Label-free imaging of hemoglobin degradation and hemosiderin formation in brain tissues with femtosecond pump-probe microscopy

Lili Zhang,[1‡] Xiang Zou,[2‡] Bohan Zhang,[1] Liyuan Cui,[3] Jiayi Zhang,[3] Ying Mao,[2] Liang Chen,[2*] Minbiao Ji[1*]

[1]State Key Laboratory of Surface Physics and Department of Physics, Collaborative Innovation Center of Genetics and Development, Key Laboratory of Micro and Nano Photonic Structures (Ministry of Education), Fudan University, Shanghai 200433, China

[2] Department of Neurosurgery, Huashan Hospital, Fudan University, Shanghai 200040, China

[3]State Key Laboratory of Medical Neurobiology, Institute of Bain Science, Department of Ophthalmology, Zhongshan Hospital, Fudan University, Shanghai 200032, China

‡These authors contributed equally to this work

***CORRESPONDENCE:** chenlianghs@126.com, minbiaoj@fudan.edu.cn

Supplementary Material

Fig. S1. Modulation transfer schemes of ESA, GSB and SRL, only the intensity changes of the detection beams are illustrated.

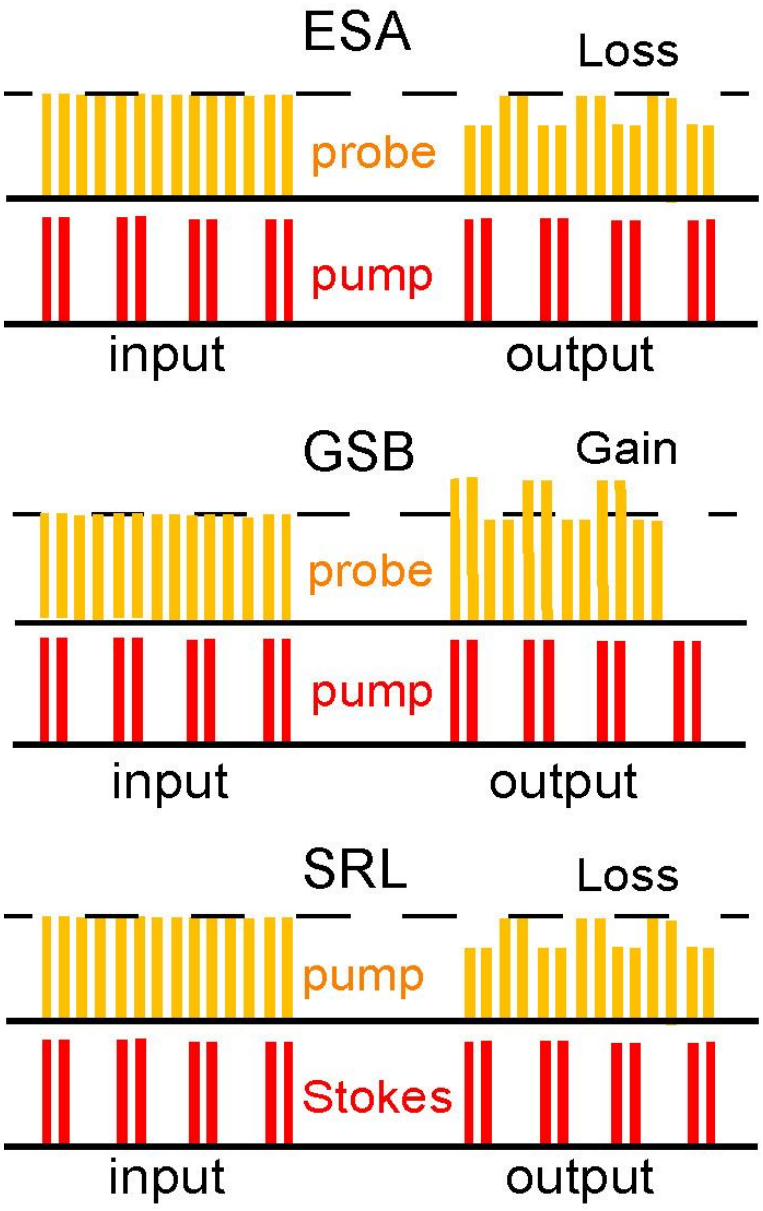


Fig. S2. TA dynamics of RBCs obtained from artery (A) and vein (V) show little differences. Two probe wavelengths were measured: 880 and 950 nm.

