Universität Hamburg
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HSFS

Die Mevalonat-unabhängige Terpenbiosynthese: Ein hervorragend geeigneter Angriffsort für neue Antiinfektiva

Regionalverbandstagung Nord, 19.02.2013

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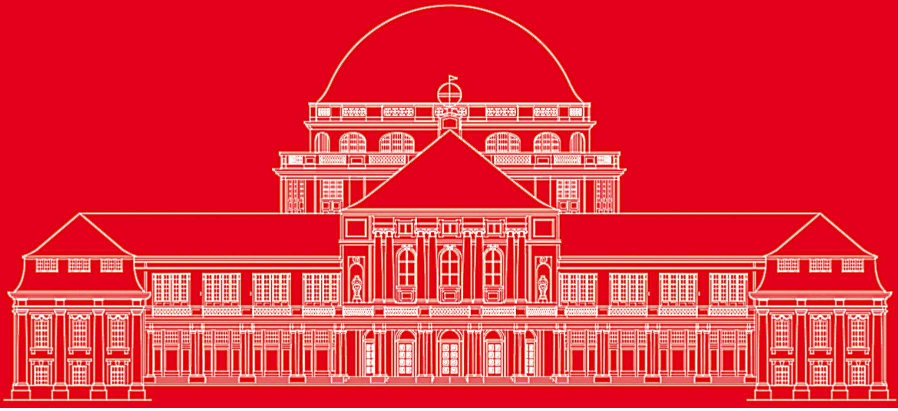
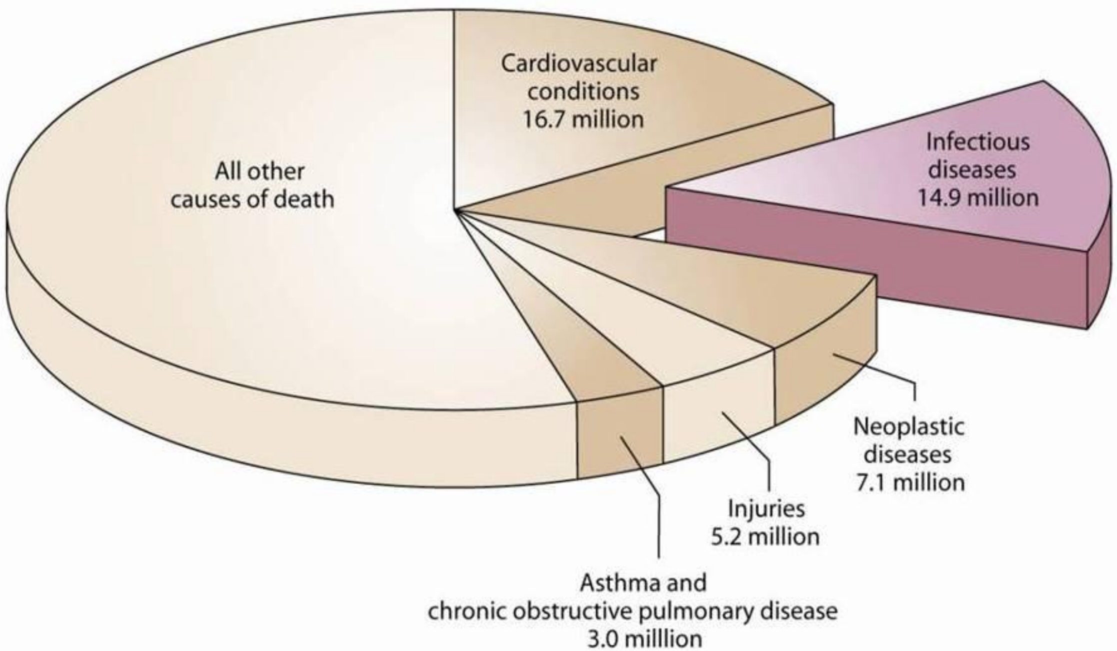


Inhalt

- Warum sind neue Antibiotika wichtig?
- Drug-Target: Terpen-Biosynthese
- Die Mevalonat-abhängige Terpenbiosynthese
- Die Mevalonat-unabhängige Terpenbiosynthese
- HTS (High Throughput Screening)

