Supplemental information

HDX-MS for epitope characterization of a therapeutic antibody candidate on the calcium-binding protein annexin-A1

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Table S1: Comparison of different parameters between the in-solution and the bead based digest.

Characteristic parameters	In solution digest	Bead based digest
Identified ANXA1 peptides	220	228
Sequence coverage ANXA1	100 %	100 %
Ab Sequence coverage LC/HC	100% / 57%	100% / 62%
Identified LC/HC peptides	65 / 70	84 / 88
Interfering Ab and ANXA1 peptides	2	3
Sequence coverage pepsin	44%	74%
Identified pepsin peptides	26	79
Interfering pepsin and ANXA1 peptides	-	-
Peptides followed for HDX	180 (81.8%)	207 (90.8%)

Table S2: HDX-MS Summary

Table HDX summary	In solution digest	Bead based digest
State	ANXA1 & ANXA1 bound by Ab	ANXA1 & ANXA1 bound by Ab
HDX reaction details	10 mM HEPES pH 7.4, 1 mM $CaCl_2$, 20 °C, 90% D_2O	10 mM HEPES pH 7.4, 1 mM $CaCl_2$, 20 °C, 90% D_2O
Time points	30s, 300s, 3000s, 30000s, 86400s,	300s, 3000s
Av. back exchange (Synthetic peptides)	29%	24 %
Digest conditions	10 min on-Ice, 1:1 pepsin:ANXA1 molar ratio	1 min on-Ice, 20 µl pepsin beads
Number of Peptides /Sequence coverage	180 / 100%	207 / 100 %
Average peptide length / average redundancy	21 (Std. Dev. 12) / 11	15 (Std. Dev. 9) / 9.1
Technical replicates (triplicate)	min 2 of 3 peptides per time point, in both states	min 2 of 3 peptides per time point, in both states
Determined ΔHX threshold for each time point	0.64 Da	0.25 Da
Significant differences in HDX	Students t-distribution on 95% confidence level	Students t-distribution on 95% confideñnce level

Table S3: List of the used systemic peptides for back exchange determination. * marks a carbamidomethylated cysteine.

#No	Sequence	Length	Retention time [min]
1	LTIEELK	7	16.3
2	FNNYQVR	7	7.9
3	MSDSVILR	8	14.5
4	SEC*HVDFFR	9	17.3
5	TVAAFGGEK	9	5.1
6	IVVLC*GQEAVK	11	14.8
7	LQDEIDAALPNK	12	15.8
8	GTTLITNLSSVLK	13	20.4
9	AAATEDATPAALEK	14	11.4
10	ISIIPQDPILFPGSLR	16	21
11	EQLDSLVC*LESAILEVLR	18	23.4
12	LSDRPQLPYLEAFILETFR	19	22.7
13	AMDSFPGPPTHWLFGHALEIQK	22	20.6
14	GDFVAVFPPMIHNDPEVFDAPK	22	20.5
15	VYGPVFTLYFGSKPTVVVHGYEAVK	25	20.7

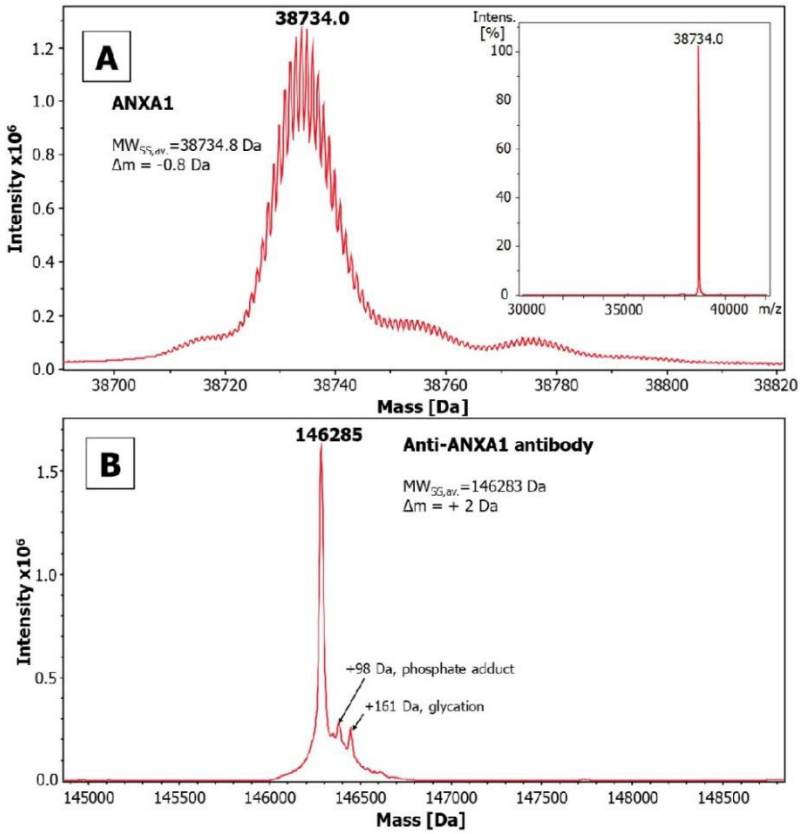


Figure S1: High resolution deconvoluted ESI QTOF MS spectrum of recombinant ANXA1 (A) and deglycosylated anti-ANXA1 antibody (B) in positive mode. The experimental mass of the molecules confirmed the theoretical mass and identity of both molecules. Integrity was good with no degradation or contamination as displayed in the inset of (A). The antibody showed 14% glycation (B).

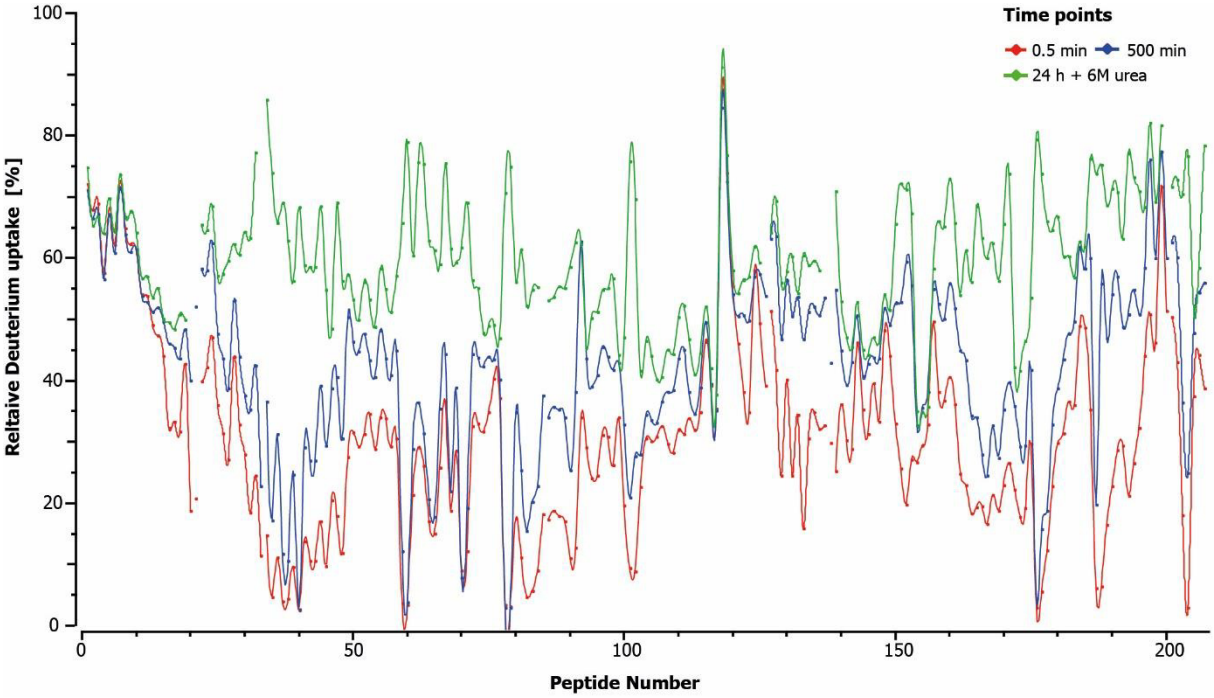


Figure S2: ANXA1 deuteration kinetics of the bead-based digest. Relative deuterium uptake of peptic peptides of ANXA1 numbered from N- to C-terminus over time.

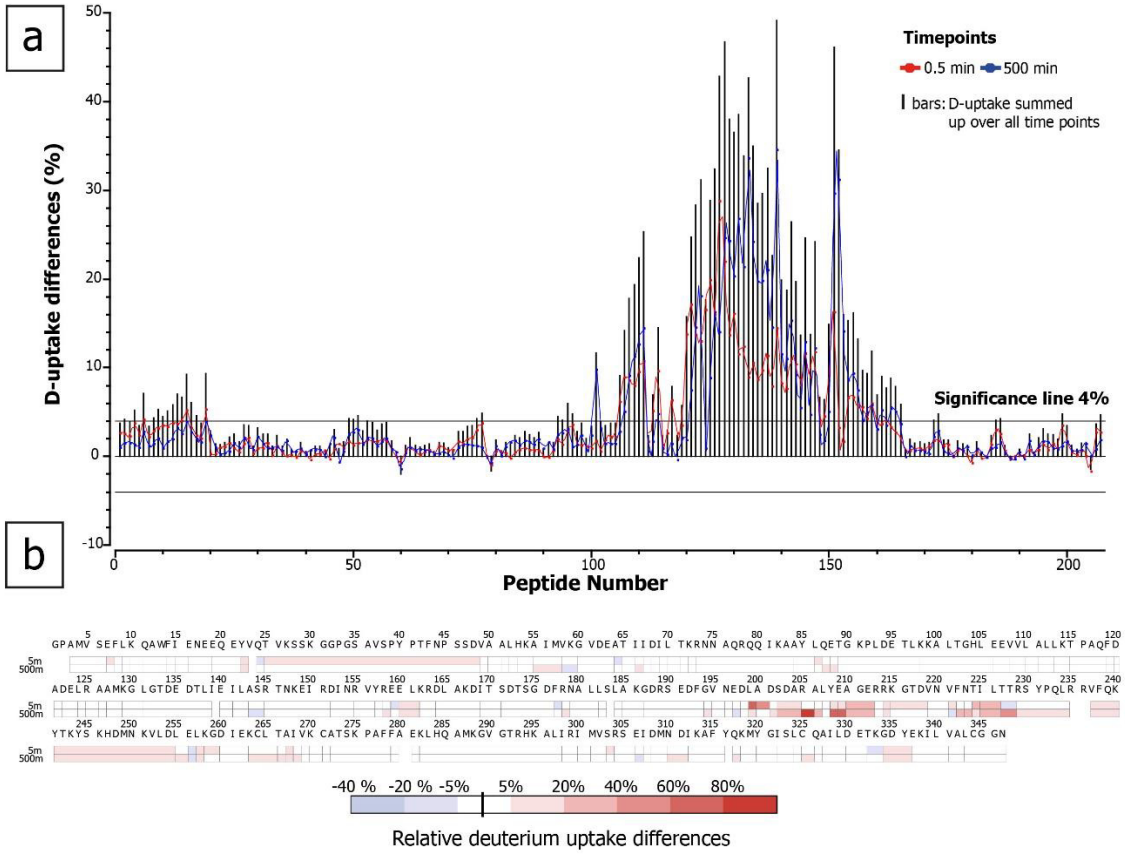


Figure S3: Epitope mapping of anti-ANXA1 by HDX mass spectrometry of the bead-based digest. (a) Differential deuterium uptake of ANXA1 alone and in complex with the anti-ANXA1. Partially overlapping peptic peptides are numbered from the N- to the C-terminus. High confidence identification by mass, retention time and charge was applied using a peptide library. (b) Heat map of anti-ANXA1 antibody epitope regions narrowed down after combining the data from all overlapping peptides and time points.

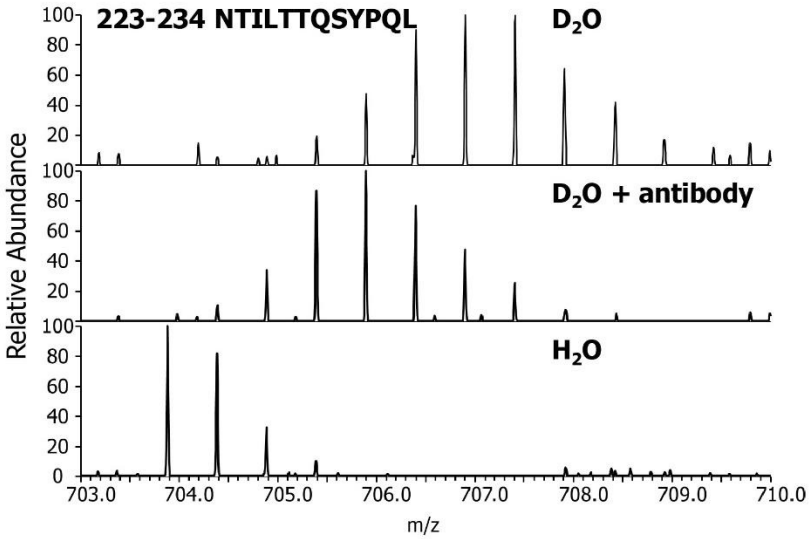


Figure S4: Mass spectra of an epitope peptide showing the isotope distribution of the control (lower panel), ANXA1 in complex with antibody (middle panel) and alone after 24 h HDX (top panel).

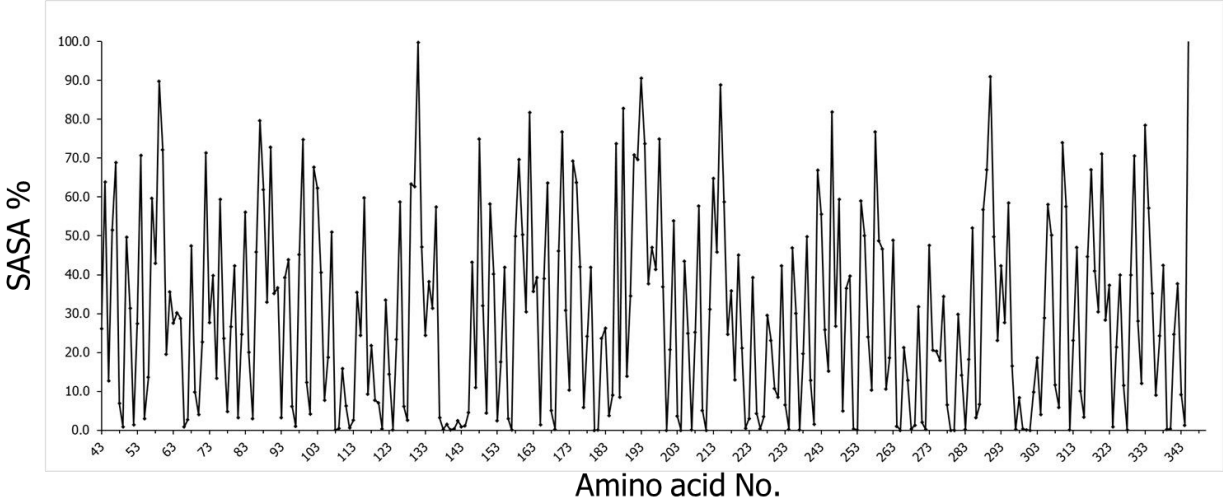
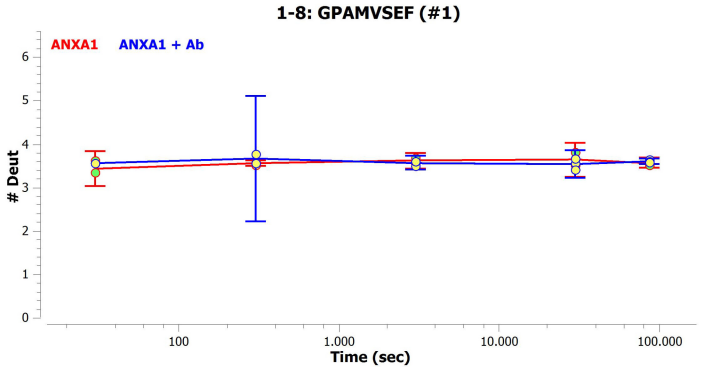
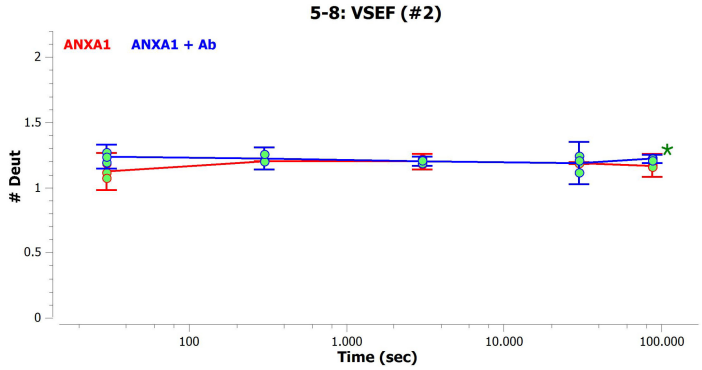
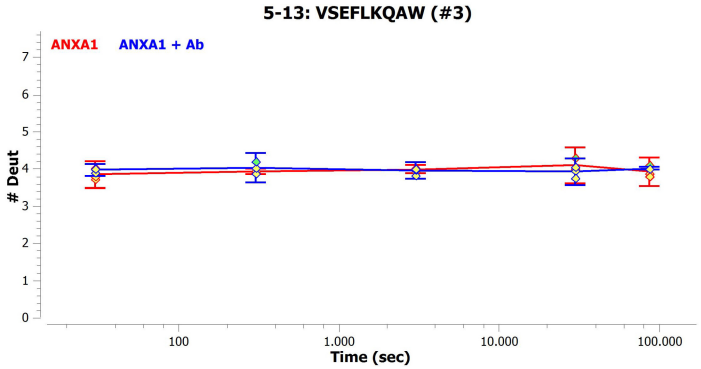
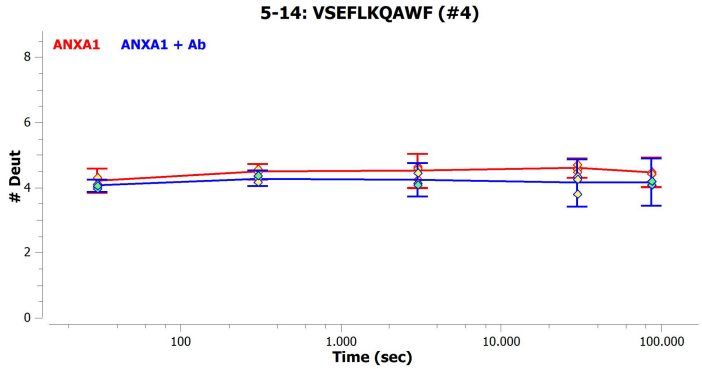


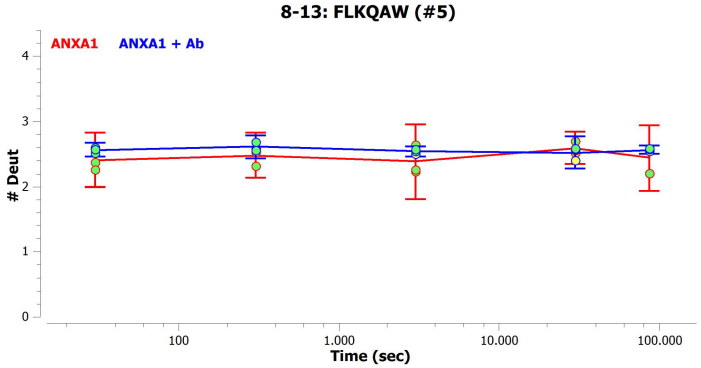
Figure S5: Relative solvent accessibility surface area (SASA) of porcine ANXA1 PDBe: 1MCX. Total SASA of each residue was calculated using PyMOL. The exposure of the residue X was then normalized to the accessibility of a GxG peptides [Miller S, Janin J, Lesk AM, Chothia C. Interior and Surface of Monomeric Proteins. J. Mol. Biol. 1987 196, 641-656]. The first 42 residues are not resolved in crystal structure. Amino acid numbers are adapted to the sequence used within this experiments.

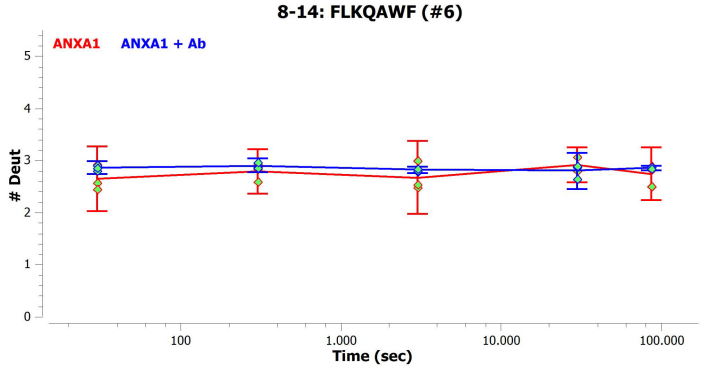

1-8: GPAMVSEF (#1)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

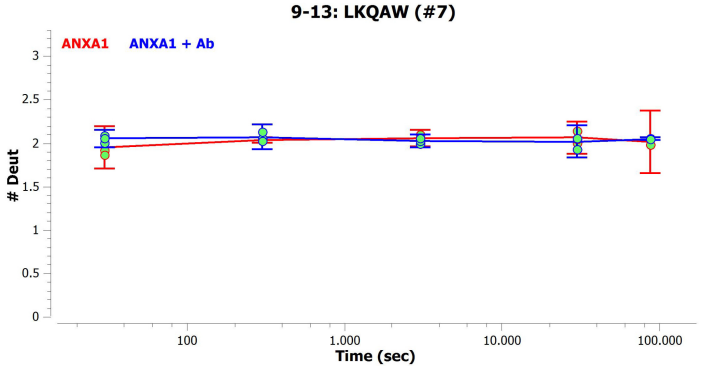

5-8: VSEF (#2)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

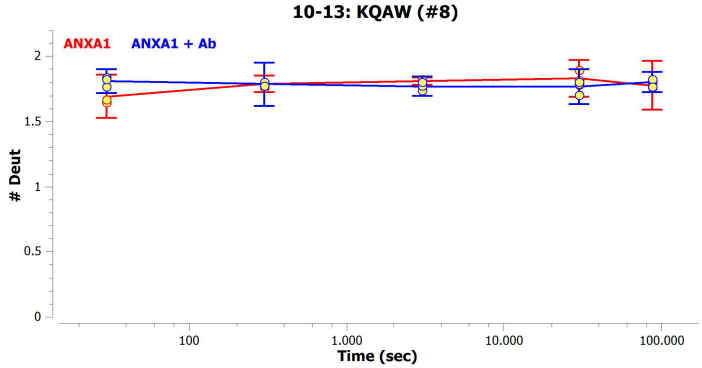

5-13: VSEFLKQAW (#3)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

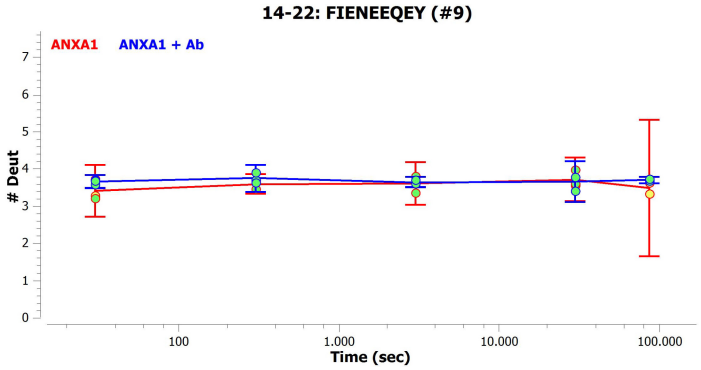

5-14: VSEFLKQAWF (#4)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

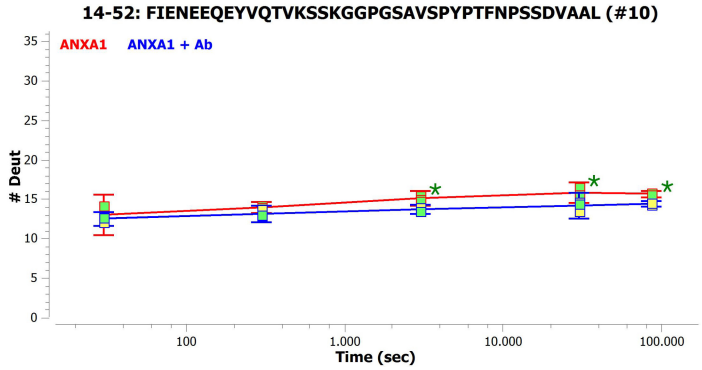

8-13: FLKQAW (#5)
ANXA1
ANXA1 + Ab
Deut
Time (sec)


8-14: FLKQAWF (#6)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

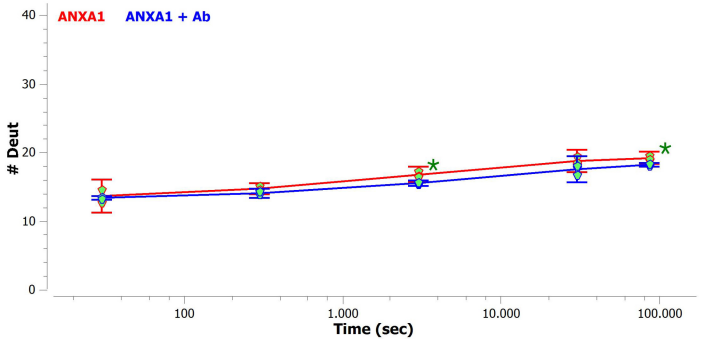

9-13: LKQAW (#7)
ANXA1
ANXA1 + Ab
Deut
Time (sec)


10-13: KQAW (#8)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

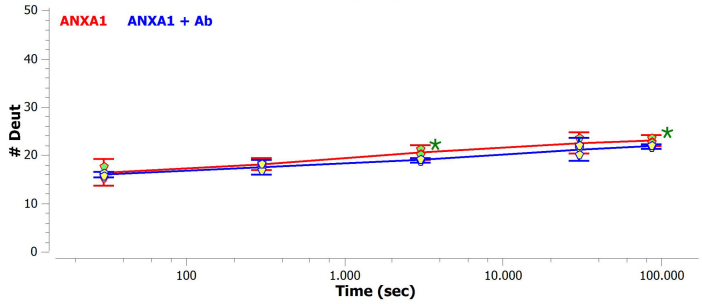

14-22: FIENEEQEY (#9)
ANXA1
ANXA1 + Ab
Deut
Time (sec)


14-52: FIENEEQEYVQTVKSSKGGPGSAVSPYPTFNPSSDVAAL (#10)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

14-57: FIENEEQEYVQTVKSSKGGPGSAVSPYPTFNPSSDVAALHKAIM (#11)



14-65: FIENEEQEYVQTVKSSKGGPGSAVSPYPTFNPSSDVAALHKAIMVKGVDEAT (#12)



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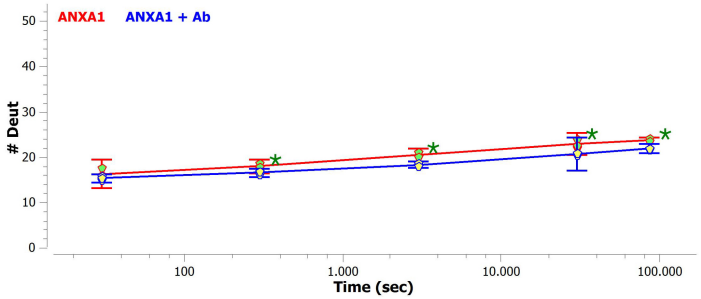
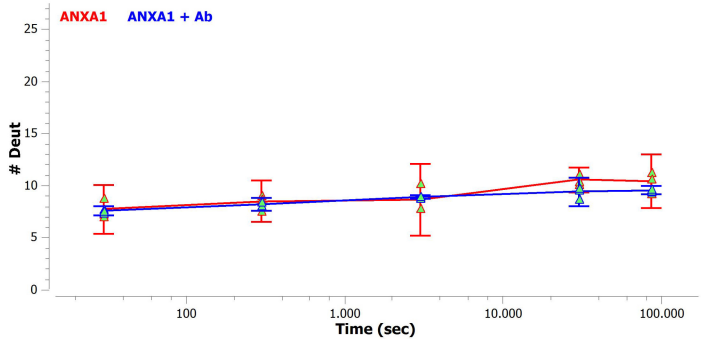
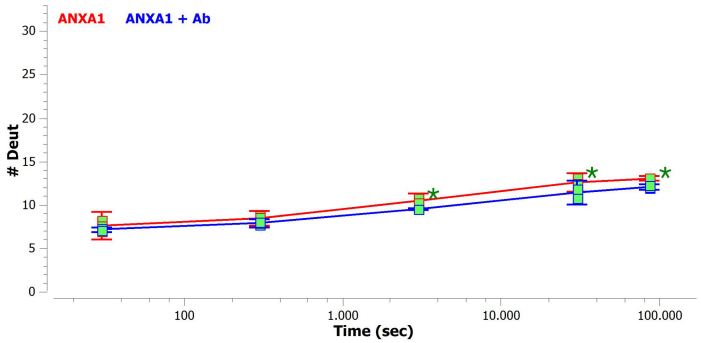


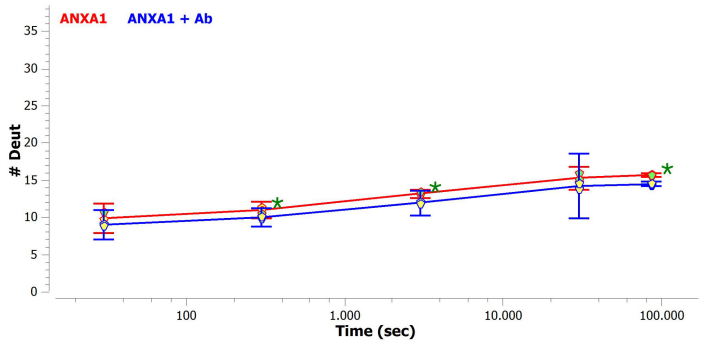
22-52: YVQTVKSSKGGPGSAVSPYPTFNPSSDVAAL (#14)



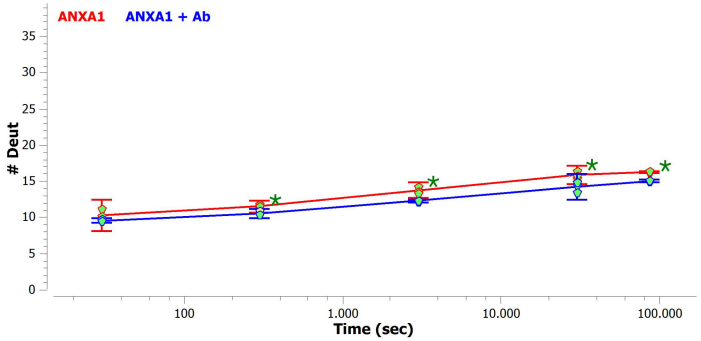
22-57: YVQTVKSSKGGPGSAVSPYPTFNPSSDVAALHKAIM (#15)



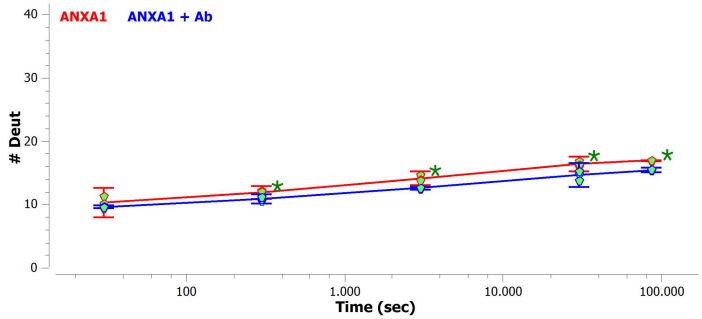
22-62: YVQTVKSSKGGPGSAVSPYPTFNPSSDVAALHKAIMVKGVD (#16)



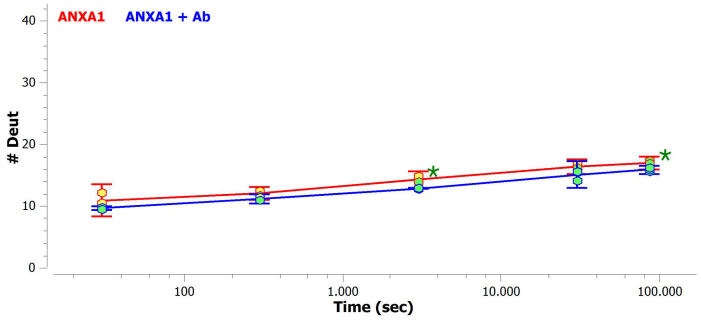
22-63: YVQTVKSSKGGPGSAVSPYPTFNPSSDVAALHKAIMVKGVDE (#17)



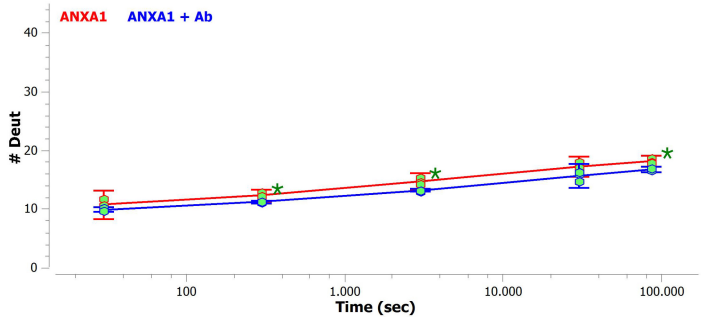
22-65: YVQTVKSSKGGPGSAVSPYPTFNPSSDVAALHKAIMVKGVDEAT (#18)



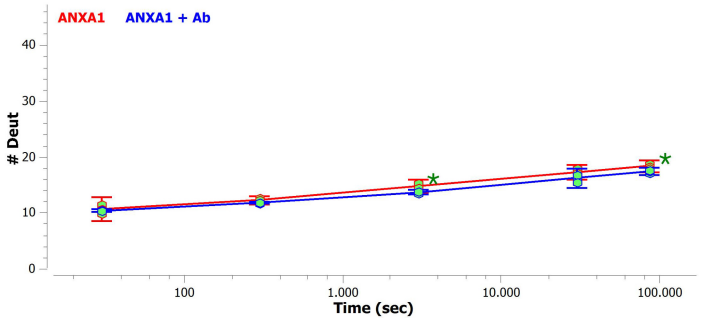
22-66: YVQTVKSSKGGPGSAVSPYPTFNPSSDVAALHKAIMVKGVDEATI (#19)



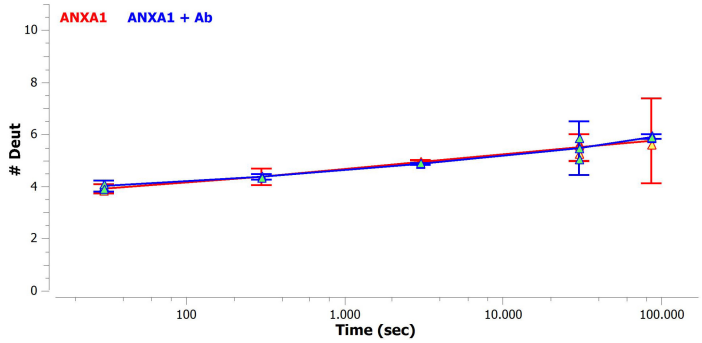
22-68: YVQTVKSSKGGPGSAVSPYPTFNPSSDVAALHKAIMVKGVDEATIID (#20)



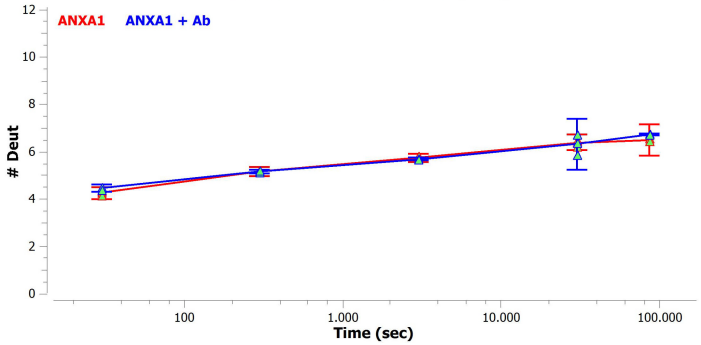
22-70: YVQTVKSSKGGPGSAVSPYPTFNPSSDVAALHKAIMVKGVDEATIIDIL (#21)



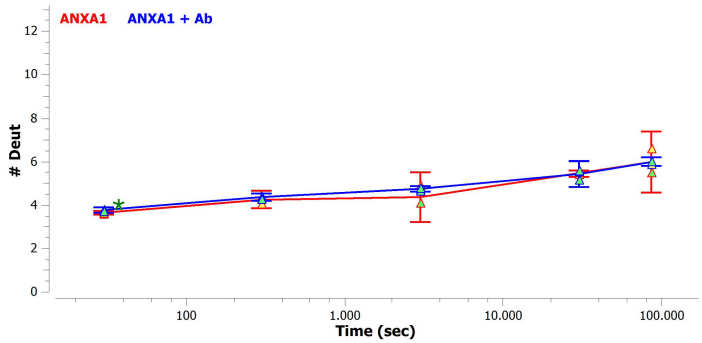
53-64: HKAIMVKGVDEA (#22)



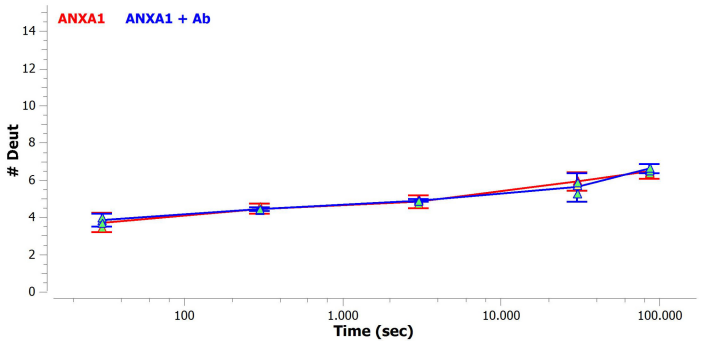
53-65: HKAIMVKGVDEAT (#23)



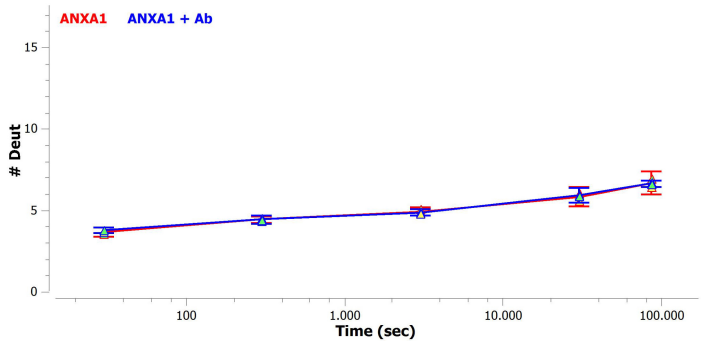
53-66: HKAIMVKGVDEATI (#24)



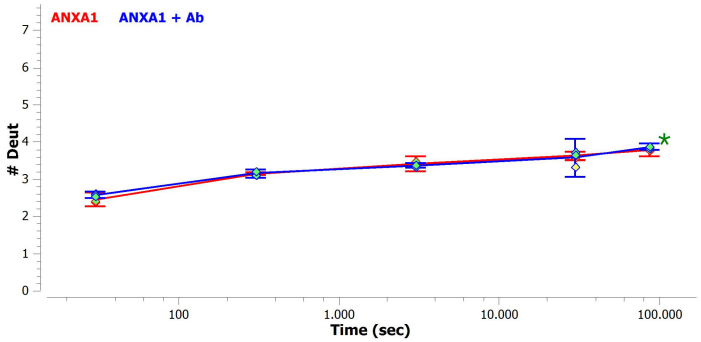
53-68: HKAIMVKGVDEATIID (#25)



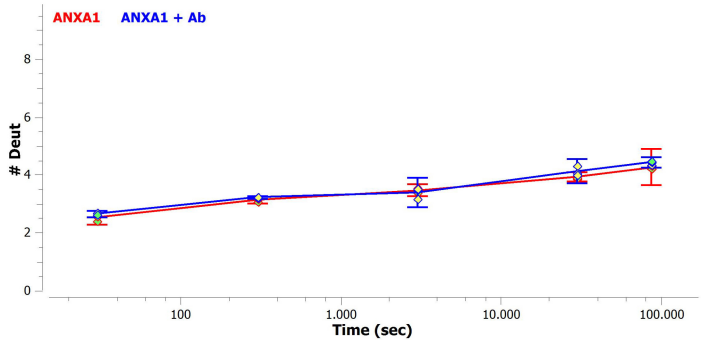
53-70: HKAIMVKGVDEATIIDIL (#26)



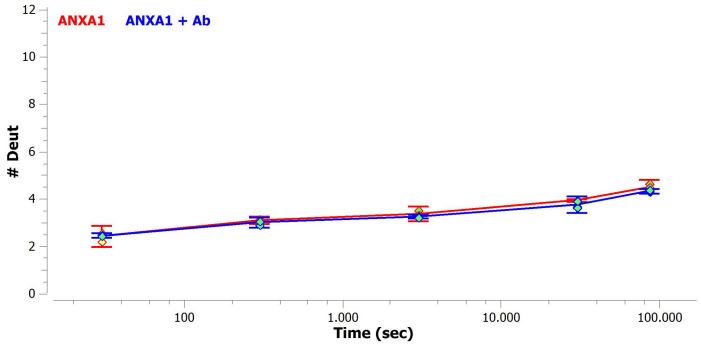
58-66: VKGVDEATI (#27)



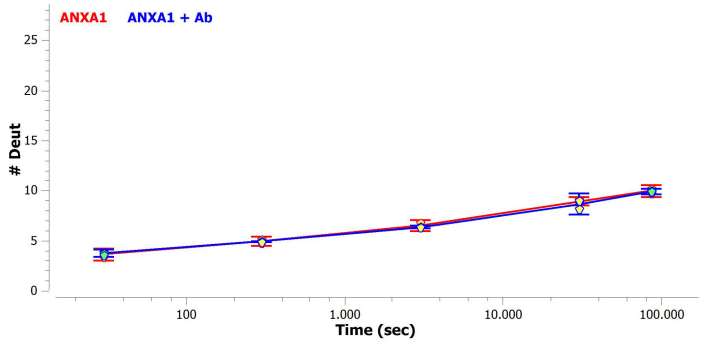
58-68: VKGVDEATIID (#28)



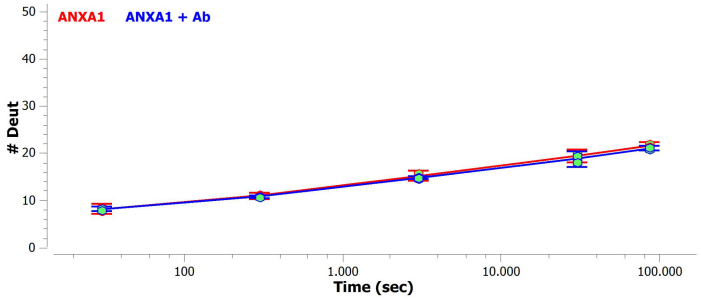
58-70: VKGVDEATIIDIL (#29)



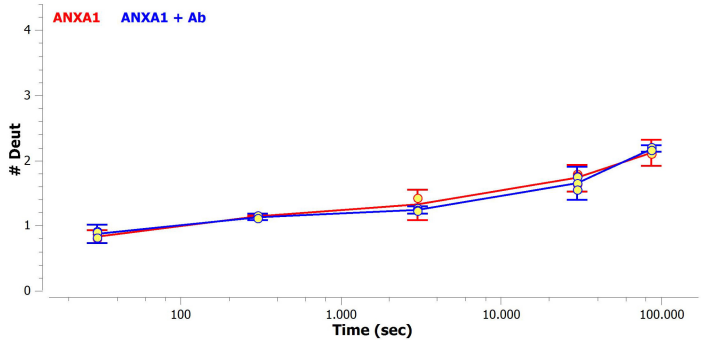
58-85: VKGVDEATIIDILTKRNNAQRQQIKAAY (#30)



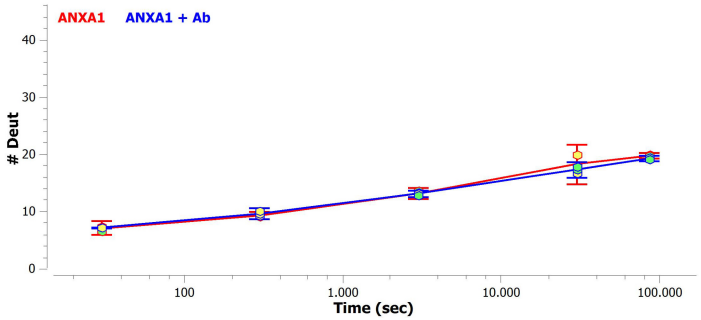
58-107: VKGVDEATIIDILTKRNNAQRQQIKAAYLQETGKPLDETLKKALTGHLEE (#31)



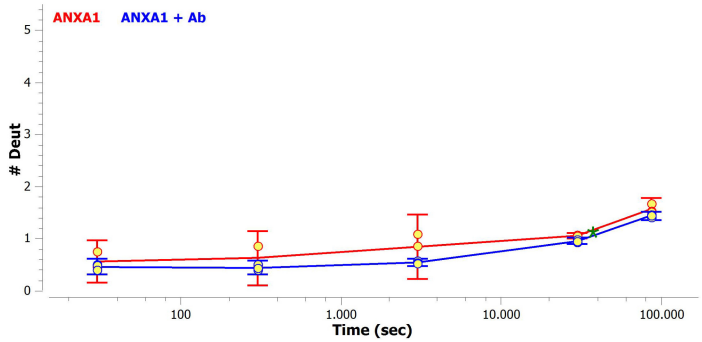
63-68: EATIID (#32)



