves of elastin under various regimes of time, temperature and hydration levels illustrate well this drastic evolution of the mechanical behaviour (Lillie and Gosline, 2002). When strained at low hydration (or low temperature, or with high strain rate), elastin behaves as a glassy and brittle material (tensile modulus reaching some tens of MPa, breaking at a strain <10%). When strained at high hydration (or high temperature or with low strain rate), elastin behaves as a rubber (tensile modulus ~0.2 MPa, breaking strain >100%).

The evolution of T_g value of the elastin-water system was found to follow the classical Fox-Flory law up to the apparition of free water. This plasticisation phenomenon by water, commonly observed for synthetic polymers like polyamides (Jaffe *et al.,* 1997), is much more marked in the case of elastin. The drastic drop of the glass transition temperature of elastin with hydration (up to the full the filling of its hydration shell) highlights the essential role of water for the viscoelastic properties of elastin, confirming the coupled dynamics of water and elastin.

As underlined in Tamburro's model (Debelle and Tamburro, 1999; Villani and Tamburro, 1999; Tamburro *et al.,* 2005), water facilitates the motions of the polypeptide chains, and the hydrated relaxed protein undergoes mainly chaotic, Brownian-like motions, behaving as a fractal system of high entropy. Above the full filling of the hydration shell, the presence of crystallisable water explains that T_g value does not fall below 0 °C (Rault *et al.,* 1995; Samouillan *et al.,* 2004).

Dynamical mechanical analysis performed on arterial elastin in physiological conditions (Lillie and Gosline, 1990) has shown that arterial elastin had to be essentially water-saturated to remain in the rubbery portion of its master curve, for example at 37 °C and at frequencies between 1 and 10 Hz (cardiac cycles). Additionally, it is established that the bulk, extracellular intra-fibrillar water plays a role in the visco-elastic properties of elastin (Weinberg *et al.,* 1995; Wang *et al.,* 2018).

Elasticity

Despite the biological importance of elastin and the use of a myriad of biophysical methods to elucidate its structure–function relationship over several decades, the molecular basis of elastin self-assembly and the structure of the self-assembled state remain poorly understood. Nevertheless, several facts are known to be critical for proper function. First, elastin must be hydrated to be elastic, for example, the coacervated state of the elastic material is essential for proper function. Second, this state must be characterised by a high entropy. Indeed, based on thermoelasticity measurements indicating that elastin's recoil force is almost entirely entropic, Hoeve and Flory postulated that elastin's structure is an isotropic, rubber-like polymer network consisting of cross-linked, random chains (Hoeve and Flory, 1958, 1974). Therefore, elastin elasticity must abide to the second law of thermodynamics which states that 'the entropy of an isolated system never decreases over time', say $\Delta S > 0$. Thus, the relaxed state should have a high entropy and the stretched one a lesser level, so that, when the application of the stretching forces ends, an entropic-driven elastic restoring force is developed by the system to recover a high entropy.

In keeping with these considerations, structural models of elastin have been proposed, which span a range from highly ordered (Venkatachalam and Urry, 1981) to maximally disordered (Hoeve and Flory, 1958, 1974) and emphasise either the hydrophobic effect (Weis-Fogh and Andersen, 1970; Gray *et al.,* 1973; Venkatachalam and Urry, 1981) or conformational entropy (Hoeve and Flory, 1958, 1974) as the dominant contribution to the elastic recoil force.

To go beyond these seemingly contradictory models, high-resolution structural information is required, notably of the hydrated state of elastin. However, the conformational heterogeneity and self-association of this macropolymer have impeded crystallographic and spectroscopic investigations and still present a significant sampling challenge to molecular simulations. Nevertheless, important advances have been made to understand the nature, structure, and molecular organisation of the elastin coacervate.

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The elastin coacervate

The coacervation process is a liquid–liquid phase separation resulting in the formation of a dense protein-rich phase, the coacervate (Johnson and Wang, 2014). For elastin, the coacervate corresponds to a functional hydrated state and its water content is in the range of 40–60% (Rauscher and Pomès, 2017; Reichheld *et al.,* 2017). The peculiar physical properties of coacervated states limit the use of structural characterisation methods because they exhibit an intrinsic disorder (Reichheld *et al.,* 2017). This phenomenon is remarkably important when the elastin coacervate is considered as elastin chains are extremely dynamics. Nevertheless, results obtained on the molecular basis for phase separation of hydrophobic elastin-like domains, block copolymer peptides mimicking alternating hydrophobic and cross-linking domains, or mini-elastin provide important insights into the structural and physicochemical basis for the elasticity of elastin (Rauscher and Pomès, 2017; Reichheld *et al.,* 2017; Vidal Ceballos *et al.,* 2022).

A computational study of self-assembled ELPs led to a detailed description of a liquid-like, disordered protein state, which may be called the liquid state of proteins and represents the first atomistic model of a protein coacervate (Rauscher and Pomès, 2017). Importantly, the detailed comparison of the equilibrium structure of hydrophobic domains successively in dilute and phase-separated states provides insight into the molecular basis of elasticity. In both states, the monomers are characterised by a high degree of conformational disorder and by significant propensities for sparse and local secondary structure. Although secondary structure propensities are essentially conserved upon phase separation, hydrophobic burial of the side chains increases. As a result, the peptides become their own solvent and the peptides expand, consistent with polymer theory and with an increase in conformational disorder. In accordance with the liquid drop model (Weis-Fogh and Andersen, 1970), peptide aggregation is driven, at least in part, by the hydrophobic effect, which results in a three-fold increase in burial of non-polar groups for each polypeptide chain compared to the monomeric form. In the coacervate, the individual polypeptide chains approach a state of maximal conformational disorder, as predicted by the Flory theorem (Flory, 1953) and as expected from a rubber-like elastomer.

As ELPs derived from the sequence of tropoelastin coacervate as tropoelastin would, Reichheld *et al.* (2017) designed such compounds and analysed their dynamic behaviour using NMR spectroscopy. Strikingly, their data show that the hydrophobic domains of their ELPs present highly dynamic and transient β-turns within disordered chains, which is consistent with the sliding turn model (Tamburro, 1990). However, they also underline that this 'disordered' organisation is retained in the coacervated state and when the coacervate is cross-linked. The direct observation of the structure and dynamics of an elastomeric material undergoing a phase transition shows that, from the structural point of view, individual sequences compare to the cross-linked polymer, which strongly support the fractal nature of elastin. Altogether, this experimental study provides additional evidence for an entropy-driven mechanism of coacervation in which transient intermolecular hydrophobic contacts are formed by disordered chains, while water is excluded from the material.

Recently, a mini-elastin mimicking the alternating domain structure of tropoelastin was used to examine the coacervation process of elastin in the absence of exogenous cross-links (Vidal Ceballos *et al.,* 2022). Using differential interference contrast, fluorescence and particle-tracking micro-rheology, the transitions occurring in mini-elastin revealed that the liquid transition presents an intermediate step where randomly distributed insoluble materials (i.e. nucleation points) exist in the liquid phase before its general collapse to the coacervated state. Coacervation therefore appears as a spontaneous, random yet hierarchical process.

Molecular basis of elasticity

The biological function of elastin is incompatible with a unique, ordered structure. In its functional coacervated state, its hydrophobic domains form a water-swollen, disordered aggregate characterised by an ensemble of degenerate conformations with significant backbone hydration and fluctuating local secondary structure. The combination of two entropic forces, the hydrophobic effect and polypeptide chain entropy, governs the elastic recoil central to elastin's function and these effects are intimately linked (Figure 8).

The hydrophobic nature of elastin chains sustains the hydrophobic effect contributing to elastin entropy. These chains are synthesised and assembled in water, say in an energetically unfavourable medium. Consequently, they try to shield their hydrophobic moieties from water molecules but the high content of glycine and proline they present prevents them from truly folding, leading to a considerable conformational entropy.

The link between elastin conformations and elasticity has proved a difficult puzzle to solve. One of the long-lasting theories concerning the elasticity mechanism is the β-spiral structure proposed by Urry (Venkatachalam and Urry, 1981) for the repeating sequence VPGVG. Molecular dynamics simulations (Li *et al.,* 2001b) show that short stretches of β-spiral are possible, but transiently. Accordingly, the β-spiral model of Urry can be incorporated into the general equilibrium folded ⇄ extended as one of the different rapidly interconverting folded structures. In this context, librational motions contribute to the internal chain dynamics and constitute an important conformational fluctuation when the β-turns are isolated (Villani and Tamburro, 1995).

The folded ⇄ extended equilibrium was modelled by Tamburro for short, glycine-rich, repeating sequences of elastin in an explicit aqueous solvent in terms of nonlinear dynamic systems (Villani *et al.,* 2000). In the relaxed state, the dynamics of the formation and breaking of intramolecular hydrogen bonds were found to be characteristic of a chaotic Brownian-like, intramolecular motion. When the simulation was performed on a stretched peptide sequence, a different dynamical behaviour was obtained, indicating a transition to low entropy, quasiperiodic soliton-like motion. As, the reductionist approach has demonstrated that folded ⇄ extended conformations were found in all exons of elastin (Tamburro *et al.,* 2003, 2006), it can be reasonably inferred that the same dynamic picture holds for the intact protein in the relaxed ⇄ stretched state.

