



# Exploring the binding pocket of Mb Pyrrolysine-tRNA ligase

**Group 11**

Group member: 张楠, 刘杨洛融, 肖康明

Speaker: 张贤睿

- Background
- Work Flow
- Data statistics
- Results
- Summary

# Background



**DNA**



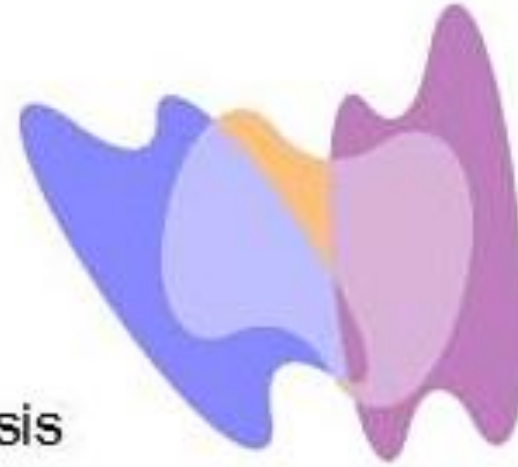
Process: Transcription  
Purpose: RNA synthesis  
Location: Nucleus



**RNA**

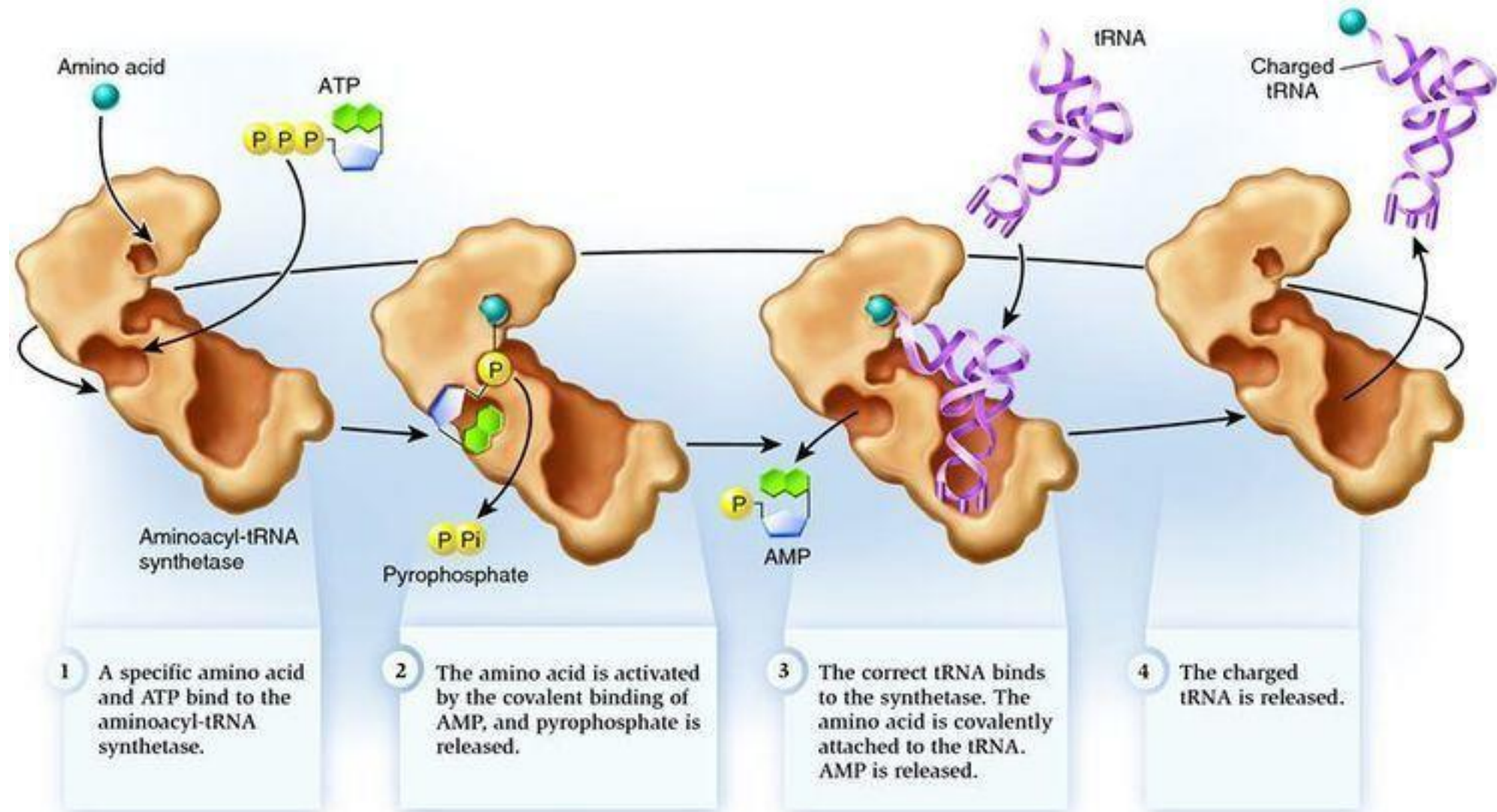


Process: Translation  
Purpose: Protein synthesis  
Location: Cytoplasm at a  
Ribosome



**Protein**

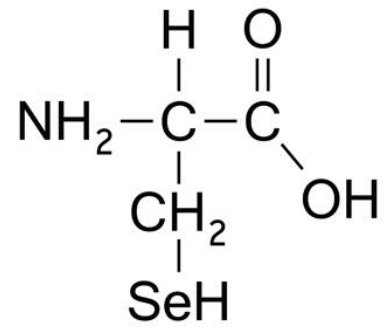
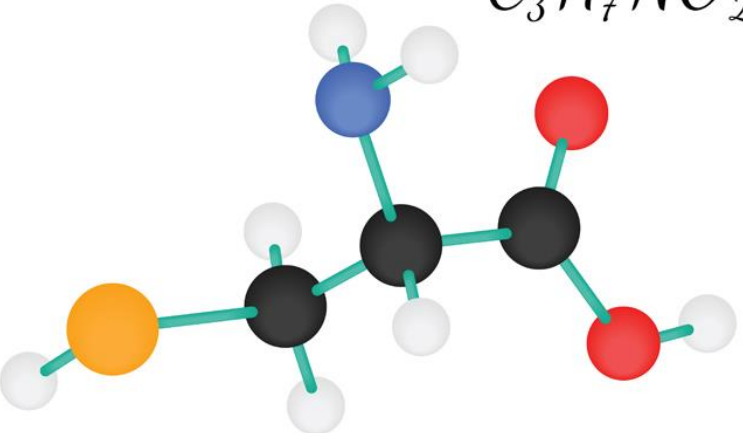
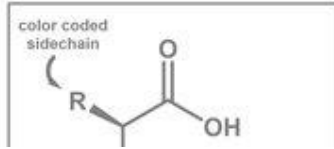
# Background



