Investigations on HAT catalyst mechanisms and its site-specificity

2016.02.13

B4 Yamaji Kyohei

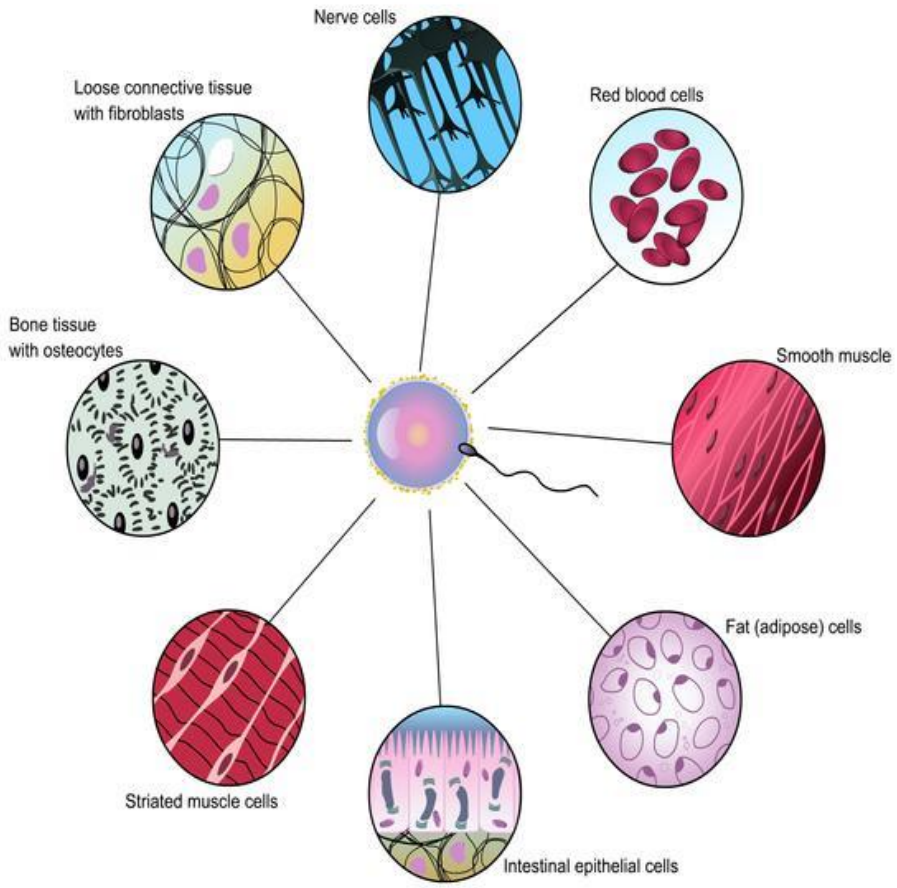
Contents

1. Introduction of chromatin modifications
2. Concept of Catalysis medicine
3. HAT introduction
4. HAT catalytic mechanisms
5. HAT site-specificity
6. Summary

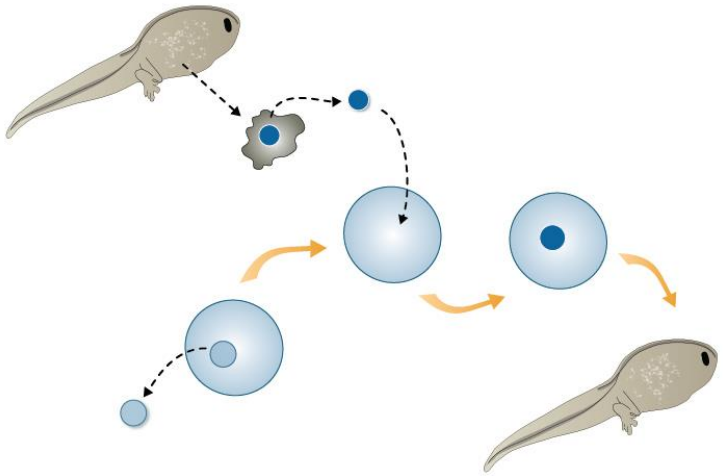
Epigenetics

The same genes, but different phenotypes

Cell differentiation from the embryo



Production of a clone frog



Differentiated cells have all the information to produce an individual.

Change in gene expression induces different phenotypes.

https://searchforbetterhealth.wikispaces.com/Genes+%26+Health

http://learn.genetics.utah.edu/content/cloning/clonezone/

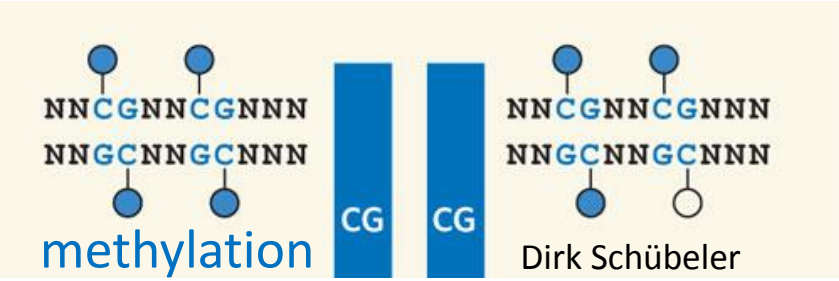
Epigenetics ; chemical modifications

DNA/Histone modifications can control its gene expression patterns.

DNA methylation pattern is different between stem cells and differentiated cells

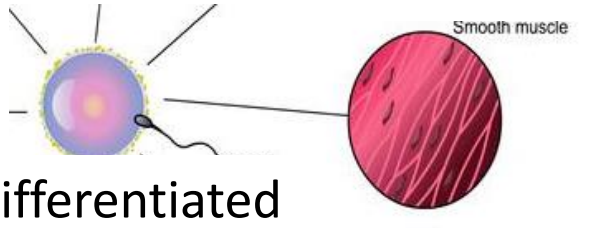
stem cells

Differentiated cells



Dirk Schübeler
Nature **462**, 296-297

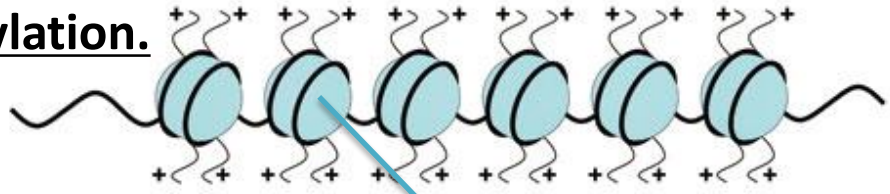
differentiated



undifferentiated

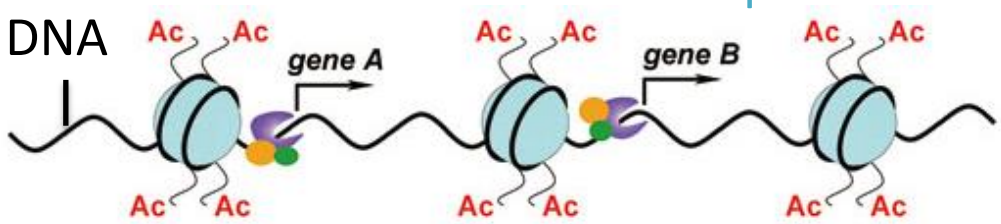
Expression of some genes is suppressed in differentiated cells with reduced global Histone acetylation.

Differentiated cells



Expression of gene A and B

stem cells



Expression of gene A and B

Loring, J F, *et al. Cell Research* (2014) **24**:143–160

Various Histone modifications

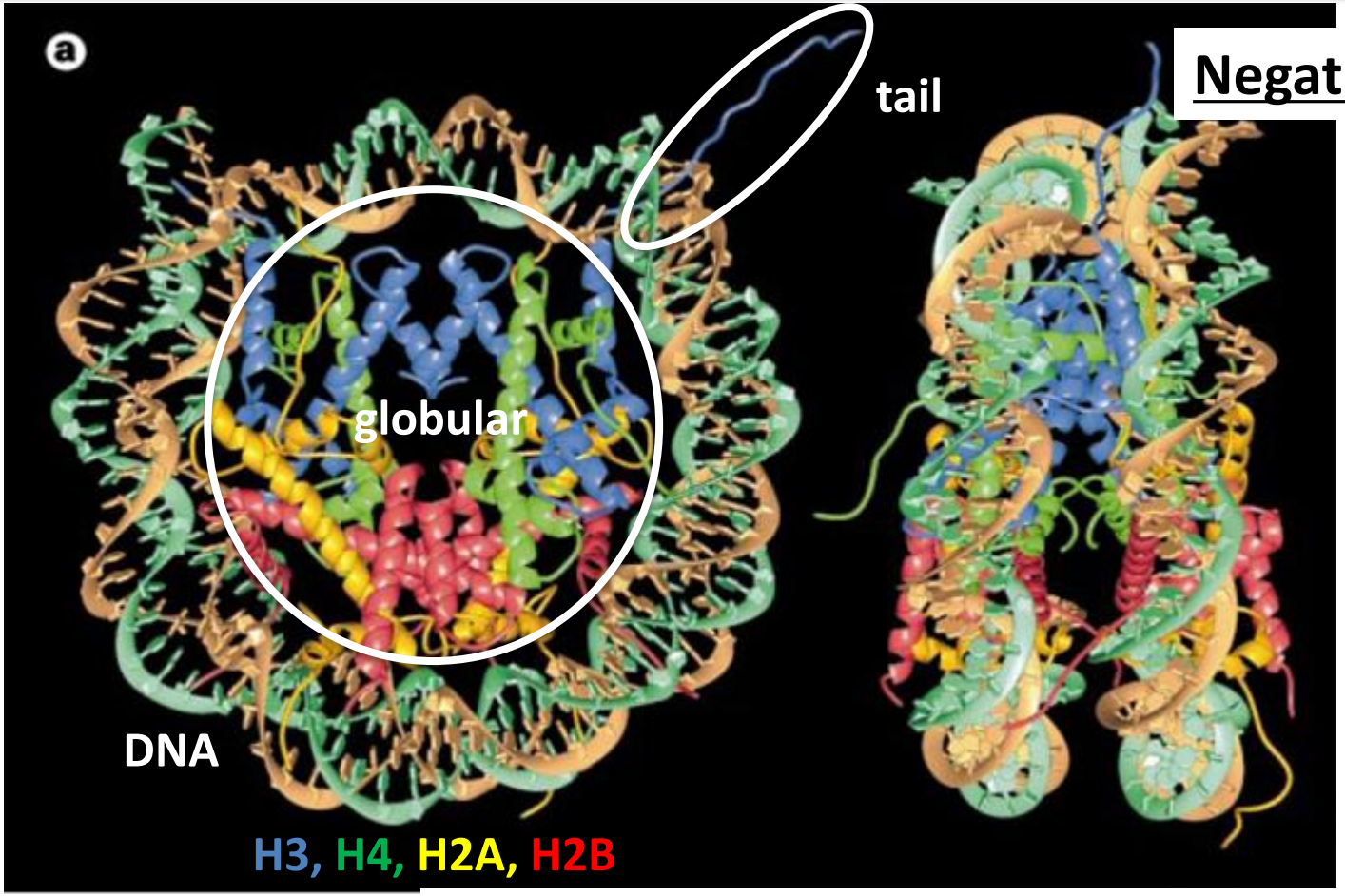
Table 1. Different Classes of Modifications Identified on Histones

Chromatin Modifications	Residues Modified	Functions Regulated
Acetylation	**K**-ac	Transcription, Repair, Replication, Condensation
Methylation (lysines)	**K**-me1 **K**-me2 **K**-me3	Transcription, Repair
Methylation (arginines)	**R**-me1 **R**-me2a **R**-me2s	Transcription
Phosphorylation	**S**-ph **T**-ph	Transcription, Repair, Condensation
Ubiquitylation	**K**-ub	Transcription, Repair
Sumoylation	**K**-su	Transcription
ADP ribosylation	**E**-ar	Transcription
Deimination	**R > Cit**	Transcription
Proline Isomerization	**P-cis > P-trans**	Transcription

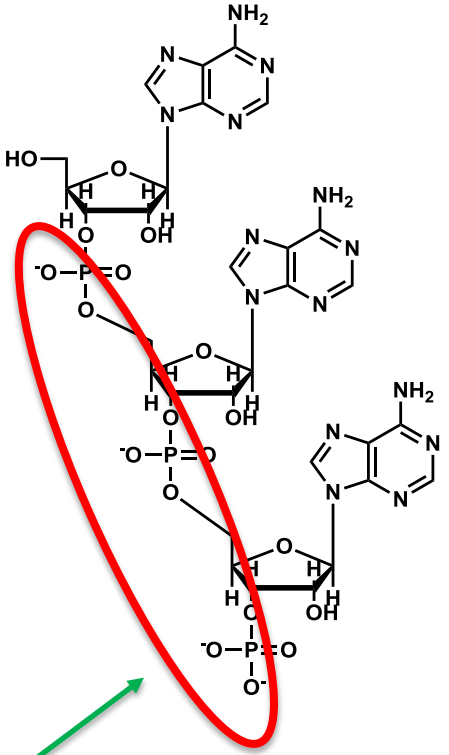
Kouzarides T, *Cell* (2007) 1**28**, 4, 693–705

Histone acetylation is reported to play vital roles in many aspects.

Interactions between Histone and DNA



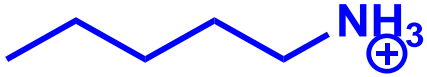
Negative charge of DNA



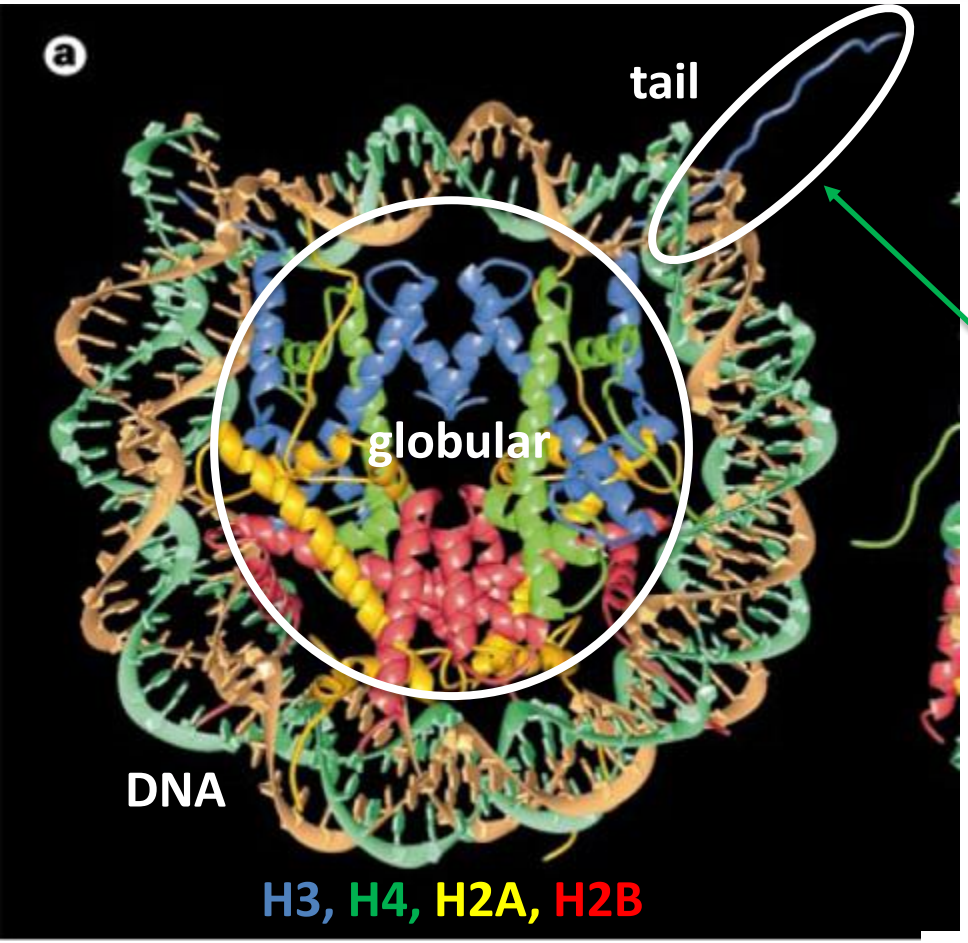
Histone rich in positive charge

Luger, K. *et al.* *Nature* **389,** 252 (1997)

interaction

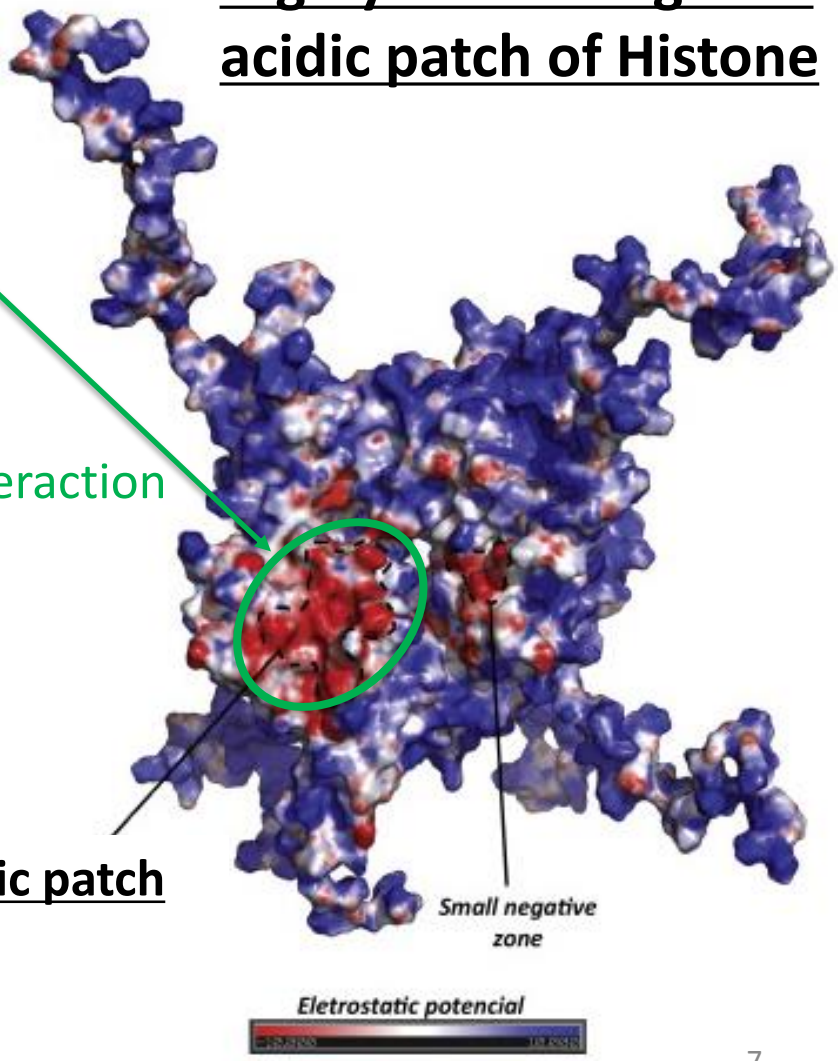


Interactions between Histone tail and acidic patch



interaction

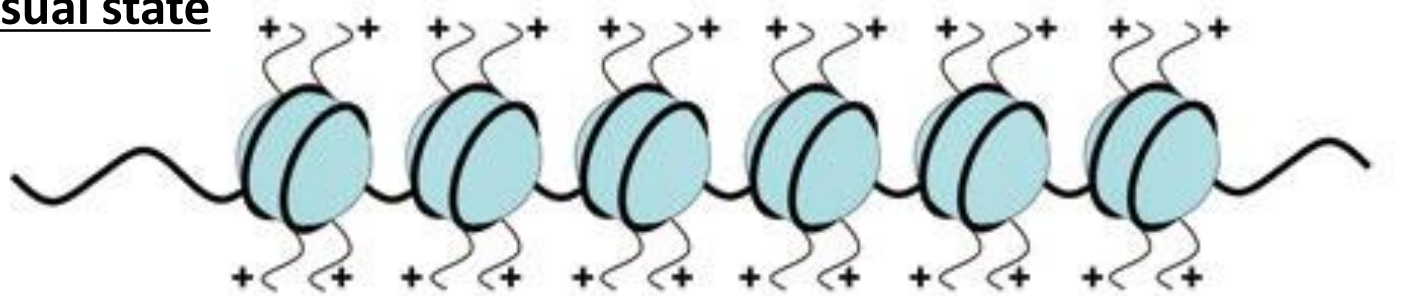
Highly electronegative acidic patch of Histone

