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Electronic Supplementary Information to:

Kinetic Evidence for Transiently Shifted Acidity Constant of Histidine Linked to Paramagnetic Tyrosine Probed by Intramolecular Electron Transfer in Oxidized Peptides

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Table S1. pK_a^* values for functional groups of peptides containing His and Tyr residues, and corresponding pK_a values calculated according to the formula pK_a=0.929pK_a^*+0.42.

Peptide	Imidazole		Amino group		Phenol	
	pK_a^*	pK_a	pK_a^*	pK_a	pK_a^*	pK_a
N-Ac-His-Tyr	7.1	7.0	n/a	n/a	10.5	10.2
N-Ac-Tyr-His	7.4	7.3	n/a	n/a	10.5	10.3
N-Ac-His-Gly-Tyr	7.1	7.0	n/a	n/a	10.6	10.3
N-Ac-Tyr-Gly-His	7.3	7.2	n/a	n/a	10.4	10.1
His-Tyr	6.1	6.1	7.8	7.7	10.6	10.3
Tyr-His	6.8	6.7	7.7	7.6	10.4	10.1
His-Gly-Tyr	5.9	5.9	7.8	7.7	10.5	10.2
Pro-His-Tyr	6.7	6.6	8.8	8.6	10.6	10.3

Simulation of CIDNP kinetics detected in photoinduced reactions between 3,3′,4,4′-tetracarboxy benzophenone and peptides containing His and Tyr residues

To determine the rate of electron transfer from tyrosine residue to histidine radical in peptides, a simulation of the CIDNP kinetics was performed. The set of equations introduced by Fischer for cyclic radical reactions[3] was modified to take into account the reduction reaction. The peculiarity of the photosystem under study is that recombination of radical pairs containing a 3,3′,4,4′-tetracarboxy benzophenone (TCBP) radical partly proceeds from the triplet state, resulting in the formation of TCBP in its protonated triplet state and His or Tyr in their ground state. 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. 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 two-component photosystem (TCBP and individual amino acid).[4] In the present investigation, the developed simulation procedure was extended to the three-component photosystem, allowing to determine the rate constant of reduction reaction.

In the reaction of triplet excited TCBP with the the mixture of derivatives of His and Tyr, three kinds of radicals are formed: the TCBP radical (concentration R_{B}), the peptide radical with radical center at His residue (concentration R_{H}), and the peptide radical with radical center at Tyr residue (concentration R_{Y}). The obsernved rate constnt of electron transfer is a sum of the first order rate constant of intramolecular reaction and the pseudo-first order intermolecular reaction, which is a product of second-order rate constant and concentration of peptide (C): $k_{\mathrm{e}}^{\mathrm{obs}} = k_{\mathrm{e}}^{\mathrm{intra}} + k_{\mathrm{e}}^{\mathrm{inter}}C$. It is assumed that the fraction of a geminate recombination is negligibly small, and the formation of radicals is instantaneous on the time scale of CIDNP. The concentrations of the radicals are described by the system of algebraic equations:

$$R_{\mathrm{B}}(t) = \frac{R_0}{1+k_{\mathrm{t}}R_0 t} \qquad \text{(s1)}$$

$$R_{\mathrm{H}}(t) = \frac{\alpha R_0 e^{-k_{\mathrm{e}}^{obs}t}}{1+k_{\mathrm{t}}R_0 t} \qquad \text{(s2)}$$

$$R_{\mathrm{Y}}(t) = \frac{R_0(1-\alpha R_0 e^{-k_{\mathrm{e}}^{obs}t})}{1+k_{\mathrm{t}}R_0 t} \qquad \text{(s3)}$$

Here, k_{t} is the rate constant of termination reaction between the dye radical and histidine radical or tyrosine radical. The initial concentrations of radicals are: R_0 for TCBP radicals, αR_0 for His radicals, and $(1-\alpha)R_0$ for Tyr radicals. The parameter α is determined by the quenching rate constants of the triplet excited dye by histidine or tyrosine (residues): $\alpha = k_q^H/(k_q^H + k_q^Y)$. This parameter was calculated from signal intensities in geminate CIDNP spectra.

