Hypothesis

Visualising the Experimentally Uncharted Territories of Membrane Protein Structures inside Protein Data Bank

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Abstract: As of January 13, 2020, there is not any direct report yet of the degree to which missing residues exist for experimentally determined membrane protein (MP) structures, which constitute more than half of current drug targets. With a chain- and position-specific visualisation and a statistical analysis of all MP structures inside PDB (as of September 25, 2019), this article argues that the experimentally uncharted territories (EUTs, i.e., consisting of missing residues) within PDB are pluggable and should be plugged with an experimental data-driven hybrid approach, and calls for continued development of MP structural determination with less and less EUTs, in light of MPs' crucial role in biological and biomedical research, both fundamental and pharmaceutical.

Keywords: Position-Specific Visualization; Experimentally Uncharted Territories; Membrane Protein Structure; Protein Data Bank

Introduction

As cellular gate-keepers, membrane proteins (MPs) account for a third of the eukaryotic proteome and are responsible for a variety of physiological functions [1–4]. For instance, cells use MPs to transmit signals into cells, transport ions and molecules, bind to cell surface or a substrate, and catalyze reactions under different conditions [5–8].

Established in 1971 [9,10], Protein Data Bank (PDB) is the global repository for experimentally obtained biomolecular structural data, constituting an enormously valuable resource for research across the biological and biomedical sciences [11–16]. With the advent of novel biophysical tools for MP structural biology, the past decade saw a rapid increase in the number of experimentally determined MP structures [17–26]. This new structural knowledge has expanded our understanding of their action mechanism [27–30], and contributed to rational drug design, too [8,31–34].

Motivation

In original PDB-format data, the experimentally determined atomic coordinates are presented in the *ATOM* records. Chances are that they do not exactly match the sequence (consisting of nucleic and/or amino acid residues) of the experimental sample *per se*, be it protein, DNA, RNA or their complexes with drugs and/or other small molecules [35–37]. In fact, this misalignment arises from the uncharted territories (i.e., missing residues) of MP experimental structures deposited in PDB. In structural biology, *hole* is usually used to describe a pore or a cavity in a biomolecular structure, like the hydrophilic lumen of Ca_v1.1 and Ca_v1.2 [26,27]. Thus, *experimentally uncharted territory* (EUT) is used instead to describe the experimentally undermined parts/regions (i.e., missing residues) of MP structures, the size of an EUT is defined as the number of residues whose atomic coordinates

are not experimentally determined and therefore missing in the original PDB-format data. Here, an N- or C-terminal flanking EUT is defined as an EUT which includes the N- or the C-terminal of a protein. Otherwise, it is called an interior or non-flanking EUT here. Take Cav1.1 as an example, which is the first (as of January 13, 2020) L-type voltage-dependent Ca²⁺ channels (VDCCs), whose structure was experimentally determined to a resolution of 3.6 Å with cryo-electron microscopy (cryo-EM) [26,38]. With a sequence alignment [39] and a close inspection of the PDB files (PDB IDs: 5GJV and 5GJW), two interior EUTs in the Cav1.1 cryo-EM structures stood out, one with 358 residues (~19.11%) and another with 119 residues (6.35%), compared to the entire length (1873) of Cav1.1 [26]. For another instance, residues 4247-4277 are not experimentally determined in the recently reported cardiac ryanodine receptor 2 (RyR2) cryo-EM structure [29], compared to the previously reported RyR2 sequences [40].

As of January 13, 2020, a total of 159230 biomolecular structures have been deposited in the Protein Data Bank [10,41]. Among the 156365 (98.20% of 159230) biomolecular structures (as of September 25, 2019) in PDB [10,41], MPs are of extraordinary functional interest due to their role in a variety of physiological functions [1–4]. Of further structural significance is EUTs in the transmembrane (TM) region and/or in the structurally proximity of the binding and action sites of drug(s) or its interacting partner(s), as such an EUT might mislead the subsequent experimental investigation into drugs' action mechanism from a structural point of view, making it even more complicated and challenging and thus even pricier for novel drugs to be developed using their traditional counterparts' structure-activity relationships [27]. Taken together, there is not any direct report yet of the degree to which MP EUTs exist throughout the 48-year-old PDB [9], of which this article aims to present a statistical analysis, visualization and overall assessment.

Statistical analysis of the MP EUTs

As of September 25, 2019, there are a total of 9666 (supplementary file **PDB1.kiwi**) experimentally determined MP (associated with 2498 ligands) structures in PDB, accounting for approximately 6.16% of all 156365 biomolecular structures in PDB [10,41]. Of the 9666, supplementary file **brief.pdf** includes a statistical update as of September 25, 2019.

As shown in Table 1, while the MP structural determination is intact (i.e., no missing residue) for only 1841 among the 9666 MPs, and a large part (75.57%, Table 1) of the chains in the MP structures are with EUTs (i.e., with missing residue), the number of missing MP residues accounts only for a small part (7.79%, Table 1) of all MP residues of the 9666.

Protein level	Variable	MPs	MPs with EUTs	Ratio
	Value	9666	7825	80.95%
Chain level	Variable	MP chains	MP chains with EUTs	Ratio
	Value	32001	24186	75.57%
Residue level	Variable	MP residues	missing MP residues	Ratio
	Value	10641563	829656	7.79%

Table 1. Statistical analysis of the MP EUTs within PDB as of September 25, 2019.

