MGB Biopharma

# Bringing True Novelty to Anti-Infective Treatment

New Class of Antibacterials Based on
a Completely New Mechanism of Action

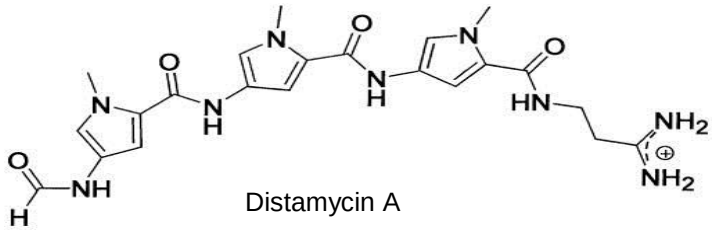
BioTrinity
London
11th-13th May 2015

# MGB Biopharma – Delivering True Novelty

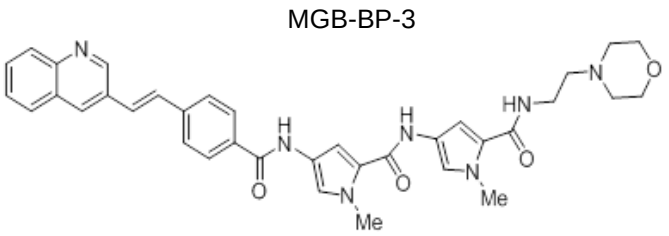
- Founded in April 2010 – HQ in Glasgow, Scotland - and funded by an Angel syndicate and the Scottish Co-Investment Fund
- Developing a truly novel class of drugs against infectious diseases based on the University of Strathclyde's DNA Minor Groove Binder (MGB) Platform Technology
- This platform provides an opportunity to develop various compounds against bacteria, viruses, fungi and parasites with a completely new mode of action which is distinct from the antimicrobial drugs used in clinical practice today
- **MGB-BP-3** is the first compound from this platform, with strong activity against Gram-positive pathogens. Currently in clinical phase I study

# Technology Platform

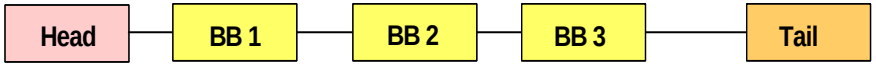
Netropsin and distamycin are naturally occurring antibiotics (*Streptomyces distallicus,* 1964) that bind reversibly to the minor groove of double helical DNA at regions with at least four consecutive AT base pairs. They are composed of a series of linked building blocks (BB) of pyrrole polyamides.



Distamycin A



MGB-BP-3




The required properties are obtained by selecting the building blocks and the way in which they are linked.

Strathclyde scientists introduced 3 new features into distamycin creating MGBs:

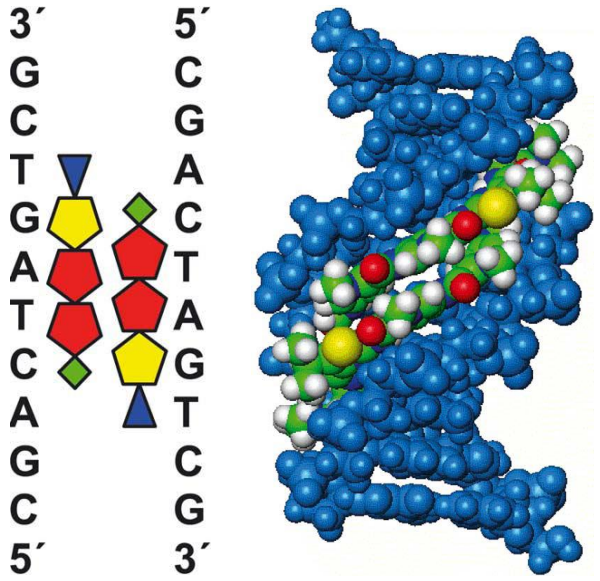
1. new building blocks in particular a thiazole;
2. short, branched alkyl chains as part of the thiazole; and
3. alkenes as links between the building blocks.

These structural features are the principal components of the patents.

