Justin Chao
jc55395

October 25, 2016

Independent Project Pre-Proposal Form

**What is your hypothesis?**
The denaturation process of a model protein, such as hemoglobin, is dependent upon secondary structural changes with temperature. The secondary structure and conformation of hemoglobin will be investigated via infrared attenuated total reflection (IR-ATR) spectroscopy.

**What steps will you take in order to make sure your results are statistically significant?**
The average of 64 spectral scans will be taken at a spectral resolution of 2 $cm^{-1}$ and in a frequency window of 4000 to 400 $cm^{-1}$, and the spectra will be processed by calculating second derivatives and the obtained peaks will be fitted using Gaussians. Parameters during the fitting process will be adjusted using initial values given by the peaks of the calculated second derivatives.
This experiment will be repeated twice.

**How the samples you plan to collect allow you to address your hypothesis?**
Given the dependence of the amide-I on backbone secondary structure, the amide-I vibration can be analyzed with respect to increasing temperature.

**Describe the sample you intend to analyze.**
Hemoglobin from bovine blood (CAS number: 9008-02-0) will be used without further purification. The BHb sample will be dissolved in $D_2O$ to avoid strong H-O absorptions within the amid-I spectral region, while preserving the protein peak position.

**What is/are your analyte(s) of interest?**
The amide-I vibrational spectrum will be analyzed with respect to increasing temperature.

**What is the expected concentration in your sample?**
BHp will be dissolved in $D_2O$ to a final concentration of 0.5% and kept for 2 days.

**Does this fall into the linear range of your instrument? If not, how will you address this issue?**
A planar silver halide fiber segment will be adapted for ATR sensing using minute sample volumes. Cylindrical silver halide fibers will be fabricated by hot extrusion from a single crystal preform, which can be prepared using the Bridgman-Stockbarger technique. Planar sections will then be created via a stainless-steel mold by pressing short cylindrical fiber sections into a planar geometry tapering.
The fiber ATR sensing element comprises of cylindrical segments at both ends facilitating radiation in-/out-coupling with a planar active sensing segment in the middle. Such a geometry ensures increased number of internal reflections and better detection densitivity compared to cylindrical fibers and conventional multi-reflection ATR crystals.

Justin Chao
jc55395
October 25, 2016

**How will you prepare your sample for analysis?**
Prior to measurement, each sample will be equilibrated for at least 5 min after reaching the target temperature.

**What technique will you use to calibrate your instrumental method (calibration curve, standard additions, etc.)?**
The IR instrument will be calibrated to a reference standard of known composition and purity. A polystyrene film will be used as a reference standard.

**What quality assurance methods will you use to validate your results? (blanks, controls, spike recovery, etc)**
Background spectra of a blank will be taken prior to measurements for baseline corrections.

FTIR questions:

**What is LOD for this technique?**
The LOD on a Cary 670 FTIR is 0.04% and 0.08% on a Cary 660 FTIR.

**What functional group peaks are you identifying and where are you expecting to find them?**
The amide-I peak can be found at around 1650 $cm^{-1}$.

**What wavelength range is used to quantify the analyte in the primary reference?**
The spectra will be region selected in the range of 1600-1700 $cm^{-1}$.

**Did the primary reference use ATR (attenuated total reflectance)?**
Attenuated Total Reflectance was used.

**What kind of cell is used in the primary reference?**
A liquid cell will be used.

**From what material are the cell windows made in the primary reference?**
The materials of the cell windows in the primary reference are unknown, but it is cell with NaCl windows may be used for this experiment.

**How will you compensate (modify your experiment) for any differences between instrument parameters in your reference and what we can do with our equipment?**
Sample concentrations and temperature parameters will need to be adjusted accordingly to the availability of specific instrument LODs and limits.