AlphaFold predictions on whole genomes at a glance

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Abstract

For model organisms, AlphaFold predictions show that 30% to 40% of amino acids have a (very) low pLDDT confidence score. This observation, combined with the method's high complexity, commands to investigate potential hallucinations and difficult cases. We do so via three contributions. First, we leverage the 3D atomic packing properties of predictions to represent a structure as a distribution. This distribution is then mapped into the so-called *2D arity map*, which simultaneously performs dimensionality reduction and clustering, effectively summarizing all structural elements across all predictions. Second, using the database of domains ECOD, we study potential biases in AlphaFold predictions at the sequence and structural levels, identifying a specific region of the arity map populated with low quality 3D domains. Third, with a focus on proteins with intrinsically disordered regions (IDRs), we identify another specific region of the arity map enriched for false positives in IDRs.

Summarizing, the arity map sheds light on the accuracy of AlphaFold predictions, both in terms of 3D domains and IDRs.

1 Introduction

AlphaFold: complexity and predictions. AlphaFold is the structure prediction program developed by Deepmind [1]. Enabling the reliable prediction of a protein structure from its amino acid sequence, AlphaFold has opened a new era in structural biology, and its leaders, D. Hassabis and J. Jumper, were co-awarded the 2024 Nobel prize in chemistry. Practically, each prediction is provided as a PDB file, with each amino acid qualified by a reliability score called the pLDDT (prediction local distance difference test) [2]. The AlphaFold developers proposed the following four confidence ranges: very high: $0.9 \leq$ pLDDT; high: $0.7 \leq$ pLDDT < 0.9; low: $0.5 \leq$ pLDDT < 0.7; very low pLDDT < 0.5. On the experimental side, the Protein Data Bank currently features $\sim 230K$ experimental structures against the $\sim 200M$ known protein sequences cataloged in UniProtKB. This disparity underscores the importance of structure prediction for whole whole genomes [3] (SWISS-MODEL Repository), a critical task to advance our understanding of proteomes. This endeavor has recently been undertaken with AlphaFold, and predictions deposited in the AlphaFold-DB database–[4] and https://alphafold.ebi.ac.uk. An exhaustive analysis of structures in AlphaFold-DB shows that for model organisms, AlphaFold predictions contain 30% to 40% of amino acids with a (very) low pLDDT (confidence) score (Section S2).

From the methods' perspective, AlphaFold uses two main modules, known as the Evoformer and the structure module, within which so-called transformers play a key role [5] to unveil long range couplings between amino acids. The overall algorithm is very complex, as evidenced by the ablation study which compares 10 variants to assess the merits of the main building blocks [1, SI]. In the context of the bias-variance trade-off in machine learning in general [6], and of the double descent phenomenon for large deep models [7], an important point is the balance between the number of parameters in AlphaFold and the pieces of information used to train it. The number of parameters has been estimated circa 100 millions. On the other hand, the number of non redundant sequences used in the self-distillation procedure is 350k [1, SI,

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Section 1.3], which, assuming a median size of 500 amino acids, results in about $\sim 1.75 \cdot 10^8$ amino acids. (Distillation is the process of transferring knowledge from a teacher to a student [8], a simplifying process bearing similarities to coreset calculations in a more algorithmic setting learning [9].)

The significant percentage of amino acids with a (very) low pLDDT and the `AlphaFold` complexity call for further investigations to better understand the quality of predictions. We undertake this endeavor using the predictions on whole genomes provided in the database `AlphaFold-DB`.

Contributions. We explore the relationship between `AlphaFold`'s complexity and the relatively high proportion of amino acids with (very) low pLDDT scores by addressing three key questions.

Q1. Predictions and structural motifs. Two related topics in analyzing `AlphaFold` predictions are the pLDDT values and the structural elements produced. The pLDDT metric has been thoroughly analyzed, revealing its effectiveness as an indicator of result robustness; high mean pLDDT values are associated with minimal structural differences between `AlphaFold` and trRosetta models [10]. However, as indicated by a lack of association with B-factors, pLDDT does not appear to correlate with flexibility [11]. Although pLDDT can predict structural disorder, alternative estimators using `AlphaFold`-predicted structures, such as the relative solvent accessibility (RSA), provide a more accurate measure of disorder [12]. In a complementary vein, the structural elements in predictions have been analyzed using the `Geometricus` algorithm [13], which encodes structural fragments based on (i) a decomposition of protein into structural fragments (based on k-mers or spherical neighborhoods), (ii) a discrete encoding of invariants (moments) these fragments into shape-mers, and (iii) a counting step of shape-mers yielding an embedding of the protein. Clustering embeddings (using non-negative matrix factorization) from 21 predicted proteomes from `AlphaFold-DB` yielded 250 clusters, with 20 main clusters/superfamilies [10]. An embedding based on shape-mers has been obtained using dimensionality reduction (t-SNE, [10, Fig. 2]). The analysis proposed by `Geometricus` is very intuitive and is appealing since the encoding can be used to query the database. On the other hand, in the context of `AlphaFold` predictions, each structure is processed as a whole regardless of individual pLDDT values.

To refine structural analysis, we develop a progressive encoding of each structure based on two filtrations, that is a sequence of nested shapes starting with the empty set and ending with the entire structure. Distributions associated to these filtrations can be used to compare structures using optimal transportation theory [14, 15]. Moreover, these distributions can be summarized by a small number of integers, yielding the *arity map*). In 2D, this map yields a natural low dimensional embedding and clustering of all structures.

