Supporting Information to

Observation of dissipating solvated protons upon hydrogel formation

E.P. van Dam[+], B. König[+], S. Ramos, E.M. Adams, G. Schwaab, M. Havenith*

Lehrstuhl für Physikalische Chemie II, Ruhr-Universität Bochum, 44780 Bochum, Germany

[+]These authors contributed equally

*Corresponding author

Content:

S1. Spectral Fitting

Difference reflection absorption spectra ($\Delta\alpha_{HA}$ and $\Delta\alpha$) were fit in Matlab R2020a (Mathworks) by a sum of damped harmonic oscillator and Gaussian functions as described by equation S1.

$$\Delta\alpha_x = y_0 + \sum_{n=1}^{4} \frac{A_n \omega_{0,n}^{\ 2} \nu^2}{4\pi^3 \left[\frac{\nu^2 \omega_{0,n}^{\ 2}}{\pi^2} + \left(\nu_{d,n}^{\ 2} + \frac{\omega_{0,n}^{\ 2}}{4\pi^2} - \nu^2\right)^2\right]} + \sum_{n=1}^{2} a_n e^{\left[-0.5\left(\frac{\nu - b_n}{c_n}\right)^2\right]} \quad \text{(S1)}$$

Where y_0 describes the baseline, A_n is related to the amplitude, and $\omega_{0,n}$, and $\nu_{d,n}$ describe the width, and center frequency of the n^{th} resonance of the damped harmonic oscillator and a_n, b_n, and c_n describe the amplitude, center frequency, and a value related to the width of the Gaussian function.[1] More specifically, the spectra were fit to four damped harmonic oscillator (DHO) contributions, DHO Peak 1 at ~100 cm^{-1}, DHO Peak 2 at ~300 cm^{-1}, DHO Peak 3 at ~490 cm^{-1}, and DHO Peak 4 at ~640 cm^{-1}, and two Gaussian (GF) contributions with GF Peak 1 at ~610 cm^{-1} and GF Peak 2 at ~623 cm^{-1}. The baseline was held at 0, as the contribution from a baseline is eliminated in the generation of the difference spectra. Fit boundaries were adjusted for each concentration of $HClO_4$, but all values were allowed to fit freely until fully converged. Example cumulative fits and fit contributions are shown in Figure S1. Peak amplitudes of DHO Peak 2 and GF Peak 2 for hyaluronan samples at different pH's are given in Table S1. Peak amplitudes of DHO Peak 2 for hyaluronan samples with urea and glucose are given in Table S2 and Table S3, respectively.