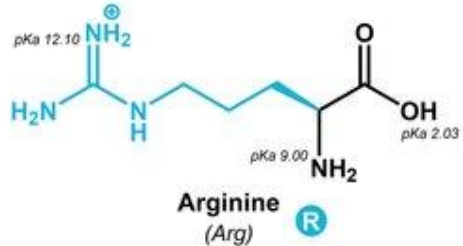
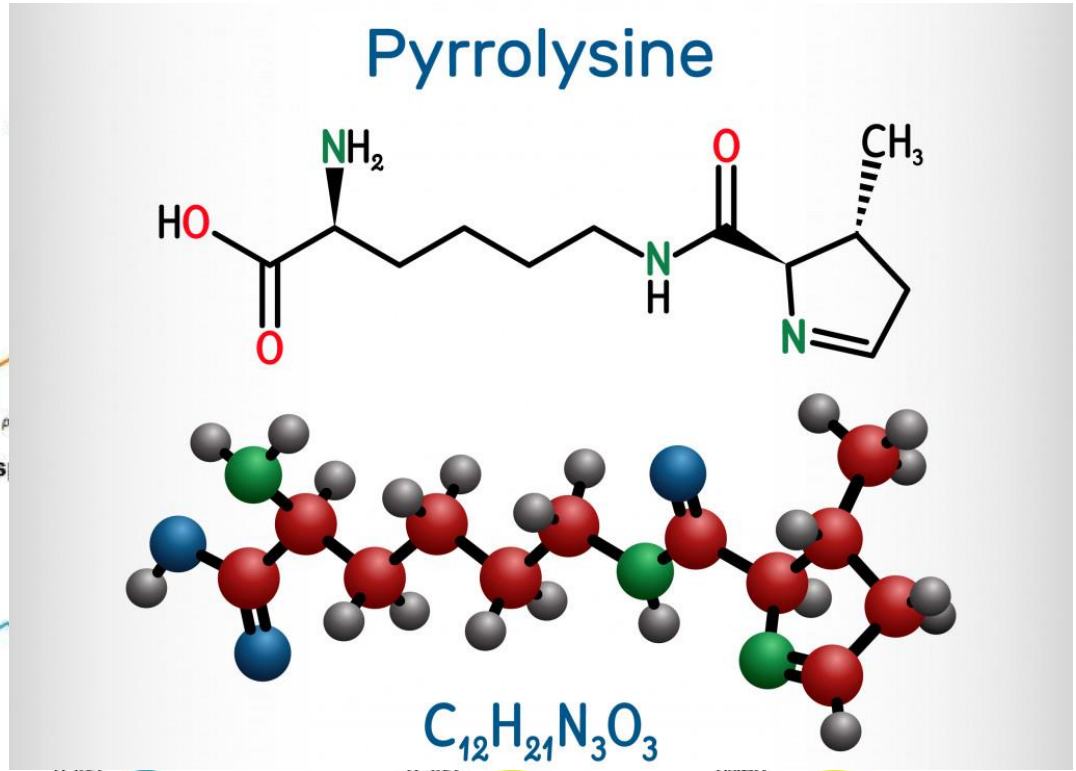
# Background

## THE 20 COMMON AMINO ACIDS

- ALIPHATIC
- AROMATIC
- AMIDIC
- HYDROXYLIC
- ⊖ CHARGED
- ⊕ CHARGED
- SULFUR CONTAINING



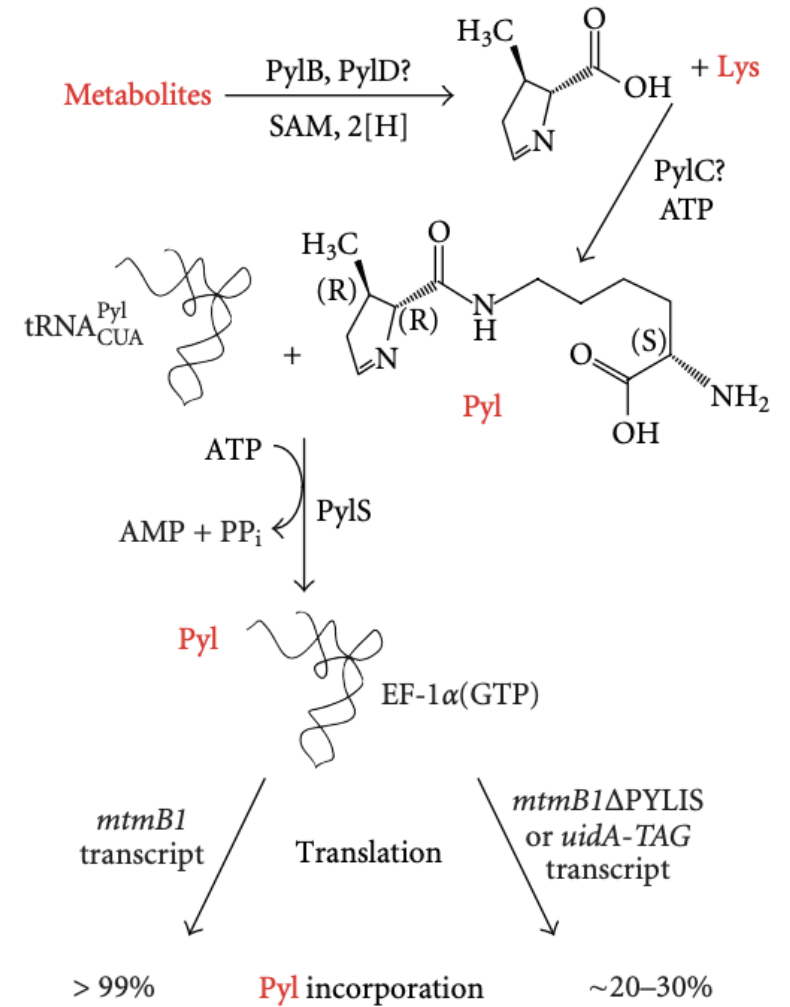
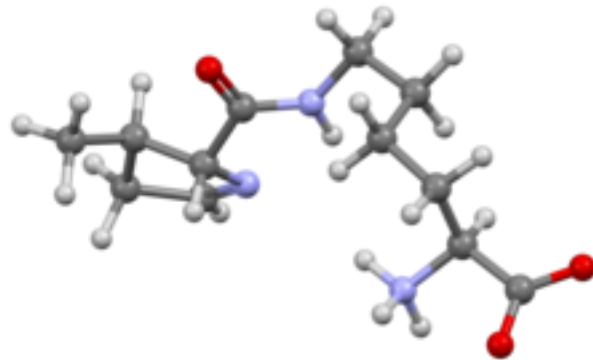
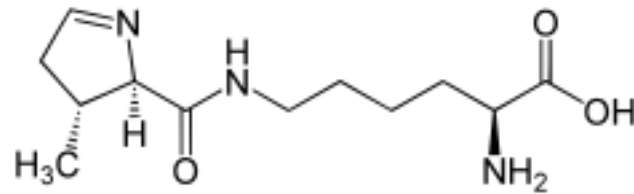
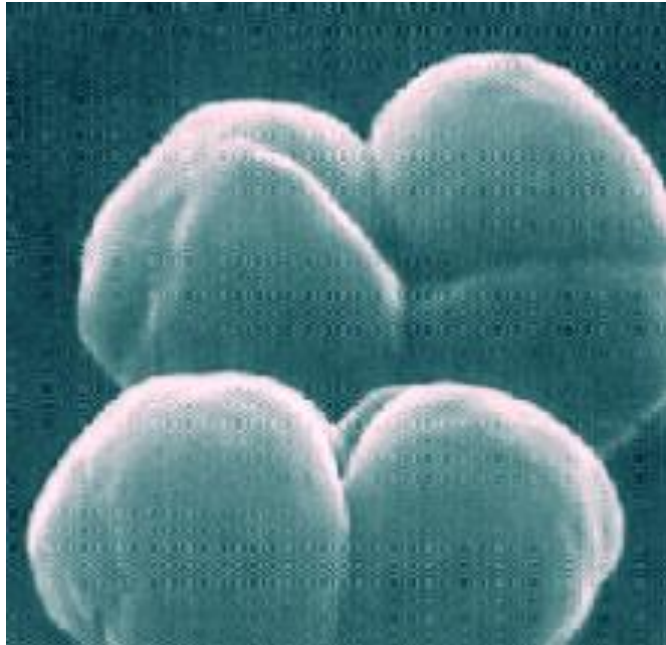
*amino acid selenocysteine*



