TUTORIAL FOR RUNNING AND ANALYZING ADEPTH RESULTS SWW Chen and JL Pellequer Version 0.3

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1) Adepth web-server address



1. Input your PDB file

Simply enter the PDB code or upload a .pdb file.

PDB code : must exist in PDB	1AY1	(Type : 1	l AY1 as a	test molecule	e)
or upload your PDB file :				Browse	э)
PDB chain ID :					
Probe radius :	1.5				
Grid size (X, Y, Z) in Å :	1.0	1.0	1.0		
Output grid file (dx format) :					
Print-out only extruded PDB :		/lin value:		Max value:	
Grid boundary (in Å) :	5				

- Enter the PDB code if available from the PDB; otherwise, select the Browse button to load a structure from your local computer.
- The grid dimension is automatically computed using both the user input "Grid size (X, Y, Z) in Å" and the size of the macromolecule. The max number of elements in the grid is 300 in one direction. Thus, users MUST ADJUST the "Grid size" values in function of their macromolecule size.
- In the above selection, the "Output grid file (dx format)" has been checked. It will provide the necessary data to display an isopotential map of the atomic depth with VMD. Small grid sizes will output very large DX files (~200 Mb).
- If one wants only the skin layer of a molecule, you have to check the box "Print-out extruded PDB", then choose the min and max depth values.
- "Grid boundary" defines the additional space between surface atoms and grid borders. The greater the number the smaller the molecule will appear on the grid.

3) Click on "Run analysis" button to start

2. Getting results

Enter a valid e-mail address where we can send a link to the results when the run is completed.

E-mail :

If you find this site useful, please cite :

Shu-wen W. Chen and Jean-Luc Pellequer *Adepth: new representation and its implications for atomic depths of macromolecules, submitted*

Run analysis	Clear input
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The email address is not required. It is only used to send result data in a zip file to your email.

Adepth

measures of atomic depths in macromolecules

Please wait...

Your request has been sent to the queue on Sun, 11 Nov 2012 16:16:16 GMT. Your job will be the next one to be processed...

Link to the result page will be send to your e-mail adress (if provided) when the job is completed.

Warning : Results are deleted from the server after 7 days.

If you did not provide your email, wait a few minutes to get the result summary message.

<u>Note:</u> Although Adepth is very fast (a few seconds), the time to obtain results may vary upon the server load or upon the size of output (remember that dx files may be very large for small grid steps).

5) Result output

Adepth

measures of atomic depths in macromolecules

Results

Check the list of WARNINGS: SDF has been calculated for: 1ay1.pdb Selected chain Id: Output PDB file : 1ay1 adepth sdf.pdb Probe radius : 1.5 (A) Grid spacing : 1.0 1.0 1.0 (A in X Y Z) Grid boundary: 5 (A) Skin min val : 0.0 (A) Skin max val : 9999.99 (A) _____ _____ When using this work, please cite: SWW Chen and JL Pellequer (2013) Adepth: new representation and its implication for atomic depths of macromolecules. Submitted.

Result files have been mailed to: You can also download results.zip with all output files or display output PDB file using JMOL applet...

For additional information please contact the coordinator on the right hand of the screen.

Result file is accessible by clicking on "result.zip", then save the file on your computer.

This file contains:

- *.out \rightarrow Log file from the run
- *.pdb \rightarrow Original PDB file
- *-adepth_sdf.pdb → Output PDB file with SDF values in b-factor column
- *.grid \rightarrow Output SDF values on the grid§
- *.dx \rightarrow Transformed .grid file into DX format for VMD§

§These files are only present when the "Output grid file" option is selected.

- Display the output PDB file using an atomic-depth colored-coded ribbon by clicking on "display output PDB file".
- A Jmol applet will display the result in a new window. By default the molecule is shown using the "Rockets" style.



6) Interpretation of results

- The atomic depth value is the distance of the atomic center from the solvent accessible surface
- The header of a sample *_sdf.pdb file is shown below:

