Evolutionary and Structural analysis of human Dyrk3

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- Background
- Evolutional analysis of Dyrk3
- Structural analysis of Dyrk3
- Structure-based drug design

Overview of DYRKs Family

- **双特异性酪氨酸磷酸化调控激酶**

  (CMGC 家族)

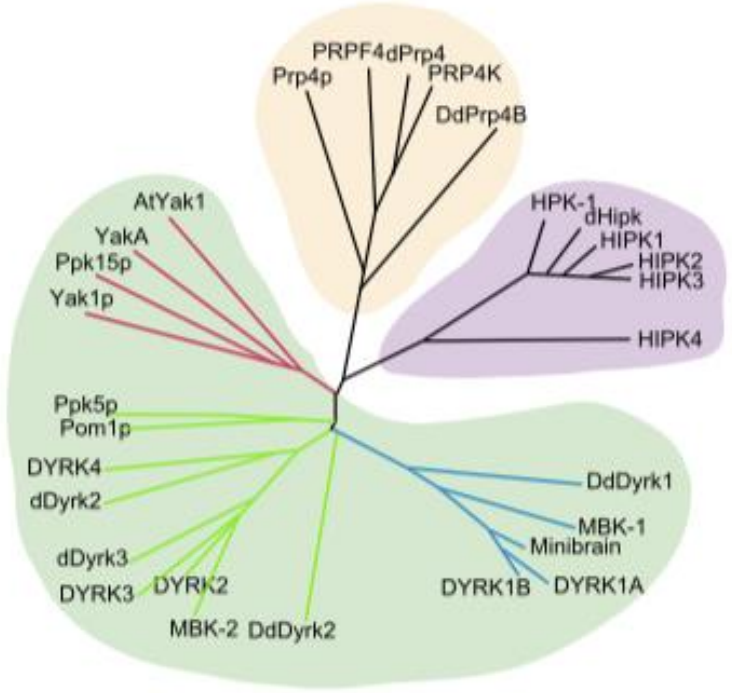
  (在 Tyr发生自磷酸化, 磷酸化底物的Ser&Thr位点)

- **在真菌、原生动物、植物、动物中十分保守**

- **DYRK1A, DYRK1B, DYRK2, DYRK3, DYRK4**

  (哺乳动物细胞)

  (在激酶结构域及其上游区域保守)

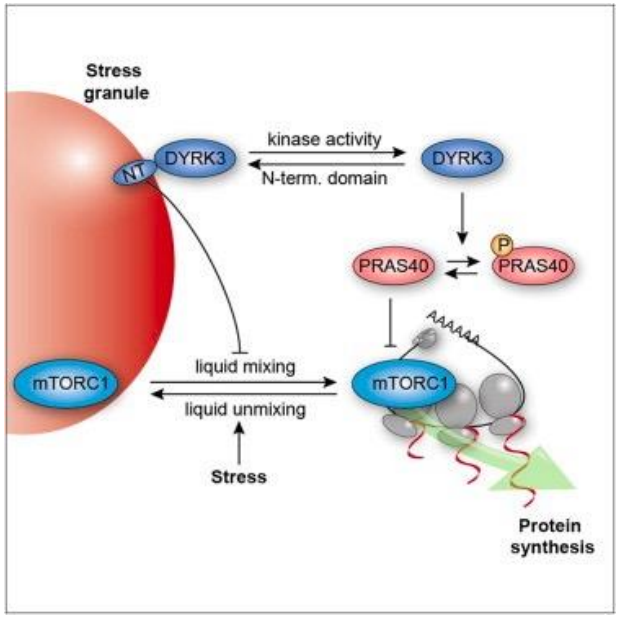


Aranda et al. 2010.

Function of DYRK3

Dyrk3 主要有以下功能：

- 在有丝分裂时期参与无膜细胞器的溶解
- 帮助无膜细胞器压力粒子的定位　(通过mTOR pathway，但其分子机制尚不清楚)

