Time-resolved CIDNP characterization of kynurenic acid radicals generated in photoinduced reactions with tryptophan and tyrosine

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Introduction

It was established that the mechanism of photoreaction of 4-hydroxy quinoline (4HQN) or kynurenic acid (KNA) in anionic form with Tyr is H-transfer, with Trp – proton-coupled electron transfer (PCET), which is confirmed by quantitative analysis of the kinetics of chemically induced dynamic nuclear polarization (CIDNP).
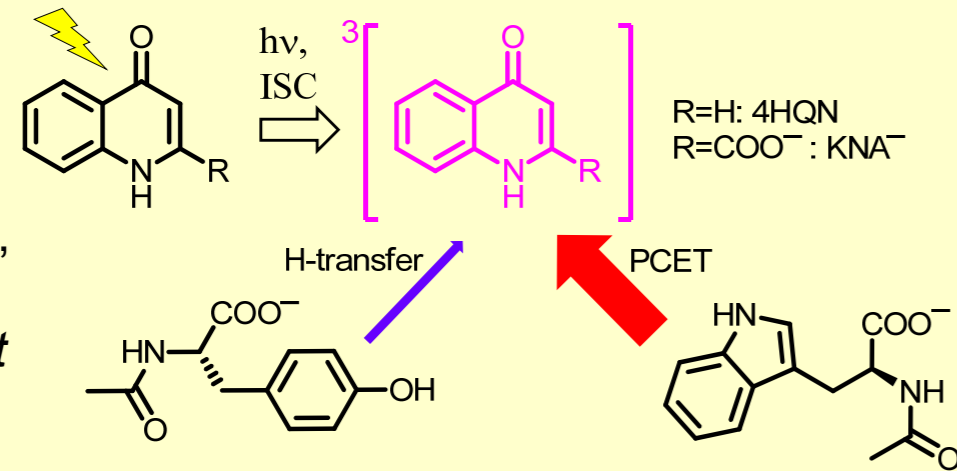
Y. S. Zhuravleva *et al.*, *J. Photochem. Photobiol. A* **2020**, *396*, 112522;
O. B. Morozova *et al.*, *PCCP* **2021**, *23*, 22483).

Objectives: identification of radical structures formed as a result of triplet quenching and determination of the pathways of H/H+ transfer

R=H: 4HQN
R=COO−: KNA−

## Methods: time-resolved CIDNP and DFT

Advantages:
- High spectral resolution of NMR spectroscopy allows for straightforward assignment of hyperfine coupling constants (HFCCs) in transient radicals to individual nuclei in diamagnetic products
- Laser flash photolysis in inconvenient because of overlapping spectra of short-lived reaction intermediates
- Comparison of NMR signal intensities in geminate CIDNP spectra and HFCCs calculated by DFT method opens the way to establish a detailed reaction scheme