In Tamburro's model, the aqueous solvent functions to facilitate the folded ⇄ extended conformational equilibria, giving rise to a noticeable internal main chain dynamics. In the model proposed by Gosline (1978), at low extensions the absorption of water onto exposed hydrophobic groups causes a large decrease in entropy, contributing to the restoring elastomeric force. In the stretched state the hydrophobic groups of polypeptide chains are exposed to the surrounding water that becomes organised in clathrate-like structures of low entropy. In this context, it is to be emphasised that the solvent presence explains the unique behaviour of elastin, the only elastomer known to require swelling

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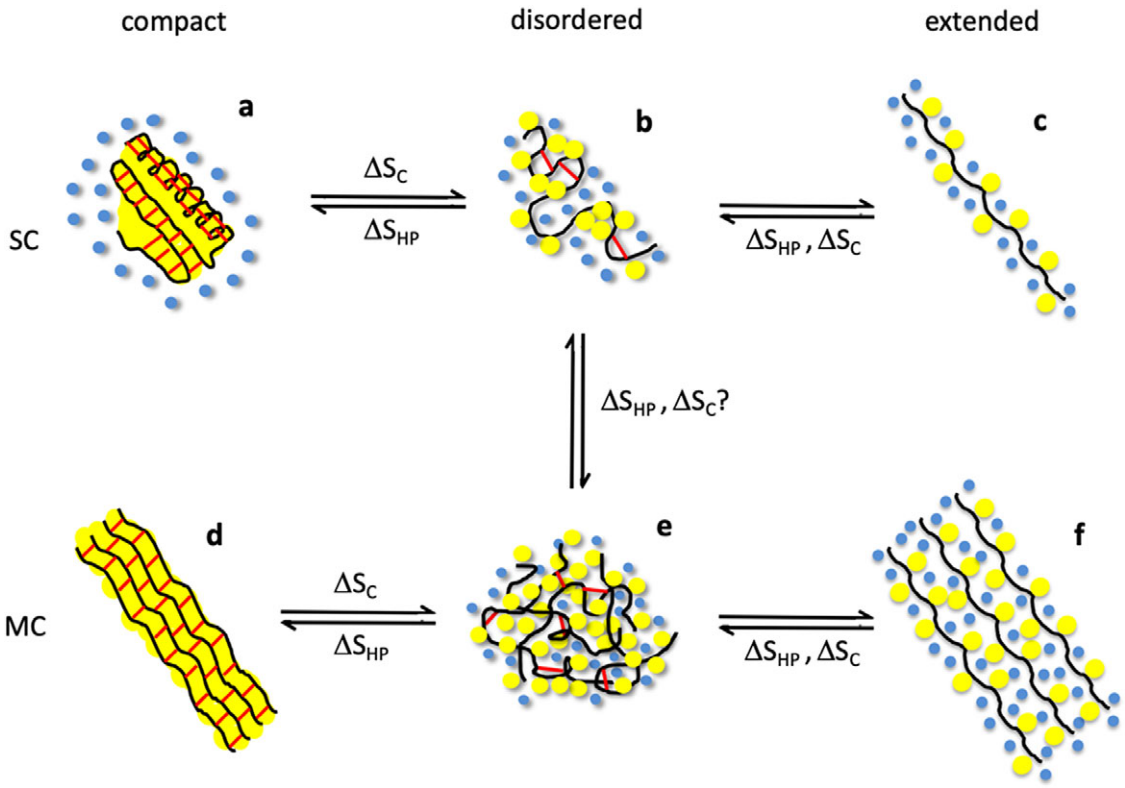

Figure 8. Structural basis of entropic elasticity in self-assembled elastomeric proteins. Schematic description of polypeptide main chains (black), non-polar side chains (yellow), solvating water molecules (blue), and peptide–peptide hydrogen bonds (red) in monomeric (SC, top row) and aggregated (MC, bottom row) states. Globular proteins that unfold or misfold are prone to aggregation, which leads to highly ordered amyloid fibrils. Both the native (*a*) and amyloid (*d*) states of globular proteins are characterised by extensive secondary structure and a water-excluding hydrophobic core. Despite their hydrophobic character, elastin and other self-assembled elastomers cannot form such compact structures due to their high content in proline and glycine (Rauscher *et al.,* 2006). Instead, they are hydrated and disordered both in their monomeric (*b*) and aggregated (*e*) states, so that they may readily undergo extension and elastic recoil (*e*,*f*). The role of the two dominant types of entropy, the hydrophobic effect (ΔS_{HP}) and chain entropy (ΔS_C), is highlighted. While the hydrophobic effect favours hydrophobic collapse (*c*→*b*, *f*→*e*), aggregation (*b*→*e*), and, if possible, compact, water-excluding states (*b*→*a*, *e*→*d*), conformational entropy favours disordered (*b*,*e*) over extended (*c*,*f*) and compact (*a*,*d*) states. As a result, both entropic effects contribute to elastic recoil. Adapted with permission from Rauscher *et al.* (2006). © 2006 Elsevier.

in water for displaying an elastomeric force. Molecular dynamics simulations (Li *et al.,* 2001b) appear to support this solvent-entropy mechanism.

In terms of nonlinear dynamic systems theory, the Gosline mechanism can be incorporated into the dynamical model theorised by Tamburro. In fact, diffusive (chaotic) motions of bulk water in the relaxed maximum entropy state would be substituted by vibrational (quasiperiodic) motions of clathrate water in the extended state. However, previous measurements (Mistrali *et al.,* 1971) have clearly demonstrated that elastin behaves as an ideal rubber even in dimethyl sulfoxide. This means that elastin's elasticity does not specifically depend on the aqueous solvent, but rather on the presence of a polar solvent, which would act as a plasticiser, favouring trans-conformational equilibria. Accordingly, internal main chain dynamics should be, at least in part, at the origin of the elasticity.

Elastin structure–function relationships defy conventional wisdom about protein folding, aggregation, and disorder due to the following considerations: (1) although the structure of the coacervated peptide chains is nearly maximally disordered, it is not random but instead contains well-defined and significantly-populated secondary structure elements in the form of hydrogen-bonded turns; (2) however, because these turns are at once local, sparse, and transient, the polypeptide backbone remains highly hydrated on average; as a result, (3) the hydrophobic side-chains cannot form a compact, water-excluding core even though they are significantly buried.

Elastin elasticity must be envisaged globally. The elastic system comprises elastin chains and its surrounding water. When both entities are considered simultaneously, the rationale for elastin elasticity can be summarised as presented in Figure 9.

One of the direct consequences of the elastic scheme presented in Figure 9 is that any effect altering chain dynamics or hydration of the coacervate in the relaxed state will modify the ability of the system to respond to deformation either by changing the amplitude or the efficiency of elasticity. Such alterations can be triggered by interactions of elastin with lipids (lipid deposition), with calcium (calcification), its cleavage, the addition of sugars (glycation), and others. Importantly, such occurrences are observed in age-related diseases affecting large elastic arteries (Wagenseil and Mecham, 2012).

Remaining questions

While the structure-elasticity relationships of hydrated elastin chains are now well understood, it must be reminded that elastin is made of various tropoelastin isoforms. The role and impact of alternative splicing in defining an efficient and functional 3D network is still a matter of debate.

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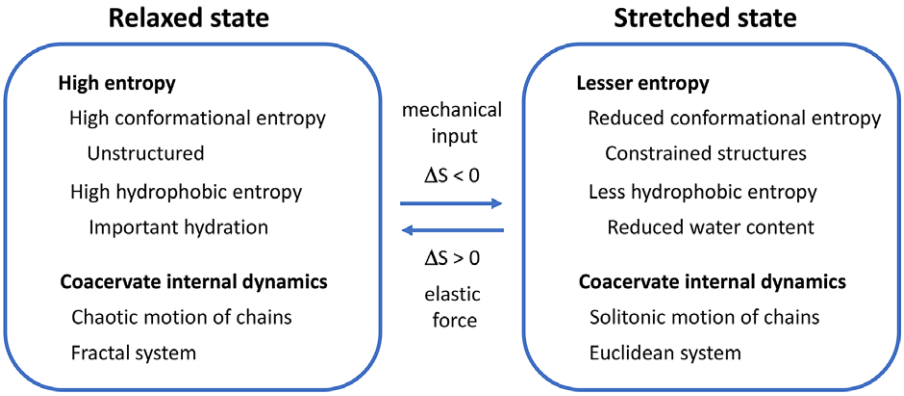

Figure 9. Structural and energy transitions underlying elasticity of the elastin coacervate. In the relaxed state, the system is highly entropic due to internal chain dynamics and its important hydration. The coacervate behaves as a fractal system. When a force is applied to the coacervate, the system stretches, conformational entropy is decreased, and water is drained from the coacervate. The entropy decreases and the system becomes Euclidean. When the stretching force is removed, an elastic force is developed to restore a high entropic level following the second principle of thermodynamics. A very recent publication (Jamhawi NM, Koder RL and Wittebort RJ (2024) Elastin recoil is driven by the hydrophobic effect. *Proceedings of the National Academy of Sciences of the United States of America* **121**, e2304009121) provides additional support to this model by showing that water molecules are increasingly ordered upon stretching.

Elastin deposition is a cell-driven process (Czirok *et al.,* 2006). However, how cells perceive the mechanical inputs during elastogenesis and orient the construction of the elastic network is still unknown. For instance, in the intervertebral disc, its shape changes according to the location within the tissue (Tavakoli *et al.,* 2020). Understanding this relationship between cells and their matrix is a critical element of tissue engineering that deserves further exploration.

Likewise, the role and importance of prolyl hydroxylation in elastin must be understood. These post-translational modifications are not randomly made (Schmelzer *et al.,* 2016). Therefore, they should have a functional role. As explained by the authors, the occurrence of prolyl-hydroxylation in elastin chains could influence both elastin stability and modulate local conformational changes. Now, the local impact of hydroxylation in elastin chains is still elusive.