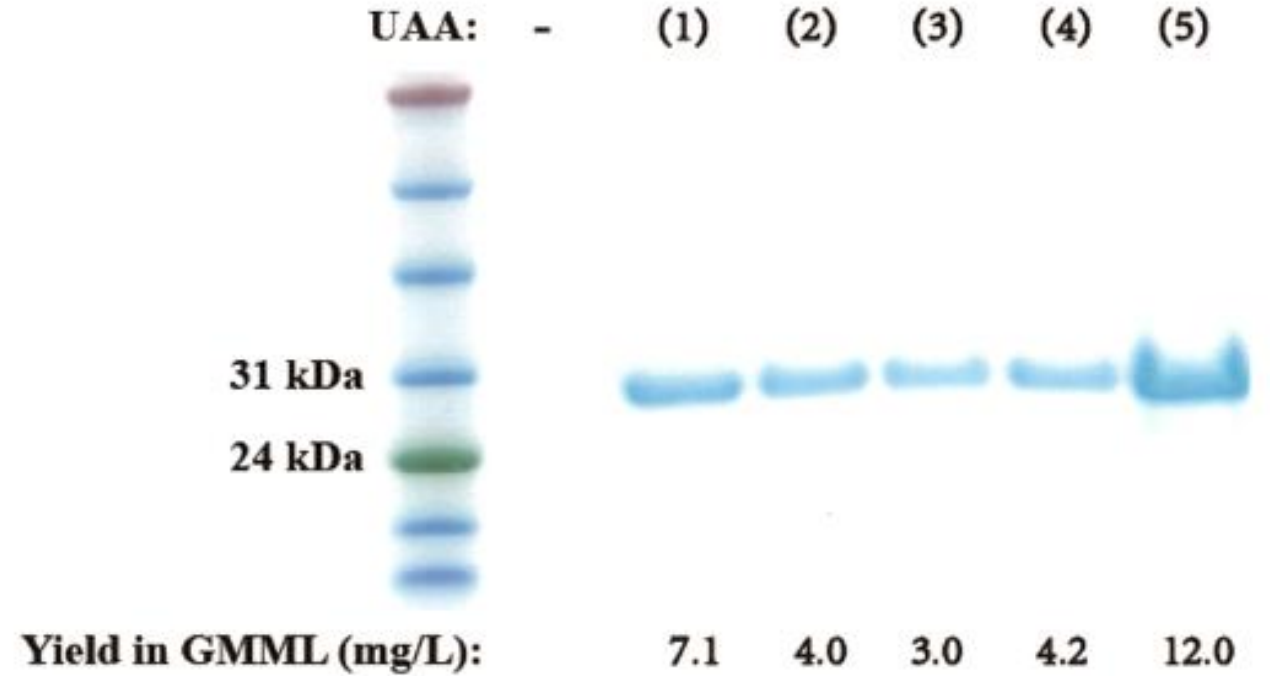
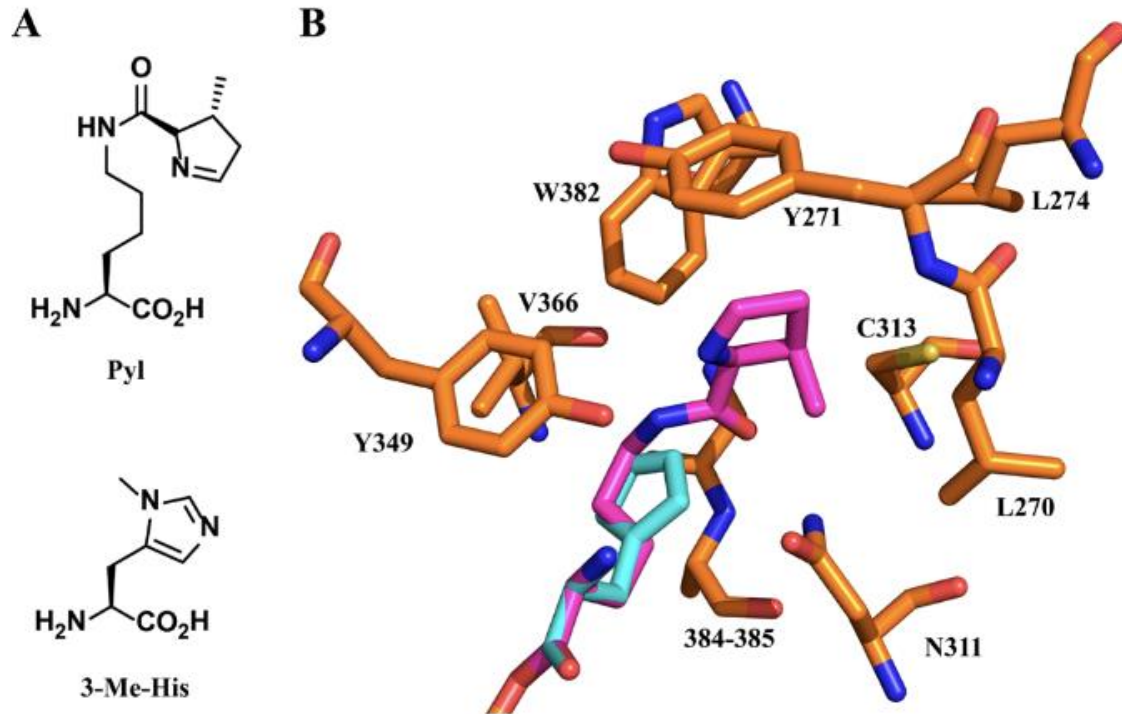


