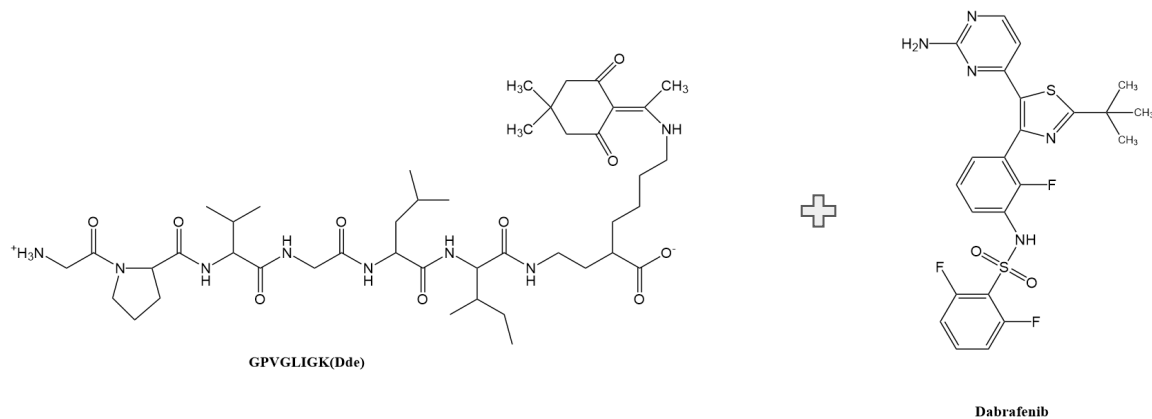


Supplemental Reactions and Conditions

11/29/2023 BME 771 Final Project Presentation

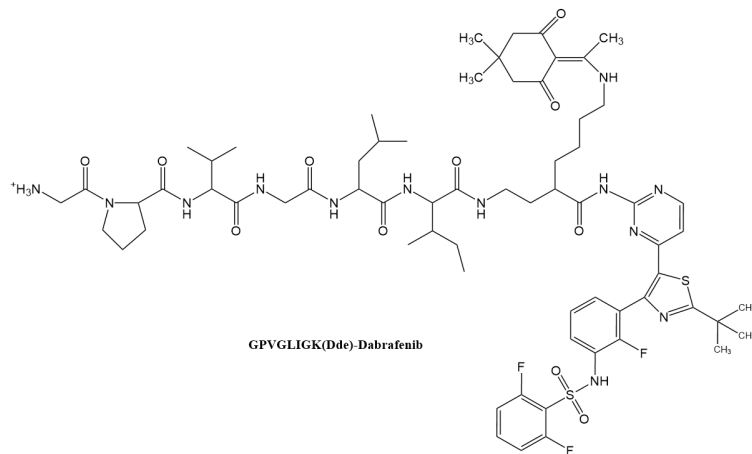
Junqi Lu, Connor Miksch
Department of Biomedical Engineering

Conjugating C-terminus of **MMP-2 degradable peptide** with free amine of dabrafenib

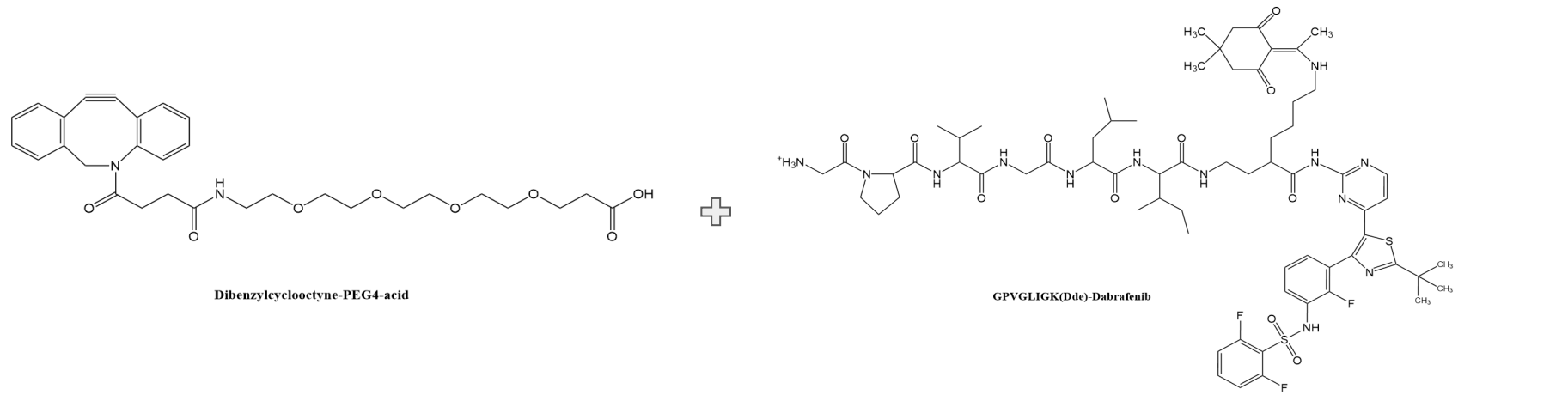


HATU coupling:

- 1.2 mmol MMP2 peptide-COOH
- 0.6 mmol 4-Methylmorpholine
- 4.8 mmol HATU
- 12 mmol Dabrafenib (excess)
- ~10 mL Dimethylformamide
- Purified by HPLC, lyophilized
- Quantified by HNMR, MALDI
- **Expected result** = increase in MW by 516.6 g/mol

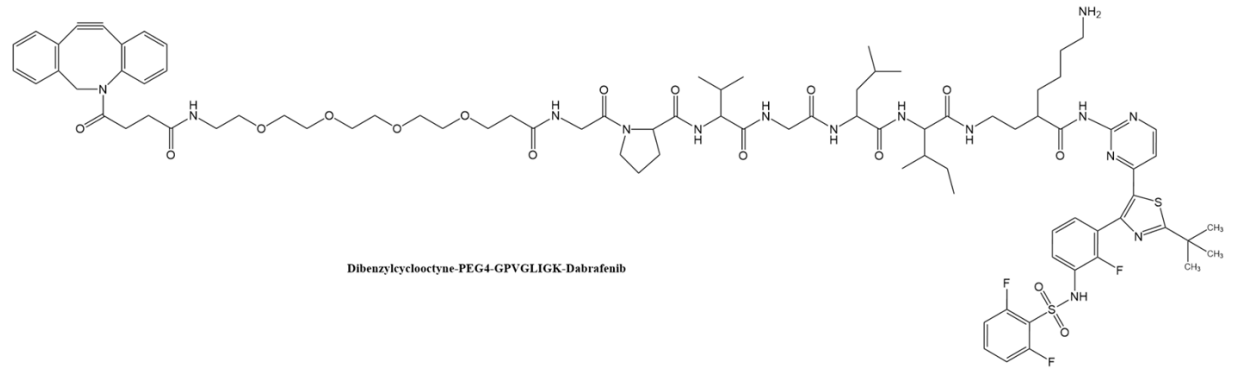


Conjugating N-terminus of **MMP-2 degradable peptide** with carboxylic acid of **DBCO**

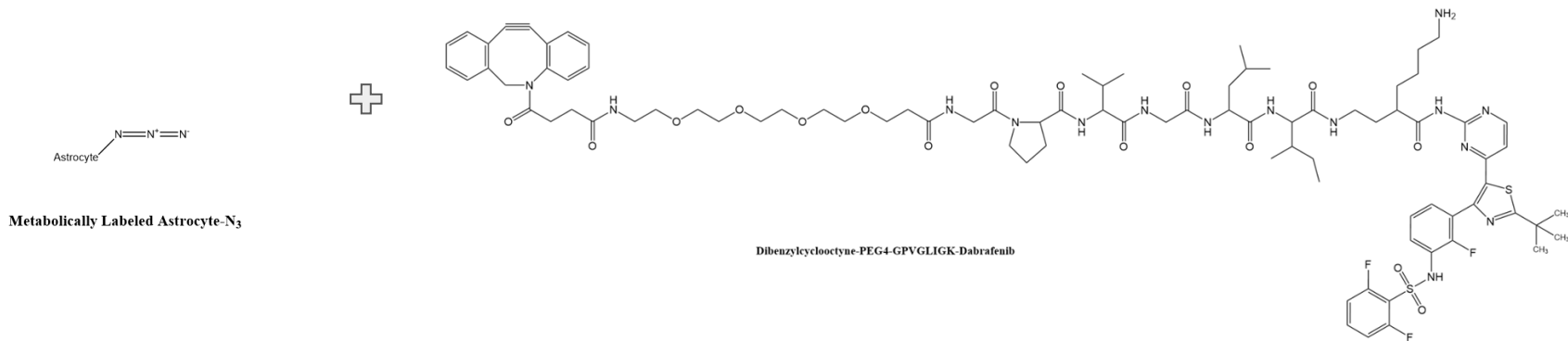


DMTMM coupling, deprotection:

- 1.2 mmol MMP2 peptide
- 1.2 mmol DMTMM
- 12 mmol DBCO-PEG4-COOH
- ~10 mL Deionized water
- Titrate with TFA for deprotection, neutralization with NaOH
- Purified by HPLC, lyophilized
- Quantified by HNMR, MALDI
- **Expected result** = increase in MW by 549.6 g/mol

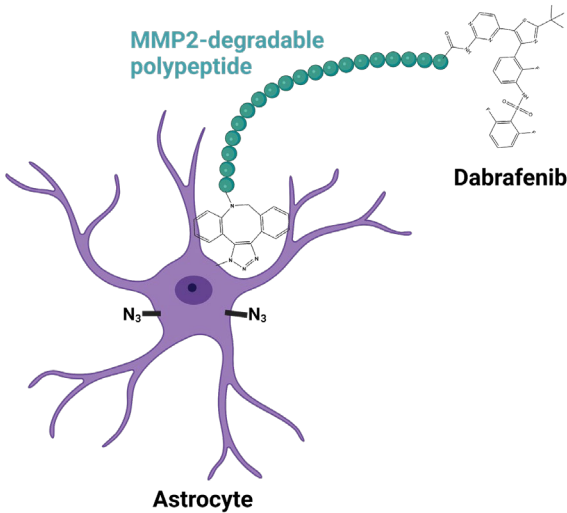


Conjugating astrocyte pendant azides with “cellular backpack” DBCO-peptide-dabrafenib

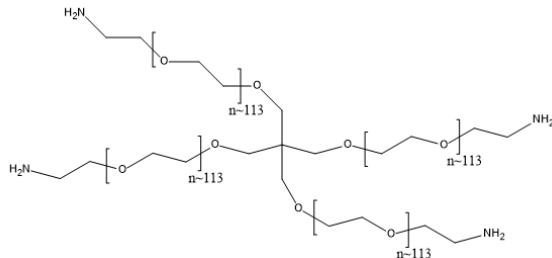


Strain promoted azide-alkyne cycloaddition:

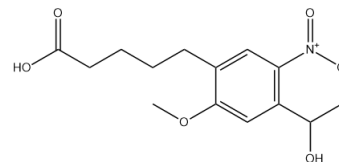
- Assess 100nM-100uM DBCO-peptide-Dabrafenib
- Dulbecco's Modified Eagle Medium (DMEM)
- 10% Fetal Bovine Serum
- 2% Penicillin-streptomycin
- 37°C, 5% CO2



Conjugating PEG-amine with Hydroxyethyl Photolinker (a nitrobenzyl derivative)



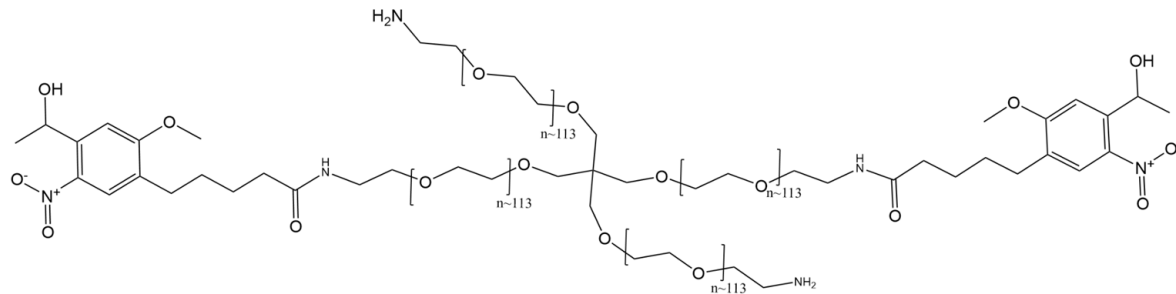
4arm PEG-NH₂ (20 kDa)



4-[4-(1-Hydroxyethyl)-2-methoxy-5-nitrophenoxy]butyric acid

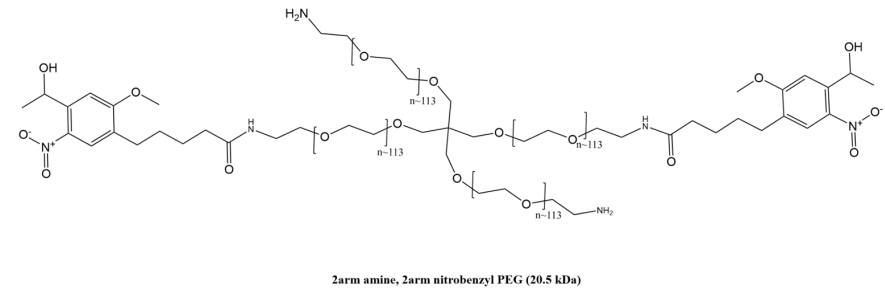
HATU coupling:

- 0.2 mmol PEG-amine
- 0.2 mmol 4-Methylmorpholine
- 3.2 mmol HATU
- 0.4 mmol Nitrobenzyl (~0.5x per amine)
- ~10 mL Dimethylformamide
- Purified by precip., dialysis
- Quantified by HNMR, MALDI
- **Expected result** = increase in MW by 592.5 g/mol



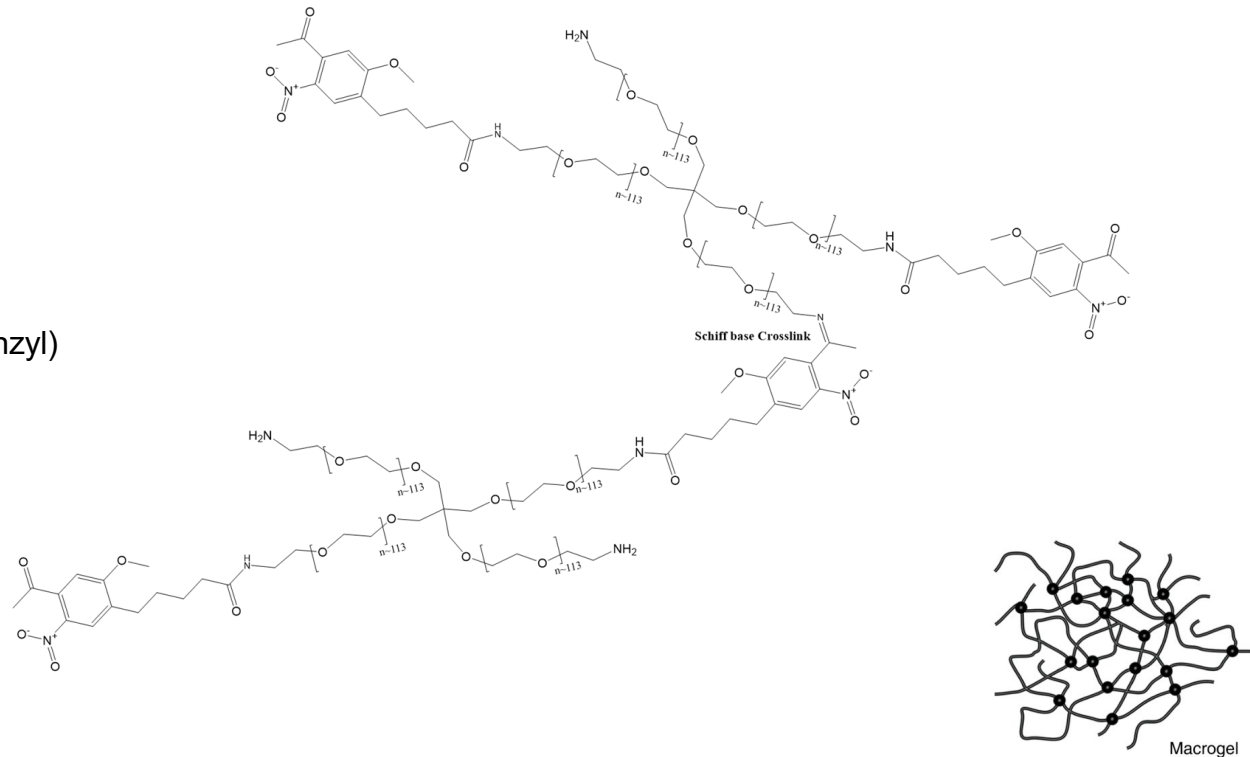
2arm amine, 2arm nitrobenzyl PEG (20.5 kDa)

Photoactivating PEG schiff base hydrogel formation



Multifunctional PEG Crosslinking

- 5wt% PEG (5mM amine, 5mM nitrobenzyl)
- Sterile PBS
- 10-20 mW/cm² 405 nm light
- RGD if necessary (1 mM CRGDL)



Photoactivating PEG schiff base microgel formation, for the encapsulation of astrocyte “cellular backpacks”

