In silico flagella modeling of the *T. cruzi* parasite

Kyle Roberts

BBSI

Virginia Commonwealth University

Richmond, Virginia

Introduction

Despite the advances that modern medicine has made to protect against disease, parasitic diseases are still a worldwide epidemic. Three prevalent diseases caused by parasites are malaria, schistosomiasis, and Chagas' disease. It is estimated that 16 to 18 million people are infected with Chagas' disease, and 50,000 people of those infected will die each year (CDC, 2004).

Trypanosoma cruzi (*T. cruzi*) is the parasite that causes Chagas' disease. It is a uniflagellate protozoan parasite that belongs to the order Kinetoplastida (Bastin, 2000). During its life cycle the parasite lives in both an insect host and a mammalian host, and has 3 developmental stages that are seen in both the invertebrate and vertebrate host (De Souza, 2002). Due to the complexity of the organism's life cycle dynamics, it is an intriguing model to study host-parasite interactions.

In particular, the flagellum of the *T. cruzi* parasite is a very interesting structure to study. The flagella of the *T. cruzi* is hypothesized to be involved with its ability to reach and invade host cells as well as be involved with the attachment to the host cell (Bastin, 2000), thereby making it an important component of the parasite's behavior. Thus, the more we understand about the flagellum of the *T. cruzi* and how the *T. cruzi* interacts with its environment, the greater ability one will have to fight diseases caused by parasites.

Currently I am developing a continuum model to describe an eukaryotic flagella in three dimensions. The model is not specific to *T. cruzi* at the moment, but will hopefully be updated in the future to include certain characteristics of the *T. cruzi* flagella. I have worked on solving equations that have been previously developed to describe the flagella (Brokaw 2002, Brokaw 1985). The model is based on whether dynein motors are on or off. The motors are switched on or off based on the local curvature of the flagella at that point. If the local curvature is greater than a critical value then the motor is turned on, and if the local curvature is less than that same critical value, then the motor is turned off.

The model first takes advantage of the physical fact that all moments acting on an element must equal zero. Thus, if s is defined as the arclength of the flagella, and t is defined as time, we have the starting equation:

$$M_A(s,t)+M_E(s,t)+M_S(s,t)+M_V(s,t)=0 \quad (1)$$

where M_A is the shear moment due to the dynein motors, M_E is the elastic bending resistance, M_s is the moment due to shear resistance (nexin links), M_V is the moment due to external viscosity. Shear moment (M_A) can be developed by finding the force at every point on each doublet of the flagella and summing those forces at each point along the length of the flagella. According to previous work, the change of force with time along a single doublet of the flagella can be described by the differential equation:

$$\frac{dm(t,s)}{dt} = -E_{SCB}\left|(m_A)\right|\dot{\sigma} + k_1(m_A - m(t,s)) \quad (2)$$

where m is the force, $-E_{SCB}$ is shear resistance, m_A is the switch value that determines whether a dynein motor is on or off, and k_1 is a rate constant at which force approaches the force of the dynein motor (Brokaw 1985). In the above equation, for a change in shear, $d\sigma/dt$, there is a resistance to that shear. Also, there is a first order recovery of m approaching m_A, the force produced by the dynein motor.

Equation (2) can be straightforwardly solved to yield an equation for force,

$$m(t,s) = -E_{SCB}\left|m_A\right| e^{-k_1 t}\int_0^t e^{k_1 t}\dot{\sigma}(s,\tau)\,d\tau + e^{-k_1 t}k_1 m_A\int_0^t e^{k_1 t}\,d\tau + e^{-k_1 t}m(0,s) \quad (3)$$

The force in the direction of each doublet can then be calculated from equation (3) and summed to obtain the total force at each point along the flagella. Thus, M_A can now be expressed as the integral of equation (3) along the length of the flagella. Bending and shear resistance can be introduced to the equation as linear resistances,

$$M_E = -E_B\kappa(s,t) \quad (4) \qquad M_s = -E_s\int_0^s \sigma(\bar{s},t)\,d\bar{s}\,ds' \quad (5)$$

where E_B and E_S are bending and shear resistance parameters, and κ is the curvature of the flagella. If there is no sliding at the base of the flagella, shear and curvature can be related by the equation,

$$\dot{\sigma}(s,t) = \int_0^s \frac{d\kappa(\bar{s},t)}{dt}\,d\bar{s} \quad (6)$$

