

Aptamer Directed Synergistic Drug Delivery

Valerie Lensch
Chemistry



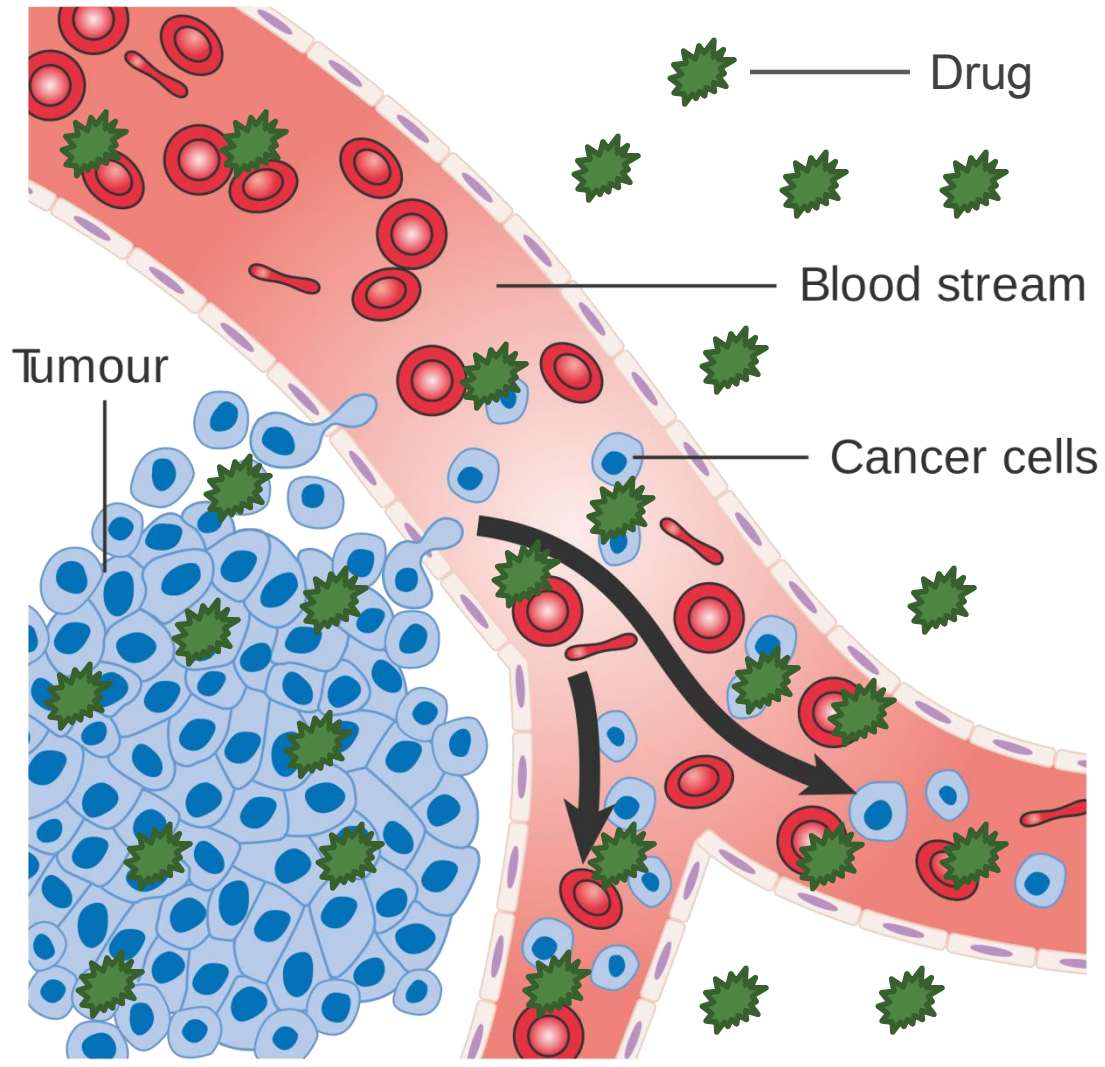
Mentor Anusha Pusuluri



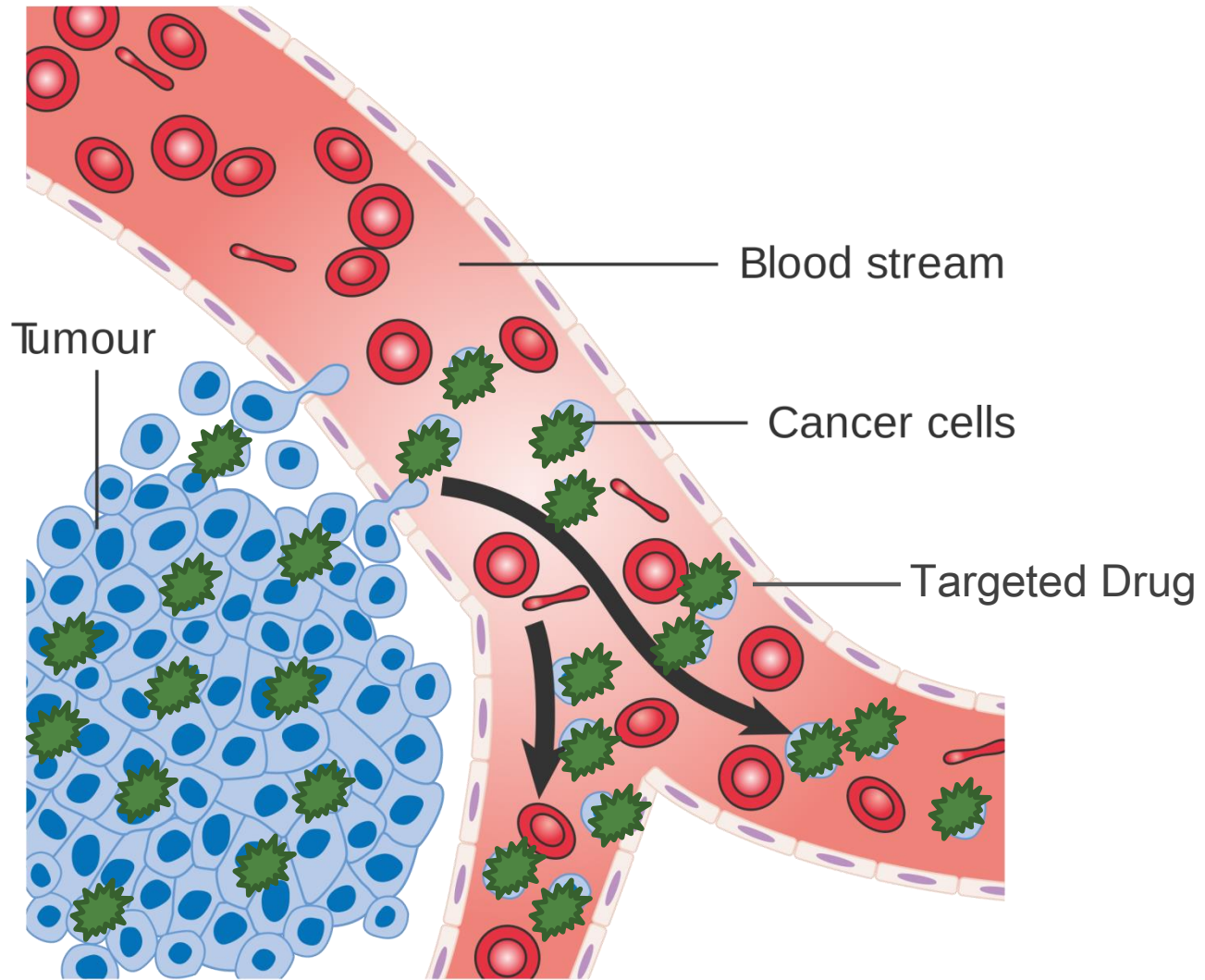
Samir Mitragotri
Department of Chemical Engineering

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Department of Mechanical Engineering

Targeted Drug Delivery Minimizes Side Effects of Chemotherapeutic Drugs



Targeted Drug Delivery Minimizes Side Effects of Chemotherapeutic Drugs



Using Unique Characteristics of Breast Cancer Cells as Targets



HR+/HER2-> aka "Luminal A"

73% of all breast cancer cases

- Best prognosis



HR-/HER2-> aka "Triple Negative"

13% of all breast cancer cases

- Worst prognosis



HR+/HER2+> aka "Luminal B"

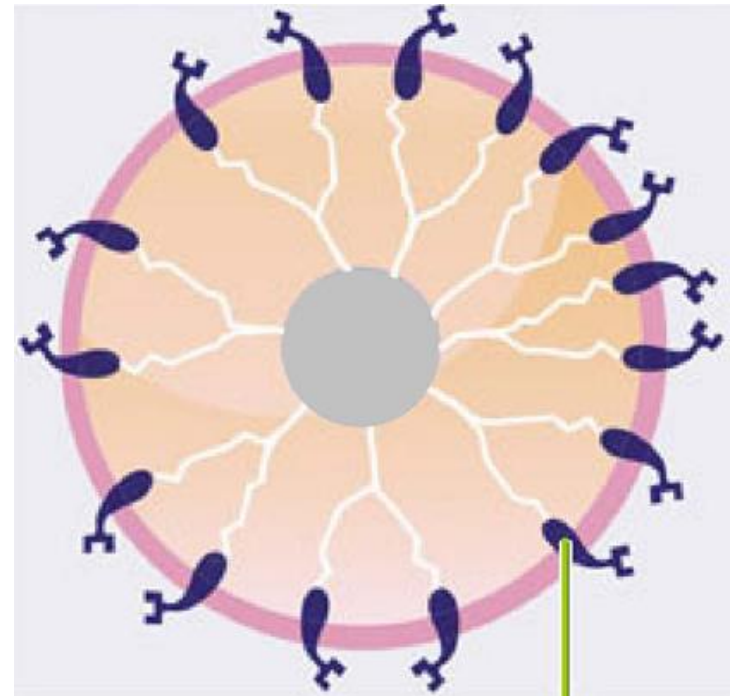
10% of all breast cancer cases



HR-/HER2+> aka "HER2-enriched"

5% of all breast cancer cases

HR=Hormone Receptor
HER2= Human Epidermal Growth Factor Receptor



HR

Using Unique Characteristics of Breast Cancer Cells as Targets



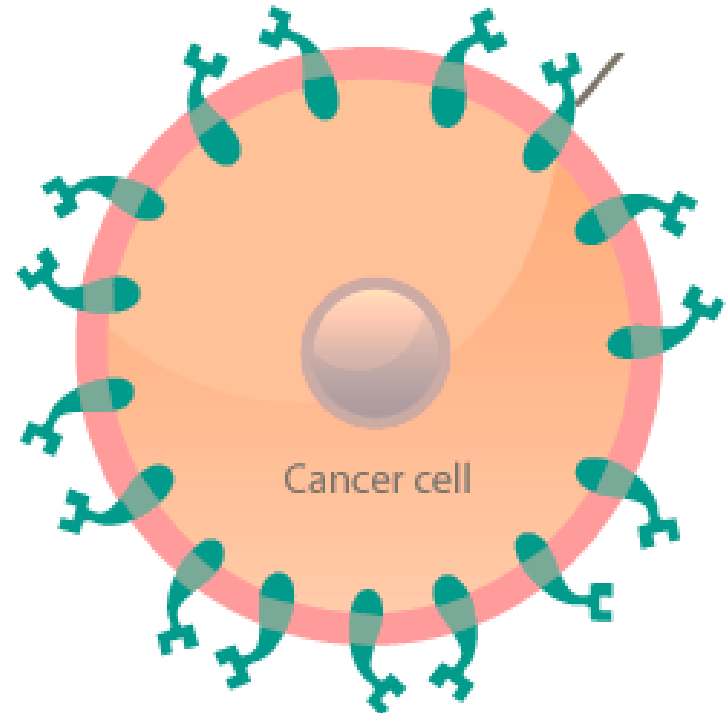
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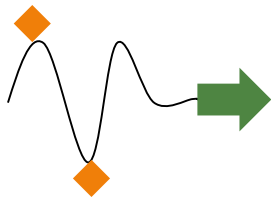
- Worst prognosis

HR=Hormone Receptor
HER2= Human Epidermal Growth
Factor Receptor

Overexpressed
Nucleolin Receptors

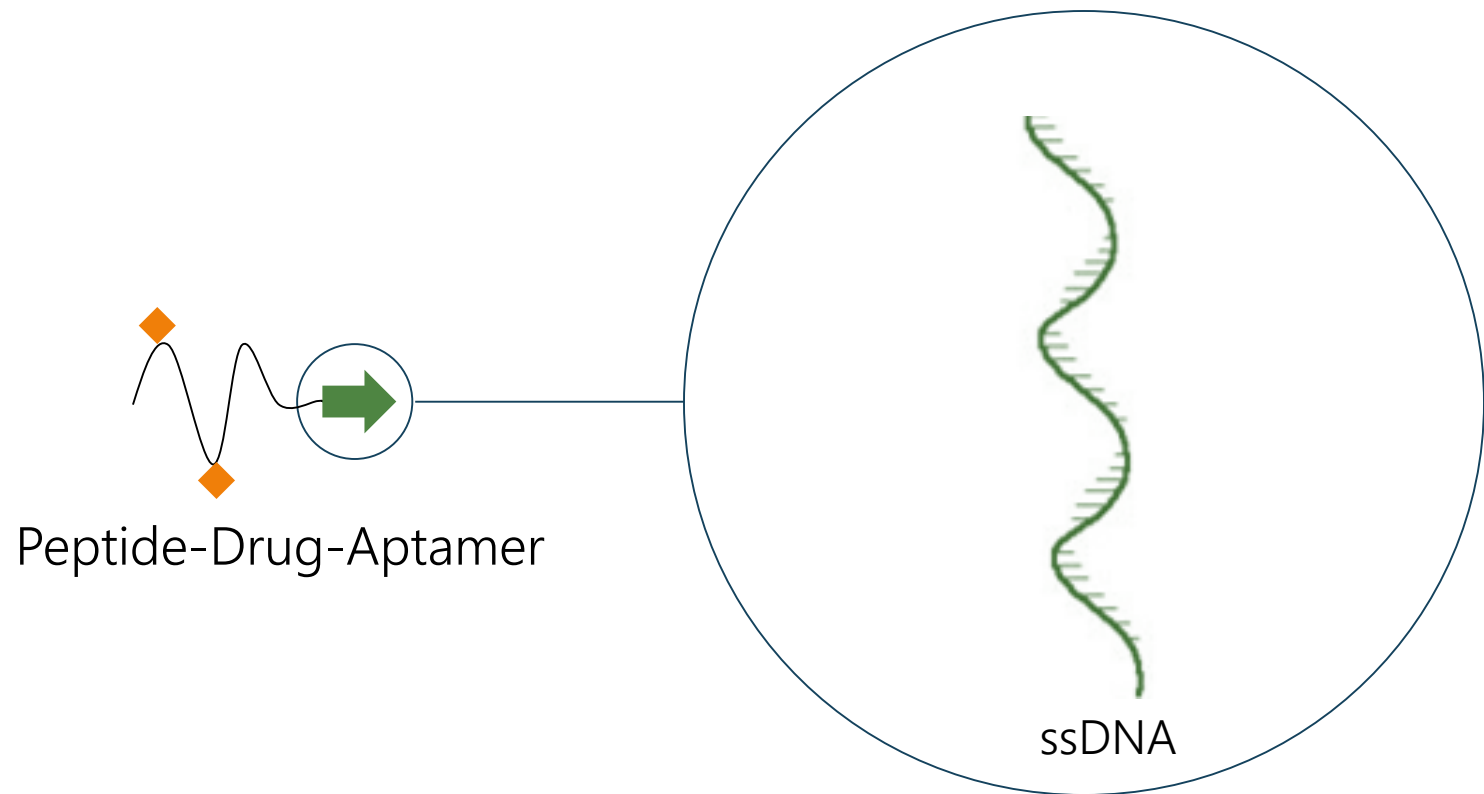


Oligonucleotide Aptamer AS1411 Targets Biomarkers on Cancer Cells

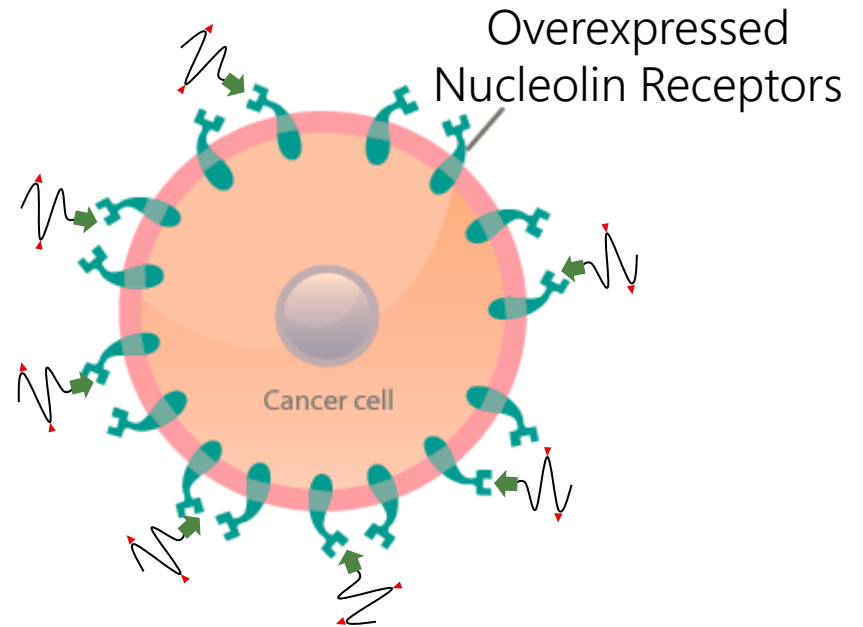
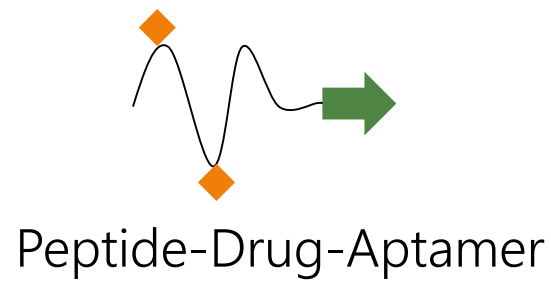


