

Peer Review

Review of: "Can AI Modelling of Protein Structures Distinguish Between Sensor and Helper NLR Immune Receptors?"

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Using AF3 analyses, the authors convincingly demonstrate that helpers consistently exhibit higher confidence scores than sensors. This finding has significant potential for the rapid identification of helper-sensor pairs in other contexts. To further enhance its practical application, I recommend establishing distinct confidence score ranges for helpers and sensors, calculated using a statistically appropriate method, such as confidence intervals (CIs). This would provide a valuable tool for researchers annotating NLRs.

- 1. Confidence Score Ranges and Statistical Rigor:** While the AF3 analyses convincingly demonstrate higher confidence scores for helpers compared to sensors, providing specific confidence score ranges for each group would significantly enhance the practical utility of this research. I recommend using a more robust statistical approach, such as confidence intervals (CIs), to define these ranges. This approach would enable other researchers to more readily annotate NLRs based on confidence scores.
- 2. Structural Wobbliness of Sensors:** The hypothesis that "sensors" exhibit greater structural flexibility to enhance interactions with diverse signaling molecules is intriguing (in this case, the pathogen-derived molecules). I encourage the authors to explore existing literature supporting this concept in other signaling assemblies. For example, the paper "Speeding molecular recognition by using the folding funnel: The fly-casting mechanism" provides relevant insights into how disorder can facilitate molecular recognition. Discussing this connection would strengthen the manuscript and extend findings beyond the immunity signaling assemblies.
- 3. Funnel-like Proteins and Lipid Bilayers:** The description of "funnel-like proteins and lipid bilayers" could be improved for a broader audience. While the current explanation is accurate,

elaborating on the physicochemical properties of the funnel region would be beneficial. Specifically, addressing whether these regions contain hydrophobic patches and whether they exhibit a preference for specific lipids (e.g., phospholipids) would enhance understanding. Emphasise that these proteins can interact with and disrupt the bilayer without necessarily forming a pore. Are the sensors richer in IDRs (not IDs)?

4. **Clarity on IDs:** The introduction of "ID" regions requires further clarification for non-specialist readers. A more detailed explanation of this concept in the introduction is crucial to ensure the broader accessibility of the paper. Throughout the manuscript, consider minimizing jargon or providing concise definitions when specialized terms are used.
5. **Stoichiometry Justification:** The rationale behind the chosen 5x and 6x stoichiometries with 50 oleic acids (do they make sense for sensors?) and three different seed values needs further justification. What experimental evidence supports this specific configuration, particularly in the context of potential differences between helpers and sensors? It's conceivable that this configuration is optimal for helpers but not for sensors. Providing a clear rationale is essential.
6. **Choice of Rice and Barley:** The selection of rice and barley as model organisms requires justification. Explaining the reasons behind this choice would strengthen the study's context and relevance.
7. **Figure 2 Legend:** The legend for Figure 2 should provide succinct descriptions of the domains depicted.

Declarations

Potential competing interests: No potential competing interests to declare.