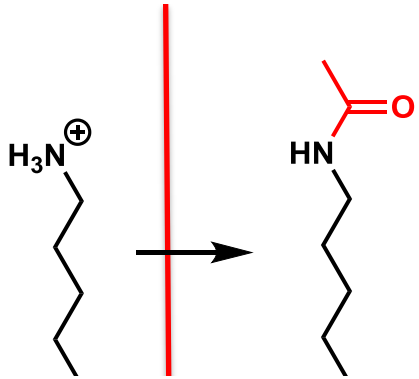


Santos, G M, *et al*. *Trends in Pharmacological Sciences,* 36, 5, 263–269, (2015)

Structural changes induced by Histone acetylation

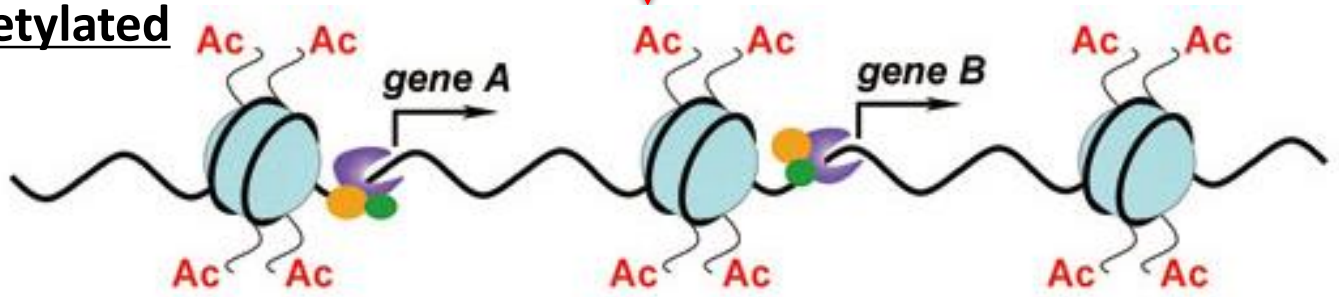
Usual state





Histone acetylation neutralizes the positive charge

acetylated

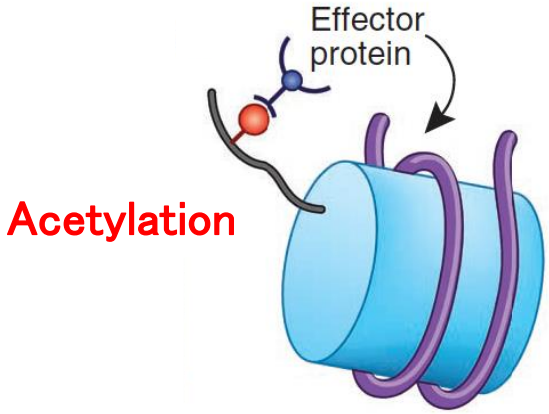


The function depends on acetylation site

Histone acetylated Lysine residues

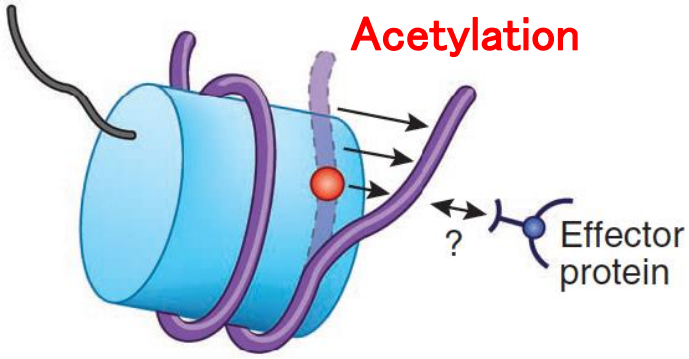
	Lysine	all
H2A	13	130
H2B	20	126
H3	13	136
H4	11	103

Acetylation at Histone tail



Work as a scaffold for effector protein

Acetylation at Histone globular



Directly induces structural change

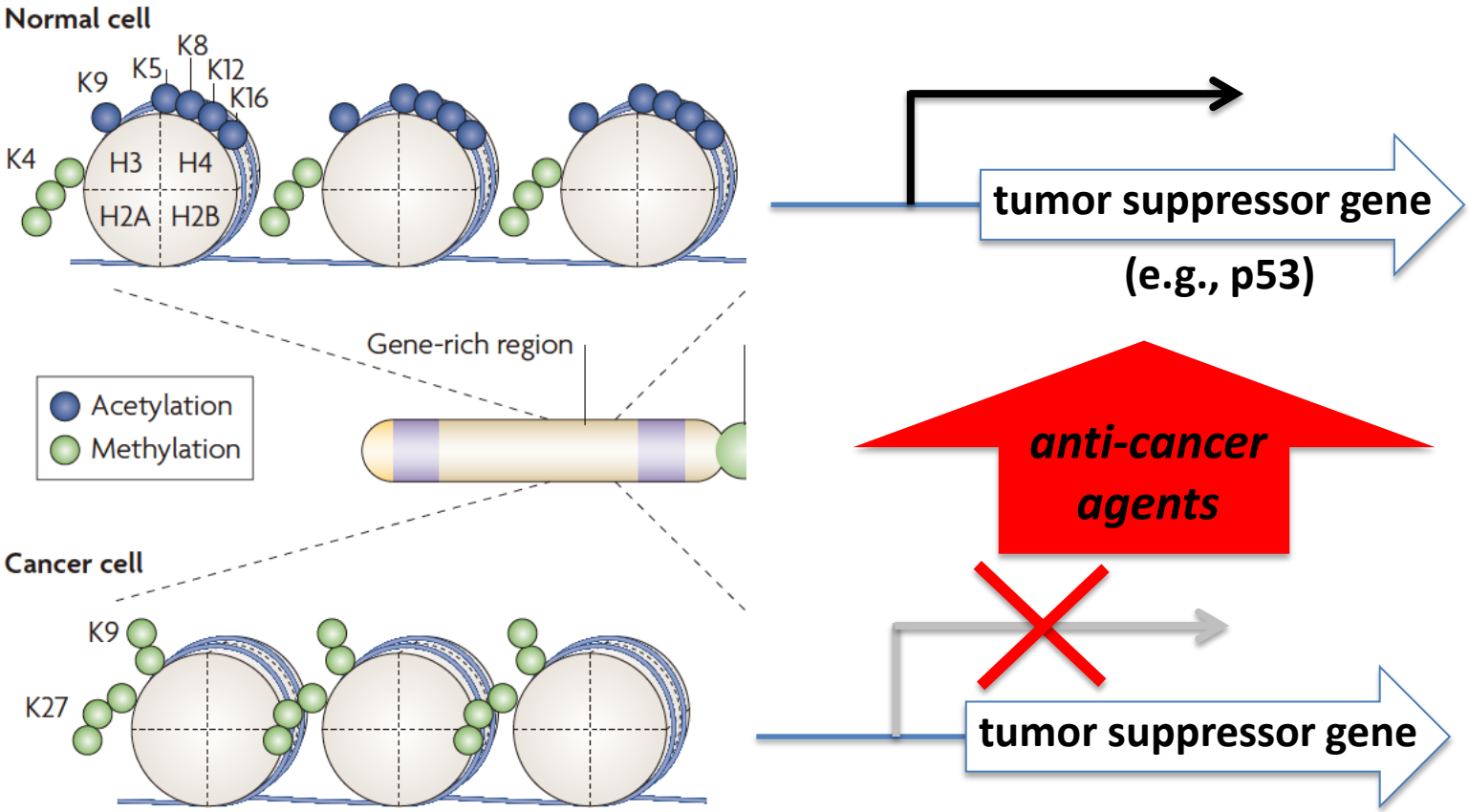
Nat. Struct. Mol. Biol. 2013, *20*, 657.

Contents

Catalysis medicine

In some cancer cells, tumor suppressor genes are suppressed with decreased Histone acetylation.

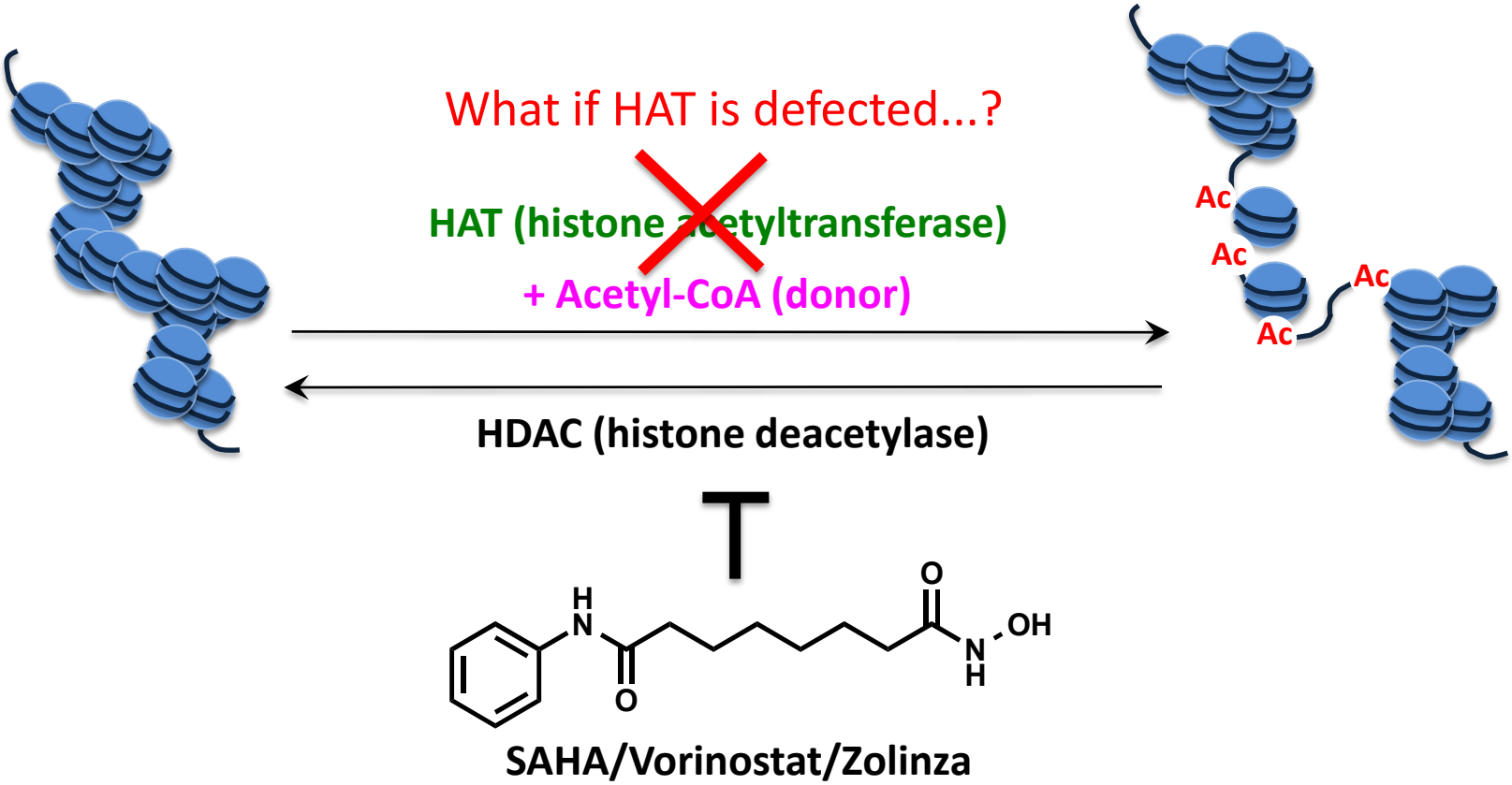
Inducing Histone acetylation can be a hopeful anti-cancer strategy.



Manel Esteller (2007)

Cataysis medicine

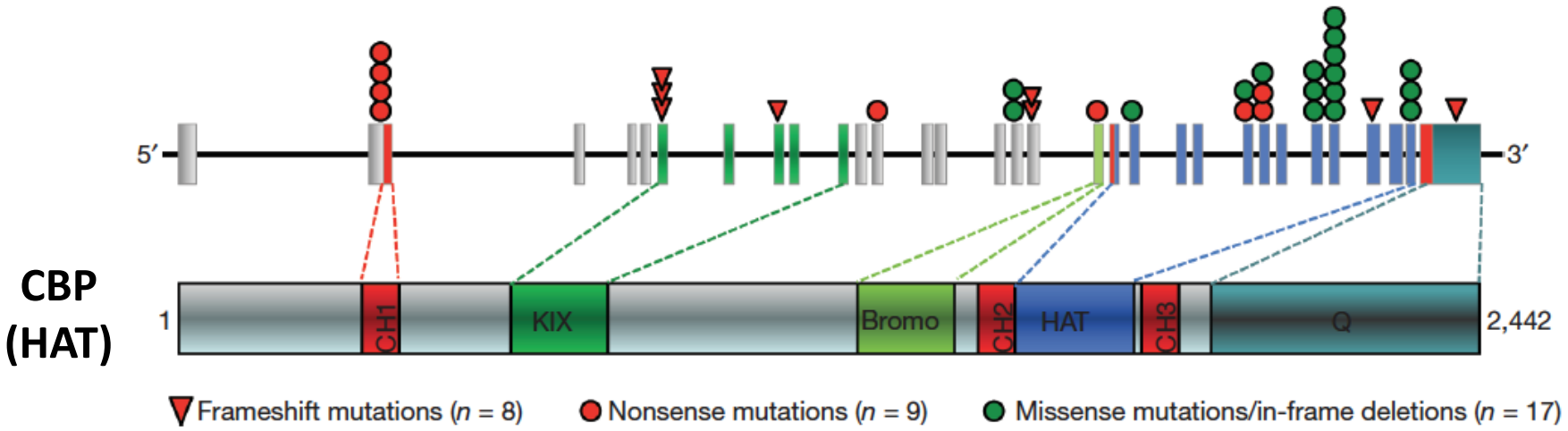
Histone acetylation level is regulated on the balance of two catalysts called HAT and HDAC.



HDAC inhibitor is actually used as an anti-cancer drug.

Cataysis medicine

Mutations of HAT are frequently found in B-cell lymphoma.

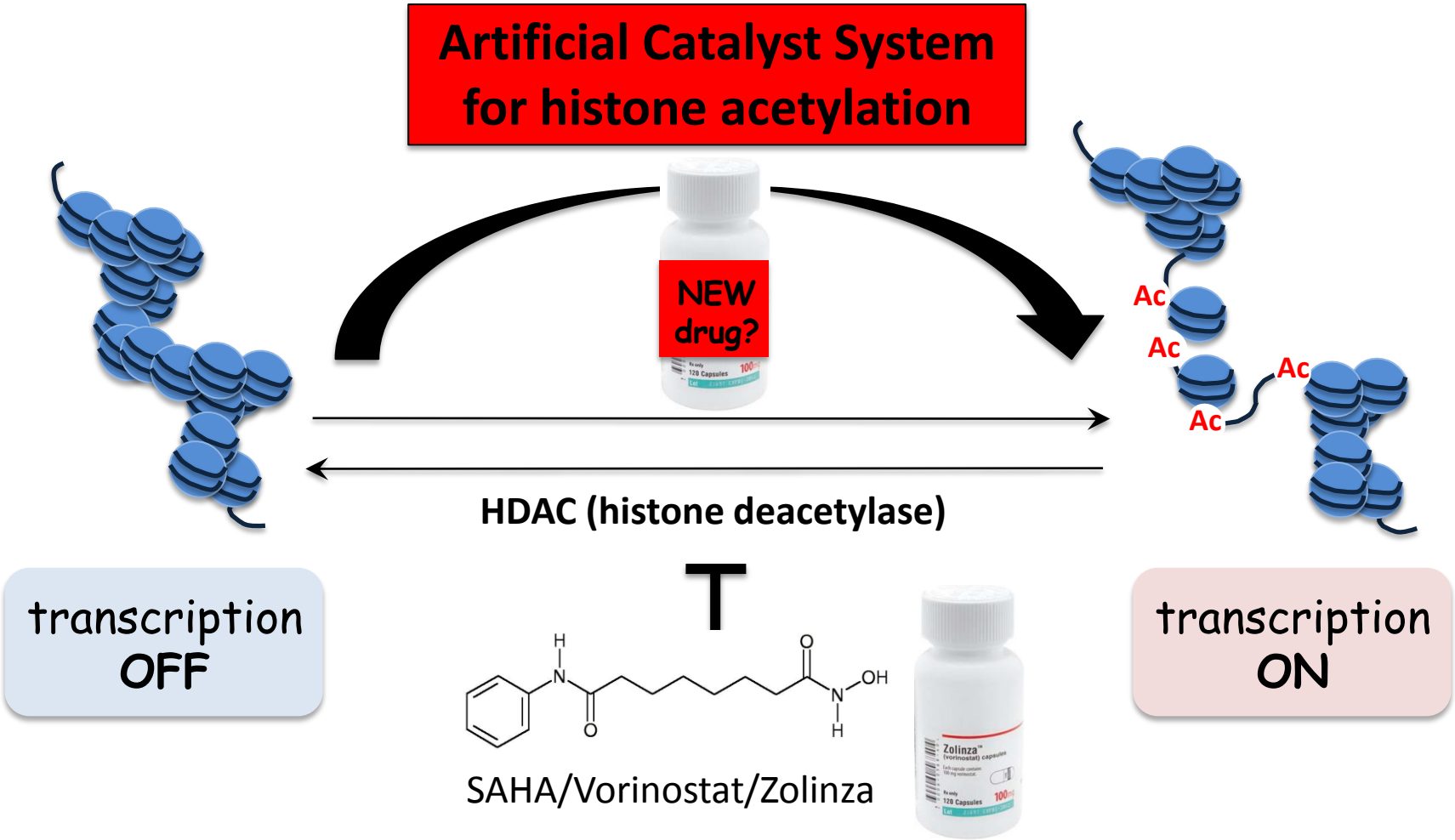


Inactivating mutations of acetyltransferase genes in B-cell lymphoma

Pasqualucci, L. *et al. Nature* 2011, *471*, 189.

HAT-independent Histone acetylation can be the solution?

HAT-independent histone acetylation by artificial catalyst system



Catalysis medicine

Requirements for the rational Histone acetylation catalyst.

1. Ability to transfer acetyl group to Lysine residues of proteins from a donor.
2. Substrate specificity (Histone↔other proteins)
3. Site specificity (ability to target residues in interest)
4. DNA sequence dependent targeting (expression of targeted genes)
5. (Specifically works in or be delivered to cancer cells)

Histone acetylation in cell is regulated to satisfy these requirements.

So, there may be something to learn from them.

