Coupling biochemistry and hydrodynamics captures hyperactivated sperm motility in a simple flagellar model

Sarah D. Olson a,*, Susan S. Suarez b, Lisa J. Fauci a

a Mathematics Department, Tulane University, 6823 St. Charles Ave., New Orleans, LA 70118, USA
b Department of Biomedical Sciences, Cornell University College of Veterinary Medicine, Ithaca, NY 14853, USA

1. Introduction

The propulsion of sperm, through an undulatory motion of the flagellum, is vital for fertilization. Sperm exhibit different types of motility in response to varying environments and chemical cues (Suarez et al., 1983; Marquez et al., 2007; Ho et al., 2002; White and Aitken, 1989). Activated motility is characterized by symmetrical flagellar bending that is associated with highly linear trajectories (Burkman, 1991). Hyperactivation is a motility pattern of mammalian sperm that is characterized by highly asymmetrical flagellar bending, leading to nonlinear trajectories, including swimming in circles. There is evidence that hyperactivation is key in enabling the sperm to swim effectively through mucus, escape from the sperm reservoir, penetrate the cumulus matrix, and penetrate the zona pellucida of the oocyte (Quill et al., 2003; Ren et al., 2001; Stauss et al., 1995; Ho and Suarez, 2001a,b; Woolley, 2010). Initiation and maintenance of hyperactivated motility is associated with a change in calcium (Ca$^{2+}$) concentration in the flagellum (Carlson et al., 2005; Quill et al., 2003; Ren et al., 2001; Stauss et al., 1995; Ho and Suarez, 2001b).

In order to transition from symmetrical to asymmetrical beating, there must be some change in the axoneme, the core of the flagellum. There are many passive and active elements of the axoneme, as well as sites where signaling molecules can bind and cause conformational changes that affect force generation (Lindemann and Lesich, 2010). The ultrastructure of the axoneme, whose cross section is shown in Fig. 1, consists of a central pair of microtubules surrounded by nine microtubule doublets. Inner and outer rows of dyneins, chemo-mechanical ATPases, emanate from each doublet and face the adjacent doublet at regularly spaced intervals (Woolley, 2010). Passive structures include the nexin links which connect the adjacent pairs of doublets and the radial spokes. Bending of the axoneme is due to the action of dynein motors causing local sliding of microtubule doublets relative to one another. This sliding is converted to bending by the restraining influence of the other passive structures, including the nexin links (Brokaw, 1971, 1972a,b). Currently, there are many hypotheses for the mechanisms that provide the switching point for activation and deactivation of dyneins (Woolley, 2010). While bending is due to the local sliding of microtubules, the overall flagellar waveform arises from the coupled nonlinear system that consists of chemical signaling, active force generation, passive elastic forces, and external fluid dynamics (Gaffney et al., 2011; Fauci and Dillon, 2006).

The length of mammalian sperm is on the order of 100 μm; typical human and bull sperm are approximately 60 μm in length,
while rat and hamster sperm are approximately 150–250 μm in length (Cummings and Woodall, 1985). Due to the small length scales, mammalian sperm swim at low Reynolds number, \( Re \sim 10^{-4} - 10^{-2} \), where viscous forces dominate. Classic fluid dynamic analyses of flagellar swimming due to prescribed kinematics has made use of the viscous fluid mechanics, and the elastic properties of the flagellum. Classic fluid dynamic analyses of flagellar swimming due to prescribed kinematics has made use of the viscous fluid mechanics, and the elastic properties of the flagellum. Classic fluid dynamic analyses of flagellar swimming due to prescribed kinematics has made use of the viscous fluid mechanics, and the elastic properties of the flagellum. Classic fluid dynamic analyses of flagellar swimming due to prescribed kinematics has made use of the viscous fluid mechanics, and the elastic properties of the flagellum.

2. Model development

Since mammalian sperm swim at a Reynolds number that is approximately zero, we assume that the fluid flow is governed by the incompressible Stokes equations:

\[
0 = -\nabla p + \mu \Delta \mathbf{u} + \mathbf{f} \quad (1a)
\]

\[
0 = \nabla \cdot \mathbf{u} \quad (1b)
\]

\[
\mathbf{f}(\mathbf{x}, t) = \int \mathbf{g}(s, t) \phi_d(\mathbf{x} - \mathbf{X}(s, t)) \, ds \quad (1c)
\]

\[
\frac{d\mathbf{X}(s, t)}{dt} = \mathbf{u}(\mathbf{X}(s, t), t) \quad (1d)
\]

where \( p \) is the pressure (Pascals, kg m\(^{-1}\) s\(^{-2}\)), \( \mathbf{u} \) is the fluid velocity (m s\(^{-1}\)), \( \mu \) is the kinematic viscosity of the fluid (kg m\(^{-1}\) s\(^{-1}\)), and \( \mathbf{f} \) is a force density that represents the force of the flagellum on the fluid (kg m\(^{-2}\) s\(^{-2}\) or N m\(^{-3}\)). The centerline of this cylindrical sperm flagellum is modeled as a neutrally-buoyant elastic filament, \( \Gamma \), that is immersed in the fluid and its position is \( \mathbf{X}(s, t) \), where \( s \) is a Lagrangian parameter that we initialize as arclength. Note that \( \mathbf{g} \) is a force per unit length that is concentrated along the length of the filament, and \( \phi_d(\mathbf{m}^{-2}) \) is a cutoff function, or regularized delta function, where

\[
\phi_d(\mathbf{x}) = \frac{15 \delta^4}{8 \pi (r^2 + \delta^2)^{3/2}}, \quad r = ||\mathbf{x}|| \quad (2)
\]

and \( \delta \) is the regularization parameter. We will discuss in Section 5 how this formulation naturally gives rise to the method of regularized Stokeslet (Cortez, 2001; Cortez et al., 2005), which allows us to numerically solve the coupled system. Eq. (1d) states that the velocity of a material point on the flagellar centerline coincides with the fluid velocity evaluated at that point. In order to focus on the effect of Ca\(^{2+}\) dynamics on motility, we ignore the presence of the sperm head and we constrain the flagellar centerline to be within a fixed plane.

2.1. Forces

The filament \( \Gamma \) is taken to be a generalized Euler elastica (Fauci and Peskin, 1988; Fauci and McDonald, 1995; Teran et al., 2010) whose energy \( E \) (N m) is given by

\[
E = \int_\Gamma (\varepsilon_{\text{Tens}} + \varepsilon_{\text{Bend}}) \, ds \quad (3)
\]

\[
\varepsilon_{\text{Tens}} = S_1 \left( \frac{||d\mathbf{X}||}{ds} - 1 \right)^2, \quad \varepsilon_{\text{Bend}} = S_2 \left( \frac{\partial \Theta}{\partial s} - \zeta(s, t) \right)^2 \quad (4)
\]

where \( \varepsilon_{\text{Tens}} \) and \( \varepsilon_{\text{Bend}} \) are the tensile and bending energy densities, respectively. The integration is taken over the filament \( \Gamma \) or \( \cdot \) is the Euclidean norm. The shear angle, \( \Theta(s, t) \), is the angle formed by the tangent to the curve \( \mathbf{X}(s, t) \) and the base of the flagellum. We define the (signed) curvature to be the rate of change of the shear angle with respect to arclength, \( \partial \Theta/\partial s \). In our model, a preferred curvature, \( \zeta(s, t) \), will be a function of the local Ca\(^{2+}\) concentration along the filament. Here, \( S_1 \) (Nm) and \( S_2 \) (Nm\(^2\)) are the tensile and bending stiffness constants, respectively. As will be discussed in Section 2.2, we will vary \( S_1 \) along the length of the flagellum and we will fix the value of \( S_2 \) to isolate the effects of the Ca\(^{2+}\) dynamics on the bending energy of the filament.

