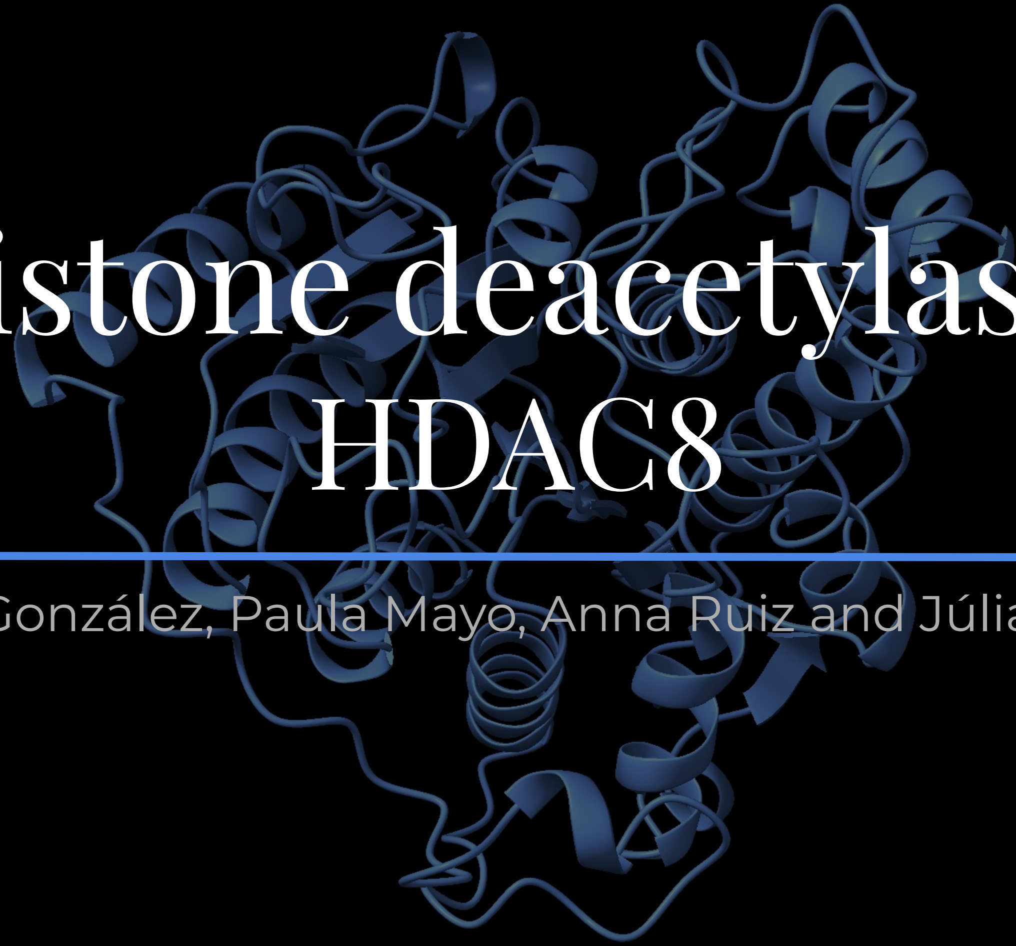


Histone deacetylases
HDAC8

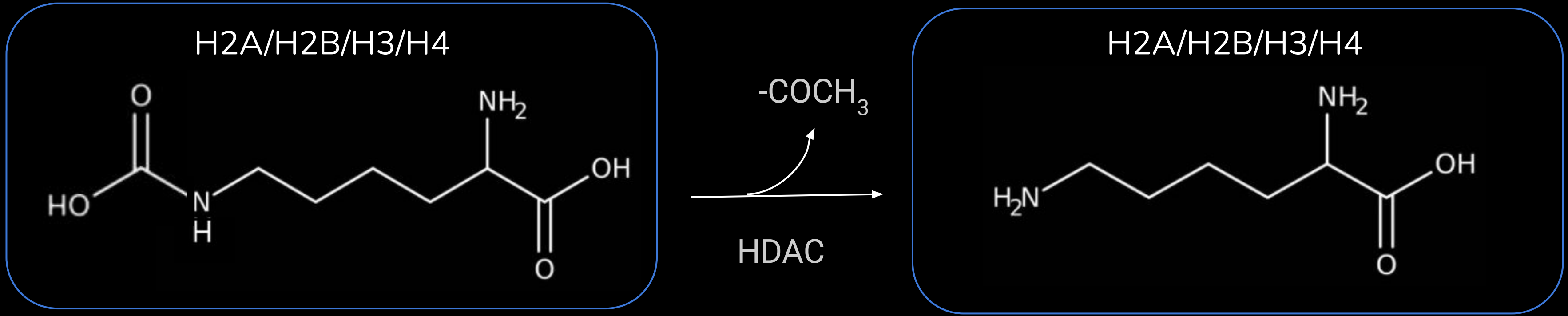
Ylenia González, Paula Mayo, Anna Ruiz and Júlia Terzulli

Index

HDACs

HDACs Physiological Role

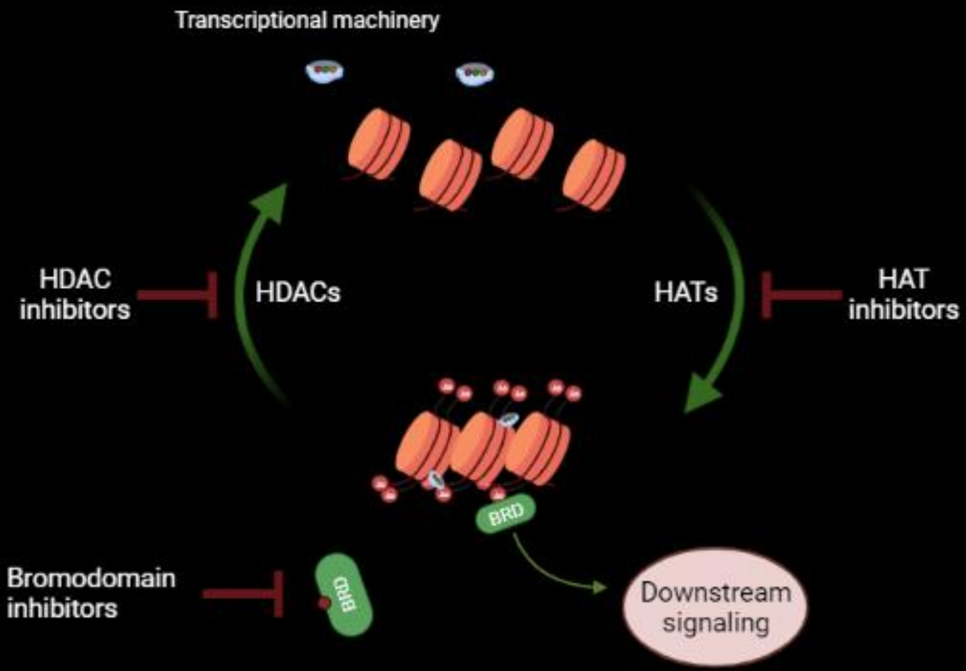
Zn^{2+}/NAD^{+}-dependent proteolytic enzyme

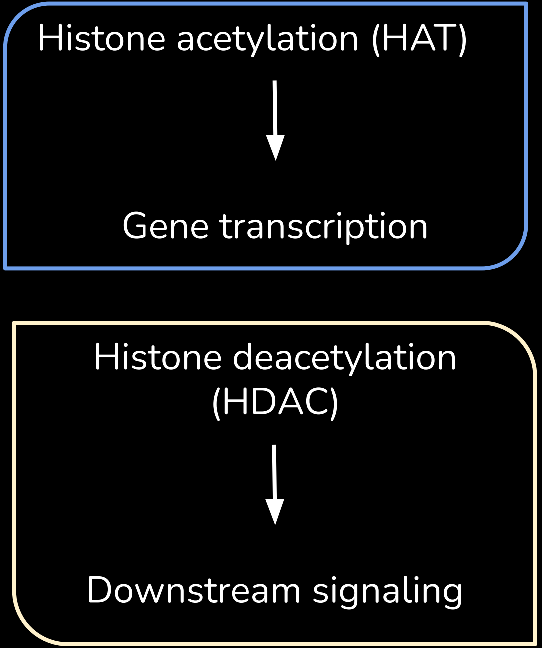


Acetylated lysine at the N-terminus

Deacetylated lysine at the N-terminus

HDACs Physiological Role





Histone acetylation (HAT)

↓

Gene transcription

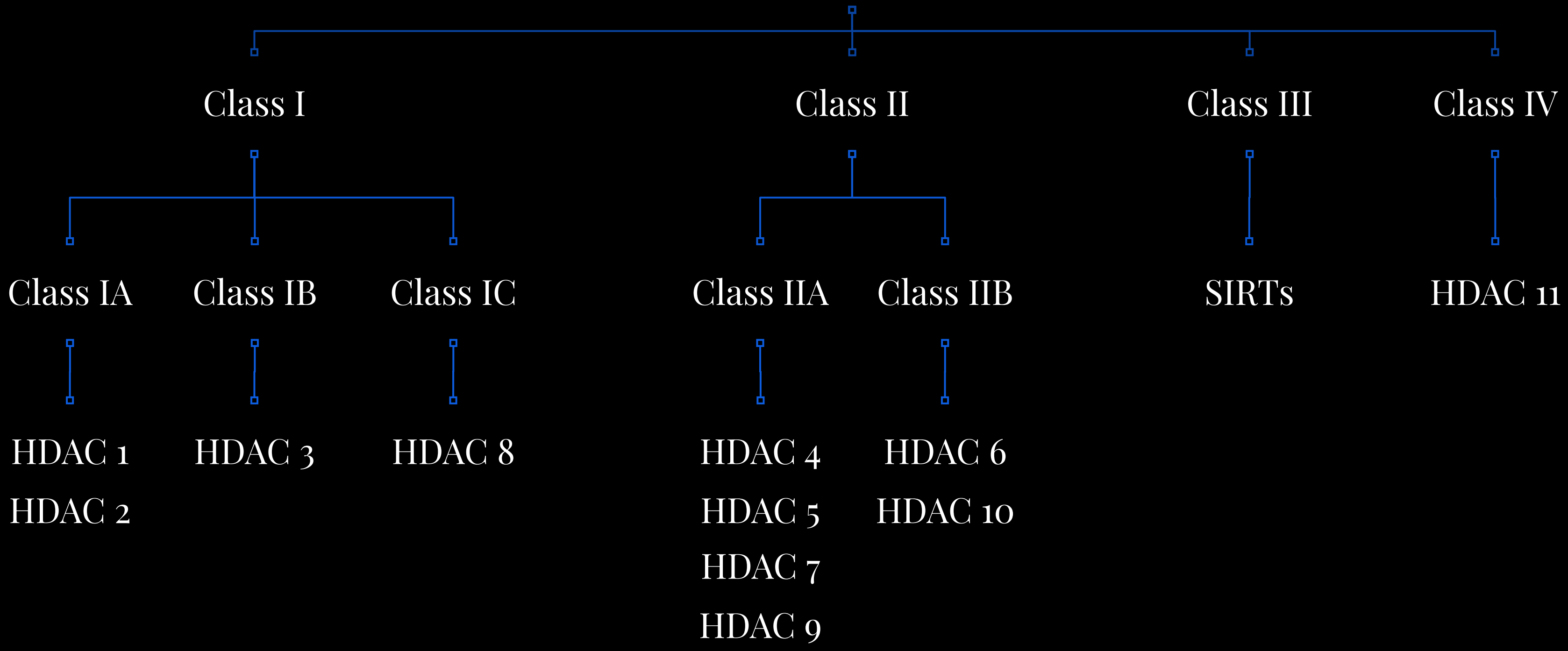
Histone deacetylation (HDAC)

↓

Downstream signaling

CLASSIFICATION

Histone deacetylases



- Class I
  - Class IA
    - HDAC 1
    - HDAC 2
  - Class IB
    - HDAC 3
  - Class IC
    - HDAC 8
- Class II
  - Class IIA
    - HDAC 4
    - HDAC 5
    - HDAC 7
    - HDAC 9
  - Class IIB
    - HDAC 6
    - HDAC 10
- Class III
  - SIRTs
- Class IV
  - HDAC 11

HDAC8

HDAC8 Physiological Role

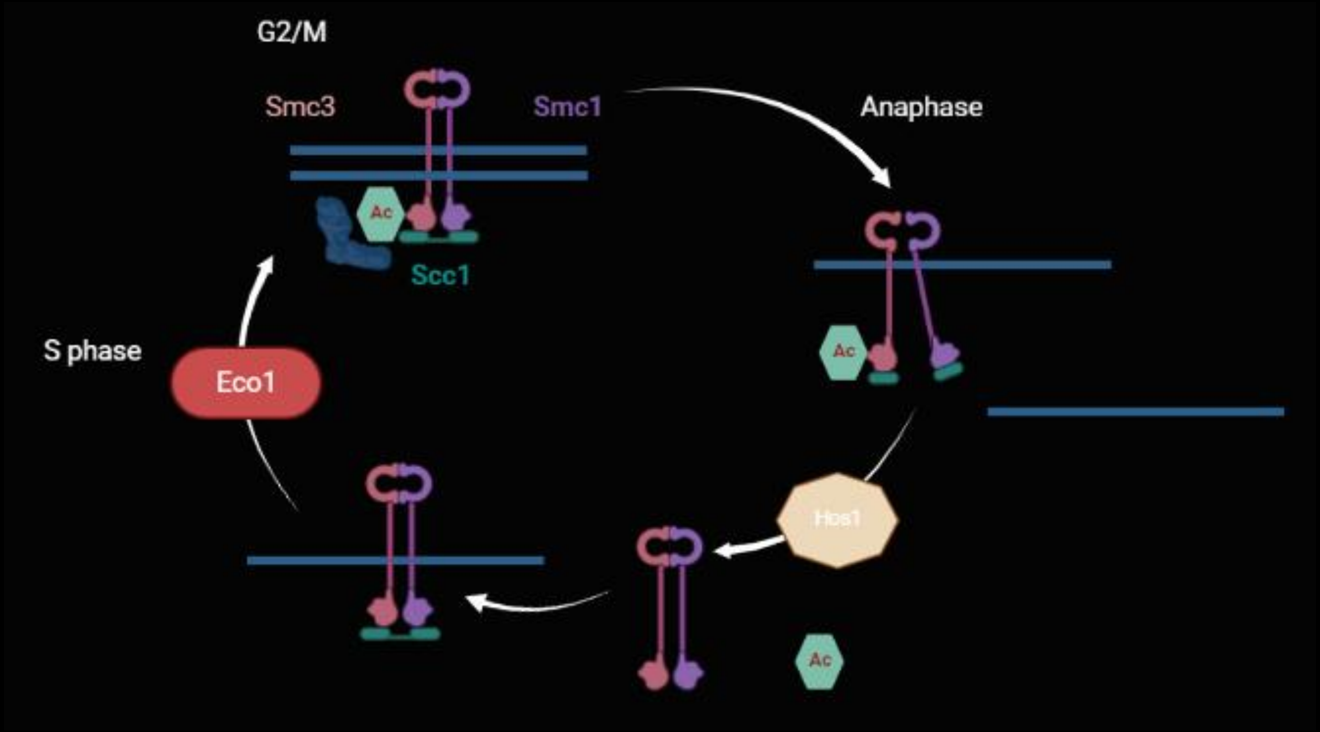
Enzyme encoded in humans by *HDAC8* gene, located in X chromosome

Also expressed in other eukaryotic organisms

→ 42 kDa, 377 residues

Histones	Non-histone proteins
H2A, H2B, H3 and H4 (Lys16 and Lys20)	p53, CREB, ERRa, SMC3

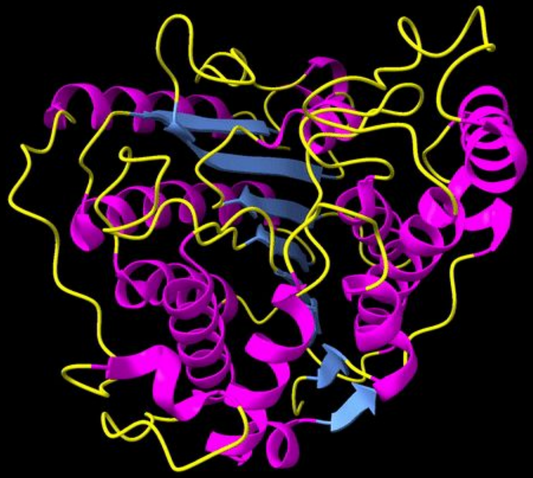
HDAC8 Physiological Role



HDAC8 description - structure, SCOP

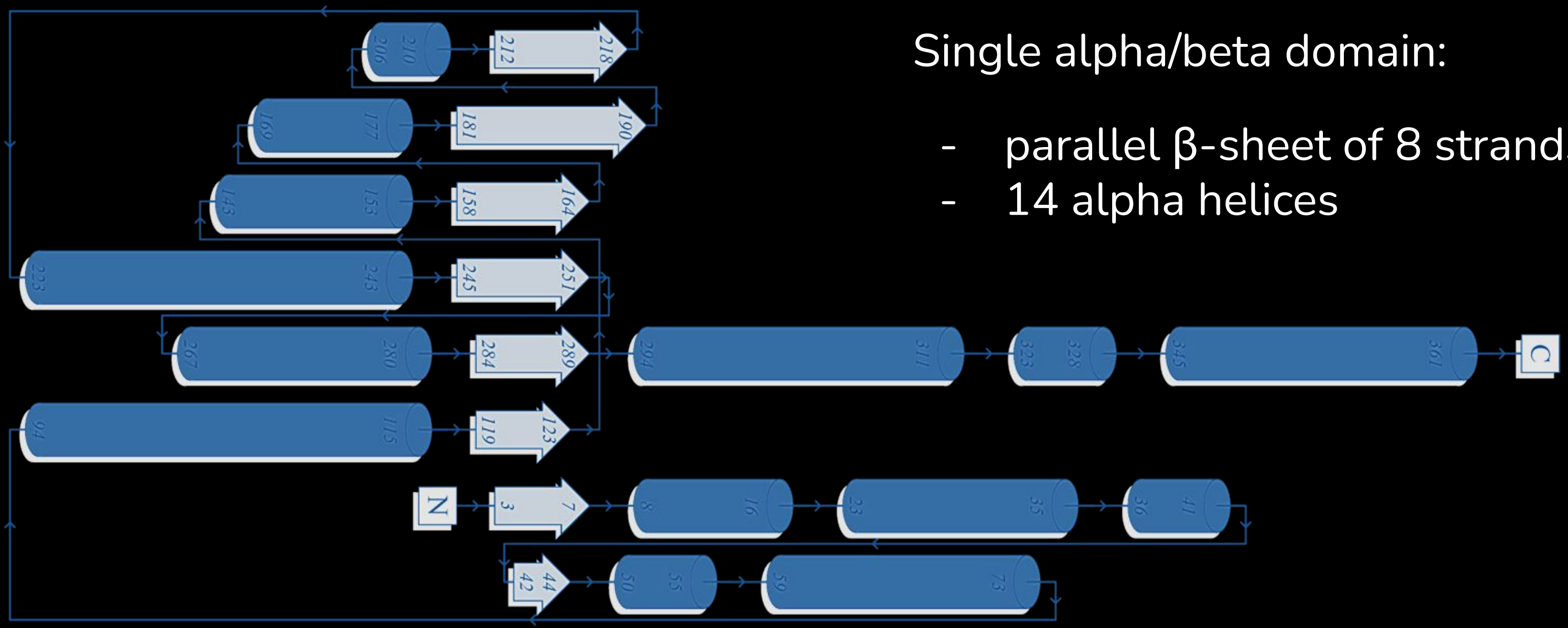
Class	Alpha and beta proteins (α/β)
Fold	Arginase/deacetylase
Superfamily	Arginase/deacetylase-like
Family	HDAC-like

