Supplementary Information

Concerted dynamics of an LPS translocon in the presence of substrate and an antimicrobial peptide

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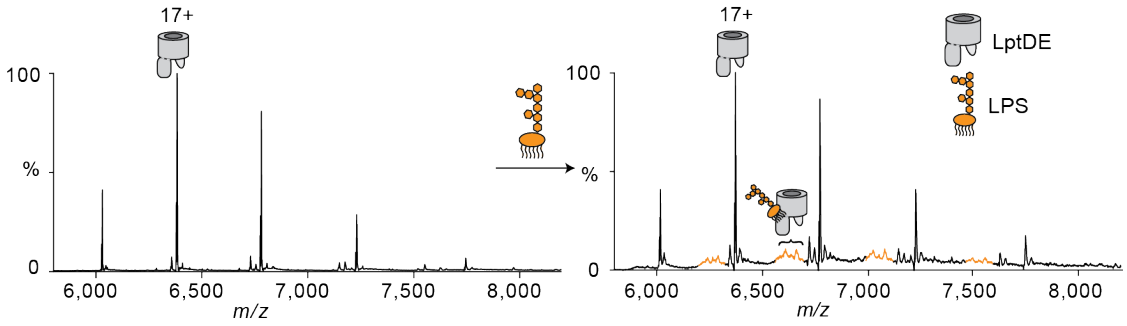
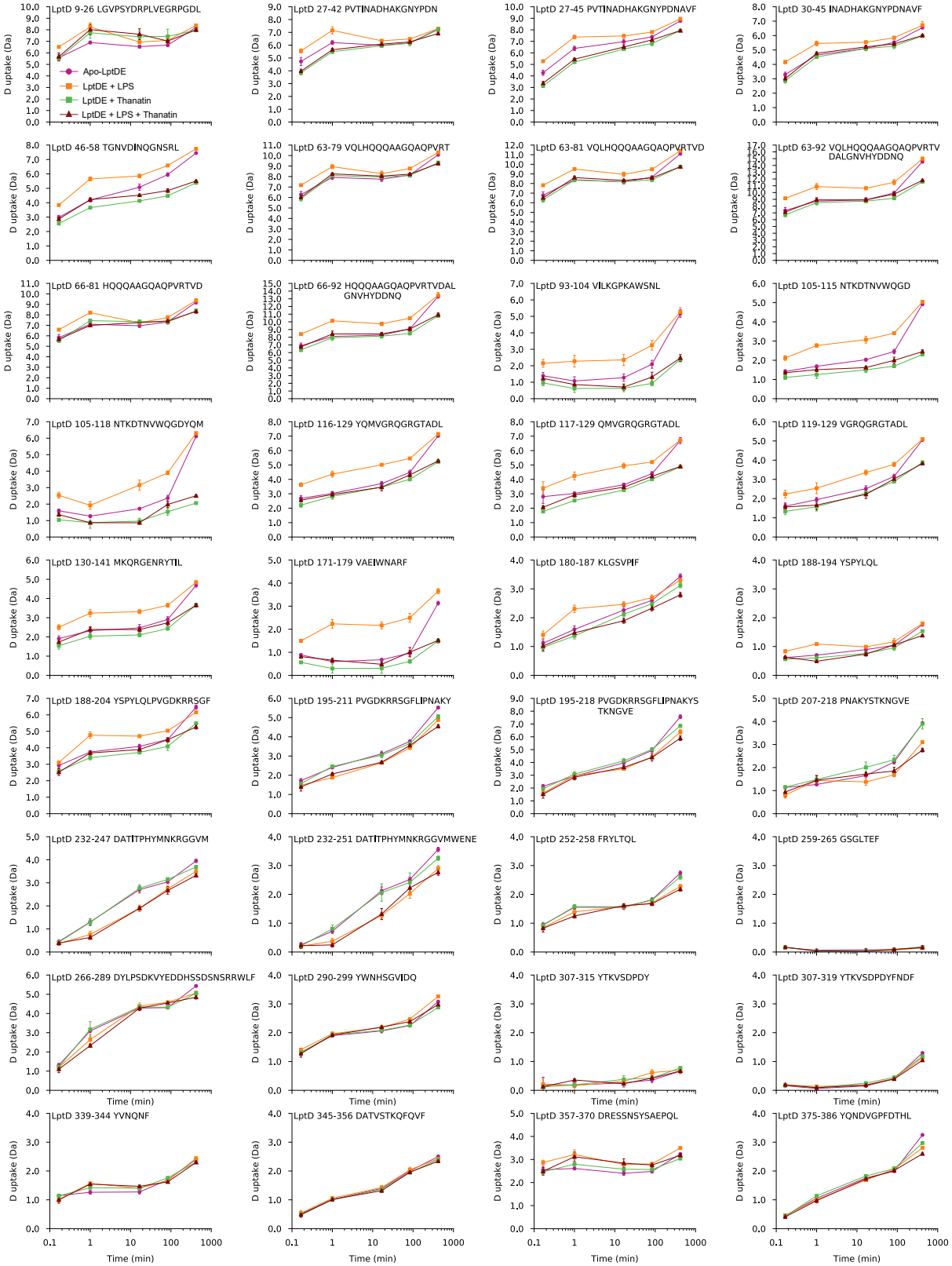
Supplementary Figures



Supplementary Figure 1. nMS analysis of LptDE-LPS interaction. LptDE (5 μM, left) in 0.5% (w/v) C_8E_4 was mixed with 10 μM LPS. A series of different adducts of molecular mass between 4 and 5 kDa was observed upon LPS addition (orange).


LptD 9-26 LGVPSYDRPLVEGRPGDL
Apo-LptDE
LptDE + LPS
LptDE + Thanatin
LptDE + LPS + Thanatin
LptD 27-42 PVTINADHAKGNYPDN
LptD 27-45 PVTINADHAKGNYPDNAVF
LptD 30-45 INADHAKGNYPDNAVF
LptD 46-58 TGNVDINQGNSRL
LptD 63-79 VQLHQQQAAGQAQPVRT
LptD 63-81 VQLHQQQAAGQAQPVRTVD
LptD 63-92 VQLHQQQAAGQAQPVRTV DALGNVHYDDNQ
LptD 66-81 HQQQAAGQAQPVRTVD
LptD 66-92 HQQQAAGQAQPVRTVDAL GNVHYDDNQ
LptD 93-104 VILKGPKAWSNL
LptD 105-115 NTKDTNVWQGD
LptD 105-118 NTKDTNVWQGDYQM
LptD 116-129 YQMVGRQGRGTADL
LptD 117-129 QMVGRQGRGTADL
LptD 119-129 VGRQGRGTADL
LptD 130-141 MKQRGENRYTIL
LptD 171-179 VAEIWNARF
LptD 180-187 KLGSVPIF
LptD 188-194 YSPYLQL
LptD 188-204 YSPYLQLPVGDKRRSGF
LptD 195-211 PVGDKRRSGFLIPNAKY
LptD 195-218 PVGDKRRSGFLIPNAKYS TKNGVE
LptD 207-218 PNAKYSTKNGVE
LptD 232-247 DATITPHYMNKRGGVM
LptD 232-251 DATITPHYMNKRGGVMWENE
LptD 252-258 FRYLTQL
LptD 259-265 GSGLTEF
LptD 266-289 DYLPSDKVYEDDHSSDSNSRRWLF
LptD 290-299 YWNHSGVIDQ
LptD 307-315 YTKVSDPDY
LptD 307-319 YTKVSDPDYFNDF
LptD 339-344 YVNQNF
LptD 345-356 DATVSTKQFQVF
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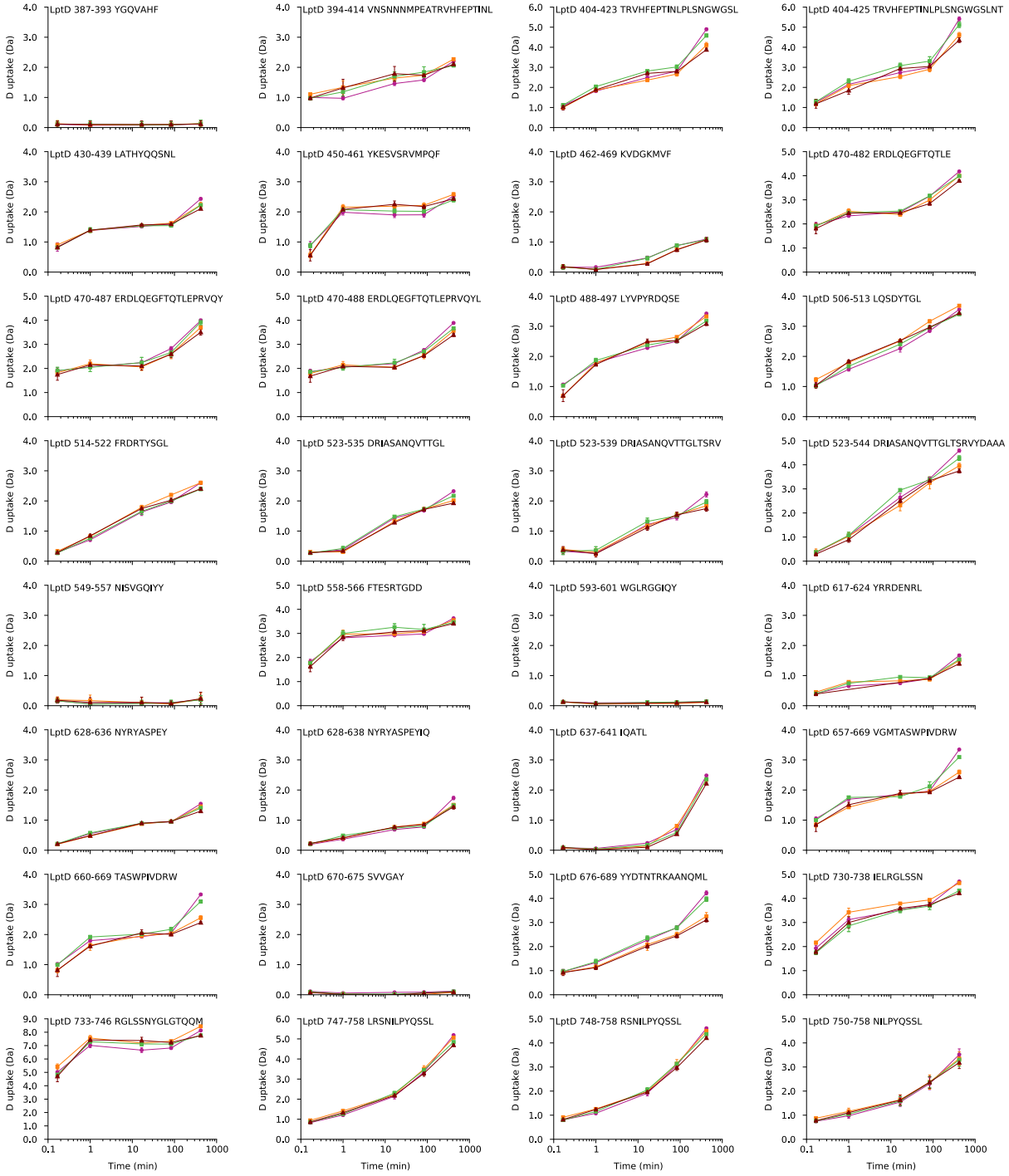
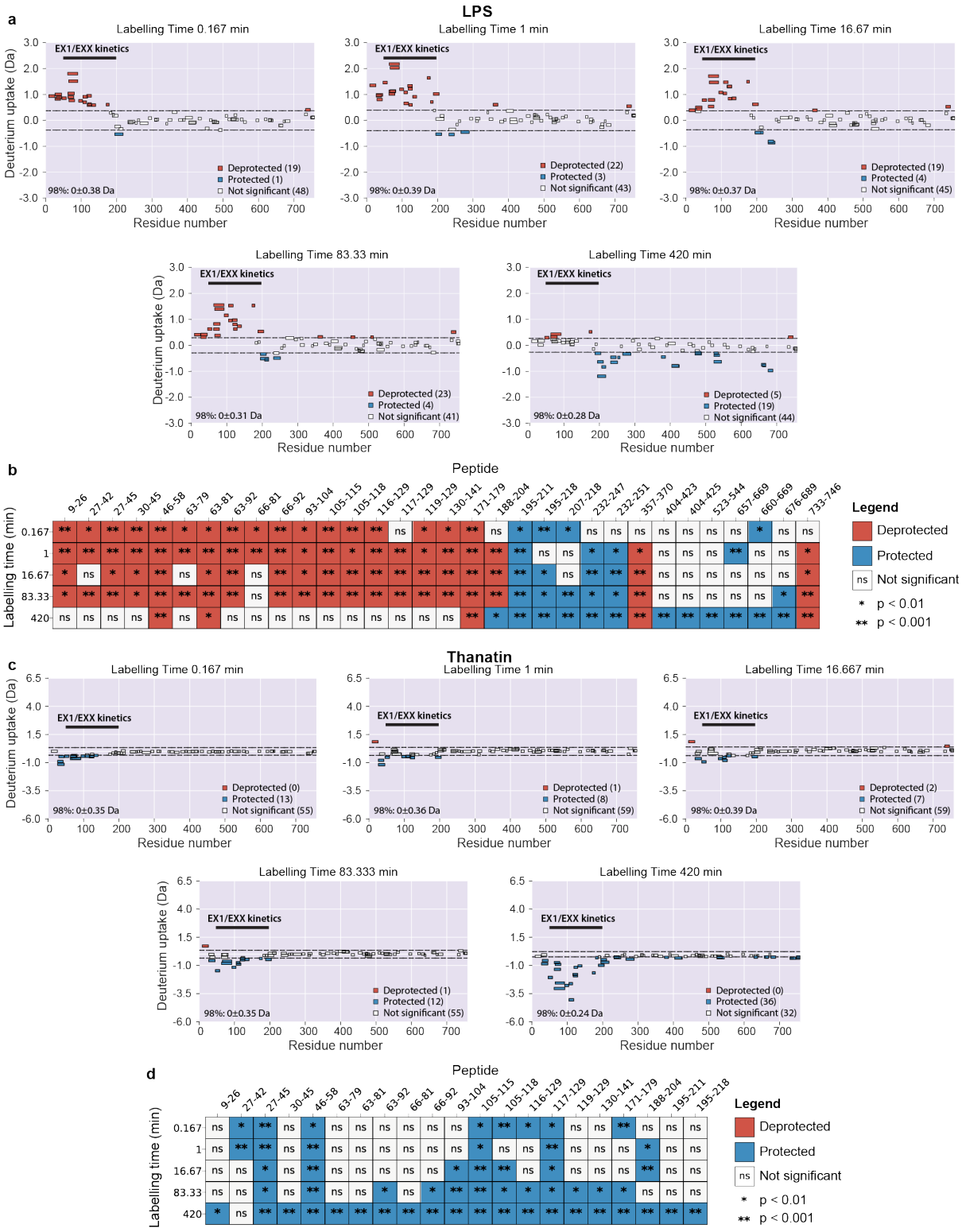
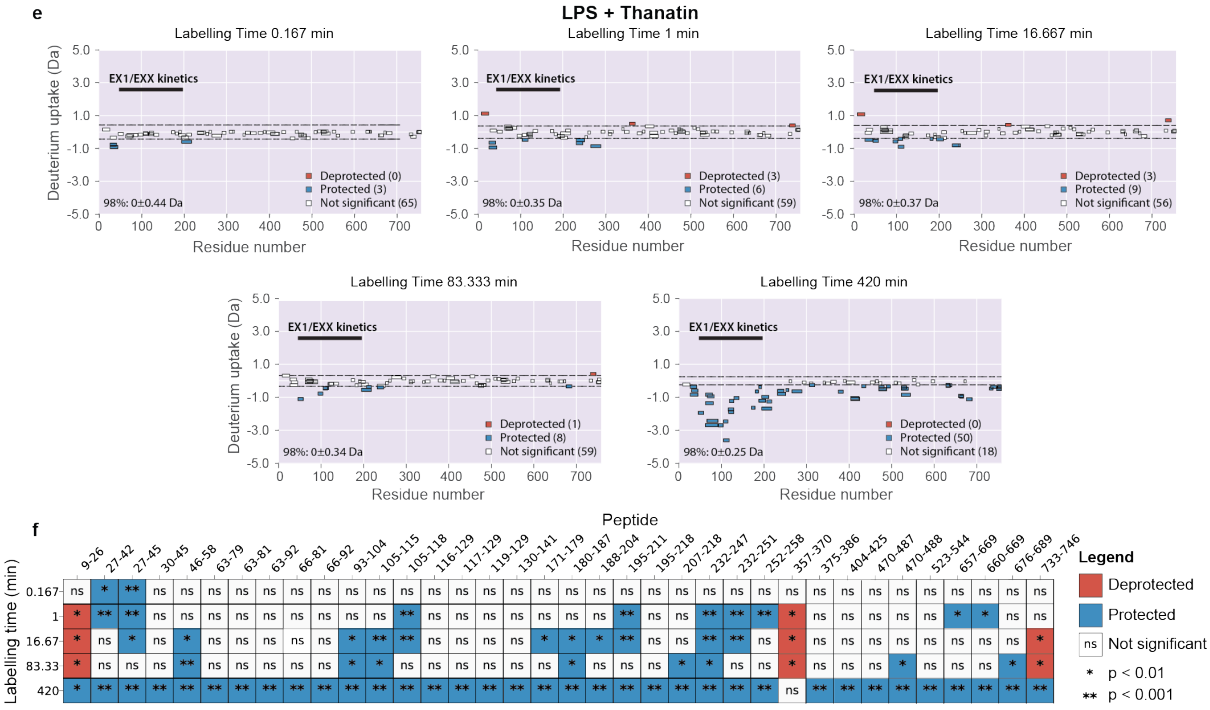
D uptake (Da)
Time (min)

