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Supplemental Information

Channeling of cAMP in PDE-PKA Complexes Promotes Signal Adaptation

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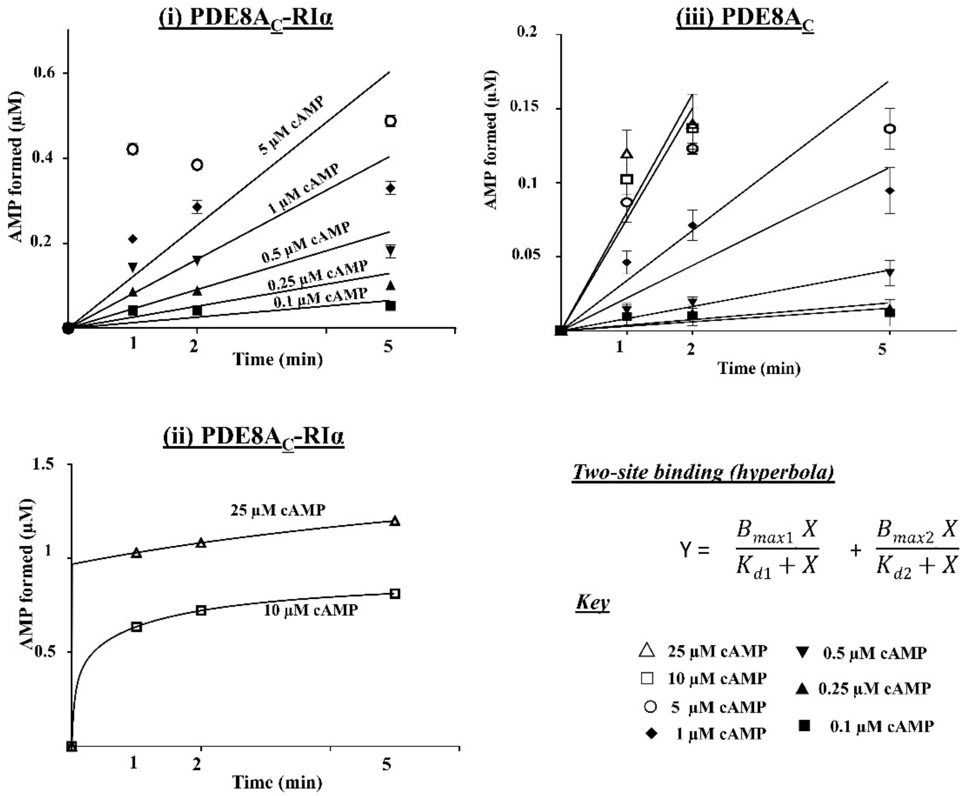
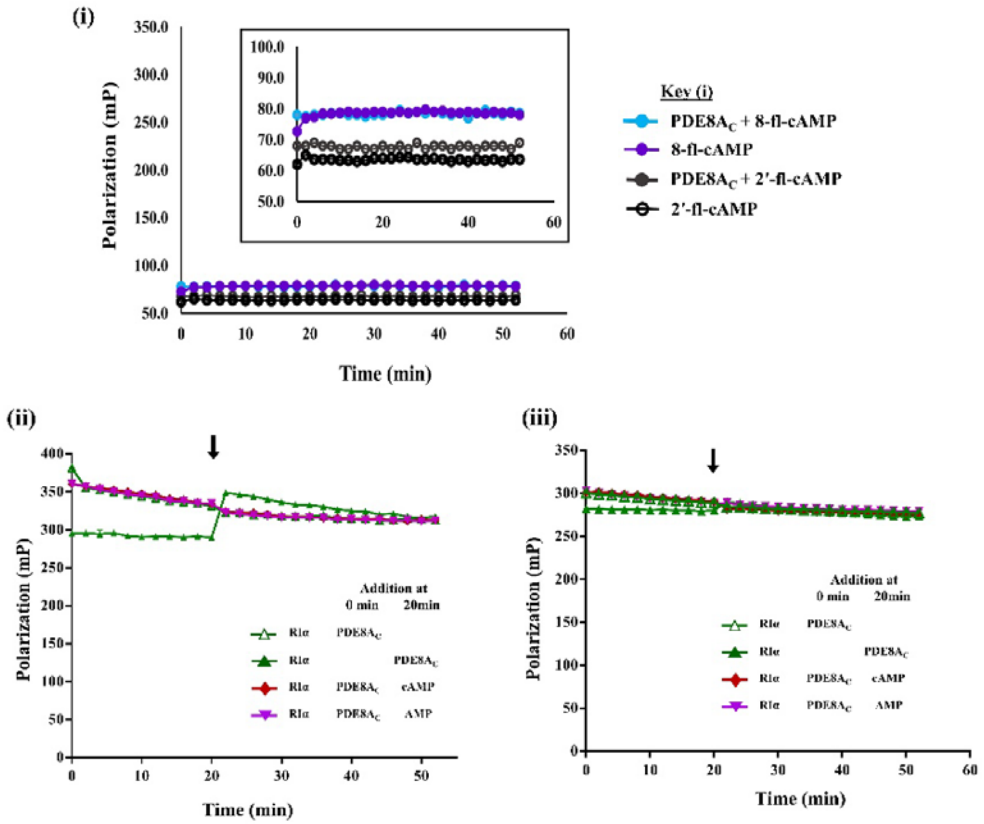
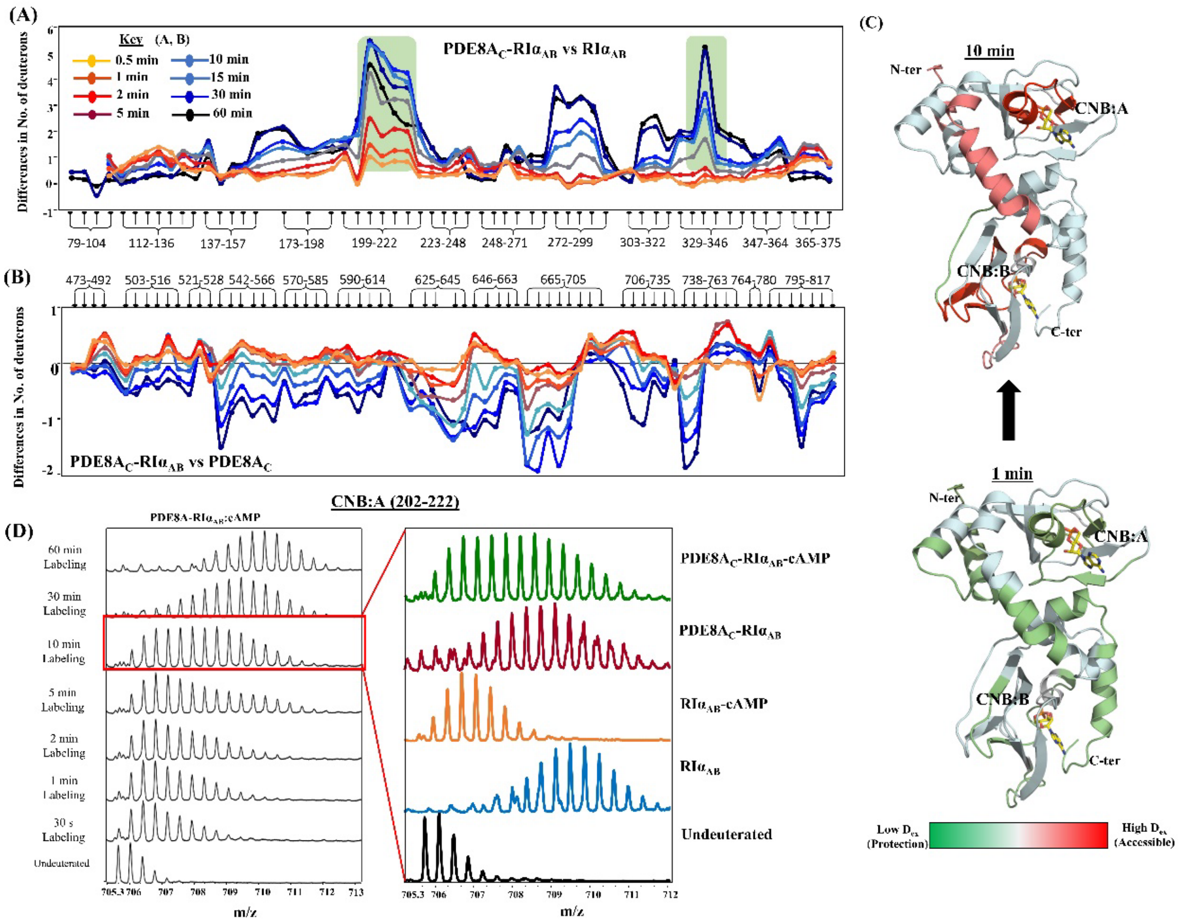


