# Adenylate Kinase

Various forms and role in cellular energy homeostasis

University of Delhi, 2023

# Introduction

- Adenylate kinase (AK, ATP:AMP phosphotransferase or more commonly Myokinase) is present in almost all eukaryotes (IMS of mitochondria) and prokaryotes (in cytoplasm & periplasm).
- It catalyzes transfer of phosphate group from a high energy doner to an acceptor substrate in a process called Phosphorylation. In case of kinase catalyzed reactions, high energy phosphate doner is usually ATP.

$$Mg^{2+}.ATP + AMP \overset{AK}{\rightleftharpoons} Mg^{2+}.ADP + ADP$$

- AK plays a crucial role in regulating and balancing production and expenditure of cellular energy by monitoring levels of ATP, ADP and AMP inside cell and thus helps in achieving homeostasis.

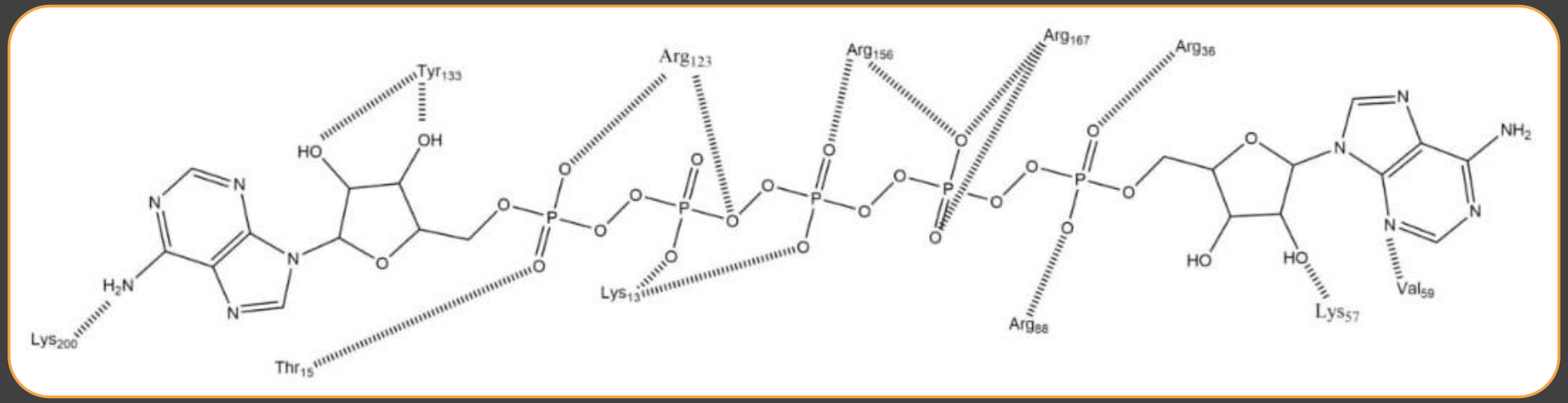
# Structure and Mechanism

- Domains and Binding Sites
- Conformational Mechanism
- Inhibition Testing
- MD simulations

# Structure of AK

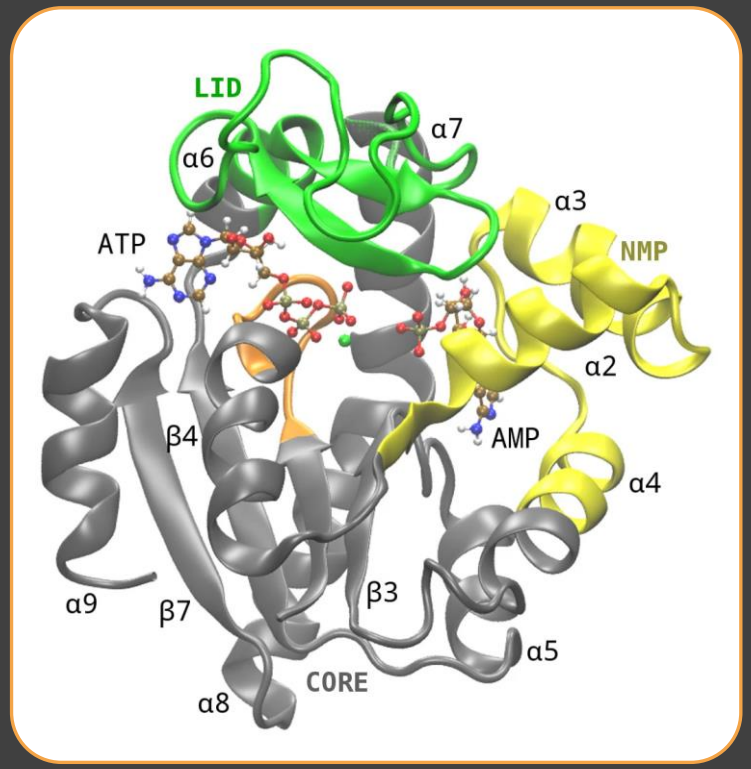
- AK in human is composed of **194** amino acid residues with molecular weight ~**22kDa**. Bacterial AK is made up of ~210-220 amino acids.
- Human AK is a globular protein composed of three domains – **CORE**, **LID** and Nucleoside Monophosphate (**NMP**) substrate binding region.
- CORE contains glycine rich region where phosphate doner binds called **P-loop**. It consists of 6 β-strands and 6 α-helices.
- The surface loop region (LID and NMP) connects the β-strands and α-helices of CORE and contains ATP & AMP binding sites.
- CORE is conformationally unaffected during enzymatic action of AK, while the surface loop region is highly flexible and undergoes significant conformational changes upon substrate binding, triggering the catalytic activity of enzyme.
- The ATP binding site is situated between CORE and LID domains (near N-terminal) and the AMP site is located between CORE and AMP (near C-terminal).

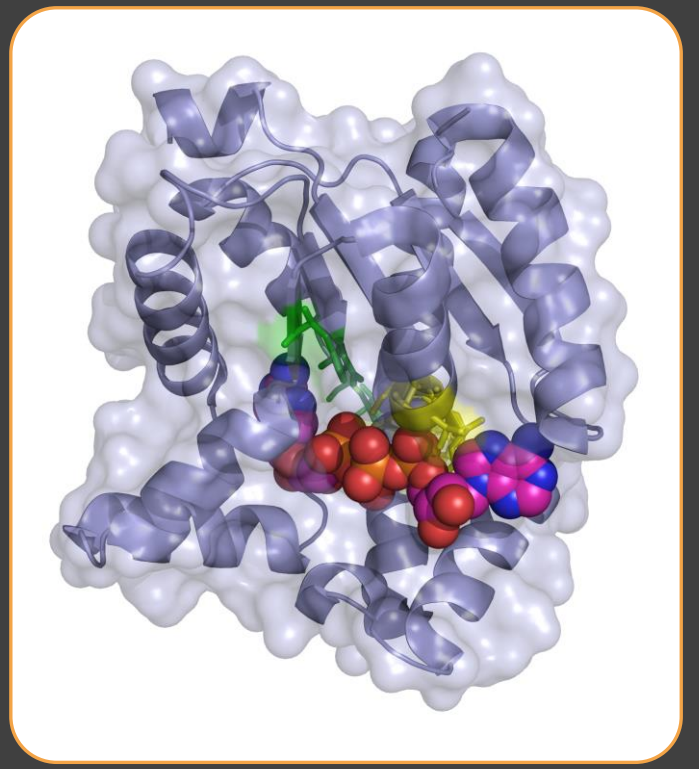
(a) Residue of AK in *Escherichia coli* involved in substrate binding




(b) AK bound to ATP and AMP

- CORE (1-29,68-117,161-214) – Gray
- LID (118-160) – Green
- NMP (30-67) – Yellow
- P-loop (7-13) – Orange



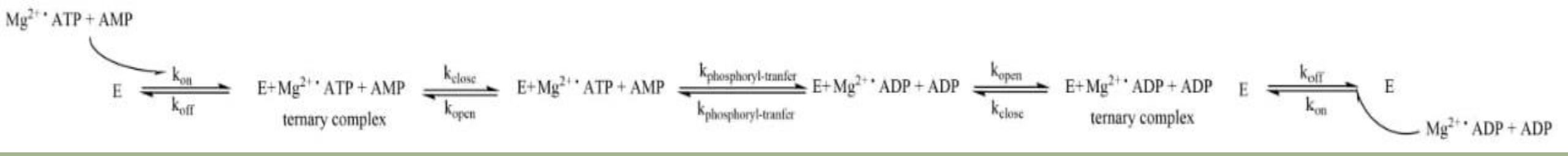



(c) 3D Ribbon model of AK complexed with 2ADP

- Active center – Yellow
- ATP binding site – Green

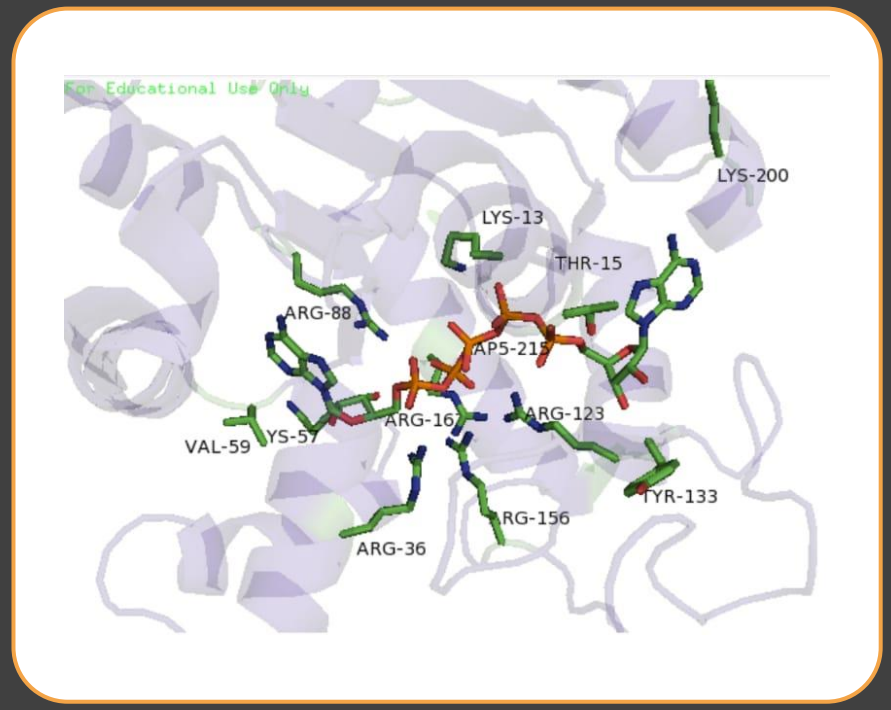
# Mechanism OF AK

- In absence of any bound ligand, enzyme is in unligated '*open*' conformation. As $Mg^{2+}$ complexed phosphate doner binds with enzyme, it triggers closing of open surface loop of LID, which signals NMP binding site to close with substrate bound within the pocket. Thus AK adopts '*closed*' conformation.
- In closed state, AK globular structure shrinks, causing water molecules to expel from enzymatic cavity, which brings the substrate in proximity with phosphate doner.
- Presence of $Mg^{2+}$ increases electrophilicity of ATP resulting in migration of γ-phosphate from ATP to AMP.
- Phosphate transfer triggers LID and NMP to open up releasing the ADP molecules. This marks completion of one catalytic cycle resulting in AK back to its '*open*' conformation.



