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Targeting Oncogenic Protein-Protein interactions to Overcome Drug Resistance

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Introduction

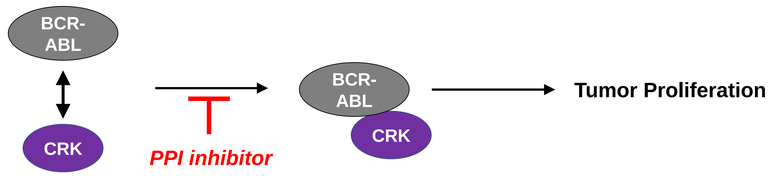
Resistance to molecularly targeted drugs is a major problem in current cancer chemotherapy. Therefore, to overcome drug resistance, there is a **critical need to develop orthogonal approaches to the currently available cancer drugs**. The non-receptor tyrosine kinase **ABL** is a proto-oncoprotein, and its abnormal chromosomal translocation produces the **BCR-ABL** fusion product is found in 95% of the patients with chronic myelogenous leukemia (CML) and in 30% of the patients with acute lymphoblastic leukemia (ALL).

Targeting protein-protein interactions (**PPIs**) has emerged as a highly promising, but challenging, area for the development of cancer therapeutics. Recent studies indicate that CT10-regulator of kinase (**CRK**) is the major phosphorylation substrate of ABL and BCR-ABL. Moreover, dysregulated CRK proteins are involved in tumor invasion and metastasis. Therefore, it was indicated that inhibition of the CRK-ABL interaction might be a highly effective strategy in overcoming drug-resistance in CML.

The goal of this proposed research is to develop *peptide-based inhibitors (peptidomimetics)* for the oncogenic CRK-ABL interaction. To design the inhibitors of the CRK-ABL interaction, we explored a cyclic structure of peptides. We have adopted split-intein circular ligation of peptides and proteins (SICLOPPS) to generate cyclic peptides. This method allows the combination of convenient sample preparation using well-established bacterial expression protocol. However, the method has suffered from difficulty in purification of cyclic peptides generated in bacteria. To overcome the barrier, we designed a construct of split-intein for conditional splicing reaction that allows the purification of cyclic peptides. We expect that our approach will enhance the application of cyclic peptides for the development of a wide range of therapeutics.

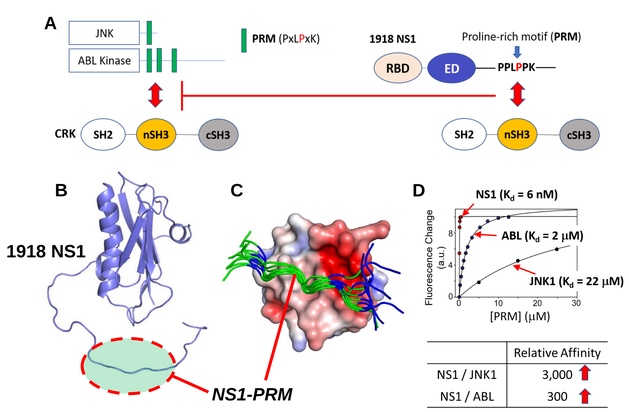
Inhibition of the interaction between CRK and BCR-ABL

BCR-ABL is the fusion product of a chromosomal translocation causative for chronic myeloid leukemia (CML). Direct binding of CRK To BCR-ABL results in the proliferation of CML cells. *Cancer Res. (2010) 70, 7325-7335*



1918 Influenza virus hijacks CRK during infection

Previously, we showed that the proline-rich motif (PRM) of 1918 NS1 protein has a high affinity to CRK. This promote us to use the 1918 NS1-PRM for the development of inhibitors of the CRK-BCR-ABL interaction in cancer cells. *ACS Chem Biol (2017) 12, 1199-1203*

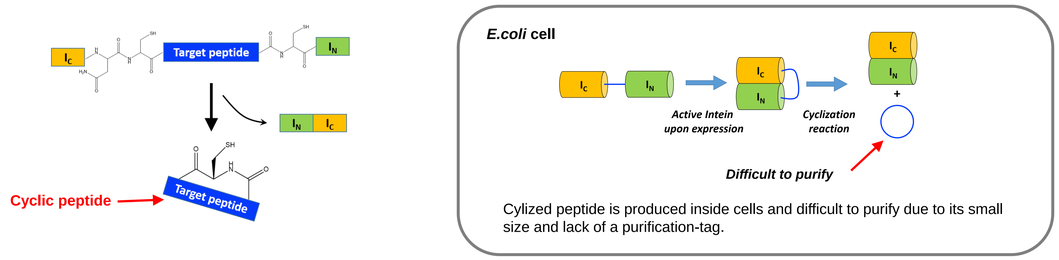


	Relative Affinity
NS1 / JNK1	3,000
NS1 / ABL	300

(A) Schimatic showing the hijacking of human CRK by 1918 NS1 protein. The high affinity of 1918 NS1-PRM outcompetes cellular binding partners of CRK including ABL and JNK. **(B)** Nuclear Magnetic Resonance (NMR) structure of the 1918 NS1 protein. **(C)** Crystal Structure of the complex between 1918 NS1-PRM and the nSH3 domain of CRK. **(D)** Binding isotherms of 1918 NS1-PRM and other cellular PRMS to CRK.

