

Photorhabdus and *Xenorhabdus*: potent producers of natural products

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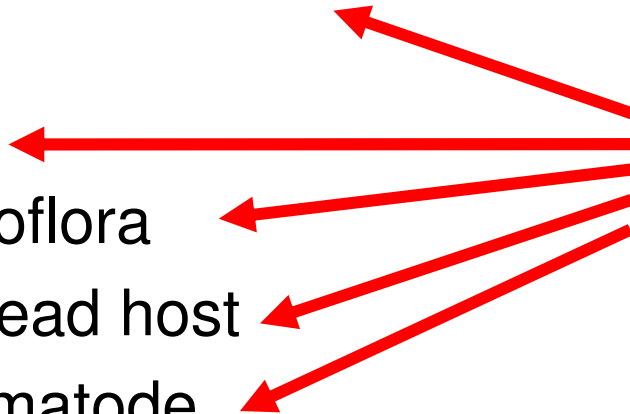
Secondary metabolism research at the Department of Pharmaceutical Biotechnology, Saarland University

Prof. Dr. Rolf Müller/Dr. Helge B. Bode

- secondary metabolites
 - isolation, structure elucidation, biosynthesis, genetics, mode of action
- regulation of secondary metabolism
 - proteomics, metabolomics, transcriptomics
- strain/compound optimization
 - heterologous expression of complete gene clusters
 - combinatorial biosynthesis
- organisms
 - myxobacteria, streptomyces, pseudomonads

Xenorhabdus and Photorhabdus

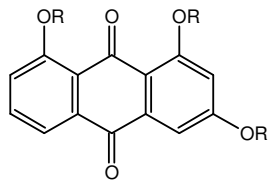
Important events for the bacteria/nematode/host system

- inhibition of the insect immune response (PLA₂ inhibition?)
 - killing of host
 - killing of host microflora
 - protection of the dead host
 - symbiosis with nematode
- secondary metabolites**
- 

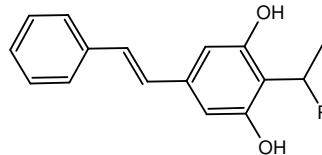
Photorhabdus and *Xenorhabdus* are potent secondary metabolite producers

- 10 different strains have been analyzed by HPLC-MS
- all but one show the production of 1-4 very lipophilic compounds which are produced in high amounts up to 400 mg/L
 - these lipophilic compounds are all very small (MW 200-350)
 - simple chemical structures
- several other compounds are produced by all strains investigated, most of them “new”

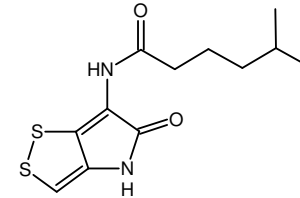
Known compounds show antibacterial, antifungal and cytotoxic activity



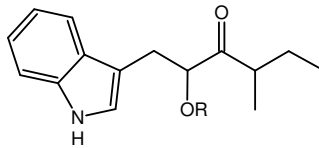
Anthraquinone (R = H oder Me)



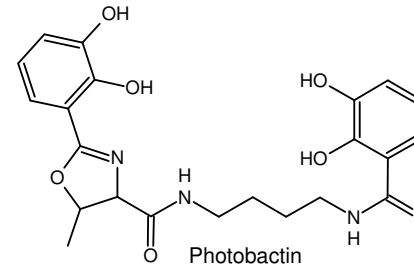
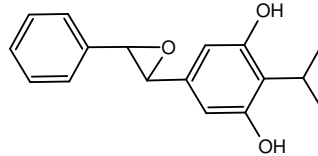
E-Hydroxystilbene R = H, CH₃



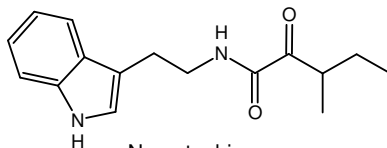
Xenorhabdin (different acyl moieties)



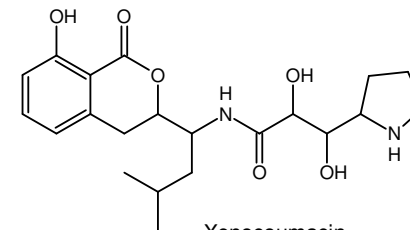
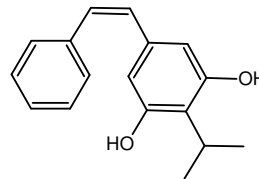
Indol derivatives (R = Ac or H)



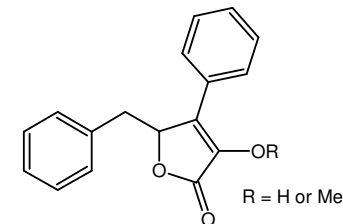
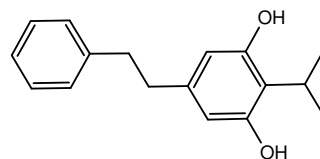
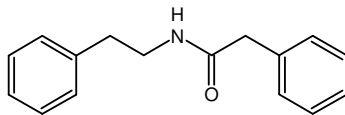
Photobactin



