Chemistry 259

Medicinal Chemistry of Modern Antibiotics

Spring 2012

Lecture 5: *Modern Target Discovery & MOA*

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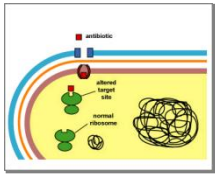
Drug Discovery & Development Process: General Overview

Pre-clinical Clinical

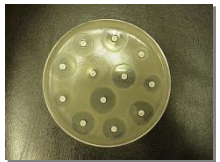
Discovery **Development**

Target Discovery | **Lead Discovery** | **Lead Optimisation** | **ADMET*** | **Clinical Trials** | **FDA Review & Approval**

- target identification
- target validation



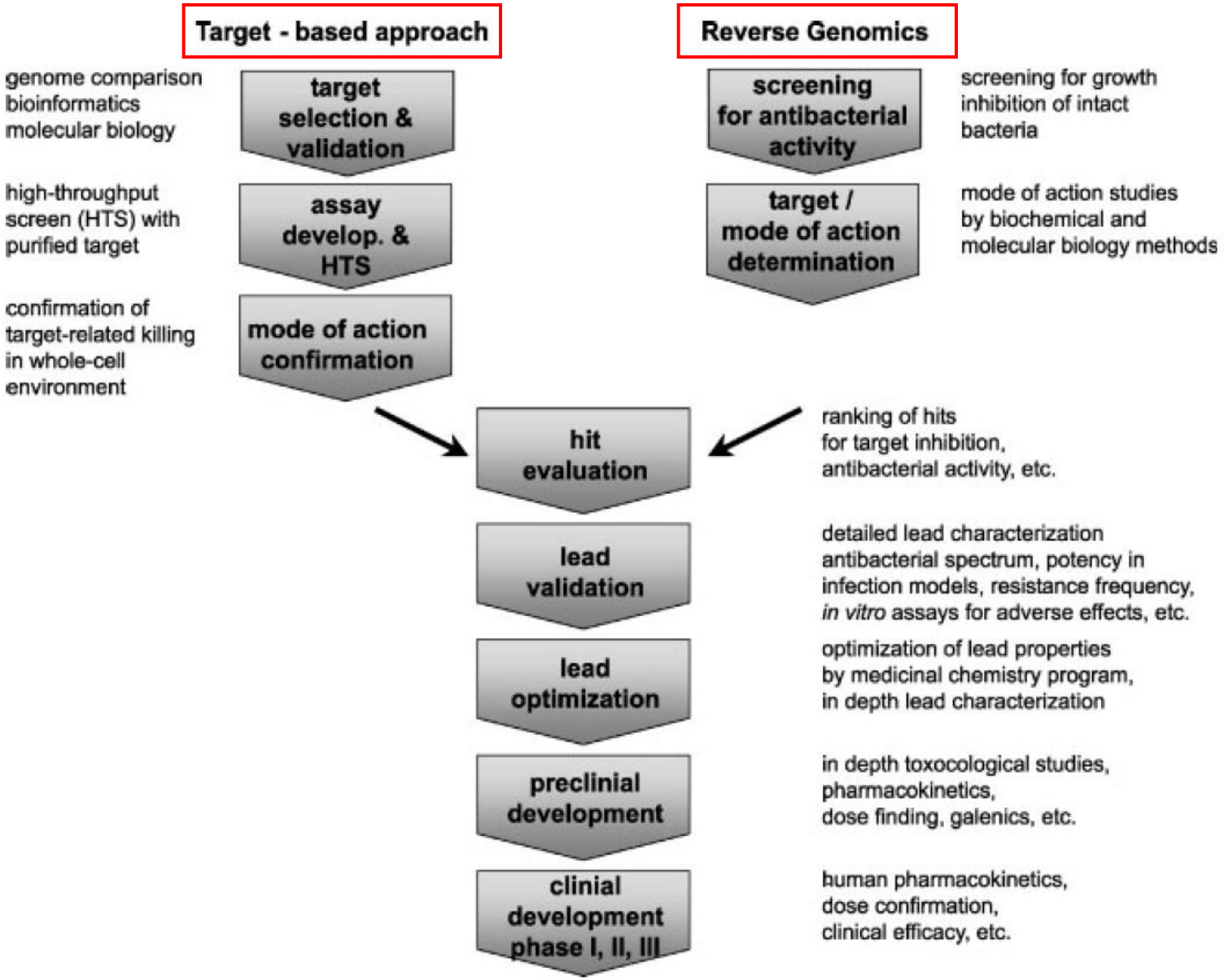
- assay development
- screening
- drug design
- medicinal chemistry ("hit to lead")
- mechanism of action (MOA)



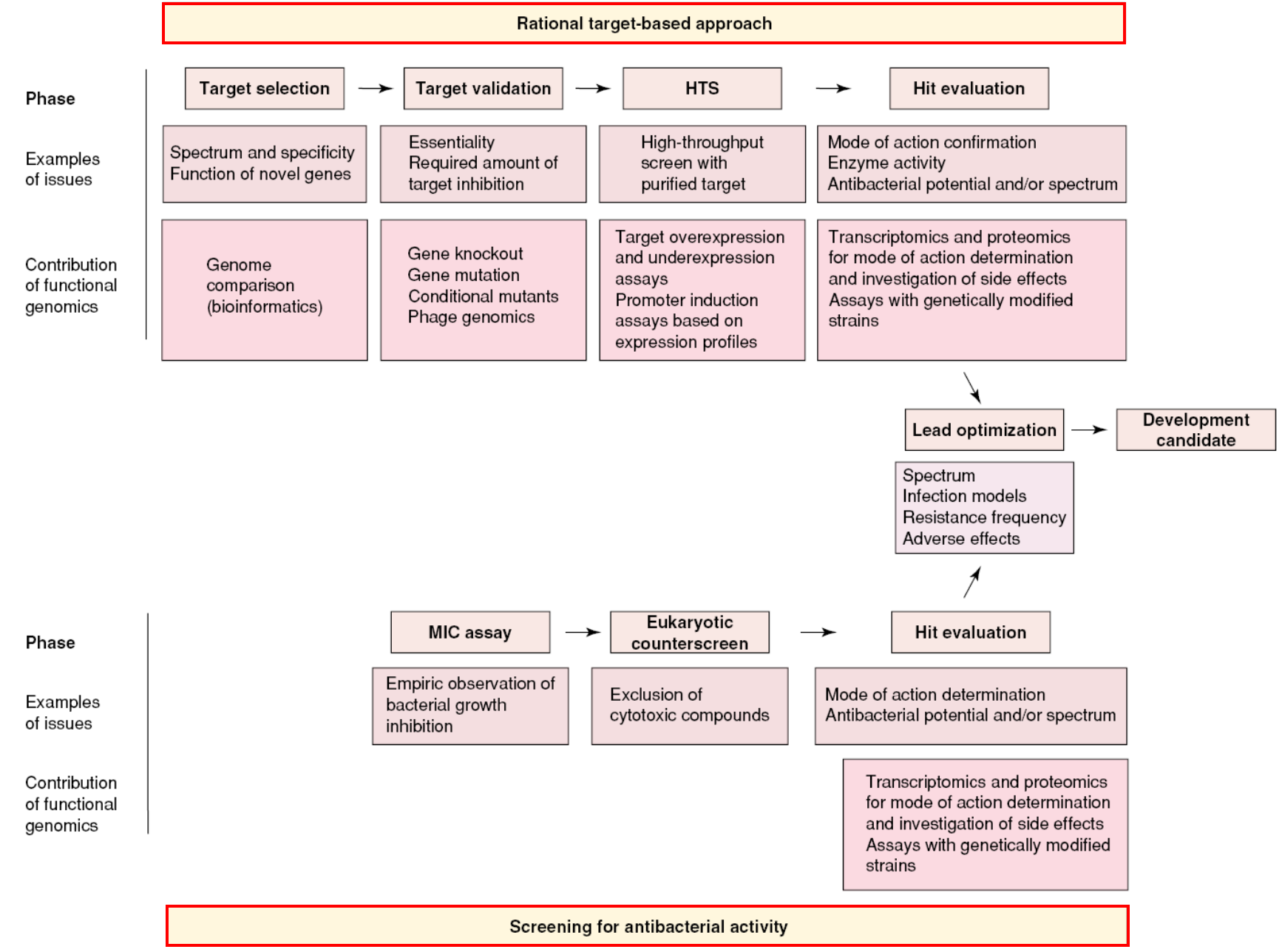
Investigational New Drug Application ("IND Filing")

More generalized process
(regulatory requirements)

Antibacterial Discovery: Target-Based & Reverse Genomics



Antibacterial Discovery: Target-Based & Reverse Genomics

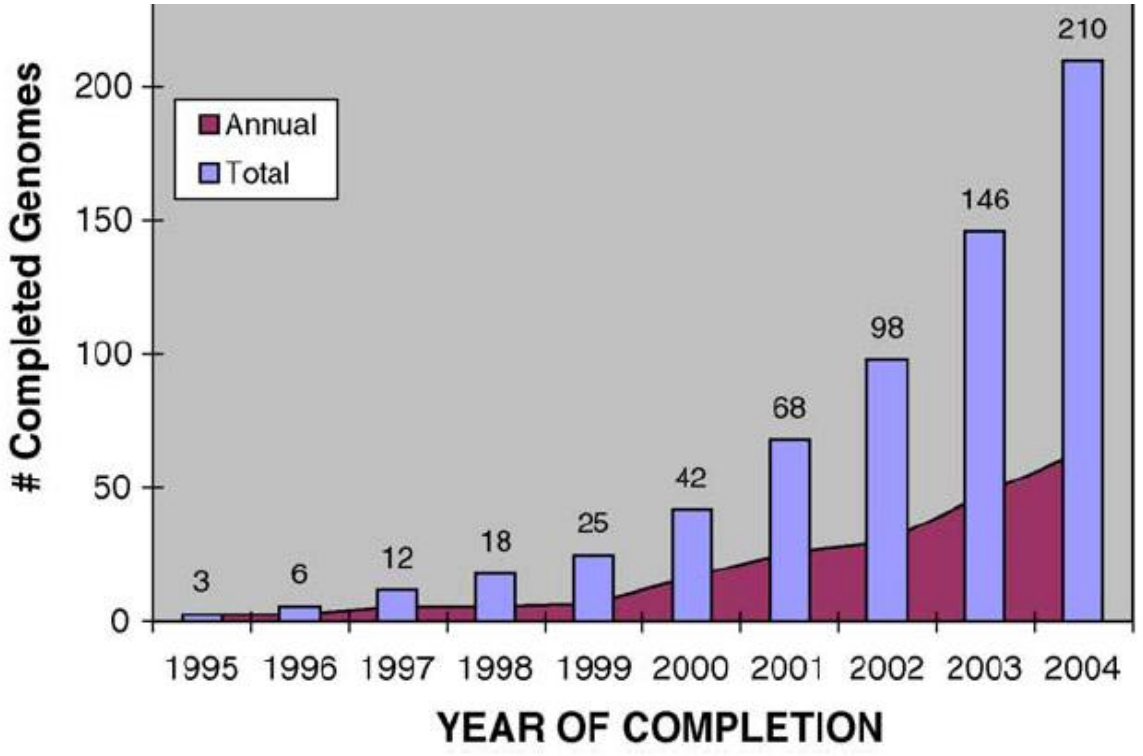


Basis for "Omics Approaches": Sequenced Genomes

Genomes available in ERGO™

Number of genomes available in ERGO™ (■), genomes available in NCBI (■) :

All genomes in ERGO	931/678
Bacteria	541/338
Archaea	35/30
Viral	224/198
Eukarya	131/112



Target Discovery: Essential Genes in Bacteria

Number of potential essential genes identified in genome-wide gene inactivation studies

Organism	Total no. of genes	No. of (potentially) essential genes[a]	Method	Refs
Bacillus subtilis	4101	271	Plasmid insertion mutagenesis Conditional mutants Estimations derived from literature study	[20]
Escherichia coli	4279	620	Transposon mutagenesis	[16]
Haemophilus influenzae	1709	256	Transposon mutagenesis	[19]
Helicobacter pylori	1552	344	Transposon mutagenesis	[17]
Mycoplasma genitalium	484	256–350	Transposon mutagenesis	[14]
Staphylococcus aureus	2595	150–658	Antisense RNA expression	[26,27]
Streptococcus pneumoniae	2043	113 out of 347 examined genes	Plasmid insertion mutagenesis	[21]

[a]For most species, the conclusion that genes might be essential has been drawn because gene inactivation was not achieved. Therefore, the genes need to be regarded as being potentially essential. Validation of essentiality will reduce the number of essential genes. The essentiality of genes has been studied *in vitro* in complex medium. Genes validated this way are also considered to be probably indispensable *in vivo*. The best-validated essentiality study has been performed in *B. subtilis* and, in this case, the number of essential genes seems to be realistic.

