Electronic Supplementary Information to:

Solvent Dynamics Plays a Decisive Role in the Molecular Recognition of Biologically Relevant Redox Proteins

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Protein Expression and Purification

Escherichia coli codon-optimized genes of *fnr*, *petF and fdx3* (N-terminal Strep-TagII) from *Chlamydomonas reinhardtii* were each cloned into pASK-IBA7plus vector (IBA Lifesciences) and heterologously expressed in *E. coli* BL21(DE3)ΔiscR as described previously.[1] In this, electrocompetent *E. coli* BL21(DE3)-ΔiscR cells were transformed using the respective expression plasmid pASK-IBA7, carrying the *E. coli* codon-optimized gene of either *fnr*, *petf or fdx3*. Transformant strains were pre-cultivated in LB-medium overnight at 37 °C before the expression culture (8×500 ml) was inoculated to an optical density at 600 nm (OD_{600}) of 0.01. For FNR, the expression culture was then grown in LB-Medium under constant shaking (180 rpm) at 37 °C up to an OD_{600} of 0.6. For ferredoxin expression, Vogel-Bonner (VB) medium was used instead and the expression cultures were grown until an OD_{550} of 0.6.[2] For both, FNR or ferredoxins, protein expression was induced by the addition of anhydrotetracycline (AHT, 0.2 µg/ml) and cultivation was carried out over night for 16 hours at 20 °C. The cells were harvested by centrifugation at 4 °C (15 min, 9000 g) and cell pellets were resuspended in Tris-buffer (pH 8). *E. coli* cell disruption was achieved by addition of lysozyme (in 1% triton X-100 and 10 mg lysozyme per liter of *E. coli* culture) and subsequent sonification. After ultracentrifugation at 4 °C (165.000 g), the recombinant strep-tagged proteins were affinity

purified with Strep-Tactin Superflow high-capacity cartridges (IBA Lifesciences) according to manufacturer's instructions and concentrated to 3-4 mM in 0.1 M Tris/HCl-buffer, pH 8.0 using Amicon ultra centrifugal filters (MERCK, Germany). SDS-PAGE was used to monitor the purity of the protein isolates. For ferredoxins, protein concentration was determined spectrophotometrically via UV/VIS spectroscopy at 420 nm using the Beer-Lambert Law and a molar extinction coefficient of 9.7 $mM^{-1} \cdot cm^{-1}$.[3] For FNR, the protein concentration was determined via Bradford assay (Bio-Rad) using bovine serum albumin as a standard (Biolabs).[4] Also, the FNR concentration was determined according to the specific absorption maximum of its cofactor Flavin adenine dinucleotide (FAD), with a molar extinction coefficient of 9,4 $mM^{-1} \cdot cm^{-1}$ at 457 nm in order to make sure that the protein was fully occupied with its cofactor.[5] All proteins were stored at −80 °C until further use.

Determination of Relaxation Constants

The complex dielectric response of the protein solutions was obtained from the simultaneously measured absorption and refractive index, as shown in Equations 1a and 1b, where ω is the angular frequency.

$$\varepsilon'(\omega) = n^2(\omega) - k^2(\omega) \tag{1a}$$

$$\varepsilon''(\omega) = 2n(\omega)k(\omega) \tag{1b}$$

The complex dielectric response can be described by a double Debye function, shown in Equation

$$\varepsilon(v) = \varepsilon'(v) - i\varepsilon''(v) = \varepsilon_\infty + \frac{\varepsilon_s - \varepsilon_1}{1 + i\omega\tau_1} ++ \frac{\varepsilon_1 - \varepsilon_\infty}{1 + i\omega\tau_2} \tag{2}$$

2, where ε_s is the static permittivity, ε_1 is the dielectric constant of the first relaxation process , ε_∞

is the extrapolated value at high frequencies, and τ_1 and τ_2 are the slow and fast relaxation times. Here, ε_s was held constant at the literature value of pure water,[7] and ε_1, ε_∞, τ_1, and τ_2 were allowed to vary.

