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Structural anomalies in a published NMR-derived structure of IRAK-M

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ABSTRACT

Signaling by Toll-Like Receptors and the Interleukin-1 Receptor (IL1-R) involves intracellular binding of MyD88, followed by assembly of IL1-R Associated Kinases (IRAKs) into the so-called Myddosome. Using NMR, Nechama et al. determined the structure of the IRAK-M death domain monomer (PDBid: 5UKE). With this structure, they performed a docking study to model the location of IRAK-M in the Myddosome. Based on this, they present a molecular basis for selectivity of IRAK-M towards IRAK1 over IRAK2 binding. When we attempted to use 5UKE as a homology modeling template, we noticed that our 5UKE-based models had structural issues, such as disallowed torsion angles and solvent exposed tryptophans. We therefore analyzed the NMR ensemble of 5UKE using structure validation tools and we compared 5UKE with homologous high-resolution X-ray structures. We identified several structural anomalies in 5UKE, including packing issues, frayed helices and improbable side chain conformations. We used Yasara to build a homology model, based on two high resolution death domain crystal structures, as an alternative model for the IRAK-M death domain (atomic coordinates, modeling details and validation are available at https://swift.cmbi.umcn.nl/gv/service/5uke/). Our model agrees better with known death domain structure information than 5UKE and also with the chemical shift data that was deposited for 5UKE.

The Myddosome is a layered, oligomeric structure consisting of six MyD88 subunits at the receptor side, four IRAK4 monomers in the middle layer and four IRAK1 or four IRAK2 monomers in the distal layer [1]. IRAK-M is thought to modulate signaling via the Myddosome [2]. The IRAK-M death domain structure presented by Nechama et al. [3] consists of a classical death domain fold with two long, partly flexible terminal loops (Fig. 1). In the Nechama study, two docking runs were performed. In one of these, 5UKE was docked into the Myddosome (PDBid:3MOP) from which one subunit of the IRAK2 tetramer was removed, while in the other run, additionally, the three remaining IRAK2 subunits were substituted for homology models for IRAK1. Only the docking run with IRAK1 in the distal layer yielded poses with 5UKE at the location of the removed subunit. From this it was concluded that IRAK-M prefers binding to IRAK1 over IRAK2. The authors propose that this selectivity is due to an interaction of IRAK-M:D33[1] with IRAK1:R61 that is absent for IRAK2, and due to repulsion between IRAK-M:E46 and IRAK2:D14 that is absent for IRAK1. The docking results are used by the authors to propose that IRAK1 binds and phosphorylates IRAK-M in a conformation of the IRAK-M death domain in which residues L20 and P21 interact with Y105. Nechama et al. suggest that the subsequent isomerization of the nearby phosphor-S110-P111 by the prolyl isomerase PIN1 will release the L20/P21–Y105 interaction. This may then lead to loss of interaction of IRAK-M with IRAK-1 and leaves the modified IRAK-M to initiate signaling.

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[1] The residue numbering in the Nechama paper accounts for an additional N-terminal pentapeptide. For consistency with later studies, we use the canonical numbering.

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Fig. 1. The classical death domain structure is shown in the middle (PDBid:1d2z.A). On the left is PDBid:5uke with its flexible loops in yellow. On the right is the homology model.

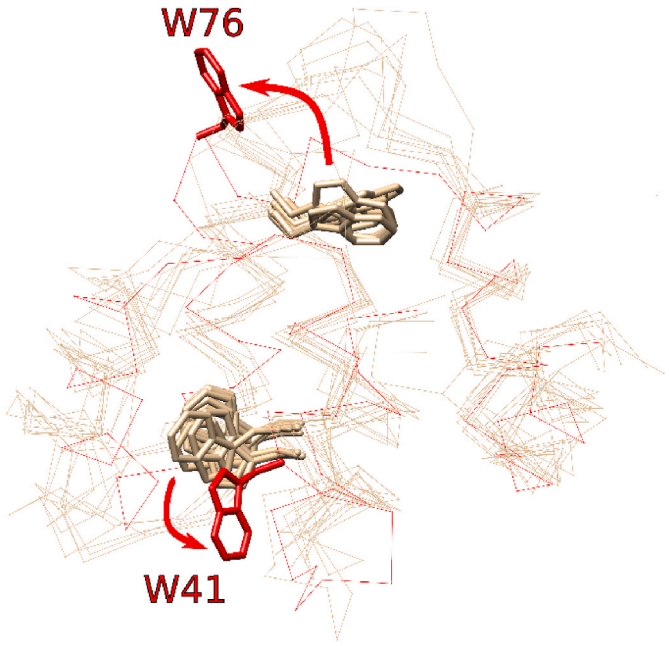

Fig. 2. Superposition of 5UKE with nine structurally related X-ray structures. 5UKE is colored red.

