ITC等温滴定微量热仪使用培训

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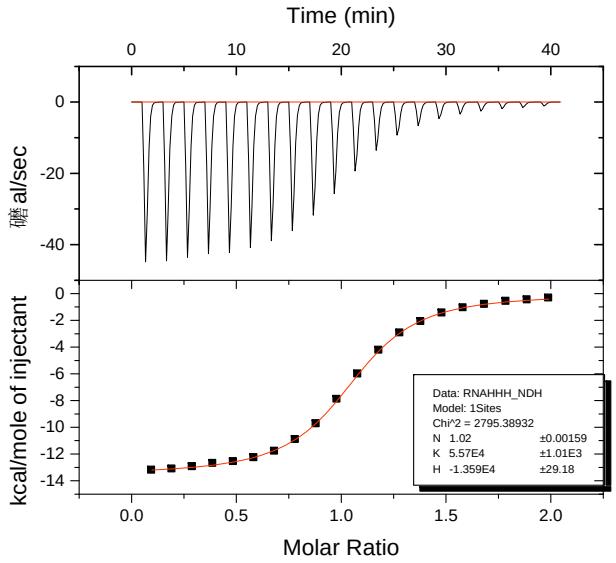
中心3台ITC仪器

仪器	噪音	样品需求量	优点
	**PEAQ-ITC** 0.15 ncal/s	滴定针60μL 样品池280μL *滴定针容量40μL 样品池容量200μL	**省时省样品** **实验操作和数据分析更简单**
	**ITC200** 0.2 ncal/s		**省时省样品** 机时费更低
	**VP-ITC** 0.2 ncal/s	滴定针0.5mL 样品池2mL *滴定针和样品池容量均大7倍	**信噪比更好** **能测出更微弱的相互作用热量**

ITC什么原理？能做什么？

ITC基本原理

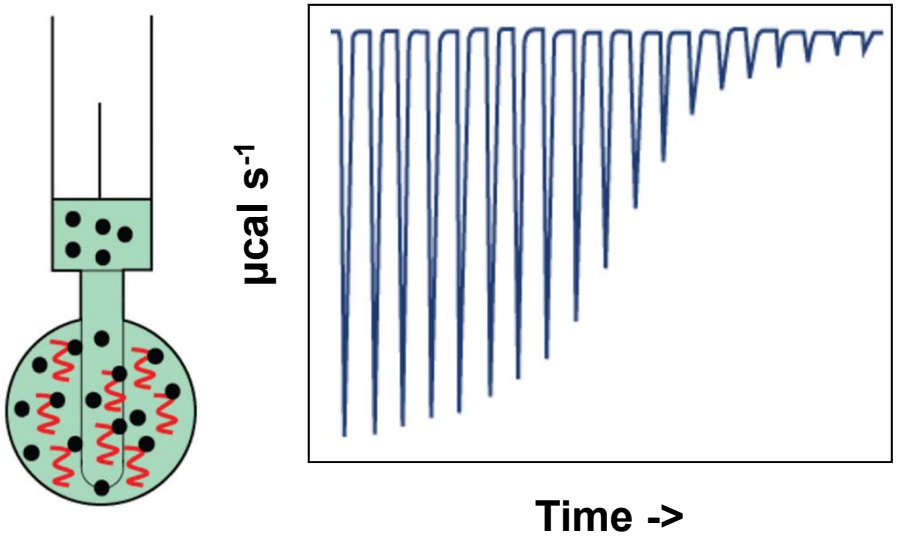


- “配体”放在滴定针中
- “大分子”放在样品池
- 测量反应热

- 单次ITC实验可获得的参数：
  - 亲和力（K_D）
  - 反应热（ΔH）
  - 结合位点的数目（N）

ITC基本原理

基于热量检测的通用技术



1. 原始数据积分；
2. 将热量进行积分并运用合适的结合模型得到：亲和力(K_D), 化学计量比 (N) 和反应焓变(ΔH);
3. 通过热力学公式，计算得到反应熵变(ΔS)。

ΔG=RT lnKD=ΔH-TΔS

K_D: Affinity
N: Stoichiometry
ΔH: Enthalpy
ΔS: Entropy

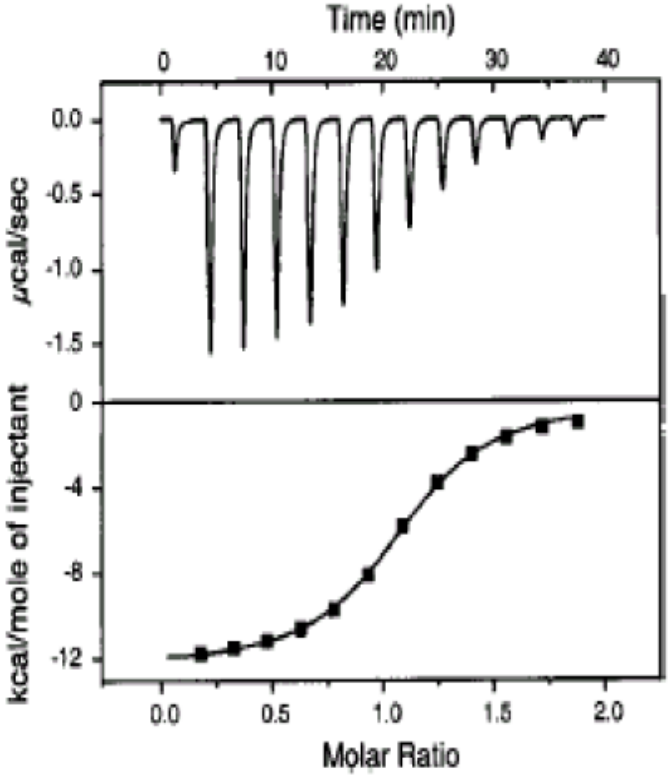
特点

- ✓ 通用的检测器
- ✓ 简单的分析设计
- ✓ 很容易操作
- ✓ **分子量范围广**
- ✓ **溶液中检测**
- ✓ **无需标记**
- ✓ 非光学检测

应用

- ■ 任意两种分子间的相互作用
  - ➢ 证实结合以及活性
  - ➢ 获得亲和力、结合比和热力学参数
- ■ 研究相互作用机制
- ■ 药物研发
  - ➢ 用热力学指导先导化合物的优化
  - ➢ 确认IC50和EC50
- ■ 酶动力学

ITC – protein small molecule interaction



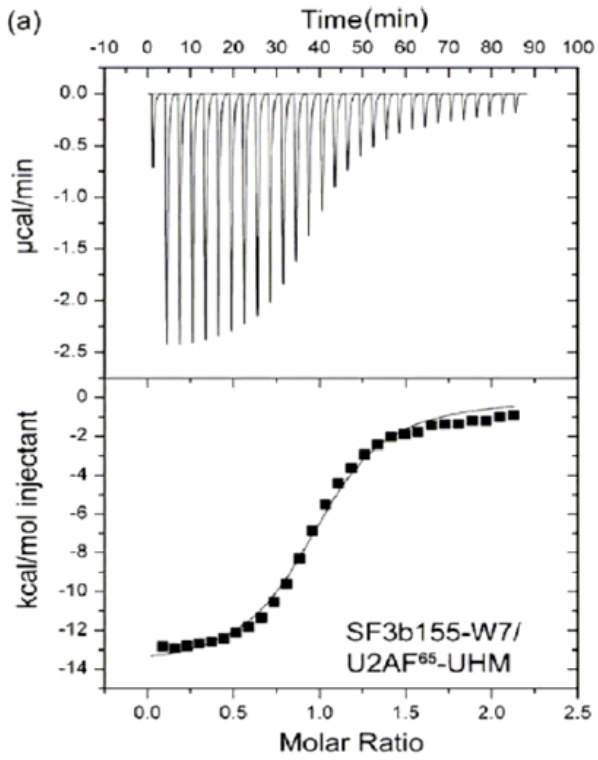
4-carboxybenzene-sulfonomide titrated into carbonic anhydrase II

N = 0.97

K_D = 730 nM

ΔH = -11.9 kcal/mol

Protein-protein interactions



C-terminal domain of nuclear RNA auxiliary factor (U2AF^{65}-UHM) binding to spliceosomal component mutant SF3b155-W7 (shown) or wild-type SF3b155

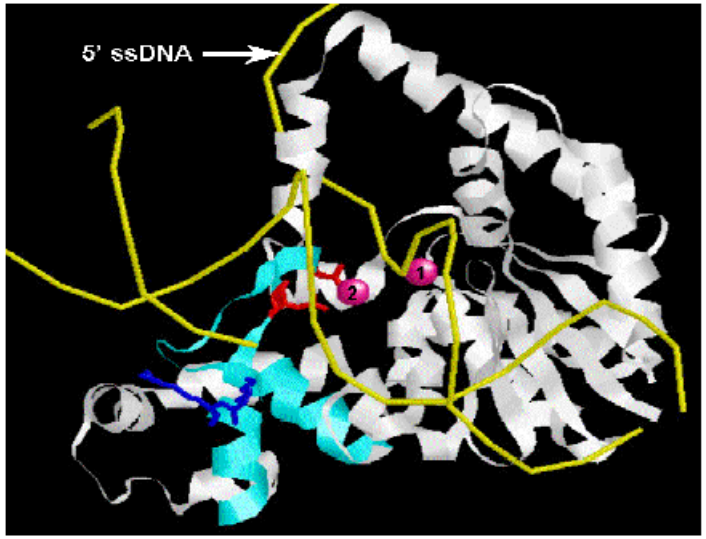
	SF3b155-W7	Wild-type SF3b155
K_D (µM)	2.50	2.83
ΔG (kcal/mol)	-7.8	-7.7
ΔH (kcal/mol)	-14.9	-9.4
ΔS (cal/mole/°K)	-23.4	-5.6

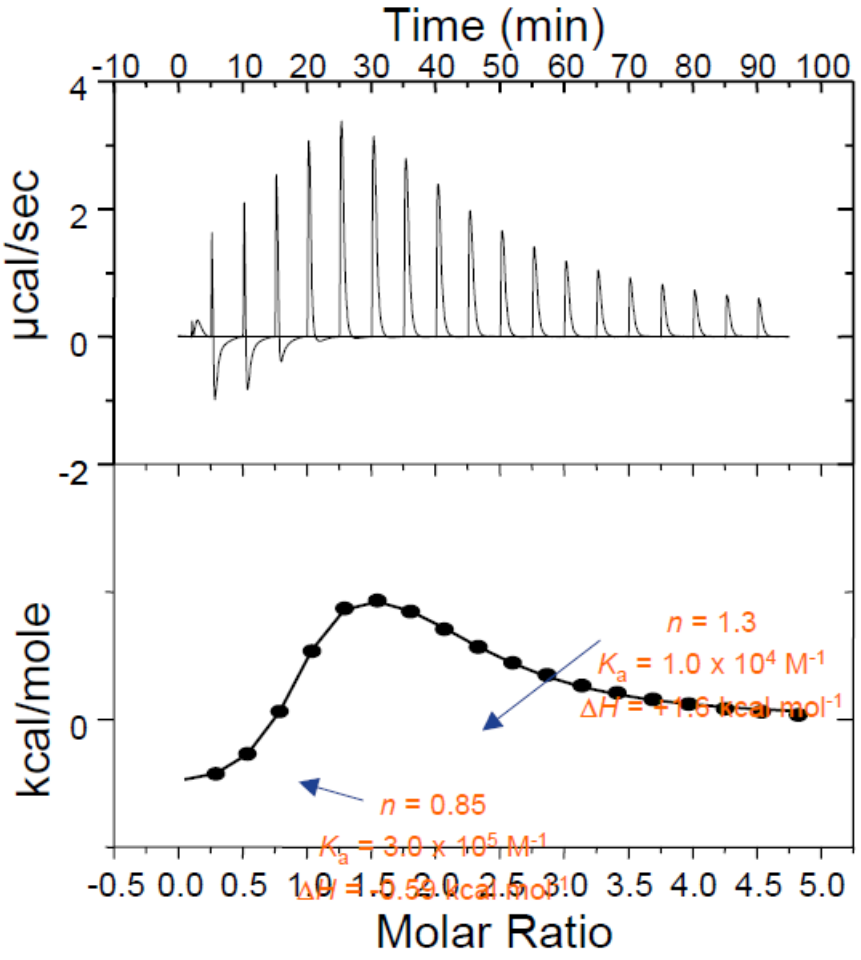
ΔH：焓变，当ΔH<0时表示反应由焓驱动，通常是通过**氢键、离子键、范德华力**产生相互作用；