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

$$\frac{\mathrm{d}P_H^R}{\mathrm{d}t} = -\, k_t P_H^R R_B - (1 - 2\chi_H) k_t \beta_H R_H R_B - \frac{P_H^R}{T_1^H} - k_e^{obs} P_H^R \qquad \text{(s9)}$$

$$\frac{\mathrm{d}P_H}{\mathrm{d}t} = k_t P_H^R R_B + (1 - 2\chi_H) k_t \beta_H R_H R_B + k_e^{obs} P_H^R \qquad \text{(s10)}$$

$$\frac{\mathrm{d}P_Y^R}{\mathrm{d}t} = -\, k_t P_Y^R R_B - (1 - 2\chi_Y) k_t \beta_Y R_Y R_B - \frac{P_Y^R}{T_1^Y} - k'_{ex} P_Y^R \qquad \text{(s11)}$$

$$\frac{\mathrm{d}P_Y}{\mathrm{d}t} = k_t P_Y^R R_B + (1 - 2\chi_Y) k_t \beta_Y R_Y R_B + k'_{ex} P_Y^R \qquad \text{(s12)}$$

Here, χ_H and χ_Y are the probabilities of triplet recombination of the radical pairs of TCBP radical, and histidine or tyrosine radical, respectively. T_1^H and T_1^Y are the relaxation times of proton H2 of His or protons H3,5 of Tyr. 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 to the geminate polarization: β_H and β_Y stand for the pairs of a TCBP radical with a His or a Tyr radical, $\beta_H = \gamma_H P_H^G/(\alpha R_0)$, $\beta_Y = \gamma_Y P_Y^G/((1-\alpha)R_0)$, with P_H^G and P_Y^G denoting the geminate polarization formed for protons of His and Tyr in the corresponding radical pairs with 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 $P_H^G = -P_H^R = 1$, $P_Y^G = -P_Y^R = 1$. The involvement of a Tyr radical in the degenerate electron exchange with the parent Tyr molecule when the latter is (partly) present in the solution with phenol in its anionic form is taken into account by using the term $k'_{ex} P_Y^R$ in equations s5 and s6; k'_{ex} is the product of the second-order exchange rate constant k_{ex} and the concentration of the tyrosinate anion. In this reaction, polarization is transferred from the Tyr radical to ground-state Tyr.

Equations s13, s14 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.

$$\frac{\mathrm{d}P_B^R}{\mathrm{d}t} = -k_t P_B^R R_H - (1 - 2\chi_H) k_t \beta_H R_H R_B - k_Y P_B^R R_Y - (1 - 2\chi_Y) k_t \beta_Y R_Y R_B - \frac{P_B^R}{T_1^B} \qquad \text{(s13)}$$

$$\frac{\mathrm{d}P_B}{\mathrm{d}t} = (1 - \chi_H) k_t P_B^R R_H + (1 - \chi_H) k_t \beta_H R_H R_B + (1 - \chi_Y) k_t P_B^R R_Y + (1 - \chi_Y) k_t \beta_Y R_Y R_B \qquad \text{(s14)}$$

The initial polarization (formed during the geminate stage) of TCBP consists of contributions of opposite sign formed in the radical pairs with a His radical and with a Tyr radical. The contributions are proportional to the concentration of the corresponding radical pairs, that are α and (1-α). The proportionality coefficients are so-called CIDNP enhancement factors F_{H} and F_{Y} (which depend on the magnetic-resonance parameters of the radicals in the radical pair):

$$P_{\mathrm{B}}^{\mathrm{G}} \sim F_{\mathrm{H}}\alpha + F_{\mathrm{Y}}(1-\alpha) \quad \text{(s15)}$$

Since the vertical scaling factor appears as fitting parameter, only ratio $F_{\mathrm{Y}}/F_{\mathrm{H}}$ should be known:

$$P_{\mathrm{B}}^{\mathrm{G}} \sim 1 + \frac{F_{\mathrm{Y}}(1-\alpha)}{F_{\mathrm{H}}\alpha} \quad \text{(s16)}$$

The parameters known from previous investigations were: T_1^{H}, T_1^{Y}, T_1^{B}. The values χ and γ were determined from the simulation of the CIDNP kinetics obtained for the photoreaction of TCBP and amino acids. The value $F_{\mathrm{Y}}/F_{\mathrm{H}}$ was taken the same as found previosly for N-acetylated amino acids. The fitting parameters for each data set including three CIDNP kinetics, for TCBP, His, and Tyr, were the vertical scaling factors, $k_{\mathrm{e}}^{\mathrm{obs}}$, $k_{\mathrm{t}}R_0$.

