Atomic structure of antifungal drug confirms unusual mechanism, opens door to less-toxic derivatives

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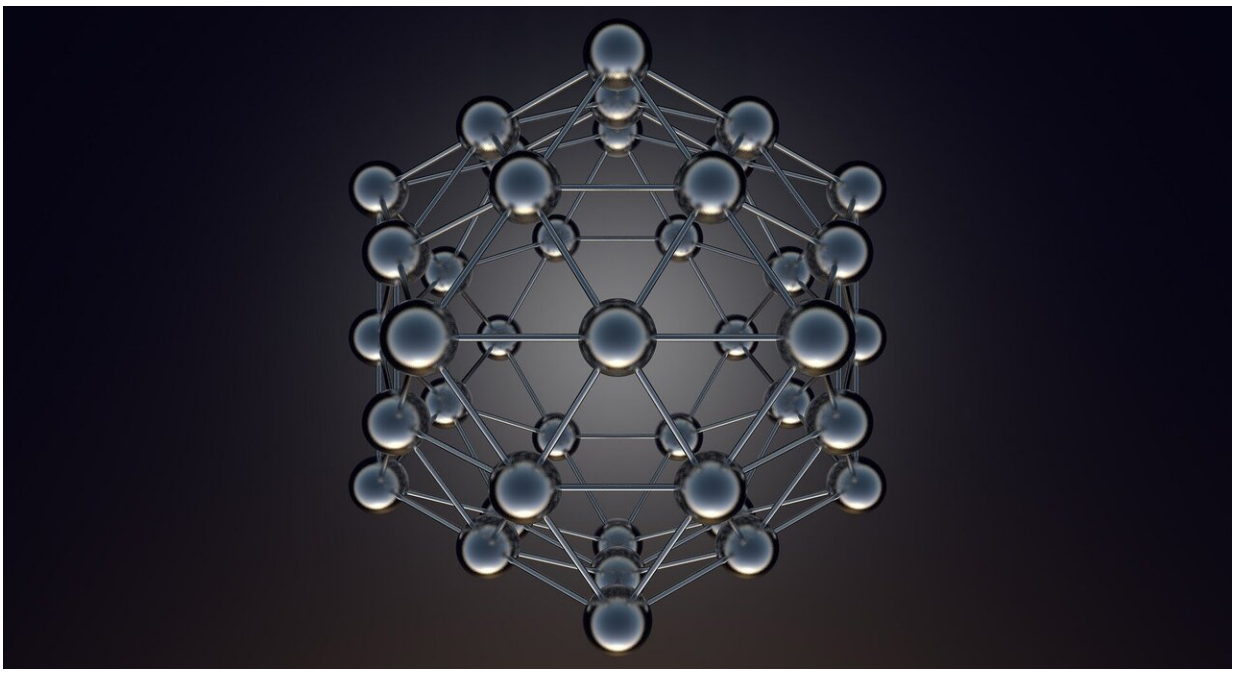

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Advanced molecular imaging technology has now mapped the structure of a drug widely used to treat fungal infections but whose workings have mystified researchers and physicians for nearly 70 years.

In a new study, researchers at the University of Illinois Urbana-

Champaign, the University of Wisconsin, Madison and the National Institutes of Health described in atomistic detail the structure of the drug amphotericin B, a powerful but toxic antifungal agent.

Seeing the structure provides illumination in the researchers' quest to formulate less-toxic AmB derivatives, said Dr. Martin D. Burke, a professor of chemistry at Illinois and a member of the Carle Illinois College of Medicine, as well as a medical doctor. Burke co-led the study with Chad Rienstra, a Wisconsin professor of biochemistry, and Taras Pogorelov, an Illinois research professor of chemistry. The researchers reported their findings in the journal *Nature Structural & Molecular Biology*.

"It's like we were driving in the dark at night, and all of a sudden we were able to put the lights on. With the clarity of this structure, we can see where we need to go to reach our goal of a less-toxic antifungal drug," Burke said.

Previously, researchers and physicians thought that AmB killed fungal cells by forming channels in the cell membrane, the outer envelope that encases the cell. However, in 2014, while Rienstra was a professor at Illinois, Burke and Rienstra's group found that amphotericin primarily kills cells by robbing the membrane of sterol molecules—cholesterol in human cells and ergosterol in fungal cells. Individual amphotericin molecules aggregated into a larger structure that absorbed sterol molecules out of cell membranes like a sponge, causing the cells to die.

"The ion channel is a secondary action to the antifungal activity. That let us disconnect the ion channel-forming function from the fungicidal activity of amphotericin," Burke said. His group has applied the channel-forming abilities of AmB as a "molecular prosthetics" approach to treat cystic fibrosis, yet greater understanding of the fungicidal sterol sponge remained elusive.

"We had some images but no details," said Agnieszka Lewandowska, a senior research scientist at Illinois and first author of the new study. "Now we can really see the part of the structure that we think is responsible for interacting with cholesterol, which we don't want. So then we could modify that and make sure it only interacts with ergosterol, which we do want."

Since AmB forms a large aggregate, the usual molecular imaging techniques such as nuclear magnetic resonance are difficult to apply. In the new study, the researchers developed novel sample preparation protocols and used an advanced molecular imaging technique called magic-angle spinning solid-state NMR. They also used advanced computational modeling methods to visualize the structures represented by the NMR data.

The result was a picture in atomistic detail of how small AmB molecules fit together in a head-to-tail configuration, staggered into a large lattice, leaving a void shaped and sized just right for sterol molecules. There was also some flexibility within the aggregate, potentially allowing it to flex a bit to accommodate cholesterol, which is slightly larger than ergosterol.

"We wanted to know how the AmB sponge fits together to accommodate ergosterol," Rienstra said. "Just like sponges that absorb water, if it's dried out and crusty, it doesn't move well and won't do a very good job of absorbing sterols. Once it's a little soft, it does a better job of absorbing because then it's flexible."

The detailed structure validates earlier work and also provides a road map for synthesizing derivatives, the researchers say.

"We are already in the process of investigating the structures of the AmB complexes with both cholesterol and ergosterol. It opens the door to finally build or find nontoxic derivatives of this important drug and

help a lot of people without the horrible side effects that AmB has right now," Lewandowska said.

Next, the researchers plan to continue collaborating to synthesize derivatives and then study their atomistic structures to see how they aggregate and interact with both cholesterol and ergosterol, as well as to explore the potential of other small molecules.

"Amphotericin works differently than any other drug we know about. It doesn't bind to a protein; it self-assembles into this interesting aggregate," Burke said. "We saw this whole new area of small molecule interactions. This imaging technique is giving us new tools to understand small molecule interactions and how they can perform higher order, proteinlike functions. We're finally in a position where we can rationally tap into AmB's huge functional potential, both for antifungal treatment and for molecular prosthetics."

More information: Lewandowska, A. et al, Fungicidal amphotericin B sponges are assemblies of staggered asymmetric homodimers encasing large void volumes, *Nat Struct Mol Biol* (2021). doi.org/10.1038/s41594-021-00685-4 , www.nature.com/articles/s41594-021-00685-4

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