Peptide-Drug-Aptamer

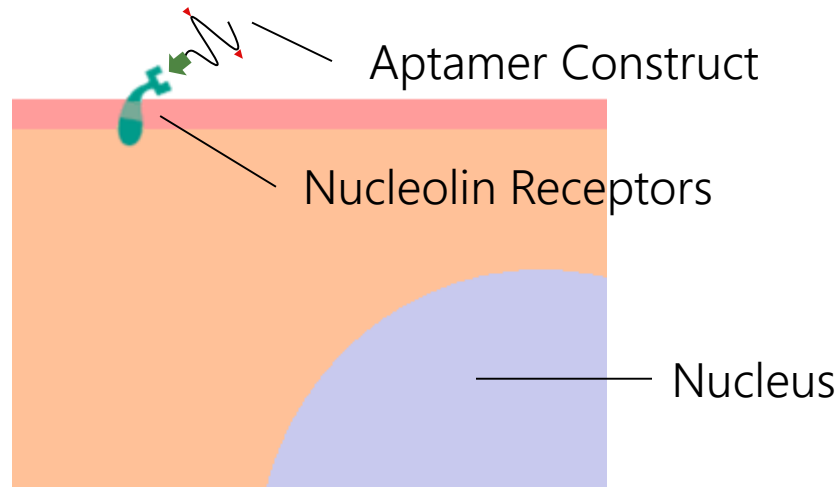
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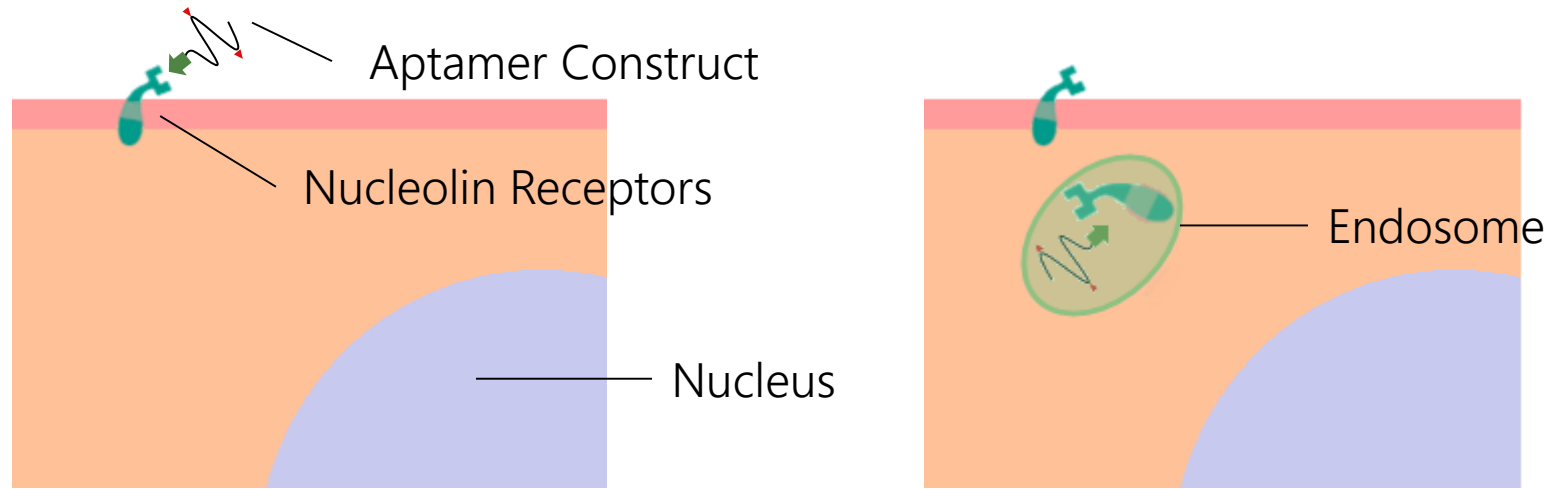
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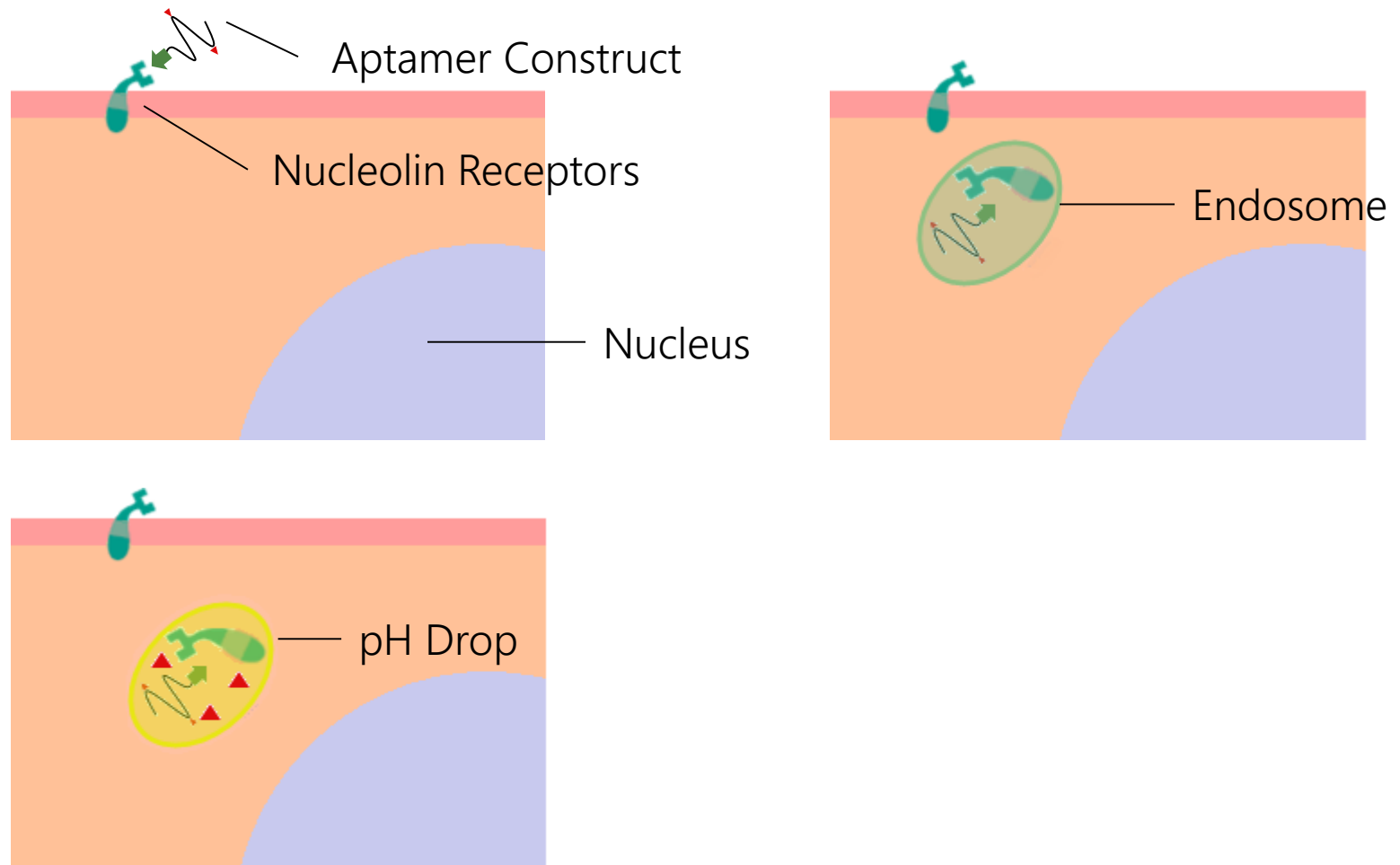
Aptamer Constructs are Taken Up Through Nucleolin Receptor-Mediated Endocytosis



