Computational Design of Oligopeptide-Based Volatile Organic Compound Probe Targeting D-limonene

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Peptide probes are promising for volatile organic compounds (VOCs) sensing applications due to their side-chain-driven binding specificity, particularly toward olfactory receptors (ORs). Here, we investigated the binding mode of D- and L-limonene with OR19a from Drosophila melanogaster through molecular docking and molecular dynamics (MD) simulations. The OR19a structure was predicted by AlphaFold3 (pTM 0.89), and molecular docking via AutoDock Vina identified a binding pocket lined by C51, I52, S55, W161, I162, P163, W164, T176, L179, H180, and 183, dominated by non-polar interactions. D-limonene showed a more favorable docking score (−5.05 ± 0.40 kcal/mol) than L-limonene (−4.94 ± 0.44 kcal/mol), consistent with MD simulation MM/GBSA binding energies (−21.72 ± 0.92 vs −21.50 ± 0.60 kcal/mol) and a more rigid, stable binding pose over 10 ns. These predictions were confirmed by the spot-synthesized peptides on the activated cellulose membrane with D-limonene binding assay combined with GC-MS analysis. Among 15 screened peptides, including the computationally derived sequence and rearranged variants, P14 showed the highest D-limonene capture (2.27 ± 1.55 AU). In comparison, the literature-reported positive-control peptide P2 (LBP3)[1] captured only 1.13 ± 0.92 AU under the same experimental condition. From this result, P14 demonstrated approximately a two-fold increase in D-limonene capture and represents a promising candidate for quartz crystal microbalance (QCM) sensing application. In addition, the VOC specificity will be evaluated using the spot membrane assay which will serve as a benchmark for validating the selectivity of the QCM sensor in future experiments.

References

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