Balanced globin protein expression and heme biosynthesis improve production of human hemoglobin in Saccharomyces cerevisiae

Due to limitations associated with whole blood for transfusions (antigen compatibility, transmission of infections, supply and storage), the use of cell-free hemoglobin as an oxygen carrier substitute has been in the center of research interest for decades. Human hemoglobin has previously been synthesized in yeast, however the challenge is to balance the expression of the two different globin subunits, as well as the supply of the prosthetic heme required for obtaining the active hemoglobin \((\alpha_2\beta_2)\). In this work we evaluated the expression of different combinations of \(\alpha\) and \(\beta\) peptides and combined this with metabolic engineering of the heme biosynthetic pathway. Through evaluation of several different strategies we showed that engineering the biosynthesis pathway can substantially increase the heme level in yeast cells, and this resulted in a significant enhancement of human hemoglobin production. Besides demonstration of improved hemoglobin production our work demonstrates a novel strategy for improving the production of complex proteins, especially multimers with a prosthetic group.