The manuscript "Exploration of the close chemical space of tryptophan and tyrosine reveals importance of hydrophobicity in photo- CIDNP performance" describes experimental results of detecting CIDNP under continuous illumination for a set of ten compounds that are analogs of tyrosine or tryptophan. Each compound was taken in the same amount and mixed with each of the two dyes, AT12 or fluorescein, and illuminated for several seconds in the probe of the NMR spectrometer. As a main result, the authors declare that the signal intensity observed under such conditions correlates with the hydrophobicity of the molecules.

This qualitative study of hydrophobicity could only be relevant, if the authors had applied time resolved detection and analyzed the CIDNP signals in the products of geminate recombination, often called geminate CIDNP. Under continuous illumination, however, the resulting CIDNP effects are formed as a result of a complex interplay of spin and molecular dynamics of free radicals, but it is wrong to predict CIDNP intensities based alone on the properties of the reacting diamagnetic molecules as it was done in the manuscript. As is known, some properties of free radicals can be very different from the properties of molecules in the diamagnetic state. For example, the pKa values of radicals (tryptophan and tyrosine) and the same molecules in the diamagnetic state differ by several units. Spin is the magnetic moment of nuclei and electrons; therefore, the “key players” in the formation of geminate CIDNP are the magnetic properties of radicals, as shown by R. Kaptein, namely the hyperfine coupling (HFC) constants, the difference in the g-factor of radicals in the spin-correlated pair, and the magnetic field strength. Polarization in an F-pair, as is also well known, depends on the competition between the rate of paramagnetic nuclear relaxation and the bimolecular rate of radical recombination. They can be different too for the set of chosen compounds. In addition, paramagnetic nuclear relaxation in radicals is determined by the anisotropy of the HFC. None of these parameters is mentioned in the manuscript for the ten compounds studied. I’d like to stress, that Kaptein's work in the field of CIDNP goes much deeper than the simple sign rules.

The CIDNP method in its continuous mode should be applied with great care when used for any quantitative analysis. The concentration of radical pairs in the reaction of a triplet dye with a quencher essentially depends on the quenching rate constant. In the cited work of Saprygina et al. J. Phys. Chem. A 2014, 118, 339-349, the quenching rate constants
were obtained from optical studies, the conditions for the time resolved experiment were carefully adjusted, and only geminate CIDNP was considered for quantitative analysis of the pH dependence. This was not done in the paper under review.

There is a well-documented study where the terms “hydrophobicity” and “hydrophobic collapse” were shown to be misleading for explanation of the absence of CIDNP signals of tryptophan residues in the unfolded HEWL protein under cw-illumination. This work is cited as reference 8 in the reviewed manuscript. In ref 8, the time-resolved CIDNP detection revealed CIDNP tryptophan signals of similar strength at the geminate stage for the unfolded and native state of HEWL. It means, that hydrophobicity was not a relevant parameter for the description of CIDNP in the native and unfolded state of HEWL. Instead, the reaction of intramolecular electron transfer from tyrosine to the tryptophan radical on a microsecond time scale in the unfolded protein was found to be the main cause of a decrease in the Trp signal and an increase in the tyrosine signal.

A direct comparison of the CIDNP data obtained under continuous illumination without measuring the quenching rate constant is inappropriate, since different concentrations of the quencher and different rate constants lead to different concentrations of the formed pair of geminate radicals.


The over-interpretation of the small data set as presented here is misleading. Also, the historical overview of Kaptein’s contribution to CIDNP theory is rather superficial, because it does not go deeper than just application his simple rule for the polarization sign.

With my regret, I recommend to reject the manuscript in its present form.