REMARK:	inpu	t pdi	bfile	e:/op	pt/app]	li/temp-0	day/adept	h-41/1ay	rl.pdb		
REMARK:	PROB	E ra	dius	: 1.	5 (A) ;	: GRII) spacing	x-y-z:	(1.0	1.0 1.	0) (A)
REMARK:	sdf	rang	e: (-11	.4098	-2.4376) (A)				
REMARK:	COLU	MIN O	CCUPA	ANCY	: scale	ed sdf ma	agnitude				
REMARK:	COLU	MIN B	-FAC	ΓOR:	sdf ma	agnitude					
АТОМ	1	Ν	ASP	L	1	27.504	-17.288	18.937	0.96	3.23	0
АТОМ	2	CA	ASP	L	1	27.334	-16.029	19.656	1.04	3.82	0
АТОМ	3	С	ASP	L	1	27.777	-14.855	18.795	0.98	3.62	0
АТОМ	4	0	ASP	L	1	28.548	-13.993	19.232	0.92	3.04	0
ATOM	5	СВ	ASP	L	1	28.128	-16.048	20.959	0.89	3.26	0
АТОМ	6	CG	ASP	L	1	27.644	-15.011	21.948	0.87	3.18	0
ATOM	7	OD1	ASP	L	1	26.551	-14.437	21.738	0.85	2.79	0
АТОМ	8	OD2	ASP	L	1	28.353	-14.782	22.949	0.78	2.56	0
ATOM	9	Ν	ILE	L	2	27.287	-14.850	17.560	1.28	4.27	0

- Red boxes indicate the values for the probe radius and the grid spacing (both in Å)
- Blue box indicates the min and max depth values of macromolecule
- Green box indicates the atomic depth. For respecting the PDB format of the B-factor column, depth values are provided as positive values.

7) Visualization of results

VMD is a molecular visualization program for displaying, animating, and analyzing large biomolecular systems using 3-D graphics and built-in scripting.

Go here to download: <u>http://www.ks.uiuc.edu/Research/vmd/</u> To cite VMD: Humphrey W, Dalke A and Schulten K (1996) VMD: visual molecular dynamics. *J. Mol. Graph.* **14**: 33-38.

1. Run VMD

🗖 AW	D Main						×
File	Molecule	Graphics	Display	Mouse	Extensions	Help	
ID -	FADEN	lolecule		Atoms	Frames	Vol	
	zoom 🗖	Loop 💌	step 🖣 1	speed	d		

The VMD main menu pop-up.

2. Click on <File> and select <New molecule...>. The "Molecule File Browser" will pop up.

Molecule File Browser		
Load files for: New Molect	ule	•
Filename:		Browse
Determine file type: Automatically	•	Load
Frames: First: Last: Stride: CLoad in background CLoad all at once	Volumetric Datasets	

3. Click on "Browse..." button to load your input file. The "Choose a molecule file" menu will pop up, select your molecule and its name will appear in the "Molecule File Browser" highlighted in yellow.

Choose a mole	cule file			? 🔀
Choose a molect Regarder dans : Mes documents récents Bureau	Cule file DONE TEST 1ay1.pdb 1ay1.pdb.dx 1ay1.pdb.grid 1ay1.pdb.grid 1ay1.ade.pth_sdf sdf_543.out	.pdb	€ € ்	
Mes documents Poste de travail Favoris réseau	Nom du fichier : Fichiers de type :	All files (*. *. *. *)		Ouvrir Annuler

Molecule File Browser	
Load files for: New Molecule	•
Filename: nsfert/cdrom/DONE/1ay1_adepth_sdf.pdb	Browse
Determine file type: PDB	Load
Frames: Volumetric Datasets First: Last: Stride: 0 -1 1 © Load in background Load all at once	

•In the above window, the "Determine file type" has been automatically selected. Click on "Load" to open the output file in PDB format from Adepth,

Molecule File Browser		
Load files for: New Molecu Filename: ts/File_transfert	ule /cdrom/DONE/1ay1.pdb.dx	▼ Browse
Determine file type:	▼	Load
Frames:	Volumetric Datasets	
First: Last: Stride: 0 -1 1 © Load in background © Load all at once	1: DX map	

•Repeat step 3 to open the corresponding DX grid output file.

 Careful, when loading multiple files with the above menu, it is necessary to SELECT "Load files for:" New Molecule each time. Now, in the main window of VMD, three molecules are loaded: Input PDB file: 1ay1.pdb Output Adepth PDB file: 1ay1_adepth_sdf.pdb DX grid file: 1ay1.pdb.dx

🗖 АМ	ID Main						×
File	Molecule	Graphics	Display	Mouse	Extensions	Help	
ID .	TADEM	olecule		Atoms	Frames	Vol	
0 1 2 ⁻	A D F 12 A D F 12 T A D F 12	ay1_adepth ay1.pdb.dx ay1.pdb	_sdf.pdb	3282 0 3282	1 0 1	0 1 0	
	zoom 🗆	Loop 💌	step 🖣 1	l ▶ speed	1		≯

You should see something like this in the Graphics window:



- 4. Wireframe view of the isosurface
- a/ Click on the VMD main window <Graphics> and select <Representations...>. The "Graphical representations" menu will pop up.

