X-ray reflectivity and grazing incidence diffraction studies of amyloidogenic and peripheral membrane-binding proteins

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Two biological applications of XR and GIXD

**Alzheimer’s Disease**

- **Fibrillar Oligomer** (β-sheet rich)
  - Nucleated fibrillation
  - Disrupted lipid packing
  - Membrane thinning

- **Non-Fibrillar Oligomer** (Globular)

**Lipoxygenase (15-LOX-2)**

- Lipid extraction

**Amyloidogenic Peptides**

**Peripheral Membrane Proteins**
Membrane Structural Biology

- Liquid Expanded (LE)
- Liquid Condensed (LC)

Complexity
Structure determination
Biophysical characterization
~70% of Pharmaceutical Drugs Target Membrane Proteins

- Membrane proteins are the most available targets for small molecule pharmaceutical drugs.
- Many diseases are membrane-mediated

https://www.proteinatlas.org/humanproteome/tissue/druggable

- Alzheimer’s disease
- Other neurodegenerative diseases
- Atherosclerosis
- Cholera
- Anemia
- Tay-Sach’s disease
Lipid Diversity is an Unsolved Puzzle

Lipids are the arguably the most diverse type of biomolecule and the least understood in biochemistry. Lipids are difficult to study because they cannot be manipulated through mutational analysis.

There are also a lot of carbohydrates, which are still mysterious.
Langmuir trough

\[ F = m_p g + \gamma + F_{buoyant} \]

\[ \Pi = \gamma_0 - \gamma \]

\( \Pi \) = Surface pressure (mN/m)
\( \gamma \) = surface tension (mN/m)
\( \gamma_0 \) = surface tension pure water (mN/m)

- Lipid phase changes – temperature, pressure, lipid composition
- Detects protein interactions with the monolayer membrane
Fluorescence microscopy

- Used to discern LE vs LC phase in lipid monolayer
- Changes in amount of each phase, distinct domain boundaries, shapes of domains

Texas-Red DHPE

0.5 mol% TR-DHPE
X-ray reflectivity (XR)

- XR is used to discern regions of electron-dense material
- Fit parameters for thickness, SLD, and roughness between each layer

<table>
<thead>
<tr>
<th></th>
<th>Thickness</th>
<th>$\rho / \rho_{\text{water}}$</th>
<th>Roughness</th>
<th>Subphase Roughness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tails</td>
<td>15.7 ± 0.3</td>
<td>0.98 ± 0.04</td>
<td>3.02 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Heads</td>
<td>9.0 ± 0.4</td>
<td>1.56 ± 0.04</td>
<td>3.4 ± 0.2</td>
<td>2.91 ± 0.10</td>
</tr>
</tbody>
</table>
Grazing incidence X-ray diffraction (GIXD)

- Diffraction is observed from any semi-crystalline species at the air/water interface

\[ d = \frac{2\pi}{q_{xy}} \]

\[ L_c = \frac{0.9 \times 2\pi}{\sqrt{FWHM^2 - 0.006^2}} \]

Peak Area \( \propto \) area of semi-crystalline material
Two biological applications of XR and GIXD

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  - (β-sheet rich)
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  - Membrane thinning

- **Non-Fibrillar Oligomer**
  - (Globular)
  - Lipid extraction

**Lipoxygogenesis (15-LOX-2)**

- Peripheral Membrane Proteins
Amyloidogenic Peptides in Neurodegenerative Disease

Intrinsically disordered

Amyloid Fibrils
(β-sheet rich)
Contributors to this project

Lois Wampler
Bella Bowers
Dr. Adeline Fanni
Dr. Erik Watkins (LANL)
Paulina Majewska

Dr. Eva Chi
Dr. Jaroslaw Majewski
Alzheimer’s Disease – Impact on Society

Early Stages
- Cognitive impairment
- Memory loss
- Mood swings

Later Stages
- Confusion
- Loss of muscle control
- Inability to communicate

5 Million People
In the US Alone

$259 Billion
Spent Annually

www.brightfocus.org/alzheimers
Neurodegeneration in Alzheimer’s disease

Chen, S. et al. (2013). Drug design, development and therapy. 7. 117-25.
Alzheimer's Disease – Amyloid Beta (Aβ) is toxic to neurons