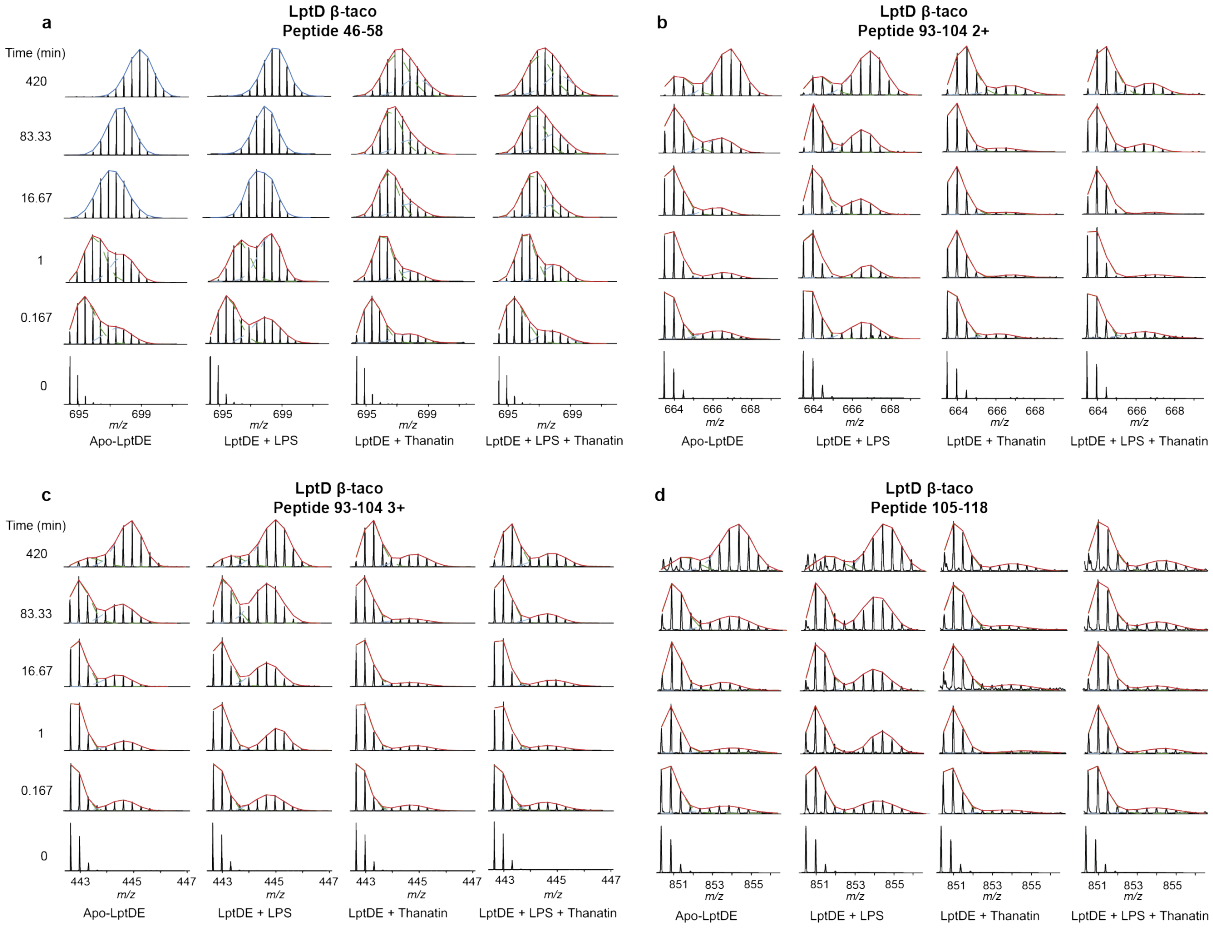


Supplementary Figure 2. Deuterium uptake plots for LptD in four states. Deuterium uptake is plotted as a function of labelling time (0.167 – 420 min) for all identified peptides. The apo state is indicated in purple, LPS-bound state in orange, thanatin-bound state in green, and LPS + thanatin state in maroon. Standard deviations are plotted as error bars ($n_{biological}$ = 2; $n_{technical}$ = 3) but are in some instances too small to be visible.

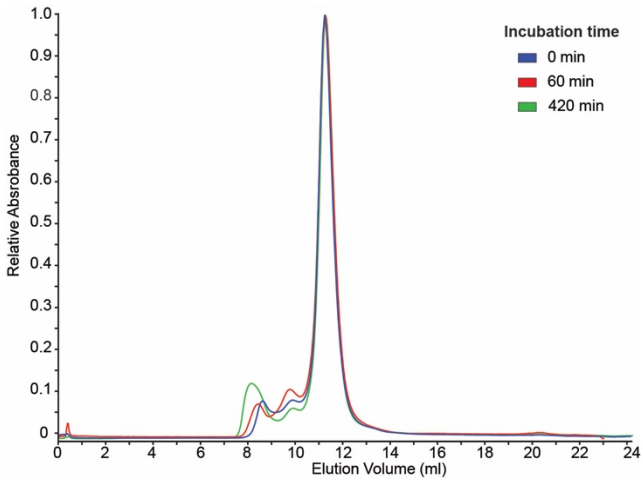

a
LPS
Labelling Time 0.167 min
Labelling Time 1 min
Labelling Time 16.67 min
Labelling Time 83.33 min
Labelling Time 420 min
EX1/EXX kinetics
Deuterium uptake (Da)
Residue number
Deprotected (19)
Protected (1)
Not significant (48)
98%: 0±0.38 Da
Deprotected (22)
Protected (3)
Not significant (43)
98%: 0±0.39 Da
Deprotected (19)
Protected (4)
Not significant (45)
98%: 0±0.37 Da
Deprotected (23)
Protected (4)
Not significant (41)
98%: 0±0.31 Da
Deprotected (5)
Protected (19)
Not significant (44)
98%: 0±0.28 Da
b
Peptide
Labelling time (min)
9-26
27-42
27-45
30-45
46-58
63-79
63-81
63-92
66-81
66-92
93-104
105-115
105-118
116-129
117-129
119-129
130-141
171-179
188-204
195-211
195-218
207-218
232-247
232-251
357-370
404-423
404-425
523-544
657-669
660-669
676-689
733-746
0.167
1
16.67
83.33
420
Legend
Deprotected
Protected
Not significant
p < 0.01
p < 0.001
c
Thanatin
Labelling Time 0.167 min
Labelling Time 1 min
Labelling Time 16.667 min
Labelling Time 83.333 min
Labelling Time 420 min
Deprotected (0)
Protected (13)
Not significant (55)
98%: 0±0.35 Da
Deprotected (1)
Protected (8)
Not significant (59)
98%: 0±0.36 Da
Deprotected (2)
Protected (7)
Not significant (59)
98%: 0±0.39 Da
Deprotected (1)
Protected (12)
Not significant (55)
98%: 0±0.35 Da
Deprotected (0)
Protected (36)
Not significant (32)
98%: 0±0.24 Da
d
Peptide
Labelling time (min)
9-26
27-42
27-45
30-45
46-58
63-79
63-81
63-92
66-81
66-92
93-104
105-115
105-118
116-129
117-129
119-129
130-141
171-179
188-204
195-211
195-218
0.167
1
16.67
83.33
420
Legend
Deprotected
Protected
Not significant
p < 0.01
p < 0.001



