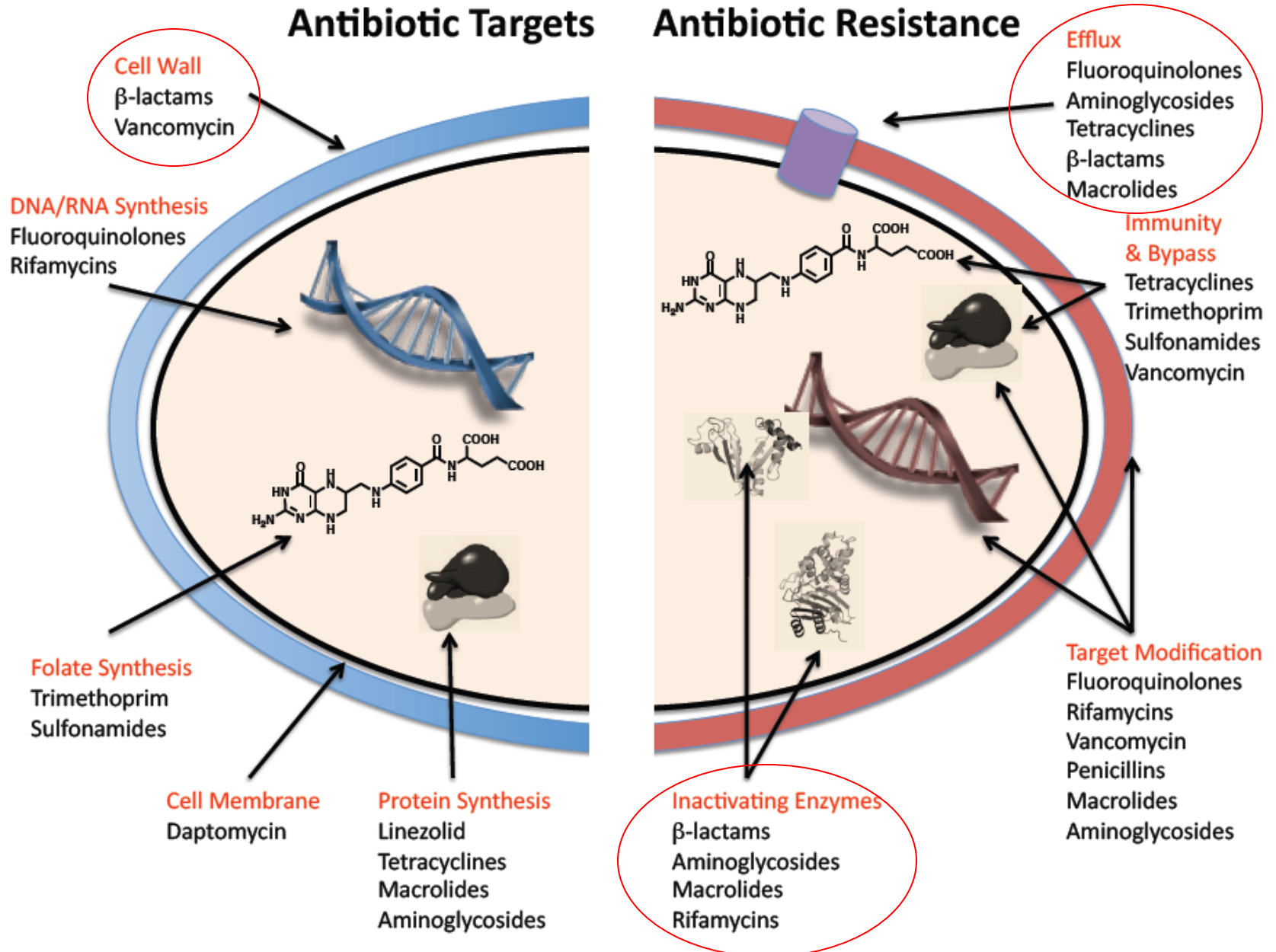
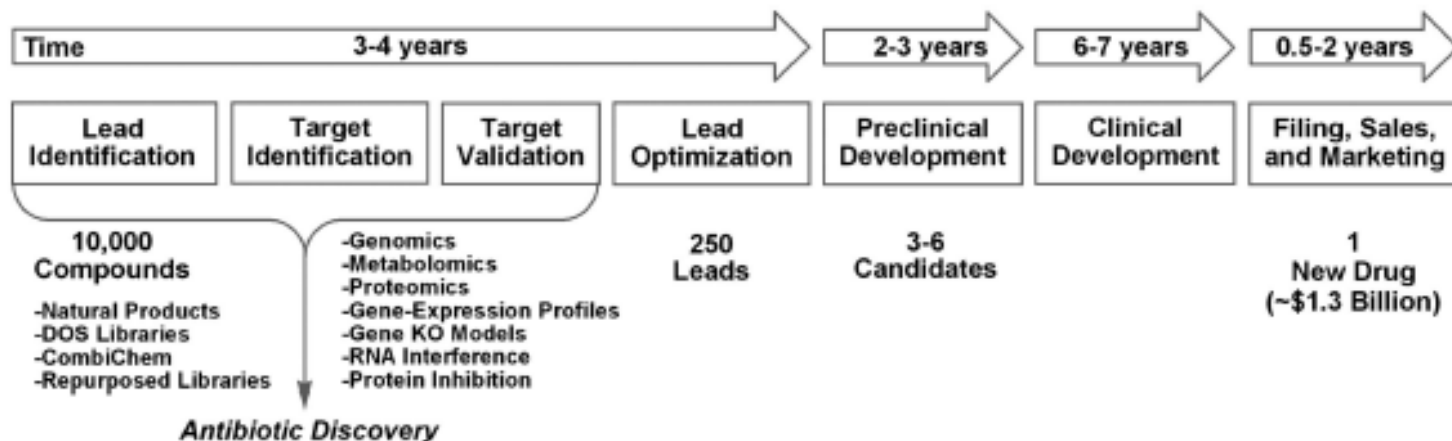
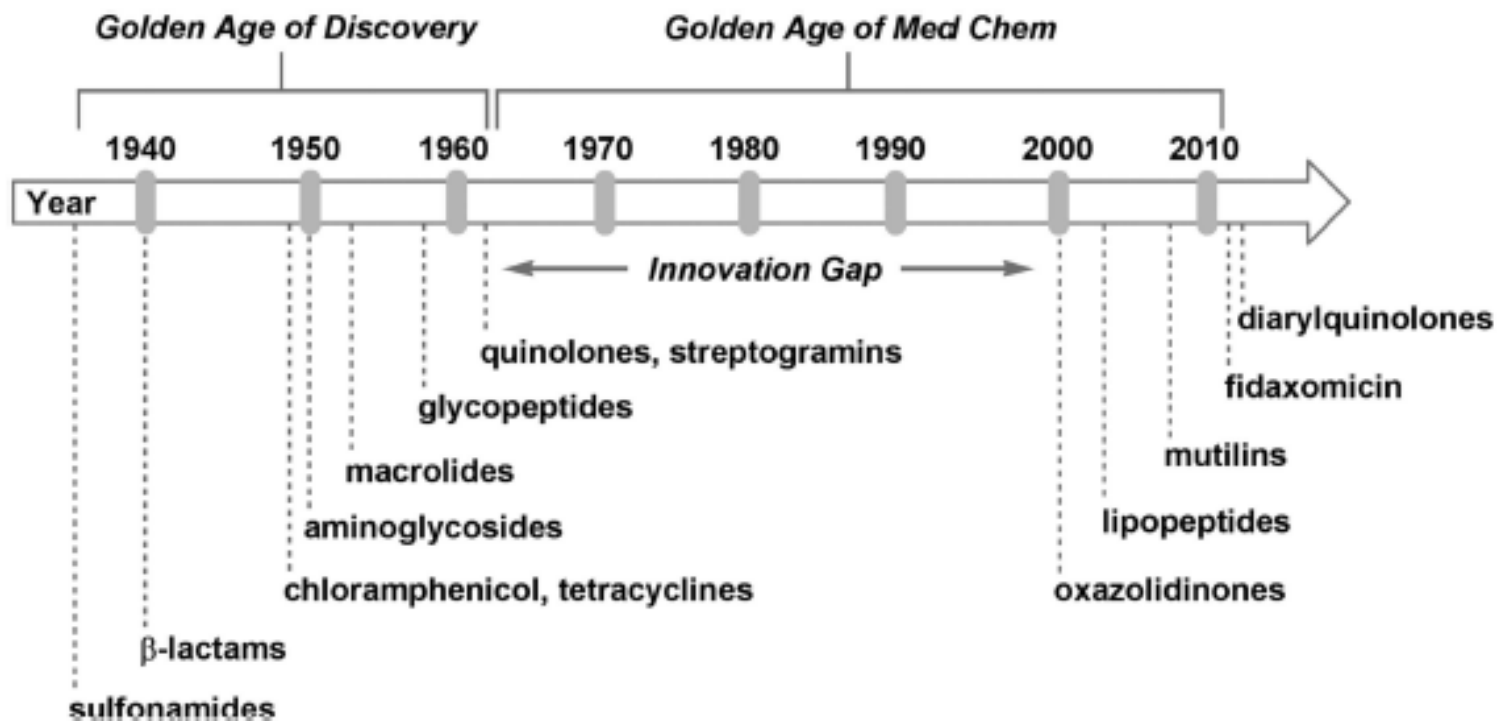


Biosintesi e modificazione degli
antibiotici β -lattamici.

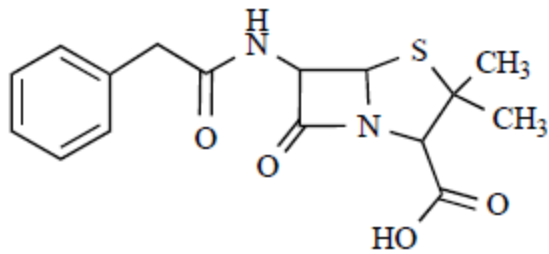
Penicilline e cefalosporine

Bersagli degli antibiotici e meccanismi di resistenza

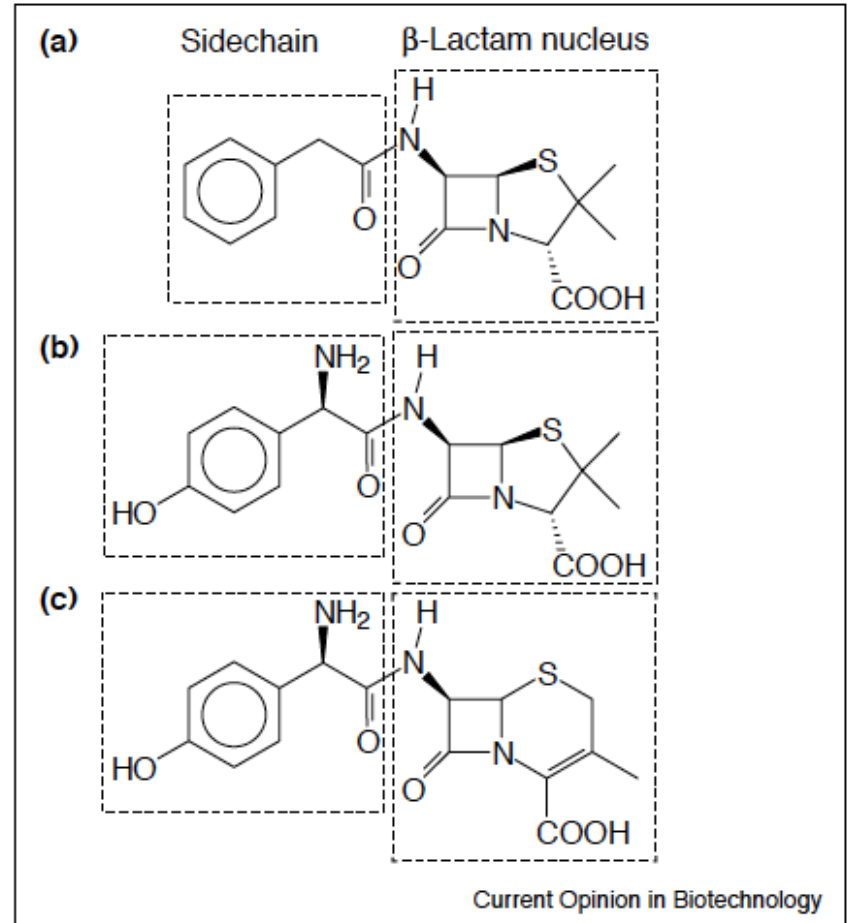
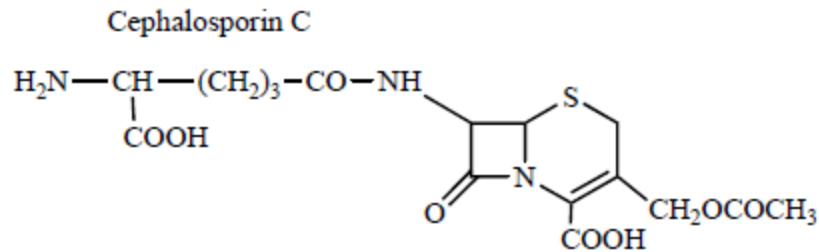




Struttura di alcuni antibiotici β -lattamici

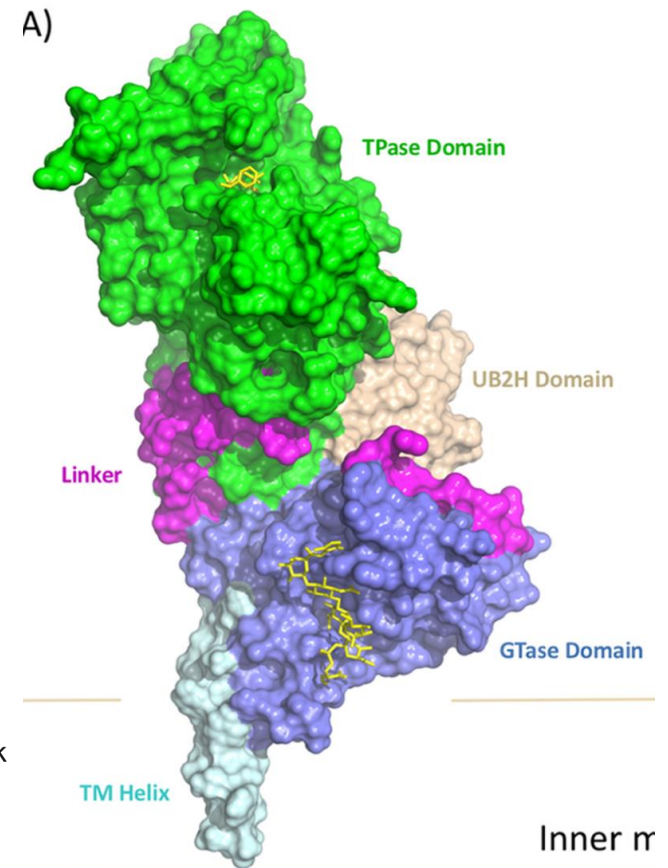
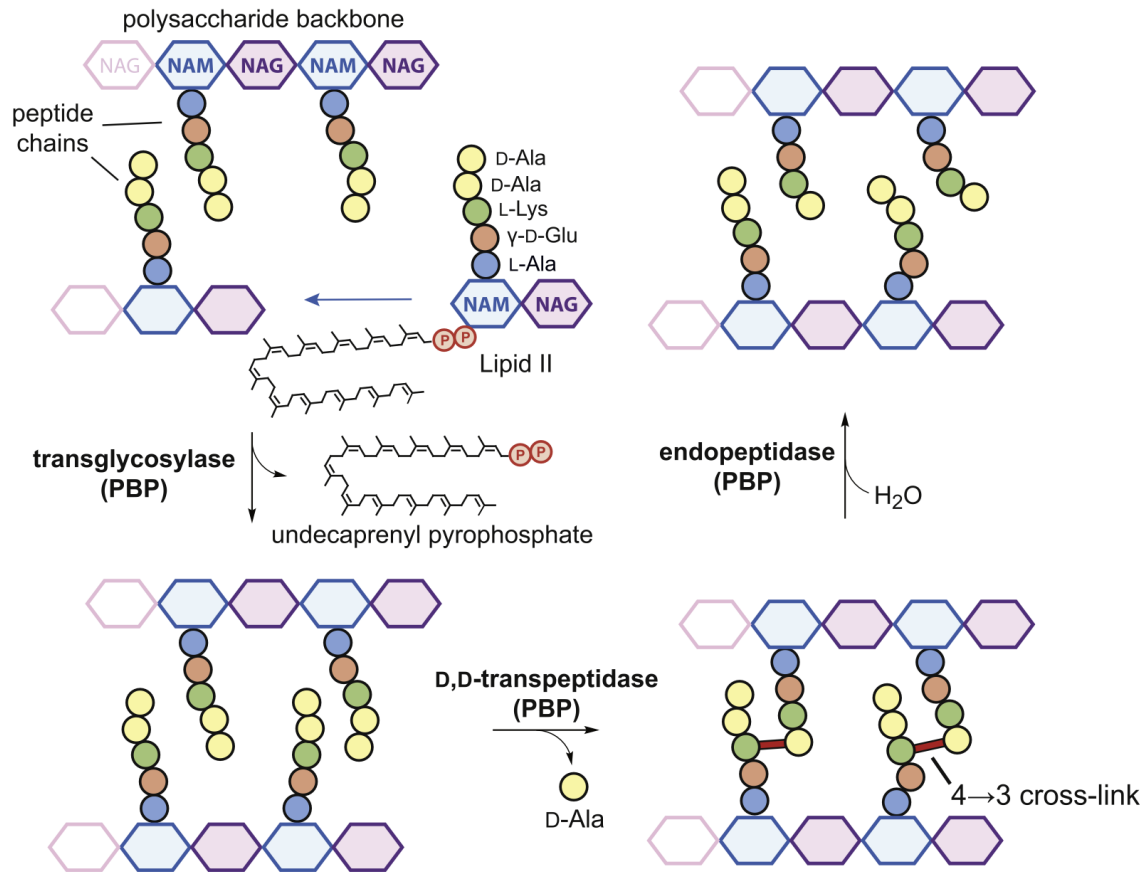


Penicillin G



β -Lactam antibiotics comprise a β -lactam nucleus coupled to a sidechain. Examples of two penicillins (a) penicillin G and (b) amoxicillin and (c) a cephalosporin, cephadroxil.

