Synchrotron and Free electron laser
Radiation: generation and application
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Study of the THz response of protein solutions at different stages of glycation

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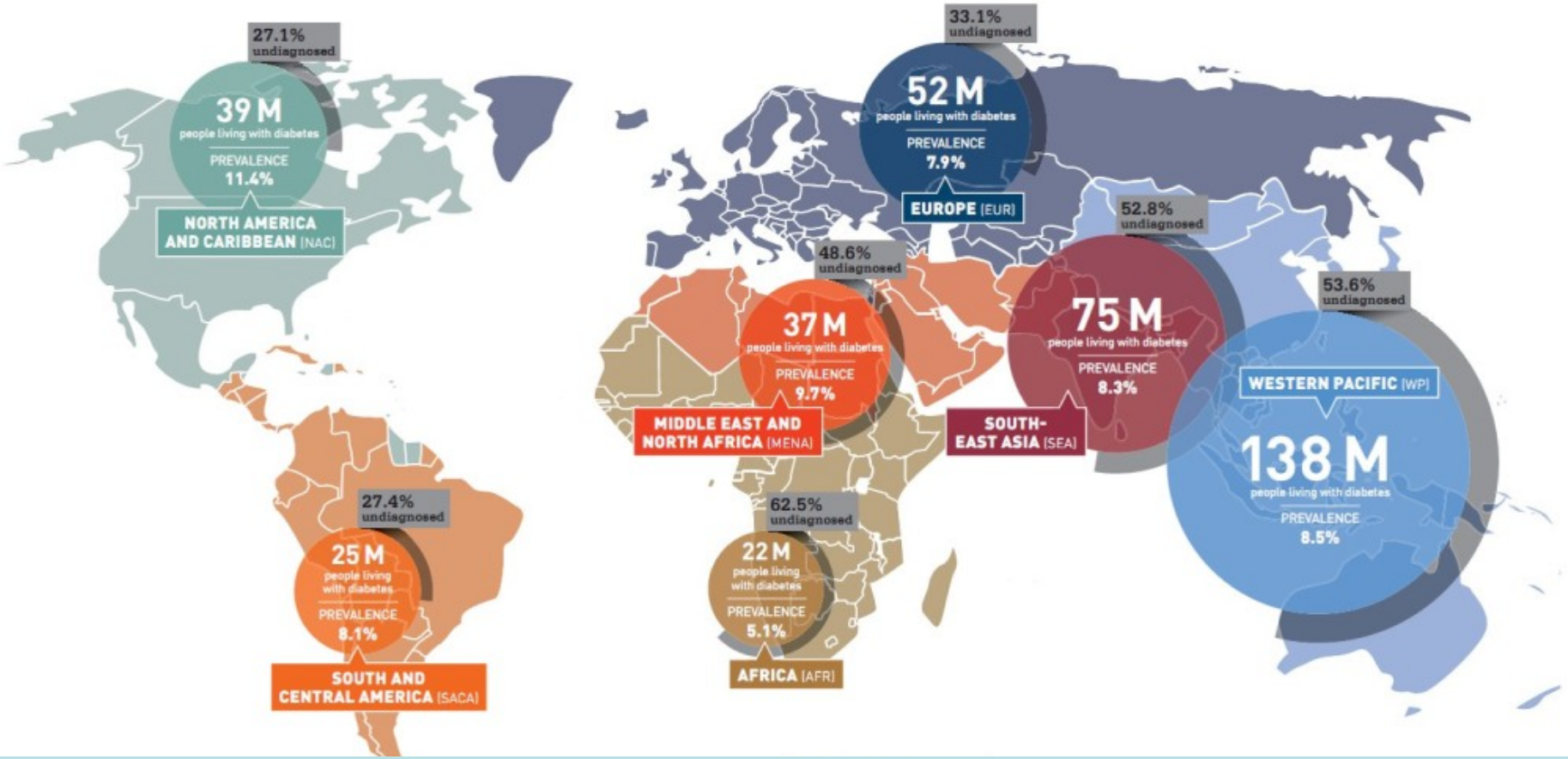
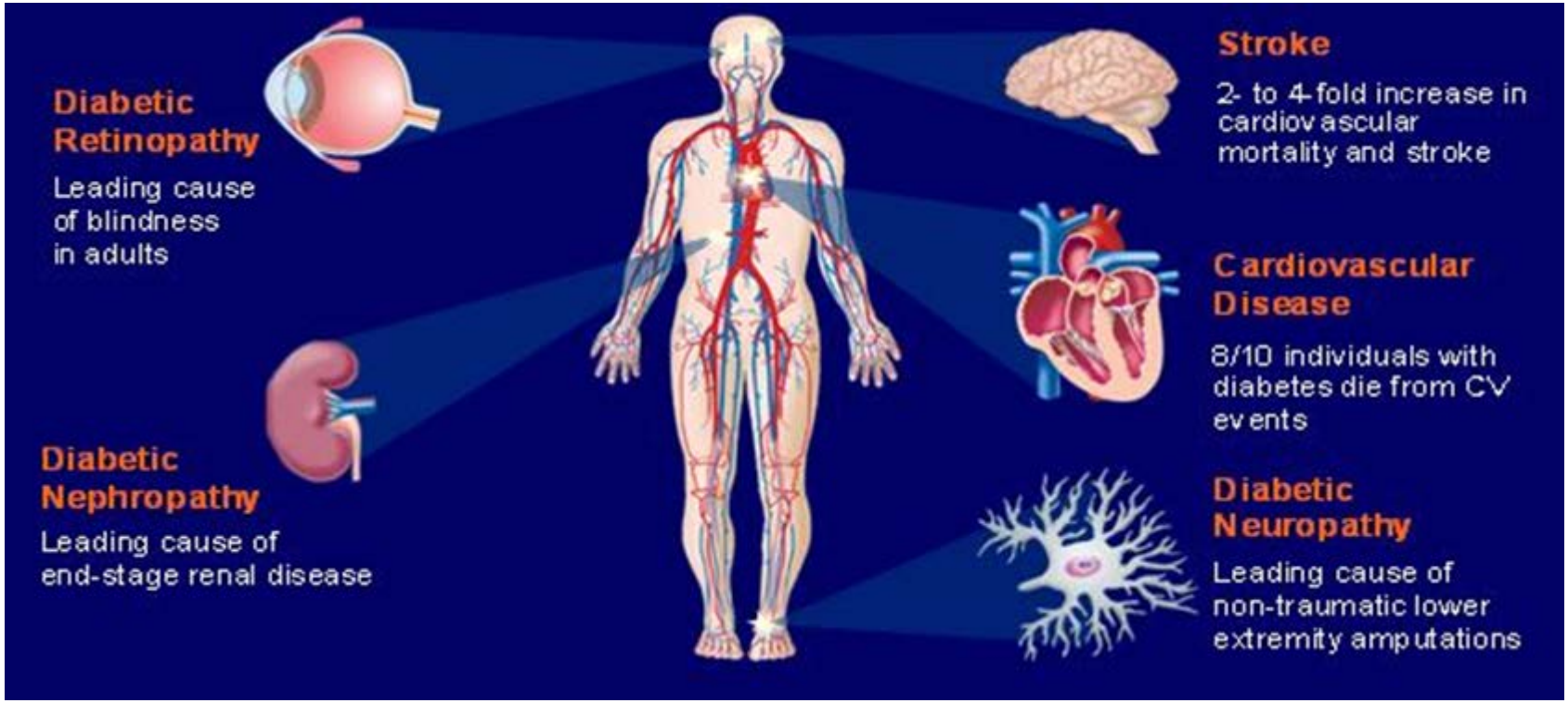
Diabetes mellitus: The epidemic of the century



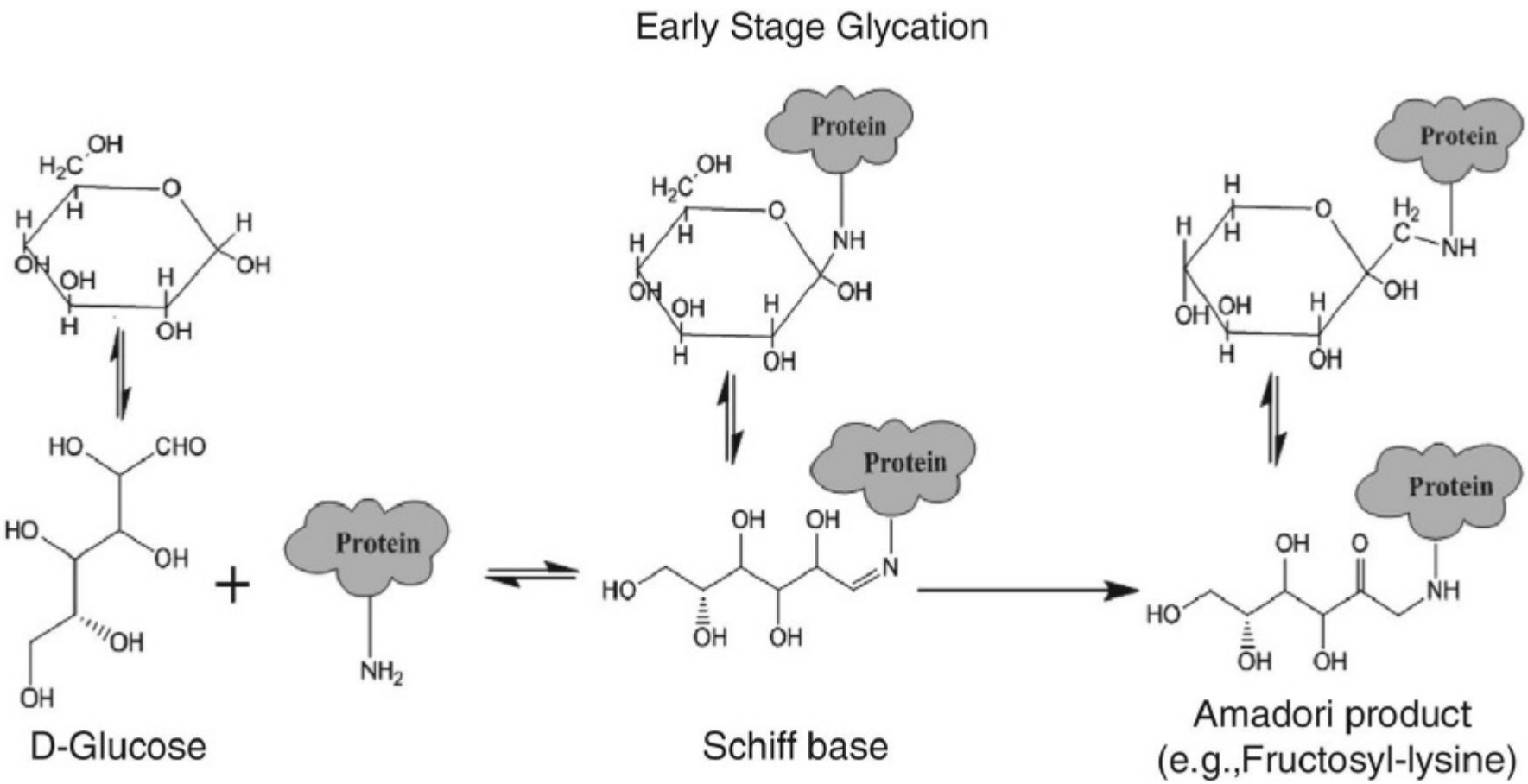
Diabetes is a group of metabolic diseases in which a person has high blood glucose level. In 2015, according to the International Diabetes Federation, at least 415 million people worldwide suffer from diabetes. Its incidence is increasing rapidly, and it is estimated that by 2030, this number will almost double. Diabetes mellitus occurs throughout the world, but is more common in the developed countries.

