

# Autodock原理及使用

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# Outline

1. 分子对接简介
2. Autodock 使用过程及实例
3. 总结

# 分子对接简介

## 分子对接定义

依据配体与受体的相互作用，模拟两分子之间通过几何和能量匹配相互识别的过程。

## 分子对接原理

通过计算方法把配体分子放在受体活性位点，利用**几何和能量互补**原则评价配体与受体相互作用好坏，通过打分函数，找到最佳结合模式。

## 分子对接用途

确定两个分子正确的相对位置和取向，研究两个分子的构象，用来**研究蛋白激活剂、抑制剂作用机制以及药物作用机制**，在新药设计中有重要的作用。

# 分子对接类型

## 刚性对接

在对接的过程中，研究体系的构象不发生变化

比较大的体系，如蛋白质和蛋白质、蛋白质和核酸之间，计算简单，考虑构象之间的契合程度。

## 半柔性对接

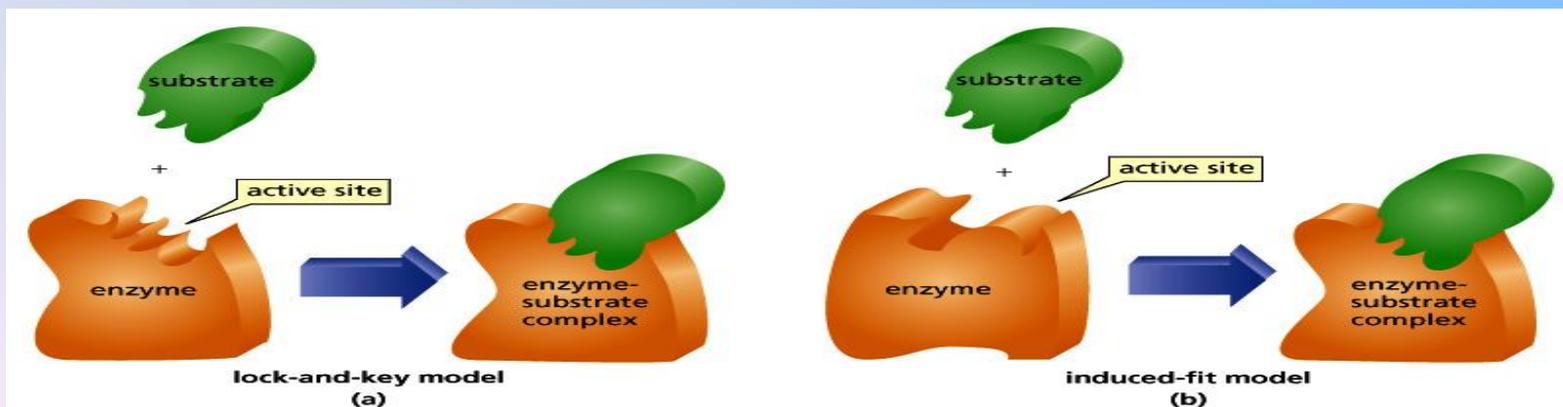
在对接过程中，研究体系尤其是配体的构象允许在一定的范围内变化

适合于处理小分子和大分子之间的对接

## 柔性对接

在对接过程中，研究体系的构象基本上是可以自由变化的

用于精确考察分子之间的识别情况



# 分子对接算法

对接类型	对接方法类型	对接方法
Flexible-Ligand Docking <b>柔性配体对接</b>	Systematic 系统方法	Conformational 构象
		Fragmentation 片段生长
		Database 数据路
	Random/stochastic 随机方法	Monte Carlo (MC) 蒙特卡罗
		Genetic algorithm (GA) 遗传算法
		Tabu Search 禁忌搜索
	Simulation methods 模拟方法	Molecular dynamics (MD) 分子动力学
		Energy minimization 能量最小化
	Flexible-Protein Docking <b>柔性蛋白对接</b>	Molecular dynamics (MD) 分子模拟
Monte Carlo (MC) 蒙特卡罗		
Rotamer libraries 旋转异构体库		
Protein-ensemble grids 蛋白集合栅格		
Soft-receptor modeling 软受体建模		

# 分子对接软件

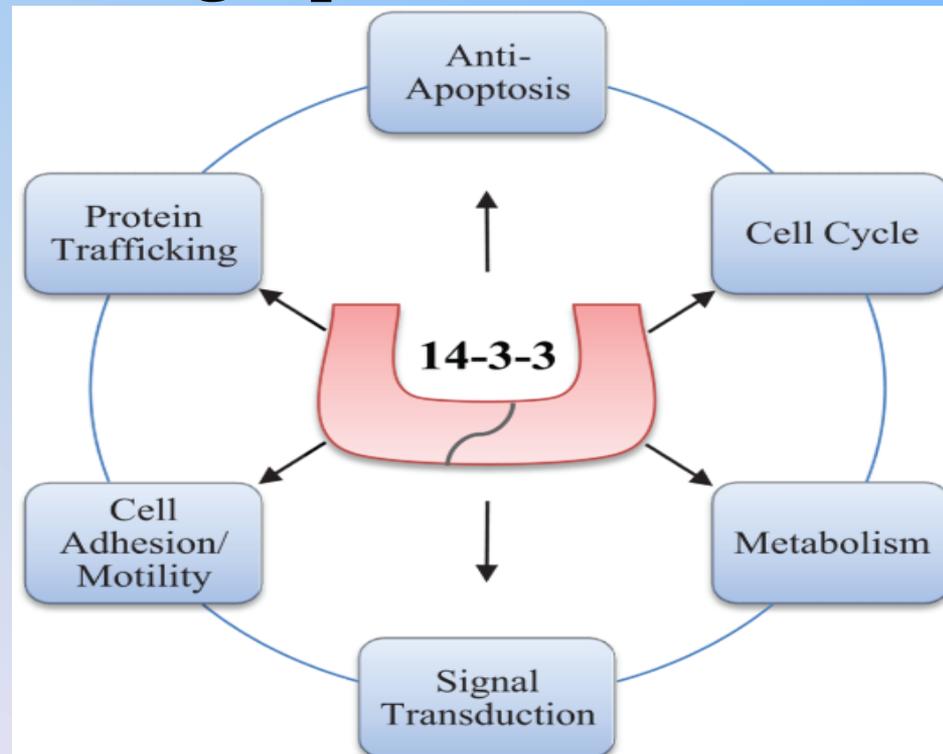
名称	优化方法	评价函数	速度	Protein-protein (peptide) docking	Protein-ligand docking
<b>DOCK</b>	片断生长	分子力场、表面匹配得分、化学环境匹配得分	快		√
<b>AutoDock</b>	遗传算法	半经验自由能评价函数	一般		√
<b>ICM-Docking</b>	随机全局优化	半经验自由能评价函数	快		√
<b>GOLD</b>	遗传算法	半经验自由能评价函数	快		√
<b>FlexX</b>	片断生长	半经验自由能评价函数	快		√
<b>Affinity</b>	蒙特卡罗/分子力学/分子动力学	分子力场	慢		√
<b>ZDock&amp;RDock</b>	几何匹配/分子动力学	CAPRI*/分子力场	慢	√	
<b>FlexiDock</b>	遗传算法	分子力场	慢		√
<b>eHiTS</b>	系统搜索	半经验自由能评价函数	快		√
<b>Hex</b>	几何匹配	*	快	√	

# **Autodock 使用过程及实例**

**Drug Design Targeting on  
14-3-3zeta for Cancer Therapy**

# Background :14-3-3 proteins

- **First discovered in 1967.**
- **In mammals, seven distinct isoforms :  $\beta$ ,  $\gamma$ ,  $\varepsilon$ ,  $\zeta$ ,  $\eta$ ,  $\sigma$ , and  $\tau$  .**
- **lack endogenous enzymatic activity; they function through their target proteins.**



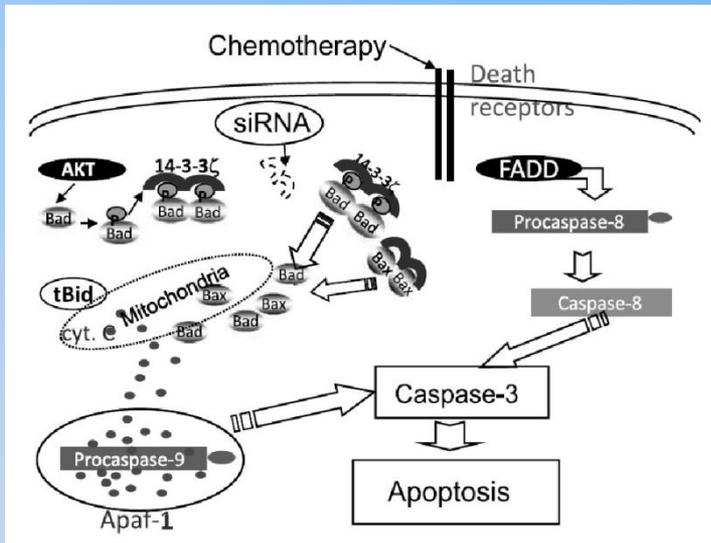
# 14-3-3 $\zeta$ as a target for anti-cancer therapy

✧ overexpression of 14-3-3 $\zeta$  is associated with diverse cancer types and regulates pathways that promote cancer initiation and progression.

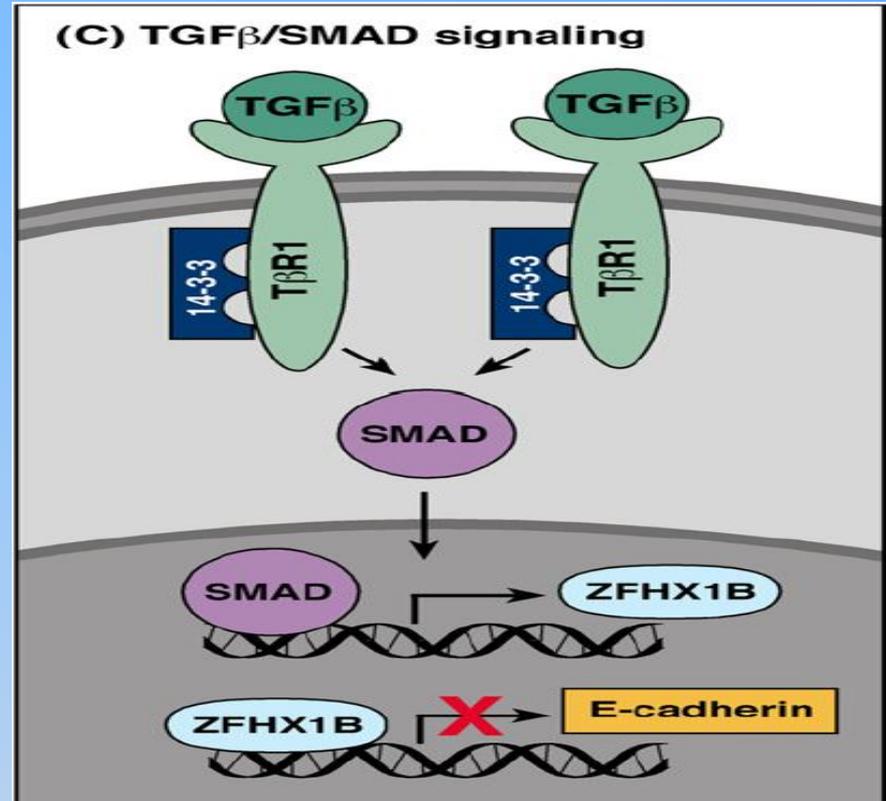
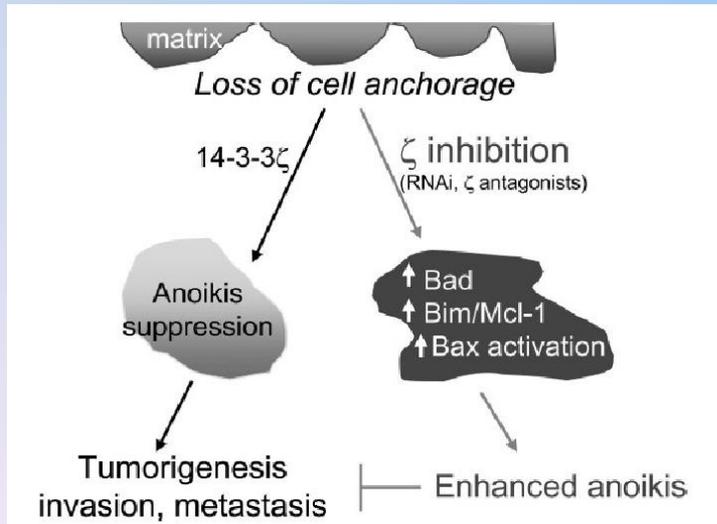
✧ 14-3-3 $\zeta$  overexpression and gene amplification are correlated with poor prognosis and chemoresistance in cancer patients.