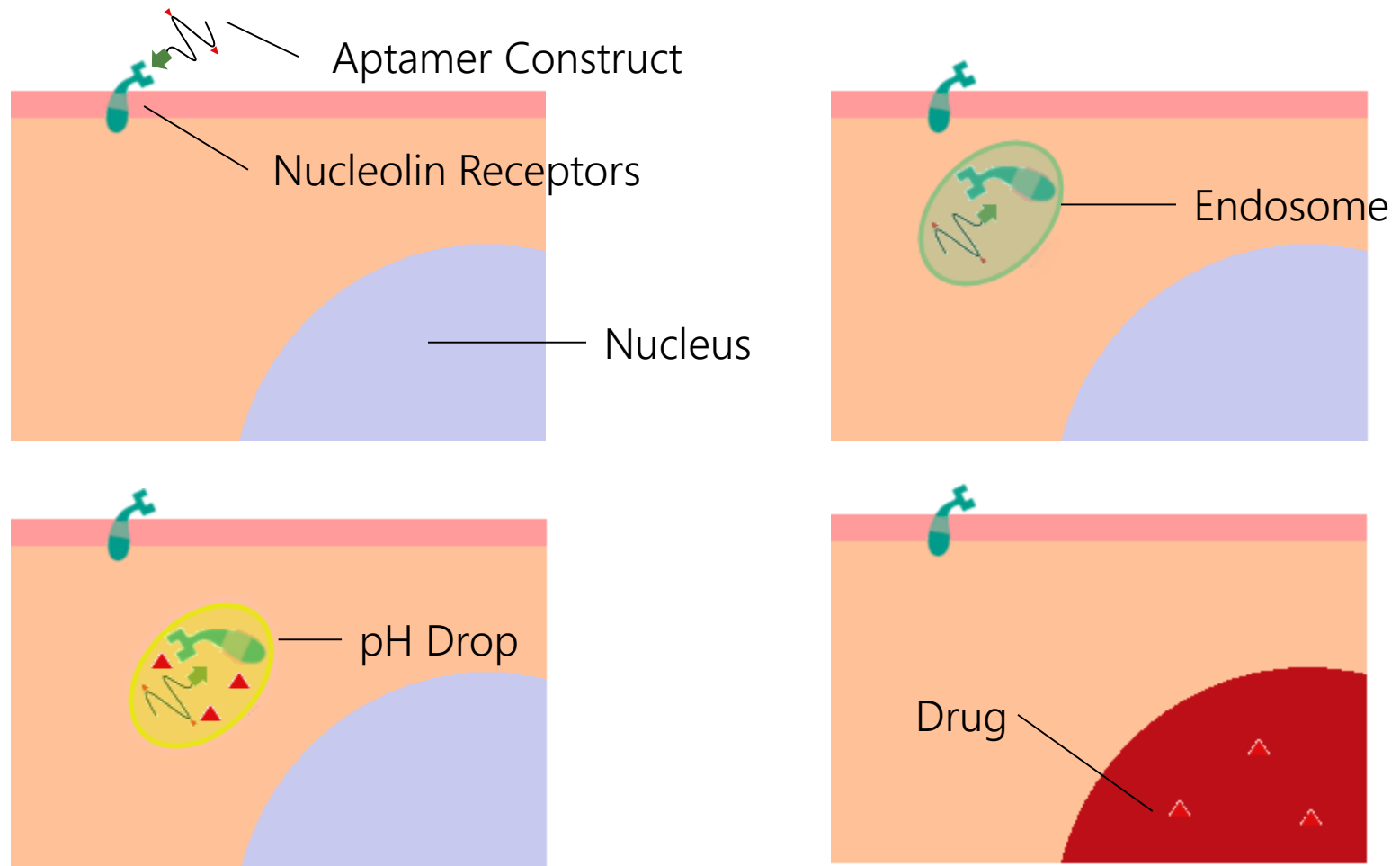
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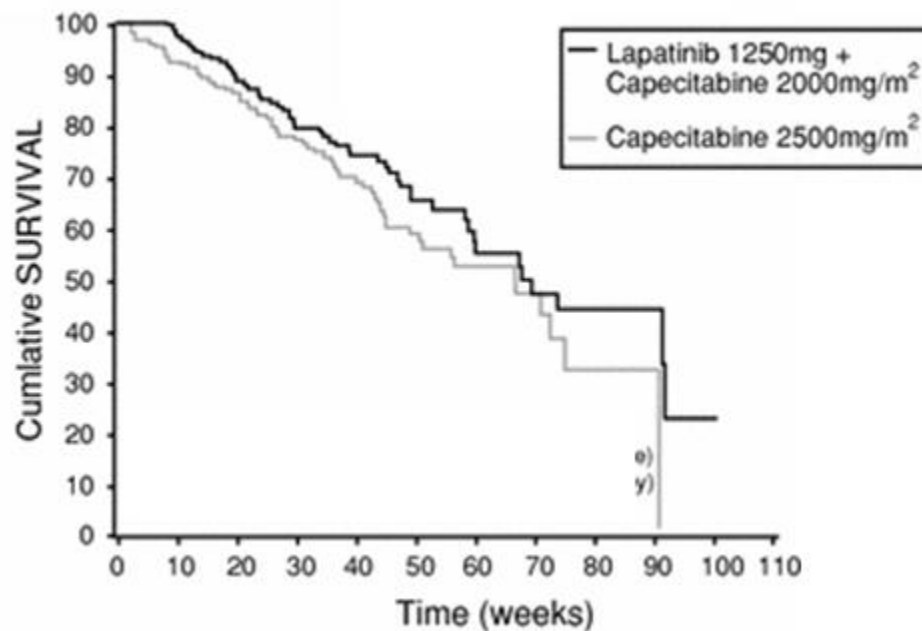
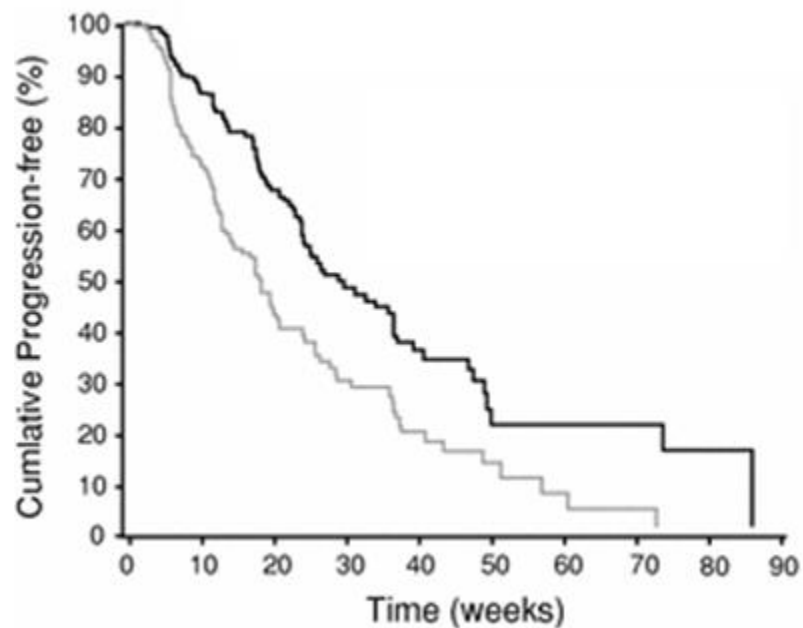
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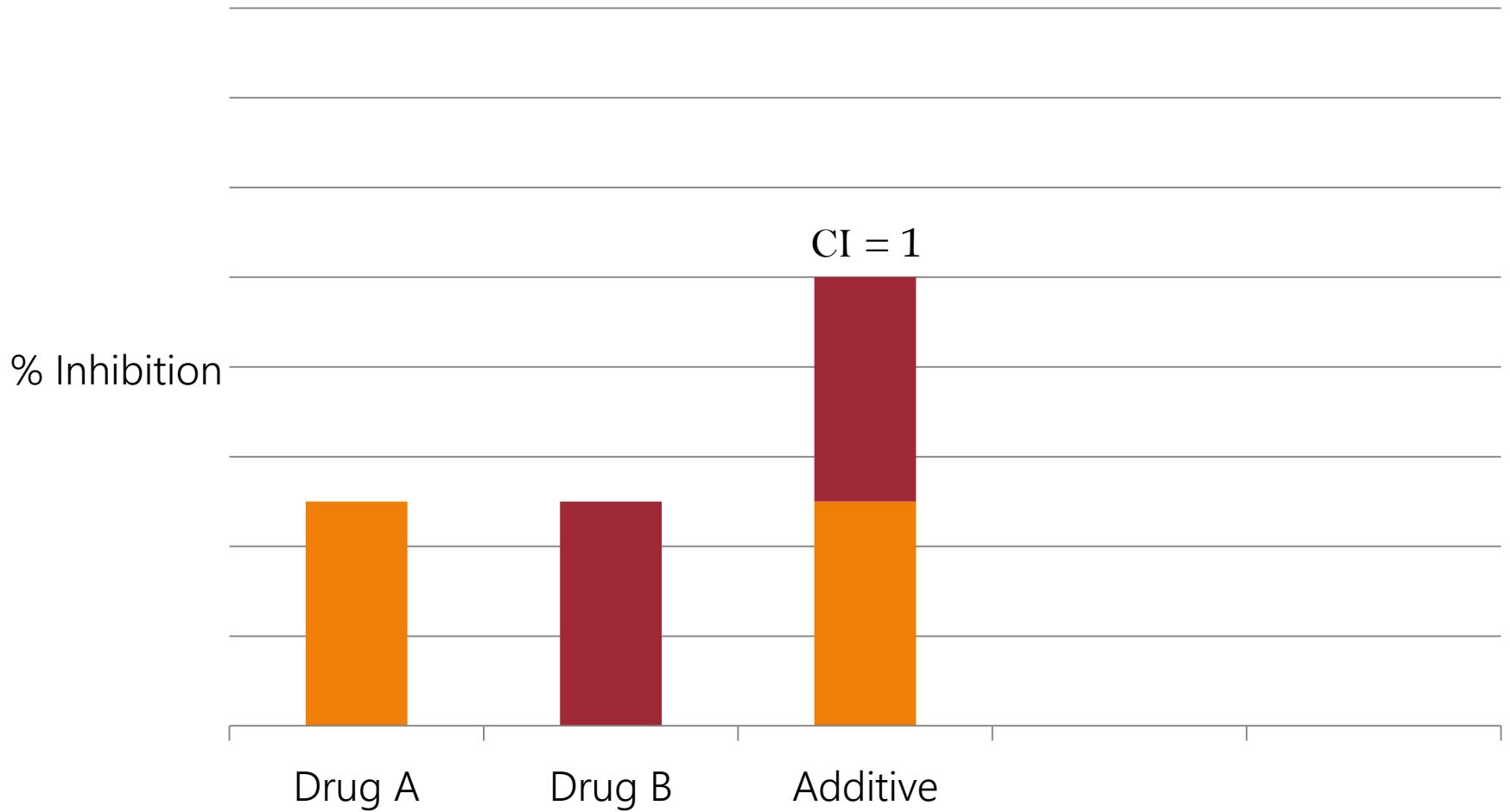
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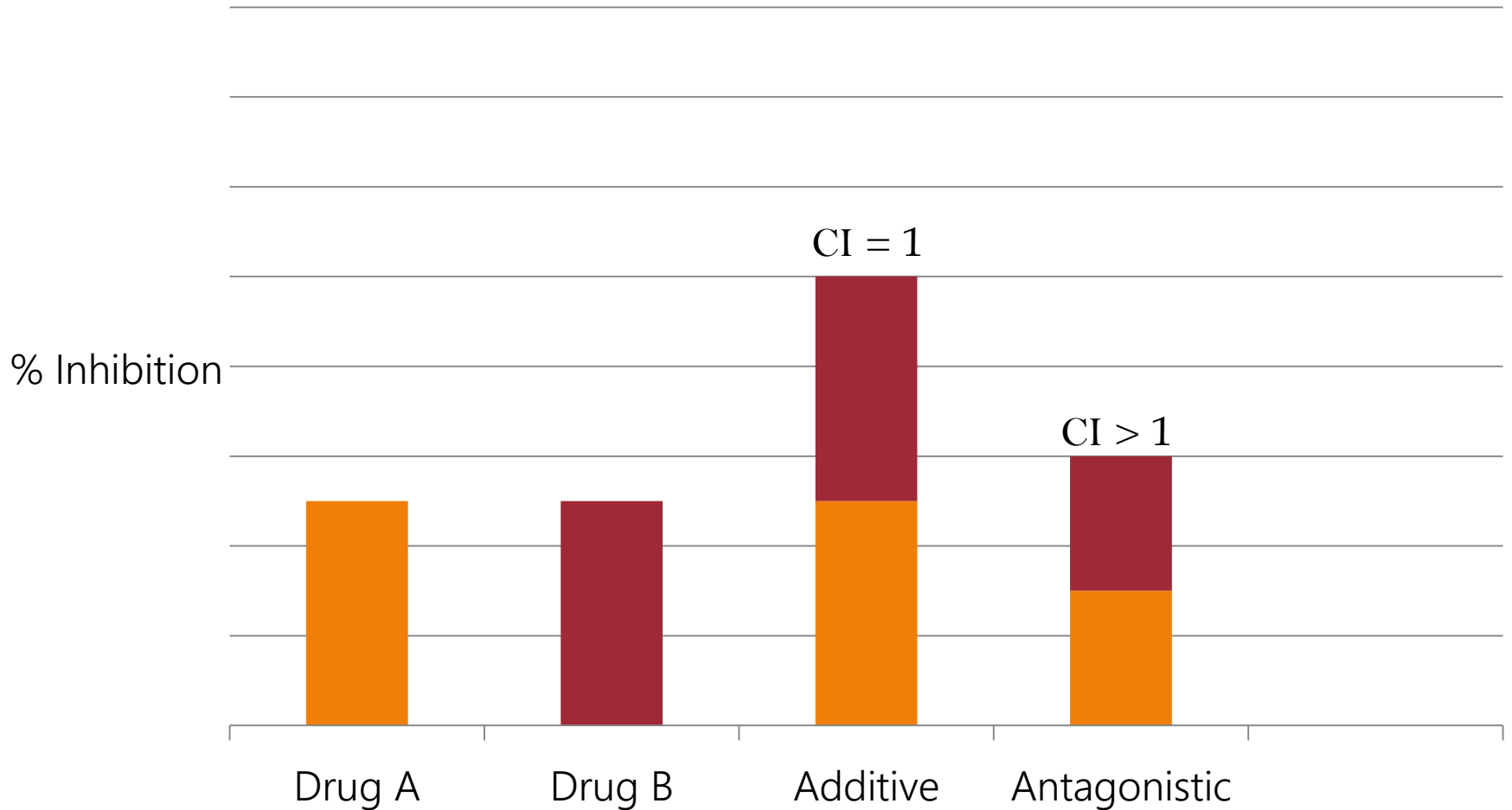
Combination Treatment Improves Efficacy of Therapy as Compared to Single Drug Treatment



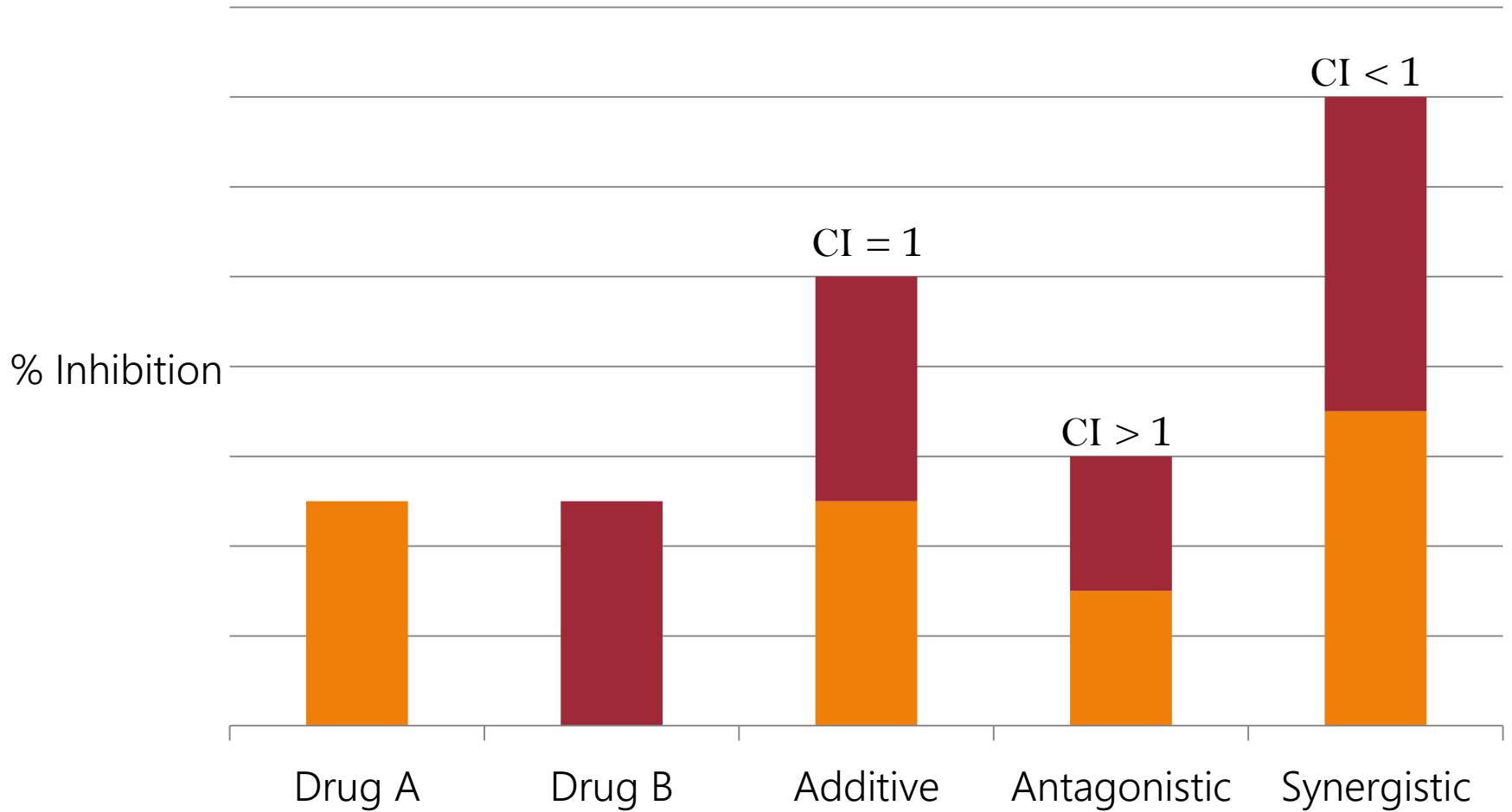
Drug Combinations Can Enhance or Undermine Drug Effect



