# Computational Insights into Fuels and Chemicals Extraction from Microbial Biorefineries

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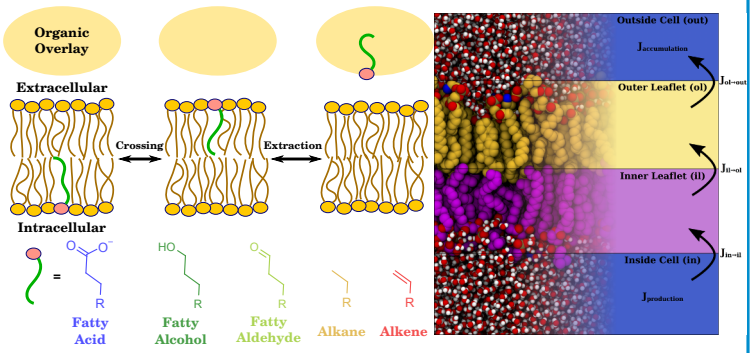
## Microbial Extraction Challenge

Through targeted engineering of microbial systems, it is possible to direct metabolism towards the production of fuels and chemicals at the industrial scale.

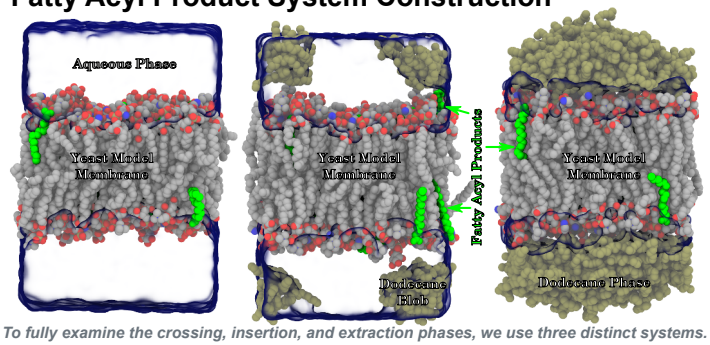
Extracting the products from the host organism poses a significant challenge. Prior benchtop experiments have observed product extraction into an organic phase from intact cells.

Through simulation, we provide mechanistic insight into rate-determining steps of product extraction in these systems, first for fatty acyl products, then for terpenoids.




*The schematic on the left shows the overall process under study, where the compound switches leaflets and then then is extracted into a dodecane overlay. Through molecular simulation, we determine which of the fluxes (right) is rate limiting for the extraction of the fatty acyl compounds in the lower left.*

## Fatty Acyl Product System Construction




*To fully examine the crossing, insertion, and extraction phases, we use three distinct systems. Membrane crossing and aqueous extraction are evaluated in the system on the left, with a yeast model membrane and 9 copies of the product in each leaflet. We extract then using a contact-based reaction coordinate into either a solid dodecane phase (left), or a dodecane blob (middle).*

*To determine permeability, we use the inhomogeneous solubility diffusion model, which is dependent principally on the free energy profile and the local diffusivity. To determine these values, we carry out replica exchange umbrella sampling simulations in addition to equilibrium simulations where we check for spontaneous membrane crossing events. Due to the flexibility of the fatty acyl products, the reaction coordinate used for extraction was based on product-lipid and product-dodecane contacts. Crossing was measured using the position of the C1 carbon relative to the membrane normal.*

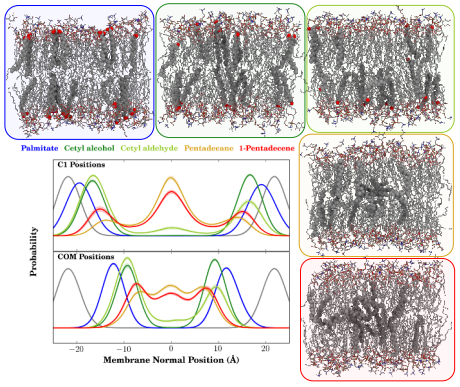
Permeability Free Energy Temperature

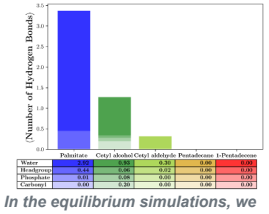
$$P^{-1} = \int_{\xi_l}^{\xi_u} \frac{\exp\left(\Delta G\left(\xi\right)\beta\right)}{D\left(\xi\right)} d\xi$$

Local diffusivity Reaction coordinate

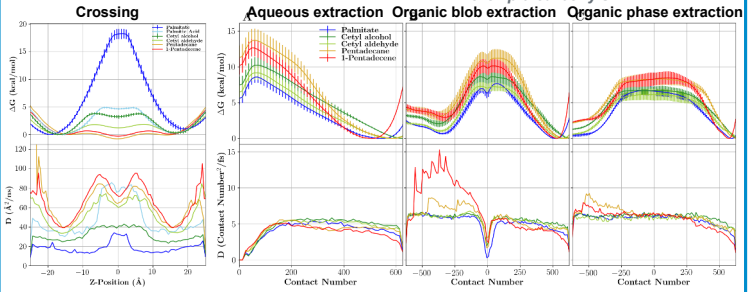
$$C\left(g_1, g_2\right) = \sum_{i \in g_1} \sum_{j \in g_2} \frac{1 - \left(\left|x_i - x_j\right| / 8\text{Å}\right)^4}{1 - \left(\left|x_i - x_j\right| / 8\text{Å}\right)^{10}}$$