The energy \( E \) is non-negative, translation and rotation invariant, and achieves its minimum when \( \frac{d||d\mathbf{X}||}{ds} = 1 \) and when the signed curvature, \( \partial \Theta/\partial s \), along the filament is \( \zeta(s, t) \). The force per
unit length, $g$ is derived from this energy:

$$g = -\frac{\partial}{\partial x}(F_{\text{passive}} + F_{\text{bend}}).$$

(5)

2.2. Passive stiffness of mammalian sperm

In mammalian sperm, the connecting piece near the head serves as the basal anchor for the flagellum and outer dense fibers (Fawcett and Phillips, 1969; Hamasaki et al., 1994). It has been well established that the diameter of the outer dense fibers decrease from the connecting piece to the tip of the tail in mammalian sperm (Telkka et al., 1961), which should affect the magnitude of the passive stiffness. Lindemann proposed to model the passive stiffness along the length of the bull sperm flagellum as

$$IE(x) = \frac{L_sE}{l}X^4$$

(6)

where $IE(x)$ is the stiffness at a given location $x$ along the flagellum, $L_sE$ is the stiffness constant determined from experiments and models that did not take into account the tapering of the flagellum, and $l$ is the length of the flagellum where $x = 0$ and $L$ correspond to the tip and base of the flagellum, respectively (Lindemann, 1973). Lindemann (1996) has also used a linear taper (proximal to distal) in the stiffness to successfully model bull sperm flagellar waveforms with the geometric clutch hypothesis. Recent experiments of bull (Lesich et al., 2008) and rat (Schmitz-Lesch and Lindemann, 2004) sperm have disabled dynein arms and measured the passive stiffness along the length of the flagellum, and it has been found to taper from the base to the tip. In our model, we choose to study the varying passive stiffness properties along the length of the mammalian sperm flagellum by varying the tensile stiffness parameter $S_i$ in Eq. (4). Here we will examine three cases for $S_i$: a flagellum whose tensile stiffness is constant along its length, one whose tensile stiffness tapers linearly proximal to distal, and one with a fourth-order taper proximal to distal. Note that $S_2$, the bending stiffness, can also vary along the length of the flagellum, but we choose to keep it fixed in order to isolate the effects of Ca$^{2+}$ dynamics on the bending energy of the filament.

2.3. Calcium-dependent curvature model

It has been well established that there exists a Ca$^{2+}$ dependence on flagellar curvature and amplitude in mammalian sperm (Lindemann and Goltz, 1988; Marquez et al., 2007; Tash and Means, 1982). In demembranated sperm experiments, increasing levels of Ca$^{2+}$ cause increased asymmetry in the flagellar waveform at relevant biological concentrations (Lindemann and Goltz, 1988; Tash and Means, 1982), where asymmetry has been found to be associated with the bend ending early in one direction (reverse bend) and growing in the other direction (principal bend) (Tash and Means, 1982). Also, if Ca$^{2+}$ is increased to very high concentrations, sperm motility is inhibited (Tash and Means, 1987; Tash et al., 1988). The ions Ni$^{2+}$ and Cd$^{2+}$ bind to Ca$^{2+}$ binding sites and selectively block dyneins on one side of the flagellum or the other (Kanous et al., 1993; Lindemann et al., 1995). This indicates that the sensitivity to divalent cations, such as Ca$^{2+}$, varies on the two sides of the flagellum.

There are many possible ways for the change in Ca$^{2+}$ concentration to affect the waveform. One mode of action is for Ca$^{2+}$ to bind directly to a subset of dynein arms, altering the probability of dyneins attaching to the corresponding microtubule doublet (Lindemann and Kanous, 1995). The second mode of action is for Ca$^{2+}$ to bind to a receptor such as calmodulin (CaM) which then activates kinases, such as CaMKII$, that phosphorylate a protein associated with the axonemal structure, resulting in a modification of the waveform (Suarez, 2008; Tash et al., 1988).

Lindemann has hypothesized that Ca$^{2+}$-induced mechanical changes such as changing the spoke length, nexin length or elasticity, or changing the elasticity or tensile strength of the spoke—central pair axis, would alter the switch point of the beat, and ultimately change the amplitude and wave propagation characteristics of the flagellum (Lindemann and Kanous, 1995).

The exact mechanism of how Ca$^{2+}$ modifies the waveform is not known, but most of the current hypotheses assume that Ca$^{2+}$ binding causes a change in the force generation, which, in turn, causes a change in the sliding distance that results in a change in the amplitude of the waveform. Here, we model hyperactivated sperm motility by choosing a preferred curvature function, $\zeta(s,t)$ (change in shear angle with respect to the material parameter $s$), in Eq. (4), as a function of the local Ca$^{2+}$ concentration. Because high speed imaging (e.g. Smith et al., 2009b) has shown that the flagellum exhibits a propagating wave of curvature, we choose an idealized sine wave as the preferred shape. The preferred (signed) curvature function corresponding to a simple sine wave with $x(s,t) = s$ and $y(s,t) = \sin(s(\pi - o))$ is

$$\zeta(s,t) = -k^2\sin(s(\pi - o))$$

(7)

for small amplitudes, $b$. The sine wave travels to the right with speed $\omega/k$ and periodicity $2\pi/\omega$, and has been used in previous models of sperm motility (Fauci and McDonald, 1995; Fauci and Peskin, 1988; Teran et al., 2010). To reflect the dependence of local dynein force generation on Ca$^{2+}$, we express the amplitude $b = b(s,t)$ in Eq. (7) in terms of a modified Hill equation,

$$b(s,t) = V_s\frac{C_a(s,t)}{C_a(s,t) + k_B}$$

(8)

that depends on the Ca$^{2+}$ concentration, $C_a$ ($\mu$m), along the length of the flagellum. The parameter $V_s$ (m) is a maximum amplitude and $k_B$ ($\mu$m) is a coefficient for receptor activation.

It has been proposed that some mammalian flagella, including rat, exhibit mostly planar beating due to the alternating activation of dyneins on microtubule doublets 1–4 and then doublets 6–9, bending the axoneme in one direction and then the other (Lindemann et al., 1992; Lindemann and Lesich, 2010). In our model, we assume that Ca$^{2+}$ affects the bending moments in these two directions differently. We can differentiate between the two bend directions of our flagellar centerline $X(s,t)$ by monitoring the sign of $\zeta - \partial^2\zeta/\partial s^2$ that identifies whether the bend is concave up or concave down with respect to arclength, a material coordinate. This allows us to designate a principal bend direction versus a reverse bend direction. We assume $V_s$ is constant and we choose

$$k_B = \begin{cases} k_{A1}, & \zeta > 0 \\ k_{A2}, & \zeta < 0 \end{cases}$$

(9a)

in order to account for an asymmetry in the model. We regard the parameter $k_A$ as the Ca$^{2+}$ concentration where half of the binding sites are occupied, and, choosing $k_{A1} \neq k_{A2}$ is equivalent to assuming there exists a difference in the number of Ca$^{2+}$ binding sites on one side of the flagellum versus the other, or assuming different Ca$^{2+}$ binding affinities on each side of the flagellum.

