Supplementary material for:

Evaluation of the *tert*-butyl group as a probe for NMR studies of macromolecular complexes

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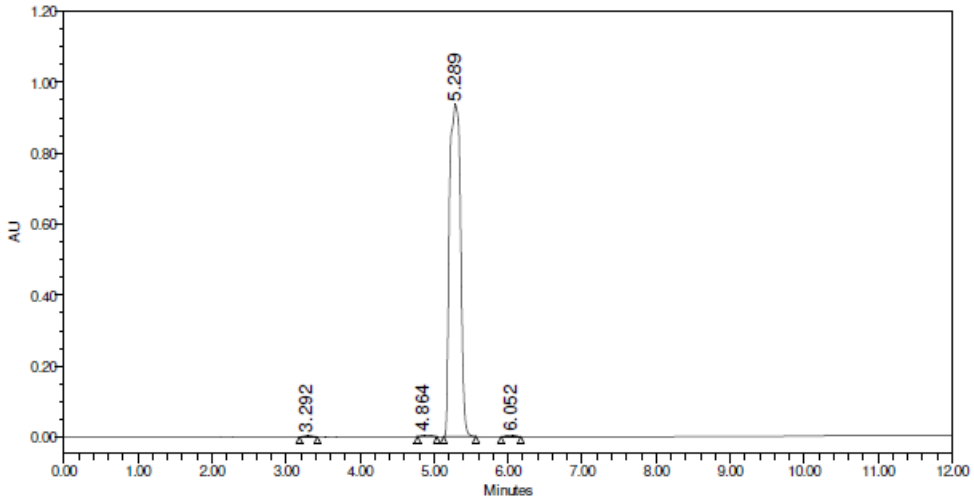
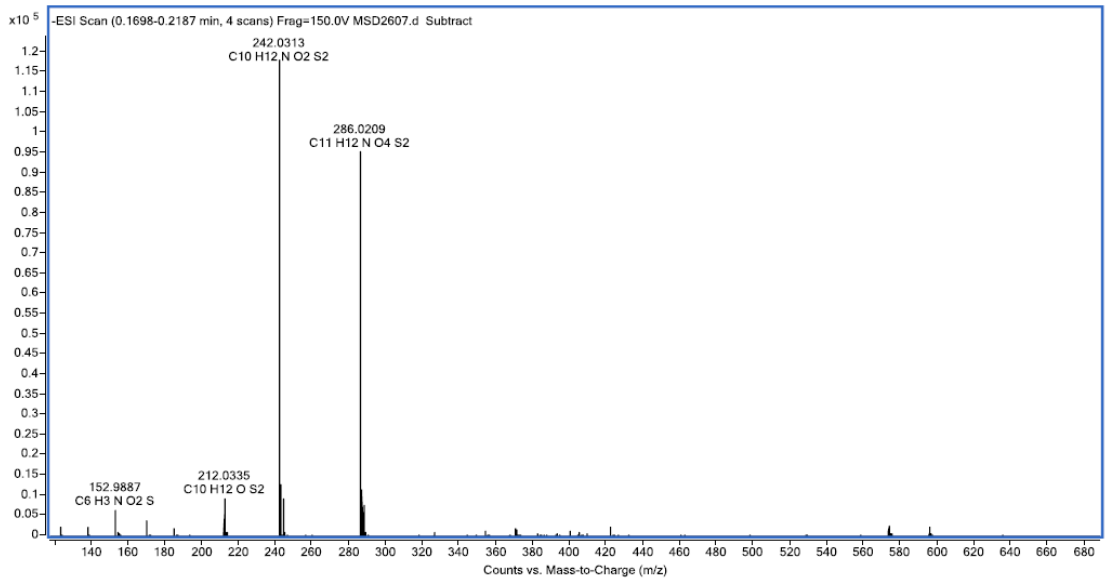
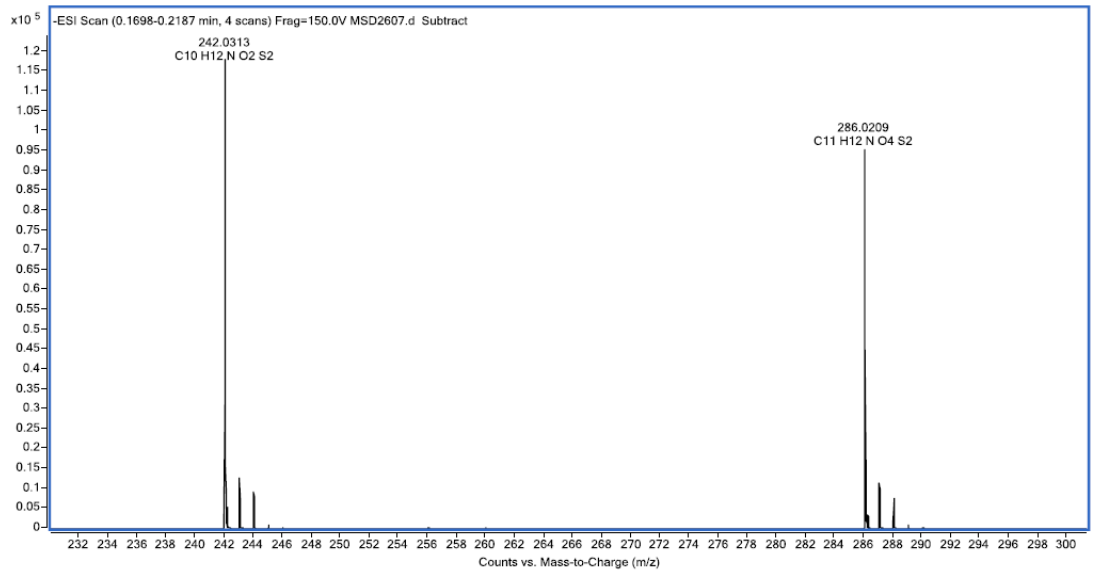


