Supplementary Information

Structural basis for Ca^{2+}-mediated interaction of the perforin C2 domain with lipid membranes

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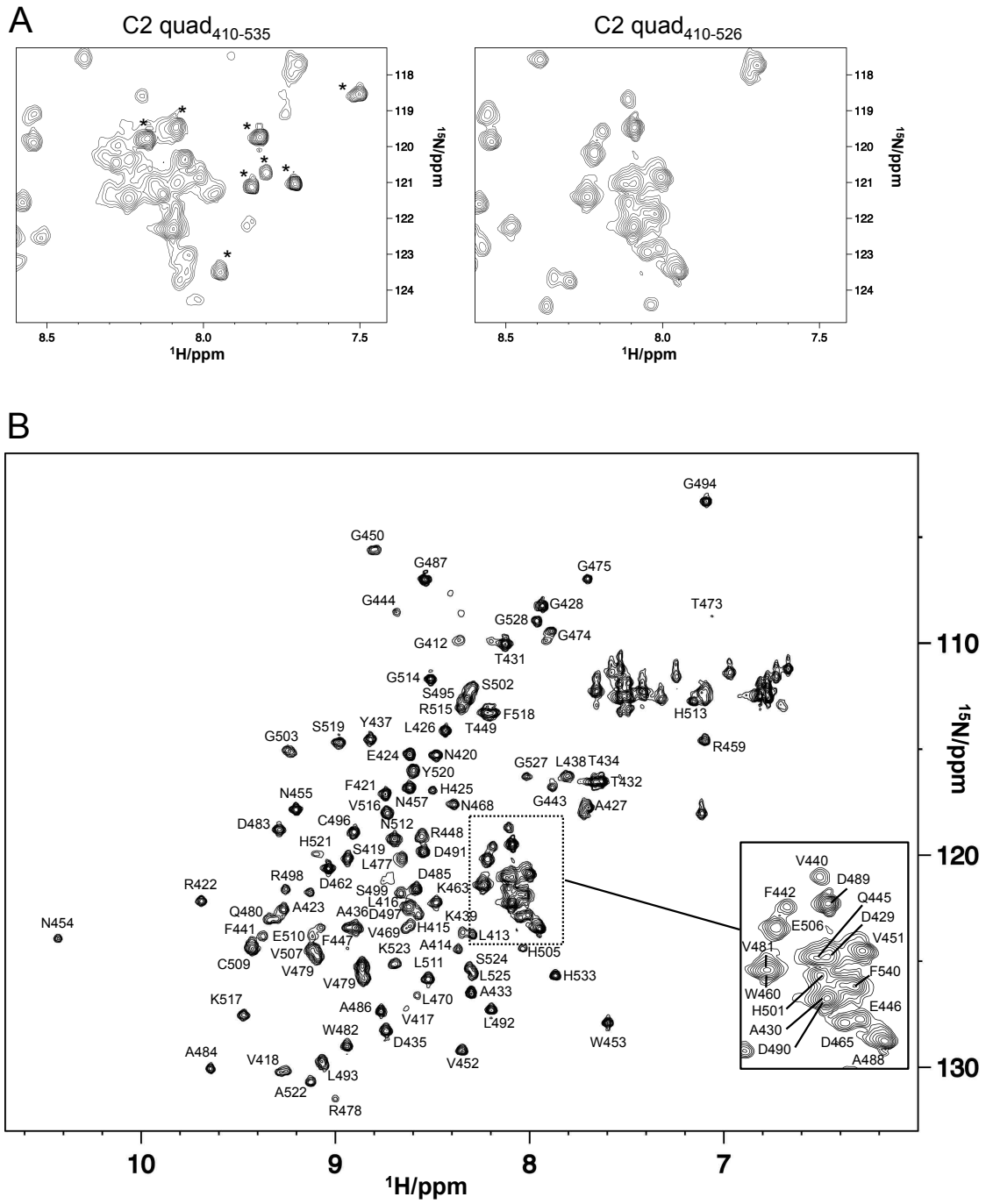

Figure S1. ^{1}H-^{15}N SOFAST-HMQC spectra of the crystal and the NMR constructs (A) Central regions of the 2D NMR spectra of the constructs for used for crystallography (C2 quad$_{410\text{-}535}$; left) and NMR (C2 quad$_{410\text{-}526}$; right) studies. In the C2 quad$_{410\text{-}535}$, the cross-peaks of the extra residues at C-terminal (marked by asterisks) are observed as intense resonances in the regions of random-coil chemical shift, indicating highly flexible. (B) Backbone resonance assignments of the C2 quad$_{410\text{-}526}$. The cross-peaks of

backbone amides are labelled with the amino acid type and sequence number; this construct consists of residues 410-526 with Gly + His_6 tag at C-terminal (Fig. 1 in the main text)