3. CatSper mediated calcium dynamics model

CatSper channels, located on the plasma membrane of the principal piece of the flagellum, are Ca$^{2+}$ channels that are sperm specific and required for hyperactivated motility (Carlson et al., 2005; Qi et al., 2007; Quill et al., 2003; Ren et al., 2001; Xia et al., 2007). (Refer to Fig. 2 for a depiction of the regions of the flagellum.) Recent experiments have also shown that CatSper channels are necessary for sperm to ascend beyond the oviductal reservoir.
(Ho et al., 2009) and to penetrate the zona pellucida of the oocyte (Ren et al., 2001; Quill et al., 2003). The relative intracellular Ca\(^{2+}\) concentration, \([\text{Ca}^{2+}]_{\text{intr}}\) was recorded in mouse sperm after the application of a cell permeable cyclic adenosine monophosphate (cAMP) analog in experiments by Xia et al. (2007). The \([\text{Ca}^{2+}]_{\text{intr}}\) first started to increase in the principal piece, the location of the CatSper channels, and was followed by an increase in \([\text{Ca}^{2+}]_{\text{intr}}\) along the length of the flagellum, with a sustained increase in the head region. After the initial increase in \([\text{Ca}^{2+}]_{\text{intr}}\), \(\text{Ca}^{2+}\) clearance mechanisms brought the \([\text{Ca}^{2+}]_{\text{intr}}\) back down to the basal, resting level. Xia et al. (2007) completed two additional experiments to assess the role of the CatSper channels. When the cAMP analog was applied to sperm from CatSper null mutants, no significant increase in \([\text{Ca}^{2+}]_{\text{intr}}\) was observed. After the application of ionomycin, which facilitates the transport of \(\text{Ca}^{2+}\) across the plasma membrane, there was an immediate increase in the \([\text{Ca}^{2+}]_{\text{intr}}\) in all regions of the sperm. Xia et al. hypothesized that the cAMP analog facilitates the opening of the CatSper channels, causing \(\text{Ca}^{2+}\) influx in the principal piece through the CatSper channels, initiating a tail to head propagation of \(\text{Ca}^{2+}\) (Xia et al., 2007).

We developed a 1-d reaction-diffusion model to describe the CatSper mediated \(\text{Ca}^{2+}\) dynamics associated with hyperactivation along a flat, stationary flagellar centerline (Olson et al., 2010). This model was based on the experimental results of Xia et al. (2007). In model, we assumed that the sustained increase in \(\text{Ca}^{2+}\) concentration in the head observed in the experiments, it was necessary to include the release of \(\text{Ca}^{2+}\) from an internal store in the model. Since this 1-d model will be used as a starting point, we will describe it below.

We track two chemical signaling molecules: \(\text{Ca}^{2+}\) and inositol 1,4,5-trisphosphate (IP\(_3\)), that are governed by the reaction-diffusion equations for \(t > 0\) and \(0 < s < L\):

\[
\frac{\partial \text{Ca}}{\partial t} = D_{\text{Ca}} \frac{\partial^2 \text{Ca}}{\partial s^2} + J_{\text{CAT}} - J_{\text{PMCA}} - J_{\text{IP3, leak}} - J_{\text{RNE, in}} + J_{\text{RNE, out}} \tag{10a}
\]

\[
\frac{\partial I}{\partial t} = D_{\text{I}} \frac{\partial^2 I}{\partial s^2} + J_{\text{IP3, out}} - J_{\text{IP3, in}} \tag{10b}
\]

where \(\text{Ca}(s, t)\) is the \(\text{Ca}^{2+}\) concentration (\(\mu\text{M}\)), \(I(s, t)\) is the IP\(_3\) concentration (\(\mu\text{M}\)), \(D_{\text{Ca}}\) and \(D_{\text{I}}\) are the effective \(\text{Ca}^{2+}\) and IP\(_3\) diffusion coefficients (\(\mu\text{m}^2\text{s}^{-1}\)), respectively, and \(J_{\text{IP3, out}}\) are the effective \(\text{Ca}^{2+}\) fluxes (\(\mu\text{m}^2\text{s}^{-1}\)). \(\text{Ca}^{2+}\) release from the redundant nuclear envelope (RNE), a \(\text{Ca}^{2+}\) store in the store, is assumed to be IP\(_3\) gated, therefore, \(J_{\text{IP3, out}}\) depends on the local \(\text{Ca}^{2+}\) and IP\(_3\) concentrations. Each of these fluxes are space and time dependent. The \(\text{Ca}^{2+}\) fluxes due to the RNE are localized to the neck, IP\(_3\) leak, and CatSper fluxes are localized to the principal piece (PP) of the flagellum. We note that subscripts of the flagellum, such as the microtubules, and \(\text{Ca}^{2+}\) buffering will cause the diffusion coefficient to vary. As a first approximation, we assume that the diffusion coefficient is constant and assume that the \(\text{Ca}^{2+}\) buffers are fast, immobile, and unsaturated (Olson et al., 2010; Sneyd et al., 2003; Wagner and Keizer, 1994).

In the experiments of Xia et al. (2007), the cAMP analog 8-Br-cAMP was applied to the medium where it then diffused into the cytosol of the sperm and underwent enzymatic conversion to cAMP. The opening of CatSper channels is facilitated by cAMP (Carlson et al., 2003) and is modeled as follows:

\[
J_{\text{CAT}}(s, t) = \begin{cases} 0 & \text{sgPP} \\ k_{\text{CAT}} \cdot \Omega(t) \cdot C_{\text{ext}} & s \in \text{PP} \end{cases} \tag{11a}
\]

\[
\frac{d\Omega}{dt} = v_1(1-\Omega) - v_2\Omega \tag{11b}
\]

\[
\frac{dA}{dt} = -a_{\text{deg}} A \tag{11c}
\]

where \(\text{Ca}^{2+}\) flux through the CatSper channels, \(J_{\text{CAT}}\) (\(\mu\text{M s}^{-1}\)), is proportional to the fraction of open CatSper channels, \(A(t)\), and the \(\text{Ca}^{2+}\) concentration in the surrounding medium, \(C_{\text{ext}}\). Note that \(k_{\text{CAT}}, v_1, v_2, a_{\text{deg}}\) are all rate constants that are defined in Olson et al. (2010). The fraction of open CatSper channels along the principal piece, \(0 \leq \Omega \leq 1\), depends on the local cAMP concentration, \(A(t)\). We assume the cAMP is being degraded throughout the cytosol of the flagellum following first order degradation kinetics. In Eq. (11a), the flux out through the PMCA, \(J_{\text{IP3, out}}\), is modeled using a Hill function and the leak flux into the cytosol, \(J_{\text{IP3, leak}}\), is modeled using a constant leak flux.

The last two \(\text{Ca}^{2+}\) fluxes are due to the redundant nuclear envelope (RNE), a \(\text{Ca}^{2+}\) store located at the base of the flagellum in the neck. The RNE is found in a variety of mammalian sperm (Ho and Suarez, 2001b, 2003) and immunolabeling has verified the presence of type I inositol 1,4,5-trisphosphate (IP\(_3\)) receptors on the RNE (Ho and Suarez, 2003). Due to the sustained increase in \(\text{Ca}^{2+}\) in the neck and head regions, it is assumed that \(\text{Ca}^{2+}\) is released from the RNE through an IP\(_3\) gated channel and is modeled as follows:

\[
J_{\text{RNE, out}}(s, t) = \begin{cases} 0 & \text{V}_{\text{RNE, IP3}(s, t) + V_{\text{RNE, leak}}} \\ s \in \text{Neck} & \text{sgNeck} \end{cases} \tag{12}
\]