Contents

HAT (Histone acetyltransferase)

Table 2. K-Acetyltransferases (KATs; Formerly Acetyltransferases)

New Name	Human	*D. melanogaster*	*S. cerevisiae*	*S. pombe*	Substrate Specificity	Function
KAT1	HAT1	CG2051	Hat1	Hat1/ Hag603	H4 (5, 12)	Histone deposition, DNA repair
KAT2		dGCN5/PCAF	Gcn5	Gcn5	H3 (9, 14, 18, 23, 36)/ H2B; yHtzl (14)	Transcription activation, DNA repair
KAT2A	hGCN5				H3 (9, 14, 18)/H2B	Transcription activation
KAT2B	PCAF				H3 (9, 14, 18)/H2B	Transcription activation
KAT3		dCBP/NEJ			H4 (5, 8); H3 (14, 18)	Transcription activation, DNA repair
KAT3A	CBP				H2A (5); H2B (12, 15)	Transcription activation
KAT3B	P300				H2A (5); H2B (12, 15)	Transcription activation
KAT4	TAF1	dTAF1	Taf1	Taf1	H3 > H4	Transcription activation
KAT5	TIP60/PLIP	dTIP60	Esa1	Mst1	H4 (5, 8, 12, 16); H2A (yeast 4, 7; chicken 5, 9, 13, 15); dH2Av/yHtzl (14)	Transcription activation, DNA repair
KAT6		(CG1894)	Sas3	(Mst2)	H3 (14, 23)	Transcription activation and elongation, DNA replication
KAT6A	MOZ/MYST3	ENOK			H3 (14)	Transcription activation
KAT6B	MORF/MYST4				H3 (14)	Transcription activation
KAT7	HBO1/MYST2	CHM		(Mst2)	H4 (5, 8, 12) > H3	Transcription, DNA replication
KAT8	HMOF/MYST1	dMOF (CG1894)	Sas2	(Mst2)	H4 (16)	Chromatin boundaries, dosage compensation, DNA repair
KAT9	ELP3	dELP3/ CG15433	Elp3	Elp3	H3	
KAT10			Hap2		H3 (14); H4	
KAT11			Rtt109		H3 (56)	Genome stability, transcription elongation
KAT12	TFIIIC90				H3 (9, 14, 18)	Pol III transcription
KAT13A	SRC1				H3/H4	Transcription activation
KAT13B	ACTR				H3/H4	Transcription activation
KAT13C	P160				H3/H4	Transcription activation
KAT13D	CLOCK				H3/H4	Transcription activation

family

GNAT

p300/CBP

MYST

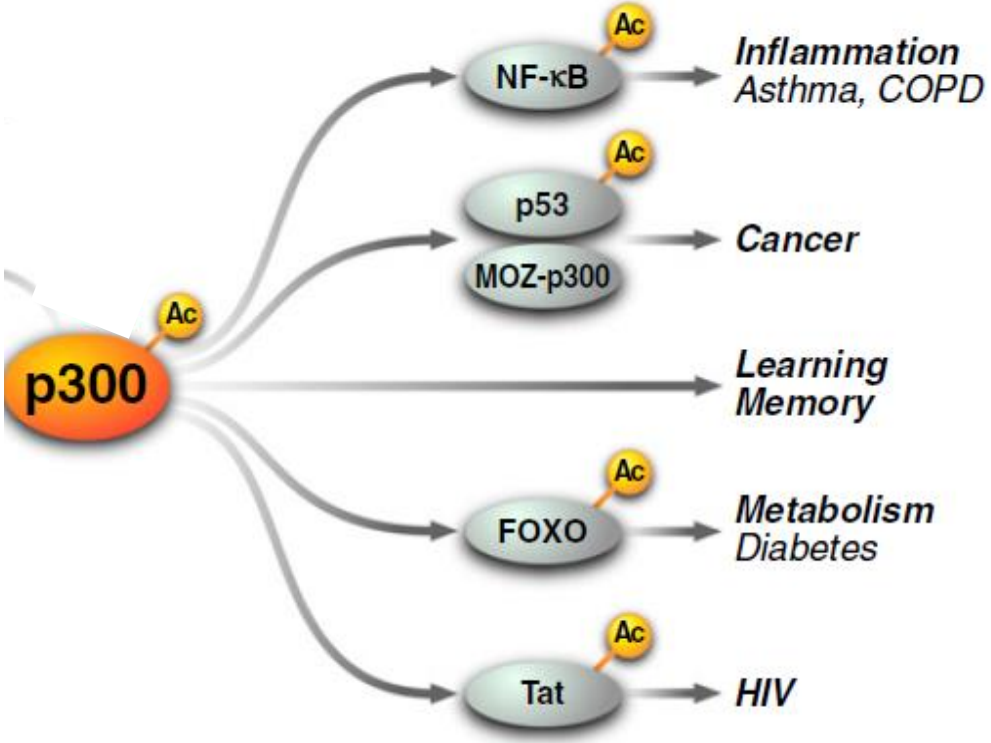
GNAT

Rtt109

C. D. Allis, *et al. Cell 2007 131, 633*

HAT (Histone acetyltransferase)

Some HAT proteins acetylate non-Histone substrates.

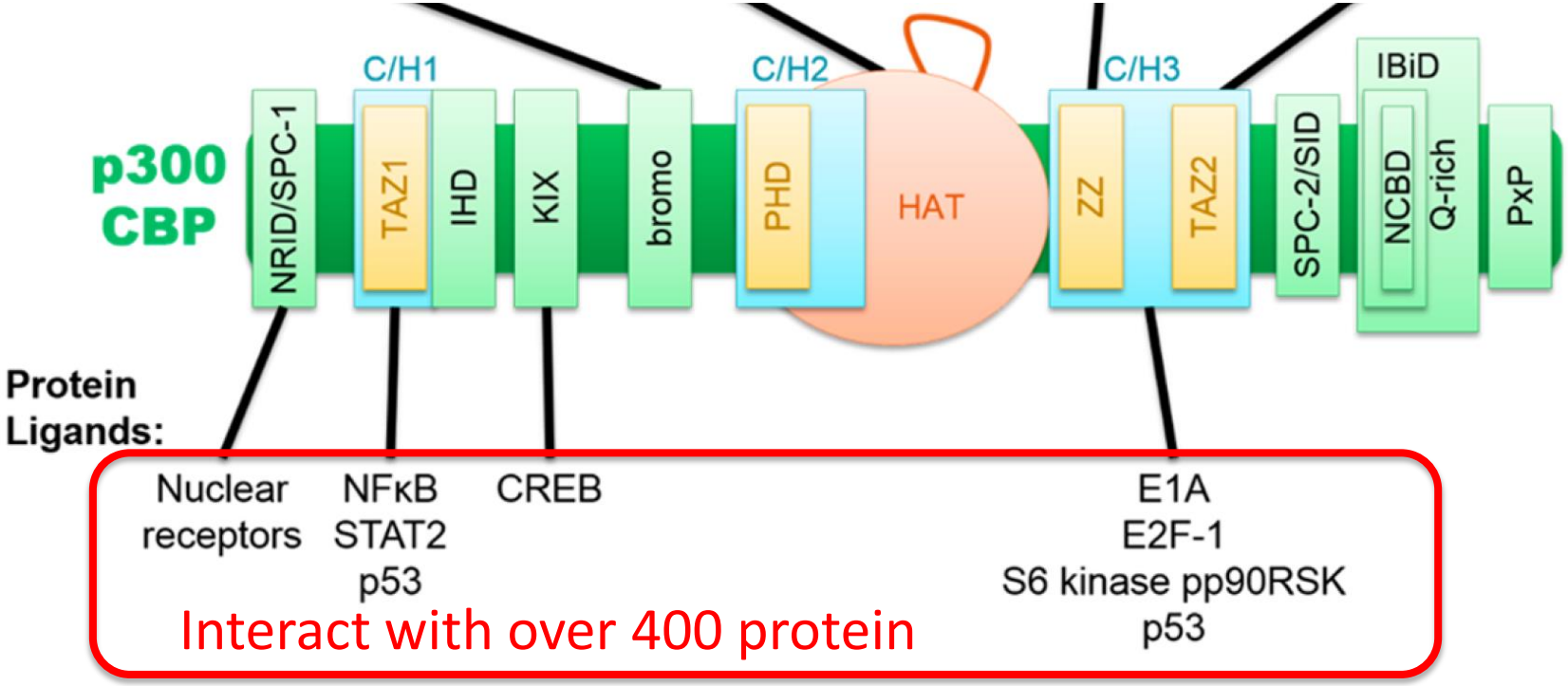


p300 acetylates 70 other proteins than Histone proteins
→HAT employs rigorous regulations as to what substrate to acetylate.

E. Verdin, *et al*. *Chemistry & Biology*, *17, 5, 471-482* (2005)

HAT (Histone acetyltransferase)

HAT proteins have various domains to interact with other proteins and DNA modifications.

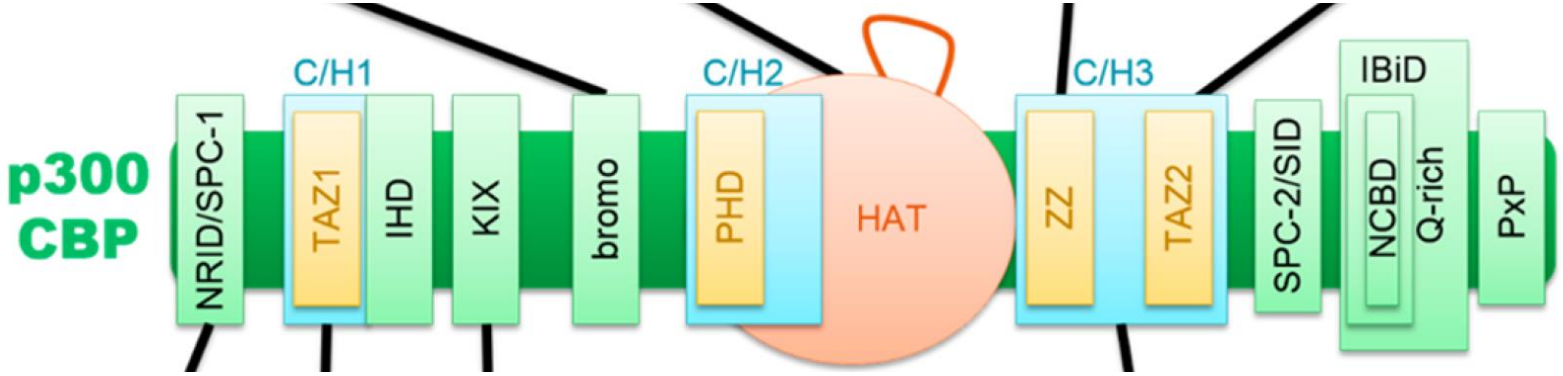


HAT domain : catalysis

Other domains : substrate recognition, scaffold and so on.

Cole P A, *et al. Chem. Rev.*, **2015**, *115* (6), pp 2419–2452

Catalysis medicine



Requirements for the rational Histone acetylation catalyst.

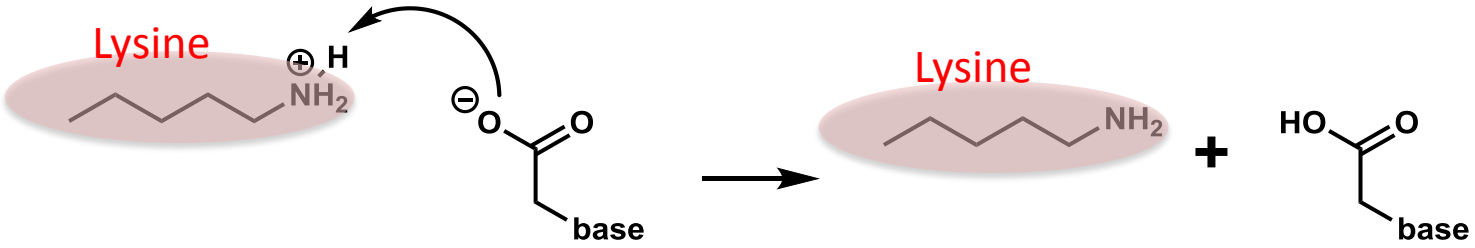
1. Ability to transfer acetyl group to Lysine residues of proteins from a donor. (HAT domain)
2. Substrate specificity (Histone↔other proteins) (co-factor)
3. Site specificity (ability to target residues in interest) (HAT domain)
4. DNA sequence dependent targeting (expression of targeted genes) (co-factor)
5. (Specifically works in or be delivered to cancer cells)

Contents

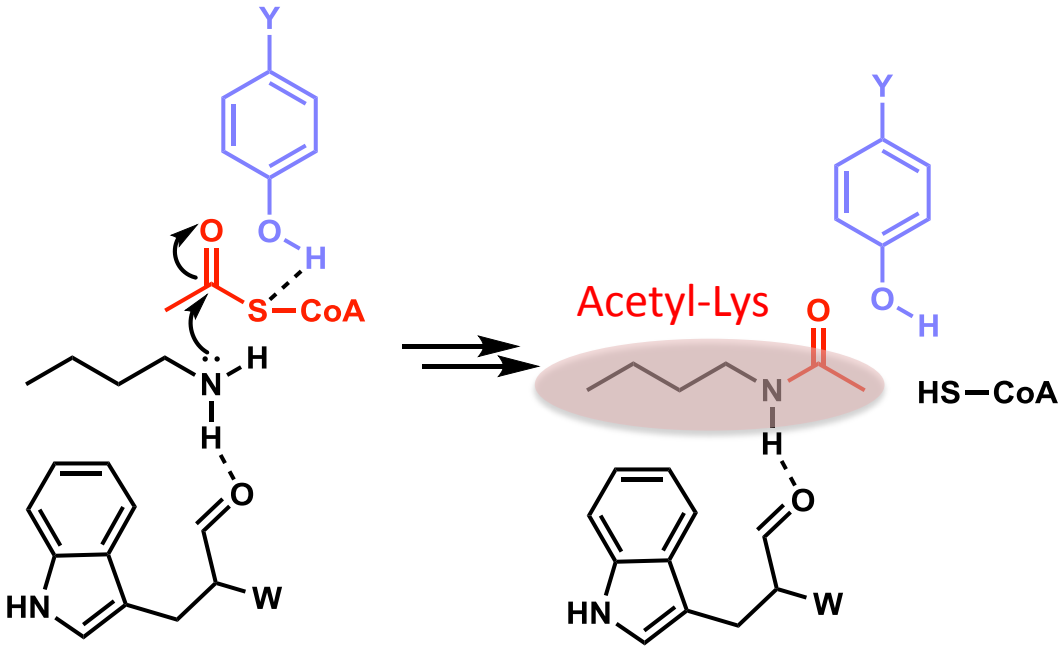
Reaction mechanisms proposed for Histone acetylation 1

Proposed for all the HAT subfamilies

1. The deprotonation of ε-amino group of the lysine substrate

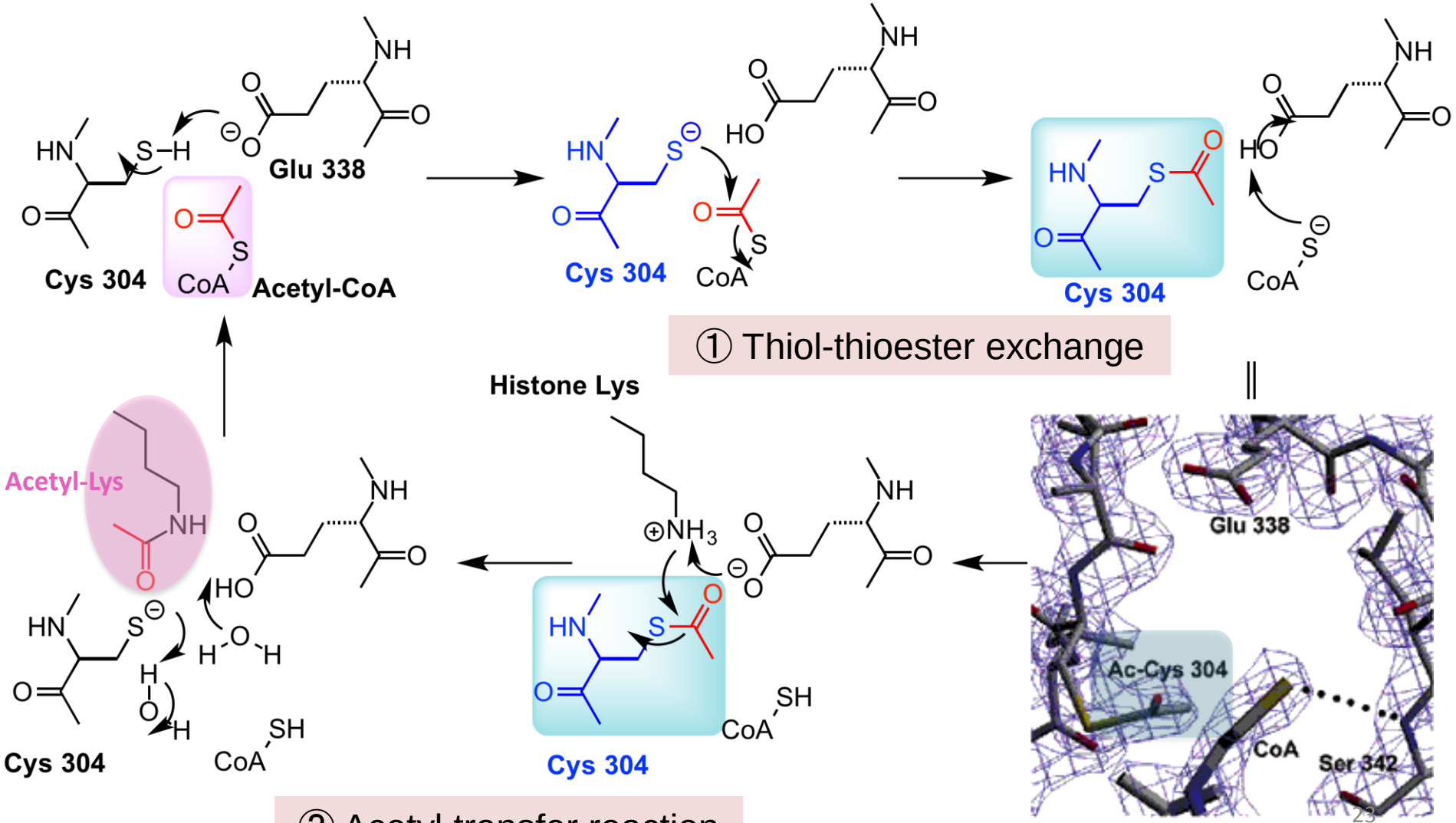


2. Activation of Ac-CoA



Reaction mechanisms proposed for Histone acetylation 2

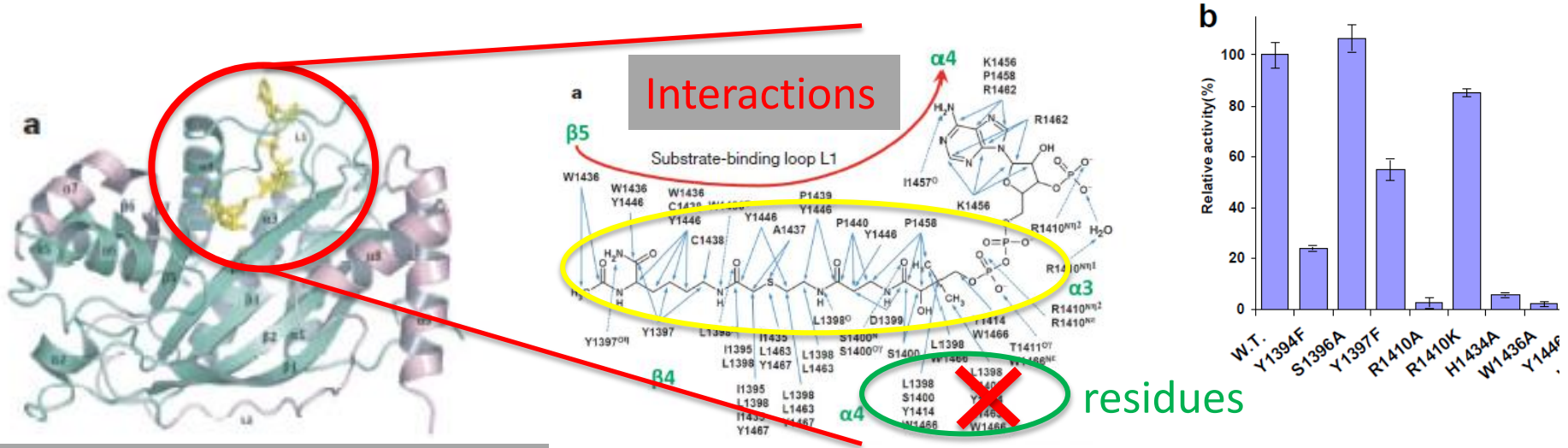
Proposed for Esa1 of MYST family (denied? afterwards)



Marmorstein, R. *et al. Nat. Struct. Biol.* **2002**, *9*, 862.

