SUPPLEMENTARY DATA

Conformational dynamics of human FXR-LBD ligand interactions studied by hydrogen/deuterium exchange mass spectrometry: Insights into the antagonism of the hypolipidemic agent *Z*-guggulsterone

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Figure S1. A, SDS PAGE of purified hFXR-LBD; B, MS spectrum of purified hFXR-LBD.

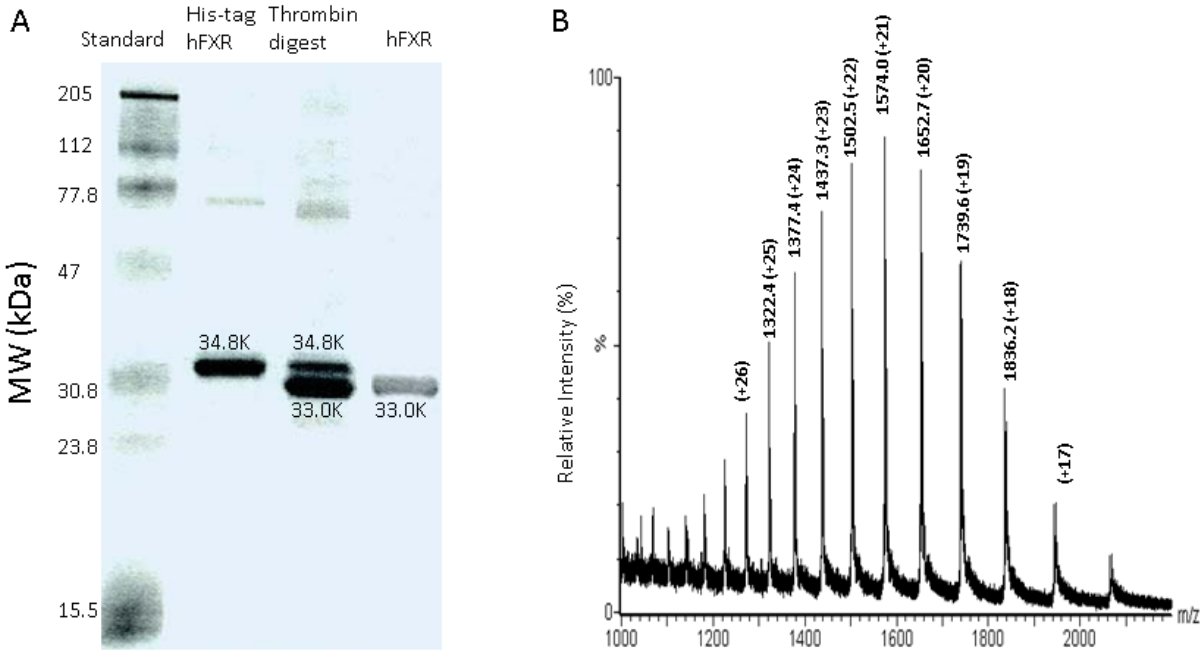


Figure S2. Binding of GG to FXR-LBD studied by intrinsic fluorescence titration.

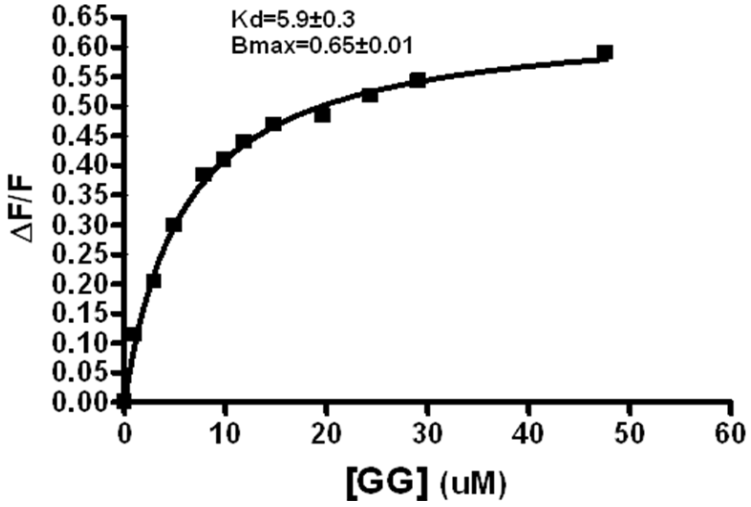


Figure S3. Design and schematic flow path diagrams of the UPLC system used in this study.

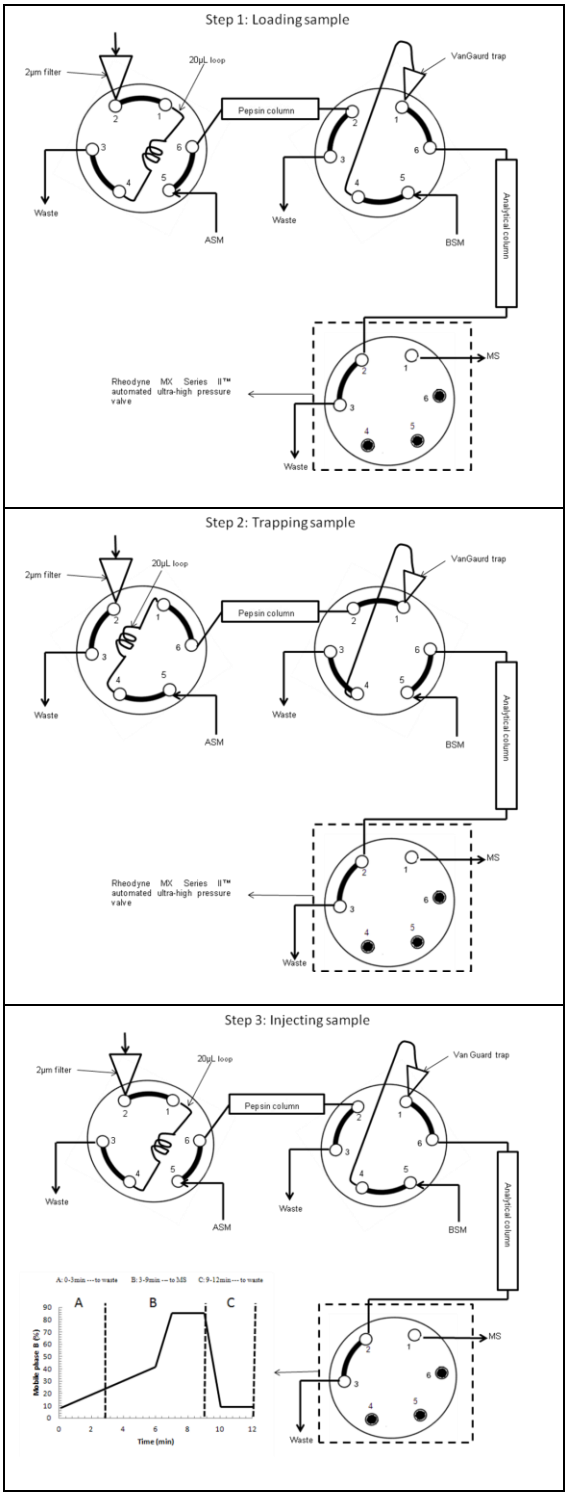


Figure S4. Peptide coverage map of FXR-LBD.

