Parallel Allostery by cAMP and PDE Coordinates Activation and Termination Phases in cAMP Signaling

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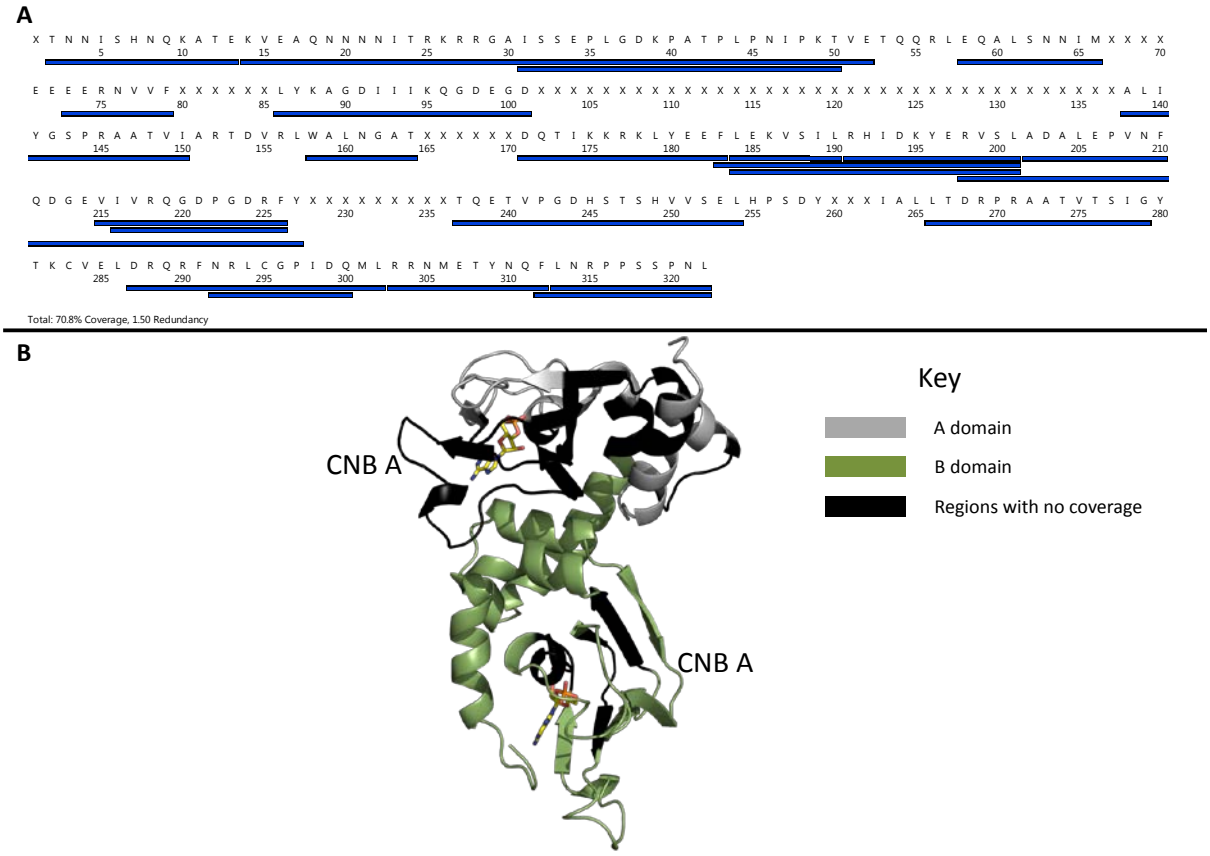
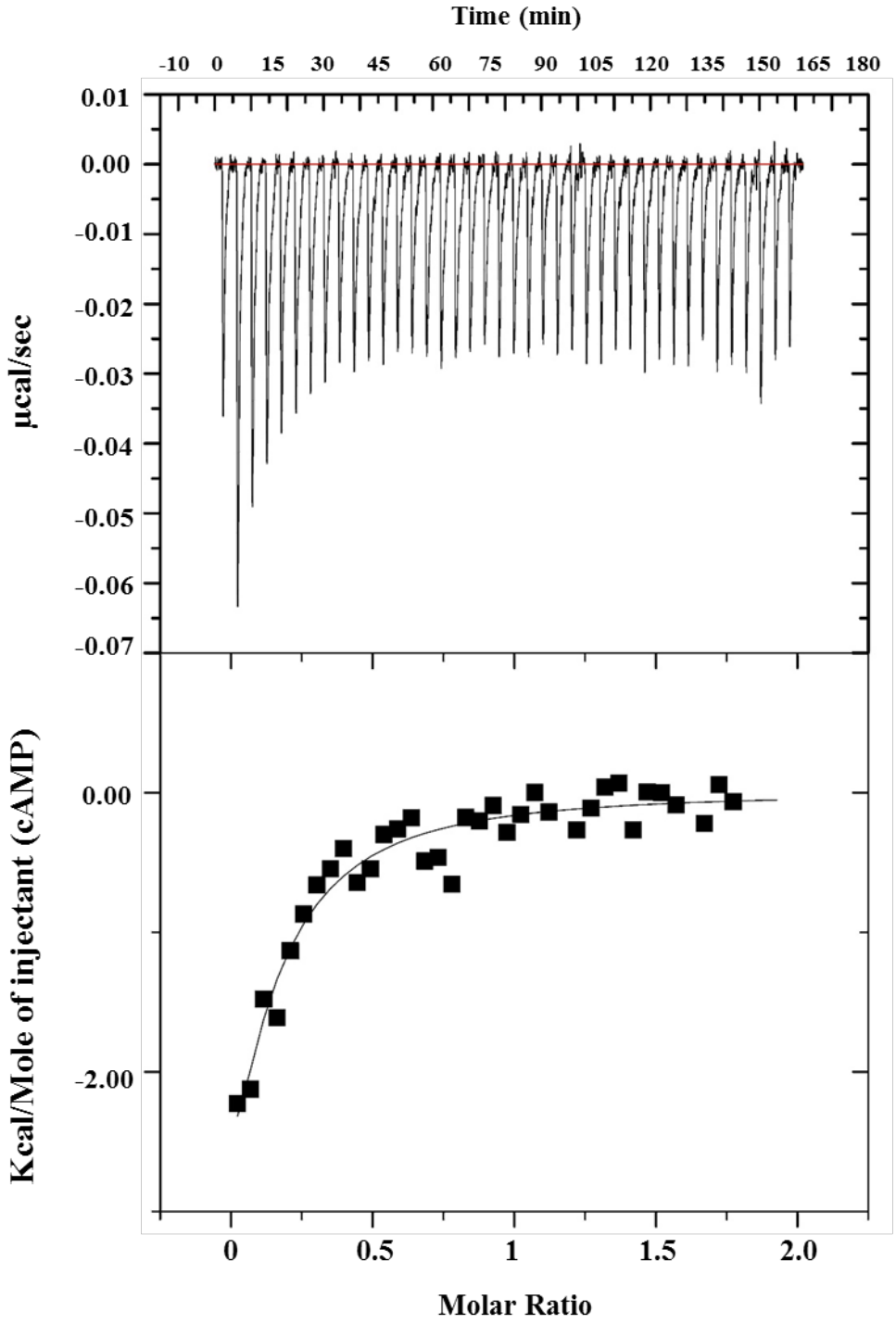
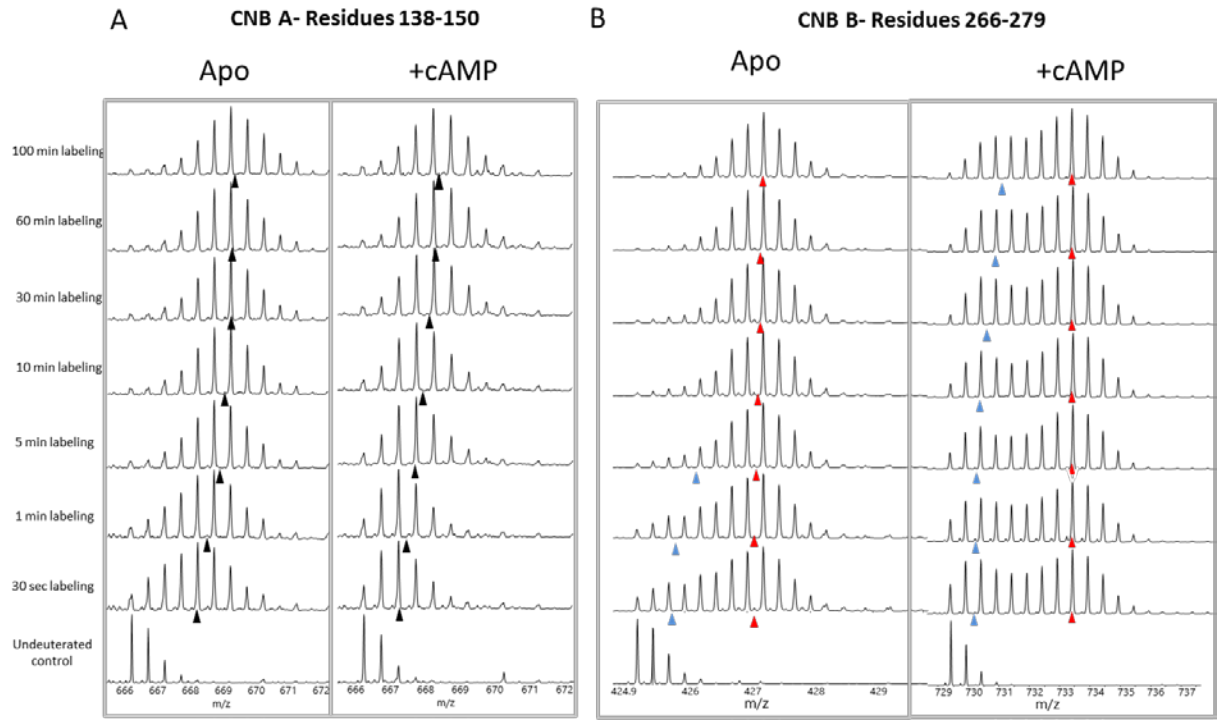
SUPPLEMENTARY INFORMATION



