Dynamics of a vesicle as a cell mimic: Effects of interior structure, cross-membrane transport, and interaction with filaments

The biological membrane is, in essence, a thermodynamically-nonequilibrium lipid bilayer [6, 30, 34, 43, 47] with a variety of molecular motors, ion pumps, or channels residing within [19, 28, 33, 46]. The membrane encapsulates cellular content such as the spectrin-actin cytoskeletal network, nucleus and other organelles in the cytoplasm. Such biologically complex structures suggest that mathematical simplification is necessary for gaining fundamental insights into the cell dynamics in simple flows, interaction between the interior structures and the cellular membrane, and/or how cross-membrane transport may be affected by the cellular dynamics. For example, as simple cell mimics [50], giant vesicles made of lipid bilayer equilibrium membrane have received much attention in recent years because they can be utilized as a biological mimics for red blood cells (RBCs) when the vesicle membrane has a shear elasticity [2, 38].

PI (Young) has investigated the dynamics of such vesicle with shear elasticity [61]. The main RBC dynamics observed in experiments have also been found for vesicle with shear elasticity: (a) tank-treading (TT), in which the RBC assumes a steady orientation and the membrane rotates, (b) swinging (SW), in which the oscillatory movement of the cell's inclination angle relative to the direction of flow, (c) tumbling (TB), in which the RBC undergoes continuous flipping motion. Theoretical analyses highlighted the membrane area-incompressibility as the source of the nonlinear dynamics. Moreover, for a vesicle with a given area-to-volume ratio, the mismatch between the encapsulated and suspending fluid viscosities selects the TT or TB mode; only vesicle containing more viscous fluid tumbles. This unusual dynamics of individual vesicle results in novel rheology: the effective viscosity of a dilute suspension of vesicles exhibits a minimum at the TT-TB transition [1, 10, 22, 23, 32].

However, most biological cells contain nucleus and organelles (Figure 1(c)). Moreover, novel engineering applications employ vesicles encapsulating large particles (Figure 1(b)). As a first step to model the cellular mechanics with such complex sub-cellular structures in the cytoplasm, it is natural to ask the greatly simplified yet fundamentally relevant question: **Does a particle in the interior fluid introduce new dynamics?**

Motivational finding 1: To answer this question, PI (Young), co-PI (Veerapaneni) and collaborators have investigated the effect of an inclusion on vesicle behavior in linear shear flow [57]. Our analytical theory and boundary integral simulations show that "internal" hydrodynamic interactions between the inclusion and the moving membrane induce TT–TB transition even if the inner and suspending fluids are the same, swinging in the presence of non–spherical inclusion, and transient vesicle slip with off-center inclusion. In a broader context, the results provide insights into the effects of internal structure in cellular hydrodynamics.

The dynamics of a single vesicle suspended in Stokes fluids are fundamental to understanding the RBC dynamics and how the release of adenosine triphosphate (ATP) (which initiates a signaling cascade resulting in the release of nitric oxide causing vasodilation [54, 55]) is correlated to the rheology of the blood.

On the sub-cellular scale, the RBC has a membrane skeleton comprised of a sprectin and actin network that is located just beneath the membrane. The Gov and Safran model links ATP release mechanism with cell deformation at this scale. It predicts that cell deformation or large changes in local membrane curvature will cause the spectrin proteins to dissociate from the actin. These topological defects in the spectrin-actin network reduce the shear modulus of cell [20]. The partially dissociated actin at these defect sites are then free to bind with and activate the cystic fibrosis transmembrane conductance regulator (CFTR) [8], which has been implicated in the ATP release cascade [14, 53]. However, the exact release mechanism for ATP has not been established. Other candidates have been proposed, such as a volume-regulated channel that may act in conjunction

with CFTR, and Pannexin 1 hemichannels (Px1). More specifically, the CFTR protein has been shown to enhance ATP release, but is not required for ATP release and is thought to activate another volume-regulated channel [5]. On the other hand, RBC express Px1 hemichannels and they have been shown to release ATP when exposed to osmotic stress [29, 48].

These findings suggest that RBCs are no longer inert bags of hemoglobin, but rather are paracine signaling the vessel wall in the field of blood research [14]. Recently the relationships between all three topics, macroscopic viscosity, ATP release, and RBC dynamics at the single cell level, are being addressed experimentally [18], see Figure 1(a).

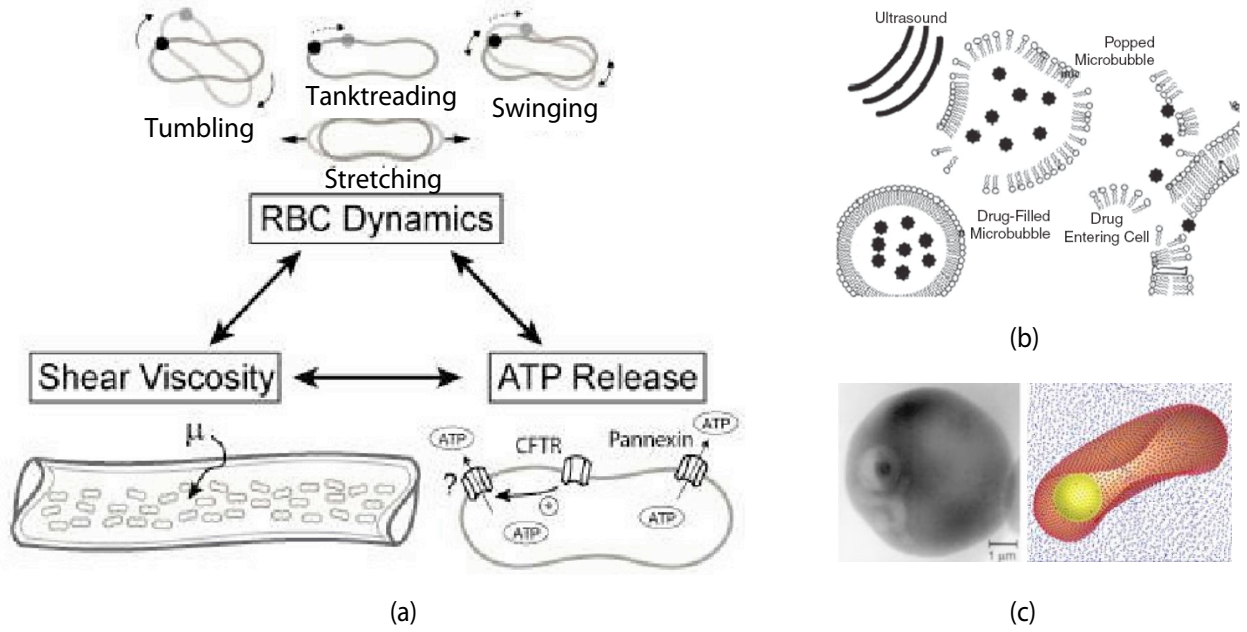

Figure 1: *(a) Sketch of the correlation between RBC dynamics, shear viscosity, and ATP release [18]. (b) Targeted drug delivery via lipid membrane enclosed sacs and ultrasound destruction (Image credit: [44]). (c) In [16], the malaria parasite is modeled as a rigid particle inside a deformable cell (RBC). Both the applications in (b) and (c) can be modeled using compound vesicles.*

Motivational finding 2: At the SW-TB transition, it is found that the cell membrane fluidizes as the shear viscosity of the RBC suspension approaches minimum. This transition also marks the point where the spectrin-actin network begins to detach from the membrane resulting in large deformation and high localized membrane curvature, freeing actin to bind with CFTR and potentially up-regulate Px1. This in turn leads to much enhanced ATP release.