# How does MGB work?

# Mode of Action

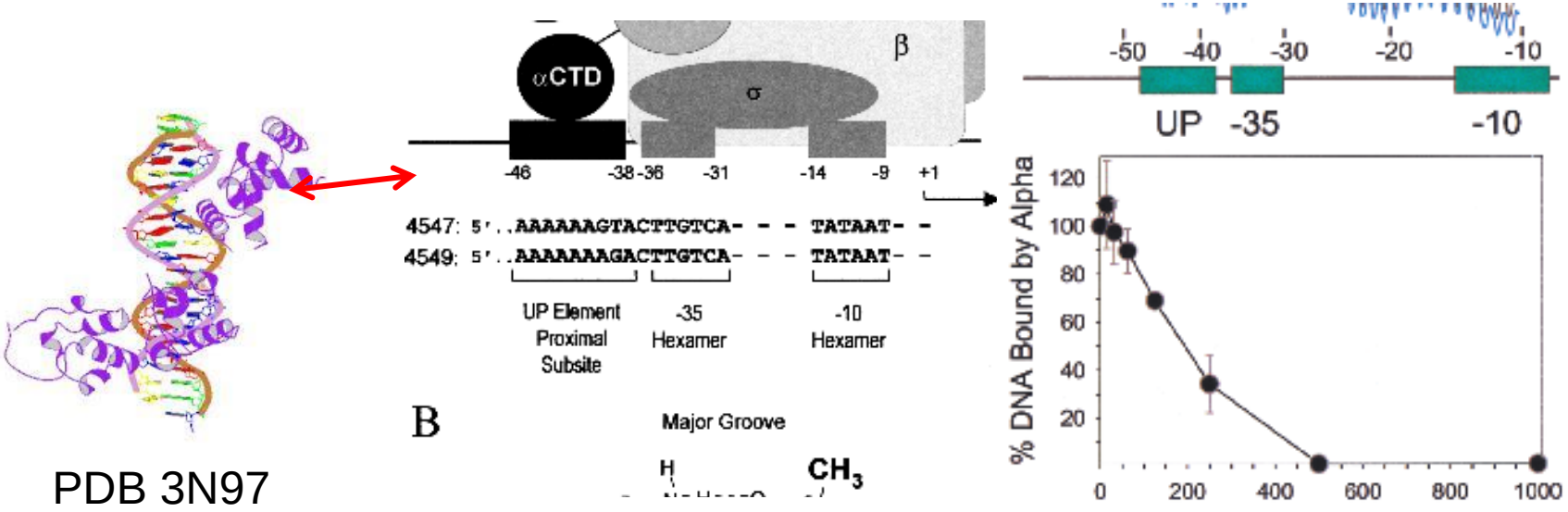
In general MGBs bind AT-rich or CG-rich sequences within the minor groove of bacterial DNA in a sequence and in a conformation-specific fashion, interfering with transcription factors and altering genetic regulation of bacteria.




*Binding of MGB-BP-3 analog compound to the DNA minor groove; NMR-derived structure of the complex between 3' and 5'-CGACTAGTCG. Green, formyl 'head'; red, N-methyl pyrrole; yellow, thiazole; blue, DMAP'tail'*

# MGBs General mechanism of action

Binding of MGB to promoter regions affects gene expression in bacteria (shown example of *E. coli*)




PDB 3N97

**Ross, W., Ernst, A. & Gourse, R. L. (2001).** Fine structure of *E. coli* RNA polymerase-promoter interactions: alpha subunit binding to the UP element minor groove. *Genes & Development* **15**, 491–506.

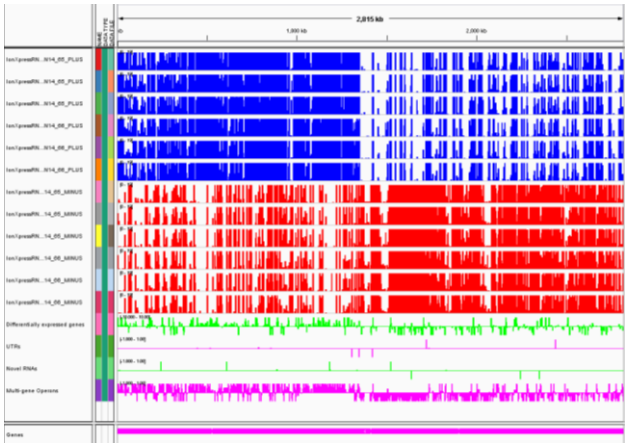
# RNA-seq. data analysis: three independent methods of MGB-BP-3 activity on S. aureus

Map reads to reference (*S. aureus* NCTC8325)

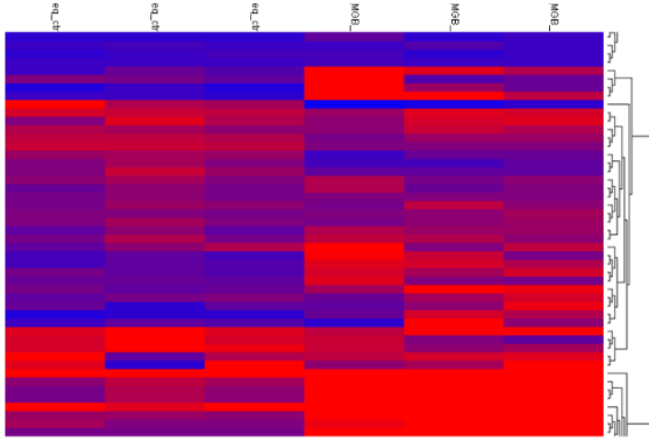
Count reads mapped to feature and normalize

