INTERNATIONAL MASTER IN SUSTAINABLE DRUG DISCOVERY

Engineering pH-sensitive folding and binder design using conditional hallucination.

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SUMMARY

The development of protein binders with conditional affinity represents a promising strategy for improving target selectivity in acidic pathological microenvironments, including the tumor extracellular space and endosomal trafficking compartments. However, current approaches largely rely on empirical mutational screening, limiting predictability and throughput. This work addresses that limitation by presenting a biophysics-informed conditional hallucination framework in which differentiable structural constraints (derived from the physical requirements of His–Asp/Glu salt bridges) are encoded as loss functions directly into an AlphaFold2 generative design pipeline.

Four custom loss functions, encoding histidine burial, acidic partner contact, sidechain orientation, and sequence preservation, were formulated and sequentially evaluated, demonstrating that their combination is required to guide gradient optimization toward His–Asp/Glu pairs capable of forming salt-bridges within the binder core. Subsequent weight calibration across nine configurations and 20 independent random seeds identified configurations w1 and w5 as the best-performing non-exclusive and exclusive settings, respectively. Both applied pH loss terms at twice the weight of standard AF2 terms, achieving all-pass hit rates of 40% and 25%, respectively, with the D/E exclusivity constraint in w5 enforcing strict 1:1 His–acidic partner pairing at the cost of a reduced but geometrically more consistent designs.

Energetic evaluation using PyRosetta showed that most selected designs (63% from w1 and 80% from w5) exhibited negative electrostatic binder energy upon histidine protonation providing computational evidence consistent with the intended pH-switch mechanism and enabling the identification of eight candidate designs for downstream experimental validation. Taken together, these results establish a proof of concept that interaction-type biophysical constraints can be embedded within gradient-based hallucination frameworks to guide the autonomous placement of pH-sensitive motifs, offering a generalizable and structure-guided route to the de novo design of conditionally active protein binders.