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November 18, 2005

Dear members of the Search Committee,

I have recently learned about a job opportunity available in your department. The job description matches very well with my experiences and interests, thus I consider myself a good candidate for this position.

I am interested in understanding how cytoskeletal structures are organized and function using advanced bioinformatics, microscopic and molecular genetics techniques. I have studied two radically different cytoskeletal complexes involved in cell motility: During my first postdoctoral position in the lab of Dr. John Murray, I studied the apical complex -- a spectacular cytoskeletal complex involved in invasion of *Toxoplasma gondii*, a parasitic protozoa and the leading cause of congenital neurological birth defects. In order to expand my understanding of eukaryotic cytoskeletal organization and cell motility, currently I am pursuing my second postdoctoral research in Dr. Waterman-Storer's lab (Scripps Research Institute, La Jolla) to study focal adhesion and actin cytoskeleton assemblies -- the essential machinery that drives the motility of mammalian cells.

For establishing my own lab, I would like to focus on protozoan biology, a field that is directly relevant to human health and rich in great experimental model systems for bioinformatics, molecular and cell biology studies. Specifically, I will focus on *Plasmodium falciparum,* the most lethal form of human malaria parasites. Both my past research and my future plans are described in detail in the accompanying Personal Statement.

From my past research experiences, I have acquired an in-depth understanding of cytoskeletal organization and dynamics in both protozoan and mammalian cell model systems, and have accumulated a powerful set of tools, including cutting edge light and electron microscopy, proteomics techniques and highly sophisticated image analysis skills, which will meet the demand of my proposed research projects. I therefore believe that I will perform well given the opportunity and hope that I would be considered a candidate. Please feel free to contact the following individuals for references:

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Sincerely,

Ke Hu

RESEARCH EXPERIENCES

I am interested in understanding how cytoskeletal structures are organized and function and have studied two radically different cytoskeletal complexes involved in cell motility: 1) the apical complex -- a spectacular cytoskeletal assembly involved in the invasion and replication of a parasitic protozoan and 2) the assemblies of focal adhesions and actin cytoskeleton -- the essential machinery that drives the motility of mammalian cells.

Cytoskeletal components of an invasion machine- the Apical Complex of *Toxoplasma gondii* (2002-2003):

Toxoplasma gondii is the leading cause of human congenital neurological defects. It is one of the 5,000 Apicomplexan parasites, a group that also includes the malaria parasite *Plasmodium falciparum.* The pathogenicity of these parasites is almost entirely due to uncontrolled cycles of host cell invasion, parasite growth and division, and host cell lysis.

Two steps in this cycle, the invasion of host cells and the parasite's cell division, rely on a unique cytoskeletal organelle, the apical complex. The apical complex is thus potentially a prime drug target, and its study is therefore strongly motivated by the sore need for better therapy for all Apicomplexan diseases. Beyond this, the major compartments of this complex: the polar rings and the conoid (a tubulin-based molecular machine that does NOT utilize conventional microtubules (Hu *et al.* 2002)) (Fig 1& 6) are fascinating structures for all cell biologists interested in the construction and function of supramolecular assemblies. Unfortunately, knowledge of the molecular composition of the apical complex was virtually non-existent, which made its function and assembly impossible to study.