Proportionality between HFCCs and CIDNP intensities

A simple proportionality relation between the HFCC of a nucleus at the radical stage and geminate CIDNP intensity of this nucleus is fulfilled for solutions of low viscosity for the situation where the number of magnetic nuclei in the system is sufficiently large (≥5-7):
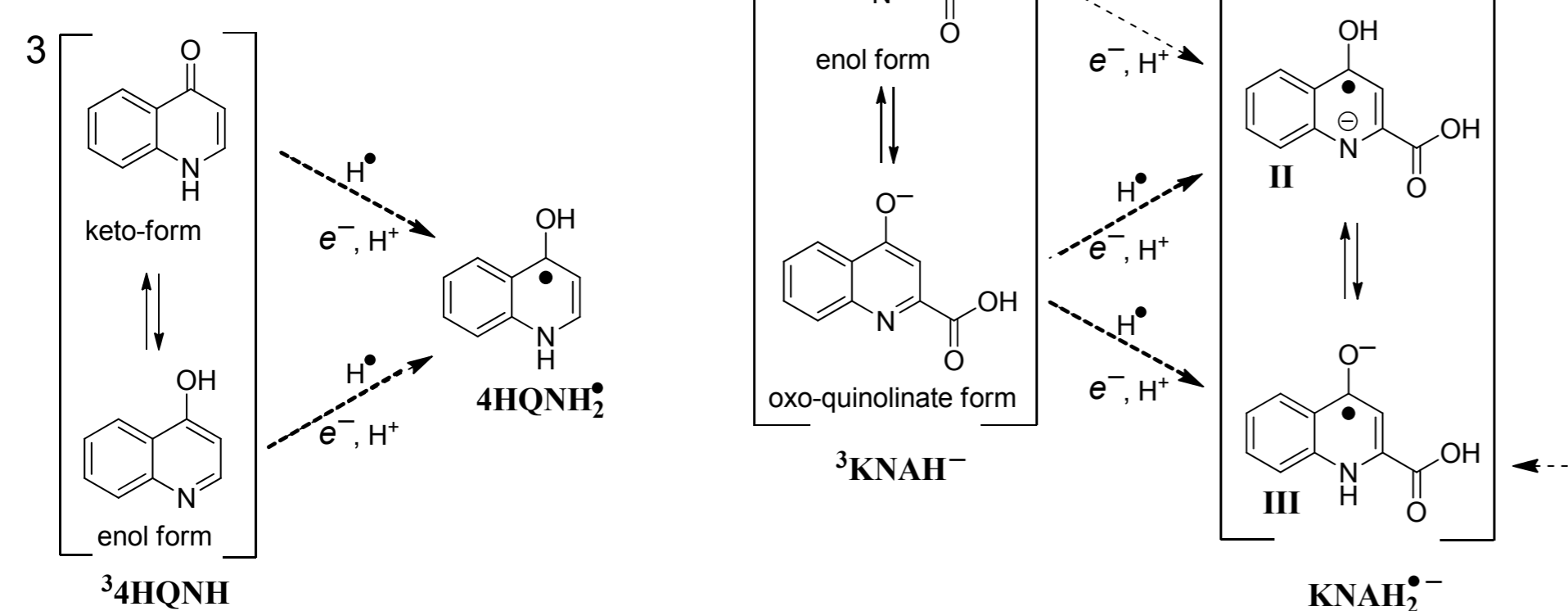
$CIDNP = sgn(\Delta g) \cdot C \cdot A$ (here C is the proportionality constant).

Thus, CIDNP pattern of diamagnetic products of radical recombination contains the 'fingerprints' of the transient radicals.

Experimental protocol

$\tau = 0 \Rightarrow$ geminate CIDNP spectrum
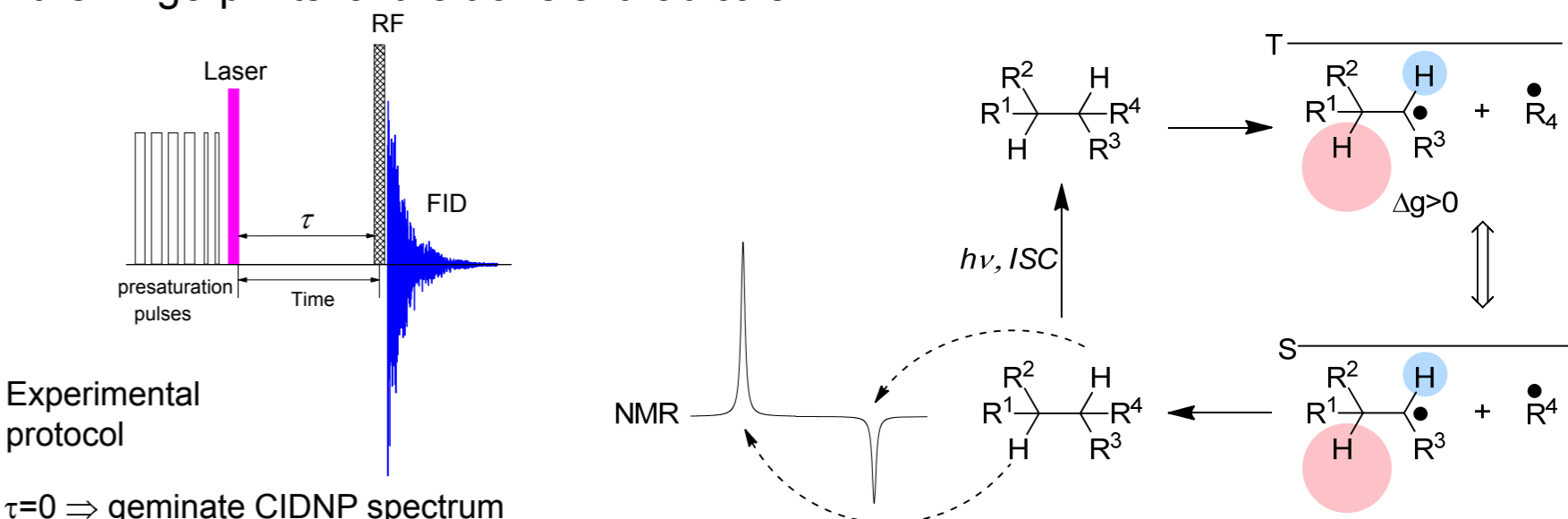
Possible pathways of PCET (e−, H+) or H-transfer (H+) to ³KNAH or ³4HQN

