Molecular Communication Among Biological Nanomachines: A Layered Architecture and Research Issues

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***Abstract*—Molecular communication is an emerging communication paradigm for biological nanomachines. It allows biological nanomachines to communicate through exchanging molecules in an aqueous environment and to perform collaborative tasks through integrating functionalities of individual biological nanomachines. This paper develops the layered architecture of molecular communication and describes research issues that molecular communication faces at each layer of the architecture. Specifically, this paper applies a layered architecture approach, traditionally used in communication networks, to molecular communication, decomposes complex molecular communication functionality into a set of manageable layers, identifies basic functionalities of each layer, and develops a descriptive model consisting of key components of the layer for each layer. This paper also discusses open research issues that need to be addressed at each layer. In addition, this paper provides an example design of targeted drug delivery, a nanomedical application, to illustrate how the layered architecture helps design an application of molecular communication. The primary contribution of this paper is to provide an in-depth architectural view of molecular communication. Establishing a layered architecture of molecular communication helps organize various research issues and design concerns into layers that are relatively independent of each other, and thus accelerates research in each layer and facilitates the design and development of applications of molecular communication.**

***Index Terms*—Biological nanomachine, layered network architecture, molecular communication, nanomedical application.**

I. Introduction

IN MOLECULAR communication, biological nanomachines communicate through exchanging molecules in an aqueous environment and collectively perform application oriented tasks [1]–[6]. Biological nanomachines, referred to as bio-nanomachines in this paper, are nano-to-micro scale devices composed of biological materials and capable of simple chemical functions. Examples of bio-nanomachines include nanoscale molecular complexes such as DNA molecules designed to perform logical operations [7], [8] and protein motors reconstructed to transport molecules [9], [10]. Examples of bio-nanomachines also include micro-scale, genetically engineered cells that are capable of sensing conditions in environments [11], [12].

Molecular communication allows a group of bio-nanomachines to communicate and perform tasks that may not be accomplished by individual bio-nanomachines. Molecular communication is expected to play a key role in a variety of application domains such as nanomedical applications [13]. Current research efforts in molecular communication, however, are primarily focused on physical layer issues in communication [14], [15], and only recently has application-oriented research been initiated. For example, molecular communication has recently been applied to the design of targeted drug delivery systems using bio-nanomachines that are capable of identifying target sites [16] or target signals [17] and releasing drug molecules at target sites [18].

The objective of this paper is to provide an in-depth architectural view of molecular communication using a layered architecture approach, traditionally used in communication networks. Similarly to the Open Systems Interconnection reference model [19] and TCP/IP Internet architecture [20], a layered architecture described in this paper organizes various research issues and design concerns in molecular communication into layers that are relatively independent of each other, and thus accelerates research in each layer and facilitates the design and development of applications of molecular communication. Once research issues and design concerns in each layer are satisfactory addressed, one may integrate findings in each layer to create a molecular communication system and also reevaluate the usefulness and validity of the layered architecture. We hope that this paper provides a starting-point for the research community to discuss and ultimately develop the standard layered architecture of molecular communication.

The remainder of this paper is organized as follows. Section II discusses potential applications of molecular communication from interdisciplinary areas of science and technology. Section III defines bio-nanomachines and introduces a layered network architecture of molecular communication. Discussions

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from Sections IV through IX focus on descriptive models for, basic functionalities of, and research issues in molecular communication on a layer-by-layer basis. Section X presents, based on the layered architecture, an illustrative example of designing a nanomedical application of molecular communication. Finally, Section XI provides concluding remarks on future research directions.

II. Applications

Molecular communication may facilitate development of varieties of applications. In this section, we briefly discuss potential application areas of molecular communication and introduce recent relevant work in molecular communication.

- **Micro-electromechanical systems (MEMS)**: The area of MEMS aims at developing small scale systems such as a lab-on-a-chip (LOC) [21], [22]. A LOC, typically a few micrometers to a millimeter in size, provides functionalities to manipulate molecules on a single chip, such as transporting molecules to specific locations, mixing one type of molecules with another type of molecules, and separating specific types of molecules from a mixture of molecules. Applications of molecular communication to LOCs is demonstrated in [23]–[26] where protein motors are integrated into molecular communication and used as carriers to transport molecules to specific locations on a chip. Several design issues in LOCs, such as addressing, switching and medium access control, are also discussed in [27]–[29].
- **Tissue engineering**: The area of tissue engineering aims at developing a tissue structure from biological cells to restore lost tissues of a patient's body [30]. In tissue engineering, stem cells (e.g., autologous cells) are extracted from a patient body, cultured *in vitro*, and returned to the lost tissue part of the patient's body. Molecular communication may provide an additional mechanism to produce spatial patterns of molecules and, thereby, help control the growth and differentiation of the stem cells into specific tissue structures. A mathematical method in [31] encodes desired spatial patterns of molecules into the initial conditions of a reaction-diffusion model in order to produce desired spatial patterns of molecules and cells in the molecular communication environment.
- **Brain machine interfaces (BMI)**: The area of BMI aims at developing a direct communication channel between a human brain and an electrical device to restore lost brain functions [32]. In BMI-based motor prostheses, for instance, motor signals generated in the brain are recorded through electrodes implanted in the brain and transmitted to an external device, which interprets the motor signals to control the artificial limb of the patient. In BMI-based brain disease treatment, signals generated from an external device are transmitted to electrodes implanted in the brain, which in turn stimulate a specific part of the brain to treat a brain disease (e.g., Parkinson's disease). For BMI applications, molecular communication may provide chemical means of interacting with the brain, a much bio-friendlier option than existing electrical means [33], [34].
- **Targeted drug delivery**: The area of targeted drug delivery aims at developing a system that performs therapy on a target site (e.g., disease cells or tumors) in a human body. Targeted drug delivery may be performed by encapsulating drug molecules in drug delivery carriers, delivering the carriers to the target site, and releasing the drug molecules from the carriers at the target site. Targeted drug delivery, therefore, reduces potential side effects of drug molecules on non-targeted sites [35], [36]. Molecular communication may provide alternative techniques to improve the accuracy of targeting and efficacy of therapy through coordination of bio-nanomachines (i.e., drug delivery carriers). For instance, bio-nanomachines may coordinate through molecular communication to identify the target site in the body [16], [37] and to control the rate of drug release in order to achieve sustained drug release [18].
- **Enhanced immune systems**: The area of enhanced immune systems aims at enhancing immune responses of the human body [13]. Artificial immune systems may be introduced into the human body and protect the human body from foreign agents. In enhanced immune systems, molecular communication may allow a group of bio-nanomachines to communicate and coordinate, for instance, to track moving targets (e.g., pathogens) and to notify other bio-nanomachines that may perform therapy, or notify external devices of the location of targets for further analysis or treatment [17].
- **Information technology**. The area of information technology may advance through integrating bio-nanomachines into currently available silicon-based electrical systems using molecular communication. For instance, future mobile phones may be integrated with bio-nanomachines capable of molecular communication for on-chip analysis of biochemical signals (e.g., molecules in blood or from sweat) [38]. A dermal display screen is another example of how molecular communication may help advance information technology. A dermal display screen is envisioned as a system of 3 billion communicating bio-nanomachines embedded below the skin surface on a human body [39]. Massively distributed bio-nanomachines capable of molecular communication may also be integrated into the Internet to form the Internet-of-nano-things [40] and body-area nanonetworks [13].

III. A Layered Architecture of Molecular Communication

In this section, we first discuss bio-nanomachines and the molecular communication environment where bio-nanomachines exist and function. We then discuss the rationale for introducing a layered architecture of molecular communication and provide an overview of the layered architecture. Detailed description of each layer of the architecture is given in subsequent sections.

A. Bio-Nanomachines

In this paper, we define a bio-nanomachine based on three criteria: material, size and functionality [5], [6]. First, a bio-nanomachine is composed of biological materials (e.g., protein, nucleic acid, lipid, biological cell) with or without non-biological materials (e.g., magnetic particles). Second, the

size of a bio-nanomachine ranges from the size of a macromolecule to that of a biological cell. Note that our definition of bio-nanomachines includes biological cells, entities typically much larger than what the term "nano" often refers to (i.e., dimensions of 1–100 nm).[1] Third, a bio-nanomachine implements a set of simple functionalities, including simple life-sustaining functionalities (e.g., acquiring and expending energy), simple actuation functionalities (e.g., moving along a protein filament), simple molecule processing functionalities (e.g., capturing/storing/releasing molecules, detecting molecules and modifying molecules).

With the above definition of bio-nanomachines, examples of bio-nanomachines include:

- A DNA sequence capable of detecting a complementary or partially complementary DNA sequence in the environment and cutting and releasing a segment of the DNA sequence using enzymes [7].
- A protein motor capable of binding to a specific type of molecules, moving along protein filaments carrying the molecules, and unbinding the molecules [10].
- A liposome capable of in-taking and releasing certain types of molecules [42], [43].
- A genetically engineered cell capable of detecting whether a certain type of molecules in its environment is within a specific range of concentration [44].
- A biological cell functionalized with non-biological materials such as photo-sensitive polymers and magnetic particles [45], [46].

B. A Molecular Communication Environment

Bio-nanomachines reside in a molecular communication environment where molecular communication takes place. The molecular communication environment, as described below, serves as a living environment, communication medium and noise source for bio-nanomachines.

1) A Living Environment: The molecular communication environment is typically a nano-to-micro scale and aqueous environment such as the inside of the human body, and it provides energy sources (such as adenosine triphosphate (ATP) molecules) for bio-nanomachines to use and an aqueous environment with suitable biochemical conditions (such as suitable temperature and a pH level) for bio-nanomachines to live in and function.

2) A Communication Medium: The communication medium that the molecular communication environment provides may be the space or fluid in the environment, and molecules may travel passively through the communication medium by stochastic diffusion in the environment [47], [48] or by causing the fluid in the environment (e.g., a blood vessel) to carry the molecules. This mode of molecule propagation is referred to as **passive transport** [Fig. 1(A)]. A typical molecular communication environment is of this type, and it requires little effort to establish this type of communication medium in the environment.

[1]We consider biological cells bio-nanomachines because they are much smaller than typical electronic devices such as sensor devices used in wireless sensor networks [41].

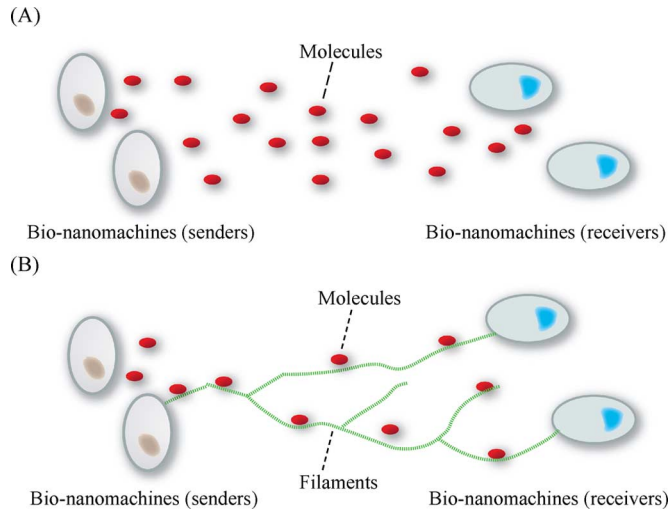

Fig. 1. (A) Passive transport and (B) active transport of molecules in the molecular communication environment.

The communication medium that the molecular communication environment provides may be a network of protein filaments or concentration gradients of certain types of molecules in the environment, and molecules propagate actively in a certain direction by using mobile bio-nanomachines as a carrier of molecules (e.g., by using protein motors carrying molecules over a network of protein filaments [29], [49] or by using self-propelling organisms [50] carrying molecules through the environment). This mode of molecule propagation is referred to as **active transport** [Fig. 1(B)]. To use this type of communication medium, connectivity between bio-nanomachines (e.g., a network of protein filaments and concentration gradients of molecules that self-propelling organisms follow) need to be established in the environment a priori either artificially or through using autonomous behaviors (e.g., dynamic instability of protein filaments [51]).

3) A Noise Source: The molecular communication environment is an aqueous environment with certain biochemical conditions (at some temperature and pH level). It also contains molecules that may naturally exist in the environment (e.g., Ca^{2+} in a biological cell), may be artificially introduced into the environment (e.g., drug molecules in a biological cell in a target drug delivery application), may be created through bio-nanomachines and energy sources decomposing in the environment, or may be created as a result of biochemical reactions among molecules in the environment. As a result, the molecular communication environment creates disturbances (i.e., noise) to bio-nanomachines and molecules that bio-nanomachines use to communicate.

Types of noise found in the molecular communication environment include:

- Biochemical noise: Biochemical conditions of the environment and molecules in the environment may biochemically interfere with bio-nanomachines and molecules that they use for communication.
- Thermal noise: Different temperatures of the environment create different activity levels of thermally activated processes and introduce the stochastic thermal motion of bio-nanomachines and molecules that they use for communication.

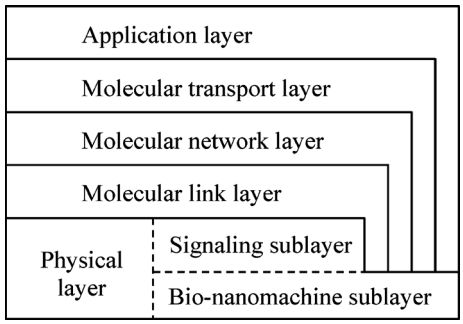

Fig. 2. A layered architecture of molecular communication.

TABLE I
A SET OF FUNCTIONALITIES PROVIDED BY EACH LAYER

Application Layer Bio-nanomachine location control, environmental monitoring, molecule concentration control, structure formation, in-network processing, multiscale messaging
Molecular Transport Layer Molecular transport data unit loss handling, molecular transport data unit flow control, molecular transport data unit congestion control, storing molecular transport data units
Molecular Network Layer Network formation, routing, molecular packet congestion control, storing molecular packets
Molecular Link Layer Framing, addressing, molecular frame transmission/reception, molecular frame loss handling, molecular frame flow control, storing molecular frames
Signaling Sublayer Signal modulation/demodulation, signal molecule transmission /reception, signal propagation/relay/multiplexing, signal molecule error handling, addressing, storing signal molecules, feedback
Bio-nanomachine Sublayer Acquire and expend energy, replicate, terminate functioning and decompose, move, capture/store/release/synthesize molecules, detect/modify molecules, remember/change the state, keep track of time, self-feedback

- Physical noise: The viscosity of the environment and fluid in the environment create physical force and make moving in a specific direction difficult for bio-nanomachines and molecules they use for communication.

C. A Layered Architecture of Molecular Communication

A layered architecture decomposes a large-scale system into a set of smaller units (i.e., layers) that are functionally independent of each other and specifies interactions among layers [19], [20]. It allows system designers to understand the working principles of the system and facilitates the design and development of the system.

Establishing a layered architecture of molecular communication may help design and develop applications of molecular communication. Fig. 2 shows an overview of the layered architecture of molecular communication considered in this paper, and Table I lists a set of functionalities provided by these layers. The main functionalities of these layers are:

- **Physical layer**: It provides functionalities to deal with physical materials. It consists of two sublayers: the **bio-nanomachine sublayer** that abstracts physical details of bio-nanomachines and the **signaling sublayer** that provides functionalities for signaling and modulating information onto molecules.
- **Molecular link layer**: It provides functionalities for communication within a direct range of communication.
- **Molecular network layer**: It provides functionalities for communication over larger distances than at the link layer.
- **Molecular transport layer**: It provides functionalities for end-to-end communication.
- **Application layer**: It provides functionalities that are useful for applications.