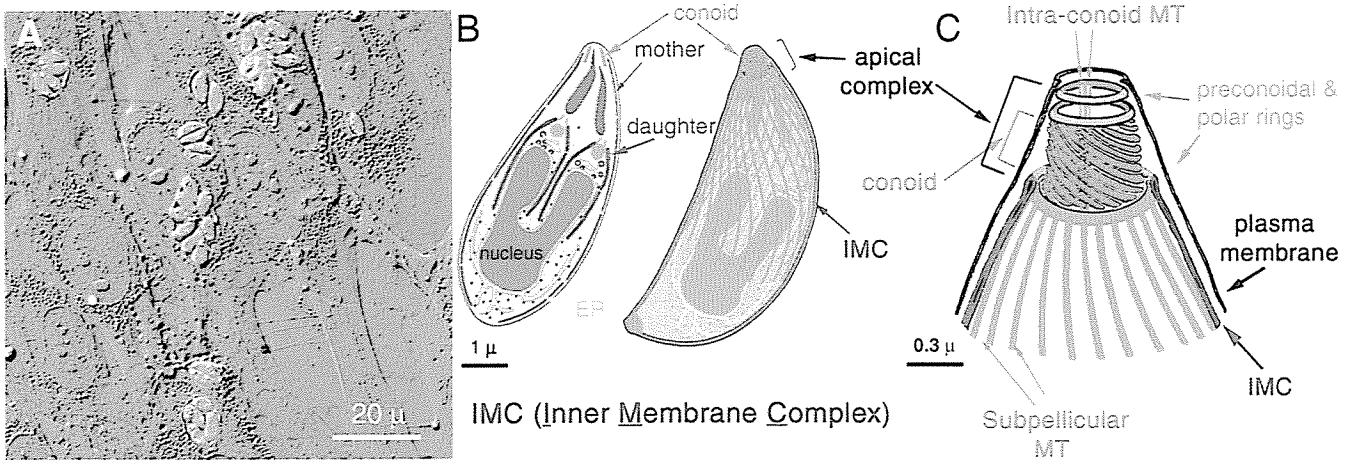

Fig 1. *(A)* Combined DIC and epifluorescence image of human fibroblasts infected with transgenic *T. gondii* expressing GFP-tubulin (green). Parasitophorous vacuoles containing 1, 2, 4, or 8 parasites are seen. *(B)* Drawings of *T. gondii*. *left*: a longitudinal section of a dividing cell. Lobes of the dividing nucleus bordered by ER, Golgi (yellow) are surrounded by the developing daughters' scaffolds (red). Maternal and daughter conoids are shown in green, secretory organelles (rhoptries) in purple. *T. gondii* has three membranes: a plasma membrane (black) and two additional layers (IMC, red) formed from a patchwork of flattened vesicles. *right*: semi-transparent view showing the 22 subpellicular MT (green) *(C)* Enlarged view of the apical complex cytoskeleton, showing the conoid (green), preconoidal and polar rings (brown) and two intra-conoid MT (green). The conoid is formed of 14 fibers of tubulin (not MT), 430 nm long, arranged in a left-handed spiral (Hu et al., 2002b)

During this postdoctoral period, I isolated the conoid/apical complex, determined its protein composition by mass spectrometry, and identified ~200 apical complex proteins, including 142 novel proteins with no characterized domains. To validate this purification and identification scheme I cloned 6 novel proteins from the final list, characterized their localization within the parasite by light and electron microscopy and followed their recruitment into the cytoskeleton during cell division. These proteins provide the first set of markers of various subcompartments within the apical complex (Fig 2). To our great surprise, several of them highlight fascinating cytoskeletal structures that were never seen before

(Fig 2 & Hu et al. 2005). Further time-lapse studies of the incorporation of these proteins by fluorescent light microscopy revealed surprisingly that the extreme apical and basal regions of this highly polarized cell originate around the centrosome at the same time during daughter formation.

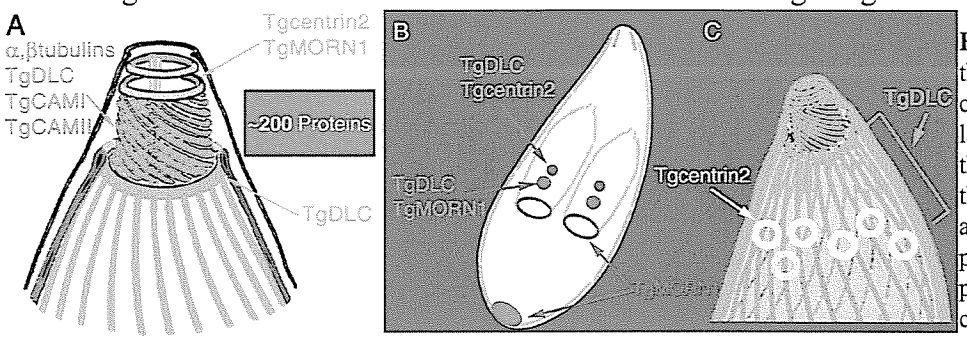

Fig 2. A. I identified ~200 proteins in the apical complex. From these, I characterized six novel proteins that label different subcompartments within the apical complex. **B&C:** Three of these proteins, TgMORN1, Tgcentrin2 and TgDLC (Dynein Light Chain), provide new markers for the spindle pole bodies (pink dots) as well as the centrioles (blue dots). In addition, they highlight several novel cytoskeletal structures that were never seen before, including "caps" on the extreme basal ends of daughter and mother scaffolds (B, red, TgMORN1); a differentiated region of the apical 1/3 of the parasite scaffold demarcated by TgDLC (C, brown shadow) and 5-6 annuli arranged in a circle at the peripheral of the parasite (C, yellow, Tgcentrin2). The Tgcentrin2 annuli are consistently associated with the lower edge of the apical cap of dynein.

This comprehensive knowledge of its components reveals the molecular complexity of this novel cytoskeletal machine, establishes a framework for studies of functional interactions among its individual proteins, and provides a wealth of new probes for studying its function and assembly, as well as for the rational selection of chemotherapeutic targets for improved control of the Apicomplexan diseases. The discovery of several novel cytoskeleton structures has greatly improved our understanding of how the entire parasite cytoskeleton is built and posed intriguing questions regarding the origin and function of these structures as well as how specialized regions can be demarcated and assembled in all cells.

