Supporting Information

Long Range Communication Between the Drug-binding Sites and Nucleotide Binding Domains of the Efflux Transporter ABCB1

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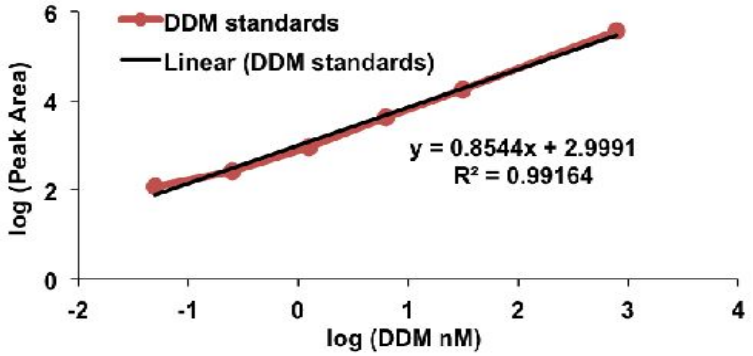


	DDM (nM)	Pgp-ND:DDM	% return from STDs
Pgp-ND prep 2.4 uM	0.90 0.77	2677 3132	
Pgp-ND prep 4.5 uM	8.96 8.72	502 516	
DDM 1.25 nM STD	0.62 0.51		49 41
DDM 31 nM STD	23.16 19.02		74 61

Figure S1. There is negligible residual DDM detergent present in P-gp nanodiscs. Different studies of P-gp in nanodiscs have shown opposing effects of zosuquidar on ATP hydrolysis, and that in a detergent environment ATP hydrolysis is stimulated. To confirm that residual DDM is not the cause of ATPase stimulation in the nanodiscs, we developed an LC-MS assay to measure residual DDM. Standard samples with known concentrations of DDM and samples from two batches of P-gp NDs were injected in duplicate and separated on a C8 column and analyzed on a Waters Xevo-Xs mass spectrometer. Micromolar samples of P-gp NDs contained nanomolar levels of residual DDM from the nanodisc formation process. Only one molecule of DDM was detected per 500 to 3000 nanodiscs. Repeat injections of standards after the Pgp-ND samples showed a decrease in recovery ("return" in above Table; 41-74% recovered). Thus, using the lowest value of recovery (41%) the estimated DDM/nanodisc corrected for recovery is below 1 DDM/1,219 P-gp NDs, or approximately 1 DDM/121, 900 DMPC molecules assuming ~100 lipids/nanodisc. The results indicate that ratios of DDM to P-gp NDs are well below a level at which DDM would impact P-gp behavior.

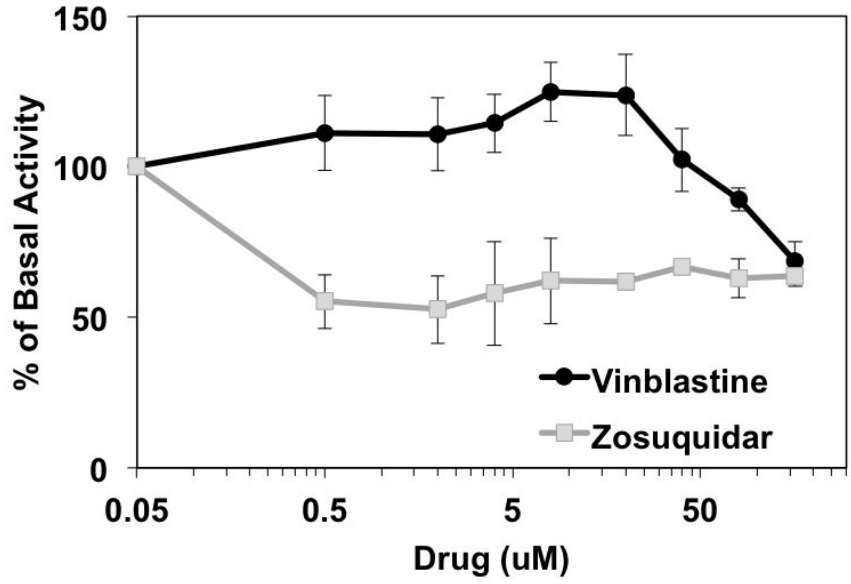


Figure S2. Drug-dependent activity of mouse P-gp in HEK293 vesicles. To probe activity in a more native membrane context, we obtained HEK293 membrane vesicles from control and wild-type mouse P-gp (MDR1a) expressing cells (SOLVO Biotechnology). We performed the same ATPase assay as in Figure 1B except for the inclusion of 1 mM EGTA to reduce background ATP hydrolysis. Vesicles containing 5 µg total protein was used in duplicate samples. Control

vesicle ATP hydrolysis is subtracted from P-gp vesicle values. Samples contain 2.5 mM ATP and are compared to basal activity at 2.5 mM ATP, which averaged 14 nmol Pi/min/mg total protein. Error bars represent the standard deviation across two independent experiments on different days. Vinblastine stimulates and inhibits P-gp ATP hydrolysis at concentrations comparable to those for P-gp in nanodiscs. Zosuquidar inhibits activity at low concentrations unlike in nanodiscs, and consistent with Shukla et al. However, modest recovery of activity is observed at higher concentrations (> 5 μM), which we observe in nanodiscs starting at a lower concentration. The combined zosuquidar results suggest possible competing effects in the more native membrane context.

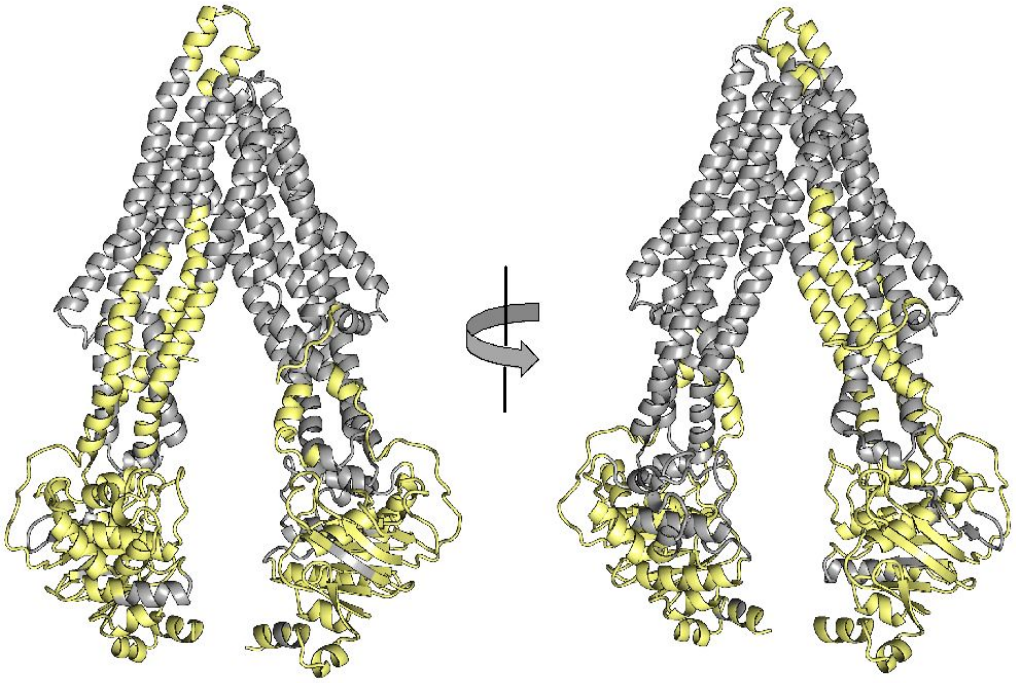


Figure S3. HDXMS sequence coverage for P-gp. 50% of the P-gp sequence was reliably analyzed in both datasets with comparable coverage. Coverage for the first dataset is shown in yellow on the apo form of P-gp (PDB ID: 5KPI).

Table S1. HDX-MS parameters for the two datasets presented.

	Dataset 1	Dataset 2
HDX reaction details	0.5 uM final Pgp nanodiscs 20 mM Tris, 100 mM NaCl, 1 mM TCEP, 5 mM $MgSO_4$, 1% DMSO pH 7.4 at 24°C	
Quench details	0.6% FA, 1 mM TCEP, 0.2% DDM	
pH of quenched undeuterated sample	2.55	2.50
Nanodisc disassembly, lipid removal details	DDM (0.1% final) 3 mg ZrO_2/sample (in quench slurry)	
HDX time course	1 minute, 8 minutes, 1 hour, 8 hours	
# of peptides	81	70
Sequence coverage	50%	
Average peptide length/ redundancy	12.7 aa/1.6	12.4 aa/1.4
Replicates	2 technical replicates	
Repeatability (average STD)	0.61%	0.54%
Significance (95% confidence interval per timepoint)	ΔHDX > 0.3% to 1.8% deuteration range across timepoints	ΔHDX > 0.4% to 1.8% deuteration range across timepoints