Investigation on catalysis mechanisms

1. Obtain crystal structure of the catalyst and its substrate
2. Observe possible interactions between the catalyst and its substrate
3. Mutational analysis to investigate the importance of a residue
4. Kinetics analysis (especially for bi-substrate catalyst)



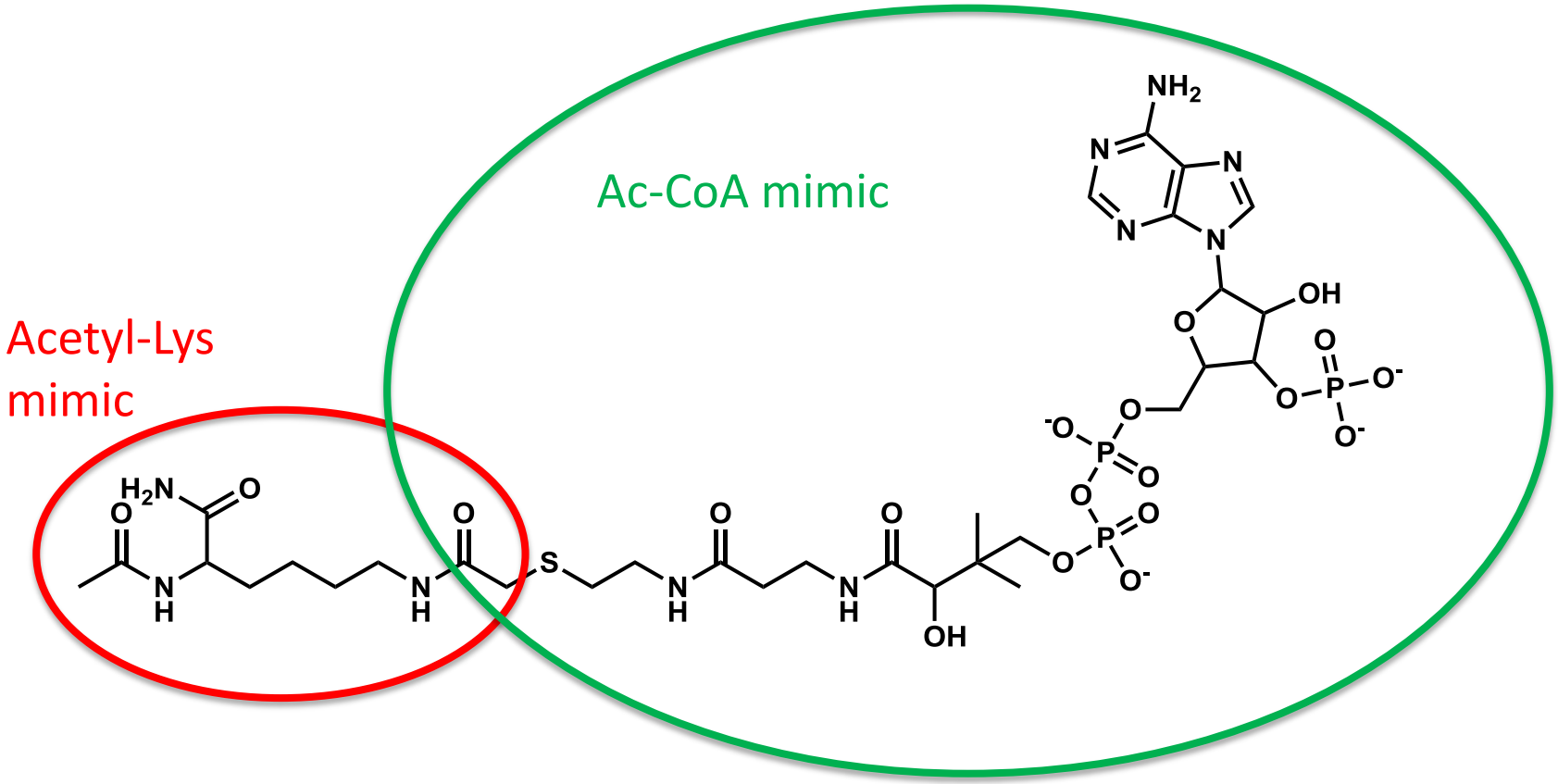
Co-Crystal with substrate

Mutation→activity?

Kinetics

Lys-CoA : bisubstrate analogue inhibitor

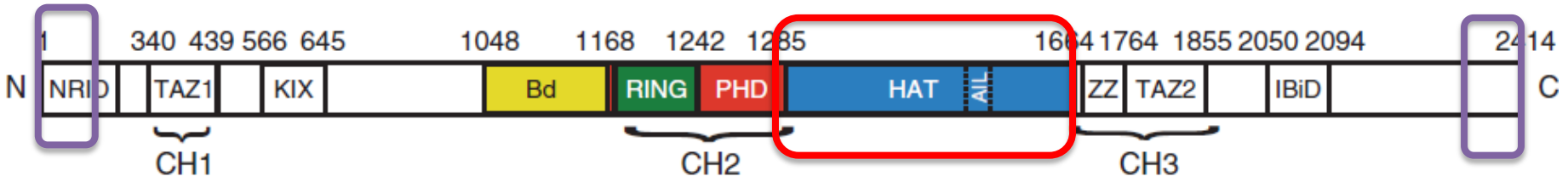
Lys-CoA : The 1st and most potent bisubstrate p300 inhibitor



Co-crystal of p300 HAT domain and Lys-CoA

HAT consists of several domains

D Panne, *et al. Nature Structural & Molecula Biology* **20**, 1040–1046 (2013)

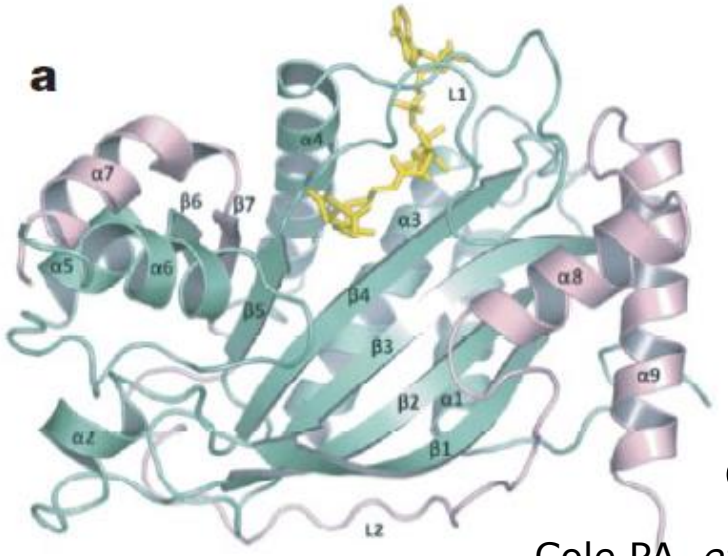


N-subdomain(28kDa)

Catalytic core

C-subdomain (10kDa)

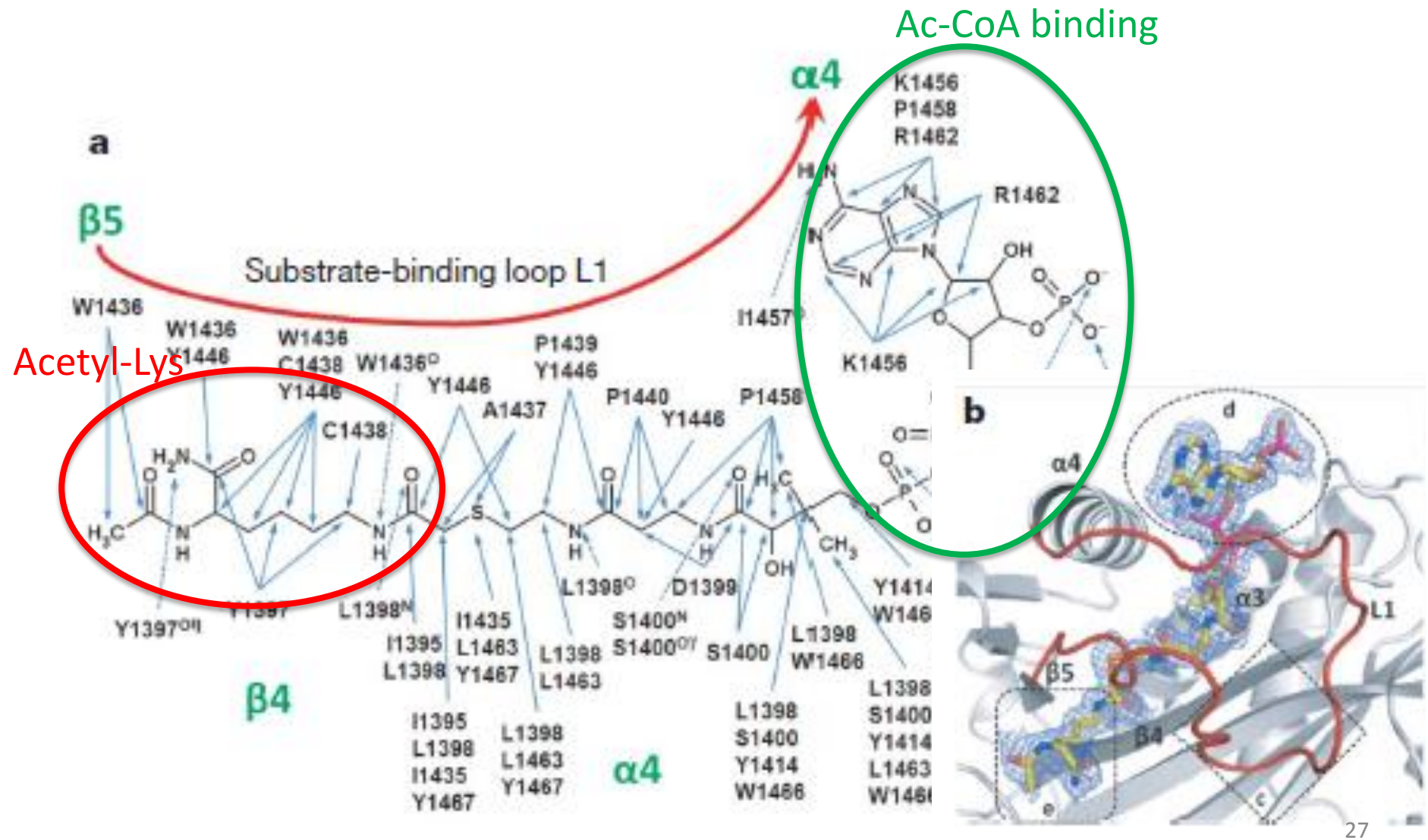
Co-crystal of p300 HAT domain with Lys-CoA



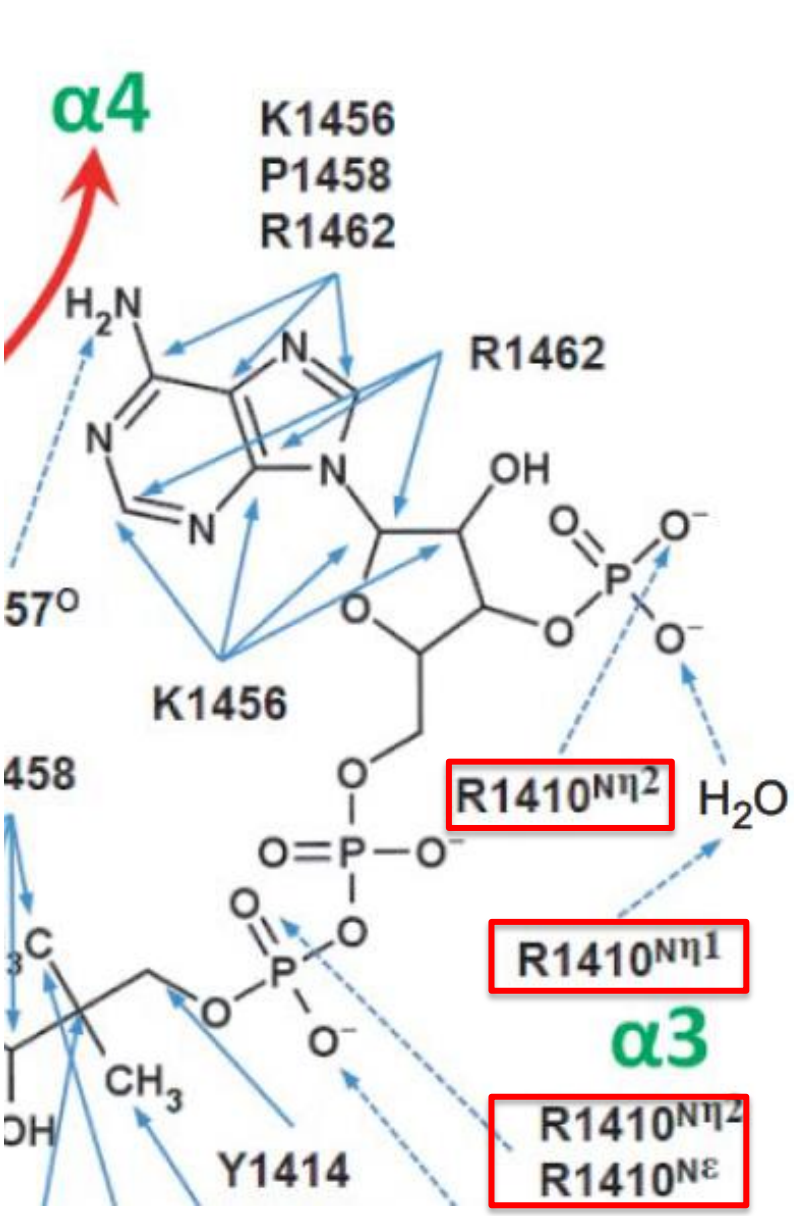
Lys-CoA
HAT domain
N-, C-subdomain (inactive, but make crystallization easy)

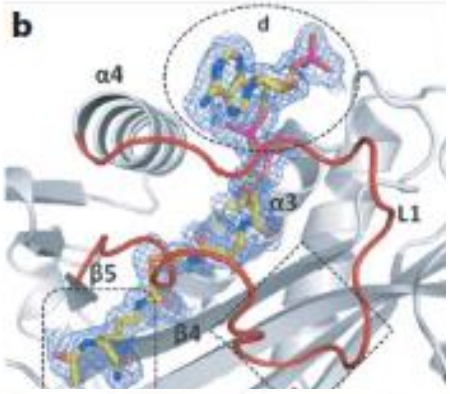
Cole PA, *et al. Nature* **451**, 846-850 (2008)

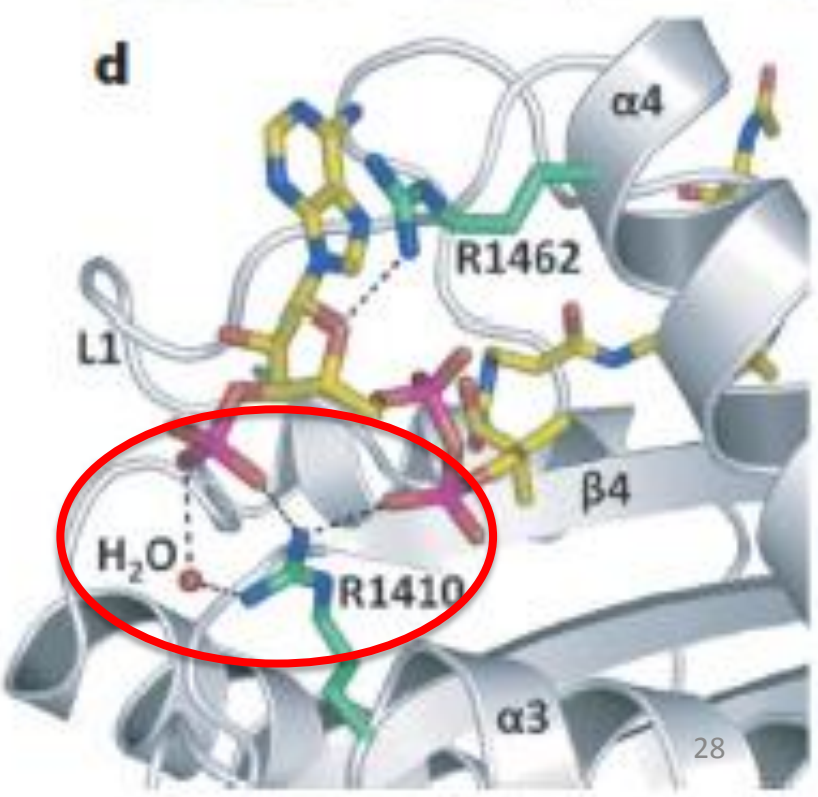
Interactions between Lys-CoA and Lys-CoA



