Complexity Mini-Project Proposal 2008

Supervisor 1: Dr Markus Kirkilionis, Mathematics, mail: mak@maths.warwick.ac.uk
Supervisor 2: Dr Luca Sbano, Mathematics

Project Title: Analysis of the flux control through the cytoplasmic stages of cell wall biosynthesis and perturbation by inhibitors: focus upon the essentiality of MurB

Project outline: The project will be the theoretical counterpart to an experimentally oriented project. Peptidoglycan is an essential component of the bacterial cell wall and its synthesis the target of numerous important antibiotics. It is this patthway where antibiotic resistance has evolved in important bacterial pathogens including MRSA, VISA, VRE and streptococci[1-10]. Cell wall biosynthesis is a three-phase process involving the cytoplasm, intracellular- and finally extracellular-face of the membrane. The **cytoplasmic phase** is initiated by *murA-murF*, with *murI* contributing D-Glu, *ddl* D-Ala-D-Ala and alanine reacemase D-Ala. A knowledge of the fluxes of intermediates through the cytoplasmic enzymatic steps in a range of organisms is essential especially as pharmaceutical determining potential efficacy of drugs that target this process. **Recently drug companies have investigated developing an inhibitor of MurB. It is interesting to note that the presence of a functional *murB* gene is essential** - as deletions of the gene need to be complemented by an extra-chromosomal copy of the gene in order for the mutant bug to live. So, why might MurB inhibitors not work? Chris Dowson's lab has found a possible explanation, i.e. that an **unreduced product of Mur A could be used by MurC by-passing MurB.**

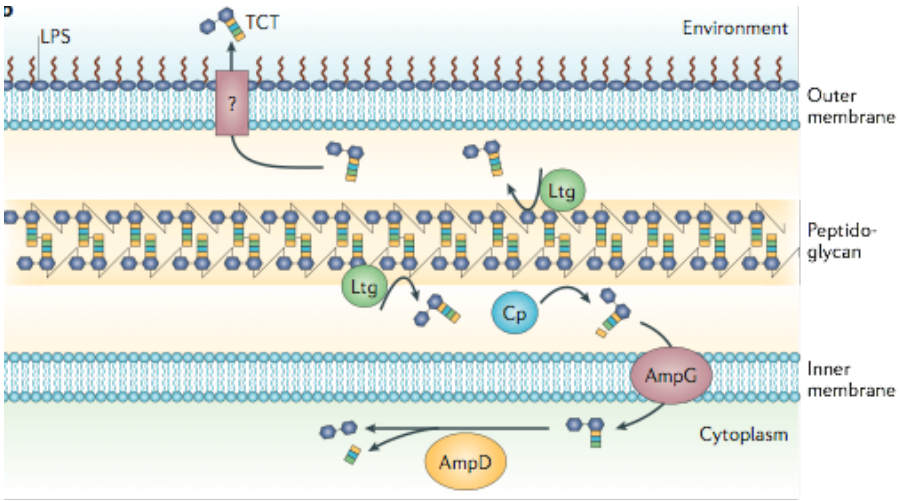

Fig: The location of the peptidoglycan cell wall in bacteria.

All the fluxes of this pathway can be measured accurately in vitro, and the student will have a knowledge how these measurements can be set up in the laboratory. In

this second mini-project we focus on different mathematical analysis tools that are available for enzyme kinetics.

Proposed Research Programme In the initial phase of the project the student will learn to understand different approaches that have currently been developed for the analysis of reaction networks in general. These are

-Stiochiometric Network Analysis (SNA)
-Graphical methods
-Metabolic flux analysis

The student will only learn to understand the fundamental mathematical basis of these methods. In a next step the student will learn how to model the different molecular machines that are involved in this process, in this case exclusively enzymes. This will be done by a new modeling approach recently developed in the group to model molecular machines, a combination of Markov chains with birth-death processes, finally looking at the deterministic limit equations after an appropriate scaling.

After this learning phase the student will set up a computational model of this pathway and perform a pathway perturbation analysis, esentially testing the *mur*B hypothesis that an alternative product serves as an inhibitor for the downstream part of the pathway.

Perturbation. This *in vitro* (A-F) system will be 'challenged' to mimic the action of specific inhibitors by altering the stochiometric ratio of these enzymes to mimic inhibition. Here we will compare the laboratory results with those generated *in silico*, i.e. by a computer simulation. The mathematical model should as an outcome show (here only numerically) which perturbations (mostly changing kinetic rates) lead to an optimal reduction of any products that can eventually serve to build up the microbes' cell walls.

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Figure 1. Cytoplasmic stages of peptidoglycan biosynthesis
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