- フランスslide(10月11日)
  - Essential Key Lysine residues
    (K9, K21, K27, K31, and K194) at MgATP binding site in N- and
    C-terminus domain of Human Adenylate Kinase (AK1)
    - Minoru Hamada[1)], Takanori Ayabe[1)2)], Hitoshi Takenaka[1)], Osamu Takenaka[3)],
      Hideharu Maruyama[1)], Masahiko Nagata[2)], Hiroyuki Nagahama[2)], Atsushi
      Yamamoto[2)],
      and Yasunori Koga[2)]
    - [1)] Dept. of Hygiene, [2)] The 2nd Dept. of Surgery, [3)] Primate Research Institute,
      Kyoto University, [4)] Nagoya Bunri College
    - [1)2)] Miyazaki Medical College, Kiyotake, Miyazaki-gun, Miyazaki, 889-16,
      JAPAN
  - FIRST INTERNATIONAL WORKSHOP on NM23/NDP KINASE
    - Institut Pasteur
    - Paris, October 11-13, 1995
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  - Adenylate Kinase (AK)
    - $MgATP^{2-} + AMP^{2-} \leftrightarrows MgADP^{-} + ADP^{3-}$
    - 1. Globular protein consisting of 194 amino acid residues.
    - 2. Widely distributed in prokaryotic and
    - eukaryotic cells.
    - 3. Consisting of three isozymes: AK1 in
    - cytosol, AK2 in mitochondrial
    - intermenbrane space, and AK3 in

- mammalian mitochondrial matrix.

1) What Function Dose Lys9 in N-terminal domain have?
- | K9 residue in N-terminal domain is existed in β-sheet after the first α-helices located in back- and downward of AK model by Pai et al. (1977)

2) Proposed Role of Lys21
- ---Participation to phosphate-binding glycine-rich loop (Res 15 - 22) in adenylate kinase: G-X-P-G-X-G-K-G from X-ray model by Pai et al. (1977)
- ---A motif as one of key residues to interact with γ-phosphate of ATP in X-ray model
- (Pai et al., 1977)
- ---Suggestion to move and interact with a-phosphate of MgATP in NMR model
- (Fry et al., 1985, 1986, Mildvan & Fry, 1987)
- ---Lys21 forms hydrogen bonds with both the γ-phosphate of ATP and the α-phosphate of
- AMP (Caldwell & Kollman, 1988)

3) Does Lys27 interact with γ-phosphate ?
- —Interaction with γ-phosphate of MgATP
- (Fry et al., 1985; Mildvan & Fry, 1987)

4) Does C-terminus involve in catalysis ?
- | C-terminal domain is denoted at the left side of AK model (Pai et al., 1977) and is shown to be close to MgATP binding site (Kim et al., 1990).
-
- | K190 in C-terminal domain of chicken AK was presumed to interact with $MgATP^{2-}$ greater than AMP^{2-}.

AK model
(Kim et al., 1990)

Materials and Method

- Items;
- 1. pMEX8-hAK1 phagemid vector carrying human cytosolic adenylate kinase (hAK1) cDNA
- 2. Purification of single stranded pMEX8-hAK1 DNA
- 3. Site-directed random mutagenesis of hAK1
- 4. Transformation and purification of double stranded pMEX8-hAK1 DNA
- 5. Screening of mutants by DNA cycle sequencing
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- 7. Kinetic analysis in the forward reaction

- Site-directed Random Mutagenesis
- Results of DNA sequence
- Results of DNA sequence
- Blue Sepharose Column Chromatography
- Km values
- Km values
- k'cat values
- k'cat values
- k'cat/Km values
- k'cat/Km values
- Km and k'cat values
- AK model
  (Kim et al., 1990)
- Results

- (1)The K9F mutant showed decreased k'cat and k'cat/Km values with almost unchanged Km values for $MgATP^{2-}$ and AMP^{2-}. The K9 residue was presumed to play an important role in catalysis.
- (2)The K21P mutant indicated decreased k'cat value, the K21 residue might play a significant role in phosphotyl transfer.
- (4)The K31F demonstrated the increased Km values for $MgATP^{2-}$, and the strikingly decreased k'cat values. The K31 residue might be appeared to contribute to catalytic reaction and substrate-binding at $MgATP^{2-}$ site.
- (5)K194L mutant showed the increased Km value for $MgATP^{2-}$, and the decreased k'cat vcalues. The K194 residue was suggested to play an important role in catalysis and of substrate-binding on $MgATP^{2-}$ interaction.

CONCLUSION

- Lys9, Lys21, Lys31, Lys27, and Lys194 residues;
- interacted with adenine nucleotide denoting each different mode at MgATP site.

Essential Key Lysine residues (K9, K21, K27, K31, and K194) at MgATP binding site in N- and C-terminus domain of Human Adenylate Kinase (AK1)

