Supplementary Information for

Determination of the Solution Structure of Antifreeze Glycoproteins using Two-Dimensional Infrared Spectroscopy

*Giulia Giubertoni [†], Konrad Meister[Ł], Arthur L. DeVries [‡] and Huib J. Bakker [†]**

[†]AMOLF, Science Park 104, 1098 XG Amsterdam, The Netherlands

[Ł]Max-Planck Institute for Polymer Research, D-55128 Mainz, Germany

[‡]University of Illinois at Urbana−Champaign, Urbana, Illinois 61801, United States

Corresponding Author:

* H.Bakker@amolf.nl, AMOLF, Science Park 104, 1098 XG Amsterdam, The Netherlands

Supplementary Methods

Sample preparation

Antifreeze glycoproteins are purified from the blood of the antarctic notothenioid toothfish, Dissostichus mawsoni, as previously described[1]. Proteins were dissolved in borate buffer, which consists of a heavy water solution of 0.3 M boric acid (purity>99.97%) and ~0.25 M NaOH to adjust the pH to 9.0, or in magnesium sulfate buffer, which consists of a heavy solution of 1 M magnesium sulfate. We chose these salt concentrations because for these added concentrations the effect on the antifreeze activity of AFGP has been reported in the literature.[2,3]

Linear spectra

All linear absorption measurements were performed using a Bruker Vertex 80v FTIR spectrometer equipped with a liquid-nitrogen-cooled-mercury-cadmium-telluride (MCT) detector. The spectra were recorded under nitrogen atmosphere at a wavelength resolution of 3 cm^{-1}. For every spectrum 100 scans were averaged. In all the measurements, a path length of 100 or 75 μm was used. The temperature-dependent FTIR measurements were performed using a Peltier-cooled temperature cell (Mid-IR Falcon, Pike technologies). The temperature was ramped from 5 to 70 ℃ at a rate of 1 ℃ /min. The background measurements on neat D2O were performed using the same ramping parameters.

2DIR spectra

In the 2DIR experiments, we perform one color experiments by pumping and probing around 1630 cm^{-1} and two color experiments by pumping at 1630 cm^{-1} and probing at 1450 cm^{-1}. The home-built setup that we use has been described before[4,5]. The excitation is performed with a pair of femtosecond mid-infrared pulses. This excitation pulse-pair induces transient absorption changes that are monitored by a probe pulse that is delayed by a time T_w. After transmission through the sample, the probe pulse is sent into an infrared spectrograph and detected with an infrared mercury-cadmium-telluride (MCT) detector array, thus yielding the transient absorption spectrum as a function of the probe frequency. The dependence of the transient absoption spectrum on the excitation frequency is determined by measuring transient spectra for many different delay times between the two excitation pulses. By Fourier transformation of these spectra, we obtain the dependence of the transient absorption spectrum on the excitation frequency. By plotting the transient absorption spectrum as a function of the excitation and the probing frequency, we obtain a two-dimensional infrared (2D-IR) transient absorption spectrum for each delay time T_w.

All the measurements are performed under N_2 atmosphere in a standard sample cell with a path length of 100 μm. The temperature of the protein is kept constant by using a Peltier element with an active feedback loop. Since the long time required for the measurement (5 hours) due

to the low signal-to-noise because of HDO absorption and low sample signal, the starting temperature was 0 ℃, and then it was raised to 5,23 and 40 ℃.

All the 2DIR spectra reported show the isotropic signal for a time delay of 0.5 ps.

Supplementary Information

Assignments

1628 cm^{-1} band

In FIG. S7 we show the linear infrared absorption spectrum of the sugar N-acetylgalactosamine in the frequency region of the amide II and amide I vibrations. It is seen that the amide I vibration of N-acetylgalactosamine absorbs near 1628 cm^{-1}, which is the same frequency as the central frequency of the second subband that is observed for solutions of AFGP, and that has been assigned to the amide I vibrations of the protein part of AFGP with an extended conformation [6–8]. The observed band for AFGP shows a distinct temperature dependence, which is not observed for the amide I vibration of N-acetylgalactosamine, as seen in Fig. S7. Hence, the observed band at 1628 cm^{-1} likely contains contributions from both the amide I vibration of N-acetylgalactosamine and the amide I vibration of the protein part of AFGP with an extended conformation.

