Terahertz mechanical vibrations in lysozyme: Raman spectroscopy vs modal analysis

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Abstract

The mechanical behaviour of proteins is receiving an increasing attention from the scientific community. Recently it has been suggested that mechanical vibrations play a crucial role in controlling structural configuration changes (folding) which govern proteins biological function. The mechanism behind protein folding is still not completely understood, and many efforts are being made to investigate this phenomenon. Complex molecular dynamics simulations and sophisticated experimental measurements are conducted to investigate protein dynamics and to perform protein structure predictions; however, these are two related, although quite distinct, approaches. Here we investigate mechanical vibrations of lysozyme by Raman spectroscopy and linear normal mode calculations (modal analysis). The input mechanical parameters to the numerical computations are taken from the literature. We first give an estimate of the order of magnitude of protein vibration frequencies by considering both classical wave mechanics and structural dynamics formulas. Afterwards, we perform modal analyses of some relevant chemical groups and of the full lysozyme protein. The numerical results are compared to experimental data, obtained from both in-house and literature Raman measurements. In particular, the attention is focused on a large peak at 0.84 THz (29.3 cm⁻¹) in the Raman spectrum obtained analyzing a lyophilized powder sample.

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1. Introduction

Understanding the mechanical behaviour of proteins has important implications in different applied sciences, from biology and medicine, to biochemistry, pharmacology, and bioengineering. The proteins three-dimensional structure controls crucial biological processes such as mitosis, mechanotransduction, immune response, cell metabolism, neural signal transmission, oxygen transportation, etc. [1–4]. In all these processes, protein dynamics plays a crucial role [5]. Moreover, several neurodegenerative diseases, as well as many allergies, are believed to be caused by incorrect folding of some proteins [6–9]. For these reasons, the mechanisms behind protein folding are objective of investigation since long time [10–21].

Nowadays, both experimental and computational techniques are used to investigate protein dynamics. As for the former, several standard non-crystallographic techniques are adopted, including Protein nuclear magnetic resonance spectroscopy (PNMRS), Dual polarisation interferometry (DPI), high time resolution measurements (including neutron scattering), Vibrational circular dichroism (VCD), Proteolysis, and Optical tweezers [22–31]. A related, although quite different, approach for studying protein vibration and structure is the use of molecular dynamics simulations. In this case, de novo or ab initio techniques are adopted to investigate protein folding [32–40]. Linear normal mode analysis (i.e., modal analysis) is also used in modelling the dynamics of biomolecular complexes: the main pros and cons of this technique are discussed in Ref. [41].

Single-molecule manipulation has allowed the forced unfolding of multidomain proteins [42–45]. Staple et al. developed a statistical mechanics theory for the stretching and unfolding of multidomain biopolymers [46]. In that paper the theory, valid essentially for any molecule that can be unfolded in the Atomic force microscope (ATM) [47], was applied to reproduce the force-extension curves of both titin and RNA hairpins as an example.

It has been suggested that mechanical vibrations that involve the whole protein play a crucial role in controlling structural...
configuration changes (folding). In particular, underdamped low-frequency collective vibrational modes in proteins have been proposed as being responsible for efficiently directing biochemical reactions and biological energy transport [48]. Therefore, the existence of delocalized vibrational modes and their involvement in biological function has become objective of investigation. Vibrational modes of proteins, both crystallized and in solution, have been found in the fields of GHZ and THz. For example, in lysozyme a 3–4 cm$^{-1}$ ($-100$ GHz) highly delocalized hinge-bending mode that opens and closes the binding cleft was found by normal mode calculations [49,50]. At the same time, some authors report that collective modes may occur also in the Terahertz range. For example, in Ref. [48] one reads that “there have been suggestions that Terahertz-frequency underdamped collective modes of the protein may direct the system along the correct path on a highly complex potential-energy surface [51], and may be responsible for the low-loss transport of energy through the protein [52], which may facilitate biological function through phonon-like modes [53]”.