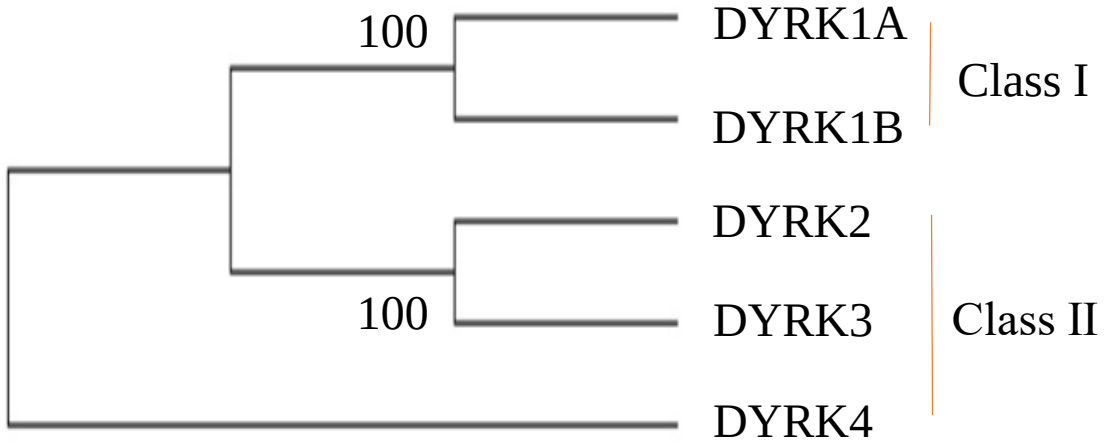


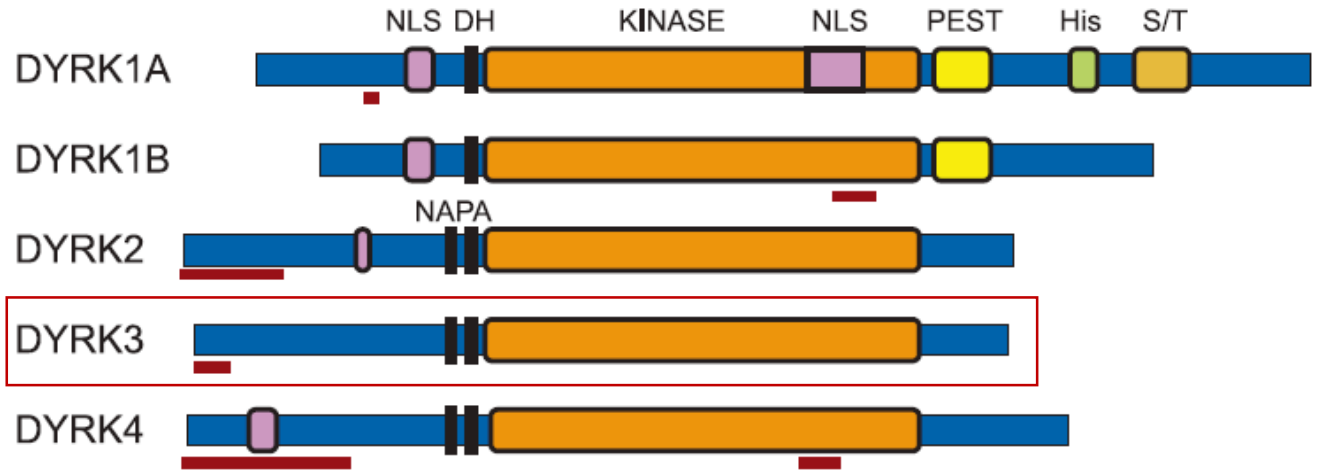
研究目的：

- 通过演化分析寻找序列中重要的突变位点，以期为Dyrk3蛋白功能的分子机制提供思路
- DYRK3 在造血细胞中选择性高表达并减弱成红细胞的发育，是导致贫血主要原因之一。本研究希望通过对Dyrk3的结构特性进行分析，以期为设计更强而有效的抑制剂提供思路，应用于贫血治疗

Kim et al al, 2018　Arpan Kumar Raiet al, 2018　Becker W. et al, 1998

Evolutional analysis of Dyrk3





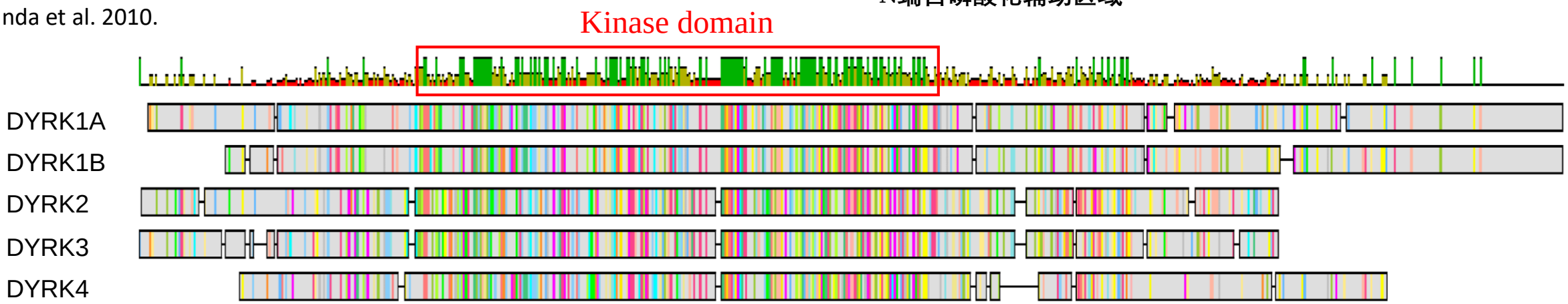
NLS: nuclear localization signal

DH: DYRK-homology box

NAPA: N-terminal autophosphorylation accessory region

N端自磷酸化辅助区域

Aranda et al. 2010.



Evolutional analysis of Dyrk3

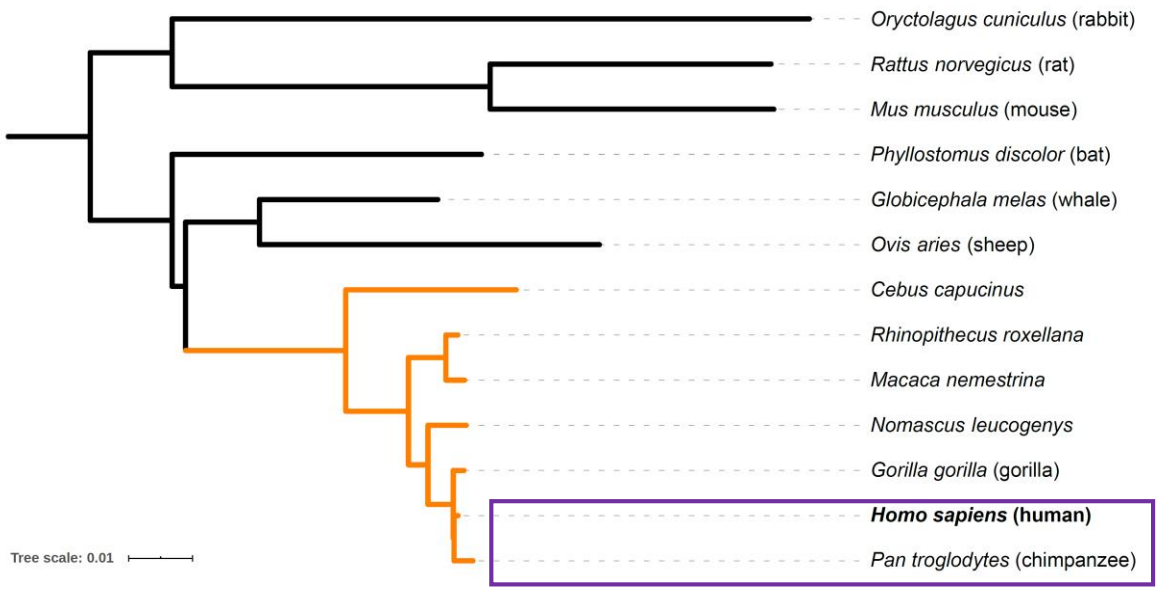
Detection of Positive Selection Using PAML