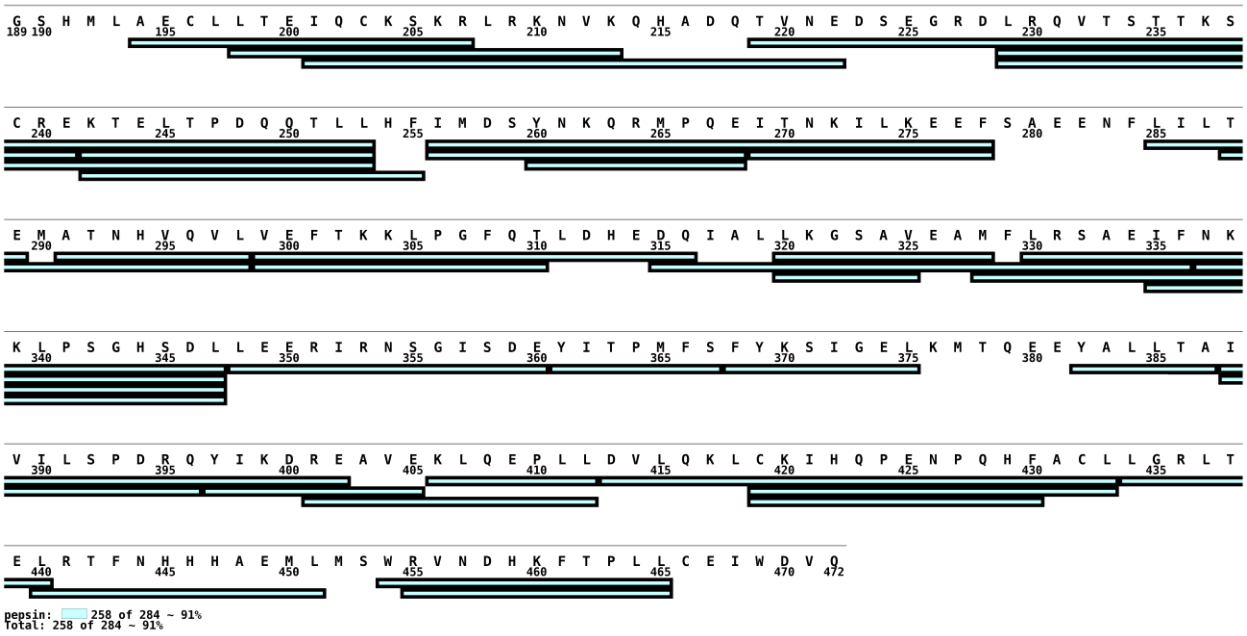
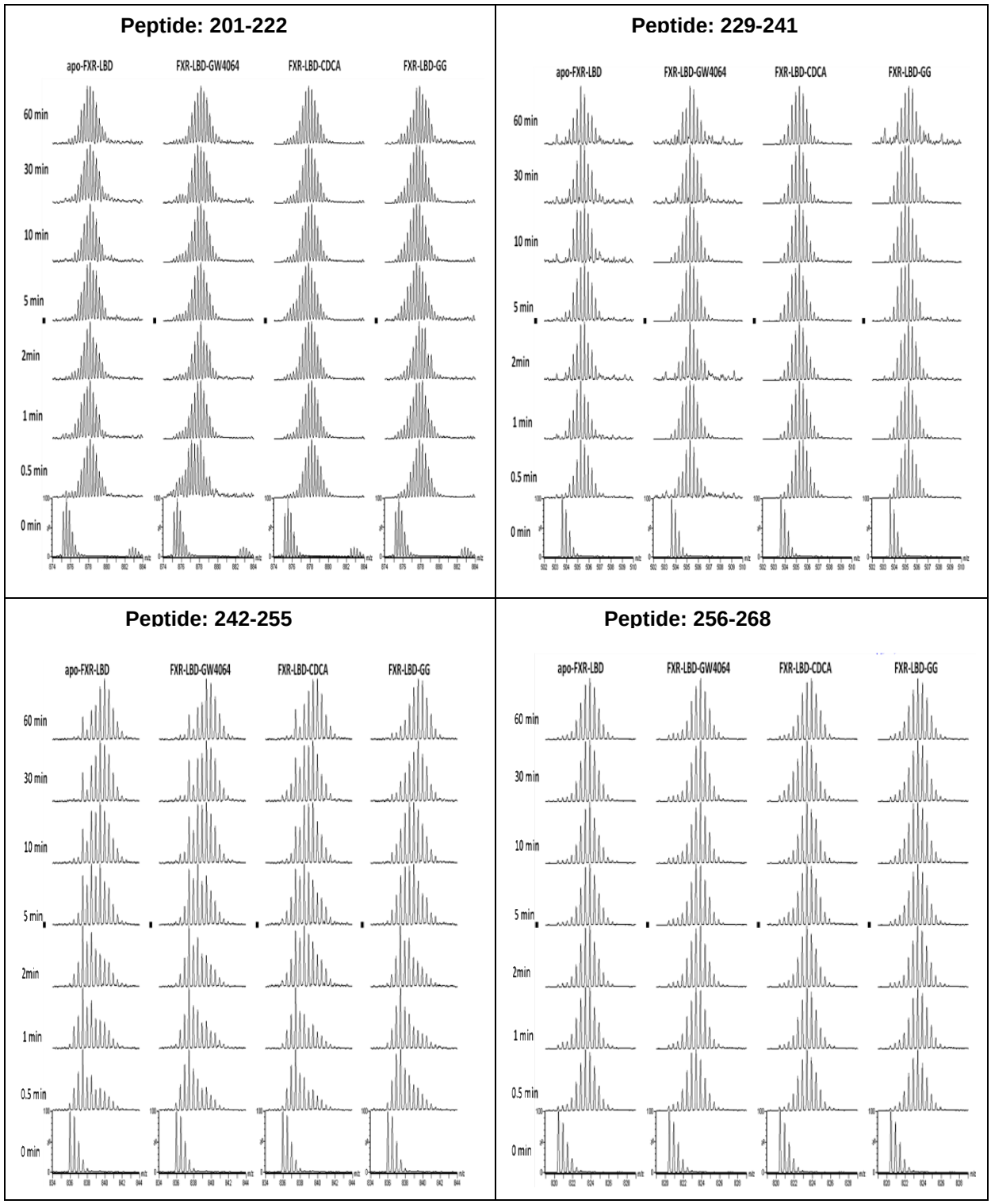
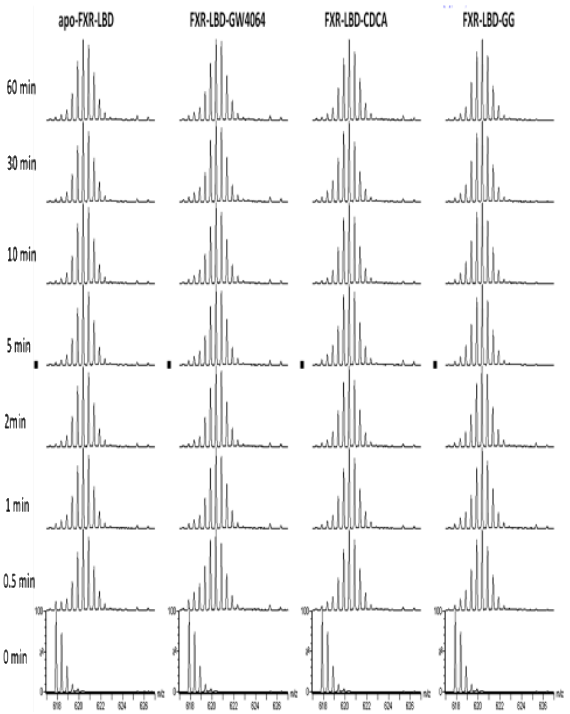


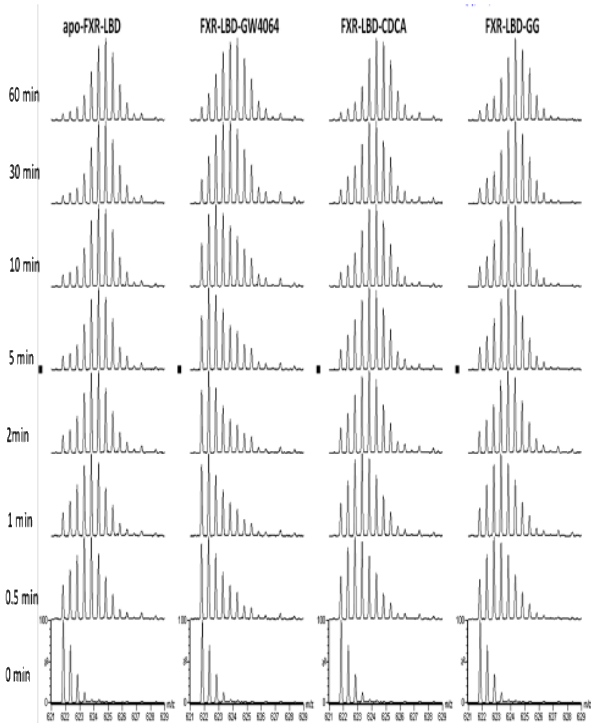
Figure S5: Mass spectra for peptides demonstrating evolution of deuterium incorporation into peptic peptides. The partial sequence of each peptide is depicted at the top of each panel. Deuteration times (0, 0.5, 1, 2, 5, 15, 30, 60 min) are denoted in each panel.



