iGEM TU/e 2014
Biomedical Engineering

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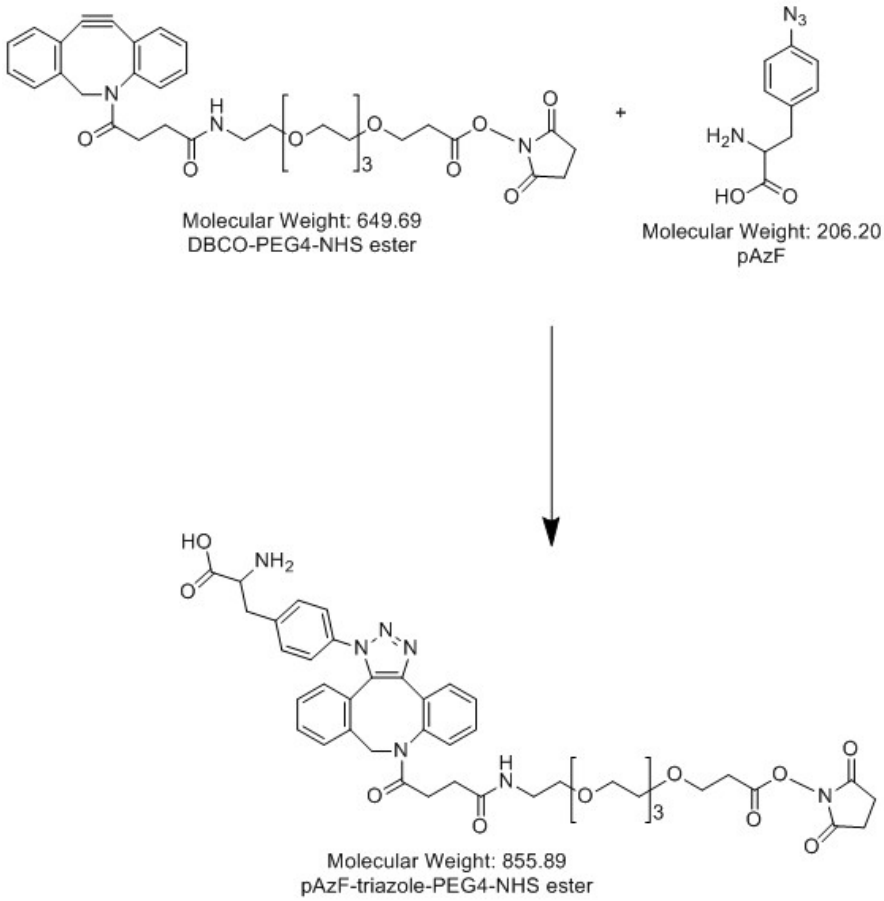
Monitoring SPAAC with UV-VIS

General protocols from the dry lab



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1 Reagents

- PBS with pH 7.2
- Unnatural amino acid p-azido-L-phenylalanine (pAzF) in DMSO (5%) and PBS dissolved (47.6 μM)
- DBCO-PEG_4-NHS ester solved in PBS (142.9 μM)

2 Settings

Software: Spectramanager
Measurement type: Spectrum measurement

Measuring absorption spectrum	
Photometric mode	Abs
Response	Medium
UV/Vis bandwidth	1.0 nm
Scan Speed	40 nm/min
Start	340 nm
End	190 nm
Data interval	1 nm
Vertical size	Auto
Scan mode	Continuous
Accumulation/cycle	Accumulation
No. Of cycles	192
Time between cycles	300 s
Temperature	20 ˚C
Stirrer	250 rpm

3 Monitoring SPAAC reaction

- Prepare blank sample by filling cuvettes with 2.5 mL PBS
- Start blank measurement
- Prepare sample in cuvette containing 1.75 mL DBCO-PEG_4-NHS ester solution and 0.75 mL pAzF solution (100 : 33.3 μM)
- Start sample measurement