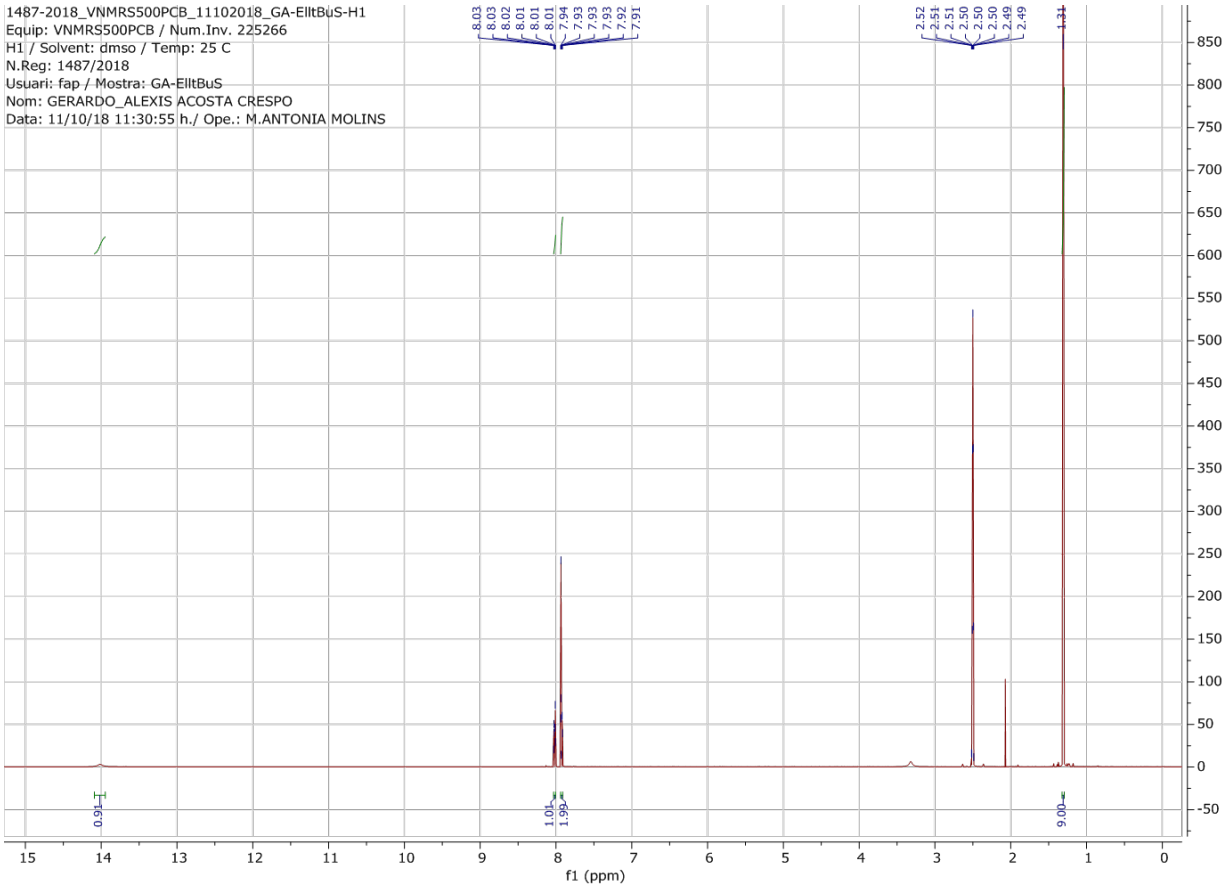
	RT	Area	% Area	Height
1	3.292	28550	0.29	3332
2	4.864	35625	0.37	3584
3	5.289	9644992	98.96	935338
4	6.052	37658	0.39	3727

