Molecular wayfinding: Mapping transport dynamics

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In a world where online shopping and same-day delivery are now the norm, smart freight, transportation, and logistics are emerging as the critical elements for the success (or failure) of companies and, to some extent, the sustainability of a city, municipality, or indeed a nation. The same paradigms exist in the molecular and cellular context with the delivery of cargo—molecules, proteins, and ions—to and from specific locations being critical to the function, viability, and sustainability of a cell, tissue, organoid, or organism. Indeed, one can readily draw analogies between inter- and intracellular transport with that of freight moving within a city, between cities, and between countries. Quantitative measurements of these transport dynamics can provide new insight into the structure, environment, and interactions that are critical to our understanding of biological mechanisms and cellular processes. In the bioengineering context, such information would be critical for understanding how diffusion through a hydrogel is impacted by the local structure and chemistry, the impact of the membrane structure on cell surface receptor dynamics, transport and clustering, and the dynamics of organelle and secretory vesicle trafficking.

Given this metaphor, let us consider some of the key questions that underpin these processes and mechanisms and, more specifically, strategies that allow us to probe the transport dynamics. Just as in the case of the movement of goods between a supplier and the customer, we need to characterize (Fig. 1):

- Route: What are starting and ending points, and are there waypoints along the way? Is the route pre-defined (i.e., along a known path or track)?
- Speed: How fast is the cargo moving? Is this a facilitated process?
- Cargo and carrier: How many items are being moved and what state are they in?
- Surroundings: How does the local environment affect or influence transport dynamics?

With this framework in place, we are faced with the following additional questions:

- How does one measure these dynamics?
- On what time scales?
- On what length scales?

Let us now look at some recent developments in platform tools that can answer these questions and consider where the future opportunities lie.

Highways and byways. While perhaps the simplest system to consider is the free diffusion of molecules in a uniform medium (i.e., isolated proteins in a homogenous solution), which would be expected to follow classic Brownian behavior, how proteins diffuse through more structurally complex environments has become of particular interest in the fields of regenerative medicine, drug delivery, and biomaterials. Hydrogels are a prime example of just such a structurally complex and ill-defined environment. Composed of hydrated polymer networks, wherein the polymers may be synthetic or natural, and either physically or chemically cross-linked, hydrogels are particularly useful because of their inherent ability to have their physical and chemical properties tuned through judicious selection of the polymer, cross-linking chemistry, and cross-link density. Fundamentally, this tunability directly impacts the ease with which a species (ion, molecule, and protein) moves through the hydrogel. What is challenging to understand in the context of a hydrogel is not the speed with which the diffusing species moves through the gel itself since that can be directly measured but rather how this diffusive process reflects the local environment within the hydrogel itself. How the local structure impacts diffusion is certainly complex and multi-faceted but ultimately comes down to two key parameters: the local porosity of the hydrogel, which defines the free path over which the diffusing species can move, and the local chemistry of the hydrogel, which can, depending on the diffusing species, hinder transport by transiently inducing local binding interactions with the hydrogel itself. The latter case often happens in the case of charged systems wherein electrostatic forces would play a significant role in influencing transport, which would normally be largely impacted by the concentration gradient across or through the hydrogel.

Congestion mapping. Direct determination of the diffusivity of a molecular species involves monitoring spatiotemporal changes in the
system. For example, one might monitor local changes in the refractive index as a consequence of changes in the protein concentration, an approach applied by Weng et al. While this is a label-less, low-cost, and relatively easily implemented approach, it is fundamentally a bulk measurement defined in large part by the imaging system used to measure the changes in RI. Interestingly, recently, researchers have used a variation on this approach of tracking changes in RI to examine hydrogel gelation dynamics. In this work, Huang et al. applied whispering gallery mode (WGM) sensors to track the dynamics of hydrogel gelation. In a WGM sensor, one exploits the self-interference of light confined within a microcavity to track changes in its local material properties, which are manifested as shifts in the WG resonance wavelengths or frequencies. The exquisite sensitivity of WGM sensors has, for instance, made them being applied in biosensing applications and, related to this context, to characterize anomalous diffusion of solvent molecules into polymer microspheres. In the work by Huang et al., cross correlation approaches applied to several WG resonances allowed the authors to more accurately track gelation dynamics and mechanics compared to conventional rheological measurements.

