Research Excellence: Simultaneous Determination of Molecular Size and Molecular Shape of Nanoparticles and Proteins in Solution Using Fluorescence Correlation Spectroscopy (FCS)

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Abstract

The development of a new instrumental capability for simultaneous determinations of molecular size and molecular shape of nanoparticles and proteins is proposed. An existing analytical technique used in the project leader's laboratory, fluorescence correlation spectroscopy (FCS), will be enhanced for this purpose. Simultaneous measurements of molecular size and shape will have broad application, but will be used initially in conjunction with ongoing and anticipated collaborative research interactions with industrial research partners interested in the development of a robust method for shape analysis of abrasive nanoparticles. This project will advance the field of FCS and will provide future external funding opportunities.

Objectives

The objectives of this project include the following:

(1) Extend the range of measurements being made with a technique used in the project leader's laboratory, fluorescence correlation spectroscopy (FCS), to include analyses of rotational diffusion coefficients for fluorescently-labelled nanoparticles and proteins. As explained in Background and Significance section of this proposal, combined measurements of rotational and translational diffusion coefficients enable the simultaneous determination of molecular size and molecular shape of nanoparticles and proteins in solution. The FCS instrument currently in use has only been applied to date for measurements of molecular size via the measurement of translational diffusion coefficients.

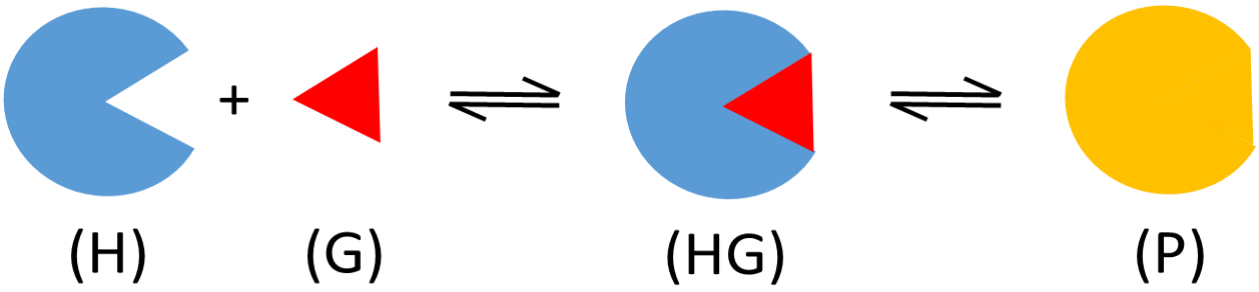
(2) Correlate FCS-measured rotational diffusion coefficients with measurements of the same parameter made using fluorescence polarization measurements. The latter measurements will be performed using the Department's steady-state spectrofluorometer. The project leader is skilled in this technique and he will instruct his research team in its use. Accomplishment of this objective will confirm the accuracy and reliability of rotational diffusion coefficients obtained from FCS.

(3) Apply the new molecular shape measurement capability to research projects with current industrial research partners interested in shape analysis for nanoparticle abrasive suspensions.

(4) Report research findings in at least one manuscript for publication and one oral presentation at a scientific conference. Use the findings as preliminary results for the preparation of research contracts with future industrial research partners and for the submission of a future grant proposal to a federal funding agency, such as the National Science Foundation (NSF).

Background and Significance

Chemical reactions occurring in a liquid solution that produce desired products depend critically on the nature of interactions between the reactants at the molecular scale. One such interaction is the molecular recognition[1] of a guest molecule (G) by a host molecule (H) which react to form a product molecule (P):