Currently, in the project I have set the external viscosity to be zero. Later models will incorporate viscosity, but in the beginning stages it is simpler to let it be zero. M_A, M_E, and M_S, have all been defined and can be substituted into equation (1). If curvature is substituted for shear, then the only unknown in the moment balance is curvature. Curvature can be solved for, and be straightforwardly used to describe the shape of the flagella. The moment balance is now as follows,

$$\begin{aligned} &-E_s\int_0^s\int_0^{s'}\kappa(\bar{s},t)\,d\bar{s}\,ds' - N E_{SCB}e^{-k_1 t}\int_0^t\int_0^s\int_0^{s'} e^{k_1 t}\dot{\kappa}(\bar{s},\tau)\,d\bar{s}\,ds'\,d\tau \\ &+e^{-k_1 t}N k_1\int_0^s\int_0^t e^{k_1\tau}\,d\tau\,d\bar{s} + \sum_{i=1}^{9}\cos(\theta_m[i])\,e^{-k_1 t}\int_0^s m(0,\bar{s})\,d\bar{s} - E_B\kappa(s,t) = 0 \end{aligned} \quad (7)$$

where N is the sum of the doublet shear moments, and θ_m is the direction of the doublet moments. The current integral for of equation (7) can't be solved exactly, but it can be turned into a differential equation that can be solved for curvature. By taking appropriate derivatives with respect to "*s*" and "*t*", we obtain the following partial differential equation for κ(s,t),

$$\kappa(s,t) + C_1\frac{\partial\kappa(s,t)}{\partial t} + C_2\frac{\partial^2\kappa(s,t)}{\partial s^2} + C_3\frac{\partial^3\kappa(s,t)}{\partial s^2\partial t} = 0 \quad (8)$$

$$\text{where} \quad C_1 = \frac{E_s + N E_{SCB}}{E_s k_1} \qquad C_2 = \frac{E_B}{E_s} \qquad C_3 = \frac{E_B}{E_S k_1}$$

We can now use the technique of separation of variables to derive a solution for equation (8). We do this as follows. If we let κ(s,t) = S(s)T(t) then equation (8) can be rewritten as

$$TS + C_1\dot{T}S + C_2\ddot{S}T + C_3\ddot{S}\dot{T} = 0 \quad (9)$$

where a dot denotes the derivative with respect to the given independent variable. Equation (9) can be rearranged and factored two different ways to yield a function that only depends on t on the left hand side, and a function that only depends on s on the right hand side. For these two equations to be equal for all *t* and *s* they must both be equal to a constant value $-\lambda^2$.

$$\frac{-(1+C_1\frac{\dot{T}}{T})}{C_2+C_3\frac{\dot{T}}{T}}=\frac{\ddot{S}}{S}=-\lambda^2 \quad (10) \qquad \frac{\dot{T}}{T}=\frac{-(1+C_2\frac{\ddot{S}}{S})}{C_1+C_3\frac{\ddot{S}}{S}}=-\lambda^2 \quad (11)$$

Equations 10 and 11 both produce straightforward differential equations for T and S. When solving for T and S, in equation 10, there is always an oscillating term, but in equation 11 there is only an oscillating term if

$$\frac{C_1\lambda^2-1}{C_2-C_3\lambda^2}<0$$

Remember that the sign of C_1 depends on the direction of the shear force on the flagella. Biologically, C_1 can be positive or negative and that should have no bearing on the motion of the flagella, except the direction in which the flagella is moving. But, in this case, the sign of C_1 will determine whether or not the flagella model oscillates. There seems to be something amiss with this result.

Applying the boundary conditions associated with equation 10 allows us to demonstrate that there are an infinite number of solutions for 10. We index these with the letter "*n.*" Combining all of our pieces and remembering that $\kappa(s,t) = S(s)T(t)$, we have that the total solution for the curvature $\kappa(s,t)$ is given by

$$\kappa(s,t)=\sum_{\substack{n=-\infty \\ n\neq 0}}^{\infty} \Omega_n e^{-(\frac{1-\lambda_n^2 C_2}{C_1-C_3\lambda_n^2})t}\sin(\lambda_n s) \quad (12)$$

where $\lambda_n = \pi\ n\ /\ l$ and l is the length of the flagellum. At this point, we have finally arrived at an equation for curvature that can be used to describe the flagella for all time. We now need to determine the value of the unkown constant Ω_n. We do this as follows. Set $t = 0$ and use the initial condition for the curvature at the beginning of the simulation. This yields the following equation.

$$\kappa(s,0)=\sum_{\substack{n=-\infty \\ n\neq 0}}^{\infty} \Omega_n \sin(\lambda_n s) \quad (13)$$

Next, we apply the method of Fourier analysis to extract the values of Ω_n. We first multiply equation (13) by $\sin(\lambda_m s)$ (assuming *m* is not equal to *n*) and integrate the equation along the length of the flagellum. This yields the following equation.

