Biosynthesis of Natural Products

Introduction to Secondary Metabolism & the Shikimate Pathway

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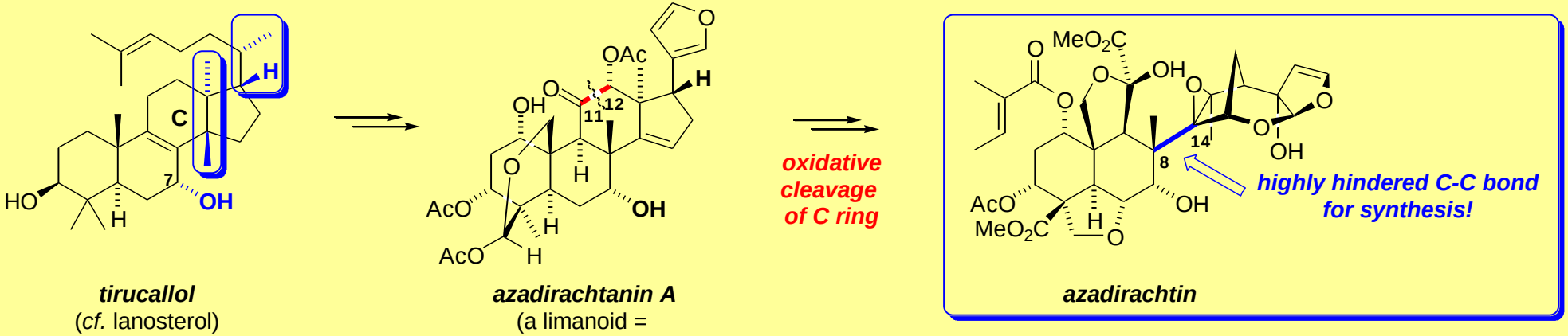
Oct 2019

Lessons in Synthesis - *Azadirachtin*

- ***Azadirachtin*** is a potent ***insect anti-feedant*** from the Indian ***neem tree:***
  - exact biogenesis unknown but certainly *via* steroid modification:



tirucallol
(*cf.* lanosterol)

azadirachtanin A
(a limanoid =
tetra-*nor*-triterpenoid)

azadirachtin

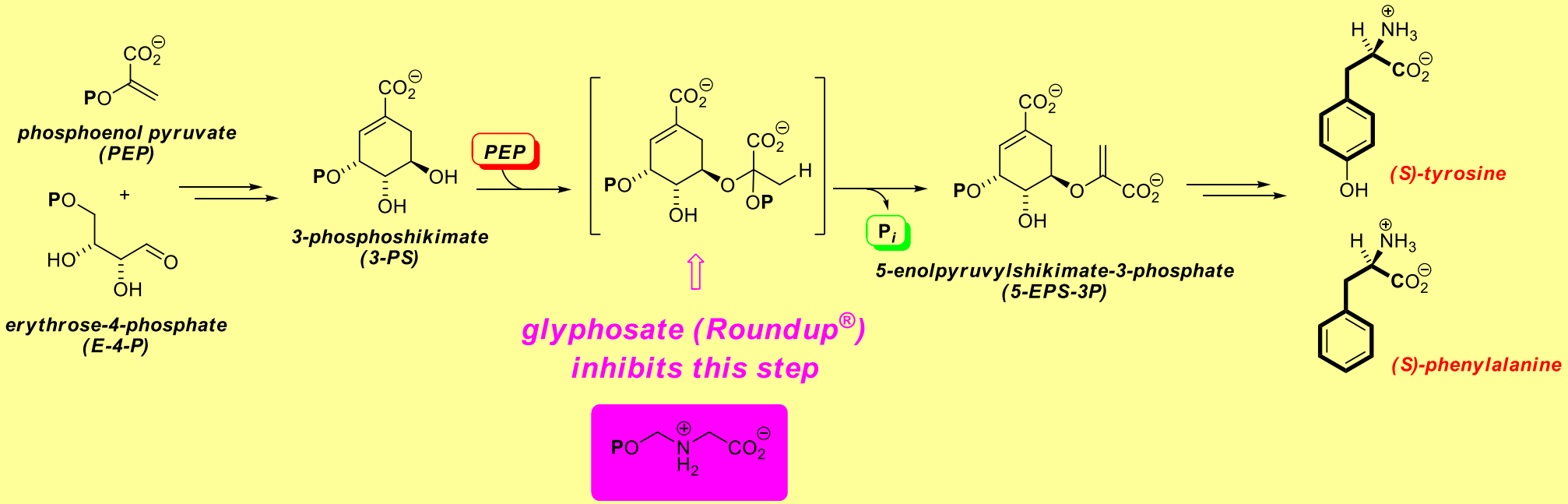
  - Intense synthetic efforts by the groups of Nicolaou, Watanabe, Ley and others since structural elucidation in 1987.
  - 1st total synthesis achieved in 2007 by Ley following 22 yrs of effort
  - **~40 researchers and over 100 person-years of research! – 64-step synthesis**

  - Veitch *Angew. Chem. Int. Ed.* **2007**, *46*, 7629 (DOI) & Veitch *Angew. Chem. Int. Ed.* **2007**, *46*, 7633 (DOI)
  - ***Review 'The azadirachtin story' see:*** Veitch *Angew. Chem. Int. Ed.* **2008**, *47*, 9402 (DOI)

Rational Agrochemical Development – *Shikimate Pathway Intervention*

- ***The shikimate biosynthetic pathway is not found in animals/humans – only in plants***
  - selective intervention in these pathways allows development of agrochemicals with minimal human toxicity

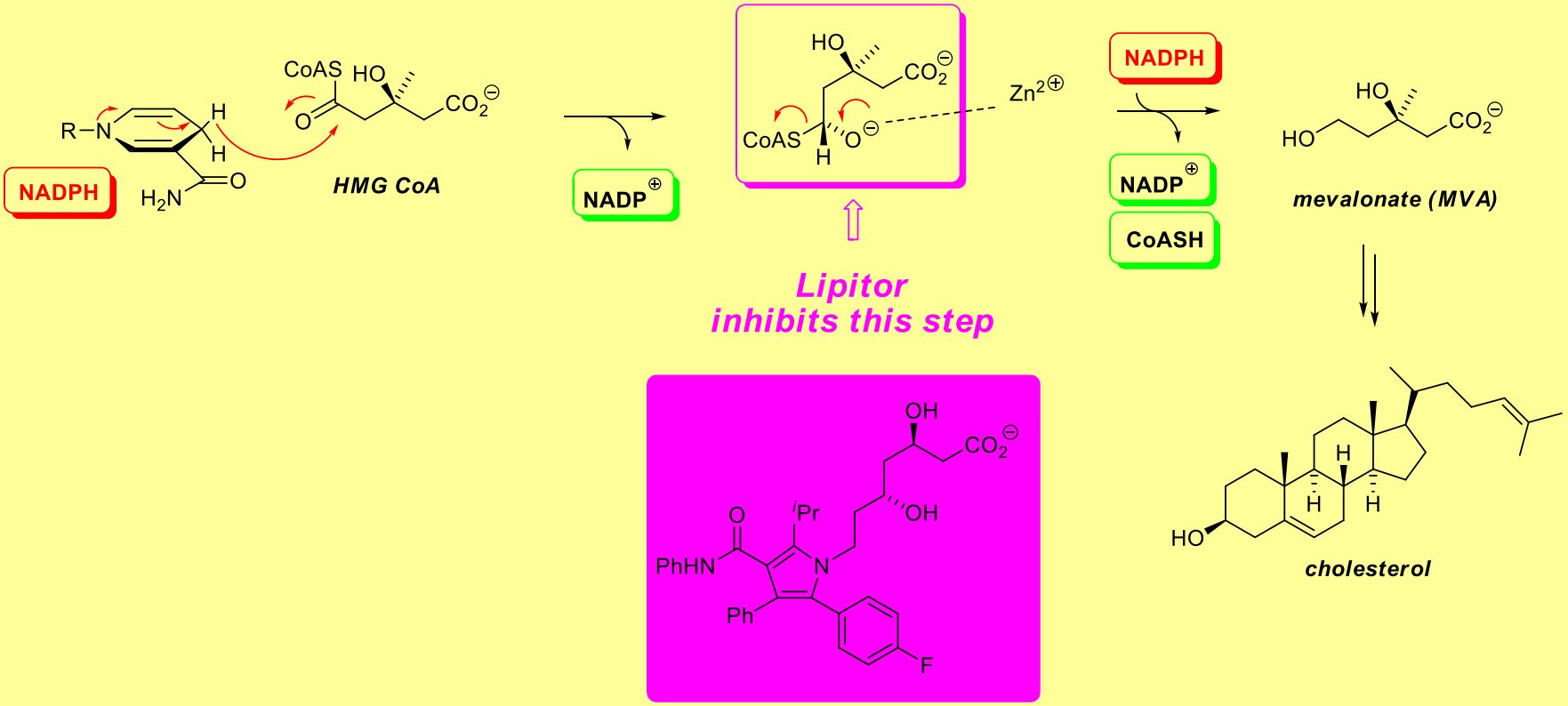
- ***Glyphosate ('Roundup') – a Monsanto agrochemical is a potent inhibitor of the conversion of 3-phosphoshikimate (3-PS) → 5-enolpyruvylshikimate-3-phosphate (5-EPS-3P)***
  - a non-selective herbicide



Inspiration for Med Chem - *Statins*

- ***HMG CoA → MVA*** is the ***rate determining step*** in the biosynthetic pathway to ***cholesterol***

