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Editorial

Special Issue: "Molecular Dynamics Simulations and Structural Analysis of Protein Domains"

Alexandre G. de Brevern [1,2]

[1] DSIMB Bioinformatics Team, BIGR, INSERM, Université Paris Cité, F-75015 Paris, France; alexandre.debrevern@univ-paris-diderot.fr; Tel.: +33-1-4449-3000
[2] DSIMB Bioinformatics Team, BIGR, INSERM, Université de la Réunion, F-97715 Saint Denis, France

The 3D protein structure is the basis for all their biological functions. For more than 50 years, obtaining this structure has been difficult, costly, and sometimes impossible. Molecular modelling approaches are therefore making it possible to increase our knowledge by providing high-quality 3D structural models [1–9]. In this context, the AlphaFold2, AlphaFold3, ESMFold, and other deep learning approaches have enabled significant progress [10–15], with hundreds of millions of 3D structural models being made available to the scientific community [16–18].

However, the 3D structure gives only a small insight into biological function [14,19–21]. The 3D protein structure or 3D protein structural model is static, whereas the different parts of proteins are rigid, flexible, highly flexible, or even completely disordered [22–25]. Similarly, biological function can be associated with major conformational changes that are not provided by knowledge of a single structure [26,27]. It is therefore essential to understand the life of protein structures through their dynamics [28–30]. Molecular dynamics simulations (MDs), both classical and advanced, provide access to many more detailed questions of critical biomedical interest [31–36]. For example, a point mutation in a structural model does not explain the functional change at the atomic level. MD simulations are needed to generate hypotheses about the effects of the mutation [37–41]. In this way, MDs allow us to see allosteric changes that are sometimes impossible to characterise experimentally [42–47].

This Special Issue had so focussed on issues ranging from the most fundamental to the most applied research on protein structures (or structural models) through the use and/or development of both classical and more innovative simulations. In the end, eight articles were accepted for publication in this Special Issue. I would like to thank the authors for their confidence at the time of submission and also those who unfortunately did not make it to the end of the process. I would also like to mention the involvement of several other editors specialising in this area, including Istvan Simon, Paulino Gómez-Puertas, Stefanie Krick, and Yuri Lyubchenko, as well as the administrative staff who ensured that the review process ran smoothly. The articles deal with very different systems and approaches, which have similarities but often very different aims. The five research articles are presented first, followed by the three review articles.

To begin with, López-Luis, Soriano-Pérez, Parada-Fabián, Torres, Maldonado-Rodríguez, and Méndez-Tenorio propose a work that may seem simple: a structural model [48]. Their work concerns the largest protein of *Helicobacter pylori* (Hp), a bacterium detected in the stomach of more than 50% of the human population [49]. In some well-documented cases, Hp can cause gastroduodenal ulcers and become a risk factor for gastric cancer (GC) [50–52]. These strains encode virulence genes such as type IV secretion system (T4SS), adhesins, and a cytotoxin [53]. The largest protein of the Hp T4SS is CagY (a little less than 2000 amino acids). Only its carboxyl C-terminal region has known homology; the rest have no simple direct links to structural homologues [54]. They split the protein into seven main domains, starting by modelling the C-terminal part, which has a structural support [54].

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They use different approaches, such as I-TASSER [6], ROSETTA [8], and combine the results with different structural supports using SWISSMODEL [55]. For the other regions, they repeat similar approaches, adding AlphaFold/CollabFold [10,55] and monitoring the results. An important point emphasised is the fact that only dimers can be approached due to computational limitations. The models are relaxed by short MDs. Classical evaluation approaches, such as ProSA-web [56], ERRAT [57], and QMEANDisCo [58], are also used to select the best models. The final model is obtained by adjusting the constraints imposed by the disulphide bridges between the different obtained chains. The article is very methodological, comparing models from different tools and confronting them with experimental data [48]. Figure 1 highlights the difficulty of the task as well as the beauty of the overall topology of the complex.