3 layers: α/β/α, parallel β-sheet of 8 strands



HDAC8 (1T64), *Homo sapiens*, 1.90Å

HDAC8 description - structure

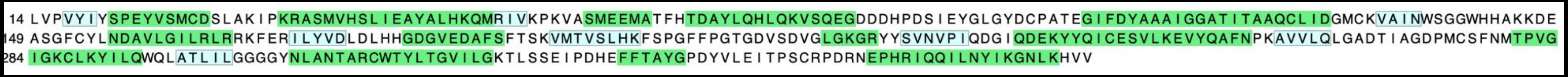


Topology diagram generated using PDBsum (EMBL-EBI)

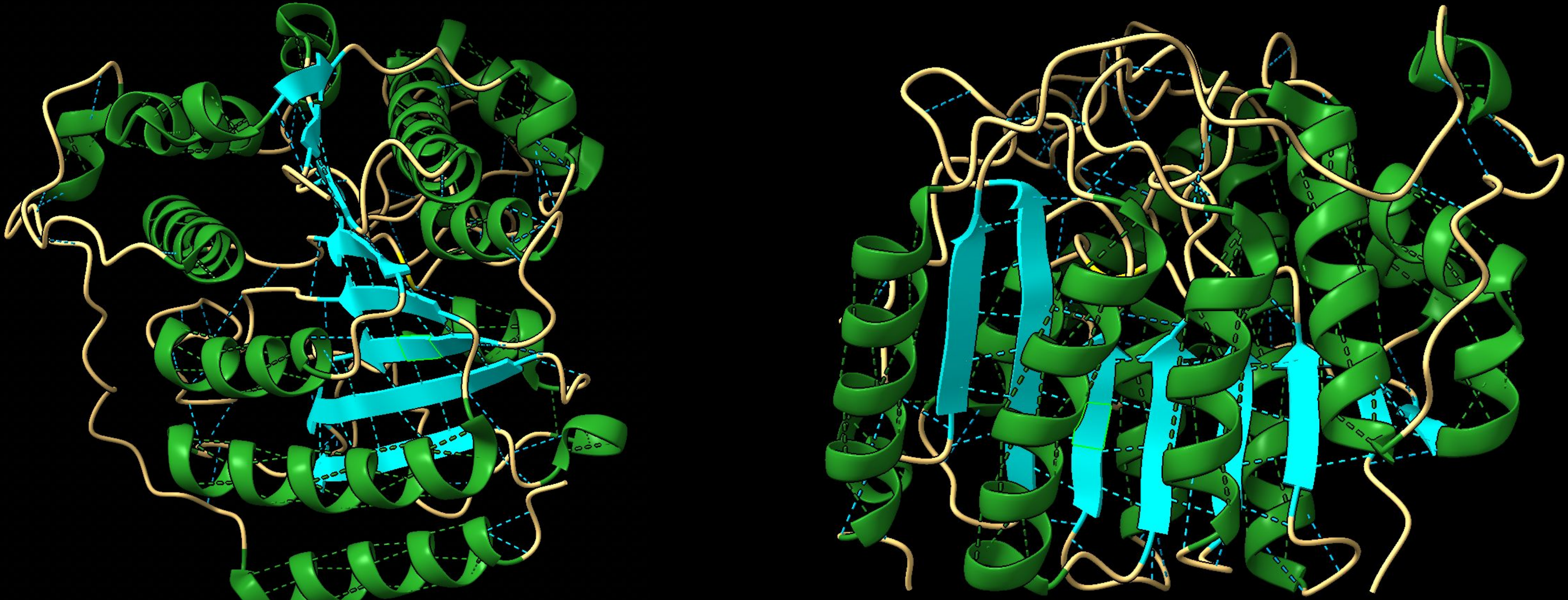
Single alpha/beta domain:

- parallel β-sheet of 8 strands
- 14 alpha helices

HDAC8 description - structure

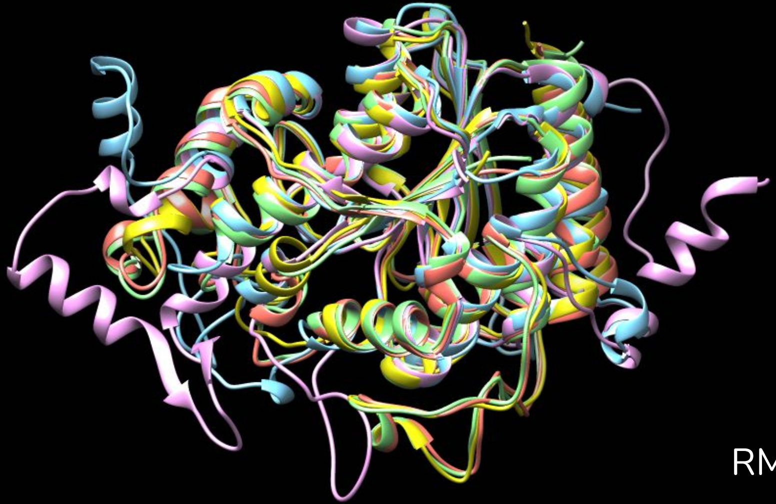


14 LVPVYIYSPEYVSMCDSLAKIPKRASMVHSLIEAYALHKQMRIVKPKVASMEEMATFHTDAYLQHLQKVSQEGDDDHPDSIEYGLGYDCPATEGIFDYAAAIGGATITAAQCLIDGMCKVAINWSGGWHHAKKDE
149 ASGFCYLNDAVLGILRLRRKFERILYVDLDLHHGDGVEDAFSFTSKVMTVSLHKFSPGFFPGTGDVSDVGLGKGRYYSVNVPIQDGIQDEKYYQICESVLKEVYQAFNPKAVVLQLGADTIAGDPMCSFNMTPVG
284 IGKCLKYILQWQLATLILGGGGYNLANTARCWTYLTGVILGKTLSSEIPDHEFFTAYGPDYVLEITPSCRPDRNEPHRIQQILNYIKGNLKHVV



HDAC8 (1T64), *Homo sapiens*, 1.90Å

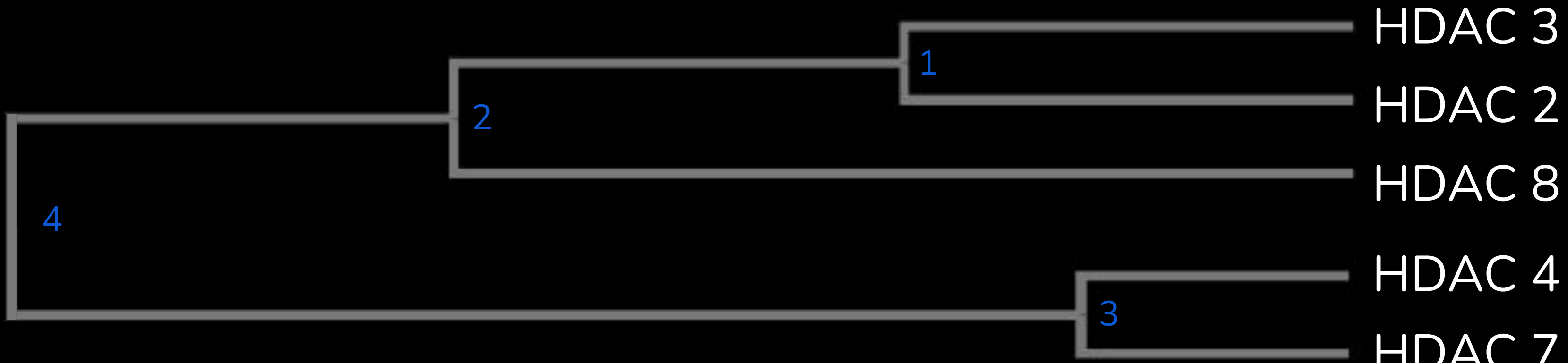
STAMP - Superimposition of human HDACs



RMSD: 1.60 Å

HDAC2 (7LTG)	HDAC 8 (1T64)	HDAC 4 (2VQJ)	HDAC 7 (3C0Y)	HDAC3 (4A69)
Homo sapiens	*Homo sapiens*	*Homo sapiens*	*Homo sapiens*	*Homo sapiens*
1.80Å	1.90Å	*2.10Å*	*2.10Å*	*2.06Å*

STAMP - HDACs dendrogram



1
RMSD: 0.76Å
Score: 9.37

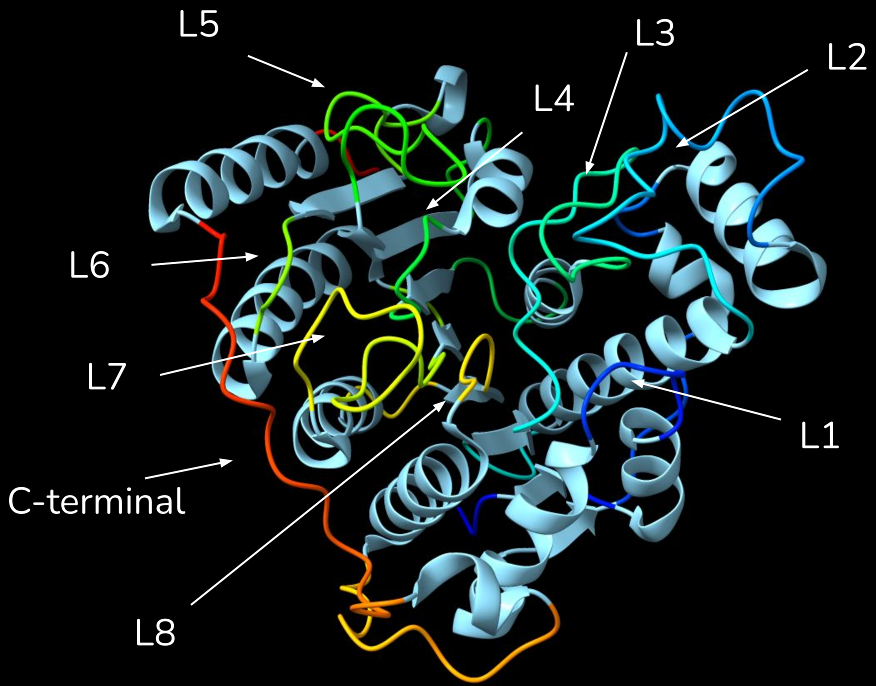
2
RMSD: 1.04Å
Score: 9.12

3
RMSD: 0.84Å
Score: 6.75

4
RMSD: 1.60Å
Score: 6.99

ACTIVE SITE

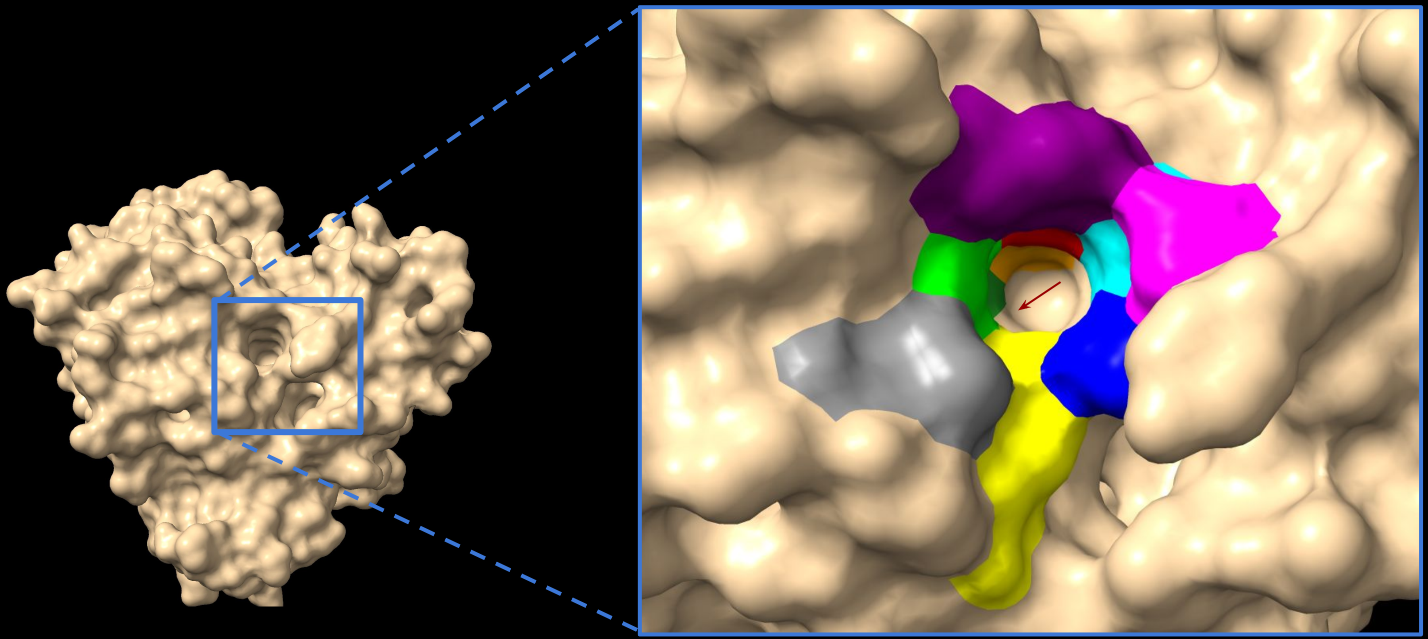
Substrate binding surface



These loops create several different conformations that allow HDAC8 to bind to different substrates

Residues in the active site

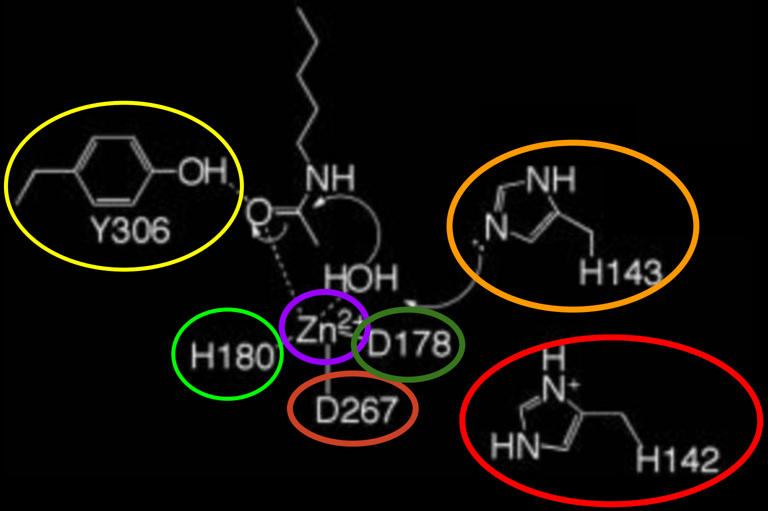
- D101
- H142
- H143
- G151
- F152
- D178
- H180
- F208
- M274
- D267
- Y306



HDAC8 (1T64), *Homo sapiens*, 1.90Å

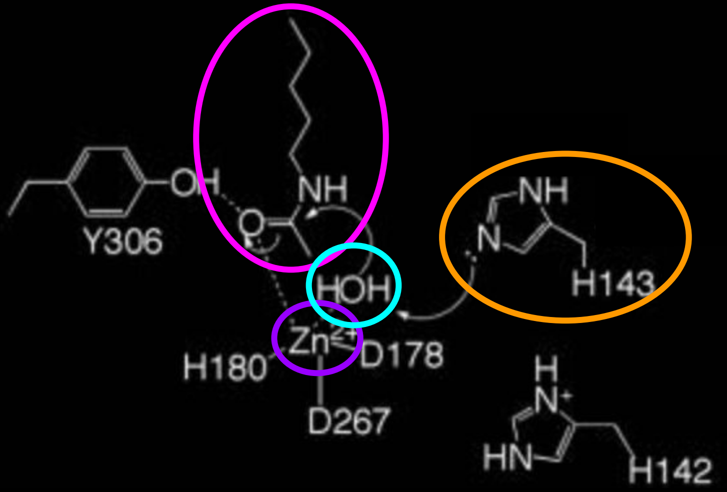
Deacetylation reaction

Deacetylation



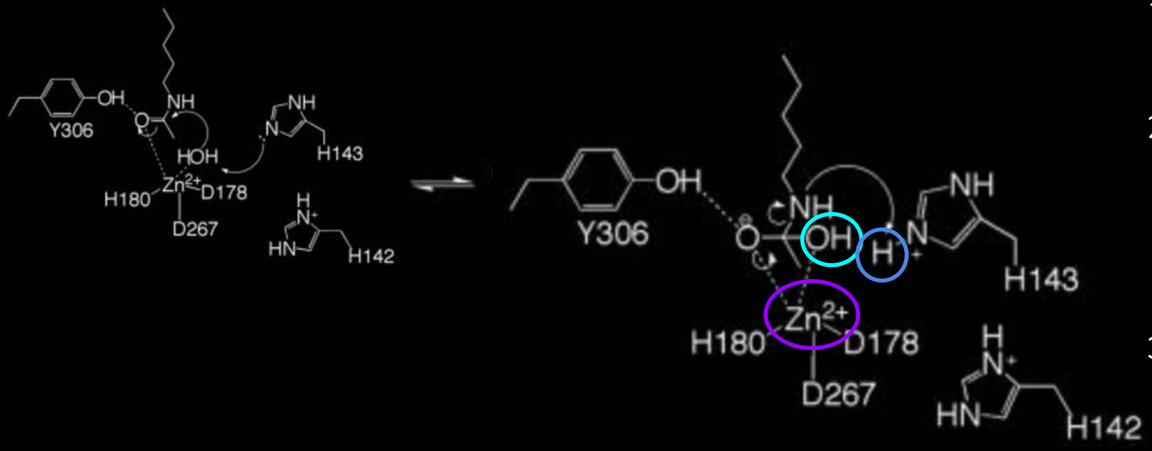
1. <u>Active site:</u> Zn^{2+} bound to H180, D267 and D178. Y306, and H142 and H143.