Supplementary Figure 1. HPLC-PDA of purified 5-(*tert*-butyldisulfanyl)-2-nitrobenzoic acid. Column: Xbridge BEH 130 C18 3,5µm 4,6x100mm; Eluent: water with 0,045% of TFA and ACN with 0,036% of TFA; Gradient: 40 to 100% of ACN in 8 minutes, Flow 1 mL/min. Purity 98,96 % at 220 nm. Retention time 5,289 min.

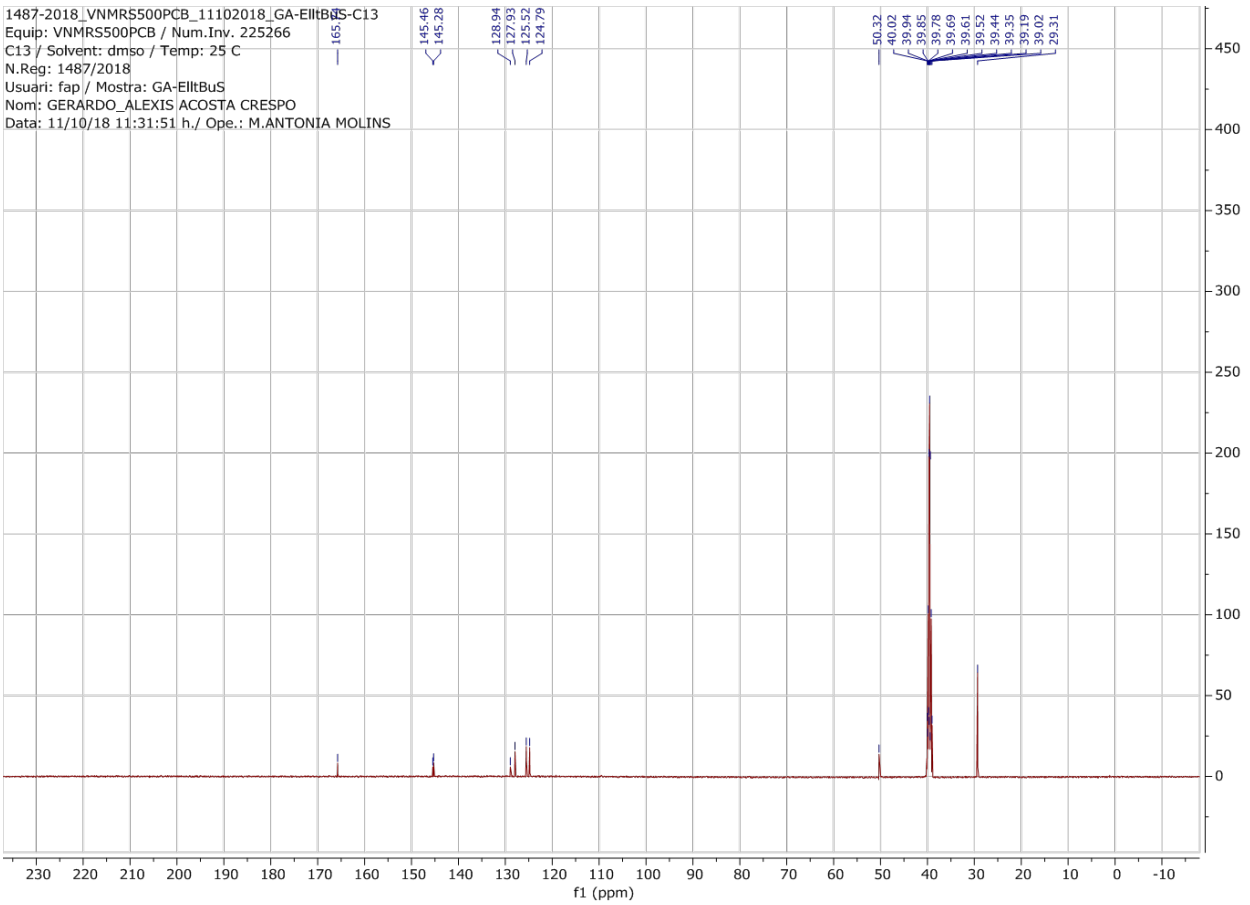




