

Targeting Proteins Computationally

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The process of programmed cell death, known as apoptosis, is an important mechanism to limit viral infection by sacrificing infected cells before the virus can replicate and spread to nearby cells. Apoptosis is regulated by both pro- and anti-apoptotic members of the B cell lymphoma-2 (Bcl-2) family of proteins. Epstein Barr virus (EBV), the causative agent of Burkitt's lymphoma, encodes a pro-survival mimic of these Bcl-2 proteins called BHRF1 that inhibits cellular apoptosis during infection and is thought to contribute to lymphomagenesis. Therefore, BHRF1 is an intriguing therapeutic target for both viral infection and anti-cancer treatments; however, previous efforts to target BHRF1 have met with limited success. In a recent *Cell* paper spanning basic research in protein structure and engineering to pre-clinical experiments in animals, a collaboration between the laboratories of Dr. David Baker (UW), Drs. David M. Hockenbery, Oliver W. Press (Clinical Research Division), and Barry L. Stoddard (Basic Sciences Division) computationally designed and tested a novel inhibitor of BHRF1 that suppresses tumor growth in an *in vivo* model system.

While computationally-based protein design has made significant progress in recent years, designing functional proteins *de novo* has remained an elusive goal. In a new and exciting approach, the researchers selected a Bcl-2 functional motif that binds to BHRF1 to serve as a folding nucleus, and then computationally assembled helical-bundle structures around this motif to scaffold it. This initial structure was then refined through multiple rounds of sequence design and structure minimization to improve monomer stability and interface quality with BHRF1. Candidate designed proteins were tested using yeast surface display, and two proteins bound BHRF1 with apparent K_{DS} as low as 58-60 nM. The researchers next used *in vitro* targeted evolution to further refine these protein-protein interactions. Ultimately, this process resulted in a protein termed BINDI (BHRF1-INhibiting Design acting Intracellularly) that binds BHRF1 at least 180-fold more specifically than other Bcl-2 homologs with a K_D of 220 +/- 50 pM. Surprisingly, the crystal structure of the BINDI-BHRF1 complex showed that the interactions extended well beyond the initial functional motif, and residues in the surrounding helical-bundles provided major contributions to the extraordinary specificity of this interaction.

The researchers next tested BINDI for biological activity in EBV infected cells. Apoptosis is mediated by release of cytochrome c, and BINDI elicited greater cytochrome c release from mitochondria isolated from some EBV infected cell lines than from mitochondria isolated from non-infected cell lines. However, BINDI did not induce cytochrome c release in all EBV-infected cell lines the researchers tested, suggesting that BHRF1 is only necessary for the survival of a subset of EBV-positive cancer cell lines. This EBV-specific BINDI-mediated cell killing was further enhanced by fusing the designed protein with antennapedia, a peptide that enhances cellular uptake *in vitro*. These results suggested that BINDI may be an effective therapeutic molecule for EBV-positive Burkitt's lymphoma. To test this hypothesis the researchers implanted EBV-positive tumors in mice and treated them with BINDI delivered by an antibody-micelle formulation designed to deliver protein drugs intracellularly. Mice treated with BINDI had substantially lower tumor volume (140 +/- 60 mm³) than untreated (1080 +/- 500 mm³), chemo only (680 +/- 410 mm³), or antibody-micelle control mice

(330 +/- 140 mm³) (BINDI v. antibody-micelle control, unpaired t test p = 0.003). BINDI treatment also significantly increased the survival time of the mice (log-rank test p = 0.006). "Eventually, it is hoped that this approach can be applied to treat human EBV-associated malignancies, such as Burkitt's Lymphoma, which is one of the most common cancers treated at the FHCRC-affiliated Uganda Cancer Institute in Kampala," said Dr. Oliver Press.

This development of a highly specific and highly active protein molecule by a computational design approach is exciting, suggesting several future avenues to develop this technology. Going forward, "the Baker, Stoddard, and Hockenbery labs are now collaborating to create and study additional designer proteins that can discriminate between a series of highly similar molecular targets (all from the same protein family as the one targeted in this Cell paper), in order to be able to create panels of unique protein drug leads for a variety of cancer types and indications," said Dr. Barry L. Stoddard.

[Procko E, Berquig GY, Shen BW, Song Y, Frayo S, Convertine AJ, Margineantu D, Booth G, Correia BE, Cheng Y, Schief WR, Hockenbery DM, Press OW, Stoddard BL, Stayton PS, Baker D.](#) 2014. A computationally designed inhibitor of an Epstein-Barr viral Bcl-2 protein induces apoptosis in infected cells. *Cell*. 1644-56.

See also: [Procko E, Hedman R, Hamilton K, Seetharaman J, Fleishman SJ, Su M, Aramini J, Kornhaber G, Hunt JF, Tong L, Montelione GT, Baker D.](#) 2013. Computational design of a protein-based enzyme inhibitor. *J Mol Biol*. 3563-75.

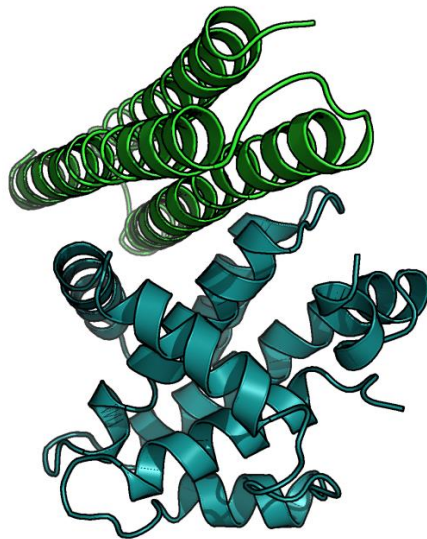


Image courtesy of Dr. Barry L. Stoddard

Ribbon diagram of BINDI (green) binding to BHRF1 (blue).