Healthy Neuron

Diseased Neuron

Diseased brains accumulate high concentrations of Aβ resulting in neurotoxicity

Amyloid Precursor Protein (APP) is cleaved to produce Aβ

Monomeric Aβ

Excessive Aβ production
Poor Aβ clearance
Increased aggregation

Amyloid plaques

Annu Rev Neurosci. 2011; 34: 185–204
Amyloid Beta (Aβ40) Aggregation Pathway

Monomer

Intrinsically disordered

Fibrillar Oligomers (FO)

Non-Fibrillar Oligomers (NFO)

β-sheet rich

Globular

Mature fibrils

Healthy

Toxic!

https://scopeblog.stanford.edu/

https://www.alzinfo.org/

Adapted from The EMBO Journal (2011) 30, 2255-2265
Lipid Membrane Catalyzes Fibrillation

Insertion into membrane → Membrane catalyzes fibrillation

Monomeric protein → Slow oligomer formation in solution → Fibril nucleation

Membrane degradation
Aβ oligomers exhibit profound disruption to cell membrane

Aβ monomers – membrane intact

Aβ oligomers – severe deformation to membrane

Dye leakage experiments also show membrane barrier is compromised

Aβ fibrils – membrane intact

Aβ40 Constructs: Structure and interaction with membranes

1. How do different Aβ40 structures interact with lipid membranes?

2. How does the structure of Aβ40 and the membrane change during/after interaction?
Preparation of Aβ constructs


Aβ_m, FO, and NFO all insert into anionic membrane

- Monomeric Aβ40, FO, and NFO all insert into anionic membranes
- NFO destabilizes anionic DMPG membrane

DMPG: 1,2-dimyristoyl-sn-glycero-3-phospho-(1’-rac-glycerol)

Aβ constructs cause disruption to lipid packing

- Aβm disrupts LC domains
- FO causes bright patches around perimeter of LC domains
- NFO caused fusion of LC domains and exhibited bright patches forming within the LC domains

X-ray reflectivity – Aβ40 insertion into DMPG membrane

- Aβm and FO form 34-37 Å layer of protein underneath lipid headgroups
- No protein is observed in the NFO + DMPG sample

### XR Fit Parameters

<table>
<thead>
<tr>
<th>Subphase</th>
<th>Slab 1 (Tails)</th>
<th>Slab 2 (Heads)</th>
<th>Slab 3 (outside layer)</th>
<th>χ²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Thickness</td>
<td>ρ / ρ_{water}</td>
<td>Roughness</td>
<td></td>
</tr>
<tr>
<td>DMPG</td>
<td>15.9 ± 0.2</td>
<td>0.97 ± 0.03</td>
<td>3.27 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>DMPG + Aβm</td>
<td>14.67 ± 0.03</td>
<td>1.057 ± 0.004</td>
<td>4.41 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>DMPG + FO</td>
<td>13.21 ± 0.04</td>
<td>0.940 ± 0.004</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>DMPG + NFO</td>
<td>16.0 ± 0.2</td>
<td>0.98 ± 0.03</td>
<td>3.32 ± 0.04</td>
<td></td>
</tr>
</tbody>
</table>

|          | Thickness     | ρ / ρ_{water} | Roughness              |     |
| DMPG     | 9.1 ± 0.3     | 1.58 ± 0.02   | 3.4 ± 0.2              |     |
| DMPG + Aβm | 9.4 ± 0.4    | 1.534 ± 0.007 | 3.7                    |     |
| DMPG + FO | 9.1 ± 0.4     | 1.552 ± 0.013 | 5.1                    |     |
| DMPG + NFO| 9.3 ± 0.3     | 1.53 ± 0.03   | 3.4 ± 0.2              |     |

|          | Roughness     |     |     |
| DMPG     | 2.8 ± 0.4     |     |     |
| DMPG + Aβm | 8.59 ± 0.17  |     |     |
| DMPG + FO | 10.5 ± 0.3    |     |     |
| DMPG + NFO| 2.93 ± 0.10   |     |     |