Testing for differential gene expression

Rockhopper:



EDGE:



RNA-Rocket:

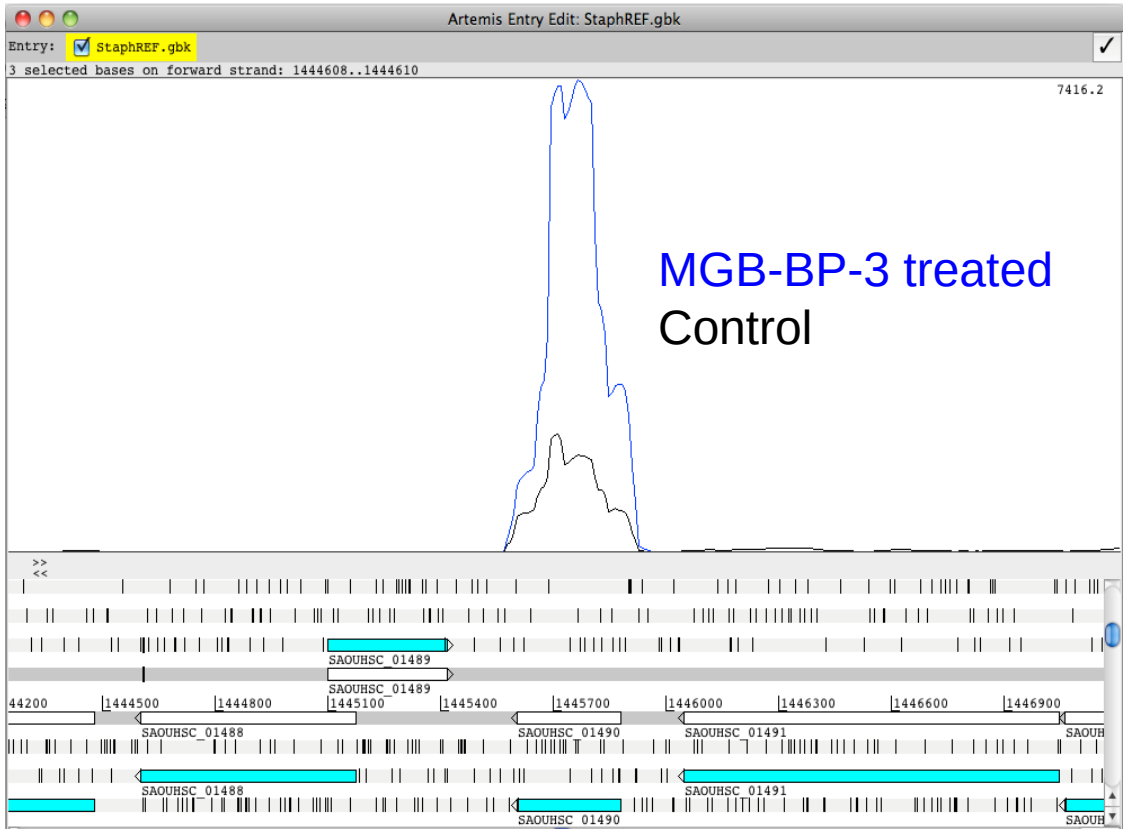
RNA-Rocket

Galaxy

[Computational analysis of bacterial RNA-seq data](). Ryan McClure, Divya Balasubramanian, Yan Sun, Maksym Bobrovskyy, Paul Sumby, Caroline A. Genco, Carin K. Vanderpool, and Brian Tjaden. *Nucleic Acids Research*, 41(14):e140, 2013.