From the analysis, intensities of the following signals were used: H2 of His, H3,5 of Tyr, H6,6′ of TCBP. In case of overlaping signals of H4 of His and H3,5 of Tyr, CIDNP kinetics of H3,5 of Tyr was reconstructed by subtracting 0.75 of signal intensity of H2 of His (signal H4 of His demonstrates the same shape in its CIDNP kinetics as H2, with the intensity of 75%). The parameters of simulations for different pairs of reactants and the examples of CIDNP kinetics are given in the next sections.

Parameters used in the simulations of CIDNP kinetics detected in photoinduced reactions between 3,3′,4,4′-tetracarboxy benzophenone and peptides containing His and Tyr residues, and best-fit values the observed electron transfer rate constant $k_\mathrm{e}^\mathrm{obs}$ at peptide concentration *C*

List of parameters:

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

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

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

χ_Y – the probability of triplet recombination of the radical pairs of TCBP radical and tyrosine radical

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

T_1^Y– nuclear paramagnetic relaxation time for H3,5 protons of tyrosine radical

$F_\mathrm{Y}/F_\mathrm{H}$ – ratio of enhanecement factors for CIDNP of H6,6′ of TCBP, formed in the pair of TCBP radical with tyrosine radical, and TCBP radical with histidine radical

Table S1.

N-AcHis-Tyr		
γ_H=2.3, γ_Y=1.4, χ_H=0.22, χ_Y=0.22, T_1^H=10 µs, T_1^Y=30 µs, $F_\mathrm{Y}/F_\mathrm{H}$=–1.3.		
pH*	*C*, mM	$k_\mathrm{e}^\mathrm{obs}$, s^{-1}
7.1	20	4.8×10^4
	40	4.6×10^4
7.6	40	9.3×10^4
8.2	20	1.2×10^5
	40	1.7×10^5
8.6	20	1.8×10^5
9.0	20	2.6×10^5
	40	4.2×10^5
9.5	20	5.1×10^5
	40	1.3×10^6
N-AcTyr-His		
7.7	20	3.3×10^4
	40	4.1×10^4
8.1	20	5.8×10^4
	40	9.5×10^4

8.7	20	1.1×10^5
	40	1.8×10^5
9.1	20	1.7×10^5
	40	3.6×10^5
9.5	20	4.0×10^5
	40	9.3×10^5
N-AcHis-Gly-Tyr		
6.9	20	3.9×10^4
	40	3.8×10^4
7.5	20	5.1×10^4
	40	6.3×10^4
8.1	20	$8,7\times10^4$
	40	1.2×10^5
8.5	20	1.4×10^5
	40	2.0×10^5
9.0	20	2.9×10^5
	40	5.3×10^5
9.5	20	6.5×10^5
	40	1.3×10^6
N-AcTyr-Gly-His		
7.6	20	3.8×10^4
	40	4.4×10^4
8.2	20	5.3×10^4
	40	8.7×10^4
9.0	20	2.1×10^5
	40	4.5×10^5
9.5	20	4.0×10^5
	40	7.5×10^5
His-Tyr		
6.1	40	8.7×10^4
6.2	20	6.6×10^4
	40	9.6×10^4
6.6	20	1.2×10^5
	40	1.4×10^5

7.4	20	2.3×10^{5}
	40	3.3×10^{5}
8.4	20	3.4×10^{5}
	40	4.6×10^{5}
9.0	20	4.0×10^{5}
	40	7.9×10^{5}
9.5	20	7.2×10^{5}
	40	1.4×10^{6}
Tyr-His		
6.6	20	1.7×10^{4}
	40	2.1×10^{4}
7.0	20	3.3×10^{4}
	40	5.4×10^{4}
7.6	20	1.0×10^{5}
	40	1.2×10^{5}
8.2	20	1.7×10^{5}
	40	2.5×10^{5}
8.9	20	2.9×10^{5}
	40	5.5×10^{5}
9.5	20	4.6×10^{5}
	40	9.5×10^{5}
His-Gly-Tyr		
6.2	20	1.1×10^{5}
	40	1.5×10^{5}
7.1	20	2.3×10^{5}
7.4	40	3.2×10^{5}
8.0	20	4.3×10^{5}
	40	5.9×10^{5}
8.5	20	4.5×10^{5}
	40	8.3×10^{5}
9.0	20	6.9×10^{5}
	40	1.3×10^{6}
9.5	20	1.0×10^{6}
Pro-His-Tyr		