ESI References

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SI Figures

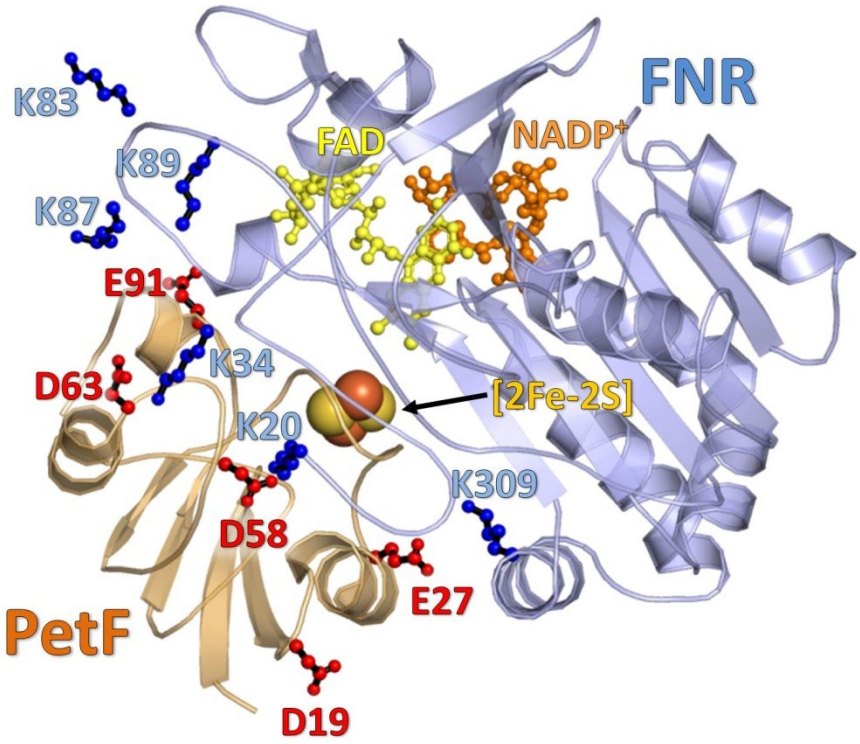


Figure S1. Structure of the transient FNR-$NADP^+$-PetF complex from *Chlamydomonas reinhardtii* as a cartoon and stick model (complex orientation based on PDB-ID: 1GAQ). PetF is shown in orange (PDB-ID: 2MH7); the structural model of FNR is based on PDB-ID 1EWY. Relevant basic (blue) and acidic (red) amino acids that are putatively involed in the complex formation via salt bridging, as well the redox-centers, are displayed as sticks and spheres.

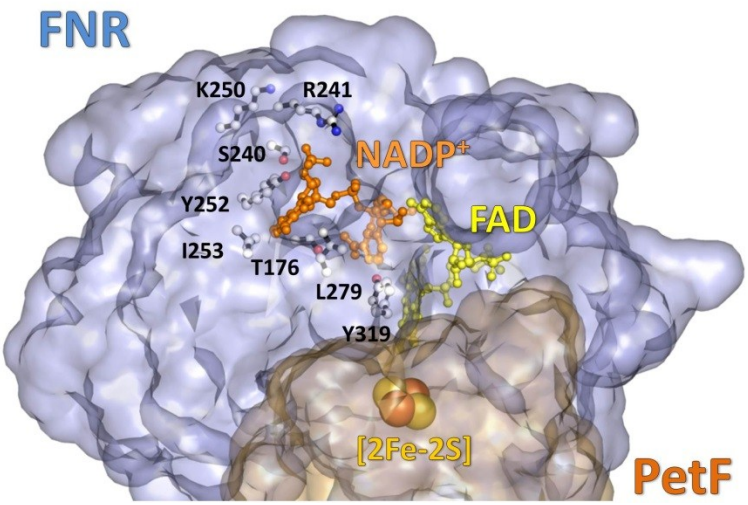


Figure S2. Structure of the transient FNR-$NADP^+$-PetF complex like in figure S1. This surface representation demonstrates that the substrate of FNR, i.e. $NADP^+$ (orange), is largely buried in the interior of the protein and close to the FAD cofactor (yellow), thus being solvent inaccessable. Relevant amino acids (ball-and-stick model) for stabilizing $NADP^+$ in its binding pocket were reported to only undergo slight conformational changes upon binding. Color code for these amino acids: gray: carbon, blue: nitrogen, red: oxygen.