Q2. Predictions and low quality domains. The aforementioned complexity of `AlphaFold` and the commensurate information size raises the question of potential biases in the model. To address this, we examine the locations of predicted low-quality domains. To understand our approach, it is important to recall that protein domain classification is a cornerstone of protein science [16, 17, 18]. A particularly noteworthy effort is the recently published ECOD structural domain classification of the entire human proteome [19], which leveraged `AlphaFold` predictions to enrich its classification with several new structural domains categorized by confidence scores as high or low. A domain is classified as low-confidence if it appears globular by its predicted aligned error (PAE) description but lacks a strong homologous hit (via a HMM calculation) within the `ECOD` reference–[19] and `prodata.swmed.edu/ecod/`.

We investigate whether the training of `AlphaFold` introduces biases in the prediction of low-quality domains by analyzing their distribution across the arity map. Notably, we identify a specific region of the arity map that is disproportionately populated by such domains.

Q3. Predictions and intrinsically disordered regions. Since its publication, it has been observed that the low-pLDDT regions in `AlphaFold` predictions correlate very well with observed or predicted intrinsic disordered regions (IDRs) [20]. Indeed `AlphaFold` outperforms state-of-the-art methods such as IUPred2 on disordered region detection on a benchmark of `DisProt` targets and PDB controls [10]. Indeed the per-residue pLDDT scores on the `AlphaFold-DB` human proteome prediction yields a bimodal distribution where the two peaks identify ordered and disordered regions [21]. Nonetheless, there are exceptions: in conditionally folded

IDRs are usually predicted as stably folded regions, and studies seems to imply that 10% of the low-pLDDT regions are not related to disorder [21].

To complement the previous finding, we show that although most of the `AlphaFold` disorder predictions are supported by experimental evidence, predictions with certain structural characteristics (a specific region in the arity map) are much richer in false positive IDR detection than others.

2 Results

2.1 Q1. Predictions and structural motifs

Charting whole genomes with the arity map. We provide a simple and readable encoding of these structural elements using the *arity* of a C_α carbon, namely the number of neighbors within a ball of fixed radius – taken as 10Å to account for non covalent contacts. In regions with classical α and β folds, we expect the arity mode to be in the range 15-20 (Section S1, Fig. S1). In unstructured regions, the mode is typically in the interval 5-7. For a given prediction, having sorted all C_αs by increasing arity value, we compute the corresponding histogram H_{C_α} and associated cumulative distribution function denoted CDF_{C_α}. The histogram provides a detailed encoding of packing properties of all residues, and two histogram can be compared using optimal transportation theory in general, and the so-called *Earth mover distance* in particular [14, 15]. This histogram can be summarized using a few specific values. Consider two quantiles, say $q_1 = 0.25$ and $q_2 = 0.75$. We define the *arity signature* of a polypeptide chain as the minimum arities for the CDF to reach q_1 and q_2 respectively, which we denote as $\texttt{arity}_{C_\alpha}(q_1) = \mathrm{CDF}^{-1}_{C_\alpha}(q_1)$ and $\texttt{arity}_{C_\alpha}(q_2) = \mathrm{CDF}^{-1}_{C_\alpha}(q_2)$ (Fig. 1). We process all proteins of a given genome and build the *arity map*, namely the heatmap counting the number of predictions with a prescribed arity signature (H. Sapiens, Fig. 3).

The analysis of this map provides a whole genome analysis at a glance. The x-axis $\texttt{arity}_{C_\alpha}(q_1)$ characterizes the extent of *loose packing*–low values $\mathrm{CDF}^{-1}_{C_\alpha}(q_1)$ is an indication of the presence of unstructured regions, while the y-axis characterizes the degree of *compactness*. For a given cell of this map, the distance to the diagonal $y = x$ encodes the coherence of the overall packing of the region of the protein selected by the quantiles q_1 and q_2. For example, for square on the diagonal, a fraction $q_2 - q_1$ of a.a. have the same arity, stressing the coherence of the overall 3D structure.

We produced the arity map for all models genomes from `AlphaFold-DB` (H. Sapiens, Fig. 3; other species, Section S4). At first sight, these maps are bimodal. The most pronounced mode corresponds to well folded proteins, in the arity map region $[14, 18] \times [20, 25]$. The second mode corresponds to the region $[4, 8] \times [10, 15]$, with in particular a dark spot in the cell 7×13. These modes are in general separated by low density regions in the heat map. The inspection of genomes beyond the model ones yields different patterns, though. For example, P. Falciparum exhibits a continuum between the previous two modes, which is consistent with the overall low quality of predictions noted above.

Notably, arity and pLDDT are found to strongly correlate (Fig. 3(B)), confirming that the arity is also a good proxy for prediction robustness. Yet, pLDDT is not able to show the finer details of the structural repartition that emerges from the arity map.

We further investigate the hot spot at 7×13 of the human genome (Fig. 4). Contrary to the rest of the $[4, 8] \times [10, 15]$ region, this hot spot does not find any counterpart neither in the arity plot of the human proteins of known structure (Fig. 3(C), in red) nor in the arity plot of experimentally-confirmed disordered proteins (Fig. 6). These `AlphaFold-DB` predictions therefore represent disordered structures or inaccurate predictions.

To further characterize these proteins, we perform a hierarchical clustering of arity histograms using the Earth Mover distance as metric [14, 22]. As already noticed, a 2D arity map reduces the arity histograms using two integers only. As expected, the clustering unveils the finer structure of proteins in this cell, with three main classes (Fig. 4(B,C,D)).

Arity based filtration. We now sketch the topological analysis methods used to bridge the gap between pLDDT values and structural aspects. See Section S1 for the details.