The revelations that have come from just these 6 new apical complex components prove that my simple protocol for apical complex purification, coupled with modern high-throughput proteomics tools, have uncovered a gold mine of new information. Exploiting this gold mine, which contains > 100 other novel proteins likely to be just as informative as the 6 that I cloned, will set the research agenda in several labs for years to come.

Molecular dynamics and composition of focal adhesion/actin assemblies (2003-Present)

To broaden my experience in cell motility and my skills of quantitative light microscopy, and to learn methodologies developed in a relatively mature field, currently I am pursuing my second postdoctoral research to study the regulation of the molecular interaction between the actin cytoskeleton and Focal Adhesion (FA) complexes, cytoskeletal machines that drive the motility of mammalian cells.

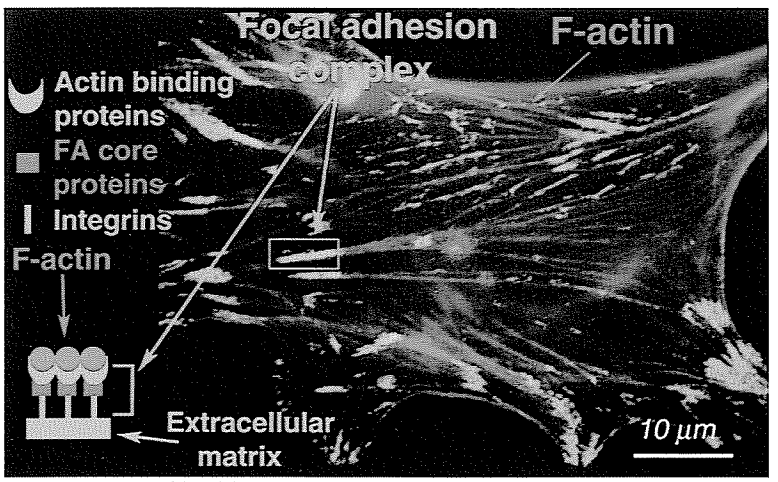

Fig.3 NIH3T3 fibroblast labeled with Alexa-568-phalloidin (F-actin, red) and anti-paxillin antibody (FA, green). Diagram: FAs link actin meshwork to EM and FA proteins can be divided into three groups: actin binding, EM binding or FA core proteins.

Cell migration in multi-cellular organisms is essential in numerous fundamental processes of life including embryonic development, cell mediated immune defense and wound healing. Ill-regulated cell migration activity results in devastating consequences, with the most notorious being cancer cell metastasis. The dynamics of the actin meshwork, driven by myosin contraction and actin polymerization, generates the forces

powering cell migration and cell morphogenesis. FAs link the actin cytoskeleton to the ExtraCellular Matrix (ECM) and are reponsible for transducing mechanical and chemical signals between the actin meshwork and the ECM (Fig 3). The dynamic interaction between FA and actin cytoskeleton is thus crucial for regulating cell migration.

Before the start of my postdoctoral study, FAs and the actin cytoskeleton had never been studied simultaneously in living cells. The molecular motion within the FA was completely obscure, therefore FAs were treated as single entities lacking intra-complex fluctuations. In order to study the interaction of these two dynamic cytoskeletal complexes in living cells, I combined multi-spectral total internal reflection fluorescence, speckle microscopy (Waterman-Storer, et al), and rigorous quantitative analysis. Using this methodology, I have visualized for the first time the molecular motion within FAs and quantitated the correlated motion between FA components and actin .

I examined 7 proteins within the FA, 1) actin binding proteins (alpha-actinin, talin, vinculin), 2) ECM binding proteins (AlphaV/beta$_3$ integrins), and 3) "FA core" proteins, which do not directly interact with either the actin cytoskeleton or the ECM (paxillin, zyxin and focal adhesion kinase) (Fig 3). I found that these proteins do not act concertedly as one unit, but instead display intriguing heterogeneity in their "coupling" with actin movement. The FA actin binding proteins exihibit the most consistent and significant correlation with actin movement, which is a strong indicator for direct or indirect interaction between these proteins and the flowing actin meshwork. In contrast, motion of the FA core proteins and integrins display only weak correlation with actin movement (Fig 4). Furthermore, the "coupling" between FA actin binding proteins and actin meshwork seems to be adjustable and varies with different signaling states.

