Modification of Campylobacter Cytolethal Distending Toxin for Selective Targeting of PD-L1 Expressing Cancer Cells

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Abstract

Cytolethal Distending Toxin (CDT), a tripartite exotoxin from *Campylobacter jejuni*, is known for inducing DNA damage and apoptosis. In this study, we engineered the A subunit (CDTA) to selectively target cancer cells by replacing its native binding region with anti-PD-L1 antibody sequences. Using sequence data from UniProt (CDTA: A1VXG4, PD-L1: Q9NZQ7), we identified and modified CDTA's residues 129-140. Structural validation using AlphaFold 2 confirmed that these modifications retained CDTA's structural stability and affinity for PD-L1, supporting its potential as a targeted cancer therapeutic. Future experimental steps include protein expression, purification, and cytotoxicity testing to confirm the toxin's selective binding and apoptotic effects on PD-L1 positive cancer cells.

Introduction

Cytolethal Distending Toxin (CDT) is a unique tripartite exotoxin produced by *Campylobacter jejuni* and other Gram-negative bacteria, recognized for its ability to induce apoptosis in eukaryotic cells [1]. CDT comprises three subunits: CDTA, CDTB, and CDTC. The A subunit (CDTA) binds to the host cell membrane, initiating toxin entry, while CDTC assists in the transport of CDTB into the nucleus by binding to the nuclear membrane [1]. Once inside, CDTB, a DNase, cleaves host DNA, causing cell cycle arrest at the G2/M checkpoint and ultimately triggering apoptosis—a regulated form of cell death that is less inflammatory than necrosis [2]. This makes CDT a promising candidate for selective cancer therapies, where targeted apoptosis could reduce damage to surrounding tissues.

Programmed Death-Ligand 1 (PD-L1) is commonly overexpressed on cancer cells, where it enables immune evasion by binding to the PD-1 receptor on T-cells and suppressing immune responses [3]. Targeting PD-L1 in cancer immunotherapy has shown promising results, making it a valuable target for cancer treatments [4]. In this study, we hypothesized that by modifying the binding region of CDTA with anti-PD-L1 antibody sequences, CDT could be engineered to selectively bind and kill PD-L1-expressing cancer cells.

Based on UniProt sequence data (CDTA: A1VXG4), we identified residues 129-140 within the Ricin B-type lectin domain of CDTA as the optimal site for modification. This region was replaced with complementarity-determining region (CDR) sequences from the heavy and light chains of an anti-PD-L1 antibody derived from human PD-L1 (UniProt: Q9NZQ7) [4, 5]. Structural modeling via AlphaFold 2 was then used to assess whether these modifications maintained the protein's stability and supported PD-L1 binding. Following these validations, we plan further experimental steps to express and purify the modified toxin for testing on PD-L1-positive cancer cell lines.

Materials and Methods

1. **CDTA Sequence Selection and Modification**

   CDTA's amino acid sequence was obtained from UniProt (A1VXG4) [1]. Residues 129-140, identified as part of the Ricin B-type lectin binding region, were selected due to their role in membrane attachment. Anti-PD-L1 antibody CDR sequences from UniProt (Q9NZQ7) were incorporated to create two modified CDTA variants [4, 5]:

   Original CDTA sequence (Yellow residues 129-140):

   MQKIIVFILCCFMTFFLYACSSKFENVNPLGRSFGEFEDTDPLKLGLEPTFPTNQEIPSLIS
   GADLVPITPITPPLTRTSNSANNNAANGINPRFKDEAFNDVLIFENRPAVSDFLTILGPSGA
   ALTVWALAQGNWIWGYTLIDSKGFGDARVWQLLLYPNDFAMIKNAKTNTCLNAYGNGIV
   HYPCDASNHAQMWKLIPMSNTAVQIKNLGNGKCIQAPITNLYGDFHKVFKIFTVECAKKD
   NFDQQWFLTTPPFTAKPLYRQGEVR

   Modified sequences:

CDTA_HC (heavy chain CDR): ALAQGNWIWGY > GYTFTRYYDMH

MQKIIVFILCCFMTFFLYACSSKFENVNPLGRSFGEFEDTDPLKLGLEPTFPTNQEIPSLIS
GADLVPITPITPPLTRTSNSANNNAANGINPRFKDEAFNDVLIFENRPAVSDFLTILGPSGA
ALTVWGYTFTRYYDMHTLIDSKGFGDARVWQLLLYPNDFAMIKNAKTNTCLNAYGNGIV
HYPCDASNHAQMWKLIPMSNTAVQIKNLGNGKCIQAPITNLYGDFHKVFKIFTVECAKKD
NFDQQWFLTTPPFTAKPLYRQGEVR

