De novo protein design for targeted binding of Zolpidem

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De novo protein design has enabled to construct the functional proteins with tailored tertiary structure from scratch. However, designing a protein with a predetermined function, particularly the ability to specifically recognize a target small molecule and stabilize the binding pose using an appropriate scaffold, remains an immensely challenging problem. In this study, we present the *de novo* designed protein binding with zolpidem, a socially problematic sedative-hypnotic drug. We designed the binding pose on 4-helix bundle and TIM-barrel protein backbone with statistically prefered interaction in the nature using Convergent Motifs for Binding Sties (COMBS)[1]. The designed proteins exhibited sub-micromolar binding affinity towards zolpidem, as confirmed by Isothermal Titration Calorimetry (ITC) and Fluorescence Polarization (FP). Through the MD simulations, we confirmed the thermodynamic stability and binding mode of the protein-small molecule complex and further optimized the binding pose by considering dynamic equilibration of interacting network. Our study suggests the potential of function-driven protein design as a strategy for developing novel proteins without the need for screening or display processes. Moreover, this approach can be extended to other substances including narcotic drugs, and applied to various biomedical research, such as clinical diagnostics, forensic analysis and drug sensing or monitoring.

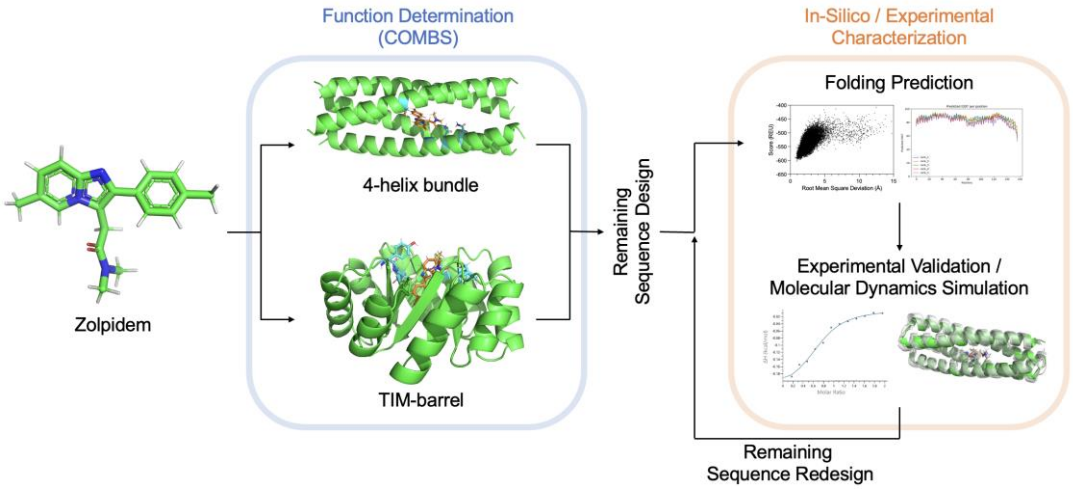

Figure 1. Design Strategy of function-driven zolpidem binding protein using COMBS.
To design zolpidem specific binding protein, we use COMBS first for binding pose, and design remaining sequence subsequently. Experimental validation confirmed the binding of the designed protein.

References

[1] Polizzi, N. F.; DeGrado, W. F. *Science* **2020**, *369 (6508),* 1227–1233.