1655 cm^{-1} band

The coupling of the amide I modes in an α-helix leads to two delocalized modes, defined as A and E**.** The transition dipole moment of A is directed parallel to the helix, while the transition dipole moment of E is oriented perpendicular to the helix [9]. In the linear infrared spectrum and in the isotropic 2DIR spectrum these two modes cannot be resolved, and give rise to a broad band centered around 1660 cm^{-1}. However, since A and E are perpendicularly oriented to each other, we can enhance the visibility of a cross-peak between the A and E modes [10] by subtracting the parallel signal from three times the perpendicular signal. This procedure reveals the presence of two bands, at 1655 cm^{-1} and at 1665 cm^{-1} (FIG.S8). The frequency splitting of the A and E modes is around 10 cm^{-1}, in agreement with values reported in the literature [11]. The relatively small frequency splitting of the 1655 cm^{-1} and at 1665 cm^{-1} modes, and their frequency values exclude an assignment of these modes to an 3_{10}-helix[12,13].

Supplementary Figures

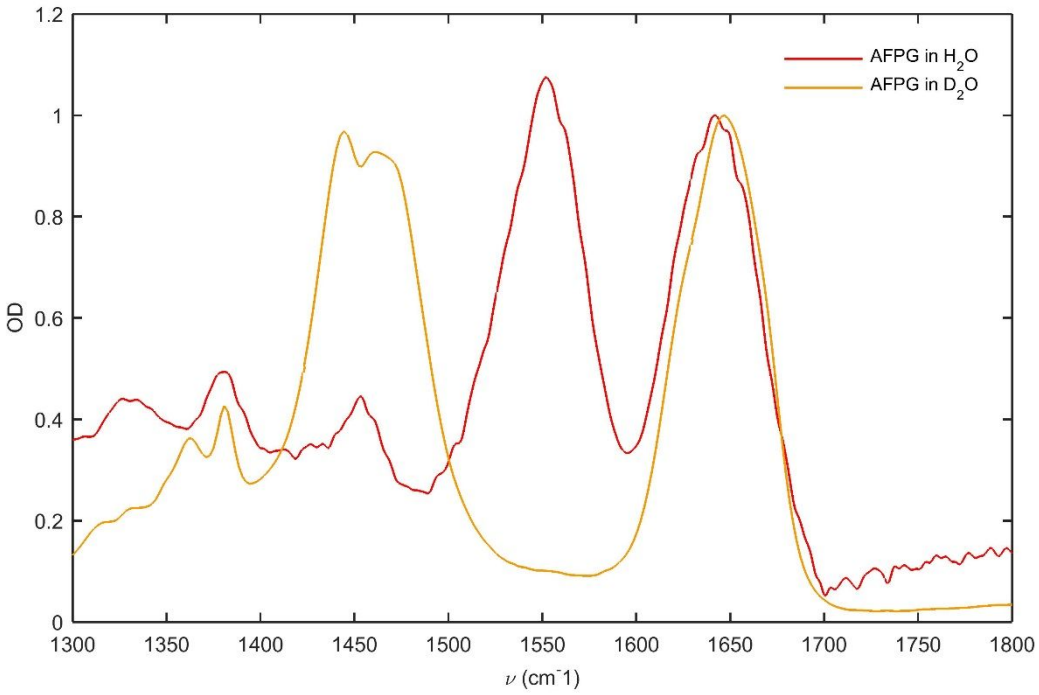

Figure S 1: Normalized infrared spectra for solutions of AFGP 1-5 at a concentration of 2% in water and heavy water solution.