Nematophin

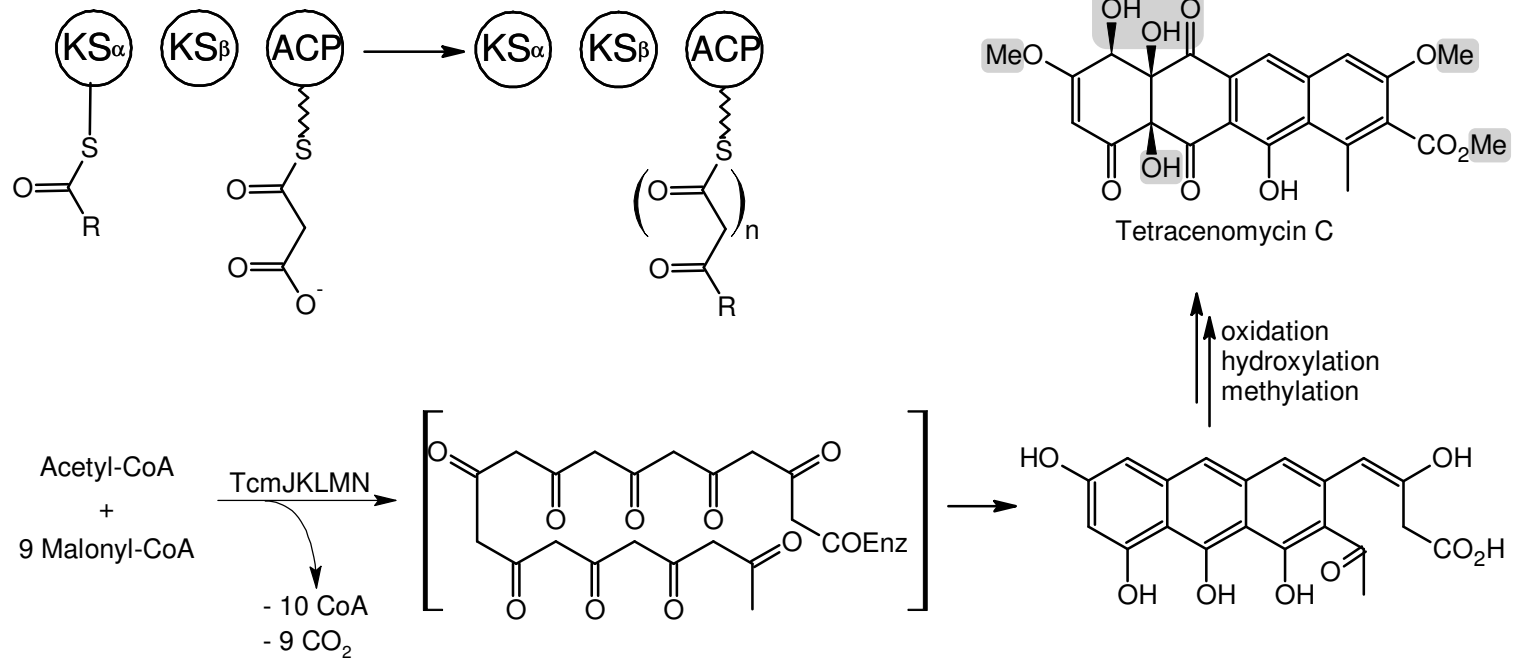


Xenocoumacin

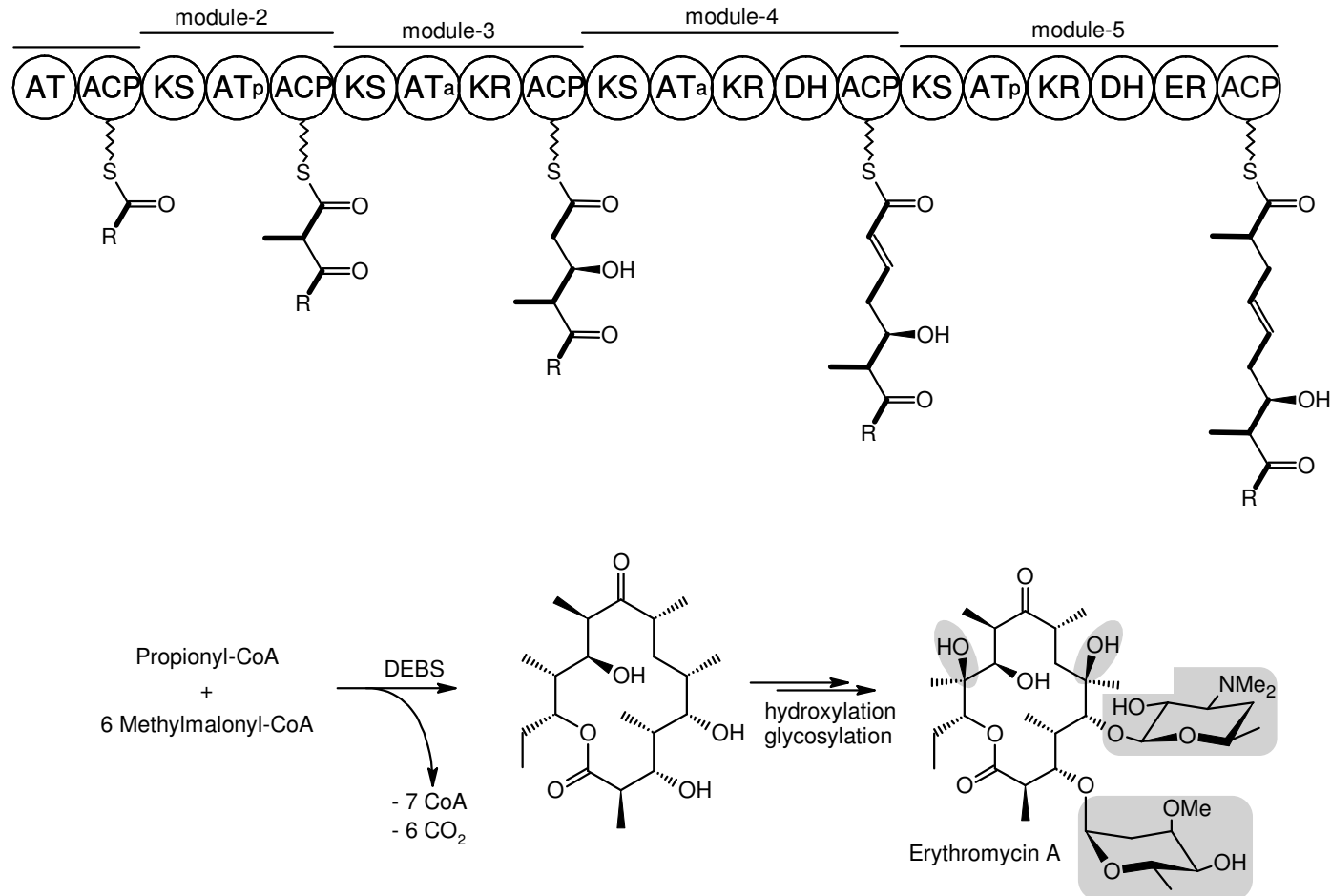


R = H or Me

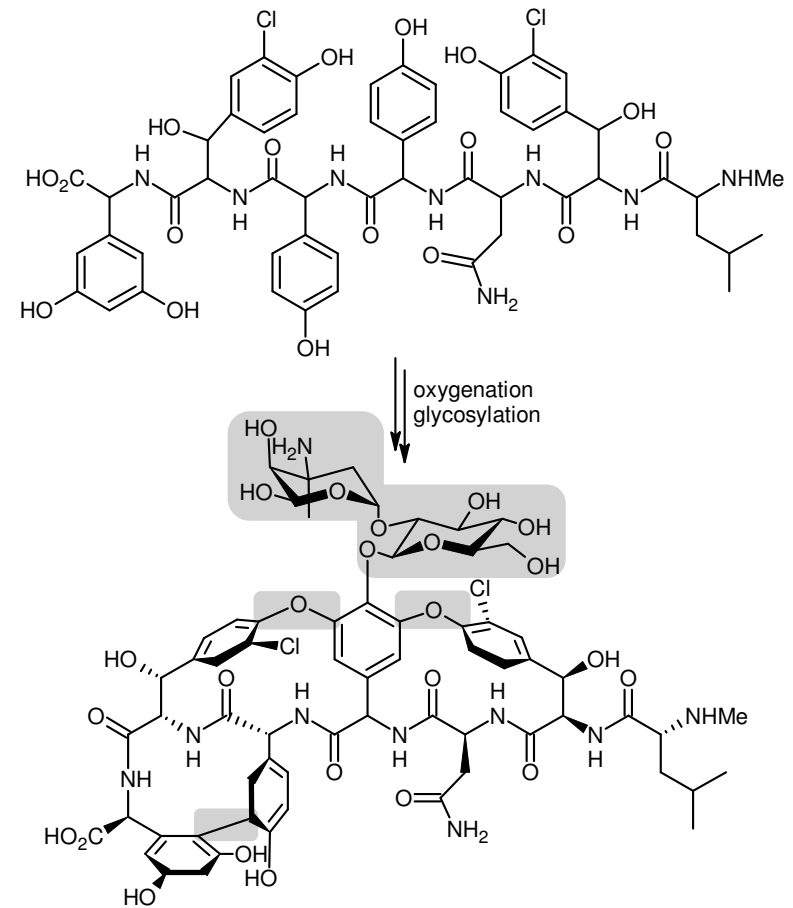
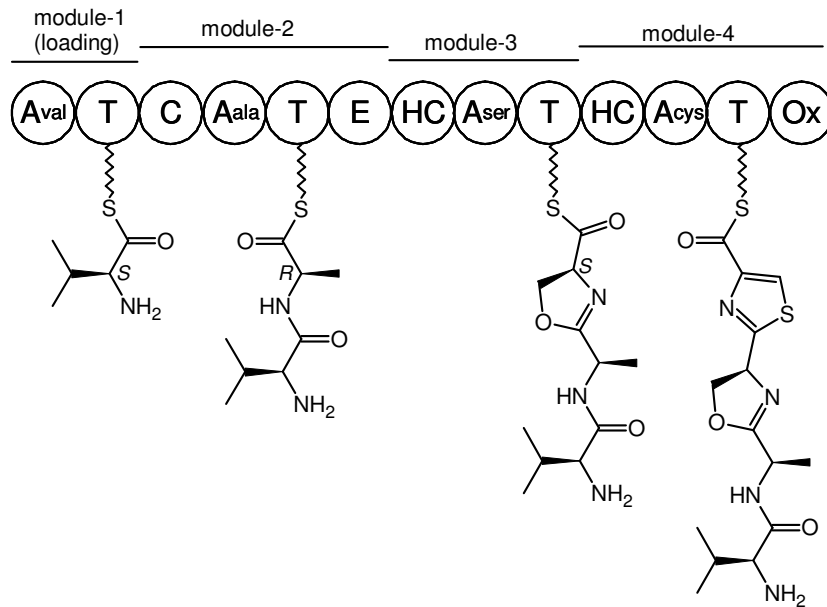
type II polyketide synthase (PKS)



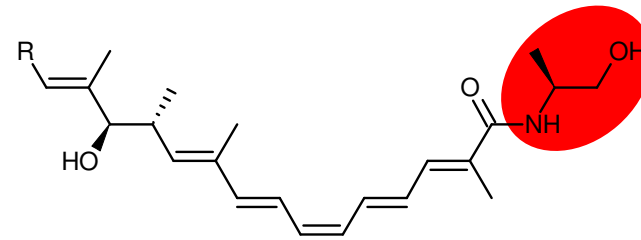
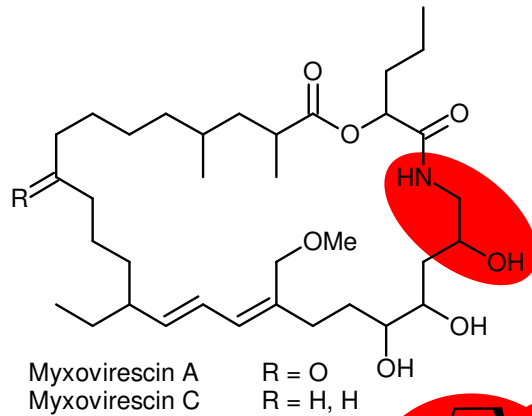
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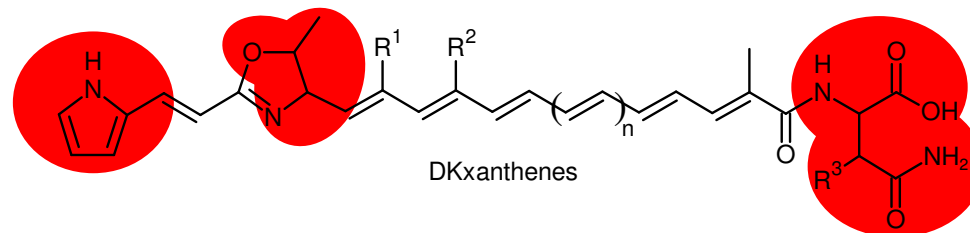
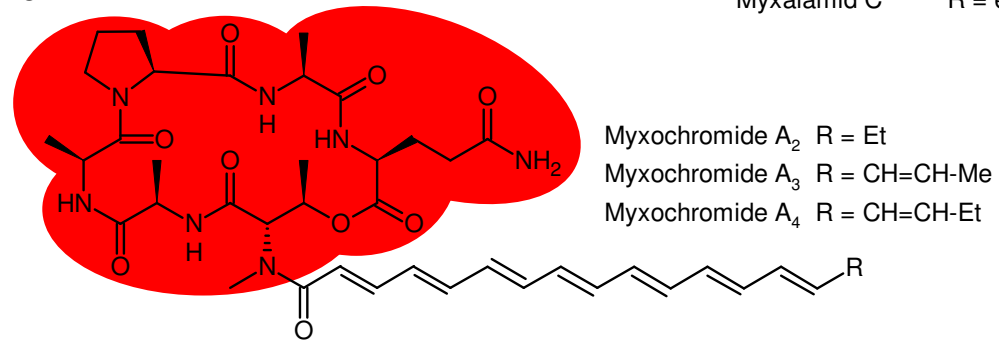
non-ribosomal peptide synthetase (NRPS)



