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

Ellenbroek, B.D.

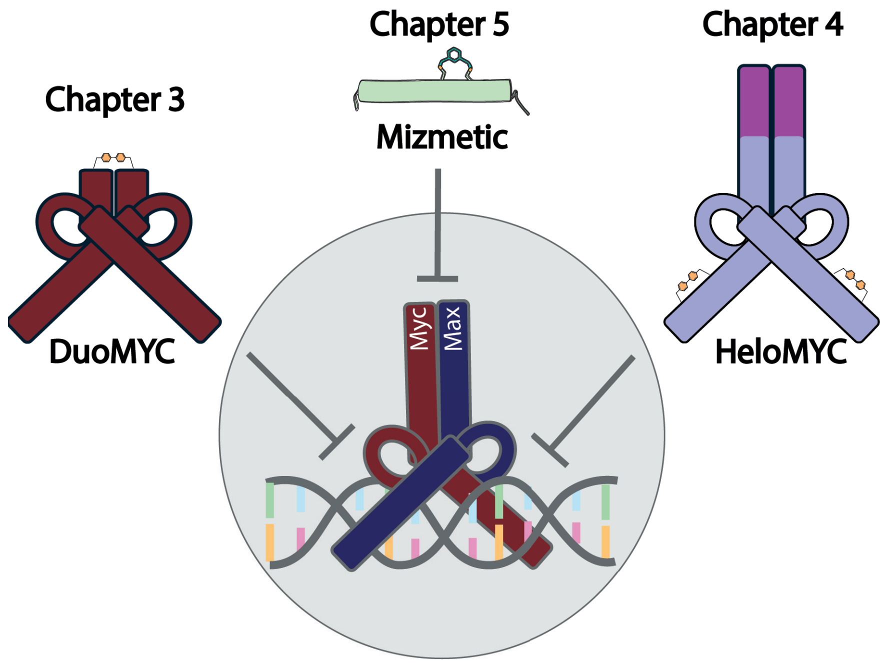
Citation
Ellenbroek, B. D. (2026, June 10). *The design of transcription factor-based inhibitors to target Myc: drop the Myc!*. Retrieved from https://hdl.handle.net/1887/4305022

Version:	Publisher's Version
License:	Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden
Downloaded from:	https://hdl.handle.net/1887/4305022

Note: To cite this publication please use the final published version (if applicable).

Chapter 6

Conclusion and future perspectives



6

The main goal of this thesis is to *'Drop the Myc'*. We explored Myc inhibition using different approaches. Firstly, we developed a cell-permeable miniprotein that inhibits Myc activity by binding to Myc's designated DNA-binding site known as the Enhancer box (E-box, sequence: CACGTG). Secondly, aiming at a similar mechanism of action, we developed a method to stabilize and increase cell permeability of recombinant proteins, able to bind the E-box site. Lastly, AlphaFold was applied to predict an unresolved protein-protein interaction (PPI) that was utilized to design peptidomimetics that could prevent Myc-Max formation. All (mini)proteins and peptidomimetics were tested by a combination of biochemical characterization and cellular assays to analyze cell permeability, stability and affinity. This chapter summarizes the most important findings of each chapter and additionally highlights future possibilities for the design of Myc targeting peptide modalities.

6.1 Peptides are promising modalities for the inhibition of disease related nucleic acids

The central dogma of life entails that the genetic flow goes from DNA to RNA to proteins.[1] This process is strictly regulated by a network of PPIs, protein-DNA interactions and protein-RNA interactions. Disruption of this network can result in the development of diseases. Strikingly, approximately 19% of all human transcription factors (TFs) are involved in a disease, such as cancer.[2] Therefore, it is important to increase our understanding on how this class of proteins can be targeted. TF inhibition is particularly challenging because they are involved in protein-DNA interactions that often entail large interfaces and occur within the nucleus. The large interface limits the use of small molecules as they are not large enough. Consequently, alternative strategies are required to effectively target TFs.

Peptide-based modalities can be used to interact with large binding surfaces. **Chapter 2** describes the development and advantages of peptides that can target disease-related DNA and RNA. Peptides are advantageous compared to small molecules due to their larger size as this allows them to recognize large surface areas. Normally, an increase in molecular size can reduce cell permeability. However, nucleic acid (NA)-binding peptides often carry multiple positive charges that promote cell penetration, a characteristic also observed in cell-penetrating peptides. We observed two primary strategies for discovering specific NA-binding peptides: library screening and structure-based design. The latter strategy was applied in **Chapter 3** and **4**.

We highlight in **Chapter 2** that staple incorporation, or cyclisation, can be beneficial for cellular uptake, thereby locking NA-binding peptidomimetics in their active conformations. We observed similar effects in **Chapter 3** and **4**. In **Chapter 3** we could replace the full dimerization domain of Omomyc with a single covalent linker, thereby locking the protein in its dimeric state. Similarly, in **Chapter 4** we showed that staples in the basic region of Omomyc increased cell permeability and serum

stability. We observed a similar effect while designing the Mizmetic compounds in **Chapter 5.** Staple incorporation resulted in an increase in binding affinity towards Myc.

Selectivity is especially important for NA binders as off-target activity can have severe consequences for transcription and translation of genes. There are currently no FDA approved peptide-based drugs that target NAs but there are examples of NA binders that are in clinical trials, for example Omomyc-103.[3] Overall, peptide and protein mimetics can be promising modalities if they are carefully designed to selectively target a single NA sequence.

6.2 Natural TFs can serve as blueprints for creating artificial transcriptional repressors with improved drug likeness

In **Chapter 3,** we applied a structure-based design strategy on Omomyc to inhibit Myc. Omomyc is a basic Helix-Loop-Helix leucine zipper (bHLH-LZ) TF and needs to dimerize to act as a transcriptional repressor.[4] Cell permeability is a major challenge when designing miniproteins. Especially for Omomyc there is contradictory data regarding its intrinsic cell permeability. To address this, we designed several truncated Omomyc variants: MiniMYC 1, MiniMYC 3 and DuoMYC.

Our goal was to decrease the overall size of Omomyc, while increasing cell permeability and retaining DNA affinity. Both MiniMYCs only contained the basic region that were covalently linked to form a dimer. MiniMYC 1 and MiniMYC 3 were unable to selectively bind DNA. A more constrained decafluorbiphenyl linker displayed a moderate binding improvement compared to a flexible lysine linker but was not sufficient for a stable protein/DNA complex. Therefore, we designed DuoMYC, which also included the loop and a small segment of the LZ of Omomyc. DuoMYC displayed low nanomolar affinity towards its canonical E-box target sequence in two different binding assays. We incorporated a flexible and a constrained linker in DuoMYC. Strikingly, there was not a major difference in affinity between both DuoMYC constructs. This suggests that the additional loop is the primary contributor to accurate DNA binding, rather than linker rigidity.

The monomer of DuoMYC, MonoMYC, could also bind as a dimer to DNA before covalent dimerization. The incorporation of a covalent linker within DuoMYC increased affinity compared to its monomer. Whereas MonoMYC exists as a monomer in solution, DuoMYC exists as a dimer. The increase in affinity is presumably attributable to a reduced entropic penalty for DuoMYC, as MonoMYC must first form a dimer prior to DNA binding. DuoMYC could inhibit Myc (IC_{50} = 464 nM) and was cell permeable. Moreover, RNA sequencing displayed that DuoMYC influenced pathways involved in cancer.