<i>Cancer type</i>	<i>14-3-3zeta expression</i>	<i>Chemoresistance</i>	<i>Poor prognosis</i>
Lung	Elevated	Yes	Yes
Breast	Elevated	Yes	Yes
Prostate	Elevated		
Diffuse large B-cell lymphoma	Elevated	Yes	
Multiple myeloma	Elevated	Yes	
Acute promyelocytic leukemia	Positive	Yes	
Liver	Positive	Yes	
Stomach	Positive		
Glioma	Elevated		Yes
Meningioma	Positive		
Esophageal	Elevated		
Head and neck squamous cell carcinoma	Elevated		Yes
Oral	Elevated		
Pancreatic	Elevated		
Ovarian	Elevated		
Skin	Elevated		

# 14-3-3 $\zeta$ as a target for anti-cancer therapy



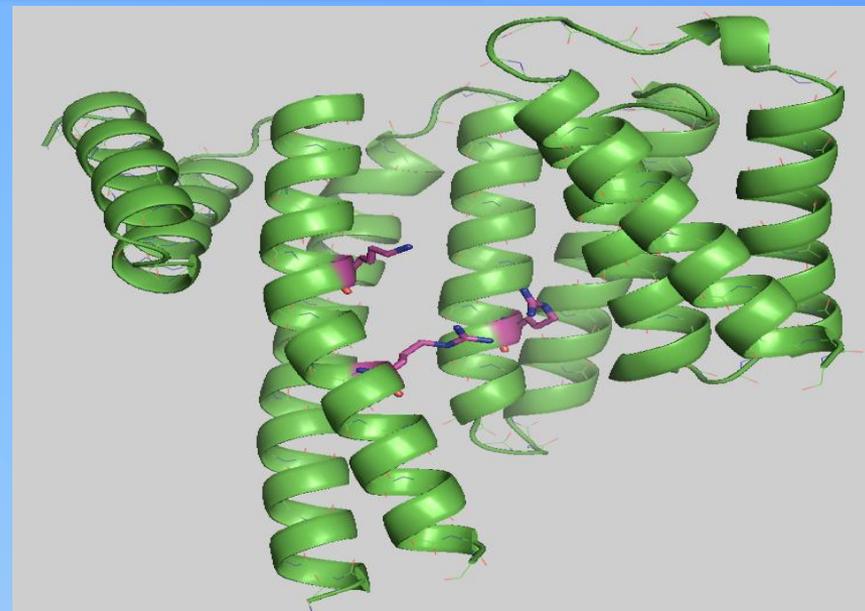
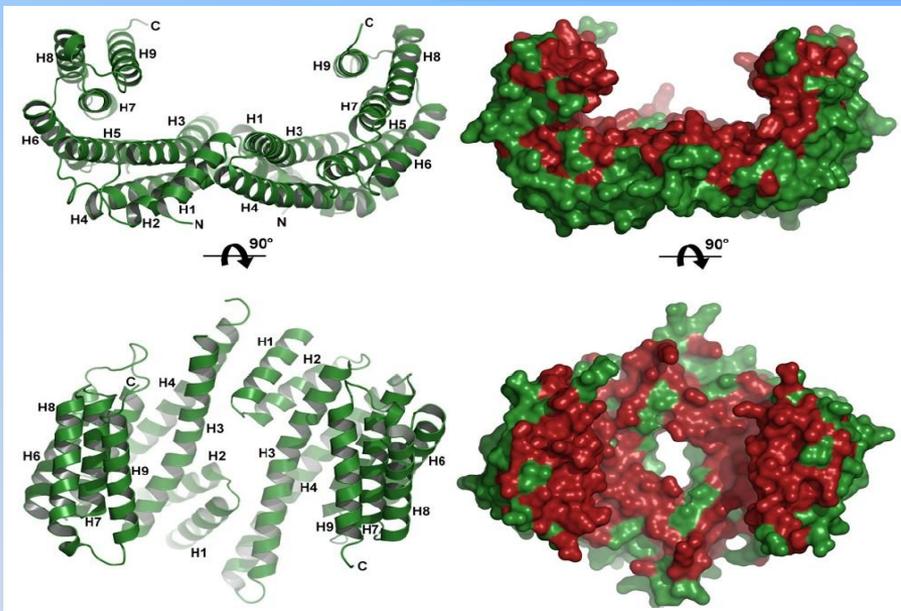
anti-apoptosis



Invade and migrate

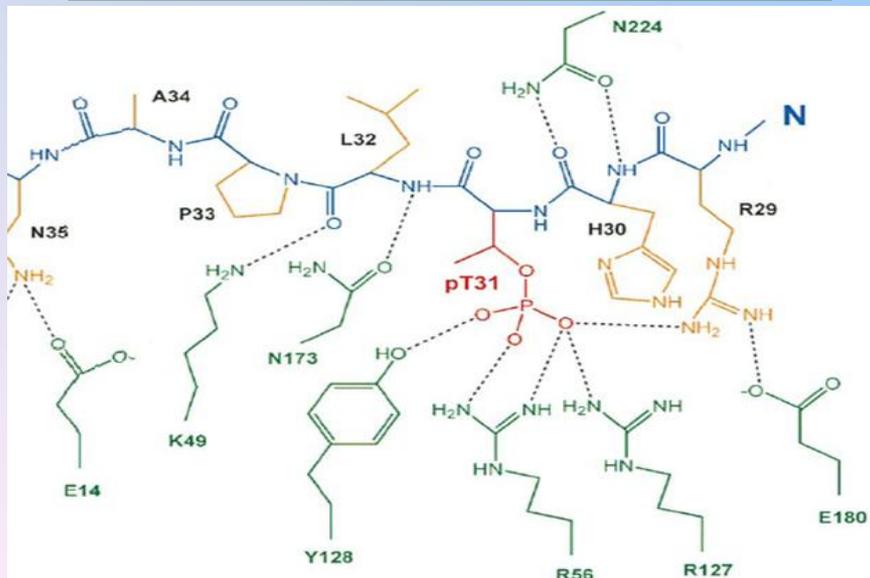
Alyson K. Freeman, et al. 14-3-3 Proteins: Diverse functions in cell proliferation and cancer progression. *Seminars in Cell & Developmental Biology*;2011,22:681-687.

# Structure of 14-3-3zeta



RSxpS/TxP OR RxxxpS/TxP

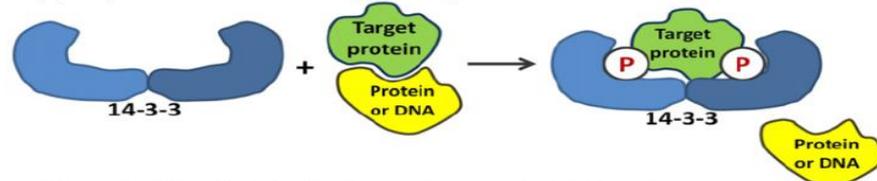
Sites: K49 R56 R127



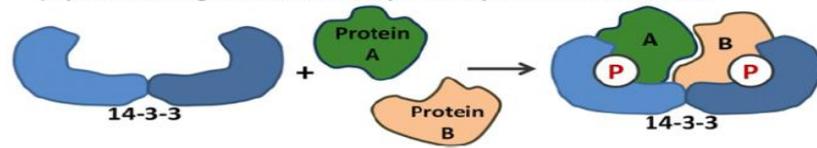
(i) Direct structural change of the target protein