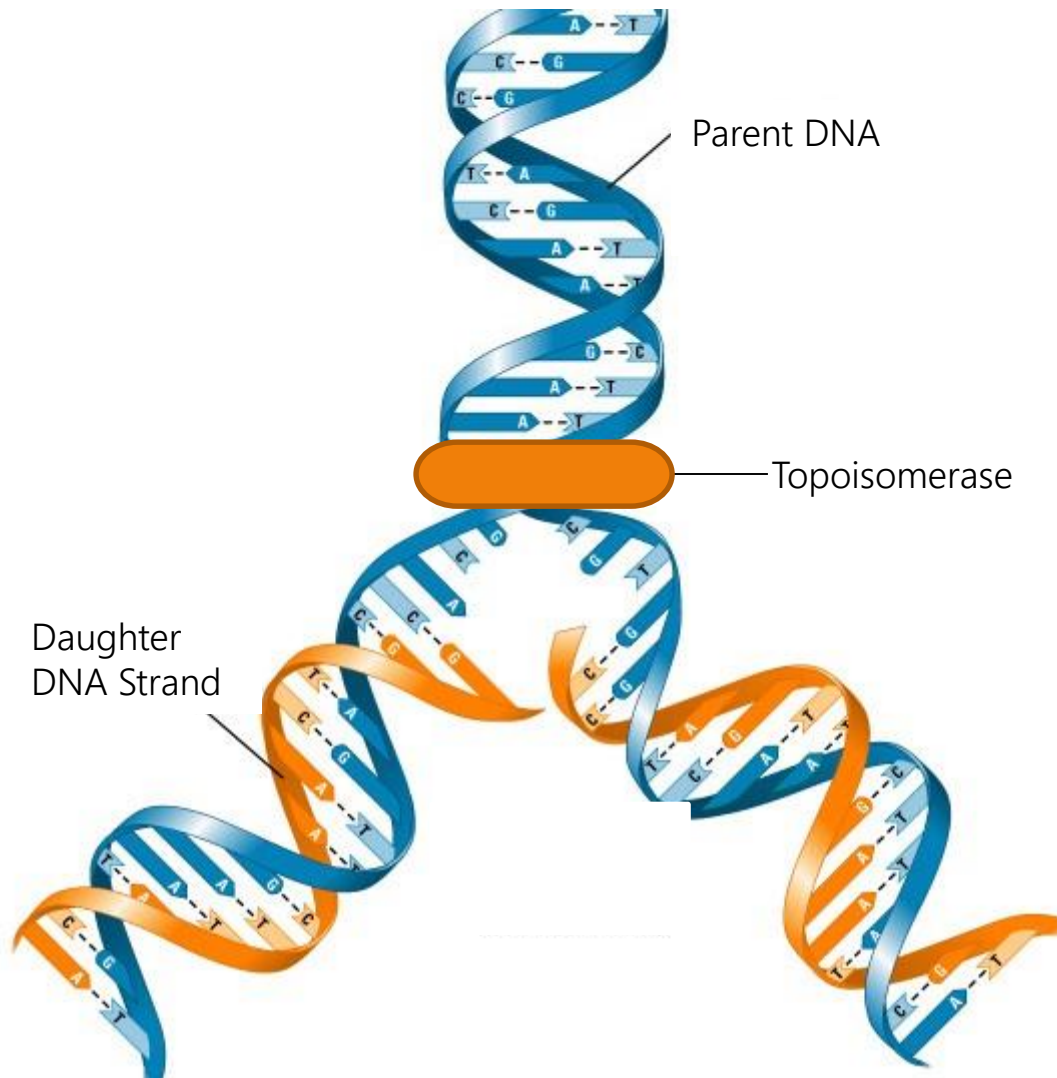
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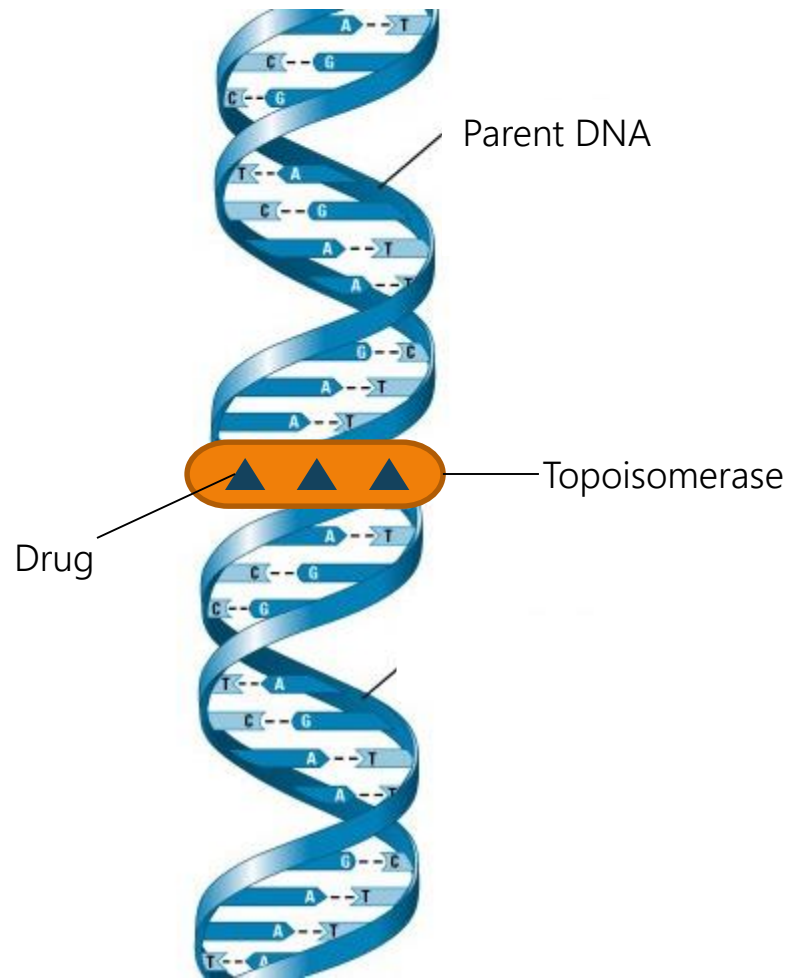
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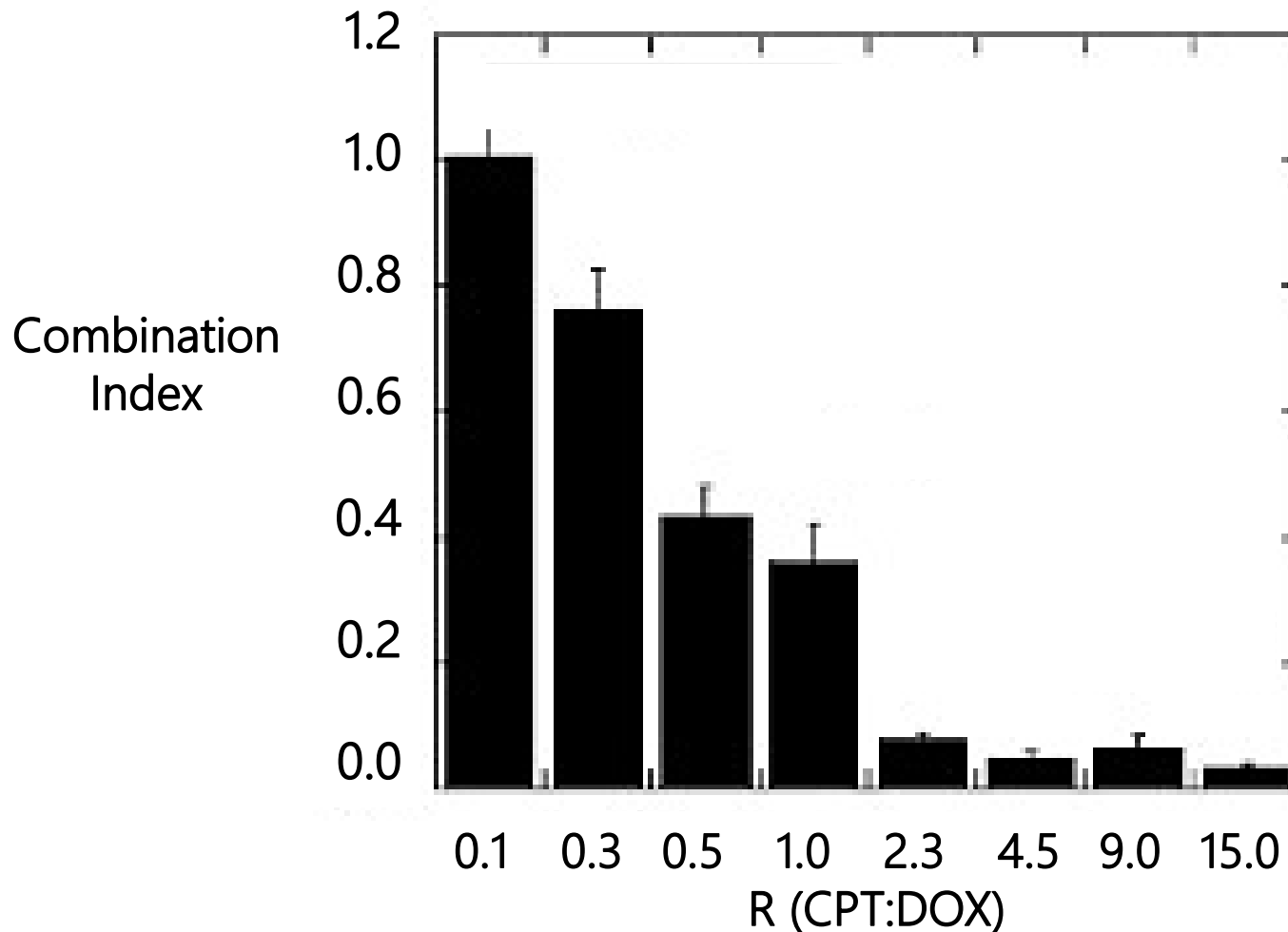
Camptothecin and Doxorubicin Inhibit Topoisomerase I and II, Leading to Apoptosis



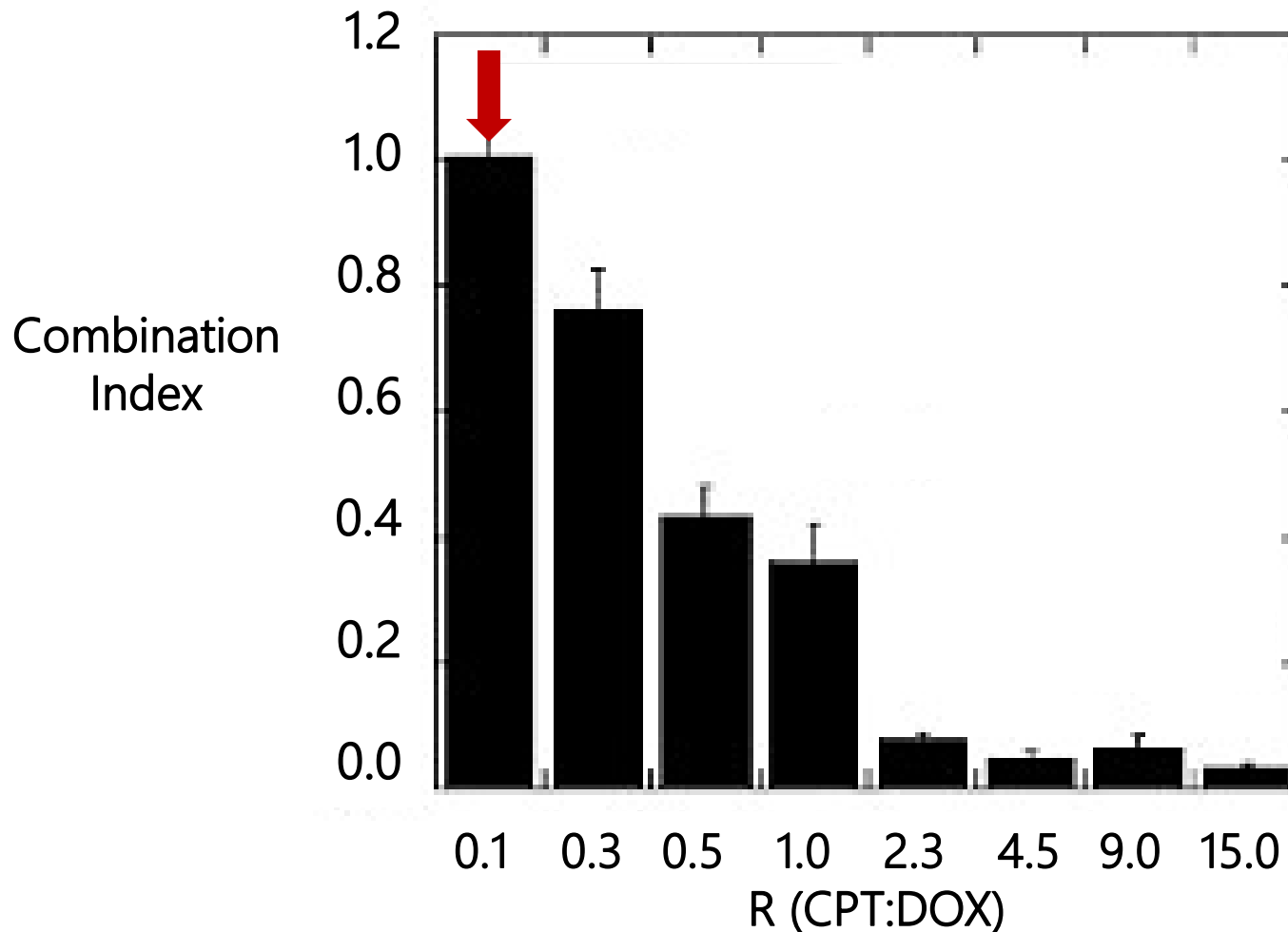
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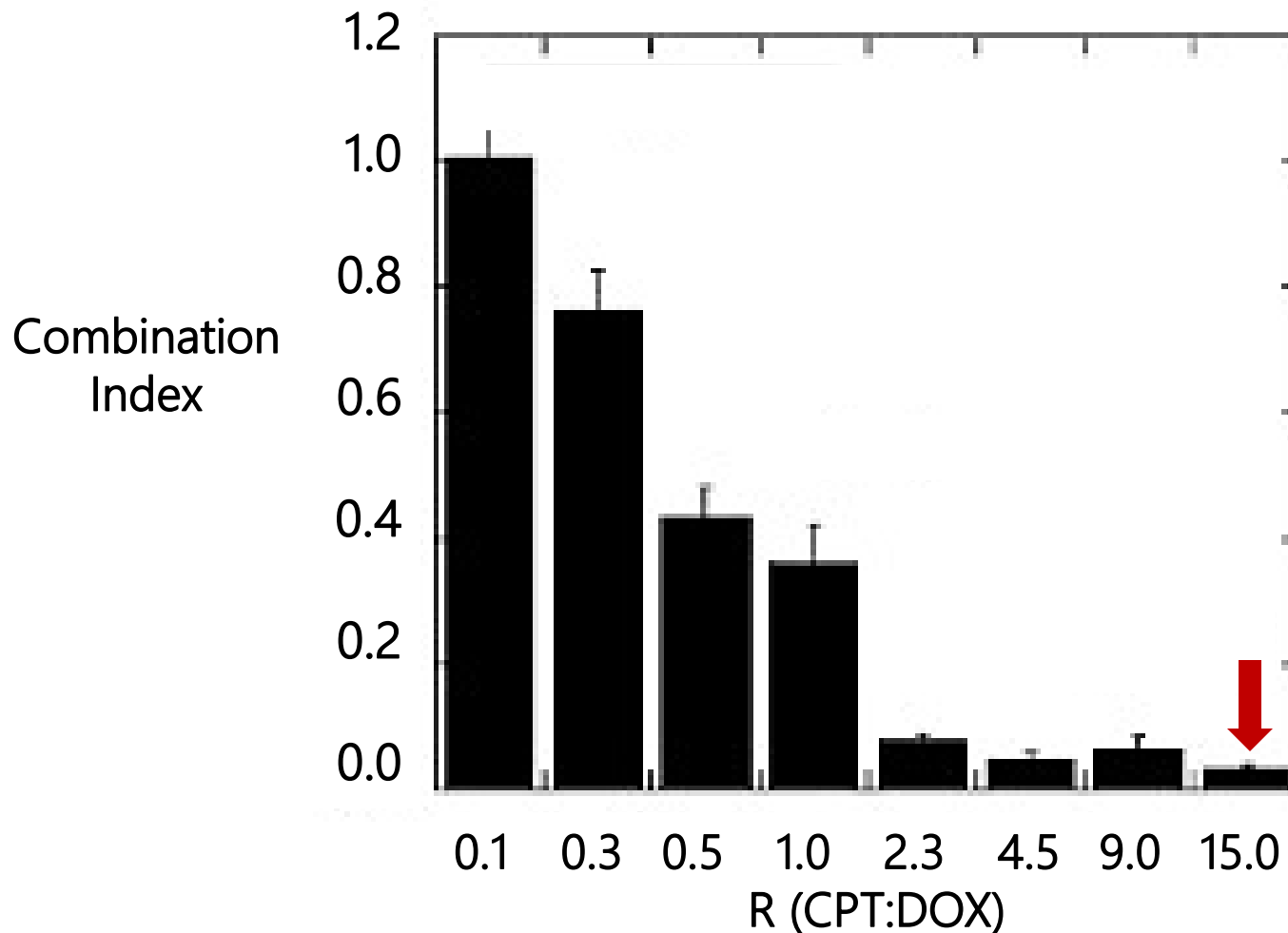
Drug Ratios Cause Synergism and Antagonism



