

# Paleotemperature trend for Precambrian life inferred from resurrected proteins

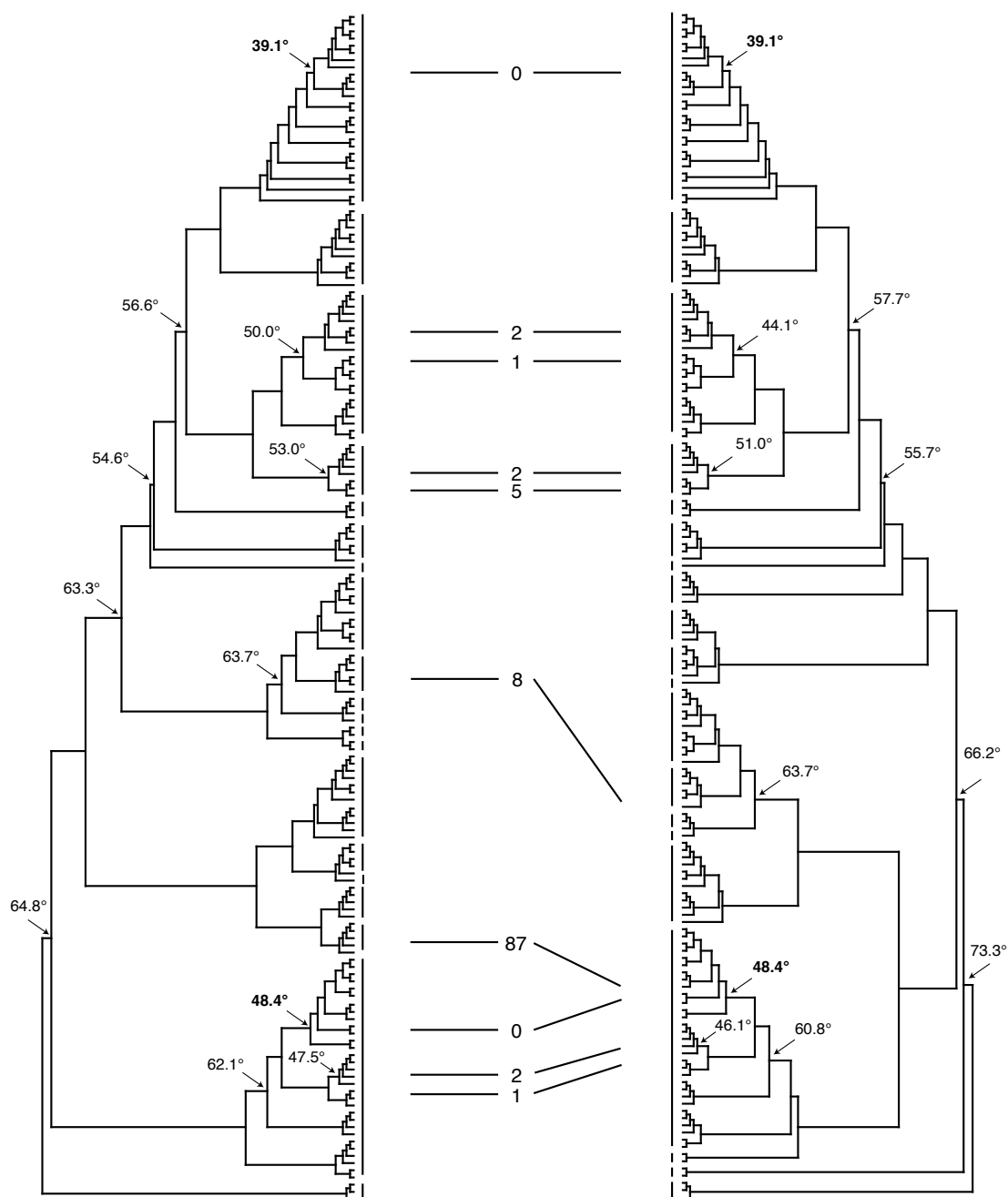
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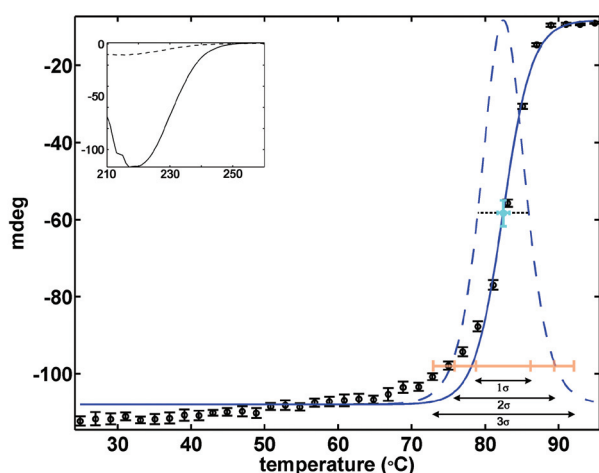
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Figure S1



