



STRUCTURAL
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Supporting information for article:

Crystal structures of human ENPP1 in apo and bound forms

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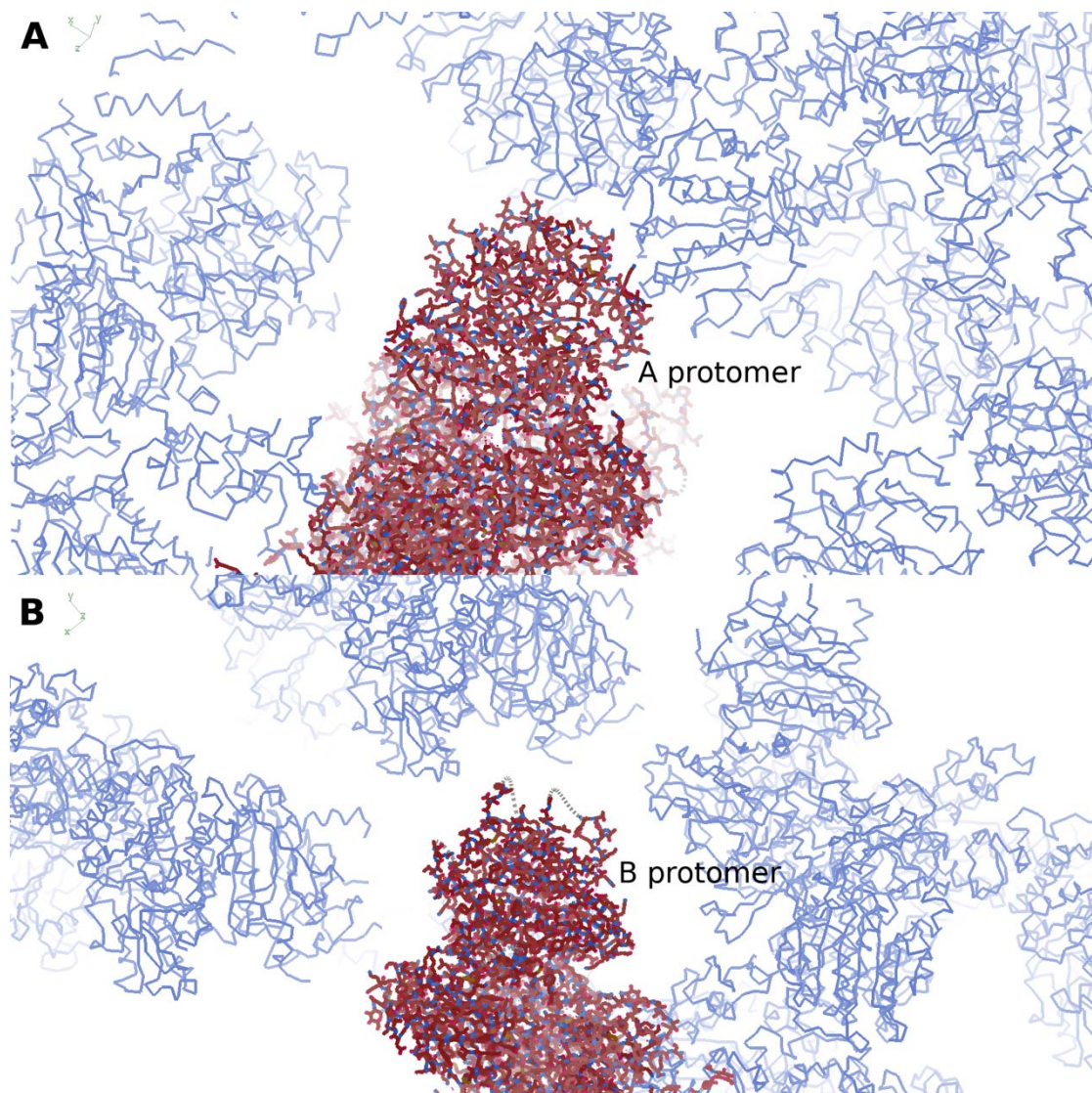


Figure S1 Crystal contacts of hENPP1 (nuclease-like domain). A: Shows the packing of the crystal around the A protomer with the nuclease-like domain in the center of the figure (in red). B: Shows the packing of the crystal around the B protomer with the nuclease-like domain in the center of the figure (in red).

A

Accession	Protein	Log Prob	Peptides	% Cov.
P22413	ENPP1 ₉₈₋₉₂₅	3757	767	96%

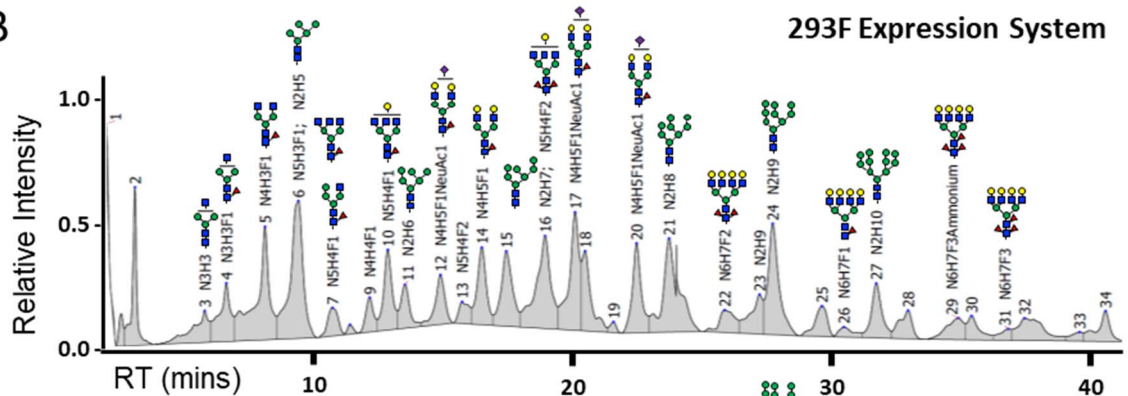
>sp|P22413|ENPP1_HUMAN Ectonucleotide pyrophosphatase/phosphodiesterase family member

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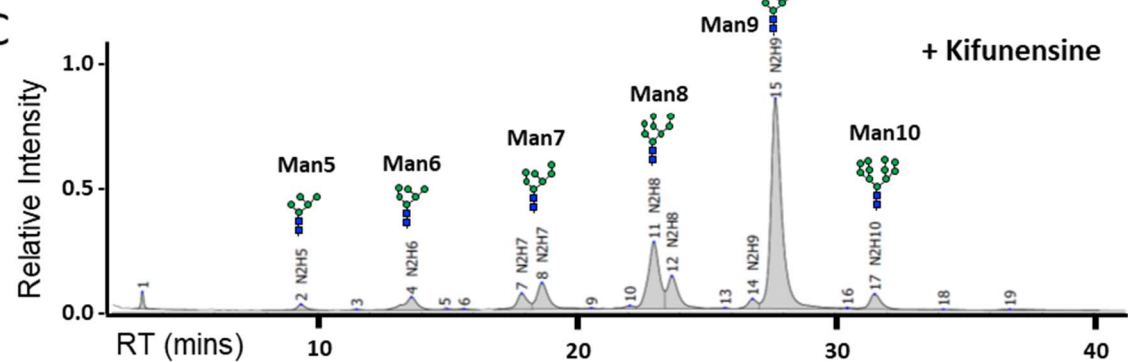
10      20      30      40      50      60      70      80      90     100
MERDGCAGGSRGEGGGRAPREGPAGNGRDRGRSHAAEAPGDQAAASLLAPMDVGEELPEKAAARTAKDPNTYKVLVLSVLCVLTILGCI FGLKPS
110     120     130     140     150     160     170     180     190     200
CAKEVKSKCGRCFERTFGNCRCDAAACVELGNCCLDYQETCIEPEHIWTCNKFRCEKRLTRSLCACSDCKDKGDCINYSVCGEKSWEPECSINE
210     220     230     240     250     260     270     280     290     300
PQCAGFETPPTLLFSLDGFRAEYLHTWGLLPVISKLKKCGTYTKNMRPVYPTKTFPNHYSIVTGLYPESHGIIDNKMYDPKMNASFSLKSKEKFNPEW
310     320     330     340     350     360     370     380     390     400
YKGEPIWVTAKYQGLKSGTFFWPGSDVEINGIFPDIYKMYNGSVPFEEERILAVLQWLQPKDERPHFYTYLLEPDSSSGHSYGPVSSEVIKALQRVDGMV
410     420     430     440     450     460     470     480     490     500
GMLMDGLKELNLHRCNLNLIISDHGMEQGSCCKYIYLNKYLGDVKNIKVIYGAARLRPSDVPDKYYSFNYEGIARNLSCREPNQHFKPYLKHFLPKRLH
510     520     530     540     550     560     570     580     590     600
FAKSDRIEPLTFYLDPQWLALNPSERKYCGSGFHGSDNVFSNMQALFVYGGPGFKHGEADTFENIEVYNLMCDLLNLTLPAPNGTHGSLNHLKKNPVY
610     620     630     640     650     660     670     680     690     700
TPKHPKEVHPLVQCPTFRNPDNLGCSNPNPILPIEDFQTQFNLTVAEEKIKHETLPGYRPRVLQKENTICLLSQHQFMSGYSQDILMPLWTSYTVDRN
710     720     730     740     750     760     770     780     790     800
DSFSTEDFSNCLYQDFRIPLSPVHKCSFYKNTKVSYGFLSPQLNKNSSGIYSEALLTNIIVPMYQSFQVIWRYPHDTLRLRYAEERNGVNVVSGPVFD
810     820     830     840     850     860     870     880     890     900
FDYDGRCDSENLRQKRRVIRNQEILIPTHFFVILVTSCKDTSQTLPHCENLDTLAFILPHRTDNSESCVHGKHDSWVEELMLHRARITDVEHITGLSF
910     920
YQQRKEFVSDILKLTLPFTFSQED