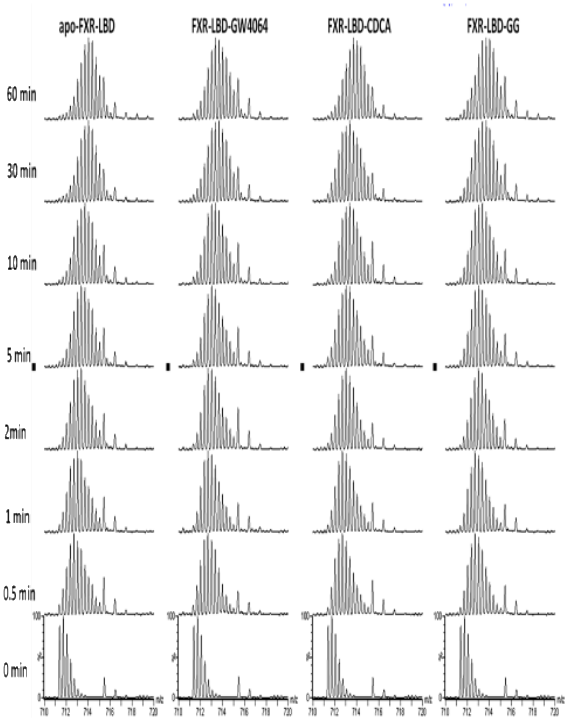
Peptide: 269-278



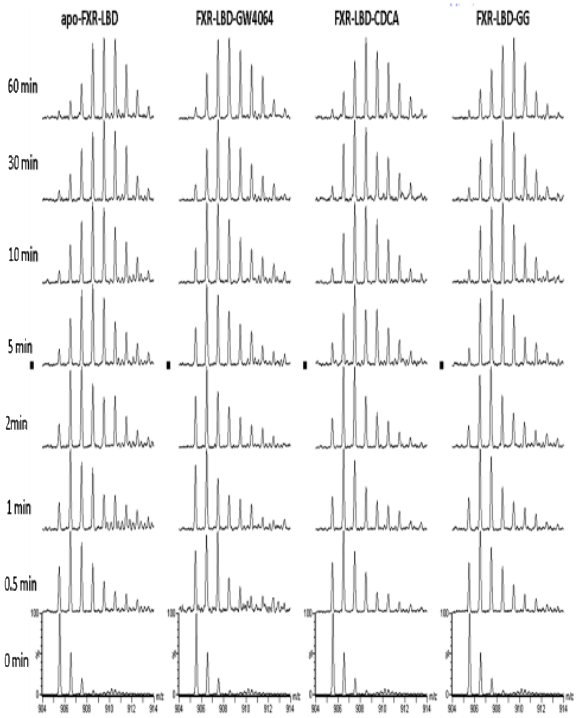
Peptide: 288-298



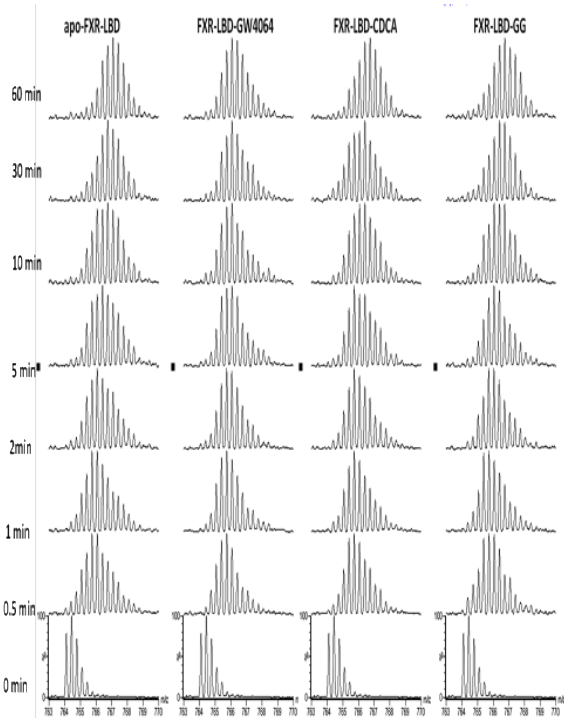
Peptide: 299-316



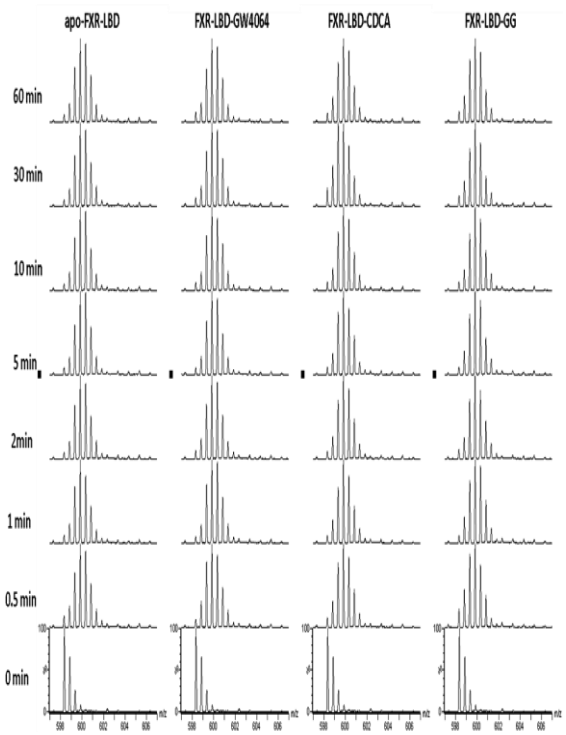
Peptide: 320-328



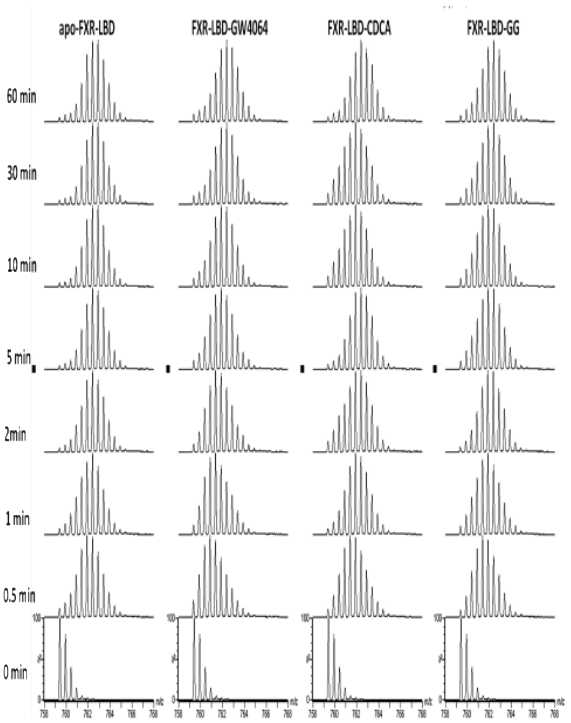
Peptide: 328-347



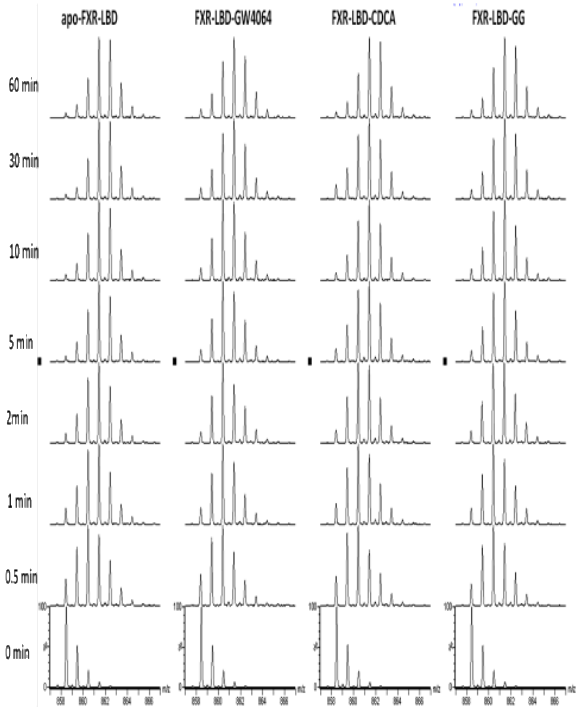
Peptide: 337-347



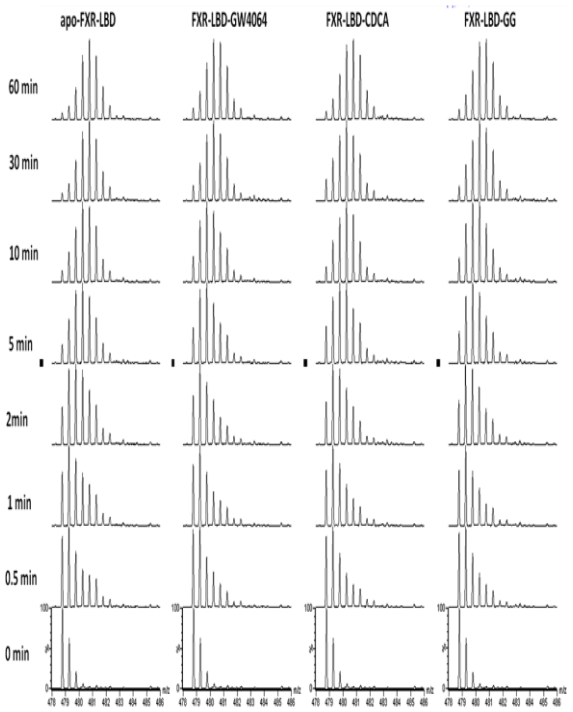
Peptide: 348-360



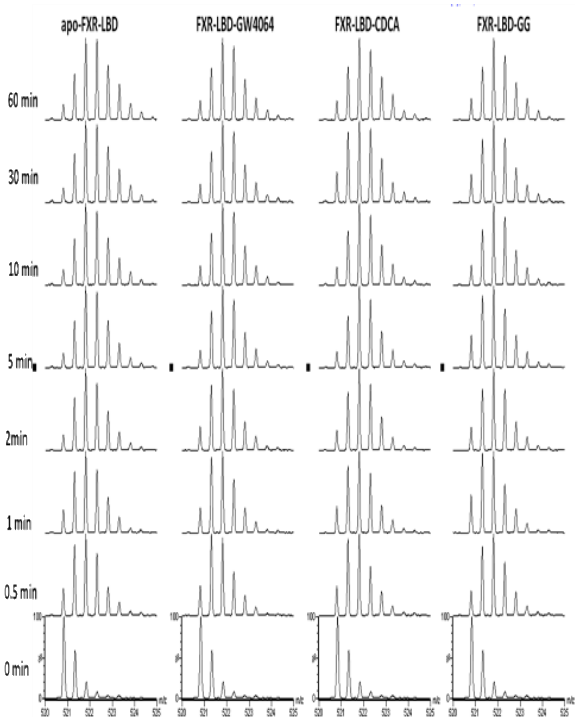
Peptide: 361-367



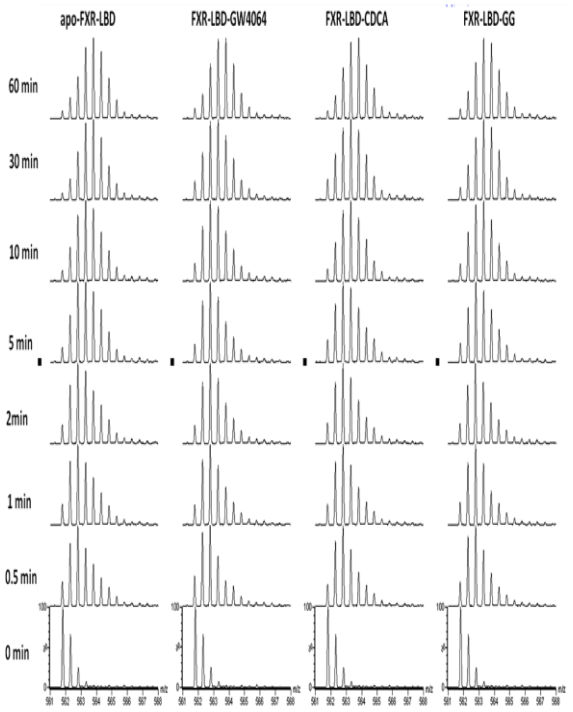