Deacetylation



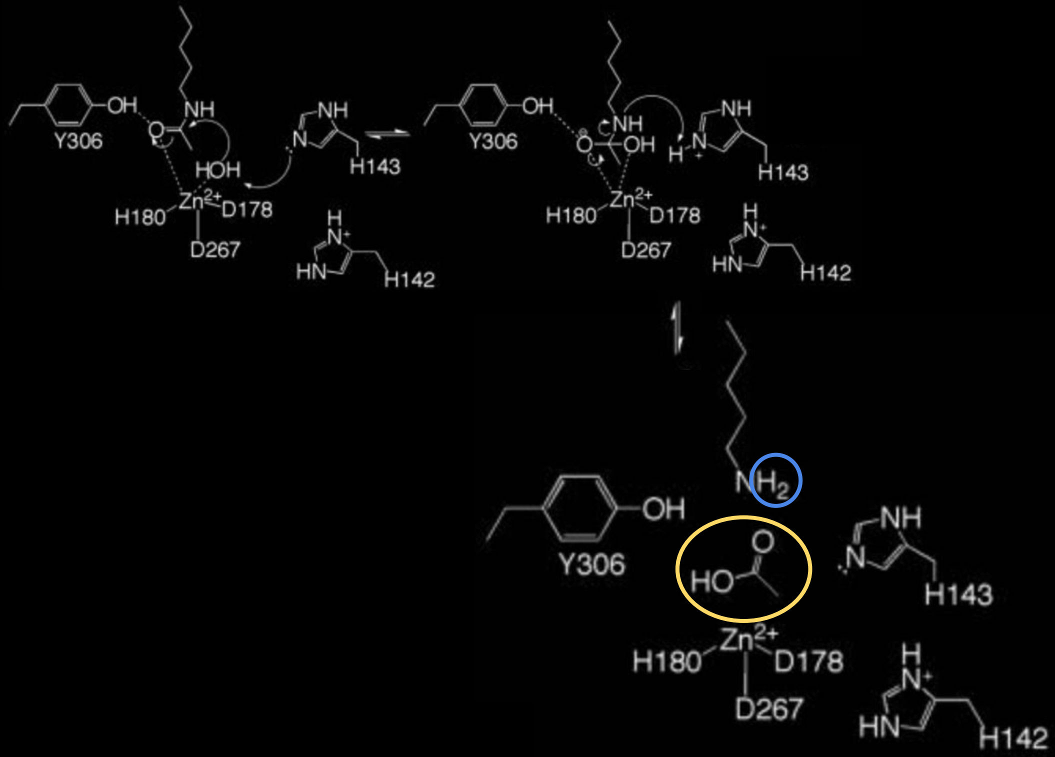
1. Active site: Zn^{2+} bound to H180, D267 and D178. Y306, and H142 and H143.
2. The Zn^{2+} and H143 promote the nucleophilic attack of the water molecule to the carbonyl group of the acetyl-lysine. The proton from the water molecule binds to H143.

Deacetylation



1. Active site: Zn^{2+} bound to H180, D267 and D178. Y306, and H142 and H143.
2. The Zn^{2+} and H143 promote the nucleophilic attack of the water molecule to the carbonyl group of the acetyl-lysine. The proton from the water molecule binds to H143.
3. The OH group binds to the carbonyl group of acetyl-lysine forming a tetrahedral intermediate, stabilized by Y306. The OH group remains attached to the metal ion.

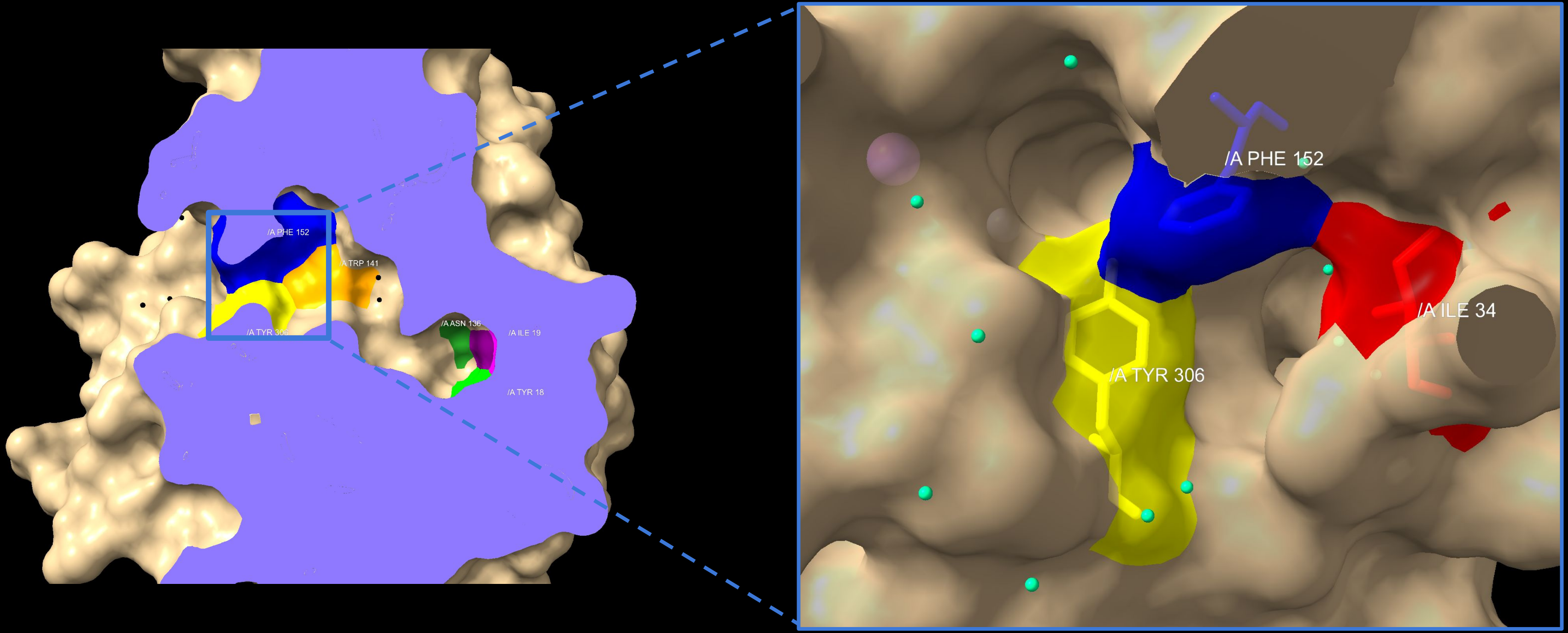
Deacetylation



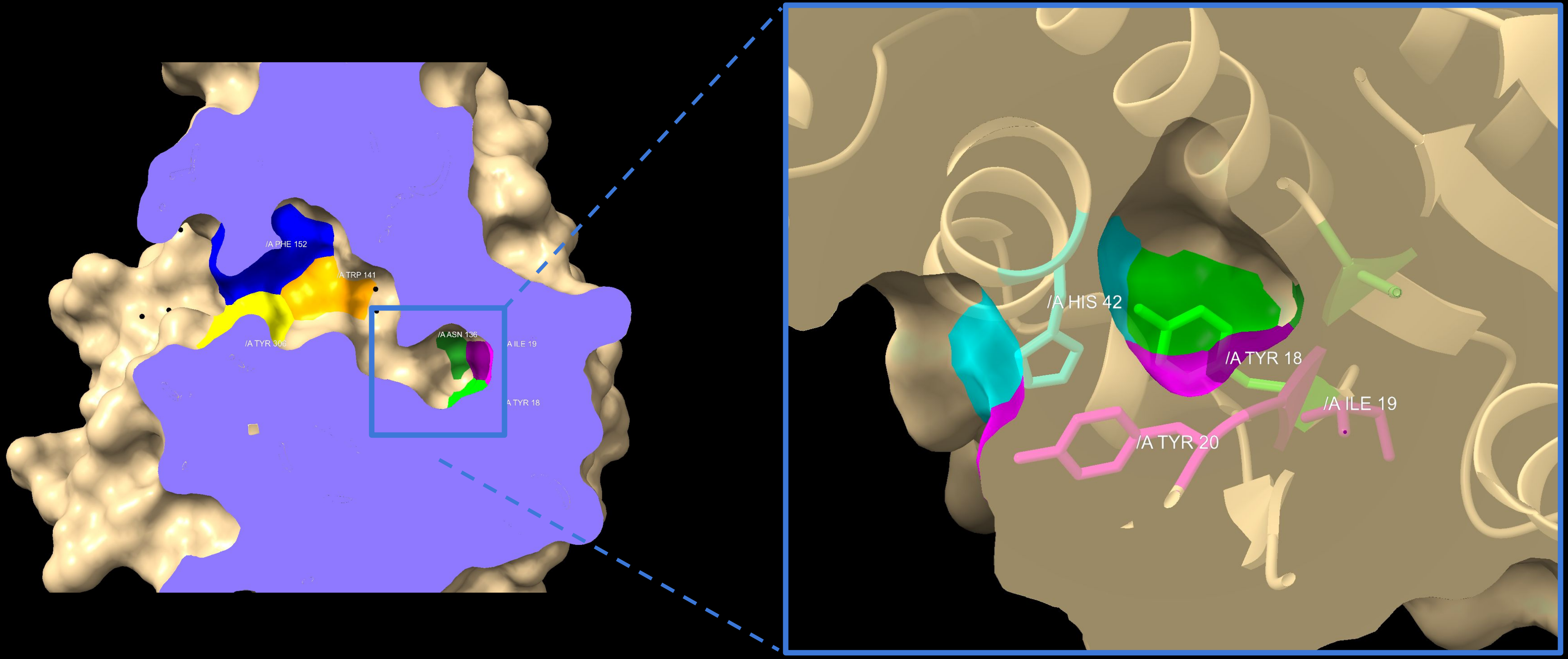
1. Active site: Zn^{2+} bound to H180, D267 and D178. Y306, and H142 and H143.
2. The Zn^{2+} and H143 promote the nucleophilic attack of the water molecule to the carbonyl group of the acetyl-lysine. The proton from the water molecule binds to H143.
3. The OH group binds to the carbonyl group of acetyl-lysine forming a tetrahedral intermediate, stabilized by Y306. The OH group remains attached to the metal ion.
4. The proton from the H143 is transferred to the leaving amino group of lysine, causing the collapse of the tetrahedral intermediate and the release of the acetate group.

LIBERATION CHANNEL

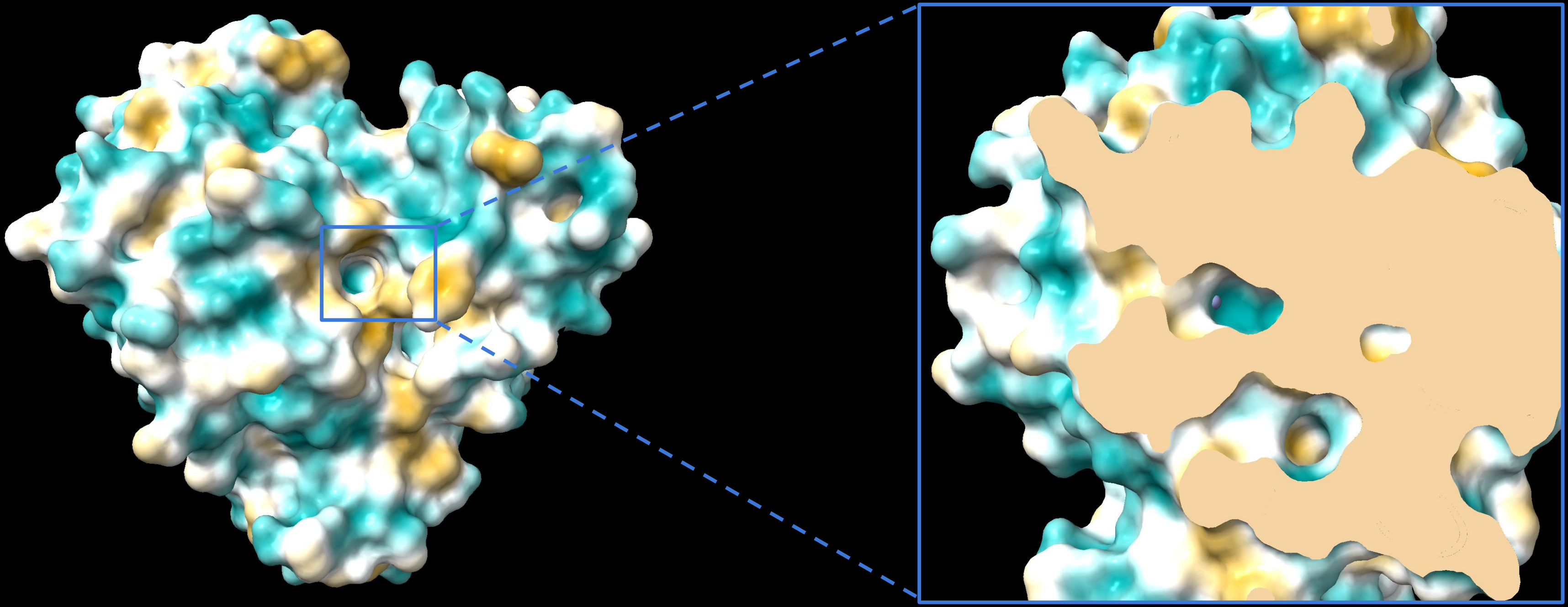
Residues in the liberation channel (I)



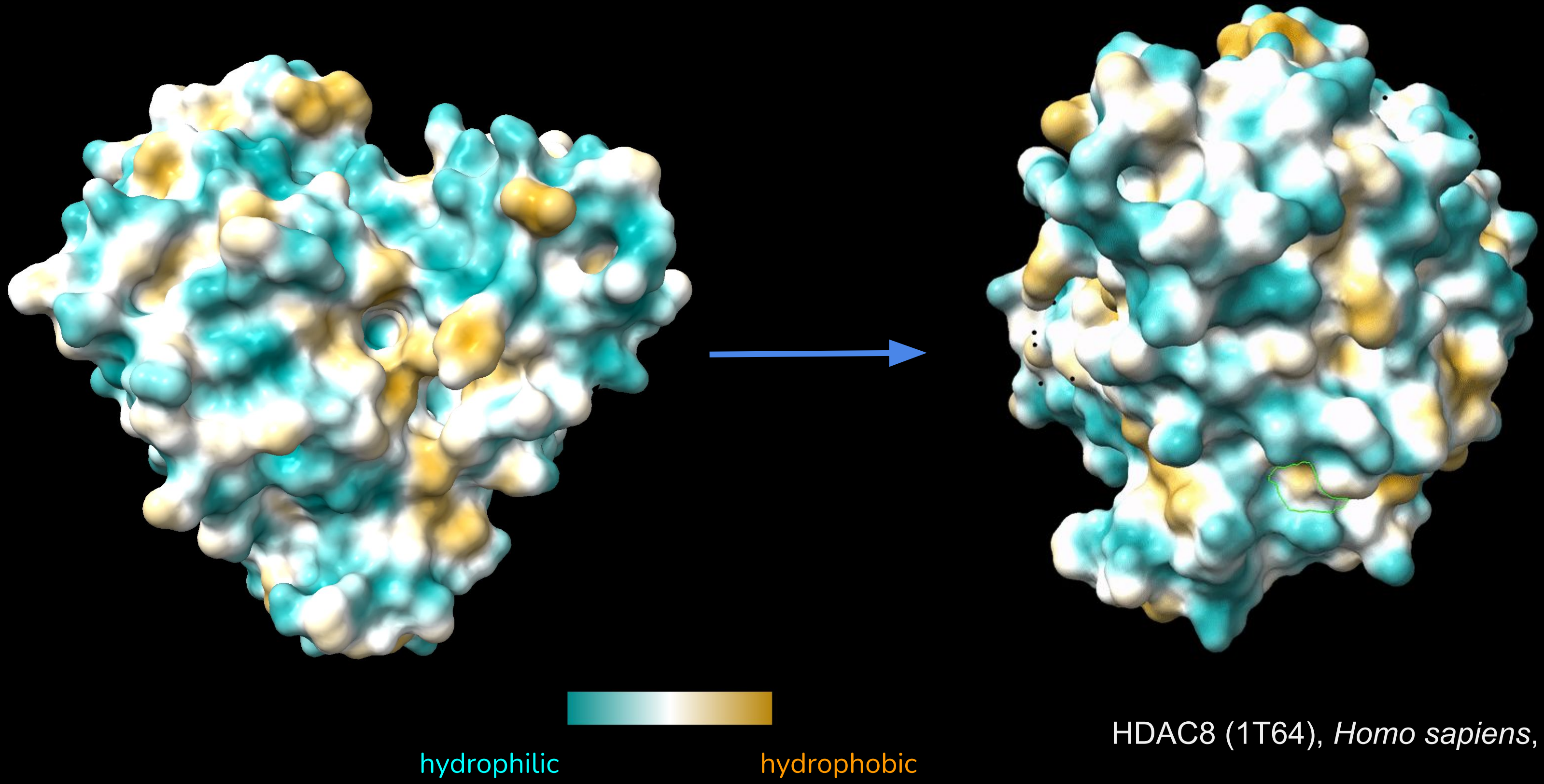
Residues in the liberation channel (I)