Weltweite Todesursachen



Infectious diseases	Annual deaths (million)
Respiratory infections	3.96
HIV/AIDS	2.77
Diarrhoeal diseases	1.80
Tuberculosis	1.56
Vaccine-preventable childhood diseases	1.12
Malaria	1.27
STDs (other than HIV)	0.18
Meningitis	0.17
Hepatitis B and C	0.16
Tropical parasitic diseases	0.13
Dengue	0.02
Other infectious diseases	1.76

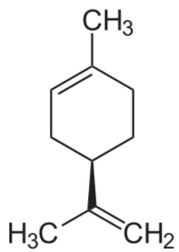
Morens *et al.*, *Nature* **430**, 242-249 (2004)

Entwicklung von Antibiotika und deren Resistenzen

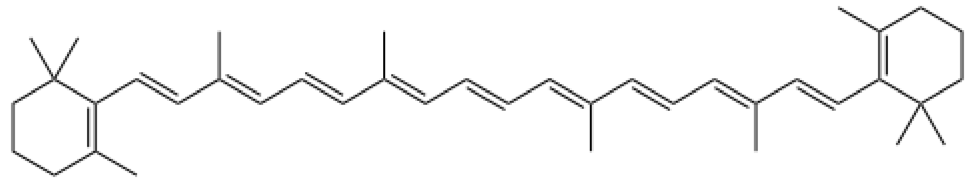
Antibiotic	Year deployed	Resistance observed
	Antibiotics	
Sulfonamides	1930s	1940s
Penicillin	1943	1946
Streptomycin	1943	1959
Chloramphenicol	1947	1959
Tetracycline	1948	1953
Erythromycin	1952	1988
Vancomycin	1956	1988
Methicillin	1960	1961
Ampicillin	1961	1973
Cephalosporins	1960s	late 1960s

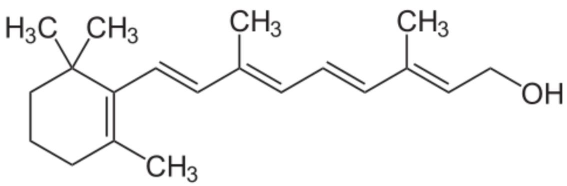
Palumbi, S.R. *Science* **293**, 1786-1790 (2001)