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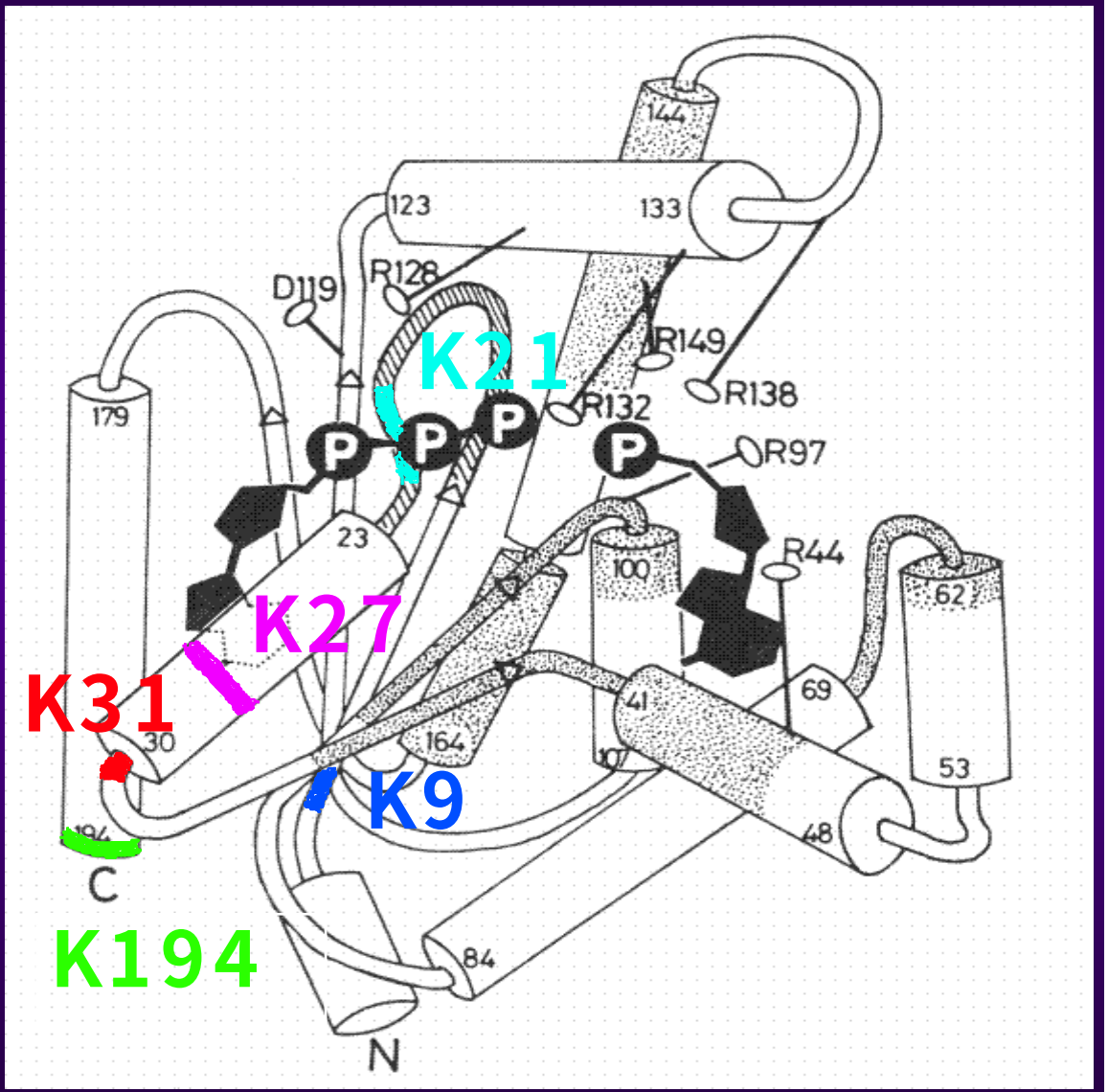

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7. Kinetic analysis in the forward reaction

DNA sequence of primers

Lys21

	15	16	17	18	19	20	21	22	23	24	25	26	27	
	Gly	Gly	Pro	Gly	Ser	Gly	Lys	Gly	Thr	Gln	Cys	Glu	Lys	
5'-	GGC	GGC	CCG	GGT	TCT	GGC	**AAA**	GGT	ACC	CAG	TGC	GAG	AAA	-3'
			3'-	CCA	AGA	CCG	**XXY**	CCA	TGG	GTC	ACG	-5'		

Lys27

	21	22	23	24	25	26	27	28	29	30	31	32	33	
	Lys	Gly	Thr	Gln	Cys	Glu	Lys	Ile	Val	Gln	Lys	Tyr	Gly	
5'-	AAA	GGT	ACC	CAG	TGC	GAG	**AAA**	ATC	GTG	CAG	AAA	TAC	GGC	-3'
		3'-	GG	GTC	ACG	CTC	**XXY**	TAG	CAC	G	-5'			

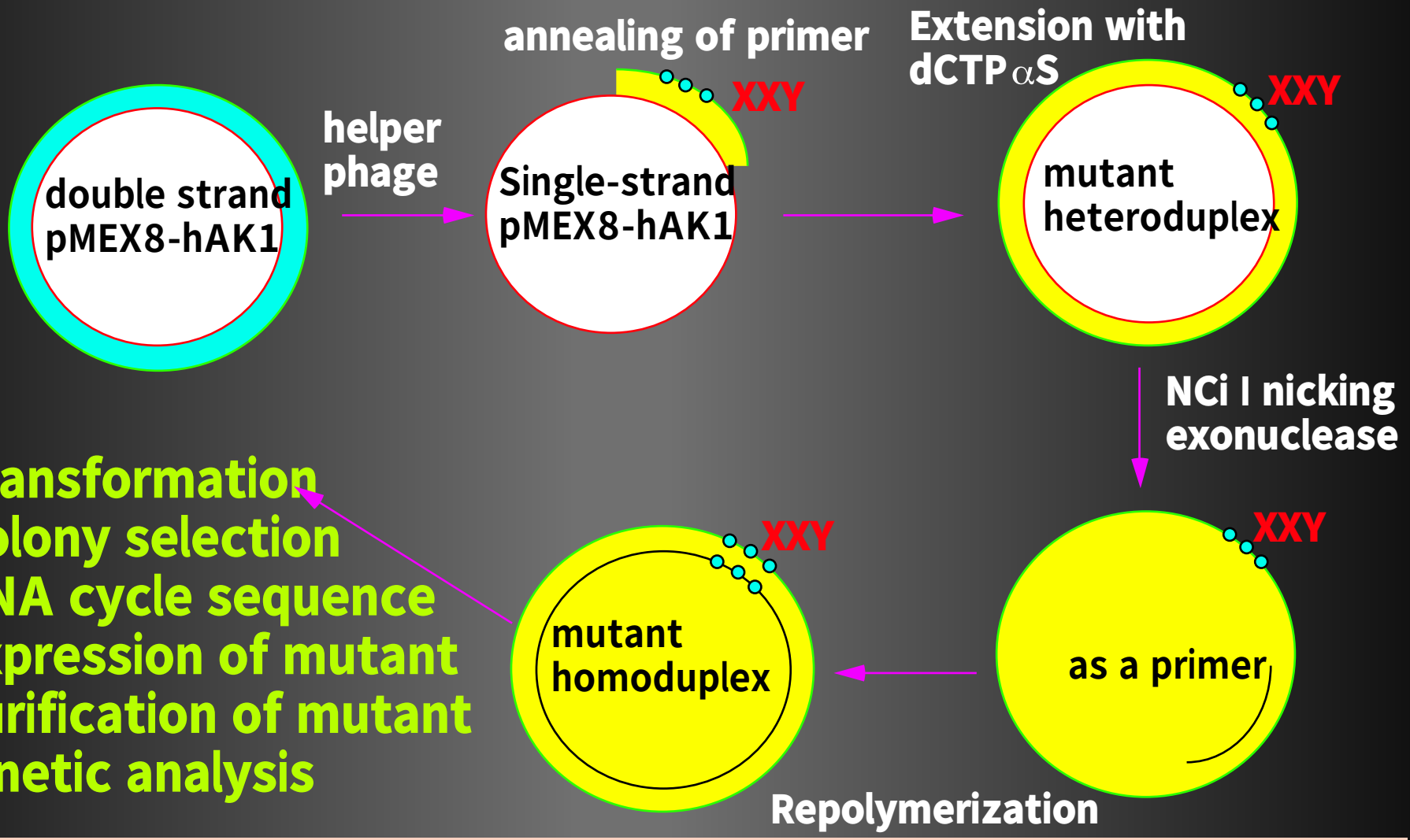
Lys9 5'- C GAA GAT GAT **YXX** AGT CTT CTT AAG C -3'