Complications of Diabetes

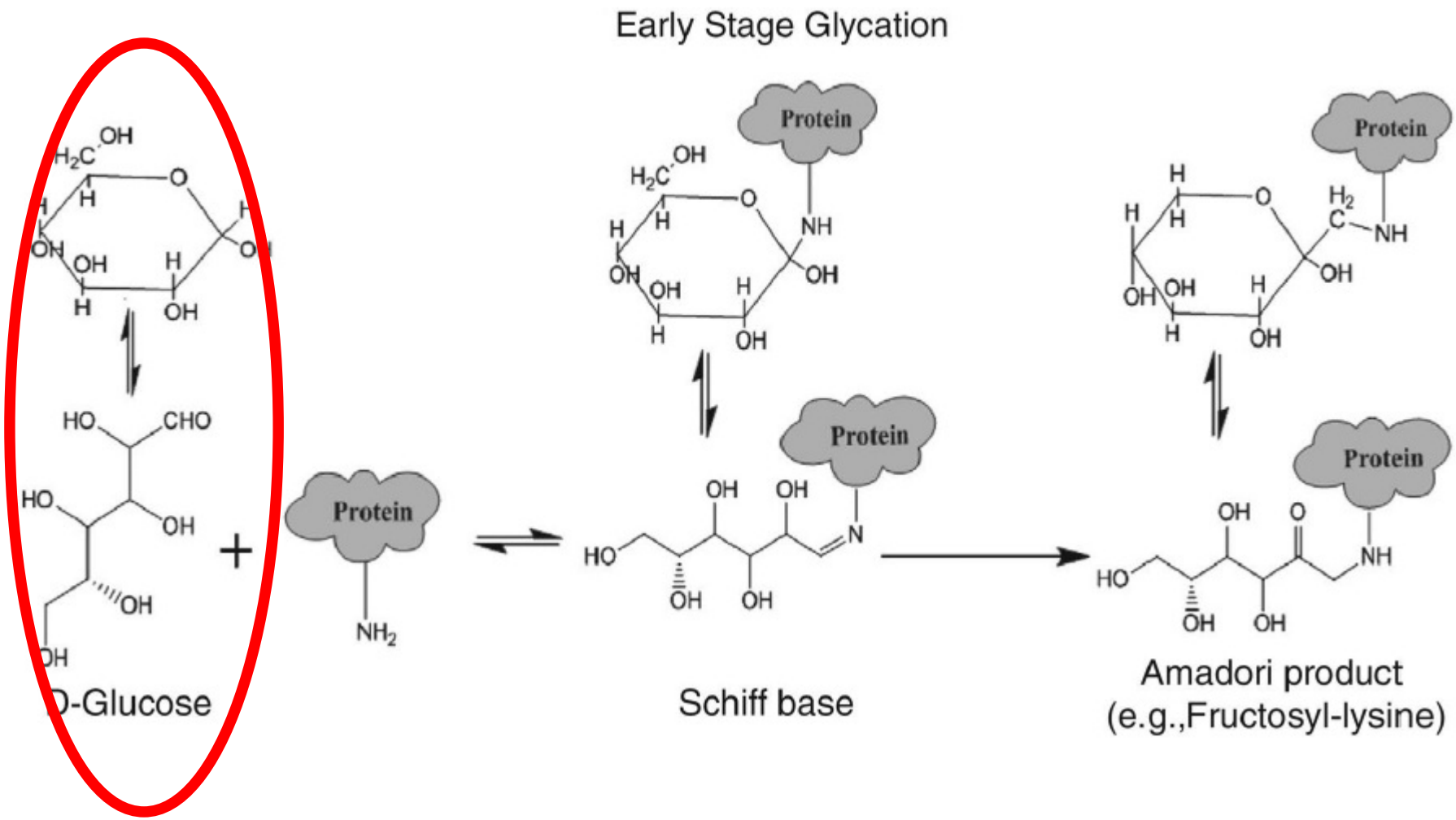


The nonenzymatic attachment of sugars to proteins, namely glycation, is accelerated under diabetic conditions. Monitoring the glycated serum albumin levels gives the short term variation of glucose concentration in diabetic patients blood. The study of proteins containing either early stage glycation products has become of great interest due to the suspected effects of glycation on protein function and tissue damage during Diabetes.

We used the transmission THz spectroscopy to study early stage of albumin glycation.



Glycation involves the non-enzymatic addition of reducing sugars and/or their reactive degradation products to primary or secondary amine groups on proteins. Early stage glycation involves the nucleophilic attack of a reducing sugar with primary amine groups on proteins to form a Schiff base that slowly rearranges to form an Amadori product or a ketoamine



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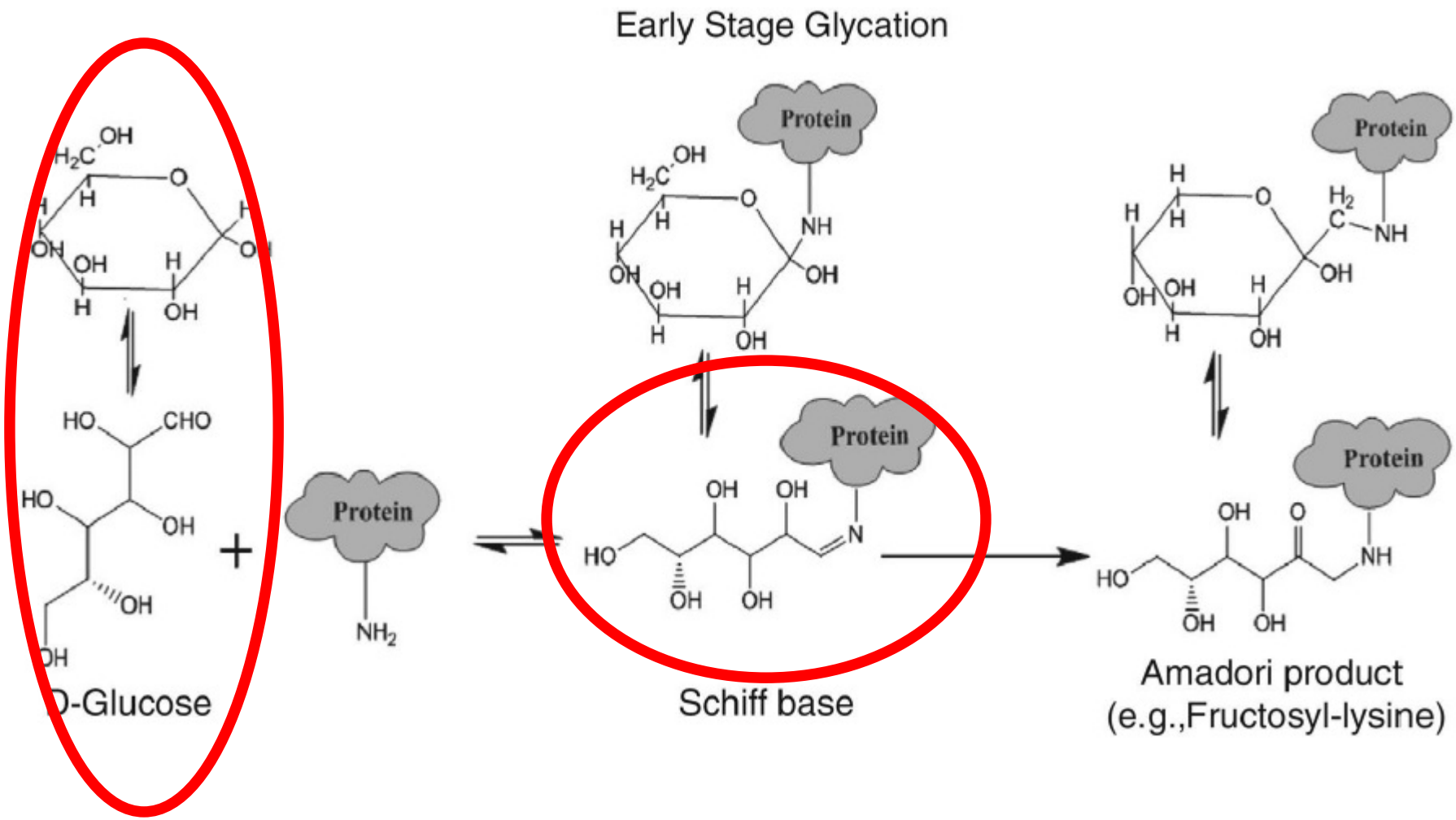