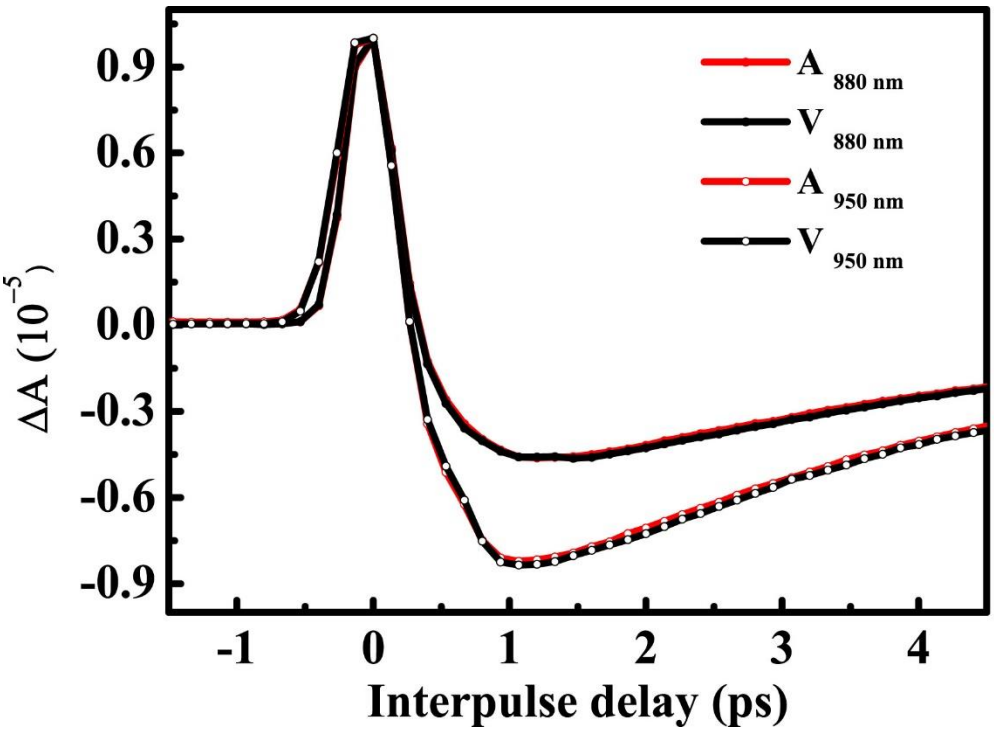
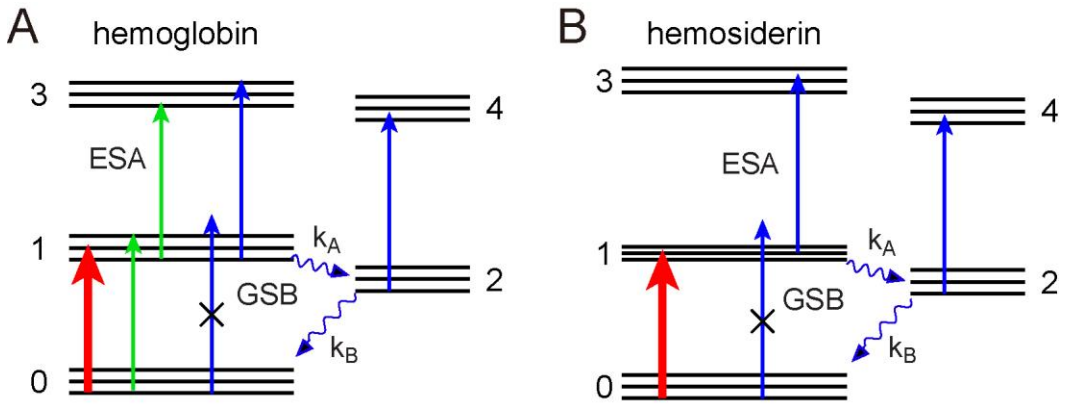


