Methylene Blue as a Means to Bypass the Compromised Complex I in Mitochondrial Disease

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Background

- *Oxidative phosphorylation* (OXPHOS), the process by which ATP is produced, is fueled by the *Electron Transport Chain* (ETC) – an electron assembly line
- Some *mitochondrial diseases* (MD) involve a mutation of Complex I, the first gateway to the rest of the ETC
- The disease pathology necessitates frequent surgical intervention due to the constant degeneration of high-energy-demand organ tissue (Sasano, et al., 2007).
- Certain anesthetics such as propofol are known inhibitors of Complex I (Vanlander, et al., 2012).

The Problem

- Propofol, isoflurane, and other common anesthetics pose significant health risks to MD patients
  - Blockage of ubiquinone-binding sites in Complex I lead to electron leakage (Pereira, 2023).
  - Unused/leaked electrons exposed to oxygen can become *reactive oxygen species* (ROS). ROS prematurely oxidize cellular components like lipids and proteins, leading to severe tissue damage.

Researchable Question	Hypothesis
Can methylene blue act as an alternative electron carrier in the Electron Transport Chain when Complex I is inhibited?	Spectrophotometric analysis of cytochrome c will show greater Complex III activity when mitochondrial tissue is exposed to methylene blue.

Procedures

Harvest | Isolation | Assay

Analysis

Results

Planning & Future Steps

Insert Analysis piece here.