R1410 : intensive interaction with phosphate moiety





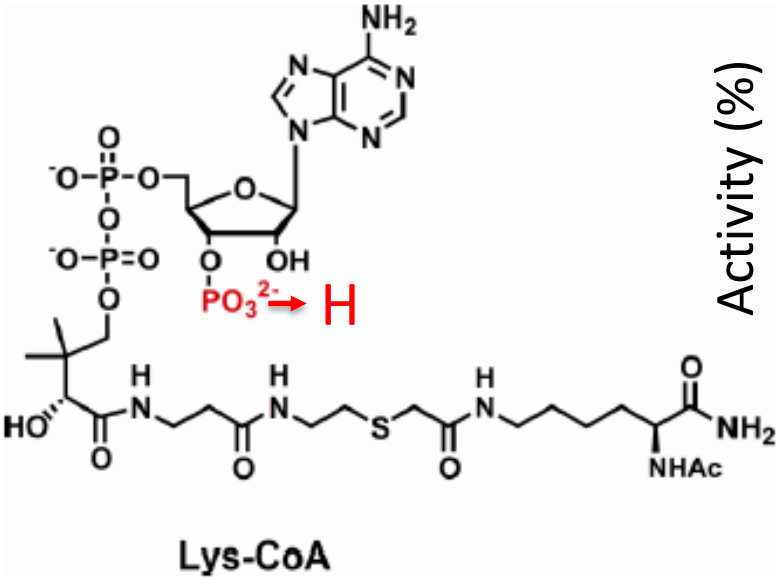


Confirmation of the importance of R1410

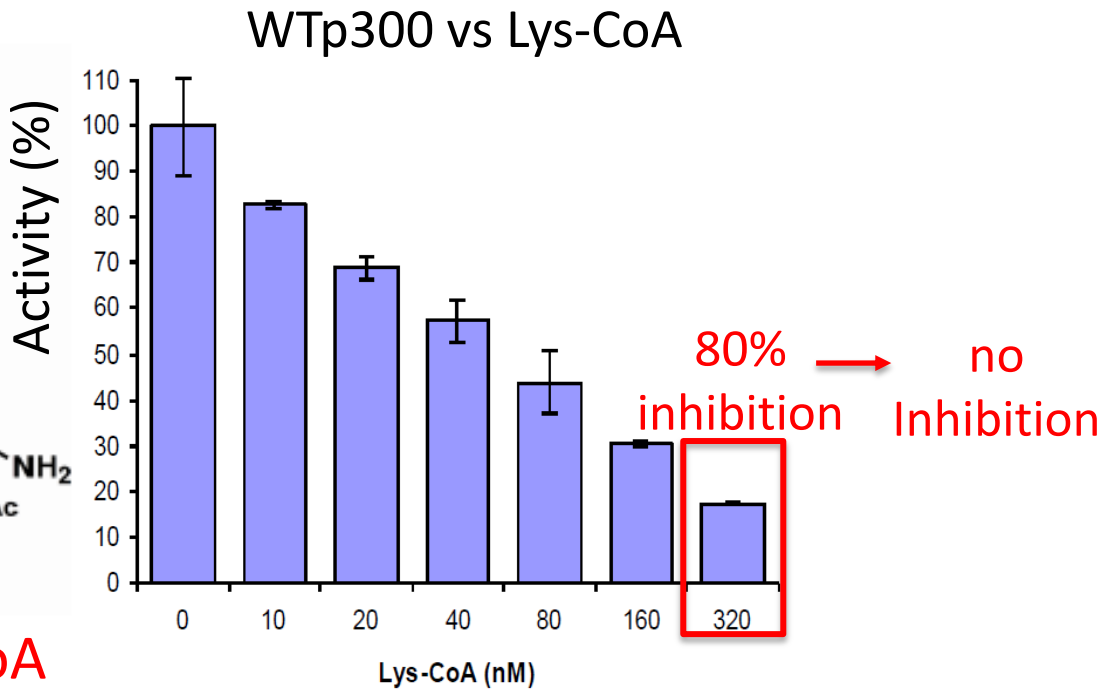
R1410A mutation shows reduced affinity for Ac-CoA

Enzyme	K_m(μM) for H4-15	K_m(μM) for AcCoA	k_{cat}(s^{-1})	V/K($M^{-1}s^{-1}$)†	Structural basis for the mutant residue
W.T.	164±10	40±6	4.1±0.1	25,000±1643	
R1410A	190±40	657±90	1.2±0.1	6263±1300	H-bond with 3' and pantetheine phosphate
R1410K	156±40	74±6	1.4±0.1	8718±2200	

Hydrogen bond between R1410 and 3'-phosphate is essential for bindin

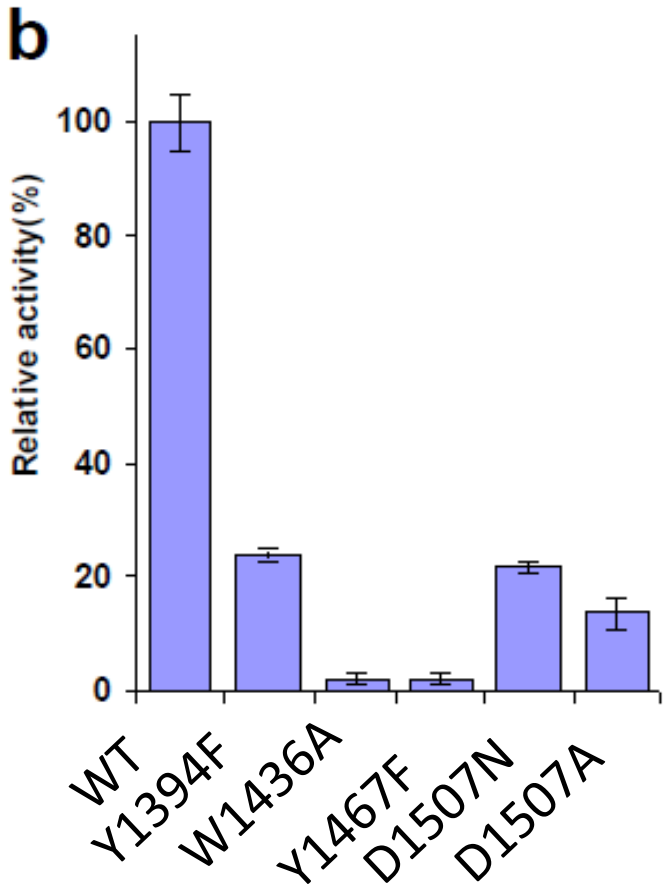


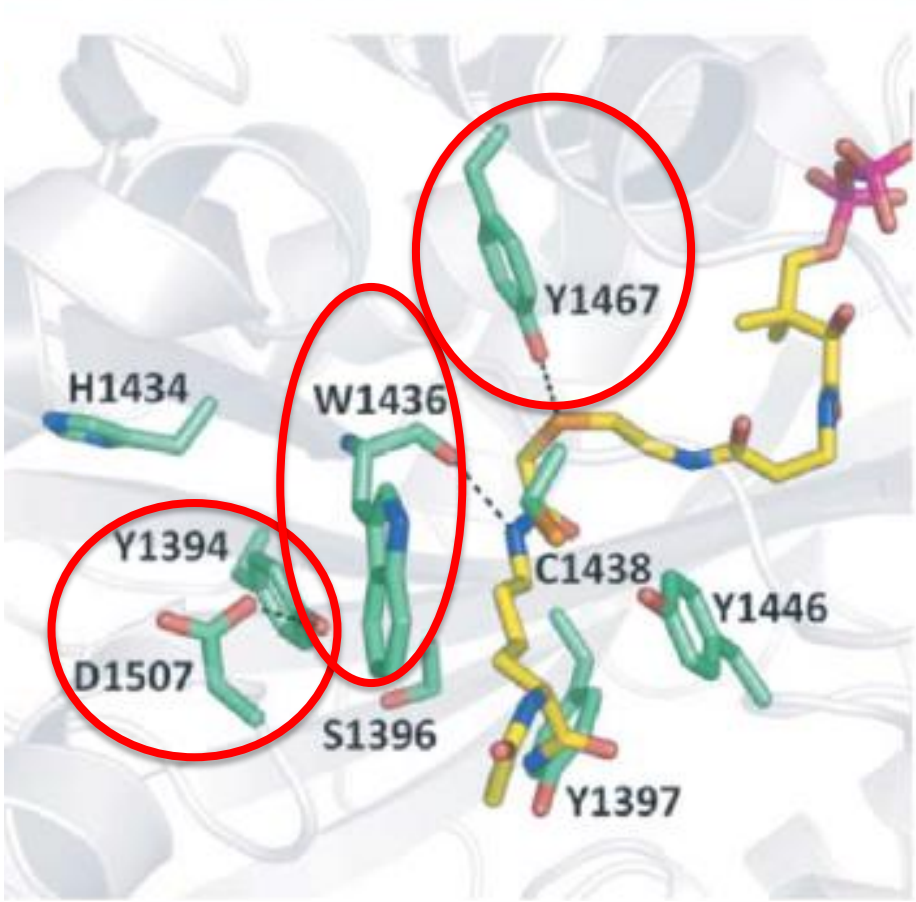
→ 3'-dephospho-Lys-CoA



Important residues interacting with Lysine moiety

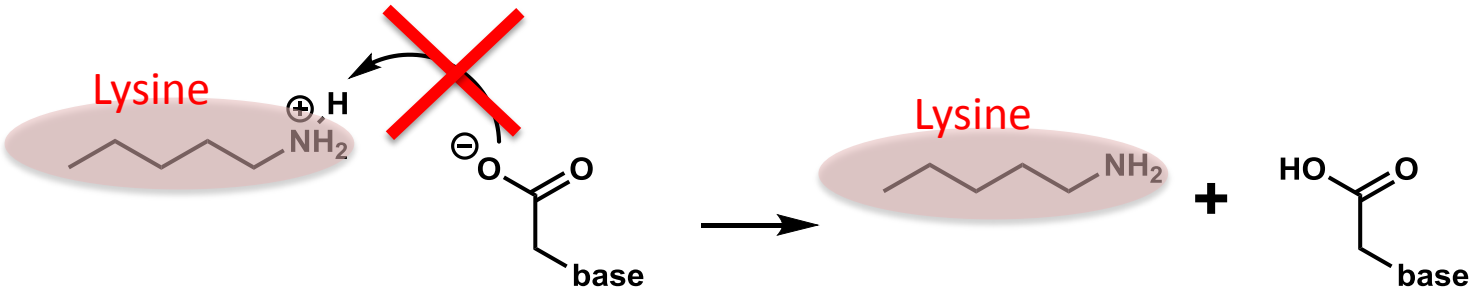
Residues essential for catalysis shown by mutational analysis





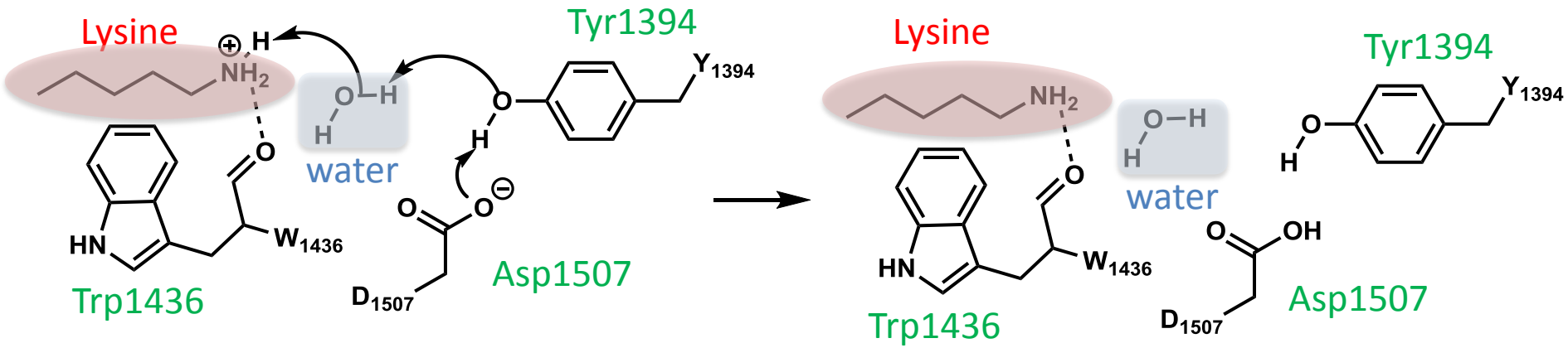
Detailed proton transfer mechanism

No general base in enough proximity for proton transfer.



Proposed mechanisms for proton transfer.

Chen L *et al. J. Phys. Chem. B*, **2014**, *118* (8), pp 2009–2019



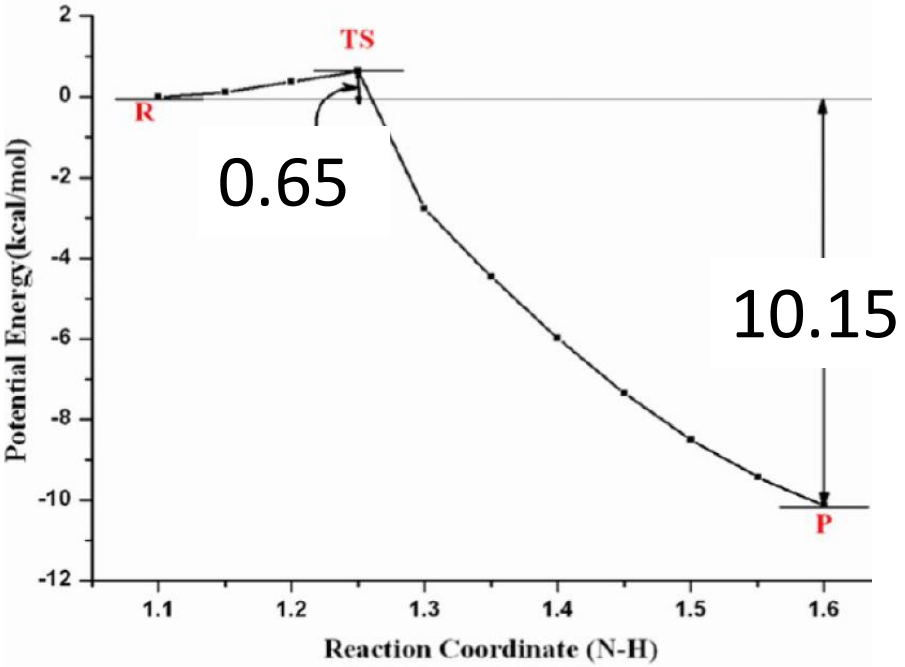
Calculations supporting the mechanism

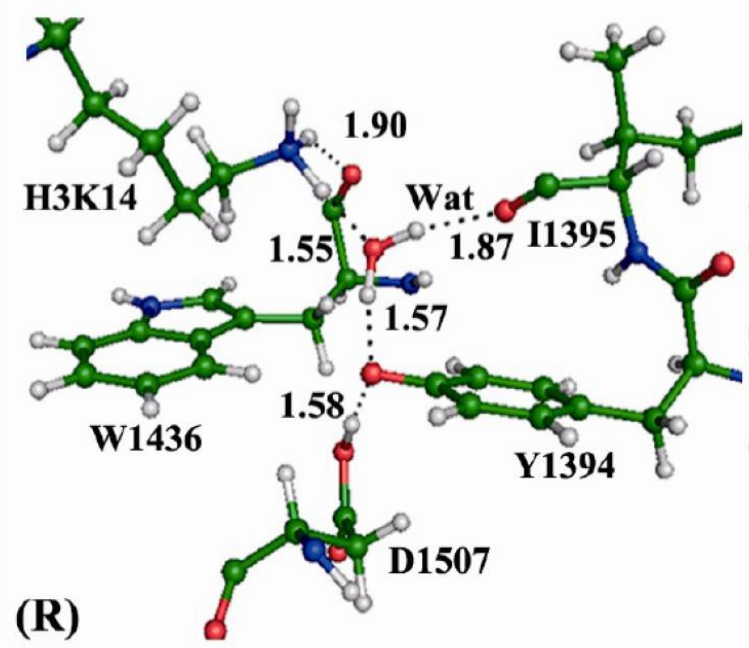
QM/MM calculations : simulation for large molecule

- QM/MM methods are the combination of QM(quantum mechanics) and MM (molecular mechanics)
- QM : accurate but high computational complexity (applied only to catalytic core)
- MM : low content but low computational complexity (applied to all the molecules)