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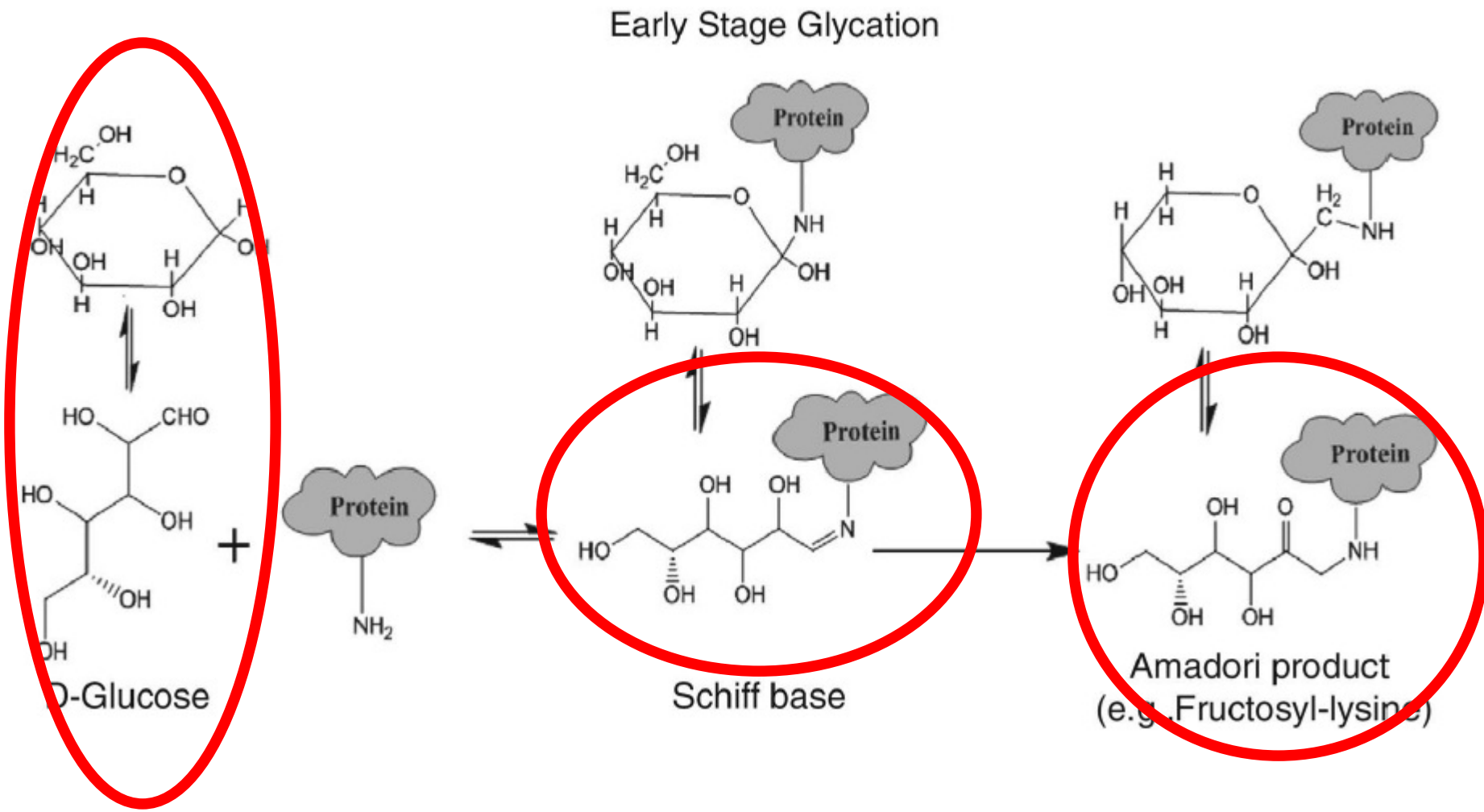

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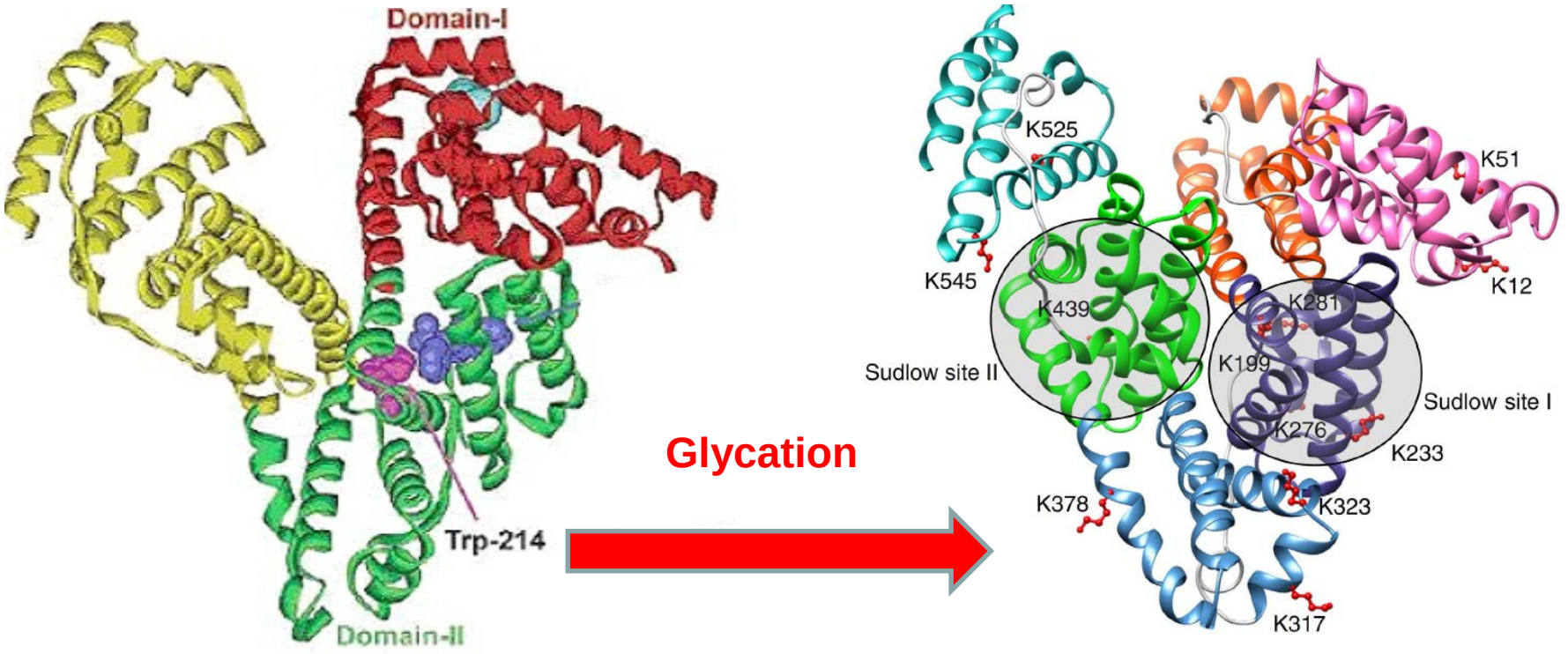
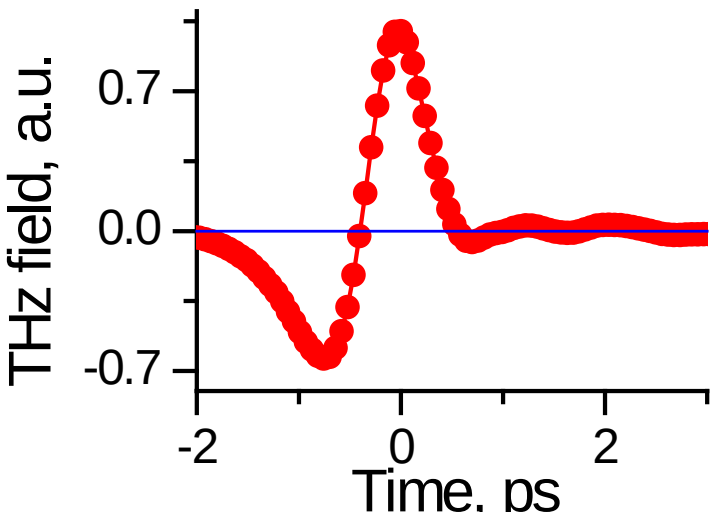
BOVINE SERUM ALBUMIN (BSA) **GLYCATED BSA**

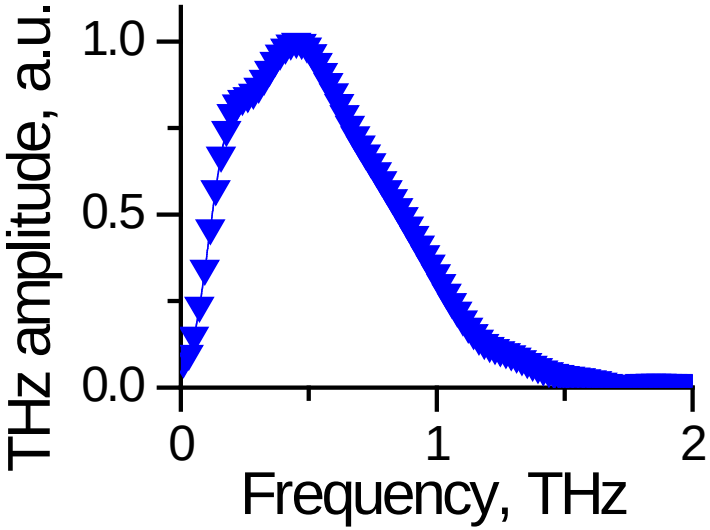


Glycation of BSA alters its structure resulting in loss of both secondary and tertiary structure. Albumin glycation alters ligand binding and plays a significant role in diabetic complications.