- Aβm and FO cause decrease in lipid tail thickness

GID: All Aβ40 Decreases Lipid Tail Packing

All proteins reduce the amount of DMPG ordered domains (NFO > FO > Monomeric Aβ40)

<table>
<thead>
<tr>
<th></th>
<th>Inter-Molecule Distance (Å)**</th>
<th>Integrated Peak Area</th>
<th>Coherence Length $L_c$ (Å)</th>
<th>Surface Pressure (mN/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMPG</td>
<td>4.87 ± 0.02</td>
<td>704 ± 12</td>
<td>469 ± 12</td>
<td>25.0</td>
</tr>
<tr>
<td>DMPG + Aβ$_m$</td>
<td>4.89 ± 0.02</td>
<td>369 ± 10</td>
<td>209 ± 9</td>
<td>35.8</td>
</tr>
<tr>
<td>DMPG + FO</td>
<td>4.87 ± 0.02</td>
<td>229 ± 9</td>
<td>269 ± 14</td>
<td>37.0</td>
</tr>
<tr>
<td>DMPG + NFO</td>
<td>4.87 ± 0.02</td>
<td>138 ± 10</td>
<td>350 ± 30</td>
<td>30.0</td>
</tr>
</tbody>
</table>

$A\beta_m$ and FO form β-sheet rich structures at the membrane

<table>
<thead>
<tr>
<th></th>
<th>$d$ Spacing (Å)</th>
<th>Integrated Peak Area</th>
<th>Coherence Length $L_c$ (Å)</th>
<th>Surface Pressure (mN/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMPG + $A\beta_m$</td>
<td>4.73 ± 0.02</td>
<td>69 ± 12</td>
<td>79 ± 11</td>
<td>35.8</td>
</tr>
<tr>
<td>DMPG + FO</td>
<td>4.73 ± 0.04</td>
<td>130 ± 13</td>
<td>67 ± 17</td>
<td>37.0</td>
</tr>
<tr>
<td>DMPG + NFO</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Source: Petkova et al. 2002

Membrane Binding Induces Structure Change for Aβₘ

- Aβₘ change secondary structure after inserting into lipid vesicles
- FO and NFO maintain their native structures in the membrane

**SUV**

**POPC**: 1-Palmitoyl-2-oleoyl-SN-glycero-3-phosphocholine

**POPG**: 1-hexadecanoyl-2-(9Z-octadecenoyl)-sn-glycero-3-phospho-(1′-rac-glycerol)

• Fibrils convert hexagonally packed LC phase lipids to a disordered LE phase
• Fibrils protrude into the aqueous subphase ~36Å
• 70Å is the size that the fibrils coherently scatter on the membrane

Non-fibrillar oligomers have detergent-like impacts on the membrane

(A) Stable Membrane
- Condensed lipid packing
- Stable membrane

(B) Disrupted Lipid Packing and Mechanical Stress
- Nucleated fibrillation
- Disrupted lipid packing
- Membrane thinning

Monomeric Aβ40
(Intrinsically Disordered)

Fibrillar Oligomer
(β-sheet rich)

(C) Lipid Extraction
- Lipid extraction

Non-Fibrillar Oligomer
(Globular)

Aβ constructs are surface active and assemble into fibrils at the air/water interface

- All Aβ construct tested are surface active, adsorbing to the air/water interface
- All constructs assembled into fibrils at the air/water interface
- A second diffraction peak was observed indication inter-sheet assembly of fibrils.
Two biological applications of XR and GIXD

**Alzheimer’s Disease**

- **Fibrillar Oligomer** (β-sheet rich)
- **Non-Fibrillar Oligomer** (Globular)
- **Amyloidogenic Peptides**
  - Nucleated fibrillation
  - Disrupted lipid packing
  - Membrane thinning