# Background

- *Methanosarcina* (甲烷八叠球菌)



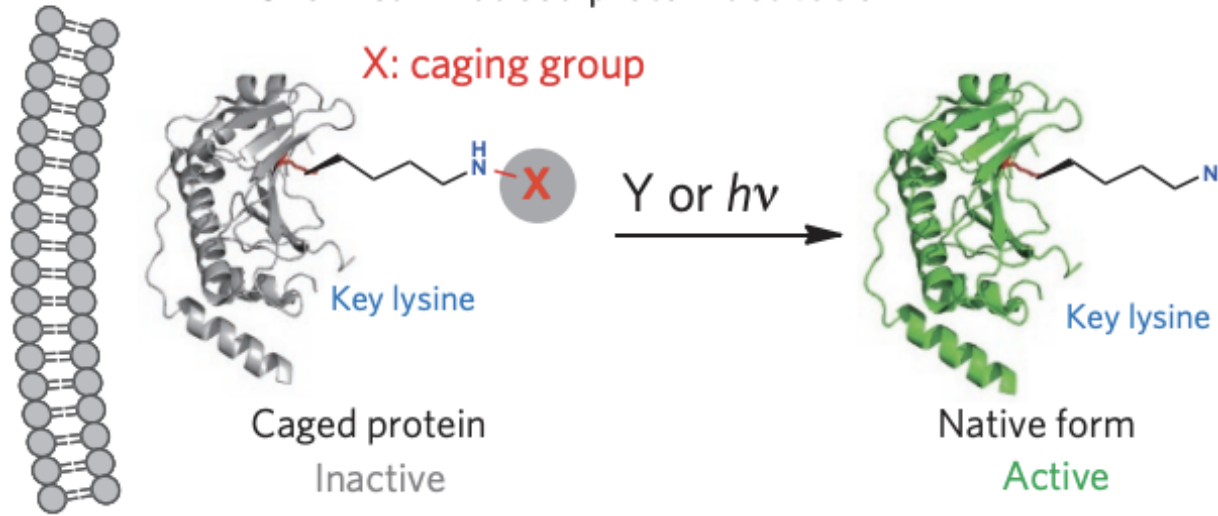
Rother, M. & Krzycki, J. A. *Archaea*, (2010).



Xiao, H. *et al.* *ACS Chem. Biol.* **9**, 1092–1096 (2014).

# Background

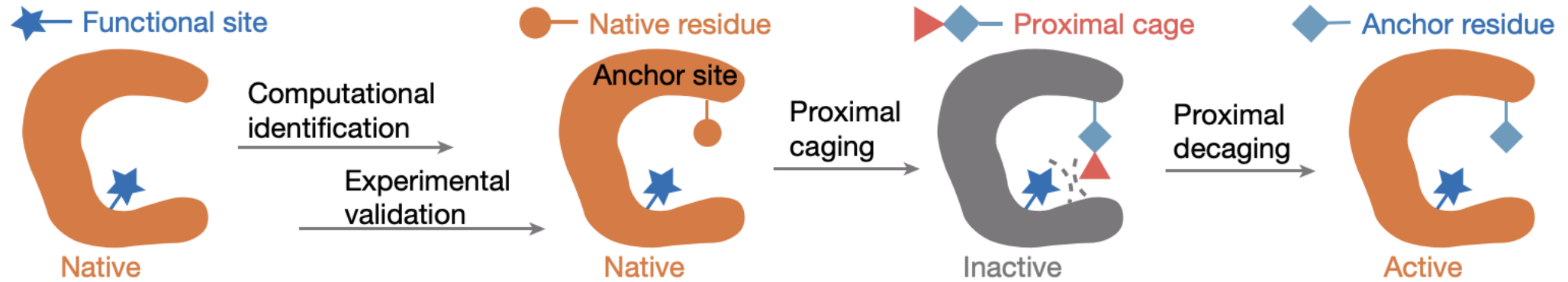
## Chemical-induced protein activation



**a**

Li, J., Chen, P. R. *Nat. Chem. Biol.* **12**, 129–137 (2016).

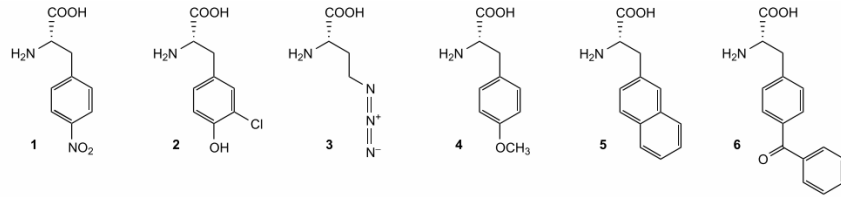
## Computationally aided and genetically encoded proximal decaging (CAGE-prox)



Wang, J. *et al. Nature* **569**, 509–513 (2019).



# Scientific Concerns



Organism

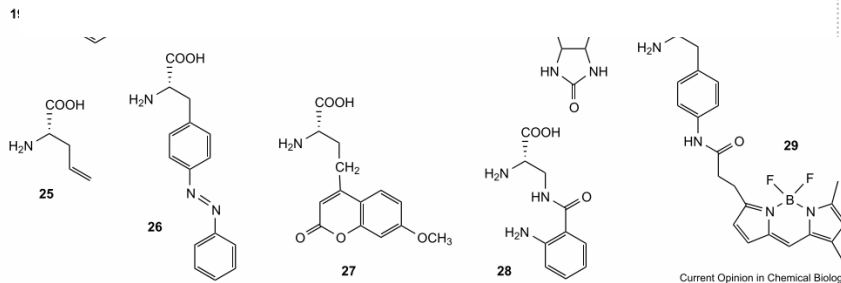
Methanosarcina barkeri

Methanosarcina barkeri (strain Fusaro / DSM 804)

Methanosarcina acetivorans (strain ATCC 35395 / DSM 2834 / JCM 12185 / C2A)

Methanosarcina mazei (strain ATCC BAA-159 / DSM 3647 / Goe1 / Go1 / JCM 11833 / OCM 88)  
(Methanosarcina frisia)

Methanosarcina thermophila



- More residue-based unnatural amino acid
- Enzyme from other organism
- Rational design of binding pocket
- Identification of catalytical residue

Hohsaka, T., Sisido, M. *Curr. Opin. Chem. Biol.* **6**, 809–815 (2002).