The variability of arities coding the compactness of the atomic environments around C_αs allows incrementally *building* the protein by inserting the a.a. by order of increasing arity and connecting each a.a. to its predecessor (N-ter) and successor (C-ter) if already inserted. This process, which again consists of incrementally inserting a.a., yields a sequence of nested shapes called a *filtration* [23]. Along the way, one maintains the connected components which appear and merge, using a Union-Find data structure [24]. The curve coding the evolution of the number of c.c. is termed the *connected components with arity* curve (c.c. curve for short). We also assume one is able to detect the *salient* (also called *persistent*) local extrema on these curves.

The case of well folded proteins follows this pattern as focusing on salient extrema yields a unimodal arity curve with a salient extremum in the range 15-20 (Fig. 1(Top, blue curve)). The same holds for unstructured proteins, excepts that the local extremum is attained at values $\sim 5-7$ (Fig. 1(Middle, blue curve)). The signature is more informative for proteins having both high and low quality regions (Fig. 1(Bottom, blue curve)). For such proteins, the curve of connected components is typically bimodal, since one successively forms the regions with low and high arity.

Concluding, the ability to detect the persistent local extrema on these curves gives a direct indication on the structural elements encountered in the predictions.

Remark 1 *For additional examples, the reader is referred to the following figures, panel (C): folded proteins: Fig. S25, Fig. S26; unfolded proteins: Fig. S27, Fig. S28); proteins with folded and unfolded regions: Fig. S29, Fig. S30).*

pLDDT based filtration. A filtration can also be built by inserting a.a. by increasing negative pLDDT, so as to favor the emergence of connected components involving a.a. with large pLDDT values. As an example, assume that all a.a. can be segregated into (i) a first group of a.a. corresponding to an unstructured region on the N-ter side, with low confidence (say pLDDT < 0.5); and (ii) a second group of a.a. corresponding to a structured region on the C-ter side, with high confidence (say pLDDT > 0.5). Along the insertion, one observes the formation of the former and the latter regions (Section S1, Fig. S2). For a region with random pLDDT values in a range, the insertion of individual a.a. results in a well behaved curve peaking at half of the amino acids, and whose maxima if conjectured to be $1/4$ of the number of a.a. (Fig. S3; conjecture 1).

The case of `AlphaFold` predictions follows these patterns (Fig. 2, left column, top and middle cases), with a clear unimodal distribution upon selecting salient extrema. The cases of high interest are those with low and high confidence regions, as their c.c. curve again exhibits a clear bimodal profile, as evidenced by the salient extrema (Fig. 2(Bottom)). On this example, the plateau at five connected components corresponds to five helices–four long ones plus a shorter one. The plot enables estimating the number of amino acids on the well folded regions as $\sim 781 - 4 \times 85 = 440$ – using the $1/4$ conjecture mentioned above. On this example, the sum of the size of helices being of ~ 400 amino acids.

The persistence diagrams associated with the construction of the filtration also exhibit the dynamics of connected components (Fig. 2, right column). For example, for the protein from D. Melanogaster, one clearly sees the dynamics for the high (resp. low) confidence region of the molecule on the left (resp. right) part of the diagram.

Remark 2 *For additional examples, the reader is referred to the following figures, panel (E): folded proteins: Fig. S25, Fig. S26; unfolded proteins: Fig. S27, Fig. S28; proteins with folded and unfolded regions: Fig. S29, Fig. S30.*

Statistics to query whole genomes. The analysis tools just presented can naturally be used to query a whole organism so as to identify proteins with specific properties.

arity signature–for which one can also vary the number of points and their positions (Section S1, Def. 1), the connected components curves (for arity and pLDDT), and their salient points, and naturally the persistence diagrams.

2.2 Q2. Predictions and low quality domains

We investigate potential learning biases in AlphaFold predictions at the sequence and structure levels.

At the sequence level, counting the number of homologous structures in UniProtKB for each arity signature exhibits a clear trend between the arity signature and the average number of homologous sequences (Section S1.4, Fig. S55(A)). However, mapping these homologs to their cluster representatives in the current release of Uniclust30 erases this bias (Fig. S55(B)), simply showing that structured proteins have more homologous sequences in nature. We also note that the quality of predictions in terms of average pLDDT shows a gradient towards high arity values (Fig. 3(B)). Since both the AlphaFold direct and distillation training algorithms construct MSAs of non-redundant sequences with sequence identity $< 30\%$, we conclude that a large and diverse input MSA is necessary but not sufficient for guaranteeing high-quality predictions.

We proceed with the investigation of potential biases at the structural level using the recently published ECOD structural domain classification of the entire human proteome [19]. Using AlphaFold-DB predictions, ECOD could enrich its classification with several new structural domains. At the level of H-groups in ECOD hierarchy, each identifier refers to a cluster of sequence-homologous, same-topology domains in tight relation with the SCOP superfamily level. For each arity signature, we thus collect the unique ECOD H-group identifiers and the corresponding folds. The resulting 2D-histogram shows a clear correlation with the protein count and the number of ECOD folds (Fig. 5(A), upper triangle for count and lower triangle for ECOD folds). In order to remove the correlation and study the residual signal, we consider the signed difference of the two rescaled histograms. (The rescaling ensures that the two maxima are equal.) This difference map shows two main domains (Fig. 5(B), red and blue regions). On the one hand, the region of the main peak *i.e.* 13×20 until the 7×13 peak (excluded) shows an enrichment in ECOD domains. On the other hand, the region of the 7×13 peak towards the bottom left of the arity map (IDP region, see next Section) gets depleted.

