MCB Exam 3 Review
Schlesinger, Amarasinghe, and Fremont Lectures

Brittany Brumback
12/16/17
THE CELL BIOLOGY OF APOPTOSIS
TO LIVE IS TO DIE – METALLICA(2007)

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<table>
<thead>
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<th>HOW CELLS ACHIEVE MORTALITY</th>
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Apoptosis – Programmed cell death, controlled part of development

Necrosis – Premature cell death caused by external factors

Autophagy – Degradation of cell components in lysosome. Type of programmed cell death, separate from apoptosis

Senescence – Cell cycle arrest
The Importance of Cell Death

- Homeostatic Programmed Cell Death
  Cellular renewal and animal size limitation are achieved.

- Effective and specific way to Remove Dangerous Cells
  DNA damage activates PARP-1 for DNA repair but depletes NAD causing apoptosis.
  Infection with Papilloma virus blocks apoptosis by degradation of p53.
THE IMPORTANCE OF CELL DEATH

- Physiologic Induction of Apoptosis
  
  *Programmed Cell Death is dominant in developmental processes.*
  
  *Programmed Cell Death is a primary regulation of immunologic function.*

- Protection From The Immune System.
  
  *Cells that fail to detect self must be destroyed.*
  
  *Cells that can self detect must survive.*
Compartmentalization versus Lysis

Kerr et al., (1972), Br. J. Cancer, 26:239-257

Apoptosis

Necrosis
A apoptotic cells shrink

Intact membranes → Volume reduction → Membrane channel activity
Bax forms Pores

Bax Pore in 10 Micron Liposome

0 min 2 min

5 min 17 min
Bax releases cytochrome c

![Graph showing the release of cytochrome c with fluorescence measurements over time.](image-url)
Cancer Surveillance

Summary

- Apoptosis a the major physiologic regulator of cell survival
  - Plays dominant roles in immune function, tumor surveillance, differentiation and tissue maintenance
- Bcl-2–family proteins are dedicated to the initiation and control of apoptosis
  - \( \approx 25 \) proteins of three types: inhibitory, regulatory and initiating
- The release of cytochrome c and other proteins from mitochondria initiates apoptosis execution
  - Cytochrome c is an intracellular messenger with a cytosolic “receptor”
Protein crystallography in practice

MCB

14 Dec 2017

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An 7-step program for protein structure determination by x-ray crystallography

1. Produce monodisperse protein either alone or as relevant complexes
2. Grow and characterize crystals
3. Collect X-ray diffraction data
4. Solve the phase problem either experimentally or computationally
5. Build and refine an atomic model using the electron density map
6. Validation: How do you know if a crystal structure is right?
7. Develop structure-based hypothesis
1. Produce monodisperse protein either alone or as relevant complexes

Methods to determine protein purity, heterogeneity, and monodispersity

- Gel electrophoresis (native, isoelectric focusing, and SDS-PAGE)
- Size exclusion chromatography

Characterize your protein using a number of biophysical methods

Establish the binding stoichiometry of interacting partners
No Xtals?

**Decrease protein heterogeneity**

- Remove purification tags and other artifacts of protein production

- Remove carbohydrate residues or consensus sites (i.e., N-x-S/T)

- Determine domain boundaries by limited proteolysis followed by mass spectrometry or amino-terminal sequencing. Make new expression constructs if necessary.

**Think about the biochemistry of the system!** Does your protein have co-factors, accessory proteins, or interacting partners to prepare as complexes? Is their an inhibitor available? Are kinases or phosphatases available that will allow for the preparation of a homogeneous sample?