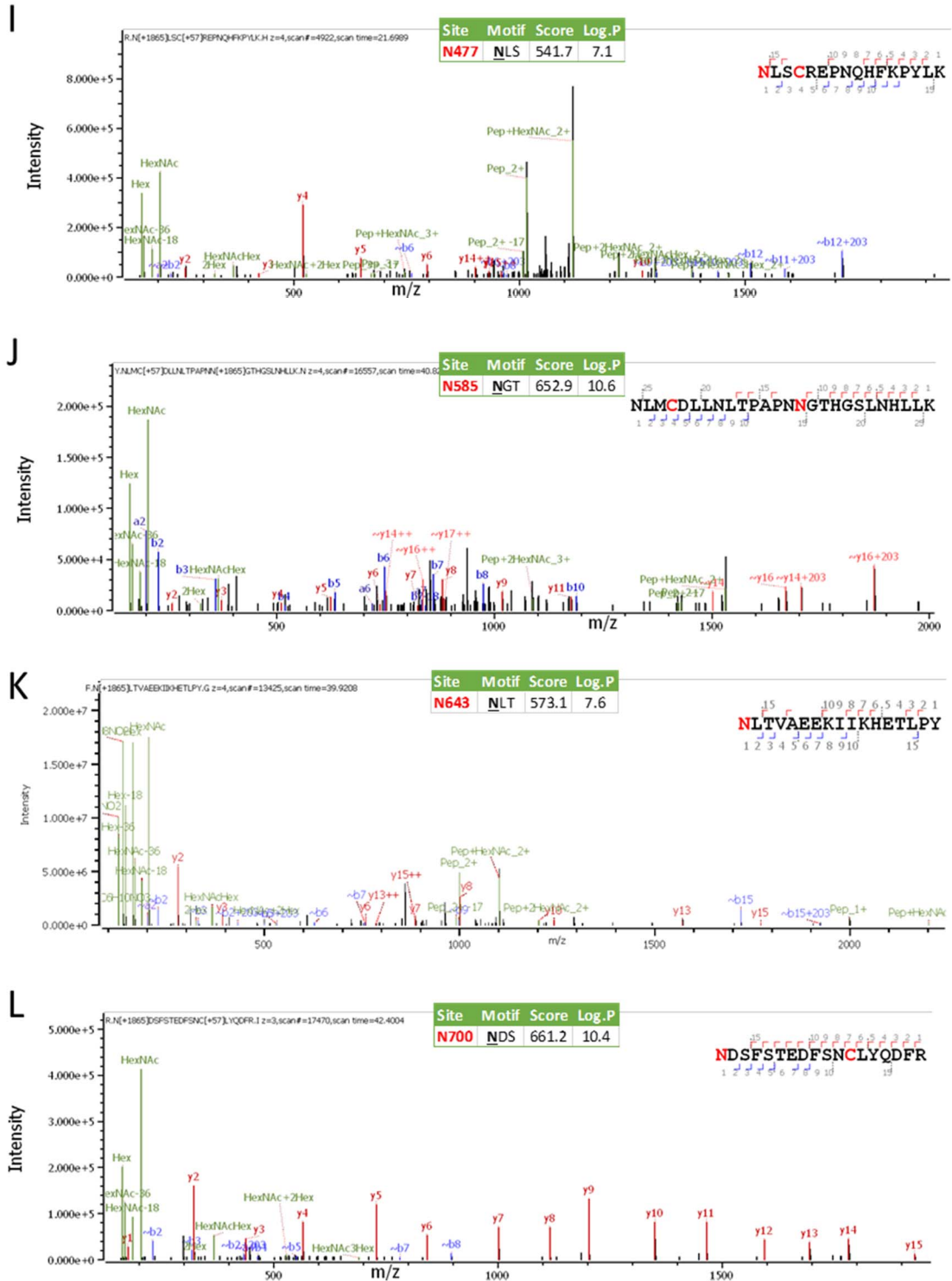
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B



C





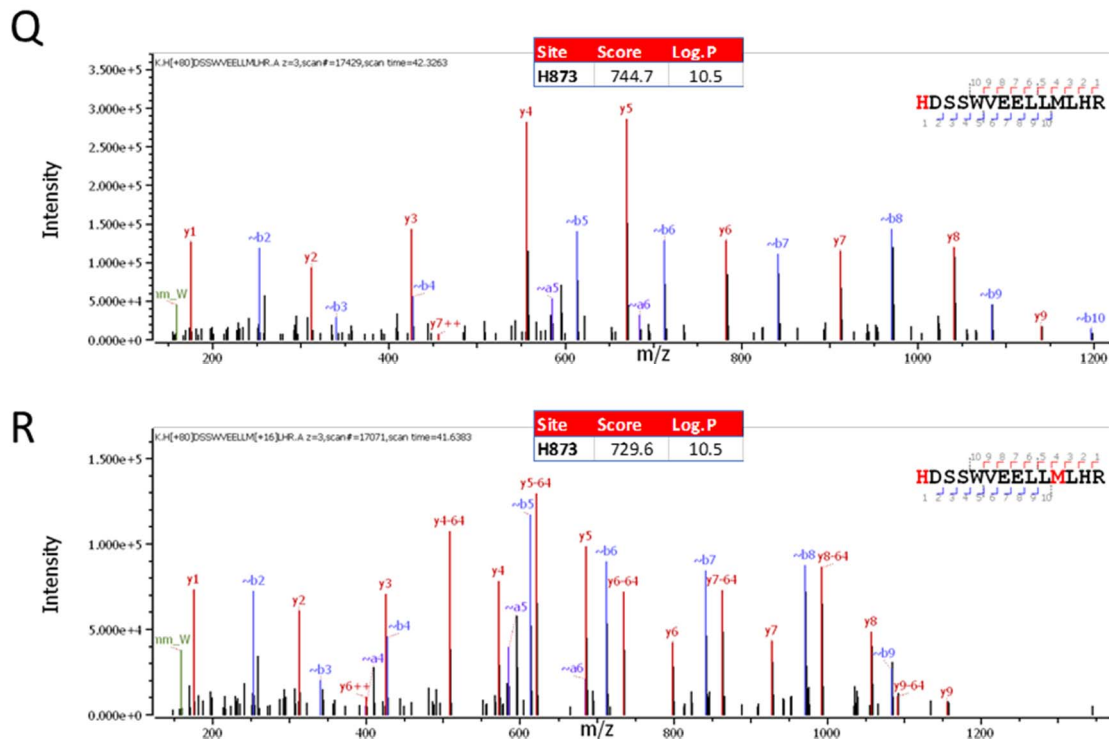


Figure S2 Mass spectrometric analysis. **A:** Peptide sequencing of a kifunensine-treated, affinity purified hENPP1-HIS preparation by LC-MSMS confidently identified 767 unique peptides with 96% sequence coverage matching of hENPP1 residues 98-925 (green). Nine predicted N-linked glycosylation sites of hENPP1 are highlighted in yellow, based on UniProt entry P22413. **B:** N-linked glycoprofiling of hENPP1 expressed in Freestyle 293 cells identified over 30 different glycan structures, including predominantly high-mannose and complex, core-fucosylated glycan structures. **C:** Kifunensine treatment significantly reduced N-glycan heterogeneity and resulted in hENPP1 decorated with high-mannose-type glycan structures of Man9 > Man8 > Man7 type. **D:** Intact mass determination of a kifunensine-treated hENPP1 154-925 construct is consistent with 13.8-15.0kDa N-linked glycan mass additions (+/- Hex). The major observed mass of 100,980Da matches the expected protein mass (MW~86,556.5Da) decorated with 4xMan8 + 4xMan9 N-linked glycans. **E:** A diagram depicting the relative occupancy of N-linked glycosylation sites (green) or phosphorylation sites (red) of hENPP1 based on spectrum counting (e.g. fraction of modified versus total peptide spectra matches per site; height of bars and whiskers correspond to average +/- standard error of three independent biological replicates). **F-N:** Highly confident LC-MSMS evidence spectra showing Man9-modified glycopeptide sequences for glycosylation sites N179, N285, N341, N477, N585, N700, N731 and N48. **O-R:** LC-MSMS evidence for peptide sequences containing phosphorylated T256 or H873.

