Structural modeling of proteins: Principle and application to an ion channel

Haruki Nakamura
PDBj, Institute for Protein Research, Osaka University

Daron M. Standley
Immunology Frontier Research Center, Osaka University

Narutoshi Kamiya
The center for Advanced Medical Engineering and Informatics, Osaka University

http://www.protein.osaka-u.ac.jp/rcsfp/pi/
http://www.pdbj.org/
Goal of the Tutorial:
To construct the homology model of hERG channel

Search for Homolog
FASTA, BLAST, psi-BLAST

Precise Alignment
3D-1D compatibility search (threading)

Backbone modeling: Loops
loop search, conf. sampling

Side-chain modeling:
Combinatorial problem
Dead End Elimination (DEE)

Step 2. Make the alignment by threading. Access to SFAS: Sequence to Function Annotation Server (http://sysimm100.protein.osaka-u.ac.jp/sfas/), and get the best alignment to the putative template structure. The query may take several minutes to hours depending on the status of the Web site, please visit the example result page: (http://sysimm100.protein.osaka-u.ac.jp/tmp/SFAS16483/hERG_top.html)

Step 3. Make the tetramer structure and see the result. Access to Quarternary Structure Service (http://sysimm100.protein.osaka-u.ac.jp/pdb_quat/), and get & see the tetramer model structure of hERG channel.
Step 1: Get amino-acid sequence of hERG channel from NCBI

(http://www.ncbi.nlm.nih.gov/)

RecName: Full=Potassium voltage-gated channel subfamily H member 2; Alternate Name: Full=Voltage-gated potassium channel subunit Kv11.1; Alternate Name: Full=Ether-a-go-go-related gene potassium channel 1; Short=H-ERG; Short=Er1; Short=Erg; Short=Ether-a-go-go-related protein 1; Short=Eag-related protein 1; Short=Hae1 homolog.

LOCUS Q12809 1159 aa linear PRI 07-JUL-2009
RecName: Full=Potassium voltage-gated channel subfamily H member 2; Alternate Name: Full=Voltage-gated potassium channel subunit Kv11.1; Alternate Name: Full=Ether-a-go-go-related gene potassium channel 1; Short=H-ERG; Short=Er1; Short=Erg; Short=Ether-a-go-go-related protein 1; Short=Eag-related protein 1; Alternate Name: Full=Hae1 homolog.

Accession: Q12809

UniprotKB: locus KCNH2_HUMAN, accession Q12809; class: standard. extrs accession: Q75418, Q75680, Q89782, Q95177, Q91160 created: May 30, 2000.

sequence updated: Nov 1, 1996.
Step 2: Get homologs in PDB and have alignments with 3D modes.

Input your e-mail address

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Step2: Result of SFAS: The best template is 2r9rB

Jalview

Start Jalview

Return to SFAS Results
Step 3: Make the tetramer structure

Template: 2r9rB (550-670)

Model structure (monomer)

(http://sysimm100.protein.osaka-u.ac.jp/pdb_quat/)

Model structure (tetramer)
Other options

Step O1. Search PDB structures with similar amino acid sequences.
Access to Sequence Navigator (http://www.pdbj.org/), and get the PDBID information for the homologs.

Step O2. Collect the structural information of the homologs.
Access to PDBj (http://www.pdbj.org/), and get the structural information for the above PDBIDs.

Step O3. Make the sequences-structures multiple alignment.
Access to MAFFTash (http://www.pdbj.org/), and input the PDBIDs and the sequence of the hERG channel, to get the multiple alignment.

Step O4. Make the homology model with the alignment.
Access to Spanner (http://www.pdbj.org/spanner/), and input the template structure with the above PDBID and the alignment information to get the homology model.
Step 01: Search for a Homolog protein in PDB

Use of Sequence Navigator at PDBj (http://www.pdbj.org/)

Sequence Navigator

About Sequence Navigator

Sequence Navigator Soap Service

To Enter Navigator, Input a PDB ID and Chain ID

OR Input an AA Sequence

Clustering Options

@ No Clustering  ➔ (more clusters)

@ Cluster by E-value 10−6  ➔ (fewer clusters)