(ii) Physical occlusion of sequence-specific or structural features



(iii) Scaffolding that facilitates protein-protein interactions

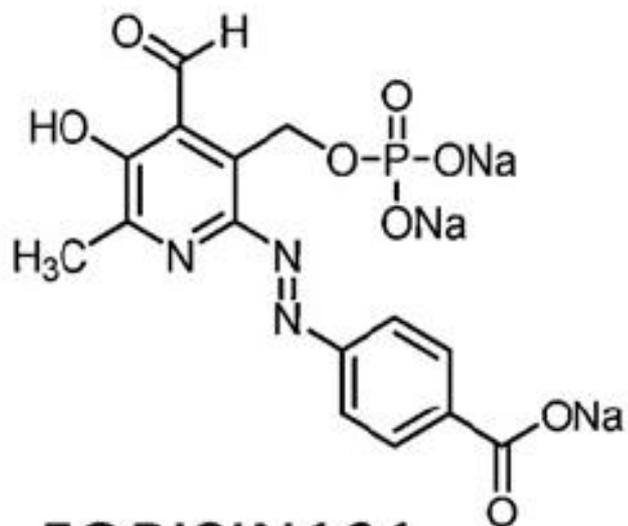


# 14-3-3ζ inhibitors

## Peptide antagonists

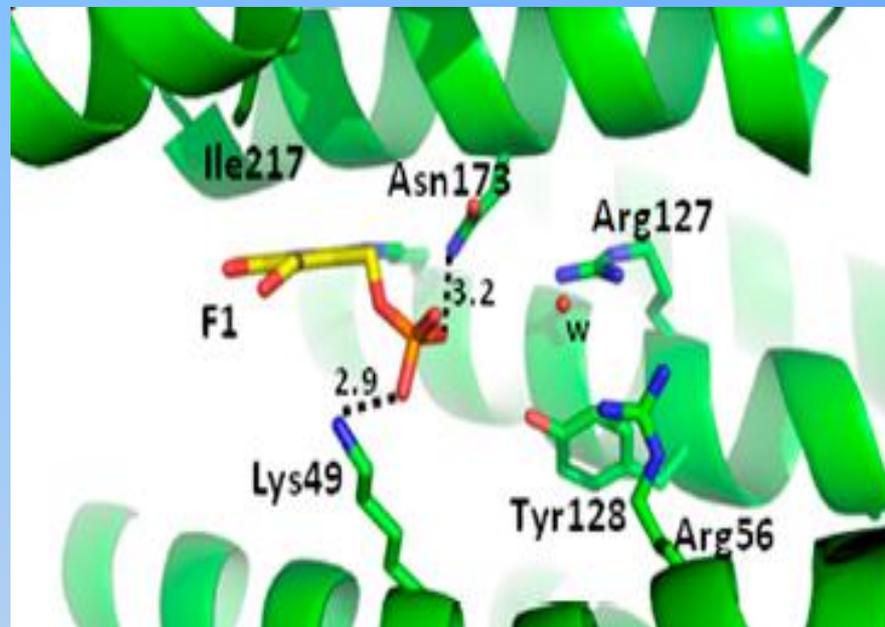
**R18:** PHCVPRDLSWLDLEANMCLP

**Difopein:** dimeric fourteen-three-three peptide inhibitor



**FOBISIN101**

**IC<sub>50</sub> : 10 μM**



van der Waals contact: Ile217

solvent molecule bridges: Arg56 and Arg127

# 流程

实验筛选获得14-3-3zeta抑制活性的抑制剂小分子



小分子衍生物模型由maestro软件通过计算化学方法生成



分子对接: Autodock4.2



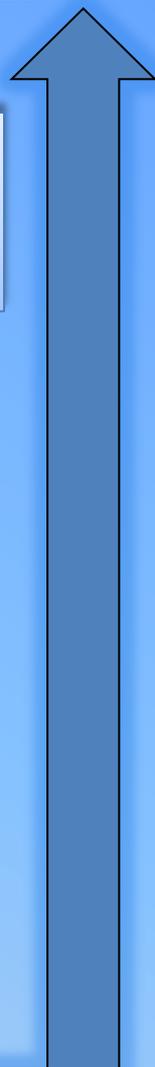
作用力分析: Poseview



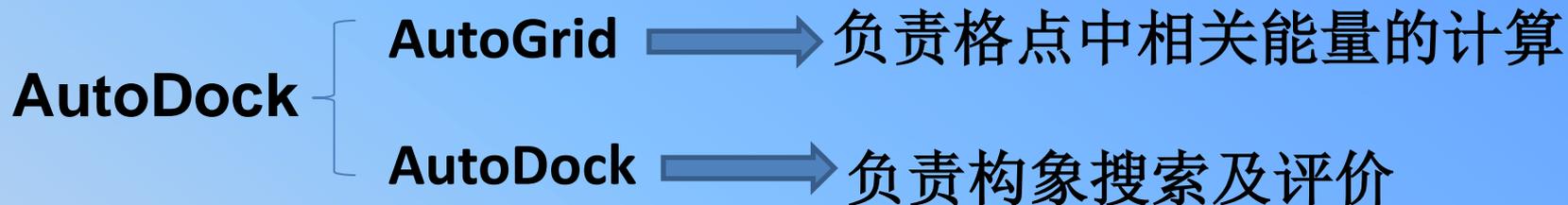
结果分析, 进一步改造, 以提高抑制活性



实验验证



# 分子对接过程



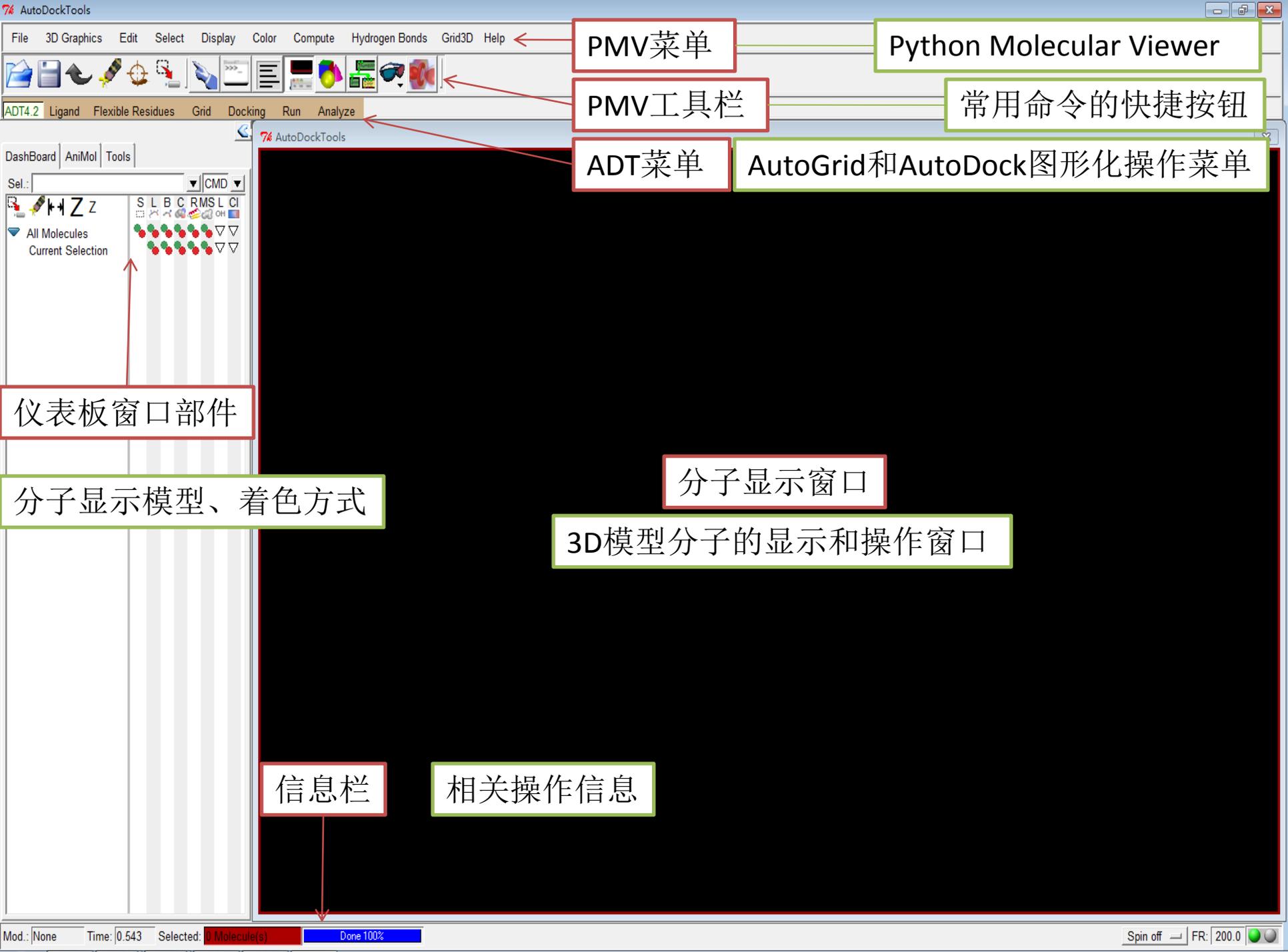
准备配体、受体分子

围绕受体活性位点的氨基酸残基形成一个范围更大的Box

用不同类型的原子作为探针进行扫描，计算格点能量

对配体在Box范围内进行构象搜索，根据配体不同构象，按方向、位置及能量进行评分，最后对结果进行排序

结果分析：找到正确对接构象，分析结果



PMV菜单

Python Molecular Viewer

PMV工具栏

常用命令的快捷按钮

ADT菜单

AutoGrid和AutoDock图形化操作菜单

仪表盘窗口部件

分子显示模型、着色方式

分子显示窗口

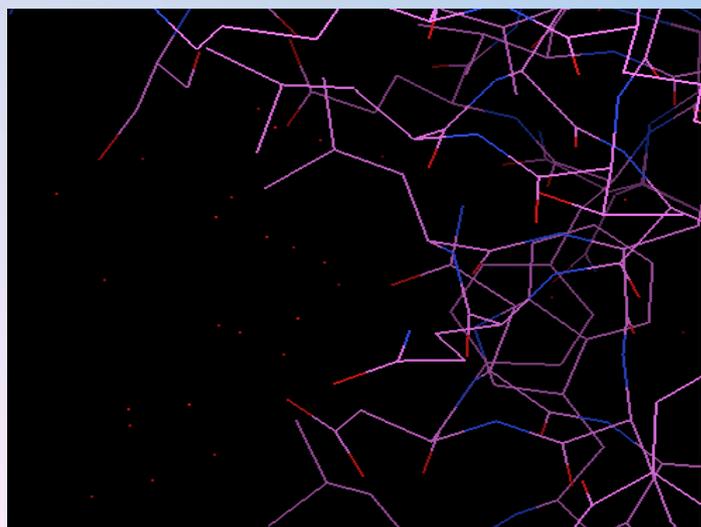
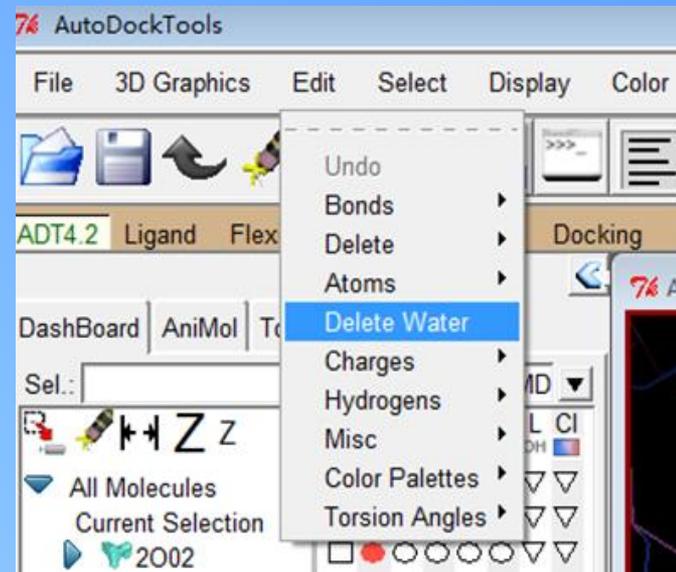
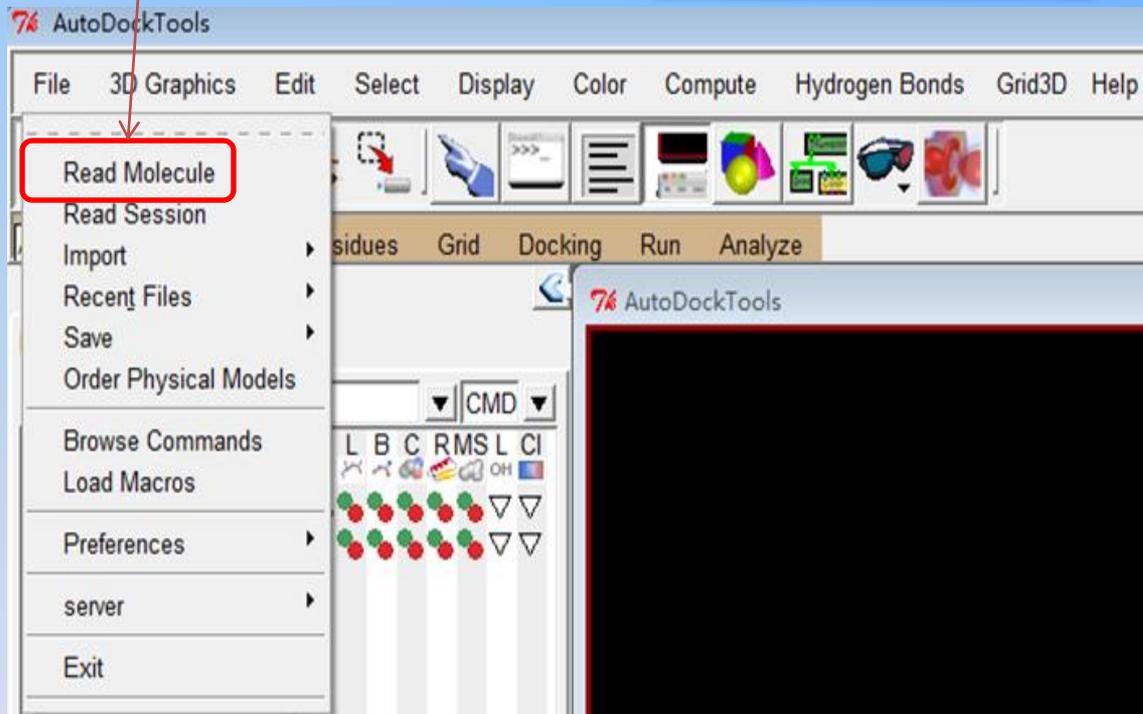
3D模型分子的显示和操作窗口

信息栏

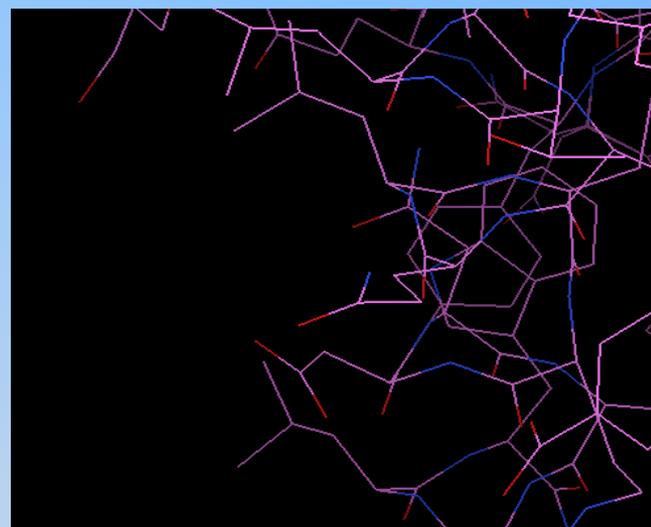
相关操作信息

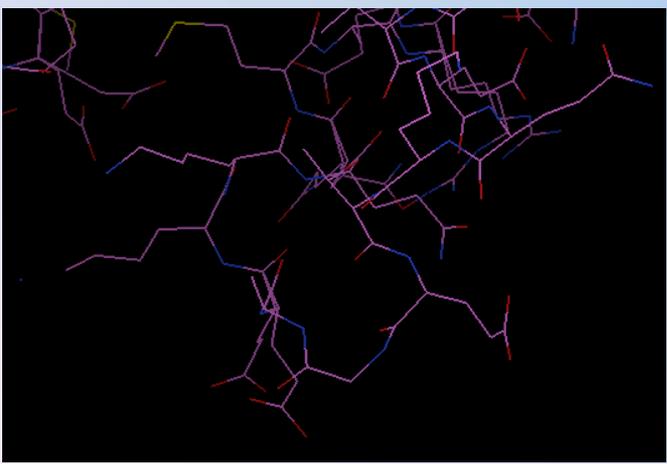
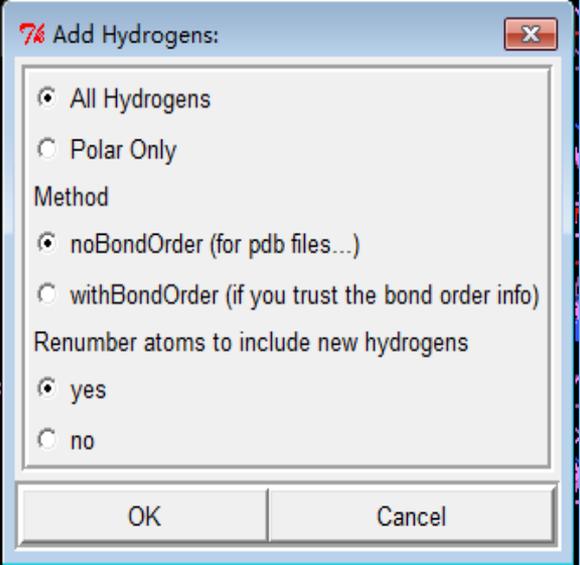
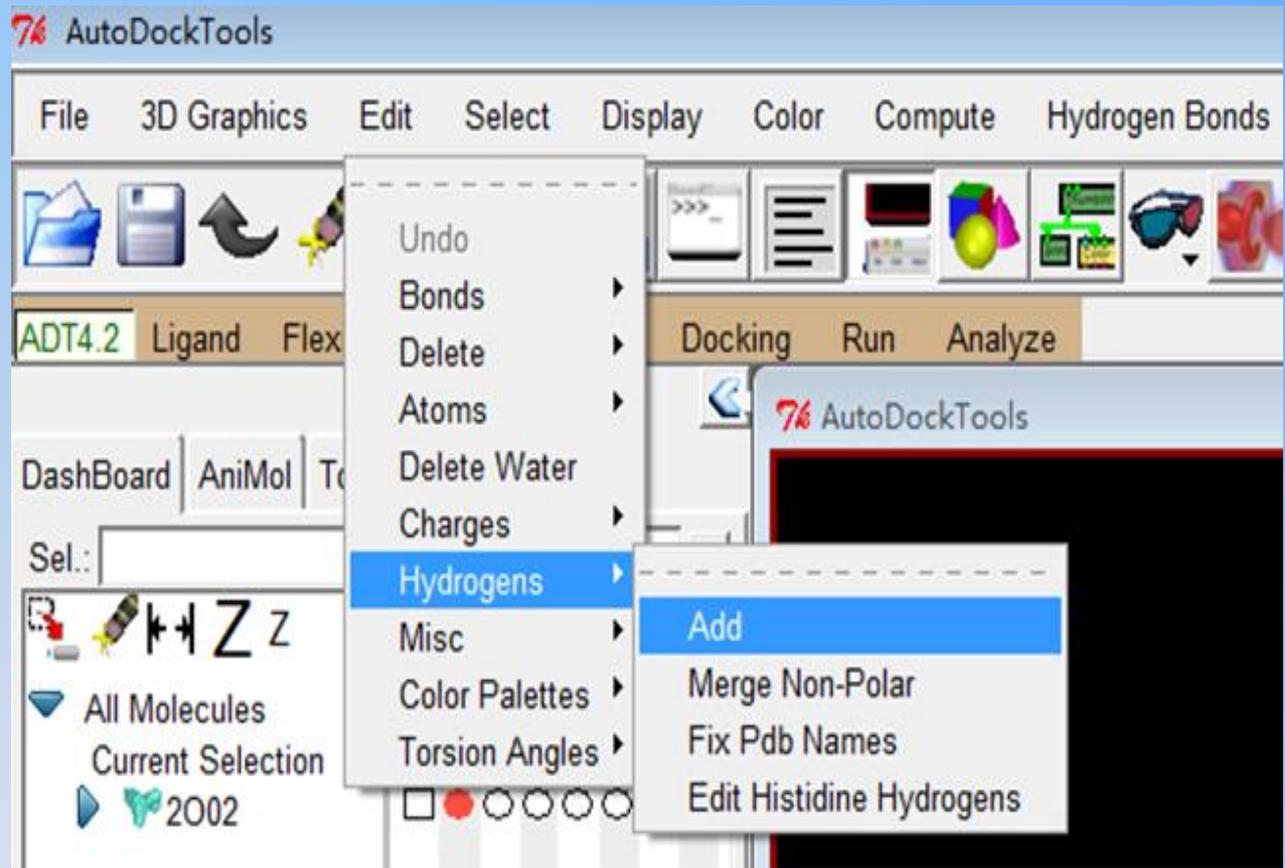
读取分子