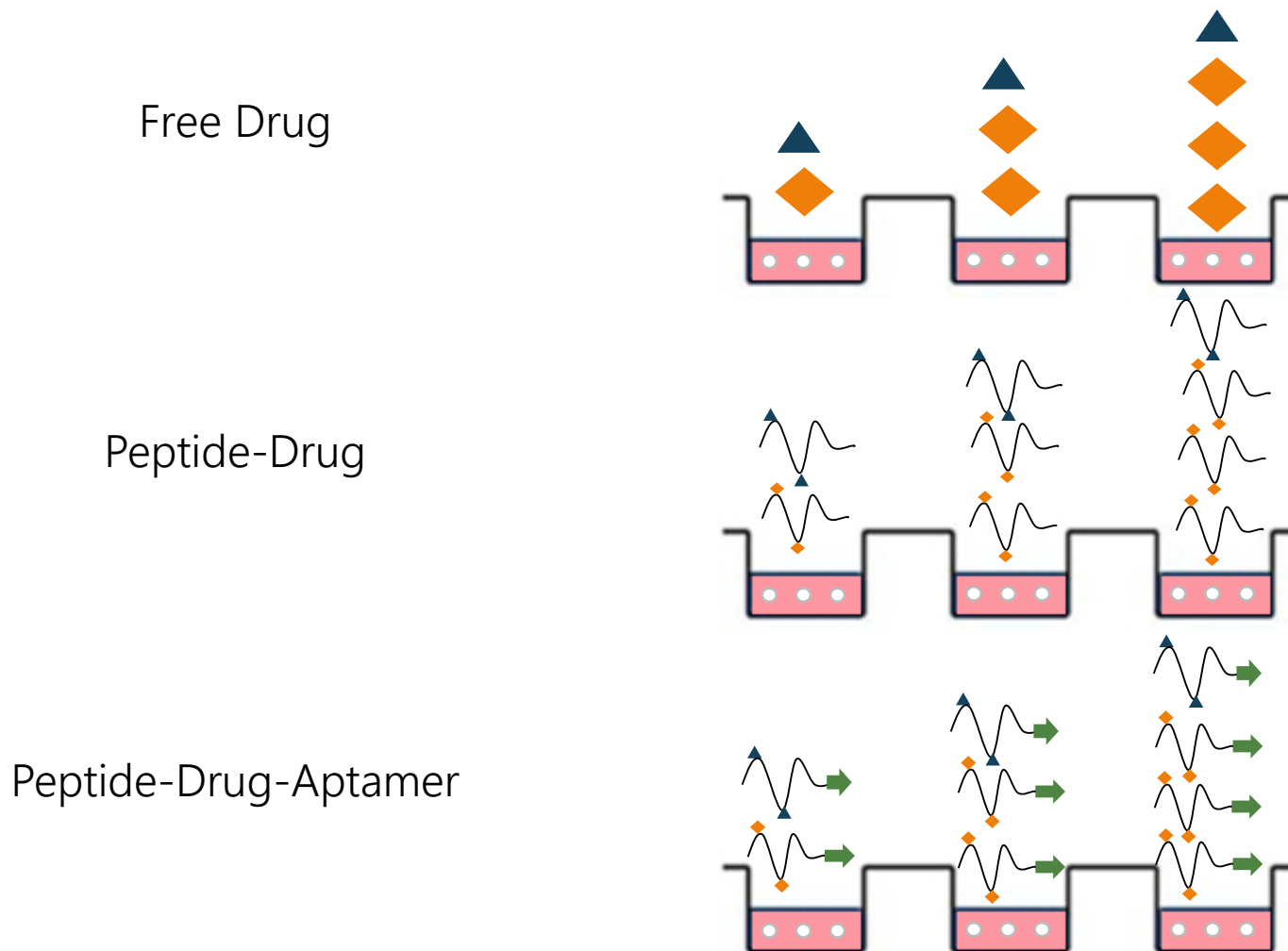
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Determining the Synergistic Ratio of Doxorubicin and Camptothecin



Using MTT Absorbance to Measure Cytotoxicity



Seed Cells in 96 Well Plate & Incubate Overnight

Using MTT Absorbance to Measure Cytotoxicity

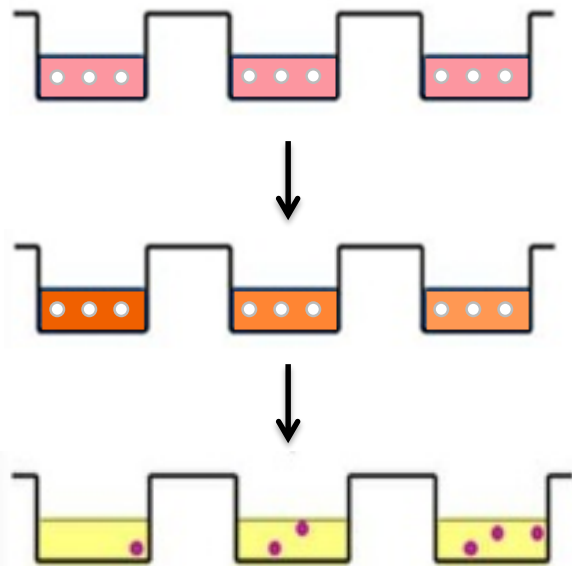


Seed Cells in 96 Well Plate & Incubate Overnight



Add Drug Combinations with Different Concentrations & Incubate for 72 Hours

Using MTT Absorbance to Measure Cytotoxicity



Seed Cells in 96 Well Plate & Incubate Overnight

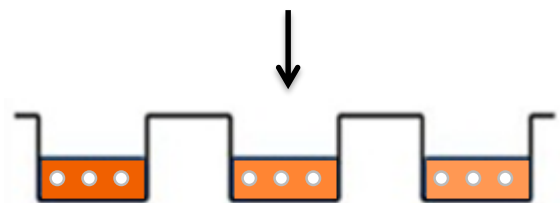
Add Drug Combinations with Different Concentrations & Incubate for 72 Hours

Remove Drug & Add Tetrazolium Dye MTT Solution until Purple Crystals Form

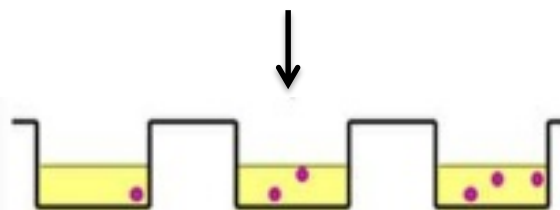
Using MTT Absorbance to Measure Cytotoxicity



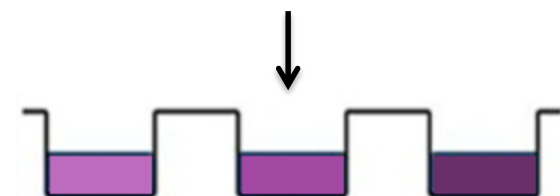
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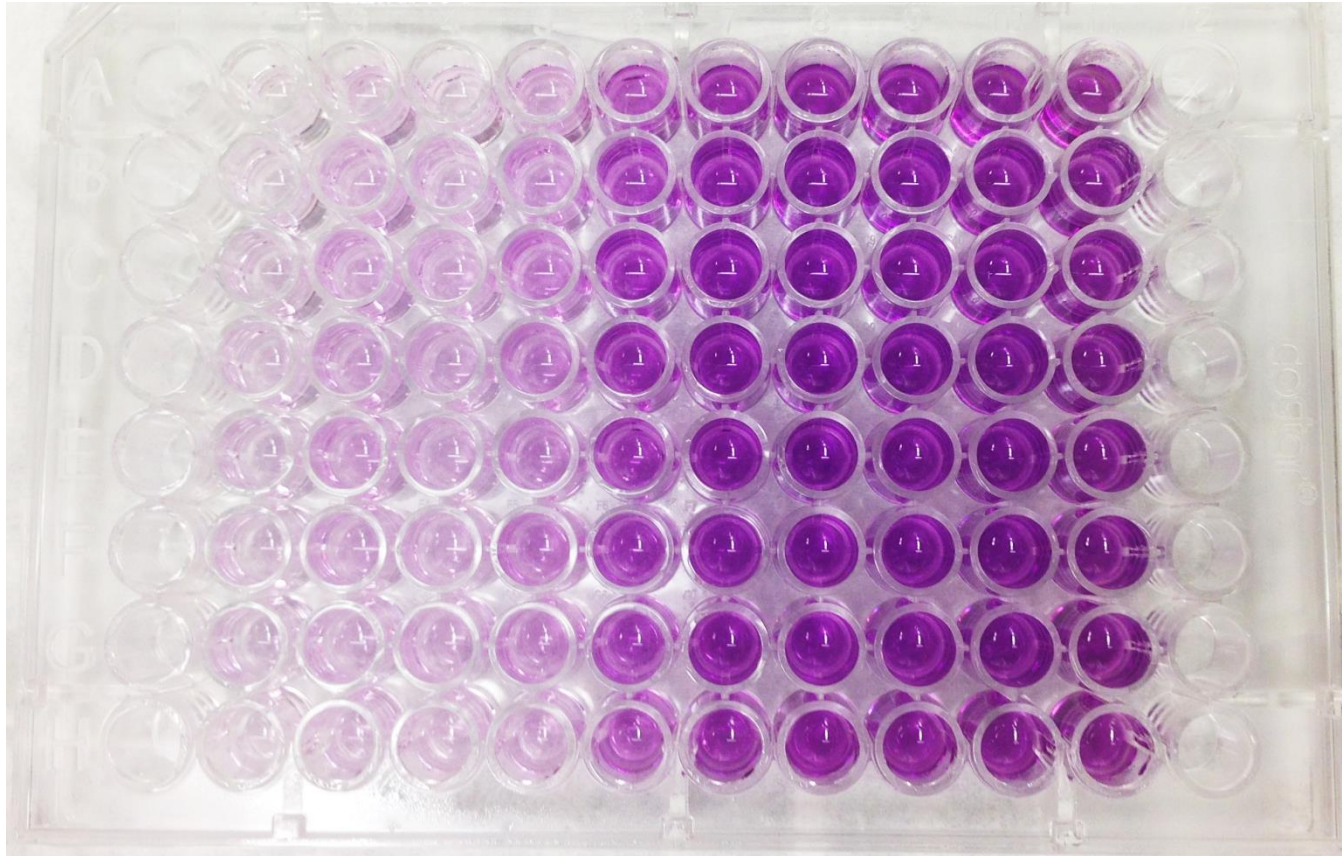


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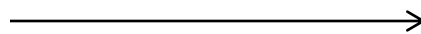