🔲 Graphical Repr					
Selected Molecule					
1: 1ay1.pdb.dx		•			
Create Rep		Delete Rep			
Style	Color	Selection			
Isosurface	Name	<volume></volume>			
<u></u>	elected Atom]			
all	icicetea Atom.				
Draw style Soler	tions Trainct	on / Deriodic			
Coloring Metho	d	Material			
Name	- Op	aque 💌			
Drawing Metho	d				
Isosurface	•	Default			
Range 8 5948 22		0: C:/Docurr ▼			
Step	Draw Po	ints Y			
Size 📢 1	Show Bo	x+lsosurface 🛉			
♦ Apply Cha	anges Automat	tically Apply			

- b/ Click on the Draw "green box" and select "Wireframe".
- c/ Click on the Show "blue box" and select "Isosurface"
- d/ Click in the Isovalue "red box" to change the depth of the isosurface

Representation of two different isosurface depths:

🗖 Graphical Repre	Graphical Representations			
Selected Molecule				
1: 1ay1.pdb.dx		•		
Create Rep		Delete Rep		
Style	Color	Selection		
Isosurface	Name	<volume></volume>		
<u>.</u>	olastad Atam			
all	elected Atom;	>		
Coloring Methor	tions Trajeci 1	tory Periodic Material		
Name		aque		
,				
Isosurface	-	Default		
Range p.5948 p2.				
Isovalue				
Step 📢 🚺 💧	🕨 Draw Wi	reframe 💌		
Size 📢 🌗 🕽	Bhow Iso	surface 💌		
A tents Obs		tion the time to		
Apply Changes Automatically Apply				

🗖 Graphical Repr						
Selected Molecule						
1: 1ay1.pdb.dx						
Create Rep		Delete Rep				
Style	Color	Selection				
Isosurface	Name	<volume></volume>				
۱ <u>ـــــ</u>	Selected Atoms	;				
all						
Draw style Seler	ctions Traiect	ory Periodic				
Coloring Metho	<u>d</u>	Material				
Name	✓ Op	aque 🔽				
Drawing Metho	d					
Isosurface	▼	Default				
Range β.5948 22	Range 8.5948 2.107 Vol Vol0: C:/Docum					
Isovalue	-9					
Step 4 1	Sten #1 1 NB Draw Wireframe					
Size						
♦ Apply Changes Automatically Apply						

Using the above settings, the graphical window should looks like:





- 5. Display of atomic depth using VMD ribbons
 - a/ Disable the display of isosurface: use the "VMD main" window and double click on the D letter of the corresponding molecule you wish to disable:

🗖 ММ	D Main						×
File	Molecule	Graphics	Display	Mouse	Extensions	Help	
ID -	TADEN	lolecule		Atoms	Frames	Vol	
0	ADF1	ay1_adepth	_sdf.pdb	3282	1	0	
1	ADF1	ay1.pdb.dx		0	0	1	
2 1	TADF1	ay1.pdb		3282	1	0	
				I			
	zoom 🗖	Loop 💌	step 🖣 1	▶ speed	l I		

- b/ In the above example, both *.pdb and *.dx files have been switched off.
- c/ Using the "Graphical Representations" window, it is possible to display the atomic depth values on a ribbon representation of the macromolecule as shown in the below graphics:

🔲 Graphical Represe	entations					
Selected Molecule						
0: 1ay1_adepth_sdf.pdb						
Create Rep		Delete Rep 1				
Style	Color	Selection				
NewRibbons B	leta	all				
Self	ected Atoms]				
all		<u>.</u>				
Draw style Selectio	Draw style Selections Trajectory Beriodic					
Coloring Method		Material				
Beta 💌	Ор	aque 💌				
Drawing Method						
NewRibbons 💌		Default				
Splin	e Style B-S	Spline 🔻				
Aspect Ratio 3.00						
Thiskness 0.47	<u> </u>					
Resolution 4 4 40						
Apply Changes Automatically Apply						

In the above window, the selected "Drawing method" is "New Ribbons" and the selected Coloring method is "beta". This option select a default blue-to-red gradient color scale for values present in the B-factor column of a PDB file.



The above image shows the "New Ribbon" drawing method colored according to values present in the bfactor column.