Le penicillin-binding proteins (PBP): enzimi bifunzionali essenziali per la biosintesi del peptidoglicano della parete batterica



Meccanismo d'azione delle penicilline e delle cefalosporine

La penicillina e la cefalosporina inibiscono la transpeptidasi, l'enzima responsabile della formazione dei legami crociati nella sintesi del peptidoglicano della parete batterica.

La penicillina e la cefalosporina sono **analoghi strutturali** del substrato naturale della transpeptidasi.

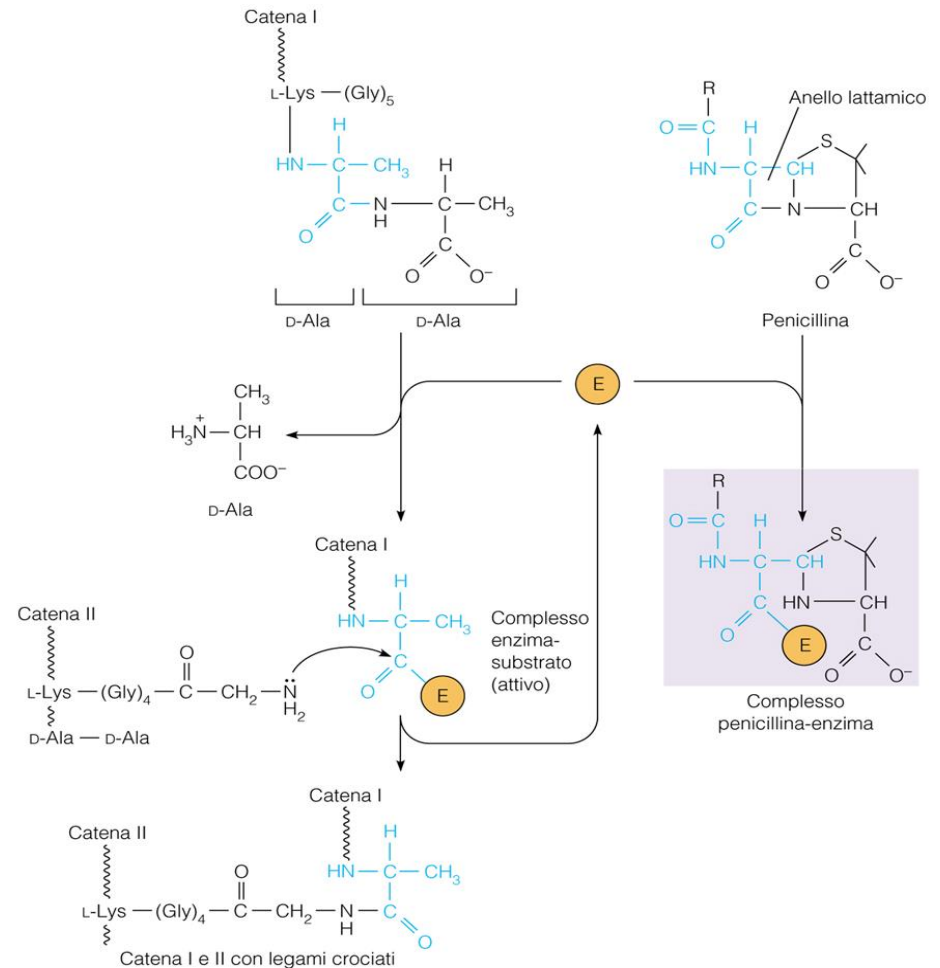
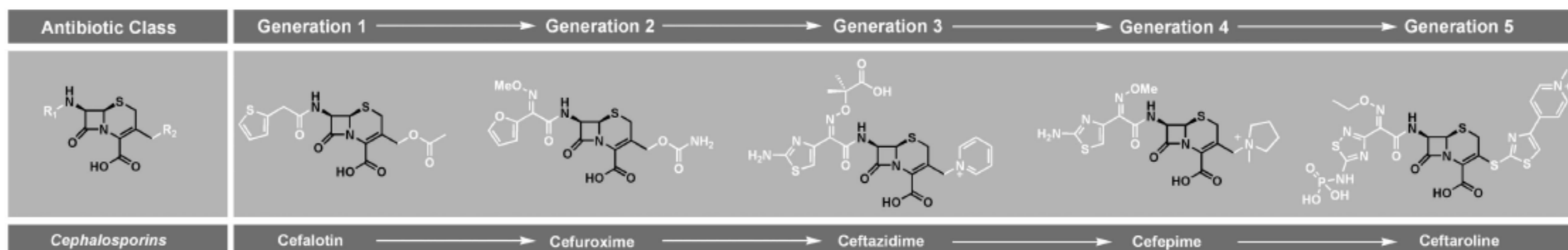


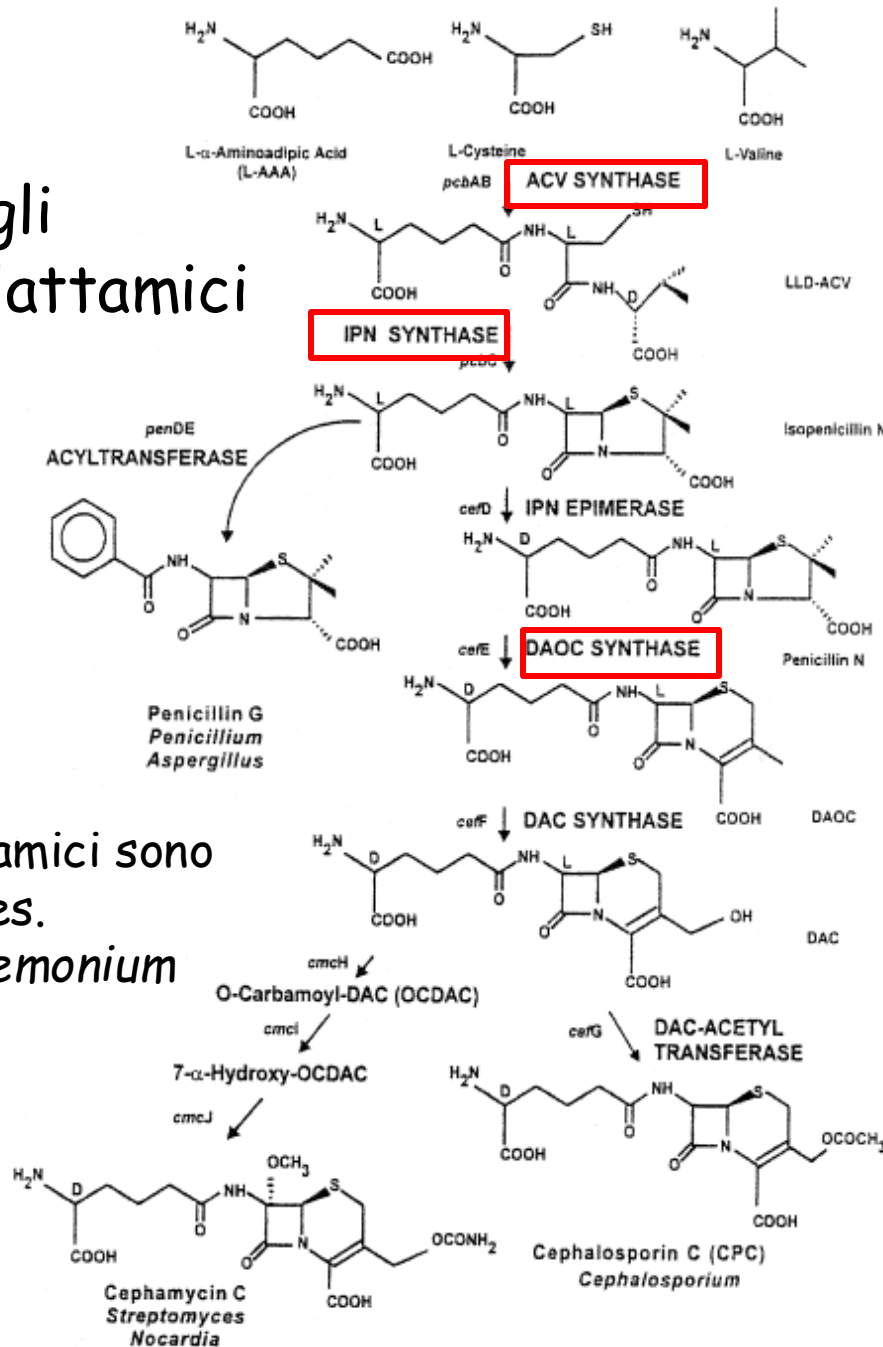
Table 1 Marketed and experimental β -lactam antibiotics. Antibiotics italicized are major commercial antibiotics

Subclass	Marketed β -lactam antibiotics
Penicillins	Ampicillin ^a , <i>amoxicillin</i> ^a , bacampicillin, cloxacillin, floxacillin, mezlocillin, nafcillin, oxacillin, penicillin G ^a , penicillin V ^a
Penicillin-resistant penicillins	<i>Methicillin</i> , dicloxacillin
Antipseudomonal penicillins	Carbenicillin, indanyl piperacillin, <i>ticarcillin</i>
First-generation cephalosporins	Cefalothin, cephradine ^a , cefadroxy ^a , cefazolin, <i>cephalexin</i> ^a
Second-generation cephalosporins	<i>Cefuroxime</i> , <i>cefaclor</i> ^a , cefotetam, cefmetazole, cefonicid
Third-generation cephalosporins	<i>Cefixime</i> ^a , <i>ceftibuten</i> , cefizoxime, ceftriaxone, cefamandol, cefoperazone, cefotaxime, proxetil, <i>cefprozil</i> ^a , ceftazidime, cefuroxime axetil, cefpodexime
Fourth-generation cephalosporins	<i>Cefepime</i>
Oxycephams	Flomoxef, latamoxef
Cefam	Cefoxitin
Carbapenems	Loracarbef ^a , <i>imipenem</i> , meropenem, panipenem
Monobactams	Aztreonam, carumonam
Clavams (β -lactamase-inhibitors)	<i>Clavulanate</i> , sulbactam, tazobactam
Penicillins/ β -lactamase inhibitors	<i>Amoxicillin/clavulanate</i> , ampicillin/sulbactam, piperacillin/tazobactam, <i>ticarcillin/clavulanate</i> , cefoperazone/sulbactam

^a Orally administered β -lactams



Biosintesi degli antibiotici β -lattamici



Gli antibiotici β -lattamici sono prodotti da funghi (es. *Penicillium* sp. e *Acremonium* sp.).

Organizzazione modulare della sintetasi peptidica NRPS dell'ACV

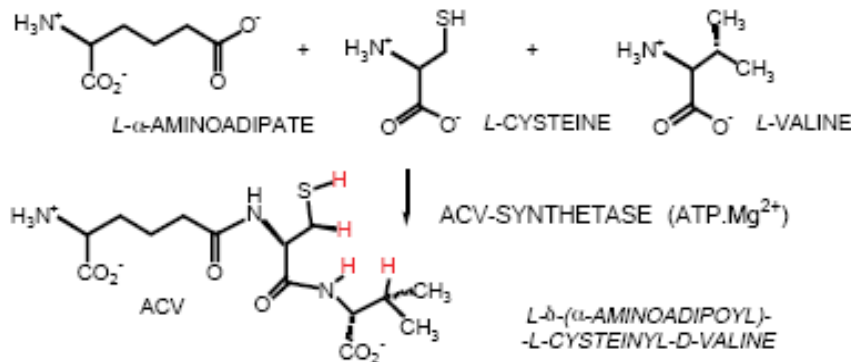
Il precursore dell'anello β -lattamico è il tripeptide
L- δ - α -aminoadipyl-L-cysteinyl-D-valina prodotto dall'ACV sintetasi.

Aad

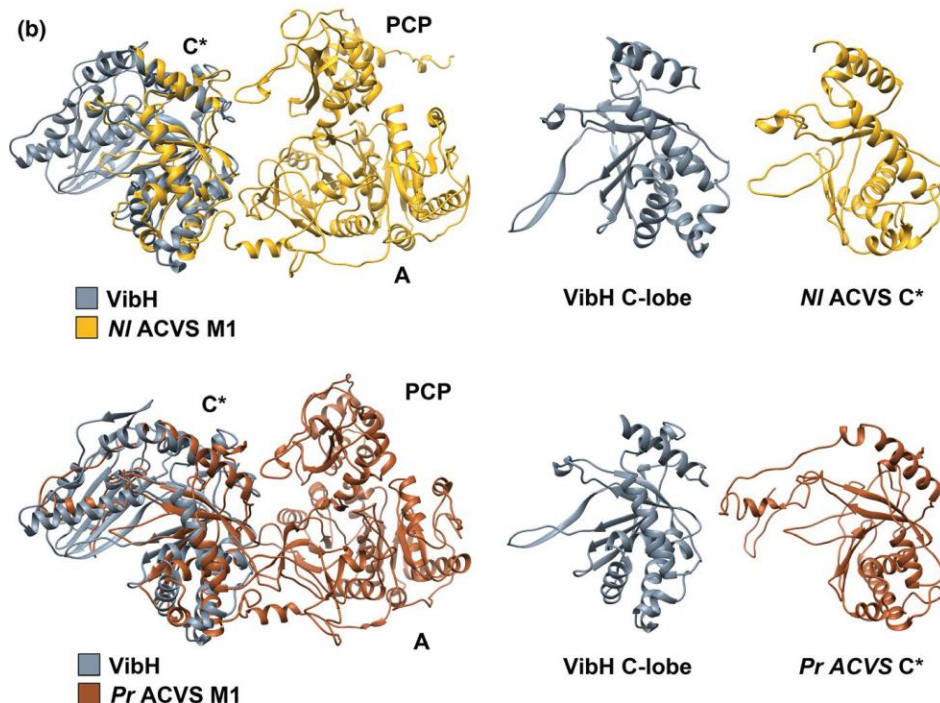
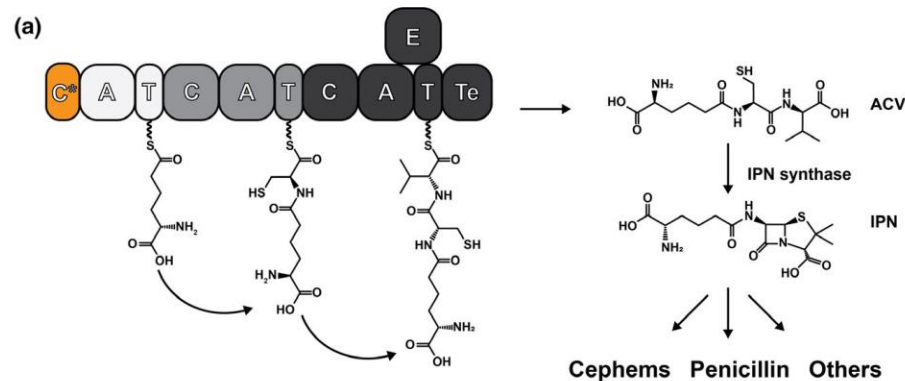
Cys