ECOD also provides a set of low-confidence domains associated with low pLDDT and PAE (predicted aligned error) values [19], enabling the calculation of the enrichment relative to this dataset. Remarkably, the former *red* region gets split, the enrichment in low confidence domains being confined to the 7×13 signature as well as the arity signatures $[5, 15] \times [14, 20]$. This extended region contains structures richer in IDRs, but still having well-formed domains. Conversely, the $[5, 7] \times [5, 12]$ region is poor both in standard and low-confidence well-formed domains, hinting at a complete dominance of IDRs over structured regions. These observations call for a further inspection of IDPs/IDRs in the arity map.

2.3 Q3. Predictions and intrinsically disordered regions (IDRs)

Several recent works assume that in AlphaFold's predictions, regions with low pLDDT and/or not displaying secondary nor tertiary structure are to be considered intrinsically disordered rather than just the product of insufficient or unexploitable information. The tools developed in the previous sections can help assessing whether and when a correlation between unstructured regions in AlphaFold's prediction correlate with experimental evidences of disordered regions. To do so, we consider the DisProt database, which collects disordered regions from more than 1300 target protein sequences. Many of the benchmarked sequences correspond to proteins of known structures, present in the PDB. For this test, we focus on the 266 targets lacking structural data and that have a predicted structure on AlphaFold-DB. We take a threshold of pLDDT $< 50\%$ to define low confidence regions and check whether they correspond to annotated IDRs in DisProt. Notably, DisProt is known not to provide complete annotations, thus in principle AlphaFold could discover new unannotated IDRs on the DisProt targets. The simplest hypothesis consists of expecting an incompleteness proportional to the extent of the predicted disordered regions–regardless of other structural characteristics of AlphaFold predictions. However, while AlphaFold predicts a large number of amino acids as part of IDRs for the region of the arity map $\texttt{arity}_{C_\alpha}(0.25) < 7$, most of these are not annotated as such in DisProt (Fig. 6). Predictions populating that part of the arity plot are thus likely to be of poor quality: this is not surprising, as the previous section details that proteins in this region often have low numbers of homologous structures.

3 Discussion and outlook

This work introduces a novel representation of structural elements in proteins by employing a filtration of amino acids depending on their 3D environment and the corresponding distribution. This distribution enables the comparison of structures using optimal transportation, and the persistent components of the filtration can be used to compare structural motifs from different proteins. To simplify this representation, the distribution can be reduced to two integers, forming the *arity map*, which simultaneously performs clustering of coherent structures and dimensionality reduction. At the genome-wide level, this clustering is used to study two important properties. First, we identify a specific region of the arity map populated with low quality structures. The region is enriched in low-confidence domains recently published in the `AlphaFold` enriched version of the `ECOD` database. Most of these domains may be termed *hallucinations* since they may appear valid, but are incorrect and/or fabricated. Second, regarding the prediction of intrinsically disordered regions, we show that the arity map exhibits another specific region with a high rate of false positive IDRs. While the performances of `AlphaFold` for IDP prediction have been praised, the notion of isolated structure for IDPs is iconoclastic since dynamics play a key role, and future work will likely address the generation of ensembles. We anticipate that these analysis will prove useful to understand limitations of `AlphaFold` regarding low quality domains and false positive IDRs.

In a broader context, the structure encoding as a distribution provides a compact and useful encoding for searching structural similarities. We anticipate that the resulting 2D encoding, by performing clustering and dimensionality reduction at once, will be used to chart important biological/biophysical properties of proteins. Our persistence-based analysis may also find broad applications at the meso-scale level, *e.g.* to study aggregation phenomena.