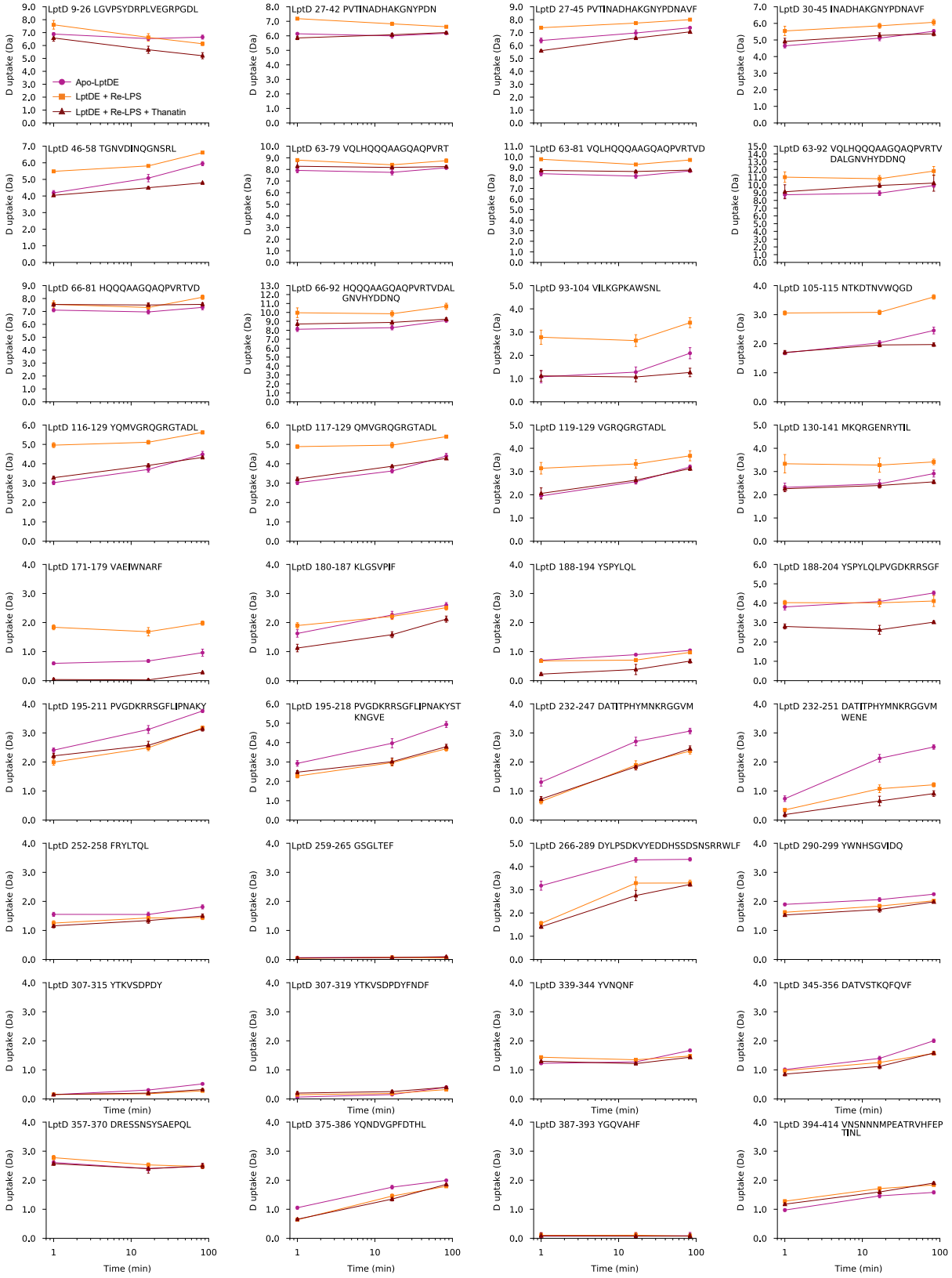
Supplementary Figure 3. Woods plots and peptide table for LptD in the presence of LPS, Thanatin or both ligands. (a) Woods plots showing the difference in deuterium uptake for each identified peptide in the presence of LPS. The length of the lines represents the length of the peptides. The confidence interval (CI) used to analyse each labelling time is 98%. The region showing EX1 kinetics is indicated in each Woods plot. (b) Peptides having a difference in deuterium uptake greater than the calculated CI according to the described methods are included in the table and a Student's t-test (two-sided, unpaired, $p < 0.01$) was performed to finally include only statistically significant differences. (c) Woods plots showing the difference in deuterium uptake for each identified peptide in the presence of thanatin. (d) Peptides having a difference in deuterium uptake greater than the calculated CI according to the described methods are included in the table and a Student's t-test (two-sided, unpaired, $p < 0.01$) was performed to finally include only statistically significant differences. (e) Woods plots showing the difference in deuterium uptake for each identified peptide in the presence of LPS and thanatin. (f) Peptides having a difference in deuterium uptake greater than the calculated CI according to the described methods are included in the table and Student's t-test (two-sided, unpaired, $p < 0.01$) was performed to finally include only statistically significant differences. Red colour indicates deprotected peptides, blue indicates protected; white and 'ns' indicate non-significant difference; * $p < 0.01$; ** $p < 0.001$. Detailed information of HDX-MS data is provided in Supplementary Table 2.

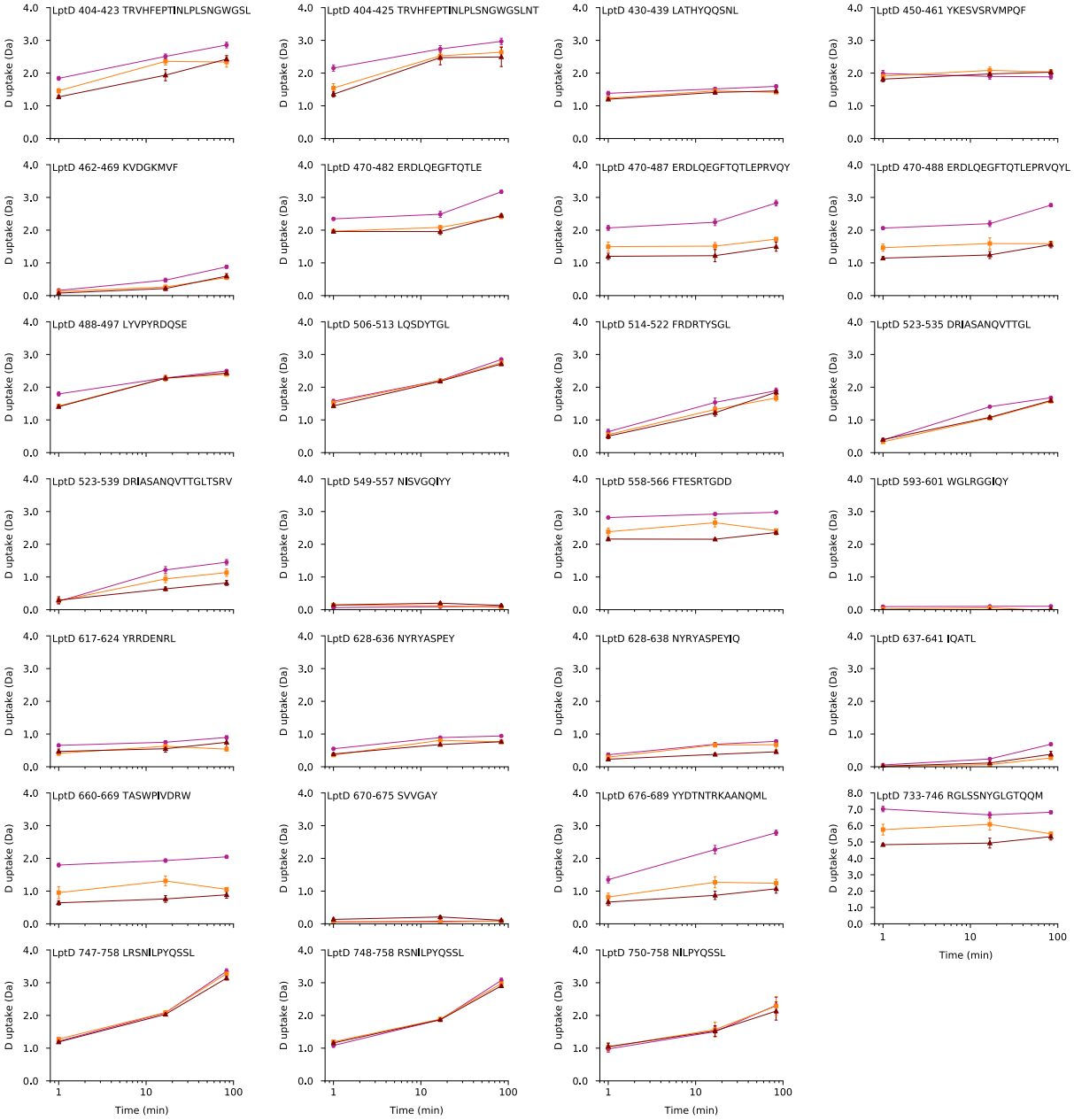


Supplementary Figure 4. Representative mass spectra for peptides showing EX1/EXX kinetics. Mass spectra are shown for apo-LptDE, LPS, thanatin or LPS + thanatin states. Two binomial isotopic envelopes produced the best fit for the spectra yielding low- (green) and high-mass (light blue) populations. The sums of the two binomial distributions are shown in red. In the case of peptide 46-58 (a) it was not always possible to unambiguously determine the two populations; therefore, the blue line represents a single binomial fit. (b) Peptide 93-104 (charge state 2+). (c) Peptide 93-104 (charge state 3+). (d) Peptide 105-118.

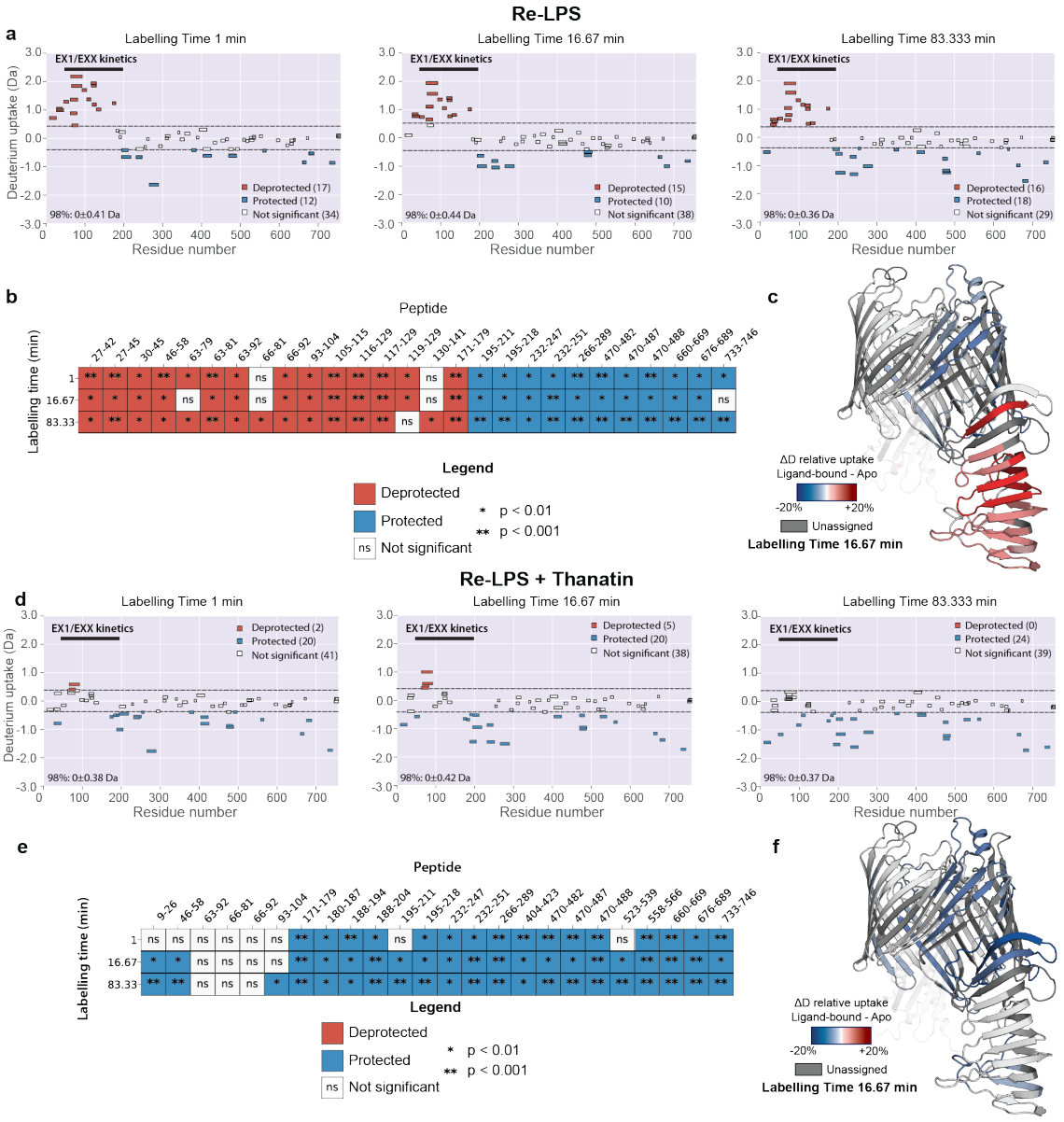


Supplementary Figure 5. Analytical SEC. Analytical SEC of LptDE performed in the buffer used for protein purification and HDX-MS experiments. The protein has been incubated at 20 °C for 0, 60, and 420 min before injection in the SEC column.

