



PTX-DCA 杂化晶体的制备与评价

指导老师：尹莉芳教授 何伟教授

答辩人：李羿

专业：药学拔尖计划 16401702

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目录

PTX-DCA 杂化晶体的制备与评价



研究背景



课题设计



工作汇报



项目结论与展望



参考文献与致谢



制备与表征



结构确证与体外性质考察



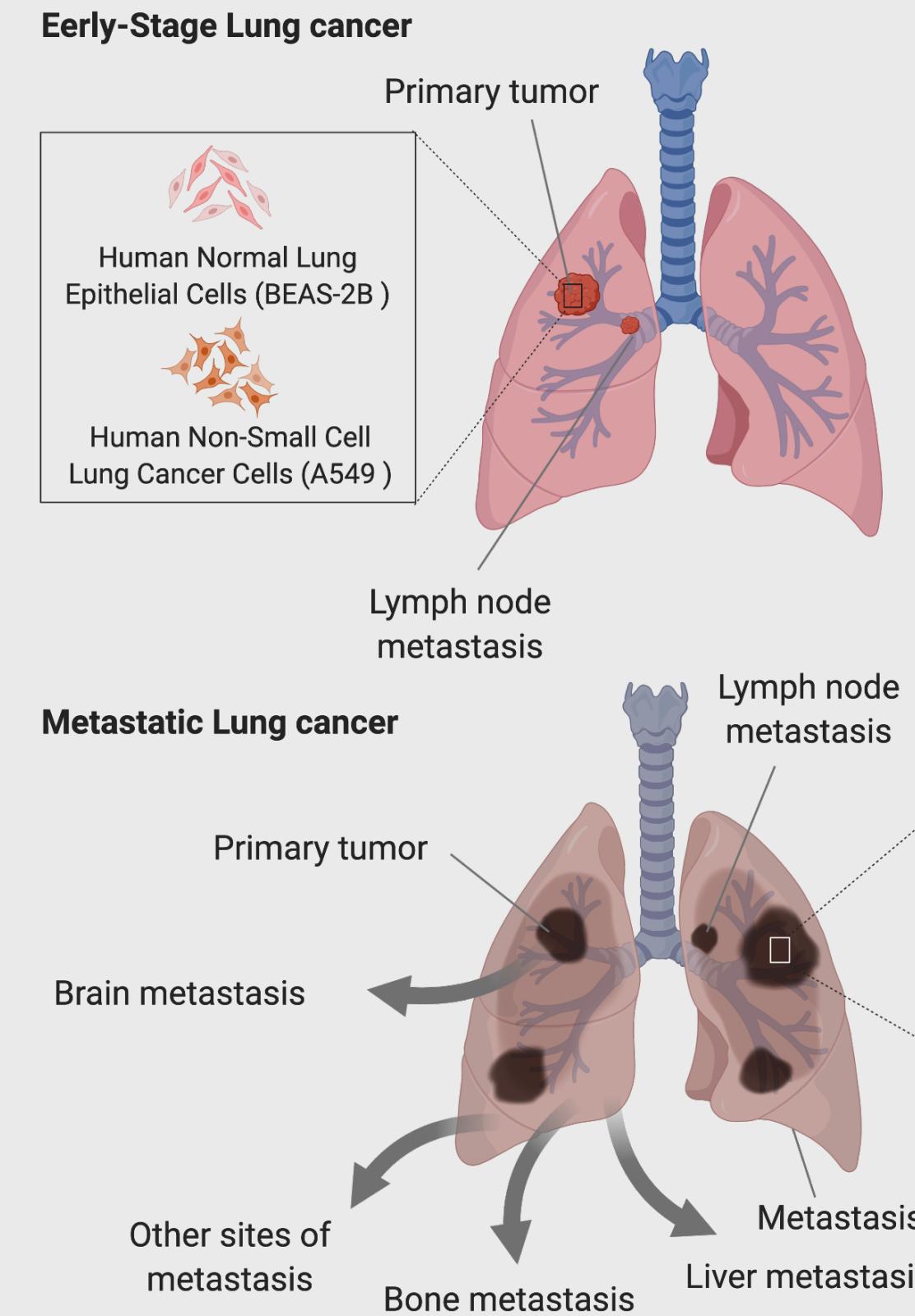
体外抗肿瘤活性研究



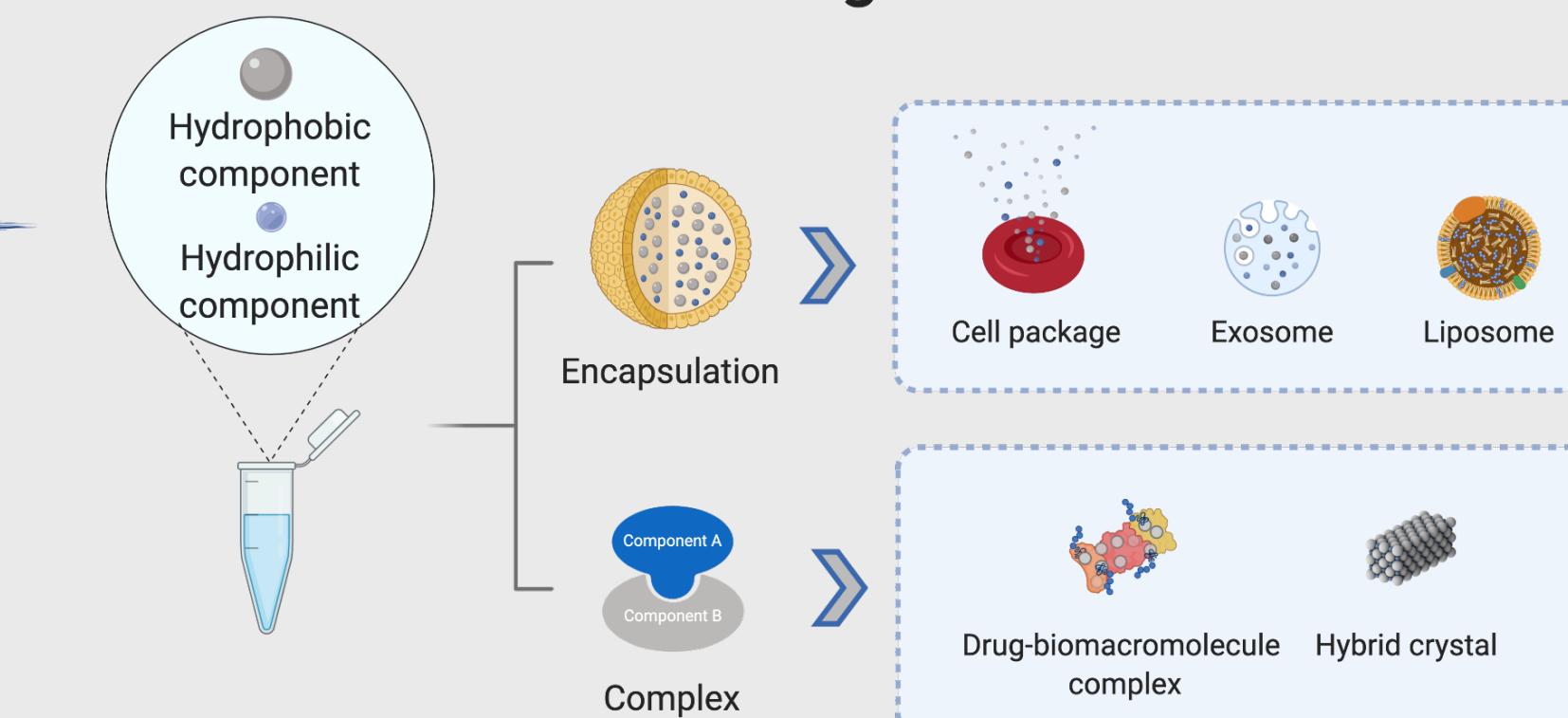


Background

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



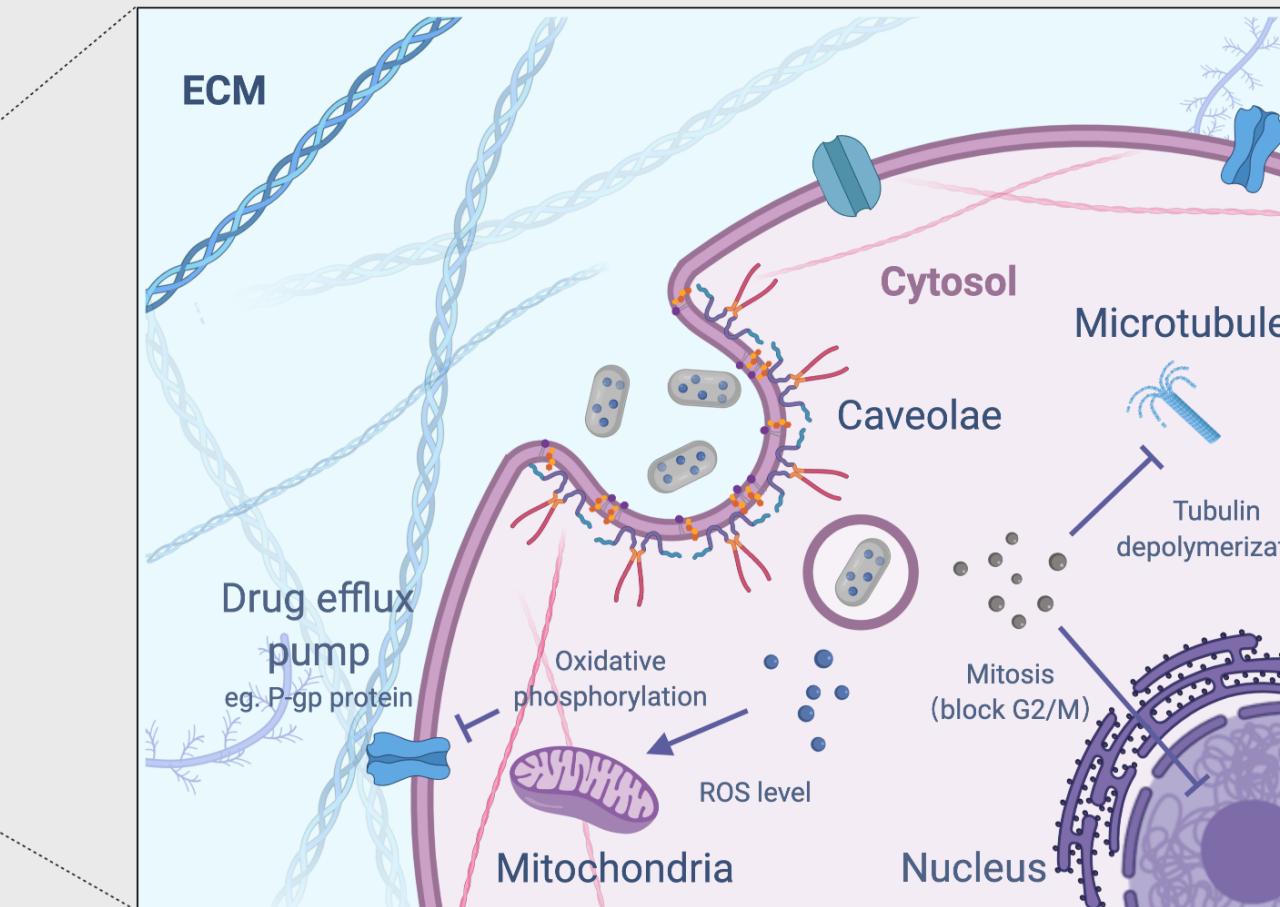
2. Drug Combination



Drug loading
Cargo ratio

Drug-delivering-drug strategy

3. Delivery efficacy



Non lysosomal endocytosis

Targeting specific organelles using surface charge



Design

Graphic abstract

PTX-DCA Ns Structure and Protein Visualization

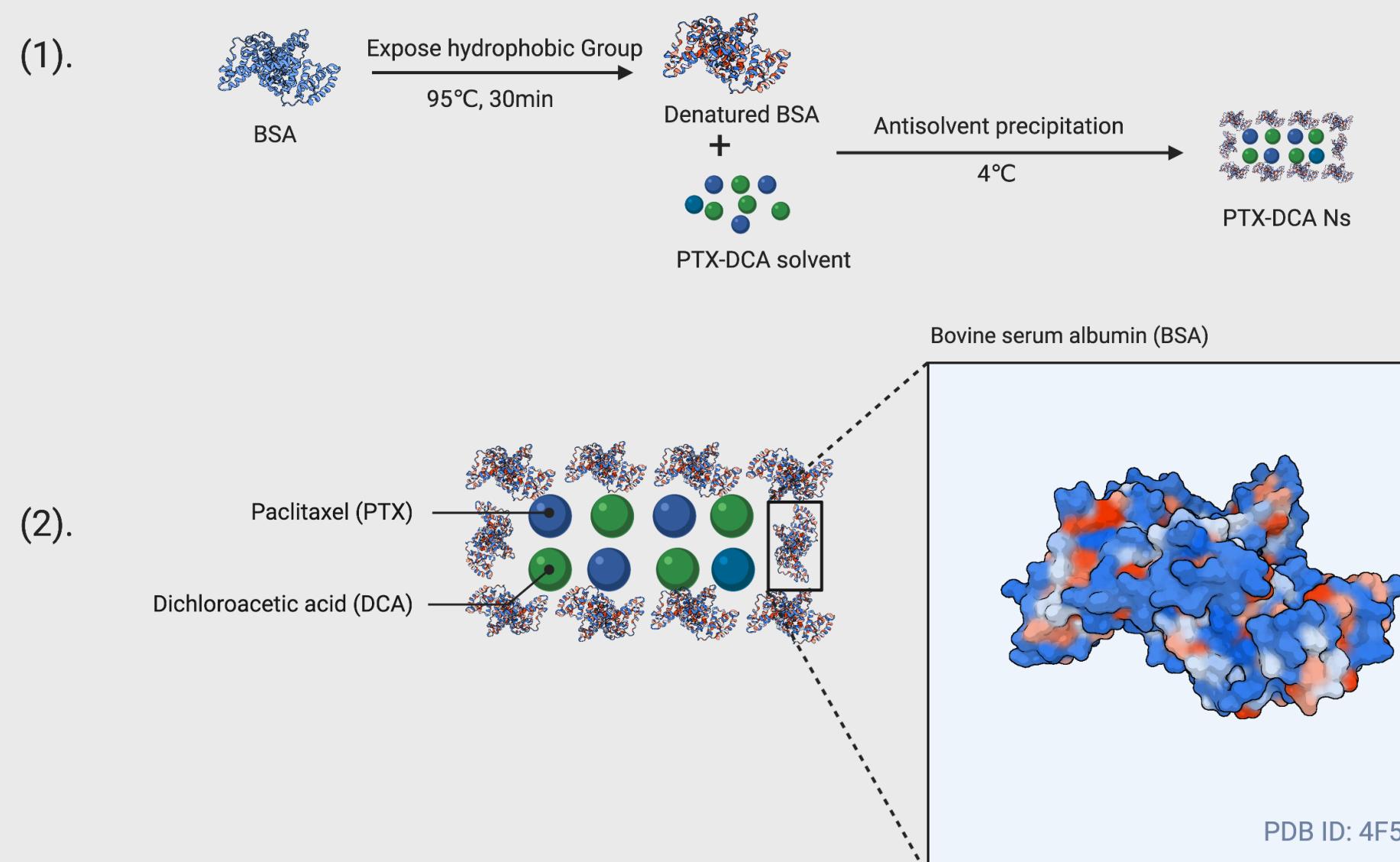


FIGURE 1: (1). PTX-DCA Ns were prepared by antisolvent precipitation method.
(2). Hydrophobicity of BSA. Hydrophilic parts were shown in blue color and lipophilic parts were shown in red.