Dissolve MTT Crystals in DMSO & Measure Absorbance

MTT Plate Gradient Correlates Concentration and Cytotoxicity

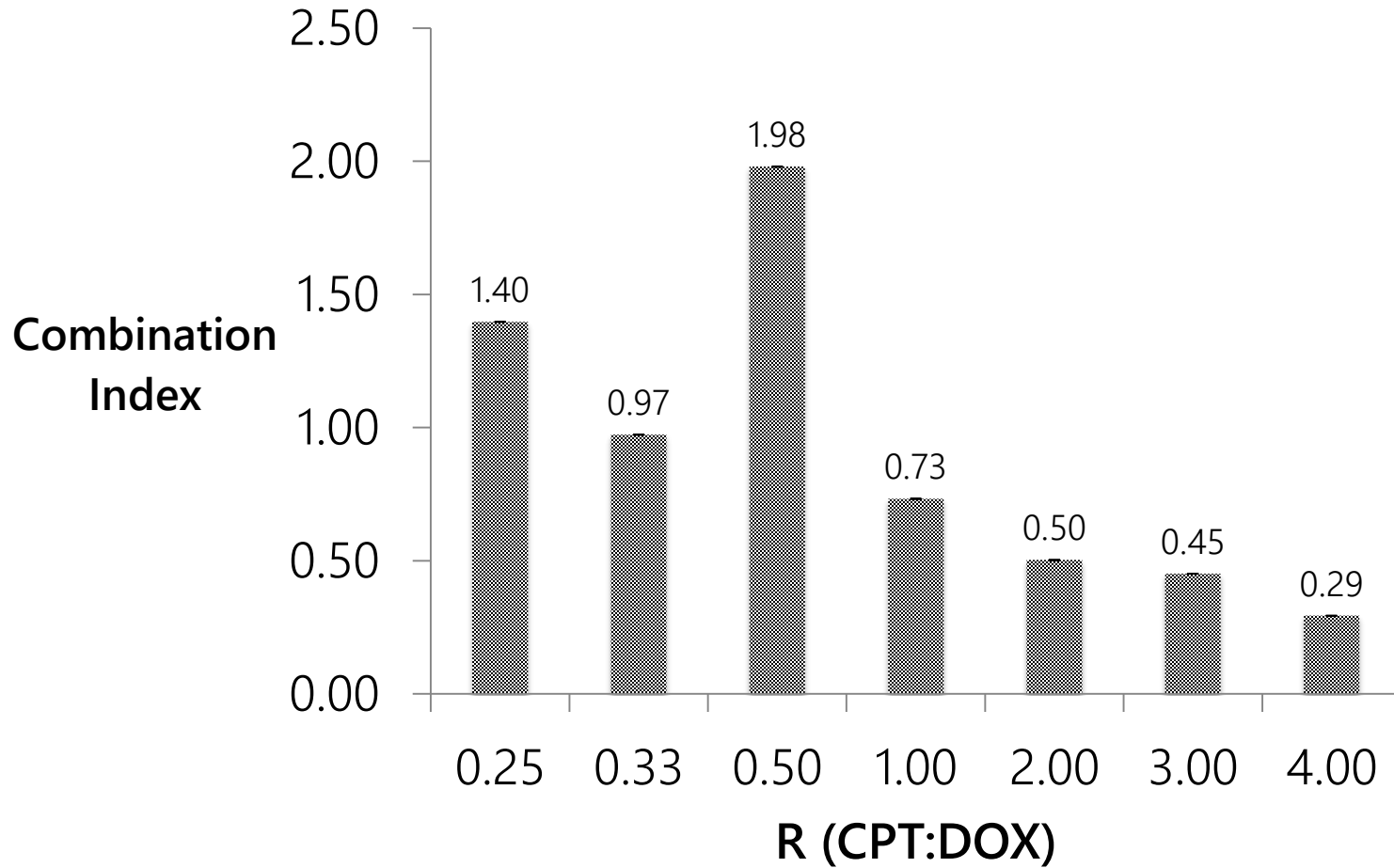


Decreasing Drug Concentration

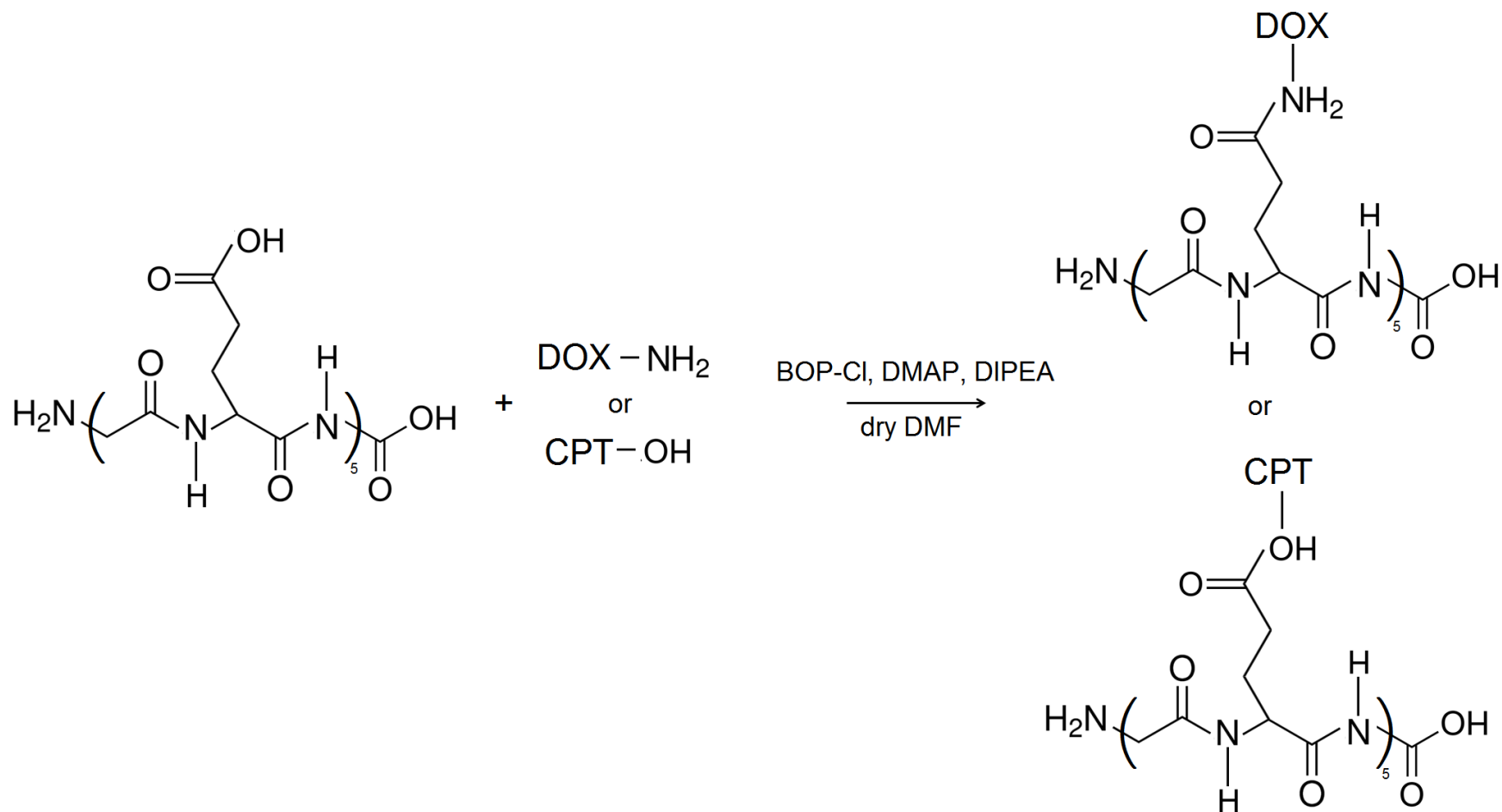


Control

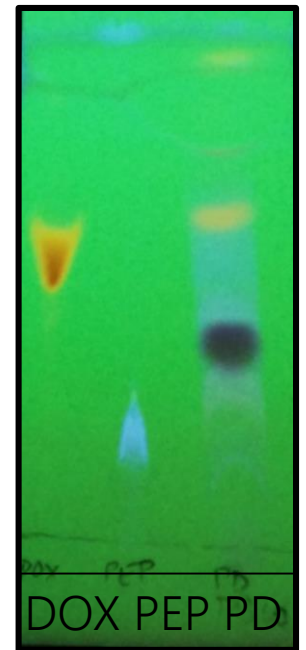
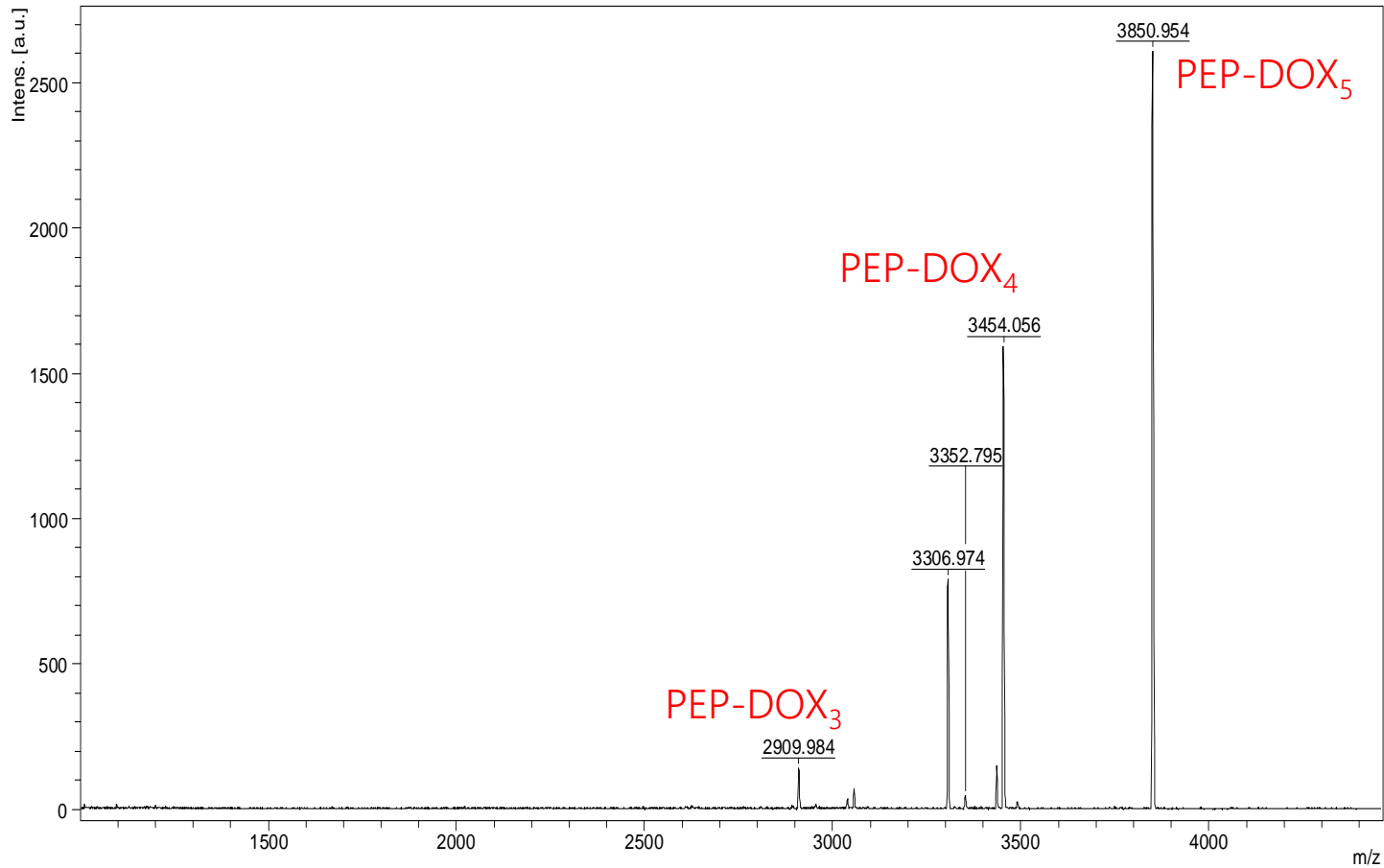
CI's for Free Drug in MDA MB 231



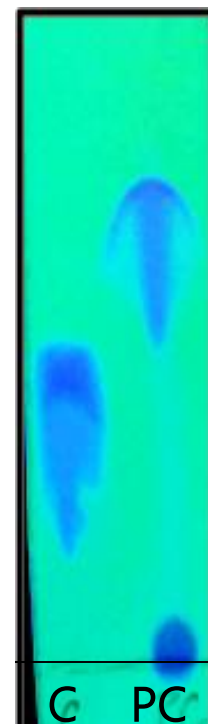
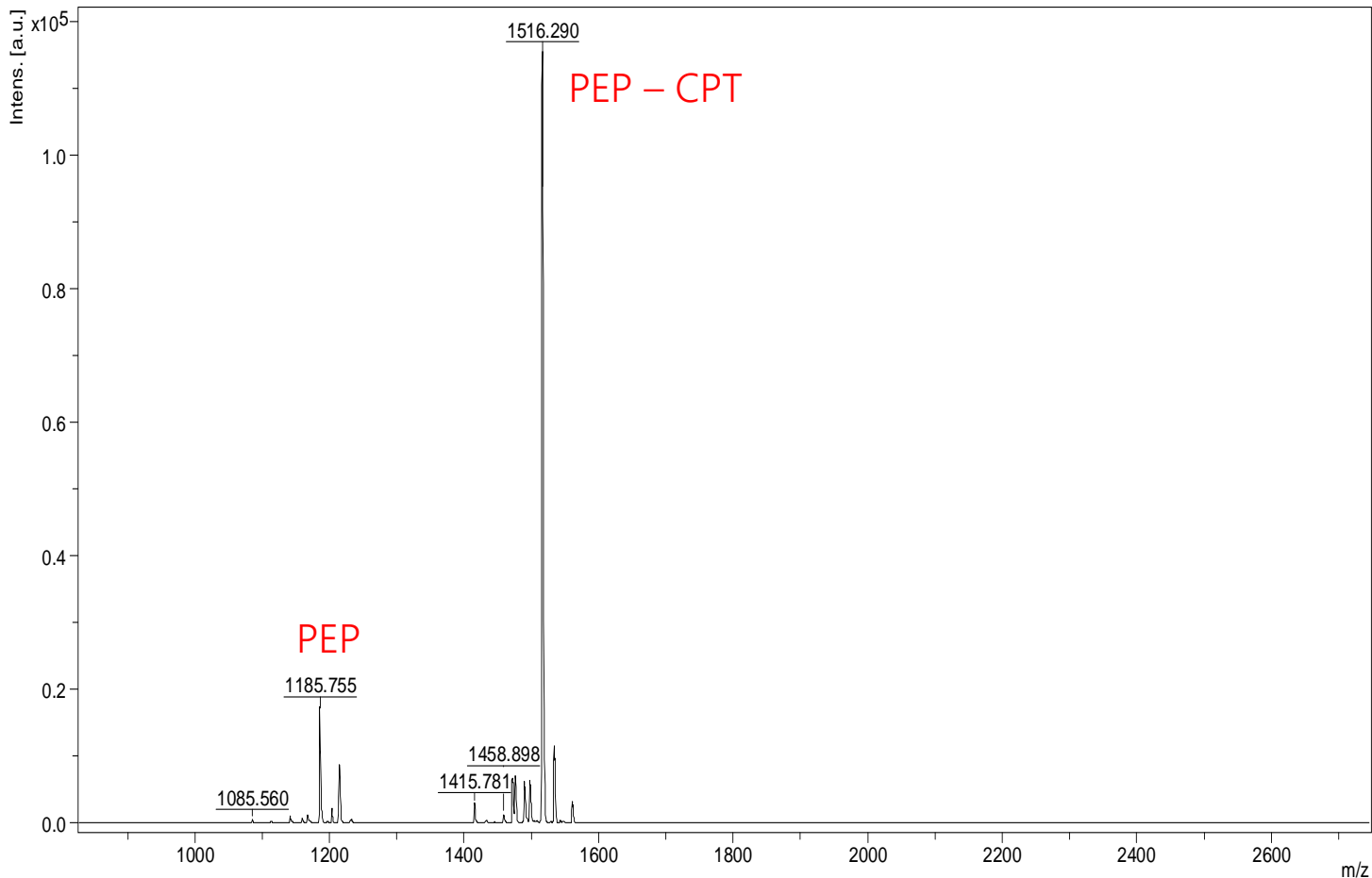
Synthesis of Peptide-Drug Constructs



