Ultrafast Heat Transport Through the Limiting Conformations of Ferric Hemoglobin

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Supporting Information

ABSTRACT: The conformational dynamics of hemoglobin are known to be strongly influenced by the hydration on both the surface of the protein and within the oxygen binding pockets. In this Communication, we probe the heat transport kinetics of the limiting conformers of methemoglobin following ultrafast visible photoexcitation of the heme group using time-resolved infrared spectroscopy. Our results indicate that anharmonic coupling of high-to-low frequency heme modes yields efficient through-space transition dipole coupling between low-frequency heme modes and low-frequency, solvent-coupled protein structural modes. We observe enhanced ballistic heat transport in T-state methemoglobin following dehydration by ethylene glycol as compared to the hydrated R-state conformer, indicating that the free energy of oxygen binding is directly coupled to, and therefore modulated by the solvent conditions of the protein.

It is well known that protein hydration plays a significant role in protein dynamics and function such as enzyme catalysis and allosteric effects,{Fenimore, 2002 #12}{Daniel, 2003 #1}{Kurkal, 2005 #2}{Frauenfelder, 2007 #3}{Colombo, 1992 #4} and hemoglobin (Hb) has been extensively investigated as model system for understanding dynamic, allosteric phenomena.{Colombo, 1992 #4}{Yonetani, 2008 #7}{Baldwin, 1979 #5}{Hundahl, 2003 #6}{Perutz, 1993 #8}{Takayanagi, 2014 #9} In transition between the oxygenated (relaxed/R) and deoxygenated (tense/T) conformations, several direct contacts between subunits are broken and exposed to solvent, which implies a difference in hydration between the two limiting conformations of Hb.{Yonetani, 2008 #7}{Hundahl, 2003 #6}{Perutz, 1993 #8}{Takayanagi, 2014 #9}{Haire, 1983 #11}{Perutz, 1979 #30} Indeed, prior work indicates that the fully oxygenated R-state of Hb is hydrated by as many as ~60 additional water molecules compared to the deoxygenated conformer, as well as an addtional ~10 water molecules in the transition state.{Colombo, 1992 #4}{Colombo, 1999 #10}{Salvay, 2003 #35}{Goldbeck, 2001 #40} With this in mind, we seek to probe how changes in hydration facilitate the transfer of the free energy of effector binding to the heme sites in Hb, thereby modulating its oxygen binding affinity.

Molecular dynamics (MD) simulations have already demonstrated existence of coupling between protein and water,{Nibali, 2014 #13;Shenogina, 2008 #14;Heyden, 2010 #15} which was suggested by THz, fluorescence and ultrafast optical Kerr effect studies.{Heyden, 2010 #15;Mazur, 2012 #17;Zhong, 2009 #20} MD simulation also predicted that low frequency modes are strongly coupled with water while intermediate and high frequency modes are decoupled with water.{Shenogina, 2008 #14} Neutron scattering and THz techniques indicated thermal coupling between protein and water is effective on picosecond timescale.{Paciaroni, 2008 #18;Paciaroni, 2008 #18;Lipps, 2012 #19} Since water on protein surface is main driving force for protein dynamics,{Combet, 2012 #22} water on protein surface has been well characterized by fluorescence, ultrafast optical Kerr effect and interfacial spectroscopy.{Mazur, 2012 #17;Zhong, 2009 #20;Engelhardt, 2014 #21;Yang, 2012 #23;Pal, 2002 #25} But not much research has focused on dynamics of the protein matrix. Although THz and far-infrared technique could provide low frequency information of protein matrix, it is hard to separate protein signal from low frequency of water since both of them have broad bands overlapped each other.{Lipps, 2012 #19}

By depositing a large excess of energy into the heme group of Hb, the heat dissipation of each limiting conformation can be directly examined via the vibrational bands of the amide backbone. Prior experiments using picosecond time-resolved infrared (TRIR) to study protein dynamics in carbonmonoxymyoglobin indicate that CO photolysis, and the subsequent energy deposition into the heme triggers an allosteric transition between the limiting conformers within 6-8 ps,{Causgrove, 1996 #47} and has been recently shown via femtosecond X-ray solution scattering measurements to induce a ballistic “proteinquake” in the initial stages of thermal equilibration.{Levantino, 2015 #49} These results have been further corroborated by our recent work, where we observed combined ballistic and diffusive heat transport in albumin complexes with Fe and Cu porphyrins using ultrafast TRIR spectroscopy. We also found that the rate of heat flow remains unchanged whether the heme chromophore is covalently or non-covalently bound to albumin. Additionally, enhanced ballistic heat transport was observed by subsequent binding of an allosteric effector, sodium myristate. {Li, 2014 #37}[ref JPCL]