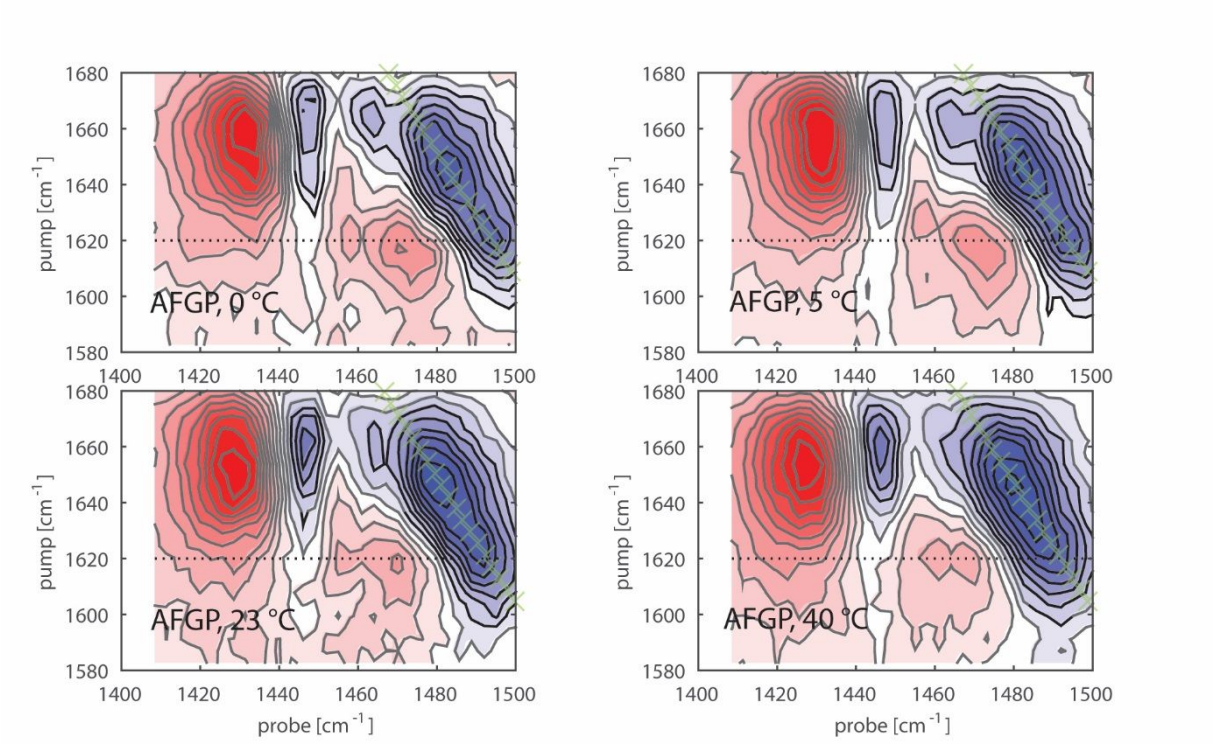

Figure S 2: 2D-IR spectra for AFGP 1-5 solutions in heavy water at a concentration of 2 wt%. The 2D-IR spectra were collected by starting the measurement at 0 °C and then increasing the temperature.

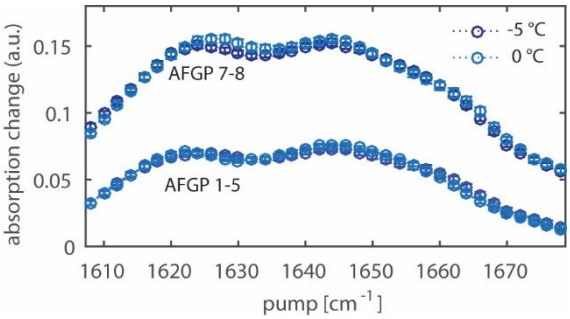

Figure S 3: Anti-diagonal slices for solutions of AFGP 1-5 and 7-8 at a concentration around 2%. The anti-diagonal slices are taken for a T_w= 0.5 ps