This study emphasized that decreasing protein size can increase cell permeability. We showed that a major part of the protein can be removed but that there is a limit to how much can be removed. Too much removal can lead to loss of selectivity or function. Therefore, a careful design approach must be performed to find a cut-off in protein size with sufficient protein activity and increased cell penetration, as displayed by DuoMYC.

To improve cell penetration without decreasing protein size, we investigated two strategies to increase cell permeability of Omomyc in **Chapter 4**. The first approach entailed an increase in arginine content. Increase in arginine and thus positive charge, can lead to increased cell permeability.[5] The second approach introduced staples inside the basic region of Omomyc. Staples can amplify intrinsic cell permeability.[6,7] They enhance the overall lipophilicity and α-helicity of the peptide by promoting intramolecular hydrogen bonding within the backbone.[8] We applied the first approach on the LZ region and increased its arginine content. Additionally, we incorporated different staples at different positions inside the basic region. To obtain our designed Omomyc variants, we used recombinant expression and strategically placed cysteines inside the basic region.

Protein expression can be a delicate process to preserve complex protein structures. However, Omomyc can dimerize upon protein contact, even following denaturation. This is a characteristic treat shared among Myc- and Max-based TFs.[9–13] For example, denatured Myc was successfully used in our native binding assay in **Chapter 5**. Similarly, we could denature Omomyc during a buffer exchange, perform a two-component stapling reaction and, subsequently, test the compounds in a native binding assay. This approach resulted in the development of the ReCHEMbinent technique. Most designed Omomyc derivatives could bind to their canonical binding site, adopted an α-helical conformation and displayed increased stability when bound to DNA. Bioactivity assays showed that HeloMYC-1421 could inhibit Myc activity in both HEK293T and HeLa cells. Moreover, labeled HeloMYC-1421 displayed increased cell permeability compared to Omomyc. Overall, we demonstrated in **Chapter 4** that the characteristic ability of Omomyc to fold upon protein-partner contact can be exploited for a beneficial outcome.

6.3 AlphaFold predicts an interaction interface on Myc where Miz-1 and MAX overlap

In **Chapter 5** we developed a direct Myc inhibitor employing the AlphaFold-Multimer software.[14,15] Myc is difficult to target using a direct inhibitor because of its partially disordered nature in the absence of binding partners. We utilized AlphaFold-Multimer to predict how Myc and Myc interacting zinc finger 1 (Miz-1) interact, as no crystal structure is currently available. Miz-1 and Myc are together involved in cancer development but it is unknown how they physically interact.[16] AlphaFold-

Multimer predicted a sequence of 28 amino acids within Miz-1, located inside Myc interacting domain 2 (MID2), to interact with the HLH domain of Myc. As this was consistent with literature, we tested and validated that this small region displayed affinity towards Myc. Incorporation of a cysteine staple increased the affinity 26-fold. An alanine-scan revealed three hotspots and five beneficial mutations that were consistent with the AlphaFold prediction. AlphaFold predicted an interacting interface overlap between Max and Miz-1 on Myc. We therefore developed an activity assay to determine if our Mizmetic compounds could inhibit Myc-Max-DNA binding. Several alanine analogues could inhibit Myc-Max formation and Mizmetic 20 displayed increased specificity towards Myc over Max. Our Mizmetic compounds highlight that peptides can be good inhibitors for disordered proteins. Similar to what was discussed in **Chapter 2**, the large contact interface of peptides, compared to small molecules, is presumably the main driver of recognition of disordered Myc.

This project highlights the impact that artificial intelligence (AI) can have on research. We could design peptidomimetics based on an unresolved PPI without the presence of a crystal structure. It also demonstrates that AI and research can complement each other and that experimental validation remains important to confirm predictions. Especially for interactions that involve IDPs or IDRs. Despite the fact that AlphaFold is generally accurate, studies have reported that it becomes less reliable for regions exceeding 100 amino acids.[17]

To conclude, peptides are promising modalities to inhibit disease-related TFs. They can adopt multiple conformations and can be designed to target large NA interfaces that are able to assume complex secondary structures. The size of peptides can be challenging, mainly for cell permeability. Therefore, careful structure-based design strategies must be applied, which can be combined with novel AI-based tools, such as AlphaFold2.

6.4 Future perspectives

The main focus of this PhD thesis is the TF Myc. Myc is responsible for approximately 15% of all gene transcription in the cell.[18] Moreover, Myc engages in more than 50% of all human cancer types. Strikingly, there is currently no drug on the clinical market that can target this oncogenic TF directly. There are however, candidates that target Myc either directly (Omomyc-103)[3] or indirectly (CX-5461)[19] that are now in clinical trials. Small molecule CX-5461 prevents Myc expression and miniprotein Omomyc-103 functions as a transcriptional repressor. These two examples demonstrate that a protein previously considered 'undruggable' is becoming druggable, thanks to an improved understanding of Myc that allows for novel and innovative inhibition strategies. The compounds discussed in this thesis may attribute to novel strategies in Myc inhibition.

6.4.1 Development of a scaffold for NA recognition.

TFs are essential for healthy cells. Especially Myc participates in numerous vital cellular processes, such as cell differentiation.[20,21] In **Chapter 2** we provided an extensive overview on peptides that are designed to bind disease-related NAs. We highlighted the potential of peptides to target and recognize NA sequences with high precision. This includes secondary structural elements and the ability to discriminate between DNA or RNA sequences. The latter has been observed for Zinc Finger (ZF) motifs.[22] The general structure of a ZF is conserved but depending on the target, a ZF motif recognizes either DNA or RNA, for example ZF5 in TFIIIA.[23] This example highlights the complexity of NA binding. It brings forth the question 'How do we design artificial modalities that can recognize one specific DNA sequence?' To answer this question Barbas III identified rules for specific DNA recognition for ZFs[24–27] and Dervan for polyamides,[28] which have been applied for the design of artificial TFs.[29,30] More recently, the Moellering lab developed an innovative design based on bHLH-LZ[31] or bzipper[32] proteins that act as transcriptional repressors. Generally, TFs contain conserved structural folds that evolved to recognize DNA with remarkable specificity. Depending on the composition of the basic region, similar folded proteins can target different DNA sequences. Indeed, in most of the previous examples, a similar structure or scaffold was transferable to design artificial TFs that target different DNA sequences.

The DuoMYC scaffold can be utilized as a starting point for the design of new synthetic TFs. In theory, we could use DuoMYC's design on other bHLH-LZ TFs to target different DNA sequences. By utilizing known crystal structures of bHLH-LZs in complex with their canonical DNA sequence, DuoMYC's design and covalent linker can provide the basis for new artificial TFs. However, modifications can always result in loss of specificity, as was observed for the reduced Omomyc derivatives (MiniMYC, **Chapter 3**). Therefore, biochemical assays need to confirm specific DNA binding. Nevertheless, before DuoMYCs design can be utilized to design other TFs, its synthesis needs to be optimized.

6.4.2 Synthesis optimization

DuoMYC's synthesis resulted in a low yield, highlighting the need for further optimization if this scaffold is to be used for other artificial TFs or late-stage modifications. Studies demonstrated that artificial TFs targeting Myc can be synthesized using different approaches and that aggregation is a common challenge during synthesis.[11–13,20,31,33–39] Longer peptide sequences tend to aggregate or adopt secondary structures, reducing coupling efficiencies during synthesis. There are strategies to overcome aggregation during synthesis that include chaotropic salts, detergent, alternative solvents, temperature adjustments and different combinations of coupling reagents can improve solubility.[40,41] With the current optimized conditions,

we could try to implement chaotropic salts (e.g. urea or guanidine hydrochloride), detergents (e.g. Tween-20 or Triton X-100) or a mixture of solvents (e.g. addition of DMSO or trifluorethanol (TFE)) during each coupling step to prevent aggregation.

