# **Structural modeling of Ember: Detailed Methods**

The following list corresponds to the steps of the analysis. It provides descriptions and URLs for the algorithms used, identifies input and output files (provided as separate supplements), and lists non-default parameters.

1. **I-TASSER:** Protein structure prediction algorithm for building 3D models based on multiple-threading alignments and iterative TASSER assembly simulations. <u>http://zhanglab.ccmb.med.umich.edu/I-TASSER/</u>

#### (i) Input:

a) Ember amino acid sequence (906 amino acid in length, GenBank accession # HQ699262)

b) Atomic coordinates derived by manually editing the dsRed structure file (PDB: 1G7K) to reflect the hypothesized order of the domains (Ember\_Template.pdb in supplementary file ember\_models.zip).

## (ii) Parameters: default.

(iii) **Output files:** Ember models in pdb formats. The top model is provided (Ember\_itasser\_top.pdb in supplementary file ember\_models.zip)

2. **CombDock:** Protein-Protein assembly algorithm based on incremental addition of the protein units such that a heuristic shape complementarity score is optimized. <u>http://bioinfo3d.cs.tau.ac.il/CombDock/download/</u>

## (i) Input:

PDB file for each individual Ember domain derived from Ember model. The data are provided in supplementary file ember\_models.zip:

- a) dom1A.pdb
- b) dom2B.pdb
- c) dom3C.pdb
- d) dom4D.pdb

(ii) Parameters: default.

(iii) Output files: Ember models in pdb formats. Top 10 models are provided (CombDock\_top10.tgz)

3. **ASSEMBLE/PIE:** Protein-protein assembly algorithms based on PIE and fast fourier transform (FFT) based approximations of the scoring function. The structure assemblies are generated exhaustively on a grid and scored using PIE. http://clsb.ices.utexas.edu/dock\_by\_pie/assemble4/

## (i) Input files:

PDB file for each individual Ember domain derived from Ember model (same as for CombDock above)

(ii) Parameters: grid size of 1.6Å, rotational sampling at every 4°

(iii) Output files: Ember models in pdb formats: Top 10 models are provided (ASSEMBLE\_top10.tgz)

4. **Score/PIE:** Scoring models based on residue contact potential learnt using Structural support vector machine (SVM) style framework to minimize false positive rates. The scoring function (PIE) scores correctly dock structures better than other random encounter complexes.

http://clsb.ices.utexas.edu/dock by pie/score/

#### (i) Input files:

- a) CombDock and ASSMBLE generated models (PDB files, contained in CombDock\_top10.tgz and ASSEMBLE\_top10.tgz)
- b) I-TASSER generated Ember model (Ember\_itasser\_top.pdb in supplementary file ember\_models.zip)
- (ii) Parameters: Default.
- (iii) Output: relative rank ordering of the input models based on relative scores

Results of Score/PIE (higher score indicates higher stability):

Top model from I-TASSER: Ember\_itasser\_top 76.999857

Top 10 models from CombDock:

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models/complex1	75.972560
models/complex140	75.837409
models/complex2	75.688685
models/complex77	75.543960
models/complex9	75.536803
models/complex108	75.393068
models/complex129	75.386804
models/complex155	75.384359
models/complex5	75.377284
models/complex435	75.365002

Top 10 models from ASSEMBLE:

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models/complex4	76.4258
models/complex10	75.6187
models/complex5	75.5243
models/complex80	75.4485
models/complex7	75.2792
models/complex96	75.195
models/complex66	75.1615
models/complex120	75.0489
models/complex19	75.0181
models/complex21	74.9078