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Supplementary information

Origin of complexity in haemoglobin evolution

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Supplemental Discussion

As shown in Fig. 1, Ancα/β does not display significant cooperativity of oxygen binding, but the confidence interval on this measurement is large (Hill coefficient *n*=1.02 ±0.24). We therefore cannot experimentally rule out the possibility that Ancα/β may have been weakly cooperative. The lack of cooperativity we observed, however, is corroborated by two independent lines of evidence. First, structural knowledge about the basis of cooperativity in extant Hb indicates that cooperativity is mediated by a conformational change in each subunit upon oxygen binding that involves reorientation of multimeric binding across IF2 – specifically, a rotation of the subunits relative to each other (63). Our data establish that Ancα/β dimers do not interact across IF2, so cooperativity of oxygen binding is not expected. Second, a lack of cooperativity in Ancα/β is the most parsimonious hypothesis, because neither extant Hbα homodimers nor Hbβ homotetramers are cooperative; for Ancα/β to have been cooperative, early gain and subsequent loss of cooperativity would have to occur in both subunit lineages.

If Ancα/β were, in fact, weakly cooperative, but our assay was unable to detect it, this would have the following implications. 1) Ancient weak cooperativity Ancα/β would have been gained during the interval between AncMH and Ancα/β, because AncMH, being a monomer, could not have been cooperative. 2) This ancient weak cooperativity would have been mediated by a structural mechanism different from that in extant Hbs. This putative mechanism would then have been lost and replaced by acquisition of IF2-mediated cooperativity during the interval between Ancα/β and Ancα+Ancβ. 3) The derived mechanism of cooperativity would be stronger than the ancient form, with this strengthening of cooperativity occurring during the interval between Ancα/β and Ancα+Ancβ, as our measurements reported in Fig. 1 show.

Supplemental Discussion Reference:

63**.** Perutz MF, Wilkinson AJ, Paoli M, Dodson GG. The stereochemical mechanism of the cooperative effects in hemoglobin revisited. Annu Rev Biophys Biomol Struct. 27:1-34, 1998.