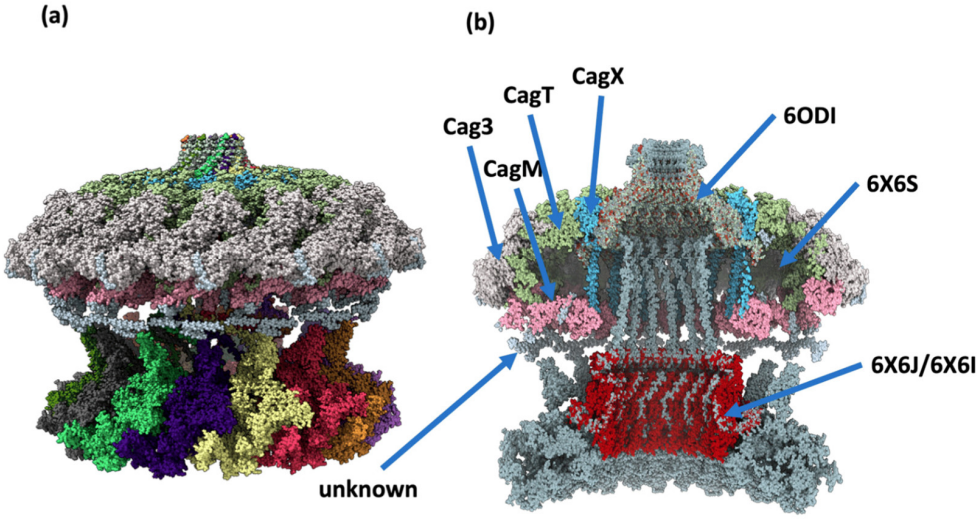

Figure 1. Assembly of the CagY protein with other protein structures of the T4SS from cryo-EM studies. (**a**) The model of CagY fitted with the structure of the T4SS from cryo-EM (PDB ID 6 × 6S [59]). (**b**) A transversal section of the assembly, showing that the most internal structure corresponds to the CagY multimer (in light grey). Other proteins of the T4SS are displayed in different colours. The structure marked as "unknown" corresponds to a non-identified protein, which is in close proximity to the protuberances of the long middle repeat region of the predicted model of CagY (taken from [48]).

Ziada, Diharce, Serillon, Bonnet, and Aci-Sèche have worked on a specific human cyclin-dependent kinase (CDK) called CDK8 [60]. CDKs are a family of serine-threonine kinases that need to bind to regulatory proteins called cyclins in order to be active. CDKs are key regulators of the cell cycle and gene transcription [61]. CDK8 is an interesting target that has recently attracted considerable attention following the publication of numerous genetic and biochemical studies highlighting its many key roles in oncogenesis [62–64]. The activity of CDK8 is controlled by a regulatory protein called cyclin C (CycC). MD simulations with binding free energy calculations allowed analysing the effect of CycC binding on the structure and dynamics of CDK8, as shown in Figure 2. The authors were able to simulate the transition between the active and inactive forms. CycC has a stabilising effect on CDK8, highlighting specific interaction hotspots in its interaction surface compared to other human CDK/Cyc pairs [60]. These results underline the importance of retaining CycC in computational studies when studying the human CDK8 protein in both its active and inactive forms.

Botnari and Tchertanov have focused on human vitamin K epoxide reductase (hVKORC1), an endoplasmic reticulum (ER) protein that reduces inactive vitamin K 2,3-epoxide to active vitamin K quinone, i.e., a protein required for blood coagulation [65]. Structurally, hVKORC1 is a small modular protein with a transmembrane domain, an intrinsically disordered L-loop that projects into the ER, and highly flexible N- and C-terminals that

float in the cytoplasm. Genetic polymorphisms of hVKORC1 are associated with low or accelerated rates of vitamin K recycling, leading to serious conditions such as bleeding and thrombosis. Surprisingly, the hVKORC1 polymorphism affects the response to doses of anti-vitamin K anticoagulants, promoting resistance to treatment. These studies follow previous studies made by the same group [66–68]. They used a de novo model [69] to perform MD simulations of the wild-type hVKORC1 and four different pathological mutants. All proteins have similar topology but different dynamics. L-loop missense mutations affect the folding and dynamic properties of the L-loop itself as well as the transmembrane domain. The extended 'open' conformation is either rarely observed (hVKORC1^{A41S} and hVKORC1^{W59R}) or never observed (hVKORC1^{S52W} and hVKORC1^{H68Y}). The compact globular conformation of the L-loop is mainly maintained by van der Waals contacts, specific for each mutant. These studies make it possible to define specific pockets during dynamics in each of these systems (see Figure 3) [65].