proposed mechanisms for proton transfer was energetically rational

Chen L *et al. J. Phys. Chem. B*, **2014**, *118* (8), pp 2009–2019

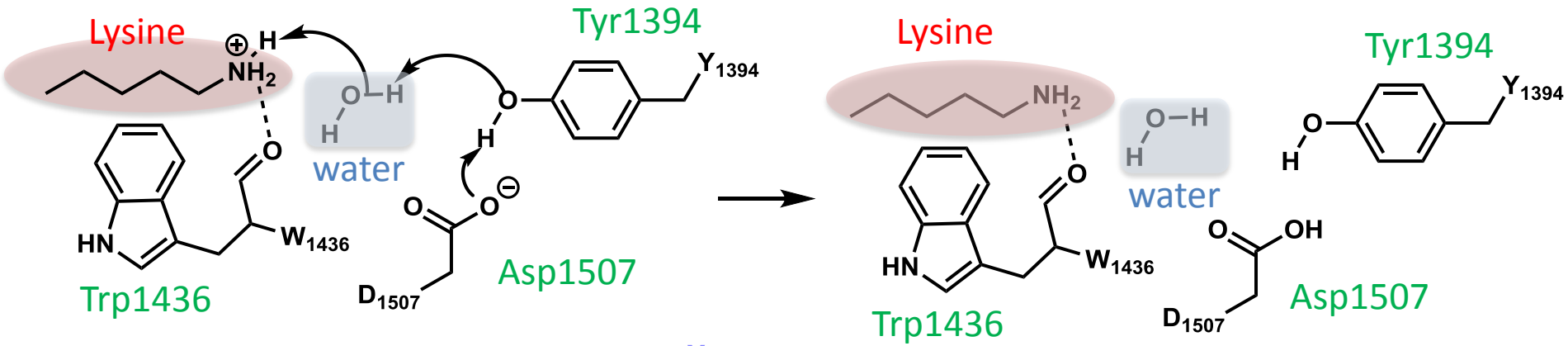




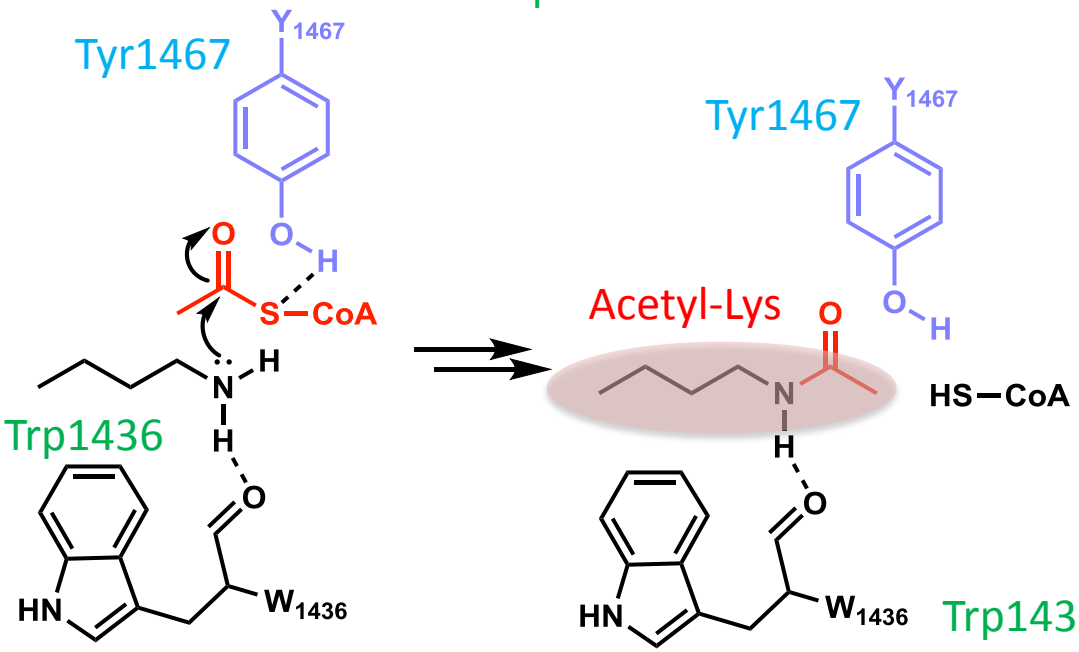
catalytic mechanisms proposed for p300

Proposed for all the HAT subfamilies

1. The deprotonation of ε-amino group of the lysine substrate

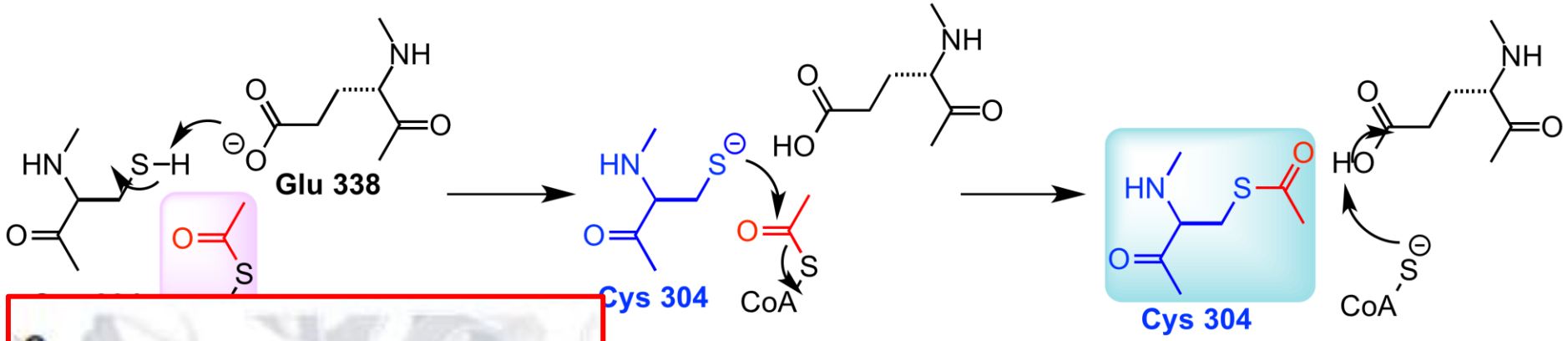


2. Activation of Ac-CoA

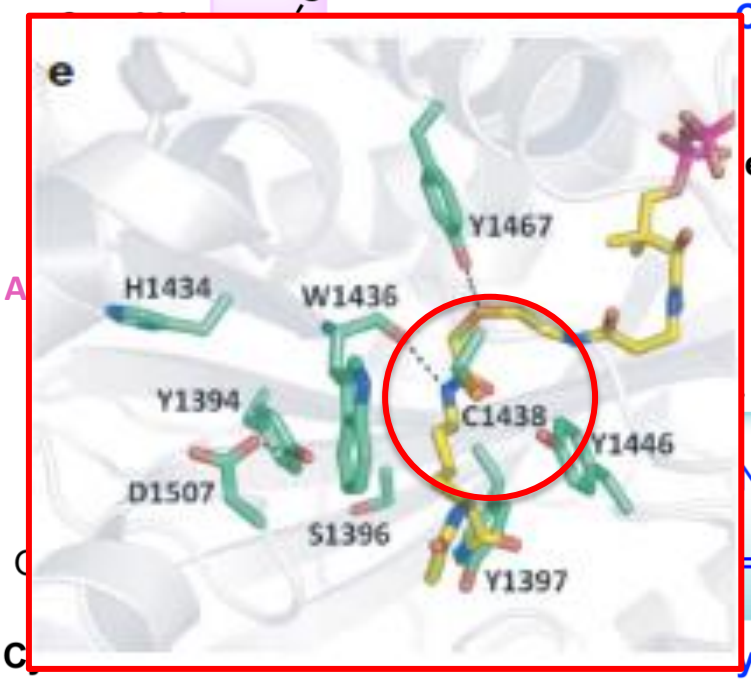


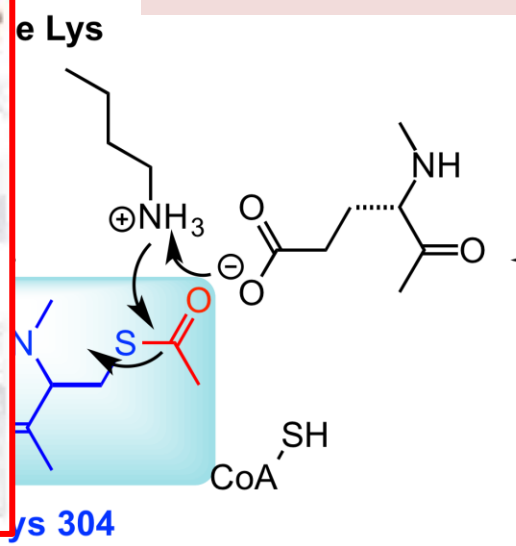
Possibility of other mechanism?

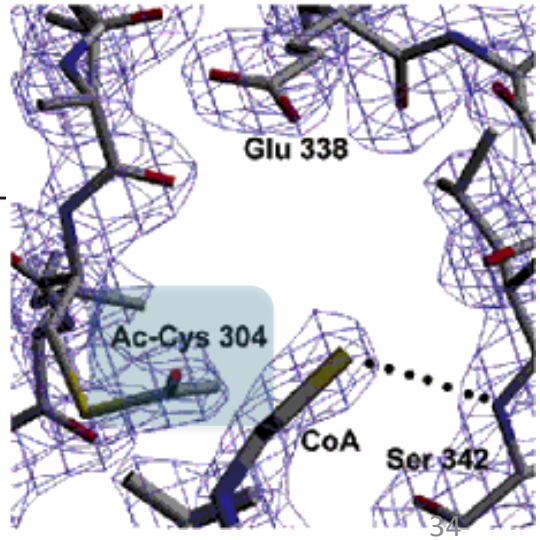
Why not this mechanisms?



① Thiol-thioester exchange







② Acetyl transfer reaction

Marmorstein, R. *et al. Nat. Struct. Biol.* **2002**, *9*, 862.

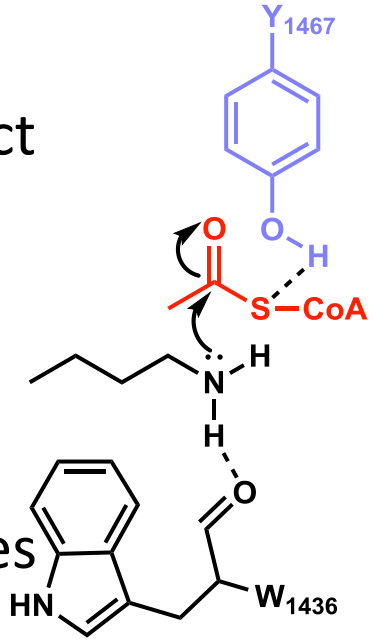
Enzyme kinetics classification

Classifications of kinetics for bisubstrate catalysts

Sequential mechanisms

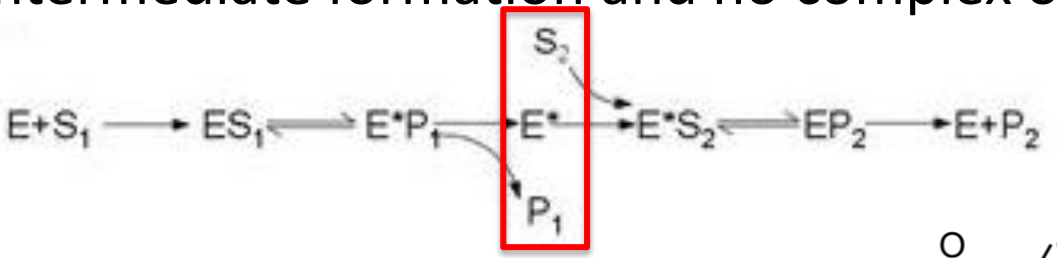
Complex formation of all the substrates before the product

E : HAT, S_1 : Ac-CoA, S_2 : Lysine, P1 : CoA-SH, P2 : Ac-Lys

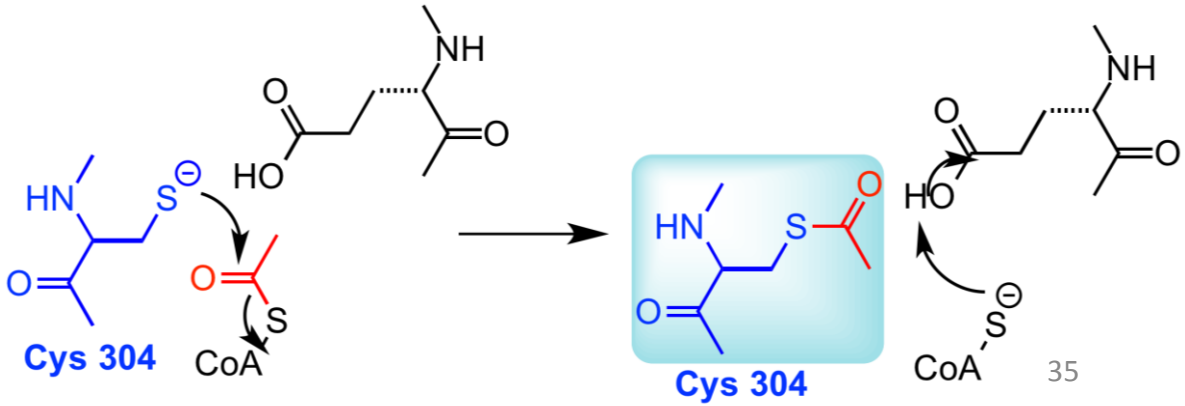


Ping-pong mechanism

Intermediate formation and no complex of all the substrates



E : HAT, S_1 : Ac-CoA, S_2 : Lysine, P1 : CoA-SH, P2 : Ac-Lys, E* : Ac-HAT

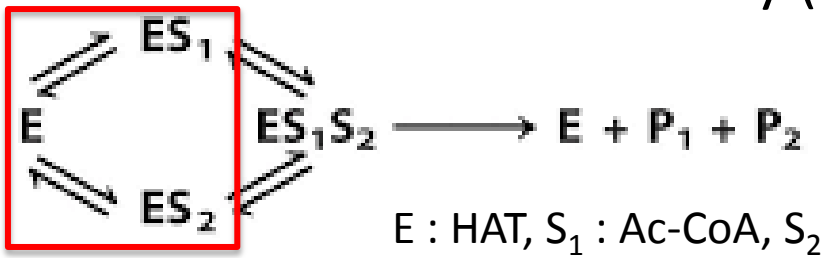


Detailed classification of sequential mechanisms

Sequential mechanism is classified into three detailed ones

Random sequential mechanisms

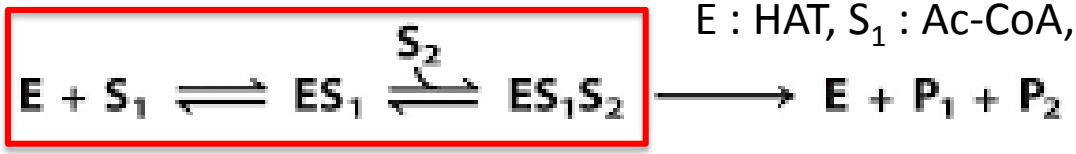
S1 and S2 bind to E randomly (no order)



E : HAT, S_1 : Ac-CoA, S_2 : Lysine, P1 : CoA-SH, P2 : Ac-Lys

Ordered sequential mechanisms

S1 binding to E is necessary before S2 binds to E

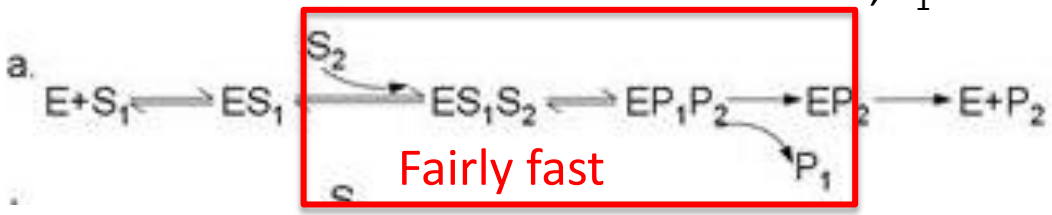


E : HAT, S_1 : Ac-CoA, S_2 : Lysine, P1 : CoA-SH, P2 : Ac-Lys

Theorell-Chance (hit-and-run) mechanisms

Ordered but no accumulation of ternary complex

E : HAT, S_1 : Ac-CoA, S_2 : Lysine, P1 : CoA-SH, P2 : Ac-Lys



Fundamentals for enzymology

Kinetics analysis of bisubstrate enzymes

At a fixed concentration of one substrate, the kinetics fits Michaelis-Menten.

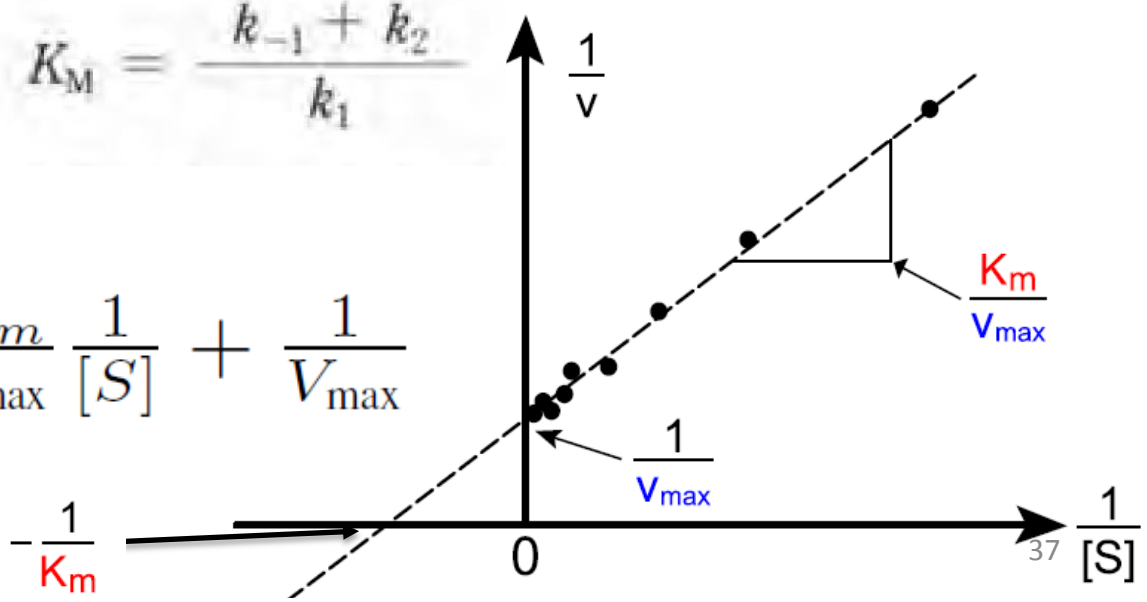
Michaelis-Menten equation and Lineweaver-Burk plot

The reaction $E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$ can be expressed as

$$V = \frac{V_{max}[S]}{K_m + [S]} \quad \text{with} \quad K_M = \frac{k_{-1} + k_2}{k_1}$$

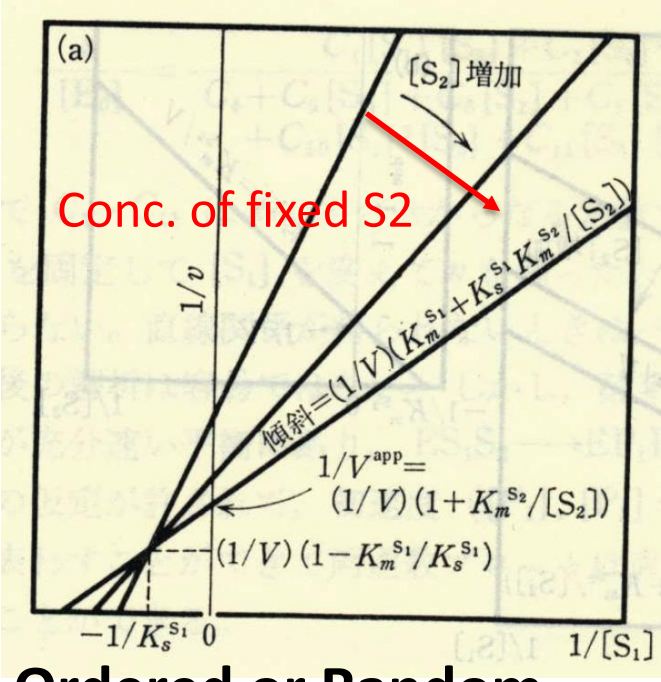
This equals

$$\frac{1}{V} = \frac{K_m + [S]}{V_{max}[S]} = \frac{K_m}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}}$$



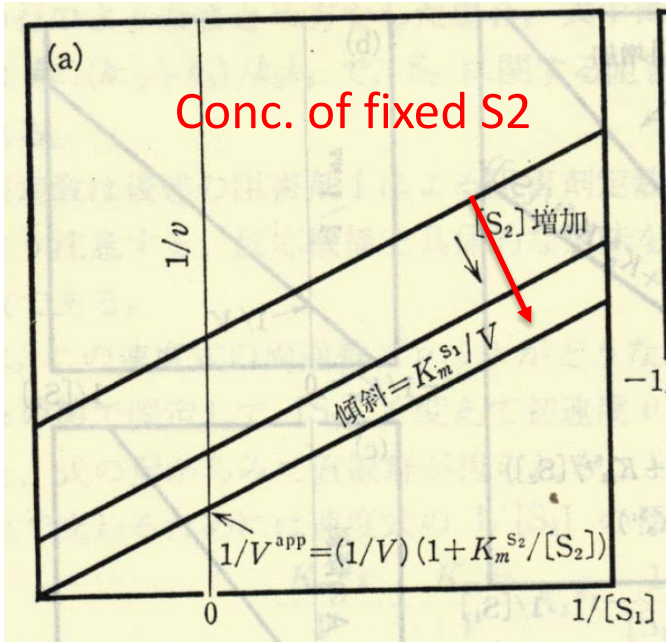
Kinetics investigation

By changing the conc. of fixed substrate, ineweaver-burk plot shows different characteristics depending on the mechanisms



Ordered or Random

crossed line patterns



Ping pong

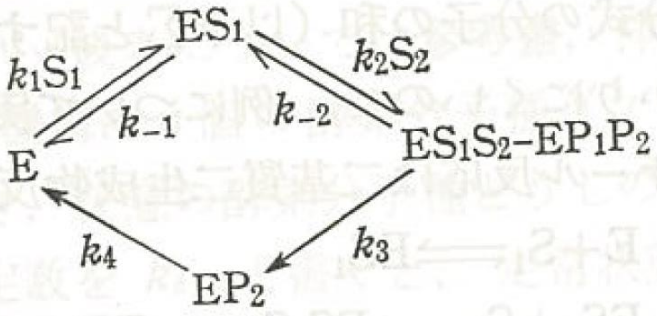
酵素キネティクス 中村隆雄(1993).

