An Overview of Biosynthesis Pathways – Inspiration for Pharmaceutical and Agrochemical Discovery

Alan C. Spivey
a.c.spivey@imperial.ac.uk

Imperial College London

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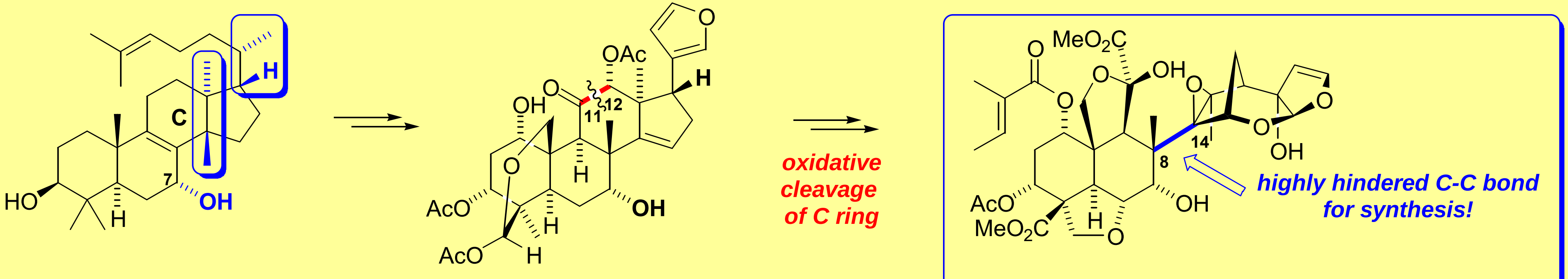
Lessons in Synthesis - *Azadirachtin*

- ***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 synhtetic 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**

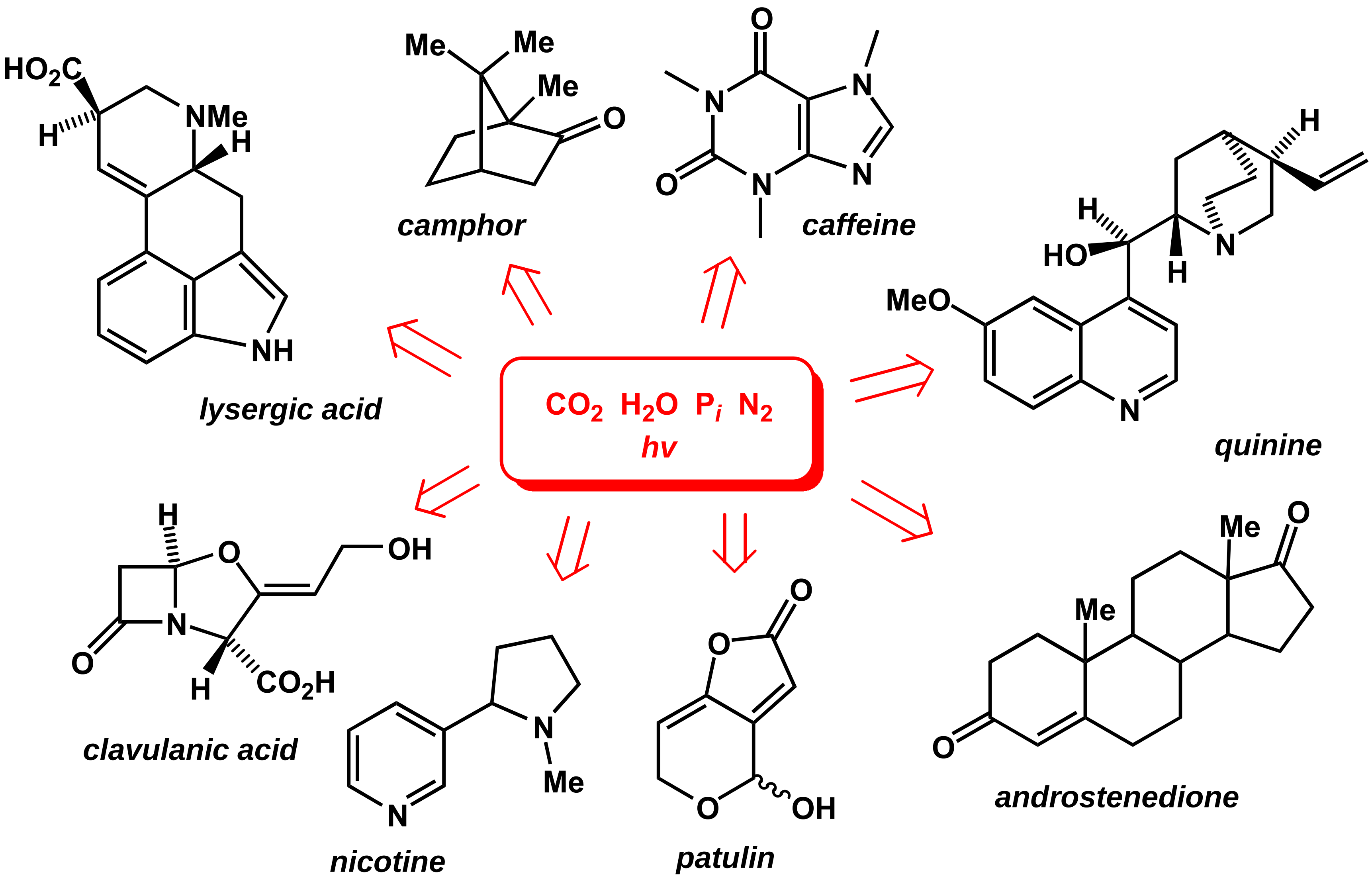
  - Veitch *Angew. Chem. Int. Ed.* **2007**, *46*, 7629 (DOI) & Veitch *Angew. Chem. Int. Ed.* **2007**, *46*, 7633 (DOI)
  - ***Review 'The azadirachtin story' see:*** Veitch *Angew. Chem. Int. Ed.* **2008**, *47*, 9402 (DOI)

Format & Scope of Presentation

- ***Metabolism & Biosynthesis***
  - some definitions, 1° & 2° metabolites
- ***Shikimate Metabolites***
  - photosynthesis & glycolysis → shikimate formation → shikimate metabolites
  - *Glyphosate* – a non-selective herbicide
- ***Alkaloids***
  - acetylCoA & the citric acid cycle → α-amino acids → alkaloids
  - *Opioids* – powerful pain killers
- ***Fatty Acids and Polyketides***
  - acetylCoA → malonylCoA → fatty acids, prostaglandins, polyketides, macrolide antibiotics
  - *NSAIDs* – anti-inflammatory's
- ***Isoprenoids/terpenes***
  - acetylCoA → mevalonate → isoprenoids, terpenoids, steroids, carotenoids
  - *Statins* – cholesterol-lowering agents

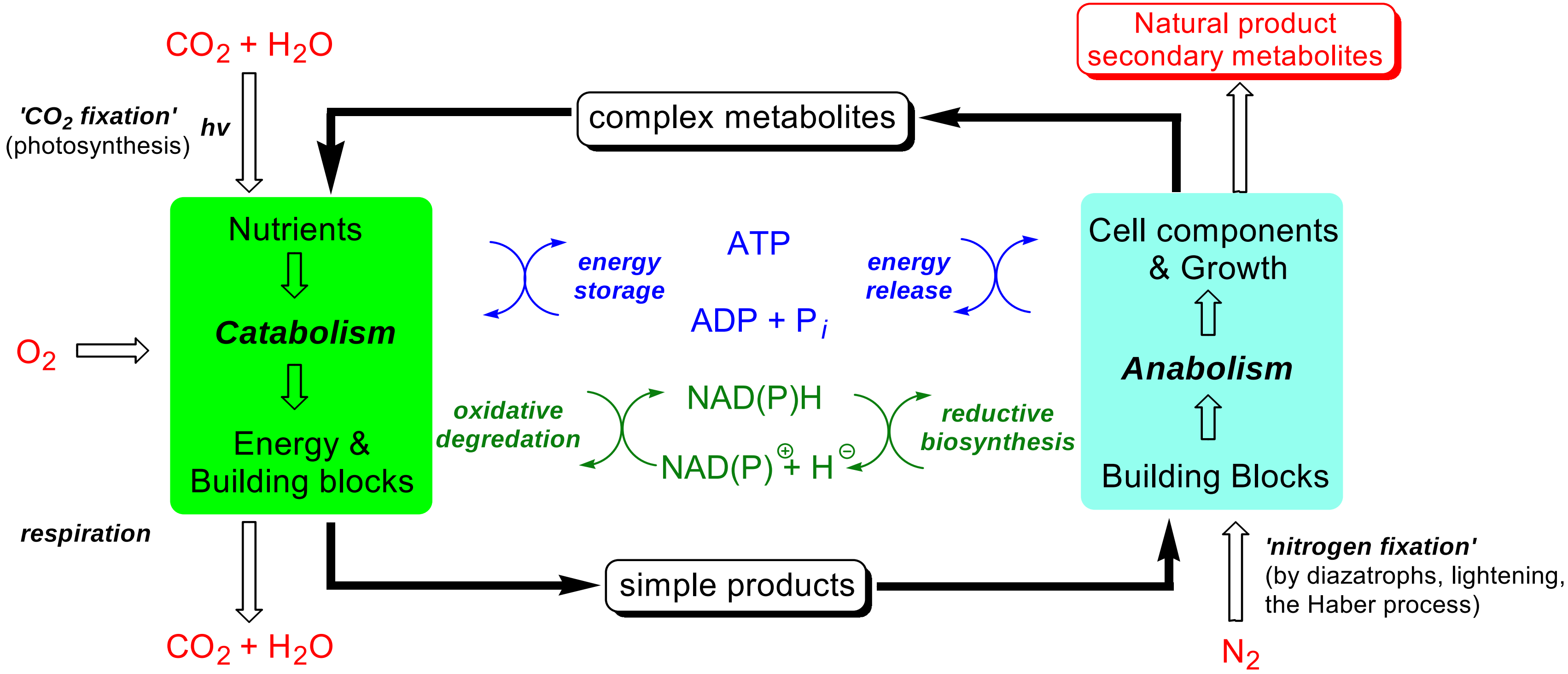
Metabolism and Biosynthesis

Metabolism & Natural Product Diversity

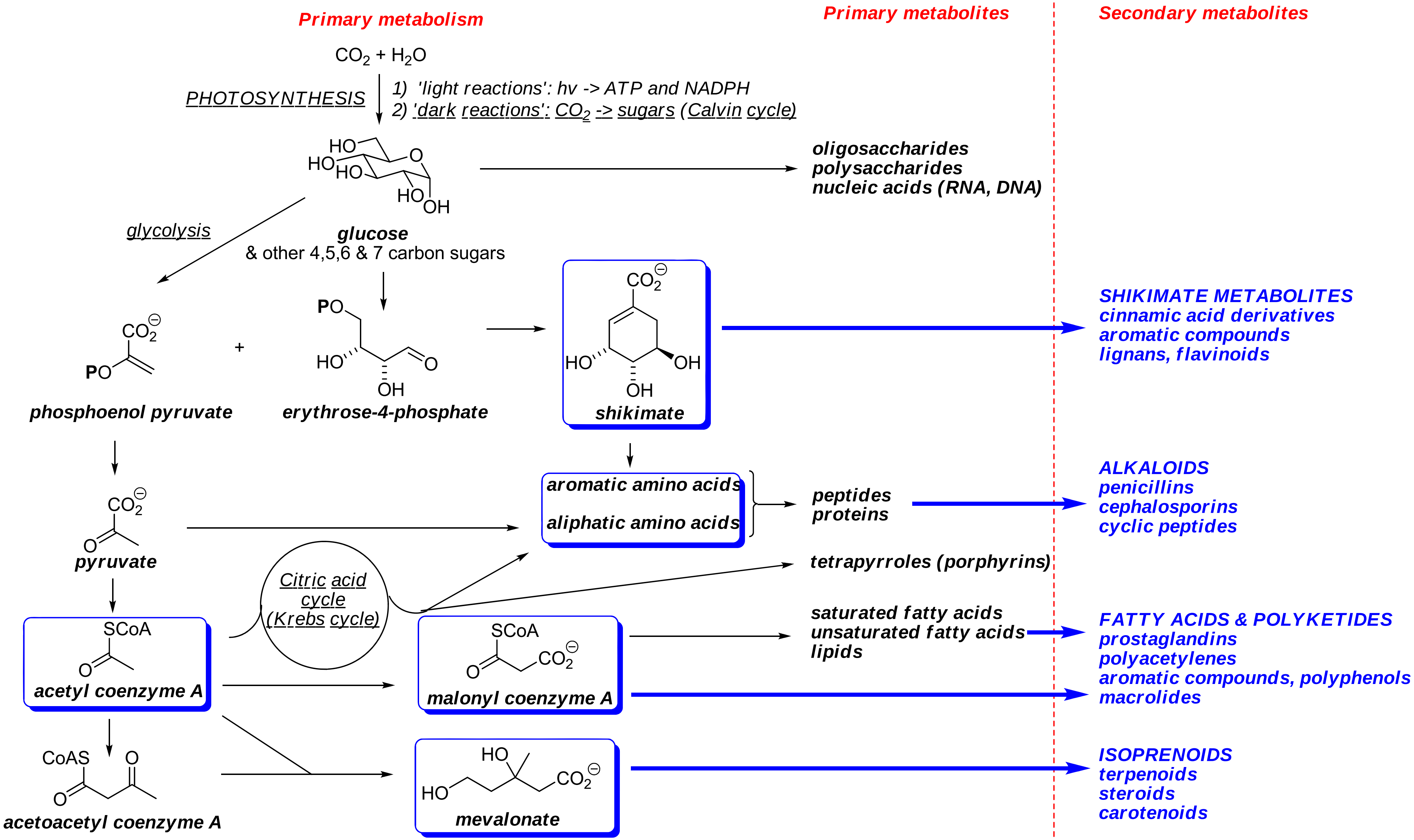


Metabolism

- ***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:



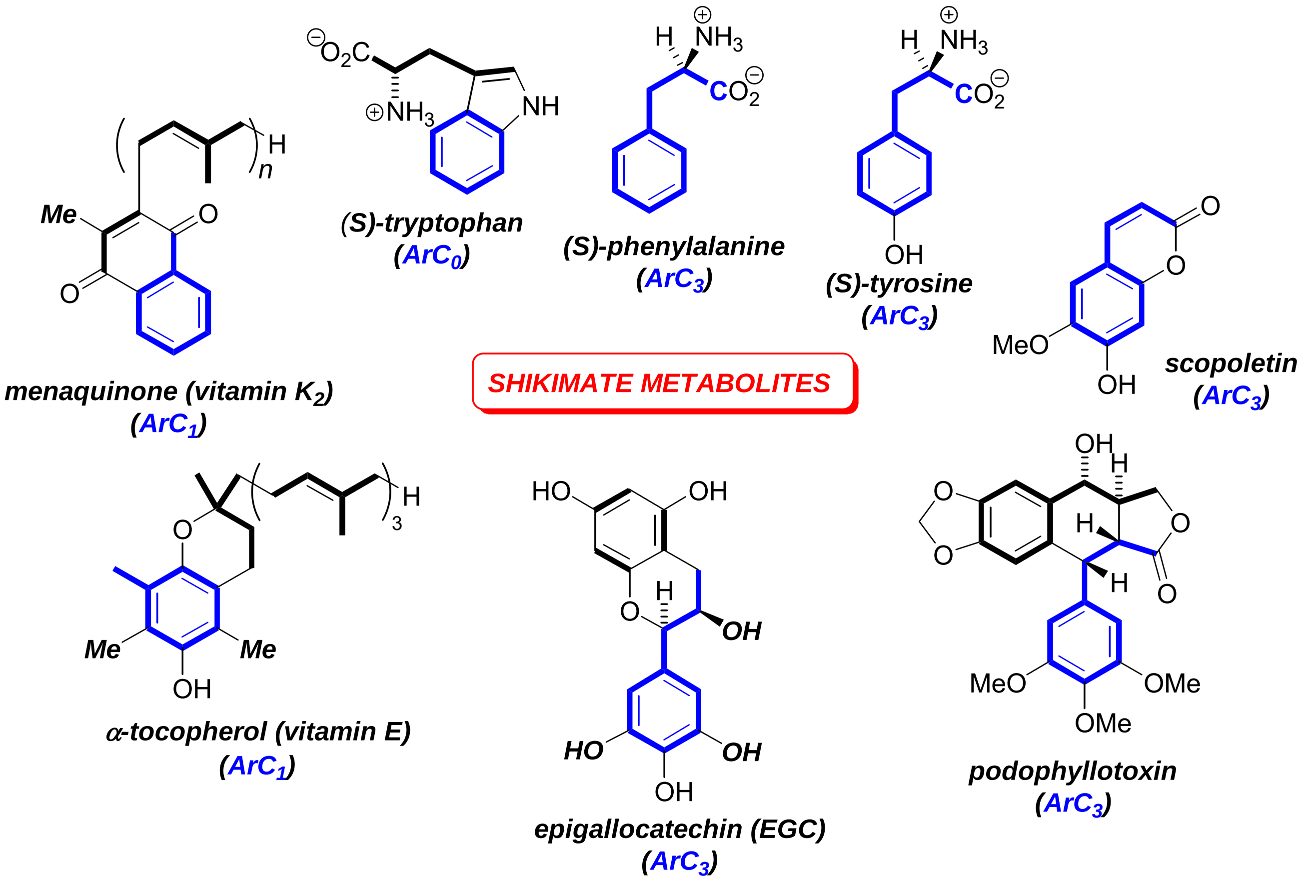
Primary Metabolism - *Overview*



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

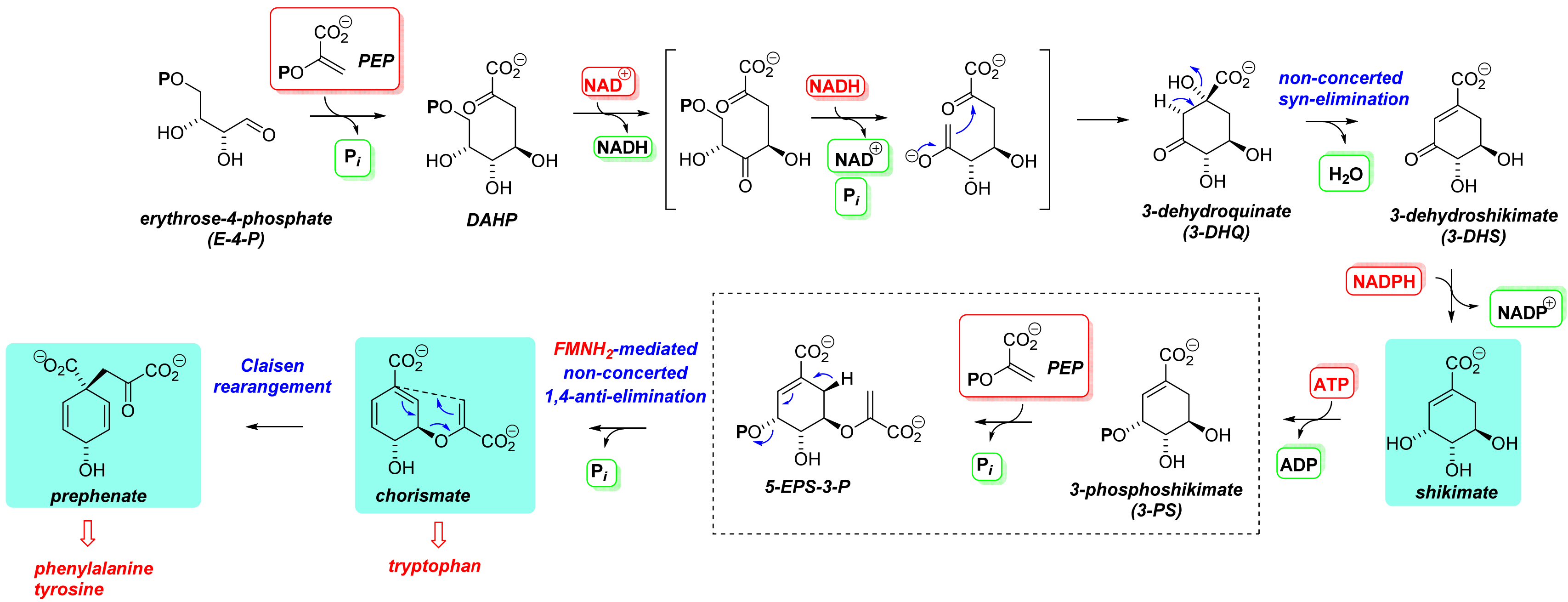
Shikimate Metabolites

Shikimate Metabolites



The Shikimate Biosynthetic Pathway - *Overview*

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

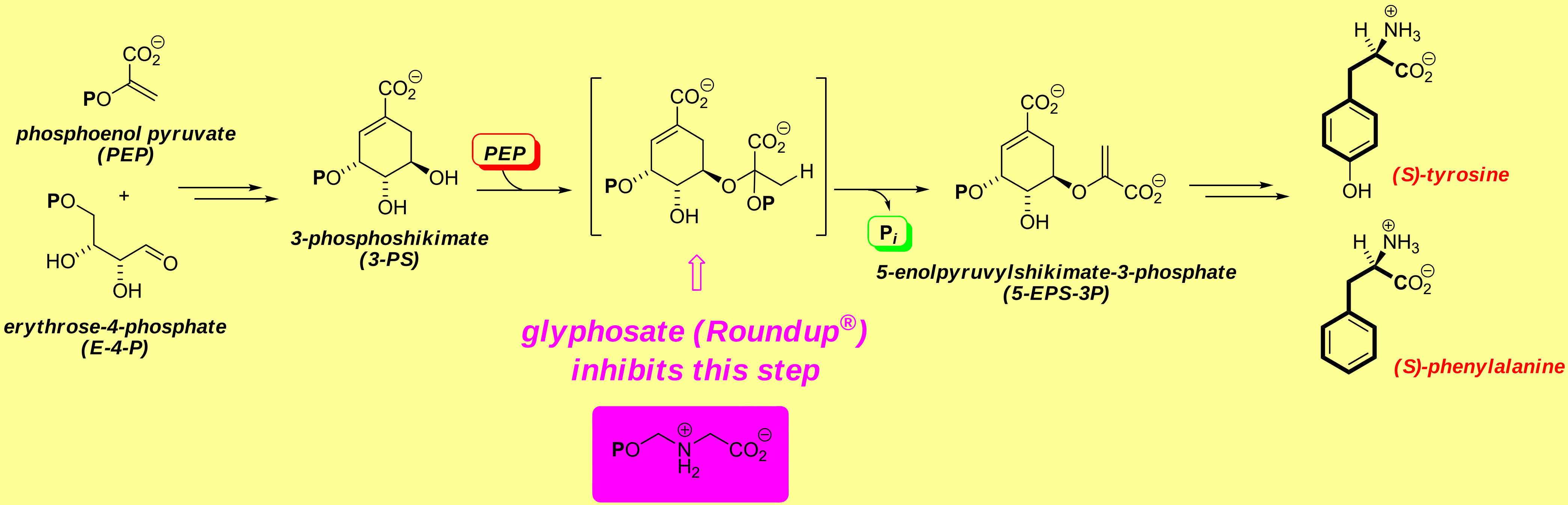


– The detailed mechanisms of these steps have been studied <u>intensively</u>. Most are chemically complex and interesting. For additional details see:
  - Mann *Chemical Aspects of Biosynthesis* Oxford Chemistry Primer No. 20, **1994** *(key details)*
  - Haslam *Shikimic Acid – Metabolism and Metabolites* 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)*

Rational Agrochemical Development – *Shikimate Pathway Intervention*

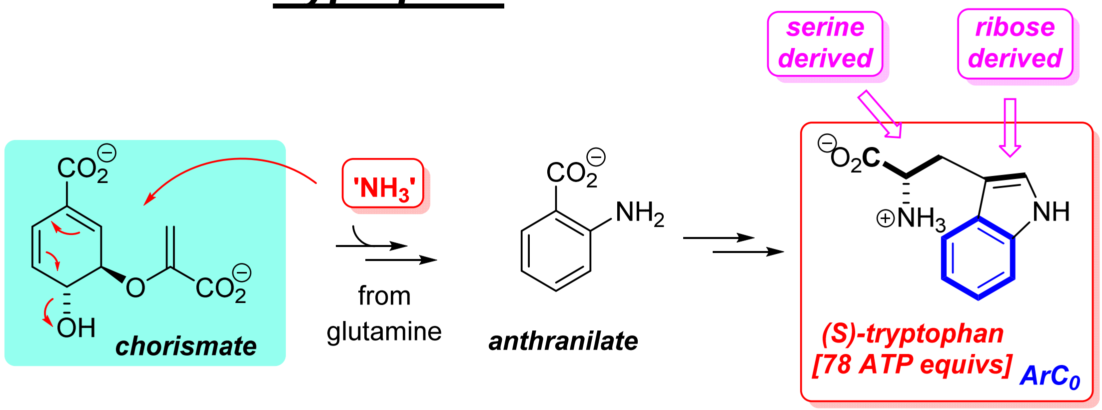
- ***The shikimate biosynthetic pathway is not found in animals/humans – only in plants***
  - selective intervention in these pathways allows development of agrochemicals with minimal human toxicity

- ***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

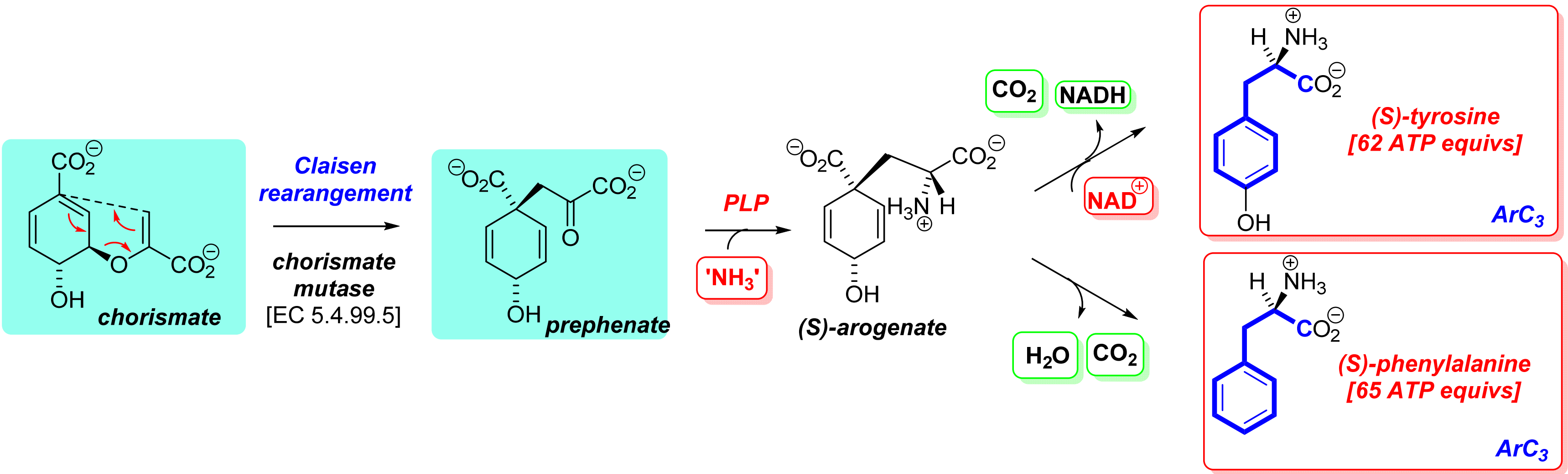


Chorismate → Tryptophan, Tyrosine & Phenylalanine

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

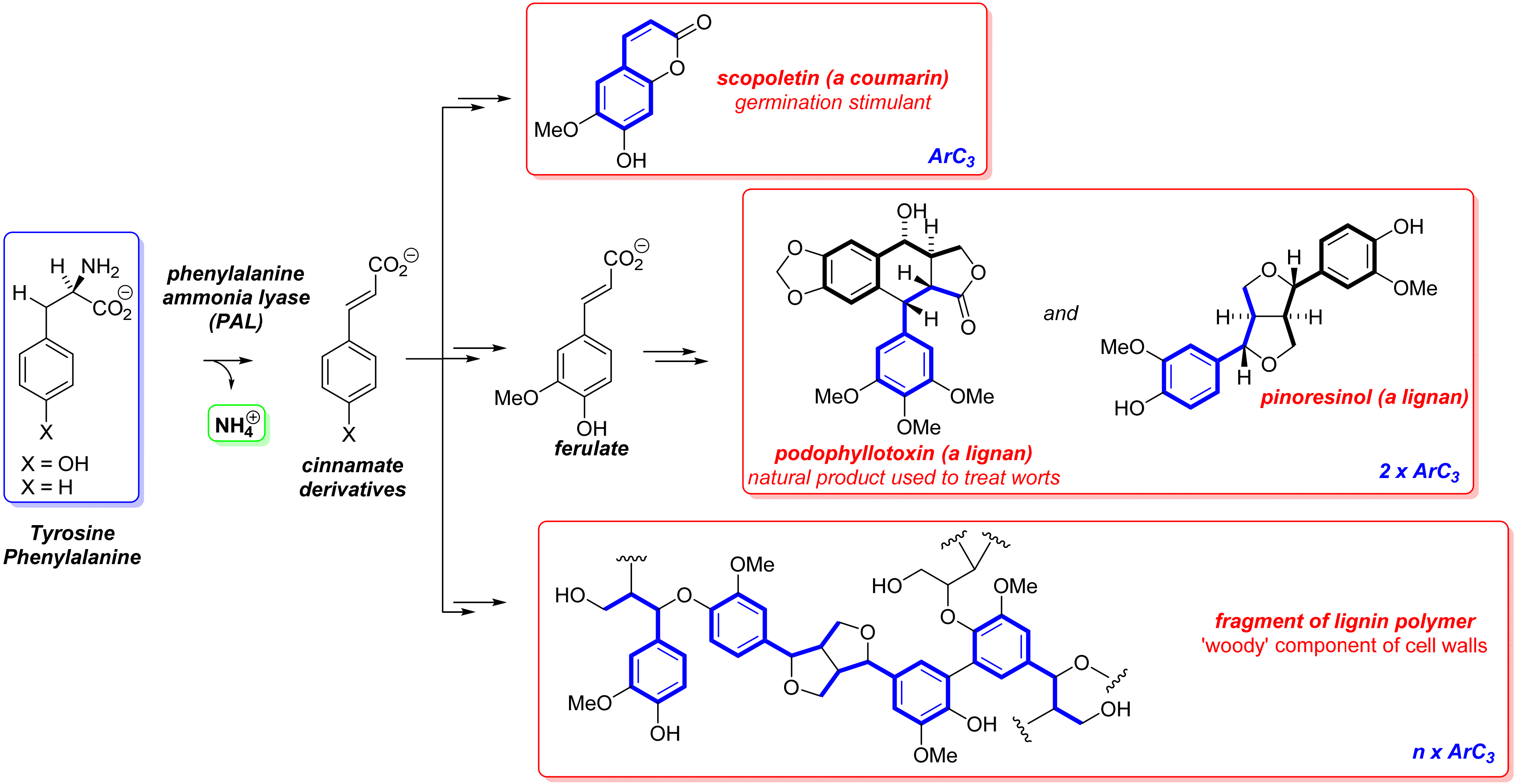


- ***Chorismate → prephenate → <u>tyrosine</u> & <u>phenylalanine</u>***
  - *NB*. The enzyme *chorismate mutase* [EC 5.4.99.5] which mediates the conversion of chorismate to prephenate is the only known '*Claisen rearrangementase*'

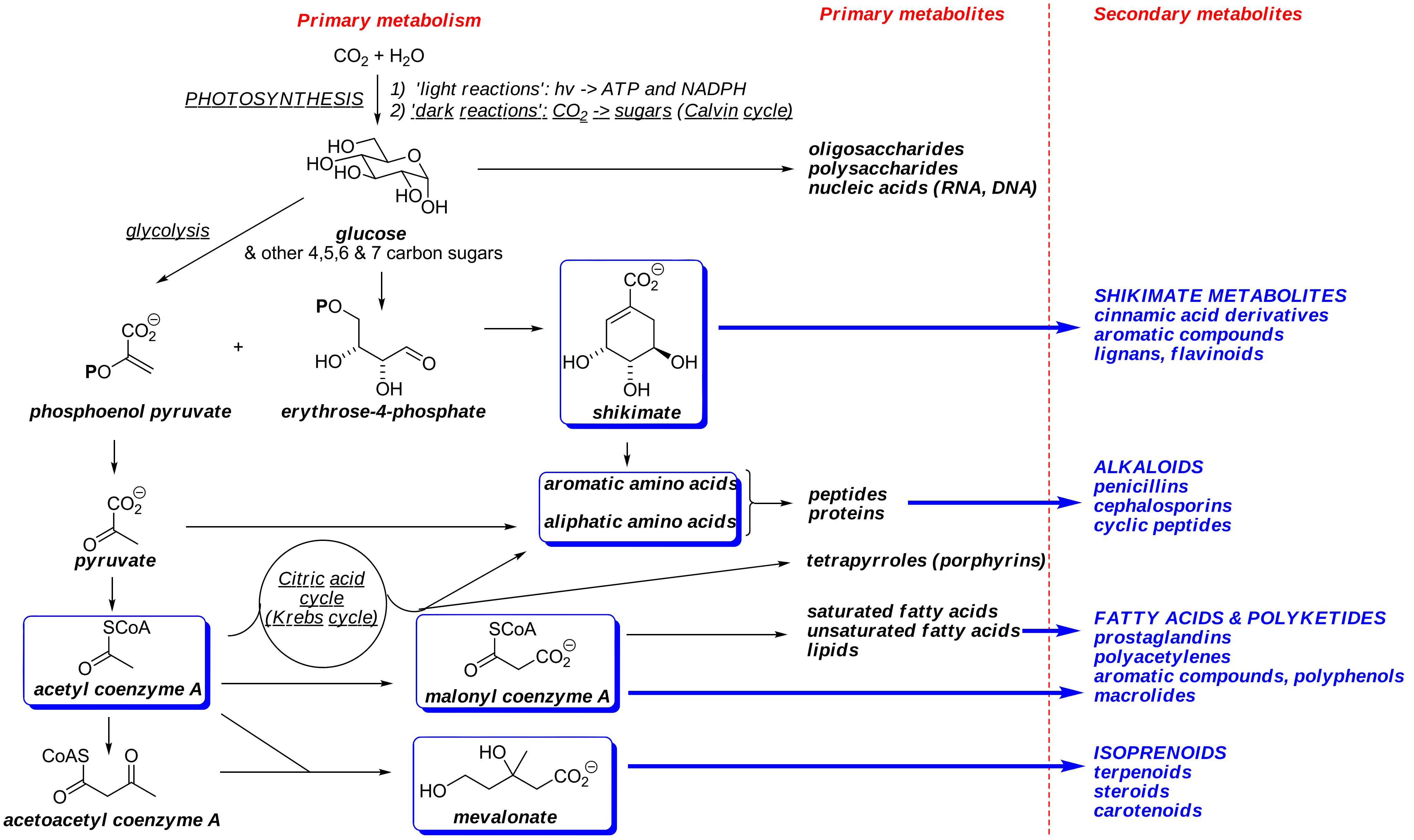


Tyrosine/Phenylalanine → ArC_3 Metabolites

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



Primary Metabolism - *Overview*

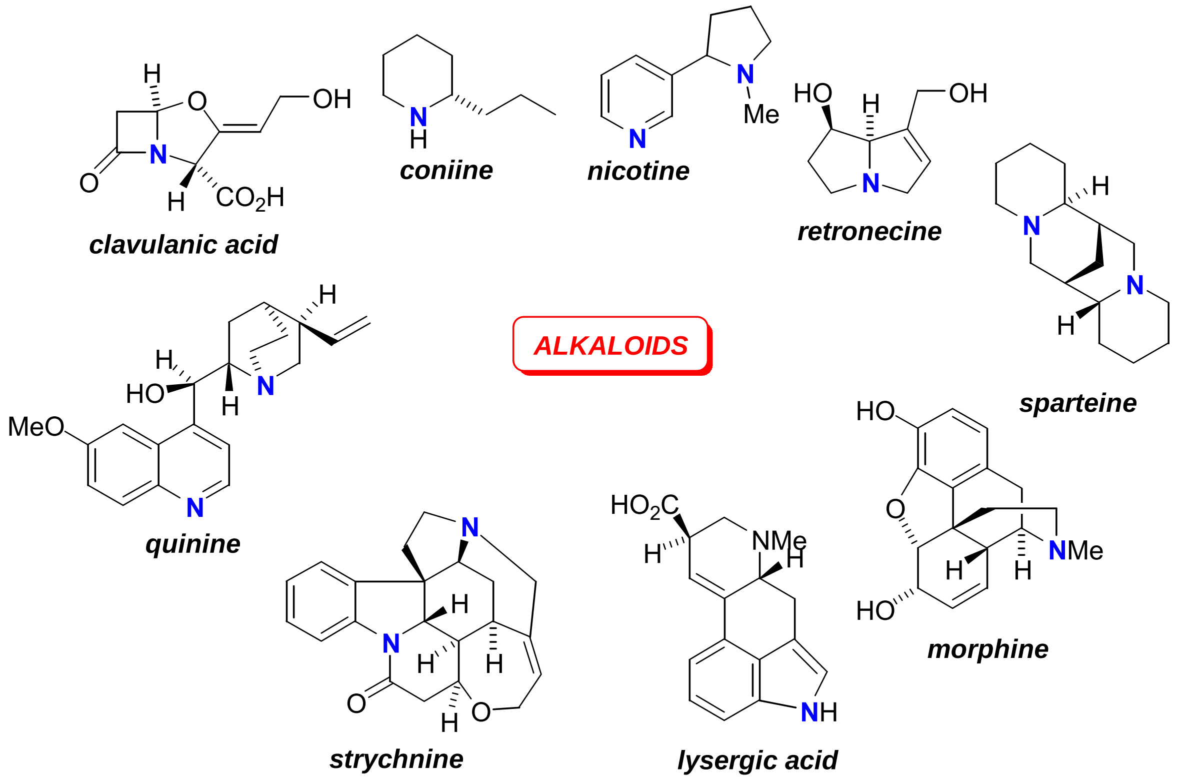


For interesting animations' of *e.g.* photosynthesis see: **http://www.johnkyrk.com/index.html**

