Structural insight into the separate roles of IP4 and DAD in activation of histone deacetylase 3

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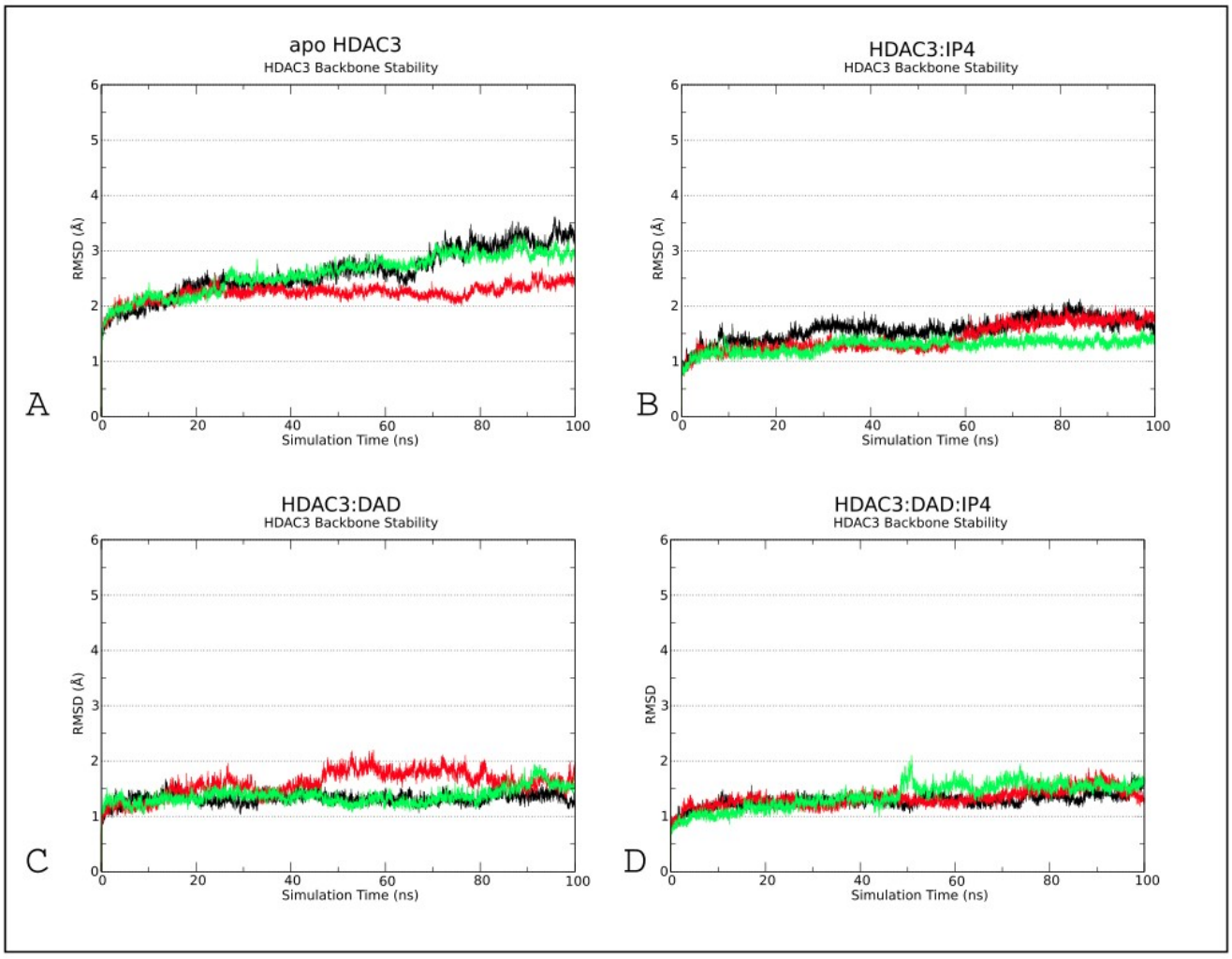
Supplementary Information



SI Figure 1: *Root mean square deviations (Å) from initial frames for MD simulations of (A) apo HDAC3, (B) HDAC3:IP4, (C) HDAC3:DAD, and (D) HDAC3:DAD:IP4. Initial frames were different snapshots from pre-production MD (once density of water box equilibrated). Three colors represent independent trials. For each trajectory, all protein backbone atoms were first aligned to its initial coordinates, and RMSD of HDAC3 backbone is plotted during the course of 100ns.*

Clustering Details for apo HDAC3 structural ensemble:

Clustering was done within Ptraj within the AmberTools package. Hierarchical, average linkage, and centroid linkage algorithms were tested. Since each algorithm requires the number of clusters to be chosen by the user, we also tested various cluster sizes (3, 4, 5) and the different algorithms on a subset of apo HDAC3 trajectories. Visual inspection of clusters as well as consideration of three different clustering metrics (DBI, pSF, and SSR/SST) were used to choose the algorithm and number of clusters, as suggested in Ref 17.