ΔS：熵变，-T ΔS<0表示是熵驱动的过程，通常是发生了**疏水相互作用**，或产生了**构象变化**等。

Multiple binding sites

ITC shows differential binding of Mn(II) ions to WT T5 5' nuclease

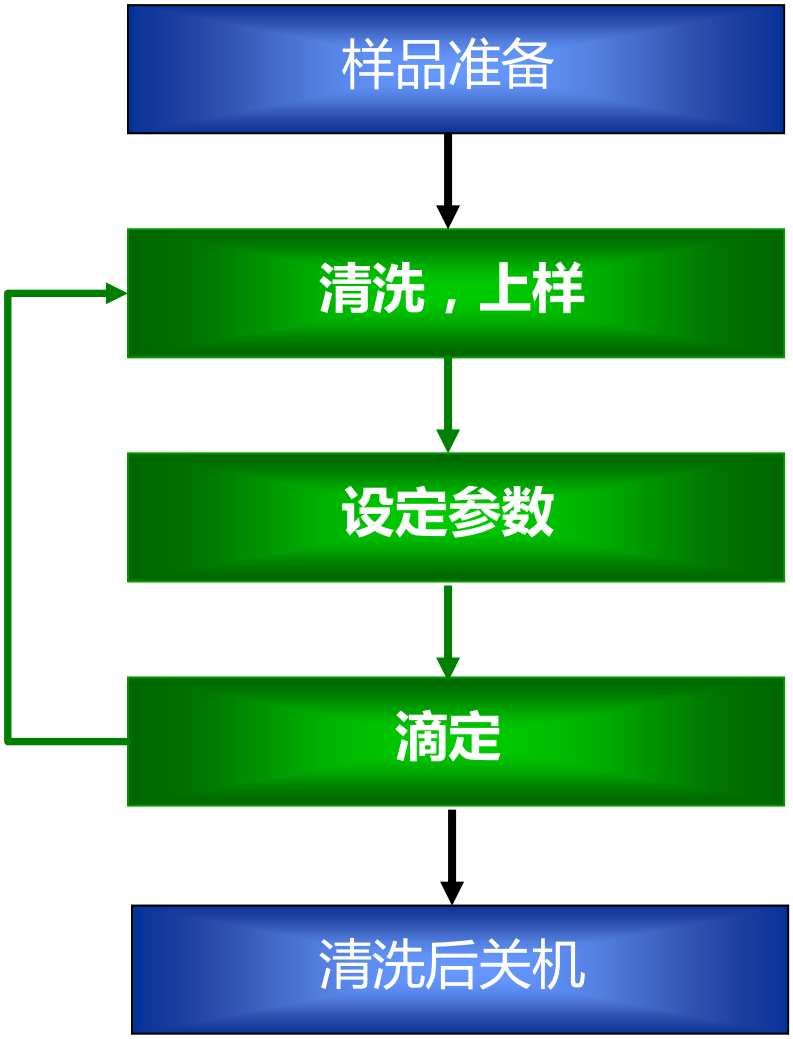




ITC实验怎么做？

（以PEAQ-ITC为例）

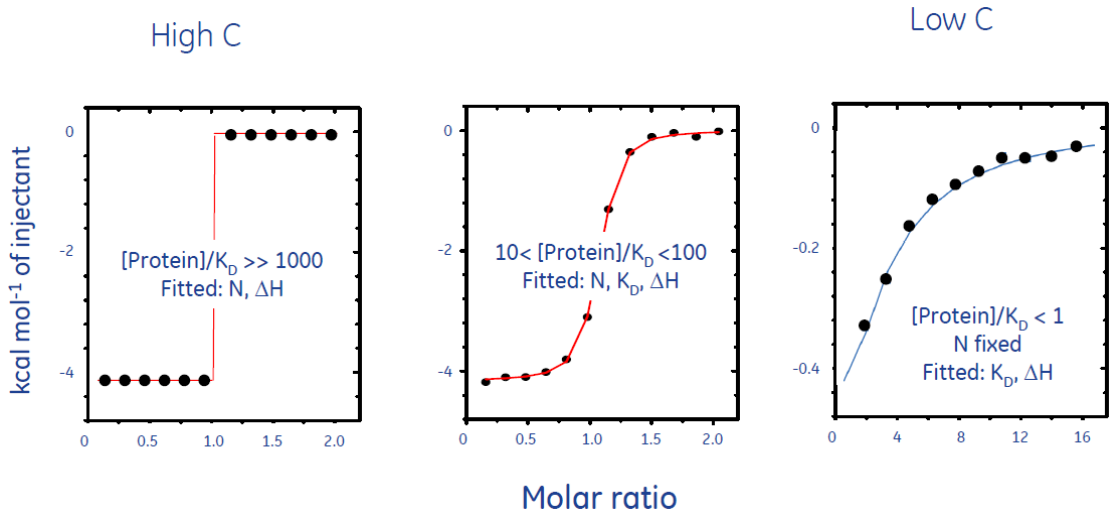
基本实验流程



样品准备-浓度与体积

- **样品浓度**：

  如果已知KD值，可根据公式估算（**样品池样品浓度=C*KD*N**，C值一般在10-100之间实验结果最好）



如果不清楚KD，可按经验浓度范围进行准备，样品池一般为**10uM~10mM**，滴定针比样品池浓度一般高10-20倍，建议初始尝试**200uM滴20uM**

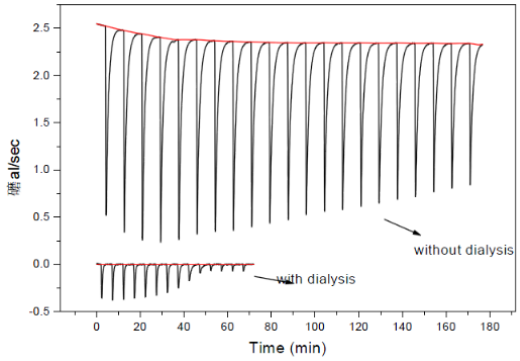
- **样品体积**：样品池280ul（建议350-400），滴定针60ul（建议100）

- 一个ITC实验一般包含A to B 和 A to Buffer两次滴定，A需要双倍用量。

样品准备-前处理

- **样品要求**：

  样品要澄清无颗粒，可通过离心或微滤处理



  两个样品所用缓冲液尽可能完全一致，pH差值＜0.05

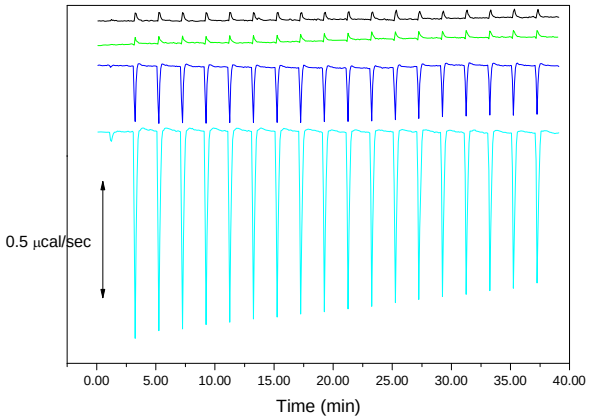
  缓冲液交换和脱盐：透析、凝胶过滤、超滤、渗滤、沉淀析出和固相萃取。
  尽可能避免使用有机溶剂！

  如需使用还原剂，最好使用：

  TCEP （ Tris (2-carboxyethylphosphine) hydrochloride,≤2mM ）

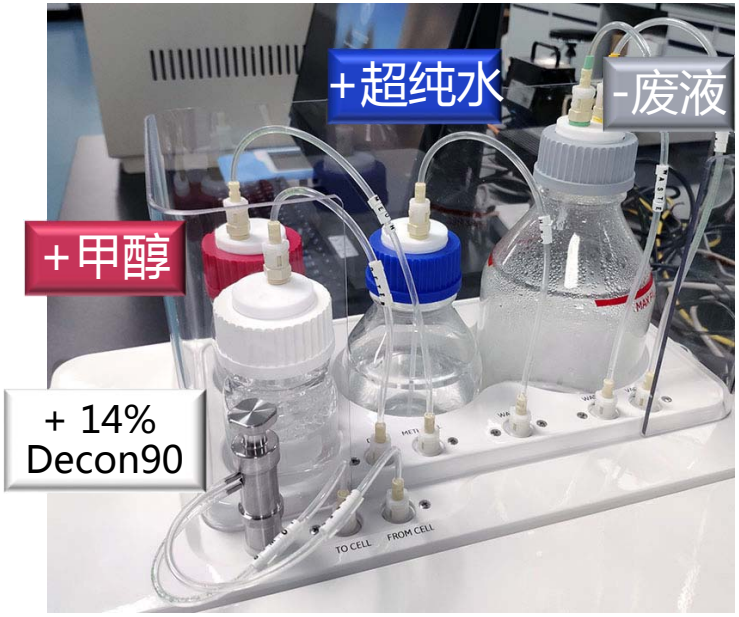
  β- 巯基乙醇（β-mercaptoethanol，β-ME，≤5mM)

