Interfacial Behavior of Fluorescent Dyes

Power and Weakness of Nanoscopic Description

Alexander P. Demchenko and Semen O. Yesylevskyy

Abstract Our macroscopic world and the world of atoms and small molecules are separated by length scales differing by seven or more orders of magnitude. Describing the latter world with fluorescence probes in terms of structure and dynamics has both merits and difficulties due to peculiarities and limitations of fluorescence method. Demonstrating unique resolution in time and very high sensitivity to interaction energies, this method generally lacks structural resolution on the level of atomic details. Therefore, presentation of fluorescent probe by its molecular structure or its derivatives (size, charge distribution, dipole moment, etc.) and of its tested molecular environment in terms of continuous medium (such as micropolarity, microfluidity, or proticity) became the common method of analysis. This description that combines molecular-level parameters and reduced to molecular-level macroscopic parameters can be termed "nanoscopic". The strong demand towards rational description of systems with molecular and nanoscale heterogeneity (surfaces of liquids and solids, liquid–liquid and liquid–solid interfaces, nanoparticles and porous nanocomposites) requires critical analysis of methodology when applied to these systems. This will be the subject of the present chapter.

Keywords Fluorescence reporters · Nanocavities and nanocomposites · Nanoscale polarity · Nanoscale viscosity · Solvatochromy · Surfaces and interfaces

A.P. Demchenko (✉)
A.V. Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Leontovicha st. 9, Kyiv 01601, Ukraine
e-mail: alexdem@ukr.net

S.O. Yesylevskyy
Institute of Physics, National Academy of Sciences of Ukraine, Prospect Nauki, 46, Kyiv 03039, Ukraine
e-mail: yesint3@yahoo.com

A.P. Demchenko (ed.), *Advanced Fluorescence Reporters in Chemistry and Biology III: Applications in Sensing and Imaging*, Springer Ser Fluoresc (2011) 10: 3–62,
DOI 10.1007/978-3-642-18035-4_1,

Contents

1 Introduction

Determination of composition, structural arrangement, and dynamics of molecules and their groups of atoms at liquid–liquid and liquid–solid interfaces are extremely important for understanding various phenomena related to adsorption and catalysis and for technologies of chemical synthesis and separation/purification of reaction products. The properties of nanoscale porous materials and of nanoparticles, possessing very high surface-to-volume ratios, can be to a dramatic extent determined by interactions at their solvent interface. Structure and stability of synthetic polymers and biopolymers (proteins, polysaccharides, and DNA) are governed by interactions with the solvent and with the adsorbed low-molecular ligands. Understanding the behavior of these materials requires molecular-size

tools integrated into these composite systems and serving as the reporters. Fluorescence probing methodology can provide such tools that are simple, highly sensitive, and nondestructive.

Fluorescence reporting focuses on *nanoscopic* properties of matter. It uses different organic dyes, luminescent metal complexes, labeled macromolecules, and different kinds of nanoparticles to evaluate local properties of their environment and of their intermolecular interactions. Sensing local field effects, the fluorescent reporter probes simultaneously the local polarity of the host medium, specific chemical interactions, and geometrical or morphological constraints. Meantime, the description of the probed system on the level of atomic details here is not available (exceptions are the formations of strong complexes and of covalent bonds by the probes, which is generally outside the probing methodology). This limitation is simply due to the size of reporters that is larger than atomic. Another limitation comes with the restricted geometry of probe location and orientation in structurally inhomogeneous systems that induces new difficulties in the understanding of their properties. Using fluorescence method, we possess a very limited number of parameters (they are intensity, anisotropy, lifetime, and the changes in excitation and/or emission spectra) that could provide informative reporter signal.

Because of these peculiarities, analysis of fluorescence data depends strongly on physical modeling leading to simplification of molecular systems or on empirical correlations relating spectroscopic parameters and intermolecular interactions [1]. Both approaches lead to quasi-continuous characterization of reporter surrounding. They allow exploration of such terms as micropolarity, microfluidity, or proticity as the *nanoscopic* analogs of parameters characterizing *macroscopic* condensed media. Such correlation of parameters that refer to macroscopic scale and molecular scale is not easy and requires different assumptions and approximations that are rarely discussed in original works.

When the averaged properties of the solvent as the "bulk medium" are of primary importance, then the quasi-continuous models (such "*continuum solvation models*") that ignore the solvent molecular structure are effective in description. Meantime, local field effects that deviate from that described in these models and a restricted geometry of probe location and orientation can be important parameters for the full understanding of spectroscopic behavior in such complex systems. Orientation of amphiphilic molecules at the polar–nonpolar interface and formation of specific interactions (such as charge-transfer complexes or H-bonds) may result in additional geometrical or morphological constraints. Then the models based on exact molecular structures (such as "*explicit solvation models*") should be applied.

The sensitivity of fluorescence emission from dye molecules to weak intermolecular interactions and their dynamics has been recognized as an important means to probe local field effects not only in homogeneous systems but also in the systems with molecular and nanoscale heterogeneity. This chapter is focused on mesoscopic description of the systems with this type of heterogeneity based on the data obtained in fluorescence probing. We analyze different methodologies in this description.

