



# PTX-DCA 杂化晶体的制备与评价

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参考文献与致谢



制备与表征



结构确证与体外性质考察



体外抗肿瘤活性研究





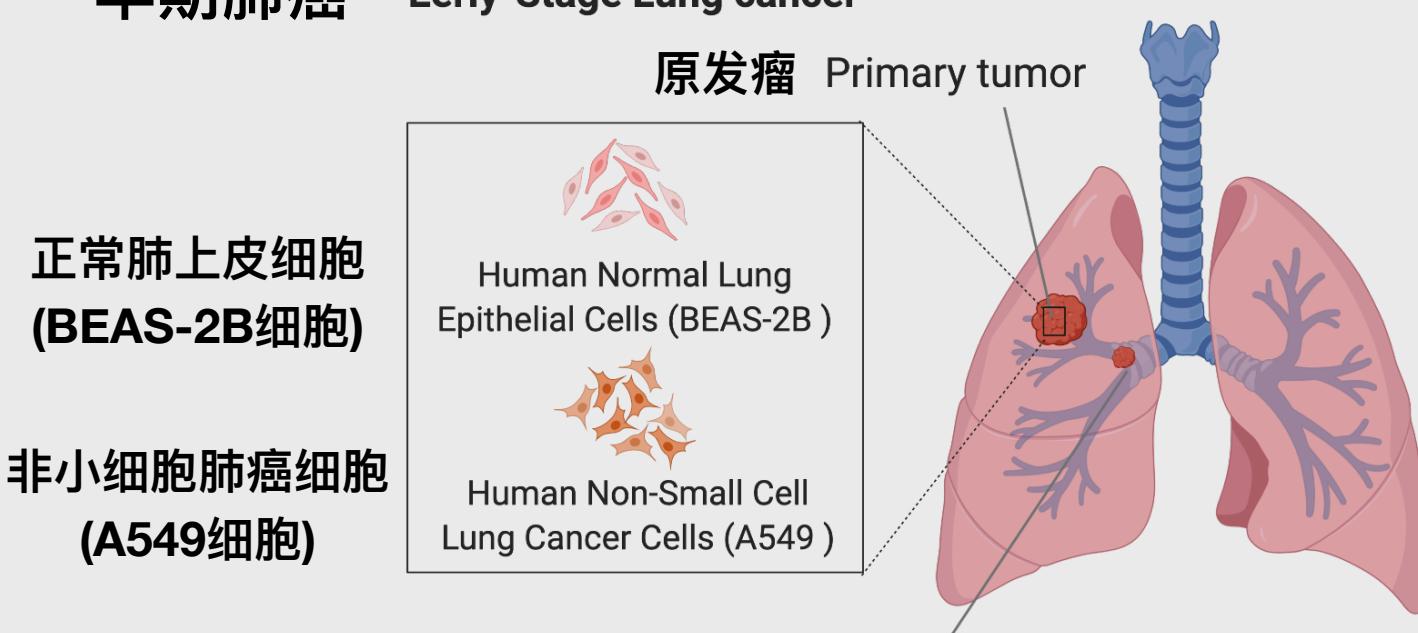
# 研究背景

## 非小细胞肺癌

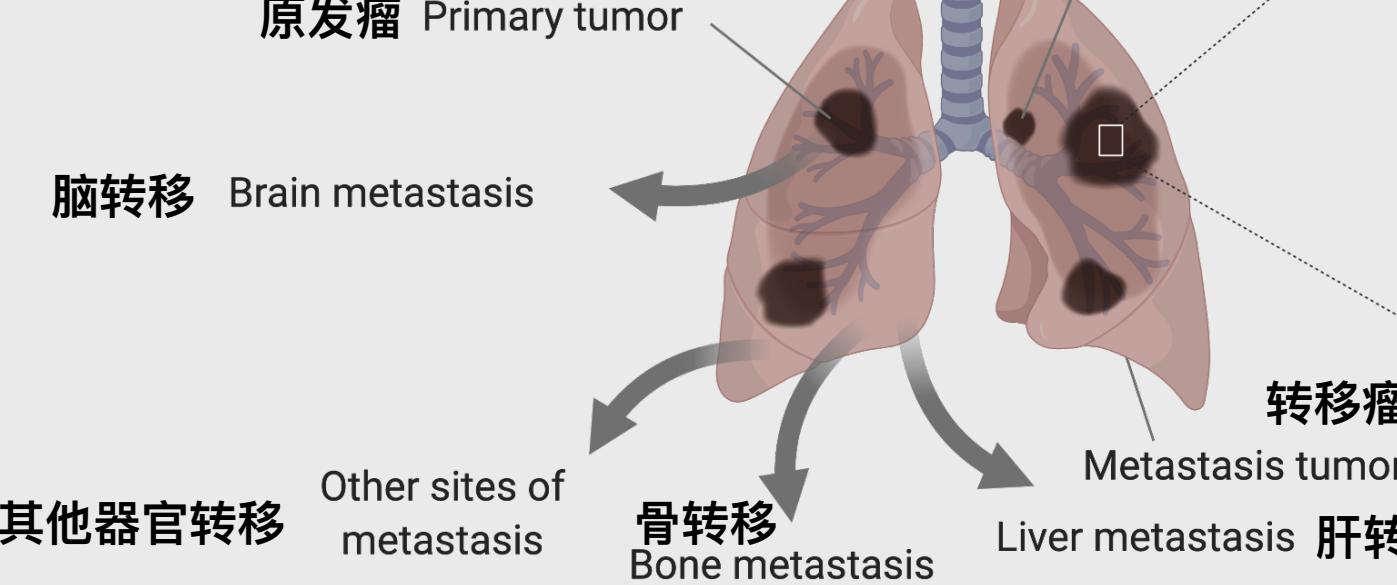
### 1. Non-small-cell lung carcinoma (NSCLC)