PKS/NRPS hybrids

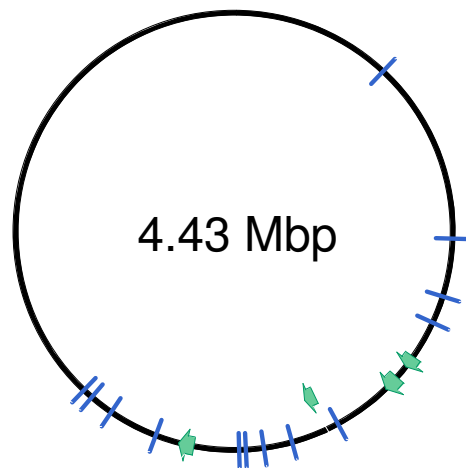


Myxalamid A R = isobutyl
Myxalamid B R = isopropyl
Myxalamid C R = ethyl



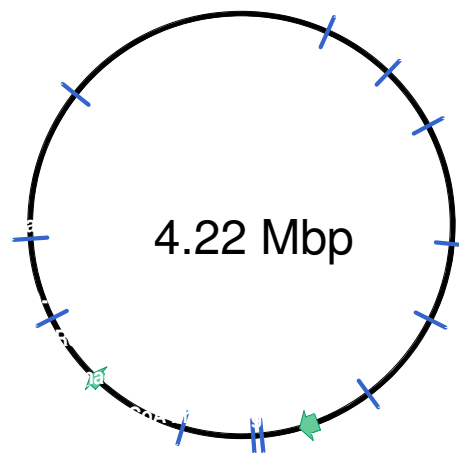
Secondary metabolism in *Xenorhabdus* and *Photorhabdus*

X. nematophila



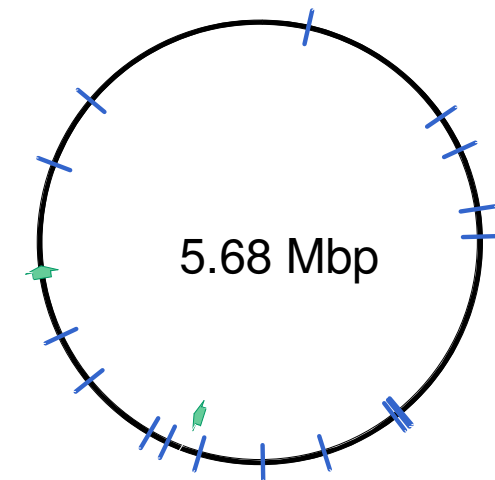
- 7.5% of the genome
- 17 biosynthesis gene cluster detected
 - 13 NRPS
 - 3 PKS/NRPS
 - 1 hydroxamate

X. bovienii



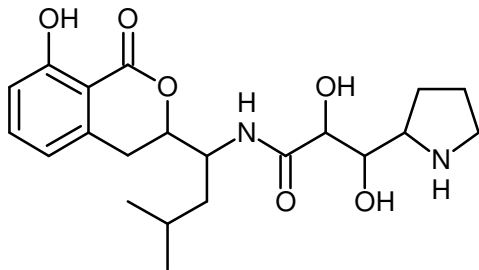
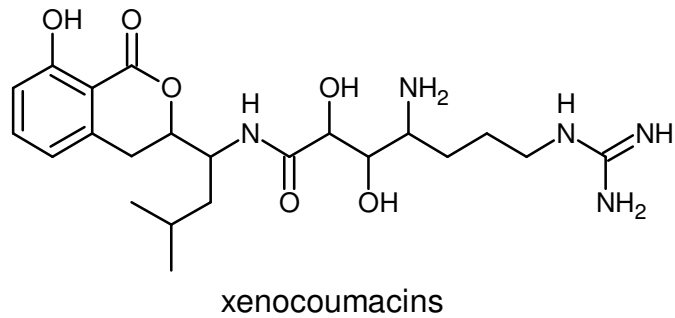
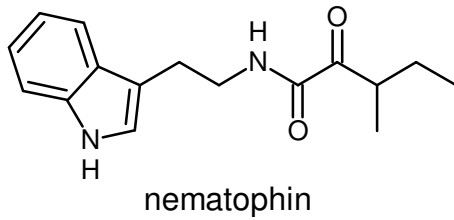
- 6.0% of the genome
- 14 biosynthesis gene cluster detected
 - 9-10 NRPS
 - 1 PKS/NRPS
 - PKS/FAS?
 - 1 hydroxamate

P. luminescence

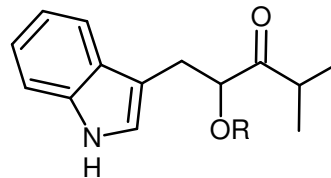
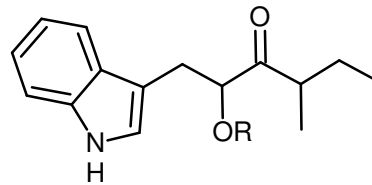


- 5.8% of the genome
- 19 biosynthesis gene cluster detected
 - several NRPS
 - some PKS/NRPS
 - TypII PKS

X. nematophila



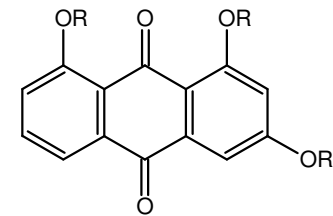
X. bovienii



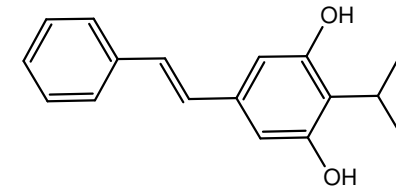
R = Ac (major products)

R = H (minor products)

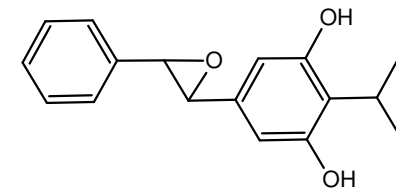
P. luminescence



Anthraquinone (R = H oder Me)