Lys194 5'- CGA CTA TTA **YXX** CAG AGC GTC C -3'

XXY : codon consisted of random bsase

X: A, G, C, or T base Y : G or C base

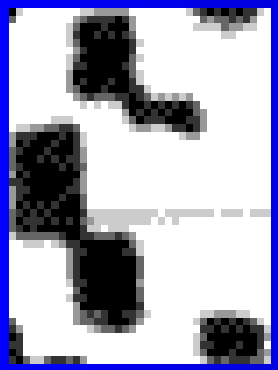
Site-directed Random Mutagenesis



1. Transformation
2. Colony selection
3. DNA cycle sequence
4. Expression of mutant
5. Purification of mutant
6. Kinetic analysis

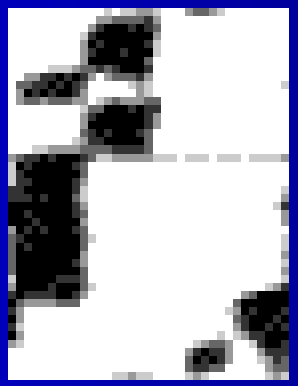
Results of DNA sequence

WTAK
A G C T



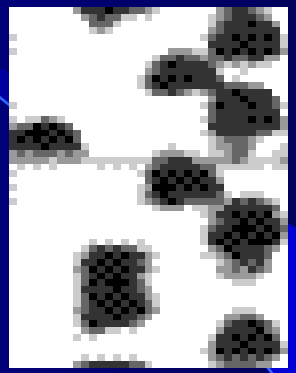
GGCAAAGGT
Gly20
Lys21
Gly22

WTAK