Each tautomeric form of ³KNAH− can be a parent for two forms of radical species. For ³4HQNH the scheme of possible reactions is greatly simplified: the number of tautomers is reduced to two, from which a single radical can be formed.

³4HQNH — keto-form / enol form — 4HQNH₂•

³KNAH− — keto-form / enol form / oxo-quinolinate form → I, II, III (KNAH₂•−)

¹H NMR spectra of 4HQN (a) and KNA (b). Geminate ¹H CIDNP spectra detected in the photoinduced reaction of 4HQN (c) and KNA (d) with N-AcTyr; 4HQN (e) and KNA (f) with N-AcTrp
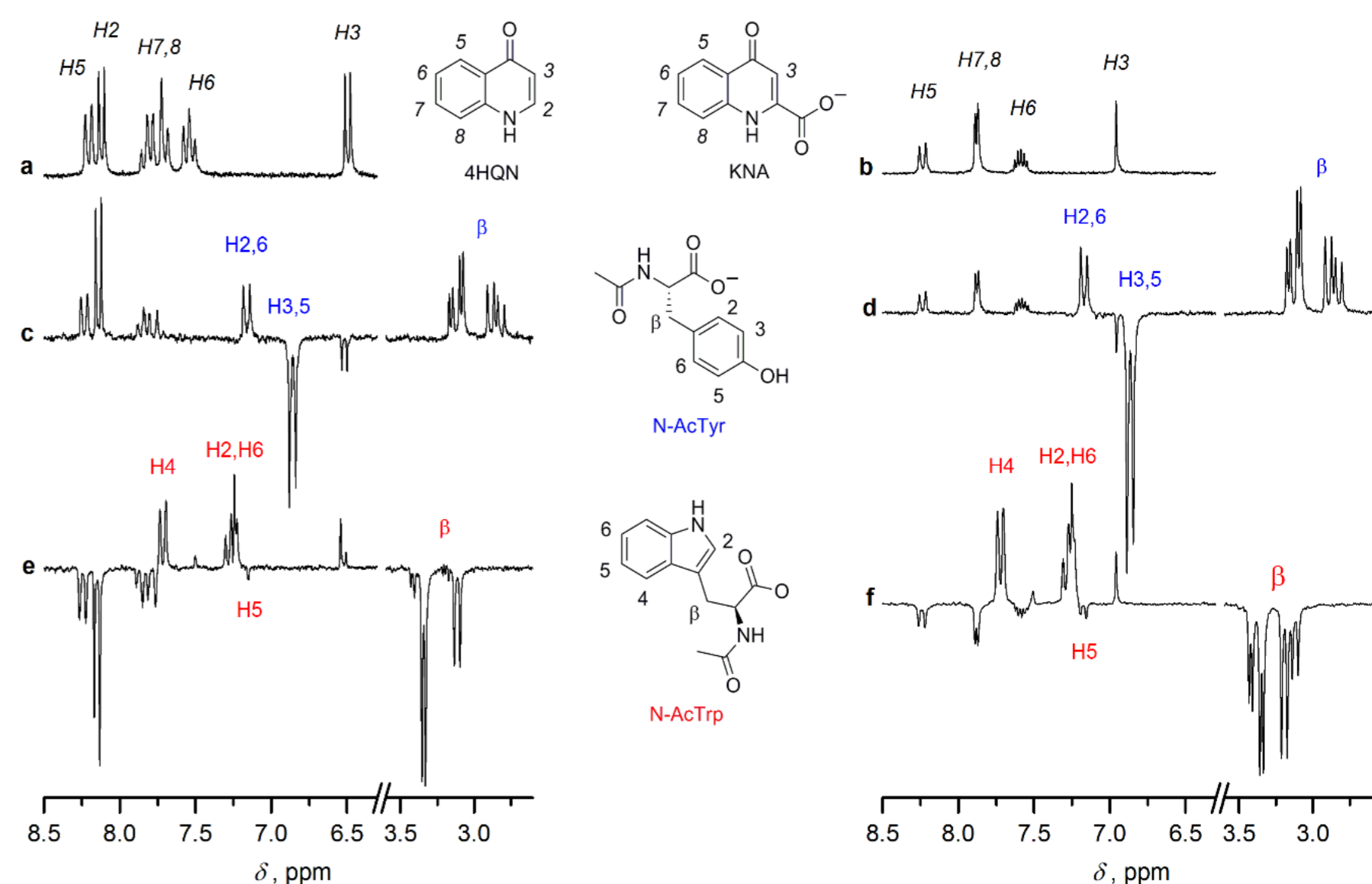
## Calculated ¹H HFCCs and g-factors of KNA and 4HQN radicals