This is an example of how cross-membrane transport is affected by the interaction between the membrane and the cytoskeletal network. In addition, pump or channel residing in the membrane are also responsible for active transport of ion/water molecules or proteins. Inspired by the above two motivational findings, we aim to address the general fundamental question: **How does cross-membrane transport correlate to the cellular dynamics and the rheological properties?** Two approaches will be undertaken to elucidate the roles of cross-membrane transport in the cellular dynamics. The first approach focuses on analysis and implementing the cross-membrane transport in the boundary-integral framework. The second approach focuses on the interaction between filaments and membrane.

First Approach During the process of active transport, the trans-located ions/molecules ex-

ert forces on the membrane. The net effects on the membrane have been modeled as non-thermal noises that lead to membrane fluctuations and deformation [27, 42]. The spectra of the resultant membrane fluctuations have been found to relate to change in membrane bending rigidity [15, 19, 33, 37]. A multi-scale continuum description of such small-scale ion-channel transport has been established using the variational principle [12, 21]. These results have been recently implemented within the framework of immersed-boundary method to simulate the macroscopic effects of ion transport on membrane dynamics. We propose to implement such continuum transport equations into the boundary-integral framework and perform perturbative analysis on the dynamics of vesicle in shear flow with active transport. In the limit of thin membrane interface, active membrane transport results in non-thermal stochastic forces at the membrane in the dipole-force approximation [31]. The microscopic ion transport will be evaluated at the interface in the boundary-integral formulation. This is an advantage over results from diffusive interface, immersed boundary, and immersed interface methods. Furthermore, the proposed modeling results will be compared against experimental data from H. Stone's microfluidic lab (Princeton University).

Second Approach Another salient ingredient in cellular dynamics is the interaction between membrane and a network of actin filaments. The enhanced ATP release is activated once a threshold in the cytoskeletal network is reached. Such stretch-activated threshold is commonly found in mechanotransducing mechanisms. For example, the primary cilium (a cellular non-motile antenna probing the extra-cellular flows) exerts a force on the membrane at the filament base when the cilium is bent under stress from the surrounding fluid flow [49]. It is hypothesized that such a localized force on the membrane is responsible for increased calcium flux and the subsequent cellular signaling [41, 51, 35].

Young has started a collaboration with C. Jacobs (Columbia University) on determining the shape of the primary cilium hinged on the cellular membrane under flow. As will be discussed in § 3, this investigation focuses on the correlation between the filament bending and the force at the filament base. As long as the force from the cilium on the membrane exceeds a threshold, the calcium channel is assumed to be open. The PI expects that results from this collaboration will elucidate the essential coupling between membrane and the elastic filament network for cross-membrane transport.

In the following we outline three proposed research subjects, integrated to elucidate the effects of important biological features on cellular dynamics modeled by giant vesicle with added physical and mechanical properties.

Objective 1: Effects of interior structures and cross-membrane transport on vesicle dynamics

Extending the theme of vesicle as a biological mimic, PI and co-PI propose to examine in-depth how vesicle dynamics may be affected by particle(s) or structures enclosed inside. These "structures" are meant to represent idealized simplification of complicated cellular organelles (such as nucleus). PI and co-PI show that the vesicle dynamics can be affected by the hydrodynamic interaction between the interior particle and the vesicle membrane. PI and co-PI will systematically investigate such dynamics using both perturbative analysis and boundary-integral simulations in three directions: (a) asymmetric inclusion particle, (b) multiple inclusion particles, and (c) hydrodynamic interaction between compound vesicles.

We propose to investigate how other biological functions of membrane may affect the well-known vesicle dynamics. The cross-membrane transport will be incorporated into the continuum membrane description in the framework of variational principles [12, 21]. The microscopic transport across the membrane may involve water molecule and ions. Using the variational principle, Chun et al. have formulated a multi-scale framework to understand the transport of water molecules and ions across channels in the membrane. PI will perform asymptotic analysis on the continuum description

of vesicle with cross-membrane transport. co-PI will perform boundary integral simulations of such vesicle in shear flow. Comparative investigation will be performed, and results from boundary integral simulations will be established to be bench-marked against results from immerse-boundary or immersed-interface simulations, where the membrane is not tracked and thus the cross-membrane transport may be less accurate.

Objective 2: Effects of filament-membrane interaction on cellular dynamics

PI and co-PI propose to investigate the filament-membrane interaction in the context of cellular signaling. As discussed in the introduction, coupling between membrane dynamics/deformation with the spectrin-actin network is the key to the sub-cellular level communication. The spectrin-actin network is dynamically coupled with the membrane. However, the full coupling is too complicated to be modeled in the continuum framework. Instead, PI proposes to investigate the interaction between primary cilium and the cellular membrane in the slender-body framework. This is meant as a prototypical problem for gaining insight into the mechano-transduction process. co-PI will perform boundary integral simulations to compare with the slender-body results.

Intellectual Merit. The proposed research combines novel analysis and state-of-the-art boundary integral simulations to investigate the dynamics of a vesicle with biological features. These include: interior structures (modeled as particles inside vesicle), cross-membrane transport (modeled as drift-diffusion with size-effects) and cellular signaling from the macroscopic membrane dynamics. The interaction between the primary cilium and the elastic membrane is proposed as an example for understanding the complicated processes involved in enhanced ATP release in RBCs. The vesicle has been a paradigm for understanding the cellular dynamics in flows. The PIs propose to include crucial biological functions into the membrane dynamics and examine how the vesicle dynamics may be affected with these features taken into account. Results from the proposed research will provide integrated insight into the underlying physics for the mechano-transduction involved for cellular signaling.

The **broader Impacts** of the proposed program lie in the integrated approaches to unveil the roles of biological functions of the membrane and cellular interior in cellular dynamics under flowing conditions. In addition, knowledge obtained from the proposed research will lead to transformative engineering applications in a wide range of interdisciplinary sciences. **1.** The work in Objective 1 will provide insights into how the cellular dynamics are affected and correlated with the cellular interior structures. These "structures" include (a) rigid particles as mimics of cellular nucleus or clusters of large molecules, and (b) an actin network (around the rigid particle) that interacts with the membrane. Within the continuum framework, the proposed multi-scale modeling and simulation will generate results that will shed light on the relationship between cellular dynamics and sub-cellular level structures. **2.** The work in Objective 2 contributes significantly to the understanding of how macroscopic filament-membrane interaction implicates the meso- or micro-scale cellular signaling. Using the primary cilium as a prototypical example of filament-membrane interaction and the subsequent cellular signaling, results from the proposed work in Objective 2 will highlight the salient ingredients in the dynamical coupling between the hinged elastic filament and the elastic membrane. Results from this work will also shed light on mammalian cells probe the extra-cellular flow via the primary cilium to regulate the calcium flux. These results will provide insights into multi-scale coupling between the elastic membrane and the underlying spectrin-actin network. **3.** The proposed interdisciplinary research will be integrated with an educational effort at both the undergraduate and graduate levels, concerted with plans to recruit under-represented minority high school students. Such efforts are further discussed in §5. **4.** Finally, the results of the work will be broadly disseminated through professional meetings, including APS/DFD, SIAM, AME, BPS; and submission to refereed journals, such as the Physical Review Letters, Journal of Fluid Mechanics, Physics of Fluids, Biophysical Journal, among others.