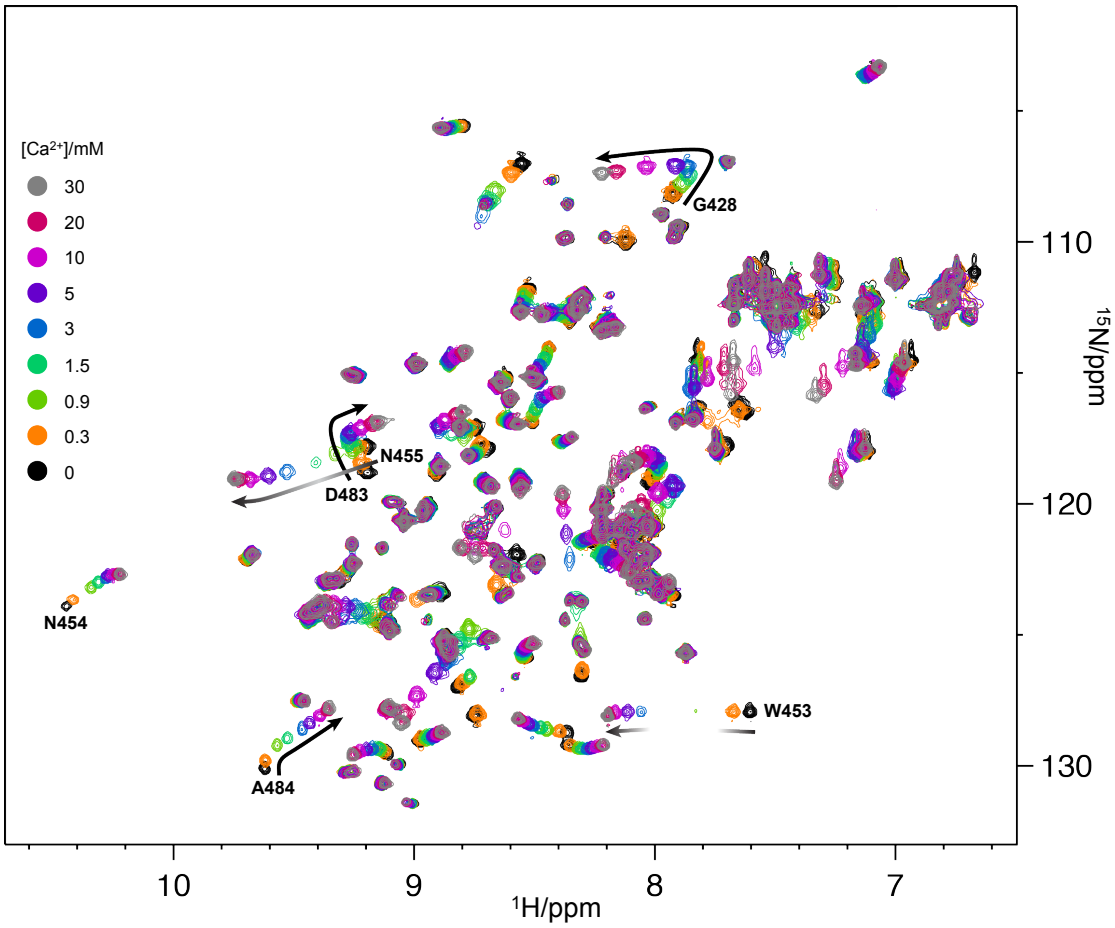

Figure S2. Titration of the C2 D491N mutant with Ca^{2+}. Overlay of ^{1}H-^{15}N SOFAST-HMQC spectra of 150 μM ^{15}N-labelled C2 D491N mutant recorded with increasing [Ca^{2+}]. Spectra were processed identically and plotted with the same contour levels. Resonance colours correspond to different [Ca^{2+}] shown in the same colour in the top-left corner. The chemical shift change patterns are shown as black arrows, and intensity changes are roughly represented by gradient.

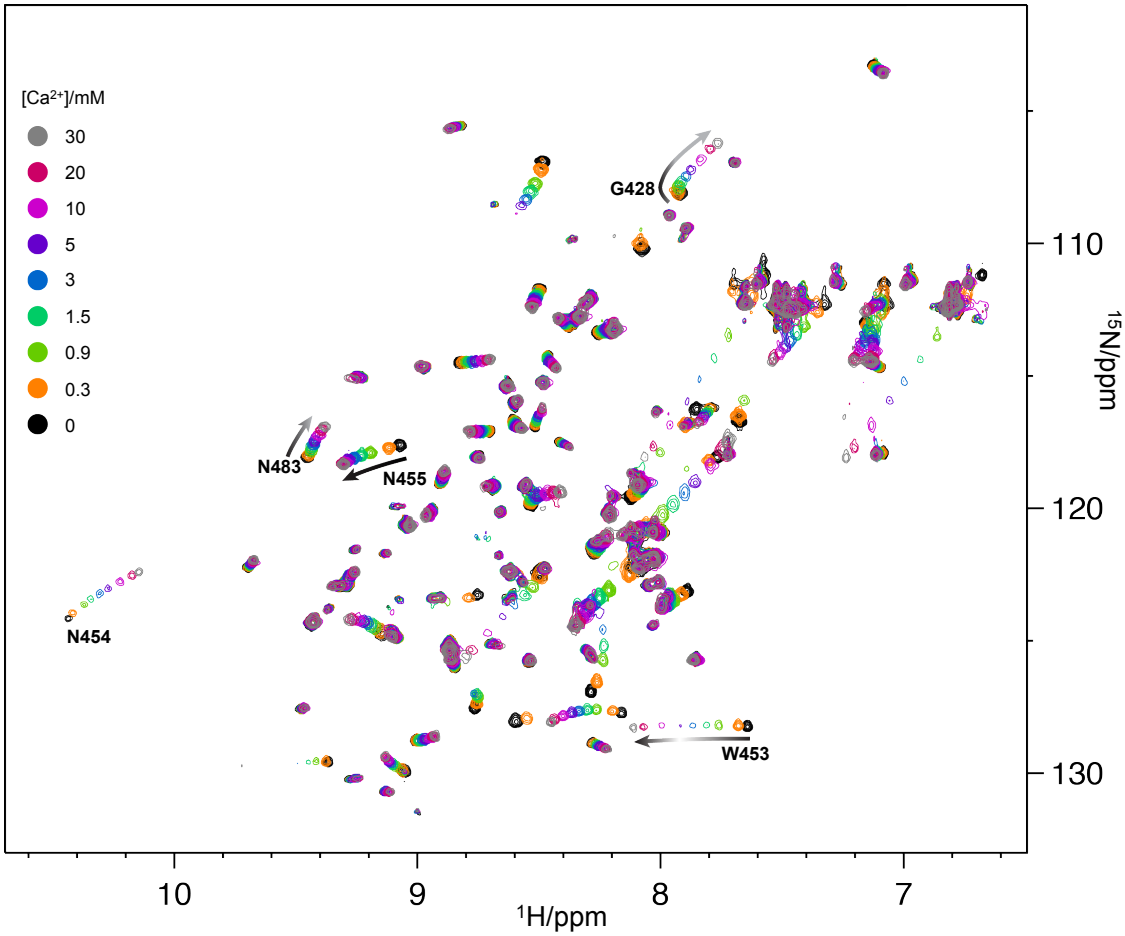

Figure S3. Titration of the C2 D483N mutant with Ca^{2+}. As per Figure S2, except for 150 μM ^{15}N-labelled C2 D483N mutant.