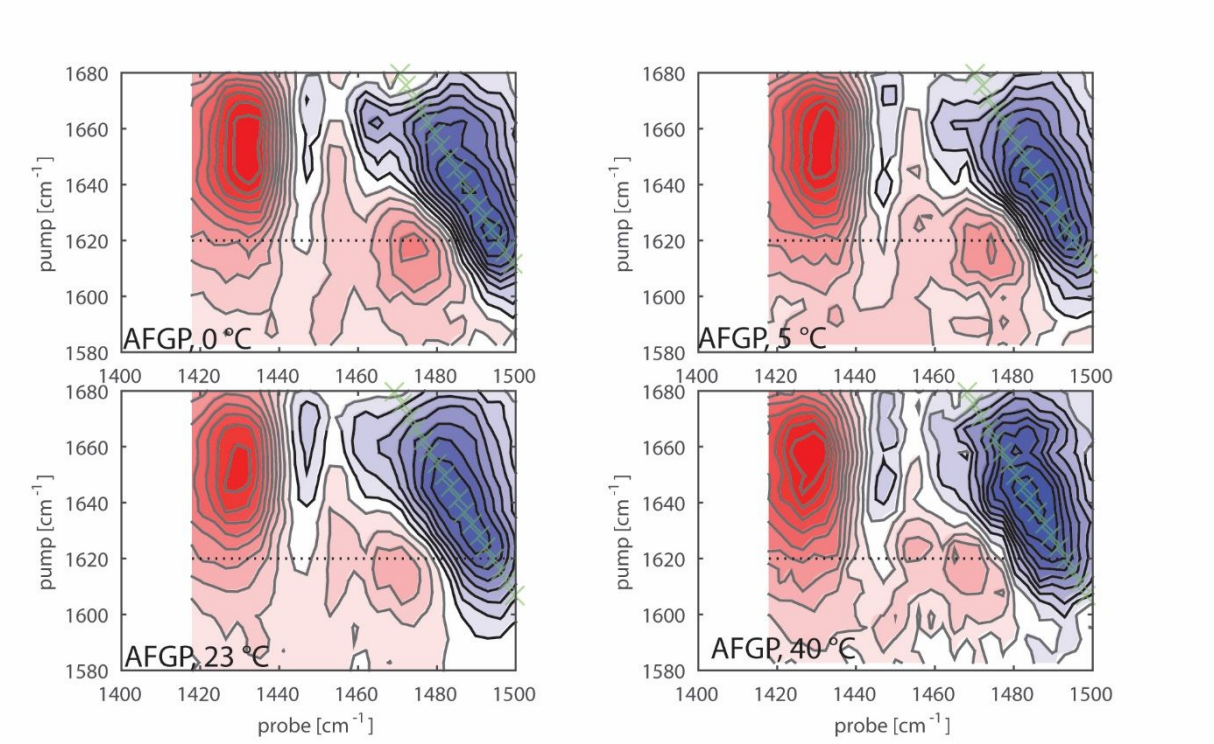

Figure S 4:2D-IR spectra for AFGP 7-8 solutions in heavy water at a concentration of 2%. The 2D-IR spectra were collected by starting the measurement at 0 °C and then increasing the temperature.