Another strategy to overcome aggregation is to use the SynTAG.[42] Bürgisser *et al.* demonstrated that, when combined with fast-flow peptide synthesis, the SynTag improved peptide solubility. This resulted in increased purity of typically difficult to synthesize peptides, including the TAD of Myc. The SynTAG combined with microwave-assisted peptide synthesis may offer a beneficial alternative approach due to shorter reaction times and generally improved yields compared to automated solid phase peptide synthesis (SPPS). Temperature can significantly influence side reactions that occur during microwave-assisted peptide synthesis, such as aspartic acid epimerization or δ-lactam formation in ariginines. Therefore, examining a temperature range could be beneficial when synthesizing DuoMYC using this approach.

Additionally, ReCHEMbinant expression could be investigated. Earlier attempts to recombinantly express DuoMYC were not viable. We were unable to remove the expression tag, presumably due to its disordered nature. This eventually limited dimerization and cell permeability. Previously, it has been demonstrated that urea combined with enterokinase could facilitate specific tag cleavage for a similar protein.[43] Therefore, urea addition may be an initial step in optimizing DuoMYC tag cleavage. Successful DuoMYC expression and tag cleavage would enable dimerization and thus the ReCHEMbinant platform.

Lastly, the Pentelute lab recently developed a strategy to synthesize proteins with SPPS followed by refolding techniques that promoted the most stable conformation.[44] Whereas incorrectly folded proteins tended to precipitate, correctly folded proteins did not. As such, their approach could be applied as an additional purification step for complicated syntheses, such as DuoMYC. However, this technique relies on protein folding and must be optimized for DuoMYC. To promote correctly folded DuoMYC, additives, such as TFE and DNA, may be incorporated to promote secondary structure formation. TFE is a helix stabilizer and is often used in circular dichroism experiments to promote helicity.[40] Similarly, we observed that DNA stabilized the overall protein structure of Omomyc in **Chapter 4**. In both examples, correctly folded DuoMYC should remain in solution, whereas misfolded proteins should precipitate out of solution.

6.4.3 Combining domains and incorporating staples may improve conformation and cell penetration

A challenge in artificial TFs design is their conformation and cell permeability. It is noteworthy that TFs contain conserved DNA binding motifs that can facilitate DNA

binding and cell permeability.[5] For example, isolated ZFs[45] and bHLH-LZs[11,13] are intrinsically cell permeable but sometimes a cell-penetrating peptide can be beneficial.[46,47] Nonetheless, removing a protein region from its native environment can lead to decreased or loss of function, as the active configuration is no longer sustained by the overall protein structure. Stabilizing the active conformation can increase specificity and cell penetration. There are approaches to guide peptides into the desired topology, such as the approach earlier described by the Pentelute lab.[44]

Combining multiple DNA binding domains can promote specificity and correct conformation.[48,49] As observed in **Chapter 3**, the incorporation of a covalent linker between two MonoMYC compounds increased affinity ~8 fold. To assess if the DuoMYC scaffold is transferable to other bHLH-LZ TFs, Myc-Max or Max-Max DuoMYC constructs would be a rational initial approach. Earlier work demonstrated that covalent Myc-Max and Max-Max dimers can bind E-box DNA.[11,13] Additionally, these constructs may have enhanced stability and helicity compared to DuoMYC, as these complexes naturally occur in cells.

TFs often bind DNA in an α-helical conformation that allows for the incorporation of staples. Staples increase helicity, protease resistance and cell permeability. Helicity is observed in Omomyc,[50] Myc-Max[51] and Max-Max[51] crystal structures in complex with their canonical binding site. Nonetheless, the basic region may adopt multiple conformations in the absence of DNA. In **Chapter 4** and literature it has been demonstrated that staples can be beneficial when incorporated into the basic region of Omomyc and Max.[12,31,52] As such, staples can also be introduced in the basic region of DuoMYC. However, as both MonoMYC dimerization and cysteine stapling rely on CLIPS™[53,54] chemistry, two synthesis strategies may be considered: performing stapling on solid support, or designing a route based on orthogonal protection groups. The latter allows for in-solution stapling and dimerization. For example, cysteine, protected with an acetamidomethyl, could be incorporated at the C-terminus for dimerization and two cysteines, protected with an acid labile trityl group, could be incorporated at the staple positions. Using this route the monomer is first stapled and subsequently dimerized after the removal of the acetamidomethyl group, all in solution. A similar strategy was applied by the Jbara group to staple Max. [52]

Helicity is also essential for Mizmetic peptides to bind Myc. The impact of different kind of staples, different size, different positions and multiple incorporated staples can be assessed in a follow-up study. Recently, Chandramohan *et al.* identified design rules for stapled peptides and suggested that an increase in hydrophobicity and rigidity is beneficial for in vivo activity.[8] All designed compounds could be assessed for helicity, Myc binding, cell penetration and Myc/Max inhibition.

6.4.4 Combining ReCHEMbinant with AI can be a powerful approach

A major difficulty when studying Myc or other TFs is their disordered nature. Whereas their disordered regions adopt a secondary conformation upon protein and DNA contact, they remain largely disordered without protein partners. This disordered character mitigates the identification of defined binding regions due to impaired structure elucidation. Before the development of AI tools, such as AlphaFold2[14] or RFDiffusion,[55] a significant part of the human proteome (~22% is IDP or IDR)[56] could not be visualized as crystal structures were not fully resolved. With AI we can predict with reasonable reliability protein structures based on their primary sequence. AI-based structure prediction software, such as RoseTTAFold[57] and AlphaFold,[14] are applied in the development of de novo protein design[55,58] and TF design.[59–61] The Baker lab are pioneers in de novo protein design. Recently, they developed a pipeline for the development of de novo designed IDR binding proteins.[55] AlphaFold on the other hand demonstrated to be a powerful tool as well, regarding IDRs.[17] AlphaFold2 allows for the incorporation of DNA which can be used for the development of artificial TF design. IDRs are extremely versatile and can adopt defined structures when they interact with protein partners.[62] Recently, a fragment scanning approach with AlphaFold2, similar to our work in **Chapter 5**, was successful in predicting IDR conformations.[17]

Using AlphaFold2 we may predict how IDRs interact with proteins. IDRs often interact with short motifs[63] that adopt secondary structures that can be stabilized through strategically placed staples. For regions larger than 60 residues the ReCHEMbinant technique may be applied. As discussed in **Chapter 4**, the ReCHEMbinant pipeline allows for the expression, purification and late-stage modification of proteins that fold upon contact. Depending on structure prediction, α-helices or β-sheets can be stabilized though the incorporation of a covalent constraint.[64,65] For example, we could apply the ReCHEMbinant approach on full length MID2. This should increase MID2 stability and affinity based on our findings in **Chapter 4** and **5**. Examples in the PDB highlight that IDPs can be crystallized when they are stabilized by protein partners (e.g. Myc-MaxDNA complex [51]). As the ReCHEMbinant pipeline produces stabilized structures this could hypothetically be utilized in combination with crystallization studies.