Using Mass Spectrometry and TLC to Verify Pep-DOX Reaction

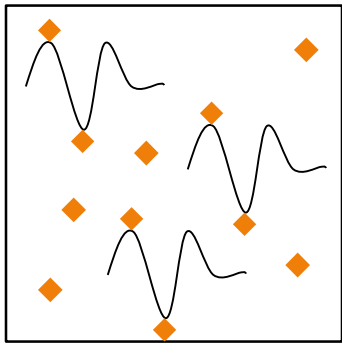


Using Mass Spectrometry and TLC to Verify Pep-CPT Reaction

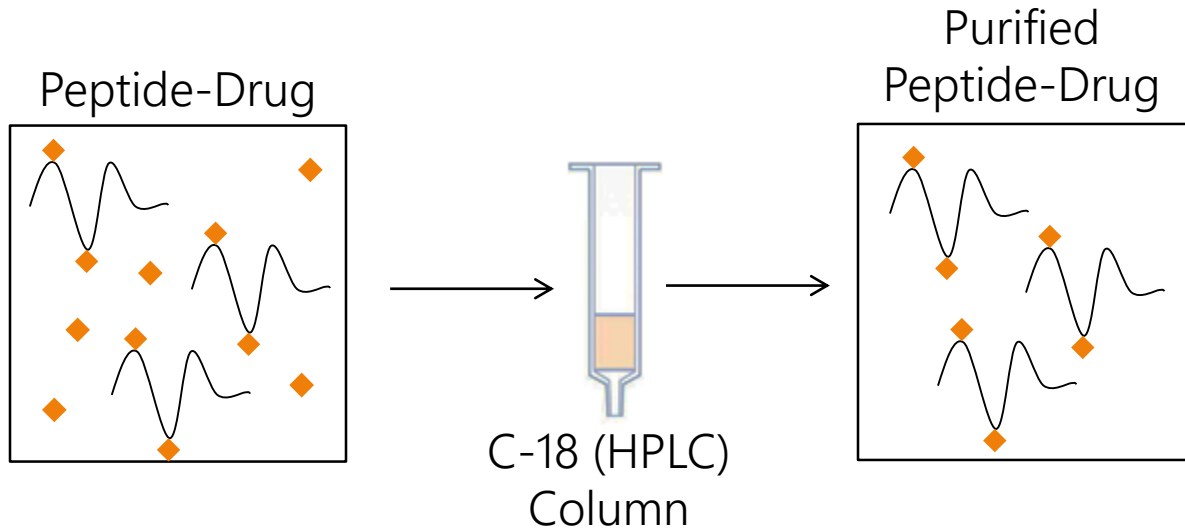


Synthesis and Purification of Aptamer Constructs Using Click Chemistry

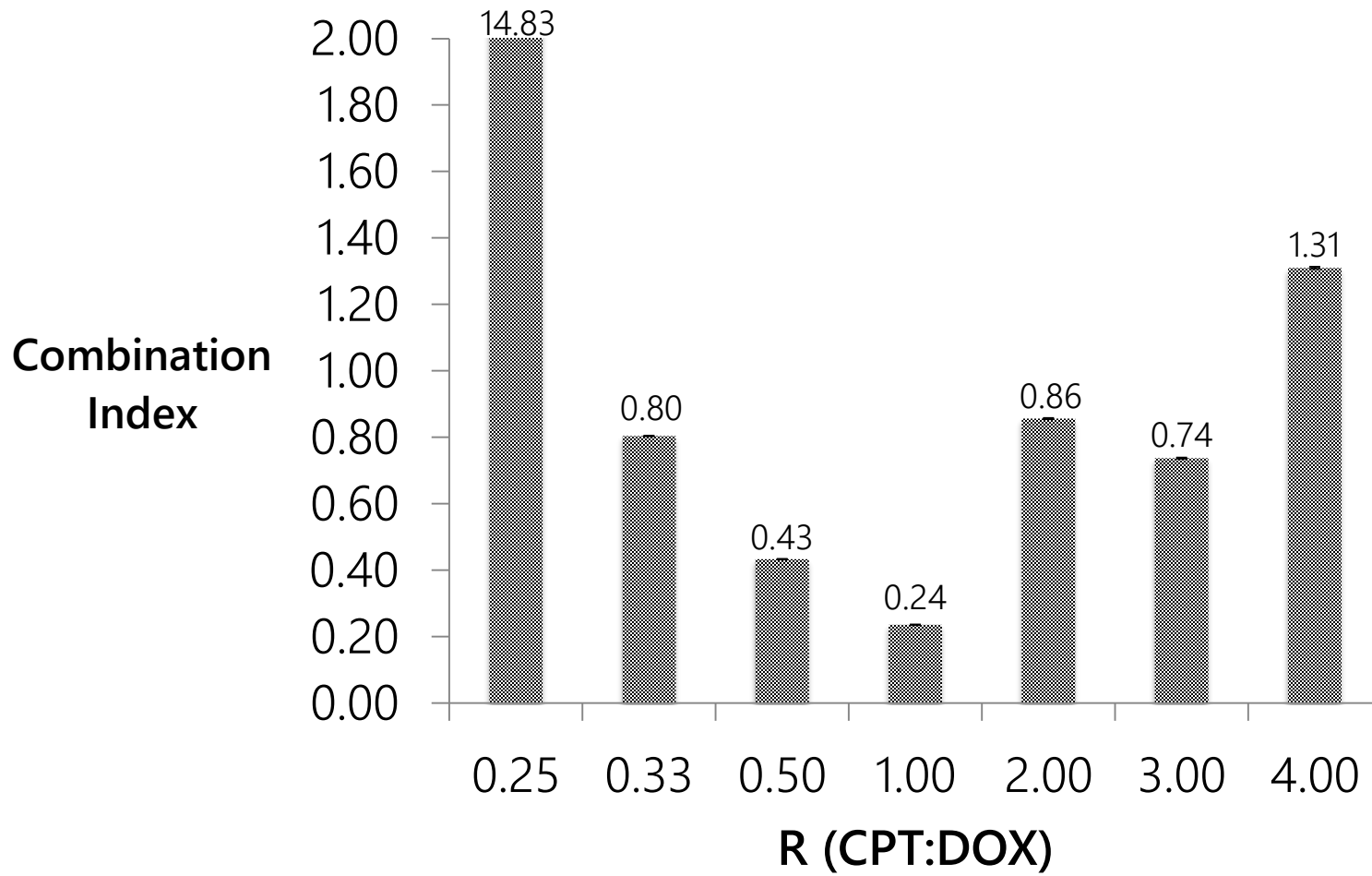
Peptide-Drug



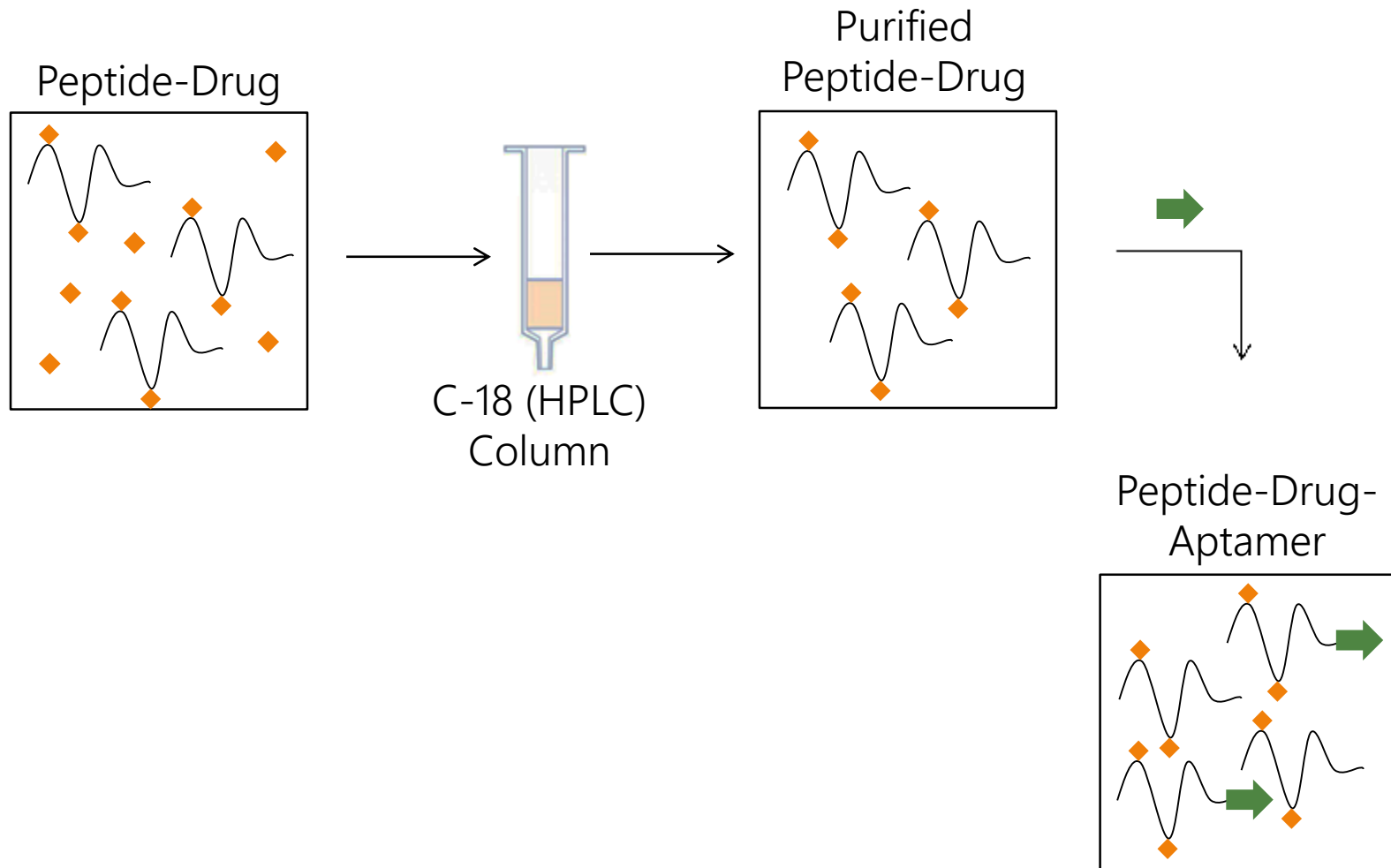
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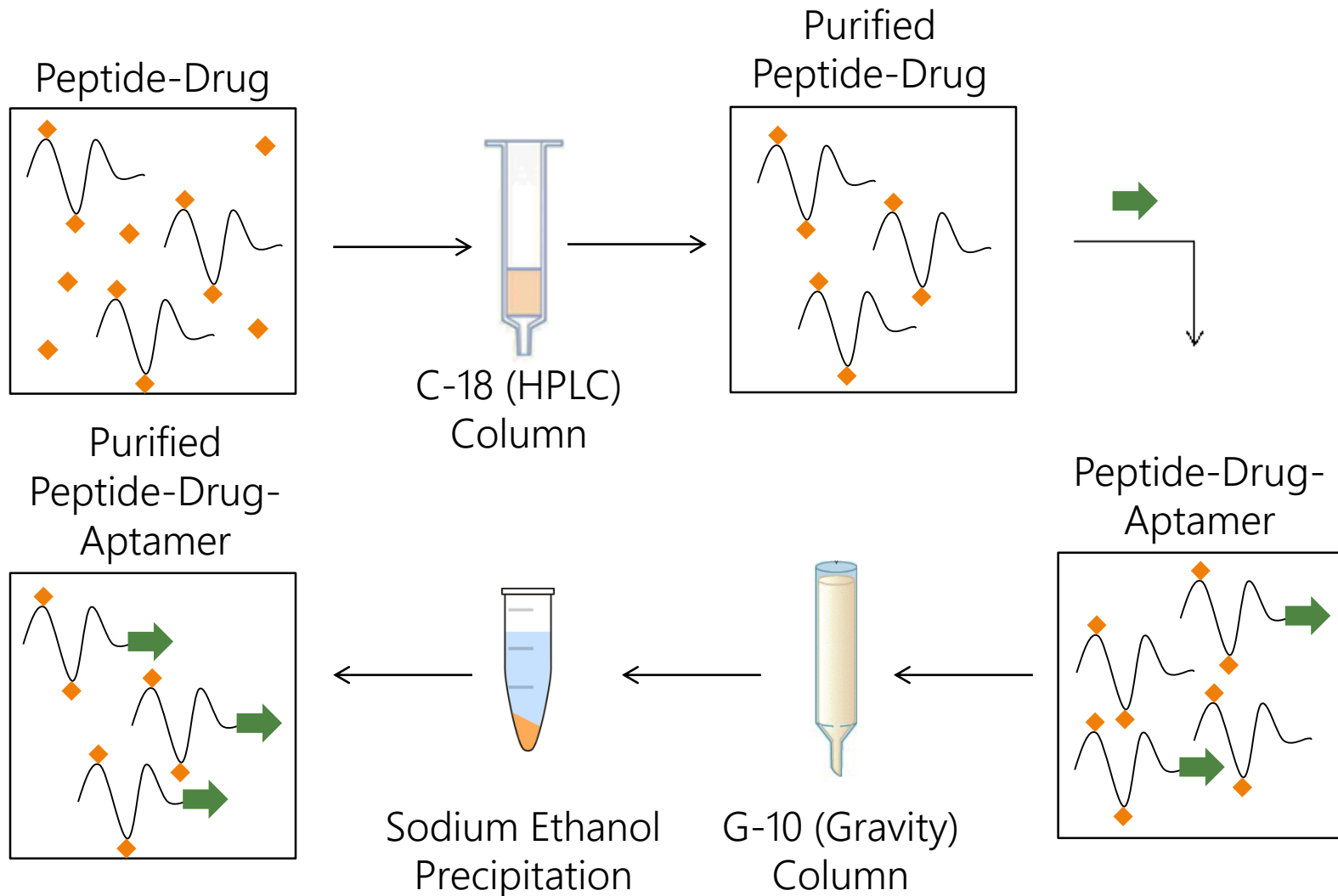
CI's for Peptide-Drug in MDA MB 231



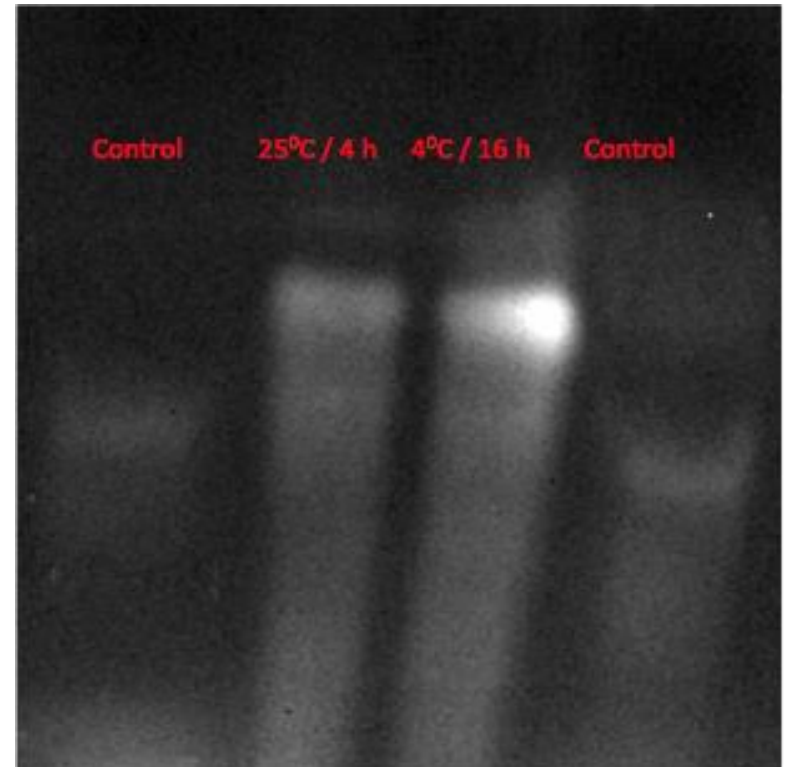
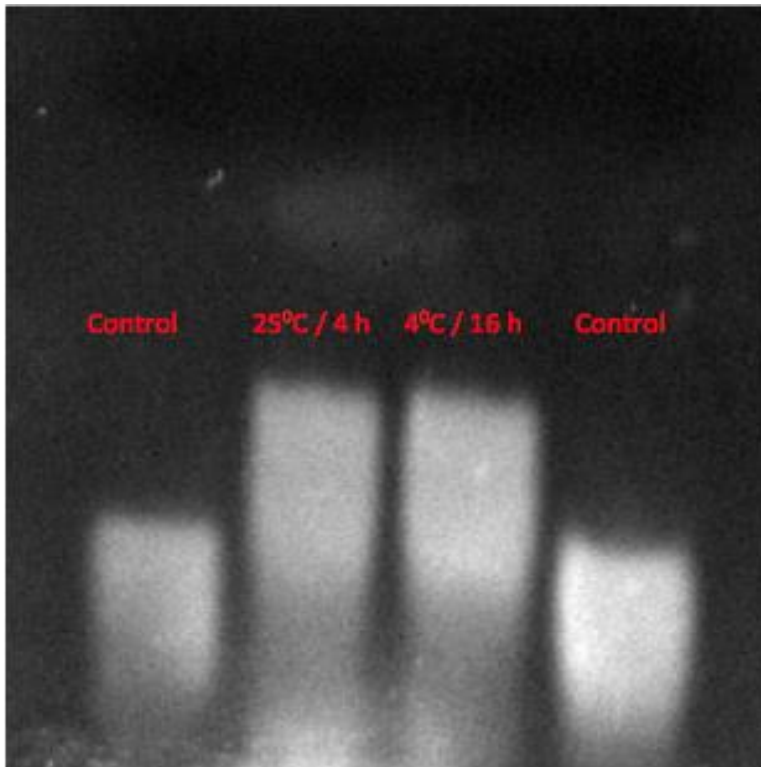
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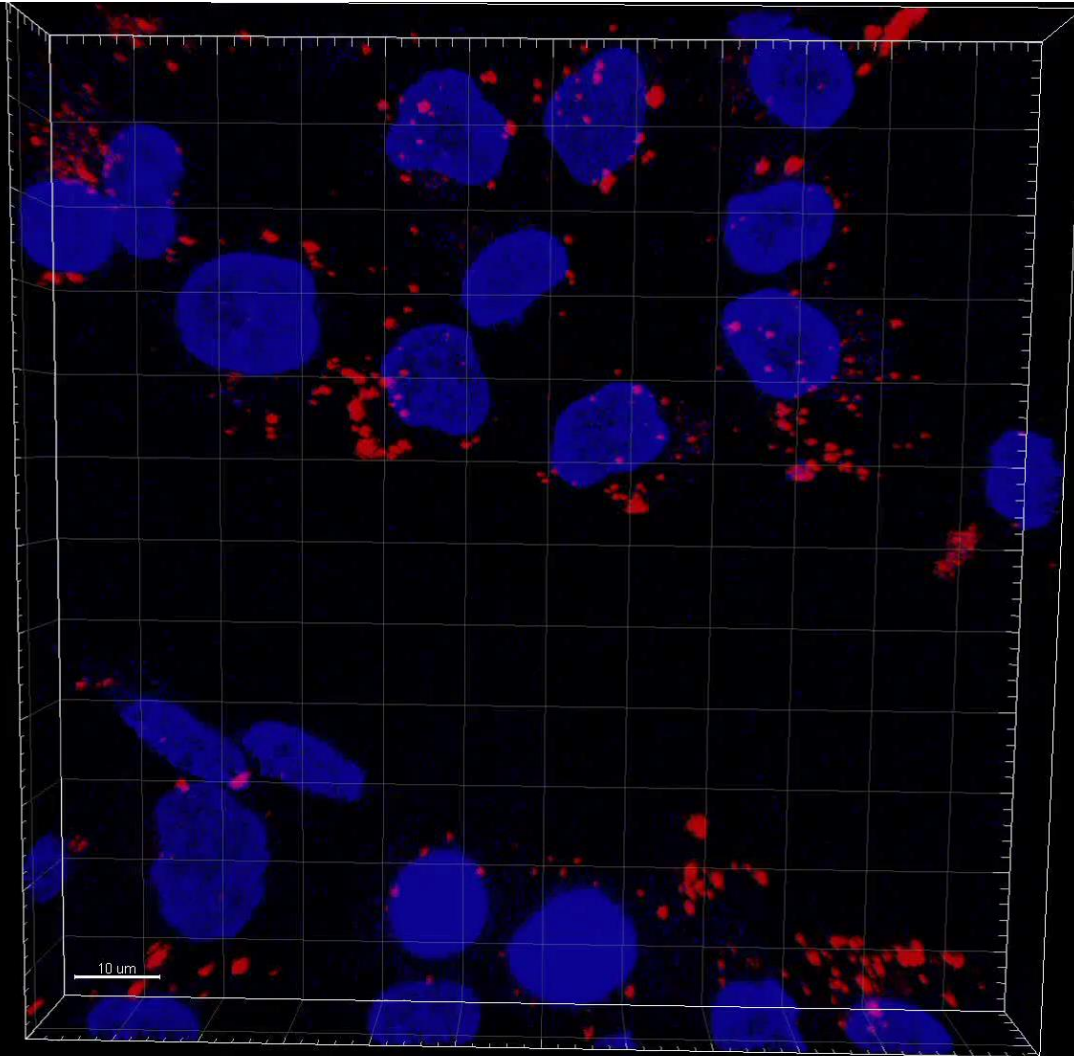
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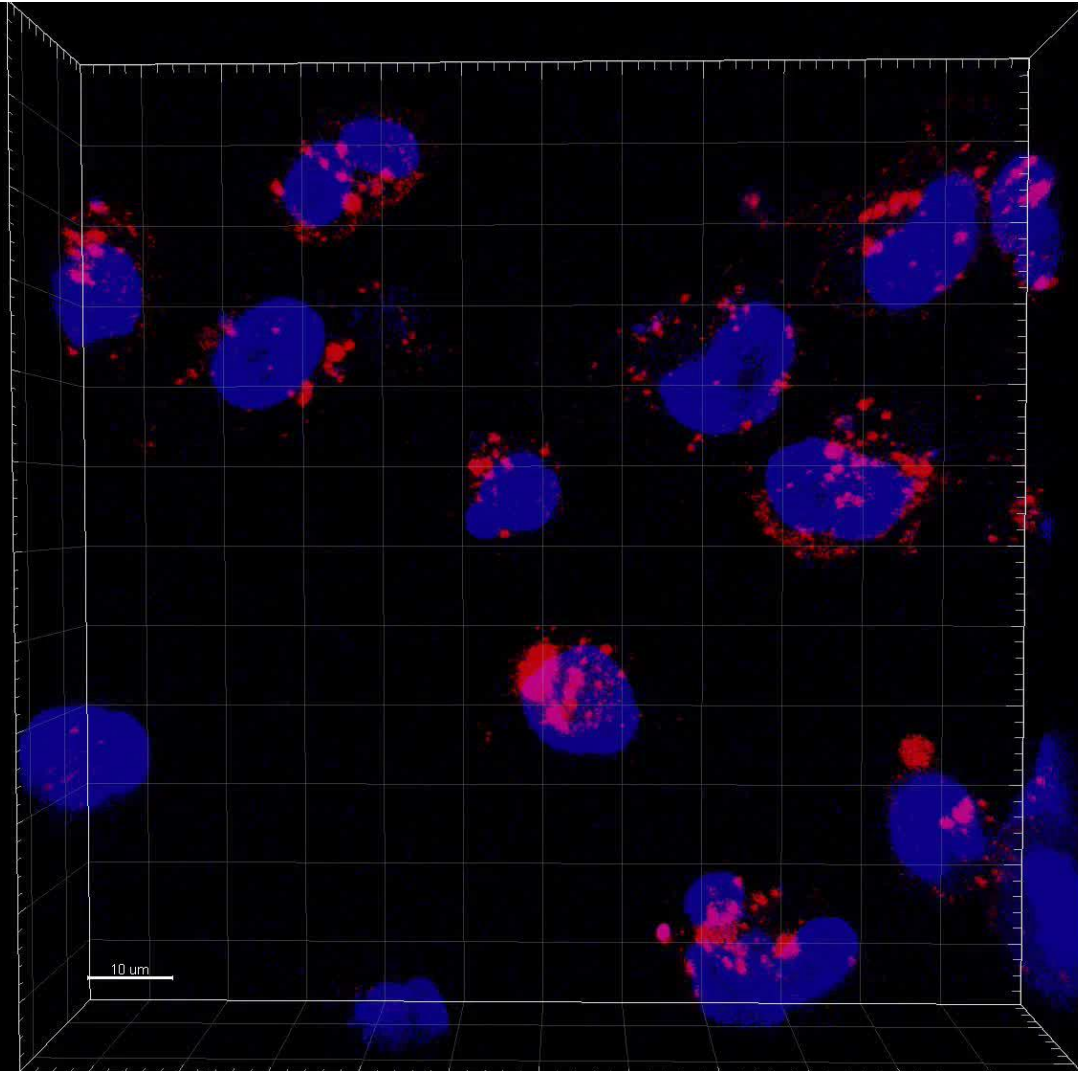
Using Gel Electrophoresis to Verify Aptamer-Peptide-Drug Conjugation