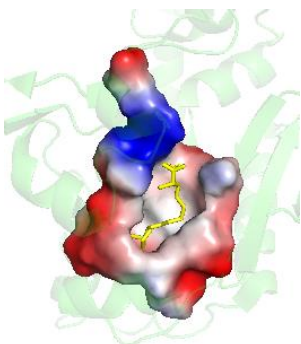
# Work flow



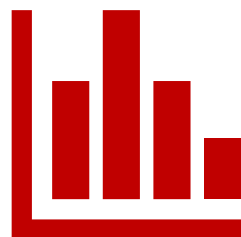
Protein Data Bank



Sequence Alignment



Pocket Fetch



Residue Statistics



Hot-Spot Finding



Pocket Prediction

```

Detected GLSL version 4.0.
OpenGL graphics engine:
GL_VENDOR: Intel
GL_RENDERER: Intel(R) HD Graphics 4000
GL_VERSION: 4.0.0 - Build 10.18.10.4358
License Expiry date: 01-aug-2022
Detected 4 CPU cores. Enabled multithreaded rendering.

```

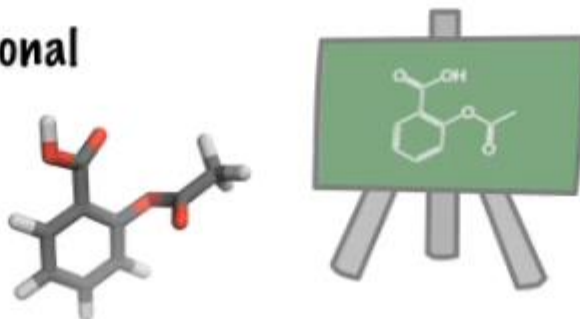
Reset Zoom Orient Draw/Ray  
 Unpick Deselect Rock Get View  
 |< < Stop Play > >| MClear  
 Builder Properties Rebuild  
 Undo Redo

PyMOL&gt;

For Educational Use Only

# Edu PyMOL

<http://pymol.org/educational>  
 v2.x



Schrödinger offers Educational-use-only PyMOL builds available at no cost to teachers and high school and college students for classroom instruction, homework assignments, and to provide a means for creating high quality figures. It is not provided for the purposes of academic research or publication.

There is no technical support from Schrödinger for "Edu" PyMOL, please use the pymol-users mailing list if you need help.

Licenses for academic: <http://pymol.org/academic>  
 Licenses for industry: <http://pymol.org/contact>

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all

A S H L C

```

Mouse Mode 3-Button Viewing
Buttons L M R Wheel
& Keys Rota Move MovZ Slab
  Shift +Box -Box Clip MovS
    Ctrl Move PkAt Pk1 MvSZ
      CtSh Sele Orig Clip MovZ
SnglClk +/- Cent Menu
DbClk Menu - PkAt
Selecting Residues
State 1/ 1

```

PyMOL&gt;\_

◀ ◁ ▢ ▷ ▶ ▶▶ S ▼ F

# Crystal structure available in PDB

UniProtKB - Q8PWF1 (PYLS\_METMA)

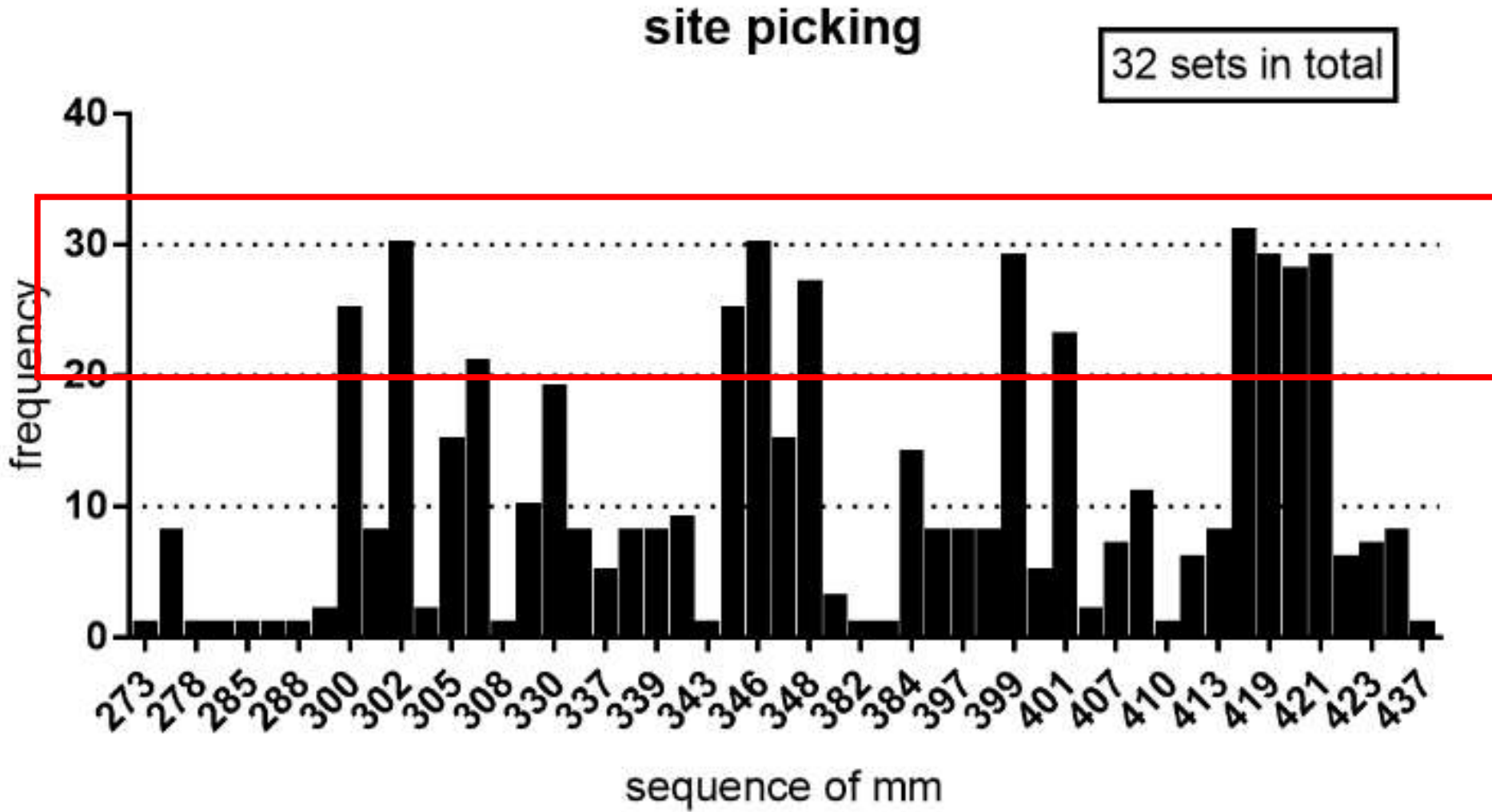
Disp	Entry	Method	Resolution (Å)	Quality	Entry	Method	Resolution (Å)	Quality	Entry	Method	Resolution (Å)	Quality
	2E3C	X-ray	2.65	A	4CH5	X-ray	2.2	A	6AAQ	X-ray	1.4	A
Entry	2Q7E	X-ray	1.8	A	4CH6	X-ray	2.05	A	6AAP	X-ray	1.5	A
Public	2Q7G	X-ray	1.9	A	4CS2	X-ray	1.9	A	6AAQ	X-ray	1.55	A
Featu	2Q7H	X-ray	2.1	A	4CS3	X-ray	1.5	A	6AAZ	X-ray	1.84	A
Featu	2ZCE	X-ray	1.8	A	4CS4	X-ray	1.35	A	6AB0	X-ray	1.44	A
Fun	2ZIM	X-ray	2.1	A	4Q6G	X-ray	2.25	A	6AB1	X-ray	1.38	A
Nam	2ZIN	X-ray	1.79	A	4TQD	X-ray	2.14	A	6AB2	X-ray	1.4	A
Sub	2ZIO	X-ray	2.06	A	4TQF	X-ray	2.71	A	6AB8	X-ray	1.75	A
Pat	3QTC	X-ray	1.75	A	4ZIB	X-ray	2.05	A	6ABK	X-ray	1.58	A
PTM	3VQV	X-ray	1.9	A	5K1P	X-ray	1.5	A	6ABL	X-ray	1.47	A
Exp	3VQW	X-ray	2.4	A	5K1X	X-ray	1.95	A	6ABM	X-ray	1.37	A
Inte	3VQX	X-ray	2.3	A/B/C/D	5UD5	X-ray	2.35	A/B	6LY3	X-ray	1.9	A
Stru	4BW9	X-ray	2.35	A	5V6X	X-ray	2.76	A/B	6LY6	X-ray	2.5	A
Fam	4BWA	X-ray	2.45	A	6AAC	X-ray	1.48	A	6LY7	X-ray	2.09	A
Seq	4CH3	X-ray	2.28	A	6AAD	X-ray	1.44	A	6LYA	X-ray	1.59	A
Sim	4CH4	X-ray	2.16	A	6AAN	X-ray	1.51	A	6LYB	X-ray	1.9	A