where \(V_{\text{RNE}}\) is the Ca\(^{2+}\) flux when all IP\(_3\) receptors are open and activated on the RNE membrane (\(\mu\text{M s}^{-1}\)) and \(V_{\text{RNE, leak}}\) is the \(\text{Ca}^{2+}\) leak across the RNE membrane (\(\mu\text{M s}^{-1}\)). It is assumed that the \(\text{Ca}^{2+}\) concentration in the RNE is large enough to sustain this flux out of the RNE. For this model, \(V_{\text{RNE, leak}}\) is assumed to be constant. The probability of the IP\(_3\) receptor being open, \(P_{\text{IP3, open}}\) is modeled in a phenomenological manner where we assume that the IP\(_3\) receptor has three binding domains, one for IP\(_3\) and two for \(\text{Ca}^{2+}\) (Olson et al., 2010; Sneyd et al., 1995). The last flux term \(J_{\text{IP3, leak}}\) is a flux term for the pumping of excess \(\text{Ca}^{2+}\) back into the RNE and is modeled using a Hill function.
We assume that production of IP3 is in the neck, the location of the RNE, and is modeled as a Hill equation,

\[
IP_{prod}(s,t) = \begin{cases} 
0 & \text{Neck} \\
v s \cdot A(t) \left( \frac{\text{Ca}^2(s,t)}{\text{Ca}^2(s,t) + k_p} \right) & s \in \text{Neck}
\end{cases}
\]  

where \( k_p \) (\( \mu \text{m} \)) is the \( \text{Ca}^{2+} \) threshold concentration for IP3 synthesis and \( v_s \) (s\(^{-1}\)) is the maximal rate of IP3 production. The degradation of IP3, \( IP_{degr} \), is assumed to follow first order degradation kinetics.

The framework of the \( \text{Ca}^{2+} \) model summarized here is based on standard \( \text{Ca}^{2+} \) models in other cell types (Olson et al., 2010). In this simple 1-d model, determined by balancing leak fluxes and using experimental values sperm were used when possible and other parameters were specified locations. Parameters from previous experiments with assumed that there was a uniform density of channels in each of the specified locations. Parameters from previous experiments with sperm were used when possible and other parameters were determined by balancing leak fluxes and using experimental values for other cell types (Olson et al., 2010). In this simple 1-d model, keeping track of two signaling molecules, \( \text{Ca}^{2+} \) and IP3, gave good agreement with the qualitative trends of the \( \text{Ca}^{2+} \) concentration observed in the experiments (Olson et al., 2010; Xia et al., 2007).

We now extend this model to capture the time and space dependent \( \text{Ca}^{2+} \) and IP3 concentration on a stretching and deforming interface, a swimming flagellum. The two reaction-diffusion Eqs. (10a)–(10b), will be rewritten in terms of the moving filament \( X(s,t) \) and will account for changes in material surface elements due to stretching and compression:

\[
\left( \frac{\partial \text{Ca}}{\partial t} \right) s + f \frac{\partial \text{Ca}}{\partial X} = D_s \frac{\partial^2 \text{Ca}}{\partial X^2} + \sum_k J_{c}(X(s,t)) \tag{14a}
\]

\[
\left( \frac{\partial IP3}{\partial t} \right) s + f \frac{\partial IP3}{\partial X} = D_s \frac{\partial^2 IP3}{\partial X^2} + \sum_k J_{k}(X(s,t)) \tag{14b}
\]

where \( J_{c} \) corresponds to the \( \text{Ca}^{2+} \) fluxes and \( J_{k} \) refers to IP3 synthesis and degradation. We account for local stretching and the immersed interface in order to accurately account for the \( \text{Ca}^{2+} \) and IP3 concentrations. These equations, derived in Lai et al. (2008) and Stone (1990), ensure that mass is conserved on a moving interface when each of the fluxes are equal to zero. Note that the CatSper channels are maintained at a constant density and they act as a local source of \( \text{Ca}^{2+} \) influx into the cytosol. This \( \text{Ca}^{2+} \) influx through the CatSper channels is independent of the local fluid velocity and stretching of the flagellum.

4. Non-dimensional model

We nondimensionalize the model using the scales and parameters in Table 1. The non-dimensional variables for the incompressible Stokes equations are defined as follows:

\[
\begin{align}
\xi &= \frac{X}{L} \\
\eta &= \frac{u}{V} \\
\tau &= \frac{p}{\rho V^2} \\
\bar{f} &= \frac{f}{f_{\text{max}}} \\
\bar{r} &= \frac{r}{r_{\text{max}}} \\
\end{align}
\]  

(15)

where the characteristic scales are based on mouse sperm. We set the length scale as \( L = 100 \mu \text{m} \), roughly the length of a mouse sperm flagellum (Cummins and Woodall, 1985). The beat frequency of hyperactivated sperm is \( \sim 10 \text{ Hz} \); we set the time scale (\( \tau \)) and velocity scale (\( \eta \)) accordingly (Wolf et al., 1986; Mukai and Okuno, 2004). The characteristic force and pressure scales are \( \bar{f} \) and \( \bar{p} \), respectively. The Stokes equations, using the non-dimensional variables in Eq. (15), simplify to

\[
0 = -\nabla \bar{p} + A \bar{u} + \bar{f} \tag{16a}
\]

\[
0 = \nabla \cdot \bar{u} \tag{16b}
\]

where \( \bar{u}, \bar{p}, \) and \( \bar{f} \) are the non-dimensional velocity, pressure, and force density, respectively. The forces given in Eq. (1c) are also appropriately nondimensionalized.

Since the forces are coupled to the \( \text{Ca}^{2+} \) concentration through the preferred curvature function, we nondimensionalize the system of reaction-diffusion equations on the deforming interface. We define the following non-dimensional variables for the \( \text{Ca}^{2+} \) model in Eqs. (14a) and (14b):

\[
\bar{c}_{\text{Ca}} = \frac{c_{\text{Ca}}}{C}, \quad \bar{r} = \frac{r}{R} \tag{17}
\]

where \( C \) is a characteristic concentration, set to 1 \( \mu \text{M} \). When we rewrite Eqs. (14a) and (14b) using the non-dimensional variables in Eqs. (15) and (17), we get

\[
\left( \frac{\partial \bar{c}_{\text{Ca}}}{\partial t} \right) s + \bar{f} \frac{\partial \bar{c}_{\text{Ca}}}{\partial X} = \frac{1}{\bar{P}_{\text{ec}}} \frac{\partial}{\partial X} \left( \frac{\partial \bar{c}_{\text{Ca}}}{\partial X} \right) + \frac{\bar{\lambda}}{\bar{C} \bar{V}} \sum_k J_{c} \tag{18a}
\]

\[
\left( \frac{\partial \bar{IP3}}{\partial t} \right) s + \bar{f} \frac{\partial \bar{IP3}}{\partial X} = \frac{1}{\bar{P}_{\text{ec}}} \frac{\partial}{\partial X} \left( \frac{\partial \bar{IP3}}{\partial X} \right) + \frac{\bar{\lambda}}{\bar{C} \bar{V}} \sum_k J_{k} \tag{18b}
\]

