Towards the Direct Measurement of Ultrafast Vibrational Energy Transfer in Proteins

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Abstract: Vibrational energy transfer (VET) within a molecule can be investigated in great detail with ultrafast IR spectroscopy. We report on progress towards mapping of VET pathways in proteins using unnatural amino acids as site-specific probes.
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A long-standing question in biophysics is the existence of networks of coupled side chains in protein domains which are believed to function as pathways for energy flow and allosteric communication [1]. The measurement of vibrational energy flow between arbitrary sites in a protein is up to today not possible in experiments. Using UAAs as site-specific vibrational probes might allow for the direct tracking of vibrational energy transfer between sites of interest and thus for the direct mapping of energy transfer pathways in proteins.

Vibrational energy transfer (VET) is a widely studied phenomenon in ultrafast spectroscopy. By 2D-IR spectroscopy or pump-probe spectroscopy it is possible to follow the energy flow between functional groups, e.g. in small molecules [2] or peptides [3]. The experimental data, especially the transfer times, provide important information about the system under investigation. They, for example, reflect the spatial proximity of functional groups [4]. The gained information can be of great importance for different applications, given that signals can be assigned to a certain functional group in the studied systems, which might be difficult in case of spectral congestion and especially for larger molecules such as proteins. A recent approach to overcome the limitation by spectral congestion in vibrational spectra of proteins is the use of unnatural amino acids (UAAs) with functional groups, which absorb well separated from protein vibrations, as site-specific vibrational probes [5]. They can be incorporated during protein expression and allow for labeling side chains at positions of interest [6].

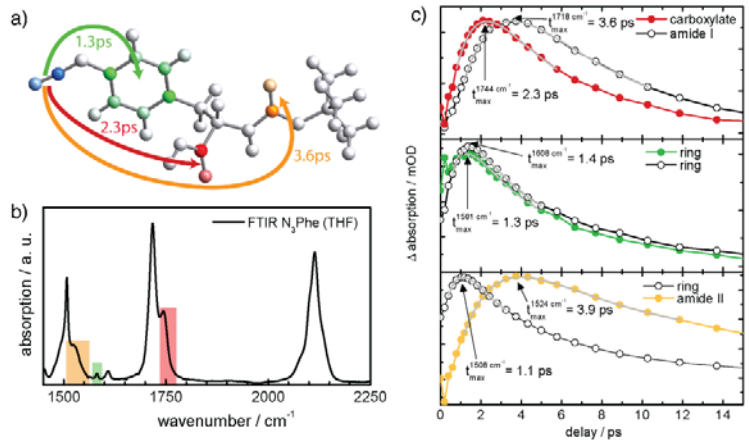

Fig. 1. Time dependence of cross-peaks in 2D-IR spectra originating from vibrational energy transfer allows unambiguous assignment of FTIR spectra. a) structure of the unnatural amino acid azidophenylalanine (N_3Phe) with VET indicated by arrows, the intensity in the colour coding reflects the kinetic energy per atom for the shown vibrations as derived by DFT calculations, b) FTIR absorption spectra of N_3Phe in tetrahydrofuran (THF), c) transients for vibrational modes in amide I region (upper panel), ring mode region (middle panel), amide II region (lower panel). Figures adapted from [2].

To test the usability of azide-containing UAAs in proteins we studied azidophenylalanine (N_3Phe) by two-colour 2D-IR spectroscopy. Even for this seemingly small system the assignment of the absorption bands (Figure 1 b) to functional groups of the molecule (Figure 1 a) is not straight forward. DFT calculations are regularly used to assist with assignment but are contradictory for this molecule. Only by using the additional information from the time-dependent VET induced cross peaks (transients shown in Figure 1 c) an unambiguous assignment of all

absorption bands functional groups is possible (shown in Figure 1 a, atoms involved in the vibrations are color coded to emphasize functional groups; color intensities are proportional to the kinetic energy of the atom, as derived by DFT calculations) [2]. The results demonstrate the concept of mapping energy flow in a molecule by the tracking of vibrational energy transfer using localized oscillators.