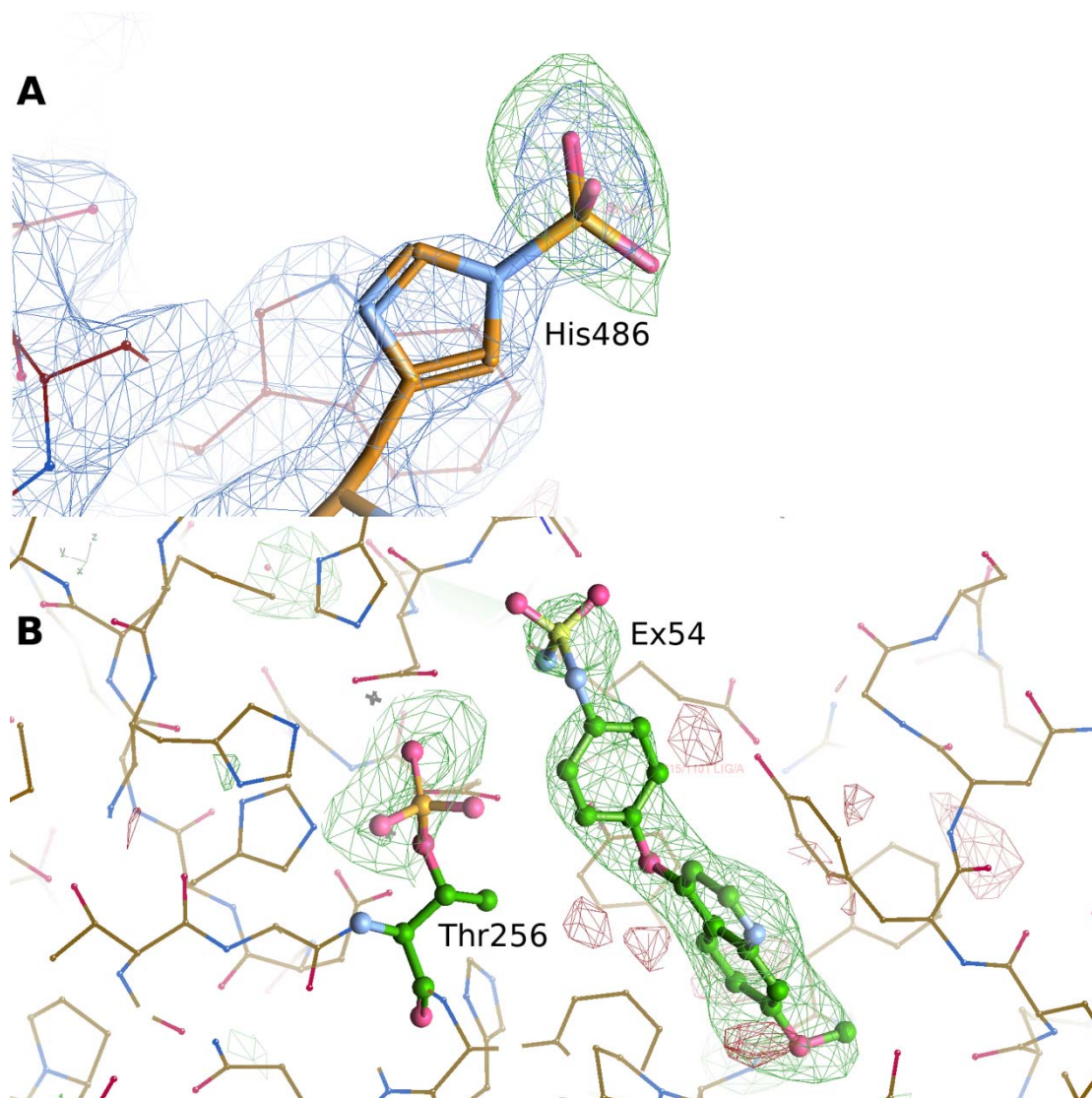


Figure S3 Difference density for phosphorylation sites A: Difference density map (green) set at 3σ for His486 showing significant difference density in the original MR map generated (difference density for the phosphate is still seen at 7σ). The blue density is set at 1.5σ . B: Difference density map set at 3σ for both Thr256 and Ex54 in the catalytic site of hENPP1. Figure was generated using Coot and The Gimp.

