

Engineering new-to-nature immune sensors for novel recognition of *Magnaporthe oryzae*



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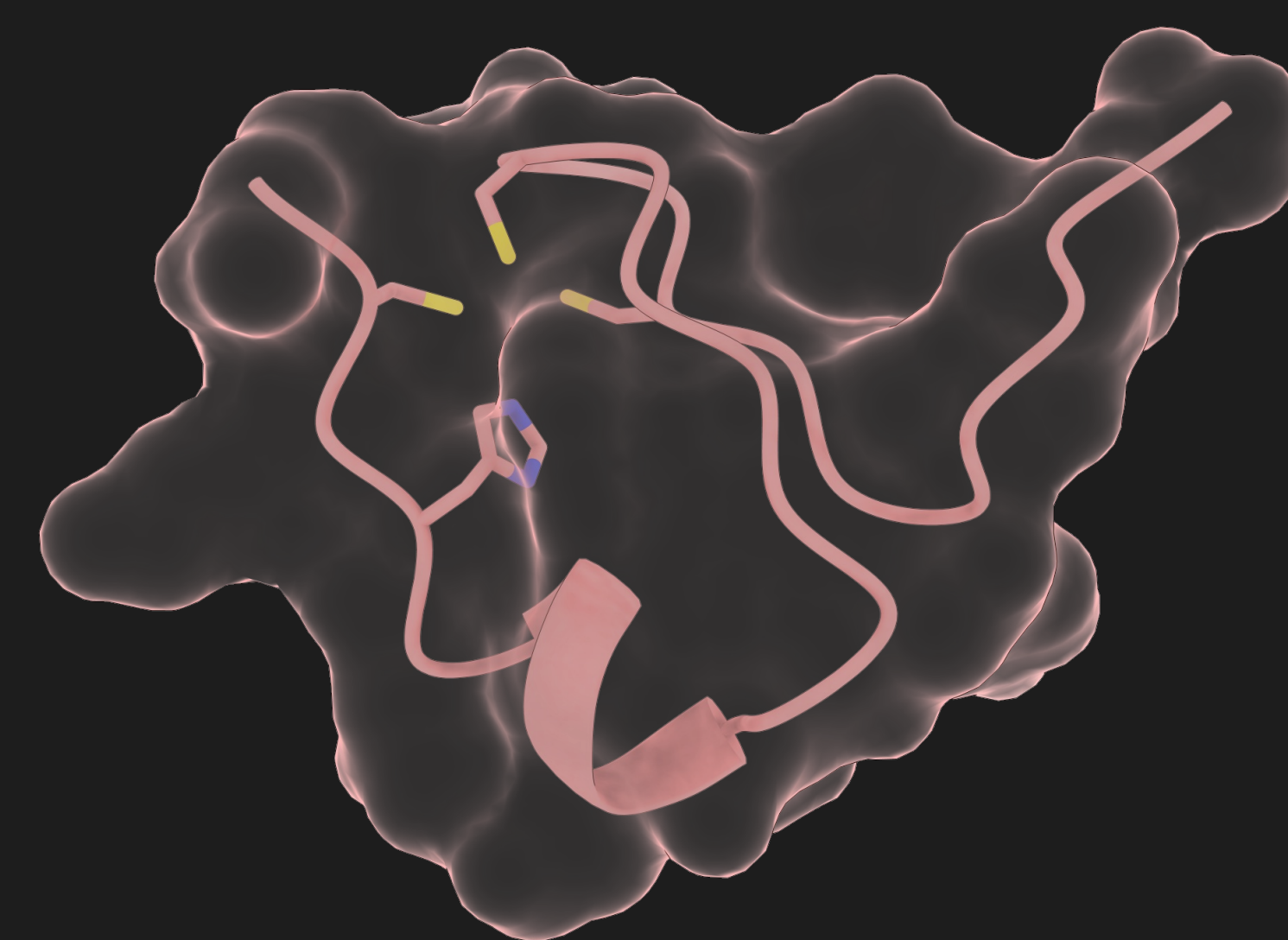
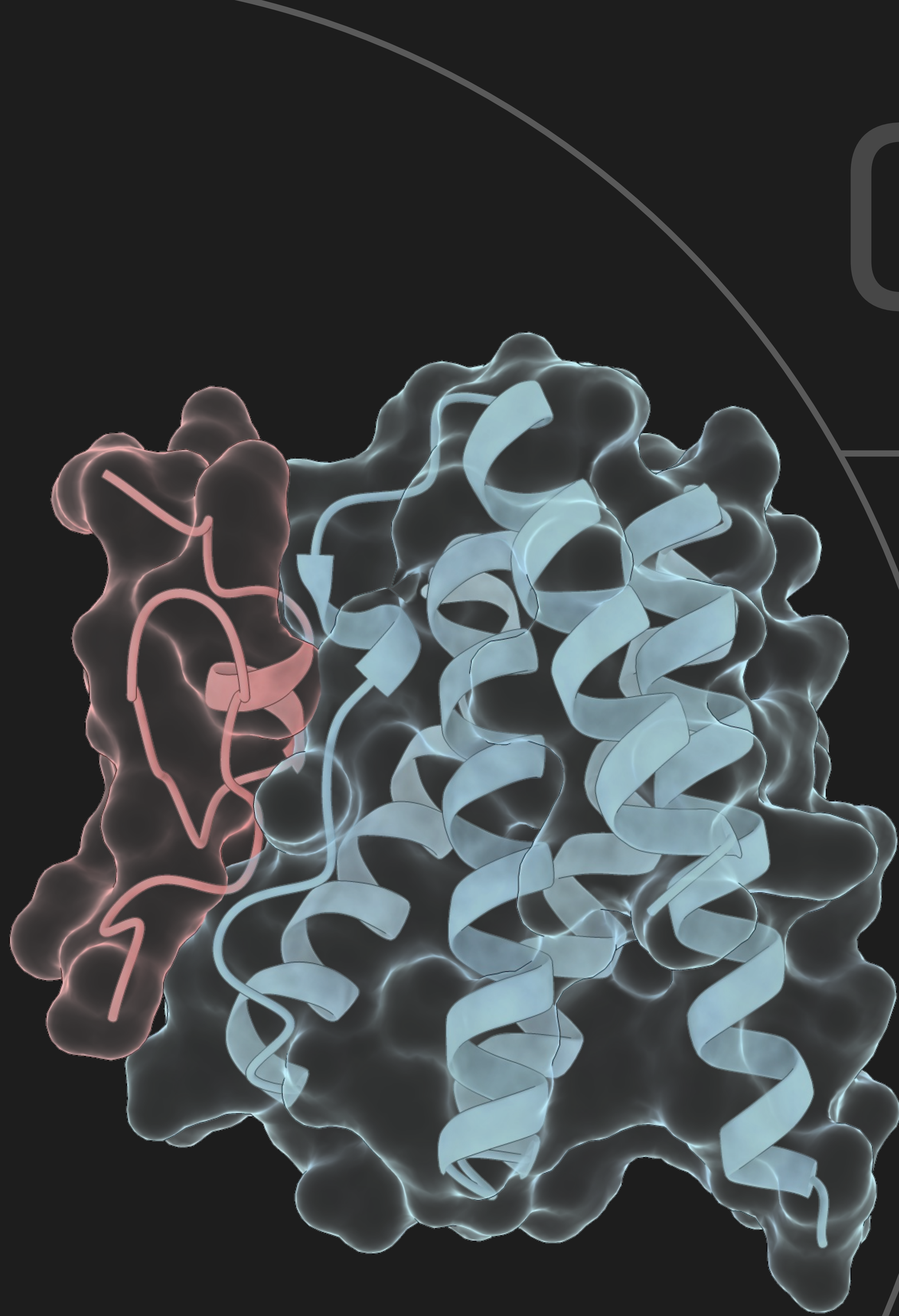