Several attempts to measure the vibrational spectra of proteins in the Terahertz range have been done with the aim of finding peaks that could then be assigned to biochemically relevant motions in the protein [48]. Before 1990 there was a gap in the electromagnetic spectrum, 0.1–1.5 THz ($3–50$ cm$^{-1}$), referred to as the “Terahertz Gap”, that was difficult to utilise due to the lack of a suitable light source. The invention of Terahertz time-domain spectroscopy (THz-TDS) enabled measurements using photons between 0.1 and 1.5 THz ($3–500$ cm$^{-1}$) that were previously impractical. Nowadays spectroscopic analysis using frequencies between 0.1 and 15 THz ($3–500$ cm$^{-1}$) is being utilized by the biochemistry community and is giving scientifically interesting information [54]. Recently, far-infrared and THz-TDS experiments on lysozyme crystals have successfully identified underdamped delocalized vibrational modes in the Terahertz range [55–57]. However, the biochemical relevance of such modes can not be proved since the crystal packing and hydration level modify the protein dynamics [48,57]. In fact, the same technique has proven to be largely unsuccessful when used for studying proteins in solution due to the very large absorption by liquid water [48,58]. In contrast, inelastic neutron scattering [59,60] and spontaneous Raman scattering [61–64] have been efficiently used to study lysozyme in solution since they do not suffer from water absorption. However, these techniques become unreliable at low frequencies (<1 THz) due to the very strong Rayleigh peak from elastic scattering, and again it has not been possible to assign biochemical relevance to any peaks observed [48]. To encompass this problems, Turton et al. [48] adopted Femtosecond optical Kerr-effect (OKE) spectroscopy for measuring the depolarized Raman spectrum in the time domain; this technique in practice is superior at low frequencies, as it does not suffer from a large Rayleigh peak [65]. Also, Xu et al. [66] successfully measured the low frequency vibrational modes of lysozyme in water by Terahertz absorption spectroscopy, using a broadband Terahertz spectrometer developed on purpose. Although broad-range spectroscopic measurements have been attempted, the challenge is to observe specific modes. As a matter of fact, calculations show that functional conformational change in many biomolecular systems can be simulated using only the first few collective vibrational modes of system [67,68]. These correlated motions are the low frequency vibrational modes that extend throughout the macromolecule. The problem lies in the selection of the most relevant modes. However, as stated in Ref. [48], the role of Terahertz-frequency vibrational modes in mediating biological functions in general is not known at present. Therefore the question is still open, and new experimental and numerical investigations are needed.

A recently proposed conceptual interpretation of protein folding suggests that it could be regarded as a dynamic nano-buckling (with snap-through) [69,70]. According to this hypothesis, the instability causing the abrupt configuration change can be induced by electro-chemical forces, acting on an oscillating structure. As it is known in structural mechanics, the force required to produce instability in an oscillating mechanical system is lower than the critical force for the same system at rest; on the other hand, if an external harmonic excitation has a frequency that matches one of the natural frequencies of the system, then resonance occurs, and the vibration amplitude increases (diverges, in linear analysis, if the system is undamped): in this case the force required to produce instability tends to zero [71–73].

Aim of the present work is to establish a comparison between experimental measurements of mechanical vibrations in crystallized lysozyme and their numerical simulation by a numerical lattice model. We focused on the Chicken egg white lysozyme (CEWL), a well-known enzyme. We performed broad-range Raman spectroscopy measurements on a lyophilized powder sample, and compared the results to literature data. Therefore, we modelled the single protein and three well-known amino acids (phenylalanine, tyrosine, and tryptophan) as three-dimensional lattices, using the Finite Element code LUSAS [76]; a classic modal analysis was run. A comparison between the resonant peaks found in the experiments and the corresponding vibration modes given by the numerical simulation was looked for. The agreement between numerical and experimental results is discussed in the paper.