COLLABORATIONS. One of the PIs (Young) is trained in physics and applied mathematics, and the other PI (Veerapaneni) is trained in computer science and engineering. Both PIs have based their research on engineering innovations and experimental findings. In Objective 1, the PIs will work with Prof. Stone (Princeton University) to investigate the interplay between RBC dynamics and the chemical signaling. In Objective 2, the PIs will continue to work with Prof. Jacobs (Columbia University) to study how the cell adjusts its chemical signaling. Both experimental collaborators provided supporting letters.

1 Prior results

The research of Veerapaneni has been supported by the NSF grants CCF-0427985, CNS-0540372, DMS-0612578 and OCI 0749285. Together with collaborations from NYU, Georgia Tech, NASA and ORNL, Veerapaneni has developed efficient large-scale computational infrastructure for simulating the dynamics of inextensible vesicles suspended in viscous fluids.

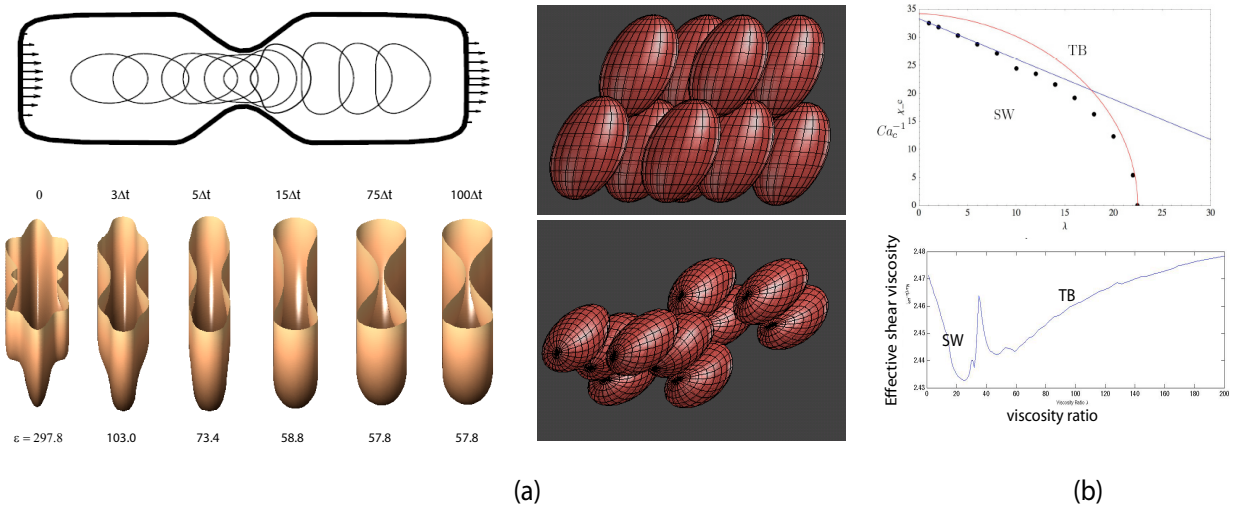

Figure 2: *(a) Examples of vesicle simulations. In the top-left panel, we show snapshots of a vesicle suspended in a microchannel fluid flow. In the bottom-left panel, we show the snapshots of a freely suspended vesicle relaxing to equilibrium by minimizing its bending energy (ε) under the constraint of constant surface area. While conventional numerical methods based on explicit time-stepping schemes require more than a million time-steps for this simulation, we were able to simulate the dynamics using fewer than 100 time-steps without compromising accuracy. In the right panel, we show two snapshots of a multiple-vesicle simulation. (b) SW-TB transition boundary as a function of shear rate and viscosity ratio, and the corresponding effective shear viscosity for the dilute suspension from [61].*

The explicit time integration schemes, which were previously used extensively for vesicle simulations, suffer a stringent constraint on the stable time steps. To circumvent this limitation, we have designed a semi-implicit scheme where the stiff terms are treated implicitly. In order to get high accuracy in space and time we haved used a spectral discretization for the spatial operators and a multistep time stepping. The resulting linearized system is resolved using a Krylov space solver and the application of the spatial operator to a vector is accelerated using the fast multipile method (FMM). We obtain a stable, accurate scheme with excellent convergence rate for the same computational cost as an explicit scheme. This method has been applied to 2D [58], 3D

axi-symmetric [60] and fully 3D [59] geometries. Sample results are shown in Figure 2(a). Based on these advancements and in contrast to previous models that simulated atmost $\mathcal{O}(10^3)$ cells, we have simulated 260 million vesicles. This corresponds to a direct numerical simulation of 50 drops of blood. For this simulation, we achieved 0.7 petaflops of sustained performance on 200K cores of the Oak Ridge National Laboratory's Jaguar supercomputer[1]. The code developed for this project has already led to significant scientific discoveries and answers for *"why do red blood cells have asymmetric shapes even in a symmetric flow?"* and *"why do compound vesicles swing?"* [24, 57].

Supported by NSF/DMS and NSF/CBET, the PI (Young) has made important contributions to the modeling of capsule and compound vesicle in shear flow, as summarized in Figures 2(b) and 3.

Figure 2(b) shows the boundary of SW-TB transition in terms of the shear rate, viscosity ratio, and the asphericity. The PI performed asymptotic analysis to understand the underlying physics of the different capsule dynamics. These results explain the behavior of the capsule as it transitions from swinging to tumbling. In addition, the effective shear viscosity of dilute capsule suspension can also be computed from the small-deformation equations. Similar dependence of shear viscosity on the viscosity ratio is found in direct numerical simulations.

Figure 3 shows the dynamics of compound vesicle in shear flow. A compound vesicle is vesicle enclosing a rigid particle (or particles) inside. The effect of an inclusion on vesicle behavior in linear shear flow is investigated. Particle dynamics in a confined geometry with dynamically evolving boundaries is a problem of fundamental interest, yet it is virtually unexplored. Our analytical theory and boundary integral simulations show that "internal" hydrodynamic interactions between the inclusion and the moving membrane induce TT–TB transition even if the inner and suspending fluids are the same, swinging in the presence of non–spherical inclusion, and transient vesicle slip with off-center inclusion. In a broader context, the results provide insights into the effects of internal structure in cellular hydrodynamics.