At the application layer, the information source (S) and information destination (D) communicate messages:

- **Message** (M): It is a concept, information, communication or statement that is understood by the information source (S) and information destination (D).
- **Information source** (S): It produces a message (M) or sequence of messages to be communicated to the information destination (D). The information source (S) may be a person, a bio-nanomachine or a conventional device at the application layer.
- **Information destination** (D): It is an entity for whom the message (M) is intended. The information destination (D) may be a person, a bio-nanomachine or a conventional device at the application layer.

As in the traditional layered architecture of communication networks, a message (M) from the information source (S) is passed downwards through the layers at the source (S), transferred through the molecular communication environment to the destination (D), passed upwards through the layers, and finally, reach the information destination (D).

For the purpose of this paper, the molecular communication environment and the environment external to the molecular communication environment are not considered as a part of the physical layer. We assume that they are given and that there is little control we can exercise except for manually controlling a limited number of conditions (e.g., manually changing the temperature and pH level of the environment).

In the subsequent sections, we describe a descriptive model for, basic functionalities of and research issues in each layer of the molecular communication layered architecture. Please note the following in the description of the basic functionalities of each layer:

- The functionality description at each layer only includes basic functionalities.
- Each layer does not necessarily implement all the functionalities described.
- Whenever possible, a description of each functionality is followed by observations from biology that may provide insights into developing mechanisms to implement the functionality.

IV. THE BIO-NANOMACHINE SUBLAYER

The bio-nanomachine sublayer of the physical layer abstracts physical details of bio-nanomachines and defines functionalities of bio-nanomachines.

A. A Descriptive Model

Bio-nanomachine i ($i \in [1, N]$) receives an input $I^i(t)$, produces an output $O^i(t+1)$, and changes its current state $S^i(t)$ to the next state $S^i(t+1)$, where N is the total number of bio-nanomachines in the molecular communication environment and t is the time parameter.[2]

1) An Input to a Bio-Nanomachine: An input $I^i(t)$ to a bio-nanomachine i at time t may consist of an input $I^i_E(t)$ from the environment and an input $I^i_j(t)$ from another bio-nanomachine j ($j \in [1, N]$). Namely,

$$I^i(t) = B^i\left(I^i_E(t), I^i_1(t), I^i_2(t), \ldots, I^i_N(t)\right), \tag{1}$$

where $B^i(\cdot)$ represents a function to aggregate all inputs to i at time t.

2) An Output From a Bio-Nanomachine: In response to input $I^i(t)$ at time t, bio-nanomachine i may produce output $O^i(t+1)$ at time $t+1$, where $O^i(t+1)$ may depend on the state $S^i(t)$ of bio-nanomachine i and input $I^i(t)$ at time t. Namely,

$$O^i(t+1) = F^i(S^i(t), I^i(t)), \tag{2}$$

where $F^i(\cdot)$ is a function to produce i's output from i's state and inputs to i at time t.[3] Note that (2) assumes output $O^i(t+1)$ is memoryless, i.e., $O^i(t+1)$ only depends on the values at time t. In reality, $O^i(t+1)$ may depend on the past; for instance, it may depend on the past k states $(S^i(t), S^i(t-1), S^i(t-2), \ldots, S^i(t-(k-1)))$ of bio-nanomachine i. It is straightforward to expand the above expression to include such cases.

Output $O^i(t+1)$ in (2) may be an action $O^i_A(t+1)$ that bio-nanomachine i may take and/or an output $O^i_I(t+1)$ that becomes an input to other bio-nanomachines and to itself (or both). Namely,

$$O^i(t+1) = \left\{O^i_A(t+1),\ O^i_I(t+1)\right\}. \tag{3}$$

3) A State of a Bio-Nanomachine: In response to input $I^i(t)$ at time t, bio-nanomachine i may change its current state $S^i(t)$ to the next state $S^i(t+1)$, where $S^i(t+1)$ may depend on the current state of a bio-nanomachine i ($S^i(t)$). Namely,

$$S^i(t+1) = G^i(S^i(t), I^i(t)), \tag{4}$$

where $G^i(\cdot)$ represents a function to change the i's state based on i's state and inputs to i at time t. Similarly to (2), (4) assumes that the next state $S^i(t+1)$ is memoryless and only depends on the values at time t.

4) Interaction Among Bio-Nanomachines: Interaction between two bio-nanomachines, j and i, is through their output and input. Output $O^j(t)$ of bio-nanomachine j determines input $I^i_j(t)$ to bio-nanomachine i. ($I^i_j(t)$ is referred to as bio-nanomachine j's contribution to the input to bio-nanomachine i). The relationship between $O^j(t)$ and $I^i_j(t)$ depends on a number of factors such as the molecular communication environment and the molecules that are being exchanged between bio-nanomachines j and i. To describe this relation, assume the following.

[2]For the simplicity of explanation, discussion in this section assumes that the time is discrete. It should be straightforward to extend the model to accommodate the continuous time.

[3]For the ease of explanation, (2) assumes that it takes 1 unit of time for a bio-nanomachine to produce an output for a given input and state.

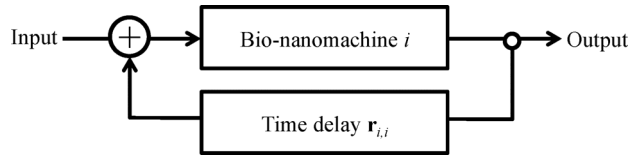

Fig. 3. Self-feedback.

- $H_{j,i}(\cdot)$ denotes the fraction of an output $O^j(t)$ from bio-nanomachine j intended for bio-nanomachine i. For instance, in active-transport, a protein motor may move from j to i on a network of protein filaments, carrying, not all the molecules that j releases, but only a fraction of molecules that j intends for i. In passive-transport, only a fraction of molecules that j releases are intended for i and may propagate towards and reach j. $H_{j,i}(\cdot)$ represents such a fraction.
- $\mathbf{r}_{j,i}$ is a random variable that represents the time delay from bio-nanomachine j to bio-nanomachine i (i.e., the time required for molecules to reach i from j). $\mathbf{r}_{j,i}$ may depend on a number of factors such as the distance and viscosity of the aqueous environment between j and i.
- $D_{j,i}(\cdot)$ denotes the function that describes loss and decay characteristics of molecules while they move from bio-nanomachine j to bio-nanomachine i. In both active-transport and passive-transport, some molecules may be lost in transit in the environment, and not all molecules that are intended for i from j reach their receiver. Characteristics of molecules may also change as they move through the environment, and molecules may lose their original characteristics when they arrive at i. $D_{j,i}(\cdot)$ describes the impact of such loss and decay onto molecules being exchanged between j and i.

With the above notations, the following equation holds to describe the relation between output $O^j(t)$ and input $I^i_j(t)$.

$$\begin{aligned} I^i_j(t) &= D_{j,i}(H_{j,i}(O^j(t-\mathbf{r}_{j,i}))) \\ &= \sum_r D_{j,i}(H_{j,i}(O^j(t-r))) \times \Pr(\mathbf{r}_{j,i} = r), \end{aligned} \tag{5}$$

where the summation is taken over possible values of $\mathbf{r}_{j,i}$. $\Pr(\mathbf{r}_{j,i} = r)$ is the probability that $\mathbf{r}_{j,i}$ is equal to r.

Note that (5) contains a special case where $j = i$, representing a feedback loop where an output of a bio-nanomachine i is fed back to itself with a time delay of $\mathbf{r}_{i,i}$ (Fig. 3).

5) Basic Components of Bio-Nanomachines: Bio-nanomachine i ($i \in [1, N]$) may embody its input $I^i(t)$, output $O^i(t+1)$, and state $S^i(t)$ using the basic components (described below) and the basic functionalities (described in Section IV-B) of the bio-nanomachine.

- **Memory**: It is a physical component that a bio-nanomachine uses to maintain a state or condition. A bio-nanomachine may embody its memory in the form of biochemical conditions (e.g., Ca^{2+} level or pH level) in its internal environment, a three-dimensional structure of molecules in its internal environment, or its own three-dimensional structure. For instance, a bio-nanomachine may be either in the state where its Ca^{2+} level is increasing or decreasing, its pH level is high or low, or its three-dimensional structure is functional or nonfunctional, implementing a 1 bit memory.

Using the Ca^{2+} level, pH level and three-dimensional structure as a memory of a bio-nanomachine may be beneficial for various molecular communication applications, as they are often a trigger of biochemical reactions in a biological cell. For instance, a biological cell may start exocytosis upon detecting an increased level of Ca^{2+}; connexins (i.e., gap junction channel proteins) will close if exposed to acidic conditions or high levels of Ca^{2+}; many proteins must fold into a functional three-dimensional structure, i.e., the most energetically favorable conformation of the protein, to perform various biochemical functions; and DNA must change its shape from tangled/compacted shape (e.g., supercoils/knots) to an untangled shape for DNA replication or transcription to start.

Biochemical conditions change as time progresses. Biological molecules have limited lifetime, and molecules used as a memory may deteriorate during their lifetime. Loss of memory at the bio-nanomachine sublayer occurs when biochemical conditions change or when molecules used as a memory deteriorate and lose their functionality as the memory.

- **Molecule storage**: It is a physical component that a bio-nanomachine uses to store molecules. Molecule storage may simply be the molecular communication environment where the molecules diffuse and wait for the bio-nanomachine to intake them. Molecule storage may be a bio-nanomachine's internal environment where molecules are held. Molecule storage may be a physical component embedded in a bio-nanomachine, for instance, a vesicle or liposome embedded in the bio-nanomachine to store molecules.

  Biological molecules have limited lifetime, and molecules stored in the molecule storage may deteriorate during their lifetime. Loss of molecules occurs when molecules in the molecule storage deteriorate and lose their functionality as the molecules. Loss of molecules also occurs when the molecular storage reaches its limit to store molecules.
- **Process and processor**: A process is a manipulation of molecules by a bio-nanomachine. A bio-nanomachine may process molecules through biochemical reactions and/or through conformation changes of itself, molecules it stores or molecules in the environment. For instance, a set of instructions "*Find if the pH level of the environment is within a certain range. If it is, create molecules of type C. Memorize that the pH level is within the given range*." may be embodied through designing a biochemical reaction that produces molecules of type C from type A and type B molecules in the environment and enzymes that become active within the given range of the pH level to help accelerate the biochemical reaction to produce type C molecules. The pH level may be memorized through designing proteins which take a biochemically functional three dimensional conformation within a given pH level range and do not fold into their functional conformations otherwise.

  A processor is a physical component that a bio-nanomachine uses to process molecules. In the above example of a process, a processor refers to a set of "molecules of type A and type B, enzymes, and proteins." Note that, in the example above, a set of instructions and what constitutes a processor are tightly coupled. Namely, when a set of instructions change, what constitutes a processor to process the instructions may change. This brings undesired dependency between a set of instructions and a processor to process it.
- **Actuator**: It is a physical component that a bio-nanomachine uses to move spontaneously and actively, consuming energy in the process. A bio-nanomachine may embody its actuator, for instance, in the form of flagellum (a whip like structure made of protein flagellin) and a rotary motor to drive a flagellum, similarly to bacteria. A bio-nanomachine may embody its actuator, for instance, in the form of leg-like motor proteins found in molecular motors (e.g., kinesins), using energy released from the ATP hydrolysis.
- **Identifier**: It identifies a bio-nanomachine or a group of bio-nanomachines. An identifier may be a unique identifier given to a bio-nanomachine or a group of bio-nanomachines or a geographic location of a bio-nanomachine or a group of bio-nanomachines.
  - **Physical identifier**: A physical identifier is an identifier physically attached to a bio-nanomachine (or a group of bio-nanomachines) and uniquely identifies a bio-nanomachine (i.e., an individual identifier) or a group of bio-nanomachines (i.e., a group identifier) that share same characteristic or characteristics (e.g., bio-nanomachines that provide same functionality such as carrying the same type of drug molecules or sensing the same type of molecules in the environment). A physical identifier may be a specific type of molecules attached to a bio-nanomachine.
  - **Location identifier**: A location identifier uniquely identifies a location (i.e., an individual identifier) or multiple locations (i.e., a group identifier) in the molecular communication environment. A location identifier may be implemented through creating an addressable space in the molecular communication environment based on concentration gradients of "guide" molecules [52]; a location in the space is distinguished (and thus identified) by a concentration degree of guide molecules. Similarly to the physical identifier, a location identifier may also be a specific type of molecules that are attached to a location or a group of locations.

Note that not all bio-nanomachines have all of the above components.

B. Functionalities

A bio-nanomachine implements a set of simple functionalities including life-sustaining, actuation, and/or molecule processing functionalities.

1) Life-Sustaining Functionalities:

- **Acquire and expend energy**: Bio-nanomachines acquire and expend energy to perform their functionalities. For instance, protein motors consume chemical energy released through adenosine triphosphate (ATP) hydrolysis to move along filamentous proteins.

- **Replicate**: Bio-nanomachines make a copy of themselves and produce bio-nanomachines capable of the same or similar functionality. One example is a biological cell that divides into two daughter cells. Another example is a self-replicating RNA sequence that encodes a specific type of enzyme which duplicates the RNA sequence.
- **Terminate functioning and decompose**: Bio-nanomachines have limited lifetime, and their functionalities may deteriorate during the lifetime. Bio-nanomachines that are no longer functional remove themselves from the environment. For instance, biological cells may decompose themselves through cell apoptosis.

2) Actuation Functionality:

- **Move**: Bio-nanomachines move through the environment. Bio-nanomachines may move passively, for instance, by stochastic diffusion in the environment or by following the fluids in the environment (e.g., in the circulatory system). Bio-nanomachines may also move actively in a certain direction. For instance, protein motors directionally move along filamentous proteins to transport molecules.

3) Molecule Processing Functionalities:

- **Capture molecules**: Bio-nanomachines capture molecules (i.e., to receive input $I^i(t)$) either directly from another bio-nanomachine j ($I^i_j(t), j \in [1, N]$) or from the environment ($I^i_E(t)$). Bio-nanomachines may capture molecules directly from other bio-nanomachines, for instance, through a direct connection with another bio-nanomachine (similarly to gap junction channels directly connecting the cytoplasm of two cells and allowing various molecules to pass between cells [53]) or through merging with another bio-nanomachine (similarly to vesicles fusing with other organelles within the cell [54], [55]). Bio-nanomachines may capture molecules from the environment, for instance, through binding to molecules of a specific structure (similarly to receptor proteins in a biological cell binding to ligands of a particular structure [56]) or through engulfing and absorbing molecules (similarly to biological cells uptaking molecules outside the cells into their cytosols through endocytosis [57]).
- **Store molecules**: Bio-nanomachines store molecules that they capture or produce in molecule storage. For instance, bio-nanomachines may be embedded with vesicles or liposomes and store molecules in vesicle or liposomes. Bio-nanomachines may use their internal environment or the molecular communication environment to store molecules.
- **Release molecules**: Bio-nanomachines release molecules (i.e., to produce output $O^i(t+1)$) into the environment or directly into other bio-nanomachines. Bio-nanomachines may release molecules into the environment, for instance, through their surface (similarly to biological cells directing the contents of vesicles out of the cell membrane and into the extracellular space through exocytosis [58]), or directly into other bio-nanomachines, for instance, through opening a direct connection with another bio-nanomachine (similarly to opening gap junction channels connecting two cells) or through merging with another bio-nanomachine (similarly to vesicle fusion).
- **Synthesize molecules**: Bio-nanomachines synthesize molecules using molecules that exist in their internal and external environments through biochemical reactions. New types of molecules may be synthesized, for instance, as signal molecules, i.e., molecules onto which information is encoded, at the signaling sublayer.
- **Detect molecules (and biochemical conditions)**: Bio-nanomachines detect the presence of a specific type of molecules in the environment. For instance, bio-nanomachines may detect the presence of a specific type of molecules through using the receptors that bind to the molecules with high specificity. Bio-nanomachines may extend this functionality to detect the biochemical conditions (e.g., the number of specific molecules or the concentration of specific molecules) in the environment, for instance, through using multiple receptors and signal transduction networks behind the receptors (similarly to bacteria detecting the concentrations of attractant and repellent molecules in the environment through using multiple transmembrane receptors and signal transduction networks [59]).
- **Modify molecules (and biochemical conditions)**: Bio-nanomachines modify their own characteristics or characteristics of molecules (such as three dimensional structure or conformation) in their internal and external environments. For instance, DNA molecules have different conformations (e.g., twist and writhe), and conformations may be modified by using topoisomerases (enzymes). Many proteins have multiple energetically stable structures, and their structures may be modified through using interactions among their amino acids. Membrane receptors have different conformations, and they may modify their structures through binding to certain molecules (e.g., IP3 receptors modify their conformations to open when they bind to IP3 enzymes). Bio-nanomachines may extend this functionality to modify the biochemical conditions (e.g., the number of specific molecules or the concentration of specific molecules) in the environment. For instance, bio-nanomachines (biological cells) may increase the Ca^{2+} level when IP_3 receptors acting as a Ca^{2+} channel bind to inositol trisphosphate (IP_3) enzyme and modify their conformation to open and release Ca^{2+} from the endoplasmic reticulum (ER).