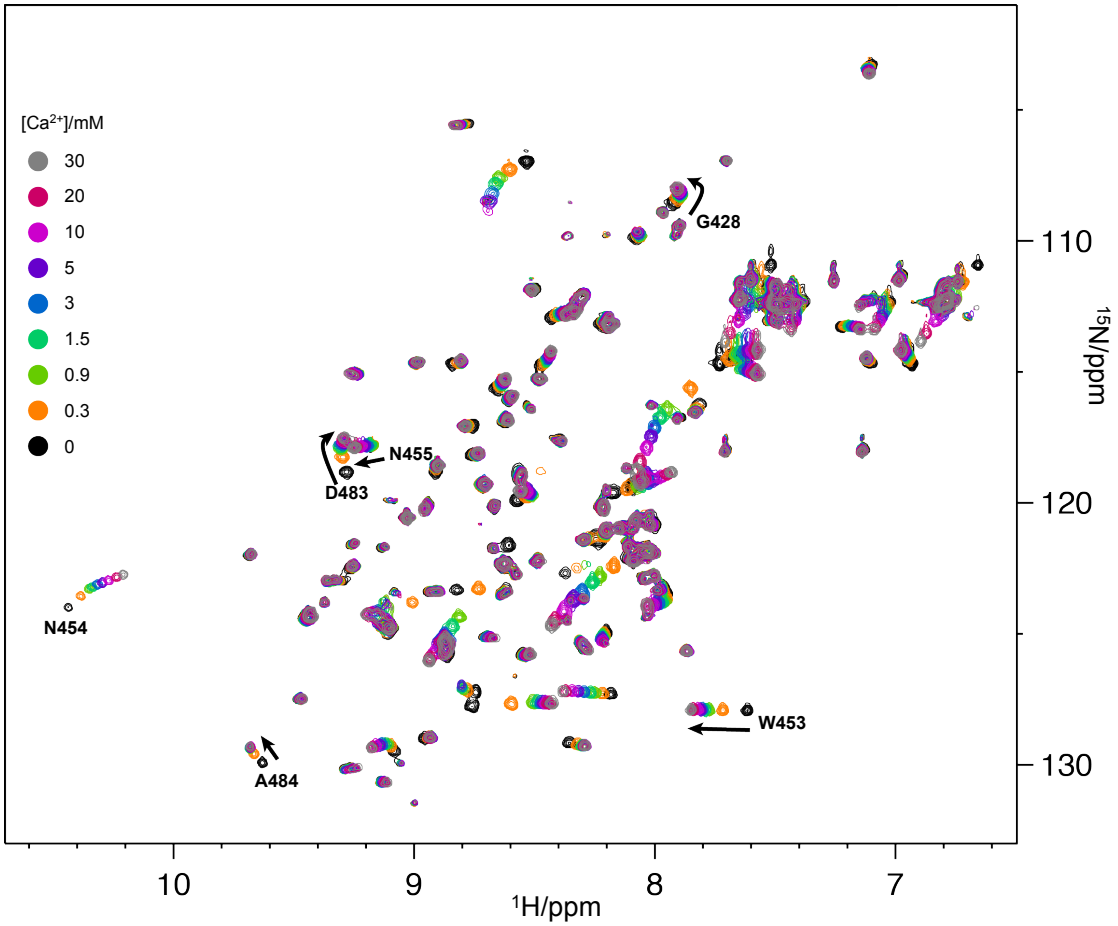

Figure S4. Titration of the C2 D429N mutant with Ca^{2+}. As per Figure S2, except for 150 μM ^{15}N-labelled C2 D429N mutant.

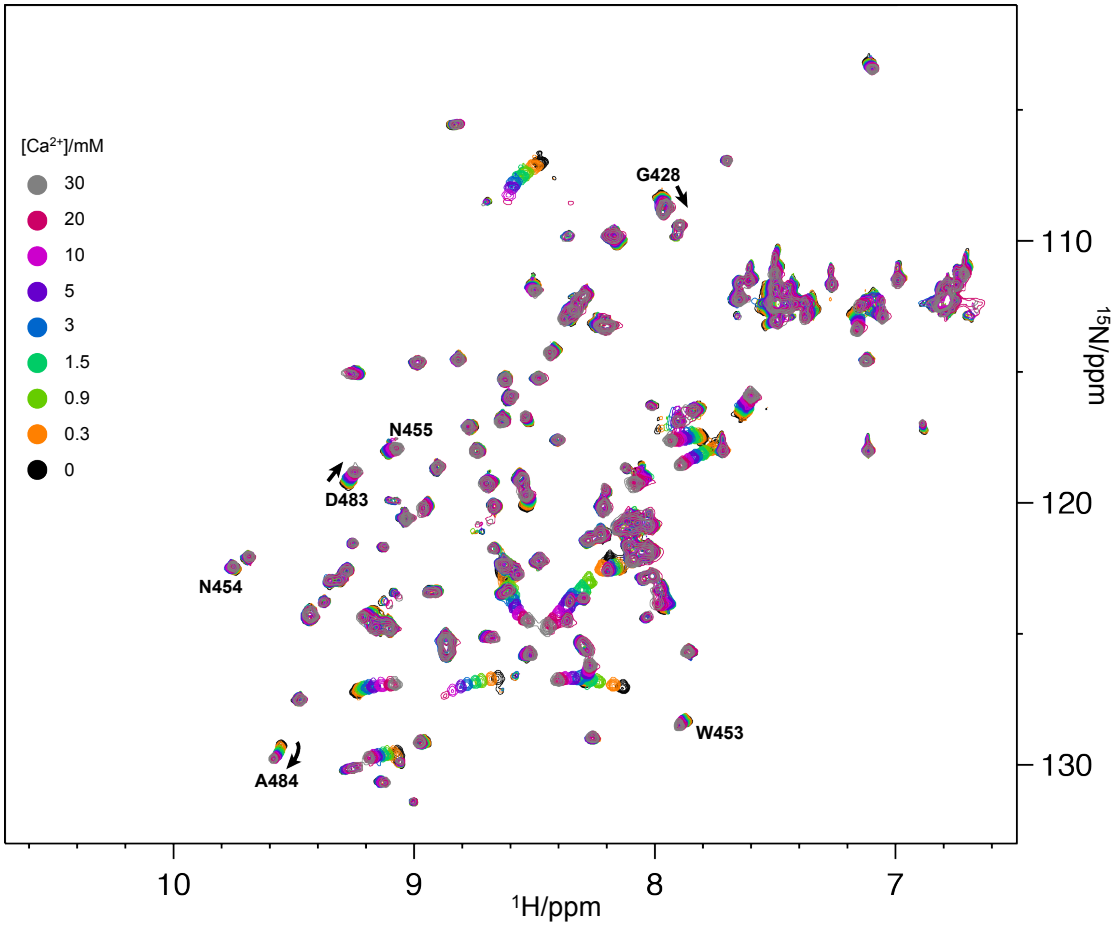

Figure S5. Titration of the C2 D435N mutant with Ca^{2+}. As per Figure S2, except for 150 μM ^{15}N-labelled C2 D435N mutant.