Parallel line patterns

Theorell-chance : depends on enzymes

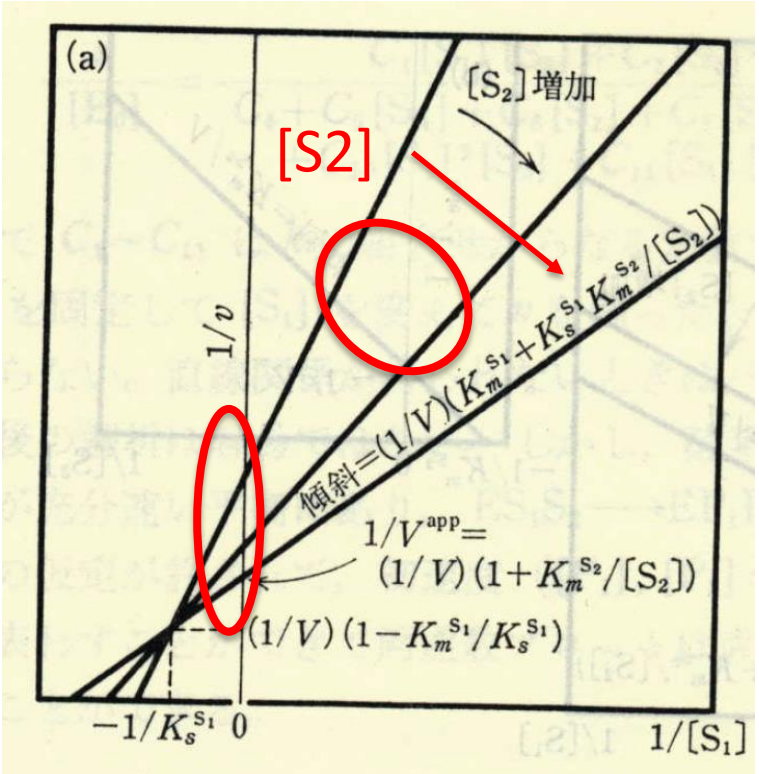
Kinetics investigation

Why ordered mechanisms show crossed line patterns



$$\frac{1}{v}=\frac{1}{V}\left(1+\frac{K_m^{S_2}}{[S_2]}\right)+\frac{1}{V}\left(K_m^{S_1}+K_s^{S_1}\frac{K_m^{S_2}}{[S_2]}\right)\frac{1}{[S_1]}$$

$$=\frac{1}{V}\left(1+\frac{K_m^{S_2}}{[S_2]}\right)+\frac{K_m^{S_1}}{V}\left(1+\frac{K_i^{S_2}}{[S_2]}\right)\frac{1}{[S_1]}$$

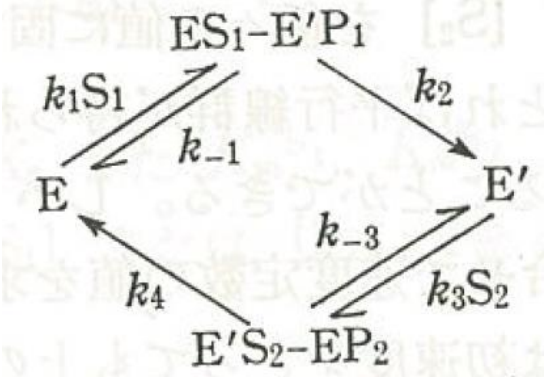


Both slope and intercept depends on [S2]

1/[S1]

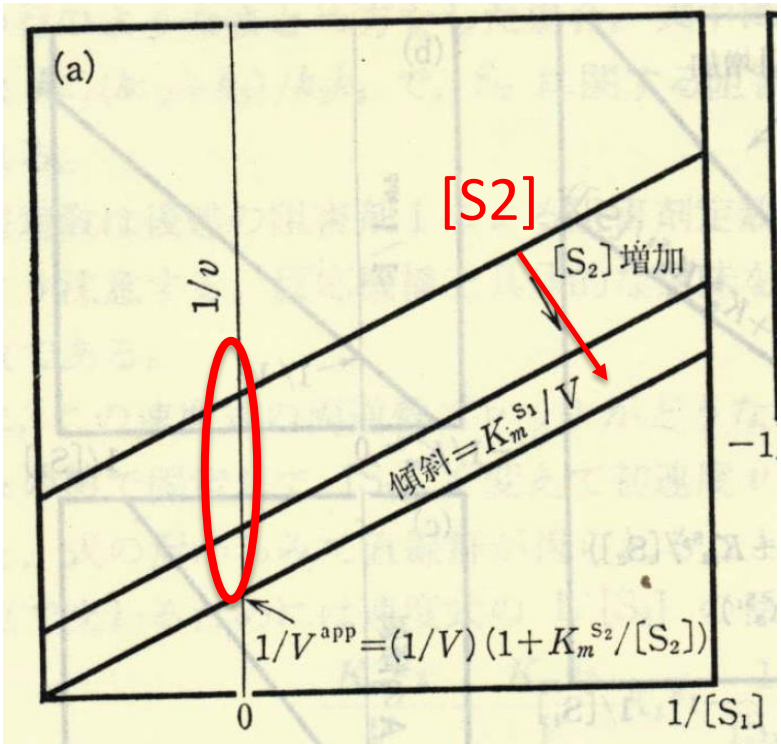
kinetics investigation

Why ping-pong mechanisms show parallel line patterns



$$\frac{1}{v}=\frac{1}{V}\left(1+\frac{K_m^{S_2}}{[S_2]}\right)+\frac{K_m^{S_1}}{V}\frac{1}{[S_1]}$$

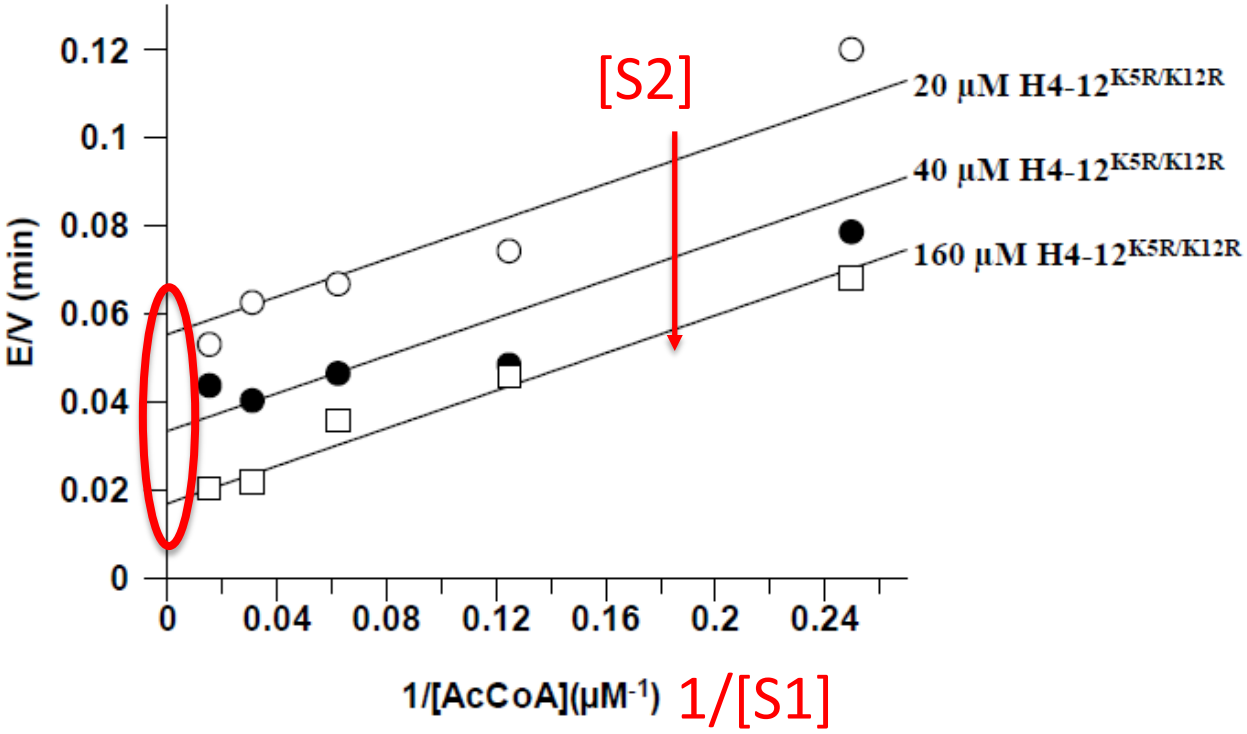
Only intercept depends on [S2]



1/[S1]

P300 kinetics

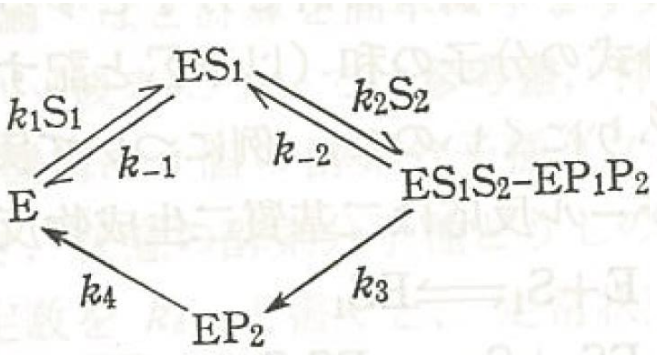
P300 showed parallel line patterns



Characteristics of ping pong mechanisms, but sometimes of Theorell-chance mechanisms

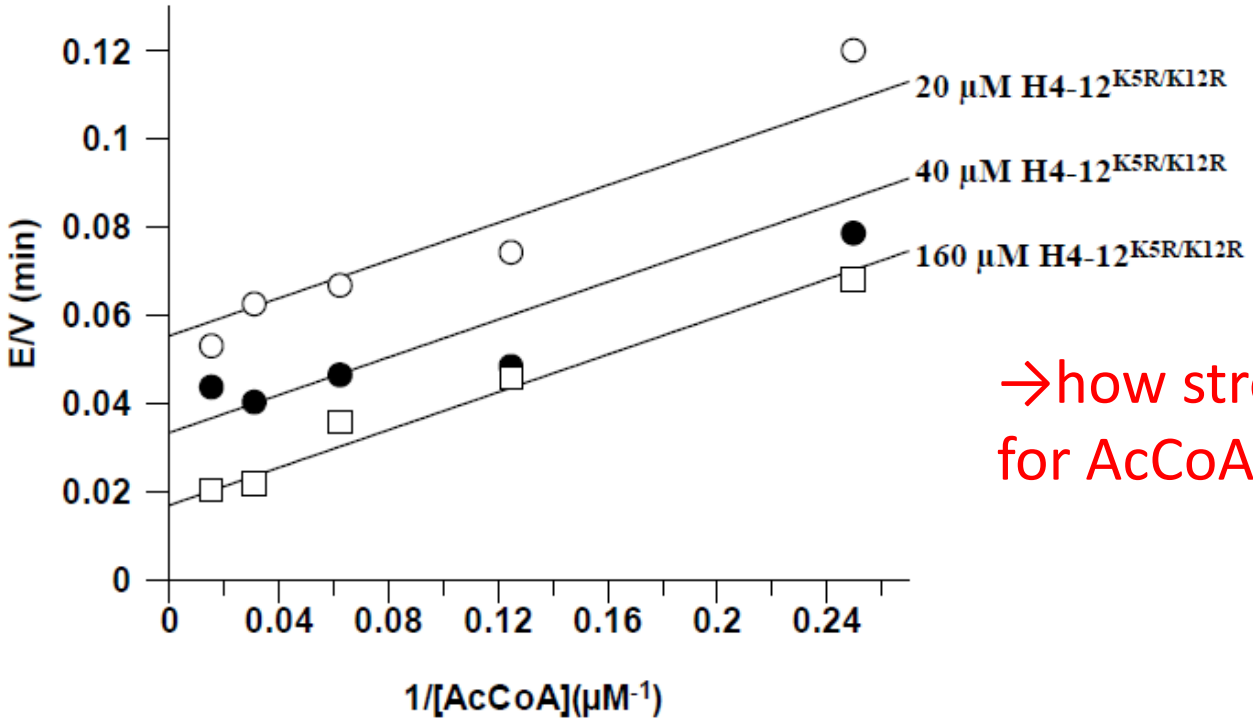
Qualifications for Theorell-chance to show parallel line patterns

Theorell-chance mechanisms can show parallel line patterns if k-1<<k1



$$\frac{1}{v}=\frac{1}{V}\left(1+\frac{K_m{}^{S_2}}{[S_2]}\right)+\frac{1}{V}\left(K_m{}^{S_1}+K_s{}^{S_1}\frac{K_m{}^{S_2}}{[S_2]}\right)\frac{1}{[S_1]}$$

$$=\frac{1}{V}\left(1+\frac{K_m{}^{S_2}}{[S_2]}\right)+\frac{K_m{}^{S_1}}{V}\left(1+\frac{\cancel{K_i{}^{S_2}}}{[S_2]}\right)\frac{1}{[S_1]}$$

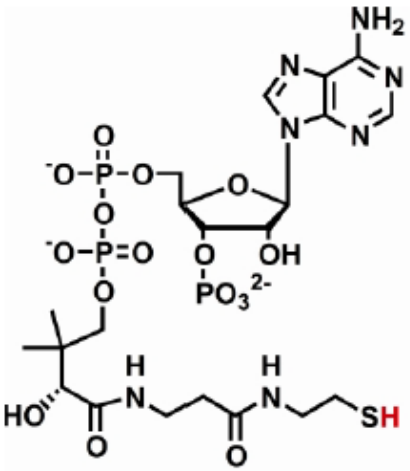


If k-1<<k,
$K_i{}^{S1}$=k-1/k1≒0
Then, only intercept depends on [S2]

→how strong is the affinity for AcCoA?

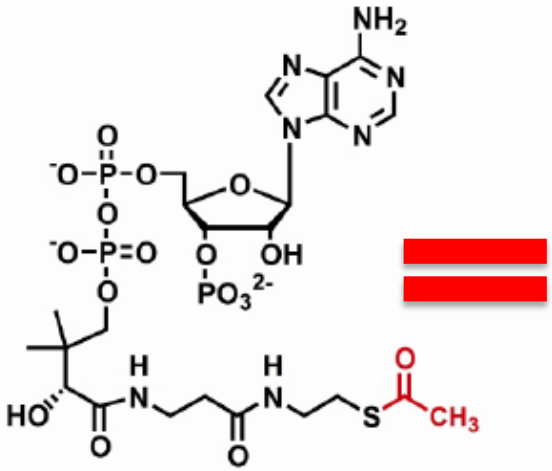
Affinity of p300 for AcCoA

p300 shows strong affinity for Ac-CoA and weak affinity for CoA-SH

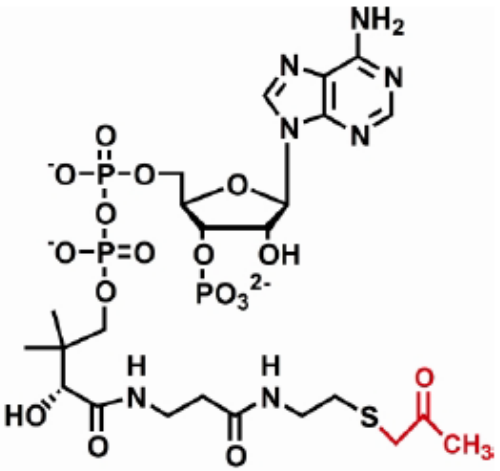


CoASH

Kd=7.3±1.4uM



acetyl-CoA



acetonyl-CoA

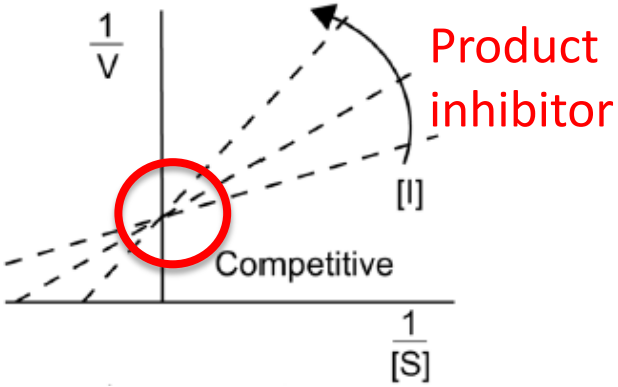
Kd=740±60nM

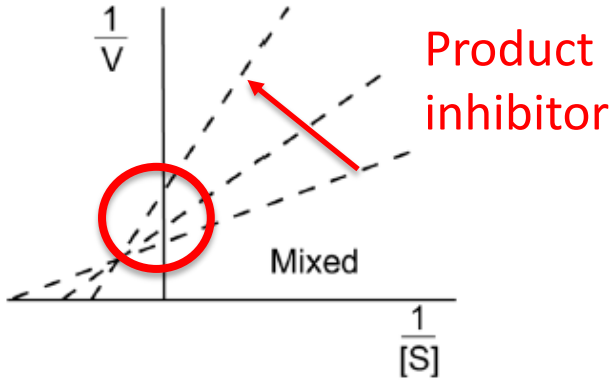
→k-1<<k1, supporting Theorell-Chance

Investigations on product inhibition

Product inhibition patterns differs among different mechanisms

Mechanism	Product inhibitor	Variable AcCoA, (unsaturated Peptide)	Variable Peptide, (unsaturated AcCoA)
Ordered Bi Bi	Ac-peptide	Mixed Type	Mixed Type
	CoASH	Competitive	Mixed Type
Theorell-Chance(T-C)	Ac-peptide	Mixed Type	Competitive
	CoASH	Competitive	Mixed Type
Ping Pong	Ac-peptide	Competitive	Mixed Type
	CoASH	Mixed Type	Competitive

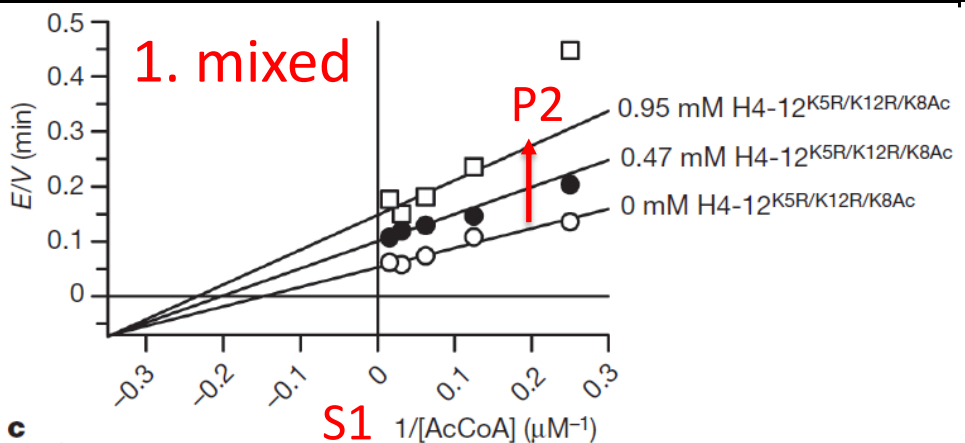


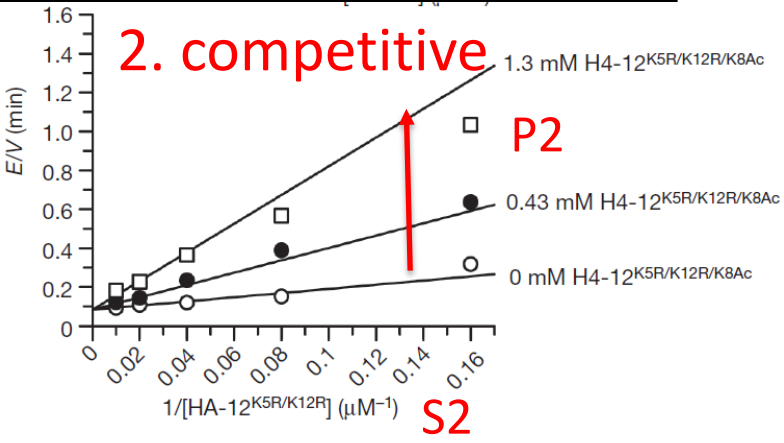


P300 fits Theorell-Chanace mechnisms

product inhibition of p300 is consistent with Theorell-Chance

Mechanism	Product inhibitor	Variable AcCoA, (unsaturated Peptide) S1	Variable Peptide, (unsaturated AcCoA) S2
Ordered Bi Bi p300	Ac-peptide	Mixed Type	Mixed Type
	CoASH	Competitive	Mixed Type
Theorell-Chance(T-C)	P2 Ac-peptide	1. Mixed Type	2. Competitive
	CoASH	Competitive	Mixed Type
Ping Pong	Ac-peptide	Competitive	Mixed Type
	CoASH	Mixed Type	Competitive





Other HATs kinetics and affinity for inhibitors

Theorell-chance mechanism is unique to p300 among HATs

Gcn5/PCAF(GNAT) : sequential (random or ordered)
Esa1(MYST) : ping-pong or sequential (random or ordered)
Rtt109 : unknown (none of the 4 classifications applied)

The relation between HAT inhibitors and catalytic mechanisms

	Esa1 (μM)	Tip60 (μM)	p300 (μM)	PCAF (μM)
H4K5CoA (**1**)	18.33 ± 1.07	143.35 ± 21.70	2.88 ± 0.46	65.93 ± 6.41
H4K8CoA (**2**)	13.94 ± 2.36	111.70 ± 19.24	8.15 ± 0.70	124.30 ± 13.61
H4K12CoA (**3**)	20.30 ± 2.70	25.87 ± 8.09	4.35 ± 0.39	53.57 ± 9.83
H4K16CoA (**4**)	5.51 ± 0.98	17.59±2.40	6.62 ± 0.56	58.47 ± 4.22
H2AK5CoA (**5**)	12.09 ± 0.30	20.91 ± 2.48	17.35 ± 1.39	60.54 ± 2.96
H3K14CoA (**6**)	4.78 ± 1.05	79.62 ± 17.22	7.54 ± 1.15	2.27 ± 0.14
Lys-CoA (**7**)	7.00 ± 1.18	29.75 ± 2.88	0.98 ± 0.01	108.30 ± 6.73
CoASH	68.58 ± 8.07	82.27 ± 16.25	45.94 ± 6.92	41.91 ± 4.20
Anacardic acid	297.23 ± 96.08	347.59 ± 55.39	>1000	667.05 ± 349.51
Curcumin	>150	>200	>40	—

Zheng, Y. G. *Et al.* Bioorg. Med. Chem. 2009, 17, 1381.