# Inhibition Testing

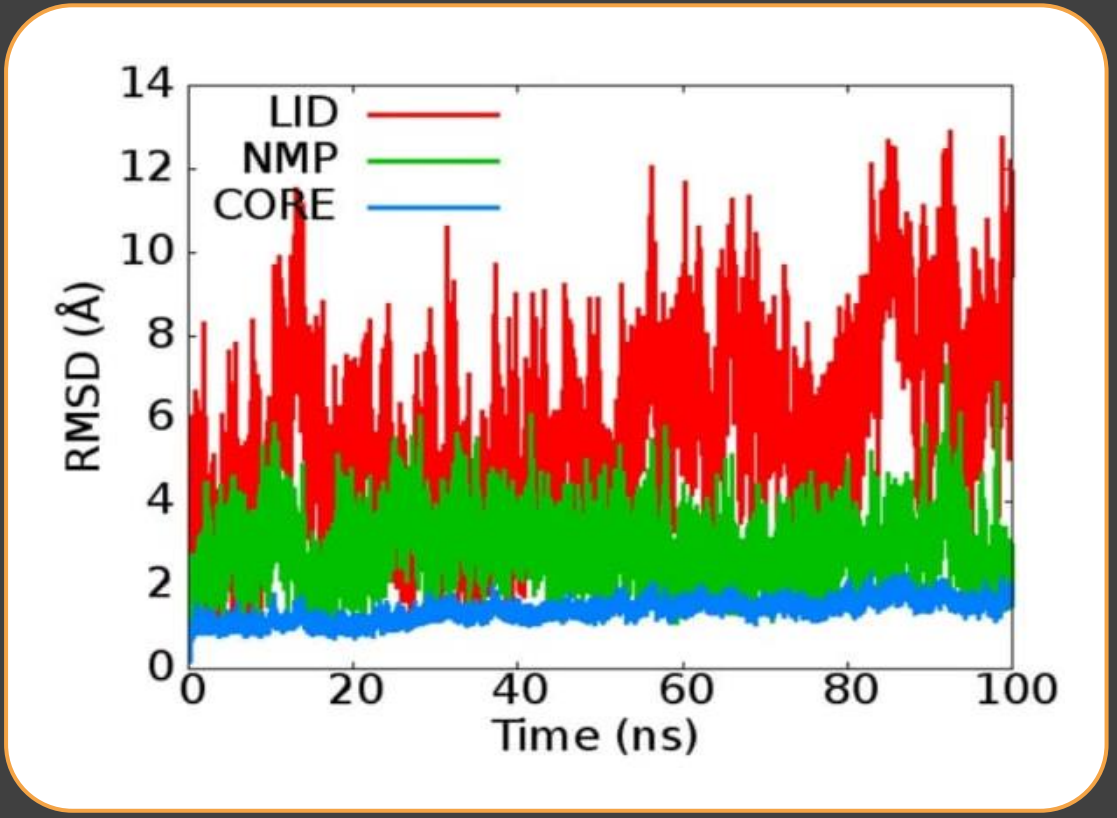
- Crystal structure of AK from *E. coli* with AP5A [ P1,P5-Di(adenosine-5') pentaphosphate] inhibitor shows that AP5A binds with Arg88 at α-phosphate group. This inhibition results 99% loss of catalytic activity suggesting Arg88 plays crucial role in phosphoryl transfer reaction.
- Another residue in CORE domain that remains highly conserved across species is Arg119, located at adenosine binding region of AK and binds with Adenine within active site.
- Other conserved residues are Lys113, Arg123, Arg156 and Arg167 which form a network of positive amino acids which stabilizes accumulation of negative charge on phosphoryl group during the transfer.
- This lysine-arginine network is also bound by two distal aspartate residues that causes the enzyme to fold and lose flexibility.



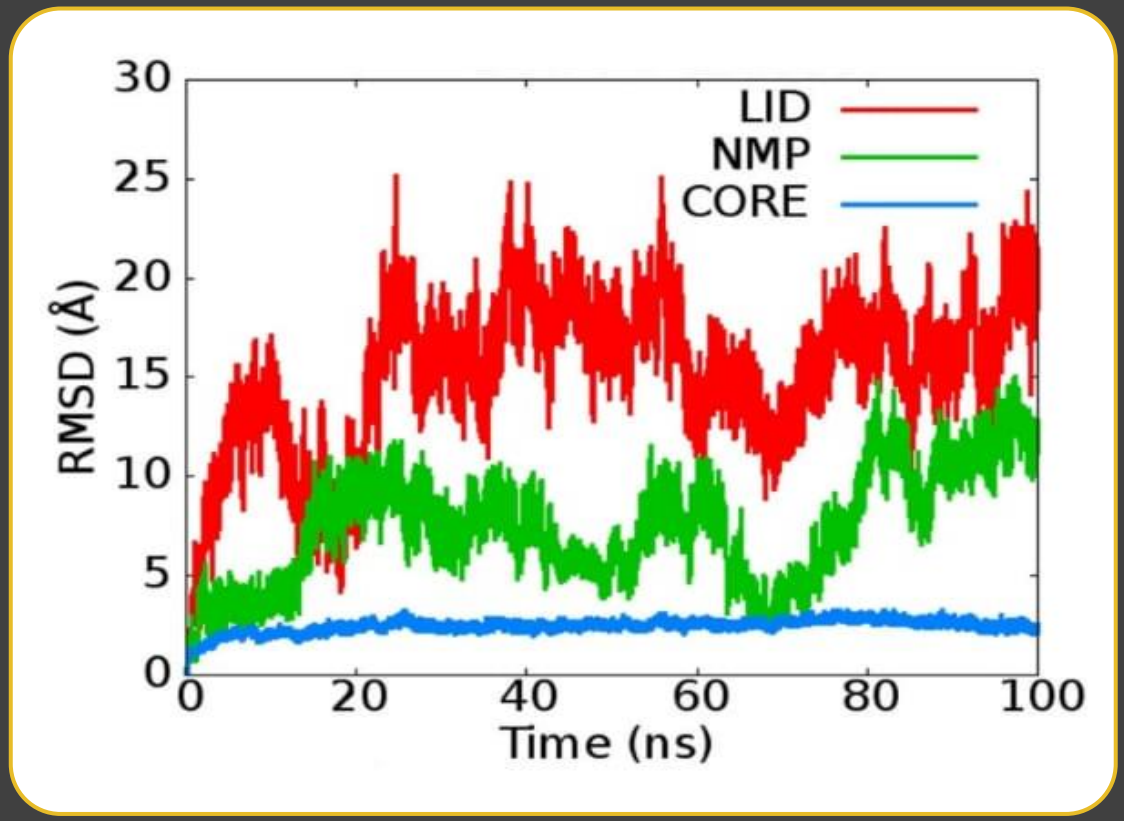

Key residue in AK of *E. coli* crystallized with AP5A inhibitor

# MD Simulation

- Conformational sampling of AK can be done using ~1μs metadynamic sampling rate, which allows for collective motion of X-ray structural functions of AK to be simulated in 1μs-ms timescale.
- From MD simulation of X-ray structure of AK, it is evident that the enzyme adopts several conformations separated by few $k_BT$.
- From Root Mean Square Deviation (RMSD) and Fluctuation (RMSF) plots, it is evident that CORE is conformationally stable, while LID transverses wider conformational space than NMP. LID is more flexible than NMP and attains several conformations from open to closed structure.
- Among these possible conformations, open and closed states bear lowest free energy, implying these are the most prominent conformations.
- Open state is 1-2 $k_BT$ lower in free energy than closed state, that's why open state corresponds to binding of ligands to AK.