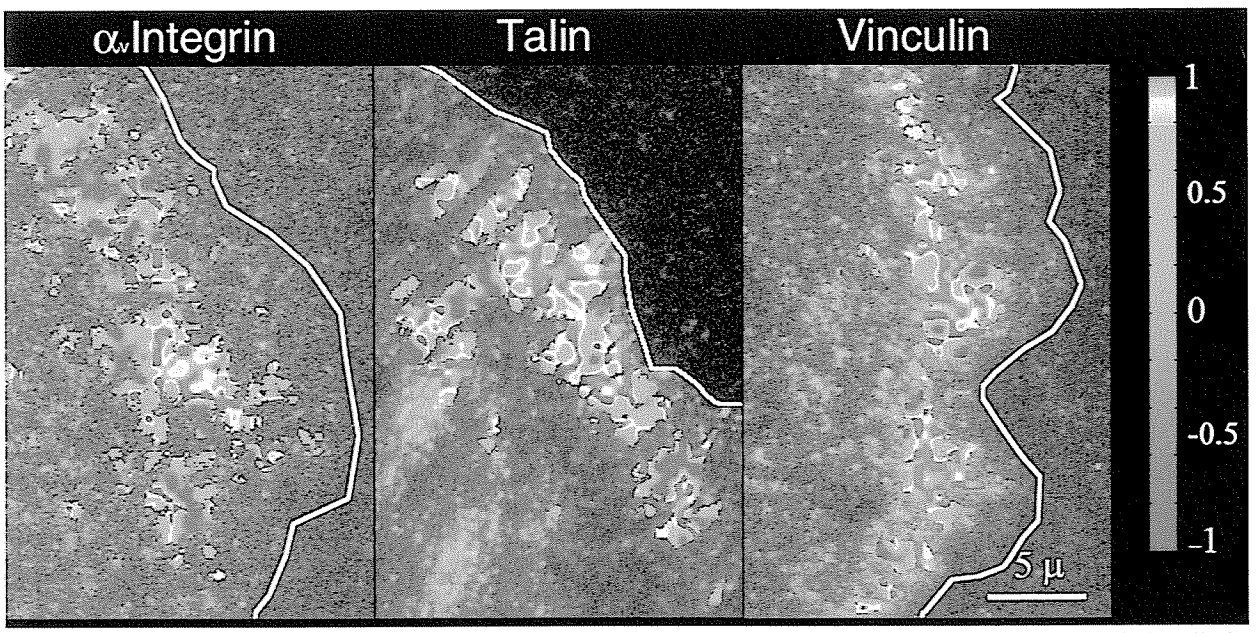

Fig 4 Colored maps of Direction Correlation Scores (DCS) between actin and alphaV integrin, talin, and vinculin in Ptk1cells. The DCS between actin and integrin is much lower than those between actin and talin, or actin and vinculin. DCS is the cosine of the angle between the actin and FA movement vectors. When DCS=1(color coded red), the two movement vectors are in the same direction.

My findings challenge prevailing assumptions in the field that FA components are largely static within the complex. In addition, my results indicate that the molecular "coupling" between FA proteins and actin in living cells is subject to rather sophisticated regulation. This regulated variability needs to be taken into account for understanding the generation and transmission of traction forces within the actin cytoskeleton through FAs . Further, the methodology we developed in this study, i.e. correlation of molecular motion within stable complexes, is also extremely valuable in detecting direct or indirect molecular interaction within all macromolecular complexes in living cells.

My second approach for studying the regulation of the function of FAs and the actin cytoskeleton has been to investigate the molecular composition of focal adhesion/actin assemblies. I initiated a proteomics project aiming to identify unknown protein components in the FA and actin cytoskeleton assemblies. I have discovered over 15 new candidate FA/actin cytoskeleton components. I have cloned and characterized several of them, two of which display interesting specific localization and dynamics in the FAs and stress fibers (Fig 5). Functional analysis of these previously unknown proteins is underway.