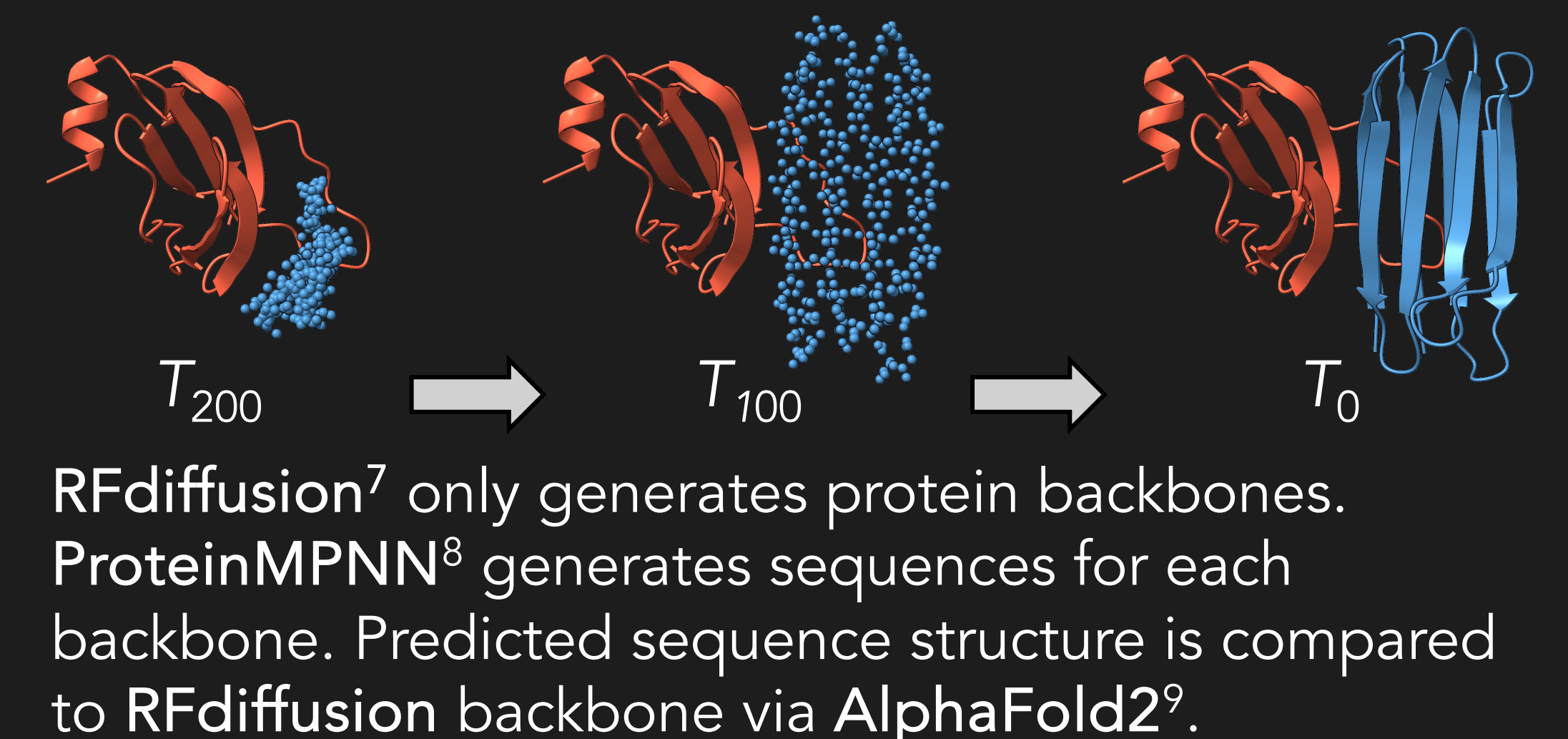
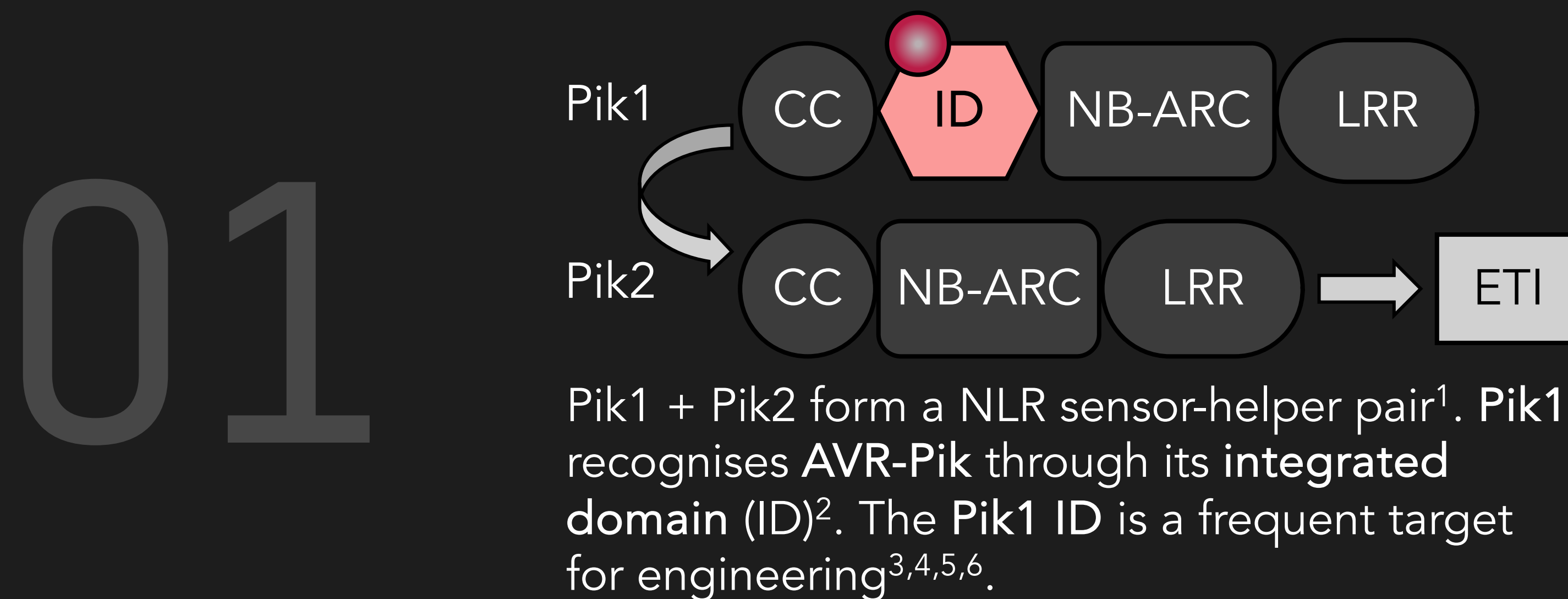
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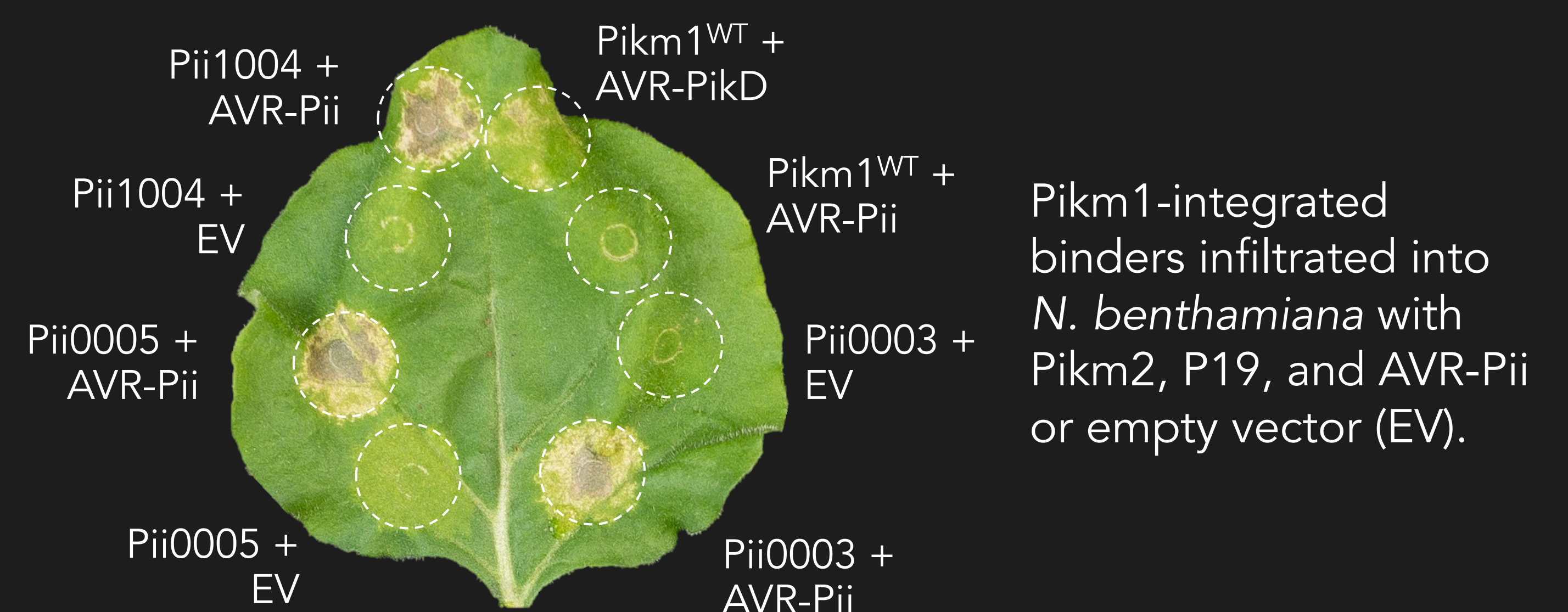
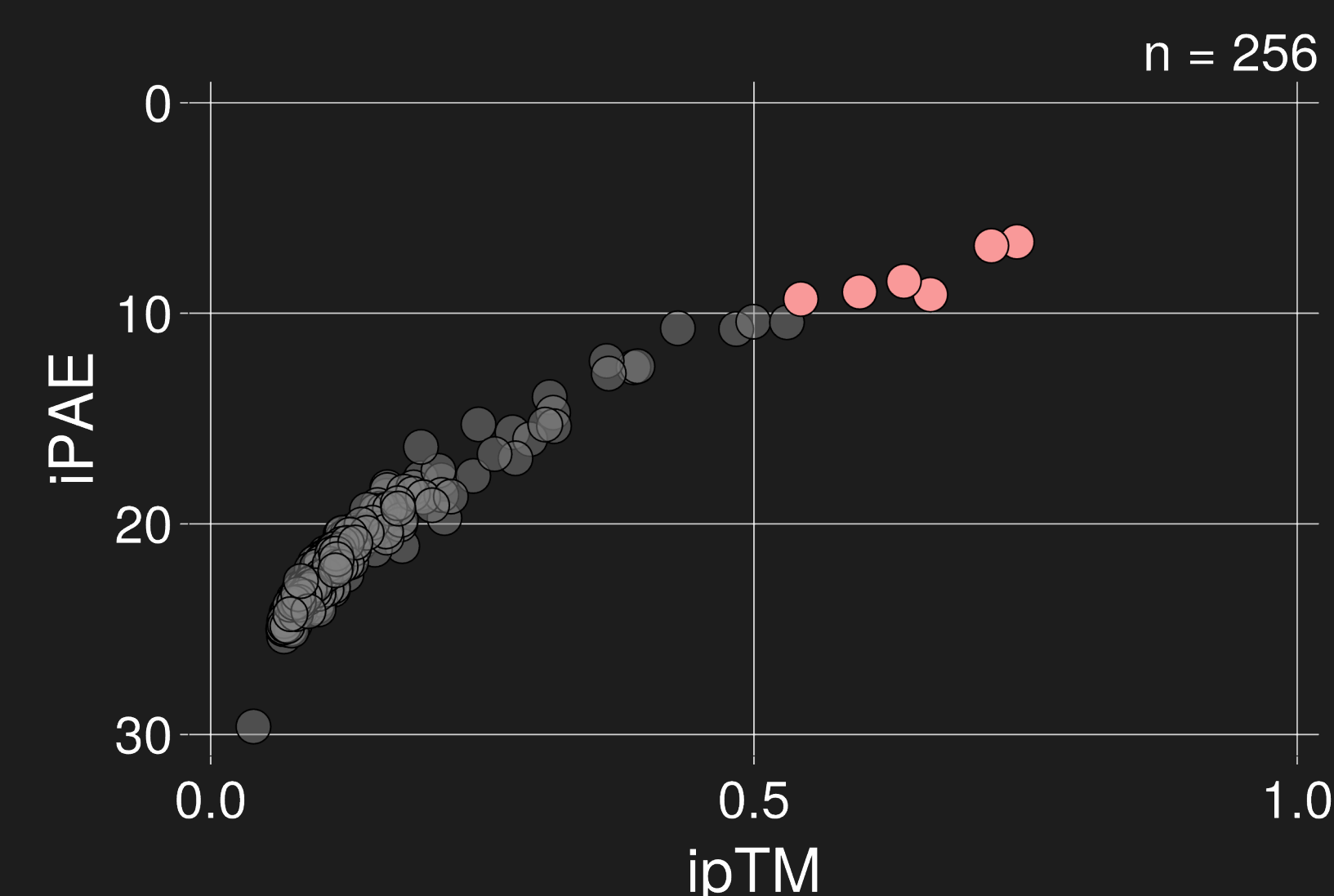
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Phytopathogens deploy intracellular virulence proteins known as effectors to induce pathogenesis via host protein interactions. Resistance occurs through effector recognition by NLRs, some of which contain modular integrated domains (IDs). This modularity has facilitated engineering attempts to expand effector recognition profiles. However, this is mostly limited to IDs where the effector-ID interface is already understood. Utilising *de novo* protein design tools, we have generated effector-binding proteins against the *Magnaporthe oryzae* effector AVR-Pii to integrate into NLRs. Our work advances previous efforts to broaden NLR recognition by allowing the design of novel effector-binding IDs based on protein structure alone.



