Supporting information

Uncovering protein conformational dynamics within two-component viral biomolecular condensates

Alice Colyer[1], Julia Acker[2], Alexander Borodavka[2]*, Antonio N. Calabrese[1]*

[1] **Astbury Centre for Structural Molecular Biology**, School of Molecular and Cellular Biology, Faculty of Biological Sciences, University of Leeds, LS2 9JT, Leeds, United Kingdom

[2] **Department of Chemical Engineering and Biotechnology,** University of Cambridge, CB2 1QW, Cambridge, United Kingdom

Correspondence to Alexander Borodavka: ab2677@cam.ac.uk (A. Borodavka) ***and Antonio N. Calabrese*:** a.calabrese@leeds.ac.uk (A.N. Calabrese)

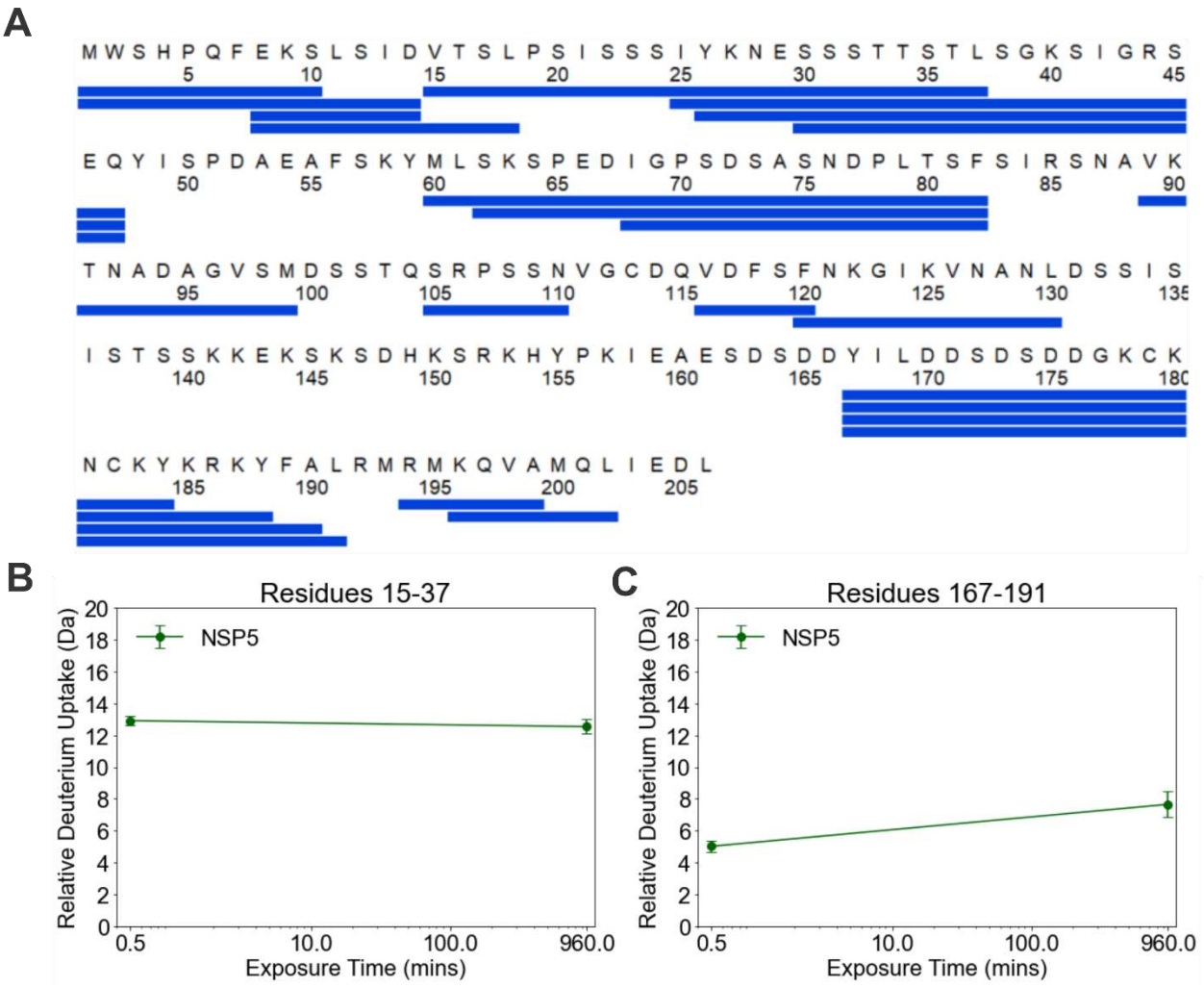

Fig. S1 (A) Coverage map of differential HDX-MS of NSP5. Each blue bar represents an individual peptic peptide identified by HDX-MS. A total of 21 NSP5-derived