

# Understand Discrete Nanoscale Transport

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Unlike macroscopic problems in mass, energy and momentum transfer, which are described by continuum equations, the movements and interactions of the nanoscale entities within a cell constitute a transport phenomenon known as discrete nanoscale transport (DNT).

Imagine yourself on an alien spaceship, watching the daily goings-on of a large city through a telescope. You would see (among other things) complex transportation systems designed to maintain proper traffic flow and efficient distribution of goods and people.

Now imagine peering at a cell under a microscope. The interior of the cell is populated with numerous nanoscale entities (Figure 1), ranging from 10–25-nm ribosomes to 100–500-nm endosomes and lysosomes. These entities, also known as cellular organelles, make up as much as 15–25% of the cell volume (see box). They are involved in important cellular functions, such as supplying power (mitochondria), manufacturing proteins (ribosomes), delivering biochemical products (endosomes, secretory vesicles), and storing indigestible materials (lysosomes).

Just like cities, cells have evolved complex transport systems to maintain proper trafficking and distribution of these organelles. Major biochemical pathways are organized along cytoskeletal filaments (*e.g.*, microtubules and actin filaments), which form the backbone of the public transport system inside cells. Cellular organelles are hauled along the cytoskeletal tracks by molecular motors and communicate with each other via specific protocols. In many aspects, the complexity of cellular transport far exceeds the complexity of urban traffic — which is not surprising, since cells have been evolving for billions of

years, but most modern metropolises are no more than a few hundred years old.

The movements and interactions of these cellular organelles constitute a distinct class of transport phenomena, known as *discrete nanoscale transport* (DNT). Here, the term nanoscale refers to the nano-morphological features of the discrete entities being transported and their relevant interactions, whose length scale is from a few to several hundred nanometers. This distinguishes nanoscale transport from the transport of molecular-scale entities, such as ions, solutes, metabolites, lipids, and proteins (1), and transport processes at the whole-cell level, such as cell motility (2). Transport of nanoscale entities is determined by thermal mobility and by their interactions with cellular structures (*e.g.*, cytoskeletal filaments), with molecular-scale entities (*e.g.*, motor proteins and signaling molecules), and with other nanoscale entities. In most cases, due to the discrete nature of the transport events and their strong dependence on the local structural and architectural properties of the biological media, discrete nanoscale transport cannot be described by the continuum approaches that are routinely used in fluid mechanics and heat and mass transfer.

Important cellular functions often involve multiple DNT processes. Disruption or malfunction of DNT is observed in many hereditary and autoimmune diseases

## NANOSCALE ENTITIES INSIDE A TYPICAL 20- $\mu\text{m}$ HUMAN CELL

**Actin Filaments (AFs)** — components of a cell's cytoskeleton that provide mechanical support for the cell, determine the cell shape, and enable cell movements. At the peripheral region, in the vicinity of the cell edge, AFs tend to point outward, whereas inside the cytoplasm, their orientation is random. In many cells, AFs are uniformly distributed over the cell surface.

**Cytoplasm** — a gel-like material that fills a cell, consisting of the cytosol and all cellular organelles, except the cell nucleus. The cytosol is made up of water, salts, organic molecules and proteins.

**Endosomes** — membranous organelles inside the cell. Endosomes are classified into early endosomes and late endosomes. Early endosomes are involved in sorting and recycling of receptors. Late endosomes are responsible for the initial breakdown of the internalized material and delivery to lysosomes.

**Endocytosis** — the process by which cells internalize extracellular entities by engulfing them with their cell membrane. The endocytic pathway consists of early endosomes, late endosomes, and lysosomes.

**Exocytosis** — the process by which a cell releases biomolecules into the extracellular space through its membrane.

**Glycogen** — a polysaccharide of glucose that serves as a cell's primary short-term energy storage.

**Golgi Apparatus** — vesicles in a cell that process and package macromolecules, such as proteins and lipids, synthesized by the cell prior to their secretion.