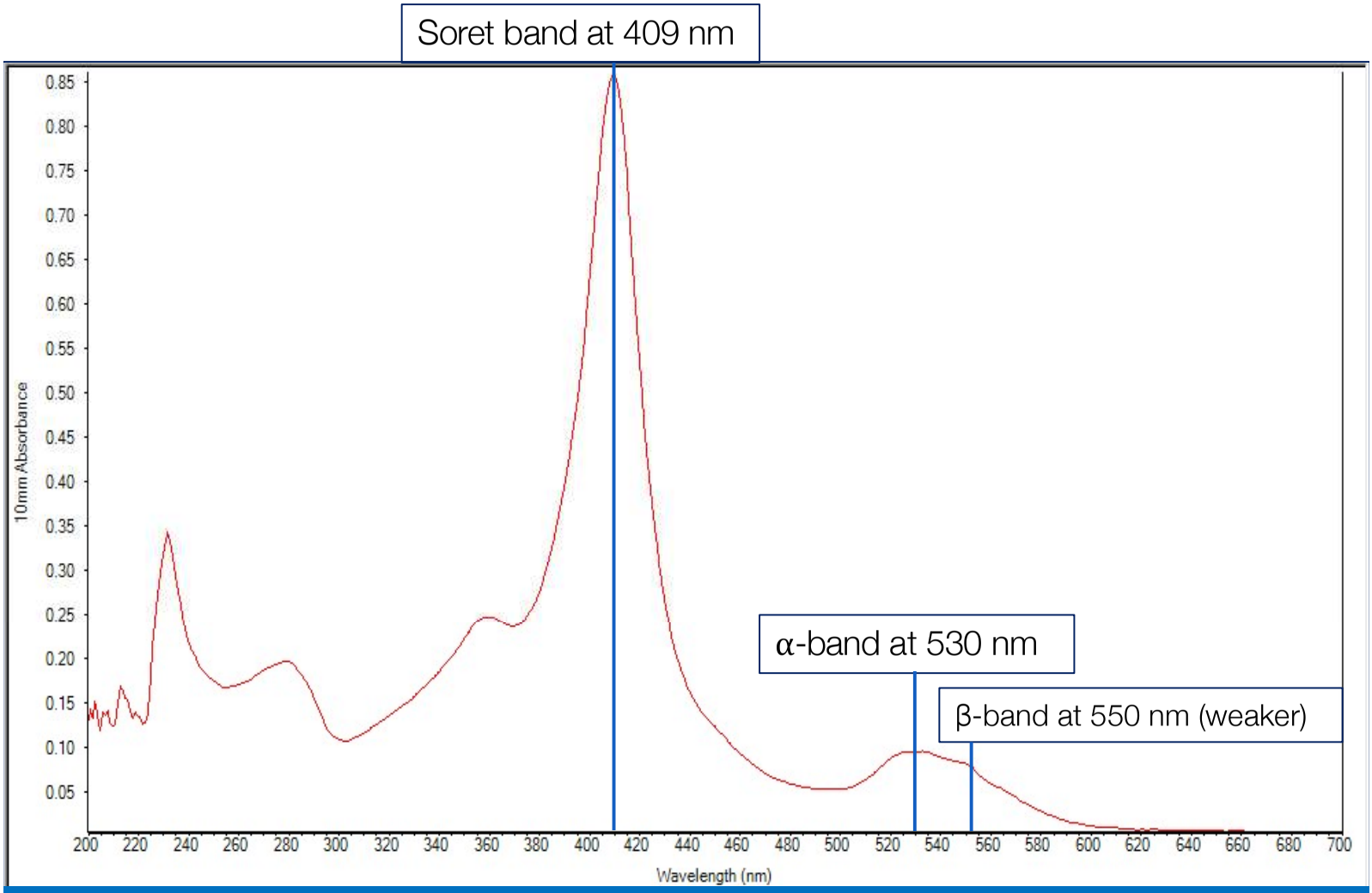

Figure 1: Cytochrome c absorption with DTT: minute 0 (oxidized)