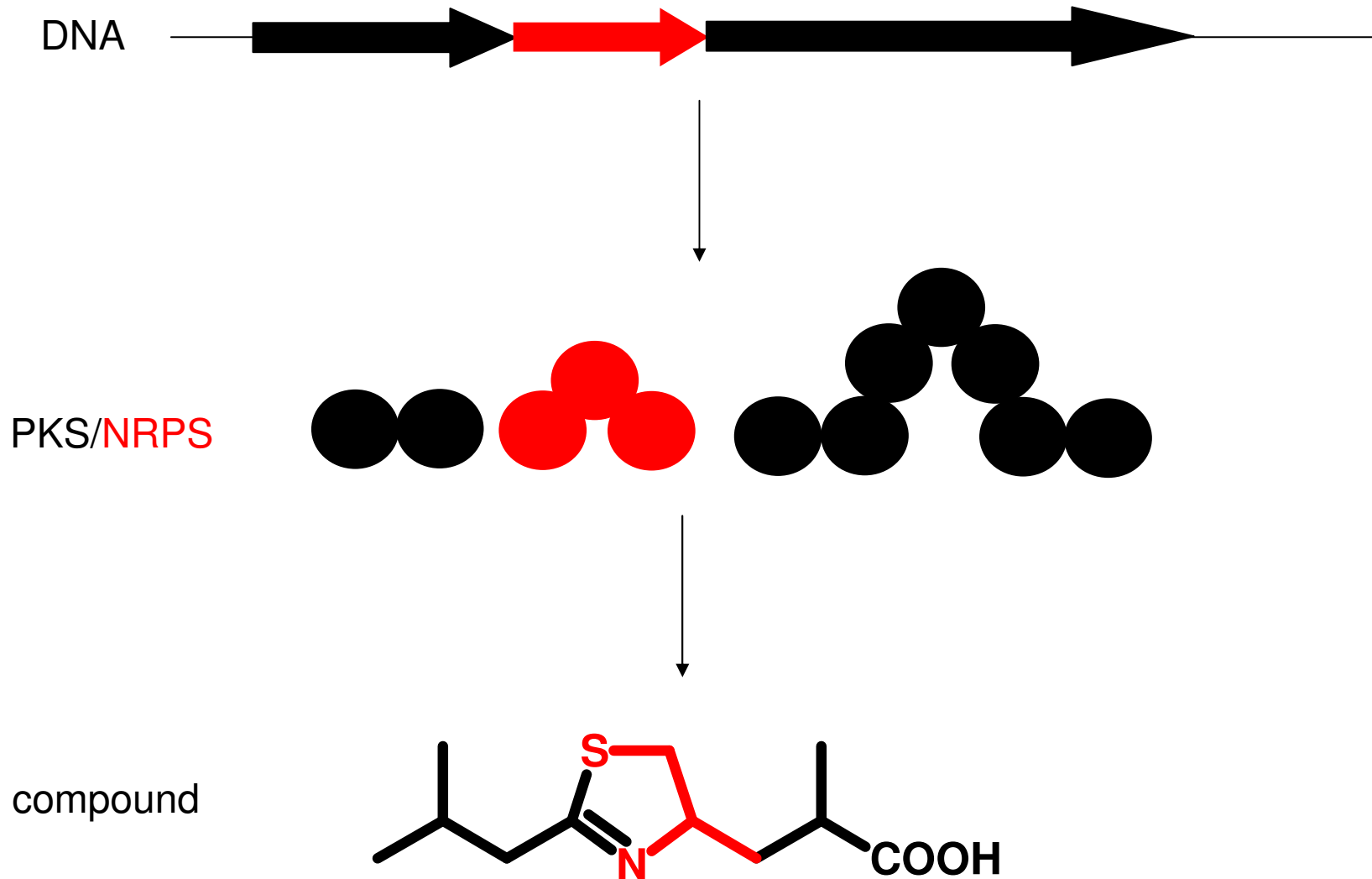
Hydroxystilbene



in silico vs. *in extract* secondary metabolism

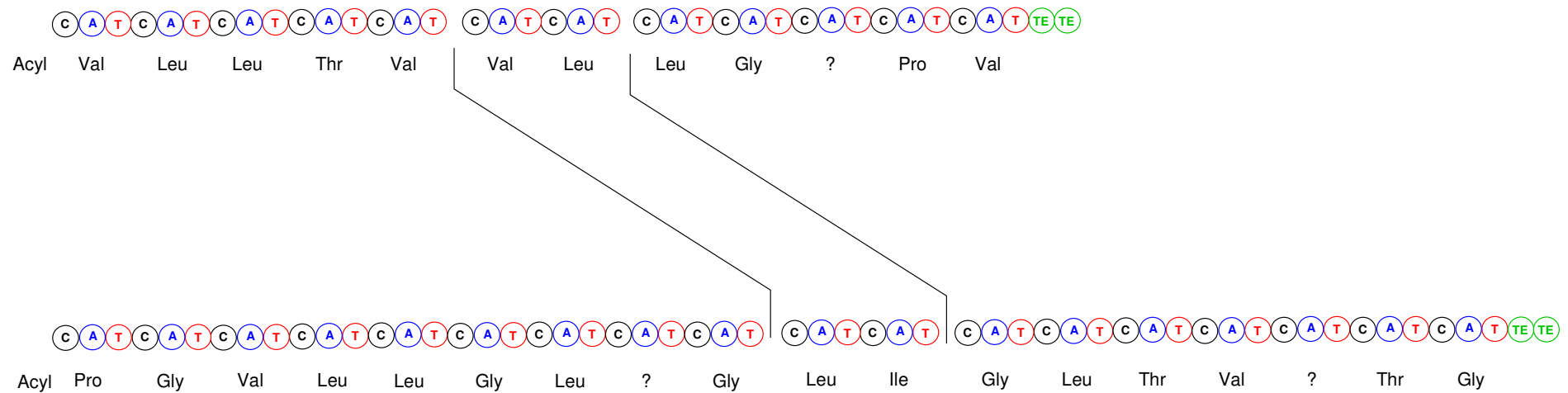
- some compounds are only produced under specific conditions
 - some gene clusters might be inactive
 - some compounds are unstable or difficult to isolate
 - some compounds are only produced in trace amounts
-
- no cluster can be correlated to the compound because the biosynthesis is unknown yet

“*in silico*” secondary metabolism



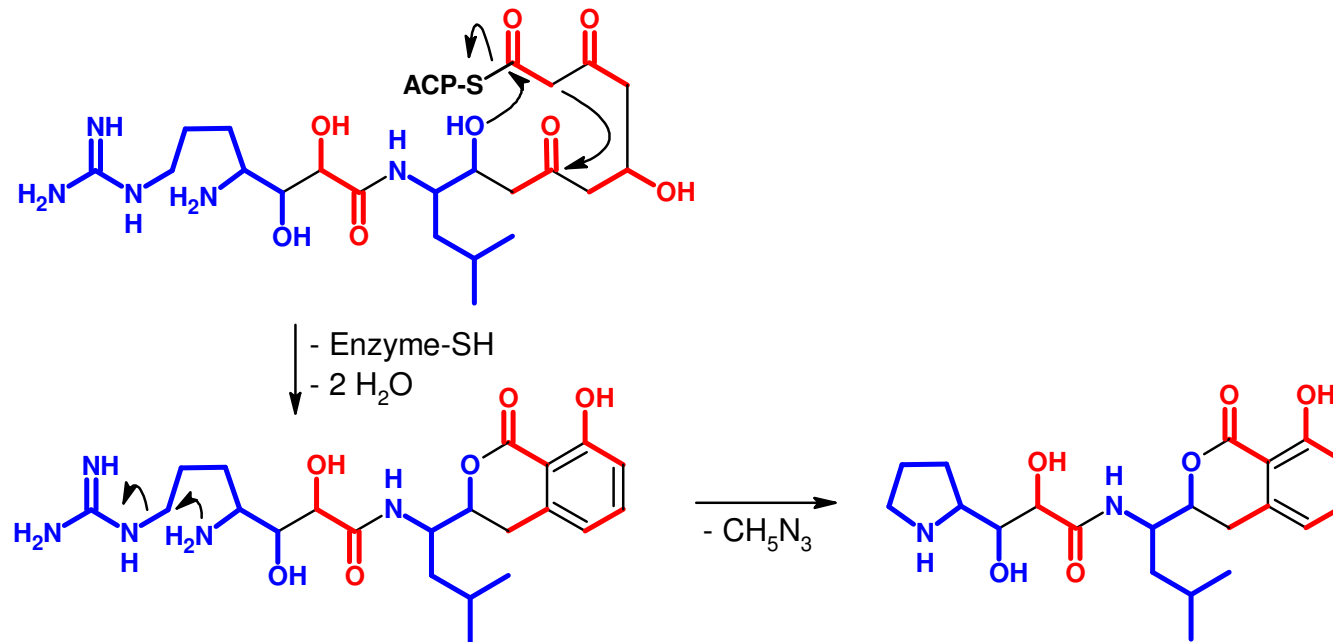
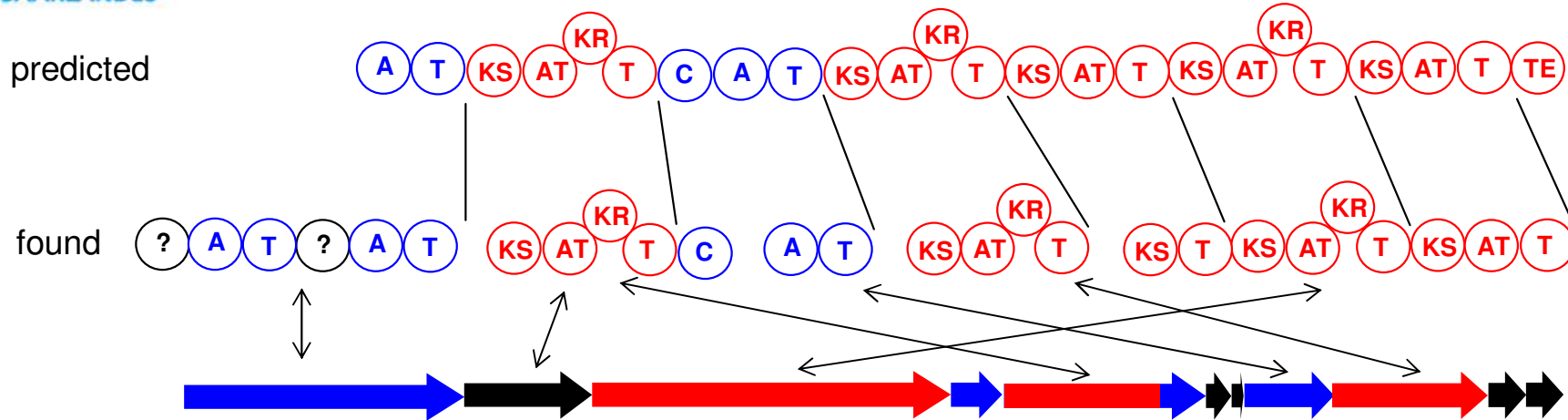
more lipophilic compounds...

X. nematophila: 43 kb @ 1.86 Mb (12 aa)



X. bovienii: 60 kb @ 1.86 Mb (18 aa)

possible xenocoumacin biosynthesis



Summary

- *Photorhabdus* and *Xenorhabdus* are very potent producers of secondary metabolites
- almost all compounds show biological activity
- all strains seem to be multiproducers of secondary metabolites also reflected by their genome sequence
- some lipophilic compounds are always produced in high amounts
- the biosynthesis of some compounds does not follow standard PKS/NRPS mechanisms



Alexander Brachmann



Gertrud Schwär



Michael Ring

Thanks to...

Barry Goldman
and the *Xenorhabdus*
genome consortium
(USDA-NSF for funding)



Rolf Müller
and all
members of
the
department



Deutsche
Forschungsgemeinschaft

DFG

Emmy
Noether-
Programm

Deutsche
Forschungsgemeinschaft

DFG

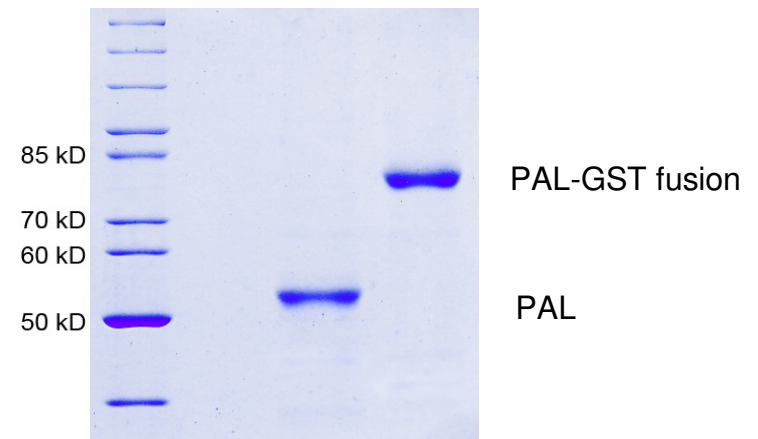
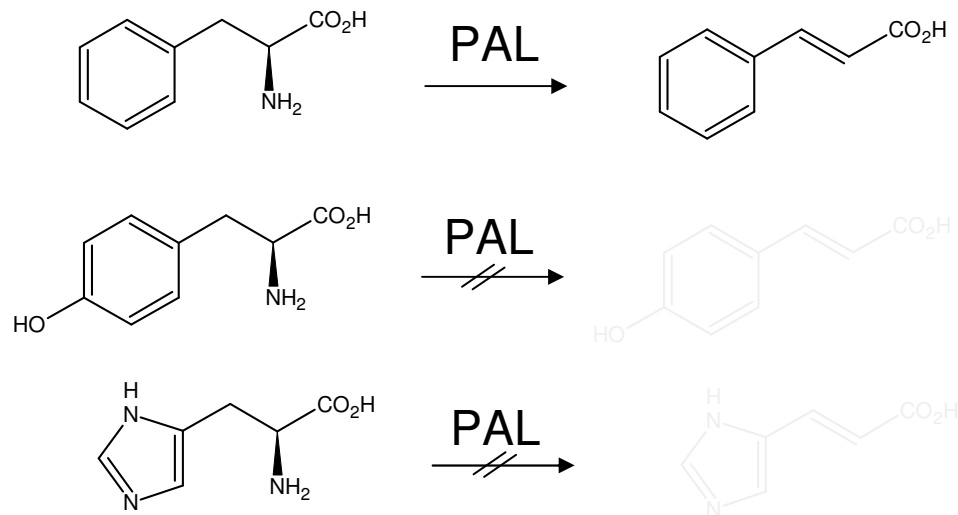




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Bacterial stilbene biosynthesis

- overexpression of the phenylalanine ammonium lyase (PAL) in *E. coli* confirmed the enzymatic activity and specificity



Why should we look for natural products...



news feature

A shot in the arm

Antibiotics are failing and drug companies have all but stopped developing new ones. Will conquered diseases come back to haunt us? Martin Leeb examines one plan to avert the crisis.

