Reviewer 1

The reviewer's comments have been included in black and our responses with proposed changes are in blue.

This study presents a statistical model for estimating numerical ages from intra-crystalline protein decomposition (IcPD) data in fossil mollusc opercula. The authors use amino acid racemization data from seven boreholes in the Pannonian Basin, Hungary where opercula are buried at depths of 5-460 m combined with independently derived ages and measured geothermal gradients. The key innovation is incorporating both time and burial temperature into a single predictive framework using Bayesian inference. The model is tested on four samples, producing age estimates with broad uncertainty distributions (several hundred thousand years), with better performance on deeper, older material. The authors identify multiple sources of unaccounted uncertainty and propose this as a pilot study requiring substantial further development.

STRENGTHS

Strong Empirical Foundation. The study builds on previous analyses of material with known ages and measured geothermal gradients (Nelson et al., 2024). This is a solid foundation for developing a new methodology. The use of multiple boreholes (n=7) with variable geothermal gradients provides natural variation in the temperature-time space.

Sound Statistical Framework. The Bayesian approach to uncertainty estimation is appropriate, although I cannot speak to the accuracy of its execution. Using three amino acids (Ala, Glx, Val) with different racemization rates to calibrate age predictions is sensible.

Realistic of Burial Temperatures. Unlike earlier isothermal heating studies, this work attempts to model actual burial conditions, including varying temperatures with depth and time. The incorporation of variable geothermal gradients (40–57 °C/km) is a step toward more realistic modeling of racemization kinetics at environmental temperatures rather than laboratory conditions.

T**ransparent Acknowledgment of Limitations.** The authors openly discuss sources of uncertainty (Püspöki age model uncertainties, geothermal gradient estimates, initial burial phase effects, climate influence near surface) and clearly identify this as preliminary work needing further refinement.

WEAKNESSES & CONCERNS

Unclear Explanation of Motivation and Significance. The paper needs additional context to better understand the motivation and significance of this study. For a non-specialist reader, the paper seems to imply that a numerical model is necessary to produce ages from amino acid data. The core contribution of developing a model that can estimate numerical ages from IcPD data without relying on direct calibration but instead using estimated temperature history is obscured. The paper would be improved by clearly explaining:

- How traditional AAG dating works as a relative technique dependent on calibration using other geochronology methods
- What problem arises when age calibration data are unavailable (the motivation for this work)
- How this modeling approach offers a potential solution by substituting temperature history for direct calibration
- Why this is a significant conceptual advance

Without this context, the significance of the modeling effort is lost, and the results (broad age distributions) appear disappointing rather than a promising first step toward a new approach.

We thank the reviewer for highlighting the strengths of this paper and providing a constructive critique. We agree that adding context will better explain the significance and motivation of our model to a wider audience. While some context is provided in the second paragraph of the introduction, we propose expanding this section to avoid implying that numerical models are necessary to derive age constraints from amino acid geochronology (AAG). Specifically, we will expand our explanation on how AAG is traditionally used for age control in Quaternary deposits. We will then include a discussion of the challenges when calibration data is lacking before reviewing previous attempts to establish age calibrations from AAG.

In the third paragraph, we propose shifting the focus to the unique conditions of the Pannonian Basin, where independent ages and known temperature gradients help establish a model for IcPD versus age and depth. We will explain how this builds on prior work. We agree, that by establishing this background the significance of this new approach will be made clearer.

Unclear Applicability. The study focuses on material buried at depths >100 m, but the paper does not clearly articulate whether this model is specifically designed only for deep burial contexts or whether it is intended as a general approach applicable to any material where temperature history can be estimated. This distinction is crucial for understanding the significance and future potential of the work. The paper notes that shallow samples produce "uninformative" distributions, suggesting the approach breaks down near the surface where climate effects are strong. However, the paper does not explain whether this is a fundamental limitation of the model or a problem that future development might overcome. If the model applies only to deeply buried material, what is its practical scope for Quaternary dating, where most studies involve shallow deposits?

We designed this model to evaluate whether subsurface temperature profiles can estimate IcPD extent, since temperature a controlling variable of the decomposition process. However, as the reviewer highlights, surface heat conduction makes modeling near-surface temperatures challenging.

To enable more accurate age predictions for shallower deposits, future research should integrate modern ground temperature data from core sampling sites to observe the depth limit of surface temperature influence. Adapting the model to account for this will require adjusting its current mathematical parameters and assumptions. We will expand the existing discussion on shallower deposit age estimation (lines 199–211) to outline this future direction.

Limited Discussion of Kinetics. A major motivation for this work is to "provide a more accurate description of the kinetic behaviour of IcPD at lower temperatures." However, this stated goal is not explicitly addressed. There is minimal comparison with high-temperature data or discussion of whether the kinetic parameters differ meaningfully from what previous studies suggest.

We thank the reviewer for this comment. Due to the unique conditions of this area, we agree that this dataset provides an excellent opportunity to study the kinetics of IcPD at lower temperatures, and is something we wish to explore. Our model uses both the geothermal gradient and the extent of IcPD to estimate age; however, its current accuracy remains limited, and key sources of uncertainty still need to be explored.

Due to these limitations, we do not believe a detailed discussion of kinetic parameters is possible at this stage, making it beyond the scope of this short communication. Comparing the kinetics of protein decomposition in this dataset with previous high-temperature experimental data would be better addressed in a dedicated study. We therefore propose reframing the introduction to focus on developing models that accurately estimate age from IcPD data, while highlighting the comparison between burial temperatures and high-temperature kinetics as a promising avenue for future research in the discussion/conclusion.

Critical Missing Figure. A figure showing the actual IcPD vs. age data that underpins the model is missing. Such a visualization is essential context for readers to comprehend the study. I suggest using separate panels for each amino acid and color-coding the plot symbols by burial temperature.

This visualisation is presented in Nelson et al. (2024), which we have cited in this work. We propose to include an adapted figure, in the format advised by the reviewer, including this as a new figure 2 and renumbering the subsequent figures accordingly.

OVERALL ASSESSMENT

This is a technically sound pilot study that tackles an understudied problem: how to extract numerical age information from amino acid data. The use of real field data, measured geothermal gradients, and Bayesian uncertainty quantification are commendable. However, the work is incomplete in its current form. The motivation and significance are unclear, the stated goal of characterizing low-temperature kinetics is underdeveloped, and the underlaying data are not clearly shown. It needs additional context and more attention to how a non-specialist might be able to comprehend and apply the approach. With these additions, this contribution would be suitable for publication.