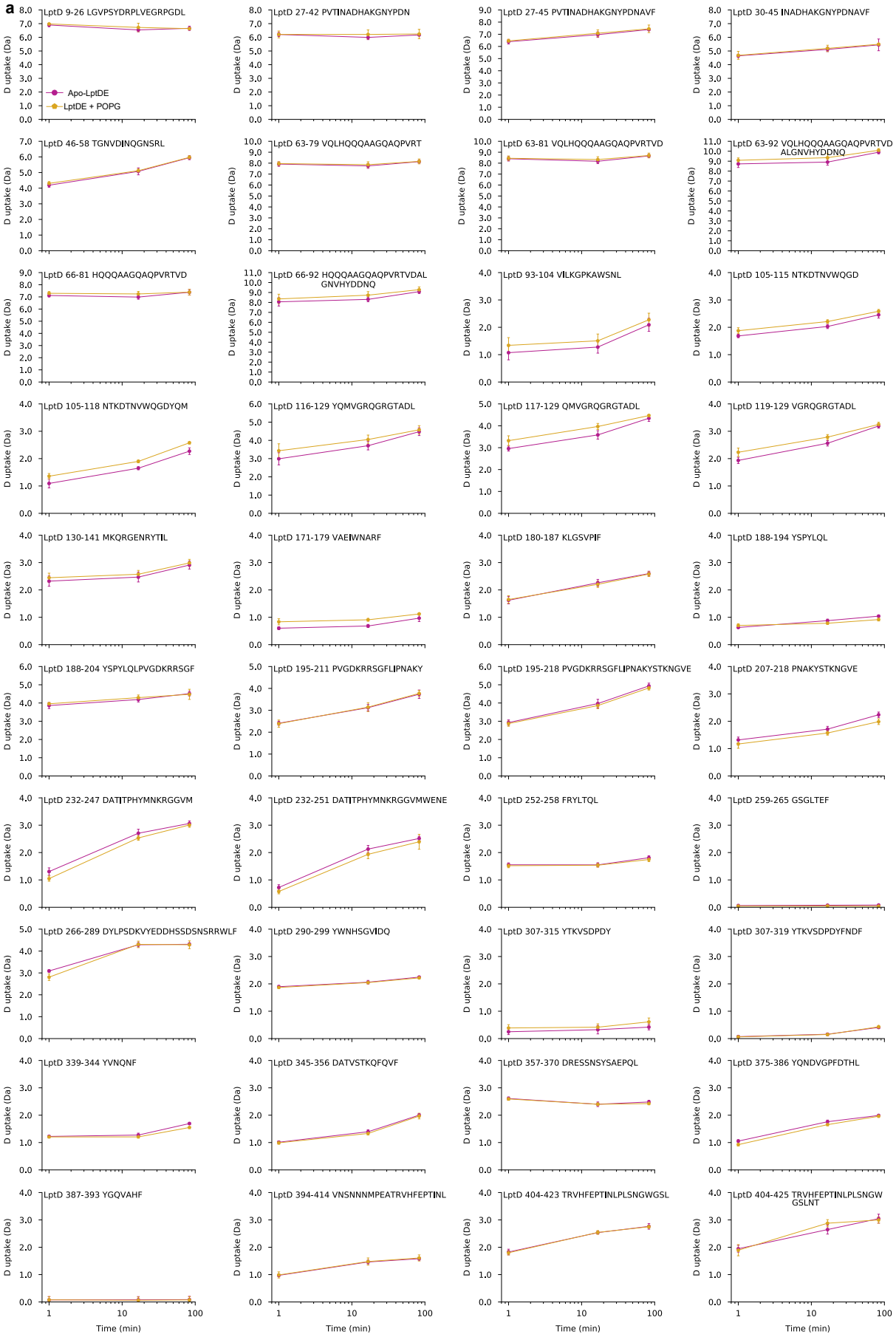

LptD 9-26 LGVPSYDRPLVEGRPGDL
LptD 27-42 PVTINADHAKGNYPDN
LptD 27-45 PVTINADHAKGNYPDNAVF
LptD 30-45 INADHAKGNYPDNAVF
LptD 46-58 TGNVDINQGNSRL
LptD 63-79 VQLHQQQAAGQAQPVRT
LptD 63-81 VQLHQQQAAGQAQPVRTVD
LptD 63-92 VQLHQQQAAGQAQPVRTV DALGNVHYDDNQ
LptD 66-81 HQQQAAGQAQPVRTVD
LptD 66-92 HQQQAAGQAQPVRTVDAL GNVHYDDNQ
LptD 93-104 VILKGPKAWSNL
LptD 105-115 NTKDTNVWQGD
LptD 116-129 YQMVGRQGRGTADL
LptD 117-129 QMVGRQGRGTADL
LptD 119-129 VGRQGRGTADL
LptD 130-141 MKQRGENRYTIL
LptD 171-179 VAEIWNARF
LptD 180-187 KLGSVPIF
LptD 188-194 YSPYLQL
LptD 188-204 YSPYLQLPVGDKRRSGF
LptD 195-211 PVGDKRRSGFLIPNAKY
LptD 195-218 PVGDKRRSGFLIPNAKYST KNGVE
LptD 232-247 DATITPHYMNKRGGVM
LptD 232-251 DATITPHYMNKRGGVM WENE
LptD 252-258 FRYLTQL
LptD 259-265 GSGLTEF
LptD 266-289 DYLPSDKVYEDDHSSDSNSRRWLF
LptD 290-299 YWNHSGVIDQ
LptD 307-315 YTKVSDPDY
LptD 307-319 YTKVSDPDYFNDF
LptD 339-344 YVNQNF
LptD 345-356 DATVSTKQFQVF
LptD 357-370 DRESSNSYSAEPQL
LptD 375-386 YQNDVGPFDTHL
LptD 387-393 YGQVAHF
LptD 394-414 VNSNNNMPEATRVHFEP TINL
D uptake (Da)
Time (min)
Apo-LptDE
LptDE + Re-LPS
LptDE + Re-LPS + Thanatin



Supplementary Figure 6. Deuterium uptake plots for LptD in three states. Deuterium uptake is plotted as a function of labelling time (1 – 83.33 min) for all identified peptides. The apo state is indicated in purple, Re-LPS-bound state in orange, and Re-LPS + thanatin state in maroon. Standard deviations are plotted as error bars ($n_{biological}$ = 1; $n_{technical}$ = 3) but are in some instances too small to be visible.



Supplementary Figure 7. Woods plots and peptide table for LptD in the presence of Re-LPS or Re-LPS+Thanain. (a) Woods plots showing the difference in deuterium uptake for each identified peptide in the presence of Re-LPS. The length of the lines represents the length of the peptides. The CI used to analyse each labelling time is 98%. The region showing EX1 kinetics is indicated in each Woods plot. (b) Peptides having a difference in deuterium uptake greater than the calculated CI according to the described methods are included in the table and Student's t-test (two-sided, unpaired, $p < 0.01$) was performed to finally include only statistically significant differences. (c) Difference in relative deuterium uptake (scaled for the number of residues of each peptide) at the 16.67 min labelling time mapped on the crystal structure of LptD (PDB ID: 5IV9). (d) Woods plots showing the difference in deuterium uptake for each identified peptide in the presence of Re-LPS and thanatin. (e) Peptides having a difference in deuterium uptake greater than the calculated CI according to the described methods are included in the table and Student's t-test (two-sided, unpaired, $p < 0.01$) was performed to finally include only statistically significant differences. (f) Difference in relative deuterium uptake (scaled for the number of residues of each peptide) at the 16.67 min labelling time mapped on the crystal structure of LptD (PDB ID: 5IV9). Only peptides showing significant difference are coloured. Red colour indicates deprotected peptides, blue indicates protected; white and 'ns' indicate non-significant difference; * $p < 0.01$; ** $p < 0.001$. Detailed information of HDX-MS data is provided in Supplementary Table 3.


a
LptD 9-26 LGVPSYDRPLVEGRPGDL
LptD 27-42 PVTINADHAKGNYPDN
LptD 27-45 PVTINADHAKGNYPDNAVF
LptD 30-45 INADHAKGNYPDNAVF
LptD 46-58 TGNVDINQGNSRL
LptD 63-79 VQLHQQQAAGQAQPVRT
LptD 63-81 VQLHQQQAAGQAQPVRTVD
LptD 63-92 VQLHQQQAAGQAQPVRTVD ALGNVHYDDNQ
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LptD 93-104 VILKGPKAWSNL
LptD 105-115 NTKDTNVWQGD
LptD 105-118 NTKDTNVWQGDYQM
LptD 116-129 YQMVGRQGRGTADL
LptD 117-129 QMVGRQGRGTADL
LptD 119-129 VGRQGRGTADL
LptD 130-141 MKQRGENRYTIL
LptD 171-179 VAEIWNARF
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LptD 188-194 YSPYLQL
LptD 188-204 YSPYLQLPVGDKRRSGF
LptD 195-211 PVGDKRRSGFLIPNAKY
LptD 195-218 PVGDKRRSGFLIPNAKYSTKNGVE
LptD 207-218 PNAKYSTKNGVE
LptD 232-247 DATITPHYMNKRGGVM
LptD 232-251 DATITPHYMNKRGGVMWENE
LptD 252-258 FRYLTQL
LptD 259-265 GSGLTEF
LptD 266-289 DYLPSDKVYEDDHSSDSNSRRWLF
LptD 290-299 YWNHSGVIDQ
LptD 307-315 YTKVSDPDY
LptD 307-319 YTKVSDPDYFNDF
LptD 339-344 YVNQNF
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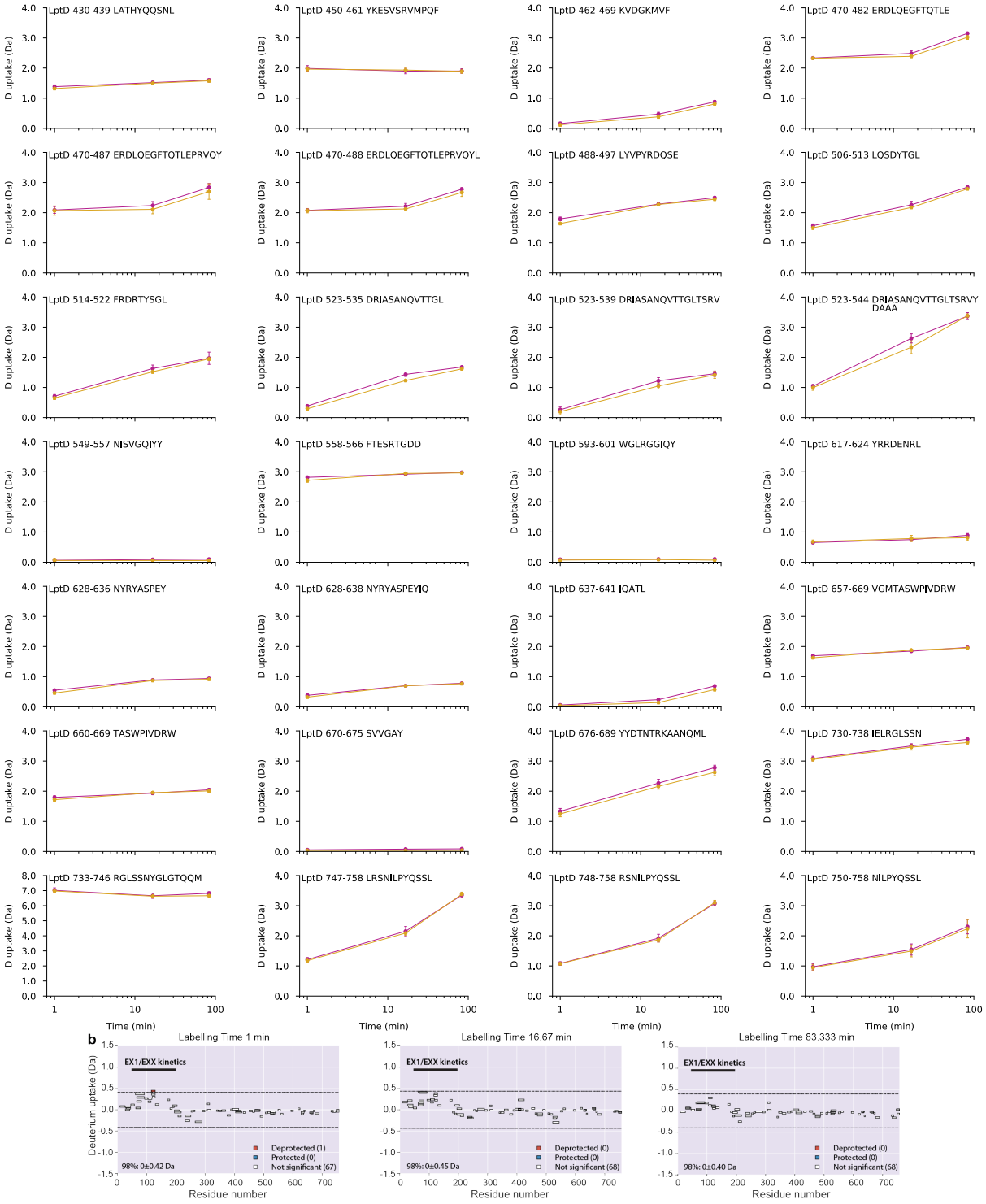
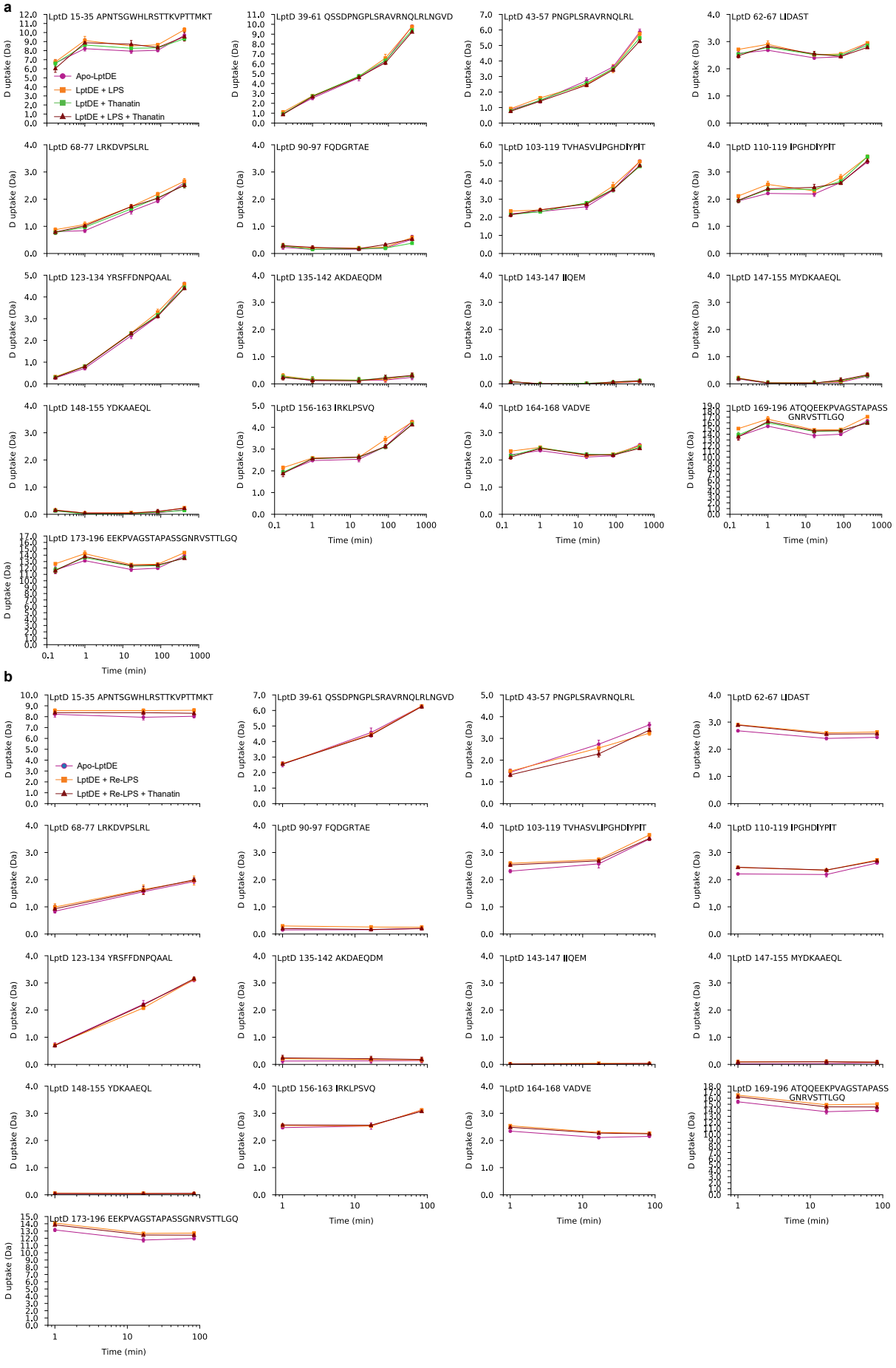
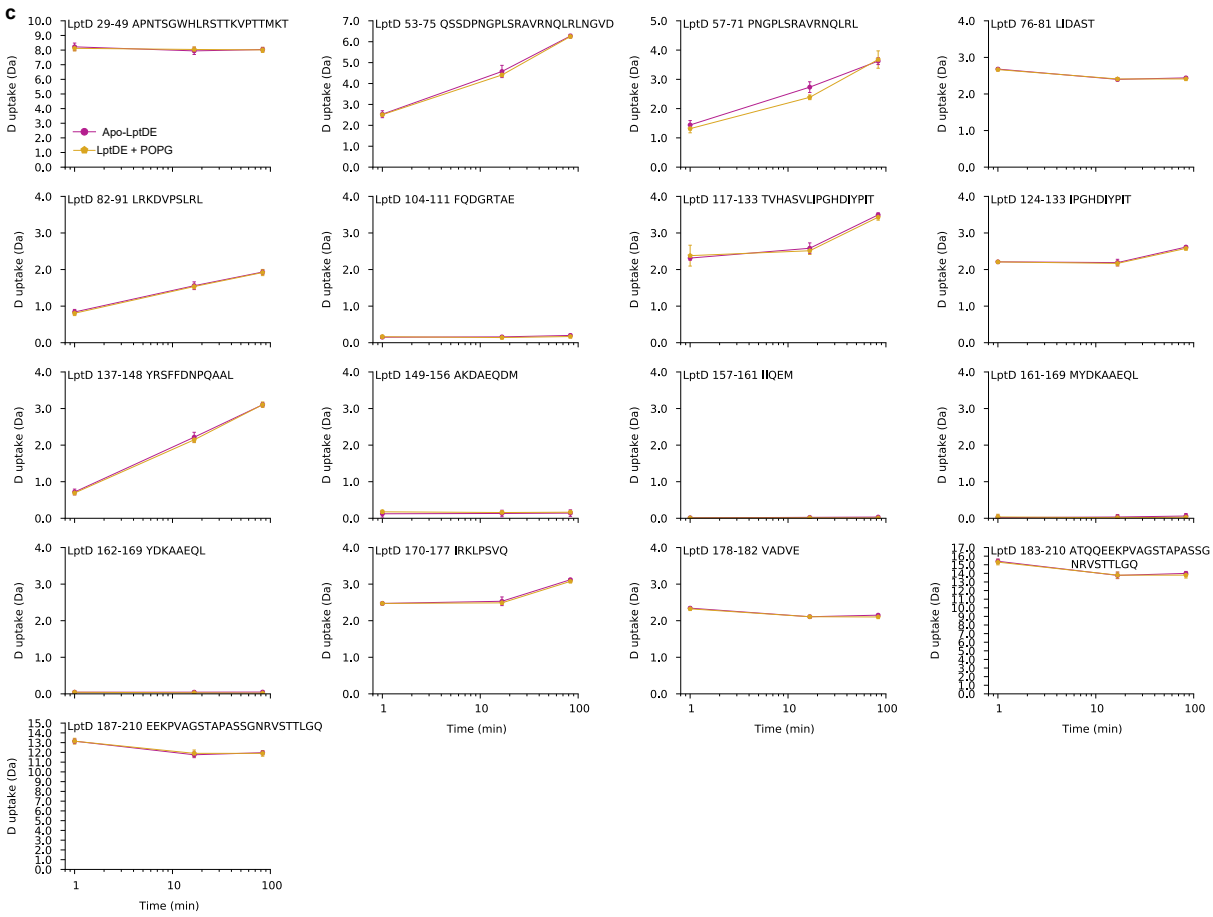
LptD 357-370 DRESSNSYSAEPQL
LptD 375-386 YQNDVGPFDTHL
LptD 387-393 YGQVAHF
LptD 394-414 VNSNNNMPEATRVHFEPTINL
LptD 404-423 TRVHFEPTINLPLSNGWGSL
LptD 404-425 TRVHFEPTINLPLSNGW GSLNT
Apo-LptDE
LptDE + POPG
D uptake (Da)
Time (min)