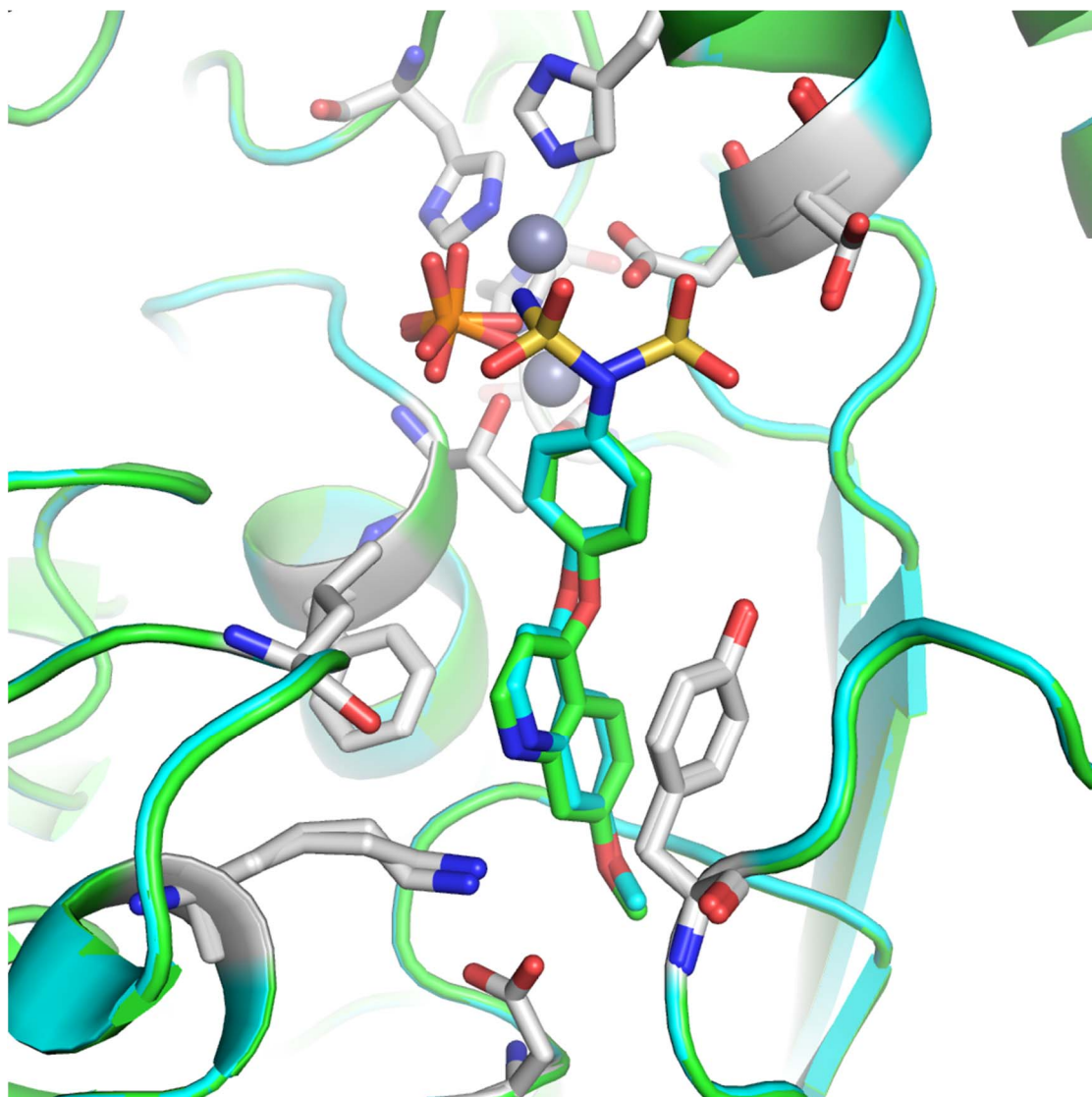
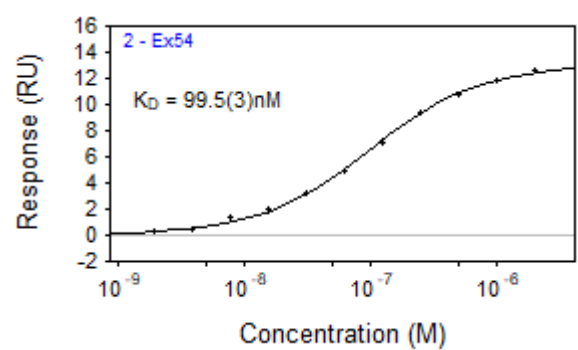
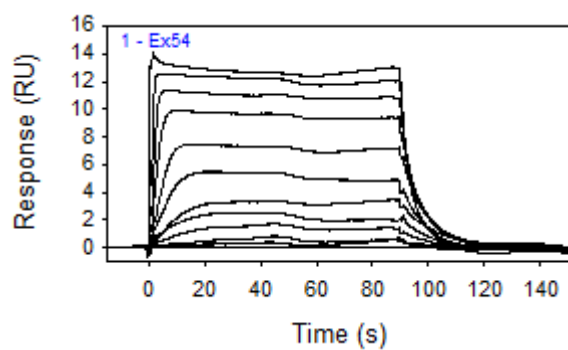
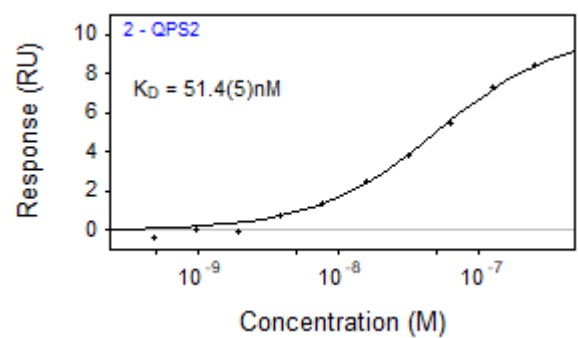
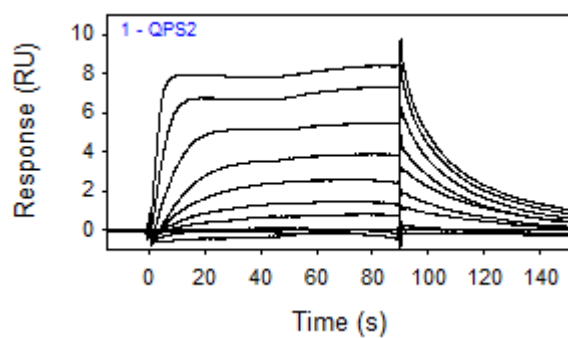
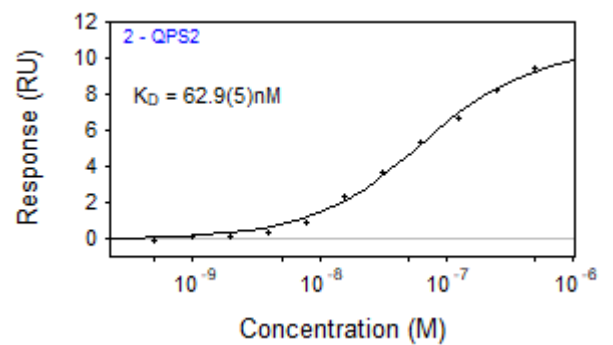
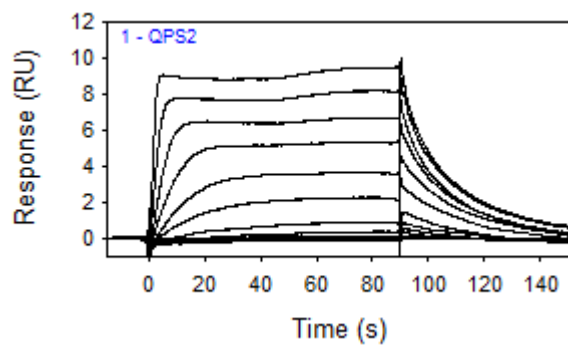
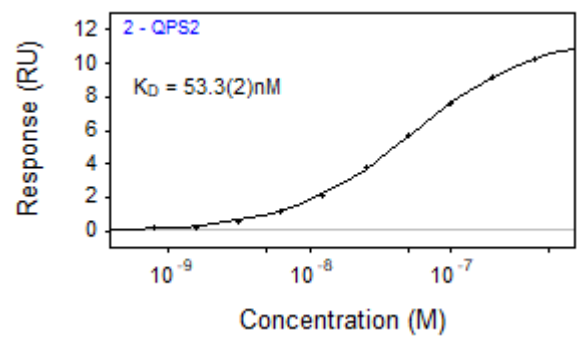
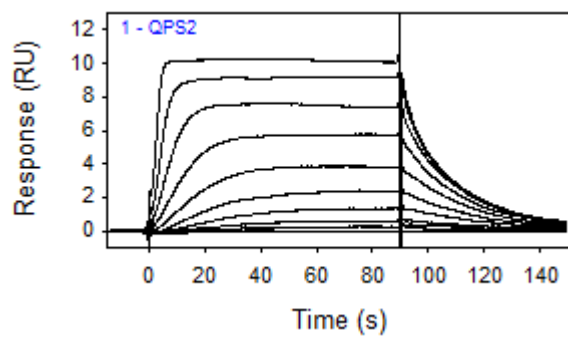
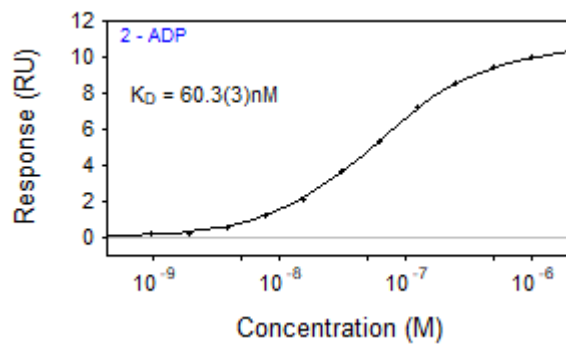
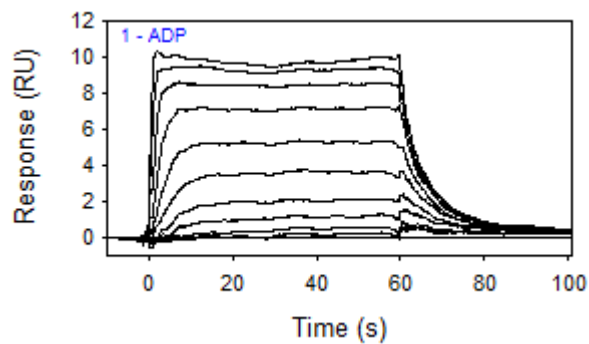
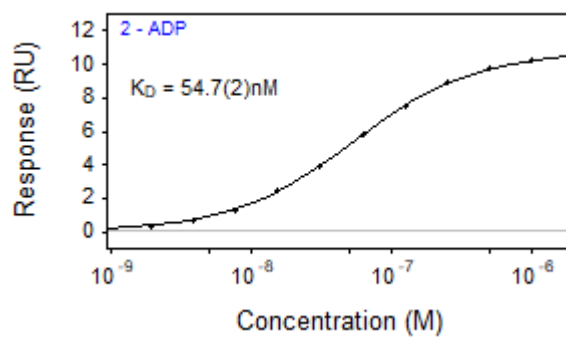
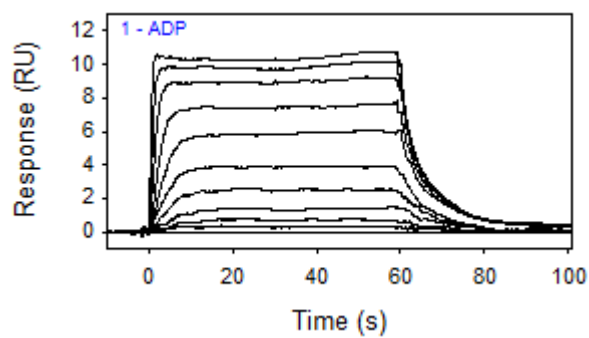
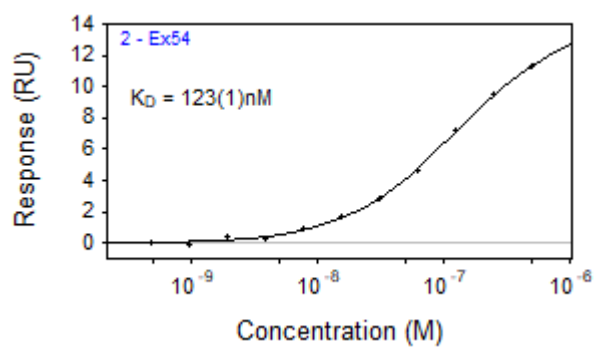
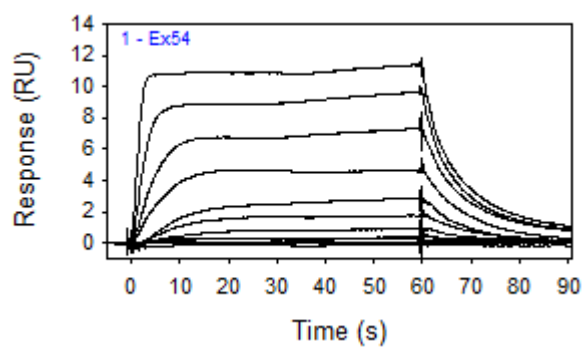
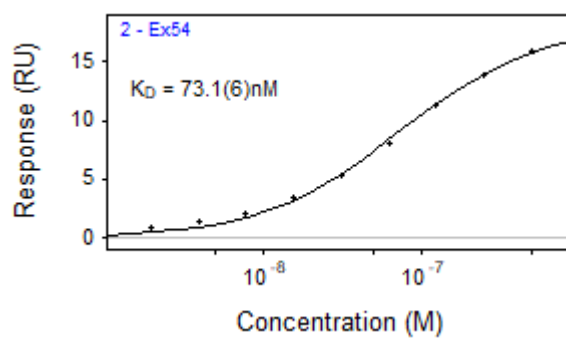
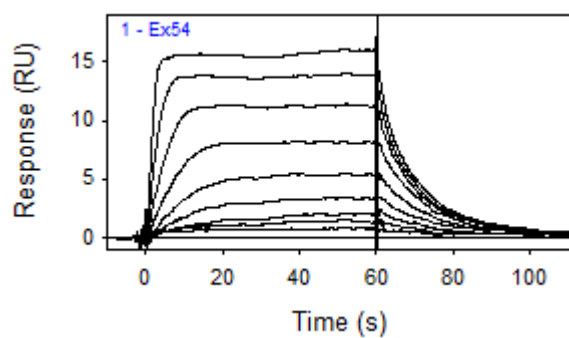
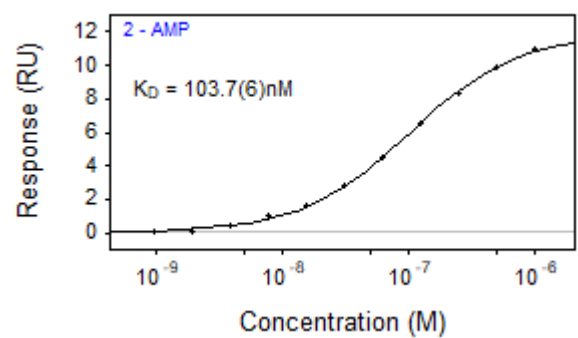
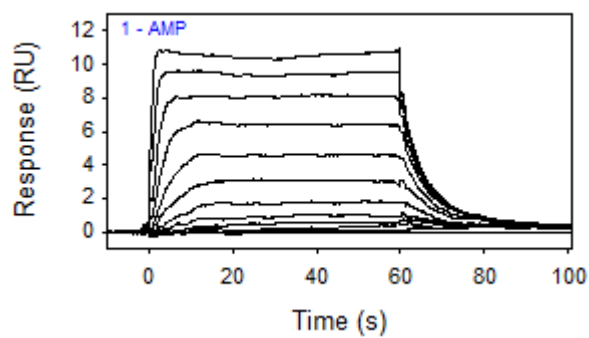
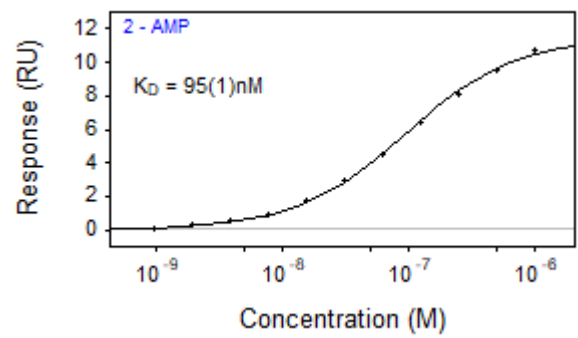
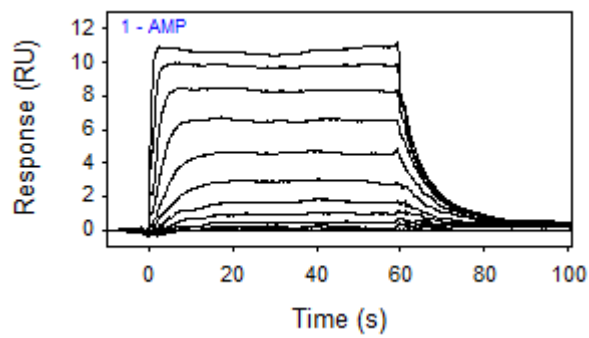
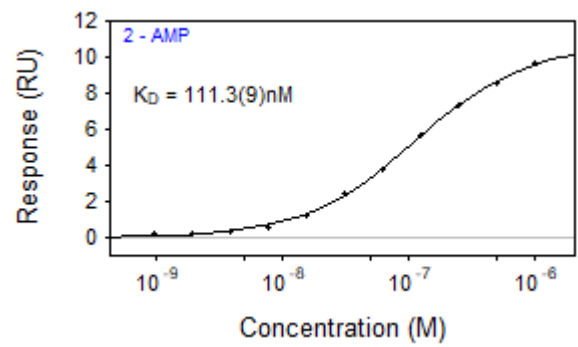
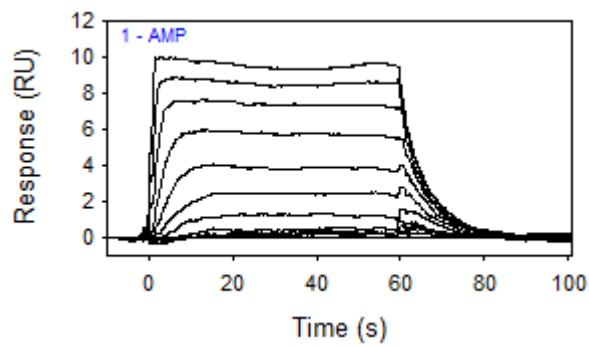
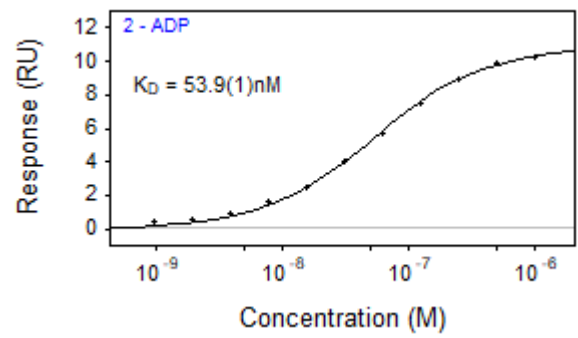
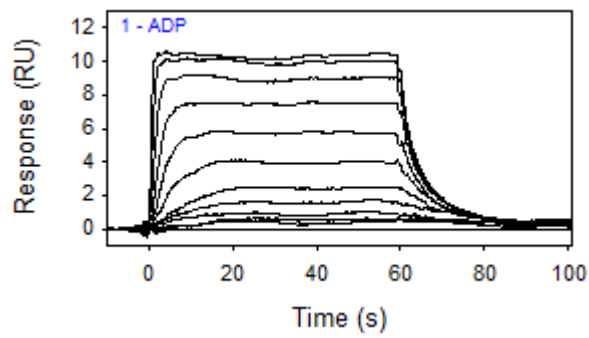
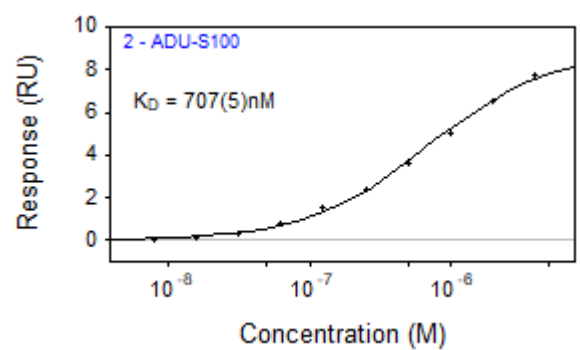
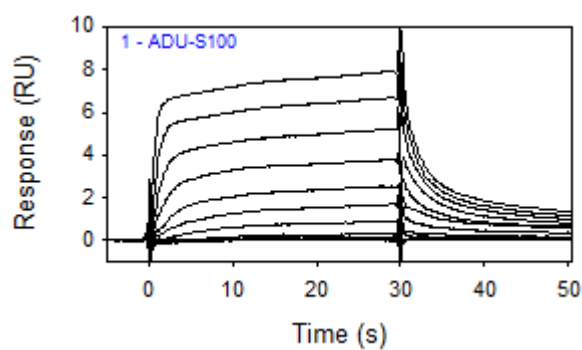
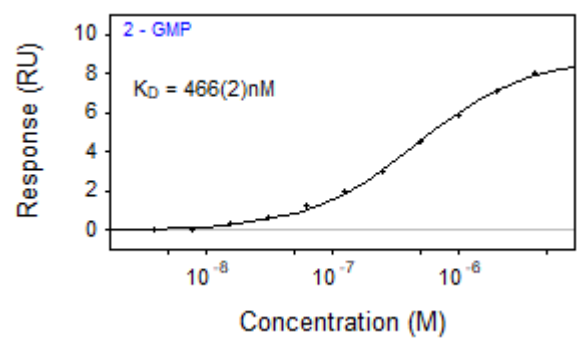
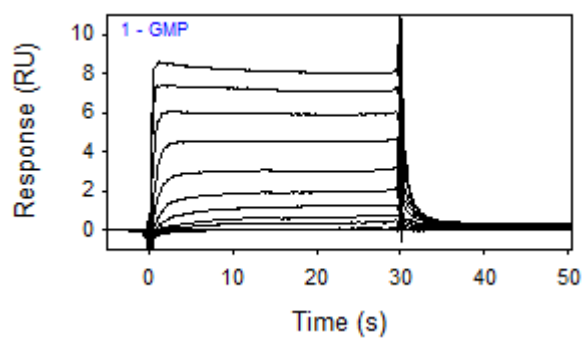
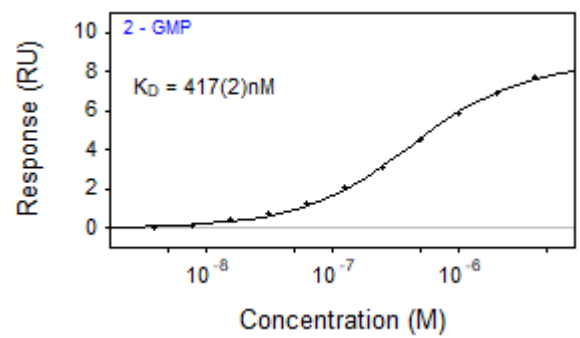
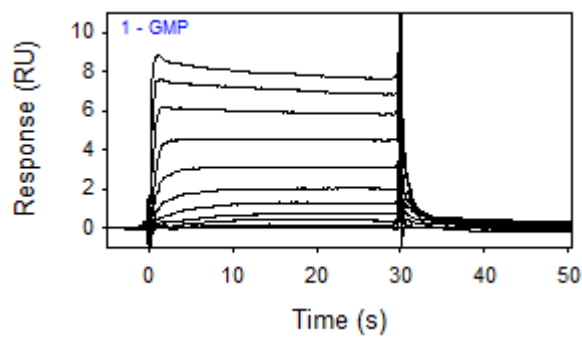
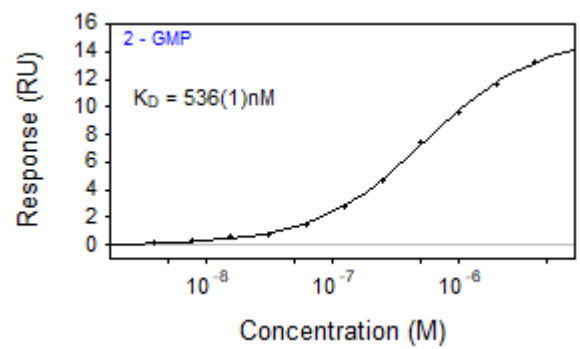
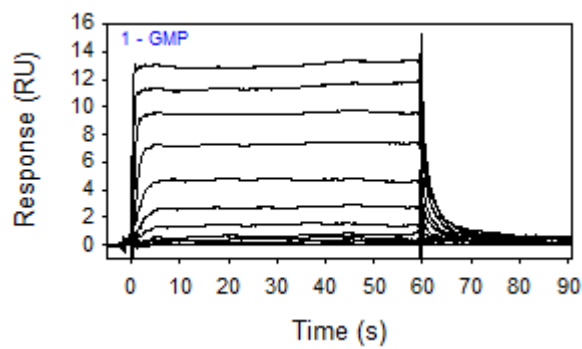


