RESEARCH ARTICLE

Outer membrane β-barrel structure prediction through the lens of AlphaFold2

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Abstract
Most proteins found in the outer membrane of gram-negative bacteria share a common domain: the transmembrane β-barrel. These outer membrane β-barrels (OMBBs) occur in multiple sizes and different families with a wide range of functions evolved independently by amplification from a pool of homologous ancestral ββ-hairpins. This is part of the reason why predicting their three-dimensional (3D) structure, especially by homology modeling, is a major challenge. Recently, DeepMind’s AlphaFold v2 (AF2) became the first structure prediction method to reach close-to-experimental atomic accuracy in CASP even for difficult targets. However, membrane proteins, especially OMBBs, were not abundant during their training, raising the question of how accurate the predictions are for these families. In this study, we assessed the performance of AF2 in the prediction of OMBBs and OMBB-like folds of various topologies using an in-house-developed tool for the analysis of OMBB 3D structures, and barrOs. In agreement with previous studies on other membrane protein classes, our results indicate that AF2 predicts transmembrane β-barrel structures at high accuracy independently of the use of templates, even for novel topologies absent from the training set. These results provide confidence on the models generated by AF2 and open the door to the structural elucidation of novel transmembrane β-barrel topologies identified in high-throughput OMBB annotation studies or designed de novo.

KEYWORDS
bacterial outer membrane proteins; computational biology; databases, protein; models, molecular; protein conformation, beta-strand; protein folding

INTRODUCTION

Protein structure prediction is an important tool to gain insights into the function and biological role of macromolecular machines from three-dimensional (3D) models. While the number of known natural protein sequences has been increasing exponentially1,2 since the first sequencing of a protein in the 1950s, the experimental determination of macromolecular 3D structures is a laborious task. For this reason, and even with the recent considerable improvements in experimental biophysical methods, the rate by which protein structures are deposited in the Protein Data Bank (PDB)3 is much lower than that by which protein-coding sequences are made available through GenBank or the UniProt Knowledgebase. One way of tightening this gap is to use computational approaches such as homology modeling, threading, or ab initio methods for protein structure prediction.4–7

The Critical Assessment of Protein Structure Prediction (CASP)8 experiment provides a platform for the benchmarking of such methods. Since its onset in the early 1990s, it has fostered the...
development of multiple approaches exploring a wide range of data sources and computational techniques. Until recently, homology modeling was the method of choice to model 3D structures of proteins with homologs of known structure in the PDB, while ab initio methods were preferred for all others. However, ab initio modeling was rarely able to reach the same level of accuracy. That changed in 2020, with DeepMind’s second version of the AlphaFold algorithm (AF2) providing, on average, close to experimental accuracy levels for most targets in the 14th round of CASP (CASP14).  

AF2 is a deep neural network with two attention-based transformation modules, where evolutionary, physical, and geometric information is used to perform end-to-end protein structure prediction. The first module, the Evoformer, uses information from multiple sequence alignments and templates to generate a pair representation, a contact map of sorts, for the input. The second module, the structure module, uses this representation and the input sequence to fold the target protein. The network has been trained with all protein structures in the PDB as of April 30, 2018, and it is not tailored to any specific class of proteins.

However, the PDB is biased toward those proteins that are “easier” to experimentally characterize, with only 10% of its content corresponding to membrane proteins. For this reason, it is not expected that AF2 is able to predict the 3D structure of transmembrane proteins as accurately as soluble ones, especially when multiple domains are present. In a recent study, Hegedüs et al. assessed AF2 structure prediction of α-helical transmembrane proteins. They observed that the models predicted by AF2 exhibited a known fold of α-helical transmembrane proteins for all 1137 test cases, suggesting that the prediction of transmembrane proteins by AF2 is as accurate as for soluble proteins.