HYDROPHOBICITY



HDAC8 (1T64), *Homo sapiens*, 1.90Å

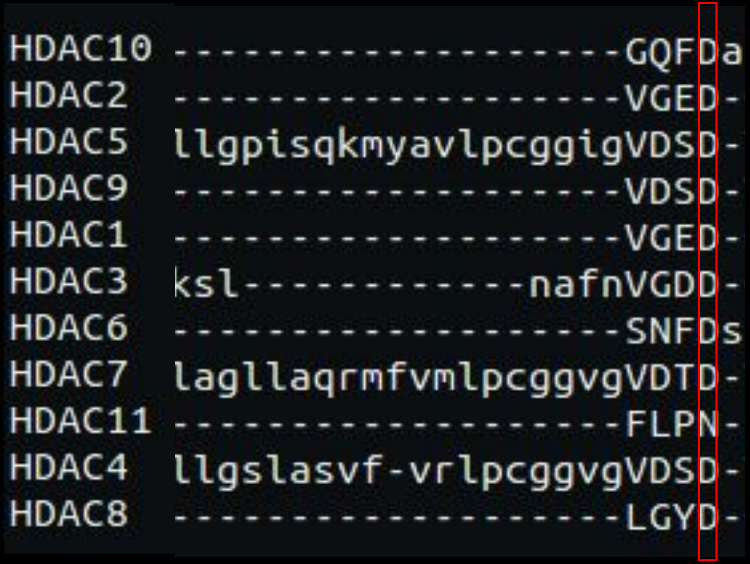


HDAC8 (1T64), *Homo sapiens*, 1.90Å

Human HDACs alignment

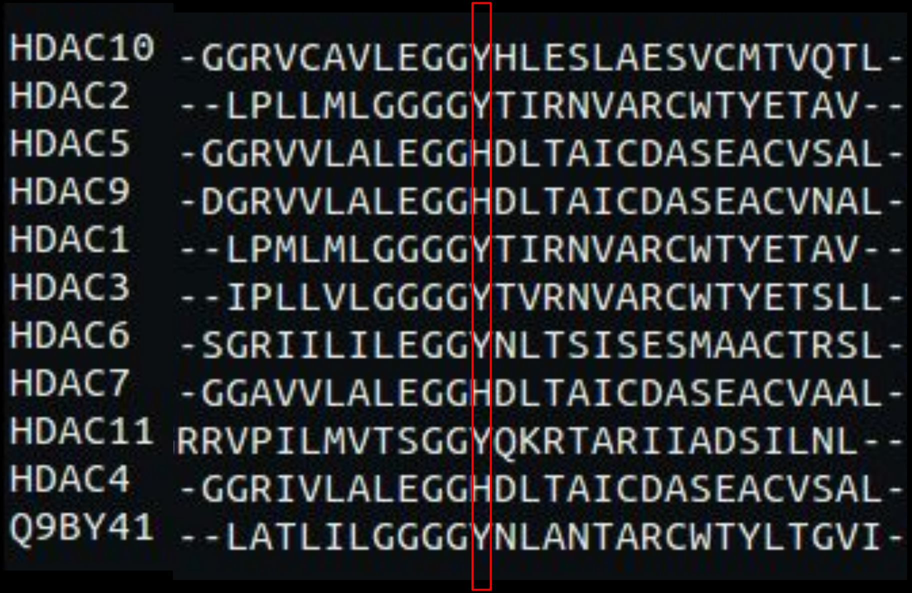
Residues conserved in human HDACs

D101 → Substrate binding



```
HDAC10 -------------------GQFDa
HDAC2  -------------------VGED-
HDAC5  llgpisqkmyavlpcggigVDSD-
HDAC9  -------------------VDSD-
HDAC1  -------------------VGED-
HDAC3  ksl------------nafnVGDD-
HDAC6  -------------------SNFDs
HDAC7  lagllaqrmfvmlpcggvgVDTD-
HDAC11 -------------------FLPN-
HDAC4  llgslasvf-vrlpcggvgVDSD-
HDAC8  -------------------LGYD-
```

Y306 → Stabilization of tetrahedral intermediate

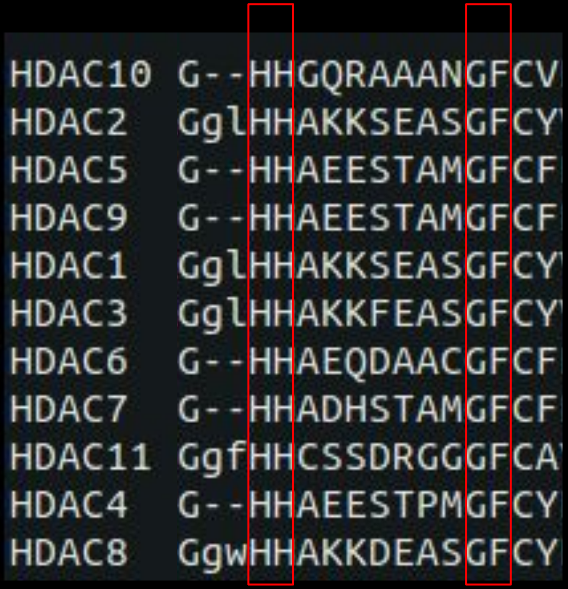


```
HDAC10 -GGRVCAVLEGGYHLESLAESVCMTVQTL-
HDAC2  --LPLLMLGGGGYTIRNVARCWTYETAV--
HDAC5  -GGRVVLALEGGHDLTAICDASEACVSAL-
HDAC9  -DGRVVLALEGGHDLTAICDASEACVNAL-
HDAC1  --LPMLMLGGGGYTIRNVARCWTYETAV--
HDAC3  --IPLLVLGGGGYTVRNVARCWTYETSLL-
HDAC6  -SGRIILILEGGYNLTSISESMAACTRSL-
HDAC7  -GGAVVLALEGGHDLTAICDASEACVAAL-
HDAC11 RRVPILMVTSGGYQKRTARIIADSILNL--
HDAC4  -GGRIVLALEGGHDLTAICDASEACVSAL-
Q9BY41 --LATLILGGGGYNLANTARCWTYLTGVI-
```

Residues conserved in human HDACs

H142 H143 G151 F152

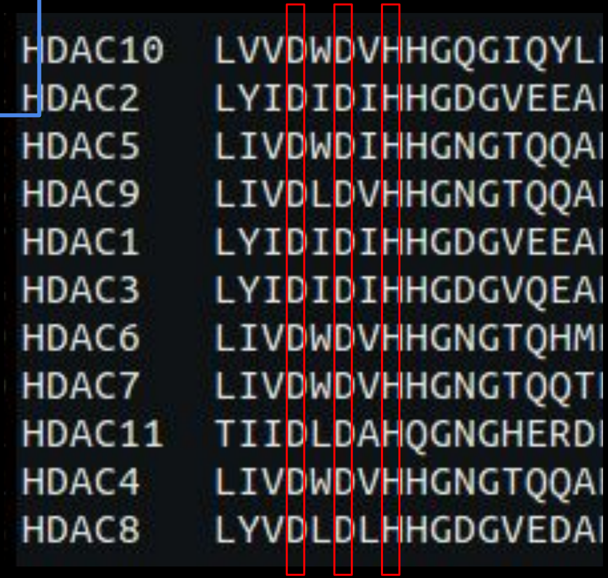
- Deacetylation reaction
- Hydrophobicity



```
HDAC10 G--HHGQRAAANGFCV
HDAC2  GglHHAKKSEASGFCY
HDAC5  G--HHAEESTAMGFCF
HDAC9  G--HHAEESTAMGFCF
HDAC1  GglHHAKKSEASGFCY
HDAC3  GglHHAKKFEASGFCY
HDAC6  G--HHAEQDAACGFCF
HDAC7  G--HHADHSTAMGFCF
HDAC11 GgfHHCSSDRGGGFCA
HDAC4  G--HHAEESTPMGFCY
HDAC8  GgwHHAKKDEASGFCY
```

D176 D178 H180

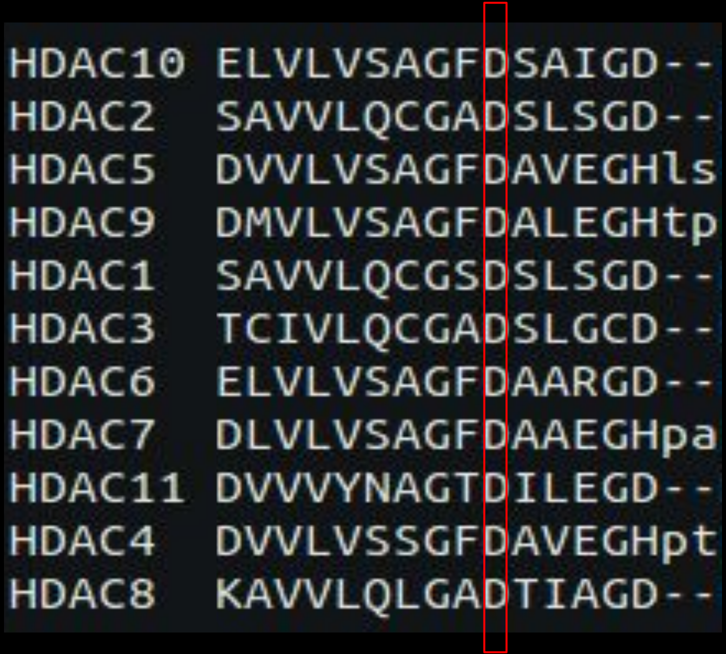
- Bound to Zn^{2+}
- Zn^{2+} coordination



```
HDAC10   LVVDWDVHHGQGIQYL
HDAC2    LYIDIDIHHGDGVEEA
HDAC5    LIVDWDIHHGNGTQQA
HDAC9    LIVDLDVHHGNGTQQA
HDAC1    LYIDIDIHHGDGVEEA
HDAC3    LYIDIDIHHGDGVQEA
HDAC6    LIVDWDVHHGNGTQHM
HDAC7    LIVDWDVHHGNGTQQT
HDAC11   TIIDLDAHQGNGHERD
HDAC4    LIVDWDVHHGNGTQQA
HDAC8    LYVDLDLHHGDGVEDA
```

Residues conserved in human HDACs

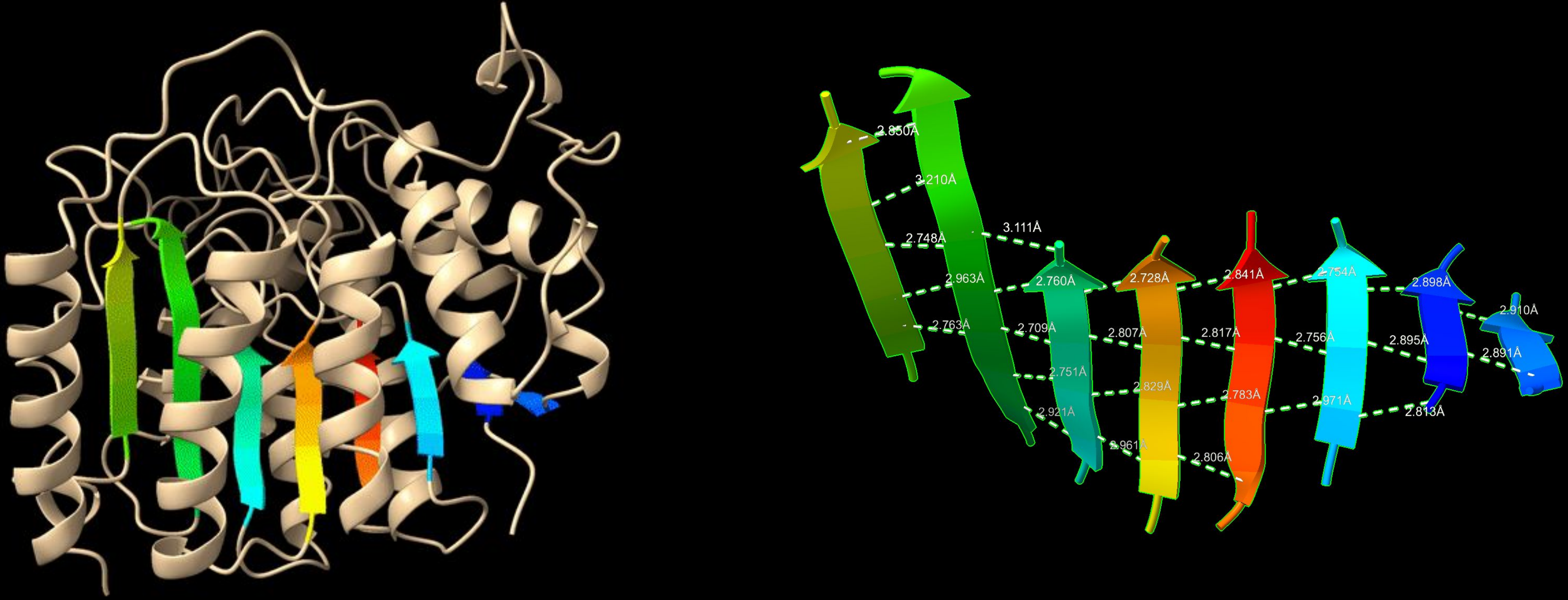
D267 → Bound to Zn^{2+}



```
HDAC10 ELVLVSAGFDSAIGD--
HDAC2  SAVVLQCGADSLSGD--
HDAC5  DVVLVSAGFDAVEGHls
HDAC9  DMVLVSAGFDALEGHtp
HDAC1  SAVVLQCGSDSLSGD--
HDAC3  TCIVLQCGADSLGCD--
HDAC6  ELVLVSAGFDAARGD--
HDAC7  DLVLVSAGFDAAEGHpa
HDAC11 DVVVYNAGTDILEGD--
HDAC4  DVVLVSSGFDAVEGHpt
HDAC8  KAVVLQLGADTIAGD--
```

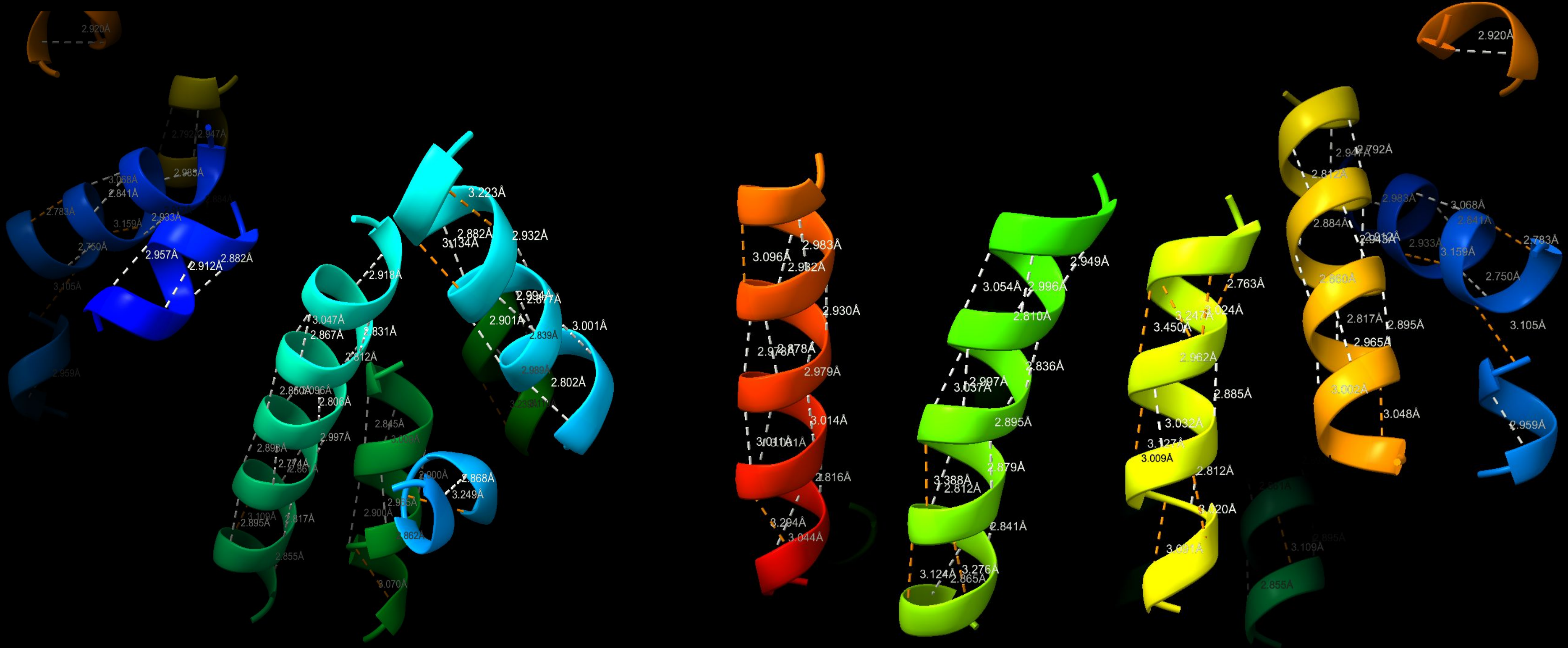
Intramolecular interactions

Hydrogen bonds - beta sheet

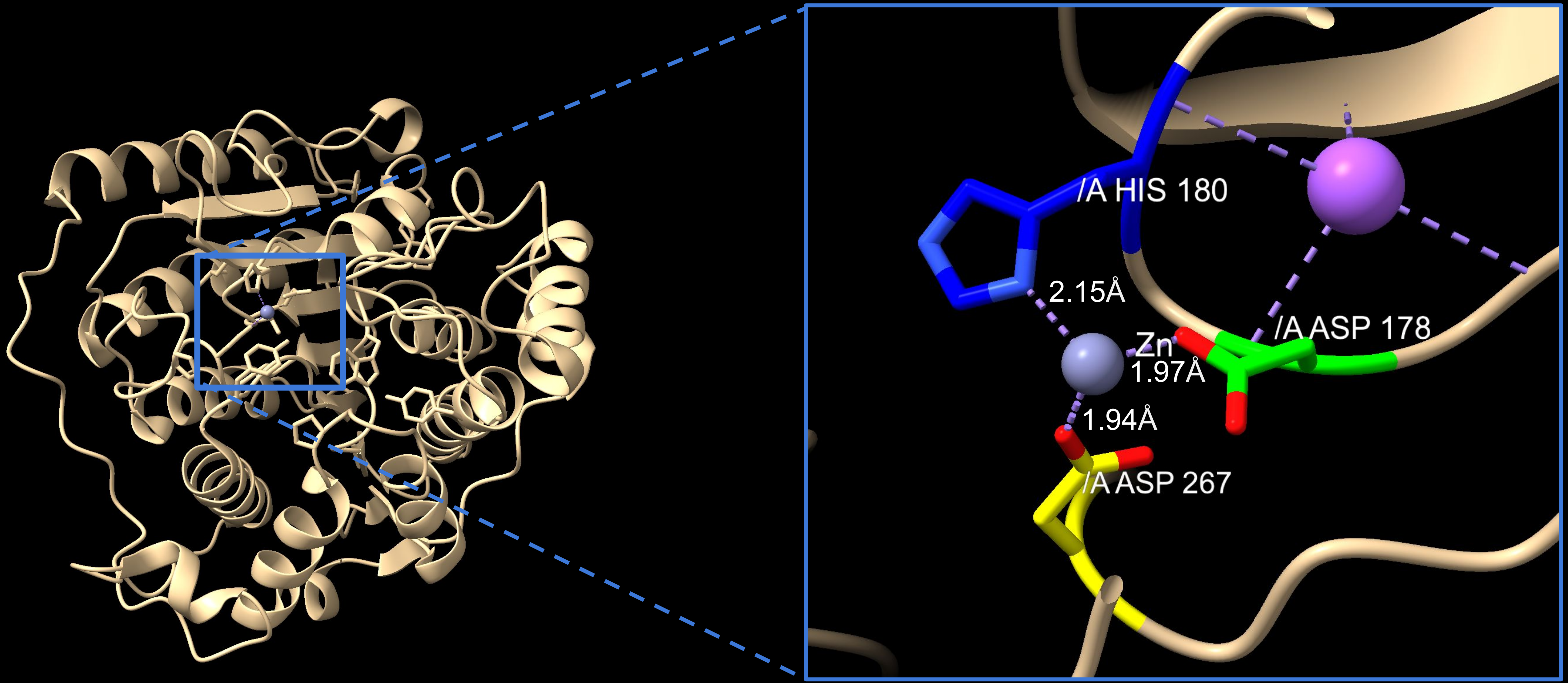


HDAC8 (1T64), *Homo sapiens*, 1.90Å

Hydrogen bonds - alpha helixes



Zn^{2+} in the active site



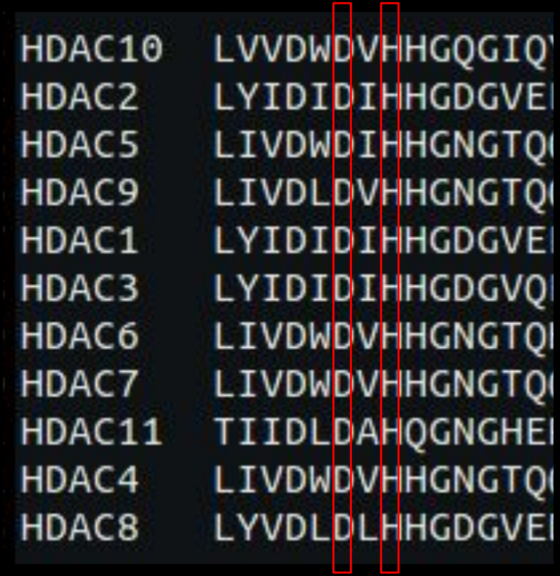
HDAC8 (1T64), *Homo sapiens*, 1.90Å

Zn^{2+} in the active site

	Atom	Distance
ASP 178	OD2	1.97 Å
HIS 180	ND1	2.15 Å
ASP 267	OD2	1.94 Å

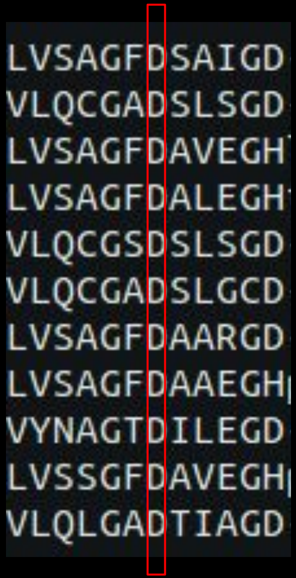
Zn^{2+} in the active site - conserved residues in HDACs