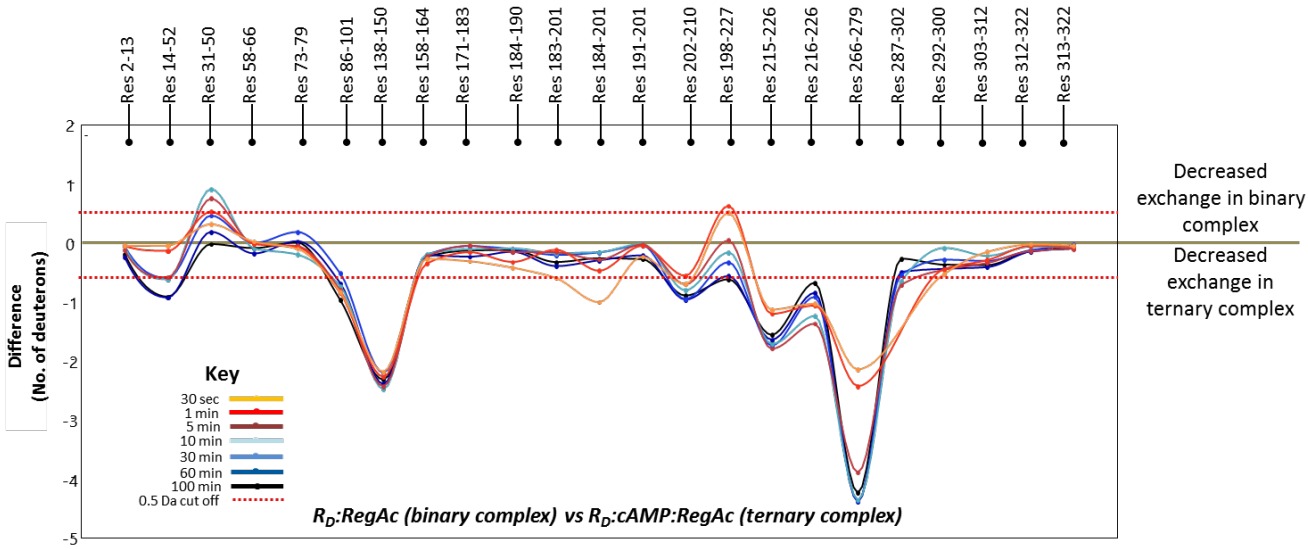
Supplementary Figure 1: ***Sequence coverage of*** R_D**.** A) The primary sequence of full length R_D is depicted. The blue boxes indicate all peptide fragments spanning regions of primary sequence obtained from our data set analysis. Approximately 71% sequence coverage was observed. Amino acids labeled as 'X' are residues which were not identified in the initial protein database search engine. B) Regions with sequence coverage was mapped onto the tertiary structure modelled structure of R_D. CNB-A domain is represented in grey, CNB-B domain is in green and the regions with no coverage in black. Cyclic AMP molecules are shown in yellow sticks.