In this report, we focus on the second-largest class of transmembrane proteins: the outer membrane β-barrels (OMBBs). OMBBs are abundant in gram-negative bacteria but are also found in chloroplasts, mitochondria, and mitochondria-associated organelles. They have both medical and biotechnological importance as they are composed of an antiparallel β-sheet that connects back to itself to form a pore that crosses the outer membrane, where they perform a large variety of biological activities essential for survival. This can be accomplished either by a single chain or by multiple chains that form the pore upon oligomerization in the membrane.

While OMBBs are not found outside gram-negative bacteria and their related eukaryotic organelles, OMBB-like folds are also adopted by gram-positive efflux pumps, some pore-forming toxins, and other innate immunity-associated proteins. OMBBs and OMBB-like proteins are found in a large spectrum of protein families either as single domains, together with other domains, or in multiple copies in the same chain. Different families are composed of different numbers of β-strands. The diameter of the barrel depends on the number of β-strands and their shear, which is, simply put, a measure of the parallel displacement of the strands relative to each other.

OMBB and OMBB-like structure prediction is a challenging task as novel families emerge by the reuse and amplification of smaller pieces from other membrane β-barrels. In the special case of homology modeling, when dealing with a novel family for which no full-length template is known or for which the full-length template corresponds to part of a larger β-barrel, the resulting model will either correspond to (1) a mix of local matches with mismatching shears that prevent the proper closing of the barrel, or (2) an incomplete, open barrel incompatible with the membrane environment. Current membrane β-barrel modeling approaches circumvent these problems by using external information specific to these proteins. These include the generation of barrel blueprints directly from a theoretical description of a barrel, and the prediction of contacts between those segments from free energy potentials based on statistical models or evolutionary couplings.

In this report, we sought to evaluate how the family-agnostic AF2 network performs for OMBBs and OMBB-like membrane β-barrels. As of the time of AF2 training, about 100 single-chained OMBBs at a maximum of 70% sequence identity (Table S1) with 8 up to 26 β-strands were deposited in the PDB. Of these, 28 form multimeric complexes (Table S1). In parallel, 21 non-redundant multimeric β-barrel pores with 12–108 β-strands distributed between 3 up to 27 chains could be found in the PDB. Some of these are members of the OMBB superfamily, such as the 12-stranded trimeric autotransporter adhesins, but they also include toxins such as the 14-stranded heptameric anthrax protective antigen (PA) pore and the 108-stranded gasdermin A3 membrane pore, composed of 27 chains.

In the meantime, the structure of a 36-stranded single-chained OMBB, the translocon of the Fibrobacteres-Chlorobi-Bacteroidetes type 9 secretion system in complex with its 14-stranded OMBB “plug,” was solved by cryo-EM, and more than 30 previously unknown OMBB families were predicted at the sequence level, including the largest ever reported single-chain OMBB, with at least 38 predicted strands. In the case of long-known OMBB and OMBB-like topologies, structural information has been fed into the network during the training phase. Thus, even without using homologous structures for modeling, membrane β-barrel models of high accuracy are expected. But since the newest topology of a 36-stranded OMBB was discovered after the date limit for inclusion in the training set, it is unclear how AF2 performs in cases where the final topology is new to AF2 and what the impact of using templates is.

2 | METHODS

2.1 | Identification of membrane β-barrels and extraction of barrel geometric features with barrOs

barrOs (for barrel circle searcher) is an in-house-developed tool that, given a PDB structure, uses a graph-based approach to identify the strands that form a barrel fold and compute its geometric features. This includes the number of strands, the barrel diameter, and the shear number of the barrel region. The method is family-agnostic and can take as input (1) a PDB structure, (2) a list of PDB IDs, or...
(3) HHsearch output files. It can be targeted specifically to transmembrane proteins by using the Orientations of Proteins in Membranes database,\(^{49}\) as a source of 3D structures, and to membrane β-barrels specifically by combining it with the results from HHsearch.

For each structure to be analyzed, barrOs start by extracting all Cα atoms and search for all β-strands. This is done by (1) running DSSP\(^{50,51}\) and, in parallel, (2) detecting what we denote as “regular regions.” Regular regions are continuous backbone segments where the angle between the Cα\(_i\)–Cα\(_{i+2}\) and Cα\(_{i+1}–\)Cα\(_{i+3}\) vectors is lower than 25°. Regular and stranded region annotations derived from the DSSP (“E”) output are then fused, and the resulting continuous intervals referred to as “strands.”