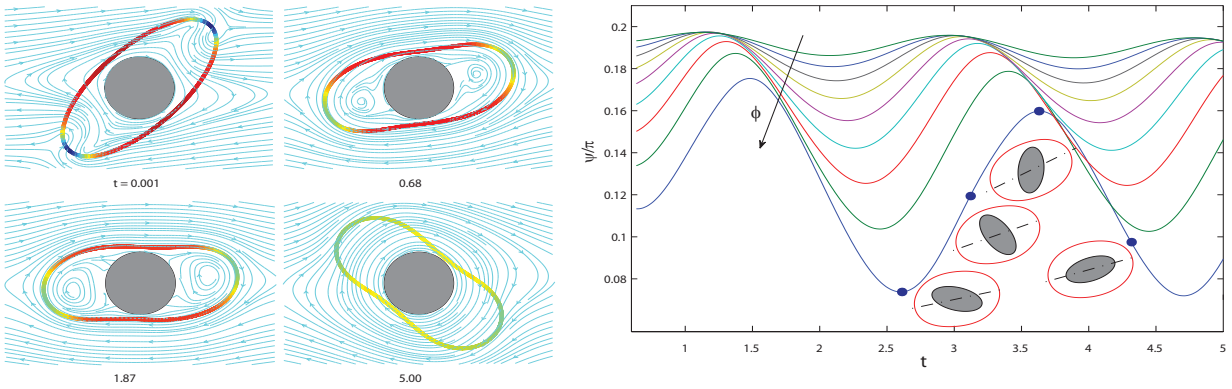

Figure 3: *Simulations of compound vesicles in shear flow. In the left panel, snapshots of the tumbling motion of a vesicle with circular inclusion is shown and in the right, the swinging motion with elliptical inclusions is depicted.*

[1]This work received the 2010 ACM Gordon Bell Prize.

2 Objective 1: Effects of interior structures and cross-membrane transport on cellular dynamics

2.1 Background

The PIs have utilized perturbative analysis (Young) and boundary-integral simulations (Veerapaneni) to study the vesicle dynamics in shear flow with a spherical rigid particle immersed in the fluid inside the vesicle membrane.

Taking into account the hydrodynamic interaction between the rigid particle and the vesicle membrane, both analysis and simulation predict TT-TB transition for particle above a critical size. Using such transition the PIs successfully predict the effective fluid viscosity inside the vesicle. The analysis also gives estimate of the effective shear viscosity and first normal stress difference for suspension of such vesicle as a function of the inclusion particle size. Direct boundary-integral simulations of the compound vesicle reveals more intriguing dynamics: (1) The inclusion particle waltzes into the concentric configuration with the vesicle via the nonlinear hydrodynamic interaction, and (2) the vesicle membrane can swing without membrane shear elasticity if the inclusion particle is a concentric ellipsoid. Furthermore, the rheology of suspension of vesicle with such inclusion also depends on the "internal" hydrodynamic interaction between the inclusion and the membrane.

Albeit a great simplification of the interior structures of the biological cell, the above results imply that the internal cellular structures may contribute to cellular dynamics and change the rheological properties of suspension of cells. These findings encourage the PIs to investigate further the dynamics of vesicles with more realistic biological features.

To quantitatively investigate how internal structures may be connected with certain macroscopic mechanical properties, the first subject focuses on the systematic studies of hydrodynamic interaction between rigid particle(s) and the enclosing elastic membrane. Through this proposed work the PIs will gain fundamental understanding of the correlation between suspension rheology and microscopic dynamics of suspension vesicles. Results from this work will pave the way for designs of biological mimics of which the suspension has the desirable rheological properties, such as the effective shear viscosity or shear-thinning of the RBC suspension.

In the limit of small membrane deformation, the complex stochastic coupling between membrane and the underlying filamentary protein network can be well-approximated by membrane resistance to shearing, or finite shear elasticity. Vesicles with finite shear elasticity are capsules like RBCs. The PIs will adopt a linear stress-strain relation for such capsule with rigid particles inside. As will be explained in § 2.2, the inclusion of non-point-symmetric particle(s) induce an effective membrane shear elasticity. The PIs will investigate the interplay between membrane shear elasticity and inclusion particles.

The PIs propose to include further the cross-membrane transport into the vesicle modeling. The dynamics of RBC membrane is found to affect the ATP release. Experimentally it is found that the ATP release is correlated with the bending rigidity of the RBC membrane. Inspired by these findings, the PIs aim to address the fundamental question: **Does cross-membrane transport couple dynamically with the vesicle membrane to achieve regulatory cellular signaling?**

Small molecules and ions transport across the cellular membrane via simple diffusion, facilitated diffusion, and active transport. Large molecules, on the other hand, achieve the cross-membrane transport through membrane trafficking. The exchange between a cell and its surroundings is an essential process that is controlled by the membrane. It is influenced by (1) the discriminating barrier of the lipid bi-layer, (2) the specific transport proteins located within the membrane, and (3) the membrane curvature and membrane dynamics.

Molecules like water may move across the membrane via the simple diffusion: The concentration gradient is a source of potential energy and simple diffusion is an energetically favorable reaction that does not require energy input. Such simple diffusion transport is a non-selective process, independent of membrane transport proteins, and is determined by the concentration contrast between inside and outside the membrane and the molecular membrane permeability. The permeability depends on the size and charge of the molecule.

For large molecules (glucose or sucrose) and charged ions, they cannot diffuse across the lipid bilayer. They transport through the membrane via facilitated diffusion, a selective process determined by the concentration contrast and the membrane transport proteins, which are carriers or channel proteins that permit the passage of large molecules and charged ions.

The channel-mediated cross-membrane transport of large molecules or ions has been modeled using the continuum variational principle. The finite-size effects can be consistently captured in the variational framework. The transport equations can be incorporated in the boundary-integral formulation and the perturbative analysis. The PIs will further investigate how microscopic cross-membrane transport may affect the cellular dynamics, such as the TT-TB transition? The proposed formulation is advantageous over other immersed interface/boundary methods because the cross-membrane transport is calculated right at the interaface in the boundary-integral formulation (see § 2.3). By incorporating the transport equations into multi-scale continuum description of the membrane dynamics under flow, the PIs aim to focus on the correlation between the cross-membrane flux and (1) the membrane dynamics, (2) the membrane stress distribution, (3) the bulk rheology of the suspension, and (4) comparison with experimental results from Stone's lab.

Results from these proposed studies on effects of cross-membrane transport will provide transformative insights into how electric fields may be optimally utilized for opening/closing the membrane for gene or drug delivery in engineering applications (electro-protation). The proposed boundary-integral simulations will be useful for bench-mark against other immersed-boundary or immersed-interface methods.

In the following we present details on the two proposed research directions in Objective 1.

2.2 Effects of interior "structures" on dynamics of vesicle

Veerapaneni performed boundary-integral simulations and found that a vesicle can swing under shear flow when it includes a rigid ellipsoid inside. This is intriguing because, without the inclusion, vesicle can swing under shear only when the membrane has finite shear elasticity. When the inclusion particle is non-spherical, it tumbles in shear flow. The interaction between the tumbling non-spherical inclusion particle and the elastic membrane leads to the swinging vesicle. Results from the boundary-integral simulations further quantifies the dependence of the swinging amplitude on the inclusion particle size. Young will conduct perturbative analysis on this system to elucidate the underlying physics of such hydrodynamic interaction between membrane and the enclosed particle.

