

Official opponent's Review of the dissertation by Hamlet Narek Khachatryan, entitled “Active site conformational analysis framework of viral proteins for structure-based antiviral drug discovery” submitted for the degree of Candidate of Biological Sciences (PhD)

It is well known that developing and translating a new therapeutic agent or vaccine into clinical practice takes years. It would seem impossible to synthesize, validate, and verify a new antiviral drug within a matter of weeks following the outbreak of an epidemic. However, the COVID-19 pandemic forced researchers and manufacturers to achieve what seemed impossible, and we have witnessed that. But is it possible to accelerate this process even further and create a universal method for identifying target protein structures for the *in silico* screening of antiviral drugs? The primary objective of the dissertation under review was the rigorous development of a system for the comparative analysis of representative protein conformations to facilitate antiviral drug discovery and development.

Historically, matching a drug to a viral protein was like trying to fit a rigid key into a lock. Instead of treating a virus's target protein as a rigid, unmoving shape, this research establishes a systematic framework to study how the main "docking station" of viral proteins moves, bends, and shifts over time (conformational analysis). This flexibility is then exploited in highly targeted structure-based drug discovery. This framework treats the lock as something dynamic, allowing bioinformaticians and medicinal chemists to design flexible "master keys". This significantly accelerates the drug discovery pipeline, lowers development costs, and prepares global health systems to react faster to future viral pandemics. Indeed, we know that protein molecules are highly dynamic in the cell and constantly switch between different micro-conformations. This concept was first discussed long ago—the self-oscillatory processes in proteins were described and studied. In 1974, Jabotinsky and Belousov published the monograph “Concentration dependent self-oscillation”, where they described that process in detail.

Thus, Hamlet Khachatryan focused on the active site of the target protein—a specific pocket involved in viral replication or infection of host cells. The framework captures the full range of shapes the pocket can take. On the other hand, when a virus mutates, it often changes the shape of its protein active site so that a drug can no longer bind to it. This framework analyzes the active site's movements to find "vulnerable," highly conserved geometries—shapes the virus must maintain to process its natural substrates and survive. Designing drugs to fit these specific shapes inflicts an insurmountable fitness cost on the virus if it tries to mutate, effectively neutralizing resistance.

Many related viruses (like different strains of coronaviruses or influenza) share similar active site mechanics. By understanding the shared conformational pathways across a viral family, this framework helps scientists design broad-spectrum inhibitors—a single drug capable of targeting the active sites of multiple current and emerging viral threats.

The thesis describes Q-Pocket as a configurable Python package with PDB retrieval, preprocessing, featurization, clustering, representative selection and visualization. The YAML

(Yet Another Markup Language) -driven workflow makes the method more reusable than a one-off analysis script.

Q-Pocket, developed in the current research as a configurable Python package with PDB retrieval, preprocessing, featurization, clustering, representative selection and visualization, does more than compare backbone coordinates. It describes the solvent-accessible cavity as a point cloud and annotates it with electrostatic, Coulomb, lipophilic, hydrogen-bond and aromatic fields. That is a richer model of a binding site than a simple RMSD-based comparison.

The use of geodesic graphs, normalized graph Laplacians, eigenvalue-based shape signatures and Graph-Fourier power spectra is one of the most technically sophisticated parts of the dissertation. This is a real computational-method contribution, not just another docking workflow.

The study works with 342 curated Mpro holo structures and 690 retrieved and 684 featurized HIV-1 protease structures. The HIV-1 PR set is particularly useful because it contains apo, inhibitor-bound, resistant and stabilized forms.

Using Vina, rDock and Glide together with EF, ROC-AUC, BEDROC and pose-reproduction metrics is a sensible attempt to reduce dependence on one scoring function. It does not solve every interpretation problem, but it strengthens the benchmark technically.

The RL design campaign proceeds to synthesis, enzymatic inhibition, cell-based antiviral testing and an animal model. For a computationally driven PhD, reaching this level of experimental translation is a major strength.

The thesis includes CPE, plaque assay, viral-NP Western blot, cytotoxicity and animal outcomes. These orthogonal readouts make the experimental section considerably stronger.

The thesis acknowledges PDB sampling bias, missing cryptic states, benchmark dependence and the fact that the causal link from Q-Pocket selection to experimental success is only partly established. That self-critique is scientifically healthy.

At the same time, I would like to make several observations that could serve as discussion points.

- The synopsis states that 32 influenza A and 23 influenza B structures were resolved into eight clusters, while Table 2 does not make sufficiently clear how the two viral groups contributed to those clusters. The full thesis clarifies that the final dataset comprised 32 IAV HxN1 and 23 IBV structures and that eight major clusters were identified, with one cluster containing only IBV structures. Nevertheless, because IAV and IBV neuraminidases share only ~35% sequence identity, it would be useful to clarify how much of the observed separation reflects conformational differences versus subtype/sequence divergence.
- There is no matched control arm demonstrating Q-Pocket's actual advantage. This may be the strongest causal-design limitation. The dissertation argues that representative-conformation selection is a decisive input and that framework-selected structures deliver experimentally confirmed gains. Yet the prospective workflows do not include a matched

- There are several discrepancies between the English dissertation text and the Russian summary. One example is the reported Mpro hit rate. The English Results section states that 7 of 16 tested compounds had $IC_{50} < 100 \mu M$ and presents this as a $>40\%$ hit rate ($7/16 = 43.75\%$), whereas the Russian summary states that only 2 of 16 compounds showed in vitro activity, corresponding to 12.5%. The full dissertation itself consistently reports 7/16, so this discrepancy appears to be in the summary and should be corrected for consistency.

In conclusion, given the significant novelty of these methodologies in optimizing the synthesis of new antiviral drugs—which rely on fundamentally new approaches to the problem—the findings successfully leverage “cloud-based” frameworks to identify new effectors. Furthermore, considering that the core findings of this research have been published in high-impact, peer-reviewed scientific journals, it is evident that the dissertation fully meets the standards required for a doctoral degree. Consequently, the candidate, Hamlet Khachatryan, highly deserves to be awarded the degree of **Doctor of Philosophy (PhD) in Biology**.

H. C. Young

I hereby certify the signature of Dr. K.B. Nazaryan

18.03.26p