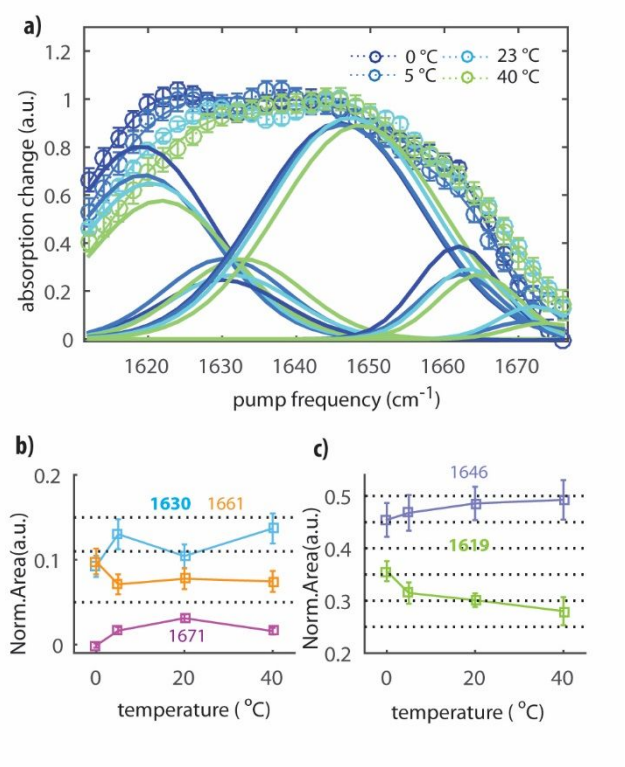

Figure S 5: a) Anti-diagonal slices for solutions of AFGP 7-8 at a concentration of 2% for different temperature. Raw data are represented by circles, and fit data by continuous line. In addition, there are reported the Gaussian-shaped five different bands used to fit the data. The centre frequencies are allowed to shift 0.05 cm^{-1}/ °C to take in account of the temperature effect[14]. The raw data shows slight differences in intensity though it is the same sample. This is due to the long time (72 hours) required for the complete set of measurements. b) and c) Normalized areas of the five bands reported in a) for different temperatures.