  Modifying the biochemical conditions such as the Ca^{2+} level may be used to trigger some bio-nanomachine functionalities. For instance, if a biological cell is used as a molecule storage, it may start exocytosis and release internal molecules upon detecting an increased level of Ca^{2+}; an increased level of Ca^{2+}, thus, triggers "release molecules."
- **Remember the state**: Bio-nanomachines remember the state that they are in. For instance, bio-nanomachines may embody a memory in the form of biochemical conditions (e.g., Ca^{2+} level or pH level) of their internal and external environments or in the form of a three-dimensional structure of themselves or of a molecule (molecules) in their internal and external environments. Bio-nanomachines may also embody a memory in the form of the number of molecules stored in their internal environment.
- **Change the state**: Bio-nanomachines change between simple states. For instance, a bio-nanomachine, either in the state where its pH level is high or low, may change

its state by decreasing or increasing the pH level. A bio-nanomachine, either in the state where its three-dimensional structure is functional or nonfunctional, may change its state through conformational changes. A bio-nanomachine may change its state through modifying the number of molecules that it stores internally, if the number of molecules represents its state.

- **Keep track of time**: Bio-nanomachines may biochemically implement a clock. For instance, bio-nanomachines may device their own clock through implementing a transcription/translation feedback loop [60], [61]. Bio-nanomachines may also use their naturally-occurring biological clock such as a circadian clock.
- **Self-feedback**: A bio-nanomachine regulates itself through feeding back its own output $(O^i(t - \mathbf{r}_{i,i}))$ as an input $(I_i^i(t))$ as shown in Fig. 3. Bio-nanomachines may use self-feedback to implement oscillatory chemical clocks to keep track of time [62].

It is important to note that all the functionalities suggested above rely on underlying biochemical reactions.

C. Research Issues

1) How Do We Learn From Biology?: In the previous subsection (Section IV-B), we discussed whenever possible how bio-nanomachine functionalities can be implemented, based on observations of biological systems. The observations are made, however, in a random manner from different disciplines in biology (e.g., cell biology, genetics, and biochemistry). It is important to develop a systematic approach to learn from biology and to apply insights learned from biology in a coherent manner to designing bio-nanomachines.

2) How Do We Design Bio-Nanomachines, Taking an Advantage of Unique Features of Biomaterials?: A design of bio-nanomachines may exploit unique features that biomaterials and biological entities inherently have. One unique feature of biomaterials and biological entities is that they respond to unknown inputs (i.e., inputs that they have not seen before). For instance, existing anti-virus agents in a human immune system respond to unknown virus and mutate themselves. Another unique feature is that they exhibit autonomous behaviors. For instance, within a biological cell, protein filaments exhibit dynamic instability, an autonomous behavior. They attach to each other, detach from each other, and stochastically grow and stabilize to create a network of protein filaments [51]. Yet another unique feature is that they adapt to new and changing conditions within a generation (adaptation) and through generations (evolution). For instance, biological cells often adapt and become less sensitive to repeated stimuli. Bacteria evolve through multiple generations; i.e., miscopying of genetic materials creates diversity in bacteria, and bacteria evolve through natural selection. It is important to incorporate unique features of biomaterials and biological entities in bio-nanomachine designs.

3) How Do We Design Bio-Nanomachines With Clear Separation of Specificity and Generality?: Bio-nanomachine implements input (I), output (O) and state (S) using their functionalities. Ideally, a design of a bio-nanomachine should clearly separate "a part that is independent of specific forms of I, O and S" and "a part that is dependent on I, O and S" with an interface to allow these two parts to work together. This allows reusing bio-nanomachines for different applications that require different forms of I, O and S. For instance, a genetically modified cell (i.e., a bio-nanomachine) may contain "a vesicle that can store different types of molecules"; this container of molecules is independent of specific forms of I, O and S and compartmentalizes application specific molecules. It is important to design bio-nanomachines with clear separation of specificity and generality.

4) How Do We Control Bio-Nanomachines?: Bio-nanomachines need to perform their functionalities in a controlled manner. Bio-nanomachine functionalities should be triggered and stopped at an appropriate time. It is also necessary to set control parameters of bio-nanomachine functionalities such as the direction of bio-nanomachine movement (in "move"), the type of molecules to capture ("capture molecules"), the number of molecules to release ("release molecules"), and if proteins should be folded or not ("modify molecules").

For instance, in "move," bio-nanomachines (e.g., protein motors, self-propelling organisms) may move actively in a certain direction. Bio-nanomachines such as protein motors may be triggered to move or to stop, for instance, by controlling the environmental conditions (e.g., the amount of energy source (ATP molecules) in the environment, temperature and pH level of the environment). Direction of bio-nanomachine movement may be controlled, for instance, by controlling the placement of protein filaments, and by creating a concentration gradient of molecules in the environment. Such control may be applied externally, or bio-nanomachines may be designed to autonomously exercise such control.

It is important to design mechanisms that control bio-nanomachine functionalities. External control of bio-nanomachine functionalities may be necessary in the initial phase of research to gain insights as to how we may design bio-nanomachines that autonomously perform functionalities in a controlled manner [45].

5) How Do We Design Bio-Nanomachines That Remember?: As discussed in (2) and (4), output $O^i(t+1)$ and the next state $S^i(t+1)$ of bio-nanomachine i may depend on the past, for instance, the past k states $(S^i(t), S^i(t-1), S^i(t-2), \ldots, S^i(t-(k-1)))$ of bio-nanomachine i. This requires bio-nanomachines to remember multitude of their past states. Some features of biomaterials and biological molecules may be useful in implementing a multi-bit memory in a bio-nanomachine. For instance, many biological molecules (such as proteins) have multiple kinetically stable three-dimensional structures and undergo reversible structural changes and fold into one or more specific spatial conformations in order to perform their biological function. Bio-nanomachines may use such multitude conformational changes of biological molecules as a multi-bit memory and associate each conformation with a specific piece of information. It is important to design memory capability of bio-nanomachines.

6) How Do We Design Bio-Nanomachines That Process Molecules?: As discussed earlier, bio-nanomachines process molecules through biochemical reactions and conformation changes of molecules, i.e., through creating changes in physical characteristics of molecules. In designing bio-nanomachines, one may carefully examine what physical characteristics

should be modified and what type of molecule processing a given physical characteristic change represents. It is also important to avoid tightly coupling a given physical characteristic change to a specific type of molecule processing so that one physical characteristic change can represent a wide range of same/similar types of molecule processing.

7) How Do We Design Bio-Nanomachines That Are Robust in Highly Dynamic and Stochastic Molecular Communication?: Biomaterials that bio-nanomachines are made of deteriorate, and bio-nanomachines also suffer from stochastic fluctuations due to their small sizes. Thus, the same bio-nanomachine may not exhibit the same behavior (i.e., may not produce the same output (O) and same state change) even when environmental conditions and its input (I) and state (S) are the same. For instance, protein motors moving on a network of protein filaments may take different paths at intersections of multiple filaments and arrive at different destinations even when the environmental conditions are the same [63], [64]. Genetically identical cells often have different gene expression levels due to the internal noise (such as Brownian motion of molecules inside a cell) [65]. Responses of neurons to external stimuli are probabilistic and often characterized using a distribution [66].

Using the notations introduced in this section, the dynamic and stochastic nature of bio-nanomachines is described as follows.

- Bio-nanomachine population (N) in molecular communication is highly dynamic and constantly changes.
- Physical location of a bio-nanomachine stochastically fluctuates, and thus, $\mathbf{r}_{i,j}$ (distance measured in time required for molecules to travel from bio-nanomachine j to bio-nanomachine i) also exhibits stochastic fluctuations.
- Physical behavior and movement of bio-nanomachines stochastically fluctuate, and thus, $D_{j,i}(\,\cdot\,)$ (decay characteristics of molecules while they move from bio-nanomachine j to bio-nanomachine i) stochastically fluctuates, and do other system factors (such as input $I^i(t)$ and output $O^i(t+1)$) that depend on $D_{j,i}(\,\cdot\,)$.
- An action $O^i_A(t+1)$ that bio-nanomachine i takes in response to the input $I^i(t)$ is subject to stochastic fluctuations.
- Outputs and state changes of a bio-nanomachine are highly dynamic. Functions F^i (to determine the output of bio-nanomachine i, (2)) and G^i (to determine the next state of bio-nanomachine i, (4)) depend on the age of bio-nanomachine i. In addition, functions F^i and G^i depend on how biochemically active bio-nanomachine i is (i.e., the amount of energy in the molecular communication environment and the conditions of the molecular communication environment such as its temperature (i.e., $I^i_E(t)$)).

It is important to design bio-nanomachines that are robust in highly dynamic and stochastic molecular communication.

8) How Do We Design Energy Efficient Bio-Nanomachines?: Bio-nanomachines need to be energy efficient, and an overall molecular communication design needs to secure energy sources for bio-nanomachines. Energy sources impact the lifetime of bio-nanomachines and the speed of deterioration of bio-nanomachines during their lifetime. One approach for securing energy sources is to exploit the molecular communication environment where bio-nanomachines are deployed. For instance, in targeted drug delivery, once bio-nanomachines are injected into a human body, they may use molecules abundant in the environment, such as ATP, as an energy source. Another approach is to have an energy source outside the molecular communication environment. For instance, external devices may be placed outside the molecular communication environment and generate light or heat to provide energy to bio-nanomachines (e.g., photo-activatable bio-nanomachines). It is important to design energy efficient bio-nanomachines and secure their energy sources.

9) How Do We Deploy Bio-Nanomachines and Remove "No Longer Functioning" Bio-Nanomachines?: It is important to develop methods for deploying bio-nanomachines in the molecular communication environment. Bio-nanomachines may be deployed randomly and move to a target site through the molecular communication environment; bio-nanomachines may be deployed at a specific site (e.g., a disease site in a human body), requiring less control once they are deployed. It is also important to develop methods for removing no-longer functioning bio-nanomachines and energy sources from the molecular communication environment. Bio-nanomachines may be removed, for instance, using "terminate functioning and decompose." For biological cell-based bio-nanomachines, programmed cell death, an inherent mechanisms of biological cells, may be induced so that components within the dying bio-nanomachines are decomposed, disposed of naturally into the environment (e.g., human body), and recycled in the environment.

10) What Are Suitable Biomaterials for Engineering Bio-Nanomachines?: Functionalities of bio-nanomachines are single-task oriented as described in Section IV-B and referred to as simple in this paper. It is very complex, however, to actually implement bio-nanomachines with required functionalities and input (I), output (O) and state (S). To implement bio-nanomachines, materials may be chosen based on their programmability. DNA molecules are highly programmable in creating a structure from DNA molecules [67], since their binding processes (i.e., DNA-base paring rules) are well-understood, and it is relatively easy to predict the structure to be made. Biological cells are less programmable with the current technology as their internal chemical processes are complex and significantly impacted by environmental noise.

Materials for engineering bio-nanomachines may also be chosen based on their biocompatibility with and biodegradability in the environment assumed in applications. For instance, targeted drug delivery may use naturally occurring biological cells such as red blood cells as bio-nanomachines to carry drug molecules [36]. Red blood cells are biocompatible and can be injected into the bloodstream. They remain in a blood stream for a relatively long period of time (e.g., 120 days), and are later removed from the blood stream by the reticuloendothelial system, making them suitable materials for bio-nanomachines that operate in the circulatory system. It is important to choose biomaterials to engineer bio-nanomachines based on their programmability and biocompatibility.

11) What Are the Methods of Engineering Bio-Nanomachines With Biomaterials Chosen?: Methods of engineering bio-nanomachines include lithography based top-down approaches, which may apply to engineering bio-nanomachines

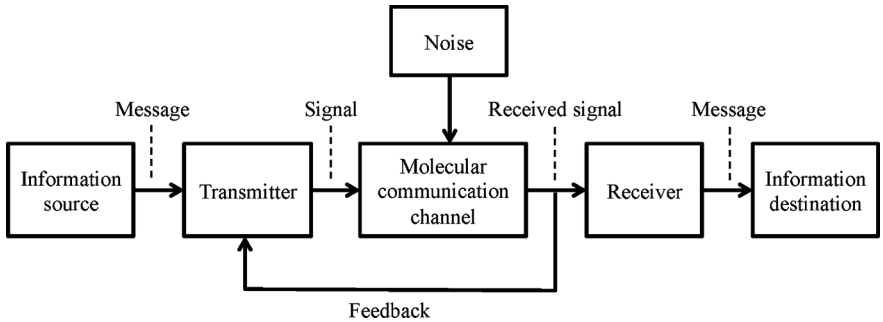

Fig. 4. Signaling sublayer in molecular communication based on Shannon model of communication.

of relatively larger (micro-scale) size. The self-assembly based bottom-up approaches may be more promising to engineer smaller-scale bio-nanomachines (e.g., in the nanometer range) [68]. The top-down and bottom-up combined approaches may also apply; for instance, chemical substances are patterned onto a surface using lithography, and then biological materials are placed on the surface to self-assemble into a specific structure based on interactions between the chemical substances and biological materials. It is important to develop methods of engineering bio-nanomachines for the biomaterials chosen.

V. The Signaling Sublayer

The signaling sublayer of the physical layer provides functionalities for a bio-nanomachine to communicate with other bio-nanomachines. At the signaling sublayer, signaling is done using biomaterials.

A. A Descriptive Model

Following Shannon model of communication [69], basic components of the signaling sublayer are the following (Fig. 4).