Alkaloids

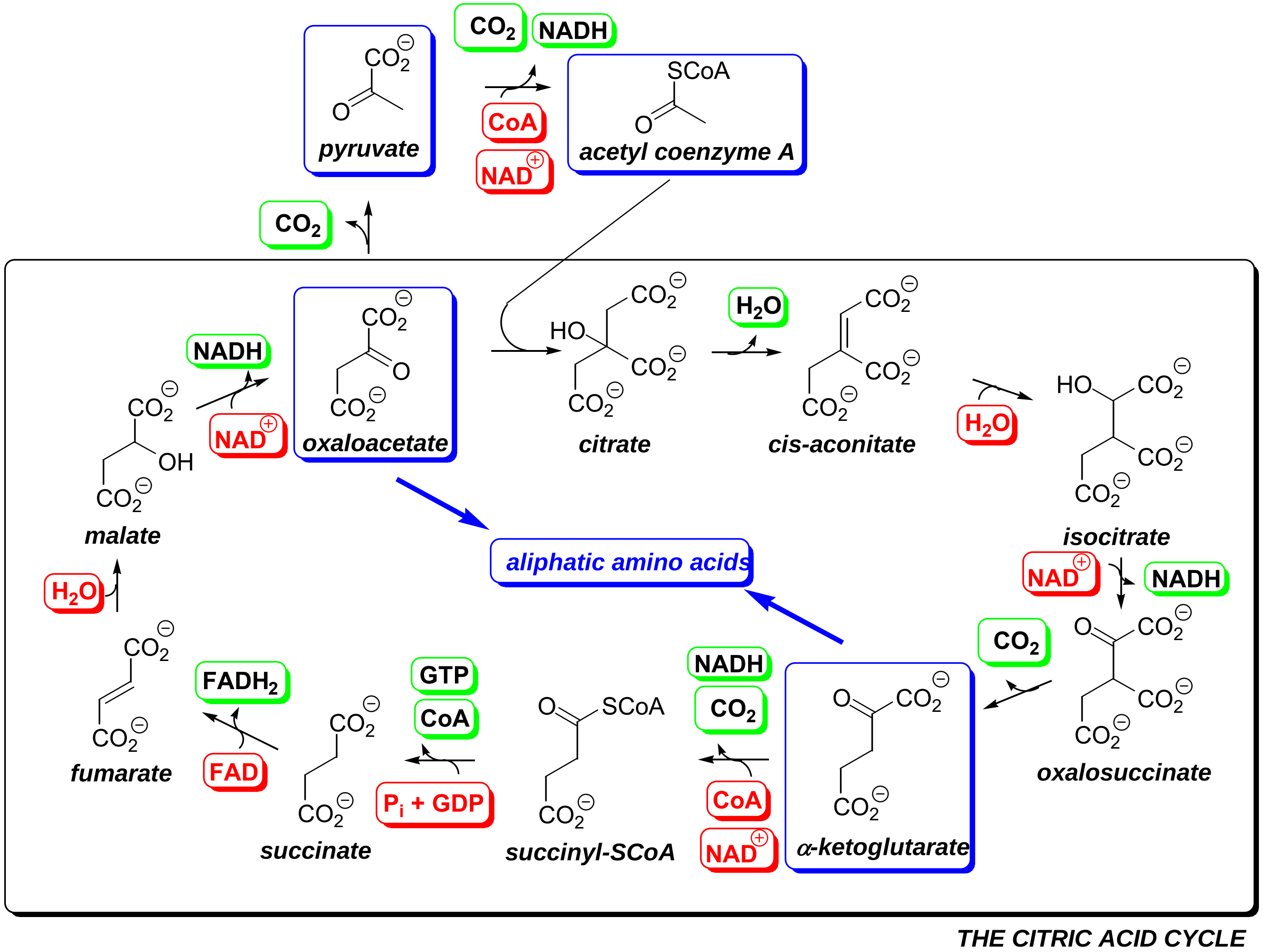
Alkaloids

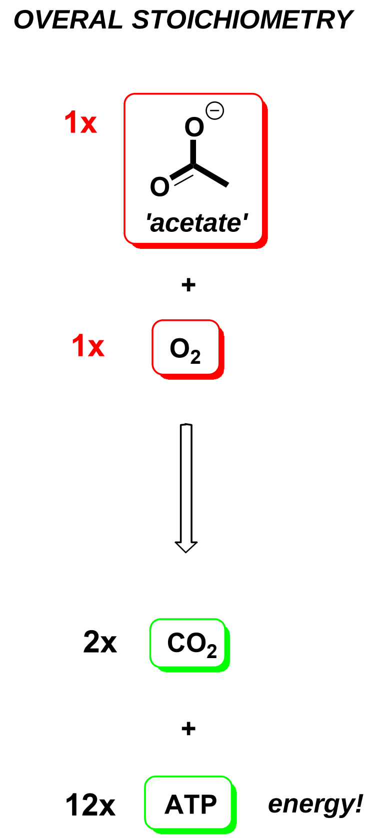
- ***Definitions:***
  - ***originally –*** '*a natural product that could be extracted out of alkaline but not acidic water*' (*i.e.* containing a basic amine function that protonated in acid)
  - ***more generally -*** '*any non-peptidic & non-nucleotide nitrogenous secondary metabolite*'



The Citric Acid Cycle

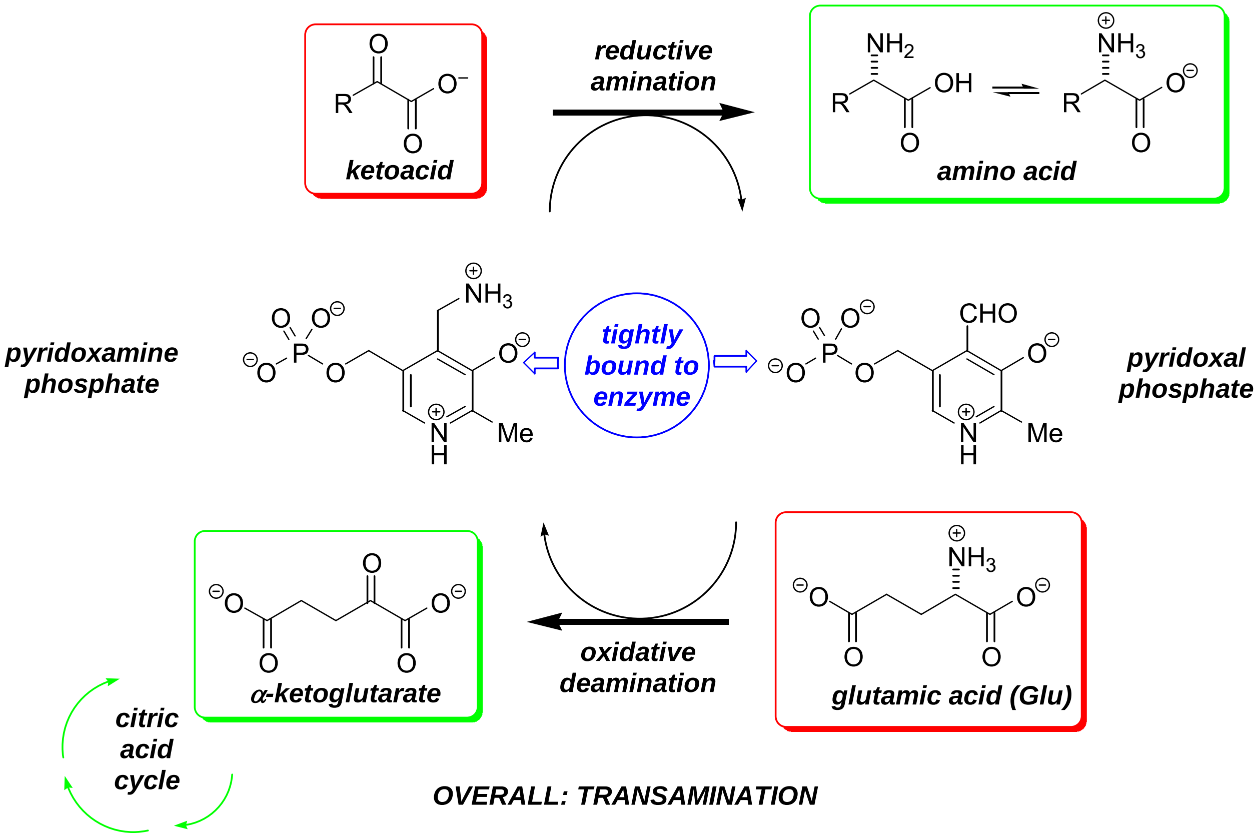
- ***The citric acid (Krebs) cycle*** is a major catabolic pathway of 1° metabolism that provides two key building blocks for aliphatic amino acid biosynthesis - ***oxaloacetate*** & ***α-ketoglutarate:***

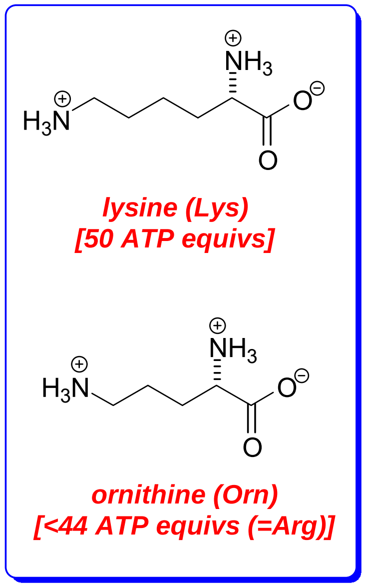




The Biosynthesis of Lysine & Ornithine

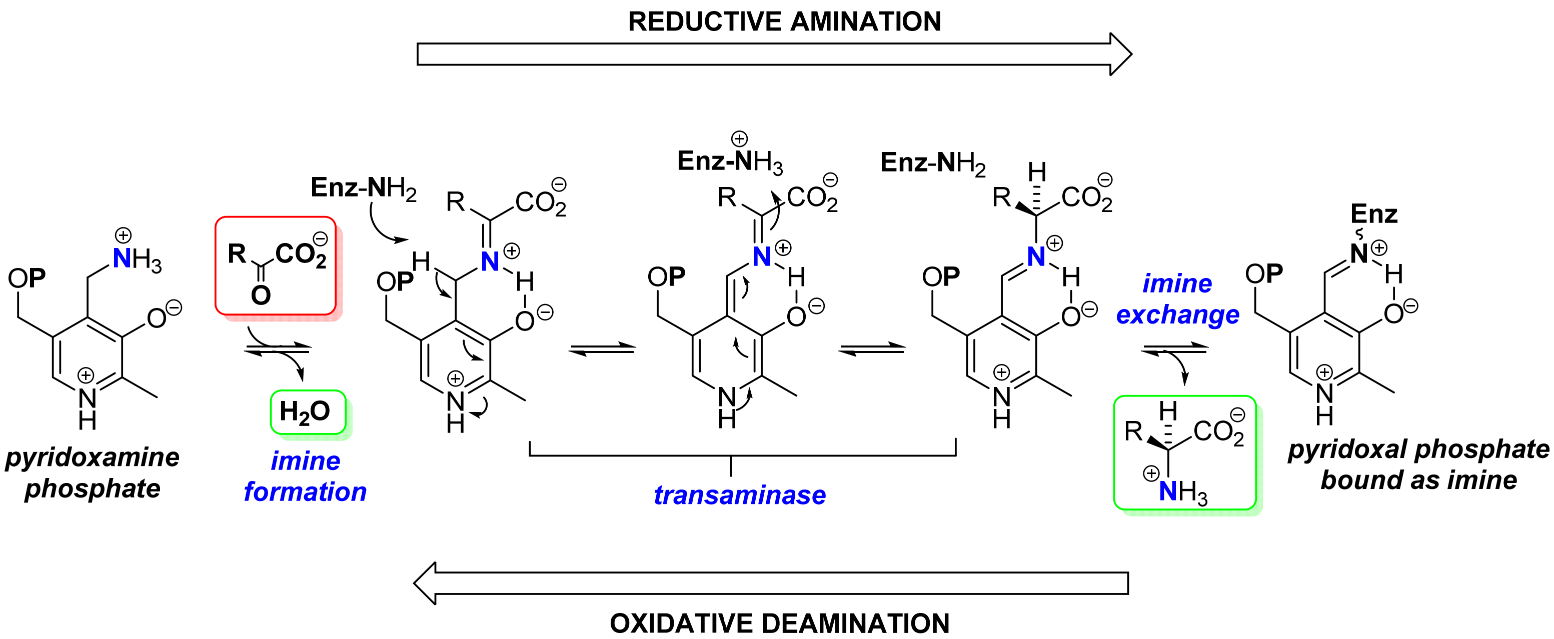
- ***Lysine & ornithine*** - the two most significant, *non-aromatic* α-amino acid ***precursors to alkaloids:***
  - *NB.* lysine (Lys) is proteinogenic whereas ornithine (Orn) is not
  - phenylalanine (Phe), tyrosine (Tyr) & tryptophan (Trp) from ***shikimate*** are the other important precursors
  - biosynthesis is *via* reductive amination of the appropriate α-ketoacid mediated by ***pyridoxal-5'-phosphate*** (***PLP***)





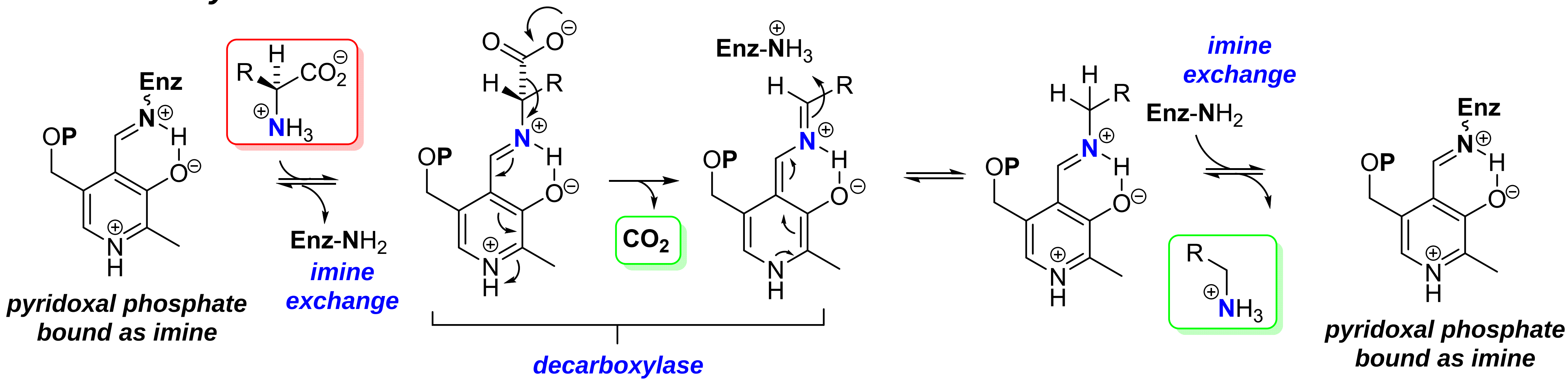
PLP Chemistry – *Transamination & Racemisation*

- ***Transamination:***

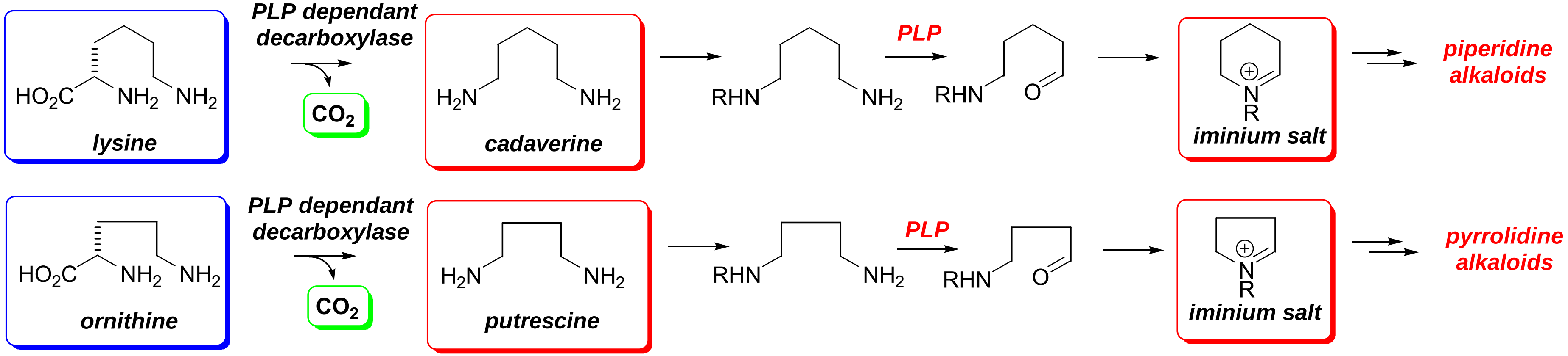


PLP Chemistry – *Decarboxylation*

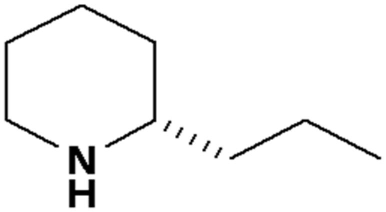
- ***Decarboxylation***:



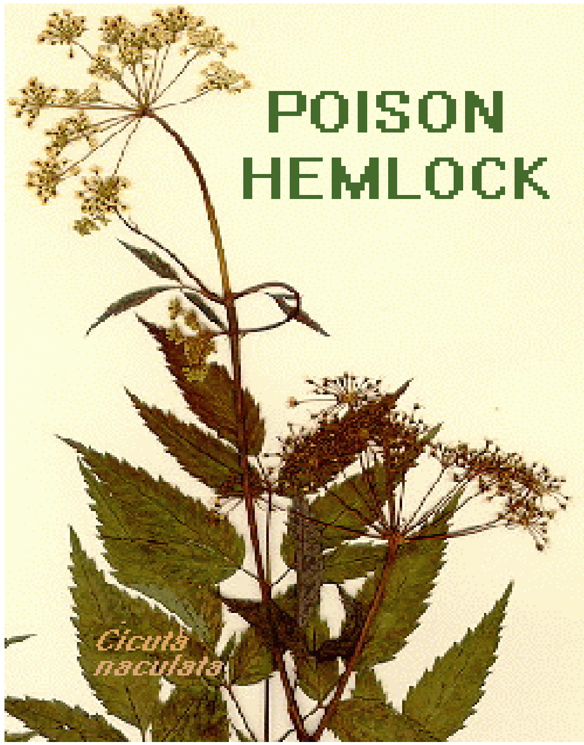
- Decarboxylation of ***lysine*** & ***ornithine***:

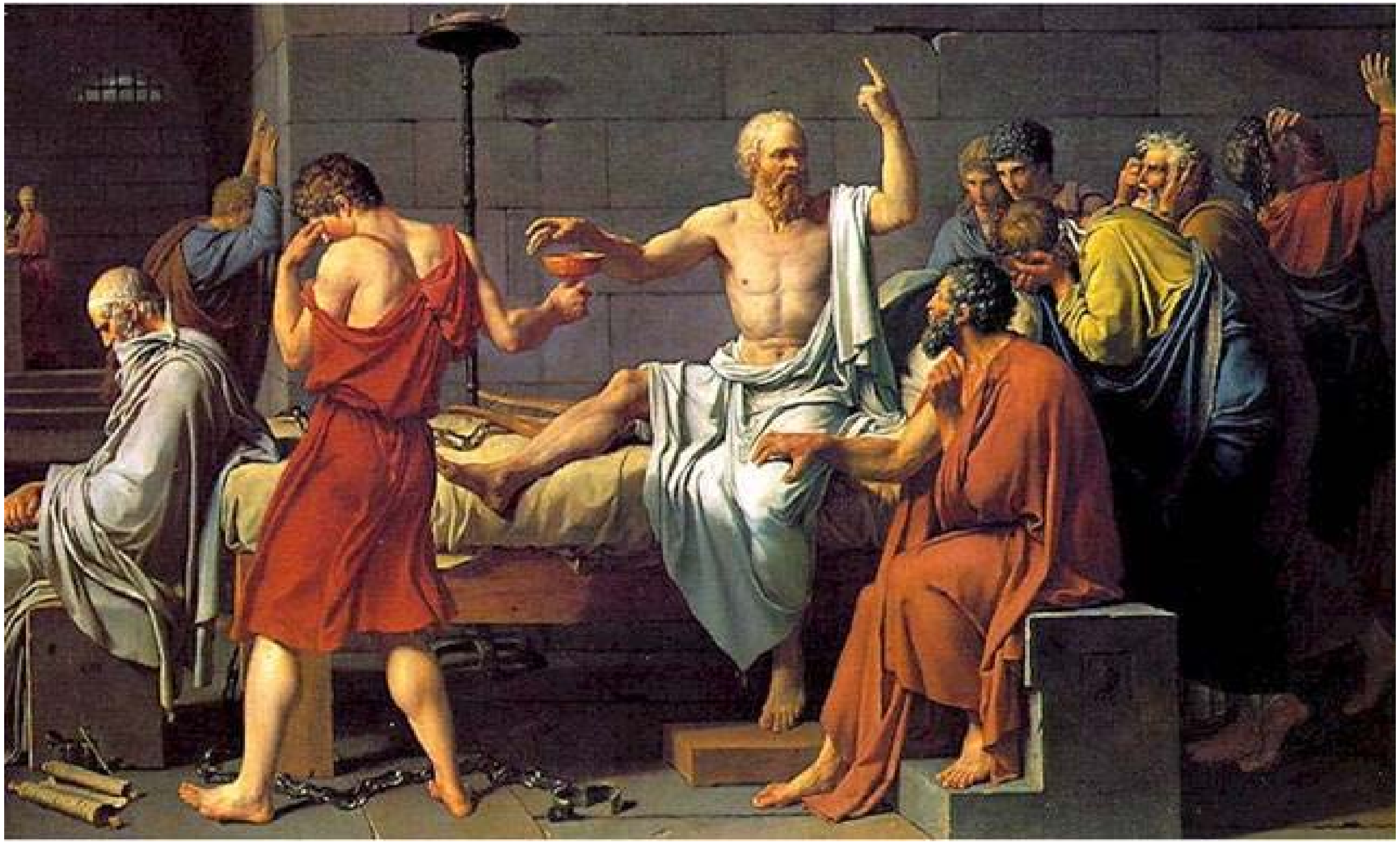


Lysine-derived Piperidine Alkaloids – *Hemlock!*



Coniine



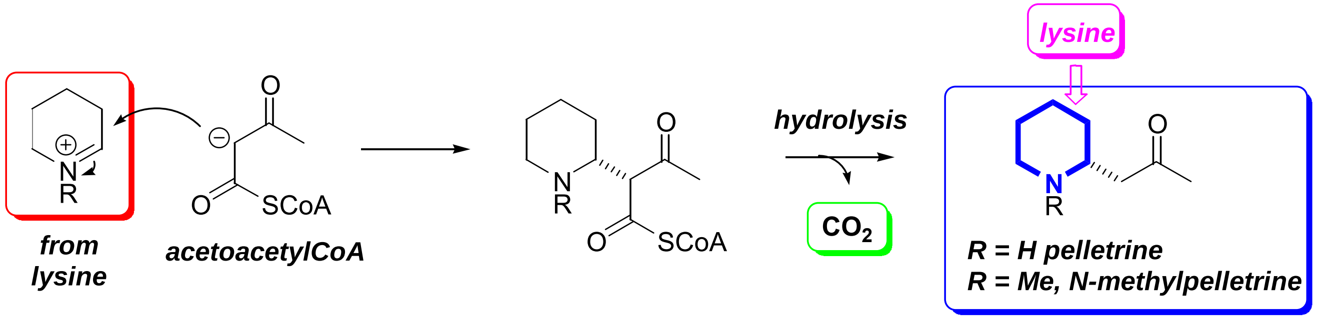


Socrates drinking poison hemlock, 399 B.C.

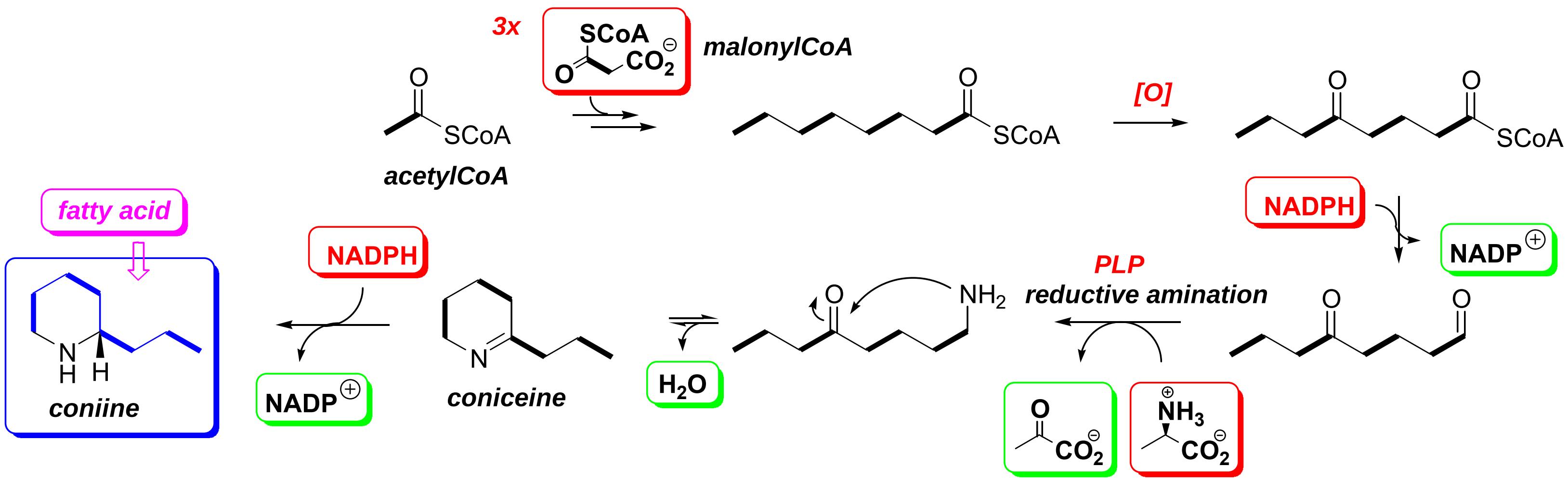
"The Death of Socrates" by Jacques-Louis David (1787)

Piperidine Alkaloids – *Pelletierine & Coniine*

- ***Pelletierine***:

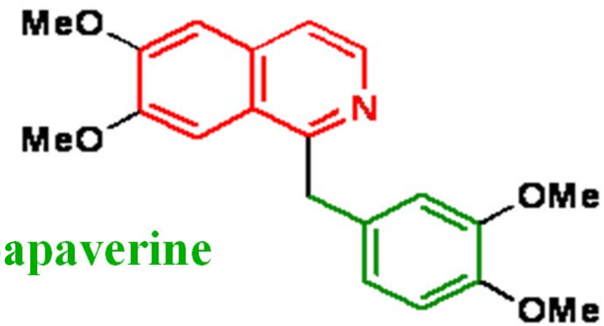


- ***Coniine***:
  - in 399 BC Socrates was sentenced to death for impiety and executed by being forced to drink a potion made from poison hemlock. The toxic component in hemlock is coniine. Although by analogy with the above pathway, biosynthesis from lysine might be suspected, it is in fact of ***fatty acid*** origin

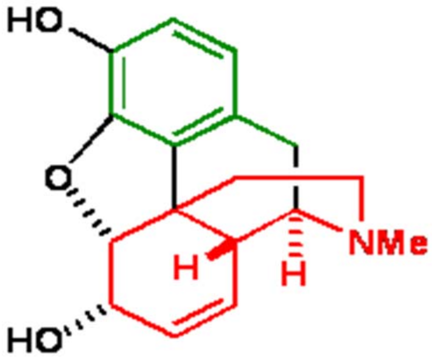


Tyrosine-derived Alkaloids - *Opium Alkaloids*

Benzylisoquinoline Alkaloids



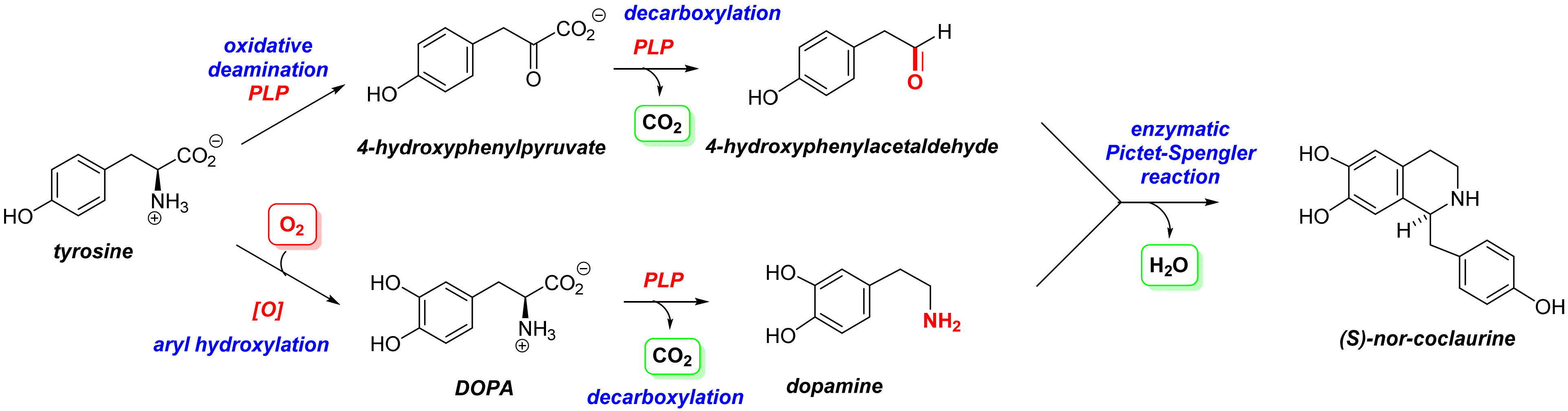
papaverine



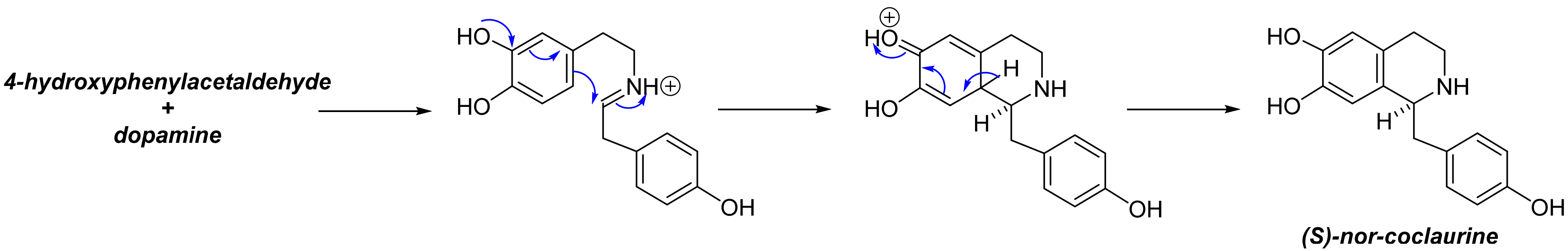
morphine

Benzylisoquinoline Alkaloids – *Ring Formation*

- ***Benzylisoquinoline alkaloids*** constitute an extremely large and varied group of alkaloids
  - many, particularly the *opium alkaloids* (*e.g.* papaverine, morphine) are ***biosynthesised*** from two molecules of tyrosine *via* ***nor-coclaurine*** (and then ***nor-laudanosoline***).