# 准备受体

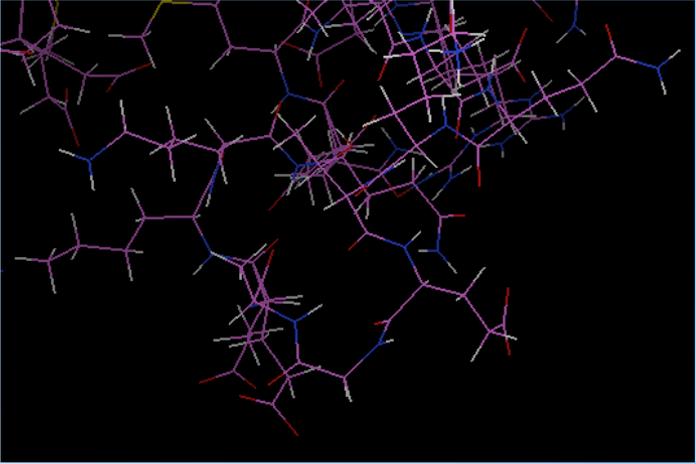


删除水分子

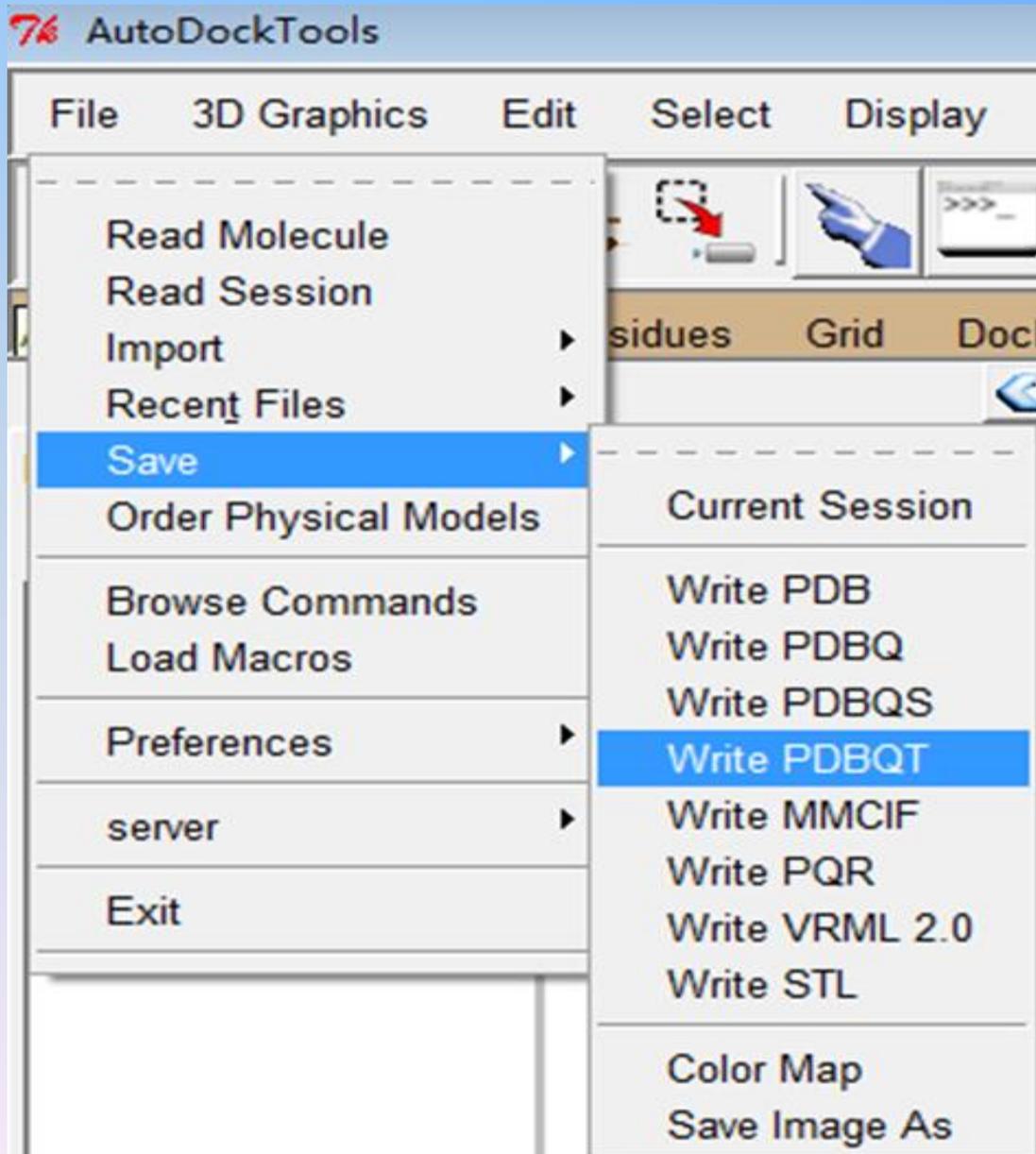




为受体分子加H



## 保存受体分子为PDBQT格式



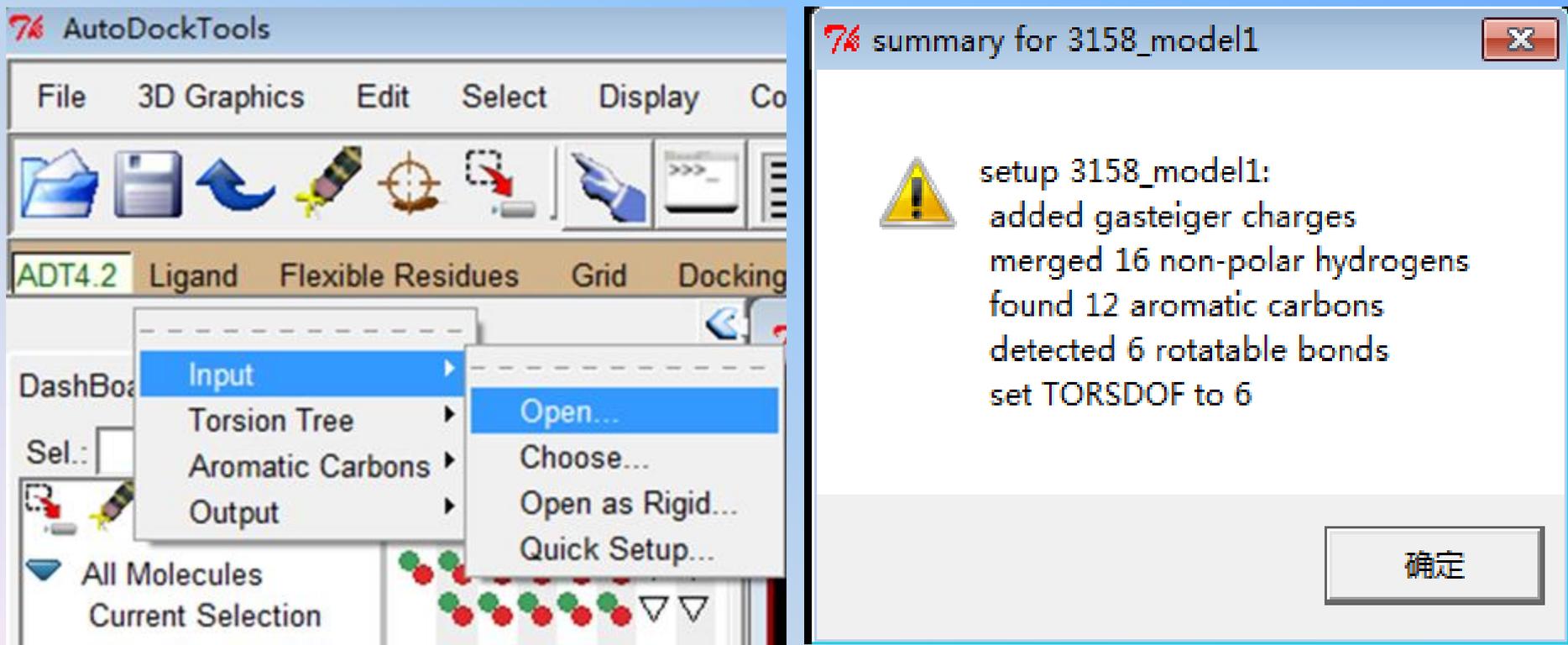
后续受体和配体的操作都需要利用PDBQT格式文件

# 准备配体

ADT检测Ligand分子是否已经加了电荷，自动加上Gasteiger电荷

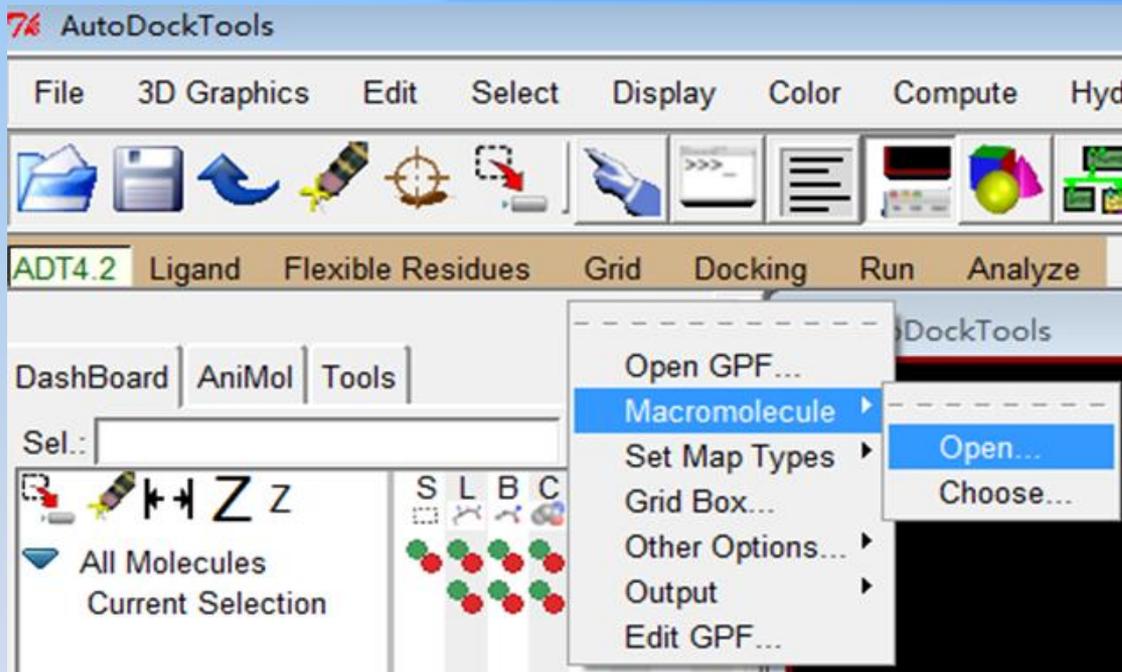
ADT检测并合并非极性的H

将Ligand中的每个原子设置为“AutoDock原子类型”

