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One for All, All for One: The Peculiar Dynamics of TNF-Receptor-Associated Factor (TRAF2) Subunits

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Abstract: TNF Receptor-Associated Factor 2 (TRAF2) is a homo-trimer belonging to the TNF-receptor-associated factor family (TRAFs). The TRAF2 oligomeric state is crucial for receptor binding, the interaction with other proteins (involved in the TNFR signaling), and the interaction with biological membranes. In this study, we present a computational analysis of the Molecular Dynamics of TRAF2-C (a truncated and soluble TRAF2 form) to identify patterns in the interactions between the three chains. We have performed a canonical analysis of the motion applied to molecular dynamics starting from the available crystal structure to identify correlated motions in TRAF2 dynamics. We have computed the displacement matrix, providing a frame-by-frame displacement for each residue in the dynamic. We provide the results in terms of the correlation matrix, which represents a detailed map of the correlated motions of residues. Eventually, we computed the so-called dynamical clusters, based on the Principal Component Analysis (PCA) of the motion (displacement) and the k means application on the first two principal components space. The results clearly indicate that, most of the time, two chains move in a strongly correlated motion, while the third chain follows a freer motion. A detailed analysis of the correlation matrix also shows that a few specific interface residues characterize the interaction of the more independent subunit with the other two. These findings suggest that the equilibrium between the trimer and the dissociated species (dimers and monomers) might be finely tuned by controlling a few critical residues in the protein quaternary structure, probably facilitating the regulation of oligomerization and dissociation in vivo.

Keywords: TNF-receptor-associated factor; protein asymmetry; TRAF dissociation/oligomerization

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1. Introduction

The biological relevance and widespread diffusion of oligomeric proteins have been very well known since the infancy of X-ray crystallography, with hemoglobin(s) being the most popular example. The peculiar structure of proteins/enzymes constituted by multiple subunits has been the object of a large number of chemical-physical studies, as it represents the most elementary paradigm for the study of macromolecular interactions in complex systems. In particular, those made up of identical subunits (homomers, homo-oligomers) are extremely interesting because they display a high degree of functional complexity yet preserve a sort of structural simplicity due to the repetition of modules that are, in principle, indistinguishable from each other.

The quaternary structure provides noteworthy advantages to these molecules, including folding efficiency [1], allosteric regulation [2], and evolutionary adaptation [3,4], just to mention a few. Most of these features are strictly correlated to the spatial and topological

properties that distinguish homo-oligomers, first and foremost their intrinsic symmetry [5]. Symmetry, in fact, dictates the efficiency of the assembling process by providing better and more stable inter-subunits surfaces, a hallmark that has considerably influenced evolution [5]. Despite the fact homomers are symmetric on a global scale, local deviations from a uniform regularity are definitely not uncommon, as systematic theoretical studies on homo-dimeric structures have revealed [6].

Recently, we reported the non-symmetrical dynamics of TRAF2-C, a truncated form of TRAF2, which is a member of the TNF receptor-associated factor family [7]. The biological activity of TRAFs mainly concerns signaling but also includes several events that play important roles in programmed cell death, immunity, and inflammation [8]. As with other members of the TRAF family, TRAF2 is a homo-trimer constituted by a globular C-terminal domain and a coiled-coil section, in which three long alpha-helices are involved [9].

Previous equilibrium-unfolding studies on TRAF2-C have provided evidence that the oligomeric structure undergoes dissociation into monomeric and dimeric species using a mild denaturant concentration or hydrostatic pressure [10]. Fluorescence correlation spectroscopy [10] has also demonstrated that the trimer spontaneously separates into subunits at a low concentration (namely in the nanomolar range), thus increasing its ability to bind synthetic [11] and natural membranes [12].

In order to better understand such a spontaneous association/dissociation process, we investigated the protein conformational dynamics by temperature-dependent spectroscopic measurements in the near UV and reproduced them in silico, starting from the available crystal structure [7,13].

We also applied Protein Contact Network (PCN) analysis to demonstrate that the local structural heterogeneity at each monomer–monomer interface is responsible for the asymmetrical formation of two distinct clusters, composed by a dimeric and monomeric subunit, respectively [13]. This association has a rather dynamic character, as, during the simulation, the cluster compositions change, with the three partners alternating themselves in forming different 2 + 1 combinations [13].

In the present study, we carried out a canonical analysis of the simulated motion and parallel B-factors to monitor the protein molecular structure over time.