A G C T



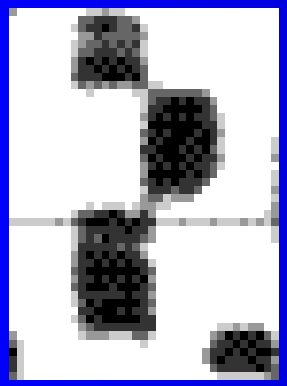
GAGAAAATC
Glu26
Lys27
Ile28

WTAK
A G C T



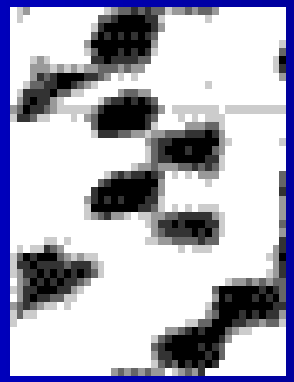
TCTACTGGT
Ser38
Thr39
Gly40

K21P
A G C T



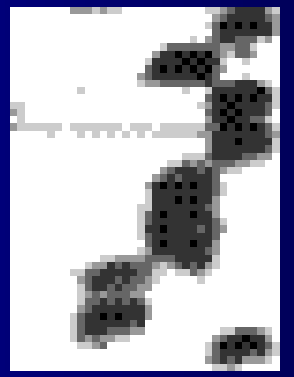
GGCCCGGGT
Gly20
Pro21
Gly22

K27R
A G C T



GAGCGCATC
Glu26
Arg27
Ile28

T39S
A G C T

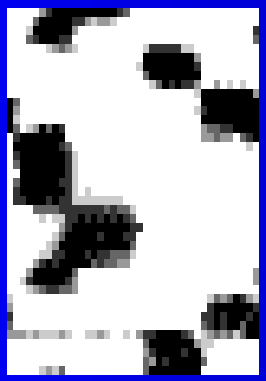


TCTTCCGGT
Ser38
Ser39
Gly40

Results of DNA sequence

WTAK

A G C T



ACTAAGATC

8Thr

9Lys

10Ile

K9F

A G C T

ACTTTCATC

8Thr

9Phe

