IBiSA_tools Documentation

Release 0.12

Kota Kasahara

Jun 22, 2016

Contents:

CHAPTER

ONE

ABOUT

1.1 Authors

IBiSA_tools version 0.12 (12 Jun. 2016)

- Kota Kasahara, Ritsumeikan University
- Kengo Kinoshita, Tohoku University

1.2 Citation

- Kasahara K and Kinoshita K, (Under review) IBiSA_tools: A Computational Toolkit for the Ion Binding State Analysis on Molecular Dynamics Trajectories of Ion Channels.
- Kasahara K, Shirota M, and Kinoshita K (2016) Ion Concentration- and Voltage-Dependent Push and Pull Mechanisms of Potassium Channel Ion Conduction. PLoS ONE, 11, e0150716.
- Kasahara K, Shirota M, and Kinoshita K (2013) Ion Concentration-Dependent Ion Conduction Mechanism of a Voltage-Sensitive Potassium Channel. PLoS ONE, 8, e56342.

1.3 Overview

IBiSA_tools, which stands for "Ion Binding State Analysis tools", provides a computational tools for analyzing ion conduction mechanisms hidden in the molecular dynamics (MD) trajectory data. In this analysis, each ion conduction event is detected and mechanisms of the events are identified. See the citations for details of theory and applications.

- The current version of IBiSA_tools can be applied only for GROMACS trajectory file (.trr). If your trajectories are written in another format, you have to convert it into .trr, by using some tools, e.g., VMD plugin and MDAnalysis.
- IBiSA_tools is consisting of a C++ program and Python (2.6 or 2.7) scripts.
- The attached tutorial files use R software (www.r-project.org) to draw plots.
- Network drawing software, e.g., Cytoscape, is required to visualize ion binding state graph.

http://kotakasahara.github.com

Only a C++ program, trachan, must be compiled as follows:

```
cd src/trachan
./configure
make
```

In this document, we assume the binary and python scripts are included at the directory indicated by ${IBISA}.

CHAPTER

TWO

TUTORIAL

2.1 Setting the path to IBiSA_tools

```
export IBISA="${HOME}/local/ibisa"
```

2.2 Preparing the configuration file

config.txt:

```
--fn-pdb                        init.pdb
--dt                            10
--site-boundary                 20.0
--site-boundary                -25.0
--fn-pore-axis-coordinates      pore_axis.txt
--fn-pore-axis-coordinates-r    pore_axis_r.txt
--pore-axis-basis-from          A 374 O
--pore-axis-basis-from          B 374 O
--pore-axis-basis-from          C 374 O
--pore-axis-basis-from          D 374 O
--pore-axis-basis-to            A 377 O
--pore-axis-basis-to            B 377 O
--pore-axis-basis-to            C 377 O
--pore-axis-basis-to            D 377 O
--site-max-radius             10.0
--site-height-margin           5.0
--channel-chain-id            ABCD
--trace-atom-name             K
--fn-trr traj.trr
```

Each line indicate a set of key and values.

- *–mode* is always "sice-occupancy". This field is for future extensions.
- *–fn-pdb* is the initial structure file.
- *–dt* is the delta-t for the trajectory file
- *–site-max-radius* is the maximum radius of ion channel pore.
- *–site-boundary* is specified two values, 20.0 and -25.0. This setting means that the range from 20.0 > z > -25.0 in the pore axis will be analyzed. The origin is set by *–pore-axis-basis-from* key.
- *–fn-pore-axis-coordinates* and *–fn-pore-axis-coordinates-r* are the output filenames.

- *–pore-axis-basis-from* specifies the origin of the pore axis. "A 374 O" means the O atom in the residue 374 of the chain A. In this tutorial, four oxigen atoms are specified. The center of these atoms is set to be the origin of the pore axis.
- *–pore-axis-basis-to* is the direction of the pore axis. The line from the center of *...-from* to the center of *...-to* defines the pore axis.
- *–site-height-margin* is the margin length along the pore axis. In this case, the range from 20.0+5.0 to -25.0-5.0 will be analyzed.
- *–channel-chain-id* specifies chain ids of a channel protein.
- *–trace-atom-name* specifies the atom name of target ions, defined in the .pdb file.
- *–fn-trr* is the file name of the trajectory.