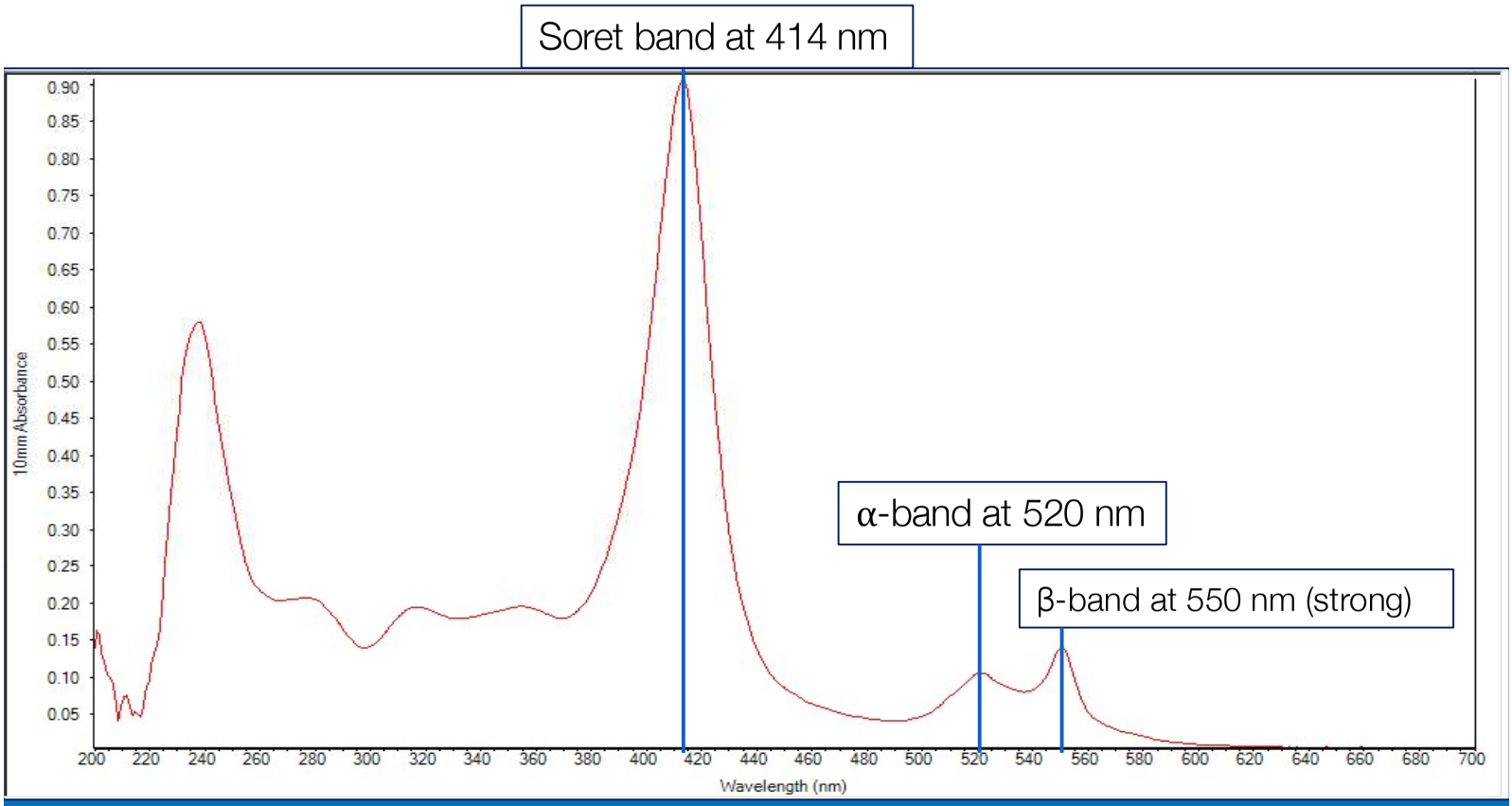

Figure 2: Cytochrome c absorption with DTT: minute 12 (reduced)

