Biophysical interactions of lipid membranes and biomanufactured chemicals

CBMNet

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The membrane properties

• Life depends on maintaining a set of strict properties of the lipid membrane
  – Impermeable to polar molecules (ions)
  – Fluidity; mobility of lipids
  – Bending (while maintaining a bilayer structure)
  – Structural integrity
  – Shielding (e.g. LPS)
  – Chemical/specific roles
    • Lipids specific for signalling
    • Lipids specific for protein function (annular lipid shells)
    • Co-enzymes (ubiquinone-10)
    • Vitamins
    • Many more
Case study:  
long-chain polyunsaturated fatty acids (LC-PUFA)

- LC-PUFA normally obtained from marine sources, but expression in plants would allow harvesting from seeds.
- Nutritional value.
- Industrial value (Δ12 FA).
- Seeds needs high amounts of LC-PUFA to be commercially relevant (>> 20%)
- Metabolically, LC-PUFA are synthesised by changing pathways that modify FA on PC.

Case study:
long-chain polyunsaturated fatty acids (LC-PUFA)

“Biochemical analyses of plants that express these introduced fatty-acid metabolic pathways have highlighted the central importance of ensuring the removal of novel fatty acids from their site of synthesis on phosphatidylcholine to enable their further modification, exclusion from membrane lipids and accumulation in seed triacylglycerols.”

Case study:
Lipid accumulation for biofuel production

• Photosynthetic organisms could be used for solar biomass production, but metabolic engineering is required to optimise fuel types. Lipids (TAG) are suitable.
• Upon nitrogen depletion, *Chlamydomonas reinhardtii* stores energy in the form of TAG in lipid droplets (oil-bodies) and starch.
• Blocking starch production by GM did not enhance TAG production.
• Metabolic engineering has been used to increase lipid production, but this has negative effects on growth.

Case study: Lipid accumulation for biofuel production

- Reducing lipid catabolism is an alternative approach.
- Reducing lipid catabolism problematic in plants as required for seedling establishment.
- Recent paper studied this approach in the diatom *Thalassiosira pseudonana* (an microalga).
- This diatom stores TAG under silicon starvation.

Case study:
Lipid accumulation for biofuel production

- Clear increase in TAG and lipid production in lipid droplets in mutants.

Case study: Lipid accumulation for biofuel production

- Total fatty acid composition is altered, but unclear what the distribution is in membranes.

Case study: n-butanol and isobutanol

- Production of n-butanol and isobutanol in strains like *Clostridium acetobutylicum* is targeted for biofuel production.
- Although production of isobutanol up to 20g/L could be achieved (in 2010), isobutanol is cytotoxic to bacteria at ~8 g/L due to the disruption of membranes and consequent disruption of bioenergetics.
- *E. coli* has been engineered to also make butanol.
- *E. coli* tolerance was enhanced by growing it on increasing amount of isobutanol and the genome sequenced.

Case study: n-butanol and isobutanol

- Five mutants identified and effects confirmed.
- Effects convoluted and difficult to explain.
- $\Delta yhbJ$ leads to enhanced expression of GlmS, which catalyzes the synthesis of GlcN-6-P. An increase in GlcN-6-P enhances the synthesis of UDP-GlcNAc, which is a precursor for peptidoglycan and LPS.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutation type</th>
<th>Position (nucleotide)</th>
<th>Region</th>
<th>Coordinates</th>
<th>Function</th>
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<td>acrA</td>
<td>IS10 (+)</td>
<td>1175</td>
<td>Coding</td>
<td>483 668</td>
<td>Membrane protein, subunit of AcrAB-ToIC multidrug efflux transport system</td>
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<td>gatY</td>
<td>IS10 (−)</td>
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<td>2125 224</td>
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<td>yhbJ</td>
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<td>437</td>
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<td>3 345 574</td>
<td>Predicted P-loop containing ATPase</td>
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<td>marCRAB</td>
<td>Deletion, hipA–flxA</td>
<td>Coding + intergenic</td>
<td>1 588 791–1 645 053</td>
<td>marR: DNA-binding transcriptional repressor</td>
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<td>marA: transcriptional dual regulator</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>marB: multiple antibiotic resistance protein</td>
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<td></td>
<td></td>
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<td>marC: predicted transporter</td>
</tr>
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</table>

*a* The direction of the IS elements. ‘+’ is forward, ‘−’ is reverse strand.

*b* Starting position of insertion sequence.
Case study:
Octanoic acid (and n-butanol)

- Exactly the same idea was repeated 5 years later with octanoic acid.
- Evolved strains showed improved resistance to octanoic acid, but also other compounds like n-butanol.
- Analysis showed that evolved strains has significantly longer lipid length.

Case study: Octanoic acid

- A separate approach was trialled, in which the membrane was reconstructed to be more resistant to short-chain fatty acids and alcohols.
- Trans unsaturated fatty acids (TUFA) were produced and incorporated into the membrane of *E. coli* by expression of cis-transisomerase (Cti) from *Pseudomonas aeruginosa*.

Case study: Octanoic acid

• Increasing TUFA, increases the membrane rigidity (reduces fluidity).
• Engineered strains producing short-chain fatty acids (TFA), including octanoic acids (C8), gave higher yields in the strains that also expressed Cti.

Case study: Octanoic acid

Case study: Intrinsically disordered proteins

- When intrinsically disordered proteins (Epsin1 and AP180) are bound to lipid membrane via his-tag-NTA bonding, tubules are formed, disrupting the membrane.

Case study: Intrinsically disordered proteins

- It is thought this is due to steric repulsion.
- For claterin-mediated uptake, disordered domains could prevent uptake.

Case study:
Intrinsically disordered proteins

Biophysical analyses of effects.

1. Stability (leak assays)
2. Transition temperature (Tm)
3. Phase separation, critical temperature (Tc)
4. The area compressibility modulus, bending modulus, lysis tension, lysis strain, and area expansion.
5. Mechanical stability and structure?
Stability (e.g. leak assay)

- CF Leak assay


Transition temperature (Tm)

- Differential scanning calorimetry is a relatively easy way to measure Tm.
- (Values of butanol in mg/ml → 5 mg/mL = 68 mM)

Critical temperature (Tc)

- Cell derived giant vesicles
- Add fluorescent probe (DiI-C₁₂, which labels the liquid disordered (Ld) phase).
- Observe phase separation as a function of temperature.

area compressibility modulus, bending modulus, lysis tension, lysis strain, and area expansion

- One experiment (micropipette aspiration).
- Not too sensitive and concentrations up to 8 M of short alcohols were added.
- Could effects of altered lipid composition or additives be studied?

Using biophysics to inform tolerance

- Recently, MD simulation and lipid diffusion measurements were used to determine if additives can stabilise model membranes impaired by butanol.
- One compound was selected (COE1-5C) that indeed reduced fluidity and membrane disruption of model membranes.
- Growth test with *E. coli* showed moderate improvements in tolerance towards butanol.
- COE1-5C is too expensive to use as an additive, while COE1-5C was developed to promote charge movement across membranes.