$$Y = \frac{B_{max1}\,X}{K_{d1}+X} + \frac{B_{max2}\,X}{K_{d2}+X}$$

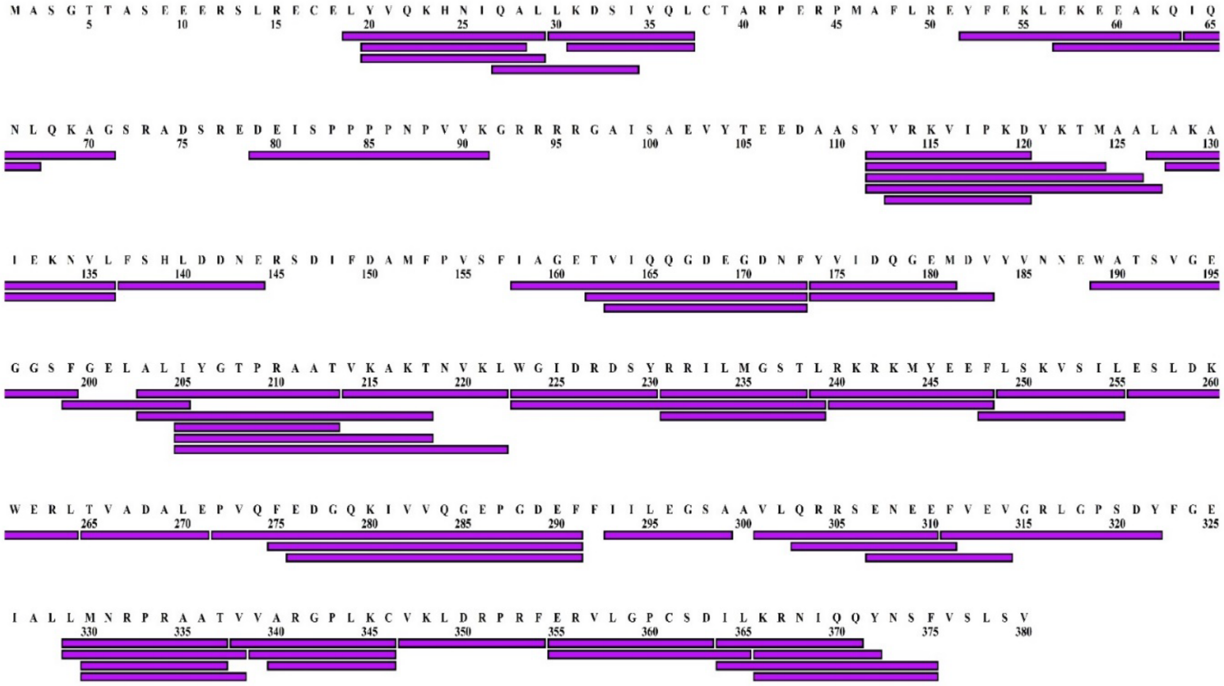
Supplementary Figure S1: ***Amount of AMP formed by hydrolysis of cAMP by PDE8A$_C$-RIα complex and PDE8A$_C$.*** The amounts of AMP formed at different substrate concentrations is plotted as a function of time (1, 2 and 5 min) for the two enzymatic sites – PDE8A$_C$-RIα complex and PDE8A$_C$. (i) For cAMP hydrolysis by PDE8A$_C$-RIα, concentration of AMP formed in the linear range of cAMP concentrations (0.1 – 5 µM) is depicted and their slopes calculated by linear regression equation. (ii) The biphasic burst and lag velocities for 10 µM and 25 µM were calculated using two-site binding equation for hyperbola, as shown. (iii) Concentration of AMP formed by cAMP hydrolysis by PDE8A at different cAMP concentrations are plotted with kinetics fitted into linear regression equation. The slopes for each graph were calculated from average of three independent measurements and their standard deviations are also shown. The graphs were plotted using GraphPad Prism 6.0 (San Diego, CA).



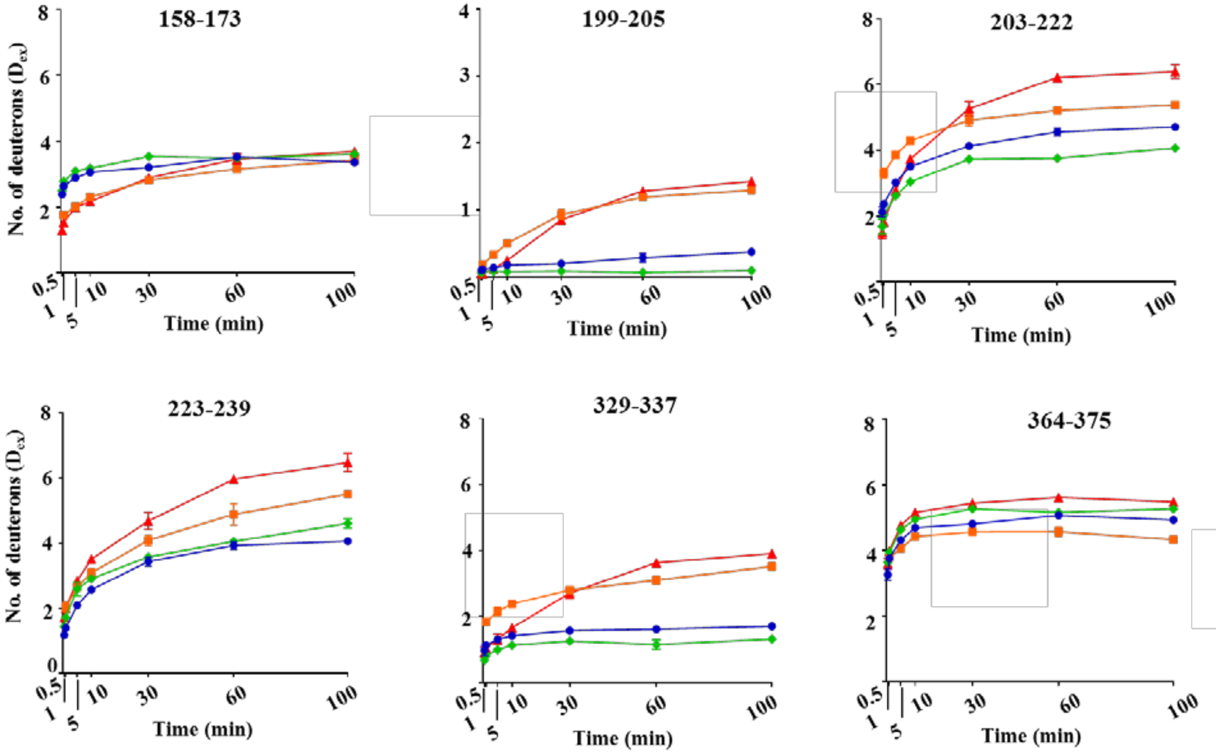
Supplementary Figure S2: ***Fluorescence Polarization of PDE8A$_C$-RIα complex and PDE8A$_C$, fluorescent cAMP analogs:*** (i) Depiction of the control fluorescence polarization experiment carried out for free PDE8A$_C$ with the two fluorescent cAMP analogs. PDE8A$_C$ (10 µM) was treated with 8fl-cAMP (10 µM, *closed circles - lilac*) and 2′fl-cAMP (10 µM, *open circles - black*) and the fluorescence polarization was recorded for 50 min. No significant changes in polarization was observed for PDE8A$_C$ with 8fl-cAMP (*closed circles, blue*) or PDE8A$_C$ with 2′fl-cAMP (*closed circles, gray*). Each point represents an average of technical triplicate and standard errors are also shown. (ii, iii) To full-length RIα saturated with either 2′fl-cAMP (ii) or 8fl-cAMP (iii), PDE8A$_C$ (10 µM) was added at 0 min (*open triangles, green*) and 20 min (*closed triangles, green*) and polarization was measured for 50 min. Competitive displacement of 2′fl-cAMP (i) and 8fl-cAMP (ii) from PDE8A$_C$-RIα complex by cAMP (diamonds, red) or 2 mM AMP (*inverted triangles, lilac*) added after 20 min (black arrow).