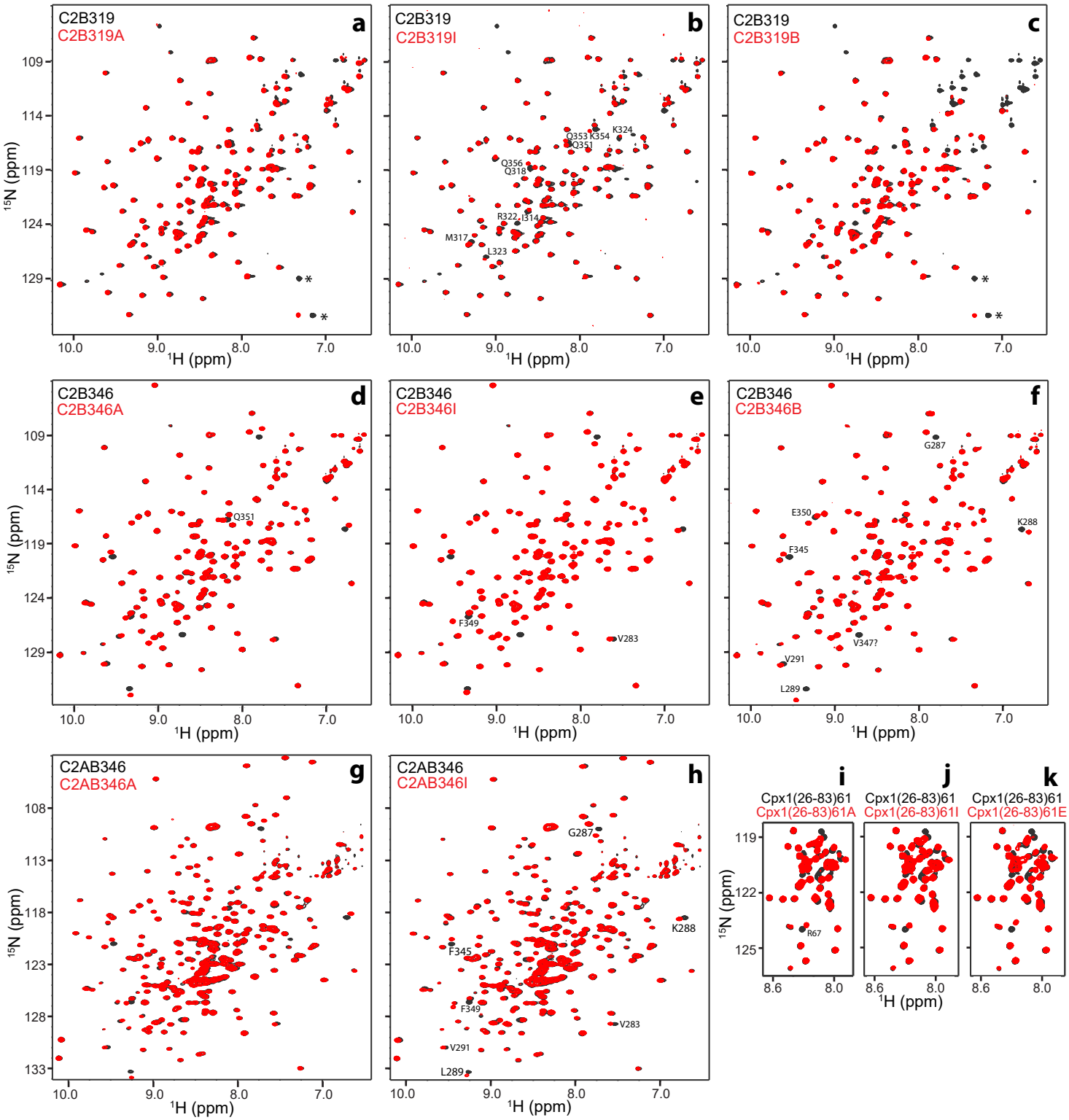
Supplementary Figure 2. HR-MS: HR-MS –ESI of 5-(tert-butyldisulfanyl)-2-nitrobenzoic acid. Calculated Mass $[M+H]^{+1}$ = 287,35; $[M\text{-}COOH]^{-1}$ = 242,33. Found Mass $[M-H]^{-1}$ = 286,0209 Diff. = 1.43 ppm; $[M\text{-}COOH]^{-1}$ = 242,0313 Diff = 0,81 ppm. Diff = (Calculated Mass- Found Mass)/Calculated Mass * 10^6.