Two strands where the minimum interstrand distance of their Cα atoms is less than 5 Å are considered adjacent, allowing the construction of a strand-connectivity matrix. This matrix is then used to build an undirected, labeled graph, and the cycle basis function implemented in networkX\(^{22}\) is used to identify the nodes, that is, the strands, that form a closed cycle.

Given that transmembrane β-barrels typically have an even number of strands (except for the 19-stranded mitochondria-specific OMBBs), if the resulting number of barrel-forming strands (the estimated topology) is uneven, this process is repeated using the regular regions or the DSSP-extracted strands until an even topology is obtained. If the result remains uneven, that topology is considered. Structures without detected structured barrels are excluded, and only those with a detected barrel fold are used for further analysis. This includes the estimation of the barrel height, the average diameter, and the shear number, as defined in Murzin et al.\(^{25}\)

In cases where an input file contains multiple chains but no barrel was detected for the target chain, if the -multimer flag is provided at input the analysis is repeated considering all chains. This enables the detection of transmembrane β-barrels that only form upon oligomerization. Conversely, the -complexes flag allows for the detection of multiple β-barrels in multiple chains of the same input PDB file, which enables the detection of β-barrel that form complexes with other β-barrel.

### 2.2 Collection of test case structures

We collected two different sets of test cases: (1) a set of single-chained OMBBs, which are all members of the OMBB superfamily and form the β-barrel domain using one single, continuous β-sheet, and (2) a set of multi-chained membrane β-barrels (MMBBs), which are all proteins that form a β-barrel domain only upon oligomerization and include members of the OMBB superfamily but also other transmembrane β-barrel forming proteins.

For the single-chained OMBBs set, 10 OMBBs of known structure covering topologies of 8- to 24-stranded barrels (Table S1) were used as input for searches against an HHM database of the PDB70 (as of February 2021) with HHpred\(^{23}\) through the MPI Bioinformatics toolkit.\(^{54}\) Default parameters were used and all PDB chains matched at a p-value better than 10 collected. These were then analyzed with barrOs in order to (1) identify the matched PDB IDs that carry a barrel fold, (2) extract geometric features of the barrel region, (3) extract the sequence of the barrel domains, and (4) identify those that form higher-order complexes with other OMBBs. With this, 129 unique single-chain OMBBs of known structure, of which 27 form higher-order complexes, were collected, and the Cα-sequences of the detected barrel regions, including the barrel-forming strands and the connecting loops, were extracted and used at later stages as input to AF2 and AF2-multimer.

For the MMBBs, 15 multimeric barrel-forming proteins of known structure covering topologies between 12- and 108-stranded barrels (Table S2) were used as input for searches against the PDB70 (as of April 2023) as described above. The resulting matches at a p-value better than 10 were further processed with barrOs using the -multimer flag. With this, 23 unique MMBBs were collected, and the Cα-sequences of the chains that form the detected barrel regions, including the barrel-forming strands but also the N- and C-terminal regions, were saved. In this case, we did not remove the N- or C-terminal regions as they emulate the loops that connect the strands in single-chained membrane β-barrels.

The targets were further matched to their corresponding models in the AlphaFold database (AFDB) or other experimental structural templates using the Structure Integration with Function, Taxonomy, and Sequence resource.\(^{55}\) This allowed for the alignment of modeled residues to the corresponding sequence in UniProt and for the linking of target UniProt IDs to all their experimental structures in the PDB and predicted models in the AFDB.\(^{11}\)

### 2.3 Running AF2

We ran AlphaFold v2.0.1\(^{10}\) and AlphaFold-multimer v2.3.0\(^{56}\) (AF2 and AF2-multimer, respectively) on a local cluster instance with three different parameter settings: The first was performed with the default pipeline, which includes the use of all templates found in the PDB (labeled “M”). In the second, AF2 was run without considering any templates by setting the --max_template_date option to 1900-01-01 (labeled “Mnotemp”). And in the third, template usage was partially allowed by setting the --max_template_date option to 1 day prior to the respective release date in order to exclude the deposited structure from being used as a template for modeling (labeled “Mrldate”).