$\omega = dN/dS$, 非同义突变率和同义突变率的比值

$\omega < 1$, 负向选择

$\omega = 1$, 中性进化

$\omega > 1$, 正向选择



分支模型（branch models）得到的 ω 值在进化树的各分支间差异较大，从而对于检测在某些谱系上发生的正向选择十分有效

- 零假设：系统发生树中的所有分支具有同样的dN/dS
- 备选假设：我们指定的分支具有和背景不同的dN/dS

P value $= 3.49 \times 10^{-3}$

分支-位点模型（branch-site models）

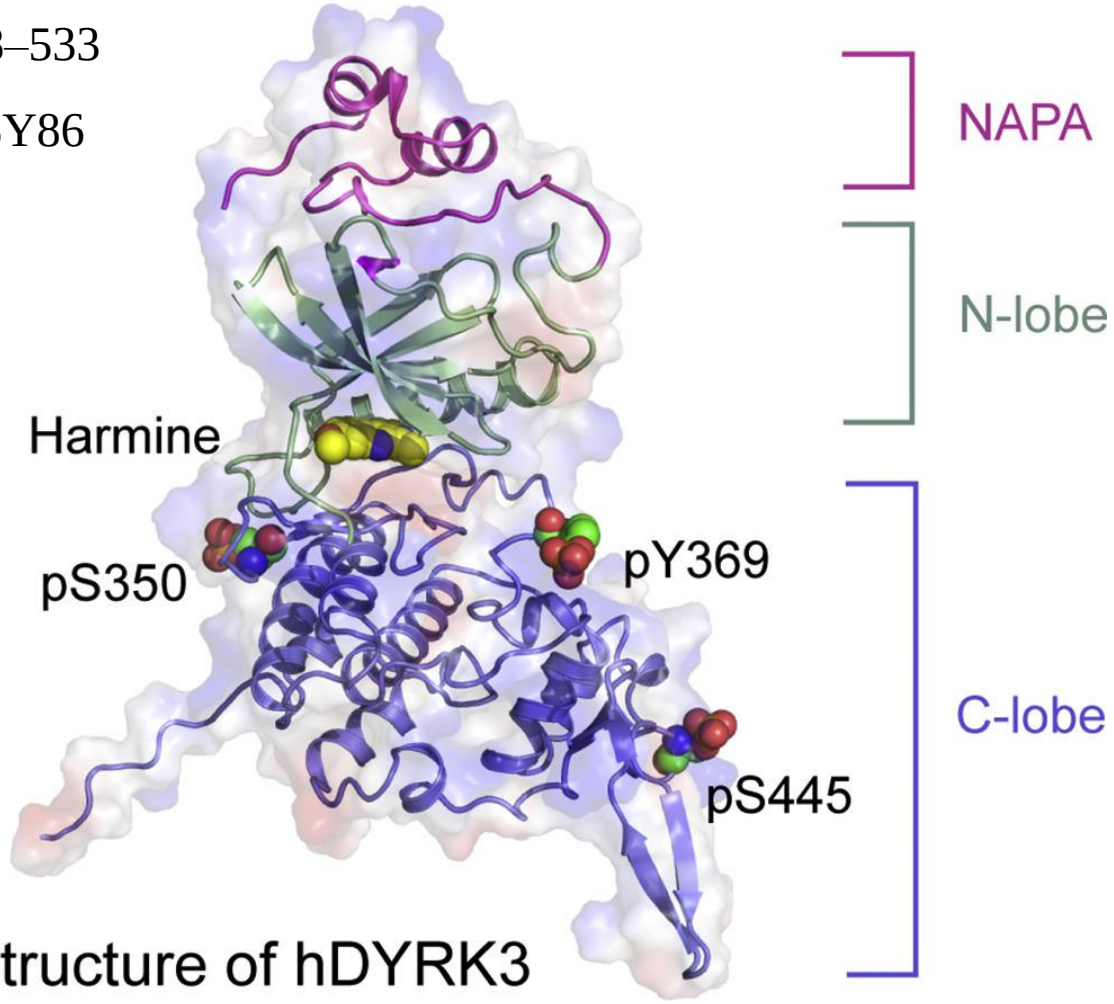
45 R 0.983

Structural analysis of Dyrk3

Residues : 138–533

PDB entry : 5Y86

1.9 Å

