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CA(t)CHEing Hits for NSP13 - Rapid Hit Identification Through Virtual Screening

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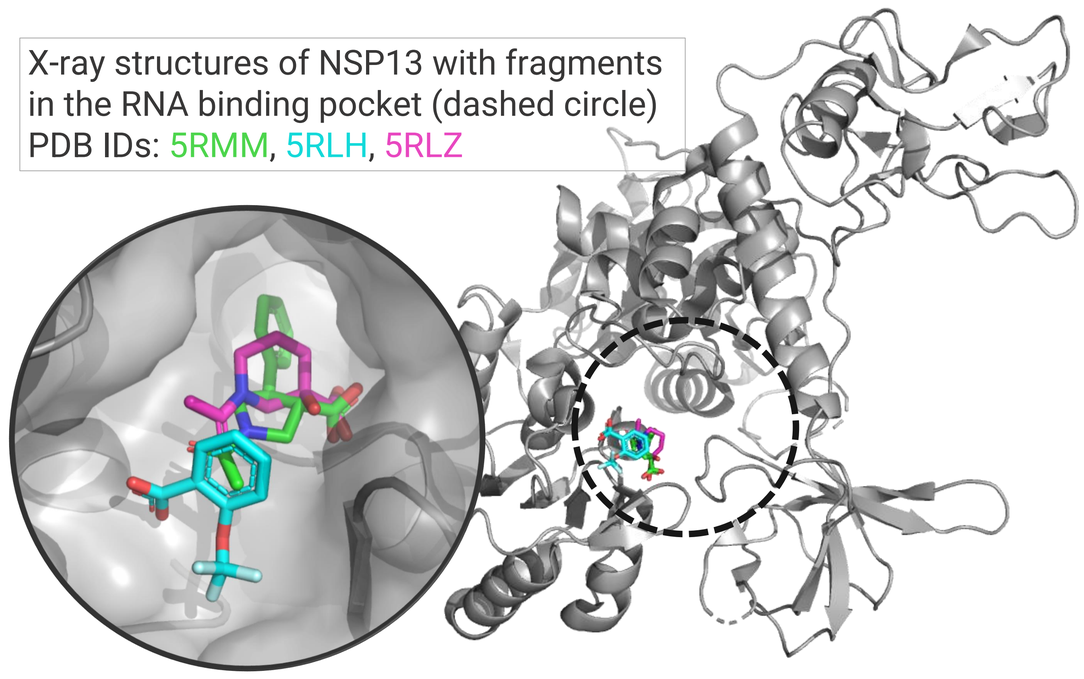
CACHE Challenge 2

The Critical Assessment of Computational Hit-finding Experiments (CACHE) consortium aims to improve in silico hit identification methods and find chemical matter for yet understudied biologically relevant targets [1]. For each CACHE challenge a target for fundamental biology or drug discovery is chosen and participants are to predict hits by *in silico* methods only. The virtual hits are then tested experimentally by CACHE for target binding, here by Surface Plasmon Resonance (SPR). A side goal is the discovery of new pharmacological tools for yet understudied targets that are eventually made publicly available including the recorded experimental data.

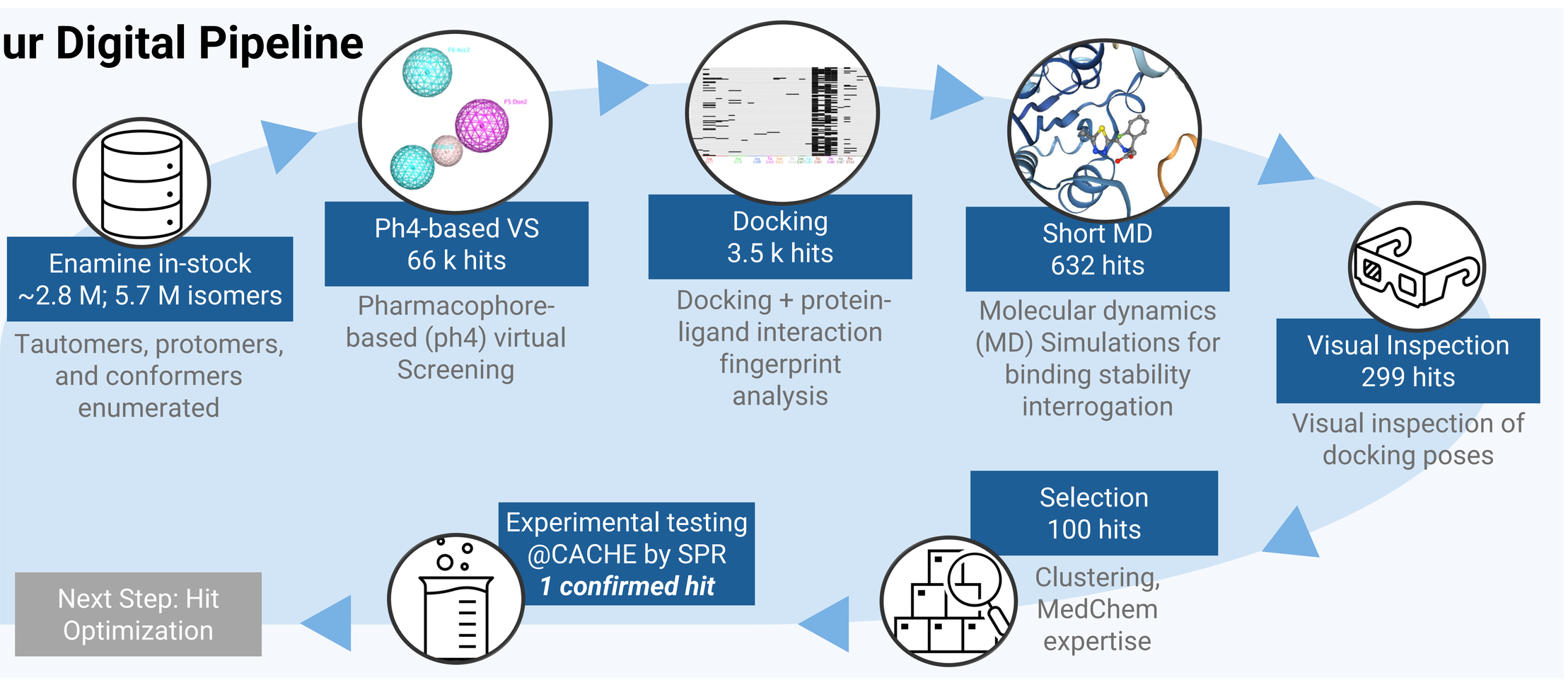
The target for the 2nd CACHE challenge was the RNA binding pocket of SARS-CoV-2 NSP13 helicase. Helicase inhibition is an established anti-viral strategy [2]. The starting points were four fragments co-crystalized to the RNA-binding site [3]. We focused the three fragments binding in the interface of the 1B and 2A domain.