Single-chain models were constructed for all 129 single-chained OMBBs, while high-order complexes were constructed for exemplary OMBB dimers and trimers. These were selected by filtering the sequences of the 129 single-chained OMBBs to a maximum sequence identity of 20% with MMseq2\(^{57}\) and selecting a maximum of three complexes per oligomeric state represented. For MMBBs, due to the large diversity and complexity of the folds found, we limited the analysis to those 12 with up to 16 β-strands.

### 2.4 Model comparison and quality assessment

All highest-ranking AF2 models were used as input for barrOs to identify barrel topologies and extract geometrical information of the
barrel domains. They were further compared to their reference (experimental or predicted) structure using superposition-based and superposition-free metrics. The superposition-based template modeling score (TM-score) and root-mean-square deviation (RMSD) were computed with TM-align for single-chain OMBBs and with US-align for multimers and complexes. The superposition-free per-residue local distance difference test score (Cα-lDDT), which evaluates local distance differences between Cα atoms in a model and its Reference, were computed with OpenStructure v2.2.0, and the interface-specific QS-best and average DockQ (DockQ-ave) scores with OpenStructure v2.5.0. QS-best quantifies the similarity between interfaces as a function of shared interfacial contacts, considering only interchain contacts between residues that can be mapped between the model and the reference. DockQ measures the quality of protein–protein docking models by providing a continuous score that combines three independent quality measures standardized by the Critical Assessment of PRedicted Interactions. In its original implementation, DockQ only operates on single interfaces, and averaging provides a combined score for higher-order oligomers.

These quality metrics were further compared to those reported by AF2 and AF2-multimer: the per-residue predicted lDDT (pIDDT) reported by AF2, which estimates how well the model would agree with the experimental structure based on the Cα-IDDT, and the combined predicted TM (pTM) and interface pTM (ipTM) reported by AF2-multimer, where ipTM estimates the TM-score of interactions between residues from different chains and, thus, the accuracy of interfaces in multimeric complexes.

To compare the models to experimental structures, the latter was used as a reference. To compare to those models in AFDDB, both our models and the experimental structures were used as references instead, so that the lack of coordinates and interactions in both the models and the reference experimental structures did not down weight local superposition-free scores.

### 3 | RESULTS

#### 3.1 | Modeling of single-chain OMBBs

As a first step, we evaluated how well AF2 captures the core geometric features of single-chain OMBBs in the presence and absence of templates, especially the topology of the domain, the average diameter of the channel, and the shear (Figure 1). For that, the 129 single-chain OMBB experimental structures and their corresponding AF2-predicted models were analyzed with barrO. The first observation is that AF2 predicted models with the correct topology for most cases; out of the 129, there were only two test cases where the number of strands in the model deviated by ±1. In these cases, visual inspection highlighted that the difference is not a result of an incorrectly modeled topology, but due to minor differences in the experimental structure and the AF2 model that misled barrO during the identification of regular regions (Figures S1A, B).

Regarding the shear and barrel diameter, there are also only marginal differences between the target structures and the models predicted by AF2, with some noteworthy exceptions. One striking case is that of Vibrio cholerae OmpT (PDB ID 6EHD), where the shear of the model generated with no templates (“Mnotemp”) was 18 residues smaller (Figure S1C). The reason here lies on an extracellular loop that in both AF2 models predicted using templates (“M” and “Mreldate”) and the experimental structure is modeled inward, facing the channel of the barrel, while in the “Mnotemp” model it faces the exterior, extending the strands that build the barrel region and leading to an incorrect value of the shear.