D178 H180



```
HDAC10   LVVDWDVHHGQGIQ
HDAC2    LYIDIDIHHGDGVE
HDAC5    LIVDWDIHHGNGTQ
HDAC9    LIVDLDVHHGNGTQ
HDAC1    LYIDIDIHHGDGVE
HDAC3    LYIDIDIHHGDGVQ
HDAC6    LIVDWDVHHGNGTQ
HDAC7    LIVDWDVHHGNGTQ
HDAC11   TIIDLDAHQGNGHE
HDAC4    LIVDWDVHHGNGTQ
HDAC8    LYVDLDLHHGDGVE
```

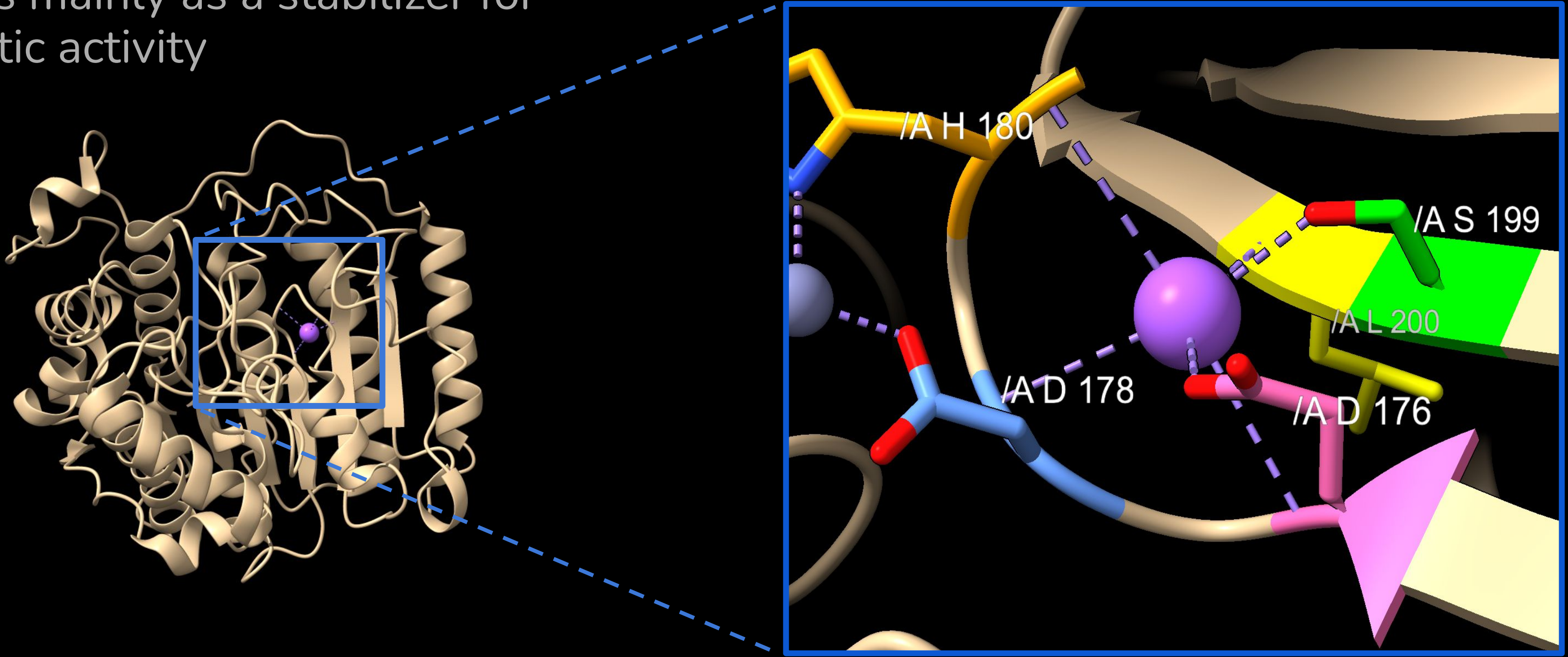
D267



```
LVSAGFDSAIGD
VLQCGADSLSGD
LVSAGFDAVEGH
LVSAGFDALEGH
VLQCGSDSLSGD
VLQCGADSLGCD
LVSAGFDAARGD
LVSAGFDAAEGH
VYNAGTDILEGD
LVSSGFDAVEGH
VLQLGADTIAGD
```

K^+ binding site

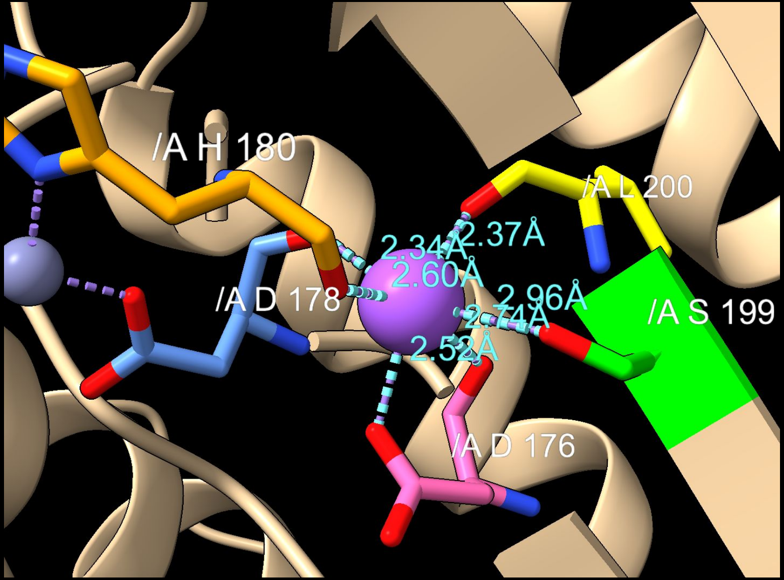
K^+ acts mainly as a stabilizer for catalytic activity



HDAC8 (1T64), *Homo sapiens*, 1.90Å

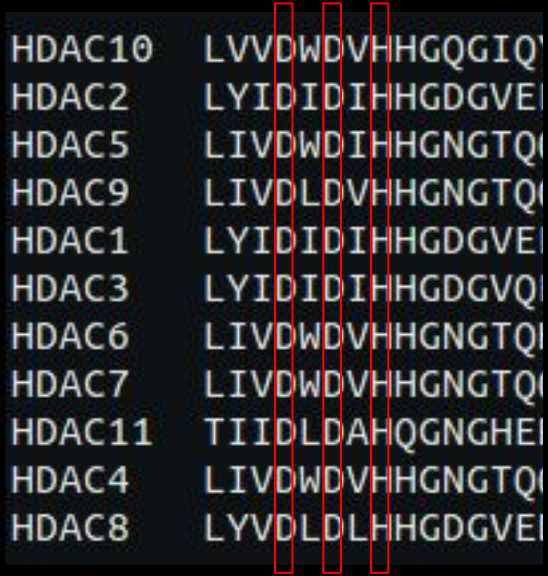
K^+ binding site

	Atom	Distance
ASP 176	OD1	2.52 Å
ASP 176	O	2.74 Å
ASP 178	O	2.34 Å
HIS 180	O	2.60 Å
LEU 200	O	2.37 Å
SER 199	O	2.96 Å



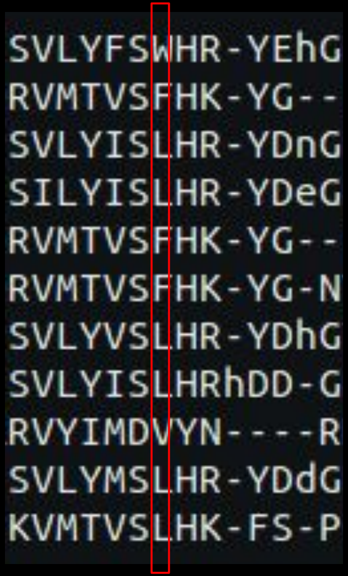
K^+ - conserved residues in HDACs

D176 D178 H180



```
HDAC10   LVVDWDVHHGQGIQ
HDAC2    LYIDIDIHHGDGVE
HDAC5    LIVDWDIHHGNGTQ
HDAC9    LIVDLDVHHGNGTQ
HDAC1    LYIDIDIHHGDGVE
HDAC3    LYIDIDIHHGDGVQ
HDAC6    LIVDWDVHHGNGTQ
HDAC7    LIVDWDVHHGNGTQ
HDAC11   TIIDLDAHQGNGHE
HDAC4    LIVDWDVHHGNGTQ
HDAC8    LYVDLDLHHGDGVE
```

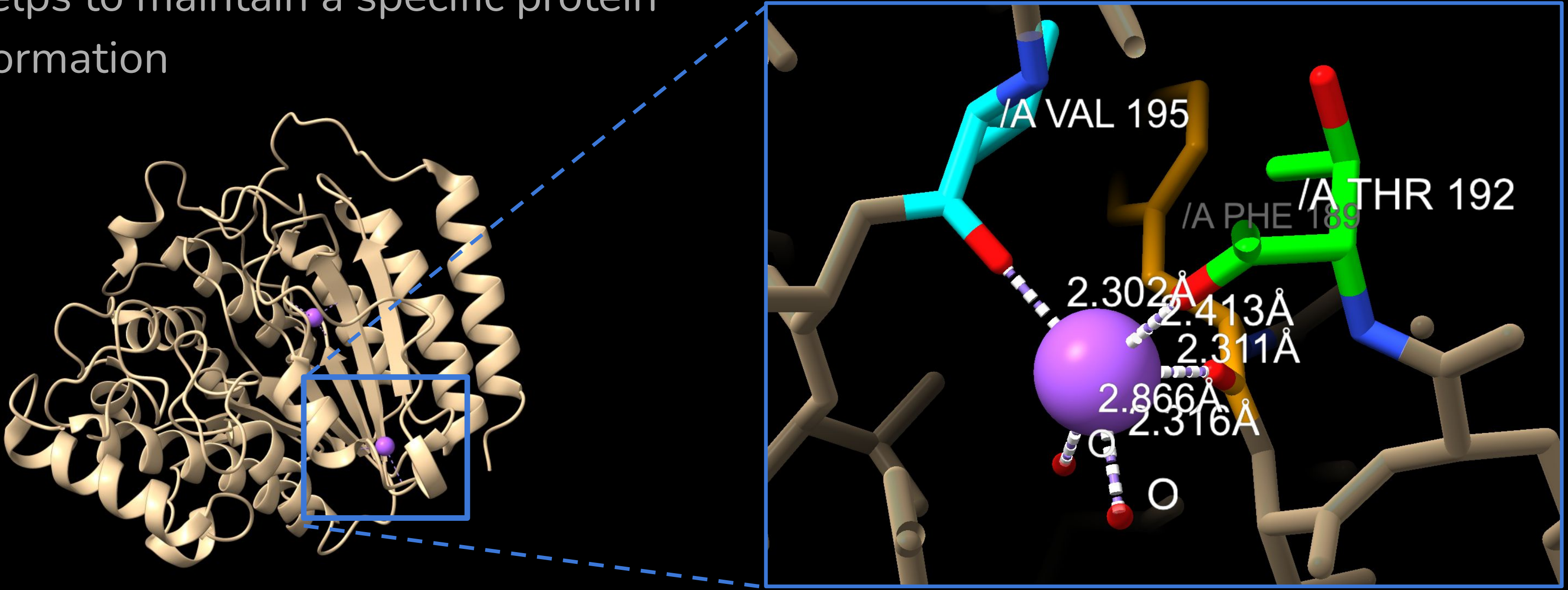
L200



```
SVLYFSWHR-YEhG
RVMTVSFHK-YG--
SVLYISLHR-YDnG
SILYISLHR-YDeG
RVMTVSFHK-YG--
RVMTVSFHK-YG-N
SVLYVSLHR-YDhG
SVLYISLHRhDD-G
RVYIMDVYN----R
SVLYMSLHR-YDdG
KVMTVSLHK-FS-P
```

K^+ binding site

K^+ helps to maintain a specific protein conformation



HDAC8 (1T64), *Homo sapiens*, 1.90Å

Post-translational modifications

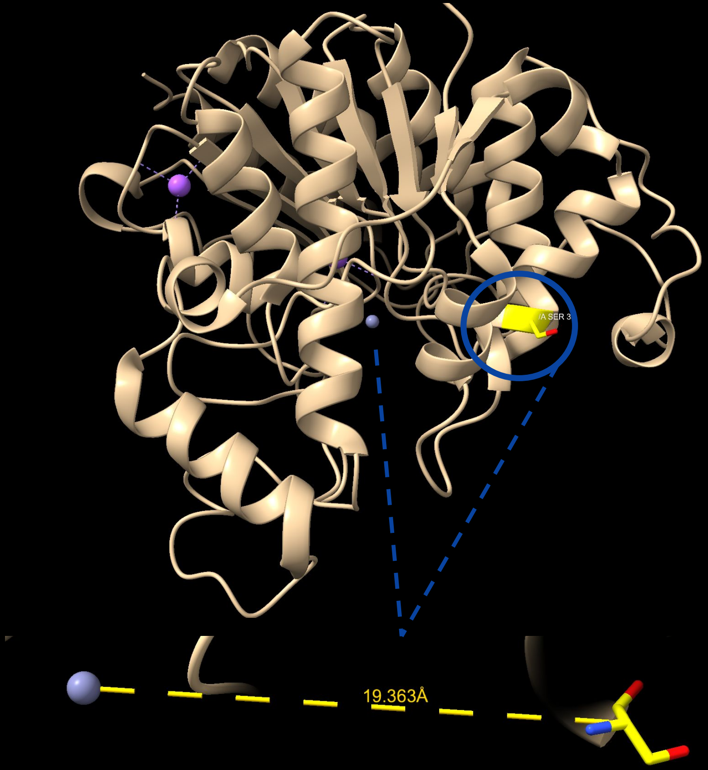
Post-translational modification

PKA phosphorylation

MEEPEEPADSGQSLVPVYIYSPEYVSMCDSLAKIP
KRASMVHSLIEAYALHKQMRIVKPK

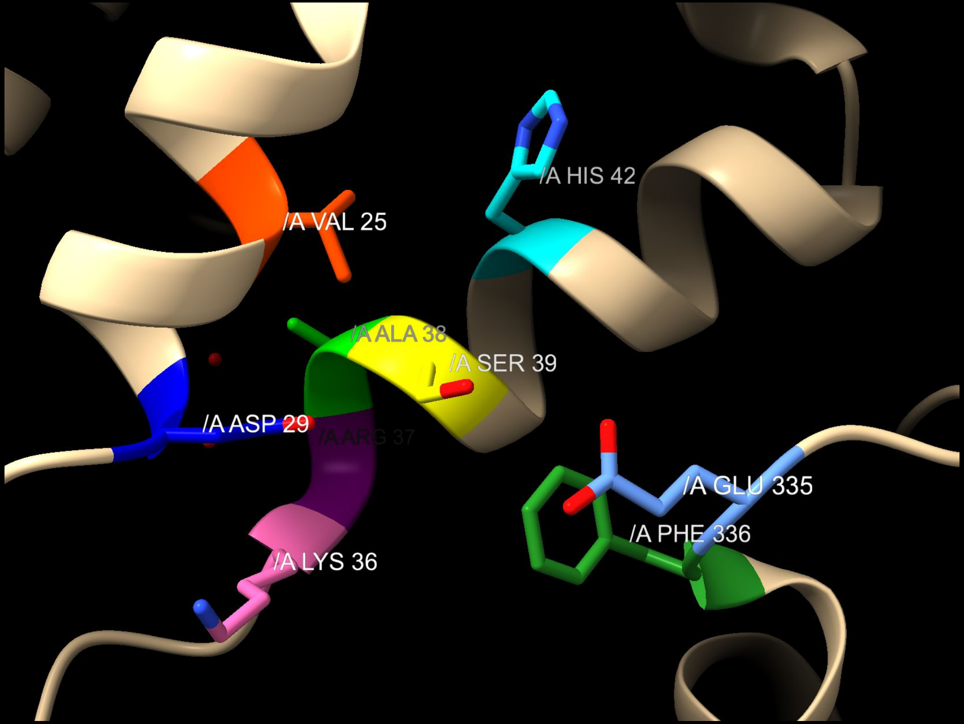
Phosphorylation has the potential to affect:

- Subcellular localization
- Protein-protein interactions
- Allosteric effects
- HDAC8 activity → conformational changes that propagate to the active site or enzyme-substrate interface



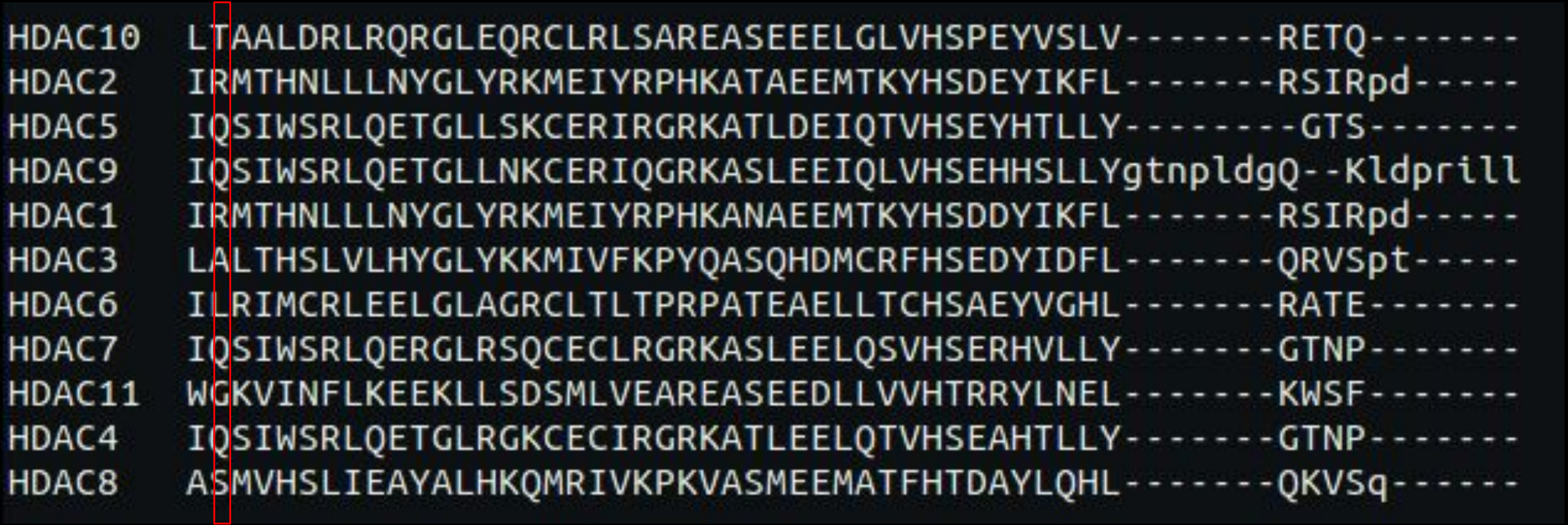
Post-translational modification

S39



Residues conserved in human HDACs

S39



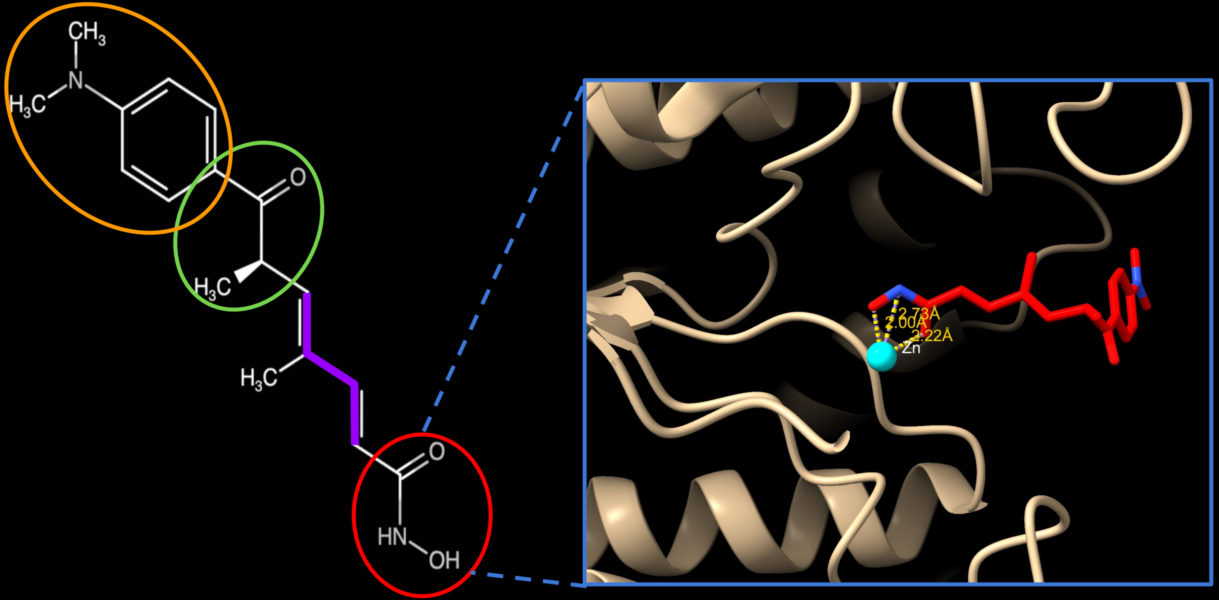
```
HDAC10   LTAALDRLRQRGLEQRCLRLSAREASEEELGLVHSPEYVSLV-------RETQ-------
HDAC2    IRMTHNLLLNYGLYRKMEIYRPHKATAEEMTKYHSDEYIKFL-------RSIRpd-----
HDAC5    IQSIWSRLQETGLLSKCERIRGRKATLDEIQTVHSEYHTLLY--------GTS-------
HDAC9    IQSIWSRLQETGLLNKCERIQGRKASLEEIQLVHSEHHSLLYgtnpldgQ--Kldprill
HDAC1    IRMTHNLLLNYGLYRKMEIYRPHKANAEEMTKYHSDDYIKFL-------RSIRpd-----
HDAC3    LALTHSLVLHYGLYKKMIVFKPYQASQHDMCRFHSEDYIDFL-------QRVSpt-----
HDAC6    ILRIMCRLEELGLAGRCLTLTPRPATEAELLTCHSAEYVGHL-------RATE-------
HDAC7    IQSIWSRLQERGLRSQCECLRGRKASLEELQSVHSERHVLLY-------GTNP-------
HDAC11   WGKVINFLKEEKLLSDSMLVEAREASEEDLLVVHTRRYLNEL-------KWSF-------
HDAC4    IQSIWSRLQETGLRGKCECIRGRKATLEELQTVHSEAHTLLY-------GTNP-------
HDAC8    ASMVHSLIEAYALHKQMRIVKPKVASMEEMATFHTDAYLQHL-------QKVSq------
```

Trichostatin A

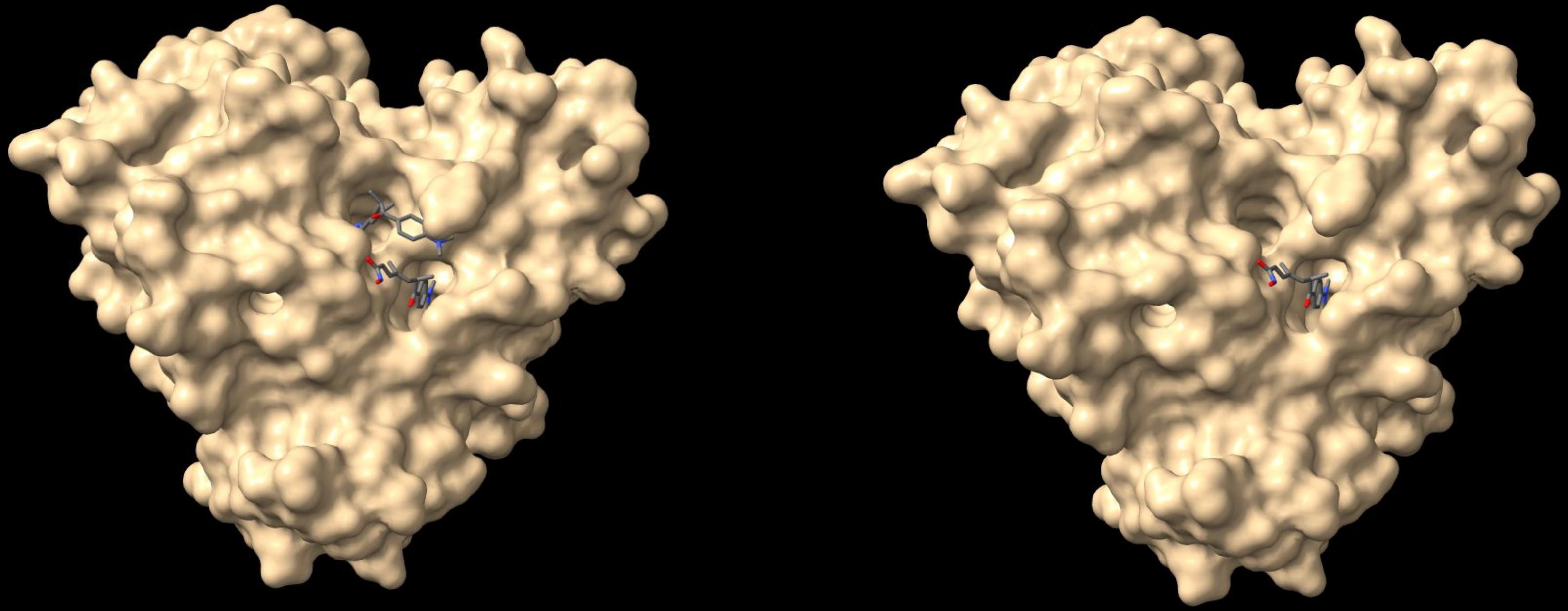
Structure

Molecular weight: 302,37 g/mol
Formula: $C_{17}H_{22}N_2O_3$

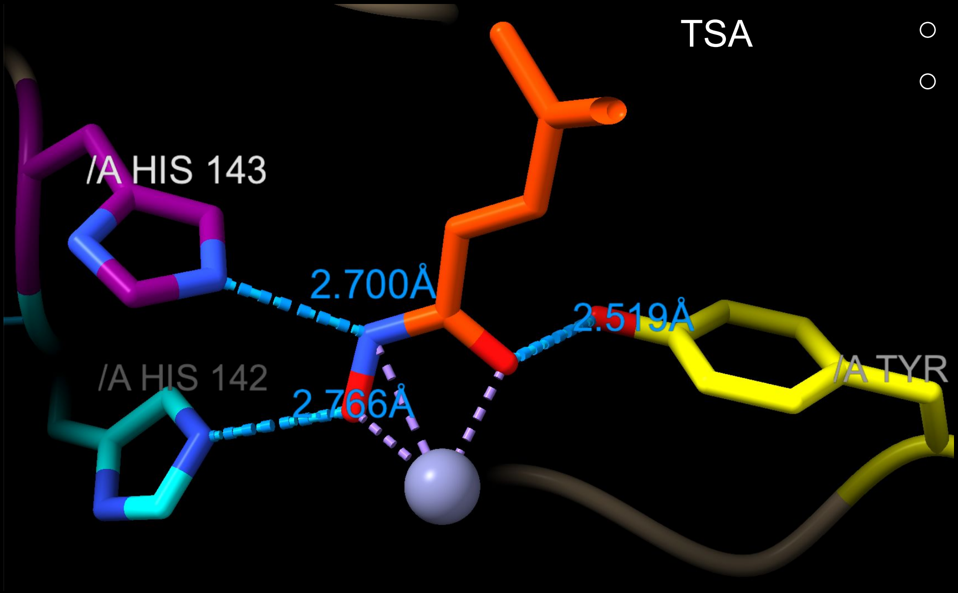
- Dimethylamine
- Ketone
- Polyene
- Hydroxamate: it binds to Zn^{2+} due to chelation



TSA in the liberation channel



Hydrogen bonds in the active site



- HIS142 (NE2) - TSA: hydrogen bond (2,766Å)
- TYR 306 (OH) - TSA: hydrogen bond (2,519Å)
- HIS143 (NE2) - TSA: hydrogen bond (2,700Å)

TSA interactions in the active site

HDAC atom	TSA atom	Distance	Bond
HIS 143-NE2	N1	2.70 Å	Hydrogen
HIS 142-NE2	N1	3.47 Å	Van der Waals
HIS 143-CD2	N1	3.23 Å	Van der Waals
Zn^{2+}	O1	2.00 Å	Covalent
HIS 142-NE2	O1	2.77 Å	Hydrogen
HIS 143-NE2	O1	3.11 Å	Van der Waals
Zn^{2+}	O2	2.22 Å	Covalent
TYR 306-CE1	O2	3.35 Å	Van der Waals
TYR 306-CZ	O2	3.35 Å	Van der Waals
TYR 306-OH	O2	2.52 Å	Van der Waals

Mutations: diseases

Cornelia de Lange disease

Cornelia de Lange syndrome (CdLS) is a dominantly **inherited congenital malformation disorder**.

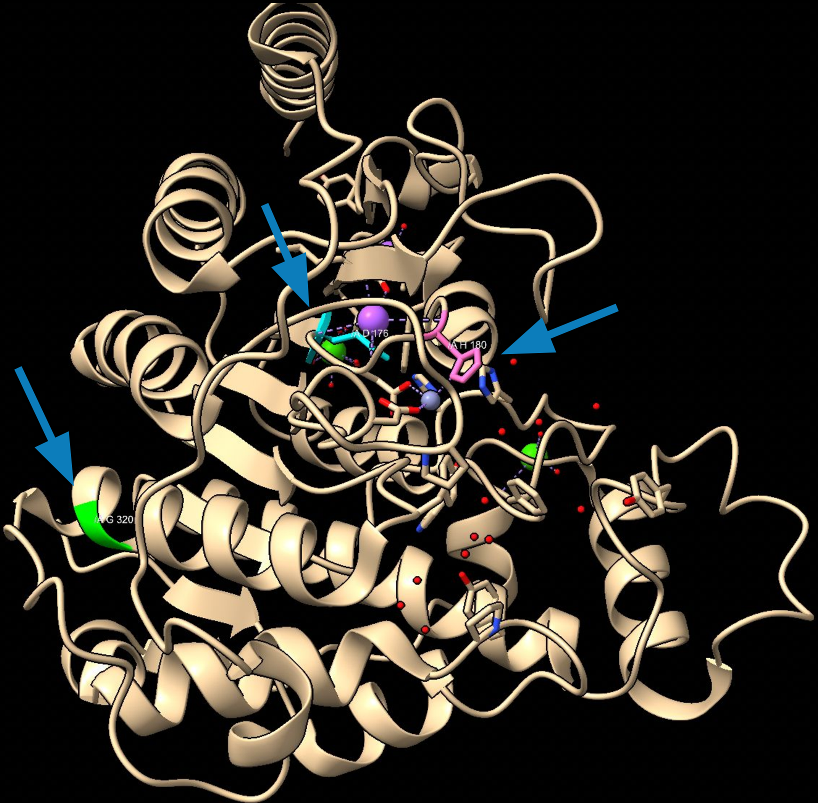
- ➔ Caused by mutations in the cohesin-loading protein NIPBL or in the core cohesin components SMC1A and SMC3.

SMC3 is acetylated during S-phase to establish cohesiveness of chromatin-loaded cohesin. **Loss of HDAC8 activity results in increased SMC3 acetylation** and inefficient dissolution of the cohesin complex.

SMC3 with retained acetylation is loaded onto chromatin = altered transcription

Mutations in HDAC8 related to Cornelia de Lange disease

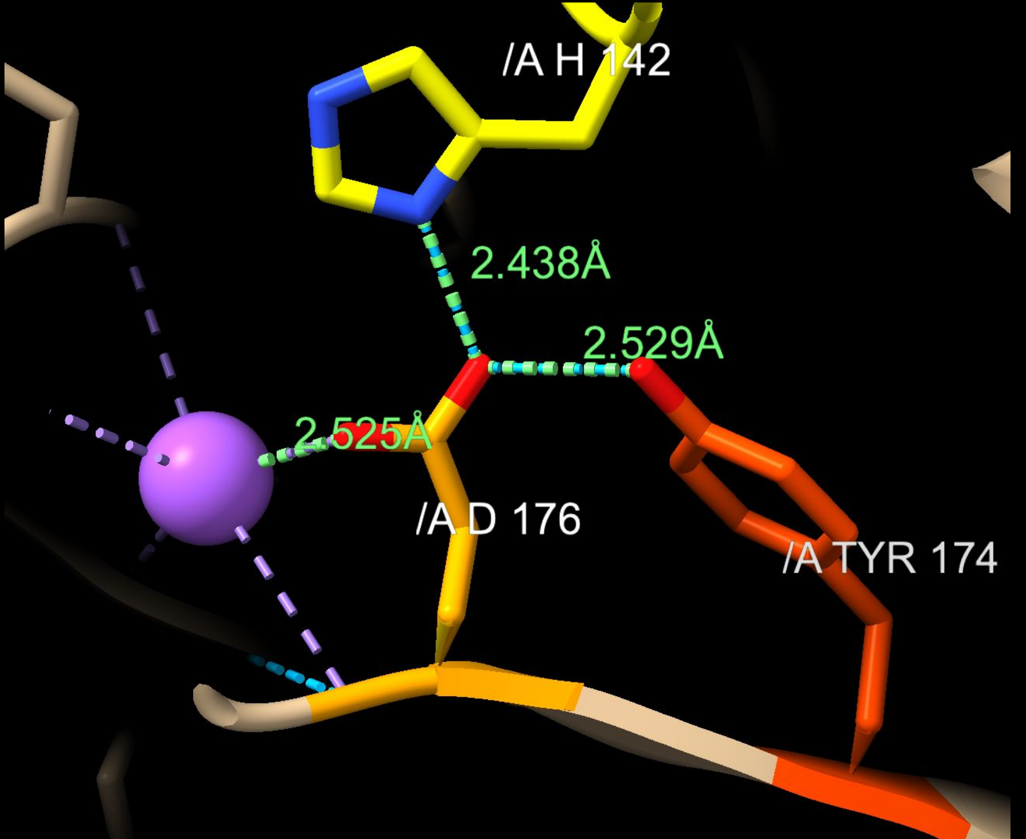
Mutation	Position
Histidine (H) → Arginine (R)	180
Glycine (G) → Arginine (R)	320
Aspartic acid (D) → Alanine (A)	176



D176A mutation

- **D176** is bound to a monovalent cation (**K^+**) at the active site.

