HADDOCK v2.4
Settings

Default settings

Input structures

- File format for the PDB files must follow the wwPDB standards wwPDB format standards
- The server supports docking from ensembles of structures. For this simply submit a PDB file containing multiple models (Note that they should all contain exactly the same number of atoms and the different models should be surrounded by MODEL and ENDMDL records).
- It is possible to either download a PDB directly from the RCSB database or to provide your own PDB. pdb_mode
- If within one submitted structures multiple unconnected bodies are detected, additional distance restraints will **automatically** be defined to keep the bodies together (e.g in an antibody).

Configuration

Maximum number of input molecules = 20
Maximum number of models allowed in the refinement interface = 100
Maximum number of atoms per input molecule (without hydrogens) = 75000
Maximum number of pair of restraints, only applied to NCS, SYM, RDCs, PCSs and DANI = 10

Number of structures generated

Number of trials for rigid body minimisation = 5
ntrials
Number of structures for rigid body docking (it0) = 1000
structures_0
Sample 180 degrees rotated solutions during rigid body EM = True
rotate180_0

Ambiguous Interaction Restraints (AIRs)

- Only active/passive residues can be used as restraints in EASY mode, no restraints file or center-of-mass restraints can be provided.

  activereslist passivereslist

- You can choose to either defined passive residues yourself, to no provide any or to let HADDOCK defined them as the closest residues from the active residues you have provided. In automatic mode, the passive residues are taken as all residues that have a solvent accessibility higher than 15% and at least one atom at less than 6.5A from any atom of an active residue.

  auto_passive auto_passive_radius

  By default, 50% of the AIRs will be randomly deleted for each docking trial (%excluded=100/number of partitions).
  Randomly exclude a fraction of the ambiguous restraints (AIRs) = True
  noecv
  Number of partitions for random exclusion = 2.0
  ncvpart

Flexibility treatment

Semi-flexible residues are **automatically** defined from an analysis of intermolecular contacts (<5.0A).
How are the flexible segments defined? = automatic
semiflex_mode

Protonation state of histidines

- The protonation state of histidines is **automatically** defined by using MolProbity/Reduce.

  his_#_state MolProbity

Co-factors and ligands

- Missing parameter and topology files for co-factors and small ligands are **automatically** obtained from PRODRG. PRODRG

DNA/RNA restraints (if applicable)

- The type of the molecule must have been explicitly set by the user. moleculetype
- *Backbone dihedral angles restraints*: values measured from input structure.
  Pucker restraints: measured from input structure.
  Planarity restraints: on a per base basis.
  Hydrogen bond restraints (DNA only): from detected base-pairs.

Hydrogen bonds restraints

- In which iteration should the restraints start being applied, default: **it0** hbond_firstit
- *Note*: The default value was changed from **it1** to **it0** on 16/Nov/2020

Clustering parameters

- Default method: **FCC** (vs RMSD) clust_meth
- Default cut-off: **0.6** clust_cutoff
- Default minimum size: **4** clust_size

Final scoring

- After itw, the reported scores and energies are averages calculated over the top four members of a cluster. The HADDOCK score is defined as:

  $$\text{HADDOCK-score}_{\text{it0}} = 0.01*E_{vdw} + 1.0*E_{elec} + 1.0*E_{desolv} + 0.01*E_{air} - 0.01*BSA$$

  $$\text{HADDOCK-score}_{\text{it1}} = 1.0*E_{vdw} + 1.0*E_{elec} + 1.0*E_{desolv} + 0.1*E_{air} - 0.01*BSA$$

  $$\mathbf{HADDOCK\text{-}score_{itw} = 1.0*E_{vdw} + 0.2*E_{elec} + 1.0*E_{desolv} + 0.1*E_{air}}$$

  w_elec w_vdw w_desolv

Restraints validation

- All restraints (distances, hbonds, dihedral angle, RDCs and diffusion anisotropy) are submitted to a strict validation by the server. They should comply to CNS syntax.

  dihedralfile unambigtblfile tblfile pcsfile rdcfile tensorfile hbondfile CNS syntax danfile

Refinement settings

Parameters

MD steps for rigid body high temperature TAD = 0
initiosteps
MD steps during first rigid body cooling stage = 0
cool1_steps
MD steps during second cooling stage with flexible side-chains at interface = 0
cool2_steps
MD steps during third cooling stage with fully flexible interface = 0
cool3_steps
Rebuild missing atoms in context of the molecule partner = False
rebuildcplx

Water Refinement

- A solvent shell is built around the complex and, subsequently, a series of short MD simulations are performed according to the parameters below, all atoms except the side-chain atoms at the interface are restrained to their original position. Next, 1250 MD steps are performed at 300 K with position restraints for heavy atoms which are not part of the PPI (residues not involved in intermolecular contacts within 5 Å). Finally, the system is cooled down (1000 MD steps at 300, 200 and 100 K) with position restraints on the backbone atoms of the protein complex, excluding the interface atoms.