2. Materials and methods

Raman spectroscopy measurements [77–87] were performed on hen egg white purified, salt free lysozyme. Lysozyme is a rather small protein, also known as N-acetylmuramidase glycanidohydrolase, consisting of 129 amino acids and having a molecular weight equal to 14.3 kDa. It has an enzymatic function [88–90] and was described for the first time in 1922 by Fleming [91]. The three-dimensional structure of hen egg white lysozyme was reconstructed for the first time in 1965 by Blake et al., who obtained a 2 Ångström (200 p.m.) resolution model via X-ray crystallography [92]. The shape of this enzyme is globular (compact), with an average diameter of 4–5 nm. It is the first enzyme containing all 20 amino acids whose 3D structure was obtained. In 2007 Steve Kent obtained the first chemical synthesis of lysozyme at the university of Chicago.

The samples ( Worthington Biochemical Corporation, Product Code: LYSF, Lot Number: 34K151516B; 100% protein, two times crystallized) were received and tested in the form of a dialyzed, lyophilized powder, containing ≥ 8000 units per mg dry weight. According to the unit definition reported in the certificate of analysis, one unit is equal to a decrease in turbidity of 0.001 A450 nm per minute at 25 °C, pH 7.0 using a 0.3 mg/ml suspension of Micrococcus lysodeikticus cells (Product Code: ML) as substrate.

The experiments were performed in the High pressure spectroscopy (HPS) laboratory at the Physics Department of Sapienza University in Rome (Italy), with the technical support of the company Horiba Scientific. The experimental apparatus consisted in a Raman spectrometer LabR AM HR evolution, a last-generation device produced by Horiba Scientific. It mounts a confocal microscope equipped with three objectives (20×, 50×, 100×), coupled to a 633 nm wavelength He-Ne laser. The spectrometer determines two diffraction gratings, having resolutions equal to 600 lines/mm (<3 cm$^{-1}$) and to 1800 lines/mm (<1 cm$^{-1}$). In order to analyze low frequencies (<10–15 THz), ultra low frequency (ULF) filters of the latest generation were adopted. The ULF module allows Raman spectroscopic information in the sub-100 cm$^{-1}$ region, with measurements down to 5 cm$^{-1}$ routinely available. This technology
claims the highest performances of the single spectrometer: measurements are obtained in just a few seconds or minutes, without any limitation in the higher wavenumber region; Stokes and Anti-Stokes spectral features can be simultaneously measured, providing additional information [93].

A preliminary calibration of the spectrometer was done prior to the measurements by means of an in-house code developed on purpose, and adopting the emission spectrum of a Neon lamp as a reference. In fact, measurements may slightly be affected by ambient conditions (air temperature and humidity) and so it is a good rule to calibrate the spectrometer before each measurement session. The calibration consists in comparing the measured and the known reference spectra, and then shifting the former so that to overlap the other in correspondence to a selected peak (typically the main one). The Neon lamp is a good reference since its spectrum has very narrow and marked peaks. The experiments were conducted in room conditions, at the temperature of 22 °C.

In addition to the experiments, simplified numerical calculations on protein vibrations were made according to the following methods.

A first estimate of the order of magnitude of the characteristic vibration frequencies of protein structures can be obtained by considering the wave propagation theory. Longitudinal expansion/contraction (tension/compression) waves are generally said pressure waves, or phonons when their particle nature is emphasized, contractions on protein vibrations were made according to the following relationship [94].

\[ f = \frac{v}{\lambda}. \]  

Eq. (1) allows to obtain the order of magnitude of the eigenfrequencies of expansion/contraction vibration modes in solids at different dimensional scales. Thus, by assuming a constant pressure wave speed \( v = 10^3 \text{ m/s} \) (\( \approx \) denotes the order of magnitude), we obtain for proteins vibration (Fig. 1): (i) \( f = 10^{13} \text{ Hz} \) for localized eigenvibrations of chemical groups or amino acids (\( \lambda = 10^{-10} \text{ m} \)); (ii) \( f = 10^{12} \text{ Hz} \) for eigenvibrations of small proteins or extended portions of large proteins (\( \lambda = 10^{-9} \text{ m} \)); (iii) \( f = 10^{11} \text{ Hz} \) global eigenvibrations of large proteins (\( \lambda = 10^{-8} \text{ m} \)).