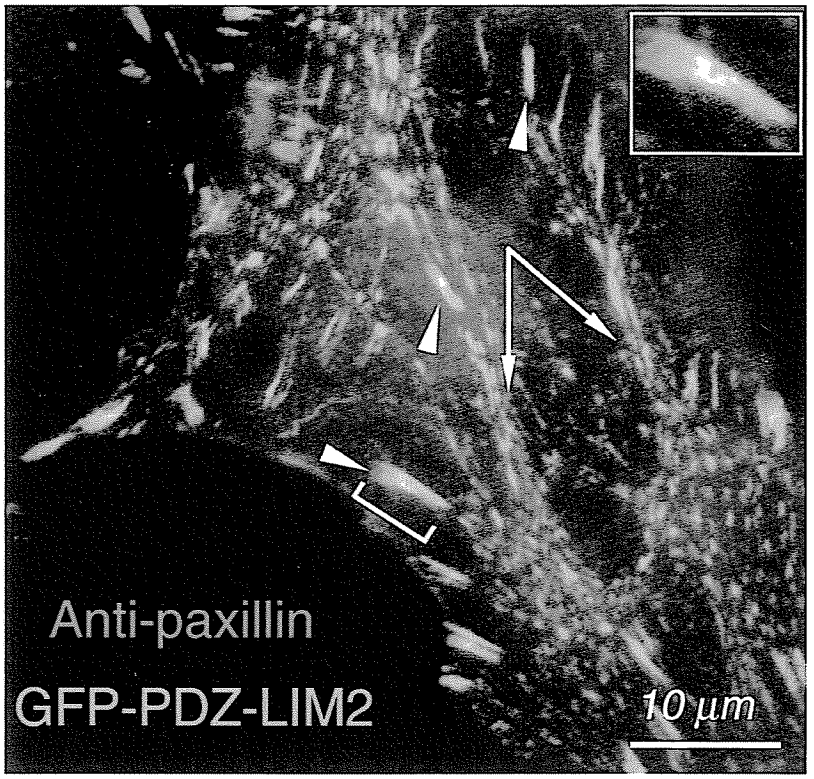

Fig 5. NIH3T3 fibroblast expressing GFP-PDZ-LIM2, a novel protein identified from the FA proteomics screen. GFP-PDZ-LIM2 is highly concentrated within the stress fiber (arrows) and FAs (arrowheads). Notice that in this cell, GFP-PDZ-LIM2 only partially colocalize with paxillin (red) in FA. *Insert*: 2-fold magnification of the FA indicated by yellow bracket.

RESEARCH PLANS: Cytoskeletal Biogenesis of Apicomplexan Parasites

My lab will study the cell biology of Apicomplexan parasites. The phylum Apicomplexa contains 5,000 parasite species that live on a vast range of hosts. Many of these parasites are immediately relevant to human health, including *Toxoplasma gondii*, the most common cause of congenital neurological defects in humans and *Plasmodium falciparum*, the most lethal cause of malaria. This phylum is also rich in fantastic experimental model systems for cell and molecular biology as well as bioinformatics and evolutionary studies.

Apicomplexan parasites as model systems

Powerful tools are available for genomics and molecular genetics studies in Apicomplexan parasites.

The genome projects of several important apicomplexan parasites, including *Toxoplasma gondii* and *Plasmodium falciparum,* are essentially complete, which makes it possible to conduct genome-wide bioinformatics analysis and large-scale comparative studies. Gene manipulation tools such as gene tagging, knockout and stable transfection are also available in these organisms and in many cases are simpler and quicker than in mammalian cells.

Apicomplexan parasites are outstanding systems for studying fundamental questions in cell biology such as organellar and cytoskeletal remodeling and dynamics.

Although under-exploited by main-stream cell biology, the apicomplexan parasites have spectacular cytoskeletal structures (Fig 6) that are highly-ordered, and have practically the identical arrangement in every cell (Fig 7). Unlike mammalian cells, *de novo* pre-formation of the daughter cytoskeleton prior to the cytokinesis is easily visualized. Understanding how the cytoskeletons of these parasites are constructed during cell division will reveal how very complicated cytoskeletal structures are built from scratch, and therefore is extremely valuable for understanding cytoskeleton biogenesis in all cells.

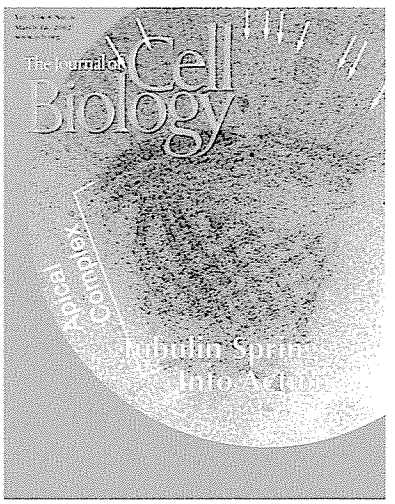

Fig 6. The Cytoskeleton of Apicomplexan parasites. CryoEM image of *T. gondii* cytoskeleton extracted by detergent (Hu et al. 2002). *Yellow arrows*: the subpellicular microtubules, *Yellow bracket:* the apical complex.

Apicomplexan parasites are also more advantageous than mammalian cells in studying organellar biogenesis due to the regularity of their subcellular structures. For example, *T. gondii* has only one well-defined contiguous ER, one Golgi with reproducible shape, one mitochondrion, and 22 microtubules, all in the same location in every cell. This regularity not only highly simplifies the study of the replication of each organelle, but also makes it possible to address how a cell is built from orderly partition of organelles and assembly of cytoskeleton. This type of study is much more difficult in a typical mammalian cell, because each cellular structure exists in numerous copies and it is very difficult to follow structural changes of the complicated cytoskeleton and the intricate rearrangements of a large number of organelles during cell division.

