

Electronic Supplementary Information to:

Reduction of Transient Carnosine Radicals Depends on β -Alanyl Amino Group Charge

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**Simulation of CIDNP kinetics detected in photoinduced reactions of the photosystem 3,3',4,4'-
tetracarboxy benzophenone/histidine/tryptophan
(TCBP/His/Trp)**

To determine the rate of the reduction reaction of histidine radicals (including that in peptides with Gly or β -Ala at N-terminus) by tryptophan (including that in Trp-Gly) a simulation of the CIDNP kinetics was performed. The set of equations introduced by Fischer for cyclic radical reactions¹ was modified to take into account the reduction reaction. The peculiarity of the photo-system under study is that recombination of radical pairs containing a TCBP radical partly proceeds from its triplet state, resulting in the formation of TCBP in its protonated triplet state, ³TCBPH, and His or Trp in their ground state.² This is a crucial point: without taking into account recombination to this triplet state, satisfactory agreement between experimental and simulated CIDNP kinetics could not be achieved. The triplet channel of recombination was a subject of a separate investigation, in which all the parameters were determined that describe the CIDNP kinetics in photoreactions of a two-component photosystem (TCBP and individual amino acid).² In the present investigation, the developed simulation procedure was applied to a three-component photosystem, allowing to determine the rate constant of the reduction reaction of the His radicals by Trp.

In the reaction of triplet excited TCBP with a mixture of derivatives of His and Trp, three kinds of radicals are formed: the TCBP radical (concentration R_B), the histidine radical (concentration R_H), and the tryptophan radical (concentration R_W). The reduction reaction was treated as pseudo-first order, described by a product of a second-order rate constant and the concentration of Trp: $k'_r = k_r \times C_W$. It is assumed that the fraction of geminate recombination is negligibly small, and the formation of radicals is instantaneous on the time scale of CIDNP. The concentrations of radicals are described by the following equations:

$$\frac{dR_B}{dt} = -k_H R_B R_H - k_W R_B R_W \quad (s1)$$

$$\frac{dR_H}{dt} = -k_H R_B R_H - k'_r R_H \quad (s2)$$

$$\frac{dR_W}{dt} = -k_W R_B R_W + k'_r R_H \quad (s3)$$

Here, different rate constants are used for the termination reaction of the dye radical with a histidine or tryptophan radical, k_H and k_W . Non-equal termination rate constants are important for the case of combination of acetylated and-non acetylated amino acids: a positive charge at the amino group speeds up the decay in the reaction with the TCBP radical, having four negatively charged carboxylic groups. The initial concentrations of radicals are: R_0 for TCBP radicals, αR_0 for His radicals, and $(1-$

$\alpha)R_0$ for Trp radicals. This parameter was calculated from relative CIDNP intensities of His (residue) and Trp (residue) in the spectra obtained with no delay after the laser pulse (geminate CIDNP spectra).

Nuclear polarization in the radicals, P_H^R, P_W^R , and in the ground state molecules, P_H and P_W , is described by the following equations:

$$\frac{dP_H^R}{dt} = -k_H P_H^R R_B - (1 - 2\chi_H)k_H \beta_H R_H R_B - \frac{P_H^R}{T_1^H} - k_r' P_H^R \quad (s4)$$

$$\frac{dP_H}{dt} = k_H P_H^R R_B + (1 - 2\chi_H)k_H \beta_H R_H R_B + k_r' P_H^R \quad (s5)$$

$$\frac{dP_W^R}{dt} = -k_W P_W^R R_B - (1 - 2\chi_W)k_W \beta_W R_W R_B - \frac{P_W^R}{T_1^W} \quad (s6)$$

$$\frac{dP_W}{dt} = k_W P_W^R R_B + (1 - 2\chi_W)k_W \beta_W R_W R_B \quad (s7)$$

Here, χ_H and χ_W are the probabilities of triplet recombination of the pairs consisting of a TCBP radical, and a histidine or tryptophan radical, respectively. T_1^H and T_1^W are the paramagnetic relaxation times of protons of His radical or or Trp radical. The parameters β represent the polarization per pair, created upon recombination of F-pairs of radicals in the bulk, and are related to the geminate polarization P^G via the quantity γ , which is the ratio of polarization created in F-pairs and geminate polarization: β_H and β_W stand for the pairs of a TCBP radical with a His or a Trp radical, $\beta_H = \gamma_H P_H^G / (\alpha R_0)$, $\beta_W = \gamma_W P_W^G / ((1 - \alpha)R_0)$, with P_H^G and P_W^G denoting the geminate polarization formed for protons of His and Trp in the corresponding radical pairs with a TCBP radical. Since we do not measure the absolute value of polarization, in the simulation geminate polarization appears as vertical scaling factor, which for convenience of presentation in most cases is scaled to unity. The initial polarizations were taken as $P_H^G = -P_H^R = 1$, $P_W^G = -P_W^R = 1$.

