The design of transcription factor-based inhibitors to target Myc: drop the Myc!

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English summary

The aim of this thesis is to inhibit the transcription factor (TF) Myc. Myc is involved in more than 50% of all cancers. However, there is currently no treatment to inhibit Myc. As such, it is important to do research towards Myc or other disease-related TFs. As Myc is intrinsically disordered without its protein partner Max and located inside the nucleus, it is a difficult pharmaceutical target.

This thesis tries to inhibit Myc using different strategies. By using crystal structures, artificial intelligence and protein expression, novel Myc inhibitors were designed, synthesized and tested. We either prevent Myc from binding to its canonical target sequence or we prevent Myc to bind to its protein partner Max.

Chapter 1 goes into detail about background information that explains fundamental topics related to artificial TF and peptidomimetic design. It begins with a general introduction about TFs, that play an important role in gene regulation. However, they are also involved in multiple diseases, such as cancer. Despite their importance, TFs are difficult to inhibit due to their location deep inside the cellular nucleus and their partly disordered nature. The introduction outlines general strategies for inhibiting TFs and discusses how peptides can be used to overcome common challenges. Peptides can be engineered into artificial TFs that mimic their natural counterpart, target the same canonical DNA binding site and function as a transcriptional repressor. Additionally, Chapter 1 highlights the biological function of the oncogenic TF Myc and reviews strategies known to target this protein. Taken together, this chapter provides the scientific content to understand the topics discussed in this thesis.

Chapter 2 provides an extensive overview of peptides designed to target disease-related DNA or RNA. Compared to small molecules, peptides can adopt multiple conformations allowing them to recognize large DNA and RNA surfaces. We explain different strategies to discover peptides that can target DNA or RNA. Often large libraries are designed and screened against DNA or RNA of interest. Large libraries increase the possibility of finding peptides that can target a specific sequence. Additionally, structure-based design studies are discussed that used endogenous TFs as a template and successfully designed peptides or miniproteins. Lastly, late-stage peptide modifications can be beneficial. For example, cyclisation or stapling can increase affinity towards a specific sequence by locking the peptide in the active conformation. The findings in this chapter provide an extra context for the following chapters discussed within this thesis.

In **Chapter 3** we perform a structure-based design study, using Omomyc as a template, to design artificial TFs to inhibit Myc. Omomyc is known to inhibit Myc. However, not all studies show that Omomyc has intrinsic cell permeability. We hypothesize that Omomycs size is the main limitation and aim to decrease the overall structure to the core elements needed for successful DNA binding. We designed multiple variants that differed in size based on Omomyc and showed that the basic region alone is not sufficient for successful DNA binding. An additional domain is needed for a stable protein/DNA complex. This designed miniprotein, DuoMYC, is able to enter cells, inhibit Myc and influence cancer related pathways.

Chapter 4 uses the full crystal structure of Omomyc. Instead of decreasing overall size, we incorporate staples and positive charge to enhance intrinsic cell permeability. Normally, protein expression is a delicate process to retain the overall protein structure. However, Omomyc can fold upon protein partner contact which allowed us to incorporate staples after we expressed the proteins in bacteria. We called this technique the ReCHEMbinant technique. Using the ReCHEMbinant platform we can modify and stabilize easy-folding proteins after protein expression. We use Omomyc as a proof of principle protein and designed HeloMYC. HeloMYC could enter cells, inactivate Myc and influence cancer related pathways. Additionally, the modified proteins were more stable compared to their parent compound.

In **Chapter 5** we inhibit Myc via a different strategy. In this chapter we do not aim to block the canonical DNA binding site of Myc but the interaction between Myc and Max. By using AlphaFold-Multimer we analyzed the interaction between Myc and another protein partner of Myc: Miz-1. AlphaFold-Multimer predicts how proteins interact based on their primary sequence. Our Miz-1/Myc prediction was supported by literature and allowed us to design Miz-1-based Myc inhibitors, Mizmetics. Biochemical assays validated an interaction between Myc and Mizmetics and, additionally, the incorporation of a staple resulted in increased affinity. Moreover, we identified positions that were essential for the interaction between Myc and Mizmetic. Other mutations, that could be explained by AlphaFold, increased affinity between our designed peptides and Myc. These mutated peptides could inhibit Myc/Max/DNA interaction and became more specific towards Myc.

In **Chapter 6** the most important findings are summarized. Minimalizing and stabilizing Omomyc resulted in novel Myc inhibitors. Additionally, the use of artificial intelligence (AI) resulted in a newly identified Myc inhibitor. The novel designs and design strategies assist with inhibiting Myc and bring us closer to a cure towards Myc-related cancer. Additionally, future perspectives are discussed. For example, how DuoMYC can be used to target other disease-related TFs, how the ReCHEMbinant approach can be used to analyze disordered proteins or other strategies to inhibit Myc. This thesis highlights its value in the struggle against Myc.

The thesis provides a concise overview of rationally designed artificial TFs and AI-assisted peptide design, always with the aim to inhibit Myc. Without a treatment for Myc-related cancers, research remains important to get a better understanding of Myc. Moreover, the work described in this thesis can also be used to target other disease-related TFs and oncogenic proteins.