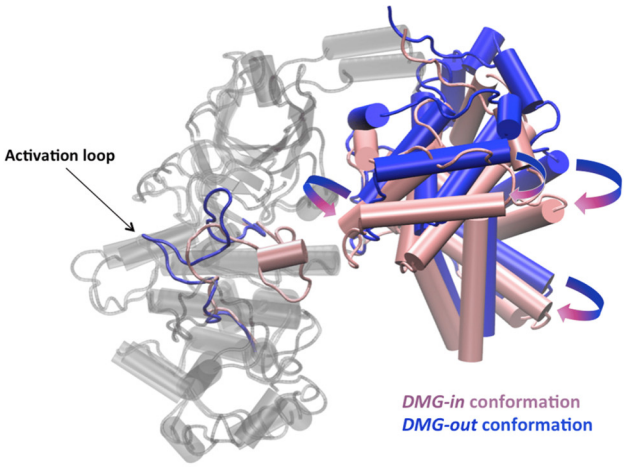

Figure 2. DMG-in and DMG-out conformations of the CDK8–CycC complexes. CDK8 structures are coloured in grey, except the activation loop. The activation loop and the CycC structures are coloured in pink in the DMG-in conformation and in blue in the DMG-out conformation (taken from [60]). DMG (Asp-Met-Gly) motif ranges from residues 173 to 175.

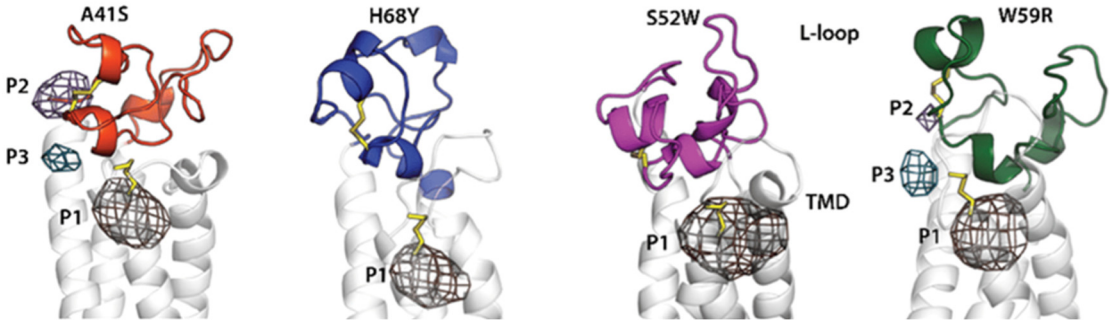

Figure 3. Pockets in hVKORC1 mutants. Pockets localised in four mutants. Protein is shown as a cartoon with the L-loop distinguished by color: hVKORC1^{A41S} in orange-red; hVKORC1^{H68Y} in dark blue; hVKORC1^{S52W} in fuchsia; and hVKORC1^{W59R} in dark green. Disulphide bonds and crucial residues are in sticks; pockets are delimited by meshed contours (adapted from [65]).

Delort, Cottone, Malliavin, and Müller attack a membrane system of biomedical interest. Botulinum neurotoxins (BoNT) [70], secreted by *Clostridium botulinum*, are among the most potent toxic compounds in the world, causing flaccid paralysis in the host [71,72]. The toxins consist of two protein chains linked by a disulphide bridge: the light chain (LC) and the heavy chain (HC). The role of the HC is to prepare the way for the catalytic region of the light chain, which is responsible for the toxicity of BoNT [73,74]. The system is complex

because it is highly sensitive to pH, and especially in the dimeric structure, the switching domain changes from α-helices to β-strands, a quite drastic evolution [75]. The work by Delort and colleagues studies the internal dynamics of the translocation domain in water and in a mixture of water and ethanol using MDs over microseconds. A combination of elasticity theory and geometry is used to describe the different configurations of the system. A very specific feature of the work presented is the use of theoretical mechanisms known as the Twister mechanism and the Darboux torque mechanism [76], which make it possible to understand how membrane deformation is induced. They have been successfully applied in [77–80]. This study is the first to link the Twister mechanism with biological interactions.