peptides were identified after digestion with pepsin, representing over 66% sequence coverage. No coverage was observed between residues 131-166, likely due to the low sequence complexity of this region (which contains a high proportion of serine, lysine and aspartic acid residues that may not be cleaved by pepsin). (B) N-terminal peptide fragment appears to reach maximum exchange at the 30 second time point (residues 15-37), (C) whereas C-terminal peptide fragments appear to increase in relative deuterium uptake between 30 seconds and 16 hour time points (residues 167-191).

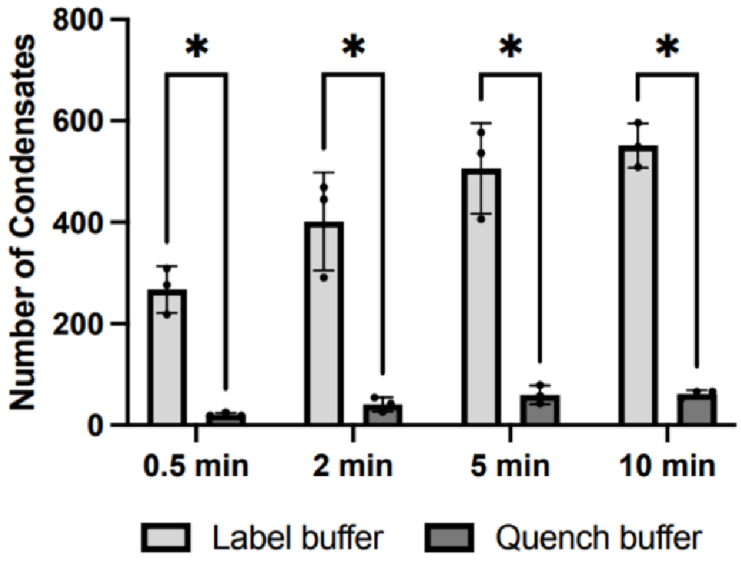

Fig. S2 Number of condensates under equilibration, label and quench conditions across HDX-MS timepoints. Significant differences in the number of condensates were calculated by performing a one-way ANOVA (** = p≤ 0.001)