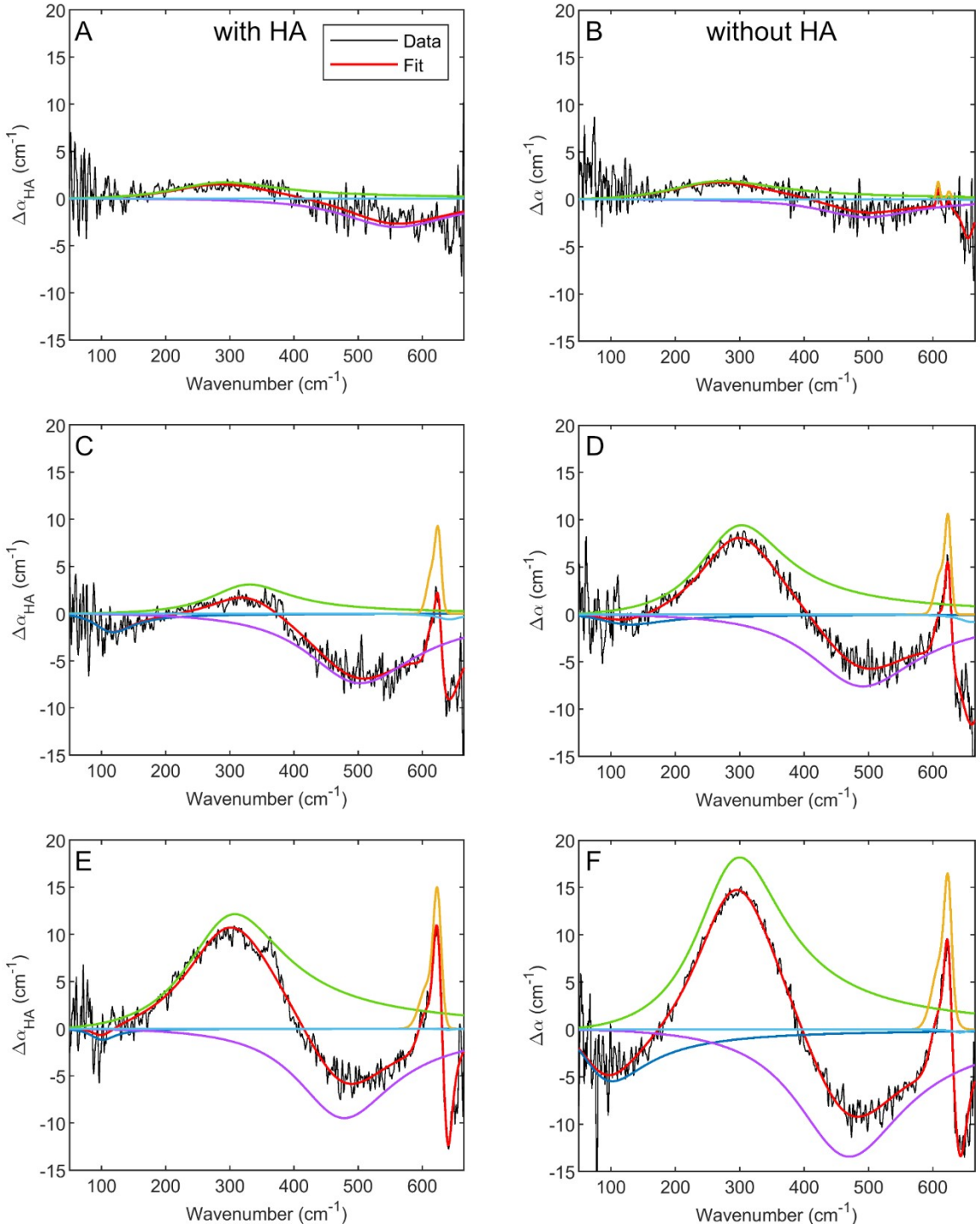

Figure S1. Fits of difference THz reflection absorption spectra of selected samples: (A, C, E) 20 mg/mL hyaluronan with 8, 44 and 83 mM $HClO_4$ respectively, after subtraction of hyaluronan with 150 mM NaCl and 0 mM $HClO_4$. (B, D, F) 8, 44 and 83 mM $HClO_4$ respectively, after subtraction of 150 mM NaCl and 0 mM $HClO_4$. The spectra (black line) were fit with a combination of damped harmonic oscillators and Gaussians (colored lines), according to the text. The fit result is shown as a red line.

Table S1. Peak amplitudes of the solvated proton band (A_{300}) and ClO_4^- band (A_{623}) of solutions with and without hyaluronan.

[$HClO_4$] (mM)	pH with HA[2]	A_{300} with HA	A_{300} without HA	A_{623} with HA	A_{623} without HA
3	4.13	20.8 ± 9.7	13.5 ± 4.4	0 ± 0	0.6 ± 0.3
8	3.67	26.1 ± 4.7	30.5 ± 5.5	0 ± 0	1.7 ± 1.4
13	3.41	48.7 ± 3.8	49.5 ± 8.4	1.5 ± 1.5	3.0 ± 1.3
22	3.08	71.9 ± 4.1	83.1 ± 1.8	4.0 ± 0.2	5.0 ± 0.9
31	2.81	31.5 ± 16.9	114.2 ± 9.4	5.5 ± 0.3	6.5 ± 2.2
38	2.61	31.8 ± 11.6	111.1 ± 22.0	6.1 ± 1.3	7.8 ± 1.0
44	2.42	34.5 ± 5.0	121.8 ± 7.6	7.8 ± 0.9	8.5± 2.2
45	2.39	74.1 ± 4.0	103.6 ± 1.9	6.4 ± 1.7	9.3 ± 0.03
51	2.20	138.7 ± 6.3	135.3 ± 7.1	7.3 ± 0.9	9.8 ± 0.7
54	2.11	132.9 ± 15.7	138.2 ± 7.0	8.4 ± 0.9	11.2 ± 0.8
60	1.93	164.9 ± 14.9	176.2 ± 12.4	9.9 ± 1.2	9.6 ± 1.0
70	1.70	177.7 ± 6.0	173.0 ± 10.8	12.1 ± 0.7	12.4 ± 0.9
83	1.54	192.5 ± 22.50	215.6 ± 12.7	14.6 ± 1.0	12.5 ± 1.1

Table S2. Peak amplitudes of the solvated proton band (A_{300}) of hyaluronan solutions in the elastic state (pH 2.4) and liquid state (pH 1.9) with different urea concentrations.