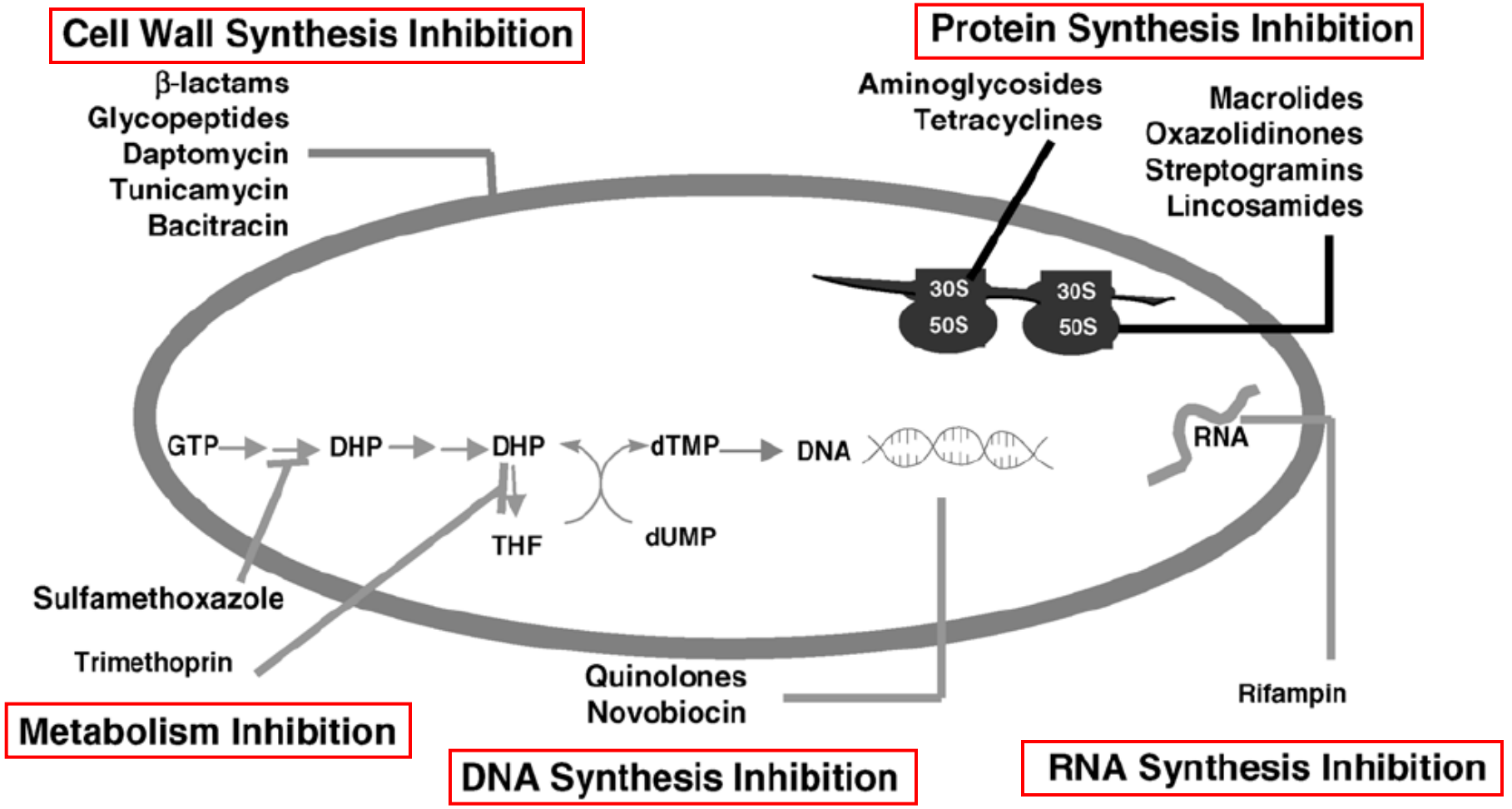
(Freiberg et al., *Drug Discov. Today* 2005, 10, 927)

Target Discovery: Essential Genes & Potential Antibiotic Targets

Table 1 – Desirable properties of a good antibacterial target

Target property	Why desirable	Alternative
Essential	Inhibition leads to bacterial stasis or death	Inhibition of virulence may also be effective
Present in multiple bacterial species	Potential for broad-spectrum inhibitor of bacterial growth	A narrower spectrum may also be desired
Selectivity	Greater selectivity for bacterial target may result in less toxicity in humans	Effective drugs are in use against targets with significant homology to human equivalents
Bactericidal	Killing bacteria is optimal	There are several effective bacteriostatic drugs on the market
In vitro functional assay	Enzymatic assay could aid drug discovery	There are alternative methods to discover inhibitors

Antibacterial Targets: Overview



Target Discovery: Essential Genes & Potential Antibiotic Targets

Number of bacterial targets present in important Gram-positive and both Gram-positive and Gram-negative pathogens[a]

Functional category of gene product	No. of targets in Gram-positive pathogens	No. of targets in Gram-positive and Gram-negative pathogens
Cell division	12	9
Nucleotide biosynthesis	12	10
Coenzyme biosynthesis	10	7
Fatty acid biosynthesis	14	14
Translation	71	68
Transcription	9	8
Replication	26	25
Cell wall biosynthesis	19	16
Others	10	10
Total number	183	167

(Freiberg et al., *Drug Discov. Today* 2005, 10, 927)

Target Discovery: Determination of Target Gene Essentiality

Method	Bacterial species	Reference
Random mutagenesis		
Plasmid insertion	*S. pneumoniae*	[11]
Conditional lethals	*E. coli*, *S. typhimurium*	[12,13]
Transposon	*E. coli*, *H. pylori*, *M. genitalium*	[14–16]
Shotgun antisense	*S. aureus*	[17]
Cassette mutagenesis	*H. influenzae*	[18]
Targeted gene disruption		
Plasmid insertion	*E. coli*, *S. pneumoniae*	[19,20]
Allelic exchange	*H. pylori*	[21]
Crossover PCR	*E. coli*	[22]
Targeted conditional lethals	*E. coli*, *S. aureus*	[12,23]
In vivo virulence		
Signature-tagged mutagenesis	*S. typhimurium*	[24]
In vivo expression technology	*S. typhimurium*	[25]
Differential fluorescence induction	*S. typhimurium*	[26]

Target Discovery: Transcriptome & Proteome Profiling

DNA → mRNA → Protein
Genome → Transcriptome → Proteome
(= all genes) (= all mRNAs) (= all proteins)

Technical advantages and restrictions of transcriptome and proteome expression profiling.

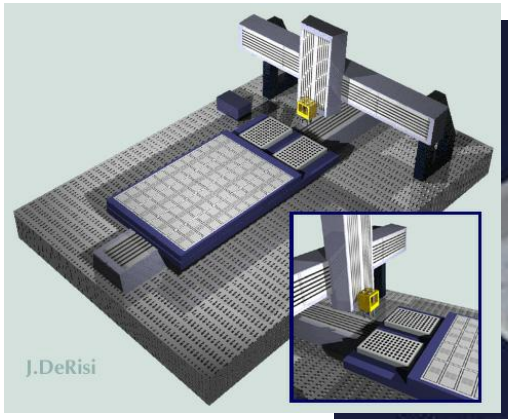
	Transcriptome	Proteome
Information content	Information on mRNA but not on protein level mRNA expression profiling on a whole genome basis possible.	Information on protein amount, synthesis rates, protein modification, protein degradation. Up to now only a subset of the proteome visualized by proteomics.
Technical demands	Position of genes fixed and known.	Gel to gel variation of protein position, protein identification required, multiple proteins per spot and spots per protein possible.
	Virtually all genes covered by a single chip for parallel analysis.	Only protein subsets detected on a single gel, basic proteins and membrane proteins require special separation conditions.
Costs per sample	Hardware costs higher than in case of 2D-gel electrophoresis.	Hardware costs lower, however preparation of gels for various sub-proteomes more labor-intensive.

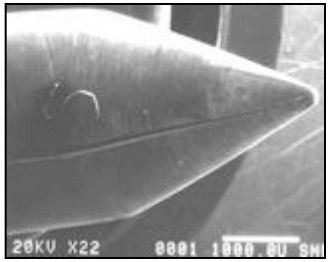
(Freiberg et al., *Drug Discov. Today* 2005, 10, 927)

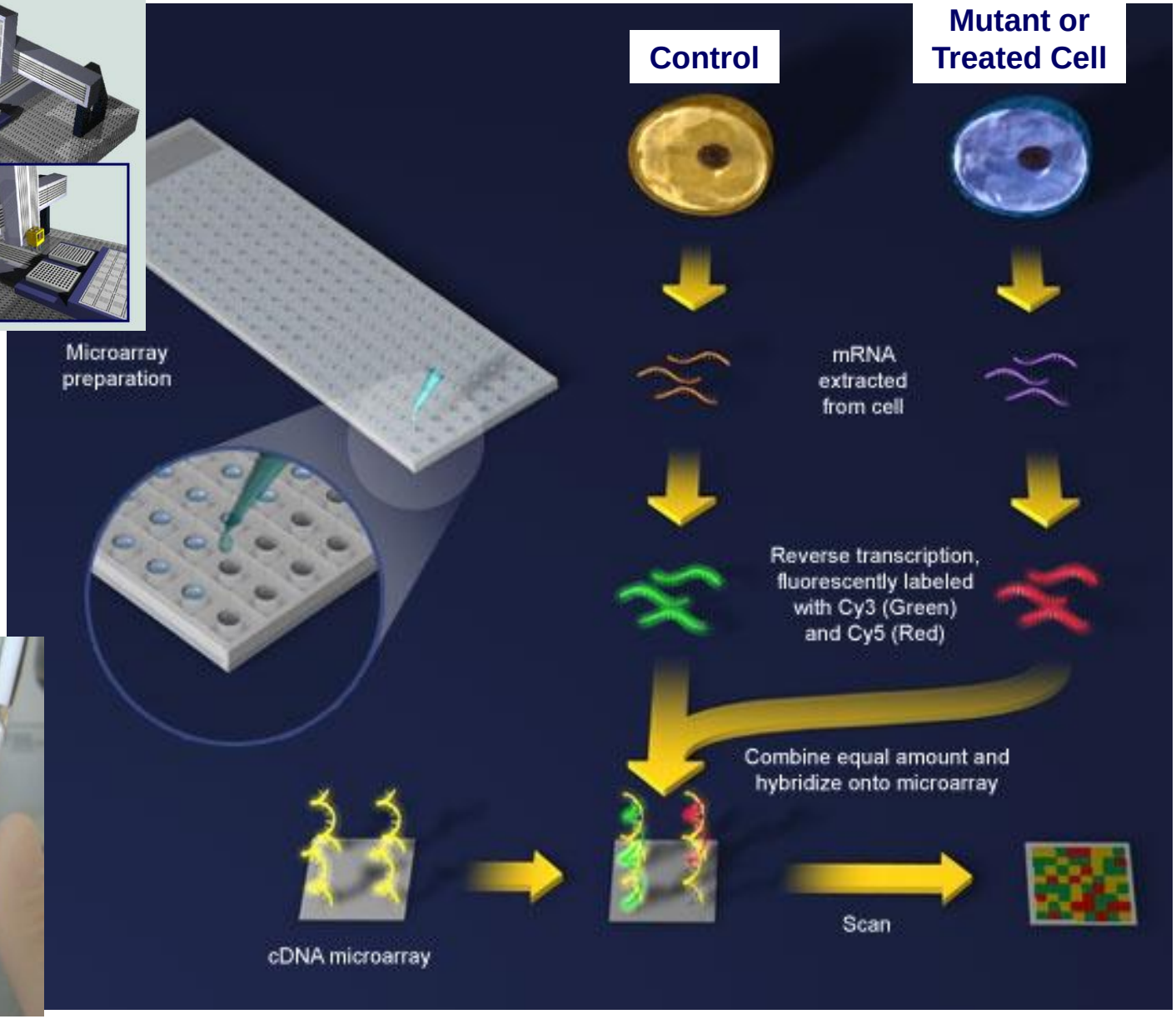
1) Essentiality of a target (study knockout mutant vs. wildtype)
2) Impact/mechanism of action (MOA) of a compound/antibiotic

Transcriptome expression profiling? →

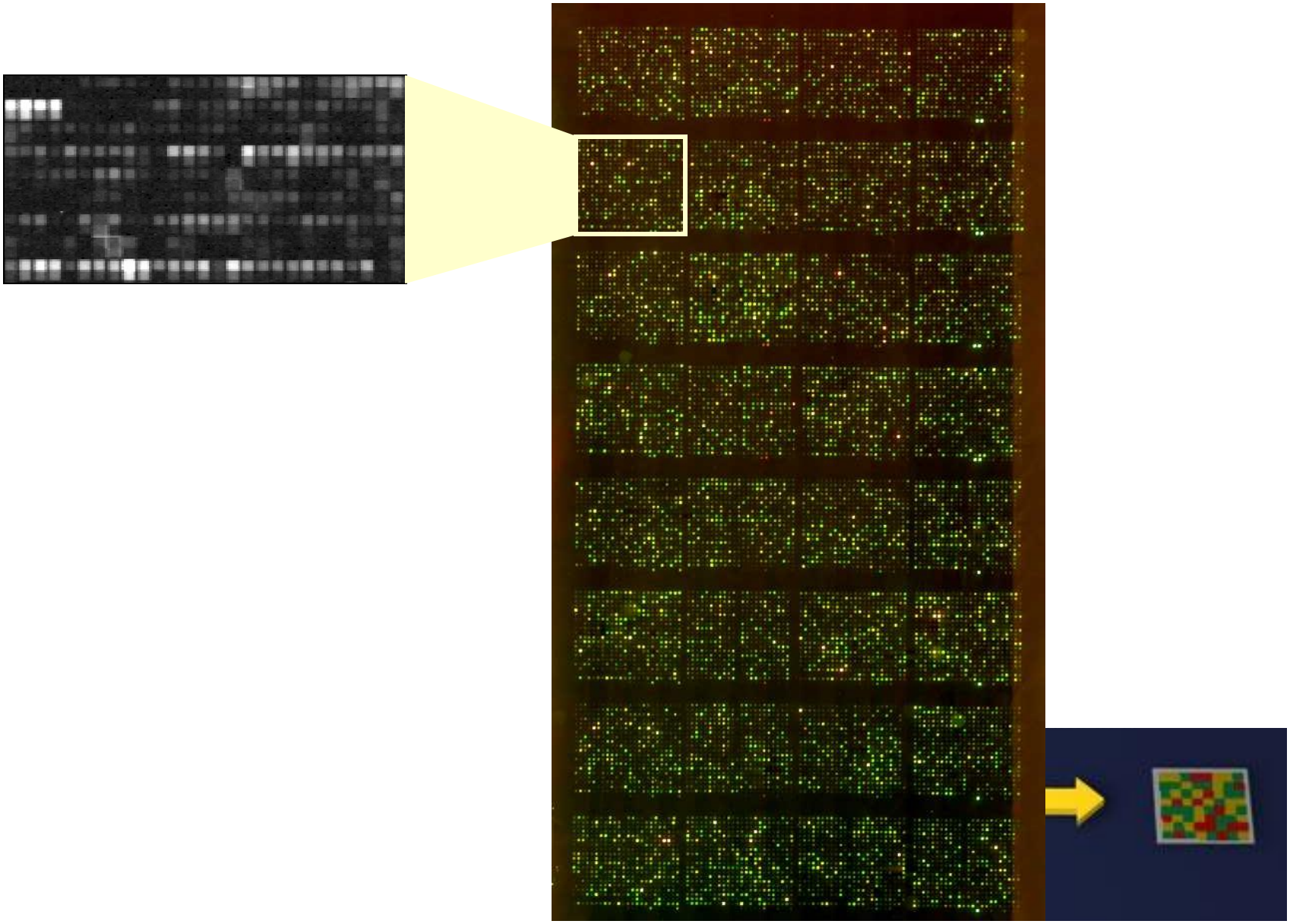
Target Discovery: Microarray Expression Profiling