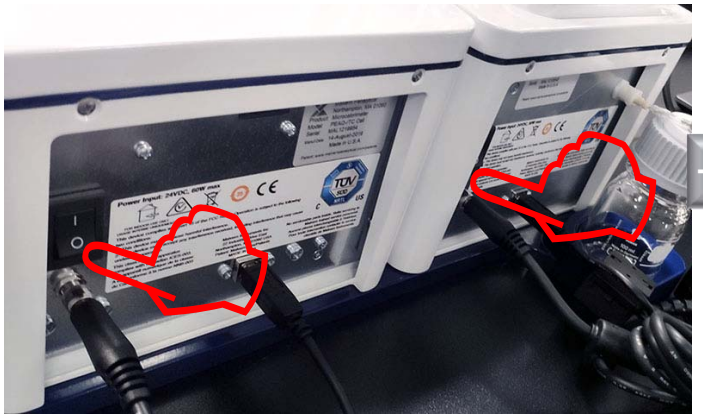
  避免使用DTT（自身氧化放热，导致背景漂移）

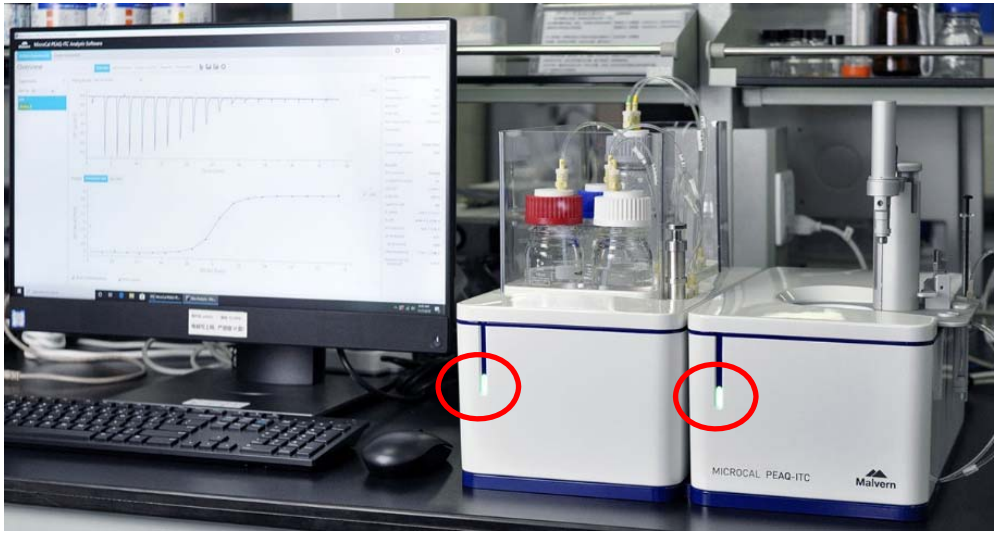


- **真空脱气5min（推荐）**或离心脱气10min（12000rpm或以上）

检查溶液瓶-刷卡开机



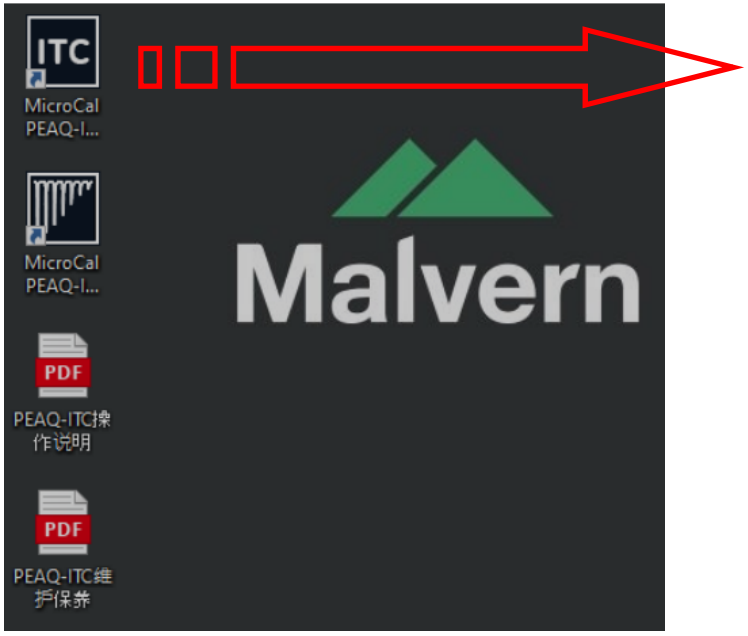


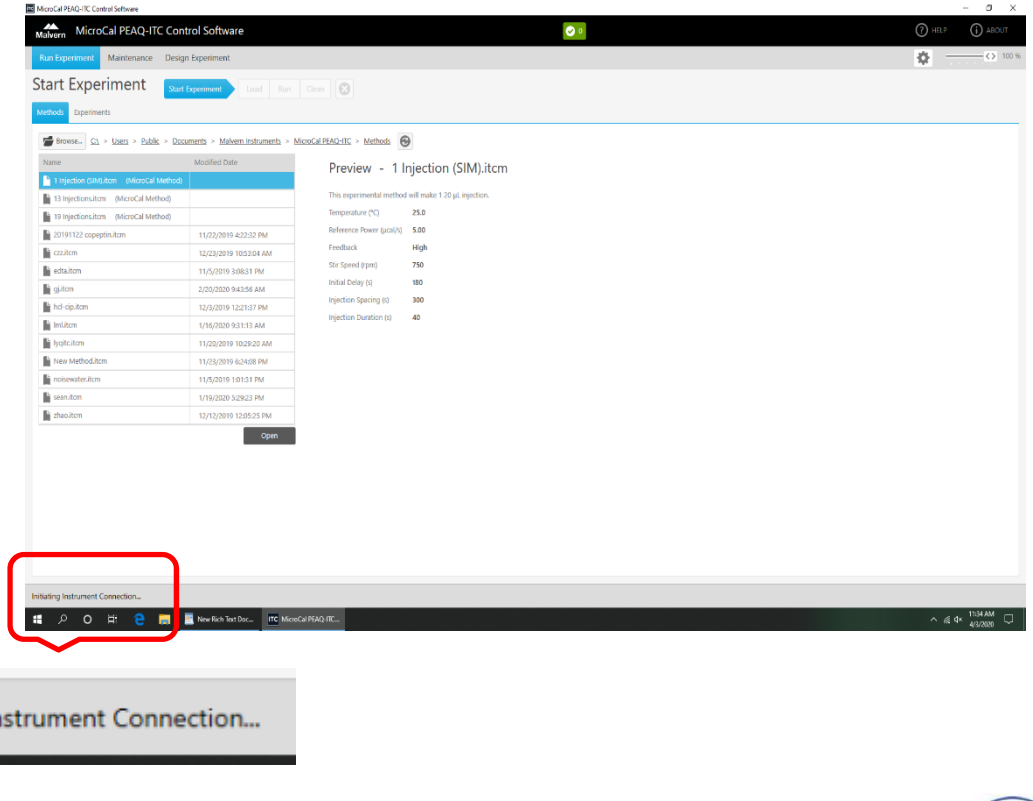


2个电源指示灯亮起

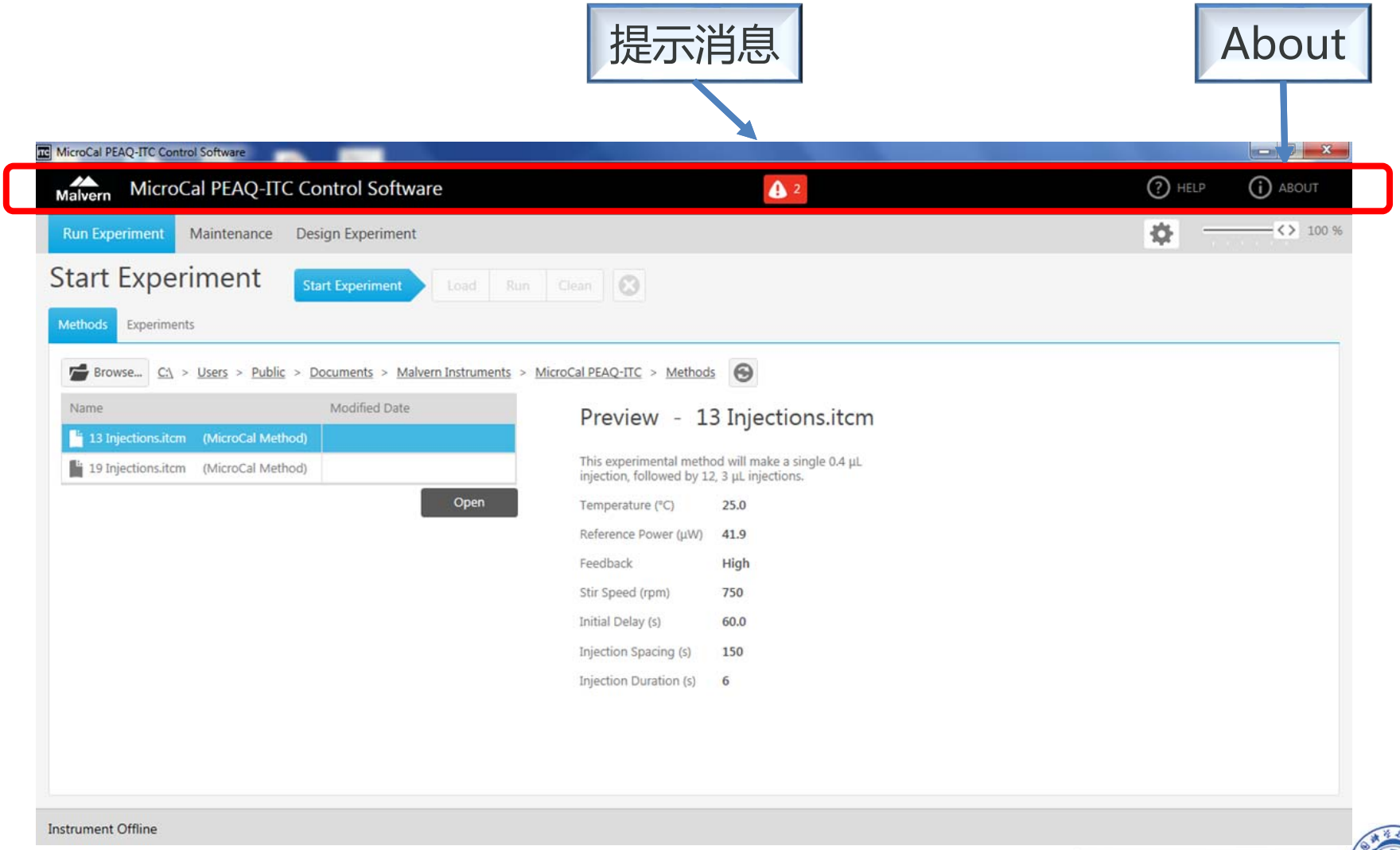
联机

- 打开控制软件（ITC 图标），注意查看联机状态Online/Offline

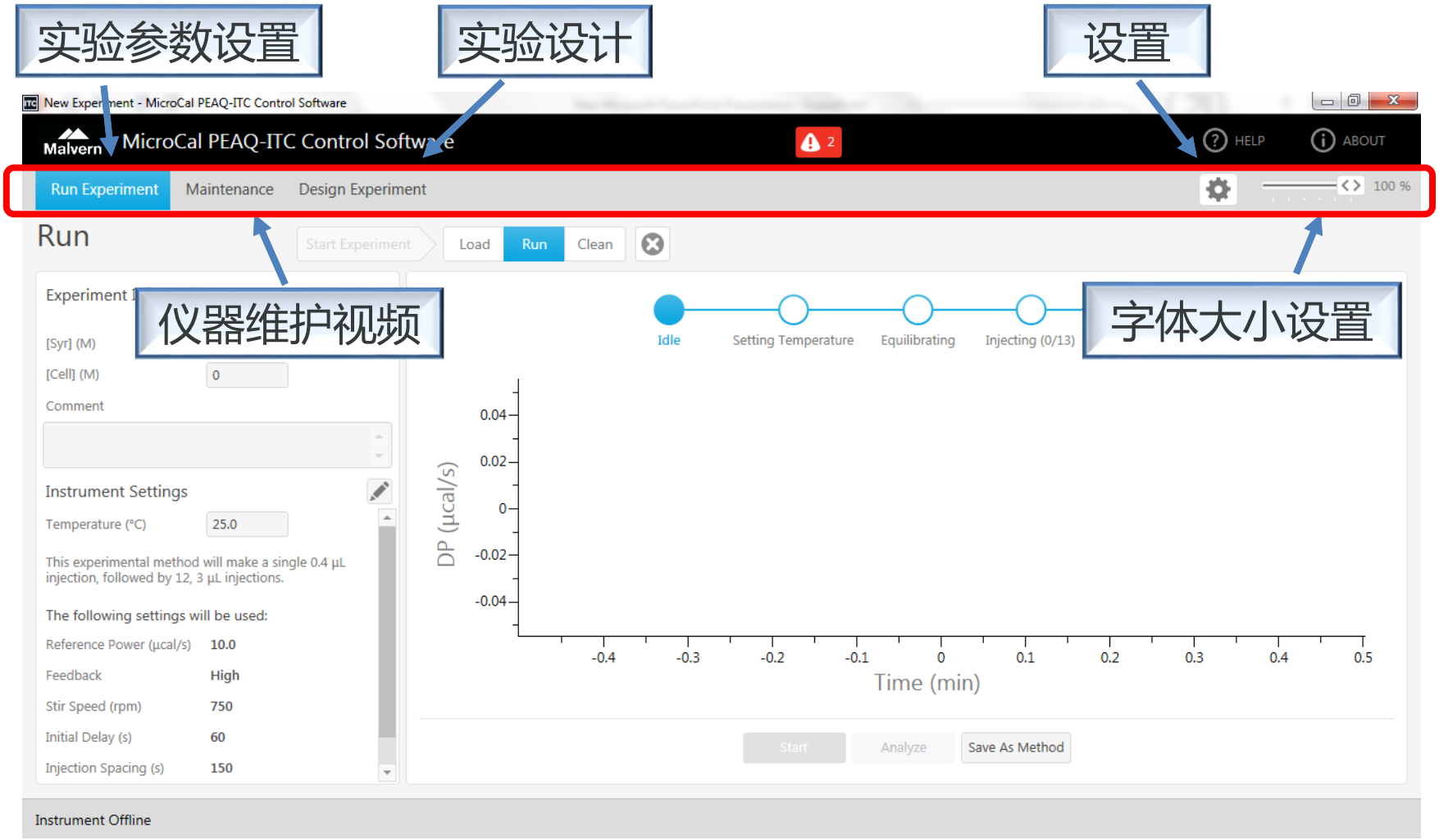




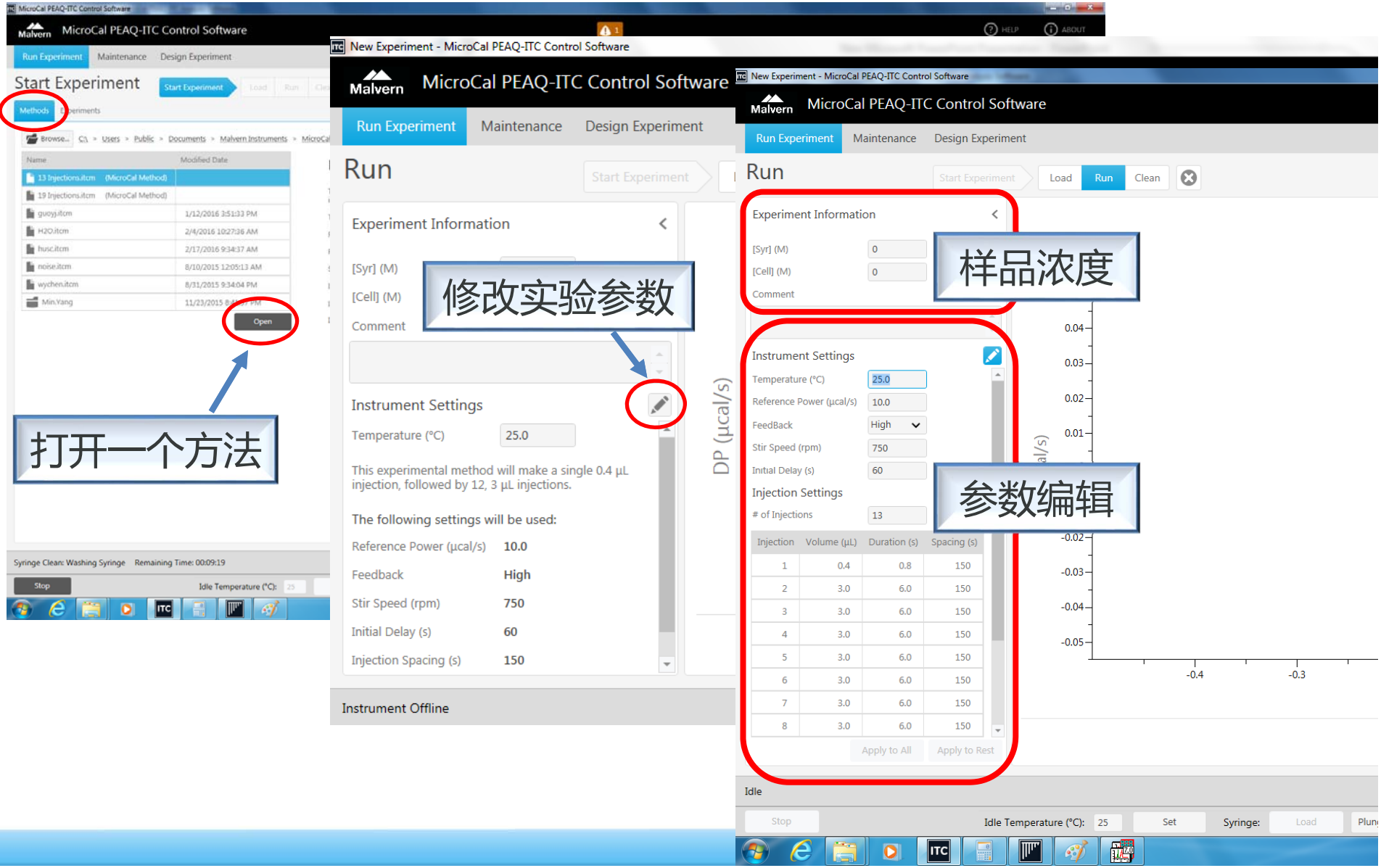
控制软件——首标


提示消息
About
MicroCal PEAQ-ITC Control Software
Malvern
MicroCal PEAQ-ITC Control Software
2
HELP
ABOUT
Run Experiment
Maintenance
Design Experiment
100 %
Start Experiment
Start Experiment
Load
Run
Clean
Methods
Experiments
Browse...
C:\ > Users > Public > Documents > Malvern Instruments > MicroCal PEAQ-ITC > Methods
Name
Modified Date
13 Injections.itcm (MicroCal Method)
19 Injections.itcm (MicroCal Method)
Open
Preview - 13 Injections.itcm
This experimental method will make a single 0.4 µL injection, followed by 12, 3 µL injections.
Temperature (°C) 25.0
Reference Power (µW) 41.9
Feedback High
Stir Speed (rpm) 750
Initial Delay (s) 60.0
Injection Spacing (s) 150
Injection Duration (s) 6
Instrument Offline

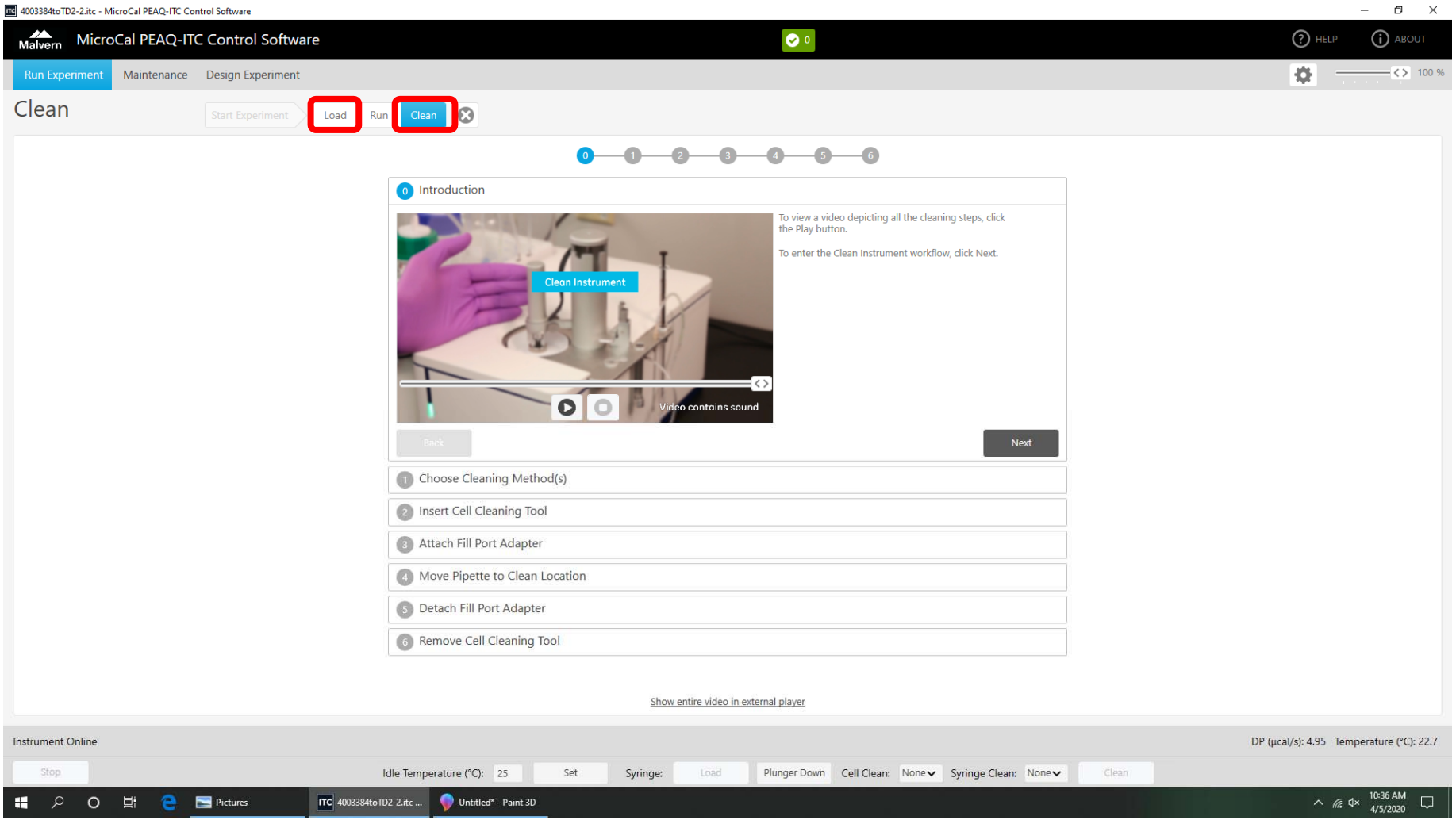
控制软件——主导航栏


实验参数设置
实验设计
设置
New Experiment - MicroCal PEAQ-ITC Control Software
Malvern
MicroCal PEAQ-ITC Control Software
2
HELP
ABOUT
Run Experiment
Maintenance
Design Experiment
100 %
Run
Start Experiment
Load
Run
Clean
仪器维护视频
字体大小设置
Experiment
[Syr] (M)
[Cell] (M)
0
Comment
Instrument Settings
Temperature (°C)
25.0
This experimental method will make a single 0.4 µL injection, followed by 12, 3 µL injections.
The following settings will be used:
Reference Power (µcal/s) 10.0
Feedback High
Stir Speed (rpm) 750
Initial Delay (s) 60
Injection Spacing (s) 150
Idle
Setting Temperature
Equilibrating
Injecting (0/13)
DP (µcal/s)
0.04
0.02
0
-0.02
-0.04
-0.4
-0.3
-0.2
-0.1
0
0.1
0.2
0.3
0.4
0.5
Time (min)
Start
Analyze
Save As Method
Instrument Offline

