Assess whether Cu induces inhibition of GapA and LpdA in vivo

Assess whether LpdA is inhibited in vitro

Copper (Cu) is an essential metal required as a cofactor for enzymes such as quinol oxidase. However, high concentrations of Cu are toxic to cells, and consequently, Cu levels are strictly regulated. Due to the rise of antibiotic resistance in organisms such as methicillin resistant *Staphylococcus aureus* (MRSA), which is one of the major nosocomially acquired bacterial infections, interest in how excess Cu causes toxicity has risen. This is highlighted by recent clinical trials which are assessing the use of Cu surfaces in hospitals as a means to prevent bacterial transmission. Our laboratory recently identified two proteins in *S. aureus*, glyceraldehyde-3-phosphate dehydrogenase (GapA) and lipoamide dehydrogenase (LpdA), which potentially bind Cu in medium supplemented with excess Cu. We hypothesise that excess Cu may cause toxicity by sequentially binding to and inhibiting enzymes involved in metabolic processes within the cell.

**Methods**

1) **Growth Analysis**

   - Dilute in defined medium
   - OD<sub>600</sub> measured every hour for 8 hours in 96 well plate

2) **Isolation of in vivo lysates**

   - Cells pelleted and washed with EDTA to remove excess Cu

3) **Isolation of Recombinant Enzyme**

   - Elution treated with TEV protease

4) **Activity Assays**

   Enzymatic activity of GapA was determined from production of NADH in the presence of its substrate G3P. LpdA activity was determined from consumption of NADH in the presence of lipopamide. Both reactions were monitored at A<sub>562</sub>nm for 5 minutes from 10µl of lysate/recombinant enzyme. Lysates from cells grown in the absence of Cu (control) and recombinant LpdA were also titrated with metal.

**Results**

1) *S. aureus* shows [Cu]-dependent growth inhibition.

2) GapA is inhibited by concentrations of Cu which do not inhibit LpdA in vivo.

3) Incubation of lysates in Zn(II) and Ag(I) but not Cu(I) or Cu(II) inhibits LpdA.

4) LpdA activity is inhibited by Zn(II), but not by Cu(I), Cu(II) or Ag(I) in vitro.

**Conclusions**

- Cu shows strong inhibition of GapA activity but only inhibits LpdA at very high Cu concentrations in vivo.
- No significant effects of Cu(I) or Cu(II) on the activity of LpdA were observed in vitro.