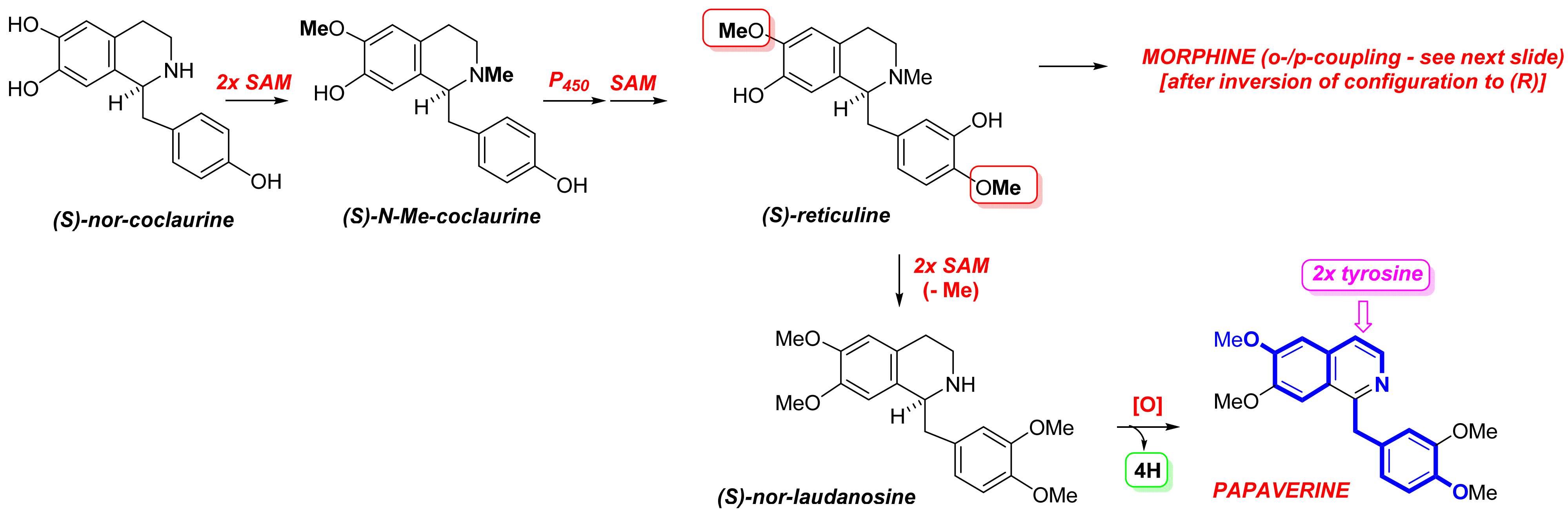


- ***Mechanism of Pictet Spengler reaction:***



Benzylisoquinoline Alkaloids - *Papaverine*

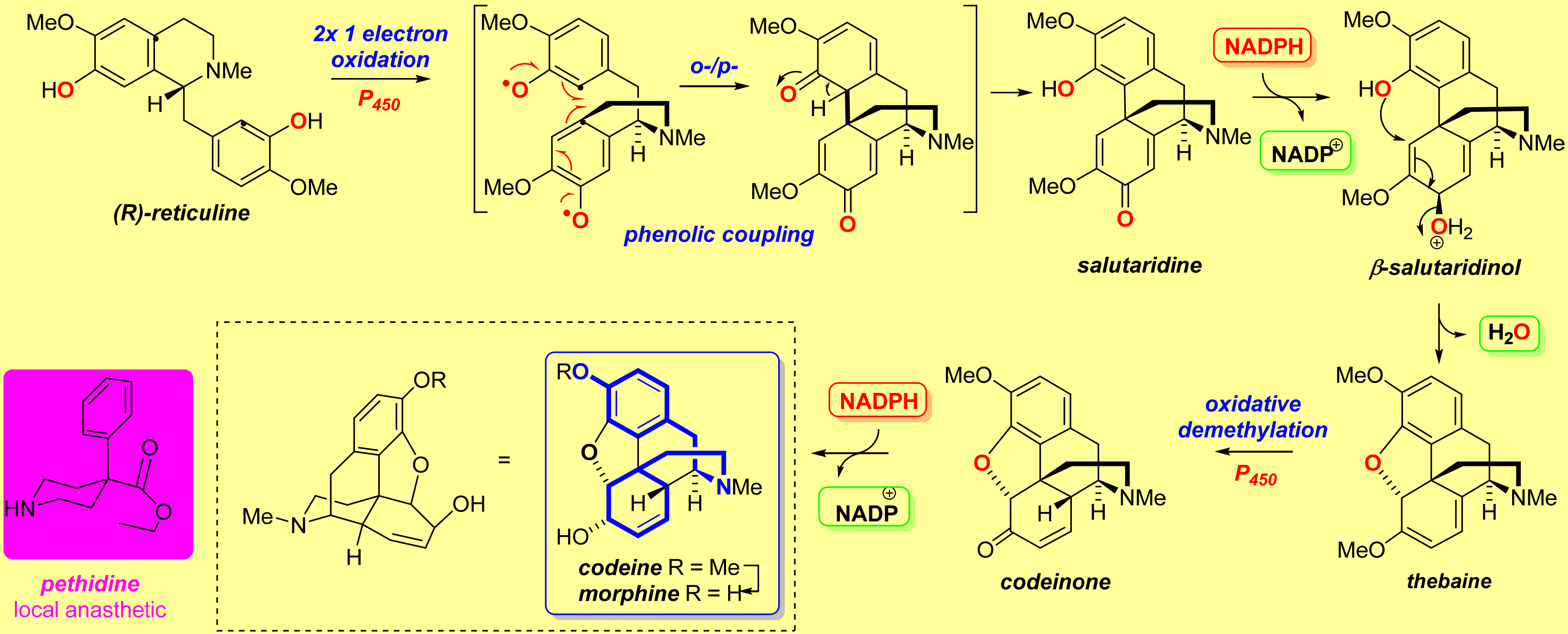
- ***Papaverine:*** analgesic contsituent of the **opium poppy** (*Papaver somniferum):*
  - ***biosynthesis:***



  - *NB.* The prefix ***nor*** means ***without a methyl group***. Coclaurine, reticuline and laudanosine are the *N*-methyl compounds

Oxidative Phenolic Coupling – *Morphine & Synthetic Opioids*

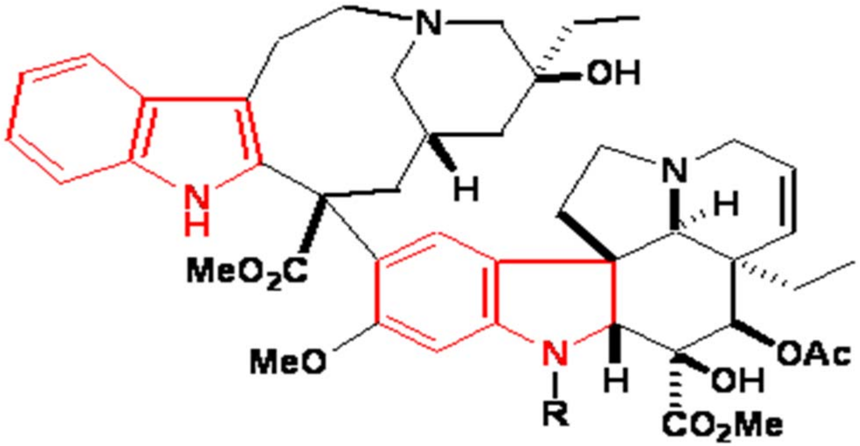
- ***Morphine:*** analgesic & sedative contsituent of the **opium poppy** (*Papaver somniferum):*
  - ***biosynthesis:*** *o-/p-* oxidative phenolic coupling of ***reticuline:***



  - Morphine acts by activating the ***opiate receptors*** in the brain (IC_{50} 3 nM)
  - The natural ligands for these receptors are peptides: *e.g.* Leu-enkephalin (Tyr–Gly–Gly–Phe–Leu) (IC_{50} 12 nM)

Dimeric Indole Alkaloids – *Vinca extracts*

Dimeric Indole Alkaloids

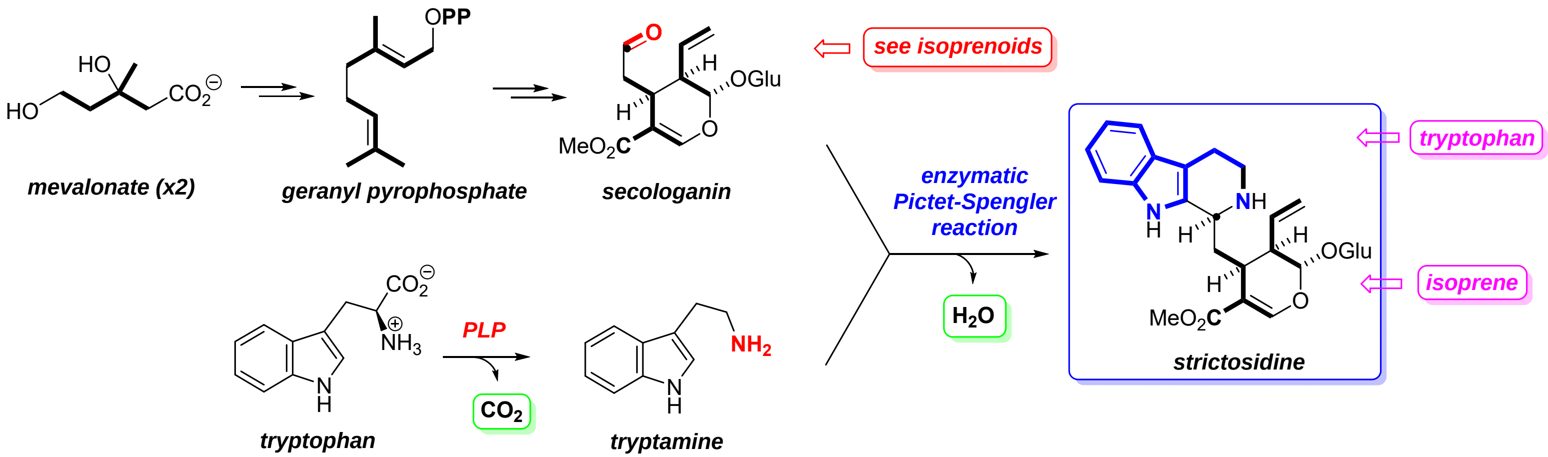


vinblastine (R = Me)
vincristine (R = CHO)

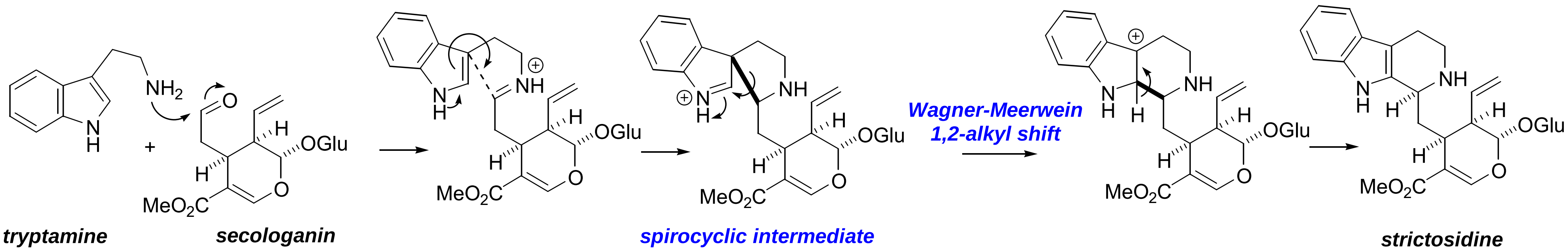
Potent **anti tumour** alkaloids used in **cancer chemotherapy**

Tryptamine + Secolaganin → Strictosidine

- Most alkaloids of ***mixed Tryptophan/mevalonate biogenesis*** (>1200) are derived from ***strictosidine:***
  - ***Strictosidine*** is derived from the condensation of ***tryptamine*** with the iridoid C_{10} monoterpene ***secologanin:***

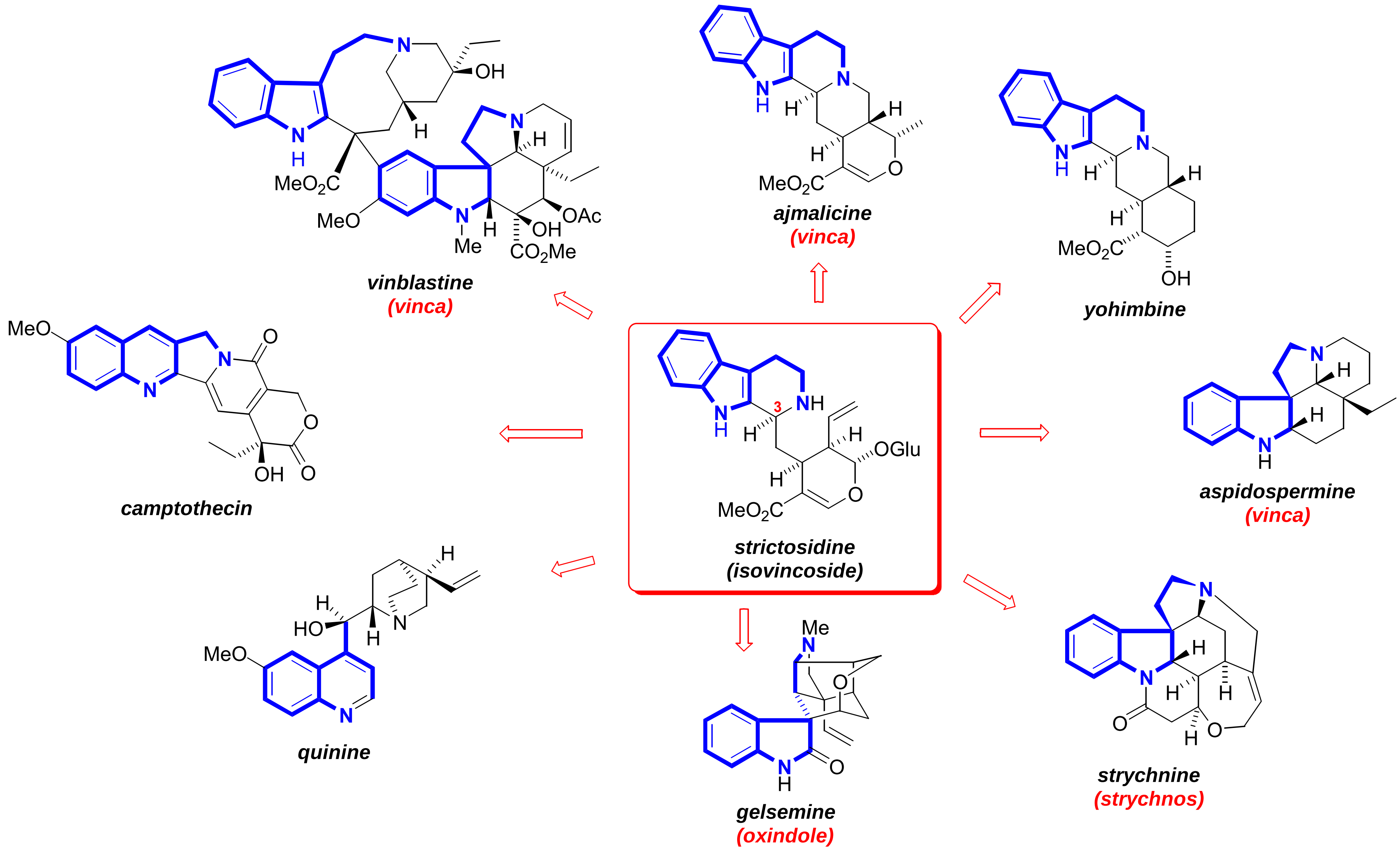


- ***Mechanism of Pictet-Spengler reaction:***
  - *via* ***spirocyclic*** intermediate then ***Wagner-Meerwein*** 1,2-alkyl shift:

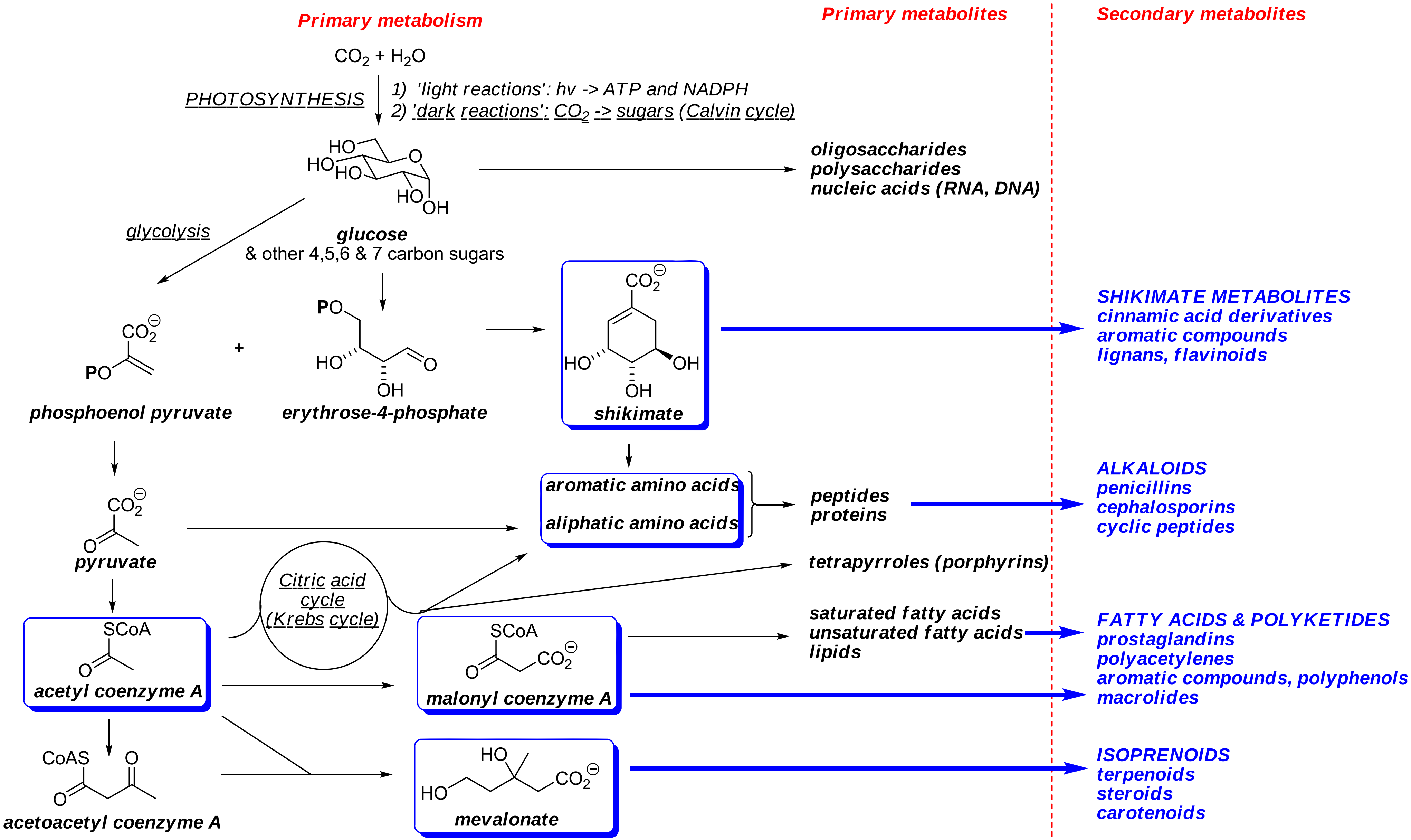


Strictosidine → *Vinca, Strychnos, Quinine etc.*

- The diversity of alkaloids derived from ***strictosidine*** is stunning and many pathways remain to be fully elucidated:



Primary Metabolism - *Overview*

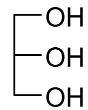


For interesting animations' of *e.g.* photosynthesis see: **http://www.johnkyrk.com/index.html**

Fatty Acids

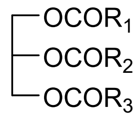
Fatty Acid Primary Metabolites

- ***Primary metabolites:***
  - ***fully saturated, linear carboxylic acids*** & derived ***(poly)unsaturated derivatives:***
    - constituents of essential natural waxes, seed oils, ***glycerides*** (fats) & phospholipids
    - ***structural role*** – ***glycerides*** & phospholipids are essential constituents of cell membranes
    - ***energy storage*** – ***glycerides*** can also be catabolised into acetate → citric acid cycle
    - ***biosynthetic precursors*** – for elaboration to secondary metabolites



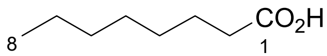
glycerol

⇩ ***3x fatty acids***

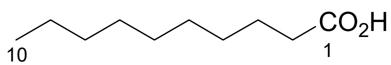


glycerides

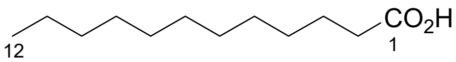
SATURATED ACIDS [$MeCH_2(CH_2CH_2)_nCH_2CO_2H$ (n = 2-8)]
e.g.



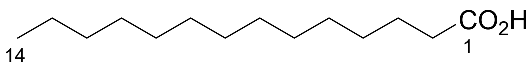
caprylic acid (C8, n = 2)



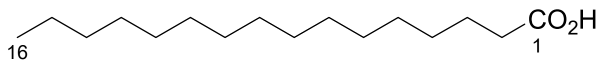
capric acid (C8, n = 3)



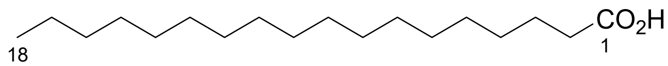
lauric acid (C12, n = 4)



myristic acid (C14, n = 5)

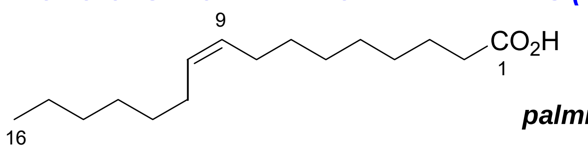


palmitic acid (C16, n = 6)

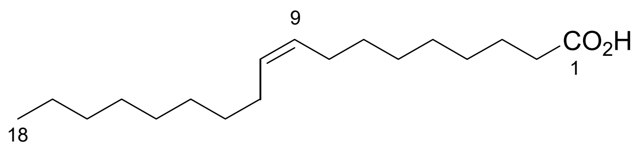


stearic acid (C18, n = 7)

MONO-UNSATURATED ACID DERIVATIVES (MUFAs) e.g.

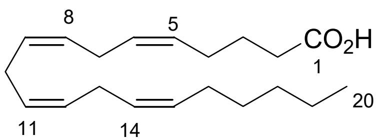


palmitoleic acid (C16, Z -Δ^9)

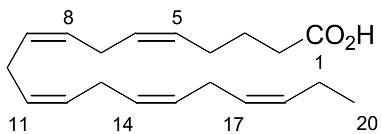


oleic acid (C18, Z-Δ^9)
(>80% of fat in olive oil)

POLY-UNSATURATED ACID DERIVATIVES (PUFAs)
e.g.



arachidonic acid (AA)
(C20, Z -Δ^5, Z -Δ^8, Z -Δ^{11}, Z -Δ^{14})

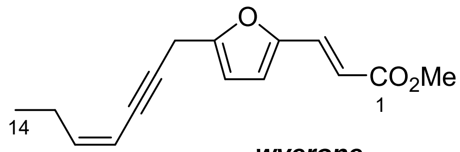


eicosapentaenoic acid (EPA)
(C20, Z-Δ^5, Z -Δ^8, Z-Δ^{11}, Z -Δ^{14}, Z -Δ^{17})
(in cod liver oil)

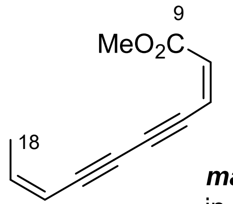
Fatty Acids Derivatives – *Secondary Metabolites*

- ***Secondary metabolites***
  - further ***elaborated*** derivatives of ***polyunsaturated fatty acids (PUFAs)***
    - *e.g.* polyacetylenes & 'eicosanoids' (prostaglandins, thromboxanes & leukotrienes)

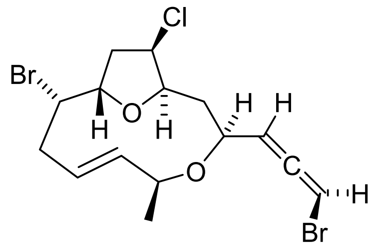
POLYACETYLENES
e.g.



wyerone
anti-fungal

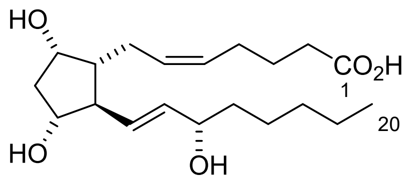


matricaria ester
in chamomile tea



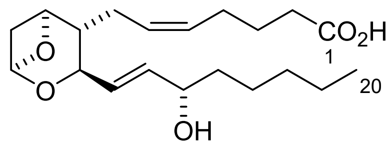
obtusallene II
marine natural product

PROSTAGLANDINS
e.g.



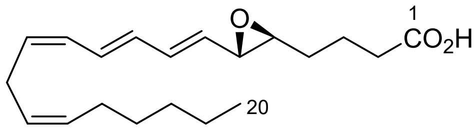
prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)

THROMBOXANES
e.g.



thromboxane A_2 (TXA_2)

LEUKOTRIENES
e.g.



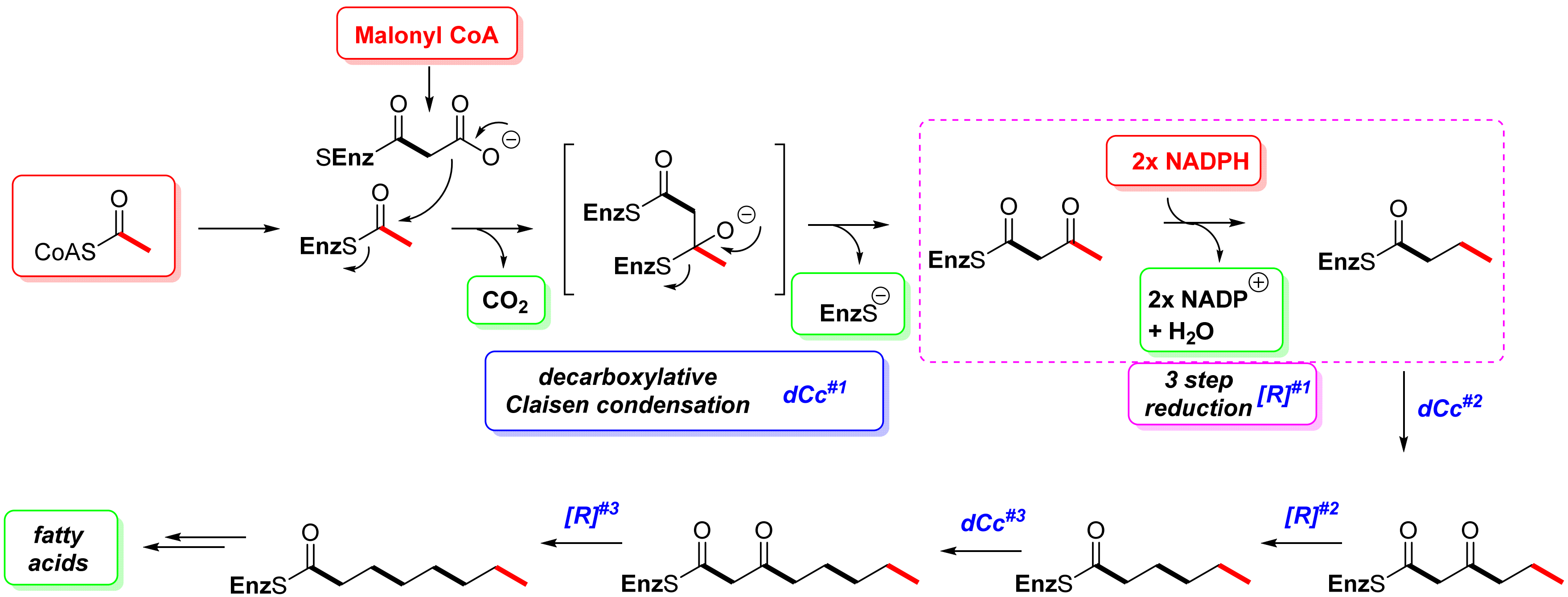
leukotriene A_4(LTA_4)

leukotriene A_4 (LTA_4)

EICOSANOIDS

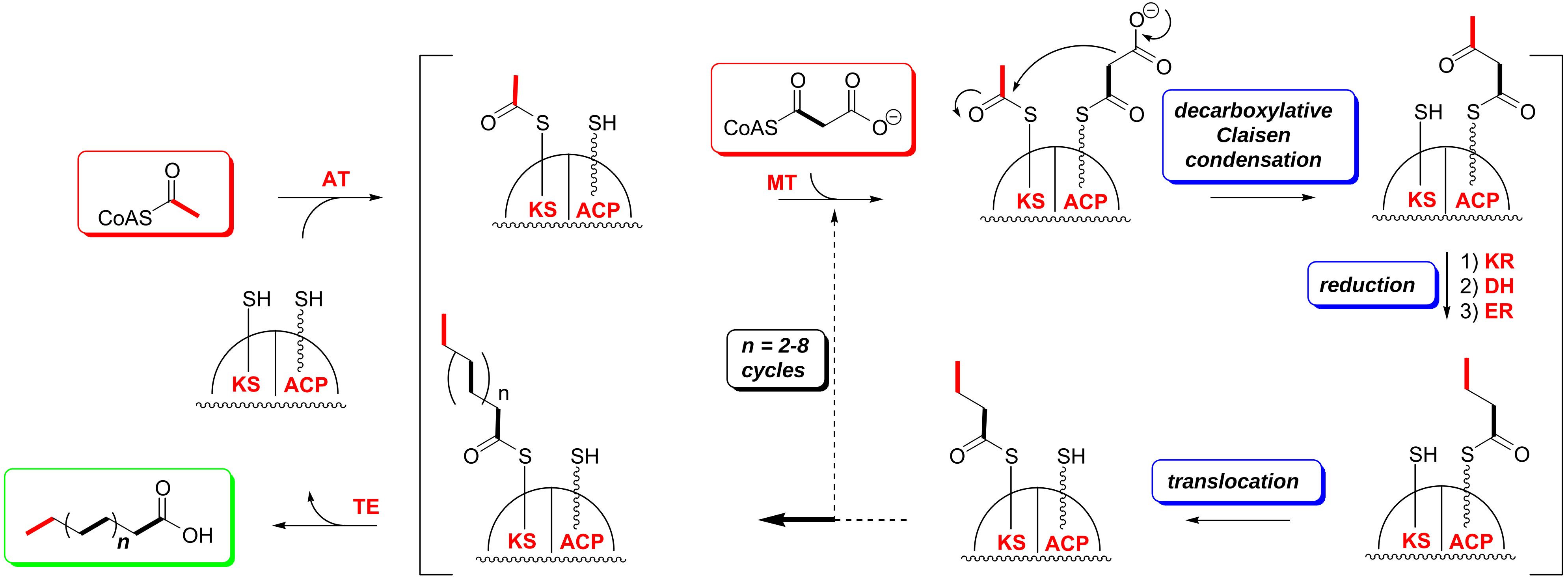
Biosynthesis of Fatty Acids – *Iterative Oligomerisation*

- ***fatty acids*** are biosynthesised from ***acetyl CoA*** as a ***starter unit*** by ***iterative*** 'head-to-tail' ***oligomerisation*** involving:
  - condensation with ***malonyl CoA*** as an ***extender unit*** (with loss of ***CO_2***) – a ***decarboxylative Claisen condensation***
  - 3-step ***reduction*** of the resulting ***ketone*** → ***methylene***
- after **n = 2-8 iterations** the ***C8-20 saturated fatty acid*** is released from the enzyme(s):



Biosynthesis of Fatty Acids – *Overview of FAS*

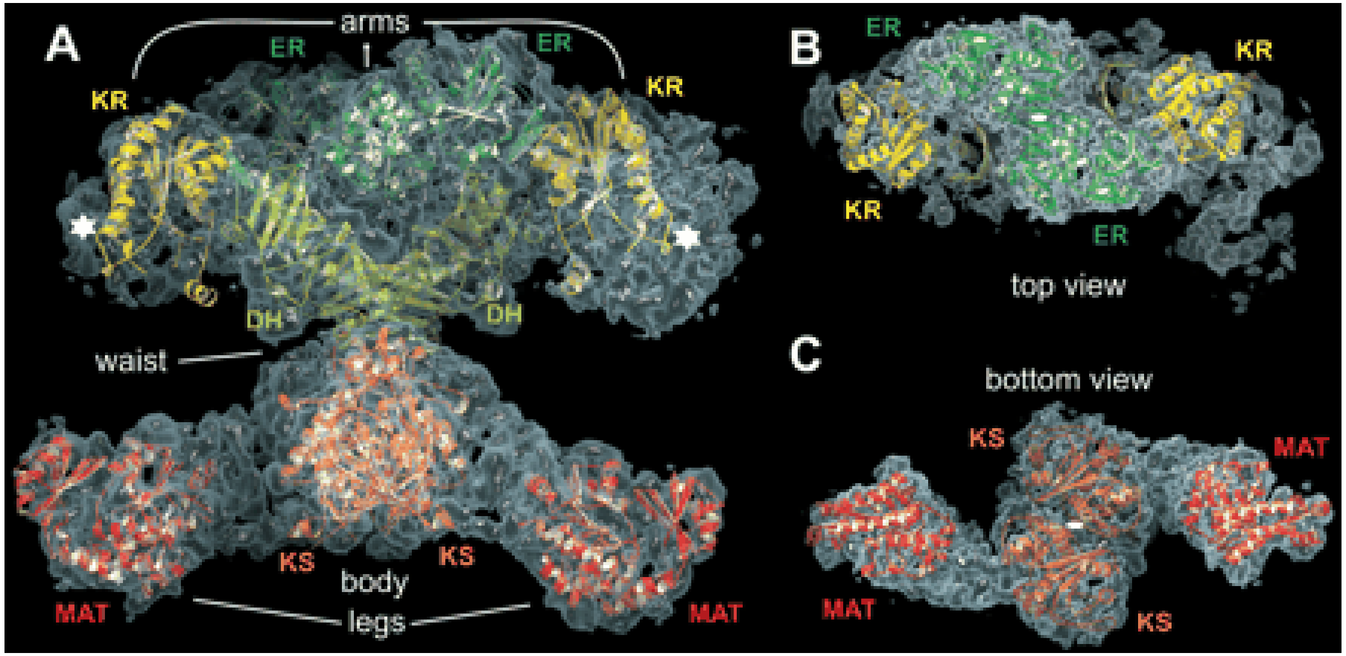
- The *in vivo* process by which all this takes place involves a 'molecular machine' - ***Fatty Acid Synthase (FAS)***
  - ***Type I FAS: single multifunctional protein complex*** (*e.g.* in mammals incl. humans)
  - ***Type II FAS: set of discrete, dissociable single-function proteins*** (*e.g.* in bacteria)
  - ***All FASs*** comprise ***8 components*** (ACP & 7× catalytic activities): ***ACP***, ***KS***, ***AT***, ***MT***, ***KR***, ***DH***, ***ER*** & [***TE***] :



KS = keto synthase (also known as **CE** = condensing enzyme); **AT** = acetyl transferase; **MT** = malonyl transferase; **KR** = keto reductase; **DH** = dehydratase; **ER** = enoyl reductase; **TE** = thioesterase; **ACP** = acyl carrier protein

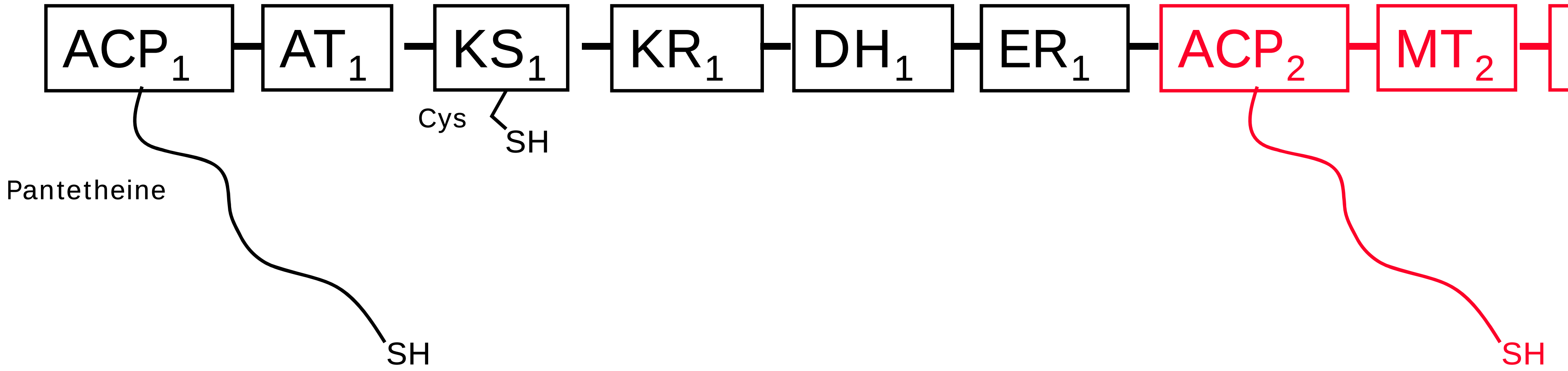
Human Fatty Acid Synthase (FAS)

- the first three-dimensional structure of human fatty acid synthase (272 kDa) at 4.5 Å resolution by X-ray crystallography:
  - Maier, Jenni & Ban *Science* **2006**, *311*, 1258 (**DOI**) ; also Fungal FAS @ 3.1 Å resolution see: Jenni *et al.* *Science* **2007**, *316*, 254 & 288