早期肺癌

Early-Stage Lung cancer

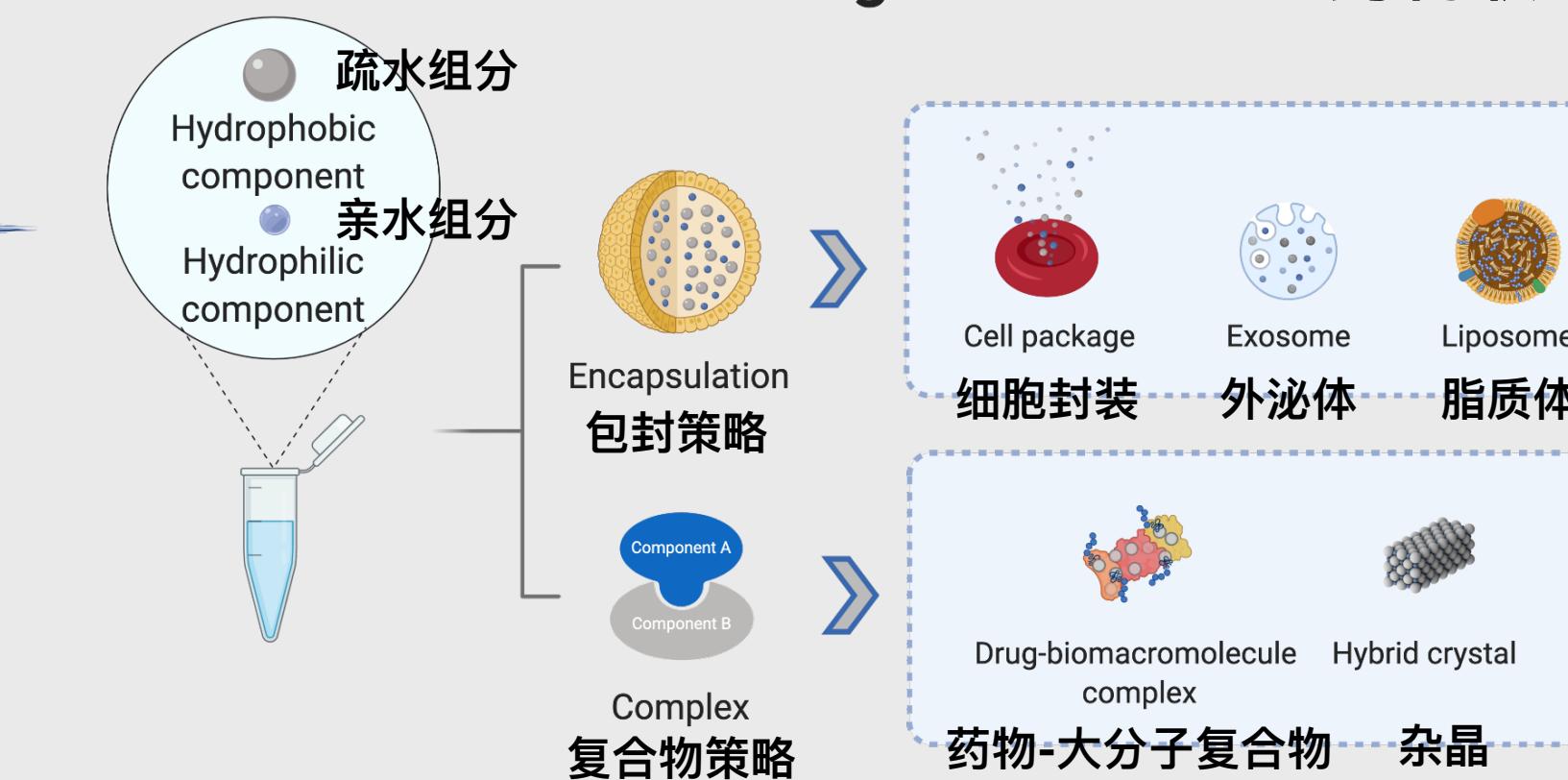


转移性肺癌 Metastatic Lung cancer



增加化疗敏感性而不引起耐药性

## 2. Drug Combination 药物联用策略

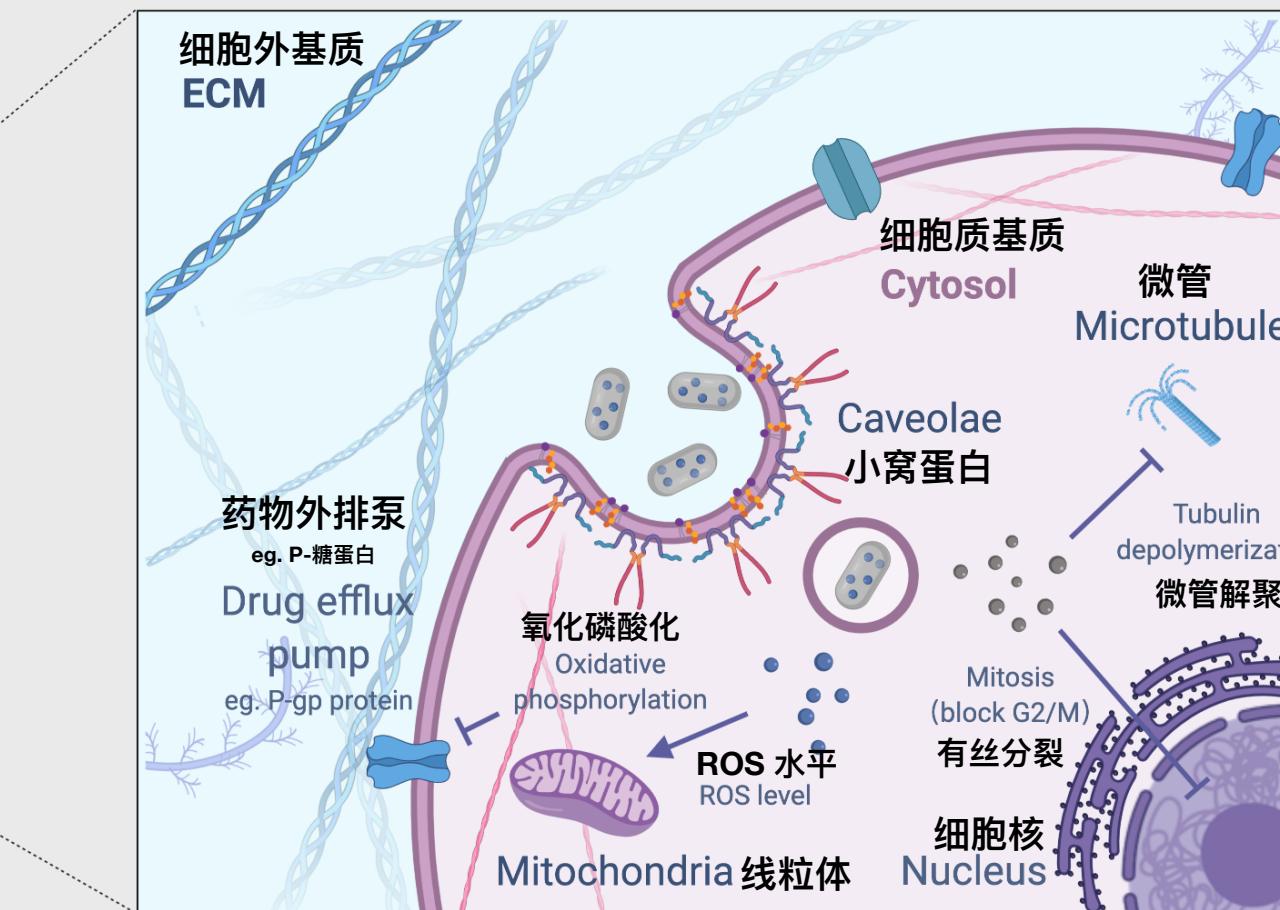


载药量较低  
药物比例难控制

药物递送药物策略构建  
杂化晶体体系

## 疏水性不同的多组分共递送

### 3. Delivery efficacy 药物递送效率



通过形貌控制介导非溶酶体入胞途径

通过表面电荷使晶体靶向特定细胞器

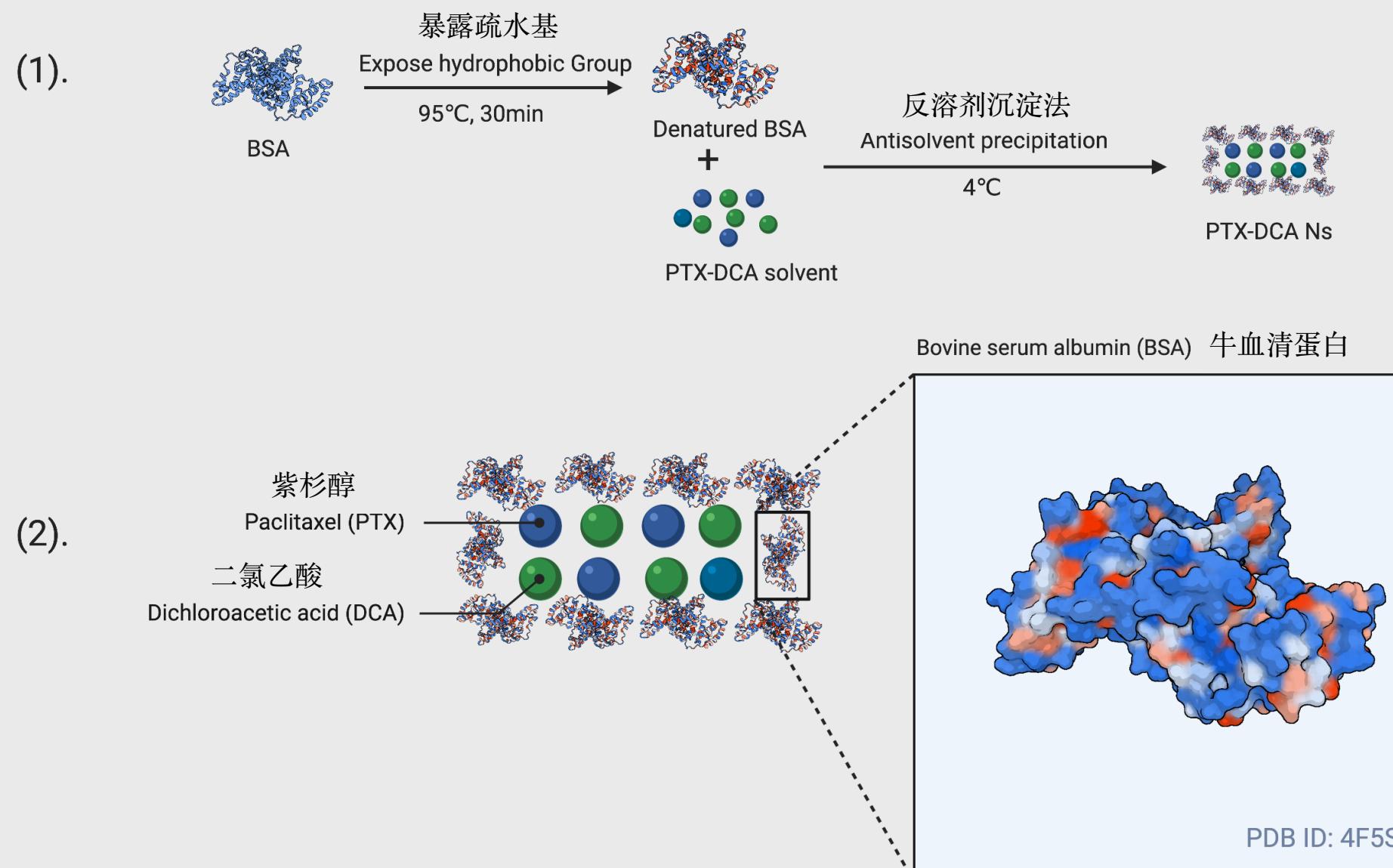
血浆清除率和细胞摄取的矛盾



# 课题设计

## 摘要图

### 紫杉醇-二氯乙酸纳米杂化晶体结构与蛋白可视化 PTX-DCA Ns Structure and Protein Visualization



(1). 通过反溶剂沉淀法制备PTX-DCA Ns (2). BSA蛋白结构可视化，  
蓝色部分为亲水基，红色部分为疏水基

通过药物递送药物策略逆转耐药性和促进细胞凋亡

### 合成与表征

#### 杂化晶体制备

反溶剂沉淀法<sup>[3]</sup>

#### 杂化晶体表征

粒径表征: DLS动态光散射

形貌表征: TEM成像

载药能力: HPLC分析

### 结构确证与体外性质考察

#### 结构确证

X射线粉末衍射 (PXRD)

荧光共振能量转移 (FRET)

圆二色光谱 (CD)

#### 体外性质

体外稳定性考察

### 体外抗肿瘤活性研究

#### 细胞毒性与药物协同效应研究

MTT法 & Chou-Talaly协同作用模型

#### 细胞凋亡

Annexin V-PI双染法流式细胞术测定

#### 细胞周期阻滞

PI单染法流式细胞术测定

## 流程图



# 工作汇报

# 1

## 制备与表征

制备

反溶剂沉淀法<sup>[3]</sup>

问题与解决

组分水溶性差异导致制备工艺需要调整：  
采用控制变量法考察单一变量；采用响应面分析法权衡多变量

表征

粒径测量

TEM成像

Zeta 电位

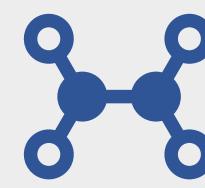
载药能力

假设与验证

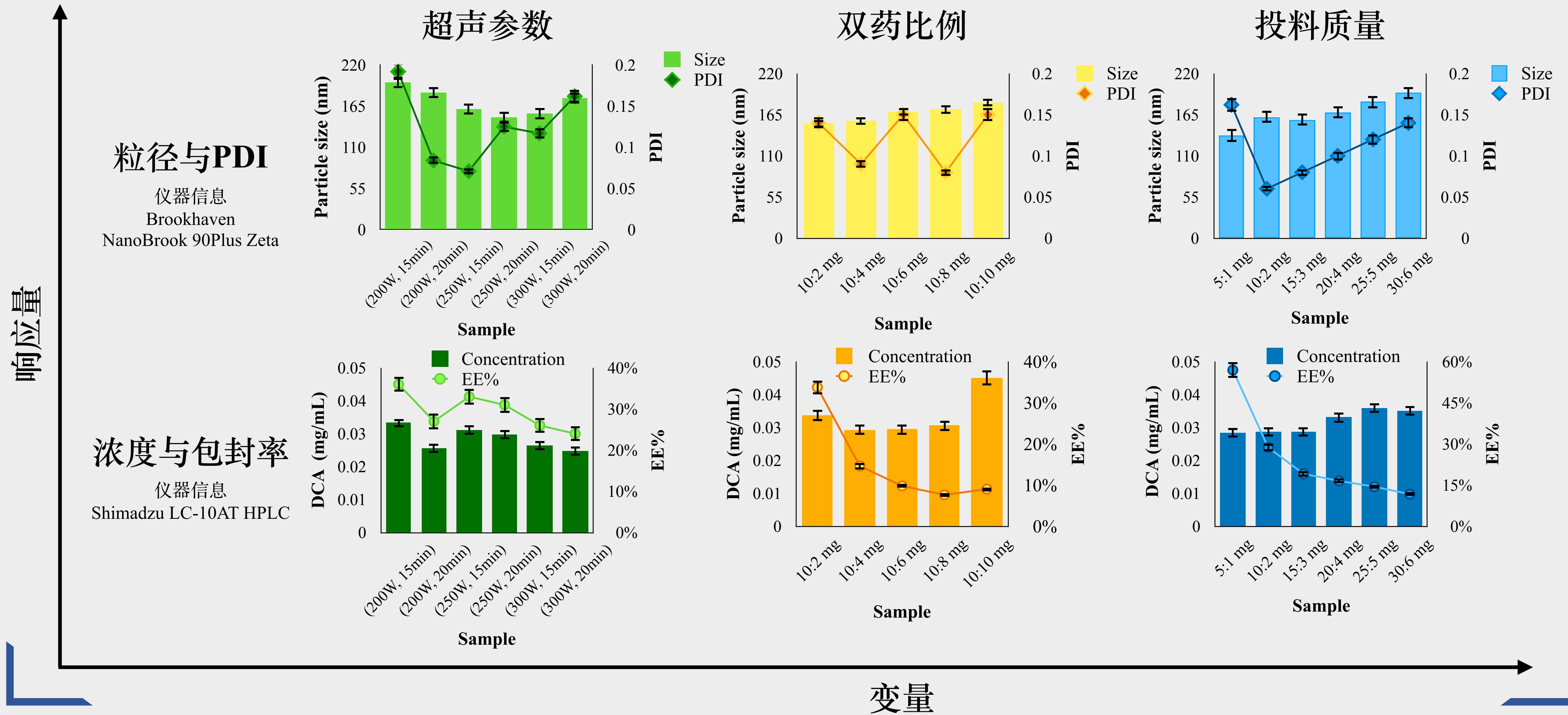
具备特定性状参数的纳米粒子可介导非溶酶体入胞途径<sup>[3, 5, 6]</sup>