(a) Open conformation



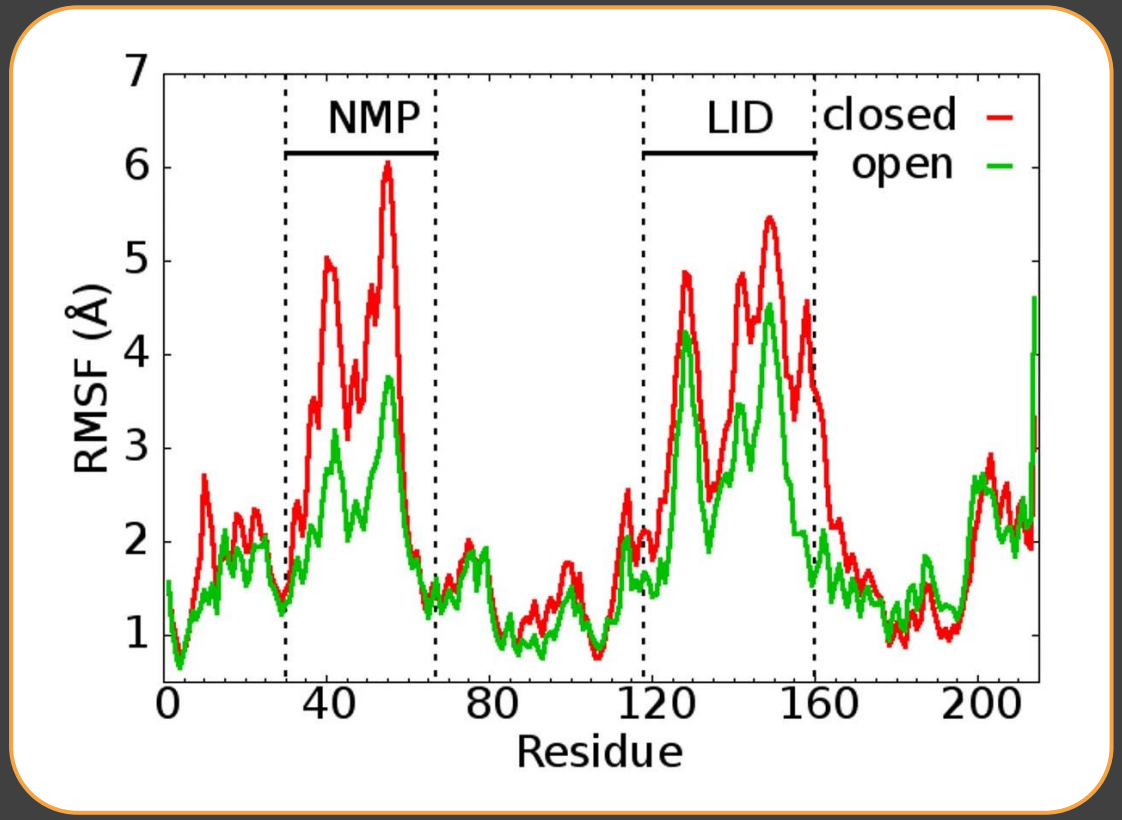

(a) Closed conformation

# RMSD Plots from MD simulations

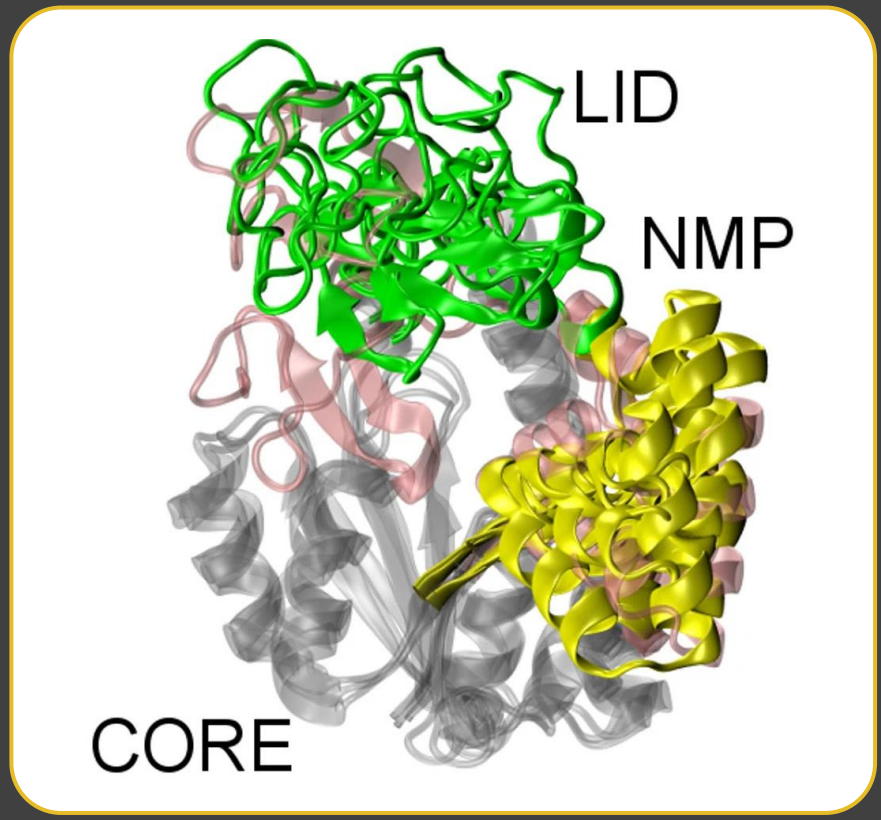
RMSD values calculated for each domain of AK in

(a) Open conformation

(b) Closed conformation




(a) RMSF Plot




(a) MD:X-ray superimposition

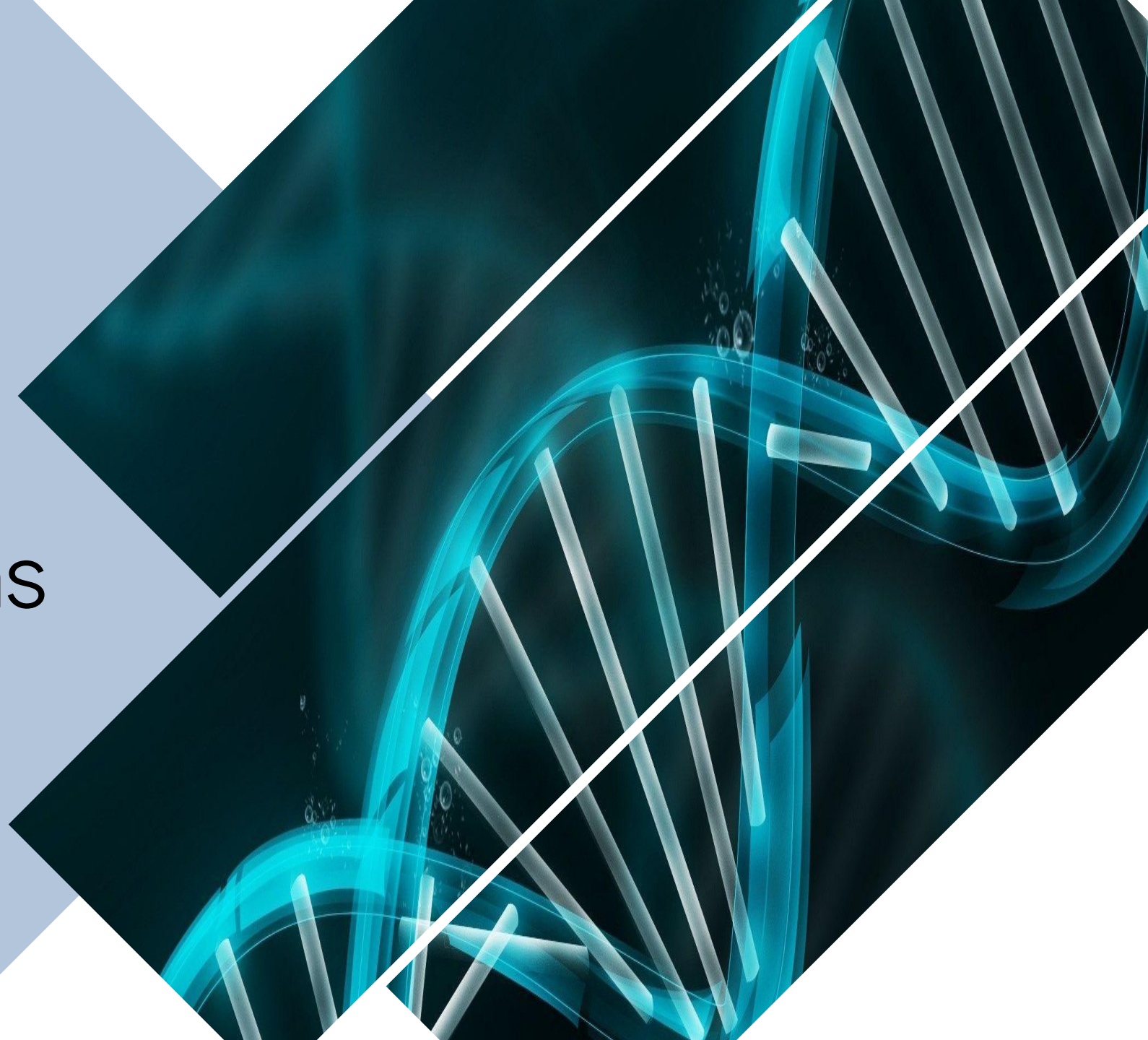
# RMSF and Superimposition Plots from MD simulations

(a) RMSF of backbone atoms calculated from MD simulation for open and closed AK conformations

(b) Selected MD simulation conformations superimposed over open and closed X-ray states of AK

# Assay and Isoforms

- AK Assays
- Human AK Isoforms



# AK Assay

- Introduction
- Conventional Assays
- Spectrophotometric Assay based on pH
- Effect of T and KCl on AK activity

# Introduction TO Assay

- Enzymatic assay is a laboratory technique to study the activity of an enzyme, and factors affecting it.
- For a simple reaction, $S \rightleftharpoons P$

$$\mathrm{EA(enzyme\ activity)} = -\frac{dN_s}{dt} = r \times V$$

where

$N_s$ : number of moles of substrate

V : reaction volume

r : rate of forward reaction

SI unit : 1 katal = 1 mol/s

Conventional unit : 1 enzyme unit (1 U) = 1 $\mu mol/\ min = 16.67$ nano katals

# Conventional Assays (Coupled Reactions)

- For the reaction: $\mathrm{ADP} + \mathrm{ADP} \leftrightharpoons \mathrm{AMP} + \mathrm{ATP}$
- By coupling the reaction with *hexokinase and glucose – 6 – phosphate dehydrogenase (G-6-P-DH), in which the final product NADPH is measured spectrophotometrically at 340 nm.*

$$\mathrm{ATP} + \mathrm{Glucose} \xrightarrow{\text{hexokinase}} \mathrm{Glucose} - 6 - \mathrm{phosphate} + \mathrm{ADP} + \mathrm{H}^{+}$$

$$\mathrm{Glucose} - 6 - \mathrm{phosphate} + \mathrm{NADP} \xrightarrow{\mathrm{G-6-P-DH}} 6 - \mathrm{phosphogluconic\ acid} + \mathrm{NADPH}$$

- For the reaction in reverse direction, $\mathrm{ATP} + \mathrm{AMP} \leftrightharpoons \mathrm{ADP} + \mathrm{ADP}$
- By coupling the reaction with *pyruvate kinase* and *lactate dehydrogenase, measuring the oxidation of NADH at 340 nm spectrophotometrically, Ak activity can be analysed.*

$$\mathrm{ADP} + \mathrm{PEP} \xrightarrow{\text{Pyruvate Kinase}} \mathrm{ATP} + \mathrm{Pyruvate}$$

$$\mathrm{Pyruvate} + \mathrm{NADH} + \mathrm{H}^{+} \xrightarrow{\text{Lactate dehydrogenase}} \mathrm{Lactate} + \mathrm{NAD}^{+}$$