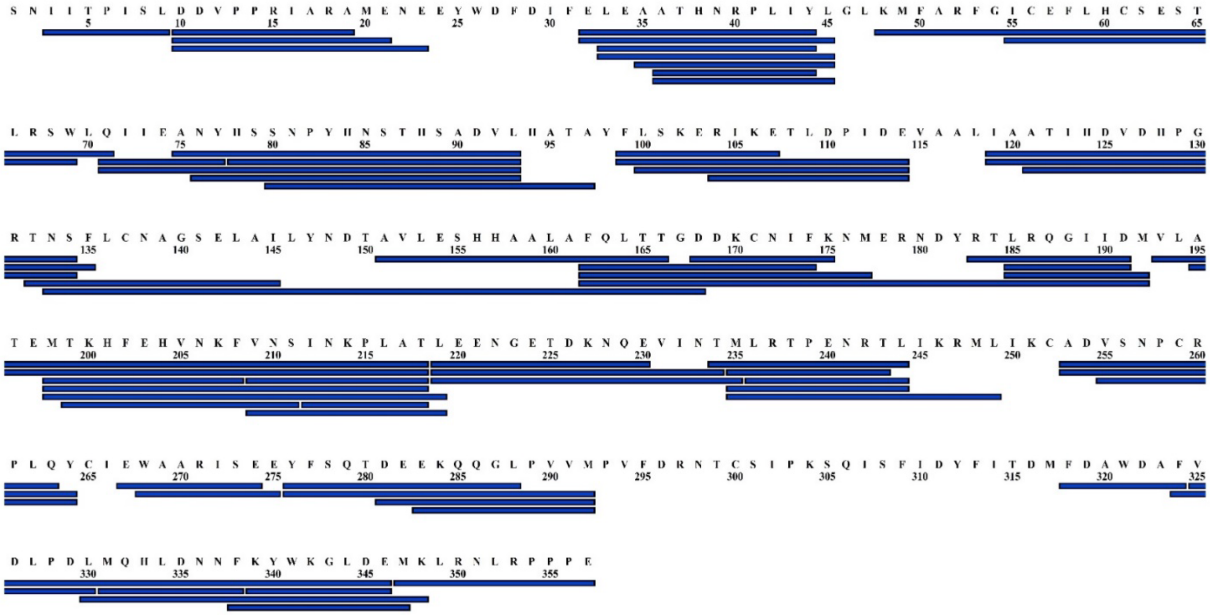
Supplementary Figure S3: ***PDE8 binding dissociates cAMP from PKA-RIα by channeling mechanism.*** (A) Differences in deuterium uptake (Y-axis) between PDE8A$_C$-RIα$_{AB}$ and RIα$_{AB}$ with residue numbers for each peptide of RIα$_{AB}$ (75-380) listed from N to C-terminus (X-axis). Positive differences indicate increased exchange while negative differences indicate decreased exchange in RIα$_{AB}$ upon complexation with PDE8A$_C$. Peptides spanning the cAMP binding sites are highlighted by green box. Deuterium labeling times are depicted according to key. *(B)* Differences in deuterium uptake for PDE8A$_C$-RIα$_{AB}$ vs PDE8A$_C$. Points in positive scale depict increased exchange while negative scale indicate decreased exchange in PDE8A$_C$-RIα$_{AB}$ complex. *(C)* Differences in deuterium exchange at 1 and 10 min are mapped on to structure of RIα$_{AB}$ as indicated in key. cAMP is represented as yellow sticks. Parts of protein with no coverage are shaded in gray. *(D)* Stacked mass spectral envelopes of peptide spanning CNB:A (202-222) for PDE8A$_C$-RIα$_{AB}$-cAMP ternary complex with increase in labeling time is indicated (left panel). The right panel highlights the isotopic distribution of mass spectra for different states of RIα$_{AB}$ after 10 min labeling time. RIα$_{AB}$ (blue) shows highest exchange, while RIα$_{AB}$ in presence of excess cAMP shows least deuterium exchange (orange). Different deuterium exchange profiles of PDE8A$_C$-RIα$_{AB}$ complex in the absence (dark red) and presence of excess cAMP (green) highlights the complexation and channeling of cAMP between the two proteins respectively.



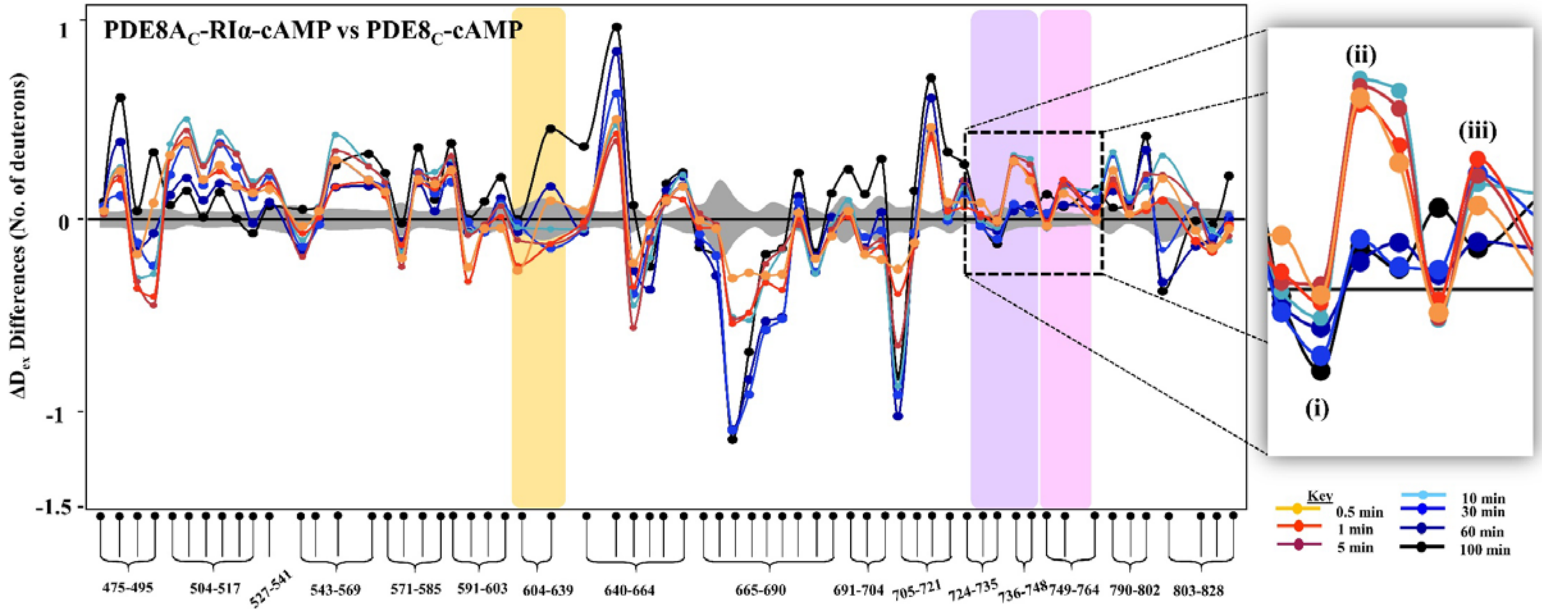
Supplementary Figure S4: ***Sequence coverage of R-subunit:*** Primary sequence of full length RIα is depicted. The lilac boxes indicate all peptide fragments spanning regions of primary sequence obtained from our analysis for describing the effects of cAMP and PDE8A using HDXMS. Approximately 85% sequence coverage was observed.



