4. Abstrakt

One of the major challenges in contemporary medicine is the accurate diagnosis, effective treatment, and prevention of autoimmune disorders. A promising therapeutic approach involves immunotherapy that targets immune checkpoints (ICPs). These molecules control the strength of the immune response by delivering activating or inhibitory signals to immune cells, thereby ensuring the maintenance of physiological balance.

In this study, I focused on the herpesvirus entry mediator (HVEM) protein and its ligand, LIGHT, both of which belong to the group of immune checkpoint–stimulating molecules. These proteins are members of the tumor necrosis factor superfamily, and their interaction triggers signaling pathways that lead to, among other effects, the activation of T cells, natural killer (NK) cells, and the maturation of dendritic cells.

Disturbances in the HVEM/LIGHT signaling pathway can result in excessive immune activation, contributing to the development of autoimmune diseases such as inflammatory bowel disease and rheumatoid arthritis. Therefore, blocking the interaction between HVEM and LIGHT using specific inhibitors may represent a viable therapeutic strategy. Such inhibitors could also be beneficial in transplantation medicine, where controlled immunosuppression is necessary to prevent graft rejection. Their application may help reduce or prevent complications such as graft-versus-host disease and host-versus-graft responses.

Recent advances in chemoinformatics have significantly accelerated drug discovery processes. In particular, computer-assisted design of inhibitors, using advanced computational tools, enables rapid screening and evaluation of numerous candidate molecules. This approach allows for the preliminary selection of promising compounds, minimizing the need for time-consuming experimental work. Integrating computational design with chemical synthesis and experimental validation represents an efficient strategy for developing targeted inhibitors.

In my research, I concentrated on designing peptide-based inhibitors that disrupt the formation of the HVEM/LIGHT complex. These peptides were derived from specific fragments of the HVEM and LIGHT proteins in terms of both sequence and structure. Initially, I performed a detailed analysis of the HVEM/LIGHT complex using molecular dynamics (MD) simulations to identify key amino acid residues involved in binding.

Based on these findings, I designed a series of peptide candidates and further evaluated their binding affinity through additional MD simulations. To better understand the interactions stabilizing the protein–peptide complexes, I carried out binding energy decomposition at the level of individual residues using the MM-GBSA method. For the most promising peptides, I proposed specific amino acid substitutions that could potentially weaken binding interactions. Additional analyses, including steered molecular dynamics simulations and thermodynamic parameter evaluation, provided further insight into the nature of protein–peptide interactions.

Subsequently, selected peptides were synthesized and subjected to experimental validation. Two main groups of peptides were obtained: (1) those derived from the HVEM protein sequence and (2) those based on the LIGHT protein sequence. Their binding affinity was assessed using microcapillary thermophoresis, while their inhibitory activity against the HVEM/LIGHT interaction was evaluated using ELISA assays and cellular models.

The cellular experiments were conducted during my internship at the Institute of Immunology at the Medical University of Vienna. Among the HVEM-derived peptides, CRD2(39–73)e and CRD2e_K54E showed the strongest binding to LIGHT and the highest inhibitory activity. In the case of LIGHT-based peptides, light3 and its analogue light3_E178L exhibited the most favorable properties.

These findings suggest that the identified peptides may serve as promising starting points for further optimization. However, additional studies are necessary to fully evaluate their immunomodulatory effects and therapeutic applicability.