**Lipid** — a fat-soluble molecule that performs one of many key biological functions, such as acting as structural components of cell membranes, serving as energy storage sources, and participating in signaling pathways.

**Lysosomes** — the final station in the endocytic pathway. They are involved in the final breakdown of internalized cargo as well as the storage of indigestible material.

**Microtubule** — a component of the cytoskeleton. Microtubules are filamentous polymers with a plus end and a minus end. They have diameter of approximately 24 nm, and lengths that vary from several micrometers to possibly millimeters in axons of nerve cells. Microtubules serve as structural components within cells and are involved in many cellular processes, including mitosis, cytokinesis, and vesicular transport.

**Mitochondria** — membrane-enclosed organelles that generate most of a cell's supply of adenosine triphosphate (ATP), which is used as a source of chemical energy.

Mitochondria are also involved in such processes as signaling, cellular differentiation, cell death, and the control of the cell cycle and cell growth.

**Molecular Motors** — proteins that can transform chemical energy directly into mechanical movements. Examples of molecular motors include kinesins, dyneins and myosins. Kinesins and dyneins typically move cargos toward the plus and the minus ends of microtubules, respectively. Myosins are responsible for transport on networks of actin filaments.

**Organelle** — a discrete structure within a cell. An organelle is to the cell what an organ is to the body (hence the name organelle). There are many types of organelles, each with its own specialized function. Examples of organelles

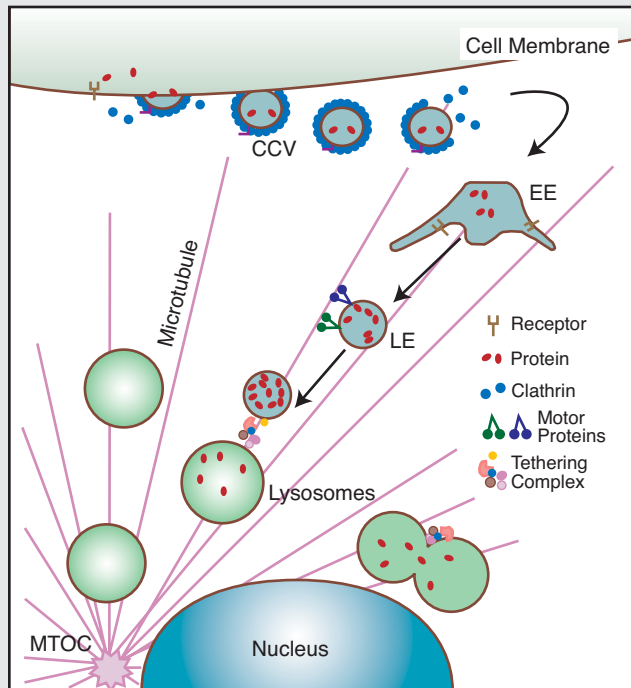


Figure 1. The endocytic pathway, a major biochemical pathway in eukaryotic cells (*i.e.*, cells with a membrane-bound nucleus), involves several transport processes.

include endosomes, lysosomes, mitochondria, secretory vesicles, and mRNA granules.

**Peroxisomes** — organelles that participate in the metabolism of fatty acids and other metabolites, and as part of the cell's secretory pathway, aid in ridding the cell of toxic peroxides.

**Proteasomes** — large protein complexes inside a cell that degrade unneeded or damaged proteins.

**Ribosome** — a minute particle consisting of RNA and associated proteins found in large numbers in the cytoplasm of living cells. They bind messenger RNA and transfer RNA to synthesize polypeptides and proteins.

