Journal of Biomedical Optics

BiomedicalOptics.SPIEDigitalLibrary.org

Low-frequency vibrational spectroscopy of proteins with different secondary structures

Irina A. Balakhnina
Nikolay N. Brandt
Andrey Yu. Chikishev
Anna A. Mankova
Irina G. Shpachenko

SPIE.

Irina A. Balakhnina, Nikolay N. Brandt, Andrey Yu. Chikishev, Anna A. Mankova, Irina G. Shpachenko, "Low-frequency vibrational spectroscopy of proteins with different secondary structures," *J. Biomed. Opt.* **22**(9), 091509 (2017), doi: 10.1117/1.JBO.22.9.091509.

Low-frequency vibrational spectroscopy of proteins with different secondary structures

Irina A. Balakhnina,[a] Nikolay N. Brandt,[a] Andrey Yu. Chikishev,[b] Anna A. Mankova,[a,*] and Irina G. Shpachenko[a]
[a]Lomonosov Moscow State University, Physics Department, Leninskie gory, Moscow, Russia
[b]Lomonosov Moscow State University, International Laser Center, Leninskie gory, Moscow, Russia

Abstract. Fourier transform infrared (FTIR) and Raman spectra of proteins with significantly different structures are measured in a spectral interval of 50 to 500 cm^{-1} and noticeable spectral differences are revealed. Intensities of several spectral bands correlate with contents of secondary structure elements. FTIR spectra of superhelical proteins exhibit developed spectral features that are absent in the spectra of globular proteins. Significant differences of the Raman spectra of proteins that are not directly related to the difference of the secondary structures can be due to differences of tertiary and/or quaternary structure of protein molecules. [DOI: 10.1117/1.JBO.22.9.091509]

Keywords: FTIR; Raman; low-frequency spectroscopy; proteins; secondary structure.

Paper 160884SSR received Jan. 5, 2017; accepted for publication Mar. 14, 2017; published online Mar. 25, 2017.

1 Introduction

Protein molecules are among the most interesting biological structures since they are involved in functioning of living organisms. Complicated spatial structure of a protein macromolecule determines its functional activity. Even minor structural changes may cause substantial variations in enzymatic activity.

Computer simulations based on molecular dynamics show that protein macromolecules may exhibit low-frequency molecular vibrations presumably related to function-related motions of large domains of molecules.[1–4] Most computations were performed for normal modes with frequencies of <100 cm^{-1}. A monotonic decrease in the number of vibrational modes with an increase in frequency to 300 cm^{-1} was demonstrated in Ref. 2. Such calculations stimulate the spectroscopic study at frequencies of several terahertz (several hundreds of wavenumbers). The recent interest in such experiments is due to the progress in THz technologies.[5,6] Amide VI and VII bands in protein spectra at frequencies of about 600 and 200 cm^{-1}, respectively, must be conformation sensitive bands.[7–9]

Raman and IR spectroscopic techniques are efficient tools for the study of molecular structures. Measurements in the fingerprint range have shown that several bands in protein spectra can be used to characterize protein structure. In particular, amide I and III bands can be used to determine secondary structure.[10–13] The bands of disulphide bridges and tyrosine doublet are sensitive to structural changes, induced by various external effects. Raman spectroscopy was used in Refs. 14–16 to study the influence of chemical reagents on protein molecules. The results show intensity redistribution of the bands of disulphide bridges related to their conformational transitions. Such transitions may cause changes of a protein molecule as a whole.

Rapid progress in THz spectroscopy necessitates the analysis of the low-frequency spectral bands that can presumably be used to additionally characterize protein structure. Note that the existing assignments based on IR and Raman data are tentative and insufficient. In one of the first works using low-frequency Raman spectroscopy, the spectra of crystalline lysozyme were compared with the spectra of the protein solution.[17] The bands at 25, 75, 115, and 160 cm^{-1} were detected in the crystalline sample. The first band was missing in the spectrum of solution. The authors assigned the bands at 25 and 75 cm^{-1} to intermolecular and torsional vibrations, respectively. Several proteins were studied in Ref. 18 using low-frequency Fourier transform infrared (FTIR) spectroscopy. An attempt was made at the assignment of spectral bands with the aid of approximation of generally similar protein spectra with Lorentzian components. *N*-methylacetamide was used in Ref. 19 to simulate a peptide bond of protein at several temperatures. The bands peaked at 190 and 280 cm^{-1} were assigned to torsional motion and in-plane bending, respectively. The analysis of a few H-bonded systems was used to assign the band at about 100 cm^{-1} to the vibrations of H-bonds. A further analysis of the low-frequency IR and Raman of *N*-methylacetamide, lysozyme, and aprotinin using theoretical models proved the assignment.[20] The band at 290 cm^{-1} appears to be sensitive to the structure of water in protein solutions. Low-frequency spectral range was also discussed in Refs. 21 and 22, in particular, the bands at 230, 280, and 310 cm^{-1} were assigned to intramolecular vibrations, and the band at 100 cm^{-1} was assigned to intermolecular vibrations. Low-frequency Raman spectra of DNA films and lysozyme were studied in Ref. 23.