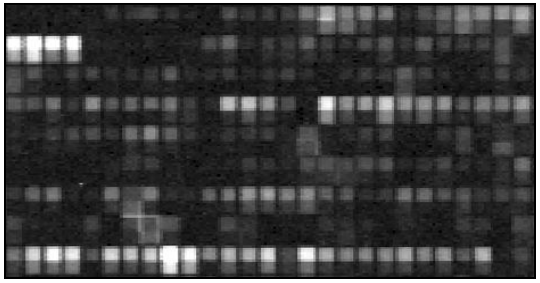




Target Discovery: Microarray Expression Profiling



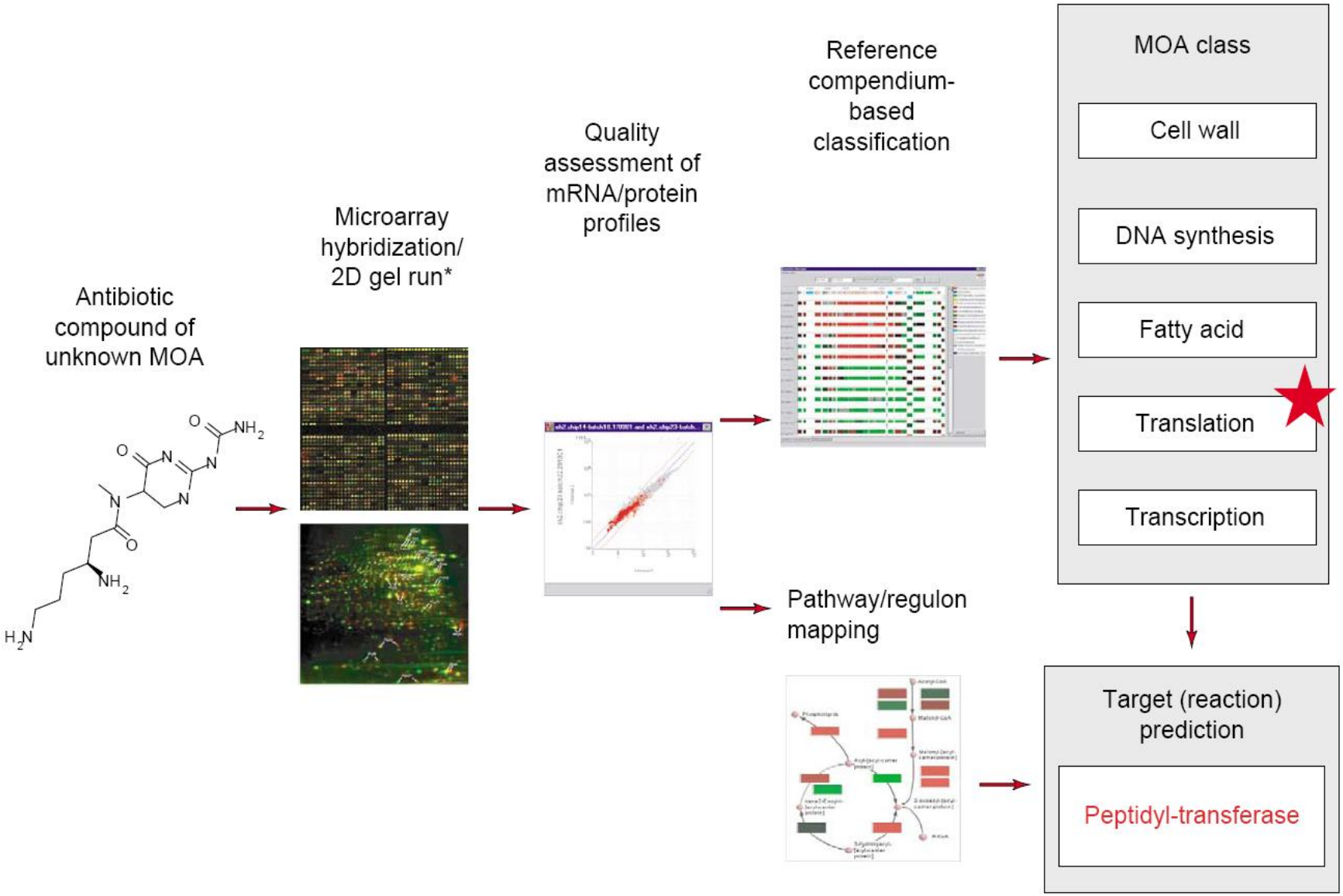
Target Discovery: Transcriptome & Proteome Profiling - MOA



Groups of gene-expression responses to antibiotic treatment

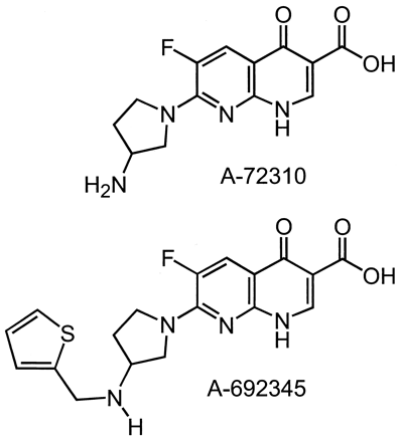
Gene-expression response group	Group characteristics and examples
1. Direct effects	Characteristic signatures of primary target inhibition, complicated by secondary effects (e.g. antibiotics targeting DNA replication machinery cause DNA damage and elicit SOS DNA-repair response; antibiotics targeting RNA synthesis inhibit transcription and elicit changes in tRNAs and nucleotides, for example).
2. Indirect effects	Triggered when primary target is inhibited, as organism attempts to compensate for changes in its environment (e.g. general stress responses, metabolic changes and resistance mechanisms).
3. Secondary effects	Downstream effects of target inhibition that have no particular role in antibiotic action and thus do not impact on the fate of antibiotic-treated bacteria.
4. Bystander effects	Changes in organism- or antibiotic-specific genes, or in generally unrelated genes.

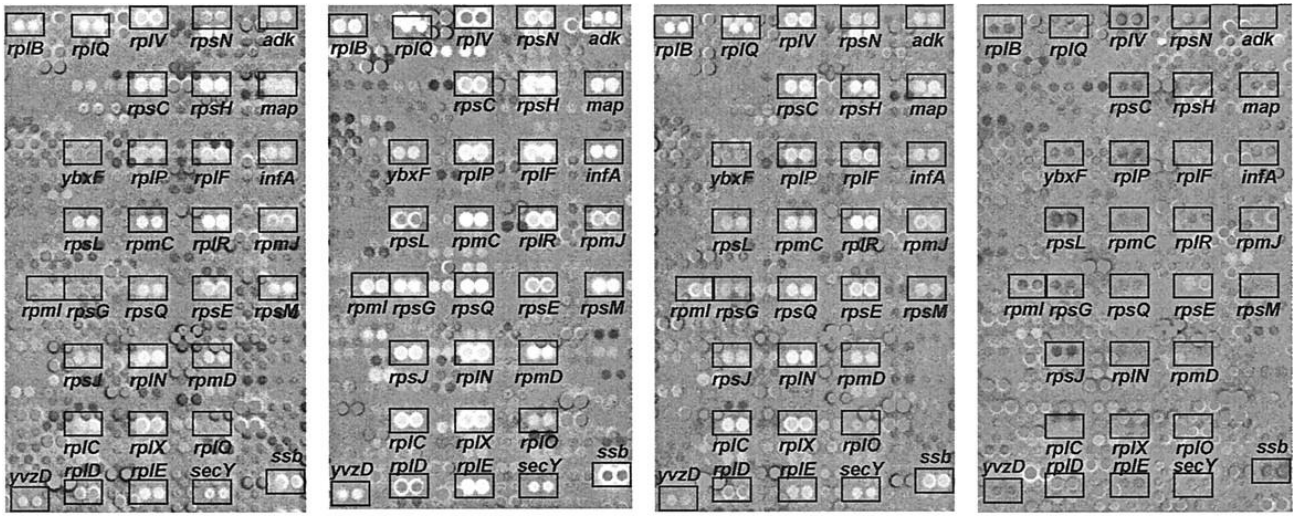
Target Discovery: Transcriptome & Proteome Profiling - MOA



(Freiberg et al., *Curr. Opin. Microbiol.* 2004, 7, 451)

Target Discovery: Transcriptome Profiling – MOA Example

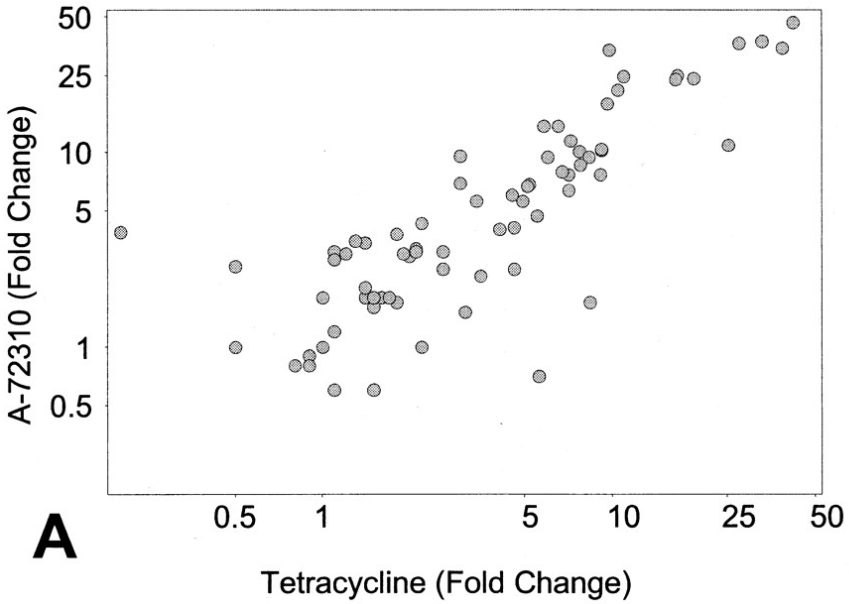


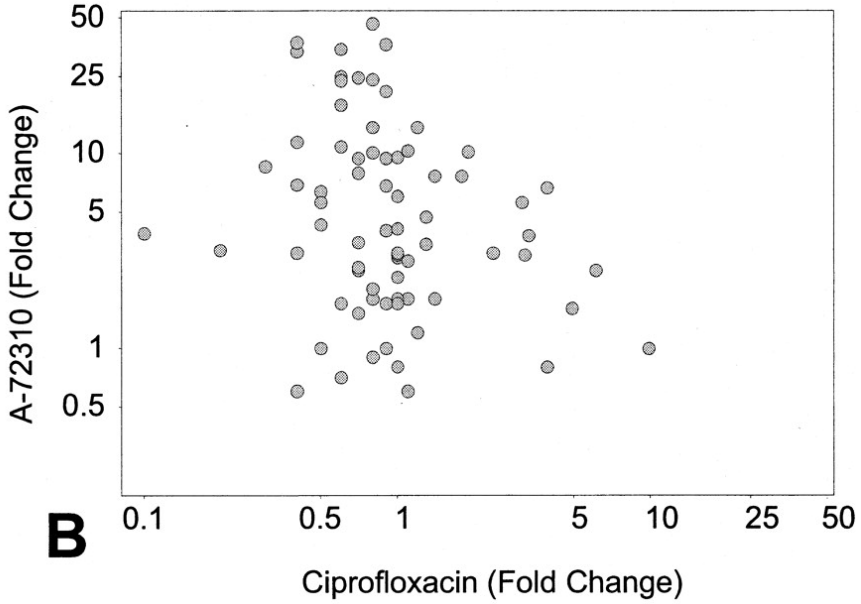


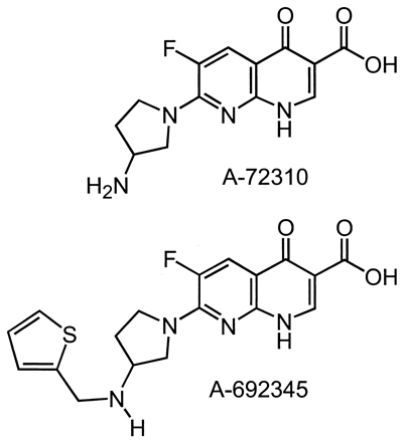
Composite *B. subtilis* gene expression array images for A-72310 (128 µg/ml), clarithromycin (10 µg/ml), tetracycline (0.1 µg/ml), and ciprofloxacin (0.1 µg/ml). Drug-induced changes in mRNA concentration are indicated by either light (upregulated), dark (downregulated), or neutral gray (unchanged) spots (in duplicate).