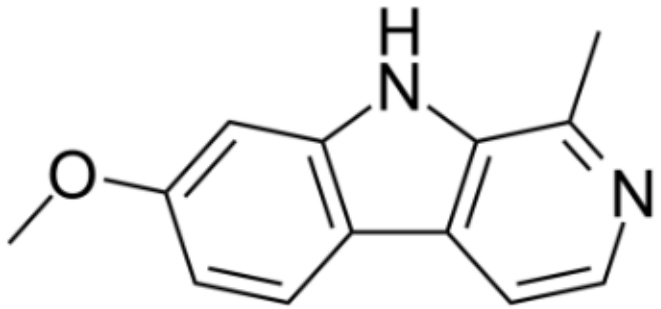


Crystal structure of hDYRK3

Similar to other DYRKs family members —— a common kinase fold

NAPA : N-terminal autophosphorylation accessory domain

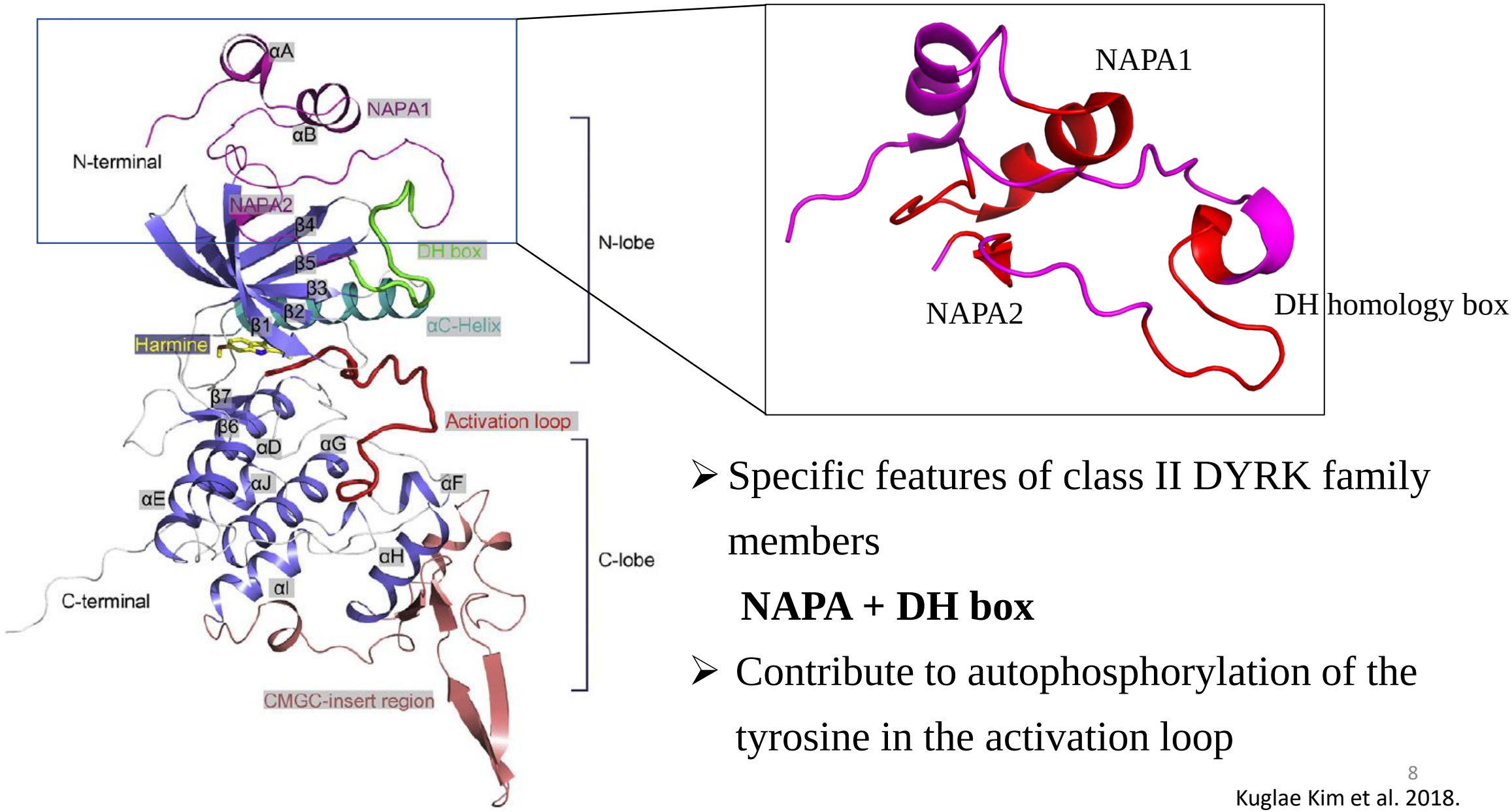
Harmine



CAS No. : 442-51-3

Kuglae Kim et al. 2018.

Structural analysis of Dyrk3



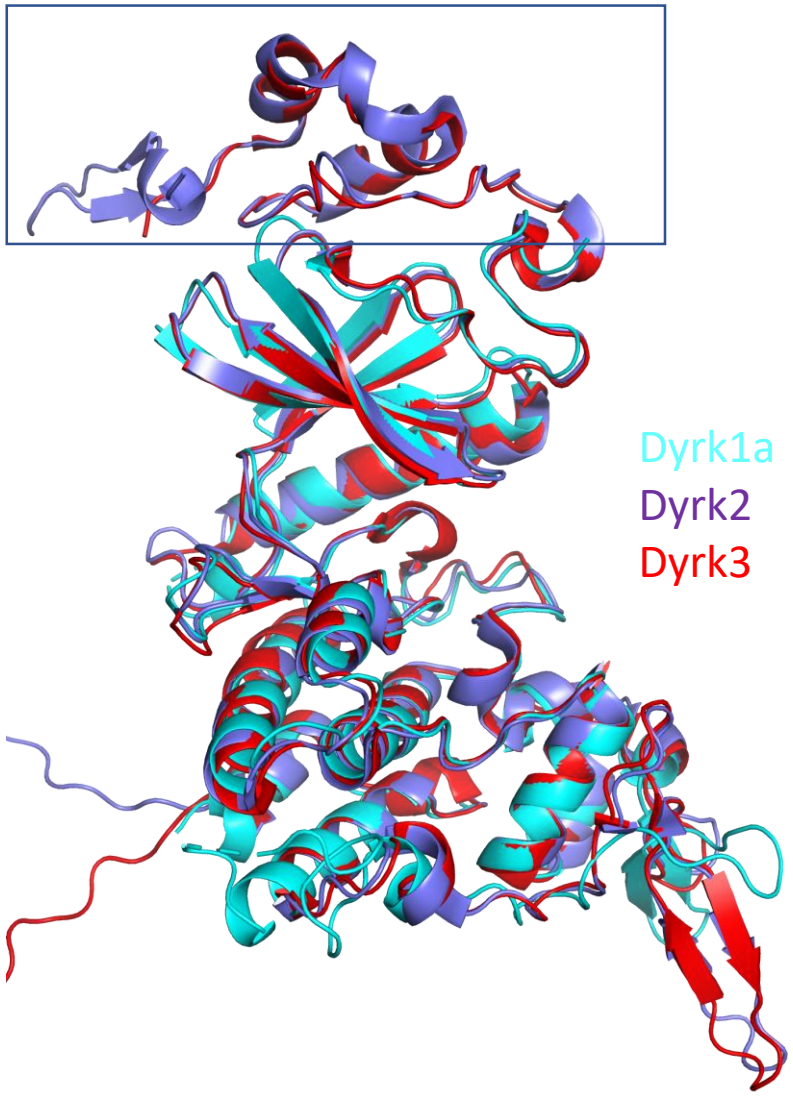
- Specific features of class II DYRK family members