where \( \bar{c}_{\text{Ca}} \) and \( \bar{r} \) are the non-dimensional \( \text{Ca}^{2+} \) and IP3 concentrations, respectively. The Peclet numbers, \( \bar{P}_{\text{ec}} \) and \( \bar{P}_{\text{ds}} \), are non-dimensional measures of the relative importance of advection to diffusion. Note that each of the flux terms are appropriately nondimensionalized and the preferred curvature function is appropriately rewritten.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Values</th>
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<tbody>
<tr>
<td>( \mathcal{L} )</td>
<td>Length scale</td>
<td>( \sim 1 \times 10^{-4} \text{m} (100 \mu \text{m}) ), mouse sperm (Cummins and Woodall, 1985)</td>
</tr>
<tr>
<td>( T )</td>
<td>Time scale</td>
<td>0.1 s (beat frequency of hyperactivated mouse sperm ( \sim 10 \text{ Hz} ) (Mukai and Okuno, 2004; Wolf et al., 1986)</td>
</tr>
<tr>
<td>( V )</td>
<td>Velocity scale (wavespeed)</td>
<td>( \mathcal{L} \cdot 10 \text{ Hz} = 1 \times 10^{-2} \text{m s}^{-1} (1000 \mu \text{m s}^{-1}) )</td>
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<tr>
<td>( \rho )</td>
<td>Viscosity</td>
<td>For water ( 1 \times 10^{-3} \text{kg m}^{-1} \text{s}^{-1} )</td>
</tr>
<tr>
<td>( f )</td>
<td>Force scale</td>
<td>( \rho V^2 )</td>
</tr>
<tr>
<td>( \bar{P} )</td>
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<tr>
<td>( \bar{C} )</td>
<td>Concentration scale</td>
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<tr>
<td>( \bar{P}_{\text{ec}} )</td>
<td>( \text{Ca}^{2+} ) Peclet number</td>
<td>( (\bar{C}V)/D_{\text{Ca}} \sim 5000 )</td>
</tr>
<tr>
<td>( \bar{P}_{\text{ds}} )</td>
<td>IP3 Peclet number</td>
<td>( (\bar{C}V)/D_{\text{IP3}} \sim 333 )</td>
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</table>
to be a function of the nondimensional Ca\(^{2+}\) concentration. For notational simplicity, all parameters and variables in the following sections are nondimensional unless specifically stated otherwise, and the bars are omitted.

5. Numerical methods and parameters

A Stokeslet is the velocity field due to a point force \(\mathbf{g}_k\) exerted at \(X_k\), in an unbounded 3-d fluid and is given by

\[
\mathbf{u}_k(x) = \frac{g_k}{8\pi\epsilon^2} \frac{[g_k \cdot (x - X_k)](x - X_k)}{\|x - X_k\|^3}
\]

where \(r = \|x - X_k\|\). Note that this solution is singular at \(x = X_k\), which motivates the use of numerical methods that regularize this singularity.

5.1. Regularized stokeslets

The method of regularized Stokeslets is a Lagrangian method for computing Stokes flow due to a distribution of forces (Cortez, 2001; Cortez et al., 2005). This method has been used to investigate the hydrodynamics of sperm swimming near surfaces (Gillies et al., 2009), flagellar bundling of bacteria (Flores et al., 2005), and the forward progression of a rigid helical tube of nonzero thickness as a model of spirochete motility (Cortez et al., 2005). We use the method of regularized Stokeslets in 3-d, to solve the nondimensional Stokes equations. The flagellar centerline is represented by \(X_k\) for \(k = 1, 2, \ldots, M\) and the force density is

\[
\mathbf{f}(x) = \sum_{k=1}^{M} g_k \phi_k(x - X_k) \Delta t
\]

where \(\phi_k\) is the radially symmetric smooth approximation to a 3-d delta distribution given in Eq. (2). Note that \(\phi_k\) satisfies \(\int \phi_k(x - X_k) \, dx = 1\). The flagellar centerline, as in Gillies et al. (2009), acts as a distribution of regularized point forces, and returns a virtual radius that we take as the regularization parameter \(\epsilon\). This regularization parameter gives the extent of the region about the centerline where the force is applied. The regularized Stokeslet formulation treats the immersed flagellum as part of the fluid domain (all of \(\mathbb{R}^3\) where smoothed forces are distributed. This is in contrast to Lighthill’s slender body theory approximation (Lighthill, 1976), where the flagellar centerline is the exterior of the cylindrical flagellum, whose centerline supports singular forces. In this model, we do not enforce boundary conditions sharply on the surface of a cylindrical tube by including higher order singularities, as in slender body theory (Lighthill, 1976). However, Gillies et al. (2009) have shown that the near-field velocity error for the ‘smeared’ flagellum is small. Requiring that the fluid velocity decays at infinity, the unique solution to the Stokes Eqs. (16a) and (16b) in all of \(\mathbb{R}^3\) due to the smoothed forces in Eq. (20) is

\[
\mathbf{u}(x) = \frac{1}{8\pi\epsilon^2} \sum_{k=1}^{M} \left[ g_k (r^2 + 2\delta^2) + g_k \cdot (x - X_k) \right] (x - X_k) \frac{1}{(r^2 + \delta^2)^{3/2}}
\]

This velocity is an exact solution of the Stokes equations and is everywhere incompressible. Note that, while the flow is three-dimensional, we restrict the motion of the flagellar centerline to a fixed plane.

5.2. Numerical algorithm

The details of the numerical time integration are outlined below. Note that \(\mathbf{u}_k^n\) denotes a variable \(\bullet\) at the \(n\)th time step at the \(k\)th point on the flagellar centerline and \(k = 1, 2, \ldots, M\).

Given \(X_k^n, \mathbf{g}_k^n, \) and \(\Gamma^n\):

1. Evaluate the forces \(g_k^n\)
   - Calculate the preferred curvature, \(\frac{\partial}{\partial \tau}\n\)
   - Compute \(g_k = -\frac{\partial g_k^{n-1}}{\partial \tau}\), discretizing the energy densities in Eq. (4) as
     \[
     \epsilon = S_1 \sum_{k=1}^{M-1} \left\{ \frac{|X_k^n - X_{k+1}^n|}{\Delta s} - 1 \right\}^2 + S_2 \sum_{k=2}^{M-1} \left\{ \frac{|z \cdot (X_k^n - X_{k-1}^n)|}{\Delta s} - \epsilon_n \right\}^2
     \]
     where \(z = (0, 0, 1)\).

2. Evaluate the fluid velocity at the flagellar centerline, \(\mathbf{u}^{n+1}(X_k^n)\) using Eq. (21)

3. Use a 4th order Runge–Kutta method to update the locations of the flagellar centerline, \(X_k^{n+1}\)

4. Solve for \(\mathbf{g}_k^{n+1}\) and \(\Gamma^{n+1}\) using a Crank–Nicolson scheme to discretize the reaction-diffusion Eqs. (18a) and (18b) (outlined in Lai et al., 2008)

5.3. Parameters, initial conditions, and boundary conditions

A mouse sperm flagellum has a length of \(\sim 100\ \mu m\) and a radius of \(1\ \mu m\) (Cummins and Woodall, 1985). In the nondimensional model, the flagellar length is set to 1 and accordingly, the regularization parameter is set to \(\delta = 0.1\). The flagellar centerline is discretized using \(M = 100\) points. The flagellum is divided into the following regions: end piece \(s = 0\), principal piece \(s = 0.07\) to 0.73, midpiece \(s = 0.73\) to 0.9, neck \(s = 0.9\) to 0.92, and the head \(s = 0.92\) to 1. The time step is \(\Delta t = 5 \times 10^{-7}\), representing \(2 \times 10^6\) time steps per beat period.

The parameters that govern the Ca\(^{2+}\) and IP\(_3\) dynamics are given in Olson et al. (2010). As described previously, we assume a constant, effective diffusion coefficient for the Ca\(^{2+}\) and IP\(_3\) that is an order of magnitude slower than diffusion in water to account for barriers to diffusion and buffering (Olson et al., 2010). We prescribe no flux boundary conditions for the Ca\(^{2+}\) and IP\(_3\) concentrations at the ends of the flagellar centerline. The Ca\(^{2+}\) concentration in the external fluid is assumed to be constant and at a nondimensional concentration of 2000 and the Ca\(^{2+}\) concentration within the cytosol of the flagellum is initialized at 0.1. In all of the simulations, the cAMP was applied at \(t = 1\) nondimensional time, initiating the CatSper mediated Ca\(^{2+}\) dynamics.

