# Sub-terahertz spectroscopy as a probe for protein stability in an ionic environment

Oleksandr Sushko[1], Junyi Qiu[1,2], Rostyslav Dubrovka[1], Richard W. Pickersgill[2] and Robert S. Donnan[1]

[1]School of EECS, Queen Mary University of London, Peter Landin Building, London E1 4FZ, UK

[2]School of SBCS, Queen Mary University of London, Mile End Road, London E1 4NS, UK

***Abstract*— The sub-terahertz (sub-THz) absorption properties of bovine serum albumin (BSA) protein solutions, are investigated at different concentrations of sodium chloride (NaCl). Initially the proposed technique is validated by tracking the unfolding process of BSA in the concentrated solution of strong denaturant – GdmCl. Measurements are performed on a quasi-optical table with frequency multiplier heads covering 0.22 – 0.325 GHz. Results show a minimum THz absorption for a 100 mM concentration of NaCl, indicating the most stable protein conformation. Sub-THz spectroscopy, therefore, facilitates identification of the salt buffer concentration that enables the least dynamically active protein conformation.**

## I. INTRODUCTION

THZ and sub-THz spectroscopy have been applied to the study of solvation dynamics of various bio-molecules ranging from amino acids to complex DNA and lipid layers [1]. THz radiation senses the *collective* dynamics of molecules and hydrogen-bond networks that are directly related to the properties of bio-molecule such as conformation state, hydration shell radius and surface hydrophobicity, etc. [2].

Naturally-solvated proteins exist in aqueous environments rich in salt ions, sugars and other bio-molecules. In many THz studies a dilute salt buffer is used to stabilize the pH of a solution and protein structure [3, 4]. For instance, Bye et. al. have shown using differential scanning calorimetry (DSC), that a NaCl solution actively stabilizes the lysozyme structure above 550 mM [5]. Some studies have already addressed the THz response of solvated ions [6]. However, only limited THz studies are available on the influence and stabilizing effect of various salt buffers on protein stability in solution. THz spectroscopy of bio-solutions is still in its early stages of development and acceptance compared to the established techniques of fluorescence spectroscopy, circular dichroism, calorimetry, etc. The technology readiness level (TRL) of THz applications in medical fields (including investigation of bio-samples and solutions) scores only 1 – 4, with 9 – being a commercially viable 'product'[7]. However growing availability of THz sources supported by high performance molecular dynamics simulations is building a foundation for THz methods and analysis.

In this study, a sub-THz quasi-optical bench is first verified by monitoring the unfolding process of BSA protein under the influence of strong chaotrope GdmCl. The THz response of BSA protein solutions, at different NaCl concentrations, has been recorded over the 0.22 – 0.325 GHz waveguide band. The molarity of the NaCl buffer is set at 0, 50, 100, 150, 200 and 500 mM, while the BSA concentration in solutions is 10 and 25 mg/ml.

## II. RESULTS

Experimental results were collected using a VNA-driven quasi-optical bench. Frequency-multiplier heads coupled to corrugated horns up-convert the frequency to 0.22 – 0.325 THz. This band of frequency corresponds to a wave period of the order of 1 ps. Such a time-scale is attributed to fast relaxation processes of bulk water dynamics. The VNA signal power is of the order of 1 mW and is continuous wave (CW) radiation. Sub-THz absorption is calculated as the inverse of the transmission coefficient $S_{21}$ integrated over the whole waveguide band. Sample solutions are injected into a Bruker liquid cell (A145) fitted with quartz windows. The cell is then positioned in the common focal point of two, fast parabolic mirrors. The thickness of solutions is set at 100 μm by a PTFE washer. The set of solution concentrations are processed for measurement as follows; for refilling, the cell is removed from the beam-line, flushed with distilled water and filled with the next solution. Combined measurement error comprises kinematic uncertainty and instrumental noise and together constitutes ± 0.2% and is invariant for each reading.

BSA protein powder was purchased from Sigma Aldrich (purity > 98 %) and used as received. Initially the system was tested for sensitivity to protein denaturation. Fig. 1 shows the relative changes in absorption of BSA protein solution at different concentrations of guanidinium chloride (GdmCl). Relative absorption of pure GdmCl is also provided as a reference. GdmCl is a strong chaotrope and is commonly used as a protein denaturant [8]. The dominating mechanism of unfolding is destabilization of protein structure via interaction of charged salt particles with charged and polar sites on the protein surface. Above 1.5–2 M of GdmCl, protein solution exhibits a clear decrease in THz transmission consistent with THz-TDS study of HSA/GdmCl solution [9]. This concentration marks a transition-point where rapid protein unfolding begins. As BSA molecules unfold and lose their tertiary structure, more hydrophobic residues from the protein core get exposed to the solvent. This process is accompanied by an increase in Gibbs free energy. It is known that hydrophilic and polar molecules possess higher THz absorptivity compared to hydrophobic [10]. Therefore a protein solution attenuates THz radiation less than GdmCl salt solution (Fig. 1) as protein molecules undergo denaturing and become more hydrophobic. The critical concentration of GdmCl determined here by THz spectroscopy is consistent with other techniques such as fluorescence spectroscopy [11] and circular dichroism [8]. For instance enzymatic activity determined by colorimetric methods reveals that glucose oxidase (a dimeric enzyme) starts unfolding at 2 M concentration of GdmCl and at 4 M it is completely unfolded [8]. In the same study other experimental methods as

fluorescence spectroscopy, circular dichroism and size-exclusion chromatography confirm thes unfolding trends. This is in agreement with data presented here. The inflexion point of the curve for GdmCl solution can also elucidate specific information regarding the size of the dynamic hydration shell [12].