Initial compound	Radical	g-factor	H3	H5	H6	H7	H8	H2
4HQNH	4HQNH₂•	2.00295	0.173	-0.44	-0.016	-0.328	-0.194	-0.826
KNAH	KNAH₂•− (I)	2.00325	0.206	-0.288	-0.11	-0.176	-0.224	-
	KNAH₂•− (II)	2.00339	0.003	-0.025	-0.348	0.067	-0.338	-
	KNAH₂•− (III)	2.00364	-0.239	-0.065	-0.14	-0.011	-0.167	-

4HQNH₂• KNAH₂•− (I) (II) (III)
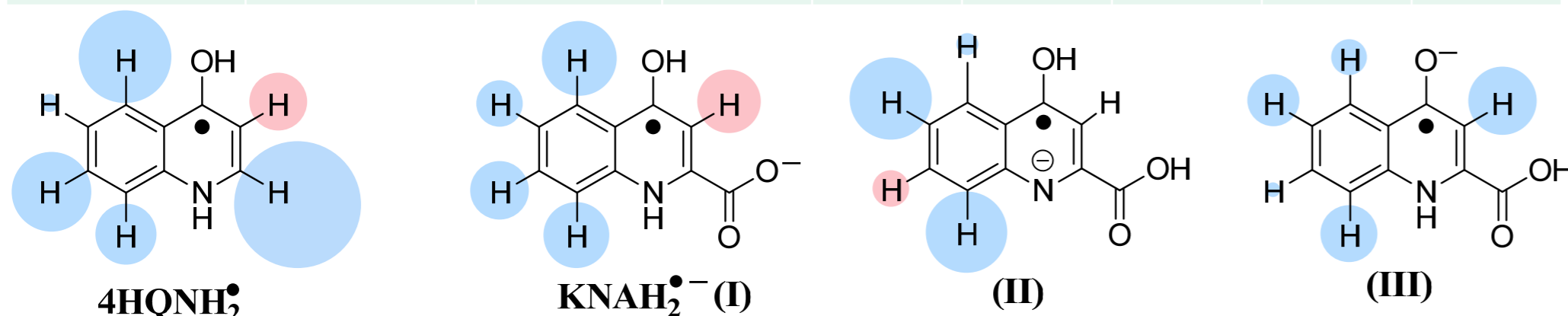
KNA radicals differ by not only spin density distribution, but also by g-factor. Thus, to reveal contributions of KNA radicals into the observed CIDNP effects, it was necessary to calculate CIDNP intensities formed in the radical pairs consisting of amino acid radical and KNAH₂•− radical I, II, or III using Adrians's model.