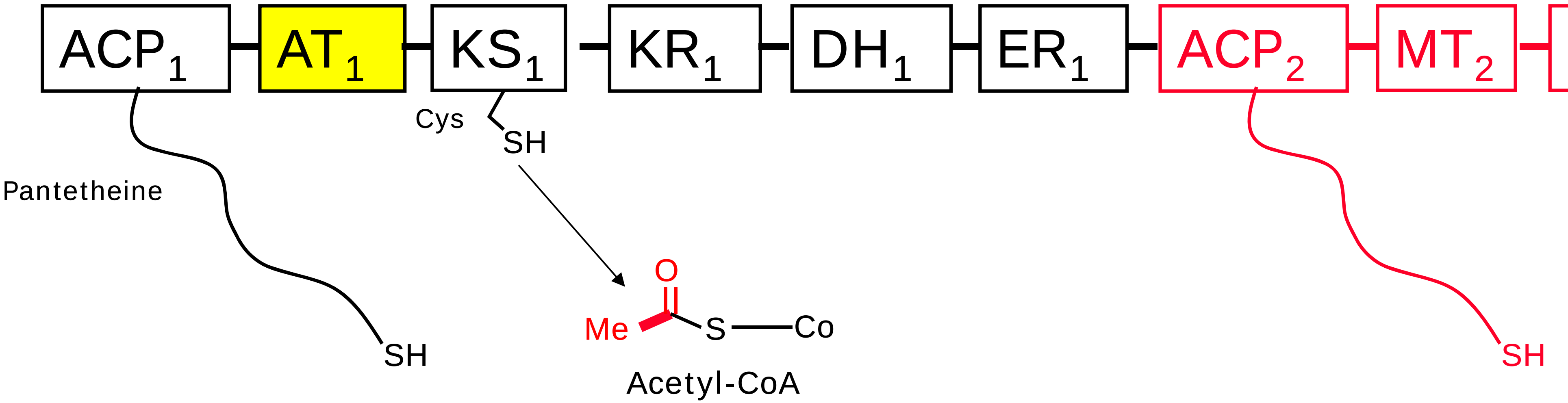
Structural overview. (**A**) Front view: FAS consists of a lower part comprising the KS (lower body) and MAT domains (legs) connected at the waist with an upper part formed by the DH, ER (upper body), and KR domains (arms). (**B**) Top view of FAS with the ER and KR domains resting on the DH domains. (**C**) Bottom view showing the arrangement of the KS and MAT domains and the continuous electron density between the KS and MAT domains

FATTY ACID BIOSYNTHESIS (type II FAS)



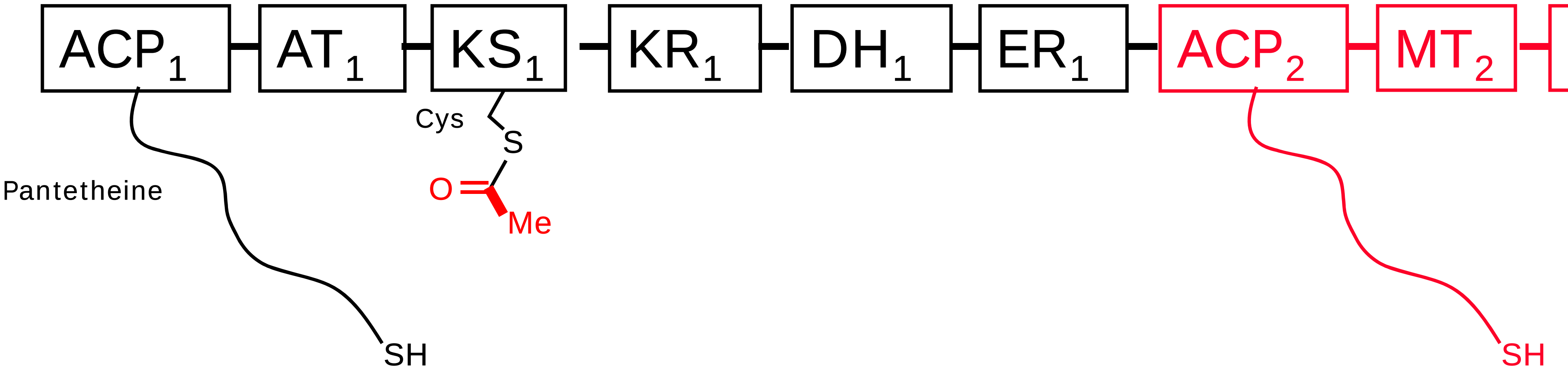
NB. the following sequence of slides have been adapted from: http://www.courses.fas.harvard.edu/%7echem27/

FATTY ACID BIOSYNTHESIS

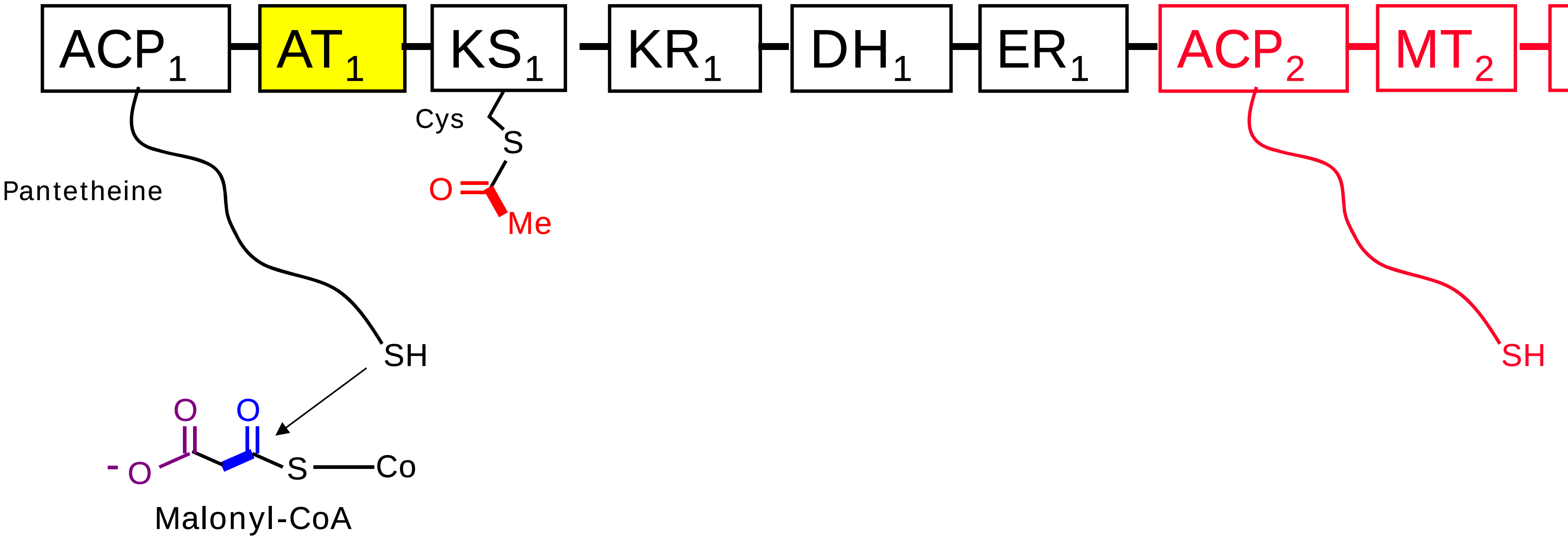


- AT_1 loads acetyl group onto KS_1

FATTY ACID BIOSYNTHESIS

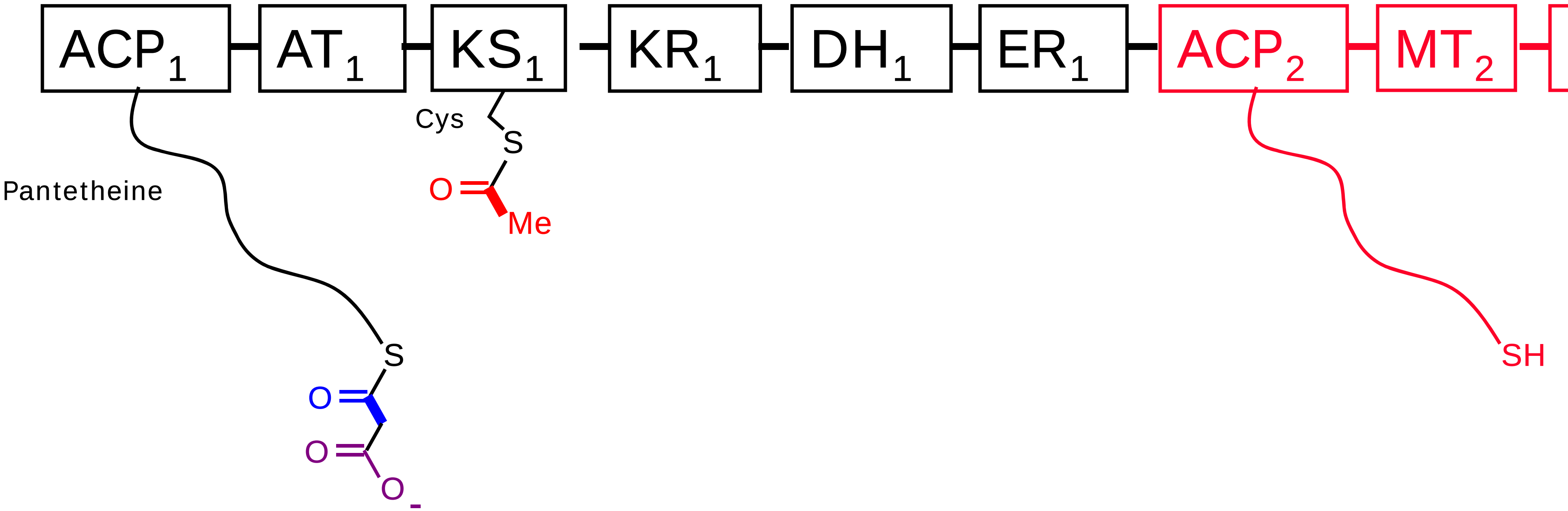


FATTY ACID BIOSYNTHESIS

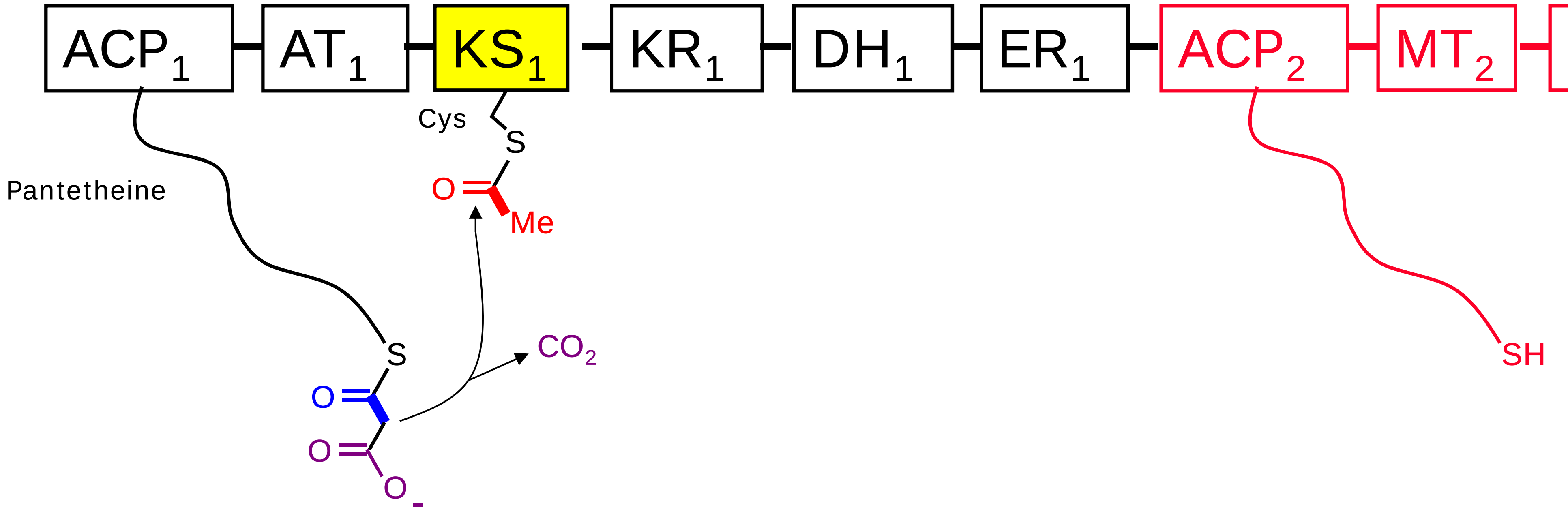


- AT_1 loads malonyl group onto ACP_1

FATTY ACID BIOSYNTHESIS

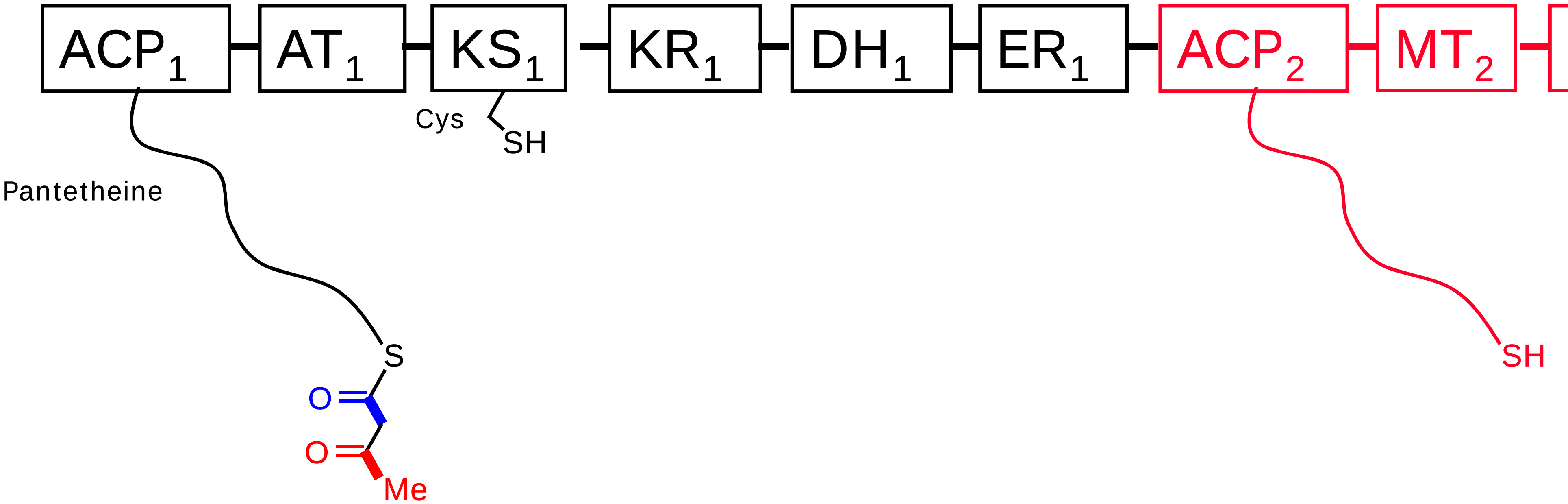


FATTY ACID BIOSYNTHESIS

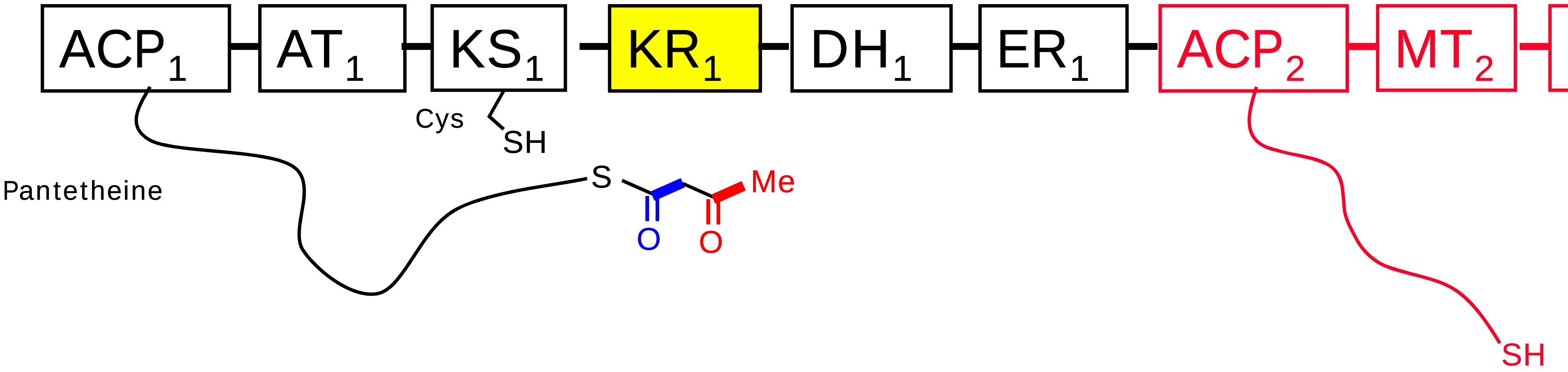


- KS_1 catalyzes Claisen condensation

FATTY ACID BIOSYNTHESIS

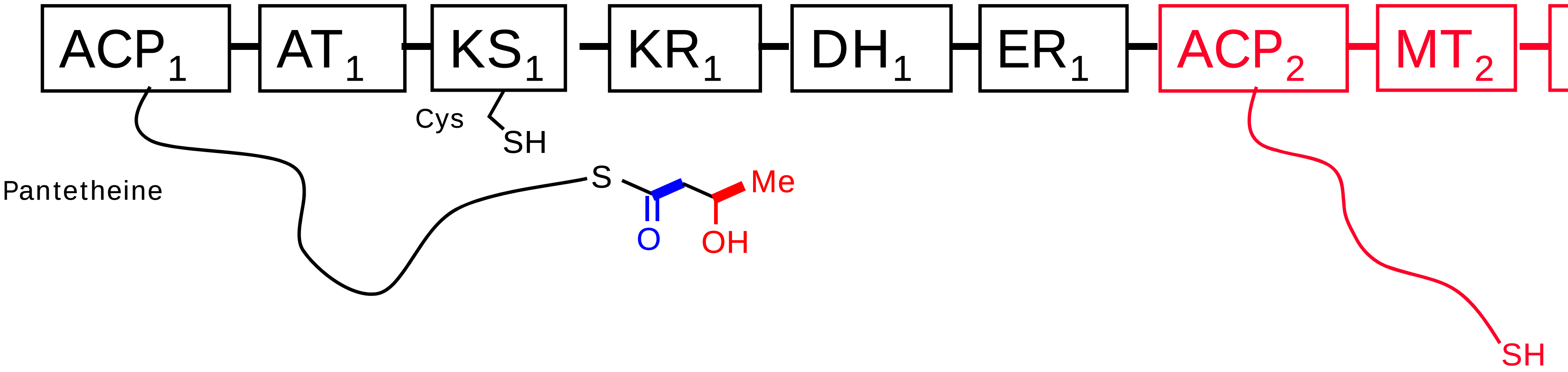


FATTY ACID BIOSYNTHESIS

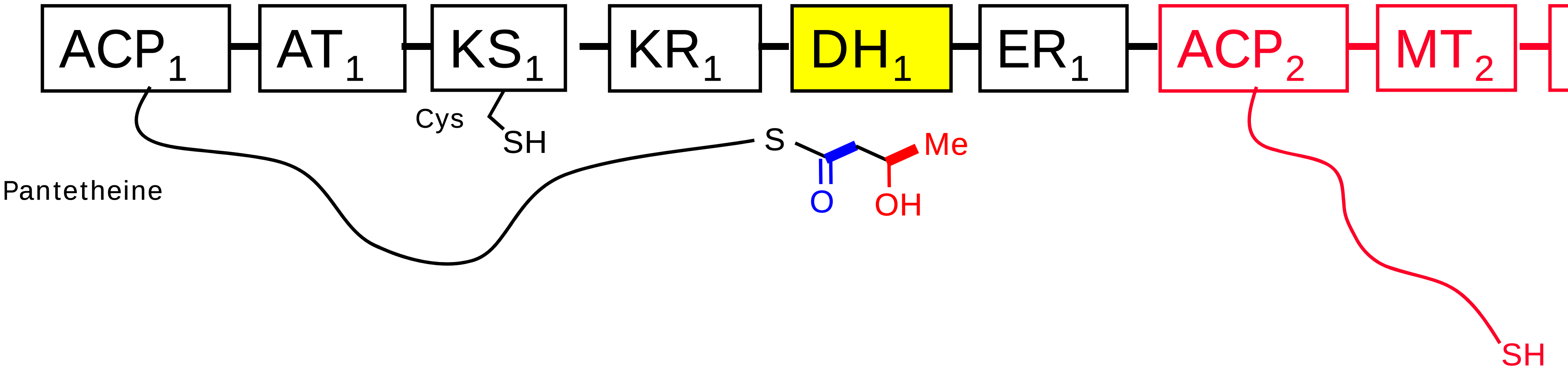


- KR_1 catalyzes reduction of ketone

FATTY ACID BIOSYNTHESIS

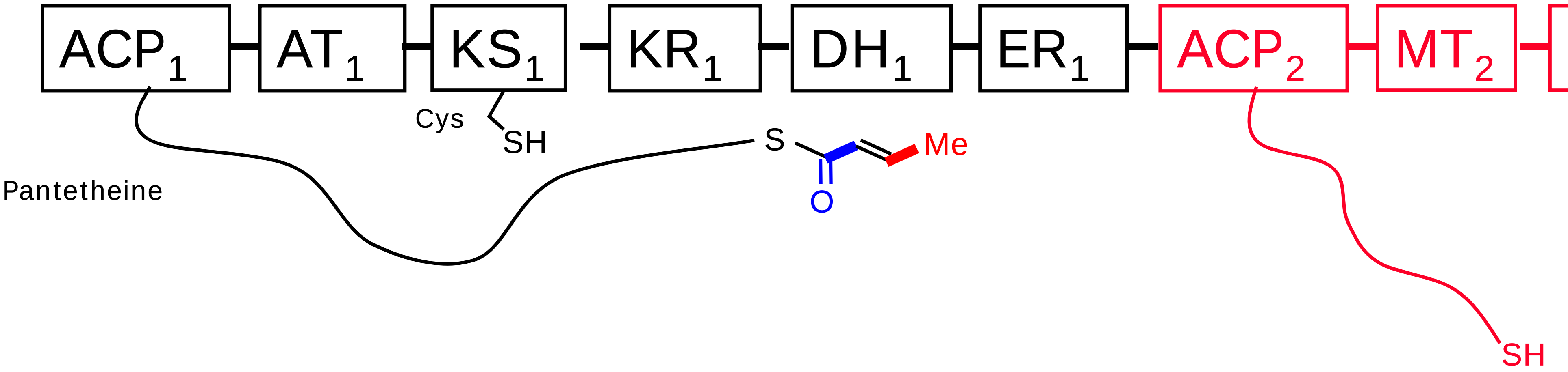


FATTY ACID BIOSYNTHESIS



- DH_1 catalyzes dehydration of alcohol

FATTY ACID BIOSYNTHESIS



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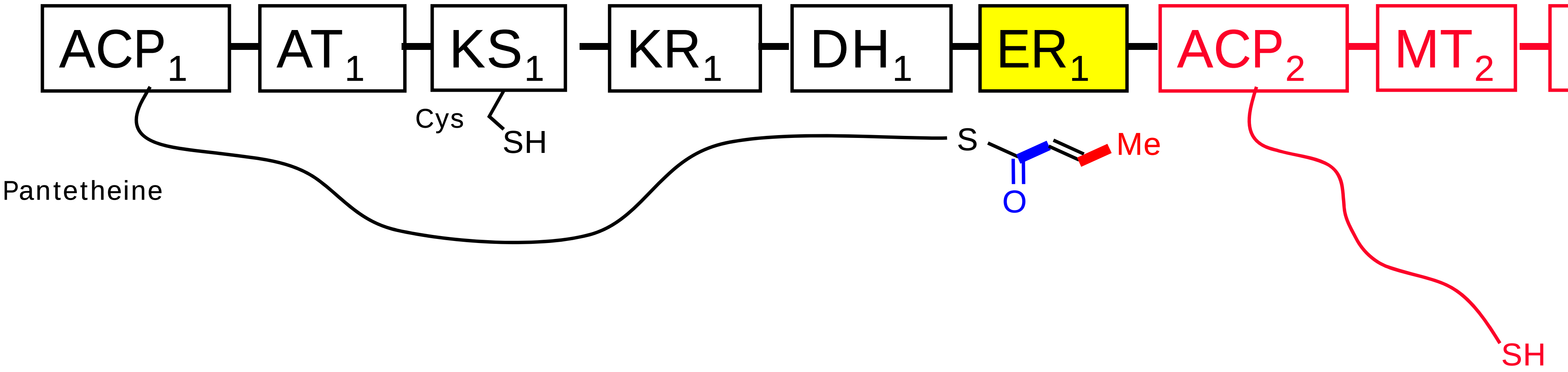
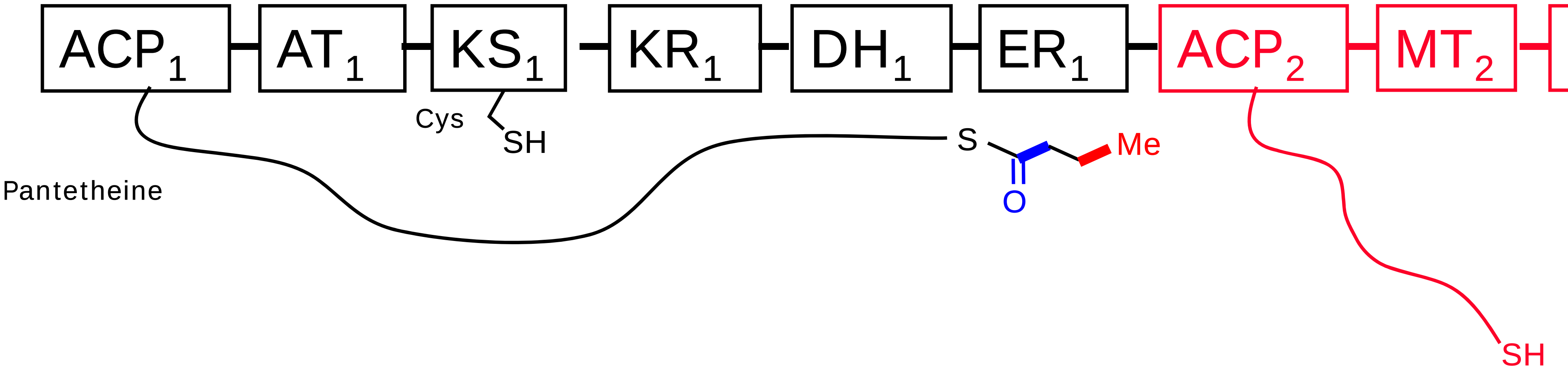


- ER_1 catalyzes reduction of alkene

FATTY ACID BIOSYNTHESIS



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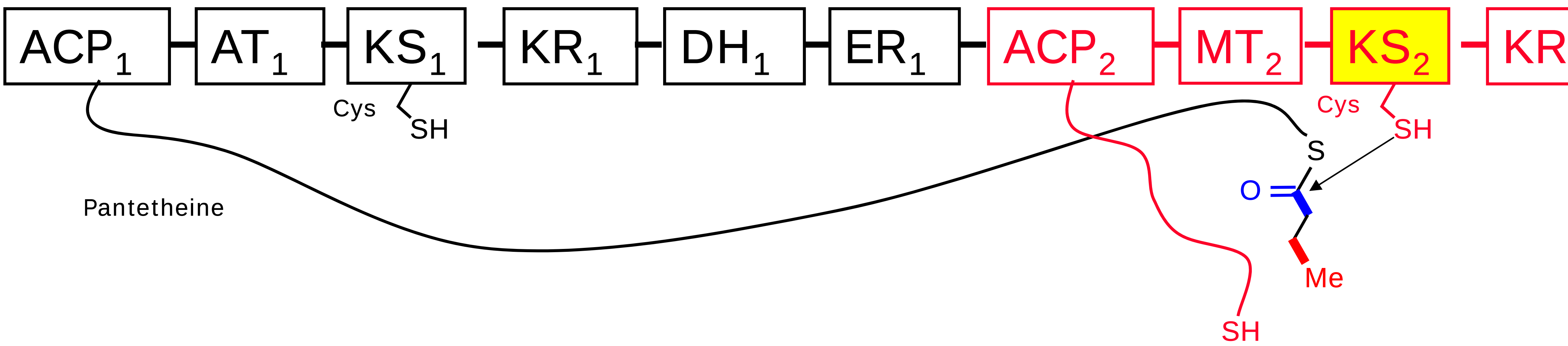
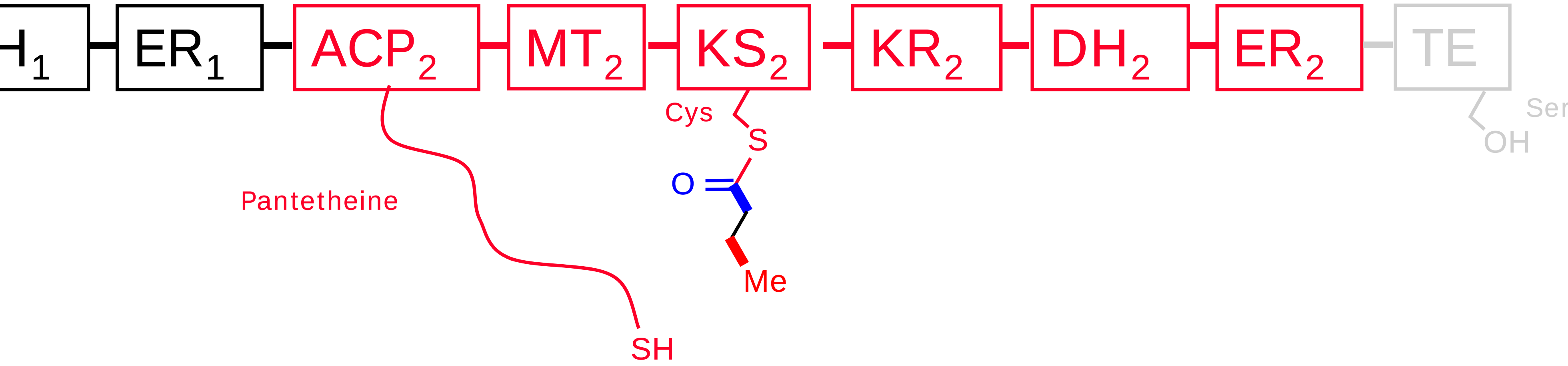


- KS_2 catalyzes translocation to module 2

FATTY ACID BIOSYNTHESIS



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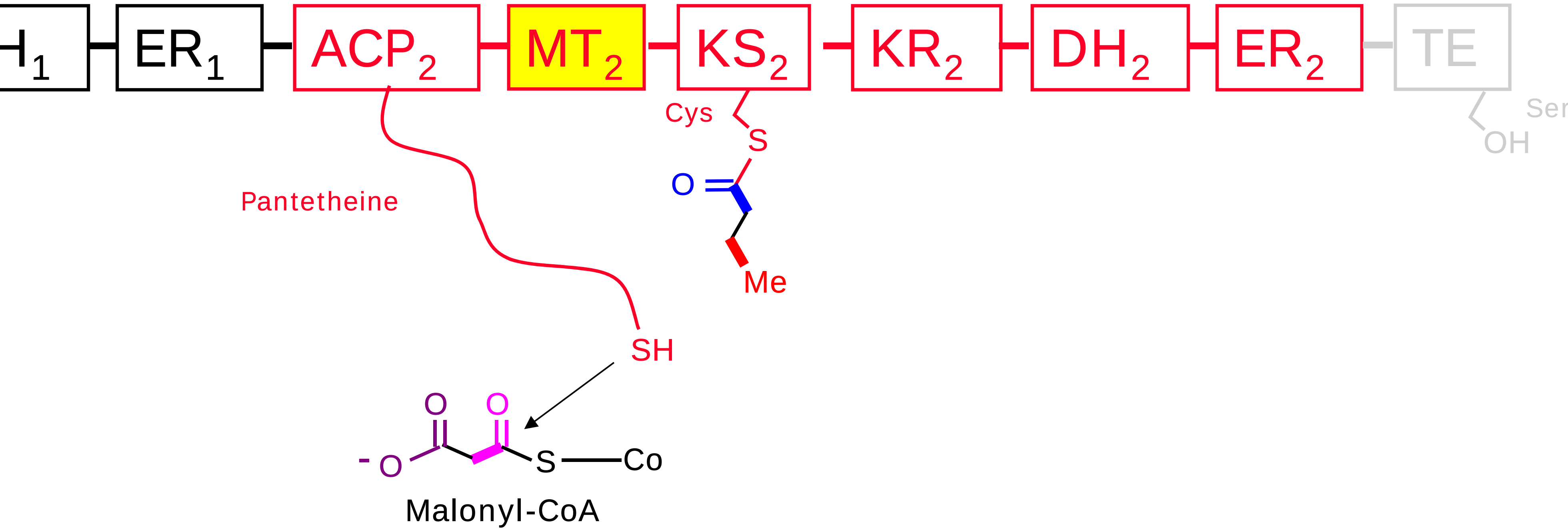
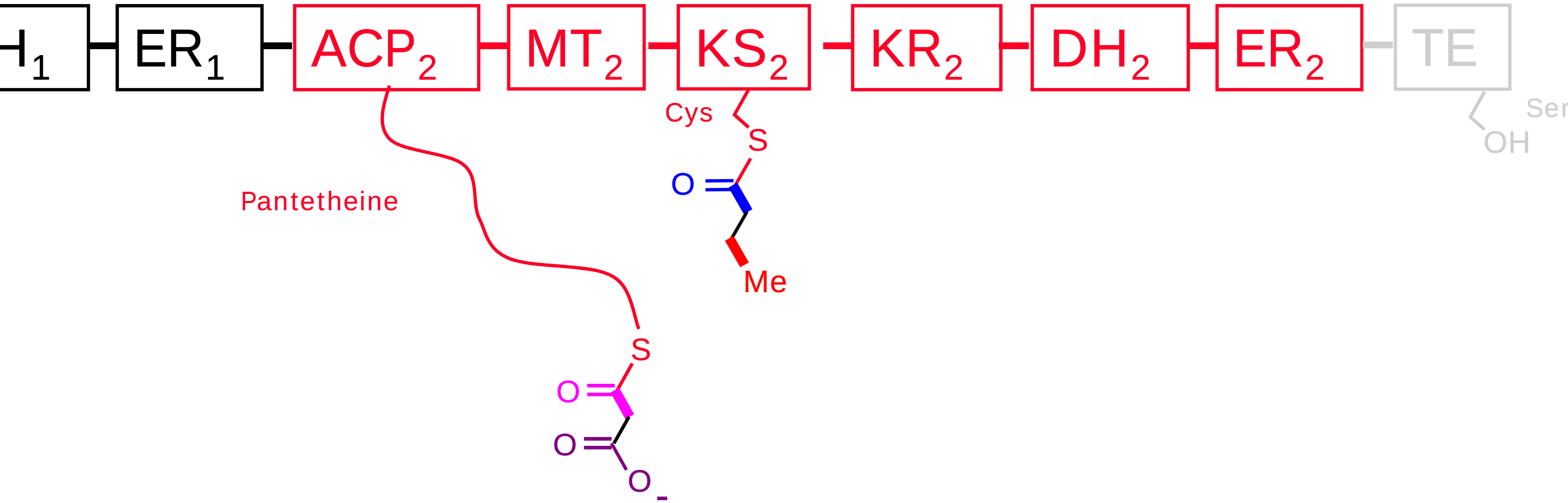