CDTA_LC (light chain CDR): ALAQGNWIWGY > RQYYSTPRTF

MQKIIVFILCCFMTFFLYACSSKFENVNPLGRSFGEFEDTDPLKLGLEPTFPTNQEIPSLIS
GADLVPITPITPPLTRTSNSANNNAANGINPRFKDEAFNDVLIFENRPAVSDFLTILGPSGA
ALTVWRQYYSTPRTFTLIDSKGFGDARVWQLLLYPNDFAMIKNAKTNTCLNAYGNGIVH
YPCDASNHAQMWKLIPMSNTAVQIKNLGNGKCIQAPITNLYGDFHKVFKIFTVECAKKDN
FDQQWFLTTPPFTAKPLYRQGEVR

2. **Structural Validation and Binding Simulation Using AlphaFold 2**

AlphaFold 2 was utilized to predict structural interactions of the modified CDTA variants (CDTA_HC and CDTA_LC) with PD-L1. The simulation showed that:

- The modified CDTA maintained structural integrity with stable protein folding.
- Binding simulations indicated favorable orientations of the CDR-modified regions toward PD-L1's binding domain.

These results supported the potential of both modified CDTA variants for specific PD-L1 binding.

3. **Future Experiments: Protein Expression and Purification**

Planned experiments will include expressing the modified CDTA variants in *E. coli*, followed by purification through affinity chromatography. The goal is to achieve high-purity proteins for reassembly with CDTB and CDTC to form complete CDT complexes. These complexes will then be tested for functionality in subsequent assays.

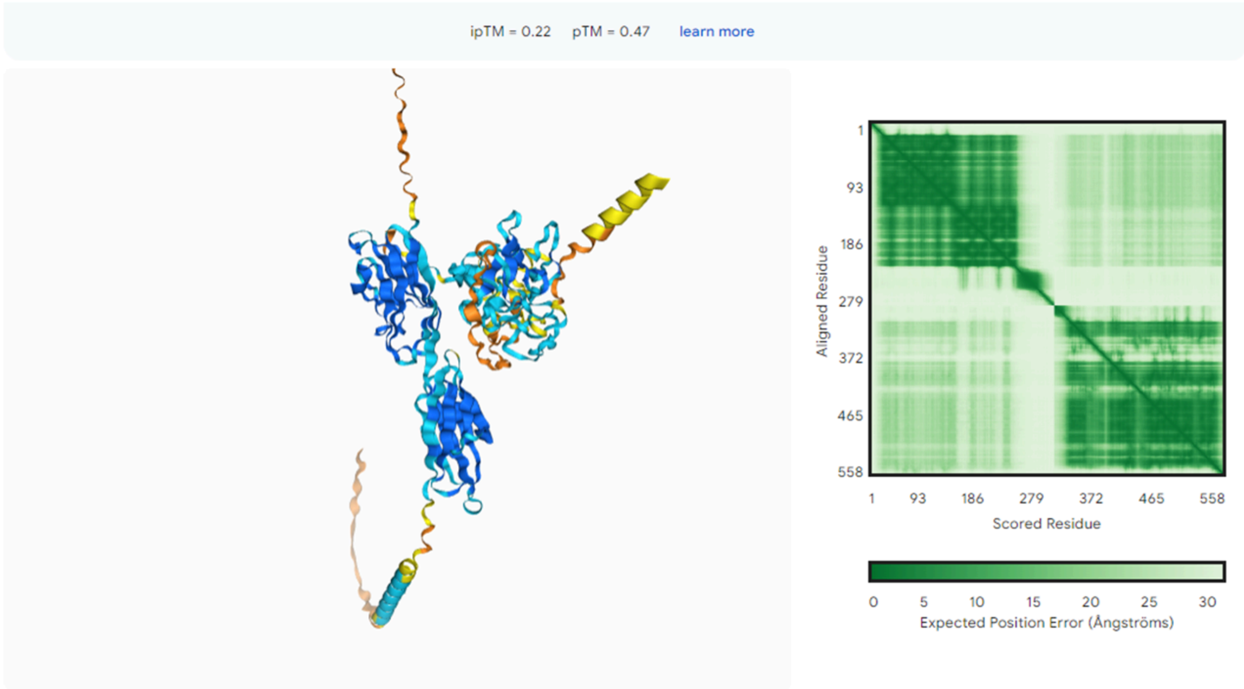
4. **Future Experiments: Cytotoxicity and Selectivity Assays**

   Cytotoxicity assays will be conducted using PD-L1-expressing cancer cell lines and PD-L1-negative controls. Flow cytometry with apoptotic markers, such as Annexin V, and MTT assays for cell viability will be used to assess the toxin's selective apoptotic effects on PD-L1-positive cells.