Val

A-T-C-A-T-C-A-T-E-Te



Il primo modulo della ACV NRPS

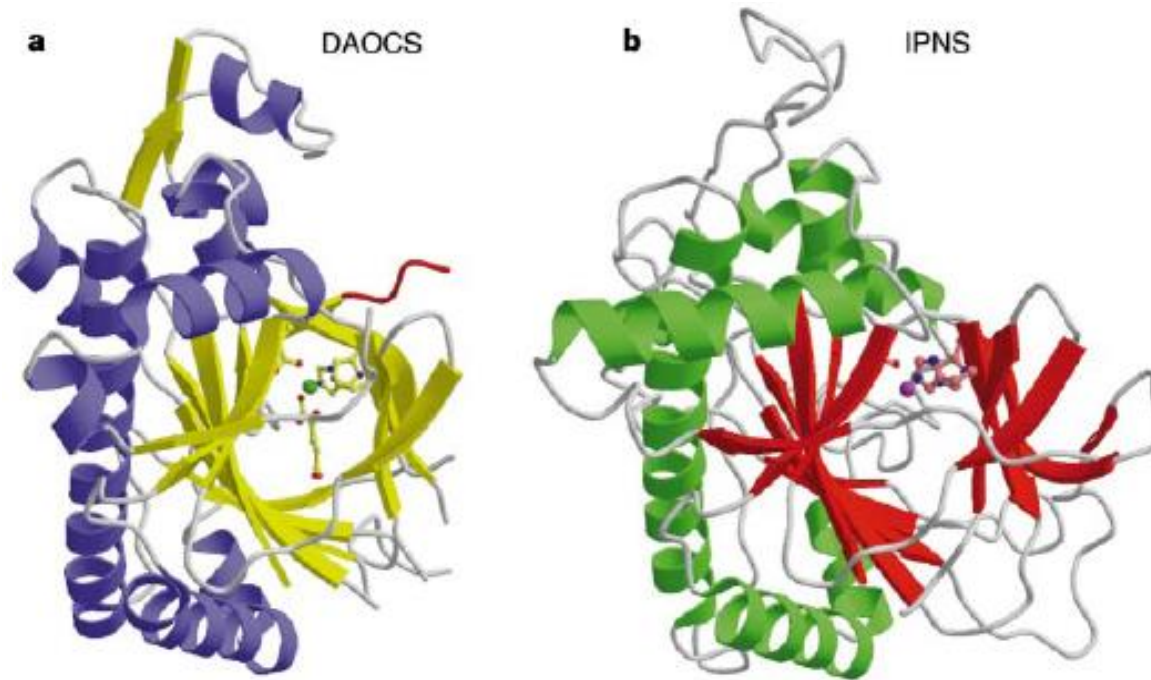


Analisi bionformatiche hanno identificato un dominio N-terminale atipico simile al lobo C dei domini di condensazione

La delezione di questo dominio abolisce la sintesi di ACV

Il dominio C* potrebbe essere coinvolto nel reclutamento e/o posizionamento del substrato per la reazione atipica

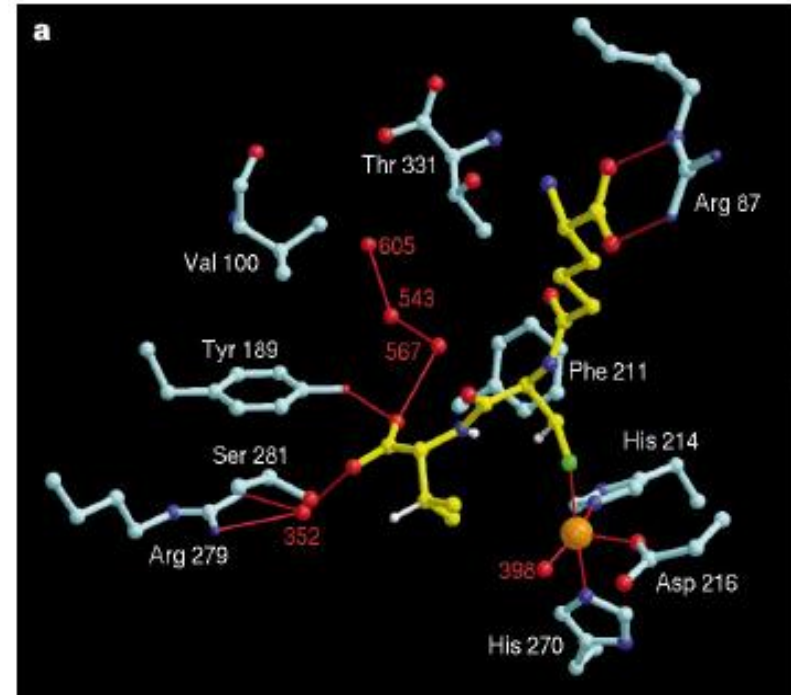
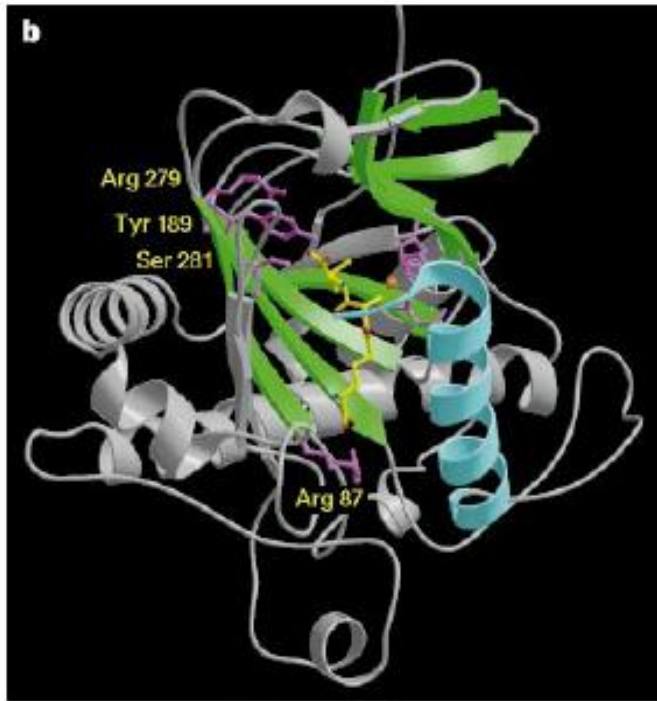
Struttura della isopenicillina N sintasi (IPNS) e della deacetossicefalosporina sintasi (DAOCS)



La IPNS e la DAOCS catalizzano le reazioni di chiusura e di espansione degli anelli del nucleo β -lattamico

Struttura della IPNS di *A. nidulans*

Sito attivo dell'enzima



La IPNS è un enzima che utilizza ferro e ossigeno per catalizzare la sintesi dell'anello β -lattamico. L'atomo di ferro nel sito attivo è coordinato da due istidine e un aspartato.

Meccanismo d'azione proposto per la IPNS

Strutture cristallografiche di IPNS in presenza di substrati che non permettono il completamento della reazione.

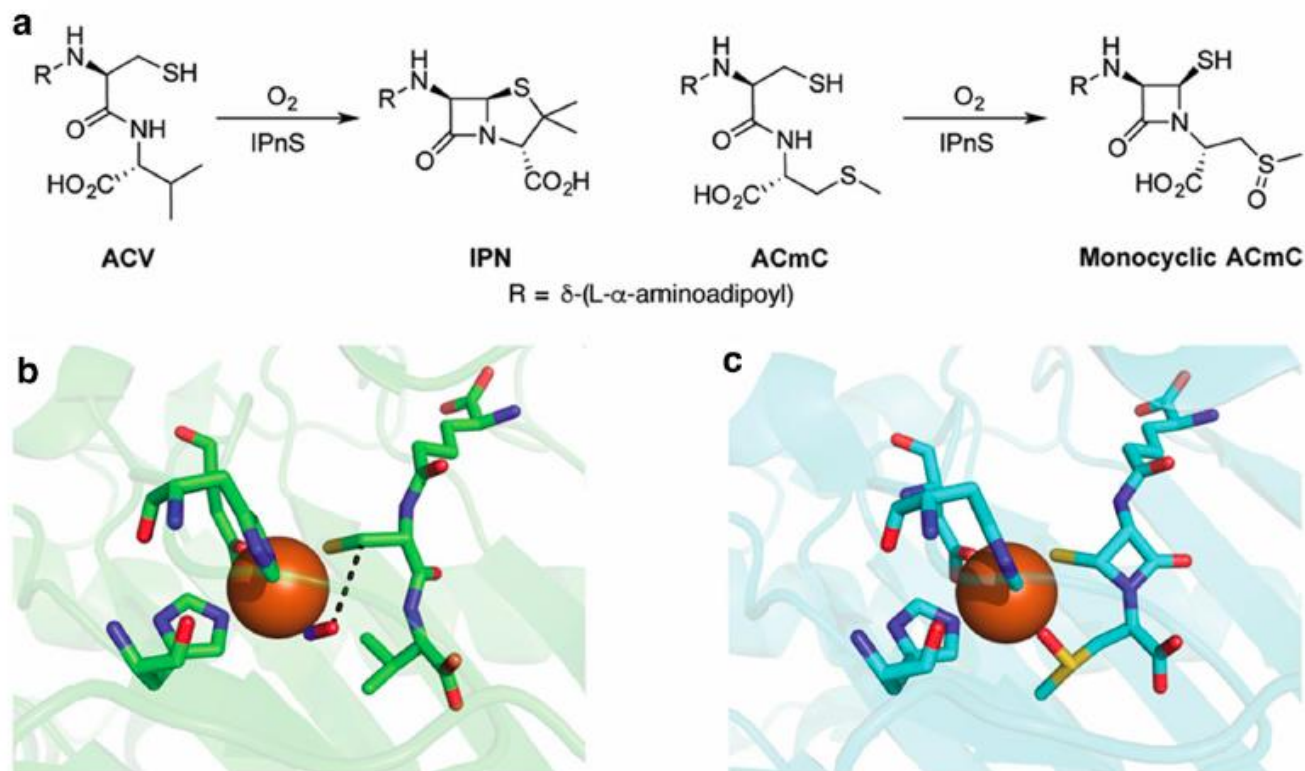
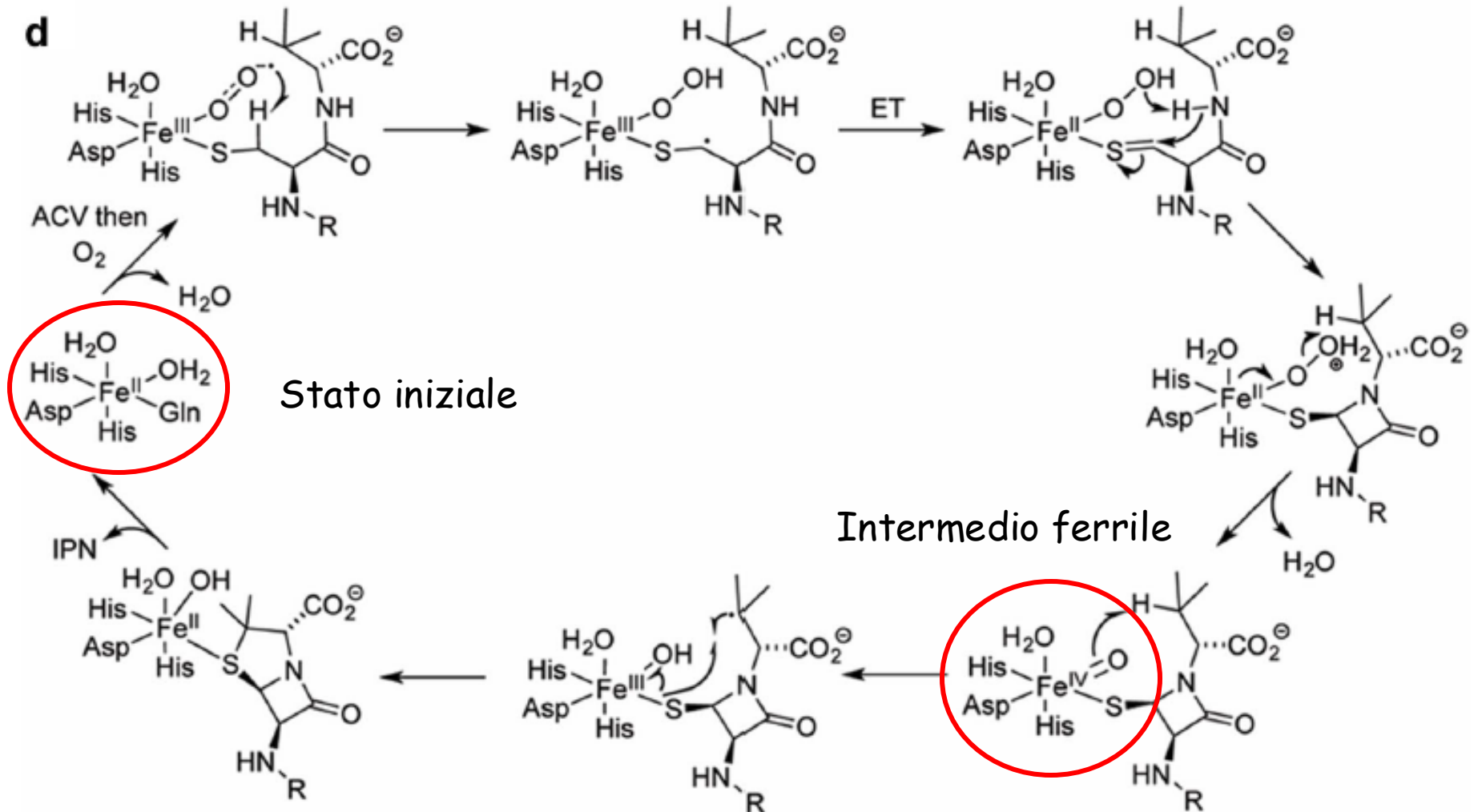
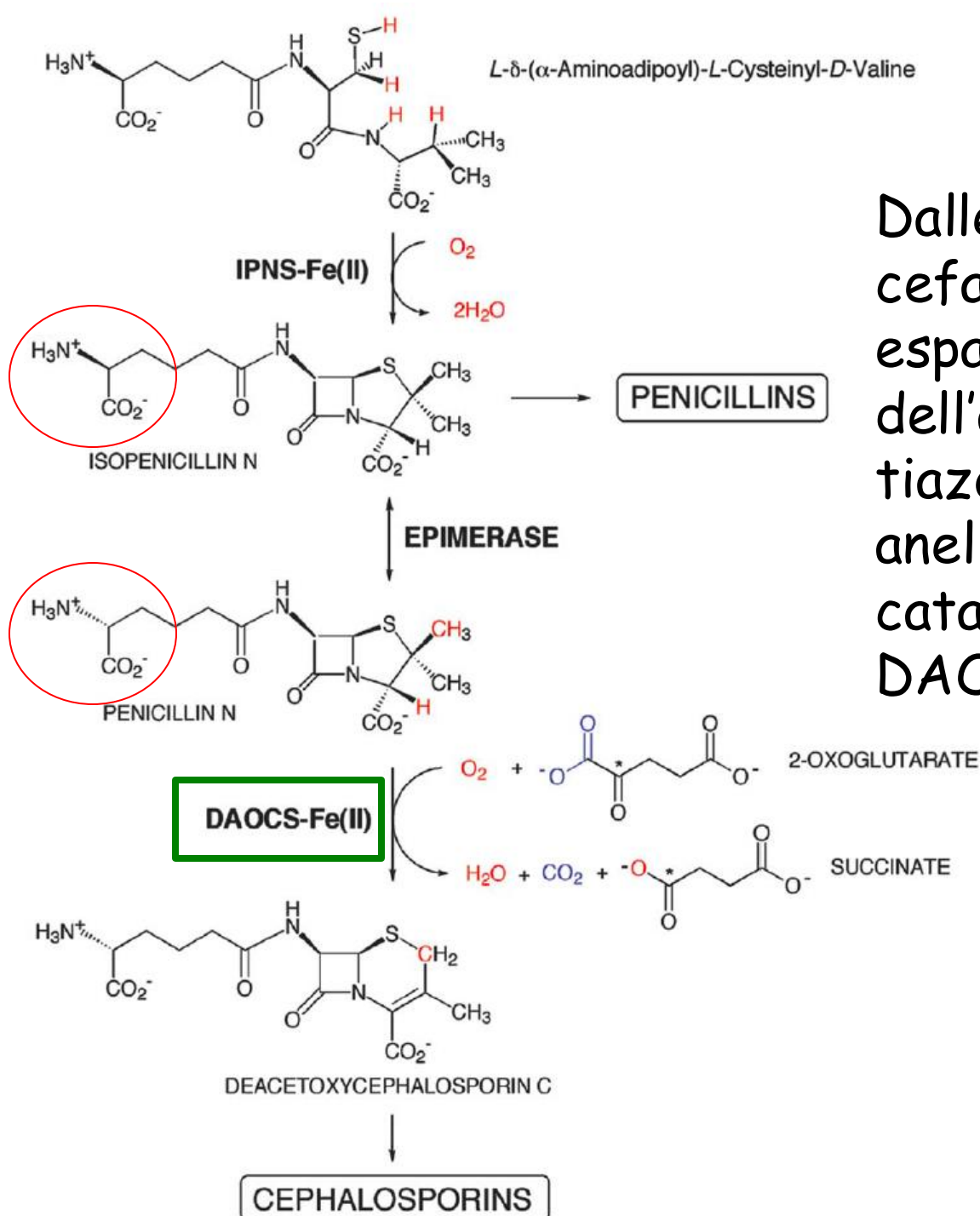