Equations s8, s9 describe nuclear polarization in the TCBP radical, P_B^R , and its ground state, P_B . Polarization formed via triplet recombination of radical pairs in the protonated triplet state of TCBP stays off resonance and is not detected by NMR.

$$\frac{dP_B^R}{dt} = -k_H P_B^R R_H - (1 - 2\chi_H)k_H \beta_H R_H R_B - k_W P_B^R R_W - (1 - 2\chi_W)k_W \beta_W R_W R_B - \frac{P_B^R}{T_1^B} \quad (s8)$$

$$\frac{dP_B}{dt} = (1 - \chi_H)k_H P_B^R R_H + (1 - \chi_H)k_H \beta_H R_H R_B + (1 - \chi_W)k_W P_B^R R_W + (1 - \chi_W)k_W \beta_W R_W R_B \quad (s9)$$

The initial polarization (formed during the geminate stage) of TCBP consists of contributions of CIDNP of the same sign formed in the radical pairs with a His radical and with a Trp radical. Because of the same sign of polarization (by contrast to the case of His and Tyr), the CIDNP kinetics for TCBP in this case is almost insensitive to the rate of the reduction reaction (reaction (1) of main text), but rather

reflects the overall radical decay in the termination of radical pairs. Thus, for determination of pseudo-first order reaction rate, k_r' , common data sets – for His and Trp – were fitted simultaneously, and in some cases data set for TCBP was also taken to check the reliability of the simulation (especially when CIDNP intensity of Trp was noticeably lower than that of His). The second order rate constants of the reduction reaction were determined at two concentrations of Trp, C_w . Rate constant k_r was taken as the average of those.

The parameters for L-His, N-AcHis, L-Trp, N-AcTrp were known from previous simulations of the corresponding CIDNP kinetics obtained for the photoreaction of the two-component system, containing TCBP and an amino acid, or its N-acetyl derivative.³ In the present study, the unknown parameters were obtained for the two-component systems TCBP/ β -Ala-His, TCBP/Gly-His, TCBP/Trp-Gly. All the parameters are given in Tables S1-S8. The fitting parameters for each data set including two CIDNP kinetics, for His, and Trp, or three CIDNP kinetics, for His, Trp and TCBP, were the vertical scaling factors, k_r' , $k_H R_0$.

For the analysis, signal intensities of H4 of His, of β of Trp, and of H6,6' of TCBP were used. The parameters of simulations for different reactants are given in the next section.

Parameters used in the simulations of CIDNP kinetics for different combinations of amino acids and their N-acetyl derivatives at listed concentrations; best-fit values of pseudo-first order rate constant of reduction reaction $k_r \times C_w$; second order rate constant of reduction reaction k_r , calculated as the mean between two values at different C_w

List of parameters:

γ_H – ratio of polarization created in F-pairs to the geminate polarization for the pair of TCBP and His radicals

γ_W – ratio of polarization created in F-pairs to the geminate polarization for the pair of TCBP and Trp radicals

χ_H – probability of triplet recombination of the pairs consisting of a TCBP radical and a histidine radical

χ_W – probability of triplet recombination of the pairs consisting of a TCBP radical and a tryptophan radical

T_1^H – paramagnetic nuclear relaxation time for proton H2 of the histidine radical

T_1^W – paramagnetic nuclear relaxation time for H β protons of the tryptophan radical

k_H/k_W – ratio of second-order rate constants for the termination reaction of the dye radical with histidine and tryptophan radicals

Table S1.