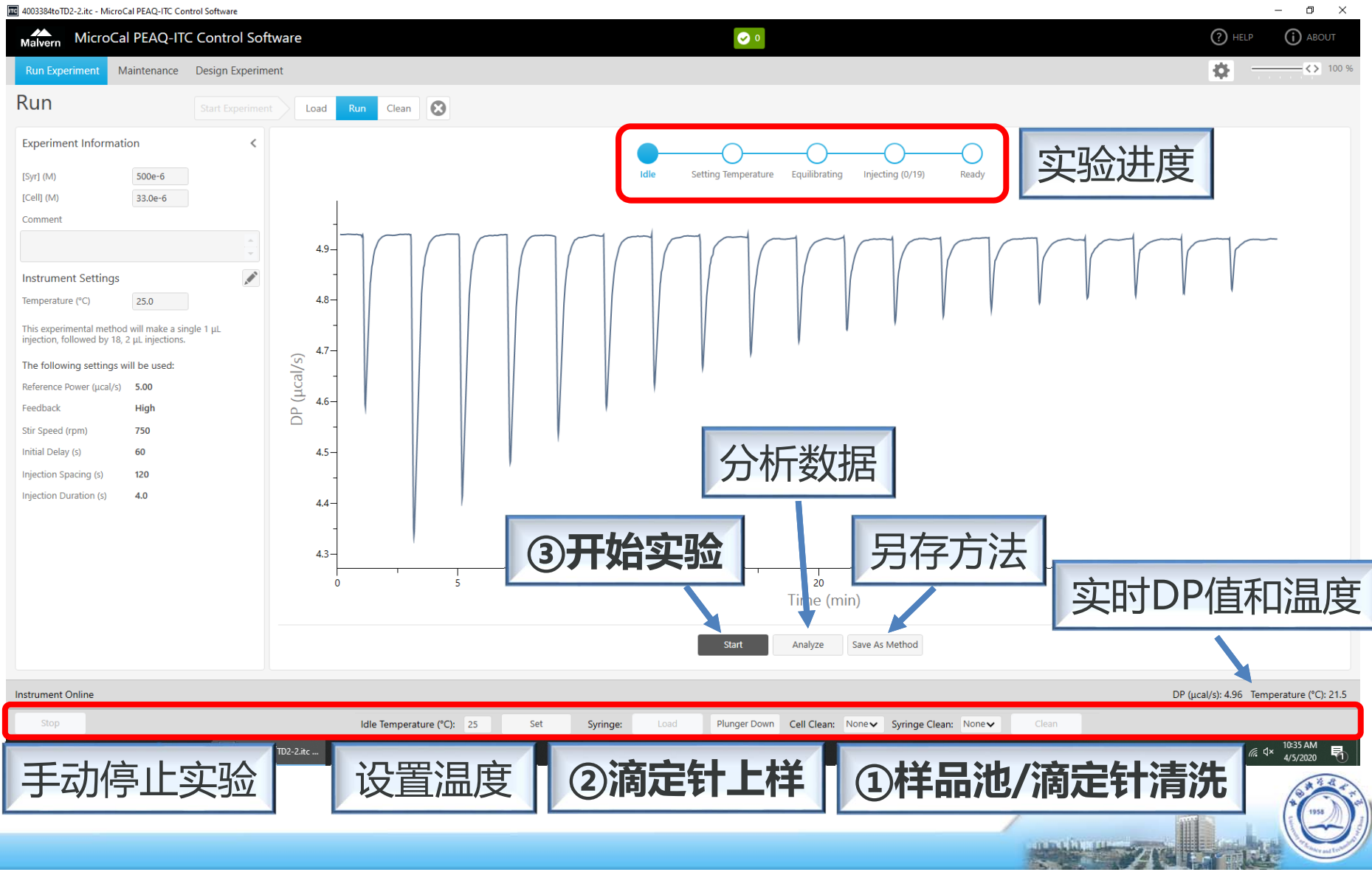
控制软件——打开和编辑实验方法



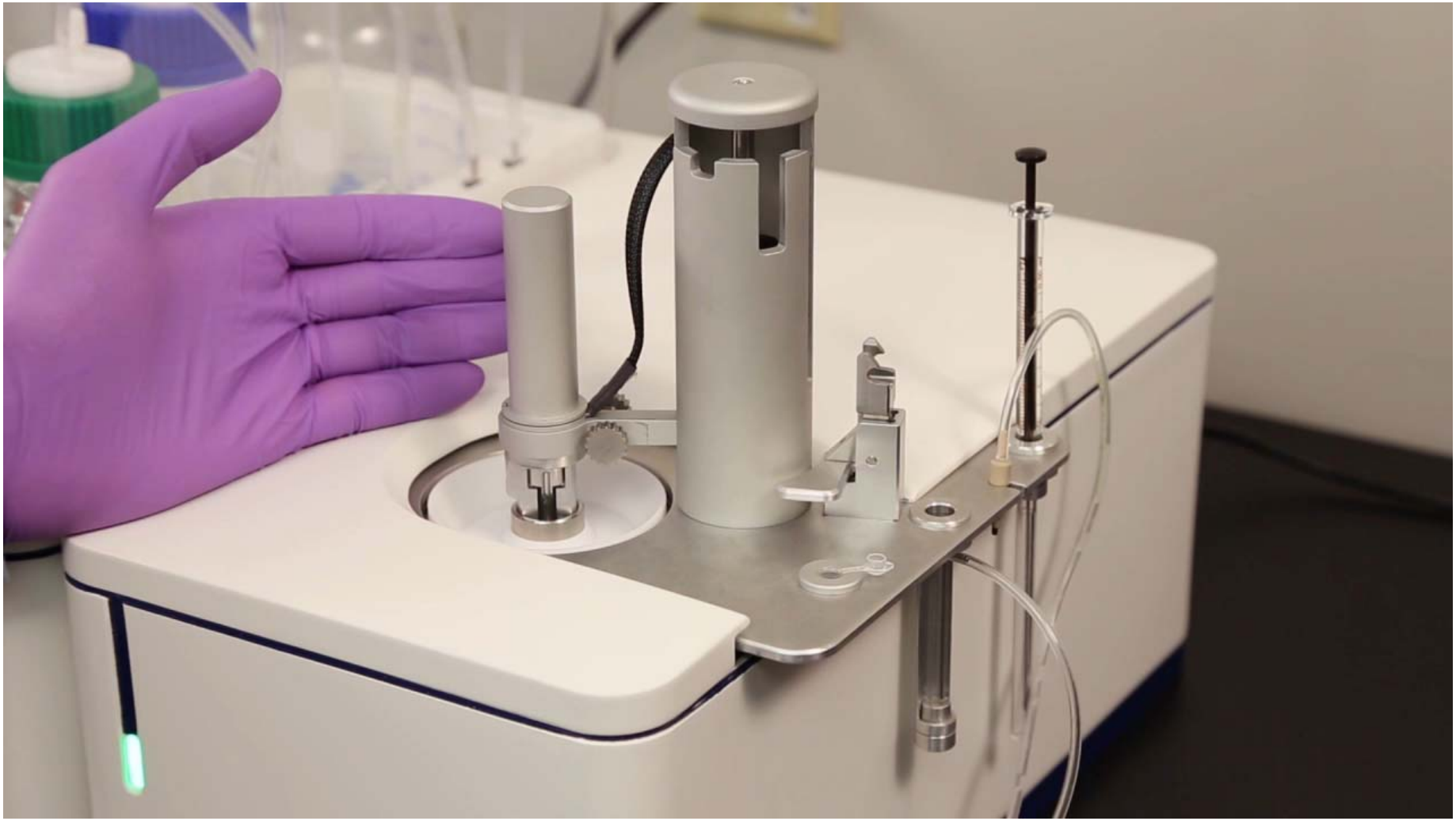
控制软件——清洗与上样操作视频



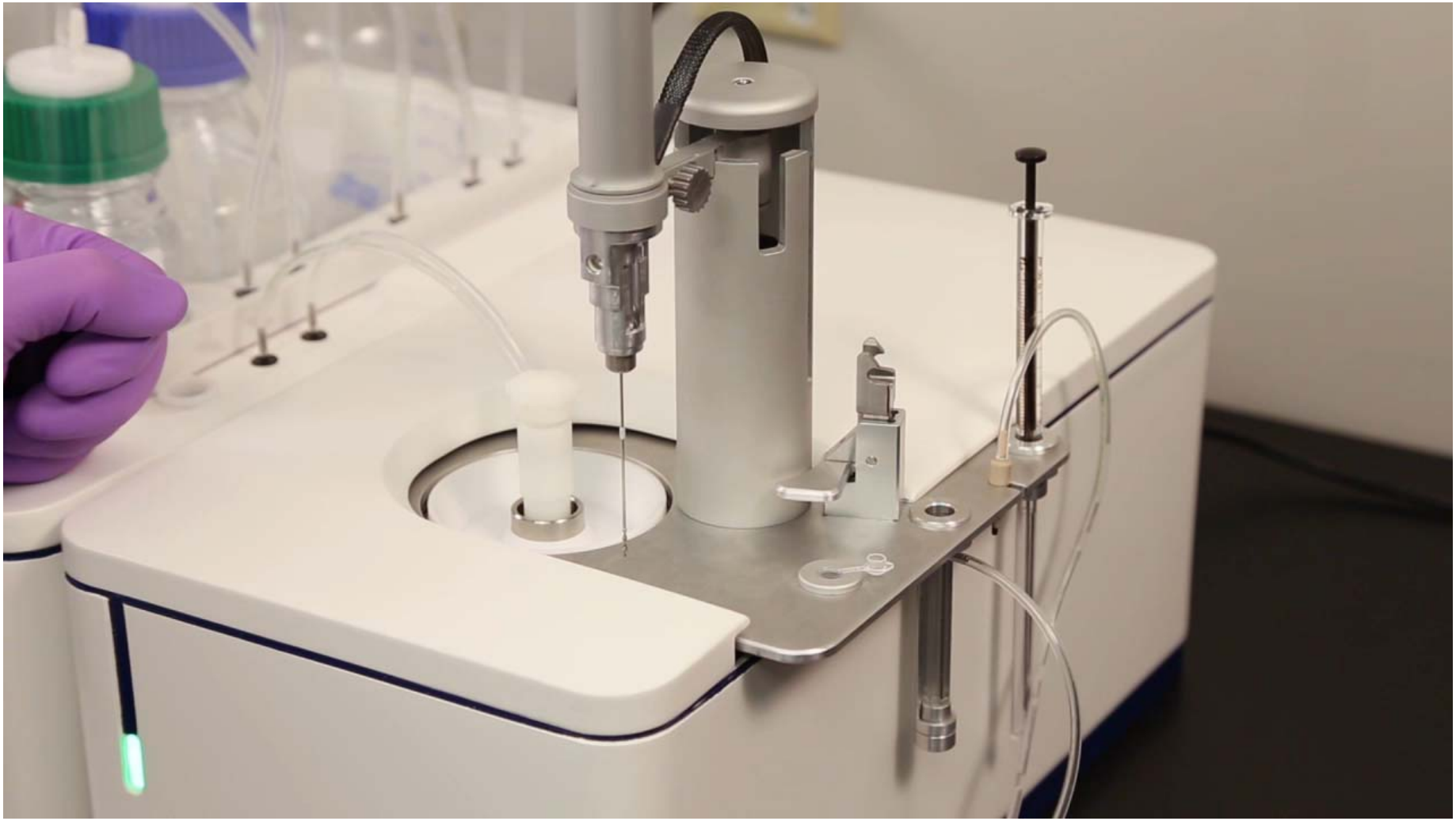
控制软件——仪器控制


4003384toTD2-2.itc - MicroCal PEAQ-ITC Control Software
Malvern MicroCal PEAQ-ITC Control Software
HELP
ABOUT
Run Experiment
Maintenance
Design Experiment
100 %
Run
Start Experiment
Load
Run
Clean
Experiment Information
[Syr] (M) 500e-6
[Cell] (M) 33.0e-6
Comment
Instrument Settings
Temperature (°C) 25.0
This experimental method will make a single 1 µL injection, followed by 18, 2 µL injections.
The following settings will be used:
Reference Power (µcal/s) 5.00
Feedback High
Stir Speed (rpm) 750
Initial Delay (s) 60
Injection Spacing (s) 120
Injection Duration (s) 4.0
Idle
Setting Temperature
Equilibrating
Injecting (0/19)
Ready
实验进度
DP (µcal/s)
Time (min)
分析数据
③开始实验
另存方法
实时DP值和温度
Start
Analyze
Save As Method
Instrument Online
DP (µcal/s): 4.96 Temperature (°C): 21.5
Stop
Idle Temperature (°C): 25
Set
Syringe:
Load
Plunger Down
Cell Clean: None
Syringe Clean: None
Clean
手动停止实验
设置温度
②滴定针上样
①样品池/滴定针清洗