## Fatty Acyl Product Permeability and Extraction



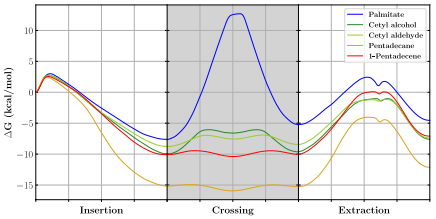

*In the equilibrium simulations, we see that the aldehyde actually has a noticeable population of fatty acids that are crossing at any given time. This is due to the reduced number of hydrogen bonds formed with the membrane environment, as the aldehyde does not have a hydrogen bond donor that can interact with the lipid carbonyls.*



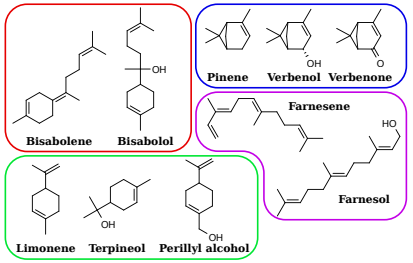

*From the replica exchange umbrella sampling calculations, we obtain the free energy profile and the local diffusivities required to determine a permeability. The trends seen in these profiles are in line with what has been reported elsewhere for lipid desorption. Since we are considering only one acyl tail, the barrier to desorption is approximately half of what it would be for a lipid to be extracted from the membrane.*

| Product | Bilayer Crossing | Extraction | | |
|---|---|---|---|---|
| | $\log_{10}\left[P\left(\frac{cm}{s}\right)\right]$ | $\log_{10}\left[P^*_{aq}\left(\frac{cm}{s}\right)\right]$ | $\log_{10}\left[P^*_{blob}\left(\frac{cm}{s}\right)\right]$ | $\log_{10}\left[P^*_{phase}\left(\frac{cm}{s}\right)\right]$ |
| Palmitate | -11.0 | -1.7 | -1.6 | -1.6 |
| Palmitic Acid | -1.6 | – | – | – |
| Cetyl Alcohol | -1.2 | -3.2 | -2.4 | -1.6 |
| Cetyl Aldehyde | 0.2 | -2.3 | -1.4 | -0.9 |
| Pentadecane | 1.4 | -5.3 | -3.9 | -2.3 |
| 1-Pentadecene | 1.1 | -4.4 | -3.1 | -2.4 |

*Since we considered each stage of the process independently, a permeability-like quantity can be determined for each step of the overall product egress process. For the compounds under consideration, typically the extraction step is rate-limiting, as bilayer crossing happens fast enough for uncharged compounds that the kinetics of desorption control the combined permeability of the whole process. Alcohols and aldehydes would therefore be the optimal product to overexpress, as they transit the bilayer quickly unlike charged species, and are hydrophillic enough to exit the bilayer at an appreciable rate. However, due to the overall energetics of the process, these products will be most concentrateeven when dodecane is present.*

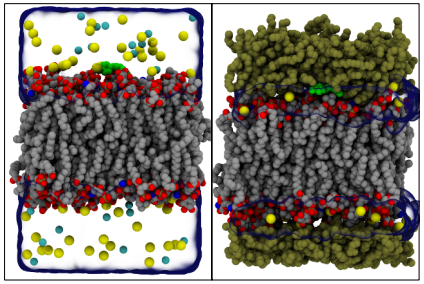


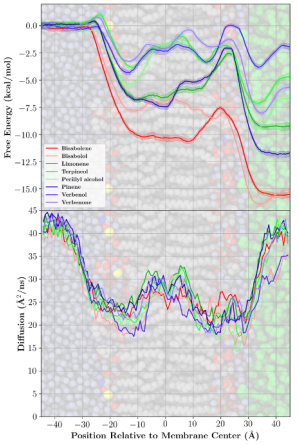

## Extension to Terpenoid Products

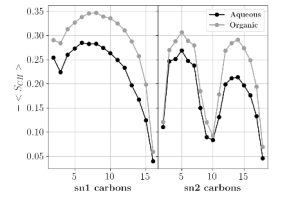



*Similar to fatty acids, terpenoids are also frequent target compounds for strain engineering efforts. Based on the fatty acyl product results, we expect that mild oxidation would improve the efficiency of terpenoid extraction.*

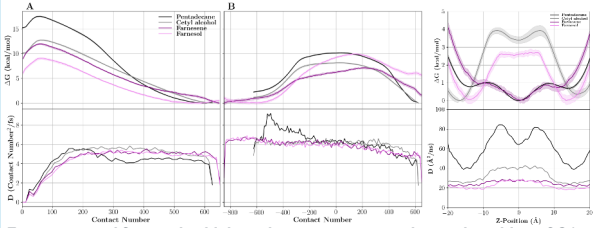
*In contrast to the fatty acid simulations, these products were not initially integrated into the membrane, but instead were placed above it. The systems were then equilibrated prior to biased simulation to determine the free energy and diffusivity profiles.*






*Rather than exclusively using contact number as we did before to measure extraction, we directly use the compound center of mass for the compounds with few rotateable bonds as our reaction coordinate. In this case, we clearly see a difference in lipid ordering when dodecane is present. Alternative simulations in a fixed-area ensemble are currently being performed to determine the magnitude of the lipid ordering effect.*




*Farnesene and farnesol, which again use contact number and position of C1 as the reaction coordinates, do not show this discontinuity at the bilayer center.*

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