(Dandliker et al., *Antimicrob. Ag. Chemother.* 2003, 47, 3831)

Target Discovery: Transcriptome Profiling – MOA Example



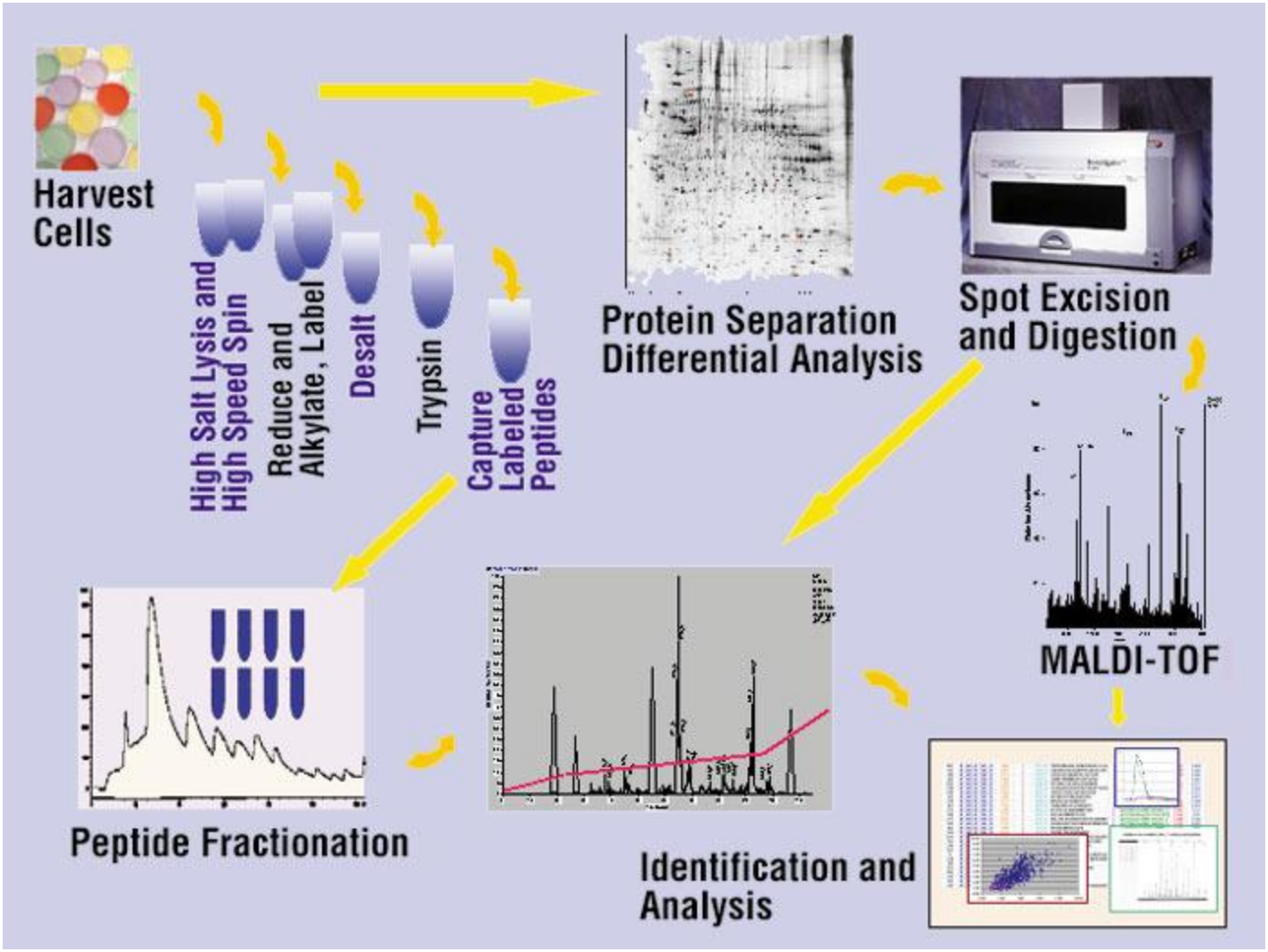




Scatter plots of the fold change in *B. subtilis* mRNA levels induced by A-72310 and tetracycline (A) and A-72310 and ciprofloxacin (B).

(Dandliker et al., *Antimicrob. Ag. Chemother.* 2003, 47, 3831)

Target Discovery: Proteome Profiling - MOA



Target Discovery: Proteome Profiling - MOA

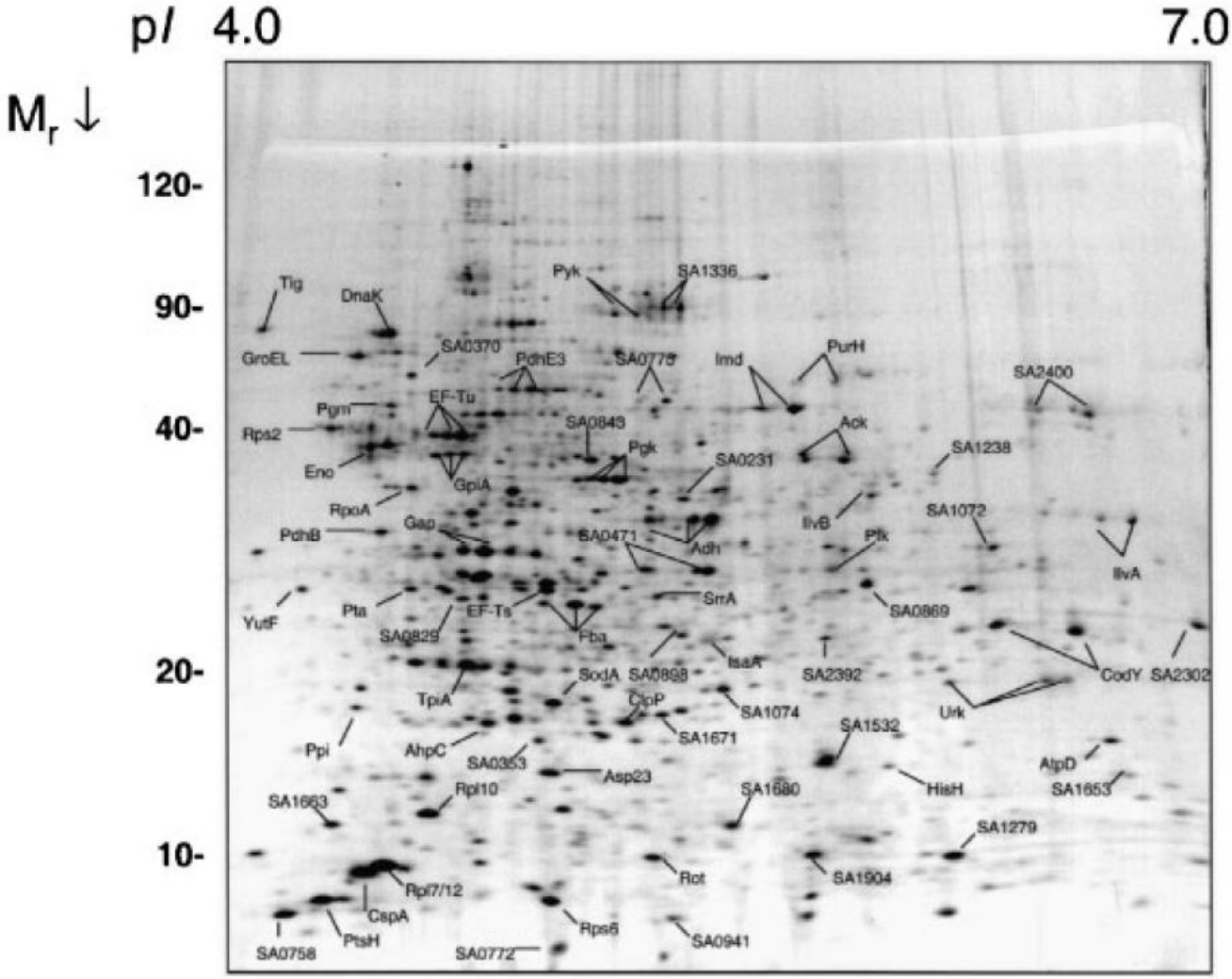
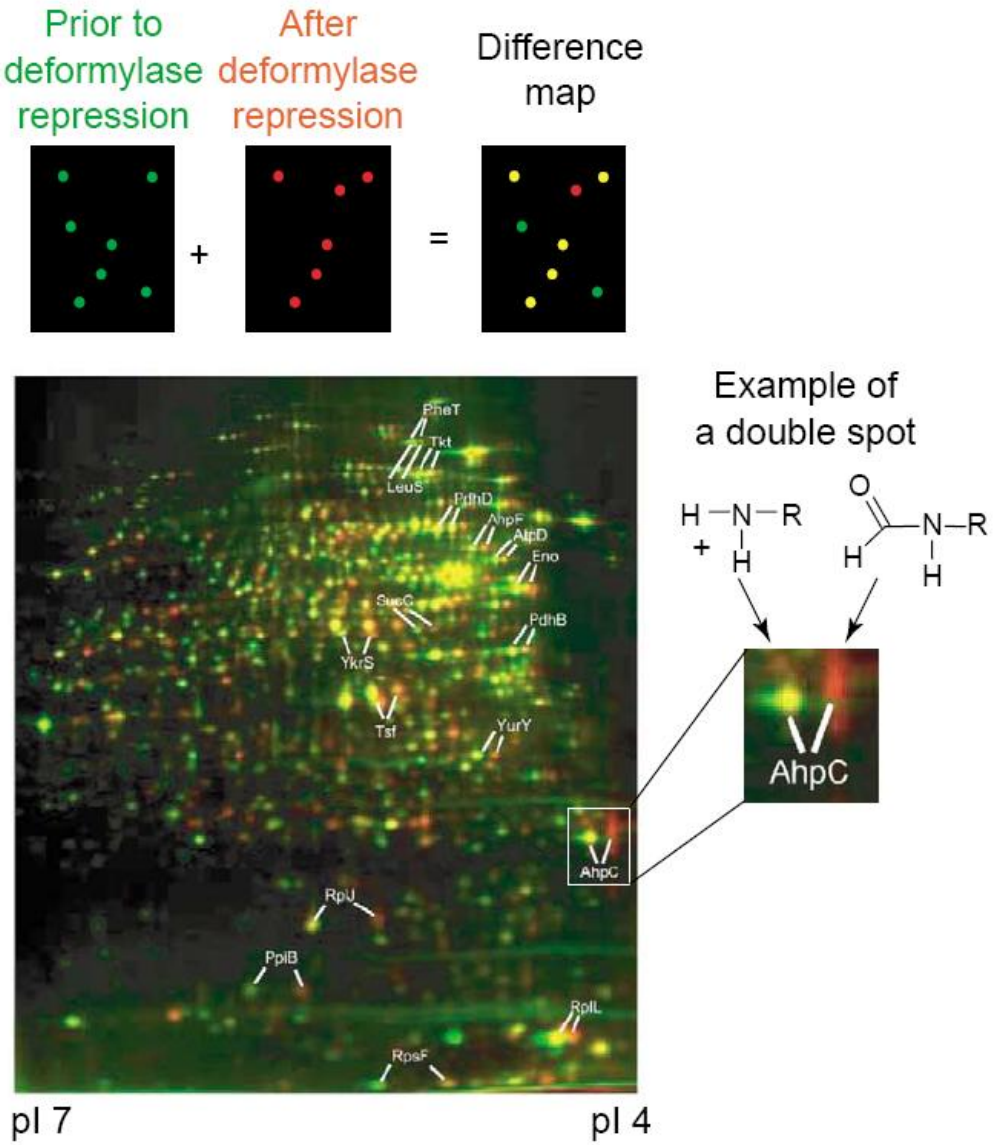