On the other hand, the same results can be achieved by the theory of mechanical vibrations of elastic systems. Molecular forces are difficult to model. However, for small displacements, attractive/reductive forces between atoms can generally be considered as linearly elastic [94]. Bonds between the same type of atom are covalent bonds, and bonds between atoms having electronegativities which differ by a little are also predominantly covalent [95,96]. For covalent bonds, bond energies and bond lengths depend on many factors; however, there is a general trend in that the shorter the bond length, the higher the bond energy. The study of small-amplitude atomic oscillations around the equilibrium distance implies considering the second-order Taylor series expansion of the potential (the so-called harmonic approximation); this yields a linear relation between interatomic force and distance, and justifies the use of elastic linear springs to model interatomic bonds [80,94–96]. Therefore, considering only expansion/contraction deformations, the order of magnitude of the vibration frequency in protein structures can be evaluated as [94,97]:

\[ f = \frac{1}{2\pi} \sqrt{\frac{k}{m}} \]  

being \( k \) and \( m \) the apparent axial (tensile/compressive) stiffness and the mass of the system, respectively. Thus, applying Eq. (2) to proteins yields again (\( k = 10^2 \text{ N m}^{-1} \)): (i) \( f = 10^{13} \text{ Hz} \) for chemical groups or amino acids (\( m = 10^{-26} \text{ kg} \)); (ii) \( f = 10^{12} \text{ Hz} \) for small proteins or extended portions of large proteins (\( m = 10^{-24} \text{ kg} \)); (iii) \( f = 10^{11} \text{ Hz} \) for large proteins (\( m = 10^{-22} \text{ kg} \)). For example, considering entire proteins, we have \( f \approx 7.8 \times 10^{11} \text{ Hz} \) for lysozyme (\( m = 2.37 \times 10^{-23} \text{ kg} \); average diameter about 4–5 nm) or \( f = 2.1 \times 10^{11} \text{ Hz} \) for Na\(^+\)/K\(^-\) ATPase (\( m = 1.78 \times 10^{-22} \text{ kg} \); average width about 4 nm, length about 16 nm). On the other hand, if we consider very small portions, like amino acids or chemical groups, we obtain frequencies of tens of Terahertz (see Sect. 3).

In order to make some more precise calculation, let us consider the simple case of a diatomic covalent bond. Taking the single C–C bond, idealized as a two-mass–spring linear system with \( m_1 = m_2 = m_c \approx 1.99 \times 10^{-26} \text{ kg} \) and \( k = k_{\text{C–C}} \approx 180 \text{ N m}^{-1} \) [98], and excluding rigid motions, the vibration frequency can be obtained as [97]:

\[ f_{\text{C–C}} = \frac{1}{2\pi} \sqrt{\frac{2k_{\text{C–C}}}{m_c}} \approx 2.14 \times 10^{13} \text{ Hz} \left( 713 \text{ cm}^{-1} \right). \]  

The previous value is in perfect agreement with the literature (see Ref. [99]). In a similar way we can also evaluate the bond

\[ \lambda = 10^{-10} \text{ m} \]

\[ f = 10^{13} \text{ Hz} \]

\[ \lambda = 10^{-9} \text{ m} \]

\[ f = 10^{12} \text{ Hz} \]

\[ \lambda = 10^{-8} \text{ m} \]

\[ f = 10^{11} \text{ Hz} \]

