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Discovery of Internal Ligand Inhibitors Targeting SHANK1 PDZ Domain Guided by Dynamic Ligation Screening Strategy

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Introduction

Protein-protein interactions have received extensive recent attention as targets for drug discovery given their key role in controlling many biological processes. In terms of the model PPI studied here, we choose the SHANK1 PDZ domain as the target protein. An accumulating body of research shows that mutation in SHANK genes often will interfere with the structure and function of their corresponding product proteins, leading to various neurological disorders and psychiatric diseases [1]. As one of the best-known and largest PPI modules, the PDZ domain is responsible for supporting the intracellular communication network at the postsynaptic site (Figure 1).

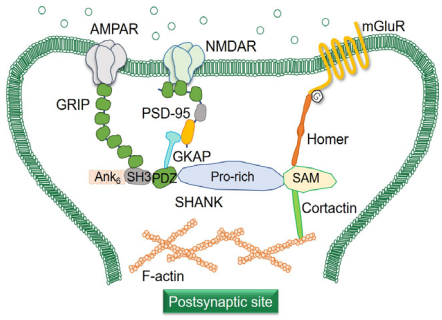

Fig. 1. Scheme illustration role of SHANK and its interacting proteins in the postsynaptic site.

Typically, SHANK PDZ binds to *C*-terminal PBMs in a β-strand conformation, such as the reported examples – *β*PIX/SHANK1 PDZ PPI (Figure 2A) [2] and GKAP/SHANK1 PDZ PPI (Figure 2B) [3]. Hegedüs et al used a dynamic ligation screening workflow (Figure 3) to identify peptide fragment hybrids bearing N-terminal modifications with strong affinity for SHANK1 PDZ (Figure 2C) [4], however no promising *C*-terminal fragments were identified. Internal ligands that bind PDZ domains have been identified as alternative motifs in modulating SHANK proteins in recent years (Figure 2D) [5] and may represent promising starting points for identification of *C*-terminal peptide-fragment hybrids.

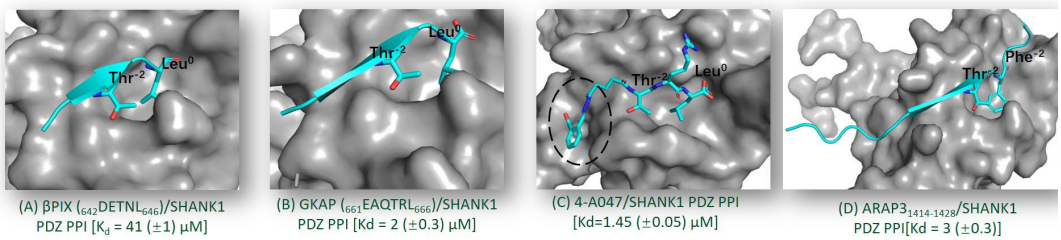

Fig. 2. Ribbon diagrams of typical PPIs of SHANK1 PDZ domain with C-terminal ligands (A-C) and with internal ligand (D).

Results and Discussion

Inspired by prior research on SHANK1 PDZ ligands, we seek to design novel internal ligand inhibitors with the SHANK1 PDZ domain as target. The main strategy is based on the dynamic ligation screening method [4] combined with fluorescent anisotropy assays.

First of all, an artificial sequence PSSMI generated through position-specific scoring experiments in a recently published paper [5] was chosen as precursor after determining the binding affinity (0.81 ± 0.08 μM) of its fluorescently labelled analogue FAM-Ahx-PSSMI and its

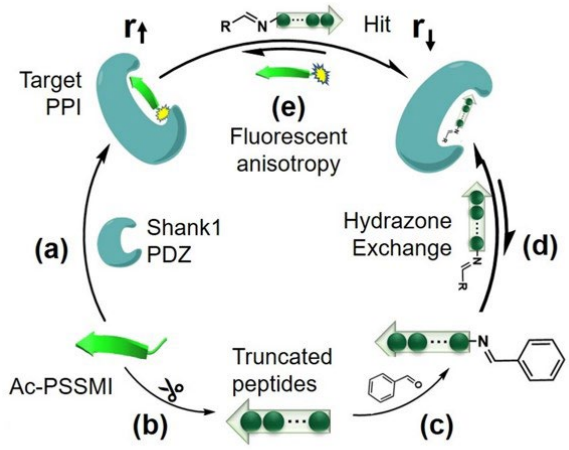

Fig. 3. Schematic illustrating the dynamic ligation screening approach for identification of peptide fragment hydrazones that inhibit strand mediated PPIs.

competition inhibitory activity (3.9 ± 0.1 μM) against FAM-Ahx-PSSMI, where the SHANK1 PDZ domain (656-762) was the target protein.

To further improve the inhibitory activity of designed peptides and explore the function of hybrid short linear motifs (SLiMs) in modulating the PPI of SHANK1 PDZ with internal ligands, we truncated the acetylated peptide Ac_PSSMI from the *N*-terminus, *C*-terminus and both sides.

Based on our truncation studies, we chose phenylhydrazone analogues of PSSMI with *N*-terminal acetamides as templates for subsequent screening. Hydrazone exchange reactions were involved in the dynamic ligation screening experiments (Figure 3). By employing a library containing 165 aldehyde fragments, as a result, a series of potential hydrazones were obtained, and inhibitory activities against FAM_Ahx_PSSMI were established. Resynthesis, purification of promising fragment peptide hydrazones and full dose response analysis confirmed this approach could identify potent *C*-terminal peptide-fragment hybrids with single digit μM IC_{50} values. These results pave the way to further develop short linear internal ligands for SHANK1 PDZ domain.

Acknowledgments

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