Supplementary Figure 3. ^{1}H-NMR spectrum of 5-(*tert*-butyldisulfanyl)-2-nitrobenzoic acid.

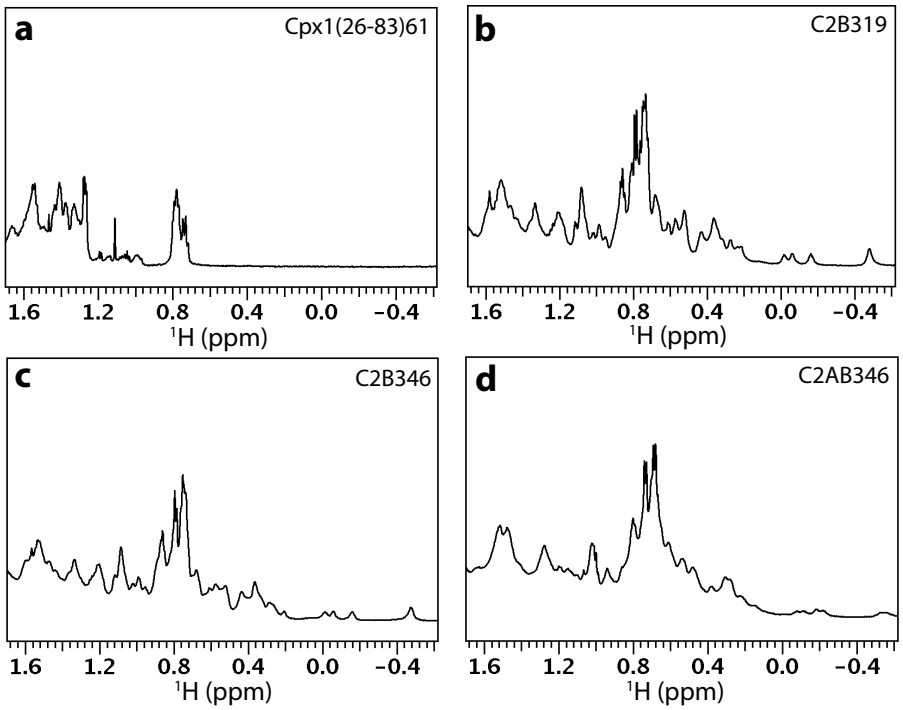


Supplementary Figure 4. ^{13}C-NMR spectrum of 5- (*tert*-butyldisulfanyl)-2-nitrobenzoic acid.

