Open Access MAGNETIC RESONANCE Discussions

Magn. Reson. Discuss., referee comment RC2
https://doi.org/10.5194/mr-2021-15-RC2, 2021
© Author(s) 2021. This work is distributed under
the Creative Commons Attribution 4.0 License.

Comment on mr-2021-15

Anonymous Referee #2

Referee comment on "Conformational features and ionization states of Lys side chains in a protein studied using the stereo-array isotope labeling (SAIL) method" by Mitsuhiro Takeda et al., Magn. Reson. Discuss., https://doi.org/10.5194/mr-2021-15-RC2, 2021

I can join the gerenal comments by referee 1. My main concern is the one-bond deuterium isotope effect on ^{15}N chemical shifts of Lys66. This is probably not fully protonated juged from the isotope effects (see below). Most interactions will lead to a decrease compared to the free lysines (Williamson, Chem.Commun. 49, 9824, 2013). On the other hand it is too large to be -ND_2 as the effets of amines are of the order or 0.7 ppm (Lycka, 23, 973, 1985). As the experiments are done a a Shigimi tube and from what I can tell no special precautions are taken to take into account the difference in pK_a values in H_2O and D_2O, one could fear that part of the large effect is caused by a change in the equilibrium due to deuteriation, as the pH is in the vicinity ot the pKa value. Therefore, I strongly recommend that the measurements are repeated in a single tube and with varying amounts of D_2O to obtain the isotope effects.