Nonetheless, it still is not certain the degree to which the 829656 missing MP residues aligns with the binding and action site(s) of MP-targeting drug(s). Hence, this articles presents below a visualization of the MP EUTs within PDB.

Visualizing the MP EUTs inside PDB

To ensure reproducibility, Table 2 outlines the entire computational analysis step by step. In Figures 1, 2 and 3, each missing MP residue is marked as a tiny blue point according to its PDB ID, its chain ID and its sequence-specific position (i.e., **Residue ID**), while each maximum **Residue ID** is marked as a red point, and each and every MP chain is represented as a straight line perpendicular

Step	Input file	Python script command	Output file(s)
1	PDB1.kiwi	RCSB.py	9666 PDB files
2	9666 PDB files	second.py	hole.neu
3	hole.neu	third.py	third.out
4	third.out	fourth.py	fourth.out
5	fourth.out	fifth.py 1 10000	Figure 1
6	fourth.out	fifth.py 10000 20000	Figure 2
7	fourth.out	fifth.py 20000 24186	Figure 3
8	fourth.out	histogram.py	Figure 4

Table 2. Computational analysis of the MP EUTs within PDB. In this table, all files and python scripts (excluding the 9666 PDB files) are submitted as supplementary files.

71 to x-axis. In case its structural determination is intact, it is represented as just one red point at the top
72 of a white straight line perpendicular to x-axis. Otherwise, the positions of all missing MP residues are
73 marked by a series of blue points under that red point along the white straight line perpendicular to
74 x-axis. In other words, in Figures 1, 2 and 3, the area under all red points represents all MP sequences
75 as of September 25, 2019, in which the white area is the experimentally charted territory, while the
76 blue area is the experimentally uncharted territory. Collectively, Figures 1, 2 and 3 put forward a
77 visual update of the accumulation of MP EUTs inside PDB during the past decade, highlighting a need
78 towards MP structural determination with less and less EUTs, considering their roles in a variety of
79 physiological functions [1–4], as above discussed.

Figure 1. Chain- and sequence position-specific visualization of the MP EUTs within PDB as of September 25, 2019. This figure accounts for the first 10000 among the 24186 (Table 1) chains of MPs with EUTs (fourth.out in Table 2).

80 Nevertheless, Figures 1, 2 and 3 might be misleading in that the size of a blue point (denoting
81 one missing residue) is much wider than the width of a column corresponding to a single MP chain,
82 leading to a slightly distorted illustration of the white area, i.e., the experimentally charted territory.
83 Unfortunately, this issue is not easy to resolve, because if the X-axis is to be enlarged so that each

Figure 2. Chain- and sequence position-specific visualization of the MP EUTs within PDB as of September 25, 2019. This figure accounts for the second 10000 among the 24186 (Table 1) chains of MPs with EUTs (fourth.out in Table 2).

Figure 3. Chain- and sequence position-specific visualization of the MP EUTs within PDB as of September 25, 2019. This figure accounts for the last 4186 among the 24186 (Table 1) chains of MPs with EUTs (fourth.out in Table 2).

structure is represented with the width of a blue point, that would lead to way too many figures to reach an accurate overall assessment of MP EUTs in the whole PDB.

From Figure 4, it can be seen that the EUT sizes range from 1 to ~1200, and that most EUTs (97.30%) are with a size of less than 200 MP residues. Notwithstanding, no evidence can be drawn from Figure 4 that the MP EUTs are negligible and harmless for subsequent functional investigation of MPs' roles in the life of a cell. Instead, Figure 4, along with Figures 1, 2 and 3, calls for MP structural determination with less and less EUTs, too.

Figure 4. A histogram of the size-specific distribution of the MP EUTs within PDB.

Conclusion and Discussion

The past decade of MP structural determination saw the gradual accumulation of MP EUTs within PDB [42–48], reaching a point where it is increasingly reasonable, if not pressing, for MP structures to be experimentally determined in an EUT-less manner.

1. MP EUTs within PDB do matter. The experimental determination of MP structures is usually achieved through lengthy and laborious experimental efforts and expensive tools [36,49,50]. On one hand, MP EUTs harms the integrity of the hard-earned biomolecular structural data within PDB [36,51–54]. On the other, it is possible that MP EUTs misleads fundamental investigation into current drugs' action mechanism when they are structurally located at the proximity of the drugs' binding site.
2. In general, the existence of the EUTs in PDB could be attributed to the technical limitations of the biophysical tools and/or difficult sample (intrinsically disordered proteins for instance) [55–59] during experimental data acquisition.
3. Technically, MP EUTs are pluggable and should be plugged an experimental data-driven hybrid approach [60–73]. In other words, the continued development of PDB with less and less MP EUTs within is within the reach of currently available tools, experimental and computational. After almost half a century since the launch of PDB [9], the time is now ripe for this aim to be included on the structural biology development agenda.
4. While the focus here is MP EUTs within the 48-year-old PDB [9], case-specifically, it is more informative to map MP EUTs to MP structures, and closely examine the spatial positions of the missing residues), as it allows us to find out whether the EUTs actually reside in the structural proximity of the binding/action sites of conventional drugs/potential drug candidates or not,

towards an intact picture of MPs' roles (as more than half of current drug targets) in the life of a cell.

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