Results

1. **AlphaFold 2 Structural Simulation Results**

   AlphaFold 2 simulations confirmed that the modified CDTA variants, CDTA_HC and CDTA_LC, retained structural integrity when modified with PD-L1 targeting sequences. The simulations indicated that the CDR regions were correctly positioned to engage PD-L1, suggesting that the engineered binding specificity could be effective in practice.



Type	Copies	Sequence
Protein	1	MRIFAVFIFM TYWHLLNAFT VTVPKDLYVV EYGSNMTIEC KFPVEKQLDL AALIVYWEME DKNIIQFVHG EEDLKVQHSS YRQRARLLKD QLSLGNAALQ ITDVKLQDAG VYRCMISYGG ADYKRITVKV NAPYNKINQR ILVVDPVTSE HELTCQAEGY PKAEVIWTSS DHQVLSGKTT TTNSKREEKL FNVTSTLRIN TTTNEIFYCT FRRLDPEENH TAELVIPELP LAHPPNERTH LVILGAILLC LGVALTFIFR LRKGRMMDVK KCGIQDTNSK KQSDTHLEET
Protein	1	MQKIIVFILC CFMTFFLYAC SSKFENVNPL GRSFGEFEDT DPLKLGLEPT FPTNQEIPSL ISGADLVPIT PITPPLTRTS NSANNNAANG INPRFKDEAF NDVLIFENRP AVSDFLTILG PSGAALTVWG YTFTRYYDMH TLIDSKGFGD ARVWQLLLYP NDFAMIKNAK TNTCLNAYGN GIVHYPCDAS NHAQMWKLIP MSNTAVQIKN LGNGKCIQAP ITNLYGDFHK VFKIFTVECA KKDNFDQQWF LTTPPFTAKP LYRQGEVR

Seed: 419772353

Figure 1. Interaction between CDTA with grafted anti-PD-L1 Heavy Chain CDR (right) and PD-L1 (left) confirmed via AlphaFold 2.