**Vector** — an entity, either biological or synthetic, used to deliver genetic material into a cell.

Nanoscale Entity	Typical Size	Typical Volume	Typical Number per Cell	Relative Volume in Cell
Ribosomes	25 nm	8,000 nm <sup>3</sup>	~10 <sup>7</sup>	1.0%
Golgi Vesicles	30–80 nm	65,000 nm <sup>3</sup>	~200,000	0.2%
Secretory Vesicles	0.1–0.4 $\mu\text{m}$	0.004 $\mu\text{m}^3$	~10,000	0.5%
Glycogen Granules	10–40 nm	10,000 nm <sup>3</sup>	~100,000	0.01%
Lipid Droplets	0.2–0.5 $\mu\text{m}$	~0.05 $\mu\text{m}^3$	~100	0.05%
Endosomes	0.2–0.5 $\mu\text{m}$	~0.05 $\mu\text{m}^3$	~1,000–2,000	1.3%
Lysosomes	0.5–1 $\mu\text{m}$	0.31 $\mu\text{m}^3$	~200–1,000	2.3%
Proteasomes	20 nm	3,400 nm <sup>3</sup>	~10 <sup>7</sup>	0.4%
Peroxisomes	0.5–1 $\mu\text{m}$	0.31 $\mu\text{m}^3$	~300	1.1%
Mitochondria	2–3 $\mu\text{m}$	~1 $\mu\text{m}^3$	~300–3,000	12.5%
Viruses	10–100 nm	10,000 nm <sup>3</sup>	10–100	0.001%
Synthetic Vectors	0.2–0.5 $\mu\text{m}$	~0.05 $\mu\text{m}^3$	~10,000–100,000	6.3%
Cell Total	20 $\mu\text{m}$	8,000 $\mu\text{m}^3$	—	—

(3, 4). For instance, several neurological diseases, including Alzheimer's, Parkinson's and Huntington's diseases, have been associated with defective molecular motors, irregular activities of microtubule-associated proteins, and failure in communication between nanoscale compartments (3, 5, 6). A quantitative understanding of nanoscale transport processes will help in identifying the abnormal trafficking step(s) and in developing the therapeutic treatments that can restore normal functions.

Such valuable information on the inner workings of the cell could also lead to innovations in nanomedicine and nanotechnology. The therapeutic success of nanoscale devices (*e.g.*, nanorobots) and nanoscale drug carriers (*e.g.*, nanoparticles, liposomes, viral capsids, nanoshells, and synthetic gene vectors) relies heavily on the ability to map the organization of the cell interior and to navigate the devices to the correct targets. By mimicking how organelles move and interact with each other, it will be possible to engineer more-efficient nanoscale systems for biomedical purposes. Ultimately, one could apply the organizing principles of intracellular transport to build a "supramolecular factory" to manufacture and assemble nanoscale products (7).

The quest to understand the principles that govern the transport and organization of nanoscale entities has attracted intensive research from many disciplines, such as molecular biology, biochemistry and biophysics. With the recent advent of quantitative fluorescence microscopy, the movements of single molecules and single particles can be tracked and the organization of living cells can be analyzed in exquisite detail. This has led to advances in intracellular dynamics of macromolecules, cytoskeleton-based transport, and membrane fusion and fission. These new developments

have made a significant impact on the understanding of basic cellular functions, including endocytosis, exocytosis, homeostasis, intracellular signaling, etc.

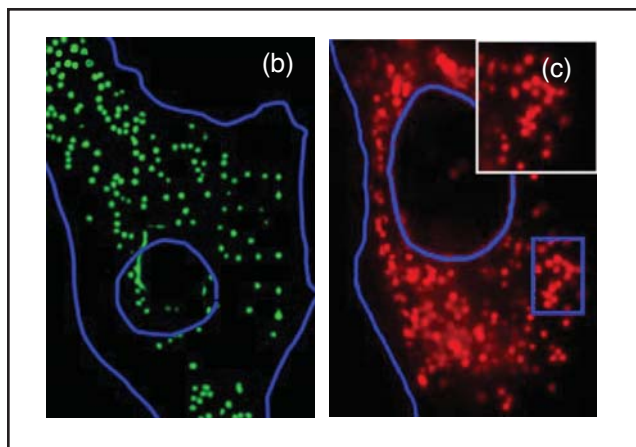
At the same time, the data produced by these experiments are rich and complex and go beyond the simple intuitive models traditionally employed to explain biological problems. It is now widely recognized that mathematical and computational models are needed to fully exploit the wealth of information made available by recent improvements in experimental techniques. The challenge is to translate qualitative observations and hypotheses into quantitative, fundamentally sound and verifiable models.