Young has studied the hydrodynamic interaction between a point-symmetric particle and the enclosing membrane. Results from this analysis are compared against Veerapaneni's boundary-integral results and summarized in [57]. When the inclusion particle is not point-symmetric, the analysis is more involved. For example, the velocity field induced by the particle is

$$\mathbf{v}^{\text{scattering}} = \sum_{jmq} \sum_{jm'q'} c_{jmq} X^{\dagger}\left(jmq|j'm'q'\right) \mathbf{u}^{-}_{j'm'q'}, \tag{1}$$

where c_{jmq} is the expansion coefficient and $X^{\dagger}\left(jmq|j'm'q'\right)$ is the scattering matrix. $\mathbf{u}^{-}_{j'm'q'}$ is the fundamental solutions to the incompressible Stokes equations inside the membrane without the inclusion particle. The scattering matrix is determined from the velocity continuity condition on

the inclusion particle surface: The flow field satisfies the no-slip boundary condition at the particle surface. Following the procedures in [57], the basic flows $\mathbf{u}^{-}_{jmq}$ and $\mathbf{u}^{+}_{jmq}$ are first determined on the particle surface and expanded in terms of vector spherical harmonics $\mathbf{y}_{jmq}$ as

$$\mathbf{u}^{\pm}_{jmq} = \mathbf{y}_{jmq} + \sum_{j_1 m_1 q_1} d_{j_1 m_1 q_1} \sum_{j_2 m_2 q_2} \alpha^{\pm}\left(jmq; j_1 m_1 q_1; j_2 m_2 q_2\right) \mathbf{y}_{j_2 m_2 q_2}. \tag{2}$$

Inserting the above basic flows into equation 1 and the velocity field outside the vesicle

$$\mathbf{v}^{\text{outside}} = \sum_{jmq} c_{jmq} \mathbf{u}^{+}_{jmq}, \tag{3}$$

the no-slip boundary velocity boundary condition

$$\mathbf{v}^{\text{outside}} + \mathbf{v}^{\text{scattering}} = \mathbf{v}|_{\text{particle translation velocity}}, \tag{4}$$

then gives the coefficients d_{jmq} by projecting onto each spherical harmonics. $\alpha^{\pm}$'s are determined from the stress-balance on the membrane. As the inclusion particle may not be point-symmetric, solving for d_{jmq} and $\alpha^{\pm}$ requires re-grouping the scalar-harmonic expansion of the product of two vector harmonics.

The above perturbative procedures will give amplitude equations that explain the transition from TT to SW, and SW to TB vesicle dynamics in shear flow, whereas in the case of point-symmetric particle inclusion where only TT-TB transition is found [57]. Without inclusion particles, the SW mode is found only for vesicle with shear elasticity (capsule) [61]. Therefore the hydrodynamic interaction between an elastic membrane and a non-point-symmetric rigid particle induces effective membrane shear elasticity.

To elucidate the underlying physics for the SW modes with non-point-symmetric particles inside, the shear elasticity will be incorporated in the modeling to quantify the induced membrane elasticity due to hydrodynamic interaction with the inclusion particles. If the capsule (vesicle with membrane shear elasticity) deformation is sufficiently small, the membrane constitutive relations reduce to a linear stress-strain relation, yielding the two-dimensional equivalent of Hookes law [3, 11]

$$\tau_{\mu} = 2\left(K_A - \mu\right)\left(\nabla_s \cdot \mathbf{d}\right) H\mathbf{n} - \left(K_A - \mu\right)\nabla_s \nabla_s \cdot \mathbf{d} - \mu \nabla_s \cdot \left[\nabla_s \mathbf{d} \cdot \mathbf{I}_s + \mathbf{I}_s \cdot \left(\nabla_s \mathbf{d}\right)^{\dagger}\right], \tag{5}$$

where $\mathbf{d}$ is the displacement of a material particle of the membrane from its ustressed position $\mathbf{d} = \mathbf{x} - \mathbf{X}$, and $H = \nabla \cdot \mathbf{n}/2$ is the mean curvature. The surface gradient operator $\nabla_s \equiv (\mathbf{I} - \mathbf{nn}) \cdot \nabla$. K_A and μ are the stretch and shear elasticity moduli, respectively. For RBC $\mu \sim 10^{-6}$ N/m and $K_A \sim 200$ N/m. Such membrane shear elasticity will also be implemented in the boundary-integral code by Veerapaneni. Comparative investigations on compound capsule (vesicle with shear elasticity and inclusion particle) between simulations and analysis will give quantification of the induced shear elasticity due to membrane-particle hydrodynamic interaction.

The PIs propose to investigate the effects of multiple particles inside the giant vesicle, which is relevant in engineering applications [36, 63]. Veerapaneni will implement multiple rigid particles inside the capsule in his fast boundary-integral code and investigate its dynamics in shear flow.

Boundary integral simulations. A crucial advantage of the boundary integral method is that a volume mesh is not required. The first step is to write boundary integral equations that describe the interfacial evolutions. Let us assume that the interior and exterior of a compound vesicle enclosing multiple rigid particles is filled with the same fluid that is governed by the incompressible Stokes equations. Let Γ denote the membrane and $\gamma = \cup_{m=1}^{M} \gamma_m$ where γ_m is the boundary of the mth rigid particle. The setup is depicted in Figure 4.

The velocity $\mathbf{v}(\mathbf{x})$ at any arbitrary point $\mathbf{x}$ in the fluid can be written as

$$\mathbf{v}(\mathbf{x}) = \mathbf{v}_\infty(\mathbf{x}) + \mathcal{S}_\Gamma[\mathbf{f}_b + \mathbf{f}_\sigma](\mathbf{x}) + \mathcal{S}_\gamma[\mathbf{f}](\mathbf{x}) + \mathcal{T}_\gamma[\mathbf{u}](\mathbf{x}) \tag{6}$$

where $\mathbf{v}_\infty$ is the far-field boundary condition, $\mathbf{f}_b$ and $\mathbf{f}_\sigma$ are the membrane tractions, and $\mathbf{f}, \mathbf{u}$ are the tractions and boundary velocities of the inclusions. $\mathcal{S}[\cdot]$ and $\mathcal{T}[\cdot]$ are respectively the convolutions with the Stokeslet and the Stresslet

$$\mathcal{S}_\Gamma[\mathbf{f}] = \frac{1}{4\pi\eta}\int_\Gamma \left(-\log\|\mathbf{r}\|\mathbf{I} + \frac{\mathbf{r}\otimes\mathbf{r}}{\|\mathbf{r}\|^2}\right)\mathbf{f}\, d\Gamma, \quad \mathbf{r} = \mathbf{x} - \mathbf{y}, \tag{7}$$

$$\mathcal{T}_\gamma[\mathbf{u}] = \frac{1}{\pi}\int_\gamma \frac{\mathbf{r}\otimes\mathbf{r}}{\|\mathbf{r}\|^4}(\mathbf{r}\cdot\mathbf{n})\mathbf{u}\, d\gamma. \tag{8}$$

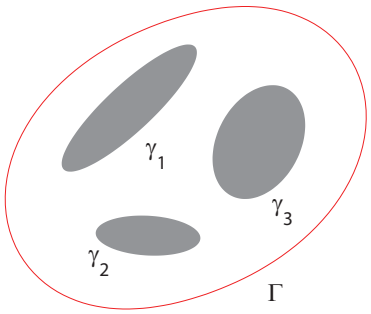

Figure 4: *A compound vesicle enclosing three rigid particles.*

In the above equations, $\mathbf{n}$ is the normal to the boundary and $\mathbf{I}$ is the unit tensor. Under load, the lipid membrane stores energy in bending. The local inextensibility constraint requires the velocity along the membrane is solenoidal, $\mathrm{div}_\gamma(\mathbf{v}) = 0$. This constraint is enforced by introducing a Lagrange multiplier σ [25]. Hence, the membrane tractions are [58, 7]

$$\mathbf{f}_b = \kappa_B\left(c_{ss} + \frac{c^3}{2}\right)\mathbf{n}, \quad \mathbf{f}_\sigma = (\sigma\mathbf{x}_s)_s\,, \tag{9}$$

where κ_B is the bending rigidity and c is the curvature, s is the arclength parameter; subscript s denotes a derivative with respect to arclength.