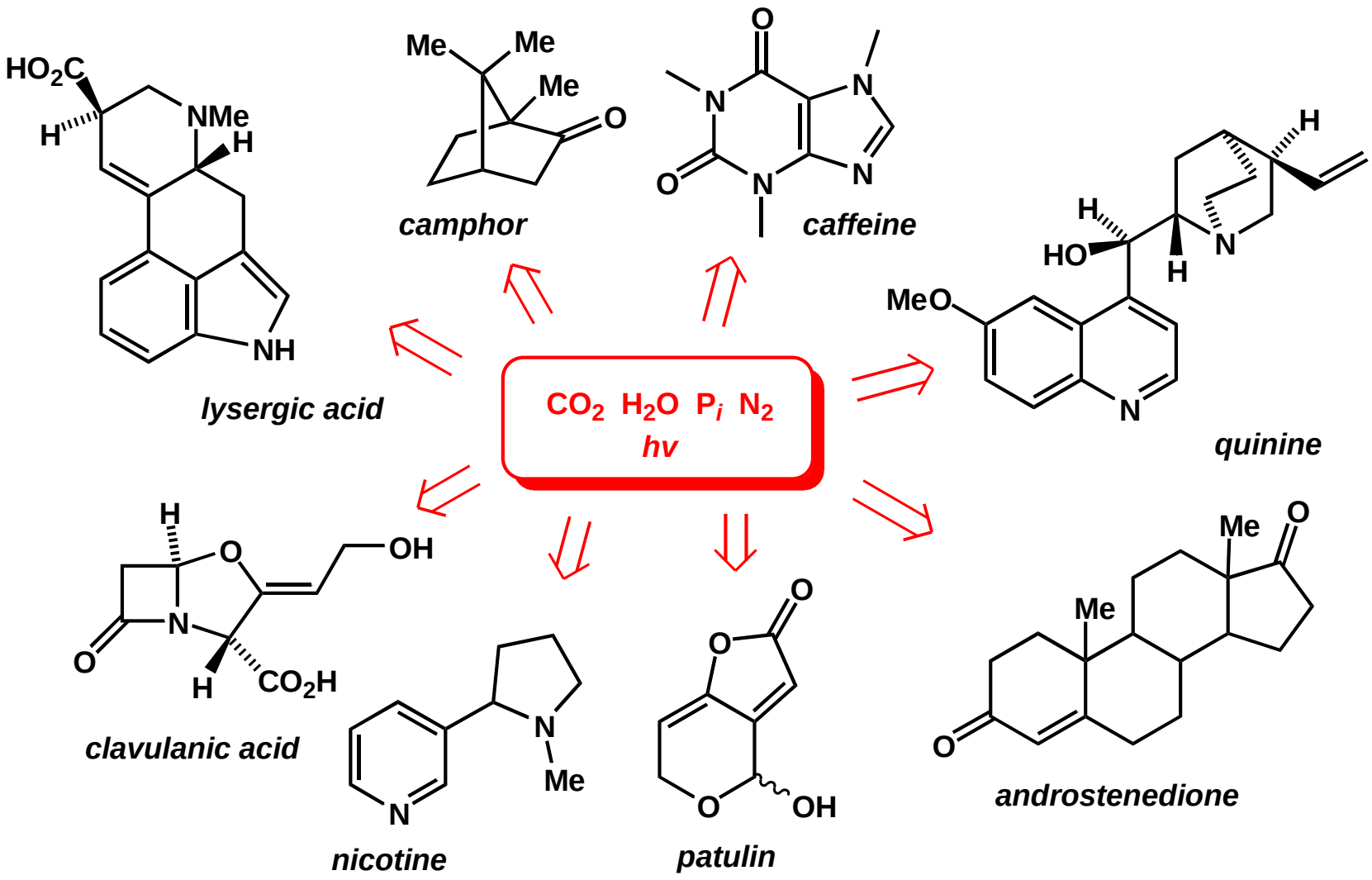
- ***'Statins'*** inhibit HMG CoA reductase and are used clinically to treat ***hypercholesteraemia*** - a causative factor in ***heart disease***
  - *e.g.* ***lipitor*** (Atorvastatin calcium, Pfizer) is a competitive inhibitior of HMG-CoA reductase and the worlds biggest selling drug [first drug to reach $10 billion sales (2004: $10.8 bn]



Format & Scope of Lecture

- ***What is biosynthesis?***
  - some definitions – phototrophs, chemotrophs; metabolism (catabolism/anabolism), 1° & 2° metabolites
- ***Overview of primary metabolism → secondary metabolites***
- ***Biological/biosynthetic reactions – enzyme & cofactor chemistry***
  - *free energy source* – ATP
  - *C-C & C-O bond formation* – CoASH, SAM, DMAPP, biotin
  - *oxidation* – NAD^+, FAD/FMN, haem iron oxo monooxygenases
  - *reduction* – NADPH
  - *C-N bond formation* – pyridoxal
- ***The Shikimate Pathway***
  - phenylalanine, tyrosine, tryptophan
  - coumarins, lignans & lignins

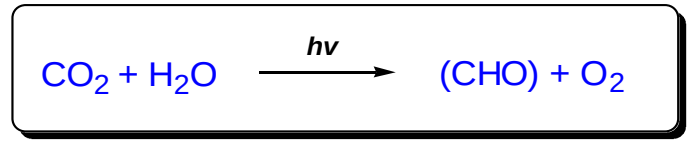
Metabolism & Natural Product Diversity



Phototrophs & Chemotrophs

- ***Living organisms*** are not at equilibrium. They ***require*** a continuous influx of ***free energy*** to perform mechanical work & for cellular growth/repair:

  - ***Phototrophs*** (*e.g.* green plants, algae & photosynthetic bacteria): derive free energy from the sun *via* *photosynthesis* ('CO_2 fixation'):
    - 10^{15} kg/year by green plants, which constitute 99% of Earths biomass (*i.e.* 10^{12} tons of dry matter)
    - 1g of carbon processed = >6250 litres of air



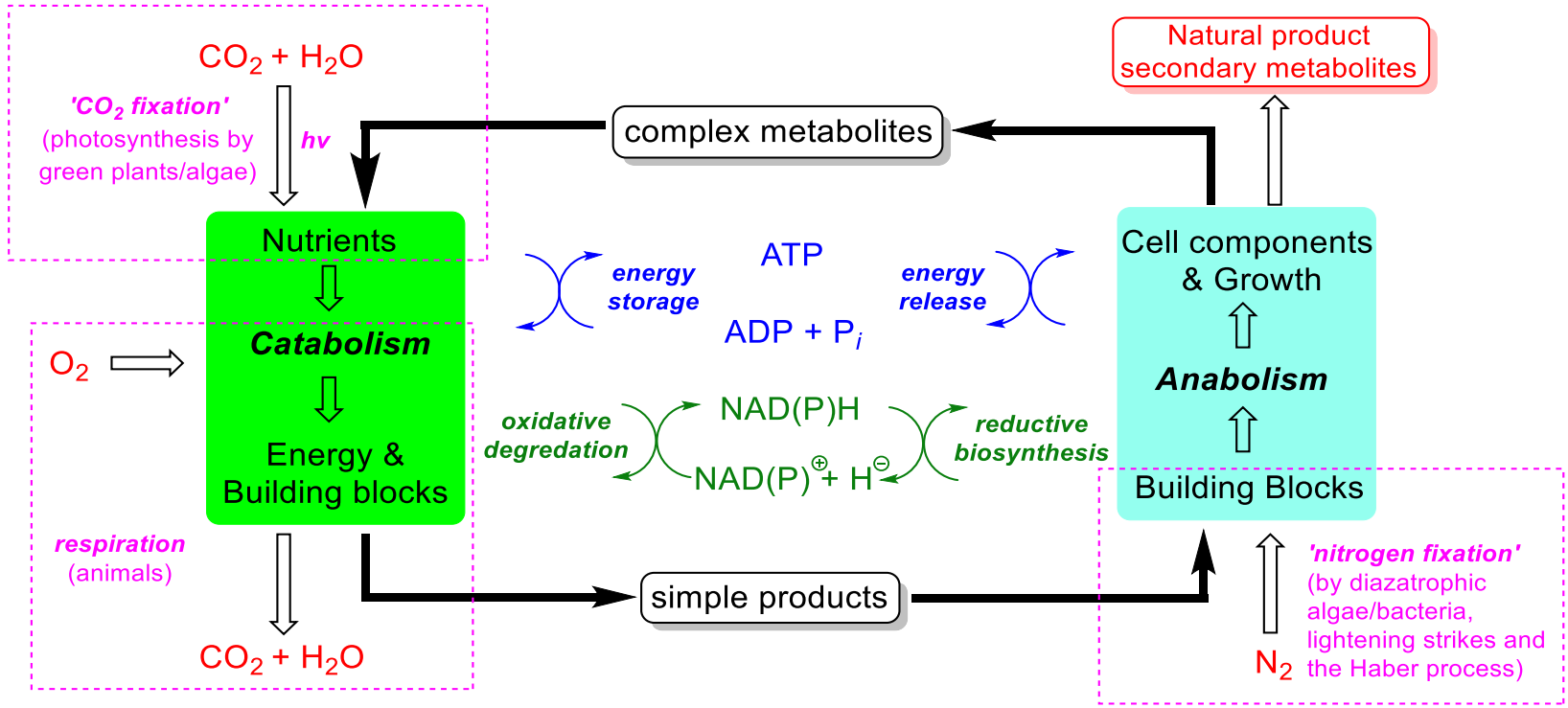
PHOTOSYNTHESIS

  - ***Chemotrophs*** (*e.g.* animals, fungi, most bacteria): derive free energy by *oxidising nutrients* (*e.g.* carbohydrates, lipids, proteins) obtained from other organisms, ultimately phototrophs
    - some bacteria & fungi require just D-glucose
    - mammals require sugars, essential amino acids & certain vitamins (enzyme co-factors or precursors)