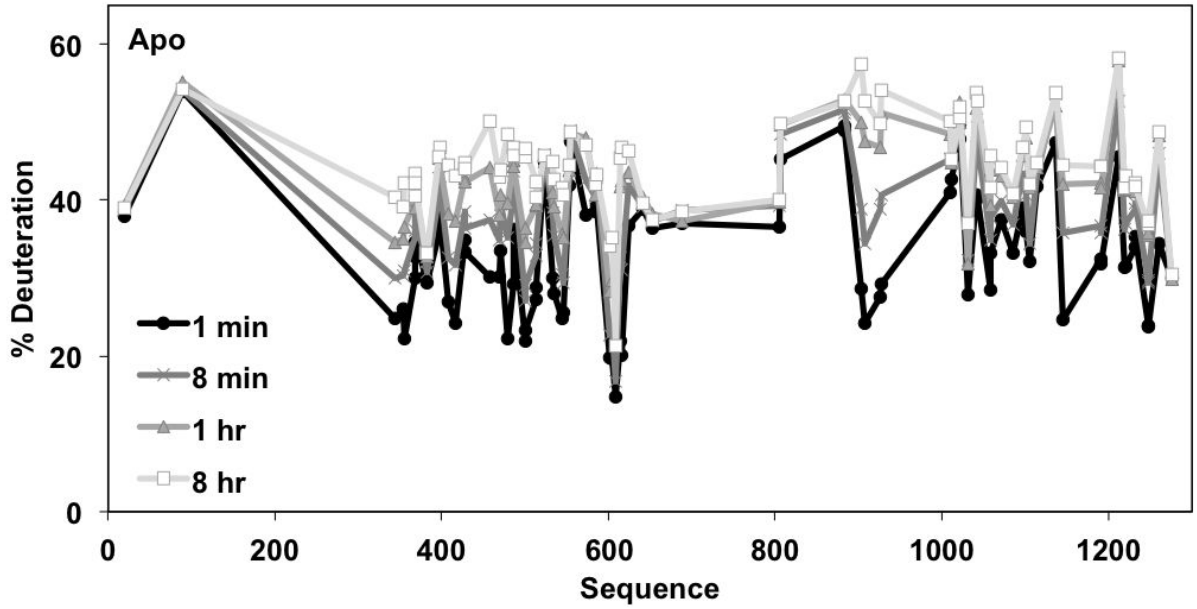


Figure S4. Percent deuteration profile of apo P-gp from HDX-MS. Absolute deuterium uptake percentages are shown for all time points and peptides from dataset 1 to show range of less-ordered, more-ordered, and dynamic regions.

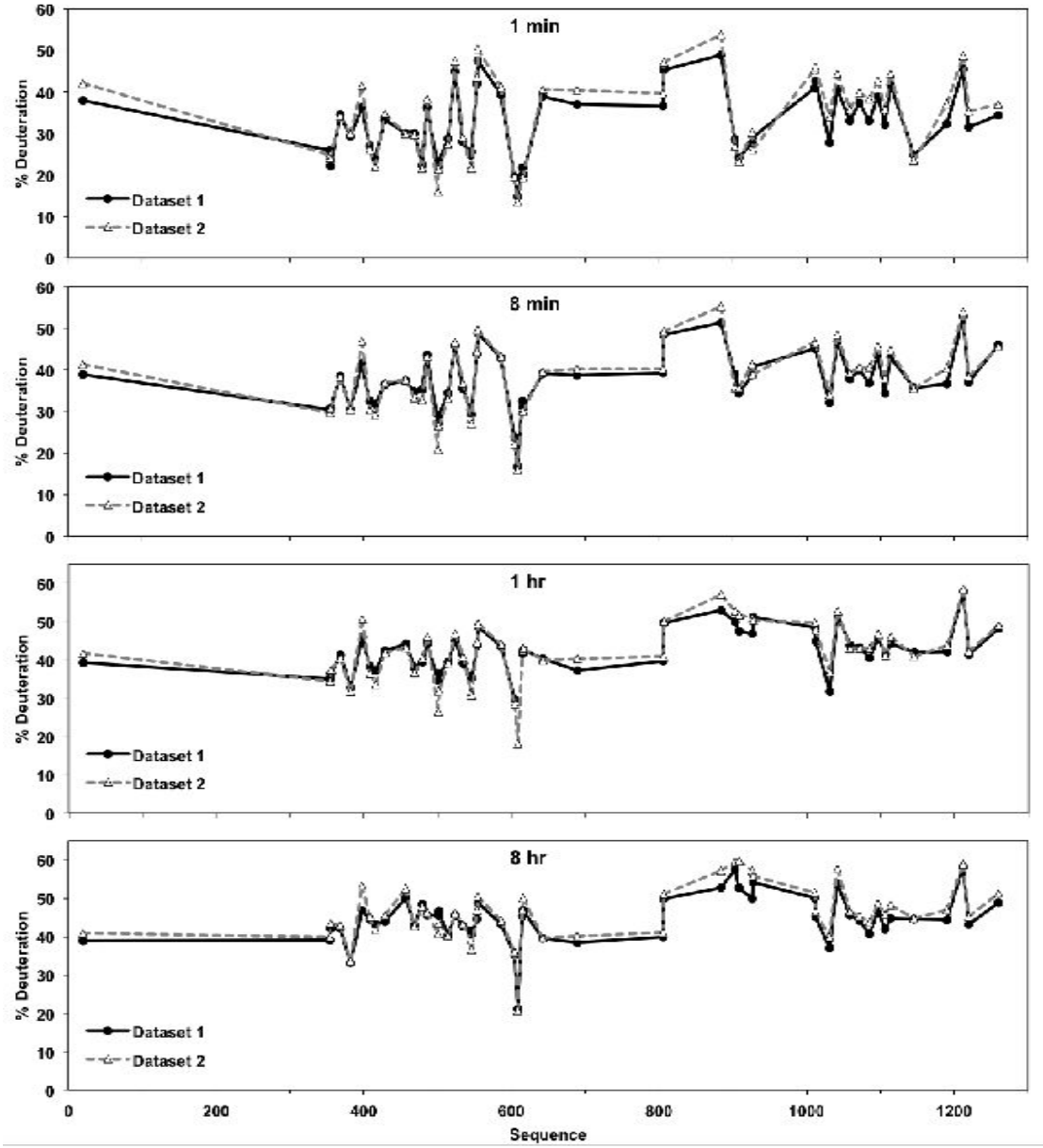
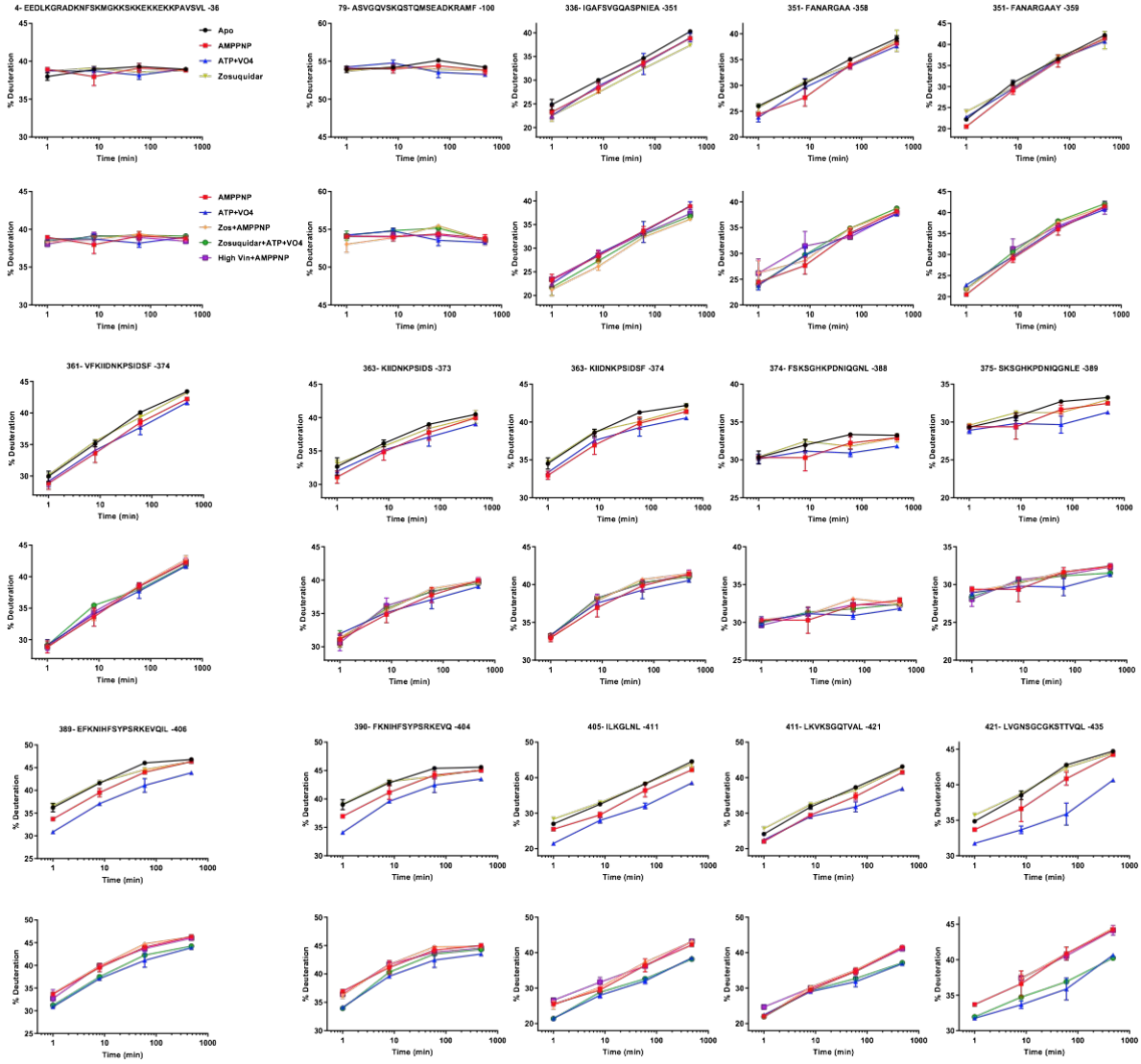


Figure S5. Comparison of absolute deuterium uptake for two datasets. All data presented originate from two datasets, with samples prepared from different batches of P-gp nanodiscs, and deuteration reaction components, and MS performed months apart. The absolute deuteration percent for identical peptides (and same charge state) is shown for each time point between the two datasets. Absolute uptake is consistent for essentially all peptides and the overall trends with time are clearly preserved between datasets. This shows a high level of reproducibility between different P-gp and nanodisc preparations.