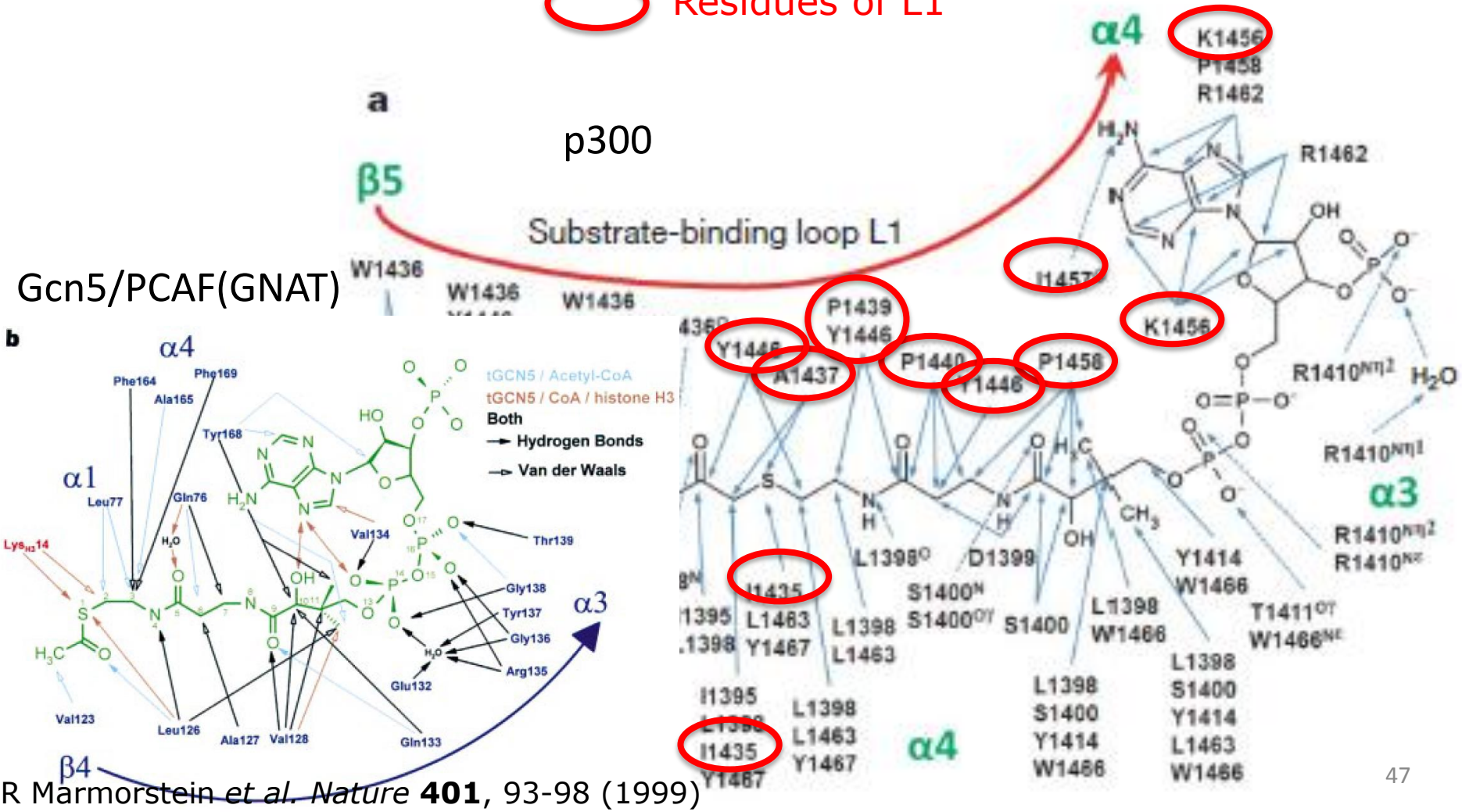
Grounds and meaning of different mechanisms

L1 loop unique to p300 realizes the strong affinity for Ac-CoA

Residues of L1

p300

Gcn5/PCAF(GNAT)

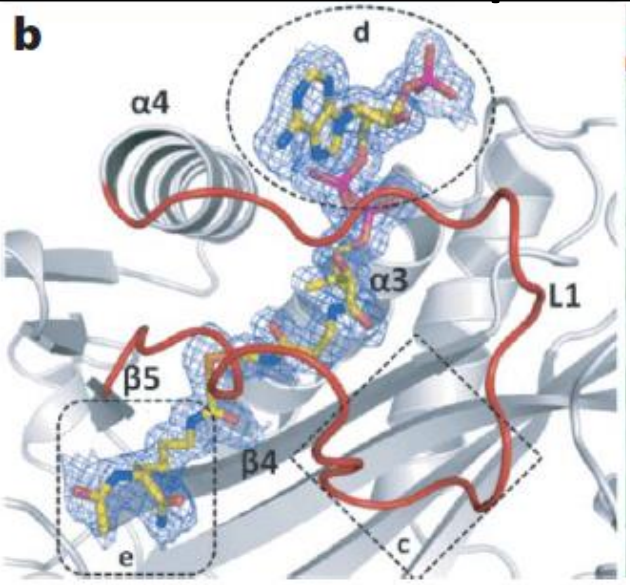


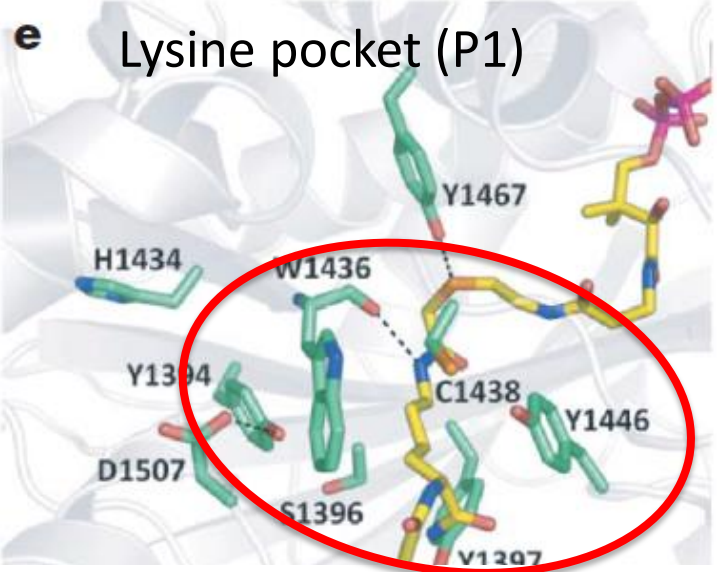
R Marmorstein *et al. Nature* **401**, 93-98 (1999)

Contents

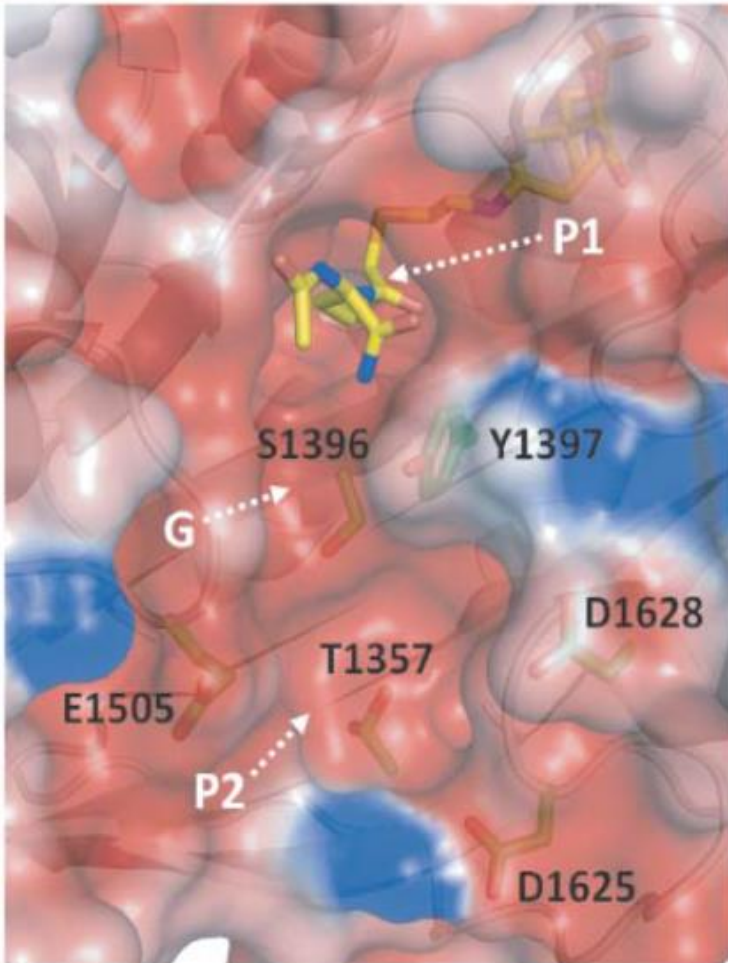
P300 site specificty

Substrate and site specificity of p300



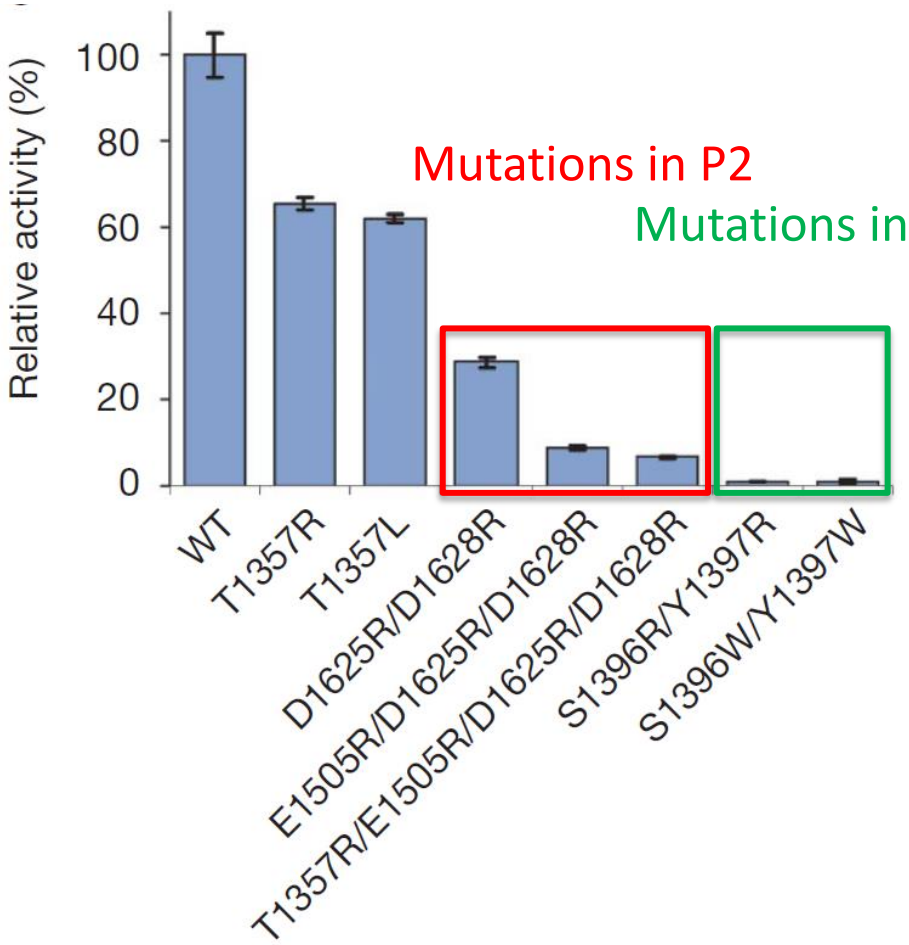


P2 and groove

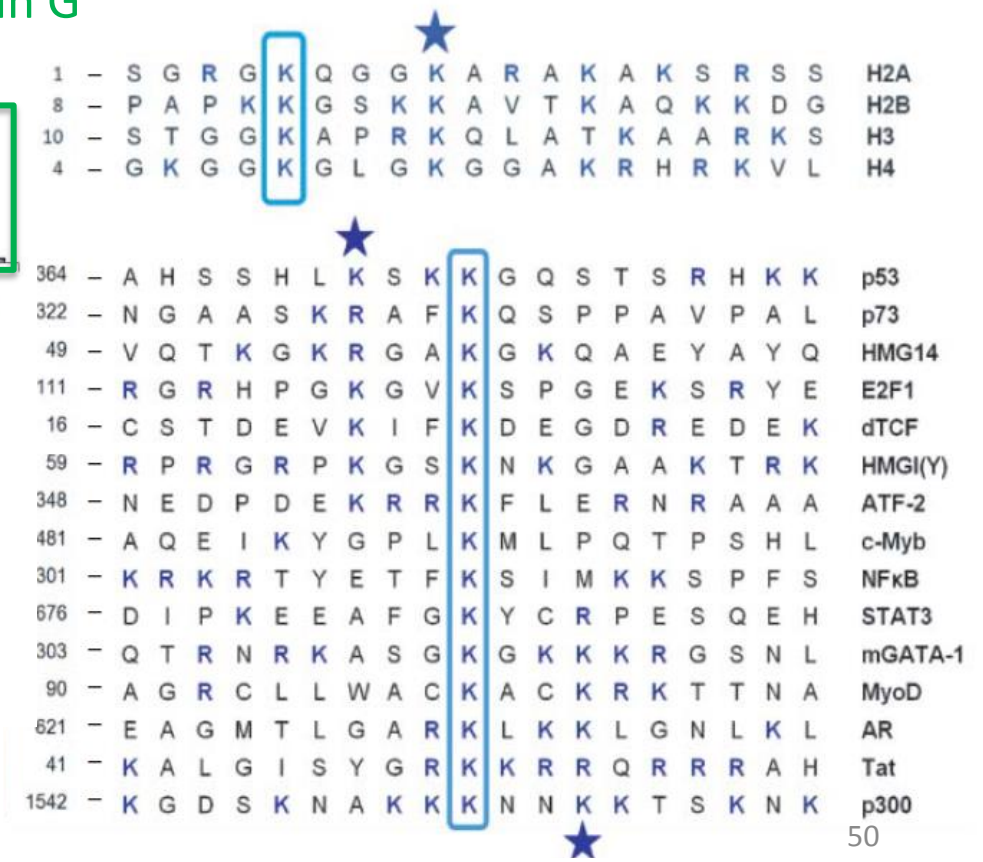


Essential residues for site specificity

Mutational analysis



All p300 substrates have positive residues 10 Å down- or up-stream of the substrate residue



Important interactions between substrate and p300 residues

Electrostatic interaction between the nearby residues and D1625/D1628 is important

Peptide	Peptide Sequence	V/K($M^{-1}s^{-1}$) for W.T.	V/K($M^{-1}s^{-1}$) for D1625R/D1628R
H4-15	GRG**K**GG**K**GLG**K**GGAK	25000±1643	1162±60
H4-15$^{K5D/K12D}$	GRG**D**GG**K**GLG**D**GGAK	636±40	422±20
H4-12$^{K5A/K12A}$	RG**A**GG**K**GLG**A**GA	3824±180	515±15
H4-12$^{K5X/K12A}$	RG**X**GG**K**GLG**A**GA*	5247±260	738±20
Ac-Lys-NH_2	CH_3CO-NH-Lys-$CONH_2$	1843±50	326±10

*X=citrulline

Mutations that maintain electrostatic interactions between peptide and p300 lower the drop in V/K.

Contents

Summary

- All HAT proteins acetylate Histone by two-step reactions; proton transfer and activation of Ac-CoA by sequential mechanisms.
- Only Esa1 is reported to catalyze the reaction by ping-pong mechanisms with enzyme nucleophile.
- p300 employs unique Theorell-chance mechanisms with the help of L1 binding loop, realizing its broad substrate recognition.
- p300 recognizes acetylation site with its acidic pocket near the catalytic pocket.

Appendix; P300 kinetics

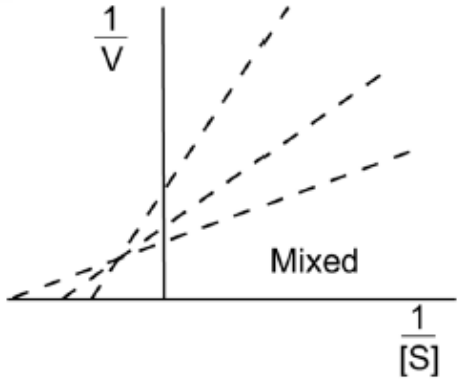
Product inhibition in ordered mechanisms

Equations of ordered mechanisms considering product inhibition

$$\frac{v}{[E]_0}=\frac{V([S_1][S_2]-[P_1][P_2]/K_{eq})}{K_s^{S_1}K_m^{S_2}+K_m^{S_2}[S_1]+K_m^{S_1}[S_2]+[S_1][S_2]+\frac{K_m^{S_2}K_m^{P_2}}{K_m^{P_1}K_s^{P_2}}[S_1][P_1]}$$
$$+\frac{K_m^{S_1}}{K_s^{P_2}}[S_2][P_2]+\frac{K_s^{S_1}K_m^{S_2}}{K_s^{P_2}K_m^{P_1}}[P_1][P_2]+\frac{K_s^{S_1}K_m^{S_2}K_m^{P_2}}{K_s^{P_2}K_m^{P_1}}[P_1]$$
$$+\frac{K_s^{S_1}}{K_s^{P_2}}K_m^{S_2}[P_2]+\frac{1}{K_i^{P_1}}[S_1][S_2][P_1]+\frac{K_s^{S_1}K_m^{S_2}}{K_i^{S_2}K_s^{P_2}K_m^{P_1}}[S_2][P_1][P_2]$$

When only P1 is added (when [P2] is 0,)

$$\frac{[E]_0}{v}=\frac{1}{V}\left(1+\frac{[P_1]}{K_i^{P_1}}+\frac{K_m^{S_2}}{[S_2]}\left(1+\frac{K_m^{P_2}}{K_m^{P_1}K_s^{S_2}}[P_1]\right)\right)$$
$$+\frac{1}{V}\left(K_m^{S_1}+\frac{K_s^{S_1}K_m^{S_2}}{[S_2]}\left(1+\frac{K_m^{P_2}[P_1]}{K_m^{P_1}K_s^{P_2}}\right)\right)\frac{1}{[S_1]}$$



For a fixed [S2], [S1] inhibition of [P1] is mixed-type
(both slope and intercept depends on [P1])