  NAPA + DH box

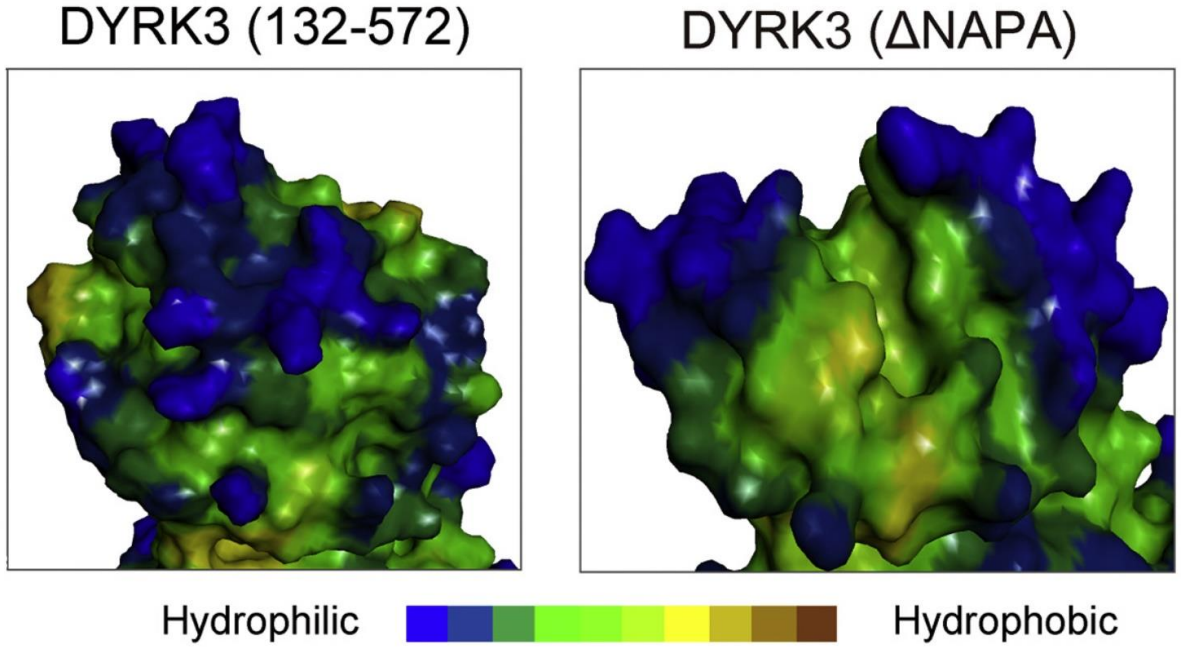
- Contribute to autophosphorylation of the tyrosine in the activation loop

Kuglae Kim et al. 2018.

Structural analysis of Dyrk3

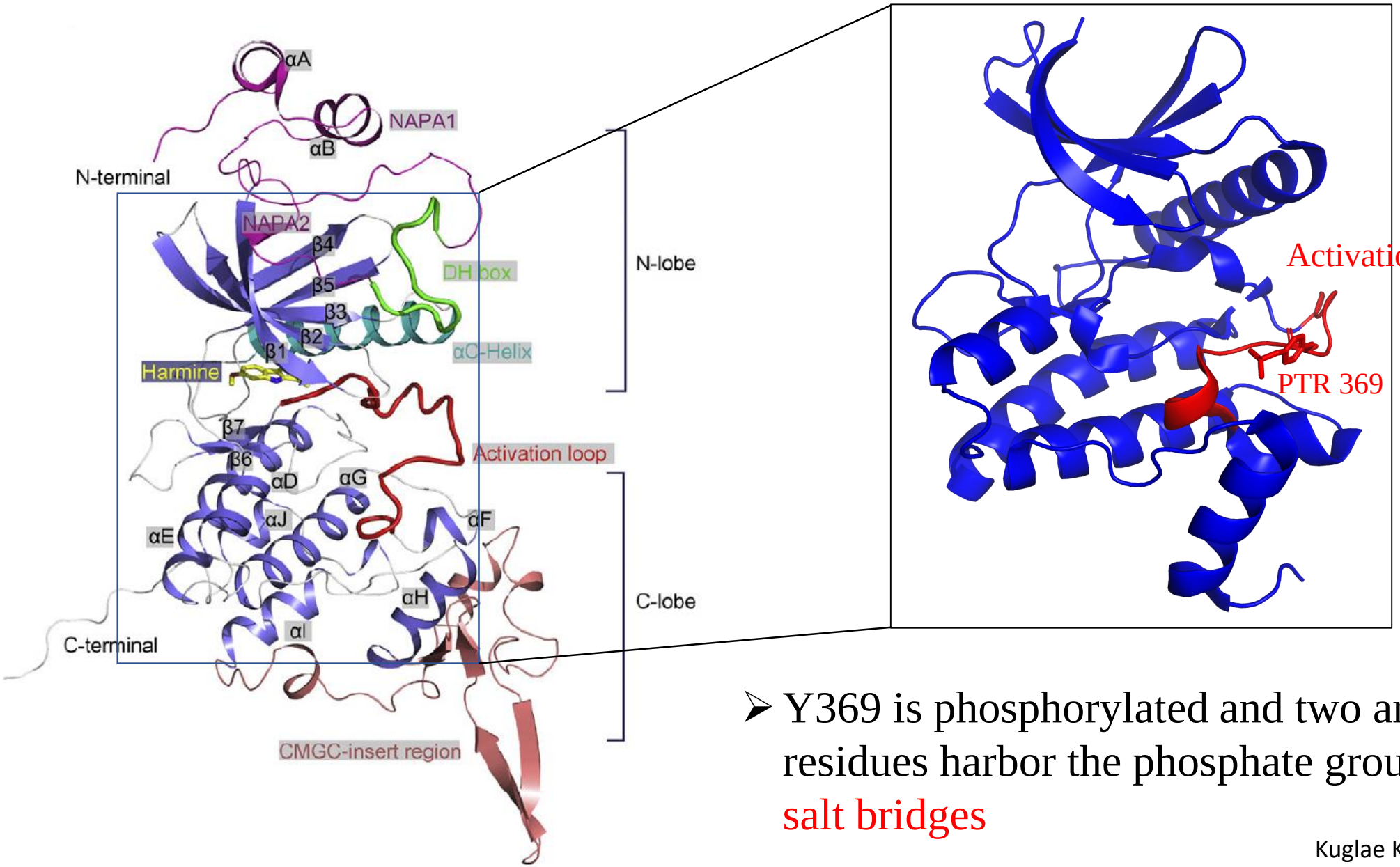


- **Structural alignment**
  Dyrk3 vs Dyrk1A (RMSD) : 0.948
  Dyrk3 vs Dyrk2 (RMSD) : 0.637
- NAPA is an **important domain** for DYRK3 protein stability



Kuglae Kim et al. 2018.