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4 Artwork

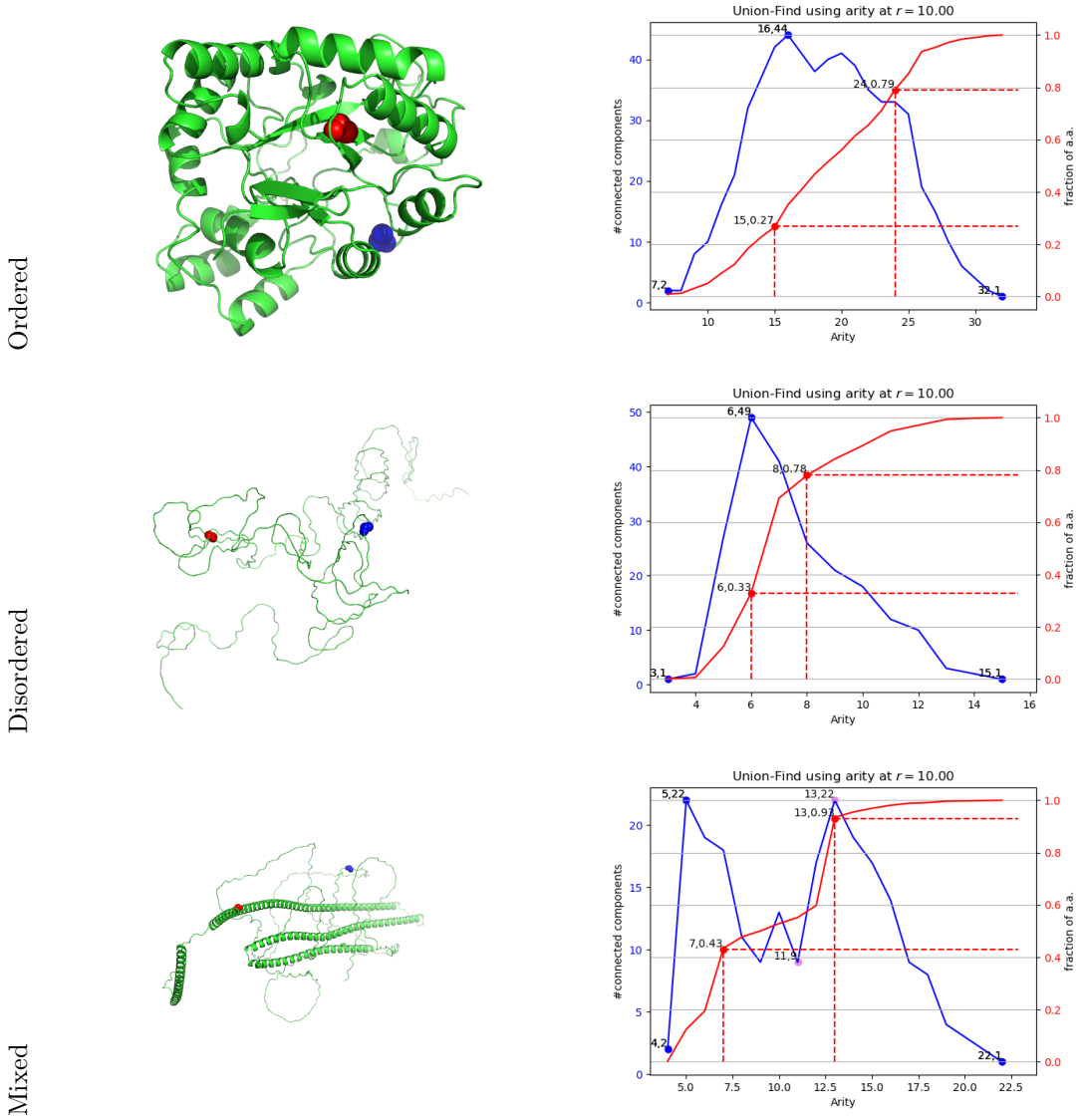
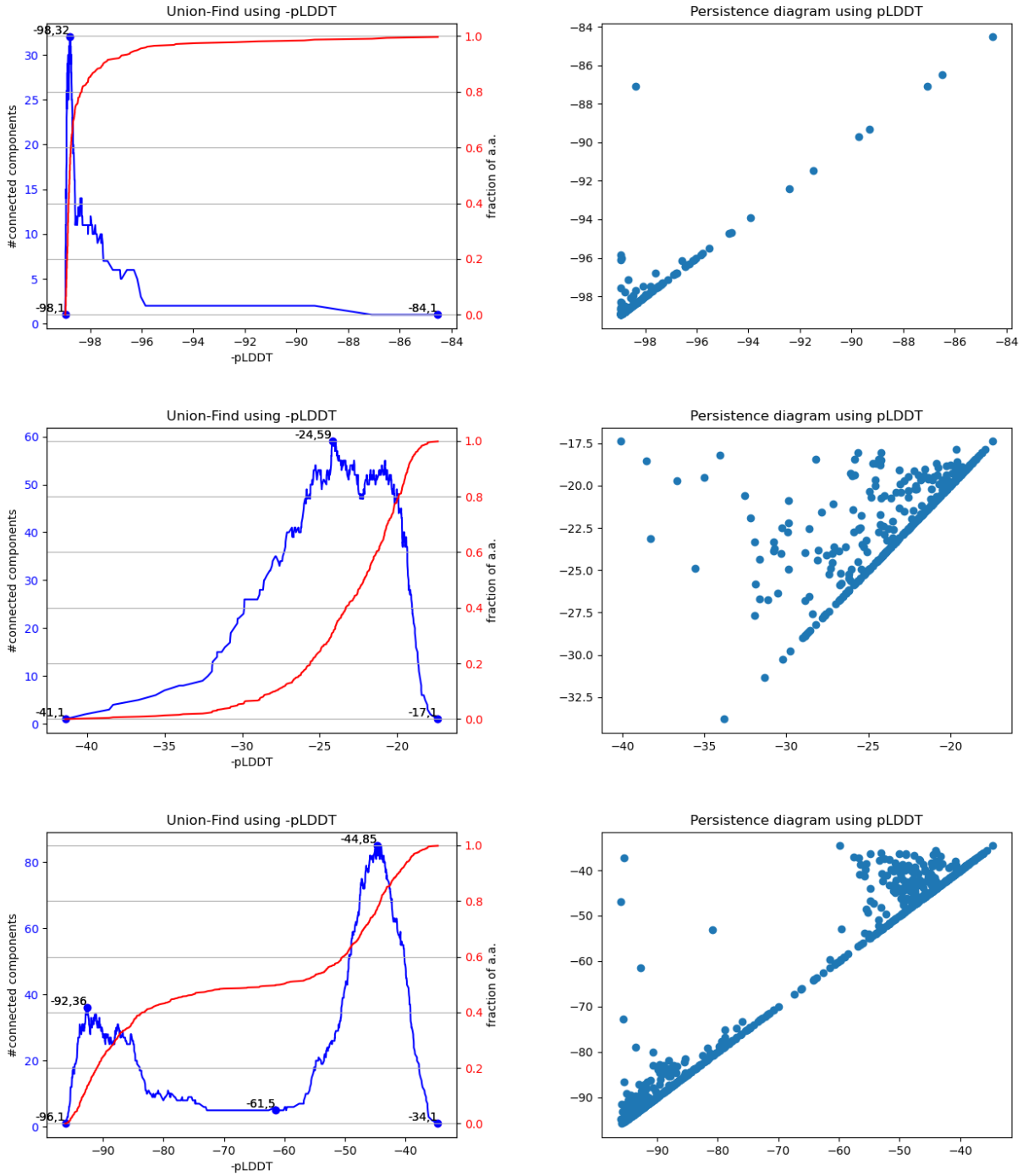


