

Magn. Reson. Discuss., referee comment RC2
<https://doi.org/10.5194/mr-2021-15-RC2>, 2021
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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 general 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 judged 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 $-\text{ND}_2$ as the effects of amines are of the order or 0.7 ppm (Lycka, 23, 973, 1985). As the experiments are done in 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 deuteration, as the pH is in the vicinity of the pK_a 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.