Parallel Session Xb, June 1, Thursday

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HERPESVIRAL HCMV UL141 ANTAGONIST DEVELOPMENT TO BLOCK TRAIL DEATH RECEPTOR BINDING

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Numerous studies have demonstrated that human cytomegalovirus (HCMV) encodes countermeasures against a spectrum of immune responses [1-5]. This arsenal of immunomodulatory functions is likely a reflection of the natural history of the virus, providing the capacity to establish lifelong infections of the host as well as to reinfect people with an existing infection despite the presence of a substantial immune response. The complexity of these immunological interactions is being studied extensively. HCMV has developed many sophisticated mechanisms targeting host immunity and has become a paradigm for viral immune evasion. Suffice it to say that HCMV-encoded gene functions target antigen presentation by major histocompatibility complex (MHC) class I and class II molecules, utilize cytokine mimicry to exert paracrine functions against immune cells and encode proteins that antagonize the range of innate immune responses directed against the virus. We have been studying several of viral immune modulatory genes over the last decade, primarily focusing on those that target and/or intersect with Ig-like- and TNF-family signaling [2-5]. The fact that ablating a single strategy can markedly impact infection suggests that multiple Achilles' heals may be exploitable for antiviral agents or biotherapeutics development.

HCMV commensurately uses own proteins to restrict NK activating ligands and receptors on the surface of infected cells. An extra-ordinarily effective strategy is encoded by the HCMV UL141 protein, which inhibits surface expression of the NK activating ligands CD155 and CD112 [5] and also binds death receptors for the TNF apoptotic ligand TRAIL [2,4]. This pleiotropy of UL141 is required for its broad and potent inhibition of NK cells, and a viral mutant lacking UL141 is highly susceptible to NK killing, revealing how disrupting the function of a single HCMV gene can tip the balance in favor of host defense.

In this work, we sought to develop the short peptide or synthetic compound (UL141 antagonist) based on our recent crystal structure that would specifically binds viral UL141 to block receptor binding thus prevent the viral action. This is relevant, as the UL141 is the most abundant HCMV protein on plasma membrane and it is also a component of the virion. Within first part, we sought to test a small library of synthetized compounds (potential UL141 antagonists) that would block the receptor binding *in vitro*, on the cell, or virion surface. Series of compounds that have been tested are of glycomimetics structures consisting of various saccharide units linked with non-saccharide. In particular; non-ionic glycolipids, 'click'-conjugates or iminosugars. The ELISA-like TMB assay has been used in combination with dynabeads^{TM} coating to test whether the compound could block the TRAIL-R2 binding. The most promising compounds are (14) and (22) (out of 23 tested) that have proven the ability to block UL141/TRAIL-R2 complex formation. SPR kinetics analysis was used to determine the binding constants (K_D). The affinities to UL141 were determined to 24 M and 29 M for compound 14 and 22, respectively. Moreover, both compounds were docked to UL141 structure to reveal specific binding