By taking the limit as $\mathbf{x}$ approaches the membrane interface in equation (6), we get an integro-differential equation for the evolution of the membrane. Similarly, taking the limit to the boundary of the rigid particle, we get an integral equation for the traction and velocity on each of the inclusion boundaries. Combined with the condition for a force–free and torque–free rigid body particle motion,

$$\int_{\gamma_m}\mathbf{f}\, d\gamma_m = 0; \quad \int_{\gamma_m}(\mathbf{y} - \mathbf{y}_c)\times\mathbf{f}\, d\gamma_m = 0 \tag{10}$$

we get three equations for the three unknowns: membrane forces $\mathbf{f}$, translational and rotational velocities for each inclusion[2]. We will extend the fast, high-order methods developed by Veerapaneni et al. in [58, 45] for simple vesicles to solve the resulting coupled set of nonlinear integro-differential equations.

This configuration is too complicated for perturbative analysis. Young will analyze simulation data and examine how hydrodynamic interactions between multiple inclusion particles and capsule can lead to different effective shear viscosity and normal stress differences of the suspension.

2.3 Cross-membrane transport

1. Diffusion across the vesicle membrane: The PIs will implement diffusion transport into both the analysis and the boundary-integral simulation. The simplest diffusion transport that couples to the cellular dynamics is the osmotic effects. The Stokes equations are coupled with the diffusion across the membrane following the formulation in [9]

$$-\nabla\left(p - \frac{1}{3}\eta\triangle\right) + \eta\nabla^2\mathbf{v} + \rho\mathbf{b} = 0, \quad \text{and} \quad \nabla\cdot\mathbf{v} = \triangle, \tag{11}$$

[2]We would like to emphasize that the inclusions are *not fixed* and are continuously evolving whose motion is obtained at every time-step by solving the aforementioned integro-differential equations.

with boundary conditions

$$\mathbf{v} \to \mathbf{v}_\infty \text{ for } |\mathbf{x}| \to \infty; \quad \mathbf{v}(\mathbf{x}) = \mathbf{v}^{\text{inside}}(\mathbf{x}) \text{ for } \mathbf{x} = \mathbf{x}_S, \tag{12}$$

where $\mathbf{v}^{\text{inside}}$ denotes the flow inside the vesicle. $\mathbf{v}$ is the Eulerian velocity field, p is the pressure field, $\mathbf{b}$ is an external body force per unit mass, η and ρ are the fluid viscosity and density, respectively. $\triangle = DV/Dt/V$ is the rate of expansion of the vesicle membrane with V the volume enclosed by the vesicle membrane.

The membrane tension is determined by the constraint of constant membrane surface area as usual, and the integral equations for the compressible Stokes equations are

$$\begin{aligned}(1+\lambda)\,\mathbf{v}(\mathbf{x}_0) &= 2\mathbf{v}_\infty(\mathbf{x}_0) - \frac{1}{4\pi\mu}\int_S \mathbf{G}(\mathbf{x})\cdot\triangle\mathbf{T}(\mathbf{x})dS_x + \frac{3(\lambda-1)}{2\pi}\int_S \mathbf{u}(\mathbf{x})\cdot\mathbf{T}(\mathbf{x})\cdot\mathbf{n}(\mathbf{x})dS_x \\ &\quad -\frac{1}{4\pi\mu}\int_S \mathbf{G}(\mathbf{x})\,(\triangle\rho\mathbf{g}\cdot\mathbf{x})\cdot\mathbf{n}(\mathbf{x})dS_x + \frac{\lambda\triangle' - \triangle}{2\pi}\int_S \frac{\mathbf{n}(\mathbf{x})}{r}dS_x.\end{aligned} \tag{13}$$

λ is the viscosity ratio, $\mathbf{G}$ is the Stokeslet, $\mathbf{T}$ is the membrane traction, $\mathbf{n}$ is the surface unit normal vector, $\triangle\rho$ is the density contrast, and $\triangle = \nabla\cdot\mathbf{u}$. The kinematic condition gives the membrane velocity as the local fluid velocity: $D\mathbf{x_0}/Dt = \mathbf{u}(\mathbf{x_0})$. The PIs will investigate the osmotic effects based on the above formulation of compressible Stokes equation: Young will perform the perturbative analysis and compare the results with the boundary-integral simulation by Veerapaneni.

2. Ion-channel mediated transport: Here we focus on the cross-membrane transport through ion channels. As long as the cell membrane is well-approximated by a thin interface, the active transport through ion channels induce a force at the membrane $\mathbf{F}_{\text{act}}$ given as

$$\mathbf{F}_{\text{act}} = \sigma_{\text{act}} + D_\alpha \mathbf{T}^\alpha_{\text{act}}, \tag{14}$$

where σ_{act} is the force per area and $\mathbf{T}^\alpha_{\text{act}}$ is the stress induced due to the active transport [27, 31]. Both σ_{act} and $\mathbf{T}^\alpha_{\text{act}}$ have been formulated in the dipole-force approximation of the force exerted on the membrane due to active transport [31]. For example, the force per area

$$\sigma_{\text{act}} = \left(w^\uparrow + w^\downarrow\right)\left(F_a n_\triangle + 2HF'_a n_\Sigma\right), \tag{15}$$

with $w^\uparrow$ and $w^\downarrow$ the constant lengths giving the distances from the membrane where the forces act, F_a and F'_a are constants representing the strength of the active force and their curvature dependence, $n_\triangle = n_+ - n_-$ and $n_\Sigma = n_+ + n_-$ are the protein (ion) densities on either side of the membrane, and H the mean curvature on the membrane. The PIs will combine this coarse-grained continuum description of active transport with the transport equations formulated in [12, 21]. At each marker (patch) on the membrane, the concentration (n) of the protein (or ion) inside (outside) the membrane satisfies the transport equation

$$\frac{\partial n_i}{\partial t} = \nabla\left(\frac{D_i}{k_B T} n_i \nabla\left(\mu_i^{PNP} + \mu_i^{HS}\right)\right), \tag{16}$$

where μ^{PNP} is the chemical potential in the Poisson-Nerst-Planck formulation, and μ^{HS} is the hard-sphere potential due to the finite-size effect, and D_i is the i-th diffusion constant. i can be $+(-)$ for outside (inside) the membrane. The gradient operator $\nabla \equiv (\partial/\partial x, \partial/\partial y, \partial/\partial h)$ with (x, y) the curvilinear coordinate on the membrane and $h(x, y)$ is the cross-membrane height.