AVR-Pii (PDB: 7PP2) is a Zn-finger effector targeting the exocyst complex. It interacts with both -F2 and -F3 forms of the host protein Exo70¹⁰.



Predicted complex of AVR-Pii (pink) and Pii1004 (blue), predicted via AlphaFold2⁹. With iPAE and ipTM scores of 6.6 and 0.74 respectively, Pii1004 was a strong *in silico* candidate. Cell death assays demonstrated Pii1004 induced a significant cell death response in the presence of AVR-Pii. Unlike Pii1005, a binder candidate with the same backbone but different sequence, Pii1004 did not exhibit any auto-activity. Image by PMFE, using ChimeraX.

Isothermal titration calorimetry

Aim: quantify binding affinity between Pii1004 and AVR-Pii.

Question: how does it compare to the AVR-Pii and Exo70 binding affinity?

X-ray diffraction

Aim: solve crystal structure of Pii1004 and AVR-Pii complex.

Question: does the Pii1004 structure match the RFdiffusion-generated backbone?

Transgenic lines

Aim: generate Pikm1-integrated Pii1004 lines in rice and barley.

Question: is observed resistance transferrable to economically significant crops?

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1. Zdrzałek et al., 2020. PLOS One, 15 (9).
2. Maqbool et al., 2015. eLife, 4.
3. Kourilis et al., 2023. Science, 379.
4. Maidment et al., 2023. eLife, 12.
5. Bentham et al., 2023. The Plant Cell, 35 (10).
6. Zdrzałek et al., 2024. PNAS, 121 (28).
7. Watson et al., 2023. Nature, 620.
8. Dauparas et al., 2022. Science, 378.
9. Jumper et al., 2021. Nature, 596.
10. De la Concepcion et al., 2022. PNAS, 119(43).
11. Bennett et al., 2023. Nature Communications, 14.

