Rapid screening of multiple detergents for extraction, purification and crystallization of membrane proteins

Debanu Das 1,*, Qian Steven Xu 1,*, Jonas Lee2, Rosalind Kim1 and Sung-Hou Kim 1,2

1Berkeley Structural Genomics Center, Lawrence Berkeley National Laboratory, Berkeley, California 94720 http://www.strgen.org
2Department of Chemistry, University of California, Berkeley, California 94720-5230 * These authors contributed equally to the work

Introduction

One of the major hurdles in the crystallography of membrane proteins is the quest for a detergent that will not only allow optimum extraction of the target from the membrane, but will also maintain protein stability throughout the purification procedure and result in diffraction quality crystals. This is manifested in the small number of crystal structures of membrane proteins (~160) compared to the thousands of crystal structures of soluble proteins (~28,000) in the Protein Data Bank. Despite recent reports recognizing the need to optimize the screening of different detergents, there is a lack of well defined and efficient protocols for this purpose and thus such screening remains largely a trial and error procedure which can be rather time consuming and specific to the particular membrane protein under study.

We describe a rapid screening protocol based on conventional methodologies of membrane extraction and affinity purification that can be used to test many different detergents, including non-ionic and zwitterionic detergents, for suitability for membrane protein extraction, purification and crystallization with results obtained in 48-72 hours. The strategy can be modified to screen their compatibility in solubilizing, purifying and crystallizing membrane proteins. The protocol can be easily modified to incorporate minor modifications to take into consideration the biochemical nature of a target, and thus can be generally applied to any membrane protein in a high-throughput implementation.

II. Table of detergents used in the screen

<table>
<thead>
<tr>
<th>Detergent</th>
<th>Mol Wt</th>
<th>CMC value (mM)</th>
<th>Micellar (Critical Micelle Concentration)</th>
<th>Final % used for target extraction</th>
<th>Optimum extraction of membrane proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ionic</td>
<td>SDS</td>
<td>288.38</td>
<td>2.6</td>
<td>0.075</td>
<td>0.75</td>
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<tr>
<td></td>
<td>Zwitter ionic</td>
<td>259.41</td>
<td>1.5</td>
<td>0.034</td>
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<td></td>
<td>CHAP</td>
<td>849.9</td>
<td>8.0</td>
<td>0.49</td>
<td>1</td>
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<td></td>
<td>Empigen</td>
<td>271.4</td>
<td>1.5</td>
<td>0.041</td>
<td>1</td>
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<tr>
<td></td>
<td>Flo-choline (FC)</td>
<td>135.5</td>
<td>1.5</td>
<td>0.053</td>
<td>0.6</td>
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<td></td>
<td>DPPE</td>
<td>481.57</td>
<td>1.4</td>
<td>0.067</td>
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<tr>
<td></td>
<td>Non-ionic</td>
<td>OG</td>
<td>306.4</td>
<td>0.20</td>
<td>0.5</td>
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<tr>
<td></td>
<td></td>
<td>n-octyl-b-D-glucoside</td>
<td>292.4</td>
<td>0.86</td>
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<tr>
<td></td>
<td></td>
<td>DM</td>
<td>482.6</td>
<td>1.8</td>
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<td></td>
<td></td>
<td>DDM</td>
<td>310.5</td>
<td>0.17</td>
<td>0.0087</td>
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<td></td>
<td></td>
<td>Trem X-100</td>
<td>447</td>
<td>0.23</td>
<td>0.015</td>
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<tr>
<td></td>
<td></td>
<td>C12 E12</td>
<td>581</td>
<td>0.05</td>
<td>0.0029</td>
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<tr>
<td></td>
<td></td>
<td>C12 E9</td>
<td>438.1</td>
<td>1.8</td>
<td>0.27</td>
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<tr>
<td></td>
<td></td>
<td>Triton X-100</td>
<td>466.5</td>
<td>1.6</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HEGA 10</td>
<td>379.5</td>
<td>7.0</td>
<td>0.27</td>
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<tr>
<td></td>
<td></td>
<td>MEGA 10</td>
<td>349.5</td>
<td>6.0</td>
<td>0.21</td>
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<tr>
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<td></td>
<td>NECAMEG</td>
<td>335.4</td>
<td>19.5</td>
<td>0.65</td>
</tr>
</tbody>
</table>

Legend: L-NS=Low-speed centrifugation pellet, U-P=Ultracentrifugation, IMAC= Immobilized Metal Affinity Chromatography, SEC=Size exclusion chromatography

III. Results of small scale detergent extraction and purification trials using the detergents listed in II. Three membrane proteins underwent the process as described in I.

Membrane protein

<table>
<thead>
<tr>
<th>Gel after extraction screen</th>
<th>Gel after purification screen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane protein screen</td>
<td>Membrane protein purification</td>
</tr>
</tbody>
</table>

STAGE 1.

1. L-SN, aliquot into 18 eppendorf tubes
2. Ultracentrifugation
3. Isolate U-P (Membrane portion)
4. Aliquot 1 ml of each Detergent Screening solution (DS 1-18) into each eppendorf tube, extract membrane target overnight
5. Ultracentrifugation after extraction, analyze extraction efficiency by SDS-PAGE (SDS extraction as control). Select 5 best detergents

STAGE 2.

Screen detergents for purification

1. L-SN, ultracentrifugation, keep U-P at 4C
2. Divide U-P into 5 portions, extract with 5 best detergents
3. Run batch IMAC purification on 5 samples (at 2x CMC detergent level)

STAGE 3.

Crystallization screen

1. Concentrate eluted protein and set up crystallization screen based on yield
2. Concentrate, do detergent exchange after purification either by microdialysis or by SEC to optimize detergent for crystallization screen

Conclusions

- The results obtained demonstrate the feasibility of implementing a rapid protocol for testing a range of detergents to screen their compatibility in solubilizing, purifying and crystallizing membrane proteins.
- The protocol has been tested on three membrane proteins (MW 60 kDa) with varying levels of success. For all three test cases, stages 1&2 were successfully implemented. In case I, yields after purification and concentration were enough to use for crystallization screening.
- Based on the results of this screen, experiments can be conducted on each of these proteins on a large scale.

Acknowledgements

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