6.5	20	5.3×10^4
	40	1.0×10^5
6.9	20	6.8×10^4
	40	1.1×10^5
7.4	20	1.1×10^5
	40	1.8×10^5
8.2	20	2.5×10^5
	40	3.6×10^5
9.0	20	5.7×10^5
	40	7.3×10^5
9.2	20	5.9×10^5
	40	8.7×10^5
9.5	20	8.1×10^5
	40	1.2×10^5

CIDNP kinetics detected in photoinduced reaction between 3,3′,4,4′-tetracarboxy benzophenone and peptide His-Tyr

For Tyr, absolute value of polarization (emission) is shown. Vertical scaling is ajusted for calculated initial polarization equal to 1.

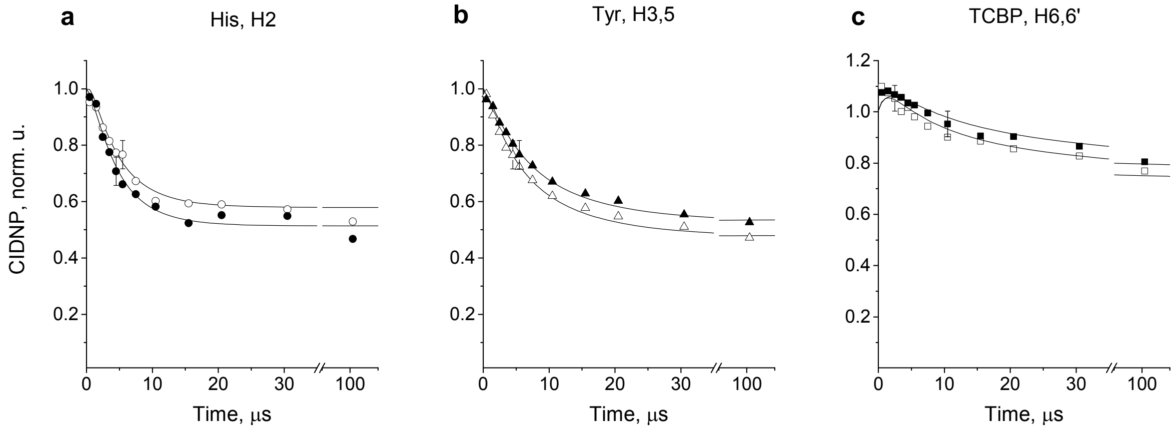

Figure S1. CIDNP kinetics for the H2 proton of His residue (a), H3,5 protons of Tyr residue, and H6,6′ protons of TCBP (c), obtained during the photoreaction between TCBP with His-Tyr at pH* 6.2 at 20 mM (open symbols) and 40 mM (solid symbols) of His-Tyr.

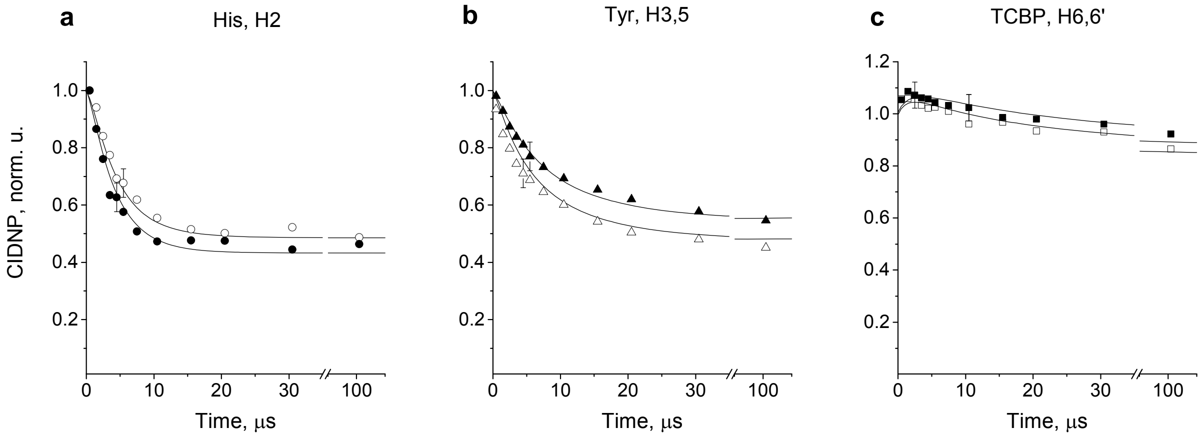

Figure S2. CIDNP kinetics for the H2 proton of His residue (a), H3,5 protons of Tyr residue, and H6,6′ protons of TCBP (c), obtained during the photoreaction between TCBP with His-Tyr at pH* 6.6 at 20 mM (open symbols) and 40 mM (solid symbols) of His-Tyr.