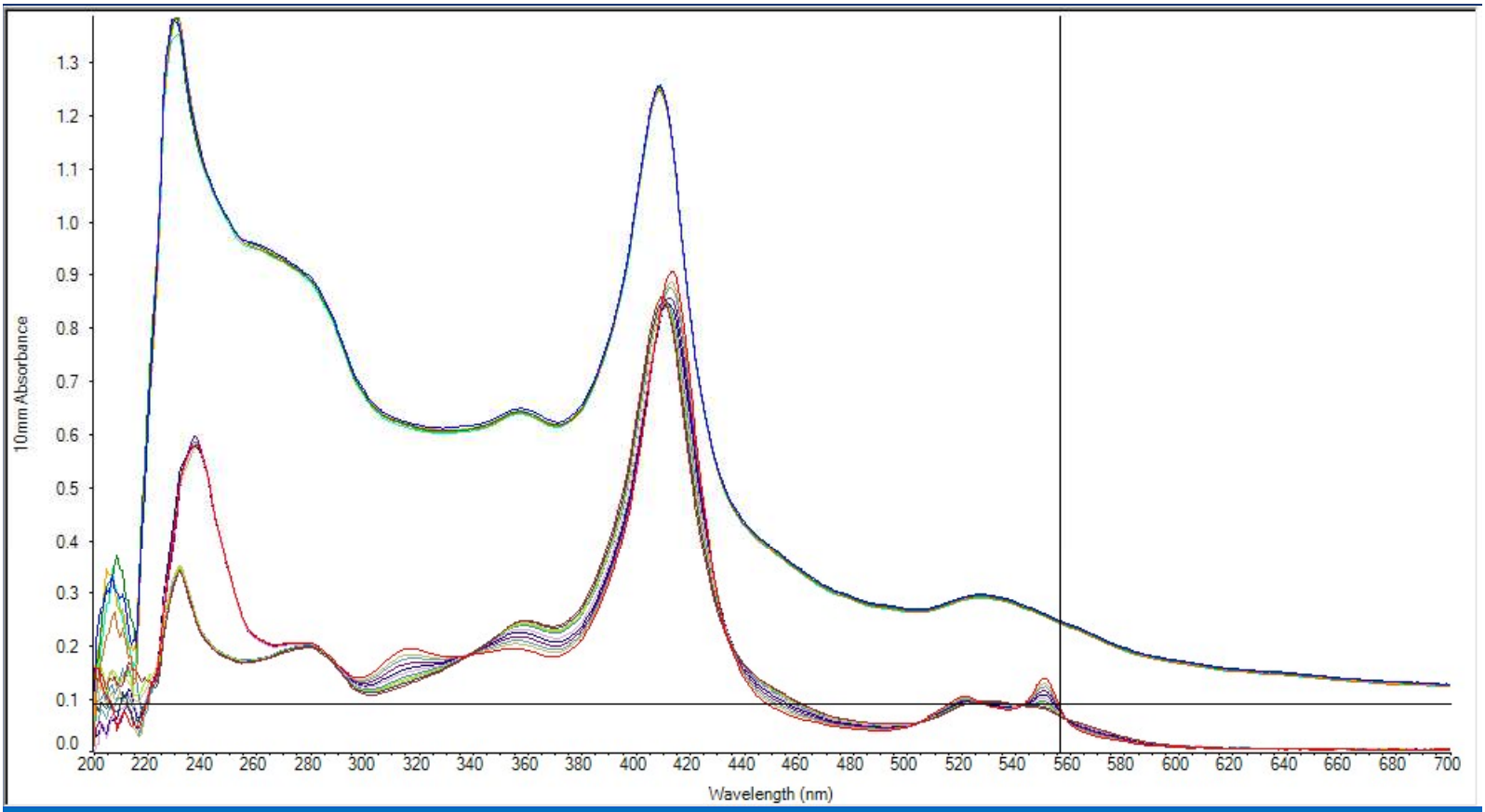

Figure 3: Cytochrome c absorption with DTT: full reaction (oxidase → reductase)