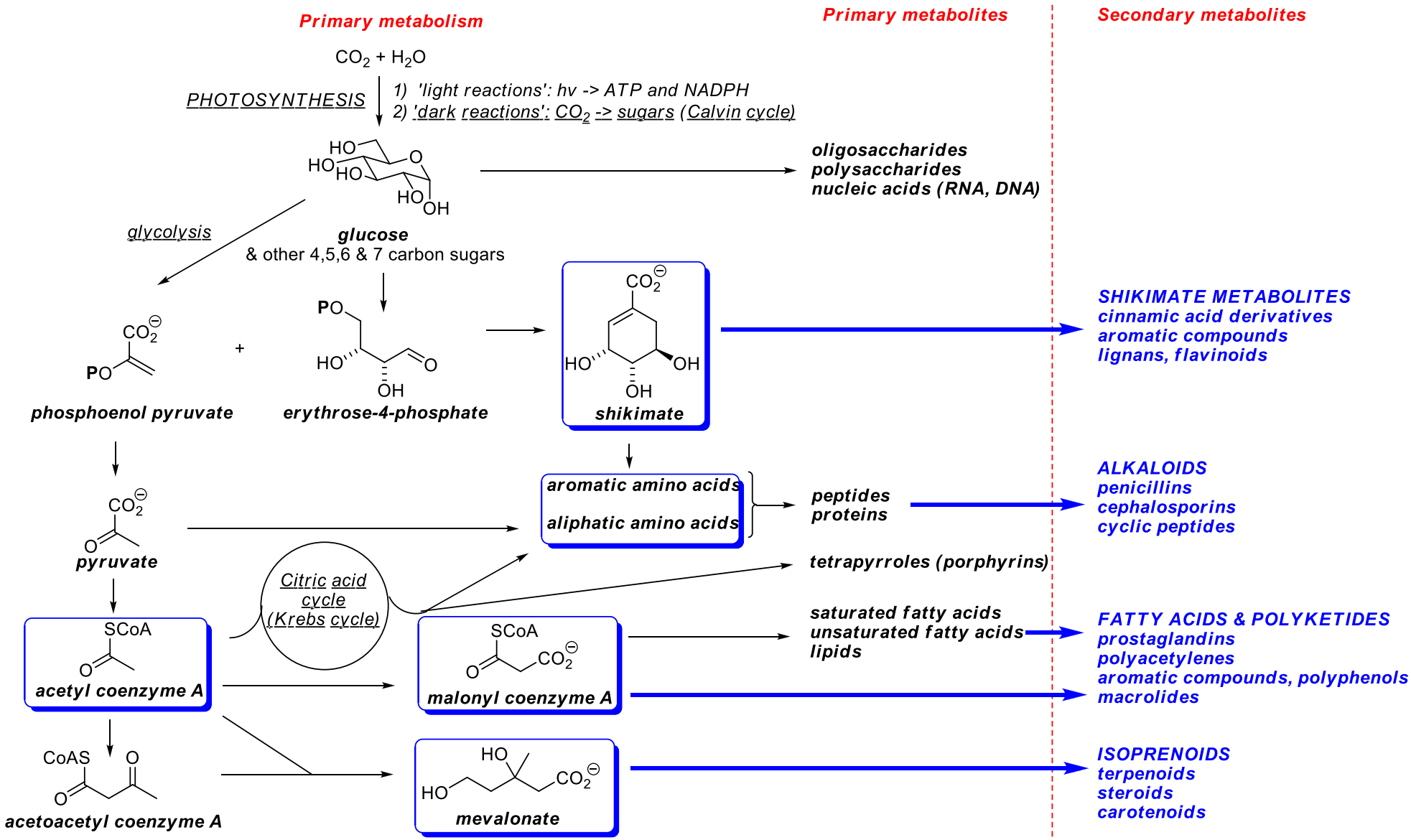
    - *Degradation of the nutrients is coupled to the stoichiometric production of 'high energy' phosphate compounds, particularly adenosine triphosphate (ATP, see later). All metabolic function is underpinned by ATP energetic coupling*
    - *By analogy with a money-based economy, the metabolic cost of production of a given metabolite from another can be quantified in terms of 'ATP equivalents' defined as the # of moles of ATP consumed/produced per mole of substrate converted in the reaction or sequence*

Metabolism

- ***Metabolism*** is the term used for *in vivo* processes by which compounds are degraded, interconverted and synthesised:
  - ***Catabolic*** or *degradative*: primarily to release energy and provide building blocks
    - generally *oxidative* processes/sequences (glycolysis, Krebs cycle)
  - ***Anabolic*** or *biosynthetic*: primarily to create new cellular materials (1° & 2° metabolites)
    - generally *reductive* processes/sequences
- These two types of process are coupled – one provides the driving force for the other:



Primary Metabolism - *Overview*



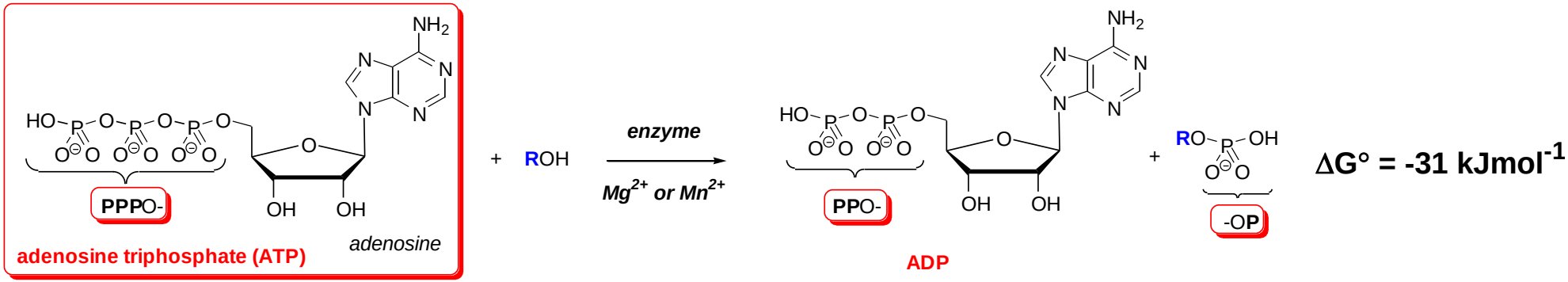
For interesting animations' of *e.g.* photosynthesis see: **http://www.johnkyrk.com/index.html**

Biological/Biosynthetic Reactions – *Enzyme Catalysis & Cofactors*

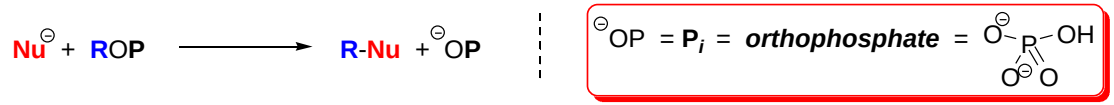
- Most biosynthetic steps are catalysed by specific, individual ***enzymes***. They generally perform familiar processes such as ***oxidation***, ***reduction***, ***alkylation***, ***hydrolysis***, ***acylation***, ***hydroxylation***, ***elimination*** *etc.*
- ***Different enzymes*** carrying out ***related reactions*** often employ ***common co-factors***: small organic functional fragments and/or metal ions. *e.g.*
  - ***FREE ENERGY RELEASING COUPLE:*** ***Adenosine triphosphate (ATP)***
  - ***C-C & C-O BOND FORMATION:*** ***Coenzyme A (CoASH); S-adenosyl methionine (SAM); dimethylallylpyrophosphate (DMAPP); biotin***
  - ***OXIDATION:*** ***NAD(P)$^{+}$; FAD/FMN; Haem iron oxo species (e.g. P_{450})***
  - ***REDUCTION:*** ***NAD(P)H; ($FADH_2$/$FMNH_2$)***
  - ***C-N BOND FORMATION:*** ***Pyridoxal***

Free Energy Releasing Couple - *ATP*

- ***Adenosine triphosphate (ATP)***
  - phosphorylation of an alcohol by adenosine diphosphate (ADP) is highly ***exothermic*** (*i.e.* liberates energy):



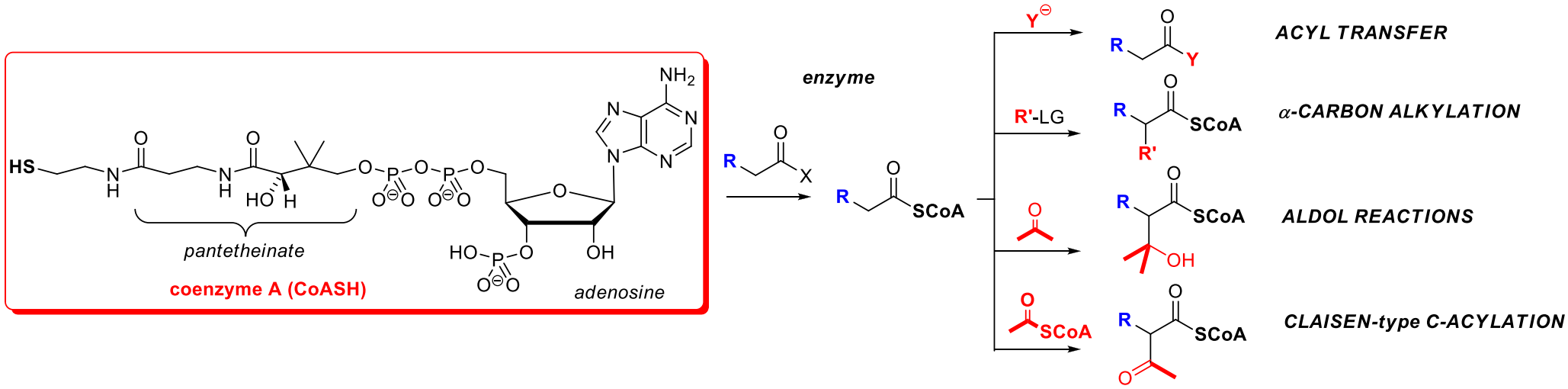
  - The phosphorylated alcohol (**R**O**P**) is then activated towards nucleophilic displacement:



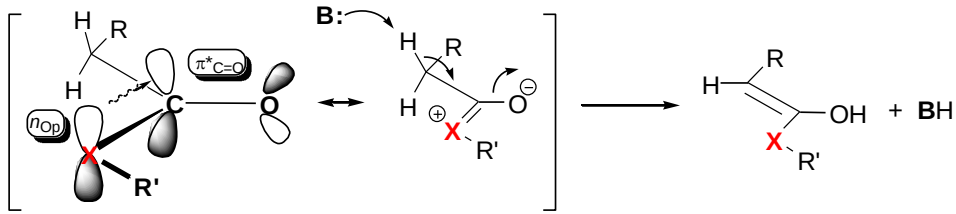
  - So, overall the ***endothermic*** process ROH + Y^- → RY + OH^- has been achieved by 'coupling' the process to the 'hydrolysis of ATP'
  - The situation is analogous to the use of tosylate activation to achieve nucleophilic displacement of an alcohol
  - In general, the exothermicity associated with phosphorylation shifts the equilibria of 'coupled' process by ***a factor of ~10^8***

Acylation & C-C Bond Formation α to C=O – *CoASH*

- ***Coenzyme A (CoASH)***
  - Coenzyme A acts as an acyl transfer/α-carbon activation reagent by forming reactive acyl thioesters:



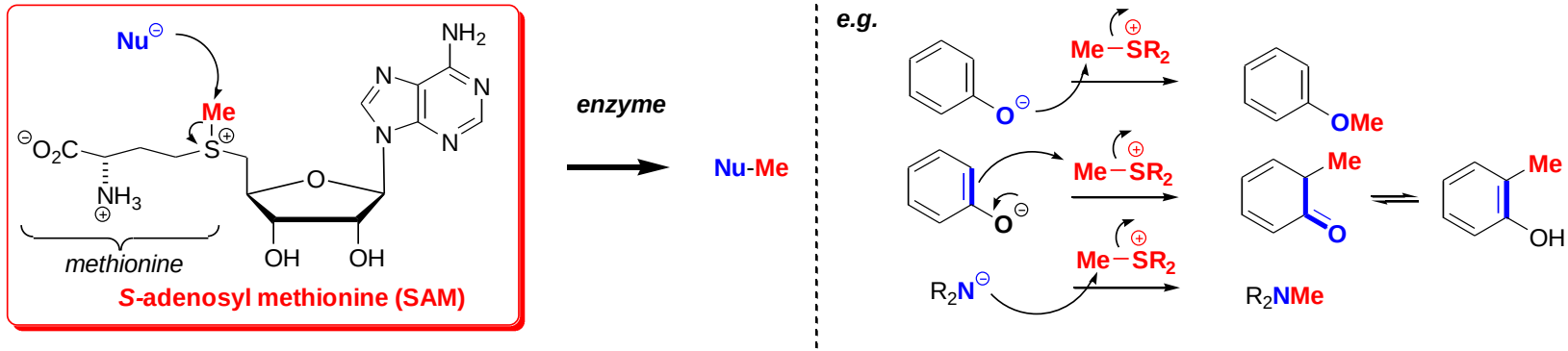
  - Acyl CoA derivatives can act as nucleophiles or electrophiles depending on the circumstances
  - These modes of reactivity reflect inherent properties of alkyl thioesters:
    - The ***good leaving group ability of RS***$^-$ (*cf.* RO^-) reflects: pK_a (RSH) ~10 *cf.* pK_a (ROH) ~16
    - The ***high electrophilic character of a thioester carbonyl carbon*** (*cf*. normal esters) reflects the poor orbital overlap between the lone pairs on sulfur (n_S) [*cf.* n_O] and the carbonyl anti bonding molecular orbital $\pi^*_{C=O}$
    - The ***enhanced acidity of protons α to the carbonyl of thioesters*** (*cf.* normal esters) reflects the same poor $n_S \leftrightarrow \pi^*_{C=O}$ resonance:



n_X-$\pi^*_{C=O}$ resonance makes carbonyl less susceptible to enolisation
Sulfur is in the 2nd period
so its lone pair has poor size/energy match with the $\pi^*_{C=O}$ orbital
Hence: $pK_a(RCH_2COSR')$ ~20 *cf.* RCH_2COOR' ~25
i.e. α to a thioester is similar to α to a ketone

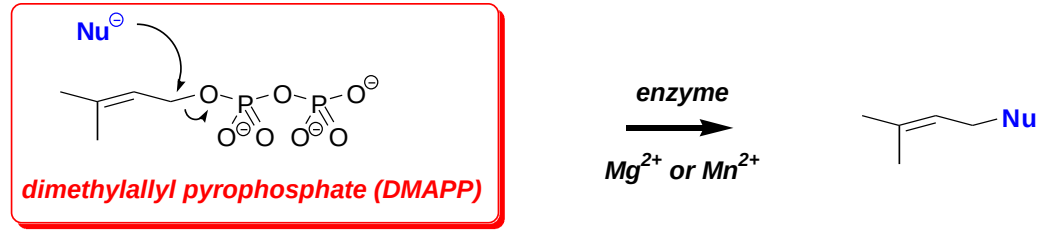
Methylation/Dimethylallylation – *SAM & DMAPP*

- ***S-Adenosyl methionine (SAM)***
  - SAM acts as a versatile *O*-, *C*-, *N*- & *S*- methylating reagent *in vivo*



  - Equivalent to performing an S_N2 methylation using MeI in the laboratory

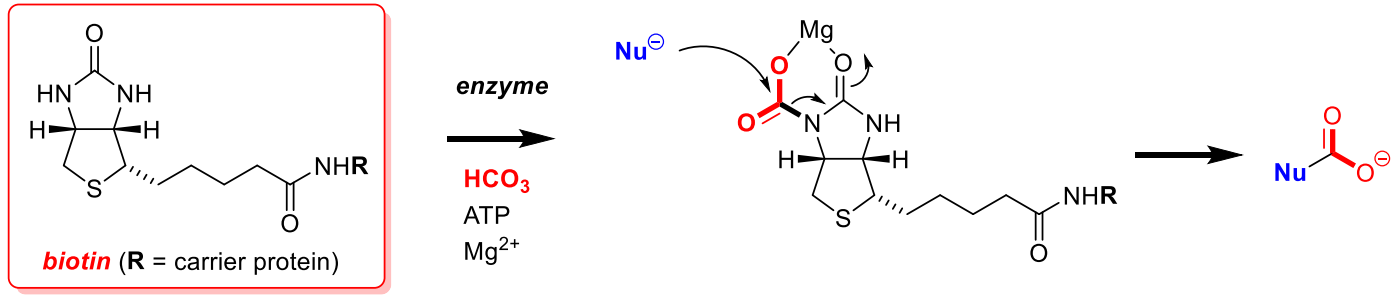
- ***Dimethylallyl pyrophosphate (DMAPP)***
  - DMAPP acts a dimethylallylating reagent – the pyrophosphate (+ Mg^{2+}/Mn^{2+}) is an excellent leaving group



  - Equivalent to performing an S_N2 allylation using allyl bromide in the laboratory

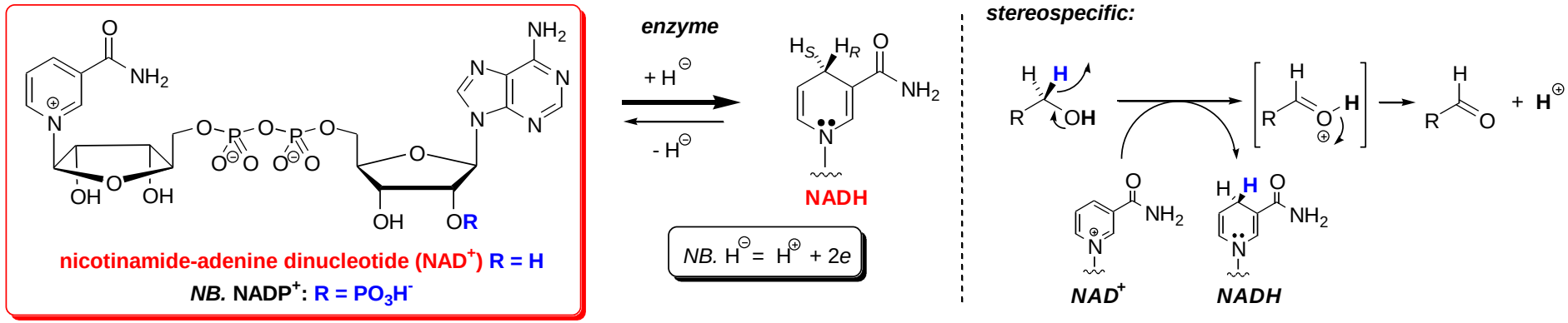
Carboxylation – *Biotin*

- ***Biotin***
  - Biotin in the presence of bicarbonate, ATP and Mg^{2+} enables nucleophile carboxylation *in vivo:*



