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Magn. Reson. Discuss., author comment AC1
https://doi.org/10.5194/mr-2021-53-AC1, 2021

Comment on mr-2021-53

Kumaran Baskaran et al.

Author comment on "Anomalous amide proton chemical shifts as signatures of hydrogen bonding to aromatic sidechains" by Kumaran Baskaran et al., Magn. Reson. Discuss., https://doi.org/10.5194/mr-2021-53-AC1, 2021

We are grateful for the insightful questions and helpful comments from both reviewers. Our responses are below.

Reviewer 1

The shifts plotted in Figure 3 are not solely due to ring current shifts. The distance dependence likely also reflects other shift effects that correlate with distance from the protein surface, not related to ring current effects. This may help explain the surprising long-range dependence.

The data in Figure 3 is derived from NMR structures, not crystal structures. Crystal structures (with only a handful of exceptions) don't have chemical shifts and thus lack Z-scores.

It has long been noted that NMR structures differ qualitatively from X-ray crystal structures by being less compact (Clore and Gronenborn; Williamson). The reasons for this are multifaceted. Crystal structures these days are typically determined at low temperatures (and proteins have a well-established thermal expansion; Frauenfelder). Most importantly, packing forces that are not present in solution can play various structure-determining roles. In contrast, NMR structures are not uniquely determined by the experimental data, especially early ones and those at low resolution, and the empirical potential energy functions used in NMR structure determination can play a more prominent role than for crystal structures.

The shift measurements don't provide insight into the nature of the energetic interaction, they merely help confirm proximity and orientation. In principle the distributions could be used to compute a potential of mean force, which could give insight into the energetics, however the discordance between predicted and measured chemical shifts for many atoms suggest that details of aromatic side-chain packing remain imprecise.

The parameterization of the aromatic ring current shift factors for different aromatic amino acid side chains implicitly accounts for the magnetic susceptibility of the protein interior. Undoubtedly this susceptibility is inhomogeneous and different locally, however, implicitly assuming homogeity has enabled good agreement between measured 1H ring current shifts and ring current shifts predicted from high-resolution protein structures. (Hoch, 1983)

These relevant statistics (excluding systems that contain paramagnetic metals) are available on the BMRB web site, https://bmrb.io/ref_info/stats.php?restype=aa&set=filt

We will note this in the revised manuscript.

We will revise multiple figures to address these and other suggestions.

Reviewer 2

We agree, there is nothing in the NMR data that proves the interaction is attractive, however Occam's Razor would imply an attractive interaction as the simplest explanation for the non-random distributions observed. In light of some of our findings, we hope that computational chemists will re-visit the energetics of aromatic interactions with NH groups.

We chose the longer cutoff to illustrate the non-random nature of the distance/orientation distribution. As noted in response to reviewer1, the is fully visible. The long tail of the distribution reflects additional effects, beyond those caused by ring currents.

The question of whether the NH vector points at the ring center or at a ring atom or bond is an interesting and important point. Indeed, in the plots in Figure 4 one can notice a peak near 25° for Phe and Tyr, which is the azimuthal angle expected for amides directly above one of the ring carbons and 3.4 Å from the ring plane. We thank referee Peter Tolstoy for this insight.