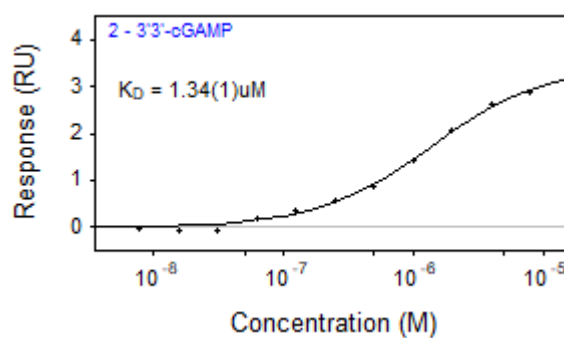
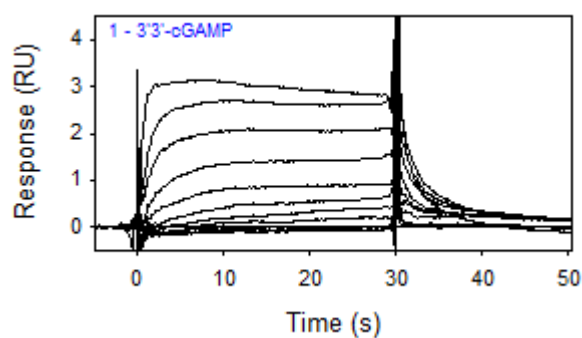
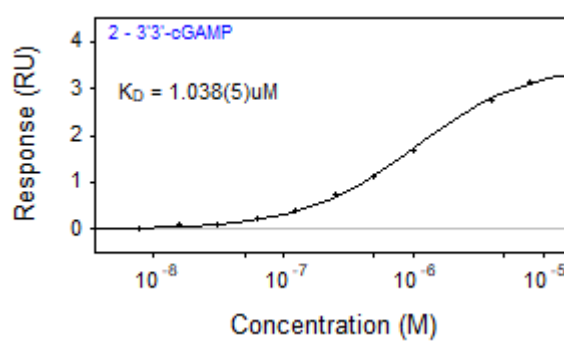
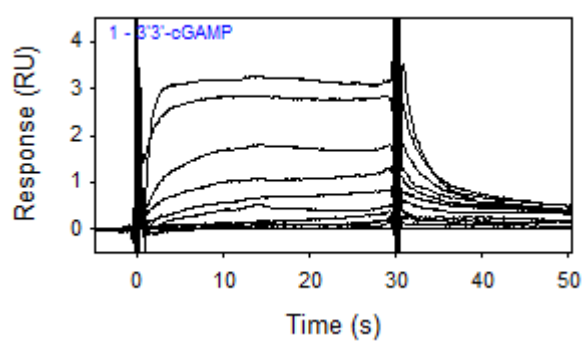
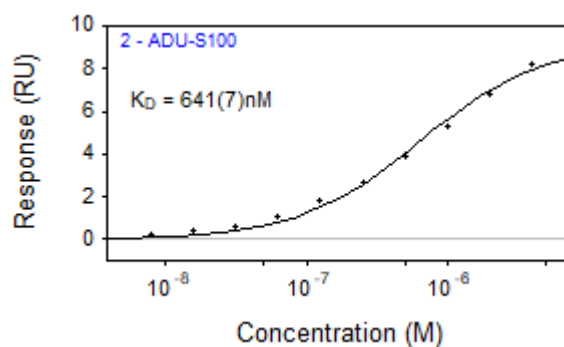
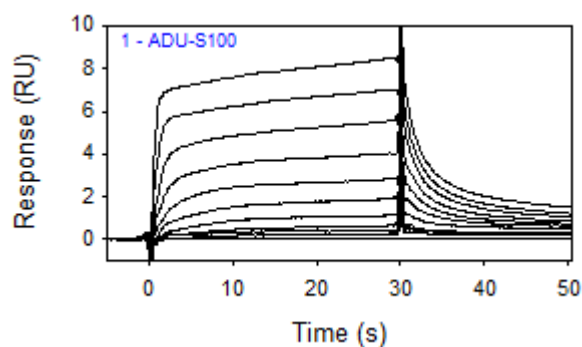
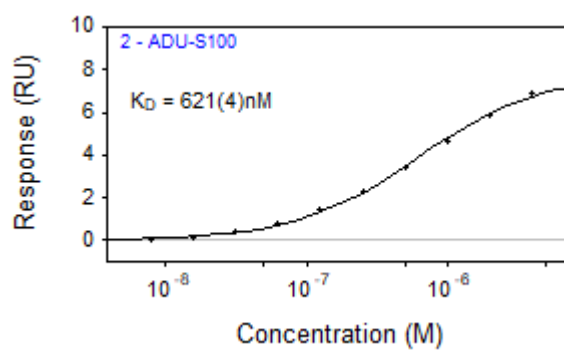
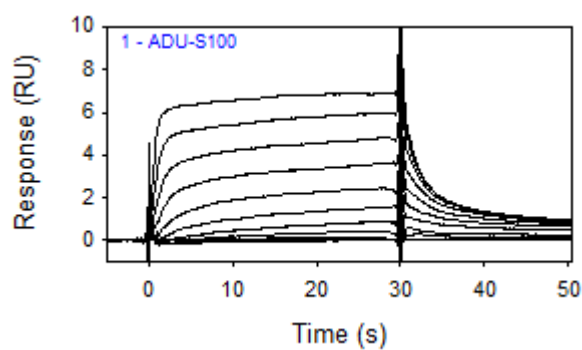
Figure S4 Ex54 sulfamide movement. Variable sulfamide position of Ex54. The A and B protomers were superposed using the SSM algorithm in Coot. There is little variation between these active sites, but one can see that the sulfamide position of the two Ex54 molecules is quite different. Figure was generated with PyMol.