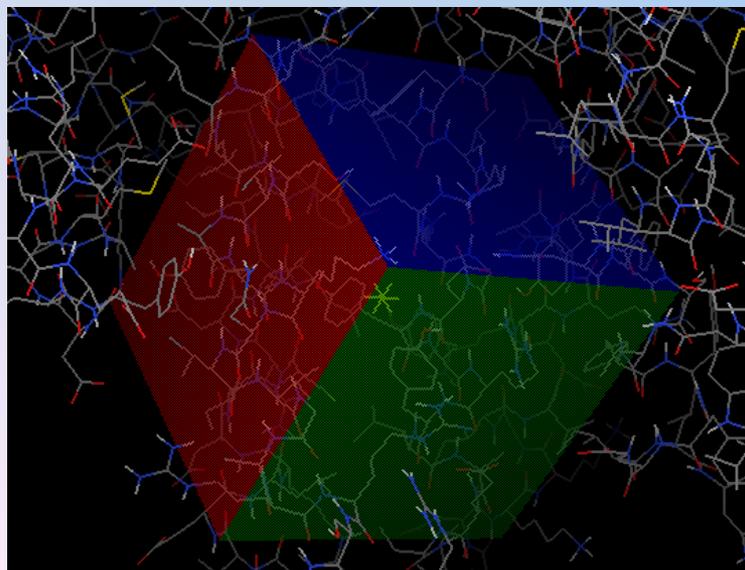


保存配体分子为PDBQT格式

# 准备AutoGrid参数文件

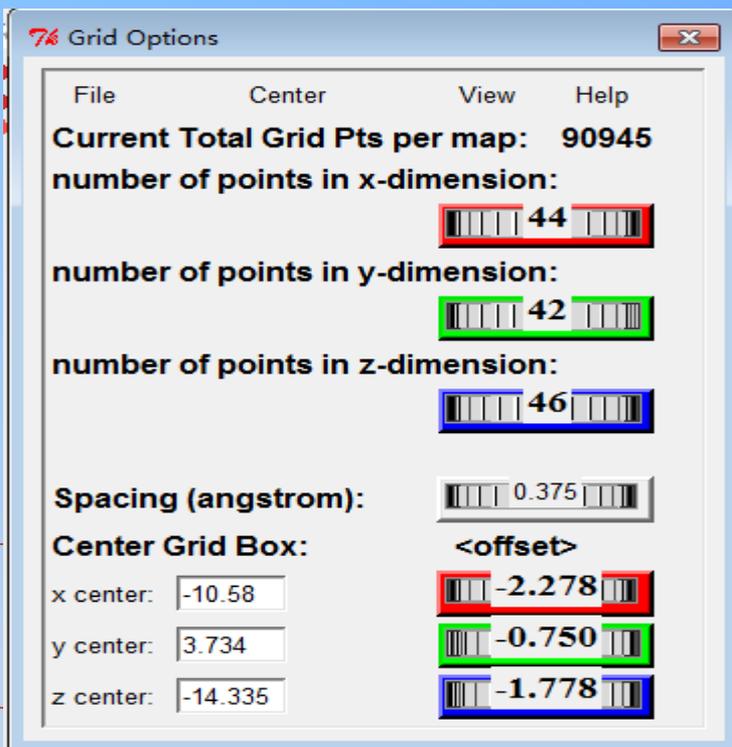


ADT检测受体分子是否已经加了电荷，自动加上Gasteiger电荷

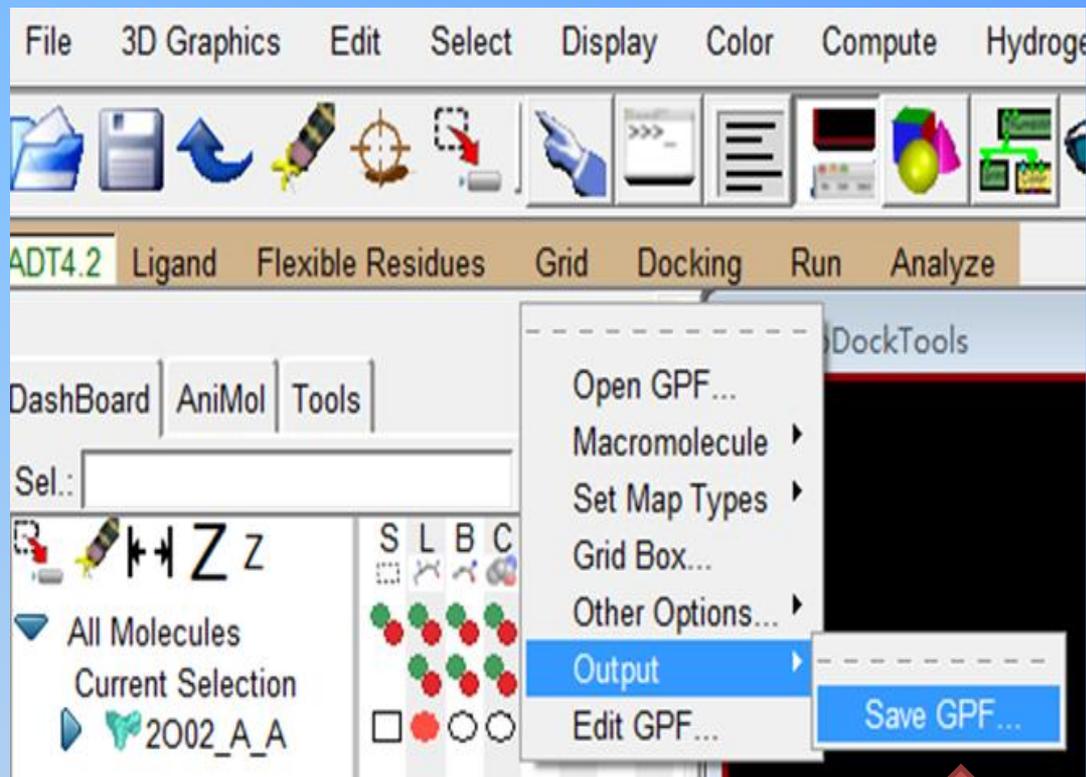
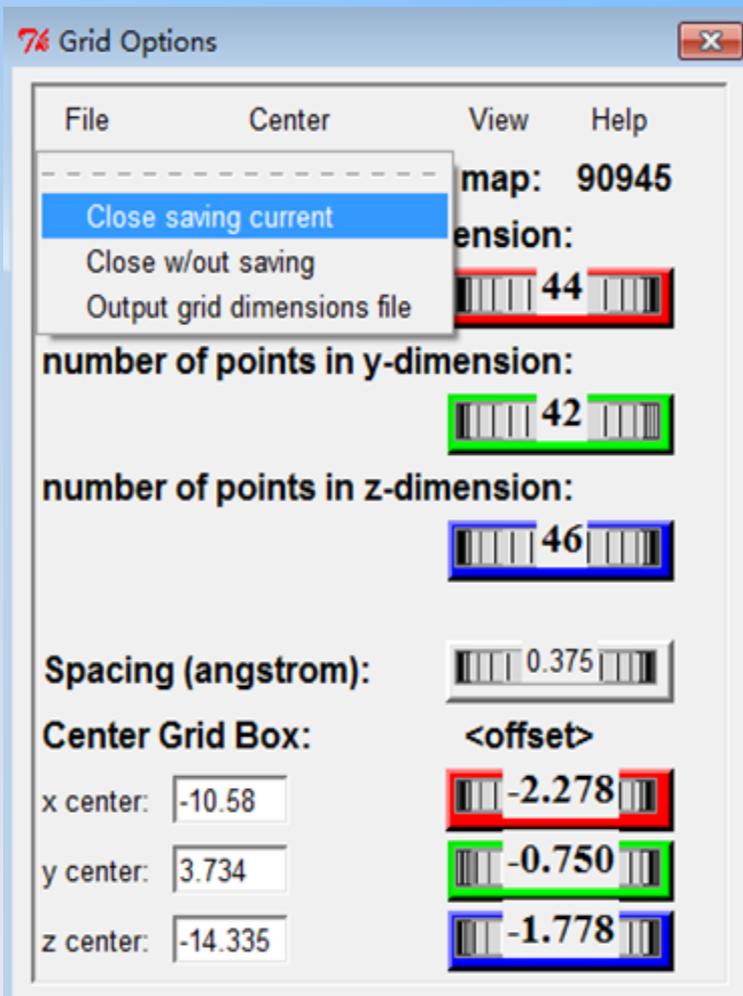


box大小

box位置



# 设置好的Grid参数保存成GPF文件

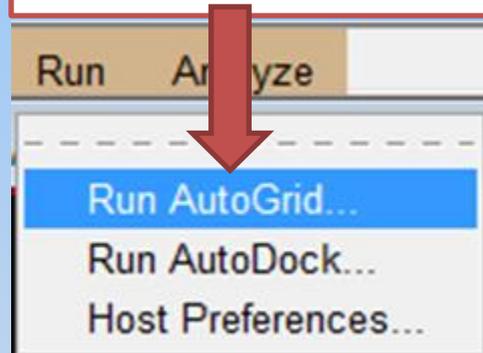


生成GPF文件

GPF文件用于运行Autogrid

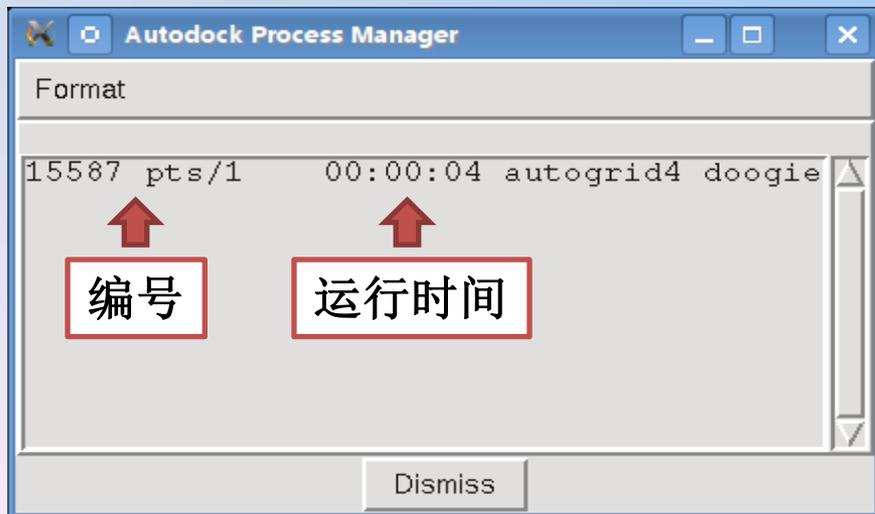
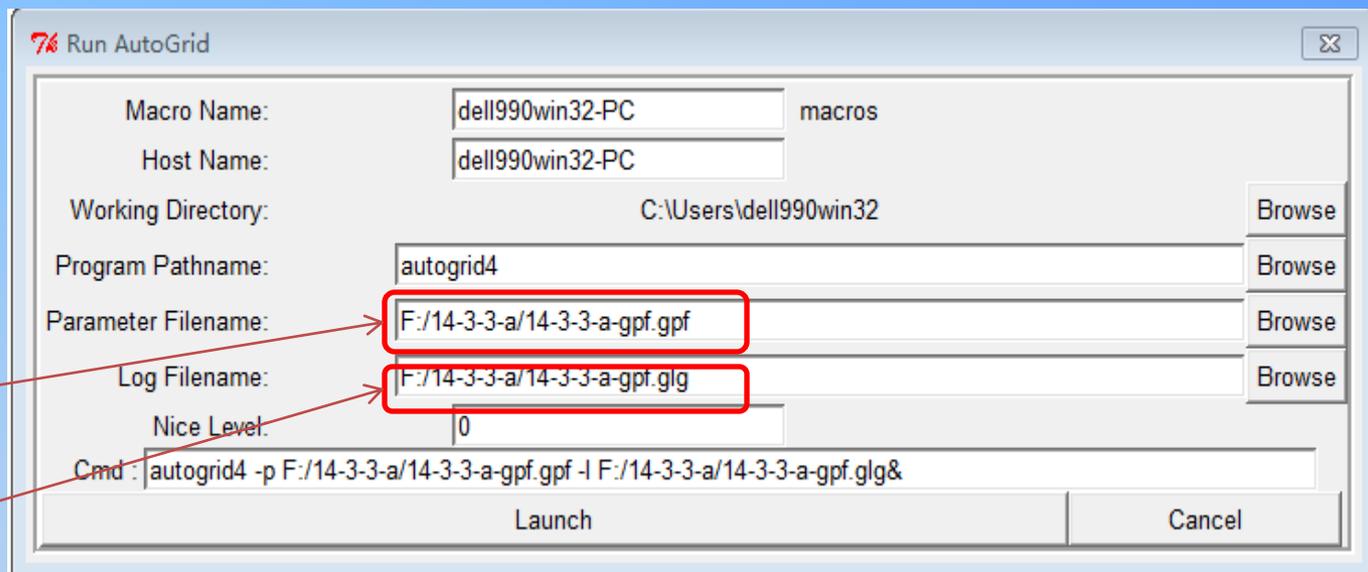
# 运行AutoGrid

运行程序进行计算



已经生成的GPF文件

结果保存为GLG文件

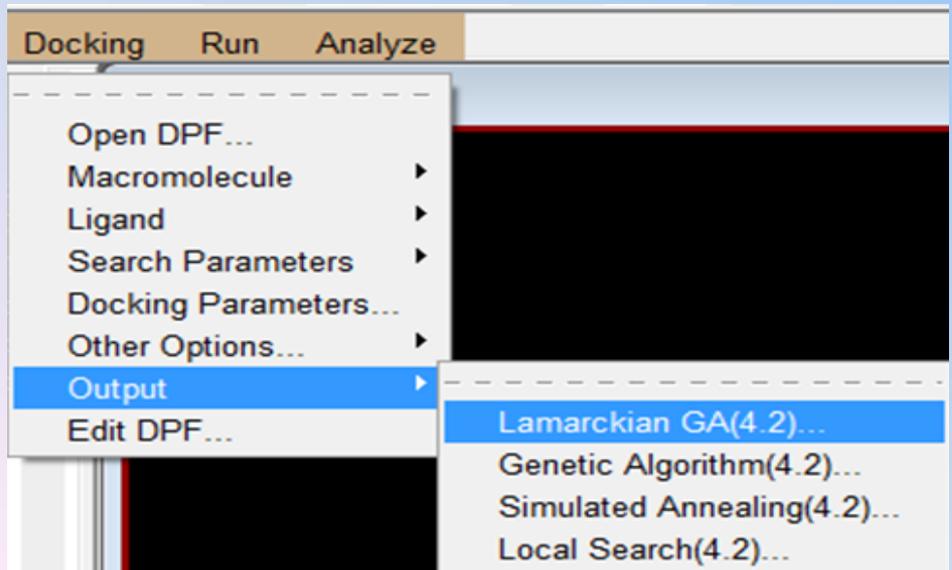
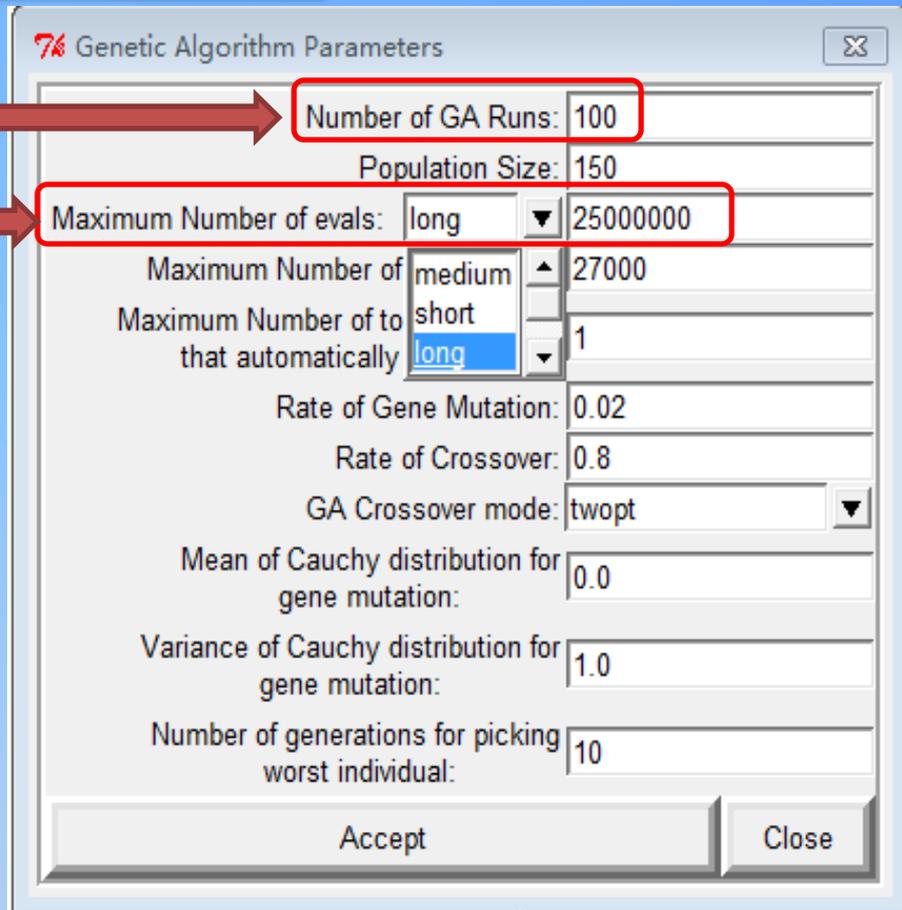
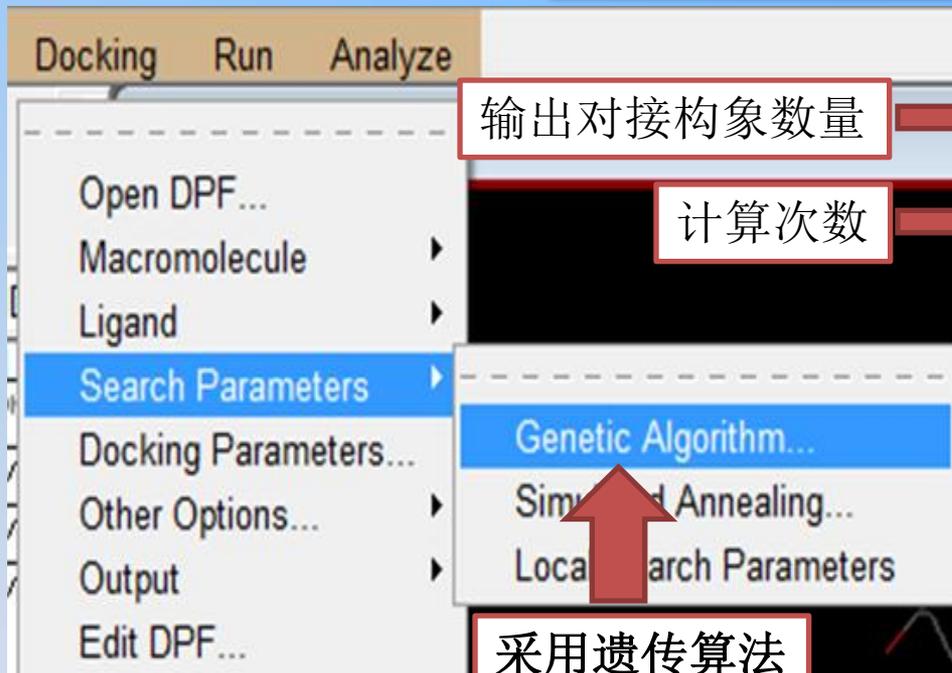


