Late-stage reshaping of phage-displayed libraries to macrocyclic landscapes for ligand discovery

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Foundational Genetically-encoded libraries (GEL) platforms like phage-, yeast-, mRNA-display construct libraries using 20 natural amino acids (20AA). GEL can be expanded by unnatural amino acids (UAA) and chemical post-translational modification (cPTM). The standard procedure involves incorporating UAA or cPTM into a "naïve" library, followed by multiple rounds selection. However, such approach uses zero knowledge of binding interactions which might have been discovered from 20AA libraries. There is currently no consensus whether libraries containing pre-existing knowledge can offer an effective path for discovery of molecular interactions. To explore this, we evaluated the feasibility of discovering macrocyclic peptide ligands from "non-zero knowledge" libraries by chemically reshaping pre-selected phage-displayed 20AA binders against the NS3aH1 protease. The re-shaping is performed using a novel C2-symmetric linchpin, 3,5-bis(bromomethyl)benzaldehyde (termed KYL). KYL diversified peptide libraries into bicyclic architecture and delineated 2 distinct sequence populations: (i) peptides with HXDMT motif retained binding upon bicyclization (ii) peptides without HXDMT motif lost binding once chemically modified. The same HXDMT family can be found in selections starting from naïve KYL-modified library. Our report provides a case study for discovering bicyclic ligands using pre-selected 20AA libraries, suggesting that other 20AA-based selections potentially could be used for discovery advanced peptide ligands.