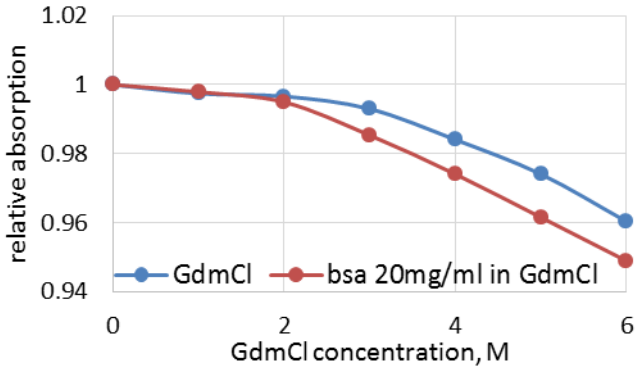


**Fig. 1.** Concentration-dependent relative absorption of pure guanidinium chloride solution (blue) and containing 20 mg/ml of BSA protein.

After verifying the above concept, lower concentration solutions of BSA and sodium chloride (a neutral salt compared to GdmCl), was studied. NaCl is often used to balance the acidity of bio-solutions. Fig. 2 shows the collected experimental results for NaCl buffer and two BSA protein solutions at 10 and 25 mg/ml concentrations. It illustrates how the absorption of the solution changes with increasing concentration of NaCl. As expected, for pure NaCl buffer, the absorption rises monotonically until 200 mM, since charged $Na^+$ and $Cl^-$ ions are more responsive to THz radiation than water molecules, and then saturates. For protein solutions, however, the trend is more complex. Both BSA solutions exhibit a well-pronounced minimum in THz response at a salt concentration of 100 mM. This feature is interpreted as stabilization of the protein structure by $Na^+$ and $Cl^-$ ions at this particular concentration. Initially, when the NaCl concentration is increased, ions start to occupy protein binding sites, thereby inhibiting its flexibility. This reduction in action is expressed as a retarded response to THz probe-radiation.

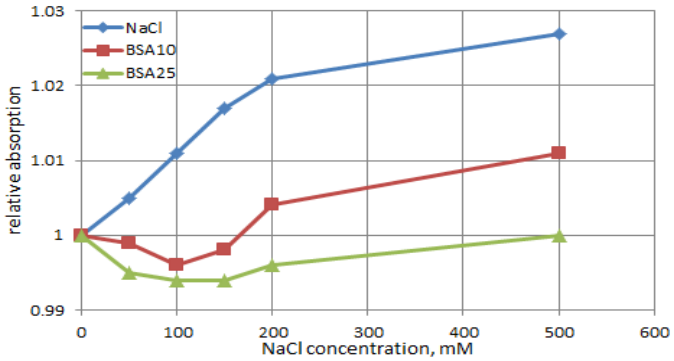


**Fig. 2.** Relative absorption of NaCl (blue), 10 mg/ml BSA solution (red) and 25 mg/ml BSA solution sampled at 0, 50, 100, 150, 200 and 500 mM of NaCl

The evolution of relative-absorption is similar from 200 to 500 mM for all three solutions considered. This implies that above 200 mM the additional ions remain mainly in the buffer and do not actively interact with protein molecules as most of the binding sites on the protein surface are already occupied. Interestingly, the investigation of thermal stability of BSA by differential scanning calorimetry (DSC) is not able to reproduce this feature at 100 mM of NaCl. DSC results show a continuous increase in melting temperature ($T_m$) of BSA up to 500 mM of NaCl, indicating that thermal stability also rises. Our DSC experiments agree with the findings obtained by the same technique for the glucose oxidase enzyme [8]. The $T_m$ of this enzyme constantly grows up 2 M of NaCl while starting to plateau after 0.5 M. Such differences between THz and DSC results might be triggered by distinct physical quantities sensed (DSC measures changes in heat capacity, while THz radiation probes collective long-range dipole reorientation dynamics), as well as much lower protein concentrations used in DSC.

## III. Summary

This study provides additional evidence that sub-THz spectroscopy is sensitive to molecular conformation changes, illustrated by the unfolding of BSA protein in GdmCl solution. The results also show that sub-THz spectroscopy can be meaningfully applied to identify the buffer concentration that ensures the most stable protein conformation. This is crucial for enabling a prolonged storage of protein solutions; and provides insights into protein-salt interaction. Future work will consider investigation of a wider range of proteins and salt concentrations; and atomistic molecular dynamics simulations of solvated BSA protein in particular to address the differences between THz and DSC results.

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