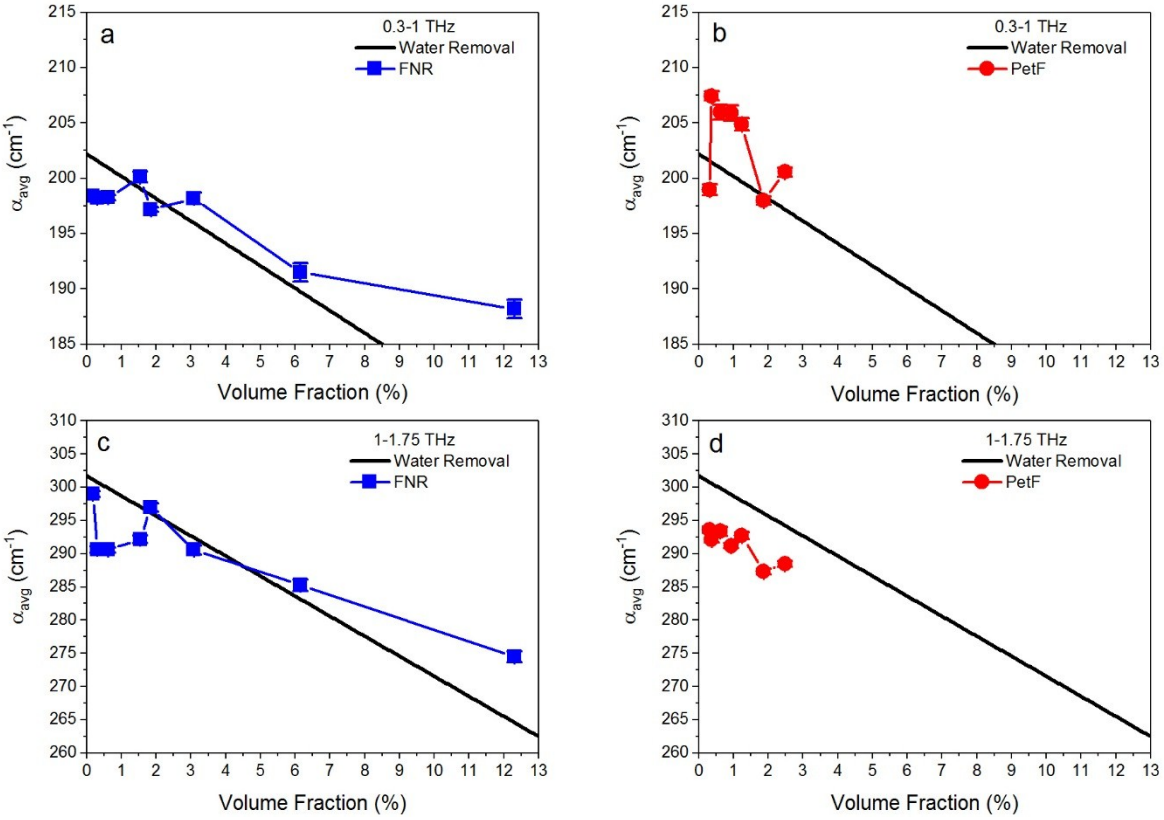


Figure S3. THz-TDS absorption spectra of aqueous solutions at pH 8 of (a) FNR and (b) PetF and the average absorption coefficient of FNR and PetF solutions in the (a,b) 0.3 -1 THz range and (c,d) 1 – 1.75 THz range.