Split-Intein Circular Ligation of Peptides and ProteinS (SICLOPPS)

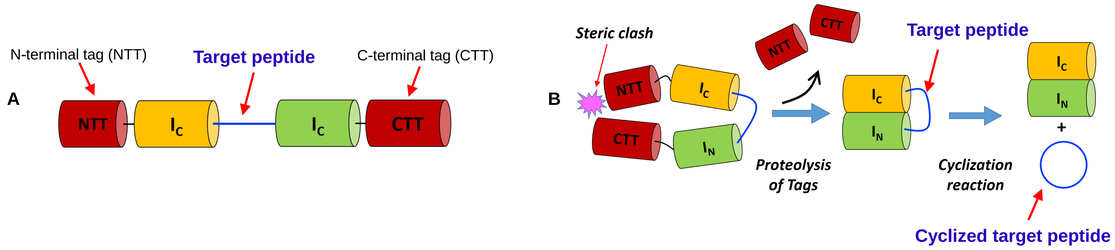
Despite the great potential of 1918 NS1-PRM as an inhibitor of the CRK-BCR-ABL interaction, peptides are easily degraded inside cells. To develop more drug-like inhibitors, we explored peptide cyclization. Cyclic peptides show better biological activity than their linear counterparts due to the rigid conformations and/or higher resistance to proteases. To make a cyclic version of NS1-PRM, we employed a biochemical approach called SICLOPPS.



(A) Simplified SICLOPPS-mediated cyclization reaction. The target peptide is cyclized upon splicing reaction of intein. **(B)** Cyclization occurs as soon as intein is expressed in E. coli cells. The cyclized peptide is difficult to purify for detailed analysis.

Design of conditional SICLOPPS

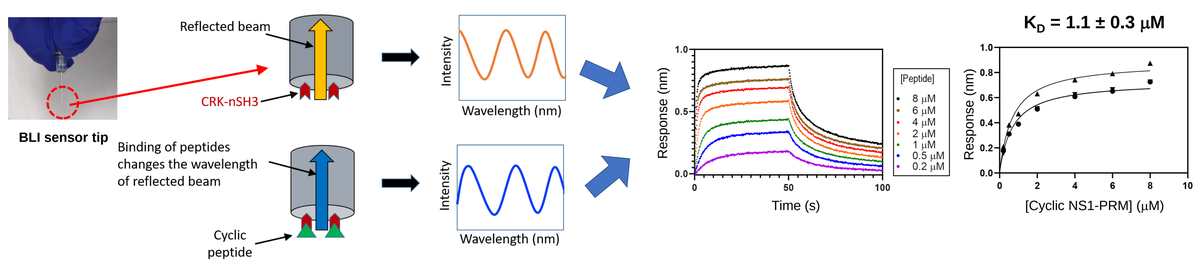
To delay the cyclization reaction, we designed a tagged split-intein construct. The N- and C-terminal tagging proteins induces steric clash, preventing association of split intein fragments. The tagged intein with encoded target peptide can be purified in vitro. Upon proteolytic digestion of the tagging proteins, cyclization reactions starts. Therefore, the cyclized peptide can be purified.



(A) Schematic showing a construct for conditional SICLOPPS. NTT and CTT represents the N- and C-terminal tag, respectively. IN and IC represent the N- and C-terminal fragments of Split-intein. **(B)** NTT and CTT prevents the association and folding of split-intein fragments. The cyclization only occurs when the tagging proteins are cleaved off by proteases.

Proof of concept

Cyclic NS1-PRM was designed and purified for in vitro characterization. Binding of the purified cyclic NS1-PRM to the nSH3-CRK domain was confirmed using a biolayer interferometry (BLI).



Conclusions and Future Direction

We demonstrated the feasibility of the conditional SICLOPPS. This approach allows detailed in vitro characterization of cyclic peptides, which is important for the rational optimization of binding properties. Combined with BLI, peptide cyclization using SICLOPPS can enhance the identification of cyclc peptides blocking oncogenic protein-protein interaction. Future studies will focus on (1) the optimization of the cyclic NS1-PRM to increase the affinity to CRK and (2) cell-based tests of the inhibitory activity of the cyclic peptides.