Supplementary Figure S5: *Deuterium uptake plots for representative peptides of RIα.* Plots showing absolute number of deuterons incorporated (Y-axis) in each peptide with increase in deuterium labeling time (X-axis) for representative peptides spanning different regions of RIα. Each plot comprises of RIα present in four different conditions – RIα (*closed circles, blue*); RIα saturated with excess cAMP (*closed circles, green*); PDE8A$_C$-RIα (squares, orange); and PDE8A$_C$-RIα-cAMP (*triangles, red*). HDXMS experiment values reported here are an average of three independent measurements, as tabulated in table ST1. The graphs were plotted using GraphPad Prism 6.0 (San Diego, CA).



Supplementary Figure S6: ***Sequence coverage of PDE8A:*** Primary sequence of catalytic fragment of PDE8A is shown. The blue boxes indicate all peptide fragments spanning regions of primary sequence obtained from our analysis for describing the differences of PDE8A in binary and ternary complexes using HDXMS. Approximately 87% sequence coverage was observed.



Supplementary Figure S7: ***Effects of PKAR on PDE8 in PDE8-RIα complex as mapped by HDXMS.*** Difference plot comparing differences in deuterium uptake (Y-axis) between PDE8A$_C$-RIα-cAMP and PDE8A$_C$-cAMP with residue numbers for each peptic peptide of catalytic domain of PDE8A listed from N to C-terminus (X-axis). Difference in deuterium exchange in positive scale denotes increased exchange, while negative scale denotes decreased exchange in PDE8A$_C$:RIα complex upon addition of excess cAMP. Peptides spanning the catalytic site of PDE8A (yellow box), cAMP binding (purple box) and cAMP recognition (pink box) are highlighted. Deuterium labeling times of each peptide are depicted according to key. Standard errors are represented in gray. Three peptides residues (i) 724-736, (ii) 740-747 and (iii) 748-764 interacting with cAMP are zoomed in inset.

Supplementary Table ST1: Summary of peptide fragments from HDXMS data for RIα present in different forms. The table summarizes the relative deuterium exchange values reported for the peptides analyzed. A comparison of absolute deuterium exchange of the peptides for two different labelling times 1 min and 30 min is tabulated.

S. No.	Peptide sequence (MH+)	Residue Nos	z[a]	MEA[b]	No. of deuterons exchanged after 1min (Mean± SD)[c]				No. of deuterons exchanged after 30 min (Mean± SD)			
					PDE8:RIα	**PDE8:RIα :cAMP**	**RIα:cAMP**	**RIα**	**PDE8:RIα**	**PDE8:RIα :cAMP**	**RIα:cAMP**	**RIα**
1	YVQKHNIQA (1100.58)	20-28	2	8	2.15 ± 0.01	2.44 ± 0.02	2.35 ± 0.09	2.50 ± 0.04	2.71 ± 0.04	3.16 ± 0.1	2.90 ± 0.1	3.05 ± 0.07
2	YVQKHNIQAL (1213.67)	20-29	2	9	2.44 ± 0.03	2.74 ± 0.05	2.50 ± 0.04	2.50 ± 0.03	3.41 ± 0.06	3.74 ± 0.04	3.44 ± 0.05	3.31 ± 0.04
3	LKDSIVQL (915.55)	30-37	2	7	0.59 ± 0.03	0.53 ± 0.03	0.42 ± 0.02	0.37 ± 0.01	1.57 ± 0.07	1.81 ± 0.02	1.74 ± 0.06	1.64 ± 0.06
4	EKEEAKQIQNL (1329.7)	57-67	2	10	4.22 ± 0.05	4.60 ± 0.06	4.36 ± 0.06	4.26 ± 0.03	4.74 ± 0.01	4.11 ± 0.05	4.39 ± 0.03	4.36 ± 0.01
5	DEISPPPPNPVVK (1388.74)	79-91	2	7	3.52 ± 0.08	3.56 ± 0.06	3.92 ± 0.03	3.96 ± 0.05	3.55 ± 0.04	3.73 ± 0.03	3.93 ± 0.04	3.9 ± 0.05
6	YVRKVIPKD (1117.67)	112-120	3	7	3.23 ± 0.09	3.55 ± 0.03	3.21 ± 0.04	2.99 ± 0.02	3.22 ± 0.09	3.53 ± 0.10	3.36 ± 0.05	3.16 ± 0.04
7	YVRKVIPKDYKTM (1640.92)	112-124	3	11	4.12 ± 0.05	4.59 ± 0.08	4.52 ± 0.05	4.08 ± 0.02	4.08 ± 0.03	4.61 ± 0.01	4.79 ± 0.04	4.27 ± 0.08
8	YVRKVIPKDYKTMAA (1782.99)	112-126	3	13	5.14 ± 0.08	5.95 ± 0.04	5.72 ± 0.04	5.46 ± 0.04	5.05 ± 0.06	6.06 ± 0.01	6.17 ± 0.04	5.73 ± 0.10
9	YVRKVIPKDYKTMAAL (1896.08)	112-127	3	14	5.41 ± 0.09	5.94 ± 0.09	5.49 ± 0.04	5.09 ± 0.04	5.91 ± 0.10	6.57 ± 0.08	6.38 ± 0.06	5.85 ± 0.10
10	VRKVIPKD (954.61)	113-120	2	6	2.49 ± 0.02	2.46 ± 0.05	2.51 ± 0.03	2.41 ± 0.02	2.54 ± 0.02	2.53 ± 0.04	2.73 ± 0.03	2.53 ± 0.04
11	LAKAIEKNVL (1098.68)	127-136	2	9	3.02 ± 0.04	3.08 ± 0.06	2.77 ± 0.00	2.72 ± 0.03	4.15 ± 0.06	4.59 ± 0.1	4.31 ± 0.04	4.08 ± 0.03
12	AKAIEKNVL (985.60)	128-136	2	8	2.66 ± 0.03	2.81 ± 0.04	2.42 ± 0.02	2.37 ± 0.03	3.52 ± 0.06	3.87 ± 0.1	3.50 ± 0.04	3.23 ± 0.07
13	FSHLDDNE (976.40)	137-144	2	7	1.19 ± 0.01	1.37 ± 0.02	1.14 ± 0.01	1.02 ± 0.01	1.16 ± 0.02	1.35 ± 0.03	1.15 ± 0.02	1.01 ± 0.01
14	IAGETVIQQGDEGDNF (1692.77)	158-173	2	15	1.76 ± 0.05	1.54 ± 0.04	2.78 ± 0.01	2.63 ± 0.03	2.82 ± 0.03	2.89 ± 0.11	3.54 ± 0.08	3.21 ± 0.04
15	TVIQQGDEGDNF (1322.58)	162-173	2	11	1.80 ± 0.01	1.51 ± 0.08	2.40 ± 0.01	2.34 ± 0.02	2.39 ± 0.06	2.38 ± 0.06	2.76 ± 0.04	2.61 ± 0.04
16	VIQQGDEGDNF (1221.54)	163-173	2	10	1.82 ± 0.03	1.69 ± 0.05	2.08 ± 0.02	2.03 ± 0.03	2.04 ± 0.03	2.21 ± 0.05	2.43 ± 0.05	2.23 ± 0.03
17	YVIDQGEM (954.42)	174-181	2	7	0.87 ± 0.01	0.72 ± 0.01	0.65 ± 0.02	0.81 ± 0.01	2.43 ± 0.05	1.6 ± 0.07	1.03 ± 0.01	1.5 ± 0.01
18	YVIDQGEMDV (1168.52)	174-183	2	9	0.78 ± 0.03	0.59 ± 0.03	0.66 ± 0.05	0.76 ± 0.05	0.99 ± 0.03	1.61 ± 0.1	0.98 ± 0.04	1.40 ± 0.07
19	WATSVGEGGSF (1097.49)	189-199	1	10	2.67 ± 0.08	2.66 ± 0.1	2.61 ± 0.01	2.52 ± 0.01	3.60 ± 0.12	4.06 ± 0.01	2.92 ± 0.05	2.82 ± 0.02
20	FGELALI (762.44)	199-205	1	6	0.36 ± 0.01	0.10 ± 0.02	0.16 ± 0.02	0.22 ± 0.01	1.86 ± 0.10	1.7 ± 0.06	0.17 ± 0.02	0.39 ± 0.04