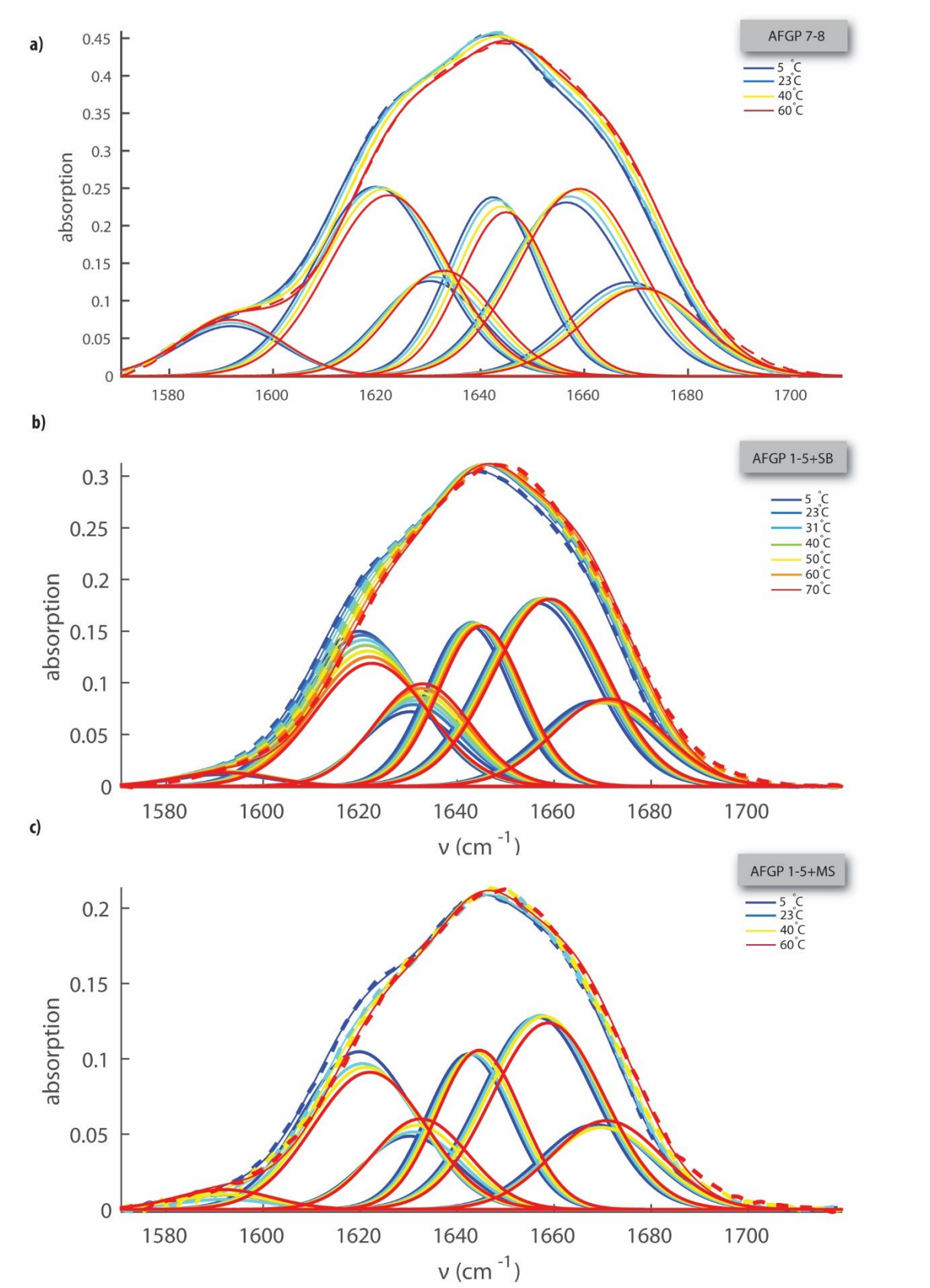

Figure S 6: Linear infrared spectra for solutions of AFGP 7-8 and AFGP 1-5 with 1 M of magnesium sulfate and AFGP 1-5 with 0.3 sodium borate at a concentration of ~2%. The raw data are represented by the dashed lines, and the fit data by the continuous lines. In addition, there are reported the six Gaussian-shaped bands used to fit the data. The centre frequencies are allowed to shift ~0.05 cm^{-1}/ °C to take in account of the temperature effect.

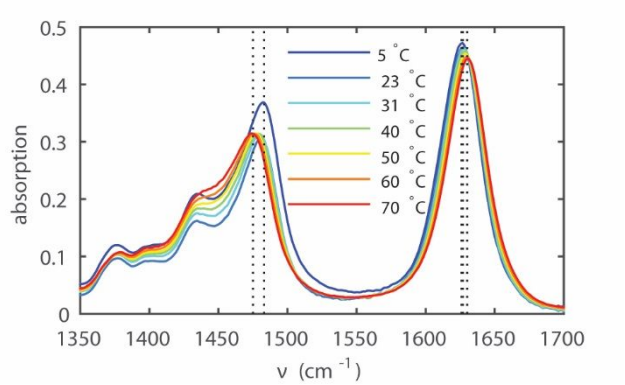

Figure S 7: Linear infrared spectra of N-acetylgalacosamine at a concentration of 6%. Dashed lines indicate the position of the amide II and the amide I vibrations.

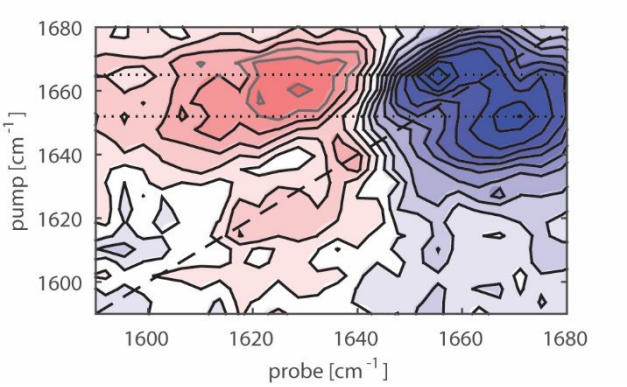

Figure S 8: 2D-IR spectrum for solution of AFGP 1-5 at a concentration of 2% at 2 °C. The 2D-IR spectrum is obtained by subtracting parallel signal from 3 times the perpendicular signal. Dashed lines indicate the two different alpha-helix modes at 1655 and 1665 cm^{-1} .