6.4.5 Other strategies to inhibit Myc

We demonstrated that the Myc-Max complex can be inhibited by a peptide identified with AlphaFold2 through an unresolved PPI first identified by Peukert *et al.* in 1997.[66] This fragment-based AI approach may also be suitable to target Myc in its complete disordered region. Myc contains a large, disordered region that with conserved Myc Homology boxes (MB).[67] The interaction between Myc and Max has been widely studied and most Myc inhibiting strategies either try to prevent DNA

binding or Myc-Max formation. For example, Struntz et al. developed a Max-Max stabilizer[68] Another approach would be to target the MBs. MBs regulate correct DNA recognition, PPIs and increase Myc stability.[69,70] There are small molecules identified that inhibit MB-protein interactions that would otherwise stabilize Myc, such as Aurora A.[71] By performing AlphaFold2 predictions with protein partners of Myc and isolated MBs, possible binding modes can be identified that could result into MB-based Myc inhibitors, such as our approach in **Chapter 5**.

Library designs are powerful for the identification of peptide-based NA binders.[72] A variety of library techniques have been used to identify new peptide modalities that target NA, such as phage display and combinatorial libraries.[73] A novel direct evolution technique called phage-assisted continuous evolution (PACE) was developed in 2011.[74] This technique utilizes the constant selection of the bacteriophage while promoting directed evolution regulated by the activity of interest.[74,75] This strategy was successfully implemented in combination with rational design on the structure of ME47.[76] ME47, similar to DuoMYC, is an artificial TF designed to target the E-box sequence.[77] PACE is a powerful technique that allows for quick protein evolution, which could be used to increase DuoMYC affinity to E-box DNA[76] or to repurpose it to target different disease-related NAs.[78] Similarly, it can be utilized to increase selectivity toward Myc when the Mizmetics are used as a template.[79]

Phage display can also be used to screen for macrocyclic peptides[80] or stapled peptides.[81] For example, recently the group of Barniol-Xicota developed a method to utilize phage display to screen for macrocyclic peptides through the incorporation and modification of strategically placed cysteines.[80] We could utilize stapled phage display to screen for stapled Mizmetic- or MB-based peptides that could function as a Myc inhibitor, provided that these peptides interact as an α-helix or β-sheet according to AlphaFold or other AI tools.

Lastly, covalent inhibition is a promising approach to target Myc. Covalent inhibitors, such as EN4,[82] form a covalent bond between reactive residues within the target. These residues often involve cysteines but other amino acids, such as lysine,[83] can be targeted.[84] The Mizmetic alanine analogues discussed in **Chapter 5** may be suitable as a starting point for a covalent inhibitor design. AlphaFold predicts Glu21 in Mizmetic WT in close proximity to Lys392 in Myc. Lysines are often utilized for their reactivity for a covalent inhibitor design.[85,86] The close proximity between Glu21 and Lys392 allows for the incorporation of a reactive warhead within Mizmetic 20. Resembling an earlier described staple protocol for DuoMYC, orthogonal protection groups can be utilized to first staple the Mizmetics on solid support and subsequently attach a warhead on position 21. An initial approach could incorporate Cys(Mmt). The Mmt group can be removed under mild acidic conditions, enabling on solid support stapling. At position 21 a Lys(alloc) could be incorporated. The alloc protection group can be removed after stapling and a reactive warhead can be attached to the amine

group of Lysine. The obtained compounds can be analyzed for covalent attachment using SDS-PAGE. Once a selective inhibitor is obtained, these can be evaluated using our developed native assay to observe Myc-Max inhibition.

6.5 Final note

In this thesis the inhibition of Myc was explored through two different strategies. Two different design strategies were investigated for the development of artificial TFs either with SPPS or recombinant expression. Additionally, in combination with AI, a structure activity relationship study was performed to design a new Myc/Max inhibitor based on an unresolved PPI. This work explored the field of protein design in combination with late-stage peptide modification that allowed for the replacement of a complete dimerization domain by a small covalent constraint. Additionally, this thesis tried to highlight the potential of peptides in the struggle against Myc. Altogether we hope that our work will be beneficial in the future development of artificial TFs, characterization of IDPs and IDRs and the complete field in protein architecture.

6.6 References

[1] F. Crick, "Central Dogma of Molecular Biology" *Nature* **1970**, *227*, 1970.

[2] S. A. Lambert, A. Jolma, L. F. Campitelli, P. K. Das, Y. Yin, M. Albu, X. Chen, J. Taipale, T. R. Hughes, M. T. Weirauch, "The Human Transcription Factors" *Cell* **2018**, *172*, 650–665.

[3] E. Garralda, M. E. Beaulieu, V. Moreno, S. Casacuberta-Serra, S. Martínez-Martín, L. Foradada, G. Alonso, D. Massó-Vallés, S. López-Estévez, T. Jauset, E. Corral de la Fuente, B. Doger, T. Hernández, R. Perez-Lopez, O. Arqués, V. Castillo Cano, J. Morales, J. R. Whitfield, M. Niewel, L. Soucek, E. Calvo, "MYC targeting by OMO-103 in solid tumors: a phase 1 trial" *Nat Med* **2024**, *30*, 762–771.

[4] C. Murre, "Helix–loop–helix proteins and the advent of cellular diversity: 30 years of discovery" *Genes Dev* **2019**, *33*, 6–25.

[5] M. E. Beaulieu, T. Jauset, D. Massó-Vallés, S. Martínez-Martín, P. Rahl, L. Maltais, M. F. Zacarias-Fluck, S. Casacuberta-Serra, E. S. Del Pozo, C. Fiore, L. Foradada, V. C. Cano, M. Sánchez-Hervás, M. Guenther, E. R. Sanz, M. Oteo, C. Tremblay, G. Martín, D. Letourneau, M. Montagne, M. Á. M. Alonso, J. R. Whitfield, P. Lavigne, L. Soucek, "Intrinsic cell-penetrating activity propels omomyc from proof of concept to viable anti-myc therapy" *Sci Transl Med* **2019**, *11*, DOI 10.1126/scitranslmed.aar5012.

[6] H. Jo, N. Meinhardt, Y. Wu, S. Kulkarni, X. Hu, K. E. Low, P. L. Davies, W. F. DeGrado, D. C. Greenbaum, "Development of α-helical calpain probes by mimicking a natural protein-protein interaction." *J Am Chem Soc* **2012**, *134*, 17704–17713.

[7] Y. H. Lau, P. De Andrade, Y. Wu, D. R. Spring, "Peptide stapling techniques based on different macrocyclisation chemistries" *Chem Soc Rev* **2015**, *44*, 91–102.

[8] A. Chandramohan, H. Josien, T. Y. Yuen, R. Duggal, D. Spiegelberg, L. Yan, Y.-C. A. Juang, L. Ge, P. G. Aronica, H. Y. K. Kaan, Y. H. Lim, A. Peier, B. Sherborne, J. Hochman, S. Lin, K. Biswas, M. Nestor, C. S. Verma, D. P. Lane, T. K. Sawyer, R. Garbaccio, B. Henry, S. Kannan, C. J. Brown, C. W. Johannes, A. W. Partridge, "Design-rules for stapled peptides with in vivo activity and their application to Mdm2/X antagonists" *Nat Commun* **2024**, *15*, 489.

[9] O. Harel, M. Jbara, "Chemical Synthesis of Bioactive Proteins" *Angewandte Chemie* **2023**, *135*, DOI 10.1002/ange.202217716.