10Ile

WTAK

A G C T

CTGAAATAATAGTCGA

193L

194K

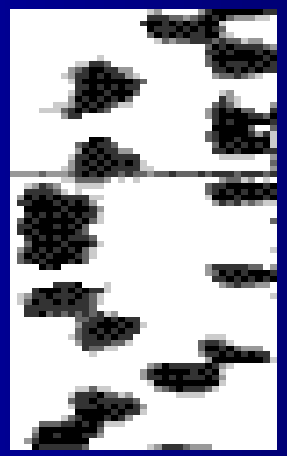
195stop

196stop

Cla I site

K194L

A G C T



CTGTTGTAATAGTCGA

193L

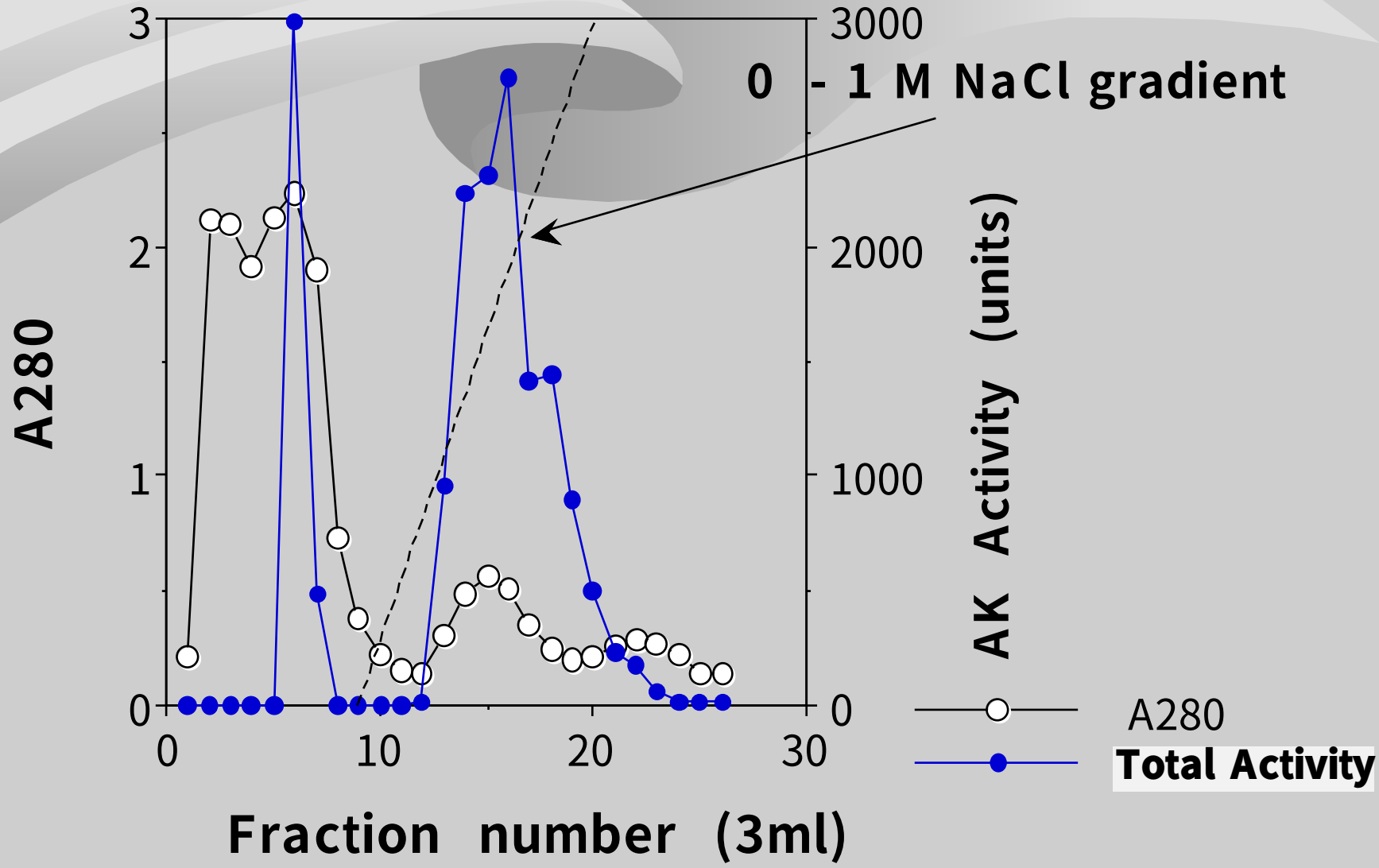
194L

195stop

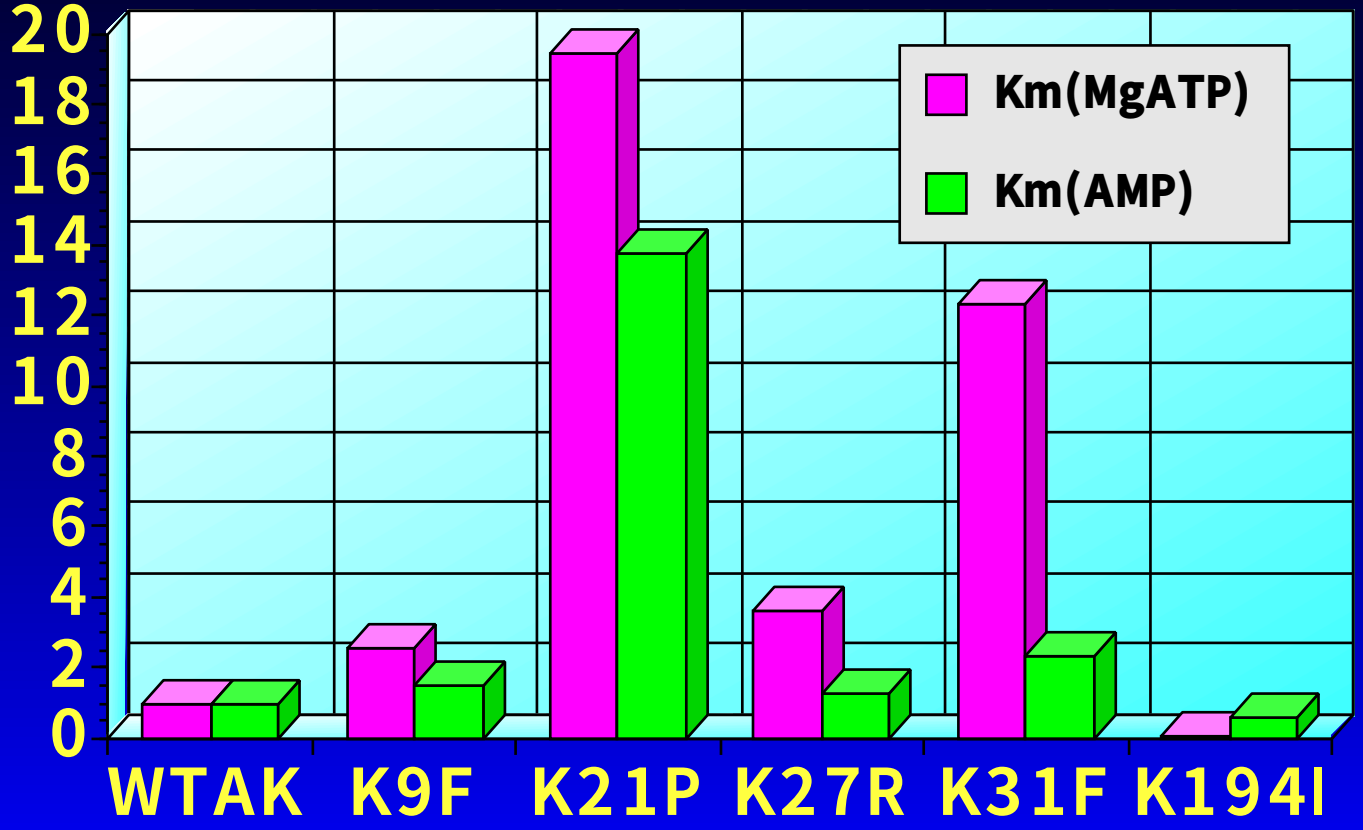
196stop

Cla I site

Blue Sepharose Column Chromatography



Comparison of Km values



Km values

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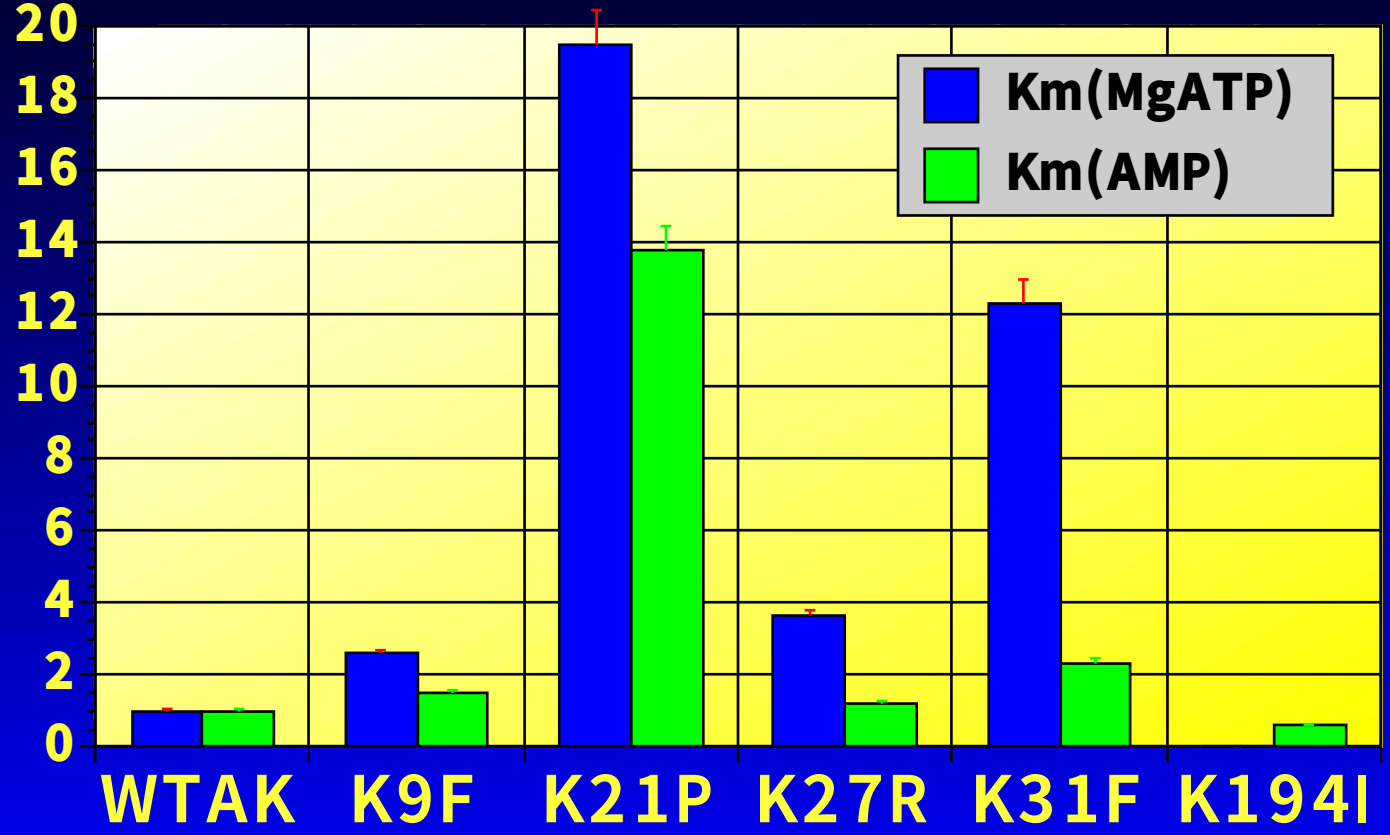
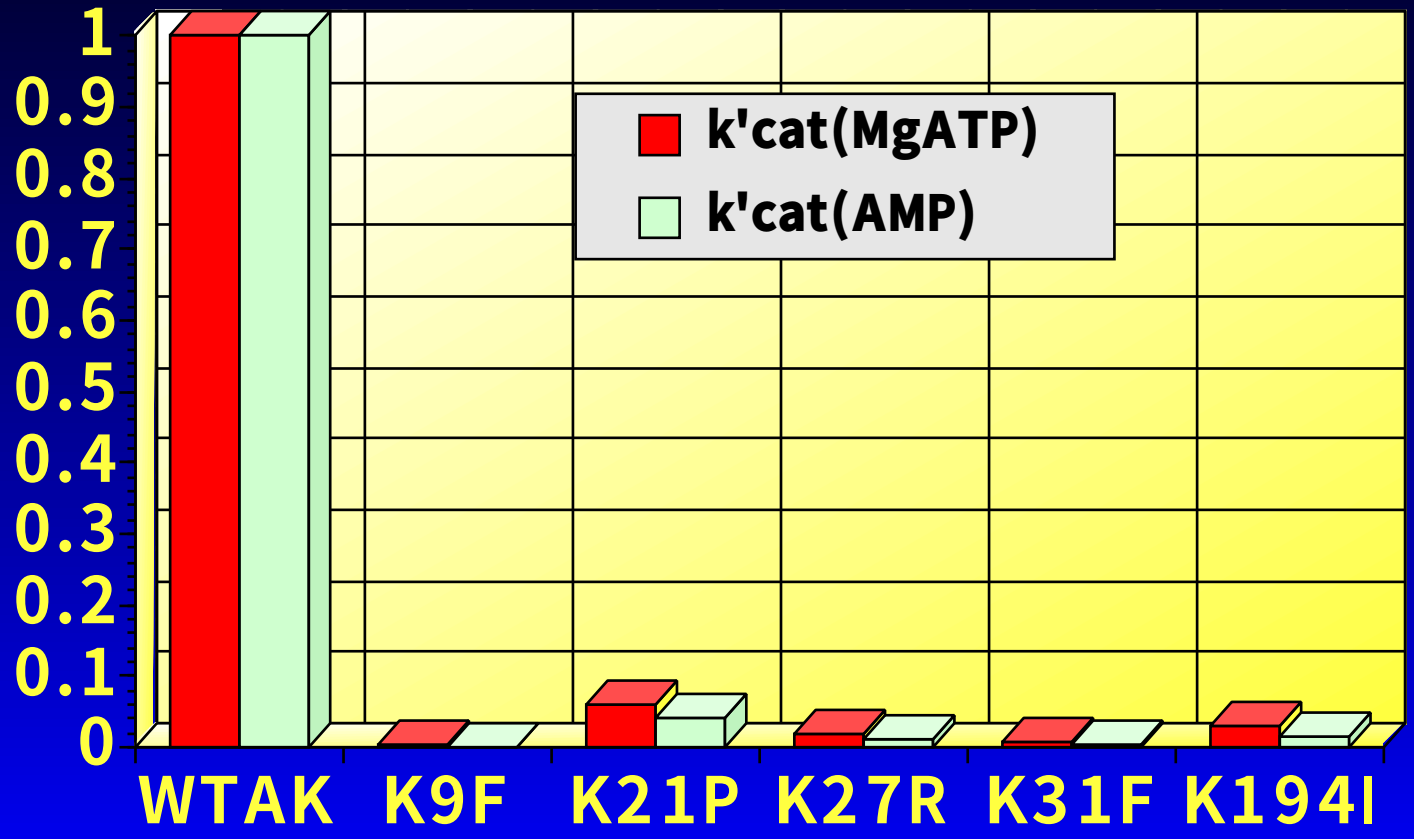