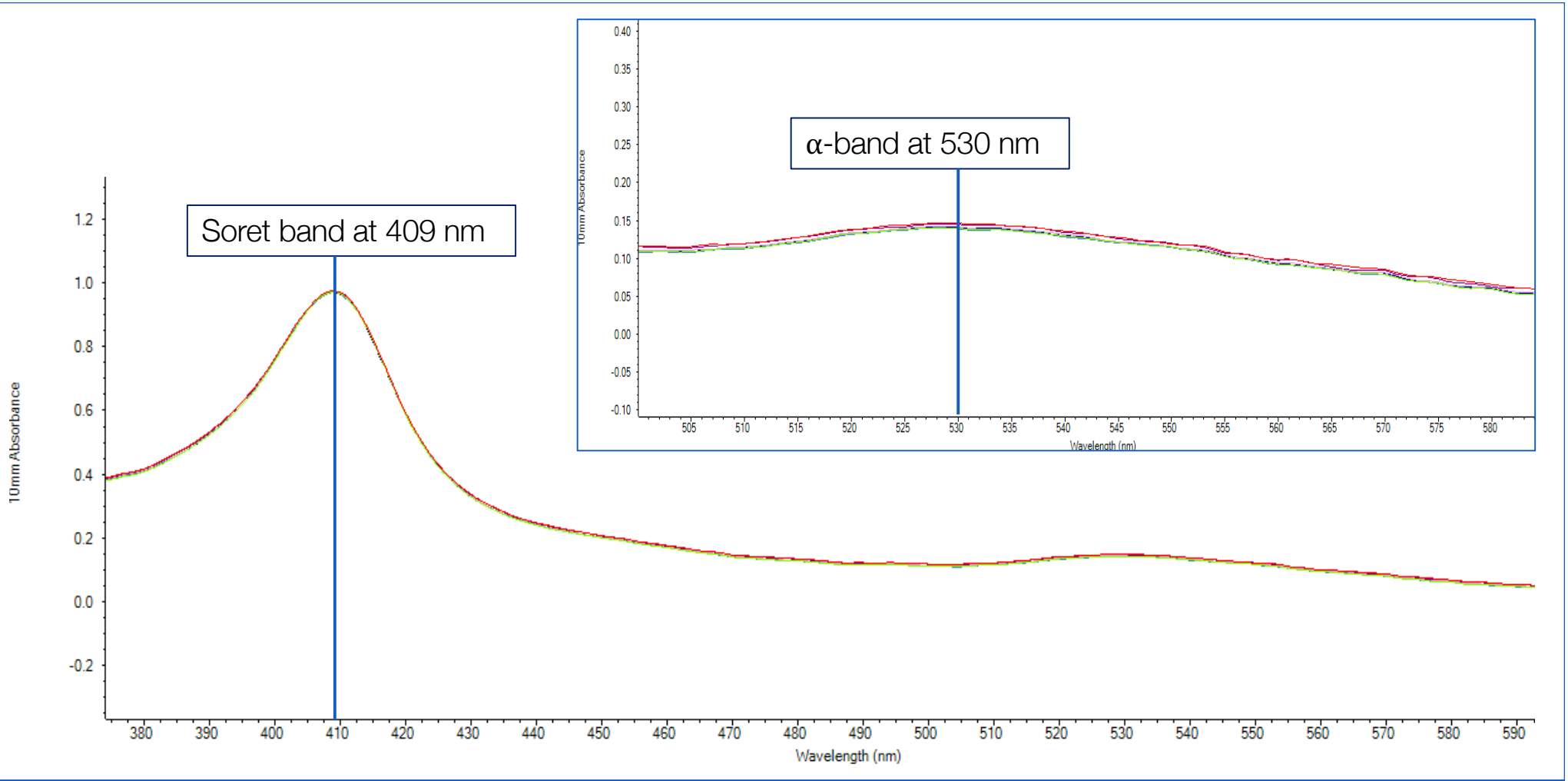

Figure 4: Cytochrome c absorption with non-MB LB10 mitochondria: minute 0 to minute 19 (minimal to no reduction)

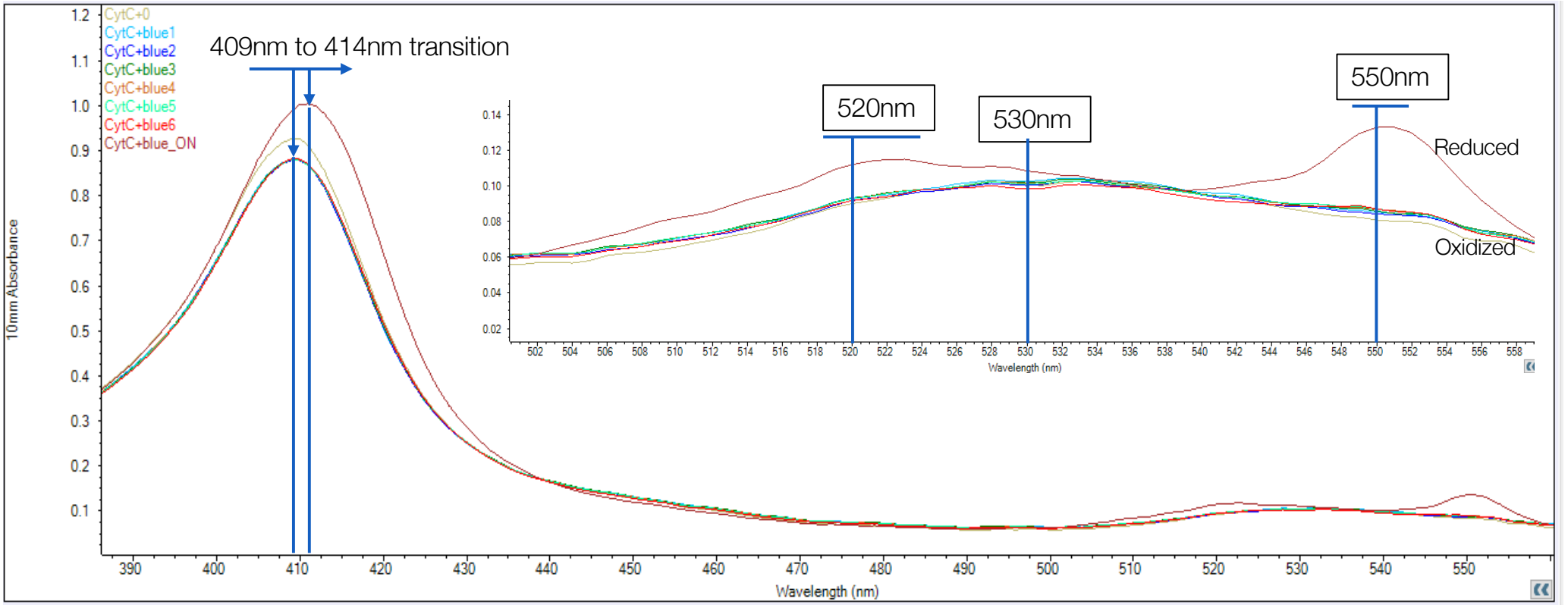

Figure 5: Cytochrome c absorption with MB-treated LB10 mitochondria: minute 0 to Overnight (reduction present)