Far-IR spectra of native and structurally modified proteins were compared in Ref. 24. Factors that lead to denaturation (pH and temperature) cause spectral changes in the low-frequency range of several proteins. Different combinations of experimental parameters lead to different spectral changes that can be due to conformational changes of the protein or its dimerization. Two proteins with substantially different secondary structures [chymotrypsin and bovine serum albumin (BSA)] were studied in Ref. 25 using low-frequency FTIR

*Address all correspondence to: Anna A. Mankova, E-mail: mankova@physics.msu.ru

1083-3668/2017/$25.00 © 2017 SPIE

and Raman spectroscopic measurements. The spectra of native proteins were compared with the spectra of thermally and chemically denatured molecules. Spectral data show that the effect of thermal denaturation is stronger than the effect of chemical agents that cause the cleavage of disulphide bonds in proteins.

Generally, protein structures are predominantly studied using spectral data of fingerprint range, whereas low-frequency data are poorly discussed in the literature. As distinct from the fingerprint range, the low-frequency range of vibrational spectra does not contain bands of single amino acids. Summarizing the existing data, we conclude that the low-frequency bands can be assigned to NH bending and out-of-plain vibrations, CN torsions, C=O out-of-plain vibrations, CCC bending vibrations, and CNC and CCN deformations. Thus, vibrations can be classified as skeletal or as vibrations that belong to almost all amino acids. Alternatively or additionally, the low-frequency bands can be assigned to correlated vibrations in large fragments (domains) of protein molecules.

The purpose of this work is to compare and analyze low-frequency Raman and FTIR spectra of several proteins with different secondary structures.

2 Experimental Methods

In the experiments, we use chymotrypsin from Samson-Med, bovine serum albumin (BSA) from MP Biomedicals (Cat. no. 160069), lysozyme from MP Biomedicals (CAS 9001-63-2), fibrinogen from Sigma (CAS 9001-32-5), ovalbumine from Worthington (Cat. no. 3056), concanavalin from MP Biomedicals (Cat. no. 150710), human serum albumin from Sigma (CAS 70024-90-7), lactalbumin from MP Biomedicals (Cat. No. 102128), collagen from MP Biomedicals (Cat. No. 160084). Table 1 shows the average content of elements of secondary structure in proteins under study. Note that fibrinogen and collagen differ from the remaining proteins, since the former can be classified as superhelical (or coiled-coil) proteins.

Table 1 The content of elements of secondary structure in proteins under study.

Protein	α-helix (%)	β-sheet (%)	Random coil (%)	Refs.	Molecular weight (kDa)
Concanavalin	2	65	33	10, 26	102
Chymotrypsin	8	50	42	10, 27–29	25
Lactalbumin	29	30	41	30, 31	14.2
Ovalbumine	31	32	37	32	45
Fibrinogen	40	20	40	33, 34	340
Lysozyme	48	20	32	10, 26, 28, 29, 35	14.5
BSA	57	0	43	28, 29, 36, 37	69
Human serum albumin	62	0	38	38	66.5
Collagen	100 (left-hand helix)	0	0	39	300

Lyophilized protein powders are used in Raman measurements. Thin pressed tablets of the powders with masses ranging from 2.5 to 10 mg and thicknesses of about 200 μm are employed in the FTIR measurements.

Raman spectra are measured with the aid of a Thermo Scientific DXR Raman confocal microscope with a 10× objective at an excitation wavelength of 532 nm, a maximum power of 10 mW, and a spectral resolution of 5 cm^{-1}.