Fig. 2 Overview of the chemistry effected by IPNS. **a** The native substrate of IPNS catalysis (*left*) and a substrate analog that does not undergo a second cyclization (*right*). **b** The crystal structure of the ternary complex of Fe(II)-IPNS-ACV-NO (PDB ID: 1BLZ). The distance between the oxygen atom of NO to the β carbon of cysteine in ACV is 3.3 Å (*black dashes*). **c** The structure of the product of the

in crystallo reaction of Fe(II)-IPNS with ACmC reveals formation of the thiazolidine ring and sulfoxidation of the methylcysteine moiety (PDB ID: 1QJF). **d** A proposed mechanism for catalysis by IPNS. Instead of a formal ET in the second step after substrate binding, the two structures could also be considered resonance forms. For details, see the text

Meccanismo d'azione proposto per la IPNS





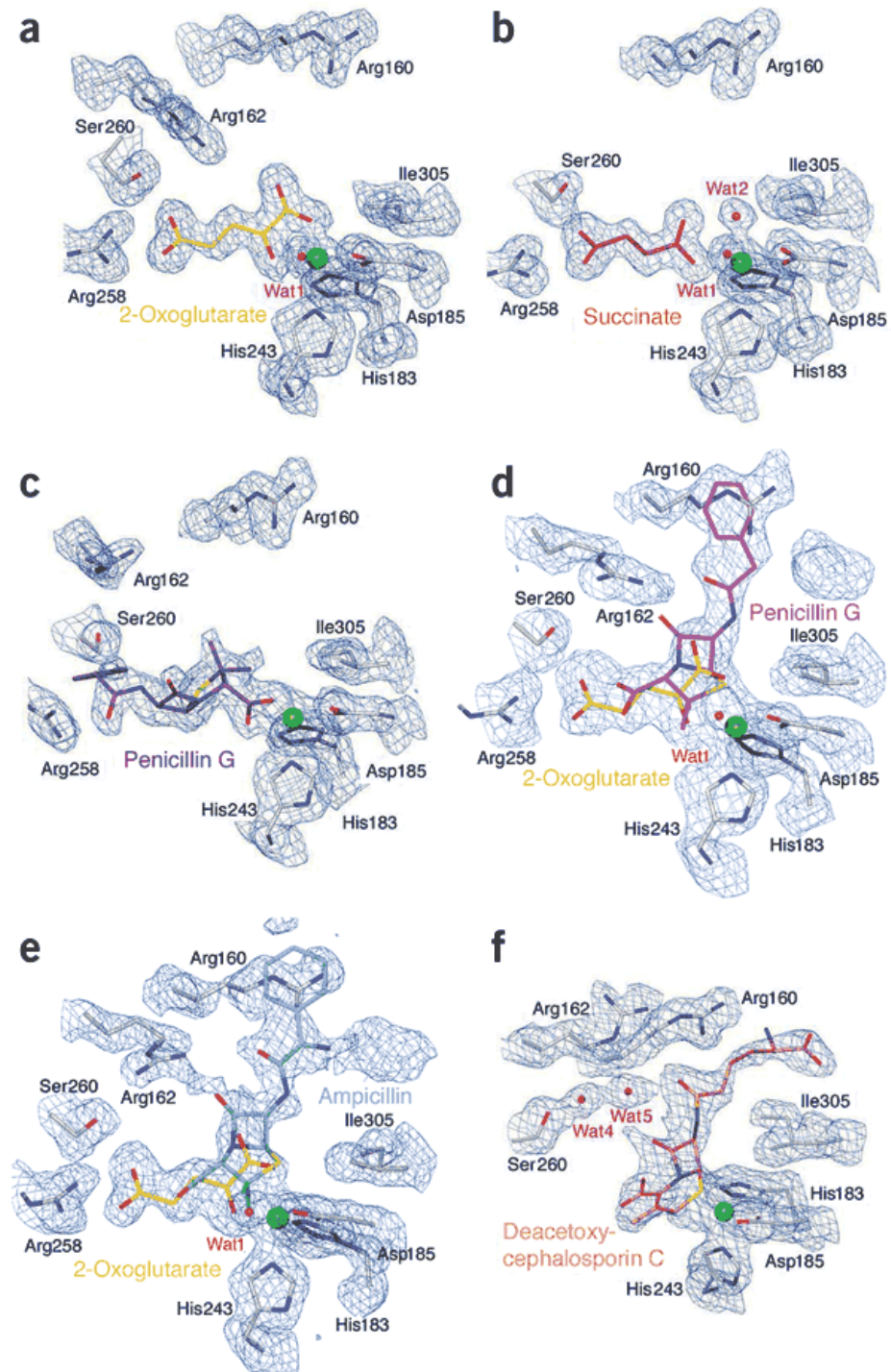
Dalle penicilline alle
 cefalosporine:
 espansione
 dell'anello
 tiazolidinico ad
 anello diidrotiazinico
 catalizzata dalla
DAOCS

Meccanismo d'azione della DAOCS, un enzima ferro- e α -chetoglutarato-dipendente.

Il sito di legame della penicillina è parzialmente sovrapposto a quello dell' α -chetoglutarato → reazione sequenziale

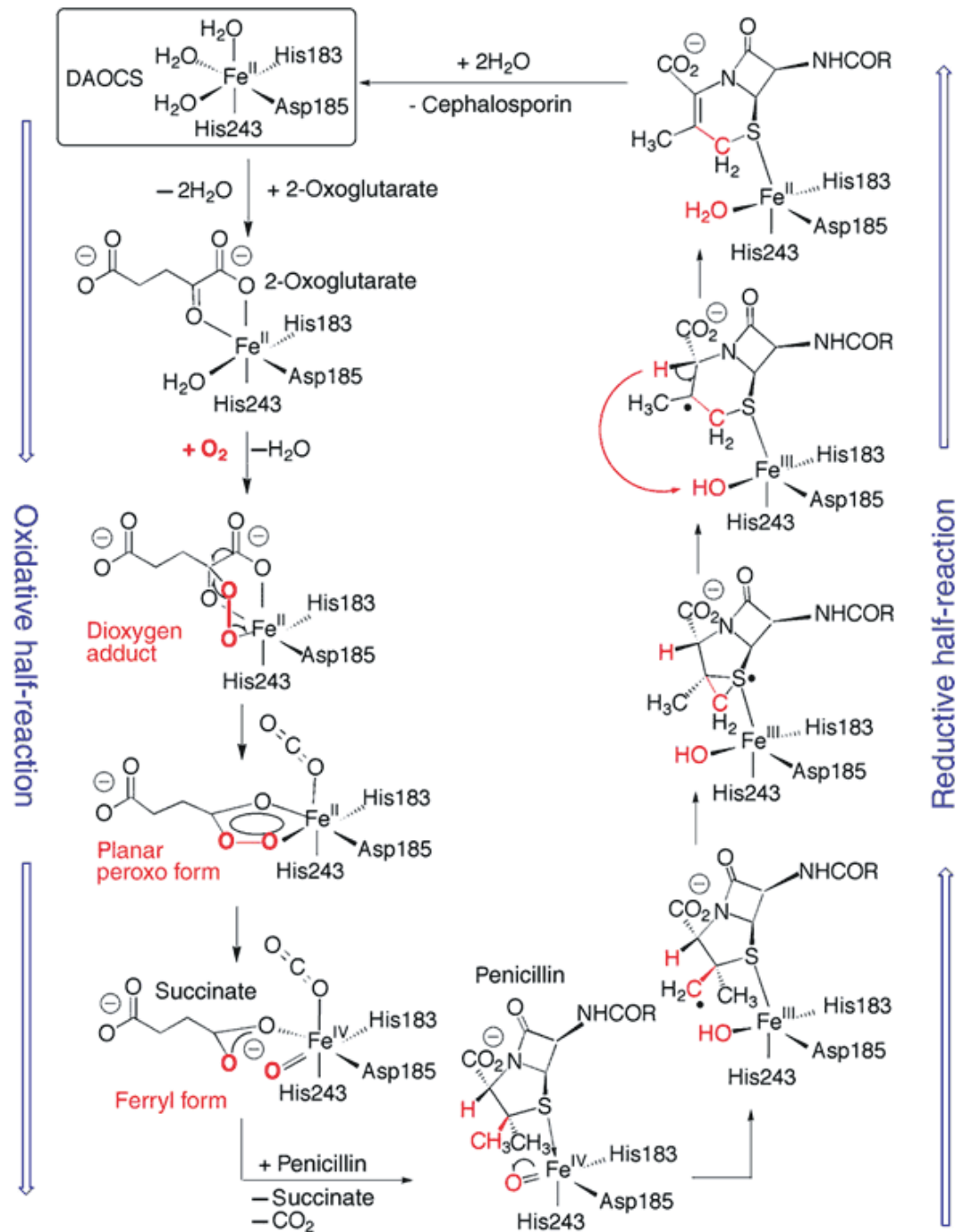
The active site region of DAOCS in complex with substrates and products.

(a) The DAOCS-Fe(II)-2-oxoglutarate complex at 1.5-Å resolution. (b) The DAOCS-Fe(II)-succinate complex at 1.5-Å resolution. (c) The DAOCS-Fe(II)-penicillin G complex at 1.6-Å resolution. (d) The DAOCS-Fe(II)-2-oxoglutarate-penicillin G complex at 1.7 Å resolution. (e) The DAOCS-Fe(II)-2-oxoglutarate-ampicillin complex at 1.5-Å resolution. (f) The DAOCS-Fe(II)-DAOC complex at 1.7-Å resolution. The density next to the penicillin side chain in d,e corresponds to a minor alternative conformation of the side chain. Dioxygen is expected to bind at the position of Wat1 in a. The oxygen of the ferryl iron would be formed at this site. The carbon atoms in 2-oxoglutarate are yellow, in succinate orange, in penicillin G magenta, in ampicillin cyan and in DAOC gold.



Meccanismo d'azione della DAOCS

- Il legame dell' α -chetoglutarato attiva il Fe permettendo il legame dell' O_2
- Con la decarbossilazione ossidativa del co-substrato si forma un intermedio ossidante stabilizzato dal succinato
- Quando la penicillina espelle il succinato scatena l'attacco ossidativo su se stessa
- La formazione di un radicale e il trasferimento di 2 elettroni all'ossigeno producono cefalosporina e H_2O



Ceppi per la produzione industriale di penicilline

Table 1 Penicillin titre various organisms

Species	Strain	Origin	Penicillin titre (g/l)	Reference
<i>Aspergillus nidulans</i>	7/142	Isolate	0.003	Simpson and Caten 1979
	A6-9	6 rounds CSI	0.012	Simpson and Caten 1979
<i>Aspergillus oryzae</i>	NS4DLDP	Isolate	0.00005	Marui et al. 2010
	OE-A	Transformant with 3 genes	0.00098	Marui et al. 2010
<i>Saccharomyces cerevisiae</i>	HpPen4	Transformant with 5 genes	0.001	Gidijala et al. 2009
<i>Penicillium nalgiovense</i>	Various	Food isolates	0.01–0.065	Laich et al. 2002
<i>Penicillium griseofulvum</i>	Various	Sausage isolates	0.04	Laich et al. 1999
<i>Penicillium notatum</i>	NRRL1249B1	Fleming's isolate	0.0012	Jami et al. 2010a
<i>Penicillium chrysogenum</i>	NRRL1951	Isolate cantaloupe	0.0155–0.150	Smidák et al. 2010b
				Jami et al. 2010a
	Wisconsin54-1255	Derivative of NRRL1951 (16 generations)	0.02 0.550–0.8	Kiel et al. 2005 Jami et al. 2010a
	DS08425	Derivative of Wisconsin54-1255 (9 generations)	0.98	Kiel et al. 2005
	DS04825-PEX11	Pex11 overexpression in DS04825	2.03	Kiel et al. 2005
	AS-P-78	Derivative of Wisconsin lineage	4.8	Jami et al. 2010a
	BW1952	Derivative of Wisconsin lineage	~10	Newbert et al. 1997
	NMU2/40	Derivative of Wisconsin lineage	6	Smidák et al. 2010b
	Best industrial strains	Derivative of Wisconsin lineage	30–55	Lein 1986
				Rowlands 1991

Miglioramento dei ceppi per la produzione industriale di penicillina

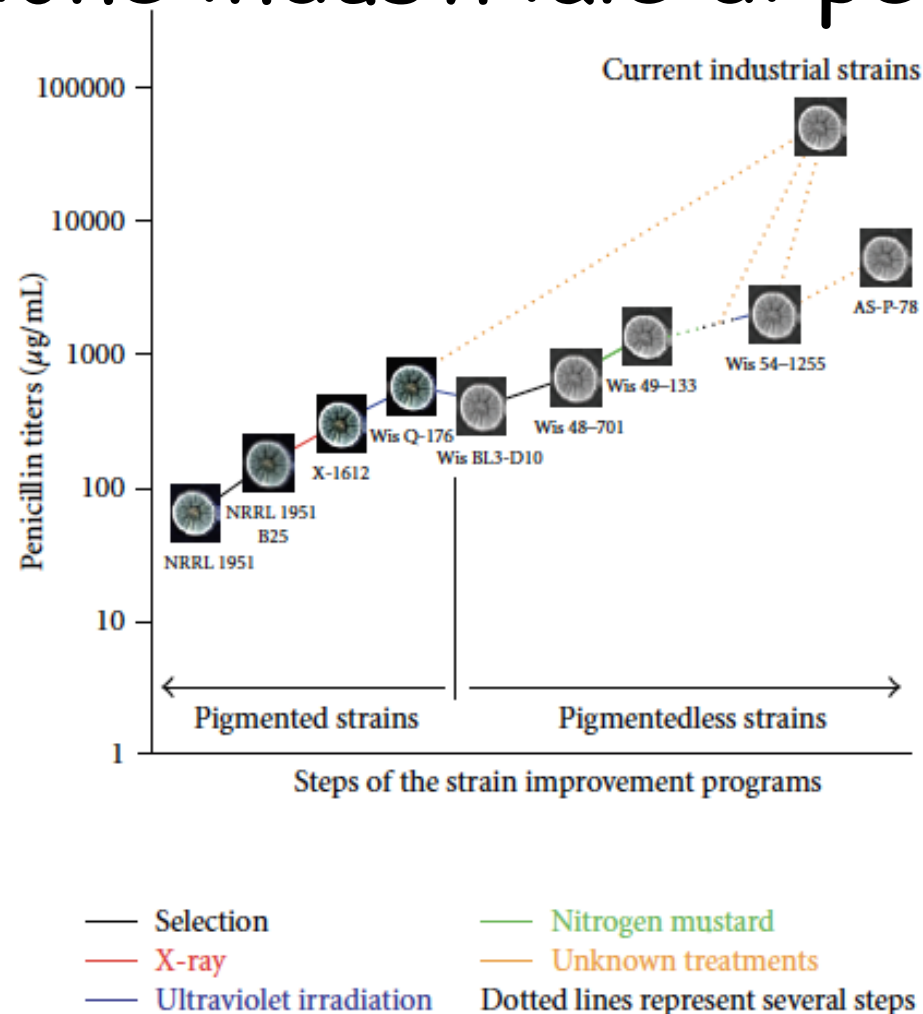


FIGURE 1: Scheme showing the industrial strain improvement program of *P. chrysogenum*, Barreiro et al., 2011.