β-Ala-His/N-AcTrp				
$\gamma_H=2.3, \gamma_W=1.5, \chi_H=0.20, \chi_W=0.19, T_1^H=16 \mu s, T_1^W=100 \mu s, k_H/k_W=2$ (NH ₃ ⁺ β -Ala-His/N-AcTrp), $k_H/k_W=1$ (NH ₂ β -Ala-His/N-AcTrp)				
pH*	C_H , mM	C_W , mM	$k_r \times C_w$, s ⁻¹	k_r , M ⁻¹ s ⁻¹
6.9	10	10	8.5×10^4	8.5×10^6
	10	20	1.7×10^5	
8.4	10	10	7.7×10^4	7.9×10^6
	10	20	1.6×10^5	
9.6	15	10	5.7×10^4	6.1×10^6
	15	20	1.3×10^4	
10.1	20	10	6.2×10^4	5.6×10^6
	20	20	1.0×10^5	
11.0	20	10	4.0×10^4	3.6×10^6
	20	20	6.5×10^4	

11.8	20	10	3.5×10^4	3.0×10^6
	20	20	4.8×10^4	

Table S2.

β-Ala-His/L-Trp				
$\gamma_H=2.3, \gamma_W=1.2, \chi_H=0.20, \chi_W=0.22, T_1^H=16 \mu\text{s}, T_1^W=63 \mu\text{s}, k_H/k_W=1$				
pH*	C_H , mM	C_W , mM	$k_r \times C_W$, s ⁻¹	k_r , M ⁻¹ s ⁻¹
6.9	20	10	9.9×10^4	1.0×10^7
	20	20	2.2×10^5	
8.4	20	10	1.1×10^5	1.0×10^7
	20	20	1.8×10^5	
9.3	20	10	1.2×10^5	1.1×10^7
	20	20	1.9×10^5	
9.7	20	10	1.1×10^5	1.0×10^7
	20	20	1.8×10^5	
10.2	20	10	8.5×10^4	8.3×10^6
	20	20	1.6×10^5	
10.6	20	10	6.1×10^4	6.1×10^6
	20	20	1.2×10^5	
11.2	20	10	6.2×10^4	5.9×10^6
	20	20	1.1×10^5	
12.2	20	10	5.2×10^4	5.1×10^6
	20	20	9.9×10^4	

Table S3.

β-Ala-His/Trp-Gly				
$\gamma_H=2.3, \gamma_W=1.6, \chi_H=0.20, \chi_W=0.25, T_1^H=16 \mu\text{s}, T_1^W=100 \mu\text{s}, k_H/k_W=2$ (NH ₃ ⁺ β -Ala-His/NH ₂ Trp-Gly), $k_H/k_W=1$ (NH ₃ ⁺ β -Ala-His/NH ₃ ⁺ Trp-Gly, NH ₂ β -Ala-His/NH ₂ Trp-Gly)				
pH*	C_H , mM	C_W , mM	$k_r \times C_W$, s ⁻¹	k_r , M ⁻¹ s ⁻¹
6.5	20	10	9.3×10^4	1.0×10^7
	20	20	2.2×10^5	
7.6	20	10	9.6×10^4	8.8×10^6
	20	20	1.6×10^5	

8.6	20	10	8.5×10^4	7.8×10^6
	20	20	1.4×10^5	
9.7	20	10	5.7×10^4	5.3×10^6
	20	20	9.8×10^5	
10.1	20	10	4.9×10^4	4.6×10^6
	20	20	8.4×10^5	
11.7	20	10	3.1×10^4	2.8×10^6
	20	20	5.0×10^4	

Table S4.

L-His/Trp-Gly				
$\gamma_H=2.3, \gamma_W=1.6, \chi_H=0.20, \chi_W=0.16, T_1^H=16 \mu s, T_1^W=100 \mu s, k_H/k_W=2$ (NH ₃ ⁺ His/NH ₂ Trp-Gly), $k_H/k_W=1$ (NH ₃ ⁺ His/NH ₃ ⁺ Trp-Gly, NH ₂ His/NH ₂ Trp-Gly)				
pH*	C _H , mM	C _W , mM	$k_r \times C_W, s^{-1}$	$k_r, M^{-1}s^{-1}$
6.0	20	10	1.8×10^5	2.0×10^7
	20	20	4.4×10^5	
6.5	20	10	2.2×10^5	2.2×10^7
	20	20	4.5×10^5	
7.1	20	10	1.7×10^5	1.9×10^7
	20	20	4.0×10^5	
7.6	20	10	1.5×10^5	1.4×10^7
	20	20	2.7×10^5	
8.6	20	10	8.1×10^4	8.6×10^6
	20	20	1.8×10^5	
9.5	20	10	7.1×10^4	6.6×10^6
	20	20	1.2×10^5	
11.7	20	10	8.0×10^4	7.3×10^6
	20	20	1.3×10^5	

Table S5.