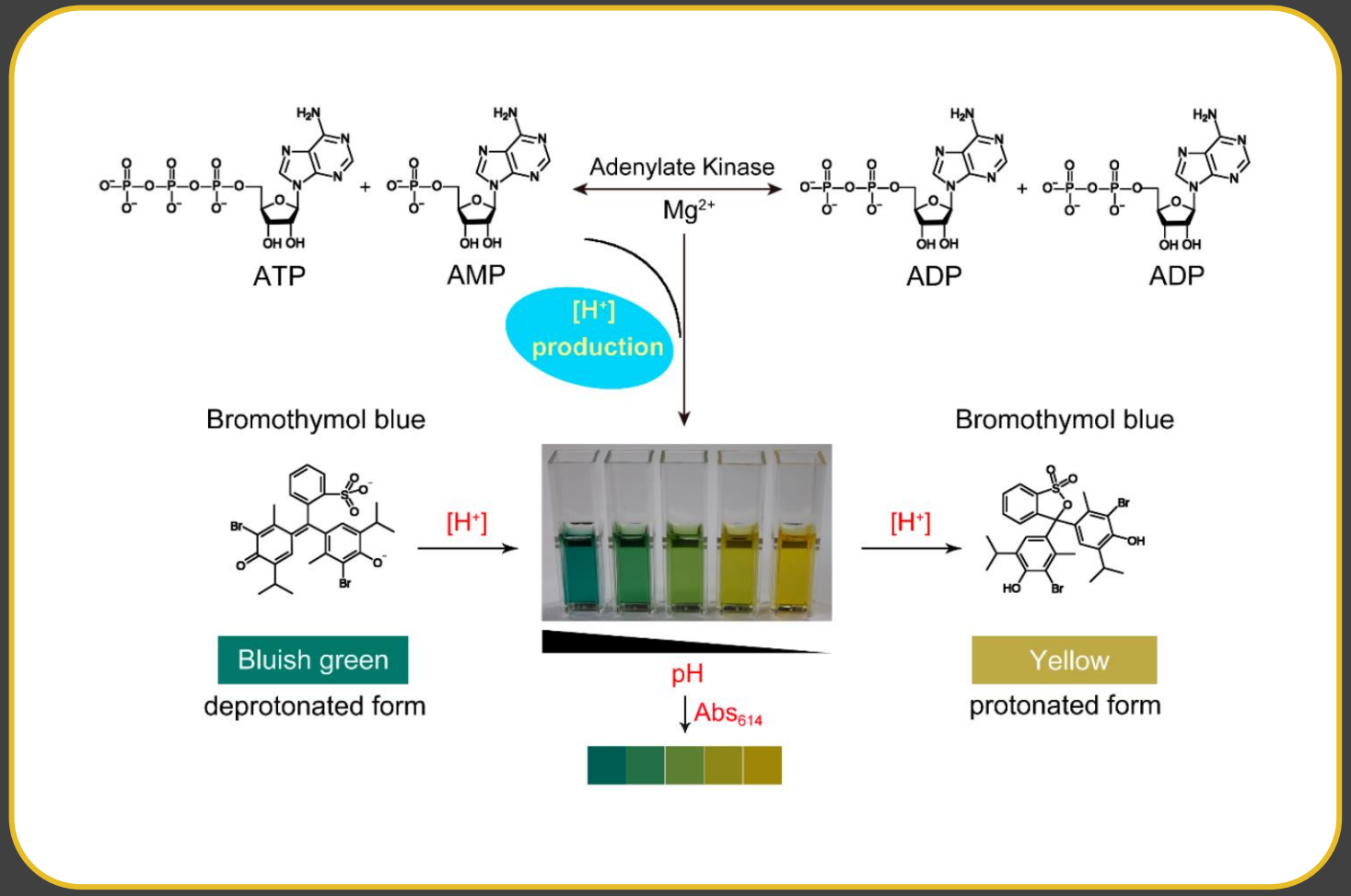
But, coupling method has some limitations, viz. multi-step process, prone to error in each step, can't analyse the effect of activator and inhibitors on AK activity in presence of other enzyme.

# Spectrophotometric Assay (based on pH)

- In a AK catalysed reaction, a proton is released when two ADP produced from ATP and AMP.

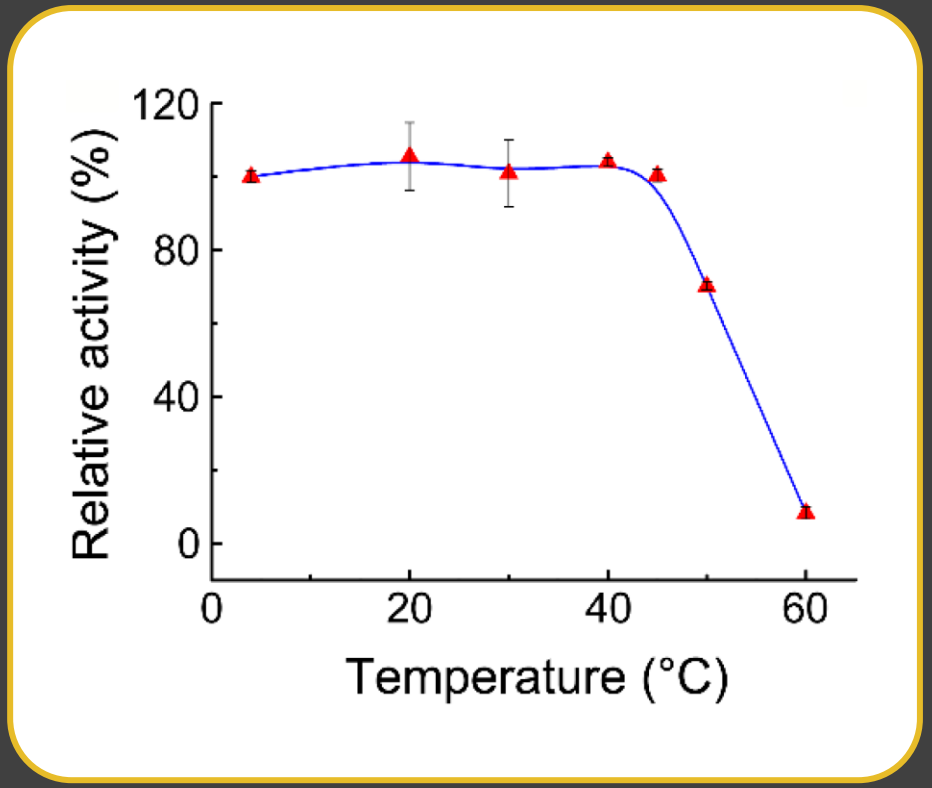
$$ATP + AMP \rightleftharpoons 2\ \text{ADP} + H^+$$

- Bromothymol blue (pH range 6 – 7.6) is an excellent pH indicator for this reaction as AK is effective at pH ~ 7.6.

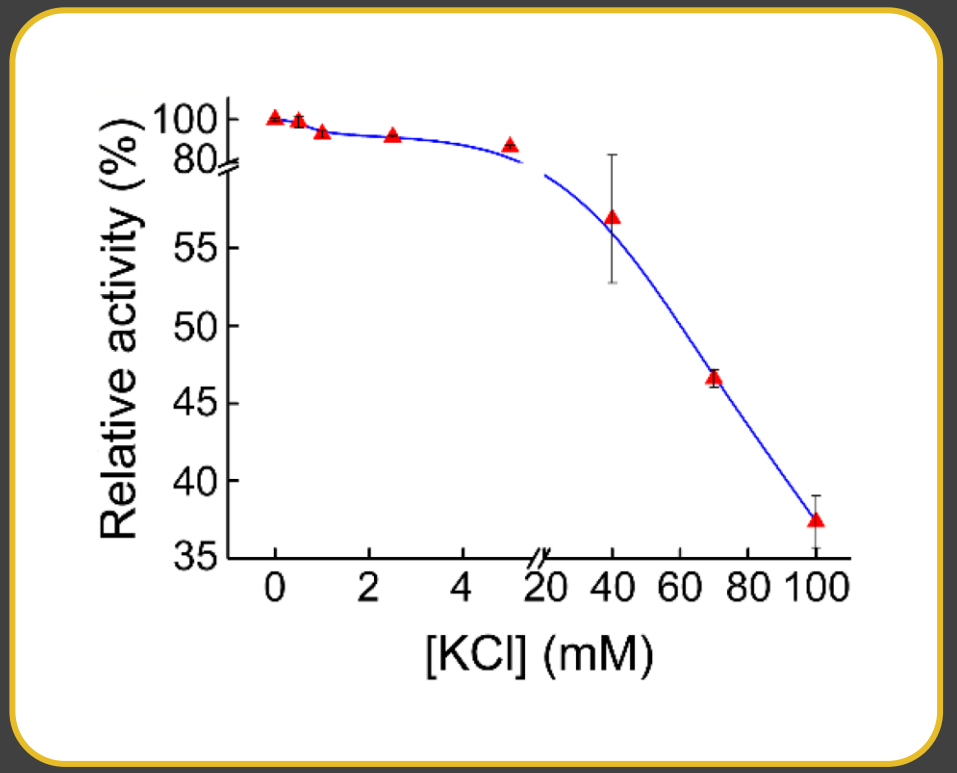



*The principle of the spectrophotometric assay for AK activity*

(a)




(b)




# Thermostability and Effect of KCl on AK activity

(a) Effect of T: AK is thermally stable up to 40° $C$ and completely denatured at 60° C

(b) Effect of KCl: KCl acts as an inhibitor for AK. The inhibitory effect is low at lower concentration. The assay suggests that 70 mM KCl reduces AK activity to almost 50%.

*Residues, Genes and Database Identifiers of human AK isoforms*

| AK | Residues | Gene | | | Accession Number |
|---|---|---|---|---|---|
| | | Chromosome | Location | NCBI Gene ID | |
| AK1 | 194 | 9 | 9q34.11 | 203 | AAH01116 |
| AK2 | 239 | 1 | 1p35.1 | 204 | AAC52061 |
| AK3 | 227 | 9 | 9p24.1 | 50808 | AAH13771 |
| AK4 | 223 | 1 | 1p31.3 | 205 | P27144 |
| AK5 | 537 | 1 | 1p31.1 | 26289 | AAH33896 |
| AK6 | 536 | 5 | 5q13.2 | 102157402 | AAO16520 |
| AK7 | 723 | 14 | 14q32.3 | 122481 | NP_689540 |
| AK8 | 479 | 9 | 9q34.13 | 158067 | NP_689785 |
| AK9 (1) | 1911 | 6 | 6q21 | 221264 | NP_001138600 |
| AK9 (2) | 421 | 6 | 6q21 | 221264 | NP_659462 |

# AK Isoforms in Humans

In human, 9 isoforms of AK has been discovered and analysed so far. They are named in the chronological order of their discovery from AK1 to AK9. They differ in their amino acid residues, subcellular location, tissue distribution, selectivity towards phosphate donor and substrate, enzymatic kinetics and therapeutic implications of dysfunction.