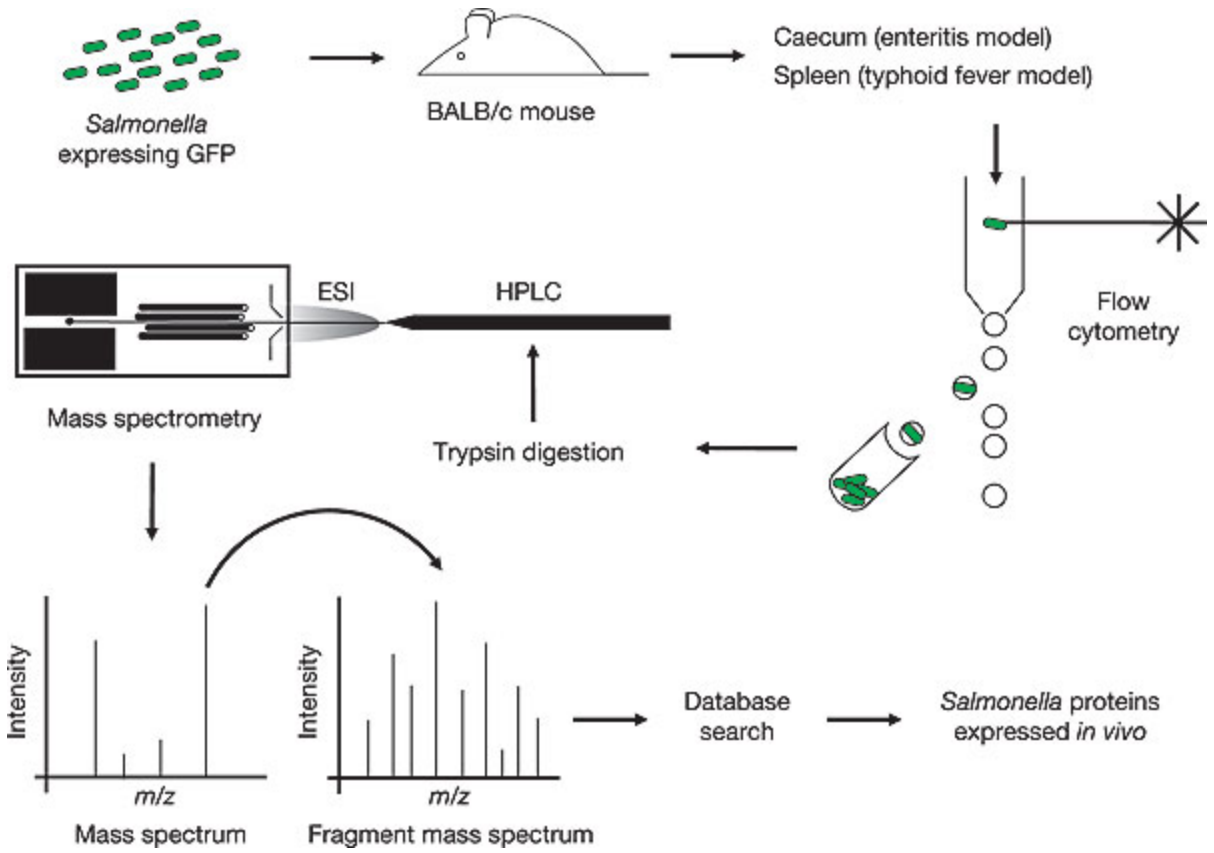
FIGURE 1. Example of a protein reference map. The proteome of *Staphylococcus aureus* 8325 was separated by 2D-gel electrophoresis, using an immobilized pH gradient in the range of pI 4–7. Proteins were stained with silver, and were identified by MALDI-MS after tryptic digestion. The identity of selected proteins that serve as landmarks on the gel are indicated. Reproduced from Hecker, Engelmann, & Cordwell (2003), with permission from Elsevier, copyright 2003.

Target Discovery: Proteome Profiling – MOA Example



(Freiberg et al., *Curr. Opin. Microbiol.* 2004, 7, 451)

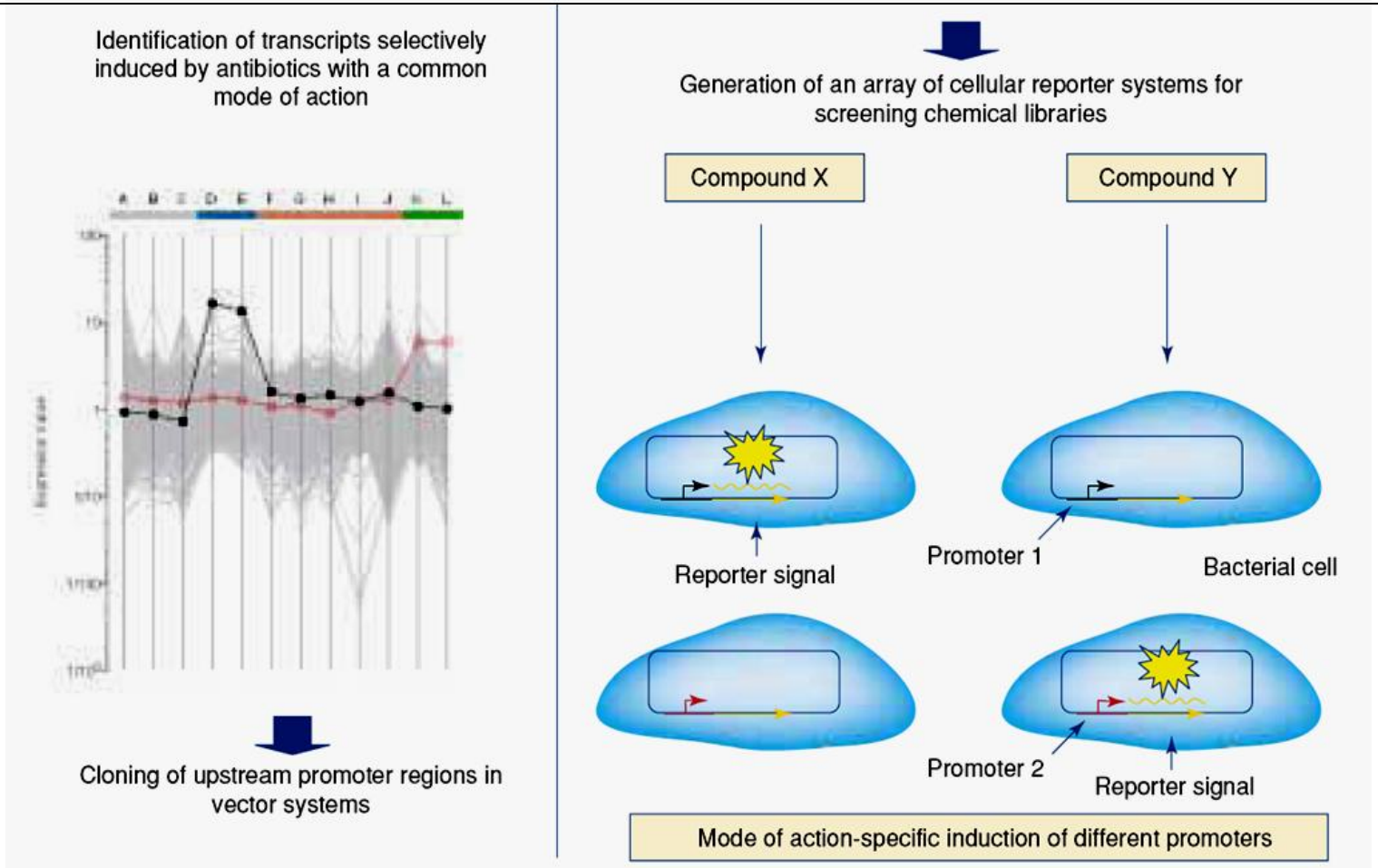
Target Discovery: *In vivo* Proteome Profiling



Mice were infected with *Salmonella* expressing GFP. After several days, fluorescent GFP-expressing *Salmonella* were purified from spleen or caecum homogenates by flow cytometry. Purified *Salmonella* were digested with trypsin and the resulting peptide mixtures were separated by high-performance liquid chromatography (HPLC). Electrospray ionization (ESI) tandem mass spectrometry of the eluted peptides yielded peptide mass spectra and fragment ion mass spectra. Comparison with databases identified the respective *Salmonella* proteins.

(Becker et al., *Nature* 2006, 440, 303)

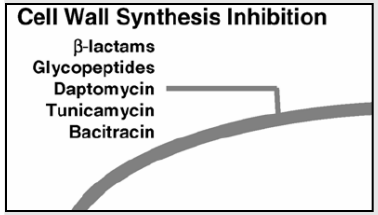
Target Discovery: Validation by Promoter Induction



Promoter induction assays based on expression profiling. Transcriptional expression profiles of all genes of a bacterial genome are represented by gray lines. In the presence of several different antibiotics (A–L with color-coded MOA), genes selectively responding to a specific type of growth inhibition can be identified. For example, one transcript is only induced by treatment with compounds D and E (black line), whereas another transcript is selectively induced by compounds K and L (red line). Upstream regions of the corresponding genes are cloned in front of reporter genes, enabling the detection of compounds with MOAs similar to D and E or K and L, respectively.Arrays of promoter induction systems represent helpful tools for the discovery of novel drug candidates.

(Freiberg et al., *Drug Discov. Today* 2005, 10, 927)

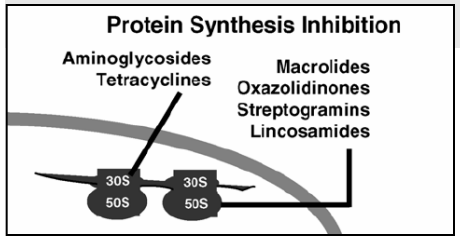
Antibacterial Discovery: Results of Target Screening

Pathway	Target protein	Function		Outcome
Peptidoglycan synthesis	Multiple	Whole pathway	[74]	MurA inhibitors found with very weak antibacterial activity (*S. aureus* MIC 16 μg/ml). Whole cell assay
	MurA	UPD-N-acetylglucosamine enolpyruvil-transferase	[75]	Enzyme inhibitors with weak antibacterial activity (*S. aureus* MIC 4 μg/ml)
			[76]	Enzyme inhibitors found without reported antibacterial activity
	MurC	UDP-N-acetylmuramyl-L-ala ligase	[77]	Enzyme inhibitors found without reported antibacterial activity
	MurG	Nucleoside diphospho-glycosyltransferase	[78]	Enzyme inhibitors found without reported antibacterial activity
	MraY	Transferase[a]	[79]	Description of methodology, no hits reported
	PBP1b	Transglycosylase/transpeptidase	[80]	Description of methodology, no hits reported

(Pelaez, *Biochem. Pharmacol.* 2006, 71, 981)

Antibacterial Discovery: Results of Target Screening

Pathway	Target protein	Function		Outcome
Protein synthesis	Phe-RS	Phenylalanyl-tRNA synthetase	[81]	Enzyme inhibitors found with in vitro and in vivo antibacterial activity antagonized by phenylalanine
	Pdf1	Peptide deformylase	[82]	Screening of focused libraries identified a lead with in vitro and modest in vivo antibacterial activity
	Multiple	Transcription–translation	[83]	Cell-free transcription–translation assay in *S. aureus* Description of methodology, no hits reported
	Multiple	Transcription–translation	[84]	Cell-free transcription–translation assay in *S. pneumoniae*. Hits found with weak antibacterial activity, slightly improved by medicinal chemistry
	Multiple[b]	Ribosome assembly	[85]	Description of methodology. Piloted with a focused library of aminoglycosides derivatives



(Pelaez, *Biochem. Pharmacol.* 2006, 71, 981)

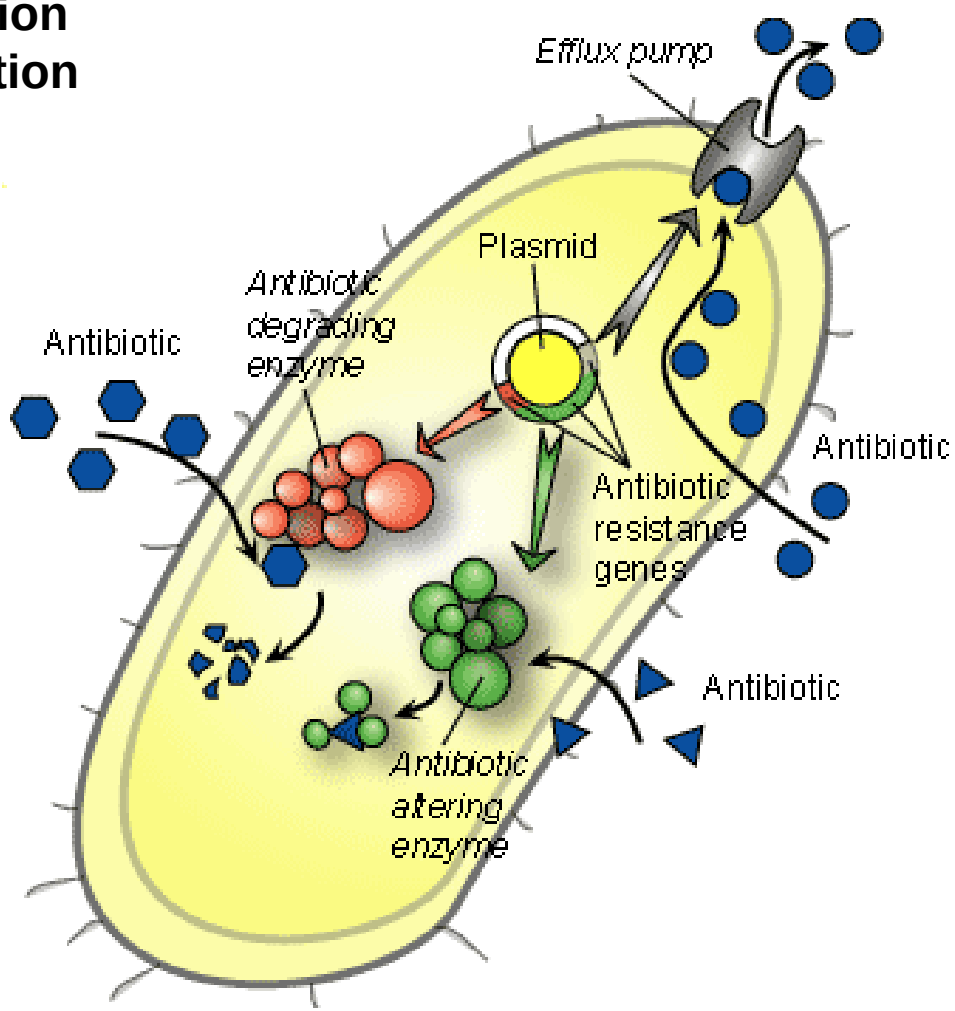
Antibacterial Discovery: Results of Target Screening