- **Message** (M): It is a concept, information, communication or statement that is understood by the information source (S) and information destination (D).
- **Information source** (S): It produces a message (M) or sequence of messages to be communicated to the information destination (D). As defined earlier, the information source (S) may be a person, a bio-nanomachine or a conventional device at the application layer. At the signaling sublayer, the information source (S) is the higher layer (i.e., the link layer) that passes the message (M) to the signaling sublayer.
- **Transmitter** (T): It operates on the message (M) to produce a signal (i.e., information-encoded molecules, referred to as **signal molecules** in the rest of this paper) suitable for transmission over the molecular communication channel (C). Transmitter (T) may be a bio-nanomachine (in the molecular communication environment) or a conventional device (in the external environment).
- **Molecular communication channel** (C): It is the medium used to transmit the signal (e.g., signal molecules) between directly communicating transmitter (T) and receiver (R). A molecular communication channel (C) may be implemented such that it inherently has broadcast nature (**a broadcast channel**). For instance, it may be the space in the environment where signal molecules diffuse and propagate stochastically from a transmitter (T) to a receiver (R) (or receivers). A molecular communication channel (C) may also be implemented such that it inherently has a point-to-point nature (**a point-to-point channel**). For instance, it may be a protein filament along which protein motors carry signal molecules from a transmitter (T) to a receiver (R).
- **Receiver** (R): It performs the inverse operation of that done by the transmitter (T) and retrieves the message (M) from the signal (e.g., signal molecules). Receiver (R) may be a bio-nanomachine (in the molecular communication environment) or a conventional device (in the external environment).
- **Information destination** (D): It is the entity for whom the message (M) is intended. As defined earlier, the information destination (D) may be a person, a conventional device, or a bio-nanomachine at the application layer. At the signaling sublayer, the information destination (D) is the higher layer (i.e., the link layer) to which the signaling sublayer passes the message (M).
- **Signal molecule storage**: It is a physical component that stores signal molecules. Similarly to the bio-nanomachine sublayer, signal molecule storage may simply be the molecular communication environment where the signal molecules diffuse and wait for the bio-nanomachine to intake them, may be a bio-nanomachine's internal environment where molecules are held, or may be a physical entity embedded in a bio-nanomachine, for instance, a vesicle or liposome embedded in the bio-nanomachine to store molecules.

  Loss of signal molecules occurs when signal molecules in the signal molecule storage deteriorate and lose their functionality as the signal molecule. Loss of signal molecules also occurs when the signal molecular storage reaches its limit to store signal molecules.
- **Noise source**: It produces an error or undesired random disturbance of a useful signal (e.g., signal molecules) in a molecular communication channel (C). At the signaling sublayer, noise is caused by the noise sources in the molecular communication environment (environmental noise) and by the signal from other communications (cross talk). Environmental noise sources include sources that cause biochemical noise, thermal noise and physical noise, as discussed earlier in Section III.

  Cross talk is caused by the signal (e.g., signal molecules) transmitted on another communication channel. Cross talk may occur, for instance, when a transmitter (T) and

a receiver (R) communicate through diffusing signal molecules in the environment. Signal molecules from a transmitter (T) diffuse in the environment, drift towards the environment where another transmitter/receiver pair is communicating and start interfering with the communication. Cross talk may also occur, for instance, when a transmitter (T) and a receiver (R) communicate using protein motors carrying signal molecules and moving along a protein filament connecting the transmitter (T) and the receiver (R). Protein motors carrying signal molecules may detach from a protein filament, diffuse in the environment, and attach to a protein filament used for communication between another transmitter/receiver pair.

B. Functionalities

- **Signal modulation** is a functionality at the transmitter (T) to vary properties of signal (e.g., signal molecules) to represent a message (M) to transmit. One mechanism for signal modulation is to choose one type of molecules out of a set of distinguishable types of molecules, each molecule type representing certain information (i.e., Molecule Shift Keying or MoSK) [70]–[73]. Another mechanism is to use one type of (indistinguishable) molecules and to modify patterns of releasing the molecules, such as changing the number of molecules to transmit (i.e., Amplitude Shift Keying or ASK) [74]–[76] and changing the time interval to transmit the molecules (i.e., Frequency Shift Keying or FSK) [77].
  Signal modulation may be supported by functionalities at the bio-nanomachine sublayer such as "release molecules" in a controlled manner, "synthesize molecules" and "modify molecules." The signaling sublayer passes information on the type of molecules to release and the patterns of releasing the molecules to the bio-nanomachine sublayer.
- **Signal demodulation** is a functionality at the receiver (R) to perform the inverse operation of signal modulation.
  Signal demodulation may be supported by molecule processing functionalities at the bio-nanomachine sublayer. As discussed earlier, through "detect molecules," bio-nanomachines may detect presence of a specific type of molecules in the environment (if the signal modulation mechanism is based on the type of molecules) and also detect concentration of molecules in the environment (if the signal modulation mechanism is based on changing the number of molecules to transmit). The bio-nanomachine sublayer passes information on the type of molecules captured and the patterns of the captured molecules to the signaling sublayer.
- **Signal molecule transmission/reception** is a functionality at the transmitter (T) to transmit signal molecules onto the molecular communication channel (C) (through "signal transmission") and at the receiver (R) to receive incoming signal molecules (through "signal reception").
  Signal transmission/reception may be supported by functionalities at the bio-nanomachine sublayer such as "release molecules" in a controlled manner and "capture molecules." When transmitting signal molecules, the signaling sublayer passes information on the type of molecules to use as signaling molecules and timing of molecule release to the bio-nanomachine sublayer. When receiving signal molecules, the bio-nanomachine sublayer passes information on the type of received signaling molecules and timing of molecule reception to the signaling sublayer.
- **Signal propagation** is a functionality in which signals travel over space in the environment [i.e., a molecular communication channel (C)]. As discussed in Section III, one mechanism for signal propagation is through passive transport. Signal molecules may diffuse and propagate stochastically in the environment. Signal molecules may be carried and transported by the fluid in the environment (e.g., a blood vessel). Another mechanism for signal propagation is through active transport. Signal molecules may propagate in a certain direction by using mobile bio-nanomachines as a carrier of signal molecules, for instance, by using protein motors or self-propelling organisms carrying signal molecules over a molecular communication channel (C).
  Signal propagation may be supported by the communication medium that molecular communication environment provides to propagate molecules (see Section III-B). The mode of signal propagation on the communication medium may be either active or passive. A molecular communication channel (C) may be either of the broadcast type or the point-to-point type Although four combinations of the type of the molecular communication channel and the mode of propagation are theoretically possible, passive propagation over a point-to-point channel may not be feasible, as propagating directionally over a point-to-point channel implies use of energy, i.e., active propagation. Passive signal propagation over a broadcast channel simply takes an advantage of the space or fluid in the environment, and it requires no or little effort to implement this mode of signal propagation over a broadcast channel. Active signal propagation (either over a broadcast channel or over a point-to-point channel) requires establishing connectivity between bio-nanomachines such as a concentration gradient that self-propelling organisms follow and a network of protein filaments that protein motors to move along. Such connectivity needs to be established a priori either artificially or through using autonomous behaviors (e.g., dynamic instability of protein filaments [51]).
  The signaling sublayer may not pass the information regarding which mode of propagation to use to the bio-nanomachine sublayer. This is because we envision that molecular communication is a priori designed to use either passive or active mode of propagation, rather than providing them as options to choose from on the fly on a single molecular communication system.
- **Signal relay** is a functionality to propagate and amplify signals (e.g., signal molecules) for long distance signal propagation [78]–[80]. One mechanism for signal relay is to introduce in the environment relay or repeater bio-nanomachines, which amplify the signal, for example, by increasing the concentration of signal molecules.
  At a signal relay bio-nanomachine, signal relay may be supported by bio-nanomachine sublayer functionalities such as "capture molecules" (to capture incoming

signal molecules), "synthesize molecules" and "release molecules" (to create new signal molecules and to release newly created signal molecules into the environment), and "release molecules" (to release signal molecules stored in an internal molecule storage into the environment). At a relay bio-nanomachine, the signaling sublayer passes information regarding the degree of amplification to the bio-nanomachine sublayer.

- **Signal multiplexing** is a functionality to aggregate multiple signals and transmit them through a shared molecular communication channel (C). One mechanism for signal multiplexing is to use time division multiplexing (TDM) in which different bio-nanomachines (or different groups of bio-nanomachines) transmit signal molecules at different times [34], [81].

  TDM-based signal multiplexing may be supported by bio-nanomachine functionalities of "keep track of time" and "release molecules" in a controlled manner. The signaling sublayer passes information regarding when to release molecules to the bio-nanomachine sublayer.
- **Signal molecule error handling** is a functionality to detect and possibly correct errors due to noise at the signaling sublayer. One mechanism for the error handling (error correction) is to embed channel codes (e.g., Hamming codes) within a pattern of transmitting molecules [82]–[84]. For example, a logical bit sequence representing a message (M) to transmit is added with redundant bits for error detection and correction, and then transmitted to the receiver (R) through signal propagation.

  Signal molecule error handling may be supported by functionalities at the bio-nanomachine sublayer such as "synthesize molecules" and "release molecules" in a controlled manner. The signaling sublayer passes information on the type of molecules to release and the patterns of releasing the molecules to the bio-nanomachine sublayer.
- **Addressing** at the signaling sublayer is a functionality to specify a receiver (or a group of receivers) that receives signal molecules or a location (or multiple locations) to which molecular frames are delivered. Addressing at the signaling sublayer may support a physical address (i.e., an address that identifies a receiver or a group of receivers) and a location address (i.e., an address that identifies a location or multiple locations). It may also support an individual address (i.e., an address that identifies a single receiver or a single location) and a group address (i.e., an address that identifies a group of receivers or multiple locations).
  - **Physical address**: A physical address uniquely identifies a receiver (i.e., an individual address) or a group of receivers that share same characteristic or characteristics (e.g., receivers that provide same functionality such as carrying the same type of drug molecules or sensing the same type of molecules in the environment) (i.e., a group address). A physical address at the signaling sublayer may be supported by a bio-nanomachine physical identifier and functionalities at the bio-nanomachine sublayer such as "capture molecules" and "detect molecules." For instance, a physical address at the signaling sublayer may be implemented using molecules that bind to molecules that are used as a physical identifier of a bio-nanomachine or a group of bio-nanomachines at the bio-nanomachine sublayer. Such molecules may be attached to signal molecules, and when signal molecules are delivered to their intended bio-nanomachine(s), molecules used as a physical address (attached to signal molecules) and molecules used as an identifier (attached to a bio-nanomachine or a group of bio-nanomachines) bind to each other. A pair of complementary DNA sequences (DNA-based address) [24] or a receptor and ligand pair (receptor-ligand based address) [74] may be used for a physical address.
  - **Location address**: A location address uniquely identifies a location (i.e., an individual address) or multiple locations (i.e., a group address) in the molecular communication environment. Similarly to the physical address, a location address at the signaling sublayer may be supported by a location identifier and functionalities at the bio-nanomachine sublayer such as "capture molecules" and "detect molecules." For instance, if the bio-nanomachine sublayer implements a location identifier through creating an addressable space based on concentration gradients of guide molecules, molecules are delivered to a location using bio-nanomachines (e.g., self-propelling organisms) capable of sensing concentrations of guide molecules. Similarly to the physical address, if the bio-nanomachine sublayer implements a location identifier using molecules, molecules that bind to the location identifier molecules at the bio-nanomachine sublayer may be used as a location address at the signaling sublayer.

  The signaling sublayer passes information on the address, what type of address it uses (physical address, location address, individual address, group address) and group membership (if it is a group address) to the bio-nanomachine sublayer.
- **Storing signal molecules**: Transmitter (T) and receiver (R) may store signal molecules in their respective signal molecule storage.
- **Feedback** is a process in which information about the past or the present influences the same phenomenon in the present or future. Feedback may be self-feedback (where an output from the signaling sublayer is given back to the signaling sublayer as an input) or between the transmitter (T) and the receiver (R).

  Self-feedback may be supported by "self-feedback" at the bio-nanomachine sublayer. The signaling sublayer does not need to pass information to the bio-nanomachine sublayer. It may simply invoke the "self-feedback" at the bio-nanomachine sublayer.

Example (Drug Delivery Application): Using the descriptive model and functionalities introduced in this section, a drug delivery application may be described as follows.

Assume that bio-nanomachines carrying anti-cancer drug molecules (signal molecules) are placed near or on cancer sites. Each of these bio-nanomachines acts as a transmitter (T). A medical doctor [information source (S)] sends a message

(M) to cancer cells [information destinations (Ds)]. The message (M) is "stop growing," and it is in the form of thermal signals or optical signals. The medical doctor [information source (S)] uses a conventional device that generates thermal or optical signals and sends the message (M) to drug-carrier bio-nanomachines [transmitters (Ts)]. Each transmitter (T) performs signal modulation and encodes the message (M) onto the type of drug molecules to release. (In this example, bio-nanomachines only carry one type of drug molecules, i.e., anti-cancer drug molecules, and thus, the signal modulation is fairly simple.) Drug molecules (signal molecules) that drug-carrier bio-nanomachines [transmitters (Ts)] released propagate through the environment [molecular communication channel (C)] and reach receptors on the membrane of cancer cells (and their associated signal transduction pathways) [receivers (Rs)]. Assuming that drug-carrier bio-nanomachines [transmitters (Ts)] and cancer cell receptors [receivers (Rs)] are within the range of direct communication and that drug-carrier bio-nanomachines carry only one type of drug molecules, signal relay, as well signal multiplexing to avoid possible interference between different types of drug molecules, is not necessary in this example. Upon receiving drug molecules (signal molecules), cancer cell receptors and their associated signal transduction pathways [receivers (Rs)] perform signal demodulation and retrieve the message (M). As a result, cancer cells [information destinations (Ds)] stop growing.

C. Research Issues

1) What Are the Fundamental Characteristics of Signal Molecules to Modulate in Signal Modulation?: In modulating electrical signals, information is modulated onto three fundamental characteristics (amplitude, frequency and phase) of a carrier wave. In modulating signal molecules, it is important to identify fundamental and orthogonal characteristics of signal molecules onto which the message (M) is modulated. As explained earlier, existing research suggests that the message (M) may be modulated onto a type of signal molecules to use (MoSK) or how the transmitter (T) releases the signal molecules into the molecular communication environment (ASK and FSK). In addition, the message (M) may be modulated onto three-dimensional conformational characteristics of signal molecules. As explained earlier, changes in three-dimensional conformations of biological molecules (e.g., proteins changing to a folded structure, DNA molecules changing to an untangled structure) are often a trigger in biological cells and play an important role in biochemistry. The message (M) can be thus modulated onto different conformational characteristics of signal molecules.

2) How Do We Decouple the Carrier and the Message (M) to Modulate in Signal Modulation?: In modulating electrical signals, a single type of carrier (i.e., electrical wave) modulates different messages (Ms), i.e., carrier is independent of the message (M) in this sense. In biological cells, the choice of carrier and information carried by the carrier are inherently tied; the type of signal molecules is the information that signal molecules carry. For instance, bacteria use acyl-homoserine-lactones as signal molecules to communicate the number of bacteria in the environment (quorum sensing) with other bacteria; information is tied to the type of signal molecules [85]. This results in a number of different types of signal molecules involved in signal transduction of biological cells, each type to control a specific aspect of cellular activities, requiring as many types of membrane receptors and signal pathways as the number of types of signal molecules in biological cells. In molecular communication, it may be desirable under some circumstances to use signal modulation mechanisms that do not rely on this dependency between the carrier and the information [the message (M)] so that one molecular communication channel (C) transmits arbitrary messages (Ms).

3) How Do We Design Signal Modulation Mechanisms That Meet the Requirement of Bio-Nanomachines?: If the transmitter (T) is a bio-nanomachine, the signal modulation mechanism at the transmitter (T) (i.e., bio-nanomachine i) must be implemented with its simple functionality and must also result in signal molecules (i.e., output $O^i(t+1)$) that conform to the characteristics of biomaterials of the transmitter (T) and the physical mechanisms of transmitting signal molecules at the transmitter (T). For instance, the signal modulation mechanism may only produce signal molecules that bind to receptor proteins and that pass through a direct connection between bio-nanomachines (e.g., gap junctions). The signal modulation mechanism may also produce signal molecules that can be compartmentalized in vesicles and liposomes and that be ejected through exocytosis. Namely, function $F^i(\cdot)$ that defines output $O^i(t+1)$ in (2) must meet these requirements. It is important to design signal modulation mechanisms that meet the requirement of bio-nanomachines.