Fig. 1. Protein eigenvibrations at different dimensional scales: correlation between wavelength, \( \lambda \), and frequency, \( f \), by assuming a constant pressure wave speed \( v = 10^3 \text{ m/s} \).
stiffness when the mass and the vibration frequency of the diatomic molecule are known. For example, for the disulfide bridge (S–S bond) we have $m_k = 5.31 \times 10^{-26}$ kg and $f_{k,S} = 1.57 \times 10^{13}$ Hz (524 cm$^{-1}$) (see Ref. [99]); thus, reversing Eq. (3) and making the pertinent substitutions gives the spring stiffness
\[
k_{S,S} = 2\pi^2 m_k f_{k,S}^2 \equiv 260 \text{ Nm}^{-1}.
\]

In this study, numerical lattice models were built for the crystal structures of both lysozyme and the following three amino acids contained in it: phenylalanine, tyrosine, and tryptophan. All models were built using LUSAS finite element code [76]. Both amino acids and lysozyme were considered isolated from the outer world. Ball-and-stick models were obtained, with masses concentrated at the nodes (atoms) and interatomic bonds replaced by linear-elastic, massless beams. Only covalent bonds were considered, neglecting all weaker bonds. The beam ends were internally clamped at each node in order to prevent rotational labilities (bending and torsion rigidities).

Venant torsion constant [100]. Starting from beam lengths
\[
i
\]

being $E (\text{F L}^{-2})$ the Young’s modulus (equal for all beams), $A_i (\text{L}^2)$ and $I_i (\text{L}^4)$ the cross-sectional area and the beam length, respectively. Each value was set so to reproduce the desired bond stiffness. Bending and (primary) torsion beam stiffness depend both on length $l_i$ and on bending, $E I_{ij}$, and torsion, $G J_i$, rigidities, respectively; $I_p (\text{L}^4)$ denotes the second moment of area (moment of inertia) of the cross-section with respect to the principal axis $j (j = 1, 2)$, $G (\text{F L}^{-2})$ is the shear modulus, and $J_f (\text{L}^4)$ denotes the St Venant torsion constant [100]. Starting from beam lengths $l_i$ and local axial, bending, and torsion rigidities, the local (element) stiffness matrix $K_i$ can be computed [100–103]. On the other hand, knowing the point masses $m_k (k = 1, 2,..., n)$ allows to obtain also the local mass matrix $M_k$ [97,100–103]. Numerical operations of rotation, expansion, and assembly of the local matrices $K_i$ and $M_i$, yield the corresponding global matrices $K$ and $M$ [100–103]. In our case, once given the required input data, all computations were automatically done by the adopted software.

Therefore, the undamped free dynamics of a structural assembly with $n$ degrees of freedom is governed by the following homogeneous linear system (generalized eigenproblem) [97,103]:
\[
\begin{bmatrix}
K_{nm} - \omega^2 M_{nm}
\end{bmatrix}
\begin{bmatrix}
x_{n+1}
\end{bmatrix}
= 0,
\]

the non-trivial solution of which gives the eigenvalues $\omega^2$ (square of the natural angular frequencies of the system) by solving the algebraic equation of order $n$ (characteristic equation) resulting from the following condition:
\[
\det(K - \omega^2 M) = 0.
\]

Once the eigenvalues have been determined, Eq. (6) allows to obtain the eigenvectors $x$ (vibration modes). As is well known, the eigenvectors are defined but for a constant. A system with $n$ degrees of freedom will therefore have $n$ natural frequencies as well as $n$ vibration modes. The solution of the eigenvalue-eigenvector problem (6) was obtained by the adopted finite element code. All models were left unconstrained in the 3D space; thus, six rigid motions had to be excluded from each analysis.

3. Results and discussion

Fig. 2 shows the Raman spectrum obtained analyzing the lysozyme samples. Data were processed with LabSpec software, supplied with the spectrometer used. Acquisition was made using the 600 lines/mm diffraction grating, 100 $\times$ objective and 200 $\mu$m confocal hole. Four different spectral ranges were analyzed, i.e. grating centered at 650, 1400, 2100 and 2900 cm$^{-1}$, and then results were collected in a unique spectrum. Each result is the mean of 10 measures with 60 s acquisition time each. Low-degree ($\leq 3$) polynomial functions were subtracted to each original spectrum in order to eliminate the background (polynomial baseline correction).