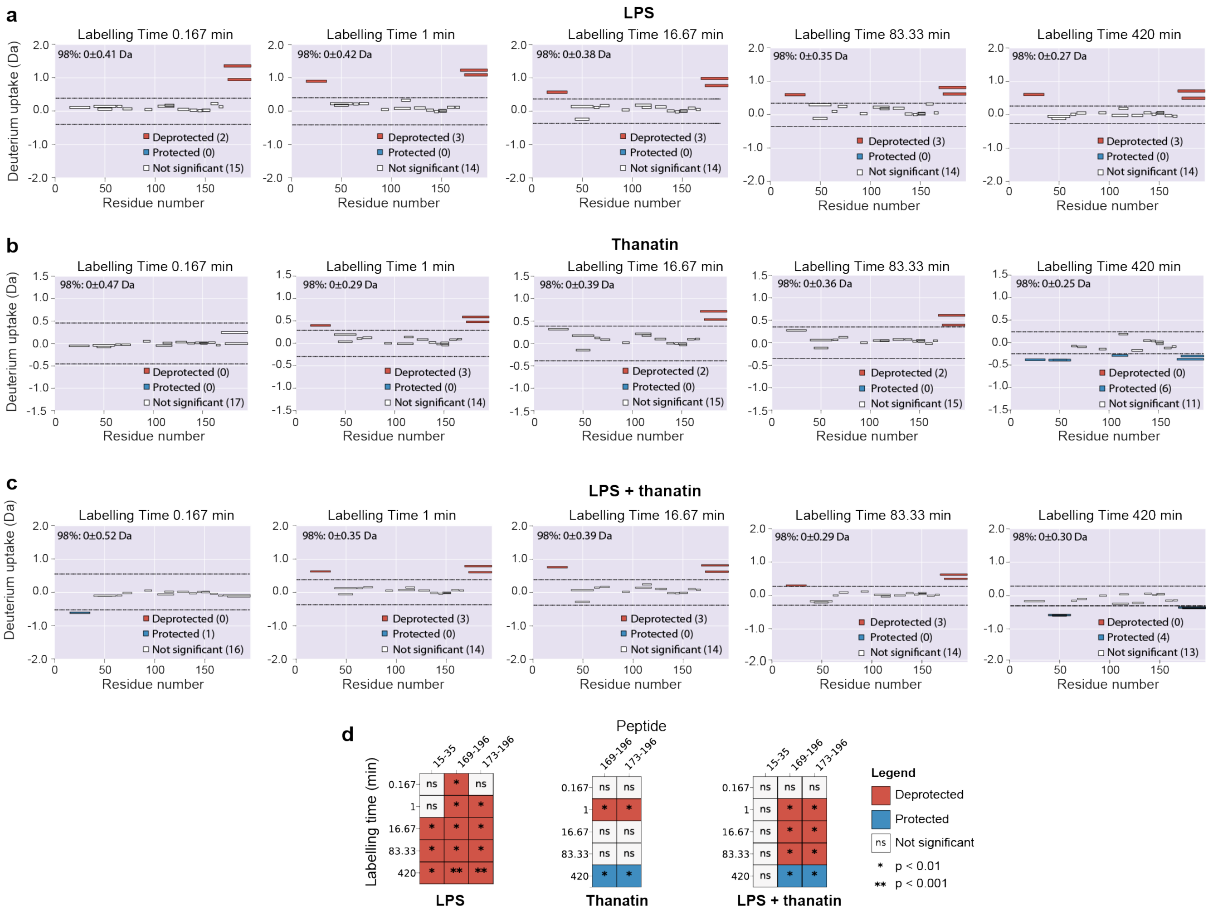


Supplementary Figure 8. Deuterium uptake and woods plots for LptD in the presence of POPG. (a) Deuterium uptake is plotted as a function of labelling time (1 – 83.33 min) for all identified peptides. The apo state is indicated in purple, and POPG state in gold. Standard deviations are plotted as error bars ($n_{biological}$ = 1; $n_{technical}$ = 3) but are in some instances too small to be visible. (b) Woods plots showing the difference in deuterium uptake for each identified peptide in the presence of Re-LPS. The length of the lines represents the length of the peptides. The CI used to analyse each labelling time is 98%. The region showing EX1 kinetics is indicated in each Woods plot. Red colour indicates deprotected peptides, blue indicates protected, white indicate non-significant difference. No statistically significant peptides were identified. Detailed information of HDX-MS data is provided in Supplementary Table 3.

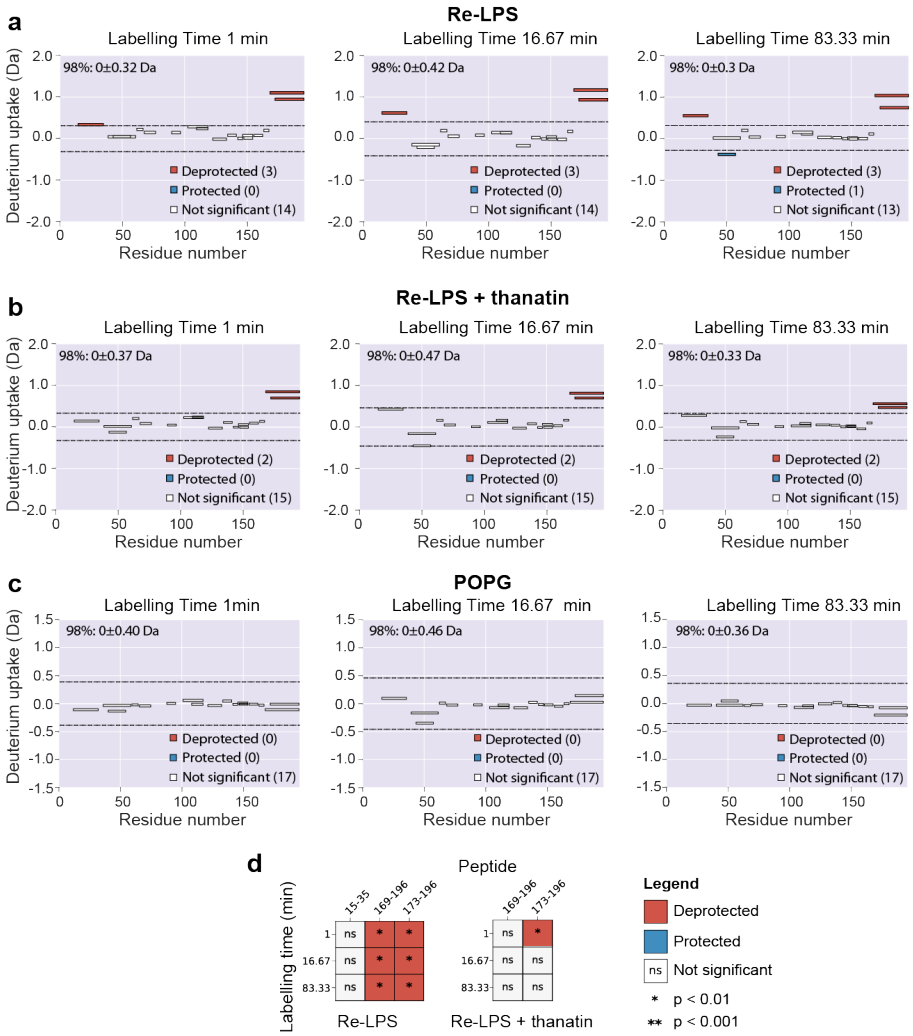

a
LptD 15-35 APNTSGWHLRSTTKVPTTMKT
LptD 39-61 QSSDPNGPLSRAVRNQLRLNGVD
LptD 43-57 PNGPLSRAVRNQLRL
LptD 62-67 LIDAST
LptD 68-77 LRKDVPSLRL
LptD 90-97 FQDGRTAE
LptD 103-119 TVHASVLIPGHDIYPIT
LptD 110-119 IPGHDIYPIT
LptD 123-134 YRSFFDNPQAAL
LptD 135-142 AKDAEQDM
LptD 143-147 IIQEM
LptD 147-155 MYDKAAEQL
LptD 148-155 YDKAAEQL
LptD 156-163 IRKLPSVQ
LptD 164-168 VADVE
LptD 169-196 ATQQEEKPVAGSTAPASS GNRVSTTLGQ
LptD 173-196 EEKPVAGSTAPASSGNRVSTTLGQ
Apo-LptDE
LptDE + LPS
LptDE + Thanatin
LptDE + LPS + Thanatin
D uptake (Da)
Time (min)
b
LptD 15-35 APNTSGWHLRSTTKVPTTMKT
LptD 39-61 QSSDPNGPLSRAVRNQLRLNGVD
LptD 43-57 PNGPLSRAVRNQLRL
LptD 62-67 LIDAST
LptD 68-77 LRKDVPSLRL
LptD 90-97 FQDGRTAE
LptD 103-119 TVHASVLIPGHDIYPIT
LptD 110-119 IPGHDIYPIT
LptD 123-134 YRSFFDNPQAAL
LptD 135-142 AKDAEQDM
LptD 143-147 IIQEM
LptD 147-155 MYDKAAEQL
LptD 148-155 YDKAAEQL
LptD 156-163 IRKLPSVQ
LptD 164-168 VADVE
LptD 169-196 ATQQEEKPVAGSTAPASS GNRVSTTLGQ
LptD 173-196 EEKPVAGSTAPASSGNRVSTTLGQ
Apo-LptDE
LptDE + Re-LPS
LptDE + Re-LPS + Thanatin
D uptake (Da)
Time (min)