Such a hypothesis suggests that only a few key residues located at the subunits' interface modulate the interaction of the more isolated subunit with the other two and that such an independent monomer displays, on average, greater mobility both at the interface and in the peripheral areas of its structure. Such peculiar cooperation among the three subunits might offer functional advantages that have been discussed in light of the dissociation/oligomerization process of TRAF2-C, also taking into account the most recent observations of the role played by the asymmetry in the structure and function of homomeric proteins [14,15].

2. Materials and Methods

2.1. Chemicals

Guanidinium hydrochloride (GdHCl) and 8-Anilino-1-naphthalenesulfonic acid (ANS) were purchased from Sigma Aldrich (St. Louis, MO, USA) and used without further purification.

Enzyme preparation TRAF2-C was expressed in *E. coli* BL21 and purified as previously reported [12]. In particular, *E. coli* BL21 (DE3) cells were transformed with the His-tagged human TRAF2-C domain (residues 310–501) construct. After TRAF2-C expression, cells were harvested and resuspended in lysis buffer (20 mM Tris-HCl pH 8.0, 150 mM NaCl, 20 mM imidazole, 10% glycerol, 1 mM DTT, and EDTA-free inhibitor of protease). The cellular extract was loaded on a 10 mL NiNTA column pre-equilibrated with buffer, and the protein was eluted using a linear imidazole gradient. Imidazole was then removed from the TRAF2-C sample by a Sephadex G25 column (GE Healthcare Life Science, Chalfont St. Giles, UK) pre-equilibrated with the kinase buffer (20 mM Tris-HCl pH 7.6 containing 150 mM NaCl and 10% glycerol). TRAF2-C was stored in a kinase buffer and used in the same buffer for all spectroscopic measurements. The protein concentration was determined by

measuring the absorbance at 280 nm and using an extinction coefficient of 17,780 M^{-1} cm^{-1} for TRAF2 monomers.

2.2. Spectroscopic Assays

Steady-state fluorescence was measured with an ISS-K2 fluorometer (ISS, Champaign, IL, USA) thermostating the samples at 20 °C by an external bath circulator. High-pressure experiments were performed on the same instrument, equipped with the ISS high-pressure cell [16]. Each measurement was repeated at least three times, and the average spectrum is reported for each set of experiments.

2.3. Molecular Graphics and Analysis of Subunits Interfaces

Protein visualization and graphical images have been obtained using VMD software [17]. Analysis of TRAF2-C hydrophobic patches and evaluation of the theoretical solvation-free energy change upon formation of each monomer–monomer interface was carried out using the Protein Interfaces, Surfaces, and Assemblies (PISA) service at the European Bioinformatics Institute. (http://www.ebi.ac.uk/pdbe/prot_int/pistart.html, accessed on 11 February 2022 [18]).

2.4. Molecular Dynamics Simulation

Classical MD simulations of the TRAF2 trimer were performed starting with the X-ray diffraction structure pdb-id:1c4a [9] and employing the GROMACS package [19] with the GROMOS43A1 force field [20]. MD simulations were carried out in the NpT ensemble at 310 K by following exactly the same equilibration procedure as already reported [7,21]. We performed two independent simulations (replicas) of the system (each starting with different velocities) lasting 250 ns each. The velocities were selected randomly from a Maxwell distribution centered around the simulation temperature.

To measure main chain flexibility, we computed the B-factors (also called temperature factors or atomic displacement parameters) of C_α atoms. The B-factor is defined by the following formula:

$$B = \frac{8\pi^2}{3} RMSF^2$$

where *RMSF* is the root mean square fluctuation of atomic positions in the trajectory.

2.5. Dynamic Cross Correlation and Canonical Correlation Analysis

The statistical analysis of MD simulations is a typical approach to identify the correlated motions of residues during protein dynamics.

At first, we computed the displacement matrix D, which is an $n \times p$ matrix where n is the number of observations (single frames) and p is the number of variables (displacement of single residues). Each column reports the displacement course for each residue (identified by its own alpha-carbon) for each frame of the dynamics, with respect to the previous frame.