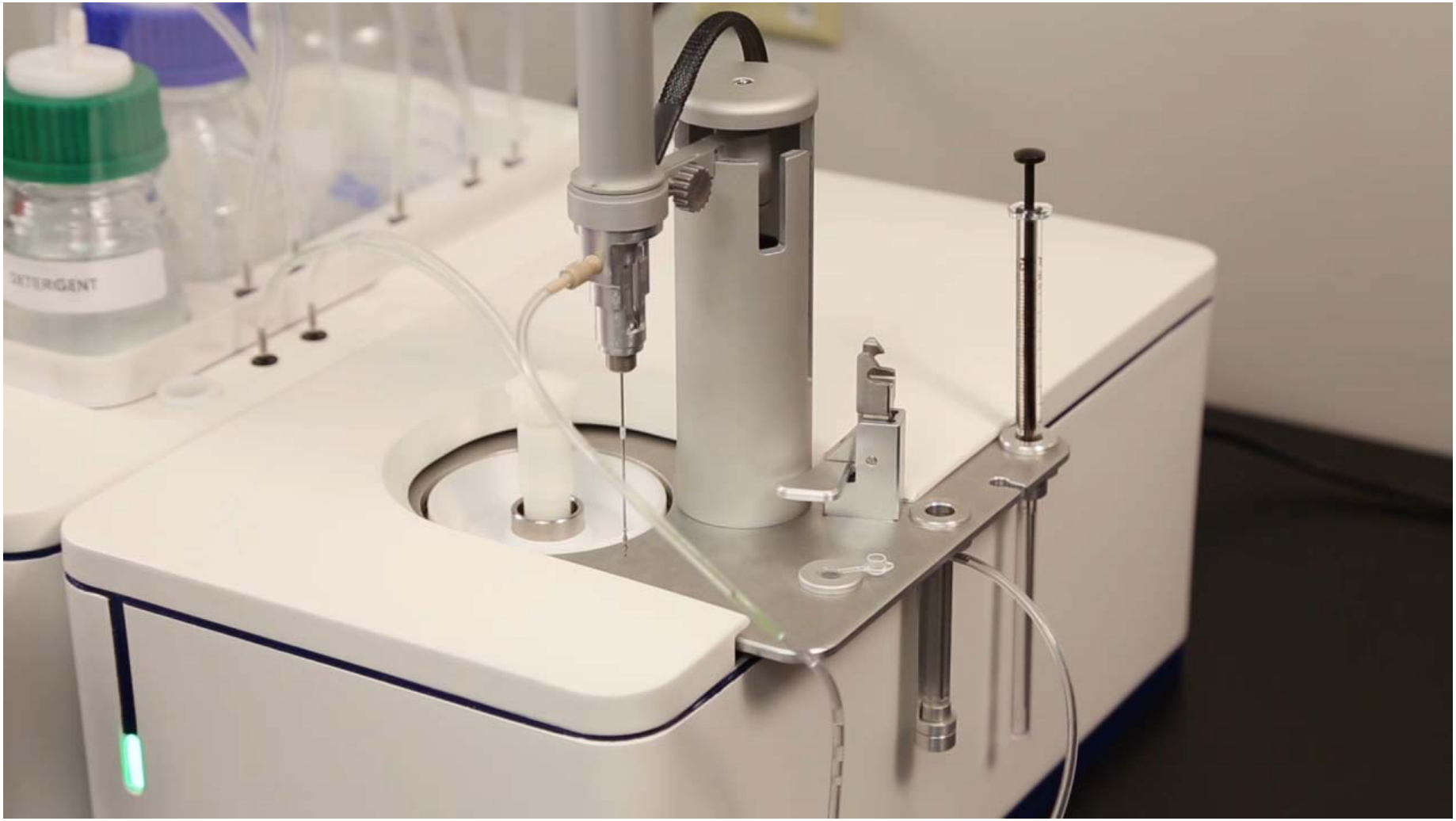
样品池清洗



滴定针清洗-连接FPA

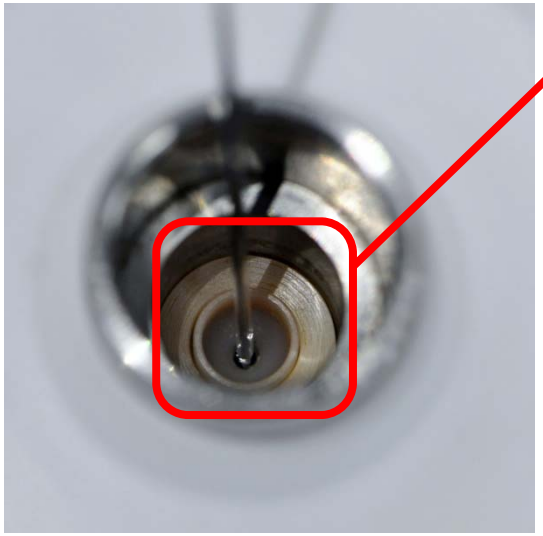


滴定针清洗-转入清洗孔



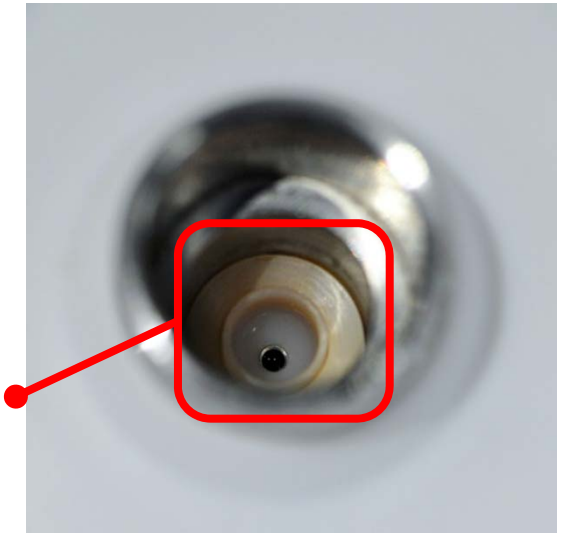
样品池上样

样品池上样



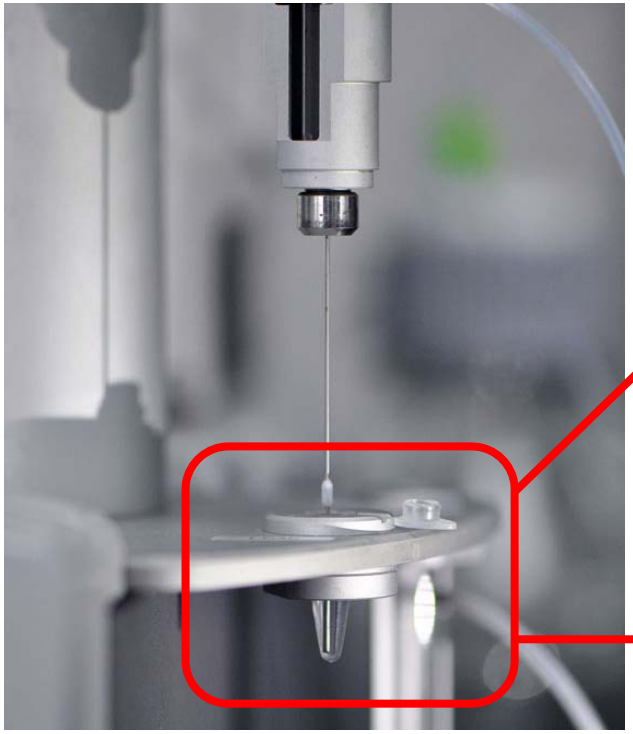
将注射器取出时，要保证样品池池口外还能看到溶液

将注射器卡在样品池池口，把池口外的溶液吸干



滴定针上样

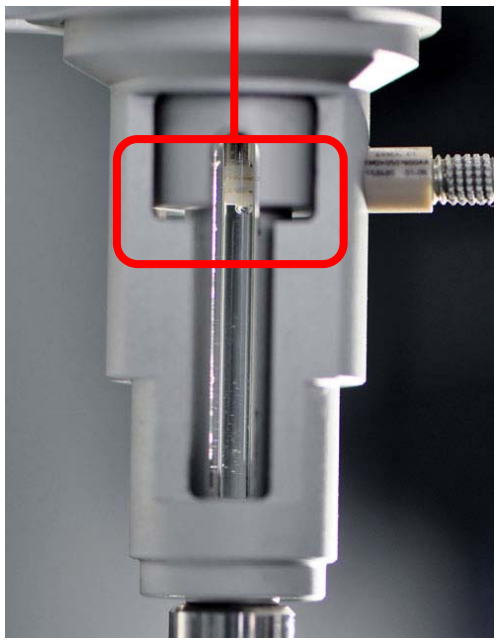
滴定针上样



至少准备60ul
建议准备100ul +

不要触底，
稍留点空间

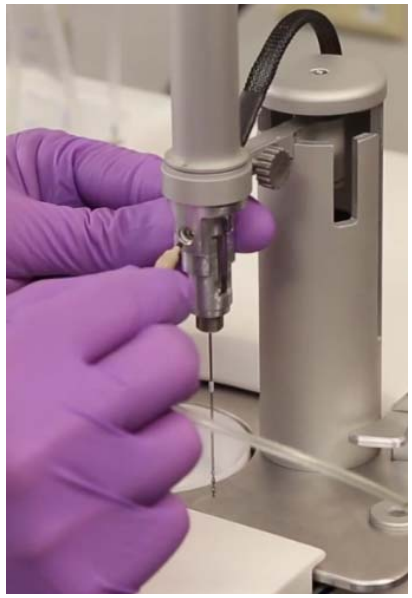
如残留气泡，直径小于
2-3mm不影响滴定

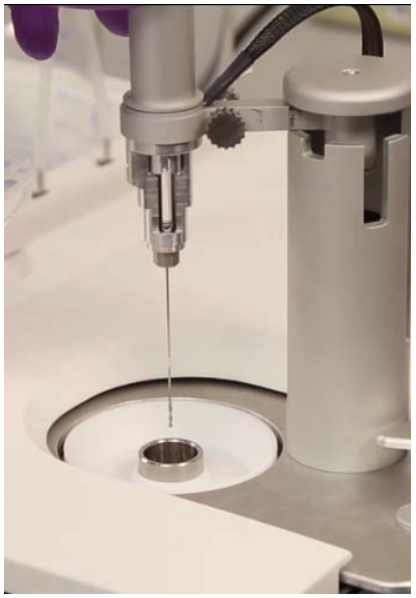


滴定针上样后-解除FPA

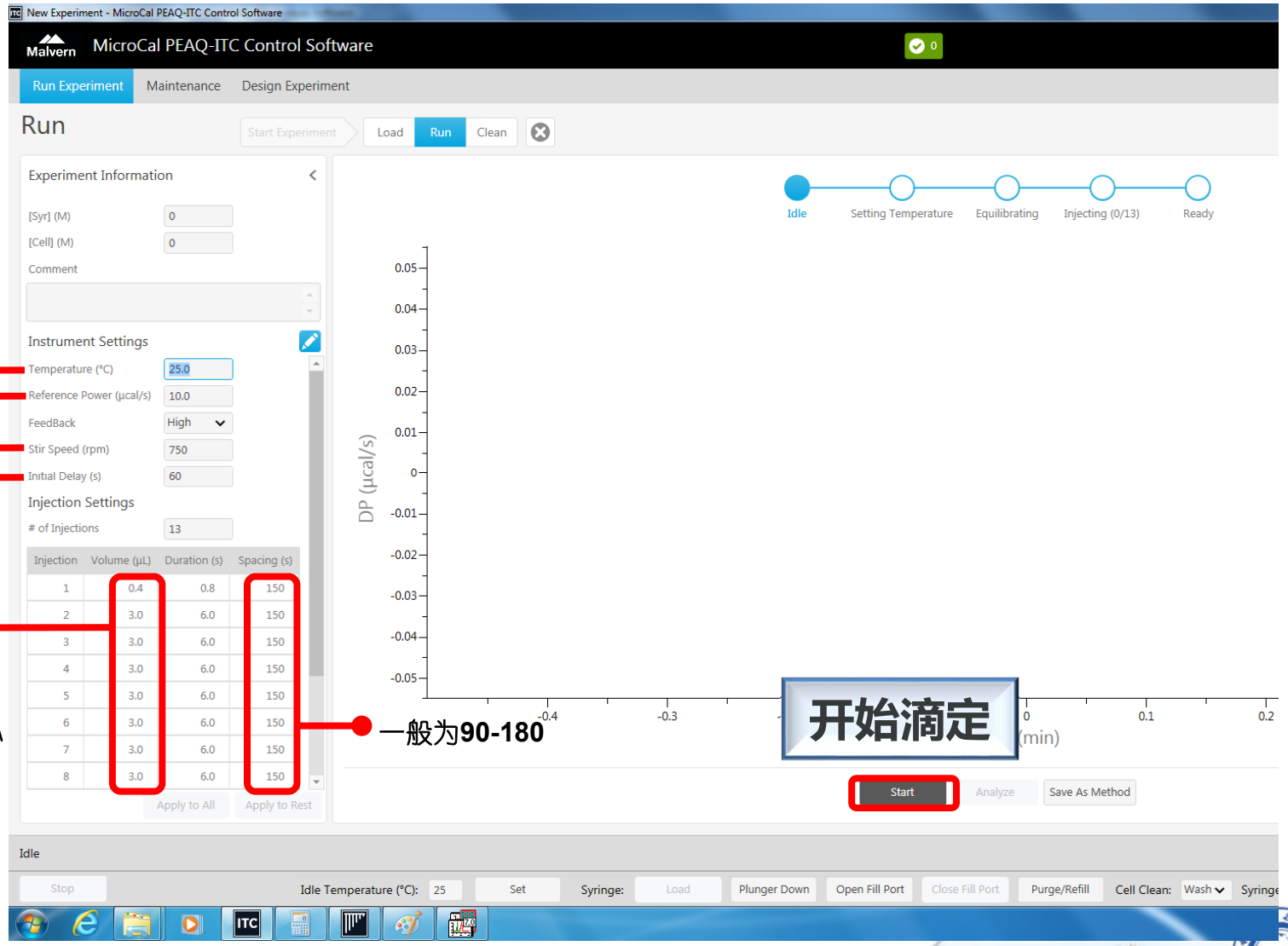
滴定针上样-转入样品池

实验全程，切勿碰到滴定针！以防折弯！！！





设定参数-开始滴定



范围是2-80
一般是5或者10

默认转速750
一般是60-120

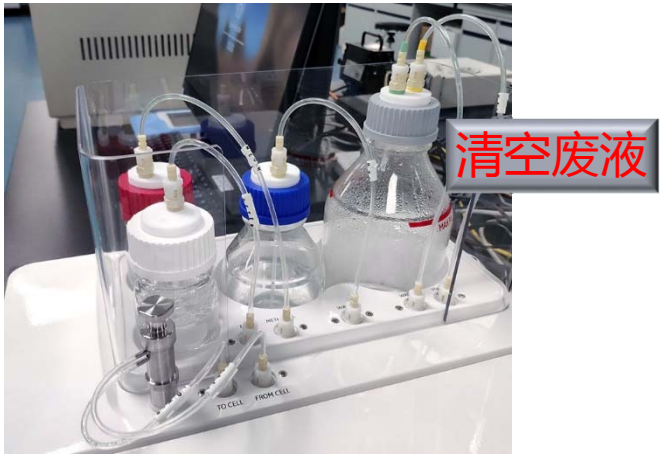
一般是2ul/滴，
不大于3ul/滴

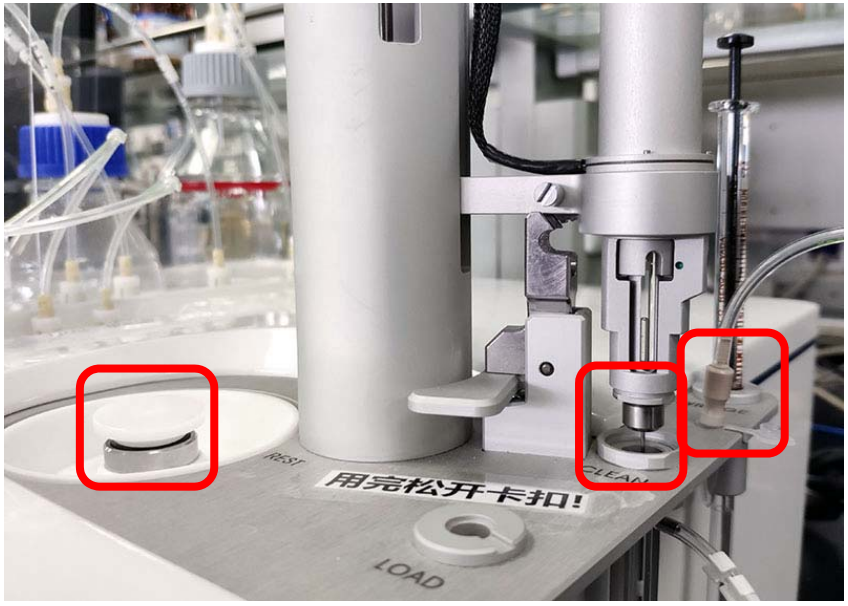
第一滴体积可以改小

一般为90-180

实验结束-清洗后关机

- 清洗样品池和滴定针，清空废液瓶
- 松开卡扣，仪器关机，刷卡
- 数据处理





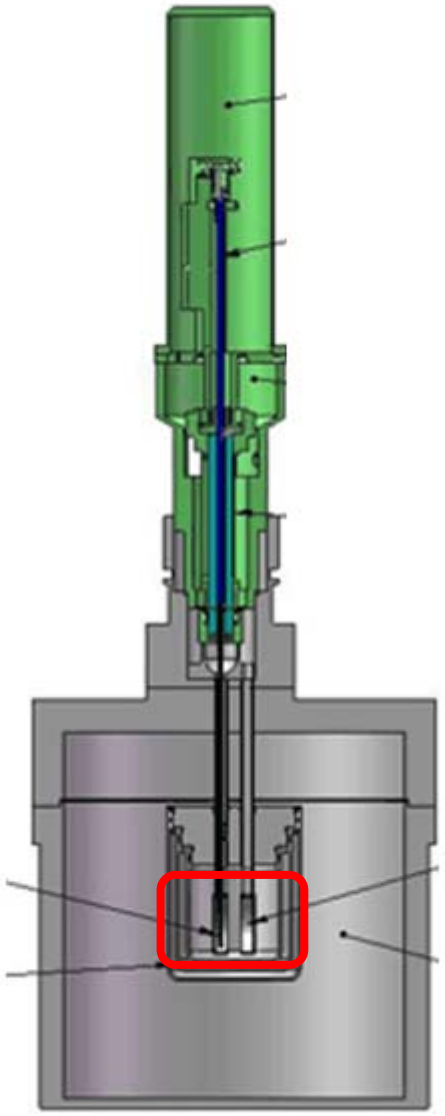
样品池与参比池

每天实验前，先做**水滴水，确认仪器状态和洁净度OK**

平衡时，DP值=“Reference power设定值±1”，
±0.5更好
若为“+”，说明参比池有问题，受污染或气泡；
若为“-”,说明样品池有问题，不干净或气泡，也可能是参比池受污染