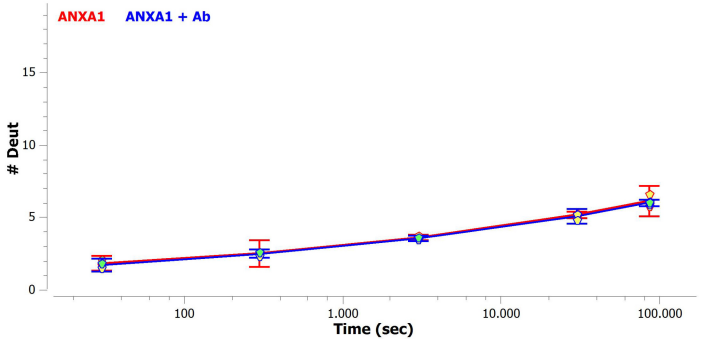
63-107: EATIIDILTKRNNAQRQQIKAAYLQETGKPLDETLKKALTGHLEE (#33)



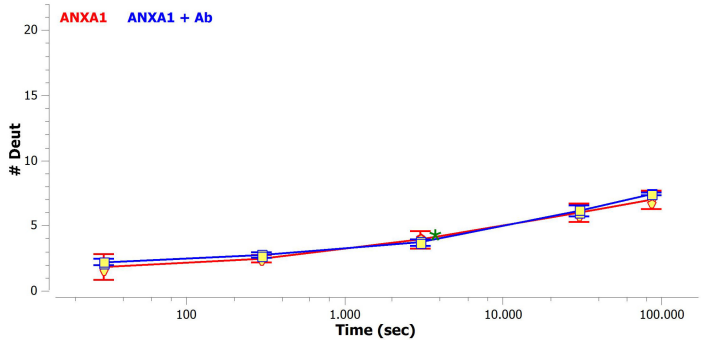
64-70: ATIIDIL (#34)



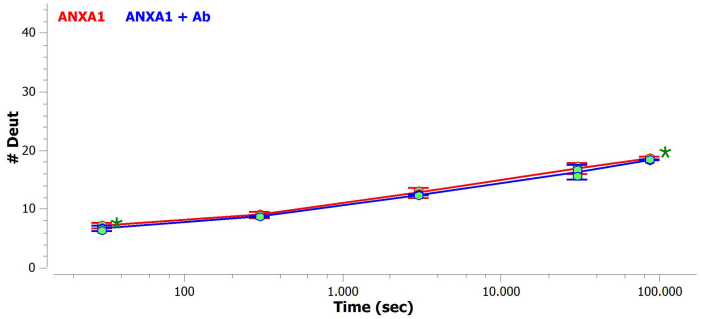
64-83: ATIIDILTKRNNAQRQQIKA (#35)



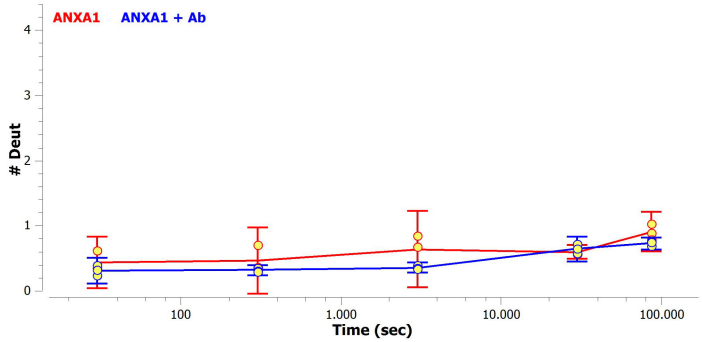
64-85: ATIIDILTKRNNAQRQQIKAAY (#36)



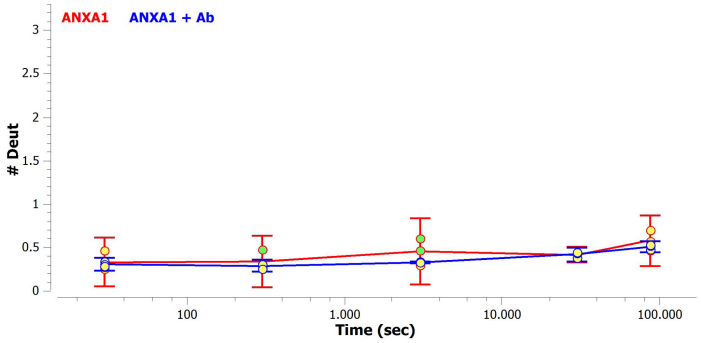
64-107: ATIIDILTKRNNAQRQQIKAAYLQETGKPLDETLKKALTGHLEE (#37)



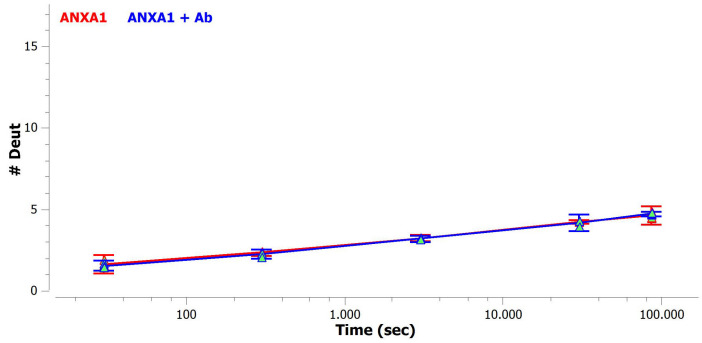
65-70: TIIDIL (#38)



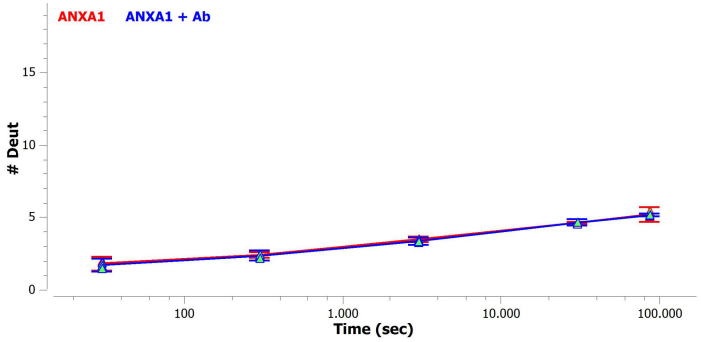
66-70: IIDIL (#39)



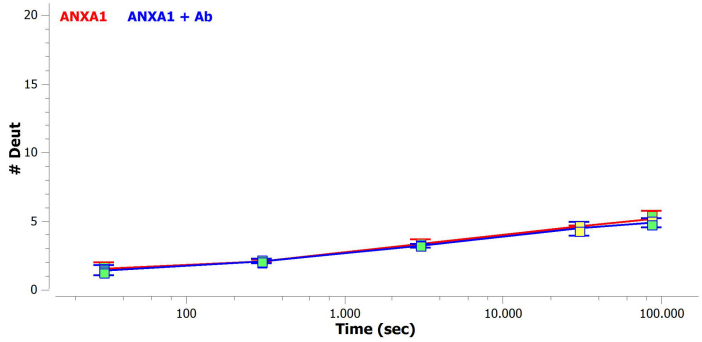
66-83: IIDILTKRNNAQRQQIKA (#40)



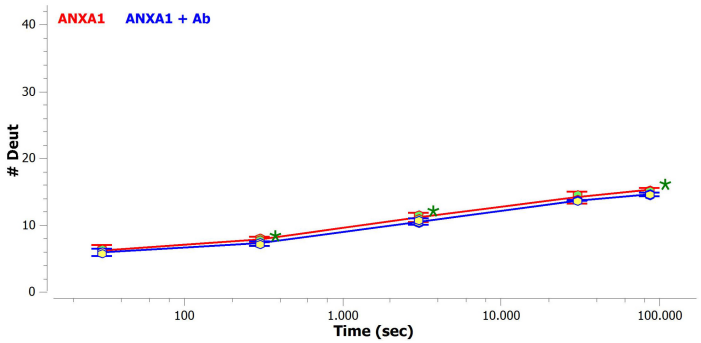
66-85: IIDILTKRNNAQRQQIKAAY (#41)



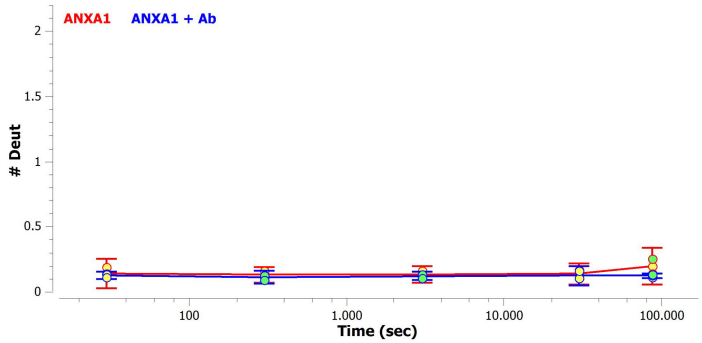
66-86: IIDILTKRNNAQRQQIKAAYL (#42)



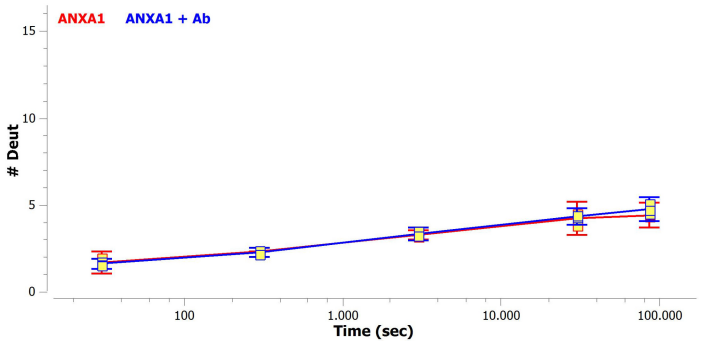
66-107: IIDILTKRNNAQRQQIKAAYLQETGKPLDETLKKALTGHLEE (#43)



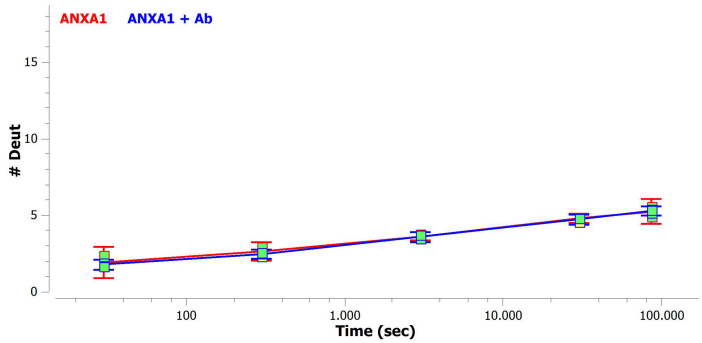
67-70: IDIL (#44)



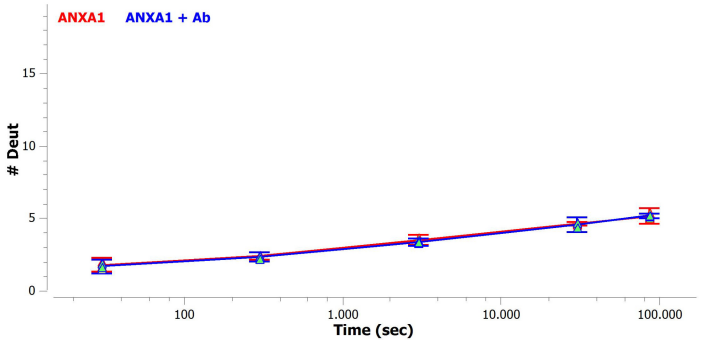
67-83: IDILTKRNNAQRQQIKA (#45)



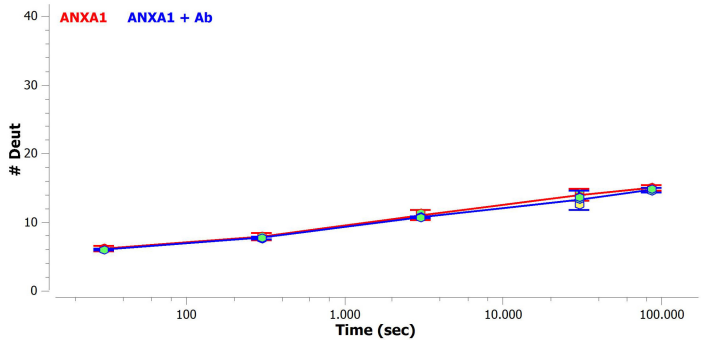
67-85: IDILTKRNNAQRQQIKAAY (#46)



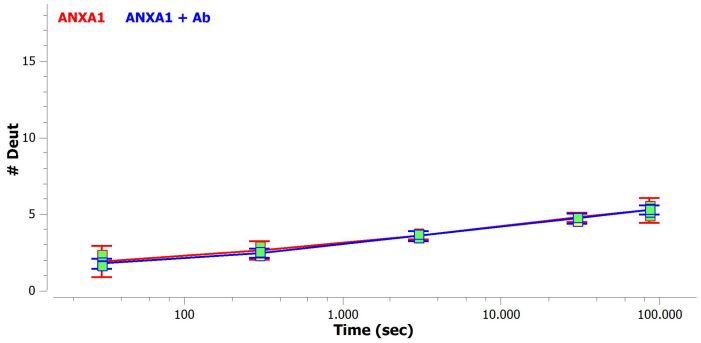
67-86: IDILTKRNNAQRQQIKAAYL (#47)



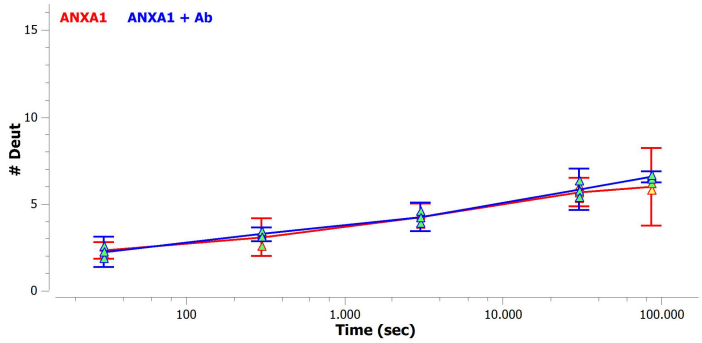
67-107: IDILTKRNNAQRQQIKAAYLQETGKPLDETLKKALTGHLEE (#48)



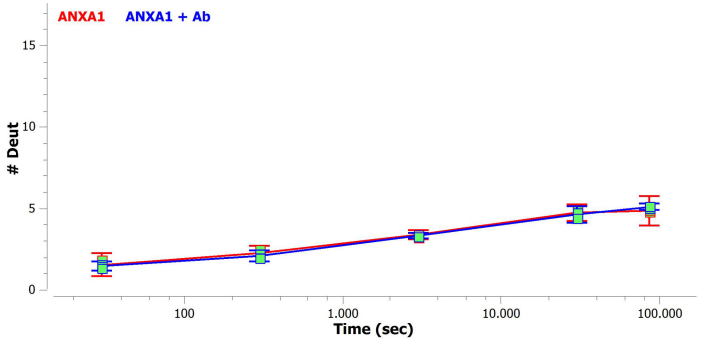
68-86: DILTKRNNAQRQQIKAAYL (#49)



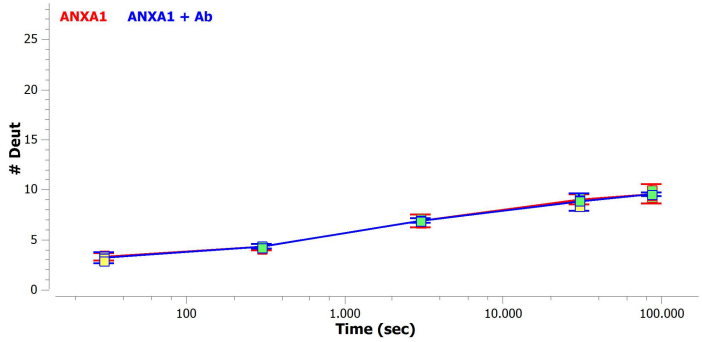
69-85: ILTKRNNAQRQQIKAAY (#50)



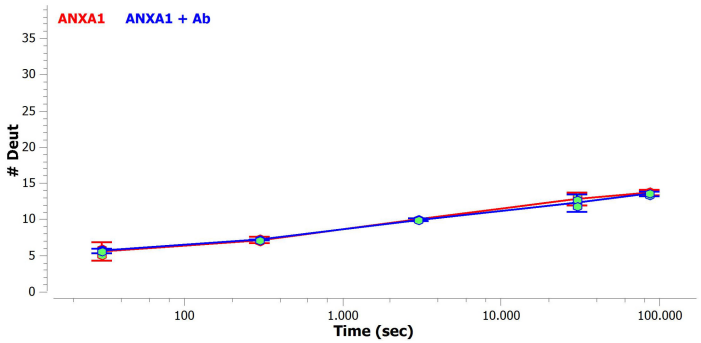
69-86: ILTKRNNAQRQQIKAAYL (#51)



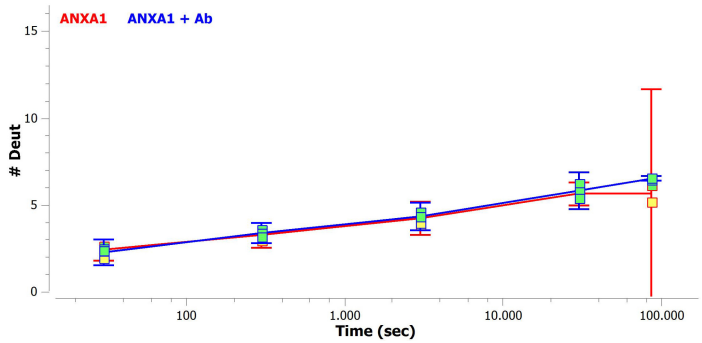
69-97: ILTKRNNAQRQQIKAAYLQETGKPLDETL (#52)



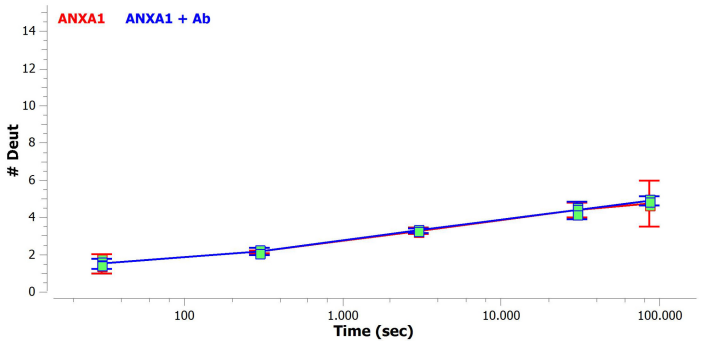
69-107: ILTKRNNAQRQQIKAAYLQETGKPLDETLKKALTGHLEE (#53)



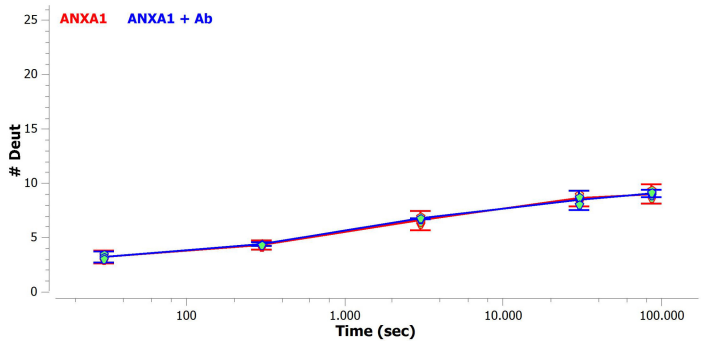
70-86: LTKRNNAQRQQIKAAYL (#54)



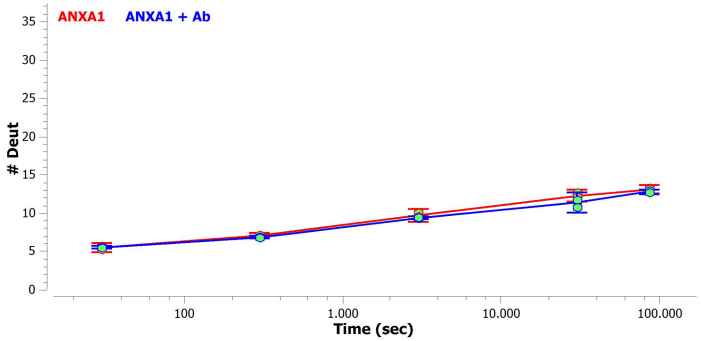
71-86: TKRNNAQRQQIKAAYL (#55)



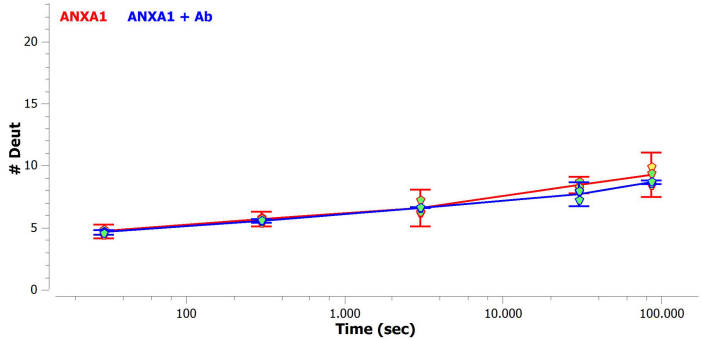
71-97: TKRNNAQRQQIKAAYLQETGKPLDETL (#56)



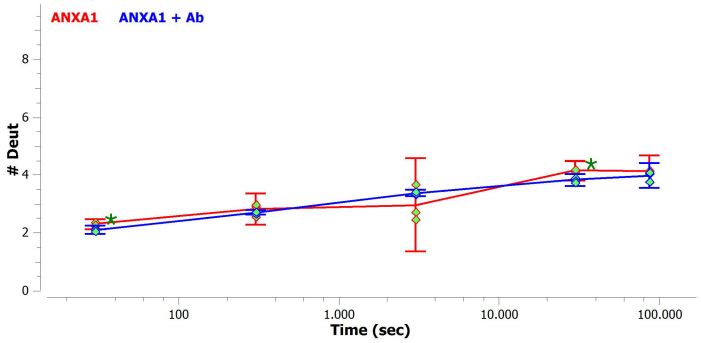
71-107: TKRNNAQRQQIKAAYLQETGKPLDETLKKALTGHLEE (#57)



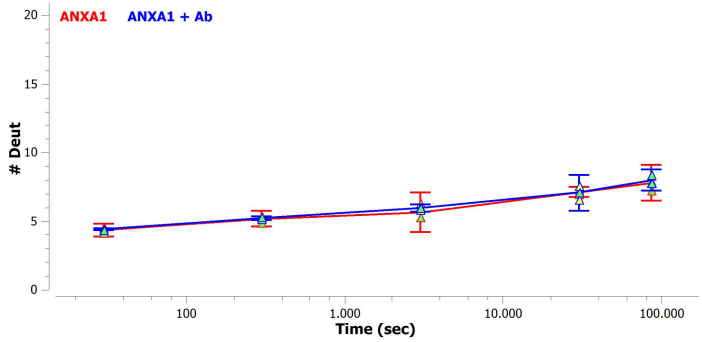
84-107: AYLQETGKPLDETLKKALTGHLEE (#58)



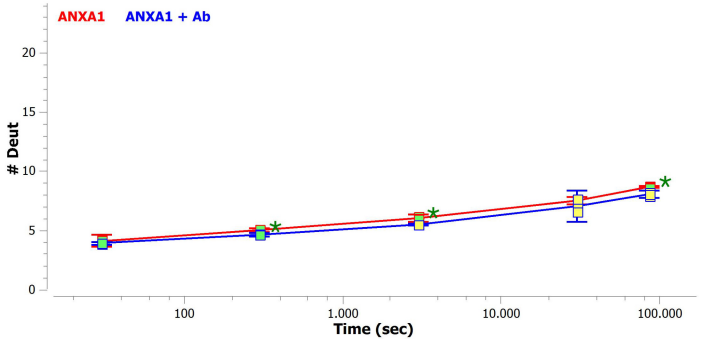
86-97: LQETGKPLDETL (#59)



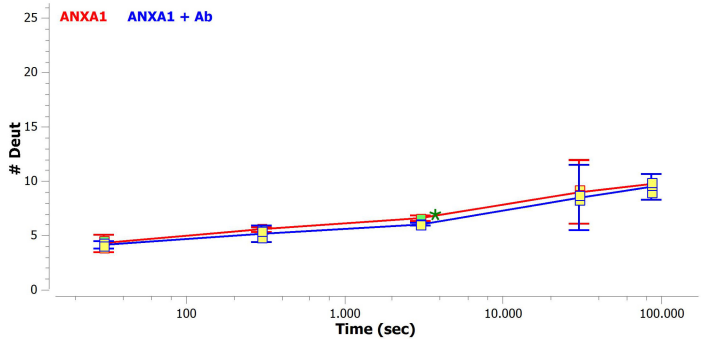
86-107: LQETGKPLDETLKKALTGHLEE (#60)



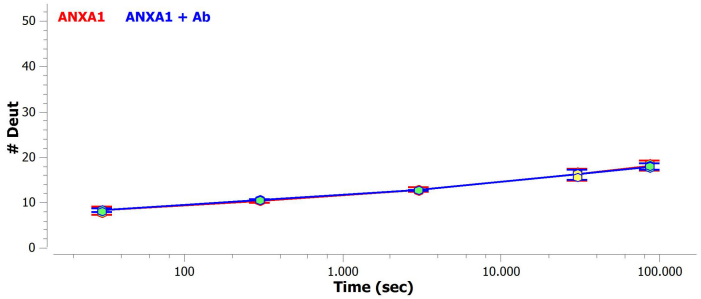
86-110: LQETGKPLDETLKKALTGHLEEVVL (#61)



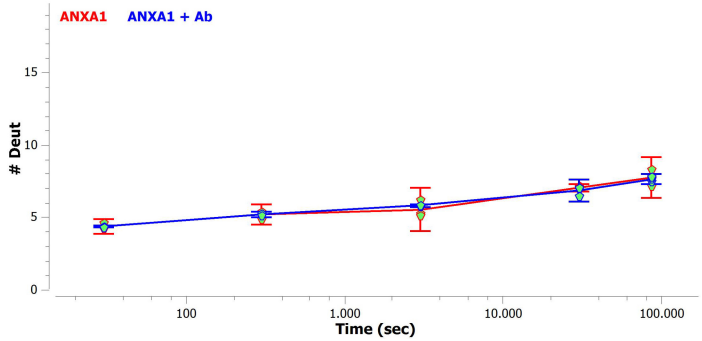
86-112: LQETGKPLDETLKKALTGHLEEVVLAL (#62)



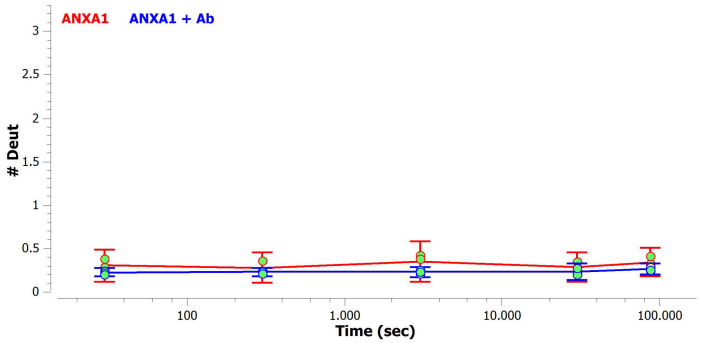
86-138: LQETGKPLDETLKKALTGHLEEVVLALLKTPAQFDADELRAAMKGLGTDEDTL (#63)



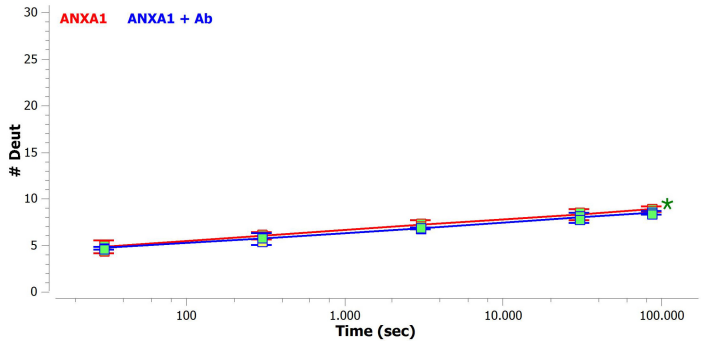
87-107: QETGKPLDETLKKALTGHLEE (#64)



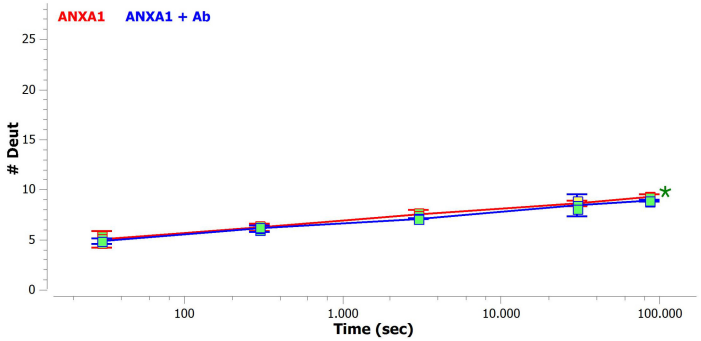
108-112: VVLAL (#65)



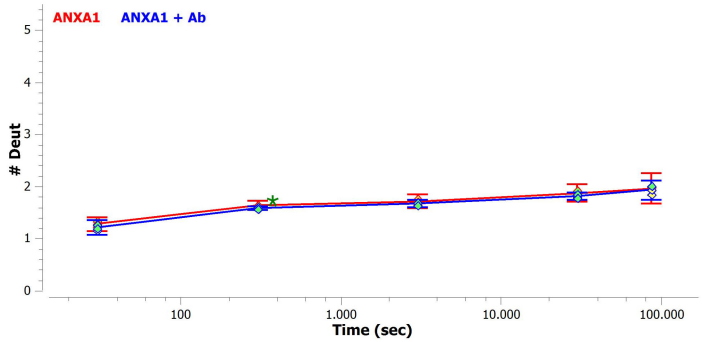
108-138: VVLALLKTPAQFDADELRAAMKGLGTDEDTL (#66)



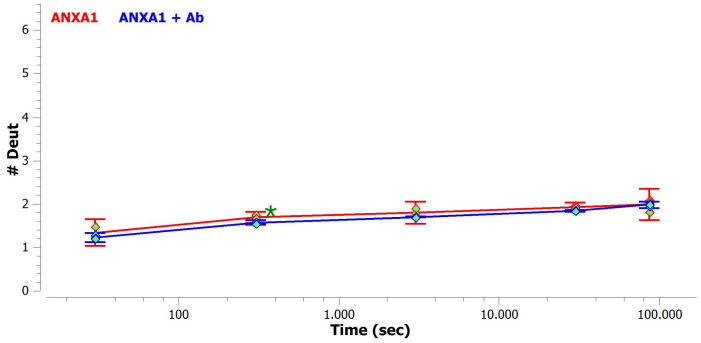
110-138: LALLKTPAQFDADELRAAMKGLGTDEDTL (#67)



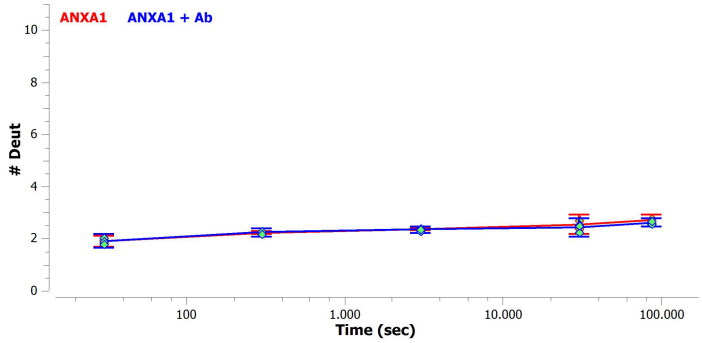
111-118: ALLKTPAQ (#68)

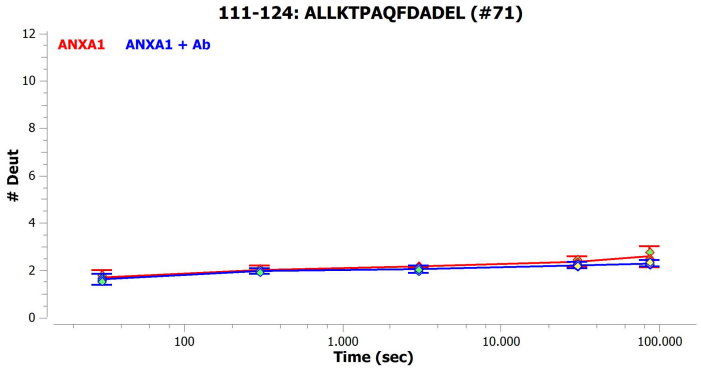


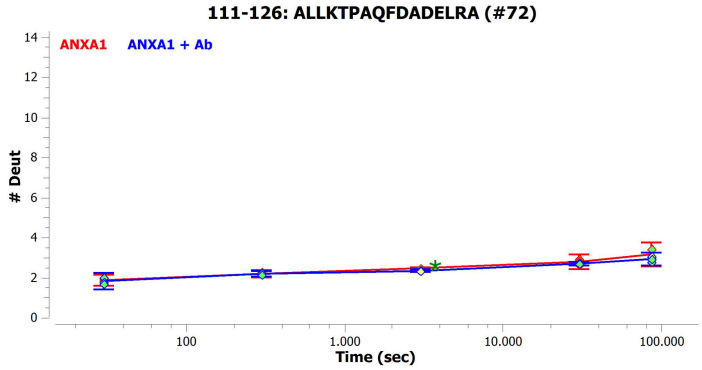
111-119: ALLKTPAQF (#69)

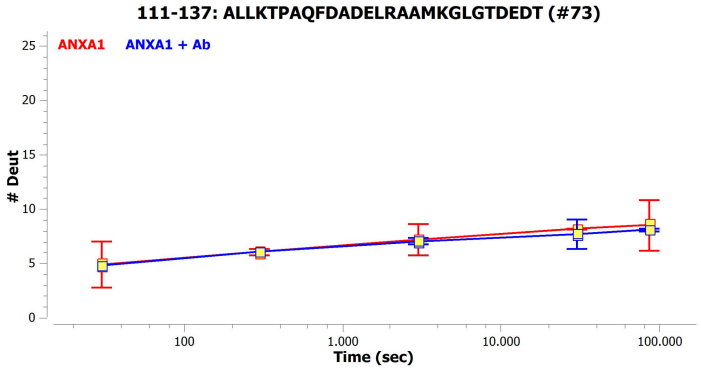


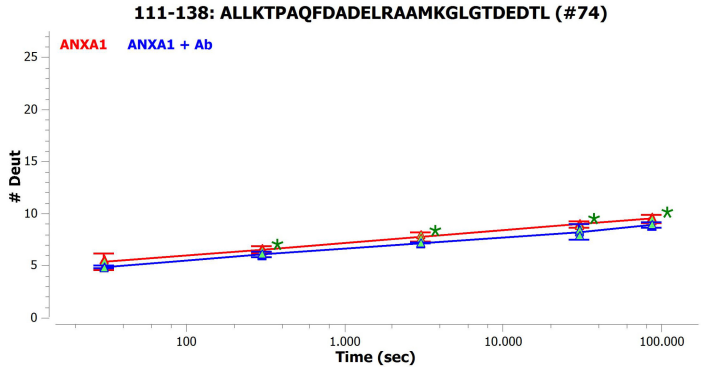
111-123: ALLKTPAQFDADE (#70)

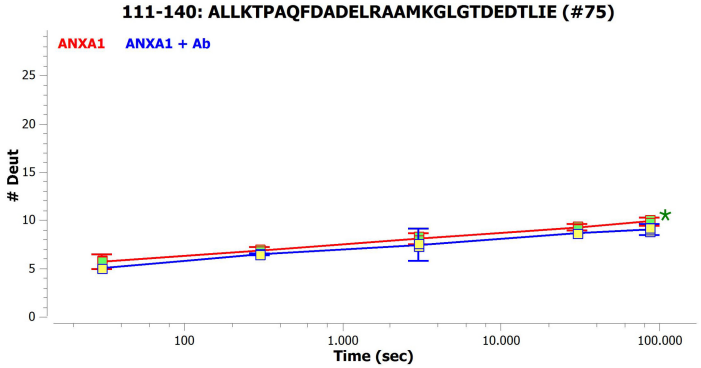


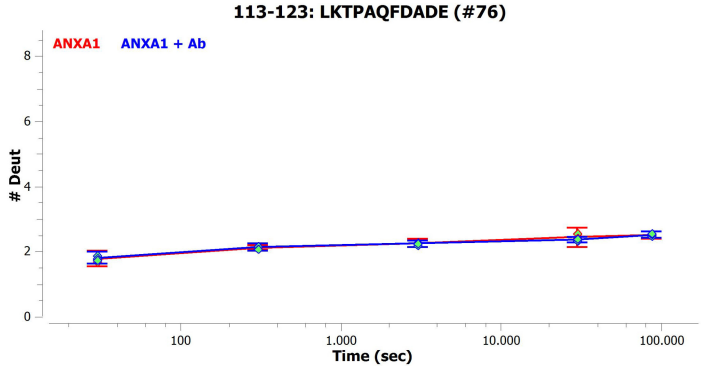

111-124: ALLKTPAQFDADEL (#71)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

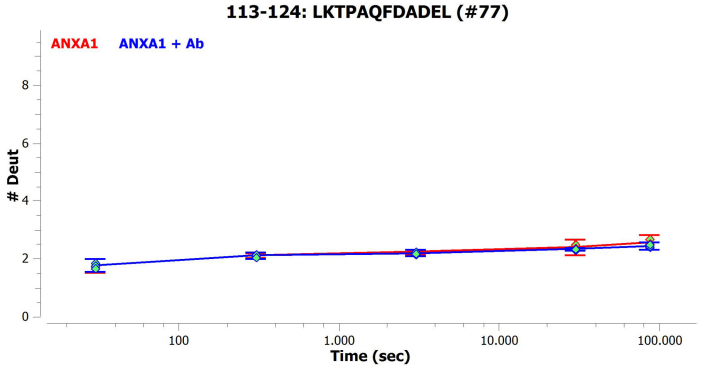

111-126: ALLKTPAQFDADELRA (#72)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

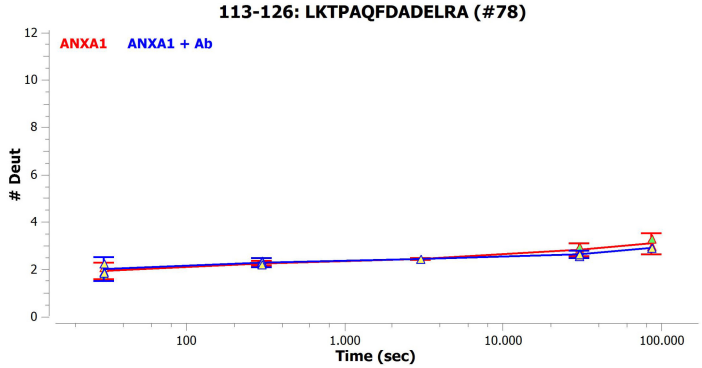

111-137: ALLKTPAQFDADELRAAMKGLGTDEDT (#73)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

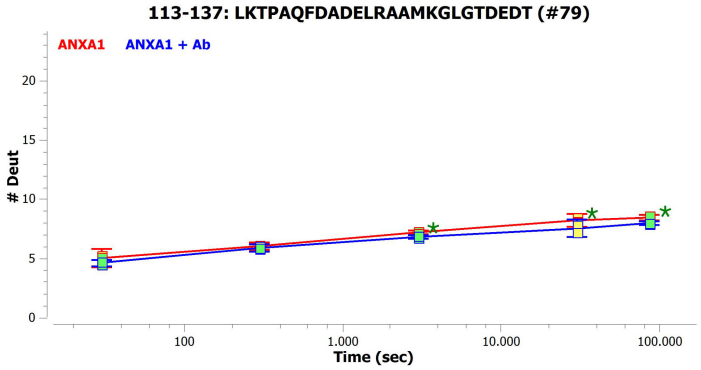

111-138: ALLKTPAQFDADELRAAMKGLGTDEDTL (#74)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

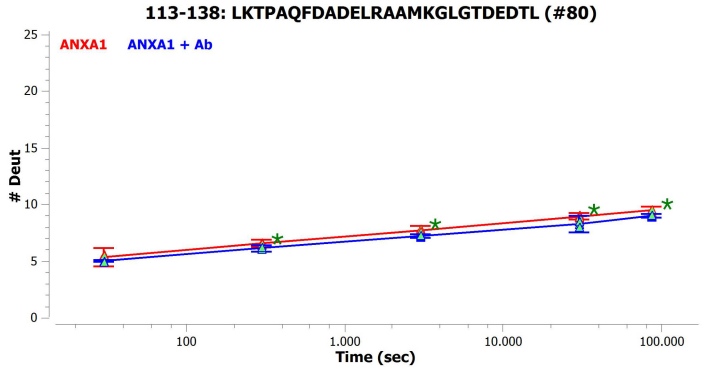

111-140: ALLKTPAQFDADELRAAMKGLGTDEDTLIE (#75)
ANXA1
ANXA1 + Ab
Deut
Time (sec)


113-123: LKTPAQFDADE (#76)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

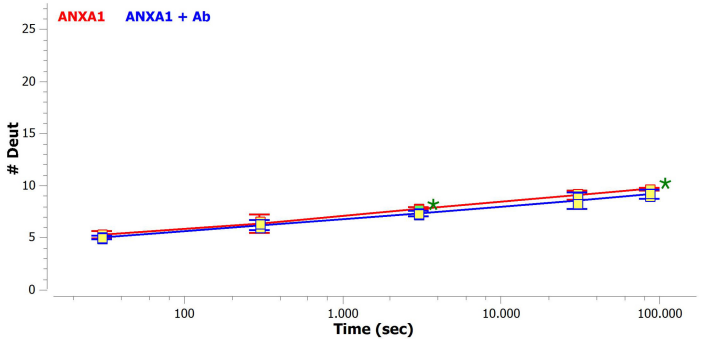

113-124: LKTPAQFDADEL (#77)
ANXA1
ANXA1 + Ab
Deut
Time (sec)


113-126: LKTPAQFDADELRA (#78)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

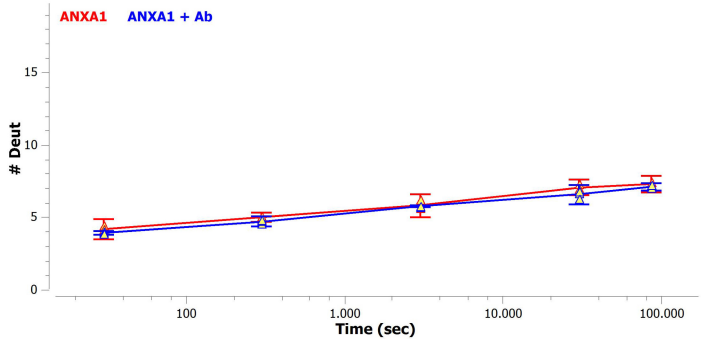

113-137: LKTPAQFDADELRAAMKGLGTDEDT (#79)
ANXA1
ANXA1 + Ab
Deut
Time (sec)


113-138: LKTPAQFDADELRAAMKGLGTDEDTL (#80)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

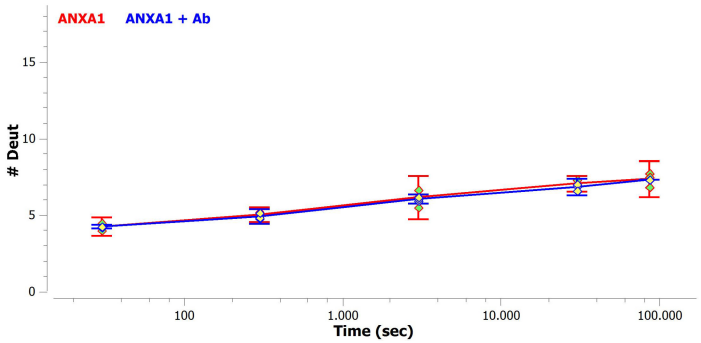
113-140: LKTPAQFDADELRAAMKGLGTDEDTLIE (#81)



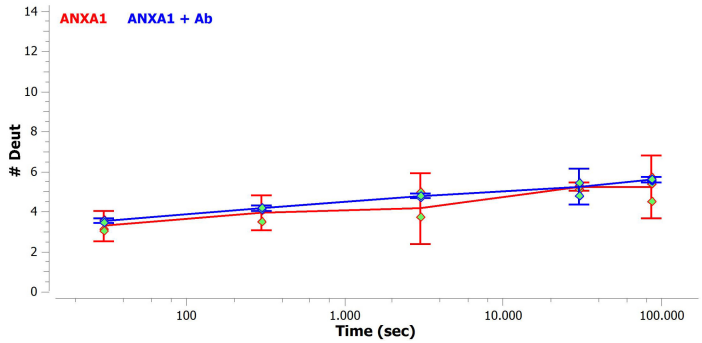
119-138: FDADELRAAMKGLGTDEDTL (#82)



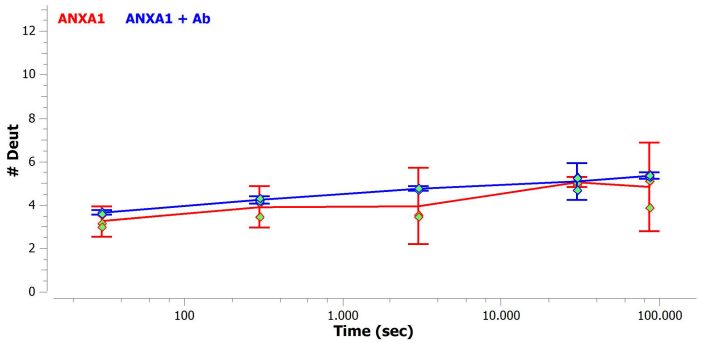
120-138: DADELRAAMKGLGTDEDTL (#83)