21	ALIYGTPRAAT (1133.63)	203-213	2	9	2.54 ± 0.05	1.96 ± 0.06	1.58 ± 0.01	1.77 ± 0.02	4.33 ± 0.10	4.67 ± 0.10	2.56 ± 0.10	3.13 ± 0.05
22	ALIYGTPRAATVKAKT (1660.97)	203-218	2	14	3.34 ± 0.11	1.83 ± 0.11	2.04 ± 0.04	2.37 ± 0.02	4.92 ± 0.10	5.35 ± 0.07	3.74 ± 0.10	4.14 ± 0.03
23	IYGTPRAAT (949.51)	205-213	2	7	2.08 ± 0.03	1.12 ± 0.03	1.24 ± 0.02	1.51 ± 0.02	2.87 ± 0.08	3.09 ± 0.14	1.81 ± 0.04	2.36 ± 0.06
24	IYGTPRAATVKAKT (1476.85)	205-218	2	12	2.93 ± 0.07	1.55 ± 0.14	1.6 ± 0.05	1.98 ± 0.04	4.06 ± 0.1	4.52 ± 0.07	2.01 ± 0.07	3.15 ± 0.06
25	IYGTPRAATVKAKTNVKL (1931.14)	205-222	3	16	3.14 ± 0.10	1.77 ± 0.09	1.59 ± 0.06	1.68 ± 0.04	4.78 ± 0.20	4.97 ± 0.20	2.74 ± 0.08	3.15 ± 0.09
26	WGIDRDSY (1011.45)	223-230	2	7	1.01 ± 0.06	0.68 ± 0.06	0.62 ± 0.03	0.66 ± 0.03	1.32 ± 0.09	1.25 ± 0.13	0.97 ± 0.03	0.89 ± 0.03
27	WGIDRDSYRRILMGSTL (2039.05)	223-239	3	16	2.05 ± 0.07	2.0 ±1 0.04	1.73 ± 0.04	1.40 ± 0.05	4.10 ± 0.09	4.72 ± 0.03	3.59 ± 0.10	3.45 ± 0.07
28	RRILMGST (933.53)	231-238	2	7	2.05 ± 0.03	1.85 ± 0.03	1.75 ± 0.02	1.73 ± 0.01	2.86 ± 0.14	2.78 ± 0.06	2.78 ± 0.06	2.59 ± 0.04
29	RRILMGSTL (1043.61)	231-239	2	8	2.19 ± 0.04	1.94 ± 0.04	1.67 ± 0.01	1.55 ± 0.01	3.02 ± 0.09	3.30 ± 0.18	2.93 ± 0.07	2.69 ± 0.03
30	LRKRKMYEEF (1399.75)	239-248	2	9	1.78 ± 0.02	1.54 ± 0.03	1.41 ± 0.02	1.34 ± 0.01	2.02 ± 0.05	2.45 ± 0.13	2.24 ± 0.03	2.00 ± 0.02
31	RKRKMYEEF (1286.67)	240-248	3	8	2.03 ± 0.02	1.74 ± 0.15	1.37 ± 0.02	1.28 ± 0.01	2.30 ± 0.06	2.77 ± 0.13	2.11 ± 0.03	1.88 ± 0.02
32	FLSKVSIL (906.56)	248-255	2	7	1.92 ± 0.01	2.03 ± 0.02	1.97 ± 0.01	1.91 ± 0.02	2.88 ± 0.05	3.22 ± 0.07	3.14 ± 0.05	2.91 ± 0.06
33	LSKVSIL (759.49)	249-255	1	6	1.76 ± 0.03	1.82 ± 0.04	1.64 ± 0.04	1.62 ± 0.05	2.76 ± 0.04	3.04 ± 0.07	2.81 ± 0.05	2.61 ± 0.1
34	ESLDKWERL (1175.6)	256-264	2	8	1.83 ± 0.06	1.58 ± 0.09	1.29 ± 0.04	1.23 ± 0.07	2.78 ± 0.06	3.03 ± 0.10	2.24 ± 0.07	2.07 ± 0.07
35	TVADALE (718.36)	265-271	1	6	1.06 ± 0.04	0.44 ± 0.01	0.61 ± 0.03	0.73 ± 0.02	2.45 ± 0.07	2.52 ± 0.03	1.71 ± 0.04	1.76 ± 0.03
36	PVQFEDGQKIVVQGEPGDEF (2218.06)	272-291	2	18	2.32 ± 0.09	2.16 ± 0.07	2.46 ± 0.11	2.59 ± 0.08	3.63 ± 0.10	3.82 ± 0.14	3.67 ± 0.08	3.8 ± 0.10
37	FEDGQKIVVQGEPGDEF (1893.88)	275-291	2	15	2.37 ± 0.08	n.a.	1.66 ± 0.01	1.7 ± 0.01	3.76 ± 0.15	n.a.	2.84 ± 0.11	2.80 ± 0.03
38	EDGQKIVVQGEPGDEF (1746.82)	276-291	2	14	2.06 ± 0.04	1.48 ± 0.04	1.66 ± 0.01	1.72 ± 0.01	3.73 ± 0.16	3.38 ± 0.17	2.72 ± 0.07	2.69 ± 0.04
39	IILEGSA (702.40)	293-299	1	6	0.86 ± 0.02	0.82 ± 0.01	0.72 ± 0.01	0.75 ± 0.01	1.09 ± 0.04	1.16 ± 0.06	1.38 ± 0.04	1.35 ± 0.03
40	VLQRRSENEE (1259.63)	301-310	2	9	2.49 ± 0.06	2.85 ± 0.15	2.52 ± 0.04	2.40 ± 0.04	2.45 ± 0.07	2.91 ± 0.03	2.56 ± 0.06	2.44 ± 0.03
41	ENEEFVEV (994.44)	307-314	2	7	0.91 ± 0.03	0.61 ± 0.02	0.57 ± 0.01	0.54 ± 0.02	1.10 ± 0.03	1.03 ± 0.09	0.87 ± 0.02	0.77 ± 0.03
42	FVEVGRLGPSDY (1138.67)	311-322	2	10	1.56 ± 0.03	1.35 ± 0.03	1.44 ± 0.07	1.49 ± 0.06	2.43 ± 0.07	2.37 ± 0.11	1.99 ± 0.04	1.95 ± 0.08
43	LMNRPRAAT (1029.56)	329-337	2	7	1.72 ± 0.05	0.99 ± 0.04	0.93 ± 0.02	1.24 ± 0.01	2.53 ± 0.08	2.53 ± 0.07	1.36 ± 0.03	1.69 ± 0.03
44	LMNRPRAATV (1128.63)	329-338	2	8	1.84 ± 0.04	1.04 ± 0.02	0.82 ± 0.01	1.14 ± 0.01	2.80 ± 0.1	1.57 ± 0.05	1.26 ± 0.12	1.57 ± 0.05