The agreement between the geometric features of AF2 models and their target experimental structure is also corroborated by superposition-based and superposition-free quality metrics. In the case of superposition-based metrics, high median TM scores, and correspondingly low RMSD values, were observed for all three experiments (Figure 2A, B). The highest median TM-scores (0.98...
± 0.02) were obtained with the AF2 default pipeline, in which template information is used for the prediction of models (“M”), but also when templates up to the release date (“Mreldate”) were allowed. Excluding templates completely (“Mnotemp”) only leads to marginally, and not statistically significant, lower TM-scores (0.97 ± 0.02).

**FIGURE 2** Full-length assessment of target single-chained structures and AF2 models. (A) The median TM-scores of the “M,” “Mnotemp,” and “Mreldate” experiments are 0.98 ± 0.02, 0.97 ± 0.02, and 0.98 ± 0.02, respectively. (B) The median RMSD values, as computed by TMalign, of the “M,” “Mnotemp,” and “Mreldate” experiments are 1.0 ± 0.5, 1.4 ± 0.5 and 1.3 ± 0.5 Å, respectively. (C) Solution NMR structure and AF2 model of PDB ID 2MLH shown in gray and blue, respectively. The backbone traces of the other 19 calculated conformers are shown in light gray.

**FIGURE 3** Predicted and calculated Ca-IDDT values per residue for two single-chained OMBB examples. (A), (B) PDB ID 1P4T, an OMBB with 8 β-strands. (C), (D) and of target PDB ID 4RL8, an OMBB with 12 β-strands. In (A) and (C) boxes and numbers indicate the β-strands, which are colored in the structural models in (B) and (D). The average correlation coefficients between predicted and calculated IDDTs over the two models shown in (A) and (C) are 0.895 ± 0.005 and 0.756 ± 0.009, respectively.
This testifies to an overall high accuracy of the AF2 models independent on the use of templates, yet there are a few outliers below and above the lower and upper quartile of the TM-score and RMSD distributions, respectively. The lowest TM-scores of <0.5 (and highest RMSD values of >4 Å) were observed for AF2 models of the 8-stranded Opa OMBB (PDB ID 2MLH), which is crucial for the recognition and engulfment of bacterial pathogens *Neisseria gonorrhoeae* or *Neisseria meningitidis* by human cells during pathogenesis. The target structure used is one of the 20 calculated conformers with the lowest energy determined by solution NMR. While the barrel region was predicted accurately, only the extracellular loops did not overlap with the target structure (Figure 2C). This is further highlighted by the superposition-free per-residue Cα-lDDT (Figure S2B), where the scores are higher (> 75) for stranded regions than for the loops (< 50). In this particular case, the flexibility of those loops is in fact essential for the function of the protein, and thus it is not surprising that the AF2 prediction does not match the selected solution NMR structure.

This trend is also observed for other cases where the TM-score is above 0.9, an uncertainty also captured by the predicted Cα-IDDT (pLDDT) computed by AF2. Examples of an 8-stranded and a 12-stranded OMBB are shown in Figure 3, highlighting the high prediction accuracy of pLDDT. Both the Cα-IDDTs and pLDDTs reach values between 95 and 100 for β-strand regions, while the loops (especially those facing the extracellular side of the outer membrane) result in lower IDDT values, with no striking differences between the three AF2 “M,” “Mnotemp,” and “Mrelate” predictions. Limiting the analysis to the β-strands forming the barrels (which we refer to as “barrel cores” for the remaining text) (Figure 4A), resulted in extremely high median IDDT values of 98.2 ± 1.6, 97.0 ± 1.9, and 97.1 ± 1.9 for the models of the “M,” “Mnotemp,” and “Mrelate” experiments, respectively; corroborating the marginal deviations observed in the different barrel geometric features.