[Urea] (% w/v)	A_{300} elastic state	A_{300} liquid state
0	34.5 ± 5.0	165.3 ± 15.0
2.5	54.2 ± 15.9	161.1 ± 6.2
5	96.7 ± 20.7	153.8 ± 14.1
10	70.6 ± 11.9	145.8 ± 13.0
20	92.3 ± 24.8	147.8 ± 12.6

Table S3. Peak amplitudes of the solvated proton band (A_{300}) of hyaluronan solutions in the elastic state (pH 2.4) and liquid state (pH 1.9) with different glucose concentrations.

[Glucose] (% w/v)	A_{300} elastic state	A_{300} liquid state
0	34.5 ± 5.0	165.3 ± 15.0
2.5	49.3 ± 6.6	159.3 ± 28.5
5	78.4 ± 14.2	126.4 ± 10.86
10	68.9 ± 11.2	137.4 ± 14.4
20	76.1 ± 17.9	98.9 ± 3.5

S2. Example absorption spectra of HA with and without $HClO_4$

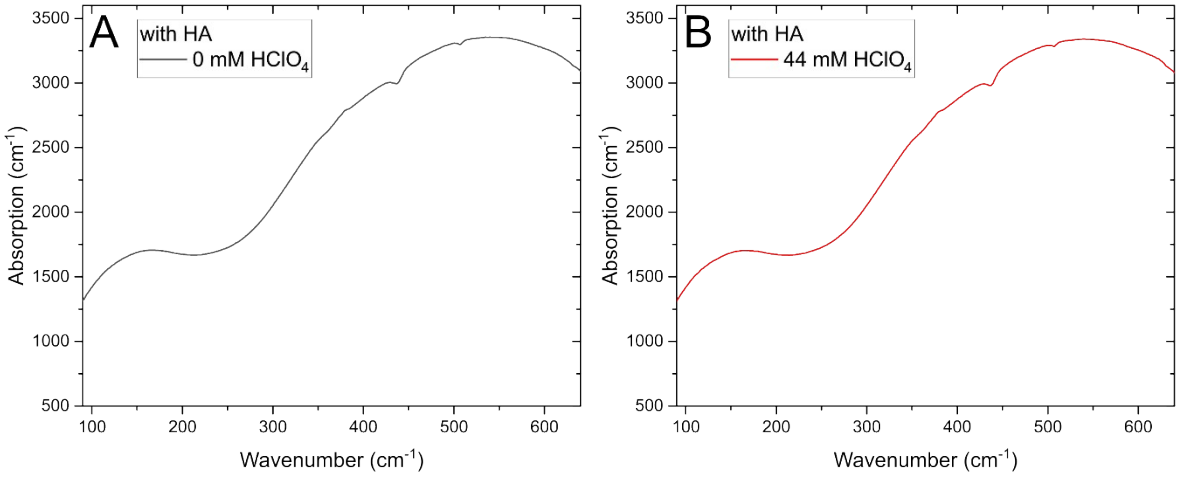

Figure S2. Example absorption spectra of HA with and without $HClO_4$ (A,B). The differences between the spectra are difficult to visualize in a pure absorbance spectrum and thus difference absorption spectra are used for analysis throughout the manuscript.

S3. THz reflection absorption spectra of hyaluronan at additional $HClO_4$ concentrations