by 张楠、刘杨洛融、肖康明





# Frequency Statistics

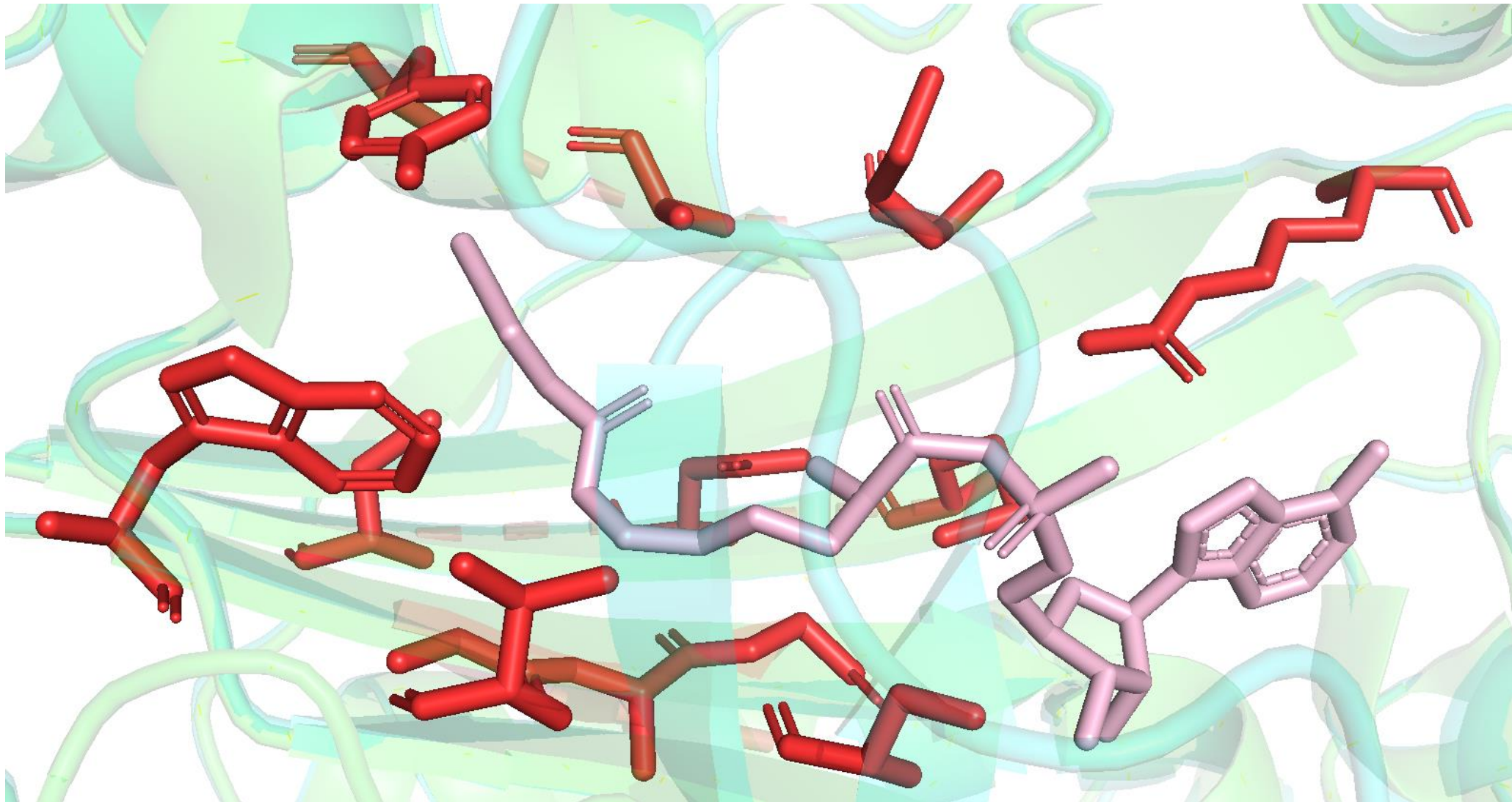




# Highlight Residue in Mm

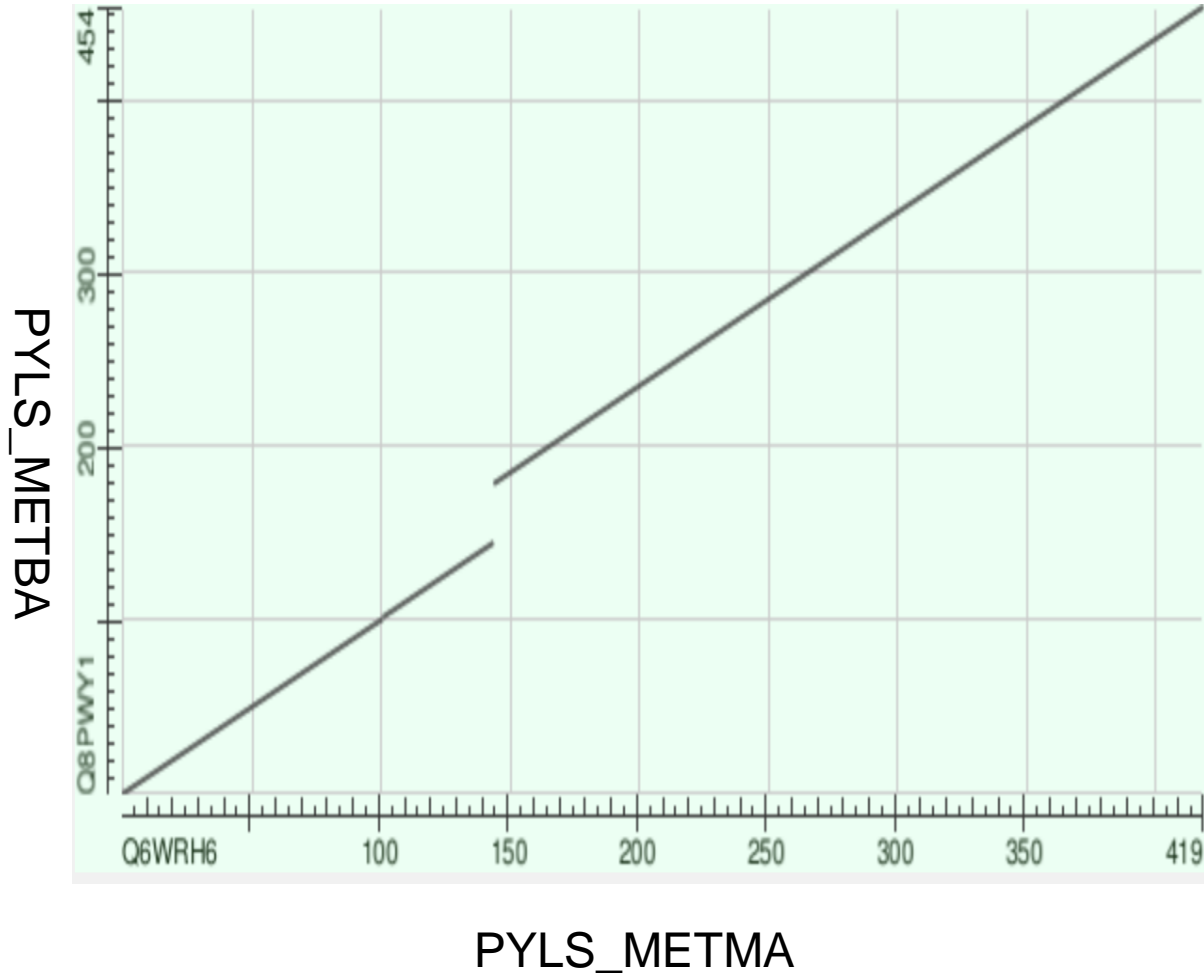
10	20	30	40	50
MDKKPLNTLI	SATGLWMSRT	GTIHKIKHHE	VSRSKIYIEM	ACGDHLVVNN
60	70	80	90	100
SRSSRTARAL	RHHKYRKTCK	RCRVSDLEDLN	KFLTKANEDQ	TSVKVKVSA
110	120	130	140	150
PTRTKKAMPK	SVARAPKPLE	NTEAAQAQPS	GSKFSPAIPV	STQESVSVPA
160	170	180	190	200
SVSTSISSIS	TGATASALVK	GNTNPITSMS	APVQASAPAL	TKSQTDRLEV
210	220	230	240	250
LLNPKDEISL	NSGKPFRELE	SELLSRRKKD	LQQIYAEERE	NYLGKLEREI
260	270	280	290	300
TRFFVDRGFL	EIKSPIL IPL	EYIERMGIDN	DTELSKQIFR	VDKNFCLRPM
310	320	330	340	350
LAPNLYNYLR	KLDRALPDPI	KIFEIGPCYR	KESDGKEHLE	EFTMLNFCQM
360	370	380	390	400
GSGCTRENLE	SIITDFLNHL	GIDFKIVGDS	CMVYGD TLDV	MHGDLELSSA
410	420	430	440	450
VVGPIPLDRE	WGIDKPWIGA	GFGLERLLKV	KHDFKNIKRA	ARSESYNGI

# Visualization of hot spot residues





# Exploring the pocket of Mb Pyl ligase



Score	Expect	Method	Identities	Positives	Gaps
576 bits(2177)	0.0	Compositional matrix adjust.	335/454(74%)	388/454(85%)	35/454(7%)
Query 1		MDKKPLDVLISATGLWMSRTGTLHKIKHHEVSRSKIYIEMACGDHLVNNRSRRTARAF			60
Sbjct 1		MDKKPL+ LISATGLWMSRTGT+HKIKHHEVSRSKIYIEMACGDHLVNNRS RTARA+ MDKKPLNTLISATGLWMSRTGTIHKIKHHEVSRSKIYIEMACGDHLVNNRSRRTARAL			60
Query 61		RHHKYRKTCKRCRVSDDEDINNFLTRSTESKNSVKVRVVSAP-KVKKAMPKSVSRAPKPLE			119