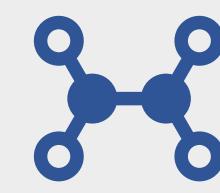
特定电荷可用于靶向特定细胞器<sup>[4]</sup>

单一组分浓度能达到上游学科文献报道的数量级<sup>[4]</sup>



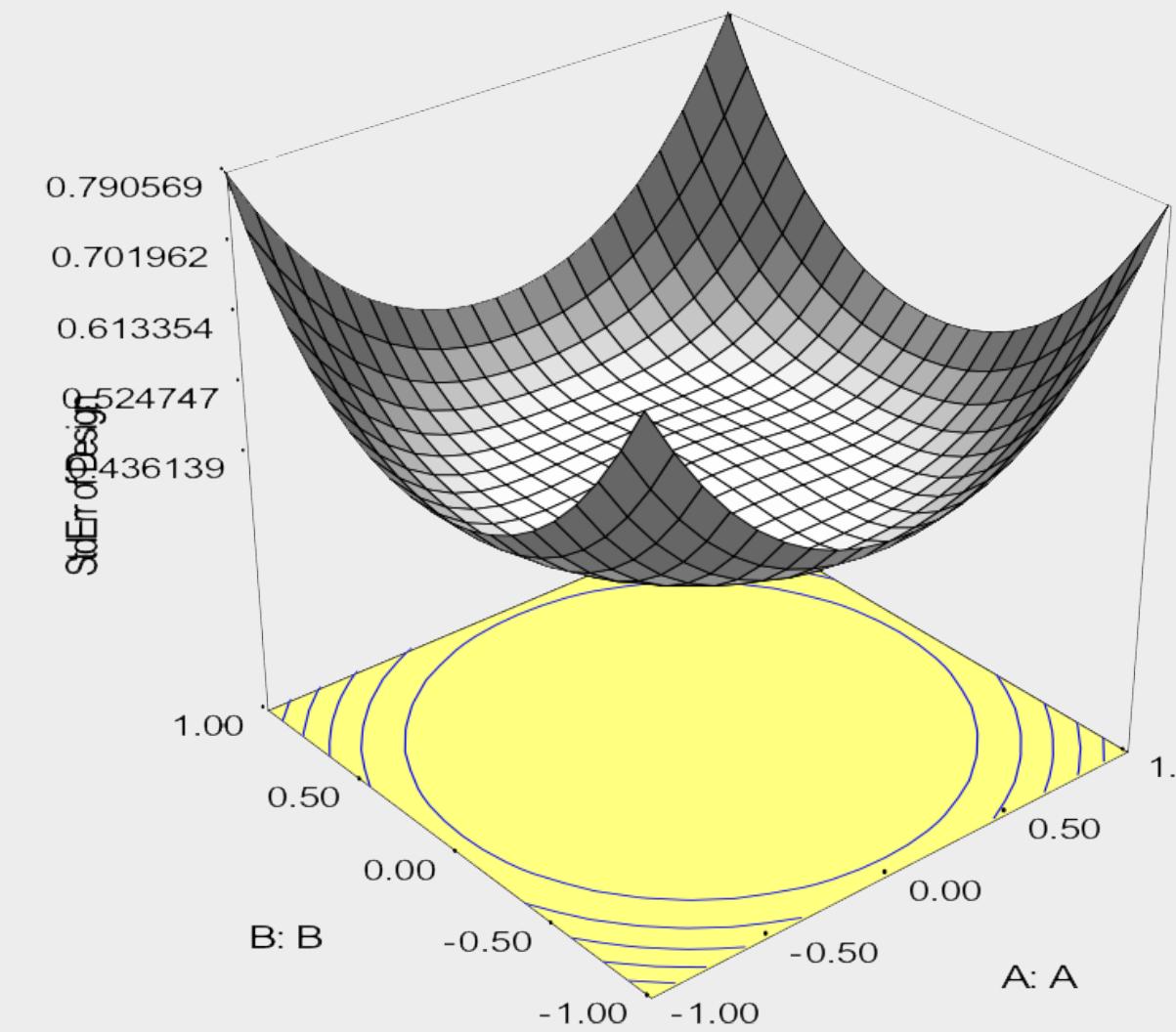
# 制备与表征 - 控制变量法优化制备工艺



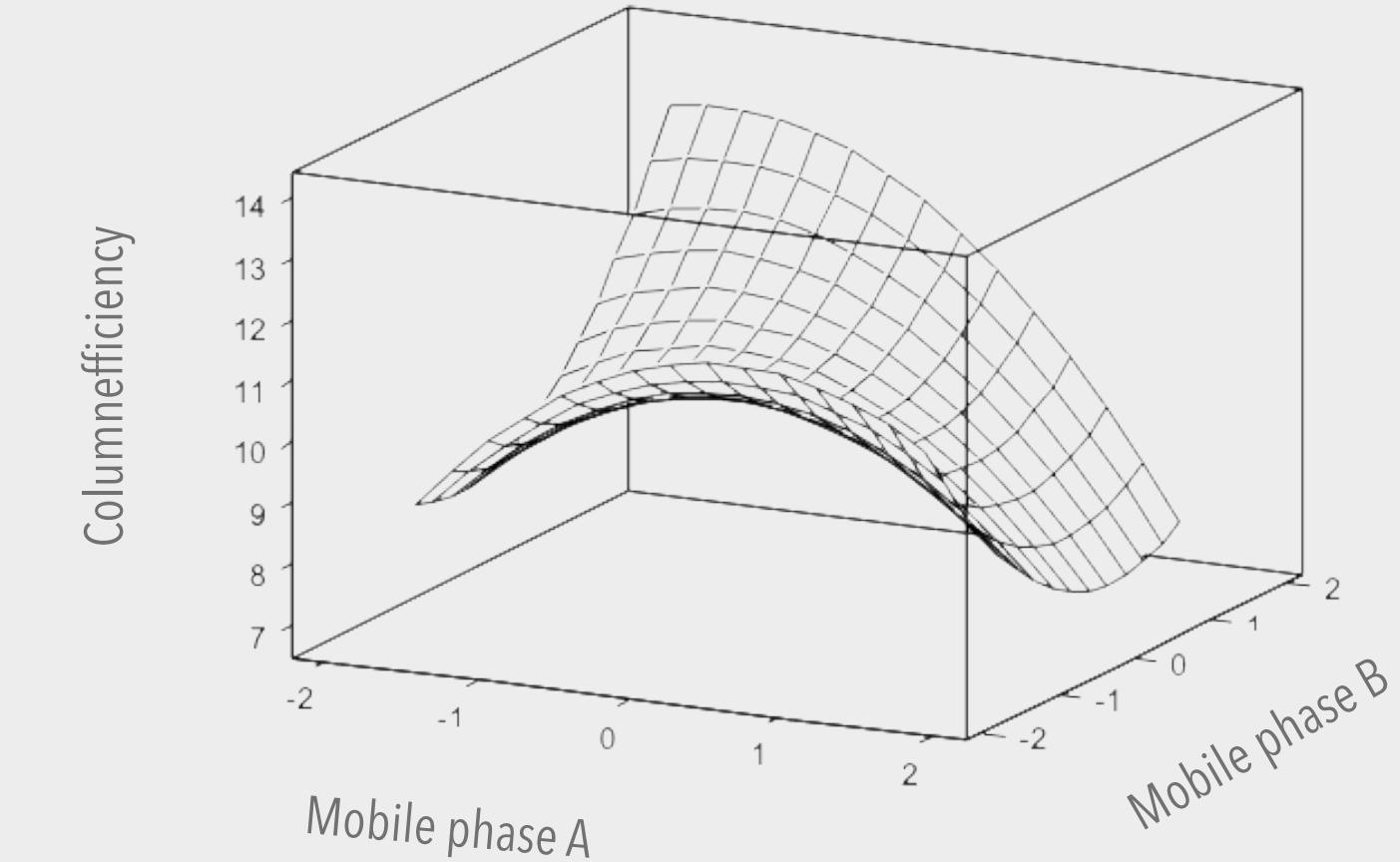


# 制备与表征 - Design Expert法分析多变量响应

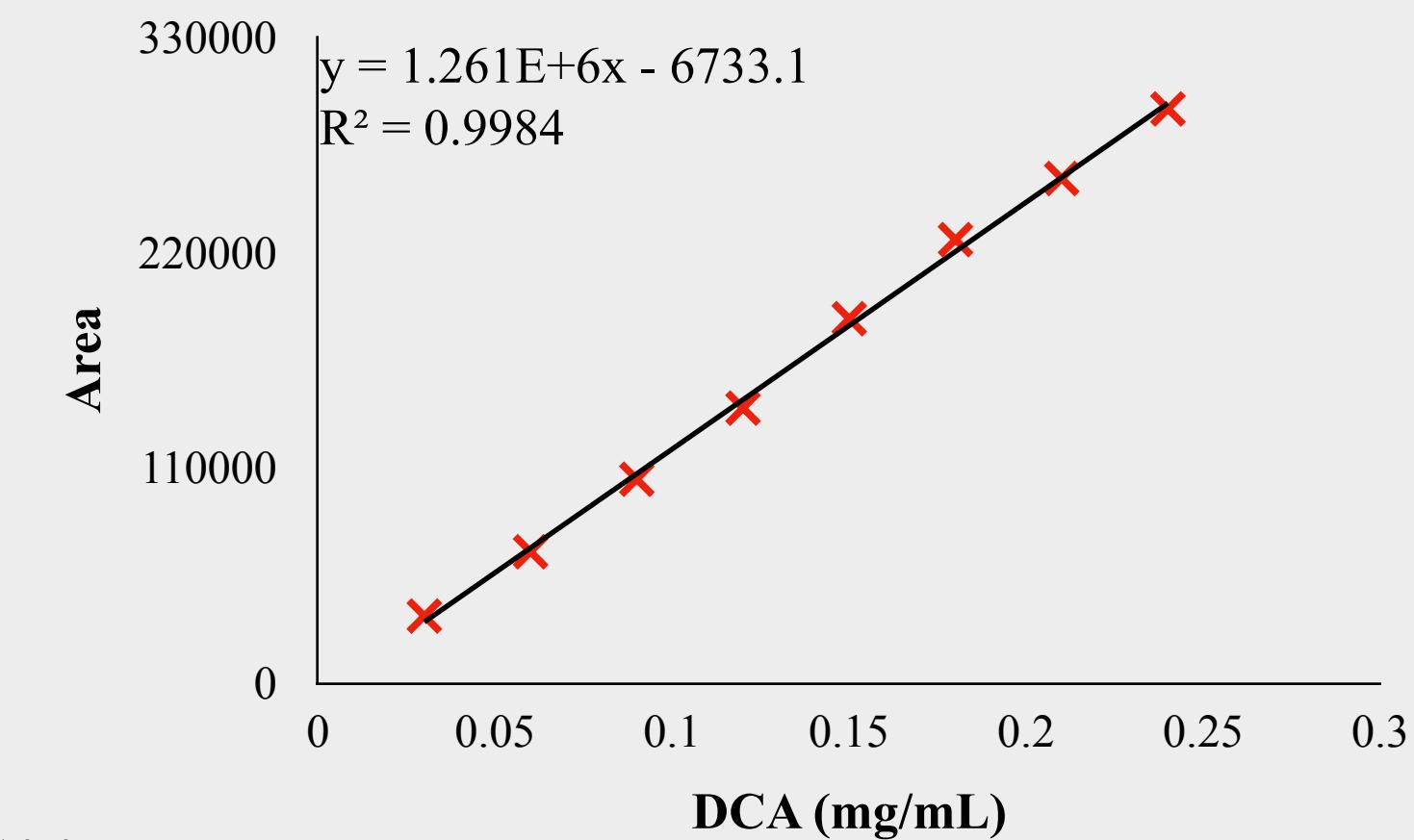
Box-Behnken 模型响应面设计法优化制备工艺



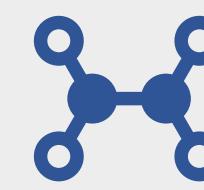
混料模型响应面设计法优化色谱条件



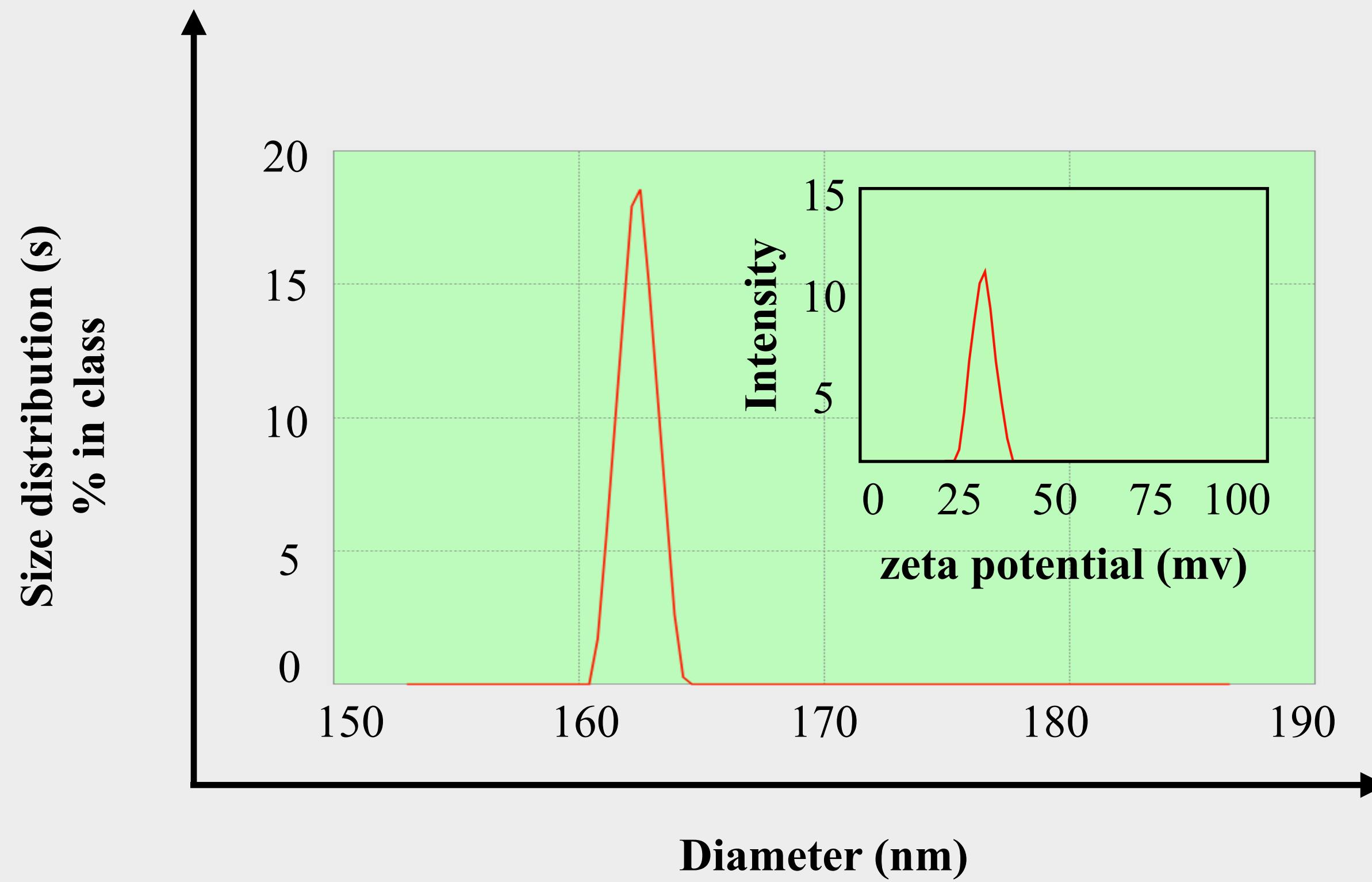
优化后HPLC 标准曲线



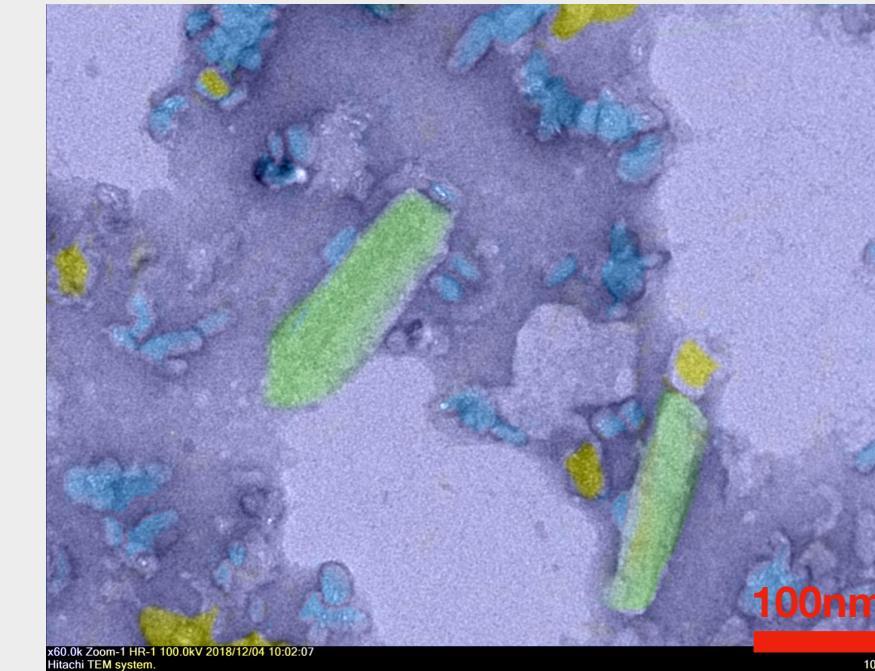
软件信息：Design Expert v10.0.7



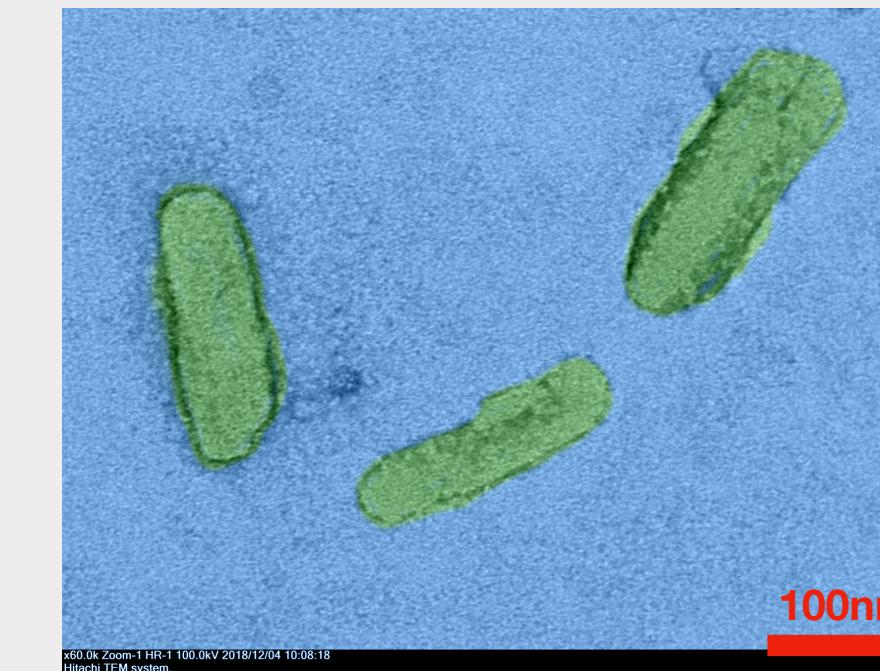
## 制备与表征 - 实验结果



制备流程优化前杂晶TEM图像  
(绿色为杂晶；蓝色为PTX单晶；黄色为蛋白碎片)



制备流程优化后杂晶TEM图像  
(绿色为杂晶)