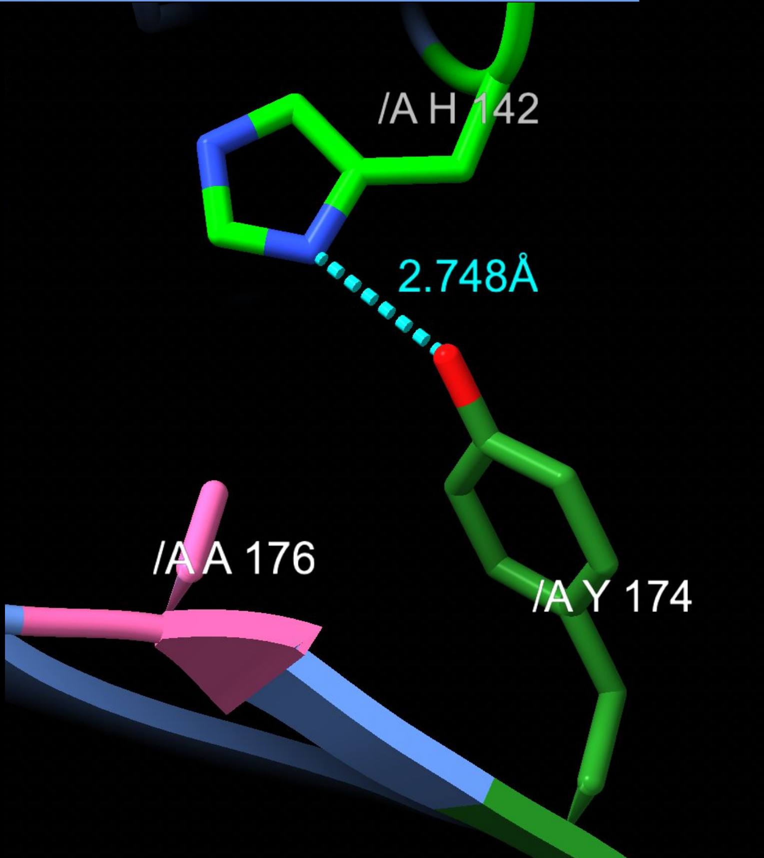
- **D176** accepts a hydrogen bond from one of the catalytic histidine residues, **H142**, and stabilizes the positively charged imidazolium cation → **key role in electrostatic catalysis**.



D176A mutation

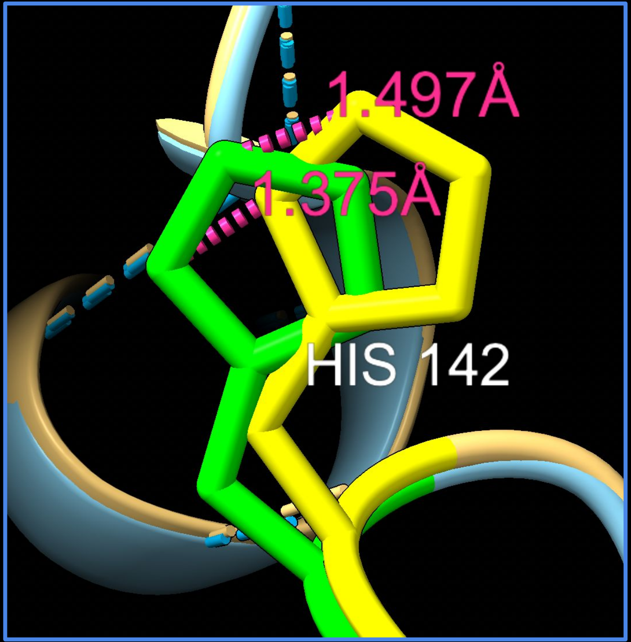
D176 → A176

- Deletion of the negatively charged carboxylate side chain of **D176** results in the dissociation of the **K**$^+$ ion to which it is coordinated.
- The side chain of **H142** rotates and shifts 1.4 Å to form a new hydrogen bond with **Y174**.
- Lower pKa of **H142** due to the loss of **D176**-**H142** hydrogen bond → proton dissociation to yield the neutral imidazole form.
- The consequences for catalysis are severe: **D176A HDAC8 exhibits a reduction in kcat.**

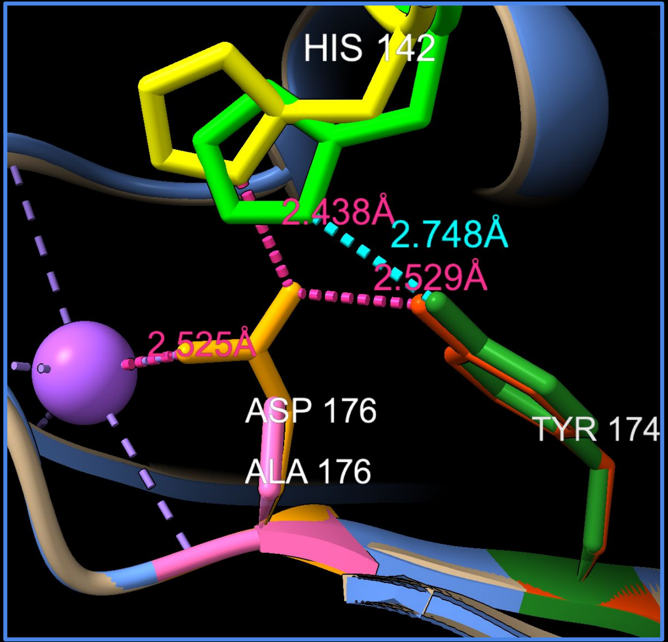


K+ binding is necessary to stabilize the protein scaffolding in a catalytically competent conformation

D176A mutation



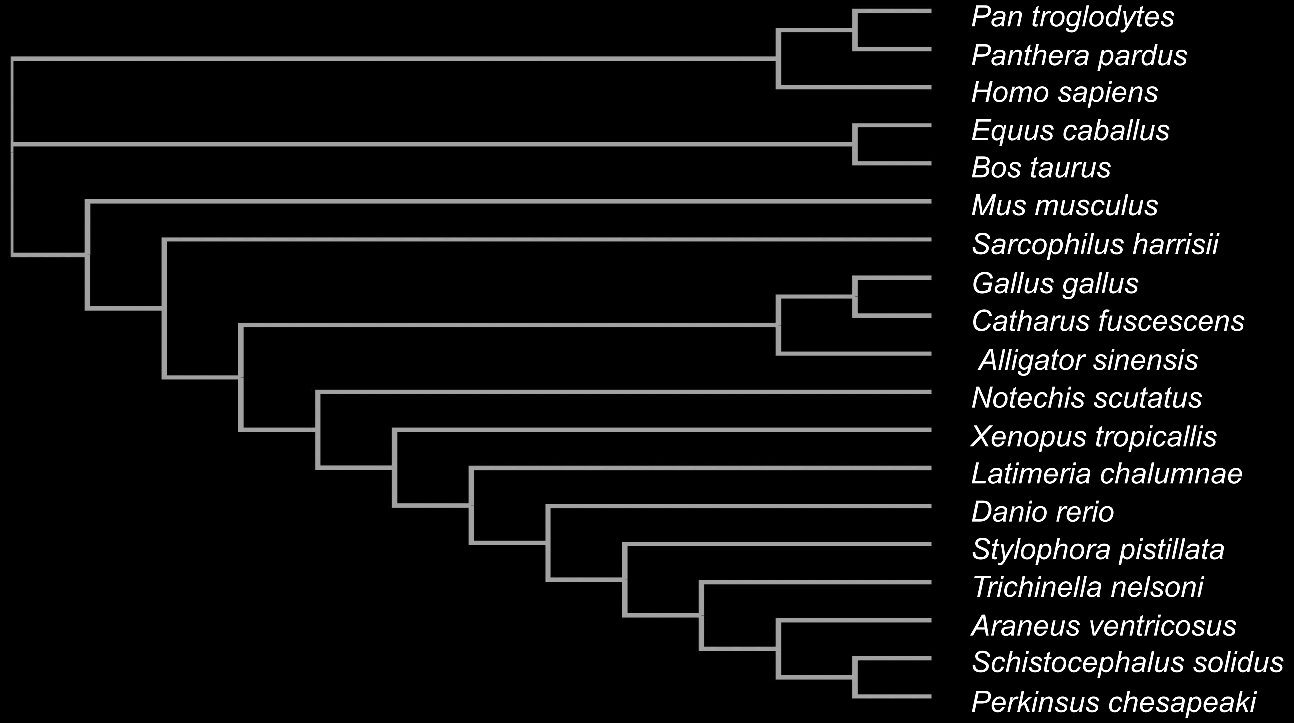
Shift of histidine 142: 1.4 Å
HIS 142: HDAC8 without mutation
HIS 142: HDAC8 with mutation



HDAC8	HDAC8 mutated
ASP 176	ALA 176
TYR 174	TYR 174
HIS 142	HIS 142

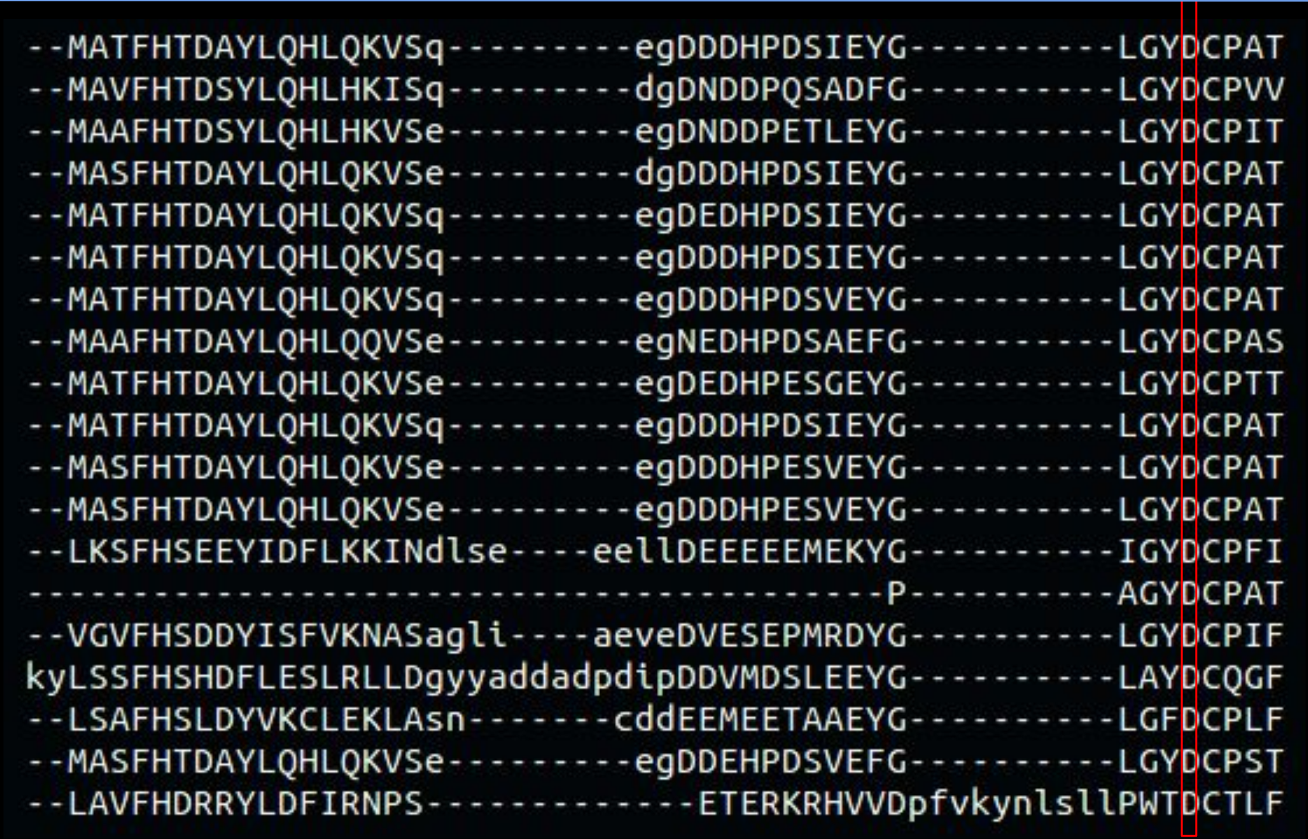
Evolution

Phylogenetic tree



D101 in different species

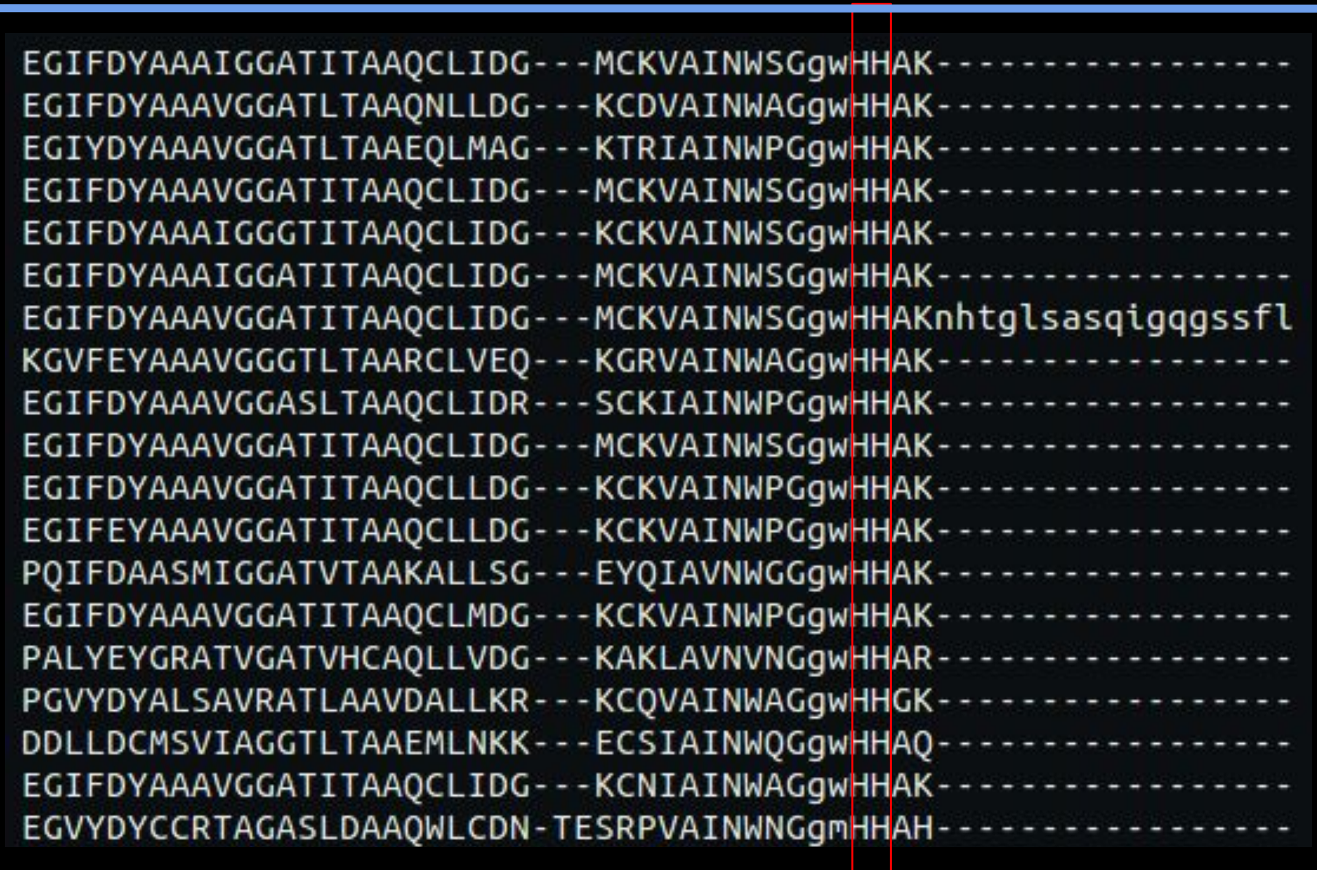
Homo sapiens
Danio rerio
Xenopus tropicallis
Bos taurus
Mus musculus
Pan troglodytes
Panthera pardus
Notechis scutatus
Latimeria chalumnae
Equus caballus
Gallus gallus
Catharus fuscescens
Araneus ventricosus
Alligator sinensis
Trichinella nelsoni
Schistocephalus solidus
Stylophora pistillata
Sarcophilus harrisii
Perkinsus chesapeaki



```
--MATFHTDAYLQHLQKVSq---------egDDDHPDSIEYG----------LGYDCPAT
--MAVFHTDSYLQHLHKISq---------dgDNDDPQSADFG----------LGYDCPVV
--MAAFHTDSYLQHLHKVSe---------egDNDDPETLEYG----------LGYDCPIT
--MASFHTDAYLQHLQKVSe---------dgDDDHPDSIEYG----------LGYDCPAT
--MATFHTDAYLQHLQKVSq---------egDEDHPDSIEYG----------LGYDCPAT
--MATFHTDAYLQHLQKVSq---------egDDDHPDSIEYG----------LGYDCPAT
--MATFHTDAYLQHLQKVSq---------egDDDHPDSVEYG----------LGYDCPAT
--MAAFHTDAYLQHLQQVSe---------egNEDHPDSAEFG----------LGYDCPAS
--MATFHTDAYLQHLQKVSe---------egDEDHPESGEYG----------LGYDCPTT
--MATFHTDAYLQHLQKVSq---------egDDDHPDSIEYG----------LGYDCPAT
--MASFHTDAYLQHLQKVSe---------egDDDHPESVEYG----------LGYDCPAT
--MASFHTDAYLQHLQKVSe---------egDDDHPESVEYG----------LGYDCPAT
--LKSFHSEEYIDFLKKINdlse----eellDEEEEEMEKYG----------IGYDCPFI
-----------------------------------------P----------AGYDCPAT
--VGVFHSDDYISFVKNASagli----aeveDVESEPMRDYG----------LGYDCPIF
kyLSSFHSHDFLESLRLLDgyyaddadpdipDDVMDSLEEYG----------LAYDCQGF
--LSAFHSLDYVKCLEKLAsn-------cddEEMEETAAEYG----------LGFDCPLF
--MASFHTDAYLQHLQKVSe---------egDDEHPDSVEFG----------LGYDCPST
--LAVFHDRRYLDFIRNPS-------------ETERKRHVVDpfvkynlsllPWTDCTLF
```

H142 and H143 in different species

Homo sapiens
Danio rerio
Xenopus tropicallis
Bos taurus
Mus musculus
Pan troglodytes
Panthera pardus
Notechis scutatus
Latimeria chalumnae
Equus caballus
Gallus gallus
Catharus fuscescens
Araneus ventricosus
Alligator sinensis
Trichinella nelsoni
Schistocephalus solidus
Stylophora pistillata
Sarcophilus harrisii
Perkinsus chesapeaki