AutoDock Process Manager

显示进程编号、运行时间及状态

运行得到针对不同原子探针的范德华作用力、静电力等作用力的Map文件

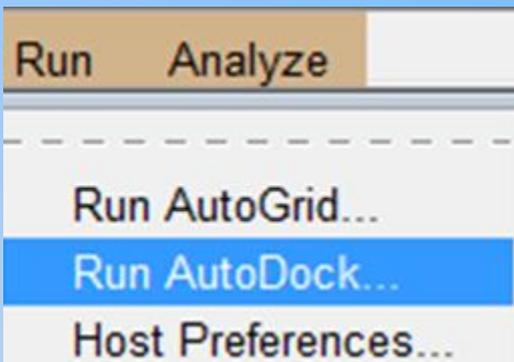
# 准备Dock参数文件



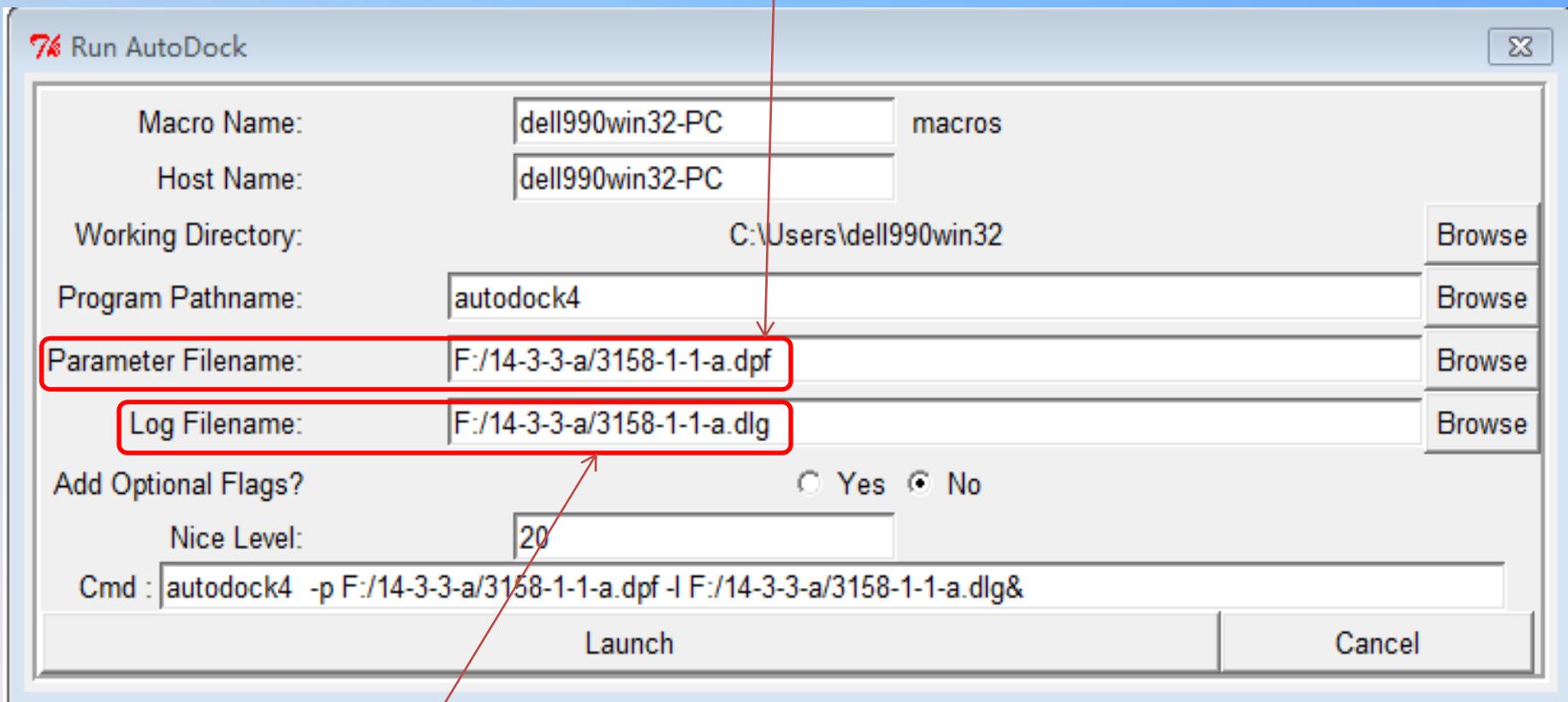
设置遗传算法搜索参数

生成DPF文件

# 运行AutoDock



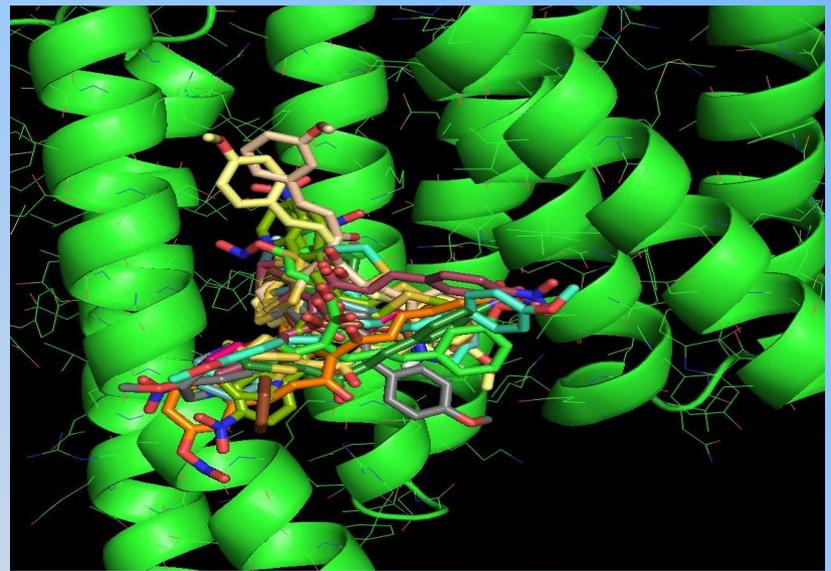
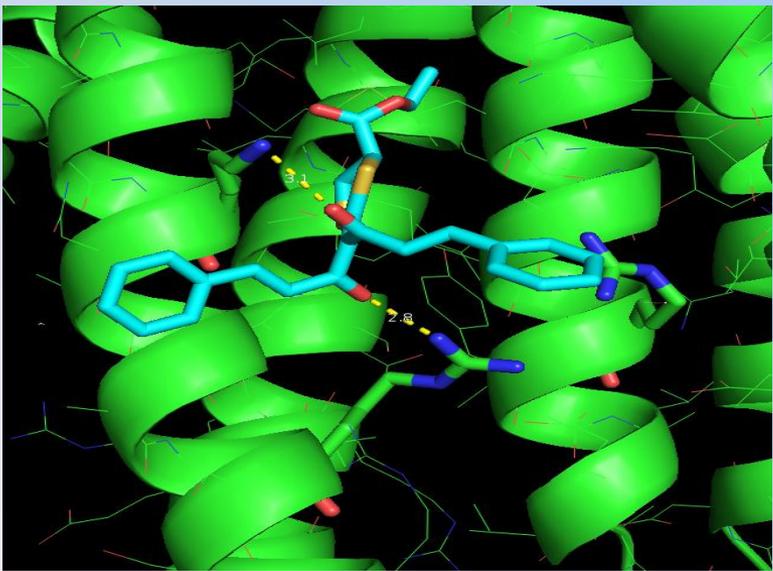
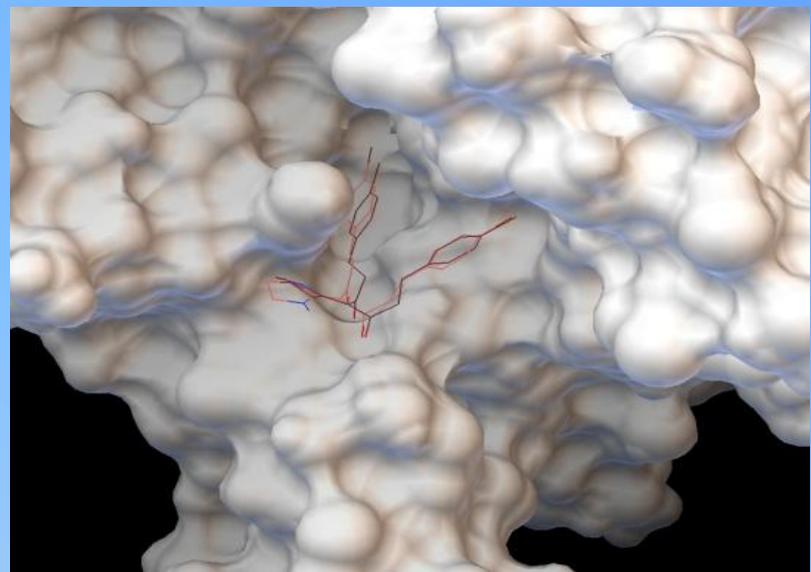
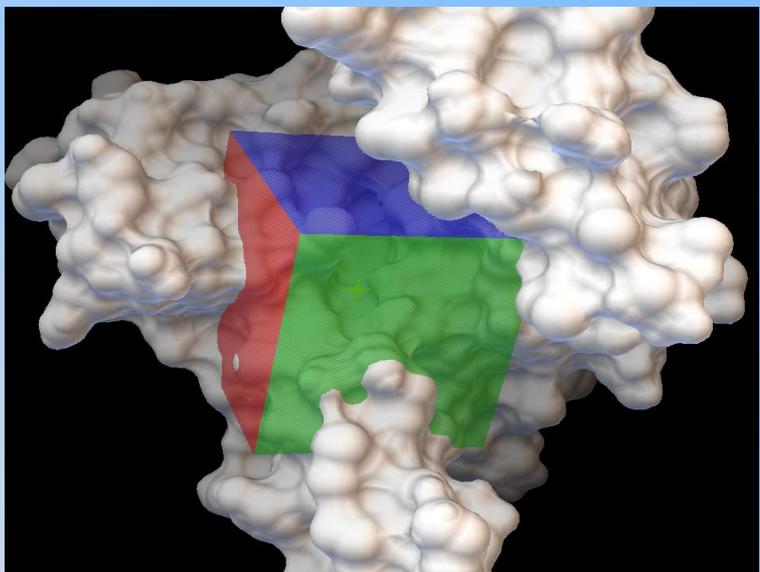
已经生成的DPF文件



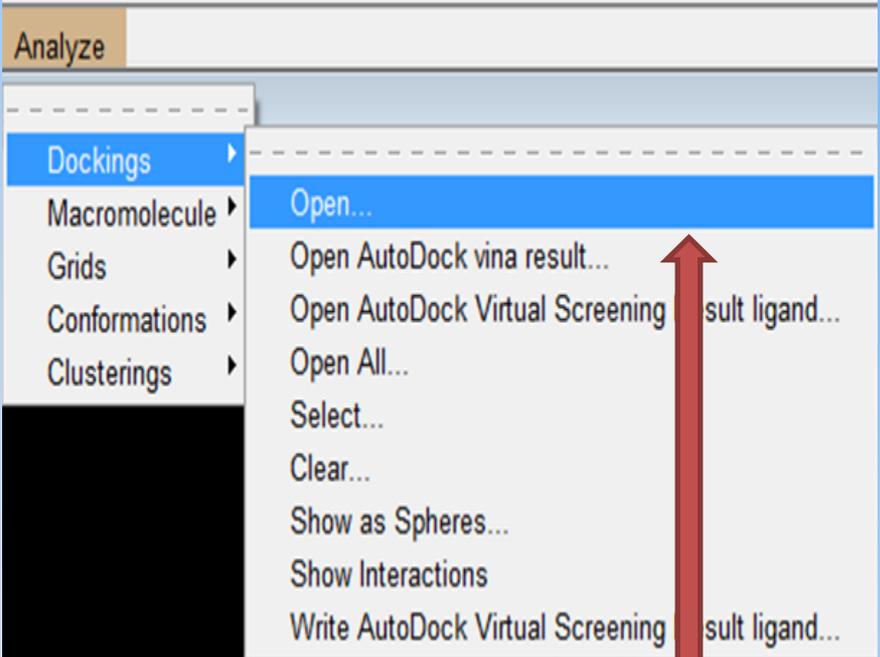
结果保存为DLG文件

将记录对接构象、对接能量、作用力等信息

# 对接结果

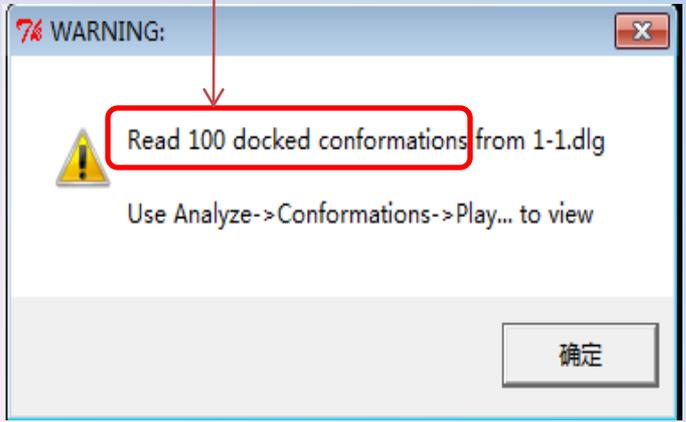


# 详细分析



100个对接结果

打开Dlg 对接文件



7% 1-1 Conformation Chooser

Rank: 1.1	
Binding Energy: -6.99	结合能
kl: 7.52uM	抑制活性
Intermolecular Energy: -7.7	
Internal Energy: -0.93	
Torsional Energy: 1.65	
Unbound Extended Energy: 0.0	
Cluster RMS: 0.0	
Ref RMS: 20.43	

select from 100 dockings:  
(double click to update coords)  
(Rank SubRank docked energy)

Rank	SubRank	docked energy
		/home/mdl/rfzhang/12yue4ri/3158_1_1_model1 input
		/home/mdl/rfzhang/12yue4ri/3158_1_1_model1 1_1 -6.99
		/home/mdl/rfzhang/12yue4ri/3158_1_1_model1 1_2 -6.93
		/home/mdl/rfzhang/12yue4ri/3158_1_1_model1 1_3 -6.73
		/home/mdl/rfzhang/12yue4ri/3158_1_1_model1 1_4 -6.67
		/home/mdl/rfzhang/12yue4ri/3158_1_1_model1 1_5 -6.67
		/home/mdl/rfzhang/12yue4ri/3158_1_1_model1 1_6 -6.6
		/home/mdl/rfzhang/12yue4ri/3158_1_1_model1 1_7 -6.58
		/home/mdl/rfzhang/12yue4ri/3158_1_1_model1 1_8 -6.53
		/home/mdl/rfzhang/12yue4ri/3158_1_1_model1 1_9 -6.53

对接构象按能量排序

# 详细分析

Analyze

Dockings

Macromolecule

Grids

Conformations

Clusterings

Open...