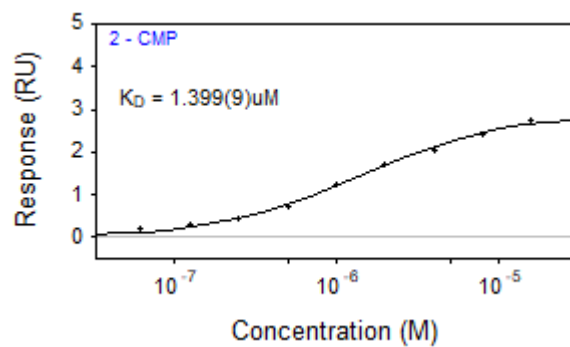
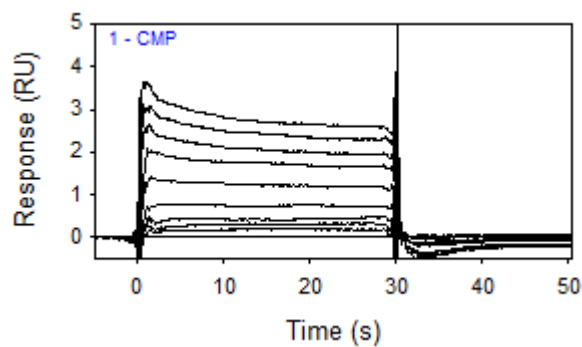
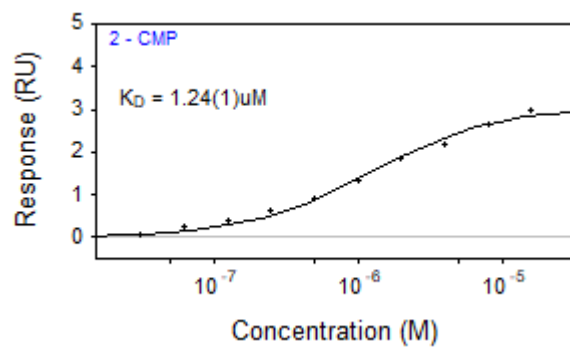
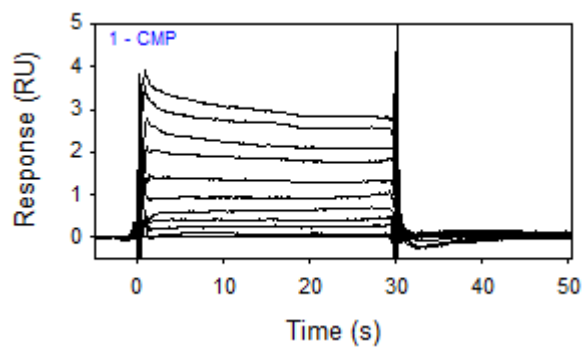
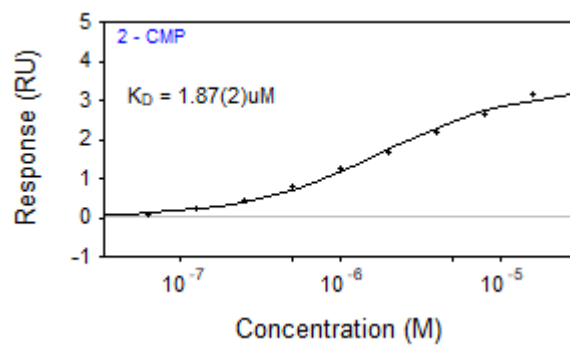
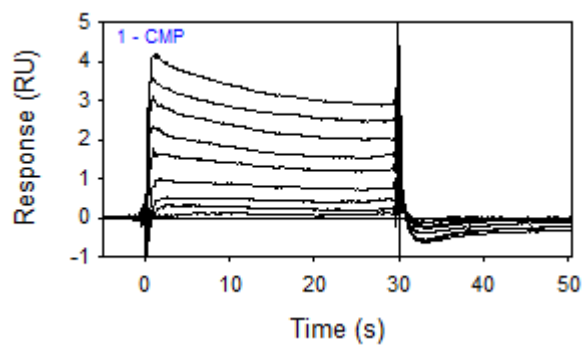
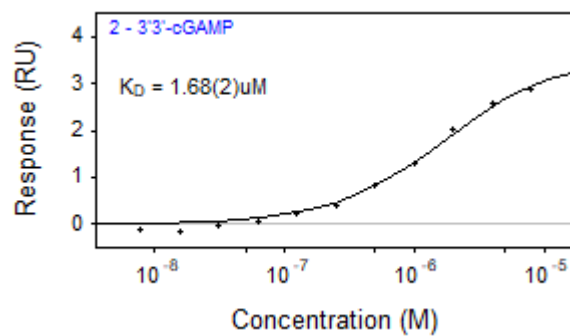
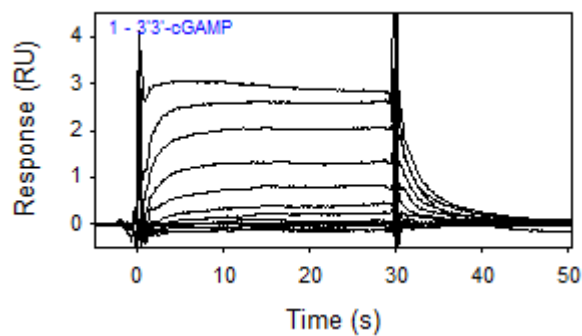