Desired Clusters	4
Clustering Algorithm	Hierarchical
Distance Metric	RMS
Davies-Bouldin Index	2.8148
Pseudo F-statistic	35311.18359
Sum of squares regression/Total sum of squares	0.91378

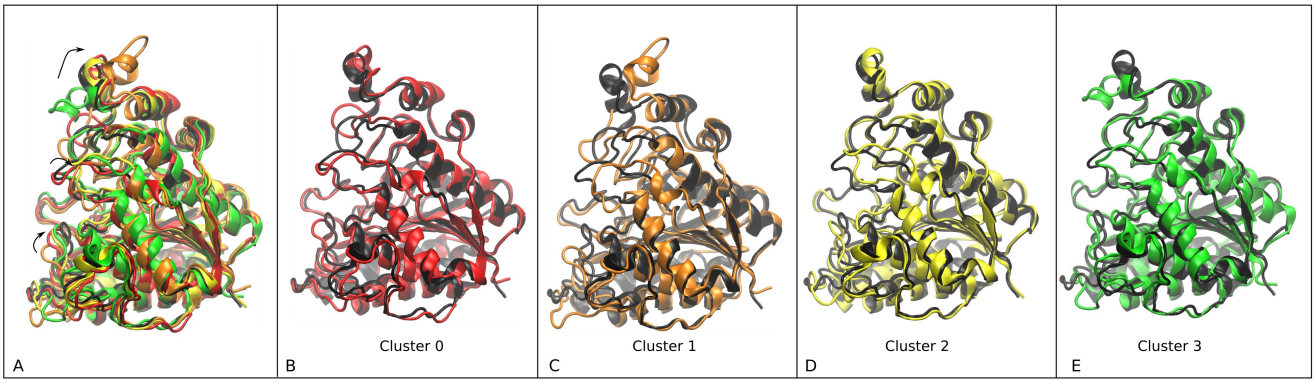
SI Table I: *Details of clustering analysis and clustering metrics for apo HDAC3 structural ensemble.*

	Avgerage distance to centroid (Å)	No. of structures	Occurence	Backbone RMSD from pdb 4A69 (Å)
Cluster0	0.714325	2042	0.204	1.277
Cluster1	0.753151	3910	0.391	2.246
Cluster2	0.734884	1376	0.138	1.175
Cluster3	0.742089	2672	0.267	1.880

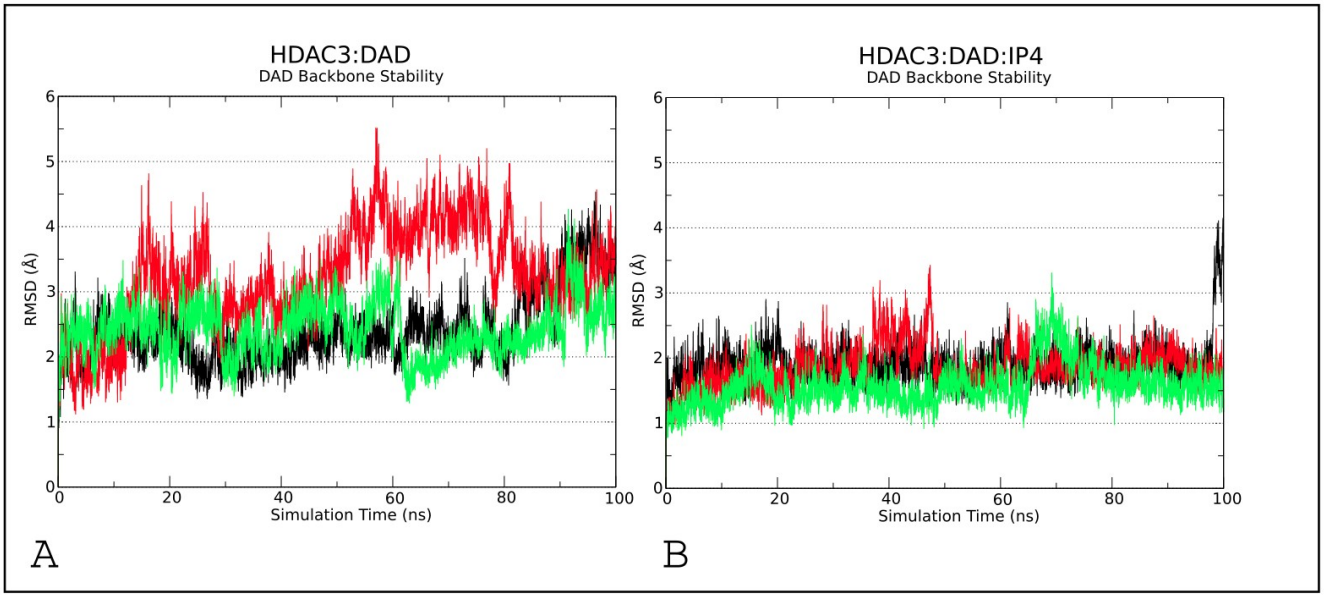
SI Table II: *Summary of four apo HDAC3 clusters. 10,000 total structures from a total of 300ns simulations (three independent 100ns trajectories) were used.*