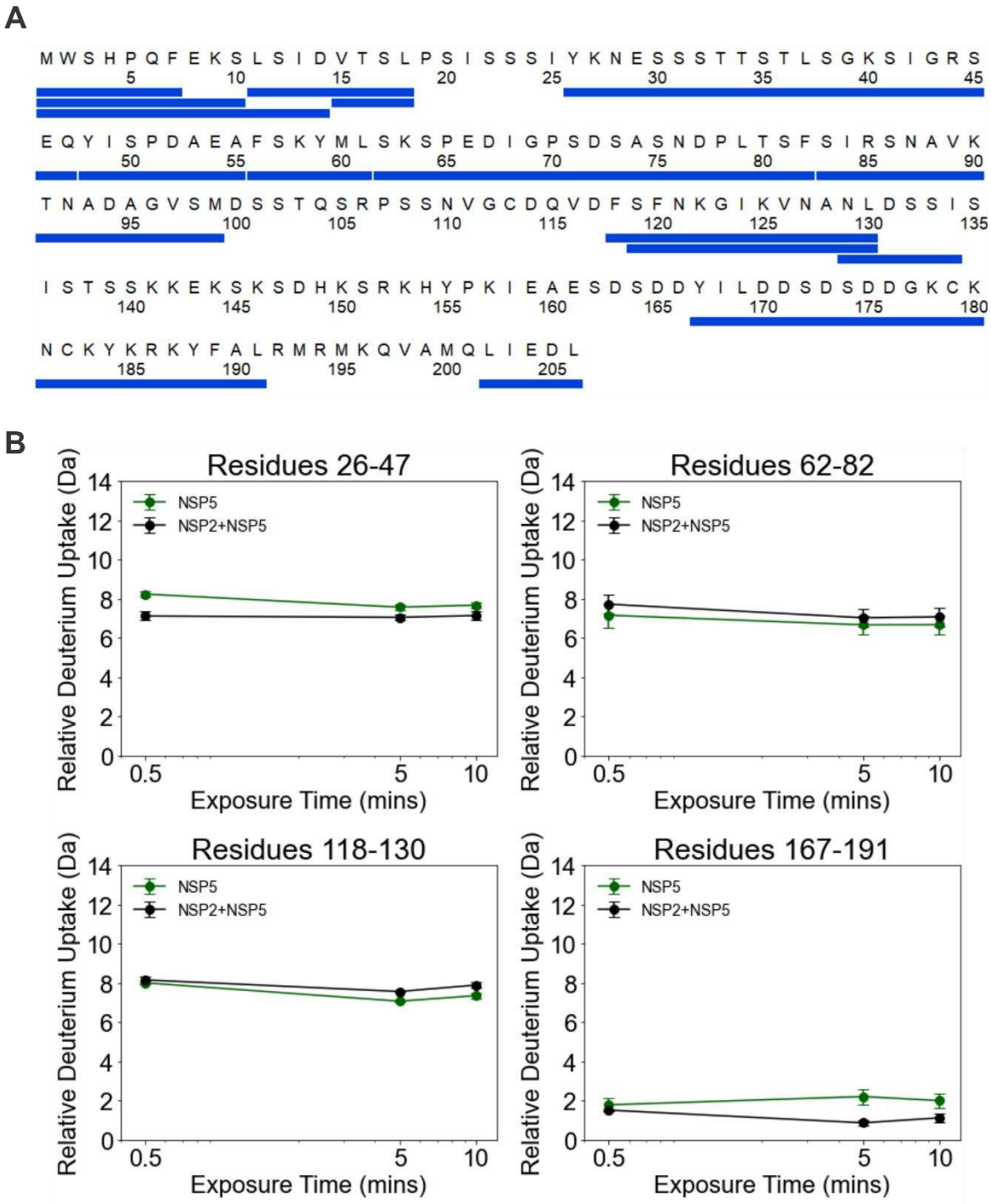

Fig. S3 (A) Coverage map for HDX-MS of NSP5 in the presence of NSP2. Each blue bar represents an individual peptic peptide identified by HDX-MS. (B) Individual uptake plots for representative significantly protected and deprotected NSP5 peptides in the presence of NSP2. A small decrease in relative deuterium uptake is observed between 30 seconds and 5 minutes, which can occur due to reduced back exchange as a result of the LEAP robot, coupled to our mass spectrometry system, omitting a step when it is utilised for sample preparation for 2-minute time points or less (Lumpkin & Komives, 2019).