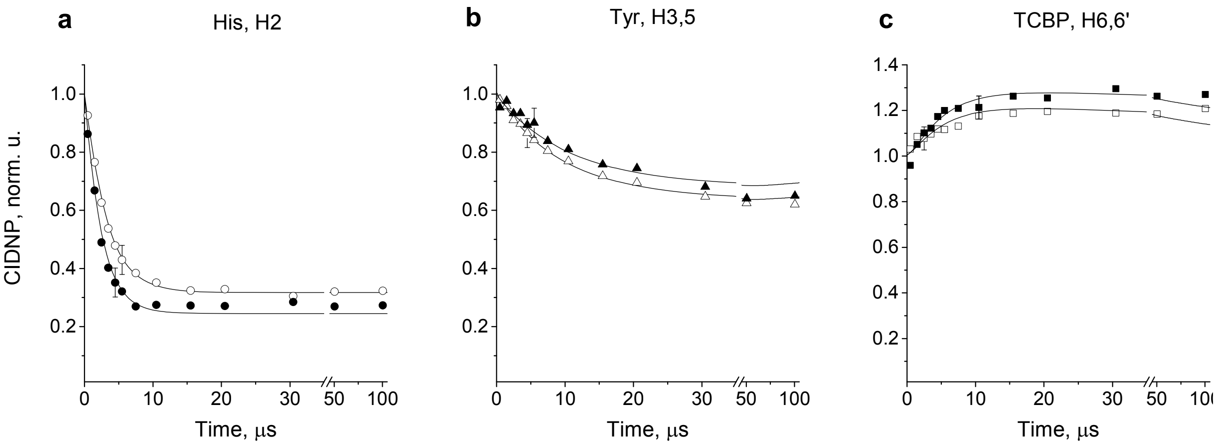

Figure S3. CIDNP kinetics for the H2 proton of His residue (a), H3,5 protons of Tyr residue, and H6,6′ protons of TCBP (c), obtained during the photoreaction between TCBP with His-Tyr at pH* 7.4 at 20 mM (open symbols) and 40 mM (solid symbols) of His-Tyr.

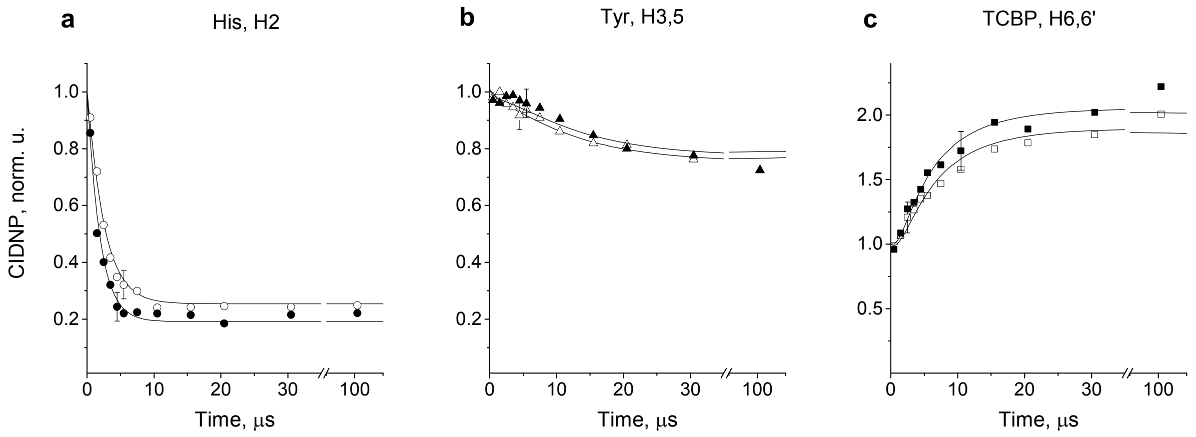

Figure S4. CIDNP kinetics for the H2 proton of His residue (a), H3,5 protons of Tyr residue, and H6,6′ protons of TCBP (c), obtained during the photoreaction between TCBP with His-Tyr at pH* 8.4 at 20 mM (open symbols) and 40 mM (solid symbols) of His-Tyr.

