**3. Docking parameters**

In this module, you need to set the docking parameters, the most important of which is the docking region. Only by setting the docking box properly can the ligand be docked to the true binding site in the protein. For batch docking tasks, docking parameters can be set in batches automatically.

1. Click "Choose saving directory" to set where the config files containing all the docking parameters will be saved.
2. Select a kind of generation mode for the docking box.
3. Set other docking parameters.
4. Enter some necessary contents in the corresponding module 3.1~3.4 according to the selected generation mode.
5. Click "Generate config files".

***3.1 Analogy docking***

Dock your ligands at the position of the co-crystal ligand in the protein. Usually, these ligands should have similar binding mechanisms, such as they are both small molecule inhibitors of a protein target, or you want to screen for competitive inhibitors of the co-crystal substrate in the protein. Of course, it’s also proper if you just want to redock the co-crystal ligand at its original position…

The box size will be set to 4.5 times the co-crystal ligand’s *R*g and the box center will be set to the geometric center of the co-crystal ligand. For more information about these docking parameters, please see our research paper.

If you want to set the center of the docking box according to the co-crystal ligand but set the box size according to your ligands so that the box size matches your ligands each time you dock, please check the option “Universal docking protocol with Vina” in module 4 before you decide to perform your docking task.

1. Click "Choose crystal ligands directory" to set where the co-crystal ligands split from the receptor (*.pdbqt*) are located. To obtain these co-crystal ligands, open the crystal structure of the ligand-receptor complex in any visualization software, save the ligand separately, and convert it into *pdbqt* format.

***3.2 Blind docking***

If you don’t know the true binding site in the protein, the blind docking method can be used. **But note that the accuracy of the blind docking method is low, it often leads to false-positive results. It means that the ligand was not docked to the true binding site. The results from blind docking are not supposed to be used as scientific bases.** This method is usually used in the Network Pharmacology researches, although it is not appropriate. Using the blind docking method, the docking box will be set to cover the whole protein.

1. Click "Choose docking receptors directory" to set where the docking receptors are located (e.g. the saving directory in module 1.2). **Note that only the docking receptors in *pdbqt* format can be recognized. These receptors should be prepared in advance.**
2. You can increase the box size to provide more space for the torsion of the ligands which bind to the protein surface, but not too much, as the blind docking box is already very large!

When the search space volume is greater than 27000 Å3, a warning will be given at the beginning of docking. The time costs of docking will increase significantly. If the blind docking method is selected, ignore this warning. **When the search space volume is greater than 1000000 Å3, the docking may fail. Please reduce the box size appropriately**.

* 1. ***Key residues docking***

The binding site is usually well-defined by the binding residues or functional residues. If it’s specific, the docking box can be determined by these key residues. In this case, the box center will be set to the geometric center of these residues. The box size needs to be set by users.

1. Click "Choose docking receptors directory" to set where the docking receptors are located (e.g. the saving directory in module 1.2). **Note that only the docking receptors in pdbqt format can be recognized.**
2. Set the box size. If it’s difficult for you to choose an appropriate box size, you can check the option “Universal docking protocol with Vina” in module 4 before you decide to perform your docking task. The box size will be determined by the Rg of your ligands automatically.
3. After you click the "Generate config files" button, the current receptor will be shown on the interface. Now you need to enter the key residue information. **Please don’t enter more than 20 residues for a protein. The length of a single chain for a protein cannot be more than 1000**.

Input format:

Capital letter of the chain in which key residues are located, *colon*, index of a residue, *underscore*, index of the next residue, …, *comma*, capital letter of the next chain, *colon*, index of a residue, *underscore*, index of the next residue, …

Some examples:

A:19\_20\_21

A:78\_89\_109,B:99

The next receptor will be selected when you click “OK”. Enter the key residue information until the binding sites of all the receptors are defined.

