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How an acid trip saves malaria: a proton-transfer mechanism for the malaria lactate-proton transporter PfFNT

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The malaria parasite *Plasmodium falciparum* relies extensively on anaerobic glycolysis for energy production in the intraerythrocytic phase of its lifecycle. The parasites depend on their formate-nitrite transporter (PfFNT) to extrude lactate and protons, the major by-products of anaerobic glycolysis, from their cytosol to prevent lethal disruptions to cytosolic pH and cell volume. However, it is not known if charged lactate and protons are transported independently, together as neutral lactic acid, or if the species convert during the transport cycle.

Cryo-EM structures have revealed each subunit of PfFNT contains a transport cavity midway through the protein, bordered by hydrophobic constrictions on each side. Inside each cavity is a histidine residue (His230) believed to be involved in substrate binding. At cytosolic pH we expect lactate to be the dominant substrate present over lactic acid. Paradoxically, our pKa calculations predict His230 to be neutral. As a charged lactate molecule is unlikely to interact with a neutral histidine residue, we hypothesise that either lactate or His230 must become protonated for binding to occur.

To test the hypothesis we used extensive molecular dynamics simulations covering all potential protonation states of the substrate and protein, and find that binding from the intracellular side only occurs between lactate and positively charged His230. Using umbrella sampling simulations we further show that lactate binding to charged His230 is more energetically favourable than lactic acid binding to neutral His230, revealing a large energy barrier for lactic acid to enter the transport cavity. As lactate binds tightly in the cavity, we suggest that lactate gets protonated to lactic acid in order to be released to the extracellular medium. This is supported by simulations in which we move the proton from His230 to lactate and observe the newly formed lactic acid dissociating to the extracellular side. Subsequently, we propose a proton-transfer mechanism as the mechanism of PfFNT transport.