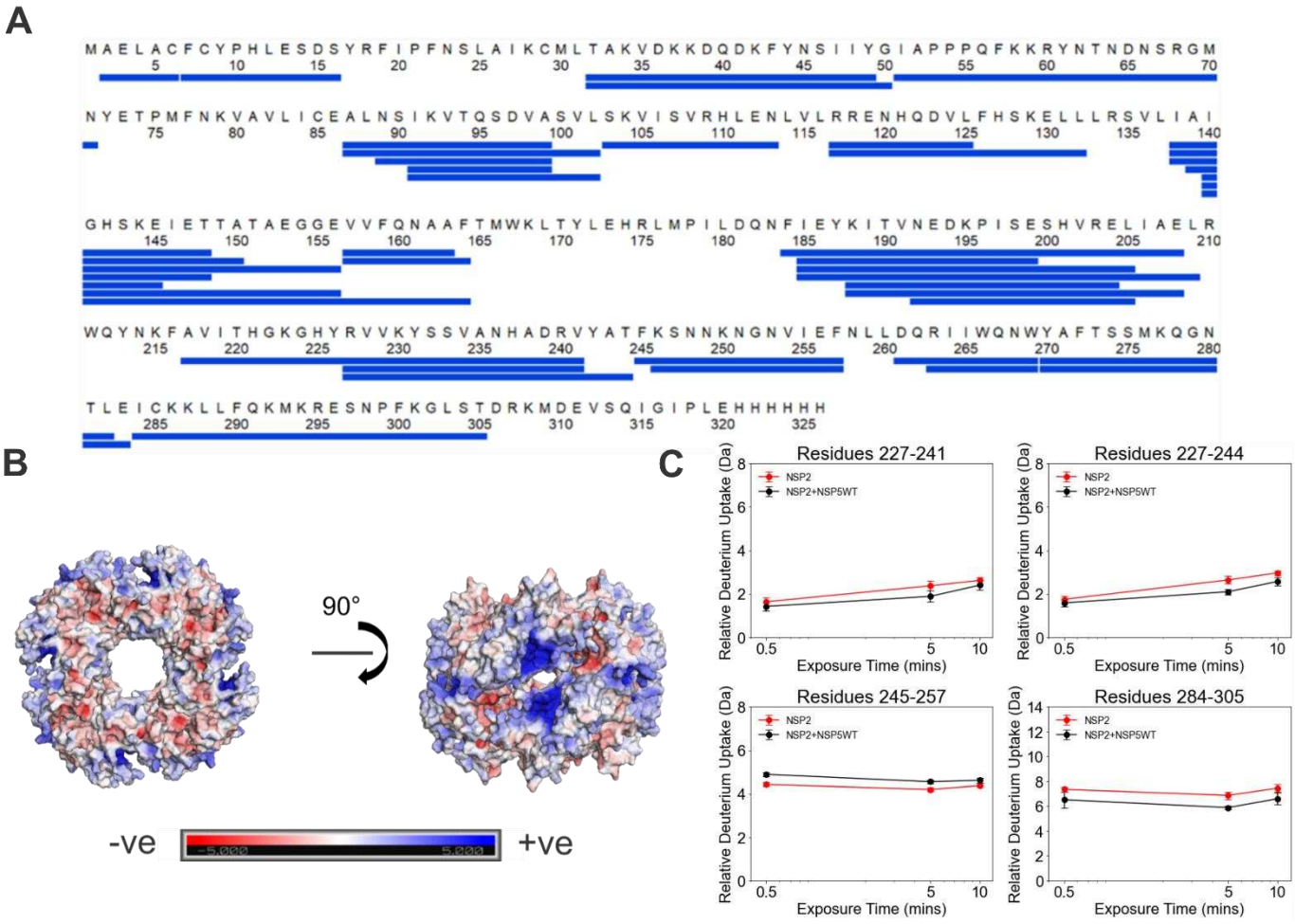

Fig. S4. (A) Coverage map for HDX-MS of NSP2 in the presence of NSP5. Each blue bar represents an individual peptic peptide identified by HDX-MS. (B) Electrostatic projection of NSP2 octamer rendered in Pymol (version 4.6) using APBS electrostatics plugin. (C) Individual uptake plots for protected peptides (residues 227-244 and 284-305) and deprotected peptides (residues 245-257). A small decrease in relative deuterium uptake is observed between 30 seconds and 5 minutes, which can occur due to reduced back exchange as a result of the LEAP robot, coupled to our mass spectrometry system, omitting a step when it is utilised for sample preparation for 2-minute time points or less (Lumpkin & Komives, 2019).

