

Introduction

Serometrix Peptimer™ Discovery Platform (SimPep™) is an *in silico* protein structure guided process that rapidly identifies a number of potential sites that regulate protein-to-protein interaction. Peptimer™ Discovery Platform Algorithms capitalize on natural selection dictated by evolution.

The Wnt/Frizzled pathway is necessary to embryonic development and tissue homeostasis. Deregulation is associated with cancer and developmental defects. The phosphoprotein dishevelled (DVL) is an upstream pathway component. DVL associates with several intracellular proteins including the multifunctional scaffolding protein β-arrestin(1), a positive modulator of the Wnt/b-catenin pathway. Bryja et al. (2) identified a trimeric DVL-β-arrestin-axin complex involved in Wnt/b-catenin signaling. Mapping the interaction interfaces of β-arrestin and DVL it appeared β-arrestin bound a region N-terminal of PDZ domain of DVL (82-267 fragment).

SimPep™ narrowed the predicted interaction of DVL with β-arrestin to fragment 246-252 of DVL2 with fragment 298-307 of β-arrestin. Selvita Life Sciences Modeling Platform was employed to validate likelihood of this predicted interaction site. Selvita's platform contains a robust core of unique structure prediction tools with high scientific credibility gained by its core algorithms. The protein-protein docking module is capable of structural filtering according to user-provided interface residues and fully flexible docking.

1. Chen W et al. 2003, Science 301:1391
2. Bryja V et al. 2007, PNAS 104:6690

Figure #1



Native β-arrestin PDB 1G4R

Methods

β-arrestin Structure (PDB code 1G4M)

Experimental Design: β-arrestin variant input for independent docking simulations:

Variant1 Native conformation. Induced activation upon interaction with DVL2 opens polar core.

Variant2, Variant3, Variant4 three separate conformations, open polar core derived manually by pushing away from each other, with different degrees, N and C domains.

Han et al. 2001 Structure 9:869

DVL2 Fragments Docked – SimPep™ Predicted Binding Site in Red

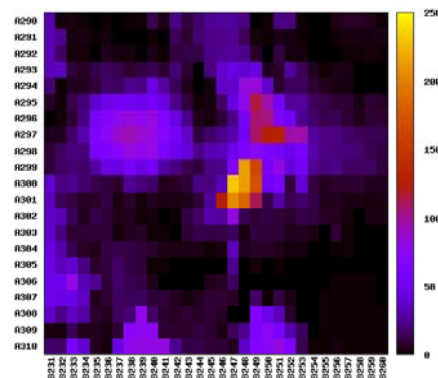
V1 (231-260): RLLKRHRRRR KQRPPRLERT
SSFSSVTDST

V2 (221-250): SSSTEQSSAS RLLKRHRRRR
KQRPPRLERT

V3 (211-241): SDEEDTMSRF SSSTEQSSAS
RLLKRHRRRR

Results

Figure #2



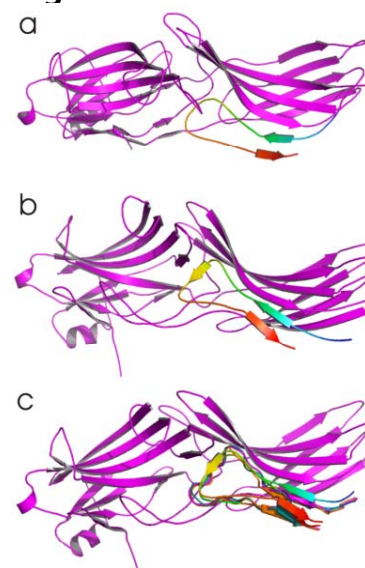
Residue-Residue Contact Map, average for 1000 low-energy models. Color indicates number of contacts

Selvita Protein Modeling Platform (SPMP)

Sixty simulations were performed that were a combination of 3 input variants of DVL2, 4 conformation input variants of β-arrestin and 5 restraint strengths levels: 0.1, 0.25, 0.7, 0.75, 1.0, by the SPMP docking technology, multi-CABS model.

Structural clustering was performed on simulation results producing 10 cluster and their model representatives. RMSD of models within cluster smaller than 2 Angstroms

Figure #3



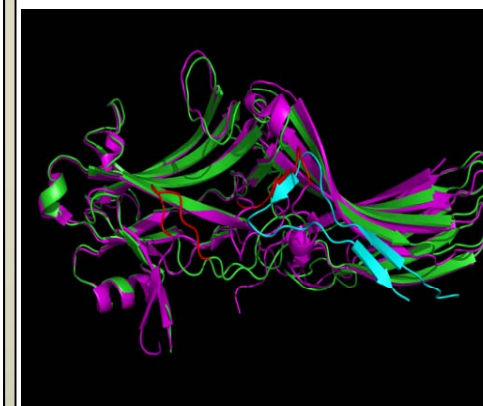
Final models of biggest clusters: a) 1-1-1.0-281-990, b) 1-1-1.0-281_750, c) all top 5 models superimposed

Potential Energy [kJ/Mol]* Final Models

1_1_1.0_281_990: -5.7055473e+04
1_1_1.0_281_750: -5.6516812e+04
1_1_1.0_281_989: -5.5631727e+04
1_1_1.0_281_988: -5.4892070e+04
1_1_1.0_281_987: -5.2463855e+04

*Energy of representative structure in OPLS force-field after optimization

Figure #4



β-arrestin-DVL2 superimposed on native β-arrestin

Conclusions

1. DVL2 variant 1 docked at SimPep™ predicted site in β-arrestin polar core
2. Serometrix prediction of binding interaction was narrowed in the final models to DVL2 246-249 with β-arrestin 299-301