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In Silico Comparative Macromolecular Modeling of the Leishmania Flagellum: 3D Perspective of an Axonemal Nanomachine

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Short Abstract: Here we introduce a few tentative computer-generated and physical models of the flagellar axoneme of the protozoan Leishmania in an attempt to represent the nanostructured complexity of the assemblies of macromolecular structures that resemble a rotatory and bending natural nanomachine. Resulting tangible models will be available at request.

Long Abstract:
BACKGROUND: Leishmania spp. are eukaryotic unicellular organisms belonging to the Kinetoplastida order and Trypanosomatidae family. Protozoa of this genus are pathogenic parasites that cause the vector-borne diseases collectively known as Leishmaniases. Currently, Leishmaniases show a wider geographic distribution and increased global incidence of human disease. These parasites exist in two major developmental stages: flagellated promastigotes in the alimentary tract of the sandfly vector and non-flagellated amastigotes residing in the phagolysosomes of macrophages in the mammalian host. The cytodifferentiation of Leishmania is accompanied by a series of morphological, molecular and biochemical changes which are often mediated by the differential expression of a variety of genes, ultimately determining a flagellar status of either flagellated or non-flagellated parasite. Cilia and flagella are among the most ancient cellular organelles that project from the surface of cells, providing motility for primitive eukaryotic cells living in an aqueous environment. During adaptation to life on land, some groups of organisms, including advanced fungi, red algae, cellular slime molds, conifers, and angiosperms, lost the ability to assemble flagella. Despite the diversity of their functions, the basic architecture of these machines is remarkably conserved. All such structures are built on an ordered assembly of microtubules known as the axoneme. The most widely distributed form of the axoneme consists of nine outer microtubule doublets (MTDs) surrounding a central pair (CP) of microtubules (the canonical 9+2 pattern). Several macromolecular complexes can be identified in the flagellum: plasma membrane, microtubules, radial spokes (RSPs), inner dynein arms (IDAs), outer dynein arms (ODAs), central pair protrusions and bipartite bridges. Flagella are constructed and maintained through the highly conserved process of

intraflagellar transport (IFT). In Chlamydomonas, the IFT particles comprise two large complexes: complex A is composed of six proteins and includes IFT-122, -139, -140 and IFT144; complex B is composed of eleven subunits including IFT-20, -52, -57, -88 and IFT172, among others. Most of the IFT proteins have yet to be identified in pathogenic uniflagellates such as Leishmania, as well as many of the 250 different proteins believed to jointly build its eukaryotic flagellum. Because of their biological and medical importance and their utility as a model for other forms of microtubule-based motility, flagella and cilia have been studied extensively. Several groups of researchers have sought to identify components of the eukaryotic flagellum by genomic, proteomic and bioinformatics strategies. Here we introduce a few tentative models of the trypanosomatid flagellar axoneme in an attempt to represent the nanostructured complexity of this organelle. The intrinsic organization of the axoneme resembles a rotatory and bending natural nanomachine and it was modeled on the dynamic basis of its known structural components (CP, MTDs, RSPs, ODAs, IDAs, etc). METHODS: Using data from various sources and different types of experiments (having in common the fact of the eukaryotic flagellum as the focus), we have applied a set of computational biology resources and tools, combined with specific visualization hardware devices and IRIX operating system, in order to built in silico physical models of the 3D structure and macromolecular organization of conserved components of the axoneme of Leishmania spp. This study started after compiling and reviewing recent literature over flagellated organisms, using 2D projection images from many different viewing angles to reconstruct objects in three dimensions and with physical 3D tangible models (Gillet et al., 2005). The distances between the structures were also based on these images. Currently, approaches with high descriptive power like 3D surface-based representations are available. However, most techniques tend to focus on 2D graph-based molecular similarity due to the complexity that accompanies reasoning with more elaborate representation. Cryo-electron tomography uses 2D projection images to reconstruct an object in three dimensions. This technique has several advantages as a method for studying cell structure, corroborating many previously described features of axoneme structure, such as periodicities and general organization. These methods of computer modeling are widely used for structures determination of biological molecules. Modeling represents a powerful experimental approach that provides understanding of numerous physical aspects of macromolecular structures. All computations analysis were carried out by using the software package EM PROGRAM (Nicastro et al., 2005) on a Fuel workstation (Silicon Graphics) running IRIX 6.5.25. Visualization, surface and volume rendering was provided by the AMIRA 3D visualization package (Mercury Computer Systems, San Diego), whose unique geometry reconstruction capabilities enables generation of three-dimensional models from image data volumes, suitable for visualization, accurate quantitative analysis and simulation tasks. RESULTS. Our computer-generated and physical models of the flagellar axoneme support the nanostructured complexity of the assemblies of macromolecular structures covering the 9+2 microtubular axis and its surroundings. Structural details and spatial distances are represented in terms of CP and MTDs radial projections, while RSPs, IDAs and CP protrusions are dynamically assessed in terms of their interface with bipartite bridges and ODAs. Models are displayed as schematic three-dimensional figures of nanostructured parts of the flagellum which are represented in different colours, longitudinal and cross sections, as well as lateral perspectives. We report here a direct application of autofabricated tangible models and augmented reality for flagellar research, as we have extended our molecular modeling environment to support the fabrication of a wide variety of physical molecular models of the axoneme, and have adapted an augmented reality system to allow virtual 3D

representations to be overlaid onto the tangible molecular models. Through that we can easily change the overlaid information, switching between different representations of the axoneme, displays of macromolecular properties, or dynamic information. The physical models provide a powerful, intuitive interface for manipulating the computer models, while analyses of these predicted models might provide key information about flagellar motility and intraflagellar transport. The knowledge about structurally implementing some (or most) of the 250 proteins (believed to jointly build the eukaryotic flagellum) in an actual predicted form in the axonemal components is, indeed, an important step to better understand the relationships between flagellar structure, functions, evolutionary origin and even virulence factors Availability: http://nugen.lcc.uece.br/lpgate/?p=flagdb