Peptide: 368-375



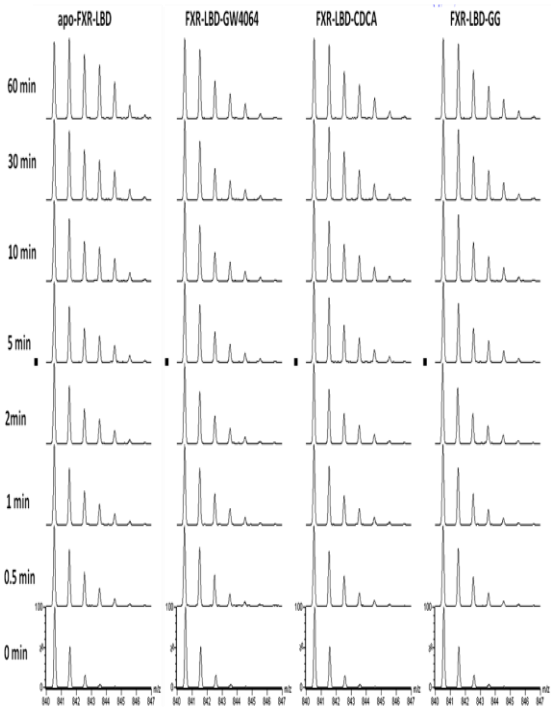
Peptide: 388-396



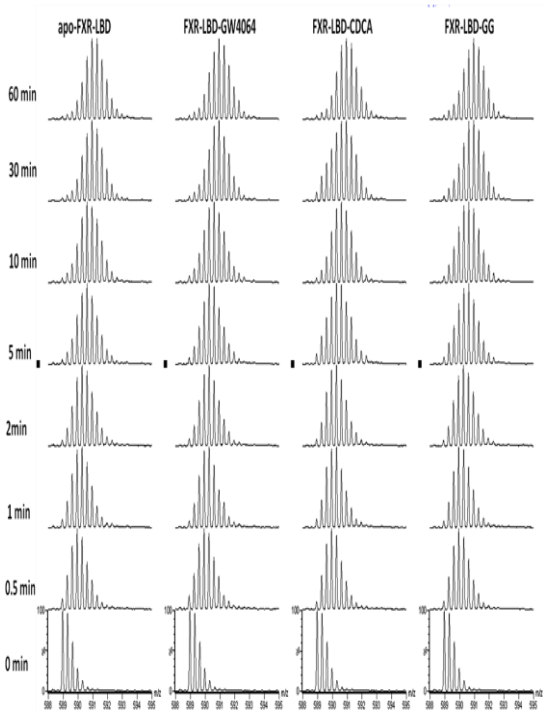
Peptide: 397-405



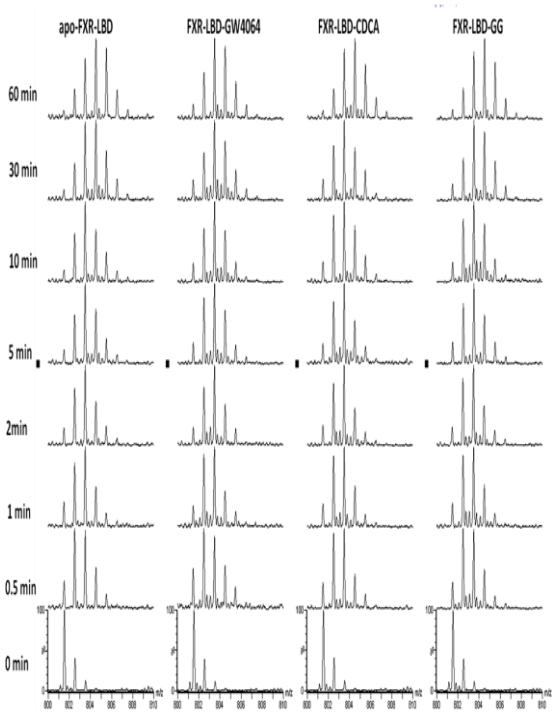
Peptide: 406-412



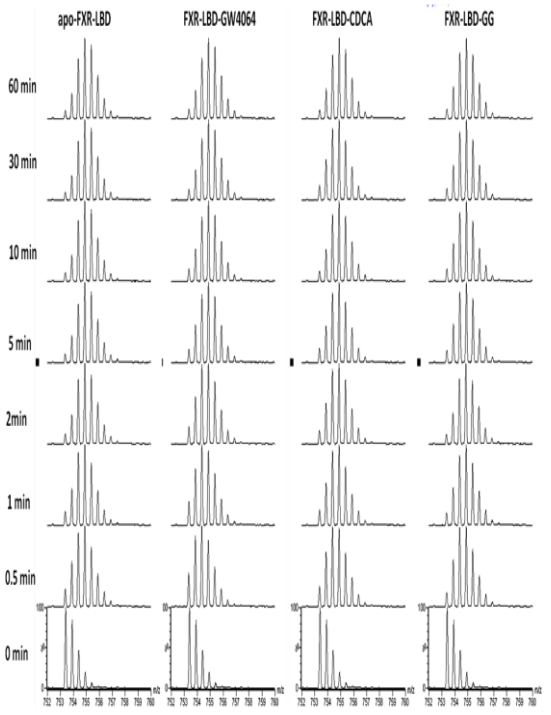
Peptide: 419-433



Peptide: 434-440



Peptide: 440-451



Peptide: 454-465

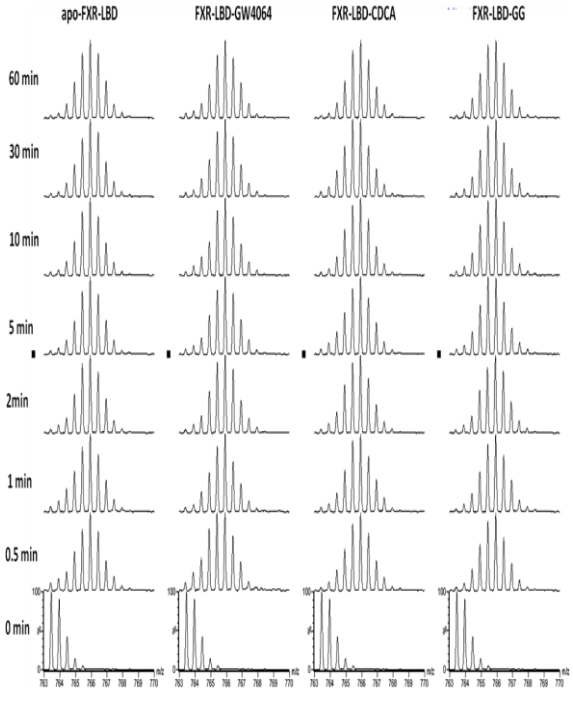
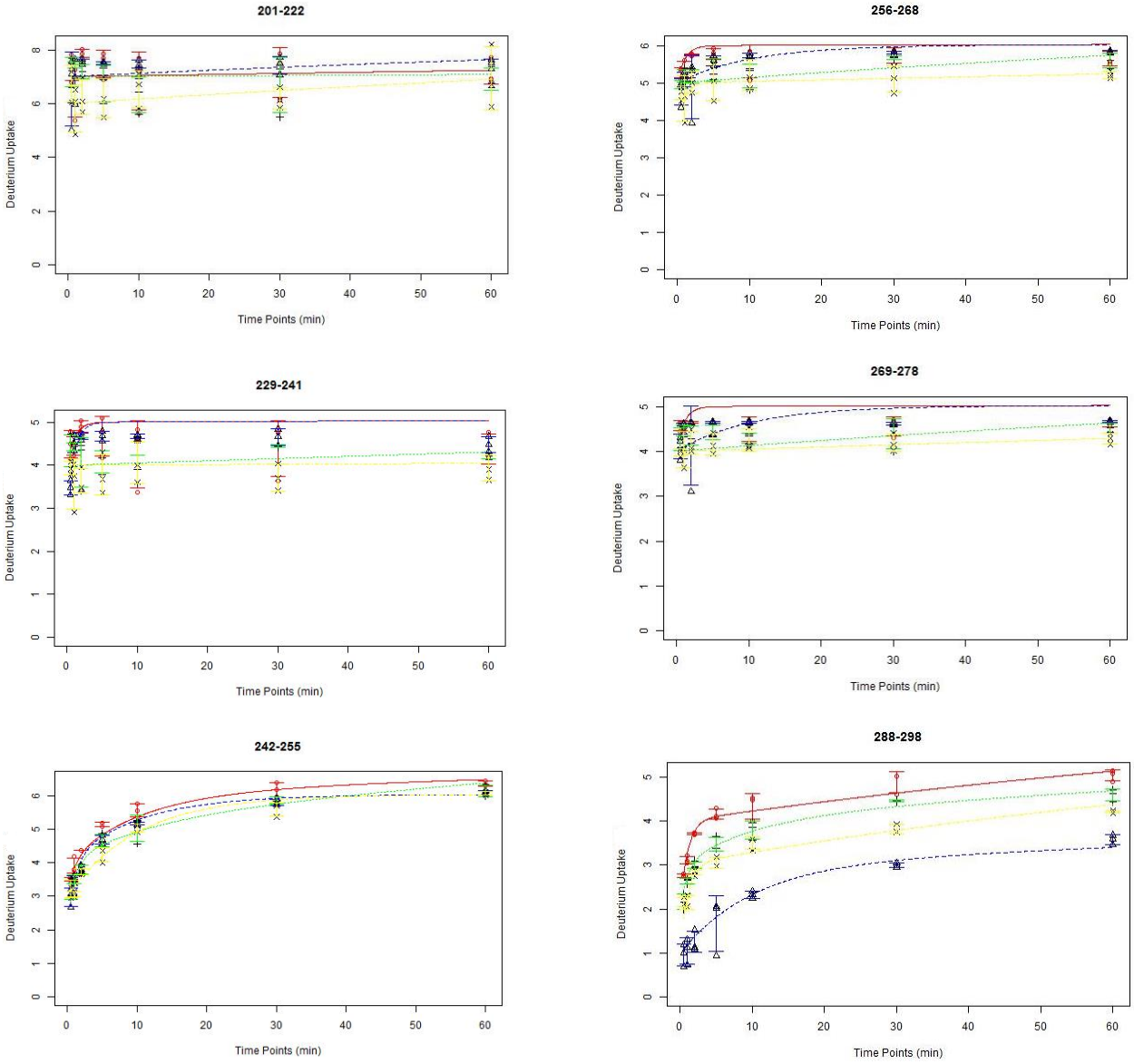
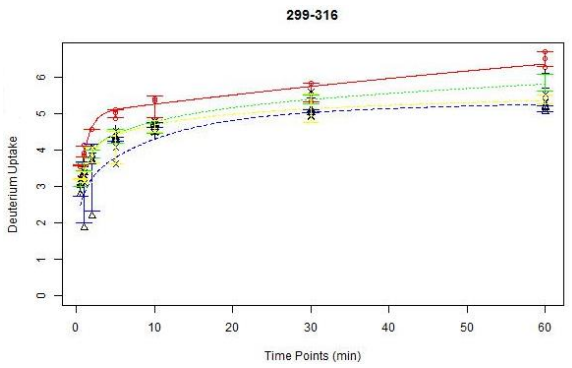
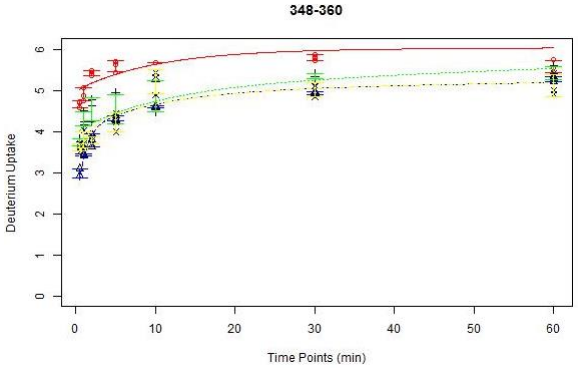
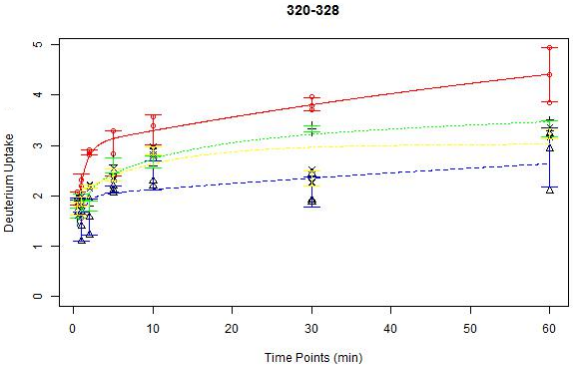