Open AutoDock vina result...

Open AutoDock Virtual Screening Result ligand...

Open All...

Select...

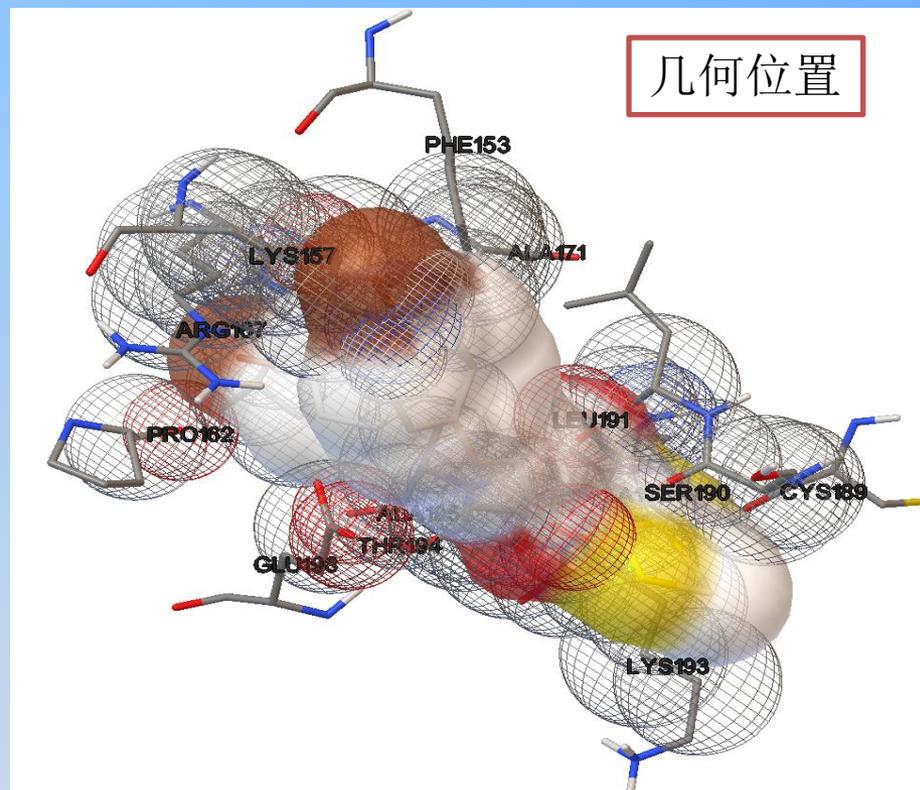
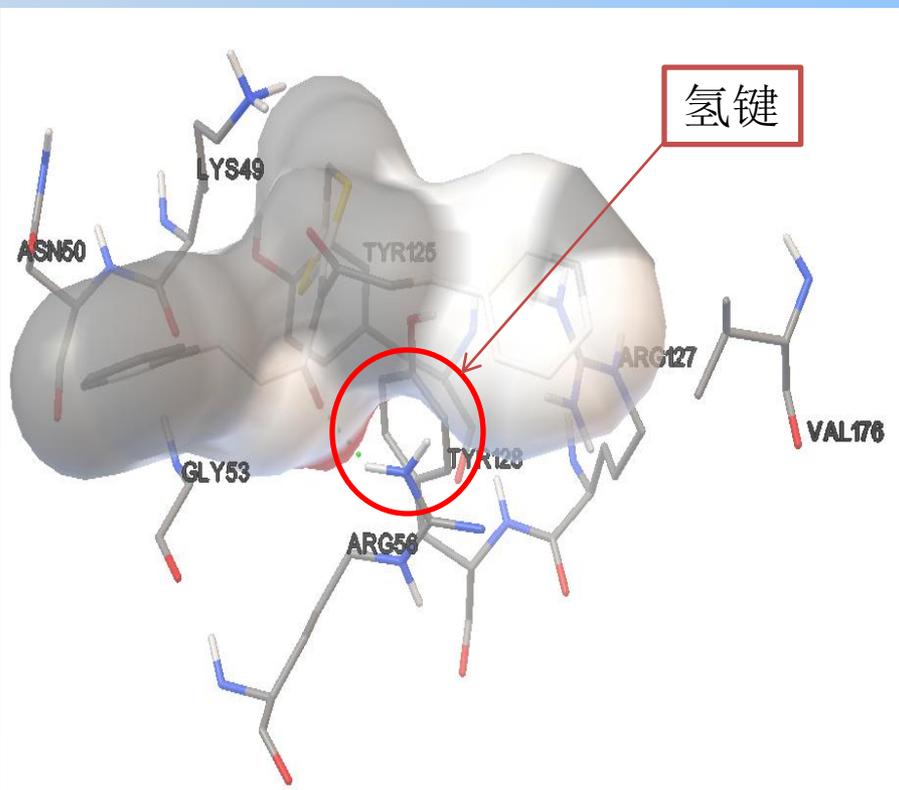
Clear...

Show as Spheres...

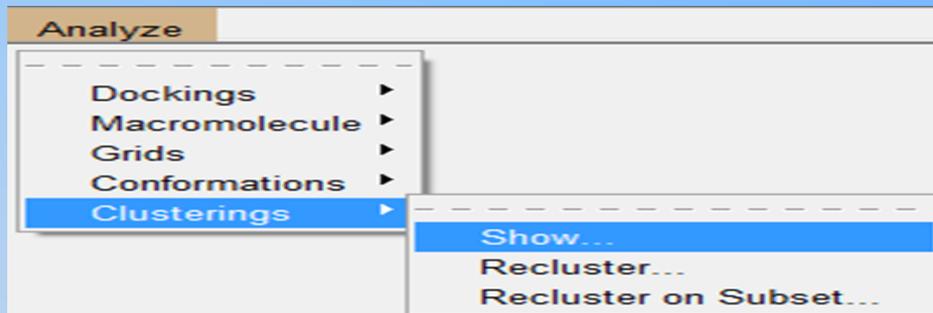
Show Interactions

Write AutoDock Virtual Screening Result ligand...

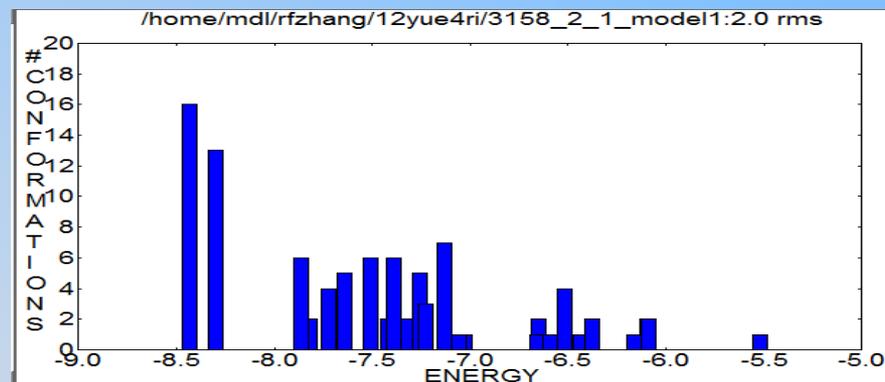
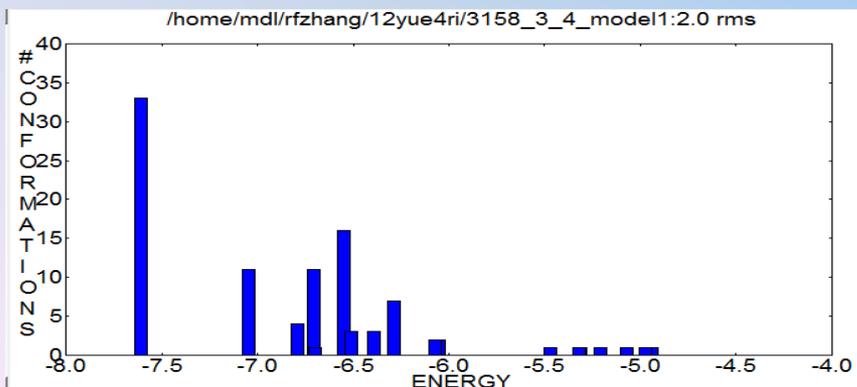
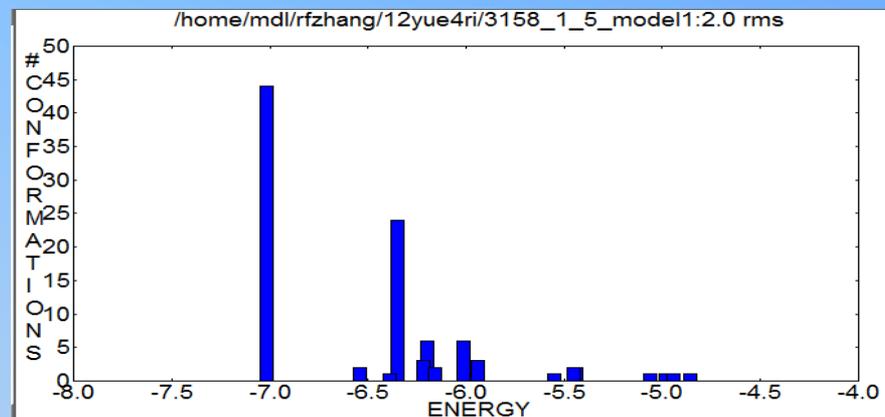
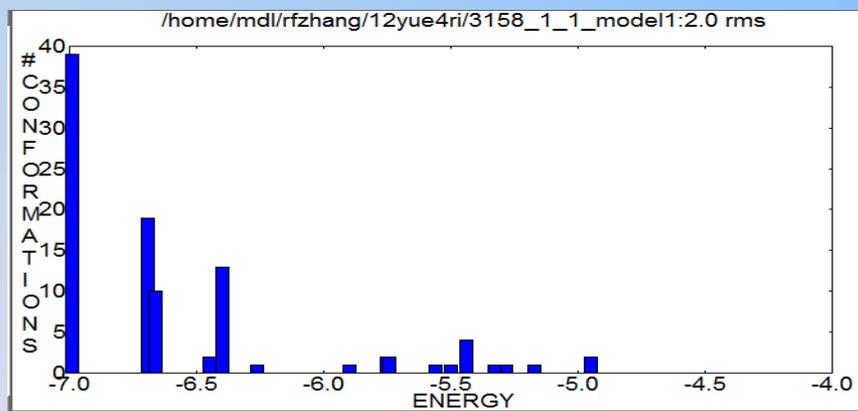
相互作用力分析

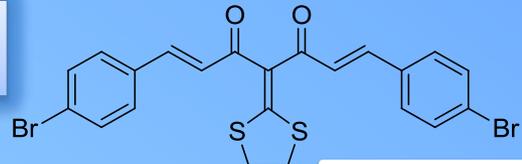


# 聚类构象

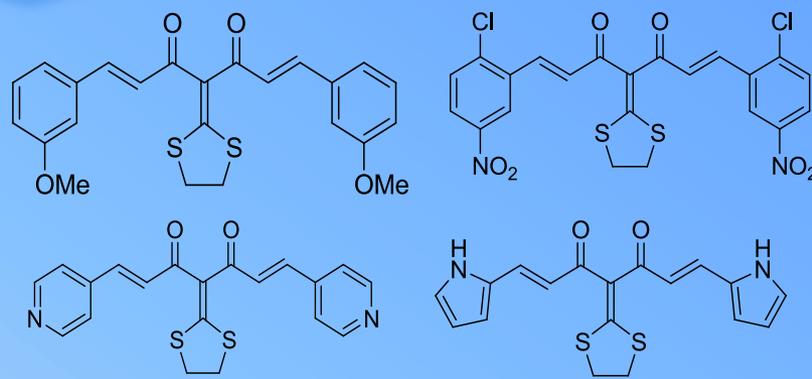
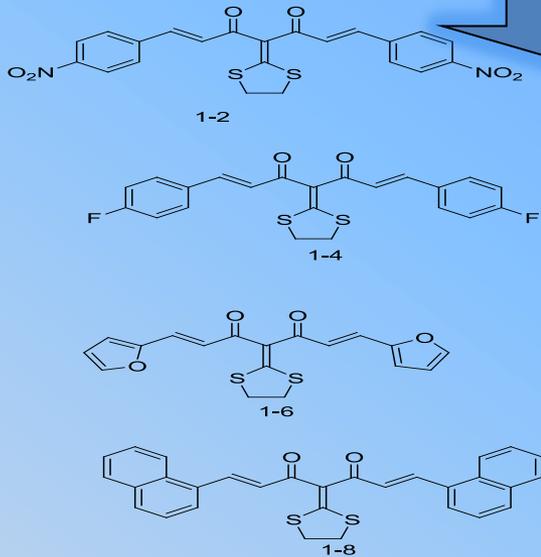
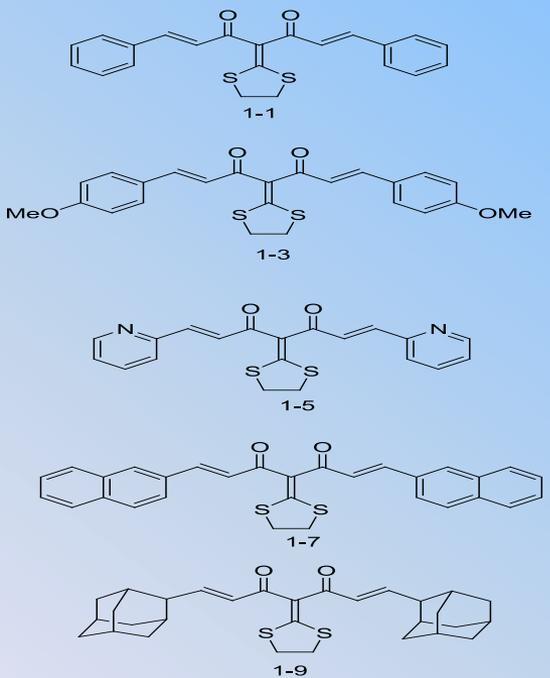