Scheme of photoinduced reaction between KNAH− and TrpH (TyrOH)

The absence of the contribution of radical KNAH₂•− (III) proves that H/H+ transfer occurs to the oxygen but not to the nitrogen of KNA, otherwise KNAH₂•− (III) could be formed after H-transfer to oxo-quinolinate form of KNA, or after H+-transfer to its reduced form. Thus, we suggest the scheme for the photoreaction of KNA with Tyr or Trp.
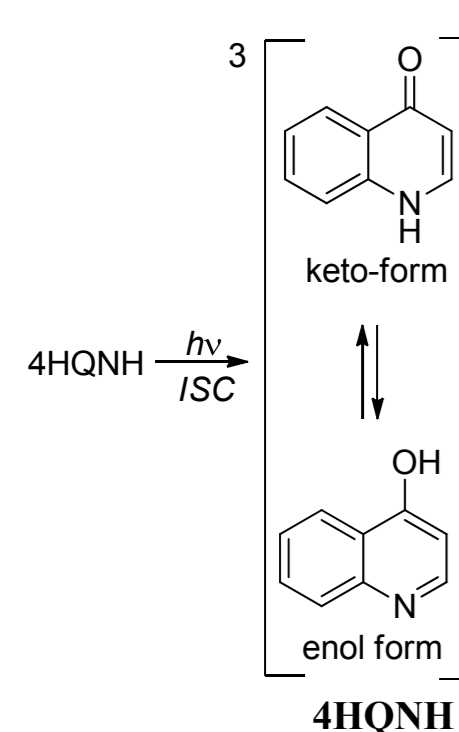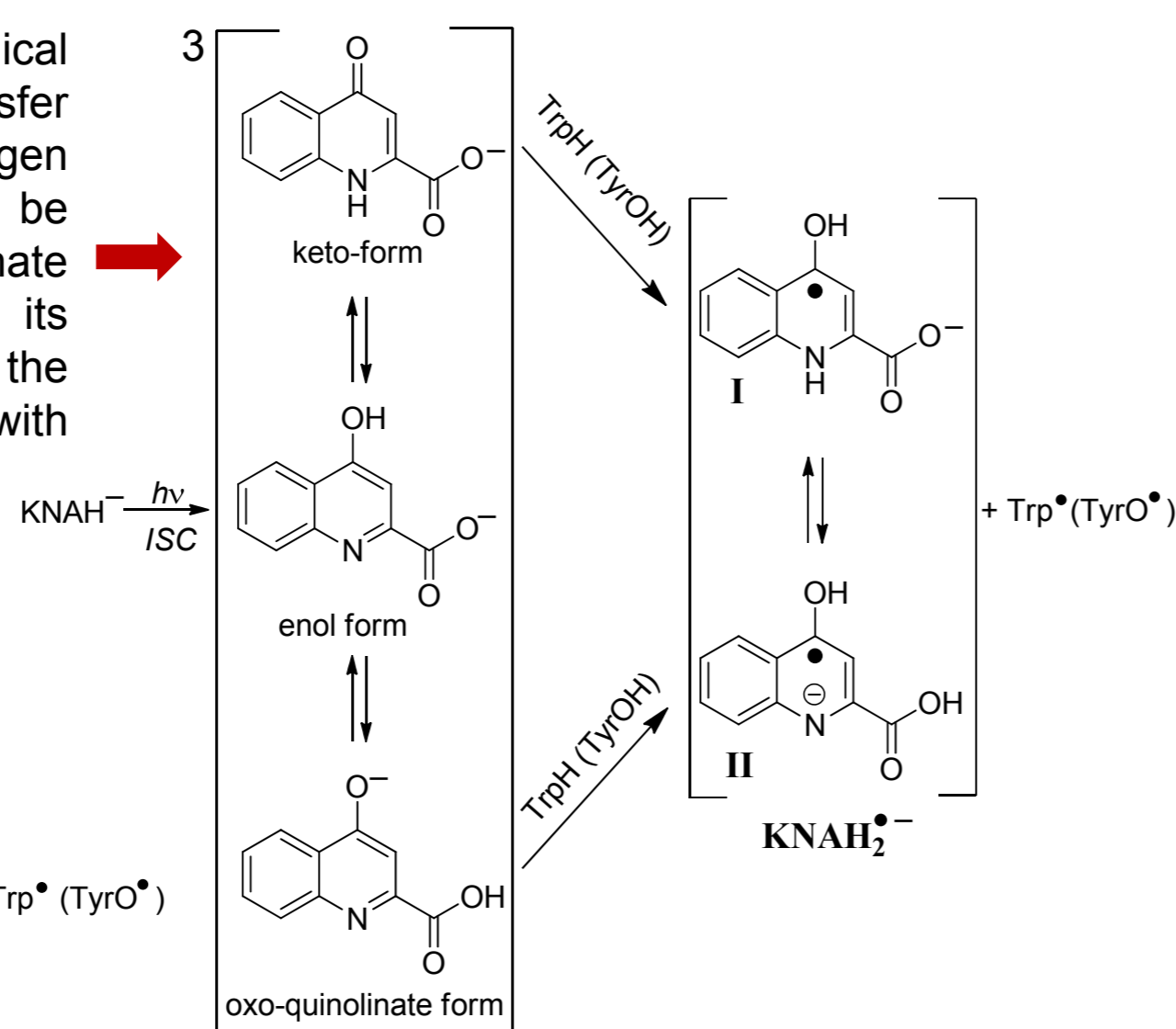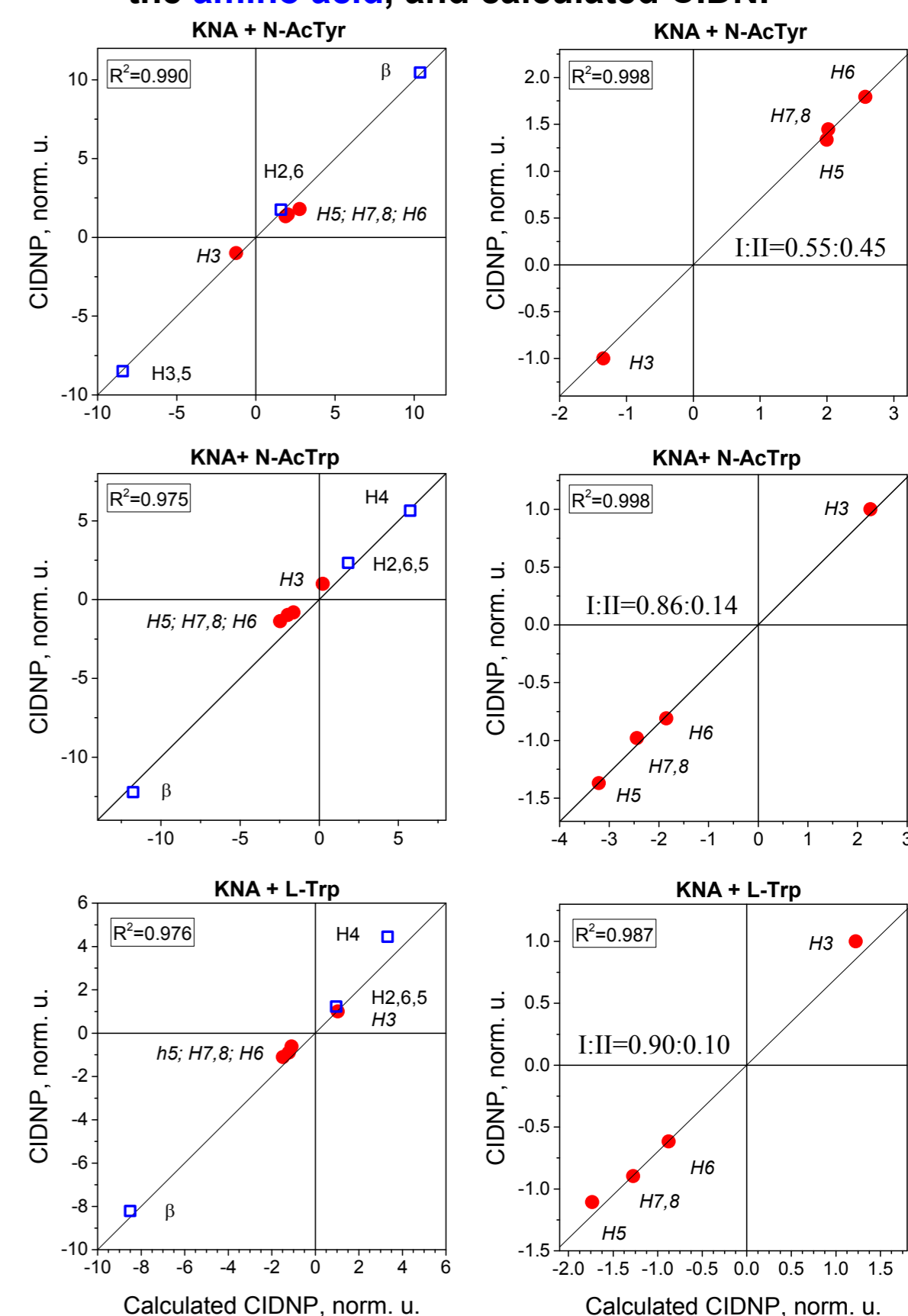
By analogy, scheme was written for the photoreaction of 4HQN with Tyr or Trp