Terahertz Time Domain Spectroscopy



Fourier

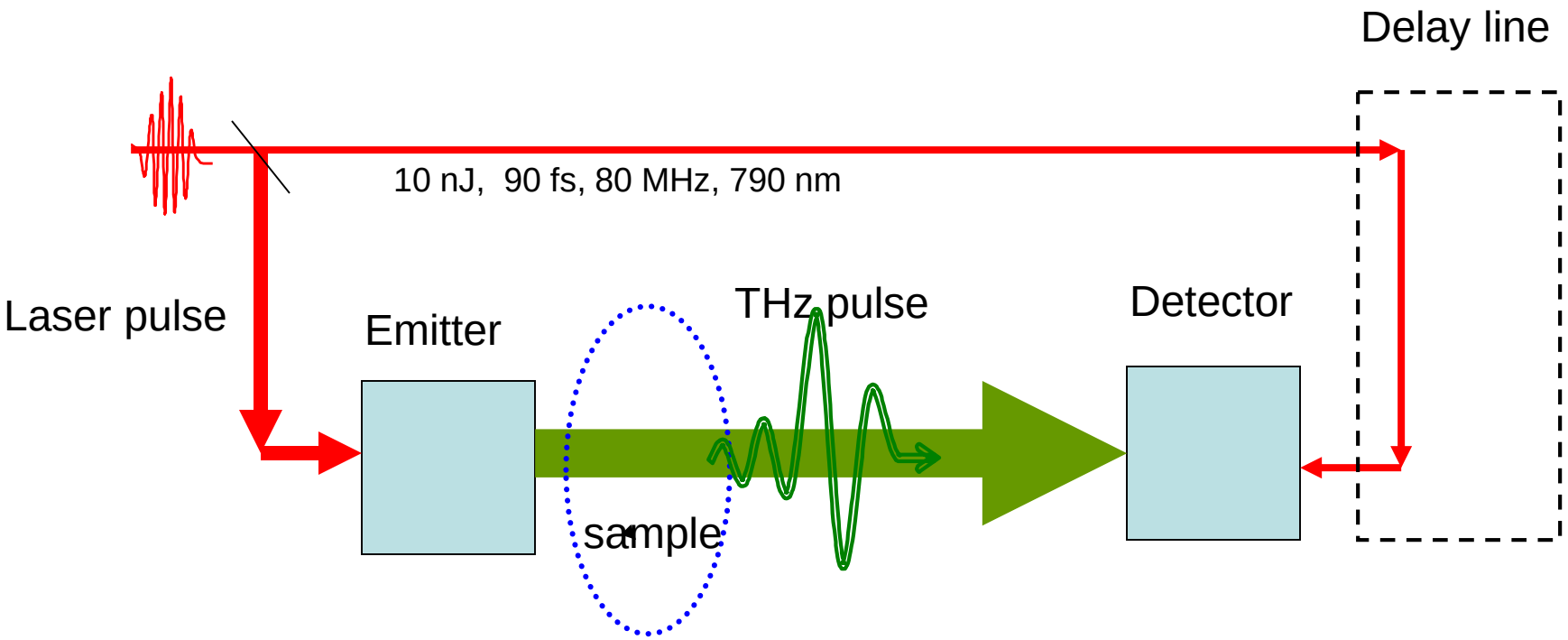


$$\left.\begin{matrix}\sqrt{T(\omega)} \\ \Delta\phi(\omega)\end{matrix}\right\} \rightarrow \left.\begin{matrix}n'(\omega) \\ \alpha(\omega)\end{matrix}\right\} \rightarrow \varepsilon(\omega) \rightarrow \varepsilon(\omega) = \varepsilon'(\omega) - i\varepsilon''(\omega)$$

THz-TDS has not yet found wide application in this field. A distinctive feature of this method is the possibility of measuring directly the refractive index and absorption coefficient and hence complex permittivity spectrum of the sample in a single scan and in a broad frequency range.

A.A. Angelutz …A.P. Shkurinov, *Quantum Electronics 44 (7) 614 – 632 (2014)*

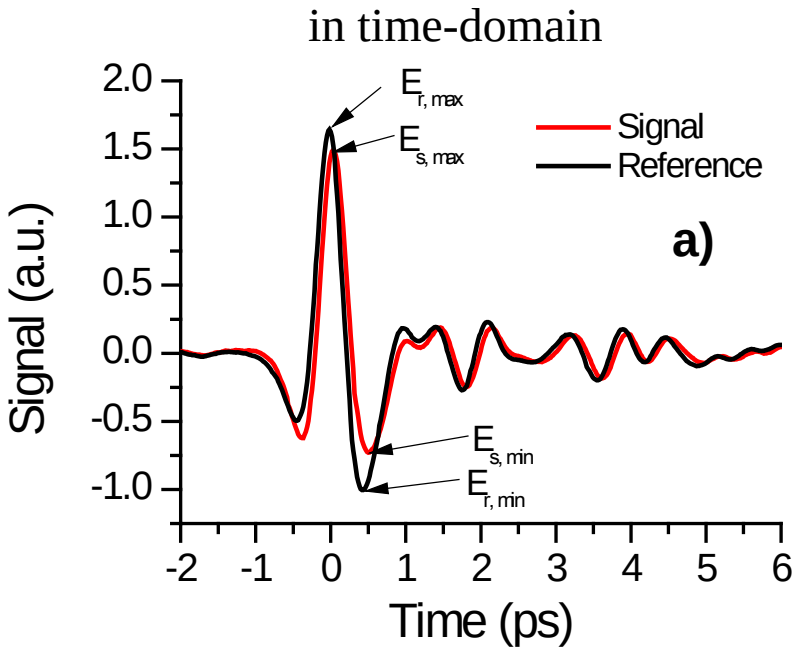
THz time-domain spectrometer

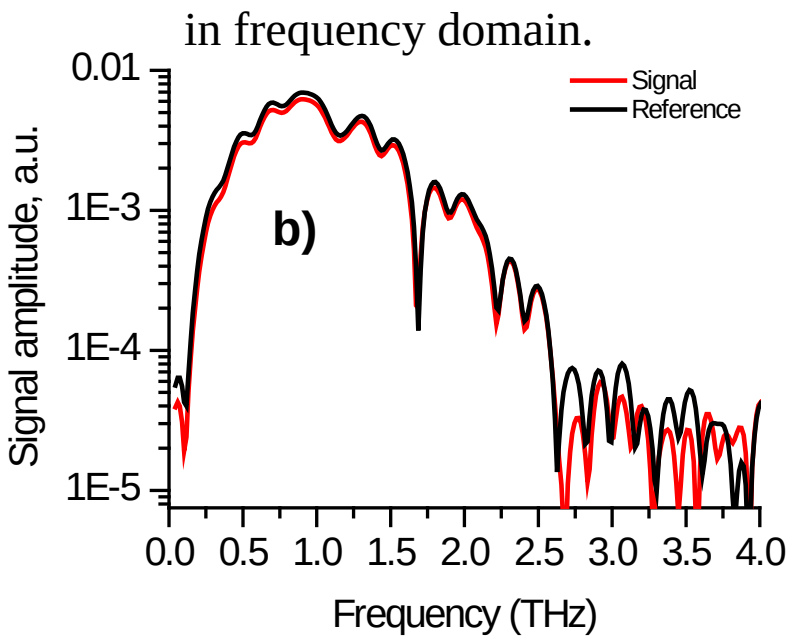


We used the radiation of a Ti:sapphire laser with a wavelength of 790 nm and a pulse duration of 90 fs with 1 Wt average power. For THz emission, the semiconductor (LT-GaAs) surface was used. For THz detection the electro-optical ZnTe crystal of 1 mm thickness was used. The spectral range of reliable measurements was between 0.05 and 1.1 THz, within which the THz pulse transmitted through 500 μm thick water layer has signal to noise ratio above one order.

M. M. Nazarov, A. P. Shkurinov, E .A. Kuleshov, V. V. Tuchin, “Terahertz time-domain spectroscopy of biological tissues”, Quantum Electronics, vol. 38, pp. 647-654 (2008)

A typical transmission spectrum





We recorded the temporal shape of a pulse transmitted through samples. A Fourier transform of a measured temporal shape of THz pulses E(t) provides a complex transmitted spectrum E(f), i.e., the amplitude |E(f)| and the phase arg(E(f)) of the spectrum. Here, E and f are, respectively, the electromagnetic-wave field intensity and frequency.