FTIR measurements are performed using a Thermo Scientific Nicolet-6700 FTIR spectrometer with a spectral resolution of 2 cm^{-1}. Each spectrum results from averaging over 500 scans.

We employ background subtraction based on rolling-circle filter.[40] The filter parameters are identical for the spectra in each figure, and subtraction is performed for each spectral interval. A typical radius of the filter with respect to wavenumbers is about 10,000 cm^{-1}. The method of Brandt et al.[41] makes it possible to approximate a single spectrum using a weighted sum of several spectra and a polynomial. The low-frequency Raman spectra are processed using $R(\nu)$ representation.[42] We choose the method or combination of methods of processing to most clearly present spectral variations.

3 Results and Discussion

We analyze FTIR spectra of proteins in three spectral intervals to reveal conformation-sensitive bands.

Figure 1 compares FTIR spectra of the proteins in a spectral interval of 70 to 200 cm^{-1}. The spectral curves result from the following sequential processing. First, background contributions are equalized in a spectral interval of 70 to 600 cm^{-1} using the method for comparison of Ref. 41. Then, almost identical linear backgrounds are subtracted in a spectral interval of 70 to 200 cm^{-1}. Finally, each spectrum is normalized by its maximum in this interval. Hereafter, averaged spectra are presented and the intensity error is 5% to 7%. Measured optical densities for the proteins are close to each other, which are seen in the inset to Fig. 1, where we compare spectra without background subtraction and normalization.

It is seen that the spectra of α-helical proteins (BSA and lysozyme) exhibit a developed shoulder at a frequency of 150 cm^{-1}, which has significantly lower intensity in the spectra of β-sheet

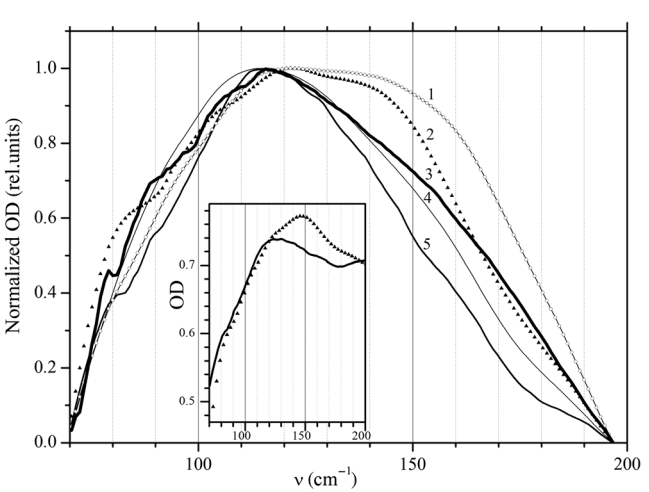

Fig. 1 Processed FTIR spectra of (1) lysozyme, (2) BSA, (3) chymotrypsin, (4) ovalbumine, and (5) concanavalin (see text for details). The inset shows the FTIR spectra of (triangles) BSA and (solid line) concanavalin without background subtraction and normalization.

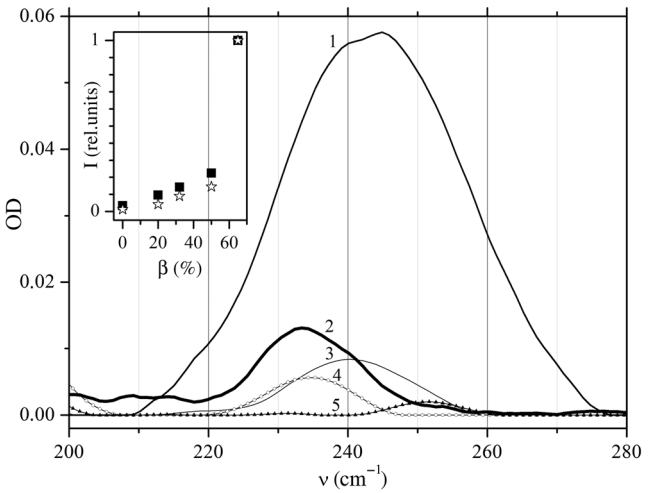

Fig. 2 Processed FTIR spectra of (1) concanavalin, (2) chymotrypsin, (3) ovalbumine, (4) lysozyme, and (5) BSA (see text for details). The inset shows plots of normalized (squares) peak and (circles) integral intensities versus relative content of β-sheets.

