Snapshots of sub-picosecond dynamics in heme-proteins captured by Femtosecond Stimulated Raman Scattering

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Abstract: The reaction pathway in photoexcited hemeproteins (ligand dissociation, energy redistribution and structural dynamics) has been unraveled by Femtosecond Stimulated Raman Scattering. The possible existence of short living intermediates as opposed to vibrational relaxation is discussed.
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1. Background and significance

Photoinduced dynamics in heme proteins embed several concurring processes: bond breaking events, excited state dynamics, vibrational energy redistribution (within the protein and to the solvent) and conformational changes. The way such different aspects syncretize on a coherent picture of the reaction pathway is a matter of open debate. Transient absorption studies provide valuable information, subject to contrasting interpretations. Specifically, the existence of short living intermediates on the way to the ground state has been hypothesized, as opposed to the direct decay from the reactive energy surface down to a cold ground state. Vibrational spectroscopy, on the other hand, is endowed with structural sensitivity, but suffers from resolution limitations. Time Resolved Resonance Raman (TR3), in particular, is a powerful technique to study protein dynamics, whose time resolution has been improved over the years from microseconds to a few picoseconds. If a sharp spectral resolution (<15cm-1) is to be maintained, however, no further improvement of the time resolution (<1ps) is obtainable due to the Fourier Transform limit.

2. Methods

Femtosecond Stimulated Raman Spectroscopy (FSRS) is a powerful method for studying ultrafast reaction dynamics, in which the simultaneous presence of two electric fields stimulates the Raman transitions [1]. It represents a smart way to "circumvent" the aforementioned time-energy transform limit, allowing for simultaneously high temporal precision and spectral resolution. Using dispersed detection, indeed, spectral resolution is fundamentally limited by the vibrational dephasing time only. Nonetheless, the time resolution is only determined by the duration of the pulse initiating the macroscopic polarization in the sample and, of course, by the photochemical pump. This "disentanglement" of time and energy resolution allows reaching values as low as ~30fs/10cm-1.
We recently combined the principles of FSRS and TR3, developing a Femtosecond Stimulated Resonance Raman (FSRRS) setup with broadly tunable Raman pulse optimized to exploit resonance enhancement in diverse biomolecules [2,3].

3. Results

We applied Femtosecond Stimulated Resonance Raman Scattering (FSRS) to study photoinduced dynamics in Myoglobin and Neuroglobin, representative cases of five and six coordinated hemes, respectively. Taking advantage of unrestricted frequency resolution (10 cm^{-1}) and time precision (30 fs), combined with broad tunability of the Raman excitation, we have been able to unveil the details of the reaction pathway. These include the early stages following ligand dissociation, ascertaining the possible existence of short living intermediates, the subsequent energy redistribution among different vibrational channels, and the underlying structural rearrangements.

FSRS data suggest that the photoexcited hemeprotein, once the ligand bond breaking is accomplished, directly evolves to a hot ground state photoproduct. Distinct signatures of anharmonicities are observed with structural sensitivity, manifesting themselves as vibrational couplings and hot bands, evolving during the cooling process. In the specific case of the six coordinated deoxy Neuroglobin, the lack of the iron-histidine stretching rules out a bond breaking event on either the proximal or distal side of the iron atom. This is in striking contrast with the case of

small exogenous ligands. Taken all together, these observations allow drawing a clear scenario for the photoinduced dynamics of heme proteins, rationalizing previous transient absorption results subject to contrasting interpretations.

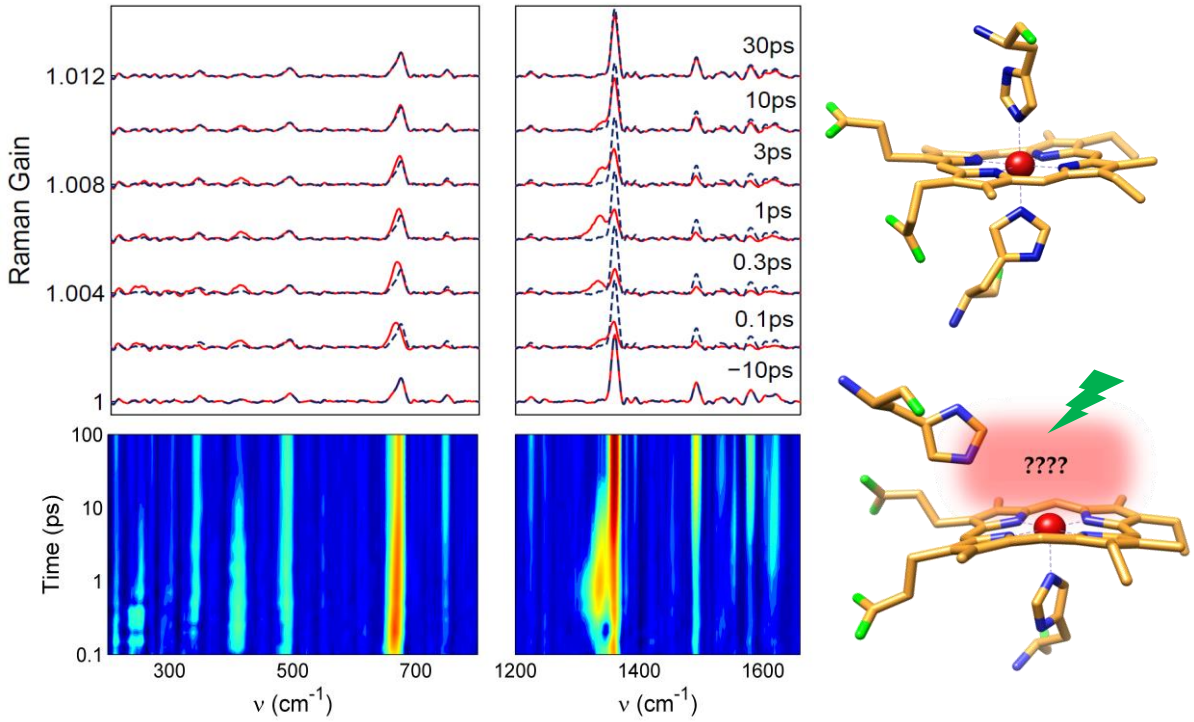

Figure 1: FSRS data of photoexcited 6-coordinated deoxy Neuroglobin. The high spectral resolution combined to sub picosecond time precision allows revealing the lack of a five coordinated photoproduct, ruling out a photolysis process. At the same time, the spectral evolution of the 1350 cm^{-1} feature indicates the energy harvesting role of the porphyrin ring breathing mode.

4. References

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