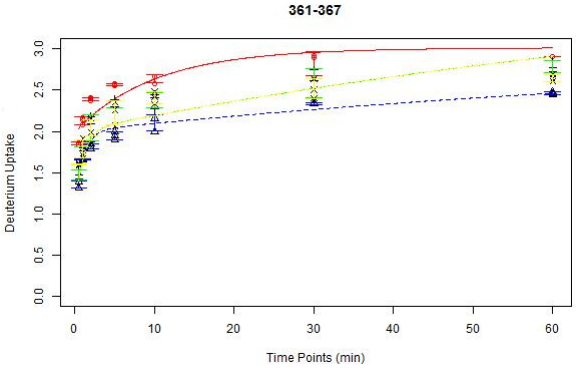
Figure S6. Deuterium incorporation levels for each peptide at seven exchange-in time points (0.5, 1, 2, 5, 10, 30, 60 min). The curves were fit with a six fixed exchange rate constants kinetic model (10, 1, 0.1, 0.01, 0.001, 0.0001 min^{-1}). The error bar for each data point is based on the three experimental replicates. Color code: apo-FXR-LBD, red line; GW4064, blue line; CDCA, green line; GG, yellow line.

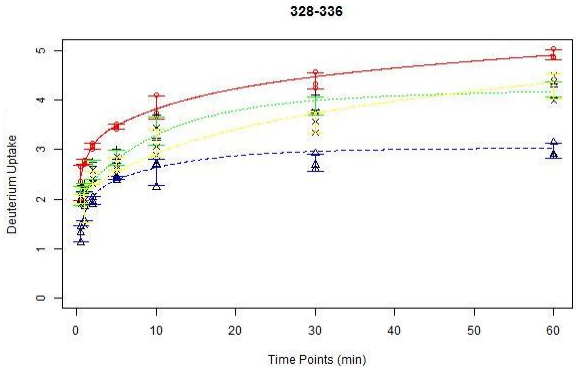


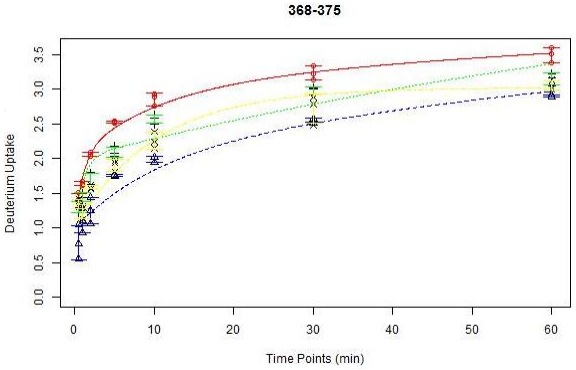

299-316
Deuterium Uptake
Time Points (min)

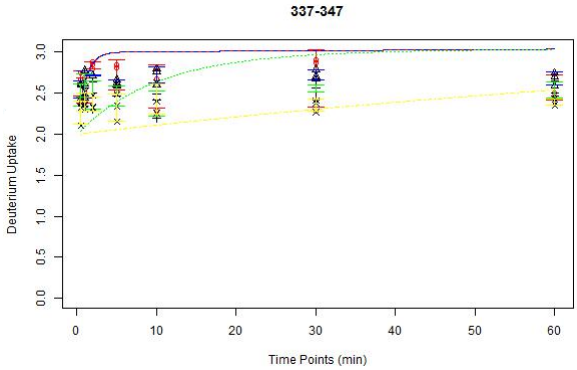

348-360
Deuterium Uptake
Time Points (min)

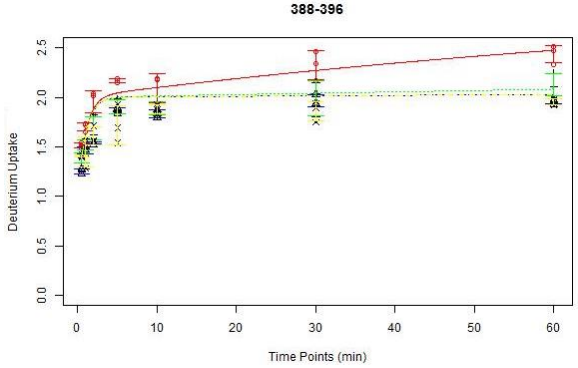

320-328
Deuterium Uptake
Time Points (min)

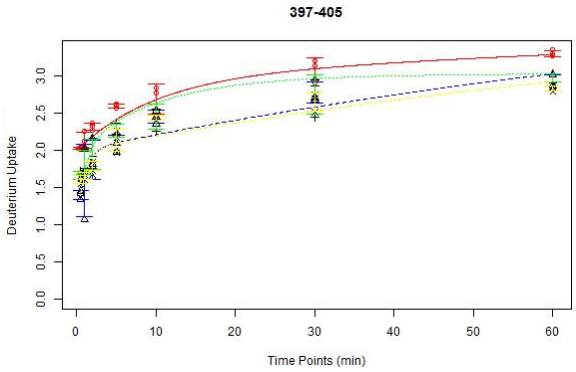

361-367
Deuterium Uptake
Time Points (min)

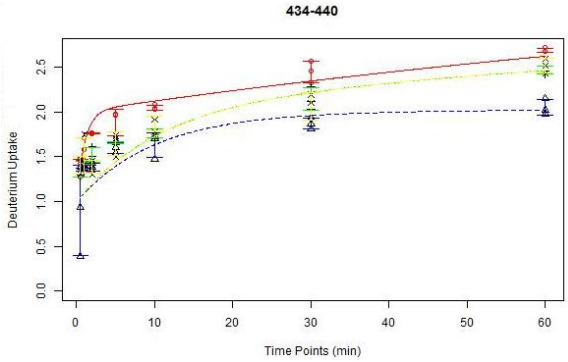

328-336
Deuterium Uptake
Time Points (min)