Interestingly, while the IDDT and pLDDT correlate well, their distribution for the barrel core regions is different independently of the use of templates (Figure 4A), with AF2 underestimating, on average, their accuracy. In four cases, however, the confidence of the AF2 models for the barrel regions was above 85 while the IDDT was lower, but still within a reasonable range of 75–80 (Figure 4B). The three first cases are PDB IDs 6QWR, 2MLH, and 2K0L, all of which are 8-stranded OMBBs whose structures were determined by NMR spectroscopy with 100 or more calculated conformers. The fourth case corresponds to PDB ID 5O8O, the first experimental structure of a 19-stranded mitochondrial import receptor subunit Tom40. Its experimental structure was determined through rigid body docking into a 6.8-Å resolution cryo-EM map of a homology model generated based on the x-ray structure of a homologous mitochondrial voltage-dependent anion channel. While the overall topology of the AF2 model matches this experimental structure and the same residues build up the barrel core, a few strands exhibit a distinct frame-shift along its axis in all three predicted models (Figure S3A), resulting in average IDDT values below 80. A more recent structure of a homologous Tom40 determined by cryo-EM at higher resolution (PDB ID...
which was also a target in this study, agrees with the AF2 model (Figure S3B).

Most of these cases, however, were either part of or had full-length homologs in the AF2 training set, thus such high accuracy could be expected a priori. Of higher interest is the performance of AF2 for cases of novel topology, unknown to AF2. Unfortunately, only one such case is available in the PDB and corresponds to the only known 36-stranded OMBB (PDB ID 6H3I). It forms the translocon of the Fibrobacteres-Chlorobi-Bacteroidetes type 9 secretion system and its structure was deposited in the PDB after April 30, 2018 (Table S1). Although AF2 had never “seen” a 36-stranded barrel, the barrel core, and its geometric features were predicted accurately, regardless of the use of templates (Figure 5). In all cases, however, local backbone conformations of the barrel region in the AF2 model are closer to standard geometries of β-sheets than those in the experimental structure. This is not a surprising result as the target is a 3.5 Å cryo-EM structure and lower resolutions lead to higher uncertainties of the atomic coordinates. In the model generated with templates, even the intra- and extracellular loops matched those in the target structure with high accuracy, as also seen in the comparison of predicted and calculated Cα-lDDT values (Figure S2A). There were only minor displacements in the loop regions of the model predicted without any template information.

3.2 | Comparison to AlphaFold database

For our study, we used the Cα-sequence as the input to AF2. Due to the difficulties often encountered when constructing loops from experimental electron density maps, especially at a lower resolution, about 50% of our test cases have missing coordinates. This leads to input sequences lacking sequence stretches that are on average 11 ± 5 residues long. Still, even in the absence of these stretches, AF2 is able to accurately capture the fold and topology of the target β-barrel core and there is no statistically significant difference between the geometry and model accuracy of the OMBBs with and without shortened loops (Figure S4). Most of our outliers do not have shortened loops.

Thanks to the large-scale efforts put together by the AlphaFold team and the EMBL-EBI, there are now over 200 million single-chain structural models for most protein sequences in UniProtKB, available through the AlphaFold database. This includes 127 of the single-chain OMBBs in our dataset and those cases where coordinates are missing from the experimental structure. AFDB models superpose to our models and the reference experimental structures with an extremely high TM-score (0.97 ± 0.04 for all cases) and low RMSD (0.9 ± 0.6 for “M” and 1.0 ± 0.6 for all others) (Figures S5A, B). This is also captured by the Cα-lDDT of the stranded regions, where for most of the cases the AFDB models agree with both our models and the experimental reference structure (Figure S5C). For the AFDB models, we observed that AF2 also underestimated its accuracy in the barrel core.

There are only a few outliers, especially in one case where the TM-scores to the AFDB models are below 0.7 and the Cα-lDDT of the strands goes below 80%. This corresponds to PDB ID 2JMM, another target whose experimental structure was determined by NMR spectroscopy with over 100 calculated conformers. Interestingly, the experimentally determined sequence, although assigned to UniProt ID P0A910, is the result of a design effort and is not a 1–1 map to the UniProt sequence and, therefore, to the model in AFDB, which may explain the differences observed between the model and our references.