仪器信息: Brookhaven NanoBrook 90Plus Zeta  
JEOL JEM-1230 TEM



# 工作汇报

## 2

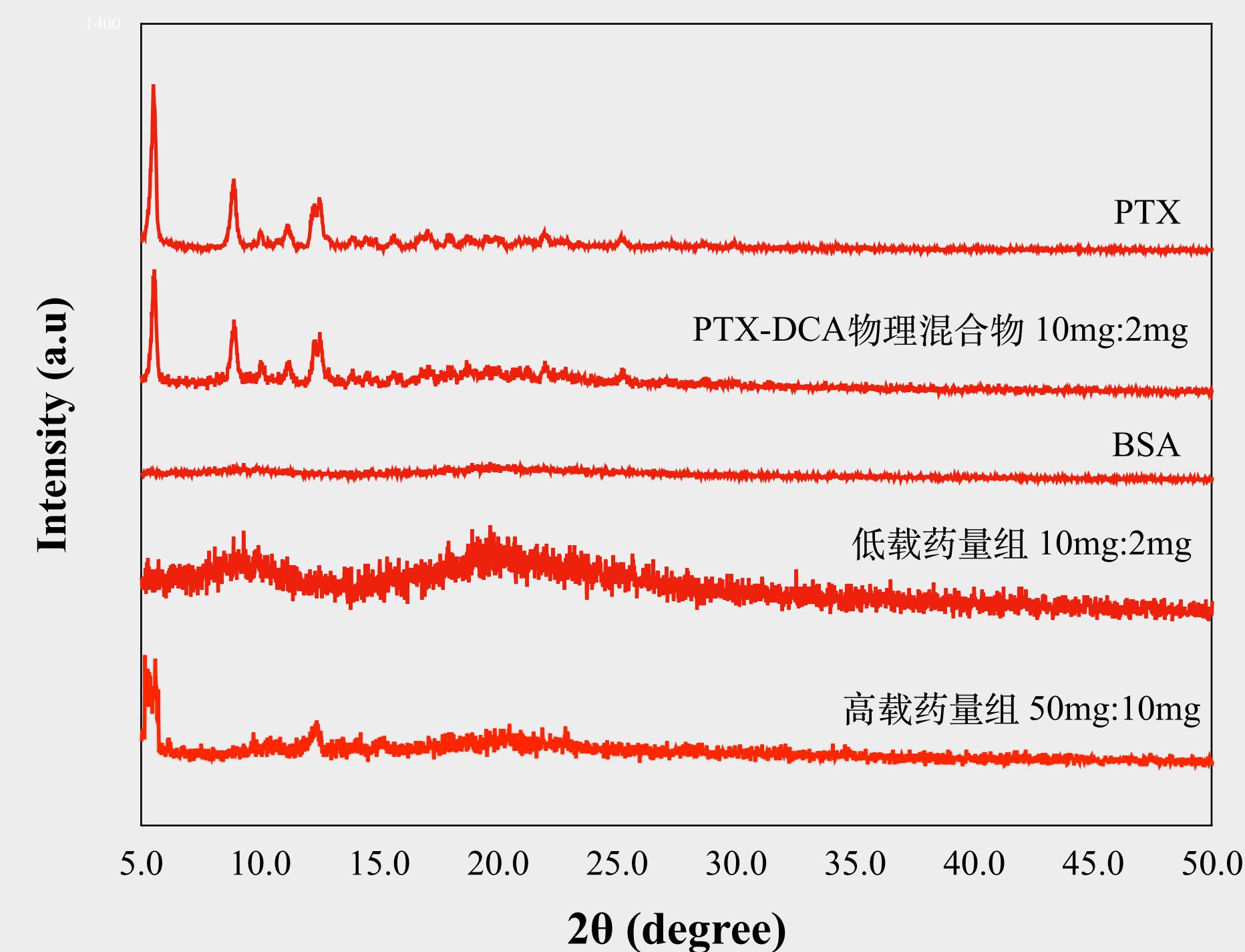
# 结构确证与体外性质考察



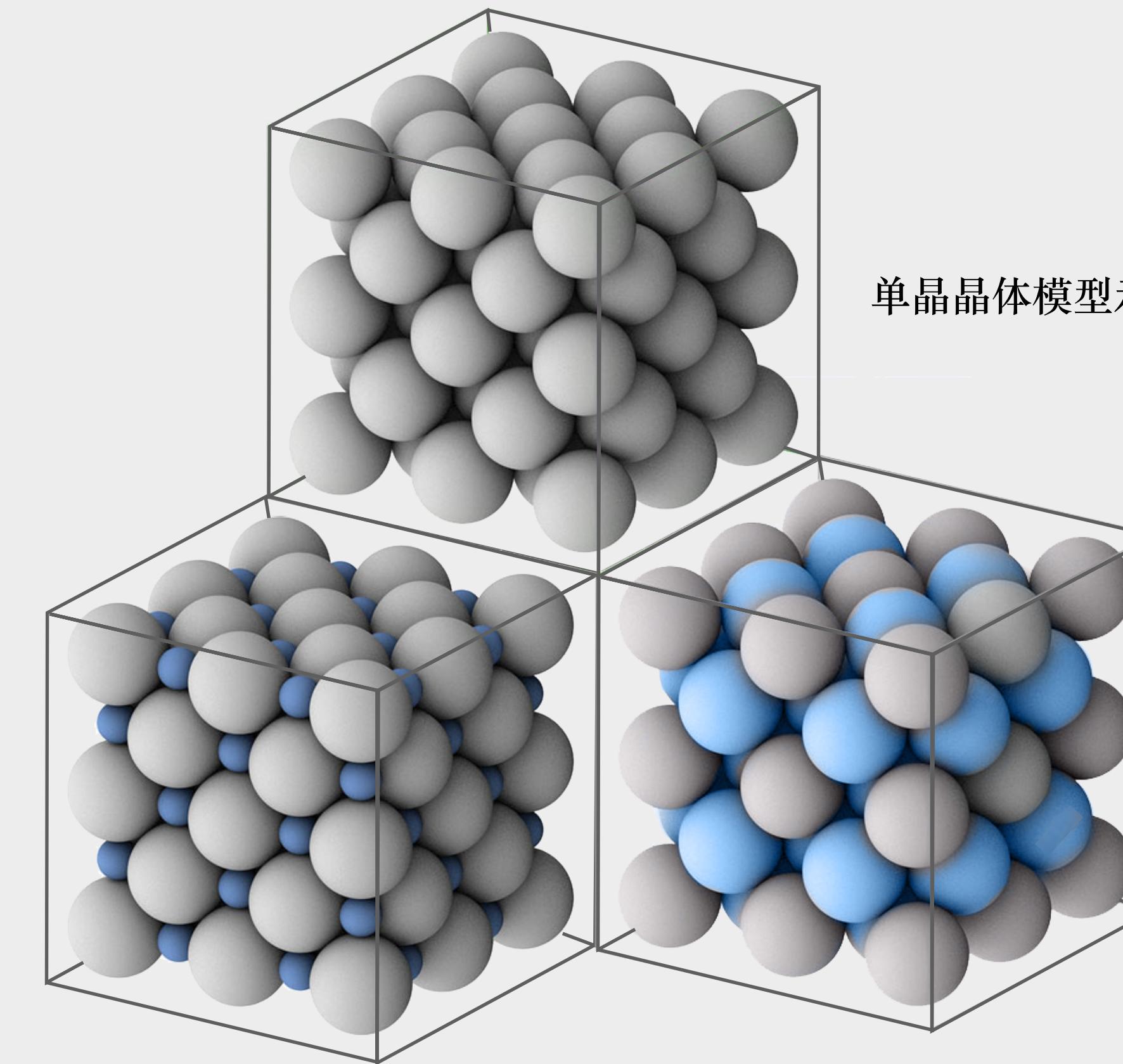


# 结构确证与体外性质考察 - X射线粉末衍射法研究单一组分与杂化晶体结构变化

PXRD图谱



单晶晶体模型示意图



吸附型杂化晶体模型示意图

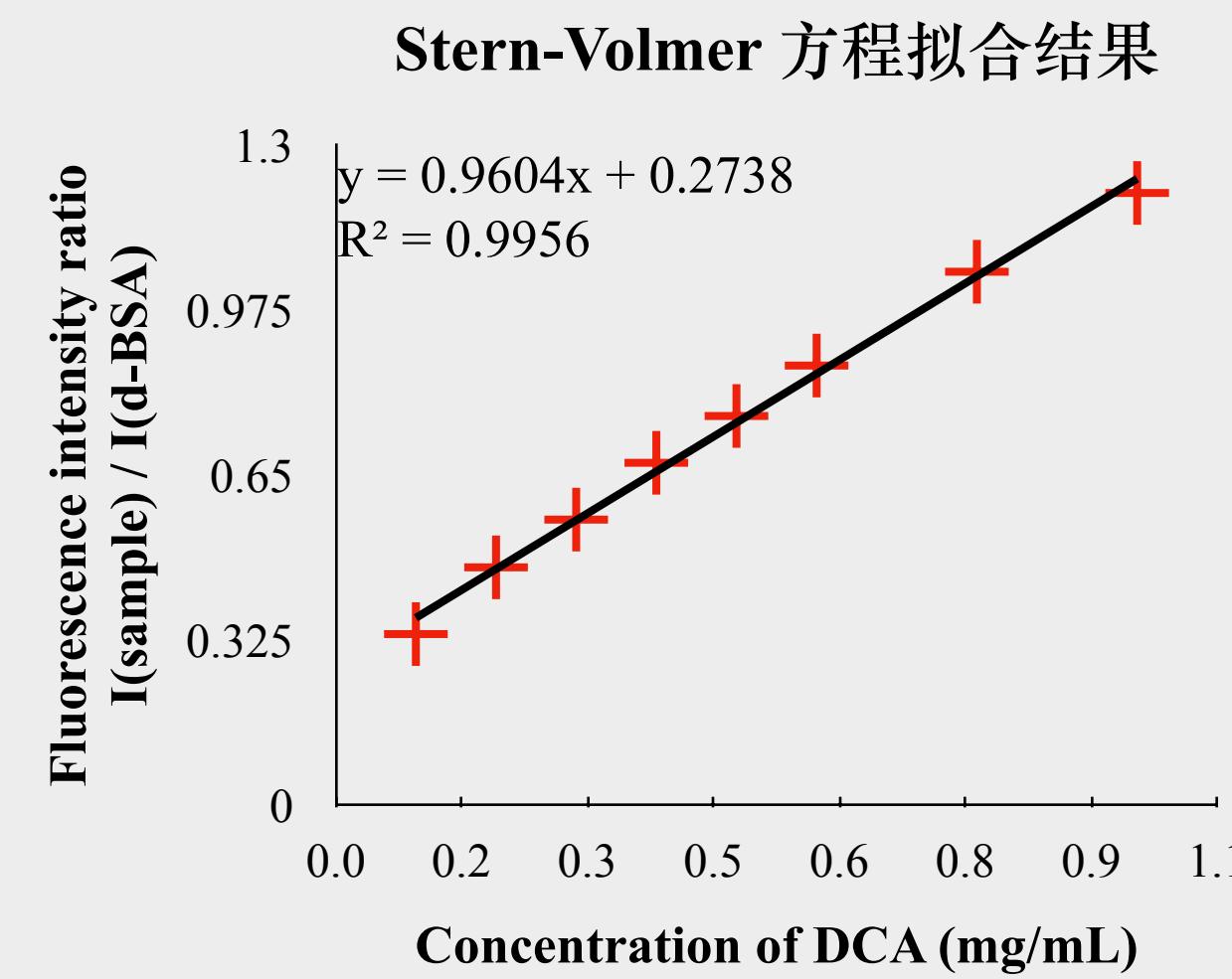
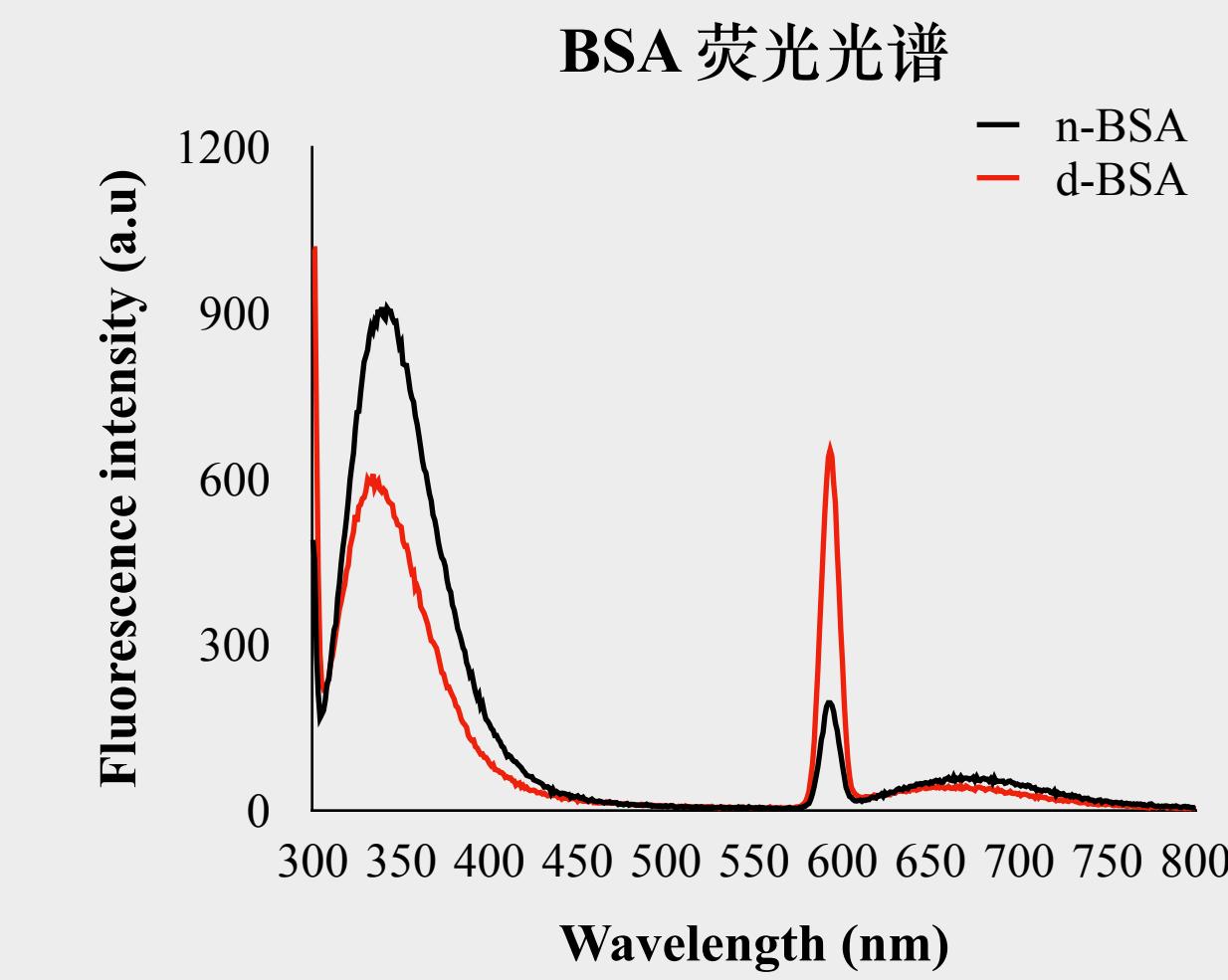
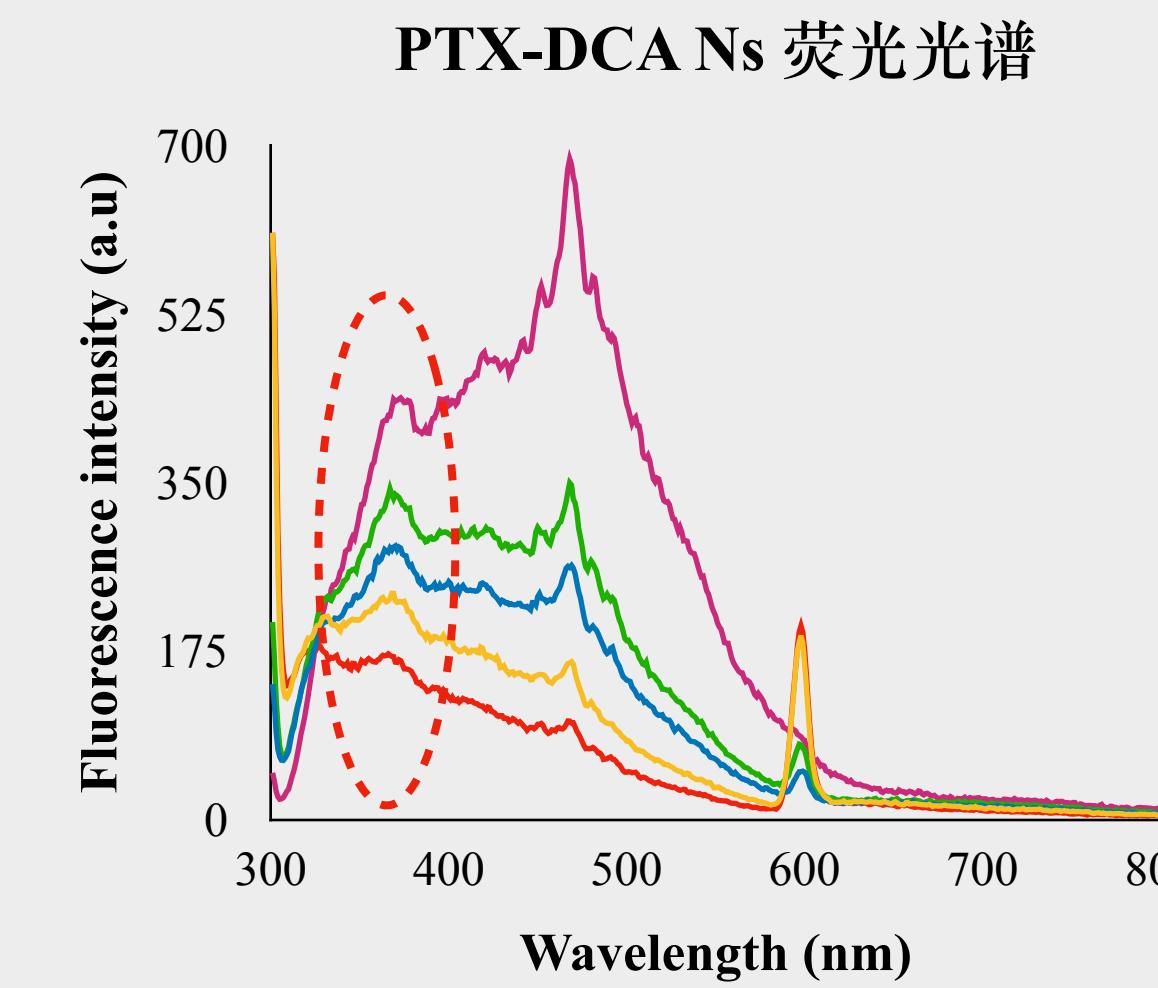
共晶型杂化晶体模型示意图