Figure S6. Deuterium uptake plots. Deuterium uptake is shown for all peptides and charge states for dataset 1 followed by dataset 2. Data is also available in excel file. For dataset 1, due to the number of conditions, two plots are shown for each peptide, with AMPPNP and ATP/VO4 data repeated in the plot below to compare the effects of drug binding to these states. Error bars represent standard deviation across duplicate samples; however, a broader statistical analysis (described in Methods) is applied across peptides and time points to identify significant differences shown in Figures 2-4.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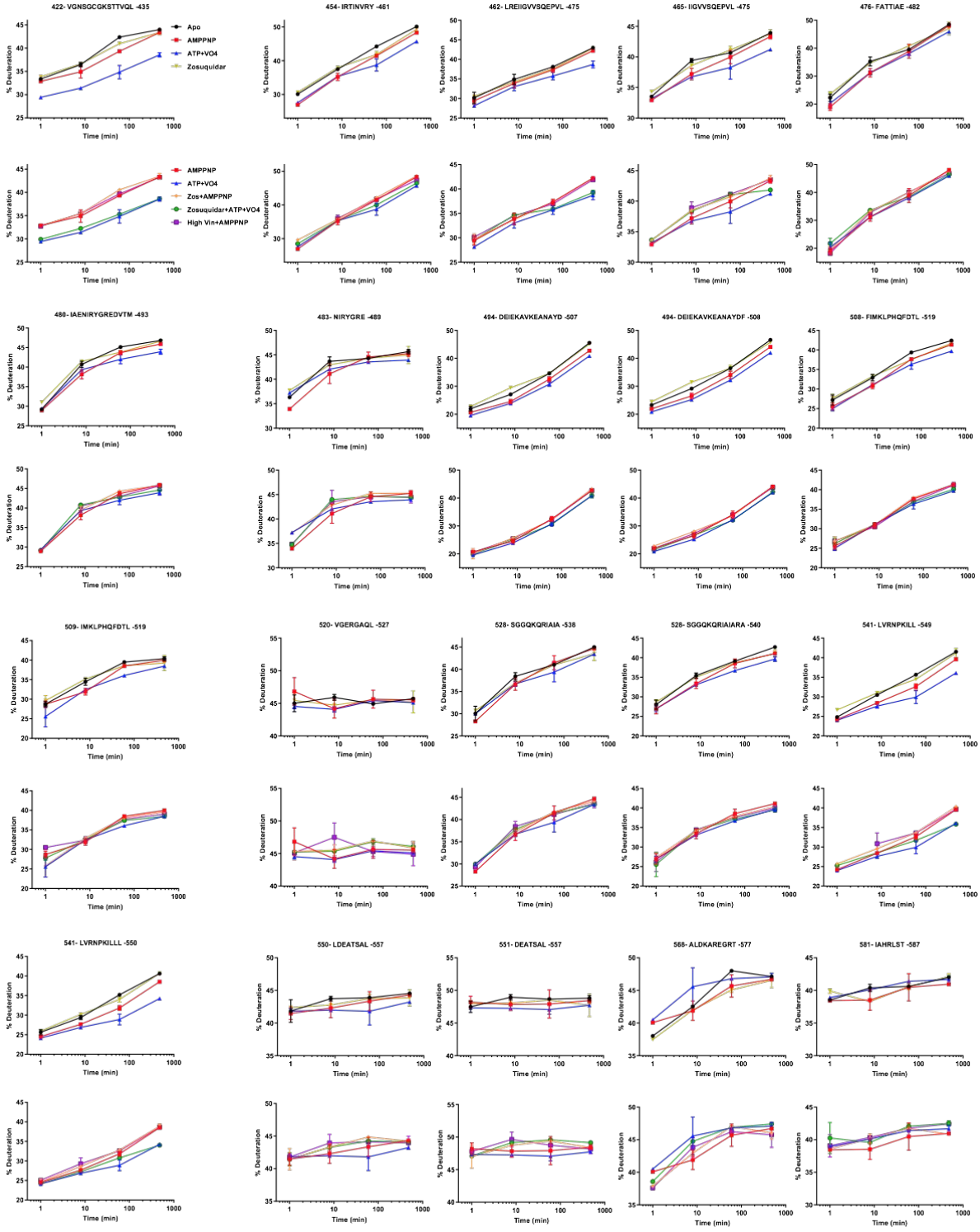
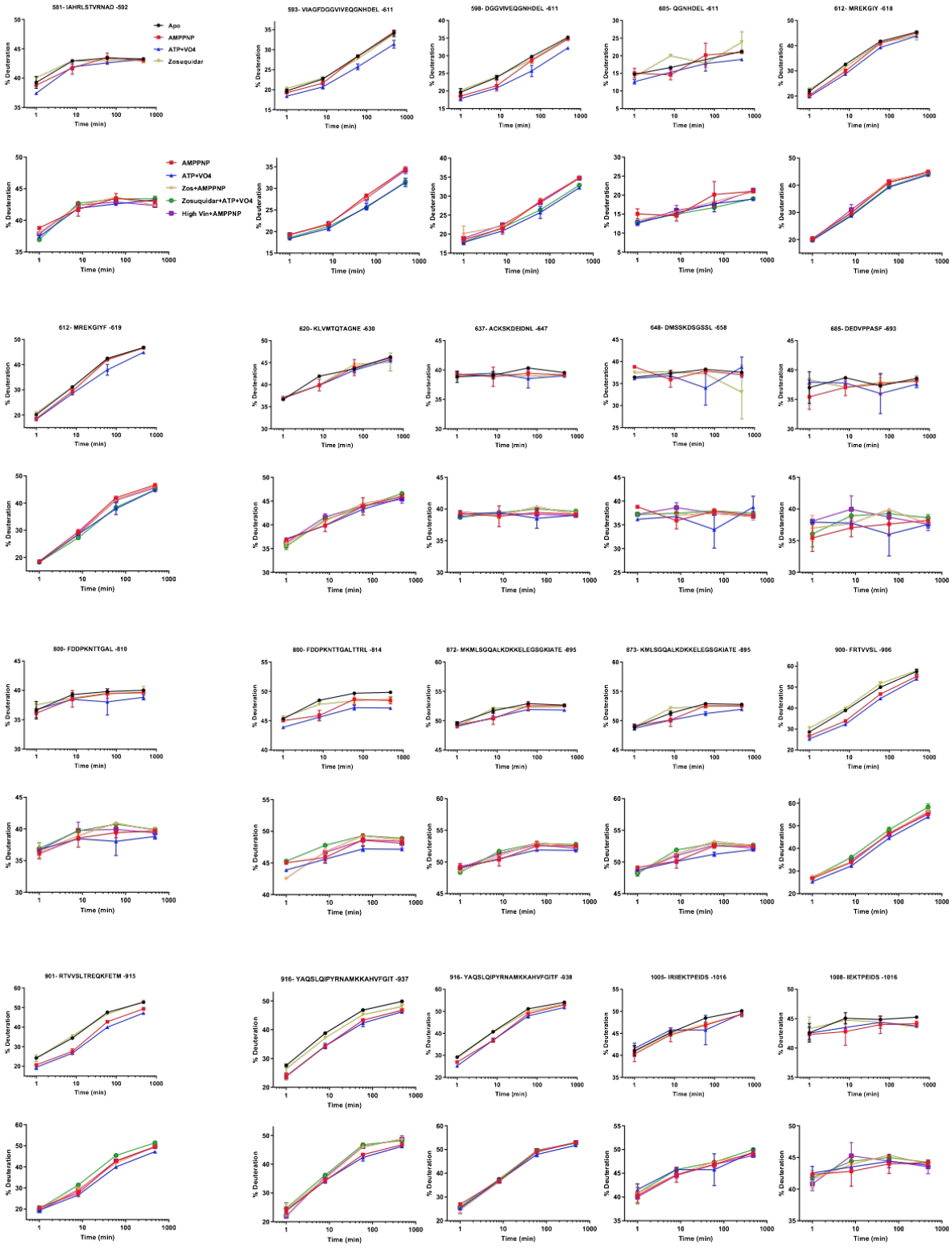
79- ASVGQVSKQSTQMSEADKRAMF -100
336- IGAFSVGQASPNIEA -351
351- FANARGAA -358
351- FANARGAAY -359
361- VFKIIDNKPSIDSF -374
363- KIIDNKPSIDS -373
363- KIIDNKPSIDSF -374
374- FSKSGHKPDNIQGNL -388
375- SKSGHKPDNIQGNLE -389
389- EFKNIHFSYPSRKEVQIL -406
390- FKNIHFSYPSRKEVQ -404
405- ILKGLNL -411
411- LKVKSGQTVAL -421
421- LVGNSGCGKSTTVQL -435
Apo
AMPPNP
ATP+VO4
Zosuquidar
Zos+AMPPNP
Zosuquidar+ATP+VO4
High Vin+AMPPNP
% Deuteration
Time (min)