Pathway	Target protein	Function		Outcome
Fatty acid synthesis	FabI	Enoyl-ACP-reductase	[26]	Enzyme inhibitors found in primary screening without antibacterial activity. Medicinal chemistry produced compounds with potent activity against *S. aureus* and in vivo activity in a rat model, though limited spectrum (substrate of efflux pumps)
	Multiple[c]	Most of the type II fatty acid synthesis pathway	[25]	Enzyme inhibitors with weak antibacterial activity identified from a natural products library
Others	FtsZ	Tubulin-like protein involved in septum formation	[86]	Description of methodology, no hits reported
	spsB	Signal peptidase I	[87]	Enzyme inhibitors with weak antibacterial activity identified from a natural products library

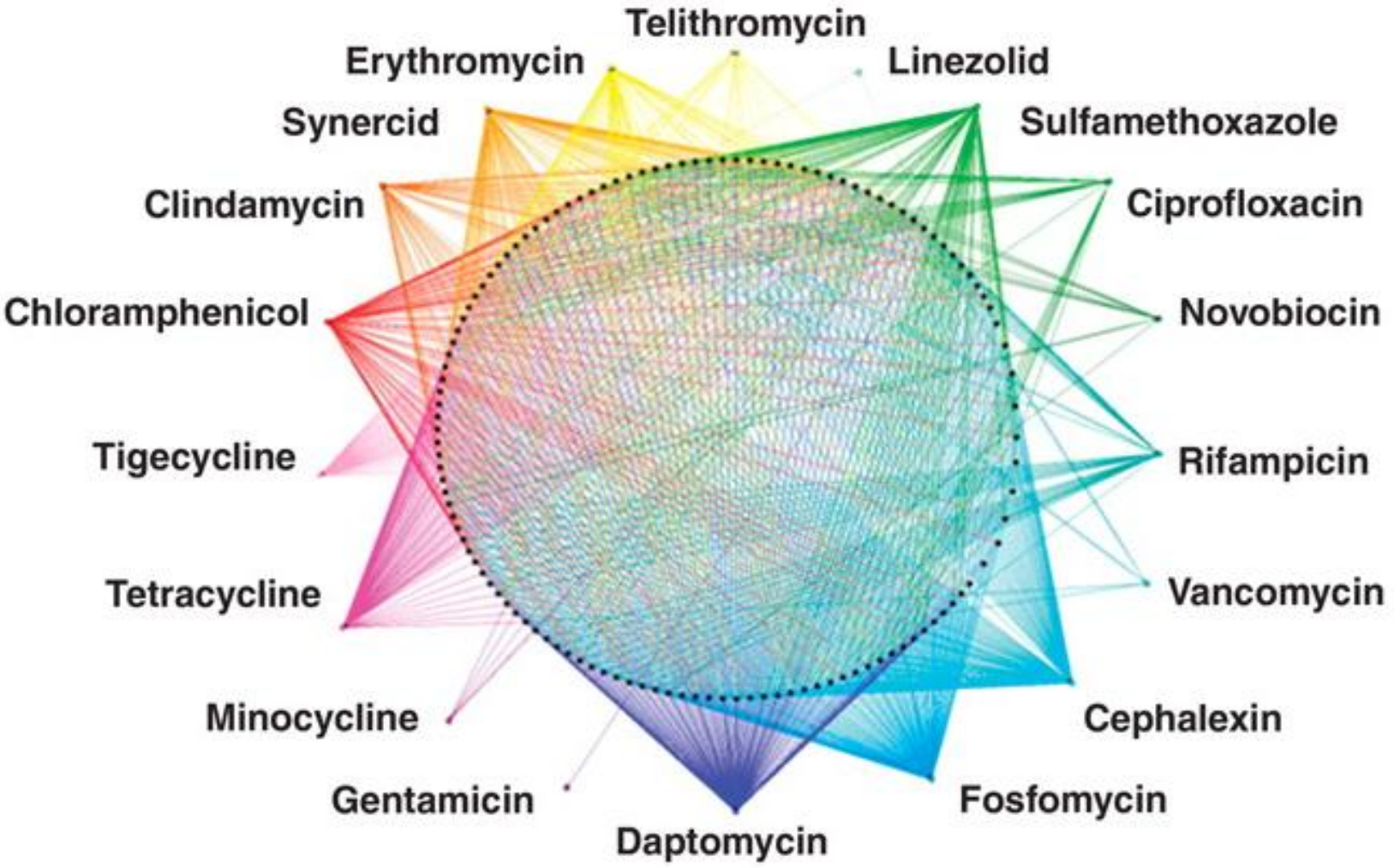
(Pelaez, *Biochem. Pharmacol.* 2006, 71, 981)

Target Discovery: Antibiotic Resistance Mechanisms

- **Membrane permeability**
- **Antibiotic degradation**
- **Antibiotic modification**
- **Target modification**
- **Efflux**



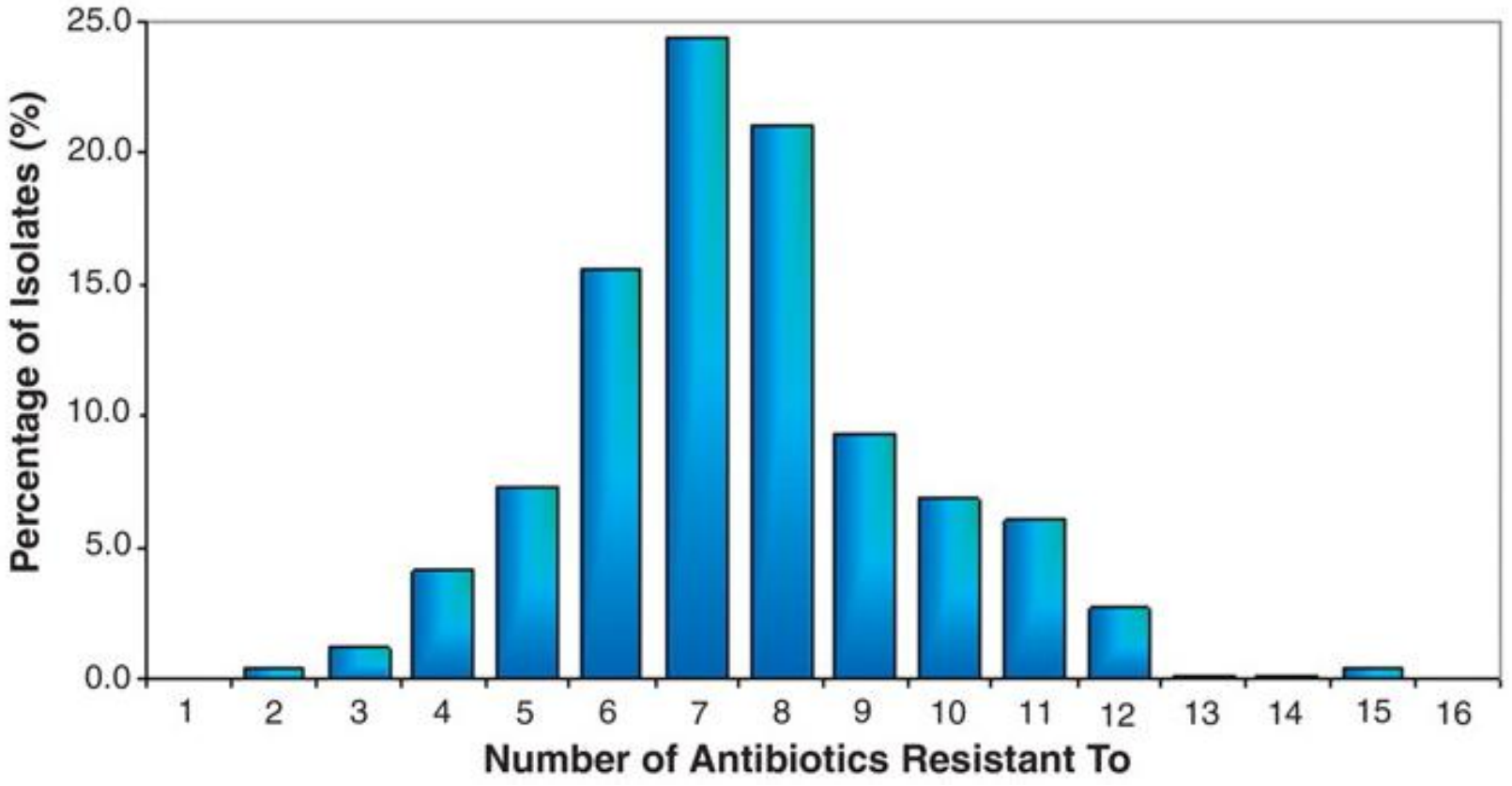
Target Discovery: Resistance Profiling ("Resistome")



Density and diversity of resistance profiles in 480 soil-derived bacterial isolates.

(D'Costa et al., *Science* 2006, 311, 374)

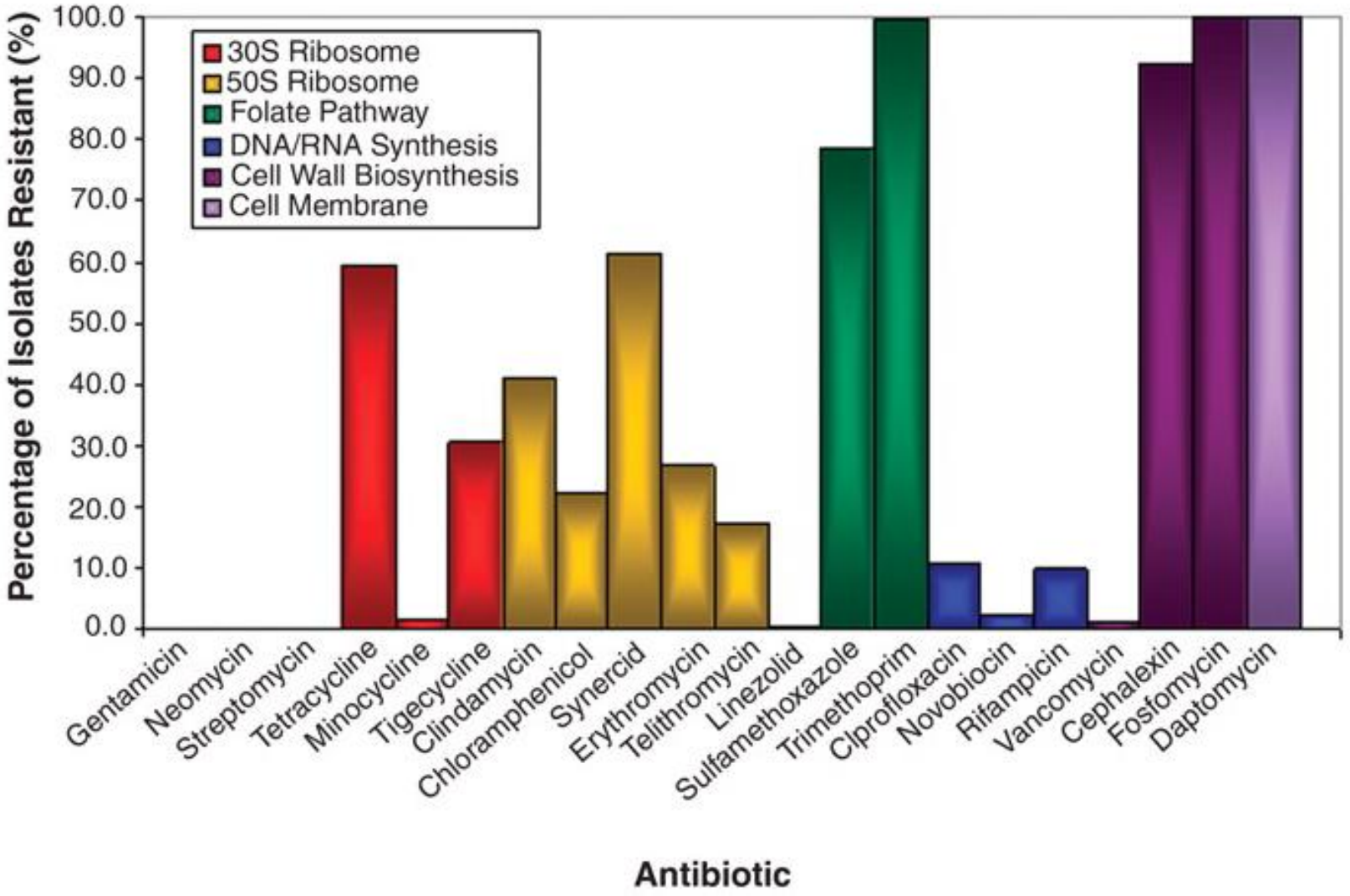
Target Discovery: Resistance Profiling



Resistance spectrum in 480 soil-derived bacterial isolates.