We, therefore, computed the Dynamic Cross Correlation (*DCC*) matrix [22] defined as:

$$DCC(i,k) = \frac{< \Delta r_i(t) \cdot \Delta r_j(t) >}{\sqrt{< \|\Delta r_i(t)\|^2 >} \cdot \sqrt{< \|\Delta r_k(t)\|^2 >}} \quad (1)$$

where $r_i(t)$ represents the i-th residue coordinates (alpha-carbon) as a function of time and $\Delta r_i(t)$ represents the residue displacement in the frame at t. *DCC* is a $p \times p$ matrix whose generic element reports the Pearson correlation coefficient $DCC(i,k)$ between the displacement vector $\Delta r_i(t)$ of the i-th residue and $\Delta r_k(t)$ of the k-th residue. High positive values of *DCC* represent proof of concerted motions of residues, values close to zero correspond to uncorrelated motion, and large negative values (close to unity) describe a hinge mechanism.

We also applied Principal Component Analysis (PCA) of the displacement matrix D to identify residues moving in a correlated way. We specifically derived the loading

matrix L whose generic element $L(i, k)$ is the Pearson correlation coefficient of the original i-th variable with the new k-th variable (principal component, remembering the original variables are the residue displacements.

We sorted as many principal components as required to reach the threshold of fixed cumulative explained variance (80%).

In our analysis, three principal components were enough to cover more than 85% of the overall explained variance. High values close to the unity of the loadings indicate that, in the given component (column), the corresponding residues participate in the collective motions the component describes. Thus, we applied a partitioning clustering algorithm to the space of loadings corresponding to significant (three) principal components to identify clusters of residues moving together.

Upon clustering, the corresponding participation coefficient for each residue was computed, defined as [23,24]:

$$P_i = 1 - \left(\frac{k_{si}}{k_i}\right) \quad (2)$$

where k_{si} is the node intra-cluster degree, i.e., the number of contacts of the node (residue) with nodes belonging to the same cluster, whereas k_i is the total node degree.

The results (clusters and participation coefficient P) have been plotted as heat maps on ribbon structures using a purposed Python script in Pymol, as previously described [7,25].

Eventually, we computed the molecular asymmetry AS, which describes the shape of protein molecules [26,27]. It assumes only positive values, and the molecule's shape is, according to AS values, prevalently globular when $AS < 0.4$, disc-shaped when $0.4 < AS < 0.6$, and rod-like when $AS > 0.6$.

3. Results and Discussion

3.1. Insights on Inter-Subunits Interface by Dissociation Measurements

The dissociation of trimeric TRAF2-C can be induced in different ways, including dilution, high pressure (between 1–1500 bar), temperature, and by means of a moderate ($\leq$1 M) amount of guanidinium hydrochloride [10,13]. The subunit distancing produces the progressive exposure of hidden hydrophobic patches, as demonstrated by an increased capability in ANS binding. This feature is shown in Figure 1 (panels A and B) reporting two examples of guanidine- and pressure-induced dissociation of TRAF2-C subunits. In both cases, the intensity of the probe's spectrum increases, and a blue shift occurs, as expected in the case of its interaction with hydrophobic surfaces [28]. The effect is protein-concentration-dependent (Figure 1A), thus confirming that the trimer is in equilibrium with species of lower molecular weight (monomers and dimers). An estimation of the total free energy change for the separation of the three subunits yielded $\Delta G_{diss} \approx 21$ kcal/mol [10], 60% of which ($\approx$12 kcal/mol) was required to detach the first monomer, while only 8–9 kcal/mol is necessary to split the remaining dimer into two monomers [7].

In the crystal structure, the theoretical solvation-free energy change upon the formation of each monomer–monomer interface is ≈ -7 kcal/mol (see Materials and Methods). As shown in Figure 1C, each subunit forms two distinct interfaces with the other partners, both characterized by a relevant percentage of hydrophobic residues, thus explaining how most of the protein quaternary structure is stabilized.