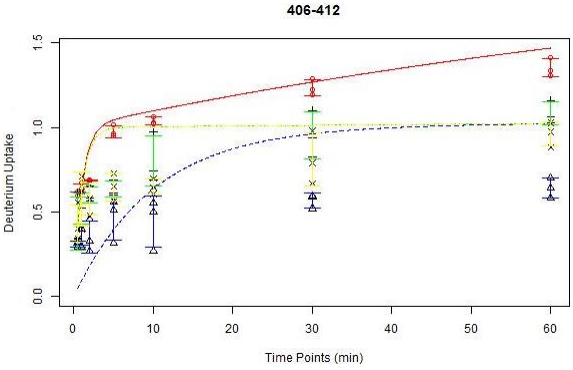

368-375
Deuterium Uptake
Time Points (min)

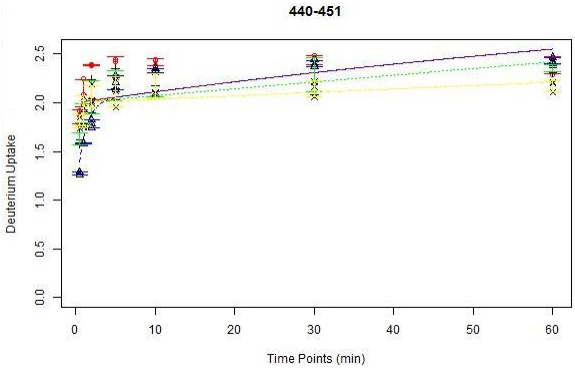

337-347
Deuterium Uptake
Time Points (min)

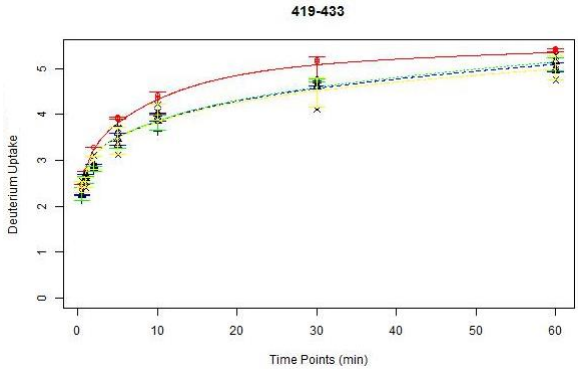

388-396
Deuterium Uptake
Time Points (min)


397-405
Deuterium Uptake
Time Points (min)


434-440
Deuterium Uptake
Time Points (min)


406-412
Deuterium Uptake
Time Points (min)


440-451
Deuterium Uptake
Time Points (min)


419-433
Deuterium Uptake
Time Points (min)

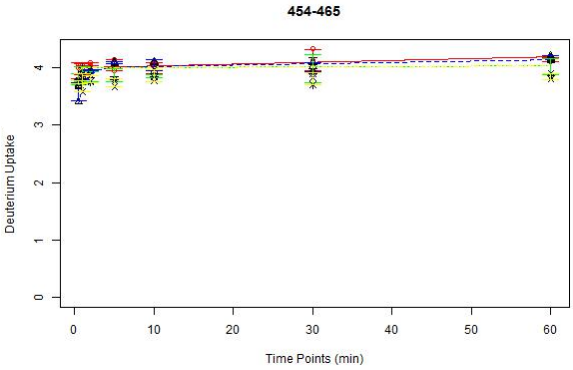

454-465
Deuterium Uptake
Time Points (min)

Figure S7. Deuterium exchange data for apo FXR-LBD.
(A) Peptic peptides HDX profiles of apo FXR-LBD. Deuterium exchange levels for each peptide (in % based on the number of backbone amides present in the peptide) are shown at different reaction time points (0.5, 1, 2, 5, 10, 30, and 60 min, from left to right). (B) HDX heat map for apo FXR-LBD. Each horizontal color block represents an analyzed peptic peptide, and each block contains seven time points (from top: 0.5, 1, 2, 5, 10, 30, 60min). The secondary structure of holo-FXR with GW4064 is based on a three-dimensional crystallographic structure (PBD ID: 3DCT) and was added for reference purposes only.

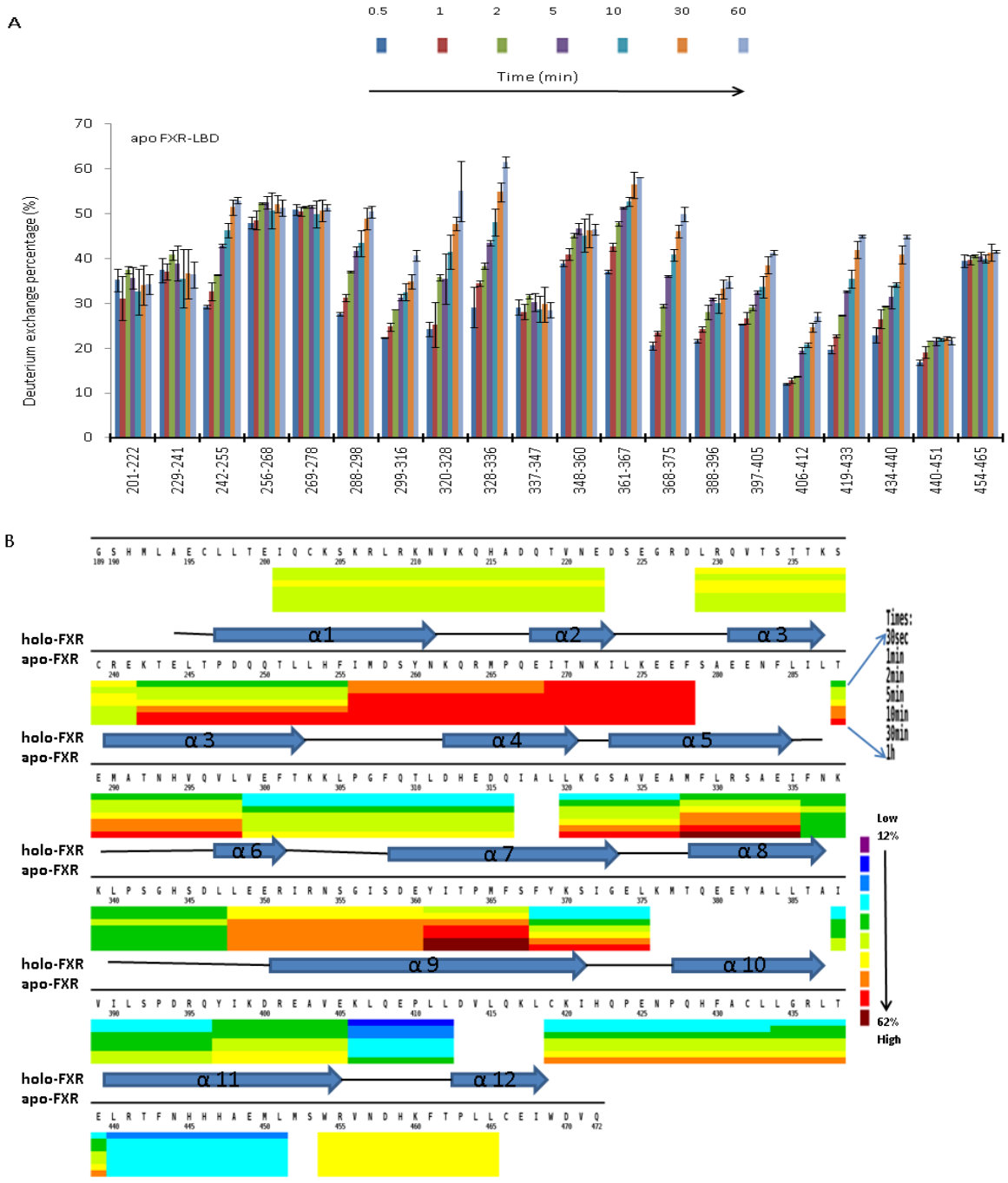


Figure S8. Peptide-level HDX profiles of FXR-LBD in the presence of GW4064, CDCA and GG(Z), respectively. Deuterium exchange for each peptide (in % based on the number of backbone amide hydrogens present in the peptide) is shown at different reaction time points (0.5, 1, 2, 5, 10, 30, and 60 min, from left to right).