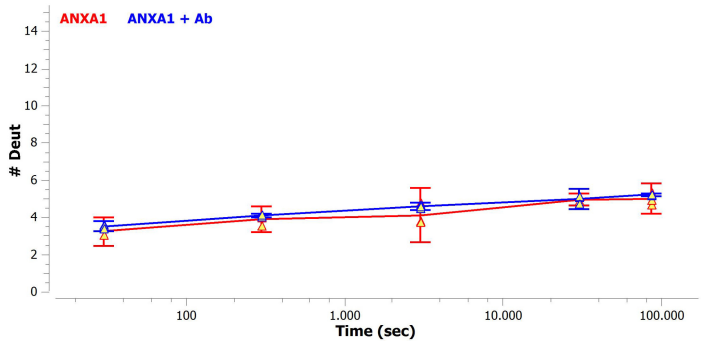
124-138: LRAAMKGLGTDEDTL (#84)



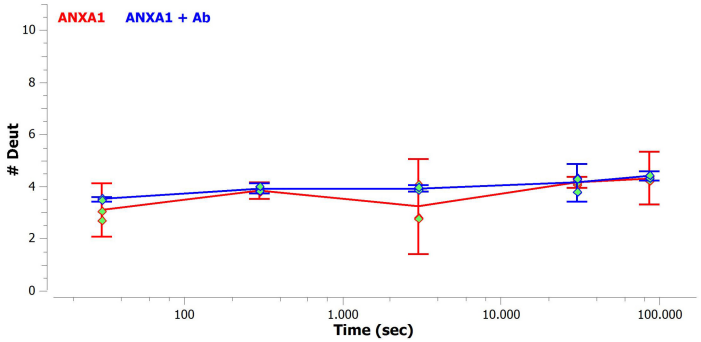
125-138: RAAMKGLGTDEDTL (#85)



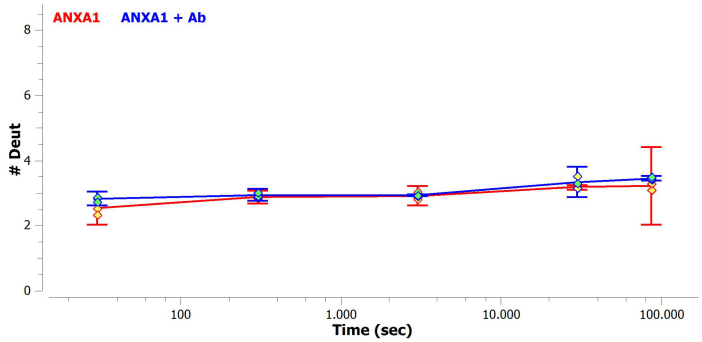
125-140: RAAMKGLGTDEDTLIE (#86)



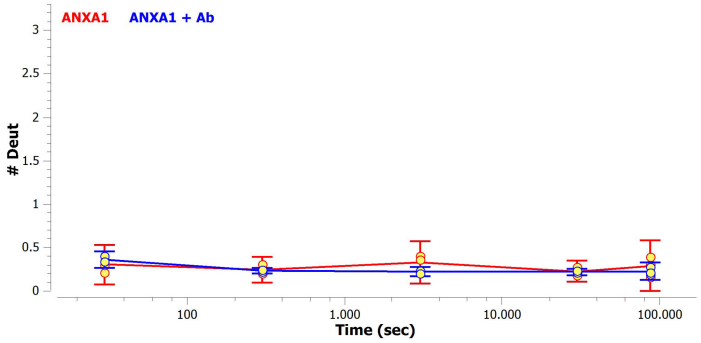
127-138: AMKGLGTDEDTL (#87)



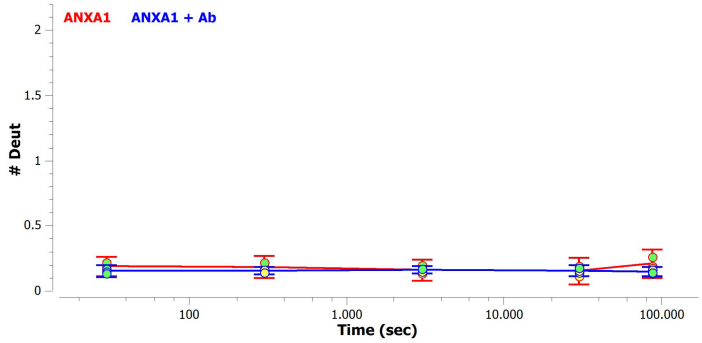
129-138: KGLGTDEDTL (#88)



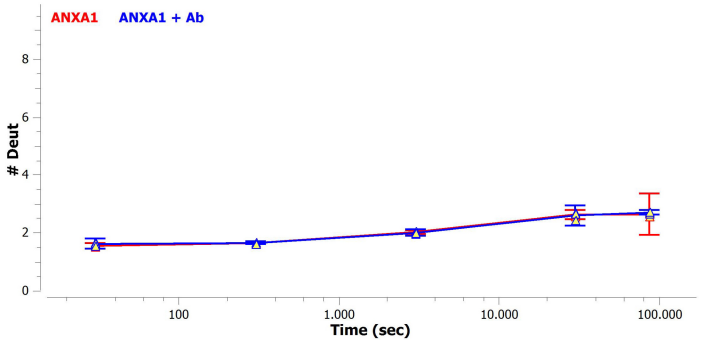
138-142: LIEIL (#89)



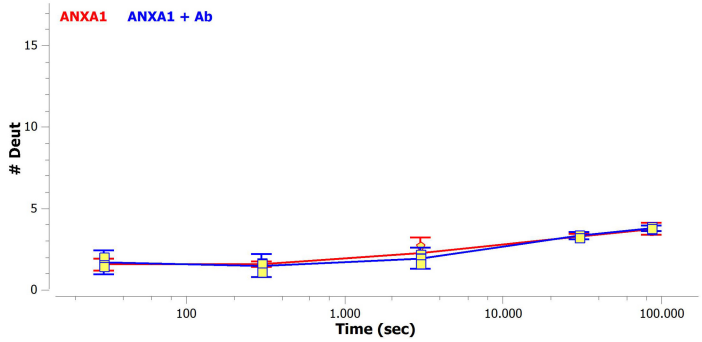
139-142: IEIL (#90)



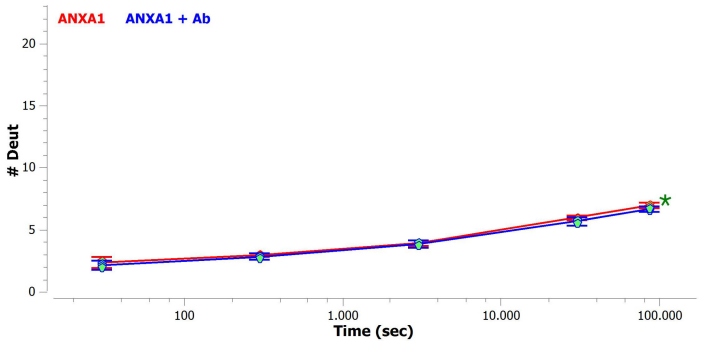
139-149: IEILASRTNKE (#91)



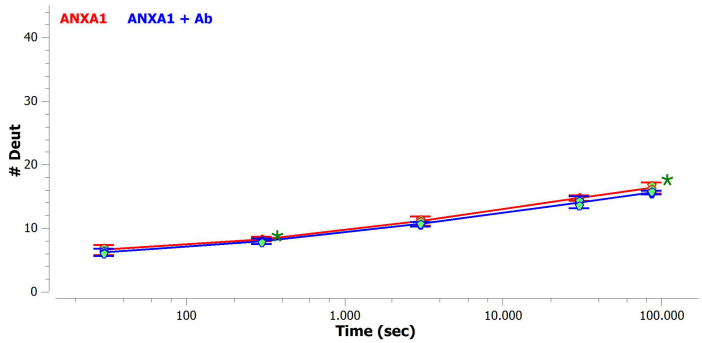
139-156: IEILASRTNKEIRDINRV (#92)



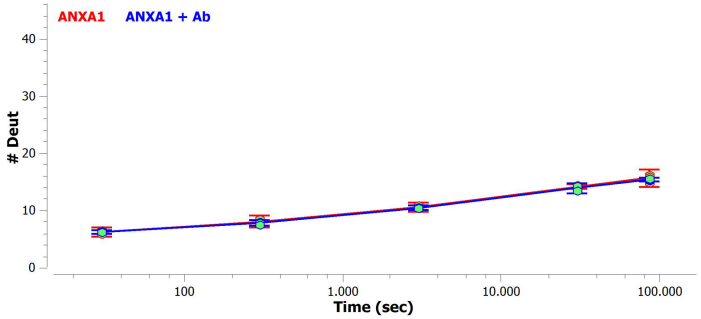
139-161: IEILASRTNKEIRDINRVYREEL (#93)



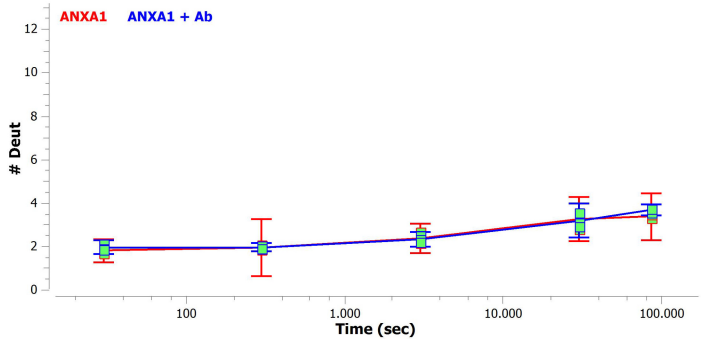
139-181: IEILASRTNKEIRDINRVYREELKRDLAKDITSDTSGDFRNAL (#94)



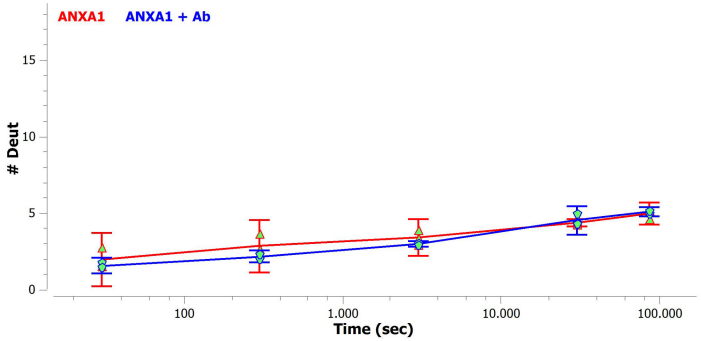
139-182: IEILASRTNKEIRDINRVYREELKRDLAKDITSDTSGDFRNALL (#95)



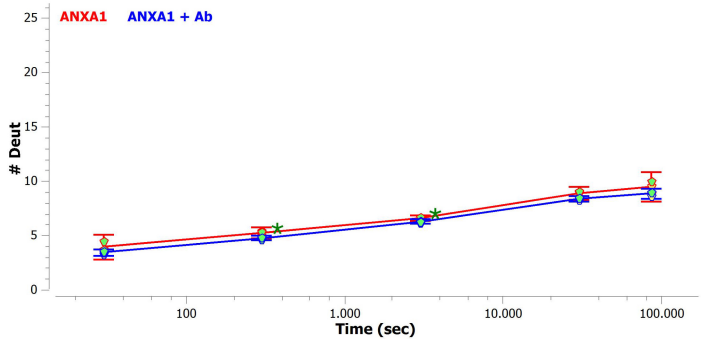
143-156: ASRTNKEIRDINRV (#96)



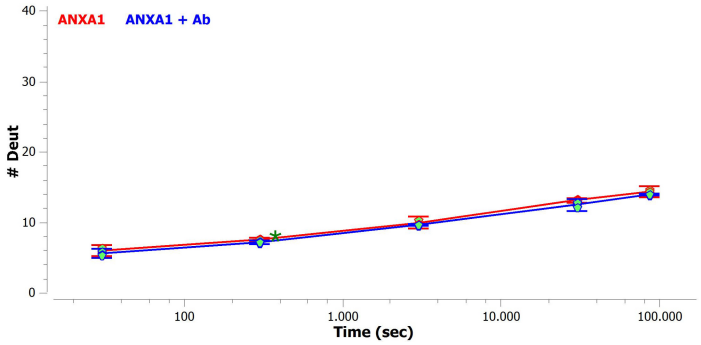
143-161: ASRTNKEIRDINRVYREEL (#97)



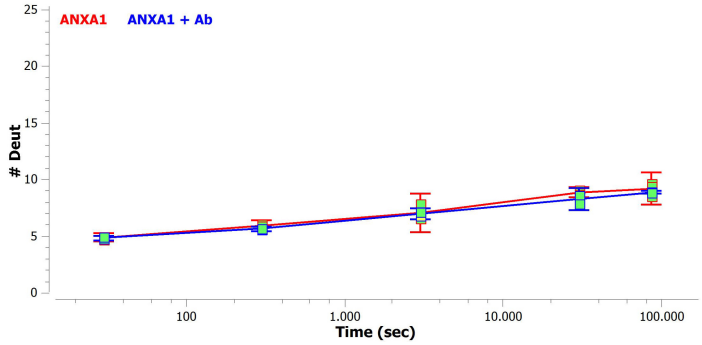
143-168: ASRTNKEIRDINRVYREELKRDLAKD (#98)



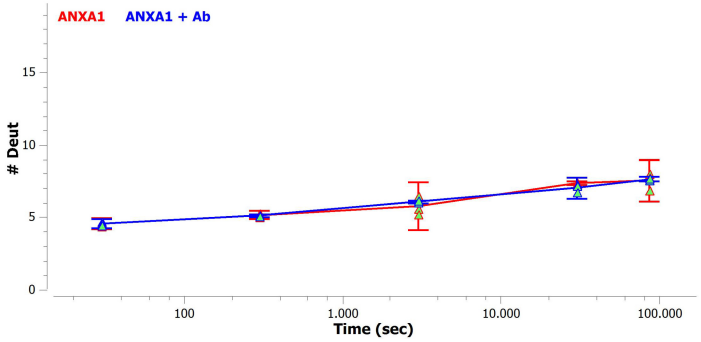
143-181: ASRTNKEIRDINRVYREELKRDLAKDITSDTSGDFRNAL (#99)



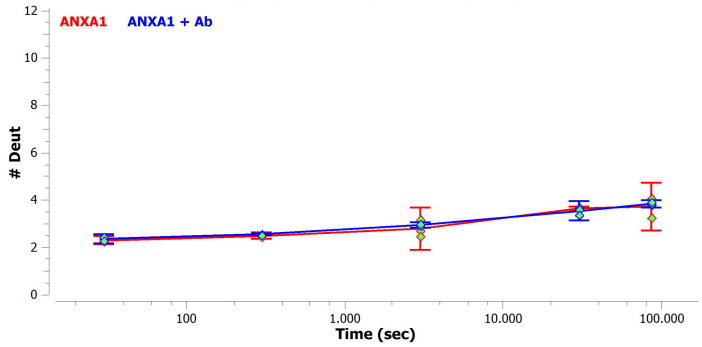
157-181: YREELKRDLAKDITSDTSGDFRNAL (#100)



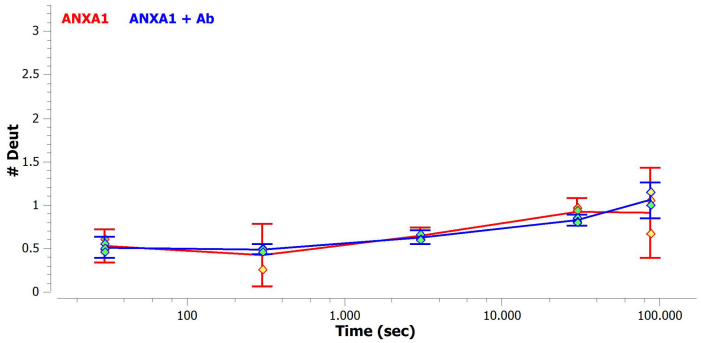
162-181: KRDLAKDITSDTSGDFRNAL (#101)



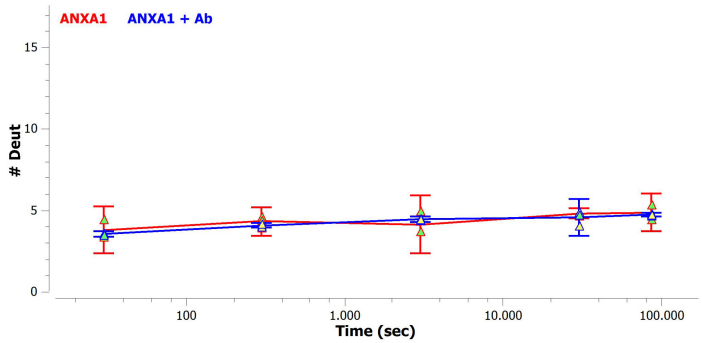
169-181: ITSDTSGDFRNAL (#102)



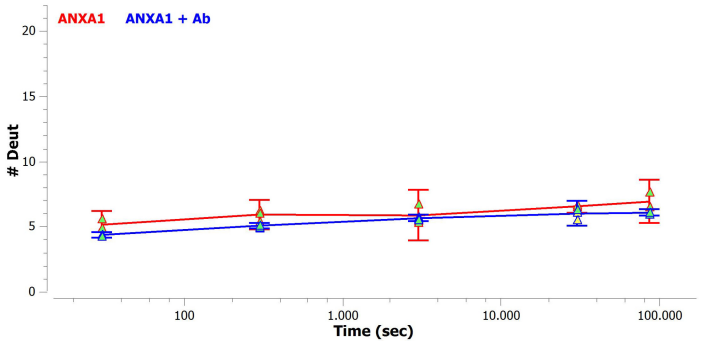
177-181: FRNAL (#103)



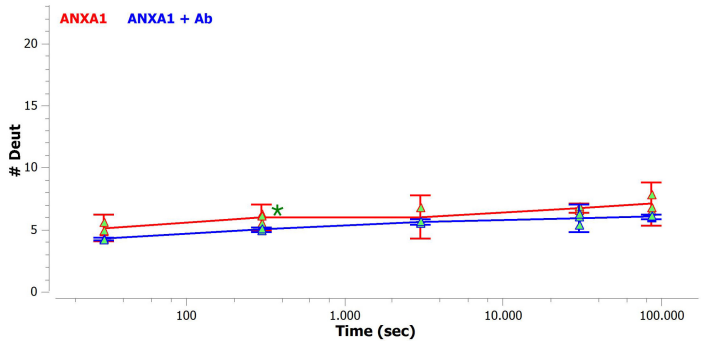
182-199: LSLAKGDRSEDFGVNEDL (#104)



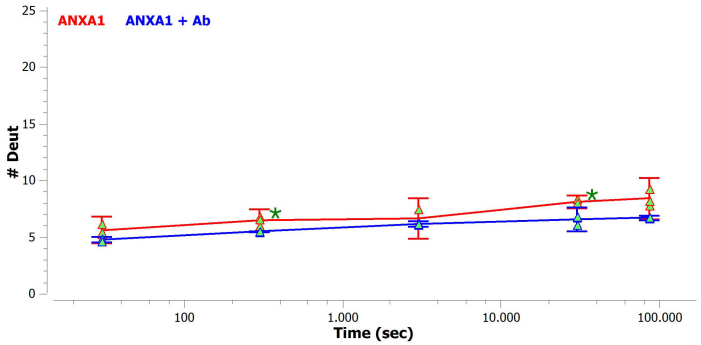
182-203: LSLAKGDRSEDFGVNEDLADSD (#105)



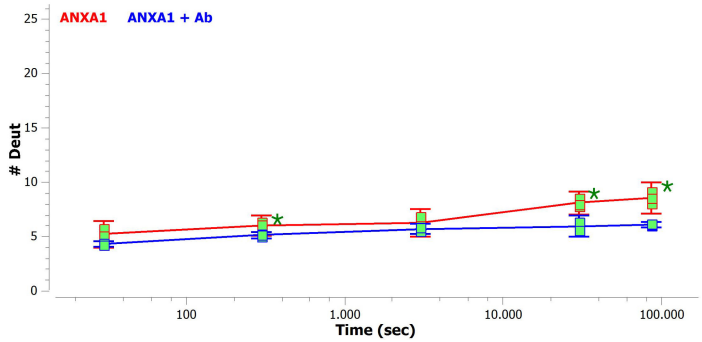
182-204: LSLAKGDRSEDFGVNEDLADSDA (#106)



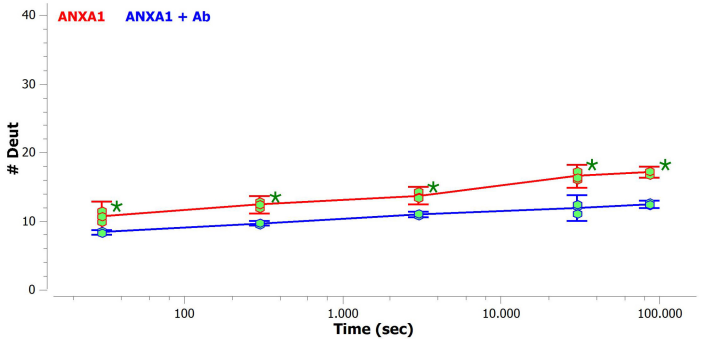
182-206: LSLAKGDRSEDFGVNEDLADSDARA (#107)



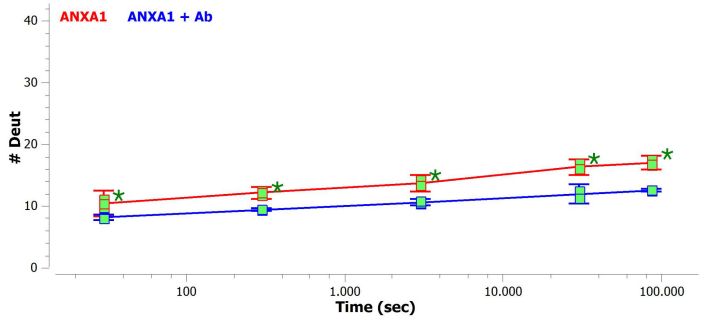
182-207: LSLAKGDRSEDFGVNEDLADSDARAL (#108)



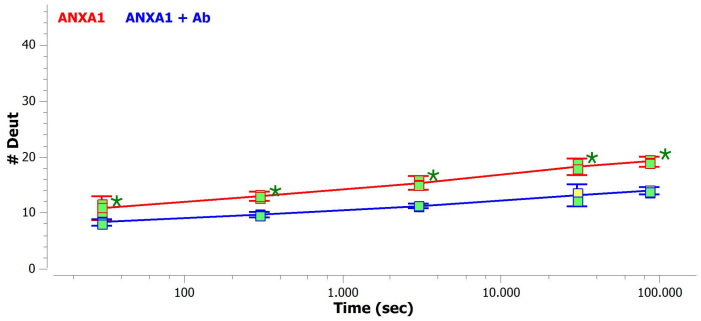
182-221: LSLAKGDRSEDFGVNEDLADSDARALYEAGERRKGTDVNV (#109)



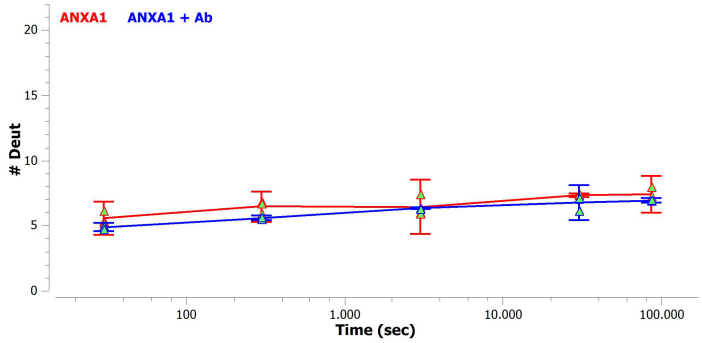
182-222: LSLAKGDRSEDFGVNEDLADSDARALYEAGERRKGTDVNVF (#110)



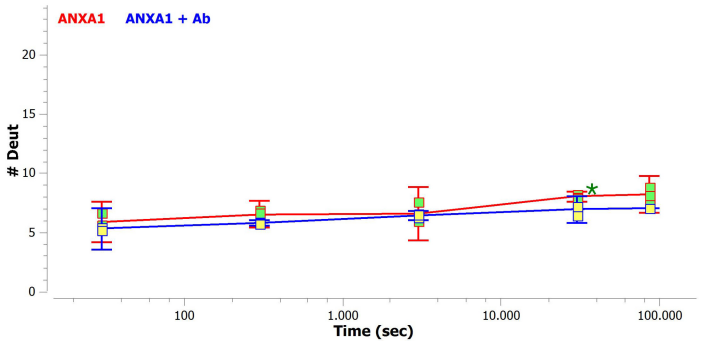
182-226: LSLAKGDRSEDFGVNEDLADSDARALYEAGERRKGTDVNVFNTIL (#111)



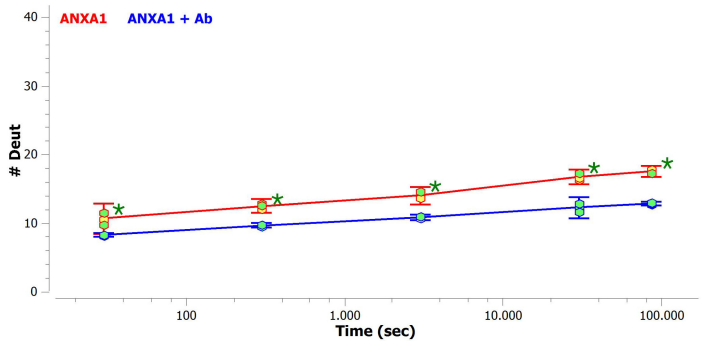
183-204: SLAKGDRSEDFGVNEDLADSDA (#112)



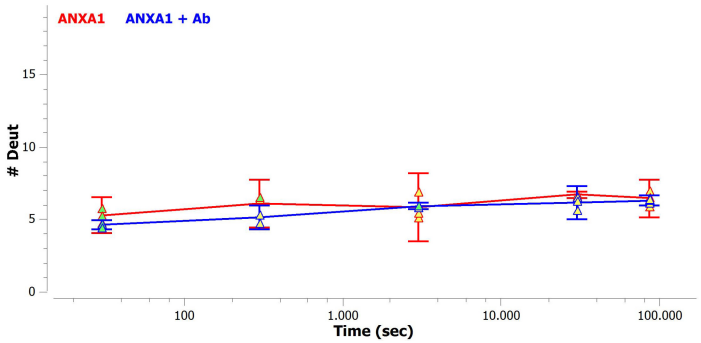
183-206: SLAKGDRSEDFGVNEDLADSDARA (#113)



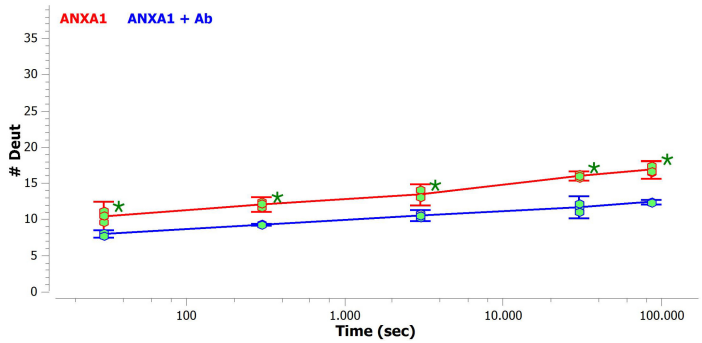
183-222: SLAKGDRSEDFGVNEDLADSDARALYEAGERRKGTDVNVF (#114)



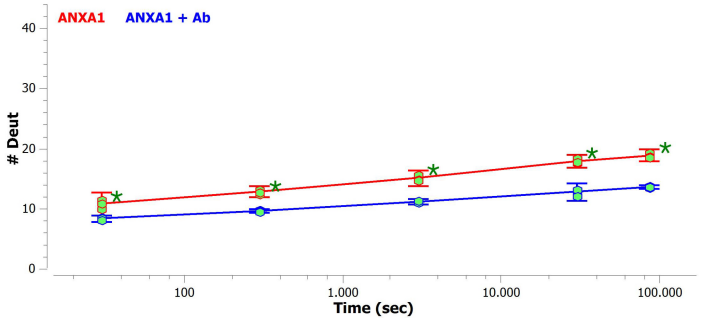
185-204: AKGDRSEDFGVNEDLADSDA (#115)



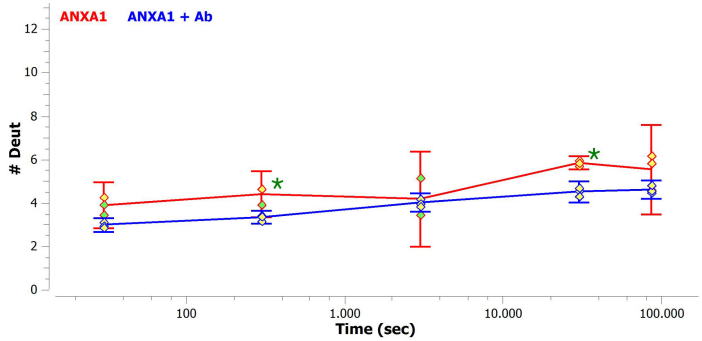
185-222: AKGDRSEDFGVNEDLADSDARALYEAGERRKGTDVNVF (#116)



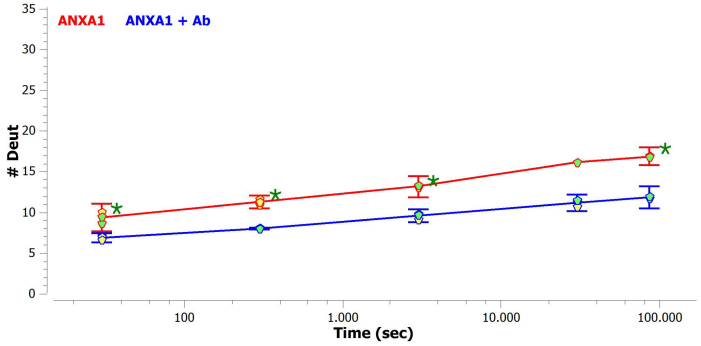
185-226: AKGDRSEDFGVNEDLADSDARALYEAGERRKGTDVNVFNTIL (#117)



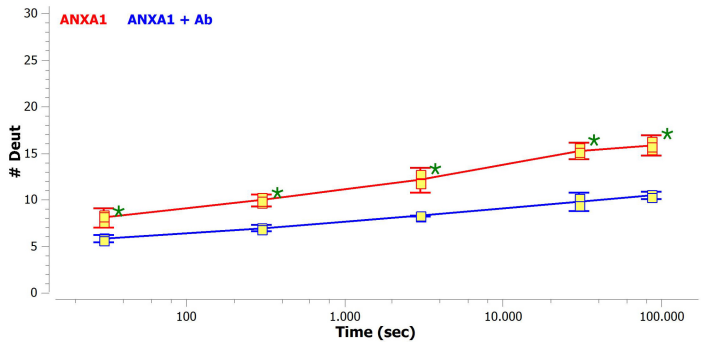
193-206: FGVNEDLADSDARA (#118)



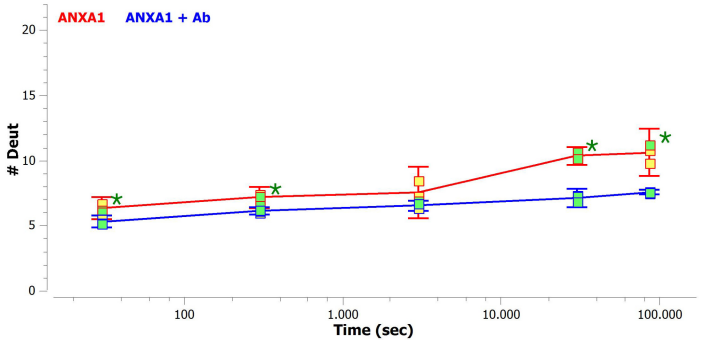
193-226: FGVNEDLADSDARALYEAGERRKGTDVNVFNTIL (#119)



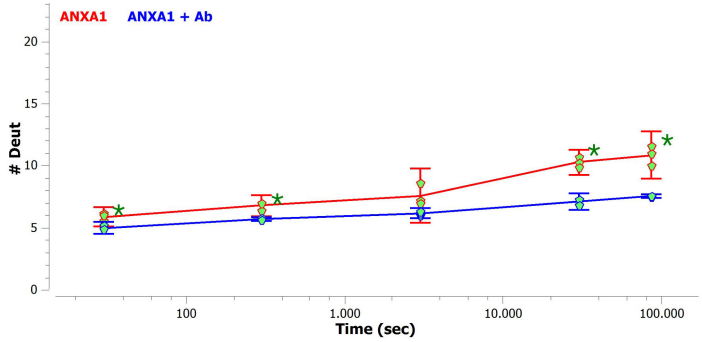
197-226: EDLADSDARALYEAGERRKGTDVNVFNTIL (#120)



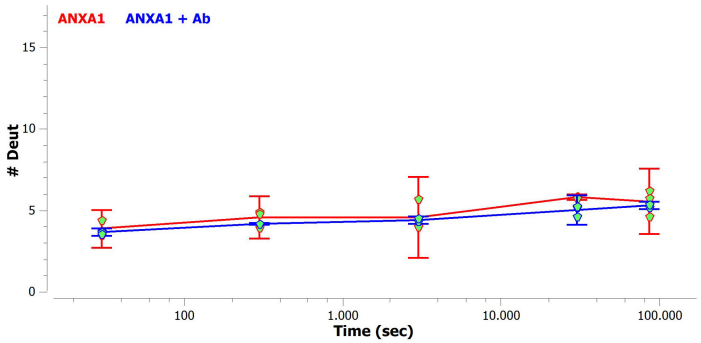
200-221: ADSDARALYEAGERRKGTDVNV (#121)



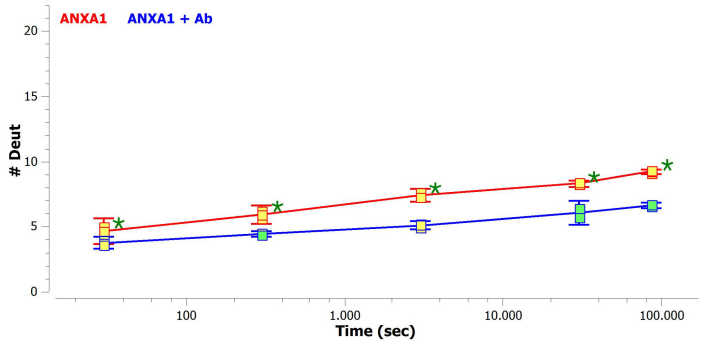
200-222: ADSDARALYEAGERRKGTDVNVF (#122)



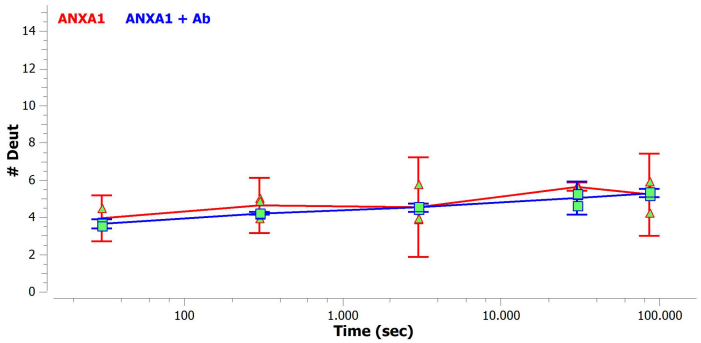
205-222: RALYEAGERRKGTDVNVF (#123)



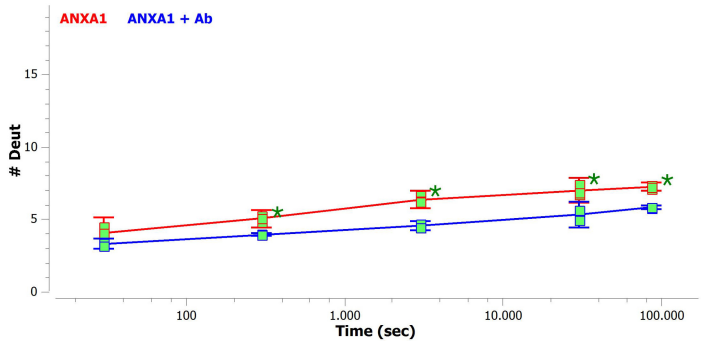
205-226: RALYEAGERRKGTDVNVFNTIL (#124)



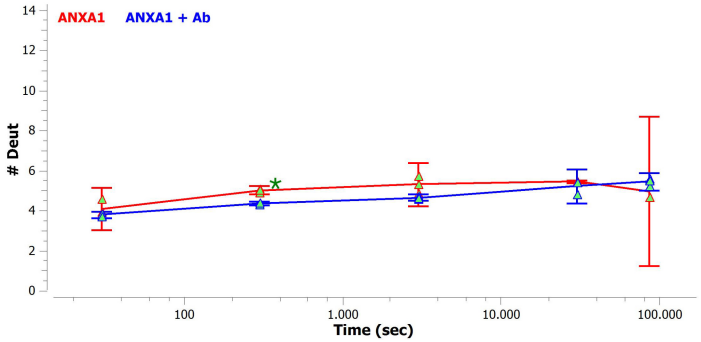
207-222: LYEAGERRKGTDVNVF (#125)



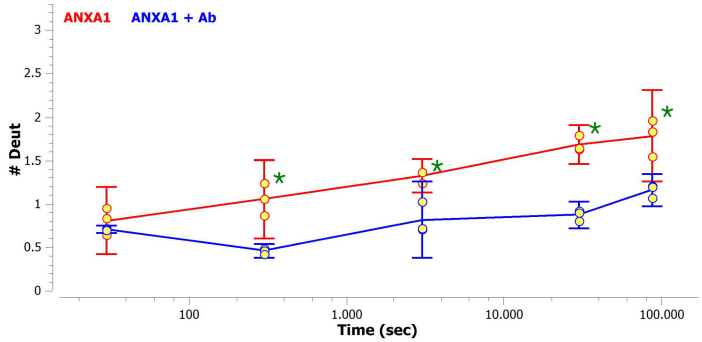
207-226: LYEAGERRKGTDVNVFNTIL (#126)



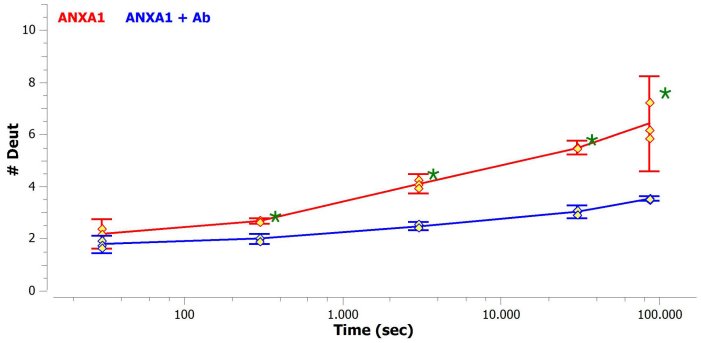
208-222: YEAGERRKGTDVNVF (#127)



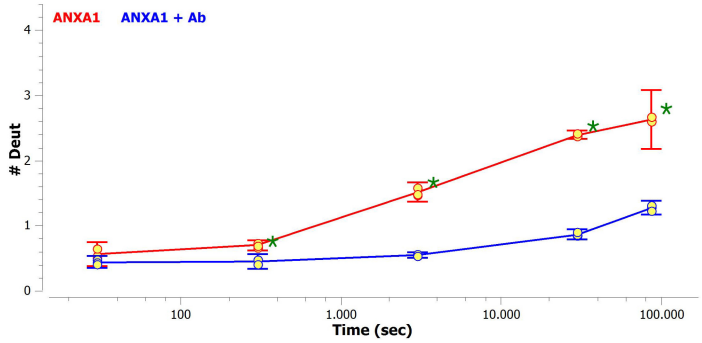
222-226: FNTIL (#128)



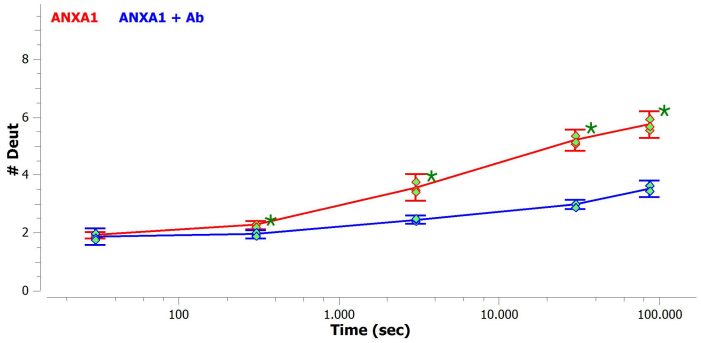
222-234: FNTILTTRSYPQL (#129)



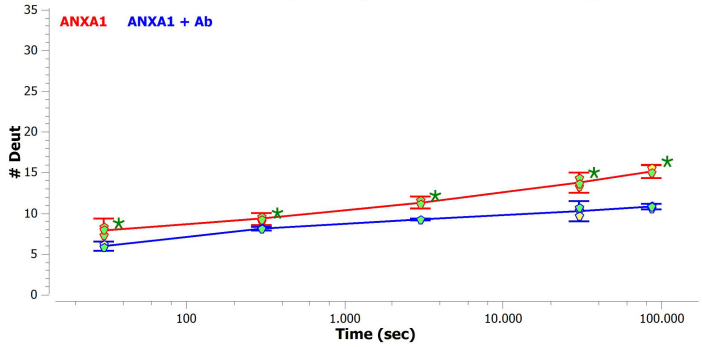
223-228: NTILTT (#130)



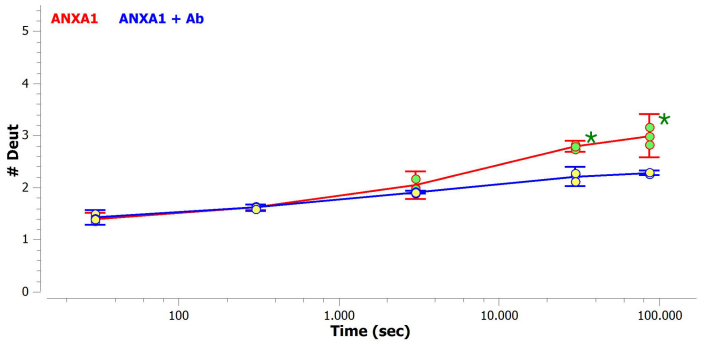
223-234: NTILTTRSYPQL (#131)



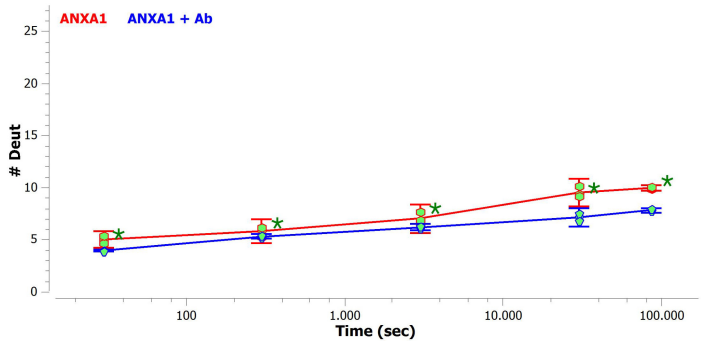
223-257: NTILTTRSYPQLRRVFQKYTKYSKHDMNKVLDLEL (#132)



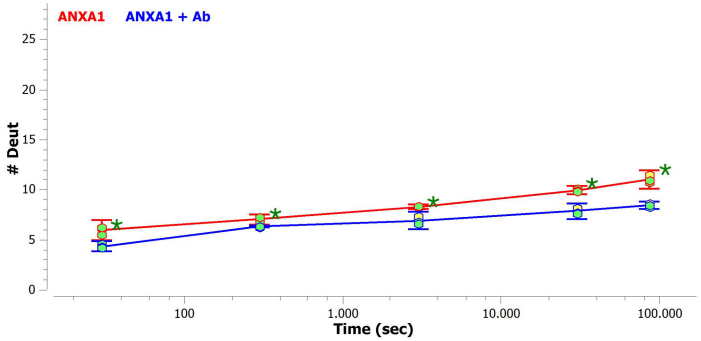
227-234: TTRSYPQL (#133)



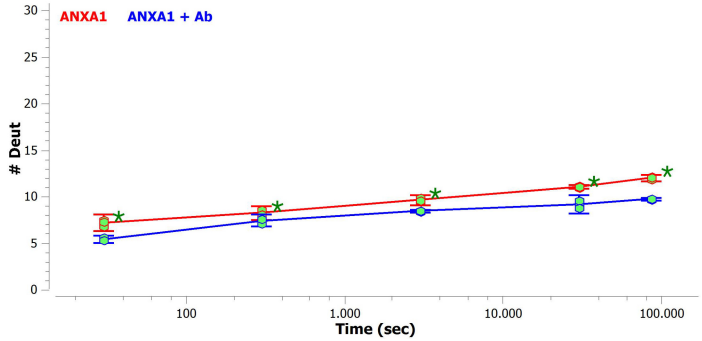
227-254: TTRSYPQLRRVFQKYTKYSKHDMNKVLD (#134)



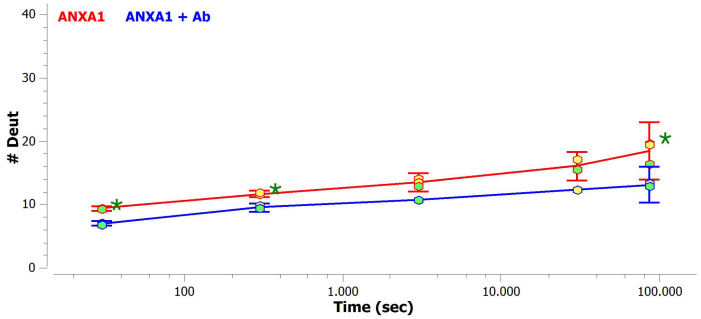
227-255: TTRSYPQLRRVFQKYTKYSKHDMNKVLDL (#135)