Cross comparison of structural ensembles

Using a RMSD cutoff of 1.4 Å between the apo structures and each of the cluster representatives, we were able to assign the same cluster occupanices for the apo trajectory as the clustering algorithm (+/- 5%). We then used the same cutoff to classify resemblance between structures from the different ensembles and the apo cluster representatives.



SI Figure 2: *Representatives from clustering analysis of apo HDAC3 simulations. (A) All four cluster representatives are superimposed on the crystal structure of HDAC3. Arrows indicate distinguishing motifs among the clusters; (B) Cluster 0 representative in red; (C) Cluster 1 representative in orange; (D) Cluster 2 representative in yellow; (E) Cluster 3 representative in green.*



SI Figure 3: *RMSD (Å) of DAD backbone atoms during MD simulations of (A) HDAC3:DAD, and (B) HDAC3:DAD:IP4 complexes. For each trajectory the entire protein backbone was aligned to its initial coordinates and then the RMSD was measured for just the DAD backbone during the 100ns simulations. Different colors represent three independent simulations.*

Model Parameters:

Bonds	k_r (kcal/mol/Å^2)	r_0 (Å)
O2-Zn	120	1.92
NB-Zn	240	2.00

SI Table III: *Parameters for bonds to zinc center in active site. Atoms are labeled according to their AMBER atom type (O2: carboxylic acid oxygen on ASP; NB is imidazole nitrogen on HIS).*

Angles	k_θ (kcal/mol/rad^2)	θ (rad)
O2-Zn-NB	50	105.00
O2-Zn-O2	50	105.00
C-O2-Zn	80	125.00
CR-NB-Zn	80	119.00
CC-NB-Zn	80	131.00

SI Table IV: *Parameters for angles involving zinc center in active site. Atoms are labeled according to their AMBER atom names.*

Atom name	Modified Charge	Atom name	Modified Charge
Zn	1.24		
ASP		**HIS**	
N	-0.5163	N	-0.4157
H	0.2936	H	0.2719
CA	0.0381	CA	-0.0581
HA	0.0880	HA	0.1360
CB	-0.4267	CB	-0.4934
HB2	0.0676	HB2	0.0961
HB3	0.0676	HB3	0.0961
CG	0.9542	CG	0.2789
OD1	-0.7929	ND1	-0.3216
OD2	-0.7929	CE1	-0.0216
C	0.5366	HE1	0.2449
O	-0.5819	NE2	-0.1949
		HE2	0.3406
		CD2	-0.3169
		HD2	0.2226
		C	0.5973
		O	-0.5679

SI Table IV: *Parameters for angles involving zinc center in active site. Atoms are labeled according to their AMBER atom type.*

Assignment of protonation states

Neutral histidine residues were simulated with either the N_ε or N_δ imidazole nitrogen protonated (HIE or HID, respectively) depending on local environments. Propka and the local environments were used to simulate certain histidines in the charged +1 state (HIP).

Residue number (pdb: 4a69)	Histidine protonation state
16	HIE
21	HIE
26	HID
32	HIE
37	HIE
55	HIE
61	HID
133	HIP
134	HID
160	HIE
172	HIE
192	HIE
233	HIE
275	HIP
338	HIE

SI Table V: *Summary of assigned protonation states for histidine residues*