Finally, recent synchrotron X-ray microtomography on aorta has evidenced the presence of dense filamentous structures inside the elastic lamellae (Ben Zemzem *et al.,* 2021). The regular 3D organisation of these filaments, and the fact that they are altered when the arterial wall ages (Ben Zemzem *et al.,* 2022), suggest a mechanical role. But further data are required to address this point and the molecular composition of these high phase contrast structures remains unknown.

Elastin-inspired technologies

Since the discovery of the first sequences corresponding to elastin elastic domains, considerable efforts have been made to understand their peculiar physicochemical behaviour, notably their coacervation process. In this aspect, D.W. Urry was a pioneer. In fact, he studied what would become the first and archetypical ELP building block, say VPGVG (Urry, 1988). Further, extensive biophysical analysis of VPGXG pentapeptide sequences led him to propose a hydrophobicity scale based on the temperature at which the inverse phase transition of the peptide was observed as a function of the residue at the X position (Urry, 1992). Undoubtedly, this work paved the way to the experimental design of tunable thermoresponsive ELPs, and further, to what we term here elastin-inspired technologies.

The ELP denomination has now passed to all artificial biopolymers harbouring elastin-inspired repeats. The versatility of ELP is acknowledged by protein chemists letting us foresee new achievements and new applications, notably in regenerative medicine (Natsume *et al.,* 2022; Goel *et al.,* 2023). This section presents some achievements in the field.

Protein purification

The inverse transition cycling purification method developed by Chilkoti's group (Meyer and Chilkoti, 1999) uses ELPs as a purification tag. This method takes advantage of the sharp and reversible phase transition the ELPs undergo, when the temperature is raised, to sequestrate the tagged recombinant protein. Indeed, as they retain their coacervation behaviour when they are fused to a protein, forcing ELPs to coacervate leads to efficient isolation of the protein. This purification method has been successfully used to separate proteins from contaminating *Escherichia coli* biomolecules by sequential and repeated steps of coacervation, centrifugation and solubilisation. This ELP-driven purification does not require chromatography, is easy to scale up and multiplex (Hassouneh *et al.,* 2010).

Health

Biomaterials

One of the first and direct use of ELPs was their incorporation in the design of biomaterials, because the cassette-like organisation of the *eln* gene facilitated the design and production of biomaterials incorporating elastin sequences (Girotti *et al.,* 2004). These biomaterials are now commonly considered in tissue engineering strategies as they present valuable properties (Chilkoti *et al.,* 2006). First, they can be genetically encoded allowing important production. Second, they are responsive to stimuli (notably temperature) and, finally, they are biocompatible. Therefore, ELPs are now commonly considered in tissue engineering strategies as they constitute a new paradigm in biomaterial design (Rodríguez-Cabello *et al.,* 2006). Further, ELP can be incorporated and used as hydrogels (Pepe *et al.,* 2021; Sharma *et al.,* 2021) or electrospun materials of various shapes (Boland *et al.,* 2004; Laezza *et al.,* 2022). The biomechanical properties of ELP-based hydrogels can be designed (Muiznieks and Keeley, 2017) and they are sensitive to the medium, notably to pH (Hollingshead and Liu, 2020). ELP-based hydrogels have been successfully used for wound care (Wen *et al.,* 2020) and to repair cartilage (Chen *et al.,* 2021). Nevertheless, their usage is more noticeable in cardiac

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(Gonzalez de Torre I *et al.,* 2020; Hume *et al.,* 2023) and vascular (Mahara *et al.,* 2017; Natsume *et al.,* 2022) regenerative medicine. Recently, a bioink containing modified and functionalised ELPs was successfully used to print scaffolds presenting excellent biocompatibility and mechanical properties (Dai *et al.,* 2021), offering new perspectives in tissue engineering.

Coating

As it is possible to tune and control the behaviour of ELP in solution (Hassouneh *et al.,* 2015), their controlled deposition as surface coating has been explored and tested, notably in the vascular context where the use of uncoated material can result in adverse effects such as restenosis. ELP coatings have been shown to improve the blood compatibility of cardiovascular devices as their presence limits platelets activation (Woodhouse *et al.,* 2004; González-Pérez *et al.,* 2022). Likewise, the deposition of genetically designed ELPs on the surface of CoCr stents enhanced endothelial cell adhesion and spreading on the metal surface (Castellanos *et al.,* 2015).

Targeting and delivery

ELP-tag have been shown to be very efficient for delivery and targeting (Jenkins *et al.,* 2021). In particular, Meyer *et al.* (2001) designed an ELP drug carrier that would coacervate at the temperature found in solid tumours, thereby targeting the drug preferably to the tumour site. Likewise, when ELP are fused with a cell-penetrating peptide, they improve the targeting and delivery of anti-tumour drugs (Walker *et al.,* 2012). In this context, the nanoparticle behaviour of ELPs and their versatility have found numerous applications. For instance, they have been used for intra-vitreal delivery to prevent age-related macular degeneration (Sreekumar *et al.,* 2018) and as vectors for the efficient delivery of cytokines in various pathological contexts (Gong *et al.,* 2022). Recently, a machine learning approach has been proposed (Cobb *et al.,* 2021) to control the size of ELP aggregates and their stability to optimise delivery.

Other applications

Although ELPs have been initially developed for applications in the health sciences, they have found interesting applications outside this field. ELPs have been shown to be excellent linkers for single-molecule force spectroscopy (Ott *et al.,* 2017). Indeed, their use improves the data quality and facilitates data analysis and interpretation because their mechanical behaviour is well-characterised and can be controlled. ELP-based compounds have also been considered in the environmental field. For instance, Sumiyoshi *et al.* (2022) have conjugated ELPs with metal chelating agents in order to sequester metal ions in ELP coacervates. As ELPs can be easily designed and bound to any metal-binding domain, the authors underline that they represent powerful heavy metal scavenging agents with potentially low environmental impacts.

The versatility of ELP has also led to astonishing applications. In 2016, Guo *et al.* (2016) demonstrated that the presence of His-tagged ELP could improve the formation and performances of lithium-O_2 battery cathodes. This finding is explained by the fact that the designed ELP form nanoparticles that can guide assembly, thereby enhancing the electric performance of the cathode. Recently, genetically engineered silk-ELP have been combined with graphene oxide binding domain (Martín-Moldes *et al.,* 2022). When graphene was bound on these compounds, the authors evidenced that their conductivity could be modulated by changing the temperature. When the critical temperature was reached the coacervate forms, graphene moieties get closer, and conductivity of the material is achieved. In summary, the presence of ELP allows an 'on–off' conductive switch driven by temperature due to hydrogel formation.

It was evidenced at the local scale, by piezoresponse force microscopy, that aortic elastin was ferroelectric (Liu *et al.,* 2014), namely that it exhibits a spontaneous electric polarisation, which can be switched by an electric field. This property, rather rare in bio-organic materials (Heredia *et al.,* 2012), is important as it could pave the way for the development of new bio-inspired molecular ferroelectrics with improved performances, peculiarly sought in the field of sensors, memory cells and energy storage.

Conclusion

Our understanding of elastin and its elasticity has improved considerably in the last decades. This improvement is due, at least in part, to the progression of computing power making it possible to realise calculations that were unachievable some years ago. Another important consideration is the fact that elastin has been studied by different scientific communities, namely biologists, chemists, and physicists. The cumulative insights gathered from various disciplines, either from theoretical or experimental data, now allow us to better understand elastin structure and the physical bases underlying its elasticity.

Elastin is a polymer of tropoelastin chains cross-linked to form beaded filaments that organise into fibres (Bressan *et al.,* 1986). As such, elastin assembly is consistent with the liquid drop model (Weis-Fogh and Andersen, 1970). In this context, tropoelastins (the beads) are organised seemingly individually. Because of their peculiar hydrophobic nature and the presence of water, tropoelastins do not fold because of their important internal chain dynamics. The described shape of tropoelastin (Baldock *et al.,* 2011) might therefore be one of the many possible shapes adopted, or their average.

As the structures of elastin in water are constantly moving and shifting, as proposed in the sliding turn model (Tamburro *et al.,* 2005), and because water sustains internal chains dynamics, the functional elastin/water system (coacervate) exhibits high entropy. This highly entropic fundamental and resting state is the prerequisite for efficient and reversible elasticity following the second principle of thermodynamics.

The last decades have seen the development of a new generation of ELPs for applications in various fields. Still, the complex behaviour of ELPs in water remains a puzzle. Indeed, by controlling their sequences, we can predict the temperature at which their coacervation will occur, but the structure and behaviour of the ensuing hydrogel remain a mystery for the moment.

This makes us humbly wonder how cells, so efficiently, manage and control tropoelastin assembly and deposition to form elastic fibres in tissues.

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References

Anfinsen CB (1972) The formation and stabilization of protein structure. *Biochemical Journal* **128**, 737–749.

Baldock C, Oberhauser AF, Ma L, Lammie D, Siegler V, Mithieux SM, Tu Y, Chow JYH, Suleman F, Malfois M, Rogers S, Guo L, Irving TC, Wess TJ and Weiss AS (2011) Shape of tropoelastin, the highly extensible protein that controls human tissue elasticity. *Proceedings of the National Academy of Sciences of the United States of America* **108**, 4322–4327.