One strategy, which has been widely used in the study of biochemical networks, is to adapt tools and concepts from the engineering sciences. Chemical engineering has a long history of addressing complex transport phenomena in physical systems. Though diverse in nature, transport processes can be studied within the unifying framework of the continuum equations of mass, energy and momentum transfer. This facilitates the application of scientific laws to complex chemical processes, with a view to analyzing, designing and controlling them. Such a framework aligns well with the need to develop a systematic engineering approach that combines quantitative experiments, theoretical modeling and systems analyses to understand and predict intracellular transport, especially discrete nanoscale transport processes.

## Basics of nanoscale transport processes: endocytosis

Endocytosis is the process by which cells internalize fluids and macromolecules. To understand the importance of DNT in the normal operations of cells, consider the transport processes involved in endocytosis (Figure 1, box).

The cell surface is decorated with coated pits that continuously fold back into the cell and break off as bud-shaped clathrin-coated vesicles (CCVs). Molecules, large or small, that happen to be present in the enclosed fluid or attached to the cell membrane or membrane receptors at the pit sites will gain entry into the cell. After being transported away from the cell membrane, the CCVs rapidly lose their clathrin coat and fuse with pre-existing early endosomes (EEs). The internalized cargo is sorted inside early endosomes, and some (*e.g.*, receptors) are returned to the cell membrane via recycling vesicles. The remaining cargo is delivered to late endosomes (LEs), which are responsible for initiating the degradation of the cargo and delivering it to lysosomes (Figure 2a). Transport of endosomes on microtubules is powered by kinesin and dyneins, which move them away from and toward the nucleus, respectively. Lysosomes, the last stop, are involved in



■ Figure 2. Degradation of a cell's internalized cargo begins in the late endosomes (left). The lysosomes complete the breakdown, reclaim the degradation products and store indigestible material (right).

the final breakdown of internalized cargo, reclamation of the degradation products, and storage of indigestible material (Figure 2b) (8, 9).

The progress of the internalized cargo along the endocytic pathway depends on three distinct nanoscale processes, namely vesicle budding, motor-assisted movements and membrane fusion, which are responsible for creation, transport and communication of nanoscale entities. These processes also serve as building blocks in other important cellular pathways.

**Vesicle budding.** Considerable progress has been made toward understanding the molecular basis of vesicle budding (10). It has been established that the key molecular components of the vesicle budding process are the proteins that coat the budding vesicle. Unique proteins participate at different stages along the endocytic and secretory pathways.

Coat proteins (such as those known as COPI and COPII) are recruited to the donor organelle membrane from the cytoplasm with the aid of specific adaptor proteins (11). The flat membrane is deformed into round buds due to the interactions between coat proteins, lipid bilayers and several cytosolic proteins (12).

The vesicle forms when the neck connecting it to the donor surface splits. This step is relatively straightforward for COPI- and COPII-coated vesicles, as the energy for membrane fission is supplied by the coat proteins. For clathrin-coated vesicles, it requires the enzymatic activity of dynamin, which forms a collar or a ring around the neck of the budding vesicle (13).

Finally, the vesicle releases the coat components, which are recycled back to the donor membrane.

**Motor-assisted transport.** The thermal diffusivity of nanoscale entities in the cytoplasm is generally much smaller than the bulk aqueous diffusivity. This is due to the higher viscosity of the cytoplasmic medium, tortuosity of the diffusion path arising from ultrastructural obstructions, and additional hydrodynamic hindrance associated with finite particle size. It often takes hours or days for nanoscale entities to traverse the length of the cell by diffusion.

