Responses to RC2

To RC2:

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.

Response: The ζ-amino group of Lys66 in the RNase variant, which is known to have a pKa of 5.7 as indicated in lines 87-88 (García-Moreno et al., 1997; Fitch et al., 2002), is *not at all protonated but completely deprotonated* at pH/pD 8.0, where all of the NMR data were obtained. The deuterium isotope shifts, $^1\Delta^{15}N$ ppm, for the $^{15}N^{\zeta}$ signals in barnase (Williamson et al., Chem. Commun., **49**, 9824-9826, 2013; we cite this paper), correspond to the differences between $\Delta\delta[\zeta\text{-}^{15}NH_3^+\text{-}\zeta\text{-}^{15}NH_2D^+]$ and $\Delta\delta[\zeta\text{-}^{15}NH_2D^+\text{-}\zeta\text{-}^{15}NHD_2^+]$. Therefore, the previously reported averaged isotope shifts, 0.357 ppm, should be tripled (i.e., 1.071 ppm) for comparison to the value of 1.1 +/- 0.1 ppm shown in Table 2 in the manuscript (*page 17*). Obviously the two values are almost identical within the errors, even though their data were obtained at pH 4.8. The deuterium isotope shifts reported by Lyčka and Hansen (Magn. Reson. Chem., **23**, 973-976, 1985) are actually not appropriate model compounds. However, as mentioned above, the ζ-amino group of Lys66 at pH/pD 8.0, which is 2.3 pH units higher than the pKa value, should be completely deprotonated as clearly demonstrated by Takayama et al. (J. Am. Chem. Soc., 130, 6714-6715, 2008). Therefore, the isotope shift for the ζ-amino group of Lys66 should correspond to $\Delta\delta[\zeta\text{-}^{15}ND_2\text{-}\zeta\text{-}^{15}NH_2]$. Since the $\zeta\text{-}^{15}N$ deuterium shifts for all 21 Lys residues measured in H_2O-D_2O (1:1) appeared exactly in the middle of the spectra obtained in 100% H_2O (no D_2O contamination since we used a dual-tube) and 100% D_2O, the fractional factors for the isotopomers are within statistically random distributions (*lines 394-401*).