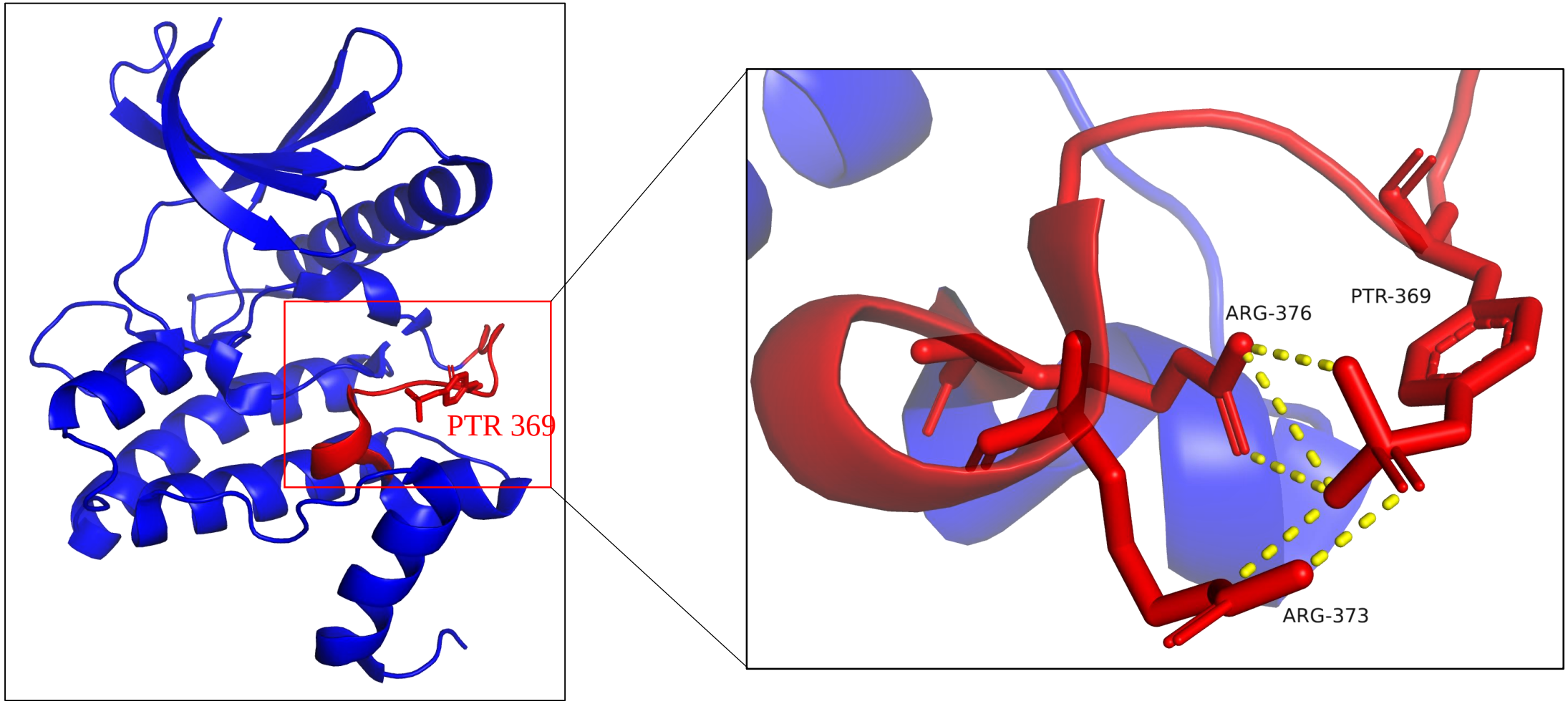
Structural analysis of Dyrk3



- Y369 is phosphorylated and two arginine residues harbor the phosphate groups by salt bridges

Kuglae Kim et al. 2018.