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^[3]

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 (FERT)
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

Time line



Report

1

Synthesis & Characterization

制备

反溶剂沉淀法^[3]

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

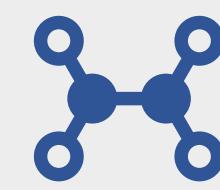
载药能力

假设与验证

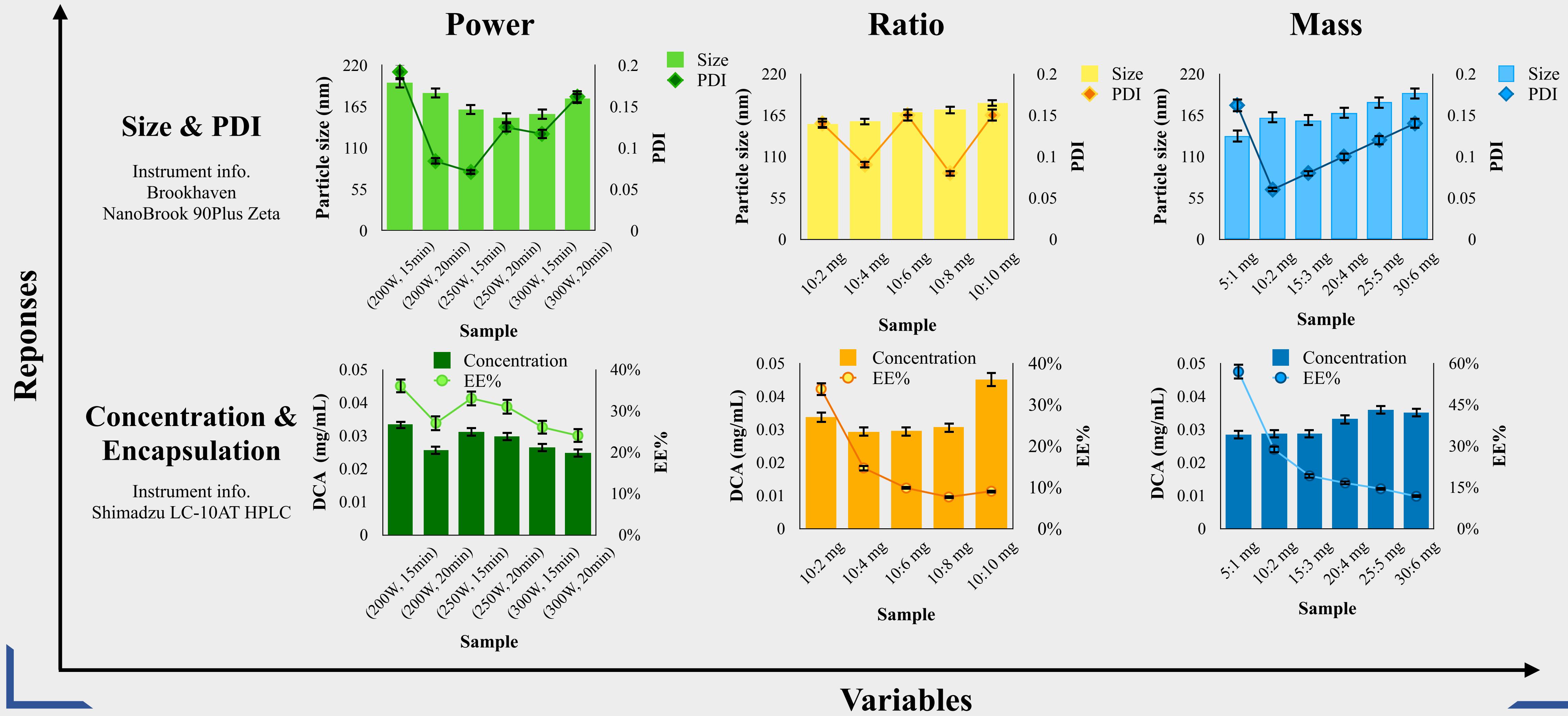
具备特定性状参数的纳米粒子可介导非溶酶体入胞途径^[3, 5, 6]

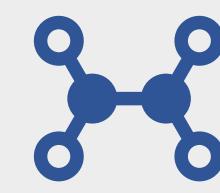
特定电荷可用于靶向特定细胞器^[4]

单一组分浓度能达到上游学科文献报道的数量级^[4]