422- VGNSGCGKSTTVQL -435
454- IRTINVRY -461
462- LREIIGVVSQEPVL -475
465- IIGVVSQEPVL -475
476- FATTIAE -482
Apo
AMPPNP
ATP+VO4
Zosuquidar
Zos+AMPPNP
Zosuquidar+ATP+VO4
High Vin+AMPPNP
480- IAENIRYGREDVTM -493
483- NIRYGRE -489
494- DEIEKAVKEANAYD -507
494- DEIEKAVKEANAYDF -508
508- FIMKLPHQFDTL -519
509- IMKLPHQFDTL -519
520- VGERGAQL -527
528- SGGQKQRIAIA -538
528- SGGQKQRIAIARA -540
541- LVRNPKILL -549
541- LVRNPKILLL -550
550- LDEATSAL -557
551- DEATSAL -557
566- ALDKAREGRT -577
581- IAHRLST -587
% Deuteration
Time (min)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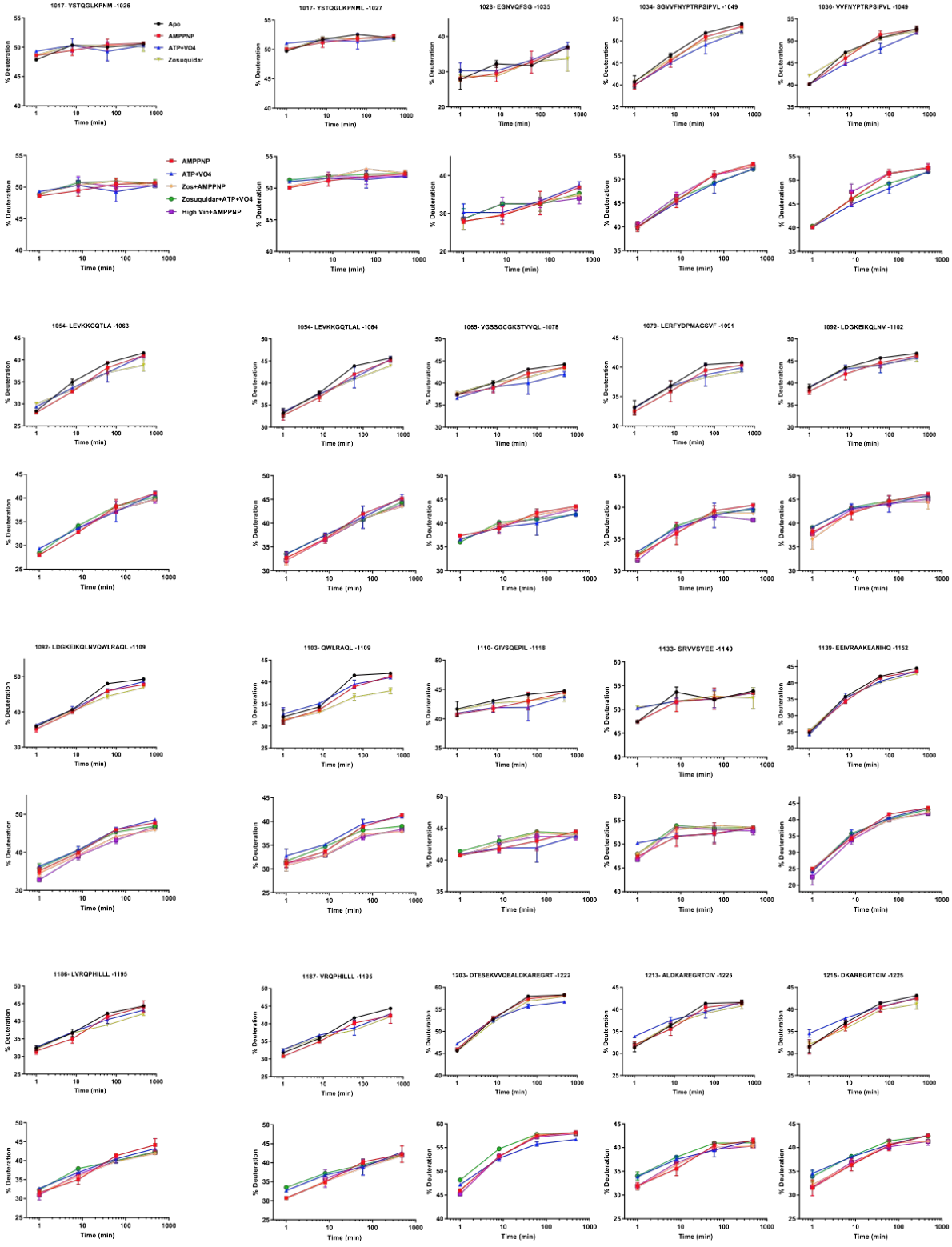
605- QGNHDEL -611
612- MREKGIY -618
612- MREKGIYF -619
620- KLVMTQTAGNE -630
637- ACKSKDEIDNL -647
648- DMSSKDSGSSL -658
685- DEDVPPASF -693
800- FDDPKNTTGAL -810
800- FDDPKNTTGALTTRL -814
872- MKMLSGQALKDKKELEGSGKIATE -895
873- KMLSGQALKDKKELEGSGKIATE -895
900- FRTVVSL -906
901- RTVVSLTREQKFETM -915
916- YAQSLQIPYRNAMKKAHVFGIT -937
916- YAQSLQIPYRNAMKKAHVFGITF -938
1005- IRIIEKTPEIDS -1016
1008- IEKTPEIDS -1016
Apo
AMPPNP
ATP+VO4
Zosuquidar
Zos+AMPPNP
Zosuquidar+ATP+VO4
High Vin+AMPPNP
% Deuteration
Time (min)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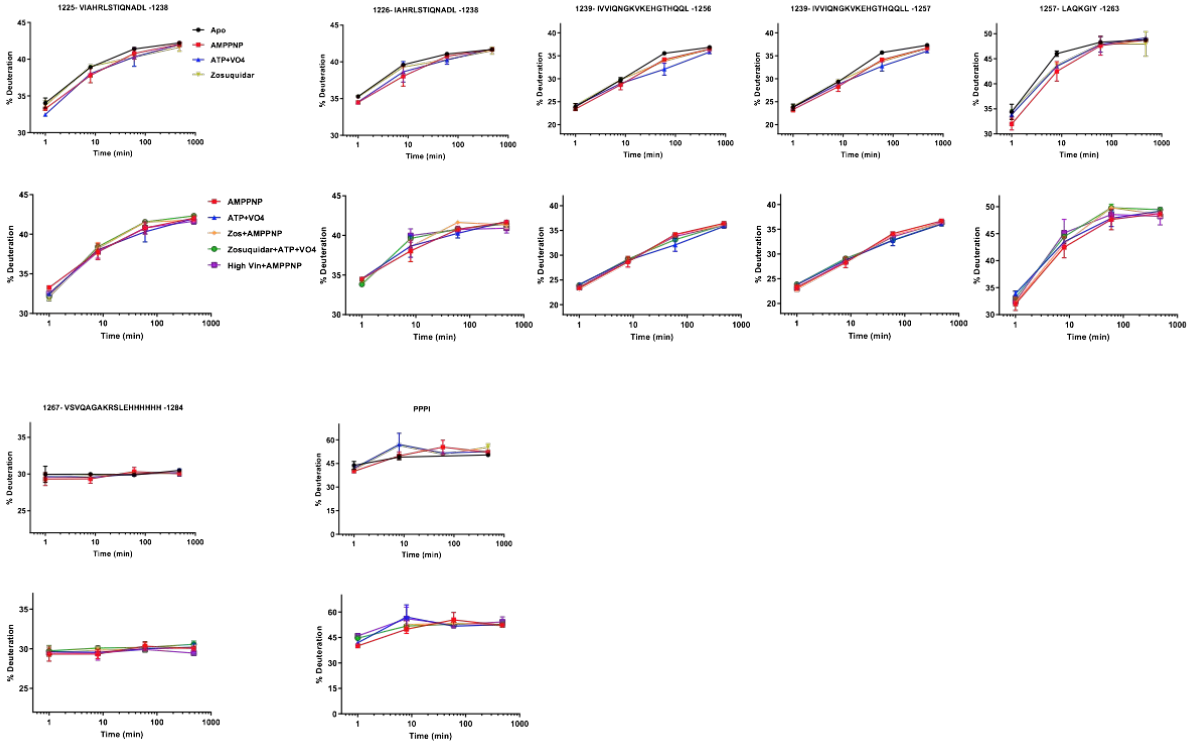
1034- SGVVFNYPTRPSIPVL -1049
1036- VVFNYPTRPSIPVL -1049
1054- LEVKKGQTLA -1063
1054- LEVKKGQTLAL -1064
1065- VGSSGCGKSTVVQL -1078
1079- LERFYDPMAGSVF -1091
1092- LDGKEIKQLNV -1102
1092- LDGKEIKQLNVQWLRAQL -1109
1103- QWLRAQL -1109
1110- GIVSQEPIL -1118
1133- SRVVSYEE -1140
1139- EEIVRAAKEANIHQ -1152
1186- LVRQPHILLL -1195
1187- VRQPHILLL -1195
1203- DTESEKVVQEALDKAREGRT -1222
1213- ALDKAREGRTCIV -1225