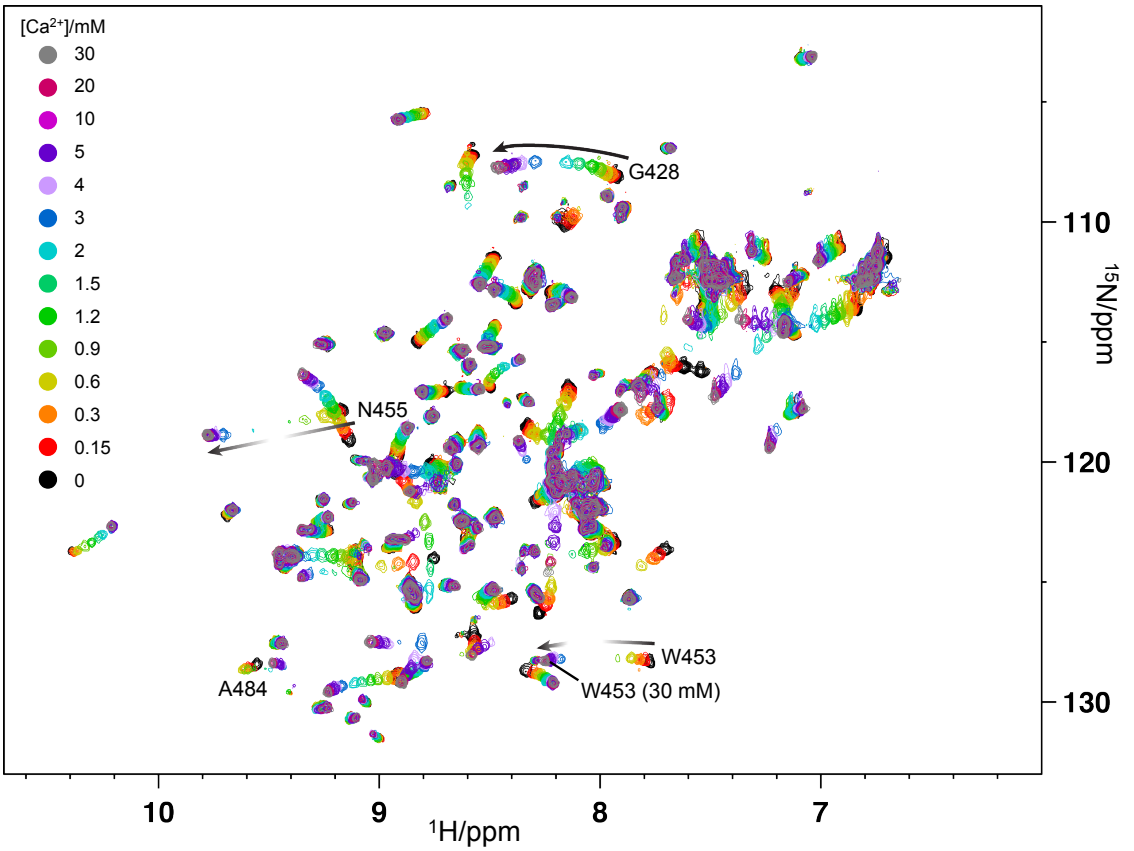

Figure S6. Titration of the C2 D490N mutant with Ca^{2+}. As per Figure S2, except for 150 μM ^{15}N-labelled C2 D490N mutant.

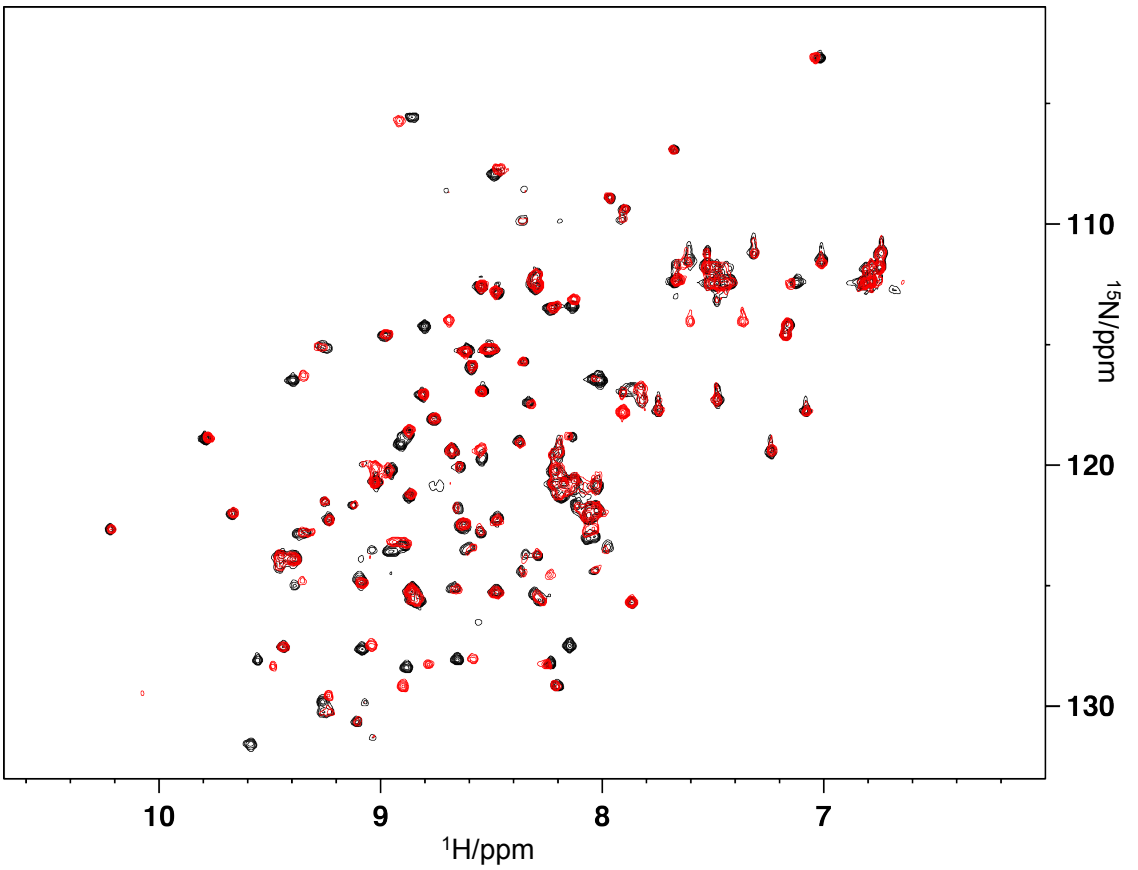

Figure S7. Comparison of C2 quad$_{410\text{-}526}$ with the C2 D490N mutant at 30 mM [Ca^{2+}]. Overlay of ^{1}H-^{15}N SOFAST-HMQC spectra of the C2 quad$_{410\text{-}526}$ (black) and the D490N mutant (red).