Comparison of k'cat values



k'cat values

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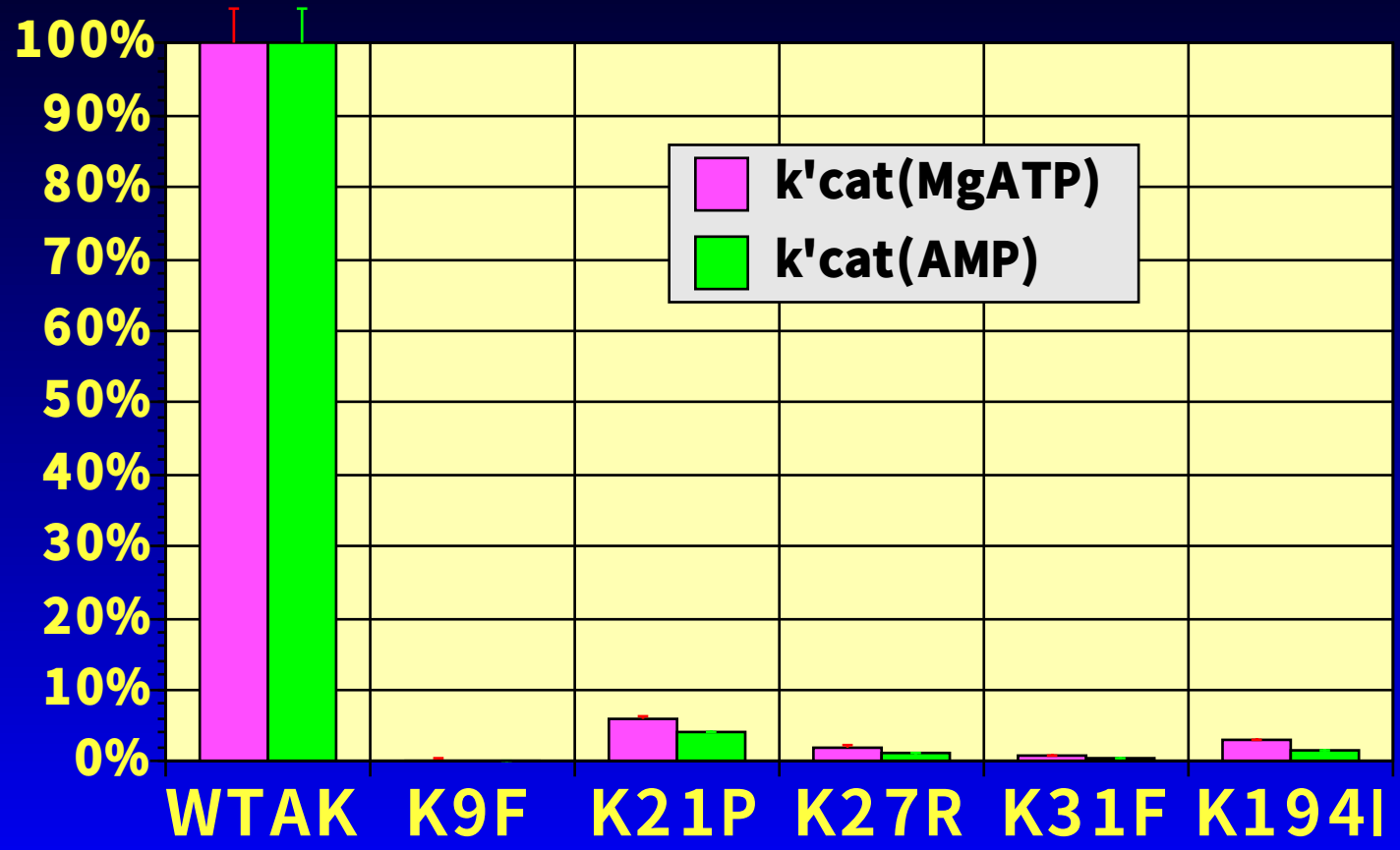
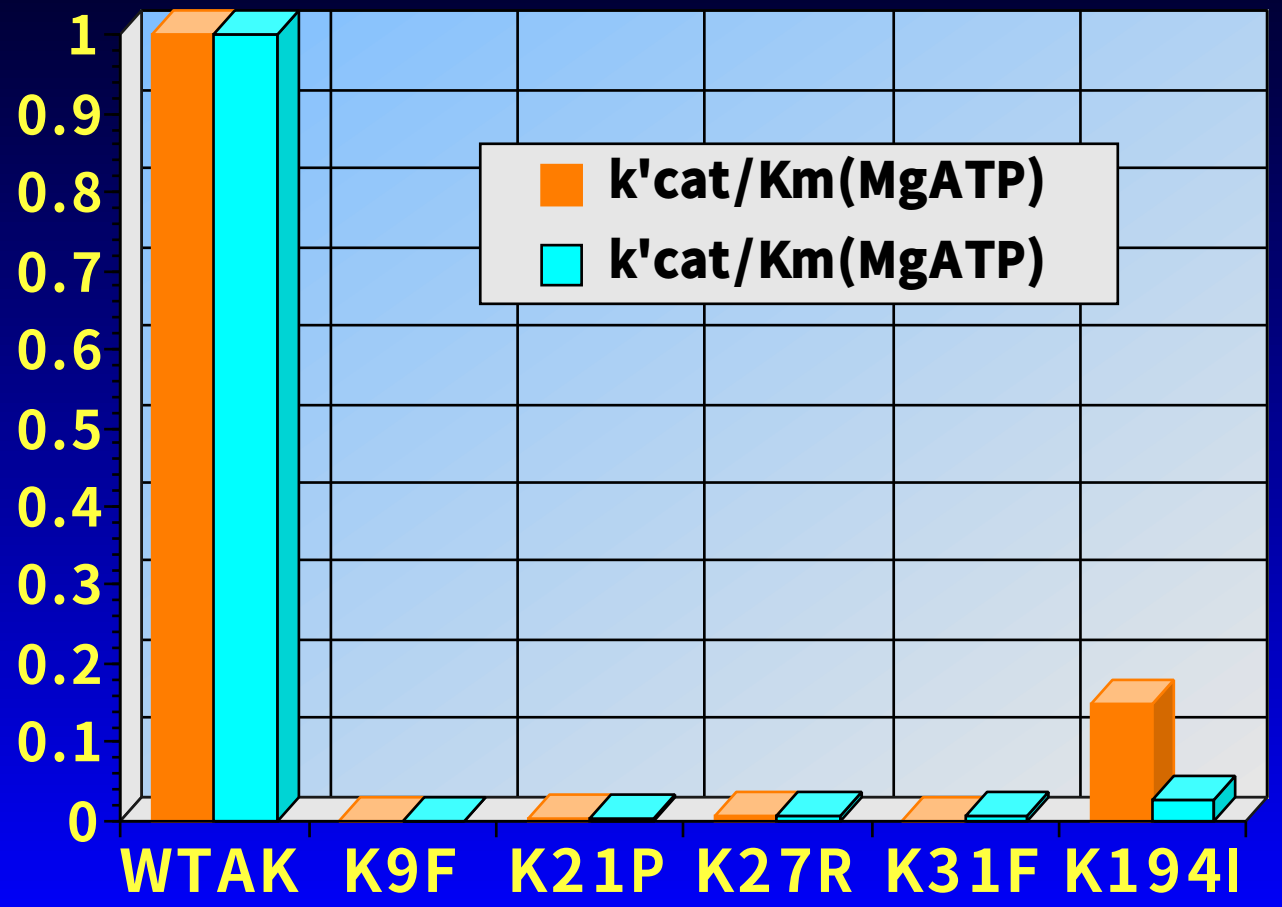