仪器信息：Brookhaven Bruker D8 Advance PXRD

软件信息：MDI Jade v6.0, 3D Studio Max 2017

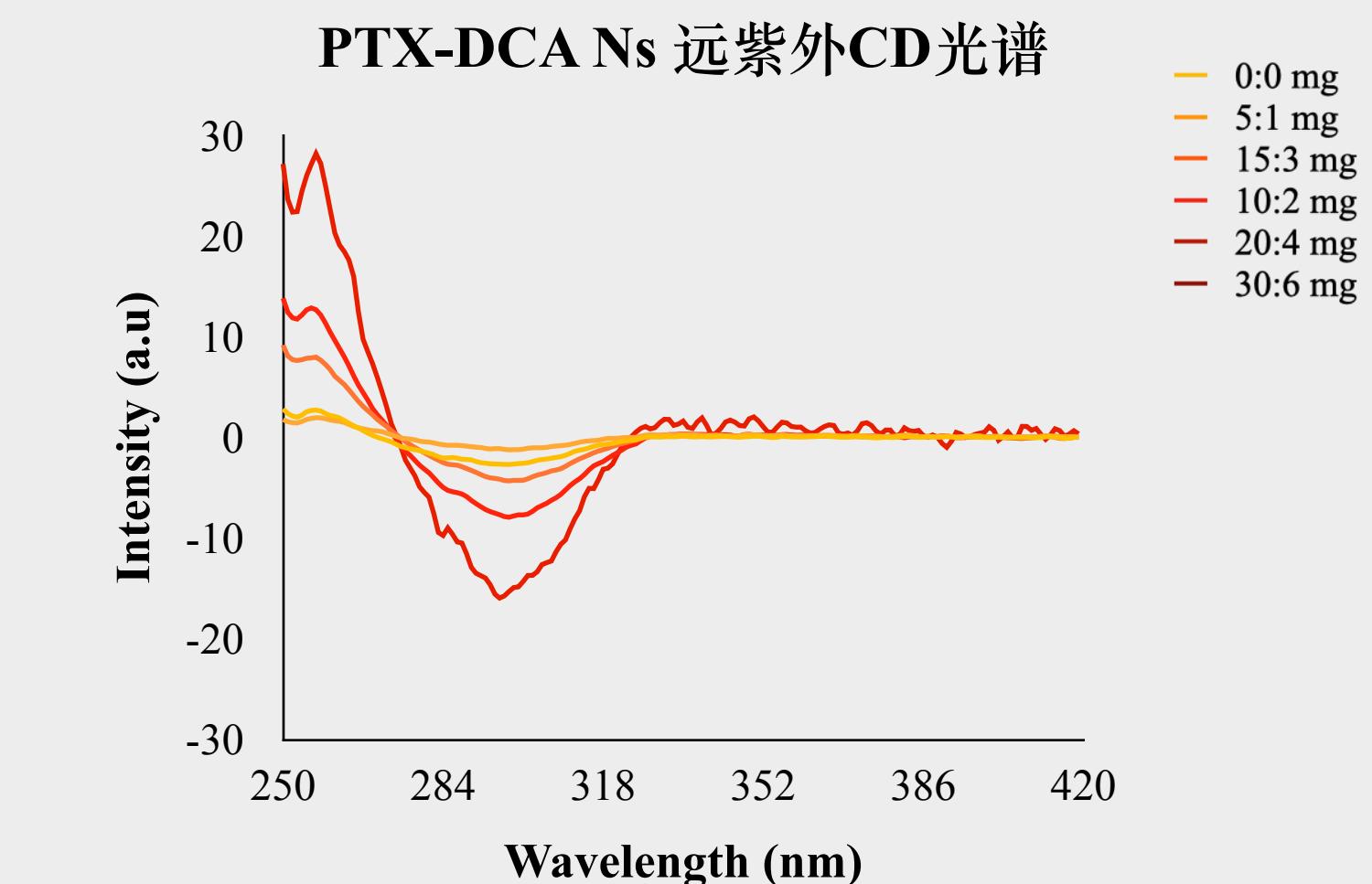
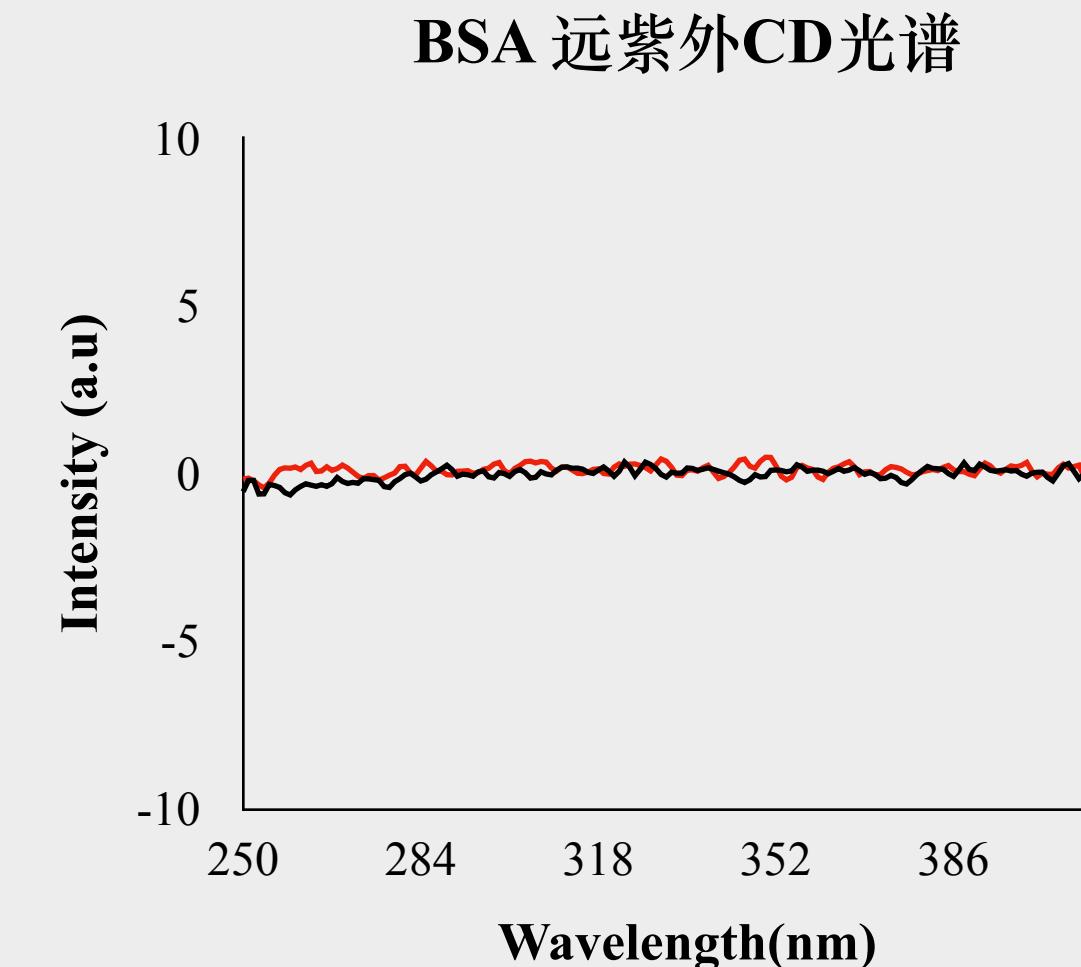
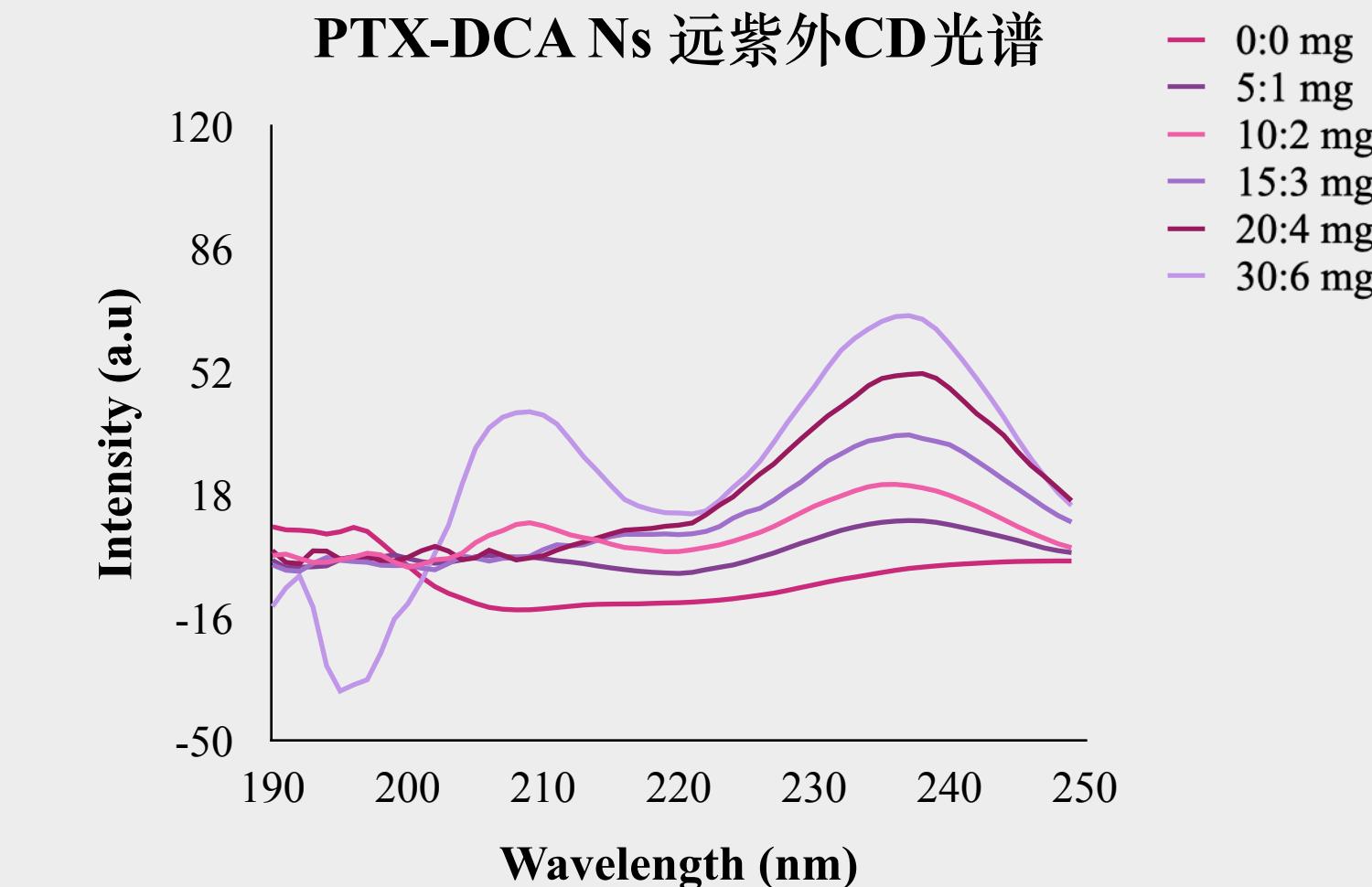
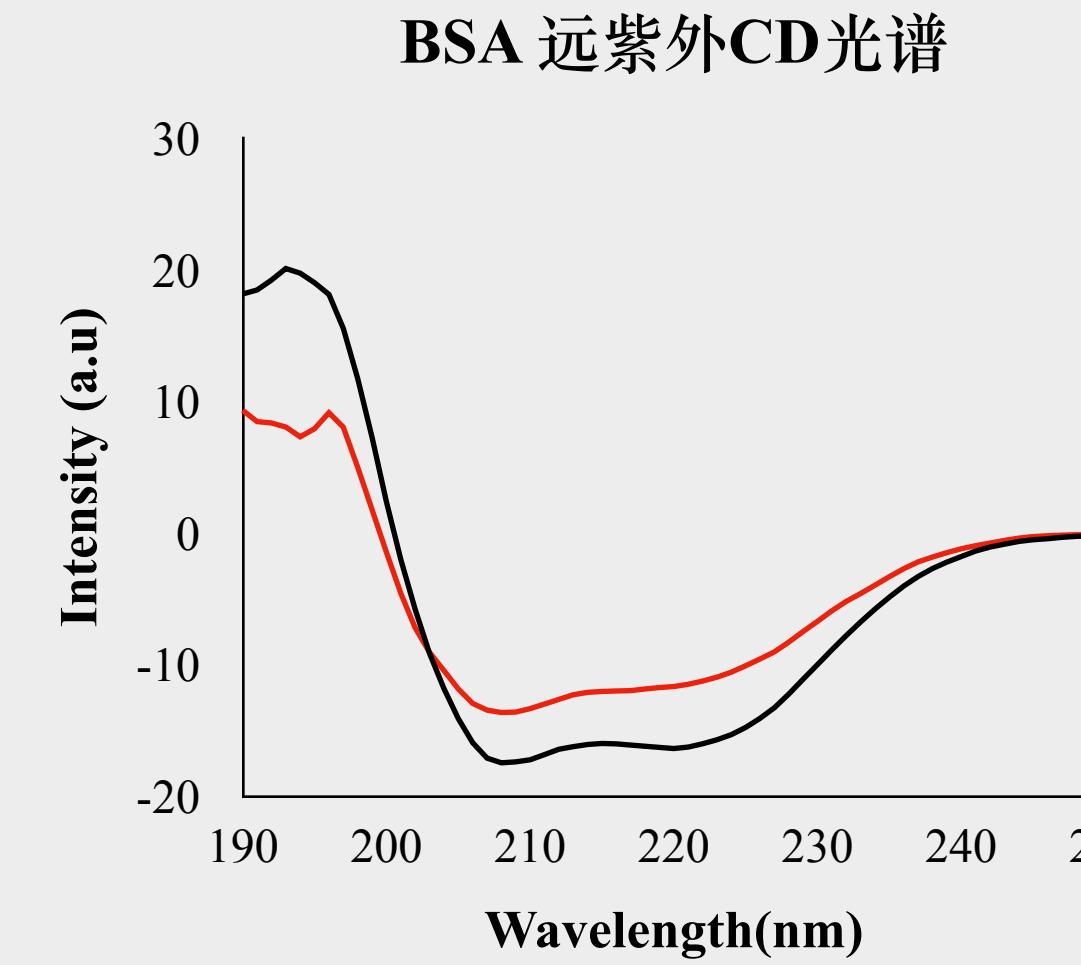


