

## **Final report on the DZIF project**

Project number: TTU 07.842  
Title of the project: Establishment and validation of a screening assay  
for pan-herpes antiviral candidates

Individual project

### **Project partner and project duration**

<u>Projekt Coordination</u>	<u>Start of term</u>	<u>End of term</u>
Medizinische Hochschule Hannover	01.07.2024	- 31.01.2025
<u>Project partner</u>	<u>Start of term</u>	<u>End of term</u>

## Chapter 1: Short report (will be published)

Please summarize the project in a generally understandable form in this Chapter.

### a) Original task and scientific/technical status built on

Please indicate the original task/objective. Add any deviations that occurred in the course of the project. Information can be taken from Chapter 4 "Project objectives" of the current project description (VHB) (no reference please).

The project aimed to identify conserved virus-virus protein-protein interactions as novel targets for pan-herpesviral inhibitor development. The specific objectives were: (1) to comprehensively predict virus-virus protein-protein interactions for HCMV and KSHV using an in-silico screening pipeline based on AlphaFold Multimer; (2) to optimize and standardize an all-in-vitro assay for rapid testing of predicted protein-protein interactions and critical interface residues; and (3) to analyze predicted interaction networks to identify conserved molecular interfaces suitable as targets for pan-herpesviral inhibitors

### b) Course of the project

Please provide information on the practical and temporal implementation of the project. If applicable, information can be taken from Chapter 6 "Work program" of the VHB (no references or results, please). Please add any adjustments/changes to the original work program, additional work packages, adjusted study starts or similar.

The project was carried out according to the proposed work packages. In Work Package 1, a comprehensive prediction of virus-virus protein-protein interactions for HCMV and KSHV was performed using AlphaFold Multimer. Work Package 2 focused on the optimization and standardization of an all-in-vitro assay for rapid testing of predicted interactions and their critical interface residues. In Work Package 3, the predicted interaction networks were analyzed to identify conserved molecular interfaces as potential targets for pan-herpesviral inhibitor development. All planned work packages were successfully completed within the intended project duration. No significant changes to the work plan, additional work packages, or delays occurred.

### c) Main results and cooperation with other research institutions, if applicable

Please provide a short, generally understandable summary of the main results. If applicable, also mention cooperation with other research institutions.

This project aimed to identify conserved protein-protein interactions within human herpesviruses as novel targets for pan-herpesviral inhibitor development. To achieve this, we first performed a comprehensive in-silico screening of potential virus-virus protein-protein interactions for human cytomegalovirus (HCMV) and Kaposi's sarcoma-associated herpesvirus (KSHV) using AlphaFold Multimer. This allowed us to predict extensive interaction networks and define conserved molecular interfaces (which are openly accessible at [www.herpesfolds.org/herpesppis](http://www.herpesfolds.org/herpesppis)).

In the second step, we optimized and standardized a fully in-vitro assay for the rapid validation of these predicted interactions. The newly established assay enables efficient testing of protein-protein interactions and their critical interface residues without the need for classical cloning and cell-based expression systems, significantly accelerating the validation process.

We were able to verify the predicted conserved interactions of the terminase subunits, an essential complex for herpesviral genome packaging. These findings confirm the accuracy of our in-silico predictions and highlight conserved viral structures that will serve as potential targets for future antiviral strategies.

## Chapter 2: Detailed description (will be published)

Please provide detailed information in Chapter 2. In subchapters 2a) - 2c), detailed information should always be presented in comparison to the current project description (VHB).

### a) Detailed presentation of the results achieved

The work carried out as part of the project must be described in detail here, particularly in comparison with the current VHB. The individual results achieved and the use of funding (with regard to content, no numbers necessary) should be comprehensible. Please structure the presentation of the results according to the work packages defined in chapter 6 of the VHB. Please also explain if there have been any changes in the objectives during the course of the project or if the objectives were not achieved during the project duration.

The project work was structured into three work packages (WP1–WP3) as defined in the current VHB. All planned objectives were fully achieved within the project duration, and no changes to the goals were necessary.

#### **Work Package 1: Comprehensive PPI Network Mapping**

In WP1, the aim was to predict all possible virus-virus protein-protein interactions (PPIs) for HCMV and KSHV using AlphaFold Multimer and a stringent scoring pipeline. This work was completed as planned. The resulting predicted interaction networks have been compiled and made publicly available through a dedicated online platform (available at [www.herpesfolds.org/herpesppis](http://www.herpesfolds.org/herpesppis)). This openly accessible resource ensures transparency and supports further scientific work by the research community.

#### **Work Package 2: Validation and Standardization of In-Vitro PPI Assay**

WP2 aimed to optimize and standardize the in-vitro assay to allow rapid experimental testing of the predicted interactions. The assay was successfully refined, achieving a higher sensitivity and dynamic range through optimization of key parameters such as wash conditions, protein concentrations, and reaction buffers. A detailed, standardized protocol for the optimized in-vitro assay was finalized and is attached to this report.

#### **Work Package 3: Identification of First Pan-Herpesviral Inhibitor Targets**

In WP3, predicted conserved molecular interfaces were analyzed to prioritize potential pan-herpesviral targets. Using the optimized assay from WP2, several predicted PPIs were experimentally validated, with a special focus on the herpesviral terminase subunits, whose interactions were successfully confirmed. These validated interactions are summarized in an attached table.

## b) Milestones and Deliverables

Please list all milestones and deliverables of the current VHB. Please select the status in each case: In the final report, the milestones and deliverables either have the status "completed" or "not achievable". Milestones/deliverables that could no longer be fulfilled within the project duration must be indicated as "not achievable".