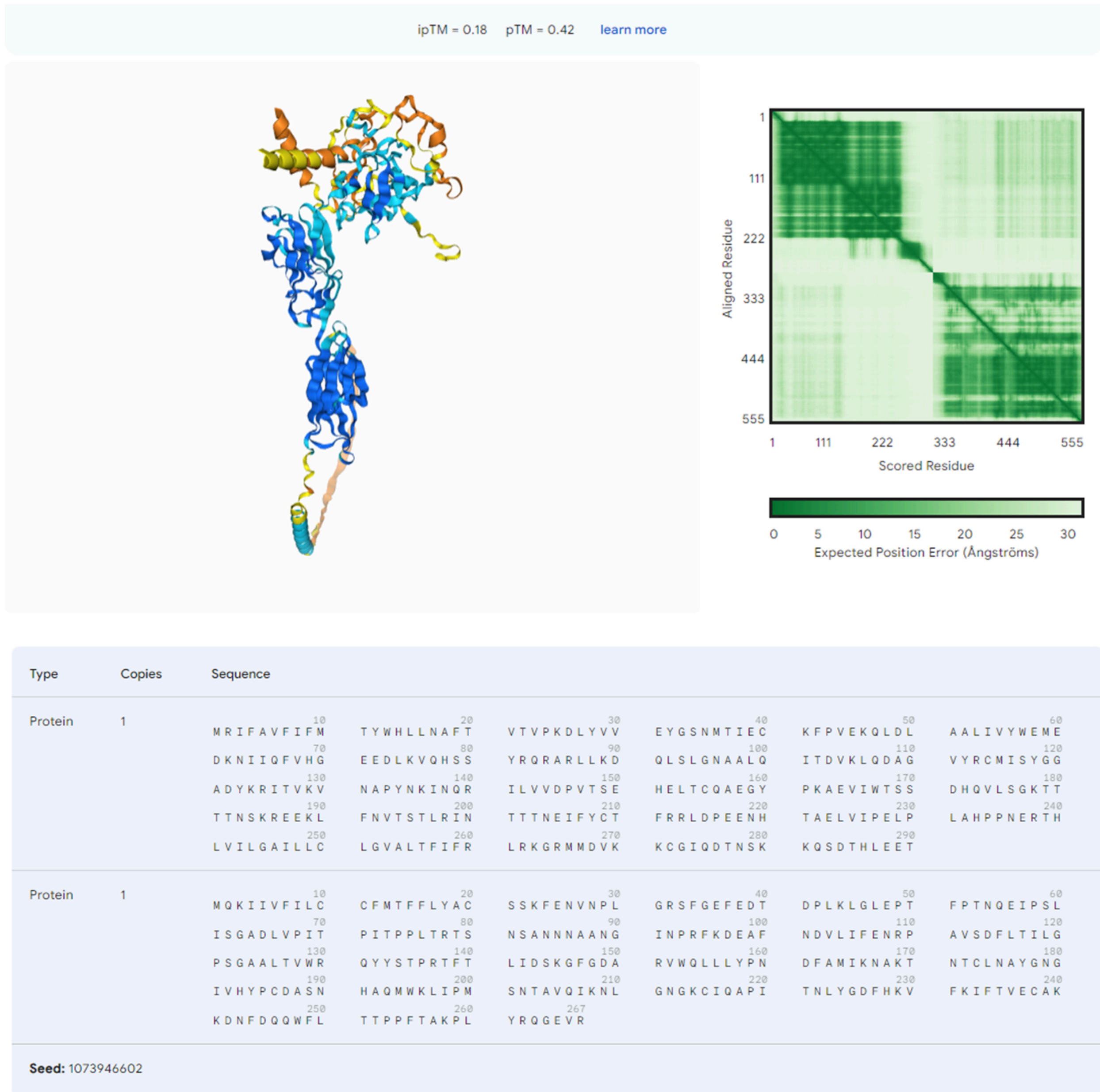


Figure 2. Interaction between CDTA with grafted anti-PD-L1 Light Chain CDR (right/top) and PD-L1 (left/bottom) confirmed via AlphaFold 2.

2. **Future Experiments: Protein Expression and Cytotoxicity Assays**

Protein expression and purification of the modified CDTA variants, as well as cytotoxicity testing on PD-L1-expressing and PD-L1-negative cell lines, will be conducted in future

experiments to validate the selective binding and apoptosis-inducing capabilities of the engineered toxin.

Discussion

The AlphaFold 2 simulation results provide a strong foundation for the feasibility of modifying CDTA to target PD-L1-expressing cells. By incorporating anti-PD-L1 antibody CDR sequences into the binding domain, the modified CDTA variants demonstrated the structural compatibility needed for PD-L1 specificity [5]. These computational findings justify the next steps in experimental validation.

Future in vitro testing will evaluate whether the modified CDT can selectively induce apoptosis in PD-L1 positive cells, providing a more targeted approach to cancer therapy with minimal off-target effects. If successful, this strategy could be extended to other cancer markers, highlighting the potential of CDT modifications as a flexible platform for targeted cancer therapeutics. Additionally, this study underscores the value of structural modeling in designing modified toxins, allowing for preliminary validation before intensive experimental work.

Keywords

Cytolethal Distending Toxin, CDTA, PD-L1, cancer targeting, apoptosis, antibody-modified toxins, AlphaFold 2, selective cytotoxicity

Reference

1. **Jinadasa, R. N., Bloom, S. E., Weiss, R. S., & Duhamel, G. E.** (2011). Cytolethal distending toxin: a conserved bacterial genotoxin that blocks cell cycle progression,

leading to apoptosis of a broad range of mammalian cell lineages. *Microbiology*, 157(7), 1851–1875.

2. **Dasti, J. I., Tareen, A. M., Lugert, R., Zautner, A. E., & Groß, U.** (2010). *Campylobacter jejuni*: A brief overview on pathogenicity-associated factors and disease-mediating mechanisms. *International Journal of Medical Microbiology*, 300(4), 205–211.
3. **Dong, H., Strome, S. E., Salomao, D. R., Tamura, H., Hirano, F., Flies, D. B., Roche, P. C., Lu, J., Zhu, G., Tamada, K., Lennon, V. A., Celis, E., & Chen, L.** (2002). Tumor-associated B7-H1 promotes T-cell apoptosis: A potential mechanism of immune evasion. *Nature Medicine*, 8(8), 793–800.
4. **Topalian, S. L., Drake, C. G., & Pardoll, D. M.** (2015). Immune checkpoint blockade: A common denominator approach to cancer therapy. *Cancer Cell*, 27(4), 450–461.
5. **Patel, S. P., & Kurzrock, R.** (2015). PD-L1 expression as a predictive biomarker in cancer immunotherapy. *Molecular Cancer Therapeutics*, 14(4), 847–856.