Suppl 1. Analysis of the three-dimensional structure of the binding site between SDHB and SDHAF2.

Spanner is a structural homology modeling pipeline that threads a query amino-acid sequence onto a template protein structure. Spanner is unique in that it handles gaps by spanning the region of interest using fragments of known structures, protein structure PDB ID 3SFE as template protein structure.

To create a model, you must provide a template structure, as well as an alignment of the query sequence you wish to model onto the template sequence. Spanner will replace mismatched residues, and fill any gaps caused by insertions or deletions.

For users that are unable to create an alignment a method for building a model starting only from sequence is also available. During this process a template search is conducted, and an alignment is built dynamically using FORTE before being passed through to the main part of the pipeline.

Spanner consists of several modules written in the Go programming language. For Spanner jobs which build a model only from sequence, the first step is a search of the PDB for possible templates using BLAST. These possible templates are then aligned and scored with FORTE.

The next step involves defining the start and end points of fragments corresponding to insertions or deletions. The start and end points are referred to as anchors because they must be equivalent in both the template and any candidate fragment. The margin parameter determines how far from the edge of a gap the fragment begins or ends. For example a margin of 0 would mean that the anchors begins at the very edge of a gap. This is usually not a good idea, and the default margin is set to 1.

A representative set of protein chains was prepared using CD-HIT at 100% sequence identity.[3](https://sysimm.org/spanner/about) All continuous fragments were then extracted from this set of chains and stored in a relational database, indexed by the internal coordinates of the fragment endpoints. A separate database is prepared for each fragment length. Currently, fragments of length 8-40, including the 8 anchor residues, are stored in the database.

**Cn3D** ("see in 3D") is a helper application for your web browser that allows researcher to view 3-dimensional structures from NCBI's Entrez Structure database. Cn3D is provided for Windows and Macintosh, and can be compiled on Unix. Cn3D simultaneously displays structure, sequence, and alignment, and now has powerful annotation and alignment editing features. *(For those who prefer to view 3D structures on the web, without the need to install a separate application, iCn3D ("I see in 3D") is available as of April 2016.)*

**Web-based Structure Viewer**iCn3D ("I see in 3D"), released in April 2016, provides interactive views of three-dimensional macromolecular structures on the web.

There is no need to install a separate application in order to use iCn3D; you just need to use a web browser that supports WebGL.

iCn3D also allows you to cutomize the display of a structure and generate a URL that allows you to share the link, and to incorporate iCn3D into your own web pages.

**New Features in Cn3D 4.3.1:**View superpositions of structures that have similar molecular complexes, as identified by the newly released VAST+ (an enhanced version of the existing Vector Alignment Search Tool). The VAST+ help document provides additional details about the tool and examples of how it can be used to learn more about proteins.

Cn3D 4.3.1 uses the MIME type: application/vnd.ncbi.cn3d.

Analyses of protein contact residues and protein buried surface areas Protein contact residues were analyzed using the **LigPlot+ program (v.1.4.5)** (<https://www.ebi.ac.uk/thornton-srv/software/>LigPlus/). Protein buried surface areas were analysed using **PDBePISA tool** (<http://pdbe.org/pisa/>) and **MOE project DB** (MOLSIS Inc. Tokyo Japan). The modeling and Docking of the protein binding region of SDHB protein and host regulating protein, SDAF1 was analyzed by **MOE project DB** with previously posted ID; SDHB and protein ID; SDAF1 (MOLSIS Inc. Shibuya, Tokyo Japan). The binding affinity between host receptor analogs and 20 loop region of H5N1 HA protein was analyzed by **MOE project DB** (MOLSIS Inc.).

To examine the extent to which the binding ability between SHDB and SDAF1 is affected by the glutamine-to-histidine mutation, we performed *in silico* analysis using protein structure PDB ID 3SFE as three dimensional template. *In silico* analysis showed that glutamine 214 of SHDB has affinity for histidine 156 of SDAF2. The affinity between glutamine 214 of SHDB and tyrosine 156 of SDAF2 was found to be -17.93 Kcal/mol. On the other hand, although the histidine 214 of the SHDB mutant has affinity for the tyrosine 156 of SDAF2, the affinity between histidine 214 of SHDB and tyrosine 156 of SDAF2 was found to be weak, at -6.31 Kcal/mol. In other words, the results of *in silico* analysis revealed that the SHDB 214His mutant may have a much weaker binding affinity to SDAF2 than the wild-type SHDB.