2.3 Executing "trachan"

```
$IBISA/bin/trachan --fn-cfg config.txt
```

Some text should appear in the standard output:

```
-----------------------------------------------------------------
                      TraChan
  TRAjectory analyzer for CHANnel pore axis
-----------------------------------------------------------------
Copyright (c) 2012 Kota Kasahara, Tohoku University
This software is distributed under the terms of the GPL license

-----------------------------------------------------------------
This program is contains some parts of the source code of GROMACS
software. Check out http://www.gromacs.org about GROMACS.
Copyright (c) 1991-2000, University of Groningen, The Netherlands
Copyright (c) 2001-2010, The GROMACS development team at
Uppsala University & The Royal Institute of Technology, Sweden.
-----------------------------------------------------------------
TraChan::mainRoutine()
site occupancy mode
n_atoms : 6997
pore axis basis a
1014 O THR 374
2735 O THR 374
4456 O THR 374
6177 O THR 374
pore axis basis b
1058 O TYR 377
2779 O TYR 377
4500 O TYR 377
6221 O TYR 377
open pore_axis.txt
open pore_axis_r.txt
average axis length = 0.945331
sd axis length = 0.000977426
```

And the two output files, *pore_axis.txt* and *pore_axis_r.txt* will be obtained.

They are tab-separated text. The first column indicate the time, and the other columns correspond to each potassium ion. Each row indicates the position of each ion at the time. *pore_axis.txt* and *pore_axis_r.txt* record the coordinates along the pore axis and those along the radial direction parpendicular to the pore axis.

2.4 Detecting ion conduction event

```
python $IBISA/bin/detect_ion_permeation.py \
  --i-pore-crd-h pore_axis.txt \
  --i-pore-crd-r pore_axis_r.txt \
  --o-permeation-event permeation_event.txt \
  --atom K --b-r 10 --b-h-up 20 --b-h-low -25
```

Trace each potassium ion travelling from -25 to 20 in z-axis. This script does not consider whether the ion through inside the pore or not. The output file *permeation_event.txt*:

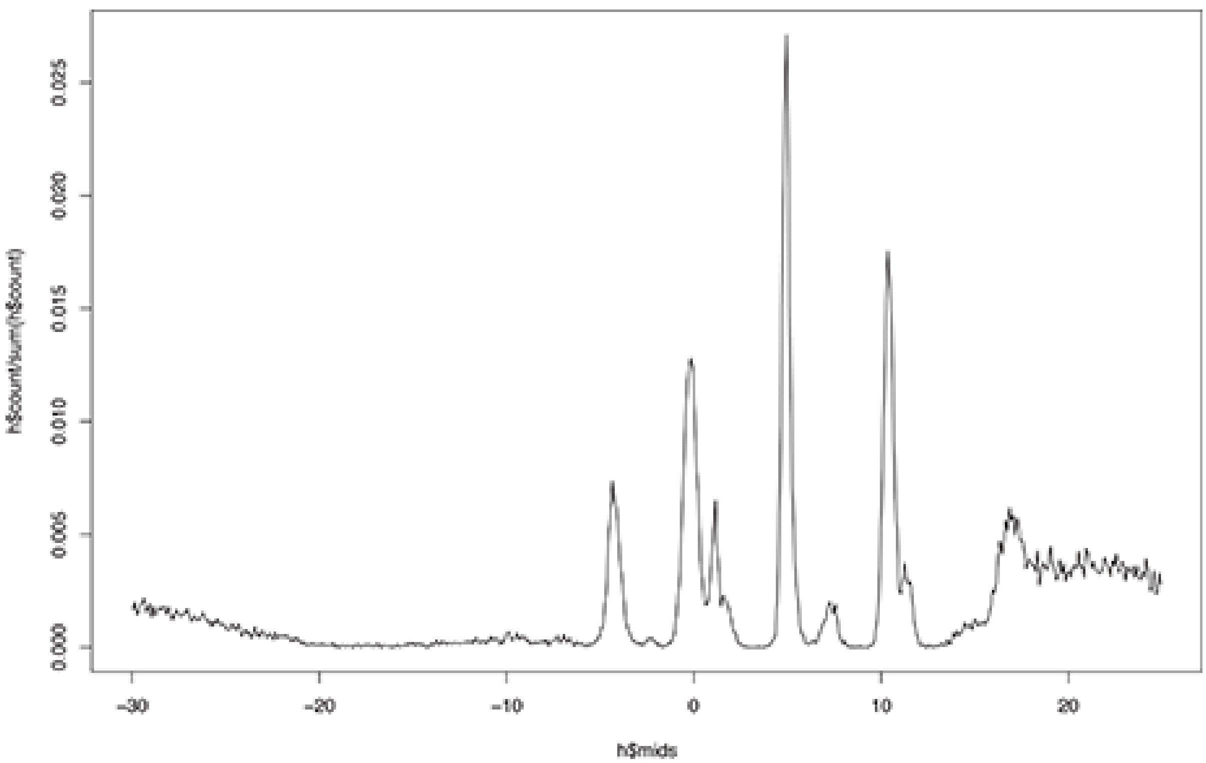
```
6961    K       -       +       5950    21400
6986    K       -       +       10370   30830
6974    K       -       +       11010   38810
6953    K       -       +       28720   44950
6968    K       -       +       36780   47280
6961    K       -       +       39150   48360
```