Oxidation – *NAD*$^+$

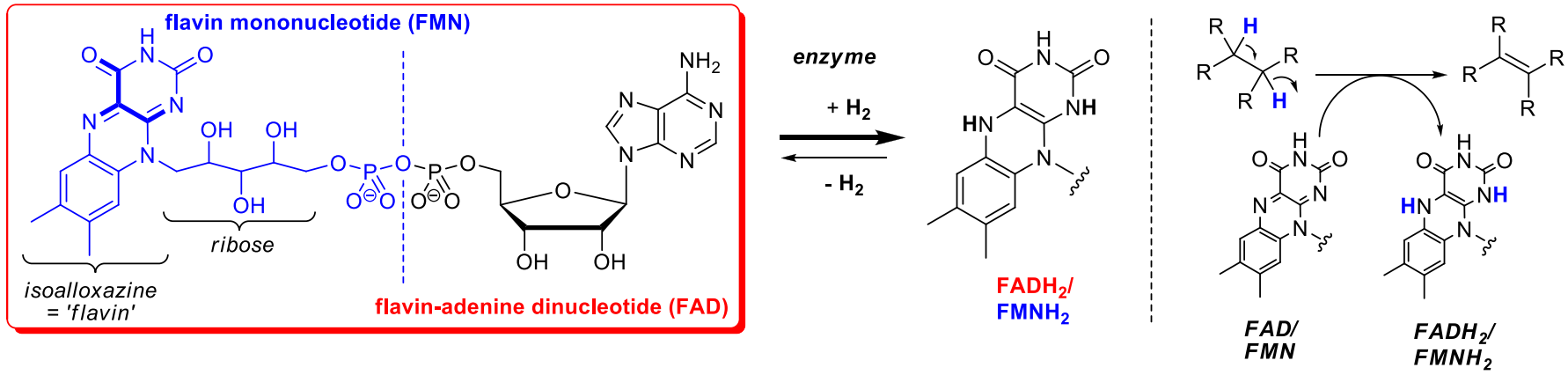
- ***Nicotinamide-adenine dinucleotide (NAD$^+$)*** [and its phosphorylated analogue ***(NADP$^+$)***] are mediators of ***biological oxidation*** (*e.g.* alcohol to ketone oxidation)
  - In general, the couple NAD$^+$/NADH is used by enzymes in ***catabolic oxidation*** (degradation)
  - The reagent is a stereospecific ***hydride acceptor***:



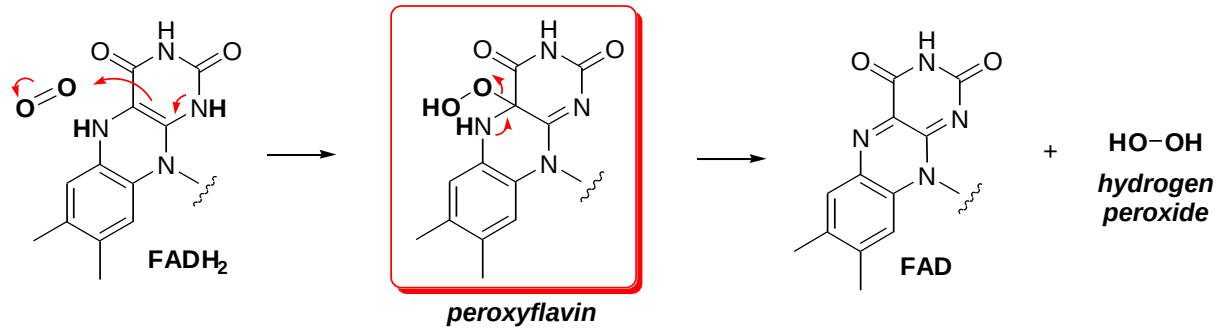
  - Different enzymes show different absolute specificities but are generally specific for the *pro-R* or *pro-S* hydrogens both for removal and delivery

Oxidation – *Flavins (FAD & FMN)*

- ***Flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN)*** are also mediators of ***biological oxidations*** (*e.g.* dehydrogenations – alkane to alkene)
  - Unlike NAD^+, which readily diffuses from enzyme to enzyme, FAD/FMN is usually tightly bound to a given enzyme, sometimes covalently

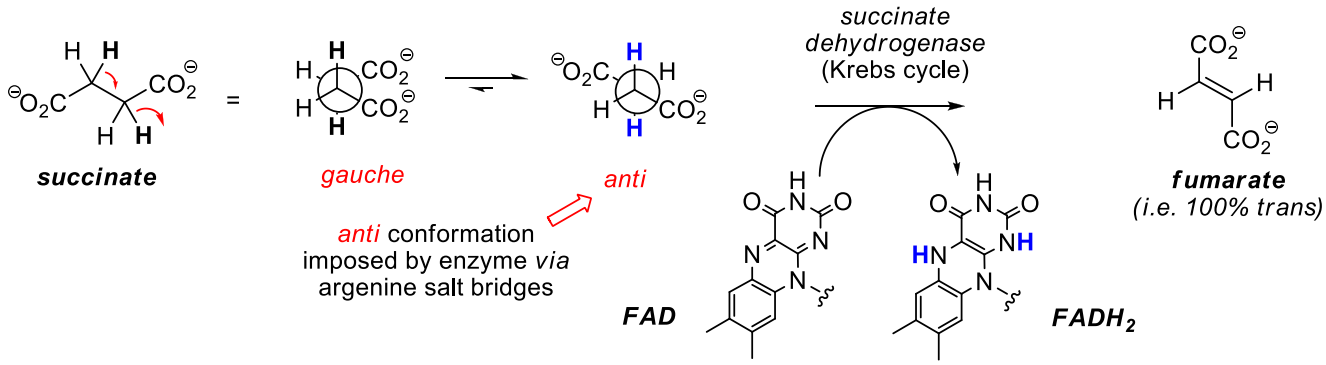


  - Re-oxidation of the $FADH_2$ back to FAD is generally by molecular ***oxygen***. The intermediate ***peroxyflavin*** can also mediate ***hydroxylation***, ***epoxidation*** & other oxygen transfer reactions (see next slide):

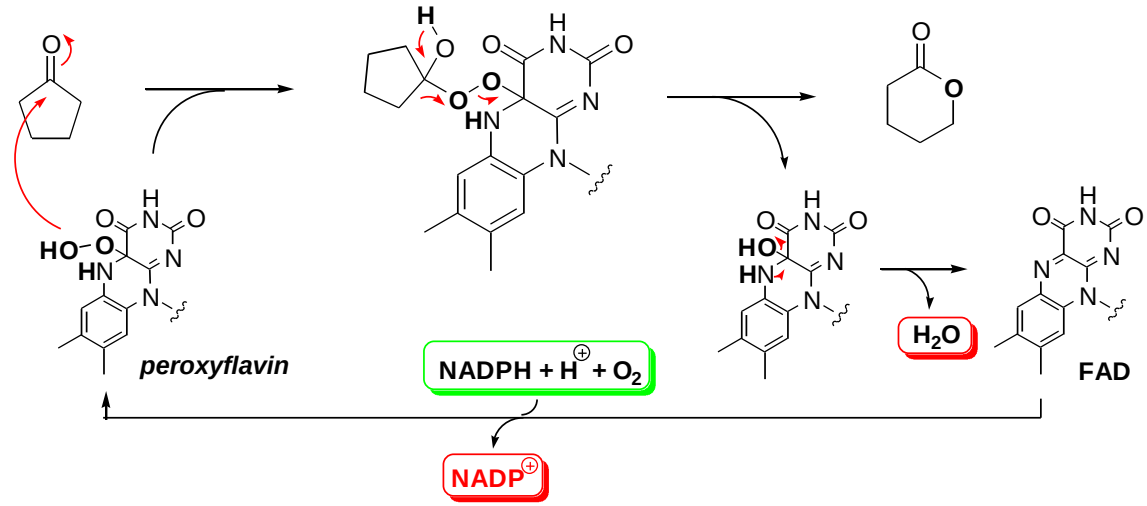


Oxidation Reactions Mediated by Flavins

- ***Dehydrogenation by flavins*** *– e.g. dehydrogenation of succinate → fumarate:*

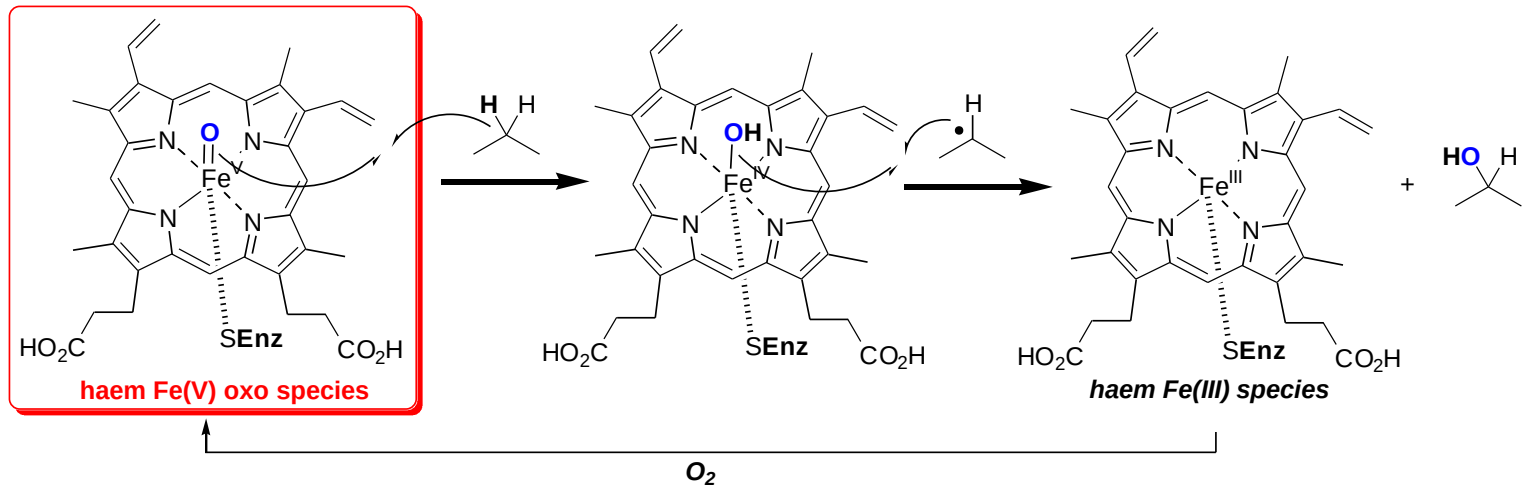


- ***Baeyer-Villiger-type oxidation by peroxyflavins*** *– e.g. ketone monooxygenase:*