样品池强力清洗：可用Decon 90浸泡
如浓度14%，温度60℃，时间10-60min
（也可用5% SDS代替）
（样品池耐碱，不耐强酸）

参比池：只能水洗！



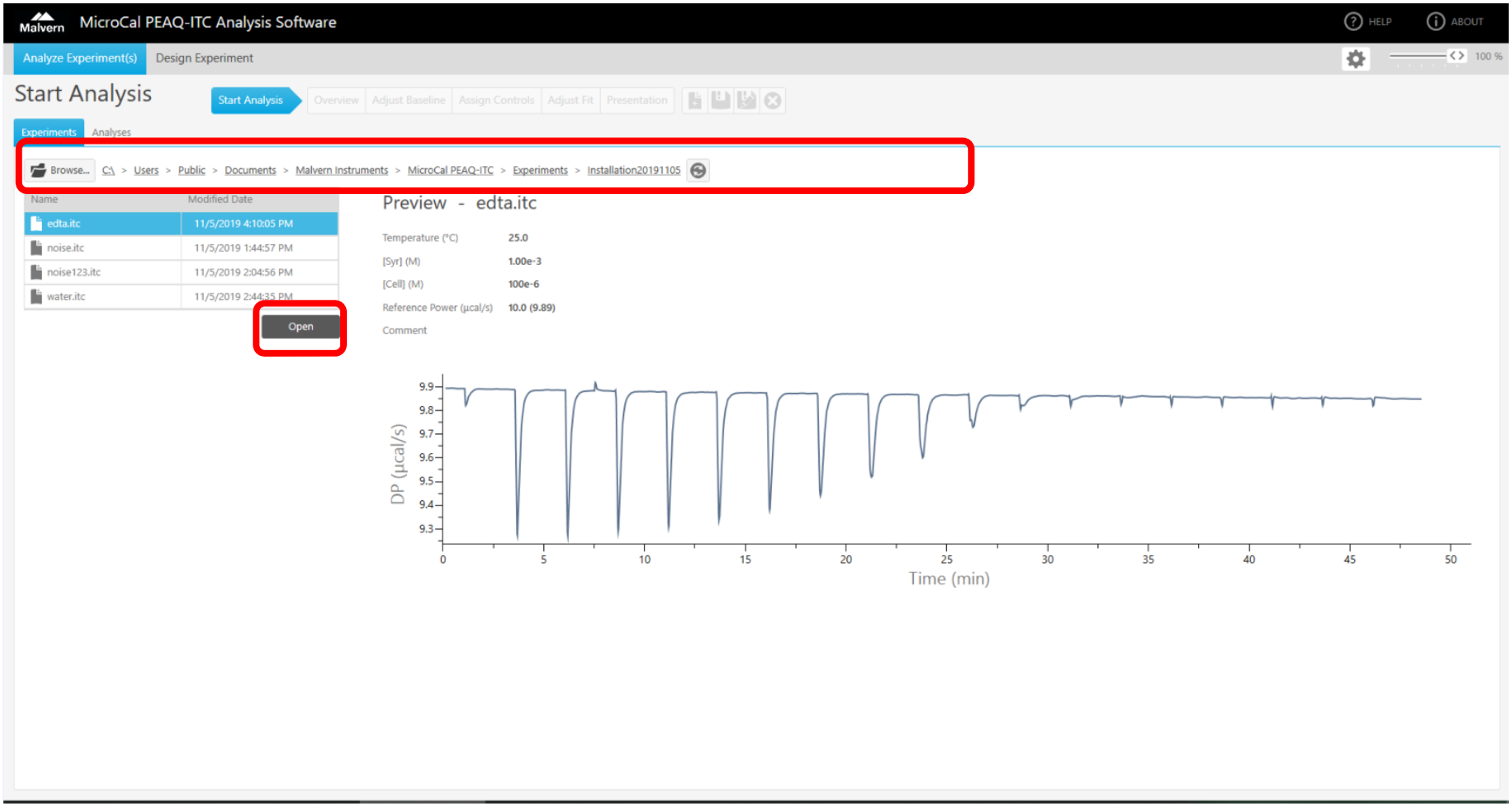
Good In - Good Out

怎么分析？

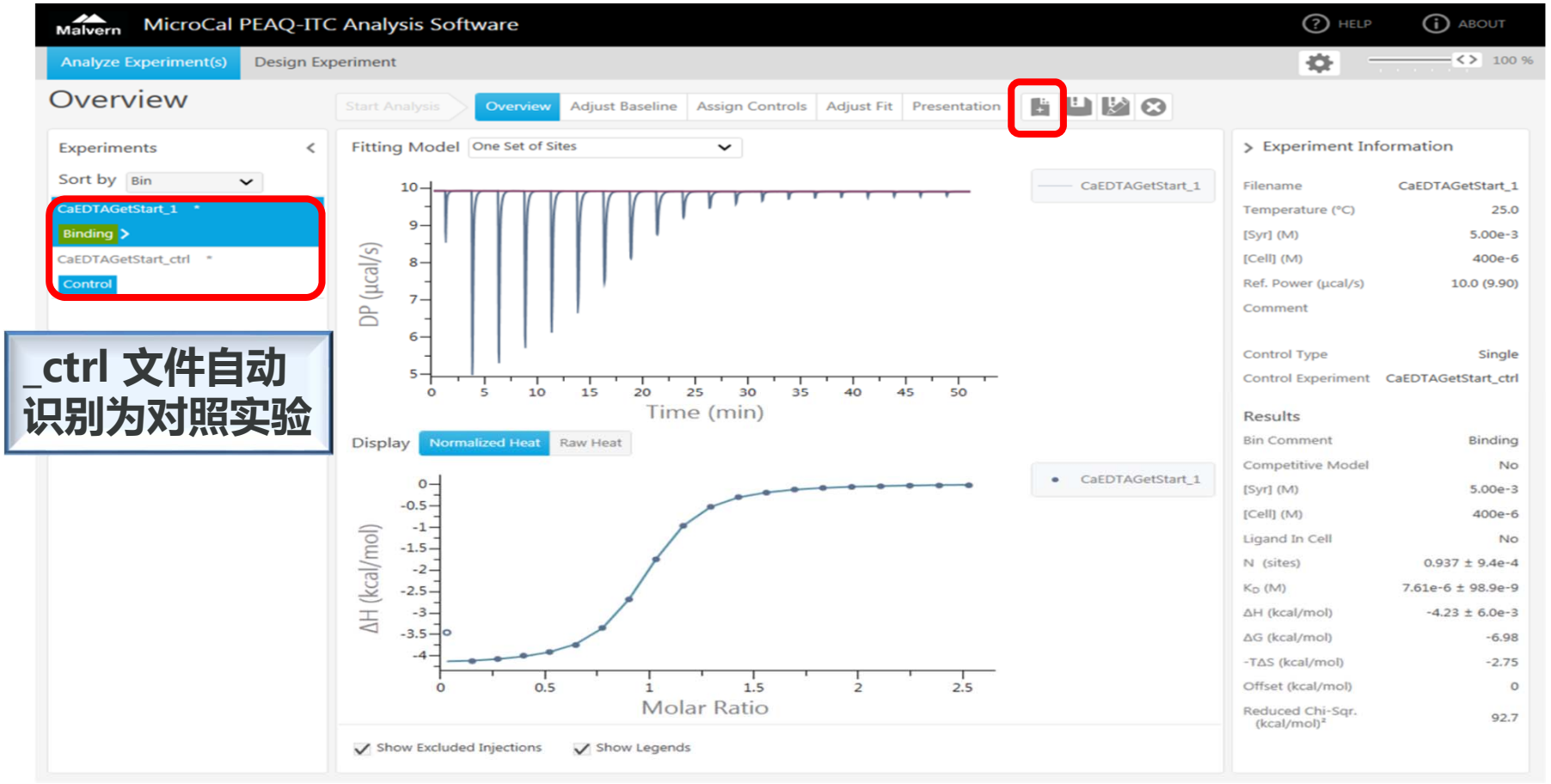
实验数据分析步骤

1. 导入数据并检查
2. 指定对照实验
3. 调整基线和积分范围（可选操作）
4. 模型拟合
5. 导出最终结果

实验数据分析——导入数据与检查

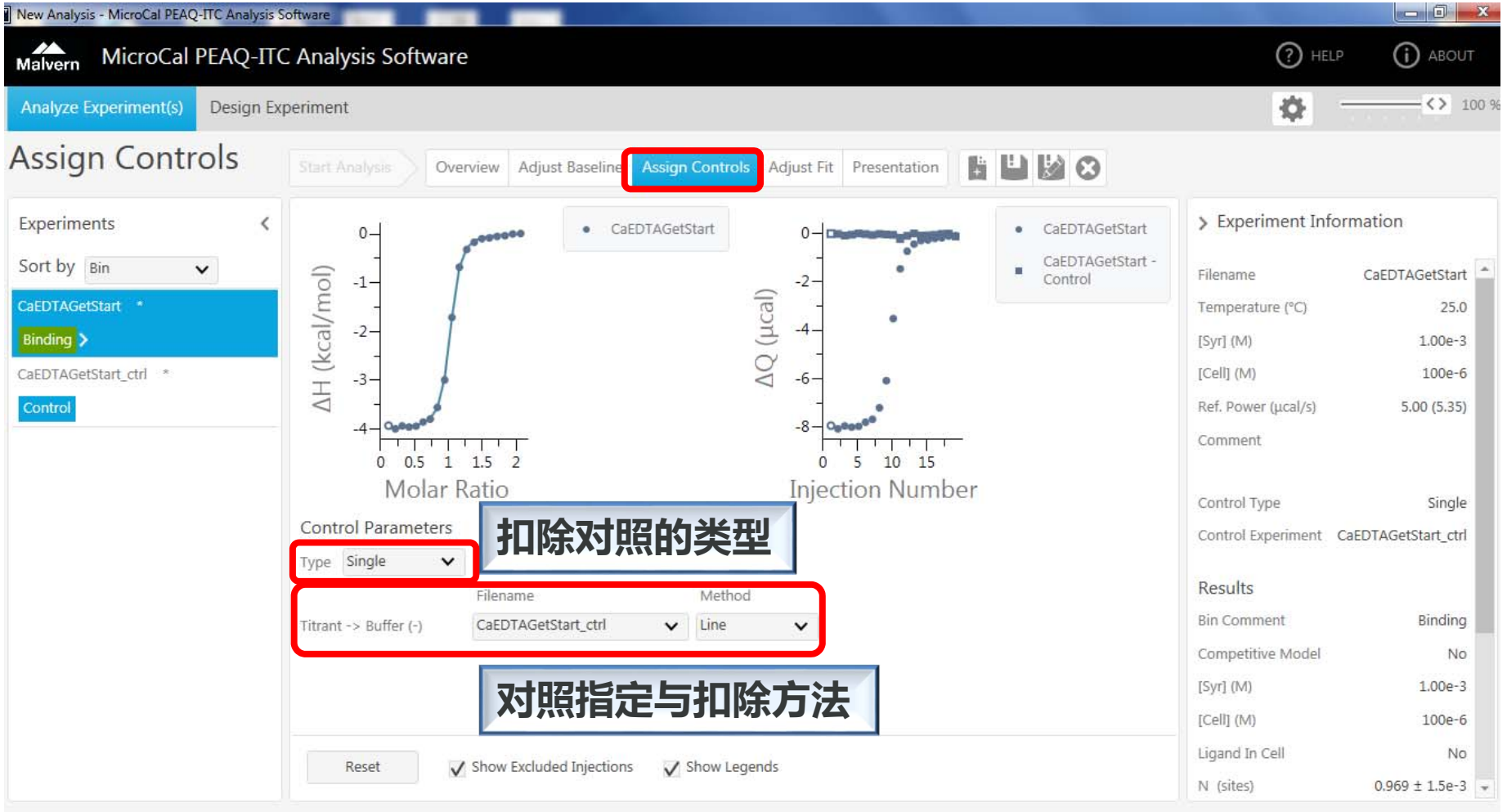


实验数据分析——可添加多个数据

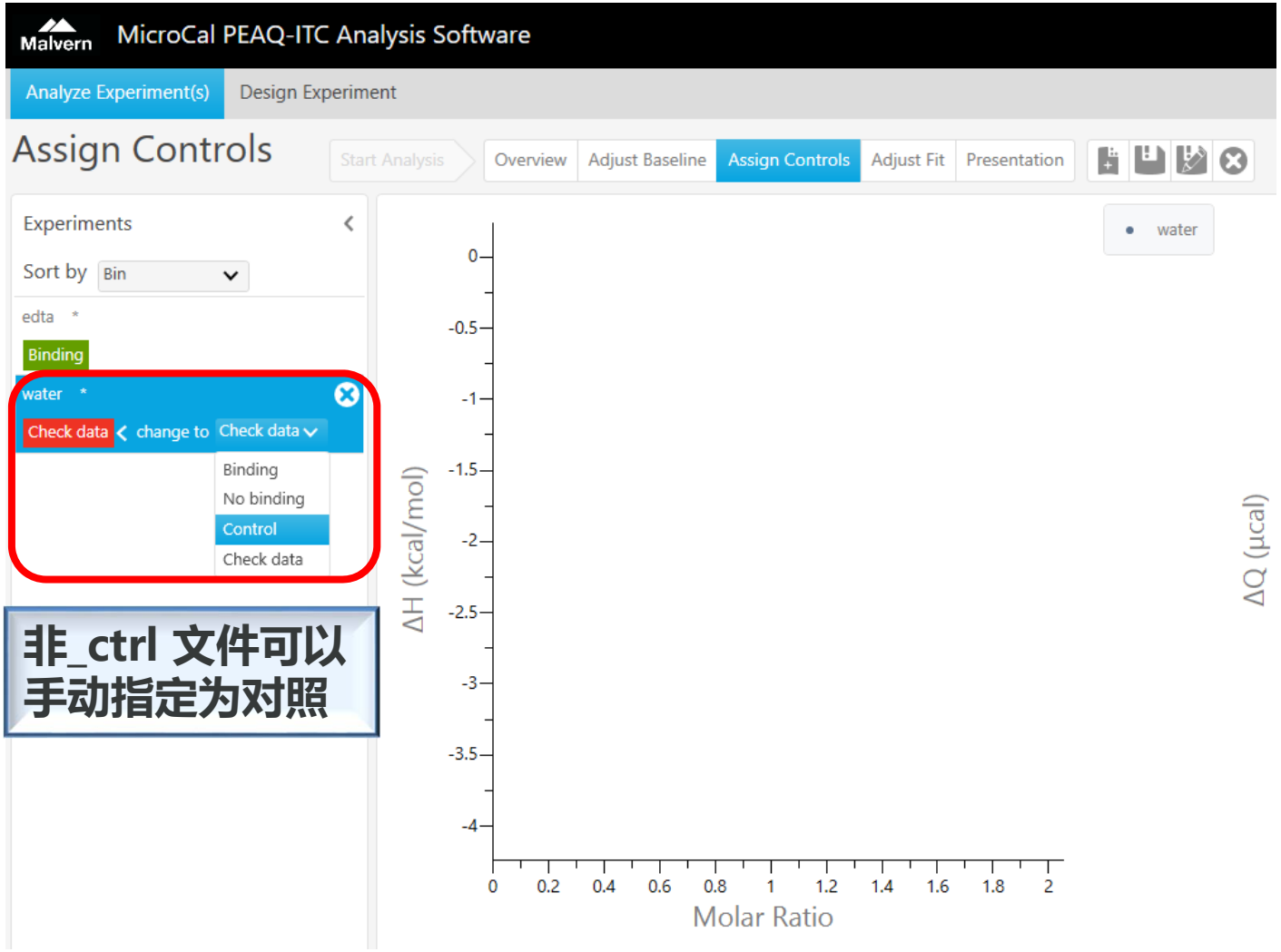


_ctrl 文件自动识别为对照实验

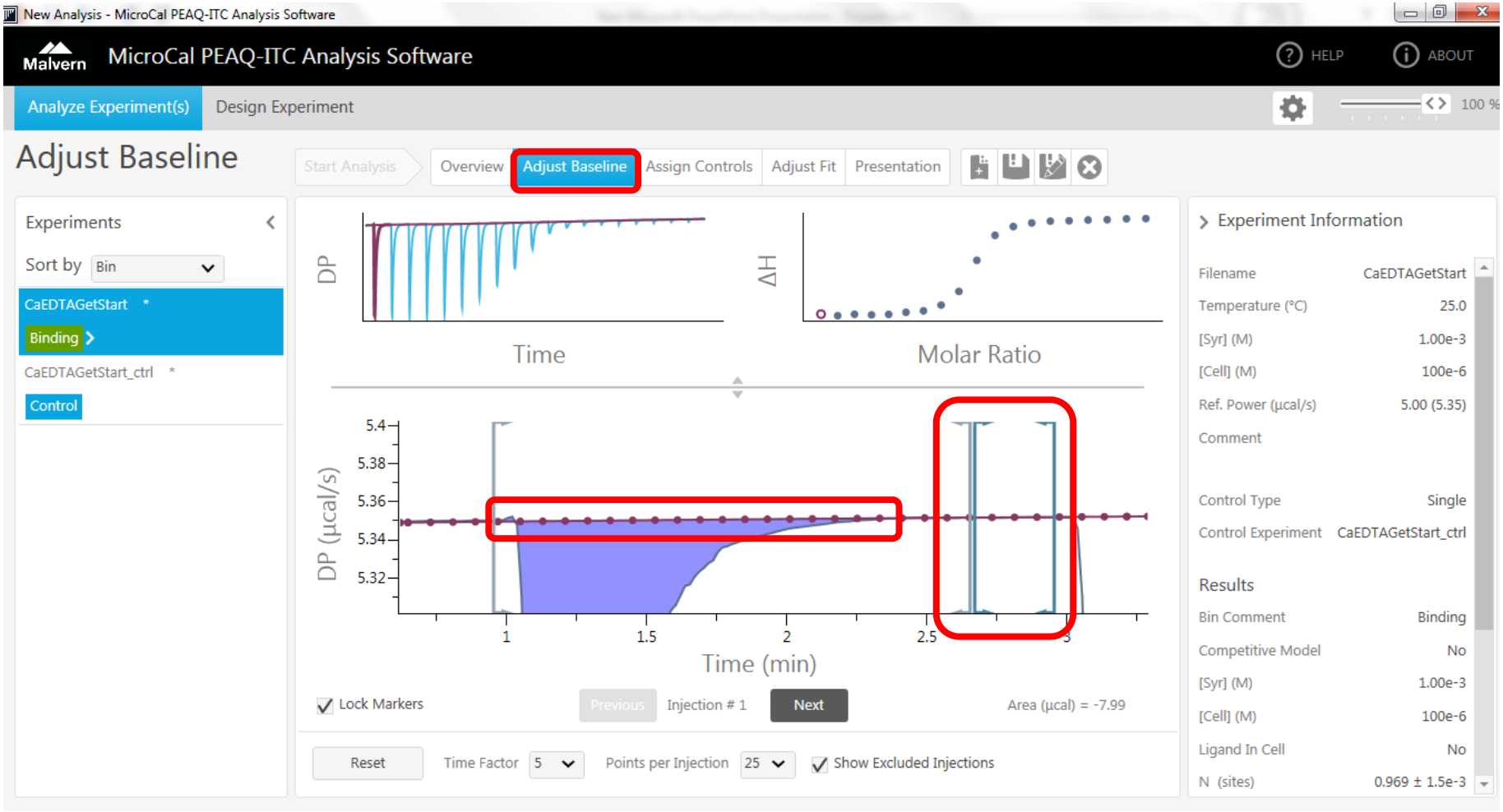
实验数据分析——指定对照实验



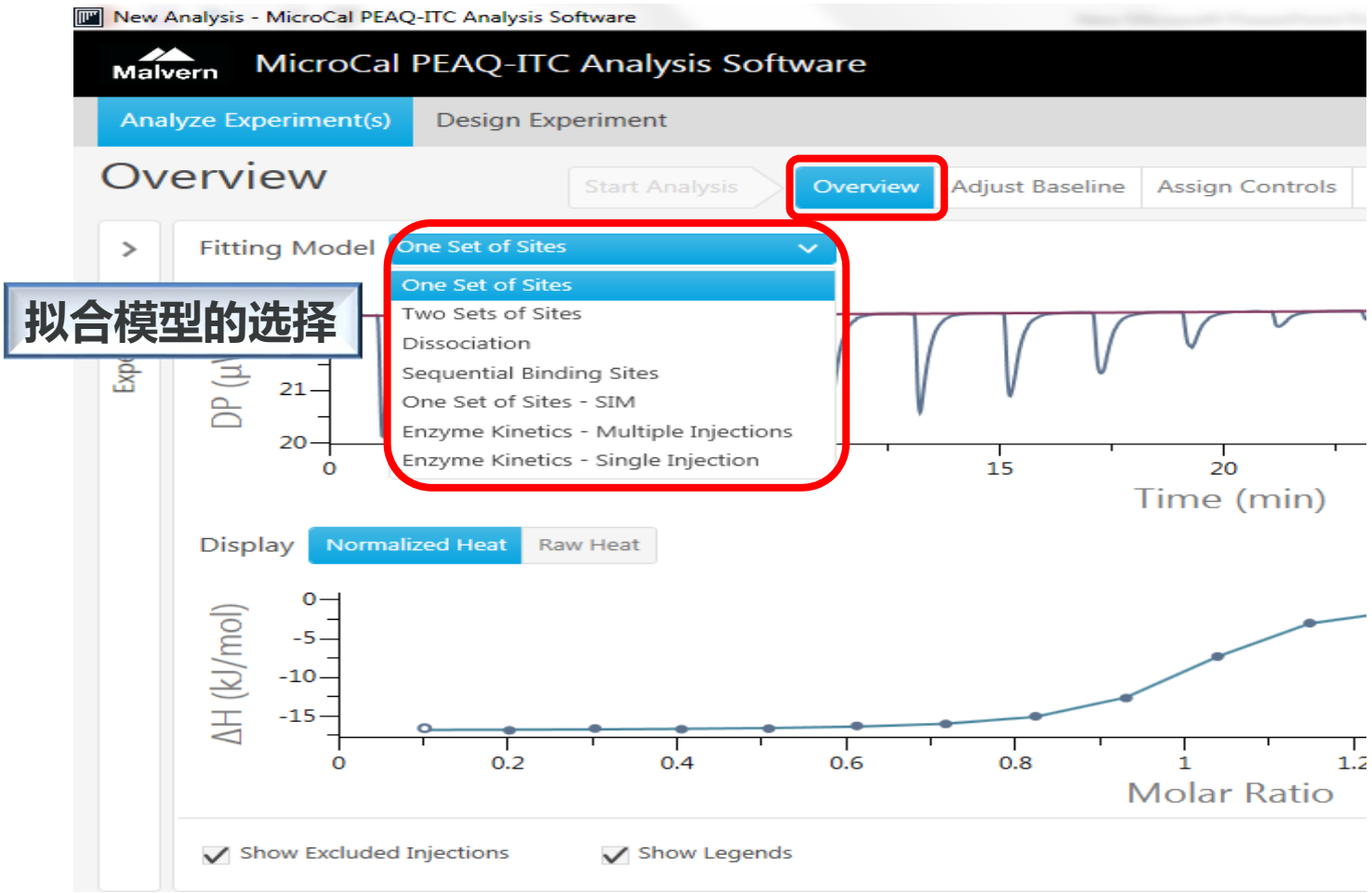
实验数据分析——指定对照实验



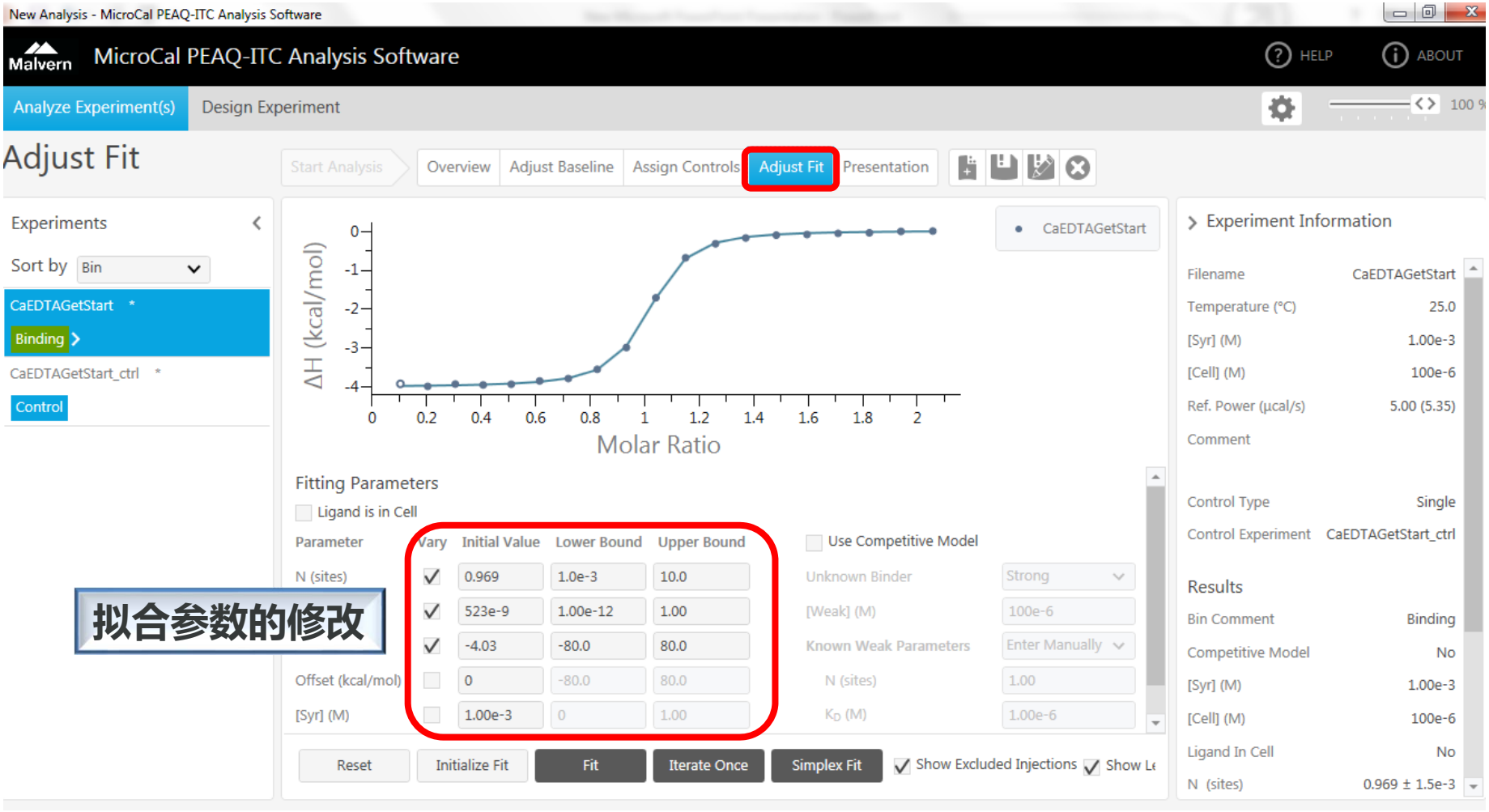
实验数据分析——调整基线和积分范围



实验数据分析——模型拟合


New Analysis - MicroCal PEAQ-ITC Analysis Software
Malvern
MicroCal PEAQ-ITC Analysis Software
Analyze Experiment(s)
Design Experiment
Overview
Start Analysis
Overview
Adjust Baseline
Assign Controls
Fitting Model
One Set of Sites