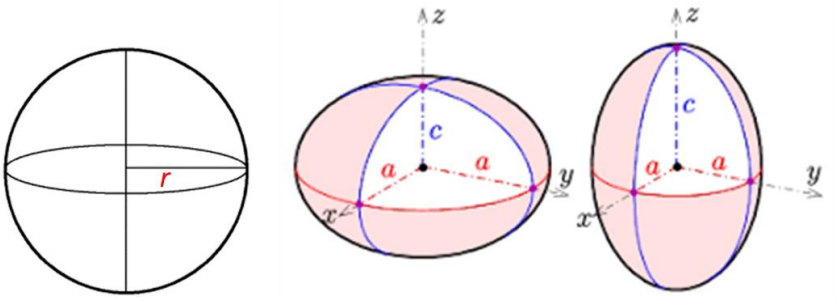


Recognition of the guest by the host is attributable to the conformal shapes of G and H that favor the formation of a host-guest complex (HG) as an intermediate in the reaction producing the product, P. This example illustrates the well-known principle that the shape of molecules can

strongly influence their reactivity. Consequently, the ability to determine the shape of molecules in solution provides an avenue to better understand and describe chemical reactivity at the molecular level.

Given the chemical importance of molecular shape, its determination is essential in all areas of chemistry. Optical spectroscopic methods of analysis stand out as particularly useful for this determination[2] due to their applicability in making measurements of molecules in solution. A single-molecule spectroscopic method used in the project leader's laboratory for the analysis of molecular size, fluorescence correlation spectroscopy (FCS), is potentially adaptable for the simultaneous determination of molecular size and shape.[3] FCS has demonstrated applicability to chemical species ranging from simple dye molecules to proteins and nanomaterials.[3] Its use in molecular size studies of both proteins and nanoparticles has been a recurring theme in the project leader's laboratory.[4-9]

The simultaneous measurement of molecular size and shape of nanoparticles and proteins in solution is proposed using FCS to determine the rotational diffusion coefficient in tandem with a determination of a translational diffusion coefficient for these species. This approach is described with the aid of the illustration shown below. If a species of interest is spherical only one dimension, its radius *r*, is required to define its shape. However, an ellipsoidal species requires two unique dimensions, commonly referred to as the major and minor axes, *a* and *c*, to define its shape. Determination of the two axes of an ellipsoid requires the measurement of two physical quantities related to the size of the ellipsoid. Because the diffusion coefficients (rotational and translational) of a species in solution depend on its size, measurement of the rotational and



translational diffusion coefficients via FCS will provide the minimum number of measured quantities needed to model the shape of a non-spherical species (nanoparticle or protein) in solution as its equivalent ellipsoid.

Implementation of this tandem measurement is feasible using the FCS instrument in the project leader's laboratory.[4] In our typical FCS experiments, when fluorescent species are present at a concentration of 1 to 10 nM (1 to 10 x 10^{-9} M) they travel one-at-a-time through a focused laser with a focal volume of less than one femtoliter (1 x 10^{-15} L). A fluorescent species transiting the focal volume produces a burst of fluorescence signals as it passes through the volume. Smaller-size fluorescent molecules or particles produce rapid bursts of fluorescence, while larger-size fluorescent molecules or particles produce slower bursts of fluorescence. By measuring the

frequency of the bursts with the FCS system's digital autocorrelator,[3] translational and rotational and diffusion coefficients of the molecules or particles can be calculated. The differentiation of signals from translational and rotational diffusion of the fluorescent particles or molecules is possible because rotational diffusion is more rapid by a factor of 100 than translational diffusion.[3]

Qualifications

A team of four researchers in the Mund-Lagowski Dept. of Chemistry and Biochemistry will collaborate on this project.

1. The project leader, E. Remsen, has 10 years of experience at Bradley in the area of FCS and related optical spectroscopic techniques. Under his guidance and since 2012, he and his students have published 8 peer-reviewed manuscripts and have presented 9 papers at scientific conferences and other universities in the area of FCS.
2. Two B.S. / M.S. students, Cody Graham and Keri Martinez, have used FCS during the past 9 months for their research projects in the areas of nano-dot imaging reagent characterization and nanoparticle abrasive analysis, respectively.
3. One B.S. student, Michael Stewart, has 3 months of experience using FCS and infrared spectroscopy in the analysis of small-molecule adsorption on a zirconia nanoparticle abrasive.