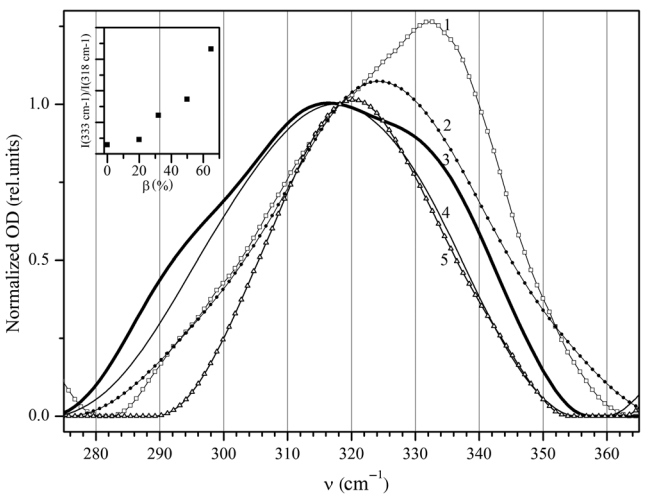

Fig. 3 Processed FTIR spectra of (1) concanavalin, (2) chymotrypsin, (3) ovalbumine, (4) lysozyme, and (5) BSA (see text for details). The inset shows plot of signal intensity at 333 cm^{-1} versus relative content of β-sheets.

proteins (concanavalin, chymotrypsin, and ovalbumine). Concanavalin has the lowest content of α-helical fragments, and the corresponding spectrum has the lowest intensity in an interval of 130 to 190 cm^{-1}. However, the percentage of secondary structure elements cannot be determined using the intensities in this spectral interval.

Figure 2 presents FTIR spectra in an interval of 200 to 280 cm^{-1}. A rolling circle filter is used to subtract backgrounds. We clearly observe a decrease in the spectral intensity in an interval of 220 to 260 cm^{-1} in a series concanavalin, chymotrypsin, ovalbumine, lysozyme, and BSA. Peak intensity (regardless of the frequency) and integral intensity (with integration over a spectral interval of 200 to 280 cm^{-1}) can be determined for each spectrum. Both peak (squares in the inset) and integral (circles in the inset) intensities of the band monotonically increase with an increase in the content of β-sheets. Significant intensity difference for concanavalin can be related to the absence of both α-helical fragments and disulphide bridges.

Rolling circle filter is also used to obtain spectral curves in an interval of 275 to 375 cm^{-1} (Fig. 3). Each spectrum is normalized by the intensity at 318 cm^{-1}, which is the peak position for the α-helical proteins. These proteins exhibit almost symmetric bands, whereas complicated band shapes are typical of β-sheet proteins. Under the above normalization, the signal intensity at 333 cm^{-1} almost monotonically increases with an increase in the content of β-sheets (see inset to Fig. 3).

Several conformation sensitive intervals can also be selected in Raman spectra of the proteins under study.

The spectra in Fig. 4 are obtained using the following procedure. First, R(ν) representation is employed.[19–21,42] Then, the background contributions are equalized in a spectral interval of 70 to 600 cm^{-1} using the method for comparison of Ref. 41. Finally, each spectrum is normalized by its integral intensity in an interval of 40 to 200 cm^{-1}. The spectra of β-sheet proteins are close to each other [panel (a)] and substantially differ from the spectra of proteins with significant contents of α-helices. Recall that collagen contains left-hand α-helices.

Each spectral curve in Fig. 5 results from background subtraction using the rolling circle filter and normalization by the integral intensity in an interval of 180 to 470 cm^{-1}. The spectra are sorted from top to bottom in ascending order with respect to

