Analyzing Spin-labeled ApoA-I via SDSL with Micro-ESR

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1 Abstract

- **Background**
  - High density lipoprotein (HDL), "good" cholesterol, helps prevent coronary artery disease (CAD).
  - Micro-ESR can measure functional HDL levels via site directed spin labeling of the protein apolipoproteinA-I (apoA-I).
- **Micro-ESR**
  - Sensitivity
    - Detection of spectral differences that depend on spin label location on the protein.
  - Quantitative Ability
    - Use of Manganese reference to provide quantitative data.

2 Background

Atherosclerosis is a major cause of coronary artery disease (CAD). High density lipoprotein (HDL), commonly known as the "good" cholesterol, serves a protective role through preventing atherosclerosis by removing cholesterol from the body. Thus, low functional HDL levels can raise the risk of CAD. ApoA-I is an important protein in high density lipoprotein (HDL). Thus, if the functional HDL levels are abnormally low, the risk of atherosclerosis and thus CAD increases.

3 SDSL

Site Directed Spin Labeling (SDSL)

- Uses electron spin resonance spectroscopy (ESR) to determine protein structure
- Uses a stable nitroxide as a spin label on the target residue
- Line shape and width of the peaks of the ESR signal reveal the structure of the protein near the residue

Broader Signal 

- Ordered protein structure which allows for less rotation of the residue
- Dipolar coupling

Narrower Signal 

- Less structured protein which allows for more rotation of the residue

4 Comparison of ESR Spectra of Spin-Labeled ApoA-I

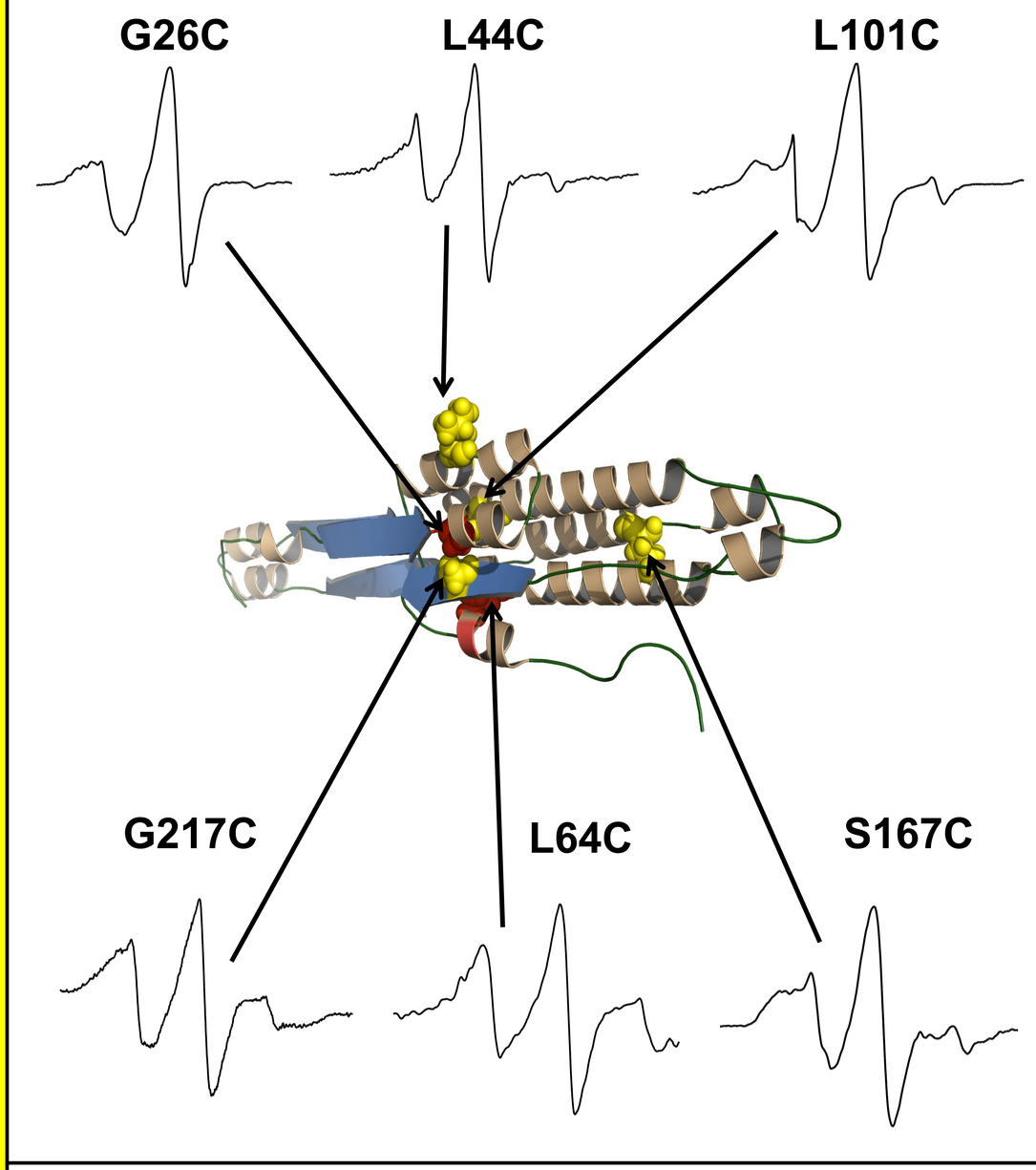

Figure 1.
Micro-ESR spectrum plots of spin labeled ApoA-I. The differences in the rotational freedom and dipolar coupling of the spin label at the different sites are reflected in the line shape and width of the spectrum plots.

6 ESR Spectrum for Quantitative Analysis

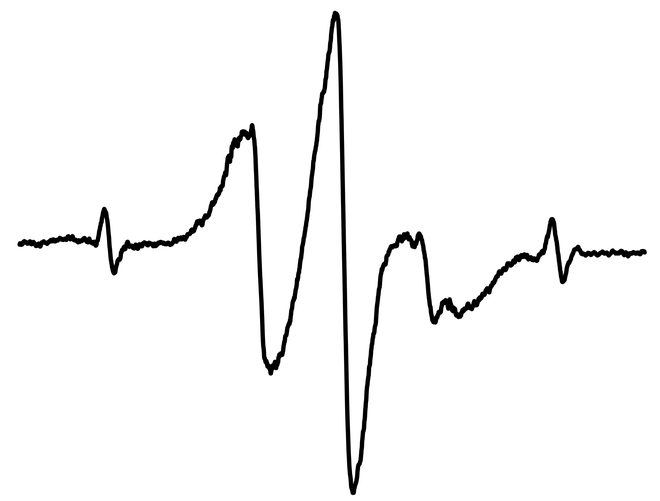

Figure 2.
Micro-ESR spectrum plot of G217C residue on apoA-I with manganese oxide as an external reference. ApoA-I signal can be quantified via comparison to the reference signal (the outermost peaks on either side).

Figure 3. Micro-ESR Spectrometer (12"x12"x12", 13.8 lbs.)

7 Acknowledgements

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References

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