**Lipoxygenase (15-LOX-2)**

- **Peripheral Membrane Proteins**
Contributors to this project

Dr. Jaroslaw Majewski
NSF Independent Research and Development Program

Dr. Marcia Newcomer (PI)
Dr. Nathan Gilbert (Scientist)

NSF/CHE- 1834750 support for APS Sector 15 ChemMatCARS
Thank you beamline scientists (Dr. Wei Bu)
Lipoxygenases and PUFAs

- Increased levels of oxidized lipids are transported by low density lipoproteins (LDL)
- Macrophages ingest LDL near blood vessel walls transforming the macrophages to foam cells
- The accumulation of localized foam cells develop plaques beneath the endothelium of the blood vessel
- **Inhibition of 15-LOX-2 could be a viable therapy for mitigating cardiovascular disease**

Structure of 15-LOX-2: Ca\textsuperscript{2+} and hydrophobic loop

- Hydrophobic loop and Ca\textsuperscript{2+} are known to increase enzyme activity

https://opm.phar.umich.edu/proteins/2345
15-LOX-2 Enzymatic Reaction

• How does 15-LOX-2 bring the lipid substrate to the active site?
• Hydrophilic peroxide associates with headgroups
• Peroxide-modified lipid is transported to extracellular leaflet and recognized by macrophages
Lipoxygenase enzyme (15-LOX-2) and lipid peroxidation

How does 15-LOX-2 bind cell membranes?

How does lipid peroxidation impact membrane structure?
Experimental Design

- **DSPC:** 18:0 PC (1,2-distearoyl-sn-glycero-3-phosphocholine)

- **SAPC:** 18:0-20:4 PC (1-stearoyl-2-arachidonoyl-sn-glycero-3-phosphocholine)

**Buffer Conditions**
- 20 mM Tris pH 7.4
- 150 mM NaCl
- 0.5 mM EDTA

**Protein Variants Used:**
- WT 15-LOX-2 (no Ca\(^{2+}\))
- WT 15-LOX-2 + Ca\(^{2+}\)
- Loop Mutant (hydrophobic loop truncated from structure)
15-LOX-2 causes surface pressure decrease when arachidonoyl substrate and hydrophobic loop are present

- Surface pressure decreases when WT 15-LOX-2 binds a membrane containing SAPC
- Loop mutant does not cause same surface pressure decrease
- Experiments will be repeated with careful control over starting surface pressure
15-LOX-2 inserts into 80:20 DSPC:SAPC

- 15-LOX-2 shows slight insertion into DSPC tails
- Insertion into SAPC causes shift in SLD
- Highest increase in electron density shown for 15-LOX-2 insertion into 8:2 DSPC:SAPC
15-LOX-2 undergoes major conformational change when binding membrane

- XR results suggest that 15-LOX-2 undergoes a major conformational change when interacting with the membrane.
- Electron density increase in tails cannot be attributed only to lipid peroxidation.

OPM Predicted Binding
Ca$^{2+}$ and Loop Mutant have small impact on XR results

- Calcium exhibits slightly higher electron density in lipid tails
- Loop mutant does not show any difference from WT
## XR fit parameters