Another specific feature of this research is the work carried out in different media, such as ethanol (see Figure 4) [70]. This rather complex approach represents the first step in coupling the mesoscopic Twister model to an atomistic approach with predictive power for protein–membrane interactions. By using the two approaches together, it has been possible to propose a hierarchy of interactions between the translocation domain and the membrane. This results in a partitioning of the protein structure into regions undergoing unfolding or separation from the protein core, and helix bundles 1 and 2 as the main players involved in insertion into the membrane.

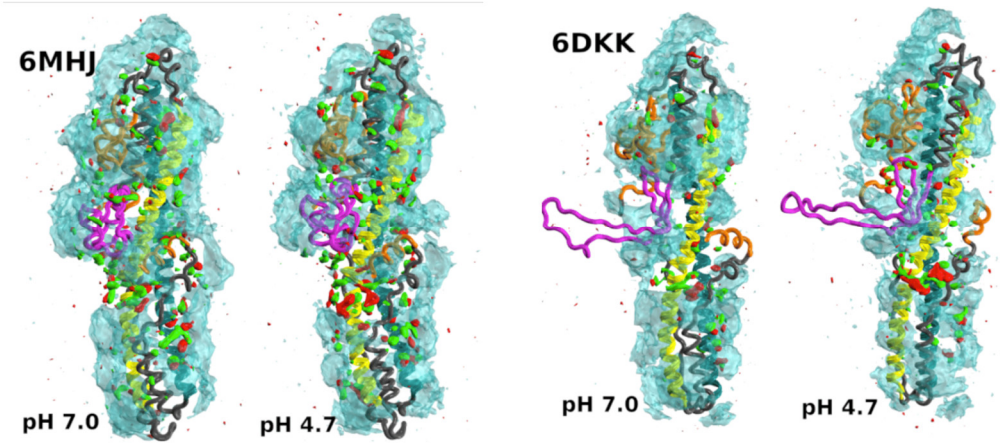

Figure 4. Isosurfaces of the spatial density function of water and ethanol atoms around the protein. Cyan isosurface: water oxygen atoms; red and green: oxygen and methyl carbon atoms, respectively. The isosurfaces are represented at the same isodensity level (0.0115) for both water and ethanol. Data were collected from the three replicates of the trajectory. The proteins are PDB entries: six MHJ determined at a pH 8.5 and six DKK were determined at a pH 5.1 [81]. The helices 1 (cyan) and 2 (yellow) are shown for the two PDB structures. Green spheres represent the hydrophobic residues in these helices. The switch is coloured in magenta, and the N-terminal domain and the C-terminal α helix in orange (taken from [70]).

Tam, Qin, Zhao, Sinha, Lei, and Wang focus on a current issue, the impact of variants on possible pathogenicity [82]. Indeed, databases often give a benign, probably benign, pathogenic, or probably pathogenic association for a given variant, but also variants of uncertain significance (VUS) due to the lack of functional evidence. Their number is far from negligible, which makes the analyses and predictions of doctors particularly complex. They are never integrated into pathogenicity prediction methods or their evaluations [83–89]. Lam and their colleagues focused on a specific case: the DNA mismatch repair (MMR) gene MLH1 is associated with Lynch syndrome (LS), an autosomal dominant inherited cancer [90,91]. Indeed, more than 80% of the data on this gene are associated with VUs. Of particular interest, the authors have developed a structure-based method called deep learning-Ramachandran plot-molecular dynamics simulation (DL-RP-MDS) [92]. This method is designed to assess the harmfulness of VUS. In practical terms, the method extracts structural information from proteins using the Ramachandran plot-molecular dynamics simulation (RP-MDS) method [93]. Thus, a point of importance is the work

carried out from the Ramachandran plot data [94], which has been evolving since 1963 but remains essential in our fields [95–98]. The crystal structure of MLH1 (PBD ID: 4P7A [99]) was used. The mutant structures for each missense variant were also built following classical procedures [23] with MODELLER [4] and best model selection by zDOPE [100]. Long MDs were performed and analysed. In a second step, the variation data are combined with an unsupervised learning model consisting of an autoencoder and a neural network classifier to identify variants that cause a significant change in protein structure. They applied the method to classify 447 MLH1 missense VUSs and predicted that 28.2% of MLH1 missense VUSs were deleterious. This approach allows us to prioritise costly experiments by searching for the most likely cases [82]. A major interest of the approach is the link with the structure that allows, as represented in Figure 5, to understand the atomic mechanism associated with a variant.