Supplementary Figure 2. cAMP binding to R_D as determined by ITC. Top panel depicts the heat released (and subsequent recovery) upon injection of cAMP from syringe to the experimental cell chamber containing apo R_D protein. For top panel, the observed heat change is plotted on Y-axis (µcal/sec) and X-axis denotes progression of the ITC experiment with time (min). Bottom panel shows the fit of the integrated and corrected heat to a binding isotherm (black line). The release of heat with every injection is plotted on Y-axis (kcal/mole of cAMP) with increase in cAMP/R_D protein molar ratio at X-axis. The fitted values were estimated as ΔH = -53.7 kcal/mol, $K_A = 9.92 \times 10^5\ M^{-1}$.



Supplementary Figure 3: ***Comparing dynamics by HDXMS of CNB pockets in cAMP-free (apo) and cAMP bound states:*** A) Stacked mass spectra of a peptide spanning residues 138-150 in CNB-A domain, comparing cAMP-free and cAMP bound state, is depicted with increasing deuterium exchange labeling time. Centroids of the spectrum are labeled in black triangles. B) Stacked mass spectra of peptide fragment spanning residues 266-279 in CNB-B domain, comparing cAMP-free and cAMP bound state, is depicted with increasing deuterium exchange labeling time. Bimodal distributions are seen for shorter labeling times (30 s and 1 min) in apo R_D. In the cAMP bound state, bimodal distribution in mass spectra are seen for all deuterium labeling times. Blue triangles indicate centroid for the lower exchanging envelope and red triangles indicate centroid for the higher exchanging envelope.



Supplementary Figure 4: A) Difference plot, plotting absolute difference in deuterons (y-axis) between R_D:$RegA_C$ binary state and R_D:cAMP:$RegA_C$ ternary state for each pepsin fragment peptide listed from the N to C-terminus (x-axis). The binary complex and ternary complex are directly compared and points in the negative scale represent a decrease in deuterium exchange in binary complex, while points in the positive scale represent decreases in deuterium exchange of the ternary complex. Each deuterium labeling time for every peptide is depicted and colored according to key. A difference of ±0.5 Da is considered significant and is represented by a red dashed line. Plots were generated using DynamX software (Version 2.0, Waters, Milford).

Supplementary Table 1: Summary of peptide fragments from HDXMS data for apo R_D, R_D:cAMP, R_D:$RegA_C$ and R_D:$RegA_C$:cAMP. The table summarizes the relative deuterium exchange values reported for the 24 peptides obtained in our analysis. 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^+)	Charge (z)	Residue No.s	MEA[a]	No. of deuterons exchanged after 1min (Mean± SD)[b]				No. of deuterons exchanged after 30min (Mean± SD)[b]			
					R_D Apo	R_D: cAMP	R_D: $RegA_C$	R_D:$RegA_C$: cAMP	R_D Apo	R_D: cAMP	R_D: $RegA_C$	R_D:$RegA_C$: cAMP
1	TNNISHNQKATE (1356.65)	2	2-13	11	5.06 ± 0.04	5.36± 0.05	5.29±0.04	5.23±0.05	5.15± 0.06	5.28± 0.08	5.34±0.02	5.27±0.02
2	KVEAQNNNNITRKRRGAISSEP LGDKPATPLPNIPKTVE (4253.311)	5	14-52	33	17.51±0.10	17.22±0.15	17.19±0.06	17.04±0.08	17.51±0.15	17.2± 0.10	17.62±0.12	17.02±0.05
3	ISSEPLGDKPATPLPNIPKT (2075.138)	2	31-50	14	2.01±0.09	2.47±0.22	2.18± 0.02	2.70±0.06	4.79±0.24	5.15±0.18	5.43±0.12	5.88±0.09
4	EQALSNNIM (1019.483)	3	58-66	8	3.36±0.15	3.48±0.08	3.90±0.06	3.87±0.05	4.42±0.15	4.31±0.12	4.67±0.02	4.66±0.01
5	EERNVVF (892.452)	2	73-79	6	1.04±0.05	1.22±0.13	1.11±0.06	1.34±0.09	2.75±0.05	2.72±0.11	2.58±0.03	2.75±0.13
6	LYKAGDIIIKQGDEGD (1734.891)	2	86-101	15	3.18±0.15	2.75±0.06	3.73±0.06	2.87±0.04	4.45±0.19	3.94±0.12	4.79±0.04	4.26±0.06
7	ALIYGSPRAATVI (1331.768)	2	138-150	11	3.67±0.08	1.76±0.05	4.19±0.12	1.91±0.08	4.88±0.07	3.22±0.13	5.21±0.01	2.99±0.17
8	WALNGAT (732.367)	2	158-164	6	2.38±0.04	2.12±0.05	2.45±0.02	2.09±0.03	2.65±0.03	2.48±0.03	2.70±0.02	2.49±0.04
9	DQTIKKRKLYEEF (1697.922)	4	171-183	12	5.51±0.02	5.67±0.07	6.65±0.15	6.48±0.07	6.53±0.09	6.41±0.02	6.90±0.05	6.84±0.04
10	FLEKVSILRHIDKYERVSL (2345.334)	4	183-201	17	6.92±0.23	6.97±0.12	8.22±0.05	8.09±0.10	7.80±0.25	7.44±0.09	9.19±0.05	8.98±0.01
11	LEKVSIL (801.508)	2	184-190	6	2.60±0.03	2.61±0.06	3.28±0.02	2.95±0.02	3.22±0.06	3.05±0.04	3.62±0.03	3.50±0.01
12	LEKVSILRHIDKYERVSL (2198.266)	2	184-201	17	6.93±0.11	6.79±0.11	7.97±0.02	7.49±0.08	7.85±0.09	7.51±0.09	8.58±0.03	8.41±0.06