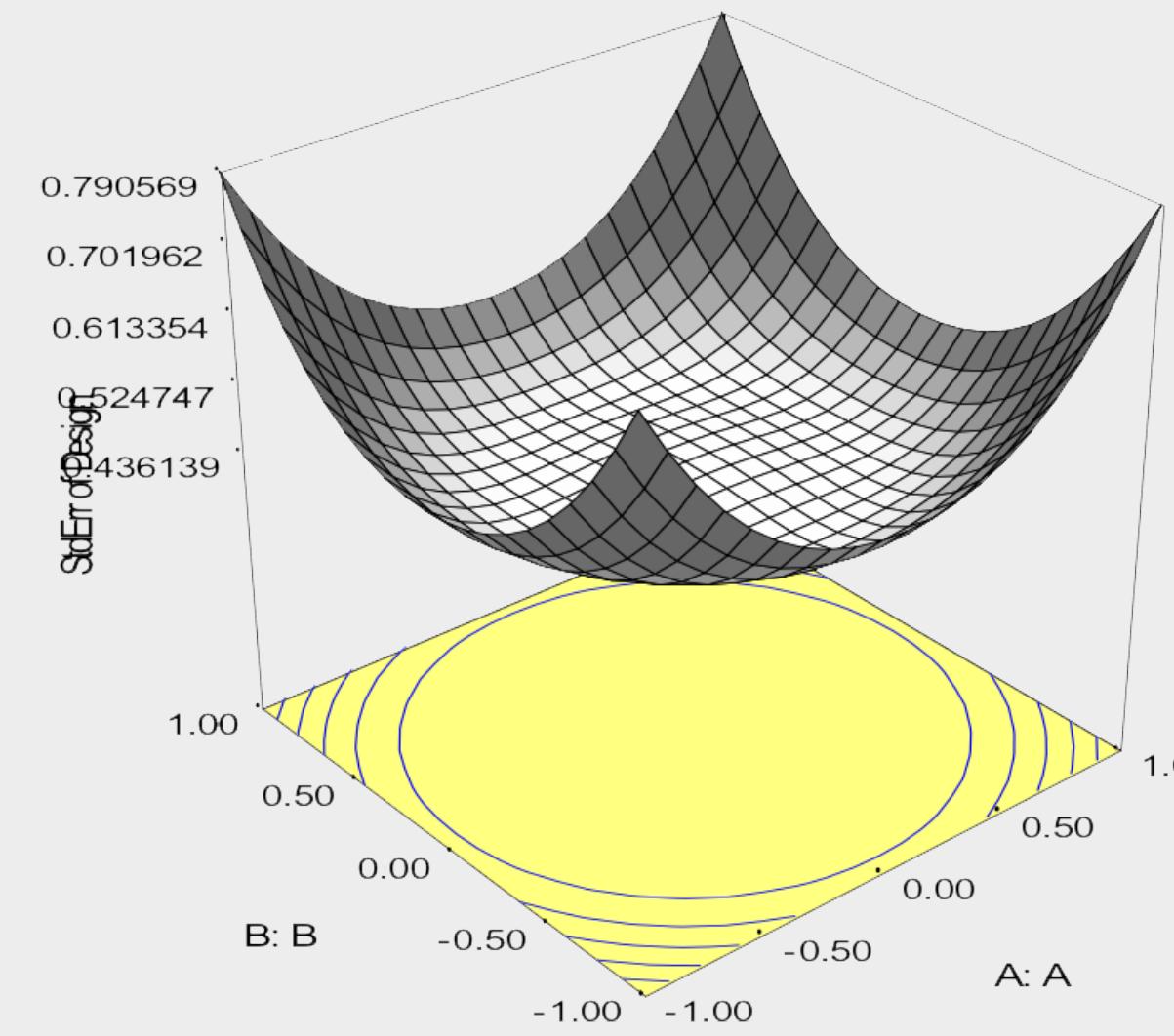
Synthesis & Characterization



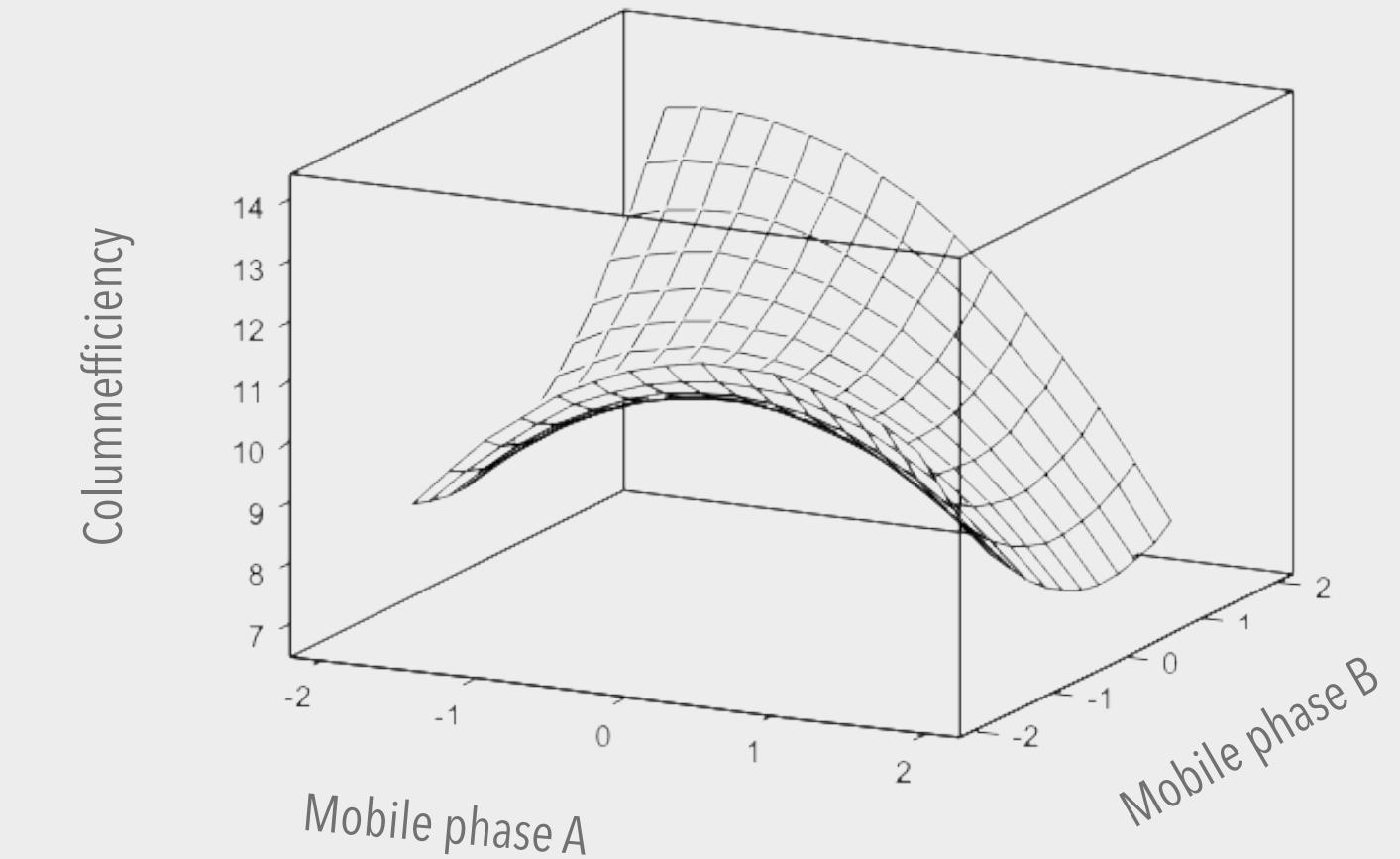


Synthesis & Characterization

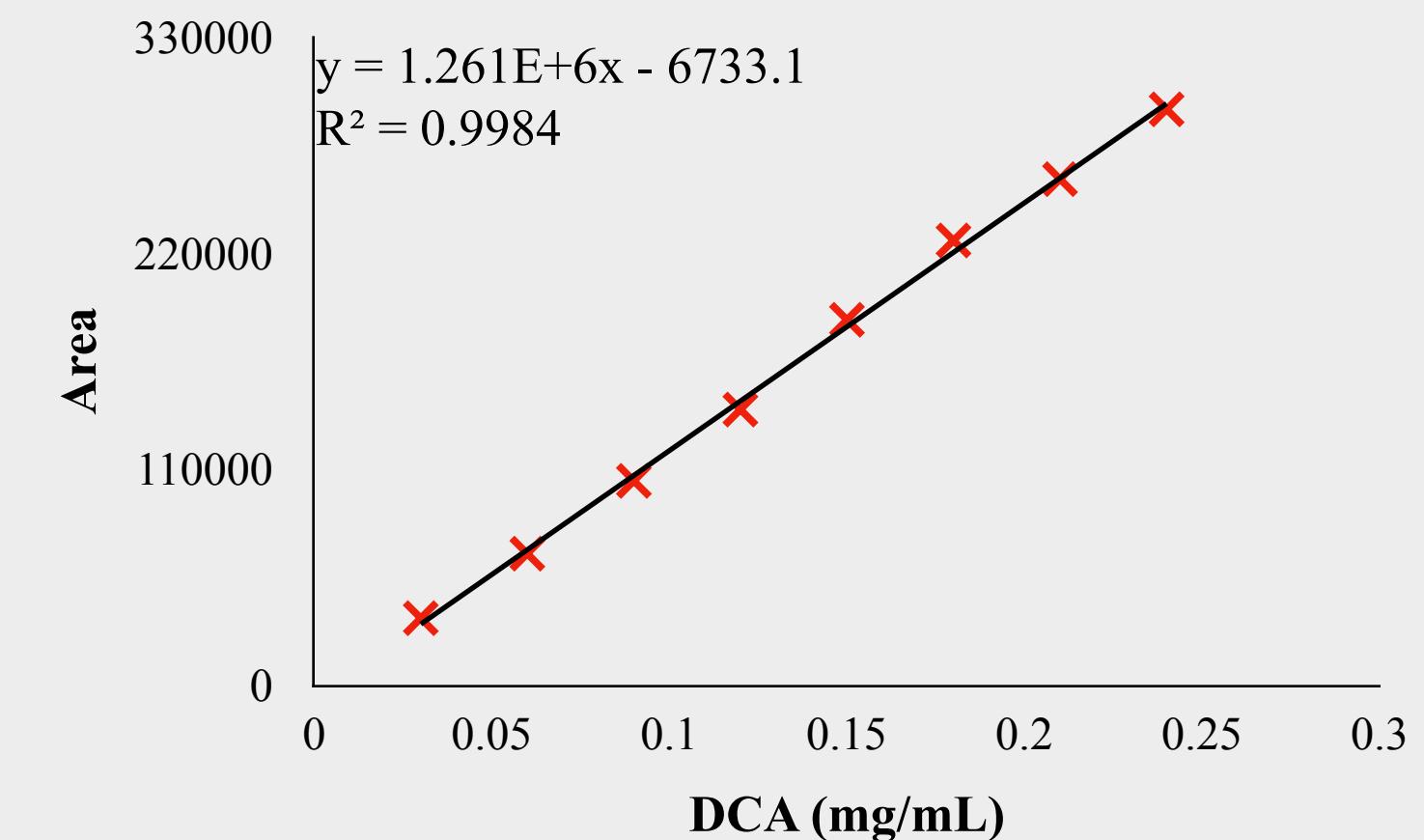
Optimization base on Box — Behnken response model



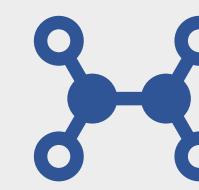
HPLC condition optimization



HPLC calibration line

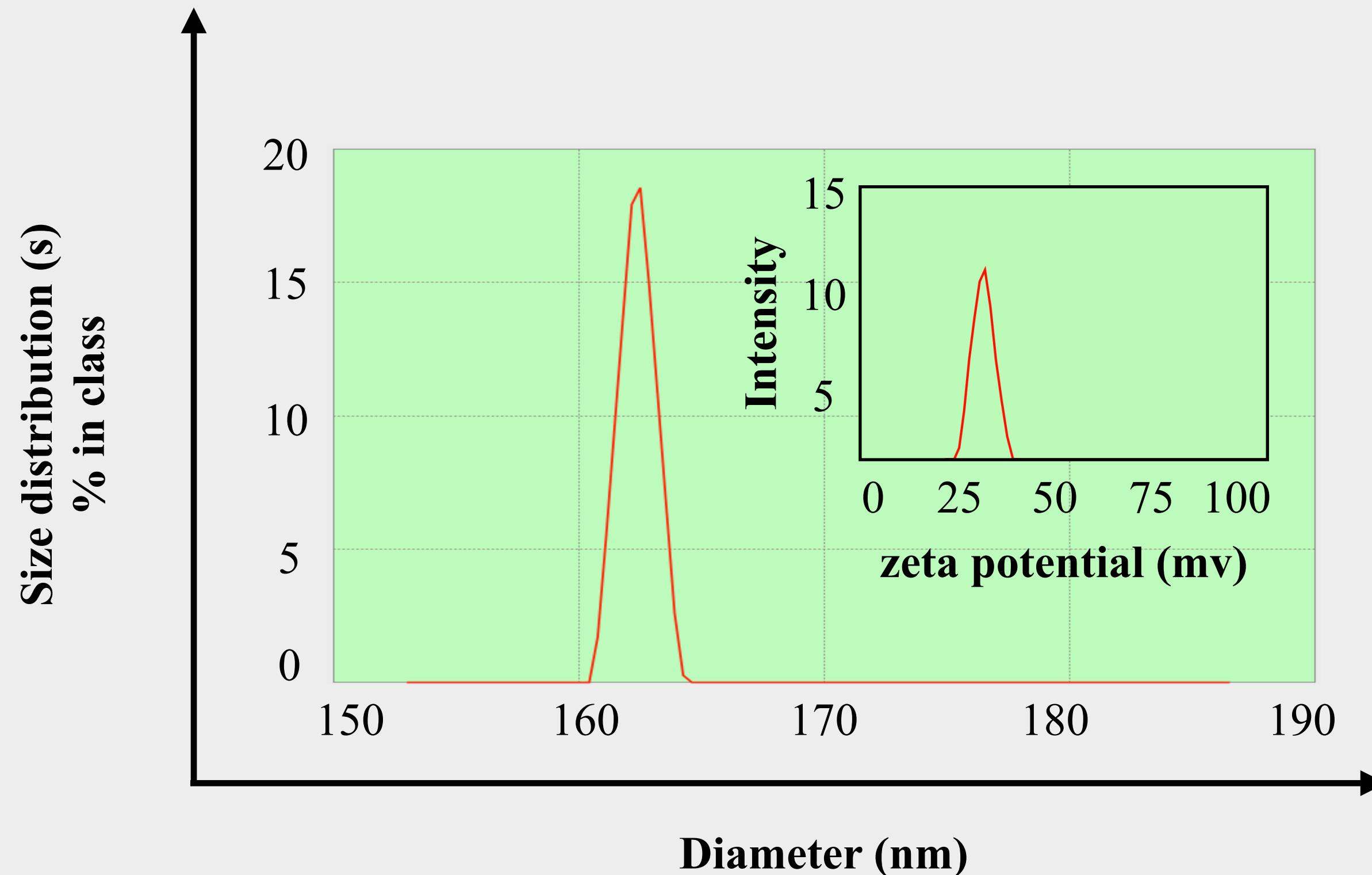


软件信息：Design Expert v10.0.7

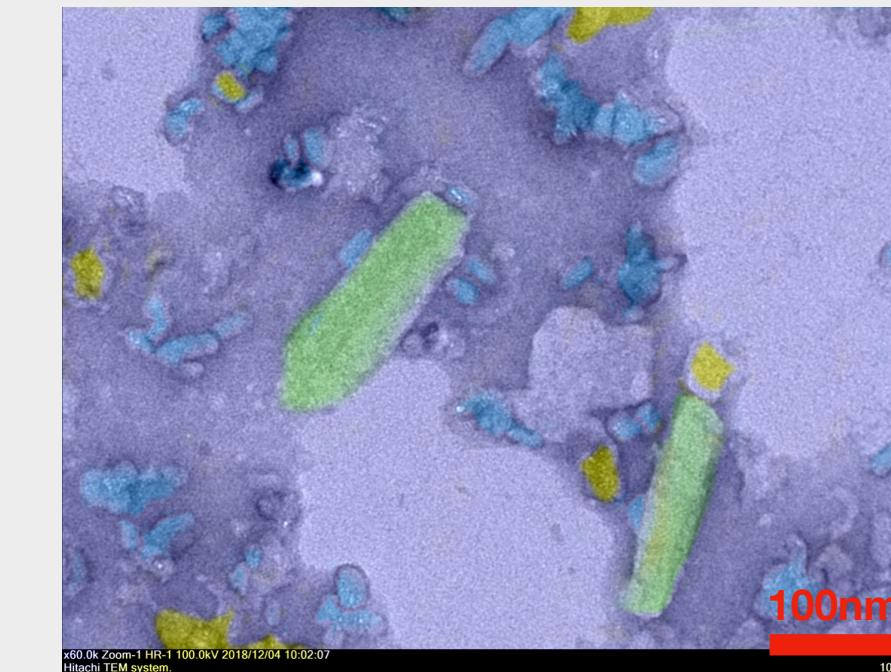


Synthesis & Characterization

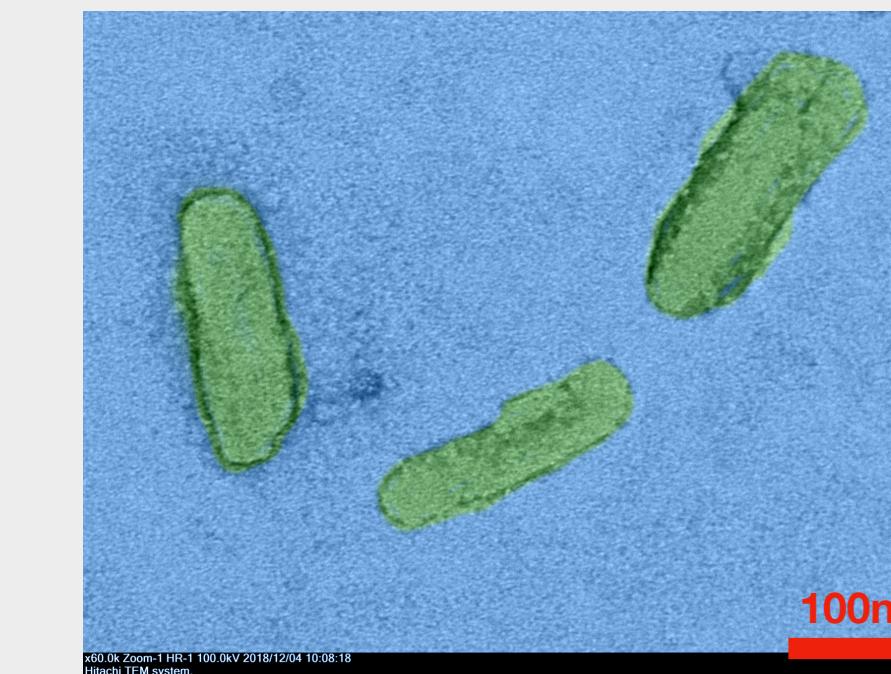
PTX-DCA Ns particle size, PDI, zeta potential measurement



TEM image BEFORE optimization
Green part is hybrid crystal; blue part is PTX mono crystal;
yellow part is protein