Centre positions (cm^{-1})	FWHM (cm^{-1})
1592±2	33±3
1620±3	39±3
1630±3	32±6
1642±2	28±3
1656±3	38±3
1668±2	38±3

Table S 1: Centre positions and widths obtained by globally fitting the linear infrared spectra of AFGP 7-8 as a function of temperature

Supplementary Biobliography

(1) Ahlgren, J. A.; DeVries, A. L. Comparison of Antifreeze Glycopeptides from Several Antarctic Fishes. *Polar Biol.* **1984**, *3*, 93–97.

(2) Ahmed, A. I.; Yeh, Y.; Osuga, Y. Y.; Feeney, R. E. Antifreeze Glycoproteins from Antarctic Fish. Inactivation by Borate. *J. Biol. Chem.* **1976**, *251*, 3033–3036.

(3) Meister, K.; Duman, J. G.; Xu, Y.; DeVries, A. L.; Leitner, D. M.; Havenith, M. The Role of Sulfates on Antifreeze Protein Activity. *J. Phys. Chem. B* **2014**, *118*, 7920–7924.

(4) Selig, O.; Siffels, R.; Rezus, Y. L. A. Ultrasensitive Ultrafast Vibrational Spectroscopy Employing the Near Field of Gold Nanoantennas. *Phys. Rev. Lett.* **2015**, *114*, 233004.

(5) Selig, O.; Cunha, A. V.; Van Eldijk, M. B.; Van Hest, J. C. M.; Jansen, T. L. C.; Bakker, H. J.; Rezus, Y. L. A. Temperature-Induced Collapse of Elastin-like Peptides Studied by 2DIR Spectroscopy. *J. Phys. Chem. B* **2018**, *122*, 8243–8254.

(6) Byler, D. M.; Susi, H. Examination of the Secondary Structure of Proteins by Deconvolved FTIR Spectra. *Biopolymers* **1986**, *25*, 469–487.

(7) Swamy, M. J.; Heimburg, T.; Marsh, D. Fourier-Transform Infrared Spectroscopic Studies on Avidin Secondary Structure and Complexation with Biotin and Biotin-Lipid Assemblies. *Biophys. J.* **1996**, *71*, 840–847.

(8) Dousseau, F.; Pézolet, M. Determination of the Secondary Structure Content of Proteins in Aqueous Solutions from Their Amide I and Amide II Infrared Bands. Comparison between Classical and Partial Least-Squares Methods. *Biochemistry* **1990**, *29*, 8771–8779.

(9) Hamm, P;Zanni, M., G. *Concepts and Methods of 2D Infrared Spectroscopy*; Cambridge University Press: Cambridge, U.K. ;2011.

(10) Woutersen, S.; Hamm, P. Time-Resolved Two-Dimensional Vibrational Spectroscopy of a Short α-Helix in Water. *J. Chem. Phys.* **2001**, *1151*, 2727–7733.

(11) Lee, S.-H.; Krimm, S. General Treatment of Vibrations of Helical Molecules and Application to Transition Dipole Coupling in Amide I and Amide II Modes of α-Helical Poly(l-Alanine). *Chem. Phys.* **1998**, *230*, 277–295.

(12) PRESTRELSKI, S. J.; BYLER, D. M.; THOMPSON, M. P. Infrared Spectroscopic Discrimination between A- and 310-helices in Globular Proteins: Reexamination of Amide I Infrared Bands of A-lactalbumin and Their Assignment to Secondary Structures. *Int. J. Pept. Protein Res.* **1991**, *37*, 508–512.

(13) Wang, J.; Hochstrasser, R. M. Characteristics of the Two-Dimensional Infrared Spectroscopy of Helices from Approximate Simulations and Analytic Models. *Chem. Phys.* **2004**, *297* , 195–219.

(14) Amunson, K. E.; Kubelka, J. On the Temperature Dependence of Amide I Frequencies of Peptides in Solution. *J. Phys. Chem. B* **2007**, *111*, 9993–999.