The results are in agreement with those of the literature, see for example [99]. Peaks related to three amino acids (tryptophan, tyrosine and phenylalanine) as well as the peak of amide I are indicated in Fig. 2. Amide I is characteristic of all proteins and is of relevance in biology since it refers to the vibration of the protein backbone. The above-mentioned three amino acids were selected as reference to validate the numerical model. Thanks to the use of ULF filters it has been possible to investigate also low-frequency vibrations. A large peak in correspondence to 0.84 THz is clearly visible in Fig. 2 (top-left).

Fig. 3 shows the Raman spectrum of the same samples around the origin. It is the result of 10 mediated 60 s acquisitions, each one obtained with the 1800 lines/mm grating, centered at 0 cm$^{-1}$. The large peak at 28 cm$^{-1}$ (0.84 THz) and a broad shoulder at 75 cm$^{-1}$ (2.25 THz) are indicated in the same diagram. Peaks in this low frequency range can also be found in the literature. For example, peaks at 25 and 75 cm$^{-1}$ were found, among others, in 1976 by Genzel et al. [104]. These peaks are not assigned in literature to any chemical group, as they should correspond to vibrations involving the entire protein or large portions of it. Such delocalized or global vibrations were investigated numerically by modelling the entire lysozyme.

Fig. 4 shows the geometry of the three amino acids implemented in LUSAS. The nodes represent concentrated masses simulating atoms. Lines represent massless beam elements simulating interatomic bonds. The structure of each amino acid was built up starting from data available in the RCSB Protein Data Bank [105]. We referred to the crystal structure of hen egg white lysozyme (access code: 4YMB) and extracted the XYZ coordinates of the three types of amino acids of interest, i.e., PHE, TYR, TRP. Files
containing all data relative to the selected crystal structure can be downloaded in different PDB formats, or simply displayed [see, for example [106]]. The structure of each amino acid (relative position of atoms) is the same, independently of its collocation in the molecule. Therefore, the choice was made without following a specific criterion. For each structure, starting from the coordinates of atoms, a .dxf file was created in MATLAB and then imported in LUSAS to create the desired geometry automatically. In Fig. 4, the atoms constituting each amino acid are also indicated; straight-line segments close to some lines connecting nodes (atoms) indicate double carbon-carbon bonds (C=\text{C}).

Atom masses and bond stiffnesses are listed in Table 1. Interatomic distances’ order of magnitude is $10^{-10}$ m. Since we are dealing with powers of ten which are not appropriate for computer calculations, a numerical scaling was introduced. We remark that this scaling must not be intended as a physical scaling, being it just an artifice introduced for ease of computation. The adopted numerical scaling is listed in Table 2. According to that scaling, the conversion between the real (subscript $r$) and the numerical (subscript $n$) frequencies (in Hz) results to be the following:

**Table 1.** Atom masses and bond stiffnesses.

<table>
<thead>
<tr>
<th>Atom</th>
<th>Atom mass (kg)</th>
<th>Bond</th>
<th>Bond stiffness (N m$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon (C)</td>
<td>$1.994475 \times 10^{-26}$</td>
<td>C-C</td>
<td>180</td>
</tr>
<tr>
<td>Nitrogen (N)</td>
<td>$2.325918 \times 10^{-26}$</td>
<td>C=\text{C}</td>
<td>320</td>
</tr>
<tr>
<td>Oxygen (O)</td>
<td>$2.656698 \times 10^{-26}$</td>
<td>C-\text{N}</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C-\text{O}</td>
<td>190</td>
</tr>
</tbody>
</table>

**Fig. 3.** Raman spectrum of crystallized lysozyme powder centered at 0 cm$^{-1}$.

**Fig. 4.** Finite element lattice model of amino acids: (a) phenylalanine; (b) tyrosine; (c) tryptophan.
\[ f_r = f_{\text{ax}} \times 10^{13}. \]  

The input parameters introduced in the analyses are listed in Table 3. Note that the values of Young’s modulus and cross-sectional area in Table 3 have no physical basis: they were chosen such that, through Eq. (5), the required axial stiffness is obtained. 3D 3-node thin beam elements were used, with 3 subdivisions per element; however, the number of subdivisions has no influence on our analysis since, as pointed out before, we are only interested in the axial behaviour (bending/torsion deformations are disregarded; see the related inertia constants in Table 3).