Since temporal shape of transmitted THz pulse E(t) does not change considerably for all studied solutions we may use pulse amplitude in time-domain as an integral characteristics of transmitted amplitude. In particular we use:

$$T_{int}=(E^{s}_{max}-E^{s}_{min})/(E^{r}_{max}-E^{r}_{min})$$

The measurement schemes
Transmission scheme

thickness is 500 μm

Measurements of the transmission spectrum of protein solution make it possible to determine more accurately properties of the solution at frequencies below 0.4 THz.

absorption coefficient

$$\alpha(f)=\ln[|E(f)|/|E_0(f)|]/d,$$

refractive index

$$n=1+\arg(E(f)/E_0(f))2\pi c/df$$

$E(f)$ and $E_0(f)$ *are spectra of the transmitted* and incident radiation,

f *is linear frequency,* d *is the sample thickness.*

Cherkasova et al. Analysis of blood plasma at terahertz frequencies, Optics and Spectroscopy 120(1):50-57 · January 2016, DOI: 10.1134/S0030400X16010069

Experiment

BSA (50.0 mg/ml) +
50 mM phosphate buffer
pH 7.4

BSA (50.0 mg/ml) +
50 mM phosphate buffer
+
0.5M glucose

BSA (50.0 mg/ml) +
50 mM phosphate buffer
+
0.5M fructose

1 t=0 min, 25ºC

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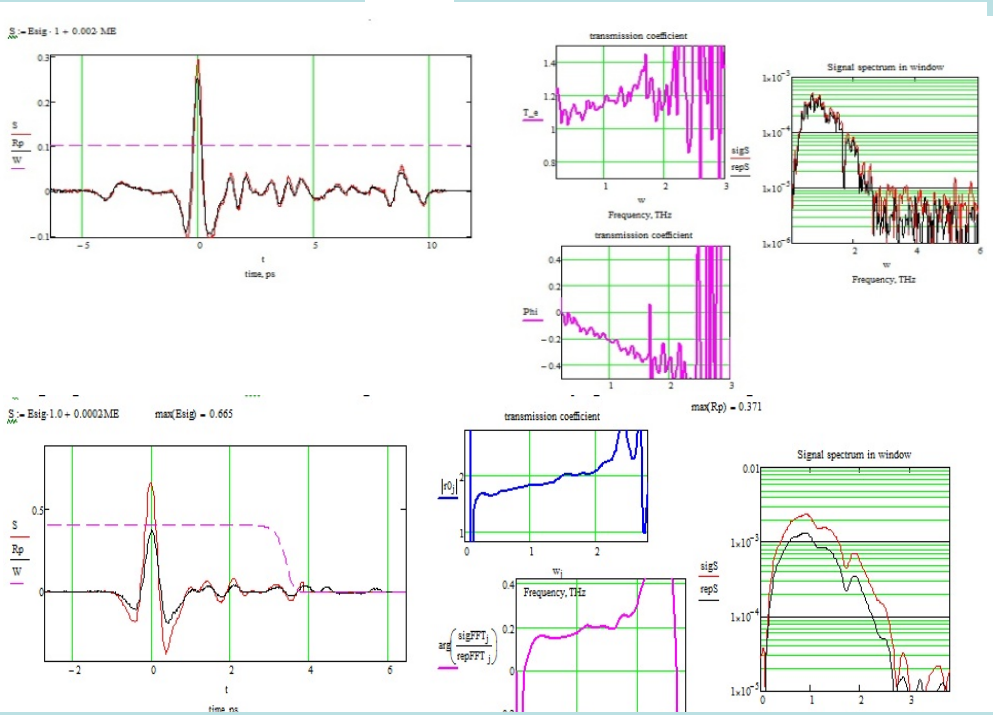
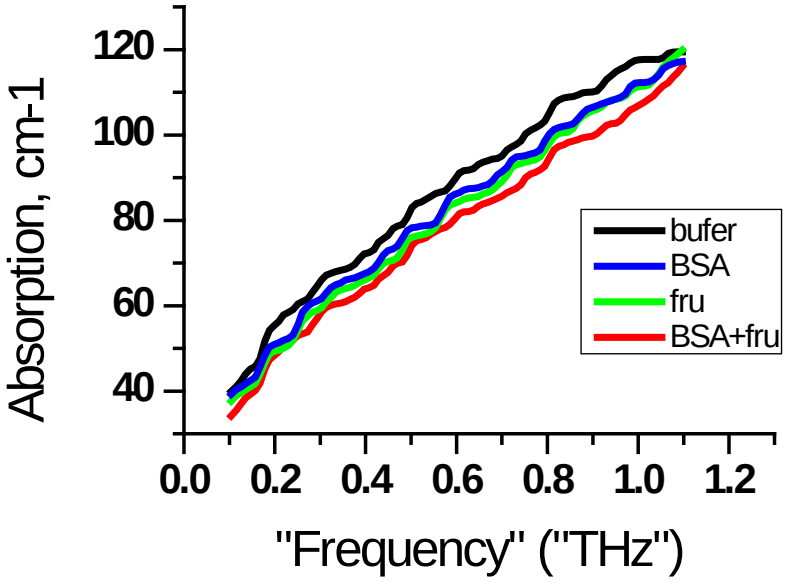
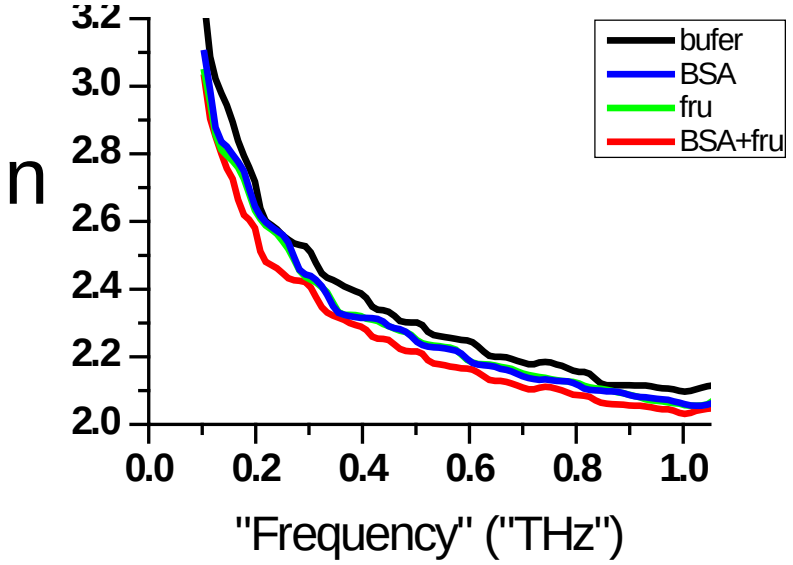
n t=0 - 96 h at 47°C



BSA was incubated with 0.5 M glucose (or 0.5 M fructose) in 50 mM phosphate buffer (pH 7.4) for 96 h at 47°C. Incubation of BSA in phosphate buffer without the presence of sugar was used as a control of spontaneous degradation of albumin during incubation. THz transmission spectra of incubation mixture were measured at 6, 24, 46 and 96 hours after the start of incubation .

Solution spectra



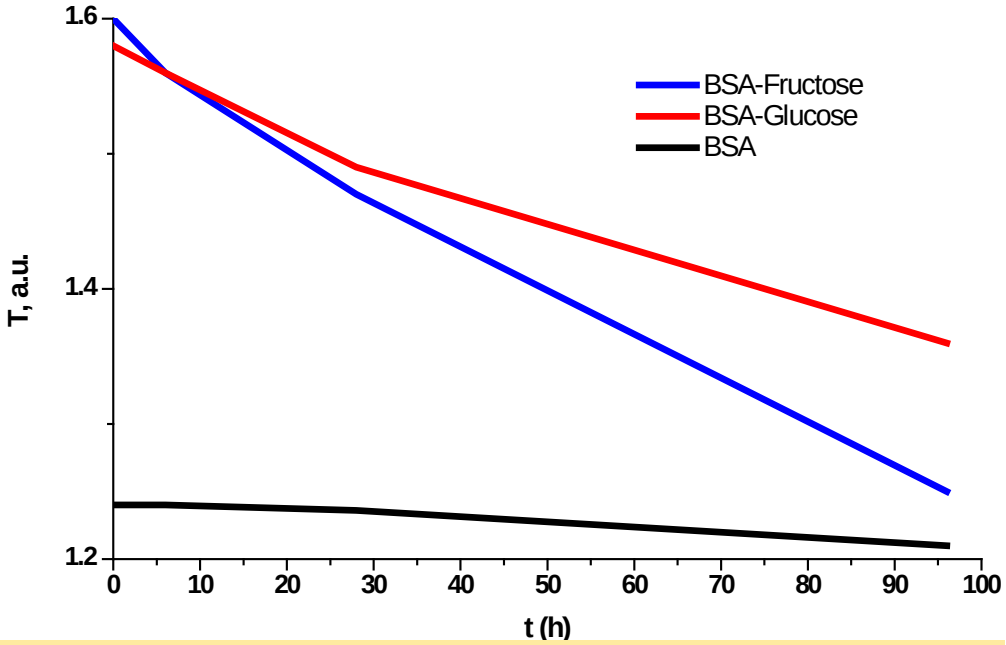


Absorption and refraction spectra of phosphate buffer, BSA solution in this buffer, fructose solution in the same buffer and a mixture containing BSA and fructose at the early stage (less than one hour) of incubation.

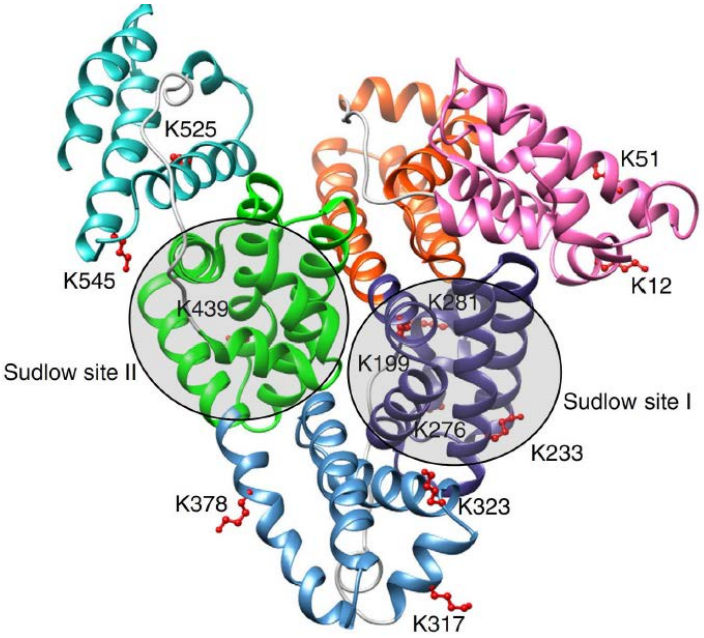
In solvents studies in THz range, there are no sharp spectral features, thus information about small changes in the shape of the broadband spectrum is important.