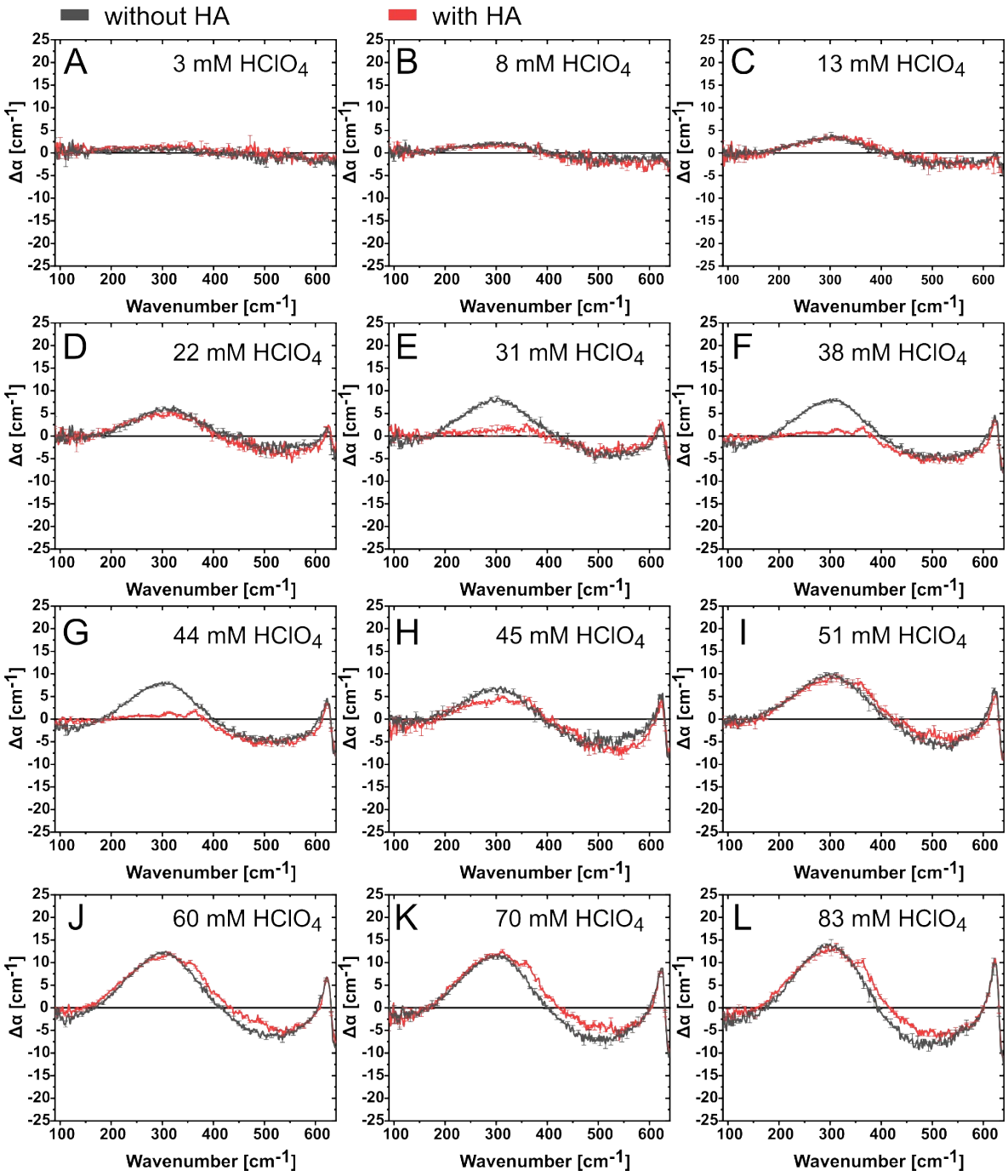

Figure S3. Difference THz reflection absorption spectra of solutions with (red) 20 mg/mL hyaluronan with different $HClO_4$ concentrations (A-L) after subtraction of hyaluronan with 150 mM NaCl and 0 mM $HClO_4$ and (grey) $HClO_4$ at different concentrations (A-L) after subtraction of 150 mM NaCl and 0 mM $HClO_4$.

S4. Example absorption spectrum of bulk water

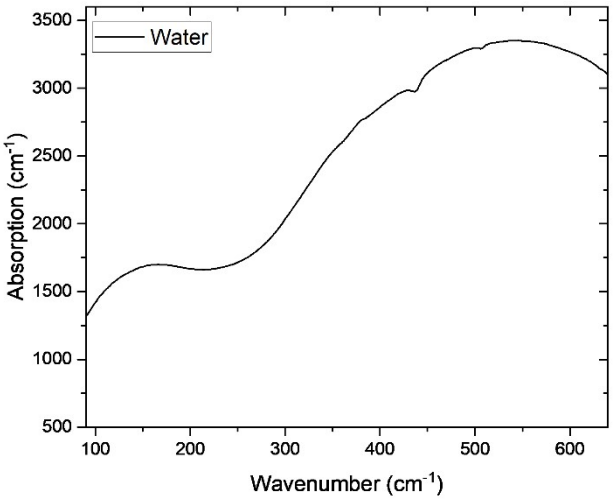

Figure S4. Example absorption spectrum of bulk water collected in an ATR geometry. It is well known that spectra in the ATR geometry exhibit a red-shift, as is shown here where the librational peak ofo bulk water is shifted to ~550 cm^{-1}.