4) How Do We Design Signal Modulation Mechanisms That Are Robust to the Noise in the Environmental?: There are a number of possible noise sources in the molecular communication environment; thermal noise sources and molecules that may biochemically interact with signal molecules as discussed in Section III-B. Different signal modulation mechanisms may be robust to such noises at different degrees. Existing research suggests using a vesicle as a container of signal molecules to protect signal molecules from noise sources in the molecular communication environment [54]. It is important to design signal modulation mechanisms that minimize the negative impact of noise.

5) To What Extent Is Signal Molecule Error Handling Necessary in Molecular Communication?: Some molecules (e.g., Ca^{2+}) are abundant in biological cells; some molecules (e.g., RNA and DNA molecules) are scarce in biological cells as they are energy expensive to create and copy. As a result, biological cells sometimes propagate a vast number of molecules (e.g., Ca^{2+}) between cells and within a cell to control their functionalities (e.g., cell growth and death). Biological cells also propagate a limited number of molecules (e.g., RNA molecules) to regulate gene expression among biological cells [86], [87].

Similarly to communication among biological cells, molecular communication may rely on a vast number of signal molecules that carry the same message (M) from a transmitter (T) to a receiver (R), making it highly redundant and thus reducing the need for error handling to a minimal. Molecular communication may also rely on a small number of signal molecules, making it highly sensitive to the environmental noise and thus requiring error handling. It is important to consider different levels of signal molecule error handling in molecular communication.

6) What Is the Communication Channel Capacity?: It is important to develop a new analytical framework and to obtain the capacity of the molecular communication channel (i.e., theoretical limit on how much information is modulated onto signal molecules and transmitted over a channel).

VI. The Molecular Link Layer

The molecular link layer provides functionalities for communication within a direct range of communication (e.g., a direct range of signal propagation).

A. A Descriptive Model

The molecular link layer is a logical layer above the signaling sublayer that deals with the physical materials (i.e., the physical layer). All components in the molecular link layer are logical components. Basic components of the molecular link layer are very similar to those of the signaling sublayer and are the following.

- **Molecular frame**: It is a concept, information, communication or statement that is understood by the sender and the receiver at the molecular link layer. It is a molecular link layer equivalent to the message (M) at the signaling sublayer.
- **Sender**: It sends out a molecular frame. It is a molecular link layer equivalent to the transmitter (T) at the signaling sublayer except that a sender at the molecular link layer does not perform signal modulation.
- **Receiver**: It receives a molecular frame sent from a sender. It is a molecular link layer equivalent to the receiver (R) at the signaling sublayer except that a molecular link layer receiver does not perform signal demodulation.[4]
- **Molecular communication link**: It is the logical medium used to transmit a molecular frame between directly communicating sender and receiver. It is a molecular link layer equivalent to the molecular communication channel (C) at the signaling sublayer. A single molecular communication link between a sender and a receiver may consist of multiple molecular communication channels (Cs) connecting the same sender/receiver pair at the signaling sublayer. Similarly to the molecular communication channel (C) at the signaling sublayer, there are two types of the molecular communication link; **shared medium links** and **point-to-point links**. A shared medium link connects multiple senders to multiple receivers. A shared medium link at the molecular link layer may be implemented, for instance, using a broadcast molecular communication channel at the physical layer (the signaling sublayer). A point-to-point link connects a single sender to a single receiver. A point-to-point link at the molecular link layer may be implemented, for instance, using a point-to-point molecular communication channel at the physical layer (the signaling sublayer).
- **Molecular frame storage**: It is a logical component to store molecular frames at a sender or at a receiver while a sender or a receiver processes another molecular frame. Molecular frame storage may be implemented using signal molecule storage at the signaling sublayer or molecule storage at the bio-nanomachine sublayer. Loss of molecular frames occurs when molecular frames in the molecular frame storage deteriorate and lose its functionality as the molecular frame, or when the molecular frame storage reaches its limit.

[4]The term "receiver" is used both at the signaling sublayer and at the link layer traditionally in communication networks, and we follow the terminology convention in this paper.

B. Functionalities

- **Framing** is a functionality to create a molecular frame from physical layer signal molecules. Framing also gives an identifier to a molecular frame it creates. Framing at the molecular link layer may be supported by the physical layer functionalities. See below.
  - — **Vesicle-based framing**: When the bio-nanomachine sublayer supports functionality of "release molecules" through releasing vesicles containing multiple types of molecules and functionality of "capture molecules" through merging with vesicles, a vesicle may naturally correspond to a molecular frame at the molecular link layer. A vesicle may include signal molecules that function as a message (M) and other molecules that function as a molecular link layer header. A vesicle may be tagged with a molecule serving as an identifier.
  - — **DNA-based framing**: When the bio-nanomachine sublayer supports functionalities of "release molecules" and "capture molecules" of DNA molecules, a DNA molecule that contains in its sequence a message (M) and an error correction code (i.e., molecular link layer information) may correspond to a molecular frame at the molecular link layer. A DNA molecule may also encode an identifier of the molecular frame in its sequence.
- **Addressing** at the molecular link layer is a functionality to specify a receiver (or a group of receivers) that receives molecular frames or a location (or multiple locations) to which molecular frames are delivered. Analogous to addressing at the signaling sublayer, addressing at the molecular link layer may support a physical address (i.e., an address that identifies a receiver or a group of receivers) and a location address (i.e., an address that identifies a location or multiple locations). It may also support an individual address (i.e., an address that identifies a single receiver or a single location) and a group address (i.e., an address that identifies a group of receivers or multiple locations).
  Addresses at the molecular link layer are logical addresses that map onto addresses at the physical layer (i.e., physical addresses and location addresses at the signaling sublayer). The molecular link layer passes information on the address, what type of address it uses (physical address, location address, individual address, group address) and group membership (if it is a group address) to the physical layer (i.e., the signaling sublayer).
- **Molecular frame transmission/reception** is a functionality to transmit (receive) a molecular frame to its receiver (from its sender) over a molecular communication link. This functionality depends on the type of the molecular communication link to use.
  - — **Point-to-point molecular communication link**: Transmitting/receiving a molecular frame on a point-to-point

link is relatively straightforward and may be supported by physical layer functionalities such as "signal molecule transmission/reception" (to transmit/receive a molecular frame), "signal propagation" (to propagate a molecular frame over a point-to-point link) and "signal relay" (to propagate a molecular frame to its receiver over long distance). The molecular link layer passes the address of the receiver to receive a molecular frame to the physical layer.

— **Shared medium molecular communication link**: Transmitting/receiving a molecular frame on a shared link requires **media access control (MAC)**. Media access control divides a shared molecular communication link among multiple senders and transmits molecular frames from multiple senders onto a shared link without causing interferences among molecular frames. One mechanism for medium access control is to use time division multiplexing (TDM) in which different senders transmit molecular frames at different times [34], [81]. Another mechanism for medium access control is to use carrier sensing where a sender senses the environment for other molecular frames, and only when there are no other molecular frames, a sender transmits a molecular frame.

Media access control at the molecular link layer may be supported by physical layer functionalities. For instance, TDM-based media access control may be supported by bio-nanomachine sublayer functionalities of "keep track of time" and "release molecules" in a controller manner. Carrier sense based media access control may be supported by bio-nanomachine sublayer functionalities such as "capture molecules" to detect presence of a specific type of molecules (i.e., a molecular frame) in the environment, as well as "keep track of time" and "release molecules" in a controlled manner. The molecular link layer passes information regarding when to release molecules to the bio-nanomachine sublayer.

- **Molecular frame loss handling** is a functionality to handle loss of a molecular frame at the molecular link layer. At the molecular link layer, loss may occur at the receiver when molecular frames in the molecular frame storage deteriorate and lose their functionality as the molecular frame, or when the molecular frame storage reaches its limit. Molecular frame loss handling involves two tasks; detecting loss and correcting loss through retransmission. Depending on who initiates the task, each of these tasks may be sender initiated or receiver initiated. Each task is described below.

  — **Molecular frame loss detection**: When loss of a molecular frame occurs at a receiver, loss may be detected by the receiver (receiver initiated loss detection). Loss may also be detected by the sender (sender initiated loss detection) in the following manner. For instance, when loss of a molecular frame occurs at a receiver, the receiver may fail to produce biochemical reactions that a (lost) molecular frame is expected to induce or may produce expected biochemical reactions at a lesser degree. Biochemical reactions that the receiver produce may propagate through the environment to the sender, and the sender may in time detect the presence or lack of expected biochemical reactions within a timeout period. The degree of the expected biochemical reactions in the environment may provide a reasonable indication of whether molecular frames that the sender transmitted have successfully reached the receiver. Sender initiated loss detection may be supported by physical layer functionalities such as "detect molecules." The molecular link layer may pass information on biochemical conditions to detect to the physical layer.

  — **Molecular frame retransmission**: When a sender detects loss of a molecular frame, it may initiate retransmission (sender initiated retransmission). Sender initiated retransmission may be supported by physical layer functionalities such as "modify molecules" and "release molecules." The molecular link layer may pass information on molecular frames to retransmit to the physical layer. When a receiver detects loss of a molecular frame, it may initiate retransmission (receiver initiated retransmission). When the receiver detects the loss, it notifies the sender of the loss. This requires the receiver to be intelligent to detect loss and to send feedback to the sender.

- **Molecular frame flow control** is a functionality for a sender to adjust the rate of transmitting molecular frames to avoid loss at a receiver. Preventing the sender from transmitting an excess number of molecular frames (or signal molecules) is especially important for applications such as targeted drug delivery where the signal molecules (e.g., drug molecules) are expensive and where excess amount of signal molecules can cause undesired side effects [18], [88].

  Similarly to the molecular frame loss handling, molecular frame flow control at the molecular link layer may be sender initiated or receiver initiated, depending on who initiates flow control.

  — **Sender initiated flow control**: The simplest form of sender initiated flow control is for a sender to use a very low rate of molecular frame transmission such that there will be no loss of molecular frames. This requires a priori knowledge regarding the receiver's capability to process incoming molecular frames. Bio-nanomachines and the molecular communication layered architecture may be designed with such knowledge, or external control may provide such knowledge with the molecular link layer.

  A more complex form of sender initiated flow control involves the sender detecting loss of a molecular frame. A sender may sense the environment for the presence or absence of the biochemical reactions that a receiver is expected to produce, and the sender adjusts the rate of molecular frame transmission based on the degree of expected biochemical reactions in the environment. Sender initiated flow control may be supported by physical layer functionalities such as "modify molecules," "detect molecules," and "release molecules" (in a controlled manner to adjust the rate of molecular frame transmission). The molecular link layer may pass infor-

mation on the rate of transmitting molecular frames to the physical layer.
  - **Receiver initiated flow control**: When the molecular frame storage at a receiver starts filling up, the receiver may notify the sender, and the sender may adjust its transmission rate. This form of flow control is most commonly implemented in the traditional layered architecture of communication networks at the link layer. This requires the receiver to be intelligent to detect the amount of available space in the molecular frame storage and to send feedback to the sender.
- **Storing molecular frames**: Sender and receiver may store molecular frames in their respective molecular frame storage.

C. Research Issues

1) What Is a Meaningful Data Unit (i.e., a Molecular Frame) at the Molecular Link Layer?: In a traditional communication network, a link layer data unit (i.e., a frame) is composed of multiple physical layer data units (i.e., bits) with a link layer header containing information specific to the layer. Analogous to the frame in a traditional communication network, a molecular frame at the molecular link layer may contain multiple signal molecules (at the physical layer) and a molecular link layer header.

Characteristics and functionalities of the physical layer may make it relatively straightforward to support molecular frames at the molecular link layer or may make it complex. For instance, as discussed earlier, if a bio-nanomachine (at the physical layer) supports vesicle-based molecule transmission and reception, a vesicle may naturally correspond to a molecular frame at the molecular link layer. On the other hand, if a bio-nanomachine (at the physical layer) releases individual signal molecules directly into the molecular communication environment through its surface, it would be difficult to group multiple signal molecules and form a molecular frame. Even if a molecular frame is successfully constructed at a sender bio-nanomachine, it would further be difficult for a receiver bio-nanomachine to recognize a group of signal molecules and to separate a header from the signal molecules in a molecular frame.

Because of the above reasons, the molecular link layer in some molecular communication may directly handle physical layer signal molecules. This may suggest a potential limitation of applying a layered architecture to molecular communication and motivate a need for cross-layer approaches. It is important to carefully consider the concept of a molecular link layer data unit and how it benefits the design of the functionalities at the molecular link layer.

2) How Do We Design Molecular Link Layer Addressing That Meets the Requirements of the Physical Layer?: Physical layer functionalities impact the design of addressing schemes at the molecular link layer (i.e., physical addresses, location addresses, individual addresses and group addresses). For instance, consider the signaling sublayer functionality of using a pair of molecules that bind to each other for addressing. This functionality may support both physical and location addresses: i.e.,

- Physical addresses by placing one of the pair molecules to a molecular frame and the other molecule of the pair at the receiver (individual address) or receivers (group address), and
- Location addresses by placing one of the pair molecules to a molecular frame and the other molecule of the pair at the location (individual address) or multiple locations (group address).

The bio-nanomachine sublayer functionality of creating an addressable space in the environment based on concentration gradients of guide molecules may be more suitable to support location based group addresses, as it is difficult to point to a single location using concentration gradients.

Type of molecular communication channel at the physical layer impacts the design of addressing schemes at the molecular link layer. For instance, if the molecular communication channel at the signaling sublayer is a broadcast channel, supporting individual addresses at the molecular link layer may be difficult, as it requires a receiver to examine the address of an incoming molecular frame and accept the molecular frame (if it is for the receiver) or discard the molecular frame (if it is for a different receiver). Bio-nanomachines may not be able to perform this task.

It is important to meet the requirements of the physical layer (the bio-nanomachine sublayer and signaling sublayer) to design addressing schemes at the molecular link layer.

3) How Do We Design Molecular Link Layer Addressing That Meets the Application Requirements?: Some applications such as targeted drug delivery may only require some molecular frames (i.e., drug molecules) to be delivered from some senders (drug-carrier bio-nanomachines) to some receivers (cancer cells). It does not necessarily require all molecular frames to be delivered from all senders to all receivers, as long as a significant number of receivers receive a significant number of molecular frames from one or more senders. Molecular communication applications such as targeted drug delivery may present new addressing requirements that are not present in a traditional communication network and necessitate a new type of addressing to support in molecular communication. It is important to reflect requirement of applications on the design of addressing at the molecular link layer.

4) How Do We Design Feedback With the Limited Functionalities of Molecular Link Layer Entities?: Feedback at the molecular link layer is between the directly communicating sender and receiver. Feedback is a critical component in any modern electrical systems, so in molecular communication. Key functionalities of the molecular link layer such as molecular frame loss handling and flow control rely of feedback.

Conceptually, feedback is simple: it is a process in which information about the past or the present influences the same phenomenon in the present or future. It is, however, very complex for bio-nanomachines to perform. In order to perform feedback, bio-nanomachine i may need to understand what changes it experienced (e.g., its current state $S^i(t)$ and the new state $S^i(t+1)$), to understand whether the change it experienced is desirable or not, to determine whether to send a positive feedback (to enhance the change) or a negative feedback (to suppress the change), to determine who should receive feedback, to secure the signal molecules (through either creating them using its internal molecules, using molecules stored within itself, or intaking molecules from the molecular communication

environment), to modulate the feedback message onto signal molecules, to add an address to the feedback signal molecules, and to transmit the feedback signal molecules. For many bio-nanomachines, this is an impressive list of tasks to perform.