The absorption and refraction spectra values in all tree solutions are smaller than those values in buffer. This is caused by the fact that some amount of water molecules, which strongly absorb THz radiation, are replaced by components (BSA or/and sugar) less absorbing in this frequency range. Noticeable is the fact that a mixture of solutions with comparable concentration of the solute demonstrates stronger difference from buffer, than any of single component solution.

Transmission coefficient T_{int} during incubation of BSA with sugars



Variations of transmission coefficient T_{int} during incubation of BSA with sugars. An averaged transmission coefficient of solution is normalized on an averaged transmission coefficient of pure water.

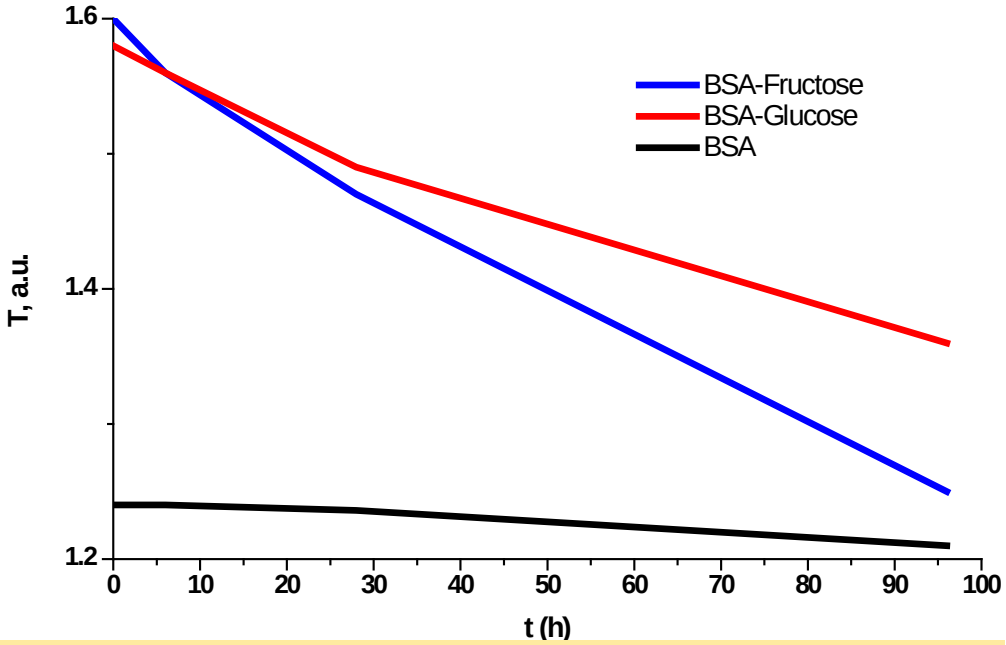


Glycation of BSA alters its structure resulting in loss of both secondary and tertiary structure.

THz absorption of BSA depends on type of sugars and incubation time

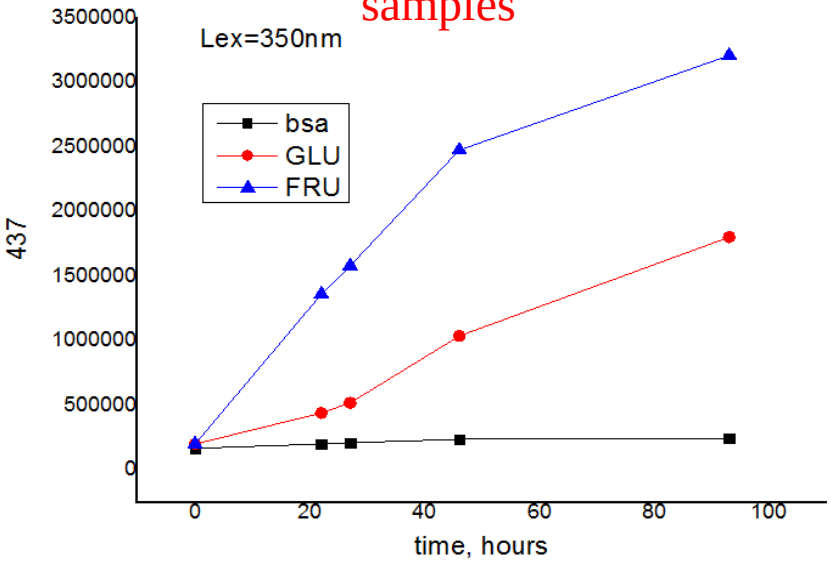
Changes were more pronounced in the case of fructose. The absorption spectra of BSA incubated in buffer alone did not change significantly.

Transmission coefficient T_{int} during incubation of BSA with sugars



Variations of transmission coefficient T_{int} during incubation of BSA with sugars. An averaged transmission coefficient of solution is normalized on an averaged transmission coefficient of pure water.

Visible range fluorescence of the same samples



350 nm excitation, 437 nm - Advanced Glycation End-products are formed by incubation of BSA and sugars

THz absorption of BSA depends on type of sugars and incubation time

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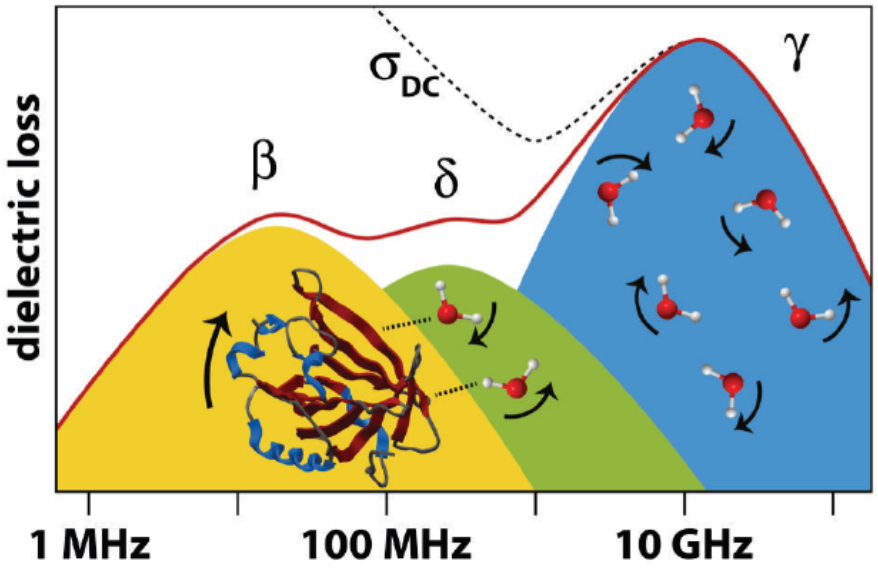
What are the reasons for the changes in THz absorption spectra of biological samples with a high glucose concentration?

We suppose that changes in the shape of THz experimental spectra are mainly due to changes in the relaxation time of water molecules in biological samples

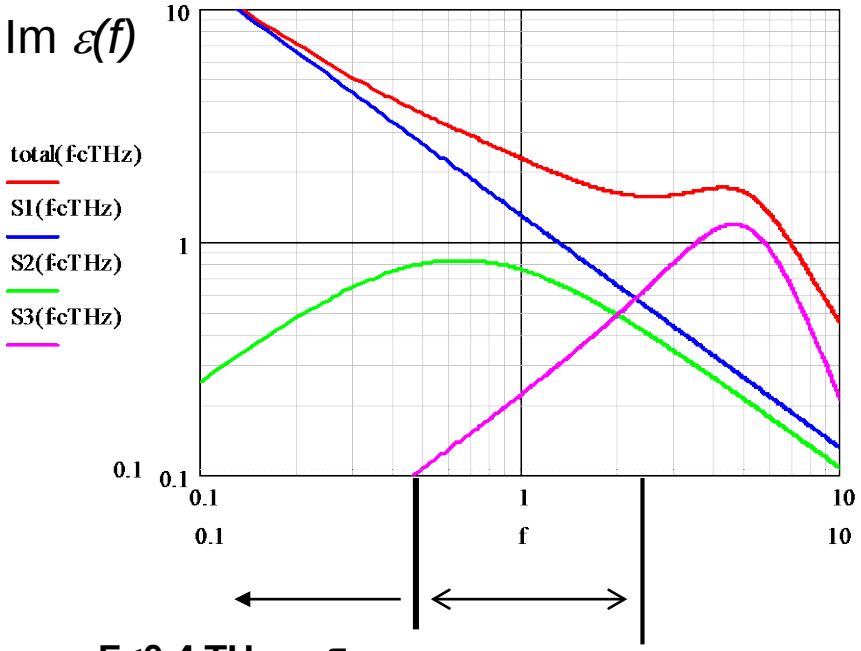
Analysis of the observed differences was performed by comparing the experimental spectra with a model of the dielectric function of water. The dielectric permittivity of water can be described by a two-component Debye model and a Lorentz term

$$\varepsilon_{water} = \varepsilon_\infty + \frac{\Delta\varepsilon_1}{1+i\omega\tau_1} + \frac{\Delta\varepsilon_2}{1+i\omega\tau_2} + \frac{A_1}{\omega_1^2 - \omega^2 + i\gamma_1\omega} + i\delta/\omega\varepsilon_0$$