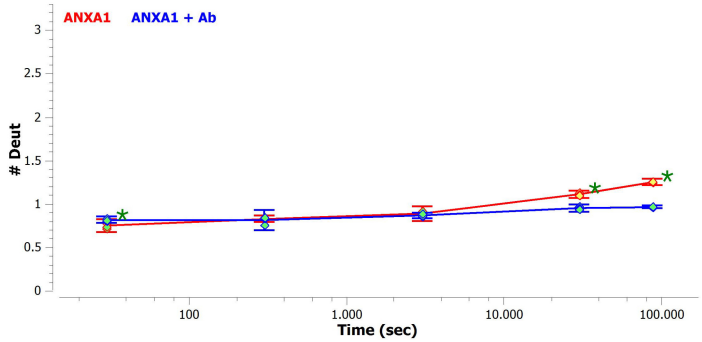
227-257: TTRSYPQLRRVFQKYTKYSKHDMNKVLDLEL (#136)



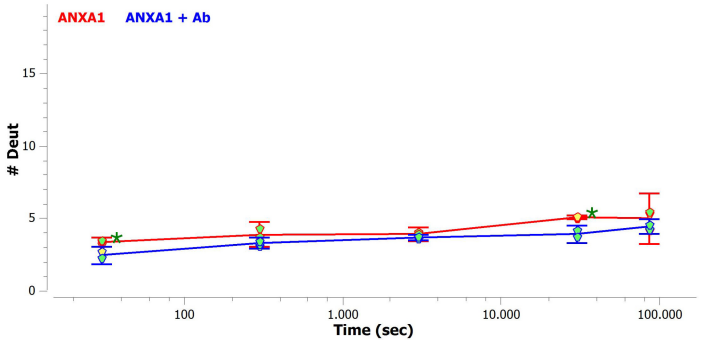
227-267: TTRSYPQLRRVFQKYTKYSKHDMNKVLDLELKGDIEKCLTA (#137)



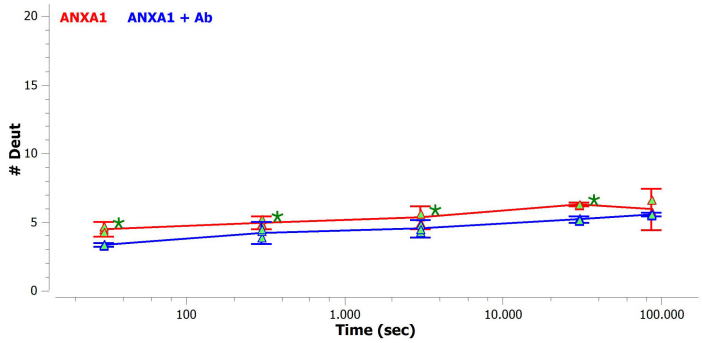
229-234: RSYPQL (#138)

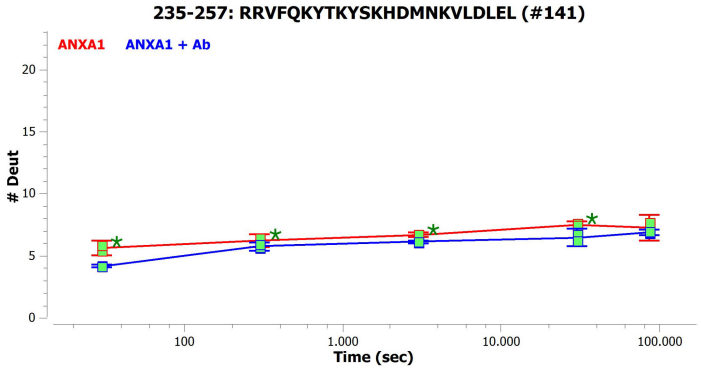


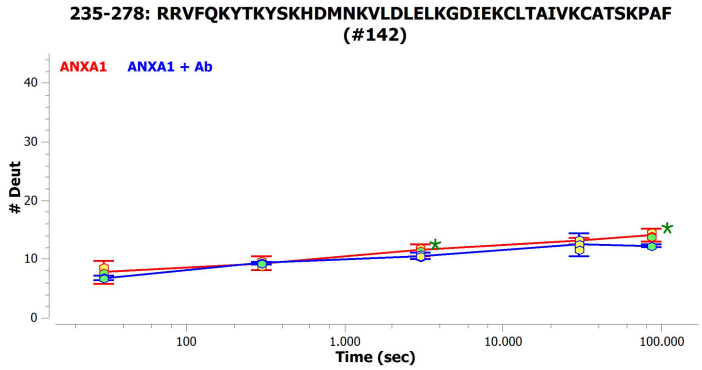
235-254: RRVFQKYTKYSKHDMNKVLD (#139)

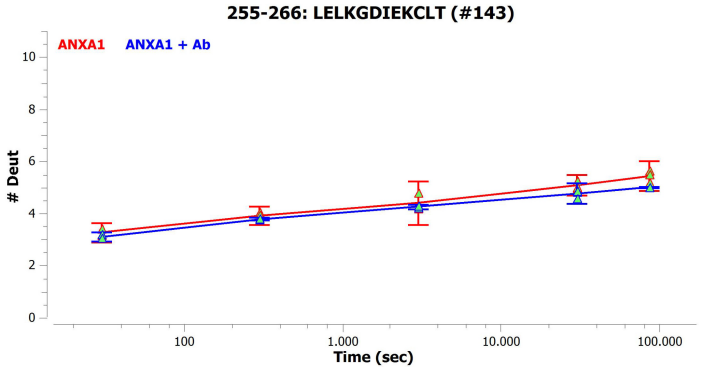


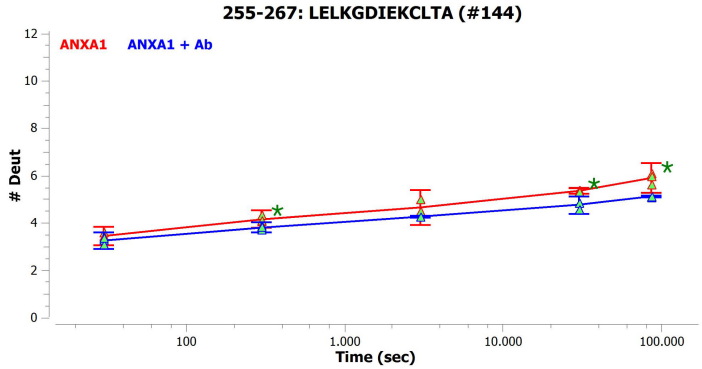
235-255: RRVFQKYTKYSKHDMNKVLDL (#140)

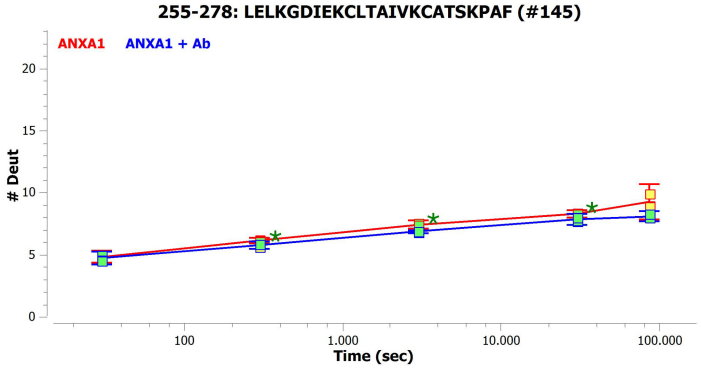


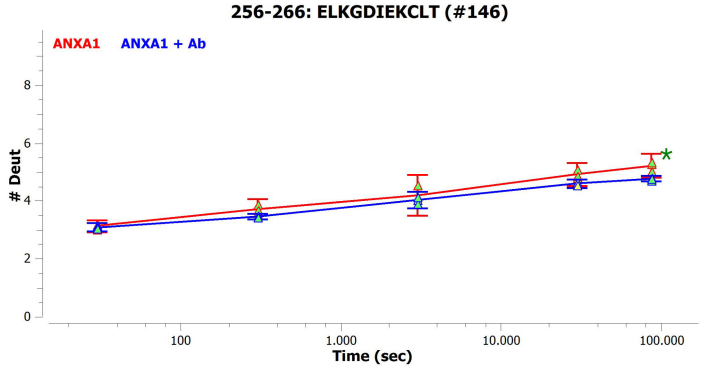

235-257: RRVFQKYTKYSKHDMNKVLDLEL (#141)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

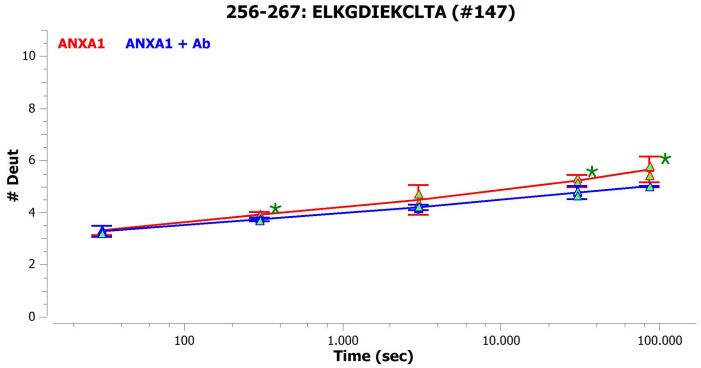

235-278: RRVFQKYTKYSKHDMNKVLDLELKGDIEKCLTAIVKCATSKPAF (#142)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

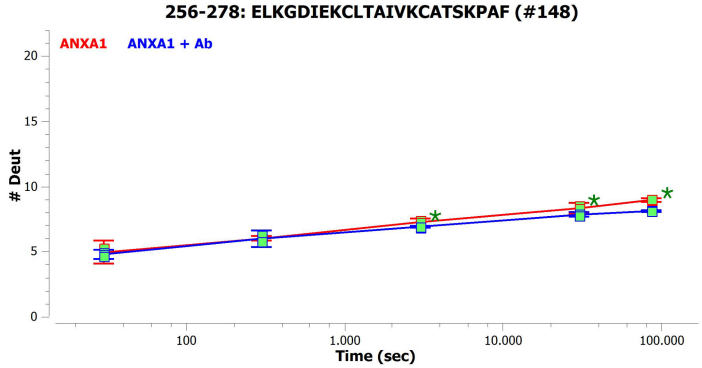

255-266: LELKGDIEKCLT (#143)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

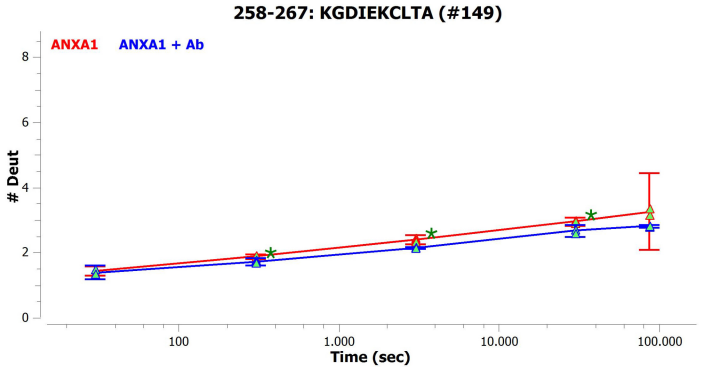

255-267: LELKGDIEKCLTA (#144)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

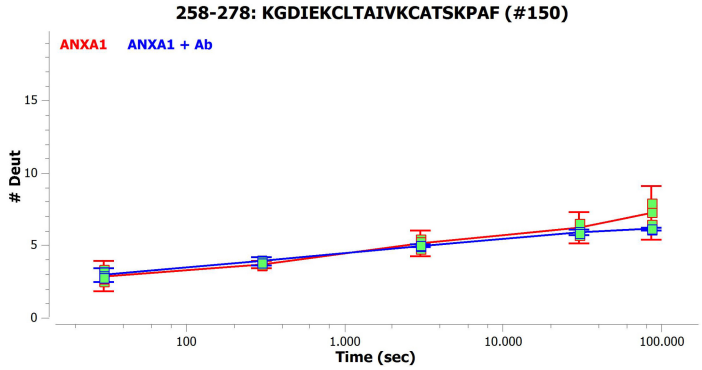

255-278: LELKGDIEKCLTAIVKCATSKPAF (#145)
ANXA1
ANXA1 + Ab
Deut
Time (sec)


256-266: ELKGDIEKCLT (#146)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

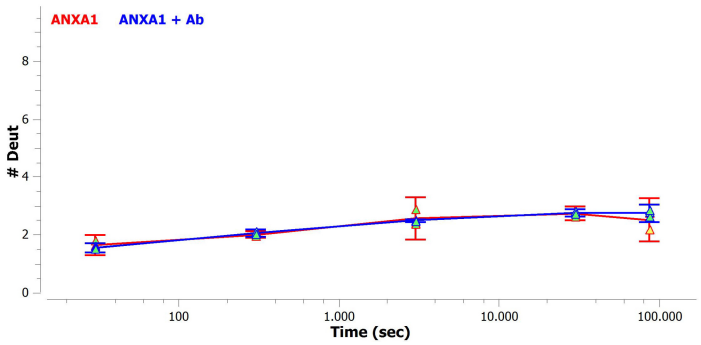

256-267: ELKGDIEKCLTA (#147)
ANXA1
ANXA1 + Ab
Deut
Time (sec)


256-278: ELKGDIEKCLTAIVKCATSKPAF (#148)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

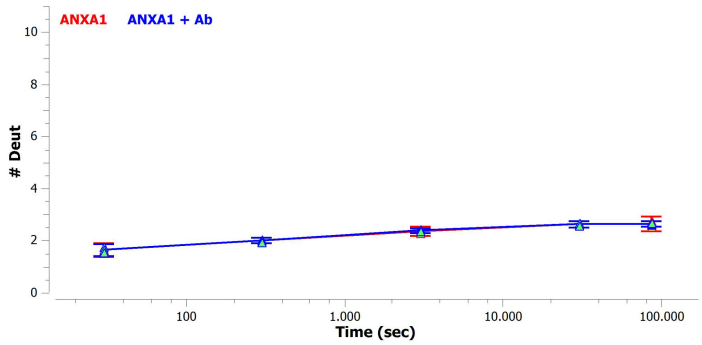

258-267: KGDIEKCLTA (#149)
ANXA1
ANXA1 + Ab
Deut
Time (sec)


258-278: KGDIEKCLTAIVKCATSKPAF (#150)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

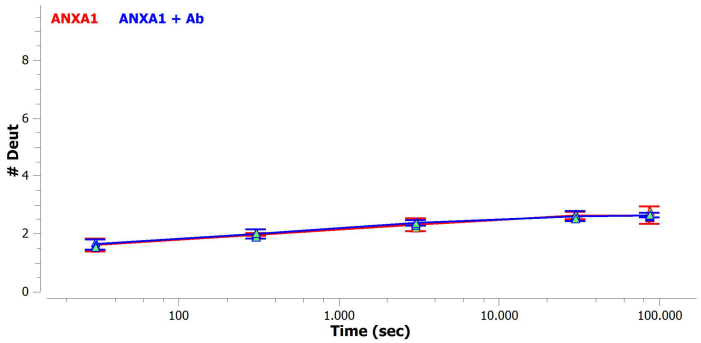
267-278: AIVKCATSKPAF (#151)



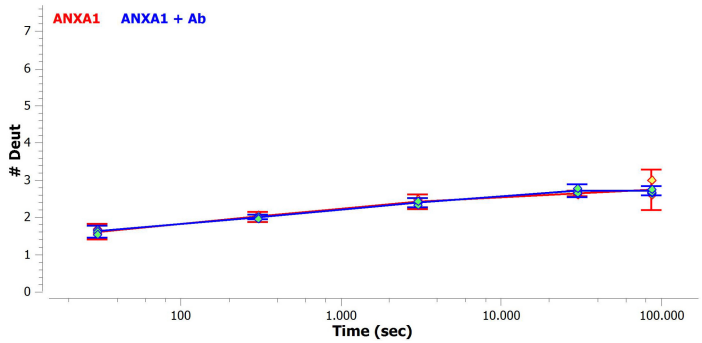
267-279: AIVKCATSKPAFF (#152)



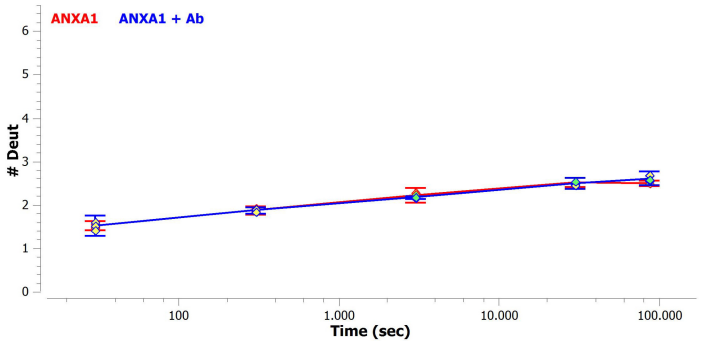
268-279: IVKCATSKPAFF (#153)



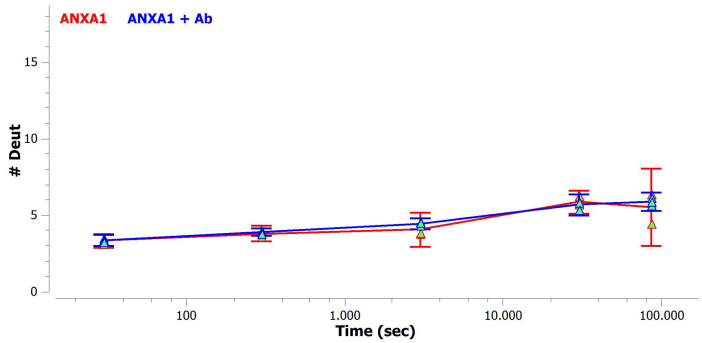
269-278: VKCATSKPAF (#154)



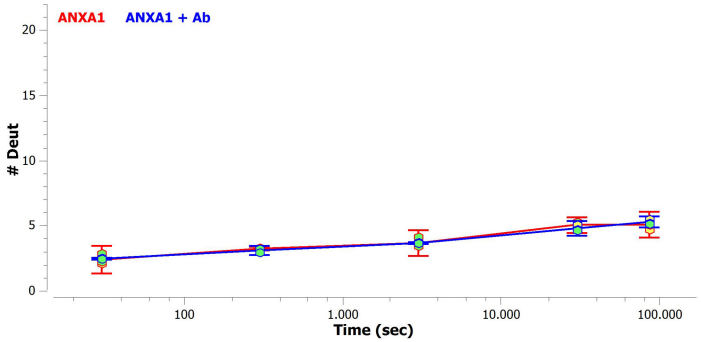
270-278: KCATSKPAF (#155)



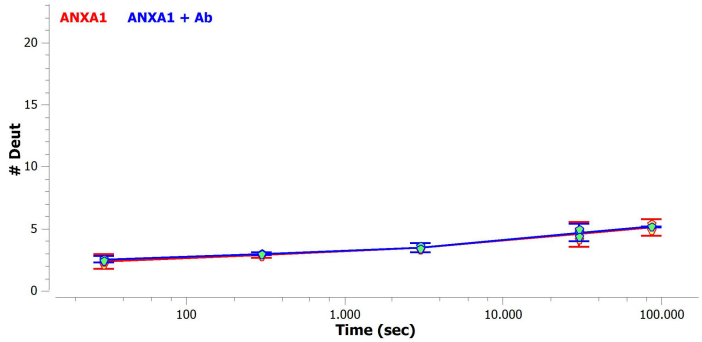
279-297: FAEKLHQAMKGVGTRHKAL (#156)



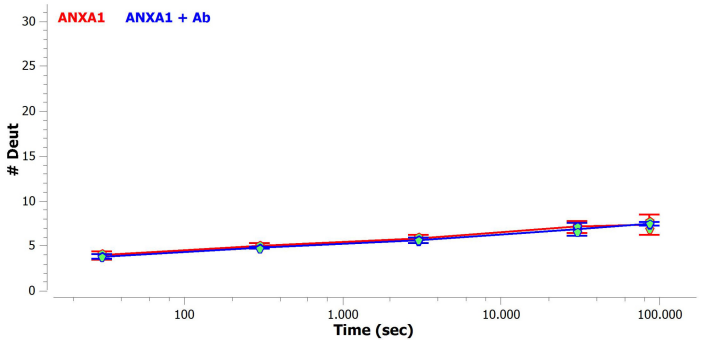
279-300: FAEKLHQAMKGVGTRHKALIRI (#157)



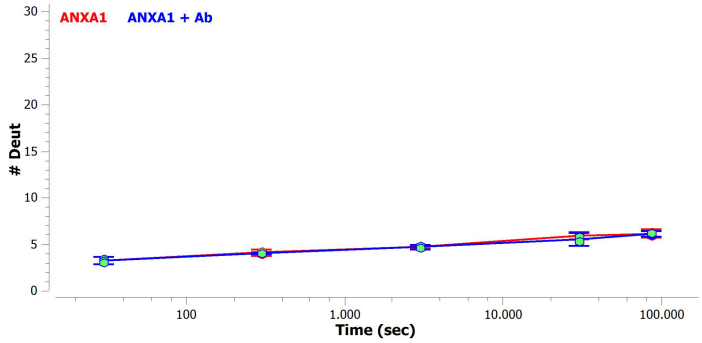
279-301: FAEKLHQAMKGVGTRHKALIRIM (#158)

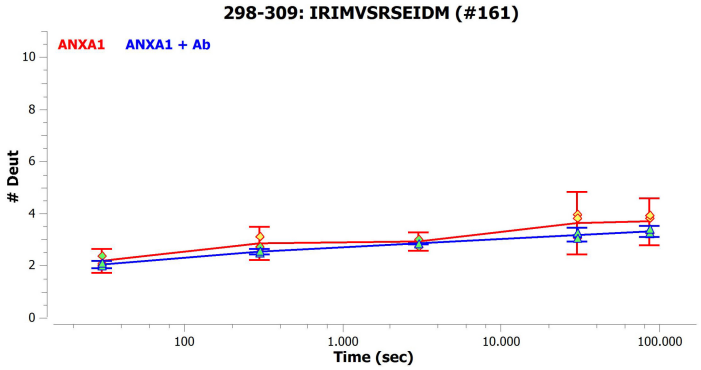


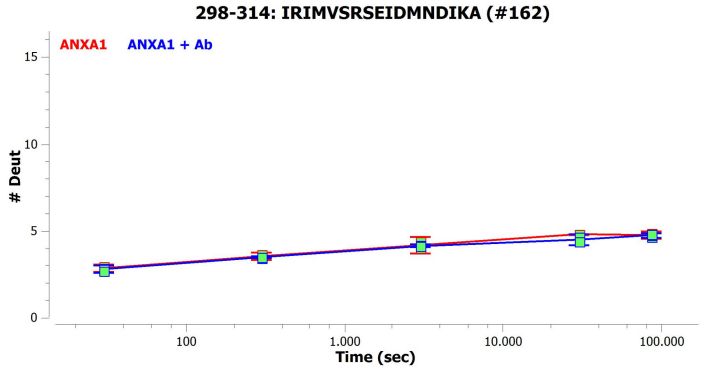
279-309: FAEKLHQAMKGVGTRHKALIRIMVSRSEIDM (#159)

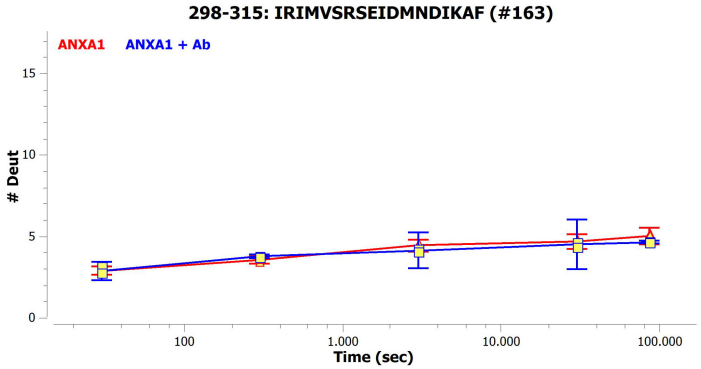


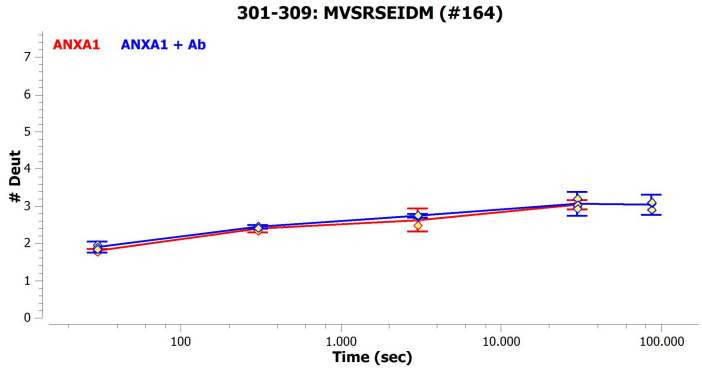
280-309: AEKLHQAMKGVGTRHKALIRIMVSRSEIDM (#160)

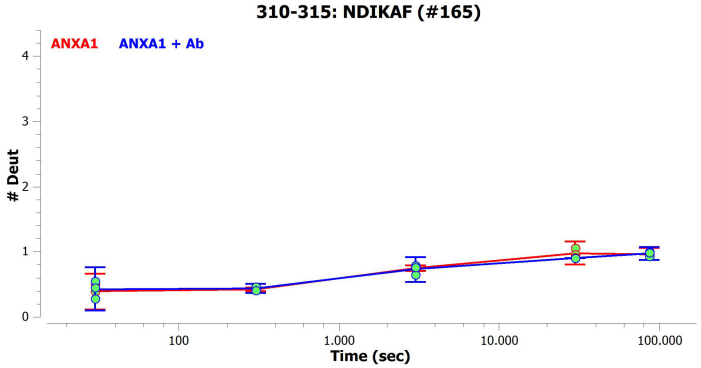


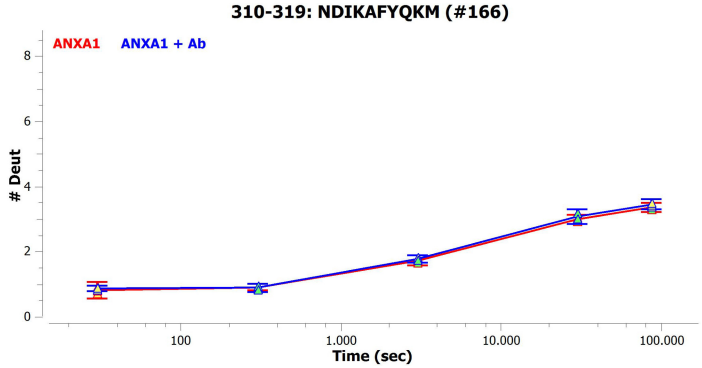

298-309: IRIMVSRSEIDM (#161)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

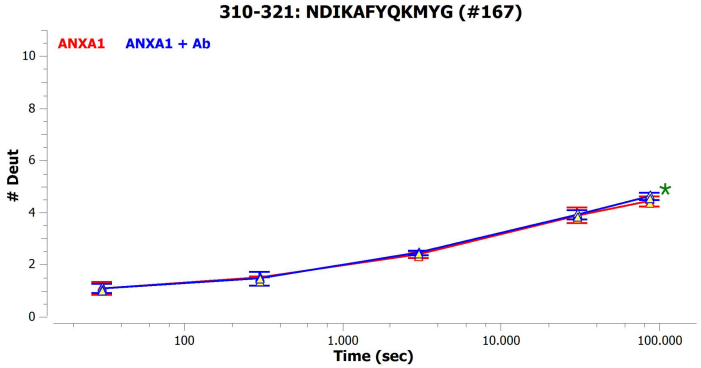

298-314: IRIMVSRSEIDMNDIKA (#162)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

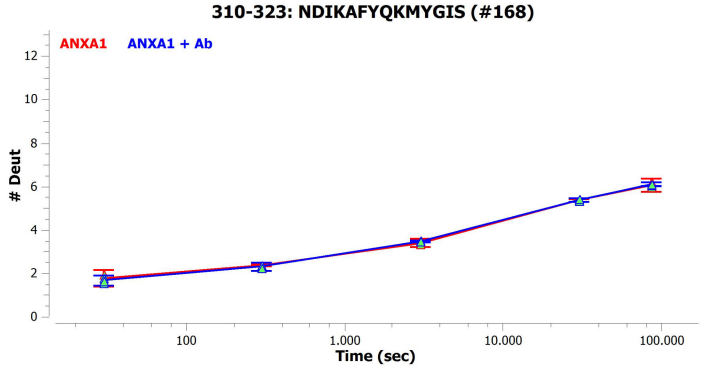

298-315: IRIMVSRSEIDMNDIKAF (#163)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

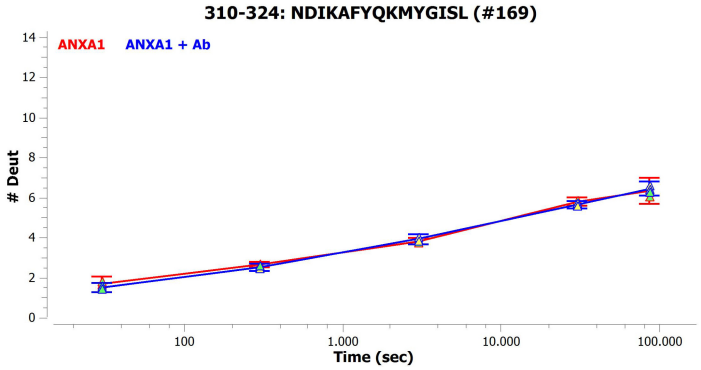

301-309: MVSRSEIDM (#164)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

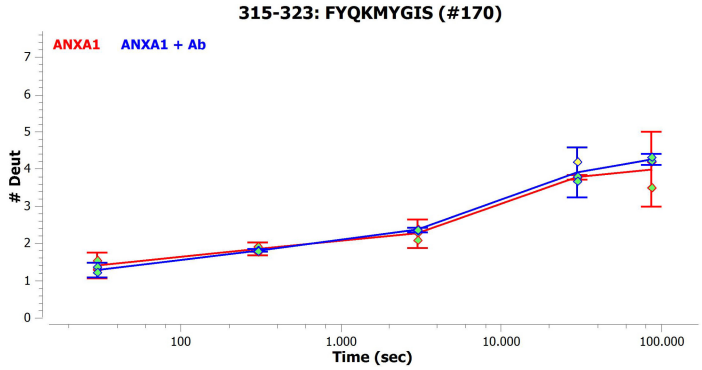

310-315: NDIKAF (#165)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

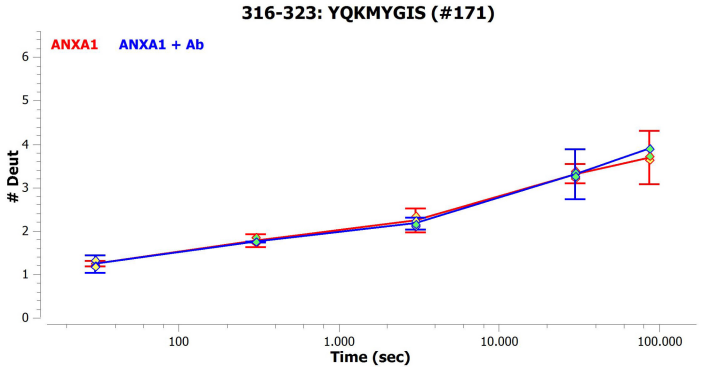

310-319: NDIKAFYQKM (#166)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

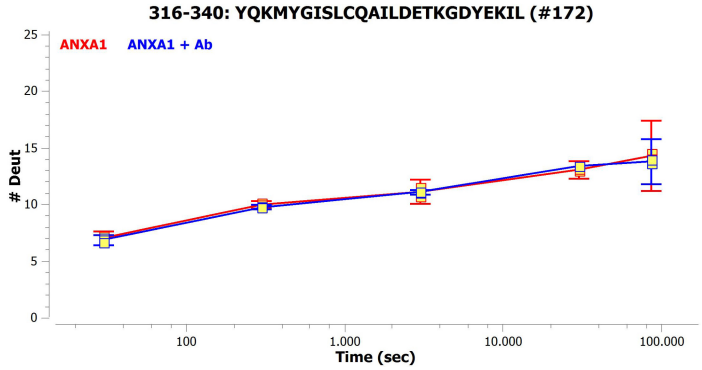

310-321: NDIKAFYQKMYG (#167)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

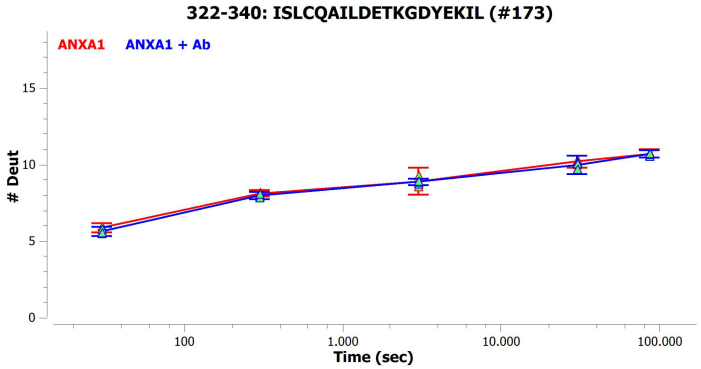

310-323: NDIKAFYQKMYGIS (#168)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

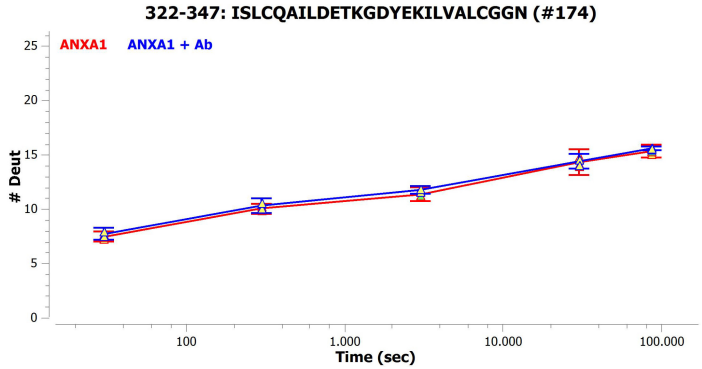

310-324: NDIKAFYQKMYGISL (#169)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

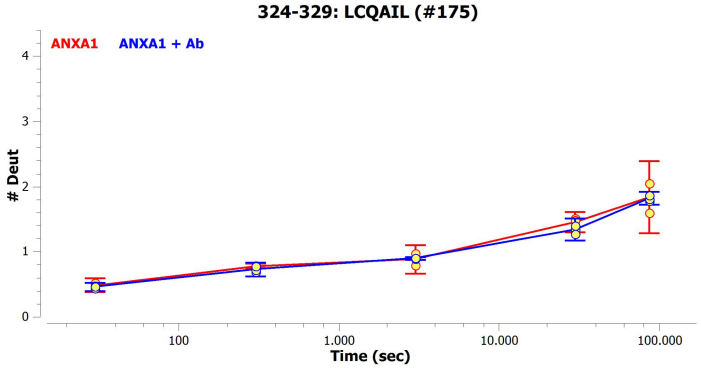

315-323: FYQKMYGIS (#170)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

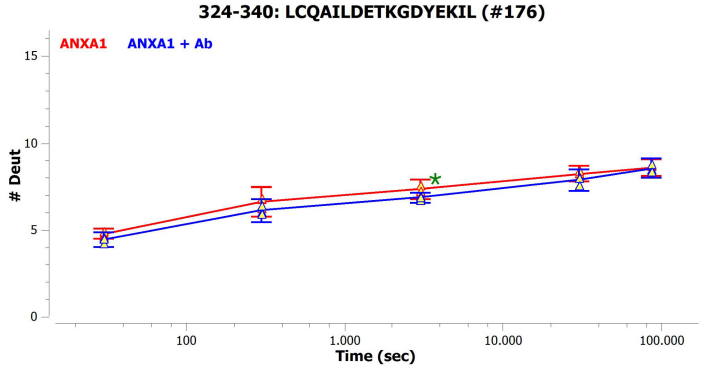

316-323: YQKMYGIS (#171)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

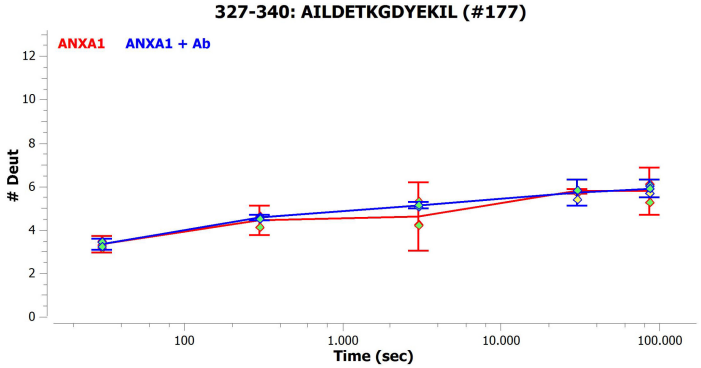

316-340: YQKMYGISLCQAILDETKGDYEKIL (#172)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

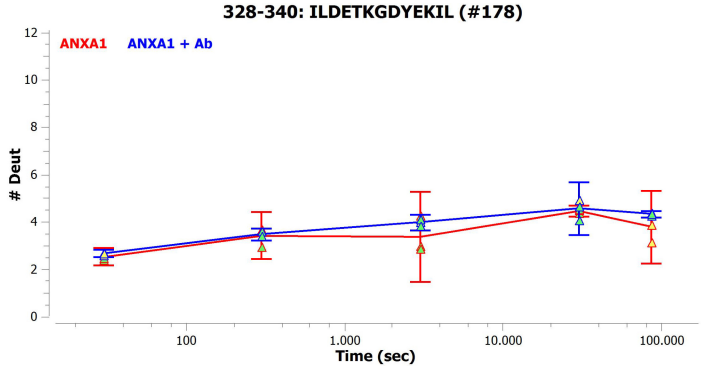

322-340: ISLCQAILDETKGDYEKIL (#173)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

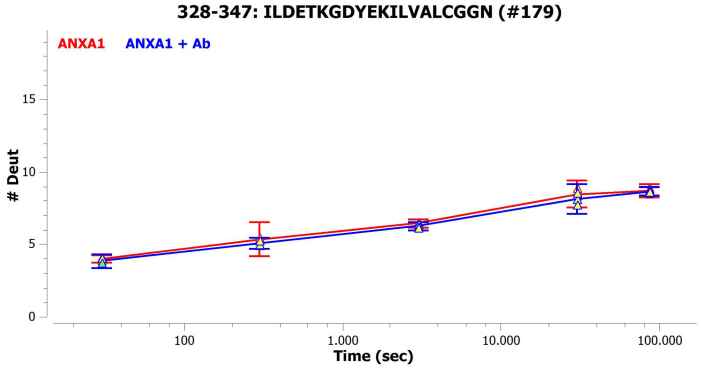

322-347: ISLCQAILDETKGDYEKILVALCGGN (#174)
ANXA1
ANXA1 + Ab
Deut
Time (sec)


324-329: LCQAIL (#175)
ANXA1
ANXA1 + Ab
Deut
Time (sec)


324-340: LCQAILDETKGDYEKIL (#176)
ANXA1
ANXA1 + Ab
Deut
Time (sec)


327-340: AILDETKGDYEKIL (#177)
ANXA1
ANXA1 + Ab
Deut
Time (sec)


328-340: ILDETKGDYEKIL (#178)
ANXA1
ANXA1 + Ab
Deut
Time (sec)


328-347: ILDETKGDYEKILVALCGGN (#179)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

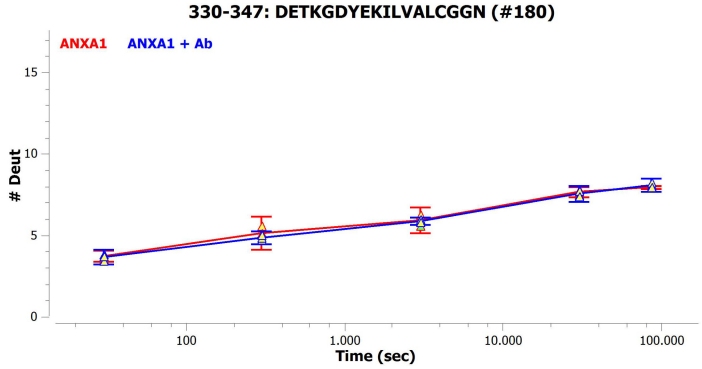
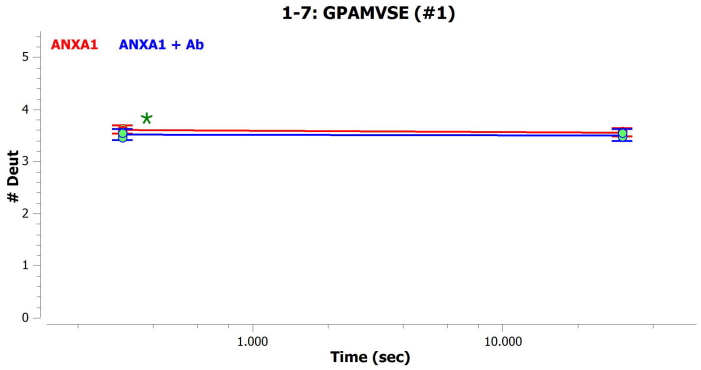
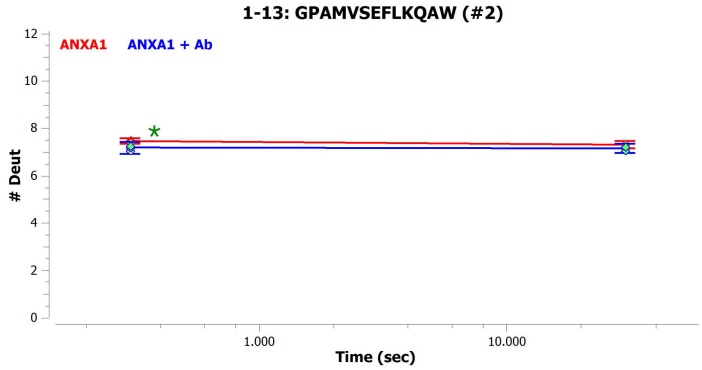
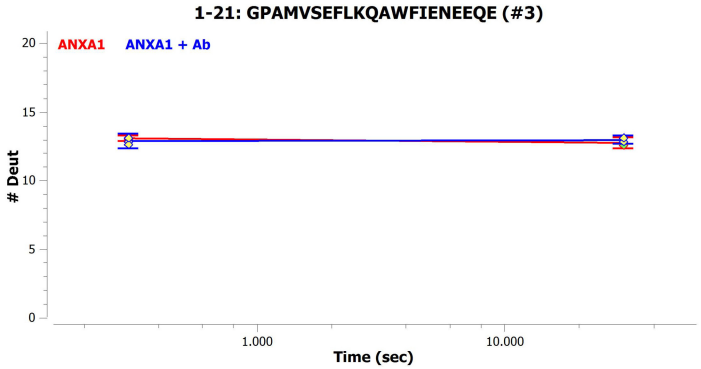
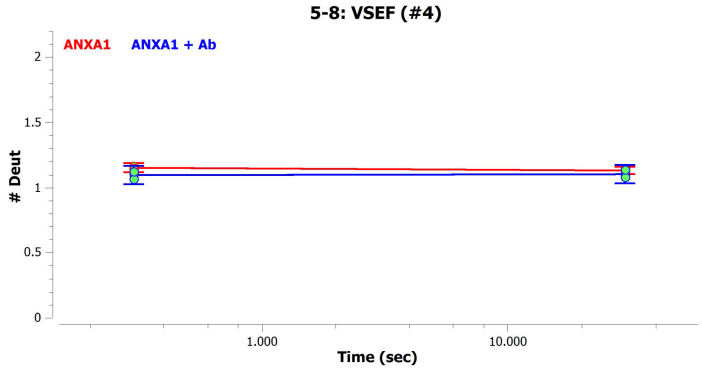

330-347: DETKGDYEKILVALCGGN (#180)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

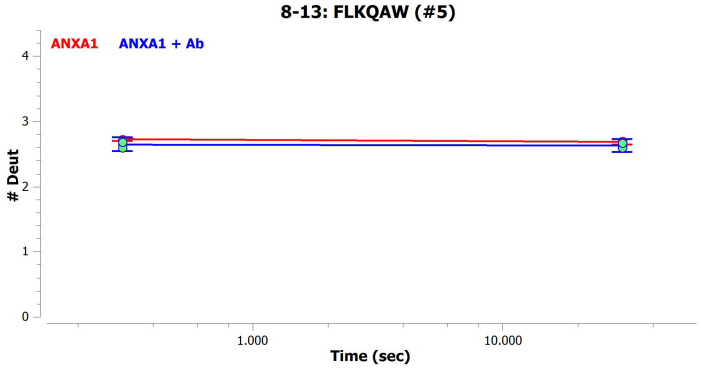
Figure S6: All peptide uptake plots of the **in-solution digest**. used in this publication of the unbound state shown in red and the bound state shown in blue. Time points marked with a star show significant differences ($p<0.05$) between both states determined by students t-test. The Y-Axis scaled to 100% possible exchange of each peptide and the error bars are based on the 95% confidence level.