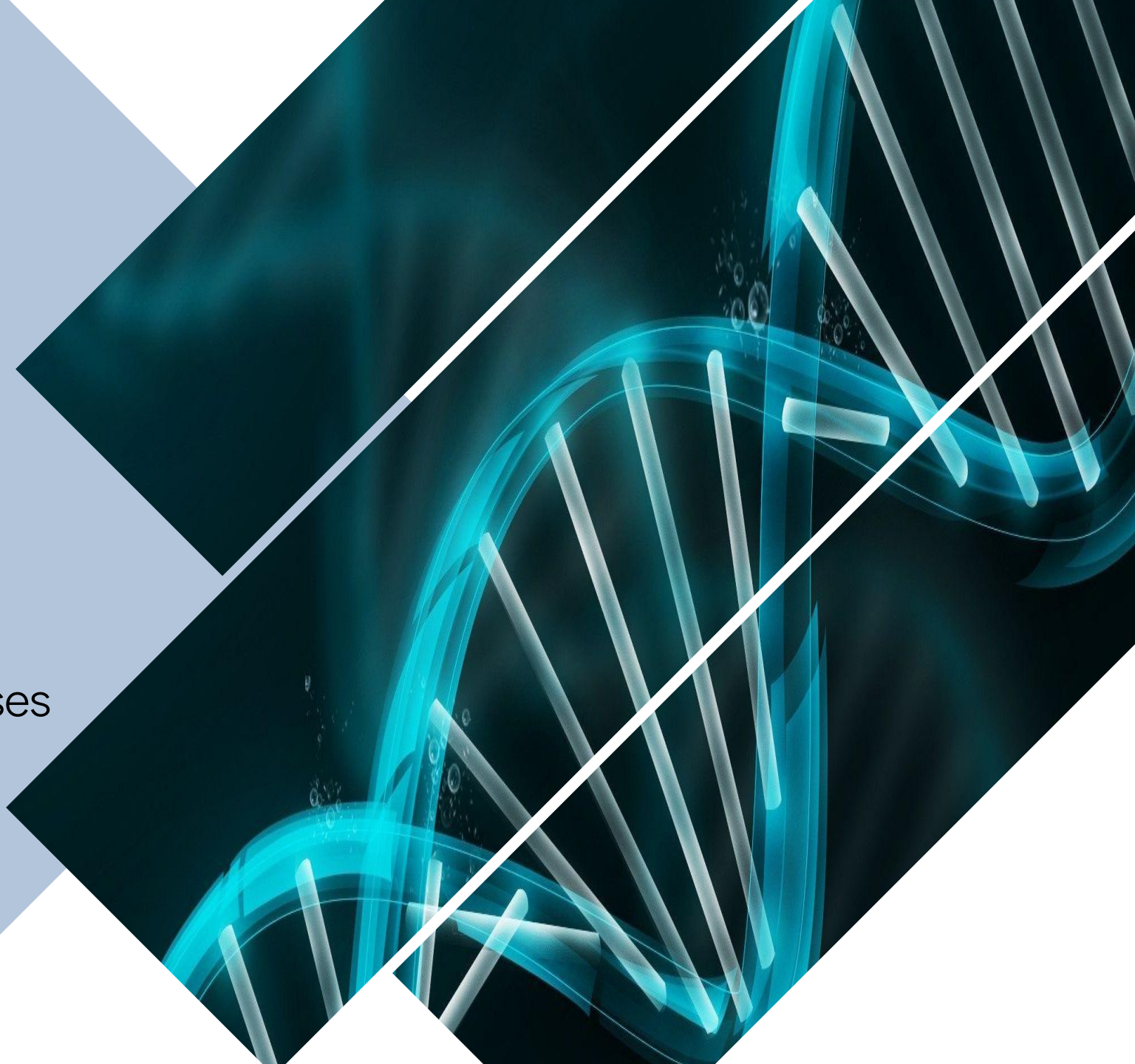
| AK | Phosphate donor | Substrate | Subcellular location | Tissue distribution |
|---|---|---|---|---|
| AK1 | All NTPs | AMP, dAMP | Cytosol | All tissues |
| AK2 | All NTPs | AMP | Mitochondria (intermembrane space) | Liver, heart, skeletal muscle, kidney, lung |
| AK3 | GTP, ITP | AMP | Mitochondria (matrix) | Liver, heart, skeletal muscle, kidney |
| AK4 | ATP, GTP | AMP, dAMP, CMP, dCMP | Mitochondria (matrix) | Liver, heart, brain, kidney |
| AK5 | ATP, GTP | AMP, dAMP, CMP, dCMP | Cytosol/nucleus | Brain |
| AK6 | All NTPs and dNTPs | AMP, dAMP, CMP, dCMP | Nucleus | All tissues |
| AK7 | ATP | AMP, dAMP, CMP, dCMP | Cytosol | Lung, trachea, testis, mammary gland, skeletal muscle |
| | GTP | AMP, CMP, dCMP | | |
| AK8 | ATP | AMP, dAMP, CMP, dCMP | Cytosol | Liver, pancreas, lung, trachea, testis |
| | GTP | AMP, CMP, dCMP | | |
| AK9 | ATP | AMP, dAMP, CMP, dCMP | Cytosol /nucleus | Pituitary gland, trachea, thymus, testis, mammary gland, brain, pharynx, uterus, spleen, lymph nodes |

# Human AK Isoforms

Subcellular location, Tissue distribution, Phosphate donor and Substrate specificity of human AK isoforms

# Functions

- AK Shuttle
- Nucleoside Diphosphate Kinases
- Purine Nucleotides Synthesis
- Nucleoside Analogues



# AK Shuttle

- Mechanism
- Bucket Brigade

# Mechanism OF AK Shuttle

Within the human cells, AK is present in 2 major isoforms which regulates the use of high energy phosphoryl's of ATP.

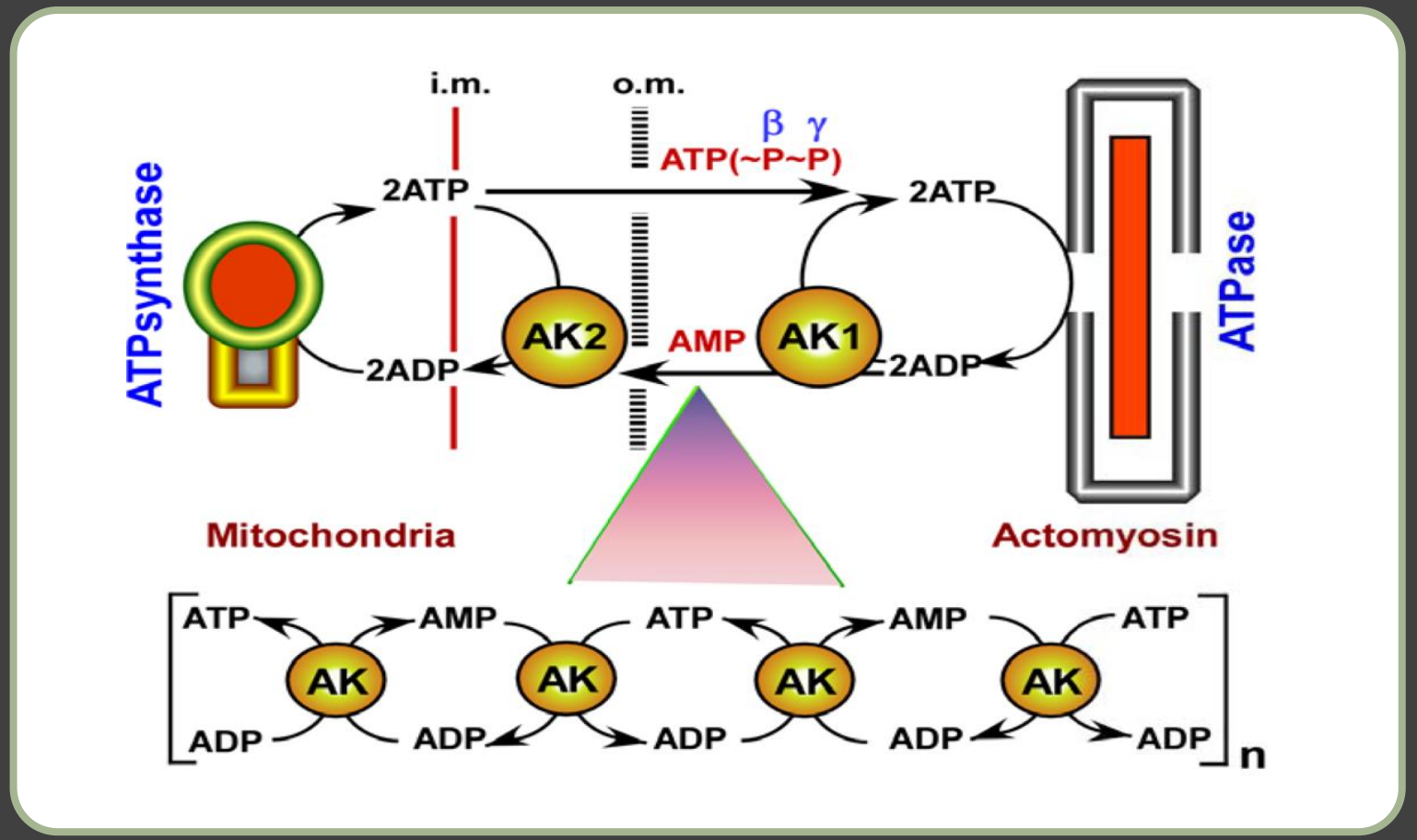
**AK1**

Cytosolic AK

**AK2**

Mitochondrial Intermembrane AK

- AK1 *shuttles* ATP to the site of high energy consumption
- ATPase hydrolyses ATP to produce energy and ADP as by product
- AK1 in cytosol converts ADP to ATP and AMP
- AMP signals AK2 to produce ADP at the inner membrane (i.m) of mitochondria
- ATP-synthase in mitochondria uses ADP to produce ATP at inner membrane