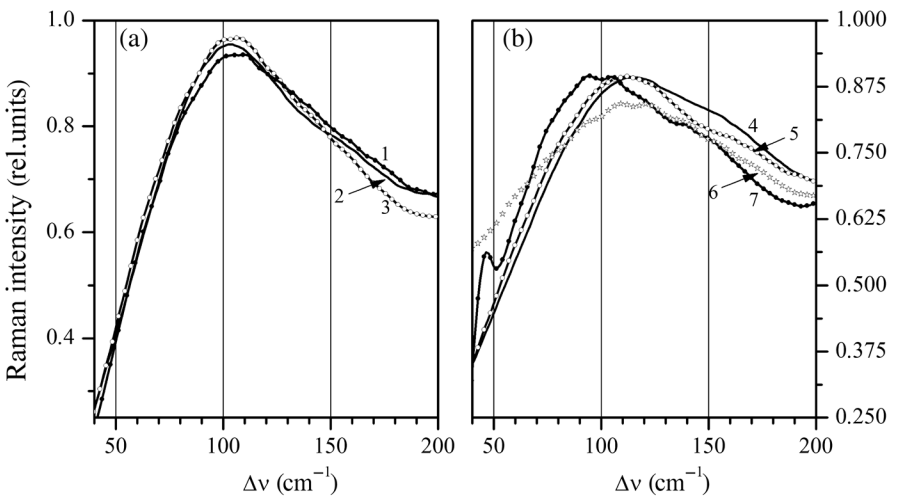

Fig. 4 Processed Raman spectra of (a) (1) ovalbumine, (2) chymotrypsin, and (3) concanavalin, and (b) (4) fibrinogen, (5) lysozyme, (6) collagen, and (7) BSA (see text for details).

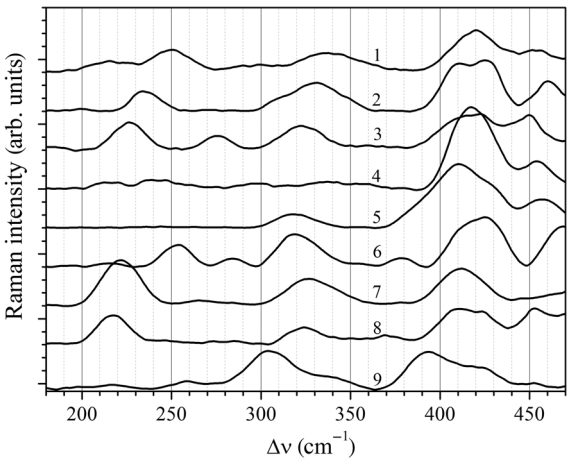

Fig. 5 Processed Raman spectra of (1) concanavalin, (2) chymotrypsin, (3) lactalbumin, (4) ovalbumin, (5) fibrinogen, (6) lysozyme, (7) BSA, (8) human serum albumin, and (9) collagen.

the content of α-helices and descending order with respect to the content of β-sheets. The spectral differences correlate with neither contents of secondary structure elements nor molecular weights (sizes) of the protein molecules. However, a general low-frequency shift of the spectra with an increase in the content of α-helices may be noted.

As was mentioned in Sec. 1, the low-frequency bands can be assigned to skeletal vibrations or vibrations of identical fragments of different amino acids. Therefore, low-frequency spectra must be insensitive to variations in amino acid composition of proteins. However, we observe significantly different Raman spectra of the proteins (Fig. 5). The spectral bands can hardly be assigned to correlated vibrations of large fragments of molecules or subglobular vibrations due to the absence of dependences on molecular weights of proteins. At the same time, the differences of Raman spectra cannot be interpreted using differences of the contents of the secondary structure elements. In this regard, we assume that such differences can be due to the differences of tertiary and/or quaternary structures.

Finally, we compare FTIR and Raman spectra of globular and nonglobular (superhelical) proteins. Figure 6 shows

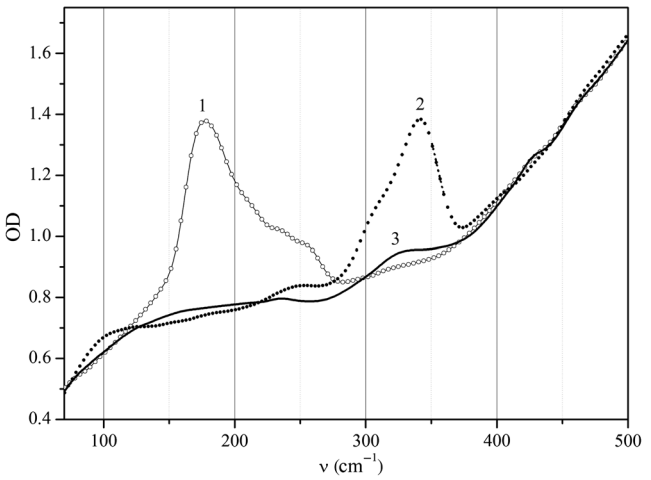

Fig. 6 FTIR spectra of (1) fibrinogen, (2) collagen, and (3) chymotrypsin.

developed low-frequency spectral features of the nonglobular proteins (fibrinogen and collagen) that are absent in the spectra of all globular proteins under study (only the spectrum of chymotrypsin is presented). Almost identical backgrounds in Fig. 6 are obtained using the method of Ref. 41.