We inspected the 5UKE structure, as we previously made and published a homology model of the IRAK-M death domain based on an X-ray structure of mouse IRAK4 [4] (PDBid:2A9I). In that model Y105 does not interact with L20 and P21. Furthermore, by mutational analysis we found that Y105 by itself is of minor importance for IRAK-M signaling, although we used different models than Nechama and coworkers [4]. When we used 5UKE in our modeling studies to attempt to explain this discrepancy, we observed several structural anomalies in this structure. We therefore analyzed the 5UKE structure ensemble using the validation suite WHAT_CHECK [5]. The Ramachandran plot of 5UKE reveals that many residues have disallowed ψ/φ torsion angles, most notably K66. WHAT_CHECK also reports poor packing quality for all chains in the ensemble. Furthermore, none of the α-helices in 5UKE display a proper hydrogen bonding pattern and several helices are unraveled at the termini or are bent relative to the corresponding one in the X-ray structures.

For further analysis chain 2 of 5UKE was used, as it has the lowest average Cα displacement to the other models in the structure ensemble. A DALI [6] search with chain 2 as the query yielded nine death domains that were structurally similar to 5UKE and had a resolution better than 3 Å (PDBids: 1WMG, 3EZQ, 1D2Z, 6ACI, 6AC5, 4O6X, 2A9I, 2YVI, 6AC0). Superposition of these nine structures and the query structure revealed a series of anomalies in 5UKE. The side chains of the tryptophans W41 and W76 in 5UKE are solvent exposed, whereas they are buried in the X-ray structures (Fig. 2). Both K66 and S67 in 5UKE have anomalous ψ/φ torsion angles. Furthermore, D33 in 5UKE is not hydrogen bonded to S67 and is more solvent exposed than in the crystal structures (SI Fig. 1). This last observation stands in contrast to the claimed role of D33 in IRAK-1 versus IRAK-2 binding selectivity. L20 is described to interact with Y105 in 5UKE, conferring stability to the IRAK-M death domain [3]. However, the related X-ray structures suggest that L20 points into a buried, hydrophobic patch. K79 in 5UKE is near the location where L20 is expected and is more buried than usual for a charged residue (SI Fig. 2).

We attempted to improve the 5UKE ensemble using the original NMR restraints that were kindly provided to us by Dr. Nicholson. However, we were not successful, possibly due to the presence of erroneous distances in the restraint list. When we compared characteristics of the distance restraints of 5UKE with those of other NMR derived death domain structures, we realized that 5UKE has very few distance restraints with a low upper limit (SI Fig. 3). A scatterplot of actual distances in the ensemble of 5UKE versus restraint upper limits has a very wide distribution (SI Fig. 4). This indicates that many distance restraints used to derive 5UKE were set too wide, so that only a few restraints contributed actively to the NMR structure calculations. Without the availability of the experimental NOE intensities, it was not possible to improve and clean up the list of NOE-based distance restraints.

Therefore, as an alternative to 5UKE, we constructed a homology model for the death domain of IRAK-M using Yasara [7]. We based this model on two high-resolution crystal structures (PDBid:2a9i and PDBid:1d2z), as detailed in the methods section. We purposefully excluded input from 5UKE or its data. The model differs substantially from PDB:5uke and displays overall better structural quality. The issues that were identified for PDB:5uke are not present in the model. W41 and W76 have conformations corresponding to those in the X-ray structures, D33 is hydrogen bonded to S67 and K66 is solvent exposed. We analyzed both 5uke and the newly made homology model with MolProbity [8]. The homology model scores better in every respect than all chains of 5uke (see SI data). We also analyzed the hydrogen bonding network with WHAT_IF. 5 unsatisfied buried hydrogen bond donors and 6 unsatisfied buried hydrogen bond acceptors are listed by WHAT_IF for the homology model, whereas for 5uke chain2: 16–105, these numbers were 26 and 12 respectively (see SI data for detailed results).