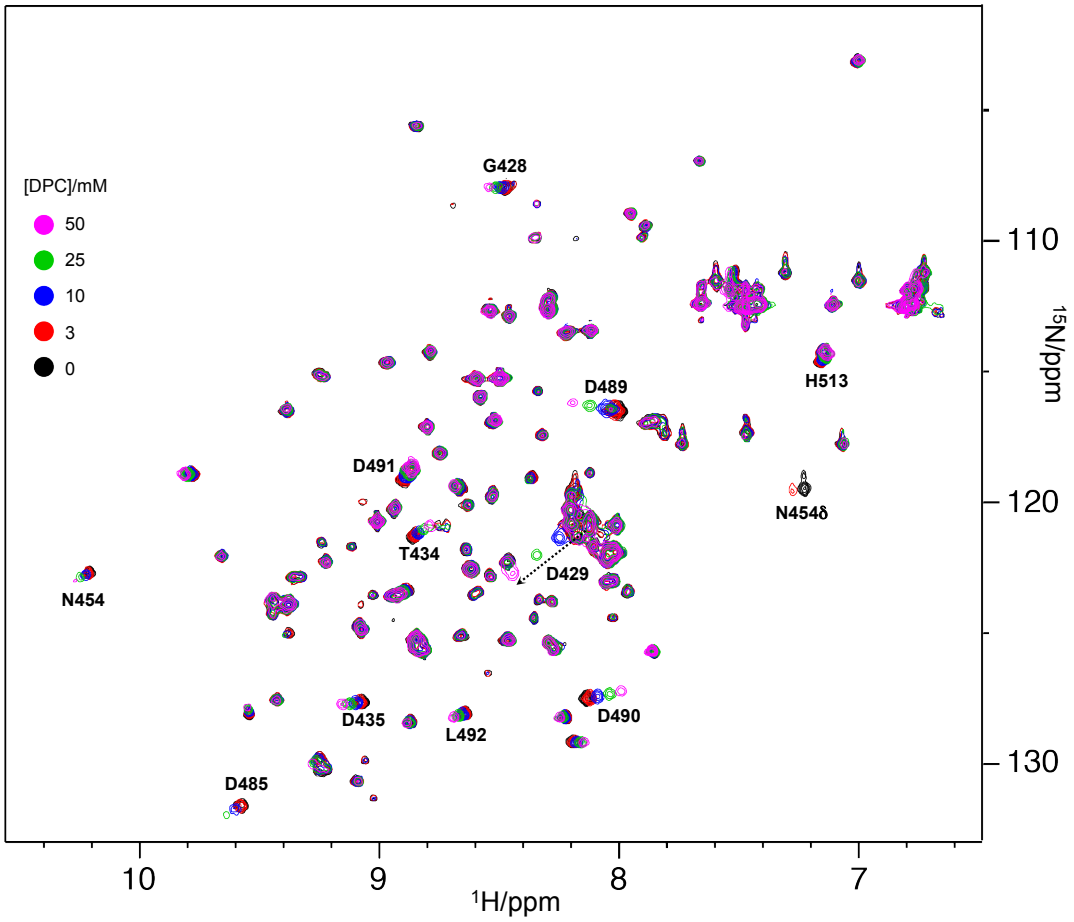

Figure S8. Titration of the C2 quad$_{410\text{-}526}$ with DPC in the presence of 30 mM [Ca^{2+}]. Overlay of ^{1}H-^{15}N SOFAST-HMQC spectra of 150 μM ^{15}N-labelled C2 quad$_{410\text{-}526}$ recorded with increasing [DPC] in the presence of 30 mM [Ca^{2+}]. Resonance colours correspond to different [DPC] as shown at the left of the spectra. Significantly perturbed resonances upon addition of DPC are labelled.

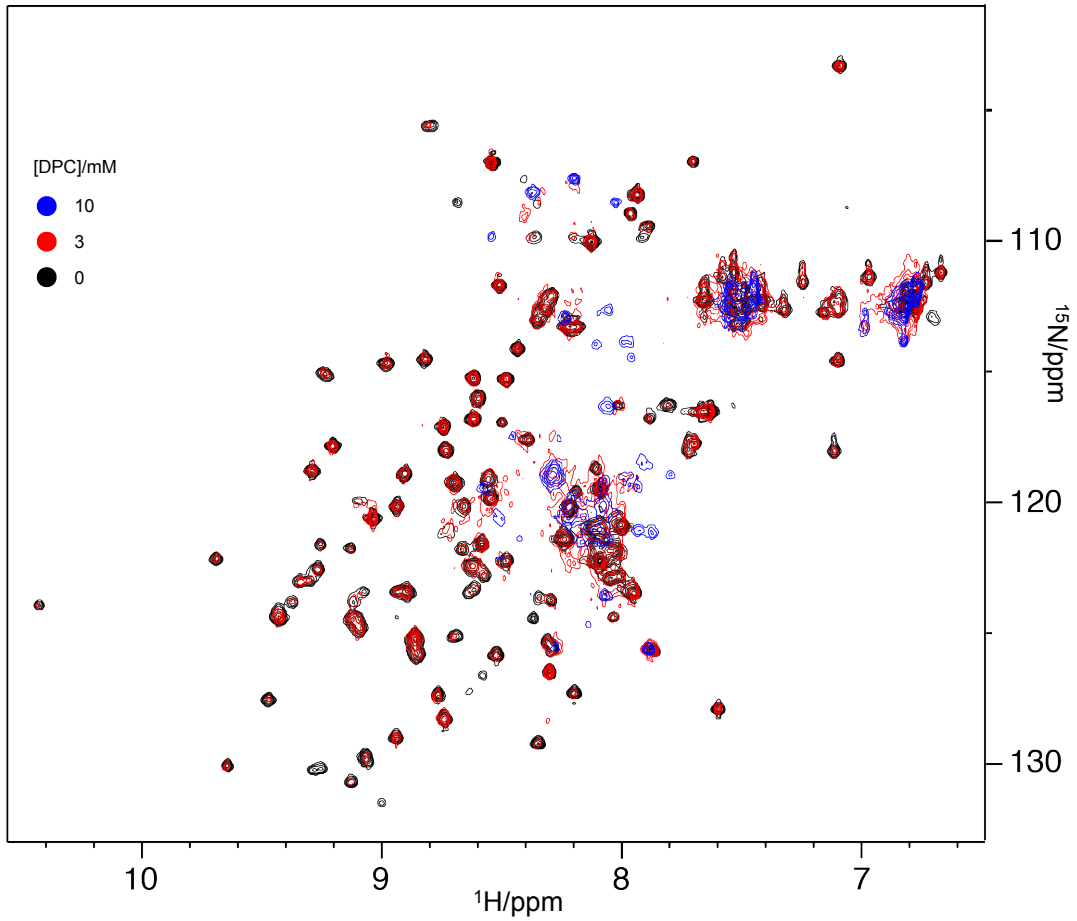

Figure S9. Titration of the C2 quad$_{410\text{-}526}$ with DPC in the absence of Ca^{2+}. Overlay of ^{1}H-^{15}N SOFAST-HMQC spectra of 150 μM ^{15}N-labelled C2 quad$_{410\text{-}526}$ recorded with increasing [DPC]. Resonance colours correspond to different [DPC] as shown at the left of the spectra. Resonances indicative of degradation or aggregation appeared in the central region of the spectra at 3 mM [DPC] and intensified at 10 mM [DPC].