Oxidation – *Haem Iron oxo Species (P_{450})*

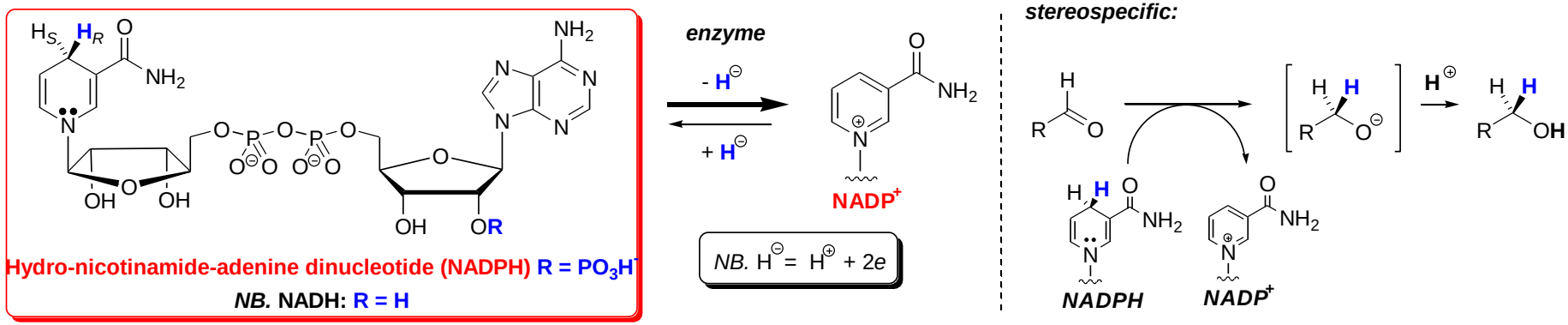
- ***Haem iron oxo species*** *e.g.* in ***cytochrome P_{450}*** (a ubiquitous ***heam monooxygenase***) are also mediators of ***biological oxidation*** (*e.g.* phenolic coupling, epoxidation, ***hydroxylation***):



  - The porphyrin ring acts as a tetradentate ligand for the octahedral iron. The two axial positions are occupied by an enzyme amino acid ligand (typically a histidine nitrogen) and hydroxy/hydroperoxy residue respectively

Reduction - *NADPH*

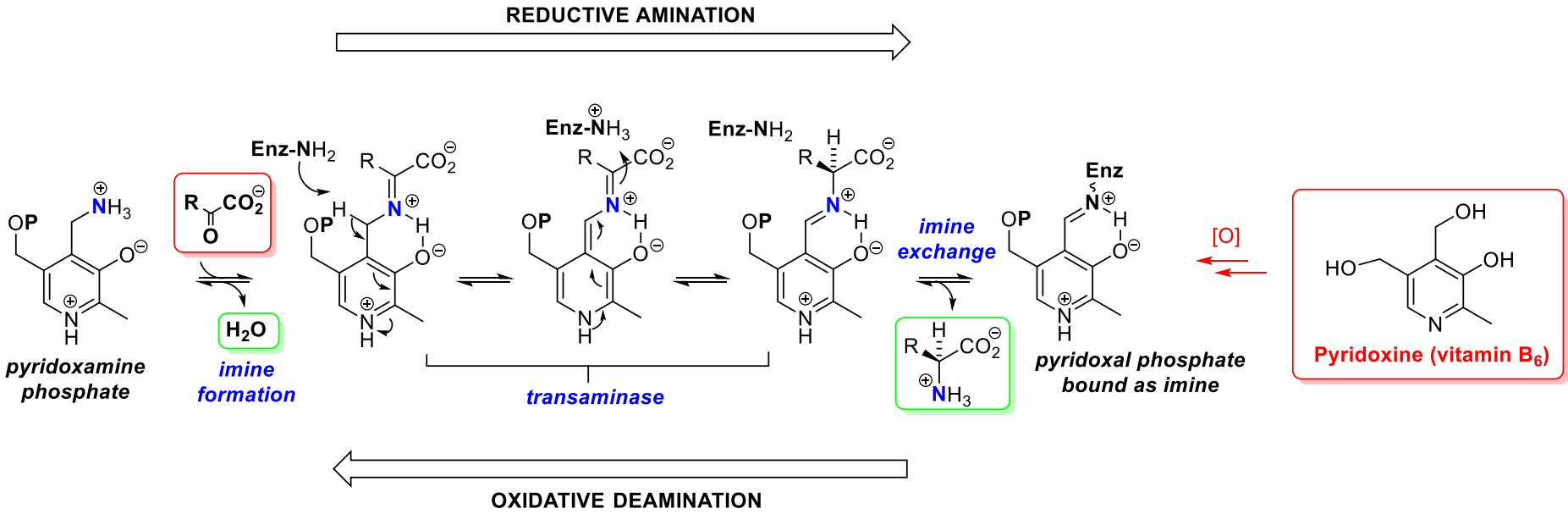
- ***Dihydro-nicotinamide-adenine dinucleotide phosphate (NADPH)*** [and its de-phosphorylated analogue ***(NADH)***] are mediators of ***biological reduction*** (*e.g.* ketone to alcohol reduction)
  - In general, the couple NAPH/$NADP^+$ is used by enzymes in ***anabolic reduction*** (biosynthesis)
  - The reagent is a stereospecific ***hydride donor***:



  - As for the reverse process, different enzymes show different absolute specificities but are generally specific for the *pro-R* or *pro-S* hydrogens both for removal and delivery

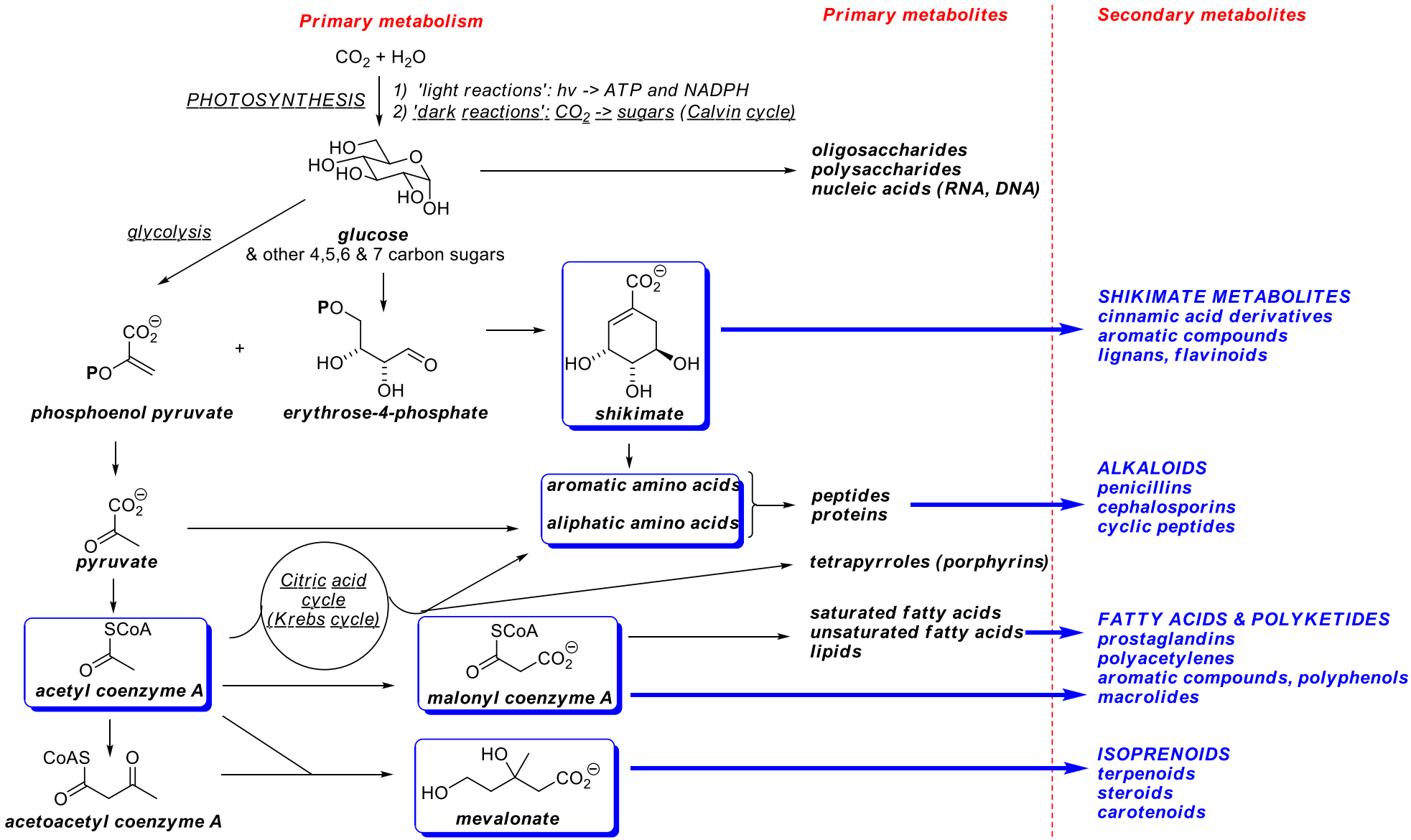
Transamination - *PLP*

- ***Pyridoxine (vitamin B_6) → pyridoxal-5'-phosphate (PLP)***
  - ***PLP*** forms ***imines*** (Schiffs bases) with ***primary amines***. This forms the basis of *in vivo* ***transamination*** of ***α-ketoacids*** to give ***α-amino acids*** (& also ***racemisation/decarboxylation*** processes, see 'alkaloids')