S5. THz reflection absorption spectra of $HClO_4$ and HCl

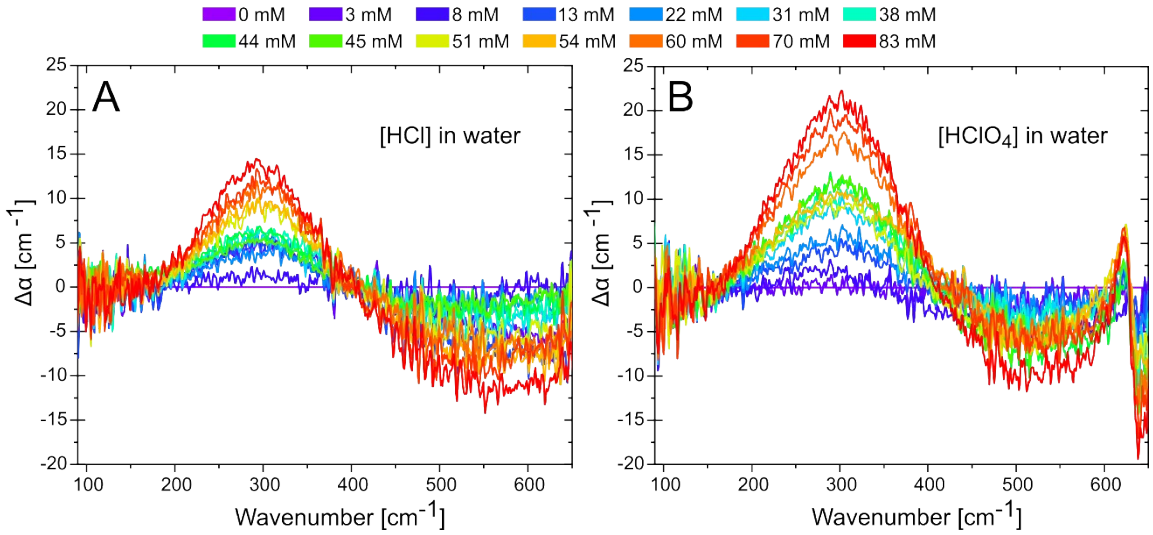

Figure S5. Difference THz reflection absorption spectra of (A) hydrochloric acid and (B) perchloric acid at different concentrations after subtraction of the water spectrum.

S6. THz reflection absorption spectra of hyaluronan with HCl

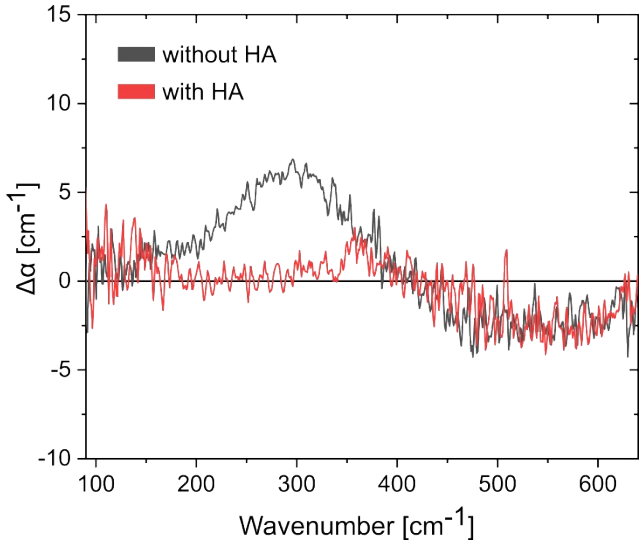

Figure S6. Difference THz reflection absorption spectra of solutions with (red) 20 mg/mL hyaluronan with 44 mM HCl (elastic state, pH 2.4) after subtraction of hyaluronan with 150 mM NaCl and 0 mM HCl and (grey) 44 mM HCl after subtraction of 150 mM NaCl and 0 mM HCl.

