**5. Result analysis**

***5.1 Analyze affinity***

Module 5.1 can be run automatically by checking the “Result Analysis” option in module 4. The affinity scores from all the docking results will be summarized in an *Excel* file (.xlsx). The affinity Heatmap (.jpg) will be also plotted.

1. Click “Choose log files directory” to select the directory where the docking results were saved in module 4.
2. Click “Choose saving directory” to set where the affinity Table and affinity Heatmap will be saved.
3. The Heatmap style can be changed to suit your preferences.
4. Click “Begin to analyze” and wait……You can click “View affinity Table” and “View affinity Heatmap” to view the results when the analysis is complete.

Note that the RMSD values in the affinity Table are taken from the *log* file of each docking. The docking pose with the lowest affinity score is used as the reference in each docking, so the **RMSD from different docking results cannot be compared, only the RMSD of different poses from a single docking result can be compared**.

Ki (Kd) values in the affinity Table are calculated according to the following equation:

In the equation, *e* is the natural constant. -*ΔG* (cal/mol) is the binding free energy, and here is the affinity score from docking. *R* is the ideal gas constant (1.986 cal/K). The temperature *T* is 298K.

**Note that if there are too many receptors and ligands in a docking project, the affinity Heatmap may display inappropriately (the affinity Table is not affected)**.

***5.2 Split docking poses***

Module 5.2 can be run automatically by checking the “Result Analysis” option in module 4. This module can be used to split the output (.pdbqt) of Vina into single pose files.

1. Click “Choose docking poses directory” to select the directory where the docking results were saved in module 4.
2. Click “Begin to split” and wait……The files will be saved in the original directory.

**Note that the docking results with special characters such as α, β, etc. in the name cannot be split. Please analyze affinity first before splitting the docking poses, otherwise the *log* file will be also moved to the subfolder after splitting the docking poses, which makes it difficult to analyze affinity.**

***5.3 Generate ligand-receptor complex (beta)***

The output of Vina contains only the ligand pose. This module is used to save the original receptor and the docking pose in a single structure file (.pdb).

1. Click “Select a ligand” to select a ligand pose (.pdbqt) to combine.
2. Click “Select a receptor” to select the receptor (.pdbqt) of the ligand.
3. Click “Choose saving directory” to set where the ligand-receptor complex will be saved.
4. Click “Generate complex”.

**After testing, the complex structure files generated by OpenBabel often contain some unknown errors, especially when the receptor is large and complex. We are looking for a better way to solve this problem. At present, please check the complex structure by visualization after it has been generated.**

***5.4 Score only***

This module is used to give the affinity score of the specific ligand pose with no docking, such as the co-crystal ligand pose or the docking results from other software.

1. Select the scoring function: Vina or Vinardo.
2. If used for comparison, remember to correct the ligands’ protonation state to keep them consistent, as different protonation states will result in different affinity scores.
3. Click “Select a receptor” to select the protein receptor (.pdbqt) without any ligand.
4. Click “Select a co-crystal ligand” to select the co-crystal ligand pose or a docking pose from another software (.pdbqt). To obtain the co-crystal ligand pose, open the crystal structure of the ligand-receptor complex in any visualization software, save the ligand separately, and convert it into pdbqt format.
5. Click “Score only”. The affinity score will be displayed in a pop-up window.

**5. 结果分析**

***5.1查看结合亲和力***

模块5.1可以通过勾选模块4中的"结果分析"选项自动执行。所有对接结果的亲和力分数将被汇总到一个*Excel*文件(.xlsx)中。亲和力热图(.jpg)也会被自动绘制。

(1) 点击"选择所有log文件所在路径"来选择对接结果所在路径，如模块4中的结果输出路径。

(2) 点击"选择保存目录"来设置结合亲和力汇总表和结合亲和力热图的保存位置。

(3) 热图风格可以根据需要设置。

(4) 点击"开始分析"等待即可。分析完成后可以点击"查看亲和力汇总表"和"查看亲和力热图"来查看结果。

请注意，亲和力汇总表中的RMSD值取自每次对接的日志文件。在每次对接中，具有最低亲和力得分的对接构象被视为参考构象，因此**不能比较不同对接结果的RMSD，只能比较单次对接结果中不同构象间的RMSD**。

亲和力汇总表中的Ki(Kd)值是按照以下公式计算的：

其中，*e*是自然常数。*-△G* (cal/mol)是结合自由能，这里是对接打分。*R*是理想气体常数(1.986cal/K)。温度*T*设为298K。

**注意，如果受体和配体过多，亲和力热图可能会不合理地显示（亲和力汇总表不会受影响）。**

***5.2分割结果构象***

模块5.2可以通过勾选模块4中的"结果分析"选项自动执行。这个模块用来将Vina的输出(.pdbqt)——包含多个排名靠前的对接构象文件，分割成单一的构象文件。

(1) 点击"选择对接结果所在路径"来选择对接结果所在路径，如模块4中的结果输出路径。

(2) 点击"开始分割"并等待，分隔后的文件将被保存在原来的路径中。

**注意，无法分隔名称中含有特殊字符的对接结果，如α，β等。另外，请先分析结合亲和力，再分割对接构象，否则分割对接构象后，日志文件也会被移到相应的子文件夹中，导致难以再分析亲和力。**

***5.3 生成配受体复合物(测试)***

Vina的输出只包含配体的结合构象。本模块用于将对接受体和配体结合构象保存在同一结构文件(.pdb)中。

(1) 点击“选择一个配体”选择用于复合的配体构象(.pdbqt)。

(2) 点击“选择一个受体”选择该配体的受体。

(3) 点击“选择保存路径”设置配受体复合物的保存位置。

(4) 点击“生成复合物”即可。

**经过测试，OpenBabel生成的复合物结构文件经常包含一些未知错误，尤其是当受体结构又大又复杂时。我们正在寻找更好的方法来解决这一问题。目前，请在生成复合物后通过可视化检查确保结构的合理性**。

***5.4 查看共晶配体打分***

该模块用于对特定的配体结合构象直接进行打分而不对接，如共晶体配体位姿或来自其他软件的对接结果。

(1) 选择打分函数：Vina或Vinardo。

(2) 如果基于比较的目的，务必修正配体的质子化状态使它们保持一致。因为不同的质子化状态会导致不同的亲和力得分。

(3) 点击"选择一个受体"，选择没有任何配体的蛋白质受体(.pdbqt)。

(4) 点击"选择共晶配体"，选择共晶配体构象或其他软件的对接结果(.pdbqt)。要获得单独的共晶配体构象，可在任意可视化软件中打开原始配受体复合物的晶体结构，单独保存配体，并将其转换为pdbqt格式。

(5) 点击"开始打分"，打分结果将弹窗显示。