[10] R. V. Nithun, Y. M. Yao, O. Harel, S. Habiballah, A. Afek, M. Jbara, "Site-Specific Acetylation of the Transcription Factor Protein Max Modulates Its DNA Binding Activity" *ACS Cent Sci* **2024**, *2024*, 1295–1303.

[11] S. Pomplun, M. Jbara, C. K. Schissel, S. Wilson Hawken, A. Boija, C. Li, I. Klein, B. L. Pentelute, "Parallel Automated Flow Synthesis of Covalent Protein Complexes That Can Inhibit MYC-Driven Transcription." *ACS Cent Sci* **2021**, *7*, 1408–1418.

[12] O. Harel, ‖ Ferran Nadal-Bufi, R. V Nithun, Y. M. Yao, A. Afek, M. Vendrell, M. Jbara, "Chemical Engineering of Transcription Factors Uncovered Cell-Permeable μMax Modulators" *J Am Chem Soc* **2025**, *15*, DOI 10.1021/JACS.5C13964.

[13] M. Jbara, S. Pomplun, C. K. Schissel, S. W. Hawken, A. Boija, I. Klein, J. Rodriguez, S. L. Buchwald, B. L. Pentelute, "Engineering Bioactive Dimeric Transcription Factor Analogs via Palladium Rebound Reagents." *J Am Chem Soc* **2021**, *143*, 11788–11798.

[14] J. Jumper, R. Evans, A. Pritzel, T. Green, M. Figurnov, O. Ronneberger, K. Tunyasuvunakool, R. Bates, A. Žídek, A. Potapenko, A. Bridgland, C. Meyer, S. A. A. Kohl, A. J. Ballard, A. Cowie, B. Romera-Paredes, S. Nikolov, R. Jain, J. Adler, T. Back, S. Petersen, D. Reiman, E. Clancy, M. Zielinski, M. Steinegger, M. Pacholska, T. Berghammer, S. Bodenstein, D. Silver, O. Vinyals, A. W. Senior, K. Kavukcuoglu, P. Kohli, D. Hassabis, "Highly accurate protein structure prediction with AlphaFold." *Nature* **2021**, *596*, 583–589.

[15] A. Omidi, M. H. Møller, N. Malhis, J. M. Bui, J. Gsponer, "AlphaFold-Multimer accurately captures interactions and dynamics of intrinsically disordered protein regions." *Proc Natl Acad Sci U S A* **2024**, *121*, e2406407121.

[16] S. Peter, J. Bultinck, K. Myant, L. A. Jaenicke, S. Walz, J. Müller, M. Gmachl, M. Treu, G. Boehmelt, C. P. Ade, W. Schmitz, A. Wiegering, C. Otto, N. Popov, O. Sansom, N. Kraut, M. Eilers, "Tumor cell-specific inhibition of MYC function using small molecule inhib-

itors of the HUWE1 ubiquitin ligase." *EMBO Mol Med* **2014**, *6*, 1525–1541.

[17] H. Bret, J. Gao, D. J. Zea, J. Andreani, R. Guerois, "From interaction networks to interfaces, scanning intrinsically disordered regions using AlphaFold2." *Nat Commun* **2024**, *15*, 597.

[18] C. V. Dang, K. A. O'Donnell, K. I. Zeller, T. Nguyen, R. C. Osthus, F. Li, "The c-Myc target gene network" *Semin Cancer Biol* **2006**, *16*, 253–264.

[19] G. C. C. Koh, S. Boushaki, S. J. Zhao, A. M. Pregnall, F. Sadiyah, C. Badja, Y. Memari, I. Georgakopoulos-Soares, S. Nik-Zainal, "The chemotherapeutic drug CX-5461 is a potent mutagen in cultured human cells" *Nature Genetics 2023 56:1* **2023**, *56*, 23–26.

[20] Z. Deng, D. R. Richardson, "The Myc Family and the Metastasis Suppressor NDRG1: Targeting Key Molecular Interactions with Innovative Therapeutics" *Pharmacol Rev* **2023**, *75*, 1007–1035.

[21] S. K. Madden, A. D. de Araujo, M. Gerhardt, D. P. Fairlie, J. M. Mason, "Taking the Myc out of cancer: toward therapeutic strategies to directly inhibit c-Myc" *Molecular Cancer 2021 20:1* **2021**, *20*, 1–18.

[22] M. Fu, P. J. Blackshear, "RNA-binding proteins in immune regulation: A focus on CCCH zinc finger proteins" *Nat Rev Immunol* **2017**, *17*, 130–143.

[23] T. M. T. Hall, "Multiple modes of RNA recognition by zinc finger proteins" *Curr Opin Struct Biol* **2005**, *15*, 367–373.

[24] D. J. Segal, B. Dreier, R. R. Beerli, C. F. Barbas, "Toward controlling gene expression at will: Selection and design of zinc finger domains recognizing each of the 5′-GNN-3′ DNA target sequences" *Proc Natl Acad Sci U S A* **1999**, *96*, 2758–2763.

[25] B. Dreier, R. P. Fuller, D. J. Segal, C. V. Lund, P. Blancafort, A. Huber, B. Koksch, C. F. Barbas, "Development of zinc finger domains for recognition of the 5′-CNN-3′ family DNA sequences and their use in the construction of artificial transcription factors" *Journal of Biological Chemistry* **2005**, *280*, 35588–35597.

[26] B. Dreier, R. R. Beerli, D. J. Segal, J. D. Flippin, C. F. Barbas, "Development of Zinc Finger Domains for Recognition of the 5′-ANN-3′ Family of DNA Sequences and Their Use in the Construction of Artificial Transcription Factors" *Journal of Biological Chemistry* **2001**, *276*, 29466–29478.

[27] B. Dreier, D. J. Segal, C. F. Barbas, "Insights into the molecular recognition of the 5′-GNN-3′ family of DNA sequences by zinc finger domains" *J Mol Biol* **2000**, *303*, 489–502.

[28] P. B. Dervan, B. S. Edelson, "Recognition of the DNA minor groove by pyrrole-imidazole polyamides" *Curr Opin Struct Biol* **2003**, *13*, 284–299.

[29] E. A. Heiderscheit, A. Eguchi, M. C. Spurgat, A. Z. Ansari, "Reprogramming cell fate with artificial transcription factors" *FEBS Lett* **2018**, *592*, 888–900.

[30] A. Eguchi, M. J. Wleklinski, M. C. Spurgat, E. A. Heiderscheit, A. S. Kropornicka, C. K. Vu, D. Bhimsaria, S. A. Swanson, R. Stewart, P. Ramanathan, T. J. Kamp, I. Slukvin, J. A. Thomson, J. R. Dutton, A. Z. Ansari, "Reprogramming cell fate with a genome-scale library of artificial transcription factors" *Proc Natl Acad Sci U S A* **2016**, *113*, E8257–E8266.

[31] T. E. Speltz, Z. Qiao, C. S. Swenson, X. Shangguan, J. S. Coukos, C. W. Lee, D. M. Thomas, J. Santana, S. W. Fanning, G. L. Greene, R. E. Moellering, "Targeting MYC with modular synthetic transcriptional repressors derived from bHLH DNA-binding domains" *Nature Biotechnology 2022* **2022**, *41*, 1–11.