S7. Additional THz reflection absorption spectra of solutions with and without hyaluronan at different urea concentrations

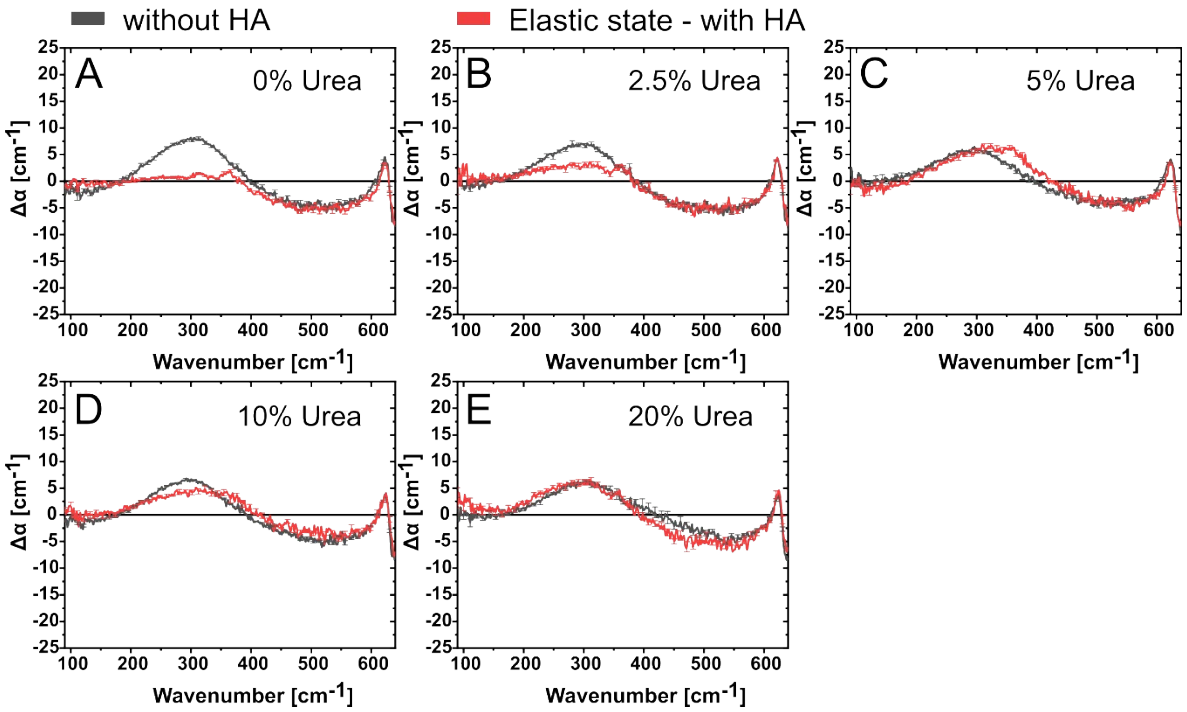

Figure S7. Difference THz reflection absorption spectra of (red) hyaluronan solutions in the elastic state (pH 2.4, 44 mM $HClO_4$) with different urea concentrations (A-E) after subtraction of hyaluronan, the same urea concentration and 150 mM NaCl. (grey) Difference spectra of 44 mM $HClO_4$ and different urea concentrations (A-E) after subtraction of the same urea concentration, 0 mM $HClO_4$ and 150 mM NaCl.