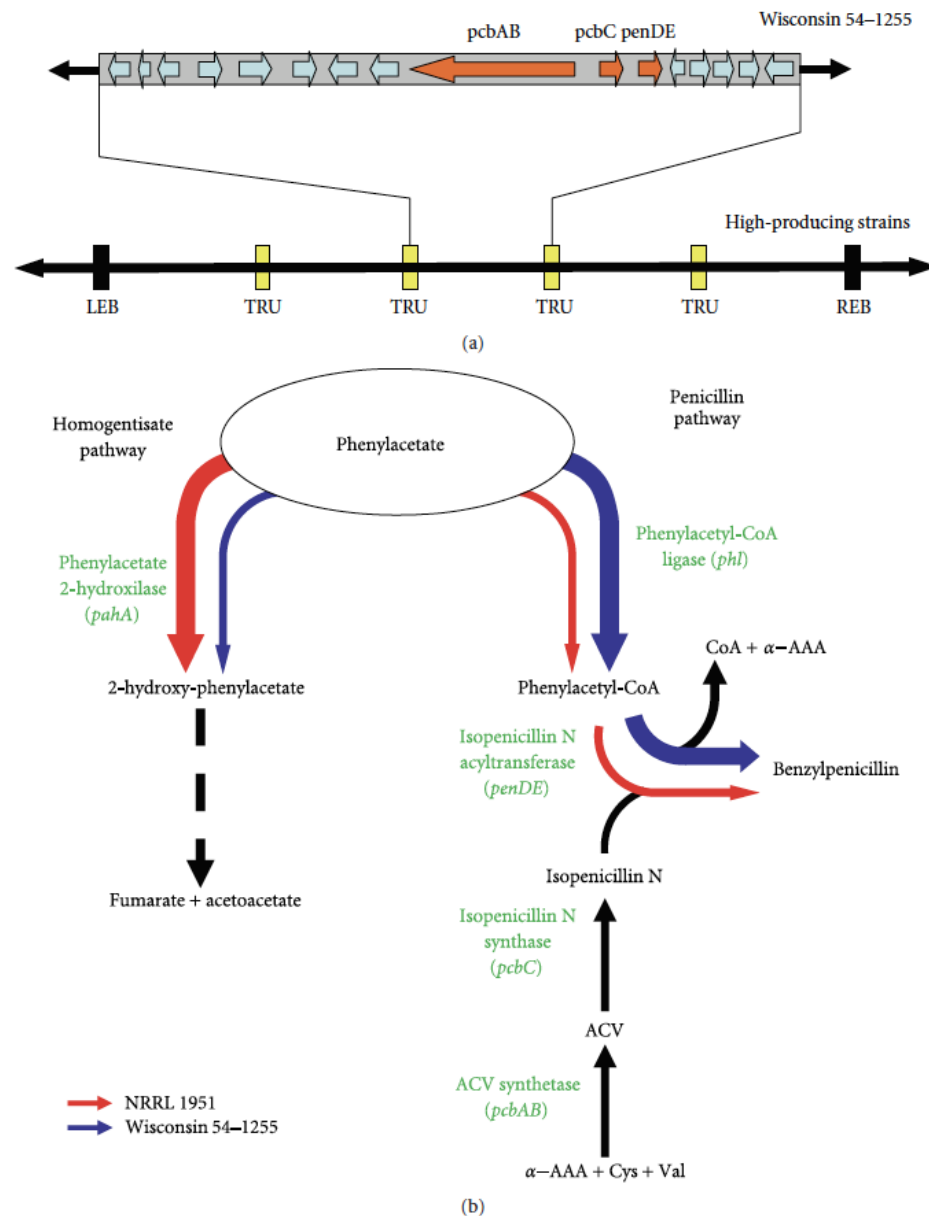


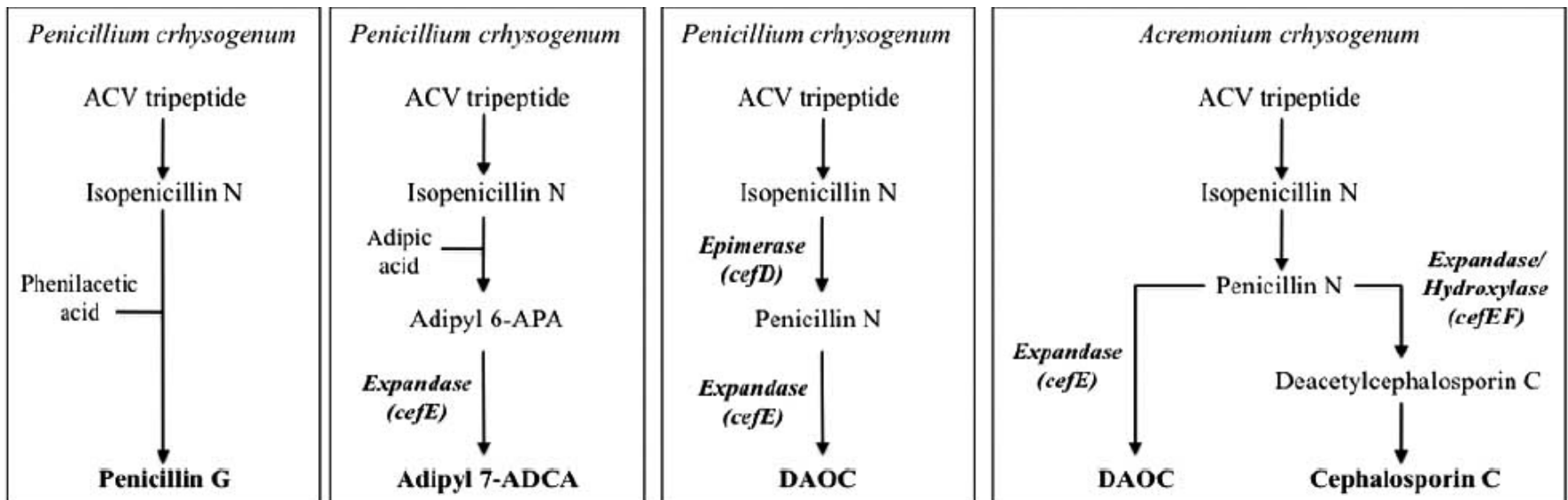
FIGURE 2: Examples of two well-known modifications that occurred during the strain improvement program. (a) Amplification of the DNA region containing the penicillin biosynthetic gene cluster in high-producing strains. LEB: left end border; REB: right end border; TRU: tandem repetition union. (b) Modification in the metabolic fluxes through the homogentisate pathway for phenylacetic acid catabolism and the penicillin biosynthetic pathway. Thickness of arrows indicates the flux rate through a specific enzyme. α -AAA: α -aminoadipic acid; ACV: δ -(L- α -aminoadipyl)-L-cysteinyl-D-valine; CoA: coenzyme A; Cys: cysteine; Val: Valine, Barreiro et al., 2011.

Table 2 Changes in penicillin manufacturing technology

Fermentation	1950	2000
Carbon source	Lactose	Glucose/sucrose
Operational mode	Batch	Fed-batch
Medium sterilization	Batch	Continuous
Air filtration	Depth filters	Membrane filters
Feeds	None	Many
Morphology	Filamentous	Pelleted
Cycle time	120 h	120–200 h
Tank volume (1,000 gallons)	10–20	20–60
Assay	Bio-assay	HPLC
Control	Temperature only	Computerized
Titer (g/l)	0.5–1.0	>40
Recovery and purification		
Mycelium removal	Filtration	Whole broth
Operational mode	Batch	Semi-continuous
Extraction stages	Many	Single to few
Precursor recovery and re-use	Discarded	Recovered and re-used
Efficiency (%)	70–80	>90
Environmental issues	Few	Many
Bulk cost	~US\$275–350/kg	~US\$15–20/kg

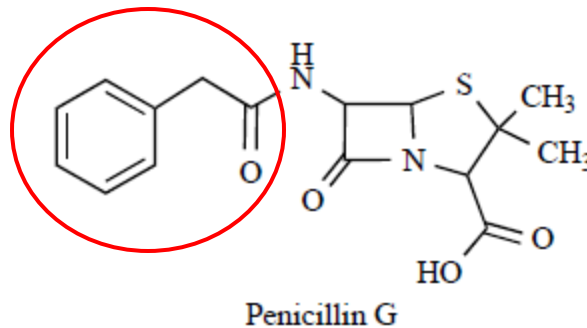
Produzione di penicilline e cefalosporine

- Ceppi attuali di *Penicillium chrysogenum*: rese fino a circa 70 gr **Penicillina G**/litro di coltura
- Fermentazione aerobica
 - Fonti di carbonio: glucosio e prodotti contenuti nell'acqua di macerazione del mais (corn steep liquor)
 - Fonte di azoto: ammoniaca
 - Aggiunta di acido fenilacetico nella fase stazionaria



Penicilline semi-sintetiche

- Le penicilline semi-sintetiche si ottengono a partire dalla penicillina G naturale
- Dalla penicillina G viene rimossa la catena laterale producendo acido 6-aminopenicillanico (6-APA)
- Il 6-APA viene acilato con una nuova catena laterale
- Le β -lattam-acilasi sono enzimi in grado di catalizzare queste due reazioni



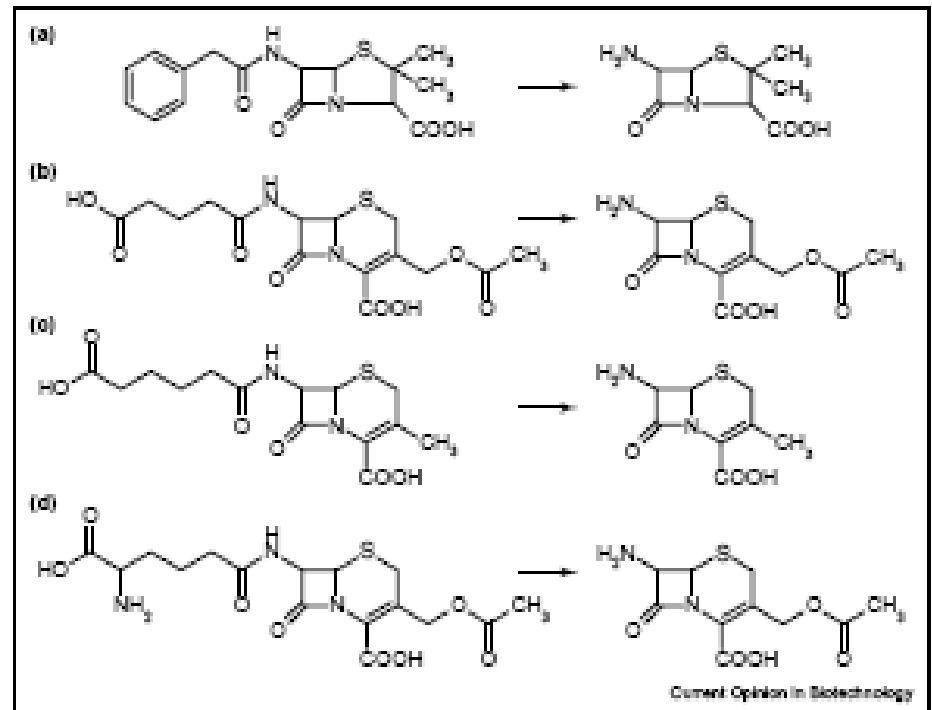
Reazioni di idrolisi catalizzate dalle β -lattam-acilasi: rimozione della catena laterale acilica

La **penicillina acilasi** idrolizza la penicillina G ad acido 6-aminopenicillanico (a).

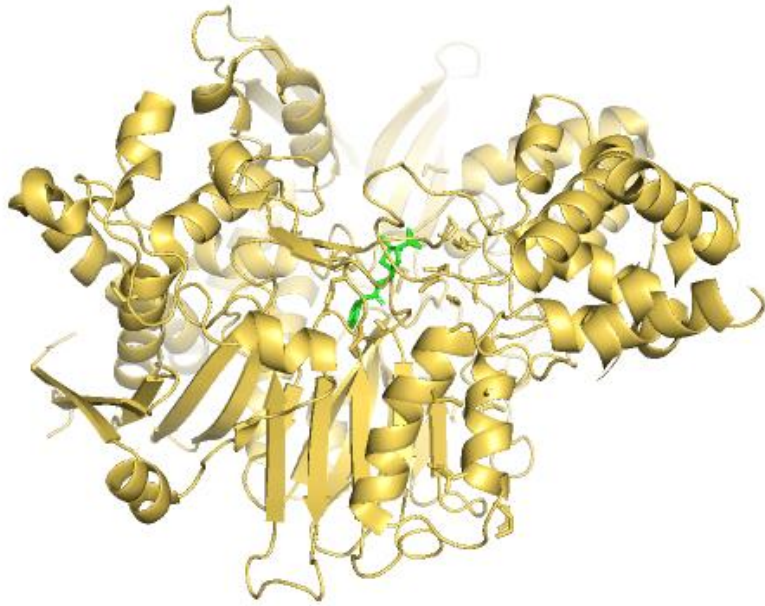
La **cefalosporina acilasi** idrolizza il glutaril-7-ACA a 7-ACA (b).

Mutanti della cefalosporina acilasi idrolizzano l'adipil-7-ADCA a 7-ADCA (c) e la cefalosporina C a 7-ACA (d).

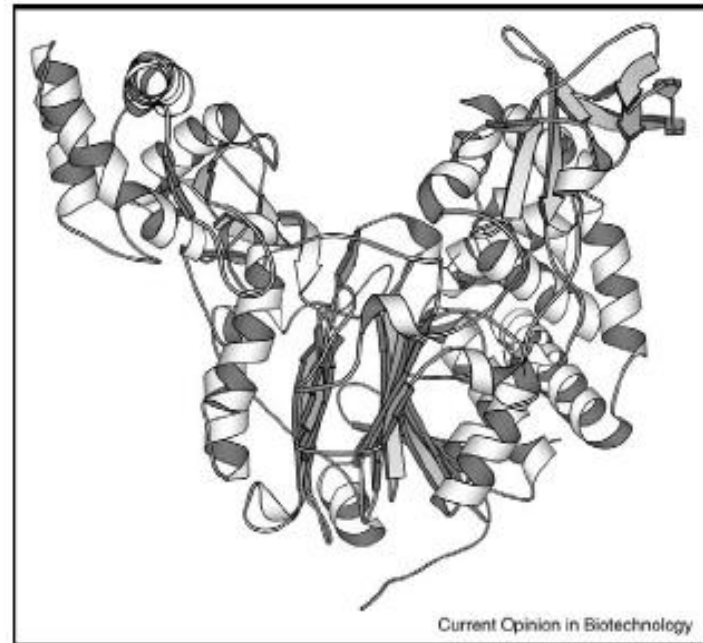
Il 6-APA e il 7-ACA sono i precursori delle penicilline e delle cefalosporine semi-sintetiche



Struttura della penicillina acilasi e della cefalosporina acilasi



Struttura della penicillina acilasi
di *Escherichia coli*

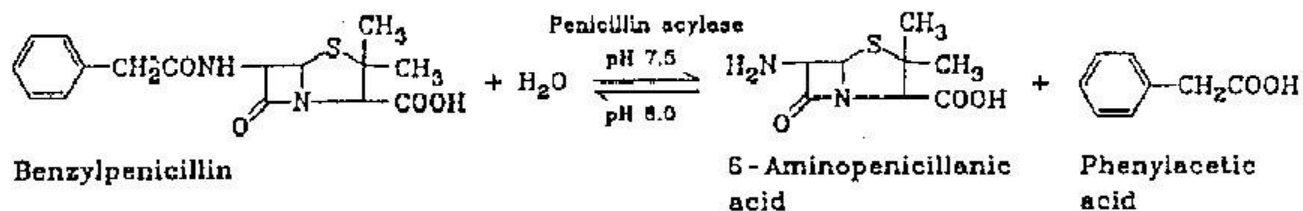


The crystal structure of *Pseudomonas diminuta* cephalosporin acylase [11]. (Figure drawn with Molscript [27].)

Organismi produttori: batteri e funghi
(*Escherichia coli*, *Bacillus megaterium*, *Kluyvera citrophila*,
Pseudomonas melanogenum, *Penicillium chrysogenum*).

Penicillina acilasi

- La penicillina acilasi catalizza l'idrolisi delle penicilline con la formazione dell'acido 6-aminopenicillanico (6-APA), precursore delle penicilline semi-sintetiche.
- La reazione è **reversibile** e la direzione dipende dal pH.
- Substrati: penicilline, alcune cefalosporine, amidi ed esteri.
- Utilizzata per la produzione di penicilline semi-sintetiche, sia per la produzione di 6-APA che nelle reazioni di sintesi.



Penicillin acylase reaction.