The first column is the ID of ion, the symbols - and + means that the ion permeated from the - side to the + side. The last two columns indicate times when the ion enters and goes out the defined region.

2.5 Analyzing The density distribution

```
R --vanilla --slave < $IBISA/r/pore_axis_density.R
```

density_distribution.eps is the distribution plot.



This plot clearly shows localization of ions in the ion binding sites. On the basis of this plot, we can define the boundary of each ion binding site.

Here, we use the definition which is defined in our previous paper. The boundaries of ion binding sites are 15.13, 12.93, 9.32, 6.25, 3.00, 0.44, -2.21, -6.08, and -20.

2.6 Discretizing the trajectory based on ion-binding sites

```
python $IBISA/bin/site_occupancy.py \
 --i-pore-crd-h pore_axis.txt \
 --i-pore-crd-r pore_axis_r.txt \
  --o-site-occ   site_occ.txt \
  --atomname K \
  -b 12.93 -b 9.32 -b 6.25 -b 3.00 -b 0.44 -b -2.21 -b -6.08  -b -20 \
  -n '-1'  -n 0     -n 1    -n 2    -n 3    -n 4    -n 5     -n 6
```

The output file *site_occ.txt* records information about what ions are retained in each ion binding sites in each snapshot.

site_ooc.txt:

```
0       1:6946:K        3:6985:K        4:6993:K        6:6935:K
10      1:6946:K        3:6985:K        4:6993:K        6:6935:K
20      1:6946:K        3:6985:K        4:6993:K        6:6935:K
30      1:6946:K        3:6985:K        4:6993:K        6:6935:K
40      1:6946:K        3:6985:K        4:6993:K        6:6935:K
```

“1:6956:K” means the ion K with the ID 6946 is bound at the site 1.

2.7 Analyzing the trajectories of each ion

```
python ${IBISA}/bin/analyze_ion_path.py \
  --i-site-occ        site_occ.txt \
  --o-all-path        site_path.txt \
  --o-count-full      site_path_count_full.txt \
  --o-count-head-tail site_path_count_ht.txt
```

The output *site_path.txt*:

```
6985    K       *:3:0:* 0:7250  *:3:2:1:0:*     0:5290:5340:7240:7250
6985    K       *:0:*   7320:7350       *:0:*   7320:7350
6985    K       *:0:*   7540:7570       *:0:*   7540:7570
```

The first column indicates the ID of the ion.

- At the third column, "*:3:0:*" means this ion got into the pore at site 3, and went out from the site 0.
- The fourth column denote the times for getting into and going out from the pore.
- The fifth column, "*:3:2:1:0:*" indicates the full trajectory of this ion from association the to pore and dissociation from the pore.

2.8 Generating the ion-binding state graph::

```
python $IBISA/bin/analyze_site_state.py \
  --i-site-occ site_occ.txt \
  --o-states   state_traj.txt \
  --o-graph    state_graph.gml \
  --atomname   K
```

The ion binding state graph can be visualized by using the output file *state_graph.gml* with a network analysis software, e.g., Cytoscape.

state_traj.txt records the ion binding state in each snapshot:

```
0       K:1:3:4:6       K:6946:6985:6993:6935
10      K:1:3:4:6       K:6946:6985:6993:6935
20      K:1:3:4:6       K:6946:6985:6993:6935
30      K:1:3:4:6       K:6946:6985:6993:6935
```

The third column indicate the IDs of ions in the ion binding sites.