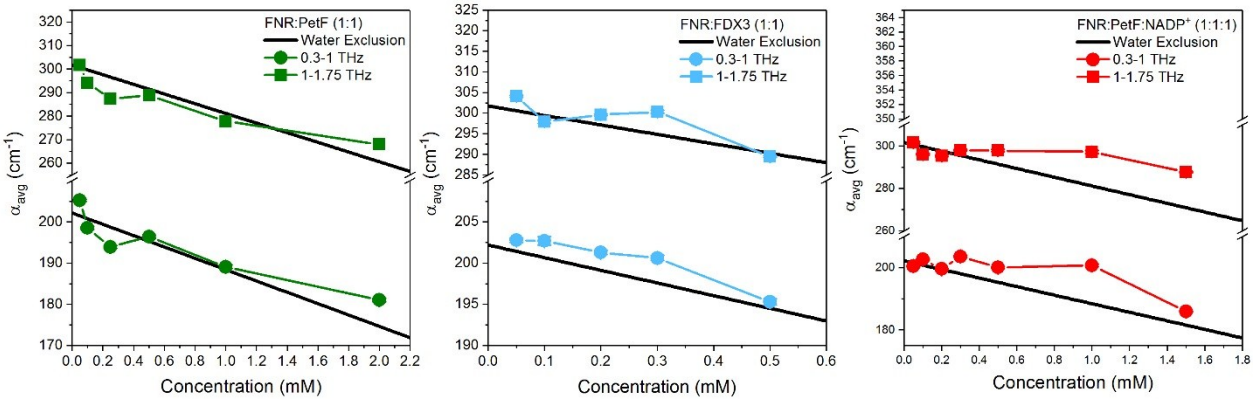
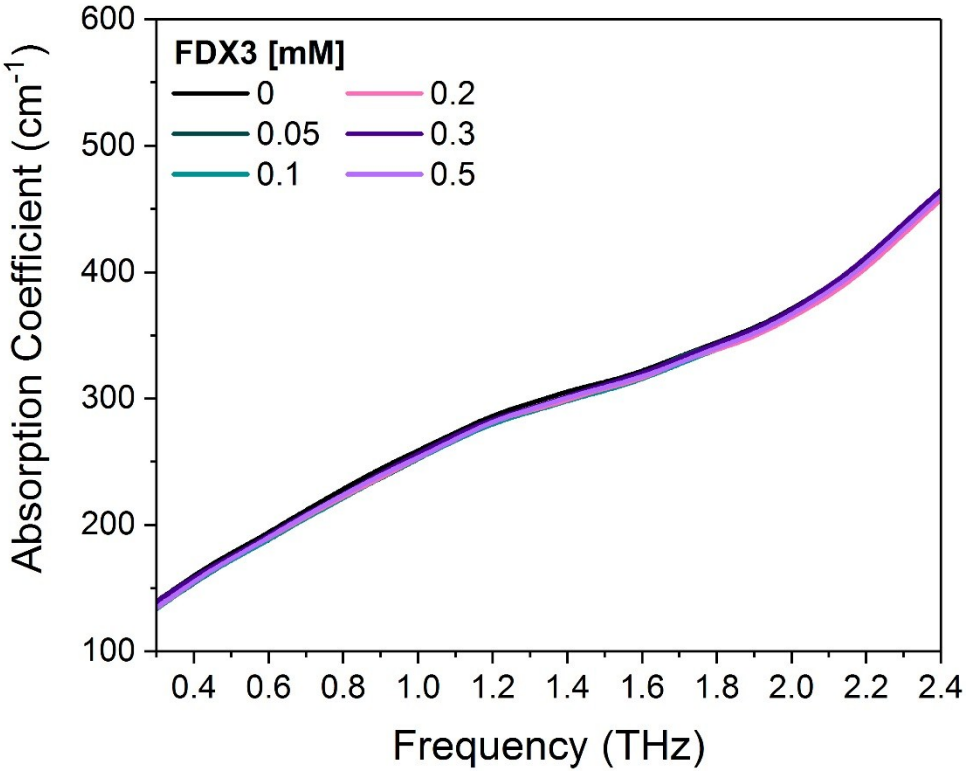


Figure S4. The average absorption coefficients of solutions of FNR:PetF, FNR:FDX3, and FNR:PetF:NADP$^+$ for frequency ranges 0.3-1 THz and 1-1.75 THz.

