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Evaluation of Dissertation of Adrine Paronyan
IN SILICO STUDY OF MODULATORS OF THE INTERACTION BETWEEN PYRIN AND 14-3-3 PROTEIN ISOFORMS
seeking a Candidate of Biological Sciences (Ph.D.) degree

Familial Mediterranean fever (FMF) is the most common of the hereditary autoinflammatory diseases and is particularly prevalent in the Armenian population, which makes the mechanisms that keep pyrin under control a subject of both fundamental and practical interest. FMF and pyrin-associated autoinflammation with neutrophilic dermatosis (PAAND) are caused by mutations in the MEFV gene. In resting cells pyrin is held in an inactive state through phosphorylation-dependent binding to 14-3-3 proteins, and the loss of this interaction is now considered the key event that releases the pyrin inflammasome and leads to uncontrolled secretion of interleukin-1β. Colchicine remains the main treatment, and a substantial proportion of patients respond insufficiently or tolerate the drug poorly, so compounds acting directly on the interaction between pyrin and 14-3-3 are of clear therapeutic interest.

Protein-protein interactions are difficult drug targets, and the 14-3-3 complexes are among the few systems for which the stabilization strategy, that is, small molecules that bind at the composite interface and strengthen the complex rather than disrupt it, has been demonstrated repeatedly. Extending this idea to pyrin is well justified. The obstacle is structural: the Protein Data Bank contains only complexes of 14-3-3 with short phosphorylated peptides of pyrin (8C2Y, 8C28, 8C30), whereas full-length pyrin is large, contains extended disordered segments and has never been resolved experimentally. Any structure-based search for modulators therefore has to begin with a model, and this is precisely the situation in which computational approaches become a preferable approach.

The A. Paronyan approached this problem consistently and carried it through to concrete candidate compounds. Full-length models of the pyrin–14-3-3τ/τ and pyrin–14-3-3ε/ε complexes were built with AlphaFold2 in multimer mode and validated against the crystallographic structures of the individual components, with RMSD below 1.2 Å for 2WL1 and below 2.6 Å for 4CG4. The models were phosphorylated at Ser208, Ser209 and Ser242 and simulated for 2 μs in explicit solvent with the ff19SB and phosaa19SB parameters, in systems of 712,000 and 805,000 atoms. This is a considerable amount of computation for complexes of this size, which was performed with a right combination of tools ensuring the accuracy of the results. Importantly, the simulations reproduce an experimental observation that was not built into the models: among the three phosphoserines, pSer242 dominates the interaction and maintains close contacts with Arg56/Arg57 and Arg127/Arg130 of 14-3-3 throughout the trajectory, in agreement with the crystallographic data showing that phosphorylation of a single residue is sufficient for complex formation of the complex and maintaining the interaction. This is a convincing internal control of the whole modeling protocol.

A separate methodological contribution is the filtering procedure developed for extracting a representative and, importantly, ligand-competent conformation from a long trajectory. Frames were selected by the presence and the volume of a pocket at the position occupied by the fragment N-[3-(aminomethyl)phenyl]acetamide in 8C30, and then ranked by RMSD to the reference structure, interaction distances and binding energies; of the 50,000 frames available for each complex, 202 and 53 passed the pocket criterion, and one representative frame was finally selected for each system. Using an experimentally determined fragment position to choose a druggable conformation from an MD ensemble is an elegant idea, and it is not specific to this particular system, which makes the approach applicable for similar problems.

On these conformations sixteen disease-relevant variants were modeled, including the PAAND-associated S242R and E244K and the common FMF substitutions of the B30.2 domain, each simulated for 100 ns and characterized by radius of gyration, RMSD, RMSF, hydrogen bonds and binding energies.

The screening part of the work is technically the most demanding. About 1.5 billion compounds of the Enamine protein-protein interaction collection were screened with PLANET on HPC cluster in 120 separate runs, using distributed computation on eight GPUs. Selected compounds were tested with a MedusaDock 2.0 flexible molecular docking method, and 340 protein-ligand complexes were filtered by PLIP contact analysis method with respect to the key binding-site residues, resulting twelve final complexes for pyrin - 14-3-3τ/τ and four for pyrin–14-3-3ε/ε. Compounds IX_8 and IX_9 for the τ/τ system and IX_17 for the ε/ε system were repeatedly selected among the native and mutant variants, which suggests that they tolerate structural variation well. The final 100 ns simulations of these complexes showed stable trajectories, persistent hydrogen bond networks and MM/PBSA ligand binding energies mostly between -30 and -80 kcal/mol, and the physicochemical and ADMET properties of the leads are reported in the Appendix. It should be mentioned that screening at this scale demonstrates that the candidate has an advanced set skills for managing such a complexes computational workflow.

