The unique allosteric regulation of crocodilian hemoglobin revealed by Cryo-EM

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Among all vertebrates, only crocodilian hemoglobin (Hb) has acquired a mechanism of allosteric regulation by bicarbonate ions (HCO_3^-), which allows the animal to stay underwater for extended periods. More than 40 years ago, it was discovered that bicarbonate ions strongly reduce the oxygen affinity of crocodilian Hb, but the molecular mechanism has remained unexplained because of difficulties with crystallization.

Using cryo-EM, we have solved the structures of crocodilian Hb in the deoxy, oxy, and carbonmonoxy states at 2.2-2.3 Å resolution. Hemoglobin has a molecular weight of only 64 kDa, still a challenging size for cryo-EM, and past cryo-EM studies of human Hb have reached a maximum resolution around 3 Å. Thanks to recent improvements in hardware and software, and the use of fresh native Hb samples from living animals, we achieved high enough resolution to observe details of the allosteric mechanism. The maps of bicarbonate ions are clearly visible, and we have unveiled the interactions between bicarbonate ions and polar or positive-charged sidechains of deoxy crocodilian Hb, at a site where no ligands are found to bind other animal Hbs. Two unique amino acid replacements are essential to form the binding site for bicarbonate ions in the T-state (deoxy) Hb, while this site is lost on the protein switching to the R-state.

Moreover, our models show some significant differences from earlier X-ray models of liganded (R-state) human Hb, indicating possible effects of crystal packing.