Terpene

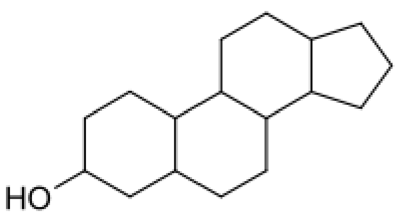


Limonen

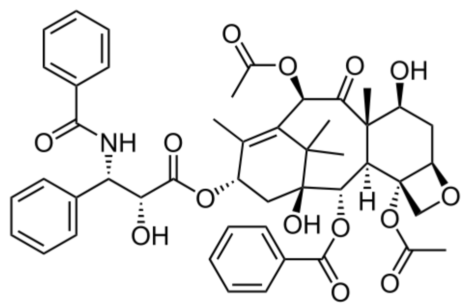

ß-Carotin



Vitamin A



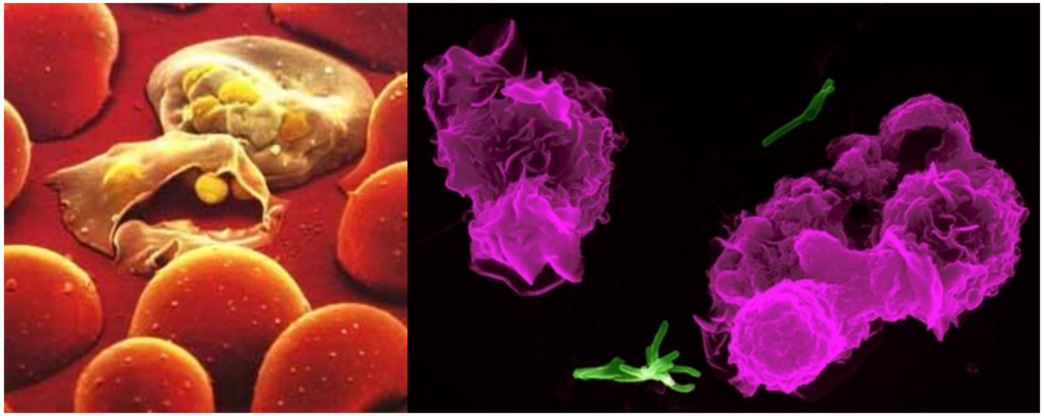
Sterin



Taxol

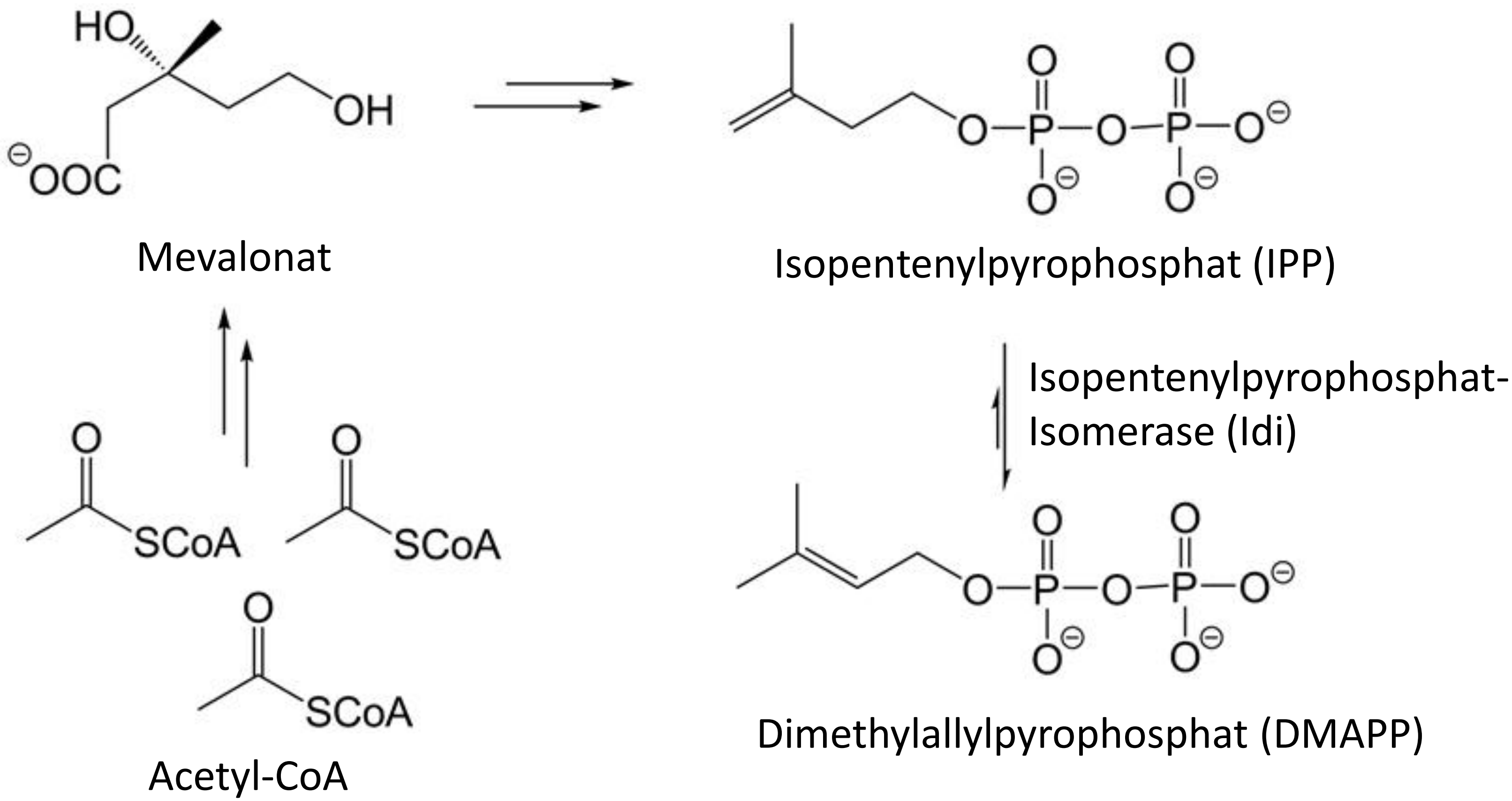
Terpen-Biosynthese

Mevalonat-Syntheseweg

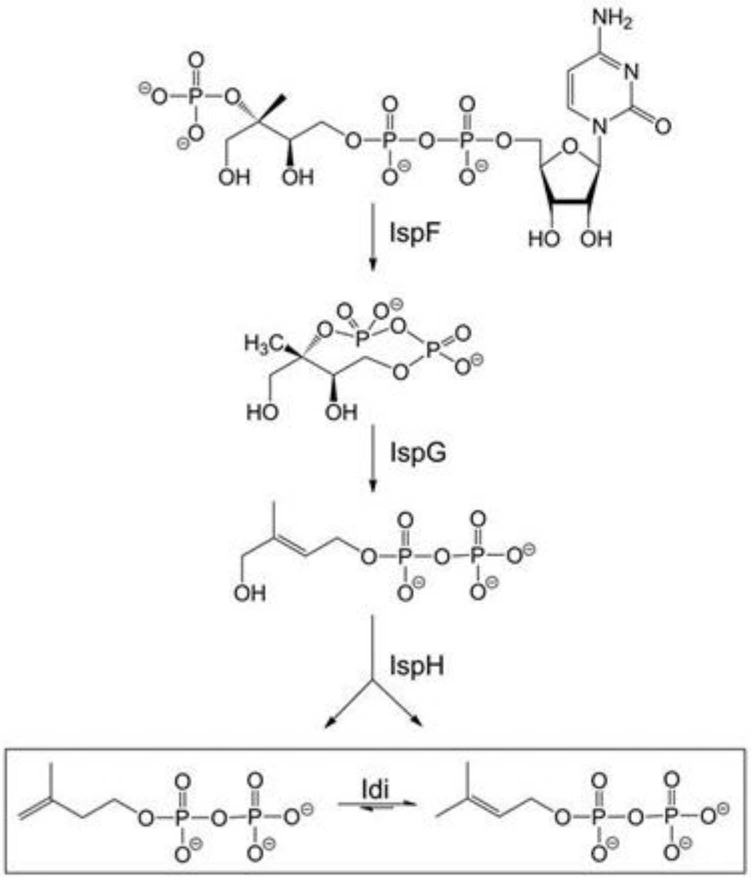


Mevalonat-unabhängiger Syntheseweg

Mevalonat-Syntheseweg



Mevalonat-unabhängiger Syntheseweg



4-Diphosphocytidyl-2C-methyl-D-erythritol-2-phosphate

2C-Methyl-D-erythritol-2,4-cyclo-diphosphate