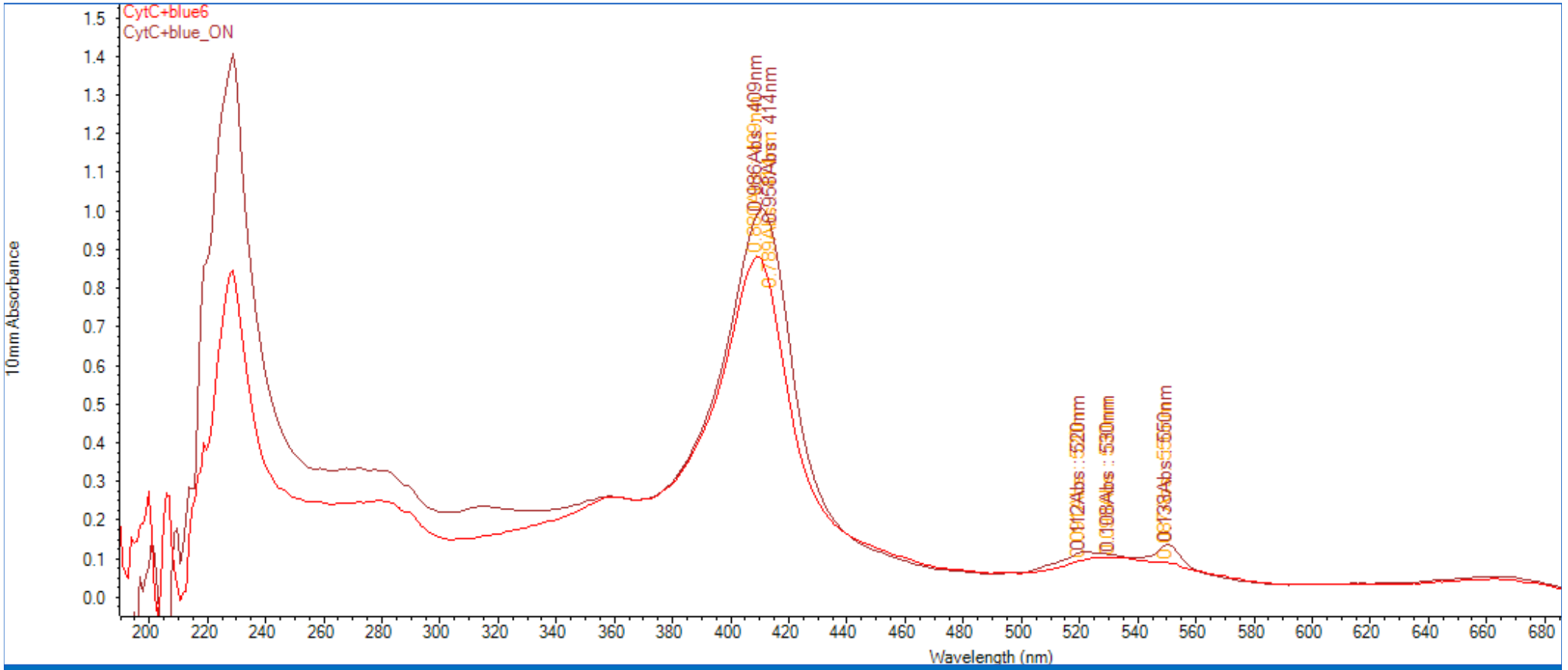

Figure 6: Cytochrome c absorption with MB-treated LB10 mitochondria: minute 0 vs. Overnight (reduction present)

Preliminary Data Collection (December)	Experimental Data Collection (February)	Future Steps
➢ Observed full reduction reaction using DTT ➢ Failed to obtain mitochondrial material ➢ Did not procure necessary equipment ➢ Insufficient prep	➢ Obtained mitochondrial material ➢ Observed reduction due to mitochondria in MB sample ➢ Verified presence of MB in mitochondria ➢ Thorough prep ➢ Failed to grow sufficient worm sample ➢ Prep & assay protocols verified; <u>need optimization</u>	➢ Grow *C. elegans* on egg plates to maximize sample ➢ Redo LB10 + Rotenone + MB run, allowing for reduction to take place over longer time course ➢ Run experiment on F22D6.4-mutated worms (Falk, 2006)

- *Went well*
- *Adjust going forward*