Figure S5. THz-TDS absorption spectra of aqueous solutions of FDX3 at pH 8.



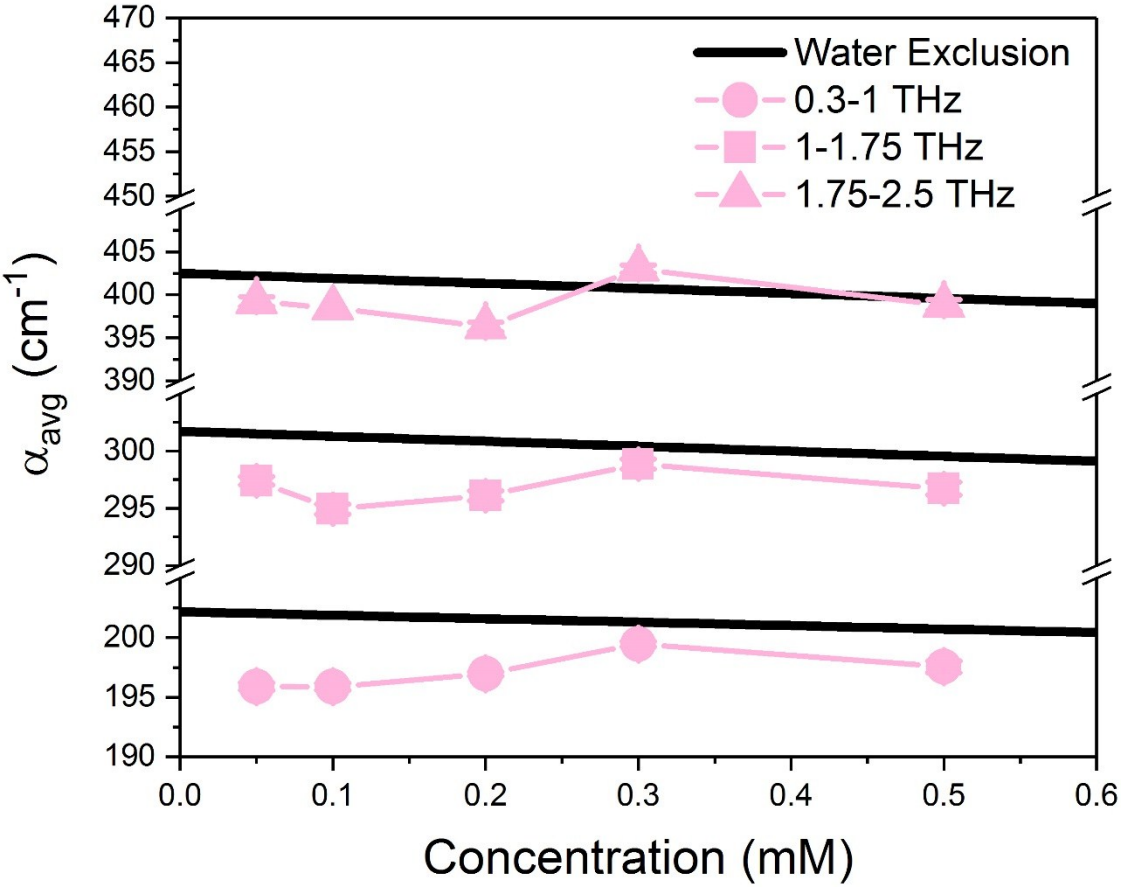


Figure S6. Average absorption coefficient in various frequency regimes for solutions of FDX3. The black line in each regime shows the expected decrease in absorption based on removal of water equal to the volume of the protein in solution.