Bellingham CM, Lillie MA, Gosline JM, Wright GM, Starcher BC, Bailey AJ, Woodhouse KA and Keeley FW (2003) Recombinant human elastin polypeptides self-assemble into biomaterials with elastin-like properties. *Biopolymers* **70**, 445–455.

Ben Zemzem A, Genevaux A, Wahart A, Bodey AJ, Blaise S, Romier-Crouzet B, Jonquet J, Bour C, Cogranne R, Beauseroy P, Dauchez M, Sherratt MJ, Debelle L and Almagro S (2021) X-ray microtomography reveals a lattice-like network within aortic elastic lamellae. *The FASEB Journal* **35**, e21844.

Ben Zemzem A, Liang X, Vanalderwiert L, Bour C, Romier-Crouzet B, Blaise S, Sherratt MJ, Weitkamp T, Dauchez M, Baud S, Passat N, Debelle L and Almagro S (2022) Early alterations of intra-mural elastic lamellae revealed by synchrotron X-ray micro-CT exploration of diabetic aortas. *International Journal of Molecular Sciences* **23**, 3250.

Bisaccia F, Castiglione-Morelli MA, Spisani S, Ostuni A, Serafini-Fracassini A, Bavoso A and Tamburro AM (1998) The amino acid sequence coded by the rarely expressed exon 26A of human elastin contains a stable beta-turn with chemotactic activity for monocytes. *Biochemistry* **37**, 11128–11135.

Blanchevoye C, Floquet N, Scandolera A, Baud S, Maurice P, Bocquet O, Blaise S, Ghoneim C, Cantarelli B, Delacoux F, Dauchez M, Efremov RG, Martiny L, Duca L and Debelle L (2013) Interaction between the elastin peptide VGVAPG and human elastin binding protein. *Journal of Biological Chemistry* **288**, 1317–1328.

Bochicchio B, Aït-Ali A, Tamburro AM and Alix AJP (2004) Spectroscopic evidence revealing polyproline II structure in hydrophobic, putatively elastomeric sequences encoded by specific exons of human tropoelastin. *Biopolymers* **73**, 484–493.

Bochicchio B and Pepe A (2011) Role of polyproline II conformation in human tropoelastin structure. *Chirality* **23**, 694–702.

Bochicchio B, Pepe A, Crudele M, Belloy N, Baud S and Dauchez M (2015) Tuning self-assembly in elastin-derived peptides. *Soft Matter* **11**, 3385–3395.

Boland ED, Matthews JA, Pawłowski KJ, Simpson DG, Wnek GE and Bowlin GL (2004) Electrospinning collagen and elastin: Preliminary vascular tissue engineering. *Frontiers in Bioscience* **9**, 1422.

Bone S and Pethig R (1985) Dielectric studies of protein hydration and hydration-induced flexibility. *Journal of Molecular Biology* **181**, 323–326.

Borman S (1991) Fractals offer mathematical tool for study of complex chemical systems: Mathematical language that uses noninteger dimensions to describe irregular shapes can be used to model catalysis, kinetics, colloid aggregation. *Chemical & Engineering News Archive* **69**, 28–35.

Bottaro S and Lindorff-Larsen K (2018) Biophysical experiments and biomolecular simulations: A perfect match? *Science* **361**, 355–360.

Boyd CD, Pierce RA, Schwarzbauer JE, Doege K and Sandell LJ (1993) Alternate exon usage is a commonly used mechanism for increasing coding diversity within genes coding for extracellular matrix proteins. *Matrix* **13**, 457–469.

Bressan GM, Pasquali-Ronchetti I, Fornieri C, Mattioli F, Castellani I and Volpin D (1986) Relevance of aggregation properties of tropoelastin to the assembly and structure of elastic fibers. *Journal of Ultrastructure and Molecular Structure Research* **94**, 209–216.

Brown-Augsburger P, Tisdale C, Broekelmann T, Sloan C and Mecham RP (1995) Identification of an elastin cross-linking domain that joins three peptide chains. *Journal of Biological Chemistry* **270**, 17778–17783.

Buchet R, Luan C-H, Prasad KU, Harris RD and Urry DW (1988) Dielectric relaxation studies on analogues of the polypentapeptlde of elastin. *The Journal of Physical Chemistry* **92**, 511–517.

Cametti C, Marchetti S, Gambi CMC and Onori G (2011) Dielectric relaxation spectroscopy of lysozyme aqueous solutions: Analysis of the δ-dispersion and the contribution of the hydration water. *The Journal of Physical Chemistry B* **115**, 7144–7153.

Castellanos MI, Zenses A-S, Grau A, Rodríguez-Cabello JC, Gil FJ, Manero JM and Pegueroles M (2015) Biofunctionalization of REDV elastin-like recombinamers improves endothelialization on CoCr alloy surfaces for cardiovascular applications. *Colloids and Surfaces B: Biointerfaces* **127**, 22–32.

Ceccorulli G, Scandola M and Pezzin G (1977) Calorimetric investigation of some elastin-solvent systems. *Biopolymers* **16**, 1505–1512.

Chang DK, Venkatachalam CM, Prasad KU and Urry DW (1989) Nuclear overhauser effect and computational characterization of the β-spiral of the polypentapeptide of elastin. *Journal of Biomolecular Structure and Dynamics* **6**, 851–858.

Chen Z, Zhang Q, Li H, Wei Q, Zhao X and Chen F (2021) Elastin-like polypeptide modified silk fibroin porous scaffold promotes osteochondral repair. *Bioactive Materials* **6**, 589–601.

Chilkoti A, Christensen T and Mackay J (2006) Stimulus responsive elastin biopolymers: Applications in medicine and biotechnology. *Current Opinion in Chemical Biology* **10**, 652–657.

Cobb JS, Engel A, Seale MA and Janorkar AV (2021) Machine learning to determine optimal conditions for controlling the size of elastin-based particles. *Scientific Reports* **11**, 6343.

Cunningham LW (1987) Structural and contractile proteins, part D: Extracellular matrix. *Methods in Enzymology* **144**, 3–561.

Czirok A, Zach J, Kozel BA, Mecham RP, Davis EC and Rongish BJ (2006) Elastic fiber macro-assembly is a hierarchical, cell motion-mediated process. *Journal of Cellular Physiology* **207**, 97–106.

Dai M, Belaïdi J-P, Fleury G, Garanger E, Rielland M, Schultze X and Lecommandoux S (2021) Elastin-like polypeptide-based bioink: A promising alternative for 3D bioprinting. *Biomacromolecules* **22**, 4956–4966.

Dandurand J, Samouillan V, Lacabanne C, Pepe A and Bochicchio B (2015) Water structure and elastin-like peptide aggregation: A differential calorimetric approach. *Journal of Thermal Analysis and Calorimetry* **120**, 419–426.

de Torre I G, Alonso M and Rodriguez-Cabello JC (2020) Elastin-based materials: Promising candidates for cardiac tissue regeneration. *Frontiers in Bioengineering and Biotechnology* **8**, 657.

Debelle L, Alix AJP, Jacob M-P, Huvenne J-P, Berjot M, Sombret B and Legrand P (1995) Bovine elastin and k-elastin secondary structure determination by optical spectroscopies. *The Journal of Biological Chemistry* **270**, 26099–26103.

Debelle L and Tamburro AM (1999) Elastin: molecular description and function. *The International Journal of Biochemistry & Cell Biology* **31**, 261–272.

Dempsey EW and Lansing AI (1954) Elastic tissue. *International Review of Cytology* **3**, 437–453.

Depenveiller C, Wong H, Crowet JM, Debelle L, Baud S, Dauchez M and Belloy N (2023) Challenging level of rigid-body approach involving numerical elements (CHLORAINE) applied to repeated elastin peptides. *Journal of Structural Biology* **215**, 107986.

Dorrington K, Grut W and McCrum NG (1975) Mechanical state of elastin. *Nature* **255**, 476–478.

Ellis GE and Packer KJ (1976) Nuclear spin-relaxation studies of hydrated elastin. *Biopolymers* **15**, 813–832.

Engeland R (1925) A new hydrolysis product from elastin. *Biochemical Journal* **19**, 850–852.

Family F (1993) Fractal structures and dynamics of cluster growth. In *On Clusters and Clustering*. Amsterdam: Elsevier, pp. 323–344.

Fenimore PW, Frauenfelder H, McMahon BH and Young RD (2004) Bulk-solvent and hydration-shell fluctuations, similar to α- and β-fluctuations in glasses, control protein motions and functions. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 14408–14413.

Floquet N, Héry-Huynh S, Dauchez M, Derreumaux P, Tamburro AM and Alix AJP (2004) Structural characterization of VGVAPG, an elastin-derived peptide. *Biopolymers* **76**, 266–280.

Flory PJ (1953) Principles of polymer chemistry. In *The George Fisher Baker Non-resident Lectureship in Chemistry at Cornell University*. Ithaca, NY: Cornell University Press.

Frauenfelder H, Chen G, Berendzen J, Fenimore PW, Jansson H, McMahon BH, Stroe IR, Swenson J and Young RD (2009) A unified model of protein dynamics. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 5129–5134.

https://doi.org/10.1017/S0033583524000040 Published online by Cambridge University Press

Fuchs PFJ, Bonvin AMJJ, Bochicchio B, Pepe A, Alix AJP and Tamburro AM (2006) Kinetics and thermodynamics of type VIII β-turn formation: A CD, NMR, and microsecond explicit molecular dynamics study of the GDNP tetrapeptide. *Biophysical Journal* **90**, 2745–2759.