Drug comp as pipeline

antibiotics o run dry

Multi-Kliniken er mehr an

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Karen M

aus nicht

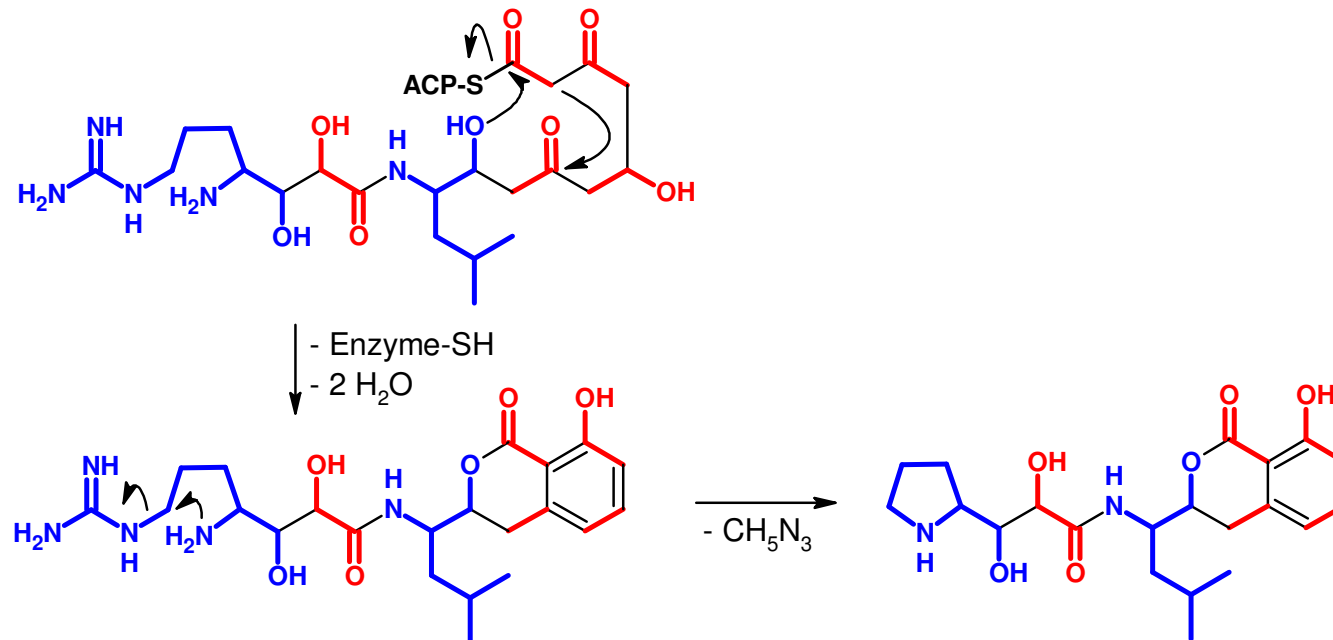
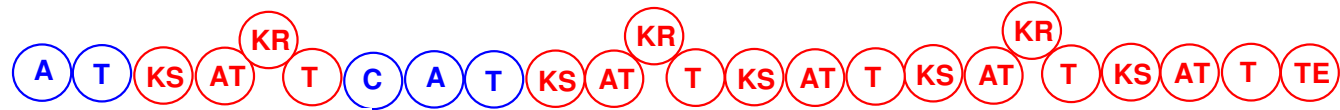
moren

Staphylokokken im menschlichen Blut (stark vergrößert). Jeder zweite trägt diese normalerweise harmlosen Bakterien in der Nase oder im Blut. Grippe-Patienten...
entweder bei geschlagartig Lunge zerstört. Staphylococcus aureus.

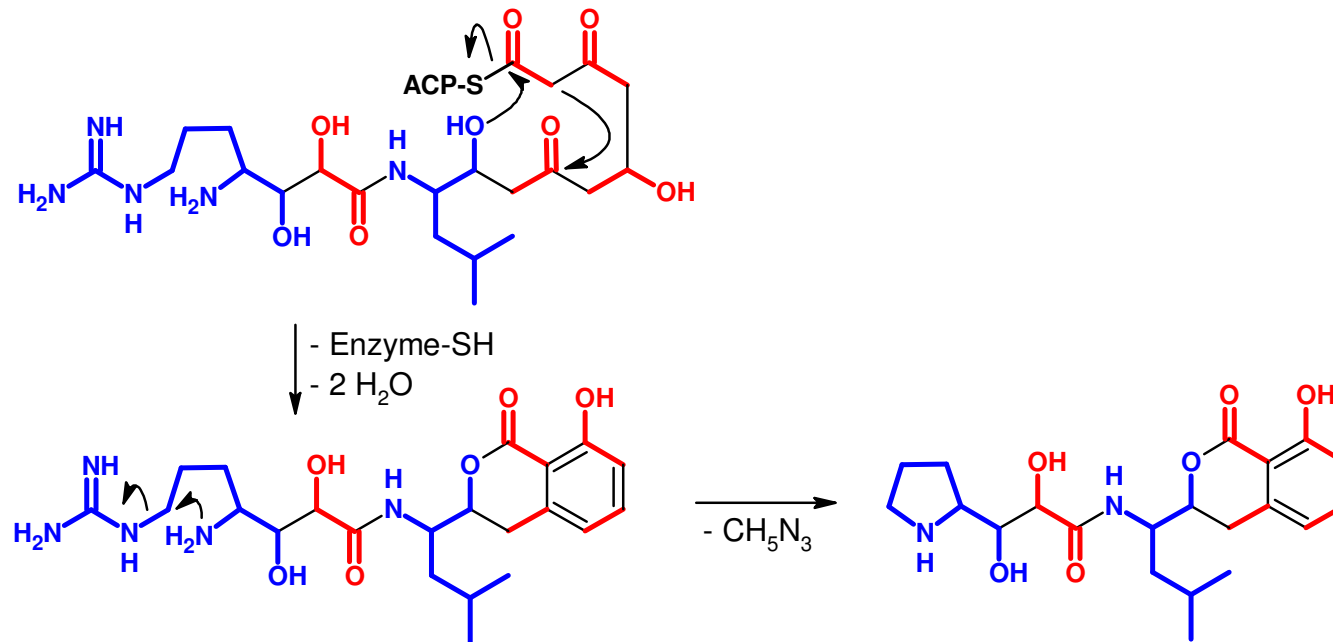
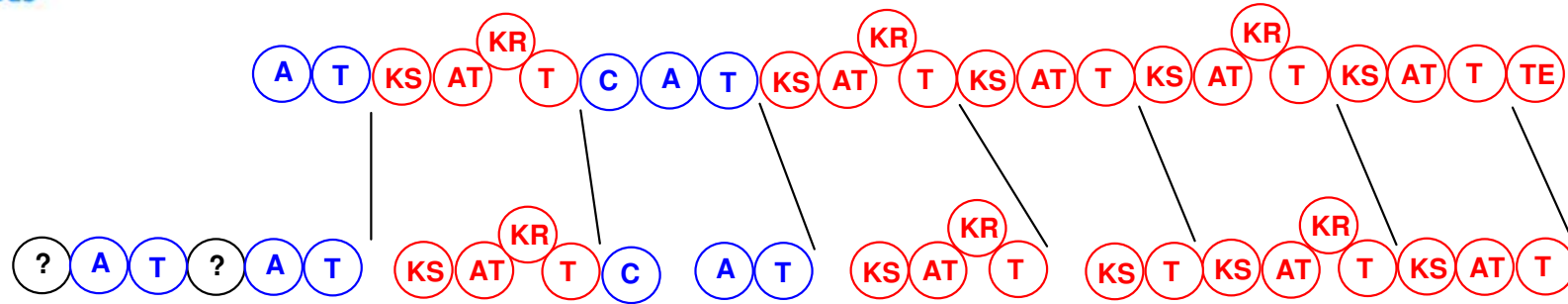
starkes Antibiotikum konnte helfen... Ursache war ein harmloser Erreger, den jeder zweite Mensch am...
 Jetzt breitet sich dieser „multiresistente“ Erreger, der bisher nur in Kliniken beobachtet wurde, weltweit aus. Alexander Tomasz, Mikrobiologe der New Yorker Rockefeller-Universität: „Wir stehen an der Grenze zu einem medizinischen Desaster!“
 Wie die Ärzte die Kontrolle verloren – Seite 9.