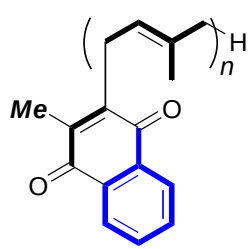
  - The α-carbon protonation is stereospecific and generally gives the (*S*) configured chiral centre

Primary Metabolism - *Overview*

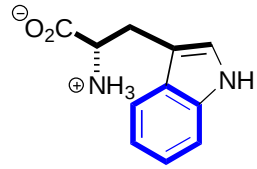


For interesting animations' of *e.g.* photosynthesis see: **http://www.johnkyrk.com/index.html**

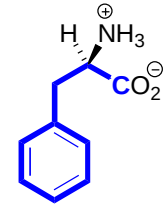
Shikimate Metabolites



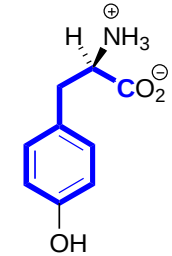
menaquinone (vitamin K_2)
(ArC_1)



(S)-tryptophan
(ArC_0)

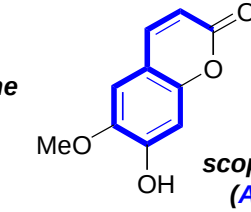


(S)-phenylalanine
(ArC_3)

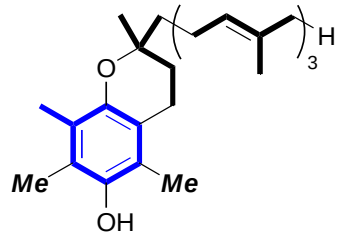


(S)-tyrosine
(ArC_3)

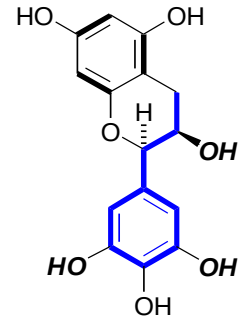
SHIKIMATE METABOLITES



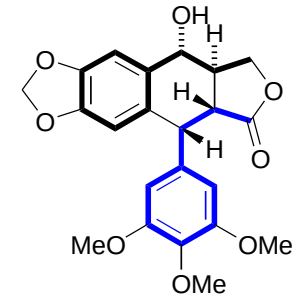
scopoletin
(ArC_3)



α-tocopherol (vitamin E)
(ArC_1)



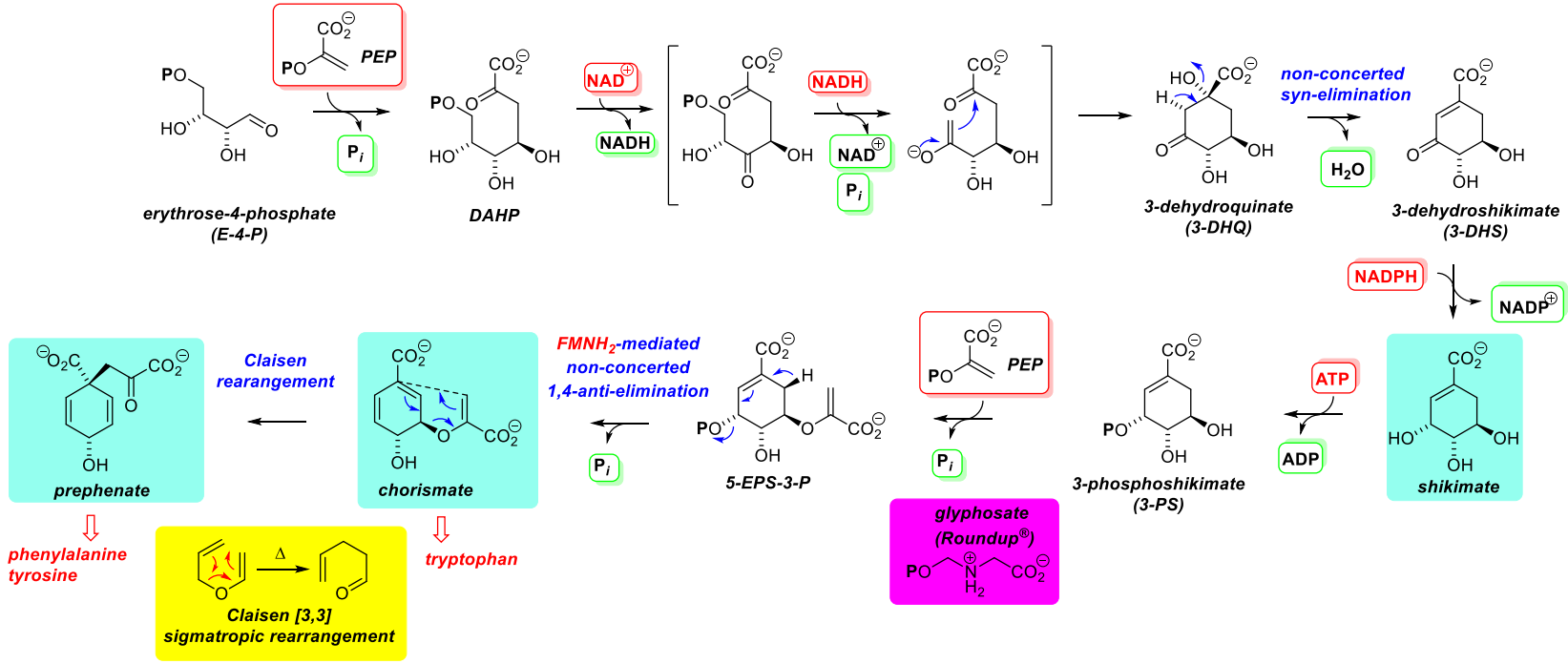
epigallocatechin (EGC)
(ArC_3)



podophyllotoxin
(ArC_3)

The Shikimate Biosynthetic Pathway - *Overview*

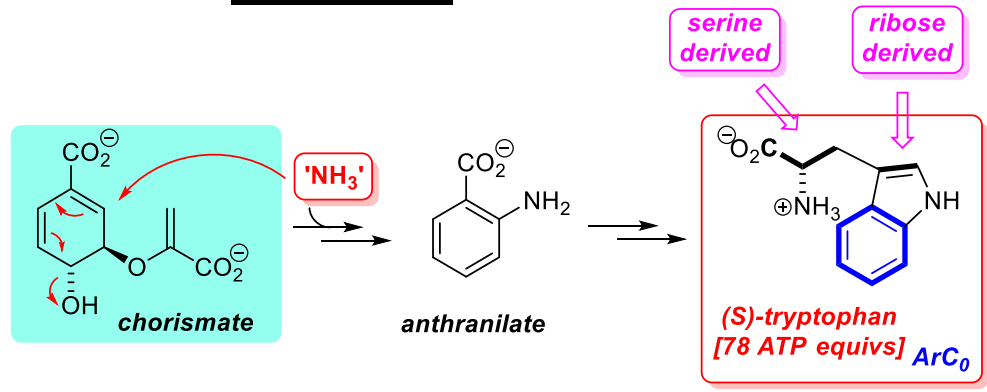
- ***Phosphoenol pyruvate*** *&* ***erythrose-4-phosphate*** → ***shikimate*** → ***chorismate*** → ***prephenate***:



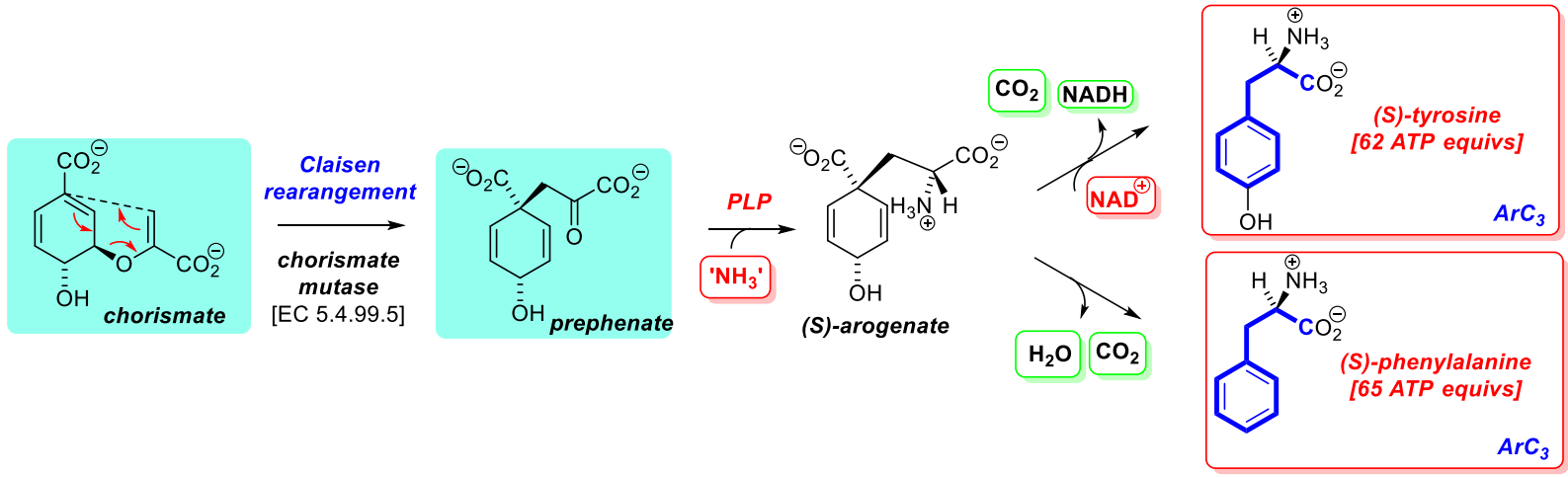
  - The detailed mechanisms of these steps have been studied intensively. Most are chemically complex and interesting. For additional details see:
    - Mann *Chemical Aspects of Biosynthesis* Oxford Chemistry Primer No. 20, **1994** *(key details)*
    - Haslam *Shikimic Acid – Metabolism and Metabolites* Wiley, **1993** *(full details and primary Lit. citations)*
    - http://www.chem.qmul.ac.uk/iubmb/enzyme/reaction/misc/shikim.html *(interesting web-site with many biosynethtic pathways)*