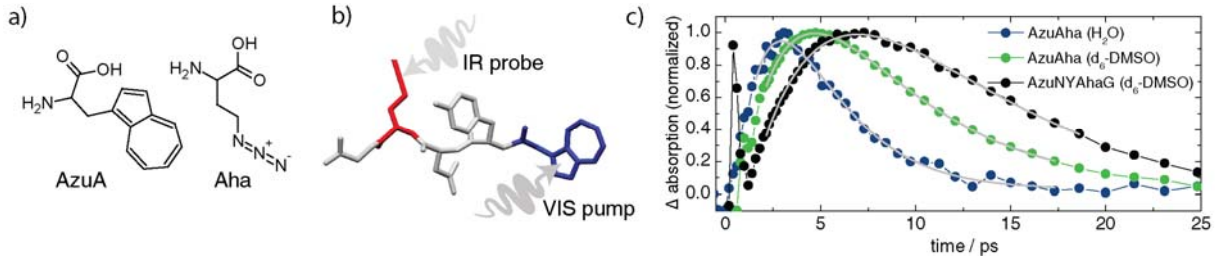

Fig. 2: A donor-acceptor pair of unnatural amino acids (UAAs) is used to track VET in model peptides. a) Structures of the UAAs azulenylalanine (AzuA), used as donor and azidohomoalanine (Aha), used as acceptor. b) Sketch of the performed experiment in the model peptide AzuNYAhaG. c) Transients for the azide stretch vibration in different systems and different solvents (see legend).

For the application of such experiments in proteins to investigate the above mentioned energy transfer pathways further requirements have to be met. To track VET in more complex systems explicit donor- and acceptor moieties need to be introduced, both absorbing separated from the native occurring modes. Additionally they need to be optically orthogonal and have decent oscillator strength.

We report here on a donor-acceptor pair specially designed to match those requirements [7]. Azulene has been successfully used before in studies of IVR or VET in smaller systems [8]. It's a famous example of the violation of Kasha's rule, meaning that upon visible excitation at 600 nm the molecule undergoes internal conversion instead of fluorescence, thereby dumping the energy in the vibrational modes of the electronic ground state within ~1 ps. This is the ideal chromophore to serve as a donor for tracking VET in proteins, because the visible excitation allows for 10 times higher energy deposition than IR excitation. Azulenylalanine (AzuA) is the corresponding UAA we have tested. As an acceptor we use the azide-containing UAA azidohomoalanine, which is compatible with AzuA in terms of independent incorporation into proteins.

Figure 2 a shows the structures of the VET donor and acceptor. We show results on experiments in model peptides: A pentamer containing additional amino acids (Tyr, Asn, Gly) was tested to demonstrate the distance-dependency and feasibility of the proposed approach for systems with the size matching the diameter of small proteins (2 nm, Figure 2 b). A dimer of the two UAAs was tested under different conditions to prove the usability in aqueous solution, an important prerequisite for applications in proteins. The transients for those systems are shown in Figure 2 c. The transfer time is not only correlated to the distance between the donor and acceptor (comparison between AzuAha and AzNYAhaG in d_6-DMSO) but also affected by the solvent with a much faster peak time observed in H_2O than in d_6-DMSO. Thus the proposed VET pair might not only be useful for the anticipated study of VET pathways in proteins, but is as well a versatile tool to study the principles of vibrational energy transfer in greater detail.

References

[1] David M. Leitner and John E. Straub, *Proteins: Energy, Heat and Signal Flow (Computations in Chemistry.(* CRC Press, 2009)
[2] H. M. Müller-Werkmeister, Y.-L. Li, E.-B. W. Lerch, D. Bigourd, J. Bredenbeck, "Ultrafast Hopping from band to band: Assigning Infrared spectra based on Vibrational Energy Transfer" Angew Chem. Int. Ed. **52,** 6214-6217 (2013).
[3] V. Botan, E. H. G. Backus, R. Pfister, A. Moretto, M. Crisma, C. Toniolo, P. H. Nguyen, G. Stock, P. Hamm, "Energy transport in peptide helices", Proc. Natl. Acad. Sci. U. S. A. **104**, 12749–12754 (2007).
[4] N. I. Rubtsova, I. V. Rubtsov, "Ballistic energy transport via perfluoroalkane linkers", Chem. Phys. **422**, 16–21 (2013).
[5] H. Kim, M. Cho, "Infrared Probes for Studying the Structure and Dynamics of Biomolecules", Chem. Rev. **113**, 5817-5847 (2013).
[6] M. G.Hoesl, N. Budisa,"In vivo incorporation of multiple noncanonical amino acids into proteins", Angew. Chem. Int. Ed., **50** 2896–2902 (2011).
[7] H. M. Müller-Werkmeister, J. Bredenbeck, "A donor-acceptor pair for the real-time study of vibrational energy transfer in proteins", Phys. Chem. Chem. Phys. **16**, 3261-3266 (2014).
[8] D. Schwarzer, C. Hanisch, P. Kutne, J. Troe, „Vibrational Energy Transfer in Highly Excited Bridged Azulene-Aryl Compounds: Direct Observation of Energy Flow through Aliphatic Chains and into the Solvent", J. Phys. Chem. A **106,** 8019–8028 (2002).