1215- DKAREGRTCIV -1225
Apo
AMPPNP
ATP+VO4
Zosuquidar
Zos+AMPPNP
Zosuquidar+ATP+VO4
High Vin+AMPPNP
% Deuteration
Time (min)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1226- IAHRLSTIQNADL -1238
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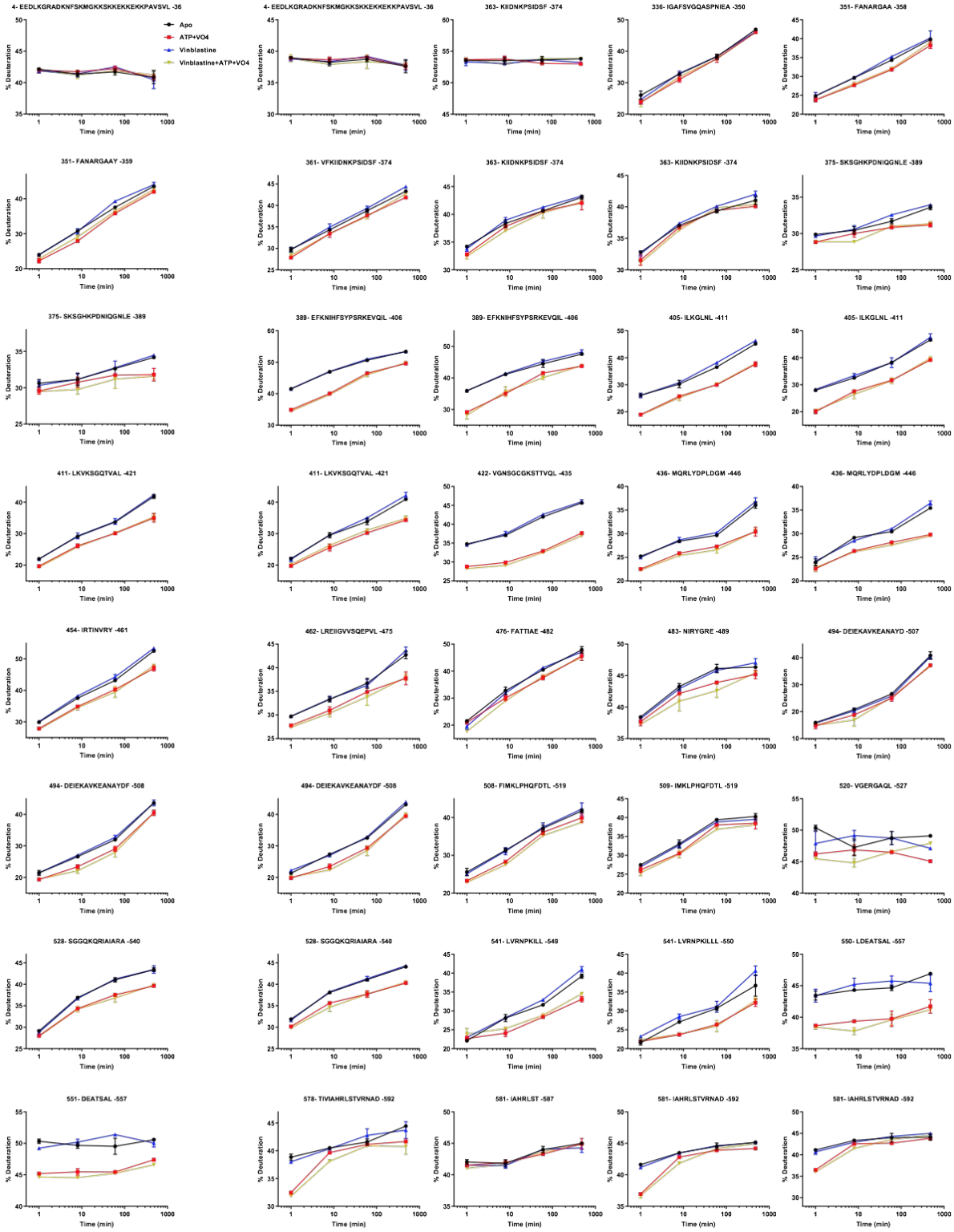
1239- IVVIQNGKVKEHGTHQQLL -1257
1257- LAQKGIY -1263
Apo
AMPPNP
ATP+VO4
Zosuquidar
Zos+AMPPNP
Zosuquidar+ATP+VO4
High Vin+AMPPNP
1267- VSVQAGAKRSLEHHHHHH -1284
PPPI
% Deuteration
Time (min)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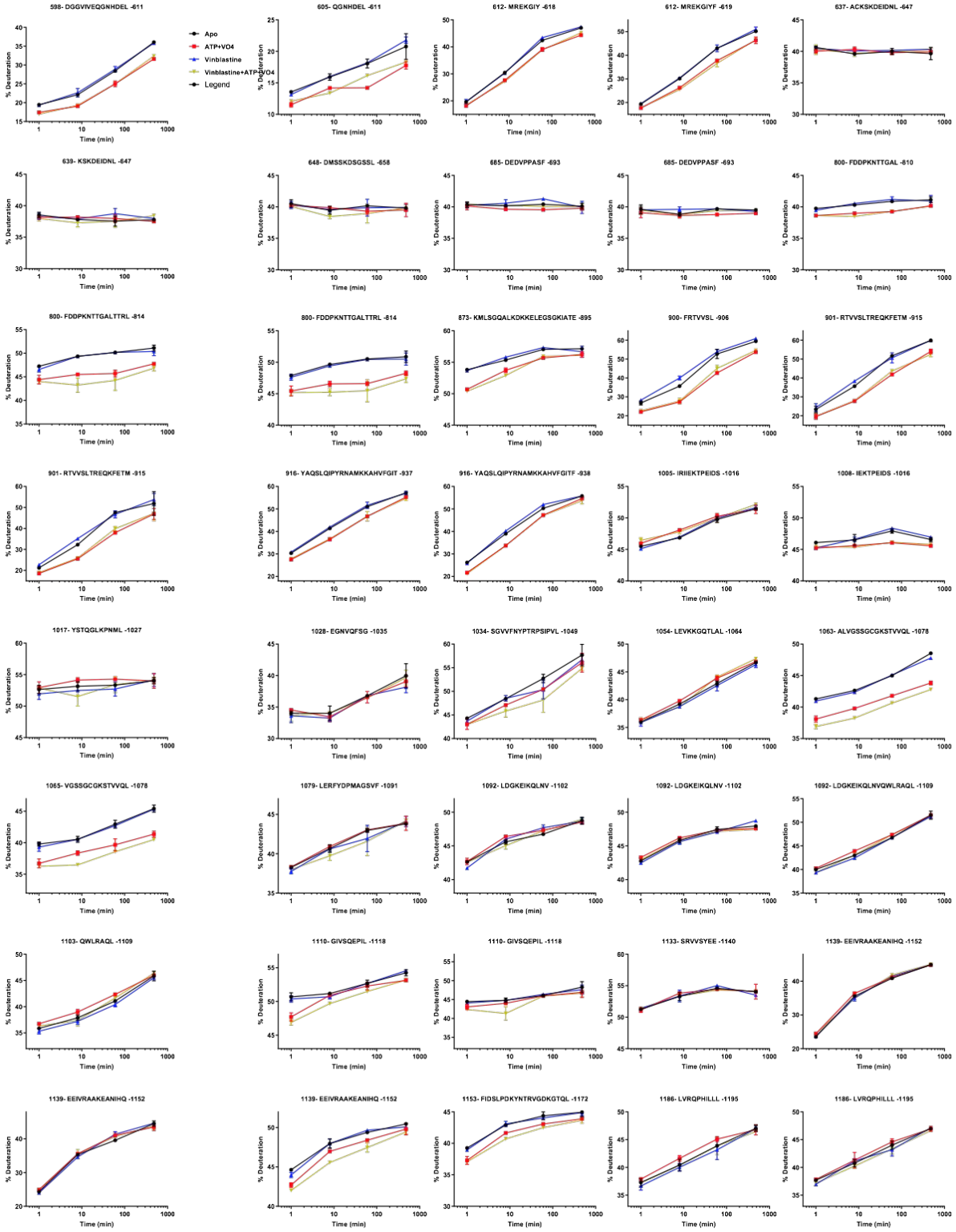
Apo
ATP+VO4
Vinblastine
Vinblastine+ATP+VO4
4- EEDLKGRADKNFSKMGKKSKKEKKEKKPAVSVL -36
363- KIIDNKPSIDSF -374
336- IGAFSVGQASPNIEA -350
351- FANARGAA -358
351- FANARGAAY -359
361- VFKIIDNKPSIDSF -374
363- KIIDNKPSIDSF -374
363- KIIDNKPSIDSF -374
375- SKSGHKPDNIQGNLE -389
375- SKSGHKPDNIQGNLE -389
389- EFKNIHFSYPSRKEVQIL -406
389- EFKNIHFSYPSRKEVQIL -406
405- ILKGLNL -411
405- ILKGLNL -411
411- LKVKSGQTVAL -421
411- LKVKSGQTVAL -421
422- VGNSGCGKSTTVQL -435
436- MQRLYDPLDGM -446
436- MQRLYDPLDGM -446
454- IRTINVRY -461
462- LREIIGVVSQEPVL -475
476- FATTIAE -482
483- NIRYGRE -489
494- DEIEKAVKEANAYD -507
494- DEIEKAVKEANAYDF -508
494- DEIEKAVKEANAYDF -508
508- FIMKLPHQFDTL -519
509- IMKLPHQFDTL -519
520- VGERGAQL -527
528- SGGQKQRIAIARA -540
528- SGGQKQRIAIARA -540
541- LVRNPKILL -549
541- LVRNPKILLL -550
550- LDEATSAL -557
551- DEATSAL -557
578- TIVIAHRLSTVRNAD -592
581- IAHRLST -587
581- IAHRLSTVRNAD -592
581- IAHRLSTVRNAD -592
% Deuteration
Time (min)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605- QGNHDEL -611
612- MREKGIY -618