Microwave ->



THz



F<0.4 THz – τ_1

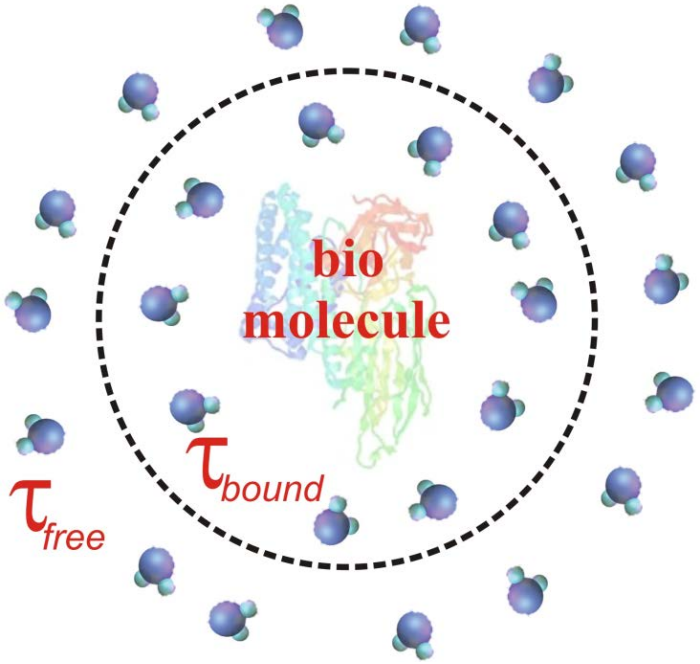
0.4-2.5 THz – τ_2

M. Nagai, H. Yada, T. Arikawa, K. Tanaka " Terahertz time-domain attenuated total reflection spectroscopy in water and biological solution" // International Journal of Infrared and Millimeter Waves, Vol. 27, 2006 No. 4 p. 505.

Analysis of the observed differences was performed by comparing the experimental spectra with a model of the dielectric function of water. The dielectric permittivity of water can be described by a two-component Debye model and a Lorentz term

$$\varepsilon_{water} = \varepsilon_\infty + \frac{\Delta\varepsilon_1}{1+i\omega\tau_1} + \frac{\Delta\varepsilon_2}{1+i\omega\tau_2} + \frac{A_1}{\omega_1^2 - \omega^2 + i\gamma_1\omega} + i\delta/\omega\varepsilon_0$$

$\Delta\varepsilon_1$ and $\Delta\varepsilon_2$ are the relaxation strengths of the two Debye relaxation modes with slow (τ_1=9.6ps) and fast (τ_2=0.25 ps) relaxation times;
ε_∞ is the dielectric constant in the high frequency limit.
A, ω_0 and γ are the amplitude, frequency and line-width of the Lorentz term, respectively.



τ_1 describes the cooperative reorganization of hydrogen-bonded water molecules, which occurs through a jump mechanism that involves the breaking and reformation of hydrogen bonds
τ_2 - dielectric relaxation of water molecules that are not involved in hydrogen bonding

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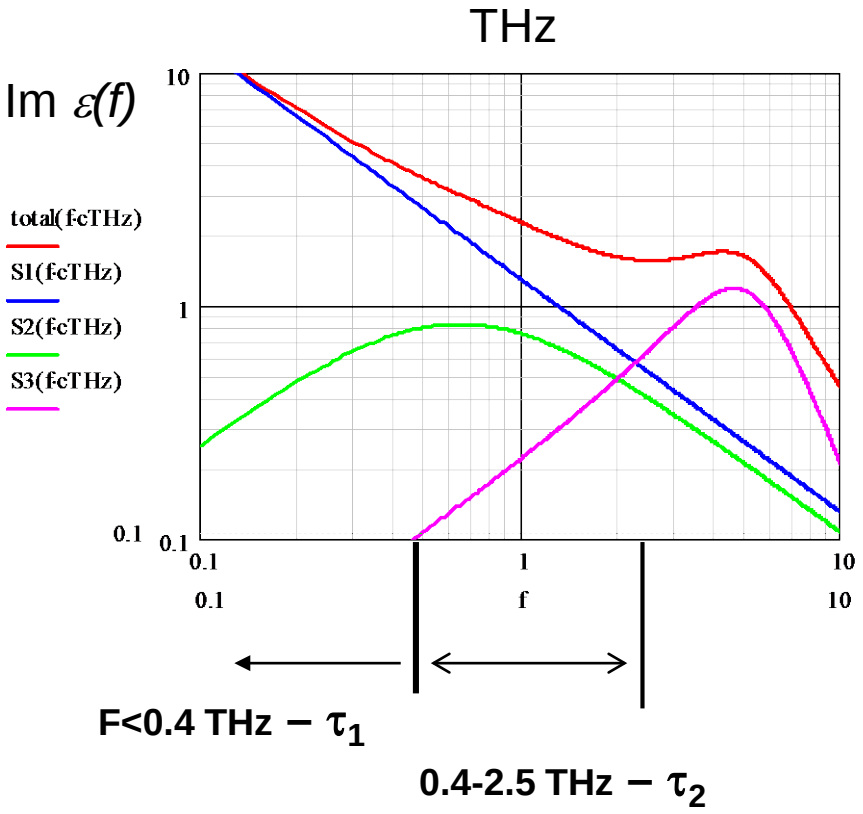
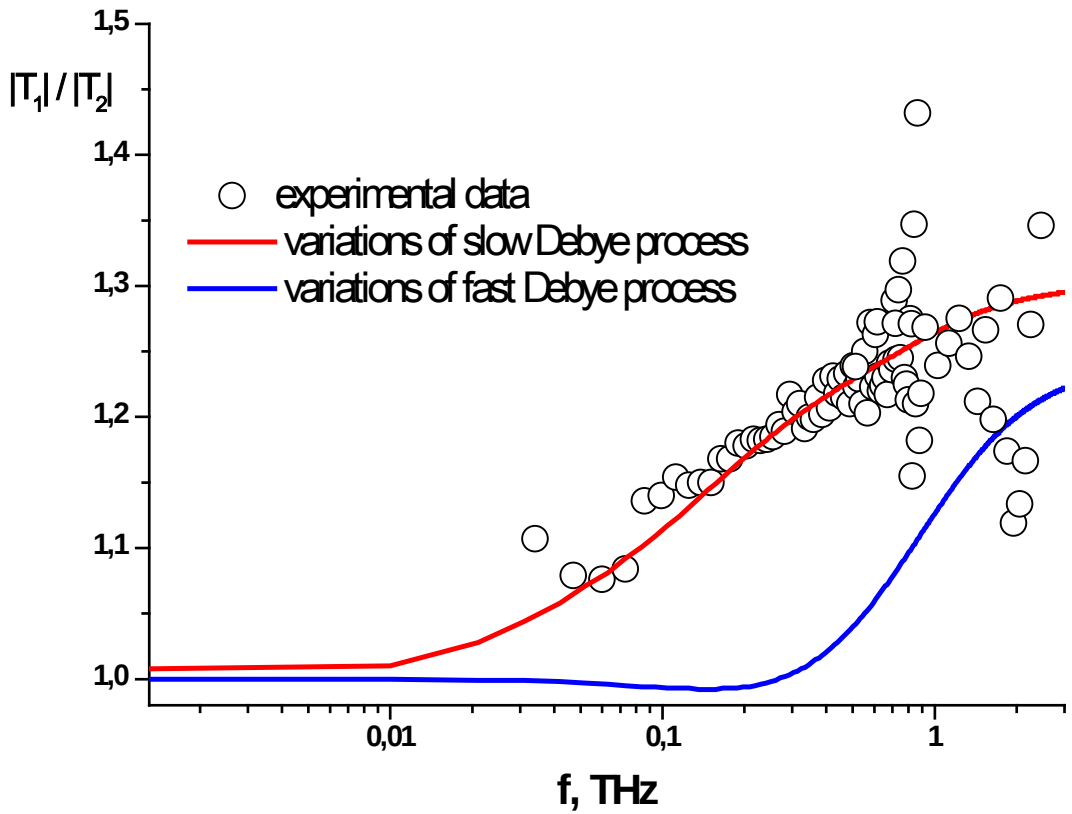
Because the first term of the Debye contains $\omega\tau_1 >> 1$, in the THz range we can accurately determine only the ratio $\Delta\varepsilon_1/\tau_1$ but not each of these values separately, that explains large variations of $\Delta\varepsilon_1$ and τ_1 values published in the literature.



Cherkasova et al. Analysis of blood plasma at terahertz frequencies, Optics and Spectroscopy 120(1):50-57 · January 2016, DOI: 10.1134/S0030400X16010069

The relative change in THz response of solution at slight variations in the model dielectric permittivity parameters , a comparison with experiment



T1 - transmission of modified sample (blood plasma), T2 – reference transmission (water); red – variations of slow Debye process (*Δε1/τ1 is decreased* 1.2 times); blue - variations of fast Debye process (*Δε2 is decreased 1.5 times)*

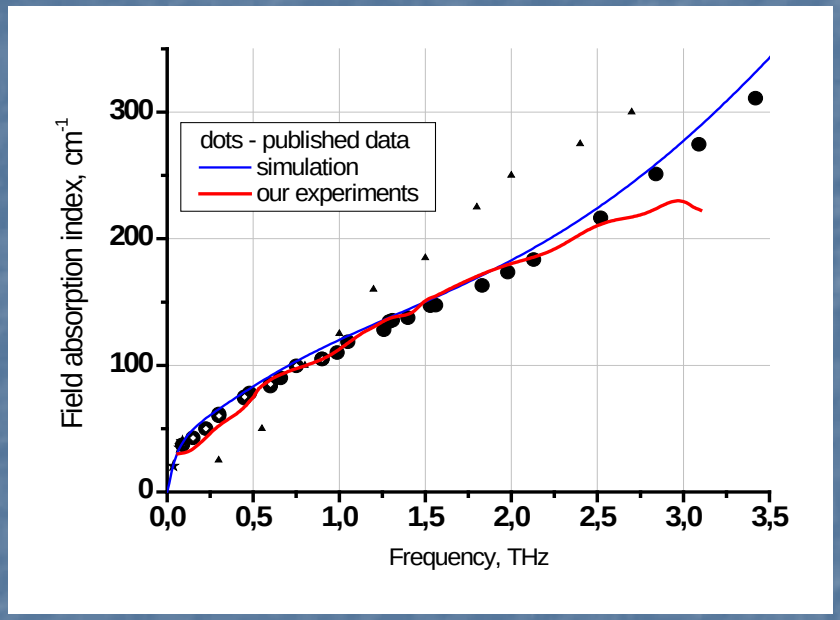
$\Delta\varepsilon_1/\tau_1=7.87\pm0.01$
water