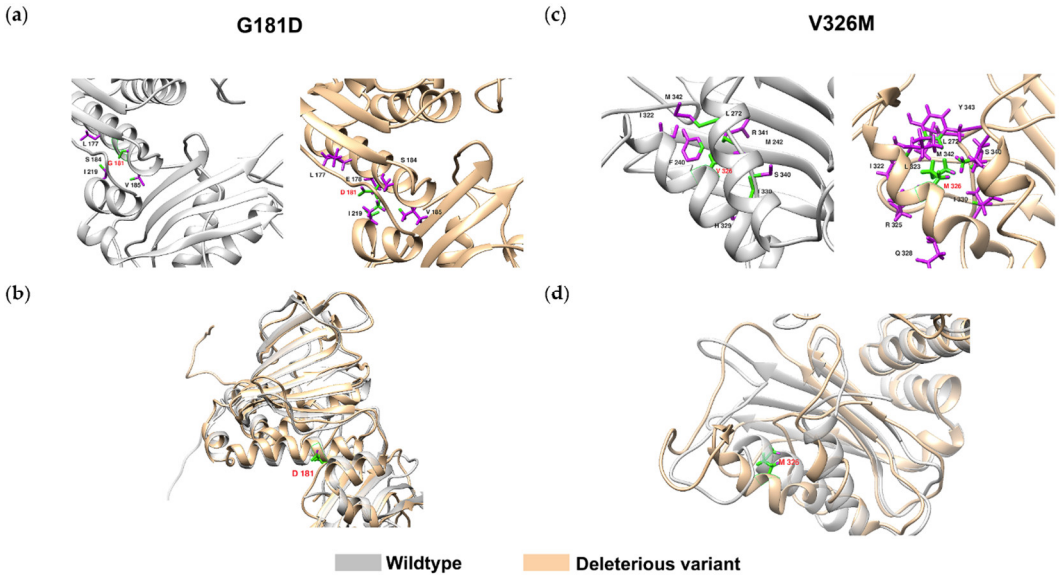

Figure 5. Structural change in MLH1 by G181D and V326M. (**a**) The wild-type G181 interacted with four residues (L177, S184, V185, and I219), whereas the variant D181 interacted with five residues (L177, E178, S184, V185, and I219). (**b**) The D181 caused instability of αG and further affected the MutS-HI domain. (**c**) The wild-typeV326 interacted with nine residues (F240, M242, L272, I322, N329, I330, S340, R341, and M342), and the M326 interacted with altered nine residues (L272, I322, L323, R325, Q328, I330, S340, M342, and Y343). (**d**) The M326 did not interact with the β sheet and caused the αI helix to detach. Grey: wild-type; peach: variant; green: interacting atoms; purple: non-interacting atoms; red label: wild-type and variants; black label: interacting residues (taken from [82]).

Qvit and colleagues then provide us with two interesting reviews on protein kinase C (PKC). Indeed, protein kinases are one of the most important drug targets in the human proteome, mainly due to the treatment of cancer, cardiovascular diseases, and a growing number of other diseases, including autoimmune and inflammatory processes. In a first review, Silnitsky, Rubin, Zerihun, and Qvit review the role of different PKC isoforms in cancer and cardiovascular diseases, with a particular focus on PKC family inhibitors [101]. They discuss translational examples and carefully examine the advantages and limitations of each compound. The review first briefly introduces kinases [102–106] and then focusses on the protein kinase C family [107–109] (see Figure 6). It highlights its regulation by lipid second messengers [110,111] and its regulations by scaffold interactions [112–114], and shows its implications in cancers [106,115–118], in cardiovascular diseases [119–123], and other important human diseases [124–127]. It ends with how to target PKC with its PKC inhibitors in clinical trials [101,128–133].