Structural analysis of Dyrk3



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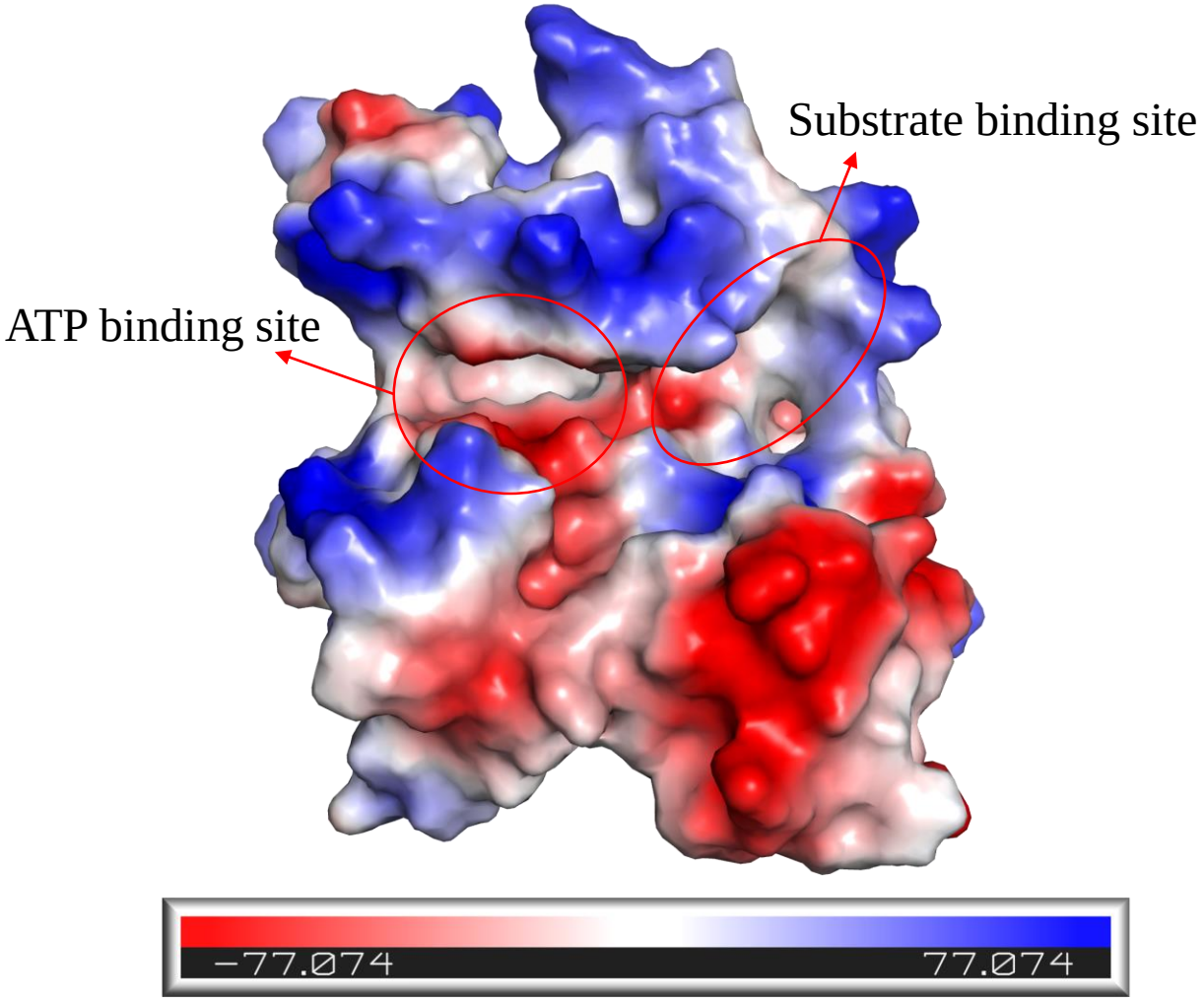
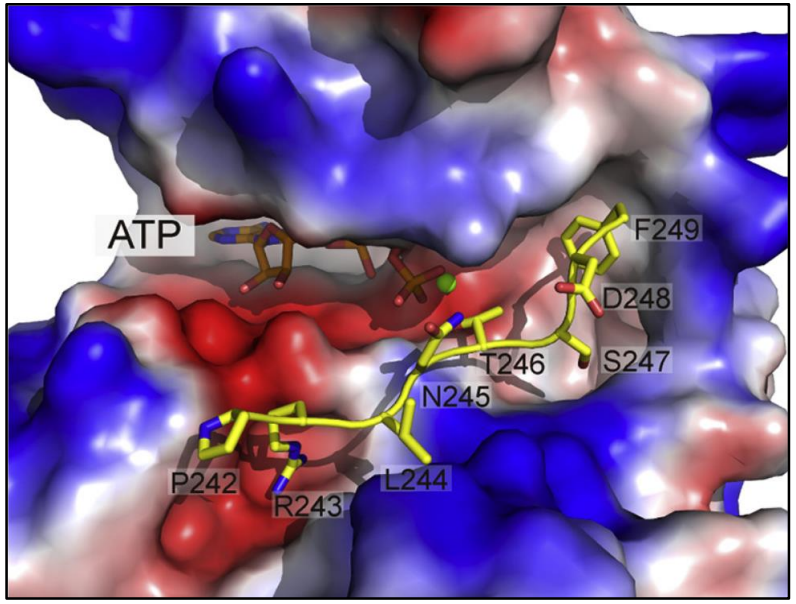
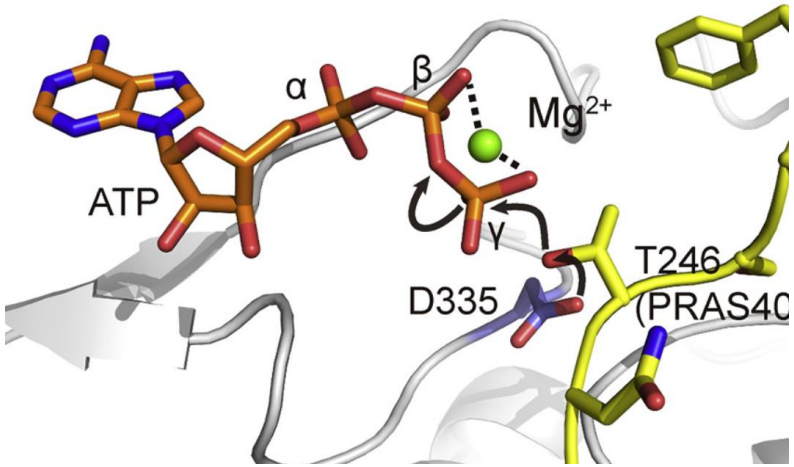
Structural analysis of Dyrk3

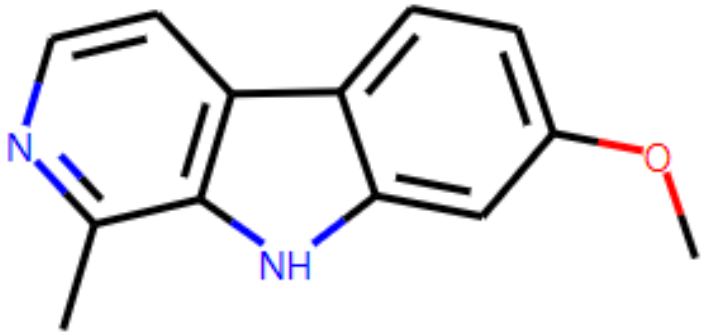






Kuglae Kim et al. 2018.