Chorismate → Tryptophan, Tyrosine & Phenylalanine

- ***Chorismate → anthranilate → <u>tryptophan</u>***

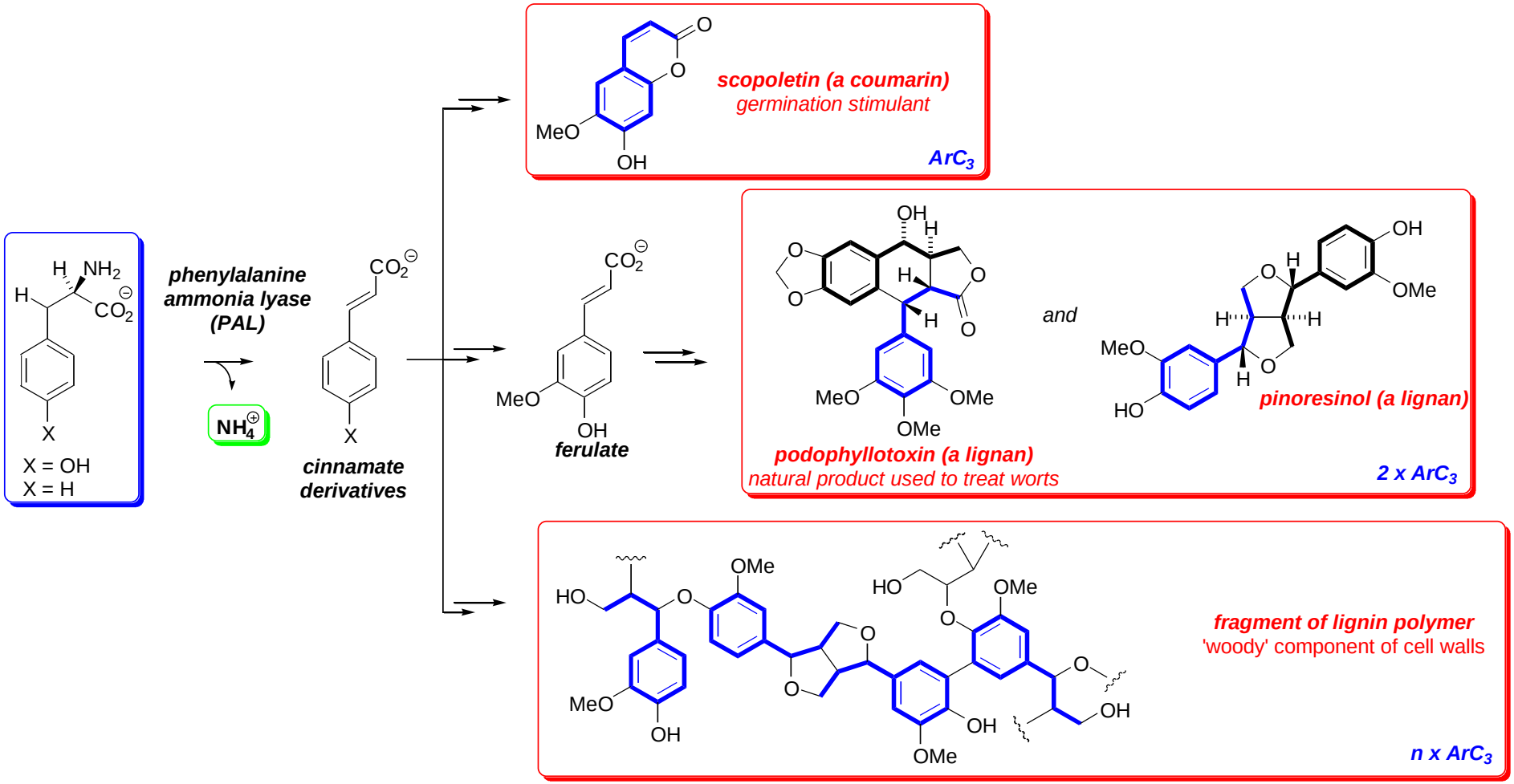


- ***Chorismate → prephenate → <u>tyrosine</u>*** & ***<u>phenylalanine</u>***
  - *NB*. The enzyme *chorismate mutase* [EC 5.4.99.5] which mediates the conversion of chorismate to prephenate is the only known '*Claisen rearrangementase*'

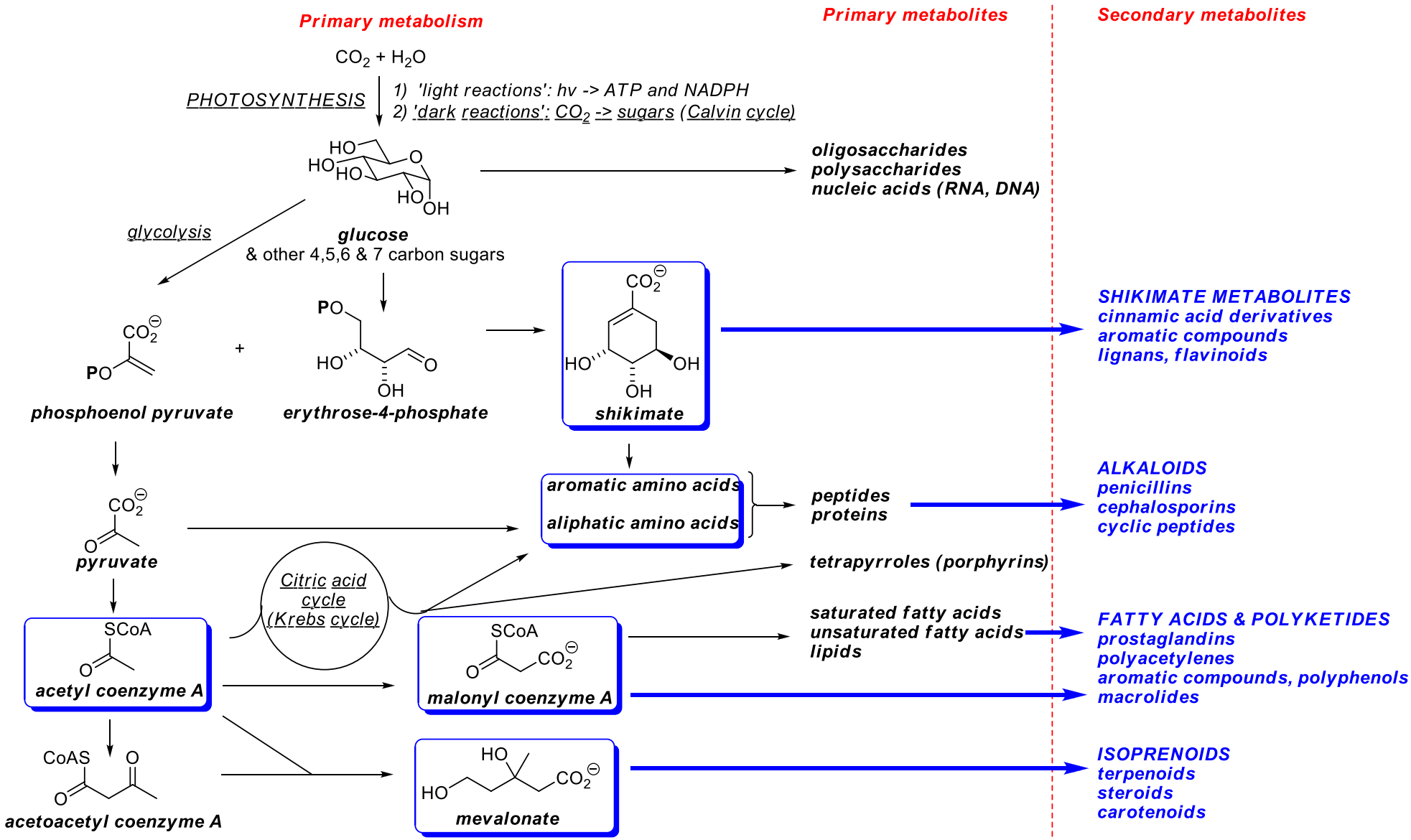


Tyrosine/Phenylalanine → ArC_3 Metabolites

- ***Tyrosine*** & ***phenylalanine*** → ***cinnamate derivatives*** → ***ArC_3 metabolites***
  - ***coumarins***, ***lignans*** (stereoselective enzymatic dimerisation) & ***lignins*** (stereorandom radical polymerisation)



Primary Metabolism - *Overview*



For interesting animations' of *e.g.* photosynthesis see: **http://www.johnkyrk.com/index.html**