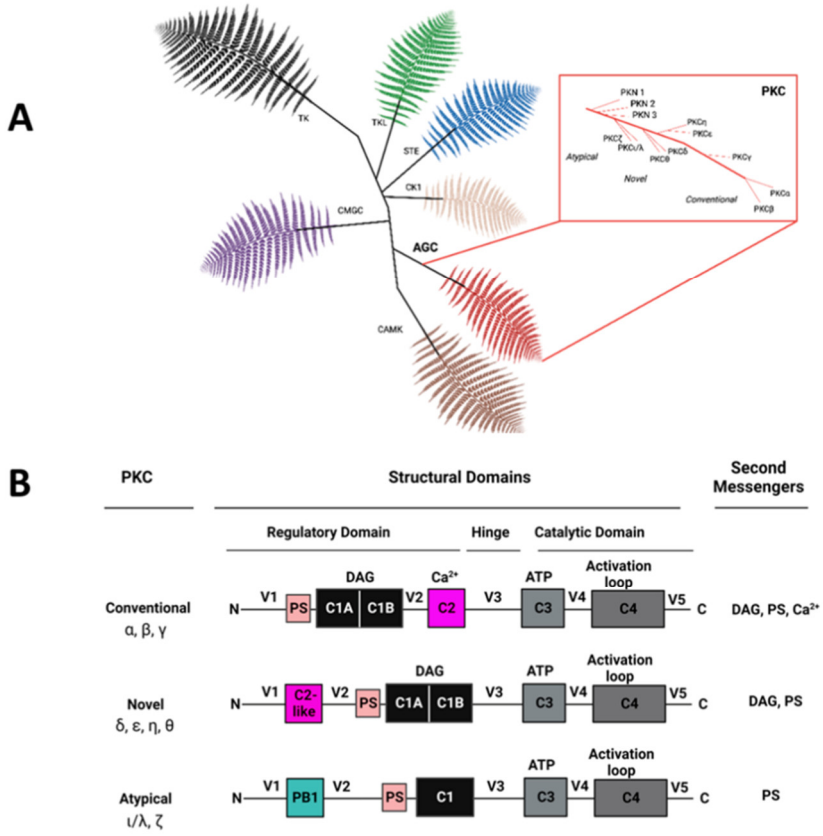

Figure 6. PKC isozyme diversity. (**A**) The human kinomephylogeny is extensive, and the PKC family is one member of the AGC superfamily (bottom right), with three groups of conventional, novel, and atypical PKC isozymes. (**B**) PKC isozymes are homologous but contain a distinct set of structural domains responsible for their diverse functions and interactions with second messengers and other binding partners. All PKC family members are constituted by four conserved domains (C1–C4) separated by a hinge region. The pseudo-substrate site (PS) keeps the protein in its inactive form. Second messengers are indicated on the right side of the picture (taken from [101]).

In the second review, Zerihun, Rubin, Silnitsky, and Qvit focus on peptides as allosteric modulators of protein kinase C targeting protein–protein interactions [134]. They are investigating alternative allosteric binding mechanisms to target PKC and new drug platforms, in particular modified peptides. The idea is to design protein kinase modulators with increased selectivity and improved pharmacological properties. In this context, molecular docking to predict the mechanisms of action of inhibitor-kinase interactions can greatly facilitate the development of next-generation PKC modulators. Pleasingly, they provide structural information on protein kinases [106,135], with protein kinases as major drug targets [136], the concept of non-catalytic domains of protein kinases [137], allosteric modulation of kinases [138], the use of allosteric sites in drug discovery [139], allosteric regulation by protein–protein interactions [140], and then move on to peptides targeting protein–protein interactions [141]. They describe the therapeutic use of peptides and approaches to developing peptides as regulators of protein kinases targeting allosteric sites [142]. They then present peptides derived from unique substrate sites [143,144] together with peptides derived from the pseudo-substrate site [145,146], those derived from substrate phosphorylation sites [147], and those derived from substrate protein–protein interaction sites [148]. Authors underline these examples by their own work on a selective inhibitor of mitofusin 1-βIIPKC associations that improves heart failure outcome in rats [149]. Figure 7 shows the principle of the dedicated approach.