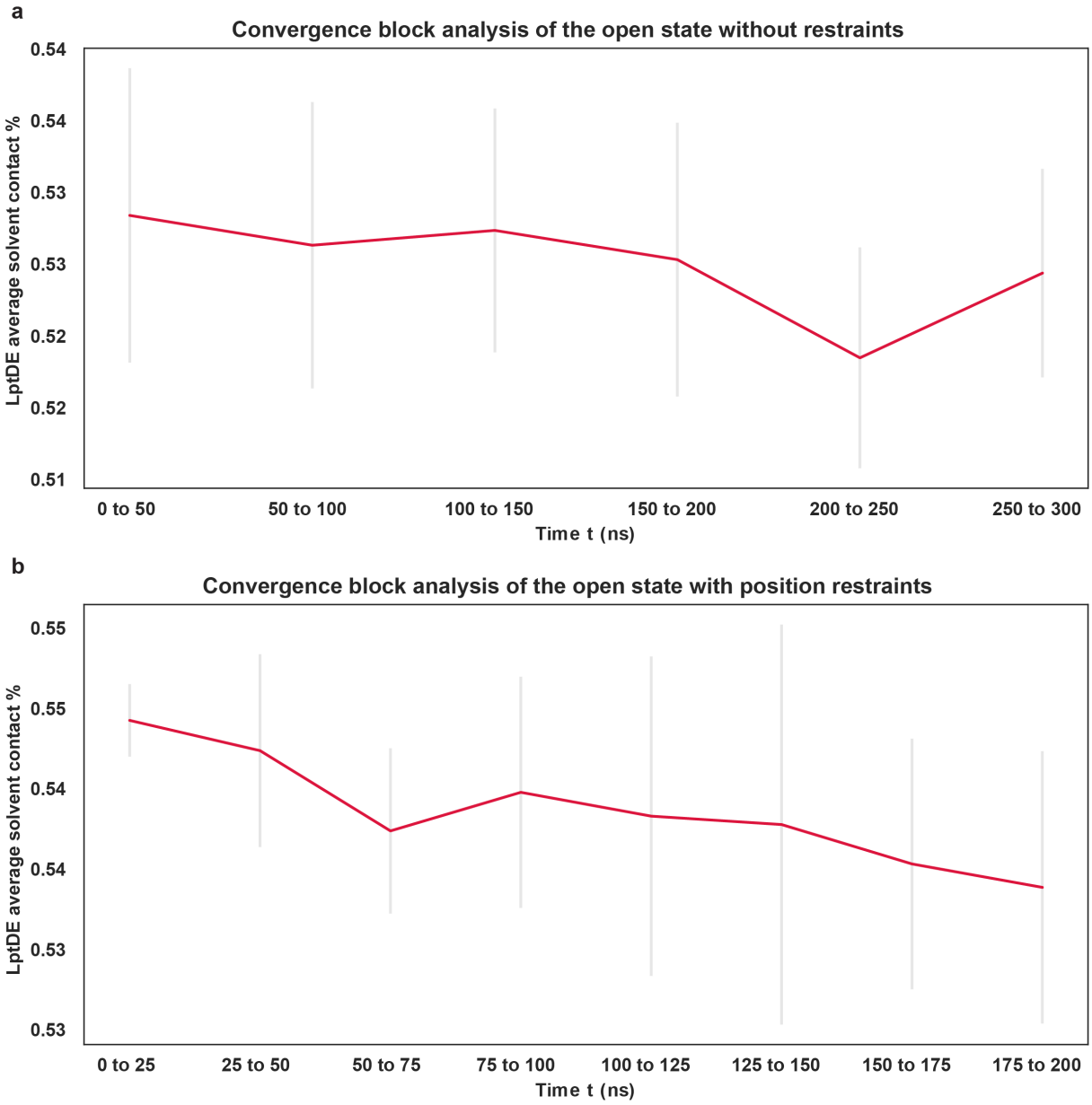
Supplementary Figure 9. Deuterium uptake plots for LptE in all states. (a) Deuterium uptake is plotted as a function of labelling time (0.167 – 420 min) for all identified peptides in the apo, LPS, thanatin and thanatin + LPS states. The apo state is indicated in purple, LPS-bound state in orange, thanatin-bound state in green, and LPS + thanatin state in maroon. Error bars indicate s.d. ($n_{biological}$ = 2; $n_{technical}$ = 3). (b) Deuterium uptake is plotted as a function of labelling time (1 – 83.33 min) for all identified peptides in apo, Re-LPS, and Re-LPS + thanatin states. The apo state is indicated in purple, Re-LPS-bound state in orange, and Re-LPS + thanatin state in maroon. Error bars indicate s.d. ($n_{biological}$ = 1; $n_{technical}$ = 3). (c) Deuterium uptake is plotted as a function of labelling time (1 – 83.33 min) for all identified peptides in apo and POPG state. The apo state is indicated in purple, and POPG state in gold. Error bars indicate s.d. ($n_{biological}$ = 1; $n_{technical}$ = 3). In some instances error bars are too small to be visible.



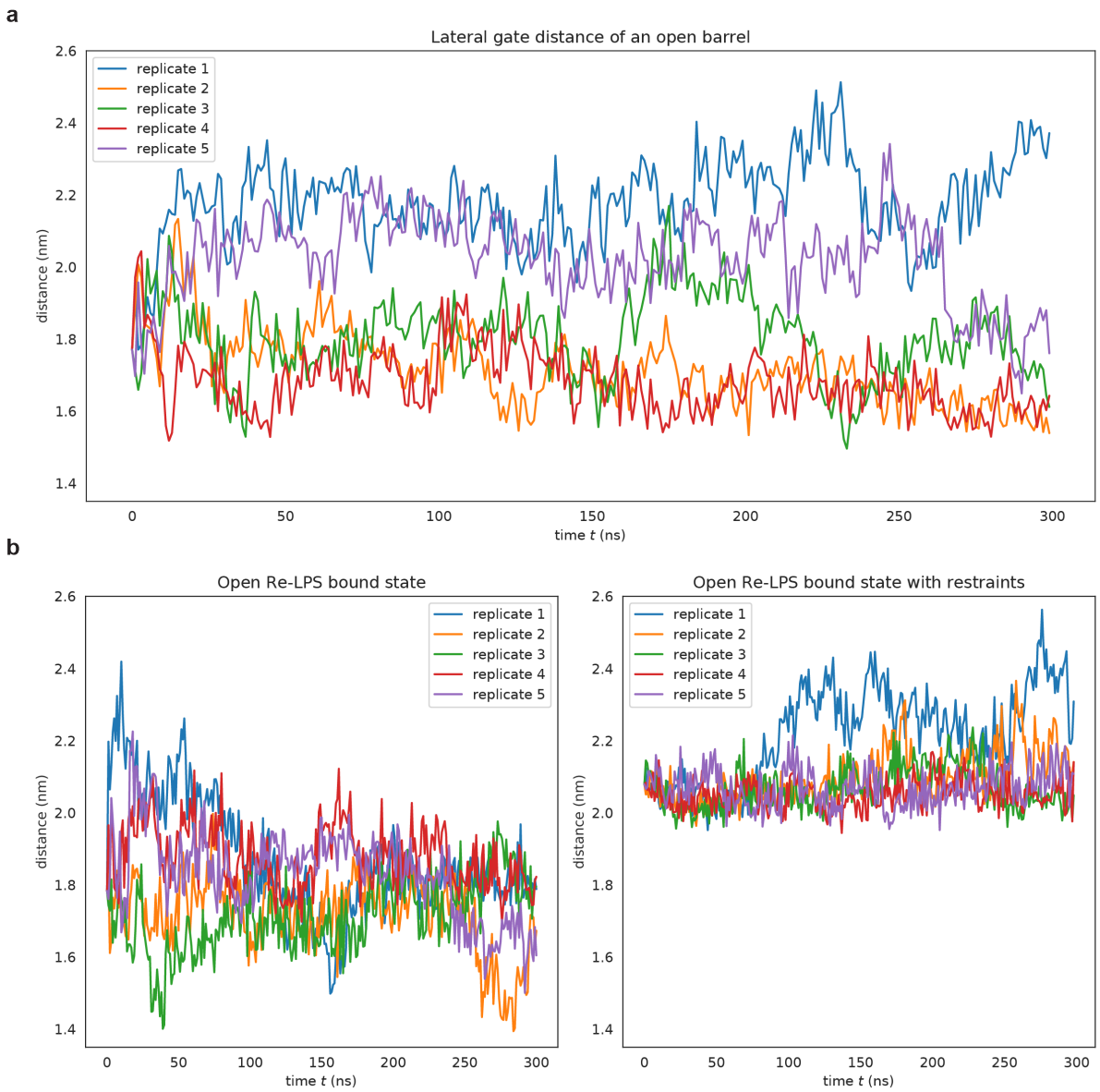
Supplementary Figure 10. Woods plots and peptide table for LptE in the presence of LPS, thanatin and LPS + thanatin. Woods plots showing the difference in deuterium uptake for each identified peptide in the presence of LPS (a), thanatin (b), and thanatin + LPS (c). The length of the lines represents the length of the peptides. The CI used to analyse each labelling time is 98%. (d) Peptides having a difference in deuterium uptake greater than the calculated CI according to the described methods are included in the table and Student's t-test (two-sided, unpaired, $p < 0.01$) was performed to finally include only statistically significant differences. Red colour indicates deprotected peptides, blue indicates protected; white and 'ns' indicate non-significant difference; * $p < 0.01$; ** $p < 0.001$. Detailed information of HDX-MS data is provided in Supplementary Table 4.



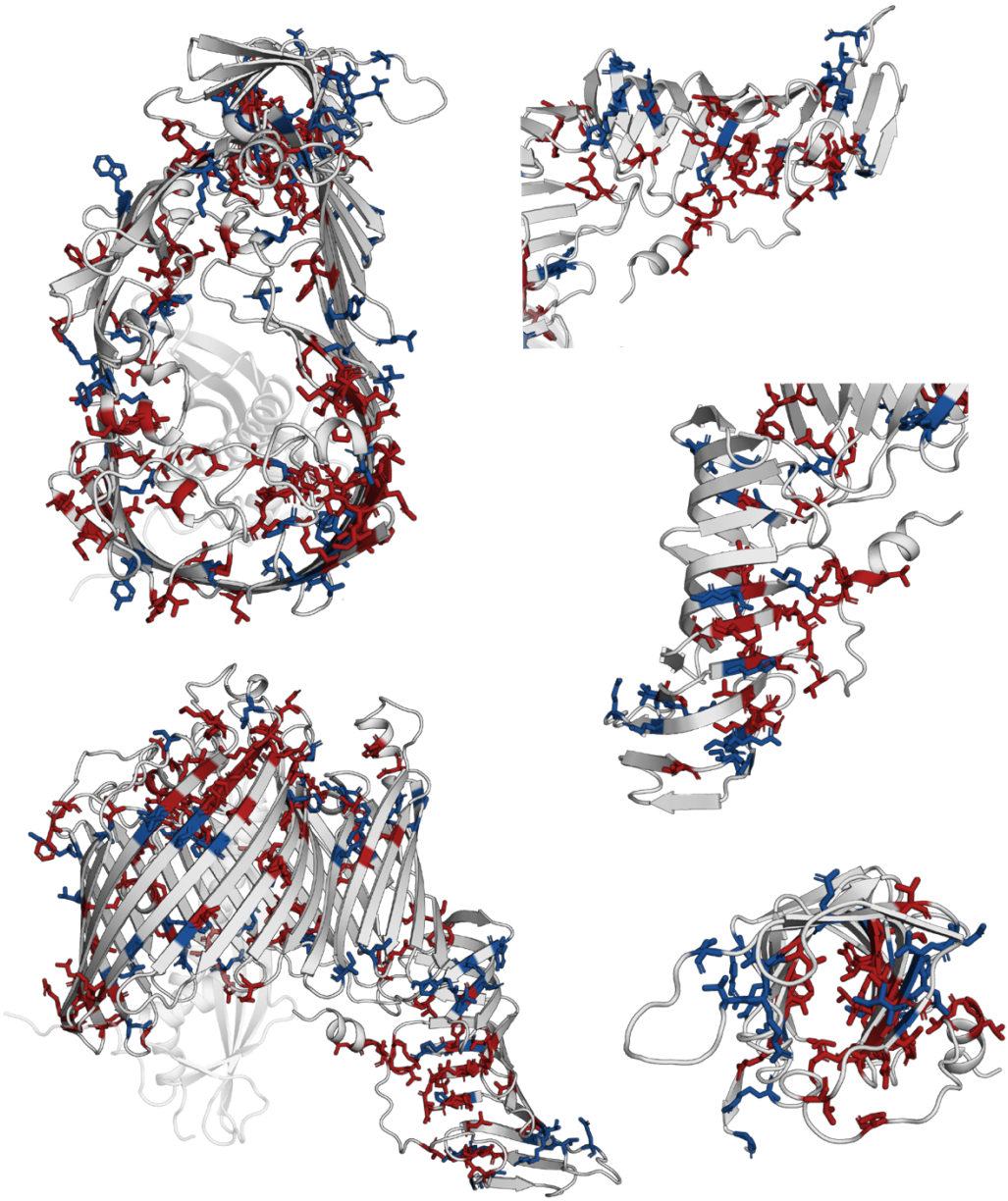
Supplementary Figure 11. Woods plots and peptide table for LptE in the presence of Re-LPS, Re-LPS + thanatin and POPG. Woods plots showing the difference in deuterium uptake for each identified peptide in the presence of Re-LPS (a), Re-LPS + thanatin (b), POPG (c). The length of the lines represents the length of the peptides. The CI used to analyse each labelling time is 98%. (d) Peptides having a difference in deuterium uptake greater than the calculated CI according to the described methods are included in the table and Student's t-test (two-sided, unpaired, $p < 0.01$) was performed to finally include only statistically significant differences. Red colour indicates deprotected peptides, blue indicates protected; white and 'ns' indicate non-significant difference; * $p < 0.01$; ** $p < 0.001$. Detailed information of HDX-MS data is provided in Supplementary Table 5.