Figure 1: **Arity signature and arity based filtration on three prototypical examples.** Each structure is represented in cartoon mode; the blue (resp. red) a.a. is that featuring the lowest (resp. highest) pLDDT value. The **arity filtration** is the evolving graph defined by the connected components of the polypeptide chain induced by all C_α carbons whose arity is less than the evolving threshold. The associated **connected components curve** (blue curve, left scale) encodes the variation of the number of connected components in the filtration, with salient extrema as blue bullets. The **arity cumulated distribution function** (red curve, right scale) represents the cumulated fraction of C_α carbons whose arity is less than a threshold. The **arity signature** is defined by the interval of arity values required to gather fractions $q_1(= 0.25)$ and $q_2(= 0.75)$ of amino acids – red bullets on the CDF curve. **(Top)** Protein with well folded regions (H. Sapiens; AF-P15121-F1-model_v4; 316 amino acids.) Quantile-arity signature [(15, 0.25), (19, 0.5), (24, 0.75)]. **(Middle)** Protein essentially disordered. (Zebrafish; AF-A0A0G2L439-F1-model_v4; 449 amino acids.) Quantile-arity signature [(6, 0.25), (7, 0.5), (8, 0.75)] **(Bottom)** Protein with ordered and disordered regions. (D. Melanogaster; AF-Q9VQS4-F1-model_v4; 781 amino acids.) Quantile-arity signature [(0.25, 7), (0.5, 9), (0.75, 13)].



Union-Find using -pLDDT

Persistence diagram using pLDDT

Figure 2: **pLDDT based filtration on the three prototypical examples of Fig. 1. (Top)** (H. Sapiens; AF-P15121-F1-model_v4; 316 amino acids.) **(Middle)** (Zebrafish; AF-A0A0G2L439-F1-model_v4; 449 amino acids.) **(Bottom)** (D. Melanogaster; AF-Q9VQS4-F1-model_v4; 781 amino acids.) Left column: the blue curve codes the variation of the number of connected components; the red one is the CDF of the number of a.a. processed at a given x-value. Right column: persistence diagram for connected components.