ADT可以将对接结果相似的分子构象进行聚类，这样会极大的方便对不同对接结果的分析 and 比较。

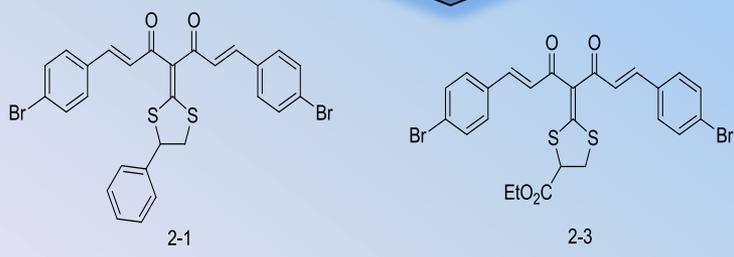




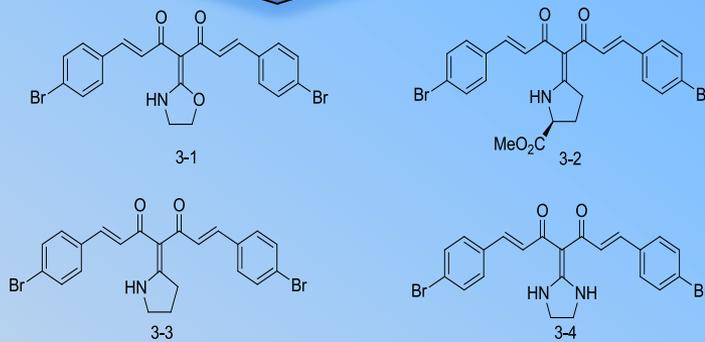
芳环取代基的衍生化



杂环上取代基的改造



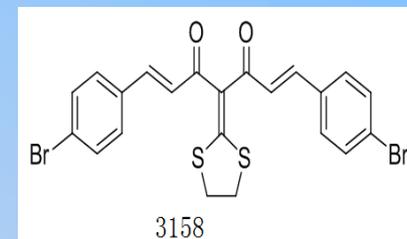
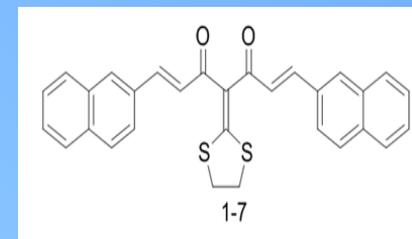
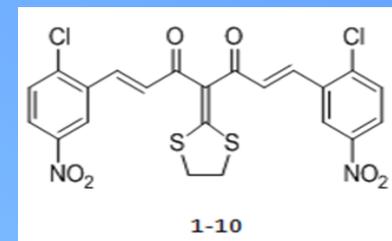
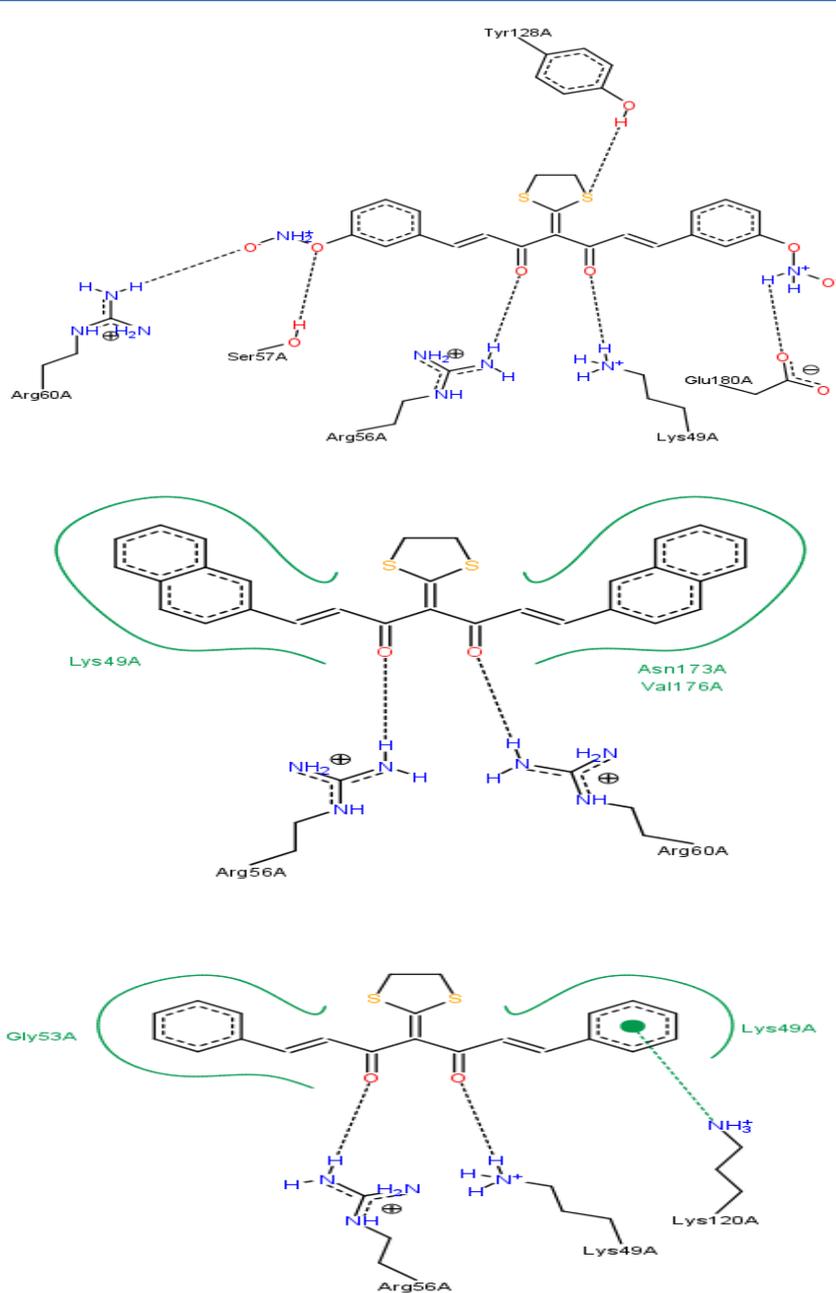
杂环的改造



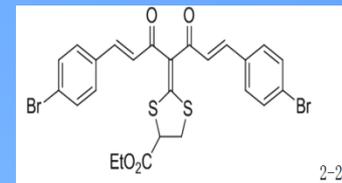
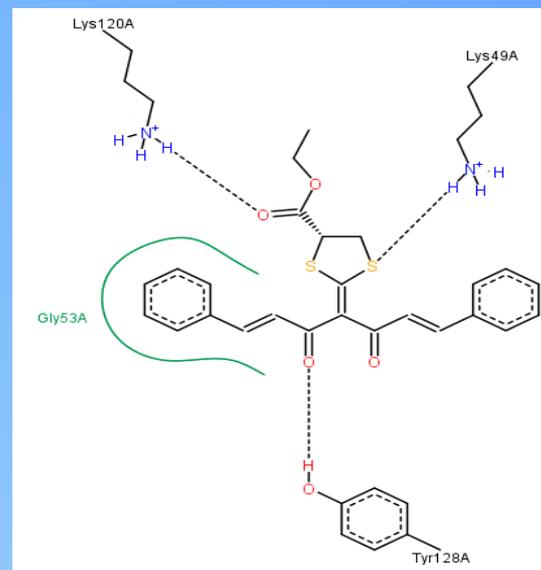
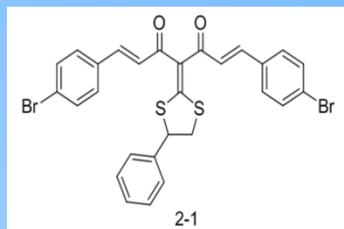
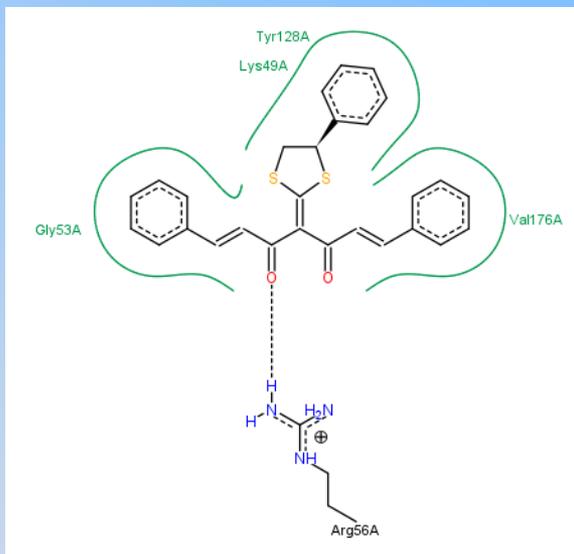
# 抑制活性

芳环取代基的衍生化抑制活性: 1-10 > 1-7 > 1-8 > 3158 > (1-1,1-2,1-5,1-9) > 1-3 > (1-6,1-11) > (1-4,1-12)

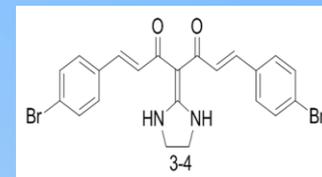
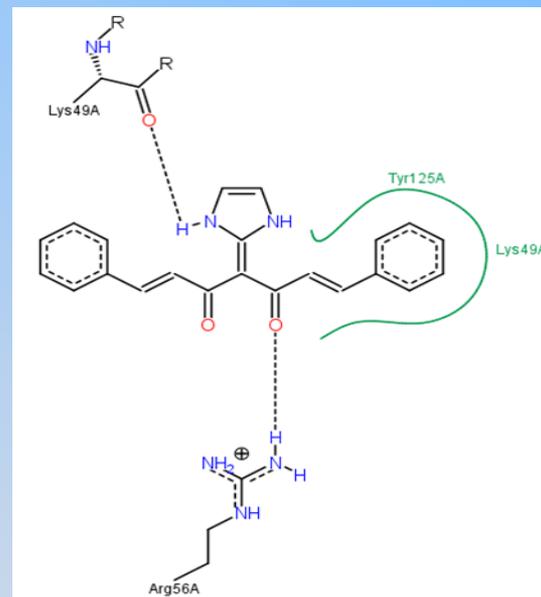
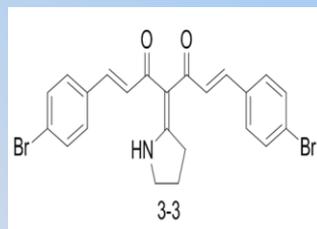
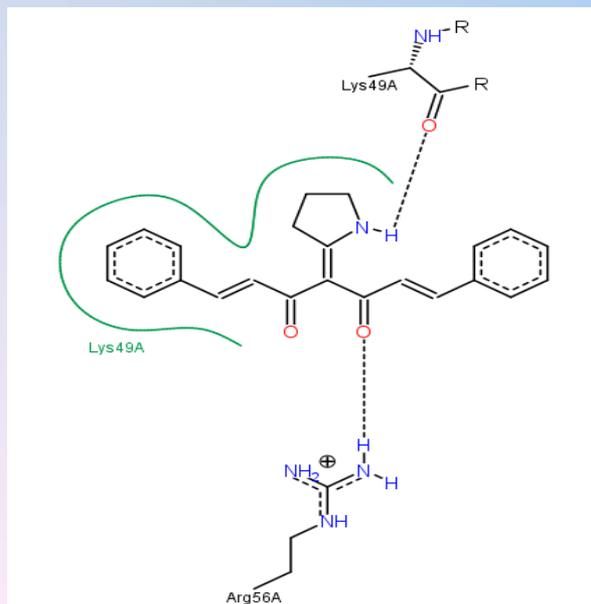
小分子	Binding energy	KI uM
3158	-7.25	4.82
1-1	-6.99	7.52
1-2	-6.99	7.51
1-3	-6.74	11.47
1-4	-6.25	26.08
1-5	-7.02	7.19
1-6	-6.57	15.38
1-7	-8.29	0.838
1-8	-7.96	1.46
1-9	-6.98	7.65
1-10	-8.31	0.816
1-11	-6.5	17.18
1-12	-6.2	28.55
2-1	-8.43	0.659
2-2	-7.38	3.91
3-1	-7.51	3.1
3-2	-7.82	1.85
3-3	-8.05	1.06
3-4	-7.61	2.46



杂环上取代基改造的两个分子抑制活性：2-1 > 2-2 > 3158

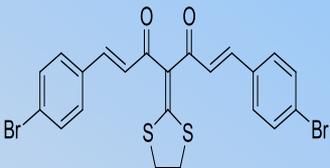


杂环改造的四个分子抑制活性：3-3 > 3-2 > 3-4 > 3-1 > 3158



# 结论

## 抑制活性

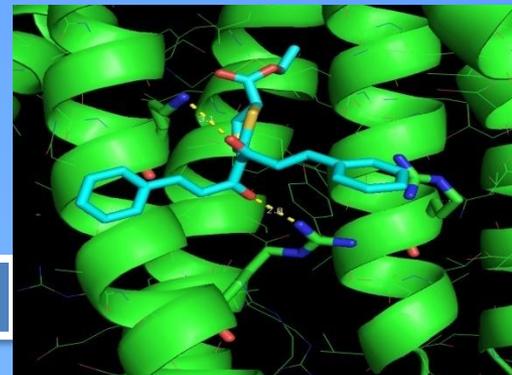


1 羰基氧与Arg56形成的氢键

2 两个芳香环与蛋白受体疏水作用

3 芳香环侧链及杂环与蛋白受体形成的氢键

4 杂环的疏水性

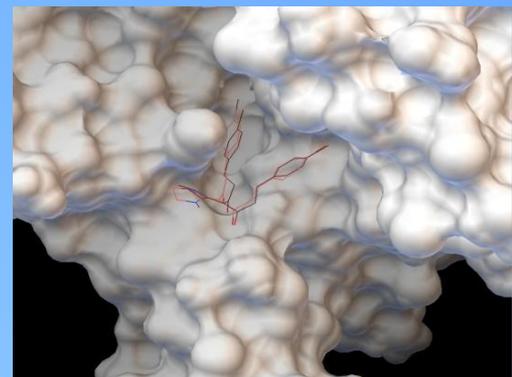


## 提高抑制活性

保留羰基氧

改变芳香环的疏水性和成氢键能力，疏水性更有效

杂环中引入氢键



细胞实验，证实部分小分子抑制活性

SPR实验，检测到部分小分子 $IC_{50} < 1\mu M$

比已报道小分子抑制剂FOBOSIN101 10 $\mu M$ 的抑制活性高10倍以上

## 意义

研究14-3-3zeta蛋白相关调控过程的研究提供手段

为癌症治疗提供新策略

# 总结

## 分子对接简介

## Autodock 使用过程及实例

研究蛋白激活剂、抑制剂作用机制以及药物作用机制，在新药设计中有重要的作用。

# 谢谢

感谢罗老师和各位同学，这学期ABC我们一起走过，收获很多！