In this Communication, we measure the differences in heat flow kinetics resulting from the allosteric R-T transition of bovine methemoglobin (metHb) via dehydration with ethylene glycol. In order to compare the allosteric contributions from hydration and ligand binding, we also probe the heat flow dynamics upon addition of exogenous ligands such as imidazole and cyanide. The results below are then put into context with differences in hydration in both ferrous hemoglobin and methemoglobin.

The comparison between the transient and equilibrium heating response is given in Figure 1A, which shows the ultrafast TRIR (10 ps delay) and temperature-dependent FTIR (T-FTIR, 23.8oC-20.3oC) difference spectra of 0.6 mM metHb in D2O. Following absorption of an ultrafast visible pulse in the TRIR experiment results in anisotropic heating of the heme via non-radiative decay to its ground electronic state on the subpicosecond time scale,{Consani, 2014 #50} yielding an excited population of vibrational modes far from thermal equilibrium. The protein and solvent are heated anisotropically as the heme thermally equilibrates with its surroundings. Conversely, isotropic solvent heating yields the pure equilibrium heating response of the protein in the T-FTIR experiment. To monitor the evolution of the transient heating response, and to characterize the heme and protein IR bands, respectively, figure 1B compares the evolution of the TRIR difference spectra of hemin and metHb at various pump delays. Both hemin and metHb exhibit ground state bleaches at 1407cm-1 and 1556 cm-1, which correspond to in-plane skeletal vibrations of the heme porphyrin ring, accompanied by broad excited state heating band at 1383 cm-1.{Hu, 1996 #60} The ground state bleach at 1466 cm-1 is assigned to amide II, with its excited state heating band centered at ~1425 cm-1, overlapping with the heme bleach. The bleach at 1630 cm-1 is assigned to the amide I backbone vibrations. A broad positive heating band at 1665 cm-1 is seen in the T-FTIR spectrum, which is absent in the TRIR spectra. This band is likely due to conformational changes at times beyond our spectral window in the TRIR experiment (i.e., ns time scale), similar to what we have reported previously for heme-albumin complexes.{Li, 2014 #37} Additionally, Fig 1A shows that the heating signals of heme and amide II in the T-FTIR spectrum are redshifted by ~10 cm-1 as compared to the 10 ps TRIR spectrum. This redshift at early times is likely due to the competition between the overlapping amide II excited state band with the heme ground state bleach as these signals evolve.