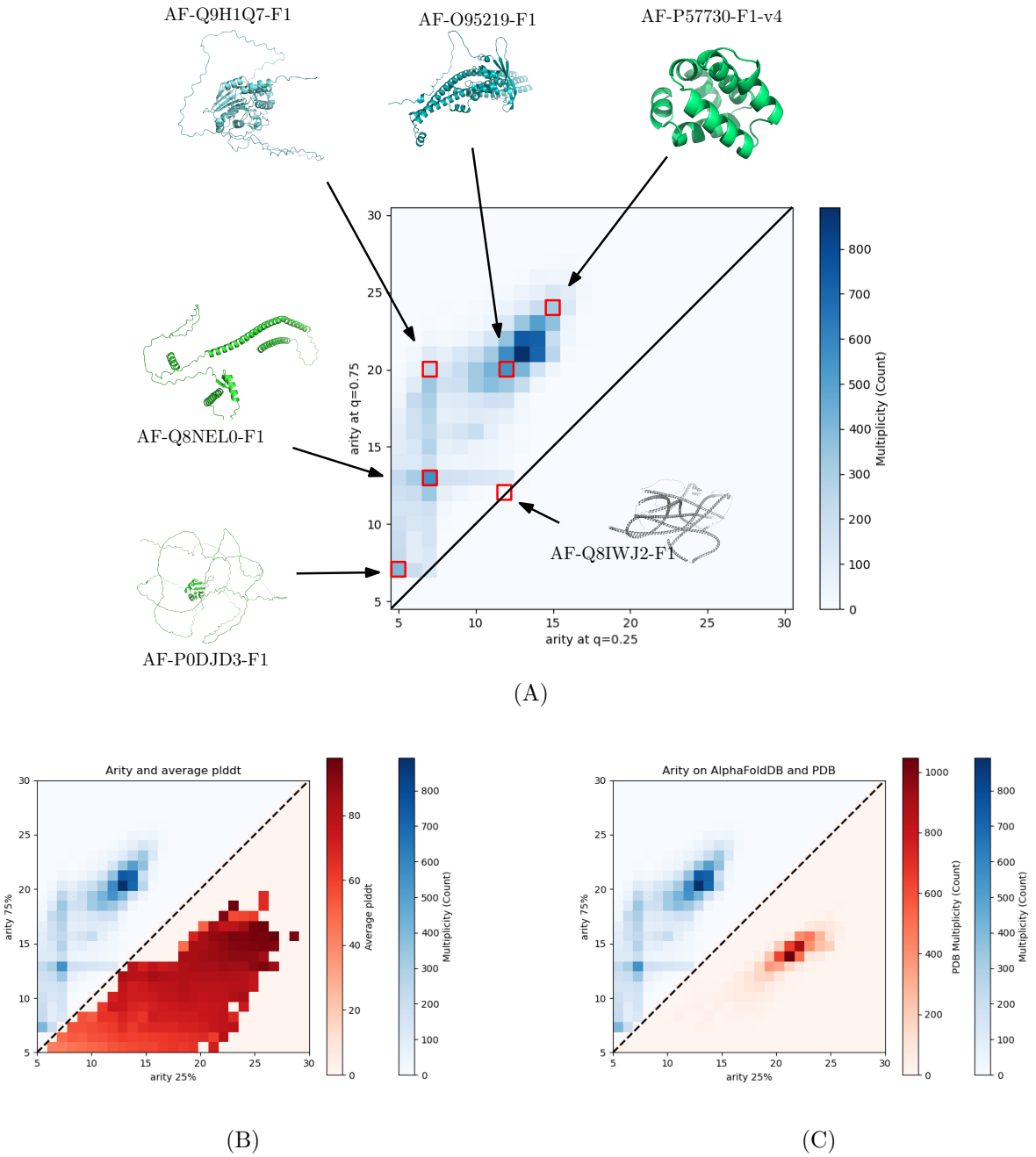


Figure 3: **H. Sapiens: arity map. (A)** Each bin corresponds to all `AlphaFold` predictions harboring a given arity signature. The upper triangle of the heatmap shows the protein count with for a prescribed arity signature. **(B)** Lower triangle: average pLDDT per bin, with mirrored coordinates. Arity and pLDDT are strongly correlated: the highes the first and last quartile are, the highest the averge pLDDT is. Arity is thus a good estimator of structural complexity. **(C)** Lower triangle: protein count from the PDB. Well-formed, ordered proteins only populate the (15,20) peak of the arity plot, confirming that the other two peaks visible in the arity plot of the `AlphaFold-DB` predictions must represent disordered structures or inaccurate predictions.

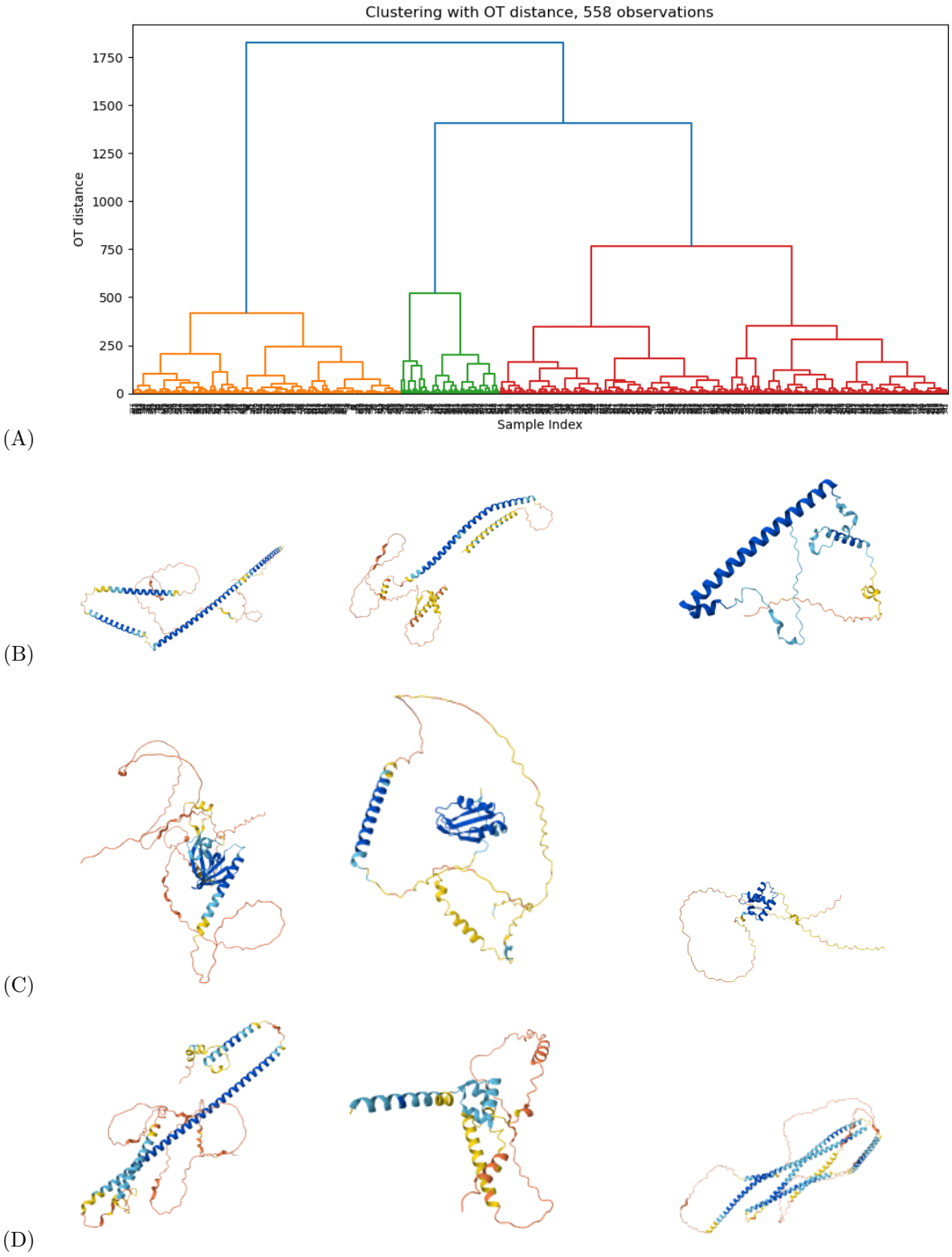
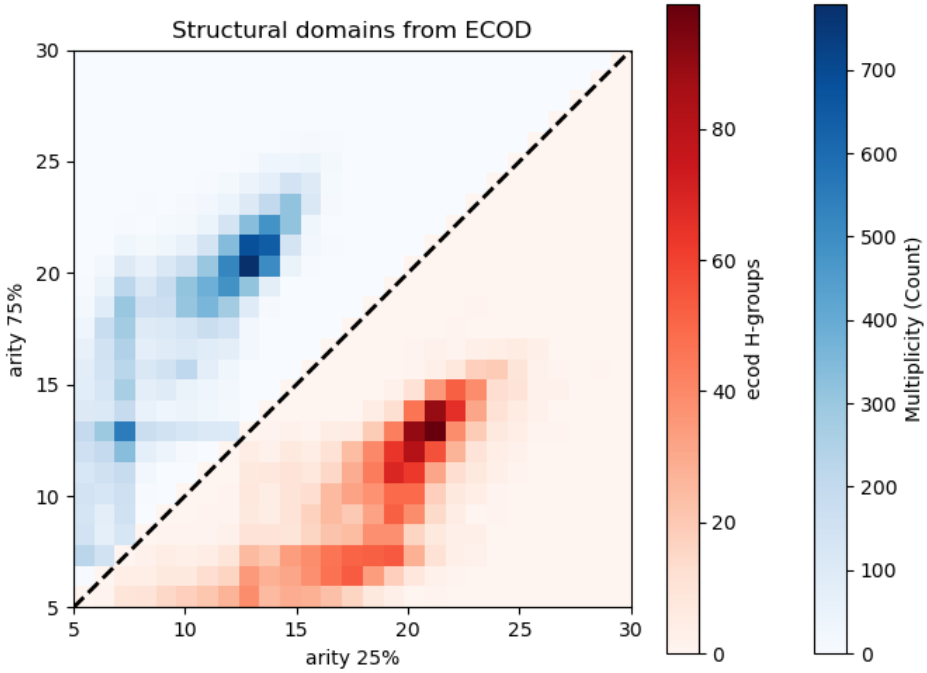
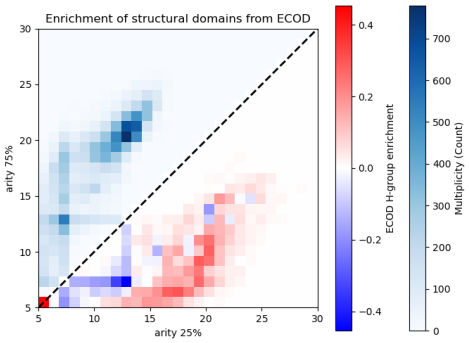