Results (1-33) / 33

Seq. Identity: 91% Seq. Positives: 97% E-value: 0.0 Score: 776 Compound: NEUROMINIDASE

New Search [2HTYC]

Query 83 385 VKL&GSUKCPSGS&WAPS&DRS&NDR&GSM&DF&FKF&FP&CS&FC&F&FL&2Q&G&K&3K&3K

2HTYC 1 385 VKL&GSUKCPSGS&WAPS&DRS&NDR&GSM&DF&FKF&FP&CS&FC&F&FL&2Q&G&K&3K

2HTYC Exact Matches: 2HU0F 2HU04 2HTYG 2HU4G 2HU0B 2HU4A 2HTYA 2HU

Seq. Identity: 91% Positives: 97% E-value: 0.0 Score: 774 Compound: NEUROMINIDASE

New Search [3CL2B]

Query 83 385 VKL&GSUKCPSGS&WAPS&DRS&NDR&GSM&DF&FKF&FP&CS&FC&F&FL&2Q&G&K&3K

3CL2B 1 385 VKL&GSUKCPSGS&WAPS&DRS&NDR&GSM&DF&FKF&FP&CS&FC&F&FL&2Q&G&K&3K

3CL2B Exact Matches: 3CL2G 3CL2F 3CL2C 3CL2H 3CL2A 3CL2D 3CL2E

Seq. Identity: 91% Positives: 97% E-value: 0.0 Score: 763 Compound: NEUROMINIDASE

New Search [3CL0A]

Query 83 385 VKL&GSUKCPSGS&WAPS&DRS&NDR&GSM&DF&FKF&FP&CS&FC&F&FL&2Q&G&K&3K

3CL0A 1 385 VKL&GSUKCPSGS&WAPS&DRS&NDR&GSM&DF&FKF&FP&CS&FC&F&FL&2Q&G&K&3K

3CL0A Exact Matches: 3CL2A

Seq. Identity: 88% Positives: 95% E-value: 0.0 Score: 763 Compound: NEUROMINIDASE

New Search [3CYEA]

Query 83 385 VKL&GSUKCPSGS&WAPS&DRS&NDR&GSM&DF&FKF&FP&CS&FC&F&FL&2Q&G&K&3K

3CYEA 1 385 VKL&GSUKCPSGS&WAPS&DRS&NDR&GSM&DF&FKF&FP&CS&FC&F&FL&2Q&G&K&3K

3CYEA Exact Matches: 3CYEA
Step O2: Get Entry Data from PDBj
(http://www.pdbj.org/)

Summary for each PDBID

Graphic viewer: jV
(http://www.pdbj.org/jV/)
Step O3: Alignment of Sequences and Structures (i)
(http://sysimm100.protein.osaka-u.ac.jp/MAFFTash.3/)

> PDBID
> 2r9rB
> 1orqC
> Q12809 | hERG; 550-671
LFLLMCTFALIAHWLACIWY
AIGNMEQPHMDSRIGWLHNL
GDQIGKPYNSSGLGGSIKD
KYVTALYFTFSSLTSVGFGN
VSPNTNSEKIFSICVMLIGS
LMYASIFGNVSAIIQRLYS
TA

PDBj
About MAFFTash
Send feedback
Step O3: Alignment of Sequences and Structures (ii)

Use "Prep-MAFFTash" for automatic search of sequences and structures.
Step O4: Homology modeling with the alignment
(http://www.pdbj.org/spanner/)

Spanner is a structural homology modeling program—that is, it threads a specific amino-acid sequence onto a specific PDB structure, patching up the gaps as best it can.

To create a model, you must provide a template structure, as well as an alignment of the sequence you wish to model onto the template sequence. Spanner will replace matching residues, fill any gaps caused by inserted or deleted residues, and thermodynamically optimize the resulting structure.

The resulting PDB, as well as a log file, will be emailed to you when the modeling task finishes. If an error prevented a homology model from being generated (for example, when the alignment you provided does not match the template structure), the log file will explain which part of the modeling sequence failed.

Template PDB structure (PDB format):

Sequence alignment (FASTA format; first sequence is the template, second sequence is the query):

Model:  (not necessary if PDB file contains only one model)

Chain:  (not necessary if PDB file contains only one chain)

Email address for results:  

START!

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