Parameters

Clustering method - Complex = FCC
clust_meth
Clustering cutoff - Complex = 0.6

clust_cutoff
Clustering method - Single structure = RMSD
clust_meth
Clustering cutoff - Single structure = 2.0
clust_cutoff
Minimum cluster size - Single = 4
clust_size
Perform cross-docking = False
crossdock
Randomly exclude a fraction of the ambiguous restraints (AIRs) = False
noecv
Number of trials for rigid body minimisation = 1
ntrials
Randomize starting orientations = False
randorien
Perform initial rigid body minimisation = False
rigidmini
Allow translation in rigid body minimisation = False
rigidtrans
Define surface contact restraints to enforce contact between the molecules = True
surfrest
Remove non-polar hydrogens = True
delenph
Refine with short molecular dynamics in explicit solvent = True
solvshell
Type of analysis = cluster
runana

Energy Minimization Refinement

- This protocol is the same as Water Refinement but here the water shell is not built, meaning only an energy minimization will be performed.

Parameters

Refine with short molecular dynamics in explicit solvent = False
solvshell

Coarse-grain Refinement

- In short, we first generate the corresponding MARTINI-based coarse-grained representation for each of the models to be refined; then, by a combination of energy-minimizations and short molecular dynamics stages, the protocol fits the atomistic structure of each of the components onto the generated CG model of the complex and optimizes the system to remove clashes. The resulting models are then scored and ranked according to the HADDOCK score.

Randomly exclude a fraction of the ambiguous restraints (AIRs) = False
noecv
Define surface contact restraints to enforce contact between the molecules = True
surfrest
Perform cross-docking = False
crossdock
Perform initial rigid body minimisation = False
rigidmini
Randomize starting orientations = False
randorien
Refine with short molecular dynamics in explicit solvent = False
solvshell

Simulated annealing with centroid restraints

- This refinement protocol consists of a semi-flexible simulated annealing refinement (it1 stage of HADDOCK) with restraints on the center of masses of the components of the complex, followed by a final energy minimization.

Randomly exclude a fraction of the ambiguous restraints (AIRs) = False
noecv
Define surface contact restraints to enforce contact between the molecules = False
surfrest
Perform cross-docking = False
crossdock
Perform initial rigid body minimisation = False
rigidmini
Randomize starting orientations = False
randorien
Allow translation in rigid body minimisation = False
rigidtrans
Refine with short molecular dynamics in explicit solvent = False
solvshell
Expand starting orientations? = True
expand
Expansion percentage = 0
expansion
Random rotation angle = 0
randangle

Number of models

- It is possible to refine single structures as well as molecular complexes. Ensembles are also supported and must be formatted using the PDBTools' **pdb_mkensemble**. The maximum number of models supported in an ensemble is **100**.
- Link to PDBTools webserver

Optimal Run Settings

Nucleotides

Epsilon constant for the electrostatic energy term in it0 = ~~10.0~~ 78.0
epsilon_0
Epsilon constant for the electrostatic energy term in it1 = ~~10.0~~ 78.0
epsilon_1

Ligands

Clustering method = ~~FCC~~ RMSD
clust_meth
Cutoff for clustering = ~~0.6~~ 1.5
clust_cutoff
Dieletric constant for it0 = ~~rdie~~ cdie
dielec_0
Dieletric constant for it1 = ~~rdie~~ cdie
dielec_1
Epsilon constant for the electrostatic energy term in it1 = ~~1.0~~ 10.0
epsilon_1
MD steps for rigid body high temperature TAD = ~~500~~ 0
initiosteps
MD steps during first rigid body cooling stage = ~~500~~ 0
cool1_steps
Initial temperature for second TAD cooling step with flexible side-chain at the interface = ~~1000~~ 500
tadinit2_t
Initial temperature for third TAD cooling step with fully flexible interface = ~~1000~~ 300
tadinit3_t
Evdw 1 = ~~0.01~~ 1.0
w_vdw_0
Eelec 3 = ~~0.2~~ 0.1
w_elec_2

Glycans

Clustering method = ~~FCC~~ RMSD
clust_meth

Cutoff for clustering = ~~0.6~~ 2.5
clust_cutoff

Peptides

Cutoff for clustering = ~~0.6~~ 5.0
clust_cutoff
Clustering method = ~~FCC~~ RMSD
clust_meth
Number of MD steps for rigid body high temperature TAD = ~~500~~ 2000
initiosteps
MD steps during first rigid body cooling stage = ~~500~~ 2000
cool1_steps
MD steps during second cooling stage with flexible side-chains at interface = ~~500~~ 4000
cool2_steps
MD steps during third cooling stage with fully flexible interface = ~~500~~ 4000
cool3_steps

Coarse-grain

Dieletric constant for it0 = ~~rdie~~ cdie
dielec_0
Dieletric constant for it1 = ~~rdie~~ cdie
dielec_1

Bioinformatics predictions

Number of partitions for random exclusion = ~~2.0~~ 1.1428
ncvpart
Number of trials for rigid body minimisation = ~~5~~ 1
ntrials
Number of structures for rigid body docking (it0) = ~~1000~~ 10000
structures_0
Number of structures for semi-flexible refinement (it1) = ~~200~~ 400
structures_1
Number of structures for the final refinement (itw) = ~~200~~ 400
waterrefine
Number of structures to analyze = ~~200~~ 400
anastruc_1