Fig. S3. Proposed relaxation pathways of hemoglobin **(A)** and hemosiderin **(B)**. For hemoglobin, at longer excitation wavelengths both ESA and GSB exist (green arrows), whereas at shorter wavelengths the GSB process vanishes (blue arrows). For hemosiderin, no GSB could be detected within our probe window.



We propose the excited states involved as shown above. Assuming instantaneous pump excitation that results in the initial population of ΔN_0 in the excited state (**1**), and a depletion of ground state (**0**) population of $-\Delta N_0$. Therefore, the kinetic equations of the three-level system can be expressed as:

$$\frac{dN_0(t)}{t} = k_B N_2(t)$$
$$\frac{dN_1(t)}{t} = -k_A N_1(t)$$
$$\frac{dN_2(t)}{t} = k_A N_1(t) - k_B N_2(t)$$

, with the initial conditions: $N_0(0) = -\Delta N_0$, $N_1(0) = \Delta N_0$, $N_2(0) = 0$

Where N_i represents the non-equilibrium differential population. The analytical solutions could be obtained:

$$N_0(t) = \Delta N_0 \cdot \frac{k_B e^{-k_A t} - k_A e^{-k_B t}}{k_A - k_B}$$
$$N_1(t) = \Delta N_0 e^{-k_A t}$$
$$N_2(t) = \Delta N_0 \cdot \frac{k_A(-e^{-k_A t} + e^{-k_B t})}{k_A - k_B}$$

The excited-state absorption signal could be calculated, given the absorption coefficients between the initial and final states,

$$S_{ESA} = \sigma_{13} N_1 + \sigma_{24} N_2 = \frac{(\sigma_{13} - \sigma_{24})k_A - \sigma_{13} k_B}{k_A - k_B} \Delta N_0 e^{-k_A t} + \frac{\sigma_{24} k_A}{k_A - k_B} \Delta N_0 e^{-k_B t},$$

Similarly, the ground-state bleaching signal could be written:

$$S_{GSB} = \sigma_{01} N_0 = \frac{\sigma_{01}(k_B e^{-k_A t} - k_A e^{-k_B t})}{k_A - k_B} \Delta N_0,$$

In addition, we include an impulsive term for the initial fast process that could not be resolved by our limited pulse width (such as cross-phase modulation), described by a single exponential decay at the time scale around the pulse duration: $S_X = A_1 e^{-t/\tau_1}$.

Therefore, the total TA signal could be expressed as the sum of the above three terms:

$$\Delta A = S_X + S_{GSB} + S_{ESA} = A_1 e^{-t/\tau_1} + A_2 e^{-t/\tau_2} + A_3 e^{-t/\tau_3},$$

$$A_2 = \frac{(\sigma_{13} - \sigma_{24})k_A - (\sigma_{13} - \sigma_{01})k_B}{k_A - k_B} \Delta N_0,$$