# Effect of MGB-BP-3 on *S. Aureus* HU protein (RNA-seq. data analysis)

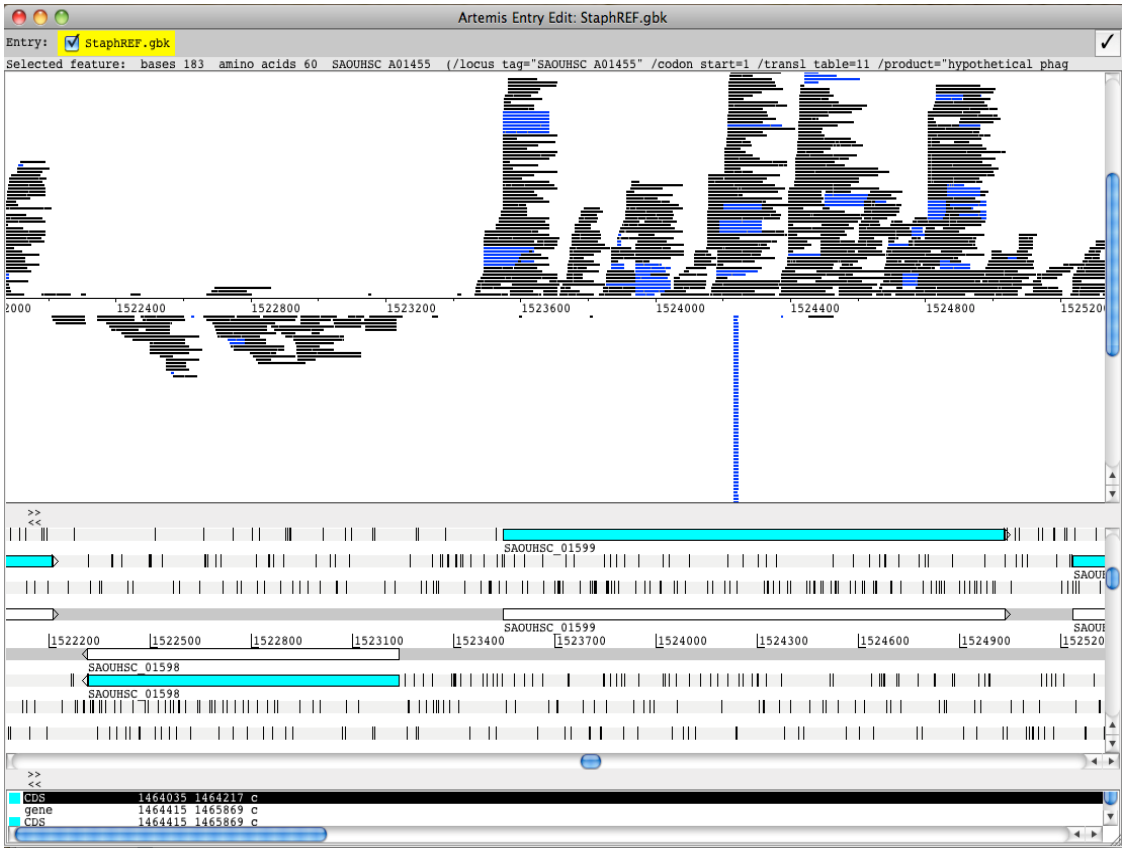
- MGB-BP-3 significantly increases expression of the histone-like protein HU (HU proteins are involved in supercoiling and DNA packaging in the prokaryotic cells, the function that is carried out by *histone protein H2A* in eukaryotic cells).
- HU protein doesn't have a well defined function in eukaryotic cells.



# Effect of MGB-BP-3 on transcripts in *S. Aureus* (RNA-seq. analysis)

MGB-BP-3 significantly affects transcripts in *S. Aureus* (RNA-seq. analysis)

**underexpressed:**
pyruvate kinase
translation elongation factor P
translation initiation facter IF-1 and IF-2
transketolase
ATP synthase F1
cell division protein FtsZ
purine nucleoside phosphorylase
GTP binding protein
thioredoxin
**methicillin resistant factor**
glycine betaine transporter
transcription antitermination protein
transcriptional regulator
DNA gyrase subunit
pyruvate carboxylase
DNA directed RNA polymerase beta prime
DNA polymerase III alpha subunit
**penicillin binding protein**
transcription-repair coupling factor
transcription termination factor Rho
transcription termination antitermination factor
DNA topoisomerase I and IV
Transcription elongation factor GreA
**drug resistance transporter**



# DNA transcription potentially affected by MGB-BP-3

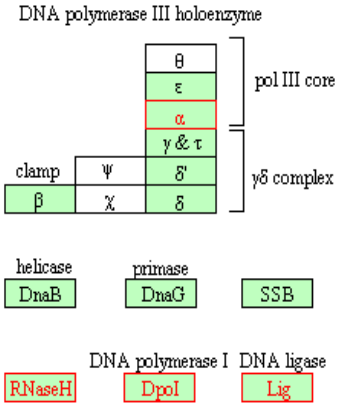
Significantly down regulated:

- DNA polymerase I
- DNA polymerase IV
- DNA polymerase III subunit alpha
- DNA ligase, RNaseH