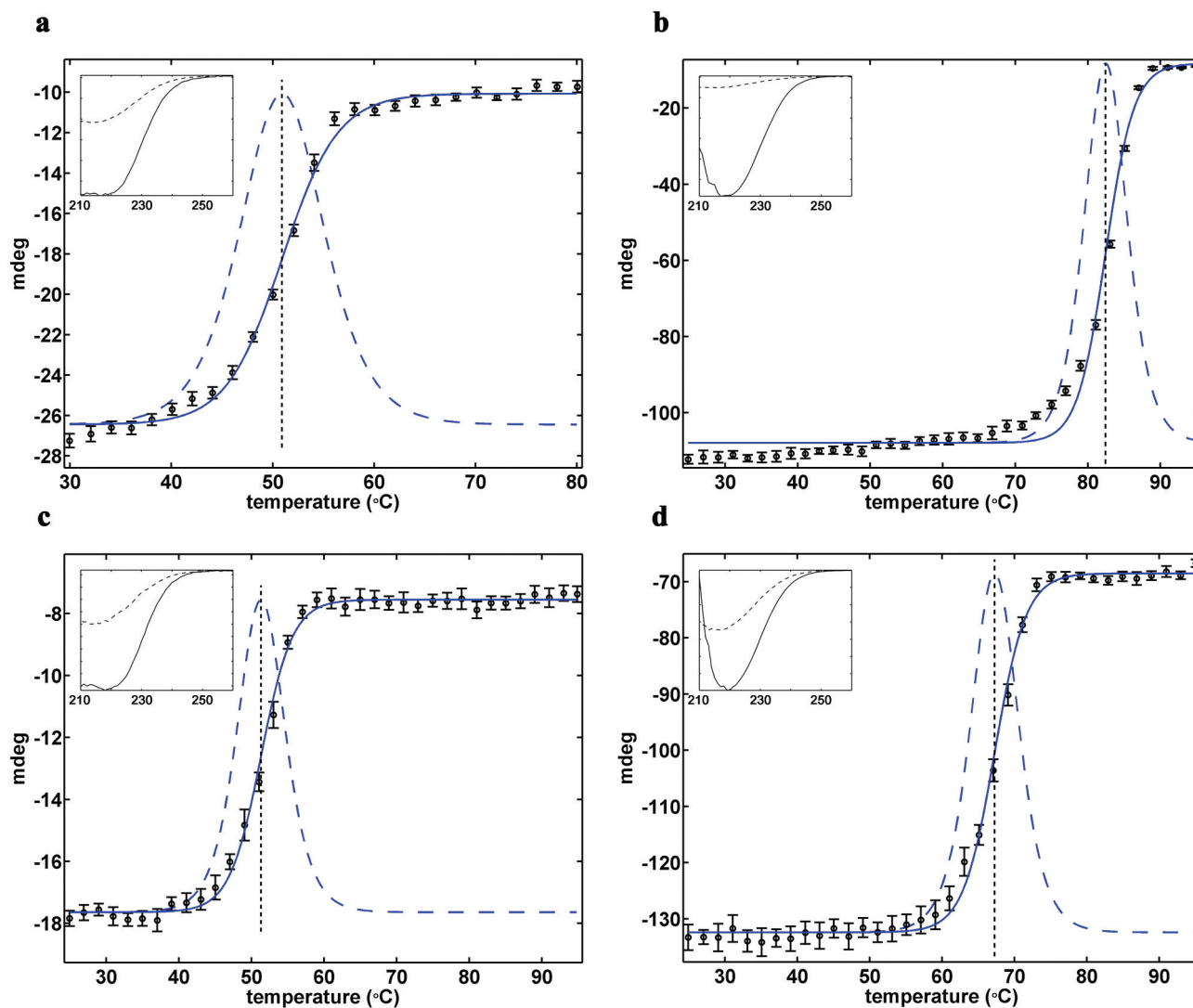
**Figure S1: Cladograms from figure 1 of the article.** This figure highlights the number of amino acid differences between analogous ancestral sequences between the two phylogenies. Melting temperatures for ancestral EF proteins are shown at their corresponding nodes. Values in bold represent identical sequences at analogous nodes between the two trees.