If feedback functionalities are not easily designed for molecular communication in general, developing feedback functionalities for specific applications may be worthwhile. For instance, consider targeted drug delivery where bio-nanomachines carry the same type of drug molecules, coordinate and release drug molecules at a disease site. Since all bio-nanomachines carry the same type of drug molecules, feedback may be sent to any or all of the bio-nanomachines, eliminating a need to identify which bio-nanomachine(s) should receive feedback. Drug-carrier bio-nanomachines may be engineered to automatically release drug molecules when a simple condition is met (e.g., when concentration of drug molecules in its molecular communication environment reaches a predetermined threshold), eliminating the need for bio-nanomachines to examine their state changes and determine if the feedback should be sent.

In an extreme case, feedback design may include use of external control. For instance, consider targeted drug delivery where drug molecules, encapsulated with materials that are sensitive to external signals (e.g., light, radio frequency signal, or ultrasound [89]), function as drug-carrier bio-nanomachines. Assume that drug-carrier bio-nanomachines also carry fluorescent molecules (e.g., green fluorescent proteins) which emit light (feedback signal) in response to excitation light under a certain condition (e.g., when concentration of drug molecules in the molecular communication environment reaches a predetermined threshold). Drug-carrier bio-nanomachines initially release drug molecules; drug molecule concentration increases and reaches the predetermined threshold; drug-carrier bio-nanomachines emit fluorescent light (in response to excitation light) as the condition is met; upon detecting the light from drug-carrier bio-nanomachines, an external device generates a signal and sends it to drug-carrier bio-nanomachines; the bio-nanomachines react to the signal from the external device and modify the kinetics to suppress the release of drug molecules.

Due to the limited functionalities of molecular link layer entities, it is important to consider requirements of applications and underlying physical layer functionalities, as well as possible use of external devices, in designing feedback at the molecular link layer. This may introduce unwanted dependency among different layers and may make the molecular communication system to lose generality. It may, however, present a first step towards gaining useful insight on how to design feedback for more general molecular communication.

5) To What Extent Is Molecular Frame Loss Handling Necessary in Molecular Communication?: Similarly to signal molecule error handling at the signaling sublayer, molecular frame loss handling at the molecular link layer depends on whether molecular frames carrying the same message are abundant or scarce. (See our previous discussions in Section V-C5.)

VII. The Molecular Network Layer

The molecular network layer provides functionalities necessary for communication over larger distances than at the molecular link layer.

A. A Descriptive Model

Basic components of the molecular network layer are similar to those of the molecular link layer and are the following.

- **Molecular packet**: It is a concept, information, communication or statement that is understood by the source and the destination (as well as the molecular router) at the molecular network layer.
- **Source**: It sends out a molecular packet. A source is either a bio-nanomachine, a group of bio-nanomachines, a location or a collection of multiple locations in the environment and is usually directly connected to the information source (S).
- **Destination**: It receives a molecular packet sent from a source. A destination is either a bio-nanomachine, a group of bio-nanomachines, a location or a collection of multiple locations in the environment and is usually directly connected to the information destination (D).
- **Molecular communication network**: It is a collection of molecular network links and routers (defined below) over which the molecular packet is transmitted from a source to a destination.

  At the physical layer, a molecular communication network may be as simple as the space in the environment where molecular packets propagate stochastically from a source to a destination. It may be more complex and consist of, for instance, a number of protein filaments forming a complex network topology connecting all possible sources and destinations.
- **Molecular network link**: It is the logical medium used to transmit a molecular packet between two adjacent molecular network nodes. (Sources, destinations and molecular routers are collectively referred to as **molecular network nodes** in this paper.) A single molecular network link between two molecular network nodes at the molecular network layer may consist of multiple molecular communication links connecting the same two molecular network nodes at the molecular link layer.

  A molecular network node may be attached to multiple molecular network links at the molecular network layer. If, at the physical layer, a molecular communication network is supported by the space in the environment where molecular packets propagate stochastically from a source to a destination, a molecular network node may directionally release a molecular packet (and hence, the molecular packet directionally propagate through the environment), presenting a choice in the directions (i.e., molecular network links) to forward a molecular packet towards. If, at the physical layer, a molecular communication network consists of a number of protein filaments forming a network topology, a molecular network node may attach multiple protein filaments, presenting a choice in molecular network links to forward a molecular packet onto.
- **Molecular router**: It is a logical component on a molecular communication network. It reads the destination address information of a molecular packet and forwards the molecular packet to one of its outgoing molecular network links towards the destination.

  A molecular router may be built upon a bio-nanomachine in the molecular communication environment where

molecular packets freely propagate and diffuse. When a molecular router receives an incoming molecular packet, it directionally forwards the molecular packet towards the destination. A molecular router may also be a bio-nanomachine placed at an intersection of protein filaments on a network, connecting sources and destinations. When a protein motor carrying a molecule packet arrives at the intersection, a molecular router creates conditions that force the arriving protein motor to take a protein filament that leads to the destination. A molecular router may also simply be a protein filament intersection on a network of protein filaments, if a protein motor carrying a molecular packet has a built-in functionality to determine which intersecting protein filament to take to reach its destination. (In this case, an arriving protein motor performs routing functionality.)

- **Molecular packet storage**: It is a logical component at a molecular network node to store molecular packets. Loss of molecular packets occurs when molecular packets in the molecular packet storage deteriorate and lose their functionality as the molecular packet, or when the molecular packet storage reaches its limit.

B. Functionalities

- **Network formation** is a functionality to form a molecular communication network between sources and destinations (i.e., between source and destination bio-nanomachines or between source and destination locations at the physical layer). The network constructed through the network formation functionality is used to transmit molecular packets from source to destination.

  Network formation at the molecular network layer may be supported by physical layer functionalities. One such mechanism for network formation is to use a self-organizing protein network [49]; bio-nanomachines attached with specific types of molecules (i.e., "seed" molecules) autonomously grow protein filaments (i.e., microtubules) through dynamic instability to establish protein filament links among bio-nanomachines. Once such links are established among bio-nanomachines, protein motors move along the links carrying signal molecules from a source to its destination. Another physical layer mechanism for network formation is to extend an addressable space at the physical layer; addressable spaces based on concentration gradients of guide molecules may be concatenated to form a molecular communication network.
- **Routing** is a functionality to control paths for propagating molecular packets between source and destination.

  Routing at the molecular network layer may be supported by functionalities at the physical layer. One mechanism at the physical layer to support routing at the molecular network layer is to use stochastic behavior of protein motors at intersections of protein filaments on a molecular communication network [63], [64] (Fig. 5.) At an intersection of protein filaments, a protein motor carrying molecular packets (or signal molecules) may switch to a crossing protein filament or continue moving along the same protein filament. Through controlling conditions such as the size of protein motors, size of protein filament links, distance between the crossing protein filament links, it may be possible to control stochastic behavior of protein motors at protein filament intersections, leading protein motors to the destination to which molecular packets (or signal molecules) are delivered.

  Another mechanism at the physical layer to support routing at the molecular network layer is to introduce a router bio-nanomachine that forwards incoming molecular packets to other bio-nanomachines [50], [90] towards the destination. For instance, motile cells (such as bacteria) that move and exchange genetic molecules upon collision with other motile cells may serve as router bio-nanomachines for opportunistic routing of genetic molecules [91]. A motile cell that carries genetic molecules (i.e., signal molecules) moves to a new location, collides with another motile cell, and transfers its signal molecules to the motile cell that it collided with. This new motile cell then moves to a new location and repeats this process until the signal molecules are passed to the destination motile cell.

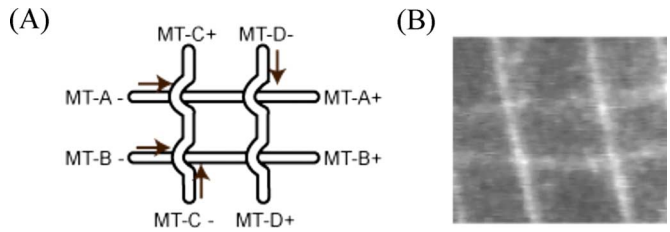

Fig. 5. Routing on a network of protein filaments. (A) Schematic indicating the directions that motor proteins move on the network. (B) An implementation of the network by microtubules (protein filaments) [63], [64].

- **Molecular packet congestion control** is a functionality to regulate the number of molecular packets that exist in the molecular communication network to avoid congestion. Limiting the number of molecular packets may be achieved through either discard molecular packets at a molecular router or regulating the rate of molecular packets entering the molecular communication network.
  - **Molecular packet discarding**: When the molecular packet storage at a molecular router either is in danger of reaching its limit or has reached its limit, the molecular router may discard new molecular packets or some molecular packets that are already in the molecular packet storage to avoid congestion within the molecular communication network. The simplest and most natural discarding mechanism is to not accept new molecular packets when the molecular storage reaches its limit. This does not require molecular network layer to differentiate molecular packets in the storage. This simplest packet discarding is easily supported by functionalities at the physical layer, as this discarding simply relies on underlying biochemical reactions of the molecule storage (e.g., biochemical reactions that govern vesicles intaking of molecules).
  - **Molecular packet rate control**: A molecular router may regulate the rate of molecular packets entering the molecular communication network. The rate of

molecular packets may be predetermined or determined through feedback from other molecular routers. For instance, a molecular router may forward molecular packets with a constant rate through inserting a predetermined amount of time lag between forwarding two successive molecular packets. Alternatively, a molecular router may adjust the length of time to insert between two successive molecular packets based on feedback (or backpressure) from other molecular routers nearby. Molecular packet rate control using the predetermined rate does not require feedback and may be supported by physical layer functionalities such as "keep track of time" and "release molecules" in an controlled manner (to insert time lag between successive molecular packet transmissions). The molecular network layer may pass information on the rate of transmitting molecular packets to the physical layer. Molecular packet rate control that adjusts the rate requires feedback.

- **Storing molecular packets**: A molecular network node (i.e., a source, a destination or a molecular router) stores molecular packets in the molecular packet storage.

C. Research Issues

1) What Is a Meaningful Data Unit (i.e., a Molecular Packet) at the Molecular Network Layer?: We earlier discussed research issues related to the molecular frame (a data unit at the molecular link layer). Research issues on the molecular packet (a molecular network layer data unit) are in parallel to those for the molecular frame (Section VI-C1). Our current thoughts include the following.

- Characteristics and functionalities of the underlying layers (i.e., the bio-nanomachine sublayer, the signaling sublayer, and the molecular link layer) may support molecular packets at the molecular network layer in a relatively straightforward manner or may make supporting molecular packets very complex.
- In some molecular communication, the molecular network layer may directly handle signal molecules (physical layer data units), instead of introducing the molecular packet, although this violates the principles of layering.
- It is important to carefully consider the concept of a molecular packet and how it benefits the design of the functionalities at the molecular network layer.

2) What Identifiable/Addressable Entities Does a Molecular Communication Network Connect?: A molecular communication network provides connectivity among sources and destinations at the molecular network layer. A source is either a bio-nanomachine, a group of bio-nanomachines, a location or a collection of multiple locations in the molecular communication environment and is usually directly connected to the information source (S). A destination similarly is either a bio-nanomachine, a group of bio-nanomachines, a location or a collection of multiple locations in the molecular communication environment and is usually directly connected to the information destination (D).

In forming a molecular communication network, one of fundamental differences between a bio-nanomachine (or a group of bio-nanomachines) and a location (or a collection of multiple locations) in the molecular communication environment is that typical bio-nanomachines move, and the locations do not.

Some applications of molecular communication such as targeted drug delivery may be designed such that molecular packets (e.g., drug molecules) are delivered from a fixed location (e.g., a part of the body where a physician injects drug molecules) to either another fixed location (a disease site in the human body) or a group of bio-nanomachines that do not move (e.g., a group of disease cells). In such a design of applications, a molecular communication network may be formed between source locations and either destination locations or a group of bio-nanomachines that is located at specific locations, not among individual bio-nanomachines. This eliminates complexity of establishing and maintaining a molecular communication network among a large number of dynamically moving, appearing and disappearing bio-nanomachines. A molecular communication network based on concentration gradients of guide molecules in the molecular communication environment, for instance, may best serve the application requirements.

Some applications of molecular communication may also be designed to rely on the abundance of bio-nanomachines and not requiring a molecular communication network to connect all sources and destinations. In targeted drug delivery, a large number of drug-carrier bio-nanomachines are injected into a human body, and drug-carrier bio-nanomachines do not necessary communicate with all other drug-carrier bio-nanomachines, nor all drug molecules need to reach all disease cells. This introduces a possibility of establishing a molecular communication network in a "best effort" manner; if a molecular communication network provides connectivity among a (randomly selected) subset of senders and sources, it may serve the application requirements. Under such scenarios, mechanisms based on autonomous nature of biological materials (such as a mechanism based on dynamic instability of protein filaments described earlier) may become attractive in forming a molecular communication network.

It is important to carefully consider application requirements and derive insights as to what entities (e.g., individual bio-nanomachines, a group of bio-nanomachines, a location or a collection of multiple locations in the environment) should be identifiable/addressable at the molecular network layer and how such entities may be connected to form a molecular communication network.

3) Is Routing Necessary in Molecular Communication?: Many traditional communication networks employ a fixed topology of physical layer links connecting sources and destinations and build atop a virtual network at the network layer. A traditional communication network does so by using either a circuit switching or a packet switching.

Circuit switching first establishes a connection (i.e., a circuit) between a source and a destination and then transmits packets using the established circuit. Circuit switching may be done on a permanent basis establishing a permanent connection between the source and the destination, or on an on-demand basis, establishing a connection when the need arises and tearing down a connection when the need disappears. Routing

in circuit switching is performed in establishing a connection; once the connection is established, there is no routing involved in transmitting packets using the connection.

Packet switching transmits packets without establishing a connection from a source to a destination, and routers in a network make routing decisions for each packet it receives and forwards the packet towards its destination.

In a traditional communication network, routing achieves the following two goals.

- Routing enables transmitting packets over a long distance beyond the range of direct communication at the link layer.
- Routing also enables diversity of communication, i.e., it enables transmitting packets carrying different messages to different destinations.

A key question is whether molecular communication always requires routing or not. The answer may depend on what applications molecular communication supports:

- When molecular communication supports an application that requires communication over a long distance and transfer of molecular packets containing different messages to different destinations (which process different messages carried in molecular packets in different manners), the answer probably is yes.
- When molecular communication supports an application that requires communication over a relatively short distance and transfer of molecular packets containing the same messages to multiple destinations (which all process the message in molecular packets in the same manner), the answer could be no. For instance, in targeted drug delivery, all drug-carrier bio-nanomachines may be injected into a human body at a location within a short distance from a disease site, and they remain close to each other; they all carry the same signal molecules (i.e., drug molecules); they all react in the same manner to the incoming signal molecules (e.g., increase or decrease the rate of releasing drug molecules). For such applications, routing may not be significant.

It is important to carefully examine application requirements in designing routing for molecular communication. Although considering application requirements may introduce unwanted dependency among different layers and may result in molecular communication without generality, it may be a viable alternative to taking upon a challenging task of designing routing for molecular communication in general.