612- MREKGIYF -619
637- ACKSKDEIDNL -647
639- KSKDEIDNL -647
648- DMSSKDSGSSL -658
685- DEDVPPASF -693
685- DEDVPPASF -693
800- FDDPKNTTGAL -810
800- FDDPKNTTGALTTRL -814
800- FDDPKNTTGALTTRL -814
873- KMLSGQALKDKKELEGSGKIATE -895
900- FRTVVSL -906
901- RTVVSLTREQKFETM -915
901- RTVVSLTREQKFETM -915
916- YAQSLQIPYRNAMKKAHVFGIT -937
916- YAQSLQIPYRNAMKKAHVFGITF -938
1005- IRIIEKTPEIDS -1016
1008- IEKTPEIDS -1016
1017- YSTQGLKPNML -1027
1028- EGNVQFSG -1035
1034- SGVVFNYPTRPSIPVL -1049
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1063- ALVGSSGCGKSTVVQL -1078
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1092- LDGKEIKQLNVQWLRAQL -1109
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1133- SRVVSYEE -1140
1139- EEIVRAAKEANIHQ -1152
1139- EEIVRAAKEANIHQ -1152
1139- EEIVRAAKEANIHQ -1152
1153- FIDSLPDKYNTRVGDKGTQL -1172
1186- LVRQPHILLL -1195
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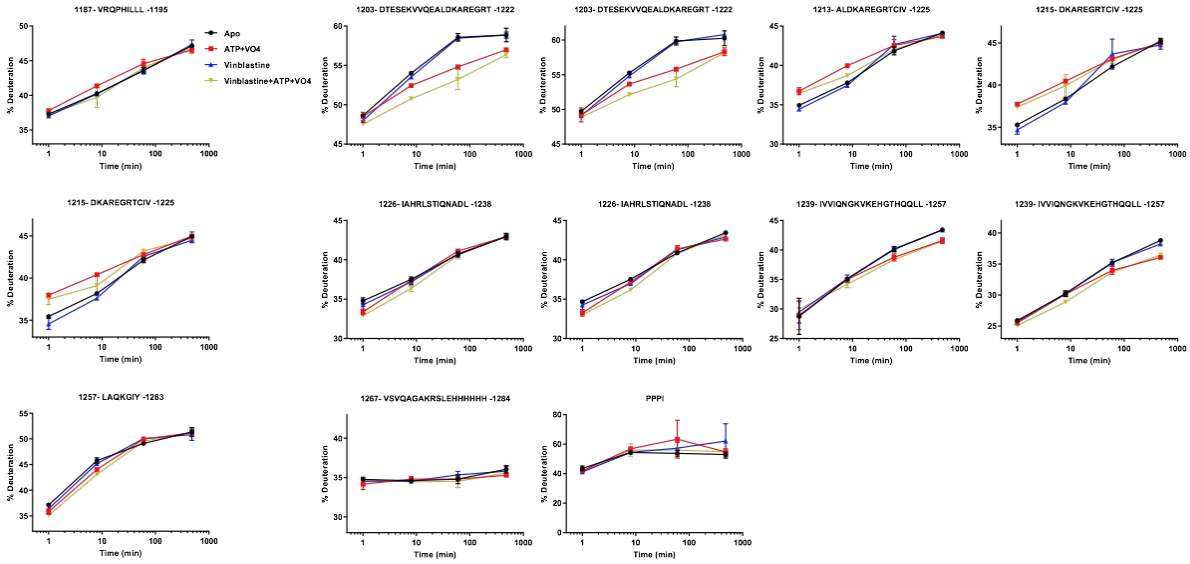
ATP+VO4
Vinblastine
Vinblastine+ATP+VO4
Legend
% Deuteration
Time (min)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1203- DTESEKVVQEALDKAREGRT -1222
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1215- DKAREGRTCIV -1225
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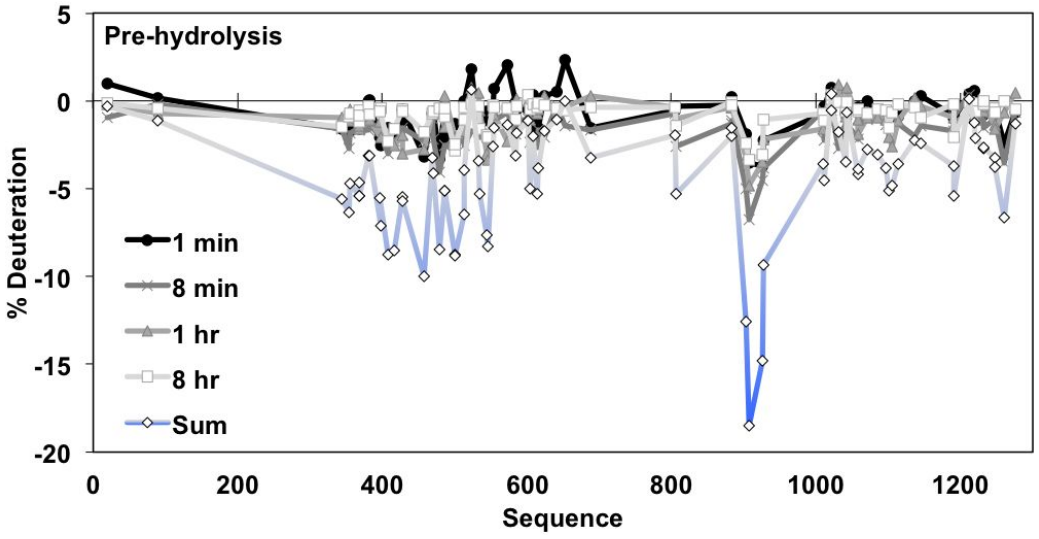
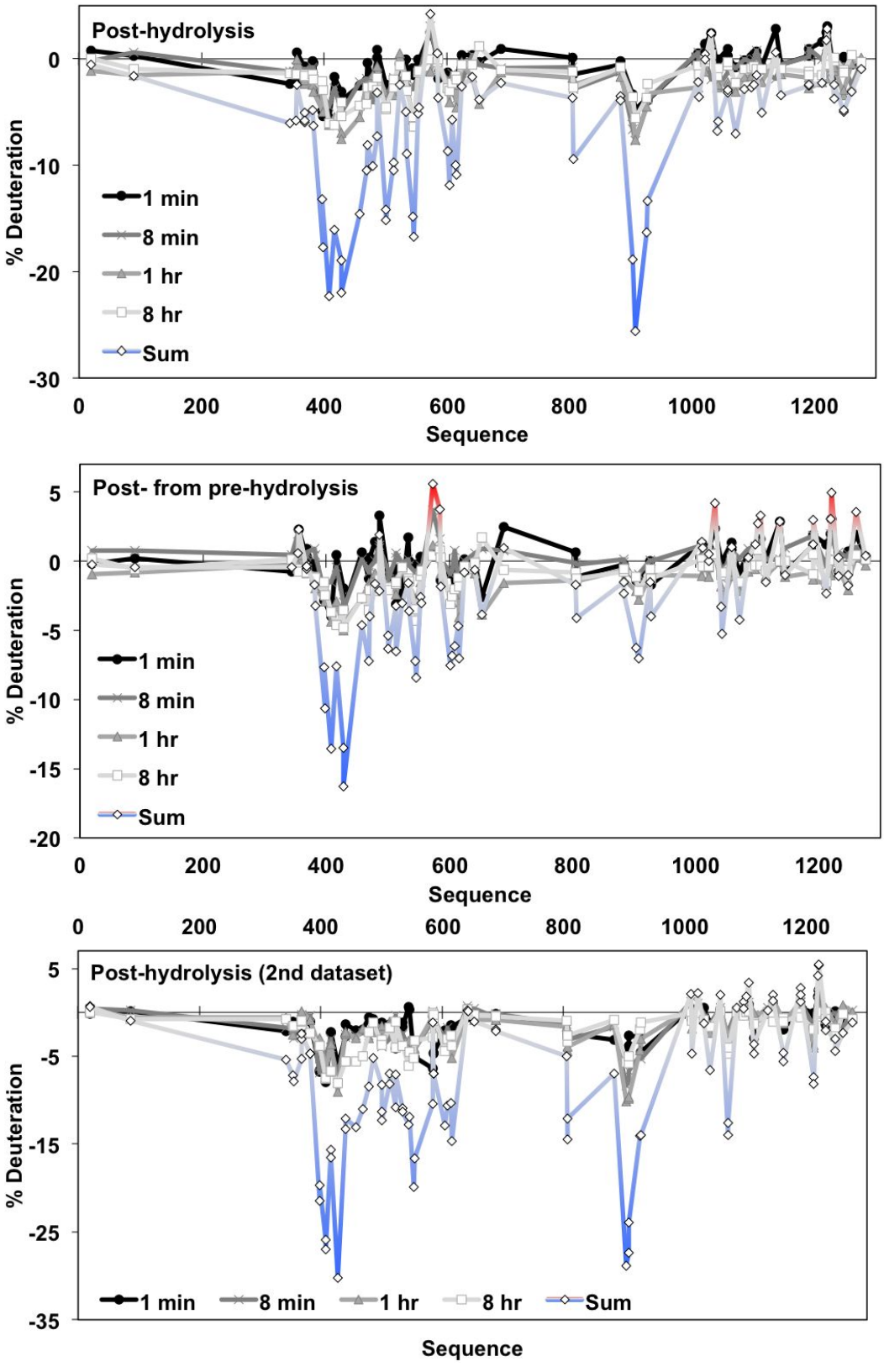
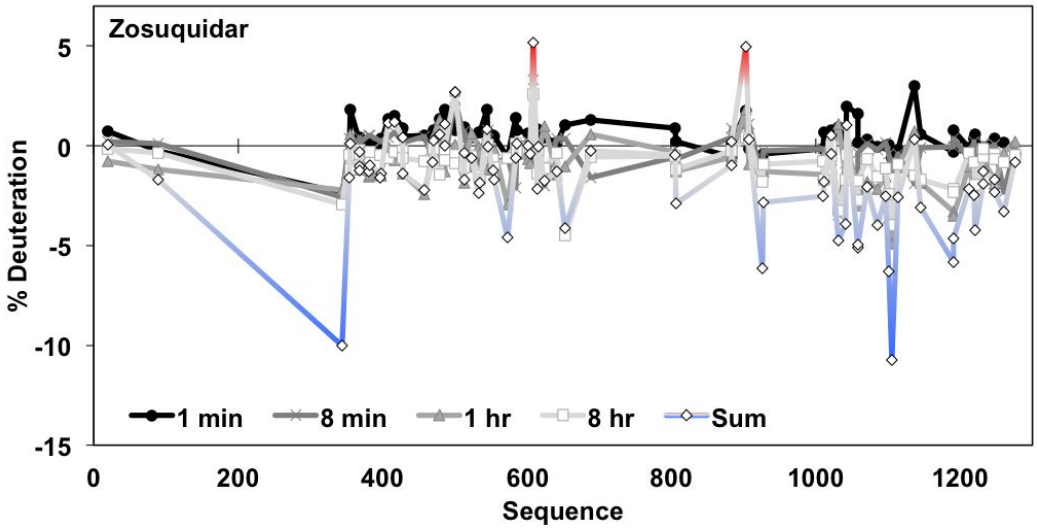
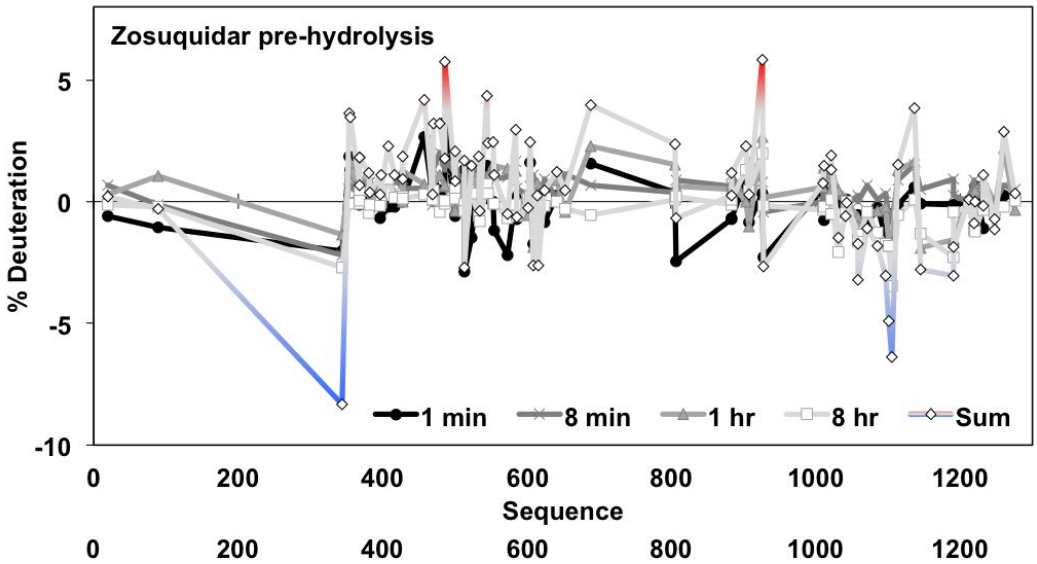
1226- IAHRLSTIQNADL -1238
1239- IVVIQNGKVKEHGTHQQLL -1257
1239- IVVIQNGKVKEHGTHQQLL -1257
1257- LAQKGIY -1263
1267- VSVQAGAKRSLEHHHHHH -1284
PPPI
Apo
ATP+VO4
Vinblastine
Vinblastine+ATP+VO4
% Deuteration
Time (min)