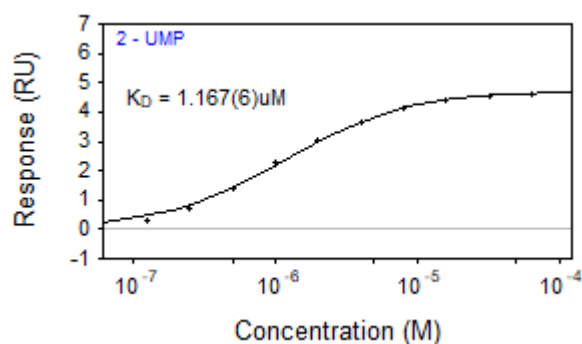
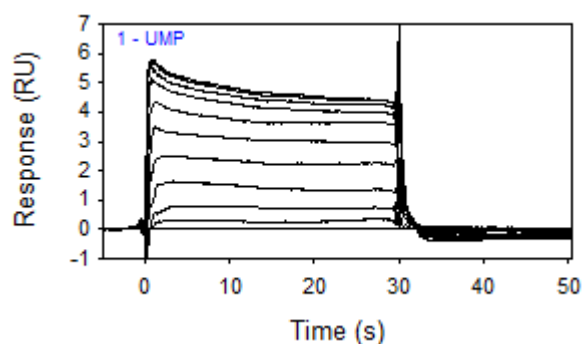
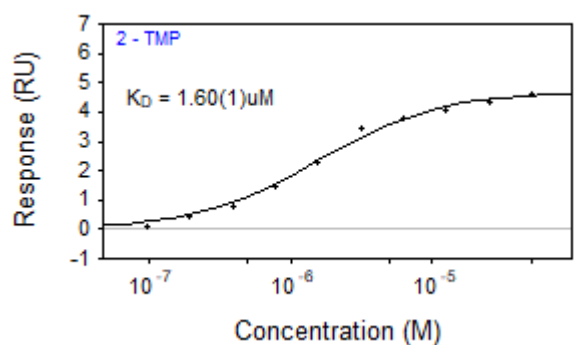
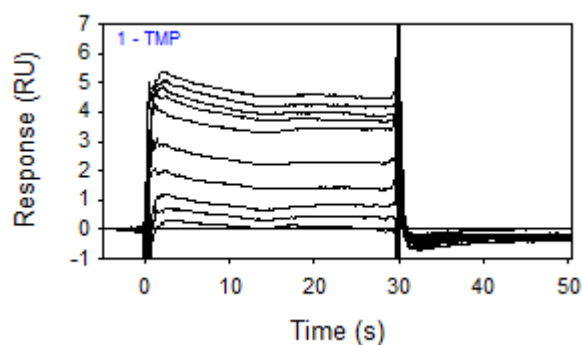
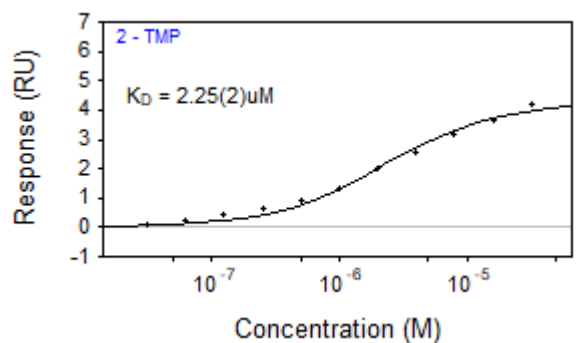
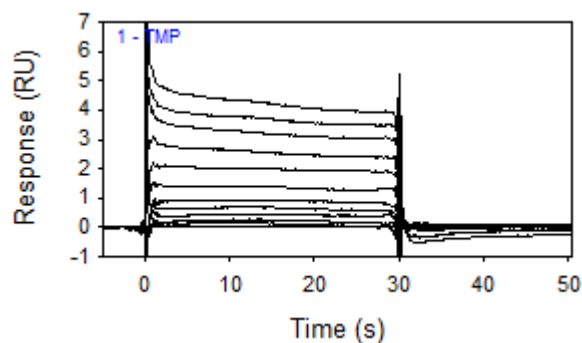
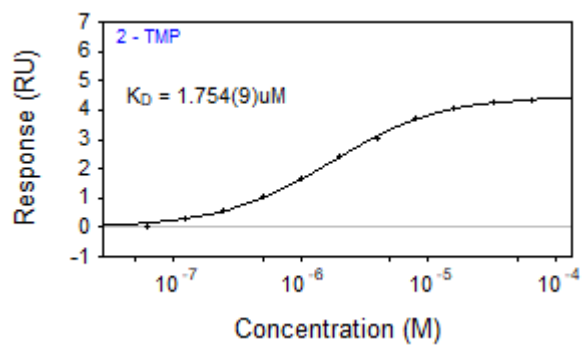
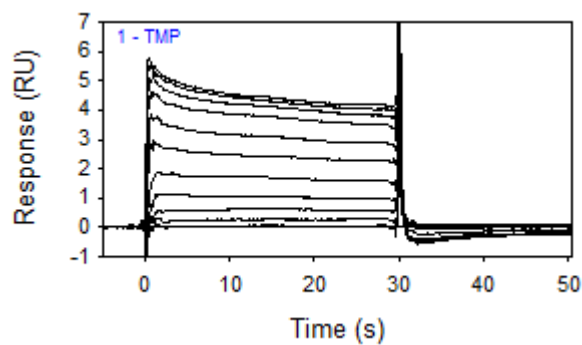