Comparison of k'cat/Km values



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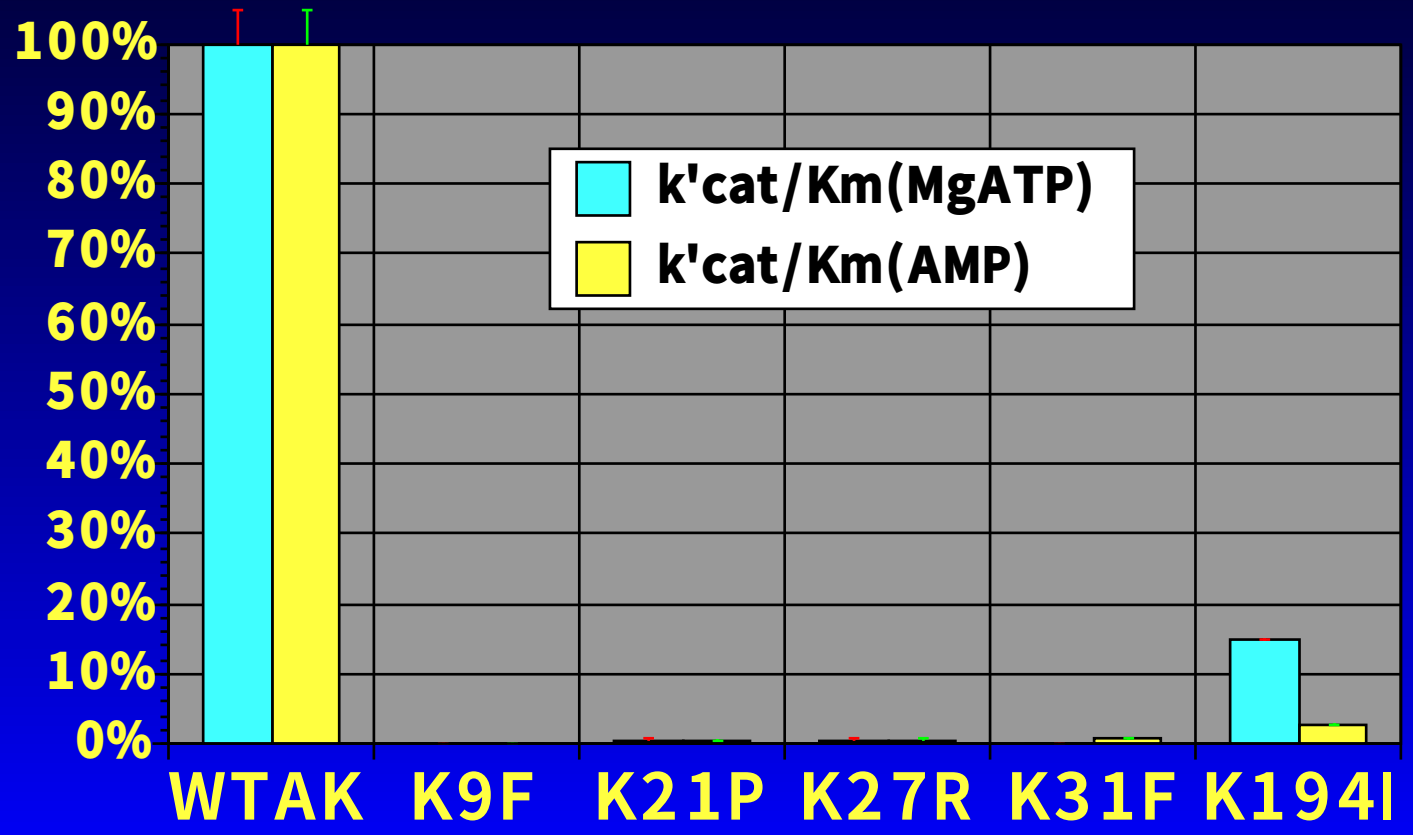
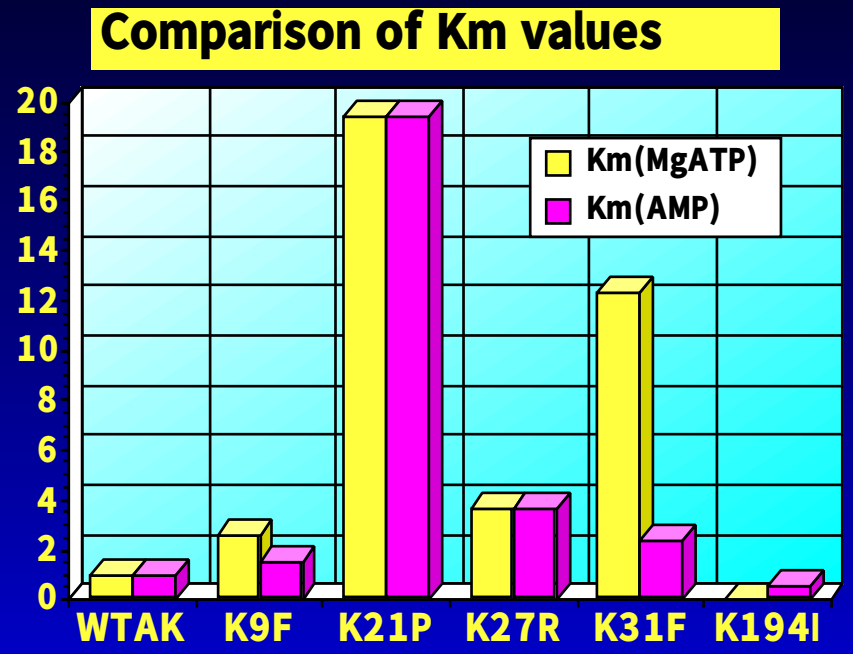
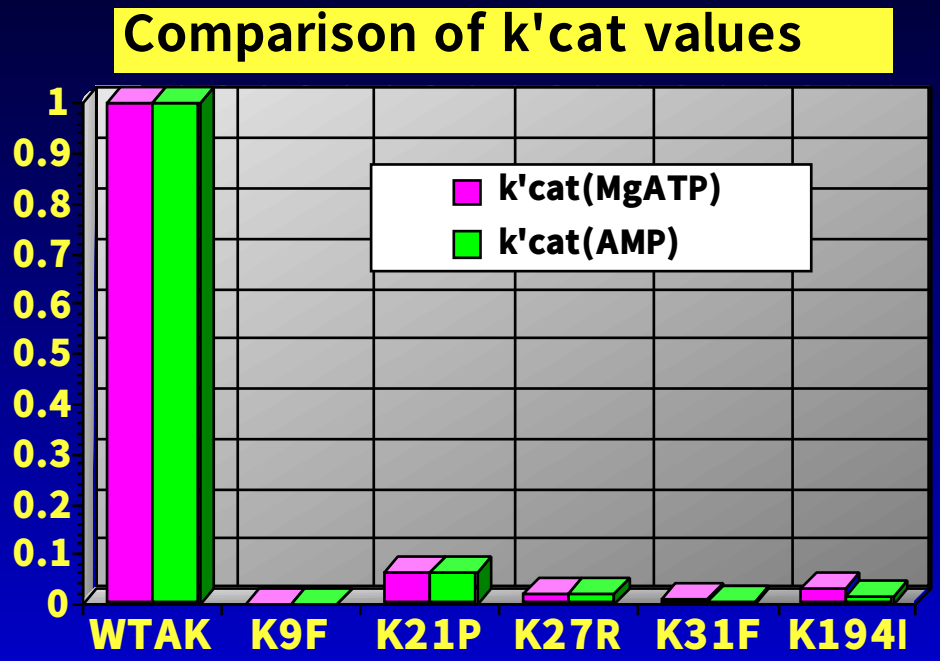