TEM image BEFORE optimization
Green part is hybrid crystal





Report

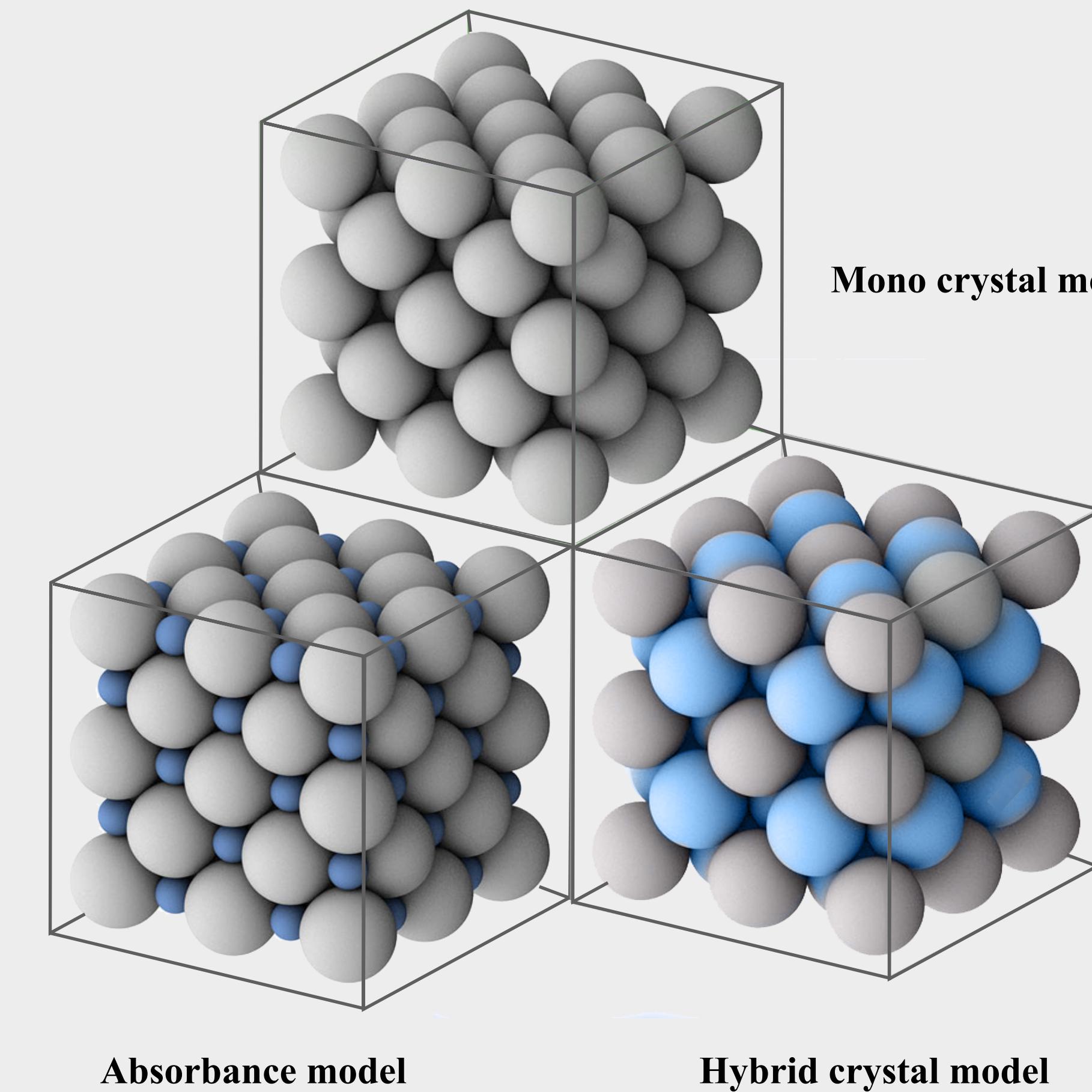
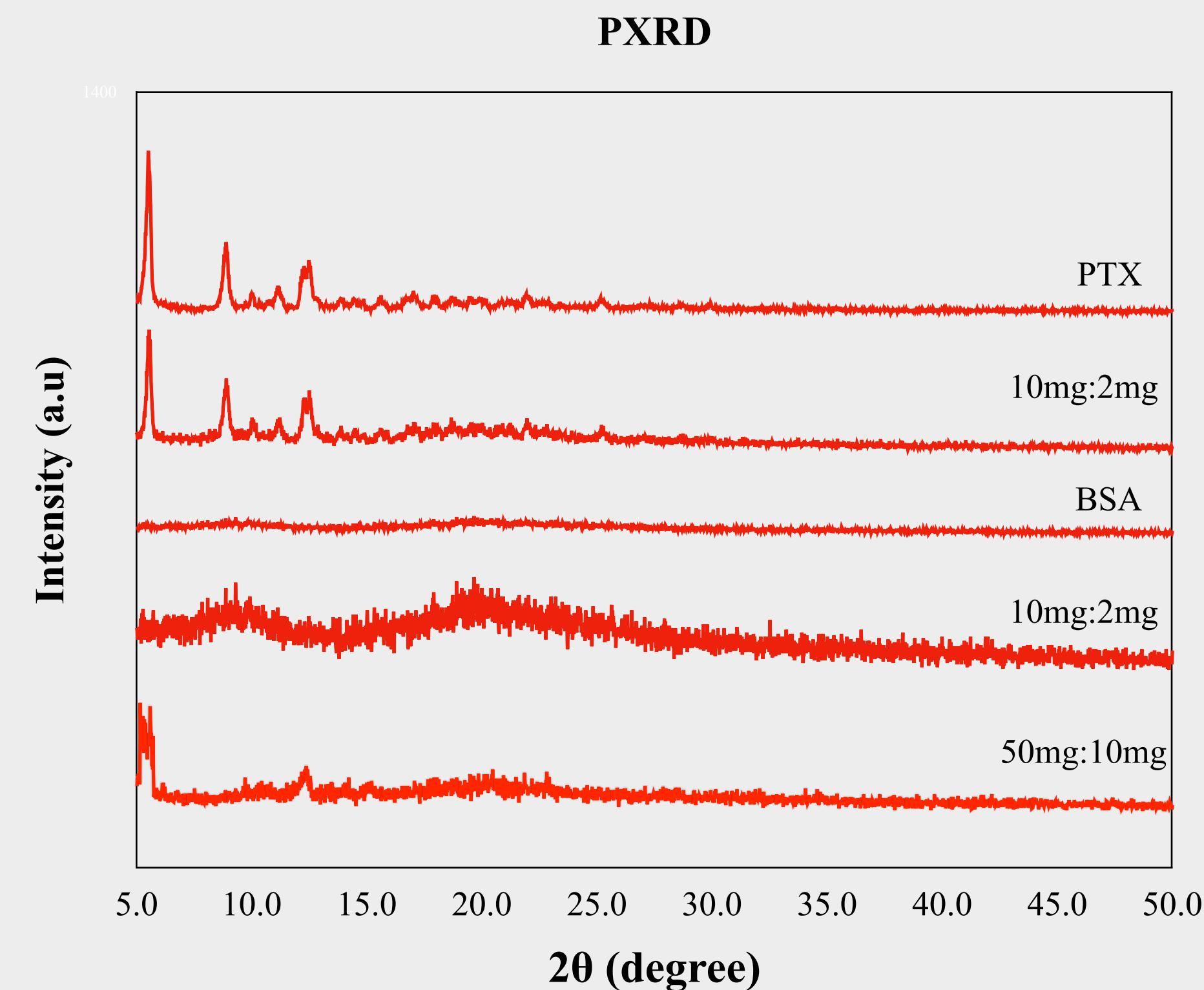
2

Structure & interaction study





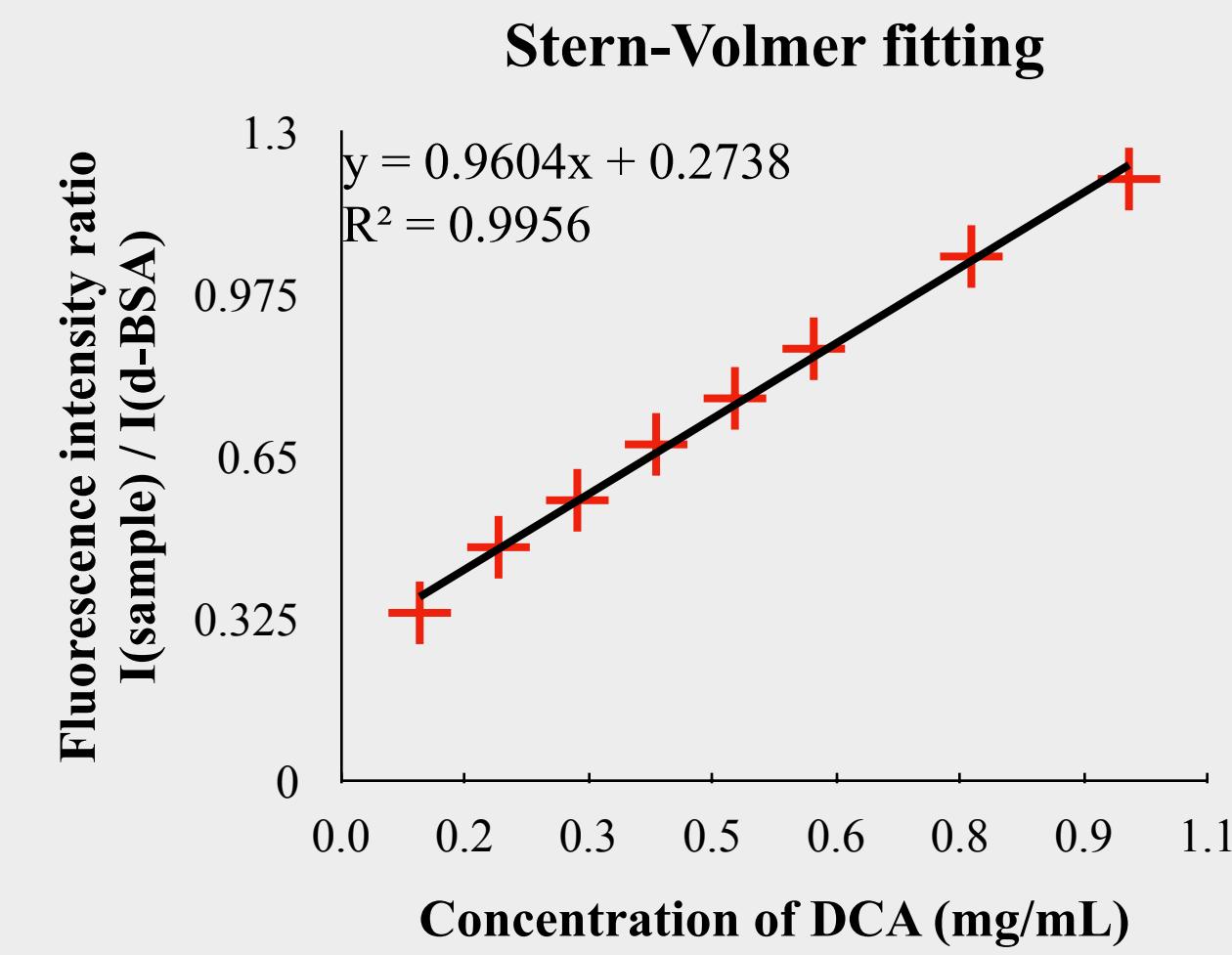
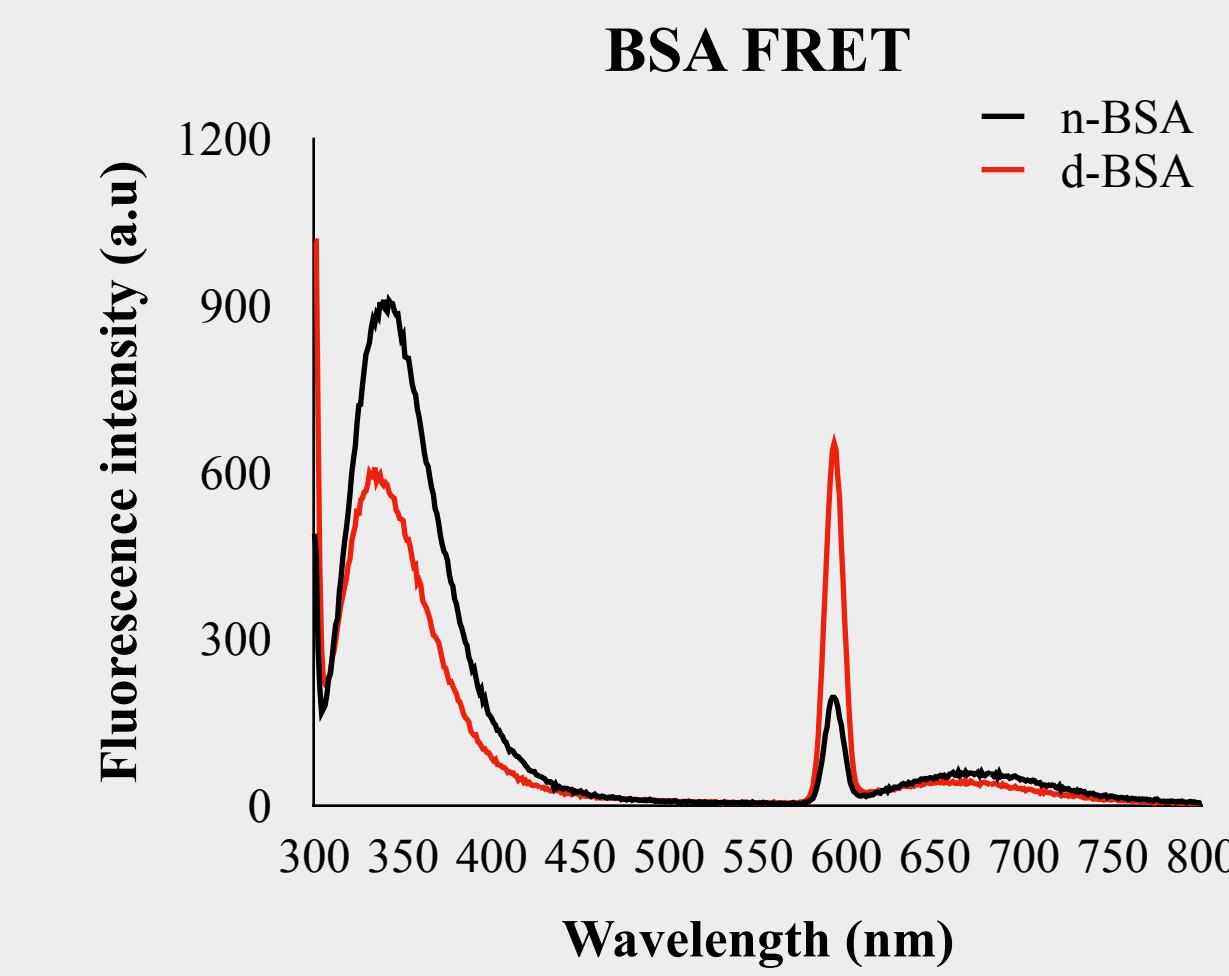
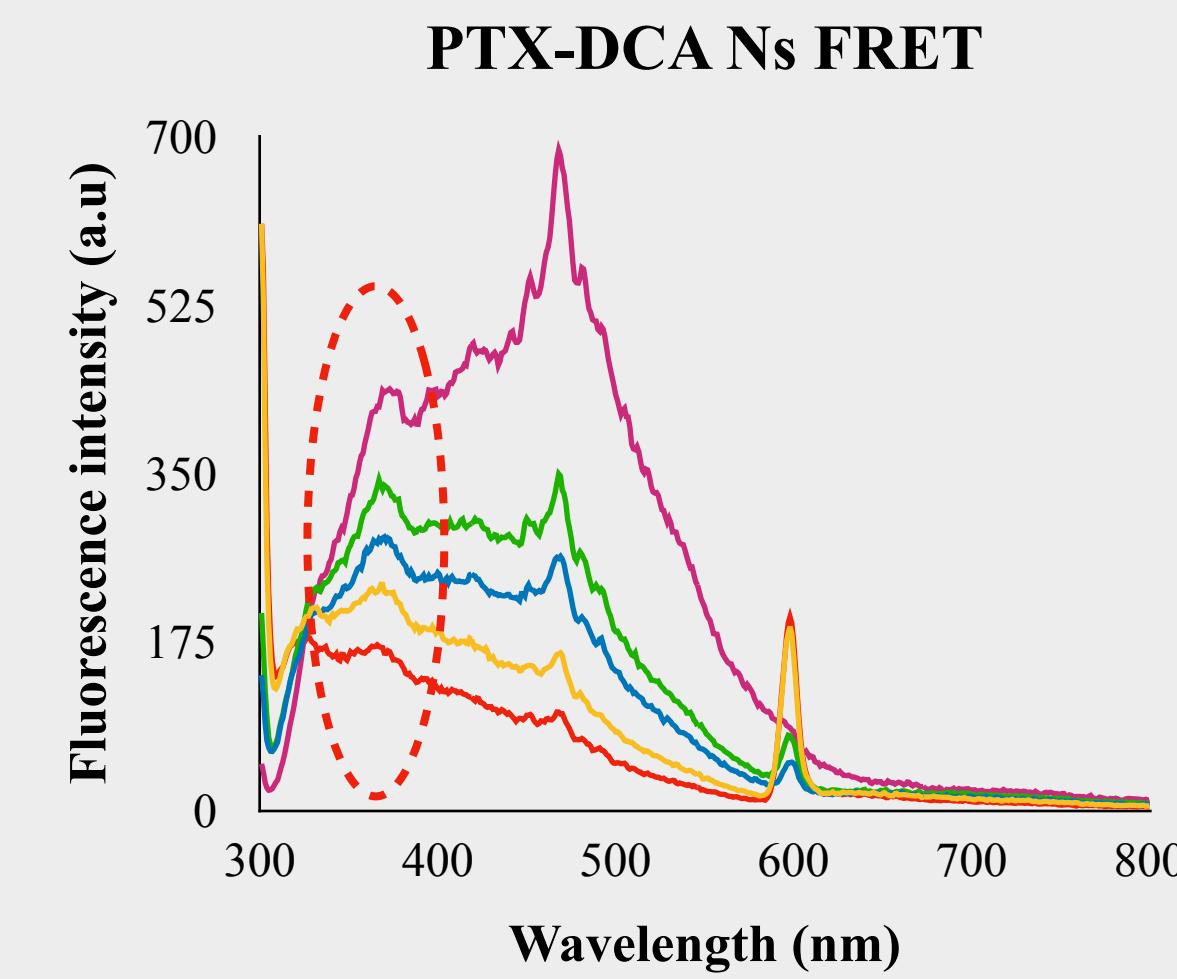
Structure & interaction study