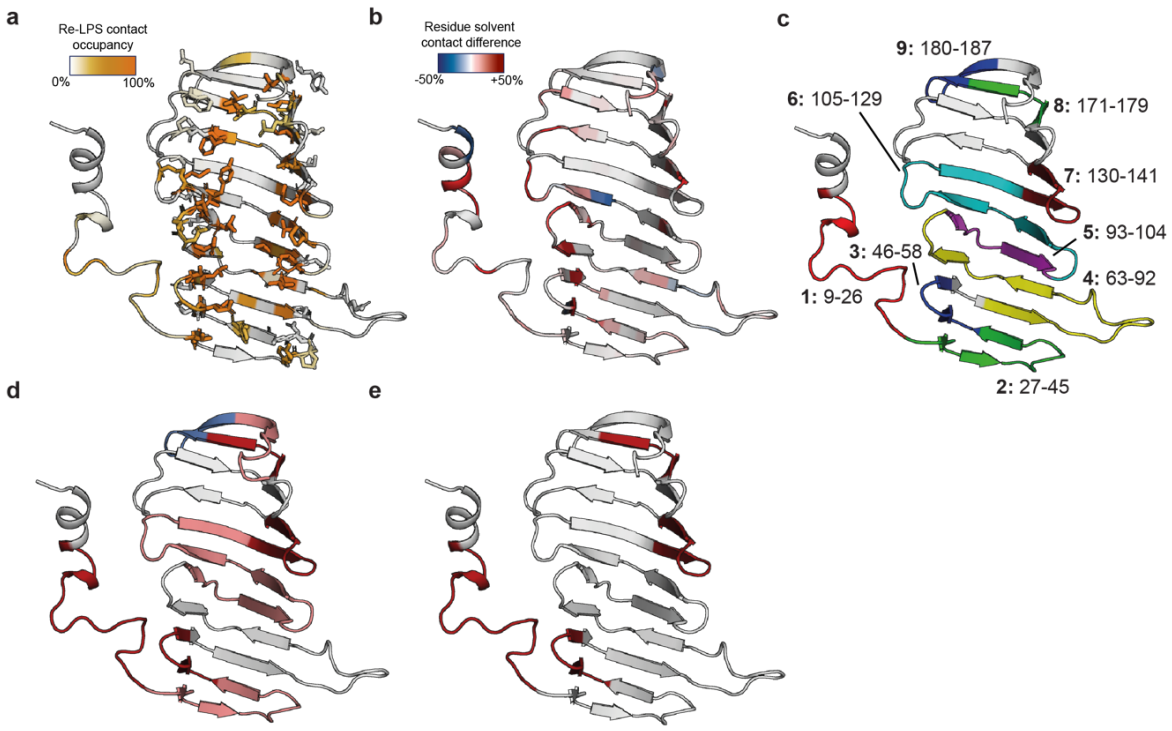
Supplementary Figure 12. Block convergence analysis. Convergence plot of the open Re-LPS bound state (a) without and with (b) PLUMED restraints, monitoring average solvent contact over all residues in LptDE. Error bars correspond to mean and s.d. of five repeats.



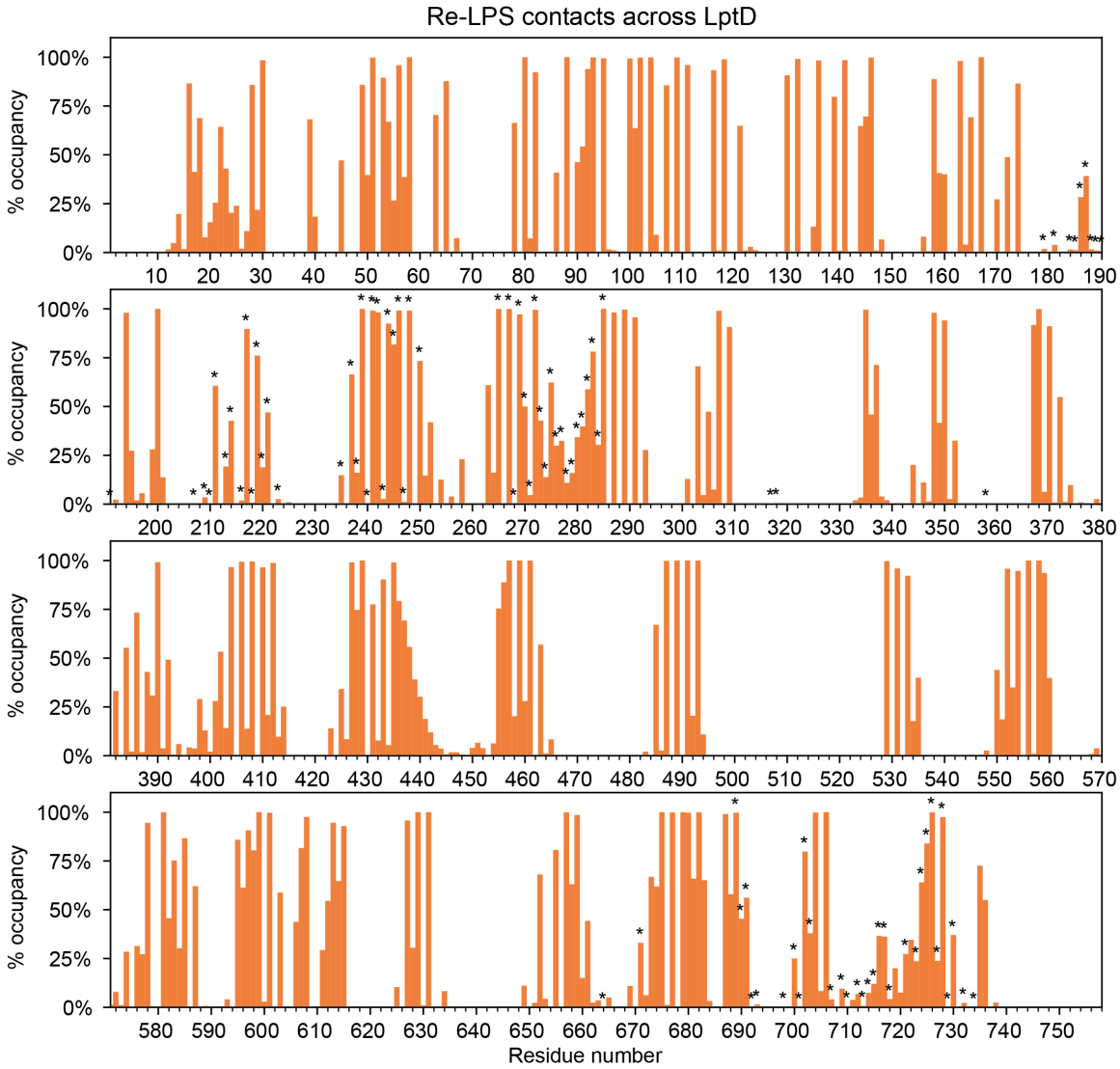
Supplementary Figure 13. Lateral gate distance. (a) Distance of putative gate on LptDE β-barrel when opened without Re-LPS bound to the β-jellyroll. Replicates 1 and 5 were found to be stable over the course of the simulation after sampling a distance above 2.25 nm. (b) Comparison of the lateral gate distance without (left) and with (right) PLUMED restraints.



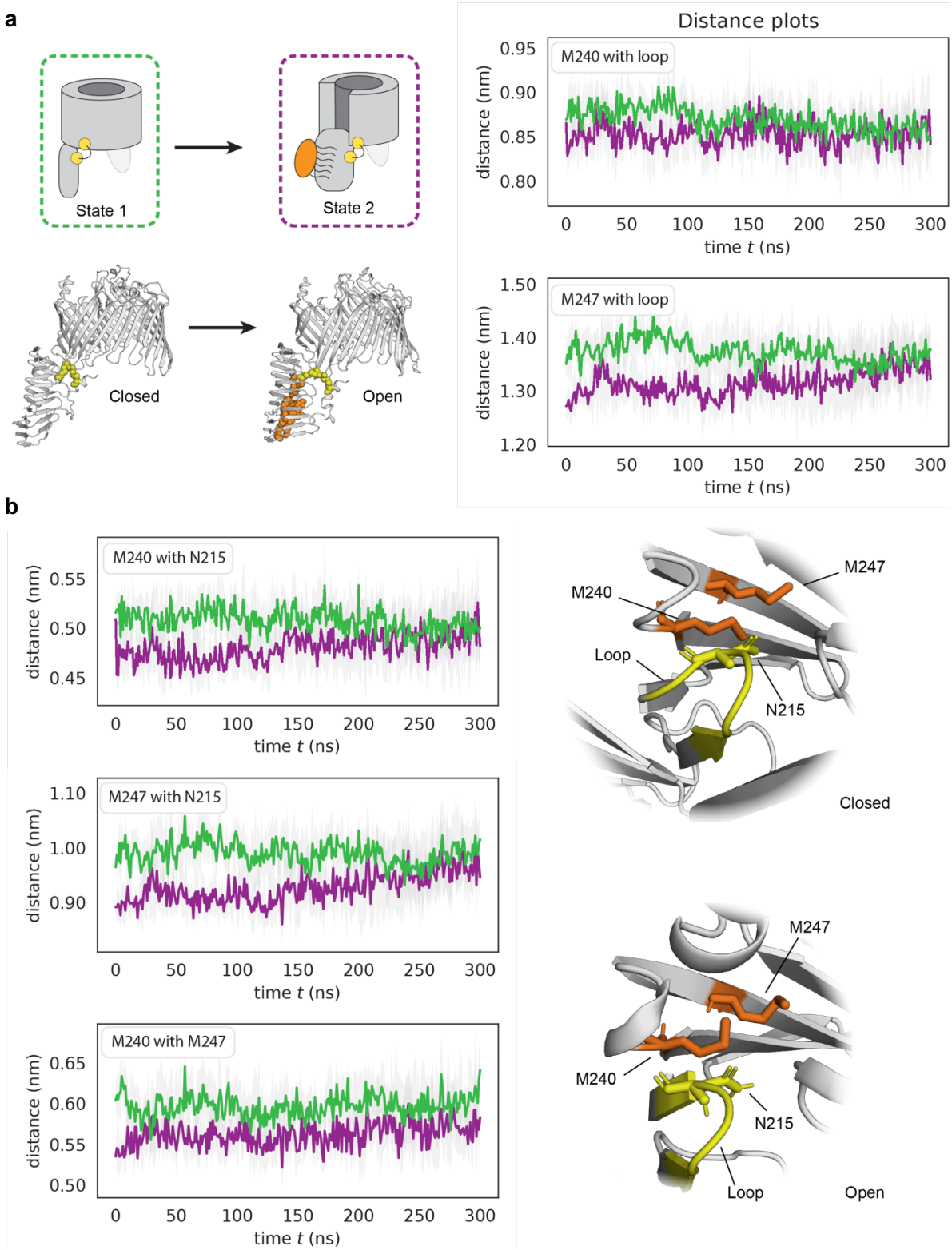
Supplementary Figure 14. LptD open (Re-LPS bound) state vs closed state differential solvent contact map. Different views of the residue by residue solvent contact map where every significant residue (CI 95%) is shown in the sticks representation. Loops pointing towards the extracellular side are highly solvated in our simulations, likely being more solvent exposed in a bilayer than in detergent, thus causing some peptides (e. g. 394-425 and 462-497) to be overly deprotected. Differences within the central peptides of the β-taco (e.g. 105-129) were found not to be statistically significant, which is likely attributed to the relatively short timescales of the simulation. Nevertheless, the binding of Re-LPS causes a substantial amount of deprotection in the β-taco by virtue of an open conformation.