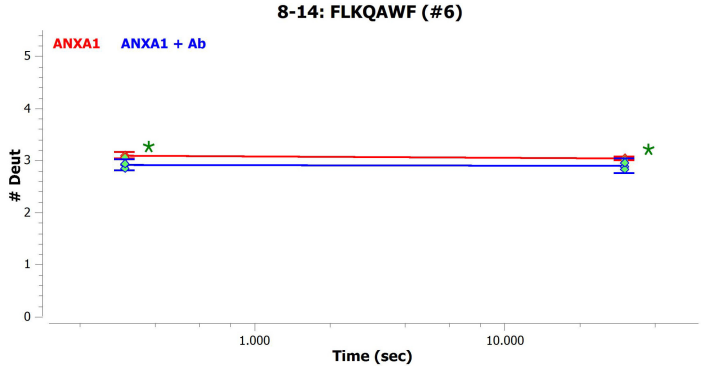

1-7: GPAMVSE (#1)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

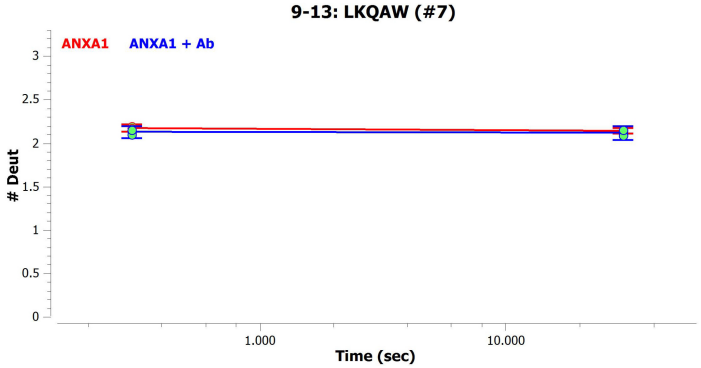

1-13: GPAMVSEFLKQAW (#2)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

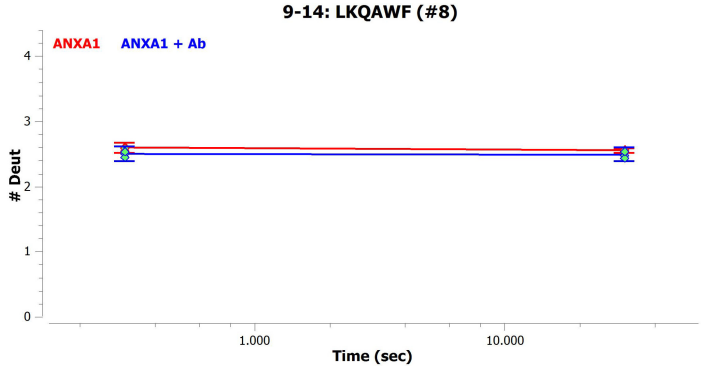

1-21: GPAMVSEFLKQAWFIENEEQE (#3)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

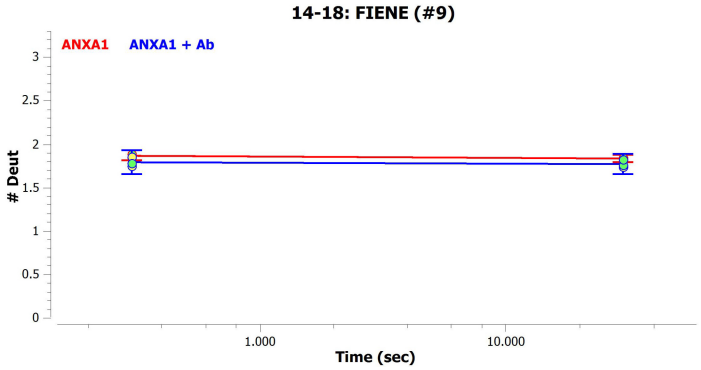

5-8: VSEF (#4)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

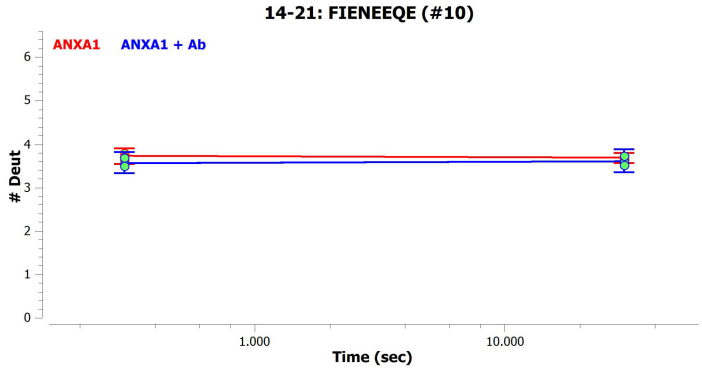

8-13: FLKQAW (#5)
ANXA1
ANXA1 + Ab
Deut
Time (sec)


8-14: FLKQAWF (#6)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

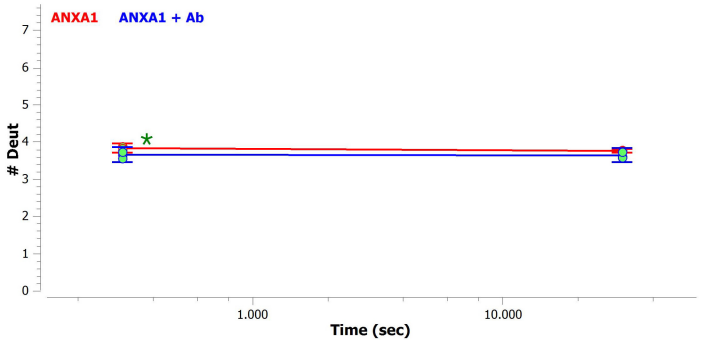

9-13: LKQAW (#7)
ANXA1
ANXA1 + Ab
Deut
Time (sec)


9-14: LKQAWF (#8)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

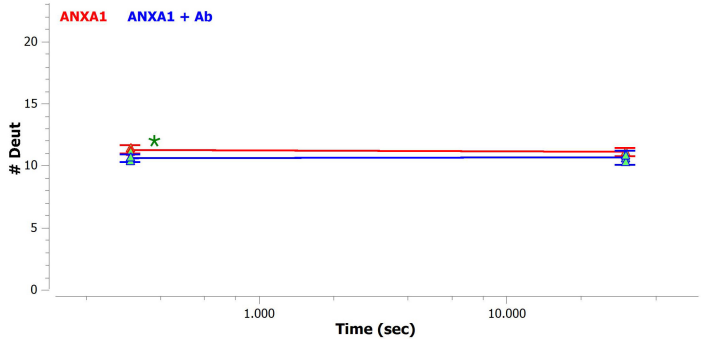

14-18: FIENE (#9)
ANXA1
ANXA1 + Ab
Deut
Time (sec)


14-21: FIENEEQE (#10)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

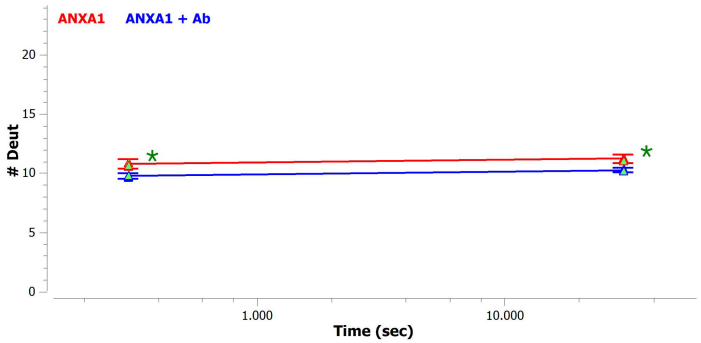
14-22: FIENEEQEY (#11)



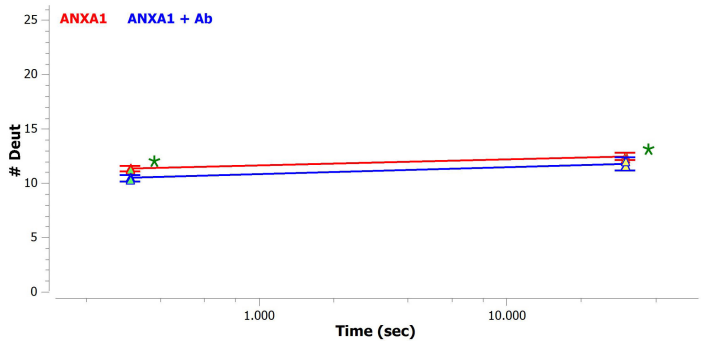
22-48: YVQTVKSSKGGPGSAVSPYPTFNPSSD (#12)



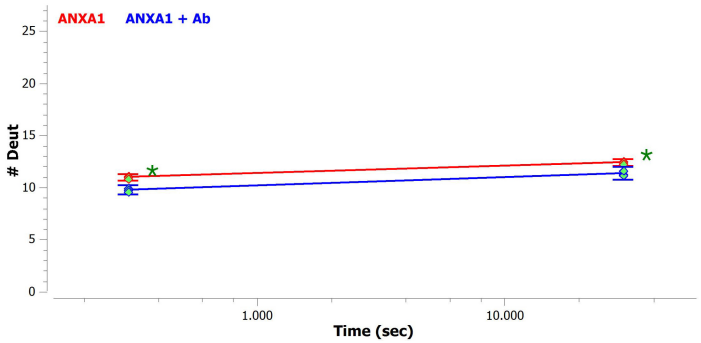
22-49: YVQTVKSSKGGPGSAVSPYPTFNPSSDV (#13)



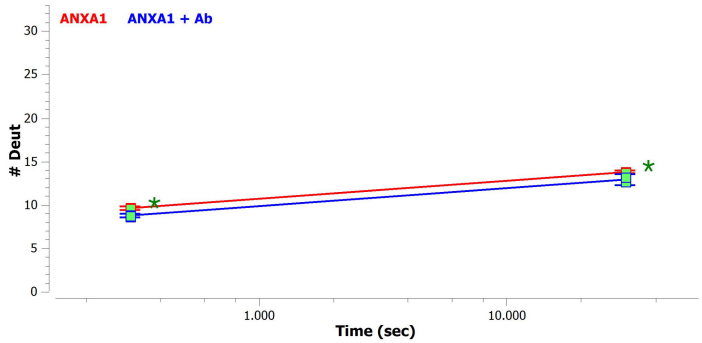
22-51: YVQTVKSSKGGPGSAVSPYPTFNPSSDVAA (#14)



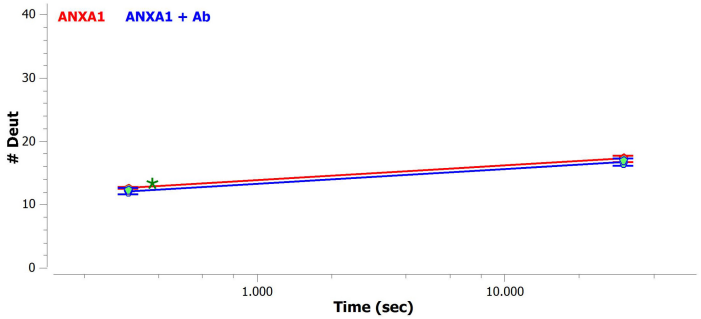
22-52: YVQTVKSSKGGPGSAVSPYPTFNPSSDVAAL (#15)



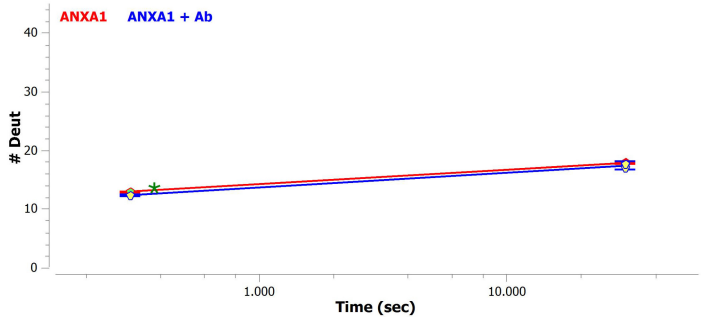
22-57: YVQTVKSSKGGPGSAVSPYPTFNPSSDVAALHKAIM (#16)



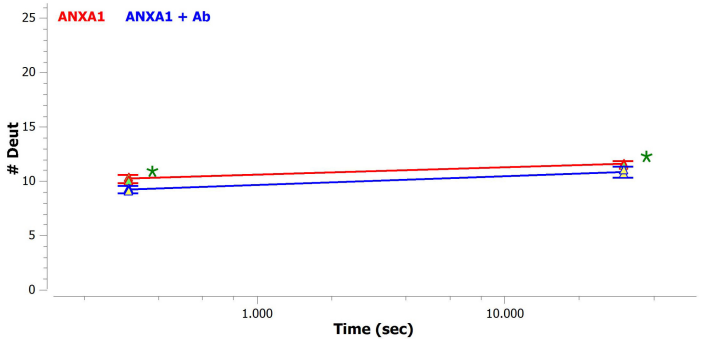
22-65: YVQTVKSSKGGPGSAVSPYPTFNPSSDVAALHKAIMVKGVDEAT (#17)



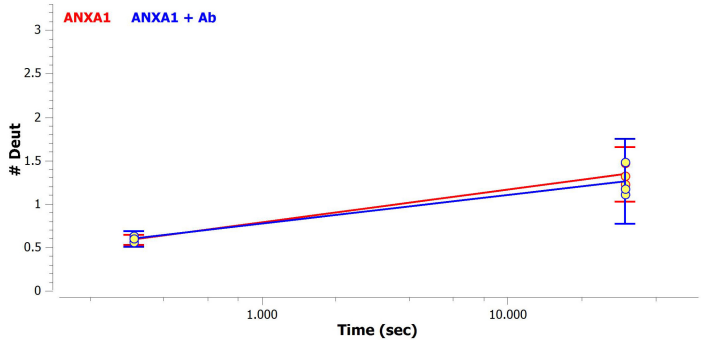
22-68: YVQTVKSSKGGPGSAVSPYPTFNPSSDVAALHKAIMVKGVDEATIID (#18)

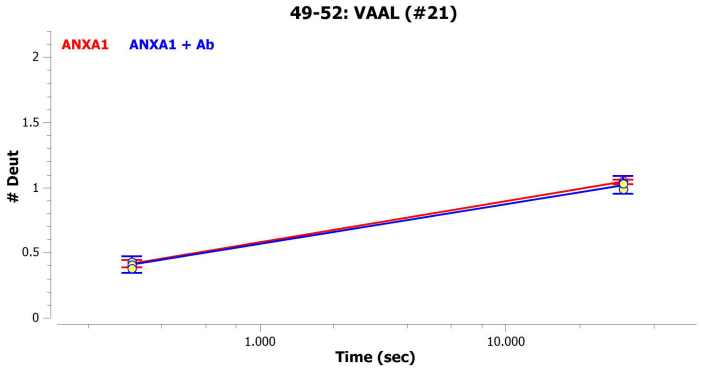


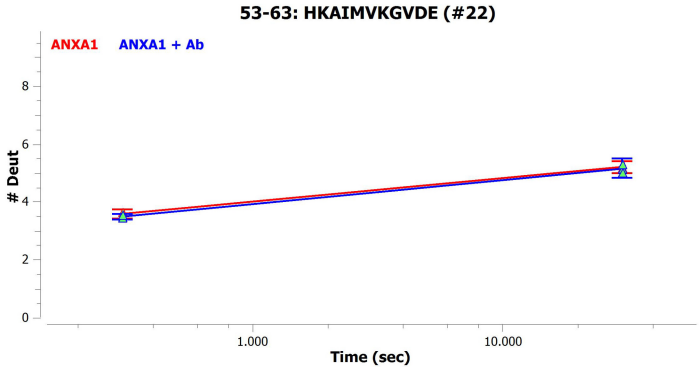
23-52: VQTVKSSKGGPGSAVSPYPTFNPSSDVAAL (#19)

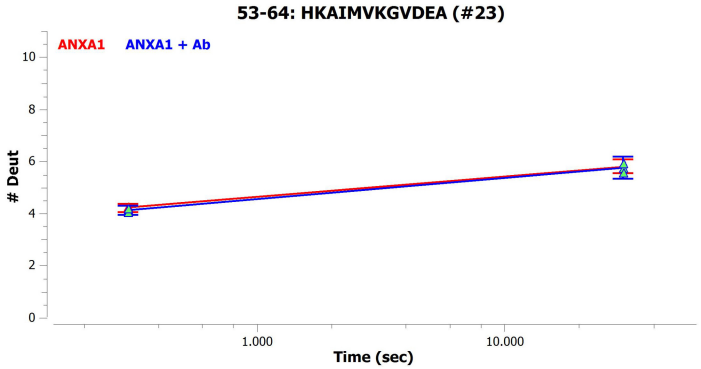


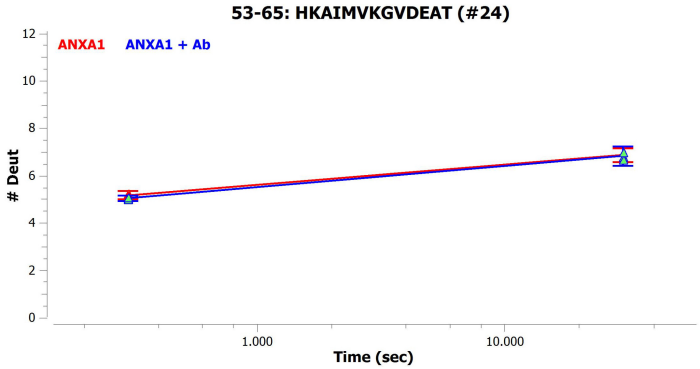
48-52: DVAAL (#20)

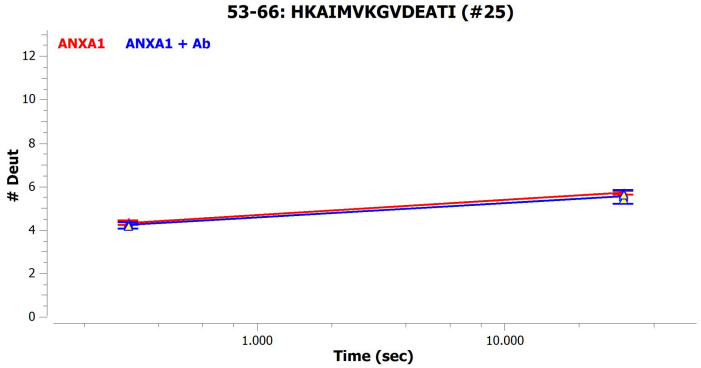


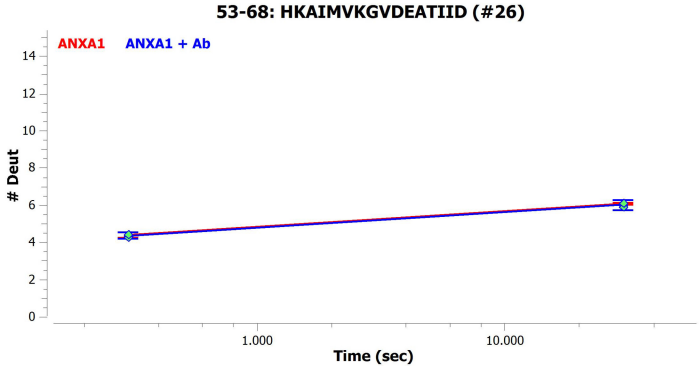

49-52: VAAL (#21)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

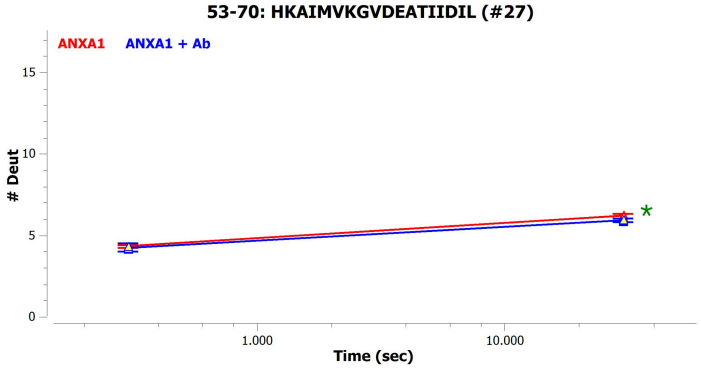

53-63: HKAIMVKGVDE (#22)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

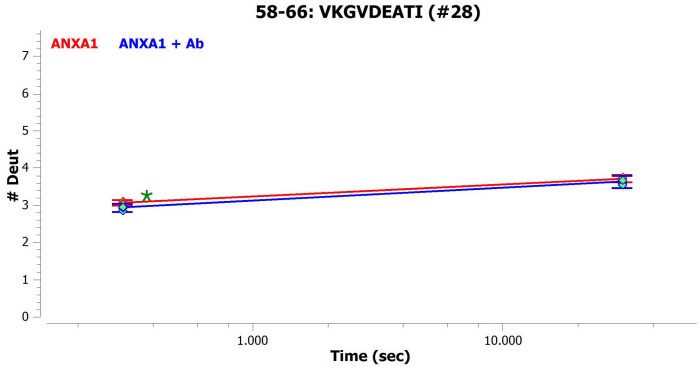

53-64: HKAIMVKGVDEA (#23)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

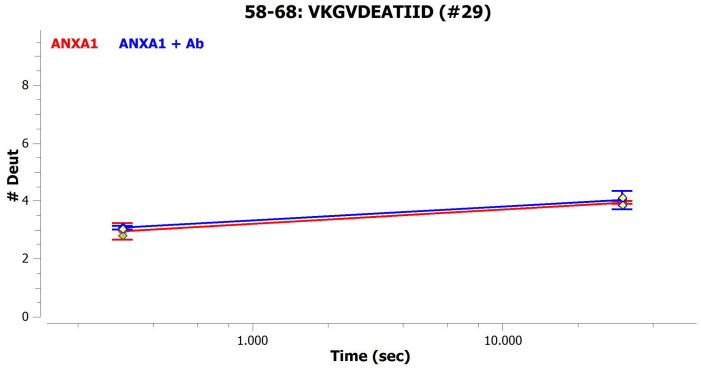

53-65: HKAIMVKGVDEAT (#24)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

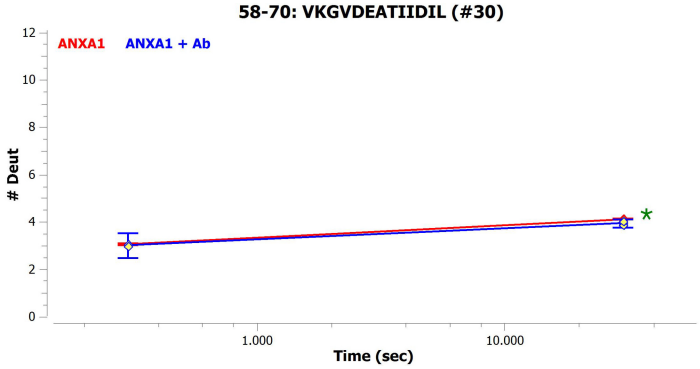

53-66: HKAIMVKGVDEATI (#25)
ANXA1
ANXA1 + Ab
Deut
Time (sec)


53-68: HKAIMVKGVDEATIID (#26)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

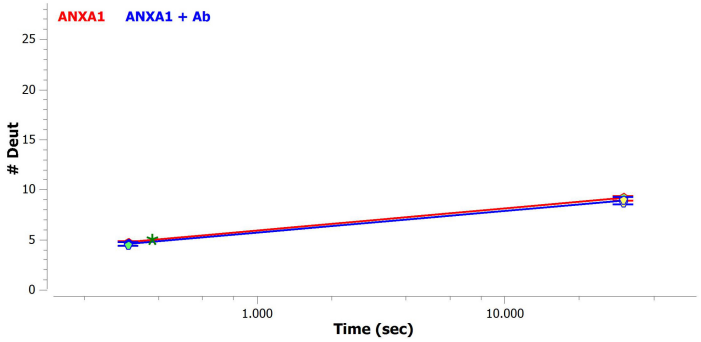

53-70: HKAIMVKGVDEATIIDIL (#27)
ANXA1
ANXA1 + Ab
Deut
Time (sec)


58-66: VKGVDEATI (#28)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

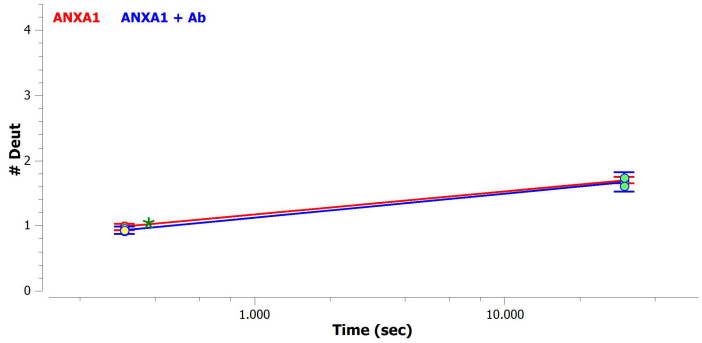

58-68: VKGVDEATIID (#29)
ANXA1
ANXA1 + Ab
Deut
Time (sec)


58-70: VKGVDEATIIDIL (#30)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

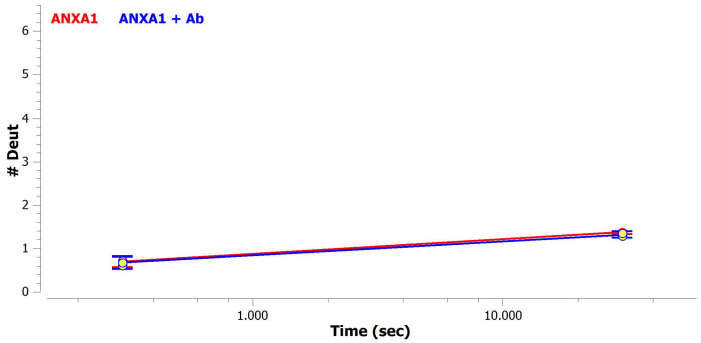
58-85: VKGVDEATIIDILTKRNNAQRQQIKAAY (#31)



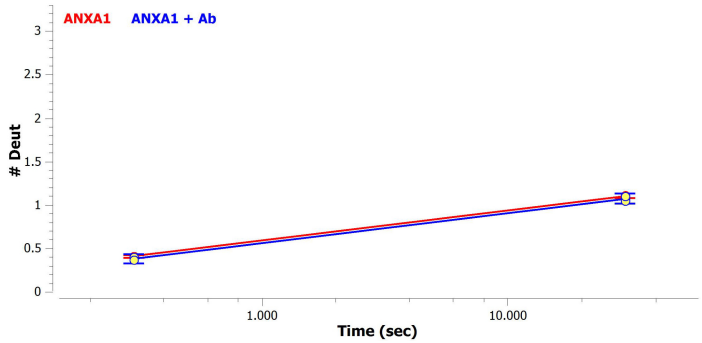
63-68: EATIID (#32)



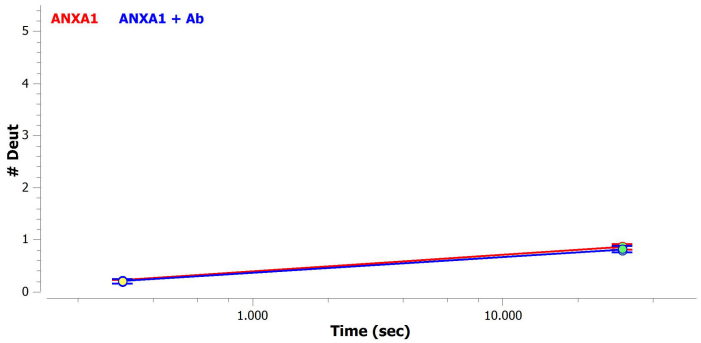
63-70: EATIIDIL (#33)



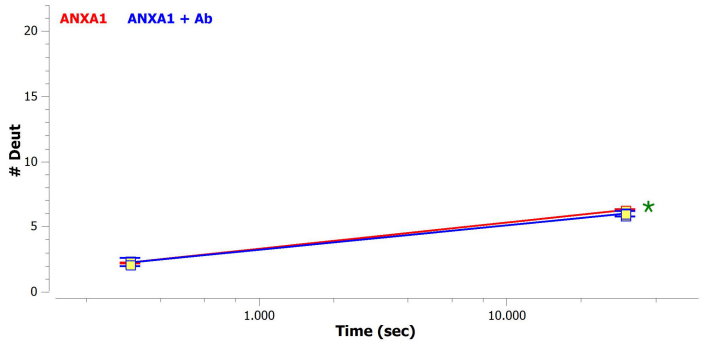
64-68: ATIID (#34)



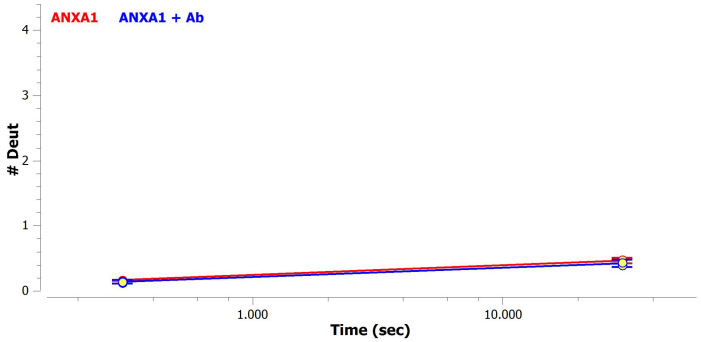
64-70: ATIIDIL (#35)



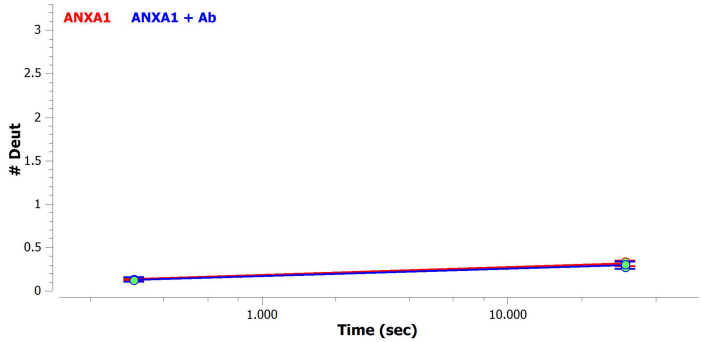
64-85: ATIIDILTKRNNAQRQQIKAAY (#36)



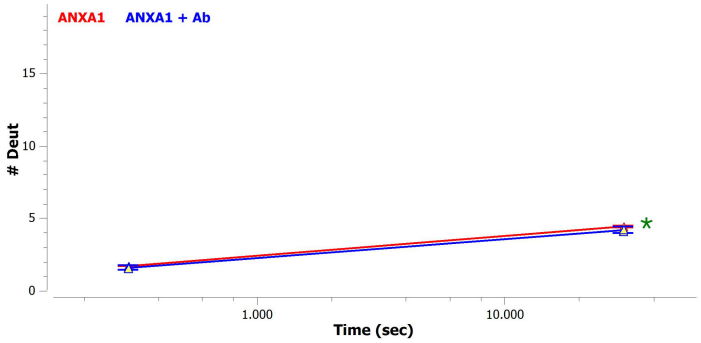
65-70: TIIDIL (#37)



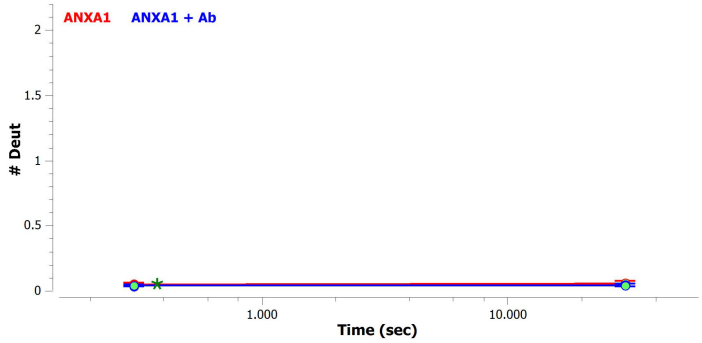
66-70: IIDIL (#38)



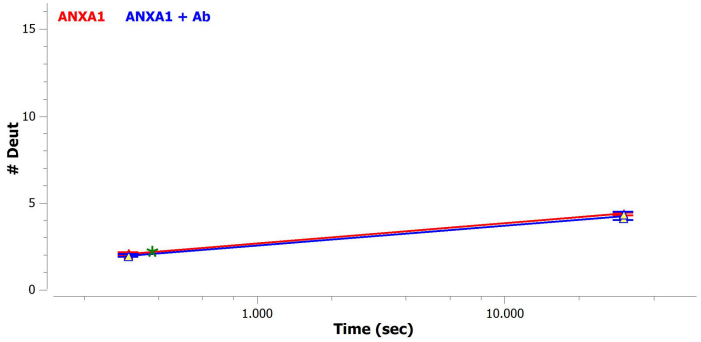
66-85: IIDILTKRNNAQRQQIKAAY (#39)



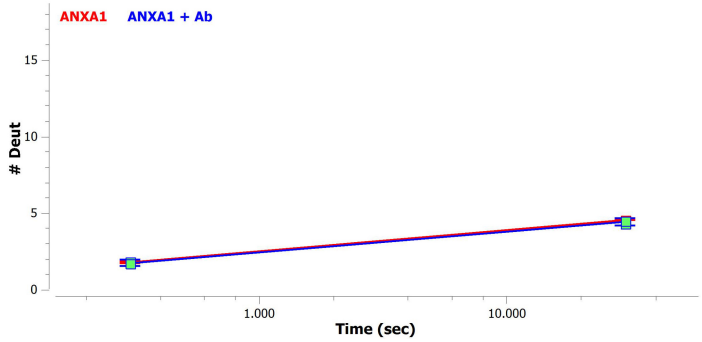
67-70: IDIL (#40)



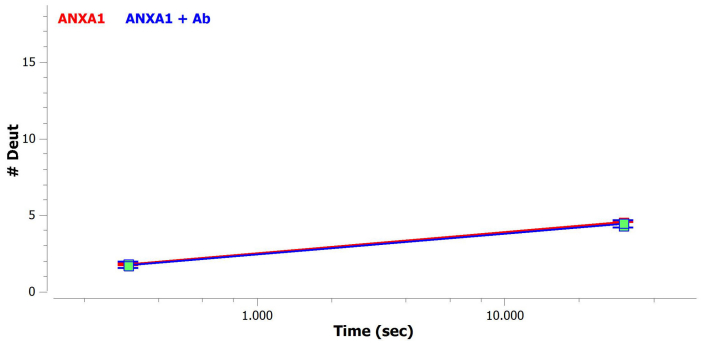
67-83: IDILTKRNNAQRQQIKA (#41)



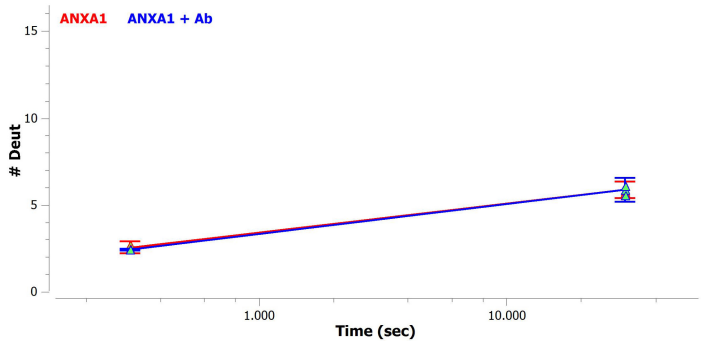
67-85: IDILTKRNNAQRQQIKAAY (#42)



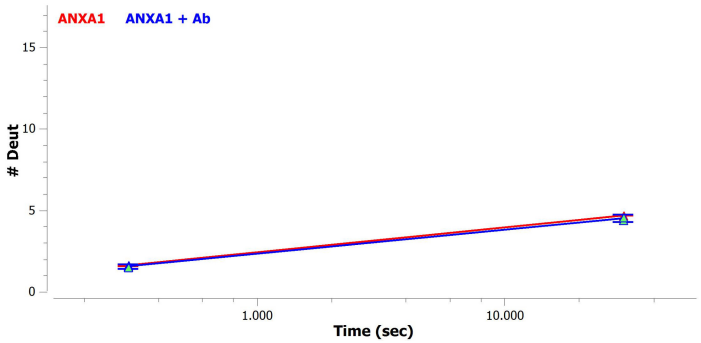
68-86: DILTKRNNAQRQQIKAAYL (#43)



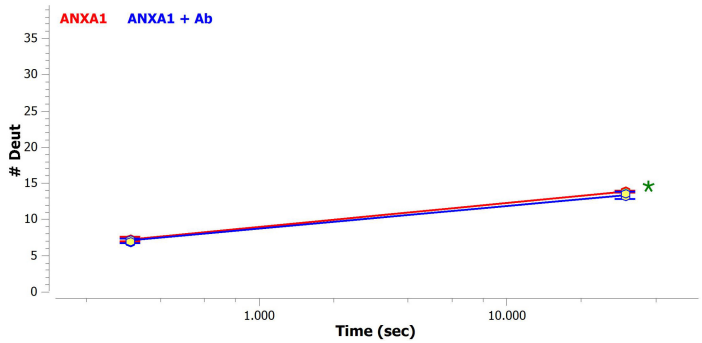
69-85: ILTKRNNAQRQQIKAAY (#44)



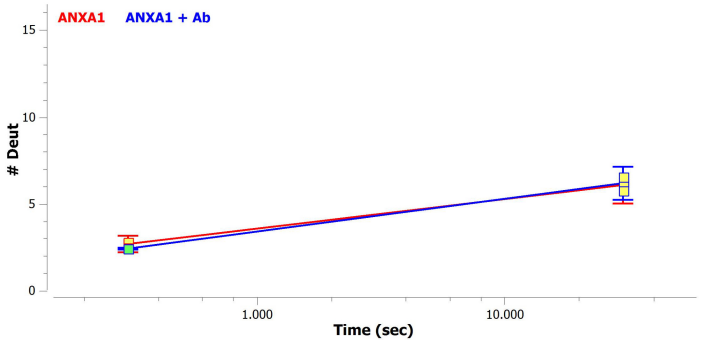
69-86: ILTKRNNAQRQQIKAAYL (#45)



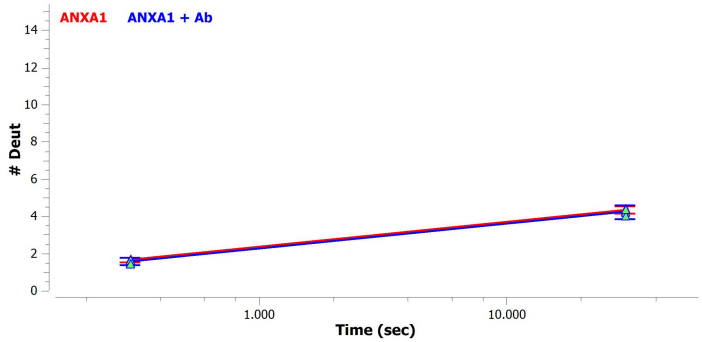
69-107: ILTKRNNAQRQQIKAAYLQETGKPLDETLKKALTGHLEE (#46)



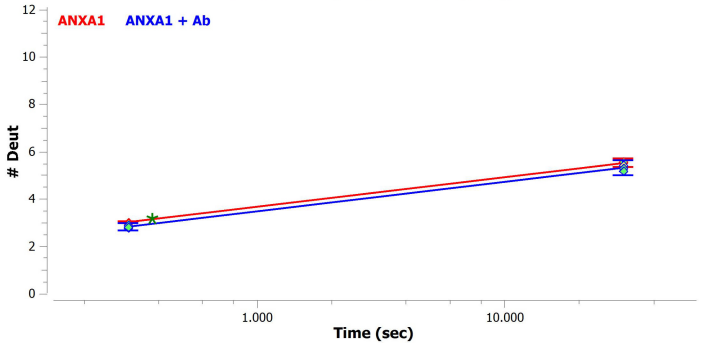
70-86: LTKRNNAQRQQIKAAYL (#47)



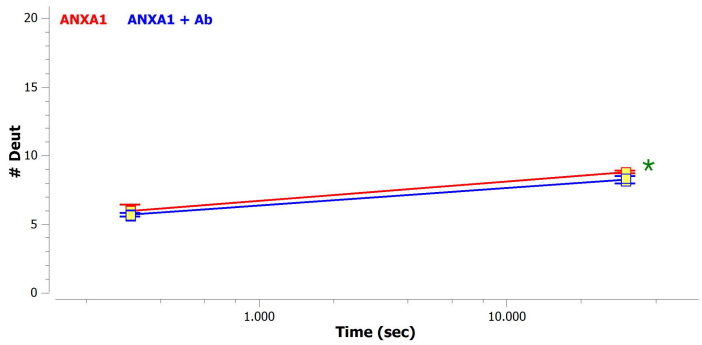
71-86: TKRNNAQRQQIKAAYL (#48)

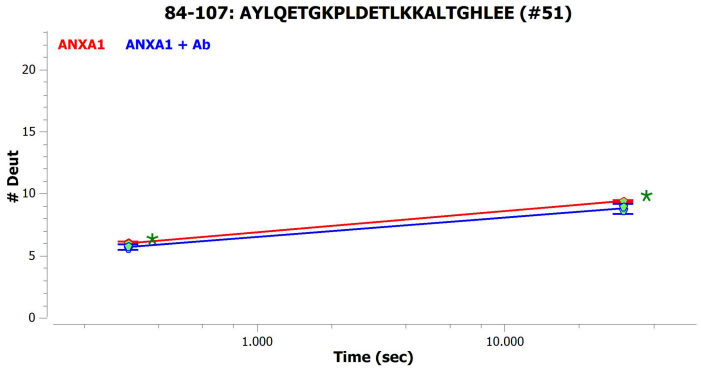


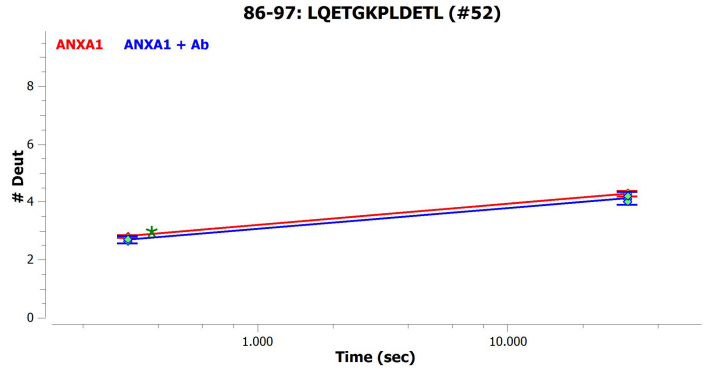
84-97: AYLQETGKPLDETL (#49)

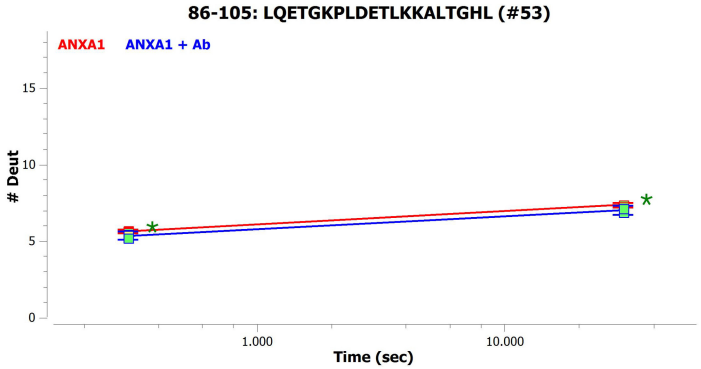


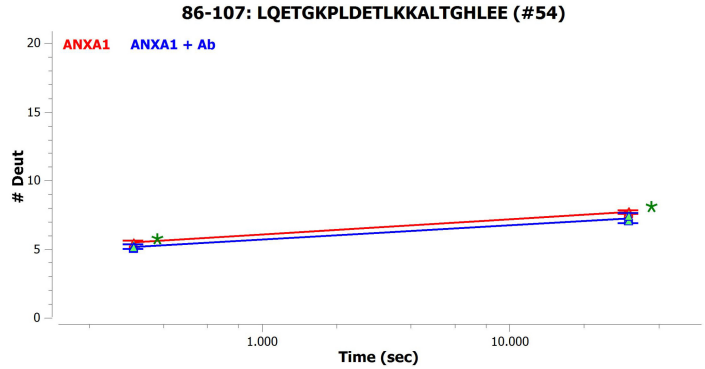
84-105: AYLQETGKPLDETLKKALTGHL (#50)

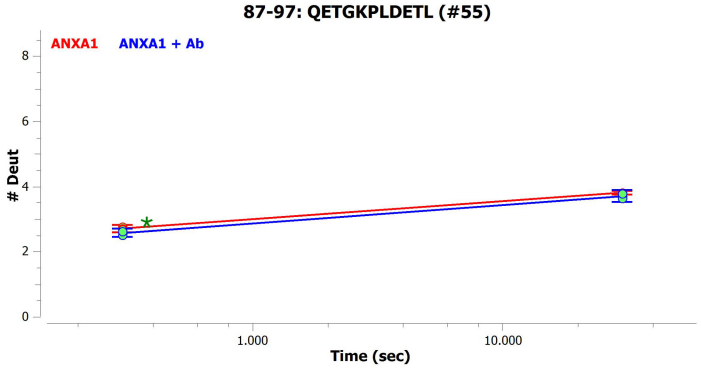


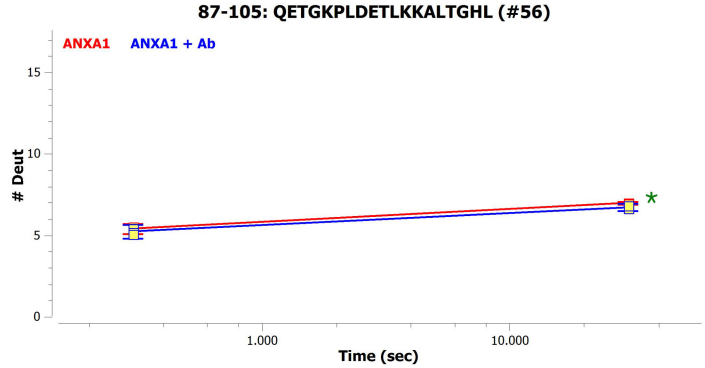

84-107: AYLQETGKPLDETLKKALTGHLEE (#51)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

