**Essential paper Summary:**



-From the graph, the haem groups are major regulators in the C5 pathway, Haem A Haem L, Haem D and Haem F in particular are linked to increasing ALA production. Whereas Hem B, HemG and Hem H diminished ALA production.

- Hem B is a key control node and might be feedback inhibited by the intermediate protoporphyrinogen IX

-C5 pathway results in the biosynthesis of ALA from glutamate.

 -We need to find a way to remove glycine and succinate

*-hemA*[17](http://www.nature.com/articles/srep08584#ref17),[18](http://www.nature.com/articles/srep08584#ref18),[19](http://www.nature.com/articles/srep08584#ref19),[20](http://www.nature.com/articles/srep08584#ref20) (encodes glutamyl-tRNA reductase, *Salmonella arizona*) and *hemL* (encodes glutamate-1-semialdehyde aminotransferase, *E. coli*) in *E. coli*

-overexpression of *rhtA* (encodes a membrane protein for threonine and homoserine exporting, *E. coli*) and optimization of minimal medium composition and cultivation process also increases ALA.

-Need to know more about haem regulation methods

-Express genes using low-copy number vector pCDFDuet-1 in*E. Coli*

-In particular, up-regulation of *hemB* resulted in reduced biomass and glucose consumption which is likely due to the accumulation of the harmful intermediate porphobilinogen (PBG) and its derivatives[12](http://www.nature.com/articles/srep08584#ref12) – So possibly downregulate Haem B?

-Interestingly, overexpression of *hemG* resulted in more biomass coupled with a long lag phase which is likely due to its direct involvement in energy generation[26](http://www.nature.com/articles/srep08584#ref26) and the toxic intermediate protoporphyrin IX[27](http://www.nature.com/articles/srep08584#ref27).

-The results suggested that high-level overexpression of the upstream genes *hemA*s and *hemL* as well as the downstream gene*hemF*, and moderate overexpression of the downstream gene *hemD* are favorable to ALA accumulation.

-Furthermore, *E*. *coli* LAG with overexpression of *hemG* gave rise to a substantial increase in HemB activity (22.21 U mg−1), which might be attributed to the direct activation by protoporphyrin IX or the weakened inhibition that caused by decreased protoporphyrinogen IX

-In this study, after investigation and identification of the four positive key genes towards ALA, the heme biosynthesis pathway was further optimized with two compatible plasmids pRSFDuet-1 (high-copy number) and pETDuet-1 (medium-copy number) according to previous results. As we expected, the production of ALA was significantly increased with an optimized combination of the increased flux towards ALA.

-One side, the results demonstrated that moderate expression of HemD is crucial to ALA accumulation since overexpression of HemD not only up-regulates the upstream genes but also draws more flux to downstream reactions from ALA.

-In comparison, the strategy of down-regulating HemB with overexpression of HemF was an alternative to improve ALA production.

- Use *E*. *coli*BL21 (DE3) was used as host for gene expression due to the T7 RNA polymerase. *E*. *coli* JM109 was used for DNA manipulations and plasmids construction.

-Luria-Bertani (LB) medium