Tiny antibodies point to vulnerability in disease-causing parasites

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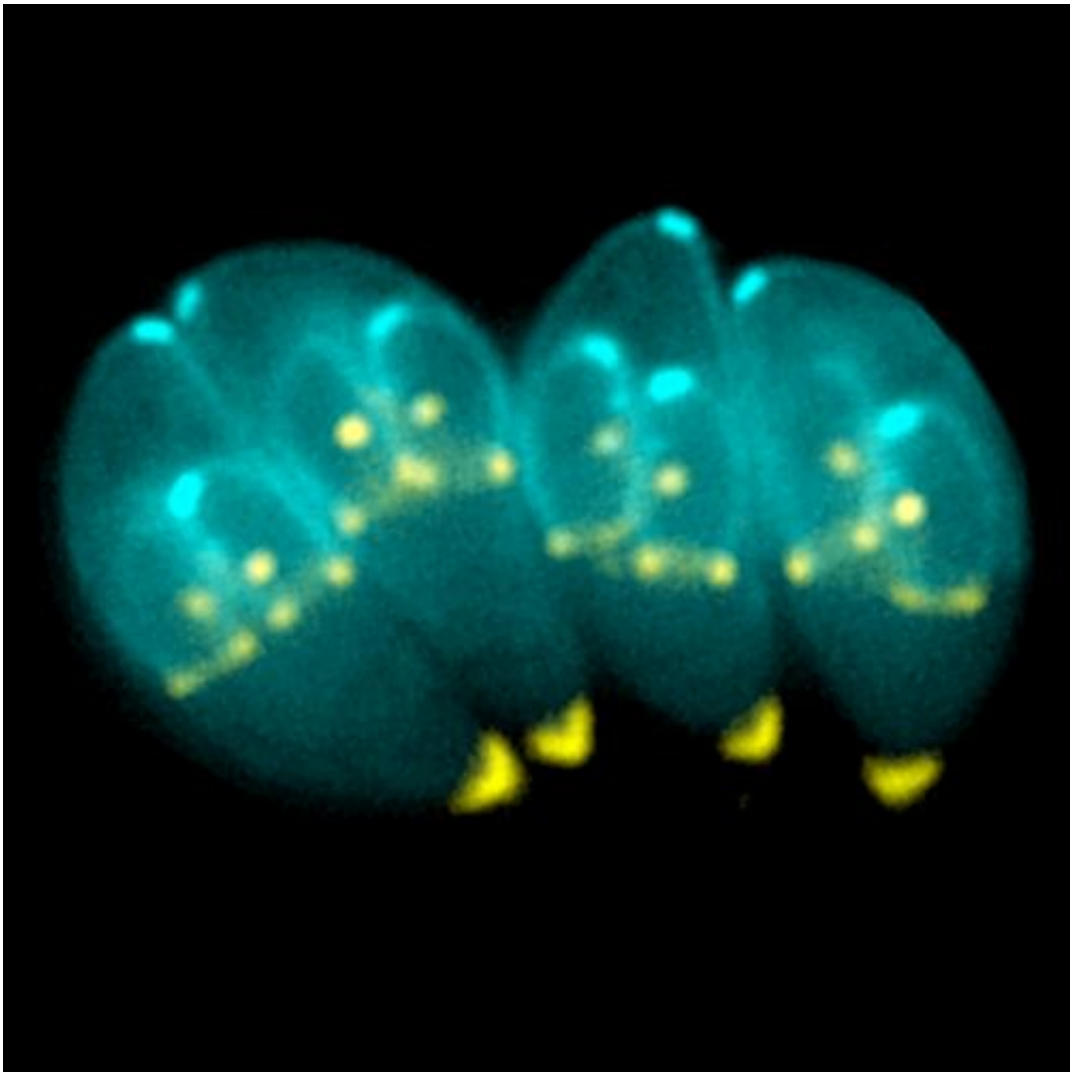

Toxoplasma gondii. Credit: Wikipedia

By teasing apart the structure of an enzyme vital to the infectious behavior of the parasites that cause toxoplasmosis and malaria, Whitehead Institute scientists have identified a potentially 'drugable' target that could prevent parasites from entering and exiting host cells.

Although toxoplasmosis causes disease only in certain individuals-including immunocompromised patients, pregnant women, and their infants, the *T. gondii* parasite is closely related to *Plasmodium*, which causes malaria. Research on *T. gondii* can provide insights into *Plasmodium*'s inner workings.

To learn more about the enzymes known as kinases, which regulate the activity of the toxoplasmosis-causing parasite *Toxoplasma gondii*, Whitehead Fellow Sebastian Lourido and his team enlisted an unlikely assistant: the alpaca. Unlike humans, whose antibodies have a heavy chain and a light chain, alpacas create heavy chain-only antibodies, which can be engineered into even smaller antibody fragments known as nanobodies. Alpaca nanobodies have a unique shape that allows them to reach into a protein's nooks and crannies, inaccessible to conventional antibodies.

Working with scientists from Whitehead Member Hidde Ploegh's lab, Thomas Schwartz's lab at MIT, and D.E. Shaw Research, Lourido identified a nanobody against the *T. gondii* enzyme CDPK1 (for "calcium-dependent protein kinase 1") that binds the kinase's regulatory domain and revealed a previously unappreciated feature of the its activation. CDPKs are essential for *T. gondii* and related parasites to invade and exit host cells, move, and reproduce. According to Lourido, this is one of the first times that nanobodies have been used to decipher the inner workings of an enzyme.

Conveniently, the nanobody, called 1B7, stabilizes CDPK1 in a conformation that allowed researchers in the Schwartz lab to solve the

kinase's structure and describe the nanobody's interaction with the molecule. With the structure in hand, the Shaw lab created long-timescale molecular dynamics simulations of the enzyme, to model the events leading to kinase inactivation. Details of the team's work are published online this week in the journal *Proceedings of the National Academy of Sciences* (*PNAS*).

Structural homology between CDPKs and the calmodulin-dependent kinases (CaMKs) found in humans led to earlier assumptions that both types of enzymes are activated in a similar fashion. But the team's work shows otherwise. A CaMK is activated when a wedge holding it in an inactive state is knocked away. In contrast, Lourido likens a CDPK's active conformation to a broken arm that must be splinted in two places to maintain its integrity. When the rigid splint is removed, the kinase loses its structural ability to function. By blocking CDPK1's regulatory domain, the 1B7 nanobody inhibits the kinase by preventing the enzyme's splint from attaching.

"This work reveals something interesting about this class of enzymes," says Lourido. "It's the first time a calcium-regulated kinase has been shown to be activated in this manner. The principle that we identify is really important: we've found a new vulnerability within an enzyme that we know is extremely important to this class of parasites—including *Plasmodium*, the parasite that causes malaria—and is absent from humans."

Because humans lack similar kinases, drugs that target CDPKs would not affect host cells.

"The location where 1B7 binds to CDPK1 is a new drug target that people had not considered before," says Jessica Ingram, a postdoctoral researcher in Ploegh's lab and one of the lead authors of the *PNAS* paper. "We'd like to do some drug screens in the presence of the nanobody to

see if we can find small molecules that bind in the same way. We could also look at other nanobodies against other kinases to see if this is applicable to other parasites and systems."

More information: Allosteric activation of apicomplexan calcium-dependent protein kinases, www.pnas.org/cgi/doi/10.1073/pnas.1505914112

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