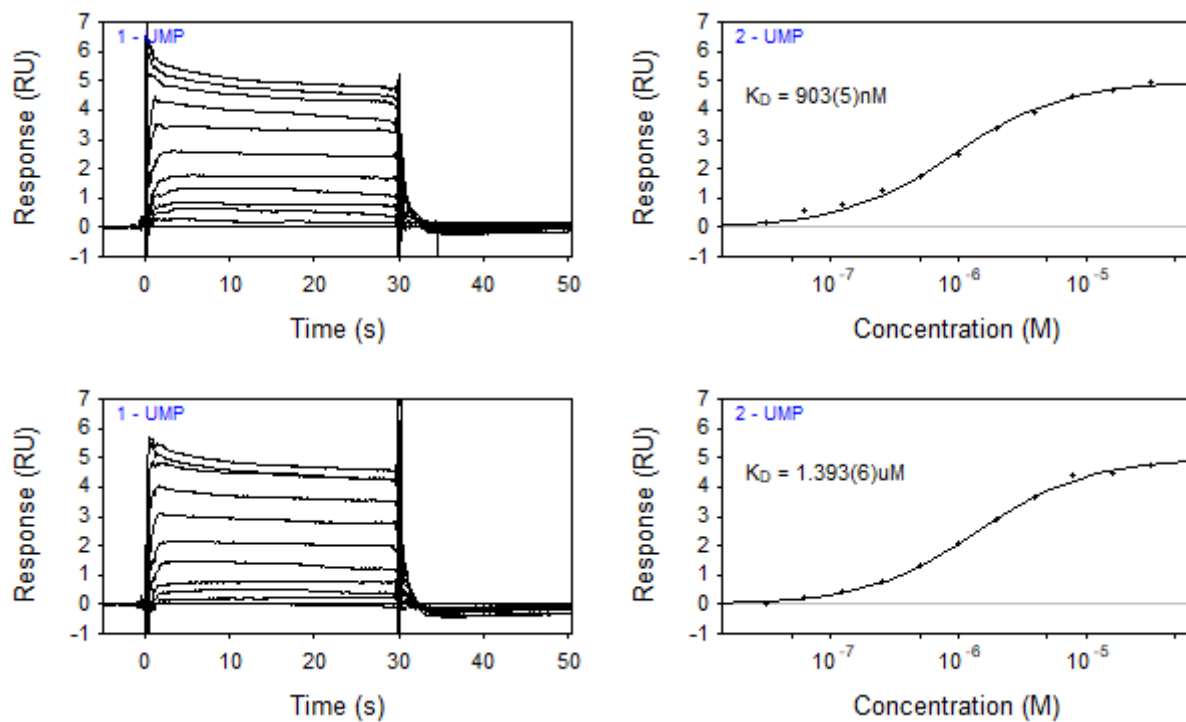


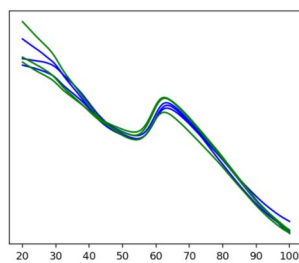
Figure S5 SPR sensorgrams and binding isotherms.

DMSO(0%)

$T_m = 59.3(+/-0.08)$

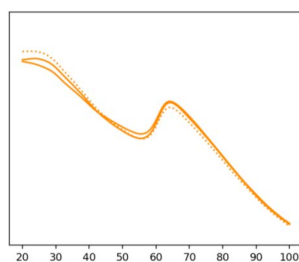
DMSO(1%),

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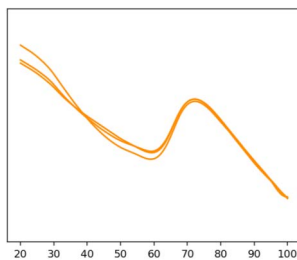


GMP

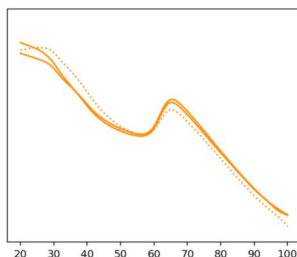
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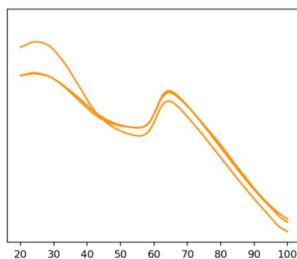
ADU-S100

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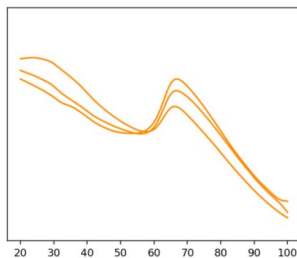
CMP

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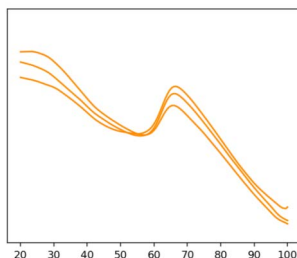
3'3'-cGAMP

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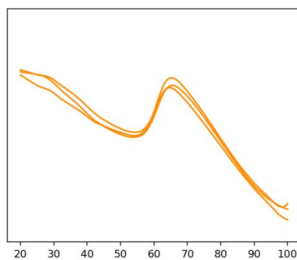
ADP

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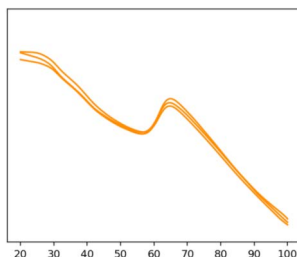
AMP

 $T_m = 62.01 (+/-0.1)$ 

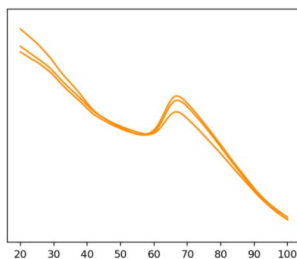
TMP

T_m = 60.54 (+/-0.1)

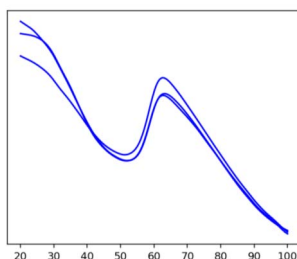
UMP

T_m = 61.05 (+/-0.05)

QPS2

T_m = 63.03 (+/-0.13)

DMSO(1%)

T_m = 58.37(+/-0.07)

Ex54

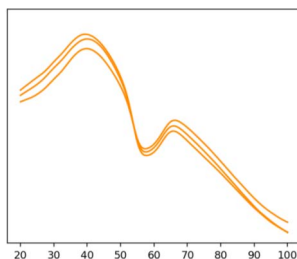
T_m = 62.15 (+/-0.03)

Figure S6 DSF melt curves. The x-axis presents temperature (°C) and the y-axis presents normalised RFU (relative fluorescence units).