Gainaru C, Fillmer A and Böhmer R (2009) Dielectric response of deeply supercooled hydration water in the connective tissue proteins collagen and elastin. *The Journal of Physical Chemistry B* **113**, 12628–12631.

Girotti A, Reguera J, Rodriguez-Cabello JC, Arias FJ, Alonso M and Testera AM (2004) Design and bioproduction of a recombinant multi(bio)functional elastin-like protein polymer containing cell adhesion sequences for tissue engineering purposes. *Journal of Materials Science: Materials in Medicine* **15**, 479–484.

Gniadecka M and Jemec GB (1998) Quantitative evaluation of chronological ageing and photoageing in vivo: Studies on skin echogenicity and thickness. *The British Journal of Dermatology* **139**, 815–821.

Goel R, Gulwani D, Upadhyay P, Sarangthem V and Singh TD (2023) Unsung versatility of elastin-like polypeptide inspired spheroid fabrication: A review. *International Journal of Biological Macromolecules* **234**, 123664.

Gong L, Yang Z, Zhang F and Gao W (2022) Cytokine conjugates to elastin-like polypeptides. *Advanced Drug Delivery Reviews* **190**, 114541.

González-Pérez F, Acosta S, Rütten S, Emonts C, Kopp A, Henke H-W, Bruners P, Gries T, Rodríguez-Cabello JC, Jockenhoevel S and Fernández-Colino A (2022) Biohybrid elastin-like venous valve with potential for in situ tissue engineering. *Frontiers in Bioengineering and Biotechnology* **10**, 988533.

Gosline JM (1978) Hydrophobic interaction and a model for the elasticity of elastin. *Biopolymers* **17**, 677–695.

Gosline J, Lillie M, Carrington E, Guerette P, Ortlepp C and Savage K (2002) Elastic proteins: Biological roles and mechanical properties. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **357**, 121–132.

Gotte L, Stern P, Elsden DF and Partridge SM (1963) The chemistry of connective tissues. 8. The composition of elastin from three bovine tissues. *Biochemical Journal* **87**, 344–351.

Gray WR, Sandberg LB and Foster JA (1973) Molecular model for elastin structure and function. *Nature* **246**, 461–466.

Guo G, Yao X, Ang H, Tan H, Zhang Y, Guo Y, Fong E and Yan Q (2016) Using elastin protein to develop highly efficient air cathodes for lithium-O_2 batteries. *Nanotechnology* **27**, 045401.

Halle B (2004) Protein hydration dynamics in solution: A critical survey. *Philosophical transactions of the Royal Society of London Series B, Biological Sciences* **359**, 1207–1223; discussion 1223–1224, 1323–1328.

Hassouneh W, Christensen T and Chilkoti A (2010) Elastin-like polypeptides as a purification tag for recombinant proteins. *Current Protocols in Protein Science* **61**, 6.11.1–6.11.16.

Hassouneh W, Zhulina EB, Chilkoti A and Rubinstein M (2015) Elastin-like polypeptide diblock copolymers self-assemble into weak micelles. *Macromolecules* **48**, 4183–4195.

Hatakeyama T, Nakamura K and Hatakeyama H (1988) Determination of bound water content in polymers by DTA, DSC and TG. *Thermochimica Acta* **123**, 153–161.

Hay JN and Laity PR (2000) Observations of water migration during thermoporometry studies of cellulose films. *Polymer* **41**, 6171–6180.

Hedtke T, Schräder CU, Heinz A, Hoehenwarter W, Brinckmann J, Groth T and Schmelzer CEH (2019) A comprehensive map of human elastin cross-linking during elastogenesis. *The FEBS Journal* **286**, 3594–3610.

Heinz A (2020) Elastases and elastokines: Elastin degradation and its significance in health and disease. *Critical Reviews in Biochemistry and Molecular Biology* **55**, 252–273.

Heinz A (2021) Elastic fibers during aging and disease. *Ageing Research Reviews* **66**, 101255.

Heredia A, Meunier V, Bdikin IK, Gracio J, Balke N, Jesse S, Tselev A, Agarwal PK, Sumpter BG, Kalinin SV and Kholkin AL (2012) Nanoscale ferroelectricity in crystalline γ-glycine. *Advanced Functional Materials* **22**, 2996–3003.

Hernández B, Crowet J-M, Thiery J, Kruglik SG, Belloy N, Baud S, Dauchez M and Debelle L (2020) Structural analysis of nonapeptides derived from elastin. *Biophysical Journal* **118**, 2755–2768.

Heys KR, Friedrich MG and Truscott RJW (2008) Free and bound water in normal and cataractous human lenses. *Investigative Ophthalmology & Visual Science* **49**, 1991–1997.

Hoeve CAJ and Flory PJ (1958) The elastic properties of elastin. *Journal of the American Chemical Society* **80**, 6523–6526.

Hoeve CAJ and Flory PJ (1974) The elastic properties of elastin. *Biopolymers* **13**, 677–686.

Hollingshead S and Liu JC (2020) pH-sensitive mechanical properties of elastin-based hydrogels. *Macromolecular Bioscience* **20**, 1900369.

Huang J, Rauscher S, Nawrocki G, Ran T, Feig M, De Groot BL, Grubmüller H and MacKerell AD (2017) CHARMM36m: An improved force field for folded and intrinsically disordered proteins. *Nature Methods* **14**, 71–73.

Hume RD, Kanagalingam S, Deshmukh T, Chen S, Mithieux SM, Rashid FN, Roohani I, Lu J, Doan T, Graham D, Clayton ZE, Slaughter E, Kizana E, Stempien-Otero AS, Brown P, Thomas L, Weiss AS and Chong JJH (2023) Tropoelastin improves post-infarct cardiac function. *Circulation Research* **132**, 72–86.

Indik Z, Yeh H, Ornstein-Goldstein N, Sheppard P, Anderson N, Rosenbloom JC, Peltonen L and Rosenbloom J (1987) Alternative splicing of human elastin mRNA indicated by sequence analysis of cloned genomic and complementary DNA. *Proceedings of the National Academy of Sciences of the United States of America* **84**, 5680–5684.

Jacob MP and Hornebeck W (1985) Isolation and characterization of insoluble and kappa-elastin. *Frontiers of Matrix Biology* **10**, 92–129.

Jaffe M, Menczel J and Bessey W (1997) Fibers. In *Thermal Characterization of Polymeric Materials*. New York: Academic Press, pp. 1767–1954.

Jenkins IC, Milligan JJ and Chilkoti A (2021) Genetically encoded elastin-like polypeptides for drug delivery. *Advanced Healthcare Materials* **10**, 2100209.

Jensen SA, Vrhovski B and Weiss AS (2000) Domain 26 of tropoelastin plays a dominant role in association by coacervation. *Journal of Biological Chemistry* **275**, 28449–28454.

Johnson NR and Wang Y (2014) Coacervate delivery systems for proteins and small molecule drugs. *Expert Opinion on Drug Delivery* **11**, 1829–1832.

Kaatze U (1997) The dielectric properties of water in its different states of interaction. *Journal of Solution Chemistry* **26**, 1049–1112.

Kaatze U, Behrends R and Pottel R (2002) Hydrogen network fluctuations and dielectric spectrometry of liquids. *Journal of Non-Crystalline Solids* **305**, 19–28.

Kakivaya SR and Hoeve CAJ (1975) The glass point of elastin. *Proceedings of the National Academy of Sciences of the United States of America* **72**, 3505–3507.

Keeley FW (2013) The evolution of elastin. In Keeley FW and Mecham RP (eds.), *Evolution of Extracellular Matrix, Biology of Extracellular Matrix*. Berlin: Springer, pp. 73–119.

Keeley FW (2021) Structural proteins | The biochemistry of elastin. In: Jez J (ed.), *Encyclopedia of Biological Chemistry III*. Amsterdam: Elsevier, pp. 668–689.

Keeley FW, Bellingham CM and Woodhouse KA (2002) Elastin as a self–organizing biomaterial: Use of recombinantly expressed human elastin polypeptides as a model for investigations of structure and self–assembly of elastin. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **357**, 185–189.

Kozel BA and Mecham RP (2019) Elastic fiber ultrastructure and assembly. *Matrix Biology* **84**, 31–40.

Kozel BA, Mecham RP and Rosenbloom J (2011) Elastin. In Mecham RP (ed.), *The Extracellular Matrix: An Overview*. Berlin: Springer, pp. 267–301.

Kozel BA, Wachi H, Davis EC and Mecham RP (2003) Domains in tropoelastin that mediate elastin depositionin vitro and in vivo. *Journal of Biological Chemistry* **278**, 18491–18498.

Kumashiro KK, Kurano TL, Niemczura WP, Martino M and Tamburro AM (2003) ^{13}C CPMAS NMR studies of the elastin-like polypeptide $(LGGVG)_n$. *Biopolymers* **70**, 221–226.

Kyriakopoulou K, Piperigkou Z, Tzaferi K and Karamanos NK (2023) Trends in extracellular matrix biology. *Molecular Biology Reports* **50**, 853–863.

Laezza A, Pepe A and Bochicchio B (2022) Elastin-hyaluronan bioconjugate as bioactive component in electrospun scaffolds. *Chemistry – A European Journal* **28**, e202201959.

Lansing AI, Rosenthal TB, Alex M and Dempsey EW (1952) The structure and chemical characterization of elastic fibers as reveled by elastase and by electron microscopy. *The Anatomical Record* **114**, 555–575.

https://doi.org/10.1017/S0033583524000040 Published online by Cambridge University Press

Lapidus LJ, Steinbach PJ, Eaton WA, Szabo A and Hofrichter J (2002) Effects of chain stiffness on the dynamics of loop formation in polypeptides. Appendix: Testing a 1-dimensional diffusion model for peptide dynamics. *The Journal of Physical Chemistry B* **106**, 11628–11640.