We tested the model using the chemical shifts as deposited for 5UKE. Chemical shifts can be used for predicting local structural features [9] and can therefore also be used to validate structures derived from mainly NOEs [10]. We uploaded the 5UKE chemical shifts to the TALOS-N web server [9] to predict backbone torsion angles. We compared the results with both 5UKE and the newly made homology

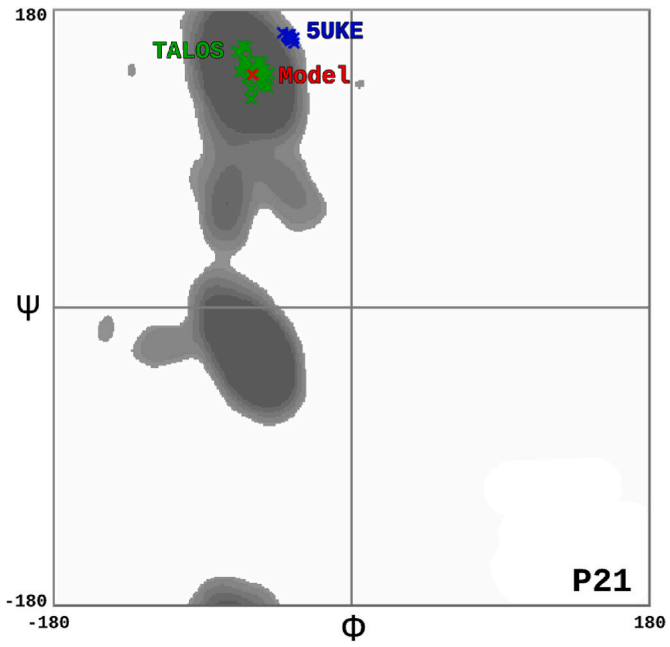

Fig. 3. For P21 the TALOS-N predicted (green) and actual (blue) torsion angles are shown. In red the corresponding torsion angle in the homology model based on 2A9I and 1D2Z is shown. The density plot was adapted from TALOS-N. See SI Fig. 5 for plots of other residues.

model. For most residues that have structural issues in 5UKE, our model is closer to the predicted values than 5UKE (Fig. 3 and SI Fig. 5).

In conclusion, 5UKE displays several important structural issues and therefore it should not be used to predict or explain biological experiments. We present here a homology model based on two high-resolution crystal structures that agrees better with currently available death domain structures, with biological knowledge about the Myddosome complex, and with chemical shift–based predictions of backbone torsion angles.

Author contributions

HP, GV and GN conceived and designed the study. HP and HI performed data acquisition and analysis. HP, HI, RB and GV interpreted the data. All authors contributed to writing the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmgm.2021.108061.

Methods

DALI [6] was used with PDB:5UKE as query. From the obtained list two high resolution crystal structures were selected (PDB:2A9I and PDB:1D2Z.A) as templates for modeling. Residues P28 to G32 were removed from PDB:2A9I. The IRAK-M death domain sequence was aligned to the template sequences by hand.

>irakm LLFDLPPALLGELCAVLDSCDGALGWRGLAERLS————————
SSWLDVRHIEKYVDQGKSGTRELLWSW-
AQKNKTIGDLLQVLQEMGHRRAIHLITNY
>T001-A AIRLLPLPVRAQLCAHLDAL-D–VWQQLATAVK————————
LYPDQVE-
QISSQKQRGRSASNEFLNIWGGQYNHTVQTLFALFKKLKLHNAMR-
LIKDY
>T002-A YIRNLNVGILRKLSDFID————————WKKLAVAIKKPSGD-
DRYNQFHIRRFEALLQTGLSPTCELLFDW-
GTTNCTVGDLVDLLVQIELFAPATLLLP-

The Yasara [7] homology modeling procedure was used to arrive at a homology model of the IRAK-M death domain monomer. The following parameters with changed in the macro file with respect to default:

- Equivalence Target = WAQKNKT, Template = WGTTNCT
- Equivalence Target = KYVDQGKSG, Template = SQKQRGRSA
- Equivalence Target = GWRGLA, Template = VWQQLA
- Equivalence Target = GWRGLA, Template = GWKKLA
- TemplateExList 5uke

The hybrid model was accepted as the final model.

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