Design, synthesis, and redesign of therapeutic peptides using SPPS, native chemical ligation, and AutoDock Crankpep

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Abstract

The use of molecular modeling is quickly becoming an essential tool for the experimentalist. In this project, the molecular docking software AutoDock CrankPep (ADCP) was utilized to analyze a variety of parameters on peptide sequences to determine which are the largest contributors in producing active compounds when synthesized experimentally and which provide the most accuracy to the computational results. Our hypothesis is that ADCP parameters (whose exactness we are trying to figure out,) can be manipulated through inspiration from wet lab data to give scores more similar to real world values. This will then lead to faster, more efficient methods for the discovery of peptide-based drugs / biologically active compounds.

Introduction

Two Projects (Figure 1).

- **Project 1**: Linking peptides utilizing native chemical ligation and a novel peptide linker.

  - Resulting dipeptide has two C-termini.

- **Project 2**: Devise peptide modeling method for ADCP.

  - Current ADCP runs produce data that strays from *in situ* data.

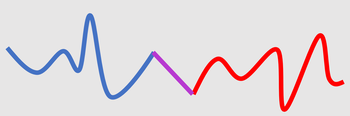

Figure 1a. (Project 1) A peptide linker (purple) linking two peptides (blue and red).

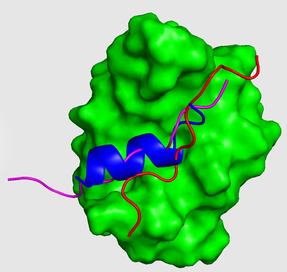

Figure 1b. (Project 2) SUMO1 (green) docked with the DAXX peptide, among other peptides.

Acknowledgments

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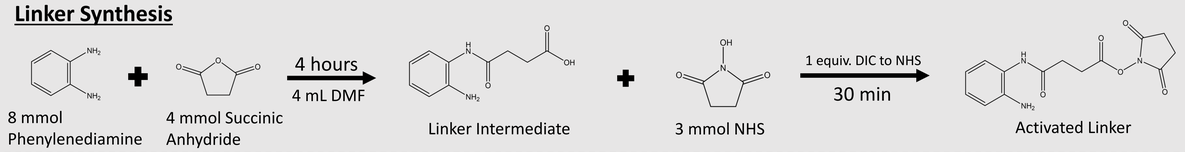
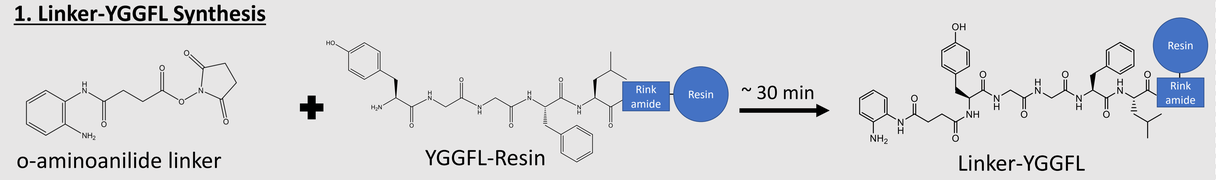
Project 1 Methods – Linker Synthesis and Native Chemical Ligation