45	MNRPRAAT (916.48)	330-337	2	6	1.50 ± 0.05	0.76 ± 0.05	0.82 ± 0.02	1.02 ± 0.02	2.20 ± 0.07	2.14 ± 0.07	1.11 ± 0.01	1.35 ± 0.04
46	MNRPRAATV (1015.55)	330-338	2	7	1.62 ± 0.04	0.80 ± 0.07	0.80 ± 0.03	0.92 ± 0.02	2.45 ± 0.09	2.21 ± 0.16	1.07 ± 0.02	1.22 ± 0.04
47	VVARGPLKC (942.55)	338-346	1	7	1.18 ± 0.05	0.56 ± 0.02	0.69 ± 0.05	0.85 ± 0.06	1.72 ± 0.06	1.56 ± 0.02	1.78 ± 0.07	1.79 ± 0.06
48	VARGPLKC (843.48)	339-346	2	6	1.35 ± 0.04	0.71 ± 0.05	0.65 ± 0.03	0.76 ± 0.01	1.78 ± 0.06	1.62 ± 0.12	1.62 ± 0.01	1.56 ± 0.02
49	ARGPLKC (744.42)	340-346	2	5	1.15 ± 0.07	0.52 ± 0.05	0.60 ± 0.02	0.63 ± 0.01	1.59 ± 0.05	1.44 ± 0.05	1.49 ± 0.03	1.38 ± 0.02
50	VKLDRPRF (1030.61)	347-354	2	6	1.05 ± 0.02	n.a	0.69 ± 0.03	0.69 ± 0.03	1.53 ± 0.04	n.a.	1.11 ± 0.03	0.97 ± 0.05
51	ERVLGPCSD (975.45)	355-363	2	7	1.89 ± 0.03	1.70 ± 0.06	1.65 ± 0.01	1.69 ± 0.05	2.52 ± 0.09	2.55 ± 0.16	2.23 ± 0.03	2.06 ± 0.02
52	ERVLGPCSDIL (1201.62)	355-365	2	9	2.19 ± 0.01	2.04 ± 0.02	2.10 ± 0.01	1.98 ± 0.03	3.24 ± 0.08	3.15 ± 0.09	2.95 ± 0.06	2.65 ± 0.05
53	ILKRNIQQ (1012.62)	364-371	2	7	2.46 ± 0.04	2.20 ± 0.08	2.22 ± 0.05	2.23 ± 0.02	2.94 ± 0.07	3.35 ± 0.14	3.37 ± 0.11	3.21 ± 0.02
54	ILKRNIQQYNSF (1523.83)	364-375	2	11	3.79 ± 0.04	3.99 ± 0.06	1.95 ± 0.01	3.76 ± 0.03	4.57 ± 0.08	5.44 ± 0.01	5.27 ± 0.07	4.82 ± 0.09
55	KRNIQQY (949.52)	366-372	2	6	2.10 ± 0.03	2.13 ± 0.08	2.08 ± 0.06	1.8 ± 0.03	2.31 ± 0.06	2.59 ± 0.10	2.58 ± 0.03	2.43 ± 0.02
56	KRNIQQYNSF (1297.66)	366-375	2	9	3.36 ± 0.03	3.76 ± 0.03	3.57 ± 0.02	3.41 ± 0.04	3.61 ± 0.04	4.26 ± 0.03	4.14 ± 0.06	3.81 ± 0.07

[a] Charge state of the peptide analyzed; [b] Number of maximum available exchangeable amides for each peptide; [c] Average and standard deviation values calculated from three independent deuterium exchange experiments. n.a. Deuteron exchange values not available.

Supplementary Table 2: Summary of peptide fragments from HDXMS data for catalytic fragment of PDE8A in different states. The table summarizes the relative deuterium exchange values reported for the peptides analyzed for PDE8A$_C$ protein. A comparison of absolute deuterium exchange of the peptides for two different labelling times 1 min and 30 min is tabulated.

S. No	**Peptide sequence (MH+)**	**Residues**	**z**[a]	**MEA**[b]	**No. of deuterons exchanged after 1min (Mean± SD)**[c]				**No. of deuterons exchanged after 30 min (Mean± SD)**[c]			
					PDE8:RIα	**PDE8:RIα:cAMP**	**PDE8:cAMP**	**PDE8**	**PDE8:RIα**	**PDE8:RIα:cAMP**	**PDE8:cAMP**	**PDE8**
1	IITPISL (756.48)	475-481	1	5	2.38 ± 0.03	2.43 ± 0.03	2.39 ± 0.04	2.29 ± 0.02	2.42 ± 0.09	2.48 ± 0.06	2.41 ± 0.05	2.36 ± 0.01
2	DDVPPRIARA (1109.61)	482-491	2	7	3.48 ± 0.04	3.44 ± 0.03	3.24 ± 0.04	3.27 ± 0.04	4.1 ± 0.01	3.96 ± 0.04	3.84 ± 0.02	3.80 ± 0.01
3	DDVPPRIARAME (1369.69)	482-493	2	9	3.46 ± 0.01	3.45 ± 0.02	3.81 ± 0.07	3.76 ± 0.06	4.61 ± 0.04	4.60 ± 0.03	4.72 ± 0.1	4.70 ± 0.02
4	ELEAATHNRPLIY (1523.79)	504-516	2	11	1.52 ± 0.01	1.68 ± 0.03	1.36 ± 0.02	1.16 ± 0.01	2.25 ± 0.05	2.18 ± 0.05	1.95 ± 0.08	2.08 ± 0.05
5	ELEAATHNRPLIYL (1639.88)	504-517	2	12	1.16 ± 0.06	1.33 ± 0.03	0.92 ± 0.03	0.72 ± 0.02	2.01 ± 0.02	1.97 ± 0.06	1.58 ± 0.07	1.67 ± 0.01
6	LEAATHNRPLIY (1397.75)	505-516	3	10	1.29 ± 0.02	1.38 ± 0.01	1.17 ± 0.02	1.05 ± 0.01	1.86 ± 0.03	1.82 ± 0.01	1.65 ± 0.01	1.78 ± 0.05
7	LEAATHNRPLIYL (1510.84)	505-517	2	11	0.93 ± 0.04	1.06 ± 0.02	0.81 ± 0.03	0.62 ± 0.01	1.69 ± 0.01	1.70 ± 0.03	1.31 ± 0.07	1.41 ± 0.01
8	AATHNRPLIYL (1268.71)	506-517	2	9	0.76 ± 0.02	0.84 ± 0.05	0.66 ± 0.02	0.51 ± 0.02	1.33 ± 0.02	1.31 ± 0.05	1.03 ± 0.06	1.09 ± 0.01
9	ATHNRPLIY (1084.59)	507-516	2	7	0.69 ± 0.04	0.83 ± 0.01	0.68 ± 0.03	0.57 ± 0.01	1.19 ± 0.04	1.14 ± 0.05	1.02 ± 0.05	1.11 ± 0.03
10	ATHNRPLIYL (1197.67)	507-517	2	8	0.60 ± 0.05	0.70 ± 0.02	0.52 ± 0.01	0.45 ± 0.02	1.13 ± 0.04	1.09 ± 0.07	0.87 ± 0.06	0.95 ± 0.01
11	KMFARFGICEFLHCSESTLRSWLQ (2889.4)	520-543	3	23	n.a.	n.a.	5.59 ± 0.04	6.18 ± 0.06	n.a.	n.a.	6.91 ± 0.01	6.51 ± 0.01
12	ICEFLHCSESTLRSW (1810.82)	527-541	2	14	1.74 ± 0.05	1.79 ± 0.05	1.86 ± 0.06	1.76 ± 0.03	2.84 ± 0.1	2.91 ± 0.1	3.06 ± 0.08	2.97 ± 0.01