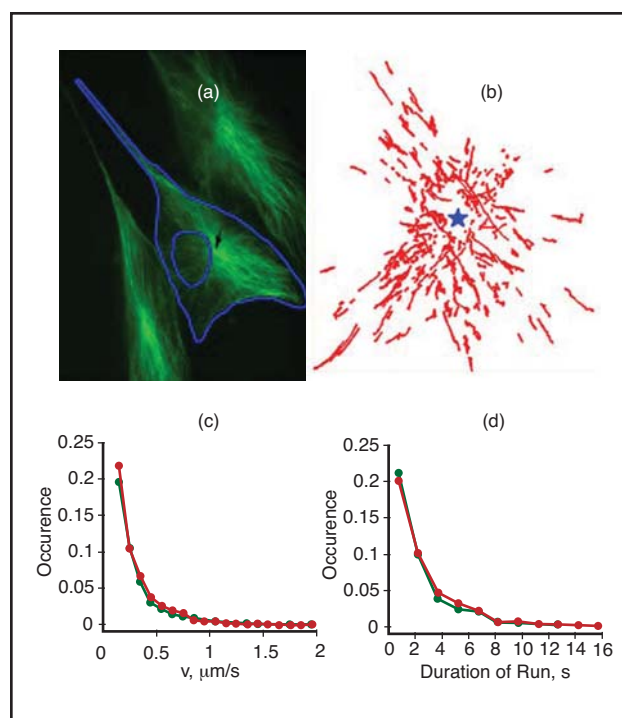
To overcome the limitations imposed by slow diffusion, cells utilize motor-driven transport on cytoskeleton networks. Two major types of cytoskeletal filaments are involved in transport, namely microtubules and actin filaments. Microtubules (MTs) are long (10–100- $\mu\text{m}$ ), polar, filamentous polymers with a plus end and a minus end. In nonpolarized cells, the minus ends of MTs are anchored to the microtubule organizing center (MTOC) located in the vicinity of the nucleus, resulting in a uniform MT array extending toward the cell cortex (Figure 3). Actin filaments (AFs) are typically shorter than microtubules, and their ori-

entation is random throughout the cytoplasm (14). In most cells, microtubules are responsible for long-distance transport while actin filaments support local delivery.

Transport on cytoskeletal filaments is driven by motor proteins, which convert chemical potential into mechanical work with high efficiency; kinesins and dyneins move cargos along microtubules, and myosins move cargos along actin filaments. The activities of motor proteins are regulated by complex signaling networks (15, 16).

**Membrane fusion.** Membrane fusion is crucial for the transfer of proteins, lipids and solutes between different compartments and for exo- and endocytic traffic of signaling molecules and receptors. Many molecular components involved in this reaction have been identified, and new proteins are continually being discovered.

Conceptually, membrane fusion consists of three major



■ Figure 3. Microtubules form an array about the microtubule organizing center (MTOC). In Figure 3a, microtubules in a human skin fibroblast are tagged by a fluorescent antibody, the nucleus and cell membrane are shown in blue, and the black arrow points to the MTOC. The measured trajectories of 350 endosomes are plotted in Figure 3b such that the geometric center of the parent cell's nucleus is shifted to the origin (star), with the motion confined to one-dimensional tracks of the microtubules. The graphs plot the distribution of the frame-to-frame velocities (Figure 3c) and the distribution of the duration of runs of endosomes in the plus (red) and minus (green) directions; the vertical coordinate indicates the frequency of occurrence of an event, *i.e.*, the probability of observing a certain velocity (c) or a certain run-length (d).

steps (17). First, the two partners must traverse the cytoplasm to meet each other. Second, the membranes must recognize and bind to each other, a protein-mediated process known as tethering. The specificity of membrane tethering is crucial to avoid undesired fusion and preserve compartmental identity. Finally, fusion occurs by forming pores connecting two apposed membranes. A pore can be transient and close after a period of time (known as kiss-and-run fusion), or it can grow larger as the two partners merge to form a new entity (direct fusion) (18, 19). As the result, the contents of the two vesicles are exchanged or accumulated in the new vesicle.

## Experimental research in discrete nanoscale transport

Experimental studies of nanoscale transport can be broadly classified into three categories: molecular, microscopic and macroscopic.

