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Mapping the Physical Interactions of

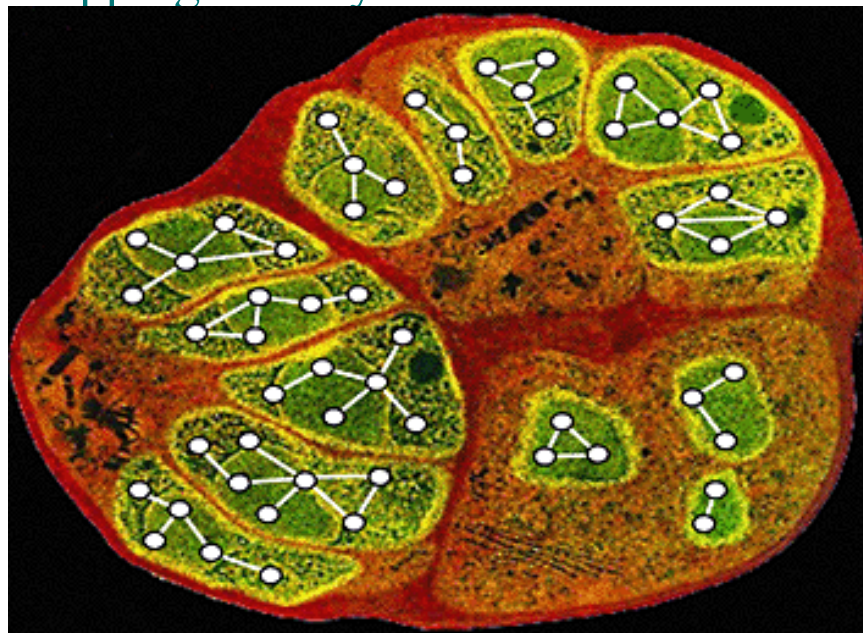


Image Title: Stylized protein interaction modules shown as white circles (proteins) connected by lines (interactions) within malaria parasites (in green) in an infected human red blood cell. - Colored transmission electron micrograph from Photo Researchers Inc., design by Marissa Vignali

Researchers have produced a detailed map that outlines thousands of physical interactions that take place between proteins found in the deadly malaria parasite, *Plasmodium falciparum*. This is the most extensive description to date of how the parasite's proteins interact.

The map provides new intelligence that will help in making decisions about the best proteins to target with drugs and vaccines in the effort to control this killer of as many as 2.7 million people a year worldwide. Human malaria is transmitted by female *Anopheles* mosquitoes, which serve as vectors for the parasites that cause the illness. *P. falciparum* causes about 80 percent of all human malaria infections and about 90 percent of the deaths.

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yielding new hypotheses and new insights about how the organism works."

— Stanley Fields

The research team, which included scientists from Prolexys Pharmaceuticals and the Howard Hughes Medical Institute (HHMI), published their findings in the November 3, 2005 issue of the journal *Nature*. Robert Hughes of Prolexys and HHMI investigator Stanley Fields at the University of Washington were senior authors of the article.

Until now, “there were only a handful of protein interactions known from directed searches in *Plasmodium falciparum*,” said Fields. “There had been no survey at the genome-wide level.” Understanding which proteins interact is crucial because interconnecting networks of proteins work together to guide the parasite's metabolism and pathogenesis. Thus, mapping the multitude of interactions among proteins is likely to lead to new insights about drug targets or vulnerabilities in the parasite's defenses.

In searching for interactions between proteins, the scientists used a technique known as the yeast two-hybrid assay, which Fields invented. In the assay used in the *Nature* study, the researchers inserted the genes for malaria protein fragments into yeast cells and induced the yeast to produce those fragments. The inserted malaria protein fragments were also engineered to be expressed together with either of two components of a regulatory protein. When malaria fragments that interact were brought together, a telltale marker gene was then triggered by the regulatory protein - detected by growth of yeast cells on a Petri plate.

Using advanced analytical systems developed by Prolexys, the researchers performed more than 32,000 yeast two-hybrid screens to identify interacting malaria proteins. The scientists also used a method for ensuring specific production of the target malaria protein fragment in the yeast, as well as highly automated machinery for performing the large-scale assays.

From these assays, the scientists identified 2,846 protein-protein interactions, most of which included a protein whose function had not yet been identified, said Fields. Knowledge of protein interactions can help reveal that function, he said.

“It's guilt by association,” he said. “When we see a protein that is uncharacterized interacting with a protein whose function is known, it's likely that the uncharacterized protein also shares that function.” What's more, as clusters of interacting proteins are mapped, multiple unknown proteins can be considered likely candidates to take part in the same biological function, he said.

The researchers analyzed the functions of known proteins involved in the interactions to reveal such clusters. That analysis revealed groups of proteins implicated in processes such as chromosome modification and gene

activation, as well as those involved in invading host cells.

“The interactions involved in host invasion will give the parasitology community another focus to study this process,” said Fields. “And some of these proteins may turn out to be useful vaccine targets.”

The researchers also identified a group of interacting proteins that the malaria parasite exports into the host cell. These interactions could provide parasitologists with insights into how the parasite modifies the host cell during infection, he said.

In the next phase of studies, the researchers are developing similar protein interaction data for the parasite *Plasmodium vivax*, another parasite that causes malaria. *P. vivax* is less virulent than *P. falciparum* and is seldom fatal. “Our aim in carrying out this analysis in a related but different parasite was that we might see interactions that would be in common between *falciparum* and *vivax*,” said Fields. Such knowledge would yield insight into the basic cell biology of the parasites, he said.

The researchers also are analyzing interactions between the malaria parasite proteins and human proteins, which could yield insights into the process of host cell infectivity, invasion and pathogenesis, said Fields.

“Our hope is that the parasitology community will find this knowledge useful to further their understanding of the basic biology of *Plasmodium* and this understanding will ultimately lead to new drugs or vaccine candidates,” said Fields. “This organism is very difficult to study. And the protein interaction data can be important - especially when combined with mass spectrometry data on which proteins are present, with protein sequence data and with genetic transcriptional profiles—in yielding new hypotheses and new insights about how the organism works.”