N-AcHis/Trp-Gly				
$\gamma_H=2.3, \gamma_W=1.7, \chi_H=0.22, \chi_W=0.16, T_1^H=16 \mu s, T_1^W=100 \mu s, k_H/k_W=1$ (N-AcHis/NH ₂ Trp-Gly)				

pH*	C_H , mM	C_W , mM	$k_r \times C_W$, s ⁻¹	k_r , M ⁻¹ s ⁻¹
11.3	20	10	3.6×10^4	3.0×10^6
	20	20	5.0×10^4	

Table S6.

Gly-His/N-AcTrp				
$\gamma_H=2.3$, $\gamma_W=1.5$, $\chi_H=0.23$, $\chi_W=0.19$, $T_1^H=16 \mu\text{s}$, $T_1^W=100 \mu\text{s}$, $k_H/k_W=2$ ($\text{NH}_3^+\text{Gly-His/N-AcTrp}$), $k_H/k_W=1$ ($\text{NH}_2\text{Gly-His/N-AcTrp}$)				
pH*	C_H , mM	C_W , mM	$k_r \times C_W$, s ⁻¹	k_r , M ⁻¹ s ⁻¹
6.9	20	10	2.8×10^4	2.7×10^6
	20	20	5.2×10^4	
7.9	20	10	2.1×10^4	2.3×10^6
	20	20	4.9×10^4	
8.2	20	10	2.7×10^4	2.7×10^6
	20	20	5.4×10^4	
8.6	20	10	3.0×10^4	2.8×10^6
	20	20	5.3×10^4	
11.1	20	10	2.9×10^4	2.6×10^6
	20	20	4.7×10^4	

Table S7.

Gly-His/L-Trp				
$\gamma_H=2.3$, $\gamma_W=1.2$, $\chi_H=0.23$, $\chi_W=0.22$, $T_1^H=16 \mu\text{s}$, $T_1^W=63 \mu\text{s}$, $k_H/k_W=1$ ($\text{NH}_3^+\text{Gly-His/NH}_3^+\text{Trp}$, $\text{NH}_2\text{Gly-His/NH}_2\text{Trp}$), $k_H/k_W=2$ ($\text{NH}_3^+\text{Gly-His/NH}_2\text{Trp}$)				
pH*	C_H , mM	C_W , mM	$k_r \times C_W$, s ⁻¹	k_r , M ⁻¹ s ⁻¹
6.8	20	10	8.3×10^4	9.2×10^6
	20	20	2.0×10^5	
7.1	20	10	8.6×10^4	8.3×10^6
	20	20	1.6×10^5	
8.3	20	10	9.2×10^4	8.4×10^6
	20	20	1.4×10^5	
9.1	20	10	9.3×10^4	8.4×10^6
	20	20	1.5×10^5	

9.7	20	10	7.7×10^4	7.1×10^6
	20	20	1.3×10^5	
10.2	20	10	6.7×10^4	6.1×10^6
	20	20	1.1×10^5	
11.2	20	10	4.6×10^4	4.7×10^6
	20	20	9.6×10^4	

Table S8.

Gly-His/Trp-Gly				
$\gamma_H=2.3, \gamma_W=1.7, \chi_H=0.23, \chi_W=0.16, T_1^H=16 \mu s, T_1^W=100 \mu s, k_H/k_W=1$ (NH ₃ ⁺ Gly-His/ NH ₃ ⁺ Trp-Gly, NH ₂ Gly-His/NH ₂ Trp-Gly), $k_H/k_W=2$ (NH ₃ ⁺ Gly-His/NH ₂ Trp-Gly), $k_H/k_W=0.5$ (NH ₂ Gly-His/NH ₃ ⁺ Trp-Gly)				
pH*	C _H , mM	C _W , mM	$k_r \times C_W, s^{-1}$	$k_r, M^{-1}s^{-1}$
6.5	20	10	7.8×10^4	8.4×10^6
	20	20	1.8×10^5	
7.6	20	10	9.2×10^4	8.6×10^6
	20	20	1.6×10^5	
8.3	20	10	7.0×10^4	6.5×10^6
	20	20	1.2×10^5	
8.6	20	10	4.8×10^4	4.7×10^6
	20	20	9.2×10^4	
9.1	20	10	4.0×10^4	3.6×10^6
	20	20	6.3×10^5	
10.7	20	10	3.3×10^4	2.8×10^6
	20	20	4.5×10^5	

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