# 结构确证与体外性质考察 - 荧光工作能量转移法研究BSA蛋白与杂晶键合模式





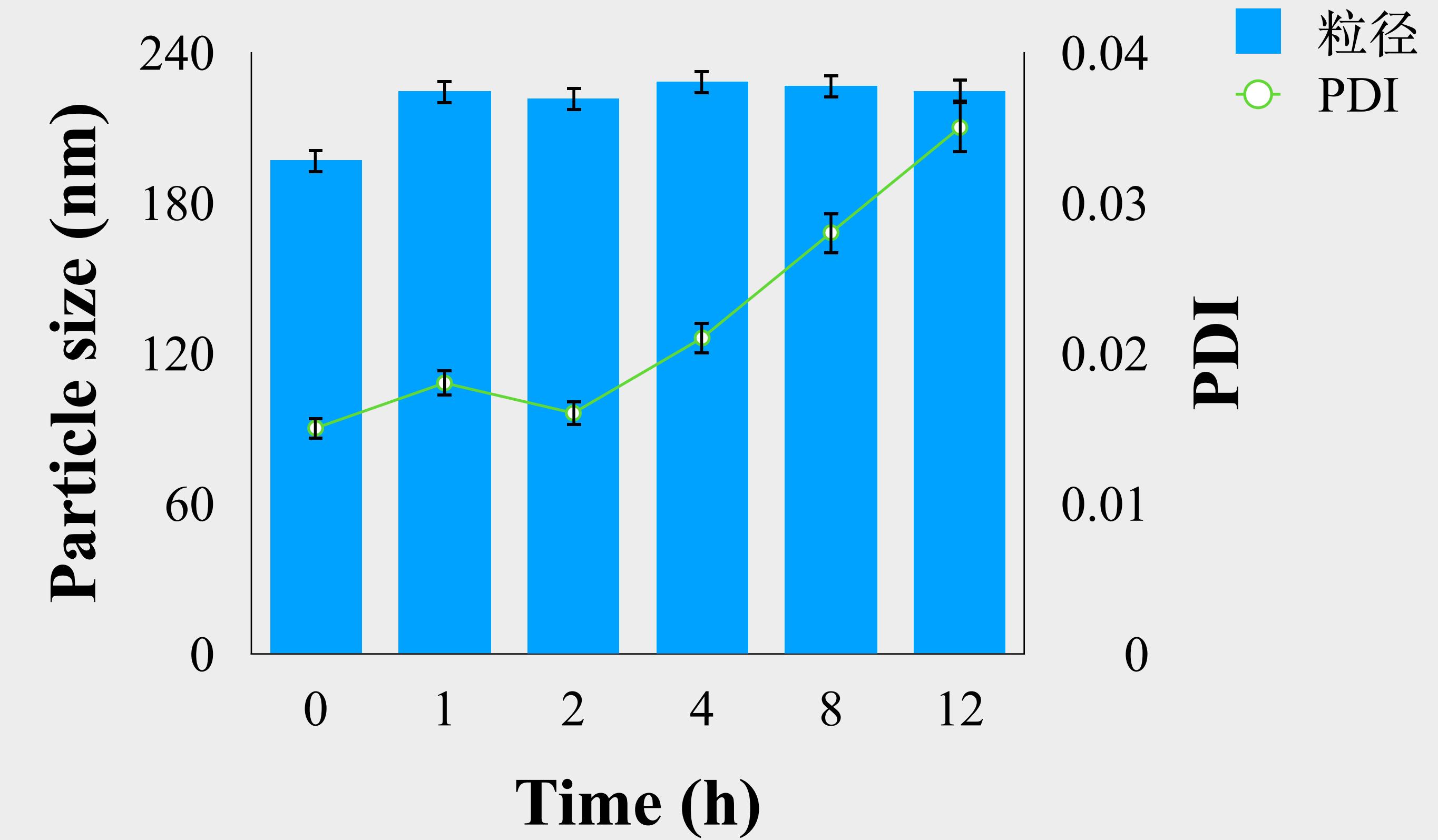
# 结构确证与体外性质考察 - 圆二色谱法验证BSA蛋白稳定剂作用



仪器信息：JASCO J-810 Circular Dichroism Spectrometer



# 结构确证与体外性质考察 - 12h 体外稳定性实验



实验条件: 37°C, 10%FBS 生理盐水溶液介质, 制剂用介质10倍稀释



## 工作汇报

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# 体外抗肿瘤活性考察

细胞毒性与药物协同效应研究

MTT法 & Chou-Talaly协同作用模型

细胞凋亡

Annexin V-PI双染法流式细胞术测定

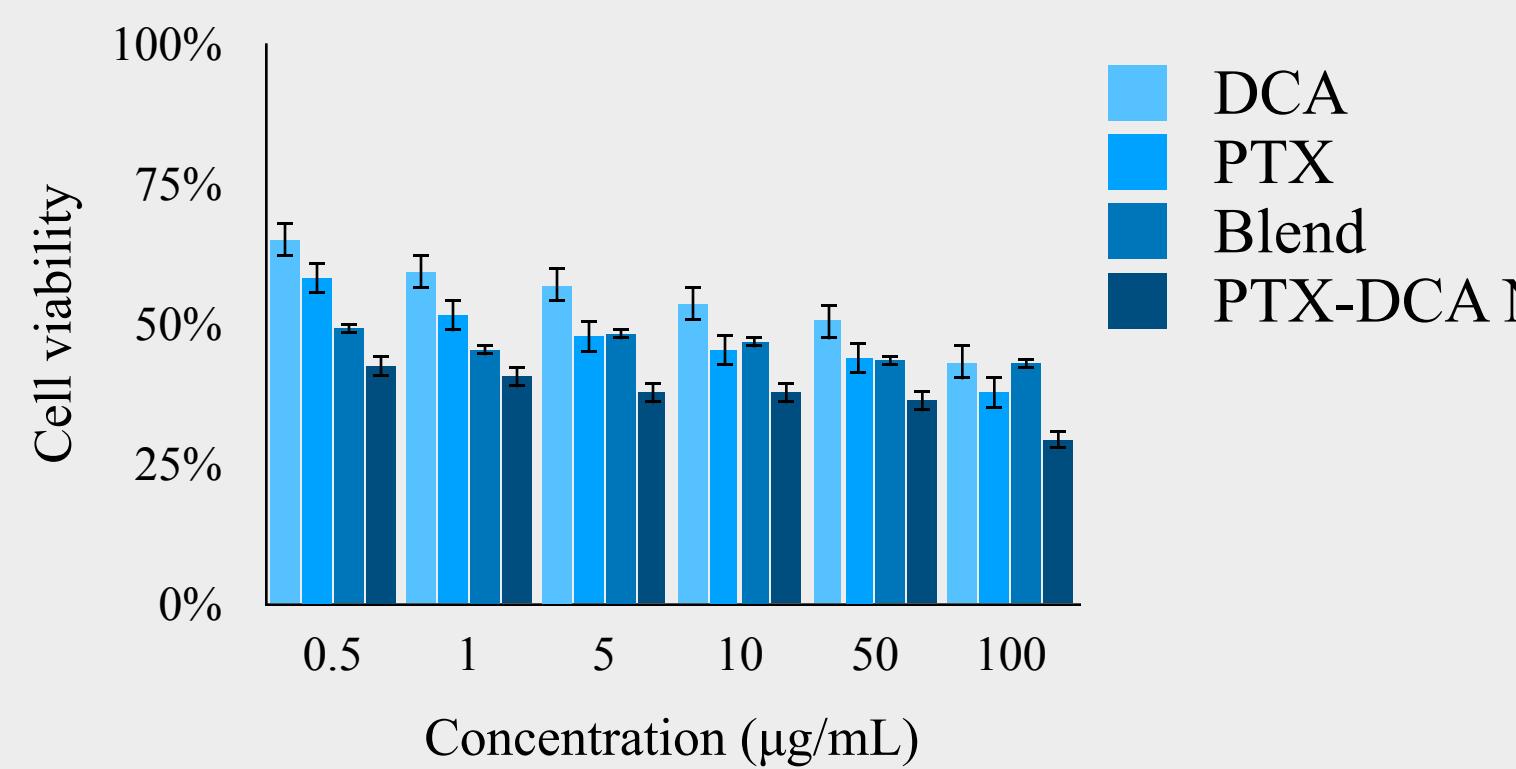
细胞周期阻滞

PI单染法流式细胞术测定

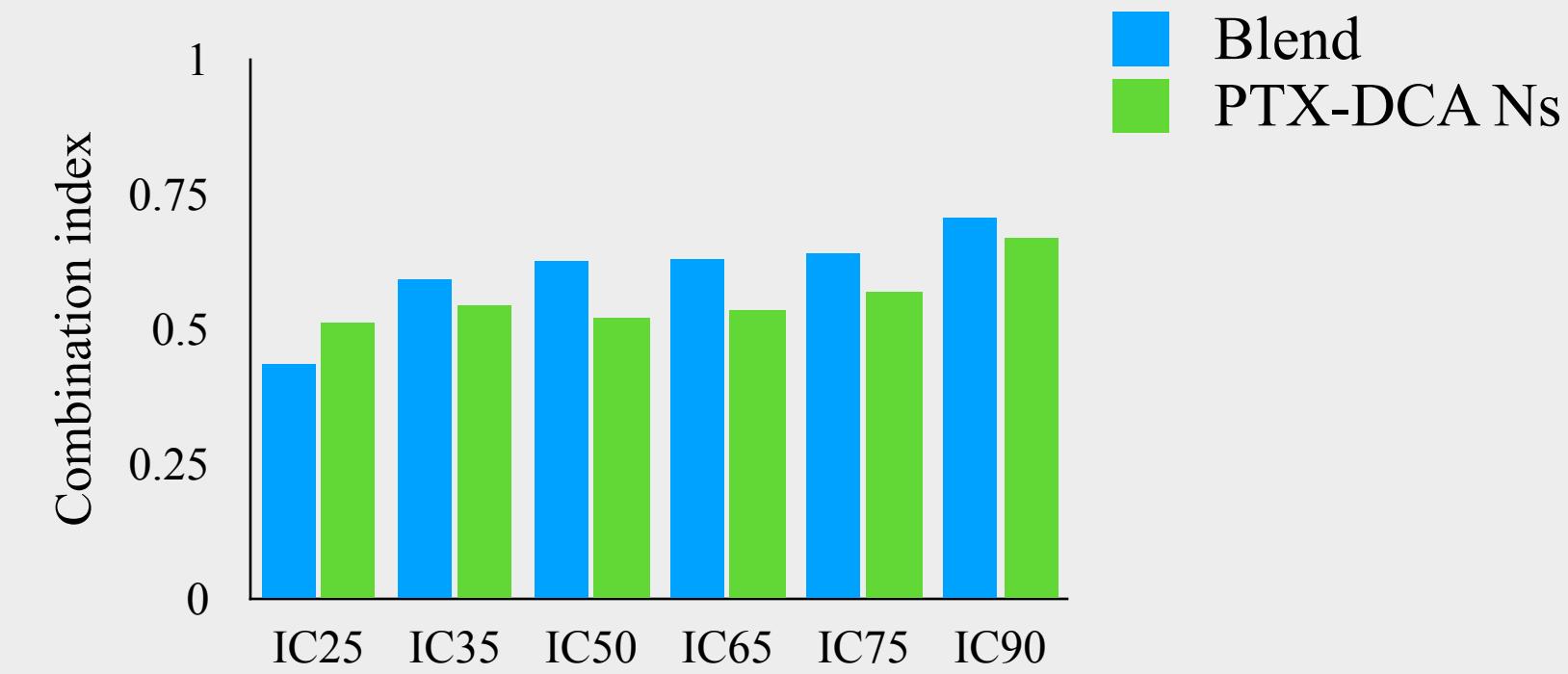


# 体外抗肿瘤活性研究 - MTT法研究细胞毒性与协同效应

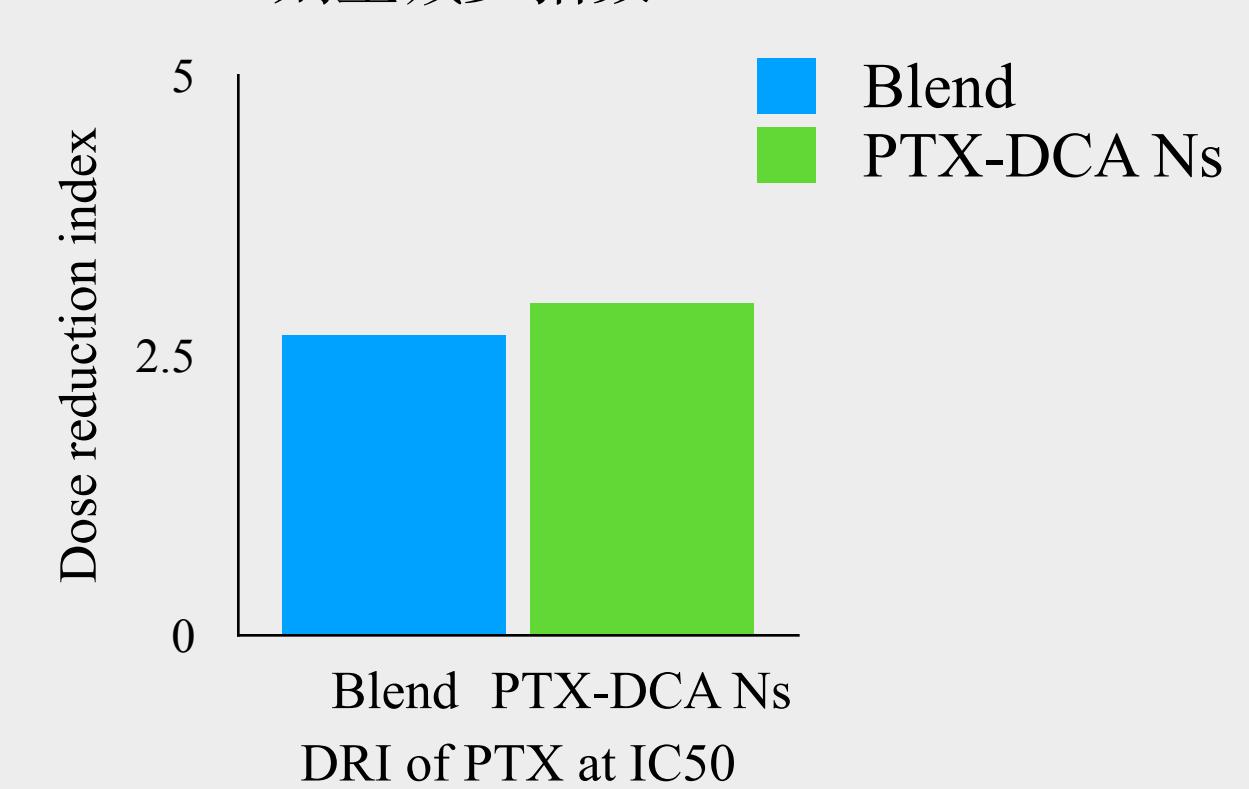
正常A549细胞毒性实验



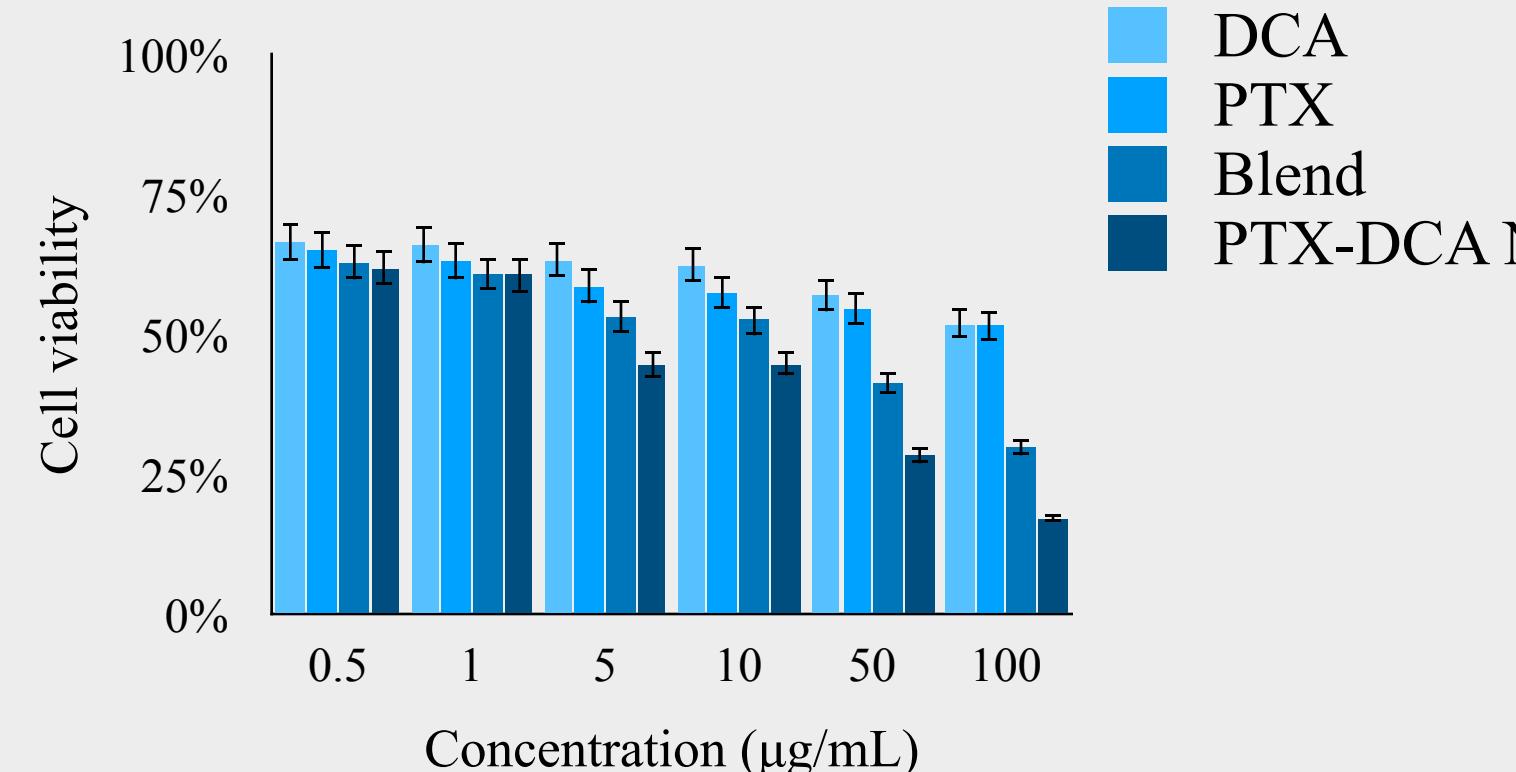
正常A549细胞  
药物联用指数CI分析



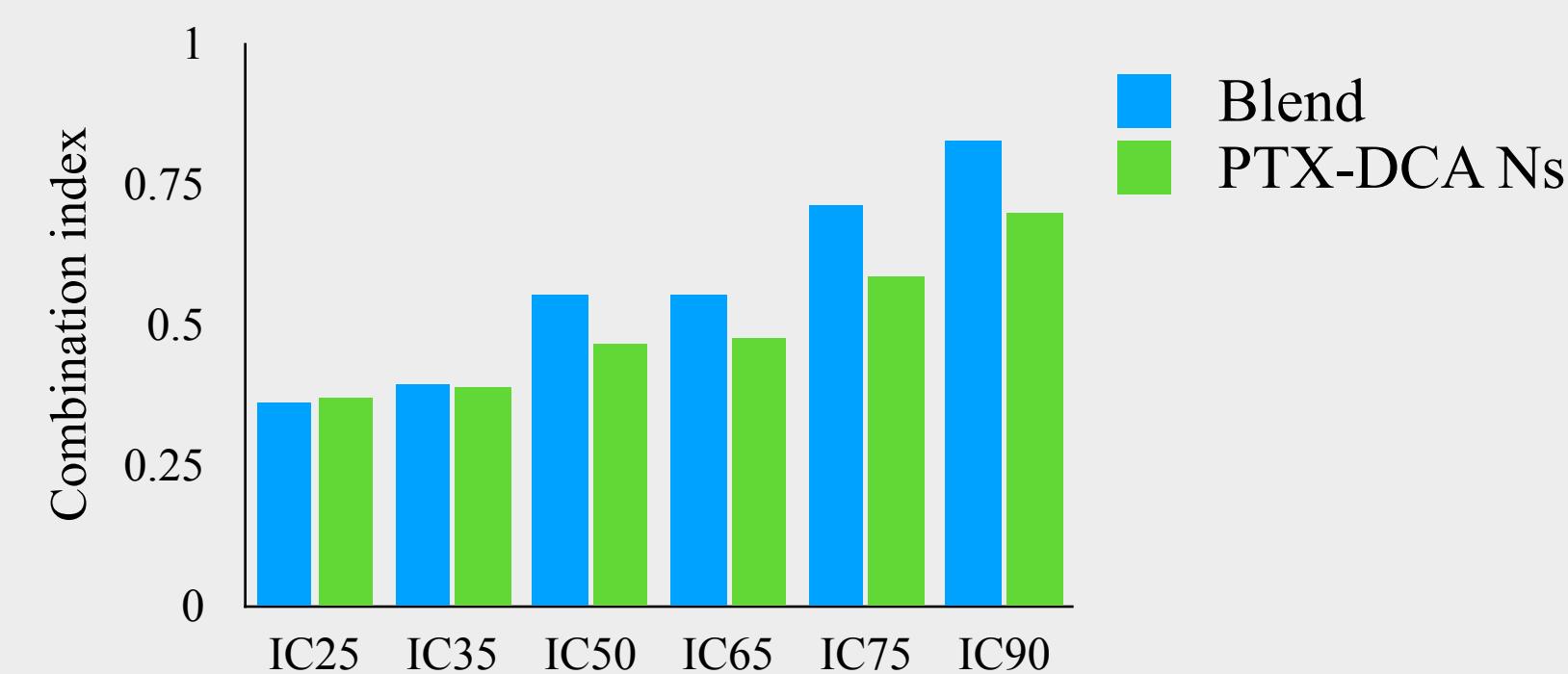
正常A549细胞  