Instrument info.: Brookhaven Bruker D8 Advance PXRD
Software info.: MDI Jade v6.0, 3D Studio Max 2017

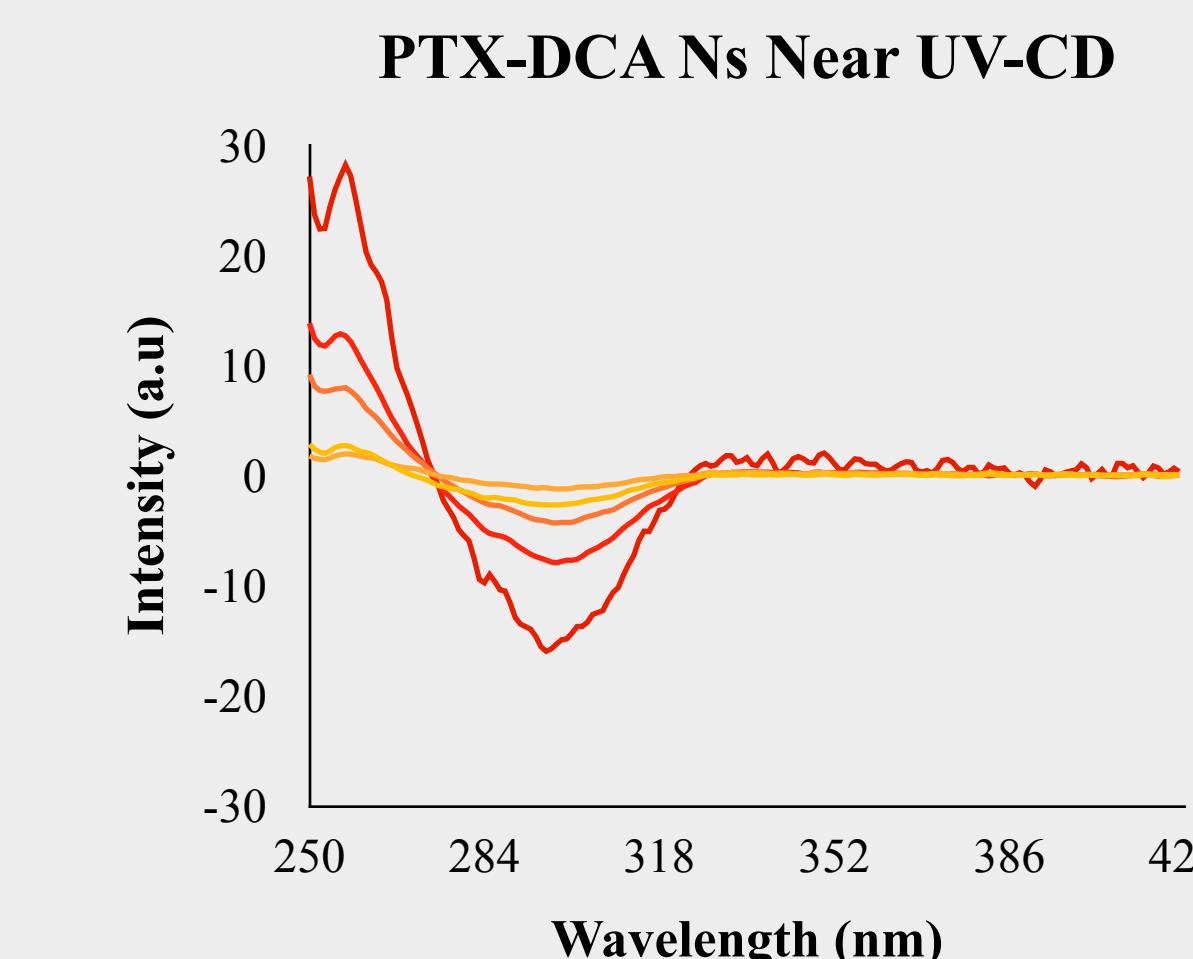
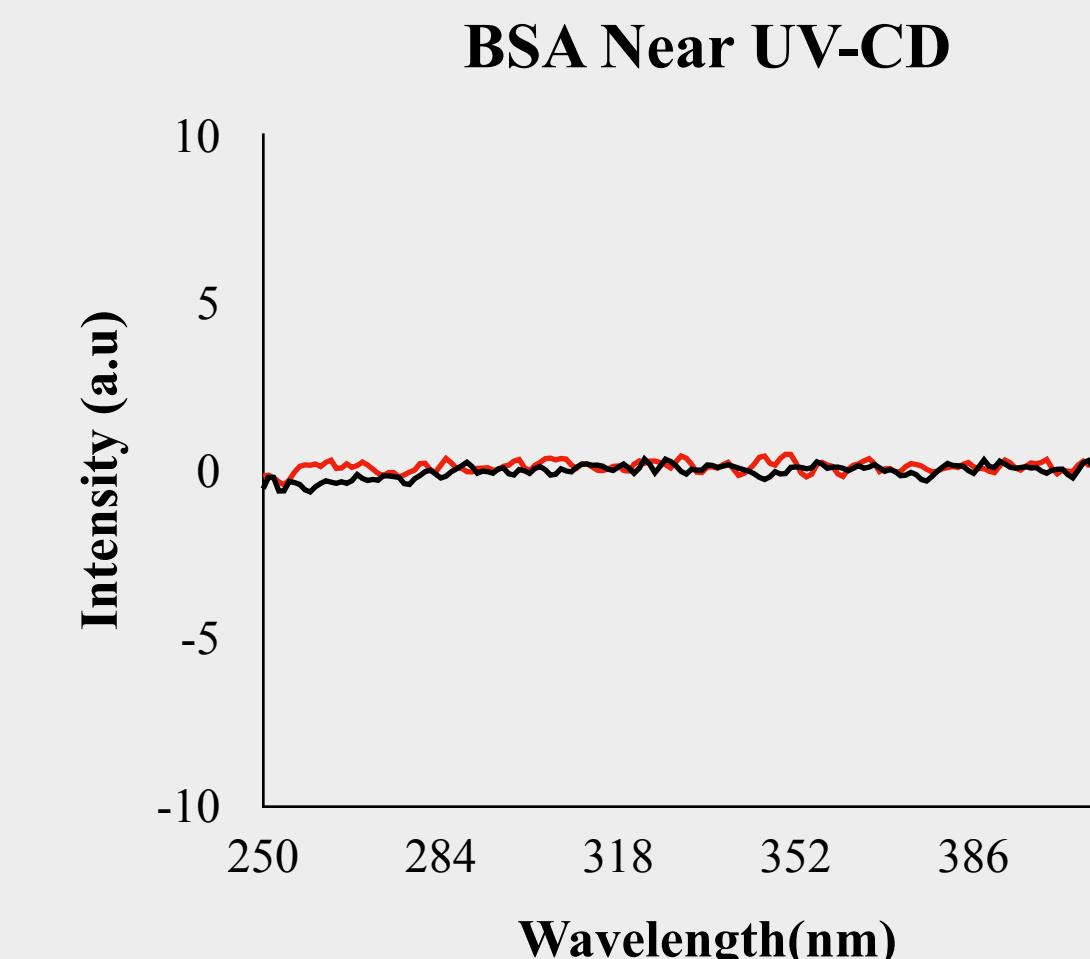
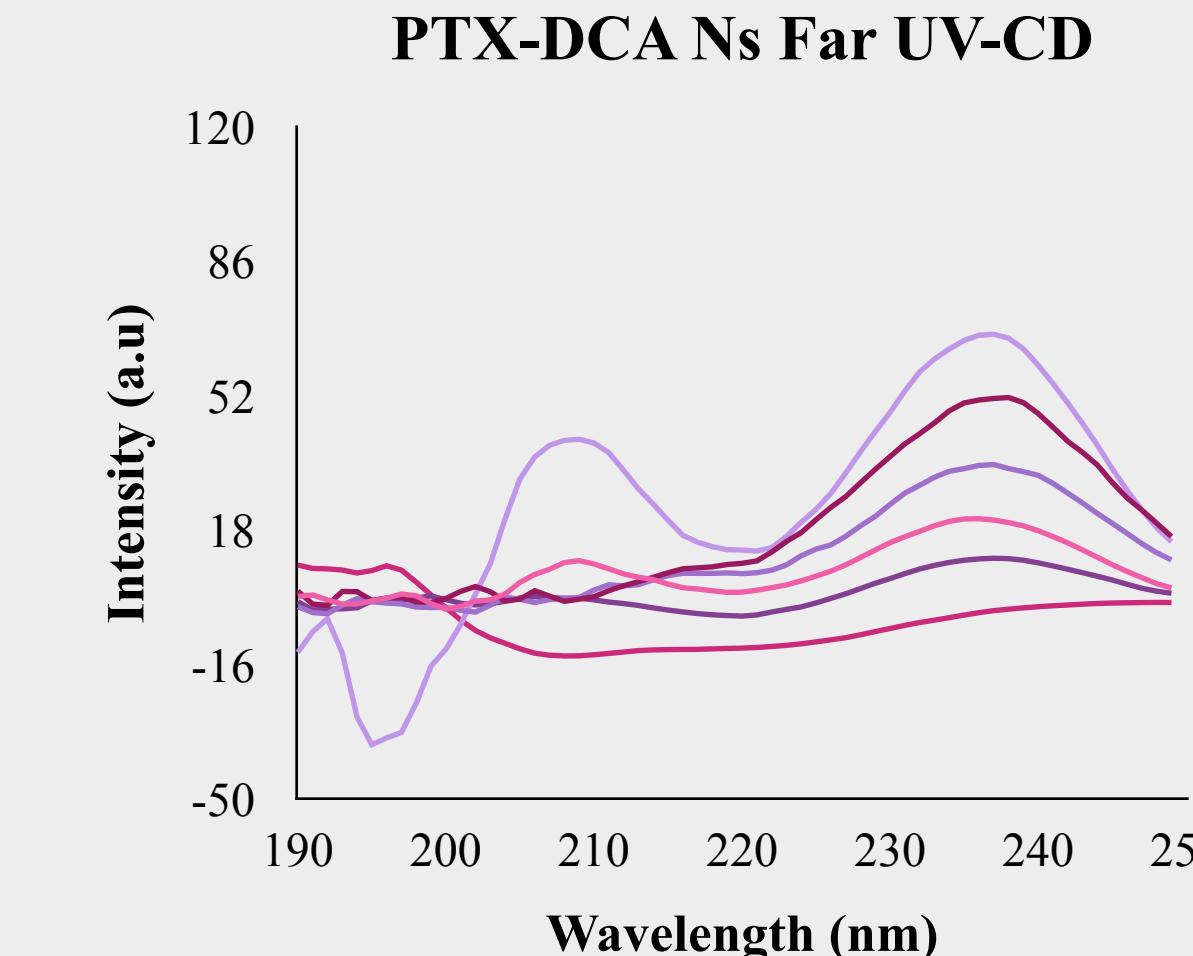
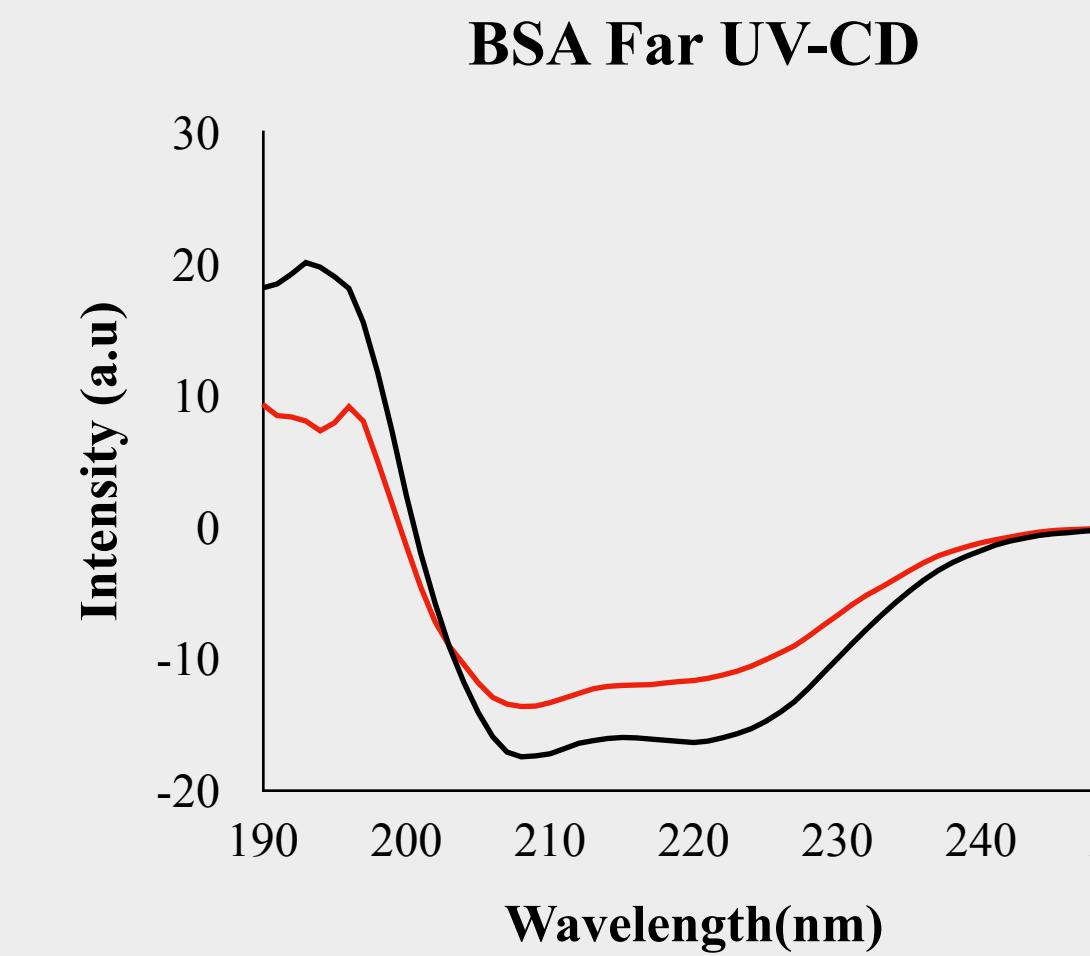