***3.4 Custom***

Customize the size (Å) and position of the docking box to suit your needs. **Note that if the size and center of the docking box have been determined using AutodockTools by visualization in advance, remember to convert those into the docking parameters for Vina. In AutodockTools, the box size is determined by the number of grid points and grid spacing while it is set directly in Vina.**

**3. 对接参数**

在这一模块中，需要设置对接参数，其中最重要的就是对接区域。只有正确设置对接盒子，配体才能被对接到蛋白质的实际结合位点。对接参数可以被批量设置。

1. 点击“选择保存路径”，设定包含对接参数的配置文件保存位置。
2. 选择一种对接盒子的生成模式。
3. 设置额外参数。
4. 根据选择的生成模式，在相应的子模块中输入必要内容。
5. 点击“生成配置文件”。

***3.1类比对接***

将你的配体对接到蛋白质中共晶配体的位置。通常这些配体应该有类似的结合机制，如他们都是蛋白质靶点的小分子抑制剂，或者要对接的配体是共晶底物的竞争性抑制剂。当然，如果你只是想把共晶配体重新对接到原来的位置，使用此方法是最好的。

对接盒子的大小会被设置为共晶配体回转半径的4.5倍，盒子中心会被设置为共晶配体的几何中心。关于这些参数的选择，请参考我们的研究论文。

1. 点击“选择对接受体所在路径”来设置单独的共晶配体(*.pdbqt*)所在位置。可以通过如下方式获得这些配体：在任意可视化软件中打开配受体晶体结构，将其中的配体单独保存，并转化为pdbqt格式。

如果你想根据共晶配体的位置设置对接盒子中心，但根据要对接的配体设置盒子大小，以便每次对接时，盒子都能够匹配对接配体，您可以在进行对接前勾选模块4中的“蛋白质-小分子半柔性对接通用方案”。

***3.2盲对接***

如果不知道蛋白质中真正的结合位点，可以使用盲对接方法。但**盲对接方法的准确性很低，经常导致假阳性的结果，即配体没有被对接到实际的结合位点，因此结果不应作为科学依据。**盲对接通常用于网络药理学的验证工作中，尽管这并不合适。盲对接的对接盒子会包裹整个蛋白质空间。

1. 点击“选择对接受体所在路径”来设置对接受体所在位置(如模块1.2中的受体结构保存路径)。**受体应被提前准备以去除无关杂质，并转化为*pdbqt*格式**。
2. 可以进一步增加盒子的大小，以为结合在蛋白质表面的配体的扭转提供更多空间，但不能太多，因为盲目对接的盒子已经很大了!

**当搜索空间大于27000 Å3时，在对接开始时将会出现警告。此时对接时间将大大增加。如果选择盲对接方法，请忽略此警告。当搜索空间体积大于1000000 Å3时，对接可能会失败，请适当减少盒子大小。**

***3.3关键残基对接***

结合位点通常由配体结合残基或蛋白质的功能性残基定义。如果结合位点是明确的，对接盒子可以由这些残基确定。此时，对接盒子中心为这些残基的几何中心，对接盒子大小需要用户定义。

1. 点击“选择对接受体所在路径” 来设置对接受体所在位置(如模块1.2中的受体结构保存路径)。**受体应为*pdbqt*格式。**
2. 设置对接盒子大小。如果您不清楚合适的尺寸，您可以在进行对接前勾选模块4中的“蛋白质-小分子半柔性对接通用方案”，盒子大小将会由配体回转半径自动计算。
3. 点击“生成配置文件”后，当前受体会显示在界面上，此时需要输入关键残基信息。**一个蛋白最多输入20个关键残基，且无法用于单链长度超过1000的蛋白质**。

输入格式：

关键残基所在链的大写字母，英文冒号，残基序号，英文下划线，下一个残基的序号，...，英文逗号，下一个链的大写字母，英文冒号，残基序号，英文下划线，下一个残基的序号，...

几个例子：

A:19\_20\_21

A:78\_89\_109,B:99

点击“OK”进入下一个受体的残基设定，直到所有受体的结合位点都被设定完成。

***3.4自定义对接***

自定义对接盒子的大小(Å)和位置以满足你的需要。**注意，如果对接盒子的大小和中心已经事先在AutodockTools中通过可视化方式确定了，务必记得将其转换为Vina的对接参数。在AutodockTools中，盒子的大小是由网格点的数量和网格间距决定的，而在Vina中，是直接设置的盒子边长(Å)。**