OPTICAL DIFFUSION-ORDERED SPECTROSCOPY: ADDING SIZE TO OPTICAL SPECTROSCOPY

We present a method to *simultaneously* characterize molecular structure and size: *Optical Diffusion-Ordered SpectroscopY (Optical-DOSY)*.[1-4] This method is inspired by concepts from NMR, and relies on the fact that the diffusion coefficient of a particle is determined by its size through the Stokes-Einstein relation.

In our approach, we create a step-function concentration profile inside an optical sample cell (Fig.1a). By measuring the time-dependent absorption spectrum in an initially solvent-filled part of the sample volume, we obtain the diffusion coefficients and the optical spectra of the species present in the sample solution. Since the diffusion coefficient is inversely proportional to the hydrodynamic radius R through the Stokes-Einstein relation, we extract simultaneously size and chemical information. From these data, we construct a two-dimensional spectrum with absorption wavelength/frequency on one axis and size on the other, in which the optical spectrum of a mixture with different molecular sizes is separated into the

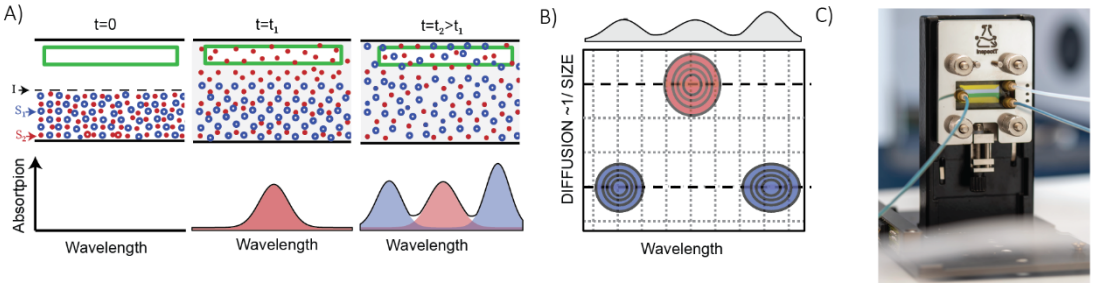

Figure 1: (A) Operation principle. At t=0 we inject a sample solution in the bottom part of a transmission sample cell, and solvent in the top part. Small molecules (red dots) diffuse faster than large ones (blue dots) into the initially solvent-filled part, so their absorption peaks appear first in the optical spectrum recorded there. Spatial selectivity is achieved using a slit in the spectrometer beam (green rectangle). (B) Schematic two-dimensional DOSY spectrum, with absorption peaks ordered by the value of the diffusion coefficient of the molecules; (C) Lab photo of IRMA, our IR-DOSY chip.

spectra of the different species, sorted by size (Fig.1b). We have demonstrated its feasibility using infrared spectroscopy (IR)[1,2], and later using Raman and UV/Vis spectroscopy[3-4], demonstrating the broad versatility of our technology. We have also developed and validated in-house prototypes of chips (Fig.1c) that can be easily integrated with common spectrometers. In this way, anyone can add size-sensitivity to their IR, UV/Vis or Raman spectrometer.

[1] Infrared Diffusion-Ordered Spectroscopy Reveals Molecular Size and Structure, Giubertoni et. al., Angewandte Chemie, 2023

[2] Multidimensional infrared diffusion-ordered spectroscopy in depletion mode distinguishes protein amyloids and monomers, Giubertoni et al., JCP, 2023

[3] Raman Diffusion-Ordered Spectroscopy, Schmidt et al, 2023, JPCA, 2023

[4] Optical Diffusion-Ordered Spectroscopy: A Simultaneous Probe of Molecular Size and UV/Visible Absorption, Analytical Chemistry, 2024