One Set of Sites
Two Sets of Sites
Dissociation
Sequential Binding Sites
One Set of Sites - SIM
Enzyme Kinetics - Multiple Injections
Enzyme Kinetics - Single Injection
拟合模型的选择
DP (µ
21
20
0
15
20
Time (min)
Display
Normalized Heat
Raw Heat
ΔH (kJ/mol)
0
-5
-10
-15
0
0.2
0.4
0.6
0.8
1
Molar Ratio
Show Excluded Injections
Show Legends

实验数据分析——模型拟合



拟合参数的修改

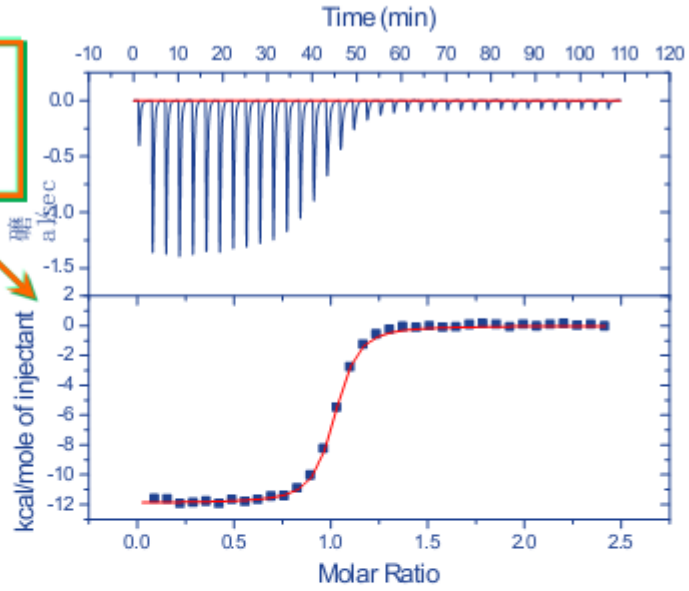
实验数据分析——导出最终结果

常见问题

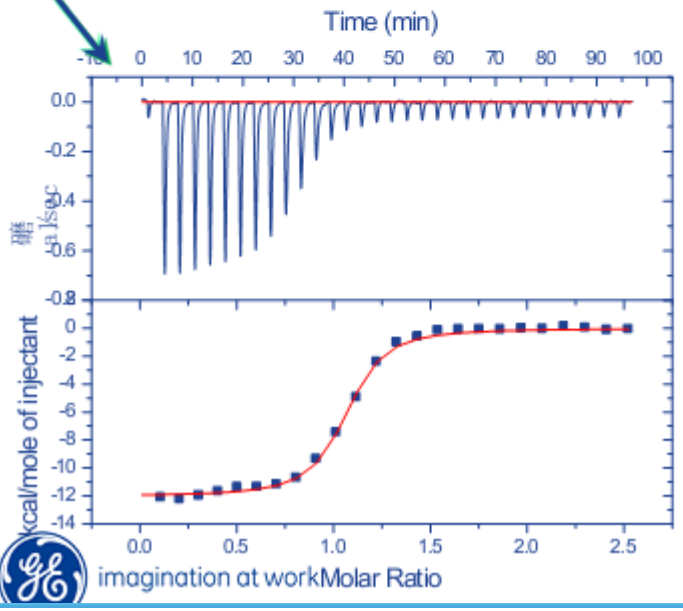
A to B 还是 B to A？

主要取决于样品浓度、溶解度、样品量、价格，以及是否有特别实验设计

29.5μM Protein titrated with
1.1mM Compound



11.5μM Compound titrated with
179μM Protein

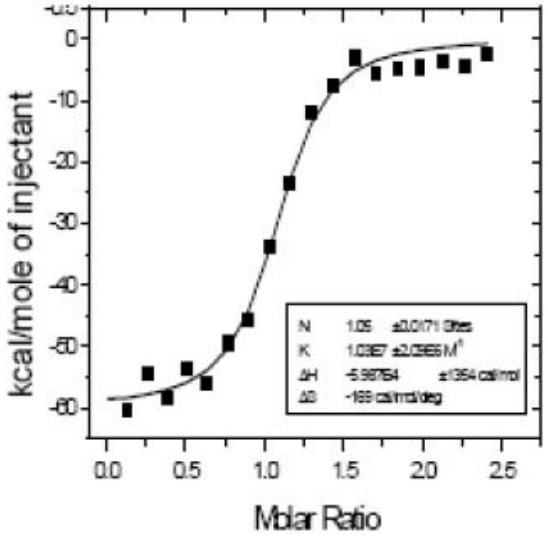


Parameter	*Ligand in syringe*	*Ligand in cell*
n	0.99	0.97
K_d	104 nM	105 nM
ΔG^o	-9.4 kcal/mol	-9.4 kcal/mol
ΔH^o	-11.9 kcal/mol	-12.4 kcal/mol
$T\Delta S^o$	-2.5 kcal/mol	-3.0 kcal/mol