13	RHIDKYERVSL (1415.775)	3	191-201	10	4.47±0.02	4.42±0.02	4.62±0.04	4.56±0.07	4.73±0.06	4.52±0.02	4.80±0.02	4.75±0.02
14	RVSLADALEPVNFQDGEVIVRQG DPGDRFY (3362.676)	3	198-227	27	6.60±0.08	6.79±0.10	6.65±0.02	7.27±0.05	9.43±0.11	9.05±0.05	9.84±0.05	9.51±0.05
15	ADALEPVNF (975.478)	4	202-210	7	3.53±0.04	3.45±0.05	3.02±0.05	2.45±0.01	3.76±0.05	3.57±0.03	3.87±0.01	2.92±0.02
16	VIVRQGDPGDRF(1358.717)	2	215-226	10	4.39±0.06	4.01±0.09	3.97±0.09	2.76±0.07	4.97±0.09	4.51±0.06	5.55±0.07	3.80±0.04
17	IVRQGDPGDRF (1259.649)	2	216-226	9	3.92±0.06	3.56±0.07	3.69±0.01	2.62±0.06	4.38±0.06	4.03±0.05	4.68±0.01	3.75±0.03
18	TQETVPGDHSTSHVVSEL (1922.909)	3	237-254	16	6.37±0.23	7.35±0.15	n/a	n/a	7.16±0.02	7.95±0.06	n/a	n/a
19	LTDRPRAATVTSIG (1457.807)	2	266-279	12	5.89±0.05	4.97±0.19	4.42±0.09	1.98±0.12	6.66±0.15	5.39±0.16	7.46±0.03	3.07±0.16
20	DRQRFNRLCGPIDQML (1961.98)	3	287-302	15	6.62±0.17	5.62±0.14	n/a	8.25±0.04	7.69±0.18	7.50±0.08	5.64±0.09	7.69±0.15
21	NRLCGPIDQ (1015.499)	2	292-300	7	3.08±0.06	2.97±0.08	3.28±0.04	2.83±0.06	3.61±0.09	3.47±0.25	3.94±0.05	3.64±0.09
22	RRNMETYNQF (1358.627)	2	303-312	9	5.13±0.05	4.98±0.06	5.23±0.04	4.93±0.08	5.37±0.09	5.13±0.05	5.53±0.06	5.22±0.07
23	FLNRPPSSPNL (1241.664)	2	312-322	7	4.06±0.03	4.04±0.03	4.01±0.04	3.95±0.05	4.10±0.04	4.02±0.03	4.06±0.01	3.93±0.06
24	LNRPPSSPNL (1094.595)	2	313-322	6	3.62±0.05	3.58±0.09	3.65±0.03	3.58±0.03	3.63±0.06	3.56±0.06	3.65±0.05	3.61±0.03

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

Supplementary Table 2. The tables list the calculated values for the amplitude intensities and respective exchange of deuterons as compared with the undeuterated control, for the two types of exchanging envelopes.