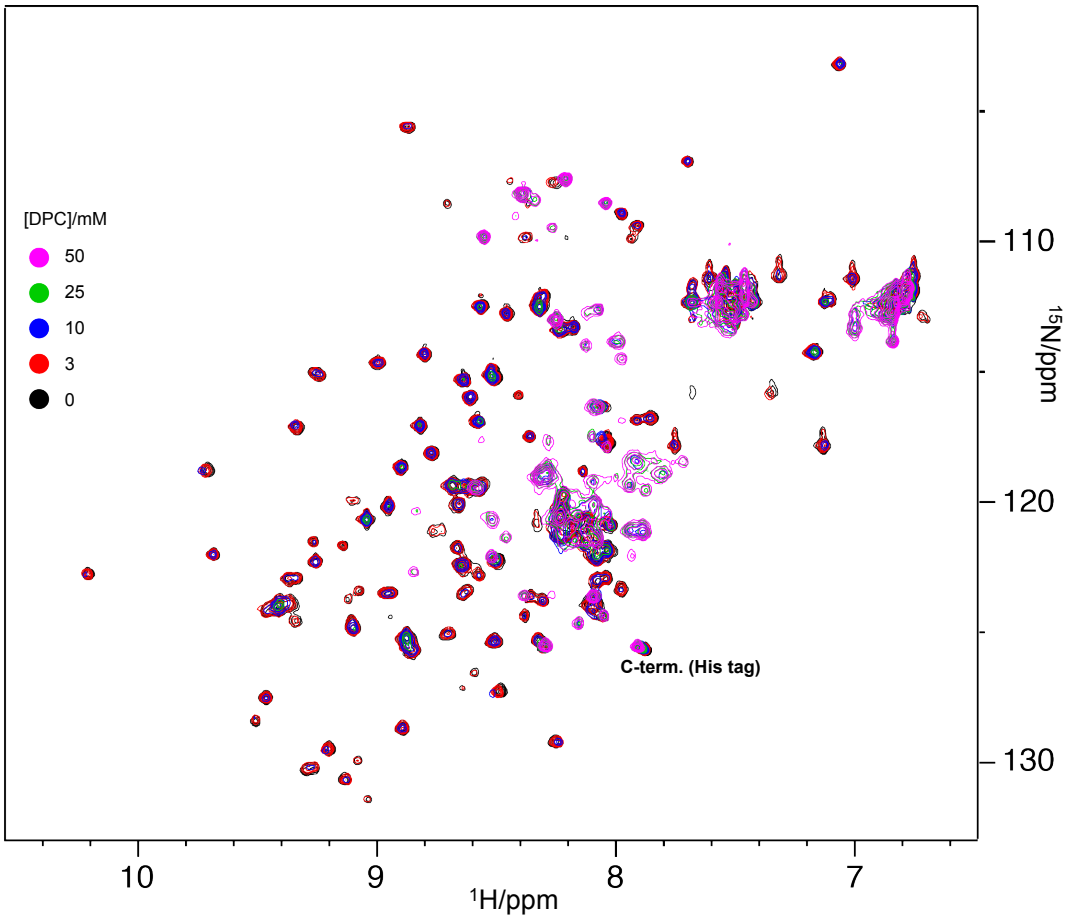

Figure S10. Titration of the C2 quad$_{410\text{-}526}$ with DPC in the presence of 2 mM [Ca^{2+}]. Overlay of ^{1}H-^{15}N SOFAST-HMQC spectra of 150 μM ^{15}N-labelled C2 quad mutant recorded with increasing [DPC]. Resonance colours correspond to different [DPC] as shown at the left of the spectra. No significant CSPs were observed upon adding DPC except for the C-terminal residue, but, the C-terminus is not near the CBRs, indicating non-specific interaction with DPC micelles. In addition, resonances indicative of degradation or aggregation gradually increased with increasing [DPC].

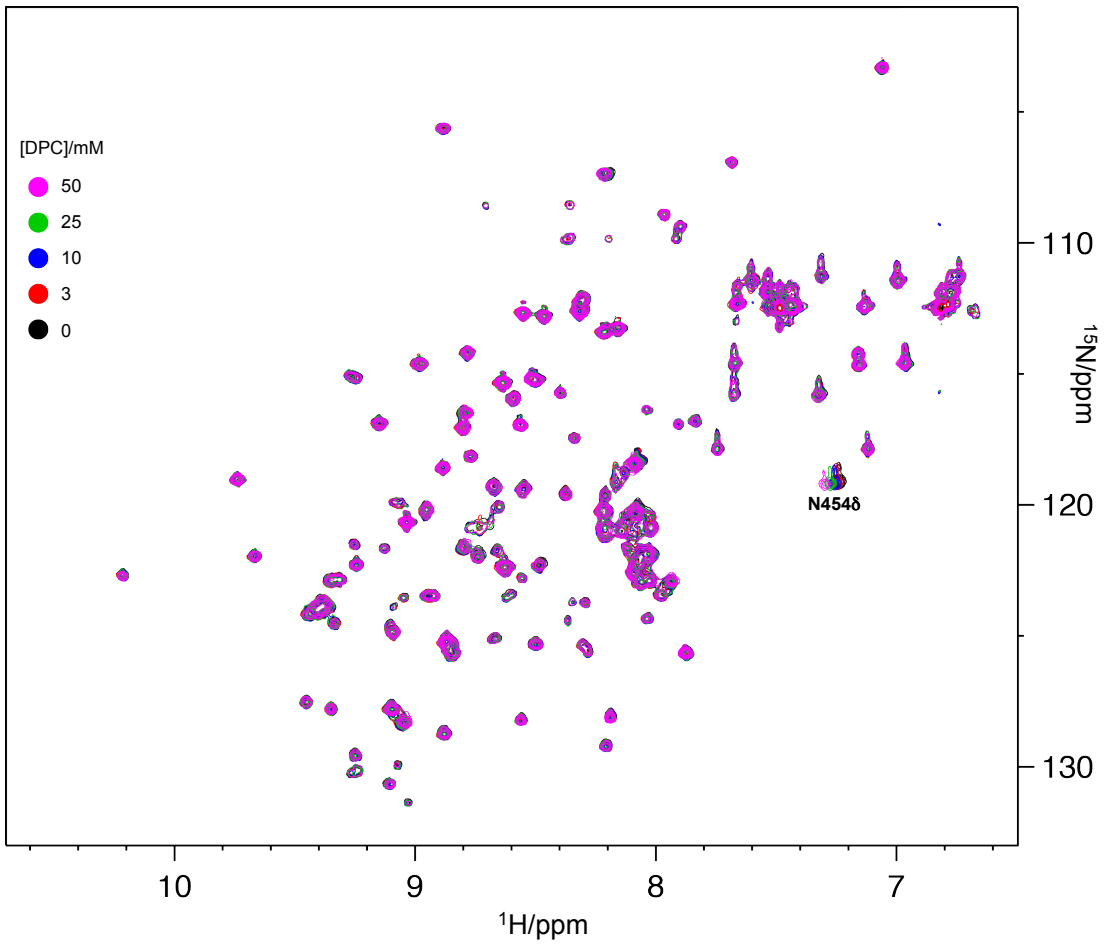

Figure S11. Interaction of the C2 D491N mutant with DPC micelles. Overlay of ^{1}H-^{15}N SOFAST-HMQC spectra of 150 μM ^{15}N-labelled C2 D491N mutant recorded with increasing [DPC] at 30 mM [Ca^{2+}]. Spectra were processed identically and plotted with the same contour levels. Resonance colours correspond to different [DPC] shown in the same colour on the left side of spectra. No significant CSPs upon adding DPC were observed in the resonances of backbone amides, but a side chain amide resonance of N454 was clearly perturbed.