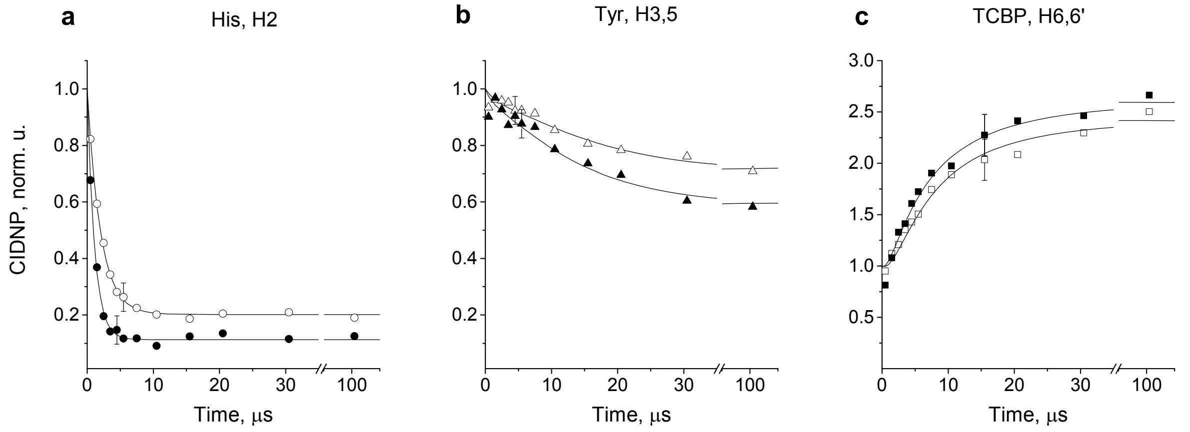

Figure S5. CIDNP kinetics for the H2 proton of His residue (a), H3,5 protons of Tyr residue, and H6,6′ protons of TCBP (c), obtained during the photoreaction between TCBP with His-Tyr at pH* 9.0 at 20 mM (open symbols) and 40 mM (solid symbols) of His-Tyr.

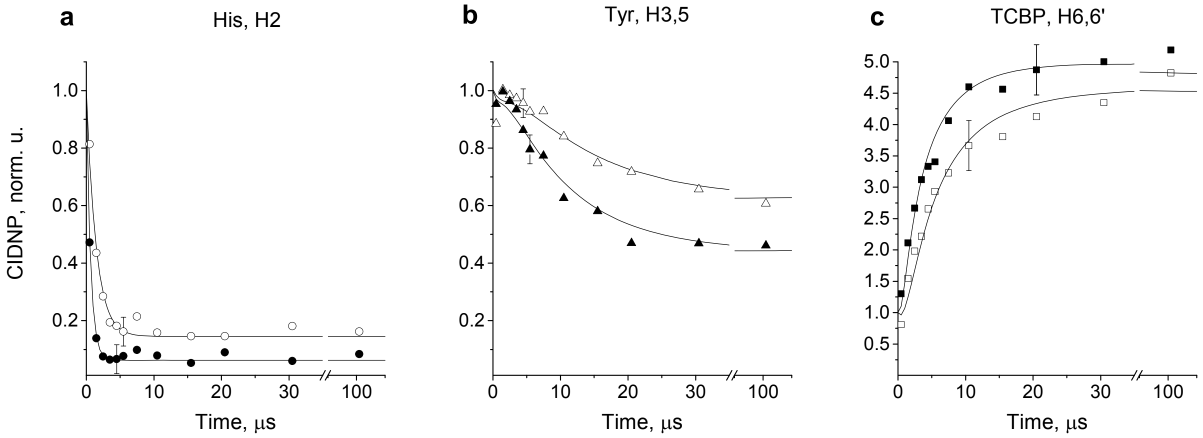

Figure S6. CIDNP kinetics for the H2 proton of His residue (a), H3,5 protons of Tyr residue, and H6,6′ protons of TCBP (c), obtained during the photoreaction between TCBP with His-Tyr at pH* 9.5 at 20 mM (open symbols) and 40 mM (solid symbols) of His-Tyr.