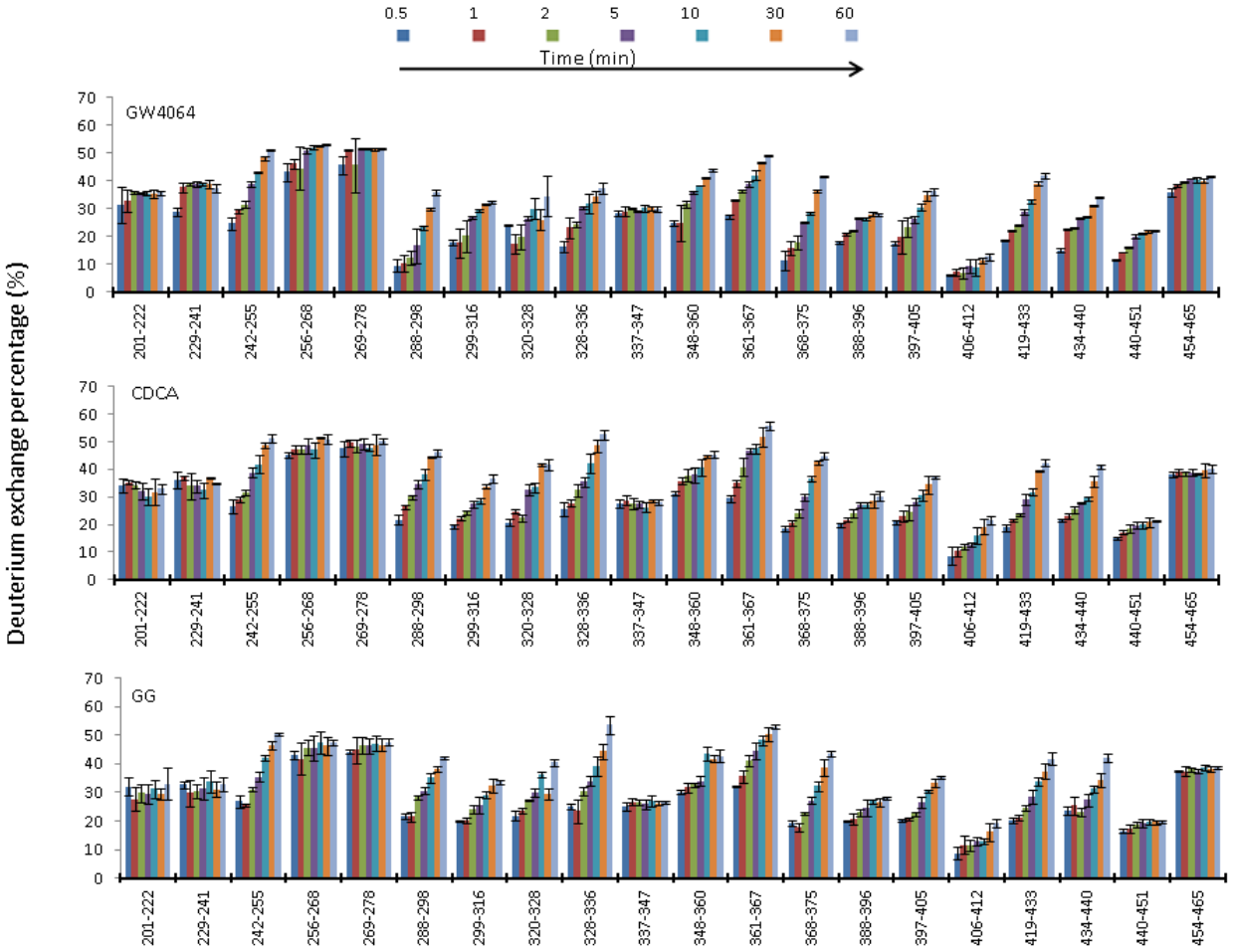


Figure S9. Ligand-dependent deuterium exchange protection plots at the peptide level. Ligand-specific deuterium exchange-in differences between ligand-free FXR-LBD and ligand-bound FXR-LBD is shown at different time points (0.5, 1, 2, 5, 10, 30, and 60 min, from left to right) for each peptide. Negative percentages indicate the increase of protection against deuterium exchange-in in a particular region of the FXR-LBD ligand complex relative to apo FXR.

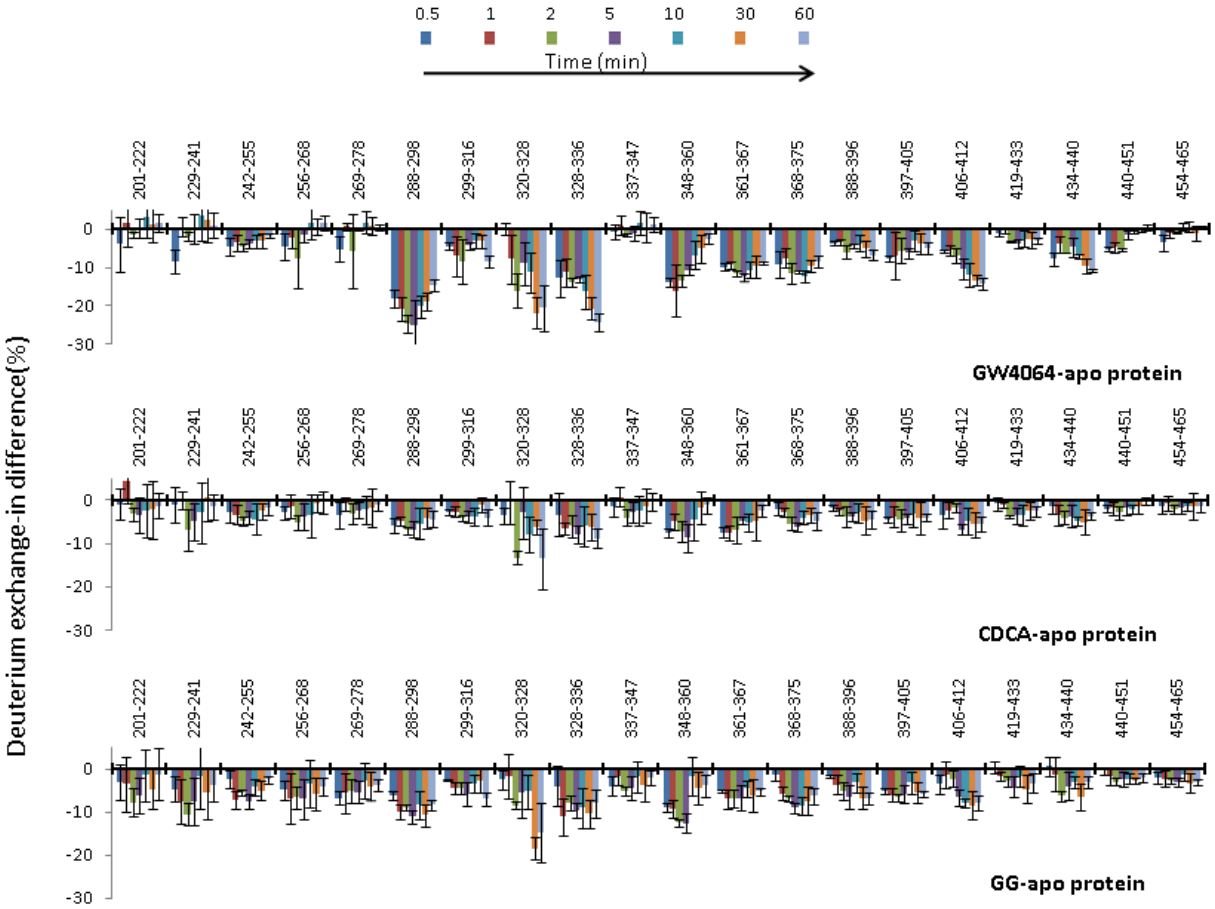


Figure S10. Overlay of the peptide deuterium levels of GW4064-bound hFXR-LBD and the B-factors of the backbone N atoms extracted from the crystal structure, PDB ID 3DCT. Asterisks mark the following: * regions containing direct interaction sites; ** regions that showed changes in exchange kinetics but showed no interacting residues in the crystal structure. For comparison the peptide deuterium levels of the apo protein are shown illustrating disparate retardation of exchange (reduced deuterium levels) in different region of the protein upon binding of the high affinity agonist GW4064.

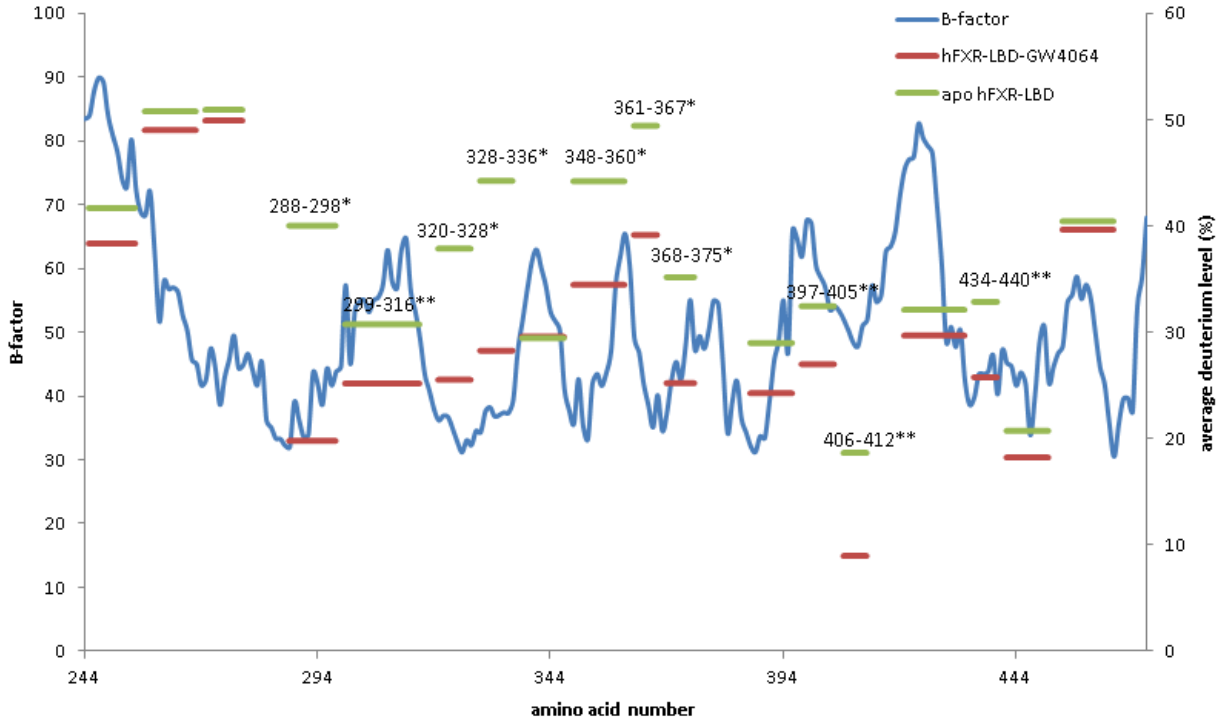


Table S1. Averaged differences in deuterium incorporation levels for each of the 20 peptides used in the current HDX-MS study. The values were obtained by averaging the differences in deuterium levels across the seven reaction time points (0.5, 1, 2, 5, 10, 30 to 60 min) used in the current time course study. A negative percentage indicates the increase of protection against deuterium exchange-in upon ligand binding and a positive number indicates less protection in a particular region of the FXR-LBD ligand complex. Deuterium level differences of regions with p<0.001, calculated by two-way ANOVA, are statistically significant.