Methodology Plan

The description of the proposed FCS technique provided in the Background and Significance section establishes the planned measurement methodology. Specific planned steps during the project are detailed below in the Project Timeline,

Possible interferences to the simultaneous determination of translational and diffusion coefficients using FCS are two-fold. FCS measurements collected on a short enough timescale to capture signals corresponding to rotational diffusion may be corrupted with an artifact common to single-photon avalanche detector (SPAD) systems used in FCS measurements. This artifact known as "after pulsing" produces a signal that overlaps the fluorescence signals corresponding to rotational diffusion.[10] This artifact can be eliminated from FCS measurements of experimental samples by making FCS measurements of blank samples and using these determinations as controls for correcting FCS data collected for the experimental samples. Another potential source of signals that might overlap signals produced by rotational diffusion are "triplet dark states" produced in fluorescent dyes when subjected to high intensity laser illumination.[3] Triplet dark state formation produces fluorescence signals also corresponding to the timescale of rotational diffusion. FCS data for experimental samples can be freed of contributions from triplet dark states by reducing the incident laser power used in an FCS experiment to a level that is insufficient for their production.[3] Based on the experiences of the project leader and his former research students with FCS, after pulsing and triplet dark state formation can be readily managed

in FCS experiments performed using the FCS instrument in the project leader's laboratory. As a result, these two sources of interference pose no significant obstacles that would inhibit the ability of the proposed FCS technique to simultaneous determine accurate translational and rotational coefficients.

Project Timeline

1. During the first month of the funding period, I will enhance the sampling algorithm used by the FCS system's data acquisition system to enable time-extended data collection suitable for capturing rotational diffusion signals. With the assistance of the students in the project, new software will be written and tested to analyze time-extended FCS data sets. These data will be used as input for calculations of rotational diffusion coefficients and subsequent modeling of the species producing the data as their equivalent ellipsoid shapes.
2. In months 2 through 9 the students will apply this new capability in analyses of model proteins of non-spherical shape (e.g., bovine serum albumin) and suspensions of nanoparticles containing mixtures of spherical and non-spherical nanoparticles (e.g., silica nanoparticle abrasives). The students will determine by trial and error, fluorescent labelling protocols that produce fluorescently labeled non-spherical proteins and heterogeneous nanoparticle suspensions suitable for the simultaneous analysis of molecular size and shape. During this time, the students will collect complementary fluorescence polarization data for fluorescently labeled species using the Department's steady-state spectroflurometer. Comparison of FCS and fluorescence polarization data will confirm the accuracy of molecular shape analyses performed using FCS.
3. During the final 3 months of the funding period, I will guide the students in the documentation of their findings for poster presentations at the Student Expo. If sufficient data are available at that time for the preparation of a manuscript for external publication, this activity will also commence during months 9 through 12.
4. After the conclusion of the project, I will write a summary report of project results and accomplishments. External funding opportunities will be sought which can employ project results as preliminary findings suitable for inclusion in either a grant proposal or a research contract proposal.

Anticipated Results

The expected major result of this project will be a new FCS analysis capability for addressing fundamental chemical problems. One of key issue that will be investigated during the project is the effect molecular shape plays in the polishing performance of nanoparticle abrasives. I receive inquiries on this subject regularly from potential collaborators working with computer chip manufacturing companies, such as Intel Corp. and suppliers of nanoparticle abrasives to these companies. Because nanoparticle abrasives are used by these companies to polish integrated circuits to near-atomic flatness, they are very interested in the characteristics of nanoparticle abrasives that drive polishing performance. This interest is rooted in their fervent need to

commercialize advanced logic and memory chips for use in next-generation computers and nearly all other commercial electronic devices.

Findings from the project will be publishable in peer-reviewed journals and will be appropriate for presentation at scientific conferences. These communication activities will additionally provide students working on the project with the opportunity to hone their writing skills and develop confidence in presenting the product of their research to scientific audiences.

Alignment with RE Priority Areas

The appropriate RE priority area under which this proposal falls is in the category of Sponsored Programs. Specifically, successful results from the project are expected to spur additional funding opportunities from one or more industrial partners (e.g., Intel Corp., Cabot Microelectronics Corp.) who have funded research contracts with my group in the past.

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