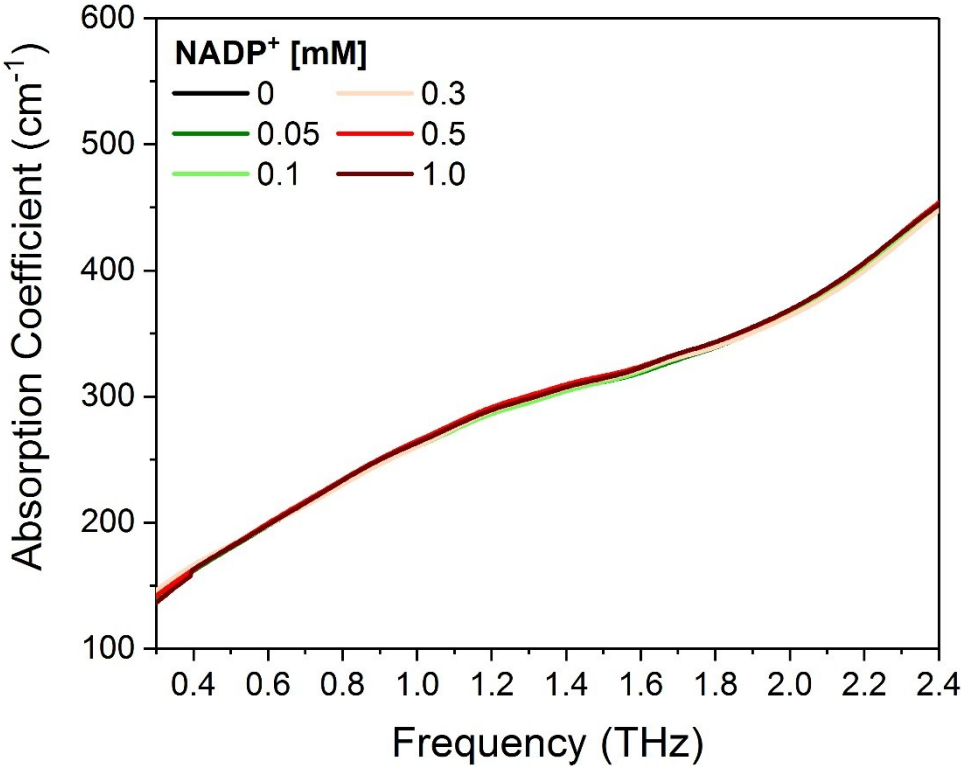


Figure S7. THz-TDS absorption spectra of aqueous solutions of $NADP^+$ at pH 8.