[32] Z. Qiao, L. C. Nguyen, D. Yang, C. Dann, D. M. Thomas, M. Henn, A. Valdespino, C. S. Swenson, S. A. Oakes, M. R. Rosner, R. E. Moellering, "Direct inhibition of tumor hypoxia response with synthetic transcriptional repressors." *Nat Chem Biol* **2025**, *21*, 247–255.

[33] L. C. Lustig, D. Dingar, W. B. Tu, C. Lourenco, M. Kalkat, I. Inamoto, R. Ponzielli, W. C. W. Chan, J. A. Shin, L. Z. Penn, "Inhibiting MYC binding to the E-box DNA motif by ME47 decreases tumour xenograft growth" *Oncogene 2017 36:49* **2017**, *36*, 6830–6837.

[34] M. J. Demma, M. J. Hohn, A. Sun, C. Mapelli, B. Hall, A. Walji, J. O'Neil, "Inhibition of Myc transcriptional activity by a mini-protein based upon Mxd1" *FEBS Lett* **2020**, *594*, 1467–1476.

[35] R. V. Nithun, Y. M. Yao, X. Lin, S. Habiballah, A. Afek, M. Jbara, "Deciphering the Role of the Ser-Phosphor-

ylation Pattern on the DNA-Binding Activity of Max Transcription Factor Using Chemical Protein Synthesis" *Angewandte Chemie International Edition* **2023**, *62*, e202310913.

[36] H. Masui, S. Fuse, "Recent Advances in the Solid- and Solution-Phase Synthesis of Peptides and Proteins Using Microflow Technology" *Org Process Res Dev* **2022**, DOI 10.1021/acs.oprd.2c00074.

[37] R. Calo-Lapido, C. Penas, A. Jiménez-Balsa, M. E. Vázquez, J. L. Mascareñas, "A chemical approach for the synthesis of the DNA-binding domain of the oncoprotein MYC" *Org Biomol Chem* **2019**, *17*, 6748–6752.

[38] Z. Z. Brown, C. Mapelli, I. Farasat, A. V Shoultz, S. A. Johnson, F. Orvieto, A. Santoprete, E. Bianchi, A. B. McCracken, K. Chen, X. Zhu, M. J. Demma, B. M. Lacey, K. A. Canada, R. M. Garbaccio, J. O'Neil, A. Walji, "Multiple Synthetic Routes to the Mini-Protein Omomyc and Coiled-Coil Domain Truncations." *J Org Chem* **2020**, *85*, 1466–1475.

[39] P. E. Dawson, T. W. Muir, I. Clark-Lewis, S. B. H. Kent, "Synthesis of Proteins by Native Chemical Ligation" *Science (1979)* **1994**, *266*, 776–779.

[40] M. Paradís-Bas, J. Tulla-Puche, F. Albericio, "The road to the synthesis of 'difficult peptides'" *Chem Soc Rev* **2016**, *45*, 631–654.

[41] A. El-Faham, F. Albericio, "Peptide Coupling Reagents, More than a Letter Soup" *Chem Rev* **2011**, *111*, 6557–6602.

[42] H. Bürgisser, E. T. Williams, A. Jeandin, R. Lescure, A. Premanand, S. Wang, N. Hartrampf, "A Versatile 'Synthesis Tag' (SynTag) for the Chemical Synthesis of Aggregating Peptides and Proteins" *J Am Chem Soc* **2024**, *146*, 34887–34899.

[43] S. H. Shahravan, X. Qu, I.-S. Chan, J. A. Shin, "Enhancing the specificity of the enterokinase cleavage reaction to promote efficient cleavage of a fusion tag" **2008**, DOI 10.1016/j.pep.2008.02.015.

[44] A. J. Callahan, A. Rondon, R. M. Reja, L. L. Salazar, S. Gandhesiri, J. Rodriguez, A. Loas, B. L. Pentelute, "Same Day Access to Folded Synthetic Proteins" *J Am Chem Soc* **2024**, *146*, 28696–28706.

[45] T. Gaj, J. Guo, Y. Kato, S. J. Sirk, C. F. Barbas, "Targeted gene knockout by direct delivery of zinc-finger nuclease proteins" *Nat Methods* **2012**, *9*, 805–807.

[46] A. F. L. Schneider, M. Kithil, M. C. Cardoso, M. Lehmann, C. P. R. Hackenberger, "Cellular uptake of large biomolecules enabled by cell-surface-reactive cell-penetrating peptide additives" *Nat Chem* **2021**, *13*, 530–539.

[47] E. Wang, A. Sorolla, P. T. Cunningham, H. M. Bogdawa, S. Beck, E. Golden, R. E. Dewhurst, L. Florez, M. N. Cruickshank, K. Hoffmann, R. M. Hopkins, J. Kim, A. J. Woo, P. M. Watt, P. Blancafort, "Tumor penetrating peptides inhibiting MYC as a potent targeted therapeutic strategy for triple-negative breast cancers" *Oncogene* **2018**, *38*, 140–150.

[48] J. Rodríguez, S. Learte-Aymamí, J. Mosquera, G. Celaya, D. Rodríguez-Larrea, M. E. Vázquez, J. L. Mascareñas, "DNA-binding miniproteins based on zinc fingers. Assessment of the interaction using nanopores" *Chem Sci* **2018**, *9*, 4118–4123.

[49] J. Rodríguez, J. Mosquera, R. García-Fandiño, M. E. Vázquez, J. L. Mascareñas, "A designed DNA binding motif that recognizes extended sites and spans two adjacent major grooves" *Chem Sci* **2016**, *7*, 3298–3303.

[50] L. A. Jung, A. Gebhardt, W. Koelmel, C. P. Ade, S. Walz, J. Kuper, B. Von Eyss, S. Letschert, C. Redel, L. D'Artista, A. Biankin, L. Zender, M. Sauer, E. Wolf, G. Evan, C. Kisker, M. Eilers, "OmoMYC blunts promoter invasion by oncogenic MYC to inhibit gene expression characteristic of MYC-dependent tumors" *Oncogene 2017 36:14* **2016**, *36*, 1911–1924.

[51] S. K. Nair, S. K. Burley, "X-ray structures of Myc-Max and Mad-Max recognizing DNA. Molecular bases of regulation by proto-oncogenic transcription factors." *Cell* **2003**, *112*, 193–205.

[52] X. Lin, O. Harel, M. Jbara, "Chemical Engineering of Artificial Transcription Factors by Orthogonal Palladium(II)-Mediated S-Arylation Reactions" *Angewandte Chemie International Edition* **2024**, *63*, e202317511.

[53] P. Timmerman, W. C. Puijk, R. H. Meloen, "Functional reconstruction and synthetic mimicry of a conformational epitope using CLIPS technology." *J Mol Recognit* **2007**, *20*, 283–299.

6

[54] P. Timmerman, J. Beld, W. C. Puijk, R. H. Meloen, "Rapid and quantitative cyclization of multiple peptide loops onto synthetic scaffolds for structural mimicry of protein surfaces." *Chembiochem* **2005**, *6*, 821–824.

[55] K. Wu, H. Jiang, D. R. Hicks, C. Liu, E. Muratspahić, T. A. Ramelot, Y. Liu, K. McNally, S. Kenny, A. Mihut, A. Gaur, B. Coventry, W. Chen, A. K. Bera, A. Kang, S. Gerben, M. Y.-L. Lamb, A. Murray, X. Li, M. A. Kennedy, W. Yang, Z. Song, G. Schober, S. M. Brierley, J. O'Neill, M. H. Gelb, G. T. Montelione, E. Derivery, D. Baker, **2024**, DOI: 10.1101/2024.07.15.603480.