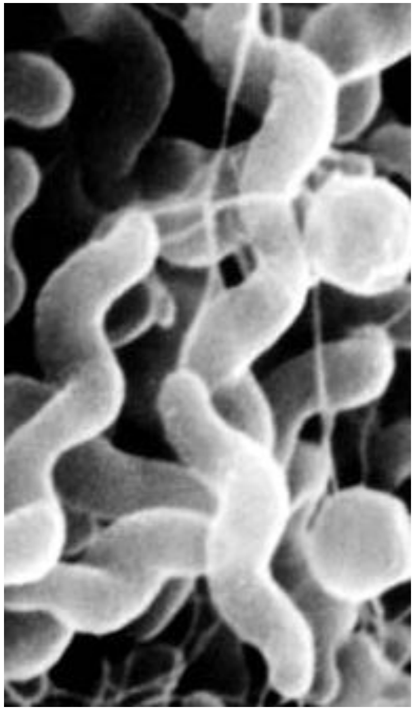
1-Hydroxy-2-methyl-2-(E)-butenyl-4-diphosphate

IPP / DMAPP

Welcher Biosyntheseweg kommt wo vor?

Organismus		Mevalonat-unabhängiger Syntheseweg	Mevalonat-Syntheseweg
Bakterien	*Aquifex aeolicus*	X	
	Chlamydophila pneumoniae	X	
	Mycobacterium tuberculosis	X	
	Staphylococcus aureus		X
	Escherichia coli	X	X
	Borrelia burgdorferi		X
	Campylobacter jejuni	X	
Archaeen	*Aeropyrum pernix*		X
Eukaryoten	*Homo sapiens*		X
	Arabidopsis thaliana	X	X
	Plasmodium falciparum	X	
	Saccharomyces cerevisiae		X