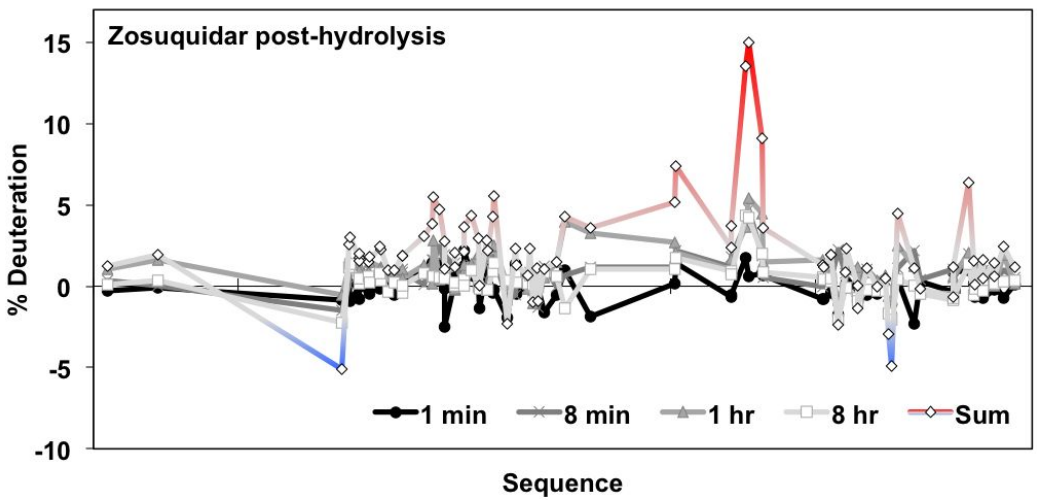
Figure S7. Change in percent deuteration for each time point for each state relative to nucleotide-free or drug-free nucleotide-bound state. (Applies to each graph) The first graph shows decreased deuteration (increased protection) for P-gp in the pre-hydrolysis (AMPPNP-bound) state relative to the nucleotide-free state. This behavior is observed across many time points, and the sum of these changes across time points (in color) emphasizes the regions experiencing a change in protection.