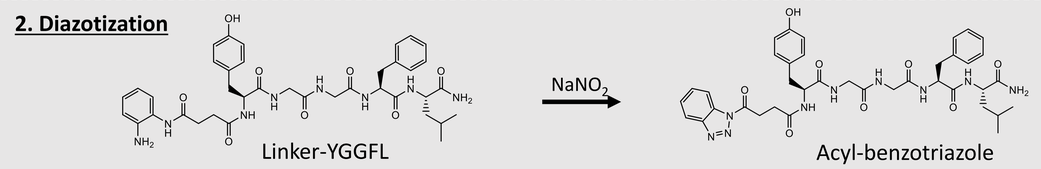
Linker Synthesis



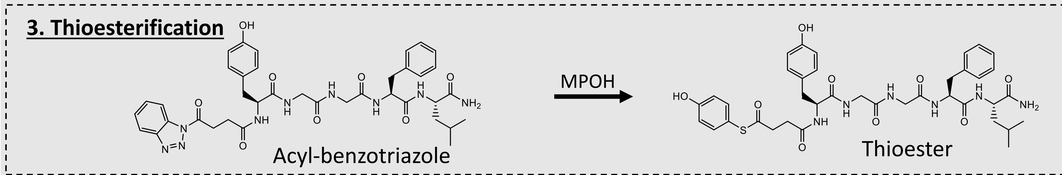
1. Linker-YGGFL Synthesis



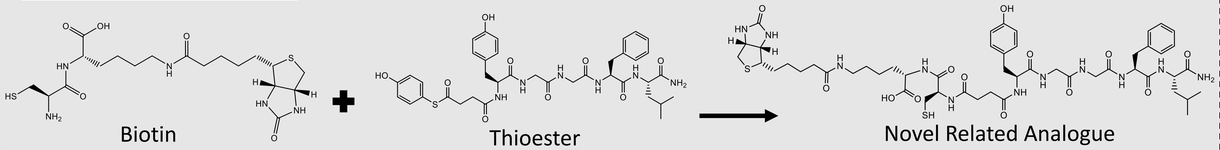
2. Diazotization



3. Thioesterification



4. Native Chemical Ligation[5]



Project 2 Methods – SUMO1, AutoDock CrankPep, and Peptide Docking

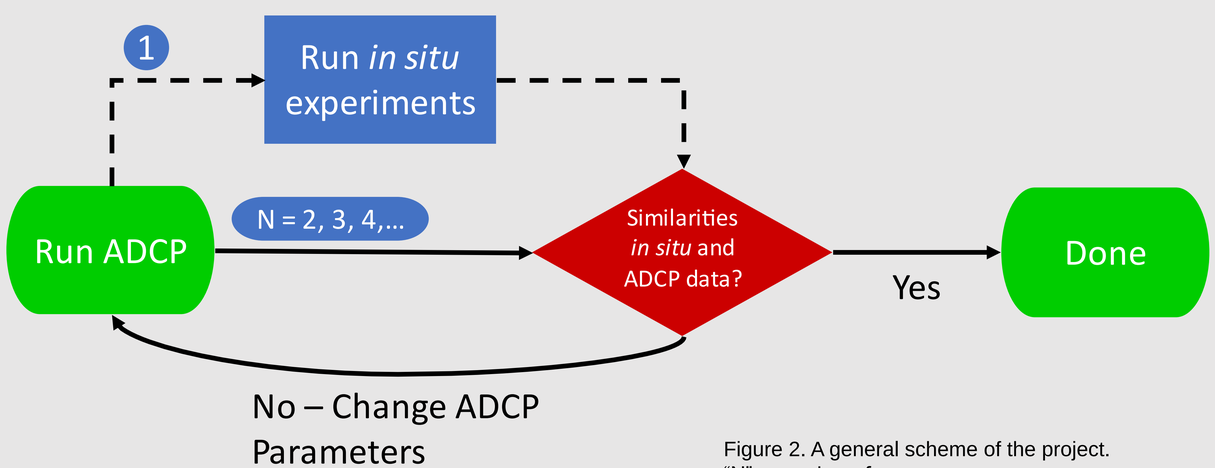


Figure 2. A general scheme of the project. "N" = number of runs.

Project 1 Results

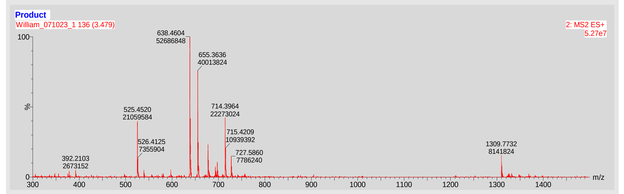


Figure 3. An example mass spectrum from the linker synthesis.

Project 2 Results

Sequence	50 Reps 7e^6 MC Steps	100 Reps 1e^6 MC Steps	100 Reps 7e^6 MC Steps	100 Reps 10e^6 MC Steps
CDPEEIIVLSDSD	-17.9	-16.1	-17.2	-18.1
FDPEEMIMLSDSD	-18.1	-16.4	-18.5	-19.5
FDPEEMIVLSDSD	-17.1	-15.8	-18.1	-18.5
FDPEEIIMLSDSD	-18.3	-16.1	-19.0	-19.7
FDPEEIIVLSDSD	-17.2	-17.2	-17.6	-18.2
CDPEEMIVLSDSD	-17.5	-17.3	-16.7	-17.7
CDPEEIIMLSDSD	-17.7	-16.2	-17.6	-18.1
CDPEEIIVMSDSD	-18.0	-15.4	-17.3	-18.3
CDPSEIIVLSDSD	-16.9	-15.3	-18.2	-17.0
CDPEENIVLSDSD	-17.7	-15.5	-17.3	-17.6
CDPEEIIGLSDSD	-17.5	-15.5	-17.6	-18.2
CDPEEIIVASDSD	-16.2	-15.5	-16.5	-16.8
CDPSEIIVASDSD	-17.0	-13.6	-17.2	-16.9
CDPEENIGASDSD	-16.7	-15.9	-17.5	-16.8

Figure 4. Comparison of several ADCP runs. The header of each column describes tested parameters of the run, these being the number of replicates and the number of Monte Carlo steps. The green highlights in the "Sequence" column represents residue mutations of the wildtype (DAXX), represented in the first row. The yellow and red highlights accentuate the highest and lowest affinity of each set of parameters/column respectively.

Discussion

Current Work:

- Currently generating *in silico* affinities using ADCP
- Currently collecting chromatograms and mass spectra for the peptide linker project

Future Work:

- Will use SPPS to synthesize peptides in ADCP work, then ITC to generate experimental binding affinities
- Finish collecting characterization data
- Finish collecting docking affinities *in situ* and *in silico*
- Compare *in silico* and *in situ* docking affinities and run different parameters to possibly determine which parameters allow for more accurate binding predictions

Work Cited

(1) *Targeting SUMO Signaling to Wrestle Cancer*. Jessie S. Kroonen 1 and Alfred C.O. Vertegaal.

(2) Cistrone, P. A.; Bird, M. J.; Flood, D. T.; Silvestri, A. P.; Hintzen, J. C. J.; Thompson, D. A.; Dawson, P. E. Native Chemical Ligation of Peptides and Proteins. *Curr. Protoc. Chem. Biol.* **2019**, *11* (1), e61. https://doi.org/10.1002/cpch.61.