Structure [a]	Residue [b]	Charge[c]	apo[d]	GW4064-apo[e]	P-valve	CDCA-apo[f]	P-valve	GG-apo[g]	P-valve	GG-GW4064[h]	P-valve	GG-CDCA[i]	P-valve	GW4064-CDCA[j]	P-valve
H1(-)	**201-222**	**3**	**34%**	**0%**	**0.789**	**-1%**	**0.178**	**-4%**	**<0.001**	**-4%**	**<0.001**	**-3%**	**0.014**	**2%**	**0.080**
H1(-)	**229-241**	**2**	**38%**	**0%**	**0.627**	**-2%**	**0.022**	**-6%**	**<0.001**	**-5%**	**<0.001**	**-3%**	**<0.001**	**2%**	**0.003**
H1	**242-255**	**2**	**42%**	**-4%**	**<0.001**	**-3%**	**<0.001**	**-5%**	**<0.001**	**-1%**	**0.002**	**-1%**	**0.007**	**0%**	**0.755**
H1-H2	**256-268**	**2**	**51%**	**-2%**	**0.040**	**-3%**	**<0.001**	**-5%**	**<0.001**	**-4%**	**<0.001**	**-3%**	**<0.001**	**1%**	**0.362**
H2	**269-278**	**2**	**51%**	**-1%**	**0.258**	**-2%**	**<0.001**	**-5%**	**<0.001**	**-4%**	**<0.001**	**-3%**	**<0.001**	**2%**	**0.280**
H3	**288-298**	**2**	**40%**	**-20%**	**<0.001**	**-6%**	**<0.001**	**-9%**	**<0.001**	**11%**	**<0.001**	**-3%**	**<0.001**	**-14%**	**<0.001**
H3-H4	**299-316**	**3**	**31%**	**-6%**	**<0.001**	**-3%**	**<0.001**	**-4%**	**<0.001**	**1%**	**0.086**	**-1%**	**0.017**	**-1%**	**0.002**
H4-H5	**320-328**	**1**	**38%**	**-12%**	**<0.001**	**-7%**	**<0.001**	**-8%**	**<0.001**	**4%**	**<0.001**	**-1%**	**0.012**	**-5%**	**<0.001**
H5	**328-336**	**2**	**44%**	**-16%**	**<0.001**	**-6%**	**<0.001**	**-9%**	**<0.001**	**7%**	**<0.001**	**-2%**	**0.012**	**-9%**	**<0.001**
H5-H6	**337-347**	**2**	**29%**	**0%**	**0.834**	**-2%**	**0.008**	**-3%**	**<0.001**	**-3%**	**<0.001**	**-2%**	**0.002**	**2%**	**<0.001**
H6	**348-360**	**2**	**44%**	**-10%**	**<0.001**	**-5%**	**<0.001**	**-8%**	**<0.001**	**2%**	**0.003**	**-2%**	**<0.001**	**-4%**	**<0.001**
H7	**361-367**	**1**	**49%**	**-10%**	**<0.001**	**-6%**	**<0.001**	**-6%**	**<0.001**	**4%**	**<0.001**	**0%**	**0.603**	**-5%**	**<0.001**
H7	**368-375**	**2**	**35%**	**-10%**	**<0.001**	**-4%**	**<0.001**	**-6%**	**<0.001**	**4%**	**<0.001**	**-2%**	**<0.001**	**-7%**	**<0.001**
H8	**388-396**	**2**	**29%**	**-5%**	**<0.001**	**-3%**	**<0.001**	**-5%**	**<0.001**	**0%**	**0.997**	**-1%**	**0.009**	**-1%**	**0.13**
H9	**397-405**	**2**	**32%**	**-5%**	**<0.001**	**-4%**	**<0.001**	**-5%**	**<0.001**	**0%**	**0.924**	**-1%**	**0.010**	**-1%**	**0.073**
H9	**406-412**	**1**	**19%**	**-10%**	**<0.001**	**-4%**	**<0.001**	**-5%**	**<0.001**	**4%**	**<0.001**	**-1%**	**0.129**	**-5%**	**<0.001**
H10	**419-433**	**3**	**32%**	**-2%**	**<0.001**	**-3%**	**<0.001**	**-3%**	**<0.001**	**0%**	**0.860**	**0%**	**0.916**	**0%**	**0.663**
H10	**434-440**	**1**	**33%**	**-7%**	**<0.001**	**-4%**	**<0.001**	**-3%**	**<0.001**	**4%**	**<0.001**	**0%**	**0.415**	**-3%**	**<0.001**
H11	**440-451**	**2**	**21%**	**-3%**	**<0.001**	**-2%**	**<0.001**	**-2%**	**<0.001**	**1%**	**0.043**	**0%**	**0.503**	**-1%**	**0.010**
H11-H12	**454-465**	**2**	**40%**	**-1%**	**0.009**	**-1%**	**0.023**	**-2%**	**<0.001**	**-2%**	**<0.001**	**-1%**	**0.009**	**1%**	**0.067**

a) The structure segment of each peptide based on the FXR-LBD-GW4064 X ray structure (PDB:3DCT) without contain the first two peptides (201-222, 229-241).

b) Residue numbers for the full-length FXR-LBD (UniRef100_B6ZGS9)

c) Charge state of each peptide observed in the LC-MS experiment.

d) Deuterium level of each peptide in apo FXR-LBD.

e) Deuterium level difference percentage between GW4064 and apo FXR-LBD.

f) Deuterium level difference percentage between CDCA and apo FXR-LBD.

g) Deuterium level difference percentage between GG and apo FXR-LBD.

h) Deuterium level difference percentage between GG and GW4064.

i) Deuterium level difference percentage between GG and CDCA.

j) Deuterium level difference percentage between GW4064 and CDCA.

Table S2. The amide hydrogen distribution of each peptide in the six-fixed-rate-constant binning model (10, 1, 0.1, 0.01, 0.001, and 0.0001 min^{-1}). Although the six-bin model provided the best fits to the deuterium uptake plots (Figure S6) we reduced the model to three categories to simply the discussion of the HDX data in terms of distributions of exchanging sites: category I (fast exchanging sites, $k = 10\ or\ 1\ min^{-1}$,), category II (medium exchanging sites, $k = 0.1\ or\ 0.01 min^{-1}$), and category III (slow exchanging sites, $k = 0.001\ or\ 0.0001\ min^{-1}$).

peptides		apo-FXR-LBD						GW4064						CDCA						GG					
		Category I		Category II		Category III		Category I		Category II		Category III		Category I		Category II		Category III		Category I		Category II		Category III	
from	to	10	1	0.1	0.01	0.001	0.0001	10	1	0.1	0.01	0.001	0.0001	10	1	0.1	0.01	0.001	0.0001	10	1	0.1	0.01	0.001	0.0001
201	222	7	0	0	1	0	13	7	0	0	1	2	11	7	0	0	0	0	14	6	0	0	1	0	14
229	241	4	1	0	0	0	7	3	2	0	0	0	7	4	0	0	0	6	2	4	0	0	0	0	8
242	255	3	1	2	1	0	5	2	2	2	0	0	6	2	2	1	3	0	4	3	0	3	0	0	6
256	268	5	1	0	0	0	5	5	0	1	0	0	5	5	0	0	1	5	0	5	0	0	0	4	2
269	278	4	1	0	0	0	4	4	1	0	0	0	4	4	0	0	1	3	1	4	0	0	0	5	0
288	298	2	2	0	2	4	0	1	0	2	1	0	6	2	1	1	1	4	1	1	2	1	0	4	2
299	316	2	3	0	2	8	1	2	2	1	0	2	9	2	2	1	1	6	4	2	2	1	0	6	5
320	328	1	2	0	4	0	1	2	0	0	1	5	0	1	1	1	1	0	4	2	0	1	0	0	5
328	336	2	1	1	2	0	2	1	1	1	0	0	5	2	0	2	0	3	1	2	0	1	3	0	2
337	347	2	1	0	0	0	6	2	1	0	0	0	6	2	0	1	0	0	6	2	0	0	1	1	5
348	360	5	0	1	0	0	6	2	2	1	0	3	4	4	0	1	1	1	5	3	1	1	0	3	4
361	367	2	0	1	0	0	2	1	1	0	1	0	2	1	1	0	2	0	1	1	1	0	2	0	1
368	375	1	1	1	1	1	2	1	0	1	2	1	2	1	1	0	3	0	2	1	0	2	0	0	4
388	396	1	1	0	1	0	4	1	1	0	0	0	5	1	1	0	0	1	4	1	1	0	0	0	5
397	405	2	0	1	0	5	0	1	1	0	2	2	2	1	1	1	0	0	5	1	1	0	2	0	4
406	412	0	1	0	1	0	3	0	0	1	0	0	4	0	1	0	0	0	4	0	1	0	0	0	4
419	433	2	1	2	0	6	1	2	1	1	2	3	3	2	1	1	2	4	2	2	1	1	2	1	5
434	440	1	1	0	1	3	0	1	0	1	0	0	4	1	0	1	1	0	3	1	0	1	1	0	3
440	451	2	0	0	1	1	7	1	1	0	1	1	7	2	0	0	0	7	2	2	0	0	0	3	6
454	465	4	0	0	0	3	3	3	1	0	0	2	4	4	0	0	0	0	6	4	0	0	0	0	6