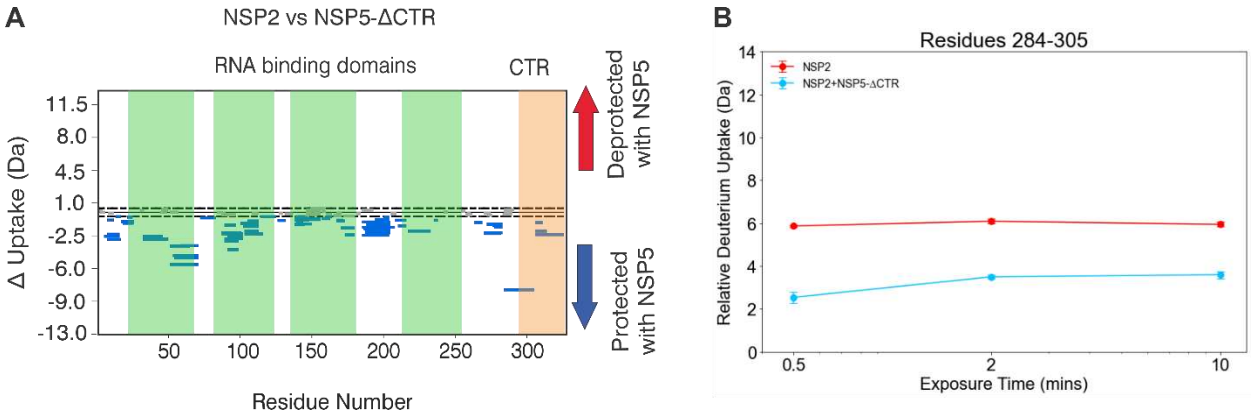

Fig. S5. (A) Cumulative Woods' plot showing the summed differences in deuterium incorporation over all timepoints for NSP2 vs NSP5-ΔCTR. Peptides from NSP2 that were significantly protected and deprotected peptides when incubated with deuterium in the presence of NSP2 are shown in blue and red, respectively (hybrid statistical test, $p < 0.02$). (B) Individual uptake plot for the protected peptide spanning residues 284-305.

Table S1. HDX-MS summary data table

	NSP5	NSP2 + NSP5	NSP5 + NSP2	NSP2 + NSP5-ΔCTR
HDX reaction details	25mM potassium phosphate, 25mM dipotassium phosphate, 300mM NaCl in 85.5% D_2O, pH 6.6, 4°C	1X PBS in 95% D_2O, pH 6.6, 4°C		
HDX time points (mins)	0.5, 960	0, 0.5, 5, 10		0, 0.5, 2, 10
HDX controls	Maximally labelled controls were not performed			
Back-exchange	N/A	N/A	N/A	N/A
No. of peptides	21	39	15	122
Sequence coverage	66%	72.7.%	67.5%	95.4%
Average peptide length / redundancy	15.29 / 2.37	14.67 / 2.41	11.87 / 1.28	9.86 / 3.87
Replicates	3 (Technical)	3 (Technical)	3 (Technical)	3 (Technical)
Repeatability	0.098 (average SD)	0.0595 (average SD)	0.0787 (average SD)	0.0354 (average SD)
Significant difference in HDX (delta HDX > XD)	p-value <0.05	98% CI: 0.26 Da / p-value <0.02	98% CI: 0.45 Da / p-value <0.02	98% CI: 0.17 Da / p-value 0.02

References

Lumpkin, R. J., & Komives, E. A. (2019). DECA, A Comprehensive, Automatic Post-processing Program for HDX-MS Data*. *Molecular & Cellular Proteomics*, *18*(12), 2516–2523. doi: 10.1074/mcp.TIR119.001731