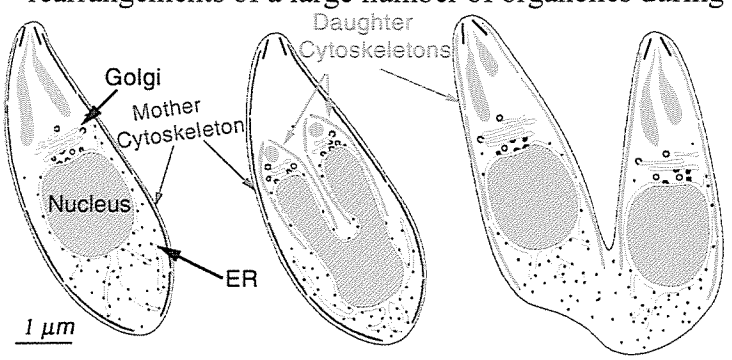

Fig 7. Cell division in Apicomplexan parasites. Apicomplexan parasites prepare for division by forming daughter cytoskeleton scaffolds within the mother. The *de novo* constructed daughter cytoskeletons (green) serve as scaffolds for organelle biogenesis and partitioning (The Mitochondrion is not included in the drawing.), while the mother cytoskeleton (red) remains intact for the most part of cell cycle. The apicomplexan parasites differ in the number of daughters formed at each cell cycle, which varies from 2 (e.g. *T. gondii* as ilustrated) to $2^n(n>1)$ (e.g. *P. falciparum*).

Significance and rationale:

I will focus on the biogenesis of the apical complex in *P. falciparum*. I choose to study the apical complex because 1) it provides a suite of targets for designing parasite specific drugs; 2) it has an intriguing structure and assembly process that will inform study of the cytoskeleton in more popular model systems; and 3) the apical complexes from different apicomplexan parasites are an array of partially conserved but significantly diverged structures, thus serving not only to reveal the evolutionary path of these ancient protozoa, also as a powerful set of positive/negative experimental controls for each other.

Each year, more than 2 million lives are claimed by the malaria parasite *P. falciparum*. In Africa, malaria is second only to AIDs in the list of the continent's killer diseases, and the interaction between these two presents great difficulties. In many regions, such as Southeast Asia, parasite strains resistant to all currently available medications have arisen, a terrifying development. Current medications tend to cause severe side effects, including abdominal pain, vomiting and anorexia. Drugs that target parasites more specifically are therefore desperately needed. The apical complex has great potential for providing unexploited drug targets, because it is a novel cytoskeletal structure (Fig 1&6) and is part of the core apparatus that drives parasite replication and invasion. My data from the *T. gondii* cytoskeletal proteomics project has shown that most apical complex components are novel proteins with no mammalian homologs, thus ideal targets for designing drugs specific for apicomplexan diseases.

Besides being a prime drug target, the *de novo* construction of the apical complex during cell division presents an extremely interesting cytoskeleton biogenesis problem that highlights important unresolved issues in cell biology. Further, comparison of the molecular composition and assembly process of the apical complexes from *T. gondii* and *P. falciparum* will yield great insights as to how these two complexes diverge in their evolutionary history, their host range and host specificity (*T.gondii* can invade and proliferate in all nucleated cells. *P. falciparum*, however, can only live in humans in liver or red blood cells and in three specialized cell types of one species of mosquito). Most of the proposed experiments will be carried out in *P. falciparum*, however, I will continue to use *T. gondii* in my lab when it is more convenient because of its greater suitability for biochemical and electron microscopy assays. Its close homology to *Plasmodium* will allow me to directly apply the results to the study of *Plasmodium sp.*

My research will have significant impact in the fields of cytoskeleton modeling and dynamics and host-pathogen interaction. From my past research experiences, I have acquired an in-depth understanding of cytoskeletal organization and dynamics in both protozoan and mamalian cell model systems and appreciation for apical complex biology, and have accumulated a powerful set of tools, including cutting edge light and electron microscopy, molecular biology, proteomics techniques and highly sophisticated image analysis skills, which will meet the demand of my proposed research projects.

Specific aims:

1. Determine the molecular composition and assembly process of the apical complex in *P. falciparum* combining high-throughput proteomics and high resolution light and electron microscopy (~ 3 – 4 years).

   I will first characterize the localization of the *Plasmodium* homologs of a subset of the *T. gondii* apical complex proteins in *P. falciparum*, to establish an initial set of molecular markers within the apical complex of *P. falciparum*. These proteins will likely mark the conserved structures shared by the apical complexes of these two species. They will also greatly facilitate later purification of the apical complex of *P. falciparum* (See appendix for details).

   I will identify the complete set of components of the apical complex of *P. falciparum*, by establishing a proteomics project in the invasive form of blood stage *P. falciparum* (merozoite) and taking advantage of the molecular markers characterized above and approaches I developed for determining the molecular composition of the apical complex of *T. gondii*, with modifications for the specific properties of *P. falciparum* (See appendix for details). The invasive form of blood

stage *P. falciparum* is chosen because it is amenable to large scale *in vitro* culture and molecular manipulation. The localization and function of identified genes, however, will be analyzed in all life stages of *P. falciparum* when accessible.

To establish the sequence of apical complex assembly in *P. falciparum*, the localization and timing of the incorporation its apical complex components will be studied combining protein dynamics studies in live parasites with high-resolution immunoEM.