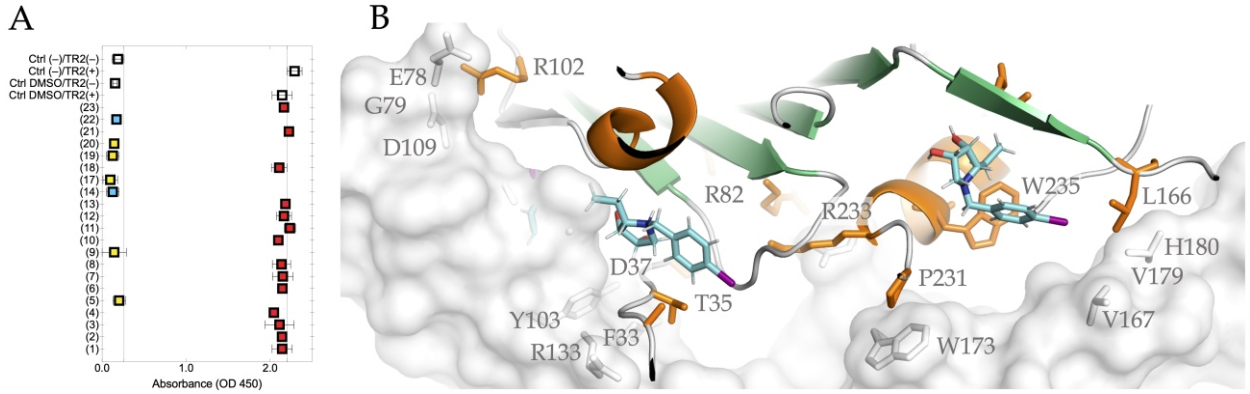

Figure 1. ELISA-like TMB assay (**A**), where dynabeads are coated by HCMV UL141, has revealed seven compounds that blocks TRAIL-R2 receptor binding (OD450 <2.0) (yellow and cyan squares). SPR binding assay revealed two compounds (14) and (22) that bind HCMV UL141 with higher affinity. Both compounds (in cyan sticks) were docked to HCMV UL141 structure to reveal specific binding site (**B**), while TRAIL-R2 receptor is also shown (in grey surface).

sites. The successful compounds will be further optimized by using *in silico* methods to target particular epitope on viral glycoprotein UL141 derived from our structural analysis.

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HYBRIDNÉ PRÍSTUPY K ŠTRUKTÚRNEJ CHARAKTERIZÁCII AGREGAČNÝCH OBLASTÍ TAU

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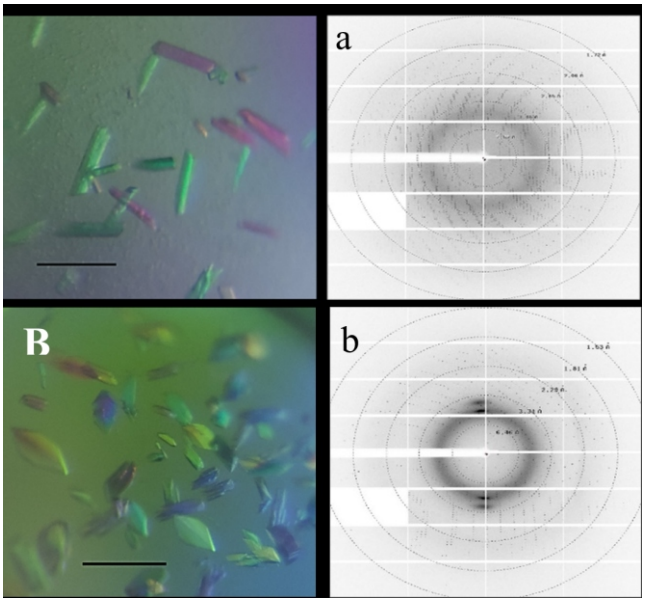
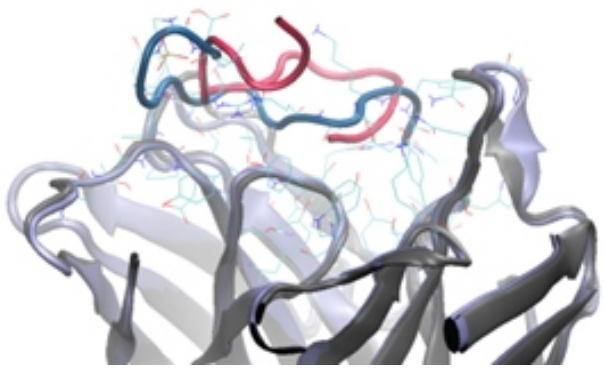
Agregácia prirodzene neusporiadaných proteínov súvisí s patogenézou mnohých neurodegeneratívnych, či nádorových ochorení. Neusporiadaným proteínom chýba stabilná trojrozmerná štruktúra, vďaka čomu majú celý rad interakčných partnerov a dokážu ovplyvňova množstvo procesov. Okrem toho častokrát dochádza k interakciám medzi molekulami samotného proteínu a vznikajú nerozpustné proteínové agregáty, vedúce k zápalu alebo až k poškodeniu buniek. Neurofibrilárne klbká sú konečným produktom agregácie neuronálneho proteínu tau a sú charakteristickým znakom Alzheimerovej choroby [1] a iných tauopátií. Proteín tau je viazaný na mikrotubuly, z ktorých v dôsledku rôznych posttranslačných úprav disociuje, čím sa zrýchľuje jeho premena z plne rozpustného monoméru na nerozpustné vlákna. V rámci primárnej štruktúry najdlhšej ľudskej izoformy tau_{1-441} sa vyznačujú oblasti, ktoré sprostredkúvajú väzbu na mikrotubuly – tzv. MTBR, pozostávajúce zo štyroch repetitívnych domén R1 - R4. Zistilo sa, že práve v tejto časti sa nachádzajú aj hexapeptidové oblasti tau zodpovedné za zvýšený obsah -štruktúr, ako charakteristickej črty amyloidovej agregácie (konkrétne ^{306}VQIVYK^{311} z R3 domény a ^{275}VQIINK^{280}v rámci R2) [2].