- Get a better talisman
4. Solve the phase problem either experimentally or computationally

- Structure factor equation: 
  \[ F_{(h,k,l)} = \sum_{j=1}^{\text{atoms}} f_{(j)} \exp[2\pi i (hx_{(j)} + ky_{(j)} + lz_{(j)})] \]

- By Fourier transform we can obtain the electron density. We know the structure factor amplitudes after successful data collection. Unfortunately, conventional x-ray diffraction doesn’t allow for direct phase measurement. This is known as the crystallographic phase problem.

\[ \rho_{(x,y,z)} = \frac{1}{V} \sum_h \sum_k \sum_l |F_{(h,k,l)}| \exp[-2\pi i (hx + ky + lz - \alpha_{(h,k,l)})] \]

- Luckily, there are a few tricks that can be used to obtain estimates of the phase \( \alpha_{(h,k,l)} \)

**Experimental Phasing Methods**

- MIR - multiple isomorphous replacement - *need heavy atom incorporation*
- MAD - multiple anomalous dispersion - *typically done with SeMet replacement*
  - MIRAS - multiple isomorphous replacement with anomalous signal
  - SIRAS - single isomorphous replacement with anomalous signal

**Computational Methods**

- MR - molecular replacement - *need related structure*
- Direct and Ab Initio methods - not yet useful for most protein crystals
Holes in rings are a good thing

Seeing a hole in a tyrosine or phenylalanine ring is universally accepted as proof of good phases. You need at least 2Å data.
6. Validation: How do you know if a crystal structure is right?

**The R-factor**

\[ R = \frac{\sum (|Fo - Fc|)}{\sum (Fo)} \]

where Fo is the observed structure factor amplitude and Fc is calculated using the atomic model.

- measure of how well the refined structure predicts the observed data

**R-free**

An unbiased, cross-validation of the R-factor. The R-free value is calculated with typically 5-10% of the observed reflections which are set aside from atomic refinement calculations.

- Main-chain torsions: the Ramachandran plot

- Geometric Distortions in bond lengths and angles

- Favorable van der Waals packing interactions

- Chemical environment of individual amino acids

- Location of insertion and deletion positions in related sequences
When things go wrong:

LETTERS
edited by Etta Kavanagh

Retraction

WE WISH TO RETRACT OUR RESEARCH ARTICLE "STRUCTURE OF MsbA from E. coli: A homolog of the multidrug resistance ATP binding cassette (ABC) transporters" and both of our Reports "Structure of the ABC transporter MsbA in complex with ADP-vanadate and lipopolysaccharide" and "X-ray structure of the EmrE multidrug transporter in complex with a substrate" (I–3).

The recently reported structure of Sav1866 (4) indicated that our MsbA structures (1, 2, 5) were incorrect in both the hand of the structure and the topology. Thus, our biological interpretations based on these inverted models for MsbA are invalid.

An in-house data reduction program introduced a change in sign for anomalous differences. This program, which was not part of a conventional data processing package, converted the anomalous pairs (I+ and I−) to (F− and F+), thereby introducing a sign change. As the diffraction data collected for each set of MsbA crystals and for the EmrE crystals were processed with the same program, the structures reported in (1–3, 5, 6) had the wrong hand.

The error in the topology of the original MsbA structure was a consequence of the low resolution of the data as well as breaks in the electron density for the connecting loop regions. Unfortunately, the use of the multicopy refinement procedure still allowed us to obtain reasonable refinement values for the wrong structures.

The Protein Data Bank (PDB) files 1JSQ, 1PF4, and 1Z2R for MsbA and 1S7B and 2F2M for EmrE have been moved to the archive of obsolete PDB entries. The MsbA and EmrE structures will be recalculated from the original data using the proper sign for the anomalous differences, and the new Cx coordinates and structure factors will be deposited.

We very sincerely regret the confusion that these papers have caused and, in particular, subsequent research efforts that were unproductive as a result of our original findings.

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References

NMR in biology: Structure, dynamics and energetics

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Main advantages of NMR for studying biomolecules:
- versatile method
- amenable to solution-state,
- physiologically relevant buffers
- superb resolving power!

Main disadvantages of NMR for studying bimolecules:
- relatively poor sensitivity!
- requires mg quantities of protein,
- high concentrations (high µM to low mM range)

NMR is an inherently local technique. It measures the properties of individual atoms (nuclei).

Global information, such as complete 3D structures, is usually obtained by summing up a large number of local measurements.
Why use NMR?