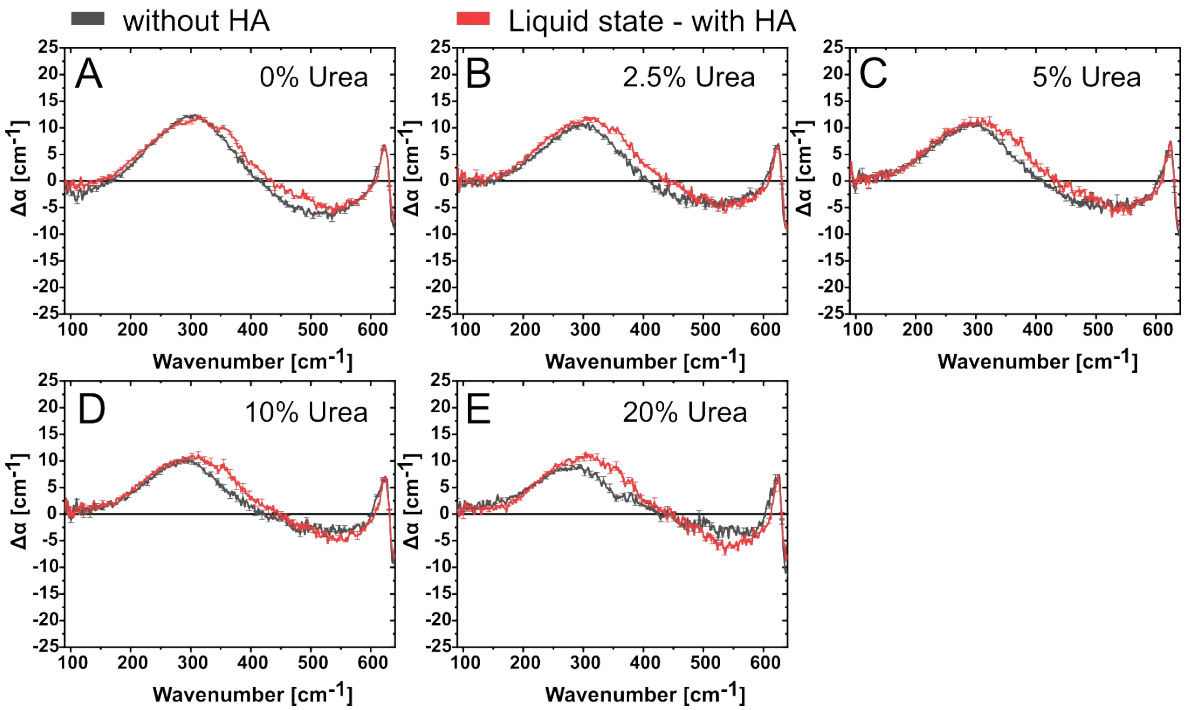

Figure S8. Difference THz reflection absorption spectra of (red) hyaluronan solutions in the liquid state (pH 1.9, 60 mM $HClO_4$) with different urea concentrations (A-E) after subtraction of hyaluronan, the same urea concentration and 150 mM NaCl. (grey) Difference spectra of 60 mM $HClO_4$ and different urea concentrations (A-E) after subtraction of the same urea concentration, 0 mM $HClO_4$ and 150 mM NaCl.

S8. Additional THz reflection absorption spectra of solutions with and without hyaluronan at different glucose concentrations

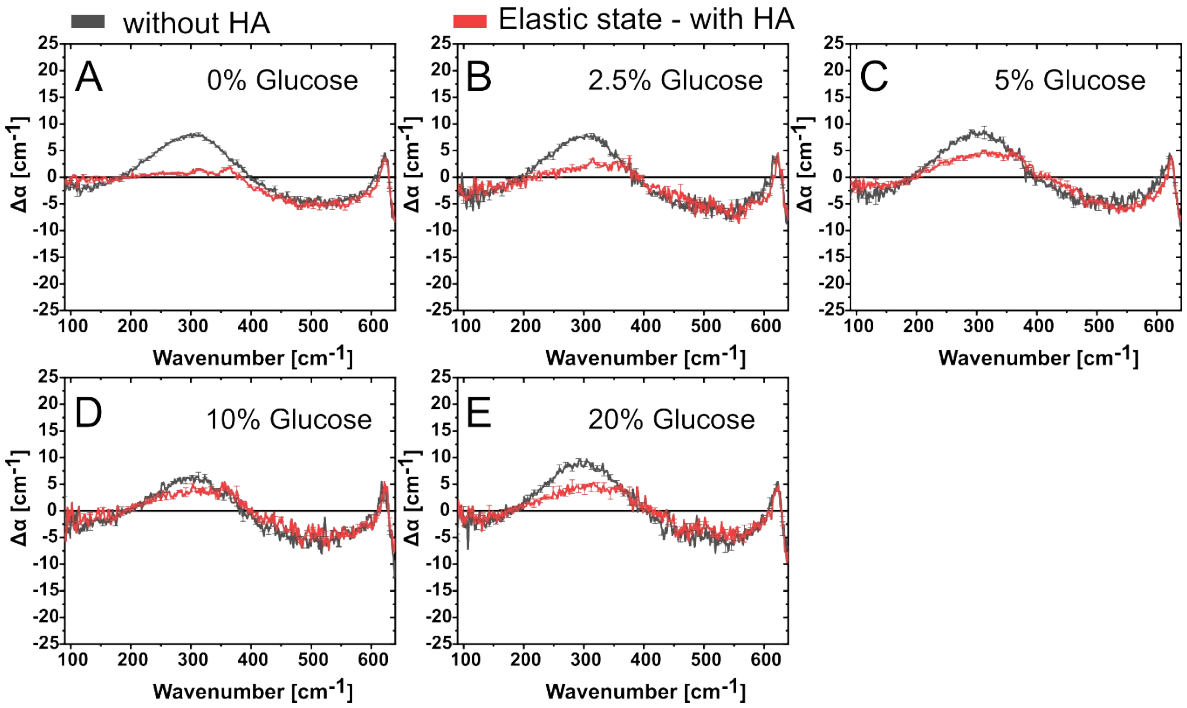

Figure S9. Difference THz reflection absorption spectra of (red) hyaluronan solutions in the elastic state (pH 2.4, 44 mM $HClO_4$) with different glucose concentrations (A-E) after subtraction of hyaluronan, the same glucose concentration and 150 mM NaCl. (grey) Difference spectra of 44 mM $HClO_4$ and different glucose concentrations (A-E) after subtraction of the same glucose concentration, 0 mM $HClO_4$ and 150 mM NaCl.

