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THz spectroscopy to probe long-range electrodynamic interactions between proteins

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Abstract— We have recently experimentally demonstrated, for the first time, the activation of long-range attractive electrodynamic forces between proteins by means of THz spectroscopies, supported by fluorescence cross correlation spectroscopy and numerical simulations. Our experimental work provides a proof of principle of out-of-equilibrium collective oscillations activation of dipole-dipole electrodynamic intermolecular forces. It paves the way for exploring the potential role of electrodynamic intermolecular forces in living matter.

I. INTRODUCTION

IN living matter, a complex network of molecular cascade events involving hundreds of different molecules, which have to diffuse, meet and interact, is responsible for the transmission of information through cells [1]. To execute their specific functions, these molecules have to be at the right place, at the right moment and in the right cascade of events of any biological action. However, biomolecular interactions of electrostatic nature, limited by Debye screening into the highly crowded cell environment, do not expand above 10 Å. Fundamentally, it is assumed that this network of interactions is dominated by random molecular diffusion, but discrepancy between the observed reaction rates in cells and the predictions of a strict random diffusion model have been reported [2, 3]. Dipole-dipole electrodynamic (ED) interactions might play a role promoting molecular attraction by being selective through resonance and acting over long distances [4]. These forces can propagate without attenuation in electrolytes of ionic strength comparable to that in the cytoplasm, provided that their oscillation frequency exceeds the Maxwell frequency at few hundreds of megahertz [5]. At such frequencies, the Debye screening is ineffective [6]. We hypothesize that, in addition to random diffusion, selective long-range attractive ED forces can bring biomolecules together to promote short-range interactions.

II. METHOD & EXPERIMENTAL RESULTS

Activation of long-range ED interactions between proteins in aqueous solution can be observed when the system is driven in a stationary out-of-equilibrium state, leading to a semi-classical phonon condensation phenomenon [4,7]. The resulting collective oscillation modes are expected in the THz domain.

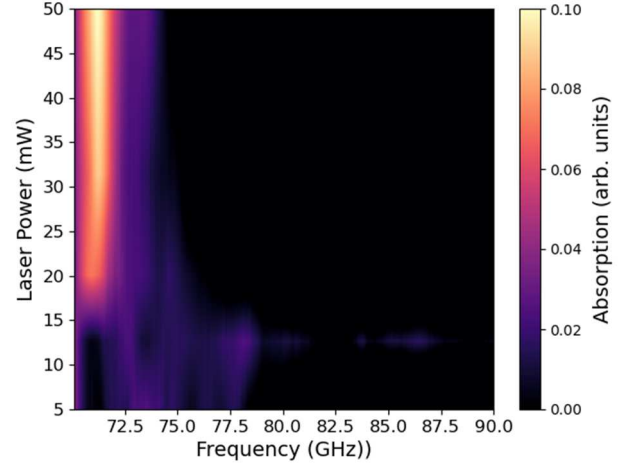


Fig. 1. R-PE collective oscillation mode assessed THz-Spectroscopy. Absorption strength of R-PE (1 mg/mL in 200 mM NaCl) as a function of detection frequency and the laser power activating the molecular collective oscillation at a frequency of 71.2 GHz (2.4 cm^{-1}). Measurements were performed with laser power of 5mW, 12.5mW, 20mW, 31.5mW and 50mW. The 488nm blue laser has been attenuated by using optical density filters.

In this work, the protein system was put in an out-of-equilibrium state by means of optical pumping. Thus, the investigated proteins have to be excitable by a light source.

To experimentally demonstrate long-range ED attractive forces between proteins, the following conditions have to be fulfilled:

(i) measurement of the activation of collective oscillation of the proteins;

(ii) measurement of a shift of the collective oscillation frequency with protein concentration, according to a " $1/\langle r \rangle^3$ " rule, where $\langle r \rangle$ is the average intermolecular distance calculated at a given molecular concentration [4];

(iii) observation of a distributed-clustering transition dependent on activation of these collective molecular oscillations.

While (iii) requires to perform Fluorescence Cross Correlation Spectroscopy (FCCS), conditions (i) and (ii) are assessed by near-field THz spectroscopy, using two different techniques.