(i) Apo R_D

Time	Amp_1	Centroid_1	DEx ± SD	Amp_2	Centroid_2	DEx ± SD
0.5	7.59	730.8	2.12 ± 0.07	12.99	733.6	7.72 ± 0.07
1	6.67	730.9	2.45 ± 0.08	12.83	733.6	7.72 ± 0.08
5	6.30	731.5	3.52 ± 0.09	11.88	733.7	7.92 ± 0.09
10	5.95	731.8	4.12 ± 0.07	11.53	733.8	8.12 ± 0.07
30	7.52	732.7	5.92 ± 0.06	7.44	733.8	8.12 ± 0.06
60	7.34	732.7	6.05 ± 0.07	6.69	733.7	7.92 ± 0.07
100	6.52	732.8	6.25 ± 0.07	7.37	733.7	8.05 ± 0.07

(ii) R_D: cAMP

Time	Amp_1	Centroid_1	DEx ± SD	Amp_2	Centroid_2	DEx ± SD
0.5	11.10	730.3	1.19 ± 0.05	13.22	733.3	7.12 ± 0.05
1	12.25	730.2	0.92 ± 0.06	13.44	733.3	7.12 ± 0.06
5	8.94	730.4	1.32 ± 0.05	12.72	733.3	7.25 ± 0.05
10	9.59	730.5	1.52 ± 0.05	13.80	733.4	7.32 ± 0.05
30	9.93	730.6	1.79 ± 0.05	13.89	733.3	7.25 ± 0.05
60	7.67	730.8	2.19 ± 0.08	13.65	733.4	7.39 ± 0.08
100	8.25	730.9	2.32 ± 0.06	13.41	733.5	7.52 ± 0.06

(iii) R_D:$RegA_C$ (Apo)

Time	Amp_1	Centroid_1	DEx ± SD	Amp_2	Centroid_2	DEx ± SD
0.5	14.11	730.9	2.32 ± 0.14	5.97	733.6	7.82 ± 0.14
1	14.26	731.1	2.72 ± 0.10	6.98	733.7	7.92 ± 0.10
5	12.05	731.6	3.65 ± 0.07	14.70	733.7	7.99 ± 0.07
10	6.59	731.7	3.99 ± 0.12	12.25	733.7	8.05 ± 0.12
30	4.89	733.1	6.79 ± 0.14	10.84	733.6	7.85 ± 0.14
60	3.16	732.9	6.39 ± 0.14	9.93	733.6	7.79 ± 0.14
100	1.03	733.1	6.72 ± 0.16	10.11	733.7	7.99 ± 0.16

(iv) RD:RegAC:cAMP

Time	Amp_1	Centroid_1	DEx ± SD	Amp_2	Centroid_2	DEx ± SD
0.5	11.70	730.1	0.65 ± 0.24	1.67	732.4	5.32 ± 0.24
1	12.64	730.1	0.79 ± 0.23	2.03	732.4	5.32 ± 0.23
5	13.24	730.2	1.05 ± 0.17	2.22	732.5	5.52 ± 0.17
10	11.09	730.3	1.19 ± 0.19	1.90	732.5	5.59 ± 0.19
30	13.45	730.6	1.85 ± 0.16	3.15	732.8	6.12 ± 0.16
60	13.44	730.8	2.12 ± 0.14	3.80	733	6.52 ± 0.14
100	13.27	730.9	2.32 ± 0.14	4.76	733	6.59 ± 0.14

All calculations were done using the sum of two Gaussians equation in GraphPad Prism 6.0.

*__Amp_1__: Amplitude, __Centroid_1__: centroid in m/z for +2 charge state mass spectral envelope, __DEx_1± SD__: number of deuterons exchanged and the standard deviations for the lower exchanging envelope; **__Amp_2__: Amplitude, __Centroid_2__: representative m/z value of centroid peak for +2 charge state, __DEx_2± SD__: number of deuterons exchanged and their standard deviations for the higher exchanging envelope. Each data point represented is an average of minimum of three independent experimental values.