Triterpenoids are secondary plant metabolites derived from squalene and consist of six isoprene units (C30). Many of them or their synthetic derivatives are currently being investigated as medicinal products for various diseases. The cyclic triterpenoid betulinic acid is of special interest for the pharmaceutical and nutritional industry as it has antiretroviral, antimalarial, and anti-inflammatory properties and has potential as an anticancer agent (Muffler et al. 2011, Mullauer et al. 2010). Despite their obvious industrial potential, the application is often hindered by their low abundance in natural plant sources. This poses challenges in a biosustainable production of such compounds due to wasteful and costly product purification.

Here, we present a novel biotechnological process for the production of betulinic acid using tailored *Saccharomyces cerevisiae* strains. The multi-scale optimization of this microbial process included:

- **Pathway engineering** by determination of optimal gene combination and dosage,
- **Compartment engineering** to increase the reaction space of the betulinic acid pathway, and
- **Strain engineering** by implementation of different push, pull and block strategies.

In parallel we developed the fermentation process and were able to boost the performance of the engineered yeast by optimization of medium composition, cultivation conditions, carbon source and mode of fermentation operation in lab scale bioreactors. Product purification was achieved by a one-step extraction with acetone.

The final process was evaluated in terms of economic and ecological efficiency and rated to be competitive with existing plant extraction procedures with potential for further performance improvement.

**Figure 1** – Strain and process optimization strategies for betulinic acid production in *S. cerevisiae*.