Medzi najčastejšie posttranslačné modifikácie tau patria hyperfosforylácia a skrátenie proteínu z oboch koncov. Ukázalo sa, že terminálne oblasti tau dokážu vytvára prechodnú štruktúru, tzv. štruktúru papierovej spinky [3], resp. štruktúru v tvare písmena S [4], čím blokujú agregačné oblasti tau a s ažujú tak interakciu molekúl proteínu. Skrátením proteínu je znemožnený vznik spomínanej protektívnej štruktúry a monoméry tau sa ochotne spájajú za vzniku dimérov, oligomérov až párových helikálnych vlákien a neurofibrilárnych klbiek. Preto sa inhibítor agregácie patologických foriem tau považuje za sľubný terapeutický cieľ, avšak návrh takéhoto inhibítora je stále obmedzený z dôvodu chýbajúcich informácií o štruktúre tau.



Obr. 1. Kryštály samostatnej DC11 Fab (B) a domnelého komplexu DC11 Fab s $tau_{321-391}$ (A) s príslušnými difrakčnými obrazcami a,b.

Na získanie prechodných konformácií tau využívame kryštalizáciu skrátených proteínov tau s Fab fragmentami monoklonálnych protilátok, ktoré ho dokážu zastabilizova v jednej konformácii [5]. Komplexy $tau_{321-391}$:DC11 Fab boli inkubované 1 hod v molárnom pomere 1,5:1. Kryštalizácia prebiehala metódou sediacej kvapky, pri 295 K v 96-jamkových kryštalizačných platničkách [6]. Zber difrakčných údajov bol uskutočnený na synchrotróne PETRA III, DESY, Hamburg v Nemecku a predbežné difrakčné údaje boli spracované v programe XDS [5]. Kryštály samostatnej Fab domény protilátky difraktovali v rozsahu až 1,4 Å , zatiaľ čo komplexy difraktovali až po 1,74 Å (Obr. 1). Na riešenie štruktúr plánujeme využi



Obr. 2. Výsledné komplexy protilátky DC25 s peptidom tau345-356 s fosforylovaným alebo nefosforylovaným SER356 získané pomocou HADDOCK serveru. (Modrý, *new cartoon* – fosfopeptid; červený, *new cartoon* – nefosforylovaný peptid tau).

metódu molekulovej náhrady a získané štruktúry budú ďalej využité v simuláciách molekulovej dynamiky a molekulovom dokovaní.

Paralelne skúmame konformačný priestor proteínu tau využitím simulácií molekulovej dynamiky. Atomistické simulácie dynamiky krátkeho peptidu $tau_{345-358}$ boli uskutočnené v explicitnom rozpúšadle v balíčku programov Gromacs 2021.3 za využitia silového poľa des-Amber. Výsledné konformácie proteínu tau boli využité na dokovanie do paratopu monoklonálnej protilátky DC25 [7] (Obr. 2). Sledovaný bol vplyv fosforylovácie na štruktúru tau a interakciu tau s protilátkou. Okrem toho boli uskutočnené aj simulácie najdlhšieho tau_{1-441} a $tau_{321-391}$ za využitia silového poľa SIRAH [8]. Získané prechodné štruktúry tau získané röntgenovou kryštalografiou, či pomocou *in silico* metód budú v budúcnosti využité na uskutočnenie virtuálneho skríningu s cieľom identifikácie potenciálneho inhibítora agregácie tau.