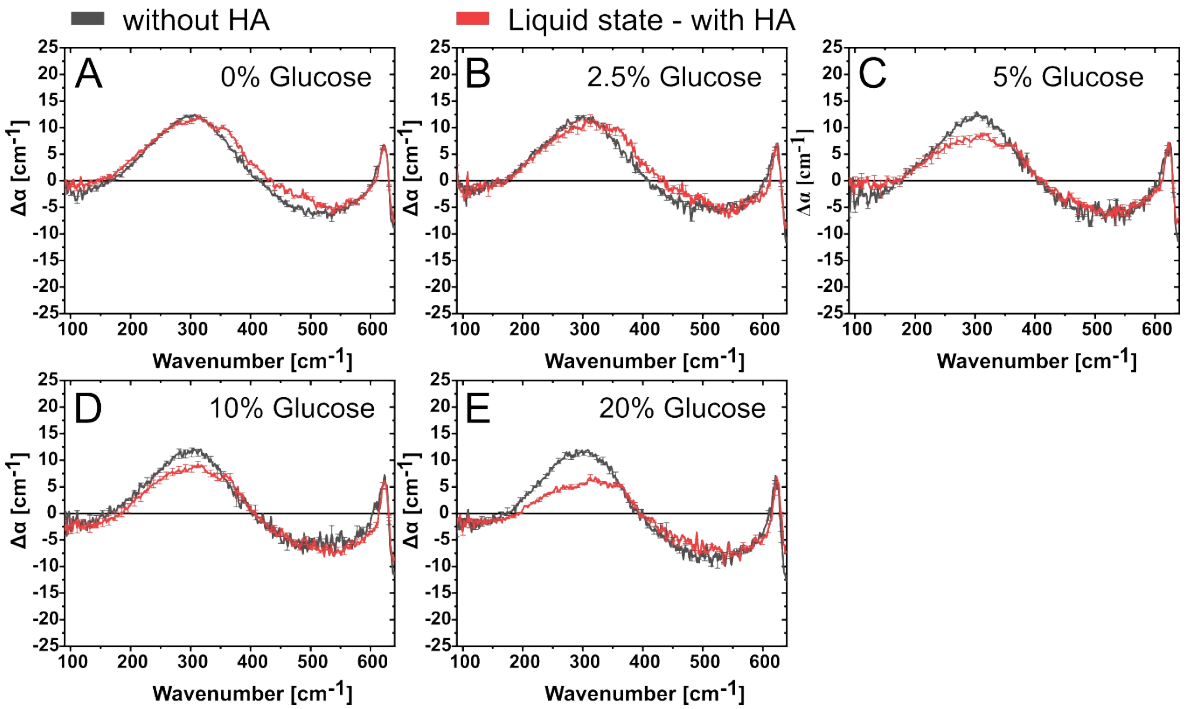

Figure S10. Difference THz reflection absorption spectra of (red) hyaluronan solutions in the liquid state (pH 1.9, 60 mM $HClO_4$) with different glucose concentrations (A-E) after subtraction of hyaluronan, the same glucose concentration and 150 mM NaCl. (grey) Difference spectra of 60 mM $HClO_4$ and different glucose concentrations (A-E) after subtraction of the same glucose concentration, 0 mM $HClO_4$ and 150 mM NaCl.

(1) Schwaab, G.; Sebastiani, F.; Havenith, M. Ion Hydration and Ion Pairing as Probed by THz Spectroscopy. *Angew. Chemie - Int. Ed.* **2019**, *58* (10), 3000–3013. https://doi.org/10.1002/anie.201805261.

(2) Giubertoni, G.; Burla, F.; Martinez-Torres, C.; Dutta, B.; Pletikapic, G.; Pelan, E.; Rezus, Y. L. A.; Koenderink, G. H.; Bakker, H. J. Molecular Origin of the Elastic State of Aqueous Hyaluronic Acid. *J. Phys. Chem. B* **2019**, *123* (14), 3043–3049. https://doi.org/10.1021/acs.jpcb.9b00982.