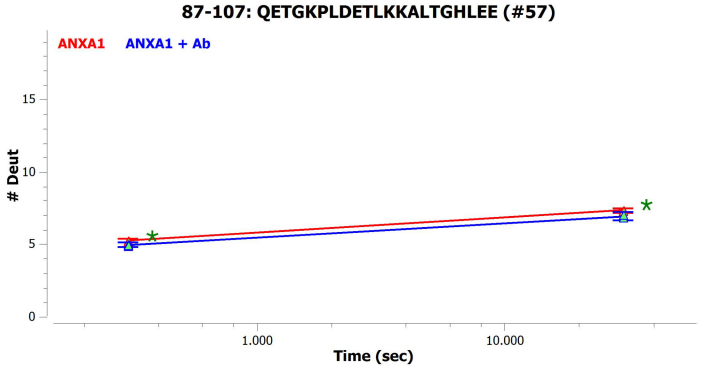

86-97: LQETGKPLDETL (#52)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

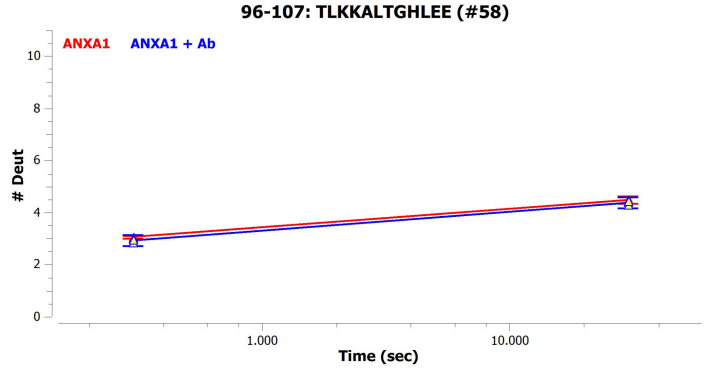

86-105: LQETGKPLDETLKKALTGHL (#53)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

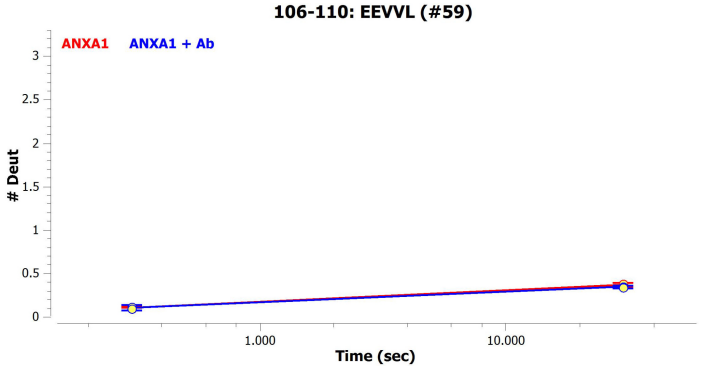

86-107: LQETGKPLDETLKKALTGHLEE (#54)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

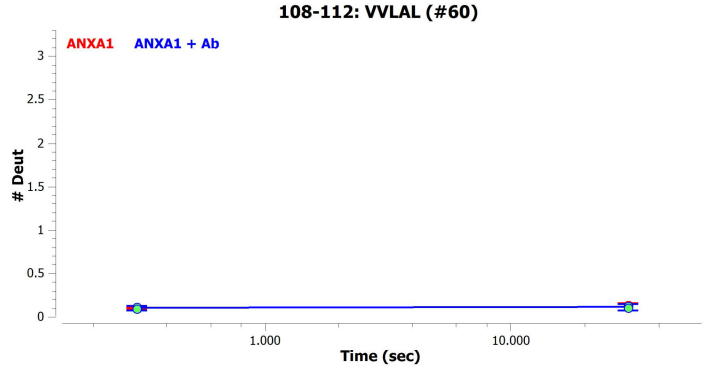

87-97: QETGKPLDETL (#55)
ANXA1
ANXA1 + Ab
Deut
Time (sec)


87-105: QETGKPLDETLKKALTGHL (#56)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

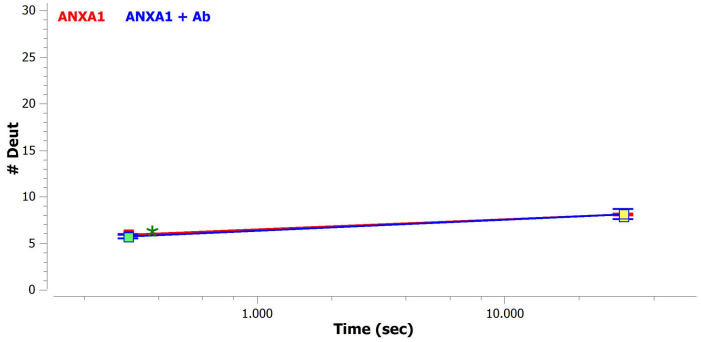

87-107: QETGKPLDETLKKALTGHLEE (#57)
ANXA1
ANXA1 + Ab
Deut
Time (sec)


96-107: TLKKALTGHLEE (#58)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

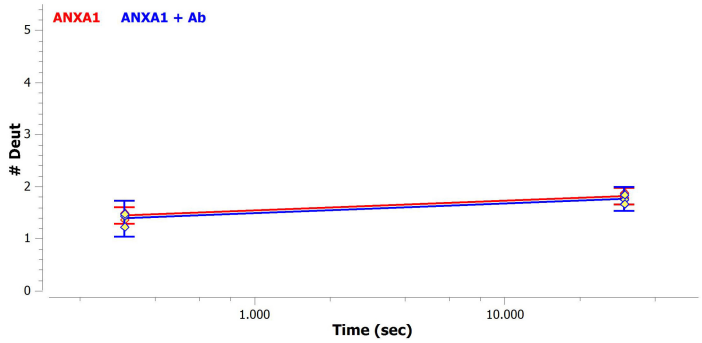

106-110: EEVVL (#59)
ANXA1
ANXA1 + Ab
Deut
Time (sec)


108-112: VVLAL (#60)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

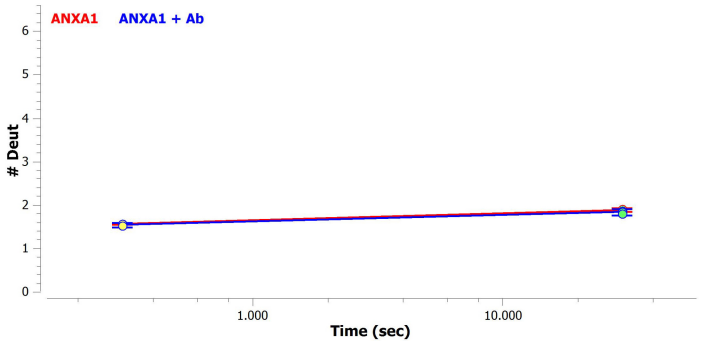
108-138: VVLALLKTPAQFDADELRAAMKGLGTDEDTL (#61)



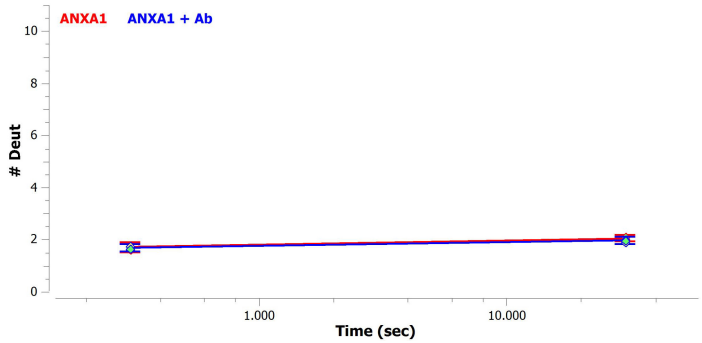
111-118: ALLKTPAQ (#62)



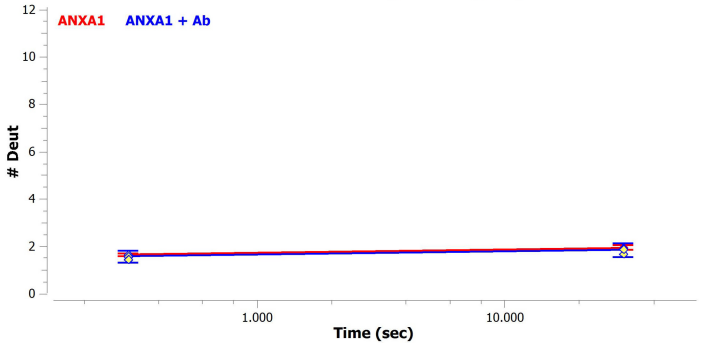
111-119: ALLKTPAQF (#63)



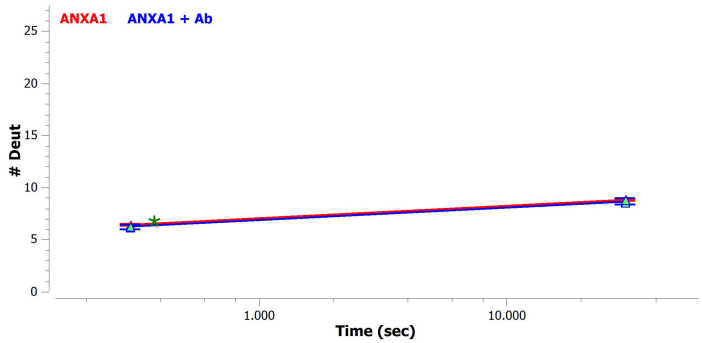
111-123: ALLKTPAQFDADE (#64)



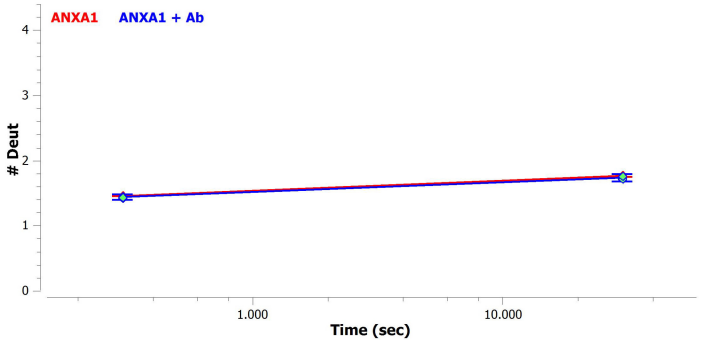
111-124: ALLKTPAQFDADEL (#65)



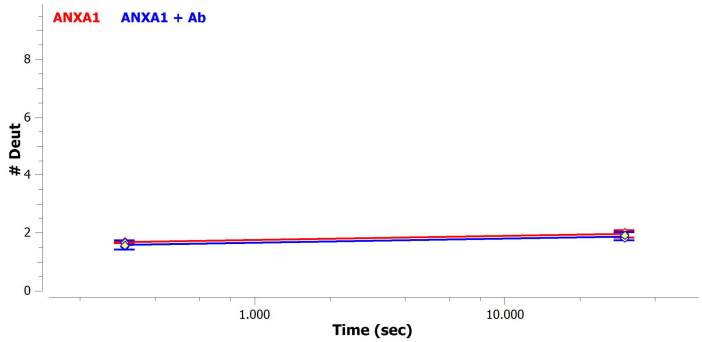
111-138: ALLKTPAQFDADELRAAMKGLGTDEDTL (#66)



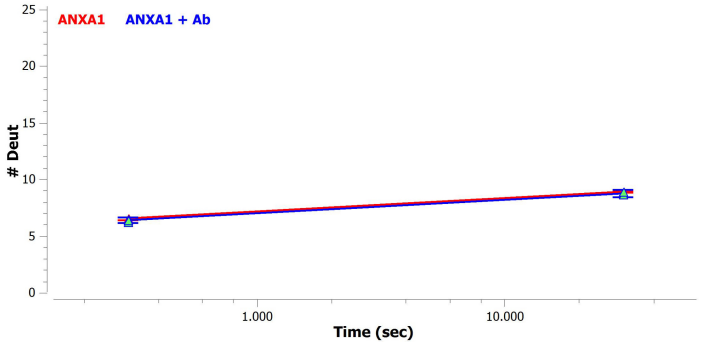
113-119: LKTPAQF (#67)



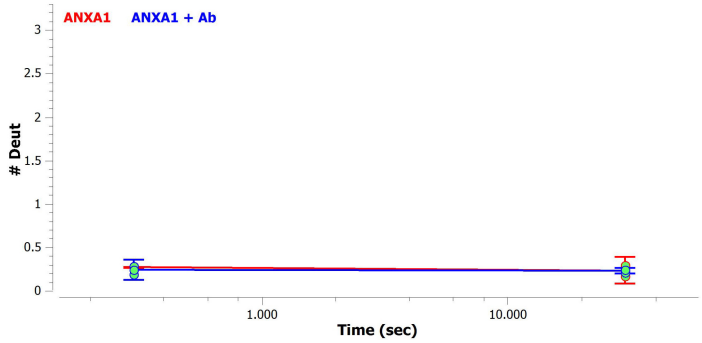
113-124: LKTPAQFDADEL (#68)



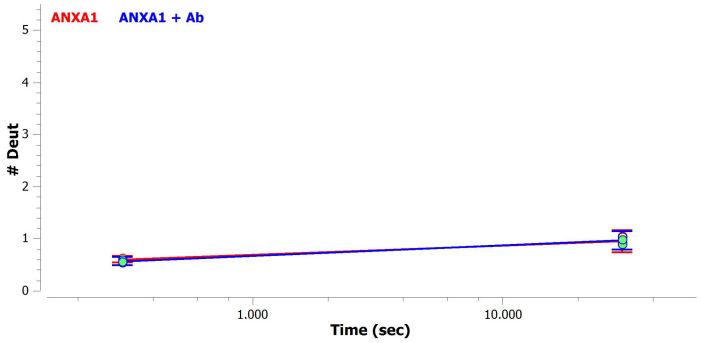
113-138: LKTPAQFDADELRAAMKGLGTDEDTL (#69)



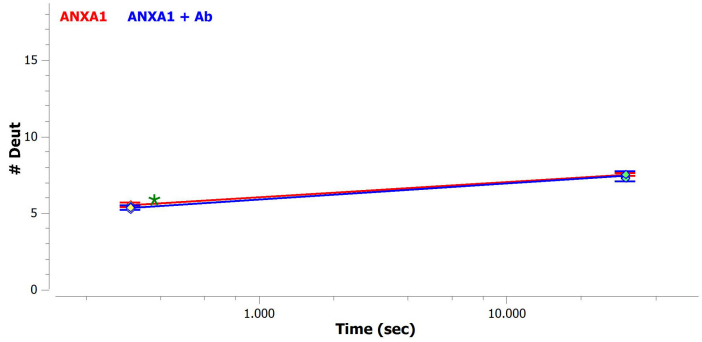
120-124: DADEL (#70)



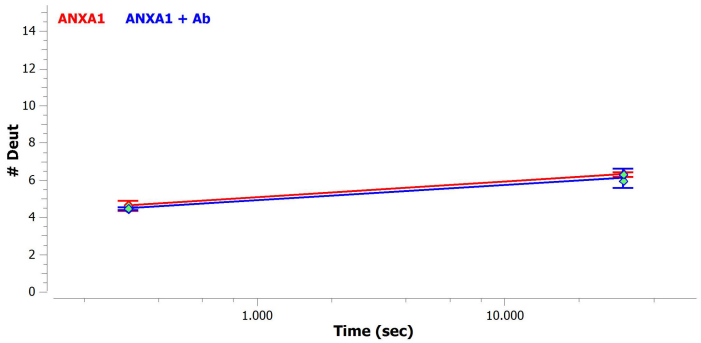
120-126: DADELRA (#71)



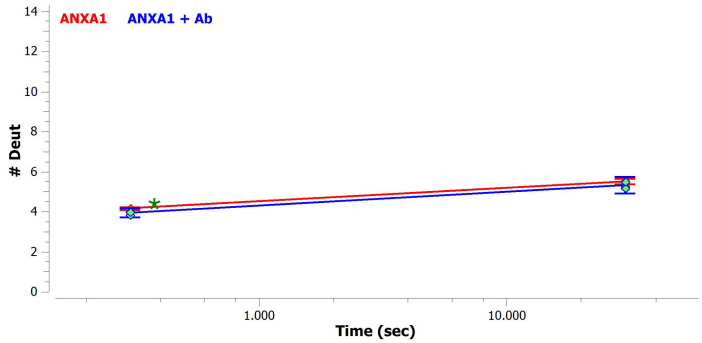
120-138: DADELRAAMKGLGTDEDTL (#72)



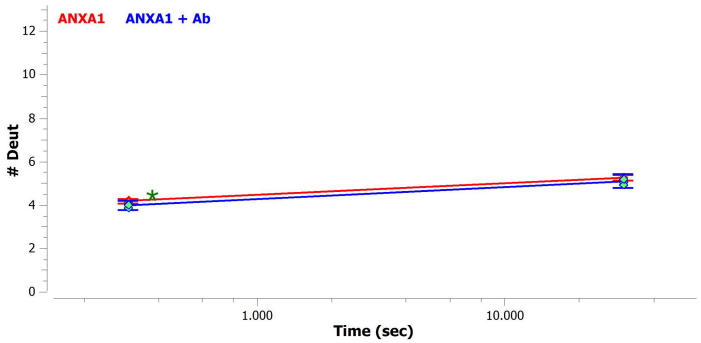
123-138: ELRAAMKGLGTDEDTL (#73)



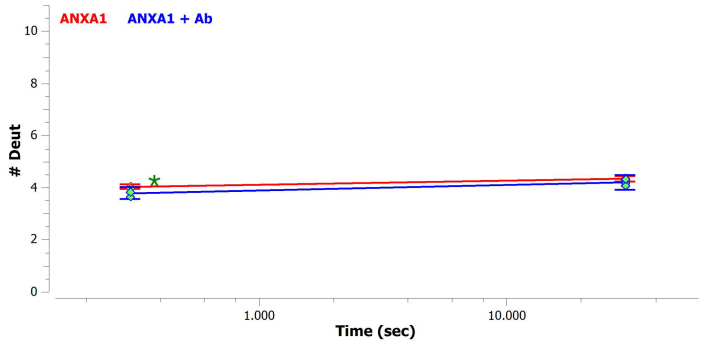
124-138: LRAAMKGLGTDEDTL (#74)



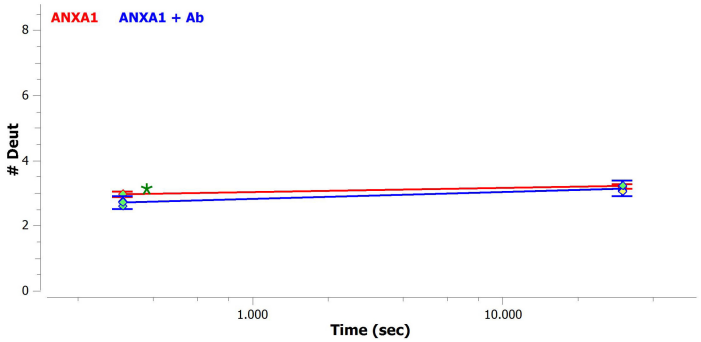
125-138: RAAMKGLGTDEDTL (#75)



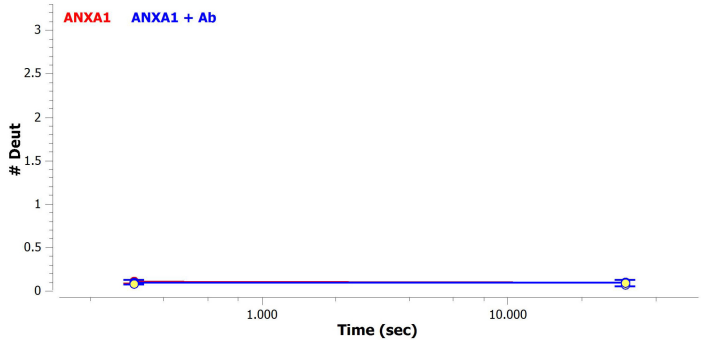
127-138: AMKGLGTDEDTL (#76)



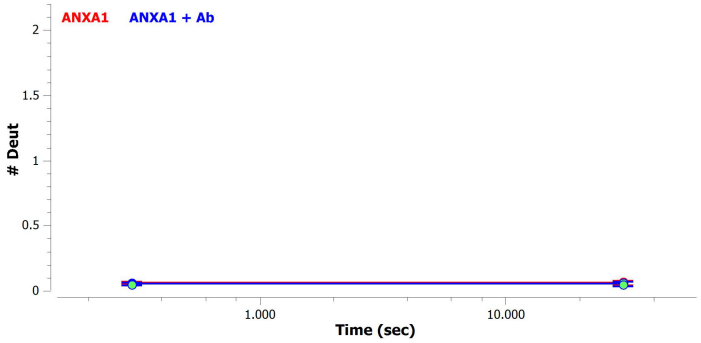
129-138: KGLGTDEDTL (#77)



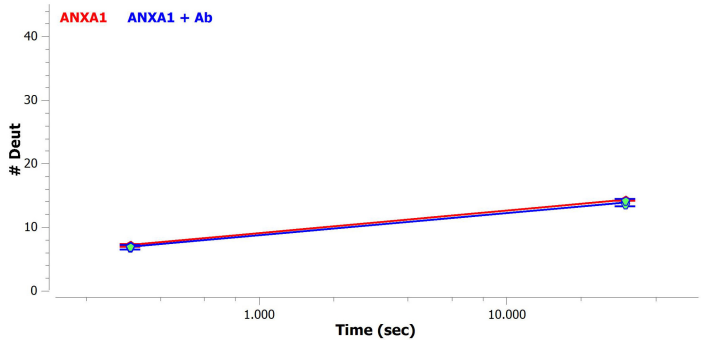
138-142: LIEIL (#78)

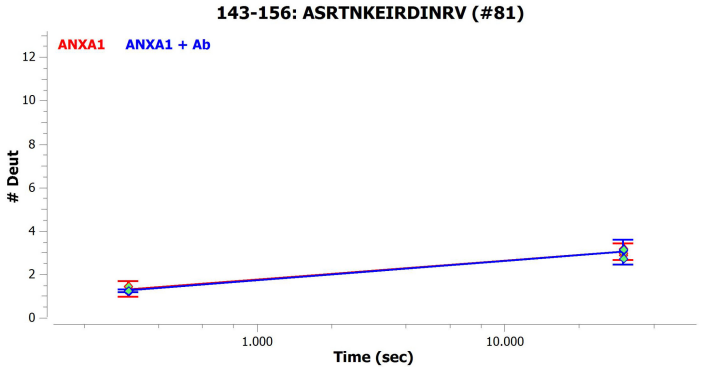


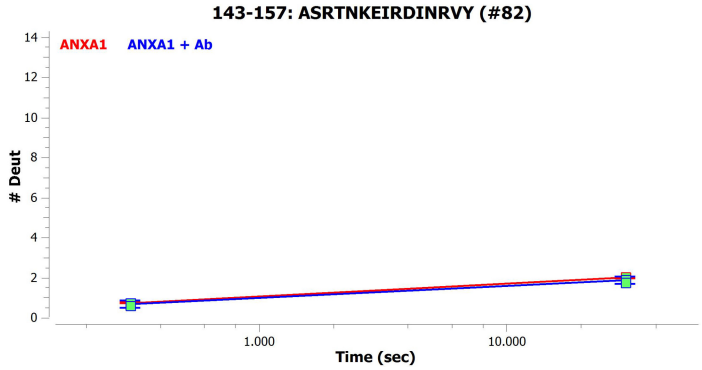
139-142: IEIL (#79)

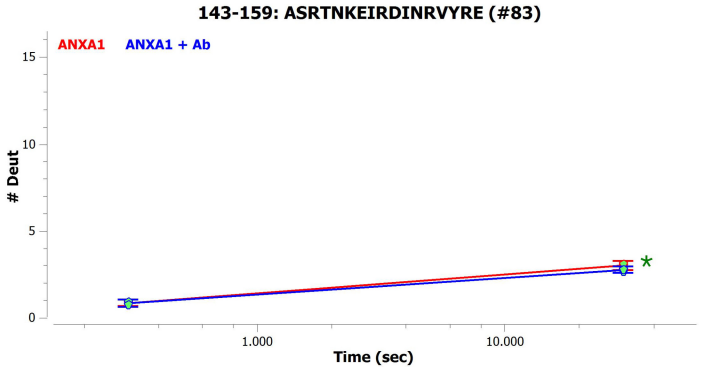


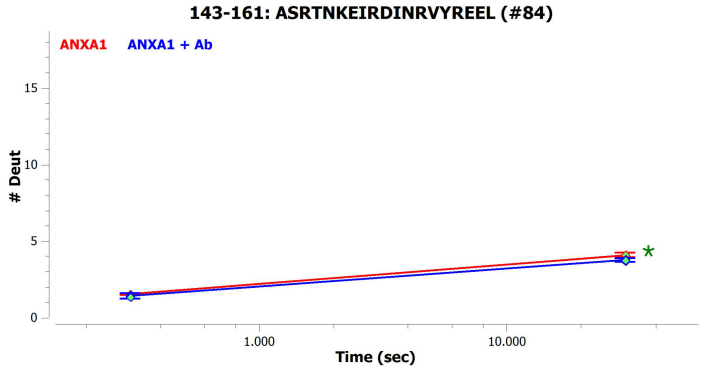
139-181: IEILASRTNKEIRDINRVYREELKRDLAKDITSDTSGDFRNAL (#80)

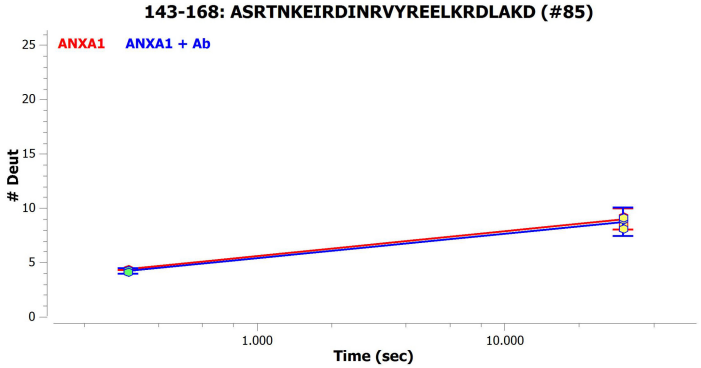


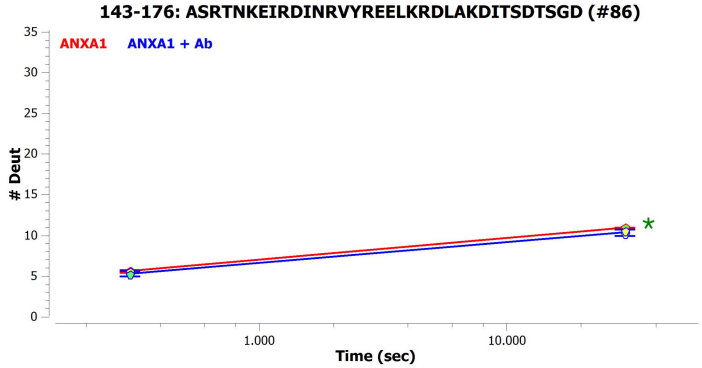

143-156: ASRTNKEIRDINRV (#81)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

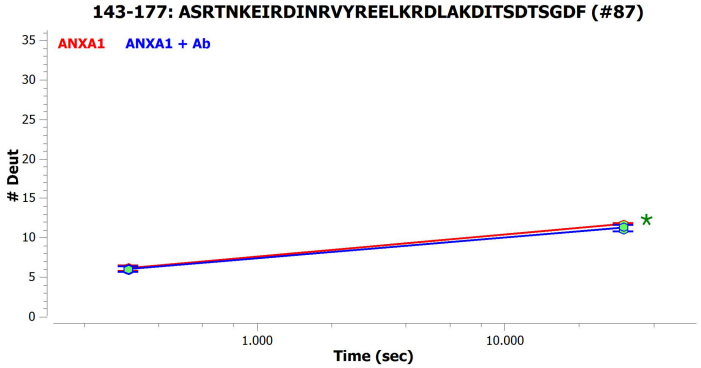

143-157: ASRTNKEIRDINRVY (#82)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

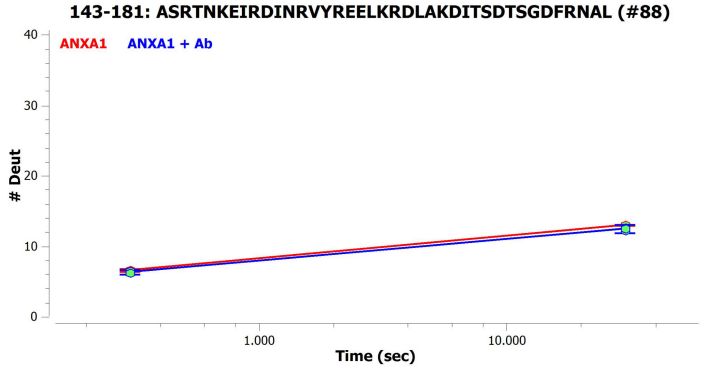

143-159: ASRTNKEIRDINRVYRE (#83)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

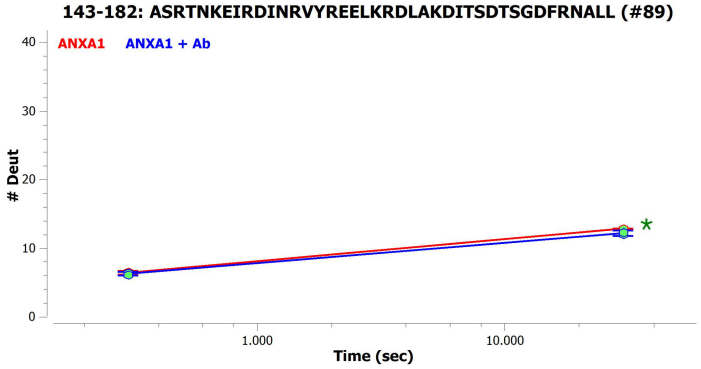

143-161: ASRTNKEIRDINRVYREEL (#84)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

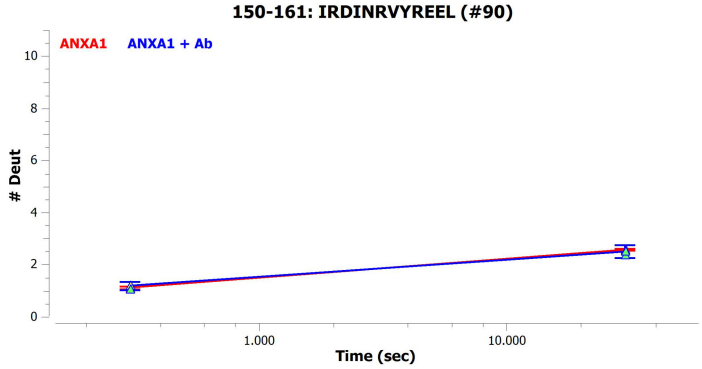

143-168: ASRTNKEIRDINRVYREELKRDLAKD (#85)
ANXA1
ANXA1 + Ab
Deut
Time (sec)


143-176: ASRTNKEIRDINRVYREELKRDLAKDITSDTSGD (#86)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

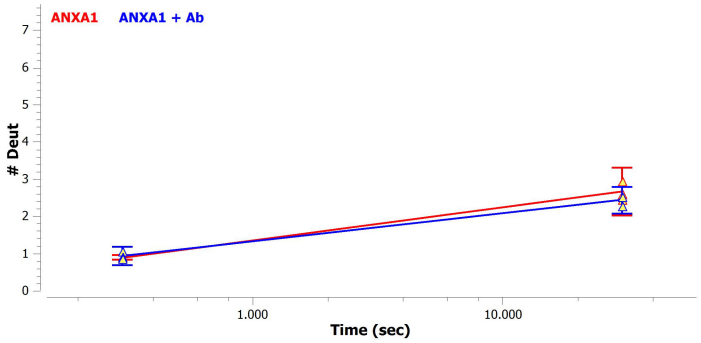

143-177: ASRTNKEIRDINRVYREELKRDLAKDITSDTSGDF (#87)
ANXA1
ANXA1 + Ab
Deut
Time (sec)


143-181: ASRTNKEIRDINRVYREELKRDLAKDITSDTSGDFRNAL (#88)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

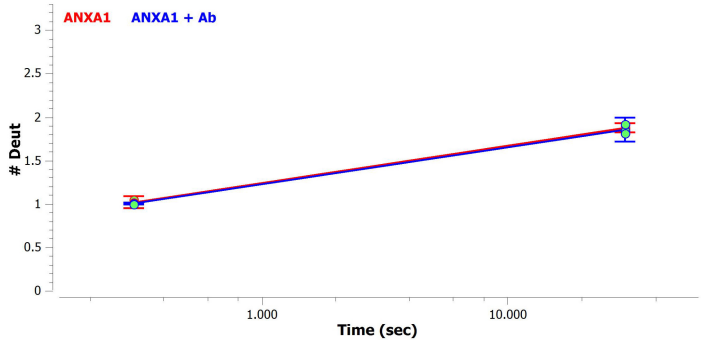

143-182: ASRTNKEIRDINRVYREELKRDLAKDITSDTSGDFRNALL (#89)
ANXA1
ANXA1 + Ab
Deut
Time (sec)


150-161: IRDINRVYREEL (#90)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

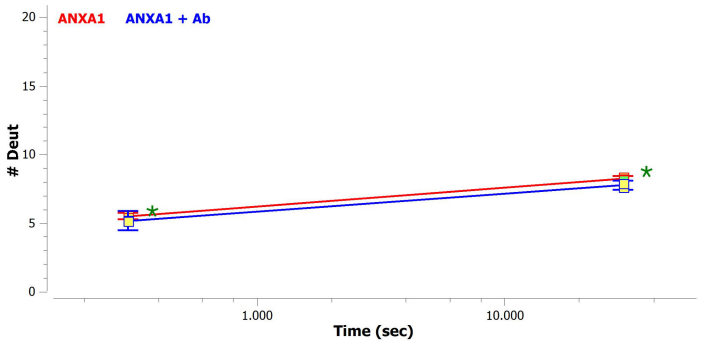
153-161: INRVYREEL (#91)



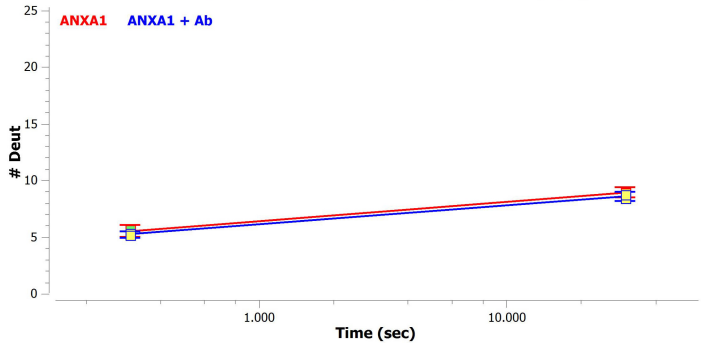
157-161: YREEL (#92)



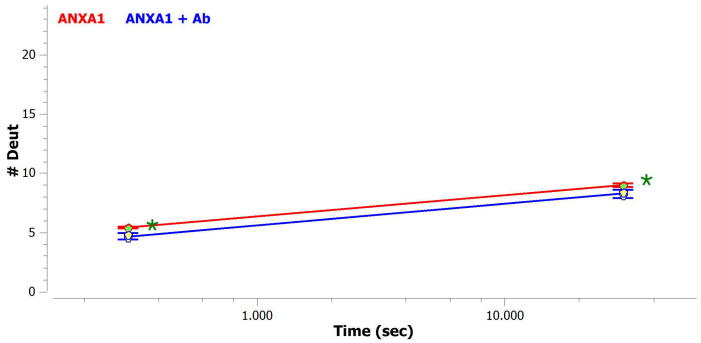
157-177: YREELKRDLAKDITSDTSGDF (#93)



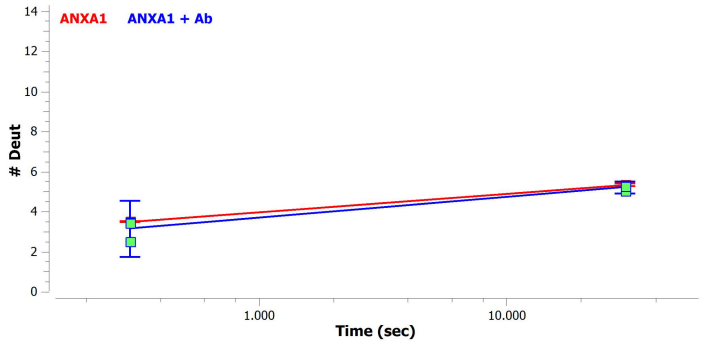
157-181: YREELKRDLAKDITSDTSGDFRNAL (#94)



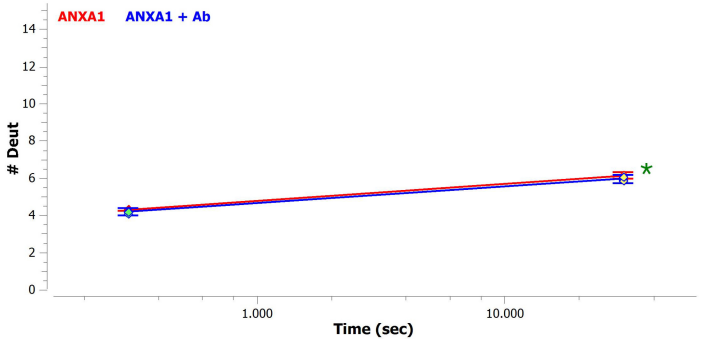
158-181: REELKRDLAKDITSDTSGDFRNAL (#95)



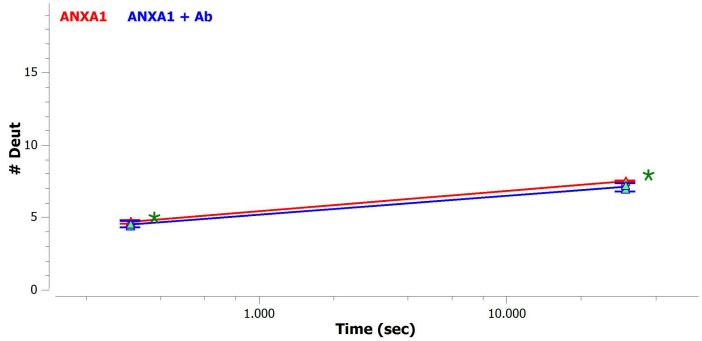
162-176: KRDLAKDITSDTSGD (#96)



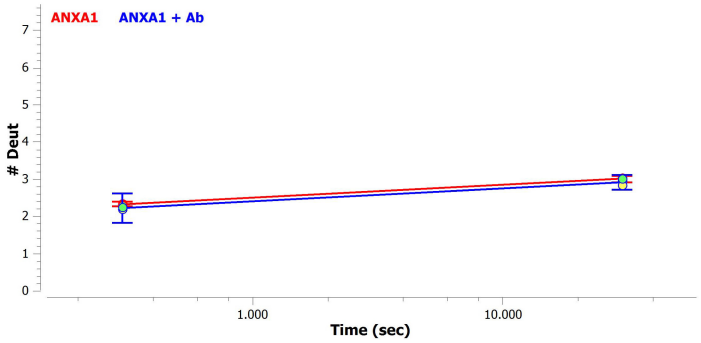
162-177: KRDLAKDITSDTSGDF (#97)



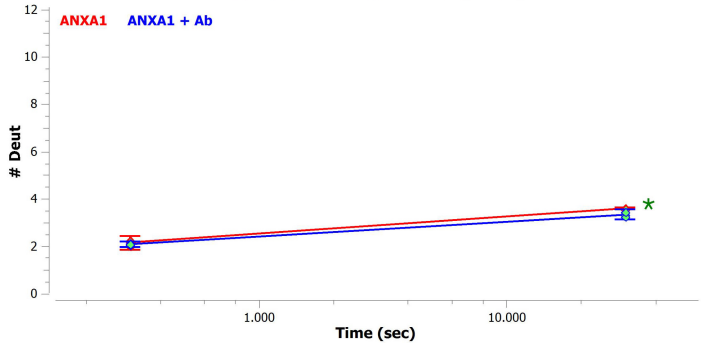
162-181: KRDLAKDITSDTSGDFRNAL (#98)



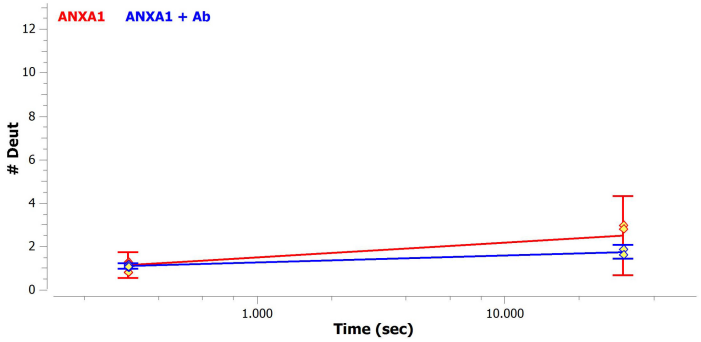
169-177: ITSDTSGDF (#99)



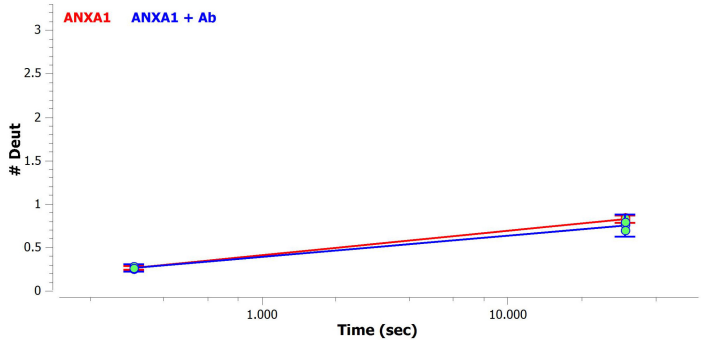
169-181: ITSDTSGDFRNAL (#100)



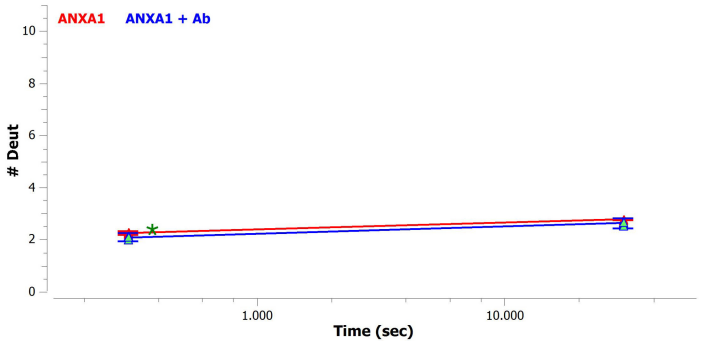
169-182: ITSDTSGDFRNALL (#101)



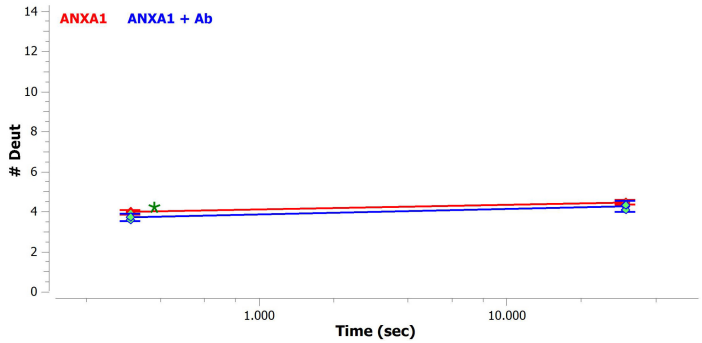
177-181: FRNAL (#102)



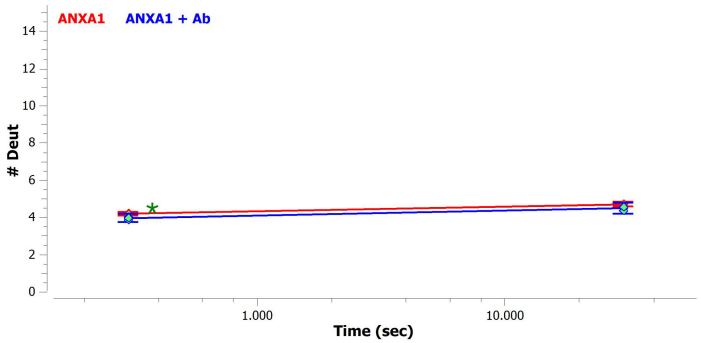
182-193: LSLAKGDRSEDF (#103)



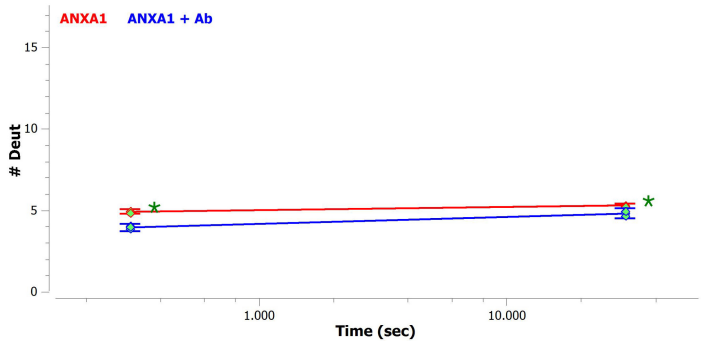
182-196: LSLAKGDRSEDFGVN (#104)



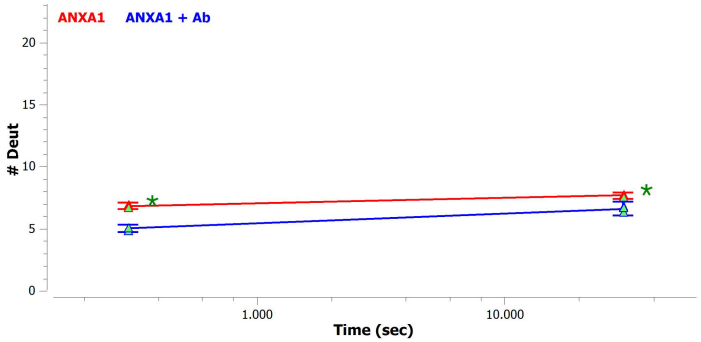
182-197: LSLAKGDRSEDFGVNE (#105)



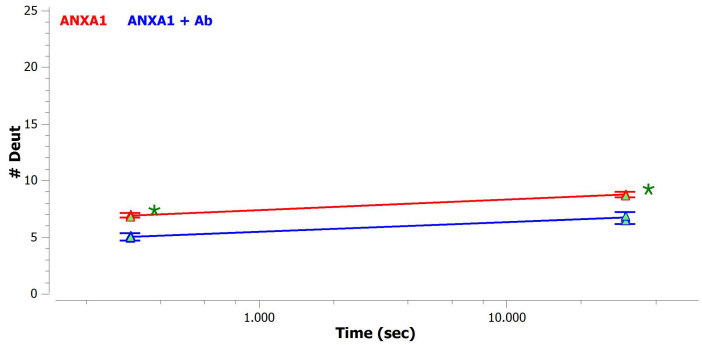
182-199: LSLAKGDRSEDFGVNEDL (#106)