Structure & interaction study





Structure & interaction study



Instrument Info.: JASCO J-810 Circular Dichroism Spectrometer



Report

3

In vitro study

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

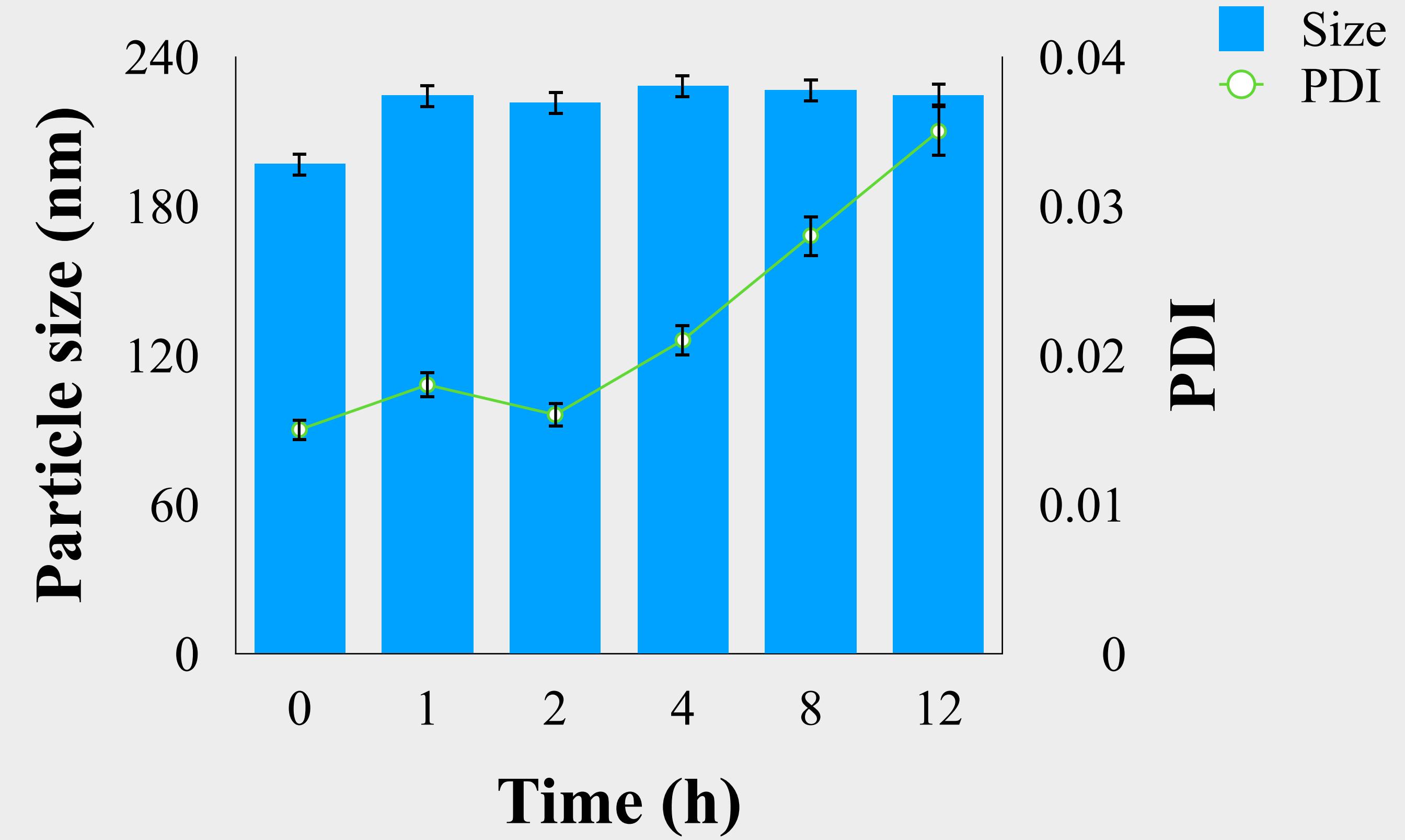
Annexin V-PI双染法流式细胞术测定

细胞周期阻滞