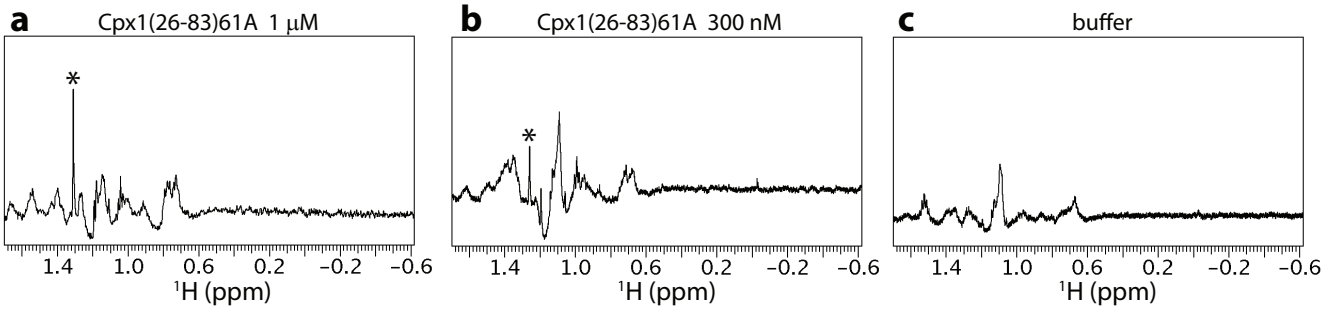


Supplementary Figure 5

Supplementary Figure 5. ^{1}H-^{15}N HSQC spectra illustrating cross-peak shifts caused by ^{t}Bu tagging. ^{1}H-^{15}N HSQC spectra of Syt1 C_2B domain N319C (**a-c**), Syt1 C_2B domain E346C (**d-f**), Syt1 C_2AB E346C (**g-h**) and Cpx1(26-83) V61c (**i-k**) before (black contours) and after (red contours) tagging with a ^{t}Bu group via the acrylate (**a, d, g, i**), iodoacetamide (**b, e, h, j**) or BDSNB (**c, f, k**) reaction. Selected cross-peaks that shifted in the spectra of the Syt1 C_2B domain or C_2AB fragment mutants due to tagging with the ^{t}Bu group using the corresponding reactions are labeled in panels (**b, f, h**), and additional cross-peaks that shifted due to tagging with other reactions are labeled in the corresponding panels. All the perturbed cross-peaks correspond to residues in close proximity to the site tagged with a ^{t}Bu group in the three-dimensional structure of the C_2B domain. For Cpx1(26-83) V61C, only one well-resolved cross-peak that shifts due to tagging (corresponding to R67) can be unambiguously assigned. The ^{1}H-^{15}N HSQC spectrum of C2B319B (red contours in panel **c**) was acquired by mistake with a TROSY-enhanced pulse sequence and hence it does not contain the cross-peaks corresponding to the Asn and Gln side chains, but the spectra is still useful to monitor shifts in the backbone NH groups. Note also that we did not acquire a ^{1}H-^{15}N HSQC spectrum from $C_2AB346B$, but tagging of the protein with ^{t}Bu was confirmed by the sharp resonance observed at the same chemical shift observed for the ^{t}Bu group of C_2B346B (Figure 4c, d).



Supplementary Figure 6. Expansions corresponding to the methyl region of 1D ^{1}H NMR spectra of Cpx1(26-83) V61C mutant [Cpx1(26-83)61)] (**a**), Syt1 C_2B domain N319C mutant (C2B319) (**b**), Syt1 C_2B domain E346C mutant (C2B346) (**c**) and Syt1 C_2AB E346C mutant (C2AB346) (**d**).



Supplementary Figure 7. 1D ^{1}H NMR spectra of 1 μM Cpx1(26-83)A (**a**), 300 nM Cpx1(26-83)A (**b**) and the buffer used to dissolve these samples (**c**), which contained 20 mM HEPES pH 7.4, 125 mM KCl and 10% D_2O. The spectra of (**a**) and (**c**) were acquired in 1 hr and were plotted at the same vertical scale. The spectrum of (**b**) was acquired in 3 hr and plotted at a vertical scale 3.3 time higher than those of (**a**, **c**).