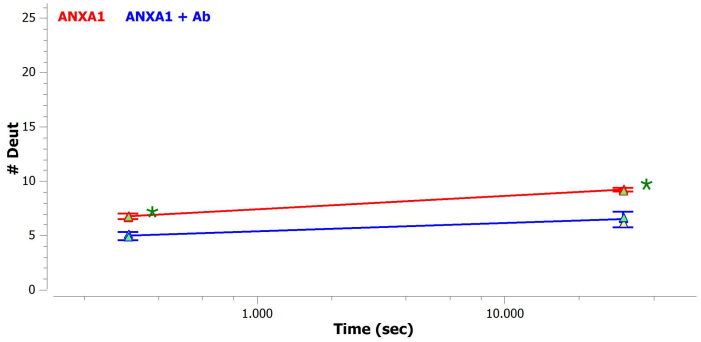
182-204: LSLAKGDRSEDFGVNEDLADSDA (#107)



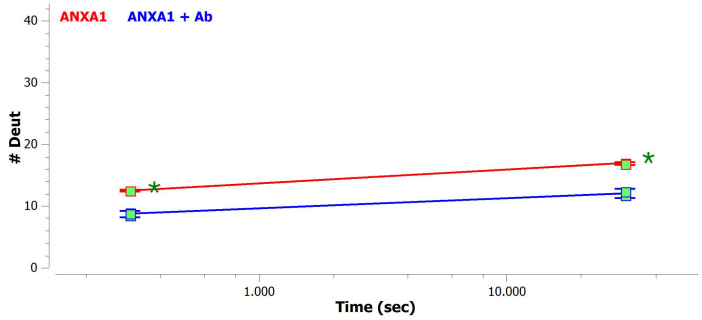
182-206: LSLAKGDRSEDFGVNEDLADSDARA (#108)



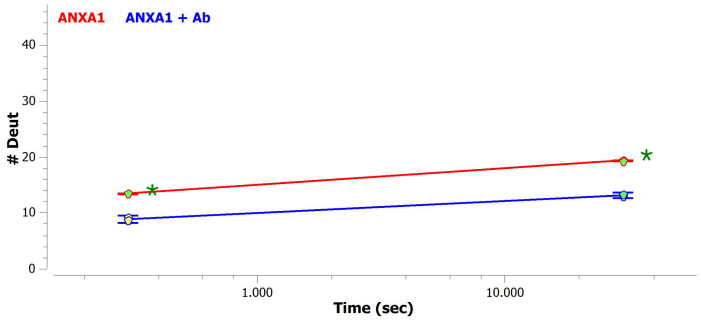
182-207: LSLAKGDRSEDFGVNEDLADSDARAL (#109)



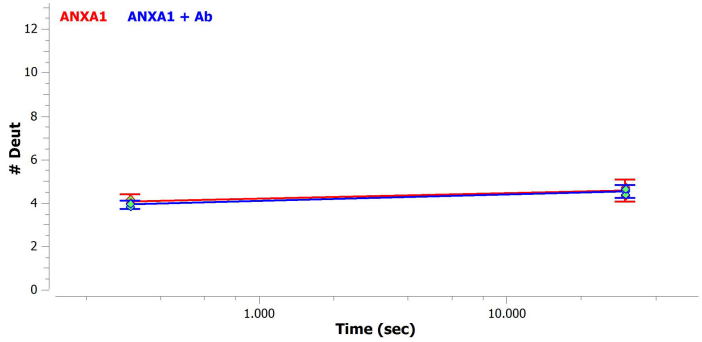
182-222: LSLAKGDRSEDFGVNEDLADSDARALYEAGERRKGTDVNVF (#110)



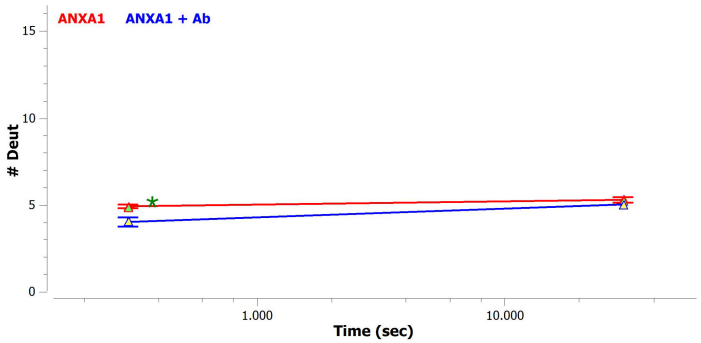
182-226: LSLAKGDRSEDFGVNEDLADSDARALYEAGERRKGTDVNVFNTIL (#111)



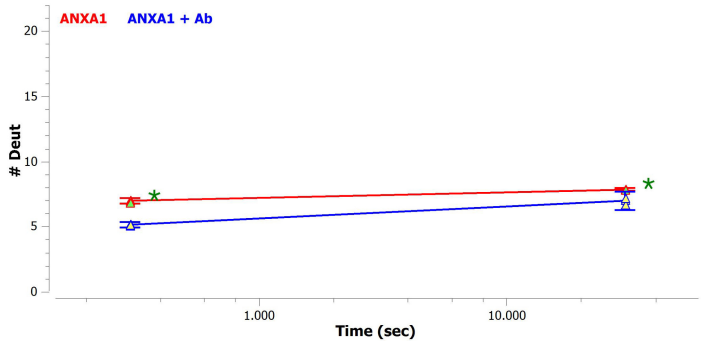
183-196: SLAKGDRSEDFGVN (#112)



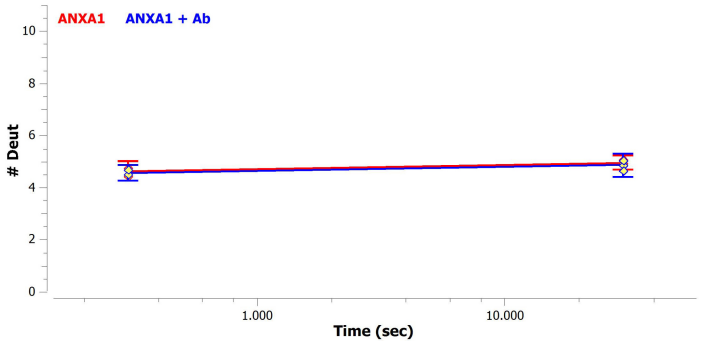
183-199: SLAKGDRSEDFGVNEDL (#113)



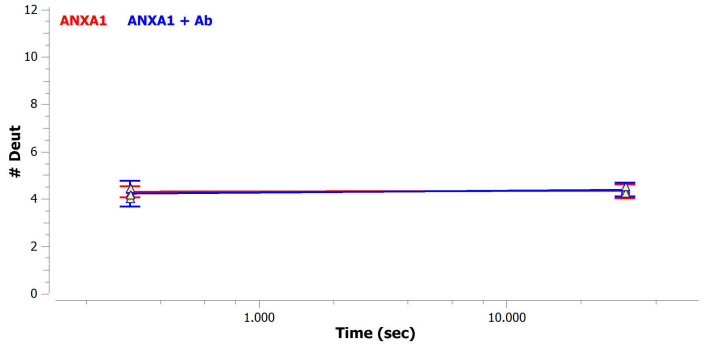
183-204: SLAKGDRSEDFGVNEDLADSDA (#114)



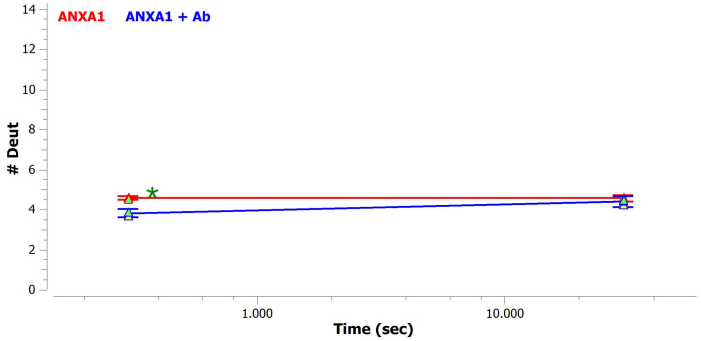
185-196: AKGDRSEDFGVN (#115)



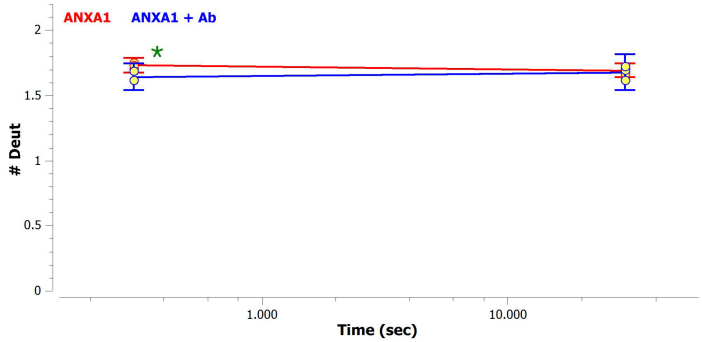
185-197: AKGDRSEDFGVNE (#116)



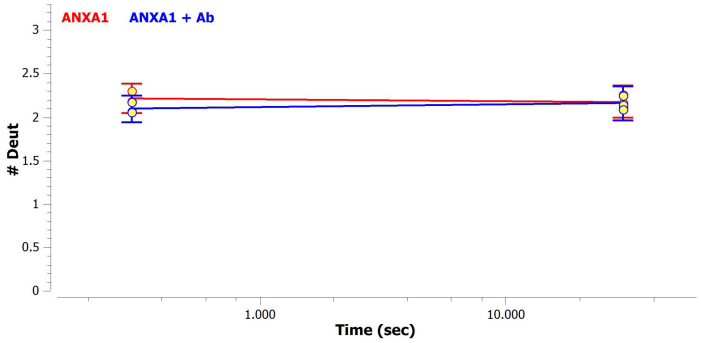
185-199: AKGDRSEDFGVNEDL (#117)



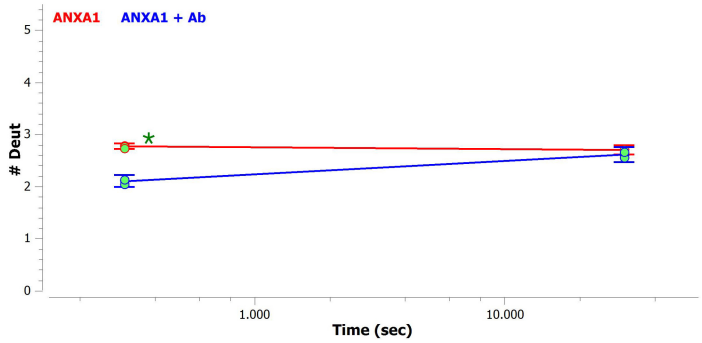
193-196: FGVN (#118)

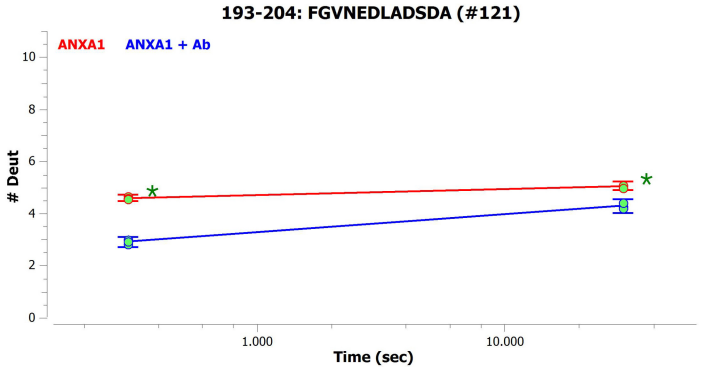


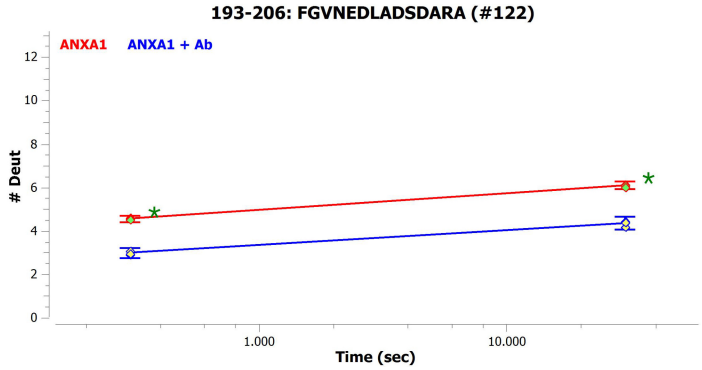
193-197: FGVNE (#119)

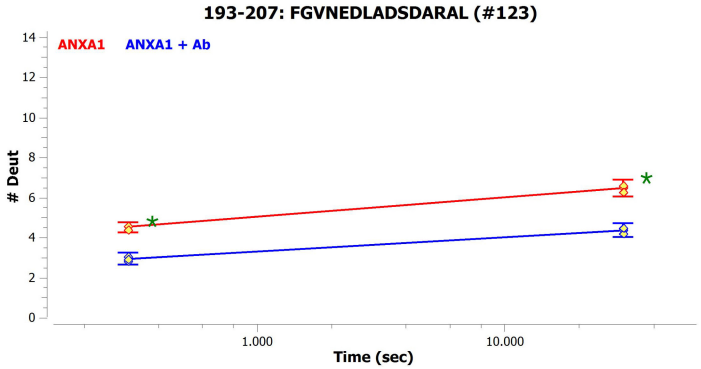


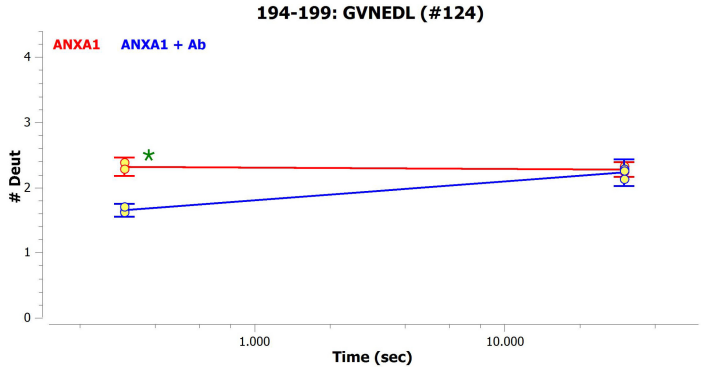
193-199: FGVNEDL (#120)

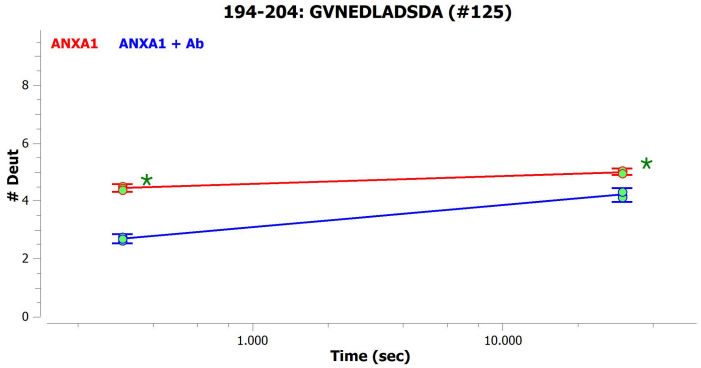


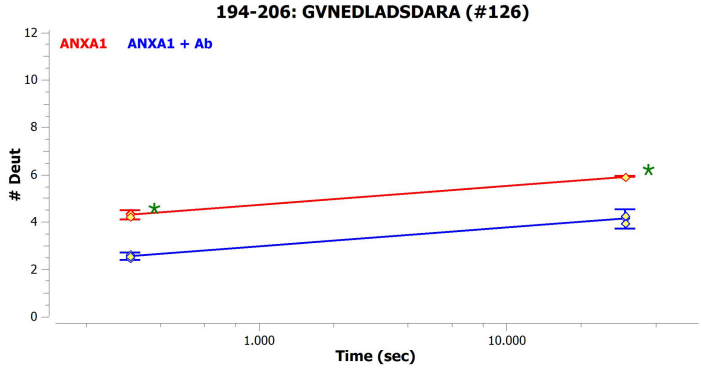

193-204: FGVNEDLADSDA (#121)
ANXA1
ANXA1 + Ab
Deut
Time (sec)
1.000
10.000

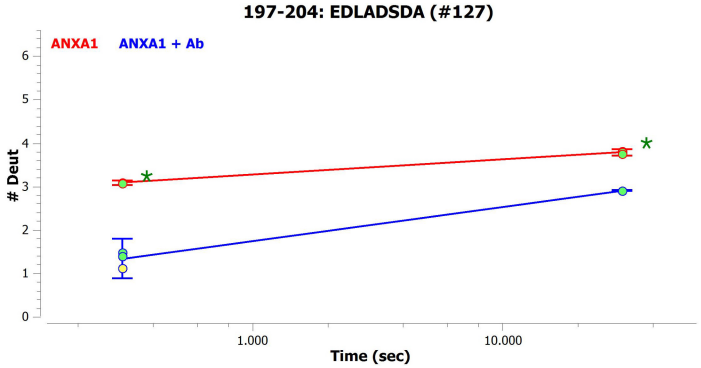

193-206: FGVNEDLADSDARA (#122)
ANXA1
ANXA1 + Ab
Deut
Time (sec)
1.000
10.000

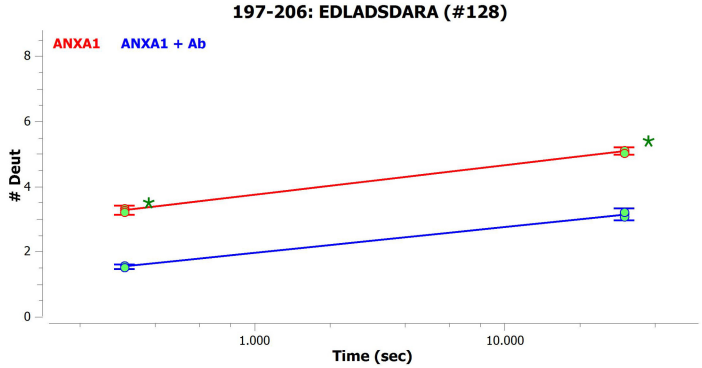

193-207: FGVNEDLADSDARAL (#123)
ANXA1
ANXA1 + Ab
Deut
Time (sec)
1.000
10.000

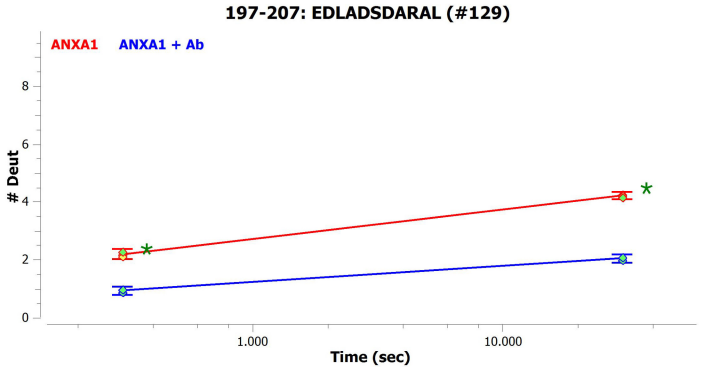

194-199: GVNEDL (#124)
ANXA1
ANXA1 + Ab
Deut
Time (sec)
1.000
10.000

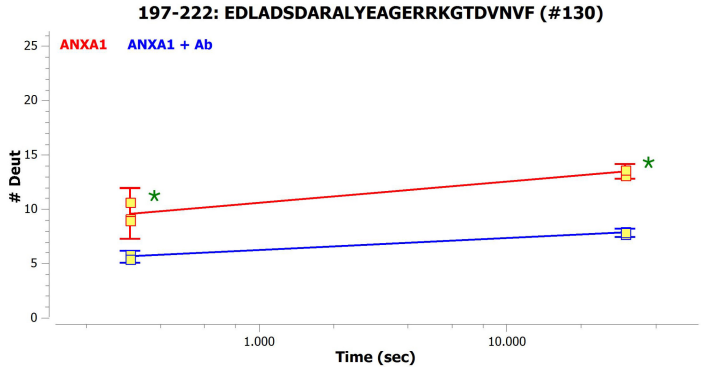

194-204: GVNEDLADSDA (#125)
ANXA1
ANXA1 + Ab
Deut
Time (sec)
1.000
10.000

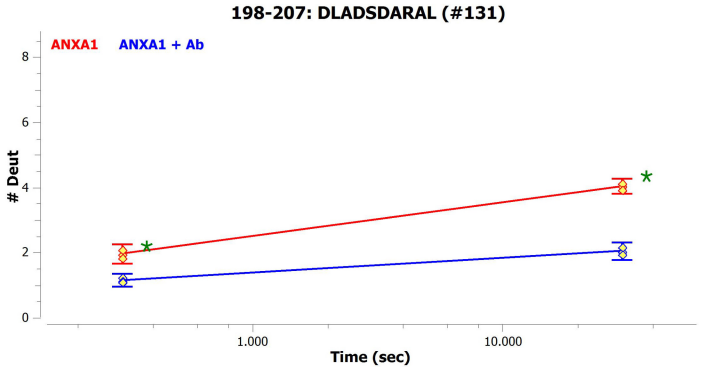

194-206: GVNEDLADSDARA (#126)
ANXA1
ANXA1 + Ab
Deut
Time (sec)
1.000
10.000

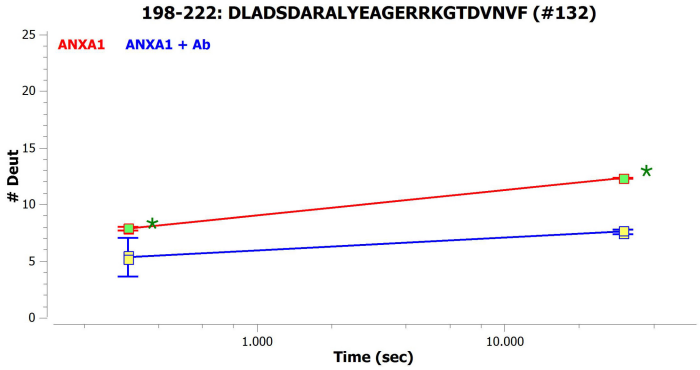

197-204: EDLADSDA (#127)
ANXA1
ANXA1 + Ab
Deut
Time (sec)
1.000
10.000

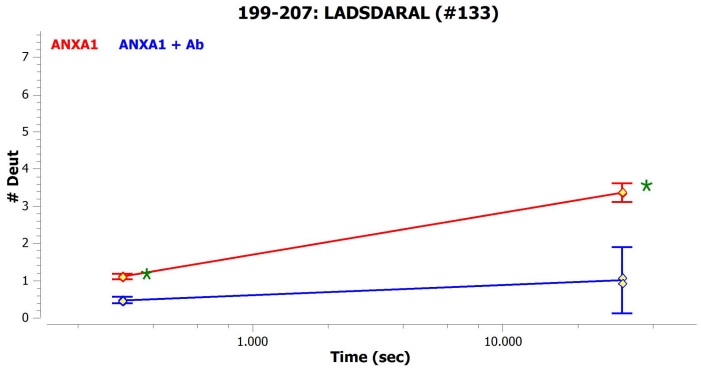

197-206: EDLADSDARA (#128)
ANXA1
ANXA1 + Ab
Deut
Time (sec)
1.000
10.000

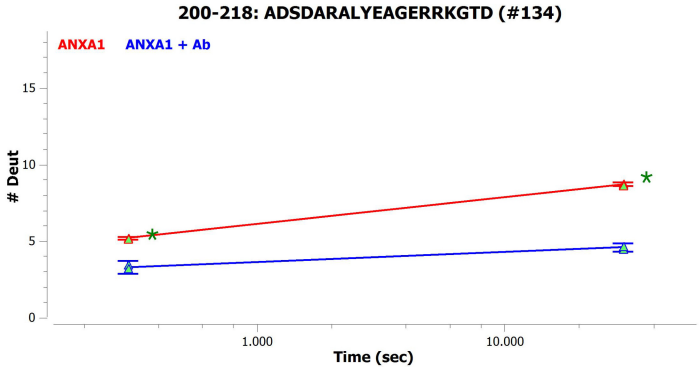

197-207: EDLADSDARAL (#129)
ANXA1
ANXA1 + Ab
Deut
Time (sec)
1.000
10.000

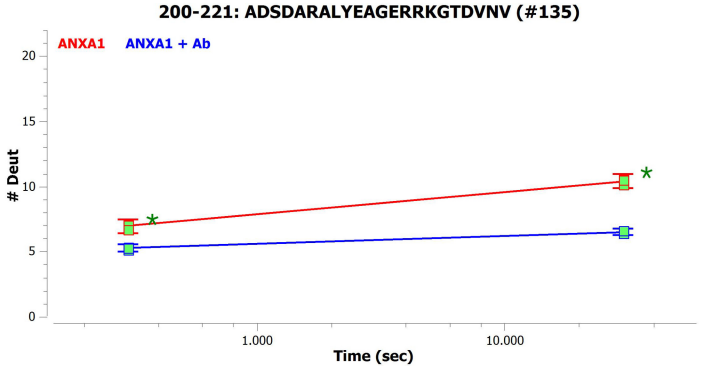

197-222: EDLADSDARALYEAGERRKGTDVNVF (#130)
ANXA1
ANXA1 + Ab
Deut
Time (sec)
1.000
10.000

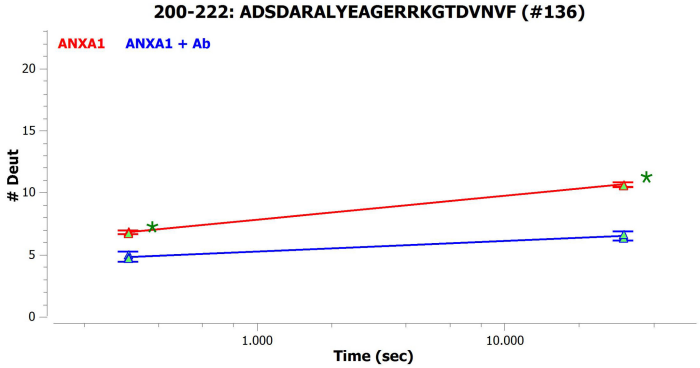

198-207: DLADSDARAL (#131)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

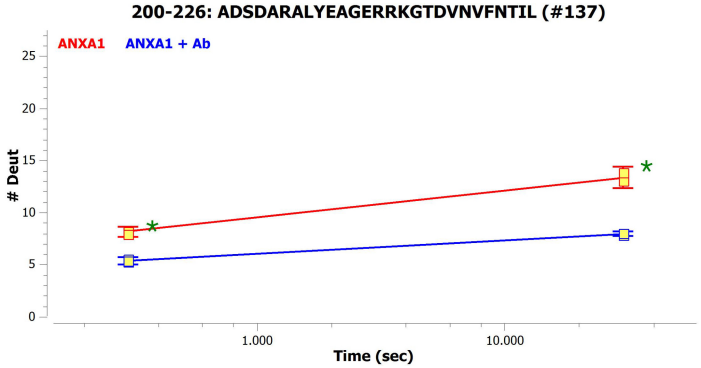

198-222: DLADSDARALYEAGERRKGTDVNVF (#132)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

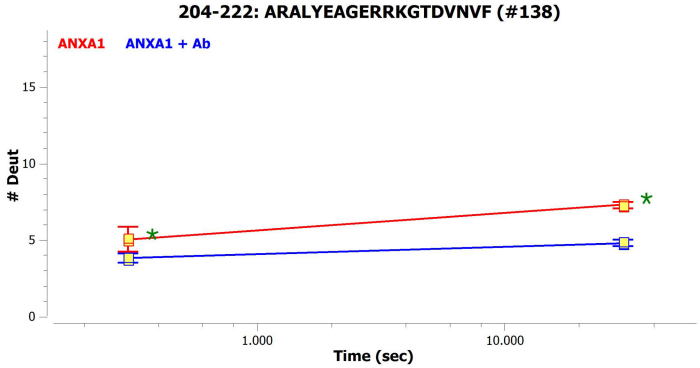

199-207: LADSDARAL (#133)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

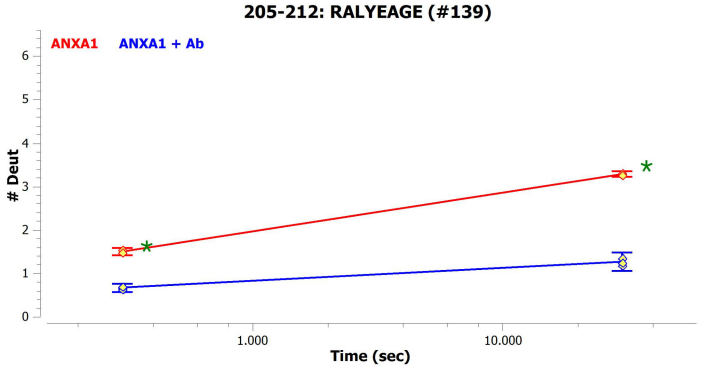

200-218: ADSDARALYEAGERRKGTD (#134)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

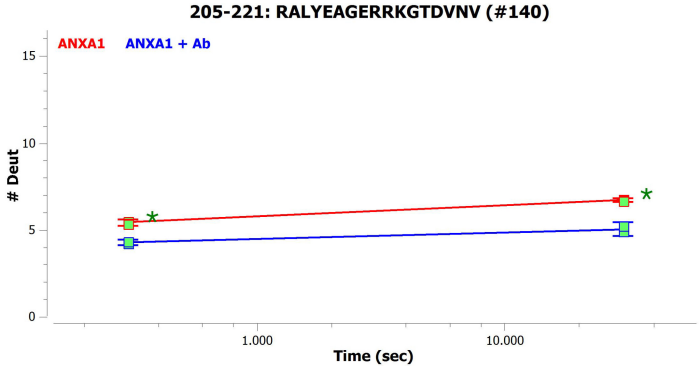

200-221: ADSDARALYEAGERRKGTDVNV (#135)
ANXA1
ANXA1 + Ab
Deut
Time (sec)


200-222: ADSDARALYEAGERRKGTDVNVF (#136)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

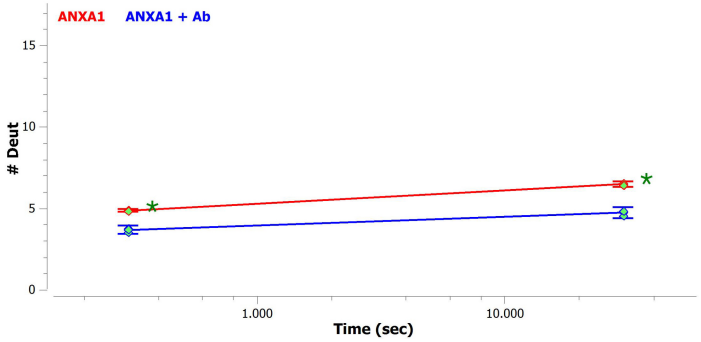

200-226: ADSDARALYEAGERRKGTDVNVFNTIL (#137)
ANXA1
ANXA1 + Ab
Deut
Time (sec)


204-222: ARALYEAGERRKGTDVNVF (#138)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

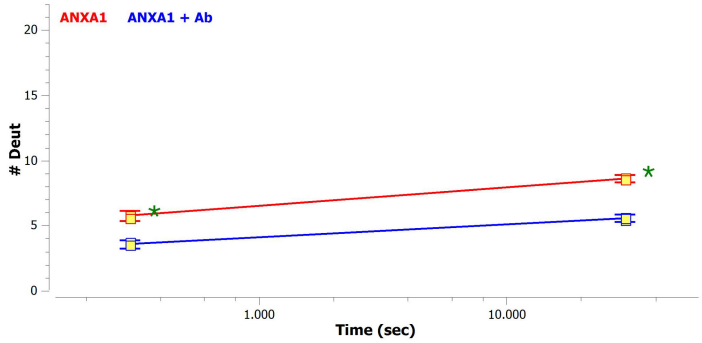

205-212: RALYEAGE (#139)
ANXA1
ANXA1 + Ab
Deut
Time (sec)


205-221: RALYEAGERRKGTDVNV (#140)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

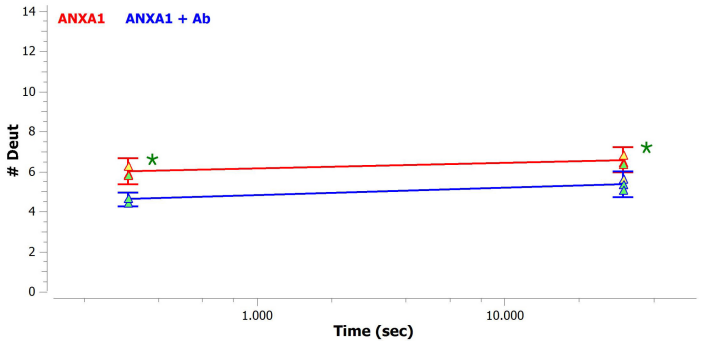
205-222: RALYEAGERRKGTDVNVF (#141)



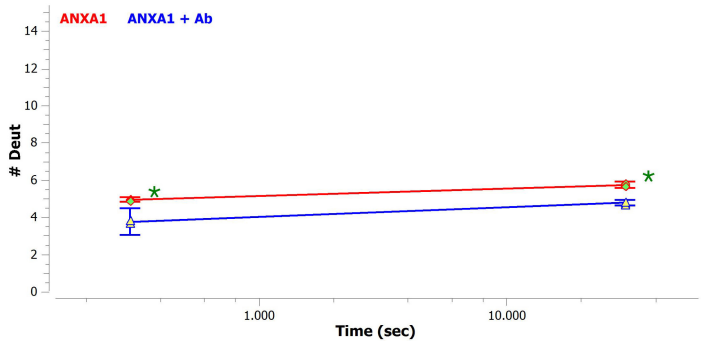
205-226: RALYEAGERRKGTDVNVFNTIL (#142)



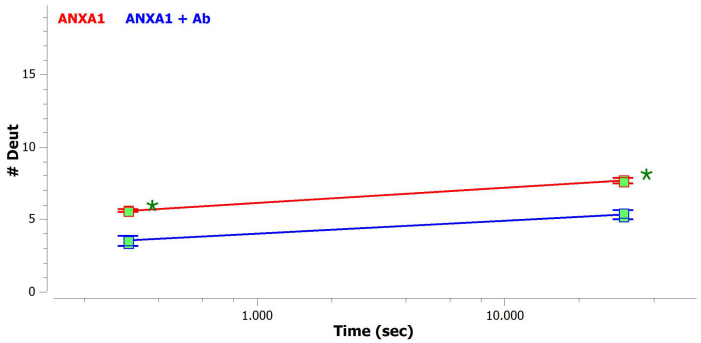
207-221: LYEAGERRKGTDVNV (#143)



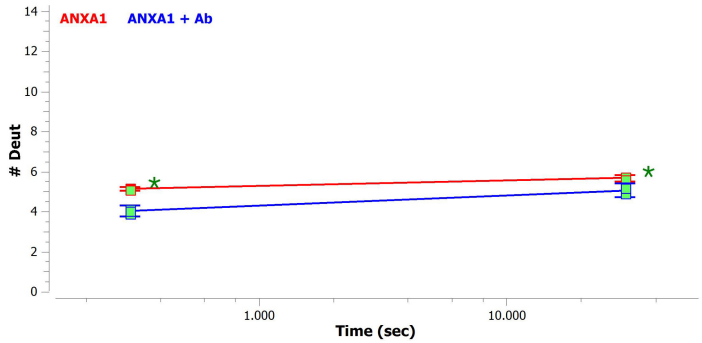
207-222: LYEAGERRKGTDVNVF (#144)



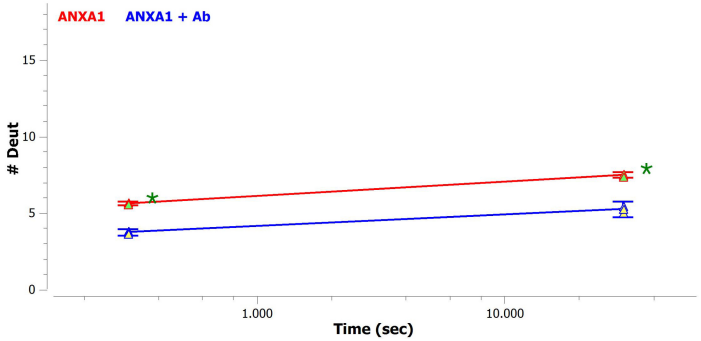
207-226: LYEAGERRKGTDVNVFNTIL (#145)



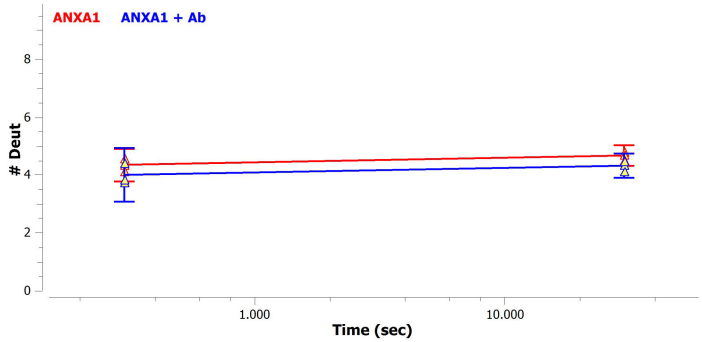
208-222: YEAGERRKGTDVNVF (#146)



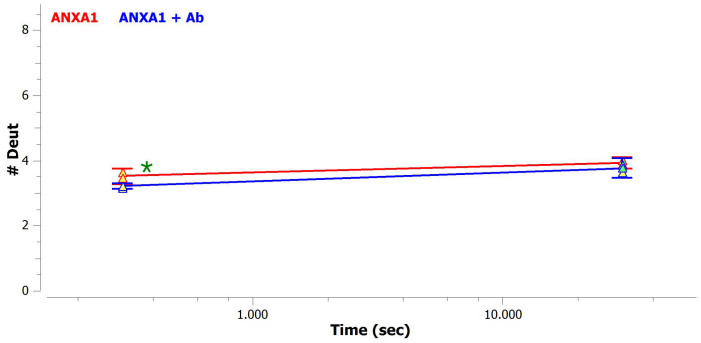
208-226: YEAGERRKGTDVNVFNTIL (#147)



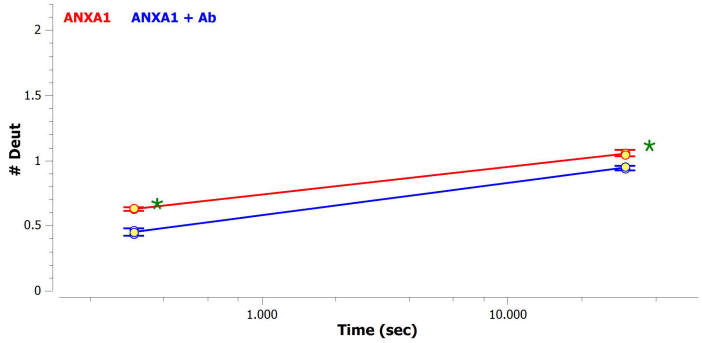
212-222: ERRKGTDVNVF (#148)



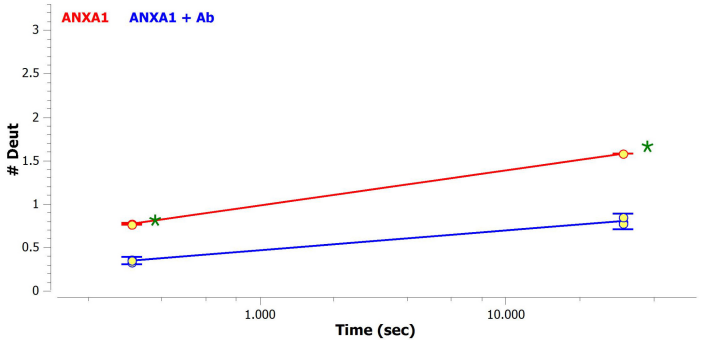
213-222: RRKGTDVNVF (#149)



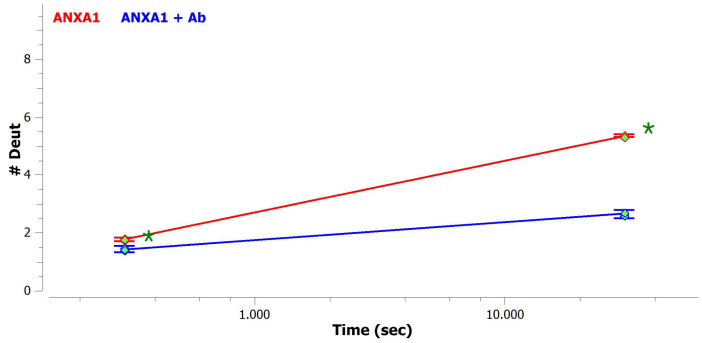
219-222: VNVF (#150)



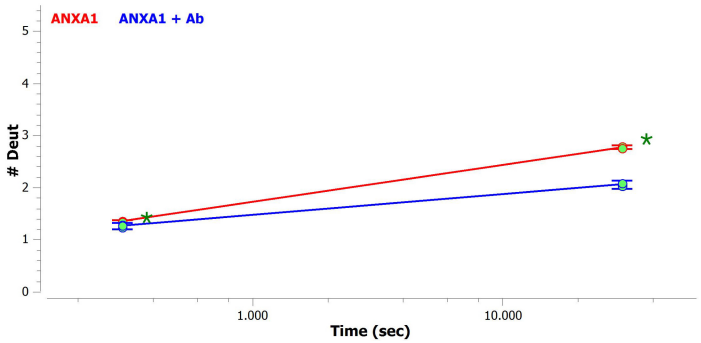
222-226: FNTIL (#151)



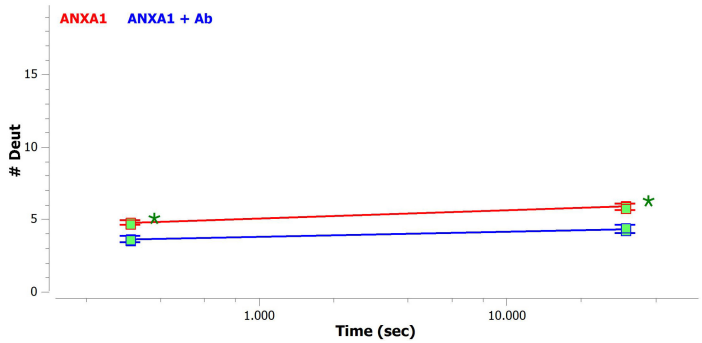
223-234: NTILTTRSYPQL (#152)



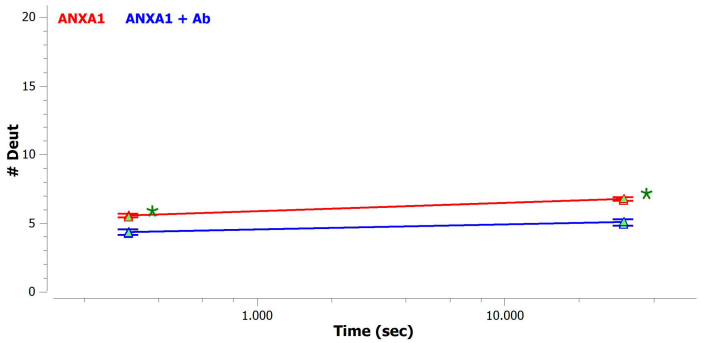
227-234: TTRSYPQL (#153)



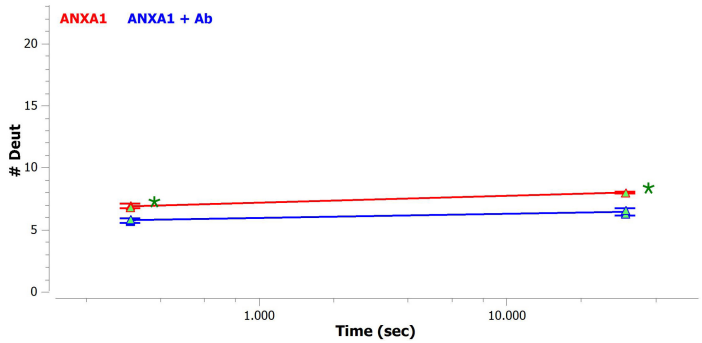
235-254: RRVFQKYTKYSKHDMNKVLD (#154)



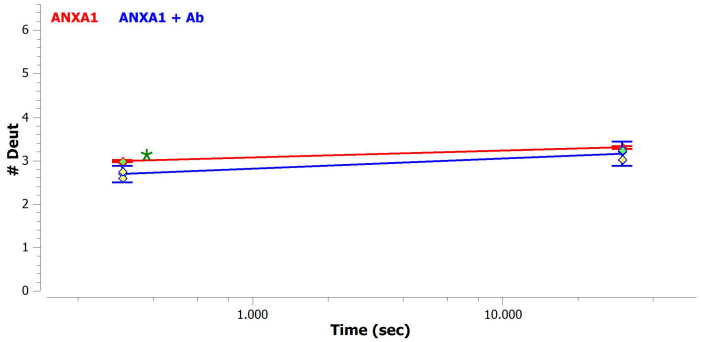
235-255: RRVFQKYTKYSKHDMNKVLDL (#155)



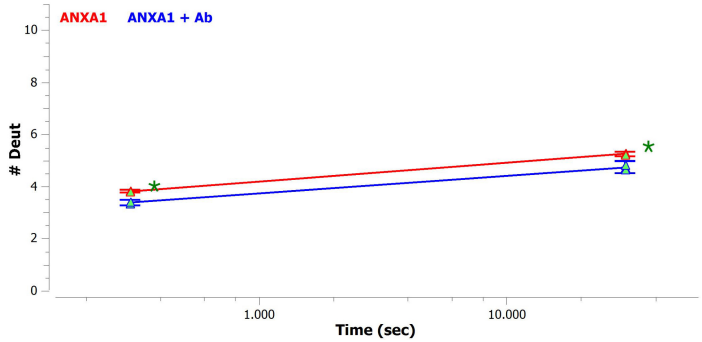
235-257: RRVFQKYTKYSKHDMNKVLDLEL (#156)