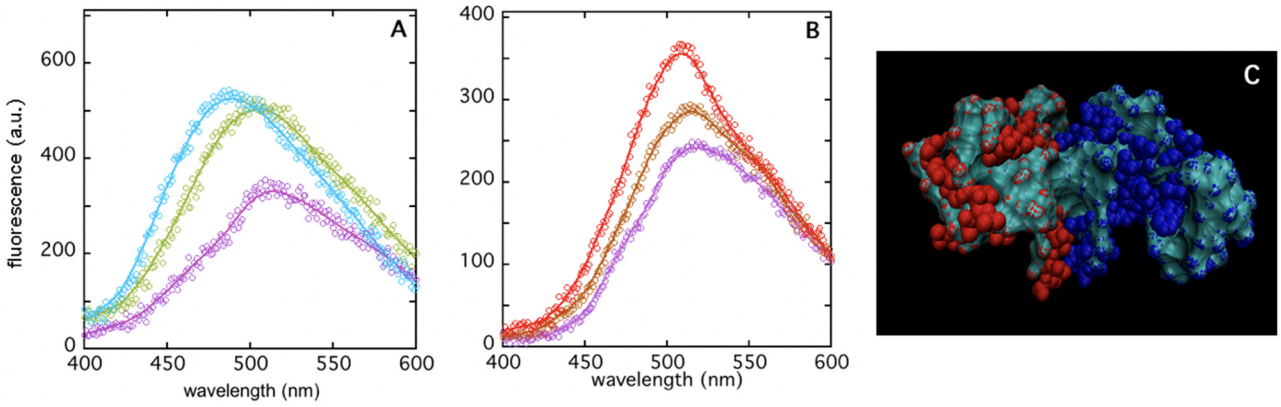

Figure 1. Panel (**A**): ANS fluorescence spectra in the presence of native TRAF2-C (2 μM, purple symbols) or TRAF2-C dissociated in 0.9 M GdHCl at two different protein concentrations, namely 2 μM (green symbols) and 0.2 μM (cyan symbols). Panel (**B**): ANS in the presence of TRAF2-C (2 μM) at 1, 1000, and 1500 bar (purple, brown, and red bullets). The solid lines are produced using a polynomial fitting function, to smooth the data. The ANS/protein ratio was 30:1 and the excitation wavelength λ_{ex} = 370 nm. Panel (**C**): Graphical representation of TRAF2-C crystallographic file (pdb entry: 1ca4) once subunit B was artificially removed. The hydrophobic residues (at the interfaces with monomer B) of subunits A and C are reported in red and blue, respectively. The rest of subunits A and C residues are in cyan.

3.2. Asymmetric Mobility of TRAF2-C Monomers

In order to obtain a more realistic picture of the protein tridimensional configuration, we also performed molecular dynamics simulations (starting from the crystallographic structure) and re-analyzed the three interfaces (AB, BC, CA) obtained at 250 ns. The results suggest that an asymmetric evolution occurs. For instance, as reported in Figure 2 for one of such simulations, the AC interface at 250 ns appears to be the favorite, involving the greatest number of side chains and displaying the largest interface area. The reason behind such a loss of symmetry originates from a slightly different overall mobility of the three subunits, as reported in Figure 3. On average, the largest fluctuations occur on monomer B, involving residues belonging to the N-terminal section, the BC interface, and the side chains of surface amino acids (Figure 3). This last change is important due to the peculiar biological role played by TRAFs, namely the binding to TNF receptors and/or to the adaptor protein TRADD [8,29,30]. Indeed, we have already reported docking simulations using a TNF-R2 peptide that suggest that binding might not necessarily take place in a symmetric way [13].

In the right panels, the displacement from t = 0 (upper picture) and t = 250 ns (lower panel) is shown, reporting the mobile residues of chain B in yellow, as VDW spheres.

Characterization of the local mobility in each TRAF2-C chain was carried out, evaluating the average B-factors values of the simulated structures. As shown in Figure 4, initially ($t \leq 50$ ns), the dynamics of the three subunits are very homogeneous and display a similar distribution of the B-factors' values. At longer simulation times, such symmetry is lost, with the more isolated subunit being characterized by enhanced mobility with respect to the monomers that form the dimeric cluster.

The asymmetric behavior of the subunits in homo-oligomers is an interesting phenomenon because it may suggest functional capabilities that cannot be estimated on the basis of the sole crystallographic structure. For instance, in 2017, Pai and co-workers [14] demonstrated, for the first time, that asymmetry has a fundamental role in driving the catalysis in a homo-dimeric enzyme. In particular, they found that the cost of an entropy decrease upon substrate binding in one subunit is compensated by increased mobility in the empty monomer [14], thus proposing a new functional advantage of dimers composed of identical subunits, with respect to single-chain enzymes. More recently, the analysis of the structural NMR data of several homomers in solution has demonstrated that, in these

systems, deviation from symmetry is the rule rather than the exception [15]. It is tempting to speculate that, also for TRAF2-C, dynamic asymmetry might play a role in regulating its association state; for instance, the fact that, in solution, one subunit is statistically more independent from the other two might explain how dissociation is initiated and equilibrium is shifted from trimers to monomeric species, as the samples are diluted in vitro from the micromolar to the nanomolar concentration range [10].