2. Comparative proteomics analysis of the apical complex of *T. gondii* and *P. falciparum* (overlapping with the 1st phase)

I will systematically compare the molecular composition and assembly process of the two apical complexes from *T. gondii* and *P. falciparum*. Although the structure of the apical complex is partially conserved within Apicomplexa, each parasite species has its own signature features. For example, the conoid, one of the major compartments of the *T. gondii* apical complex, is not normally seen in *P. falciparum*. This comparison will greatly improve our understanding of how these apical complexes are built, why they are built differently, and the evolutionary origin of the apical complex within the phylum Apicomplexa.

3. Apical complex protein function analysis (5 - 7 years, overlapping with the 1st and 2nd phase).

I will examine the effect of gene function disruption of apical complex components on parasite growth, division, invasion and differentiation at various life stages of *P. falciparum*. Gene function disruption will be achieved by conditional knockout or expression of dominant positive/negative alleles when available. Because a high-throughput, rapid gene function disruption technique is not yet available, in the absence of other information, genes characterized above (specific aim 1) will be initially prioritized according to their molecular and localization conservation within the phylum Apicomplexa.

In order to understand the function of the apical complex as a whole, I will block the apical complex assembly and study the effect on parasite invasion and division using various tools that allow for site-specific structural disruption in living cells, including pulsed-laser ablation as well as the continued development and recent refinement of CALI (chromophore assisted light inactivation) in Tsien's lab that allows focused, specific inactivation of tagged proteins in live cells.

4. Determination of the role of the centrioles/centrosome in the initiation of apical complex assembly

One of the fundamental questions in apical complex assembly concerns the initiation of the construction of the complex, i.e. is the apical complex constructed *de novo* as it appears to be, or patterned upon a preexisting template? If the latter is true, then where is the structural information stored? In *T. gondii*, at the light microscope level, the daughter apical complexes seem to originate near the replicated centrioles at the beginning of cell division and move further away from the centrioles as the daughter scaffolds develop (Hu et al. 2005). The centriole/centrosome is thus a good candidate for serving as an "organizer" for the apical complex (though perhaps not a direct template), because 1) it is known to reproduce itself, 2) it co-localizes with the daughter apical complex at the beginning of cell division. Consistent with this hypothesis, I have already identified three *T. gondii* proteins that localize to both apical complex and centrosome (Fig 2).

To further test this hypothesis, in parallel with the specific aims described above, I will 1) carry out high-resolution electron microscopy, including serial thin-section and 3-D tomography EM to examine the structural relationship between the apical complex and centrosome at early stages of apical complex assembly, 2) disrupt functions of centrosome specific genes to see if the apical complex assembly is impaired. If the results of the above two sets of experiments suggest

an important role for the centrosome in apical complex biogenesis, I will then try to reconstitute apical complex assembly *in vitro* from purified *T. gondii* centrosomes and soluble cell extracts.

Timeline:

I expect that it might take 10 years or more to conclude the projects described above with currently available technologies. However, in the past 5 years, technologies such as MudPIT have matured at an amazing speed, which along with the exponential growth of genome information, has enabled me to carry out experiments I never foresaw 5 years ago when I started my Ph.D. Five years from now, the perfection of bioinformatics technologies such as *in silico* protein interaction prediction, along with the explosive growth of protein structures in the PDB (Protein Data Bank) and the accompanying rapid maturation of algorithms for protein structure prediction by homology, will again greatly accelerate the progress of our work, thus shorten the time needed to achieve my goals.

Funding opportunities:

There are many sources of funding for malaria research; for example, the NIH and the Wellcome trust both have major initiatives in promoting malaria research. In addition, because apicomplexan parasites are outstanding cell biology model systems, my proposed studies will be highly competitive for general cell biology funding as well.

Appendix: Proteomics analysis of *P. falciparum* apical complex

I. Culture and purification of merozoites of *P. falciparum* blood stage

I will use *P. falciparum* merozoites for the purification of apical complex, because the merozoite is the invasive form of *P. falciparum* in its blood stage and it has a complete set of cytoskeletal and membrane-bound invasion organelles. Synchronization and purification of merozoites has been well established and will be conducted as described before (Blackman, MJ. 1994; Laurence Florens *et al.* 2002). In addition, recent development of large scale malaria culture in Hollow Fiber Bioreactors (FiberCell systems Inc.) makes it a relatively trivial task to harvest malaria parasites at the scale of 10^9(Li *et al.* 2002). (For my *T. gondii* apical complex proteomics project, I used a total of ~ 2-4x10^9parasites, from 8xT175 flasks, to conduct 10 replicate analyses by MudPIT).