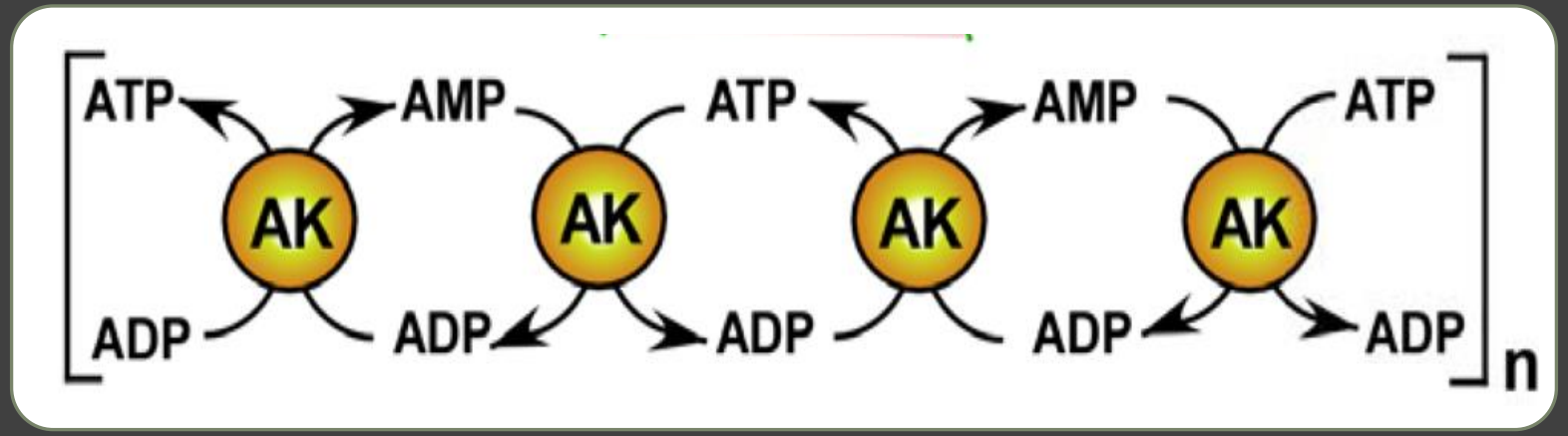



# AK Shuttle

Transfer of high energy β and γ phosphoryls of ATP from production to consumption site via ATP shuttle.

*i.m* : inner membrane, *o.m* : outer membrane *(Petras Dzeja and Andre Terzic, 2009)*

# Bucket Brigade IN AK Shuttle




- The intracellular transfer of ATP and AMP from energy production to consumption site (and reverse) may involve multiple phosphotransfer stages in a sequential manner (like a sequential relay).
- This results in propagation of ATP-AMP flux (or metabolic flux) through a series of AK molecules, known as "*bucket-brigade*"
- This type of conduction may change local metabolic flux without any apparent change in global metabolic concentration of the cell.
- This whole process is crucial for overall energy homeostasis of the cell.

# NDPKs (Nucleoside Diphosphate Kinases)

- Introduction
- Mechanism
- AK as NDPKs

# Introduction TO NDPKs

- NDPKs are enzymes that catalyze the reversible phosphoryl transfer from a donor to nucleoside diphosphate (NDP) substrate, producing corresponding nucleoside triphosphate (NTP).

$$NTP_{(d)} + NDP_{(s)} \xleftrightarrow{NDPK} NDP_{(d)} + NTP_{(s)}$$

- Donor (d)
- Substrate (s)

  - Family of genes that encode NDPKs are called nm23. So far, ten such genes have been identified in humans and are designated nm23-H1 to nm23-H9, with the last gene known as RP2.
  - NDPKs are shown to play an important role in many biological processes such as protein synthesis, metabolic signaling, cellular development, proliferation, differentiation and apoptosis.

The two major features that differentiates NDPKs from nucleoside kinases (NKs) and nucleoside monophosphate kinases (NMPKs) involved in NTP (or dNTP) synthesis are

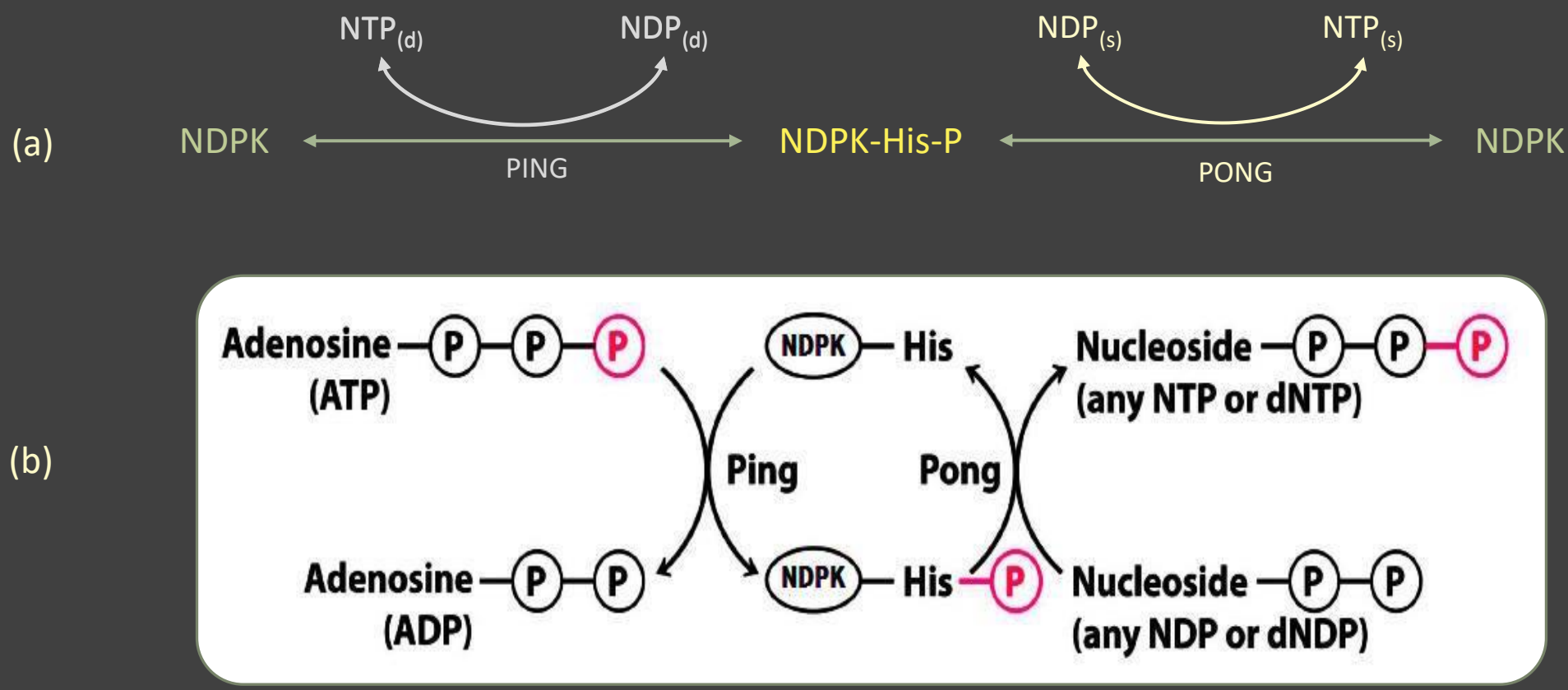
i. NDPKs do *not* have a p-loop motif in phosphate donor binding site.

ii. NDPKs are *not* specific to any particular substrate. They can accept pyrimidine and purine, ribo and deoxyribo nucleoside diphosphates as substrates.

# Mechanism OF NDPKs

NDPKs follow a *ping-pong* mechanism with the following scheme…

1. Phosphate donor $NTP_{(d)}$ binds with NDPK and transfers γ-phosphate to histidine residue of the enzyme resulting in high energy NDPK-His-P phosphoenzyme intermediate.
2. $NDP_{(d)}$ detaches from the enzyme which triggers conformational change that facilitates the binding of substrate $NDP_{(s)}$ (or $dNDP_{(s)}$) to the phosphoenzyme intermediate.
3. Transfer of phosphoryl group from NDPK-His-P to the $NDP_{(s)}$ (or $dNDP_{(s)}$) results in product $NTP_{(s)}$ (or $dNTP_{(s)}$)
4. Product is released, resulting in enzyme back to its initial open conformation.




# NDPK Mechanism

Ping-Pong mechanism followed by NDPKs for the phosphorylation of NDP (or dNDP) substrate

(a) General scheme

(b) Catalytic loop with ATP as phosphate donor *(D.L. Nelson, 2004)*

# AK as NDPKs

- In addition to NMPK activity, AK9 also shows NDPK activity. Substrate specificity slightly varies in order: CDP > UDP > ADP > GDP > TDP  irrespective of the phosphate donor.


- Likewise, other human AK isoenzymes can also convert majority of NDPs and dNDPs to corresponding NTPs and dNTPs.
- Some AK shows restricted NDP binding ability. For ex, AK8 cannot phosphorylate UDP with ATP as phosphate donor. Using alternate donor (such as GTP) can bypass this problem.
- AKs are highly selective towards NMPs (NMPK activity), but have significantly lower selectively towards NDPs (NDPK activity). This is because AK active sites are optimized for NMPs. NDPs do not fit well in those sites and can easily attack γ-phosphoryl group of the donor molecule.
- Deficiency of NDPKs can be compensated by the presence of NMPKs such as AK. This is shown in case of bacteria, *Ureaplasma parvum* that lacks NDPK gene. Mutated *Escherichia coli* with disrupted NDPK activity when injected with AK from *Ureaplasma parvum* showed renewed NDPK activity.

# Intracellular Synthesis

## of Purine Nucleotides

- Importance of Nucleotides
- The *De-novo* Pathway
- The *Salvage* Pathway

# Importance OF Nucleotides

## Nucleic Acids

- RNA and DNA are polymers of ribo- and deoxyribo-nucleotides.
- Coenzymes FAD, NAD and CoA are derivates of adenine nucleotide, which play important role in $e^-$, $H^-$ and acyl group transfer reactions.

## Energy Carriers

- ATP, NADH and FADH2 are primary energy carriers of the cell.
- ATP acts as a high-energy phosphate donor and as driving force for oxidative phosphorylation.

## Metabolic Signaling

- AMP and cAMP level changes in response to various chemical, physiological and hormonal stimuli.
- Regulates many enzymes (kinases like AK2), genetic expressions, $K^+$ and $Ca^{+2}$ channels with AMP/cAMP receptors, metabolic processes such as glycogenesis and glycogenolysis.

FAD: Flavin Adenine Dinucleotide, NAD: Nicotinamide Adenine Dinucleotide, CoA: Coenzyme-A, cAMP: cyclic AMP

## Pharmacology

- Blood coagulation via platelet aggregation is greatly affected by intracellular ADP levels. Drugs targeting ADP can treat blood clotting disorders such as Hemophilia.
- Nucleoside analogues resemble natural nucleosides and depend on intracellular phosphorylation for their activation. Useful in viral infections, cancer and other auto-immune diseases.