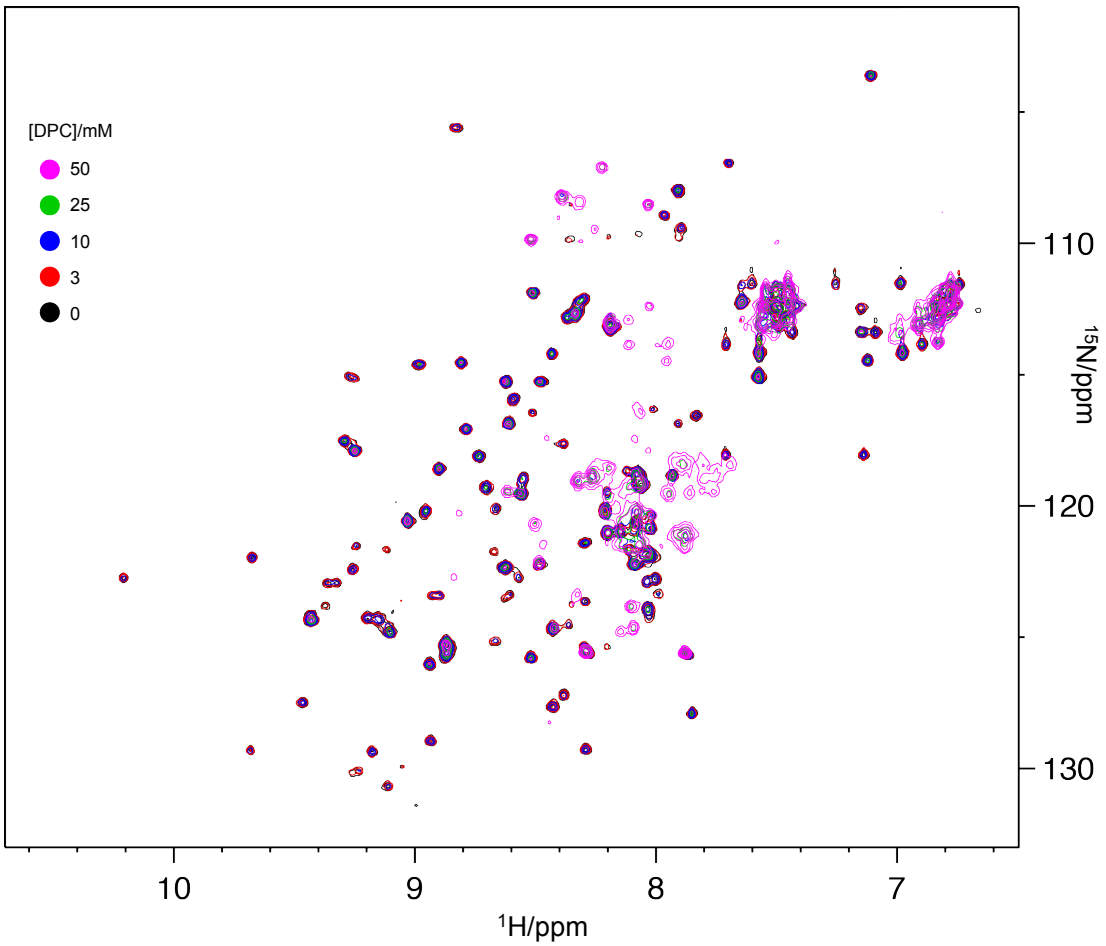

Figure S12. Interaction of the C2 D429N mutant with DPC micelles. Overlay of ^{1}H-^{15}N SOFAST-HMQC spectra of 150 μM ^{15}N-labelled C2 D429N mutant recorded with increasing [DPC] at 30 mM [Ca^{2+}]. Spectra were processed identically and plotted with the same contour levels. Resonance colours correspond to different [DPC] shown in the same colour on the left side of spectra. No significant CSPs upon adding DPC were observed and resonances indicative of degradation or aggregation gradually increased with increasing [DPC].

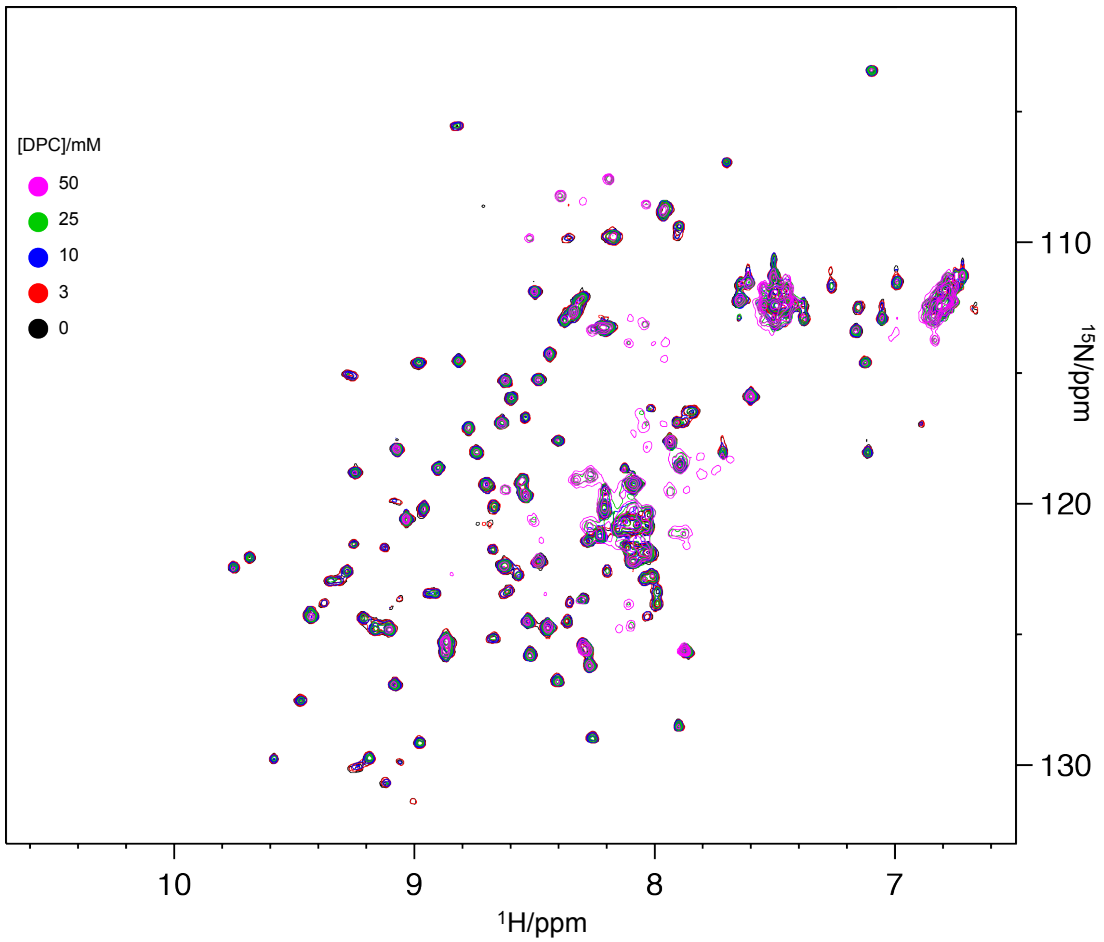

Figure S13. Interaction of the C2 D435N mutant with DPC micelles. As per Figure S12, except for 150 μM ^{15}N-labelled C2 D435N mutant.