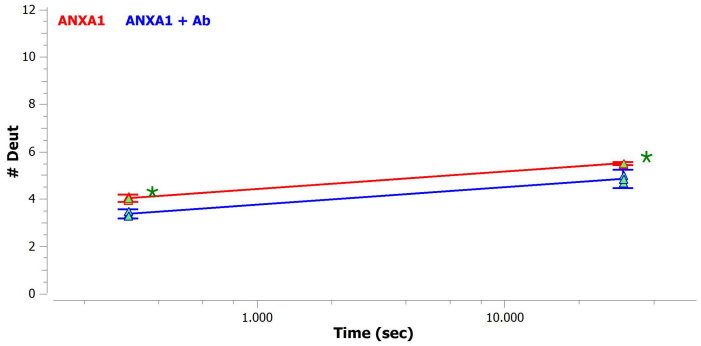
255-262: LELKGDIE (#157)



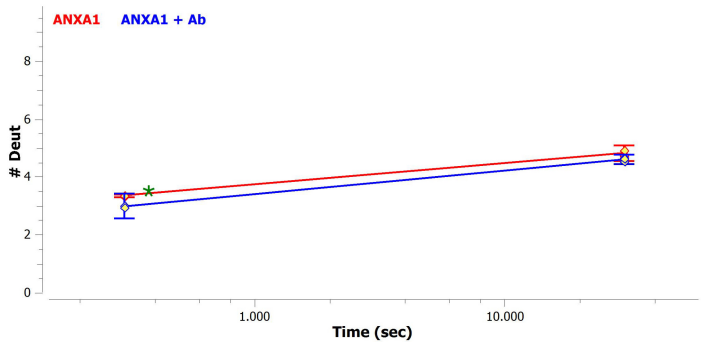
255-266: LELKGDIEKCLT (#158)



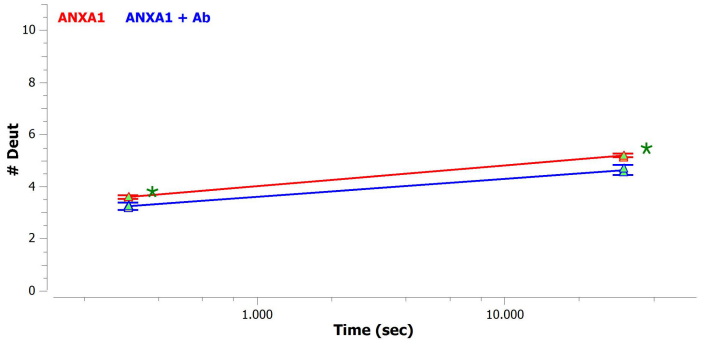
255-267: LELKGDIEKCLTA (#159)



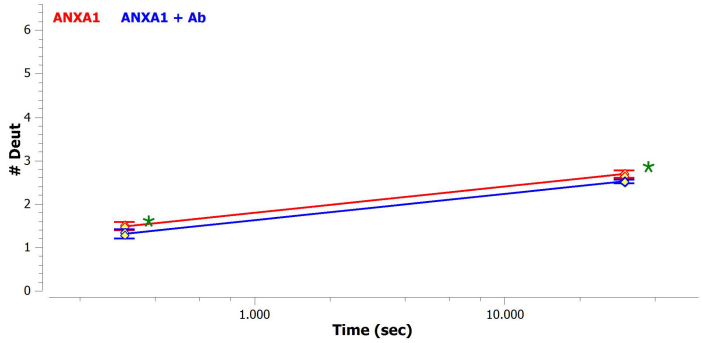
256-266: ELKGDIEKCLT (#160)



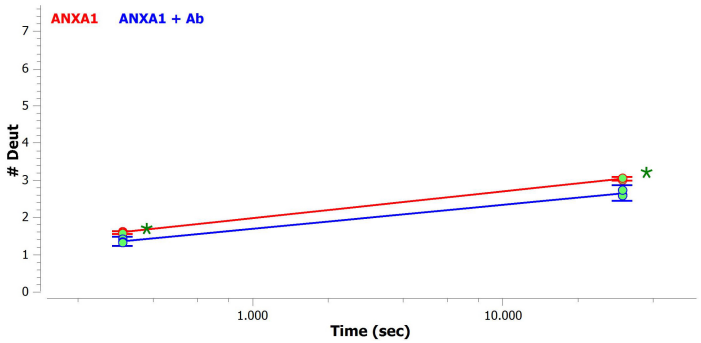
256-267: ELKGDIEKCLTA (#161)



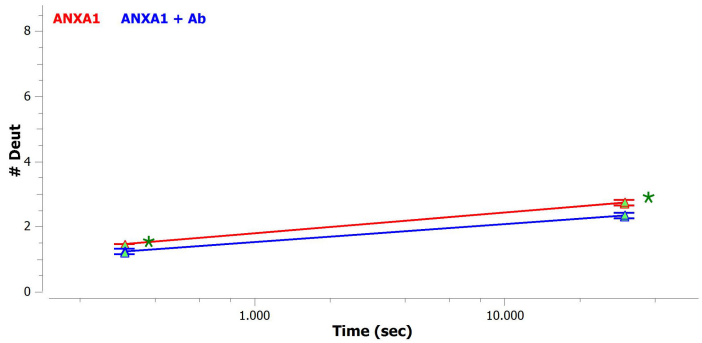
258-265: KGDIEKCL (#162)



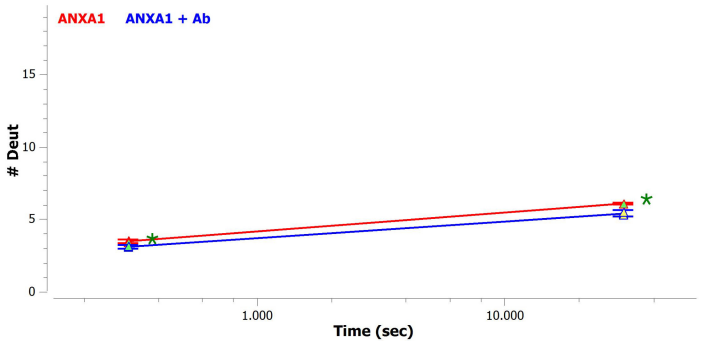
258-266: KGDIEKCLT (#163)



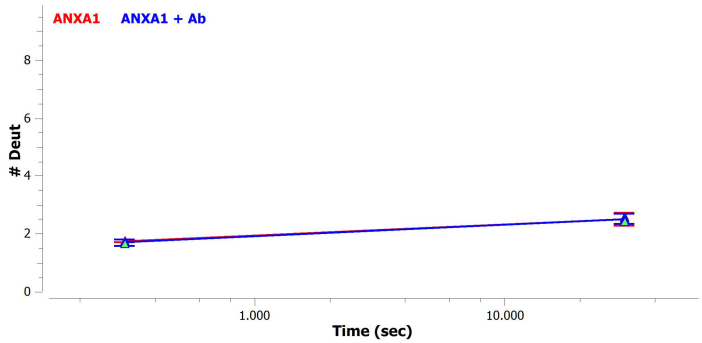
258-267: KGDIEKCLTA (#164)



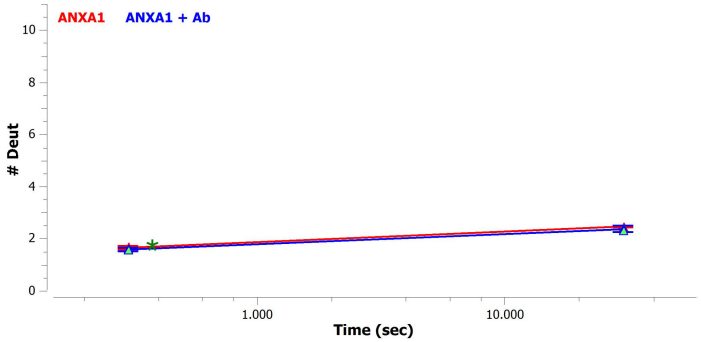
258-278: KGDIEKCLTAIVKCATSKPAF (#165)



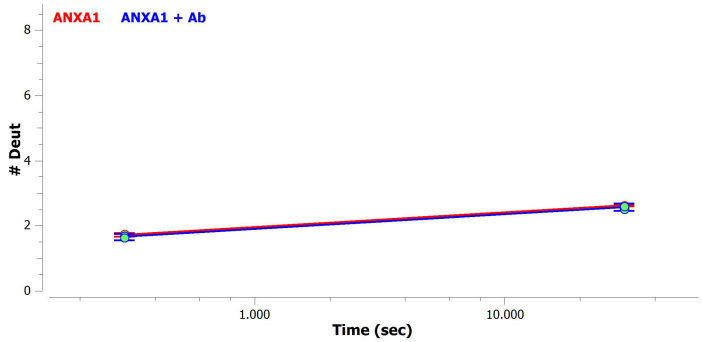
267-278: AIVKCATSKPAF (#166)



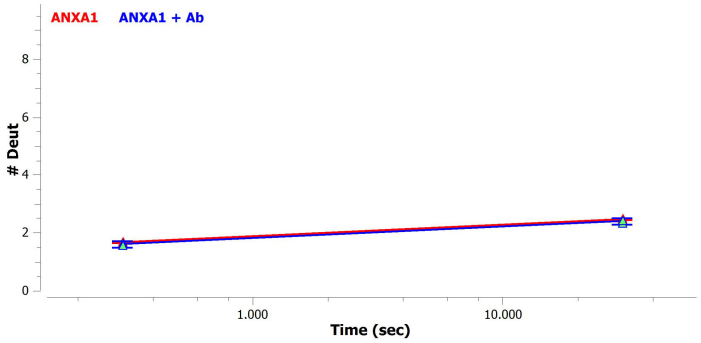
267-279: AIVKCATSKPAFF (#167)



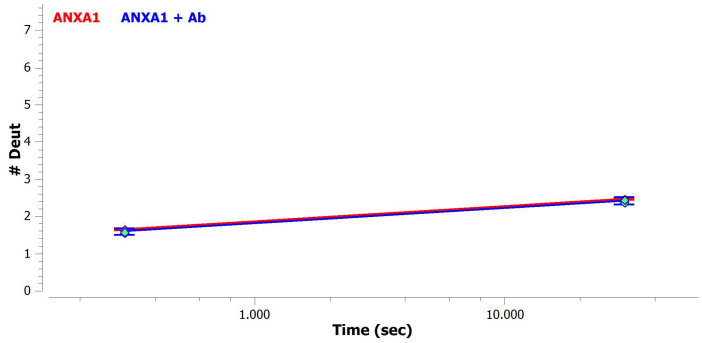
268-278: IVKCATSKPAF (#168)



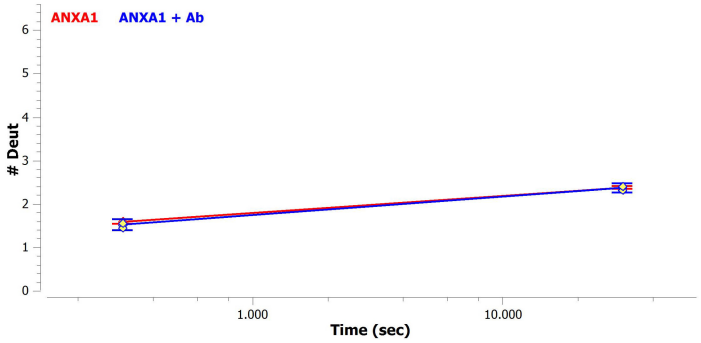
268-279: IVKCATSKPAFF (#169)



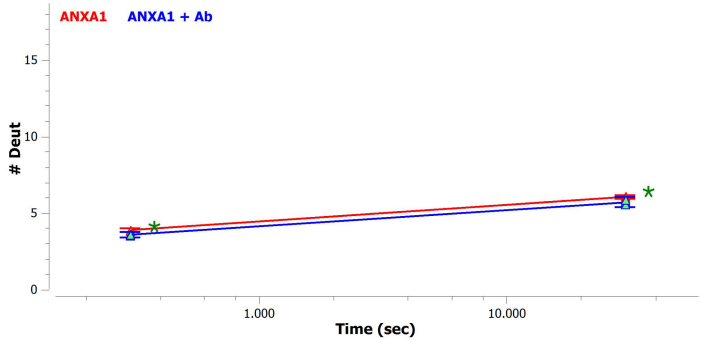
269-278: VKCATSKPAF (#170)



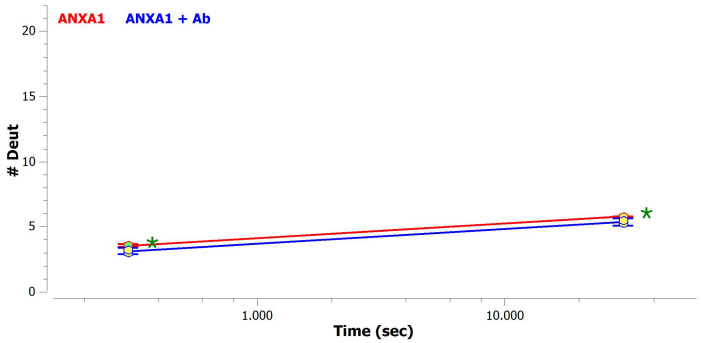
270-278: KCATSKPAF (#171)



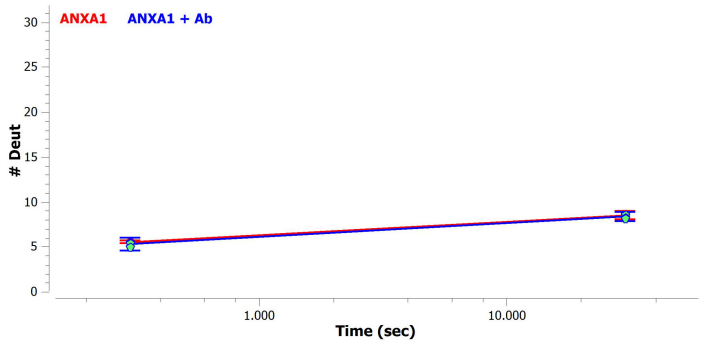
279-297: FAEKLHQAMKGVGTRHKAL (#172)



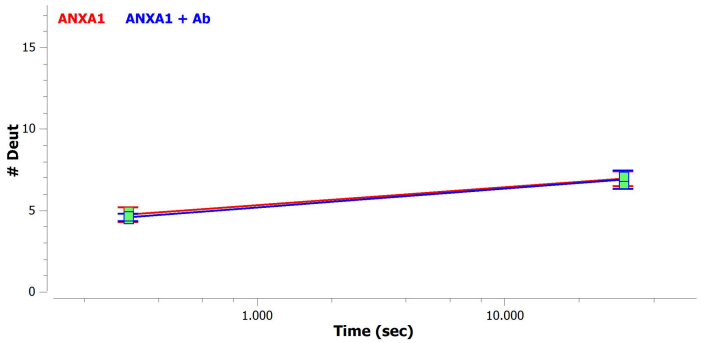
279-300: FAEKLHQAMKGVGTRHKALIRI (#173)



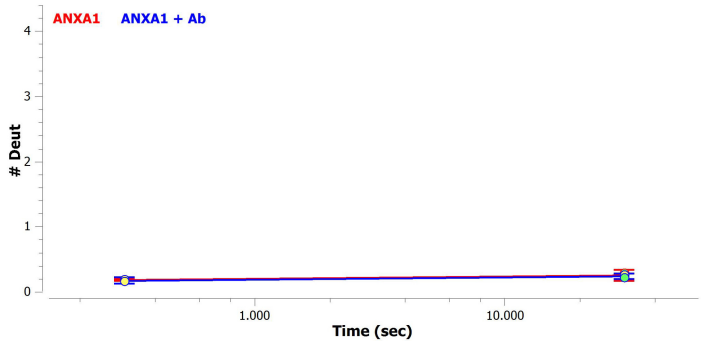
279-309: FAEKLHQAMKGVGTRHKALIRIMVSRSEIDM (#174)



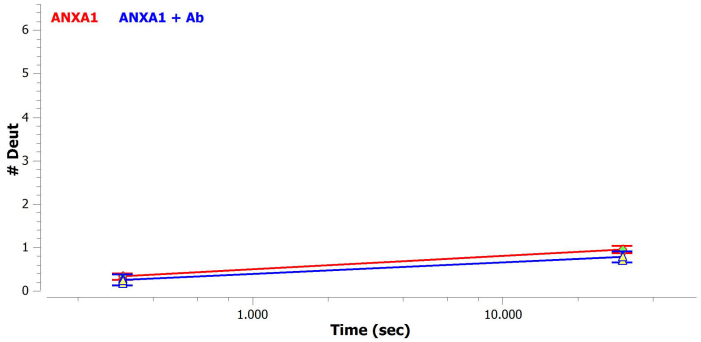
280-297: AEKLHQAMKGVGTRHKAL (#175)



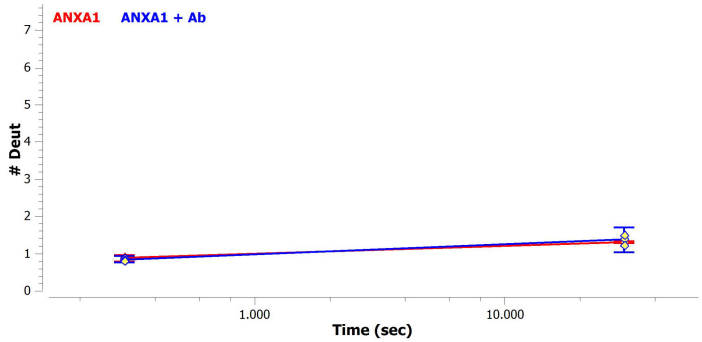
298-303: IRIMVS (#176)



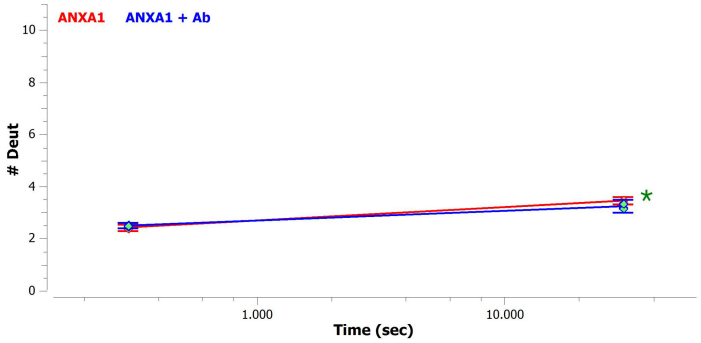
298-305: IRIMVSRS (#177)



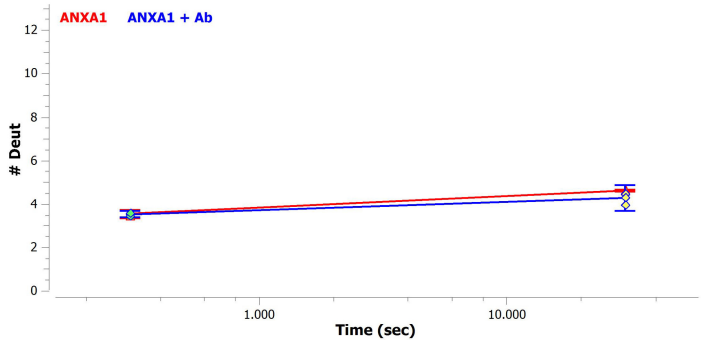
298-306: IRIMVSRSE (#178)

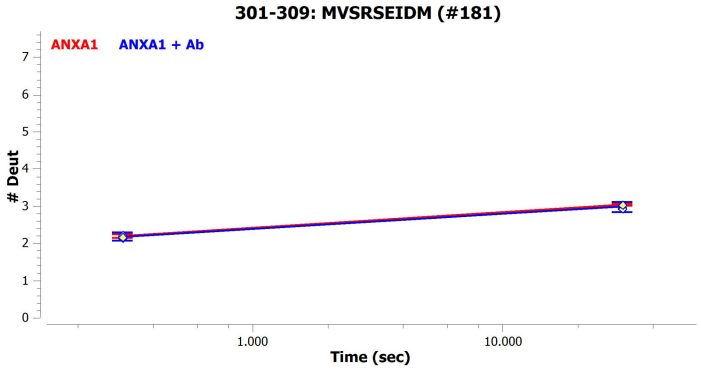


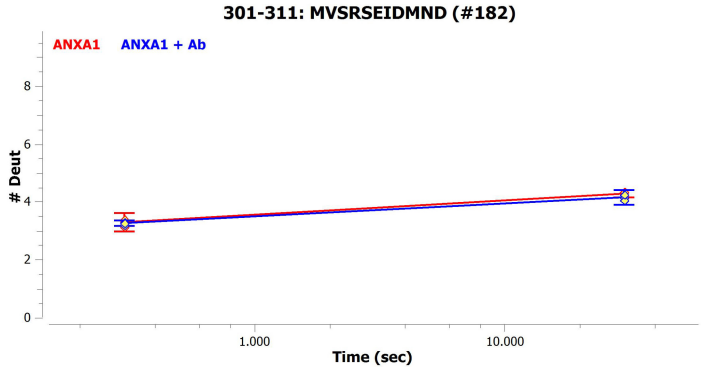
298-309: IRIMVSRSEIDM (#179)

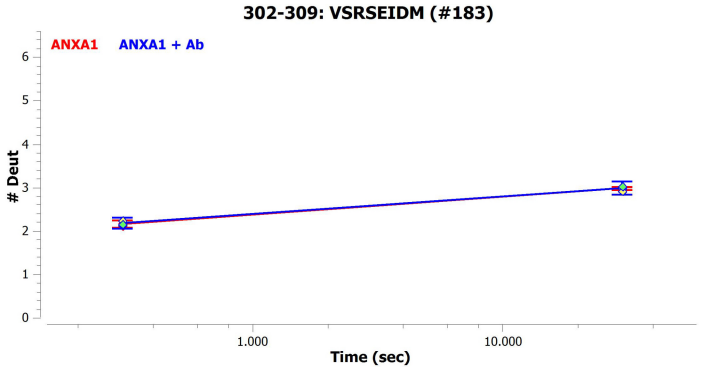


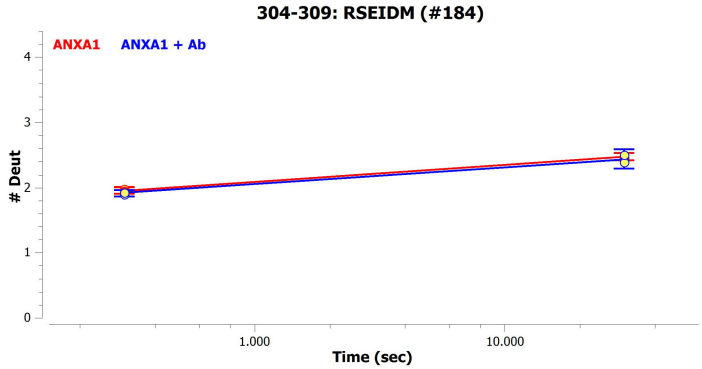
298-311: IRIMVSRSEIDMND (#180)

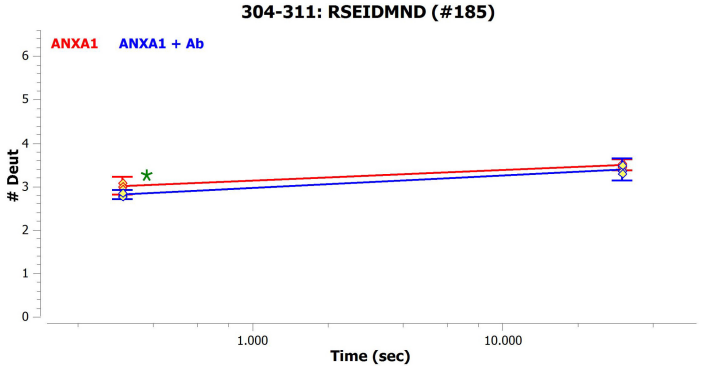


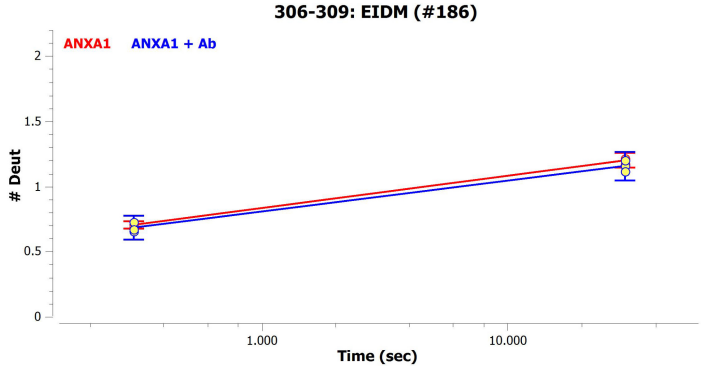

301-309: MVSRSEIDM (#181)
ANXA1
ANXA1 + Ab
Deut
Time (sec)
1.000
10.000

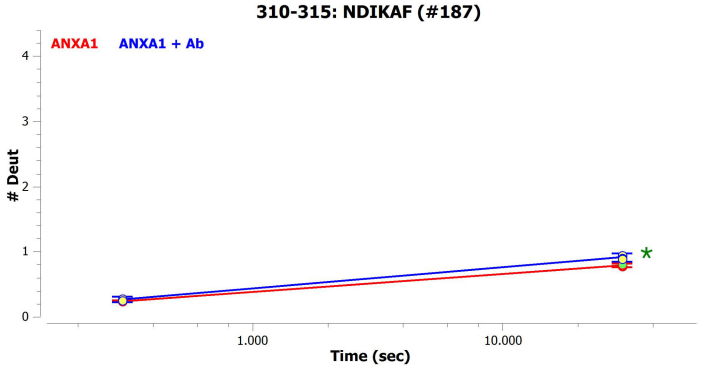

301-311: MVSRSEIDMND (#182)
ANXA1
ANXA1 + Ab
Deut
Time (sec)
1.000
10.000

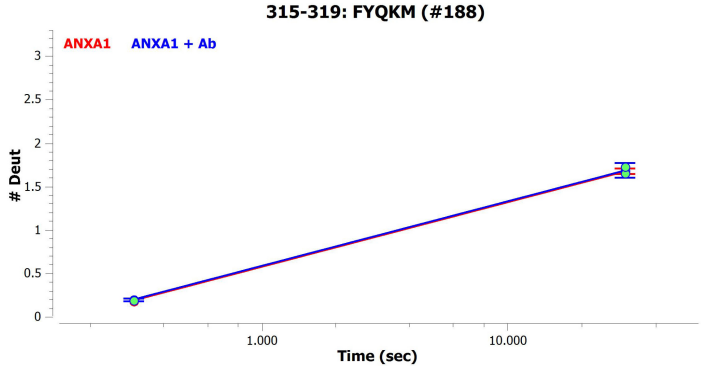

302-309: VSRSEIDM (#183)
ANXA1
ANXA1 + Ab
Deut
Time (sec)
1.000
10.000

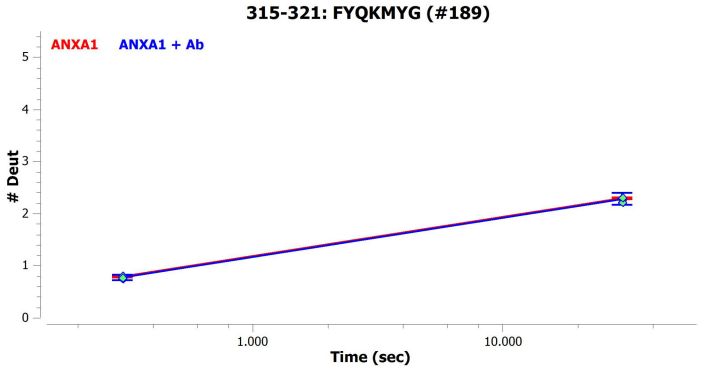

304-309: RSEIDM (#184)
ANXA1
ANXA1 + Ab
Deut
Time (sec)
1.000
10.000

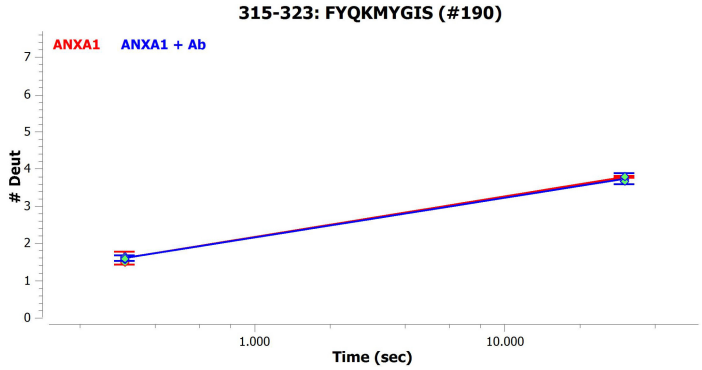

304-311: RSEIDMND (#185)
ANXA1
ANXA1 + Ab
*
Deut
Time (sec)
1.000
10.000

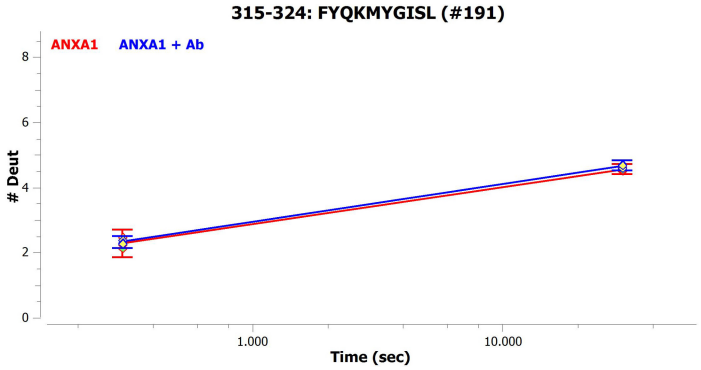

306-309: EIDM (#186)
ANXA1
ANXA1 + Ab
Deut
Time (sec)
1.000
10.000

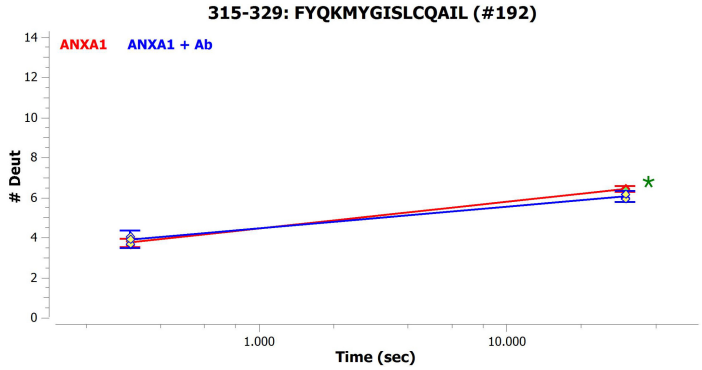

310-315: NDIKAF (#187)
ANXA1
ANXA1 + Ab
*
Deut
Time (sec)
1.000
10.000

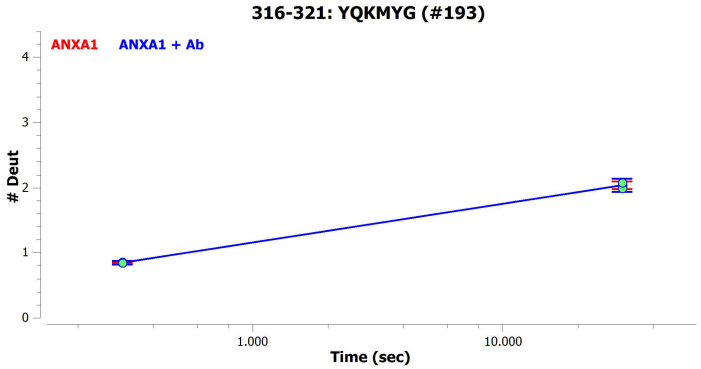

315-319: FYQKM (#188)
ANXA1
ANXA1 + Ab
Deut
Time (sec)
1.000
10.000

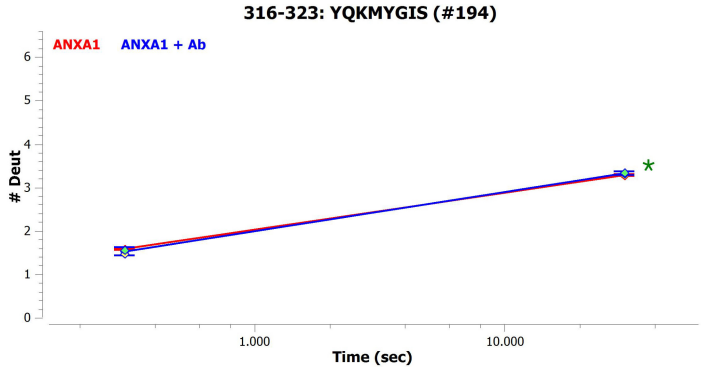

315-321: FYQKMYG (#189)
ANXA1
ANXA1 + Ab
Deut
Time (sec)
1.000
10.000

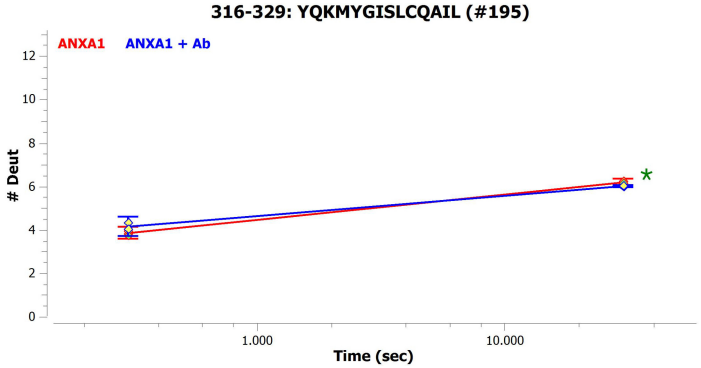

315-323: FYQKMYGIS (#190)
ANXA1
ANXA1 + Ab
Deut
Time (sec)
1.000
10.000

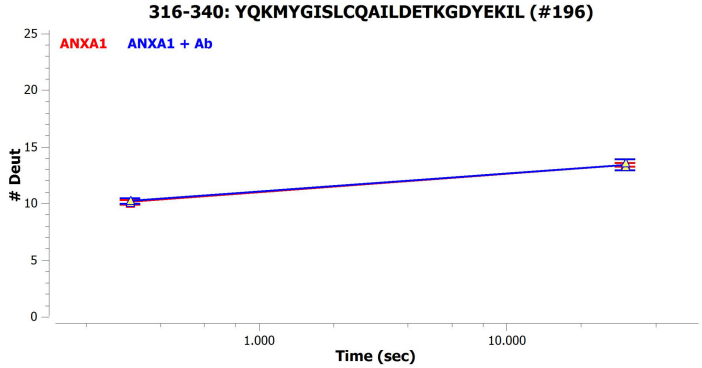

315-324: FYQKMYGISL (#191)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

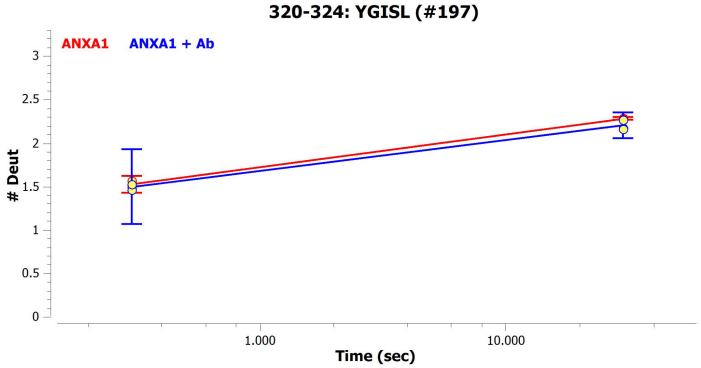

315-329: FYQKMYGISLCQAIL (#192)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

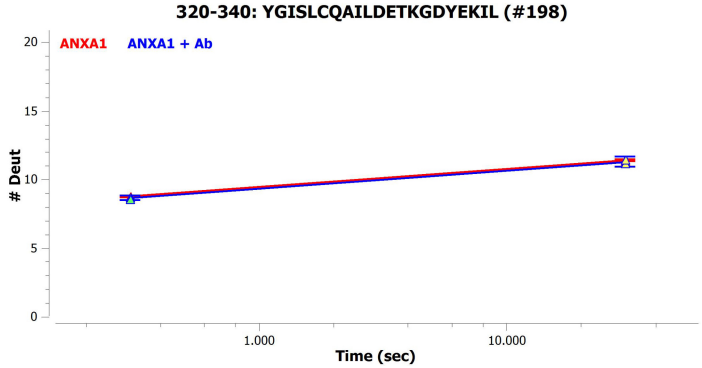

316-321: YQKMYG (#193)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

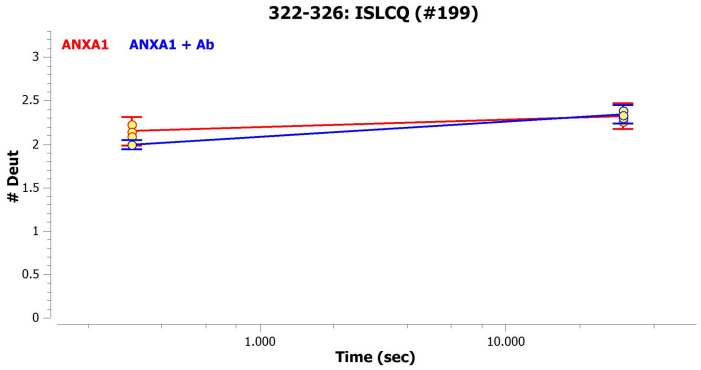

316-323: YQKMYGIS (#194)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

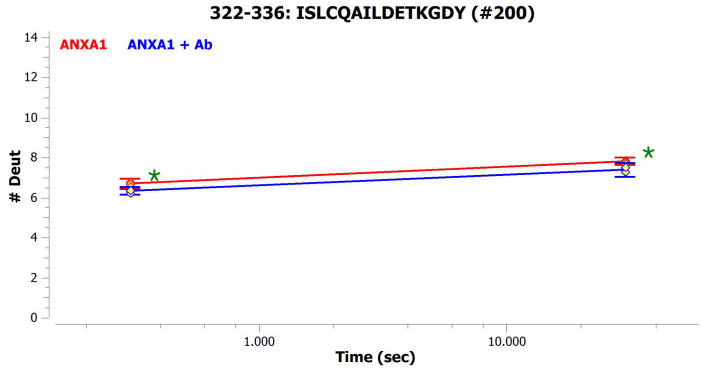

316-329: YQKMYGISLCQAIL (#195)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

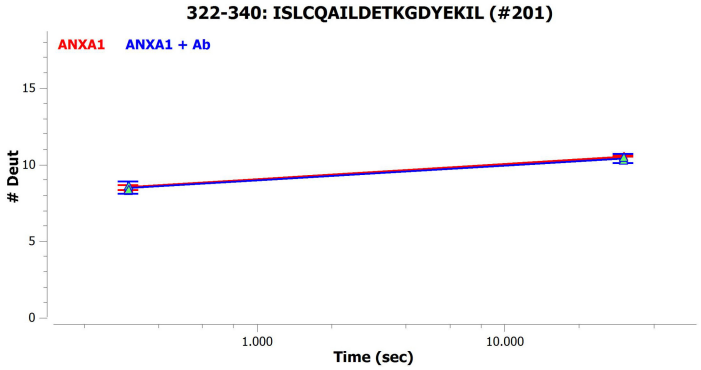

316-340: YQKMYGISLCQAILDETKGDYEKIL (#196)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

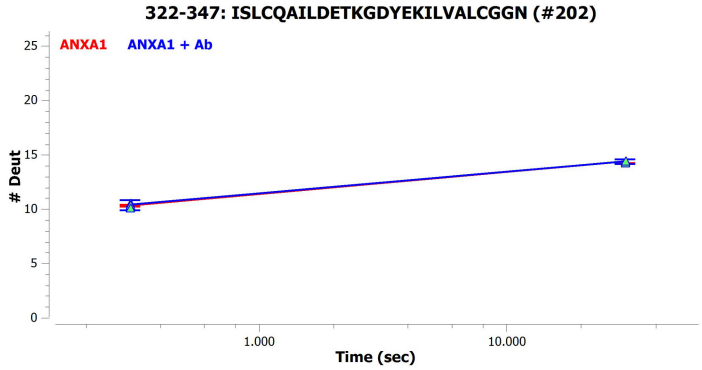

320-324: YGISL (#197)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

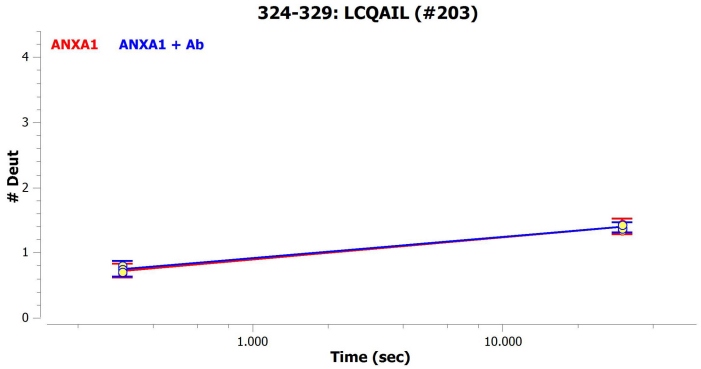

320-340: YGISLCQAILDETKGDYEKIL (#198)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

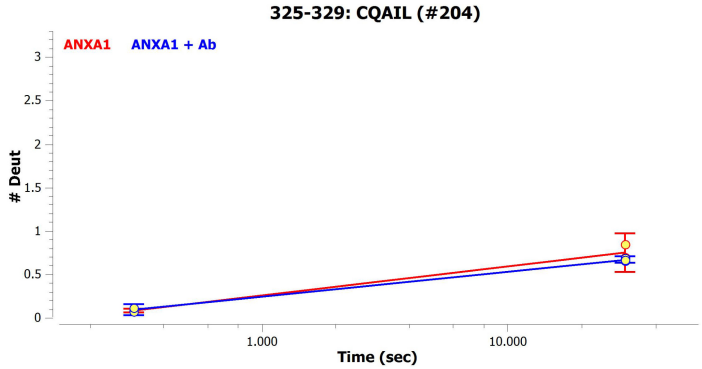

322-326: ISLCQ (#199)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

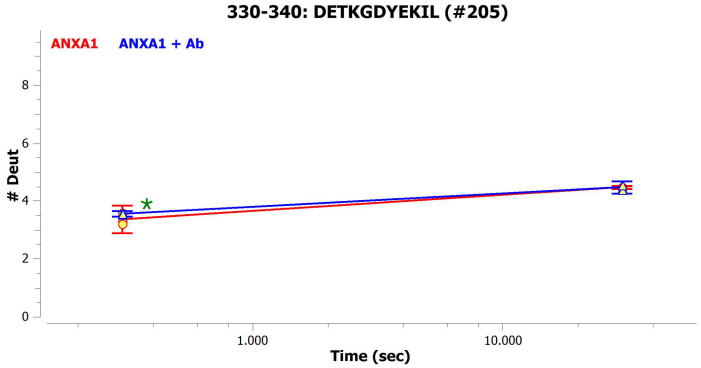

322-336: ISLCQAILDETKGDY (#200)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

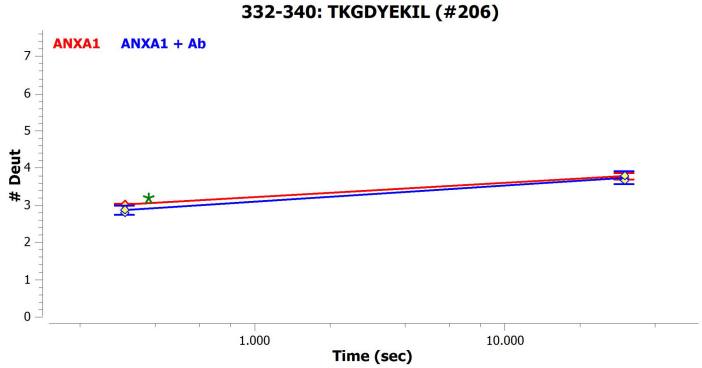

322-340: ISLCQAILDETKGDYEKIL (#201)
ANXA1
ANXA1 + Ab
Deut
Time (sec)


322-347: ISLCQAILDETKGDYEKILVALCGGN (#202)
ANXA1
ANXA1 + Ab
Deut
Time (sec)


324-329: LCQAIL (#203)
ANXA1
ANXA1 + Ab
Deut
Time (sec)


325-329: CQAIL (#204)
ANXA1
ANXA1 + Ab
Deut
Time (sec)


330-340: DETKGDYEKIL (#205)
ANXA1
ANXA1 + Ab
Deut
Time (sec)


332-340: TKGDYEKIL (#206)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

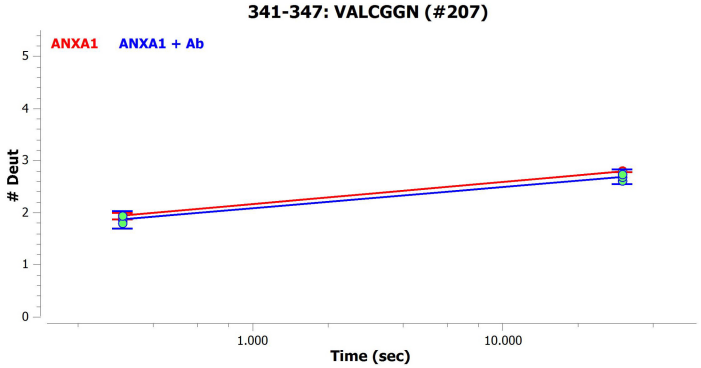

341-347: VALCGGN (#207)
ANXA1
ANXA1 + Ab
Deut
Time (sec)

Figure S7: All peptide uptake plots of the **bead based digest**. used in this publication of the unbound state shown in red and the bound state shown in blue. Time points marked with a star show significant differences ($p<0.05$) between both states determined by students t-test. The Y-Axis scaled to 100% possible exchange of each peptide and the error bars are based on the 95% confidence level.