In macroscopic experiments, the end points of the transport process are measured, often over a large population of cells (typically more than 10,000) and entities. Macroscopic experiments are often designed to test the involvement of a chosen protein in a certain cellular process, to study the fate of a certain molecule in the biochemical pathways, or to study the properties of a particular type of organelle.

Cell-free methods have also been used to study organelle biology at the population level. Cellular organelles labeled with different tracers can be extracted in an intact form and mixed in a cell-free systems, and then the mixing between the tracers can be assayed. The main advantage of such systems is the ease with which the environment can be controlled — including the concentrations of ions and pH. Cell-free assays have been the workhorse used by several major laboratories to study interactions between organelles along the endocytic and secretory pathways (20, 21).

The population-based methods have been, and remain, the primary mode of probing important intracellular processes. However, their use in studying intracellular transport is limited, primarily because they do not yield quantitative data about the behaviors of nanoscale entities at the individual level.

Recent advances in visualization techniques at the microscopic level have allowed researchers to follow real-time movements of organelles inside living cells with sub-micrometer three-dimensional spatial resolution and with time resolutions as fast as milliseconds. Useful data on transport properties, such as velocities and frequencies of directed transport, have been reported for various systems (22–24). Interestingly, many organelles tend to move bidi-

rectionally on microtubules by employing kinesins and dyneins in rapid succession (25). Most strikingly, it has been recently reported that microtubule-dependent transport of endosomes is not only bidirectional but also symmetric, as movements driven by kinesins and dyneins toward the opposite ends of MTs are approximately identical (in an ensemble average sense), as shown in Figure 3 (22). These random walks of endosomes toward both ends give rise to a form of facilitated diffusion, and lead to the uniform dispersion of endosomes along the radial coordinate of the cell.

The objective of molecular experiments is to identify, to characterize and to manipulate the molecular components (*e.g.*, motor proteins, mediating proteins, signaling molecules, etc.) that govern the processes of interest. Molecular experiments are a powerful way to unravel the underlying mechanisms of cellular processes, and can be integrated with macroscopic and microscopic experiments to reveal a holistic understanding of nanoscale transport.

## Theoretical research in discrete nanoscale transport

Currently, the prevailing modeling paradigm of nanoscale transport is pharmacokinetic models, which approximate trafficking events, including transport-related steps, as kinetic processes characterized by rate constants. This yields ordinary differential equations (ODEs) that describe concentrations of species of interest in different cellular compartments.

Kinetic models have been used successfully to describe intracellular trafficking of several nanoscale entities, including viruses and synthetic vectors. However, many characteristic dynamic events, such as microtubule-dependent transport, are not described adequately when transport processes are imposed on kinetic equations.

Spatial organization within cells is also not properly accounted for in kinetic models. Since the real reaction rates depend on the local concentration of nanoscale entities (rather than the compartmentally averaged concentration as defined in kinetic models), a proper description of spatial organization is necessary to reflect gradients in concentrations brought about by transport and cellular organization.

Therefore, there is interest in developing a new modeling approach that captures the inherent properties of transport dynamics and predicts spatiotemporal behavior, and that can be directly compared to data obtained from microscopic experiments.

Recently, phenomenological models of discrete nanoscale transport have received considerable attention (22, 26–35). At the heart of these models is the multi-state

transport concept, originally developed by Smith and Simmons (30) and Maly (28), which approximated movements of nanoscale entities as stochastic trajectories of discrete particles that intermittently switch between distinct transport modes, *e.g.*, diffusion and directed transport. The rates of switching and the properties of the transport modes are directly estimated from particle-tracking experiments. The multi-state transport theory has been validated on a wide range of systems (22, 34, 35). Most notably, four classes of organelle patterns have been identified — aggregation, hyper-dispersion, radial dispersion, and areal dispersion — and the occurrence of each pattern can be predicted using two Peclet numbers (35).