4) How Do We Design Routing Functionality for Molecular Communication Applications That Require Routing?: When a molecular communication application requires routing, molecular packets need to be routed from a source to a destination over a fixed topology of molecular communication network. Among the three switching techniques (permanent circuit switching, on-demand circuit switching and packet switching) mentioned earlier, the permanent circuit switching may be the least complex to design for molecular communication, although it is still very complex and difficult. The permanent circuit switching requires routing to be performed only once in establishing a permanent connection. Introducing external control may be easier with the permanent circuit switching, if molecular network layer entities alone cannot support routing; the external control only needs to be performed once in establishing a connection. Once a connection is established, the permanent circuit switching uses a permanent connection ID, not a destination address, to identify the path to a given destination; association is between a molecular packet (which carries a permanent connection ID) and a path, not between a molecular packet and its destination. This association between a molecular packet and a path only requires a molecular router (or a routing decision point) to maintain mapping between incoming links and outgoing links, i.e., mapping among the links that are physically connected to a molecular router (or a routing decision point). This may be less complex to perform than maintaining mapping between sources and destinations of molecular packets, i.e., mapping among entities which are not physically connected to a molecular router (or a routing decision point). Mapping between incoming links and outgoing links may be implemented using structural or biochemical characteristics of the links and of the molecular packets that use the links.

5) How Do We Design Feedback With the Limited Functionalities of Molecular Network Layer Entities?: Feedback is a critical component at the molecular network layer. For instance, molecular packet congestion control may require feedback between molecular network nodes (i.e., sources, destinations and molecular routers), requesting retransmission of the lost molecular packets.

We earlier discussed research issues related to feedback and handling of molecular frame loss at the molecular link layer. Research issues on the feedback and handling of molecular packet loss at the molecular network layer are in parallel to those at the molecular link layer. Our current thoughts are:

- Many bio-nanomachines may lack the capability to perform tasks involved in feedback.
- Instead of developing feedback mechanisms for molecular communication in general, developing feedback mechanisms for specific applications using specific mechanisms of underlying layers may be worthwhile.
- In an extreme case, use of external control may be sought after in designing feedback.
- It is important to consider requirements of applications and underlying layers, as well as possible use of external devices, in designing feedback at the molecular network layer. This may introduce unwanted dependency among different layers and may make the molecular communication system to lose generality. It may, however, present a first step towards designing feedback for general molecular communication.

VIII. The Molecular Transport Layer

At the layer above the molecular network layer, the molecular transport layer provides a set of functionalities for end-to-end communication between the source and the destination.

A. A Descriptive Model

Unlike the lower layers, the molecular transport layer is an end-to-end layer, i.e., the layer that deals with the source and the destination of the message (M) and is not concerned with

entities in between. Basic components of the molecular transport layer are very similar to those of the link layer and are the following.

- **Molecular transport data unit**: It is concept, information, communication of statement that is understood by the source and the destination at the molecular transport layer.
- **Source**: It sends out a molecular transport data unit.
- **Destination**: It receives a molecular transport data unit sent from a source. Similarly to the molecular network layer, a source (a destination) at the molecular transport layer is either a bio-nanomachine, a group of bio-nanomachines, a location or a collection of multiple locations in the molecular communication environment and is usually directly connected to the information source (S) [information destination (D)].
- **Molecular transport pipe**: It is the logical medium used to transmit a molecular transport data unit between the two ends of communication (a source and a destination). A molecular transport pipe is a collection of molecular network layer links over which the molecular transport data unit is transmitted from one end of communication (a source) to the other end of communication (a destination). There are two types of the molecular transport pipe; a **reliable pipe** and an **unreliable pipe**. A reliable pipe transfers molecular transport data units without loss and in sequence. An unreliable pipe may lose molecular transport data units and/or deliver them out of sequence.
- **Molecular transport data unit storage**: It is a logical component to store molecular transport data units. Similarly to the storage at the molecular link layer and at the molecular network layer, molecular transport data unit storage may simply be the molecular communication environment where the molecular transport data units diffuse and wait for the destination to process them. Molecular transport data unit storage may also be, for instance, a vesicle or liposome embedded in the destination to store incoming molecular transport data units. Loss of molecular transport data units occurs when molecular transport data units in the storage deteriorate and lose their functionality as the molecular transport data unit, or when the storage reaches its limit.

B. Functionalities

- **Molecular transport data unit loss handling** is a functionality to handle loss of a molecular transport data unit at the molecular transport layer. At the molecular transport layer, loss of a molecular transport data unit may occur either at a destination when the molecular transport data unit storage reaches its limit or inside the molecular transport pipe when congestion occurs in the molecular communication pipe. Molecular transport data unit loss handling involves three tasks; detecting loss, correcting loss through retransmissions and sequencing molecular transport data units. This functionality is almost identical to the loss handling at the molecular link layer.
  - **Molecular transport data unit loss detection**: Loss may be detected by the destination (destination initiated loss detection) or by the source (source initiated loss detection). With the source initiated loss detection, the source detects the degree of the biochemical reactions that a (lost) molecular transport data unit is expected to produce. Note that, since the source and destination at the molecular transport layer can be far from each other unlike at the molecular link layer, the degree of the expected biochemical reactions that propagate through the environment to the sender may not provide an accurate indication of whether molecular transport data units have successfully reached the receiver.
  - **Molecular transport data unit retransmission**: Retransmission of a molecular transport data unit may be initiated by the source (source initiated retransmission) or by the destination (destination initiated retransmission). Source initiated retransmission does not require feedback, and destination initiated retransmission does require feedback.
  - **Molecular transport data unit sequencing**: For a reliable molecular transport pipe, molecular transport data units must be delivered in sequence to the molecular transport layer at the destination [71]. Sequence control becomes important for applications such as tissue engineering, where molecular transport data units containing various proteins must be delivered to stem cells in a specific sequence to trigger a series of the growth and differentiation of the stem cells in a desired order.
    A simple approach for sequenced delivery of molecular transport data units is to follow a protocol similar to Stop-and-Wait between the source and the destination. A source transmits a molecular transport data unit, waits for the biochemical reactions that the transmitted molecular transport data unit is expected to cause to propagate back to the source, and transmits the next molecular transport data unit upon detecting the expected biochemical reactions in the environment. More complex, but perhaps more efficient, approach is that a source transmits molecular transport data units at its own rate and that the molecular transport layer at the destination resequences the data units in the storage. This requires each molecular transport data unit to have a unique sequence number. This also requires either the molecular transport layer to have ability to sort molecular transport data units in the storage or the molecular transport data storage to have ability to deliver its contents in sequence.
- **Molecular transport data unit flow control** is a functionality for a source to adjust the rate of transmitting molecular transport data units to avoid loss at a destination [18], [88]. This functionality is almost identical to the flow control at the molecular link layer.
  - **Source initiated flow control**: A source may simply use a very low transmission rate to avoid any loss of molecular transport data units at the destination. Alternatively, a source may detect loss through the source initiated "molecular transport data unit loss detection" and adjust the transmission rate.
  - **Destination initiated flow control**: When the molecular transport data storage at a destination starts filling up, the destination notifies the source, and the source adjusts its transmission rate based on the size of the available molecular transport data unit storage at the

destination. This form of flow control is most common in the traditional layered architecture of communication networks. This requires the destination to detect the amount of available space in the molecular transport data storage and to send feedback to the source.

- **Molecular transport data unit congestion control** is a functionality to regulate the number of molecular transport data units that exist in the molecular transport pipe to avoid congestion. Limiting the number of molecular transport data units may be achieved through regulating the transmission rate of the source.
  Upon detecting loss through "molecular transport data unit loss detection," a source may reduce its rate of transmitting molecular transport data units. The transmission rate may be reduced to a pre-determined and constant rate, requiring only a simple memory to store the value of the constant. The rate may also be reduced to a value that depends on the degree of congestion, requiring the ability to detect the degree of congestion (for instance, through "molecular transport data unit loss detection") and ability to calculate the new transmission rate based on the degree of congestion. Once the rate is reduced upon detecting loss, the source may start increasing the transmission rate to take a full advantage of the capacity of the molecular transport pipe. The transmission rate may be increased by a constant amount, requiring only a simple memory, or by a variable amount that depends on, for instance, how long loss has not occurred in the past, requiring ability to remember history.
- **Storing molecular transport data units**: A source or destination stores molecular transport data units in the molecular transport data unit storage.

C. Research Issues

Many of the research issues at the molecular transport layer are similar to those at the molecular link layer and molecular network layer. For instance, discussions of the following research issues are in parallel to those at the two layers.

- Meaningful data unit (i.e., a molecular transport data unit) at the molecular transport layer,
- Feedback at the molecular transport layer,
- Molecular transport data unit loss handling,
- Molecular transport data unit flow control, and
- Molecular transport data unit congestion control.

IX. The Application Layer

The application layer provides a set of functionalities for molecular communication applications.

A. A Descriptive Model

As molecular communication applications are diverse, it is difficult to develop a single descriptive model that applies to a large number of molecular communication applications. We do not attempt to provide a general descriptive model for the application layer.

B. Functionalities

The application layer may provide a set of basic functionalities that may benefit a large segment of molecular communication applications such as those described in Section II. The following are examples of such functionalities.

- **Bio-nanomachine location control** is a functionality to control the distribution of bio-nanomachines over the molecular communication environment to perform application oriented tasks (e.g., placing bio-nanomachines at disease sites for therapy). This functionality may be supported by functionalities at the physical layer. One mechanism for bio-nanomachine location control is to use mobile bio-nanomachines (i.e., bio-nanomachines with "move") and molecules that function as attractants or repellents for bio-nanomachines [17]. Such molecules may be injected into the molecular communication environment from an external device (i.e., a device outside of the molecular communication environment) or may be pre-stored in a molecule storage (with "store molecules") and released by bio-nanomachines (with "release molecules") in the molecular communication environment, forming gradients of attractants/repellents in the environment. Mobile bio-nanomachines (with "move") detect the concentration gradients (with "modify molecules" and "detect molecules") and adjust their locations according to the gradients of attractants/repellents in the environment.
- **Environmental monitoring** is a functionality to monitor the molecular communication environment and detect a specific event that may occur in the molecular communication environment. This functionality may be supported by functionalities at the physical layer. One mechanism for environmental monitoring is to distribute a number of bio-nanomachines that are capable of detecting a specific signal of interest (with "modify molecules" and "detect molecules") such as disease-indicating signal molecules released from biological cells, the concentration of specific types of molecules, and environmental disturbances caused by intrusion of foreign agents from outside of the molecular communication environment [92].
- **Molecule concentration control** is a functionality to control the concentration of a specific type of molecules in the molecular communication environment. Such functionality may be useful, for instance, to maintain the concentration of drug molecules within a specific range. This functionality may be supported by functionalities at the physical layer. One mechanism for molecular concentration control is that bio-nanomachines monitor the concentration level of a specific type of molecules (with "modify molecules" and "detect molecules") and release molecules (with "release molecules") that are stored in molecule storage (with "store molecules"), when the concentration level of the molecules goes below the threshold. Molecule concentration control may be combined with bio-nanomachine location control to control the concentration of molecules at specific locations in the molecular communication environment. Bio-nanomachines may be placed at specific locations using "bio-nanomachine location control" and "molecule concentration control," the bio-nanomachines at the specified locations adjust the concentration of molecules using "molecule concentration control" [18].

- **Structure formation** is a functionality to form a certain spatial relationship of bio-nanomachines (e.g., to form a three-dimensional structure of a human organ from bio-nanomachines). At the application layer, a desired structure may be formed through applying reaction-diffusion models based on activator and inhibitor molecules [31]. This functionality may be supported by functionalities at the physical layer. Bio-nanomachines may store activator and inhibitor molecules in molecule storage (with "store molecules") and release these molecules (with "release molecules") in a controlled manner. Bio-nanomachines detect concentrations of activators and inhibitors created in the environment (with "modify molecules" and "detect molecules") and position themselves (with "move") based on the created spatial patterns to form a structure.
- **In-network processing** is a functionality to perform some computation within a network, such as aggregating environmental conditions sensed by a group of bio-nanomachines. This functionality may be supported by functionalities at the physical layer. One mechanism for in-network processing is that individual bio-nanomachines sense an environmental condition (with "modify molecules" and "detect molecules") such as a concentration of certain type of molecules, communicate their sensed environmental conditions within a group (with "release molecules" and "capture molecules"), and identify the average environmental condition (e.g., average concentration of molecules) [93].
- **Multiscale messaging** is a functionality to transmit information from bio-nanomachines to external macro-scale devices (e.g., external electronic devices) and vise versa. This functionality may be supported by functionalities at the physical layer. One mechanism for multiscale messaging is through florescence proteins: bio-nanomachines may be implemented with fluorescent compounds that are sensitive to excitation light (a message from an external device to bio-nanomachines) and emit fluorescence light (a message from bio-nanomachines to the external device) upon receiving excitation light [45].

C. Research Issues

1) What Are Functionalities That Molecular Communication Applications Require at the Application Layer?: The application layer provides a set of value-added functionalities, namely, a set of functionalities that are shared by many molecular communication applications, not just by one application. Functionalities at the application layer are aware of applications and provide more "values" than the generic (i.e., non-application oriented) functionalities provided by the lower layers. Some examples of such functionalities are discussed above. It is important to identify and design additional functionalities that the application layer provides.

2) How Do We Design Applications Using Functionalities at the Application Layer and at the Lower Layers?: Functionalities at the application layer need to be mapped onto the lower layer functionalities so that they are actually supported by the lower layer functionalities and bio-nanomachines. This mapping involves dividing each application layer functionality to a finer level of "application level atomic tasks," and for each application atomic task, identifying functionalities at the lower layers to support the application level atomic task. At the lowest layer, i.e., at the physical layer, one must also identify a set of functions that are implementable on individual bio-nanomachines. It is important to identify and implement a set of functionalities at each layer to design a molecular communication application.

3) How Do We Design Collective Behavior of Bio-Nanomachines to Aid Functionalities at the Application Layer?: Collective behavior or swarm behavior refers to the coordinated behavior of a large number of simple individuals (bio-nanomachines) [94]. When a large number of simple individuals communicate and coordinate, complex and often useful behavior emerges out of a multiplicity of relatively simple behaviors and interactions. It is important to understand collective behavior of bio-nanomachines and design lower-layer functionalities such that useful behavior emerges at the application layer.

4) How Do We Design Application Layer Functionalities That Adapt to the Dynamic Environment and Evolve?: One of the research issues at the bio-nanomachine sublayer is to design bio-nanomachines that adapt to new and changing conditions within a generation (adaptation) and through generations (evolution). We may be able to design application layer functionalities that adapt and evolve, when underlying bio-nanomachines adapt and evolve. This eliminates the need for precise and perfect designs for bio-nanomachines and functionalities of the lower layers.

5) How Do We Interface Molecular Communication With Its External Environment?: Many applications of molecular communication involve interactions with the environment external to the molecular communication environment. For instance, targeted drug delivery requires interaction with a physician (in the external environment) who monitors how a patient is responding to drug therapy and adjusts the amount of drug or a type of drug to administer. Multiscale messaging and the interface that enables multiscale messaging are critical to transmit information from bio-nanomachines to external macro-scale devices (e.g., external electronic devices) and vice versa [45].

X. An Application Design Example: Targeted Drug Delivery

Targeted drug delivery is a promising nanomedical application of molecular communication [95]. In this section, we illustrate an example design of targeted drug delivery, where a group of bio-nanomachines communicates and cooperates through molecular communication, searches for a target site (e.g., the site of disease cells) and releases drug molecules at the target site. In the example design, we assume the following.

- **Assumption 1**: All drug-carrier bio-nanomachines are within a short distance (i.e., within a single hop communication distance) from a disease site and from each other.
- **Assumption 2**: Bio-nanomachines are uniform, i.e., all drug-carrier bio-nanomachines have the same biochemical characteristics and react in the same manner to the incoming signal molecules.
- **Assumption 3**: All drug-carrier bio-nanomachines carry the same signal molecules (i.e., drug molecules).