R-phycoerythrin (R-PE), a protein naturally excitable by light, was chosen, as textbook case. It contains about 38 fluorochromes, highly sensitive to the $\lambda=488 \text{ nm}$ laser-light

used in these experiments to provide energy to the proteins. R-PE was studied in the 70 - 110 GHz

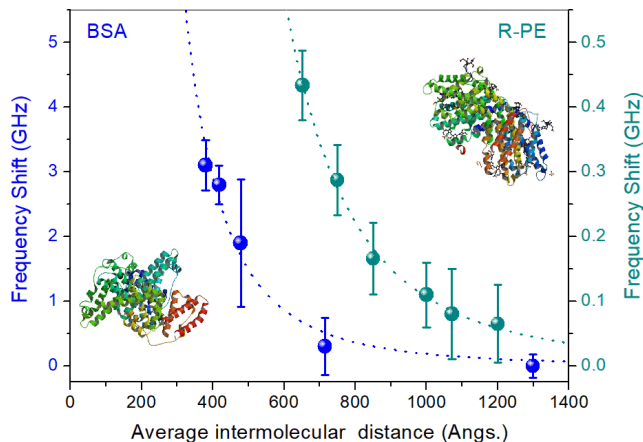


Fig. 2. Frequency shifts of the intramolecular collective vibrations of R-PE and BSA, in aqueous solution, at different concentrations [8]. Measurements were performed using THz spectroscopies. Optical pumping was achieved by using a blue-laser. The frequency shift is relative to the reference frequency measured at the lowest protein concentration. The dashed lines correspond to the fit of the experimental data with a function proportional to $1/\langle r \rangle^3$, where $\langle r \rangle$ stands for the average intermolecular and $\langle r \rangle = C^{-1/3}$, C being the protein concentration. The collective oscillation mode for R-PE lies around 71 GHz, and around 313 GHz for BSA.

Insets: Tertiary structures of the proteins.

frequency range, by using a rectenna-based THz spectroscopy setup. The signal detection is performed by a high-electron-mobility transistor connected to a bow-tie antenna (forming the rectenna), protected by a hydrophobic but biocompatible varnish, transparent to THz radiations. The protein solution is deposited on top of the rectenna. Bovine Serum Albumin (BSA) was also investigated. However, since BSA is not naturally sensitive to light, it had to be conjugated with fluorochromes (Alexa Fluor 488, 5 to 8 dyes per protein). BSA was studied in the 250 – 325 GHz frequency domain by using a microwire-based probe. In this setup, a microwire is inserted inside a metallic rectangular waveguide to allow a modal transition from TM₀₁ Sommerfeld's mode to TE₀₁ waveguide mode. This probe is capable of highly localized detection of the longitudinal component of the electric field due to the sub-wave diameter of the wire. The probe is mounted on an external mixer of a 40 GHz spectrum analyzer. The wire is immersed in the protein solution. For both R-PE and BSA, a saline buffer solution was used and about 40 μ L protein solution was needed per experiment. In the case of BSA, additional lightening was performed by using a UV lamp.

By illuminating the R-PE molecules in saline solution each molecule enters a coherent vibrational state (Fig. 1), displaying similar phenomena to those reported for BSA protein in [7]: the existence of a threshold for the energy input rate to activate the collective mode, and a saturation of the oscillation amplitude at high values of the energy input rate. Condition (i) is fulfilled.

For both BSA and RPE, we have performed THz spectroscopy measurements for various protein concentrations and observed a shift of the frequency of the collective oscillation with concentration. As showed in Fig.2, this

frequency shift evolves as a function of $1/\langle r \rangle^3$. Condition (ii) is also fulfilled.

Additionally, FCCS measurements were carried out under similar experimental conditions than THz spectroscopies. They validated the attractive nature of the observed interactions [8], fulfilling condition (iii).

III. CONCLUSION

By combining theoretical studies, THz spectroscopies and FCCS, we provide the first experimental demonstration of the existence of long-range attractive forces between proteins [8]. We anticipate that our results will pave the way for the experimental searching of ED forces in the more complex environment encountered in cell biology.

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