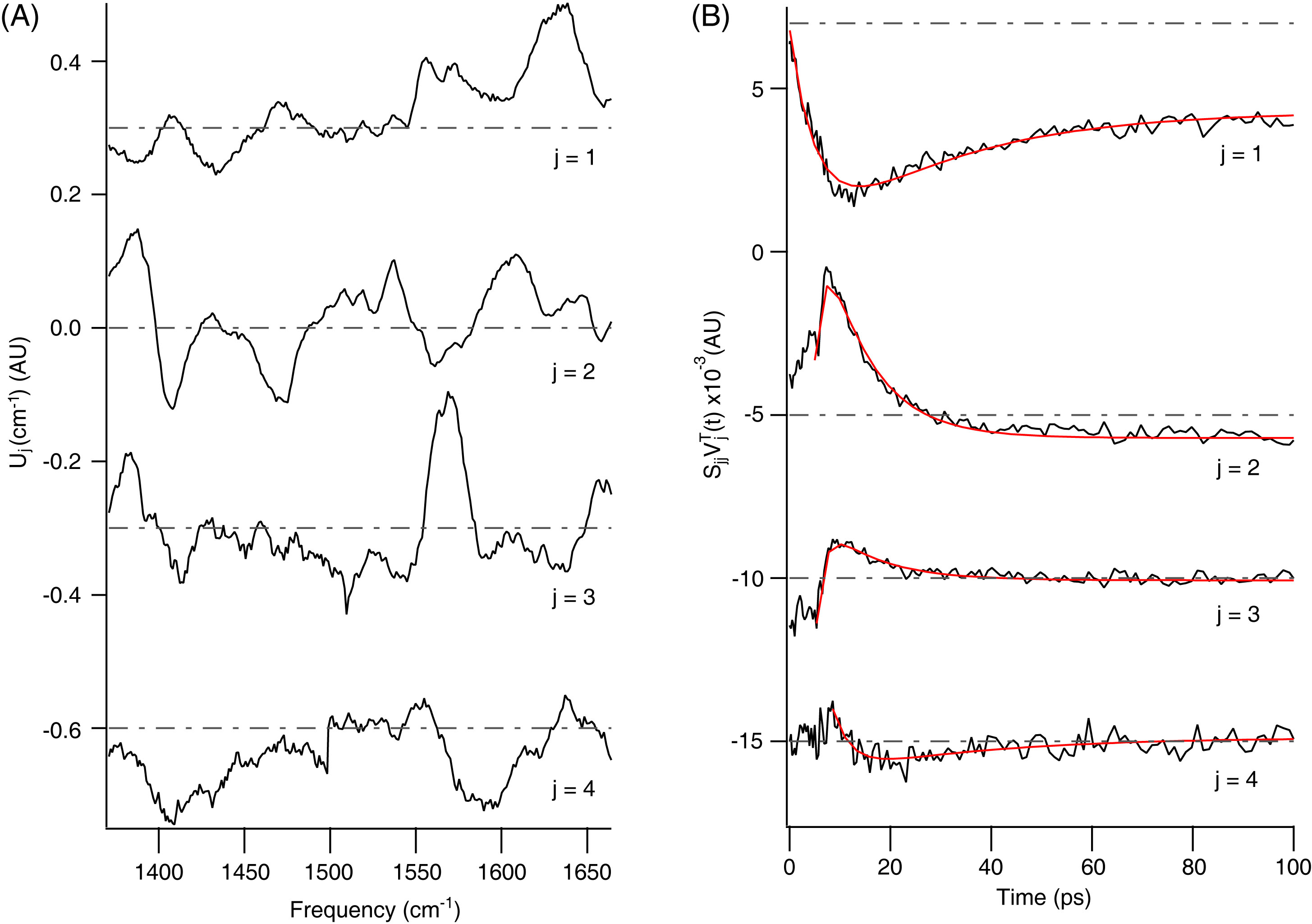


Figure 2 Singular Value Decomposition analysis of metHb in D2O truncated to j = 4 components. (A) Uj vectors corresponding to the basis spectra comprising the observed signal. (B) vectors, weighted by their singular values Sjj, corresponding to the transient components of the observed signal. Uj and Sjj curves offset for clarity. Transient fit curves to the Sjj components are overlaid in red. Gray dot-dashed lines indicate zero position for each offset curve.

Figure 1 (A) comparison of differential FTIR spectra (23.8oC-20.3oC) and TRIR spectra (10 ps) of 0.6mM methemoglobin in D2O; (B) TRIR spectral comparison of 2.5mM hemin (black) and 0.6mM methemoglonbin (red) in D2O at different time scales. TRIR spectra in (B) are offset for clarity.

In addition to the above-mentioned characterization, we can observe the overall heat flow path via the TRIR spectra. At early times (3 ps), the heme signal contributes significantly to the transient IR response. Within 10 ps, the heme signal has decayed almost completely, while the protein bands increase in intensity between 10-20 ps. The presence of a substantial ground state bleach at ~1556 cm-1 at times >10 ps is likely due to the carboxylate groups of the protein (i.e., Asp/Glu residues). By 100 ps, most of the heat has flowed out of the protein and into the solvent. The heat transport dynamics of metHb in D2O is illustrated by singular value decomposition (SVD) analysis, given below. Fitting of the raw transient signals is included in the Supporting Information.

Given the complexity of the observed signals, SVD analysis was employed to separate the frequency vs. time data matrix into a number of significant spectral and transient components (indexed by j) given by Uj(cm-1) and (t), respectively. Figure 2 illustrates the basis spectra and transient dynamics (the latter weighted by their singular values Sjj) truncated to the first four SVD components, which accounts for ~37% of the total measured signal (where S11-S44 are 22.4%, 8.3%, 3.8% and 2.5% of the measured signal, respectively); additionally, the matrix **M**(cm-1,t)= reproduces the raw data matrix (given by **A**(cm-1,t)) well, with zero lag cross-correlation of the **A** and **M** transients versus frequency >0.9 for each characteristic frequency (c.f. Supporting Information).