(D'Costa et al., *Science* 2006, 311, 374)

Target Discovery: Resistance Profiling



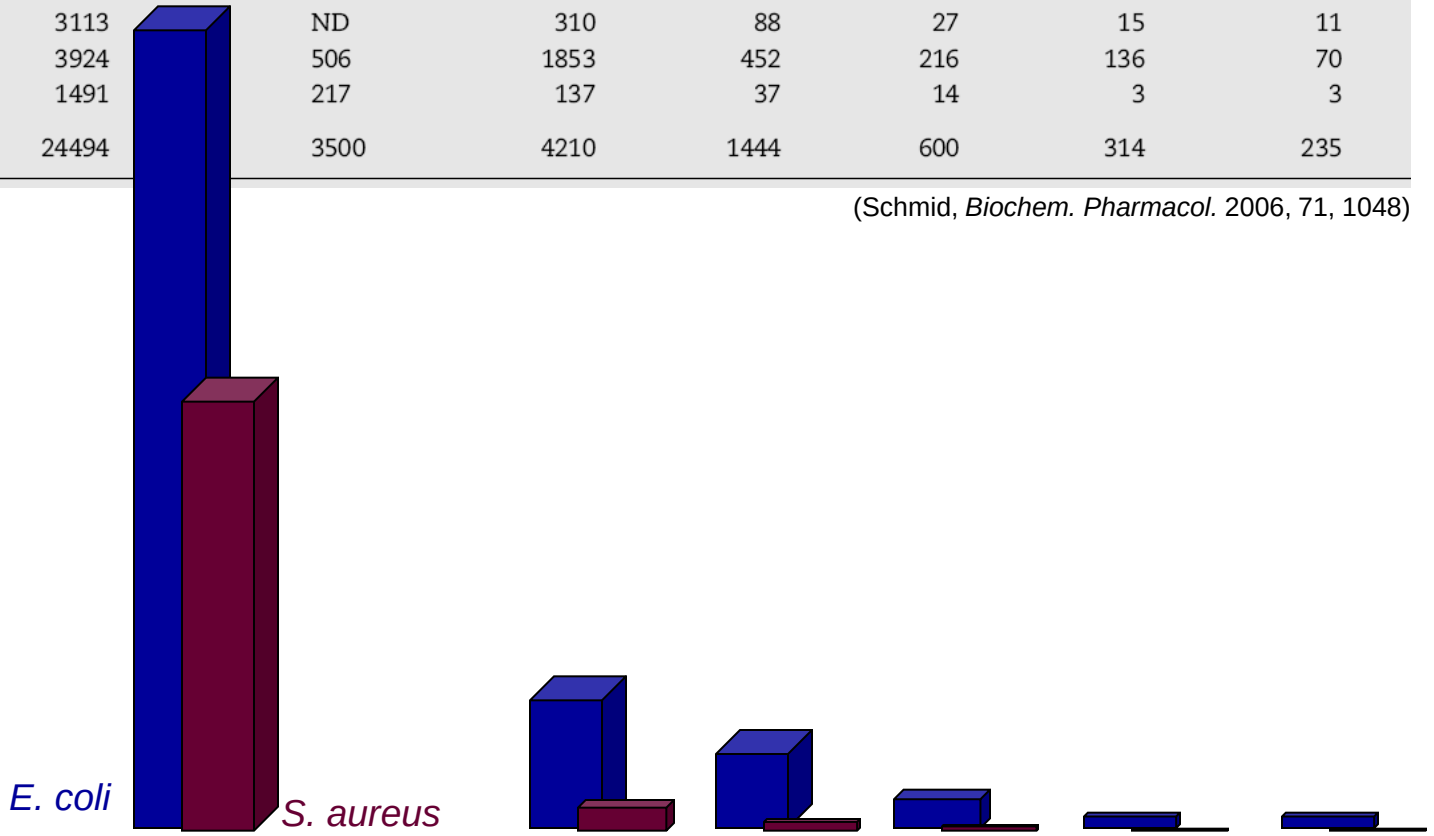
Resistance levels and MOA in 480 soil-derived bacterial isolates.

(D'Costa et al., *Science* 2006, 311, 374)

Antibacterial Discovery: Structural Genomics

	Total number genome ORFs[a]	Number of proteins with >1 trans-membrane segment[b]	Number cloned[b]	Number of purified proteins[b]	Number of protein crystals[b]	Diffraction quality crystals[b]	Number of solved structures in PDB[b]
Escherichia coli	4289	776	792	516	204	88	86
Pseudomonas aeruginosa	5565	875	379	140	34	34	36
Haemophilus influenzae	1709	259	281	89	35	12	7
Staphylococcus aureus	2360	510	169	69	38	16	14
Streptococcus pneumoniae	2043	357	289	53	32	10	8
Entercoccus faecalis	3113	ND	310	88	27	15	11
Mycobacterium tuberculosis	3924	506	1853	452	216	136	70
Helicobacter pylori	1491	217	137	37	14	3	3
Total	24494	3500	4210	1444	600	314	235

(Schmid, *Biochem. Pharmacol.* 2006, 71, 1048)



Antibacterial Discovery: Structural Genomics – Structures & Homologs

Table 3 – Structures of essential proteins of *E. coli*[a]

Gene class	Total proteins in each class[b]	Number of proteins or homologs in PDB (% of proteins in each class with a structure)[c]
Essential	250	179 (71.6)
Non-essential	3253	1614 (49.6)
Unknown	906	330 (36.4)
Total	4413	2123 (48.1)

[a] This information was generated by linking information from the PEC database (http://www.shigen.nig.ac.jp/ecoli/pec/index.jsp) with information from PEDANT (http://pedant.gsf.de/), using the "GI number" as the link between information in PEC and information in PEDANT. While not perfect, this method captured 2123 of the 2150 proteins of *E. coli* having a PDB code in the PEDANT database.

[b] PEC database (http://www.shigen.nig.ac.jp/ecoli/pec/index.jsp).

[c] PEDANT database (http://pedant.gsf.de/).

(Schmid, *Biochem. Pharmacol.* 2006, 71, 1048)

Antibacterial Discovery: Structural Genomics

Table 4 – Novel target-directed antibacterial agents

Pathway	Target	PDB	References to protein structure and SGDD efforts on the target	Programs on the target without SGDD
Cell surface, membrane, peptidoglycan, cell wall	LpxC	1XXE	[31,32]	
	MurA	1UAE	[41,44]	
	MurB	1MBB	[14]	[52]
	YjeE	1FL9	[53,54]	
	FtsZ	1RQ7		[55]
	Signal peptidase	1T7D		[56]
Fatty acid biosynthesis	Acc (acetyl-coA carboxylase)		[57]	[58,59]
	FabF	1OX0	[60,61]	
	FabH	1MZS	[33,37,62]	
	FabI	1LXC		[63]
	YacE (coaE)	1N3B	[57]	
Nucleic acid management	TopoIV (ParC, ParE)	1S16, 1ZVU	[64]	
	Gyrase	1AB4, 1EI1		[65–69]
	RNA polymerase	*1IW7*	[70]	[71]
	MvaS HMG coA sythase	1TVZ	[29,72]	
Regulation	YycF	1NXO	[73]	
Translation	PheRS	*1EIY*		[74,75]
	Met tRNA synthetase	1PG2		[76]
	Peptidyl deformylase	2AIA	[77]	[78,79]

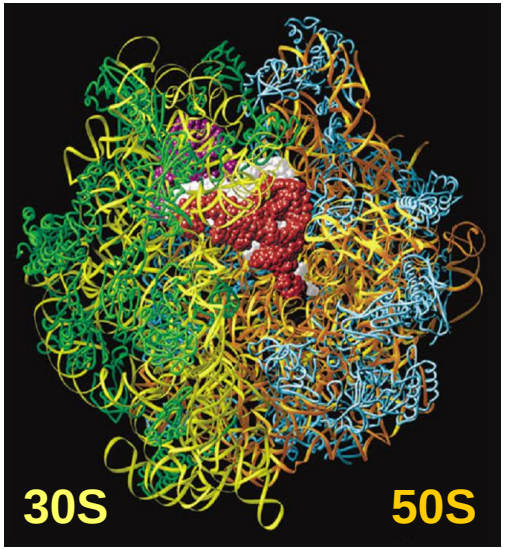
Several recent reports of new inhibitors of bacterial essential proteins have relied on high resolution protein structures to guide the drug discovery efforts. Several other projects have not been structure guided efforts, sometimes because the structure came out after the work identifying the inhibitors. The PDB code for the protein is listed in the third column; all protein structures are from clinically relevant species, except those that are italicized.

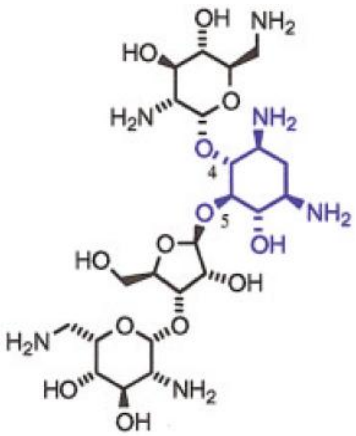
(Schmid, *Biochem. Pharmacol.* 2006, 71, 1048)

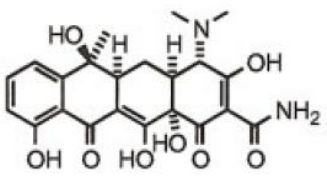
Antibacterial Discovery: Structural Genomics of the Bacterial Ribosome

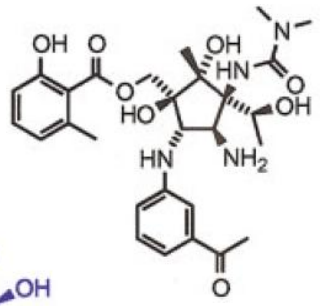
Table 1a – Available structures of antibiotics targeting the small ribosomal subunit (30S)

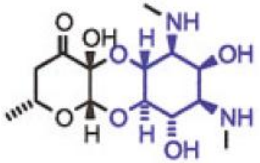
Proposed mechanism of action	Antibiotic class	Antibiotic	Refs.	PDB ID	System used for structural determination
Bind to A- or P-sites and affect decoding.	Aminoglycosides	Apramycin	[66]	1YRJ	RNA fragment
		Geneticin	[67]	1MWL	RNA fragment
		Hygromycin B	[68]	1HNZ	*T. thermophilus*
		Paromomycin	[26]	1FJG	*T. thermophilus*
		Paromomycin	[48]	1IBK	*T. thermophilus*
		Paromomycin	[25]	1J7T	RNA fragment
		Tobramycin	[50]	1LC4	RNA fragment
		Streptomycin	[26]	1FJG	*T. thermophilus*
Block binding of A-site tRNA	Tetracyclines	Tetracycline	[68]	1HNW	T. thermophilus
		Tetracycline	[69]	1I97	*T. thermophilus*
Inhibit translocation	Various	Edeine	[69]	1I95	*T. thermophilus*
		Pactamycin	[68]	1HNX	*T. thermophilus*
		Spectinomycin	[26]	1FJG	*T. thermophilus*







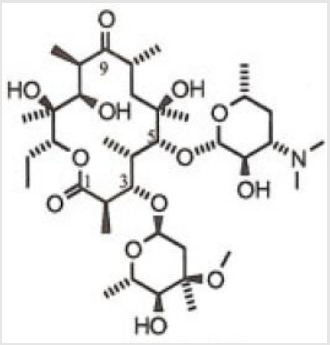
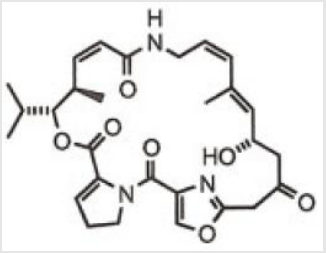
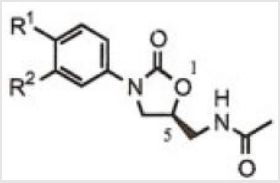




(Franceschi & Duffy, *Biochem. Pharmacol.* 2006, 71, 1016)

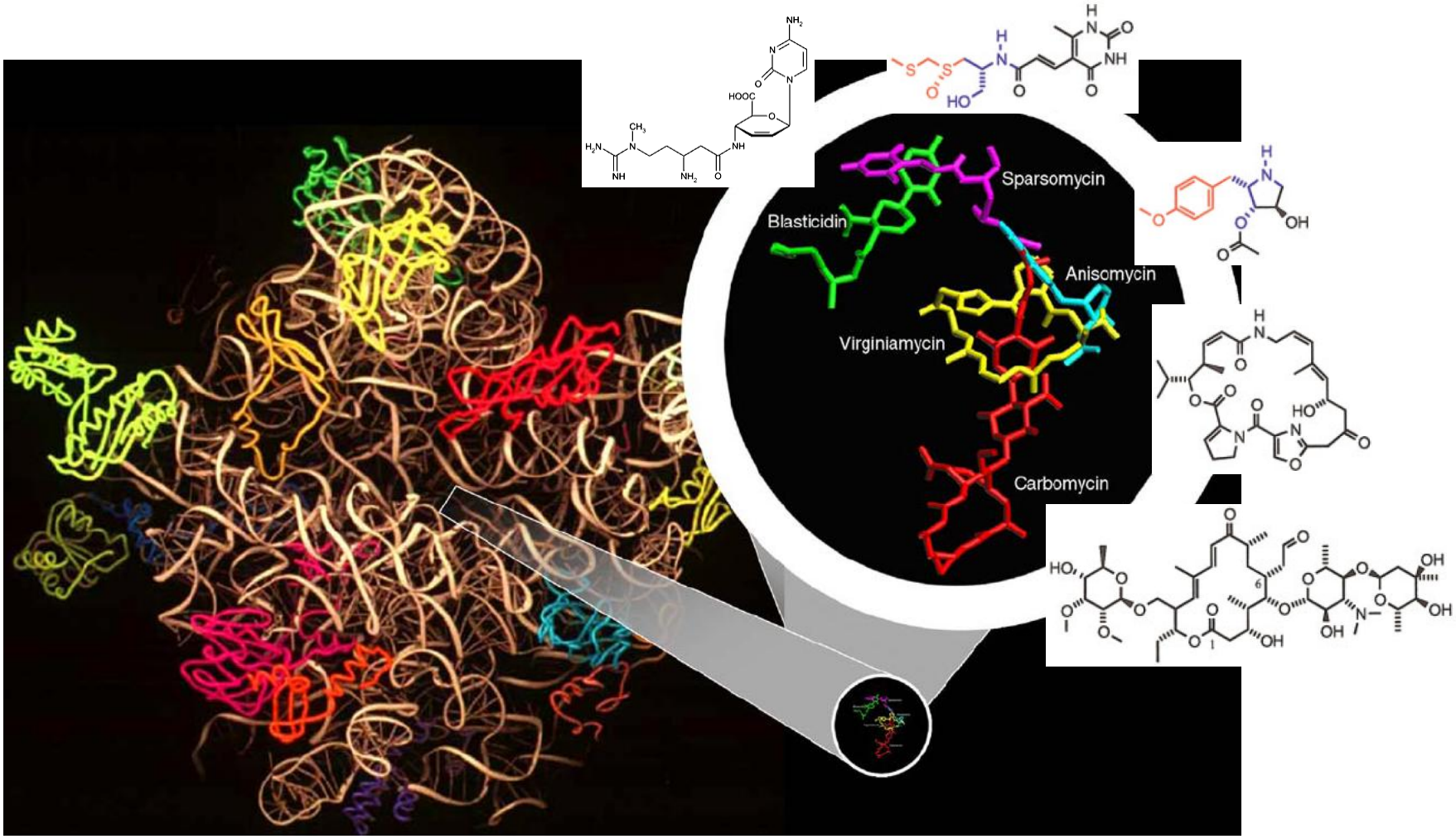
Antibacterial Discovery: Structural Genomics of the Bacterial Ribosome