The Raman spectra of nonglobular (superhelical) proteins (fibrinogen and collagen) generally differ from the spectra of globular proteins although the spectrum of ovalbumine is similar to the spectrum of fibrinogen.

It is known that amide bands, which can be used to estimate contents of secondary structure elements, dominate in the FTIR spectra of proteins in the fingerprint range. Only amide I and amide III bands are observed in Raman spectra of proteins. However, Raman spectra contain many bands assigned to single amino acids or atomic groups. Such features of the two spectroscopic techniques are manifested in this work, since the FTIR results can be interpreted in terms of contents of secondary structure elements.

Finally, note that the FTIR and Raman spectra in this work are in general agreement with the results for few proteins.[18,43–46]

4 Conclusions

Differences of the vibrational spectra of proteins under study in several spectral intervals can be interpreted using the differences of the secondary structures.

In particular, α-helical proteins exhibit an increase in spectral intensity in an interval of 130 to 190 cm^{-1} in both FTIR and Raman spectra.

For the FTIR spectra, integral intensity in an interval of 220 to 260 cm^{-1} and intensity at a frequency of 333 cm^{-1} almost monotonically increase with an increase in the content of β-sheets.

Low-frequency FTIR spectra of fibrinogen and collagen (nonglobular proteins) exhibit developed spectral features that are absent in the spectra of globular proteins. Such spectral features can be related to superhelical structure of the proteins.

Noticeable differences of the Raman spectra of proteins in an interval of 190 to 470 cm^{-1} do not correspond to the differences of secondary structures or molecular weights (sizes) of the protein molecules (on the assumption on linear dependences of Raman intensities on contents of secondary structure elements).

Disclosures

No conflicts of interest, financial or otherwise, are declared by the authors.

Acknowledgments

This work was supported in part by the Lomonosov Moscow State University Program of Development and Russian Foundation for Basic Research (project no. 15-02-06512-a).

References

1. P. Dauber-Osguthorpe et al., "Low frequency motion in proteins: comparison of normal mode and molecular dynamics of streptomyces griseus protease A," *J. Comp. Phys.* **151**, 169–189 (1999).
2. M. Levitt, C. Sander, and P. S. Stern, "Protein normal-mode dynamics: trypsin inhibitor, crambin, ribonuclease and lysozyme," *J. Mol. Biol.* **181**, 423–447 (1985).