pH*-dependences of the observed rate constant of electron transfer $k_\mathrm{e}^\mathrm{obs}$ from tyrosine residue to histidine radical in peptides

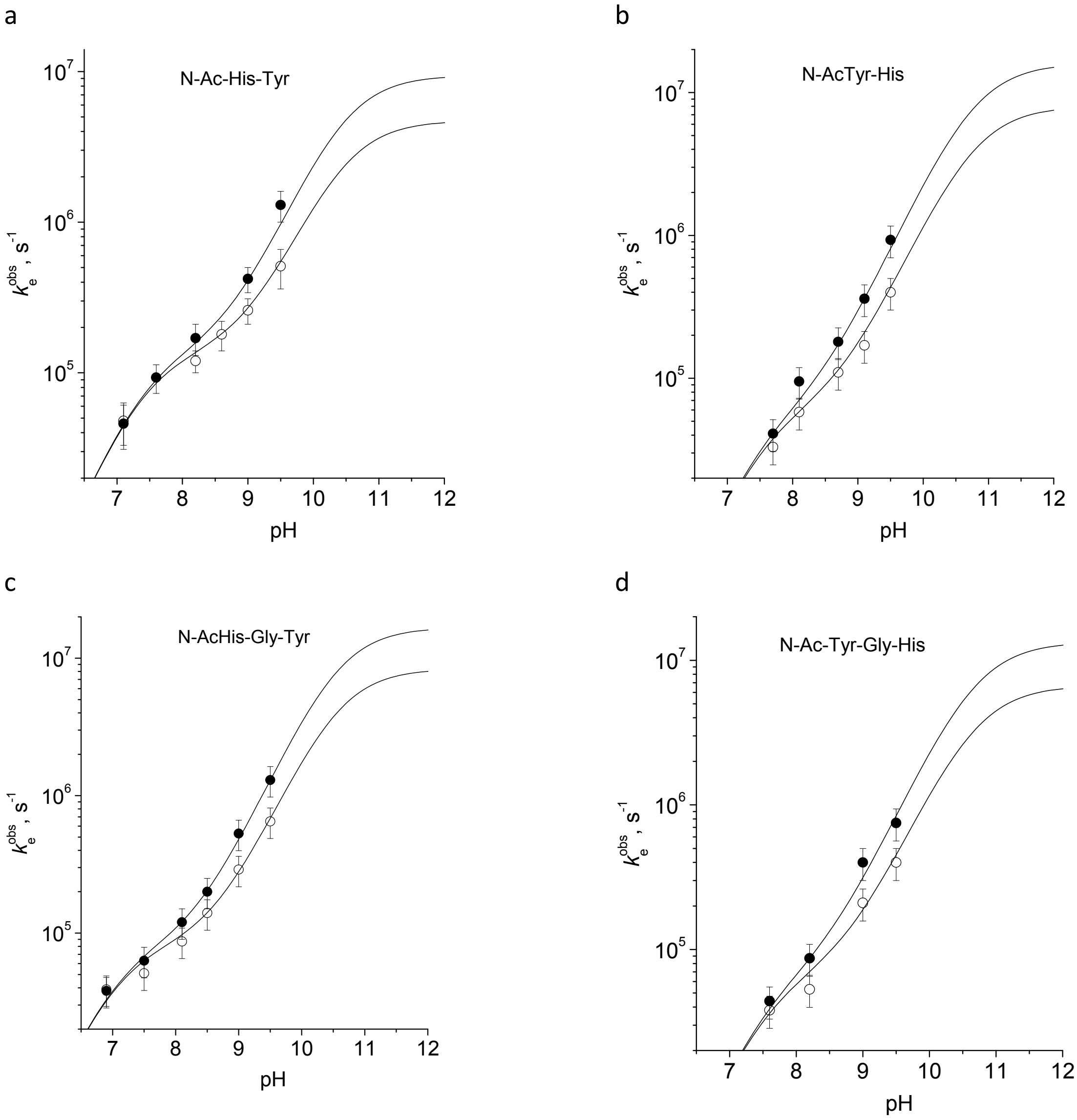

Fig. S7. pH*-dependence of the observed rate constant of electron transfer from tyrosine resudue to histidine radical in N-acetylated peptides: a – NacHis-Tyr, b – N-AcTyr-His, c – N-AcHis-Gly-Tyr, d – N-AcTyr-Gly-His.