Confocal Microscopy Z Stack of Aptamer Construct Uptake by MCF 10A

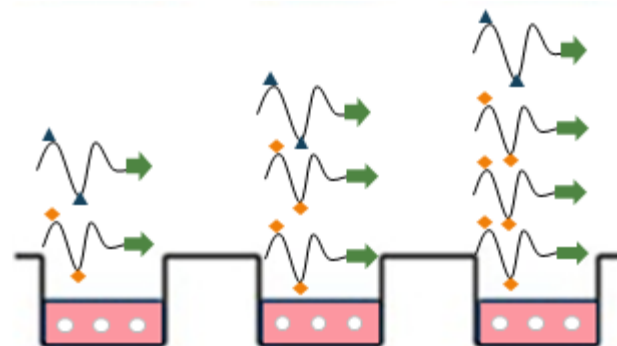


Confocal Microscopy Z Stack of Aptamer Construct Uptake by MDA MB 231

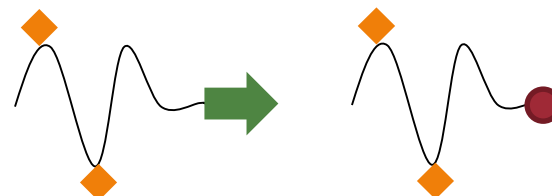


Future Work

Determining the Synergistic Ratio of Doxorubicin to Camptothecin for the Peptide-Drug-Aptamer



Comparing Cytotoxicity of Peptide-Drug-Aptamer with Nonspecific Peptide-Drug-Aptamer



Acknowledgements

Anusha Pusuluri

Mellichamp Chair Funds

Samir Mitragotri
Chemical Engineering

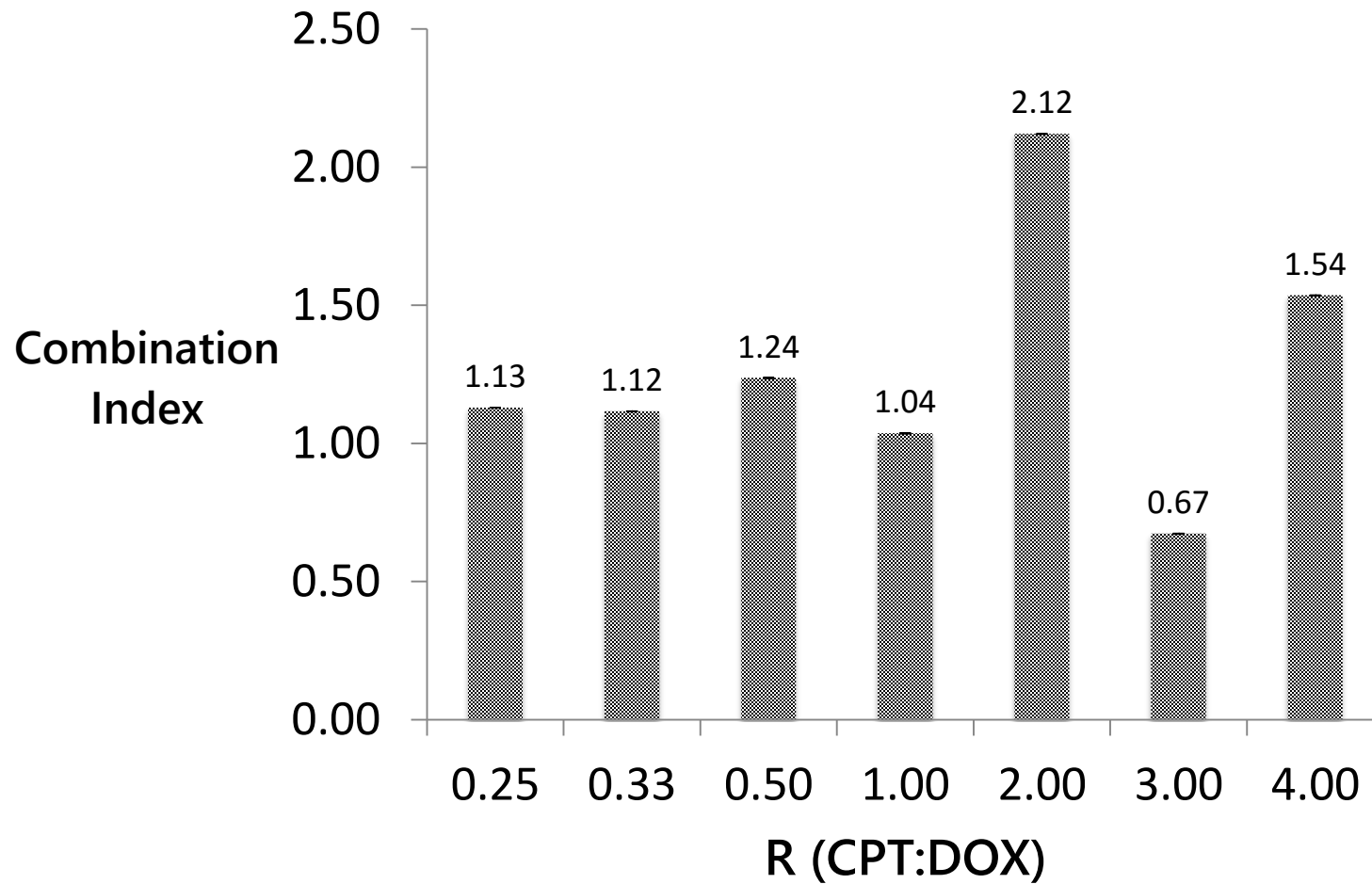
Mitragotri Group Members

Tom Soh
Mechanical Engineering

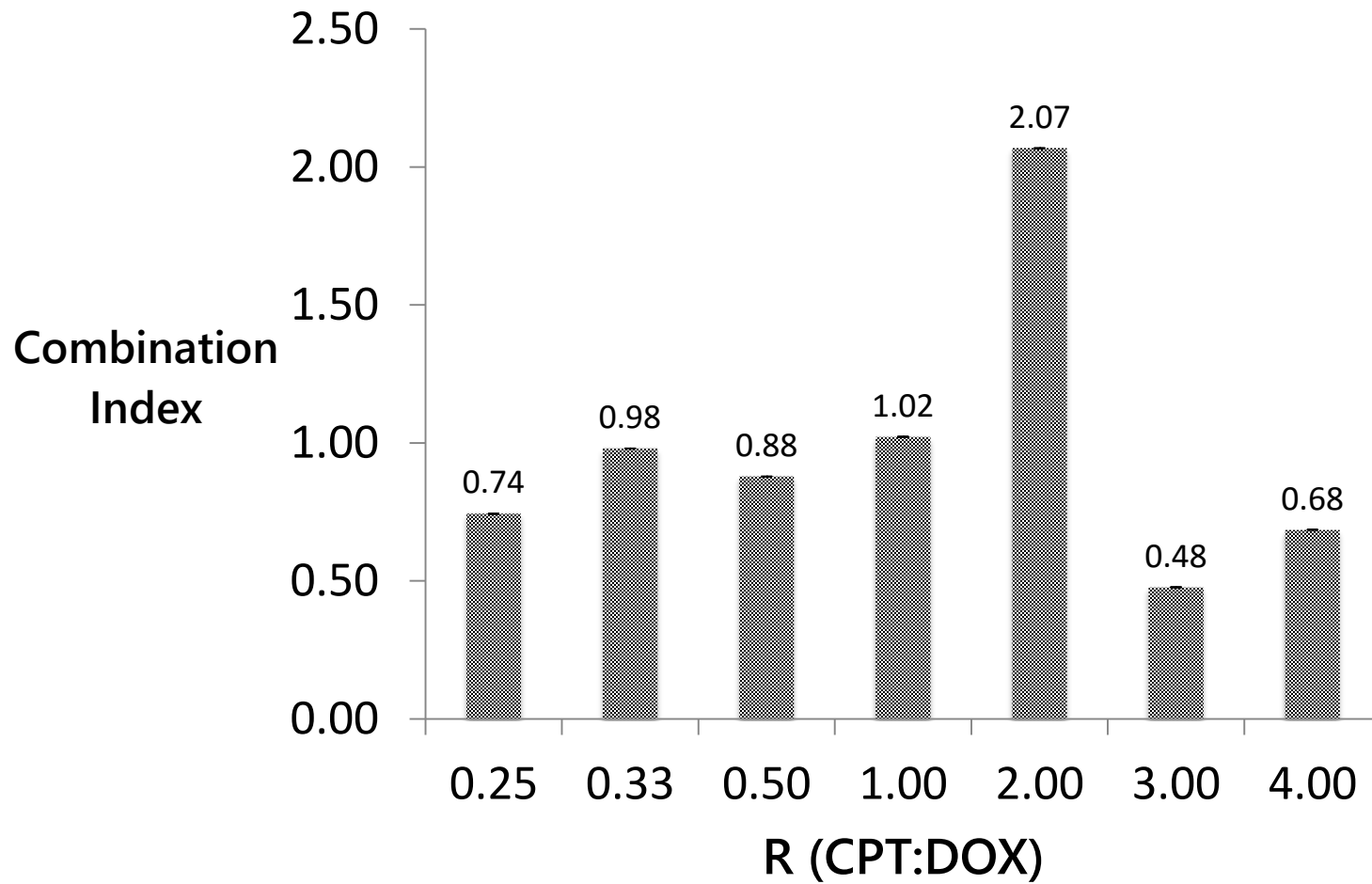
Eureka Supermentors,
Mentors, Peer Mentors



CI's for Free Drug in MCF 10A



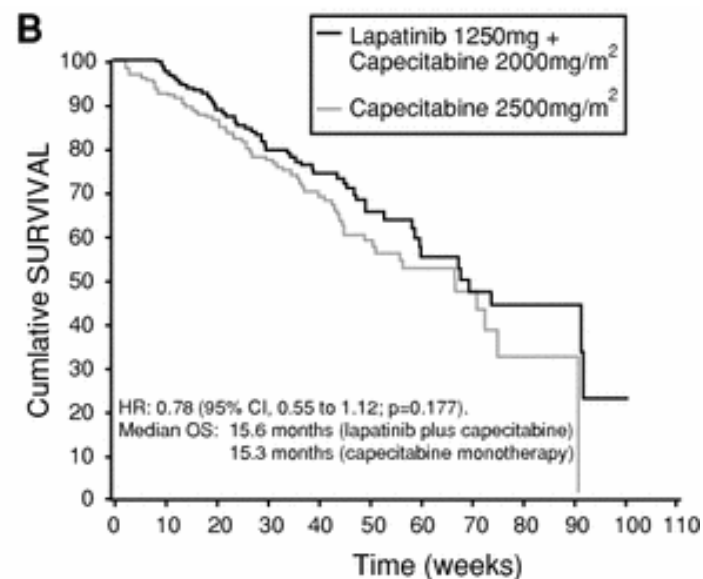
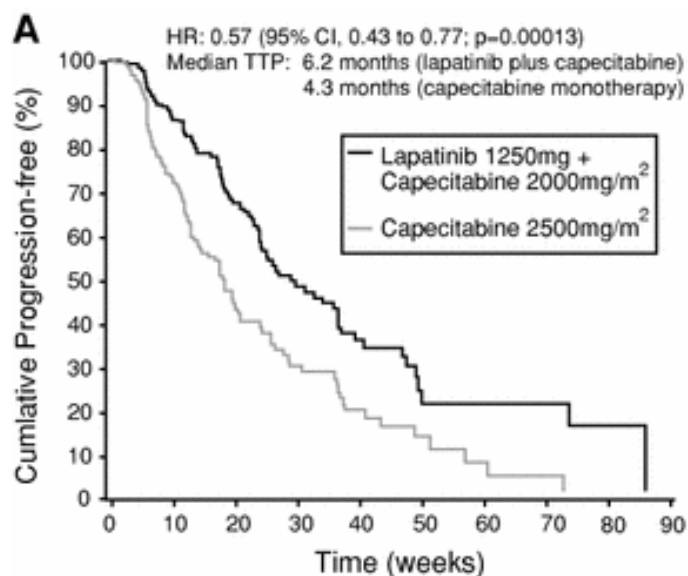
CI's for Peptide-Drug in MCF 10A



Chou-Talalay Method

$$CI = \frac{(D)_1}{(D_m)_1 [f_a / (1 - f_a)]^{1/m_1}} + \frac{(D)_2}{(D_m)_2 [f_a / (1 - f_a)]^{1/m_2}}$$

Combination Treatment Improves Efficacy of Therapy as Compared to Single Drug Treatment



Verification of Multiple Camptothecin Conjugation