<table>
<thead>
<tr>
<th>Sample</th>
<th>Slab 1 (Tails)</th>
<th>Slab 2 (Heads)</th>
<th>Subphase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Thickness</td>
<td>$\rho / \rho_{\text{water}}$</td>
<td>Roughness</td>
</tr>
<tr>
<td></td>
<td>DSPC</td>
<td>18.0 ± 0.2</td>
<td>0.998 ± 0.002</td>
</tr>
<tr>
<td>+ WT 15-LOX-2</td>
<td>18.72 ± 0.12</td>
<td>1.031 ± 0.002</td>
<td>3.330 ± 0.015</td>
</tr>
<tr>
<td></td>
<td>SAPC</td>
<td>11.8 ± 0.2</td>
<td>0.957 ± 0.001</td>
</tr>
<tr>
<td>+ WT 15-LOX-2</td>
<td>8.64 ± 0.02</td>
<td>0.857 ± 0.007</td>
<td>3.11*</td>
</tr>
<tr>
<td></td>
<td>8:2 DSPC:SAPC</td>
<td>17.4 ± 0.6</td>
<td>0.969 ± 0.004</td>
</tr>
<tr>
<td>+ WT 15-LOX-2</td>
<td>19.1 ± 0.3</td>
<td>1.095 ± 0.001</td>
<td>3.157 ± 0.007</td>
</tr>
<tr>
<td>+ WT 15-LOX-2 + Ca$^{2+}$</td>
<td>19.14 ± 0.03</td>
<td>1.111 ± 0.001</td>
<td>3.054 ± 0.007</td>
</tr>
<tr>
<td>+ Loop Mutant</td>
<td>18.9 ± 0.2</td>
<td>1.088 ± 0.001</td>
<td>3.088 ± 0.007</td>
</tr>
</tbody>
</table>

*This value was fixed to reduce the number of parameters in fitting

- 15-LOX-2 insertion results in longer tails when DSPC is present in the membrane
15-LOX-2 causes compaction in membranes with DSPC

Even in pure DSPC, 15-LOX-2 causes compaction of semi-crystalline lattice

15-LOX-2 causes lipids to convert from LC to LE phase

<table>
<thead>
<tr>
<th>Lipid Composition</th>
<th>Area per lipid ($\text{Å}^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSPC</td>
<td>46.26 ± 0.01</td>
</tr>
<tr>
<td>+ WT LOX</td>
<td>45.72 ± 0.01</td>
</tr>
<tr>
<td>80:20 DSPC:SAPC + WT LOX</td>
<td>47.28 ± 0.01</td>
</tr>
<tr>
<td>8:2 DSPC:SAPC + WT LOX</td>
<td>45.78 ± 0.01</td>
</tr>
</tbody>
</table>
Loop Mutant and Ca\textsuperscript{2+} cause lipid conversion to LE phase

- WT 15-LOX-2 causes less compaction to lipid unit cell when Ca\textsuperscript{2+} was present
- Surprisingly, Loop Mutant causes similar lipid compaction as WT.
- More lipids are converted to LE phase when Ca\textsuperscript{2+} is added, or when the Loop Mutant was used.

<table>
<thead>
<tr>
<th></th>
<th>Area per lipid (Å\textsuperscript{2})</th>
<th>Peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:2 DSPC:SAPC</td>
<td>47.28 ± 0.01</td>
<td>2509 (100%)</td>
</tr>
<tr>
<td>+ WT LOX</td>
<td>45.78 ± 0.01</td>
<td>2228 (89%)</td>
</tr>
<tr>
<td>+ WT LOX + Ca\textsuperscript{2+}</td>
<td>46.19 ± 0.01</td>
<td>1648 (66%)</td>
</tr>
<tr>
<td>+ Loop Mutant</td>
<td>45.51 ± 0.01</td>
<td>1668 (66%)</td>
</tr>
</tbody>
</table>
Conclusions and Future Directions

Conclusions

• 15-LOX-2 binds membranes and interacts with lipids, regardless of presence of hydrophobic loop or Ca$^{2+}$
• Surface pressure decrease is not observed in absence of hydrophobic loop and arachidonoyl substrate
• 15-LOX-2 interacts with lipid tails, causing tails to pack together more tightly
• Overall, 15-LOX-2 causes lipids to convert from LC to LE phase
• The membrane-bound conformation of 15-LOX-2 likely undergoes major structural rearrangement

Future Directions

• Repeat Langmuir trough studies, including fluorescence microscopy
• Perform mass spectrometry to verify presence of peroxide-modified lipid product
• Perform X-ray scattering with inactive Mn-bound 15-LOX-2
• Control studies with chemically oxidized lipid
Acknowledgements – Amyloidogenic Peptides

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Bella Bowers
Dr. Adeline Fanni
Dr. Erik Watkins (LANL)
Paulina Majewska

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