Le Page A, Khalil A, Vermette P, Frost EH, Larbi A, Witkowski JM and Fulop T (2019) The role of elastin-derived peptides in human physiology and diseases. *Matrix Biology* **84**, 81–96.

Ledvina M and Bartoš F (1968) Differences in elastase hydrolysates of elastin from ligamentum nuchae. *FEBS Letters* **1**, 9–12.

Lelj F, Tamburro AM, Villani V, Grimaldi P and Guantieri V (1992) Molecular dynamics study of the conformational behavior of a representative elastin building block: Boc-Gly-Val-Gly-Gly-Leu-OMe. *Biopolymers* **32**, 161–172.

Li B, Alonso DOV, Bennion BJ and Daggett V (2001a) Hydrophobic hydration is an important source of elasticity in elastin-based biopolymers. *Journal of the American Chemical Society* **123**, 11991–11998.

Li B, Alonso DOV and Daggett V (2001b) The molecular basis for the inverse temperature transition of elastin. *Journal of Molecular Biology* **305**, 581–592.

Li B and Daggett V (2002) Molecular basis for the extensibility of elastin. *Journal of Muscle Research and Cell Motility* **23**, 561–573.

Lillie MA and Gosline JM (1990) The effects of hydration on the dynamic mechanical properties of elastin. *Biopolymers* **29**, 1147–1160.

Lillie MA and Gosline JM (2002) Effects of lipids on elastin's viscoelastic properties. *Biopolymers* **64**, 127–138.

Liu Y, Cai H-L, Zelisko M, Wang Y, Sun J, Yan F, Ma F, Wang P, Chen QN, Zheng H, Meng X, Sharma P, Zhang Y and Li J (2014) Ferroelectric switching of elastin. *Proceedings of the National Academy of Sciences of the United States of America* **111**, E2780–E2786.

Liu WG and Yao KD (2001) What causes the unfrozen water in polymers: Hydrogen bonds between water and polymer chains? *Polymer* **42**, 3943–3947.

Long MM, King VJ, Prasad KU and Urry DW (1988) Chemotaxis of fibroblasts toward nonapeptide of elastin. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research* **968**, 300–311.

Long M, Rapaka RS, Volpin D, Pasquali-Ronchetti I and Urry DW (1980) Spectroscopic and electron micrographic studies on the repeat tetrapeptide of tropoelastin: (Val-Pro-Gly-Gly). *Archives of Biochemistry and Biophysics* **201**, 445–452.

López-Bueno C, Herreros-Lucas C, Suárez-Rodríguez M, Bittermann MR, Amigo A, Woutersen S, Giménez-López M and Rivadulla F (2021) A new type of supramolecular fluid based on H_2O–alkylammonium/phosphonium solutions. *Angewandte Chemie International Edition* **60**, 7540–7546.

Luan CH, Parker TM, Gowda DC and Urry DW (1992) Hydrophobicity of amino acid residues: Differential scanning calorimetry and synthesis of the aromatic analogues of the polypentapeptide of elastin. *Biopolymers* **32**, 1251–1261.

Lusceac SA, Vogel MR and Herbers CR (2010) 2H and 13C NMR studies on the temperature-dependent water and protein dynamics in hydrated elastin, myoglobin and collagen. *Biochimica et Biophysica Acta (BBA) – Proteins and Proteomics* **1804**, 41–48.

Maeda I, Mizoiri N, Briones MPP and Okamoto K (2007) Induction of macrophage migration through lactose-insensitive receptor by elastin-derived nonapeptides and their analog. *Journal of Peptide Science* **13**, 263–268.

Mahara A, Kiick KL and Yamaoka T (2017) *In vivo* guided vascular regeneration with a non-porous elastin-like polypeptide hydrogel tubular scaffold. *Journal of Biomedical Materials Research Part A* **105**, 1746–1755.

Martín-Moldes Z, Spey Q, Bhatacharya T and Kaplan DL (2022) Silk-elastin-like-protein/graphene-oxide composites for dynamic electronic biomaterials. *Macromolecular Bioscience* **22**, 2200122.

Maurice P, Blaise S, Gayral S, Debelle L, Laffargue M, Hornebeck W and Duca L (2013) Elastin fragmentation and atherosclerosis progression: The elastokine concept. *Trends in Cardiovascular Medicine* **23**, 211–221.

Mecham RP (2008) Methods in elastic tissue biology: Elastin isolation and purification. *Methods* **45**, 32–41.

Megret C, Lamure A, Pieraggi MT, Lacabanne C, Guantieri V and Tamburro AM (1993) Solid-state studies on synthetic fragments and analogues of elastin. *International Journal of Biological Macromolecules* **15**, 305–312.

Meyer DE and Chilkoti A (1999) Purification of recombinant proteins by fusion with thermally-responsive polypeptides. *Nature Biotechnology* **17**, 1112–1115.

Meyer KH and Ferri C (1937) Die elastischen Eigenschaften der elastischen und der kollagenen Fasern und ihre molekulare Deutung. *Pflüger's Archiv für die gesamte Physiologie des Menschen und der Tiere* **238**, 78–90.

Meyer DE, Kong GA, Dewhirst MW, Zalutsky MR and Chilkoti A (2001) Targeting a genetically engineered elastin-like polypeptide to solid tumors by local hyperthermia. *Cancer Research* **61**, 1548–1554.

Miao M, Cirulis JT, Lee S and Keeley FW (2005) Structural determinants of cross-linking and hydrophobic domains for self-assembly of elastin-like polypeptides. *Biochemistry* **44**, 14367–14375.

Mistrali F, Volpin D, Garibaldo GB and Ciferri A (1971) Thermodynamics of elasticity in open systems. Elastin. *The Journal of Physical Chemistry* **75**, 142–150.

Moroy G, Alix AJP and Héry-Huynh S (2005) Structural characterization of human elastin derived peptides containing the GXXP sequence. *Biopolymers* **78**, 206–220.

Muiznieks LD and Keeley FW (2017) Biomechanical design of elastic protein biomaterials: A balance of protein structure and conformational disorder. *ACS Biomaterials Science & Engineering* **3**, 661–679.

Muiznieks LD, Sharpe S, Pomès R and Keeley FW (2018) Role of liquid–liquid phase separation in assembly of elastin and other extracellular matrix proteins. *Journal of Molecular Biology* **430**, 4741–4753.

Nandi N, Bhattacharyya K and Bagchi B (2000) Dielectric relaxation and solvation dynamics of water in complex chemical and biological systems. *Chemical Reviews* **100**, 2013–2046.

Natsume K, Nakamura J, Sato K, Ohtsuki C and Sugawara-Narutaki A (2022) Biological properties of self-assembled nanofibers of elastin-like block polypeptides for tissue-engineered vascular grafts: Platelet inhibition, endothelial cell activation and smooth muscle cell maintenance. *Regenerative Biomaterials* **10**, rbac111.

Nguyen NN, Diger Berger R, Wagner M, Thiel R, Rgen Butt H-J and Graf R (2021) "Liquid-like" water in clathrates induced by host−guest hydrogen bonding. *The Journal of Physical Chemistry C* **125**, 15751–15757.

Ott W, Jobst MA, Bauer MS, Durner E, Milles LF, Nash MA and Gaub HE (2017) Elastin-like polypeptide linkers for single-molecule force spectroscopy. *ACS Nano* **11**, 6346–6354.

Panagopoulou A, Kyritsis A, Vodina M and Pissis P (2013) Dynamics of uncrystallized water and protein in hydrated elastin studied by thermal and dielectric techniques. *Biochimica et Biophysica Acta (BBA) – Proteins and Proteomics* **1834**, 977–988.

Partridge SM (1963) Elastin. *Advances in protein chemistry* **17**, 227–302.

Partridge SM (1967) Gel filtration using a column packed with elastin fibres. *Nature (London)* **213**, 1123–1125.

Partridge SM, Davis HF and Adair GS (1955) The chemistry of connective tissues. 2. Soluble proteins derived from partial hydrolysis of elastin. *Biochemical Journal* **61**, 11–21.

Partridge SM, Elsden DF and Thomas J (1963) Constitution of the cross-linkages in elastin. *Nature* **197**, 1297–1298.

Pasquali Ronchetti I, Fornieri C, Baccarani-Contri M and Volpin D (1979) The ultrastructure of elastin revealed by freeze-fracture electron microscopy. *Micron* **10**, 89–99.

Pepe A, Flamia R, Guerra D, Quaglino D, Bochicchio B, Pasquali-Ronchetti I and Tamburro AM (2008) Exon 26-coded polypeptide: An isolated hydrophobic domain of human tropoelastin able to self-assemble in vitro. *Matrix Biology: Journal of the International Society for Matrix Biology* **27**, 441–450.

Pepe A, Maio L, Bracalello A, Quintanilla-Sierra L, Arias FJ, Girotti A and Bochicchio B (2021) Soft hydrogel inspired by elastomeric proteins. *ACS Biomaterials Science & Engineering* **7**, 5028–5038.

Perry A, Stypa MP, Tenn BK and Kumashiro KK (2002) Solid-state 13C NMR reveals effects of temperature and hydrationon elastin. *Biophysical Journal* **82**, 1086–1095.

