We recorded a [^{19}F,^{19}F]-TOCSY spectrum of GB1 made with deuterated 5,5'-difluoro-L-leucine (where all protons of the fluorinated isopropyl groups are replaced by deuterons). The spectrum, shown underneath, shows all the strong cross-peaks of the spectrum of Figure 6a in the main text.

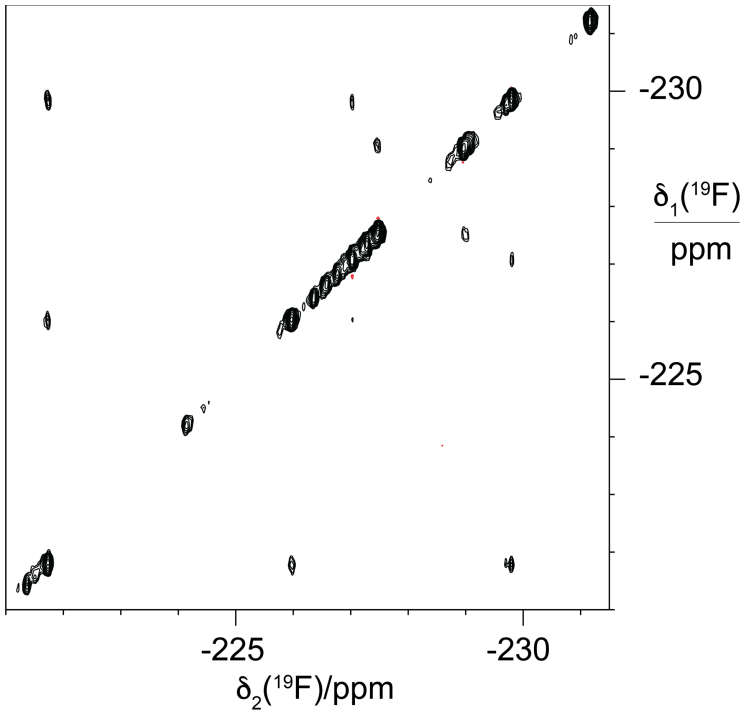

Figure 1. [^{19}F,^{19}F]-TOCSY spectrum of a 0.5 mM solution of GB1 made with deuterated 5,5'-difluoro-L-leucine. Parameters: mixing time 55 ms, t_{1max} = 6 ms, t_{2max} = 97 ms, total recording time 15 h, 400 MHz NMR spectrometer.

The article by Blake et al. (J. Biomol. NMR, 2, 527, https://doi.org/10.1007/BF02192814, 1992) reported small through-space scalar couplings in rubredoxin between the methyl protons of Ala 43 and heavy metal ions (^{113}Cd, ^{199}Hg). In our case, we find no sign of through-space couplings between ^{19}F and other spins. If they were a common occurrence, we would expect to observe through-space ^{19}F-^{1}H couplings for some of the protons that show the strongest NOEs in the HOESY spectra. Neither the COSY spectrum of Figure 5 nor the short-delay ^{1}H,^{19}F correlation experiments showed any evidence for this. Fluorine bonded to carbon features more hydrophobic than H-bond acceptor characteristics. The leucine methyls of GB1 are not near H-bond donors or heavy atoms.

To the best of our knowledge, scalar ^{19}F-^{19}F couplings mediated by electrons of an intervening chemical group have been reported only once, where the intervening group was the π-bond system of a phenyl ring, see Mallory et al. (J. Am. Chem. Soc., 112, 2577, https://doi.org/10.1021/ja00163a015, 1990).