[56] K. Kosoglu, Z. Aydin, N. Tuncbag, A. Gursoy, O. Keskin, "Structural coverage of the human interactome." *Brief Bioinform* **2023**, *25*, 1–11.

[57] M. Baek, F. DiMaio, I. Anishchenko, J. Dauparas, S. Ovchinnikov, G. R. Lee, J. Wang, Q. Cong, L. N. Kinch, R. Dustin Schaeffer, C. Millán, H. Park, C. Adams, C. R. Glassman, A. DeGiovanni, J. H. Pereira, A. V. Rodrigues, A. A. Van Dijk, A. C. Ebrecht, D. J. Opperman, T. Sagmeister, C. Buhlheller, T. Pavkov-Keller, M. K. Rathinaswamy, U. Dalwadi, C. K. Yip, J. E. Burke, K. Christopher Garcia, N. V. Grishin, P. D. Adams, R. J. Read, D. Baker, "Accurate prediction of protein structures and interactions using a three-track neural network" *Science (1979)* **2021**, *373*, 871–876.

[58] J. L. Watson, D. Juergens, N. R. Bennett, B. L. Trippe, J. Yim, H. E. Eisenach, W. Ahern, A. J. Borst, R. J. Ragotte, L. F. Milles, B. I. M. Wicky, N. Hanikel, S. J. Pellock, A. Courbet, W. Sheffler, J. Wang, P. Venkatesh, I. Sappington, S. V. Torres, A. Lauko, V. De Bortoli, E. Mathieu, S. Ovchinnikov, R. Barzilay, T. S. Jaakkola, F. DiMaio, M. Baek, D. Baker, "De novo design of protein structure and function with RFdiffusion" *Nature 2023 620:7976* **2023**, *620*, 1089–1100.

[59] L. Ledesma-Dominguez, E. Carbajal-Degante, G. Moreno-Hagelsieb, E. Perez-Rueda, "DeepReg: a deep learning hybrid model for predicting transcription factors in eukaryotic and prokaryotic genomes" *Sci Rep* **2024**, *14*, 1–11.

[60] G. B. Kim, Y. Gao, B. O. Palsson, S. Y. Lee, "DeepTFactor: A deep learning-based tool for the prediction of transcription factors" *Proc Natl Acad Sci U S A* **2021**, *118*, e2021171118.

[61] J. Liebold, F. Neuhaus, J. Geiser, S. Kurtz, J. Baumbach, K. Newaz, "Transcription factor prediction using protein 3D secondary structures" *Bioinformatics* **2024**, *41*, DOI 10.1093/BIOINFORMATICS/BTAE762.

[62] T. Orand, M. R. Jensen, "Binding mechanisms of intrinsically disordered proteins: Insights from experimental studies and structural predictions" *Curr Opin Struct Biol* **2025**, *90*, 102958.

[63] K. Van Roey, B. Uyar, R. J. Weatheritt, H. Dinkel, M. Seiler, A. Budd, T. J. Gibson, N. E. Davey, "Short linear motifs: Ubiquitous and functionally diverse protein interaction modules directing cell regulation" *Chem Rev* **2014**, *114*, 6733–6778.

[64] C. S. Swenson, G. Mandava, D. M. Thomas, R. E. Moellering, "Tackling Undruggable Targets with Designer Peptidomimetics and Synthetic Biologics" *Chem Rev* **2024**, *124*, 13020–13093.

[65] A. Dangi, I. J. Angera, J. R. Del Valle, "Intrastrand Peptide Staples That Promote β-Sheet Folding, Self-Assembly, and Amyloid Seeding" *J Am Chem Soc* **2025**, *147*, 29930–29938.

[66] K. Peukert, P. Staller, A. Schneider, G. Carmichael, F. Hänel, M. Eilers, "An alternative pathway for gene regulation by Myc." *EMBO J* **1997**, *16*, 5672–5686.

[67] Y. Zhou, X. Gao, M. Yuan, B. Yang, Q. He, J. Cao, "Targeting Myc Interacting Proteins as a Winding Path in Cancer Therapy" *Front Pharmacol* **2021**, *12*, 748852.

[68] N. B. Struntz, A. Chen, A. Deutzmann, R. M. Wilson, E. Stefan, H. L. Evans, M. A. Ramirez, T. Liang, F. Caballero, M. H. E. Wildschut, D. V Neel, D. B. Freeman, M. S. Pop, M. McConkey, S. Muller, B. H. Curtin, H. Tseng, K. R. Frombach, V. L. Butty, S. S. Levine, C. Feau, S. Elmiligy, J. A. Hong, T. A. Lewis, A. Vetere, P. A. Clemons, S. E. Malstrom, B. L. Ebert, C. Y. Lin, D. W. Felsher, A. N. Koehler, "Stabilization of the Max Homodimer with a Small Molecule Attenuates Myc-Driven Transcription." *Cell Chem Biol* **2019**, *26*, 711-723.e14.

[69] X. X. Sun, Y. Li, R. C. Sears, M. S. Dai, "Targeting the MYC Ubiquitination-Proteasome Degradation Pathway for Cancer Therapy" *Front Oncol* **2021**, *11*, 679445.

[70] S. Schütz, C. Bergsdorf, S. Hänni-Holzinger, A. Lingel, M. Renatus, A. D. Gossert, W. Jahnke, "Intrinsically Disordered Regions in the Transcription Factor MYC:MAX Modulate DNA Binding via Intramolecular Interactions" *Biochemistry* **2024**, *63*, 511.

[71] Y. Li, X. Li, J. Pu, Q. Yang, H. Guan, M. Ji, B. Shi, M. Chen, P. Hou, "C-Myc Is a Major Determinant for Antitumor Activity of Aurora A Kinase Inhibitor MLN8237 in Thyroid Cancer" *Thyroid* **2018**, *28*, 1642–1654.

[72] J. M. Mata, E. van der Nol, S. J. Pomplun, "Advances in Ultrahigh Throughput Hit Discovery with Tandem Mass Spectrometry Encoded Libraries" *J Am Chem Soc* **2023**, *145*, 19129–19139.

[73] S. Pomplun, Z. P. Gates, G. Zhang, A. J. Quartararo, B. L. Pentelute, "Discovery of Nucleic Acid Binding Molecules from Combinatorial Biohybrid Nucleobase Peptide Libraries" *J Am Chem Soc* **2020**, *142*, 19642–19651.

[74] K. M. Esvelt, J. C. Carlson, D. R. Liu, "A system for the continuous directed evolution of biomolecules" *Nature* **2011**, *472*, 499–503.

[75] S. M. Miller, T. Wang, D. R. Liu, "Phage-assisted continuous and non-continuous evolution" *Nat Protoc* **2020**, *15*, 4101–4127.

[76] I. Inamoto, I. Sheoran, S. C. Popa, M. Hussain, J. A. Shin, "Combining Rational Design and Continuous Evolution on Minimalist Proteins That Target the E-box DNA Site." *ACS Chem Biol* **2021**, *16*, 35–44.

[77] J. Xu, G. Chen, A. T. De Jong, S. H. Shahravan, J. A. Shin, "Max-E47, a designed minimalist protein that targets the E-box DNA site in vivo and in vitro" *J Am Chem Soc* **2009**, *131*, 7839–7848.

[78] M. S. Packer, H. A. Rees, D. R. Liu, "Phage-assisted continuous evolution of proteases with altered substrate specificity" **n.d.**, DOI 10.1038/s41467-017-01055-9.