Raju K and Anwar RA (1987) Primary structures of bovine elastin a, b, and c deduced from the sequences of cDNA clones. *Journal of Biological Chemistry* **262**, 5755–5762.

https://doi.org/10.1017/S0033583524000040 Published online by Cambridge University Press

Rault J, **Ping H and Nguyen T** (1995) Glass transition temperature regulation effect in a poly(vinyl alcohol)-water system. *Polymer* **36**, 1655–1661.

Rauscher S, **Baud S**, **Miao M**, **Keeley FW and Pomès R** (2006) Proline and glycine control protein self-organization into elastomeric or amyloid fibrils. *Structure* **14**, 1667–1676.

Rauscher S, **Gapsys V**, **Gajda MJ**, **Zweckstetter M**, **De Groot BL and Grubmüller H** (2015) Structural ensembles of intrinsically disordered proteins depend strongly on force field: A comparison to experiment. *Journal of Chemical Theory and Computation* **11**, 5513–5524.

Rauscher S and Pomès R (2012) Structural disorder and protein elasticity. In Fuxreiter M and Tompa P (eds.), *Fuzziness, Advances in Experimental Medicine and Biology*. New York, NY: Springer US, pp. 159–183.

Rauscher S and Pomès R (2017) The liquid structure of elastin. *eLife* **6**, e26526.

Reichheld SE, **Muiznieks LD**, **Huynh Q**, **Wang N**, **Ing C**, **Miao M**, **Sitarz EE**, **Pomès R**, **Sharpe S and Keeley FW** (2021) The evolutionary background and functional consequences of the rs2071307 polymorphism in human tropoelastin. *Biopolymers* **112**, e23414.

Reichheld SE, **Muiznieks LD**, **Keeley FW and Sharpe S** (2017) Direct observation of structure and dynamics during phase separation of an elastomeric protein. *Proceedings of the National Academy of Sciences of the United States of America* **114**, E4408–E4415.

Reichheld SE, **Muiznieks LD**, **Stahl R**, **Simonetti K**, **Sharpe S and Keeley FW** (2014) Conformational transitions of the cross-linking domains of elastin during self-assembly. *Journal of Biological Chemistry* **289**, 10057–10068.

Reuhl M and Vogel M (2022) Temperature-dependent dynamics at protein–solvent interfaces. *The Journal of Chemical Physics* **157**, 074705.

Rezus YLA and Bakker HJ (2007) Observation of immobilized water molecules around hydrophobic groups. *Physical Review Letters* **99**, 148301.

Ribeiro A, **Arias FJ**, **Reguera J**, **Alonso M and Rodríguez-Cabello JC** (2009) Influence of the amino-acid sequence on the inverse temperature transition of elastin-like polymers. *Biophysical Journal* **97**, 312–320.

Roberts S, **Dzuricky M and Chilkoti A** (2015) Elastin-like polypeptides as models of intrinsically disordered proteins. *FEBS Letters* **589**, 2477–2486.

Roberts S, **Harmon TS**, **Schaal JL**, **Miao V**, **Li K**, **Hunt A**, **Wen Y**, **Oas TG**, **Collier JH**, **Pappu RV and Chilkoti A** (2018) Injectable tissue integrating networks from recombinant polypeptides with tunable order. *Nature Materials* **17**, 1154–1163.

Rodriguez-Cabello JC, **Alonso M**, **Diez MI**, **Caballero MI and Herguedas MM** (1999) Structural investigation of the poly(pentapeptide) of elastin, poly(GVGVP), in the solid state. *Macromolecular Chemistry and Physics* **200**, 1831–1838.

Rodríguez-Cabello JC, **Arias FJ**, **Rodrigo MA and Girotti A** (2016) Elastin-like polypeptides in drug delivery. *Advanced Drug Delivery Reviews* **97**, 85–100.

Rodriguez-Cabello JC, **Gonzalez De Torre I**, **González-Pérez M**, **González-Pérez F and Montequi I** (2021) Fibrous scaffolds from elastin-based materials. *Frontiers in Bioengineering and Biotechnology* **9**, 652384.

Rodríguez-Cabello JC, **Prieto S**, **Arias F**, **Reguera J and Ribeiro A** (2006) Nanobiotechnological approach to engineered biomaterial design: The example of elastin-like polymers. *Nanomedicine* **1**, 267–280.

Rodríguez-Cabello JC, **Reguera J**, **Alonso M**, **Parker TM**, **McPherson DT and Urry DW** (2004) Endothermic and exothermic components of an inverse temperature transition for hydrophobic association by TMDSC. *Chemical Physics Letters* **388**, 127–131.

Salvi AM, **Moscarelli P**, **Bochicchio B**, **Lanza G and Castle JE** (2013) Combined effects of solvation and aggregation propensity on the final supramolecular structures adopted by hydrophobic, glycine-rich, elastin-like polypeptides. *Biopolymers* **99**, 292–313.

Salvi N, **Zapletal V**, **Jaseňáková Z**, **Zachrdla M**, **Padrta P**, **Narasimhan S**, **Marquardsen T**, **Tyburn J-M**, **Žídek L**, **Blackledge M**, **Ferrage F and Kadeřávek P** (2022) Convergent views on disordered protein dynamics from NMR and computational approaches. *Biophysical Journal* **121**, 3785–3794.

Samouillan V, **André C**, **Dandurand J and Lacabanne C** (2004) Effect of water on the molecular mobility of elastin. *Biomacromolecules* **5**, 958–964.

Samouillan V, **Tintar D and Lacabanne C** (2011) Hydrated elastin: Dynamics of water and protein followed by dielectric spectroscopies. *Chemical Physics* **385**, 19–26.

Sandberg LB (1976) Elastin structure in health and disease. In *International Review of Connective Tissue Research*. Amsterdam: Elsevier, pp. 159–210.

Sander E, **Sander LM and Ziff RM** (1994) Fractals and fractal correlations. *Computers in Physics* **8**, 420.

Schmelzer CEH and Duca L (2022) Elastic fibers: Formation, function, and fate during aging and disease. *The FEBS Journal* **289**, 3704–3730.

Schmelzer CEH, **Nagel MBM**, **Dziomba S**, **Merkher Y**, **Sivan SS and Heinz A** (2016) Prolyl hydroxylation in elastin is not random. *Biochimica et Biophysica Acta (BBA) – General Subjects* **1860**, 2169–2177.

Schräder CU, **Heinz A**, **Majovsky P**, **Karaman Mayack B**, **Brinckmann J**, **Sippl W and Schmelzer CEH** (2018) Elastin is heterogeneously cross-linked. *Journal of Biological Chemistry* **293**, 15107–15119.

Shapiro SD, **Endicott SK**, **Province MA**, **Pierce JA and Campbell EJ** (1991) Marked longevity of human lung parenchymal elastic fibers deduced from prevalence of D-aspartate and nuclear weapons-related radiocarbon. *Journal of Clinical Investigation* **87**, 1828–1834.

Sharma A, **Sharma P and Roy S** (2021) Elastin-inspired supramolecular hydrogels: A multifaceted extracellular matrix protein in biomedical engineering. *Soft Matter* **17**, 3266–3290.

Shewry PR, **Tatham AS and Bailey AJ** (eds.) (2003) *Elastomeric Proteins: Structures, Biomechanical Properties, and Biological Roles*, 1st edn. Cambridge: Cambridge University Press.

Silverstein MC, **Bilici K**, **Morgan SW**, **Wang Y**, **Zhang Y and Boutis GS** (2015) 13C, 2H NMR studies of structural and dynamical modifications of glucose-exposed porcine aortic elastin. *Biophysical Journal* **108**, 1758–1772.

Singh M, **Becker M**, **Godwin ARF and Baldock C** (2021) Structural studies of elastic fibre and microfibrillar proteins. *Matrix Biology Plus* **12**, 100078.

Sreekumar PG, **Li Z**, **Wang W**, **Spee C**, **Hinton DR**, **Kannan R and MacKay JA** (2018) Intra-vitreal αB crystallin fused to elastin-like polypeptide provides neuroprotection in a mouse model of age-related macular degeneration. *Journal of Controlled Release* **283**, 94–104.

Sudo S and Yagihara S (2009) Universality of separation behavior of relaxation processes in supercooled aqueous solutions as revealed by broadband dielectric measurements. *The Journal of Physical Chemistry B* **113**, 11448–11452.

Sumiyoshi S, **Suyama K**, **Tatsubo D**, **Tanaka N**, **Tomohara K**, **Taniguchi S**, **Maeda I and Nose T** (2022) Metal ion scavenging activity of elastin-like peptide analogues containing a cadmium ion binding sequence. *Scientific Reports* **12**, 1861.

Sun C and Boutis GS (2010) Investigation of the dynamical properties of water in elastin by deuterium double quantum filtered NMR. *Journal of Magnetic Resonance* **205**, 86–92.

Sun C and Boutis GS (2011) Measurement of the exchange rate of waters of hydration in elastin by 2D T(2)-T(2) correlation nuclear magnetic resonance spectroscopy. *New Journal of Physics* **13**, 025026.

Sun C, **Mitchell O**, **Huang J and Boutis GS** (2011) NMR studies of localized water and protein backbone dynamics in mechanically strained elastin. *The Journal of Physical Chemistry B* **115**, 13935–13942.

Tamburro AM (1990) Order-disorder in the structure of elastin: A synthetic approach. In *Elastin: Chemical and Biological Aspects*. Galatina: Congedo Editore, pp. 125–146.

Tamburro AM (2009) A never-ending love story with elastin: A scientific autobiography. *Nanomedicine (London, England)* **4**, 469–487.