Veerapaneni will implement the cross-membrane transport equations and the forces into the boundary-integral code to simulate the dynamics of vesicle with active transport. Young will

perform the perturbative analysis with the non-thermal noises in the membrane traction due to the active transport. The analytical results will be compared against results for thermal fluctuations ([17], for example) and simulation results, with specific focuses on the TT-SW, TT-TB, and SW-TB transitions of vesicle dynamics in shear flow. The effective shear viscosity of the dilute suspension of such vesicle will be computed to quantify the effects of active transport. This will facilitate the comparison with experimental results on RBC suspension from Stone' lab (Princeton University).

3 Objective 2: Effects of filament-membrane interaction on cellular dynamics

3.1 Background

As described in **Motivational finding 2**, the interaction between the bi-lipid elastic membrane and the spectrin-actin filament network is very complicated: The spectrin-actin filaments fluctuate underneath the membrane except at the cites where the filaments are pinned. The connectivity of the network depends on the local curvature of the membrane, and the membrane bending rigidity depends dynamically on the strength of filament binding in the network. As complicated as membrane-filament network interaction can be, little is found in the literature even for the dynamical filament-membrane dynamical interaction without thermal fluctuations. Pozrikidis studied the interaction between a rod hinged on a solid wall in the slender-body framework [39, 40].

Inspired by the above findings, the PIs propose to study the interaction between a primary cilium and the elastic cell membrane as a starting point, with the long-term goal set on understanding the interaction between cell membrane and the spectrin-actin filament network. The primary cilium has emerged as a cellular antenna, collecting both biochemical and biomechanical information from the extracellular environment even though it was first discovered almost a century ago [4, 26, 56]. Found in almost every cell in the body, the biological role of the primary cilium has been increasing as experimental techniques and modeling approaches advance. Due to its "relatively simpler" structures than the spectrin-actin network and yet equally complex biological functions, the PIs will focus on understanding how primary cilium reflects under flow, and how such bent shape serves as a cell mechanotransduction mechanism for regulating mechanical and chemical signals.

Shinar *et al.* [52] showed that (1) the drag forces from the stochastic MTs growth initiated by the pronucleus are sufficient for the translocation of the pronucleus, and (2) the interaction between the shell and MTs are essential for the centering process. In their simplified model the cell boundary (shell of the embryo) is rigid and the MTs are also modeled as straight polar rods, being pulled by the motor proteins distributed throughout the cytoplasm during the stochastic MT growth [52]. How will the cytoplasmatic flow be affected by the buckling dynamics of long MTs? Furthermore, what happens if the shell is deformable? These two questions will be addressed by the PIs to elucidate the underlying physics in the filament-membrane interaction.

In the following we present details of the two proposed research directions in Objective 2.

3.2 Interaction between elastic filament and elastic membrane

Figure 5(a) shows the sketch of the structure and the dimensions of the cellular antenna primary cilium. Opposed to the motile cilia driven by motor proteins for cellular motions, the primary cilium is the im-motile $9+0$ microtubule bundle wrapped in the lipid bi-layer membrane. Young has modeled the primary cilium as a hinged elastica on the solid wall in the slender-body framework. In three-dimensional space, the primary cilium is described by a centerline profile $\mathbf{x}(\mathbf{s})$, parametrized by the arc-length s. Assumed to have a uniform circular cross section of radius r_0 and total contour

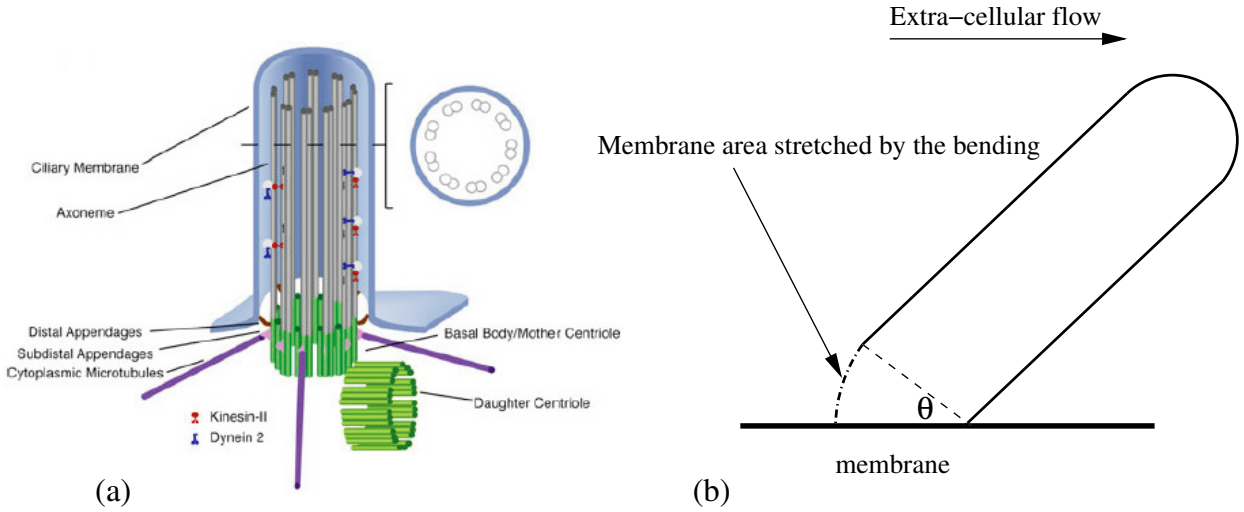

Figure 5: *(a) Schematic of the structure of the non-motile primary cilium. (b) Sketch of the stretching of the membrane when the cilium is bent due to the fluid flow.*

length L_0 such that the aspect ratio $\epsilon \equiv r_0/L_0 \ll 1$, the cilium is a slender cylinder with a constant bending rigidity. The persistence length of the cilium is comparable with the filament contour length under general conditions. Therefore the primary cilium is considered semi-flexible. Even though experiments show that the contour length of the primary cilium may change slightly, the filament can be assumed to be fixed length without loss of generality.

In the slender-body framework, the filament center line position satisfies the following equation at the leading order in ϵ

$$\eta' \left(\mathbf{x}_t - U\left(\mathbf{x}\right)\right) \quad = \quad \left(\mathbf{I} + \mathbf{x}_s\mathbf{x}_s\right)\left[-\mathbf{x}_{ssss} + \left(\sigma\mathbf{x}_s\right)_s\right], \tag{17}$$

where η' is the effective fluid viscosity scaled to the bending rigidity, U is the background velocity field, σ is the line tension determined from the constraint that the filament length is conserved locally: $\mathbf{x}_s \cdot \mathbf{x}_s = 1$. $\mathbf{I}$ is the unit tensor, and subscripts with respect to t and s denote differentiation with respect to time and arclength, respectively. The arclength s is in the range $s \in [0, 1]$ after length is scaled to L_0. The elastica is hinged at $s = 0$ and free at $s = 1$. The boundary conditions at $s = 1$ are $\mathbf{x}_{ss} = \mathbf{x}_{sss} = 0$. The boundary conditions at $s = 0$ are $\mathbf{x} = 0$ and $\mathbf{x}_s = \mathbf{n}_\theta$, where $\mathbf{n}_\theta$ is the unit vector with filament deflection angle θ at $s = 0$. θ has to be determined by the torque balance at the hinged end. As the cilium is deflected from the stress exerted by the fluid flow, its hinged end will rotate. However, because the primary cilium is wrapped in the lipid membrane, the deflection of the cilium implies a stretch in the membrane at the base, as illustrated in Figure 5(b). Assuming that the cilium base spans out a circular corner when it rotates, the increase in the surface area around the filament base (dash-dotted line in Figure 5(b)) is $\delta A \propto \theta \sin(\theta/2)$.