DISCOVERY TODAY

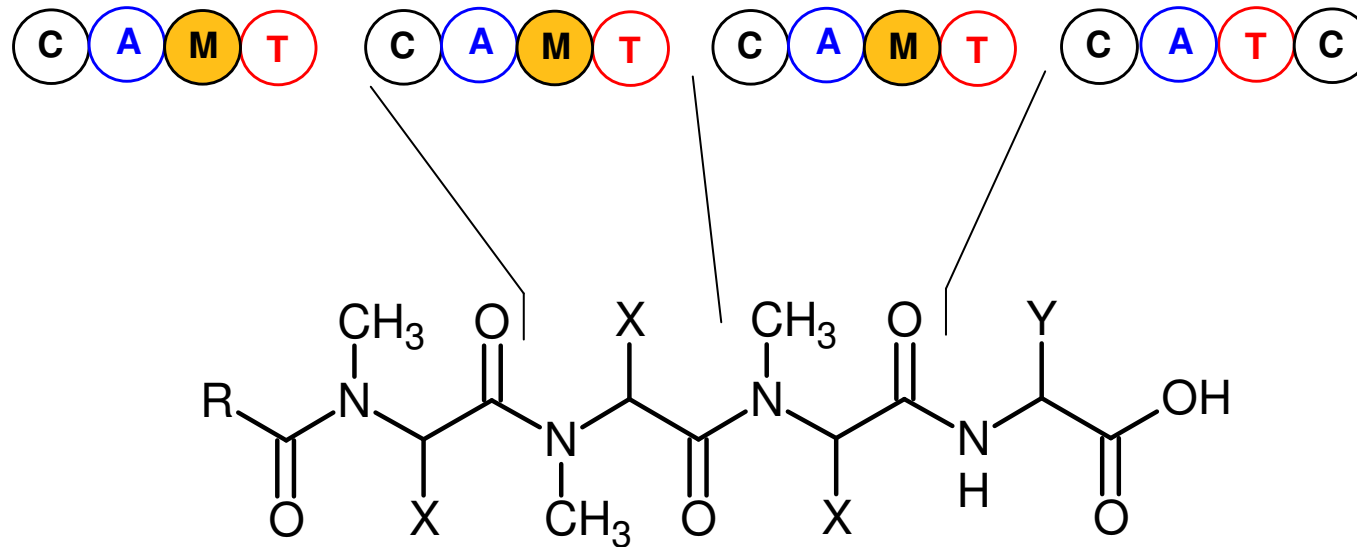
possible xenocoumacin biosynthesis



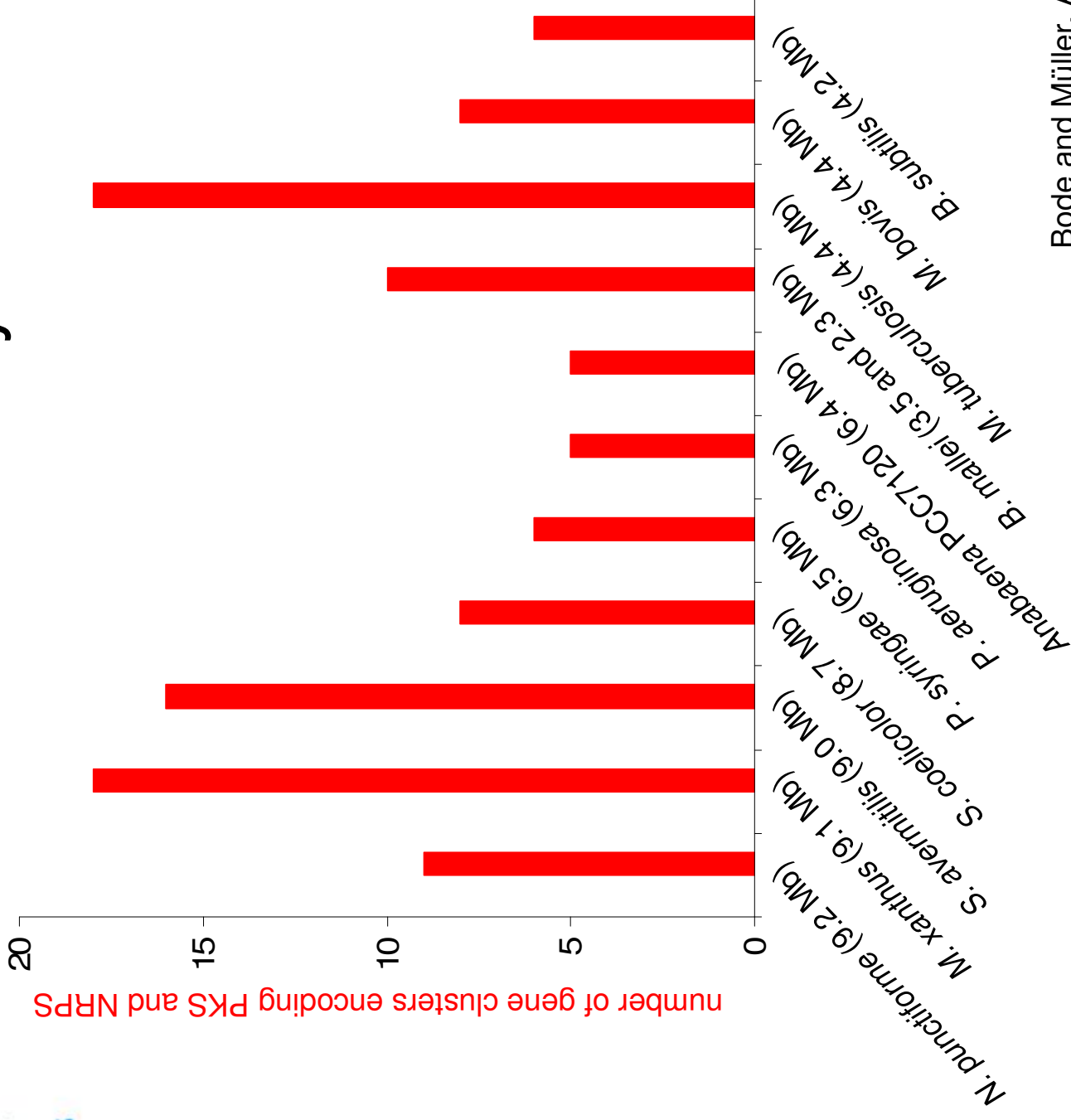
possible xenocoumacin biosynthesis



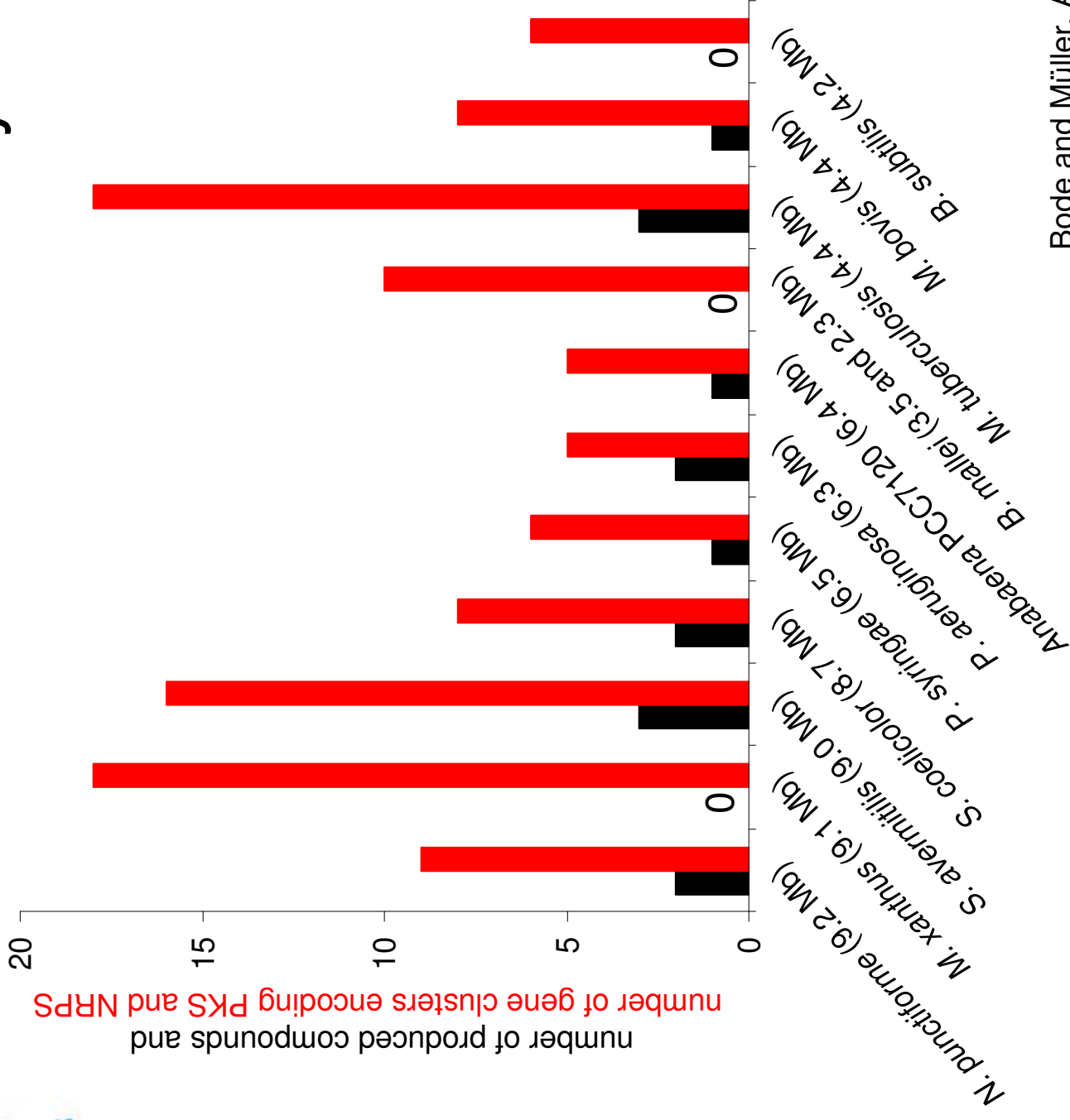
more lipophilic compounds...