Figure S2



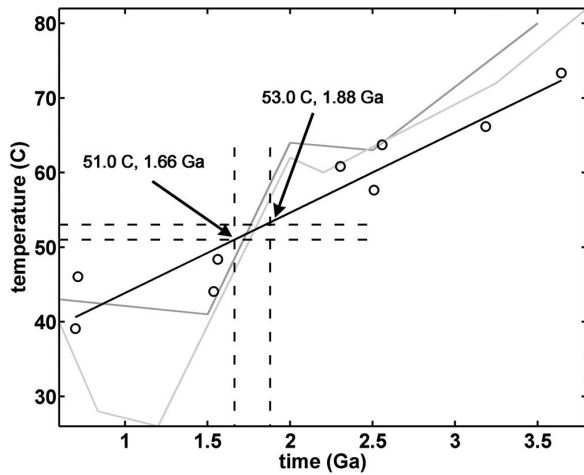
**Figure S2: Melting temperature ( $T_m$ ) analysis of ancestral and modern EF proteins.** Black circles represent the ellipticity (in millidegrees, y-axis) at 222 nm across a range of temperatures (x-axis), and their associated errors. Solid blue line shows the sigmoid line fit to the data. Light blue represents the inflection point ( $T_{1/2}$ ), and its error, of the sigmoid line. Dashed blue line is the scaled derivative of the sigmoid curve. Dashed black line is the full-width at half-maximum, while solid black lines with arrowheads show the standard deviations within the derivative. Inset highlights the wavelength scans before (solid line) and after temperature (dashed line) scans. The inset highlights the change in alpha helical confirmation at 222 nm, and thus the unfolding of the EF proteins in response to increased temperatures (solid and dashed lines show wavelength scans pre- and post-temperature scans). CD analysis of *Thermus* EF is shown as an example.

Figure S3



**Figure S3: Representative CD scans of ancestral and modern EFs.** Melting profiles for a, *E. coli*, b, *Thermus*, c & d, two ancestral EFs. Detailed description is provided in the legend to supplementary figure 2. Dashed vertical line highlights the inflection point ( $T_{1/2}$ ) of the sigmoid lines.

Figure S4



**Figure S4: Time estimate for last common ancestor of mitochondrial bacteria inferred from thermostability of ancestral proteins.** See corresponding node in figure 1 of article. Time is inferred from a linear fit to the temperature data. This inference assumes a linear decrease in the paleotemperature trend. A non-linear decrease could potentially translate to earlier or later time estimates for the last common ancestor of mitochondria. Additional details are provided in figure 3 of the main article.