$$\int_0^l \sin(\lambda_m s)\kappa(s,0)\,ds=\sum_{\substack{n=-\infty \\ n\neq 0}}^{\infty} \Omega_n \int_0^l \sin(\lambda_m s)\sin(\lambda_n s)\,ds \quad (14)$$

Equation (14) can be solved for all values of n. There are three separate cases that must be considered: (1) for $n \neq m$ the integral on the rhs is equal to zero, (2) for $n = m$ the integral is l/2 and for (3) $n = m = 0$ the integral is equal to 0. Using this information it is straightforward to solve for Ω_n and we can now express the final equation for the curvature $\kappa(s,t)$,

$$\kappa(t,s)=\sum_{\substack{n=-\infty \\ n\neq 0}}^{\infty} \frac{2}{l}\int_0^l \sin(\lambda_n s)\kappa(0,s)ds\, e^{\frac{-(1-\lambda_n^2 C_2)}{C_1-C_3\lambda_n^2}t} \sin(\lambda_n s) \quad (15)$$

Equation (15) describes the curvature of the flagella for $\forall t \in [0, \infty)$ and $\forall s \in [0, l]$. Curvature can then be used to convert the flagella coordinates into a global coordinate system, which allows the flagella to be graphically visualized. However, there is a problem with equation (15). All of the biological modeling parameters that are involved in the model appear in the exponential term. When biologically realistic values are used for these model parameters, the exponential term in equation (15) causes the summation to be zero for any value of t not equal to zero. Consequently, there seems to be something wrong with this model, our method of solving the model, or the biological assumptions that went into constructing the model..

In addition to deriving this continuum model for the flagella, an iterative approach is being developed. Also a program has been developed that can take the curvature vectors for a flagella and translate those curvatures into Cartesian coordinates and visually represent the flagella in three dimensions. The program is written in C++ using OpenGL.

Goals and Plan for Academic Year

At the moment the continuum model of the eukaryotic flagella is not working. This suggests that there must either be something wrong with some assumptions that were made while developing the model, or that the model itself is incorrect. My first goal of the academic year is to meet with Dr. Charles Brokaw, an expert in this field of work. It is his model that I am using to simulate the flagella, and his advice will be very beneficial to my project. Based on the input I get from Dr. Brokaw, there will be different directions that I go in.

If there are simple assumptions that are wrong in my model I will correct these assumptions and continue to develop this model. If there is something fundamentally wrong with solving this model continuously I will continue to develop the iterative approach to accurately describe the flagella. Or, it could be possible that I will have to start over from the beginning and develop a completely new model.

Regardless of which path I take, I will develop the model to contain all the factors that are currently incorporated into the model (shear moment, shear resistance, and bending resistance). The next step will be to incorporate viscosity into the model. Currently, viscosity is considered to be zero, which is not realistic. After viscosity is introduced, the cell body will be added to the model, so the flagella will be affected by the cell itself. The *T. cruzi* flagella is attached to the cell body for much of its length, so this is an important factor when building the model.

In parallel with this project I will continue to develop a way to visualize the flagella using curvature vectors. The working model will be made into a program that will be able to compute the motion of the flagella using the user specified parameters. This will involve not only programming the model, but developing a user interface that will allow the user to input the desired model parameters.

Budget

Given the variable nature of my project at the moment it is difficult to envision the specifics about what my project needs will be. As I begin to do more and more programming I will most likely need books about numerical and computation analysis to be able to produce effective and mathematically accurate code. In addition, depending on the magnitude of the computations, I might need to gain access to a computer that will be able to visualize and compute the calculations in a reasonable time. Lastly, I will most likely be producing lots of data, and it might be necessary to buy resources to store, visualize, and analyze this data such as computer software or hardware.

References

Bastin, P., Pullen T. J., Moreira-Leite F. F., Gull K. (2000). "Inside and outside of the trypanosome flagellum: a multifunctional organelle." *Microbes and Infection*. 2: 1865-1874.

C. J. Brokaw. "Computer simulation of flagellar movement VIII: Coordination of dynein by local curvature control can generate helical bending waves", *Cell Motil. Cytoskel.*, vol. 53, pp. 103-124, 2002.

C. J. Brokaw. "Computer simulation of flagellar movement VI. Simple Curvature-Controlled Models Are Incompletely Specified" Biophysical Journal. Vol. 48 October 1985

CDC (2004). "Fact Sheet for the general public: Chagas Disease."

De Souza, Wanderley (2002). "Basic Cell Biology of *Trypanosoma cruzi*." *Current Pharmaceutical Design*. 8: 269-285.