Sbjct 61		RHHKYRKTCKRCRVSDDED+N+FLT++ E + SVKV+VVSAP + KKAMPKSV+RAPKPLE RHHKYRKTCKRCRVSDEDLNKFLTKANEDQTSVKVKVVSAPTRTKKAMPKSVARAPKPLE			120
Query 120		NSVSAKASTNTSRSPSPAKSTPNS-----S			145
Sbjct 121		N+ +A+A + S+ P+ + ST +S NTEAAQAQPSGSKFSPAIPVSTQESVSPASVSTSISSISTGATASALVKGNTNPITSMS			180
Query 146		VPASAPAPSLTRSQDRVEALLSPEDKISLNMAKPFRELEPELVTRRKNDFQRLYTNDRE			205
Sbjct 181		P A+AP+LT+SQ DR+E LL+P D ISLN +KPFRELE+EL++RRK+D+Q++Y+++RE APVQASAPALTKSQDRLEVLNPKDEISLNSGKPFRELESELLSRRKKDLQQIYAEEERE			240
Query 206		DYLGKLERDITKFFVDRGFLEIKSPILIPAEYVERMGINNDTELSKQIFRVDKNLCLRPM			265
Sbjct 241		+YLGKLER+IT+FFVDRGFLEIKSPILIP EY+ERMGI+NDTELSKQIFRVDKN+CLRPM NYLGKLEREITRFFVDRGFLEIKSPILIPLEYIERMGIDNDTELSKQIFRVDKNFCLRPM			300
Query 266		LAPTLYNYLRKLDRIPLGPIKIFEVGPYRKESDGKEHLEEFMVFQMGSGCTRENLE			325
Sbjct 301		LAP LYNLYRKLDR LP+PIKIFE+GPCYRKESDGKEHLEEFM+VFCQMGSGCTRENLE LAPNLYNYLRKLDRALPDPIKIFEIGPCYRKESDGKEHLEEFMVFQMGSGCTRENLE			360
Query 326		ALIKEFLDYLEIDFEIVGDSCMVYGDITLDIMHGDELSSAVVGPVSLDREWGIDKWPWIGA			385
Sbjct 361		++I +FL+ L IDF IVGDSCMVYGDITLD+MHGDELSSAVVGP++LDREWGIDKWPWIGA SIITDFLNHLGIDFKIVGDSCMVYGDITLDVMHGDELSSAVVGPVPLDREWGIDKWPWIGA			420
Query 386		GFGLERLLKVMHGFKNIKRASRSSESYNGISTNL		419	
Sbjct 421		GFGLERLLKV H+FKNIKRA+RSESYNGISTNL GFGLERLLKVKHDFKNIKRAARSESYNGISTNL		454	