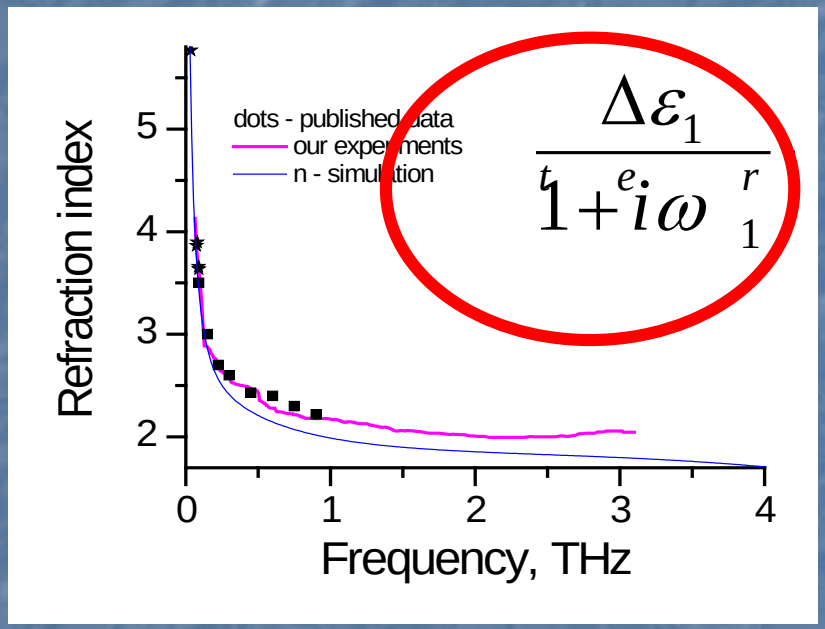
$\Delta\varepsilon_1/\tau_1=7.03\pm0.01$
diabetic samples

Changes in transmission of blood plasma diabetic rats (25 mM glucose) are determined only by variations of slow Debye process

Cherkasova et al. Application of time-domain THz spectroscopy for studying blood plasma of rats with experimental diabetes, Physics of Wave Phenomena 22(3):185-188 · July 2014, DOI: 10.3103/S1541308X14030042

Assuming that the observed spectral changes are due to changes in the state of the water, we have selected one of the parameters of the Debye model aqueous solution - $\Delta\varepsilon_1/\tau_1$, leading to the spectral features observed in the experiment

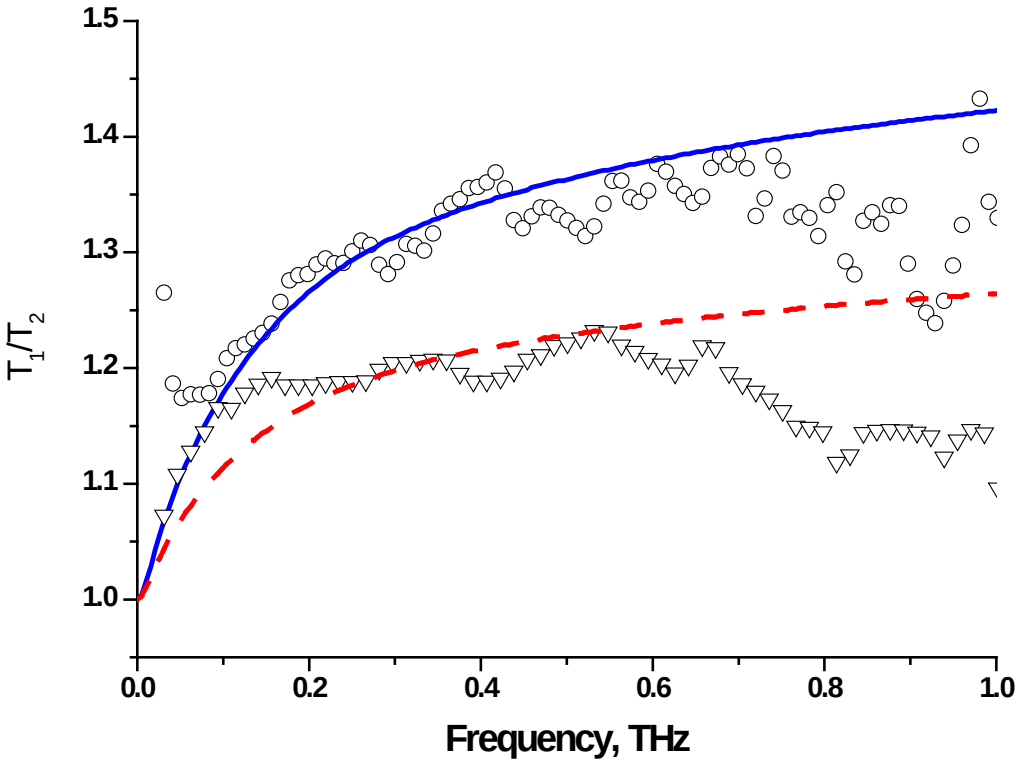




We fit our experimental spectra to model spectra of water. And in this way we obtain the optimal parameters. Model spectra were constructed using formulas from paper [1]

[1] Cherkasova, O.P., Nazarov, M. M., Angeluts, A.A., Shkurinov, A.P., “The Investigation of blood plasma in the terahertz frequency range”, Optics and Spectroscopy 120(1), 50-57(2016).

The ratio of the amplitudes of transmission spectra of solution (T_1) and buffer (T_2) at the early stage (solid line, circles) and the end (dash line, triangles) of incubation BSA with fructose. Lines are model spectra, the experimental date are points.



It was found that, for a mixture containing BSA and fructose at **the early stage of incubation**, the amplitude of the first Debye term **$\Delta\varepsilon_1$ was 88% relative to the value for pure water.** This means that 12% of the water ceased to be free and water molecules became bound with sugar or protein. **After 96 hours** of incubation BSA with fructose the amplitude of the first Debye term **$\Delta\varepsilon 1$ was 92% relative to that for pure water.**

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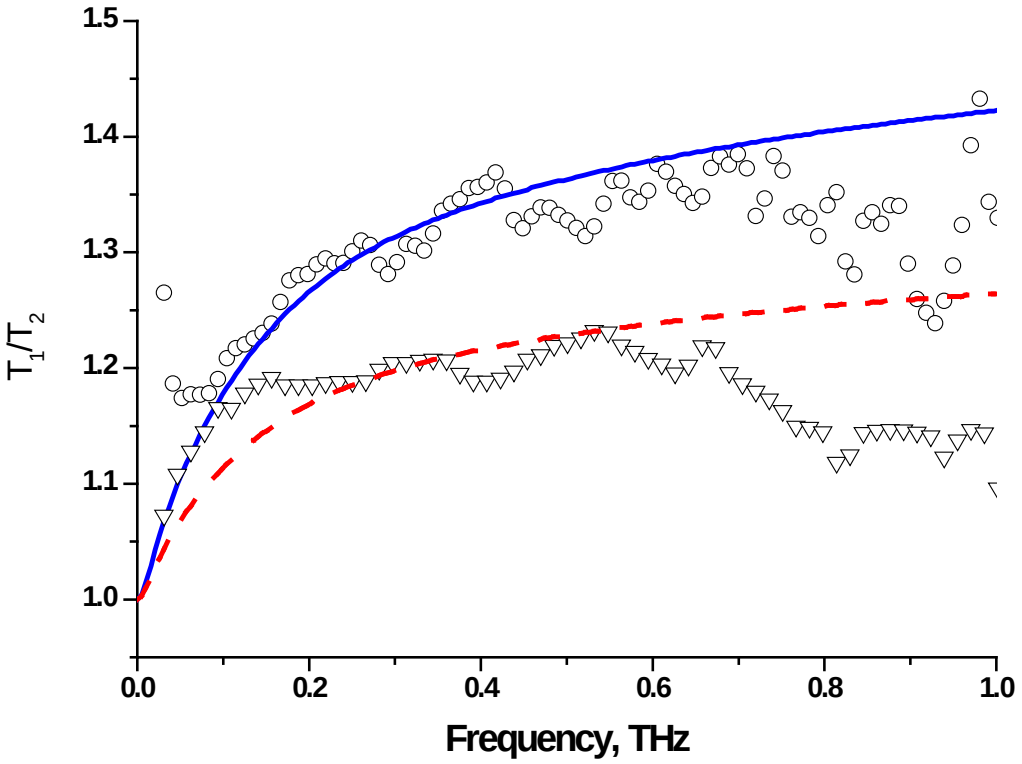

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During incubation fructose molecules form covalent bonds with protein molecule. The incubation mixture contains a significantly smaller part of sugar molecules bound with water molecules. In other words, the **amount of free water molecules is increased at the final stage of incubation.** As a result, the imaginary part of the dielectric constant is increased and this in turn leads to a reduction in the transmission of incubation mixture at 96 hour of incubation

Conclusions

- We used THz spectroscopy to study glycation dynamics of bovine serum albumin (BSA).
- THz difference between glycated and normal BSA solution decreases with incubation time. That is opposite to fluorescence signal.
- Incubating BSA with fructose causes to the largest changes in albumin THz absorption spectrum .
- All these results show that THz spectroscopy is a useful tool for monitoring the progression of glycation in time.

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Thank you for your attention