$$\tau_2 = k_A^{-1},$$

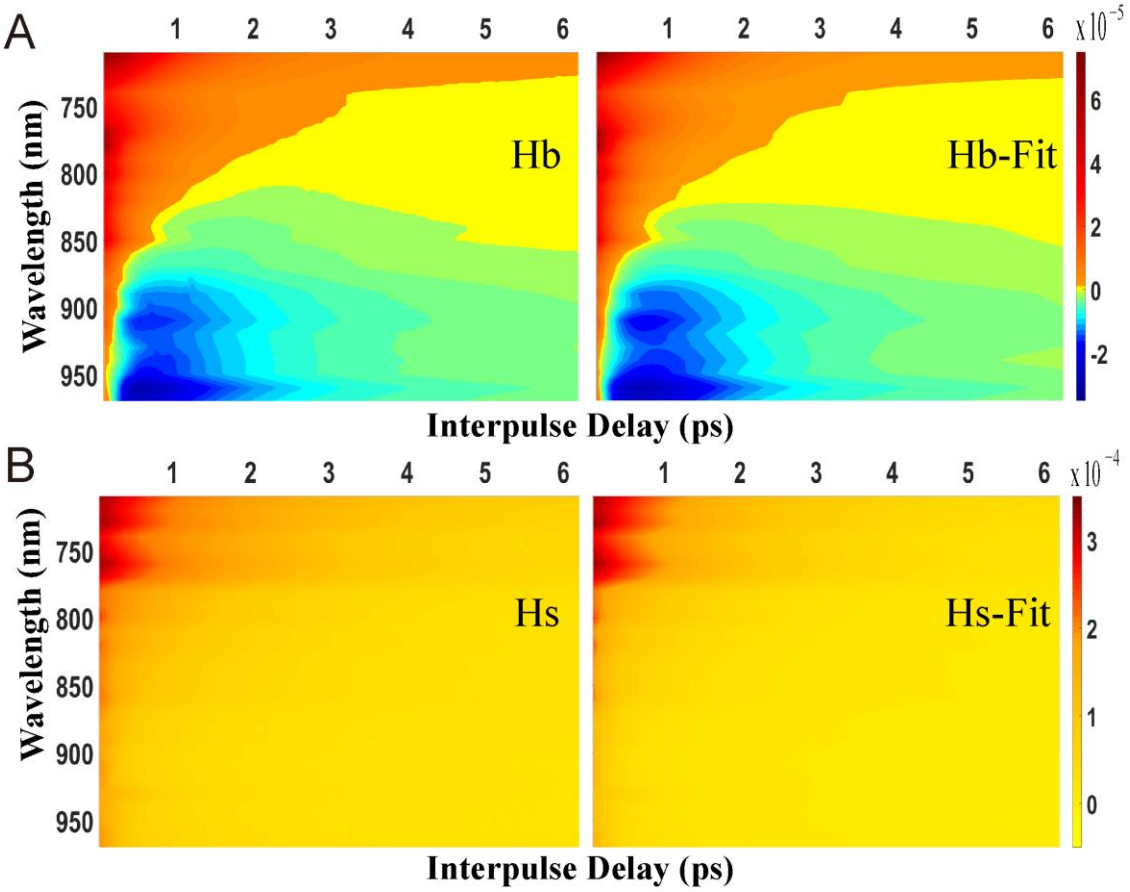
$$A_3 = \frac{(\sigma_{24} - \sigma_{01})k_A}{k_A - k_B} \Delta N_0,$$

$$\tau_3 = k_B^{-1},$$

These parameters are experimentally determined by fitting our TA data, and results are shown in **Table 1**. As can be seen, for both compounds, $k_A > k_B$, indicating state (**2**) is relatively longer lived.

Interestingly, the solution of A_3 could explain the sign flip of hemoglobin TA signals at different probe wavelengths. At short wavelengths, $\sigma_{01} < \sigma_{24}$, thus $A_3 > 0$. And at longer wavelengths, $\sigma_{01} > \sigma_{24}$, and $A_3 < 0$. For hemosiderin, σ_{01} is always small within our detection window, and hence A_3 stays positive. These could explain our measured A_3 as shown in **Fig. 3F**.

Fig. S4. Global fitting of the 2D TA spectra for **(A)** hemoglobin and **(B)** hemosiderin.



Movie S1. Raw TA images of a rat brain tissue with both red blood cells and hemosiderin probed at 880 nm.

Movie S2. Time-resolved images of the brain tissue in Figure 3.

Movie S3. Depth-resolved three-color images of the brain tissue in Figure 3.