- MT_2 loads malonyl group onto ACP_2

FATTY ACID BIOSYNTHESIS



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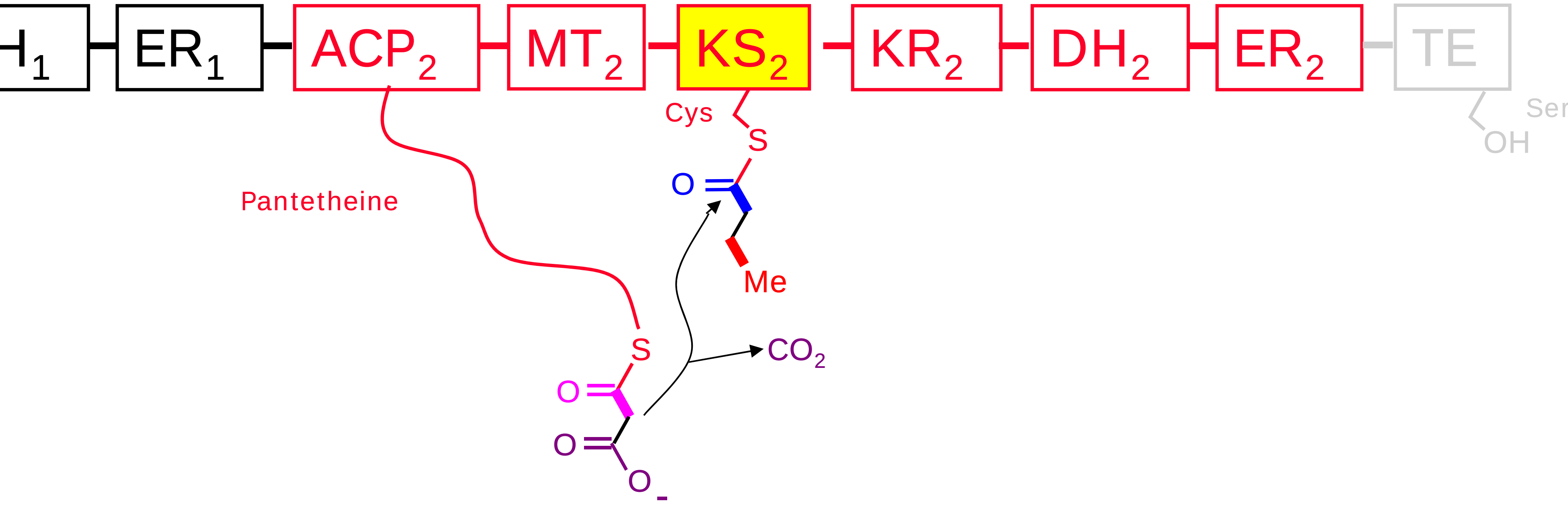
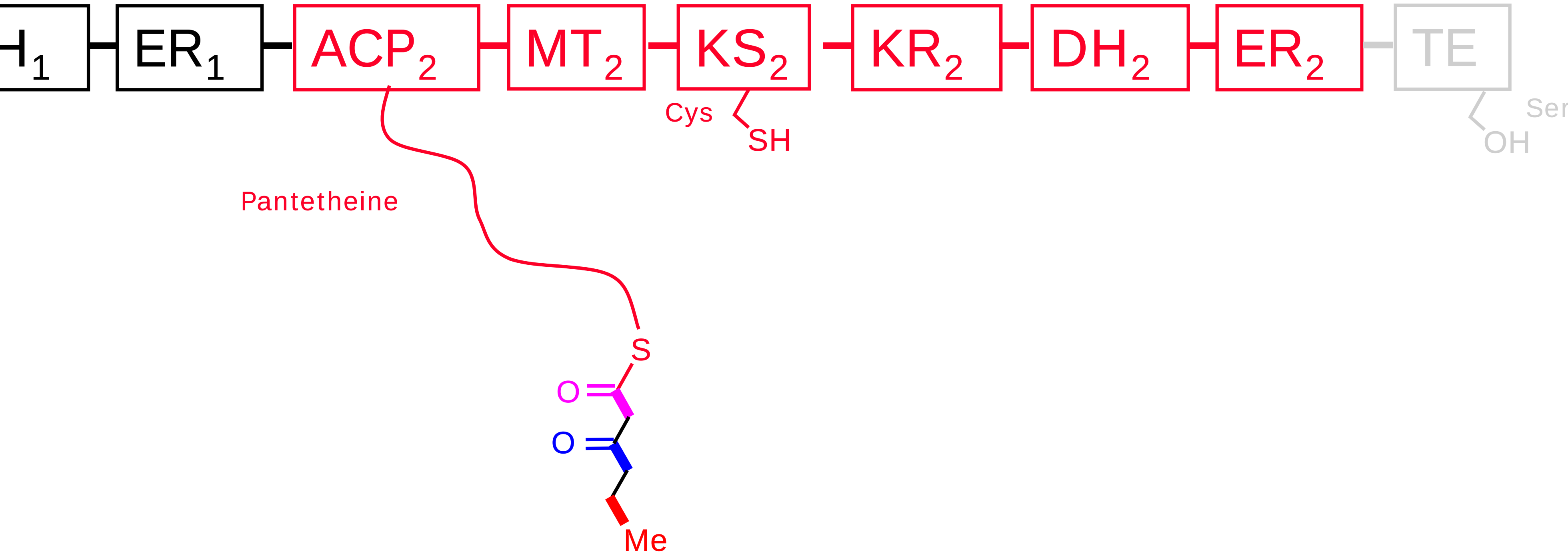


- KS_2 catalyzes Claisen condensation

FATTY ACID BIOSYNTHESIS



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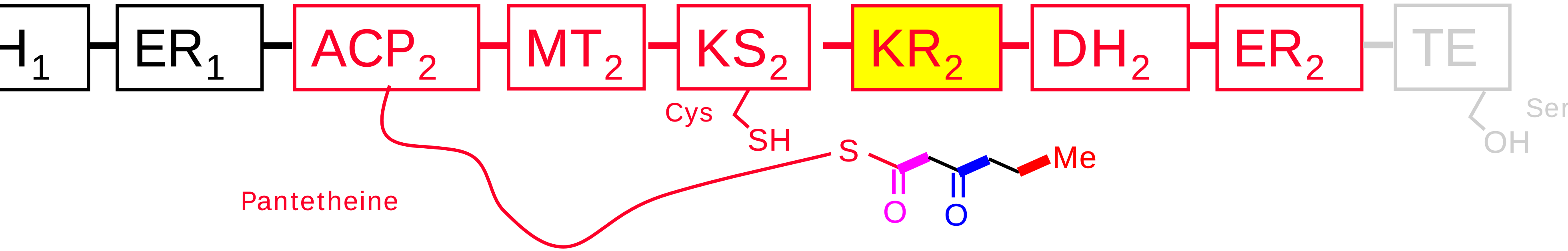
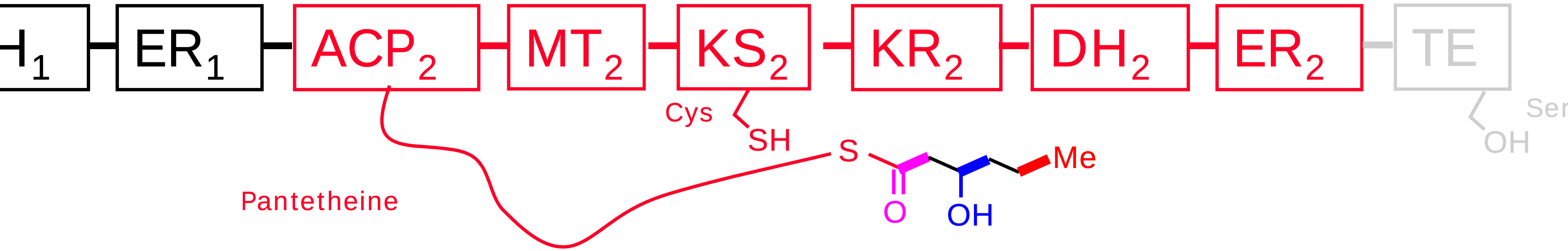


- KR_2 catalyzes reduction of ketone

FATTY ACID BIOSYNTHESIS



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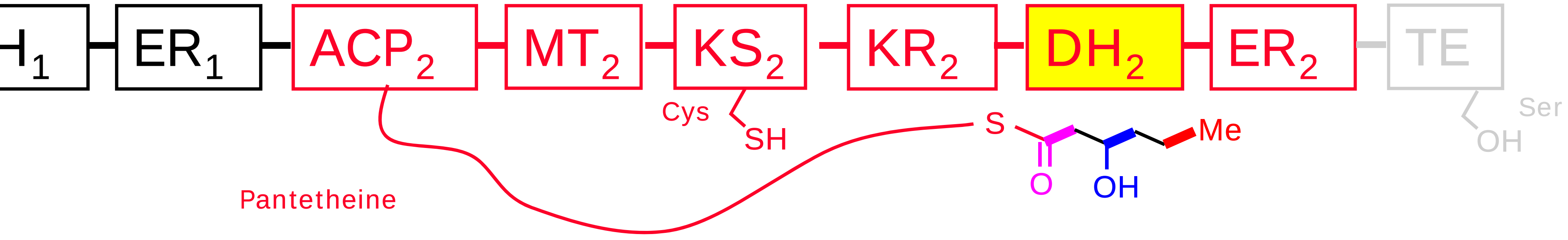
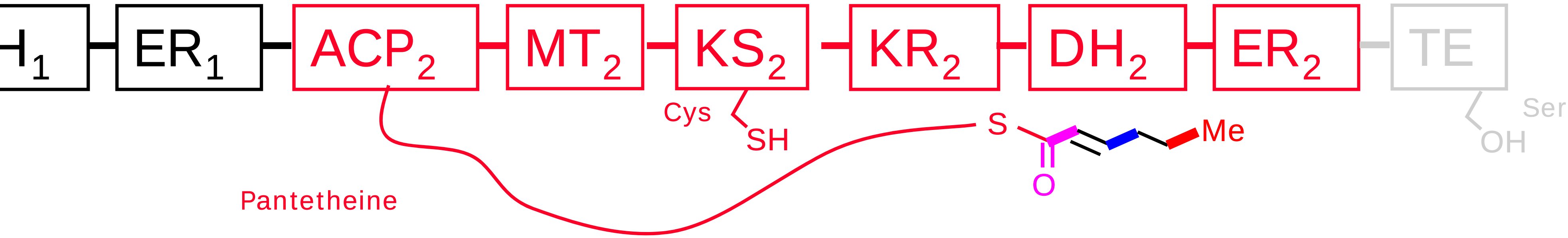


- DH_2 catalyzes dehydration of alcohol

FATTY ACID BIOSYNTHESIS



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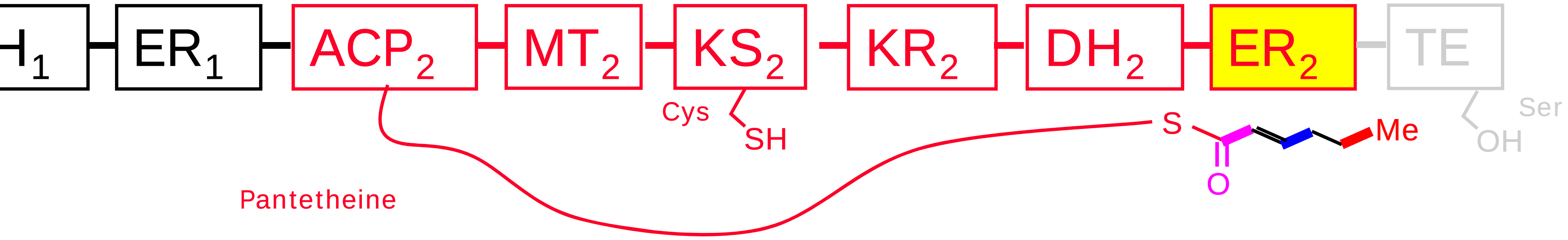
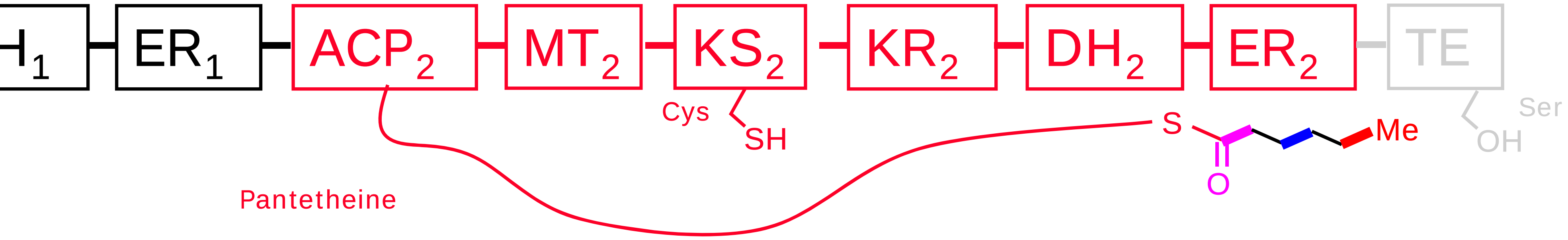


- ER_2 catalyzes reduction of alkene

FATTY ACID BIOSYNTHESIS



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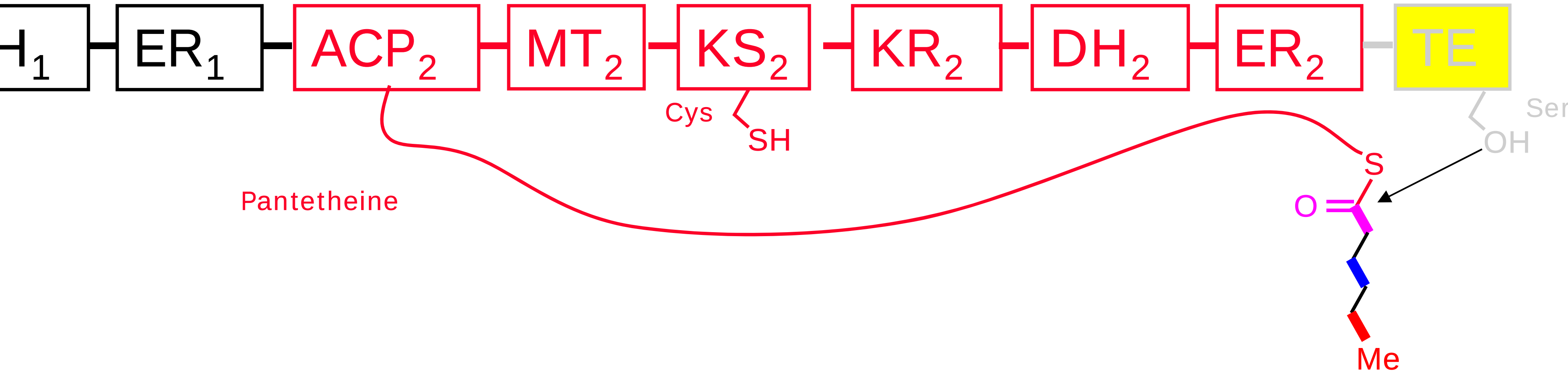
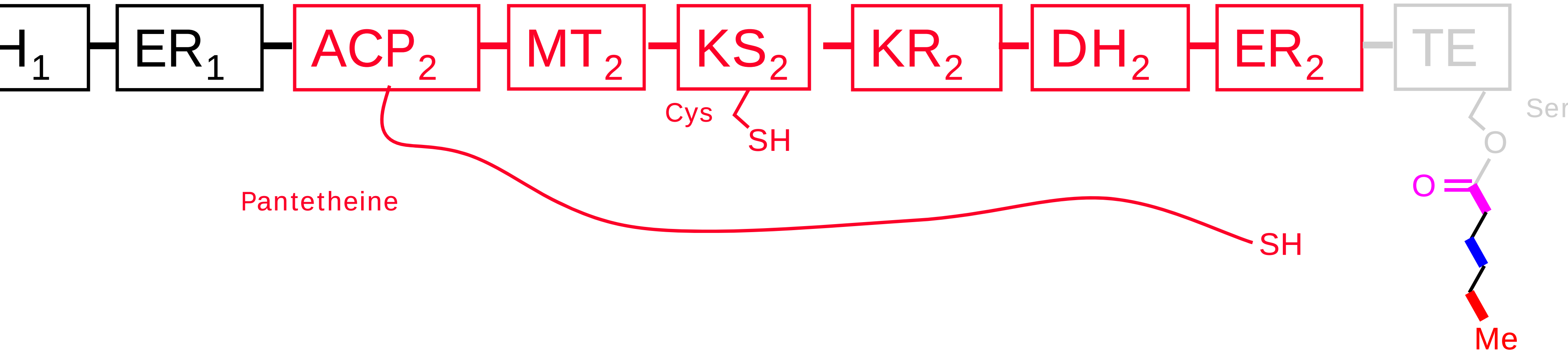


- TE catalyzes transesterification

FATTY ACID BIOSYNTHESIS



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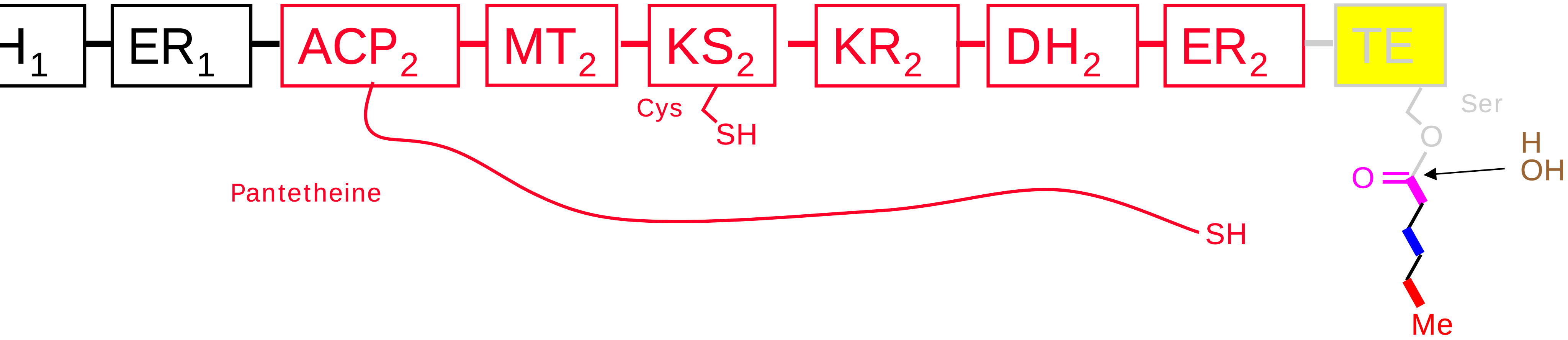
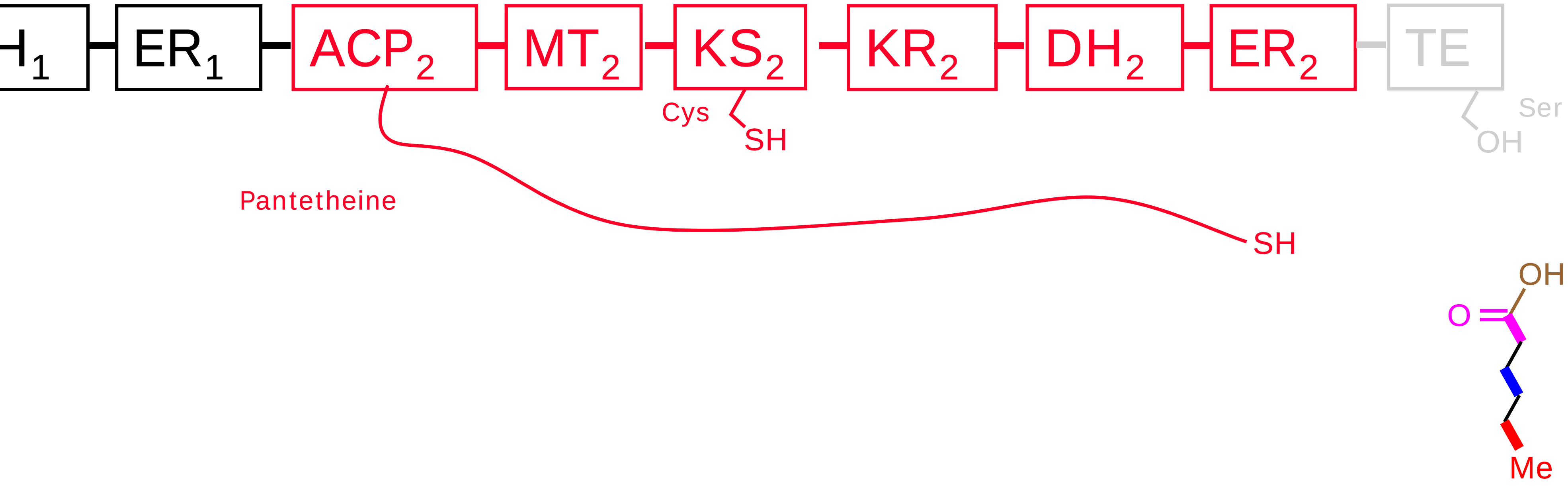


- TE catalyzes hydrolysis

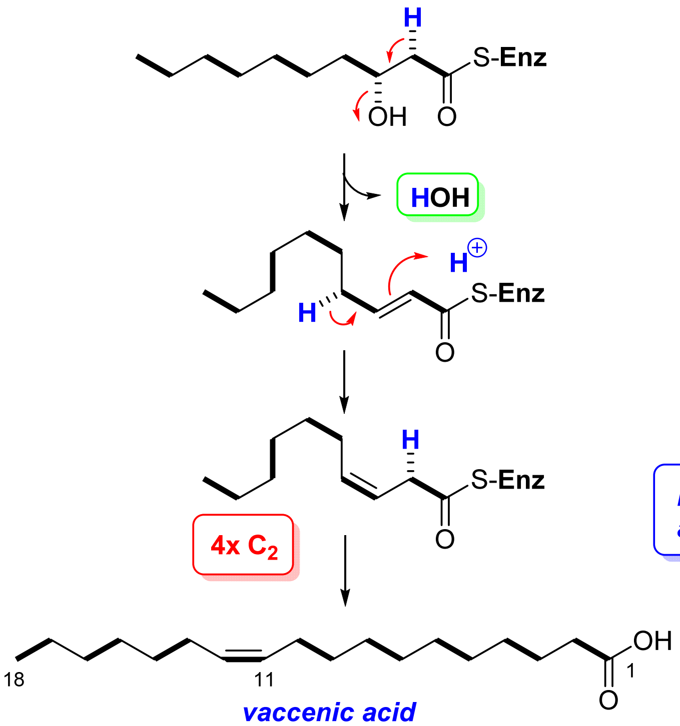
FATTY ACID BIOSYNTHESIS



Biosynthesis of Unsaturated Fatty Acids

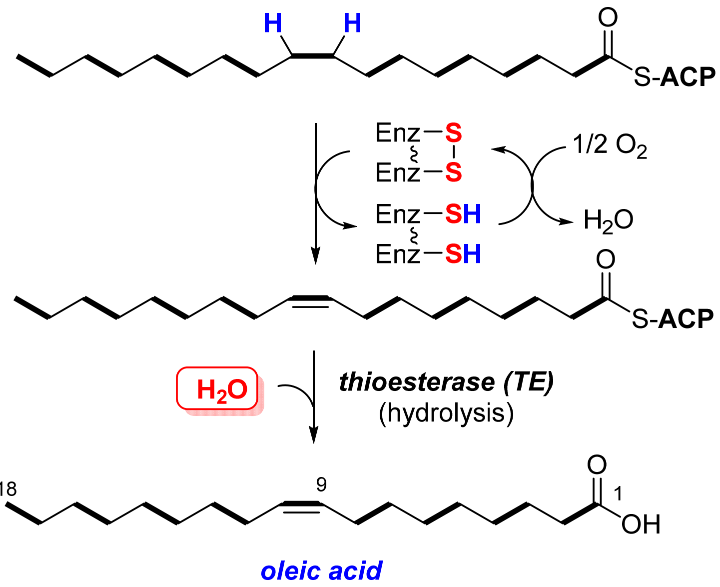
- ***two mechanisms*** are known for the introduction of double bonds into fatty acids:
  - in ***BACTERIA: anaerobic [O]*** → monounsaturated FAs (***MUFAs***)
  - in ***MAMMALS***, ***INSECTS*** & ***PLANTS: aerobic [O]*** → ***MUFAs*** & polyunsaturated FAs (***PUFAs***)

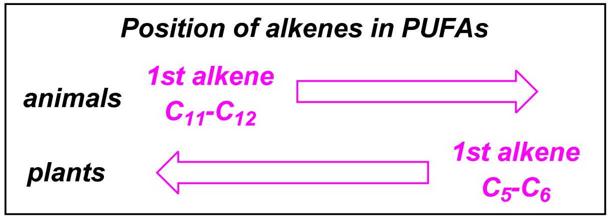
ANAEROBIC ROUTE (bacteria)
(dehydrogenation occurs during chain elongation)
mainly MUFAs but some PUFAs



NB. in both cases cis - alkenes are produced

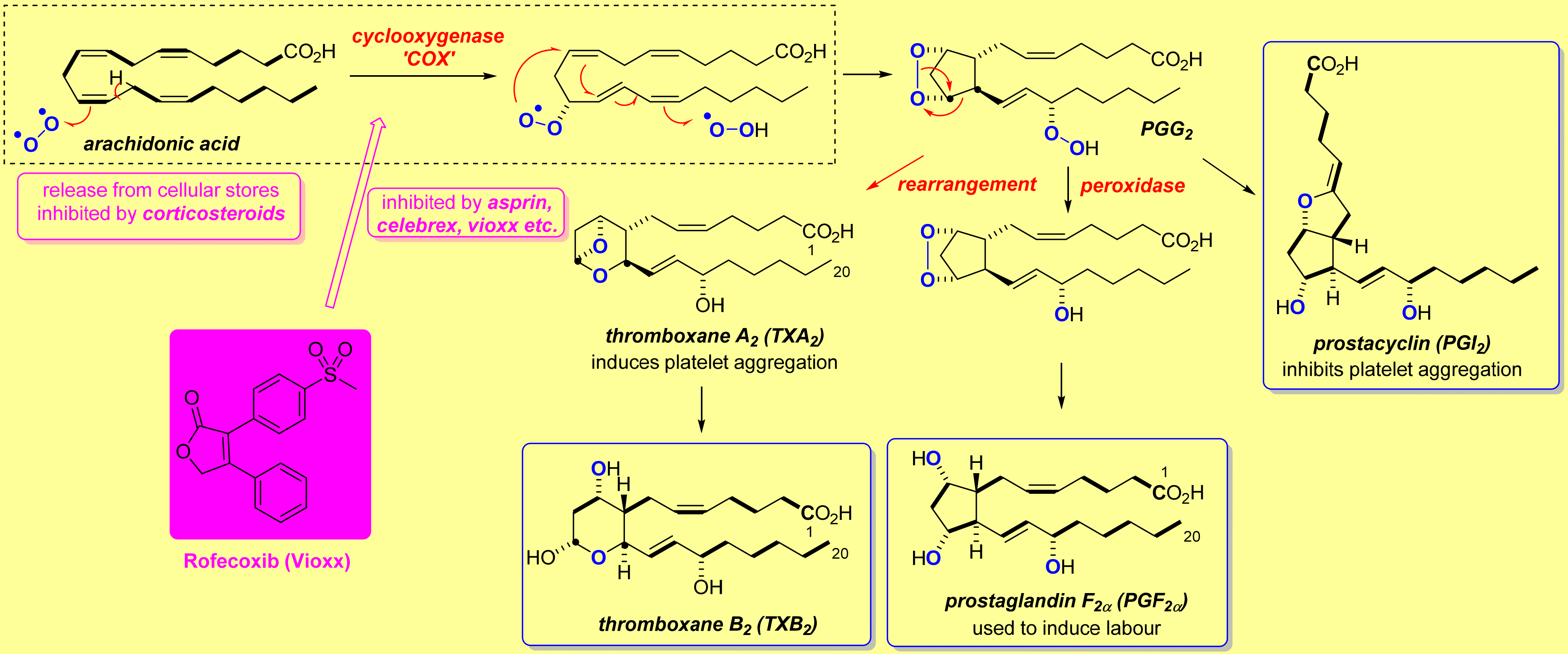
AEROBIC ROUTE (mammals, insects & plants)
(dehydrogenation occurs after chain elongation)
MUFAs & PUFAs





Rational Anti-inflammatory Development – *Prostaglandin & Thromboxane Pathway Intervention*

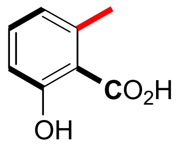
- ***prostaglandins*** & ***thromboxanes*** are derived from further oxidative processing of arachiodonic acid
- both are important ***hormones*** which control *e.g.* smooth ***muscle contractility*** (blood pressure), ***gastric secretion***, ***platelet aggregation*** & ***inflammation*** (<nM activity)
  - various pharmaceuticals including ***corticosteroids*** & ***asprin*** inhibit biosynthethetic steps in these pathways



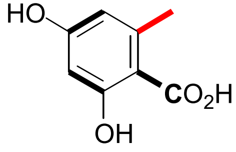
Polyketides

Polyketides

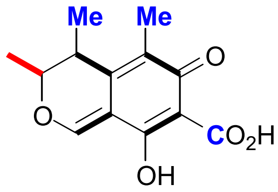
- the structural variety of ***polyketide secondary metabolites*** is very wide:
  - *NB.* starter units marked in red; extender units in bold black; post oligomerisation appended groups in blue



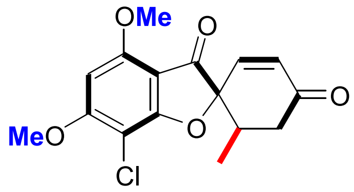
6-methylsalicylic acid
(antibiotic)



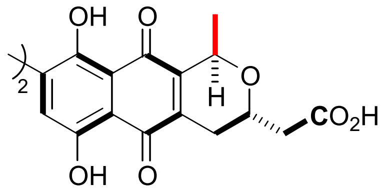
orsellinic acid



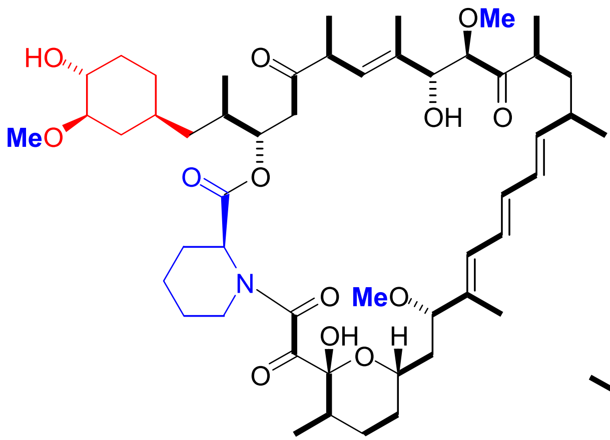
citrinin
(kidney toxin 'yellow rice disease')



Griseofulvin
(treatment for ring worm infections)

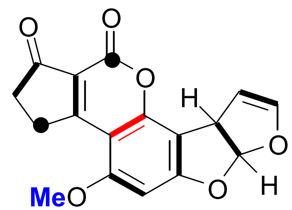


actinorhodin
(antibiotic)

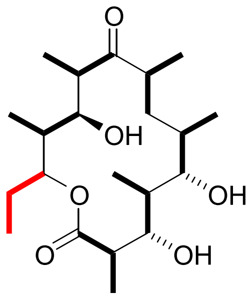


rapamycin
(immunosuppressant)
NB. a mixed polypropionate/acetate

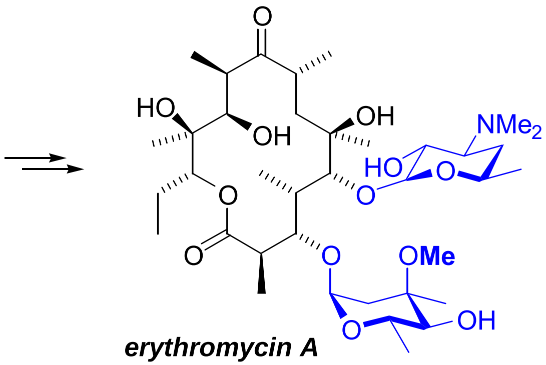
POLYKETIDES



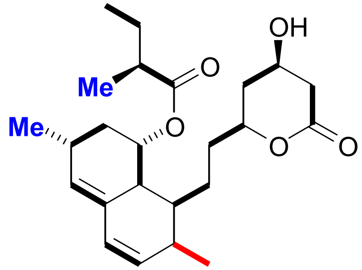
aflatoxin B1
(mycotoxic carcingen)



6-deoxyerythronolide B
NB. a polypropionate



erythromycin A
(antibiotic)

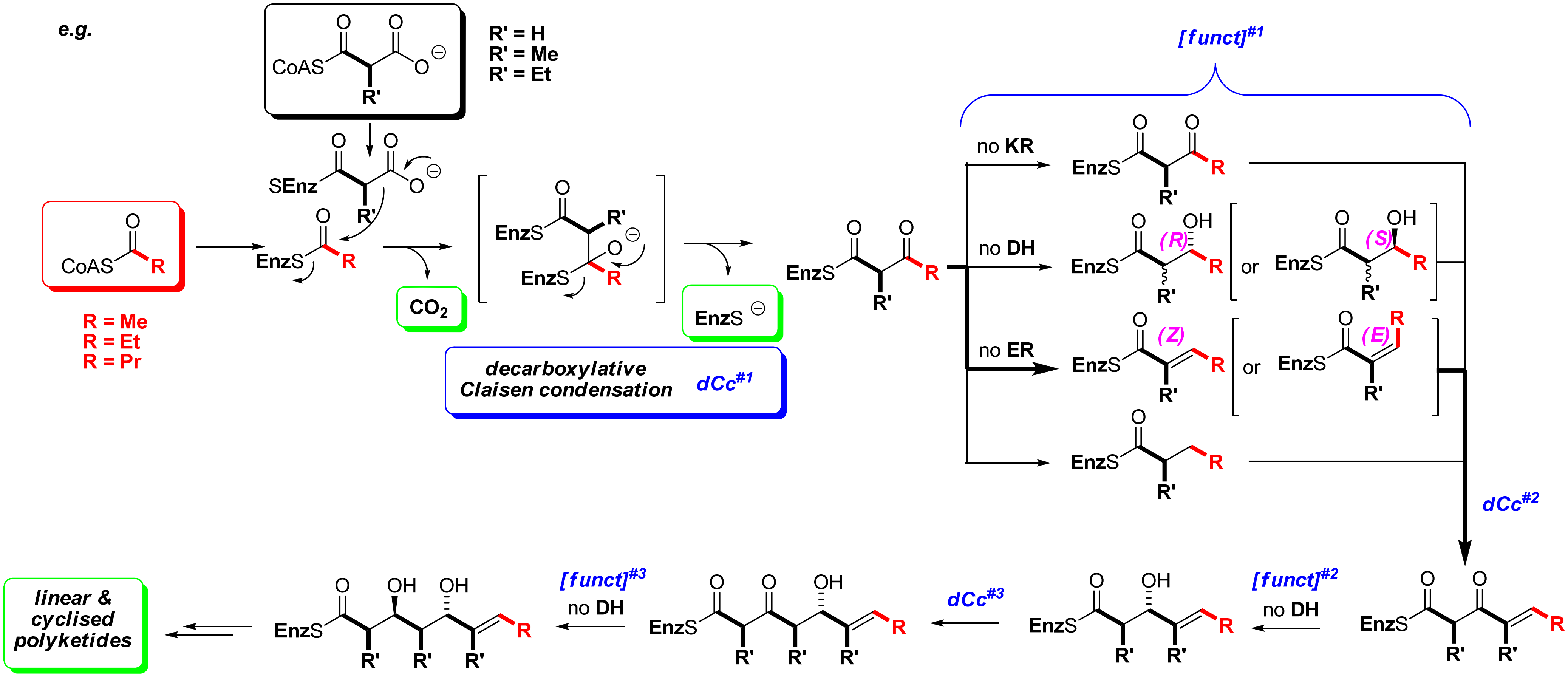


mevinolin
(=lovastatin®)
(anti-cholesterol)

Biosynthesis of Polyketides – *Oligomerisation Steps*

- ***polyketides*** are biosynthesised by a process very similar to that for ***fatty acids***
  - the key ***differences*** are:
    - ***greater variety*** of ***starter units, extender units*** & ***termination processes***
    - ***absent or incomplete reduction of the iteratively introduced β-carbonyl groups: ie. each cycle may differ in terms of KR, DH & ER modules & stereochemistry***

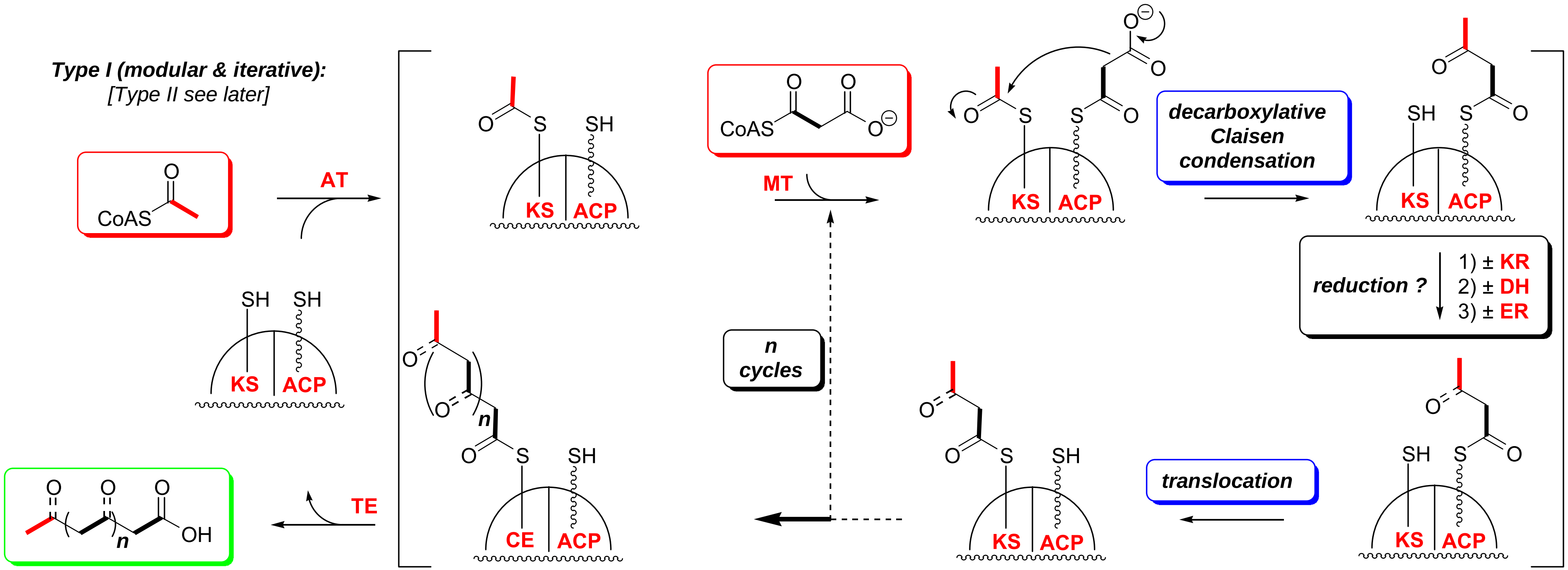
e.g.