13	QIIEANY (850.43)	543-549	1	6	0.80 ± 0.02	1.26 ± 0.03	0.75 ± 0.02	0.85 ± 0.02	1.41 ± 0.03	1.43 ± 0.02	1.46 ± 0.04	1.42 ± 0.03
14	QIIEANYHSSNPYHNSTHSADVL (2597.20)	543-565	3	21	1.46 ± 0.05	1.57 ± 0.03	1.41 ± 0.05	1.32 ± 0.01	2.38 ± 0.04	2.48 ± 0.03	2.14 ± 0.10	2.37 ± 0.08
15	ANYHSSNPYHNSTHSADVL (2113.93)	547-565	2	17	n.a.	n.a.	1.84 ± 0.03	1.73 ± 0.02	n.a.	n.a.	2.06 ± 0.07	2.05 ± 0.07
16	NYHSSNPYHNSTHSADVL (2042.89)	548-565	3	16	1.70 ± 0.01	1.85 ± 0.03	1.66 ± 0.04	1.54 ± 0.01	1.97 ± 0.03	2.06 ± 0.01	1.85 ± 0.09	1.84 ± 0.07
17	HSSNPYHNSTHSADVL (1765.79)	550-565	2	14	1.65 ± 0.01	1.67 ± 0.02	1.55 ± 0.03	1.45 ± 0.01	1.92 ± 0.03	1.87 ± 0.02	1.71 ± 0.08	1.78 ± 0.01
18	SNPYHNSTHSADVLHATA (1921.88)	552-569	3	16	1.74 ± 0.10	1.77 ± 0.09	1.90 ± 0.09	1.74 ± 0.04	2.94 ± 0.09	3.05 ± 0.10	3.19 ± 0.09	3.13 ± 0.01
19	FLSKERIKE (1149.66)	570-579	2	8	1.55 ± 0.01	1.71 ± 0.04	1.52 ± 0.01	1.40 ± 0.01	1.71 ± 0.04	1.87 ± 0.06	1.63 ± 0.05	1.78 ± 0.04
20	FLSKERIKETLDPIDE (1933.02)	571-586	2	14	2.88 ± 0.06	3.02 ± 0.03	2.87 ± 0.03	2.59 ± 0.02	3.48 ± 0.02	3.54 ± 0.01	3.41 ± 0.08	3.36 ± 0.07
21	LSKERIKETLDPIDE (1785.96)	572-586	3	13	3.14 ± 0.03	3.36 ± 0.04	3.12 ± 0.04	2.89 ± 0.03	3.62 ± 0.03	3.73 ± 0.11	3.53 ± 0.01	3.53 ± 0.02
22	IAATIHDVDHPGRTNS (1703.84)	591-606	2	14	2.75 ± 0.02	2.81 ± 0.03	2.86 ± 0.02	2.69 ± 0.03	2.96 ± 0.02	2.99 ± 0.01	3.07 ± 0.08	2.99 ± 0.05
23	IAATIHDVDHPGRTNSF (1850.91)	591-607	2	15	2.88 ± 0.04	3.01 ± 0.03	3.01 ± 0.01	2.79 ± 0.02	3.19 ± 0.07	3.26 ± 0.04	3.18 ± 0.11	3.04 ± 0.10
24	ATIHDVDHPGRTNS (1519.72)	593-606	2	12	2.25 ± 0.04	2.33 ± 0.03	2.39 ± 0.03	2.57 ± 0.02	2.56 ± 0.01	2.64 ± 0.01	2.66 ± 0.09	2.66 ± 0.06
25	TNSFLCNAGSELAI (1439.68)	604-617	2	13	n.a.	n.a.	1.39 ± 0.05	1.47 ± 0.06	n.a.	n.a.	1.58 ± 0.08	1.69 ± 0.08
26	AVLESHHAALAFQLTT (1708.90)	623-638	2	15	n.a.	n.a.	3.17 ± 0.07	3.01 ± 0.09	n.a.	n.a.	3.73 ± 0.08	3.60 ± 0.03
27	FQLTTGDDKCNIF (1501.69)	634-646	2	12	2.43 ± 0.08	2.57 ± 0.04	2.59 ± 0.03	2.53 ± 0.04	3.20 ± 0.06	3.28 ± 0.03	3.33 ± 0.07	3.21 ± 0.01

28	FQLTTGDDKCNIFKNM (1874.88)	634-649	3	15	n.a.	n.a.	2.90 ± 0.1	2.92 ± 0.09	n.a.	n.a.	4.17 ± 0.08	4.11 ± 0.01
29	DDKCNIFK (982.46)	639-647	2	7	2.47 ± 0.04	2.46 ± 0.02	2.01 ± 0.03	2.19 ± 0.02	2.85 ± 0.04	2.93 ± 0.01	2.28 ± 0.02	2.29 ± 0.04
30	KNMERNDYRT (1326.62)	647-656	2	9	5.57 ± 0.12	5.82 ± 0.06	2.78 ± 0.02	2.63 ± 0.03	5.64 ± 0.09	6.02 ± 0.14	2.88 ± 0.09	2.69 ± 0.11
31	RTLRQGIID (1071.62)	655-663	2	8	1.41 ± 0.04	1.54 ± 0.03	1.53 ± 0.03	1.37 ± 0.02	1.84 ± 0.08	1.94 ± 0.11	2.04 ± 0.04	1.98 ± 0.12
32	LRQGIID (814.48)	657-663	1	6	0.88 ± 0.04	0.92 ± 0.01	0.81 ± 0.01	0.76 ± 0.01	1.26 ± 0.03	1.23 ± 0.02	1.13 ± 0.03	1.07 ± 0.09
33	LRQGIIDM (945.51)	657-664	2	7	0.76 ± 0.06	0.81 ± 0.03	0.71 ± 0.02	0.54 ± 0.01	1.27 ± 0.03	1.29 ± 0.05	1.12 ± 0.03	1.08 ± 0.01
34	VLATEMTKHFEHVNKFVNSINKPLAT (2968.57)	665-690	4	24	4.27 ± 0.11	4.08 ± 0.07	4.62 ± 0.12	4.64 ± 0.12	6.06 ± 0.13	5.82 ± 0.06	6.92 ± 0.12	6.93 ± 0.07
35	ATEMTKHFEHVNKFVNSINKPLAT (2756.42)	667-690	3	22	3.88 ± 0.07	3.75 ± 0.01	4.24 ± 0.05	4.30 ± 0.06	5.28 ± 0.10	5.05 ± 0.14	5.96 ± 0.05	5.96 ± 0.05
36	MTKHFEHVNKF (1417.70)	670-680	2	10	1.22 ± 0.03	1.17 ± 0.01	1.21 ± 0.01	1.28 ± 0.02	1.39 ± 0.02	1.29 ± 0.03	1.36 ± 0.05	1.52 ± 0.05
37	MTKHFEHVNKFVNSINKPLAT (2455.29)	670-690	3	19	3.79 ± 0.09	3.93 ± 0.03	4.26 ± 0.05	4.14 ± 0.05	4.90 ± 0.04	4.85 ± 0.02	5.42 ± 0.01	5.48 ± 0.02
38	MTKHFEHVNKFVNSINKPLATL (2568.37)	670-691	3	20	4.03 ± 0.07	4.16 ± 0.04	4.53 ± 0.05	4.37 ± 0.04	5.12 ± 0.04	5.15 ± 0.02	5.66 ± 0.17	5.77 ± 0.05
39	TKHFEHVNKFVNS (1586.81)	671-683	2	12	2.08 ± 0.09	2.08 ± 0.03	2.12 ± 0.03	2.14 ± 0.02	2.48 ± 0.10	2.33 ± 0.09	2.52 ± 0.11	2.71 ± 0.03
40	VNSINKPLAT (1056.60)	681-691	2	8	3.08 ± 0.04	3.18 ± 0.03	3.18 ± 0.06	3.18 ± 0.03	3.66 ± 0.01	3.86 ± 0.05	3.77 ± 0.09	3.73 ± 0.06
41	VNSINKPLATL (1169.69)	681-692	2	9	3.29 ± 0.07	3.44 ± 0.03	3.65 ± 0.06	3.59 ± 0.04	3.91 ± 0.02	4.02 ± 0.08	4.29 ± 0.08	4.19 ± 0.05
42	INKPLAT (756.46)	684-690	2	5	2.01 ± 0.02	2.04 ± 0.01	2.11 ± 0.03	2.10 ± 0.03	2.26 ± 0.01	2.29 ± 0.03	2.36 ± 0.03	2.32 ± 0.01
43	LEENGETDKNQE (1405.61)	691-702	2	11	3.14 ± 0.04	3.22 ± 0.03	3.21 ± 0.04	3.13 ± 0.04	2.98 ± 0.10	3.19 ± 0.20	3.17 ± 0.03	3.16 ± 0.06