剂量减少指数DRI



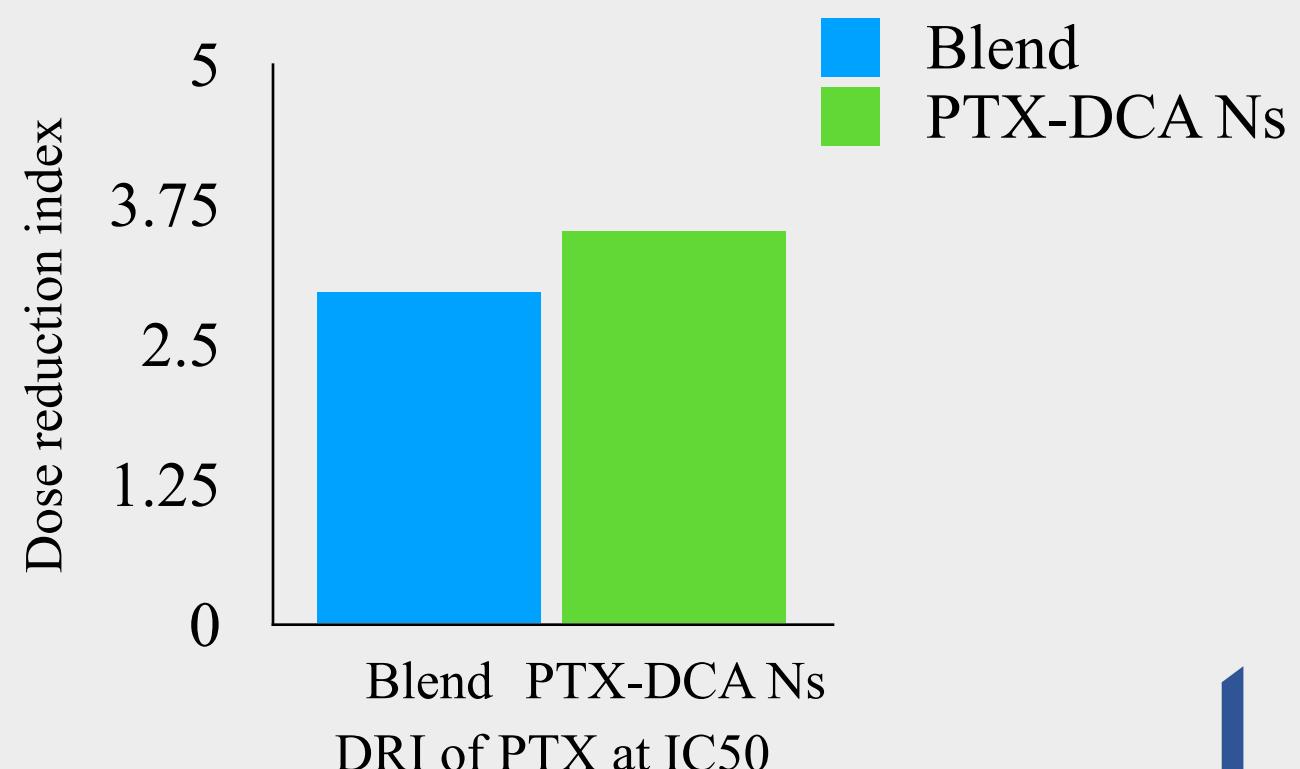
耐药A549细胞毒性实验



耐药A549细胞  
药物联用指数CI分析



耐药A549细胞  
剂量减少指数DRI

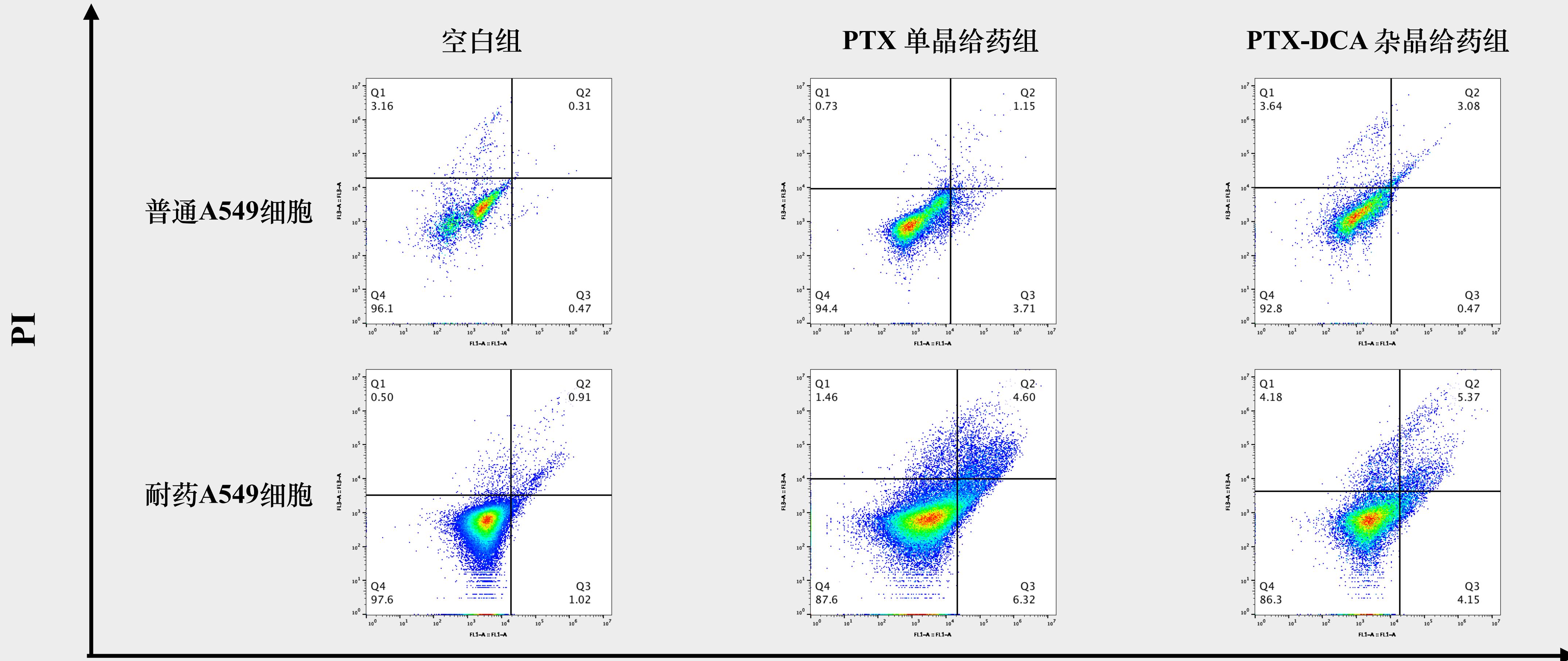


仪器信息: Thermo Scientific™ Multiskan™ FC Microplate reader

软件信息: CompuSyn v1.0.1, Chou-Talaly协同作用模型



# 体外抗肿瘤活性研究 - 双染法研究细胞凋亡



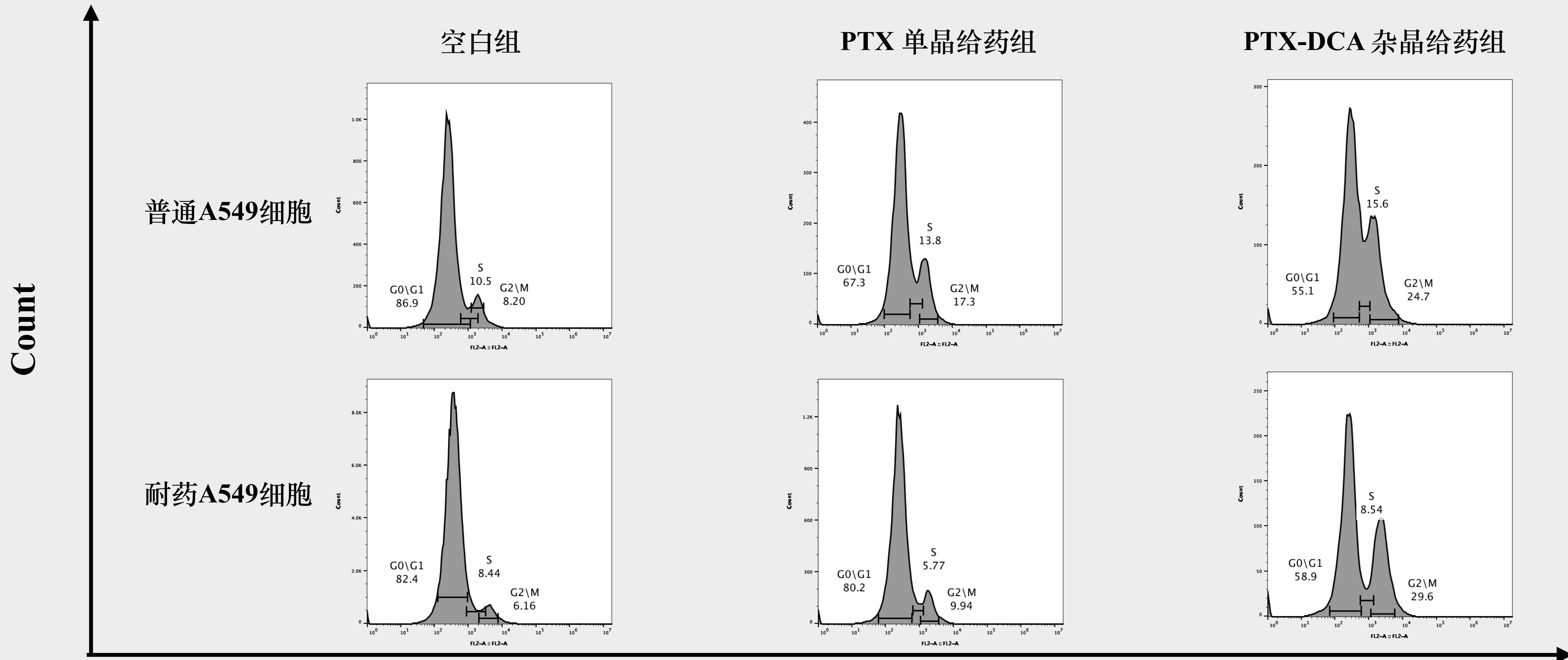
Annexin V

仪器信息：BD Accuri™ C6 Plus Cytometer

软件信息：FlowJo v10.0.0



# 体外抗肿瘤活性研究 - 单染法研究细胞细胞周期阻滞

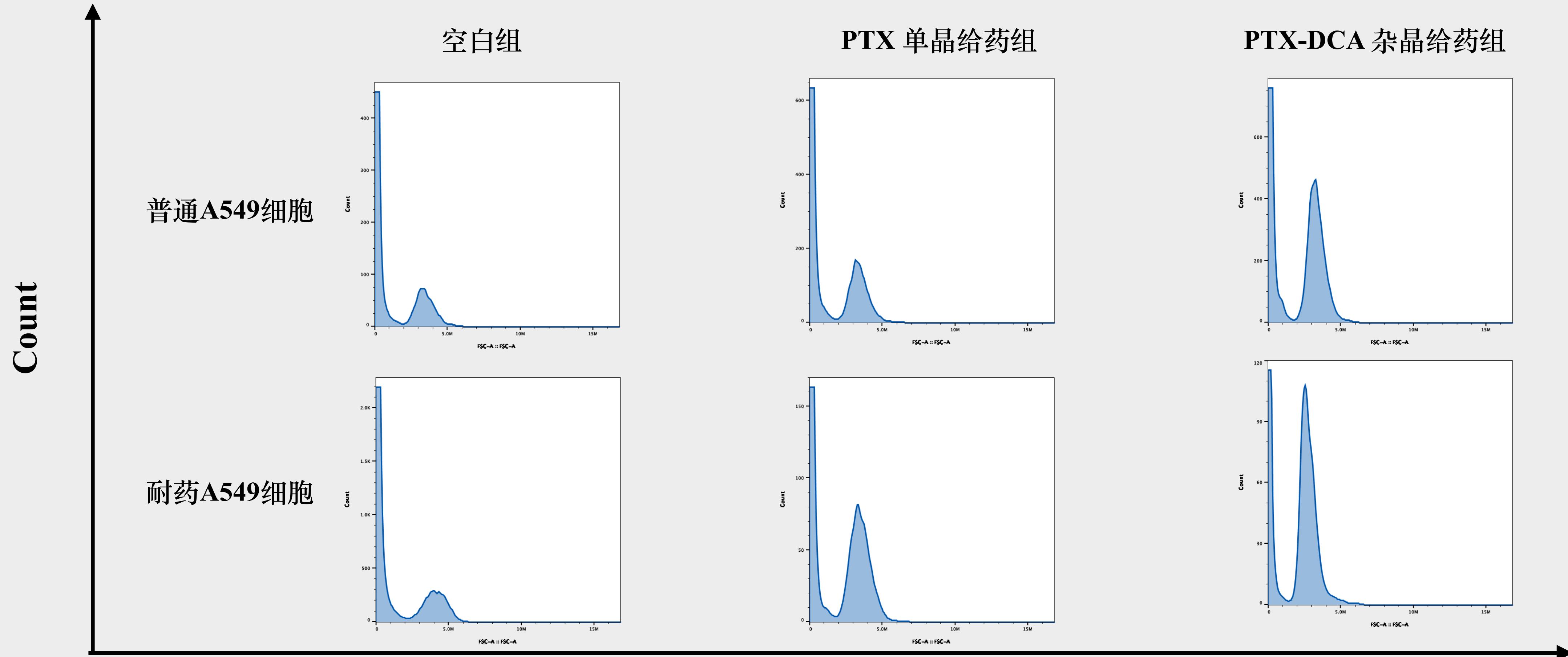


仪器信息：BD Accuri™ C6 Plus Cytometer

软件信息：FlowJo v10.0.0



# 体外抗肿瘤活性研究 - DCFH-DA染色法研究细胞线粒体ROS水平



**DCFH-DA**

仪器信息: BD Accuri™ C6 Plus Cytometer

软件信息: FlowJo v10.0.0



# 项目结论与展望

## 项目结果

利用药物递送药物策略制备了一种可递送疏水性不同组分的纳米杂化晶体，用于增加凋亡诱导效应而不引起多药耐药性，并在细胞水平验证体系有效性

