Takeda, Kainosho and co-workers present an in-depth investigation of lysine residues in a variant of SNase that has been engineered to harbor a lysine residue in its core, of which the pKa constants are shifted by about 5 units to below physiological pH. They make elegant use of their SAIL technology for stereo-specific protiation/deuteriation of the side chain, and demonstrate several approaches for the investigation of side chain conformation and charge state. The work displays a high degree of technical rigor and is well-documented. The paper is also exemplary when it comes to scholarly presentation and quality of illustrations. At the same time it is unclear to what extent the presented methods are going to alter the ways in which Lys pKa constants are studied, and the suggestion that isotope shifts on the $^{13}$Cε might be used as a proxy for Lys charge state is not yet sufficiently substantiated. It is clear from published data, as well as from results in the report that the chemical shifts of various side chain $^{13}$C and $^{15}$N atoms emerge as very powerful reporters. This has previously convincingly been demonstrated by André et al (Andre et al. JACS 2007; https://doi.org/10.1021/ja0721824), and also by the group of R.E. London (Gao et al. JACS 2006; doi: 10.1021/ja061473u) and it would appear that these methods would remain those of choice? The current work does, however, clearly point a path forward. As the Kainosho group has clearly demonstrated, access to partially deuteriated side chains displays significant improvements for higher molecular weight proteins, where the methods mentioned before will probably fail. There, utilization of SAIL Lys can present significant advances. Gauging charge state of amino acids based on chemical shifts alone presents a small uncertainty, but it is unclear at this point that isotope shifts would prove more reliable. Ultimately, either (a) the method put forward by McIntosh and co-workers (Poon et al. JACS 2006; https://doi: 10.1021/ja065766z) where the multiplet pattern of the amino groups is observed, or (b) observing a titration in the $^{13}$C or $^{15}$N shifts in the HECENZ NMR experiment would remain the unambiguous approaches. Therefore, I think that the title might do better justice when "revealed" would be replaced by "studied" or "investigated". In similar vein, the abstract might be overly optimistic to state that the isotope shift "will" be a powerful tool - possibly it "might" (Indeed on p18, the authors use a more cautious formulation). Also the $^{13}$C 1D spectrum
of ε-[13C,d2]-Lys is not as dispersed as one would wish, and for larger proteins than SNase may present severe shortcomings when compared with 2D NMR. It is unfortunate that the authors were not more successful with HECENZ experiments using SAIL-Lys (as judged from Table 1, where several 15Nζ shifts are missing).

Although the manuscript is very clearly written, I have a few textual comments:

p5 l111 - when referring to "standard protocol", a reference should be given
p5 l111 - labelling "rates"; do the authors refer to incorporation level?
Methods section - the chemical shift referencing procedure is missing. Was it IUPAC (Markley et al) or Bruker?
p6 l196 - "outrageous" probably means "outlier"? I urge the authors to be more transparent about how this was done
p12 Could the ring currents that are discussed possibly be predicted from the structure, and utilized?
p12 l257 - rather than being "quite useful" is would appear that 15Nζ shifts would be "decisive" or "unambiguous"?