The Target: SARS-CoV-2 NSP13

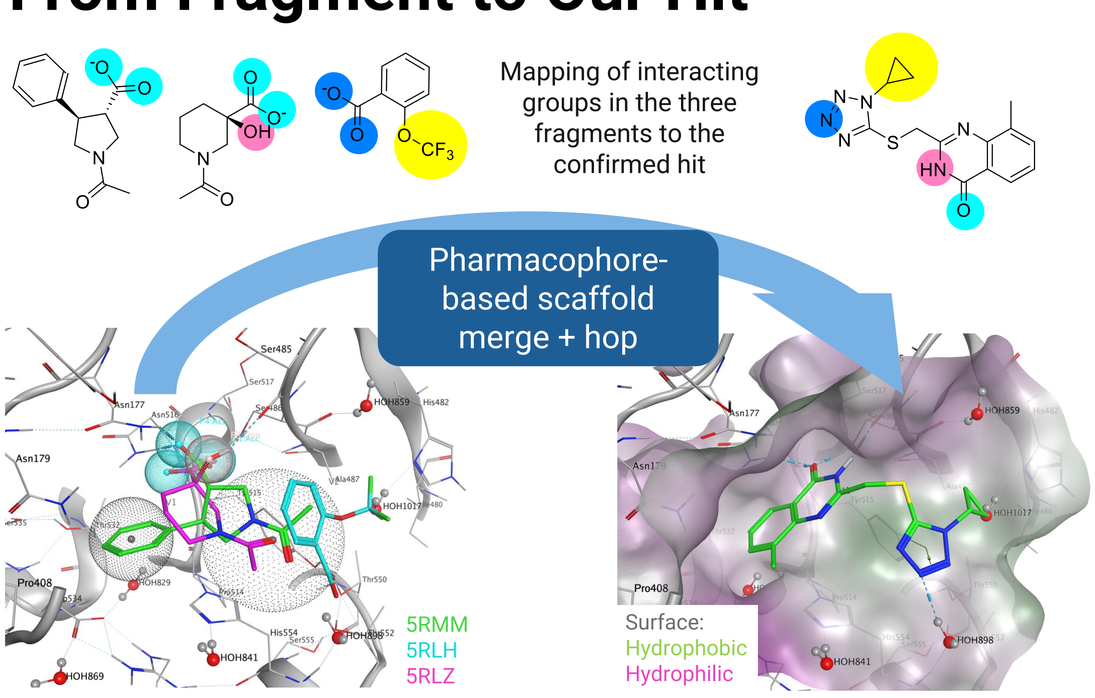
X-ray structures of NSP13 with fragments in the RNA binding pocket (dashed circle)
PDB IDs: 5RMM, 5RLH, 5RLZ



Our Digital Pipeline



From Fragment to Our Hit



Based on 3 fragments co-crystalized to the RNA-binding site and hydration site analysis (not shown) of NSP13 we developed a pharmacophore model that was subsequently used in virtual screening of the ENAMINE in-stock library. Subsequent docking followed by interaction-based filtering and in-house filters for chemical attractiveness yielded 3.5 k hits. Hydration site analysis suggested three proximal waters to be stable, which were included into the docking model. Automated molecular dynamics (MD) simulations were employed to monitor the strength of putative key H-bonds, which brought the hitlist down to 632. Visual pose inspection, clustering, and selection by a senior medicinal chemist yielded a list of 100 compounds. SPR experiments identified one out of 100 compounds as a hit. Ongoing hit optimization aims for 50 derivatives that will enter the next step of SPR experiments with CACHE again. Eventually, Nuvisan and other successful participants will contribute a set of new in silico identified pharmacological probes targeting the RNA-binding site of NSP13 to the public domain.

References: [1] Ackloo, Suzanne, et al. "CACHE (Critical Assessment of Computational Hit-finding Experiments): A public–private partnership benchmarking initiative to enable the development of computational methods for hit-finding." Nature Reviews Chemistry 6.4 (2022): 287-295. [2] Shadrick, William R., et al. "Discovering new medicines targeting helicases: challenges and recent progress." Journal of biomolecular screening 18.7 (2013): 761-781. [3] Newman, Joseph A., et al. "Structure, mechanism and crystallographic fragment screening of the SARS-CoV-2 NSP13 helicase." Nature communications 12.1 (2021): 4848.