Penicillina acilasi

- La penicillina acilasi di *E. coli* è formata da due subunità α (24 kDa) e β (62 kDa) che derivano da un precursore di 95 kDa processato proteoliticamente, con rimozione di un peptide leader di 26 aa che serve a dirigere la proteina nel periplasma e di un peptide interno di 54 aa che dà origine alle subunità e attiva l'enzima.
- La specificità per il substrato (catena laterale della penicillina) si trova nella subunità α , mentre nella subunità β si trova la serina B1 cataliticamente attiva.

Meccanismo d'azione della penicillina acilasi

- La serina B1 viene attivata come nucleofilo dal suo stesso gruppo amminico libero.
- Il meccanismo catalitico prevede la formazione di un **intermedio covalente acil-enzima**.
- L'intermedio acil-enzima viene idrolizzato da una molecola di acqua

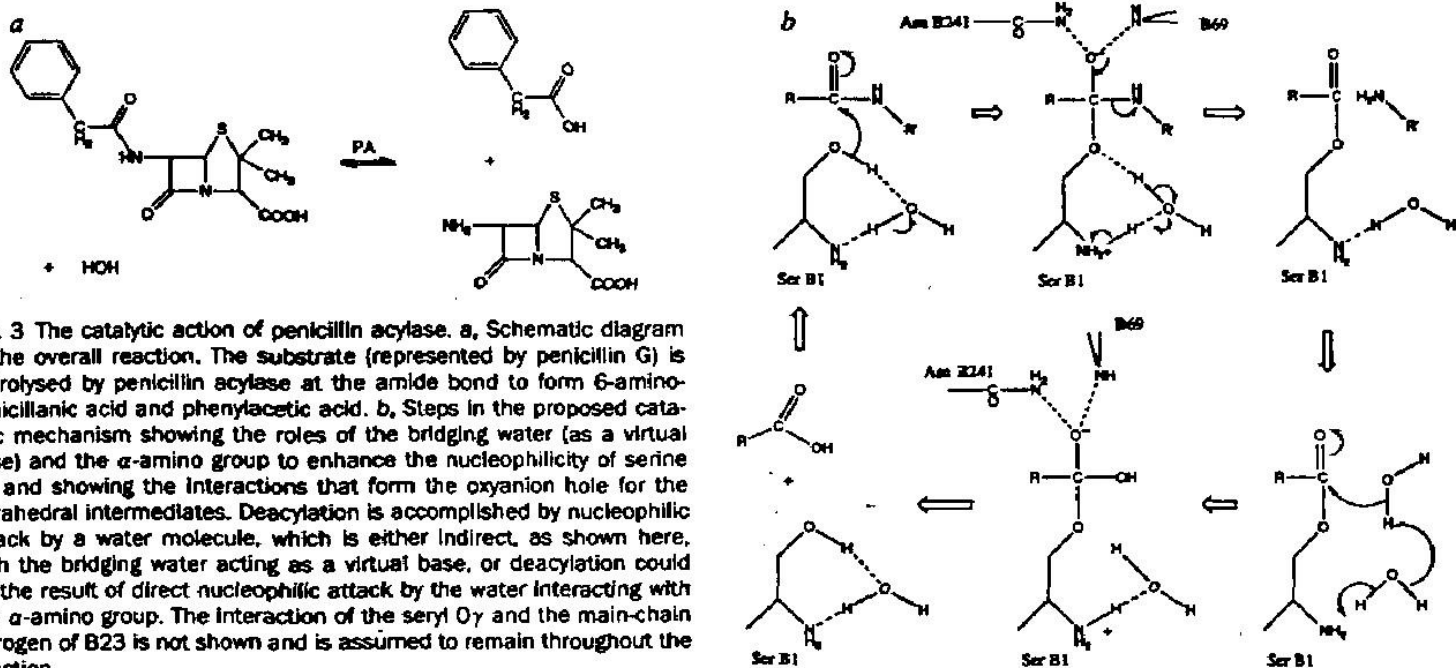
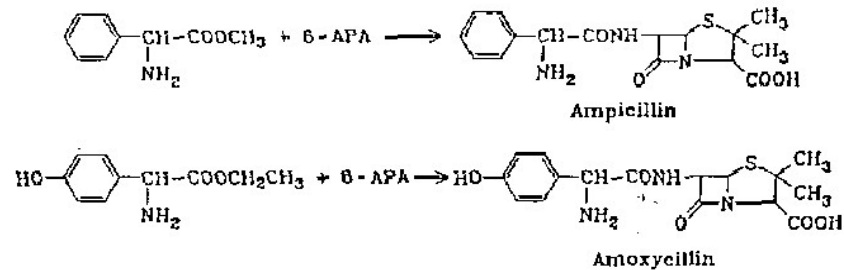


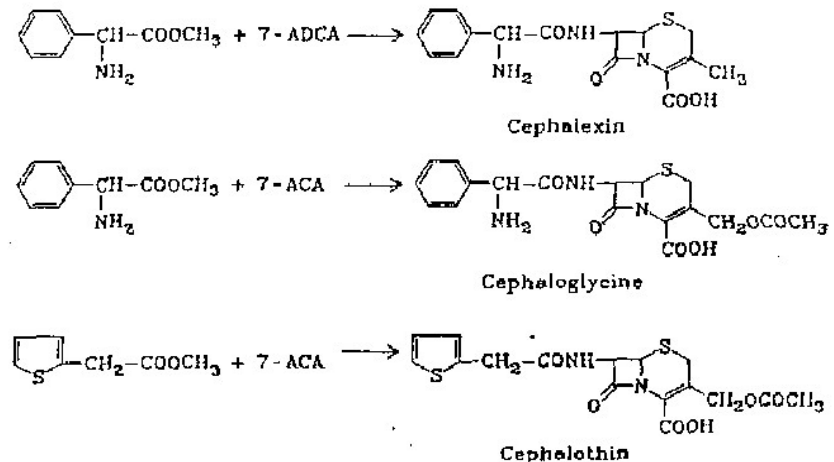
FIG. 3 The catalytic action of penicillin acylase. *a*, Schematic diagram of the overall reaction. The substrate (represented by penicillin G) is hydrolysed by penicillin acylase at the amide bond to form 6-aminopenicillanic acid and phenylacetic acid. *b*, Steps in the proposed catalytic mechanism showing the roles of the bridging water (as a virtual base) and the α -amino group to enhance the nucleophilicity of serine B1 and showing the interactions that form the oxyanion hole for the tetrahedral intermediates. Deacylation is accomplished by nucleophilic attack by a water molecule, which is either indirect, as shown here, with the bridging water acting as a virtual base, or deacylation could be the result of direct nucleophilic attack by the water interacting with the α -amino group. The interaction of the seryl O γ and the main-chain nitrogen of B23 is not shown and is assumed to remain throughout the reaction.

Penicillina acilasi per la produzione di β -lattamici semi-sintetici

Sintesi di penicilline semi-sintetiche a partire da **esteri** della catena laterale R (agenti acilanti) e 6-APA.



Sintesi di cefalosporine semi-sintetiche a partire da **esteri** della catena laterale R (agenti acilanti) e 7-ACA/7-ADCA.



Sintesi dell'amoxicillina

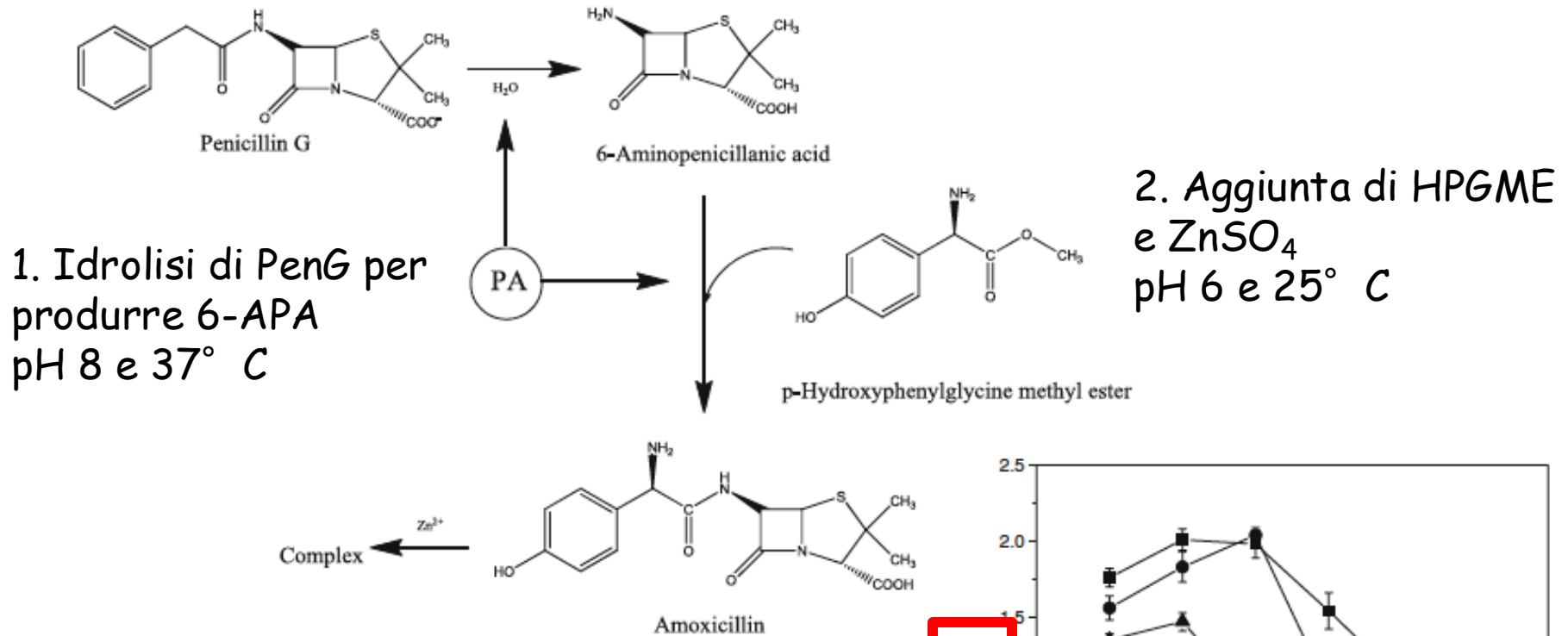


Fig. 1 Scheme of one-pot enzymatic synthesis of amoxicillin in the presence of zinc ions

In presenza di **zinco** l'amoxicillina precipita, favorendo la reazione di sintesi

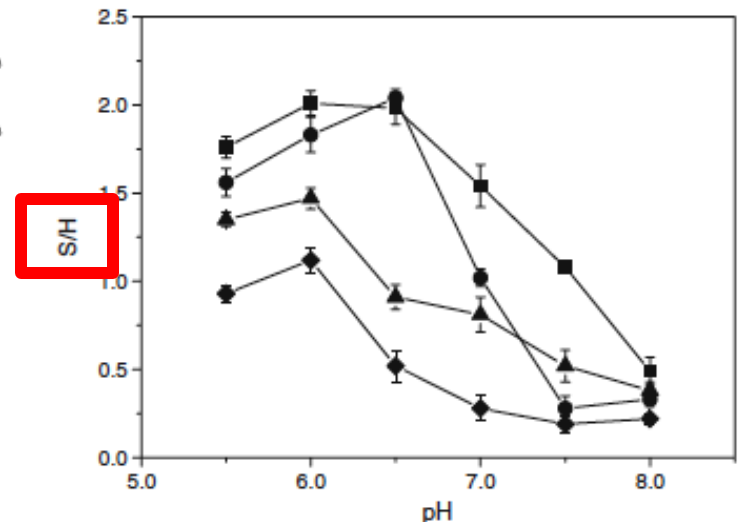


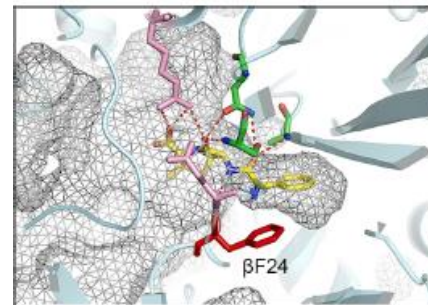
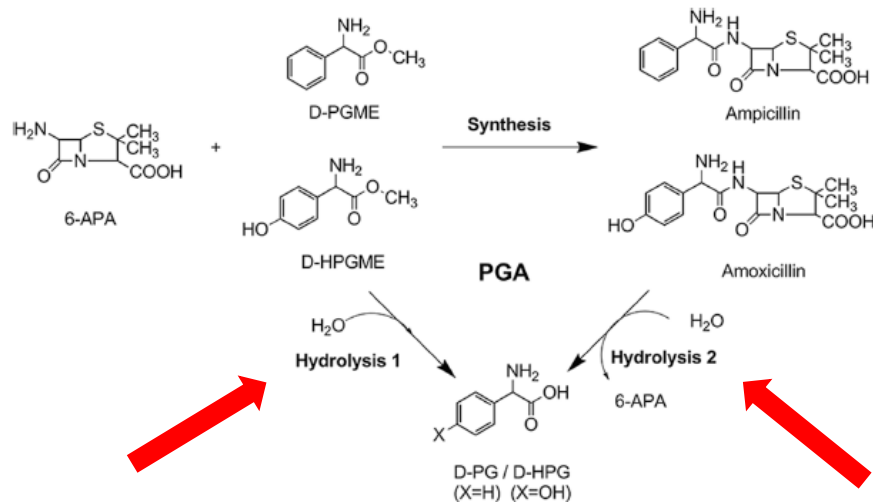
Fig. 4 Effect of pH on the S/H of the immobilized penicillin acylase. Square: AIPA; circle: KcPA; upright triangle: PCA; diamond: IPA

Ingegnerizzazione della penicillina acilasi per migliorare la sintesi

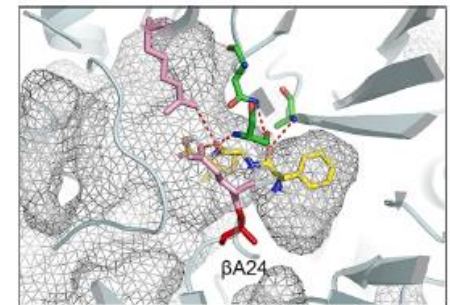
Reazioni di **idrolisi** dell'agente acilante o del prodotto della reazione diminuiscono la resa della sintesi

Aumento del rapporto $V_{\text{synthesis}}/V_{\text{hydrolysis}}$: β F24 e α F146

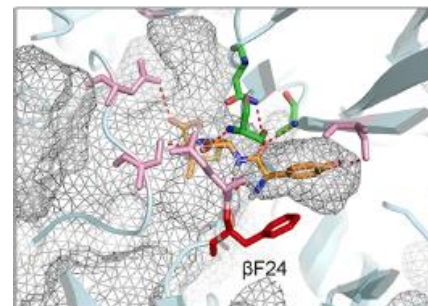
l'enzima deve avere maggiore affinità per l'agente acilante che per l'antibiotico



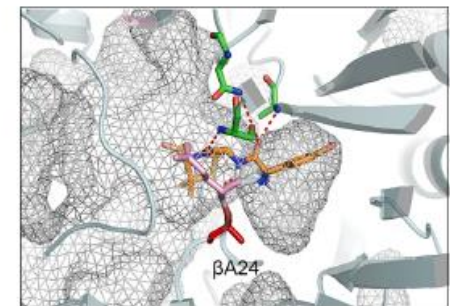
A (Ampicillin)



B (Ampicillin)