[79] A. H. Badran, V. M. Guzov, Q. Huai, M. M. Kemp, P. Vishwanath, W. Kain, A. M. Nance, A. Evdokimov, F. Moshiri, K. H. Turner, P. Wang, T. Malvar, D. R. Liu, "Continuous evolution of Bacillus thuringiensis toxins overcomes insect resistance" *Nature* **2016**, *533*, 58–63.

[80] H. Xiang, L. Bai, X. Zhang, T. Dan, P. Cheng, X. Yang, H. Ai, K. Li, X. Lei, "A facile strategy for the construction of a phage display cyclic peptide library for the selection of functional macrocycles" *Chem Sci* **2024**, *15*, 11847–11855.

[81] F. F. Faucher, K. Kristýna, B. Ková, S. Lovell, M. Bertolini, J. Herrero-Bourdieu, E. D. Cosco, M. Bogyo, M. Barniol-Xicota, "Macrocyclic Phage Display for Identification of Selective Protease Substrates" *J Am Chem Soc* **2025**, DOI 10.1021/JACS.5C04424.

[82] L. Boike, A. G. Cioffi, F. C. Majewski, J. Co, N. J. Henning, M. D. Jones, G. Liu, J. M. McKenna, J. A. Tallarico, M. Schirle, D. K. Nomura, "Discovery of a Functional Covalent Ligand Targeting an Intrinsically Disordered Cysteine within MYC." *Cell Chem Biol* **2021**, *28*, 4-13.e17.

[83] F. M. Paulussen, G. K. Schouten, C. Moertl, J. Verheul, I. Hoekstra, G. M. Koningstein, G. H. Hutchins, A. Alkir, R. A. Luirink, D. P. Geerke, P. Van Ulsen, T. Den Blaauwen, J. Luirink, T. N. Grossmann, "Covalent Proteomimetic Inhibitor of the Bacterial FtsQB Divisome Complex" *J Am Chem Soc* **2022**, *144*, 15303–15313.

[84] L. Petri, P. Ábrányi-Balogh, D. Vagrys, T. Imre, N. Varró, I. Mándity, A. Rácz, L. Wittner, K. Tóth, E. Z. Tóth, T. Juhász, B. Davis, G. M. Keserű, "A covalent strategy to target intrinsically disordered proteins: Discovery of novel tau aggregation inhibitors" *Eur J Med Chem* **2022**, *231*, 114163.

[85] M. Gehringer, S. A. Laufer, "Emerging and Re-Emerging Warheads for Targeted Covalent Inhibitors: Applications in Medicinal Chemistry and Chemical Biology" *J Med Chem* **2018**, *62*, 5673–5724.

[86] J. Pettinger, K. Jones, M. D. Cheeseman, "Lysine-Targeting Covalent Inhibitors" *Angewandte Chemie International Edition* **2017**, *56*, 15200–15209.

Appendix A

List of abbreviations

A	Adenine
AI	Artificial intelligence
ARM	Arginine-righ motif
ASO	Antisense oligonucleotide
AUC	Area under the curve
b	basic region
bHL-HL-LZ	basic helix-loop-helix leucine zipper
BIV	Bovine immunodeficiency virus
BLI	Biolayer interferometry
BLM	Bloom syndrome protein helicase domain
BRD4	Bromodomain protein 4
bZIP	basic Zipper
C	Cytosine
CD	Circular Dichroism
cDNA	Complementary DNA
cpp	cell-penetrating peptide
CTG	Cell titer glow
CV	Colume volume
DBD	DNA binding domain
DCM	Dichloromethane
DIPEA	N,N-Diisopropylethylamine
DMF	N,N-Dimethylformamide
DMSO	Dimethylsulfoxide
DNA	Deoxynucleic Acid
DPBS	Dulbecco's phosphate buffered saline
dsDNA	Double stranded DNA
dsRNA	Double stranded RNA
E-box	Enhancer box
EDTA	Ethylenediaminetetraacetic acid
EK	Enterokinase
EMSA	Electrophoretic mobility shift assay
EPO	Erythropoietin
FITC	Fluorescein isothiocyanate
Fmoc	Fluorenylmethyloxycarbonyl
G	Guanine
G4	G-quadruplex
Gly	Glysine
GSEA	gene set enrichment analysis
HATU	O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate
HCAC3	Histone Deacetylase 3
HCV	Hepatitic C virus
HIV-1	Human Immunodeficiency Virus type-1
HLH	Helix-loop-helix
HLHLD	Helix-loophelix-domain
HPLC	High-pressure Liquid chromatography
HTP	Helix-threading peptide
IDP	Intrinsically disordered protein
IDR	Intrinsically disordered region
IRES	Internal ribosome entry site
IS	Internal standard
LC-MS	Liquic Chromatography Mass spectroscopy
LTR	Long terminal repeat
Lys	Lysine
LZ	Leucine Zipper
Max	Myc-associated factor X
MB	Myc box
MD	Molecular Dynamics
MeCN	Acetonitrile
Mga	Max gene-associated
MID1/2	Myc interacting Domain 1/2

miR-21	miRNA-21
miRNA	Micro RNA
Miz-1	Myc interacting Zinc Finger 1
Mlx	Max-like protein X
MNT	Max-binding protein
mRNA	Messenger RNA
MSA	Multiple sequence alignment
MSigDB	Molecular Signatures Database
MW	Molecular weigth
NA	Nucleic acid
NCL	Native chemical ligation
ncRNA	Non-coding RNA
NEAA	Non-essential amino acids
NLS	Nuclear localization sequence
OBTC	One-bead-two-compounds
Omo-103	Omomyc-103
PACE	Phage-assisted continuous evolution
pal-RNA	Palyndromic RNA
PBS	Phosphate-Buffered Saline
PG	Protection group
PNA	Peptide nucleic acid
PNAI	Protein-nucleic acid interaction
Pol II	RNA polymerase II
POZ	Poxvirus and Zinc finger
PPI	Protein-protein interaction
pre-miRNA	precursor miRNA
pri-miRNA	Primary miRNA
PROTAC	Proteolysis Targeting Chimera
P-TEFb	Elongation factor b
QTOF	Quadrupole Time-of-Flight
RBD	RNA binding domain
RBS	Ribosome binding site
RISC	RNA-induced silencing complex
RMSD	Root-mean-square-deviation
RMSF	Root-mean-square-fluctuation
RNA	Ribonucleic Acid
RP-FC	Reversed phase flash chromatograpy
RRE	Rev Response Element
RRM	RNA recognition motif
RT	Room temperature
SEC	Superelongation complex
SPPS	Solid phase peptide synthesis
ssRNA	Single stranded DNA
T	Thymine
TAD	Transactivation domain
TALEN	Transcription activator-like effecotr nuclease
TAR	Transactivation response
TAV2b	*Tomato Aspermy virus 2b*
TBP	TAR-binding-protein
TCEP	Tris(2-carboxyethyl)phosphine hydrochloride
TF	Transcription factor
TFA	Trifluoroacteic acid
TFE	Trifluorethanol
TIC	Total-ion chromatogram
tRNA	Transfer RNA
TRRAP	Transactivation/Transformation-Domain Associated Protein
UTR	Untranslated region
XIC	Extracted-ion chromatogram
ZF	Zinc Finger
γ-AA	γ-substituted-N-acylated-N-aminoethylamino acids