3.3 | Complexes and oligomers

Out of the 129 single-chain OMBBs in our test set, 27 are deposited in the PDB as part of higher-order complexes with OMBBs, with 21 being trimers and 6 dimers. From these, we selected three non-redundant trimers and three non-redundant dimers, which include the
homotrimer of the sucrose-specific porin ScrY from *Salmonella enterica* (PDB ID 1A0S), the OmpF homotrimer from *Salmonella enterica typhi* (PDB ID 3NSG), the homotrimer of a mutant of the *Fusovulum blasticum* OpmA porin (3PRN), the homodimer of *Escherichia coli* Phospholipase A (PDB ID 1QD6), the homodimer of the RagA subunit of *Porphysomonas gingivalis* RagAB peptide transporter (PDB ID 6SLN), and the heterodimer formed by the 36-stranded translocon of type 9 secretion system and its 14-stranded OMBB “plug” (PDB ID 6H3I). For all cases except the heterodimer, templates were available when the training set of AF2-multimer was generated.

As for the single chains, AF2-multimer captures the overall interfaces accurately for all cases, with average QS-best scores higher than 0.9 and a DockQ score higher than 0.8 independently of the use of templates (Figure 6A, B). However, the quality of the interfaces tends to be lower when no template information is used, with a DockQ score of 0.82 ± 0.11 for “Mnotemp” compared to 0.88 ± 0.10 for “M” and 0.84 ± 0.13 for “Mreldate,” a trend that is also somewhat also captured by the reported pTM + ipTM score (Figure 6C). The only outlier is PDB ID 6H3I (Figure 6D), whose represented interface was never seen by AF2-multimer. In this case, the model generated using default parameters (model “M”) is closer to the reference than those generated in their absence (“Mnotemp”) or when using only templates released before the target was released in the PDB (“Mreldate”).

Globally, the “M” model superposes to the reference with an RMSD of 0.8 Å, while the other two have an RMSD of 1.7 and 1.6 Å. Despite the overall good superposition, the DockQ-ave and QS-best scores around 0.8 and 0.6 for these two models reveal that the interactions at the interface are modeled differently. Given the relatively low resolution of the experimental model, it is not straightforward to evaluate which modeling protocol resulted in the most biologically representative interface.

In the case of multimeric membrane β-barrels, the accuracy of AF2-multimer is equally high, with both the full complex and the β-barrel core modeled correctly (Figure 7A–C). However, one interesting outlier stood out, the 14-stranded heptameric anthrax PA pore (PDB ID 3J9C). For this case, barrOls did not find any barrel in the “M” and “Mreldate” models, and for both cases, the TM-score was below 0.8 and the Cα-lDDT of the expected barrel core below 30%. When no templates were used, the model generated agreed with the reference (TM-score of 0.98 and Cα-lDDT of 96.4%) (Figure 7D). Inspection of these models revealed that AF2-multimer constructed different known conformational states of the anthrax PA. Indeed, this is a dynamic complex, and the pore forms by the extension of a stretch of almost 100 residues upon interaction with a receptor at the cell surface.67 What AF2-multimer constructed in the presence of templates was the “prepore” form of the complex, while in their
absence it constructed the target “pore” form. While the PDB contains over 30 experimentally determined structures for the target sequence representing the multiple conformational states of the heptamer, most of the templates selected by AF2 corresponded to the “prepore” form, which may explain why all models constructed by AF2 for this target converged into this conformational state. When template information was absent, AF2-multimer knew from its training set that an extended, “pore” form could exist.

4 | DISCUSSION

Given the under-representation of transmembrane proteins in the PDB, and consequently in the training set of AF2, it is imperative to evaluate how the algorithm performs for such an important class of proteins. While a study was previously carried out for α-helical transmembrane proteins,\textsuperscript{14} we focused on the second-largest category: the OMBBs, especially those found at the surface of gram-negative bacteria and their eukaryotic homologs but also multimeric transmembrane β-barrels that include the analogous gram-positive, multimeric transmembrane β-barrels\textsuperscript{68} and the multimeric OMBBs that are formed by separate polypeptide/protein chains upon oligomerization.\textsuperscript{69}