2 Essentials of Mesoscopic (Nanoscopic) Analysis Based on Computational Approach

Mesoscopic objects are the objects dealt in physics of condensed systems that are larger than atoms but small enough to observe fluctuations of statistically averaged variables. Fluorescent dyes always probe their *local* molecular environment, whatever is the dimension of studied object, in which they are incorporated. The system composed of the dye and of its local environment is always mesoscopic. Because of nanometer size of such systems they are often called *nanoscopic*. The description of nanoscopic systems and, particularly, those properties that give rise to spectroscopic changes should require some combination of classical and quantum-mechanical variables. Electronic excitations and redistributions of electronic density that accompany them are described by the laws of quantum mechanics. Meantime, there is a possibility to use intermolecular potentials derived from classical mechanics to describe intermolecular interactions in the ground and excited states. This allows considering the change of energies of electronic transitions as information on these interactions.

Why we are not satisfied by just empirical correlations between spectroscopic and macroscopic-like properties and need going deeper in the analysis of molecular and electronic structures? Our first aim is to reduce ambiguity in interpretation that commonly exists even in neat solvents. For instance, the strong shifts in fluorescence spectra could be due to the change of polarity or of H-bonding potential in the dye environment. But it can be also due to some photophysical reaction in the dye coupled with the dynamics in this environment. Even more difficult is the analysis of structure and dynamics in heterogeneous systems. Imagine that we study the properties of two interacting media of macroscopic dimensions. We can describe them in *macroscopic* terms, such as polarity and viscosity. However, this does not allow for the understanding of the properties of interface, such as the sorption of amphiphilic molecules (e.g., detergents), aggregation of nanoparticles, and the interfacial catalysis. On the other hand, *atomistic* description of these systems is very hard to achieve experimentally due to limitations of the methods that are commonly used for structural analysis, such as X-ray diffraction and NMR. But even if we do so by overcoming the problems of sample crystallization or isotope enrichment, we get a huge amount of extra structural information that is hard to analyze. We will then search the possibilities for *nanoscopic* description.

An "in silico" experiment with molecular dynamic (MD) simulations [2, 3] has a much broader applicability. This approach is based on application of classical mechanics and allows computing the forces between all atoms in the system and equilibrating the structure in chosen thermodynamic ensemble. This provides the atomistic detail in structure together with realistic dynamics of individual atoms. Meantime, it is often hard to operate with such large massive of information. Numerous atomic details can mask the general picture of physical and chemical processes that depend on statistical behavior of molecular ensembles rather than on detailed atom–atom interactions. Therefore, the technique of MD simulations is

moving toward the *coarse-grained models*. In these models, the groups of adjacent atoms are combined into the "beads", which interact with each other by means of empirical potentials. Since the number of beads is much smaller than the number of individual atoms, significant speed up of computations could be achieved. The coarse-grained models could describe slow collective motions of complex molecules (such as large proteins) or macromolecular assemblies like the membranes of liposomes [4]. The coarse graining provides the *nanoscopic* description of the studied systems instead of purely atomistic treatment.

The difference in the characteristic times for different components of the studied system is crucial for adequate interpretation of MD results. Very slow rotational degrees of freedom of large solutes, such as proteins, DNA, and membranes, are never sampled adequately in MD simulations. However, the dynamics of solvent molecules is usually so fast that the solvent could be considered in local thermodynamic equilibrium for each given position and orientation of the solute. This allows averaging the solvent properties effectively and obtaining integral characteristics, such as local effective dielectric constant, local charge density, local hydrogen bonding propensity, local ionic concentrations, local electric field, etc. Such local properties could be computed around active sites of the protein and even in the vicinity of individual amino acids exposed to the solvent. As a result, unnecessary details of fast solvent motion are averaged out, while the solute is still described with atomic resolution. This represents another facet of the nanoscopic description of the system.

One of the most significant drawbacks of classical MD simulations that deal with atoms as classical bodies but not the electrons is the inability to handle the excited states, redistributions of the electronic density inside the molecules, and chemical reactions. Thus, the development of hybrid simulation techniques, which combine MD with the quantum mechanics [5, 6], has boosted in recent years. Such combined techniques allow computing electronic properties of small critical subsystems (such as organic dye molecules together with their binding sites) in realistic dynamic environment, which is described in the terms of classical mechanics. The interactions of fluorophores with their environment become dependent on their electronic state in this approach.

All MD or hybrid simulations provide the trajectories of individual atoms and, in the case of the hybrid simulations, the electronic densities of the quantum subsystems. These quantities should be integrated over the statistical ensemble to obtain useful characteristics of the system, such as viscosity, polarity, diffusion coefficient, interaction or solvation free energies, etc.

In contrast to the methods that provide atomic structural resolution, fluorescence spectroscopy allows achieving response to intermolecular interactions already in integrated manner. A *nanoscopic* level of details can be addressed here by describing the studied object as an integral but nonhomogeneous system with some of its essential properties presented on molecular level. The other properties appear as integrated over elementary interactions and their dynamics, which requires introduction of quasi-continuous description in such terms as "polarity" or "viscosity". Essentials in spatial resolution here are not lost if they are determined by the

http://www.springer.com/978-3-642-18034-7

Advanced Fluorescence Reporters in Chemistry and Biology III
Applications in Sensing and Imaging
Demchenko, A.P. (Ed.)
2011, X, 352 p., Hardcover
ISBN: 978-3-642-18034-7