2.9 Extracting cyclic paths from the state trajectory::

```
python $IBISA/bin/extract_cycles.py \
  --i-state  state_traj.txt \
  --o-cycles state_traj_cycles.txt \
  --o-state-dict state_dict_pre.txt \
  --title    "sample"
```

- option –*title* is an arbitrary string.

state_traj_cycles.txt stores the cyclic paths:

```
>       1       6010    7830    sample
6010    K:0:2:4 K:6985:6993:6935
6630    K:0:2:4:6       K:6985:6993:6935:6961
7190    K:0:2:4:5       K:6985:6993:6935:6961
7230    K:0:1:3:5       K:6985:6993:6935:6961
7820    K:1:3:5 K:6993:6935:6961
7830    K:0:2:4 K:6993:6935:6961
```

- The line begining with ">" is the header line. The cyclic phat "1" starts at 6010 and ends at 7830.
- The resting state, K:0:2:4, is the most stable state in the trajectory.

2.10 Converting states into characters. A cyclic parts transformed into a sequence::

```
python $IBISA/bin/cycle_to_sequence.py \
  --i-cycles     state_traj_cycles.txt \
  --i-state-dict state_dict_pre.txt \
  --o-state-dict state_dict.txt \
  --o-sequence   sequences.fsa
```

- *state_dict.txt* describes the correspondence between a state and a character.
- *sequences.fsa* is the sequences of cyclic paths.:

  ```
  > 0     0       *POMFB* 5       28990   31650   sample
  ```

  POMFB > 1 0 *POMFE* 8 44310 45210 sample *POMFE* > 2 0 *PJHF* 7 39280 42200 sample *PJHF* > 3 0 *POKLF* 6 36850 38640 sample *POKLF*

2.11 Generating score matrix of states

```
python $IBISA/bin/make_score_matrix.py \
   --i-state-dict  state_dict.txt \
   --o-score       score_matrix.txt
```

The similarity between states are simply defined by the number of binding ions. When the two states have the same number of ions, the score is 0.5. Otherwise, teh score is 0.0.

2.12 Performing the sequence alignment

```
python $IBISA/bin/dp_align.py \
   --i-score-matrix score_matrix.txt \
   --i-sequence     sequences.fsa \
   --o-align        align.txt -a\
   --min-len   4 \
   -g 1.0 \
   -m 1.0 \
   --ignore *
```

- *-g* and *-m* are gap score and match score, respectively.

- The output file *align.txt* shows the pairwise alignments:

```
> 2     3       0.0     2       0       *PJHF*  7       39280   42200   sample  3       0       *POKLF* 6       36850   38640   sample
```

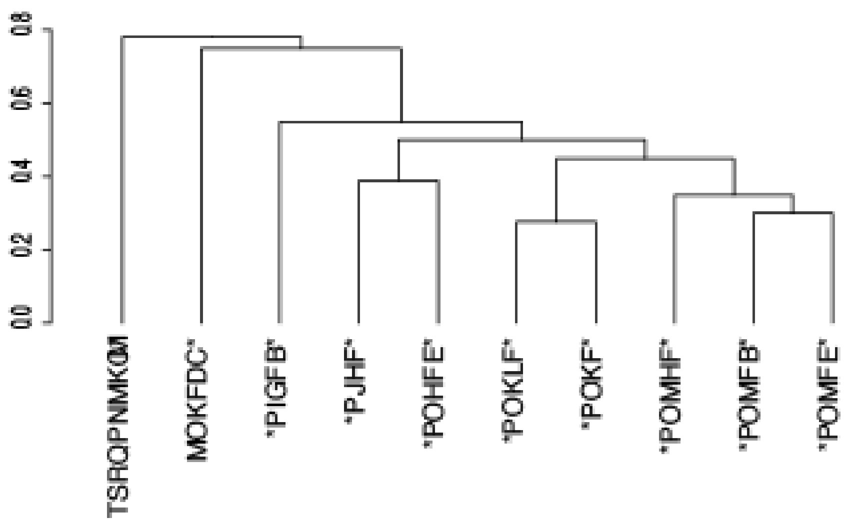
PJH-F POKLF > 0 3 1.0 0 0 *POMFB* 5 28990 31650 sample 3 0 *POKLF* 6 36850 38640 sample *POM-FB POKLF-*

2.13 Make the similarity matrix of cyclic paths

```
python $IBISA/bin/align_similarity.py \
  --i-align     align.txt \
  --i-sequence  sequences.fsa \
  --o-sim       align_sim.txt \
  -g 1 -m 1
```

2.14 Clustering aligned sequences by using R

```
R --vanilla --slave < $IBISA/r/clustering_seq.R > clustering_seq.log
```



CHAPTER

THREE

INDICES AND TABLES

- genindex
- modindex
- search