The dissertation is presented on 149 pages, contains 51 figures and 8 tables and cites 145 references, with an appendix of 2 figures and 11 tables. It follows the classical structure. The literature review covers FMF and PAAND, the domain organization of pyrin, the 14-3-3 family and its isoforms, and the mechanisms of modulation of protein-protein interactions; the methods chapter describes the modeling, simulation and screening tools in sufficient detail; and the results chapter follows the logic of the work from the initial models to the final simulations. The conclusions correspond to the aims and to the data presented. The main results have been published in five papers, including a first-authored article in ACS Omega and two articles in Molecular Biology, and were reported at international conferences, so they have already been reviewed independently by specialists in the field.

Overall, this is a complete and technically well-executed study that brings structure prediction, microsecond simulations of very large systems, ultra-large-scale virtual screening and flexible docking together into a single workflow, and that provides the first full-length structural models of pyrin in complex with 14-3-3 dimers.

As a student I worked on pyrin phosphorylation and related structural rearrangements using molecular modeling methods. However, resources and methods were limiting our ability to make applicable observations. A. Paronyan's work, in contrast, is incomparable in its depth and scale, showing the progress that she made in deepening the understanding or this problem and offering prominent solutions.

The following remarks are intended mainly as suggestions for the discussion and for the continuation of this work:

1. Because of the extended disordered segments of pyrin, the RMSD of the entire complex is dominated by their motion. Reporting RMSD separately for the structured domains and for the binding interface would show the stability of the complexes even more clearly, and a second independent trajectory for each system would make the conclusions about equilibration even stronger.

2. It would be useful to state explicitly that the MM/GBSA and MM/PBSA values are used as relative descriptors rather than as absolute binding free energies, since they do not include the entropic contribution, and to accompany them, as well as the ΔΔG values obtained for the mutants, with error estimates from averaging or from independent runs.

3. The conclusion that the selected compounds stabilize the pyrin - 14-3-3 interaction would be supported most directly by comparing the pyrin - 14-3-3 binding energy calculated with the same protocol in the

presence and in the absence of the ligand, together with the contact analysis at the pSer242–Arg interface in both cases. This is a natural next step and would turn a well-founded interpretation into a direct demonstration.

4. A short description of how many compounds were finally submitted to docking after the PLANET filtering, and of the criteria applied at that step, would make the screening funnel easier to follow and to reproduce. In the same spirit, listing the SMILES strings and Enamine catalogue numbers of the leading compounds and depositing the models and scripts in a public repository such as GitHub or Zenodo would allow others to take these molecules to the experimental stage, which the work certainly deserves.

5. The ADMET predictions given in the Appendix could be discussed briefly in the main text, since some of the leads show an elevated predicted risk of hERG inhibition and hepatotoxicity, and this information would help to prioritize among them in further work.

These remarks do not affect the overall assessment. The dissertation solves a clearly formulated problem, the results are novel and have been published in peer-reviewed journals, and the conclusions follow from the data presented. The candidate demonstrates a confident command of a broad range of modern computational methods, from structure prediction and molecular dynamics to ultra-large-scale virtual screening, and the ability to combine them into a coherent workflow on high-performance computing resources.

In summary, the work of Adrine Paronyan provides the first full-length structural models of the pyrin–14-3-3 complexes, characterizes the effect of disease-associated mutations on them and identifies small molecules with the potential to stabilize this interaction, providing a solid foundation for further experimental work on FMF and PAAND. There is no doubt that the dissertation fully meets the requirements for a dissertation seeking the degree of Candidate of Biological Sciences, and I am pleased to recommend awarding Adrine Paronyan the degree of Candidate of Biological Sciences (Ph.D.) in the field of 03.00.02, "Biophysics and Bioinformatics."

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