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EGIFDYAAAIGGATITAAQCLIDG---MCKVAINWSGgwHHAK-----------------
EGIFDYAAAVGGATLTAAQNLLDG---KCDVAINWAGgwHHAK-----------------
EGIYDYAAAVGGATLTAAEQLMAG---KTRIAINWPGgwHHAK-----------------
EGIFDYAAAVGGATITAAQCLIDG---MCKVAINWSGgwHHAK-----------------
EGIFDYAAAIGGGTITAAQCLIDG---KCKVAINWSGgwHHAK-----------------
EGIFDYAAAIGGATITAAQCLIDG---MCKVAINWSGgwHHAK-----------------
EGIFDYAAAVGGATITAAQCLIDG---MCKVAINWSGgwHHAKnhtglsasqigqgssfl
KGVFEYAAAVGGGTLTAARCLVEQ---KGRVAINWAGgwHHAK-----------------
EGIFDYAAAVGGASLTAAQCLIDR---SCKIAINWPGgwHHAK-----------------
EGIFDYAAAVGGATITAAQCLIDG---MCKVAINWSGgwHHAK-----------------
EGIFDYAAAVGGATITAAQCLLDG---KCKVAINWPGgwHHAK-----------------
EGIFEYAAAVGGATITAAQCLLDG---KCKVAINWPGgwHHAK-----------------
PQIFDAASMIGGATVTAAKALLSG---EYQIAVNWGGgwHHAK-----------------
EGIFDYAAAVGGATITAAQCLMDG---KCKVAINWPGgwHHAK-----------------
PALYEYGRATVGATVHCAQLLVDG---KAKLAVNVNGgwHHAR-----------------
PGVYDYALSAVRATLAAVDALLKR---KCQVAINWAGgwHHGK-----------------
DDLLDCMSVIAGGTLTAAEMLNKK---ECSIAINWQGgwHHAQ-----------------
EGIFDYAAAVGGATITAAQCLIDG---KCNIAINWAGgwHHAK-----------------
EGVYDYCCRTAGASLDAAQWLCDN-TESRPVAINWNGgmHHAH-----------------
```

G151, F152, D176, D178 and H180 in different species

Homo sapiens
Danio rerio
Xenopus tropicallis
Bos taurus
Mus musculus
Pan troglodytes
Panthera pardus
Notechis scutatus
Latimeria chalumnae
Equus caballus
Gallus gallus
Catharus fuscescens
Araneus ventricosus
Alligator sinensis
Trichinella nelsoni
Schistocephalus solidus
Stylophora pistillata
Sarcophilus harrisii
Perkinsus chesapeaki

```
-------------------KDEASGFCYLNDAVLGILRLRR----K--FERILYVDLDLH
-------------------KDEASGSCYVNDAVLGILKLRE----KY--DRVLYVDVDLH
-------------------KDEASGFCYLNDAVLGILKLRE----K--FDRVLYVDMDLH
-------------------KDEASGFCYLNDAVLGILRLRR----K--FDRILYVDLDLH
-------------------KDEASGFCYLNDAVLGILRLRR----K--FDRILYVDLDLH
-------------------KDEASGFCYLNDAVLGILRLRR----K--FERILYVDLDLH
pqsfcnaalsawktspsqyGDEASGFCYLNDAVLGILRLRR----K--FDRILYVDLDLH
-------------------KDEASGFCYLNDAVLGILQLRR----R--FDRVLYVDLDLH
-------------------KDEASGFCYINDAVLGILKLRQ----KYE--RVLYVDLDLH
-------------------KDEASGFCYLNDAVLGILRLRR----K--FDRILYVDLDLH
-------------------KDEASGFCYLNDAVLGILRLRQ----K--FDRILYIDLDLH
-------------------KDEASGFCYLNDAVLGILRLRQ----K--FDRVLYIDLDLH
-------------------KDQADGFCYVNDIVLGILHLLK----KYK--RVLYVDLDLH
-------------------KDEASGFCYLNDVVLGILKLRQ----K--FDRILYIDLDLH
-------------------RSAAAGFCYFNDCVIGILKLRE----R--FKRVLYIDLDAH
-------------------RAEASGFCYLNDVVIGLNYLLSsaafQDSRKRVIYLDFDLH
-------------------RNEASGFCYINDVVLGILKLRE----K--FDRILYVDIDLH
-------------------KDEASGFCYLNDAVLGILHLRR----K--FDRILYIDLDLH
-------------------CANAAGFCYVNDIVLAIVKLLE----VY--DRVLYVDLDYH
```

D183 in different species

Homo sapiens
Danio rerio
Xenopus tropicallis
Bos taurus
Mus musculus
Pan troglodytes
Panthera pardus
Notechis scutatus
Latimeria chalumnae
Equus caballus
Gallus gallus
Catharus fuscescens
Araneus ventricosus
Alligator sinensis
Trichinella nelsoni
Schistocephalus solidus
Stylophora pistillata
Sarcophilus harrisii
Perkinsus chesapeaki

```
HGDGVEDAFSFTSKVMTVSLHK----FSPGFFPGT------GDV-SDVG----L-GK-GR
HGDGVEDAFSFTSKVMTVSLHK----FSPGFFPGT------GDV-TDTG----L-GK-GR
HGDGVEDAFSFTSKVMTVSLHK----FSPGFFPGT------GDV-SDIG----L-GK-GR
HGDGVEDAFSFTSKVMTVSLHK----FSPGFFPGT------GDV-SDVG----L-GK-GR
HGDGVEDAFSFTSKVMTVSLHK----FSPGFFPGT------GDM-SDVG----L-GK-GR
HGDGVEDAFSFTSKVMTVSLHK----FSPGFFPGT------GDV-SDVG----L-GK-GR
HGDGVEDAFSFTSKVMTVSLHK----FSPGFFPGT------GDV-SDVG----L-GK-GR
HGDGVEDAFSFTSKVMTVSLHK----FGPGFFPGT------GDV-TEVG----L-GK-GR
HGDGVEDAFSFTSKVMTVSFHK----YSFGFFPGT------GDV-TDIG----L-GK-GR
HGDGVEDAFSFTSKVMTVSLHK----FSPGFFPGT------GDV-SDVG----L-GK-GR
HGDGVEDAFSFTSKVMTVSLHK----FSPGFFPGT------GDV-TDIG----L-GK-GR
HGDGVEDAFSFTSKVMTVSLHK----FSPGFFPGKlnlqgtGDV-TDVG----L-GK-GR
HGDGVEEAFAHTSRVLCFSVHK----NEIGFYPGT------GLL-NDIG----Y-GK-GK
HGDGVEDAFSFTSKVMTVSLHK----FSPGFFPGT------GDV-SEVG----L-GK-GR
HGDAVEDAFCSTRSVLTVSLHC----YEAGFYPCS------GSV-DDVG----V-GS-GK
HGDGVEEAFAYSSRVVTFSVHH----ASPGFFPGT------GDItPDSSgfftG-ARgGR
HGDGVEDAFSFTSKVMSVSFHK----FSPGFFPGT------GGC-HDVG----L-GK-GK
HGDGVEDAFSFTSKVMTVSLHK----FSPGFFPGT------GDV-SDVG----L-GK-GR
HGDAVEEAFYSCPRVVTLSIHSapskSSGNSFPGT------GAV-YDIG----PdGTpGK
```

D267 in different species

Homo sapiens	`YYSVNVPIQDGIQDEKYYQICESVLKEVYQAFNPKAVVLQLGADTIAGDPMC---SFNM-`
Danio rerio	`WYAVNVPFEDGVRDDRYCQTFTSVMQEVKALFNPEAVVMQLGADTMAGDPMC---SFNM-`
Xenopus tropicallis	`YYSVNVPLQDGIQDEKYYQICEGVLKEVFTTFNPEAVVLQLGADTIAGDPMC---SFNM-`
Bos taurus	`YYSVNVPIQDCIQDERYYHICESVLKEVYIAFNPKAVVLQLGADTIAGDPMC---SFNM-`
Mus musculus	`YYSVNVPIQDGIQDEKYYHICESVLKEVYQAFNPKAVVLQLGADTIAGDPMC---SFNM-`
Pan troglodytes	`YYSVNVPIQDGIQDEKYYQICESVLKEVYQAFNPKAVVLQLGADTIAGDPMC---SFNM-`
Panthera pardus	`YYSVNVPIQDGIQDEKYYHICESVLKEVYIAFNPKAVVLQLGADTIAGDPMC---SFNM-`
Notechis scutatus	`YYSVNVPLQDGIKNETYYQLCAAVLKDVYAAFHPGAVVLQLGADTIAGDPMC---AFNL-`
Latimeria chalumnae	`CYAVNVPLQDGIQDDKYFQICESILKEVYTAFSPQAVVLQLGADTLAGDPMC---SFNM-`
Equus caballus	`YYSVNVPIQDGIQDEKYYHICESVLKEVYIAFNPKAVVLQLGADTIAGDPMC---SFNM-`
Gallus gallus	`YYSVNVPIQDGIQDEKYYQICETVLKEVYAAFNPEAVVLQLGADTIAGDPMC---SFNM-`
Catharus fuscescens	`YYSVNVPIQDGIRDEKYYQICESVLEEVYAAFNPDAVVLQLGADTIAGDPMC---SFNM-`
Araneus ventricosus	`HYTINVPLYDGIQDSQYIELIIPLLSKTQKKFQPDIVVCQCGADTLAGDPFA---AFNL-`
Alligator sinensis	`YYTVNVPIQDGIQDEKYYQICETVLKEVYAAFNPEAVVLQLGADTIAGDPMC---SFNM-`
Trichinella nelsoni	`YYAVNVPFRQGLVDEQLLSTFDALVPKIVHLYRPEVVFLQLGTDGLAGDPVA---AFNL-`
Schistocephalus solidus	`YSCFNLPLAEGTGDETWLSTVKPILSALHASLQPSFIVVQCGADGLTSDPHR---VFNLs`
Stylophora pistillata	`YYTVNVPLKDGITDKPFIEIFSRVMSEVKKRFKPSIVVCQCGVDTLAGDPMA---SFNL-`
Sarcophilus harrisii	`YYSVNVPIQDGIRDEKYYQVCKSVLKEVYVAFNPKAVVLQLGADTIAGDPMC---SFNL-`
Perkinsus chesapeaki	`GHAVNLPMKPGLTDDLFLYALRTTLEALLQRFRPSCLVVQSGSDSLAGDLLTshsGFNL-`

Y306 in different species

Homo sapiens	-TPVGIGK-CLKYILQWQ---LATLILGGGGYNLANTARCWTYLTGVI-lgktlsseipd
Danio rerio	-TPVGVAK-CLTYILGWE---LPTLLLGGGGYNLANTARCWTYLTGT--vlgqtlsseip
Xenopus tropicallis	-TPQGIGK-CLKYVLQWQ---LPTLILGGGGYHLPNTARCWTYLTALI-vgrtlsseipd
Bos taurus	-TPVGIGK-CLKYILQWE---LATLILGGGGYNLANTARCWTYLTGVI-lgktlsseipd
Mus musculus	-TPVGIGK-CLKYVLQWQ---LATLILGGGGYNLANTARCWTYLTGVI-lgktlsseipd
Pan troglodytes	-TPVGIGK-CLKYILQWQ---LATLILGGGGYNLANTARCWTYLTGVI-lgktlsseipd
Panthera pardus	-TPVGIGK-CLKYILQWQ---LATLILGGGGYNLANTARCWTYLTGVI-lgktlsseipd
Notechis scutatus	-TPEGIGK-CLNYVLQWQ---LPTLILGGGGYHLANTARCWTYLTGVI-lgktlpseipd
Latimeria chalumnae	-TPLGVEK-CLKYVLQWE---LPTLILGGGGYNLANTARCWTYLTGVI-lgktlsseipd
Equus caballus	-TPVGIGK-CLKYILQWQ---LATLILGGGGYNLANTARCWTYLTGVI-lgktlsseipd
Gallus gallus	-TPEGVGK-CLKYVLQWQ---LATLILGGGGYNLANTARCWTYLTGVI-lgrtlsseipd
Catharus fuscescens	-TPEGVGK-CLKYVLQWQ---LATLVLGGGGYNLANTARCWTYLTGVI-lgrtlsseipd
Araneus ventricosus	-TLKAPAE-CIKLLKSWN---IPLLALGGGGYNAVNTARCWTYLLSIL-ldktvesdipd
Alligator sinensis	-TPVGVGK-CLKYVLQWQ---LATLILGGGGYNLANTARCWTYLTGVI-lgrtlsseipd
Trichinella nelsoni	-TPSAYAG-VVCRVLGFG---KPCLLVGGGGYMPTNVSRCWALVLGAL-lgqnldddipe
Schistocephalus solidus	vDQNCAHAgAVRQVLSWG---LPTLLLGGGGYHFPDTARLWALLTSI--tlsavrgniye
Stylophora pistillata	-TQYSIGE-CVKYLMEWN---LPLLLLGGGGYNVKNSARCWAYLTGV--vlnqqlspdip
Sarcophilus harrisii	-TPVGLGK-CLKYILQWQ---LATLILGGGGYHLANTARCWTYLTGVI-lgrtlsseipd
Perkinsus chesapeaki	-STRGHAT-AVQELRRLG---IPTLVLGGGGYSLTSVAKCWSMETAV--wlnrgepflsp

Conclusions

Conclusions

1. HDAC plays an important role in gene expression as it is responsible for histone deacetylation.
2. Residues of the active site are conserved among HDAC classes
3. Residues of the active site are conserved throughout evolution
4. Mutations in HDAC8 cause the molecule to malfunction, leading to disease.

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PEM Questions

PEM Questions

HDACs are classified into:

1. 3 classes
2. 5 classes
3. 2 classes
4. 4 classes ✓

PEM Questions

HDAC8 is a … histone deacetylase

a. Class IV
b. Class III
c. Class II
d. Class I ✓
e. Class V

PEM Questions

HDAC8 has:

a. 0 loops
b. 7 loops
c. 9 loops ✓
d. 6 loops
e. 11 loops

PEM Questions

Which ion is important in the deacetylation reaction?

1. Na
2. Pb
3. K
4. Zn

a) 1, 2 and 3
b) 1 and 3
c) 2 and 4
d) 1, 2, 3 and 4
e) 4 ✓

PEM Questions

What parts of the HDAC8 have an important role in the deacetylation reaction?

1. The active site
2. N-terminal region
3. The liberation channel
4. C-terminal loop

a) 1, 2 and 3
b) 1 and 3 ✓
c) 2 and 4
d) 1, 2, 3 and 4
e) 4

PEM Questions

Which residues are more conserved in HDACs family?

a) Residues of the active site ✓
b) Residues close to K^+
c) Loop 3 residues
d) N-terminal residues
e) C-terminal residues

PEM Questions

TSA is an inhibitor of HDAC8 because it binds to:

a) N-terminal residues
b) S39
c) Specifically to Zn^{2+}
d) The active site ✓
e) It does not inhibit HDAC8

PEM Questions

Mutations in HDAC8 causes different pathologies such as:

1. Cornelia de Lange disease
2. Talassemia
3. Patau syndrome
4. Down syndrome

a) 1 ✓
b) 2 and 4
c) 1, 2 and 3
d) 4
e) 1,2,3 and 4

PEM Questions

In Cornelia de Lange disease, which binding site is affected?

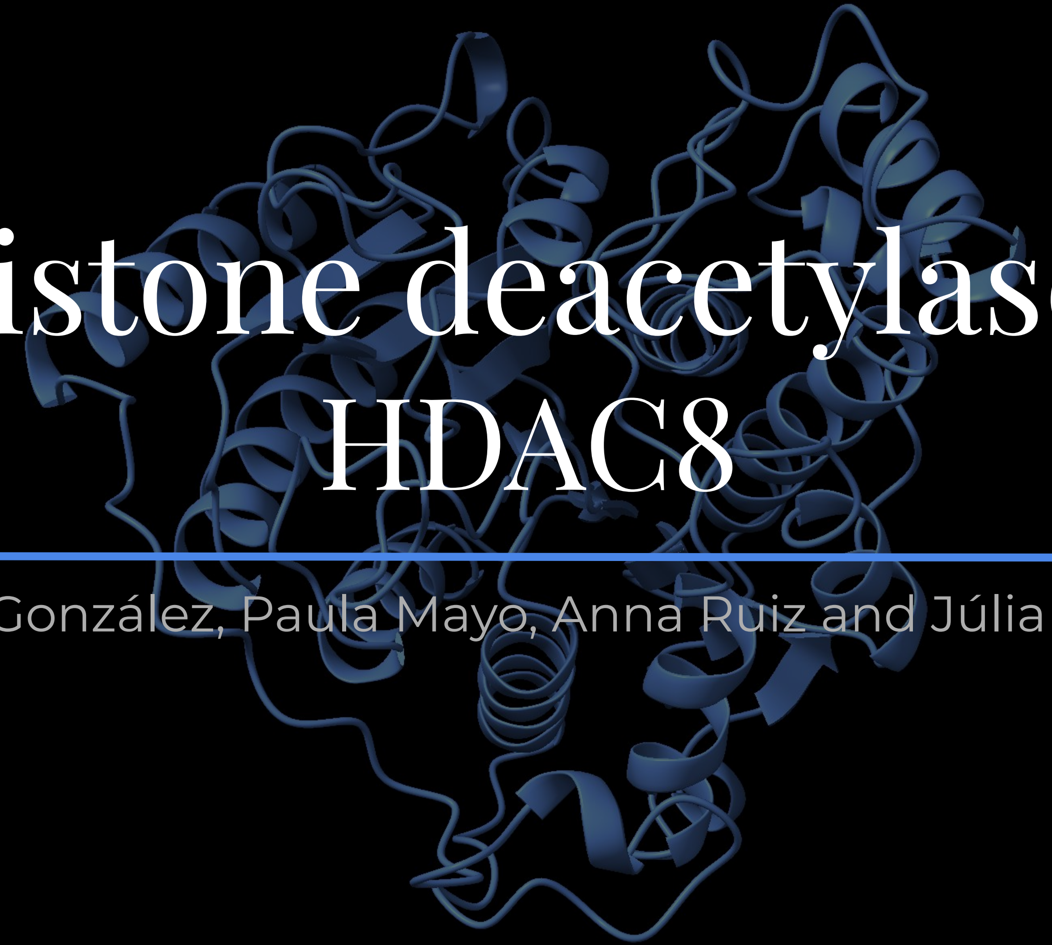
1. K^+ binding site
2. Fe^{2+} binding site
3. Zn^{+2} binding site
4. P binding site

a) 1 ✓
b) 2 and 4
c) 1, 2 and 3
d) 4
e) 1,2,3 and 4

PEM Questions

Phosphorylation of this residue inhibit the enzymatic activity of HDAC8:

a) K36
b) S39 ✓
c) H42
d) D29
e) P42



Histone deacetylases HDAC8

Ylenia González, Paula Mayo, Anna Ruiz and Júlia Terzulli