Since the nondimensional Ca\(^{2+}\) concentration varies between \(0.1\) and 1, we choose to vary \(k_a\), the nondimensional concentration where half of the receptors are bound by Ca\(^{2+}\), in this range. The nondimensional maximum amplitude, \(V_s\), is chosen to be 0.45 (unless stated otherwise), which corresponds to a dimensional amplitude of 45 \(\mu m\). The exact flagellar bend amplitude of mouse sperm is difficult to measure due to the high degree of asymmetry in the bending. The amplitude of lateral head displacement (ALH) is often reported as an indicator of how the flagellar amplitude is changing. In mouse sperm, hyperactivated sperm have an ALH of \(\sim 18\ \mu m\) (Marquez et al., 2007), which is smaller than the amplitude in the flagellum. The flagellum is initialized as a sine wave and we prescribe a periodic amplitude in the flagellum.

5.4. Calculation of flexural rigidity

The stiffness parameters chosen should reflect the material properties of mammalian sperm. In order to estimate the flexural
rigidity of our elastic model flagellum, we follow the procedure of Lim and Peskin (2004). The flagellum is initialized as an arc of a circle with curvature $K$. The total elastic energy in the system stored is calculated using Eq. (4) for the given initialized curve with the preferred curvature $\zeta(s,t)$ set to 0. That is, we are thinking of the flagellum as an elastic beam that wants to be flat with zero curvature and we are bending it in a series of preset curvatures. The energy $E$ that we are calculating for the generalized Euler elastica is computed for the various curvatures. For a homogeneous elastic beam, the energy is

$$E = \frac{Y}{K} \int_0^L ds$$

(23)

where $Y$ is the flexural rigidity, $K$ is curvature, and $L$ is the length of the beam. The flexural rigidity is defined as the product of the Young’s modulus and the second moment of the area. We calculate the energy $E$ for several values of curvature using constant stiffness coefficients $S_1 = 75$ and $S_2 = 0.075$. From these calculations, we determine that the flexural rigidity is $Y = 1.485 \times 10^{-15}$ Nm. In order to compare this with experimental results of a 3-d flagellum, we need to multiply by a length scale to achieve a flexural rigidity of the order of the flexural rigidity measured for the model flagellum is of the order of the flexural rigidity measured for mammalian sperm.

We choose to fix the bending stiffness at $S_2 = 0.075$ for all simulations to isolate the effect of $Ca^{2+}$ dynamics on the bending energy. As mentioned in Section 2.2, we wish to study different cases for the passive tensile stiffness $S_1$. We study the case of a constant tensile coefficient using $S_1 = 75$. We also explore a taper in passive tensile coefficient along the length of the flagellum for bull sperm was found to be $\sim 4-27 \times 10^{-12}$ Nm$^2$ (Lesich et al., 2008) and was in the range of $1-10 \times 10^{-10}$ Nm$^2$ for rat sperm (demembranated and dynein arms disabled) (Schmitz-Lesich and Lindemann, 2004). Therefore, the flexural rigidity of our model flagellum is of the order of the flexural rigidity measured for mammalian sperm.

6. Computational results of integrative model

6.1. CatSper mediated $Ca^{2+}$ dynamics and emergent waveforms

Representative results of the CatSper-mediated $Ca^{2+}$ dynamics along the beating flagellum with a constant tensile stiffness $S_1$ are shown in Fig. 3(a) and (b). The initial nondimensional $Ca^{2+}$ concentration is set to 0.1 along the length of the flagellum and there is assumed to be a constant, high concentration of $Ca^{2+}$ in the external fluid. As seen in Fig. 3(a), application of cAMP at $t = 1$ allows $Ca^{2+}$ to enter the principal piece through the CatSper channels. The inset zooms in on the $Ca^{2+}$ concentration at $t = 5, 5.4,$ and 5.8. Note that the $Ca^{2+}$ concentration has small oscillations in concentration due to the flagellar undulations, which cause local stretching and compression of the flagellar centerline, accounted for in Eqs. (18a) and (18b).

In Fig. 3(b), the IP$_3$ concentration initially starts at 0 along the length of the flagellum, and is being synthesized locally at the RNE, the $Ca^{2+}$ store, in the neck. Once the IP$_3$ reaches a threshold concentration, $Ca^{2+}$ is released from the RNE as seen in Fig. 3(a). At later time points, the $Ca^{2+}$ concentration reaches a maximum of about 1, the CatSper channels close, and $Ca^{2+}$ clearance mechanisms pump $Ca^{2+}$ out of the principal piece to the surrounding fluid and sequester $Ca^{2+}$ back into the RNE (results not shown). This allows for $Ca^{2+}$ and IP$_3$ to return to their resting or initial concentrations.

Fig. 4 shows snapshots of the velocities in the fixed plane of the flagellar centerline and the $Ca^{2+}$ concentration along the flagellum for a representative simulation with a constant tensile stiffness $S_1$. The flagellum is initialized as a sine wave with the prescribed nondimensional wavenumber $k = 2\pi$ and amplitude as given by the nondimensional $Ca^{2+}$ concentration and maximum amplitude $V_a$. The $Ca^{2+}$ concentration initially starts at a resting level of 0.1 and increases in the principal piece at nondimensional time $t = 1$ when the cAMP is applied and CatSper channels subsequently open. By time $t = 22.125$, shown in Fig. 4(b), there is $Ca^{2+}$ release from the RNE, the $Ca^{2+}$ store in the neck. The evolving $Ca^{2+}$ concentration along the length of the flagellum is coupled to the preferred amplitude of the waveform through Eq. (8). As time progresses to $t = 51.75$ in Fig. 4(c), the higher concentrations of $Ca^{2+}$ are observed along with a highly asymmetrical waveform with increased amplitude.

The $Ca^{2+}$ concentration evolves in time and depends on the local configuration of the flagellum as well as the local IP$_3$ concentration. In turn, the emergent waveform is driven by the preferred curvature function. In Fig. 5, flagellar waveforms over