II. Partial purification of *P. falciparum* apical complex

1. Initial identification of apical complex markers of *P. falciparum*

By studying the localization of homologs of *T. gondii* apical complex components in *P. falciparum*, I will identify proteins that have specific and concentrated localization within the apical complex of *P. falciparum*. *P. falciparum* cell lines stably expressing apical complex proteins tagged with epitopes and fluorescent proteins will be generated and used for both the purification and the assessment of the enrichment of the apical complex in the biochemical and microscopy assays outlined below.

2. Extraction and fragmentation of merozoite cytoskeleton.

This step will be performed similarly as the protocol I developed in *T. gondii*. In brief, *P. falciparum* merozoites will be extracted with detergents. The resulting parasite "ghost", consisting of apical complex, attached subpellicular microtubules and enclosing fragments of other subcellular organelles, will be fragmented using sonication. The mechanical stress from sonication will break and shear off most of the subpellicular microtubules from the apical complex and release the entrapped fragments of other organelles. The integrity of the apical complex will be examined by light microscope using the apical complex markers tagged with fluorescent proteins

3. Enrichment of the apical complex of *P. falciparum*

Strategy A. Differential centrifugation

The apical complex of *T. gondii* can be easily separated from other cytoskeletal fragment by differential centrifugation, which is a fast, easy, low cost and high yield process that allows for the recovery of more than 90% of the apical complex in the starting material. This method will thus be tested in *P. falciparum* first, however it might not be as effective in isolating the apical complex of *P. falciparum*, because of the distinct features of *P. falciparum* apical complex (e.g. conoid can not be detected in this organism), therefore alternative strategies have to be considered.

Strategy B. Affinity purification

Antibody to an epitope or fluorescent protein tagged onto the apical complex proteins is conjugated to Dyna-450 Epoxy bead (Dyna Inc.). Detergent extracted and fragmented parasite cytoskeleton will be incubated with the antibody-conjugated beads to separate the apical complex from the rest of the cytoskeletal fragments. Antibodies to endogenous proteins can also be used in this strategy when available.

Strategy C. Enrichment of apical complex by flow cytometry

Taking advantage of fluorescently labeled apical complex markers, the apical complex can be separated from other cytoskeletal fragments based on its fluorescence using flow cytometry. This method is likely to yield a high purity apical complex preparation, however, it is more costly and lower yield.

III. MudPIT analysis to identify protein components of the apical complex

Multiple replicates (~10) of the apical complex enriched and depleted fractions will be used for MudPIT analysis and comparative proteomics will be conducted between the two fractions. Due to the extreme sensitivity of MudPIT and its highly non-uniform response to protein concentration among different protein species, the use of multiple replicates is essential to distinguish *bona fide* apical complex components from chance contaminants. The bioinformatics methodology to distinguish true components from contaminants has been well established by my work in *T. gondii*.

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STATEMENT OF TEACHING EXPERIENCES AND INTERESTS

I taught for three years (September, 1997 – July, 2000) in five different courses as a teaching assistant during my Ph.D study at the University of Pennsylvania. I taught various levels of students, from freshman introductory biology (Biology 101, Biology121 and Biology 122), Principles of Microbiology (Biology 175) to Molecular Biology and Genetics (Biology 475) for advanced students. My teaching duties included preparing experimental protocols for the students, lecturing before the lab; supervising and rating the students' experimental and course work.

Since 2001, I have been teaching as an assistant instructor at Cold Spring Harbor Laboratory, in an annual course entitled "Immunocytochemistry, In Situ Hybridization, and Live Cell Imaging." This is one of the most prestigious courses in the country for theoretical and practical training in advanced imaging techniques and the subcellular localization of genes, transcripts, and proteins. This two-week intensive course has a highly competitive admission process. The selected students come from all over the world and at all levels, from early pre-doctoral students, postdoctoral researchers to established faculty members. My teaching duties for this course, which are shared among six instructors, include giving lecture on principles of microscopy, designing experiments, preparing the course manual, and supervising the logistics for experimental materials. I particular enjoy this course, because each year I have the opportunity of closely working with an amazingly diverse group of highly motivated and curious students from different scientific and cultural backgrounds. In addition, by working side by side with several superb cell biologists and great teachers, including Jason Swedlow, John Murray, Abby Dernberg and Rich Day etc., I myself have sharpened my own teaching skills.

In sum, my teaching experience has provided me with extremely valuable first-hand experience in interacting with students at very different levels. As a result, I consider myself well-versed in their motivations and needs and well-equipped for effective conveying of complicated concepts and experimental approaches.

I am therefore looking forward to contributing to high quality education of future generations of scientists and am committed to building a strong undergraduate teaching program to educate students in modern concepts of cell biology, in what we can learn from its history of progress, how old problems were solved, and more importantly, how to set the stage for probing new emerging questions and explorations in entirely new fields. Furthermore, from my past research and teaching experience, I am well-trained in cutting-edge quantitative high resolution microscopy and image analysis techniques, a set of important and widely-sought skills to pass on to students, undergraduate and graduate students alike.