Correlation between CIDNP intensities of 4HQN and the amino acid, and HFCCs of the radicals

4HQN + N-AcTyr R²=0.985
4HQN + N-AcTrp R²=0.970
KNA + L-Trp R²=0.980
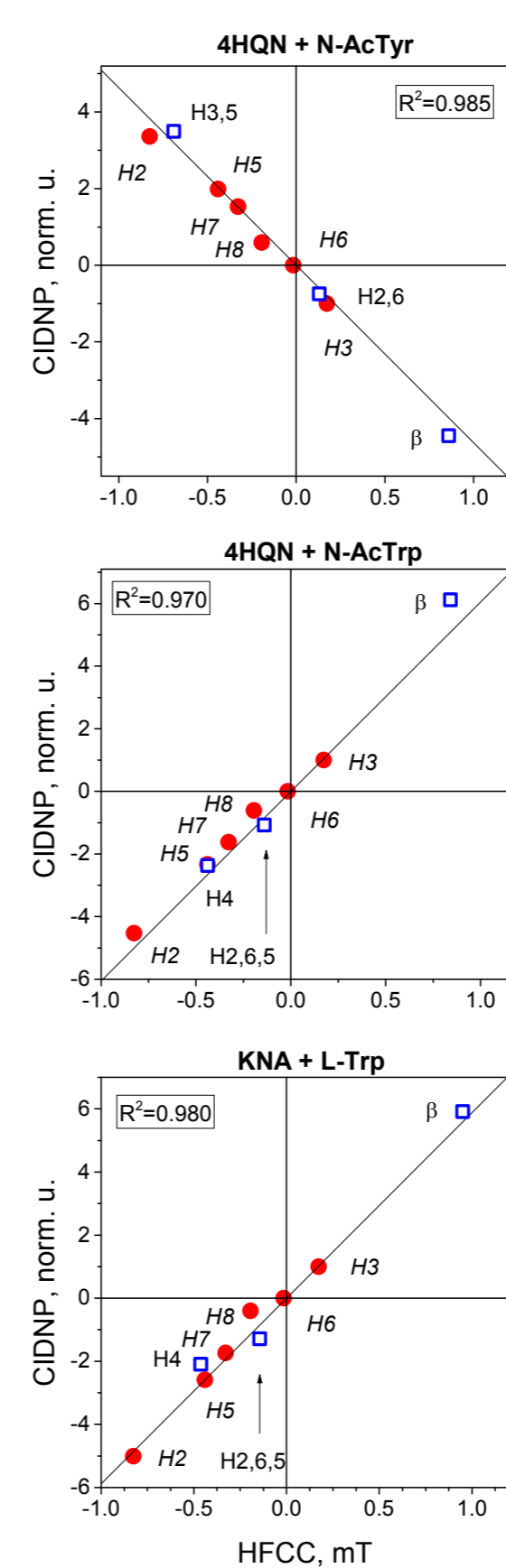
Radicals: 4HQNH₂•, TyrO•, Trp•

Correlation between CIDNP intensities of KNA and the amino acid, and calculated CIDNP

KNA + N-AcTyr R²=0.990
KNA + N-AcTyr R²=0.998 I:II=0.55:0.45
KNA+ N-AcTrp R²=0.975
KNA+ N-AcTrp R²=0.998 I:II=0.86:0.14
KNA + L-Trp R²=0.976
KNA + L-Trp R²=0.987 I:II=0.90:0.10

Analysis of KNA pattern only (left) revealed that H-transfer from Tyr to KNAH− yields comparable shares of KNAH₂•− radicals I and II (ratios are listed in the plot) whereas after PCET from Trp radical I is predominantly formed.

Conclusion

From the best-fit proportionality relashionship between HFCCs and CIDNP intensities is was found that two tautomeric forms of KNAH₂•− are formed as a result from H/H+ transfer to carbonyl oxygen of keto-form or oxo-quinolinate form. H/H+ transfer to nitrogen of enol form of was ruled out.