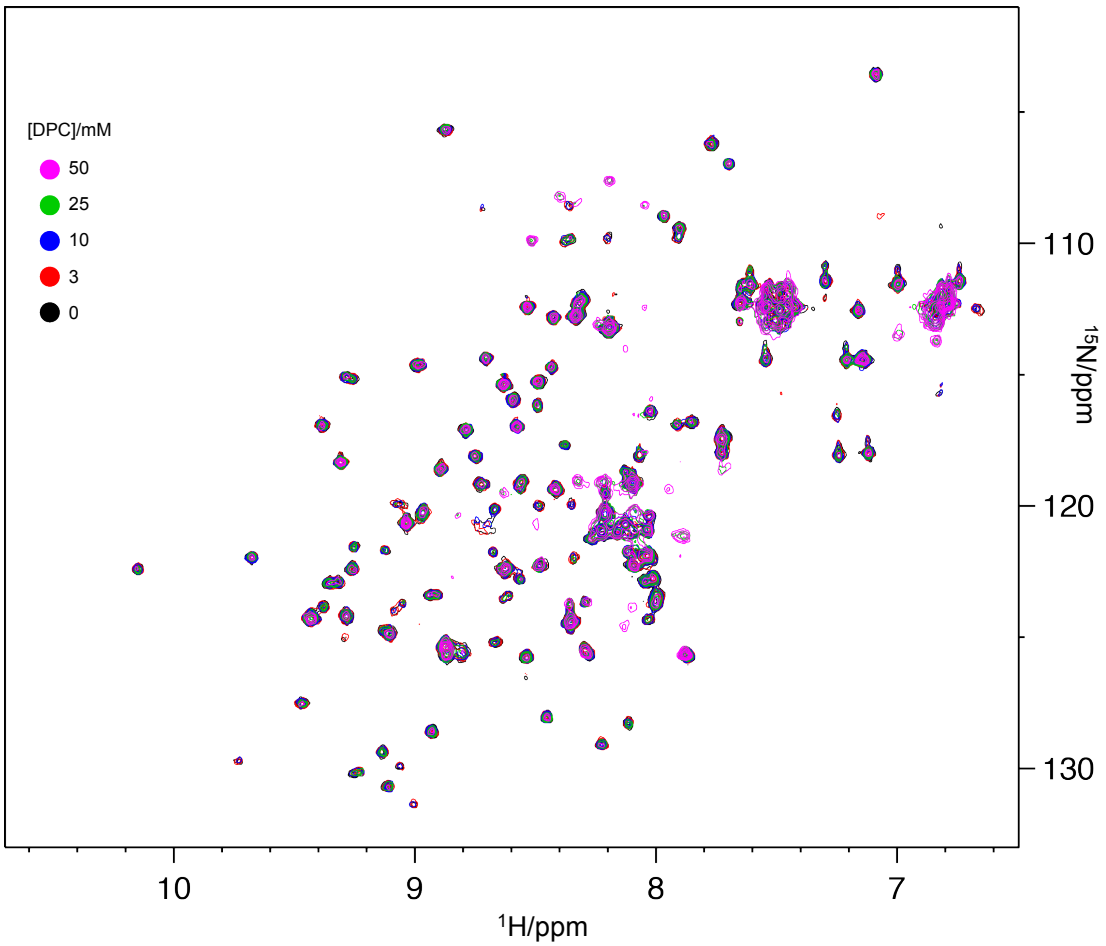

Figure S14. Interaction of the C2 D483N mutant with DPC micelles. As per Figure S12, except for 150 μM ^{15}N-labelled C2 D483N mutant.

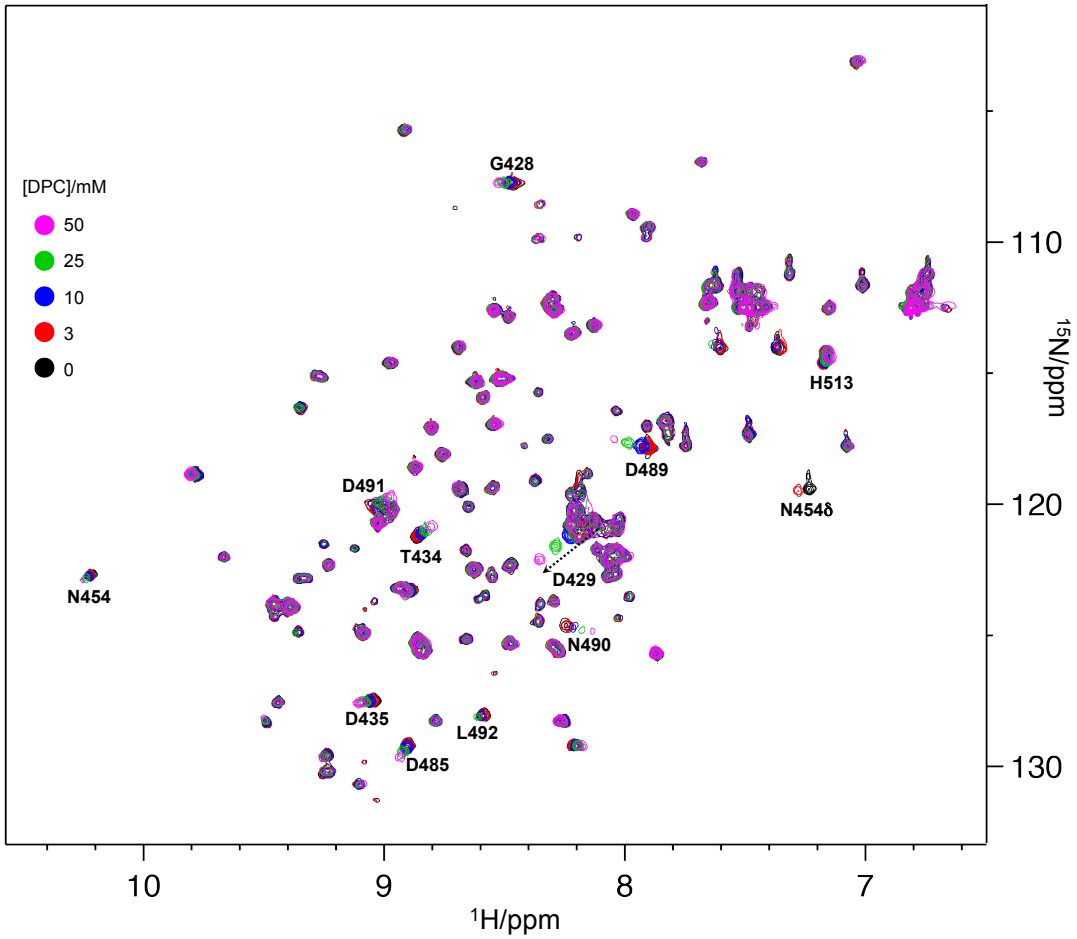

Figure S15. Interaction of the C2 D490N mutant with DPC micelles. Overlay of ^{1}H-^{15}N SOFAST-HMQC spectra of 150 µM ^{15}N-labelled C2 D490N mutant recorded with increasing [DPC] at 30 mM [Ca^{2+}] concentration. Spectra were processed identically and plotted with the same contour levels. Resonance colours correspond to different [DPC] shown in the same colour on the left side of spectra. Significantly perturbed resonances upon addition of DPC are labelled. The same resonances of the C2 $quad_{410-526}$ were perturbed with increasing [DPC].