44	LEENGETDKNQEVINT (1832.85)	691-706	2	15	5.38 ± 0.05	5.49 ± 0.06	5.67 ± 0.04	5.47 ± 0.06	5.52 ± 0.06	5.65 ± 0.09	5.74 ± 0.03	5.46 ± 0.13
45	LEENGETDKNQEVINTM (1963.89)	691-707	2	16	5.44 ± 0.01	5.48 ± 0.08	5.63 ± 0.04	5.43 ± 0.06	5.52 ± 0.09	5.61 ± 0.09	5.67 ± 0.08	5.46 ± 0.1
46	MLRTPENRT (1117.58)	707-715	2	7	2.24 ± 0.06	2.29 ± 0.02	2.43 ± 0.05	2.36 ± 0.04	2.60 ± 0.06	2.76 ± 0.09	2.83 ± 0.08	2.76 ± 0.03
47	MLRTPENRTL (1230.66)	707-716	3	8	2.65 ± 0.11	2.94 ± 0.03	2.52 ± 0.04	2.10 ± 0.06	3.27 ± 0.05	3.38 ± 0.10	2.92 ± 0.07	2.81 ± 0.11
48	MLRTPENRTLIKRML (1872.07)	707-721	3	13	1.78 ± 0.11	1.99 ± 0.02	1.93 ± 0.05	1.69 ± 0.03	2.41 ± 0.08	2.50 ± 0.11	2.37 ± 0.06	2.34 ± 0.16
49	LRTPENRTL (1099.62)	708-716	2	7	2.10 ± 0.03	2.15 ± 0.03	2.02 ± 0.01	1.99 ± 0.02	2.38 ± 0.02	2.42 ± 0.03	2.43 ± 0.06	2.42 ± 0.01
50	ADVSNPCRPLQ (1199.58)	725-735	2	8	2.42 ± 0.01	2.41 ± 0.01	2.38 ± 0.01	2.40 ± 0.01	2.52 ± 0.03	2.43 ± 0.01	2.46 ± 0.01	2.53 ± 0.01
51	ADVSNPCRPLQY (1362.65)	725-736	2	9	2.42 ± 0.03	2.42 ± 0.01	2.44 ± 0.02	2.46 ± 0.01	2.77 ± 0.03	2.52 ± 0.01	2.62 ± 0.01	2.86 ± 0.01
52	VSNPCRPLQY (1176.58)	727-736	2	7	0.91 ± 0.07	0.93 ± 0.01	2.35 ± 0.05	2.17 ± 0.08	1.79 ± 0.06	1.59 ± 0.07	2.50 ± 0.09	2.73 ± 0.02
53	EWAARISE (961.47)	739-746	1	7	1.14 ± 0.03	1.22 ± 0.04	0.93 ± 0.01	0.96 ± 0.01	1.78 ± 0.06	1.61 ± 0.03	1.53 ± 0.03	1.85 ± 0.01
54	WAARISEE (961.47)	740-747	2	7	1.17 ± 0.04	1.26 ± 0.02	1.04 ± 0.02	1.00 ± 0.03	1.73 ± 0.05	1.51 ± 0.03	1.5 ±0 0.03	1.84 ± 0.01
55	YFSQTDEEKQQGLPVVM (1998.95)	748-764	2	15	5.29 ± 0.05	5.66 ± 0.01	5.45 ± 0.05	5.14 ± 0.02	5.61 ± 0.04	5.76 ± 0.01	5.57 ± 0.01	5.28 ± 0.01
56	FSQTDEEKQQGLPVVM (1835.88)	749-764	2	14	5.39 ± 0.05	5.58 ± 0.10	5.65 ± 0.04	5.36 ± 0.03	5.70 ± 0.10	5.89 ± 0.04	5.82 ± 0.05	5.68 ± 0.01
58	EKQQGLPVVM (1128.61)	755-764	2	8	3.64 ± 0.03	3.79 ± 0.01	3.76 ± 0.04	3.72 ± 0.02	3.78 ± 0.02	3.87 ± 0.03	3.76 ± 0.03	3.73 ± 0.01
59	FDAWDAF (871.36)	790-796	1	6	0.53 ± 0.04	0.49 ± 0.06	0.31 ± 0.01	0.22 ± 0.01	0.78 ± 0.08	0.77 ± 0.07	0.44 ± 0.02	0.41 ± 0.07
60	FVDLPDL (818.43)	796-802	1	5	0.68 ± 0.02	0.70 ± 0.01	0.68 ± 0.02	0.62 ± 0.01	0.98 ± 0.03	0.99 ± 0.03	0.89 ± 0.03	0.88 ± 0.01
61	VDLPDLMQHLDNNFKYWKGLDE (2690.29)	797-818	3	20	2.86 ± 0.07	2.79 ± 0.11	2.69 ± 0.09	2.66 ± 0.06	3.88 ± 0.10	3.76 ± 0.10	3.92 ± 0.03	4.10 ± 0.01

62	LMQHLDNNFKYWKGLDEMK (2410.17)	802-820	3	18	2.93 ± 0.09	3.16 ± 0.07	0.94 ± 0.02	0.81 ± 0.09	4.04 ± 0.07	4.03 ± 0.12	1.07 ± 0.08	1.12 ± 0.07
63	MQHLDNNF (1018.44)	803-810	2	7	0.81 ± 0.09	0.94 ± 0.02	0.89 ± 0.04	0.80 ± 0.01	1.12 ± 0.07	1.07 ± 0.08	0.86 ± 0.04	0.94 ± 0.04
64	FKYWKGLDEM (1316.63)	810-819	2	9	1.71 ± 0.06	1.75 ± 0.05	1.86 ± 0.01	1.81 ± 0.01	2.04 ± 0.12	2.11 ± 0.06	2.04 ± 0.08	2.19 ± 0.07
65	KYWKGLDE (1038.52)	811-818	1	7	1.47 ± 0.08	1.36 ± 0.03	1.54 ± 0.01	1.55 ± 0.01	1.71 ± 0.06	1.64 ± 0.06	1.72 ± 0.08	1.78 ± 0.01
66	MKLRNLRPPPE (1350.77)	819-829	3	7	2.59 ± 0.05	2.70 ± 0.06	2.74 ± 0.03	2.66 ± 0.03	2.60 ±0.04	2.77 ± 0.05	2.75 ± 0.07	2.73 ± 0.01

[a] Charge state of the peptide analyzed; [b] Number of maximum available exchangeable amides for each peptide; [c] Average and standard deviation values calculated from three independent deuterium exchange experiments. n.a. Deuteron exchange values not available