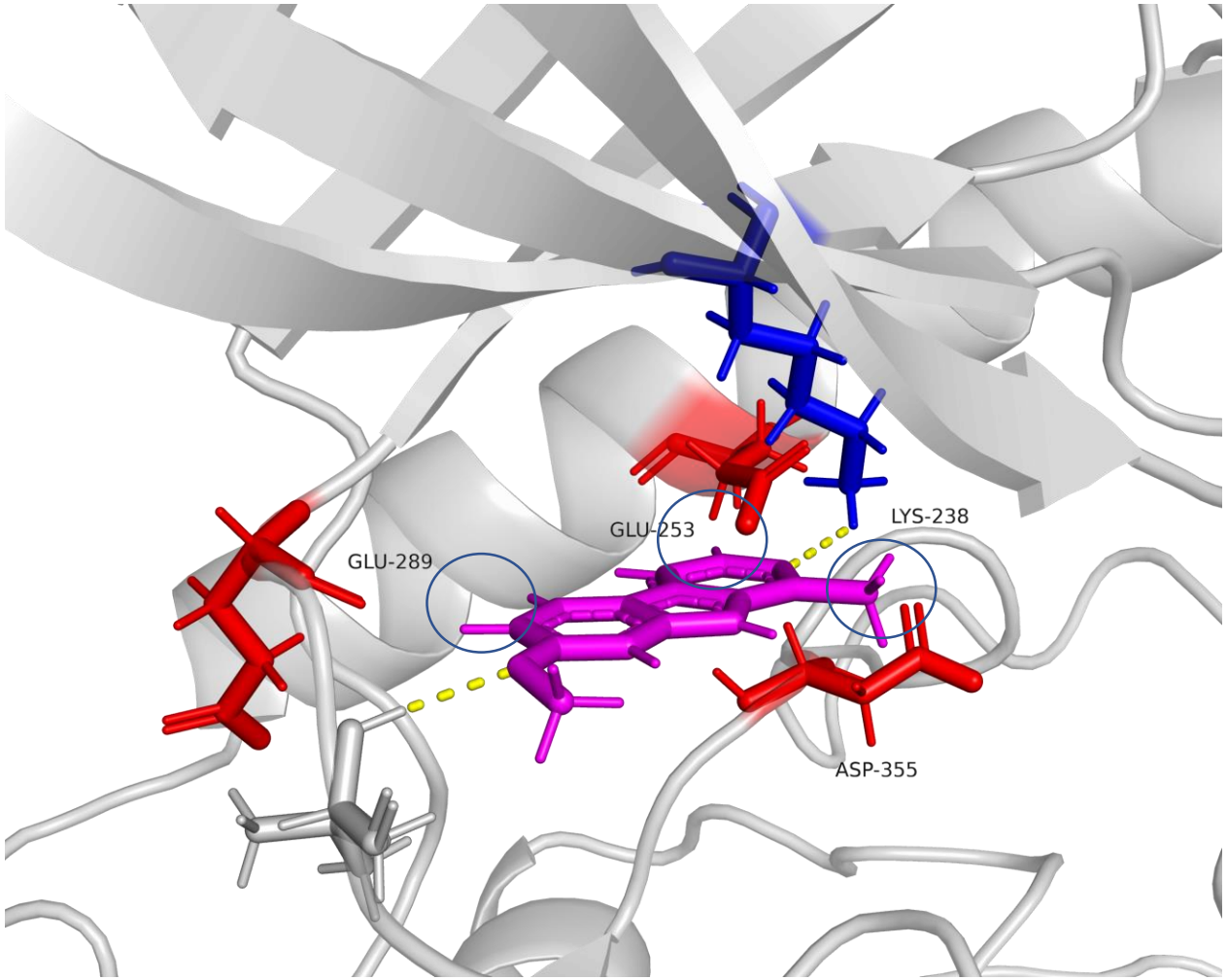
Structure-based drug design



Harmine
IC50 0.8 μM

Molecular structure optimization

- Add more polar contacts
- Increase hydrophobic interaction



Structure-based drug design

Molecular structure optimization

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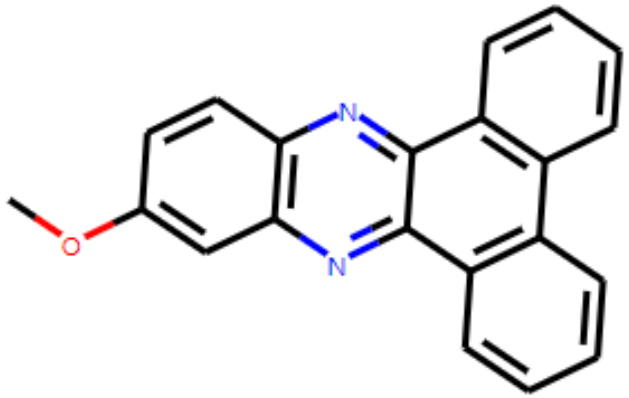

11-methoxydibenzo[a,c]phenazine

SMILES:c12c(nc3c(n1)cc(cc3)OC)c1c(c3c2cccc3)cccc1

Similarity Searching

Query Molecule : Ligand of PDB:5Y86

Target Database : Specs (210776 commercial compounds)

Method : Tanimoto Similarity

Figerprint : FP2 (1024 bit) Calculated by Open Bable

Tanimoto : 0.833333

Ref: https://www.specs.net/

Ref: http://openbabel.org/wiki/Main_Page

下一步工作计划

演化分析：

- 针对45R 设计系列突变体，研究该位点与压力粒子定位的关系，以为其分子机制提供更多的信息。

结构特性分析：

- 基于上述结构分析，针对Harmine进行增加极性相互作用的侧链修饰
- 合成增加疏水相互作用的新小分子，如有可能则继续对该分子进行修饰，增加其亲和力
- 对合成的小分子进行亲和力测定: ITC（等温量热滴定）SPR（表面等离子共振）MST（微量热涌动），筛选出最具有成药性的分子。

THANKS

Contribute:

报告人：刘番

背景资料收集：王治、张莹、刘番

演化分析：滕德群、孟德兰

结构分析：刘番、邓喆方

基于结构的药物设计：刘番、徐聪颖

Half day on the Web, saves you half month in the lab!

Supplementary

SMI	Name	Tanimoto
c12c(nccc2C)c(ccc1OC)OC	5,8-dimethoxy-4-methylquinoline	0.555556
c1(c2ncccc2ncc1Br)O	3-bromo[1,5]naphthyridin-4-ol	0.557143
c12c(c(cc(n1)OC)OC)cc(c(c2)OC)OC	2,4,6,7-tetramethoxyquinoline	0.56338
n1(c2c(cc1)cc(cc2)OC)CCN	2-(5-methoxy-1H-indol-1-yl)ethanamine	0.571429
c12c(cc(nc1cccc2OC)OC)OC	2,4,5-trimethoxyquinoline	0.634921
c12c(nc3c(n1)cc(Oc1ccc(N)cc1)cc3)c1c(c3c2cc(cc3)I)cccc1	4-[(7-iododibenzo[a,c]phenazin-11-yl)oxy]phenylamine	0.652174
c12c(cc(cc1)Cl)nccc2OC	7-chloro-4-quinolinyl methyl ether	0.655738
c1(=O)c2c([nH]c3c1cccc3)cccc2OC	1-methoxy-9(10H)-acridinone	0.666667
[n+]12c3cc(ccc3ccc1cccc2)OC	9-methoxypyrido[1,2-a]quinolinium	0.8
c12c(nc3c(n1)cc(cc3)OC)c1c(c3c2cccc3)cccc1	11-methoxydibenzo[a,c]phenazine	0.833333

Supplementary

Properties	Value	Probability
Human Intestinal Absorption	+	0.9966
Human oral bioavailability	+	0.8000
Honey bee toxicity	+	0.6911
Ames mutagenesis	+	0.8600
CYP1A2 inhibition	+	0.9551
P-glycoprotein inhibitior	+	0.6419

Ref: http://lmmd.ecust.edu.cn/admetsar2/result/?tid=46757