Fig. 5 shows the numerical mode shapes corresponding to vibration frequencies (indicated in the same file) close to those of peaks Trp, Tyr, and Phe in Fig. 2. The modes shown in Fig. 5 mainly involve deformations of the rings (the deformation has been magnified for ease of visualization). Actually, several ring breathing modes of the same amino acids are reported in the literature for frequencies close to the values indicated in Fig. 2 [99]. Of course, one must remember that amino acids have several characteristic frequencies corresponding to different vibration modes. In fact, a molecule with \( N \) atoms has, in general, 3\( N \)–6 normal modes of vibration [107].

Fig. 6 shows the numerical model of lysozyme implemented in LUSAS. A connectivity matrix was created, in MATLAB, based on atom coordinates taken from the RCSB Protein Data Bank, access code 4YM8 [105,106]; each atom was connected to all the others which are far from it no more than 0.2 nm, whereas no connection was created with atoms at longer distances. The result is a tree-like three-dimensional structure. 1020 connections were created among the 1000 point masses (atoms) constituting the enzyme. The average interatomic distance is equal to 0.141 nm. A .dxf file was created by MATLAB and therefore imported in LUSAS to construct the structural geometry.

A constant value of the point mass \( m_a = 2.37 \text{ kg} \) was used for all atoms (actual average atom mass equal to \( 2.37 \times 10^{-26} \text{ kg} \)); the beam mass density \( \rho \) was set equal to zero. The elastic properties and the bending and torsion constants are the same as in Table 3.

The cross-sectional area \( A \) was set equal to \( 9.4 \times 10^{-4} \text{ m}^2 \), constant for all beams. The default value of 4 subdivisions per beam element was used. The adopted numerical scaling is the same as before (see Table 2), so Eq. (8) still holds. Using the area \( A = 9.4 \times 10^{-4} \text{ m}^2 \), Young’s modulus \( E = 3 \times 10^5 \text{ N m}^{-2} \), and the average distance \( l = 1.41 \text{ m} \), Eq. (5) yields an average axial (bond) stiffness \( k \) equal to 200 N m\(^{-1}\).

Fig. 7 shows the mode shape found by the numerical model for a frequency close to the large peak on the left of the Raman spectrum shown in Fig. 2. The experimental and numerical frequencies are respectively equal to 0.84 THz (28.0 cm\(^{-1}\)) and 0.88 THz (29.3 cm\(^{-1}\)). This mode is clearly a global one, that represents a sort of contraction/expansion of the protein. The numerical frequency spectrum is broad and dense: other global modes were found, as well as many localized modes. In general, the higher frequencies are related to localized vibration modes.

For both amino acids and lysozyme, differences between experimental results and numerical predictions may be attributed to both the input values and the modelling simplifications introduced, such as: (i) assuming the structure as isolated, therefore neglecting any structural interaction with the surrounding environment; (ii) considering only covalent bonds, neglecting all weaker interactions—this is also correlated with the bond stiffness values—; (iii) modelling the interatomic bond as a uniaxial elastic force. On the other hand, regarding the input parameters, higher values of bond stiffness would result in a stiffer response, i.e., in higher frequency values. However, despite all the mentioned simplifications, the elementary models implemented were able to predict more than the order of magnitude of the vibration frequencies of amino acids and lysozyme structure, allowing to investigate the corresponding vibration modes at the same time. In particular, the numerical model of lysozyme showed that global protein vibrations seem to be possible at frequencies around 0.8–0.9 THz.

