

Homework assignment for Lecture 12:

Analyze one of the datasets in the BIOPHYSICS multi-wavelength AUC experiment as described below (the “selected” dataset, identify it by column name in the spectral data file, and by cell/channel in the AUC data):

1. Spectral analysis:

Using the sfGFP-BIOPHYSICS.csv and the DNA-BIOPHYSICS.csv spreadsheets (in the 12-Spectra subfolder), derive an intrinsic absorbance profile for sfGFP and DNA, normalized for 1 OD at 488 and 260 nm, respectively. Analyze the corresponding selected dataset UV spectrum (in the 12-Spectra subfolder, called BIOPHYSICS.csv). Note the angle between the two, and the percentage of protein and DNA for your selected dataset. Unfortunately, some of the datasets were not named correctly on the spectrophotometer, so the column names may be unreliable, just do any one of them and report your results.

2. Using your 2DSA-IT models, time-synchronize your multi-wavelength data with the “Multiwavelength:Optima MWL Fit Simulation” module

3. Import the resulting ISSF-* data into the LIMS and edit it.

4. Use the edited ISSF file and the newly generated intrinsic extinction spectra as basis files, and the “Multiwavelength:MWL Species Fit” module to generate a SSF-ISSF (simulated scan files from the initial simulated scan files) for both protein and DNA.

5. Import the resulting SSF-ISSF files into the LIMS system, taking care to use Water for the buffer in a call cases, and making water-based solutions with sfGFP and DNA.

6. Edit the SSF-ISSF data.

7. Analyze both protein and DNA components with the 2DSA-IT method

8. Review the total concentration for each analyte and calculate the percentage of each.

9. Compare this percentage with the percentage reported in the spectral deconvolution module.

There are two separate methods to get concentration:

1. open the 2DSA-IT models in the “Velocity:Initialize Genetic Algorithm” and integrate species of interest

2. In the “Velocity:Finite Element Model Viewer” load the data and simulate the 2DSA-IT model overlay and read the total concentration in the Analysis report for both the deconvoluted sfGFP and the deconvoluted DNA file.

Report the percentages of the total signal.

It is OK to collaborate on this homework, but if you do, please analyze a different dataset. Write this up in a PDF file and send it to me. Identify the samples as indicated above. Send me the screenshot of the analysis report section with the total concentration listed for the deconvoluted sfGFP and DNA, together with the RMSD of the final 2DSA-IT fit of the SSF-ISSF deconvoluted files.

Due date: Sunday, 2/23, 5:00 pm. 50 points total possible.