Eukaryotic and Prokaryotic xMALPs composition: a comparative study

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Rhomboids are fragile intramembrane proteases

Undercharacterized group of intramembrane proteases

Substrate cleavage in the membrane

Linked to: Alzheimer’s disease, Malaria, Parkinson’s disease
xMA stabilize fragile intramembrane proteases

Challenging expression & purification:

- Self-process in detergent micelles
- Loss of activity

Barniol-Xicota, M. & Verhelst, S. H. L. JACS. 2018, 140, 44, 14557
Activity level changes depending on xMA used

Activity measured using activity based probe TAMRA-FP + in gel resolution

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Do xMALPs resemble the native membrane?

xMAs used in this work:

- SMA
  \[ m = 2.3 \text{ or } 3 \]
  \[ n = 1 \]

- DIBMA
  \[ n = 1 \]

- SMA-QA
  \[ m = 2.3 \]
  \[ n = 1 \]

Membranes solubilized:

- Prokaryotic: \textit{E. coli}
- Eukaryotic: \textit{Jurkat cells}

- \textbf{Solubilization efficiency}
  compared to non ionic detergent DDM

- \textbf{Protein content}
  SDS-PAGE

- \textbf{Lipid content:}
  lipidomics
xMA are efficient solubilizing agents ***

**E. coli** solubilization efficiency

In **Jurkat** membranes SMAs and DIBMA as efficient as DDM

**E. coli** prot content:

Poor solubilization of high MW proteins by all xMAs in **Jurkat** membranes
xMA preferentially solubilize lipid species

Lipid composition (Jurkat)
Analyzed by LC-MS/MS

Cholesterol content
Data from colorimetric assay
Lipid charge does not influence solubilization

Phospholipid headgroups (Jurkat)
Analyzed by LC-MS/MS

- Phosphatidylglycerol - PG
- Phosphatidylinositol - PI
- Phosphatidylcholine - PC
- Phosphatidylethanolamine - PE
Saturation has little effect on xMA solubilization

Fatty acid chain saturation in total lipid content (Jurkat)

Analyzed by LC-MS/MS
Saturation has little effect on xMA solubilization

Fatty acid chain saturation in **specific** lipid classes (Jurkat) - Analyzed by LC-MS/MS

**Phosphatidylcholine (PC)**

**Phosphatidylinositol (PI)**
FA chain length does not guide xMA solubilization

Fatty acid chain length total carbons in total phospholipid (Jurkat) Analyzed by LC-MS/MS
FA chain length does not guide xMA solubilization

Fatty acid chain total carbons in total phospholipid (Jurkat) - Analyzed by LC-MS/MS

Fatty acid chain total carbons in total phosphatidylinositol (Jurkat) - Analyzed by LC-MS/MS
Conclusions

- **xMA efficient** solubilizing agents

- **Membrane structure** seems to influence solubilization preferences

- Select xMA depending on **protein** of interest and **expression system**

- Not all xMAs are membrane like.

  **SMA(3:1)** most **membrane like**.

- Caution when determining lipid **environment/activity** of xMALPed proteins

- Future: **novel polymers** with improved membrane disruption properties
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https://chemrxiv.org/s/6ccbf5fddacc5887a89d

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