Km and k'cat values





AK model
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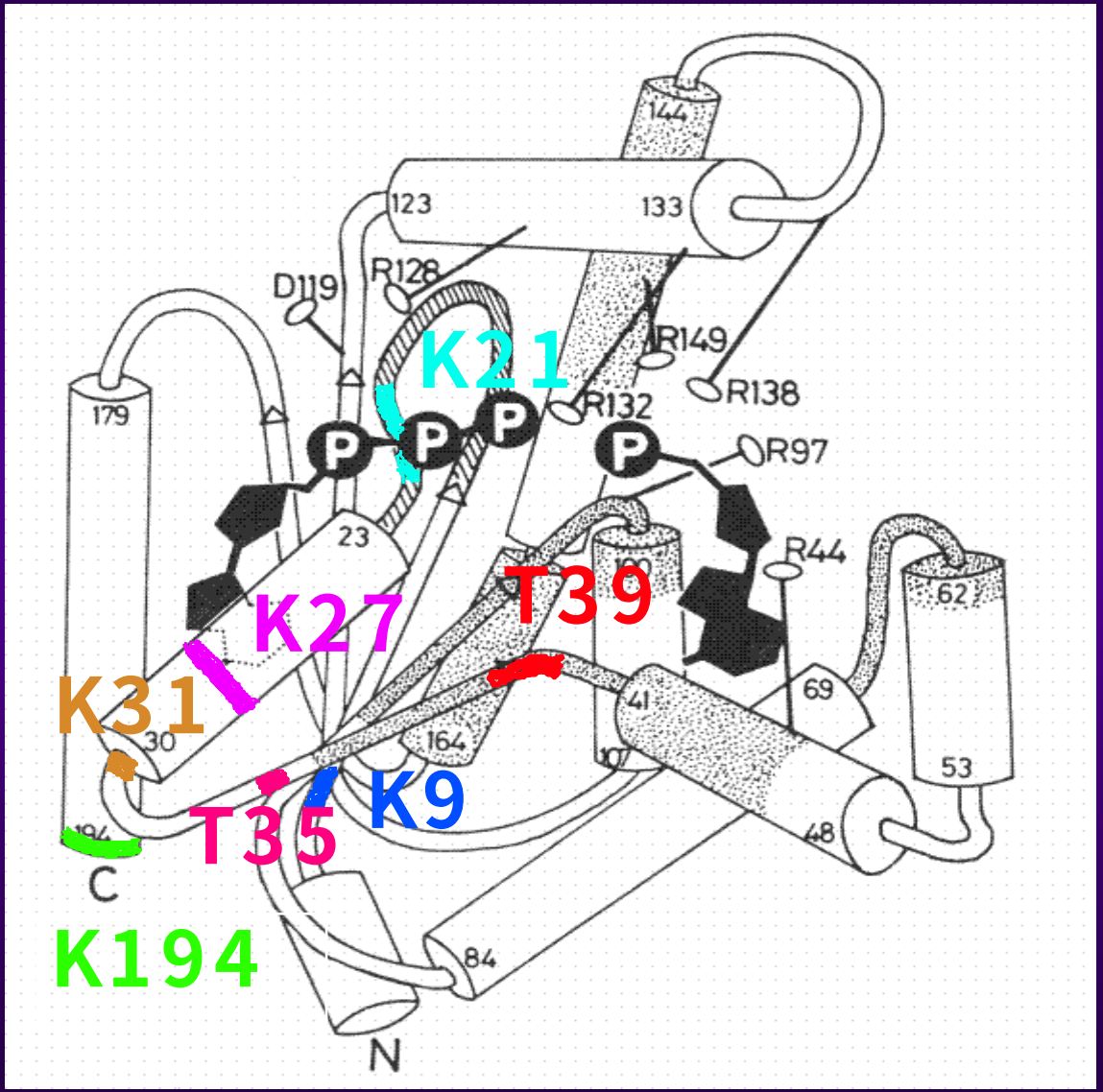

Results

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