### 4. Conclusions

Important protein biological functions are driven by structural configuration changes, i.e. folding. This is a complex phenomenon not completely understood at present. Recent studies have argued that protein mechanical vibrations can play a significant role: we can not exclude that large conformational changes may occur as the result of nano-instabilities induced by resonant mechanical oscillations. The most relevant modes are thought to be the low-frequency ones, i.e. those related to collective vibrations.

In this paper, structural vibrations of lysozyme are analyzed experimentally and numerically. Raman spectroscopy measurements were conducted on crystallized powder samples, and the results are compared to the corresponding ones of the literature. The use of ULF filters allowed to investigate also the lower frequencies, i.e., those corresponding to delocalized/global vibrations. In particular, a large peak at 0.84 THz (28 cm\(^{-1}\)) was found experimentally and investigated numerically by a simplified model of the single protein. The linear normal modes of well-known amino acids contained in proteins (phenylalanine, tyrosine, tryptophan), as well as those of the whole lysozyme protein, were investigated by modal analysis. The approach adopted here belongs

<table>
<thead>
<tr>
<th>Physical quantity</th>
<th>Scaling</th>
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<tbody>
<tr>
<td>Mass, M (kg)</td>
<td>numerical ( \times 10^{26} )</td>
</tr>
<tr>
<td>Distance, L (m)</td>
<td>numerical ( \times 10^{10} )</td>
</tr>
<tr>
<td>Stiffness, F L(^{-1}) (N m(^{-1}))</td>
<td>numerical ( \times 10^{10} )</td>
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</table>

<table>
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<tr>
<th>Elastic properties of beams</th>
<th>Geometric properties of beams cross-section</th>
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<tr>
<td>Young modulus, N m(^{-2})</td>
<td>Area, m(^2)</td>
</tr>
<tr>
<td>Poisson ratio ( \nu ) ( = -0.3 )</td>
<td>( A_{c,c} ) ( = 0.9240 \times 10^{-3} )</td>
</tr>
<tr>
<td>( \nu ) irrelevant for present analysis</td>
<td>( A_{c,N} ) ( = 1.4293 \times 10^{-3} )</td>
</tr>
<tr>
<td>Principal second moments of area, m(^4)</td>
<td>( A_{c,o} ) ( = 0.9057 \times 10^{-3} )</td>
</tr>
<tr>
<td>( I_l = I_l = 1 \times 10^6 )</td>
<td>Principal second moments of area, m(^4)</td>
</tr>
<tr>
<td>Torsion constant, m(^3)</td>
<td>( J = 1 \times 10^5 )</td>
</tr>
</tbody>
</table>
to classical mechanics. More precisely, we referred to crystallized biological materials (i.e., not in solution) and, starting from data of mechanical properties taken from the literature (e.g., mass, bond stiffness/energy), we evaluated the natural frequencies and mode shapes corresponding to small-amplitude vibrations around a given equilibrium configuration. For both amino acids and full lysozyme, simplified three-dimensional lattice models were built considering: (i) only primary (covalent) bonds between atoms, modelled as uniaxial elastic springs; (ii) point masses simulating atoms (for the lysozyme model, the average atomic mass was used); (iii) the structure under investigation unconstrained and isolated from the outer world (packing effects were not analyzed). Despite the modelling simplifications introduced, the obtained results show a good correspondence with the experimental evidences. The resonant frequencies found range from hundreds of GHz (entire protein) to tens of THz (chemical groups/amino acids). In particular, the lysozyme full model allowed to associate a mode at 0.88 THz (29.3 cm$^{-1}$) i.e. a frequency close to the large peak found experimentally to a global vibration of the single protein. Even if protein-protein interaction due to crystal packing (not considered in the model) should produce a shift between the experimental and the numerical frequencies, the main conclusion from the numerical results is that global vibrations of the lysozyme near 1 THz seem to be plausible. However, we have no indication about their possible biological relevance: this is beyond the scope of the present study.

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