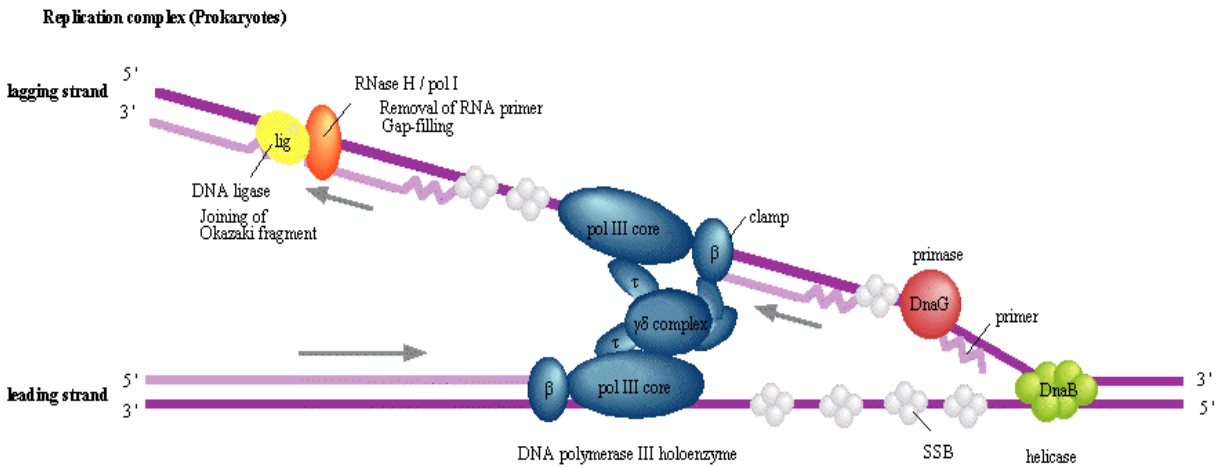
Significantly up regulated:

- DNA polymerase III subunit beta
- DNA polymerase III subunit delta'
- DNA polymerase III subunit gamma (γ) and tau (τ)
- Helicase, Primase, SSB, RNaseH

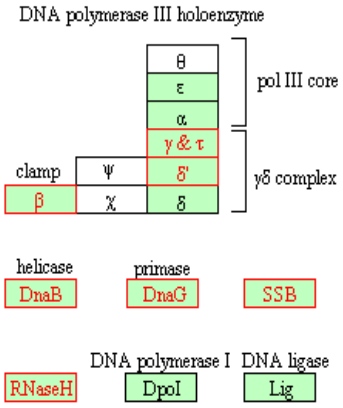
## sign. down regulated:




DNA REPLICATION



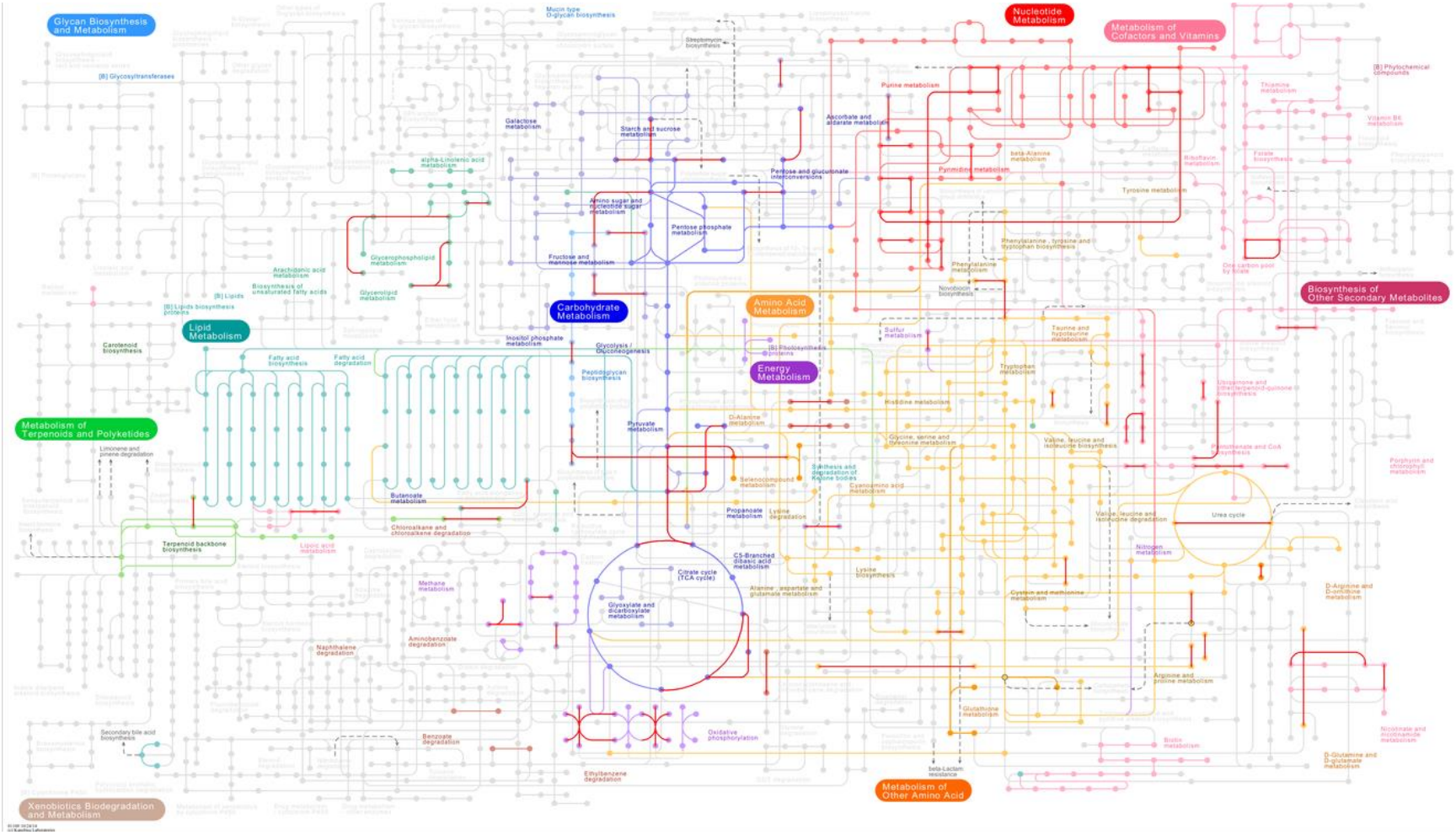

## sign. up regulated:




*Kyoto Encyclopedia of Genes and Genomes*

# Genes significantly down regulated by MGB-BP-3 highlighted in red (Rockhopper-318)

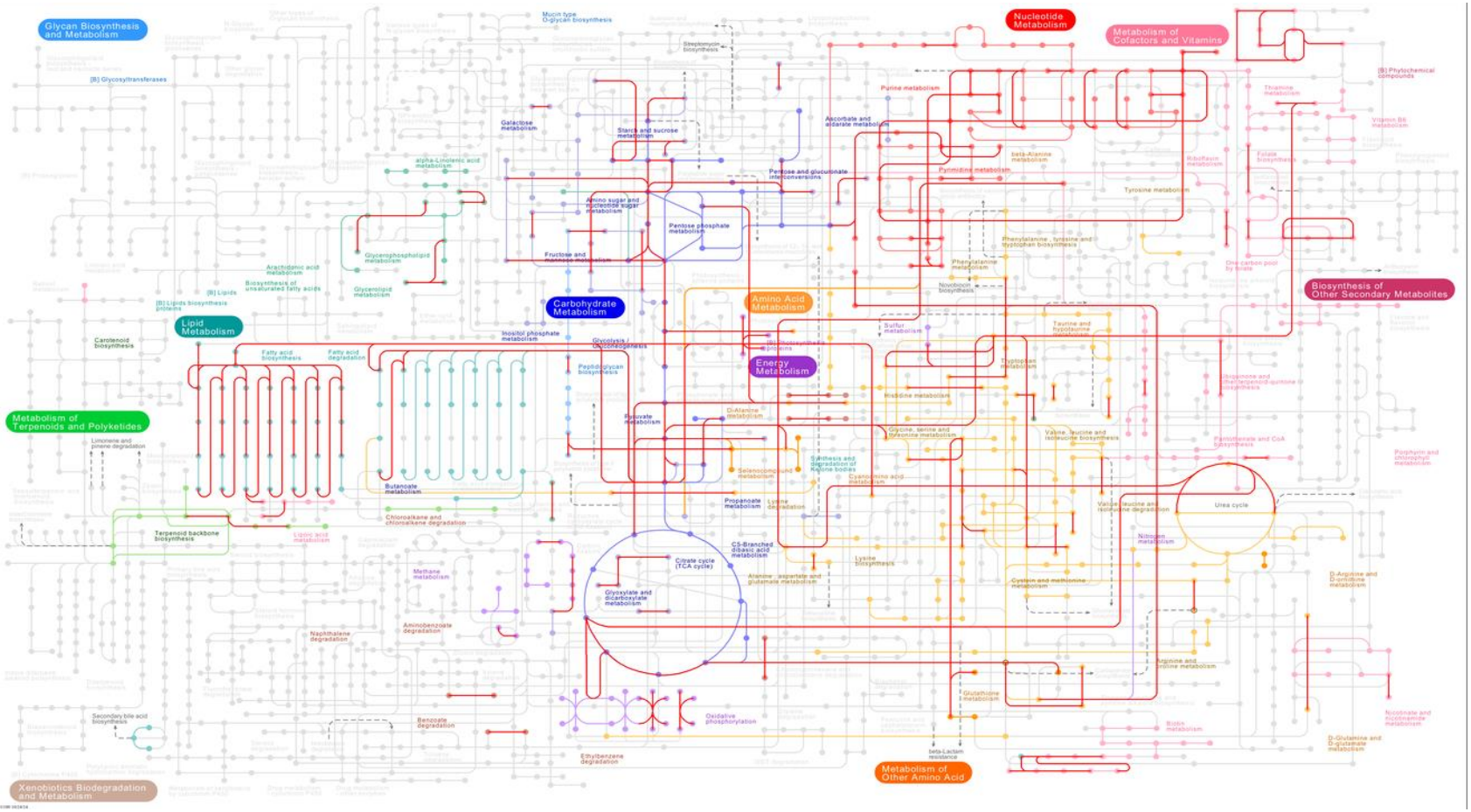
Transcriptomics data does not directly illustrate metabolic or proteomic data.