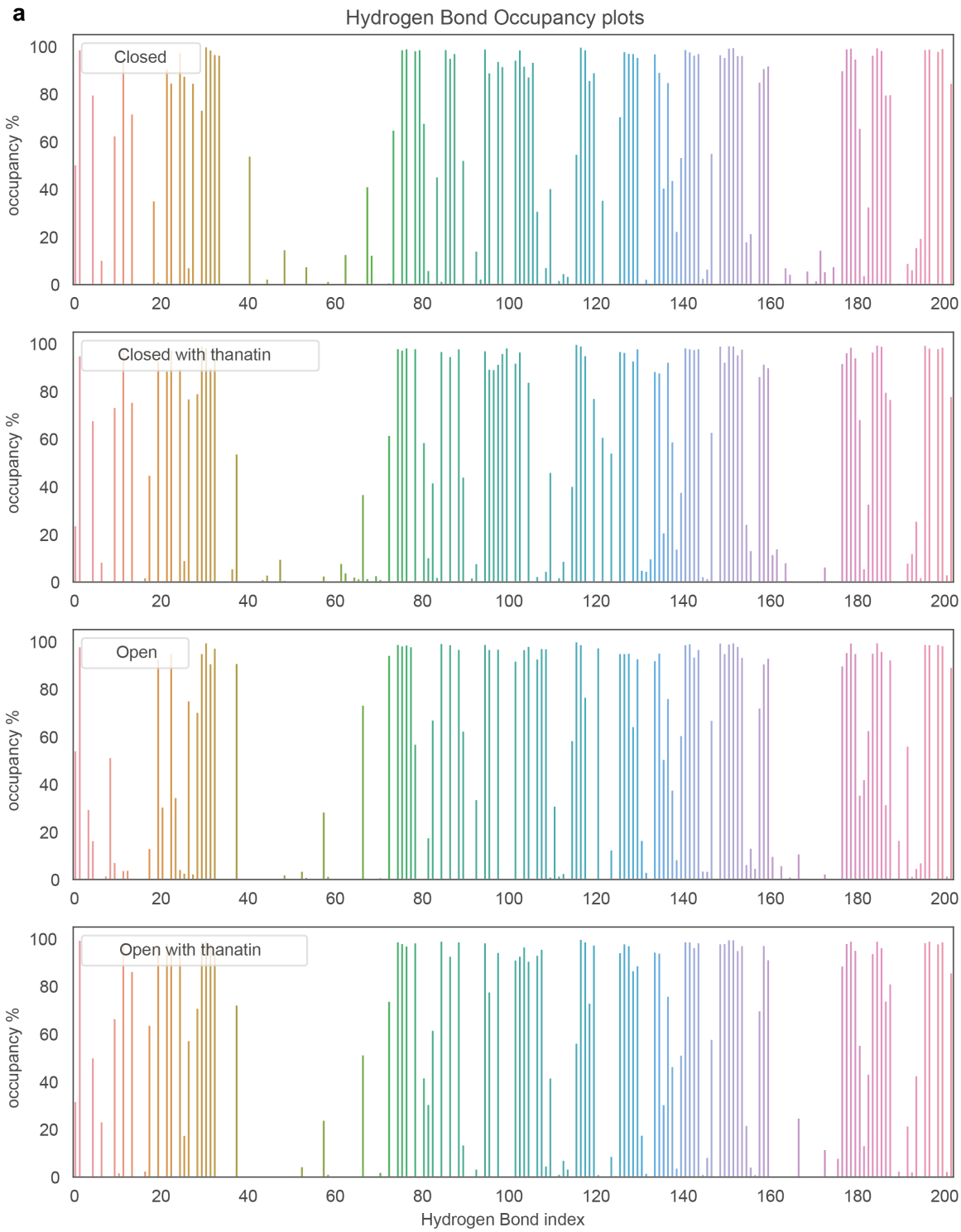
Supplementary Figure 15. Comparison of Re-LPS contacts inside the β-taco with differential solvent uptake maps. (a) Map of Re-LPS occupancy contacts with the β-taco, residues associated with increased solvent accessibility shown as sticks. (b) Residue differential solvent uptake map by percentage occupancy difference including all <95% significant values. (c) Peptide coverage of β-taco where peptides are coloured and number 1 to 9. Averaging the differential solvent values of each residue along these peptides produces (d) the peptide differential solvent uptake map including all <95% significant values. Peptides which pass the 95% CI are shown in red, those that do not are shown in pink or light blue). Peptide 4 shows no average increase in solvent uptake at all which is due to very few Re-LPS contacts correlated with an increase in solvent access, especially being averaged over comparatively large residue coverage (29 residues). This implies that this area would become more solvent exposed given more Re-LPS sampling in that region, however this is not possible due to the short timescales of the simulation as well as the rigidity of Re-LPS in the β-taco (see **Extended Data Fig. 6**). Also note that peptide 9 shows a slight overall protection owing to infrequent interactions with the inner leaflet of the outer membrane (**Extended Data Fig. 7**). (e) The final the peptide differential solvent uptake map after applying the 95% CI.

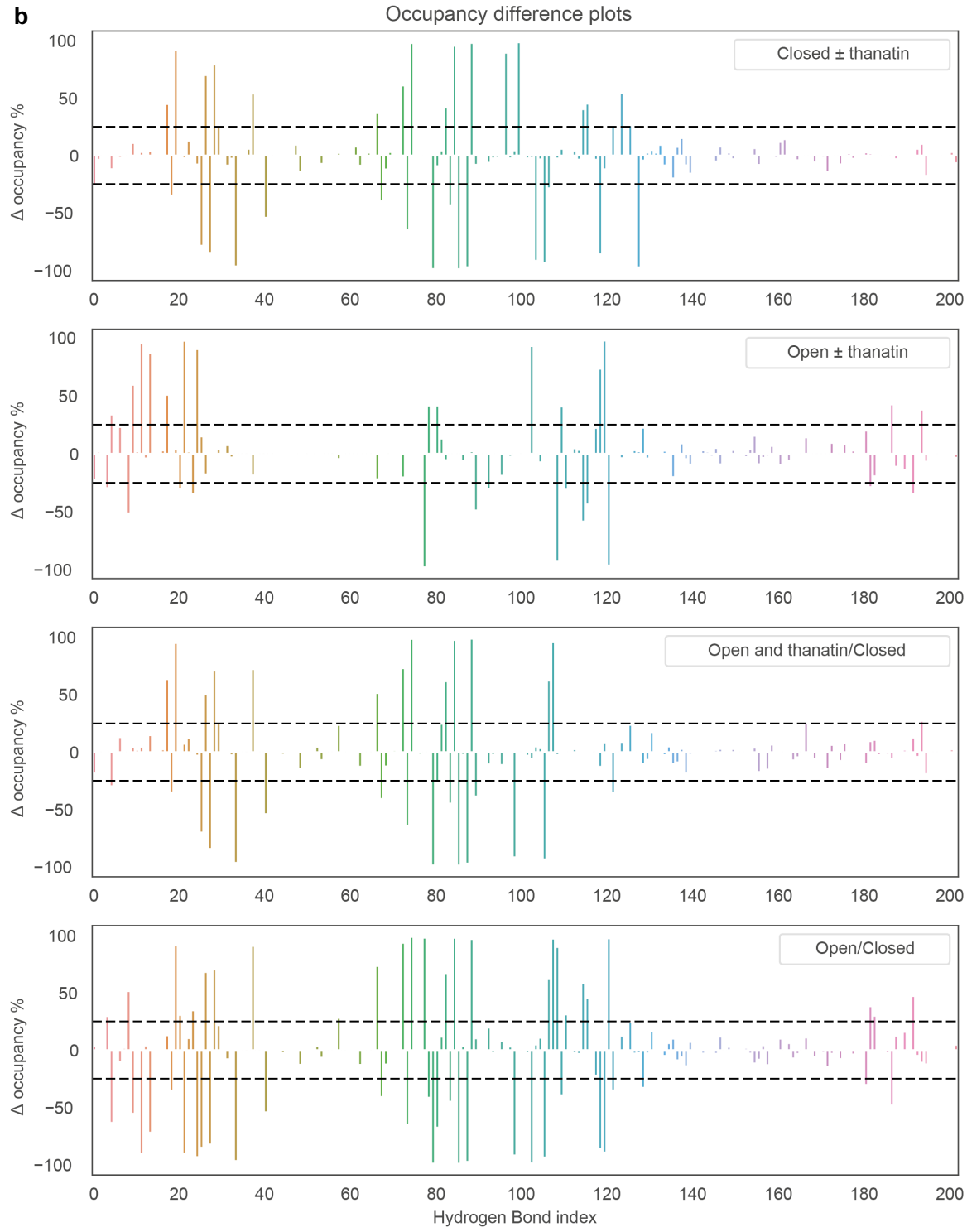


Supplementary Figure 16. Re-LPS contacts with LptD. The average Re-LPS contacts on LptD by percentage occupancy over the course of the simulation with the contacts made by the three Re-LPS lipids referenced in **Figure 2c** (Re-LPS(1-3)) highlighted (with asterisks) on the contact plot.



Supplementary Figure 17. Residue distance plots. (a) Left: cartoon schematic illustrating the difference between the apo closed state (green) and the Re-LPS bound state (purple). Right: distance plots between Met240 and Met247 from the loop located between β1 and β2 of the β-barrel. (b) Left: Residue distance plots between Met240 and Met247 as well as N215. Right: Schematic of the open and closed state conformations; methionines coloured in orange with the asparagine and loop coloured yellow.


a
Hydrogen Bond Occupancy plots
Closed
Closed with thanatin
Open
Open with thanatin
occupancy %
Hydrogen Bond index



Supplementary Figure 18. Hydrogen bond occupancy. (a) Percentage occupancy of hydrogen bonds on different states of the β-taco, considering only the backbone hydrogen bonds between residues 28 to 182 (excluding the first strand which has direct contact with thanatin). Occupancy of the hydrogen bonds was calculated over all five replicates of the four different states of the β-taco: closed, closed with thanatin docked, open (with Re-LPS) and open (with Re-LPS) and thanatin (plots listed from top to bottom respectively). (b) Difference plots of hydrogen bond occupancy between states. From top to bottom, difference plot between: closed with thanatin and closed states, open (Re-LPS bound) with thanatin and open (Re-LPS bound) states, open (Re-LPS bound) with thanatin and closed states, open (Re-LPS bound) and closed states. In each chart, respectively arbitrary cut-offs at 25% and -25% occupancy difference are plotted.

Supplementary Tables

Complex	Experimental molecular mass ± s.d. (Da)	Theoretical molecular mass (Da)
LptDE	108,472 ± 1.0	108,473
LptDE + 1x Re-LPS	110,712 ± 1.2	110,711
LptDE + 2x Re-LPS	112,949 ± 3.7	112,950
LptDE + thanatin	110,907 ± 4.1	110,909
LptDE + Re-LPS + thanatin	113,145 ± 0.7	113,147
LptDE + 2x Re-LPS + thanatin	115,382 ± 11.9	115,385
LptDE + LPS (peak 1)	112,489 ± 13	N/A
LptDE + LPS (peak 2)	113,373 ± 3.9	N/A
LptDE + 1x POPG	109,221 ± 2.2	109,222
LptDE + 2x POPG	109,972 ± 4.3	109,971
LptDE + 3x POPG	110,718 ± 2.2	110,720
LptDE + 1x CDL	109,930 ± 0.5	109,931
LptDE + 2x CDL	111,388 ± 1.6	111,389
LptDE + 3x CDL	112,845 ± 4.5	112,847

Supplementary Table 1. Molecular masses determined by nMS.

Data set	Apo-LptDE	LptDE+ LPS	LptDE + thanatin	LptDE+ LPS+thanatin
HDX reaction details	200 mM NaCl, 25 mM Tris-HCl (pH 7.5), 10% (v/v) glycerol, 0.03% (w/v) DDM. Percent deuterium: 91.7%. Temperature: 20°C			
HDX time course	0.167, 1, 16.67, 83.33, 420 min			
Back-exchange (mean/IQR)	ND			
Number of peptides	68			
Sequence coverage	70.5%			
Average peptide length/redundancy	Average peptide length = 13.4 residues Redundancy = 1.66			
Replicates	$n_{biological}$ = 2; $n_{technical}$ = 3			
Repeatability (average SD)	0.079	0.087	0.083	0.088
Significant differences in HDX (16.67 min time point)	Reference state	CI 98% = 0.37	CI 98% = 0.39	CI 98% = 0.37

Supplementary Table 2. HDX-MS data summary table for LptD in apo state and LPS, thanatin or LPS + thanatin states.

Data set	LptDE + Re-LPS	LptDE+ Re-LPS + thanatin	LptDE + POPG
HDX reaction details	200 mM NaCl, 25 mM Tris-HCl (pH 7.5), 10% (v/v) glycerol, 0.03% (w/v) DDM. Percent deuterium: 91.7%. Temperature: 20°C		
HDX time course	1, 16.67, 83.33 min		
Back-exchange (mean/IQR)		ND	
Number of peptides	63	63	68
Sequence coverage	69.1%	69.1%	70.5%
Average peptide length/redundancy	13.3/1.56	13.3/1.56	13.4/1.66
Replicates	$n_{biological}$ = 1 $n_{technical}$ = 3	$n_{biological}$ = 1 $n_{technical}$ = 3	$n_{biological}$ = 1 $n_{technical}$ = 3
Repeatability (average SD)	0.082	0.076	0.080
Significant differences in HDX (16.67 min time point)	CI 98% = 0.44	CI 98% = 0.42	CI 98% = 0.45

Supplementary Table 3. HDX-MS data summary table for LptD in Re-LPS, Re-LPS + thanatin, and POPG states.

Data set	Apo-LptDE	LptDE+ LPS	LptDE + thanatin	LptDE+ LPS+thanatin
HDX reaction details	200 mM NaCl, 25 mM Tris-HCl (pH 7.5), 10% (v/v) glycerol, 0.03% (w/v) DDM. Percent deuterium: 91.7%. Temperature: 20°C			
HDX time course	0.167, 1, 16.67, 83.33, 420 min			
Back-exchange (mean/IQR)	ND			
Number of peptides	17			
Sequence coverage	87.4%			
Average peptide length/redundancy	12.8/1.36			
Replicates	$n_{biological}$ = 2; $n_{technical}$ = 3			
Repeatability (average SD)	0.090	0.083	0.079	0.088
Significant differences in HDX (16.67 min time point)	Reference state	CI 98% = 0.38	CI 98% = 0.39	CI 98% = 0.39

Supplementary Table 4. HDX-MS data summary table for LptE in apo state and LPS, thanatin or LPS + thanatin states.

Data set	LptDE + Re-LPS	LptDE+ Re-LPS + thanatin	LptDE + POPG
HDX reaction details	200 mM NaCl, 25 mM Tris-HCl (pH 7.5), 10% (v/v) glycerol, 0.03% (w/v) DDM. Percent deuterium: 91.7%. Temperature: 20°C		
HDX time course	1, 16.67, 83.33 min		
Back-exchange (mean/IQR)	ND		
Number of peptides	17		
Sequence coverage	87.4%		
Average peptide length/redundancy	12.8/1.36		
Replicates	$n_{biological}$ = 1 $n_{technical}$ = 3	$n_{biological}$ = 1 $n_{technical}$ = 3	$n_{biological}$ = 1 $n_{technical}$ = 3
Repeatability (average SD)	0.052	0.063	0.073
Significant differences in HDX (16.67 min time point)	CI 98% = 0.42	CI 98% = 0.47	CI 98% = 0.46

Supplementary Table 5. HDX-MS data summary table for LptE in Re-LPS, Re-LPS + thanatin, and POPG states.