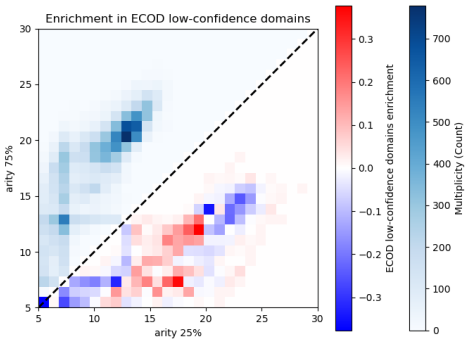
Figure 4: **H. Sapiens: hierarchical clustering of the 558 structures in the 7x13 cell of the arity map, with three random structures from every cluster. (A)** Ward hierarchical clustering of the structures using the optimal transportation distance on the normalized arity histograms H_{C_α} for each pair. **(B)** First cluster independent helices; **(C)** Second cluster: independent helices and a small β core; **(D)** Third cluster: packed helices.



(A)



(B)



(C)

Figure 5: **H. Sapiens: arity map of the human proteome vs ECOD domain enrichment in human proteins**. The upper triangle always reports the human proteome prevalence for reference. **(A)** Number of unique ECOD H-groups for each arity signature. **(B)** Enrichment difference (with respect to the proteome prevalence) of unique ECOD H-groups. **(C)** Enrichment difference (with respect to the proteome prevalence) of non-redundant ECOD low-confidence domains.

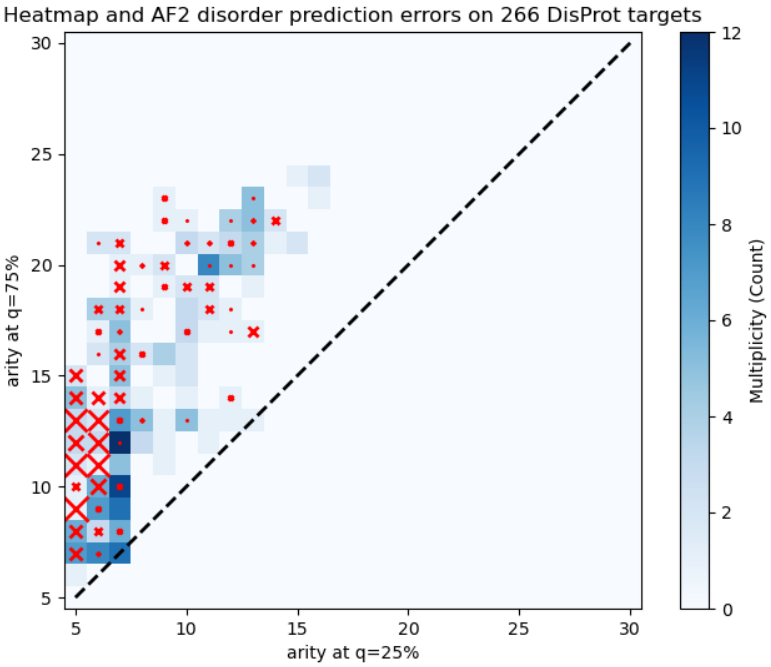


Figure 6: `DisProt` **dataset, 266 proteins lacking structural data.** The size of the red crosses over the heatmap indicates how frequently a pLDDT of less than 50 does not identify an amino acid in an experimentally annotated disordered region (no cross: never, pizel-size cross: more than 70% of the times. The leftmost region of the plot shows a high enrichment in such cases.