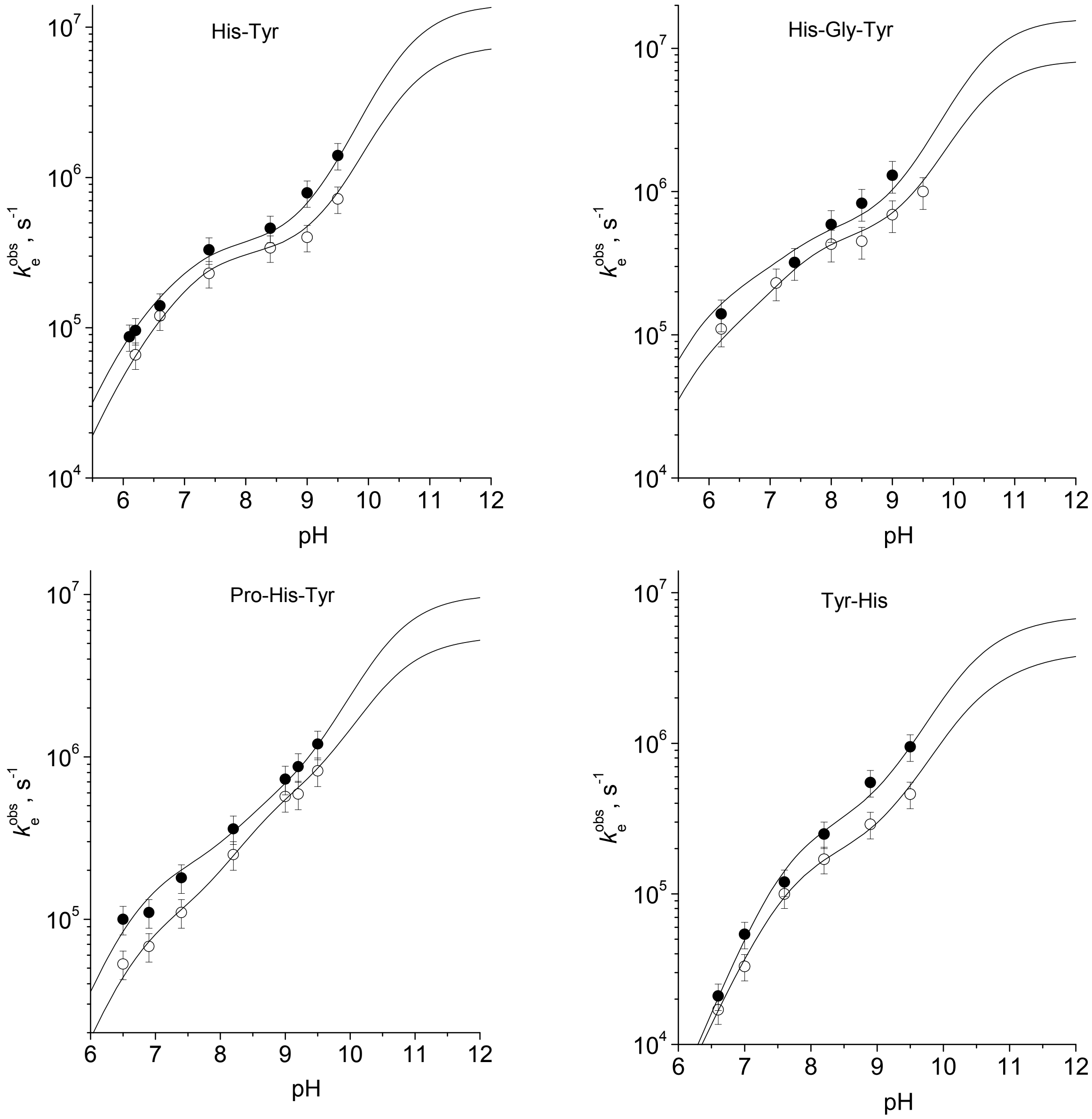

Fig. S8. pH*-dependence of the observed rate constant of electron transfer from tyrosine resudue to histidine radical in non-acetylated peptides: a –His-Tyr, b – His-Gly-Tyr, c – Pro-His-Tyr, d – Tyr-His.

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