  - this leads to ***enormous diversity...***

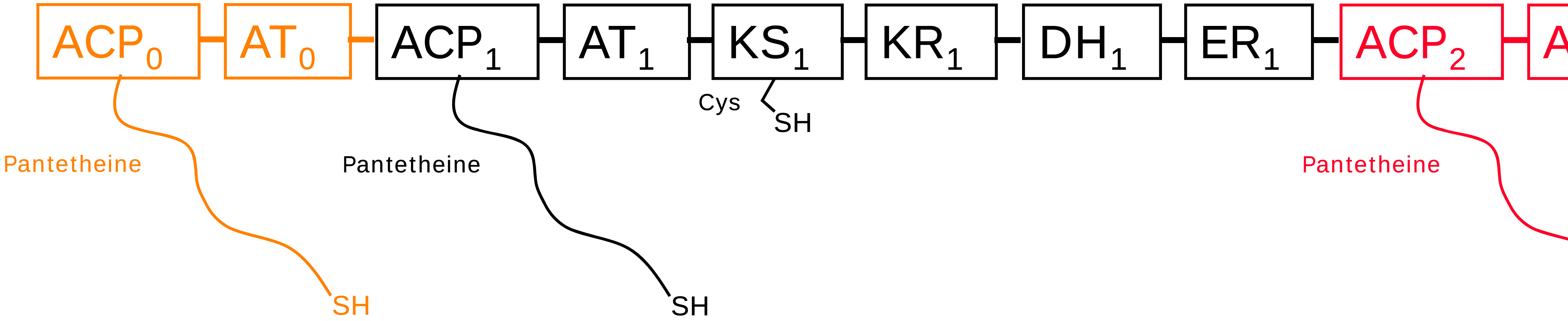
Biosynthesis of Polyketides – *Overview of PKS*

- the *in vivo* process of polyketide synthesis involves ***PolyKetide Synthases (PKSs):***
  - ***PKSs*** (except Type II, see later) comprise the same ***8 components*** as ***FASs***. *i.e.* (ACP & 7× catalytic activities): ***ACP***, ***KS***, ***AT***, ***MT***, [***KR***, ***DH***, ***ER*** & ***TE]***
  - ***Type I PKSs: single (or small set of) multifunctional protein complex(es)***
    - ***modular (microbial)*** - each 'step' has a dedicated catalytic site (→ ***macrolides***)
    - ***iterative (fungal)*** – single set of catalytic sites, each of which *may* operate in each iteration (*cf.* FASs) (→ ***aromatics/polyphenols*** - generally)
  - ***Type II PKSs: single set of discrete, dissociable single-function proteins***
    - ***iterative (microbial***) - each catalytic module *may* operate in each iteration (*cf.* FASs) (→ ***aromatics/polyphenols***)



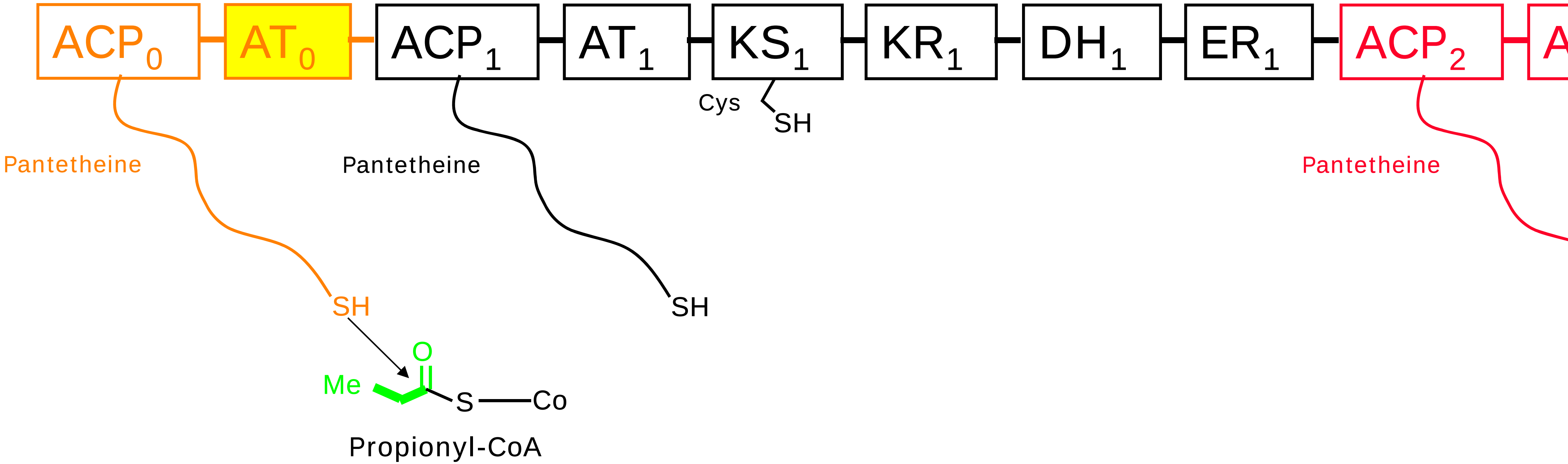
KS = keto synthase; **AT** = acetyl transferase; **MT** = malonyl transferase;
KR = keto reductase; **DH** = dehydratase; **ER** = enoyl reductase; **TE** = thioesterase; **ACP** = acyl carrier protein

POLYKETIDE BIOSYNTHESIS [Type I – (modular)]



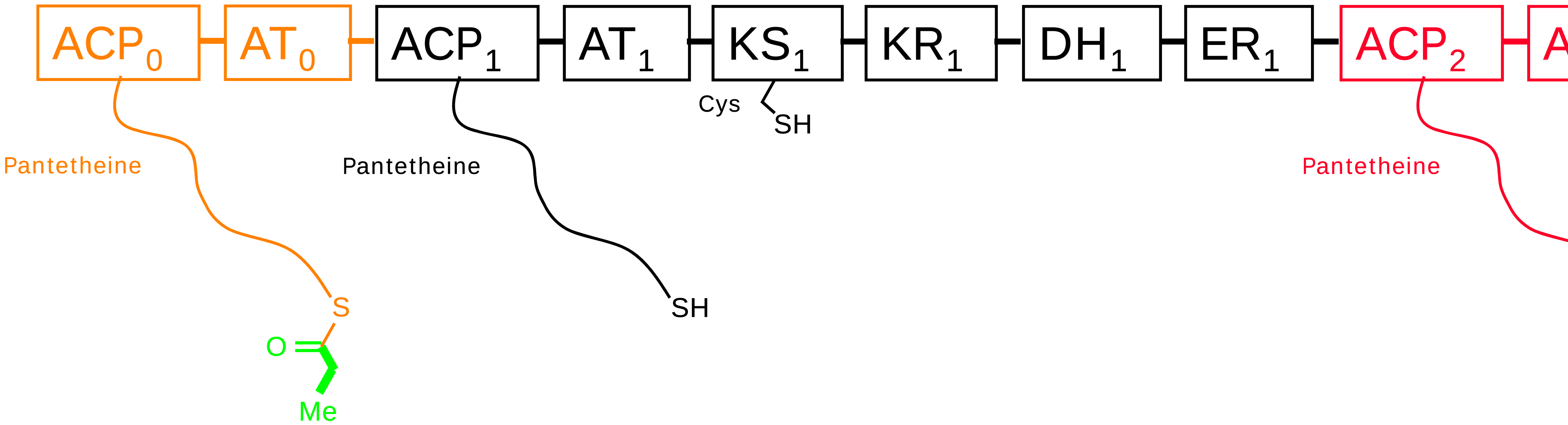
NB. the following sequence of slides has also been adapted from: http://www.courses.fas.harvard.edu/%7echem27/

POLYKETIDE BIOSYNTHESIS

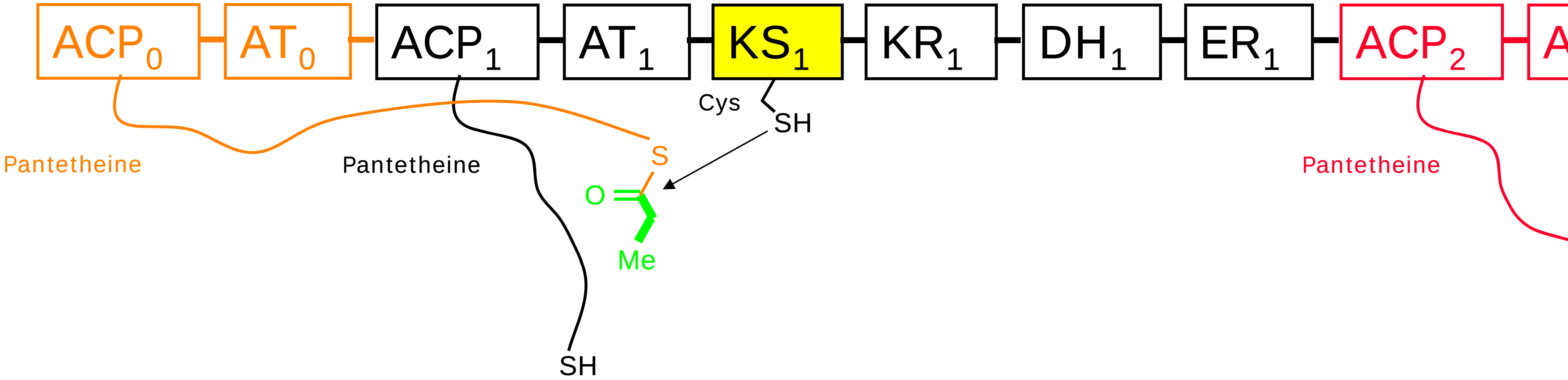


- AT_0 loads starting group (propionyl) onto ACP_0

POLYKETIDE BIOSYNTHESIS

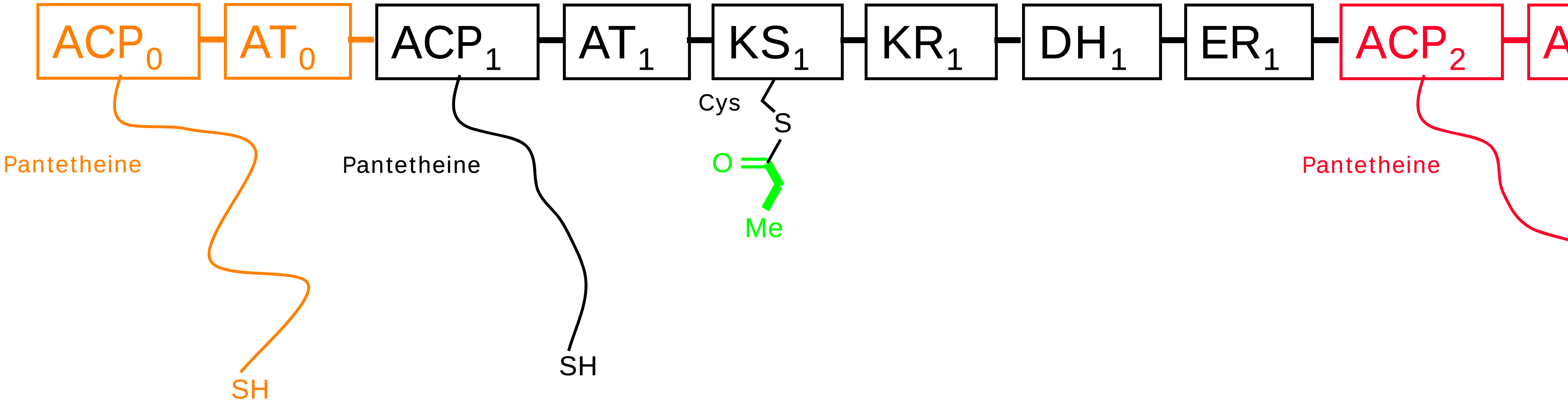


POLYKETIDE BIOSYNTHESIS

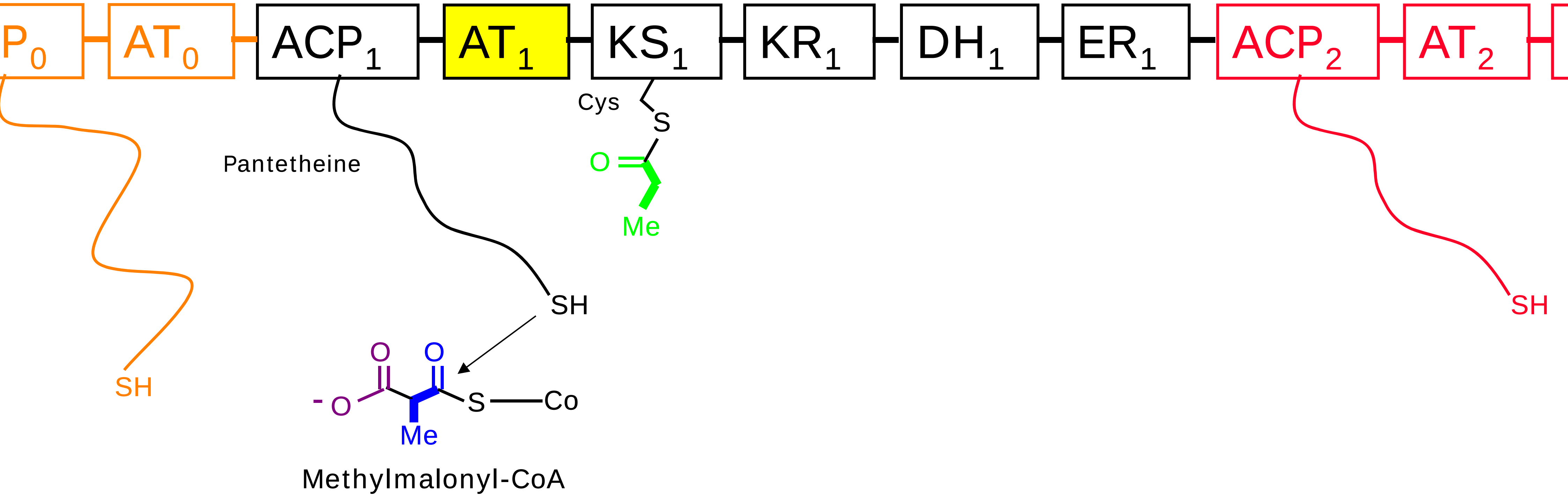


- KS_1 catalyzes translocation to module 1

POLYKETIDE BIOSYNTHESIS

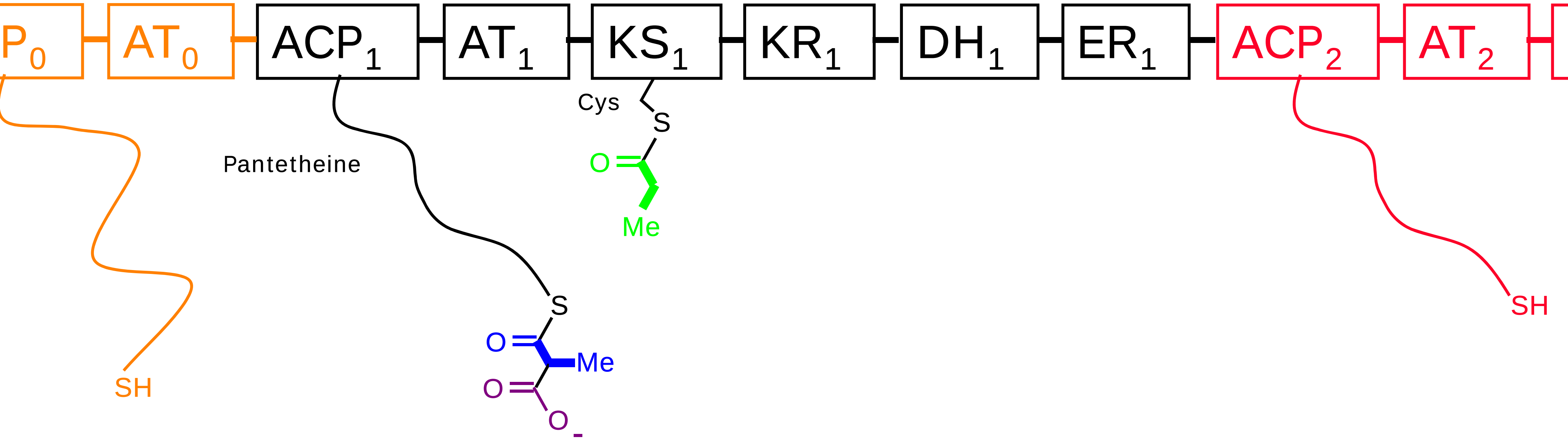


POLYKETIDE BIOSYNTHESIS

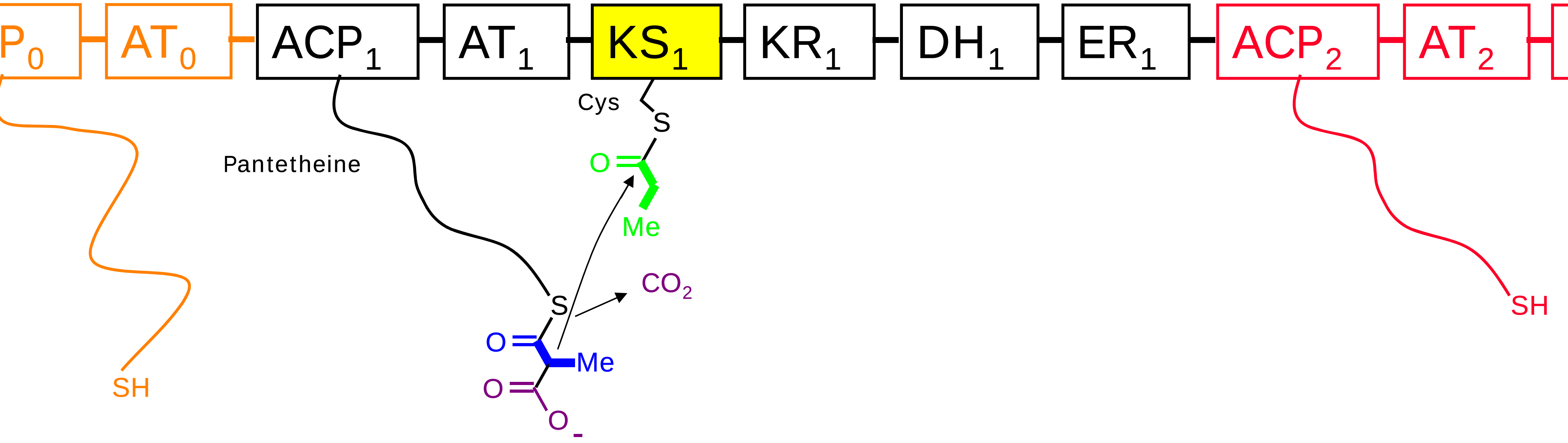


- AT_1 loads methylmalonyl group onto ACP_1

POLYKETIDE BIOSYNTHESIS

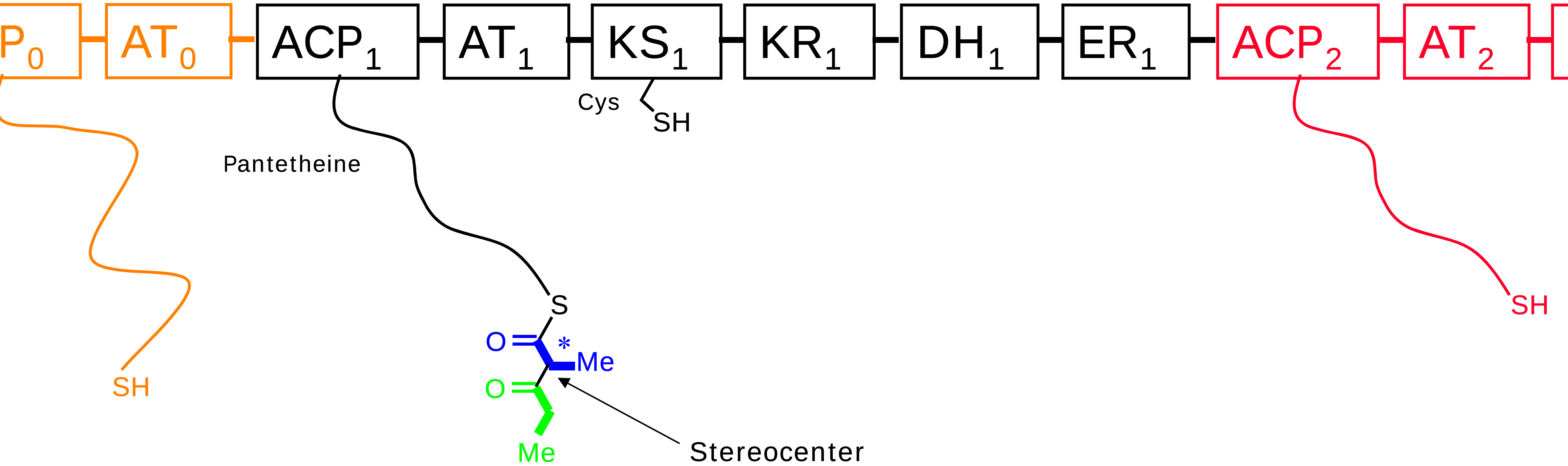


POLYKETIDE BIOSYNTHESIS

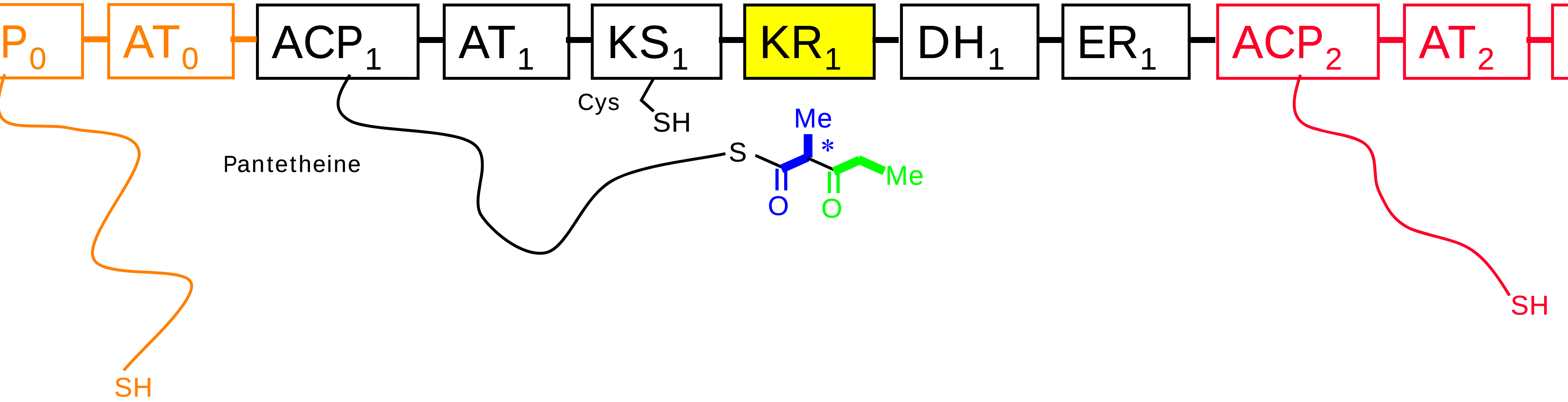


- KS_1 catalyzes Claisen condensation

POLYKETIDE BIOSYNTHESIS

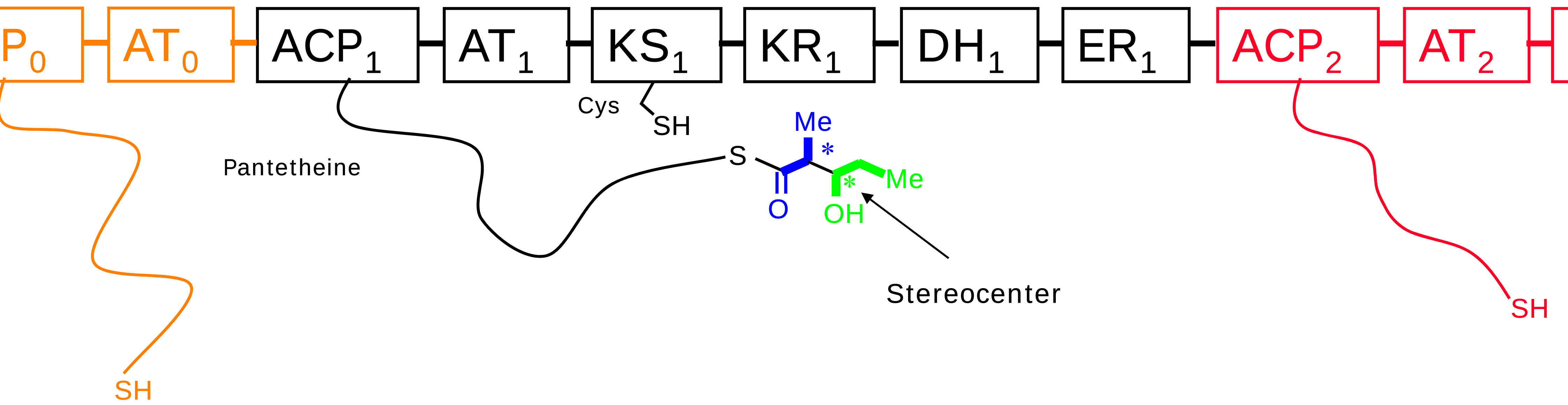


POLYKETIDE BIOSYNTHESIS

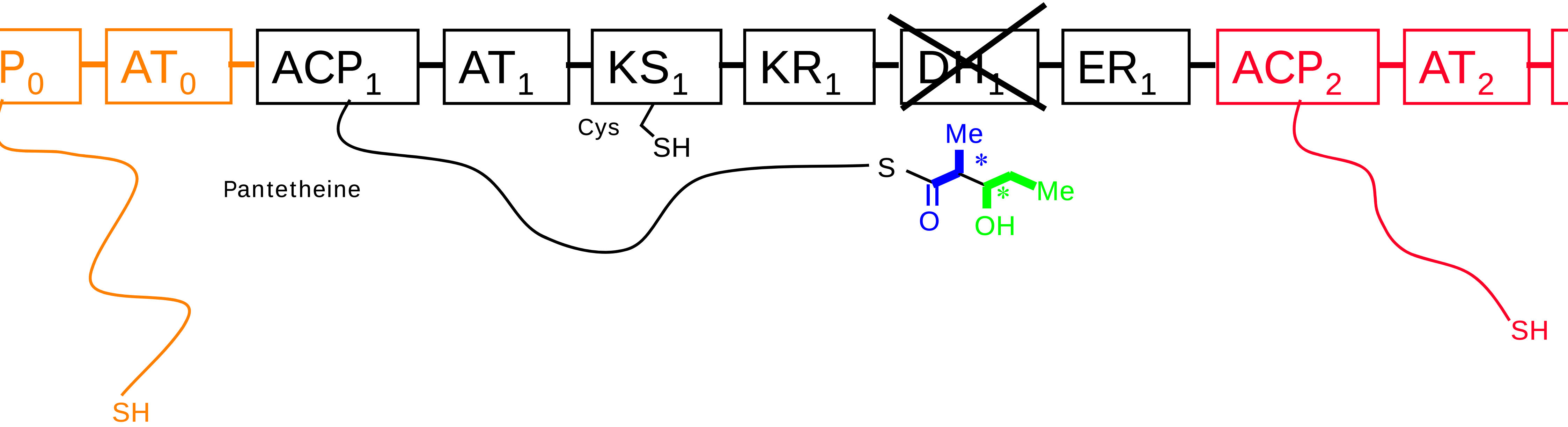


- KR_1 catalyzes reduction of ketone

POLYKETIDE BIOSYNTHESIS

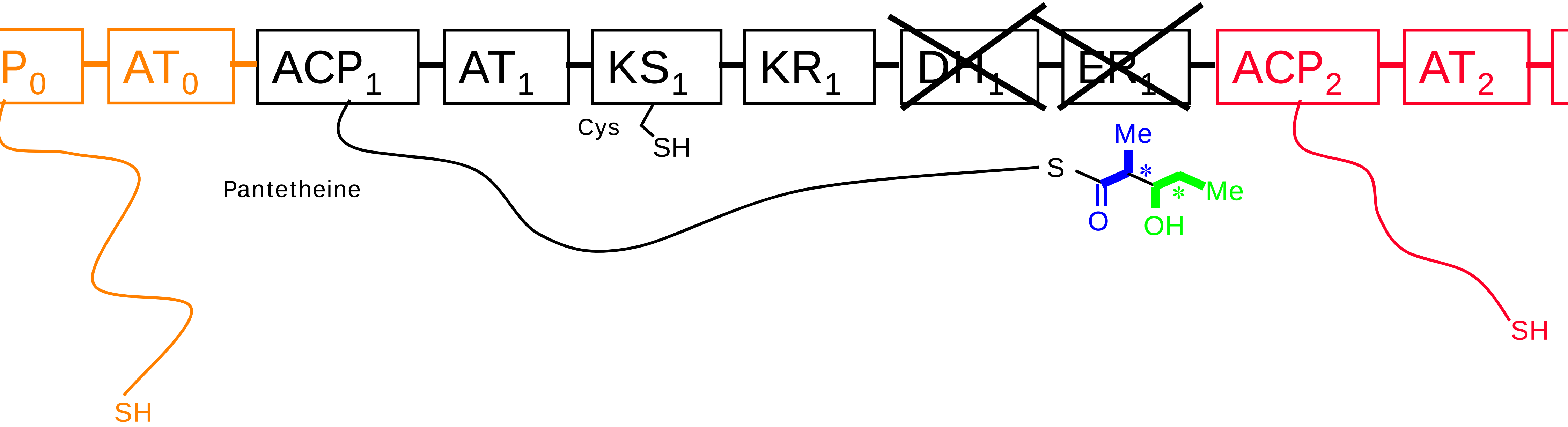


POLYKETIDE BIOSYNTHESIS



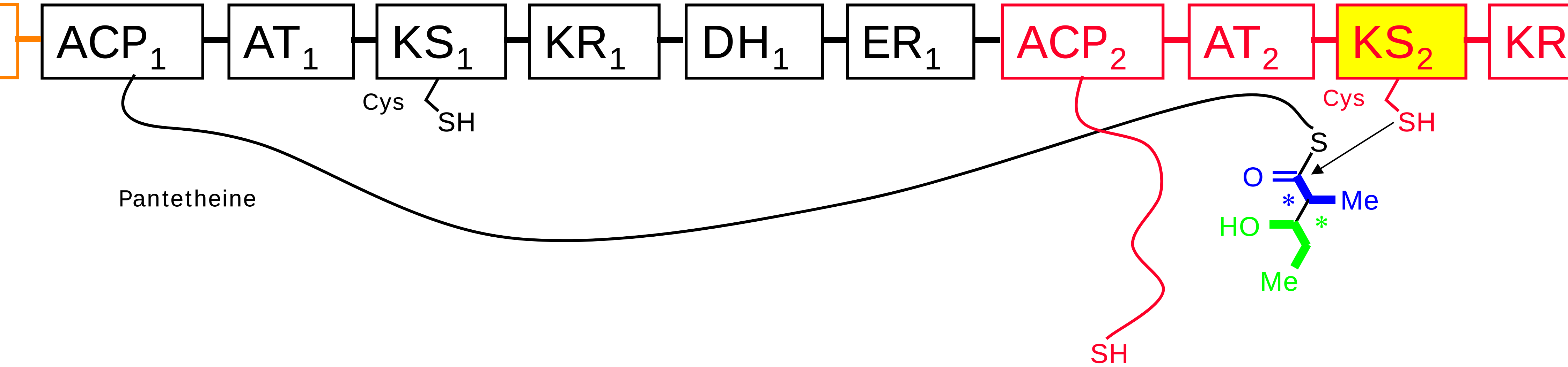
- no DH_1 activity

POLYKETIDE BIOSYNTHESIS



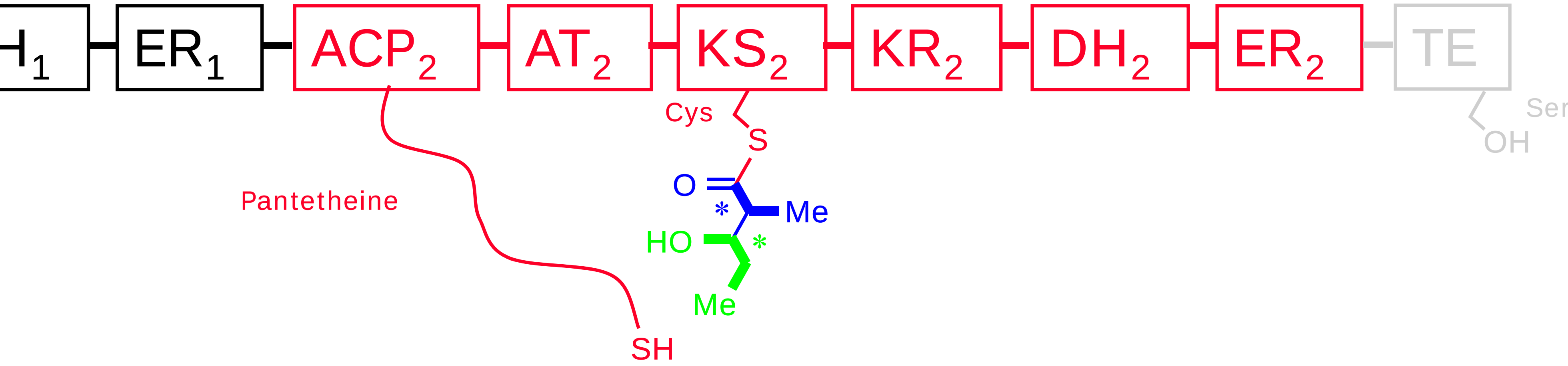
- no ER_1 activity

POLYKETIDE BIOSYNTHESIS



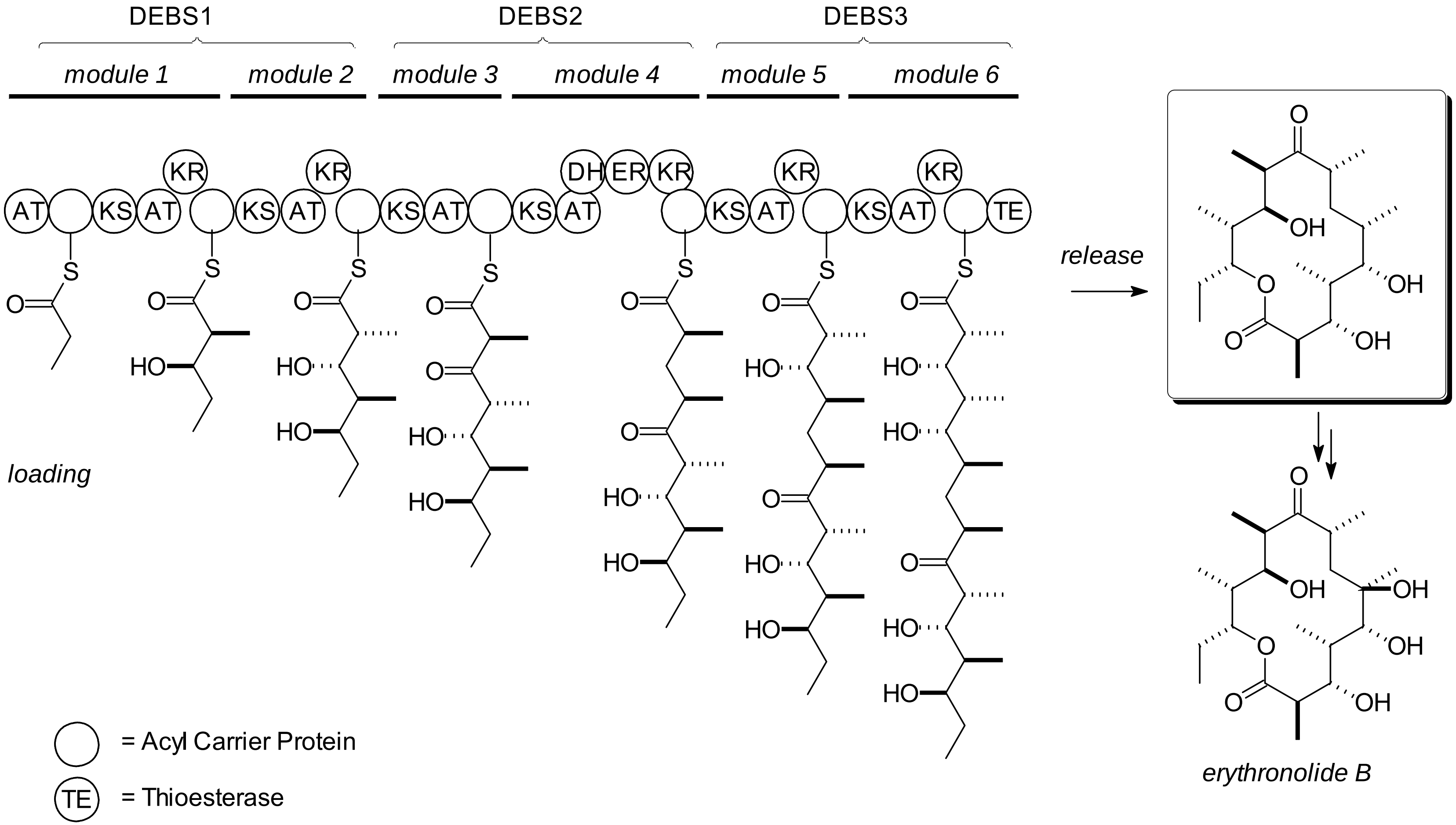
- KS_2 catalyzes translocation to module 2

