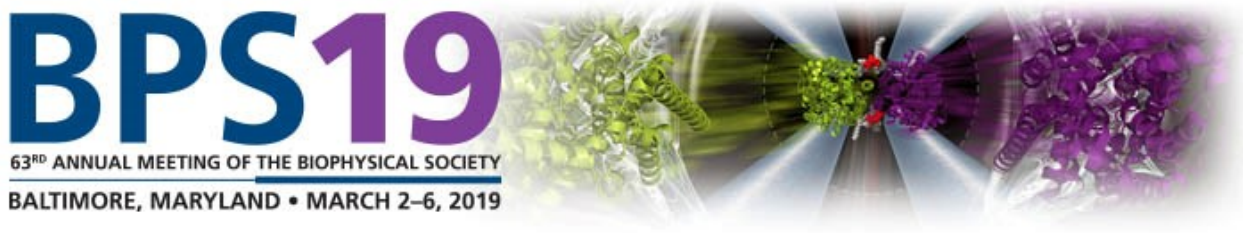

Sunday, March 3
10:30 am – 12:00 pm
Room 301
HORIBA Scientific

UNIQUE FLUORESCENCE MOLECULAR FINGERPRINTING IN ACTION: WHAT CAN CCD DETECTION DO FOR YOU?

Fluorescence is a standard tool for the study of changes on the molecular level, but it is now also becoming an emerging technique for molecular fingerprinting and spectral kinetics. The Duetta™ 2-in-1 fluorescence and absorbance spectrometer from HORIBA Scientific is a unique and powerful benchtop instrument that provides so much more than standard PMT-based scanning benchtop fluorometers. CCD detection technology, and incorporated absorbance measurements, provide more data, with more accuracy, and in less time. In this presentation, HORIBA Scientific will demonstrate two of many methods for which Duetta is uniquely equipped to measure fluorescent samples. First, Duetta can measure protein binding and FRET over the full emission range (250-1100 nm), demonstrating the effects of both donor and acceptor spectra over time with true spectral kinetics. In addition, the method of measuring Absorbance-Transmittance Excitation Emission Matrices (A-TEEMs) gives information about the molecular fingerprint of a mixture for use in component analysis of mixtures. The use of the absorbance detector enables inner-filter effect correction, which can easily be overlooked using standard fluorometers.

<u>Full Spectral Kinetics and FRET</u>

Because Duetta uses a CCD detector for emission detection, kinetics over the entire emission spectrum (250-1100 nm) instead of only at one or two different emission wavelengths. We will demonstrate the binding of a small molecule, 1,8-anilinonaphthalene sulfonate (ANS), to bovine serum albumin protein (BSA) that shows both the decrease in donor emission (BSA) and the increase of the acceptor emission (ANS) as an example of FRET kinetics. The binding of ANS to hydrophobic pockets in BSA is a known phenomenon, but is typically only measured as a kinetics experiment at the ANS emission wavelength of 475 nm. Historically, concentration-dependent experiments where emission spectra are collected over a range of ANS or protein concentrations, or both, are used to show binding kinetics or FRET as well. Duetta easily measures both the donor BSA (tryptophan) emission as well as the acceptor ANS emission during binding and shows that energy transfer occurs over the full spectral range. This is a unique capability for a benchtop fluorometer in the field of biological fluorescence.

<u>A-TEEM Molecular Fingerprinting</u>

The use of fluorescence for molecular fingerprinting is a relatively new concept and just as exciting if not more so than spectral kinetics. In most applications, changes in fluorescence intensity, or wavelength, or both, correlate to changes in physical properties of a sample. A-TEEM is a method of measuring the full fluorescence contour plot of a sample at all excitation wavelengths and all emission wavelengths. The matrix is then corrected for effects of high concentration (inner-filter effect) using the absorbance spectrum. The resulting A-TEEM gives an accurate profile of all emitting species and in turn, gives more information about the content of the sample in question, thus making it a better data set for chemometric and quantitative analysis. Solutions of tryptophan and 2-aminopurine, a fluorescent derivative of adenine, are used to demonstrate 1.) Effects of high absorbance/concentration on the fluorescence profile; and 2.) The A-TEEM profile for detection of multiple components.

Speaker
Karen Gall, Applications Scientist, HORIBA Scientific