This implies that, if we model the restoring force from stretching the membrane on the deflected cilium as a rotational spring with spring constant k, the restoring force is then proportional to $\theta \sin(\theta/2)$. The force balance at the base thus completes the boundary conditions for the line tension: At $s = 0$, $\sigma(0)\mathbf{x}_s(0) = \mathbf{x}_{sss}(0) +$ force on the hinged end. At $s = 1$, $\sigma(1) = 0$. The torque balance determines the cilium deflection angle θ from rotational torque at the base $= \mathbf{x}_s(0) \otimes \mathbf{x}_{ss}(0)$. These boundary conditions at the hinged end then close the system with the deflection angle determined consistently.

The force at the cilium base is responsible for the mechanotransduction: the primary cilium experiences force from the fluid flow and deflects, and exerts a force onto the membrane as a result. In the above simplified model, the force from the bent filament on the base is purely from stretching the area at the filament base. This force is then expected to be responsible for opening the channel by pulling the molecular motor or proteins at the base when the cilium deflects. Based on the force exerted on the membrane from the bent filament, we can estimate if a stretch-activated channel can be activated.

The above mechanical modeling of the primary cilium allows an estimation of the force at the hinged end. The amplitude of such force (transduced from the extra-cellular flow to the cell by the cilium) can be utilized to determine if a stretch-activated channel can be opened by comparing to the load that is required to stretch-activate a cross-membrane channel. To our knowledge, this is the first mechanical modeling of primary cilium that couples the macroscopic mechanical cilium deformation with the sub-cellular channel transport across membrane. Results from this work will shed light on cellular mechanotransduction, which is essential to understanding how RBCs dynamics in flow relate to their ATP release. With the results from proposed research on cross-membrane transport in § 2.3, an integrated, albeit simplified, view on cellular dynamics and their mechanotransduction will emerge.

An essential simplification in the above modeling of the primary cilium is the interaction between the cilium and the membrane, which is assumed to be a flat wall with no dynamic of its own. **What if the wall is elastic and has dynamics of its own under flow? How would the force at the hinged cilium base be affected?** We will extend Pozrikidis' boundary integral formulation to take into account the dynamics of the membrane.

3.3 Interaction between MTs and elastic membrane

In the biological cell with a nucleus, the cell membrane interacts with the interior via complicated filament network. The simplified version of the pronucleus-microtubule interaction in [52] models the MTs as rigid rods emanating from the pronucleus and grow toward the cell boundary by a stochastic polymerization process. The MTs interact with the motor proteins in the cytoplasm and exert drag forces on the fluid. The confinement provided by the rigid shell is shown to significantly affect the internal dynamics of the cytoplasm. At the filament-membrane contact point, MT stops polymerization and the force on the membrane is the anti-force of the polar pull from the motor proteins. We will examine how the interplay between membrane deformability and the nucleus-filament interaction may lead to different vesicle dynamics in flow by boundary-integral simulations of elastic membrane interacting with MT inside (similar to the experiments in [13]). Furthermore we will also allow MT to buckle upon contact with the membrane as observed in the experiments. Based on modeling results in [62, 64] it is possible that the growing MTs may buckle significantly depending on the ATP concentration in the cytoplasm. Very different dynamics of the pronucleus (the inclusion particle), and consequently the membrane, may be expected.

4 Management

The proposed research agenda requires substantial effort in mathematical analysis, algorithms research and software development, and we expect to leverage additional resources to augment requested funding. In addition to the PIs, we expect to add two graduate students, partially supported by the requested funding. The overall management will be the responsibility of Young and Veerapaneni. We expect to perform all work in close collaboration, and regular weekly meetings will be held between NYU and NJIT.

In **year 1** the systematic investigation on compound vesicle and the primary cilium modeling (Objective 2) are the main goals. Veerapaneni has started the boundary-integral simulations on compound vesicle in shear flow with multiple particles inside and shear elasticity. Young has started the perturbative analysis for non point-symmetric particle. In addition, Veerapaneni will implement several routines for simulating multiple particles within a vesicle. Mainly, we need a reliable collision detection scheme and an algorithm to compute nearly singular integrals accurately. The primary cilium modeling and comparison with the experiments will be completed by Young in year 1 as well. In **years 2 & 3** the focus of the research will be on the active membrane transport and the filament interaction with the membrane. The PIs will begin exploring the cross-membrane transport and integrating these results with those from studying the membrane-filament interaction. In addition, Veerapaneni will develop high-order surface representations, singular integral schemes, mesh-quality preservation schemes and incorporate fast algorithms such as the fast multipole method (FMM) and the fast spherical harmonic transforms. All of these components are essential for simulating large number of compound vesicles with each containing multiple inclusions. Significant resources would be required to implement these schemes and we envision one graduate student being involved full-time.

5 Educational Impacts and Outreach Program

The proposed research will be integrated into an educational effort directed toward undergraduate and graduate students in Applied Mathematics and Computer Science, as well as an outreach effort aimed at encouraging women and under-represented minority students to the study of Computational Sciences disciplines. The inherently interdisciplinary nature of the proposed research is valuable for both Applied Mathematics and Computer Science students. For those on the Applied Mathematics side, the research will enhance their training in fluid mechanics and mathematical modeling. This work will demonstrate how efficient numerical algorithm and novel modeling can be critically important and leveraged to innovative tools for engineering problems and applications. For Computer Science students, the proposed research program will provide exposure and training in aspects of biological fluids.

The immediate educational impact of the proposed research will be on undergraduate and graduate students involved in the research. As a co-Investigator in CSUMS: Research and Education in Computational Mathematics for undergraduates in the Mathematical Sciences at NJIT (funded by NSF) and the lecturer for Capstone Applied Mathematics Lab at NJIT (also funded by NSF), the PI has track records in involving undergraduate students in research that emphasizes both numerical computations and desktop experiments. The PI expects to involve more undergraduate students in this project through the PI's continuing and active involvement in CSUMS. On the graduate-level education, the PI expects the projects to lead to one PhD thesis. Herve Nganguia, a graduate student who works with the PI on a project on capsule dynamics during the summer of 2010, has passed the candidacy exam and will join the PI in the proposed research soon. The PI will foster close interaction between the students and collaborators to enhance the interdisciplinary learning environment. Research outcomes from the projects will be broadly disseminated by publications in journals and presentations in conferences.

In addition, the PI will continue to make outreach efforts to actively recruite female and under-represented minority high school students through the PI's participation in the outreach program of TECHS-NJ, Teacher Education Collaboration for High-Need Schools-NJ (which is constantly recruiting under-represented students to pursue teaching careers in New Jersey high schools). In the past half year, with colleague Prof. Bukiet (PI of TECHS-NJ), the PI has regularly participated the outreach program at New Jersey Institute of Technology.