POLYKETIDE BIOSYNTHESIS



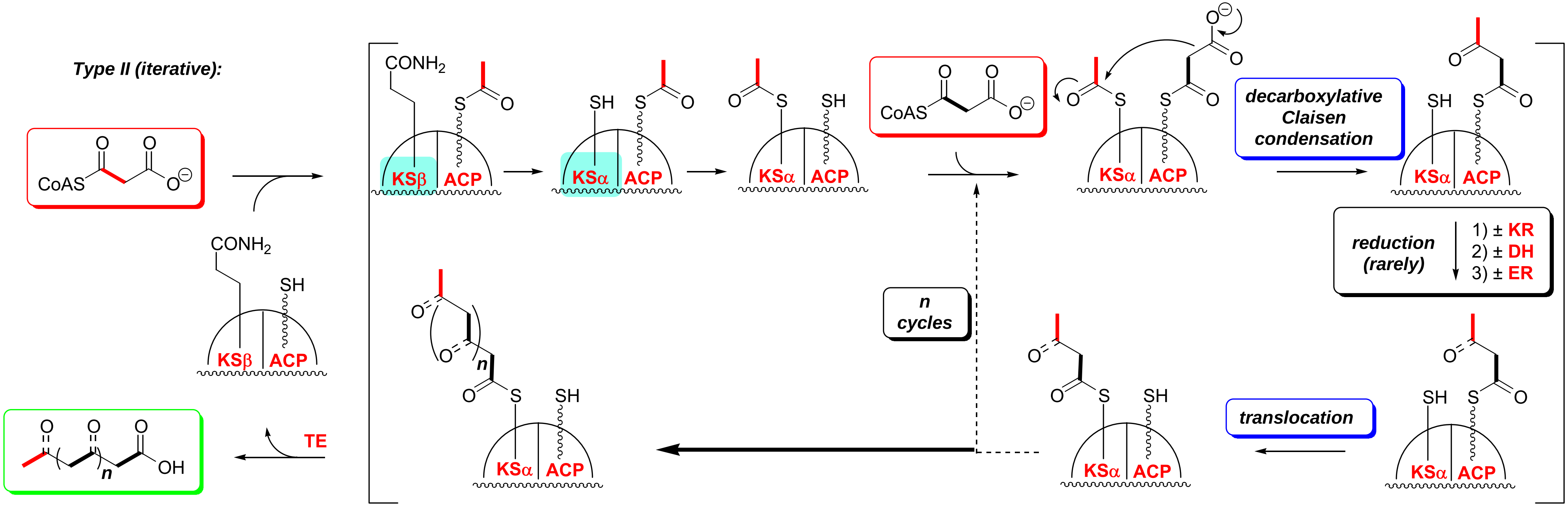
Biosynthesis of Erythromycin – *Type I(modular) PKS*

- ***6-deoxyerthronolide*** is a precursor to ***erythromycin A*** – ***bacterial*** antibiotic (*Streptomyces erythreus*):
  - ***propionate*** based ***heptaketide***; 3 multifunctional polypeptides (DEBS1, DEBS2 & DEBS3, all ~350 kDa)
  - Katz *et al. Science* **1991**, *252*, 675 (**DOI**); Staunton, Leadley *et al. Science* **1995**, *268*, 1487 (**DOI**); Khosla *et al. J. Am. Chem. Soc.* **1995**, 9105 (**DOI**); ***review:*** Staunton & Weissman *Nat. Prod. Rep.* **2001**, *18*, 380 (**DOI**)



Type II PKSs – *Enzyme Clusters (Microbial)*

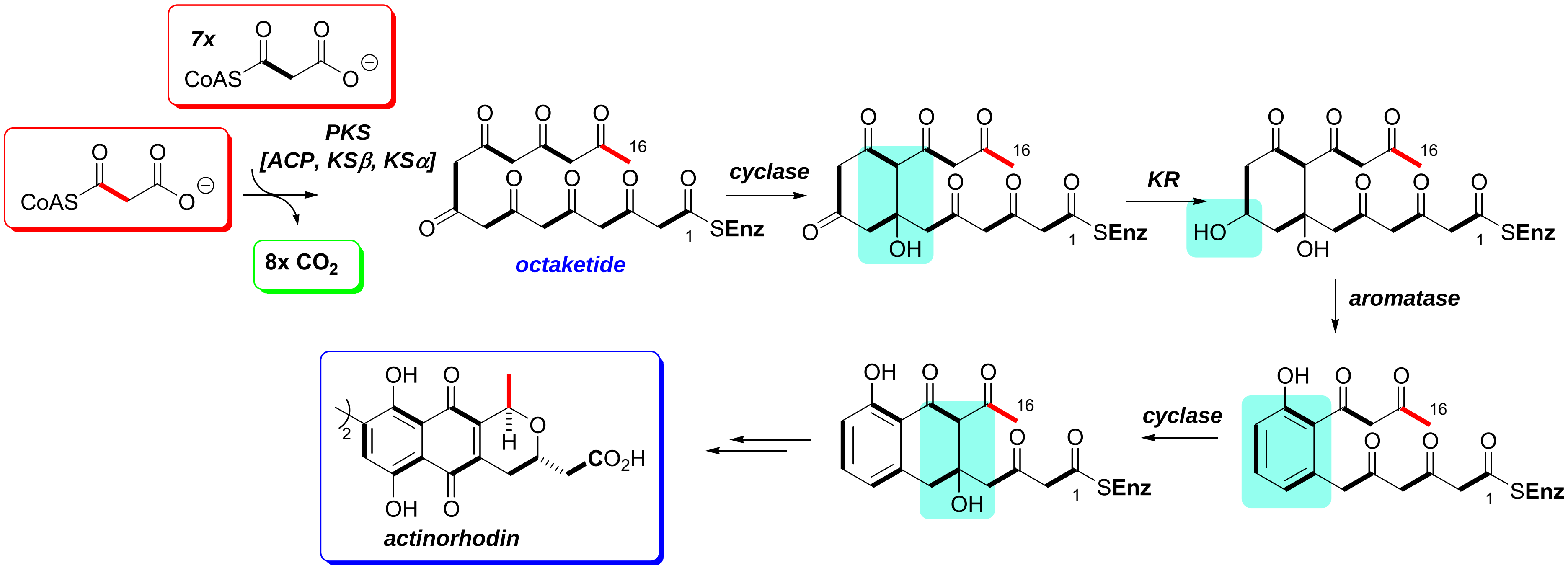
- ***Type II PKSs:*** single set of discrete, dissociable single-function proteins (ACP & 6× catalytic functions): ***ACP***, ***KS***$_{\alpha}$, ***KS***$_{\beta}$, [***KR***, ***DH***, ***ER***, & ***TE***] [*NB.* NO acetyl or malonyl transferases (AT, MT)]
  - ***iterative*** - each catalytic module *may* operate in each iteration (*cf.* FASs) (→ ***aromatics/polyphenols***)
- these clusters (generally) use ***malonate*** as BOTH ***starter*** & ***extender*** unit
- their ***ACP proteins*** are able to load malonate direct from malonyl CoA (no MT required)
  - the ***starter malonate*** is ***decarboxylated*** by '***ketosynthase' β*** (***KS***$_{\beta}$) to give ***S-acetyl-ACP***
  - the ***extender malonates*** undergo ***decarboxylative Claisen condensations*** by ***ketosynthase α*** (***KS***$_{\alpha}$)
- these clusters rarely utilise ***KR***, ***DH*** or ***ER*** activities and produce 'true' polyketides:



KS$_{\beta}$ = 'keto synthase β' (=decarboxylase!); KS$_{\alpha}$ = 'keto synthase α' (=ketosynthase!); KR = keto reductase; DH = dehydratase; ER = enoyl reductase; TE = thioesterase; ACP = acyl carrier protein

Biosynthesis of Actinorhodin – *Type II PKS*

- ***actinorhodin –*** octaketide ***bacterial antibiotic*** (*Streptomyces coelicolor*)
  – Hopwood *Chem. Rev.* **1997**, *97*, 2465 (**DOI**)



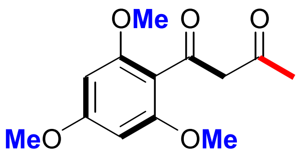
– ***timing*** of ***1st cyclisation*** and mechanism of ***control of chain length*** uncertain
  - ***octaketide*** synthesis then cyclisation? (as shown above)
  - ***hexaketide*** synthesis then cyclisation then two further rounds of extension?

– indications can sometimes be gleaned from ***biomimetic syntheses***.

Scope of Structures - *Type II PKS*

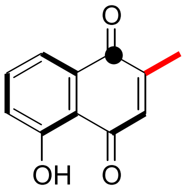
- ***microbial polyphenolic*** metabolites:

pentaketides (5x C_2)



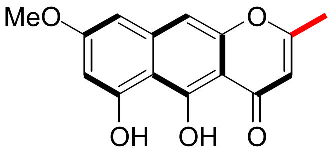
eugenone

hexaketides (6x C_2)



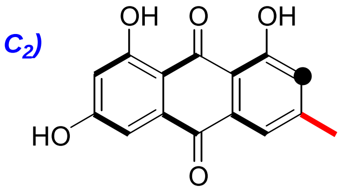
plumbagin

heptaketides (7x C_2)



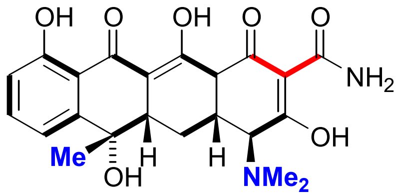
rubrofusarin

octaketides (8x C_2)



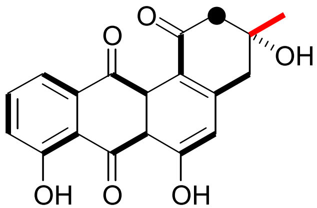
emodin

nonaketides (9x C_2)



tetracycline

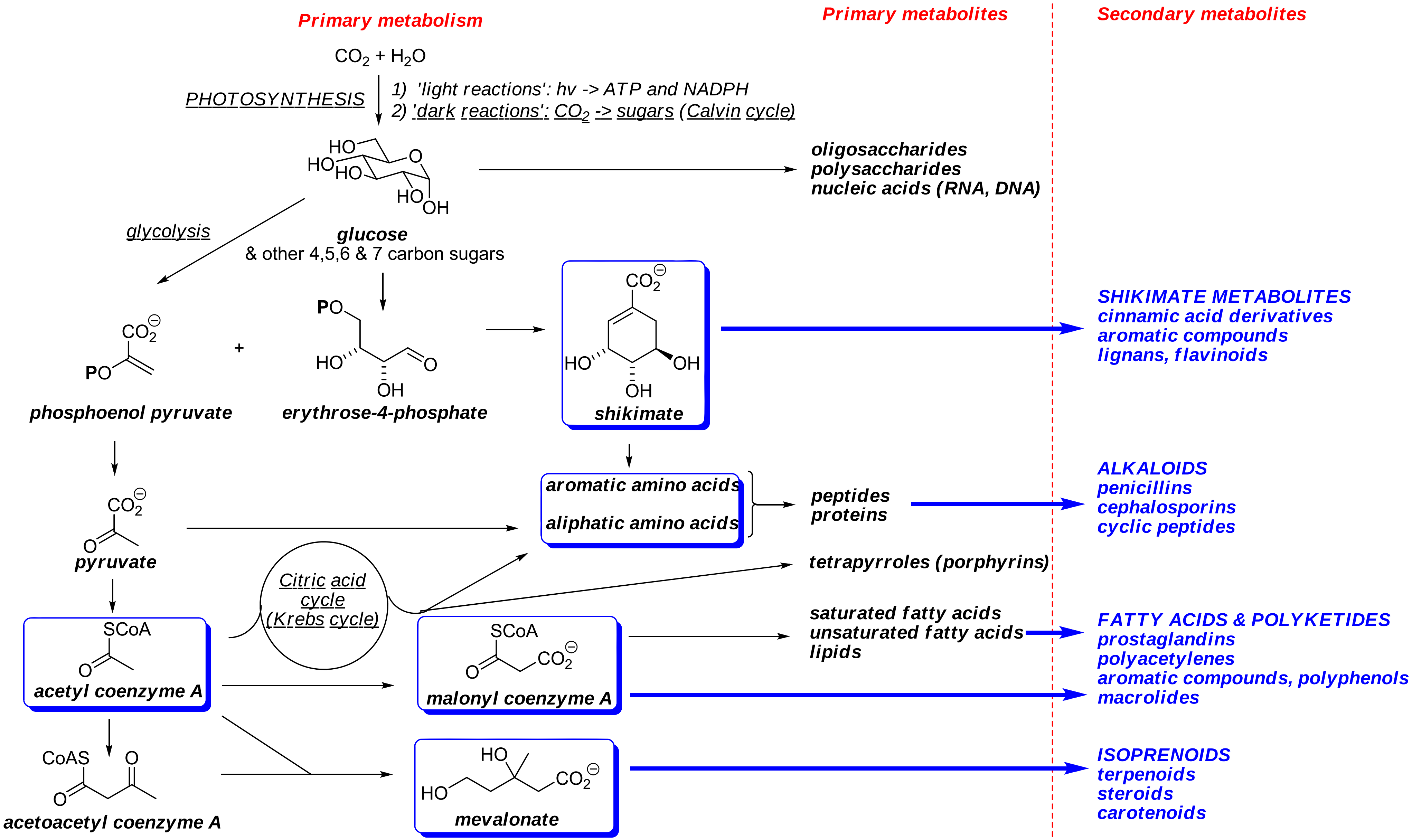
decaketides (10x C_2)



rabelomycine

- many display interesting biological activities...

Primary Metabolism - *Overview*

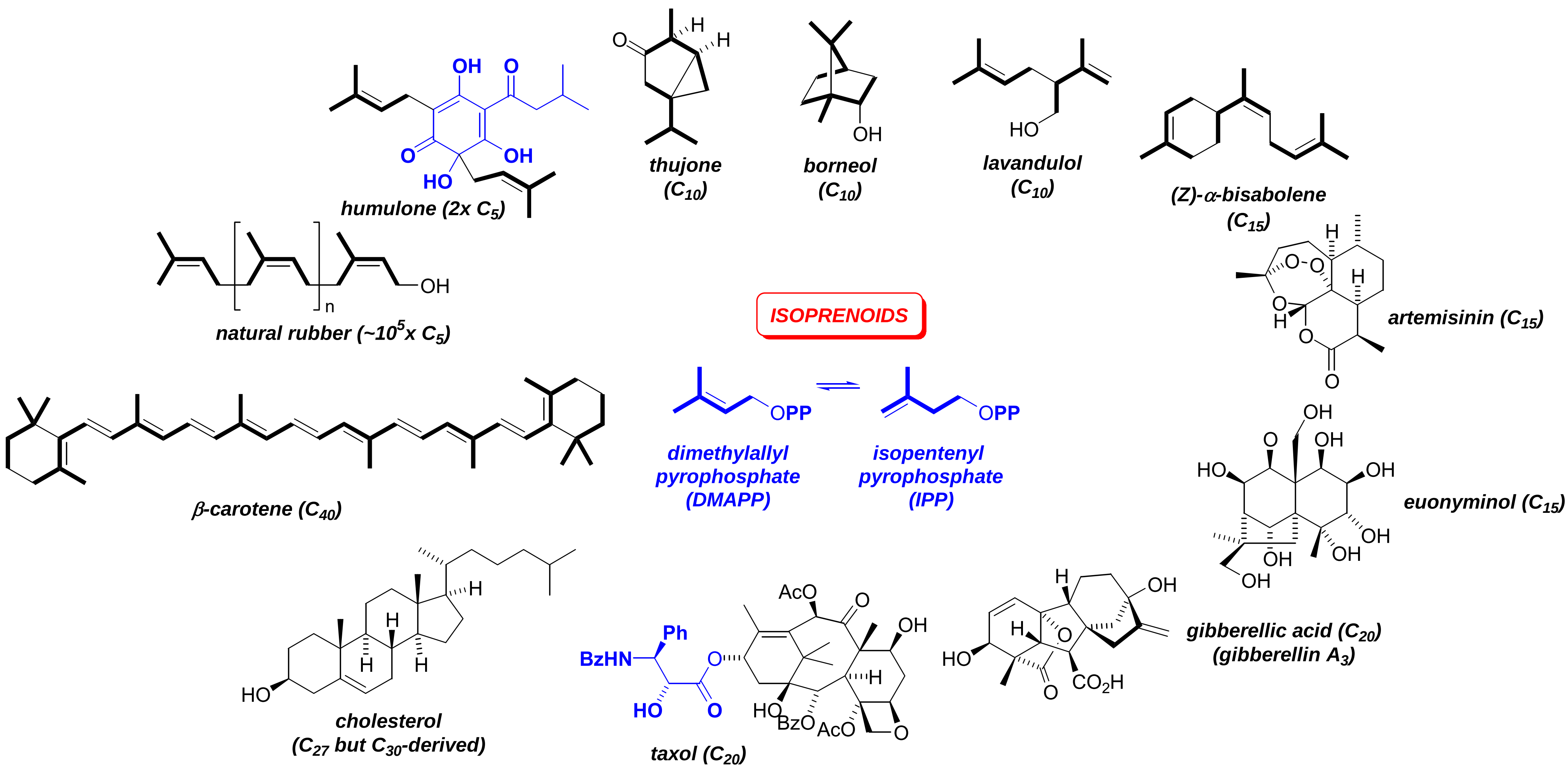


For interesting animations' of *e.g.* photosynthesis see: **http://www.johnkyrk.com/index.html**

Isoprenoids

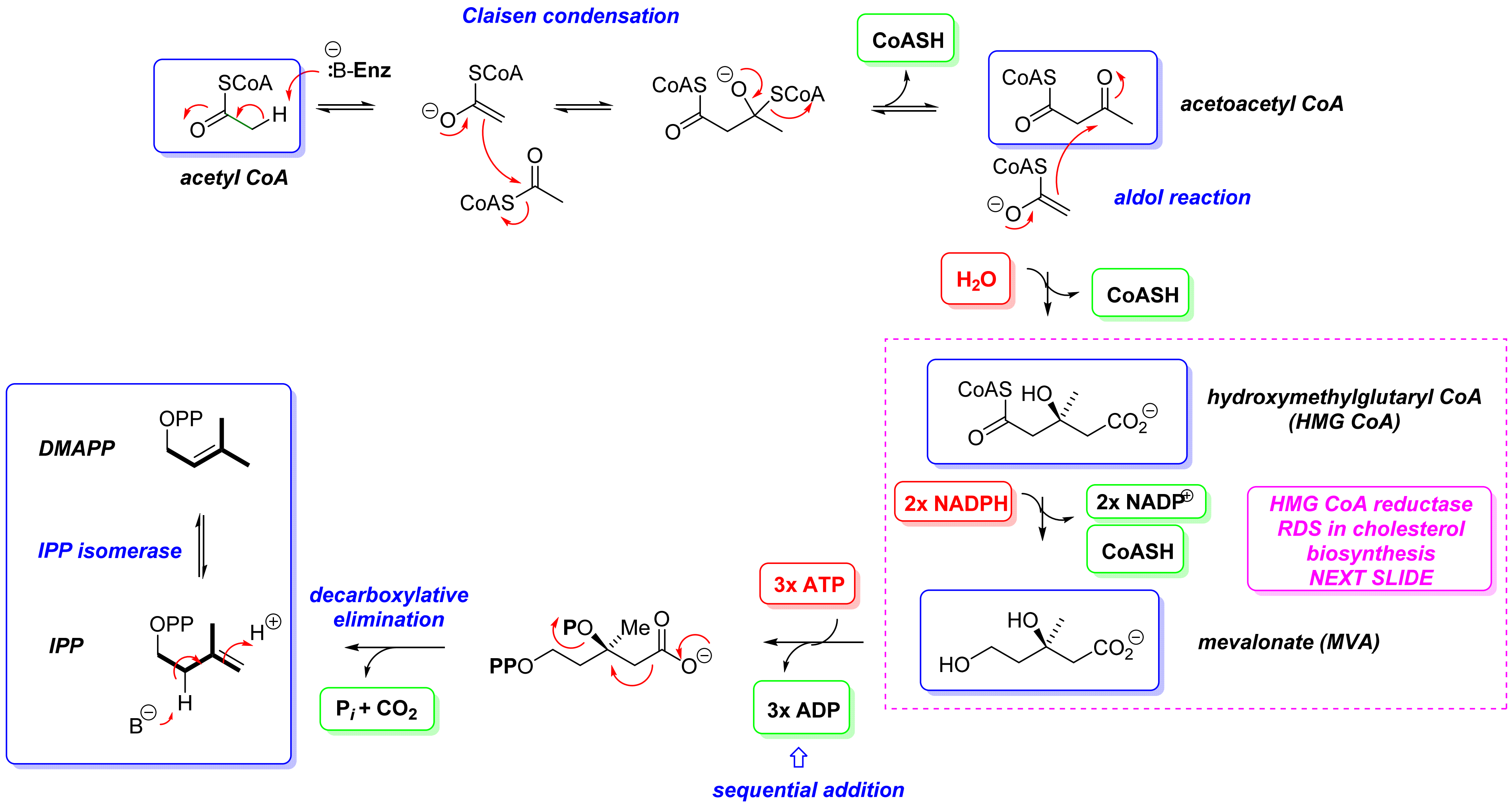
Isoprenoids

- ***isoprenoids*** are widely distributed in the natural world
  - particularly prevalent in plants and least common in insects; >30,000 known
  - composed of integral numbers of C_5 'isoprene' units:
    - ***mono***terpenes (C_{10}); ***sesqui***terpenes (C_{15}); ***di***terpenes (C_{20}); ***ses***terpenes (C_{25}, *rare*); ***tri***terpenes (C_{30}); ***carotenoids*** (C_{40})



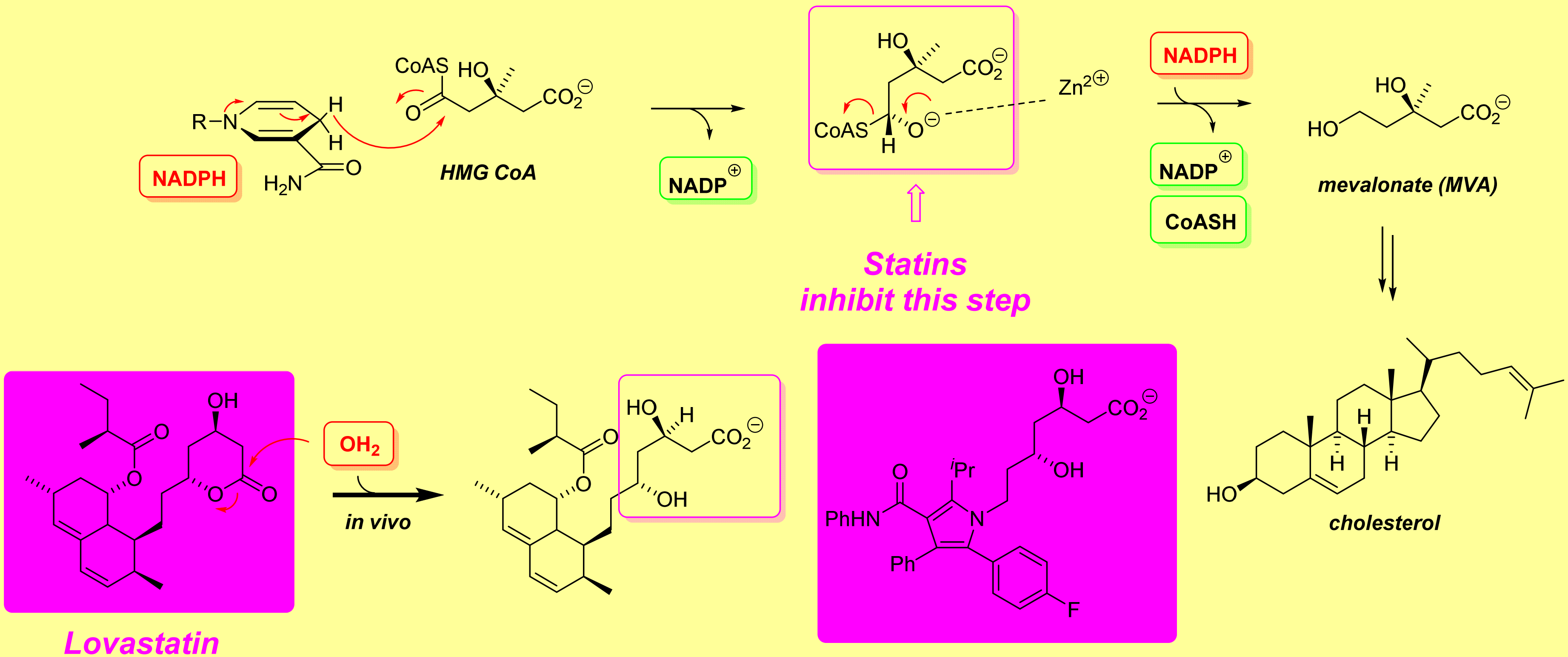
Biosynthesis of IPP & DMAPP - *via Mevalonate*

- ***IPP*** & ***DMAPP*** are the key ***C_5 precursors*** to ***all isoprenoids***
  - the ***main pathway*** is *via*: ***acetyl CoA → acetoacetyl CoA → HMG CoA → mevalonate → IPP → DMAPP:***



Rational Anti-cholesterol Development - *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***, *see:* Wu *et al. Tetrahedron* **2015**, *71*, 8487 (**DOI**)
  - *e.g.* ***mevinolin*** (=lovastatin®, Merck) from *Aspergillus terreus is a* competitive inhibitior of HMG-CoA reductase
  - *e.g.* ***lipitor*** (Atorvastatin calcium, Pfizer) is also a competitive inhibitior of HMG-CoA reductase and the worlds biggest selling drug [first drug to reach $10 billion sales (2004: $10.8 bn]



Lovastatin

PRO-DRUG

NB. type I (iterative) PKS natural product

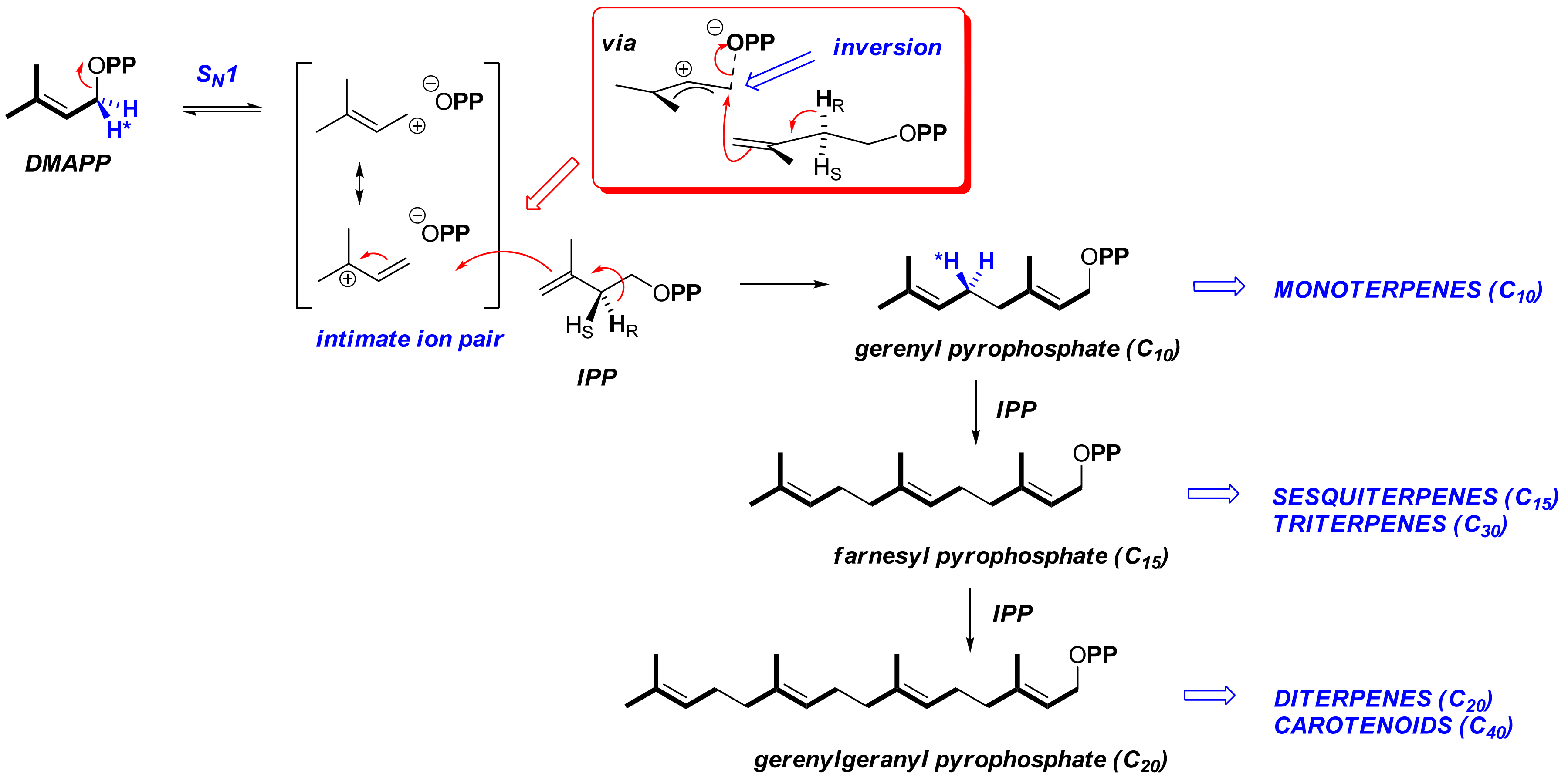
ACTIVE DRUG

mimic of tetrahedral intermediate in HMG reduction by NADPH

Lipitor

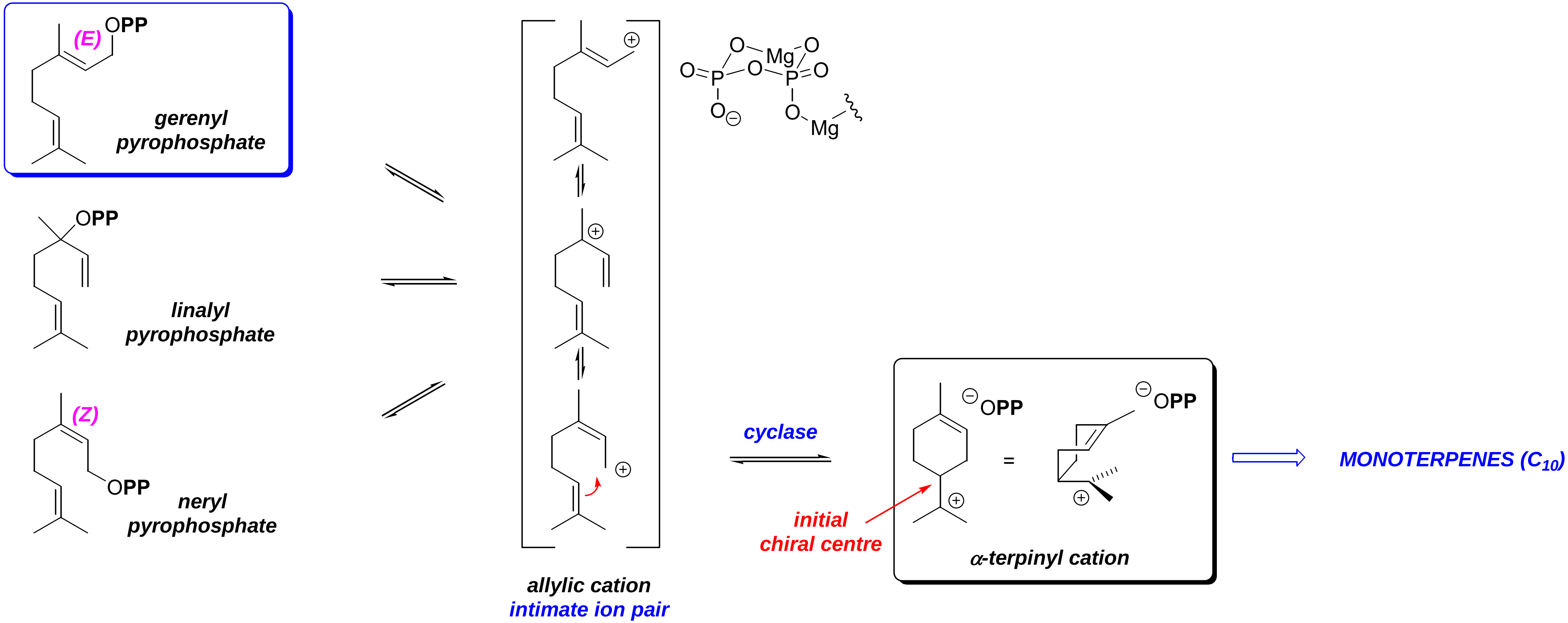
Linear C_{5n} 'head-to-tail' Pyrophosphates