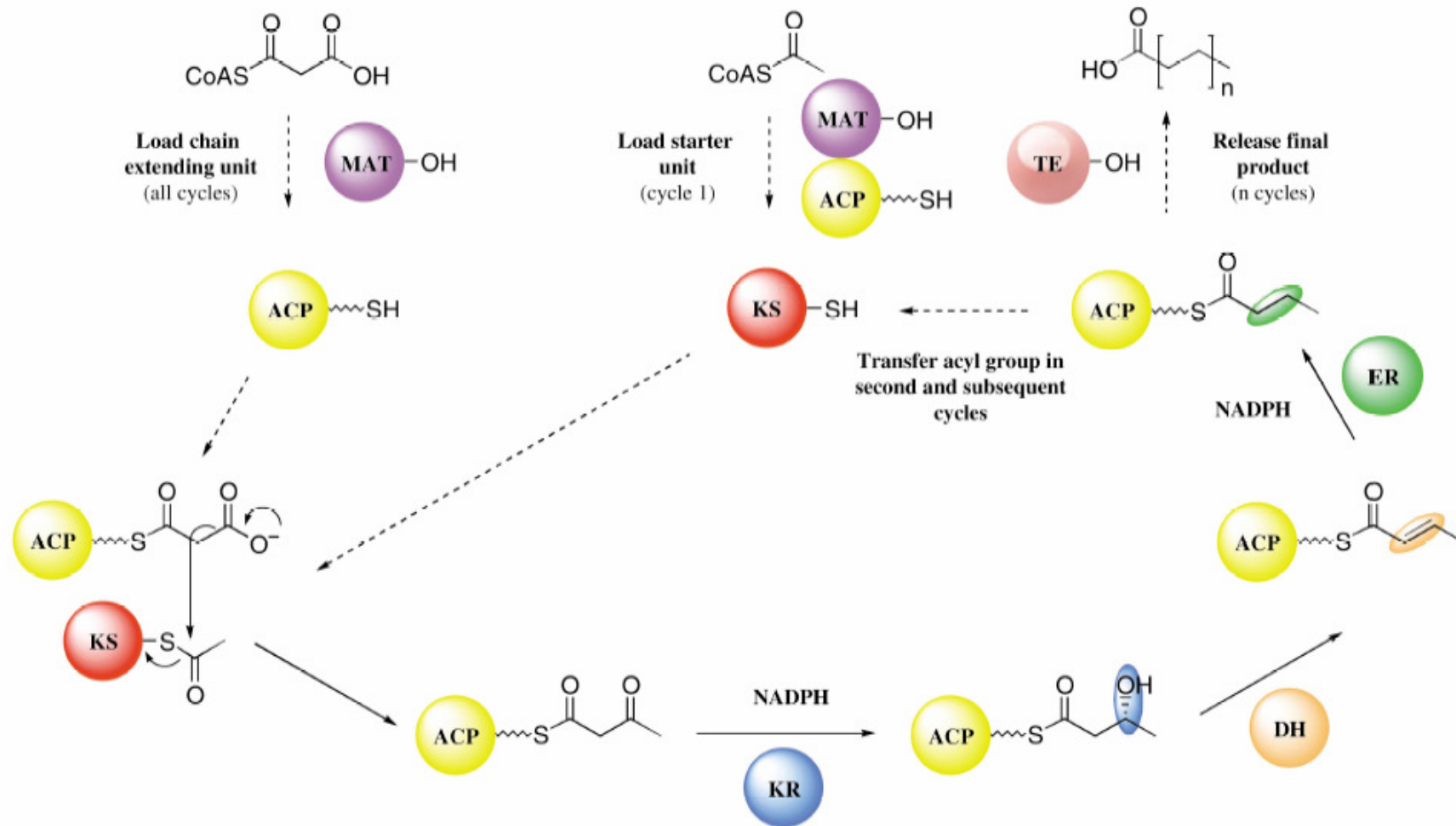
in silico secondary metabolism



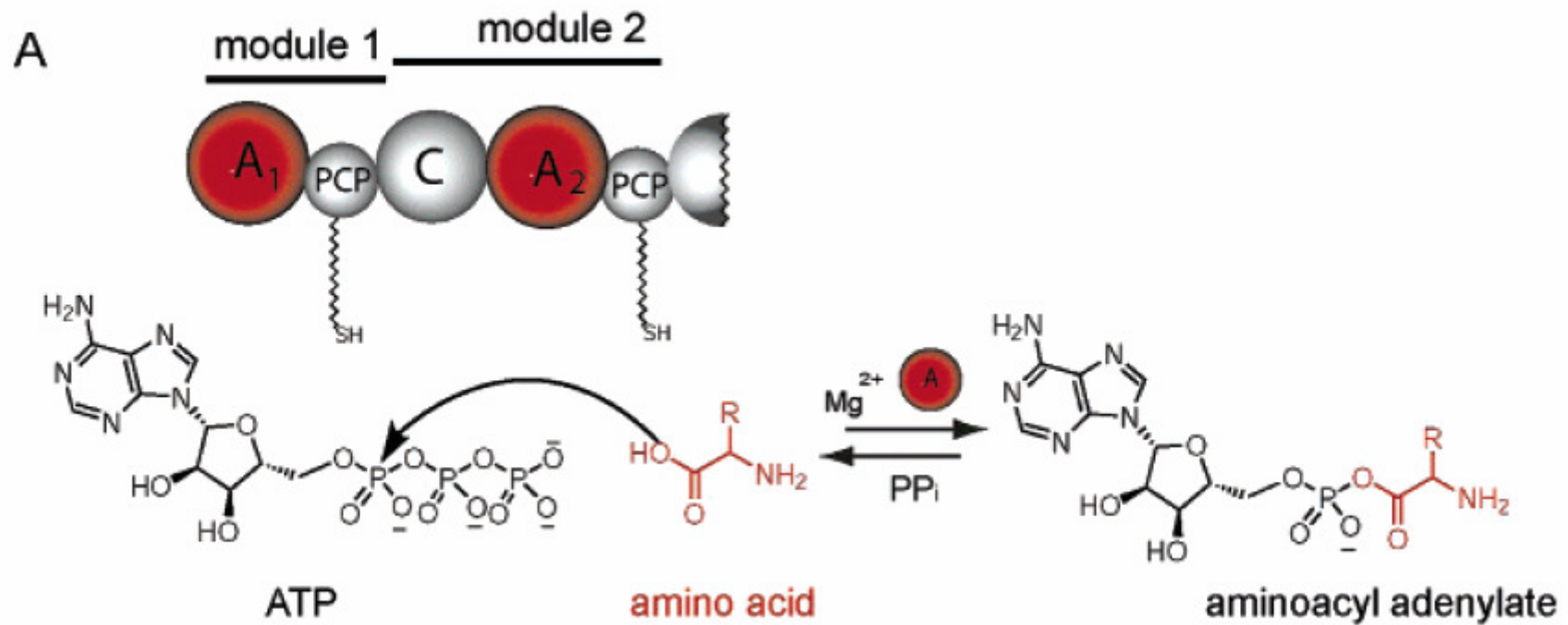
in silico vs. *in extract* secondary metabolism



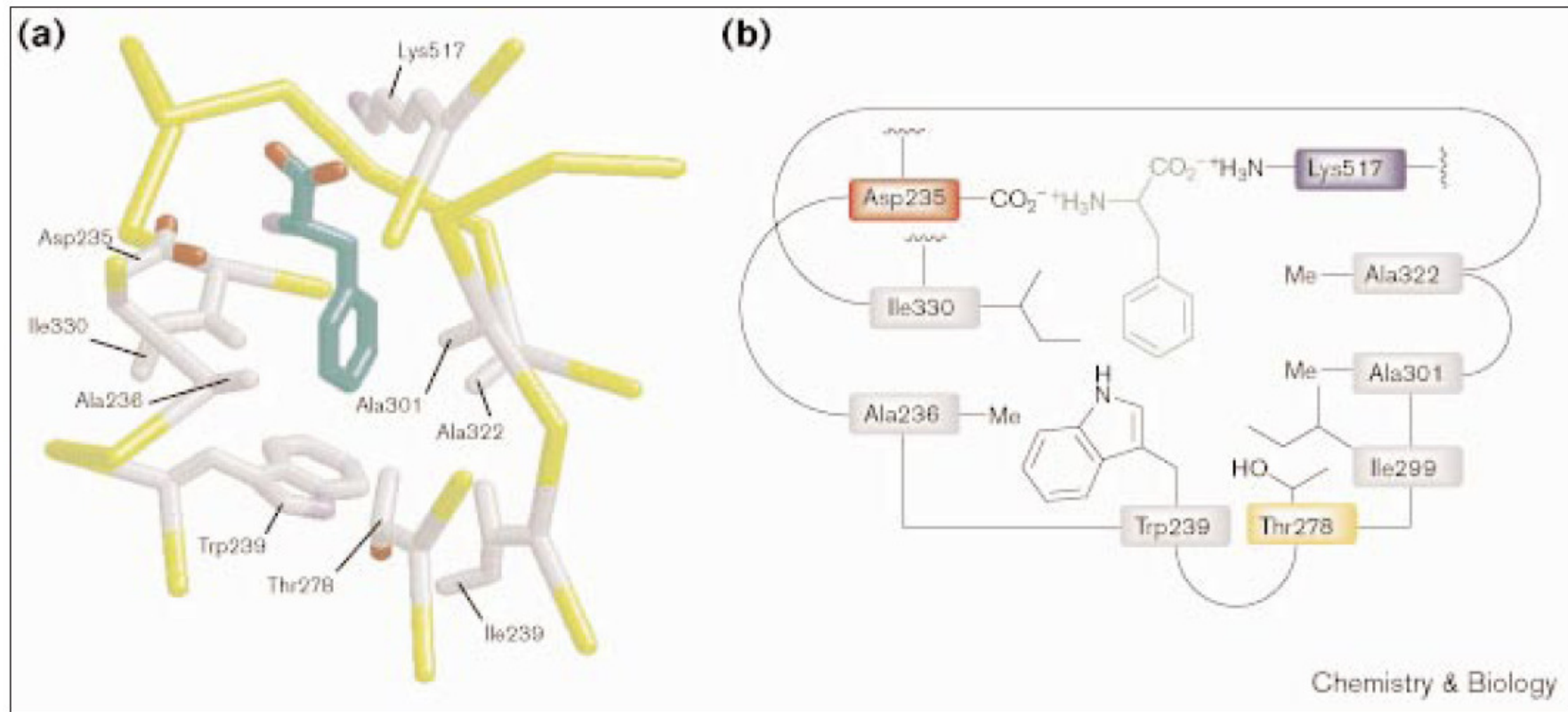
Fatty acid and polyketid biosynthesis are closely related