While this suggests an interesting opportunity to track solute diffusion through a hydrogel during gelation and permanent cross-linking, it also raises another intriguing context in which to examine these questions in the context of how diffusion through a hydrogel is impacted by both the scale and the local structure. Another key complication is how does one decouple the convective and diffusive transport in these hydrogels. The context here is that these materials can be subject to physical conditions that could induce convective flow, which certainly could dramatically affect solute transport. This very relationship was explored in a study of dextran diffusion in polyethylene glycol.
hydrogels using an indentation-based approach to characterize how intrinsic permeability can report on both diffusive and convective transport.12

In related work, Hettiaratchi et al. recently developed a capillary tube-based parallel array platform for tracking diffusion in hydrogels.9 This fluorescence imaging-based design allow for facile measurements of one-dimensional diffusion through capillary tubes containing homogeneous hydrogels. Intriguingly, since the intensity data as a function of position and time in the tubes are correlated with the local concentration, it then became relatively easy to compare these experimental data with computed data using classic mass transport modeling approaches. Expanding on this approach, Su et al. looked at molecular transport in the context of diffusion through and across a heterogeneous interface in an effort to understand how partition coefficients between phases and structures impact diffusion dynamics.14 Such work is relevant to not only fundamental studies of diffusion and transport at interfaces but also for the design of materials for drug delivery applications. In this work, a series of model cases, hydrogel-free liquid, hydrogel–hydrogel, and hydrogel–impermeable boundary (glass), were studied with intriguing insight into the role that the thin-film fluid phase present at the hydrogel surface plays in determining the final concentration profile and behavior. What is interesting about this thin film is that, at one extreme (hydrogel-free fluid), the system can be modeled as transport into a continuum bulk fluid phase, whereas in the case of the hydrogel–hydrogel or hydrogel-impermeable surface (glass), this film develops as a consequence of the contact between these phases. This is in contrast to conventional multi-layer diffusion models where the interfaces are seen as hard boundaries.

Let us now look at a slightly different orientation—namely, transport along or within an interface—and the classic example is that of molecular dynamics in the cell membrane. Understanding how the transport of membrane and membrane-associated proteins is influenced by the local structure of the membrane, ligand binding, and even association state is key to unlocking complex signaling pathways. Fluorescence-based approaches such as FRAP, single particle tracking (SPT), and image correlation strategies are commonly applied in this context.15–17 In a recent review, Sankaran and Wohland comprehensively described how these tools can be applied to provide a wide range of both spatial and temporal scales, including local microenvironment, diffusion coefficients, diffusion mechanism, concentration, and oligomerization state.18 In their perspectives article, they not only focused on the pure fluorescence-based approaches but also described how combinatorial approaches could be used to provide additional quantitative measurements for local order and structure in membranes. Interestingly, while many of these optical approaches remain far-field diffraction limited, the integration of fluorescence correlation spectroscopy (FCS) with super-resolution optical fluctuations imaging (SOFI) (fCS-SOFI) has been shown to now accurately generate sub-diffraction limit diffusion maps in hydrogels and other porous materials.19 What has been particularly exciting about this development is the co-application of fCS-SOFI with SPT to probe transport in a tunable hydrogel.20 In this work, the authors showed how pH-induced changes in a pH-sensitive hydrogel altered the balance between a model protein being confined to and diffusing within the pore structure of the hydrogel and actively adsorbing/desorbing from the hydrogel itself.

Package tracking. Another particular compelling example of the application of particle tracking and correlation spectroscopy to map out the local dynamics and transport behavior in cellular organelles was recently reported by Appelhans and Busch.21 In this work, which focused on mitochondria, the authors argued that, while techniques such as FCS can have single molecule sensitivity, they do not provide generate individual trajectory maps. Rather, in order to fully characterize the dynamics of molecules in complex organelles, such as the mitochondria, it becomes necessary to exploit high-resolution tracking and localization platforms. In this way, it becomes possible to calculate not only individual diffusion coefficients but also localize these trajectory maps to specific sub-structures within the organelle itself. What was remarkable about this work was that how the authors were able to combine and complement the insight obtained from FRAP with their tracking and localization microscopy (TALM) approaches. Most notably, the authors clearly showed that some of the key limitations of FRAP (diffraction-limited, only appropriate for systems with relatively fast recovery times) could be addressed through the TALM approach.

This last example allows us to cycle back to our opening metaphor. We need to be able to accurately track trajectories, examine the effect of the local environment, and do so over a range of both spatial and temporal scales in order to develop a comprehensive understanding of molecular transport and dynamics. We have seen how we can directly probe local dynamics within a host matrix, how correlated and combined approaches can help resolve discrepancies between bulk behavior and localized interactions, and how emerging tools such as localization microscopies are starting to tease out intricate details around confined diffusion on the nanoscale.

Smart shipping. Yet many questions and indeed opportunities remain unanswered. In many of the approaches described herein, the focus has been on a single species. We need tools that allow us to track the state and nature of the cargo that is being transported. We need the ability to simultaneously track multiple species, which would allow one to dynamically probe intermolecular interactions, the role of the host matrix/membrane/structure in influencing these interactions, and their functional implications. We need tools that allow us to probe longer time scales but with finer spatial, temporal, and compositional resolution. Addressing these challenges will lead to entirely new perspectives on the mechanisms and dynamics of molecular transport and their functional implications.

DATA AVAILABILITY

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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