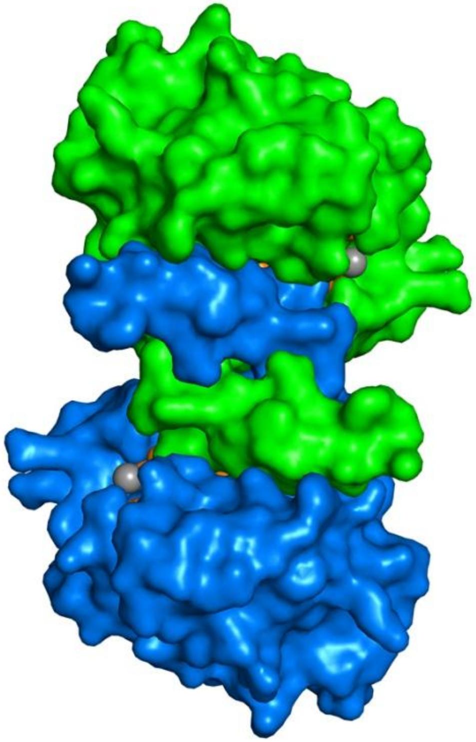
Campylobacter jejuni



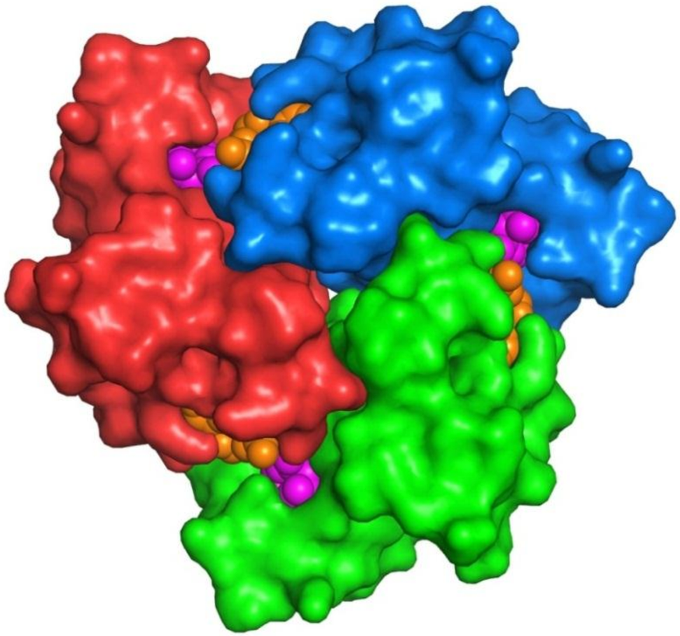
http://www.wpsa-foodsafety.com/UserFiles/Image/hoofdstukken/Foodborne%20diseases/Campylobacter.jpg

- gramnegatives Bakterium
- Übertragung über Tiere, Trinkwasser und Lebensmittel auf den Menschen
  - Krankheitsbild = schwerer Durchfall, Fieber etc.
  - Campylobacter Enteritis
- Therapie mittels Antibiotika