It is evident from inspection of the basis spectra that SVD does not cleanly separate the contributions from each individual transport process occurring in metHb. However, this is likely explained by the fact that heat flow in metHb (and similar heme proteins) is a rate-coupled process, where heat flows from the heme to protein, and from protein to solvent, with similar rates of transport (c.f. Fig 1B).[ref JPCL] It follows then that each basis spectrum contains contributions from the heme, protein, and solvent frequencies with varying amplitudes. What remains consistent is the similarity in energy transport rates between pairs of components with similarities in Uj (i.e., j = 1,4, and j = 2,3), discussed below.

The U1 and U4 spectra predominantly contain peak signals from the protein and heme, which is substantiated by the and transients. The fit results to both transients yield similar transport rates with single exponential rises of 5.4 ps and 3.6 ps, followed by single exponential decays of 28 ps and 30 ps, respectively. The fast time constants correspond to anisotropic (ballistic) heat transport out of the heme and into the protein ( ~5 ps, typ.), which has been demonstrated by ultrafast small-angle x-ray scattering studies of myoglobin to be due to coupled low-frequency vibrations between the heme group and the protein structure.{Levantino, 2015 #49} Additionally, the longer time constants correspond to isotropic (diffusive) heat flow out of the protein and into solvent ( ~30 ps, typ.). U2 and U3 contain peak signals from all three major contributing species (heme, protein, and solvent), where the fit results to and yield single-exponential rises of 1.7 ps and 1.5 ps, followed by single exponential decays of 9.2 ps and 10.9 ps, respectively. These fast time constants are characteristic of the time scale of ballistic heat transport,{Lin, 2012 #62} where the faster path is likely due to coherent energy transfer between heme and solvent and/or protein and solvent through low-frequency vibrational modes, while the slower path is due to anisotropic incoherent energy transfer from protein out to the solvent. We have previously observed similar rates of energy transport in metalloporphyrin-albumin complexes.{Li, 2014 #37}[ref JPCL]

It is well established that the transition between the oxy (R) and deoxy (T) states of Hb can be induced by modulating the degree of protein hydration via addition of ethylene glycol (EG), sucrose, stachyose, or glycine.{Colombo, 1992 #4;Hundahl, 2003 #6;Haire, 1983 #11;Colombo, 1996 #31} Since the EG cosolvent interacts weakly with Hb, modulation of oxygen binding affinity can only be observed at high EG concentrations (0.2–0.4 mole fraction, 47–70% EG w/w). Within this range, the oxygen binding affinity of Hb has been shown to decrease.{Haire, 1983 #11} In the TRIR experiments discussed below, we dehydrate the protein with the lowest mole fraction of 0.2, which effectively induces the structural transition from the R-state to the T-state, while maintaining stability of the protein.

Figure 3A shows the TRIR spectra at 2 ps of metHb in D2O and in 0.2 mole fraction EG/D2O. Since TRIR spectra change around 1630 cm-1 is only related to amide I heating, hydration effect on energy flow in hemoglobin should be disclosed by amide I. It is interesting to notice that heating band of amide I at EG/water (0.2 mole fraction) becomes narrow and is shifted to low wavenumber, which suggests structure of hemoglobin’ T state is more order and compact than its R state. Therefore, energy flow in T state is expected to be faster than R state. Figure 3B clearly shows equilibrium of energy flow in T state is 19.4 ps which is faster than R state (37.3 ps). Energy flow rate from heme to globin is extremely fast (< 1 ps). From Fig.3B, we can notice the heating curves features immediately after exciting heme. In our previous energy flow work on albumin, we discussed low frequency mode coupling between heme and protein matrix is main channel of energy flow no matter if covalent bond exists between chromophore and protein matrix. MD simulation has also demonstrated only low frequency mode of protein could strongly couple with interfacial water while high frequency mode weakly couples with water on protein.{Shenogina, 2008 #14} Unfortunately, we can’t detect energy flow rate difference due to low frequency mode coupling at different hydrations since time scale of this coupling is extremely fast. Leitner’ group predicted low frequency mode damping rates of chromophore is around subpicosecond level.{Leitner, 2012 #33}

It has been shown that both oxyHb and deoxyHb have characteristic features in their circular dichroism (CD) spectra around 280 nm, which characterize the R-to-T transition. To definitively ascribe any alteration in heat flow dynamics to an R-to-T transition, we have measured CD spectra for metHb. MetHb has been shown to undergo a less pronounced R-to- T transition in its CD spectra via addition of the allosteric effector inositol hexaphosphate,{Perutz, 1974 #59} and we have obtained similar results using EG (c.f. Supporting Information).