PI单染法流式细胞术测定



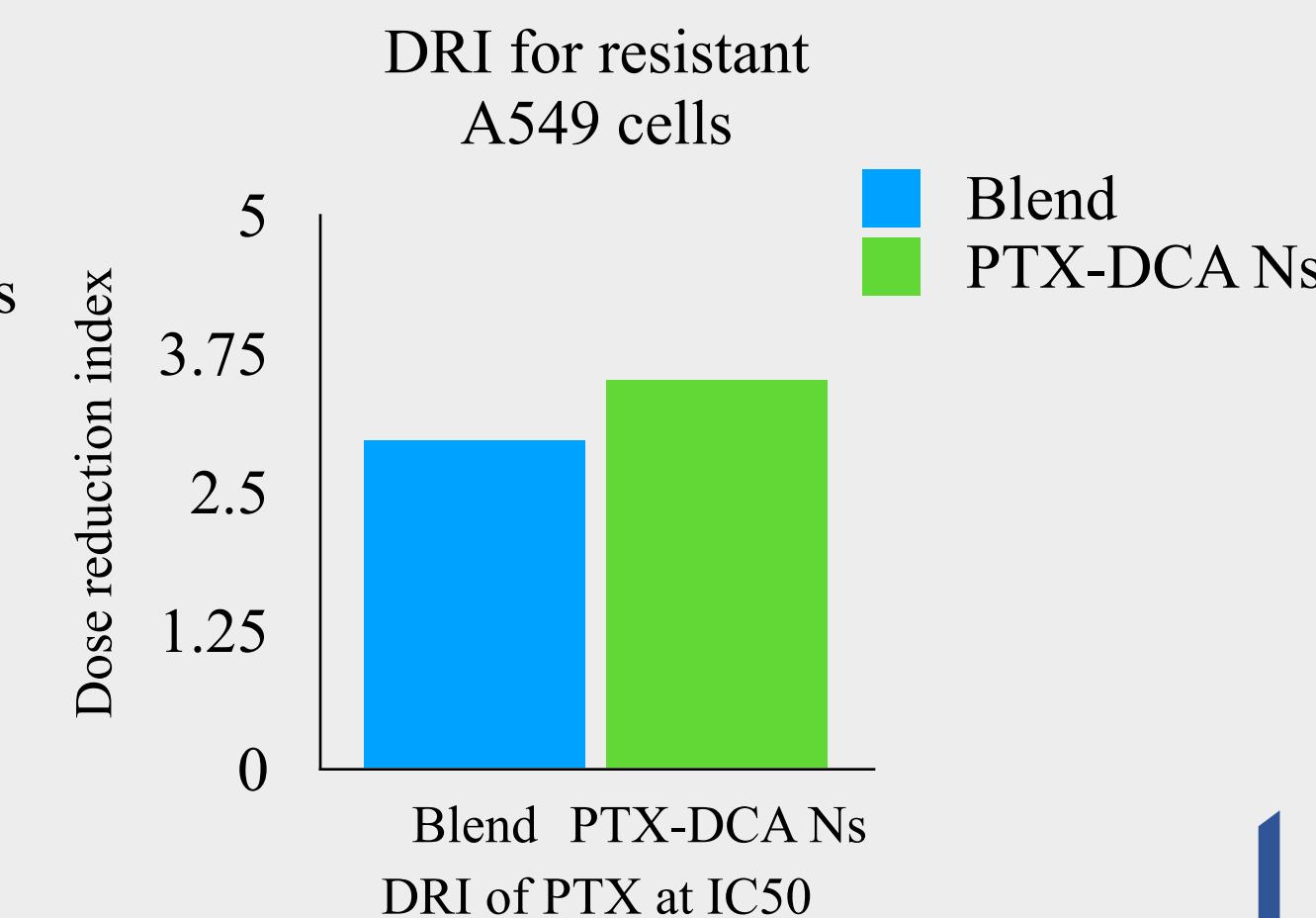
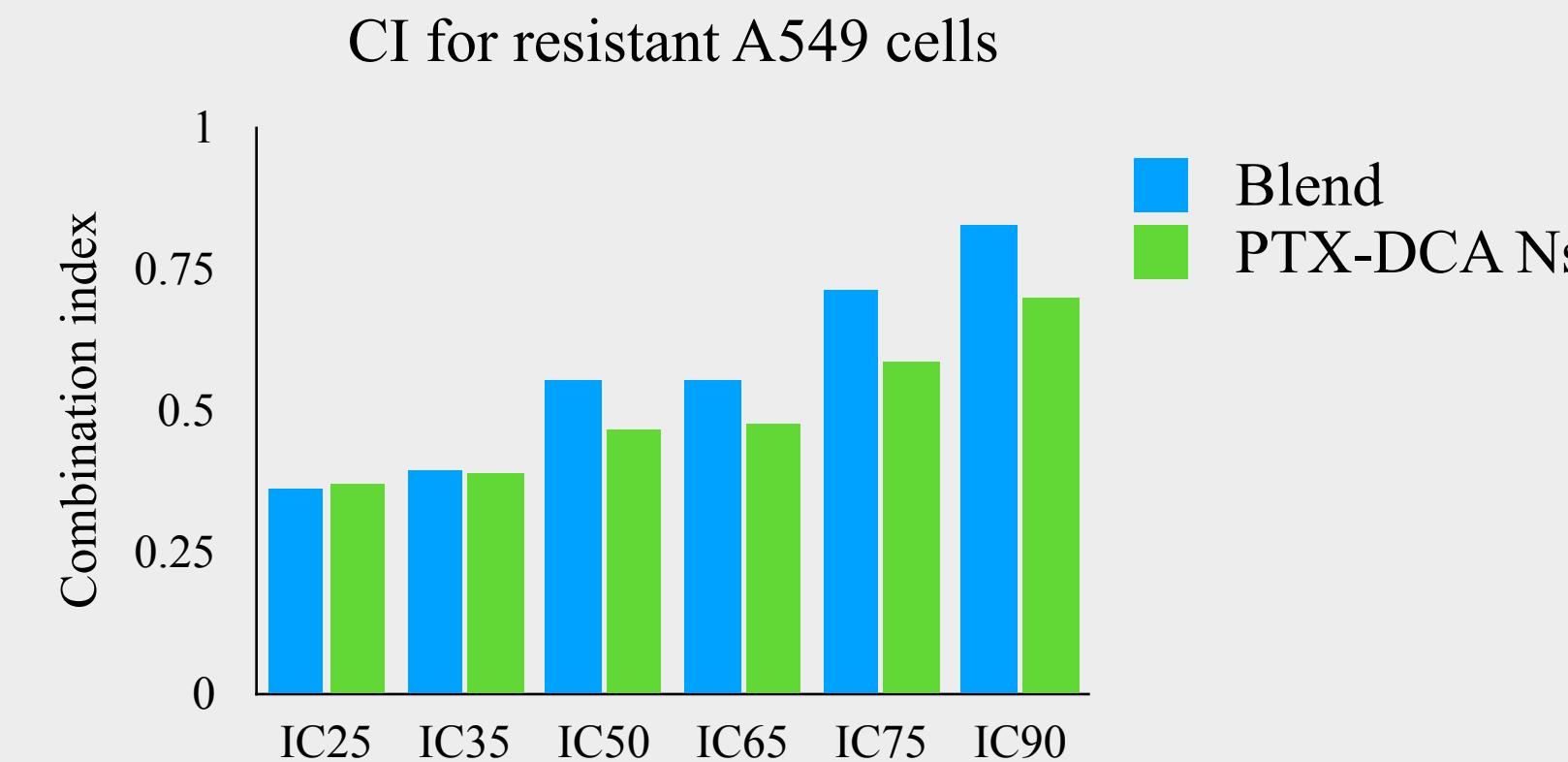
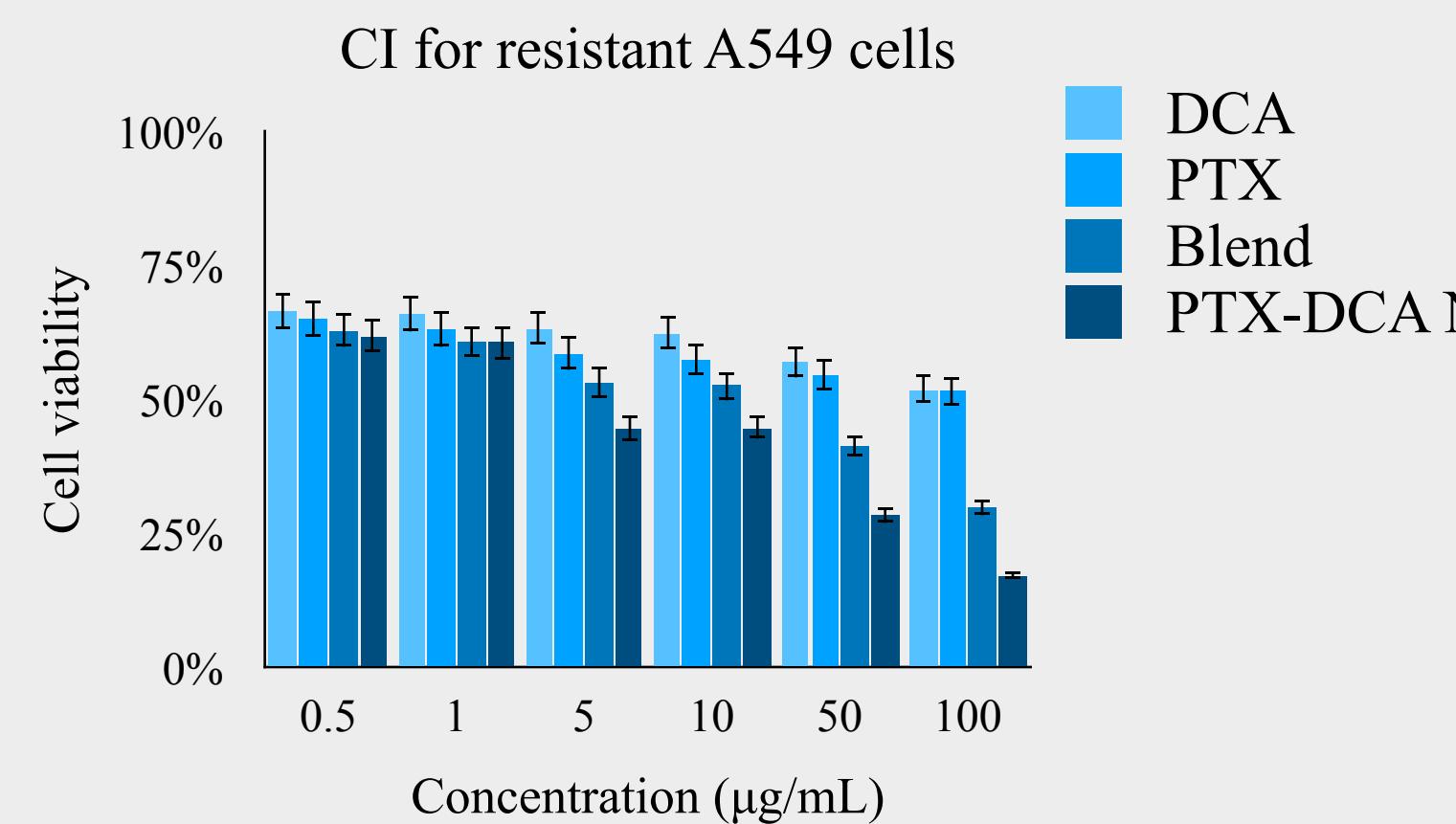
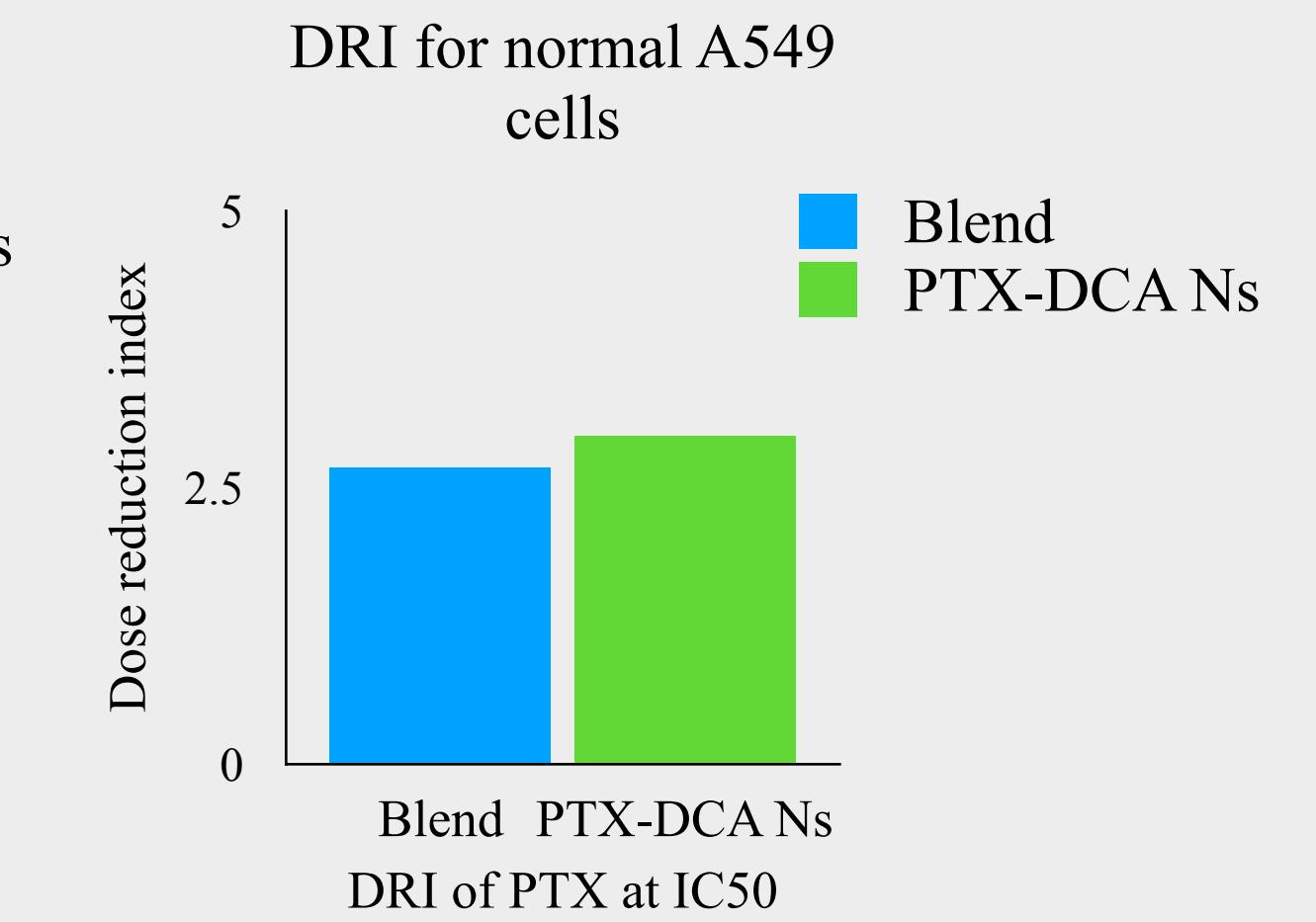
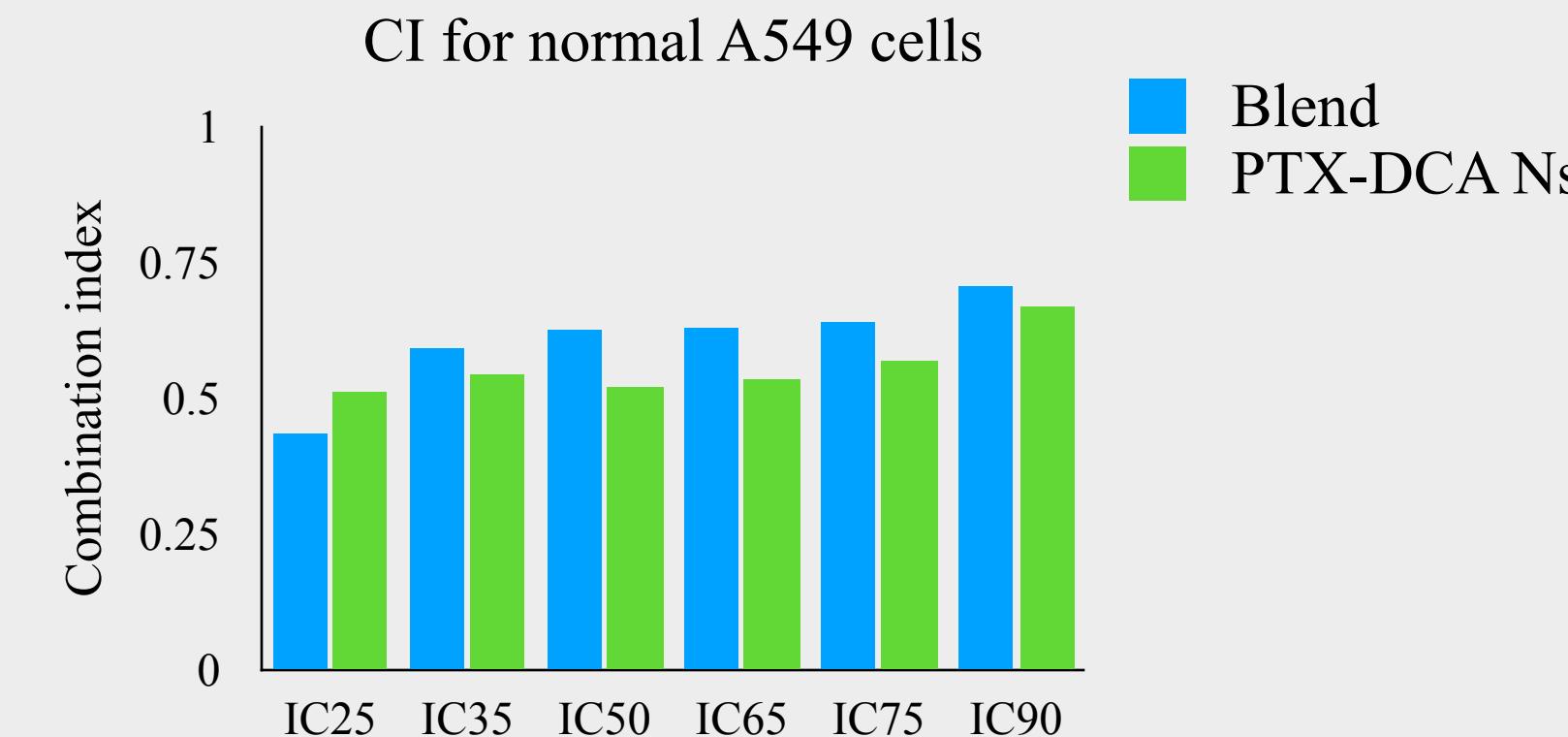
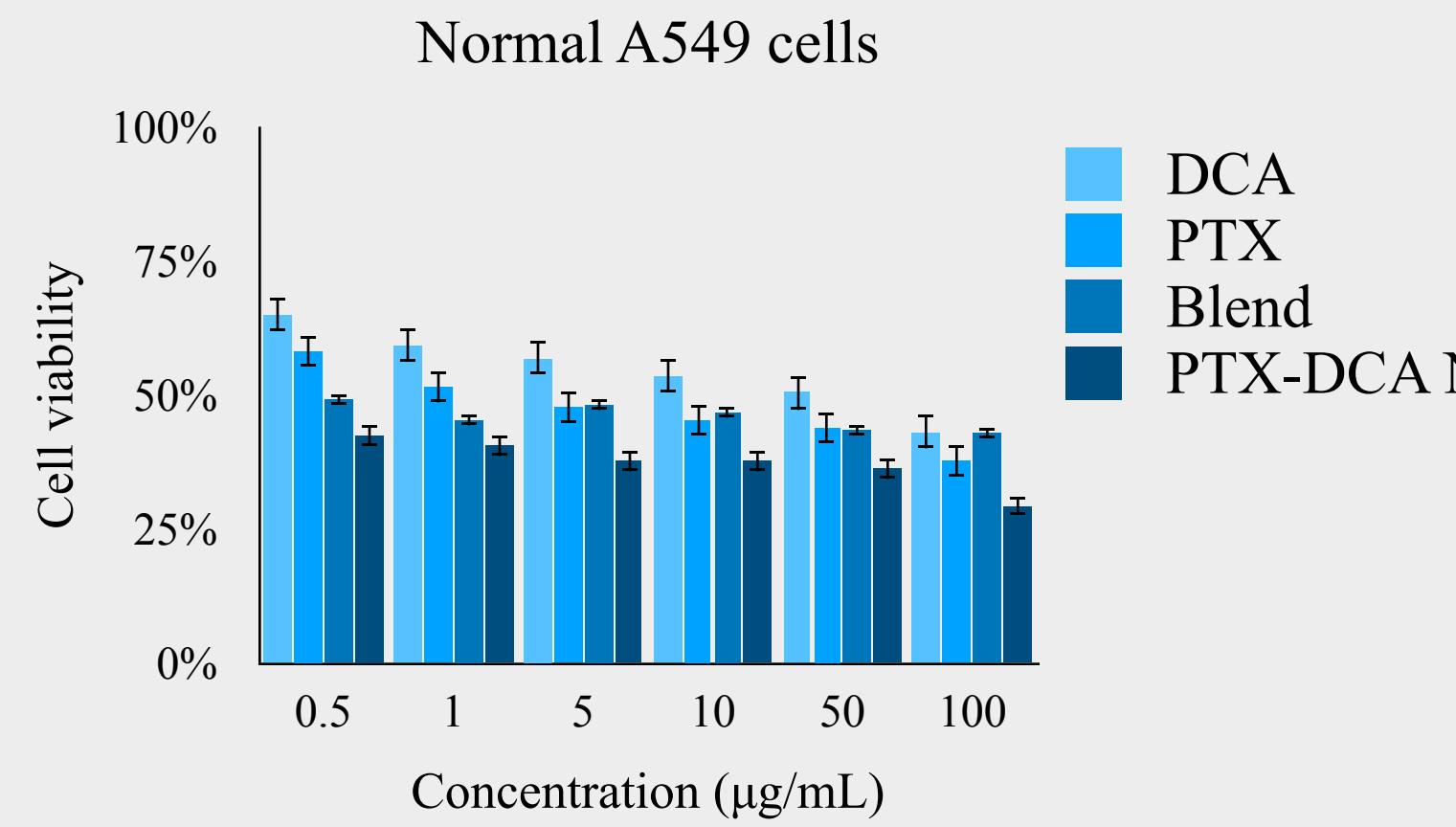
Stability study