N值不是0.5的倍数？

通常是蛋白质量问题，或定量不准

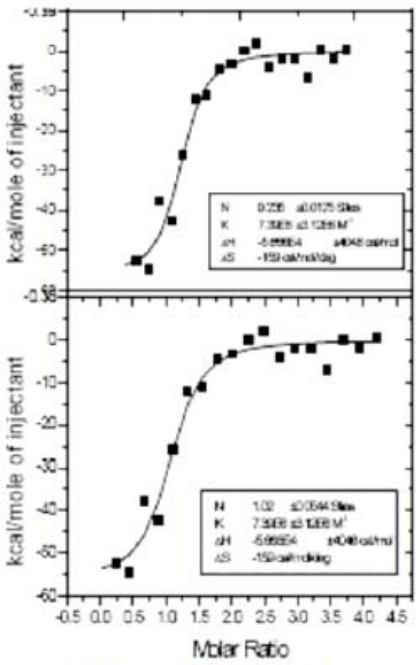
Peptide binding to Batch 1 Protein



N = 1.05
K_D = 97 nM

100% of Batch 1 protein Active based on Stoichiometry

Peptide binding to Batch 2 Protein

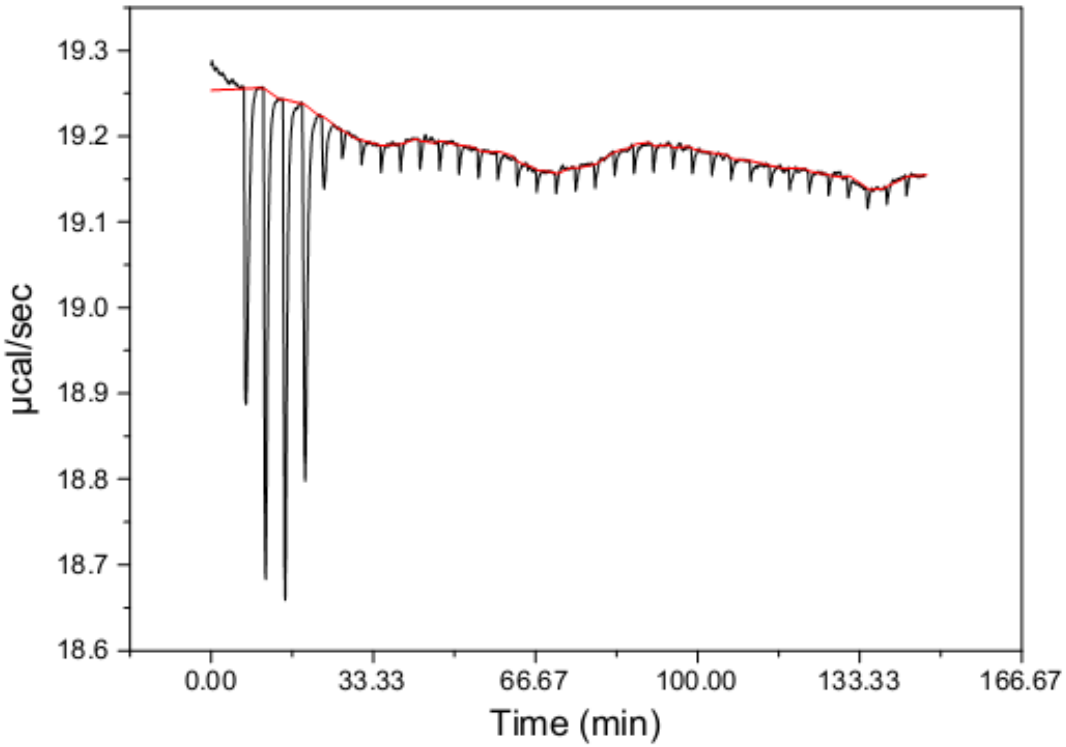


50 µM Peptide
10 µM Protein X
N = 0.235
K_D = 135 nM

Re-analyzed as
2.3 µM Protein X
N = 1.02
K_D = 135 nM

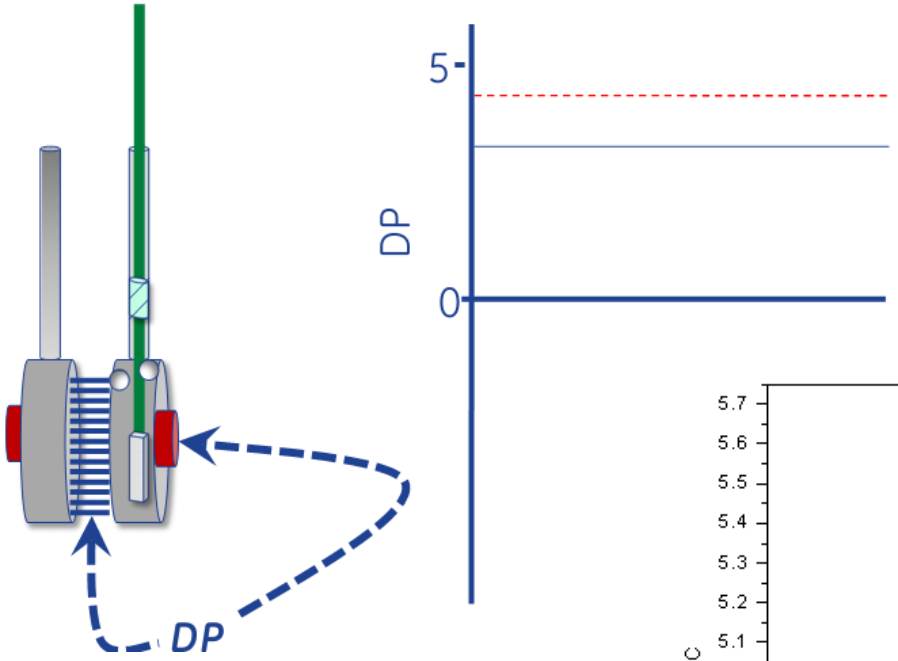
23% of Batch 2 protein Active based on Stoichiometry

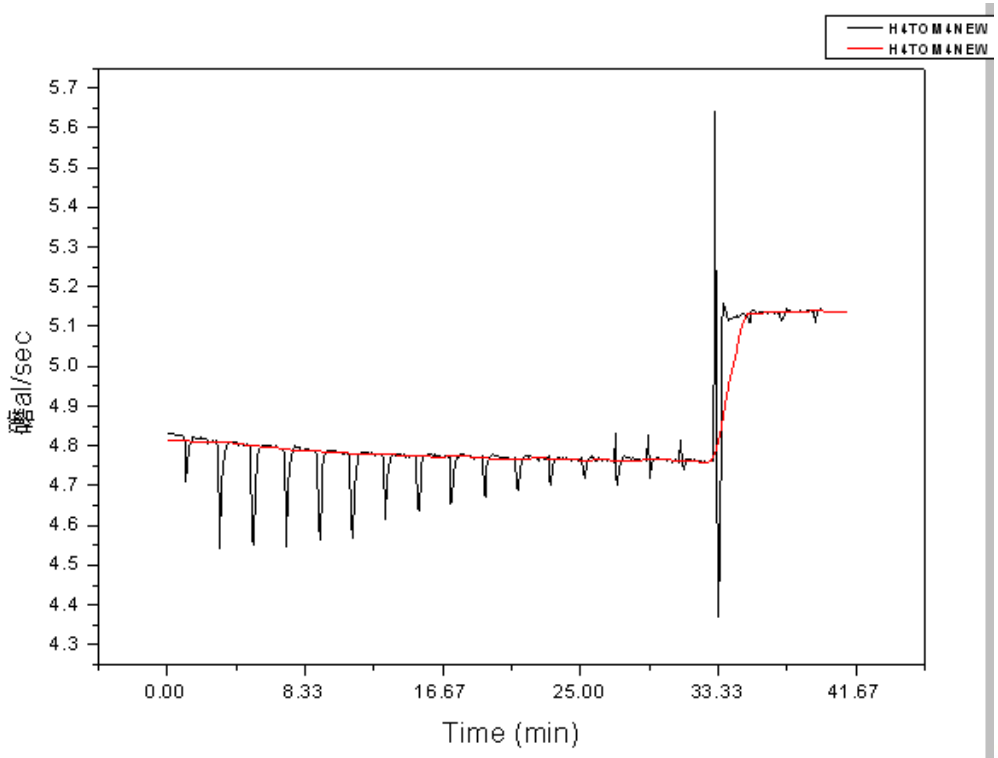
图不好看？



需调整浓度，比如上图，应该增加样品池浓度

异常峰

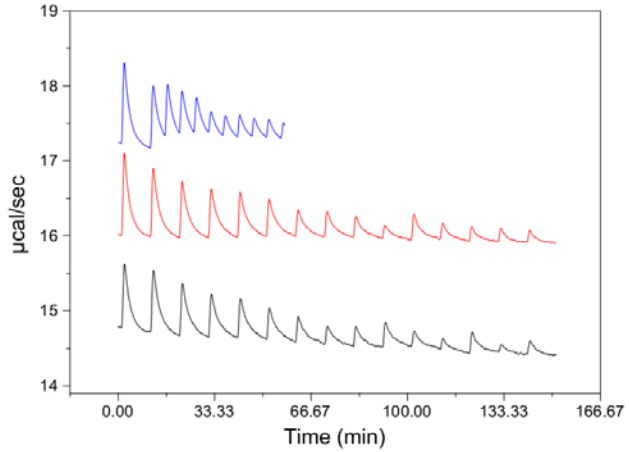


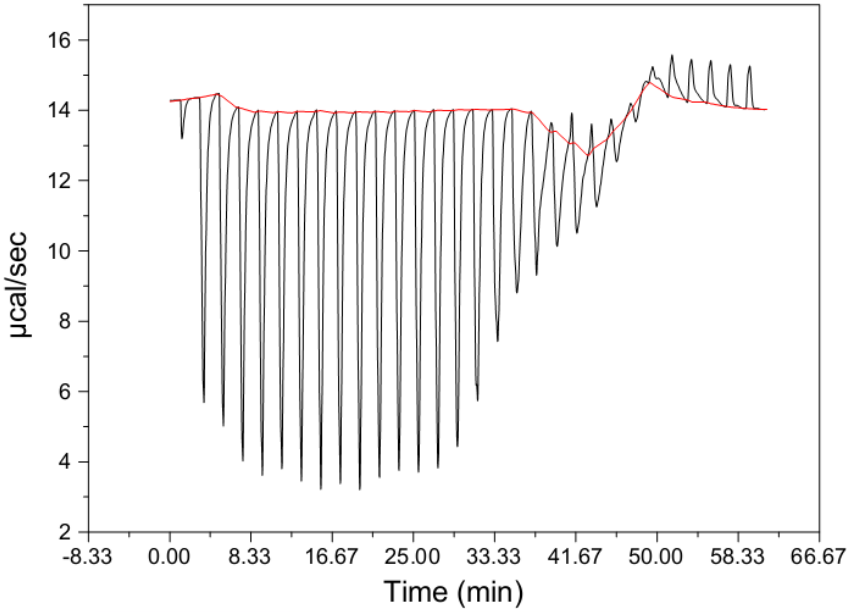


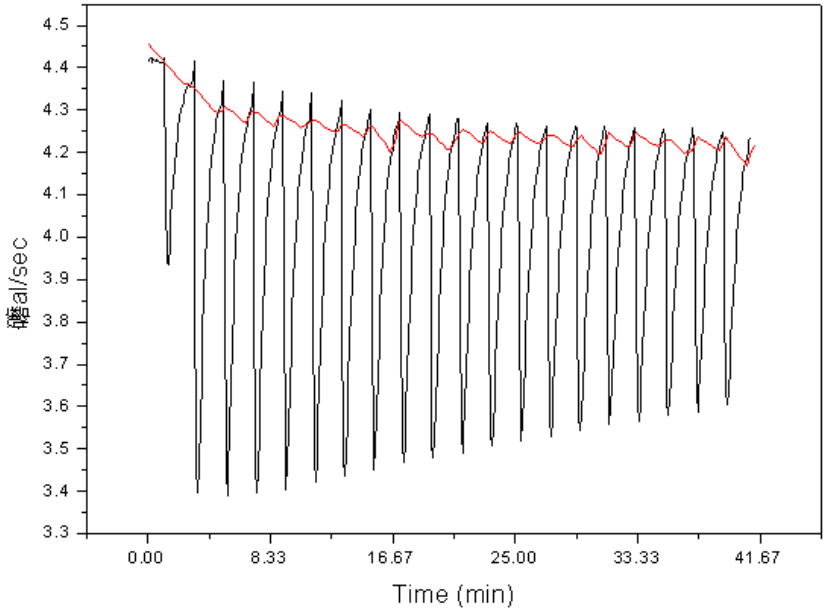
往往是气泡，或沉淀（颗粒）所导致

热量回不到基线

间隔时间不够







小结1 - 常见问题原因

- 没有结合/热量太小（浓度太低）
- 浓度比不合适
- Buffer不匹配
- 样品池不干净、上样未满、有气泡
- 参比池污染
- 间隔时间不够
- 蛋白聚集/沉淀
- Reference power太低或太高
- 仪器滴定针问题（弯曲、磨损、滑出等）

小结2 - 获取高质量数据的关键步骤

1. 实验设计合理，样品浓度合适
2. 好的样品准备
3. 准确的浓度测定
4. 实验操作：彻底清洗样品池和滴定针，水滴水确认仪器状态与洁净度，合理的滴定参数
5. 合适的空白对照
6. 合理的数据分析

更多内容我们在上机操作时进行交流

谢谢！