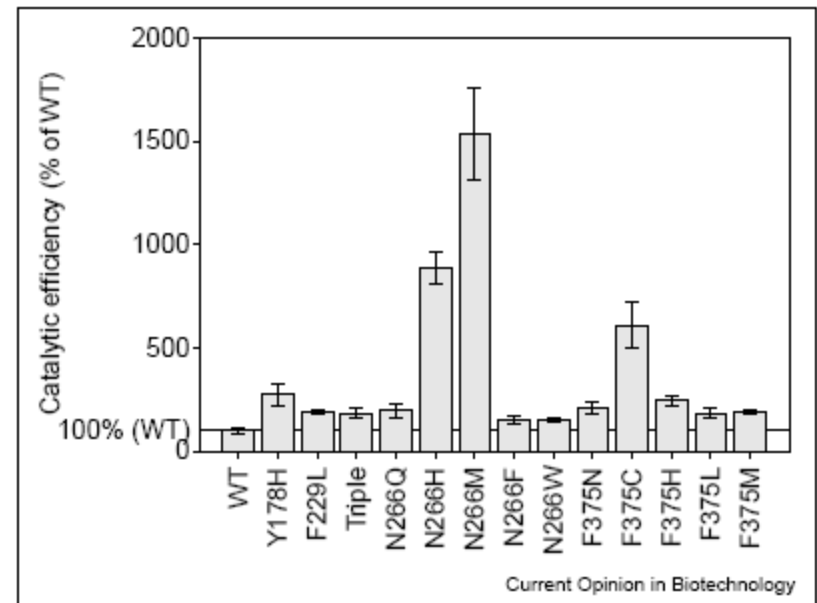
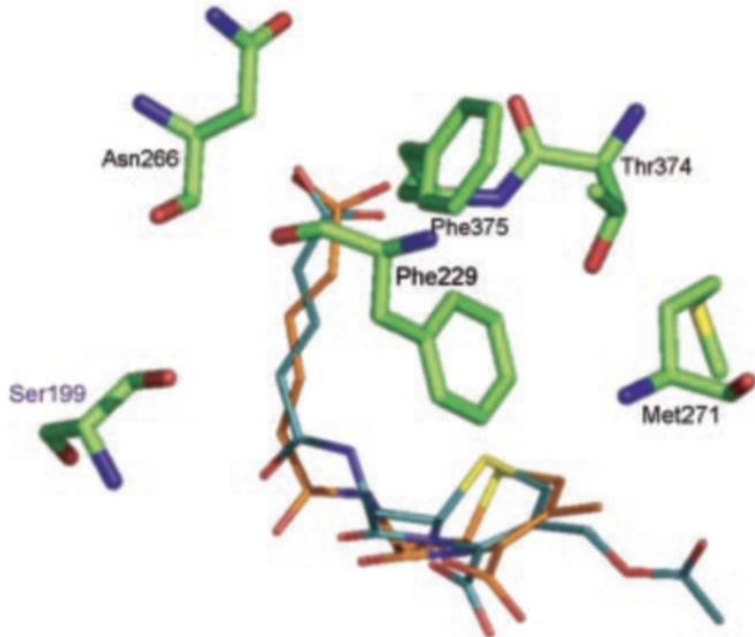
C (Amoxicillin)



D (Amoxicillin)

Ingegnerizzazione della cefalosporina acilasi

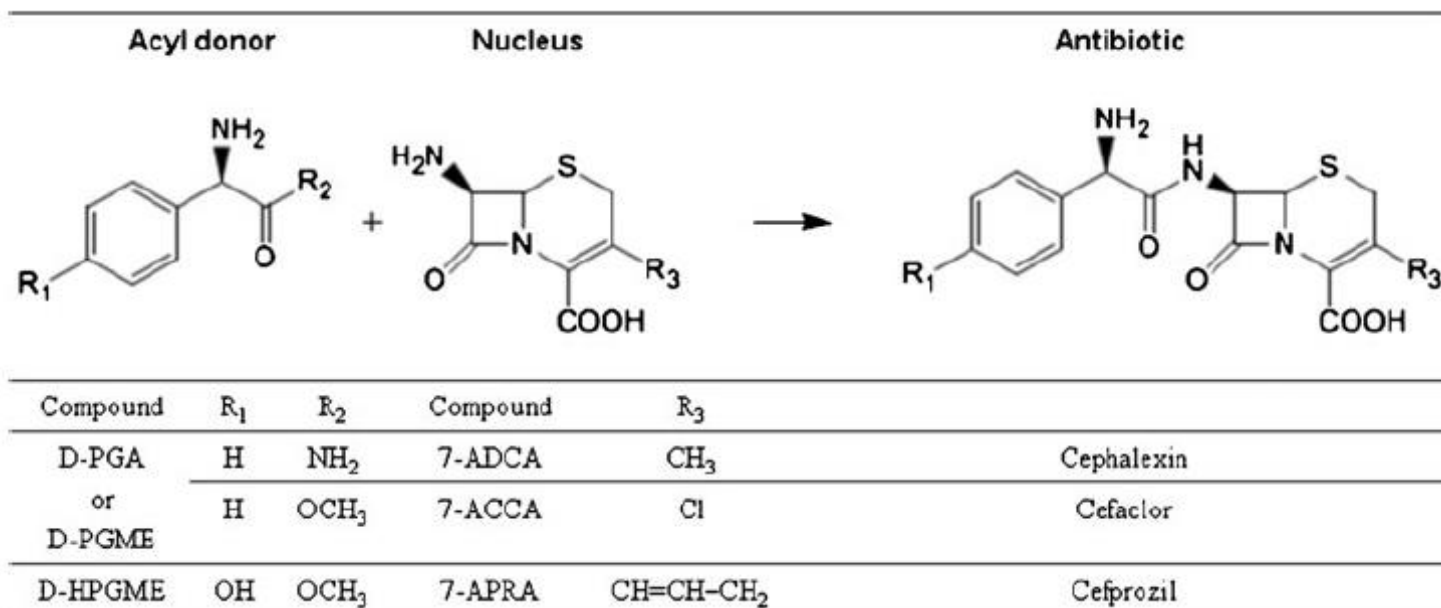
Alterazione della specificità di substrato per migliorare l'idrolisi della cefalosporina C e di adipyl-7-ADCA per la produzione di 7-ACA and 7-ADCA



Mutagenesis alters the substrate specificity of a cephalosporin acylase. The preferred substrate of cephalosporin acylase is glutaryl-7-ACA and activity towards the novel β -lactam fermentation product adipyl-7-ADCA is much lower. Mutants that exhibit an increased catalytic efficiency towards adipyl-7-ADCA were obtained by directed evolution of a cephalosporin acylase. Shown here are the best mutants found by random mutagenesis [17,18**] and saturation mutagenesis of selected residues [20,21]. A greater than 15-fold increase in activity was observed for the single mutant N266M (in single-letter amino acid code). WT, wild type; triple, the multiple mutant M271V/Q291K/T374S.

Efficient biocatalyst for large-scale synthesis of cephalosporins, obtained by combining immobilization and site-directed mutagenesis of penicillin acylase

Davide A. Cecchini • Roberto Pavesi • Sara Sanna •
 Simona Daly • Roberto Xaiz • Massimo Pregnolato •
 Marco Terreni



Ingegnerizzazione della penicillina acilasi

Identificazione di β F24 e α F146 coinvolti nel riconoscimento del substrato. La sostituzione β F24A migliora il rapporto V_S/V_H (bassa attività amidasica). La sostituzione α F146Y mantiene elevata attività nel confronti dell'agente acilante.

Immobilizzazione covalente del biocatalizzatore su Eupergit C

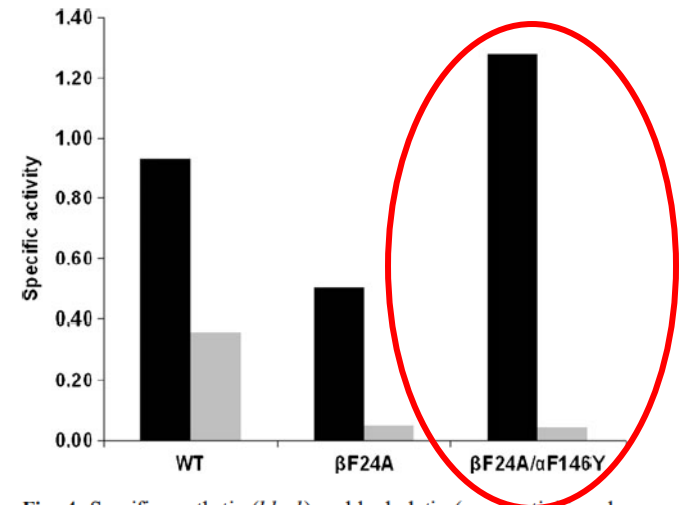
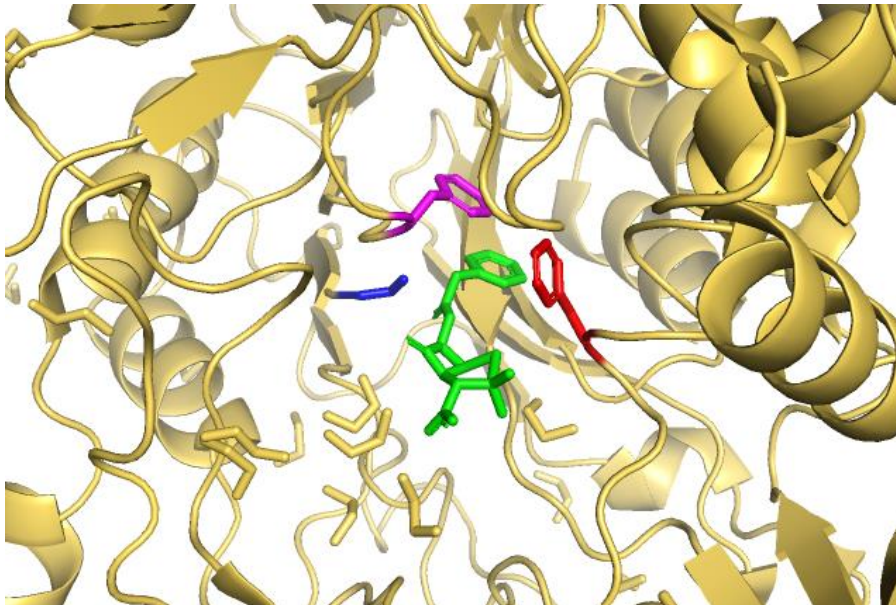


Fig. 4 Specific synthetic (black) and hydrolytic (grey) activities observed in the reactions performed using the immobilized WT and mutated acylases at 28 °C. Synthesis was performed with D-PGME. Activities are expressed as micromole per minute ($\mu\text{mol min}^{-1}$) of cephalixin synthesized or hydrolyzed per milligram of enzyme

Table 3 Loading capacity of glyoxyl Eupergit C250L toward WT-PA and immobilization of PA mutants at the optimal enzyme concentration

Enzyme	Specific activity (U _{PGK} /mg)	Offered enzyme (mg/g)	Yields (%) ^a	Activity (%) ^b	Derivative specific activity ^c
WT	38	5	42	80 (42%)	36
WT	38	10	38	140 (37%)	37
WT	38	15	30	160 (30%)	36
βF24A	11	10	38	18 (16%)	4.7
βF24A/αF146Y	0.7	10	40	2.3 (33%)	0.6

The results reported are the average of three experiments (SD<10%)

^a Percentage of the total protein evaluated by Bradford assay

^b Activity toward penicillin G potassium salt expressed per gram of support (U_{PGK}/g) and percentage of activity retained after immobilization

^c Specific activity of the immobilized protein expressed as U_{PGK}/mg of protein

Table 4 Enzymatic synthesis of cephalixin, cefaclor and cefprozil catalyzed by the immobilized wild type and PA mutants with different activated acyl donors and at different temperatures

Enzyme	Derivative (g)	Acyl donor	Product	Temperature (°C)	vs ^a (μmol min ⁻¹)	Time ^b (min)	Conversion (%)
WT	1.5	D-PGA	Cephalexin	28	4.4	20	67
βF24A	1.5		Cephalexin	28	0.1	630	68
βF24A/αF146Y	1.5		Cephalexin	28	0.3	570	77
WT	1	D-PGME	Cephalexin	4	0.4	240	90
	1		Cephalexin	28	3.7	30	76
βF24A	1		Cephalexin	4	0.3	360	99
	1		Cephalexin	28	1.9	60	98
βF24A/αF146Y	1		Cephalexin	4	0.8	240	99
	1		Cephalexin	28	4.0	40	99
WT	1	D-PGME	Cefaclor	4	0.4	300	84
	1		Cefaclor	28	2.0	50	65
βF24A	1		Cefaclor	4	0.1	1,500	98
	1		Cefaclor	28	1.5	140	98
βF24A/αF146Y	1		Cefaclor	4	0.5	360	99
	1		Cefaclor	28	3.7	60	99
WT	1	D-HPGME	Cefprozil	28	0.2	160	59
βF24A	1		Cefprozil	28	0.5	100	98
βF24A/αF146Y	1		Cefprozil	28	0.6	100	99

^a Reaction rate (vs) was measured monitoring the formation of the acylation product (μmol min⁻¹) by HPLC analysis

^b Time employed to reach the maximal conversion

Efficient cascade synthesis of ampicillin from penicillin G potassium salt using wild and mutant penicillin G acylase from *Alcaligenes faecalis*

Senwen Deng^{a,c,1}, Xiaoqiang Ma^{c,1}, Erzhen Su^{b,*}, Dongzhi Wei^{c,**}

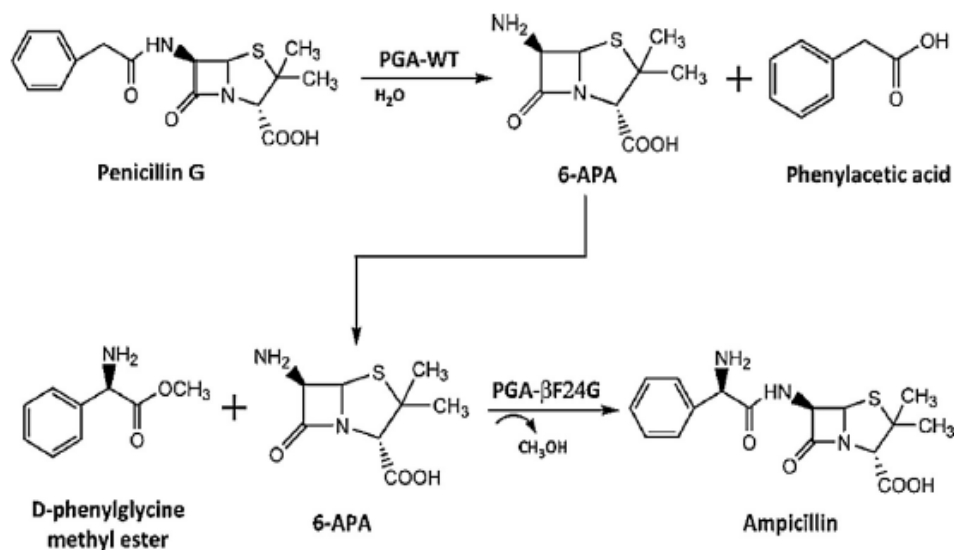


Fig. 1. Scheme of two-enzyme two-step cascade synthesis of ampicillin.

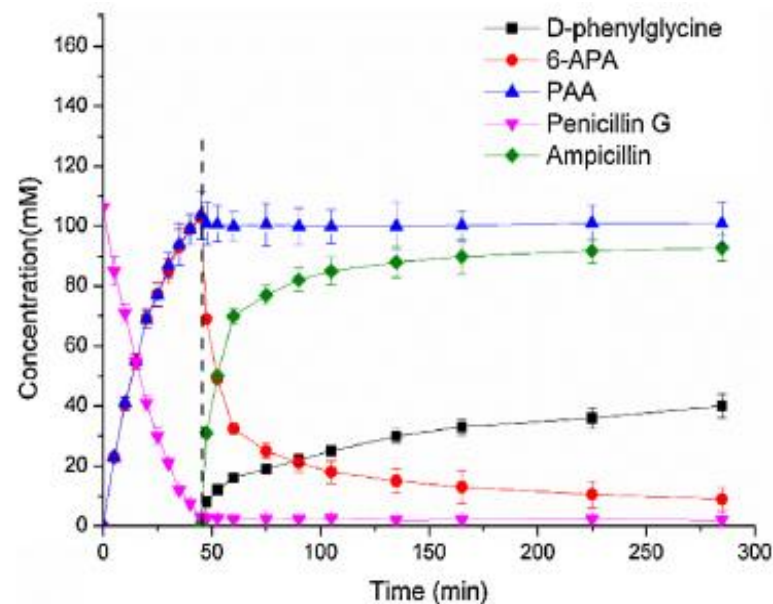
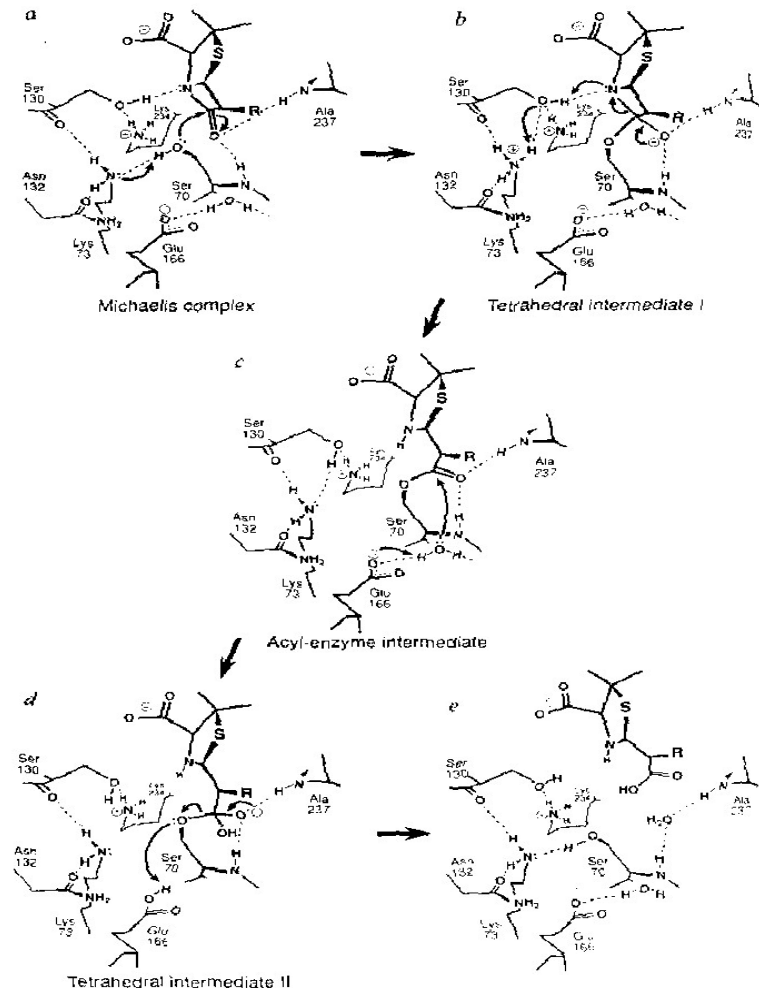


Fig. 6. Time course of two-enzyme two-step cascade synthesis of ampicillin. Dotted line is the demarcation line of the first step and the second step. Conditions of the first step: pH 8.0, 28 °C, 4% (w/v) PGK, 0.03 g/mL immobilized wild Af PGA; Conditions of the second step: initial pH 6.3, 28 °C, 150 mM D-PGME, 0.19 g/mL immobilized βF24 G mutant Af PGA.