Condition: 37°C, 10%FBS, dilution factor = 10



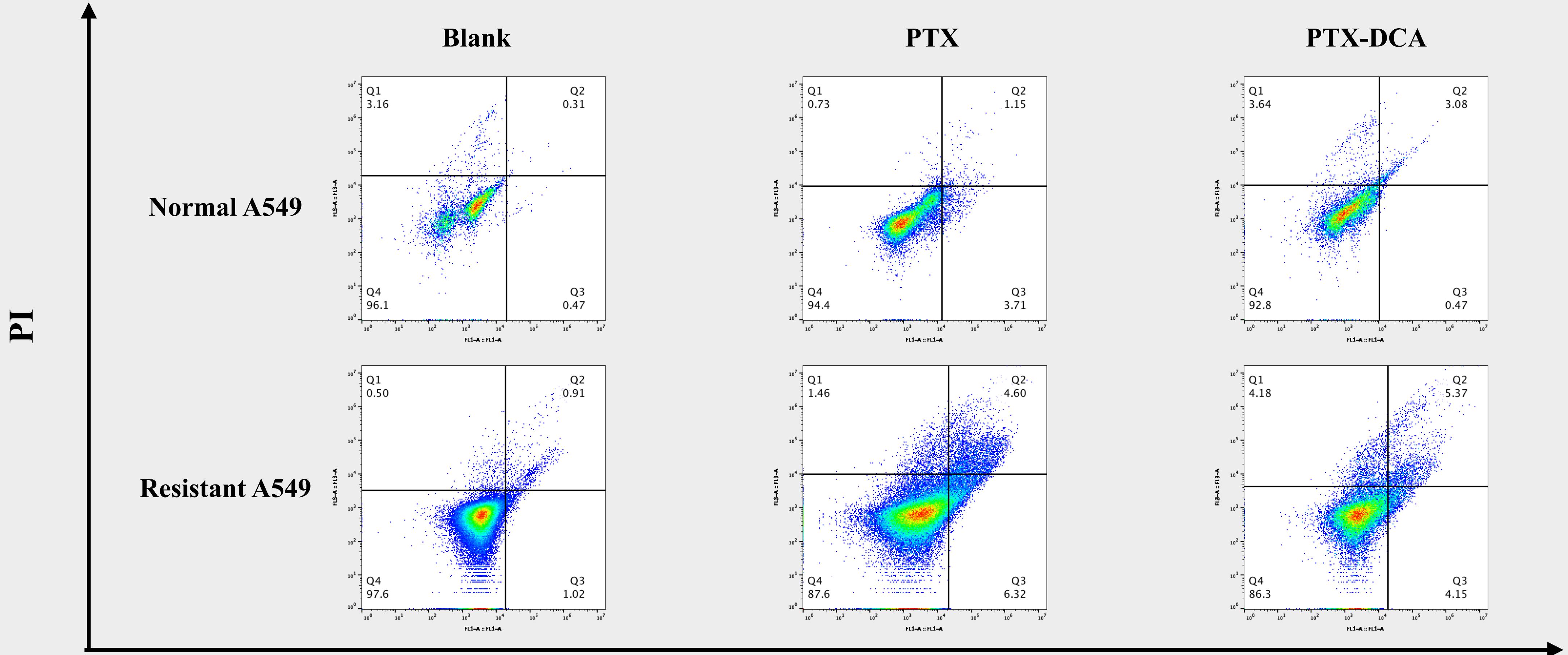
Anti-tumor effect study via MTT method



Instrument info.: Thermo Scientific™ Multiskan™ FC Microplate reader
Software info.: CompuSyn v1.0.1, Chou-Talaly model



Cell apoptosis study

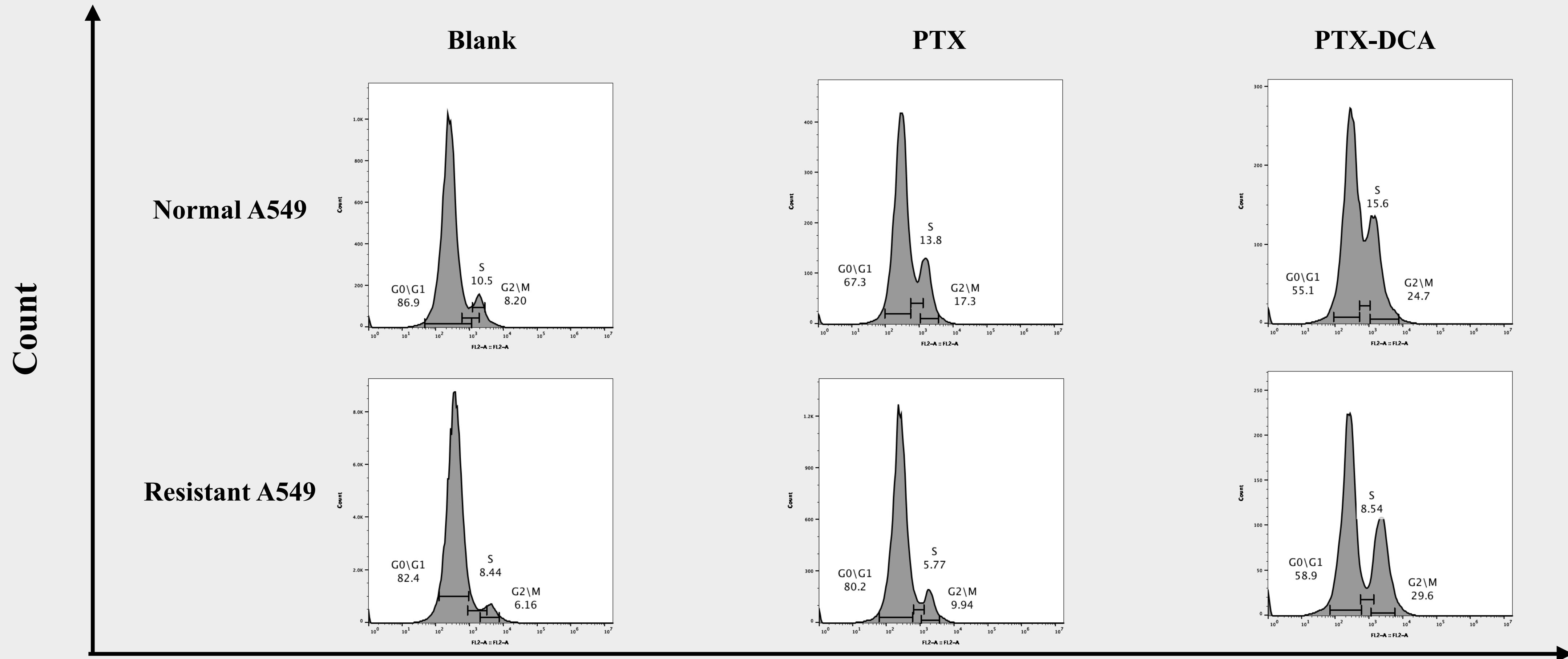


Instrument info.: BD Accuri™ C6 Plus Cytometer

Software info.: FlowJo v10.0.0



Cell cycle study

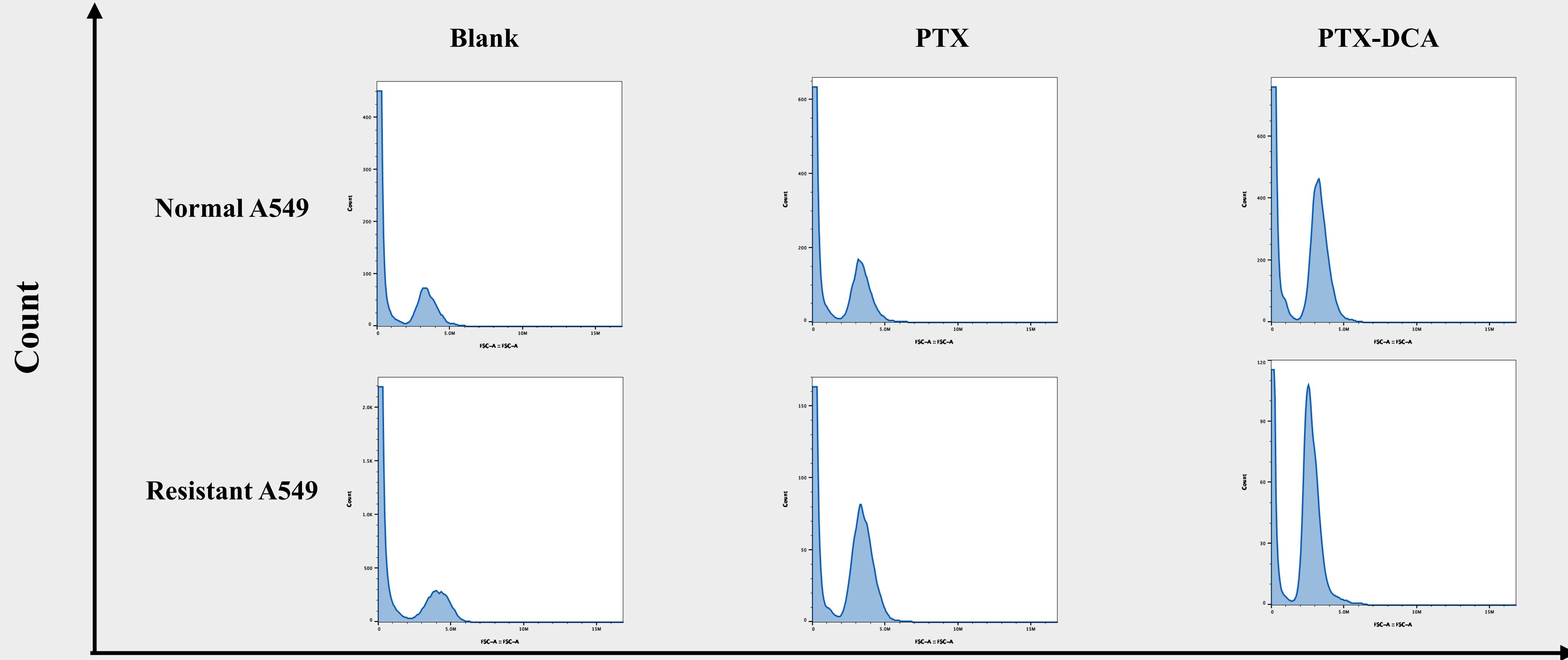


PI

Instrument info.: BD Accuri™ C6 Plus Cytometer
Software info.: FlowJo v10.0.0



Mitochondria ROS level study



DCFH-DA

Instrument info.: BD Accuri™ C6 Plus Cytometer

Software info.: 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

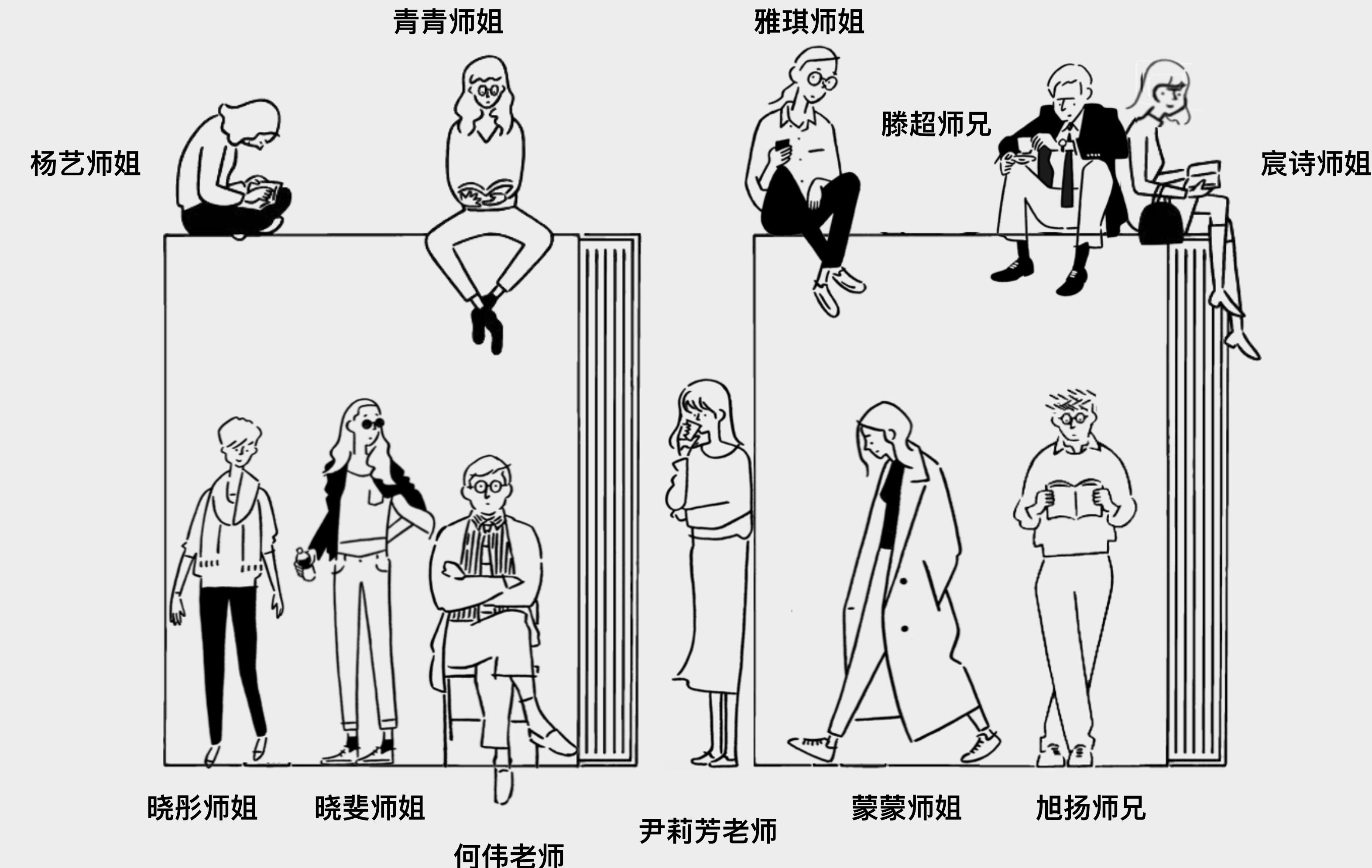


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Acknowledgement



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指导老师：尹莉芳教授 何伟教授

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