*Kyoto Encyclopedia of Genes and Genomes*

# Genes significantly up regulated by MGB-BP-3 highlighted in red (Rockhopper-318)

- Fatty acid biosynthesis: malonyl-CoA <-> beta-alanine metabolism, pyruvate metabolism
- MGB-BP-3 significantly affects the oxidative phosphorylation potential of the tricarboxylic acid cycle (**TCA cycle,** also called the **Krebs cycle**) in bacteria.




*Kyoto Encyclopedia of Genes and Genomes*

# Does MGB-BP-3 affect mammalian cells?

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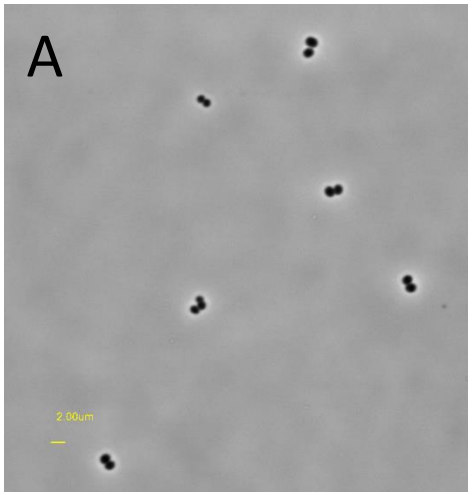
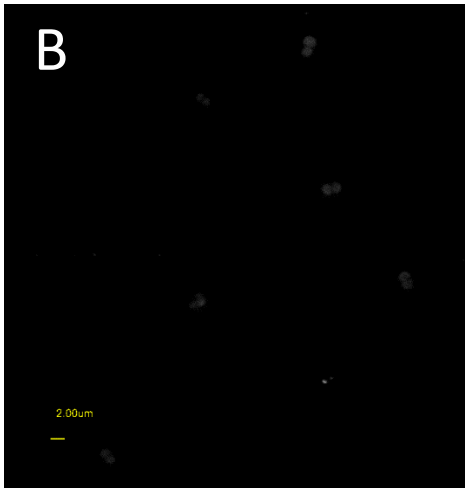
- MGB-BP-3 was internalised into all the Gram-positive bacteria tested and elicited its bactericidal effect

- MGB-BP-3 was not internalised into mammalian cells or Gram-negative bacteria (with the exception of *Neisseria meningitides* and *Moraxella catarrhalis*) and did not show any effect on DNA transcription in these cells