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BIOINFORMATIC ANALYSIS OF TREHALOSE SYNTHASES CONTAINING MALTOKINASE DOMAIN

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The present *in-silico* study is focused on trehalose synthases (TreS), enzymes converting maltose to trehalose and vice versa [1], which (at least some of them) may be part of the four-step metabolic pathway of glycogen formation from trehalose. They are classified in the Carbohydrate-Active enZymes Database (CAZy; http://www.cazy.org) [2] in the family of glycoside hydrolases GH13, known as the main -amylase family [3,4]. Family GH13 covering more than 30 different specificities [5] have been divided into 44 official subfamilies [6] and trehalose synthases belong to subfamily GH13_16. Typically, they consist of the three-domain family GH13 canonical arrangement with a catalytic $\left(\frac{\beta}{\alpha}\right)_8$ -barrel domain A, domain B (mostly of irregular structure) protruding out of the barrel in the place of the loop 3 and domain C (a 7-stranded antiparallel -sandwich) at the C-terminus [4]. In some GH13_16 enzymes, however, the domain C is succeeded by a C-terminal extension which in many cases exhibits clear sequence features of a maltokinase (MaK) [7,8]. True MaKs are single-domain enzymes that catalyze ATP-dependent phosphorylation of maltose at position 1 [8].

One of our goals was analysis of GH13_16 enzymes for the presence of maltokinase domain. Hence of total 5,933 GH13_16 members available (October 14, 2021), a set of 3,325 unique sequences with a standard TreS domain was retrieved. These were subsequently divided into two main groups: (i) 1,425 fused TreS-MaKs with a long C-terminal extension (at least 400 residues) where the full-length MaK domain was detected; and (ii) 1,900 simple TreSs with a

standard TreS domains followed by a short C-terminal extension (< 110 residues) where no additional domain was found.

The sequences of MaK domains of fused TreS-MaKs and 17 characterized true MaKs (i.e. those without a GH13_16 TreS) were aligned, conserved sequence regions were identified and members with identical CSRs were excluded. Similarly, also simple TreSs were aligned and those with identical CSRs (seven CSRs typical for GH13 family) were excluded. As a result, 604 fused TreS-MaKs and 597 simple TreSs were selected for further study. Detailed analysis revealed that only 467 MaK domains from 604 fused TreS-MaKs may represent standard MaKs with conserved catalytic machinery. In contrary, mutations in residues directly binding maltose or in catalytic aspartates were found in 79 and 58 MaK domains, respectively. Their proper catalytic function as maltokinases is thus questionable which opens up speculations about their possible new role. The group of 597 simple TreSs was used to prepare a logo based on CSRs, and as a control set in the aim to find possible differences between TreS domains of simple TreS and fused TreS-MaK enzymes. Analysis of the linkers connecting the TreS and MaK domains revealed the unusual high content of aromatic residues, tryptophans and phenylalanines, in comparison with C-terminal parts of simple TreS enzymes. The tertiary structure of any fused TreS-Mak is currently unknown, therefore the structures modeled by Alphafold [9] and available in UniProt database [10] were analyzed with a special focus on the parts connecting the two domains. The results support the hypothesis that part of the linker actually belongs to the MaK domain. Thus, the Mak domains of TreS-MaK fused enzymes are larger than those of true MaKs.

Since the presented work delivers a detailed bioinformatics analysis of fused TreS-MaK enzymes for the first time, it might help in their quick identification and contribute to better characterization of both TreSs and MaKs, and thus help in the study of the role of MaK/MaK-like domains in the subfamily GH13_16 enzymes.

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