3. K.-C. Chou, "Low-frequency motions in protein molecules, β-sheet and β-barrel," *Biophys. J.* **48**, 289–297 (1985).
4. F. Tama and Y.-H. Sanejouand, "Conformational change of proteins arising from normal mode calculations," *Protein Eng.* **14**(1), 1–6 (2001).
5. E. J. Heilweil and D. F. Plusquellic, "Terahertz spectroscopy of biomolecules," Chapter 7 in *Terahertz Spectroscopy: Principles and Applications*, S. L. Dexheimer, Ed., pp. 269–298, Optical Science and Engineering, CRC Press, Boca Raton, Florida (2007).
6. P. U. Jepsen, D. G. Cooke, and M. Koch, "Terahertz spectroscopy and imaging—modern techniques and applications," *Laser Photonics Rev.* **5**(1), 124–166 (2011).
7. Y. El Khoury et al., "On the specificity of the amide VI band for the secondary structure of proteins," *Vib. Spectrosc.* **55**, 258–266 (2011).
8. T. C. Chaam and S. Krimm, "Infrared intensities of amide modes in N-methylacetamide and poly(glycine I) from ab initio calculations of dipole moment derivatives of N-methylacetamide," *J. Chem. Phys.* **82**(4), 1631–1641 (1985).
9. M. Tsuboi and G. J. Thomas, "Polarized Raman and polarized FTIR spectroscopy," Chapter 4 in *Methods in Protein Structure and Stability Analysis: Vibrational Spectroscopy*, V. N. Uversky and E. A. Permiakov, Eds., pp. 153–194, Nova science publishers, New York (2007).
10. J. Kong and S. Yu, "Fourier transform infrared spectroscopic analysis of protein secondary structures," *Acta Biochim. et Biophys. Sin.* **39**(8), 549–559 (2007).
11. M. S. Celej et al., "Superactivity and conformational changes on α-chymotrypsin upon interfacial binding to cationic micelles," *Biochem. J.* **378**, 1059–1066 (2004).
12. A. Dong et al., "Infrared spectroscopic studies of lyophilization- and temperature-induced protein aggregation," *J. Pharm. Sci.* **84**(4), 415–424 (1995).
13. N. C. Maiti et al., "Raman spectroscopic characterization of secondary structure in natively unfolded proteins: r-synuclein," *J. Am. Chem. Soc.* **126**, 2399–2408 (2004).
14. C. David, S. Foley, and M. Enescu, "Protein S-S bridge reduction: a Raman and computational study of lysozyme interaction with TCEP," *Phys. Chem. Chem. Phys.* **11**, 2532–2542 (2009).
15. E. B. Getz et al., "A comparison between the sulfhydryl reductants tris (2-carboxyethyl)phosphine and dithiothreitol for use in protein biochemistry," *Analyt. Biochem.* **273**(1), 73–80 (1999).
16. C. David et al., "Reductive unfolding of serum albumins uncovered by Raman spectroscopy," *Biopolymers* **89**(7), 623–634 (2008).
17. L. Genzel et al., "Low-frequency Raman spectra of lysozyme," *Biopolymers* **15**, 219–225 (1976).
18. C. U. Stehle et al., "Far-infrared spectroscopy on free-standing protein films under defined temperature and hydration control," *J. Chem. Phys.* **136**, 075102 (2012).
19. O. F. Nielsen and I. J. Bigio, "Low frequency (20 – 400 cm^{-1}) vibrational spectra of n-methylacetamide in the liquid state," *Chem. Phys. Lett.* **132**(6), 502–506 (1986).
20. S. E. May Colaianni and O. F. Nielsen, "Low frequency Raman spectroscopy," *J. Mol. Struct.* **347**, 267–283 (1995).
21. O. F. Nielsen, "Hydrogen bonding in liquid amides studied by low frequency Raman spectroscopy," *J. Mol. Struct.* **175**, 251–256 (1988).
22. P. C. Painter, L. E. Mosher, and C. Rhoads, "Low-frequency modes in the Raman spectra of proteins," *Biopolymers* **21**(7), 1469–1472 (1982).
23. H. Urabe et al., "Low-frequency Raman spectra of lysozyme crystals and oriented DNA films: dynamics of crystal water," *Biophys. J.* **74**, 1533–1540 (1998).
24. R. J. Falconer et al., "Far-infrared spectroscopy of protein higher-order structures," *Appl. Spectrosc.* **64**(11), 1259–1264 (2010).
25. N. N. Brandt et al., "Effect of thermal denaturation, inhibition, and cleavage of disulfide bonds on the low-frequency Raman and FTIR spectra of chymotrypsin and albumin," *J. Biomed. Opt.* **20**(5), 051015 (2015).
26. R. W. Williams and A. K. Dunker, "A new method for determining protein secondary structure by laser Raman spectroscopy applied to FD phage," *Biophys. J.* **32**(1), 232–234 (1980).
27. M. Levitt and J. Greer, "Automatic identification of secondary structure in globular proteins," *J. Mol. Biol.* **114**(2), 181–239 (1977).
28. A. Yu et al., "Polarisation sensitive coherent anti-stokes Raman scattering spectroscopy of the amide I band of proteins in solutions," *Biophys. J.* **63**(4), 976–985 (1992).
29. F.-N. Fu et al., "Secondary structure estimation of proteins using the amide III region of FTIR spectroscopy: application to analyze calcium binding induced structural changes in calsequestrin," *Appl. Spectrosc.* **48**(11), 1432–1441 (1994).
30. S. Pasche et al., "Effects of ionic strength and surface charge on protein adsorption at PEGylated surfaces," *J. Phys. Chem. B* **109**(37), 17545–17552 (2005).
31. W. R. Krigbaum and S. P. Knlttton, "Prediction of the amount of secondary structure in a globular protein from its aminoacid composition j," *Proc. Nat. Acad. Sci. U. S. A.* **70**(10), 2809–2813 (1973).
32. J. A. Huntincjton, P. A. Patston, and P. G. Gettins, "S-ovalbumin, an ovalbumin conformer with properties analogous to those of loop-inserted serpins," *Protein Sci.* **4**(4), 613–621 (1995).
33. R. F. Doolittle, "A detailed consideration of a principal domain of vertebrate fibrinogen and its relatives," *Protein Sci.* **1**(12), 1563–1577 (1992).
34. V. Wahl et al., "The influence of residual water on the secondary structure and crystallinity of freeze-dried fibrinogen," *Int. J. Pharm.* **484**(1–2), 95–102 (2015).
35. J. Marx et al., "Characteization and conformational analysis by Raman spectroscopy of human airway lysozyme," *Biochim. Biophys. Acta* **870**(3), 488–494 (1986).
36. R. J. Jakobsen and F. M. Wasacz, "IR spectra-structure correlations and adsorption behavior for helix proteins," *Appl. Spectrosc.* **44**(9), 1478–1490 (1990).
37. K. A. Oberg and V. N. Uversky, "Secondary structure of the homologous proteins, alpha-fetoprotein and serum albumin, from their circular dichroism and infrared spectra," *Protein Pept. Lett.* **8**(4), 297–302 (2001).
38. D. Ajloo et al., "Thermodynamic and structural studies on the human serum albumin in the presence of a polyoxometalate," *Bull. Korean Chem. Soc.* **28**(5), 730–736 (2007).
39. M. D. Shoulders and R. T. Raines, "Collagen structure and stability," *Annu. Rev. Biochem.* **78**, 929–958 (2009).
40. N. N. Brandt et al., "Optimization of the rolling-circle filter," *Appl. Spec.* **60**(3), 288–293 (2006).
41. N. N. Brandt et al., "A method of comparing Raman spectra," *Laser Phys.* **14**(11), 1386–1392 (2004).
42. O. F. Nielsen, "Low-frequency spectroscopic studies of interactions in liquids," *Annu. Rep. Prog. Chem. Sect. C* **90**, 3–44 (1993).
43. R. J. Falconer, "Far-infrared spectroscopy of protein higher-order structures," *Appl. Spectrosc.* **64**(11), 1259–1264 (2010).
44. G. Giraud, J. Karolin, and K. Wynne, "Low-frequency modes of peptides and globular proteins in solution observed by ultrafast OHD-RIKES spectroscopy," *Biophys. J.* **85**(3), 1903–1913 (2003).
45. A. V. Frontzek et al., "Study of the phase transition in lysozyme crystals by Raman spectroscopy," *Biochim. Biophys. Acta* **1860**, 412–423 (2016).
46. A. Hédoux et al., "Thermostabilization mechanism of bovine serum albumin by trehalose," *J. Phys. Chem. B* **113**, 6119–6126 (2009).