Figure 3 (A) TRIR spectral comparison at 2 ps of 0.6mM hemoglobin in D2O and ethylene glycol/D2O (0.2 mole fraction); (B) IR transient comparison at 1630 cm-1 of 0.6 mM methemoglobin in D2O and ethylene glycol/D2O (0.2 mole fraction) [remeasure MetHb in EG and/or IHP and do SVD]

Numerous results indicate that water plays a critical role in protein structure and function, whether it is associated on the protein surface,{Fenimore, 2002 #12}{Combet, 2012 #22}, or within a binding pocket as an external ligand.{Goldbeck, 2001 #40} We hypothesize that the enhanced ballistic heat flow in metHb in the presence of EG is likely due to a net dehydration of the binding pocket due to the dehydrating effects of EG, which would result in the heme group adopting a domed conformation, in closer proximity to the proximal histidine when external ligands are absent.{Park, 2006 #52} Our previous results on heme-albumin complexes indicate that excess thermal energy is efficiently dissipated through the protein and out to the bulk solvent via anharmonic coupling of high-frequency in-plane heme modes to high-quanta, low-frequency out-of-plane heme modes (i.e. doming and ruffling modes 80 cm-1), whereupon a mixture of coherent and incoherent energy transfer occurs through-space to the low-frequency protein structural modes that couple out to the solvent.[ref JPCL]{Ferrante, 2016 #63}{Kubo, 2008 #58}{Deng, 2002 #56}{Galinato, 2012 #61} Additonally, due to the net dehydration of the protein surface in the T-state, the protein structure becomes more rigid due to surface dehydration, which should result in more efficient coupling of heme low-frequency modes to the low-frequency protein structural vibrations.

In ferrous hemoglobin, is well-known that the protein surface in the R-state (oxy) is hydrated by ~60 additional water molecules as compared to the T-state (deoxy), regardless of the presence of allosteric effectors.{Colombo, 1992 #4}{Colombo, 1999 #10}{Salvay, 2003 #35} Additionally, nanosecond protein relaxation dynamics of HbCO following CO photolysis indicate that the transition state from R-to-T is hydrated by an additional ~10 water molecules prior to the net surface dehydration as the T-state equilibrates. {Goldbeck, 2001 #40} In contrast to the protein surface, the hydration state of the heme pocket shows the opposite trend, in that the heme pockets are not hydrated in oxyhemoglobin, due to bound oxygen excluding water from the binding pocket, whereas the **-subunits of deoxyhemoglobin contain water.{Esquerra, 2010 #51}{Park, 2006 #52} Thus, for ferrous hemoglobin, there is a net hydration of the heme pockets in the R-T allosteric transition. However, in metHb, both the *-* and **-subunits are hydrated in the R-state (since metHb does not bind O2), and assuming similar T-state hydration to deoxyhemoglobin, the R-T transition in metHb should yield a net dehydration of the binding pocket and consequently, more efficient coupling of the low-frequency heme modes through the protein and out to the solvent.{Deatherage, 1976 #54}

In summary, we observe ultrafast (ballistic) heat flow in metHb due to anharmonic coupling of high-to-low frequency heme vibrational modes, which leads to mixtures of coherent and incoherent energy transfer between low-frequency protein and heme modes. Enhanced ballistic heat flow is observed in the T-state of metHb relative to the R-state, the structural basis of which is the net dehydration of both the protein surface and binding pockets due to the ethylene glycol cosolvent, resulting in underdamping of coupled vibrations between the heme and protein in the T-state conformer. Singular value decomposition of the TRIR results indicates rate coupling along the heat flow paths by the basis spectra (both heme-solvent and heme-protein-solvent). Our results show a direct connection of energy flow from the ligand binding site to solvent, which is modulated by the solvent conditions, implicating that the oxygen binding affinity of hemoglobin is also modulated by the solvent conditions of the protein.

ASSOCIATED CONTENT

Supporting Information

The materials and sample preparation, description fs laser system, details of the UV-Vis and T-FTIR measurements, and related spectra are given as Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes  
The authors declare no competing financial interest.

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