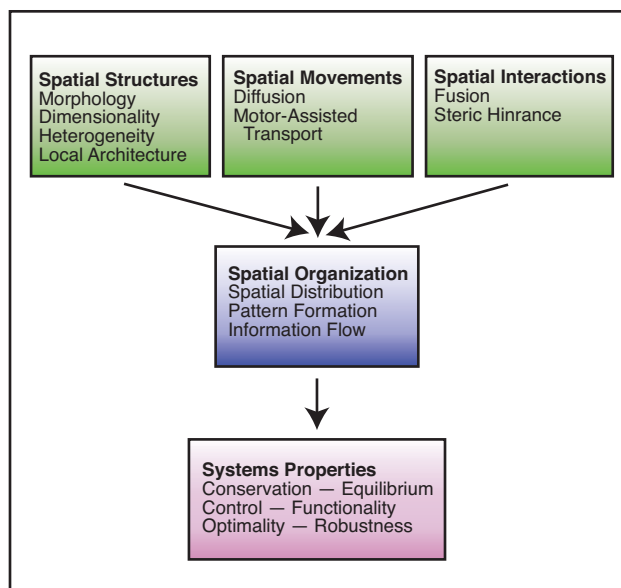
Although it is important to understand the individual transport processes and interactions between organelles, it is equally important to integrate and to study these processes within a systems context and observe how they influence cellular functions as a whole. Recently, several attempts to integrate the multi-state transport theory with mathematical models of other cellular processes (*e.g.*, signaling pathways, gene expression) have led to a better understanding of several phenomena at the cellular level, such as color adaptation, viral infection and synthetic gene delivery. In particular, systems tools (such as optimization and sensitivity analysis (36)) can be applied to provide useful insights into how a certain system configuration is selected by evolution.

### Opportunities for chemical engineers

Research on nanoscale transport has experienced explosive growth in the last two decades. However, the studies are highly fragmented, and a systematic understanding of the subject has not yet emerged. Furthermore, little attention has been paid to the spatial and organizational aspects, both of which hold the key to both diagnosis and control of nanoscale transport.

We believe it is important to develop a spatial systems view of nanoscale transport, as depicted in Figure 4. Such an approach can bring out the causal relationship between the interactions at the nano- and molecular-scales, the spatial organization that emerges from these interactions, and the functionality of the organization.

Chemical engineers are ideally suited to carry out such inquiries. We are not only well-trained in fluid mechanics, mass transfer, reaction kinetics and mathematical analysis, but we are also accustomed to dealing with research topics of a multidisciplinary nature. Furthermore, familiar chemical engineering concepts, such as reaction-diffusion-advection, population balance, optimal control, sensitivity, etc., are directly applicable to many situations. By combining quantitative experiments, theoretical models,



■ Figure 4. Nanoscale transport processes can be viewed from a spatial systems perspective.

and systems analyses, chemical engineers can find a common set of rules, general constitutive laws, and even universal principles of these complex, yet fascinating, transport phenomena.

The study of nanoscale transport also opens up numerous opportunities in nanotechnology. Recently, several groups used microtubules on micro-patterned surfaces to construct nanoscale railroad systems, complete with trains, tracks and stations (37, 38). Molecular shuttles based on principles of motor-assisted transport have the potential to extend the lab-on-a-chip paradigm to nanofluidics by enabling the active, directed and selective transport of molecules and nanoparticles.

A methodology that integrates quantitative fluorescent microscopy, image reconstruction, stochastic simulation, sensitivity analysis, and numerical optimization has been developed to identify and to quantify the rate-limiting steps along the delivery pathway of synthetic gene carriers (36). The simulation framework serves as a three-dimensional dynamic map of the intracellular space. The analysis highlights the effects of several cell-specific properties, such as topology (size, circularity and dimensionality), on the delivery efficiency of synthetic vectors. Based on comparison with gene delivery by viruses, several strategies were proposed to significantly improve transfection efficiencies of synthetic vectors. Ultimately, a better understanding of discrete nanoscale transport in different cell lines could transform how nanoscale drug-delivery systems are designed and optimized.

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