Table 1b – Available structures of antibiotics targeting the large ribosomal subunit (50S)

Proposed mechanism of action	Antibiotic class	Antibiotic	Refs.	PDB ID	System used for structural determination
	Macrolides	Azithromycin	[70]	1M1K	*H. marismortui*
		Azithromycin	[71]	1NWY	*D. radiodurans*
		Azithromycin	[19]	1YHQ	*H. marismortui* (G2058A)
		Erythromycin	[72]	1JZY	*D. radiodurans*
		Carbomycin	[70]	1K8A	*H. marismortui*
		Erythromycin	[19,79]	1YI2	*H. marismortui* (G2058A)
		Clarithromycin	[72]	1J5A	*D. radiodurans*
		Roxithromycin	[72]	1JZZ	*D. radiodurans*
		Spiramycin	[70]	1KD1	*H. marismortui*
		Troleandomycin	[73]	1OND	*D. radiodurans*
		Tylosin	[70]	1K9M	*H. marismortui*
	Ketolides	ABT-773	[71]	1NWX	*D. radiodurans*
Block peptide bond formation by interfering with A-site or P-site tRNA and/or prevent the elongation of the nascent peptide		Telithromycin	[74,79]	1P9X	*D. radiodurans*
		Telithromycin	[19]	1YIJ	*H. marismortui* (G2058A)
	Streptogramins	Dalfopristin	[75]	1SM1	*D. radiodurans*
		Quinupristin	[75]	1SM1	*D. radiodurans*
		Quinupristin	[19]	1YJW	*H. marismortui* (G2058A)
		Virginiamycin S	[19]	1YIT	*H. marismortui* (G2058A)
		Virginiamycin M	[76]	1N8R	*H. marismortui*
		Virginiamycin M	[19]	1YIT	*H. marismortui* (G2058A)
	Lincosamides	Clindamycin	[72,79]	1JZX	*D. radiodurans*
		Clindamycin	[19]	1YJN	*H. marismortui* (G2058A)
	Pleuromutilins	Tiamulin	[77]	1XBP	*D. radiodurans*
	Phenyl propanoids	Chloramphenicol	[72]	1K01	*D. radiodurans*
		Chloramphenicol	[76]	1NJ1	*H. marismortui*
	Oxazolidinones	Linezolid	[61]	Not available	*H. marismortui*
	Various	Puromycin	[78]	1FFZ	*H. marismortui*
		Sparsomycin	[76]	1M90	*H. marismortui*
		Anisomycin	[76]	1K73	*H. marismortui*
		Blasticidin S	[76]	1KC8	*H. marismortui*

(Franceschi & Duffy, *Biochem. Pharmacol.* 2006, 71, 1016)

Structure-Based Drug Discovery at the Bacterial Ribosome



(Franceschi & Duffy, *Biochem. Pharmacol.* 2006, 71, 1016)

Structure-Based Drug Discovery at the Bacterial Ribosome

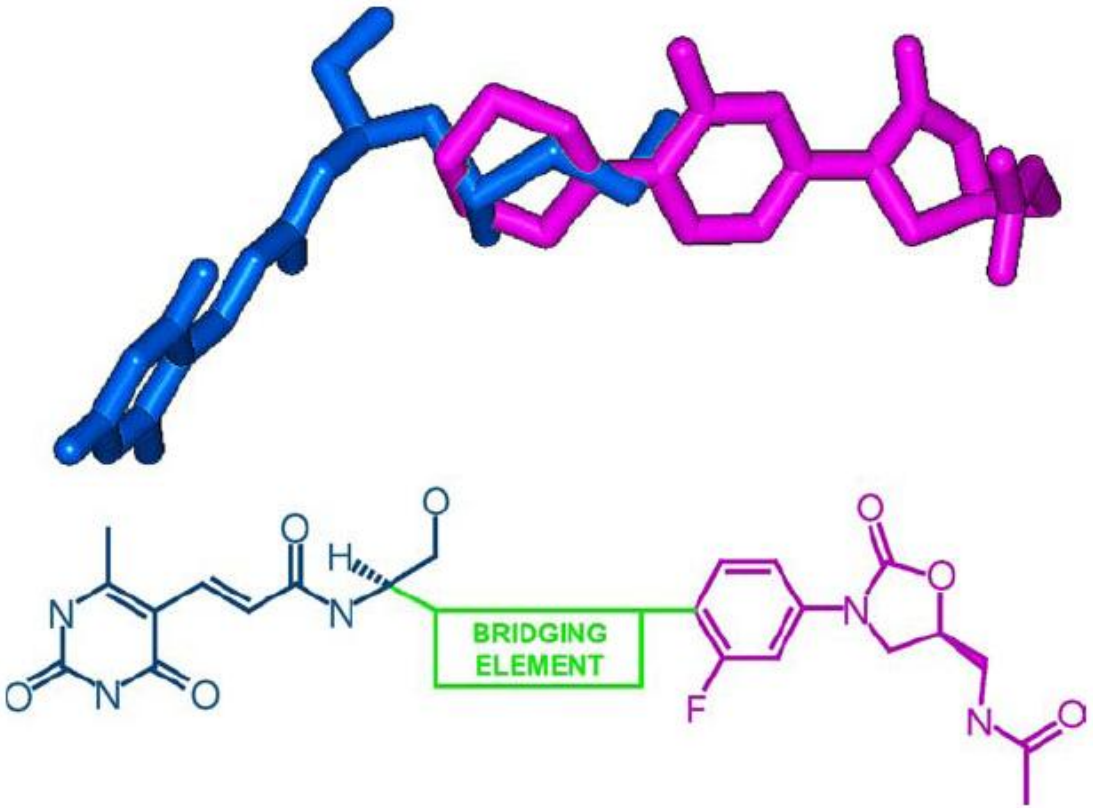
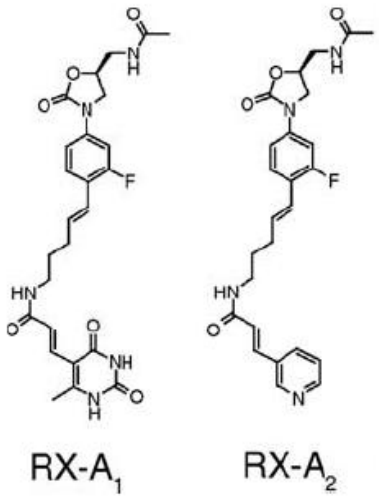


Fig. 3 – (Top) Relative binding orientations of sparsomycin (magenta) and linezolid (blue) in the 50S ribosomal subunit, with the rRNA stripped away for clarity. (Bottom) Original design hypothesis.

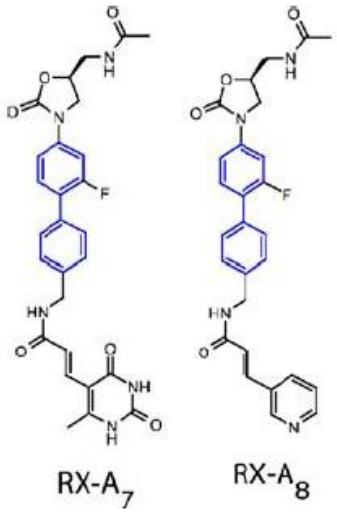
(Franceschi & Duffy, *Biochem. Pharmacol.* 2006, 71, 1016)

Structure-Based Drug Discovery at the Bacterial Ribosome



RX-A_1 RX-A_2

Strain MIC (in µg/ml)	RX-A_1	RX-A_2
S. aureus QC	32	64
S. pneumoniae 1175 (*mefa*)	1	8
S. pyogenes Msr610 (*ermB*)	1	4
E.faecalis P5 (LNZ-R G2576U)	32	128
H. influenzae parent strain RD1	>128	>128
H. influenzae 895 (*acrB* KO)	32	1
Translation IC_{50} (µM) in prokaryotes	0.92	14.6
Translation IC_{50} (µM) in eukaryotes	0.23	>200

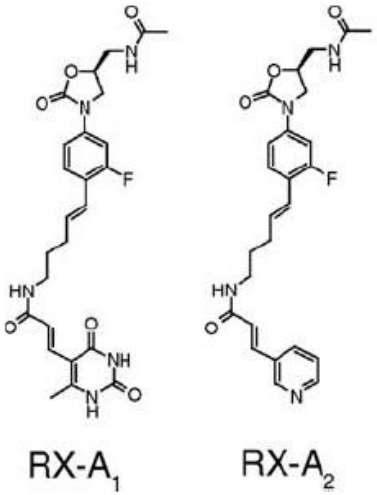


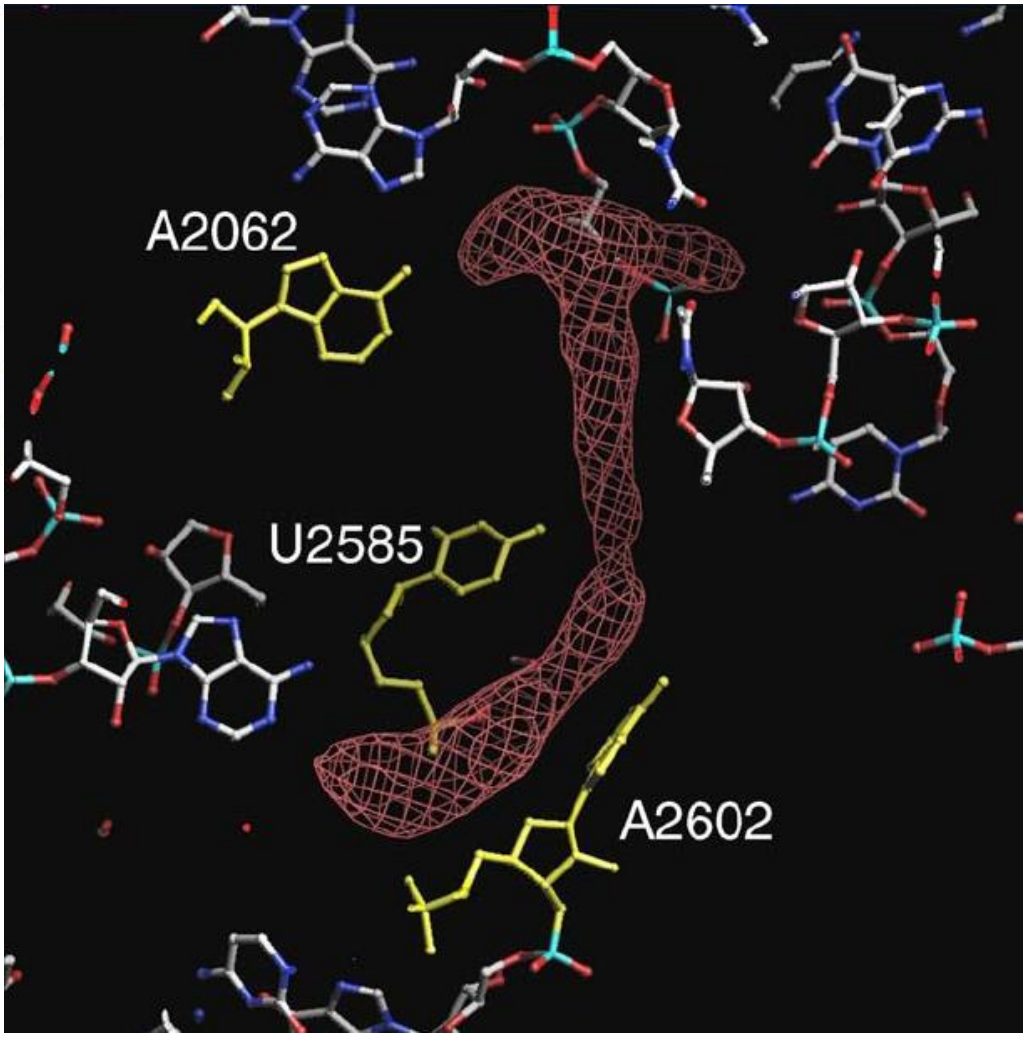
RX-A_7 RX-A_8

Strain MIC (in µg/ml)	RX-A_7	RX-A_8
S. aureus QC	0.25	4
S. pneumoniae 1175 (*mefa*)	0.25	0.5
S. pyogenes Msr610 (*ermB*)	0.25	0.5
E.faecalis P5 (LNZ-R G2576U)	16	16
H. influenzae parent strain RD1	>128	>128
H. influenzae 895 (*acrB* KO)	0.25	2
Translation IC_{50} (µM) in prokaryotes	<0.02	6.8
Translation IC_{50} (µM) in eukaryotes	1.5	>100

(Franceschi & Duffy, *Biochem. Pharmacol.* 2006, 71, 1016)

Structure-Based Drug Discovery at the Bacterial Ribosome





(Franceschi & Duffy, *Biochem. Pharmacol.* 2006, 71, 1016)