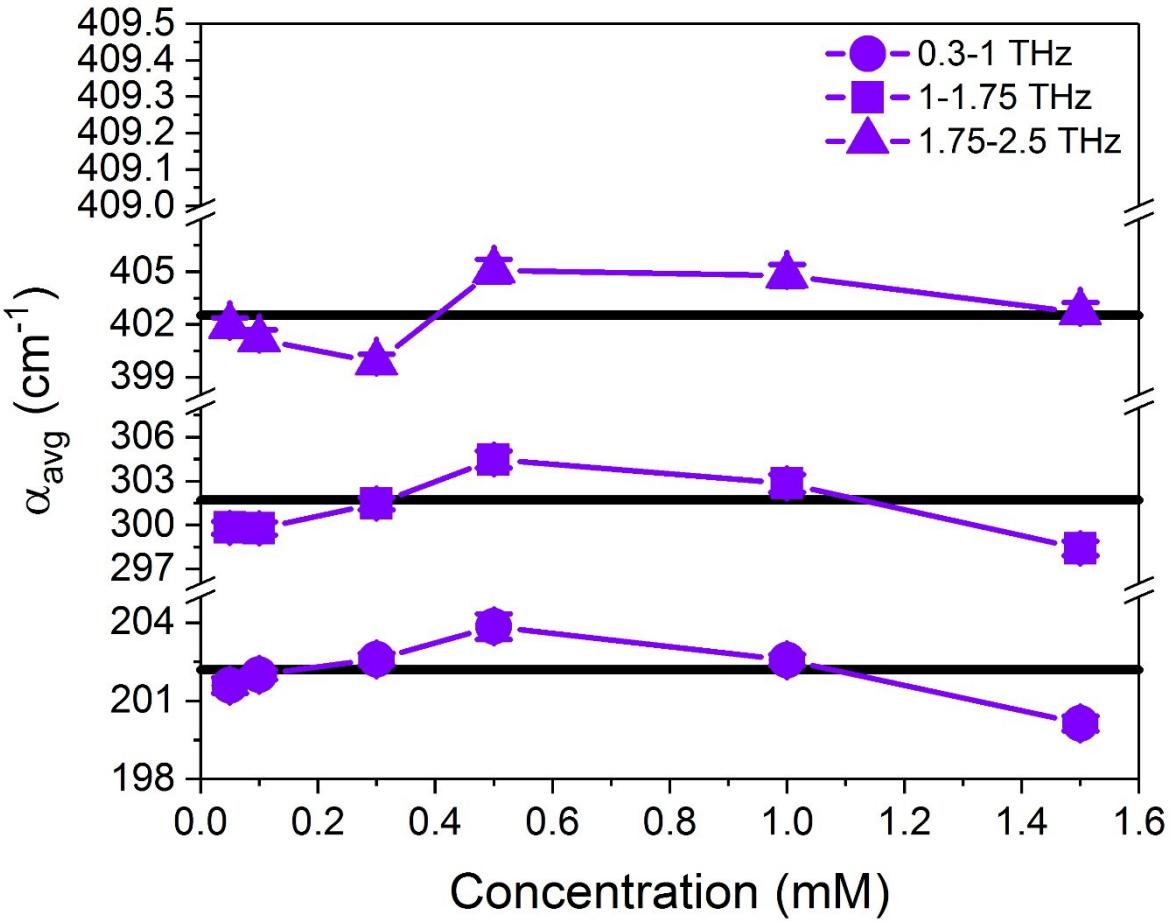


Figure S8. Average absorption coefficient in various frequency regimes for solutions of $NADP^+$. The black line in each regime shows the average absorption coefficient of the buffer solution in the same frequency regime.

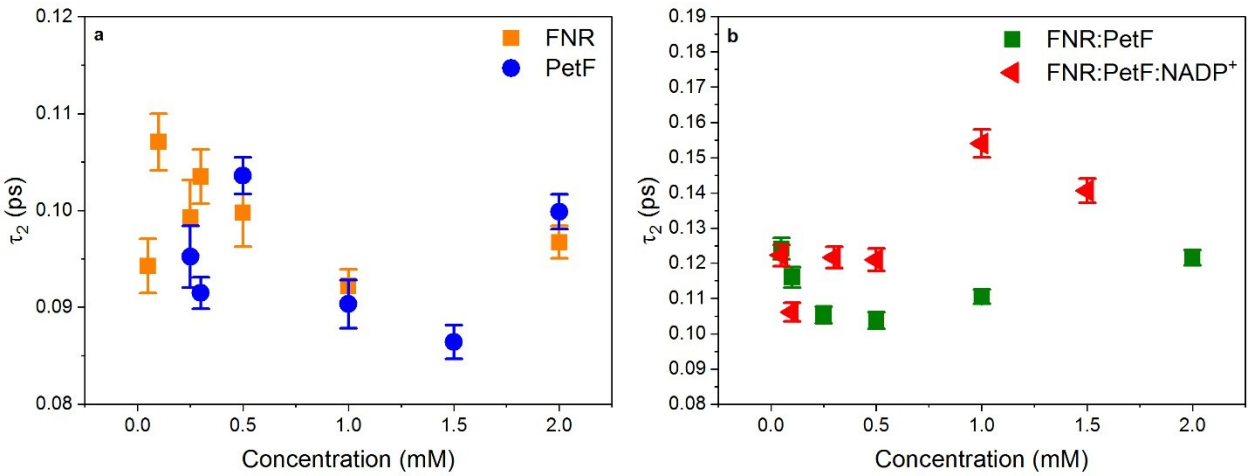


Figure S9. Relaxation time constants τ_2 of aqueous solutions of (a) FNR and PetF (b) FNR:PetF and FNR:PetF:NADP$^+$.