We identified 129 nonredundant single-chained OMBBs in the PDB, with topologies ranging from 8 to 36 strands, a number that is representative of the OMBB families defined in the OMP database (Roumia et al. 2021). In all cases, AF2 predictions were highly accurate; all experiments resulted in extremely high median TM-scores above 0.97 and low median RMSD values below 1.4 Å. Overall, no significant differences were observed. For all cases, AF2 correctly predicted the topology of the domain as well as the shear and average diameter, demonstrating that in the case of OMBBs, the accuracy of the prediction is not substantially affected by the use or omission of templates. The same trend was observed for their models in the AFDB, their complexes with other OMBBs, and those β-barrels that form only upon oligomerization.
However, targets with structures deposited in the PDB prior to April 30, 2018, were part of the training set. So even when removing the experimental structure from the template list, structural information might still be used to predict the model as it is stored in the network. One striking example is that of the heptameric anthrax PA pore, where the presence or omission of templates drove modeling into two different conformational states of the β-barrel. The only case in our test set with a topology completely new to the AF2 network was the 36-stranded OMBB from the Fibrobacteres-Chlorobi-Bacteroidetes type 9 secretion system translocon, as well as its heterodimer with a 14-stranded OMBB “side plug.” Although the network has never seen a 36-stranded OMBB, its predictions were highly accurate, even improving on the geometry of the backbone of an experimental low-resolution structure. AF2 predicted correctly the 36-stranded topology, as well as the diameter and the shear of the barrel, but also the intricate folds of the extracellular loops and the interactions with its OMBB plug at an extremely high level of detail. The models for this test case were of the same accuracy as for those of well-known topologies, indicating that structural information of templates or close homologs is not essential for a correct prediction.

On average, the per-residue IDDT is lower for loops, especially those facing the extracellular side of the outer membrane and independently of the use of templates. This is likely the result of the higher flexibility of extracellular loops observed in experimental structures, which is important for protein function. Such flexibility makes it difficult to predict a static snapshot of those regions at an atomic level of detail, which in turn decreases their pLDDT. Larger differences were also observed for cases where the target was either solved by solution NMR or low-resolution cryo-EM. The same was observed in CASP14, where AF2 also performed worst for NMR structures. More recently, Fowler et al. examined this by measuring the accuracy of solution NMR structures and comparing them to AF2 predictions. They concluded that, in general, AF2 models are more accurate than NMR ensembles. This is especially the case for β-sheet proteins, which include OMBBs, providing a consistent explanation for the observed low IDDT values when comparing AF2 models with NMR structures. However, when evaluating these values, it must be noted that the IDDT scores are merely a measure of how similar the AF2 models and the experimental structures are, without providing information on which structure is closer to the truth.

For our study, we used the amino acid sequence as extracted from the CO of the target reference structure. This means that residue stretches present in the corresponding protein sequence but unresolved in the 3D structure were excluded from modeling. While this could have caused major differences between the modeled and reference structures, this was not the case, highlighting that AF2 was able to accurately capture the OMBB sequence signals and accurately identify the correct register of interactions that make the β-barrel core. This result is of particular interest when the goal is to explore existing transmembrane β-barrels for protein design, especially at the external loop regions. Although the test set is small, our results provide confidence in the models generated by AF2 for single-chain OMBBs, multimeric transmembrane β-barrels, and their higher order complexes, especially for those cases with previously unknown topologies or novel designed sequences.

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CONFLICT OF INTEREST STATEMENT
The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT
The models generated, as well as the structural features extracted for them and the reference structures, are available in: https://www.modelarchive.org/doi/10.5452/ma-ombbaf2. barrOs can be downloaded from: https://git.scicore.unibas.ch/schwede/barrOs. In the repository, detailed instructions on how to use it for the general analysis of proteins with an expected barrel fold are provided, and the HHsearch results used in this work are provided in the Examples and Examples_multimers folders.

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SUPPORTING INFORMATION
Additional supporting information can be found online in the Supporting Information section at the end of this article.

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