### Nucleotides must be produced within the cell

1. Cell membrane does not contain any nucleotide carrier protein

2. Negatively charged phosphate groups resists diffusion across the phospholipid membrane

### Purine nucleotide synthetic pathways

*1. De-novo pathway*

Chemical synthesis of complex molecules from simple ones

*2. Salvage pathway*

Uses left over intermediates and by-products from other destructive processes of similar molecule

# The *de-novo* Pathway

Major source of DNA transcription and replication in developing cells. Steps involved are

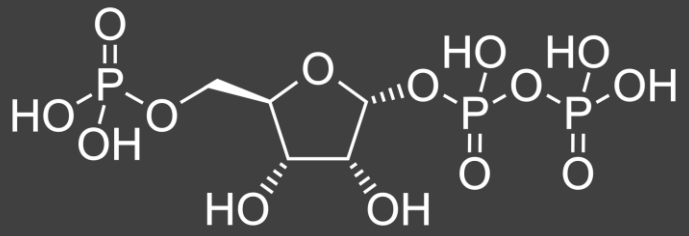
Formation of IMP (precursor for AMP and GMP) using simple molecules: PRPP as the source of ribose 5'-phosphate, amino acids, $CO_2$ and N10-formyltetrahydrofolate.

AMP and GMP are phosphorylated to ADP and GDP by AK and GUK respectively.

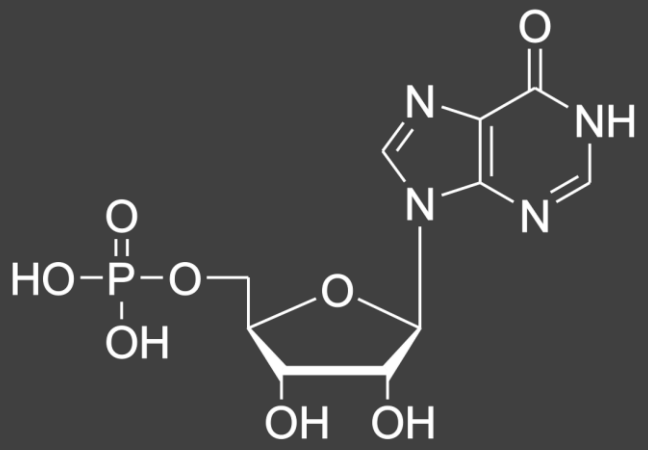
ADP and GDP can be

- phosphorylated by NDPKs to ATP and GTP.
- reduced by Ribonucleoside Reductase (RNR) to deoxyribo forms: dADP, dGDP. Phosphorylation gives dATP and dGTP.

IMP: Inosine Monophosphate, PRPP: Phosphoribosyl Pyrophosphate, GUK: Guanylate Kinase

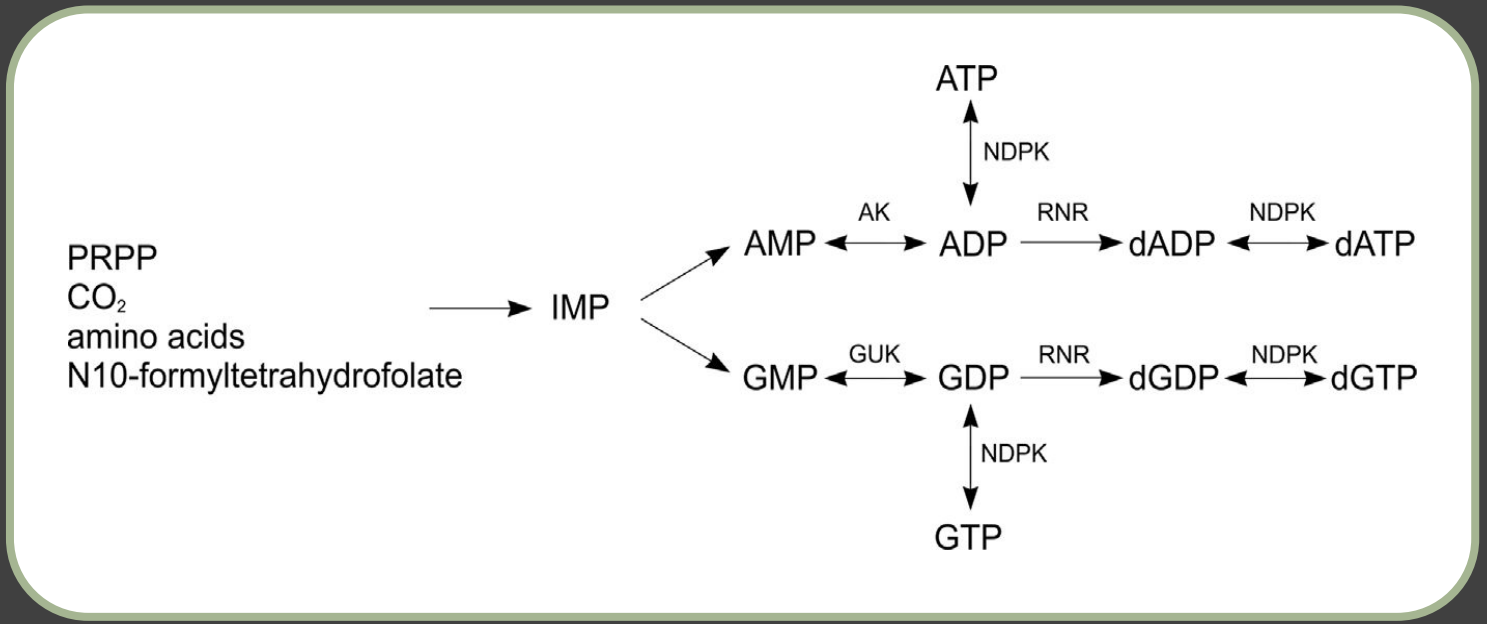


(a) 5’-Phosphoribosyl Pyrophosphate (PRPP)



(b) Inosine Monophosphate (IMP)

(c) *De-novo* pathway




# The *de-novo* Pathway

Synthesis of purine nucleotides inside a mammalian cell

# The *salvage* Pathway

Essential for DNA repair, replication and transcription in damaged cells. It uses nucleotides left as by products of RNA and DNA degradation as nucleoside precursors after dephosphorylation.

If nucleoside precursors are outside the cell, they are either actively transported or passively diffused across the cell membrane via nucleoside carrier proteins.

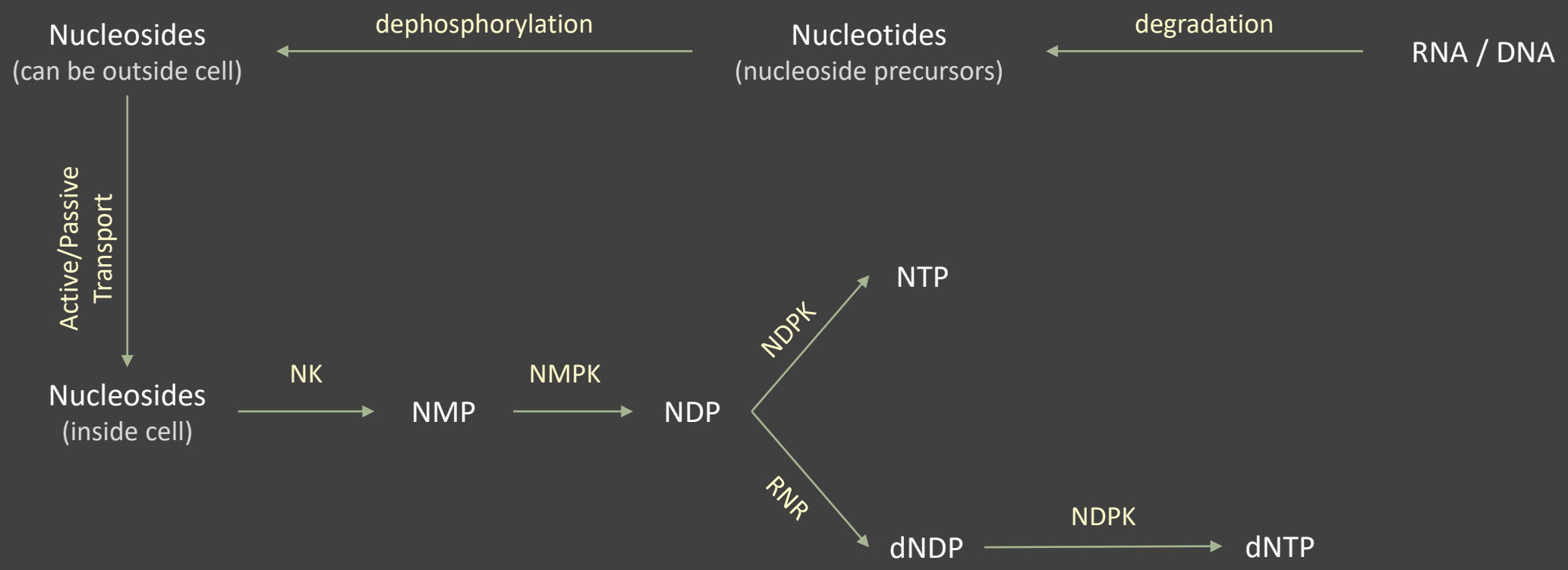
Inside the cell, NKs phosphorylate nucleosides to NMP and dNMP.

NMP (and dNMP) are phosphorylated to NDP (and dNDP) by NMPKs such as AK.

NDP can be

- phosphorylated by NDPKs to NTP.
- reduced by Ribonucleoside Reductase (RNR) to dNDP. Subsequent phosphorylation by NDPKs gives dNTP.

NKs: Nucleoside Kinases, NMPKs: Nucleoside Monophosphate Kinases




# The *salvage* pathway

Synthesis of purine nucleotides inside a mammalian cell

# Nucleoside Analogues (NAs)

- Introduction
- Mechanism
- Examples

# Introduction TO NAs

## Definition

- NAs are a class of novel drugs that resemble the structure of natural nucleosides.
- Within the cell, activated NAs mimic natural nucleotides (building blocks of DNA and RNA).

## Usage

- NAs are used as cytotoxins (ability to kill cells)
- Treatment of viral infections (influenza, hepatitis B and C, HIV, herpes etc).
- Against malignant diseases (leukaemia, lung cancer, solid tumors) and other autoimmune diseases.