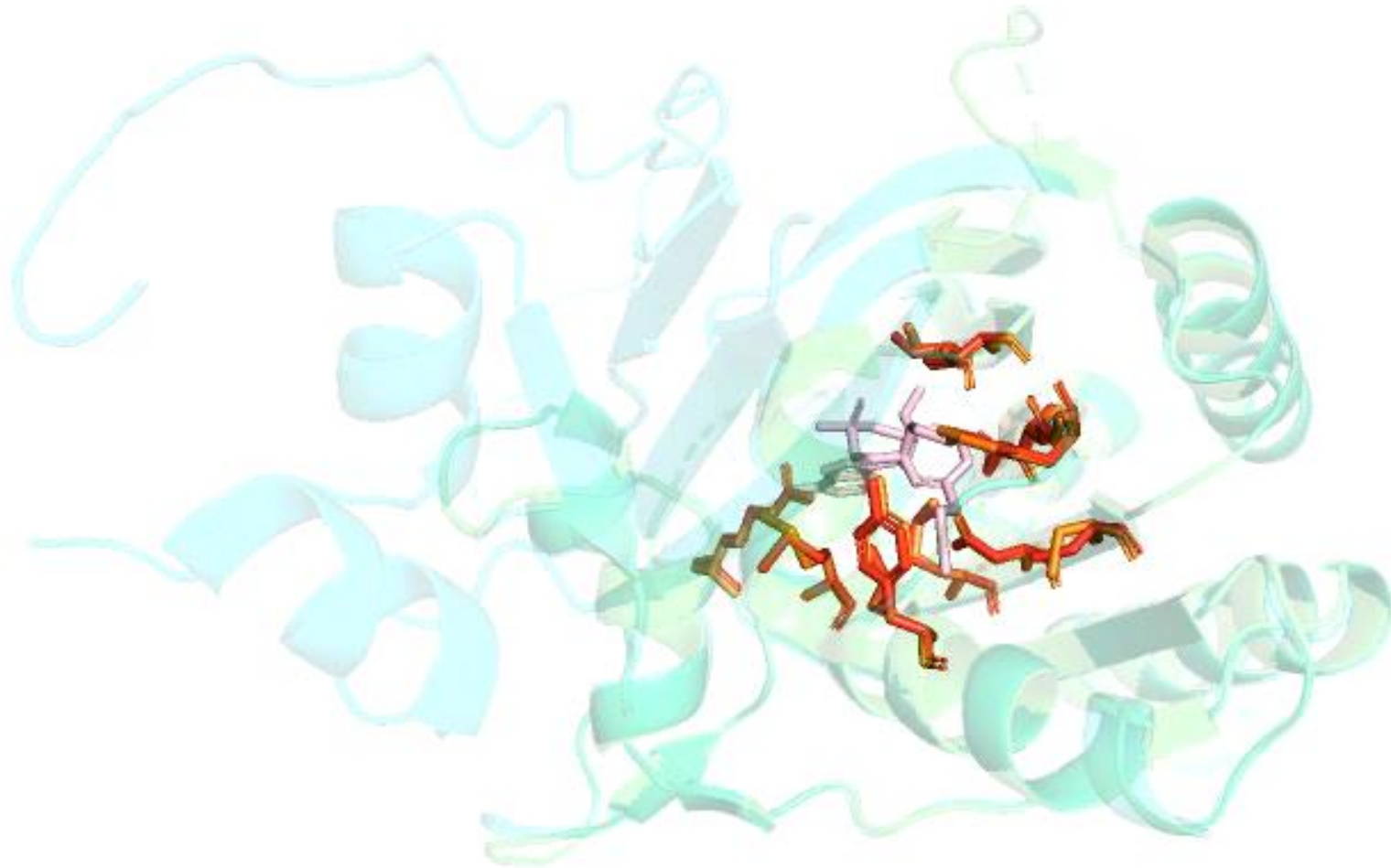
# Alignment to Mb

	10	20	30	40	50
	MDKKPLDVLI	SATGLWMSRT	GTLHKIKHHE	VSRSKIYIEM	ACGDHLVVNN
	60	70	80	90	100
	SRSCRTARAF	RHHKYRKTCK	RCRVSEDEDIN	NFLTRSTESK	NSVKVRVWSA
	110	120	130	140	150
	PKVKKAMPKS	VSRAPKPLEN	SVSAKASTNT	SRSVPSPAKS	TPNSSVPASA
	160	170	180	190	200
	PAPSLTRSQR	DRVEALLSPE	DKISLNMAKP	FRELEPELVT	RRKNDFQRLY
	210	220	230	240	250
	TNDREDYLGK	LERDITKFFV	DRGFLEIKSP	ILIPAEYVER	MGINNDTELS
	260	270	280	290	300
	KQIFRVDKNL	CLRPM <b>L</b> A <b>P</b> T <b>L</b>	<b>Y</b> NYLRKLDRI	LPGPIKIFEV	GPCY <b>R</b> KESDG
	310	320	330	340	350
	KEHLEEFT <b>M</b> V	<b>N</b> <b>F</b> <b>C</b> QMGSGC T	RENLEALIKE	FLDYLEIDFE	IVGDSCMVYG
	360	370	380	390	400
	DTLDIMHGDL	ELSS <b>A</b> V <b>V</b> GPV	SLDREWGIDK	<b>P</b> W <b>I</b> G <b>A</b> G <b>F</b> GLE	RLLKVMHGFK
	410				
	NIKRASRSES	YYNGISTNL	Enzyme Engineering		



# Align Mm with Mb

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- **Potential pocket residues discovery by pymol visualizing**
- **Hot spot residue identification through statics**
- **Sequence alignments to non-structured enzyme**
- **Rational suggestion for further enzyme engineering**

- 张贤睿：背景、pymol经验分享、整合汇报
- 刘杨洛融：演示视频制作、流程图、位点统计
- 张楠：位点统计、数据汇总及可视化
- 肖康明：位点统计、序列比对及动画制作

(排名不分先后，按照汇报中出现顺序整理)



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# Thanks for listening