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Filling the Structural Knowledge Gap in Protein Design via Native Mass Spectrometry

Enzymes are powerful molecules for highly efficient and sustainable chemical synthesis. However, natural enzymes often have limitations and require optimization for large-scale industrial applications. Propelled by artificial intelligence, computational advances such as AlphaFold opened new venues for structure-based enzyme design. However, experimental validation still largely relies on high throughput screening (HTS) for functional and phenotypical data. HTS methods for structural screening only offer limited data and cannot readily resolve multi-component or heterogeneous systems. Only a few successful candidates will have the chance for in-depth structural biology analysis to interpret the mechanism due to the low throughput.
Furthermore, the majority of the "failed"designs can't be easily formulated into meaningful knowledge to improve the computational models, leading to significant losses of research resources. Native mass spectrometry (native MS) can characterize molecular structures and interactions with fast speed, potentially filling the missing mechanistic knowledge in HTS methods. When combined with top-down MS techniques (native top-down, complex-up, etc.), subunit and residue level information can also be extracted. Recently, native MS have been integrated with computational drug design workflows for finding drug candidates. But more fundamental studies and method developments are still needed to accurately define the correlation of subtle structure features with native MS data, especially for weak interactions.
Herein we selected an artificial triplet photoenzyme RamR with two of its tailored triplet quenchers, and investigated their interactions. The two molecules has different potency in enzyme inhibition, but only differ by the presence/absence of a carboxyl group. Our preliminary data suggested the enzyme-inhibitor interaction was significant but likely non-selective on protein surface. Further experiments are ongoing to examine the enzyme-inhibitor interaction in presence of the native substrate. In summary, the native MS data improve our understanding of the biophysical mechanism of the molecular interactions, and help inform rationale design for enzymes.

User consent

yes

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