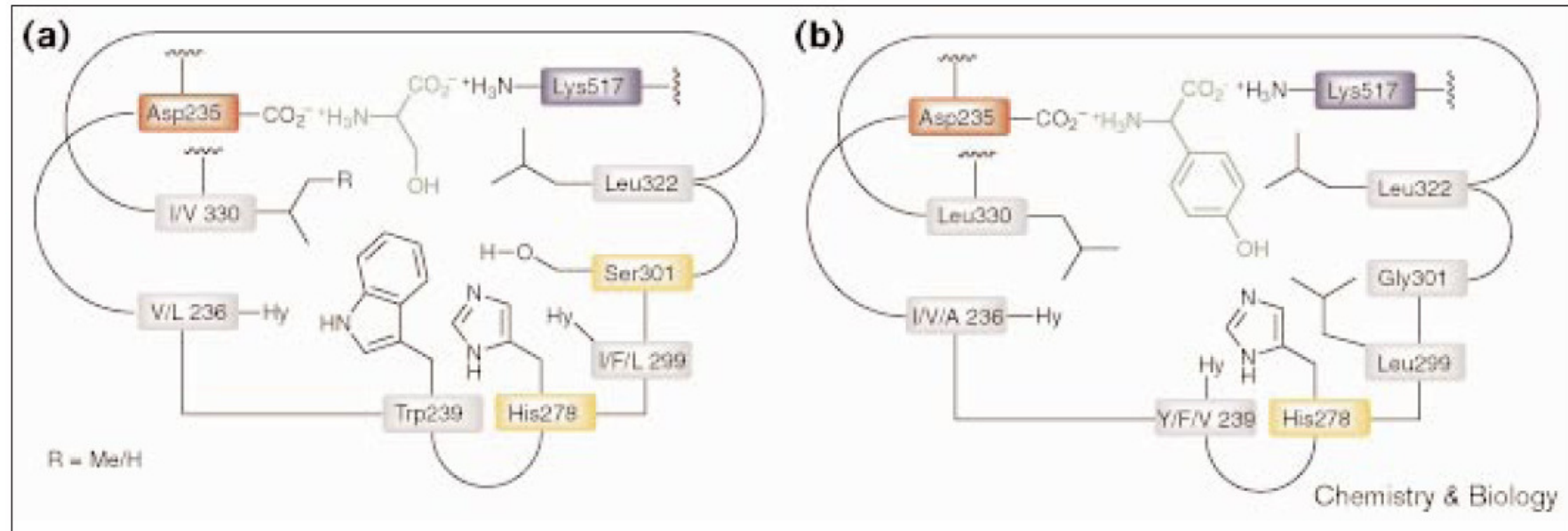
non-ribosomal peptide synthetase



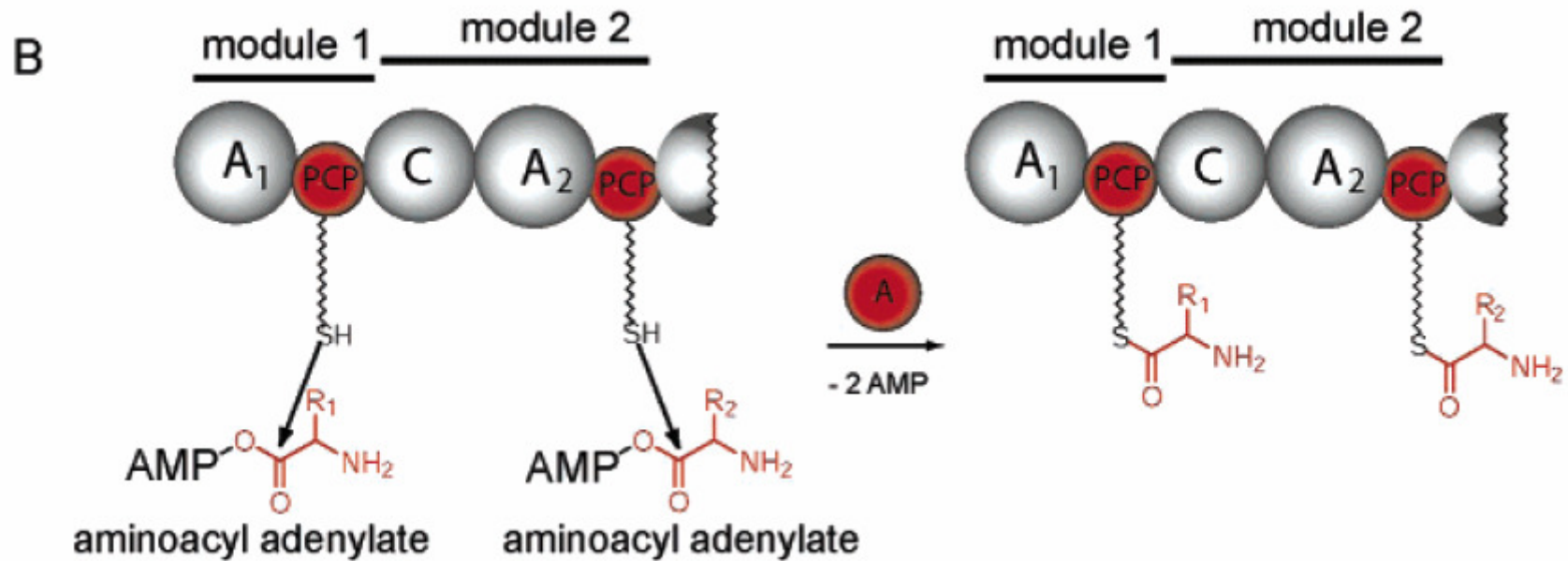
Prediction of precursors



Prediction of precursors

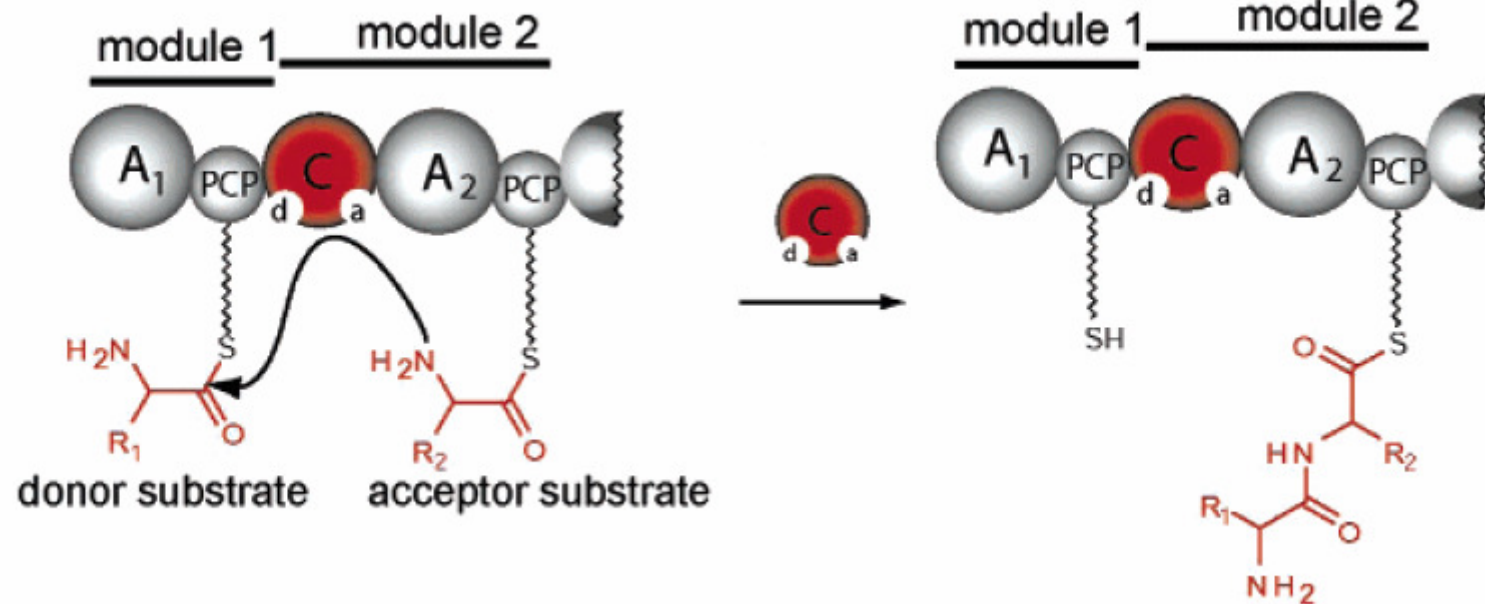


non-ribosomal peptide synthetase



non-ribosomal peptide synthetase

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non-ribosomal peptide synthetase