- Some proteins do not crystallize (unstructured, multidomain)
- Crystals do not diffract well
- Crystallization cannot solve the phase problem
- Functional differences in crystal vs in solution
- Can get information about dynamics
NUCLEAR MAGNETIC MOMENT

Fundamental properties of nuclei: mass, charge, "spin" (angular momentum)

Nuclei with both spin and charge possess a magnetic moment:

\[ \mu_n = \gamma I (\hbar/2\pi) \]

where \( \mu_n \) is the magnetic moment, \( \gamma \) is the gyromagnetic ratio, \( I \) is the spin quantum number, and \( \hbar \) is the reduced Planck constant.

If \( I = 0 \):
no magnetic moment, no NMR signal!
(nuclei with even #s of protons and neutrons)
e.g., \( ^{12}\text{C} \), \( ^{16}\text{O} \), \( ^{32}\text{S} \)
NMR invisible

TABLE 2-1 Nuclear Properties of Selected Isotopes

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Mass</th>
<th>Charge</th>
<th>( I )</th>
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</thead>
<tbody>
<tr>
<td>Even</td>
<td>Even</td>
<td>( l=0 )</td>
<td></td>
</tr>
<tr>
<td>Even</td>
<td>Odd</td>
<td>( l= ) integer</td>
<td></td>
</tr>
<tr>
<td>Odd</td>
<td></td>
<td>( l= ) half integer</td>
<td></td>
</tr>
</tbody>
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1 Abstracted in part from The 66th CRC Handbook of Chemistry and Physics, CRC Press, 1984.
2 Relative (to proton) sensitivity for equal numbers of nuclei at constant field: \( S = 7.652 \times 10^{-4} \mu^2/\hbar^2 \) where \( \mu \) is the magnetic moment of the nucleus.
Protein Structure Determination by NMR

• Stage I—Sequence specific resonance assignment

• State II – Conformational restraints

• Stage III – Calculate and refine structure
Figure 1.1. Information content of $^1$H–$^1$H NOE's in a polypeptide chain with and without sequence-specific resonance assignments. Open circles represent hydrogen atoms of the polypeptide. The polypeptide chain is represented by the horizontal line in the center. (Wüthrich, 1986)
Protein Structure Determination by NMR

• Stage I—Sequence specific resonance assignment

• State II – Conformational restraints

• Stage III – Calculate and refine structure
NMR Structure Determination

**NOE**
- a through space correlation (<5Å)
- distance constraint

**Coupling Constant (J)**
- through bond correlation
- dihedral angle constraint

**Chemical Shift**
- very sensitive to local changes in environment
- dihedral angle constraint

**Dipolar coupling constants (D)**
- bond vector orientation relative to magnetic field
- alignment with bicelles or viruses
Protein Structure Determination by NMR

• Stage I—Sequence specific resonance assignment

• State II – Conformational restraints

• Stage III – Calculate and refine structure
Analysis of the Quality of NMR Protein Structures

As we have seen before, the Quality of X-ray Structures can be monitored by an R-factor

• No comparable function for NMR
• Requires a more exhaustive analysis of NMR structures

\[
R\text{-factor} = \frac{\sum_{h,k,l} |F(h, k, l)| - k|F(h, k, l)^{\text{calc}}|}{\sum_{h,k,l} |F(h, k, l)|}
\]
Analysis of the Quality of NMR Protein Structures

Root-Mean Square Distance (RMSD) Analysis of Protein Structures

• A very common approach to assess the quality of NMR structures and to determine the relative difference between structures is to calculate an rmsd
  ➢ an rmsd is a measure of the distance separation between equivalent atoms

\[
\text{rmsd} = \sqrt{\frac{\sum_i d_i^2}{n}}
\]

➢ two identical structures will have an rmsd of 0 Å
➢ the larger the rmsd the more dissimilar the structures

0.43 ± 0.06 Å for the backbone atoms
0.81 ± 0.09 Å for all atoms
Analysis of the Quality of NMR Protein Structures

Is the “Average” NMR Structure a Real Structure?

- **No-it is a distorted structure**
  - level of distortions depends on the similarity between the structures in the ensemble
  - provides a means to measure the variability in atom positions between an ensemble of structures

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**Expanded View of an “Average” Structure**

- Some very long, stretched bonds
- Position of atoms are so scrambled the graphics program does not know which atoms to draw bonds between
- Some regions of the structure can appear relatively normal