Mevalonat-unabhängiger Syntheseweg: Struktur IspD und IspF



IspD aus *Escherichia coli*

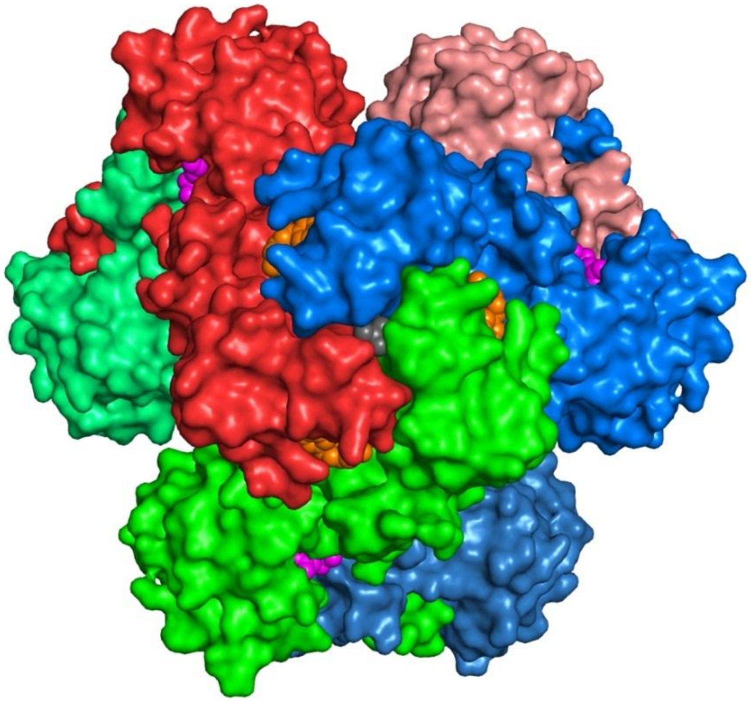


IspF aus *Escherichia coli*

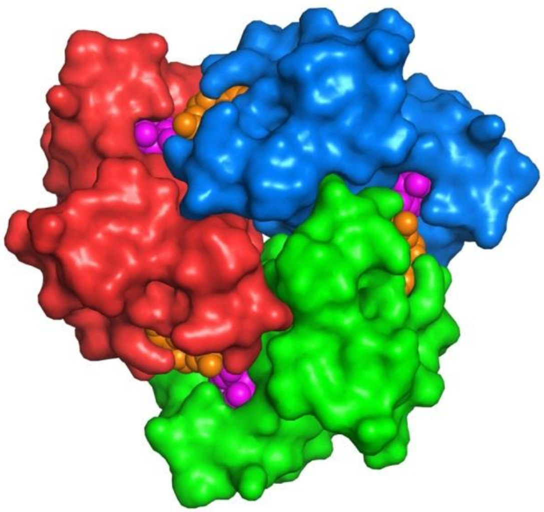
Kemp LE, Bond CS, Hunter WN , Acta Crystallogr D Biol Crystallogr 57:1189–1191 (2001)

Steinbacher S, Kaiser J, Wungsintaweekul J, Hecht S, Eisenr- eich W, Gerhardt S, Bacher A, Rohdich F, J Mol Biol 316:79–88 (2002)