# Internalisation of MGB-BP-3 into *S. aureus* NCTC8325

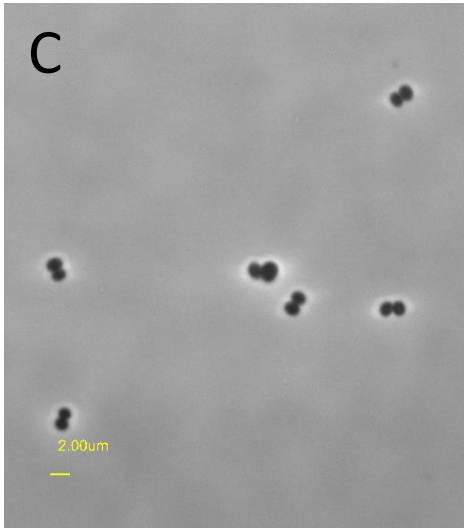


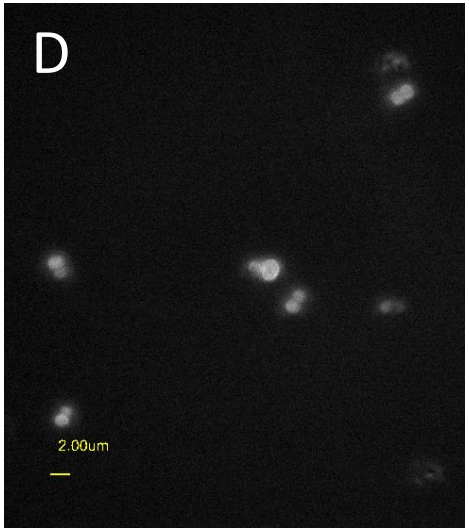




*S. aureus* NCTC8325 without MGB-BP3

**A. Brightfield**

**B. under UV**



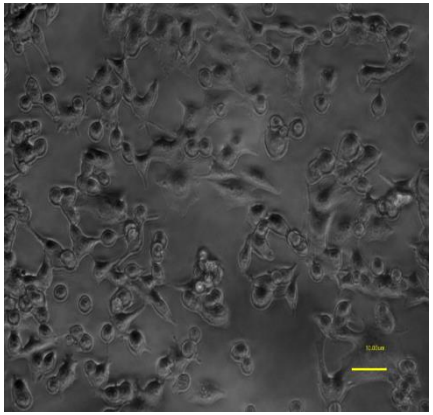
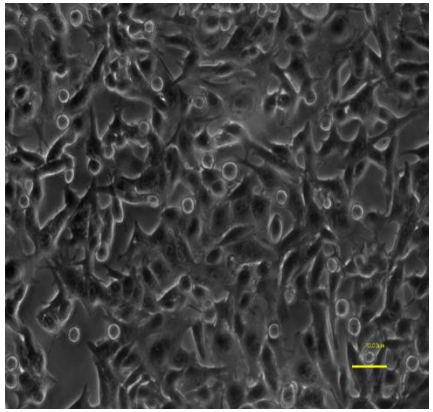
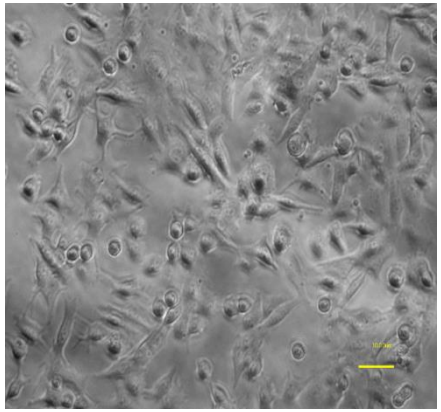
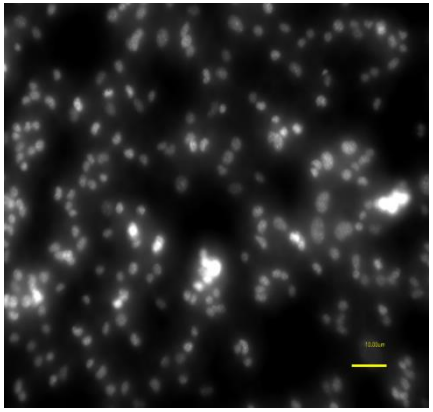
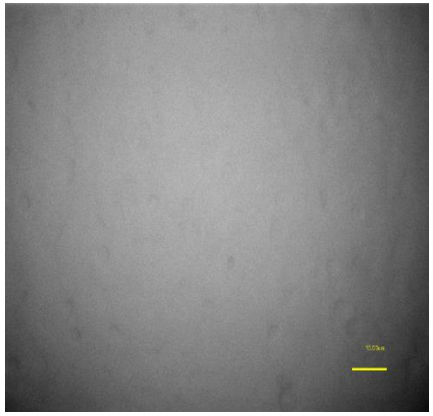
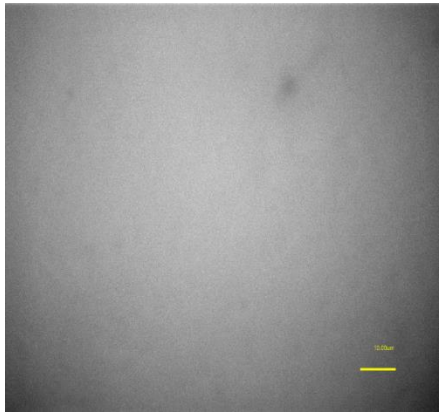




*S. aureus* NCTC8325 with MGB-BP3

**C. Brightfield**

**D. under UV**

# Absence of internalisation of MGB-BP-3 in mammalian cells B16FOluc

| | Hoechst stain (pos. ctrl) | DMSO (neg. ctrl) | MGB-BP-3 |
|---|---|---|---|
| Light microscopy |  |  |  |
| Fluorescent microscopy |  |  |  |

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