![Fig. 3. Concentration along the length of the flagellum for a few time points: (a) $Ca^{2+}$ concentration, (b) IP$_3$ concentration. In (a), the $Ca^{2+}$ concentrations start at a resting level and then increases in the principal pieces as the CatSper channels open. When the $Ca^{2+}$ and IP$_3$ concentration reach a threshold concentration, the RNE in the neck releases additional $Ca^{2+}$. The arrow and inset zooms in on the $Ca^{2+}$ concentrations in the principal piece at time points $t = 5, 5.4,$ and 5.8. Note that the $Ca^{2+}$ concentration has small oscillations in concentration due to the local stretching and compression of the flagellum, accounted for in the left hand side of Eq. (18a). Note that the sperm is divided into the following regions: end piece $s = 0-0.07$, principal piece $s = 0.07-0.73$, midpiece $s = 0.73-0.9$, neck $s = 0.9-0.92$, and the head $s = 0.92-1$. This simulation had a constant tensile stiffness $S_1$, and $K_a = 0.3$ if $\zeta(s,t) > 0$ and $K_a = 0.5$ if $\zeta(s,t) < 0$.](image-url)
the given nondimensional time period are shown and the first material point of the flagellar centerlines is superimposed for simulations with a linear taper in the tensile stiffness $S_1$. Initially, the waveforms resemble sine waves of constant amplitude, as seen at earlier time points in Fig. 5(a). The Ca\textsuperscript{2+} concentration is increasing due to the CatSper channels opening and the resulting Ca\textsuperscript{2+} release from the RNE. This causes the amplitude of the sine wave to increase largely in the principal piece as time increases. Since we also include an asymmetry in the model, the amplitude is slightly larger in one direction (the principal bend). The sperm flagellum initially swims to the right, but then its trajectory assumes a counter-clockwise, circular path. In Fig. 5(f), the overlaid emergent waveforms are qualitatively similar to those obtained by Carlson et al. (2003) for hyperactivated mouse sperm. In Fig. 6, we compare our simulated tracings of flagella to the experimental tracings and micrographs of Marquez and Suarez (2004) and Suarez and Dai (1992). The top row depicts tracings of mouse sperm, the middle row are the results of the computational model including CatSper mediated Ca\textsuperscript{2+} dynamics, and the bottom row shows micrographs of bull sperm. Each frame shows tracings that are approximately one-fourth of a beat apart. When the Ca\textsuperscript{2+} concentration is low and fairly uniform, our simulated model with a 4th order taper in tensile stiffness $S_1$ has waveforms similar to that of mouse activated motility (compare Fig. 6(a) and (c)). As the Ca\textsuperscript{2+} concentration increases via the CatSper mediated Ca\textsuperscript{2+} influx, the waveform is modified to be asymmetric and has a larger bend amplitude (Fig. 6(d)). When the Ca\textsuperscript{2+} reaches its maximal concentration in Fig. 6(e), we see that the fully hyperactivated waveform is qualitatively similar to hyperactivated mouse sperm in Fig. 6(b). Coupling the Ca\textsuperscript{2+} dynamics to the preferred curvature, we observe that the emergent flagellar waveforms have an asymmetry that is evolving throughout the simulations. We also compare our simulated results to bull sperm, shown in the bottom row. Even though bull sperm are approximately half of the length of mouse sperm, they exhibit the same asymmetrical beating in the hyperactivated state.
6.2. Effect of Ca\textsuperscript{2+} dynamics on trajectories and swimming speeds

Often, three types of motility are defined: progressive forward motility or activated motility, a transitional motility, and hyperactivated motility. Progressive motility is characterized by highly linear forward trajectories and a symmetrical waveform. Hyperactivated motility occurs when the flagellum develops an asymmetrical waveform, characterized by highly nonlinear and often times circular trajectories. In general, hyperactivated motility is associated with a decrease in beat frequency and an increase in the bend angle of the most proximal bend (Suarez et al., 1991; Ohmuro and Ishijima, 2006). In order to assess our model for capturing these types of motility, we look at trajectories of the last immersed boundary point at the end of the flagellum over the course of a simulation.

In Fig. 7, trajectories of the flagellum are shown for the three passive tensile stiffness coefficients $S_1$, grouped by column. The top row accounts for the full model, where the CatSper mediated Ca\textsuperscript{2+} dynamics of hyperactivation is used. The bottom row assumes there is an asymmetry, but there is a constant Ca\textsuperscript{2+} concentration along the length of the flagellum. The first column, graphs Fig. 7(a) and (d) are for the case of constant tensile stiffness $S_1$ along the length of the flagellum. In Fig. 7(a), the hyperactivated Ca\textsuperscript{2+} dynamics are included and the tail starts off with a low amplitude undulation. As the Ca\textsuperscript{2+} concentration increases, the tail maps out a larger amplitude trajectory and starts to move in a counter-clockwise circle. The radius of the circle the sperm is swimming decreases at first as the Ca\textsuperscript{2+} concentration is increasing. After this transitional motility, the sperm is fully hyperactivated and the sperm then swims at a circle of a fairly constant radius as the Ca\textsuperscript{2+} receptors saturate in the Hill function for the target amplitude. In comparison, Fig. 7(d) shows the same simulation assuming a basal or resting level of Ca\textsuperscript{2+} throughout the entire simulation. For the same length of time, accounting for asymmetry with a constant Ca\textsuperscript{2+} concentration gives a circular counter-clockwise path with the

![Fig. 6.](image-url)
constant and linear taper of forward progressive motility or activated motility. In the cases of swimming in a linear trajectory, which would be classified as in the tensile stiffness coefficient (Fig. 6(d) and (e)). Since this is a fully coupled system, a change that are very different from a simple sine wave (refer to to bend a larger amount, causing complex flagellar waveforms that then interacts with the viscous fluid. As time progresses, we can see large differences in the trajectories of the sperm and to determine the kinematics (Mortimer, 2000; Zhu et al., 1994; Grunert et al., 1990). The following metrics are used to characterize and identify hyperactivated motility (Mortimer, 2000):

- \( \text{VCL} \) = curvilinear velocity, the total distance the sperm head covers in a given observation period (usually the max of VCL, VSL, VAP)
- \( \text{VSL} \) = straight line velocity, the straight line distance between the first and last points of a given trajectory over a given observation period
- \( \text{VAP} \) = average path velocity, the distance the sperm has traveled in the average path direction of movement in a given observation period, i.e. to determine the average path direction, we have to create an averaged smooth path first by interpolation and then find the distance of that curve over a given observation period
- \( \text{LIN} \) = linearity = \( \frac{\text{VSL}}{\text{VCL}} \times 100 \)

Since these metrics are reported in the literature to distinguish hyperactivated motility, they serve as a benchmark comparison between theory and experiment.

In general, most of the experimental data calculations are performed by tracking the center of the sperm head, as it is easiest to follow. Therefore, all of the velocity and linearity results from our computations track a material point near the head of the flagellar centerline. In order to compare our nondimensional computer simulated model to experimental results, we return to dimensional units.

In Fig. 8(a), we compare the swimming speeds for different tensile stiffnesses (\( S_1 \)) and model parameters. The calculations are done at 30 Hz, i.e. we determine the velocities based on sampling
30 times per second. For a given set of parameters, the swimming speeds vary for the three cases of tensile stiffness coefficients used. As shown in Fig. 8, when the CatSper mediated Ca\(^{2+}\) dynamics are included, the sperm starts off with an initial average path velocity VAP of 25–75 \(\mu m\) s\(^{-1}\), which increases initially and then levels off to a VAP of 60–200 \(\mu m\) s\(^{-1}\). We can think of the increasing region as the transitional motility and the maximal velocity as the hyperactivated VAP. As shown in Table 2, the experimentally measured average path velocity VAP for hyperactivated mouse sperm is approximately 135 \(\mu m\) s\(^{-1}\), Fig. 8(a), indicates that simulations that do not account for Ca\(^{2+}\) dynamics resulted in fairly constant VAP values that are considerably lower than those observed experimentally. It is interesting to note that the case of a linear taper in tensile stiffness \(S_1\) had the highest average path velocity VAP. In Fig. 8(b), the results for the curvilinear velocity VCL are shown for the different stiffness cases and two values of \(V_{\text{Va}}\), the maximal amplitude. As expected, a smaller maximal amplitude results in a smaller curvilinear velocity VCL for all passive stiffness coefficients. As with the average path velocity VAP, we observe that the linear taper in tensile stiffness \(S_1\) has the highest VCL. The curvilinear velocity VCL in our simulations are also in line with experimental data; experiments have reported mouse VCL at approximately 393 \(\mu m\) s\(^{-1}\) (Marquez et al., 2007).