## 项目不足

尚未协同机制机理研究

代谢模式改变、细胞周期阻滞和ROS水平测量

尚未非溶酶体入胞途径确证研究

细胞摄取实验与共聚焦显微镜观察

尚未完成动物水平抗肿瘤活性研究

建立合适动物模型并进行后续研究

## 项目潜力

蛋白外壳可为小分子肽提供良好附着面

靶向性修饰潜力

表面电荷可吸附miRNA分子

治疗性修饰潜力

可用于递送疏水性不同的药物分子

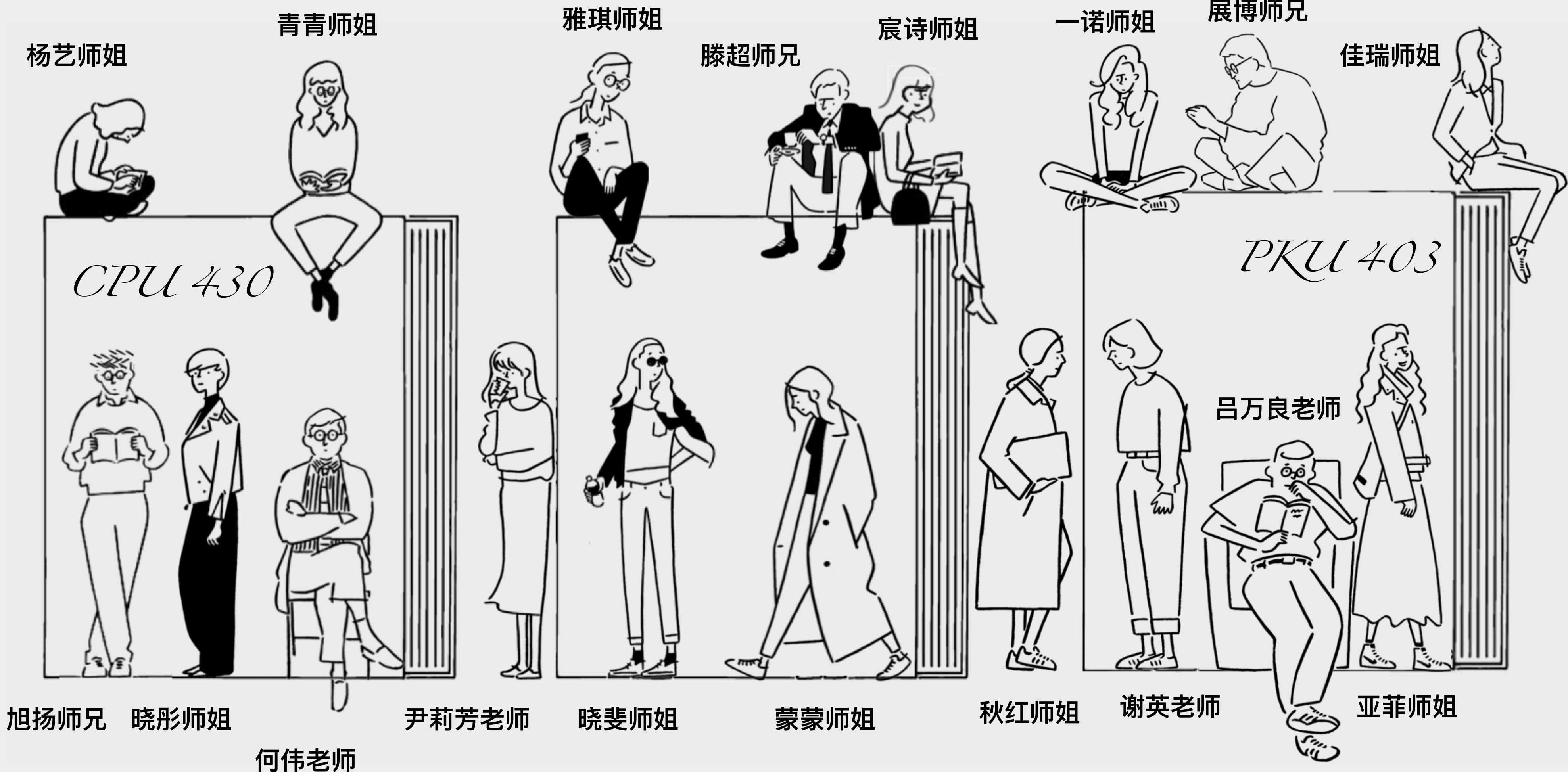
药物联用潜力



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- [2]. Mitragotri, S., Burke, P. A., & Langer, R.(2014) Overcoming the challenges in administering biopharmaceuticals: formulation and delivery strategies. *Nat reviews. Drug discovery*, 13(9), 655672. <https://doi.org/10.1038/nrd4363>
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# 致谢



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感谢聆听

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