Resistenza alle penicilline: Meccanismo d'azione delle β -lattamasi di classe A

Le β -lattamasi inattivano le penicilline perchè catalizzano l'idrolisi dell'anello β -lattamico.

Le β -lattamasi di classe A appartengono alla famiglia delle serina-idrolasi. **Ser70** è il nucleofilo che attacca l'atomo di carbonio dell'anello β -lattamico. Lys73 attiva Ser70 sottraendo il protone e trasferendolo a Ser130, che a sua volta lo trasferisce all'atomo di azoto dell'anello, aprendolo. Si forma un intermedio acil-enzima che viene deacilato attraverso l'azione di una molecola d'acqua assistita da Glu166.



Struttura di analoghi dello stato di transizione che agiscono da inibitori delle β -lattamasi di classe A

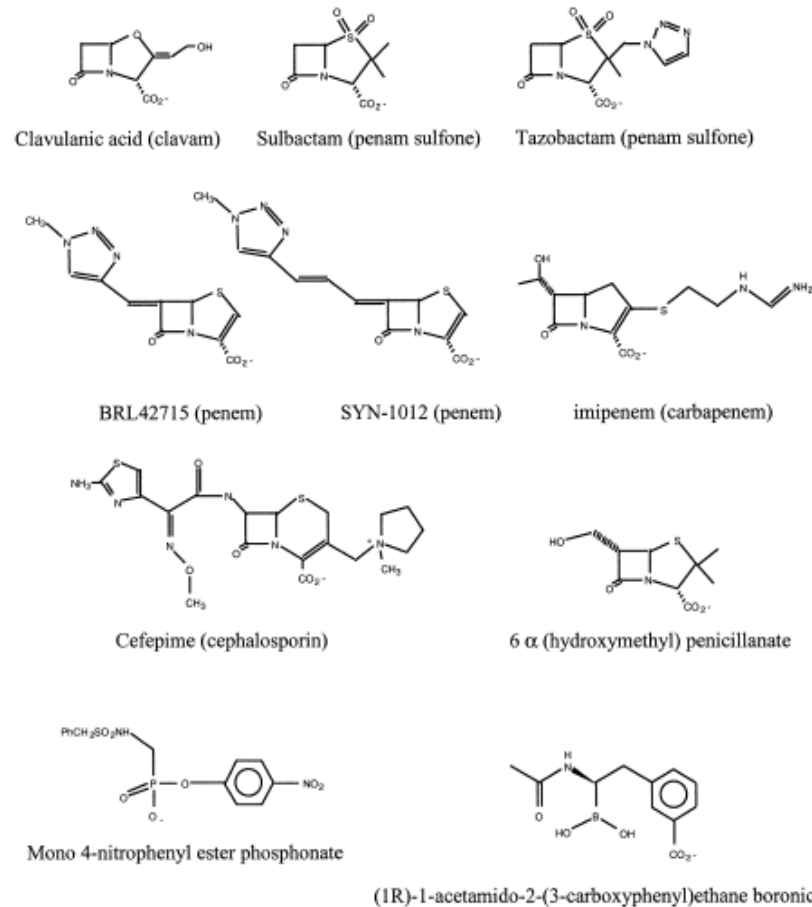


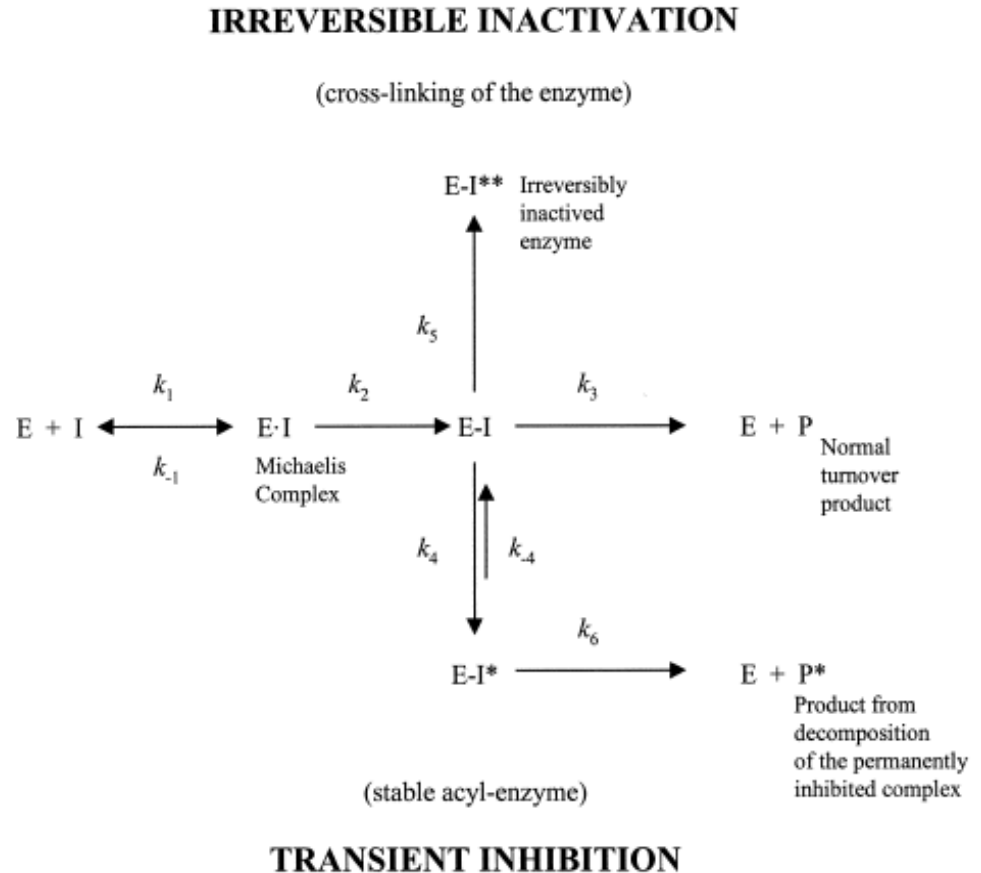
Fig. 1. Structure of β -lactams and transition state analogs with inhibitory activity against serine β -lactamases.

Schema dell'inattivazione delle β -lattamasi

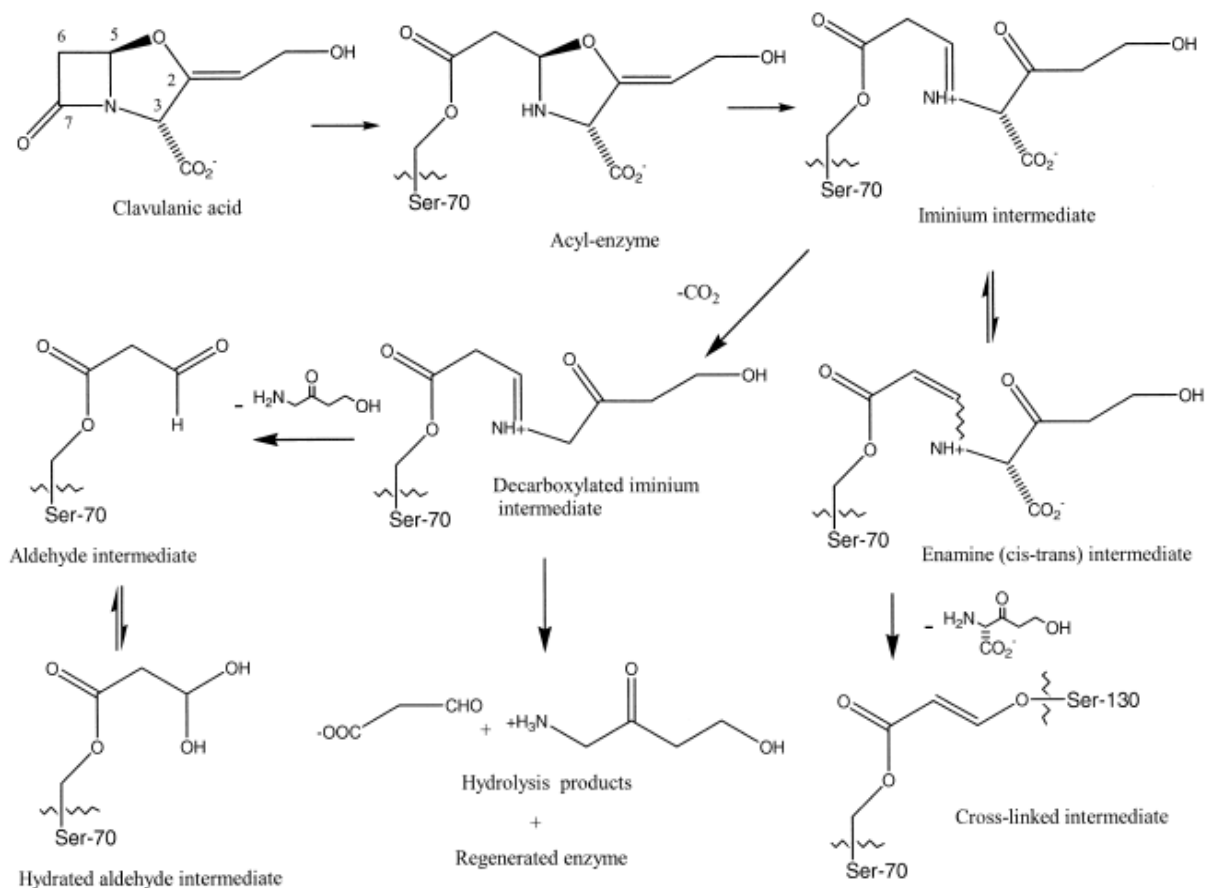
L'intermedio acil-enzima può ripartirsi attraverso due vie, che portano all'inibizione transiente o irreversibile.

In realtà, l'idrolisi dell'intermedio modificato $E-I^*$ è molto lenta e quindi l'enzima si può considerare permanentemente inibito.

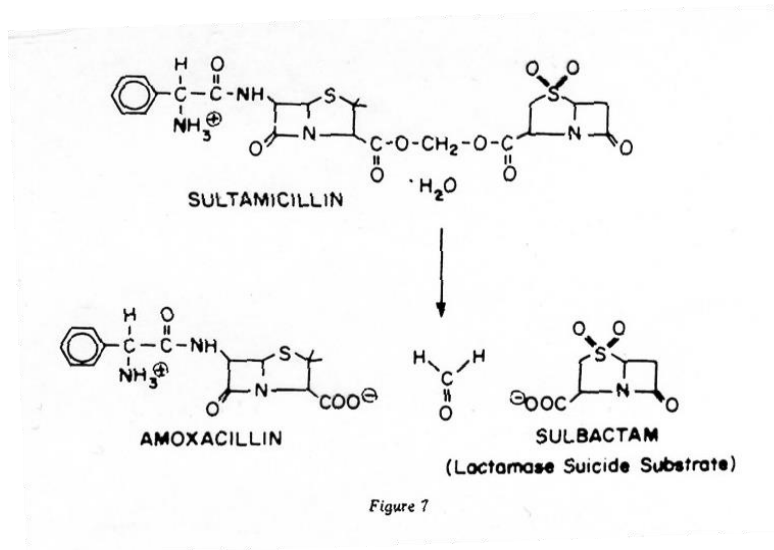
Il cross-linking porta all'inattivazione irreversibile dell'enzima.



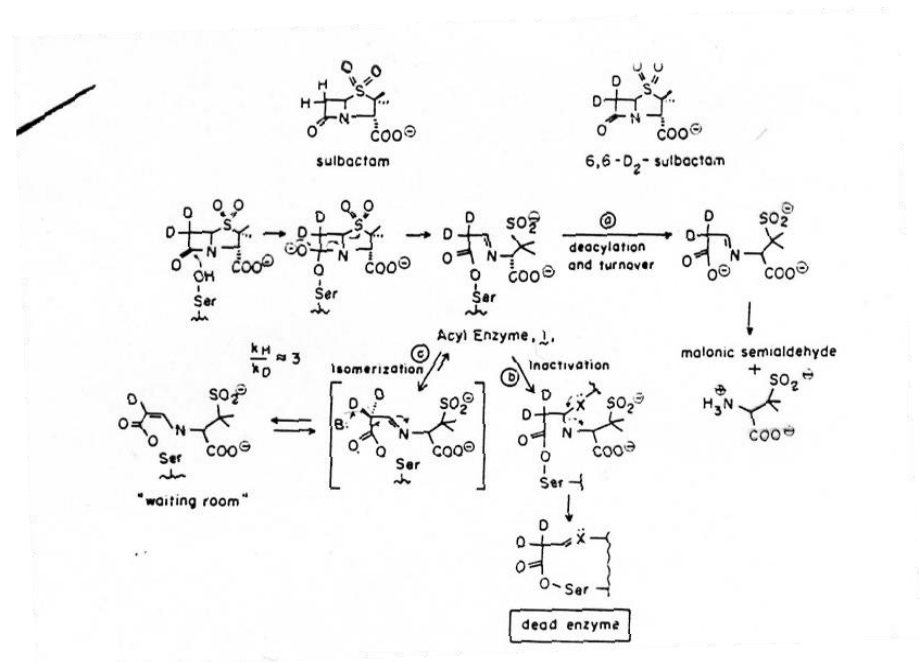
Meccanismo di inattivazione delle β -lattamasi di classe A da parte dell'acido clavulanico, un inibitore suicida.



La **sultamicillina**: un farmaco che potenzia l'azione dell'antibiotico (amoxicillina) con quella dell'inibitore delle β -lattamasi (sulbactam)



Meccanismo d'azione del sulbactam



Nuovi inibitori delle β -lattamasi di classe A

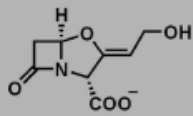
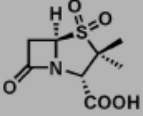
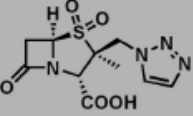
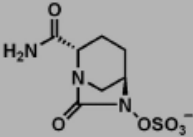
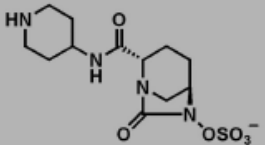
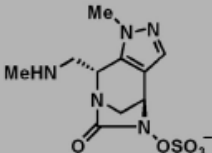
Generations:	1: Clavulanates	2: Penicillin Sulfones		3: Diazabicyclooctanes (DBOs)		
β -Lactamase Inhibitors						
	Clavulanate	Sulbactam	Tazobactam	Avibactam	MK-7655	NXL105
Development Status	Approved (Augmentin) GlaxoSmithKline	Approved (Cefobid) Pfizer	Approved (Zosyn) Pfizer	Phase II/III AstraZeneca/Forest	Phase I/II Merck	Preclinical AstraZeneca

Figure 6 Next generation 'non- β -lactam' β -lactamase inhibitors (diazabicyclooctanes; DBOs) in clinical development.

Ceftazidime-Avibactam: A Novel Cephalosporin/ β -Lactamase Inhibitor Combination for the Treatment of Resistant Gram-negative Organisms