### Milestones

No.	Title	Work package	Institution	Date as per project description	Corrected date	Status	Comment/reason
1	AlphaFold Multimer Predictions Completed	1	MHH	31.12.2024	n/a	completed	
2	In-Vitro Assay Optimization Completed	2	MHH	30.11.2024	n/a	completed	
3	Initial PPI validation	3	MHH	01.12.2024	n/a	completed	

### Deliverables

No.	Title	Work package	Institution	Date as per project description	Corrected date	Status	Comment/reason
1	Predicted PPI Network Report	1	MHH	31.12.2024	n/a	completed	<a href="http://www.herpesfolds.org/herpesppis">www.herpesfolds.org/herpesppis</a>
2	Standardized In-Vitro Assay Protocol	2	MHH	30.11.2024	n/a	completed	
3	List of Validated PPIs	3	MHH	31.01.2025	n/a	completed	

### c) Most important items of the financial report

Please refer to the entire duration of the project. Here, key words or enumerations are sufficient, coherent text and concrete numbers (in EUR) are not necessary.

Please note that the information in this chapter should correspond to the information in the numerical proof (“Beleglisten”).

#### Personnel costs

In the interests of data minimization, it is not necessary to provide people's real names. Please use function descriptions including classification/pay level, job scope and duration and, if available, personnel numbers (e.g. employment of technical staff 1, E9/level 3, 50%, three years (01/2020-12/2022)).

**Use:** none

**Circumstances:** none

#### Consumables (also contracts)

Please use general terms, e.g. mouse husbandry, antibodies, NGS, chemicals or similar. Please also indicate the awarding of contracts here.

**Use:** For the establishment and execution of the in-vitro protein-protein interaction screen, several consumables were essential. The NEBuilder HiFi DNA Assembly Kit enabled fast and accurate generation of DNA constructs, providing the basis for the production of viral proteins. Rolling Circle Amplification (RCA) kits were subsequently used to amplify these DNA templates in sufficient quantity for protein expression. The TXTL Cell-Free Expression system, which represented the main cost item, enabled the rapid and efficient production of viral proteins directly from the DNA templates. Only through the use of the TXTL system was it possible to verify predicted protein-protein interactions within a few days and entirely without classical cloning and cell-based expression, which would otherwise have taken weeks. In addition, EM grids were used for electron microscopy based quality control of the expressed proteins.

**Circumstances:** The consumables purchased were indispensable for achieving the project goals within the planned timeframe. The NEBuilder HiFi and RCA kits enabled rapid preparation of high-quality DNA substrates, a critical step for high-throughput expression workflows. The TXTL system was essential to produce functional viral proteins quickly and reproducibly, making it possible to perform interaction assays entirely in vitro and validate predicted interactions within a few days. Without these specialized consumables, it would not have been feasible to implement the planned rapid validation pipeline or to reach the project's milestones successfully.

#### Investment funds

Please check the approved equipment list, if applicable.

**Use:** none

**Circumstances:** none

## Travel expenses

E.g. meetings with project partners, DZIF annual meetings, TTU/site meetings, scientific symposia.

**Use:** none

**Circumstances:** none

## d) Necessity and adequacy of the work done

Please explain whether the use of resources was appropriate and necessary for the work performed or the results achieved (or whether the objective would have been achieved without the funding).

The procurement of consumables, particularly the NEBuilder HiFi DNA Assembly Kit, Rolling Circle Amplification kits, and the TXTL Cell-Free Expression system, was essential for the timely establishment of the in-vitro validation platform. The specific methodologies enabled the rapid and efficient production of DNA templates and proteins, which formed the basis for the verification of predicted protein-protein interactions within the available timeframe. The project objectives, in particular the high-throughput experimental validation of predicted interactions, could not have been achieved without the targeted use of these consumables. Conventional expression systems would not have allowed for the necessary speed and flexibility. Thus, the funding provided was indispensable for the implementation of the project and the achievement of the defined milestones and deliverables.

## e) Prospective benefit of the project, in particular the usability of the results according to the updated exploitation plan

Please also consider specific plans for the near future (e.g., use of results in follow-up projects/further development, who needs the result).

The project has generated key resources that provide a strong basis for further scientific and translational activities. The publicly available virus-virus protein-protein interaction networks (published at [herpesfolds.org/herpesppis](https://herpesfolds.org/herpesppis)) will serve the scientific community as a valuable reference for understanding conserved molecular mechanisms across human herpesviruses. The standardized in-vitro assay protocol enables the rapid and reproducible validation of additional predicted interactions and can be adapted for other viral families. In the near future, the validated interactions— particularly the conserved interfaces between terminase subunits — will be further investigated in follow-up projects. These projects aim to develop small-molecule inhibitors targeting these interfaces, with the long-term goal of generating broad-spectrum herpesvirus therapeutics. The results will thus be directly utilized in further funding applications and research initiatives, and are of relevance to both academic researchers focusing on herpesvirus biology and pharmaceutical development programs aiming to address unmet medical needs in antiviral therapy.

## f) Progress/Advances in the field of the project by third parties during implementation of the project

Please comment if research results relevant to this DZIF project were made known by third parties that had a (possibly direct) influence on the course of the project.

none

g) Successful or planned publication of the results according to No. 5 of the  
“Nebenbestimmungen für Zuwendungen” (NABF/NKBF 2017)

The results of the project are planned to be published in a peer-reviewed scientific journal. A manuscript focusing on the predicted and experimentally validated conserved protein-protein interactions between herpesviral terminase subunits is currently in preparation. Additionally, the predicted interaction networks have been made openly accessible to the scientific community via the online platform [www.herpesfolds.org/herpesppis](http://www.herpesfolds.org/herpesppis).