Mevalonat-unabhängiger Syntheseweg: Struktur IspDF



IspDF aus *Campylobacter jejuni*



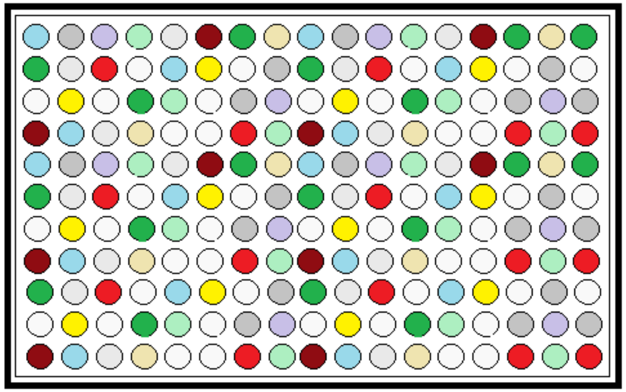
IspF aus *Escherichia coli*

Gabrielsen, M., Bond, C.S., Hallyburton, I., Hecht, S., Bacher, A., Eisenreich, W., Rohdich, F., Hunter, W.N., J.Biol.Chem. 279: 52753 (2004)

High Throughput Screening (HTS)

Pipettierrobotor

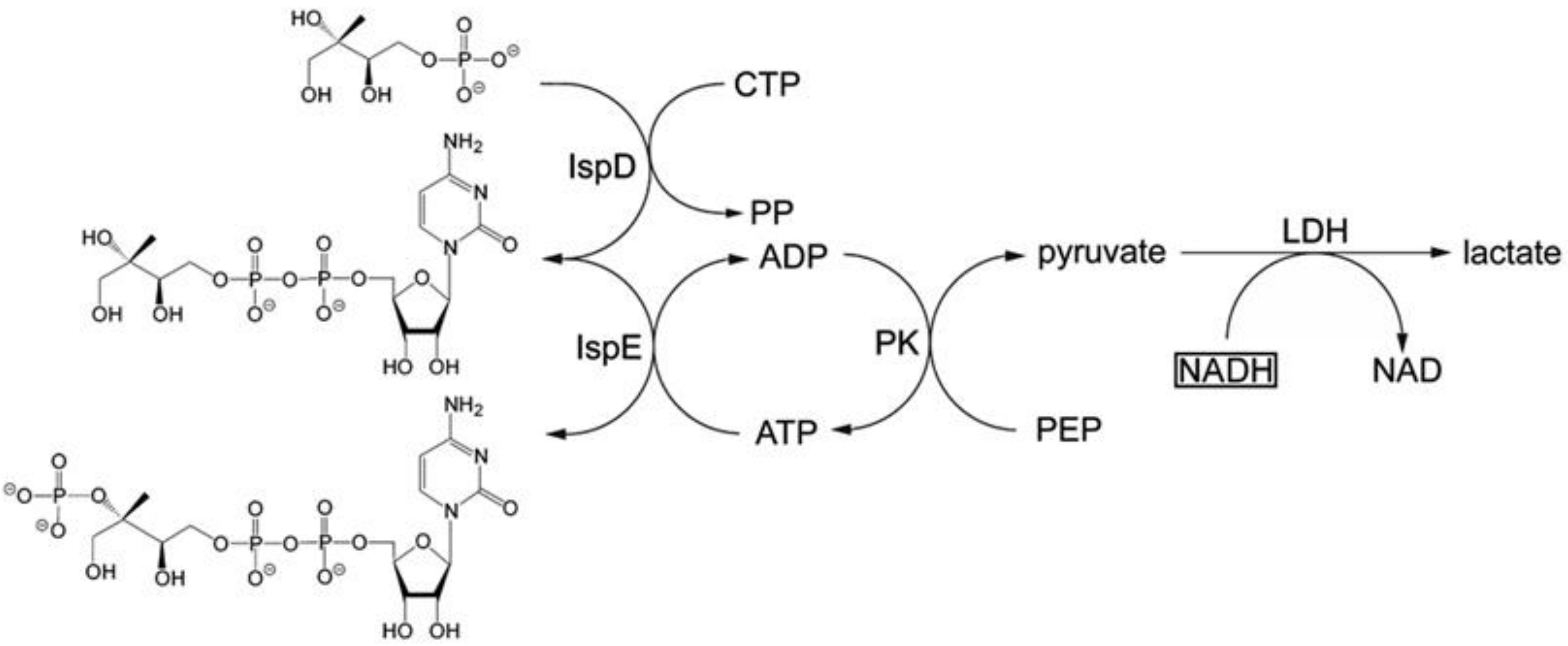
http://www.postina-pr.de/system/files/press_release_file/STARlet_Autoload.jpg