Tamburro AM, **Bochicchio B and Pepe A** (2003) Dissection of human tropoelastin: Exon-by-exon chemical synthesis and related conformational studies. *Biochemistry* **42**, 13347–13362.

Tamburro A, **Bochicchio B and Pepe A** (2005) The dissection of human tropoelastin: from the molecular structure to the self-assembly to the elasticity mechanism. *Pathologie Biologie* **53**, 383–389.

Tamburro AM, **De Stradis A and D'Alessio L** (1995) Fractal aspects of elastin supramolecular organization. *Journal of Biomolecular Structure & Dynamics* **12**, 1161–1172.

Tamburro AM, **Guantieri V**, **Daga-Gordini D and Abatangelo G** (1978) Concentration-dependent conformational transition of alpha-elastin in aqueous solution. *The Journal of Biological Chemistry* **253**, 2893–2894.

Tamburro AM, **Guantieri V and Gordini DD** (1992) Synthesis and structural studies of a pentapeptide sequence of elastin. Poly (Val-Gly-Gly-Leu-Gly). *Journal of Biomolecular Structure and Dynamics* **10**, 441–454.

Tamburro AM, **Guantieri V**, **Pandolfo L and Scopa A** (1990) Synthetic fragments and analogues of elastin. II. Conformational studies. *Biopolymers* **29**, 855–870.

https://doi.org/10.1017/S0033583524000040 Published online by Cambridge University Press

Tamburro AM, **Pepe A and Bochicchio B** (2006) Localizing alpha-helices in human tropoelastin: Assembly of the elastin "puzzle." *Biochemistry* **45**, 9518–9530.

Tamburro AM, **Pepe A**, **Bochicchio B**, **Quaglino D and Ronchetti IP** (2005) Supramolecular amyloid-like assembly of the polypeptide sequence coded by exon 30 of human tropoelastin. *The Journal of Biological Chemistry* **280**, 2682–2690.

Tang R, **Samouillan V**, **Dandurand J**, **Lacabanne C**, **Lacoste-Ferre M-H**, **Bogdanowicz P**, **Bianchi P**, **Villaret A and Nadal-Wollbold F** (2017) Identification of ageing biomarkers in human dermis biopsies by thermal analysis (DSC) combined with Fourier transform infrared spectroscopy (FTIR/ATR). *Skin Research and Technology* **23**, 573–580.

Tarakanova A, **Yeo GC**, **Baldock C**, **Weiss AS and Buehler MJ** (2018) Molecular model of human tropoelastin and implications of associated mutations. *Proceedings of the National Academy of Sciences of the United States of America* **115**, 7338–7343.

Tavakoli J, **Diwan AD and Tipper JL** (2020) The ultrastructural organization of elastic fibers at the interface of the nucleus and annulus of the intervertebral disk. *Acta Biomaterialia* **114**, 323–332.

Teeter MM (1984) Water structure of a hydrophobic protein at atomic resolution: Pentagon rings of water molecules in crystals of crambin. *Proceedings of the National Academy of Sciences of the United States of America* **81**, 6014–6018.

Timmermann EO (1989) A B. E. T.-like three sorption stage isotherm. *Journal of the Chemical Society, Faraday Transactions 1: Physical Chemistry in Condensed Phases* **85**, 1631–1645.

Urry DW (1974) On the molecular basis for vascular calcification. *Perspectives in Biology and Medicine* **18**, 68–85.

Urry DW (1988) Entropic elastic processes in protein mechanisms. I. Elastic structure due to an inverse temperature transition and elasticity due to internal chain dynamics. *Journal of Protein Chemistry* **7**, 1–34.

Urry DW (1992) Free energy transduction in polypeptides and proteins based on inverse temperature transitions. *Progress in Biophysics and Molecular Biology* 57, 23–**57**.

Urry DW (2004) The change in Gibbs free energy for hydrophobic association. *Chemical Physics Letters* **399**, 177–183.

Urry DW, **Henze R**, **Redington P**, **Long MM and Prasad KU** (1985) Temperature dependence of dielectric relaxations in α-elastin coacervate: Evidence for a peptide librational mode. *Biochemical and Biophysical Research Communications* **128**, 1000–1006.

Urry DW, **Hugel T**, **Seitz M**, **Gaub HE**, **Sheiba L**, **Dea J**, **Xu J and Parker T** (2002) Elastin: a representative ideal protein elastomer. *Philosophical Transactions of the Royal Society B: Biological Sciences* **357**, 169–184.

Urry DW and Long MM (1977) On the conformation, coacervation and function of polymeric models of elastin. In Sandberg LB, Gray WR and Franzblau C (eds.), Elastin and Elastic Tissue, Advances in Experimental Medicine and Biology. Boston, MA: Springer US, pp. 685–714.

Urry DW, **Luan CH**, **Parker TM**, **Gowda DC**, **Prasad KU**, **Reid MC and Safavy A** (1991) Temperature of polypeptide inverse temperature transition depends on mean residue hydrophobicity. *Journal of the American Chemical Society* **113**, 4346–4348.

Urry DW, **Peng XJ and McPherson DT** (1997) Characterization of waters of hydrophobic hydration by microwave dielectric relaxation. *Journal of the American Chemical Society* **119**, 1161–1162.

Urry DW, **Starcher B and Partridge SM** (1969) Coacervation of solubilized elastin effects a notable conformational change. *Nature* **222**, 795–796.

Venkatachalam CM and Urry DW (1981) Development of a linear helical conformation from its cyclic correlate. β-spiral model of the elastin poly(pentapeptide) (VPGVG)n. *Macromolecules* **14**, 1225–1229.

Vidal Ceballos A, **Díaz A JA**, **Preston JM**, **Vairamon C**, **Shen C**, **Koder RL and Elbaum-Garfinkle S** (2022) Liquid to solid transition of elastin condensates. *Proceedings of the National Academy of Sciences* **119**, e2202240119.

Villani V, **D'Alessio L and Tamburro AM** (1997) Conformational non-linear dynamical behavior of the peptide Boc-Gly-Leu-Gly-Gly-NMe. *Journal of the Chemical Society, Perkin Transactions* **2** 2375–2386.

Villani V and Tamburro AM (1995) Conformational modeling of elastin tetrapeptide Boc-Gly-Leu-Gly-Gly-NMe by molecular dynamics simulations with improvements to the thermalization procedure. *Journal of Biomolecular Structure and Dynamics* **12**, 1173–1202.

Villani V and Tamburro AM (1999) Conformational chaos of an elastin-related peptide in aqueous solution. *Annals of the New York Academy of Sciences* **879**, 284–287.

Villani V, **Tamburro AM and Zaldivar Comenges JM** (2000) Conformational chaos and biomolecular instability in aqueous solution. *Journal of the Chemical Society, Perkin Transactions* **2**, 2177–2184.

Vrhovski B, **Jensen S and Weiss AS** (1997) Coacervation characteristics of recombinant human tropoelastin. *European Journal of Biochemistry* **250**, 92–98.

Wagenseil JE and Mecham RP (2007) New insights into elastic fiber assembly. *Birth Defects Research Part C: Embryo Today: Reviews* **81**, 229–240.

Wagenseil JE and Mecham RP (2012) Elastin in large artery stiffness and hypertension. *Journal of Cardiovascular Translational Research* **5**, 264–273.

Walker GH and Ford J (1969) Amplitude instability and ergodic behavior for conservative nonlinear oscillator systems. *Physical Review* **188**, 416–432.

Walker L, **Perkins E**, **Kratz F and Raucher D** (2012) Cell penetrating peptides fused to a thermally targeted biopolymer drug carrier improve the delivery and antitumor efficacy of an acid-sensitive doxorubicin derivative. *International Journal of Pharmaceutics* **436**, 825–832.

Wang J, **Dzuricky M and Chilkoti A** (2017) The weak link: Optimization of the ligand–nanoparticle interface to enhance cancer cell targeting by polymer micelles. *Nano Letters* **17**, 5995–6005.

Wang Y, **Hahn J and Zhang Y** (2018) Mechanical properties of arterial elastin with water loss. *Journal of Biomechanical Engineering* **140**, 0410121.

Wasserman ZR and Salemme FR (1990) A molecular dynamics investigation of the elastomeric restoring force in elastin. *Biopolymers* **29**, 1613–1631.

Weinberg PD, **Winlove CP and Parker KH** (1995) The distribution of water in arterial elastin: Effects of mechanical stress, osmotic pressure, and temperature. *Biopolymers* **35**, 161–169.

Weis-Fogh T and Andersen SO (1970) New molecular model for the long-range elasticity of elastin. *Nature* **227**, 718–721.

Wen Q, **Mithieux SM and Weiss AS** (2020) Elastin biomaterials in dermal repair. *Trends in Biotechnology* **38**, 280–291.

Wilmot CM and Thornton JM (1988) Analysis and prediction of the different types of β-turn in proteins. *Journal of Molecular Biology* **203**, 221–232.

Woodhouse KA, **Klement P**, **Chen V**, **Gorbet MB**, **Keeley FW**, **Stahl R**, **Fromstein JD and Bellingham CM** (2004) Investigation of recombinant human elastin polypeptides as non-thrombogenic coatings. *Biomaterials* **25**, 4543–4553.

Yeo GC, **Keeley FW and Weiss AS** (2011) Coacervation of tropoelastin. *Advances in Colloid and Interface Science* **167**, 94–103.

https://doi.org/10.1017/S0033583524000040 Published online by Cambridge University Press