- head-to-tail ***C_5 oligomers*** are the key precursors to isoprenoids
  - ***geranyl*** pyrophosphate (C_{10}) is formed by ***S_N1 alkylation*** of ***DMAPP*** by ***IPP*** → ***monoterpenes***
  - ***farnesyl*** (C_{15}) & ***geranylgeranyl*** (C_{20}) pyrophosphates are formed by ***further S_N1 alkylations*** with ***IPP***:



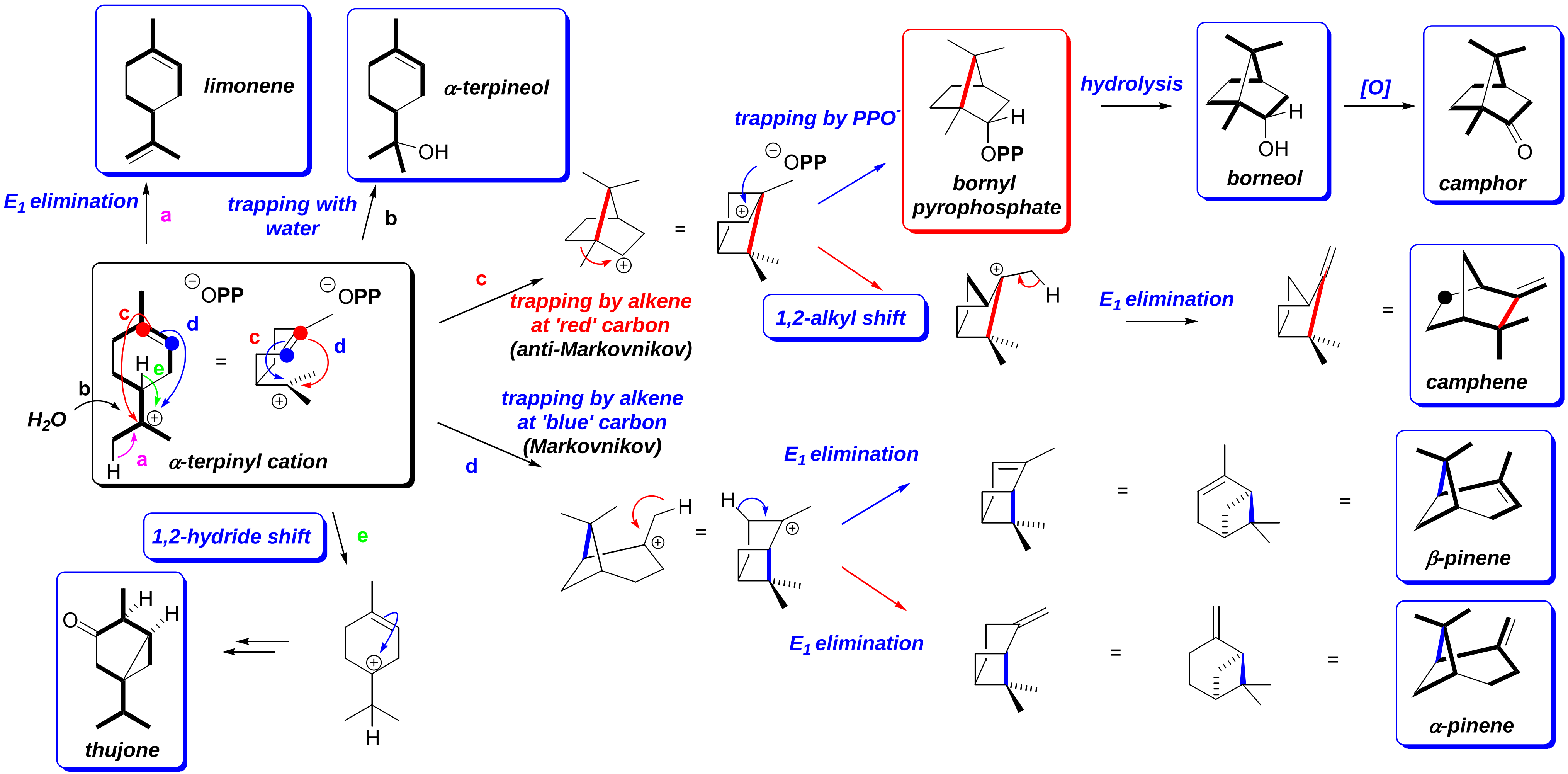
Monoterpenes – α-*Terpinyl Cation Formation*

- ***geranyl*** pyrophosphate isomerises readily via an allylic cation to ***linalyl*** & ***neryl*** pyrophosphates
  - the leaving group abilty of pyrophosphate is enhanced by coordination to Mg^{2+} ions
  - all three pyrophosphates are substrates for ***cyclases*** *via* an ***α-terpinyl cation***:



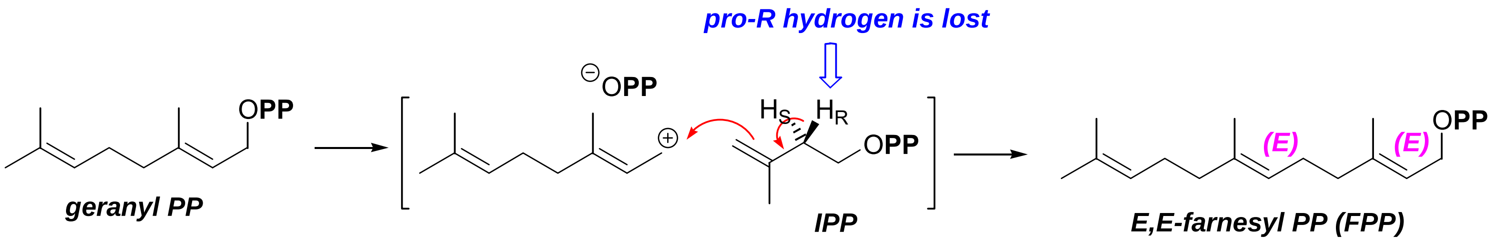
Monoterpenes – *Fate of the α-Terpinyl Cation*

- The ***α-terpinyl cation*** undergoes a rich variety of further chemistry to give a diverse array of ***monoterpenes***
- Some important enzyme catalysed pathways are shown below
  - – *NB. intervention of **Wagner-Meerwein 1,2-hydride-** & **1,2-alkyl shifts***

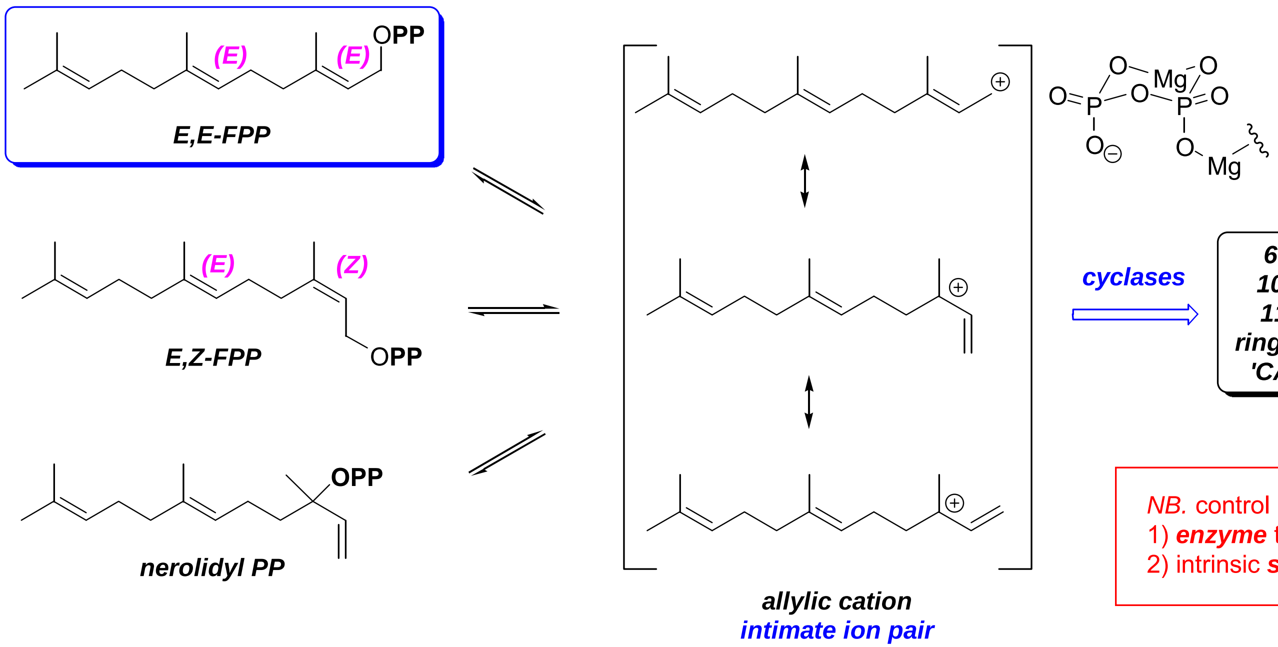


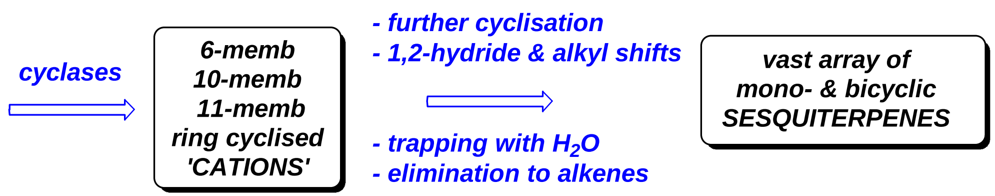
Sesquiterpenes – *Farnesyl Pyrophosphate (FPP)*

- ***'S_N2'-like alkylation*** of ***geranyl PP*** by ***IPP*** gives ***farnesyl PP***:



- just as ***geranyl PP*** readily isomerises to neryl & linaly PPs so ***farnesyl PP*** readily isomerises to equivalent compounds – allowing many modes of cyclisation & bicyclisation

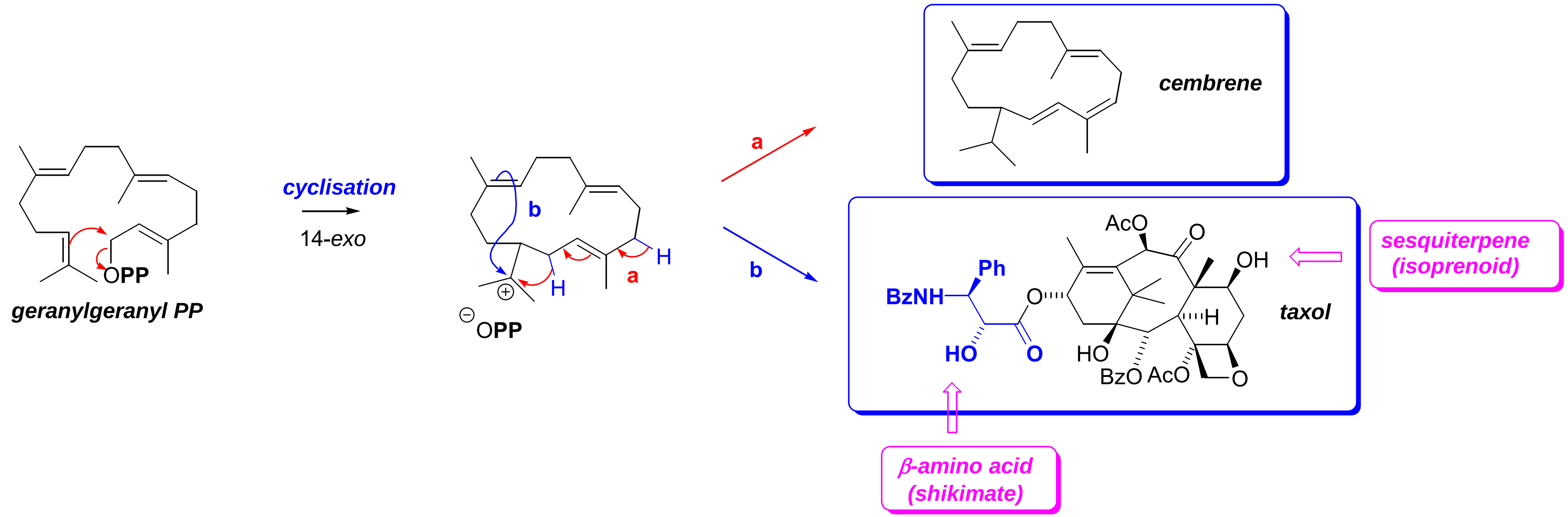




NB. control by:
1) ***enzyme*** to enforce conformation & sequestration of reactive intermediates
2) intrinsic ***stereoelectronic***s of participating orbitals

Diterpenes – *Geranylgeranyl PP* → *Taxol*

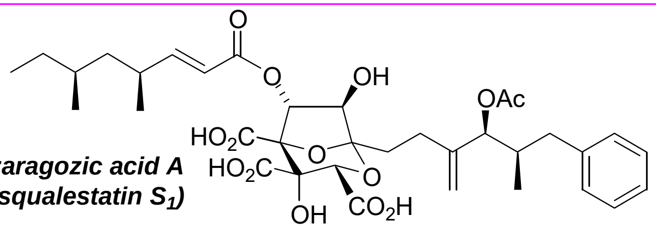
- ***Taxol*** is a potent ***anti-cancer agent*** used in the treatment of ***breast*** & ***ovarian cancers***
  - comes from the bark of the ***pacific yew*** (*Taxus brevifolia*)
  - binds to tubulin and intereferes with the assembly of microtubules
- biosynthesis is from ***geranylgeranyl PP:***



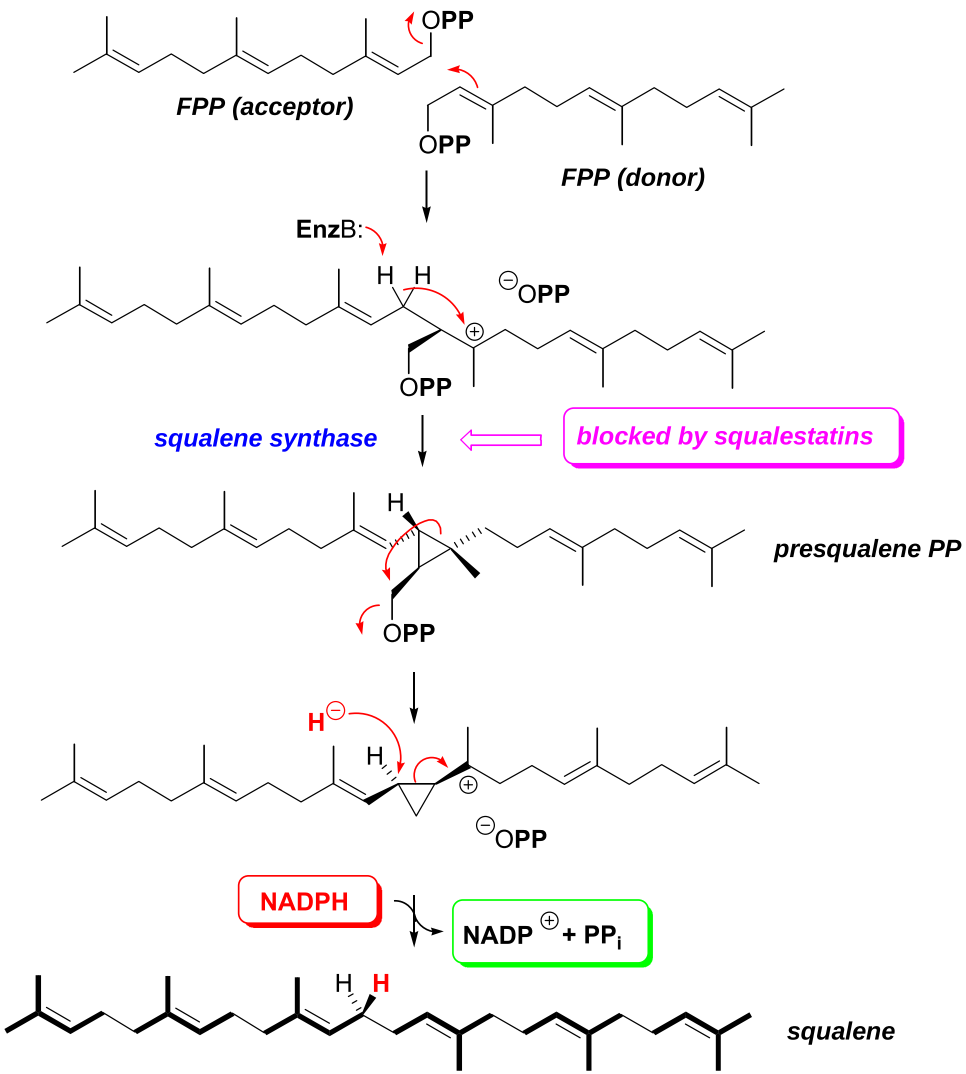
- for details see: http://www.chem.qmul.ac.uk/iubmb/enzyme/reaction/terp/taxadiene.html
- home page is: http://www.chem.qmul.ac.uk/iubmb/enzyme/
  - recommendations of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology on the Nomenclature and Classification of Enzyme-Catalysed Reactions
  - based at Department of Chemistry, Queen Mary University of London

Triterpenes – *FPP* → *Squalene*

- ***triterpenes*** (C_{30}) arise from the ***'head to head' coupling*** of ***two fanesyl PP units*** to give ***squalene*** catalysed by ***squalene synthase:***
  - squalene was first identified as a steroid precursor from ***shark liver oil***
  - the dimerisation proceeds *via* an unusual mechanism involving electrophilic cyclopropane formation - rearrangement to a tertiary cyclopropylmethyl cation and reductive cyclopropane ring-opening by NADPH (*NB.* exact mechanism disputed)
  - ***Zaragozic acids*** (***squalestatins***) mimic a rearrangement intermediate and inhibit squalene synthase. They constitute interesting leads for development of new treatments for ***hypercholesteraemia*** & ***heart disease*** (*cf.* statins)

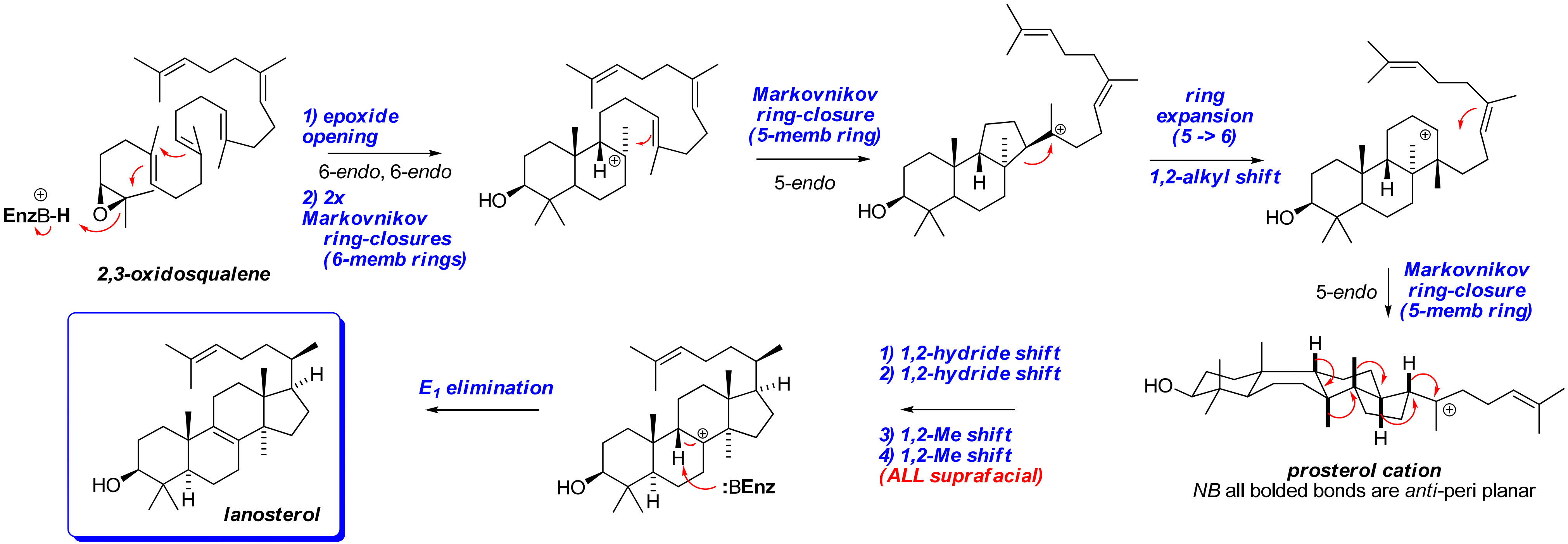


zaragozic acid A (squalestatin S_1)



Oxidosqualene-Lanosterol Cyclase – *Mechanism*

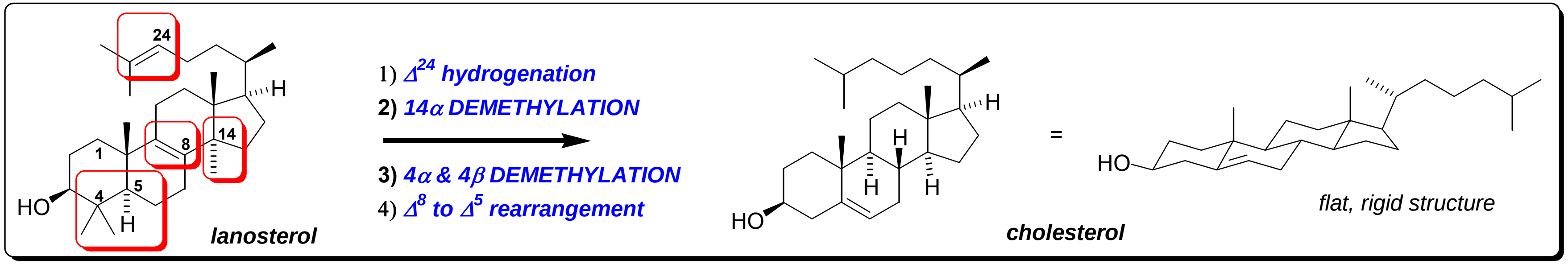
- ***oxidosqualene-lanosterol cyclase*** catalyses the formation of ***lanosterol*** from ***2,3-oxidosqualene***:
  - this cascade establishes the characteristic ring system of *ALL* ***steroids***
  - ring-expansion sequence to establish the C ring
  - the process is ***NOT concerted***, discrete ***cationic intermediates*** are involved ***& stereoelectronics dictate*** the ***regio- & stereoselectivity*** although the enzyme undoubtedly lays a role in pre-organising the ~chair-boat-chair conformation

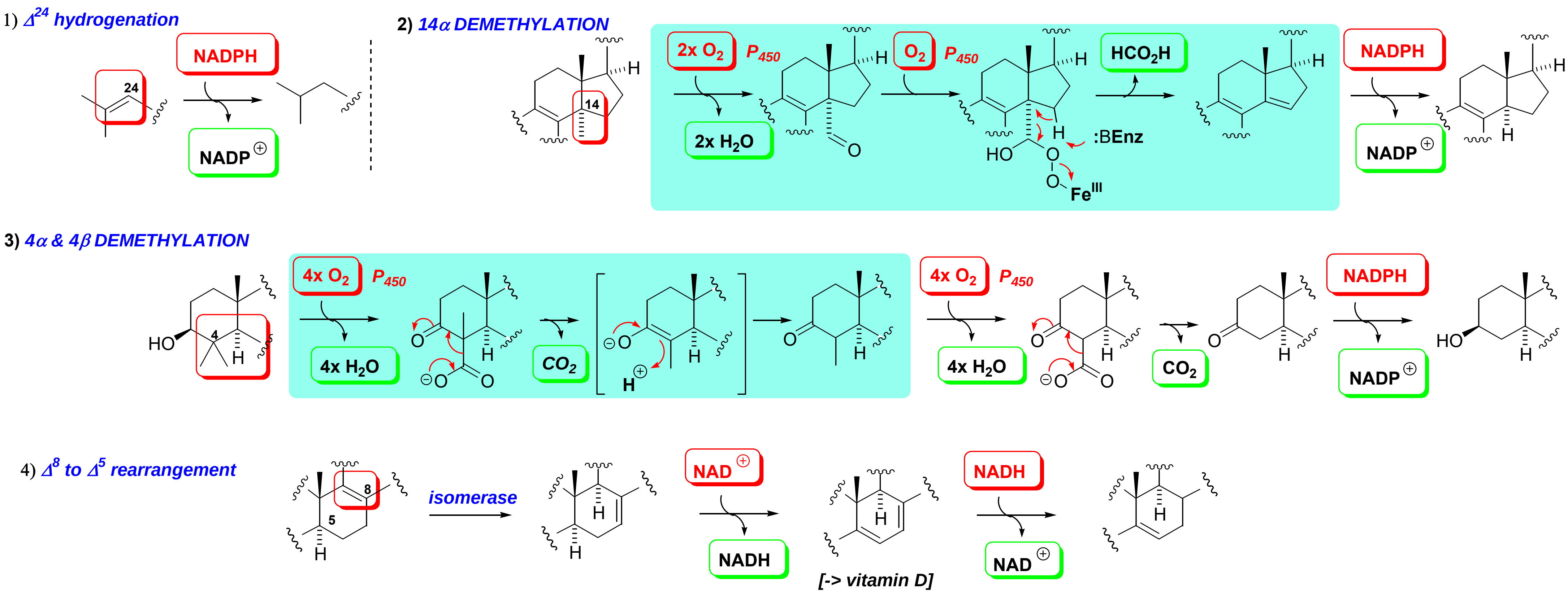


  - "*The enzyme's role is most likely to shield intermediate carbocations… thereby allowing the hydride and methyl group migrations to proceed down a thermodynamically favorable and kinetically facile cascade*"
    - Wendt *et al. Angew. Chem. Int. Ed.* **2000**, *39*, 2812 (**DOI**) & Wendt *ibid* **2005**, *44*, 3966 (**DOI**)

Lanosterol → Cholesterol – *Oxidative Demethylation*

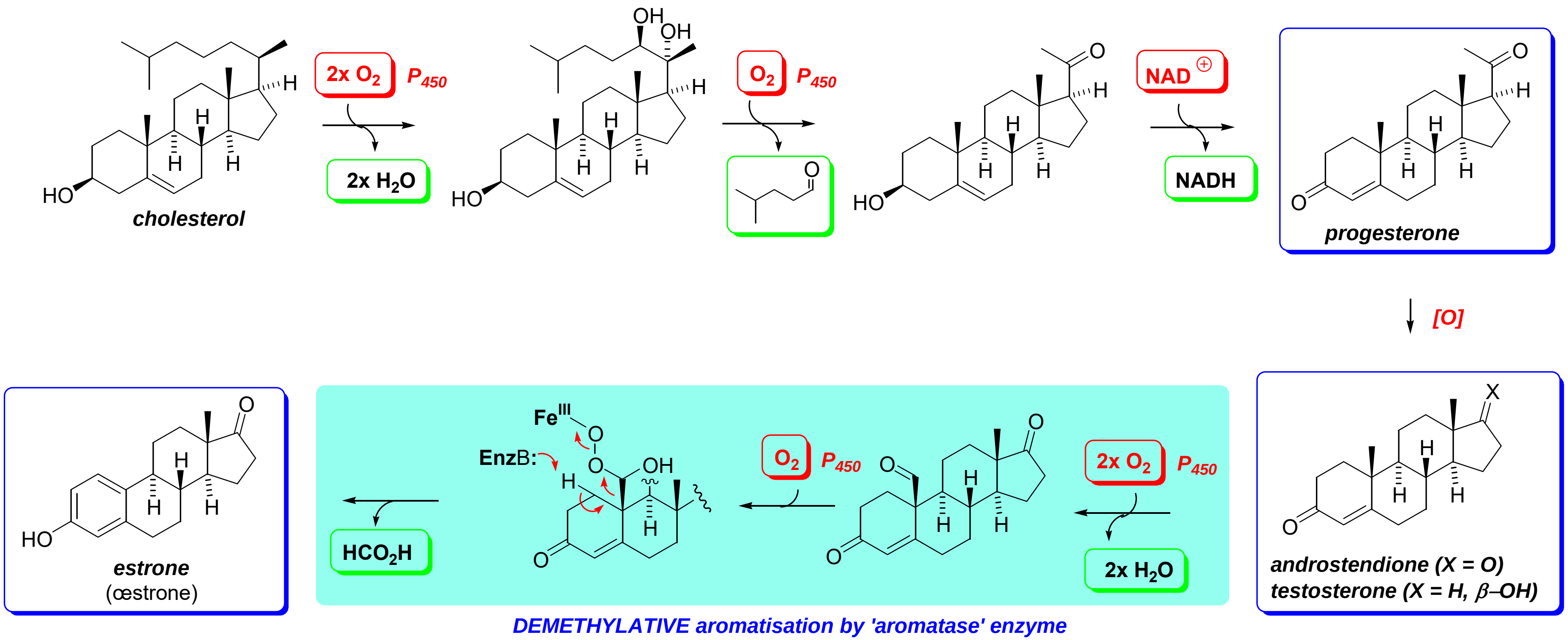
- Several steps are required for conversion of ***lanosterol*** to ***cholesterol:***





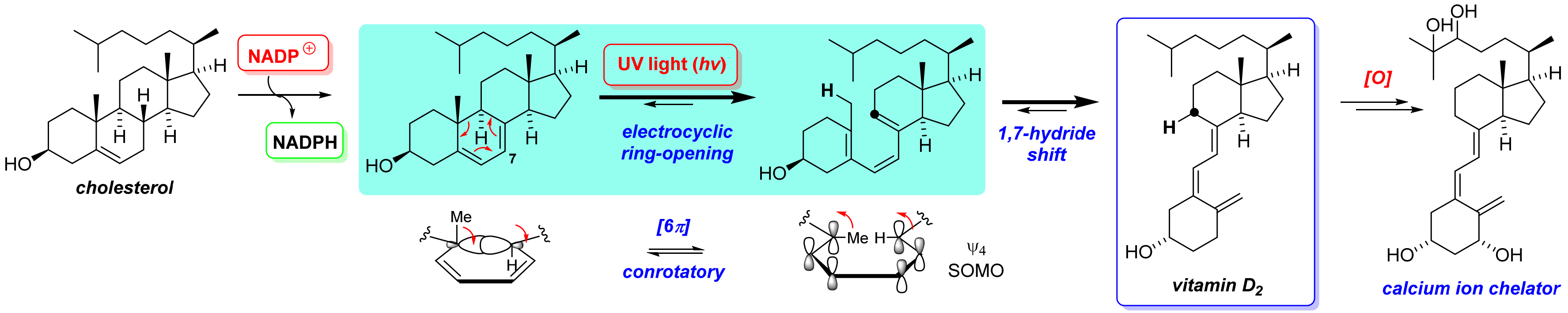
Cholesterol → Human Sex Hormones

- ***cholesterol*** is the precursor to the human sex hormones – ***progesterone, testosterone*** & ***estrone***
  – the pathway is characterised by ***extensive oxidative processing*** by ***P_{450} enzymes***
  – ***estrone*** is produced from ***androstendione*** by ***oxidative demethylation*** with ***concomitant aromatisation:***



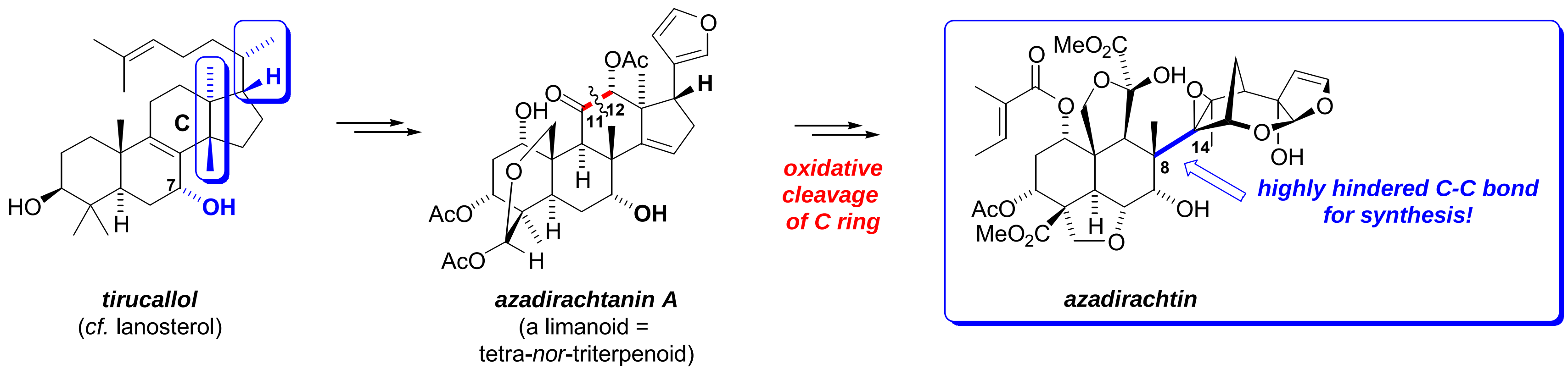
Steroid Ring Cleavage - *Vitamin D & Azadirachtin*

- ***vitamin D_2*** is biosynthesised by the ***photolytic cleavage*** of ***Δ^7-dehydrocholesterol*** by UV light:
  - a classic example of ***photo-allowed, conrotatory electrocyclic ring-opening:***



  - D vitamins are involved in ***calcium absorption; defficiency*** leads to ***rickets*** (brittle/deformed bones)

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



Summary of Presentation

- ***Metabolism & Biosynthesis***
  - some definitions, 1° & 2° metabolites
- ***Shikimate Metabolites***
  - photosynthesis & glycolysis → shikimate formation → shikimate metabolites
  - *Glyphosate* – a non-selective herbicide
- ***Alkaloids***
  - acetylCoA & the citric acid cycle → α-amino acids → alkaloids
  - *Opioids* – powerful pain killers
- ***Fatty Acids and Polyketides***
  - acetylCoA → malonylCoA → fatty acids, prostaglandins, polyketides, macrolide antibiotics
  - *NSAIDs* – anti-inflammatory's
- ***Isoprenoids/terpenes***
  - acetylCoA → mevalonate → isoprenoids, terpenoids, steroids, carotenoids
  - *Statins* – cholesterol-lowering agents