Substanzbank

Dispenser und Plattenleser

High Throughput Screening (HTS)



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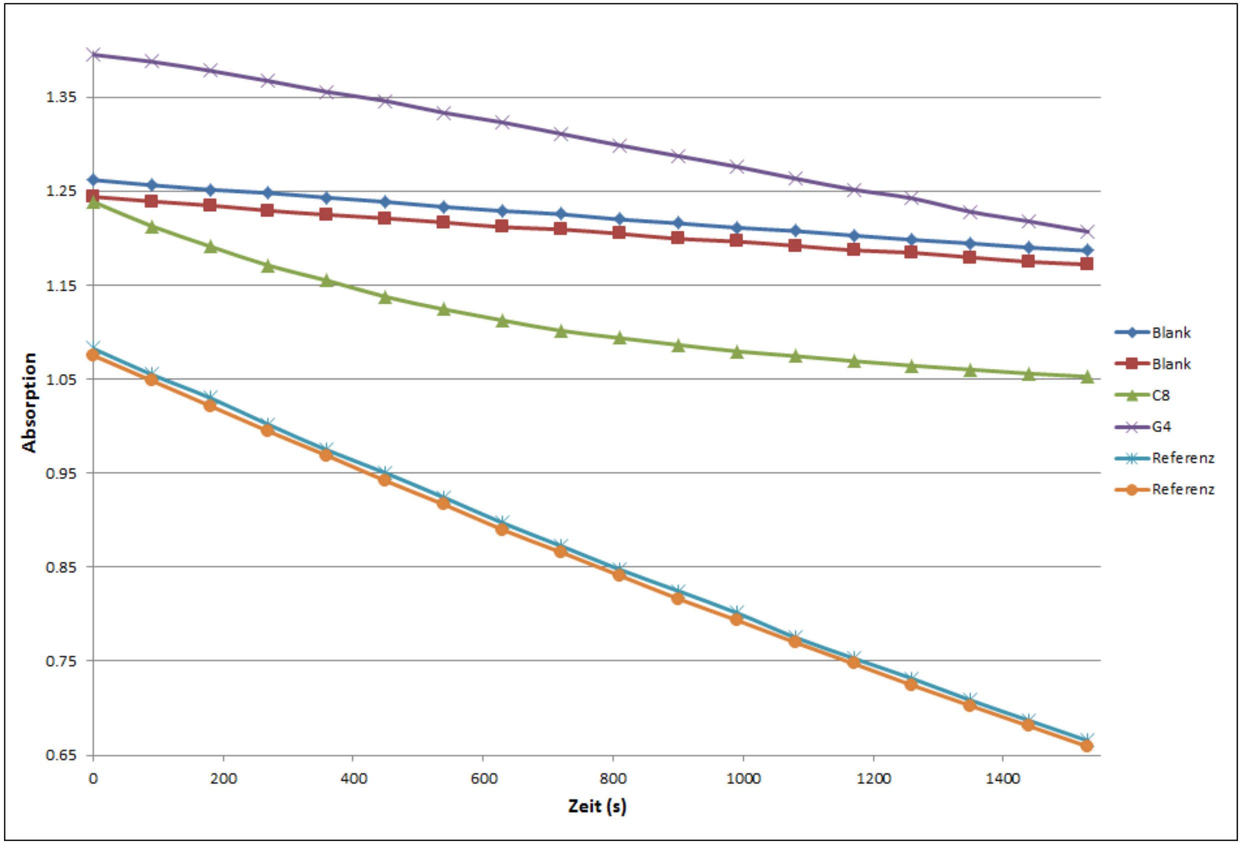
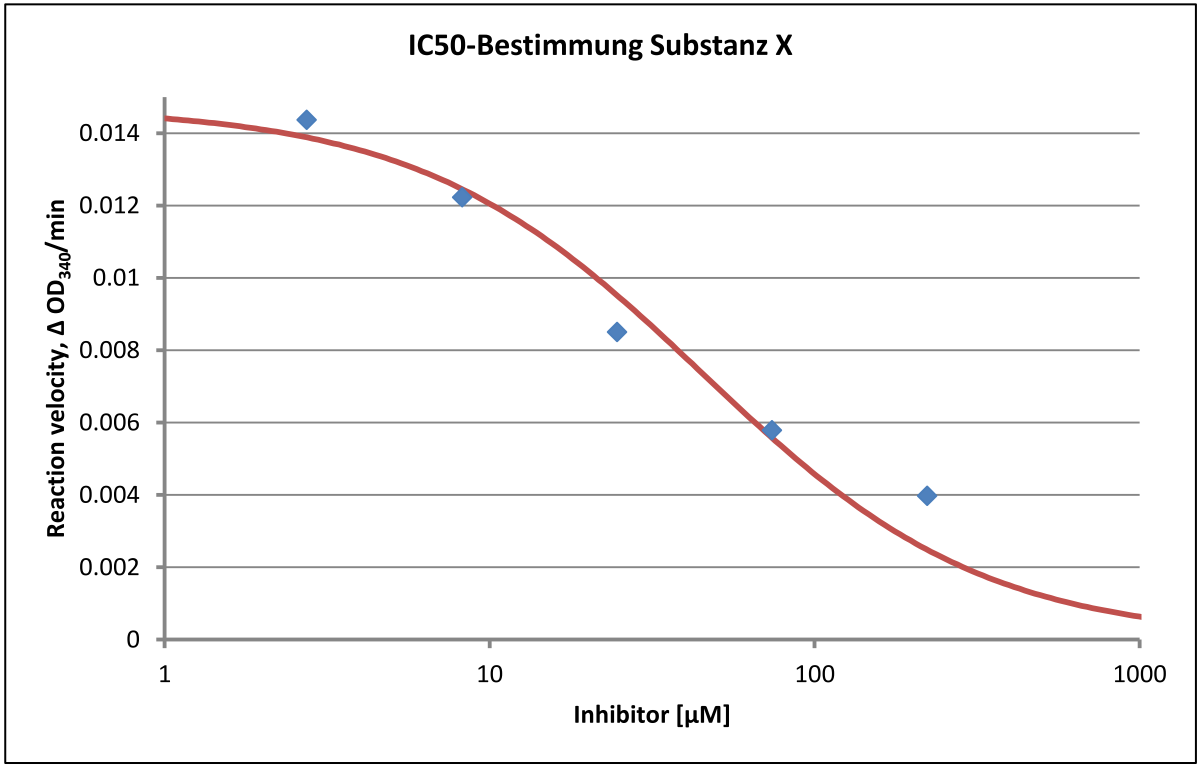


IC_{50} (mittlere inhibitorische Konzentration)

- Konzentration des Inhibitors, welche die Enzymaktivität auf die Hälfte reduziert



→ IC_{50}= 45 µM

Zusammenfassung

- Expression des Proteins
- Proteinreinigung
- High Throughput Screening
- IC_{50} – Bestimmung

Ausblick

- Kristallisation des Enzyms mit Inhibitor
- Strukturaufklärung des Komplexes (Enzym + Inhibitor)
- Optimierung der gefundenen Leitstrukturen hinsichtlich ADME/T

Vielen Dank für Ihre Aufmerksamkeit!