Irina A. Balakhnina graduated from the Faculty of Physics, Moscow State University, in 2012. Since 2012, she has been a PhD student at the Faculty of Physics, Moscow State University, and University of Palermo (Italy). Her area of interest is spectroscopy of organic molecules and cultural heritage objects.

Nikolay N. Brandt graduated and received his PhD from the Faculty of Physics, Moscow State University, in 1998 and 2001, respectively. He is a participant of International Research Projects in Tokyo and Berlin. Also, he is an associate professor at the Faculty of Physics, Moscow State University. His area of expertise is Raman, IR, and fluorescence spectroscopy of biological macromolecules aimed at the analysis of the relationship of conformational dynamics and functional activity.

Andrey Yu. Chikishev graduated and received his PhD and DrSci degrees from the Faculty of Physics, Moscow State University, in 1982, 1986, and 2002, respectively. He is a leader and participant of International Research Projects on laser spectroscopy of

biomolecules and organic molecules in the Netherlands, Germany, Japan, and Italy. He is also the head of the Laboratory of Laser Diagnostics of Biomolecules and Methods of Photonics in the Study of Objects of Cultural Heritage, International Laser Center, Moscow State University.

Anna A. Mankova graduated from the Faculty of Physics, Moscow State University, in 2012. Since 2012, she has been a PhD student at the Faculty of Physics, Moscow State University. Her area of interest is low-frequency spectroscopy of biological molecules.

Irina G. Shpachenko graduated from the Faculty of Physics, Moscow State University, in 2016. Since 2016, she has been a PhD student at the Faculty of Physics, Moscow State University. Her area of interest is low-frequency spectroscopy of biological molecules.