The linearity, a ratio of the straight line velocity to the curvilinear velocity, decreases from activated to hyperactivated motility as the flagellum switches from linear to circular trajectories. In Table 3, the linearity are reported for the three tensile stiffness coefficients \(S_1\) used in the model along with experimental values for mouse sperm. We stay within the range of experimental values of linearity for mouse data and we note that the case of the 4th order taper in tensile stiffness \(S_1\) exhibited the lowest linearity values. In comparison to CASA values for velocities and linearity, results from our computational model are within the range of experimental results for mouse sperm. We note that all of the velocity and linearity calculations were highly sensitive to the frequency of sampling per dimensional time when the results are post-processed. To be consistent, all velocities were taken at a sampling frequency of 60 Hz after \(t=1\) s dimensional time. When the results were post-processed at a sampling rate of 60 Hz, the results varied greatly with the curvilinear velocity VCL having the largest variation. Since VCL is calculated as the total distance the sperm head covers in an observation period (Mortimer, 2000), the distance covered per observation period increased at the higher sampling frequency. All of the calculations also varied slightly based on the location that they were sampled. Since CASA results are generally taken from the center of the head, our results may not be consistent since we do not accurately account for head morphology.

### Table 2

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<td>VAP ((\mu m) s(^{-1}))</td>
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### Table 3

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<td>65.9</td>
<td>Fresh (activated, mouse) (Suarez and Dai, 1992)</td>
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<tr>
<td>37.6</td>
<td>HA (mouse) (Suarez and Dai, 1992)</td>
</tr>
<tr>
<td>54 ± 12</td>
<td>Forward progressive (human) (Sukcharoen et al., 1995)</td>
</tr>
<tr>
<td>30 ± 10</td>
<td>Transition (human) (Robertson et al., 1988; Sukcharoen et al., 1995; Zhu et al., 1994)</td>
</tr>
<tr>
<td>11 ± 7</td>
<td>HA (human) (Burkman, 1991; Gruenert et al., 1990; Mortimer and Mortimer, 1990; Robertson et al., 1988; Sukcharoen et al., 1995)</td>
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<td>31.39</td>
<td>Constant (S_1) and CatSper/Constant Ca(^{2+})</td>
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<td>44.13</td>
<td>Linear taper of (S_1) and CatSper Ca(^{2+})</td>
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<td>15.5</td>
<td>4th order taper of (S_1) and CatSper Ca(^{2+})</td>
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#### 7. Discussion

The details of how Ca\(^{2+}\) affects the dynein-microtubule force generation is not known. There are several possible Ca\(^{2+}\) binding sites that could initiate a chain of events to alter the flagellar beat. One candidate is calmodulin (CaM), a Ca\(^{2+}\) receptor that has been localized to the principal piece of the flagellum (Schiøtt et al., 2007). CaM is a plausible place for the Ca\(^{2+}\) to act since CaM inhibitors have been observed to reduce percentages of activated and hyperactivated sperm (Si and Olds-Clarke, 2000), decrease the
swimming speed of sperm (White and Aitken, 1989; Ahmad et al.,
1995), and alter the beat patterns (Aoki et al., 1999; Tash and Means,
1982). After Ca$^{2+}$ binds to CaM, kinases such as CaMKIV and CaMKII $\alpha$ may be activated, which could cause a conformational change to alter the waveform. CaM kinases have been identified in the flagella of human (Ignozit and Suarez, 2005) and bovine sperm (Marin-
Briggler et al., 2005) and, in the presence of CaMKII inhibitors,
hyperactivation was inhibited (Ignozit and Suarez, 2005). Asymme-
try of the waveform could be due to an asymmetric distribution of
calmodulin along the flagellum.

In this model, we differentiate between two ‘sides’ of the flagellum by designating a principal bend direction and a reverse bend direction, that correspond to the sign of the preferred curvature. For each side of the flagellum, we define the parameter $k_A$ as the number of available Ca$^{2+}$ receptors. By choosing a slightly different value of $k_A$ on each side, we achieve asymmetric flagellar beatforms. The asymmetry in waveform is minimal at early simulation times, but increases as the Ca$^{2+}$ dynamics evolve. Here, the preferred amplitude was chosen to be a continuous function of Ca$^{2+}$, but this can easily be modified so that the preferred amplitude responds differently to values of Ca$^{2+}$ beyond a specified threshold.

Different flagellar bend directions are easily identified in mouse sperm because of their hook-shaped head. Recent experi-
ments by Chang and Suarez (2010) have shown that mouse sperm can achieve asymmetric bends in both the pro-hook and anti-
hook direction. Mouse sperm incubated under capacitating con-
ditions, once hyperactivated, exhibit pro-hook bends. In contrast, capacitated mouse sperm that are treated with thimerisol exhibit pronounced anti-hook bends (Chang and Suarez, 2010). It has been shown that thimerisol induces Ca$^{2+}$ release from internal stores (Marquez et al., 2007). The change in bend direction could, therefore, be due to different time courses of Ca$^{2+}$ evolution. In the pro-hook case, Ca$^{2+}$ first enters the flagellum through the CatSper channels on the principal piece, but in the anti-hook case, Ca$^{2+}$ first enters the flagellum through the internal stores near the head. We remark that our simple model does not require a change in the origin of the influx of Ca$^{2+}$ along the flagellum to change principal bend direction. This change in principal bend direction can be achieved just by altering the binding affinities for Ca$^{2+}$ on either side of the flagellum.

The preferred curvature of our model flagellum is driven by the evolving Ca$^{2+}$ dynamics. In the current model we do not include any Ca$^{2+}$ dependence on preferred wavelength or beat frequency. In fact, the flagellar beat frequency decreases when sperm switch from activated to hyperactivated motility (Suarez et al.,
1991; Ohmuro and Ishijima, 2006). We hope to examine the sensitivity of the emergent waveforms and swimming progression to the choice of parameters in the CatSper mediated Ca$^{2+}$ dynamics model and the Ca$^{2+}$ parameters in the target amplitude function (Olson et al., 2010). Moreover, this model does not take into account the internal structure of the axoneme, but phenomenologically expresses the coupling of Ca$^{2+}$ to a preferred curvature. We plan to extend this model to one that includes discrete representations of dynein motors (Dillon et al., 2003), whose activation kinetics depends upon the evolving Ca$^{2+}$ dynamics, and may be governed by mechanisms such as curvature control (Brokaw, 1971, 1972a,b), the geometric clutch hypothesis (Lindemann, 1994a,b), or dynein cross bridge cycle models (Sugino and Naitoh, 1982; Murase and Shimizu, 1986; Camelet et al., 1999).

8. Conclusions

Calcium dynamics play a large role in initiation and main-
tenance of hyperactivated motility, which is vital for mammalian spermatooza to reach and fertilize the egg. We have presented a model that couples CatSper mediated Ca$^{2+}$ dynamics to a simple mechanical model of an actuated, elastic flagellum that is, in turn, coupled to a viscous, incompressible fluid. While the flagellar model is structurally simple, we use 3-d Stokes equations to describe the fluid dynamics, and accurately account for the diffusion and fluxes of Ca$^{2+}$ and IP$_3$ on a moving interface. The simplicity of the flagellar model, along with the use of the grid-
free method of regularized Stokeslets, provides an efficient computational framework that allows us to resolve the fast time scale of a single flagellar beat and to simulate many such beats to capture the much longer time scale of the Ca$^{2+}$ dynamics.

We have investigated how these flagellar waveforms give rise to the nonlinear trajectories characteristic of hyperactivated sperm. In particular, this model elucidates the importance of CatSper mediated Ca$^{2+}$ dynamics in generating hyperactivated flagellar motion. When preferred curvature was specified as a function of asymmetry in Ca$^{2+}$ binding affinities, but not Ca$^{2+}$ dynamics, the resulting waveforms are not characteristic of hyperactivation. However, adding the Ca$^{2+}$ dynamics to drive the preferred curva-
ture did give rise to emergent waveforms and trajectories that closely match experimental data for hyperactivated motility. In addition, this model shows that motility properties are dependent upon the distribution of tensile stiffness along the flagellum. We view this model as a starting point to investigate the biochemistry and mechanics of sperm hyperactivation and there are many avenues yet to explore.

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