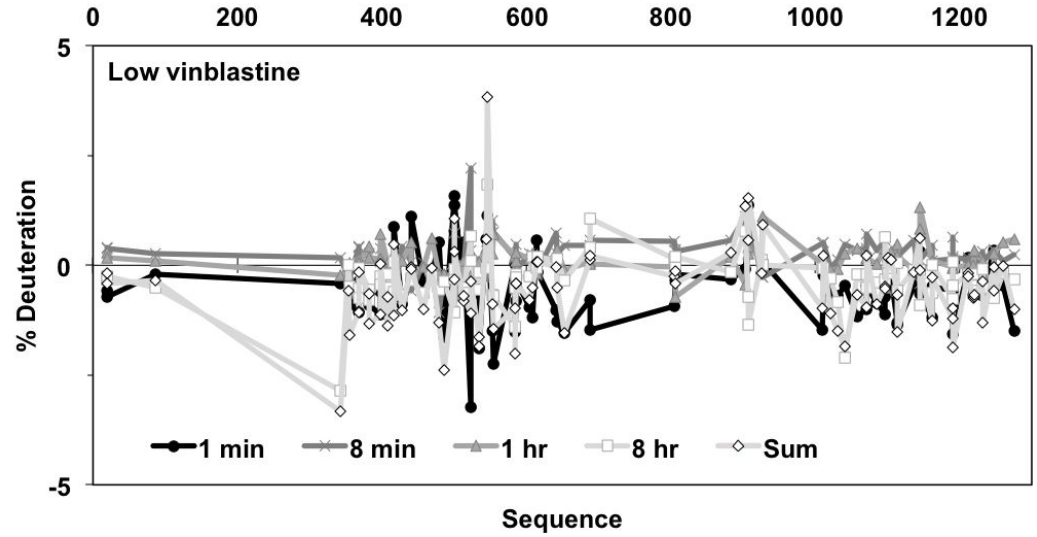


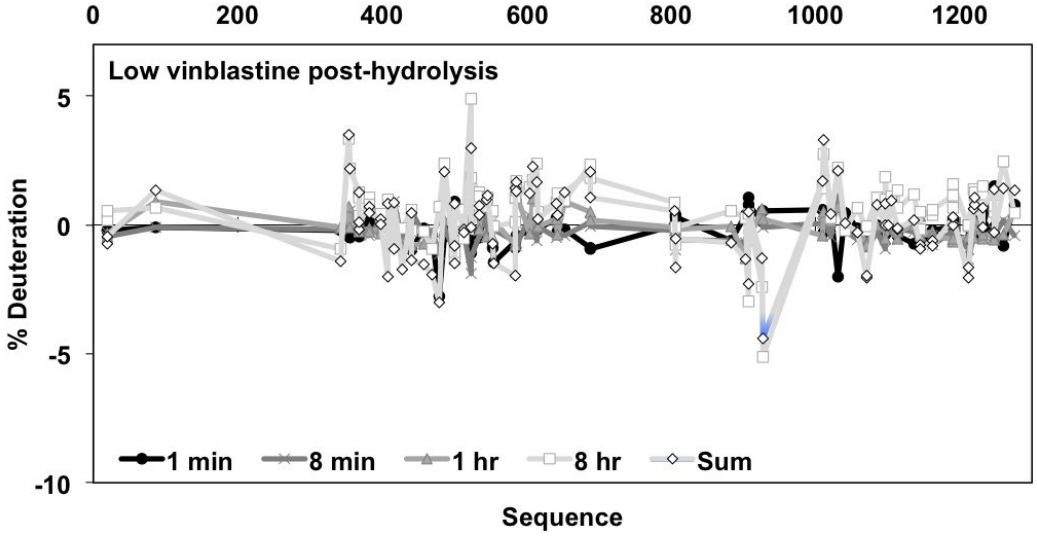

Post-hydrolysis
% Deuteration
0
-10
-20
-30
1 min
8 min
1 hr
8 hr
Sum
0
200
400
600
800
1000
1200
Sequence
Post- from pre-hydrolysis
% Deuteration
5
0
-5
-10
-15
-20
1 min
8 min
1 hr
8 hr
Sum
0
200
400
600
800
1000
1200
Sequence
Post-hydrolysis (2nd dataset)
% Deuteration
5
-5
-15
-25
-35
1 min
8 min
1 hr
8 hr
Sum
0
200
400
600
800
1000
1200
Sequence

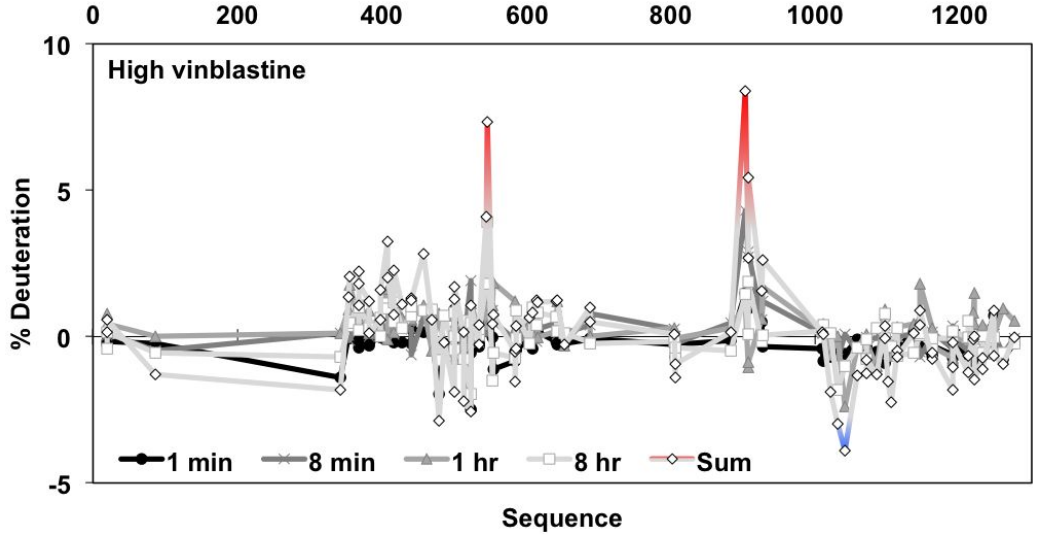

Zosuquidar
% Deuteration
5
0
-5
-10
-15
1 min
8 min
1 hr
8 hr
Sum
0
200
400
600
800
1000
1200
Sequence

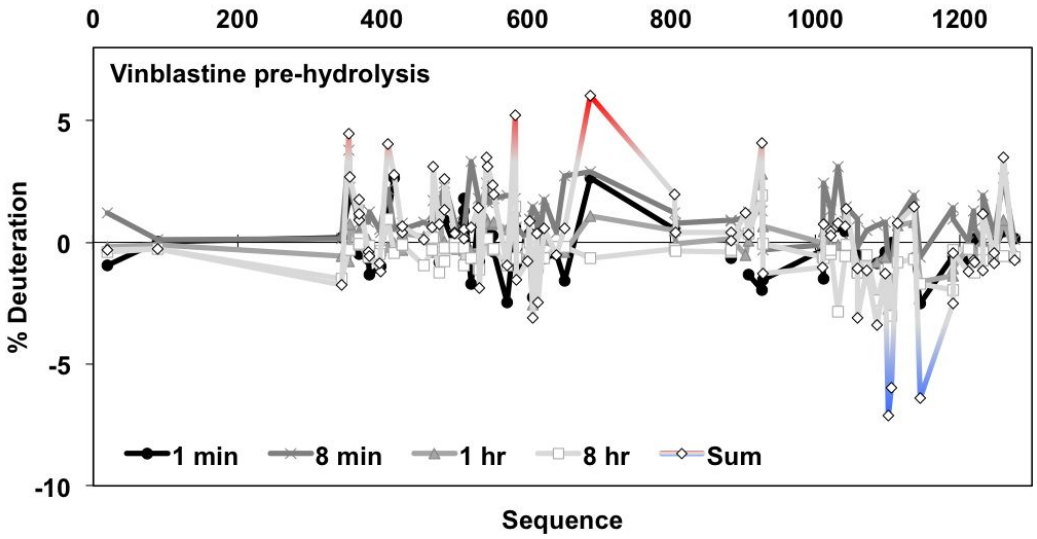

Zosuquidar pre-hydrolysis
% Deuteration
5
0
-5
-10
1 min
8 min
1 hr
8 hr
Sum
0
200
400
600
800
1000
1200
Sequence

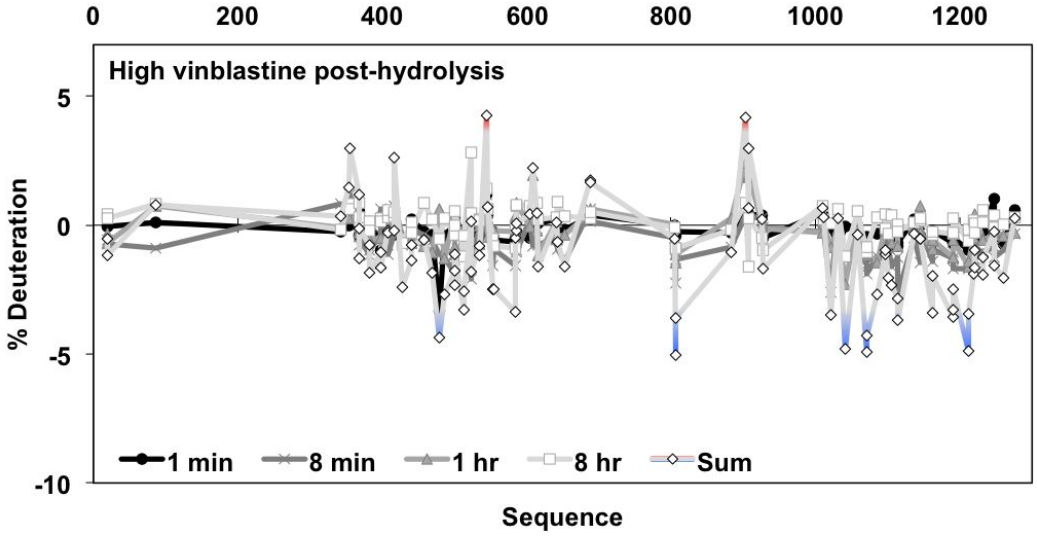

Zosuquidar post-hydrolysis
% Deuteration
15
10
5
0
-5
-10
1 min
8 min
1 hr
8 hr
Sum
Sequence


Low vinblastine
% Deuteration
Sequence
1 min
8 min
1 hr
8 hr
Sum


Low vinblastine post-hydrolysis
% Deuteration
Sequence
1 min
8 min
1 hr
8 hr
Sum


High vinblastine
% Deuteration
Sequence
1 min
8 min
1 hr
8 hr
Sum


Vinblastine pre-hydrolysis
% Deuteration
Sequence
0
200
400
600
800
1000
1200
5
0
-5
-10
1 min
8 min
1 hr
8 hr
Sum


High vinblastine post-hydrolysis
% Deuteration
Sequence
0
200
400
600
800
1000
1200
5
0
-5
-10
1 min
8 min
1 hr
8 hr
Sum