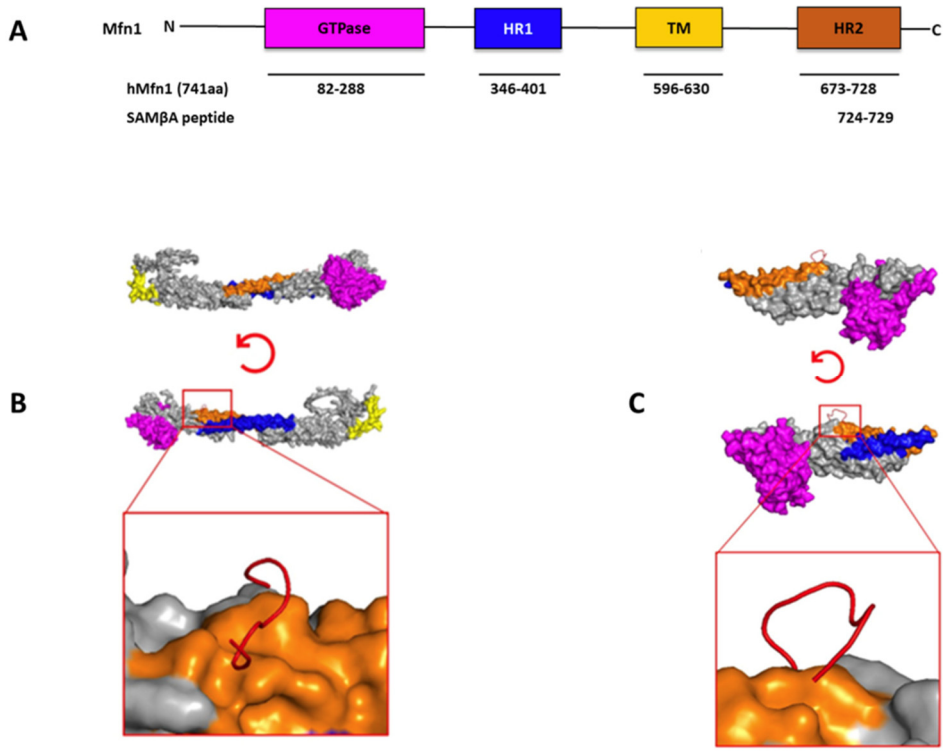

Figure 7. Structural domains of Mfn1 and peptides derived from substrate protein–protein interaction sites. A sequence corresponding to the homologous domain between PKCβII (P05771-2; residues 625–629) and Mfn1 (26251799; residues 724–729) was identified, and the SAMβA peptide corresponding to this sequence was developed. (**A**) The structural and functional domains of full-length mitofusin-1 (Mfn1) (741 AAs). The GTPase domain is shown in magenta, heptad repeat (HR1) coiled-coil regions 1 in blue, the transmembrane (TM) domain is shown in yellow, and heptad repeat (HR2) coiled-coil regions 2 in orange. (**B**) Molecular docking results for the interaction of SAMβA (RNAENFDRF) and Mfn1 to the available crystal structure (PDB: 5GO4 [118]) or (**C**) AlphaFold predicted model (Q8IWA4). To help stabilise the α-helix structure, amino acids were added at the C- and N-terminus of the peptide. Mfn1 is shown by cartoon representation coloured in (GTPase—magenta; HR1—blue; HR2—orange; and TM—yellow) and the peptide is shown in red cartoon structure. Based on the docking analysis, the peptide is docked to the HR2, which is source domain for its rational design (taken from [134]).

These results then raise the more complex issue of peptides derived from similar sequences in binding proteins. Indeed, in some cases, signalling enzymes interact with several proteins that are unrelated to each other [150]. In many cases, these unrelated proteins share a short sequence of homology that could represent the enzyme-binding site [151]. They discuss peptides derived from sequences involved in intramolecular interactions [152]. Following these examples, using advanced computational biology approaches, they highlight evolutionarily conserved peptides and, again, complex examples of peptides derived from conserved sequences in homologous but unrelated domains of proteins [153,154]. To limit the possibility of cross-reactions, peptides derived from unique protein kinase sequences have been developed, sometimes even targeting a specific isoform [155].

Finally, Zheng, and Liu conclude the Special Issue with a new view on the study of Fos-related antigen-2 (Fra-2) in respiratory diseases [156]. Fra-2 is a member of the AP-1 (activator protein 1) family of transcription factors. It is involved in the control of cell growth and differentiation by regulating the production of the extracellular matrix and coordinating the balance of signals inside and outside the cell. The authors present the structure of AP-1/Fra-2 [157], its expression, the importance of Fra-2 in tissue development,

and its regulation. They then review the role of Fra-2 in the development of respiratory diseases, chronic obstructive pulmonary disease, pulmonary fibrosis, asthma, and non-small cell lung cancer.

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