- **Assumption 4**: There are a large number of drug-carrier bio-nanomachines, and all drug-carrier bio-nanomachines carry a large number of signal molecules (i.e., drug molecules).

A. System Design

In the following, we first identify a set of application layer functionalities to support a targeted drug delivery application. We then examine lower layer functionalities necessary to support the application layer functionalities.

1) Application Layer Functionalities: In the example design considered in this section, drug-carrier bio-nanomachines that are near a disease site detect the disease site (using "environment monitoring"). They release diffusive guide molecules into the molecular communication environment and form a concentration gradient in the environment (using "molecular concentration control") with the highest concentration at the disease site and with a decreasing guide molecule concentration as moving away from the disease site due to decay of guide molecules during propagation in the environment. Other drug-carrier bio-nanomachines sense the environment (using "environmental monitoring") and detect concentration gradient of guide molecules. They then move following the concentration gradient toward a higher concentration of the guide molecule (using "bio-nanomachine location control"). When drug-carrier bio-nanomachines reach the disease site, they detect the highest concentration of guide molecules (using "environment monitoring") and start releasing drug molecules (using "molecular concentration control").

The following application functionalities are therefore necessary.

- "Bio-nanomachine location control" is necessary to control the distribution of bio-nanomachines and to place drug-carrier bio-nanomachines at a disease site for therapy.
- "Environmental monitoring" is necessary to monitor the molecular communication environment and sense the concentration of drug molecules as well as the concentration of guide molecules that control location of bio-nanomachines.
- "Molecule concentration control" is necessary to control the concentration of drug molecules at a disease site and the concentration of guide molecules in the environment.

2) Molecular Transport and Network Layer Functionalities: No functionalities are necessary at the molecular transport and network layers. This is because of Assumption 1. All entities are within a direct communication range, and thus, no end-to-end functionalities (at the molecular transport layer) and no functionalities to transmit beyond a direct communication range (at the molecular network layer) are necessary.

3) Molecular Link and Physical Layer Functionalities: The lower layer functionalities necessary to support the application layer functionalities are the following. Note that, because of Assumptions 1–4, some of the lower layer functionalities described in this paper are not necessary. See supplemental materials for details.

- At the molecular link layer:
  - "Addressing" is necessary to guide drug-carrier bio-nanomachines to the disease site. The example design adopts addressing based on locations and group addresses. In the example design, as the molecular link layer directly handles physical layer signal molecules, not frames (molecular link layer data units), addressing at the molecular link layer simply uses the addressing at the signaling sublayer.
  - "Storing molecular frames" is necessary to store signal molecules (i.e., drug molecules) and to store guide molecules (to create an addressable space). As the example design adopts the cross-layer approach, "storing molecular frames" at the molecular link layer simply uses the functionalities to store signal molecules (at the signaling sublayer) and molecules (at the bio-nanomachine sublayer).
- At the signaling sublayer:
  - "Signal molecule transmission" is necessary for drug-carrier bio-nanomachines to transmit signal molecules (i.e., drug molecules) and guide molecules (to create an addressable space).
  - "Signal propagation" is necessary to propagate signal molecules (i.e., drug molecules) and guide molecules that drug-carrier bio-nanomachines release.
  - "Addressing" is necessary to guide drug-carrier bio-nanomachines to the disease site. The example design adopts addressing based on locations and group addresses at the signaling sublayer.
  - "Storing signal molecules" is necessary to store signal molecules (i.e., drug molecules) and guide molecules in the signal molecule storage.
- At the bio-nanomachine sublayer:
  - "Identifier" (a basic component of a bio-nanomachine) is necessary for drug-carrier bio-nanomachines to move towards a disease site. In the example design, drug-carrier bio-nanomachines and a disease site are identified through their locations in the molecular communication environment. The location identifier in the example design only supports group identifier, and thus, individual drug-carrier bio-nanomachines are indistinguishable. In the example design, location identifiers are implemented through creating an addressable space in the molecular communication environment based on concentration gradients of guide molecules, and drug-carrier bio-nanomachines actively move following the concentration gradients.
  - "Acquire and expend energy" is necessary for drug-carrier bio-nanomachine to perform their functionalities.
  - "Move" is necessary for drug-carrier bio-nanomachines to move actively and directionally following the concentration gradient of guide molecules.
  - "Capture molecules" and "detect molecules (and biochemical conditions)" are necessary for drug-carrier bio-nanomachines to detect the concentration of drug molecules and guide molecules in the environment.
  - "Store molecules" is necessary for drug-carrier bio-nanomachines to store drug molecules and guide molecules in their molecule storage. In the example design, at the disease site, the environment stores drug molecules before they are intaken into disease cells.

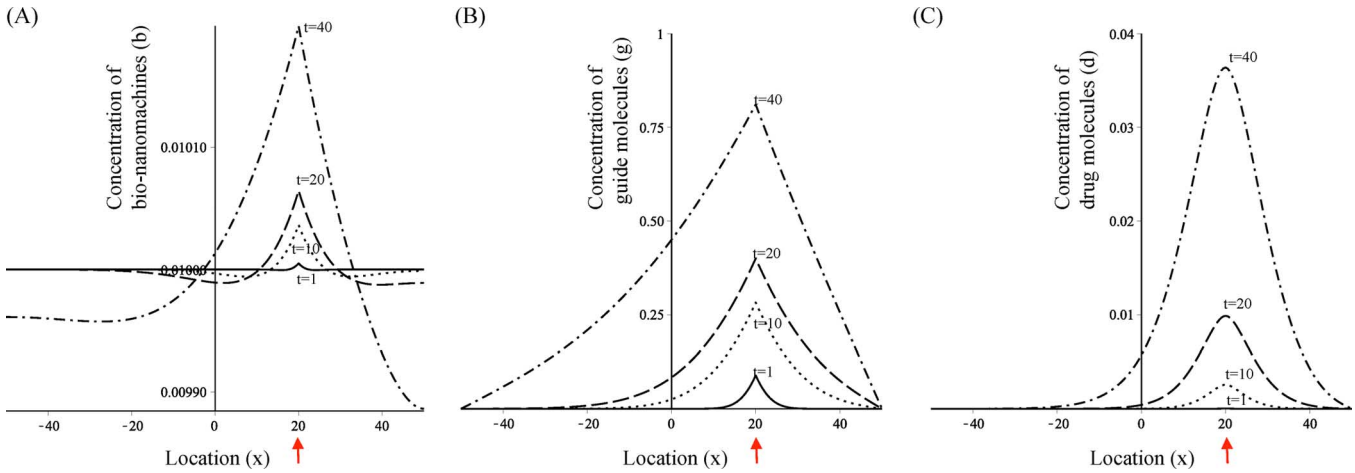

Fig. 6. Distributions of (A) bio-nanomachines, (B) guide molecules and (C) drug molecules. The arrow indicates the target site ($x = -20$).

— "Release molecules" is necessary for drug-carrier bio-nanomachines to release drug molecules and guide molecules from their molecule storage to the environment.

— "Self-feedback" is necessary to control movement of bio-nanomachines along the concentration gradients of guide molecules. A bio-nanomachine may adjust the direction of its movement based on whether the previous movement led to a higher (or lower) concentration of guide molecules.

B. An Analytic Model

To illustrate how a group of bio-nanomachines move to a target site and deliver drug molecules in the example design of targeted drug delivery, we use a simple one-dimensional model to analytically obtain the efficiency of the designed targeted drug delivery application in this subsection. The molecular communication environment is modeled as a one-dimensional line of interval $[-L, L]$ containing one target site at $x = x_T$. Bio-nanomachines are initially distributed uniformly over the interval at the same concentration $C/2L$. Bio-nanomachines bias their movement towards a higher concentration of guide molecules, and bio-nanomachine movement is modeled with the diffusion coefficient D_b and two additional parameters (V_b and K_b) that impact their mobility [96], [37]. There are no guide and drug molecules initially distributed in the environment. Bio-nanomachines release guide molecules at a constant rate of R (molecules per time unit) at the target site. Bio-nanomachines release drug molecules according to the concentration of the guide molecule. The rate of release of guide and drug molecules is modeled using the Hill function with three parameters (n, V_d, and K_d). Once released from bio-nanomachines, the guide and drug molecules diffuse in the environment with the diffusion coefficients D_g and D_d, respectively.

The following set of partial differential equations describes the rates of change in concentration of bio-nanomachines $b(x,t)$, concentration of guide molecules $g(x,t)$, and concentration of drug molecules $d(x,t)$ at location x at time t:

$$\frac{\partial b}{\partial t} = D_b \frac{\partial^2 b}{\partial x^2} - \frac{\partial}{\partial x}\left(\frac{V_b\, g}{K_b + g}\frac{\partial g}{\partial x} b\right), \tag{6}$$

$$\frac{\partial g}{\partial t} = D_g \frac{\partial^2 g}{\partial x^2} + R\, b\, \delta(x - x_T), \tag{7}$$

$$\frac{\partial d}{\partial t} = D_d \frac{\partial^2 d}{\partial x^2} + \frac{V_d\, g^n\, b}{{K_d}^n + g^n}, \tag{8}$$

where $\delta(\cdot)$ is the Dirac delta function.

In the numerical results described in Section X-C, environmental boundary conditions are such that bio-nanomachines are reflected back, and guide/drug molecules are absorbed at the environment boundaries; namely, $(\partial b)/(\partial x) = 0$, $g = 0$, $d = 0$ at $x = L$ and $-L$. Initial conditions are as described above and are: $b = C/2L$, $g = 0$, $d = 0$ at $t = 0$. Parameter values chosen and units are arbitrary: $L = 50$, $x_T = -20$, $C = 1$, $D_b = 10$, $V_b = 1$, $K_b = 0.5$, $D_g = 10$, $R = 50$, $D_d = 1$, $V_d = 1$, $K_d = 1$, and $n = 2$.

C. Numerical Results

Fig. 6 shows how the concentrations of bio-nanomachines, guide molecules and drug molecules change with time ($t = 1, 10, 20, 40$). Bio-nanomachines are initially distributed uniformly in the environment. Bio-nanomachines then move towards the target site ($x = -20$) as time progresses [Fig. 6(A)]. This is because bio-nanomachines at the target site release guide molecules, released guide molecules form a concentration gradient with its highest value at the target site [Fig. 6(B)], and bio-nanomachines in the environment move towards the target site according to the gradient. As more bio-nanomachines move towards and arrive at the target site, more guide molecules are released at the target site, and more bio-nanomachines are attracted towards the target site. Upon detecting a high concentration of guide molecules near the target site, bio-nanomachines release drug molecules, resulting in a high concentration of drug molecules near the target site while maintaining low concentrations at non-target sites [Fig. 6(C)].

Fig. 7 shows the spatio-temporal dynamics of bio-nanomachines, guide molecules, and drug molecules at location $x \in [-50, 50]$ for a longer time duration ($t \in [0, 1000]$). A concentration at (x,t) (at location x at time t) in the figure is indicated by the color at (x,t). Different colors on this figures corresponds to different concentrations, as indicated on the color code bar on the figure showing the mapping between a concentration value

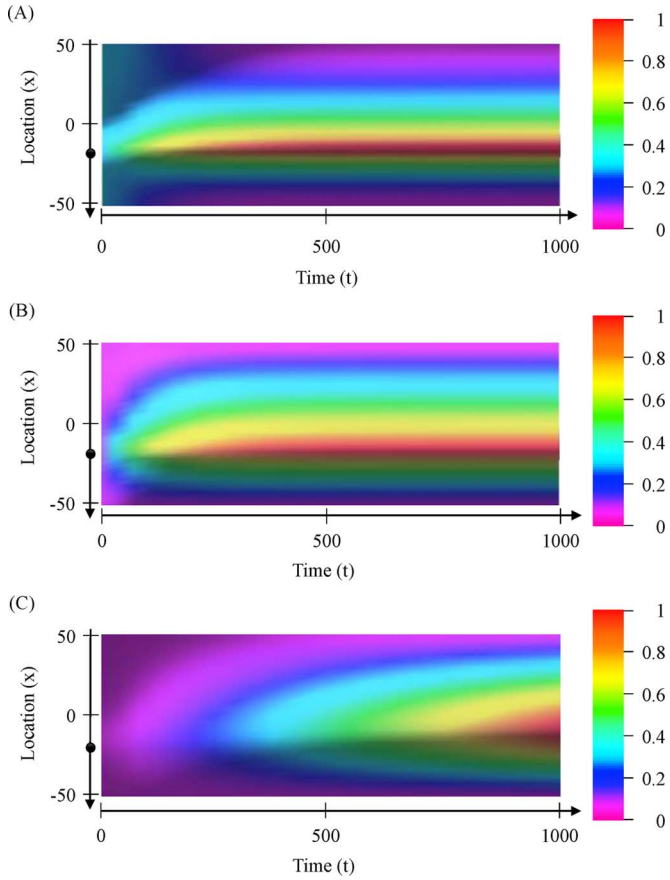

Fig. 7. Distributions of (A) bio-nanomachines, (B) guide molecules and (C) drug molecules at location $x \in [-50, 50]$ and time $t \in [0, 1000]$. The filled circle on location axis x indicates the target site ($x = -20$).

and a particular color. Note that concentration values are normalized between 0 and 1 based on the lowest and highest concentrations in Fig. 7.

The numerical results on Figs. 6 and 7 show that targeted drug delivery using bio-nanomachines increases the concentration of drug molecules at the target site. This reduces the side effects of drug molecules, since fewer drug molecules will remain at non-target sites. Side-effects may also be reduced since fewer drug molecules need to be administered in order to achieve the same concentration of drug molecules at the target site as the concentration that is achieved without targeted drug delivery.

XI. Conclusion

Following the traditional layered architecture of communication networks, this paper presented a layered architecture of molecular communication and discussed open research issues in molecular communication in each layer of the architecture. Molecular communication is highly dynamic with stochastic fluctuations due to the size, limited lifetime, and movement of bio-nanomachines; it is also of large scale, not in size, but with a large number of entities (e.g., bio-nanomachines) deployed; it has potential to adapt, evolve and exhibit emergent behavior. Fruitful future research in molecular communication addresses these unique features of molecular communication through the interplay among theoretical, simulation and empirical studies.

- Theoretical study has made significant progress. It has, however, focused on rather simplistic models and assumptions that may not fully reflect underlying biochemical and biophysical constraints. Additional theoretical study is necessary to build and analyze models that describe molecular communication with more realistic assumptions. Additional theoretical study is also necessary to establish theoretical limit on various metrics of molecular communication (such as capacity of molecular communication links and networks).
- Simulation study is necessary to obtain insights into the behavior of molecular communication under assumptions that fully reflect underlying biochemical and biophysical constraints. Such study is necessary to complement theoretical study, as the current theoretical approaches are often weak to address realistic assumptions. It is important to develop simulation tools that are computationally tractable and yet capable of handling realistic assumptions that reflect underlying biochemical, biophysical and biological constraints, as well as unique features of molecular communication (a system that is highly dynamic, of large scale in the number of entities, and with entities that adapt, evolve, and exhibit emergent behavior).
- Empirical study has been limited to creating components of molecular communication and often to reproducing functionality already available in biological systems (e.g., functionality of transporting molecules *in vitro* using molecular motors purified from biological cells). It is important to address system aspects of molecular communication through empirical approaches.

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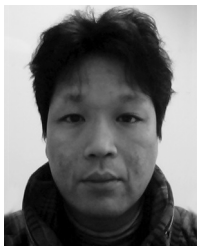

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