## Side Effects

- Due to cytotoxicity and incorporation of NAs in nucleic acids, long term usage can cause disorders similar to genetic mutations in DNA and mtDNA.
- Multi-organ disorders such as lactic acidosis, neuropathy, cardiomyopathy, and organ (liver, bone marrow, pancreas) failure.

NAs: Nucleoside Analogues, mtDNA: mitochondrial DNA


Administration

Cellular Transport

Intracellular Activation

Incorporation to DNA

# Mechanism OF NAs

## Administration

- NAs are administered as monophosphates to increase their solubility.
- Before entering the cell, they are dephosphorylated back to nucleoside by nucleotidases.

## Transport into cell

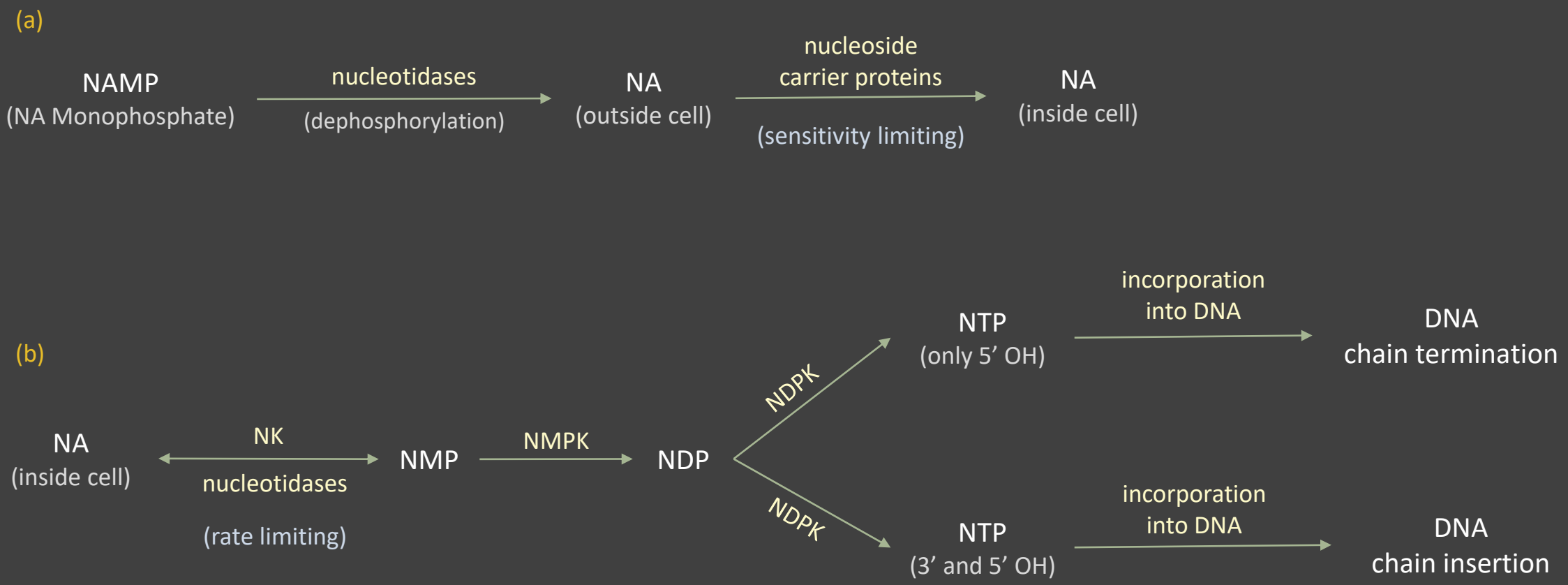
- NAs are usually hydrophilic, and require special Nucleoside Carrier Proteins (NCPs)
- Intramembrane concentration and tissue distribution of NCPs controls sensitivity towards NAs.

## Intracellular Activation to NTPs

- Sequential action of NKs, NMPKs and NDPKs. First step is rate-limiting.

## Incorporation into DNA

- After activation to NTPs, NAs are incorporated into DNA and RNA of the viral or malignant cells.
- Driven by the action of nucleic acid synthesis, replication and repair mechanisms
- Irreparable DNA mutation causes cell apoptosis

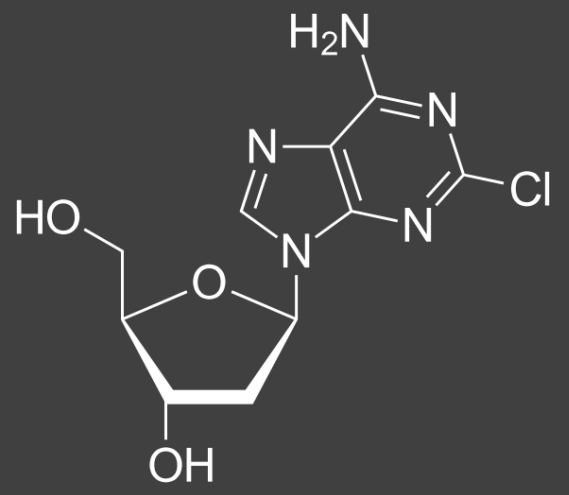



# Mechanism of NAs

(a) Administration and Transport into cell

(b) Intracellular activation and Incorporation into DNA

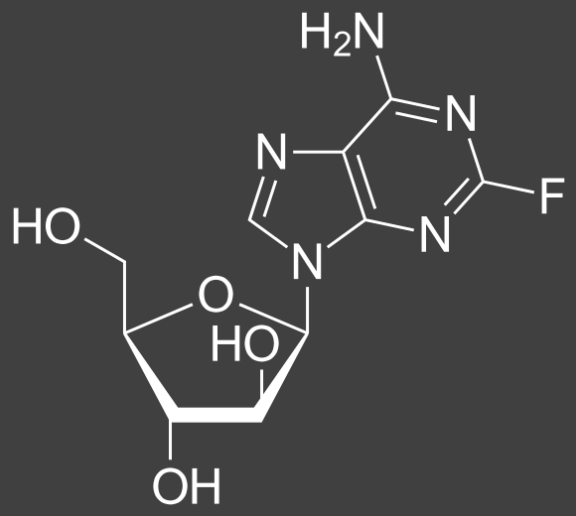
(a)




Cladribine (CdA)

2-Chloro, 2-deoxy-Adenosine

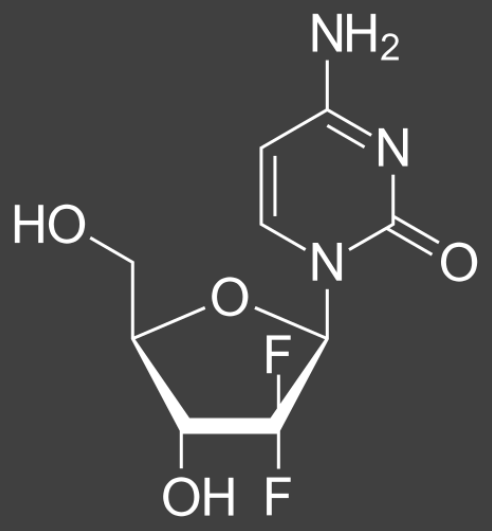
(b)




Fludarabine (FaraA)

2-Fluoro, 9-β-arabinofuranosyl-Adenine

(c)




Gemcitabine (dFdC)

2’,2’-Difluorodeoxycytidine

# Examples of Nucleoside Analogues

(a) Cladribine (CdA): analogue of *deoxy-Adenosine* (dAdo)

(b) Fludarabine (FaraA): analogue of *Adenosine* (Ado)

(c) Gemcitabine (dFdC): analogue of *deoxy-Cytidine* (dCyd)

# Conclusion

AKs are the only intracellular enzyme that can catalyze the interconversion of various adenine nucleotides, essential for nucleic acid synthesis and repair, cellular energy metabolism and homeostasis.

## Structure

- AK is composed of 3 domains: CORE, LID and NMP
  - CORE: contains the active site, highly conserved, conformationally stable
  - LID and NMP: binding sites for phosphate donor and substrate, highly flexible, significant conformation changes
- MD simulations are useful in tracking the structure and thermodynamics of various conformations acquired by AK during the phosphoryl transfer

## Assays

- Various assays have been developed to measure AK activity. Conventional assays use coupled enzymatic reactions.
- Spectrophotometric assays employing pH based indicators provide one-step method for sensitive, continuous and real time monitoring of AK activity

## Isoforms

- Nine human AK isoforms have been identified so far, which differ in substrate and phosphate donor specificity, enzyme kinetics, subcellular localization, tissue distribution, physiological functions and therapeutic impact
- Many AK isoforms indicate its important role in diverse array of cellular processes

## Function

- AK shuttle: necessary for cellular metabolism and energy homeostasis
- Nucleoside mono and di-phosphate kinases (NMPKs and NDPKs)
- Intracellular synthesis of purine nucleotides

## Impact of Dysfunction

- Leads to heart failure, neurodegenerative disorders and malignant diseases (AK1: *haemolytic anaemia*, AK2: *reticular dysgenesis*, AK7: *primary ciliary dyskinesia*)
- therapeutic interventions targeting AK activity have potential to treat such diseases. One such example is Nucleoside Analogues

Further studies in AK are therefore crucial to better understand its complex phosphoryl transfer mechanism and role in cellular processes, which can provide insights for the development of novel therapeutic strategies.

# References


- Panayiotou, C; Solaroli, N; Karlsson, A. *The many isoforms of human adenylate kinases*. The international journal of biochemistry and cell biology **2014**
- Formoso, E; Limongelli, V; Parrinello, M. *Energetics and structural characterization of the large-scale functional motion of adenylate kinase*. Scientific reports **2015**
- Dzeja, P.; Terzic, A. *Adenylate kinase and AMP signaling networks: metabolic monitoring, signal communication and body energy sensing*. International journal of molecular sciences **2009**
- Ionescu, M. I. *Adenylate kinase: a ubiquitous enzyme correlated with medical conditions*. The Protein Journal **2019**
- Song, K; Wang, Y. *A Convenient, Rapid, Sensitive, and Reliable Spectrophotometric Assay for Adenylate Kinase Activity*. Molecules **2019**
- Chang, H.-Y; Fu, C.-Y. *Adenylate Kinase. Encyclopedia of Food Microbiology*: Second Edition **2014**, 18–23
- Stryer, L ; Berg, J; Tymoczko, J. *Biochemistry, Fifth Edition*; [NCBI bookshelf]; W. H. Freeman: **2002**