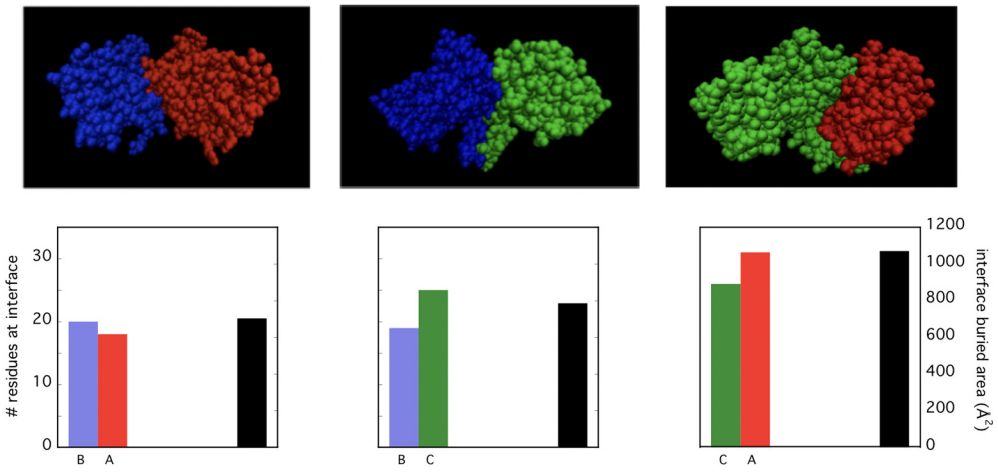

Figure 2. (**Upper**) panels: Side views of each TRAF2-C pair of monomer–monomer interfaces obtained after a 250 ns molecular dynamic simulation (red = subunit A; blue = subunit B; green = subunit C). In the (**lower**) panel, the histograms report the number of interface residues made available by each monomer in that specific association. The value of the hidden surface upon each dimer formation is also reported as a separate, black bar.

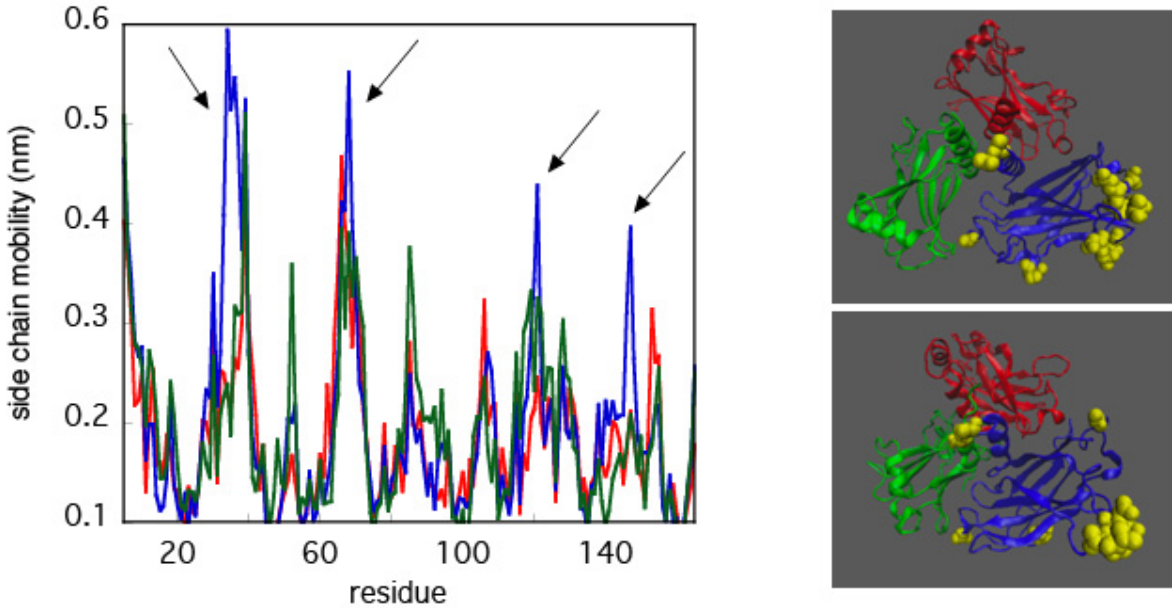

Figure 3. Left panel: Fluctuations in the side-chain positions of amino acids belonging to the A (red), B (blue), and C (green) TRAF2-C subunits, evaluated as root mean square deviation from the position at t = 0, along a 250 ns in silico simulation. The arrows indicate some of the most mobile residues of chain B.

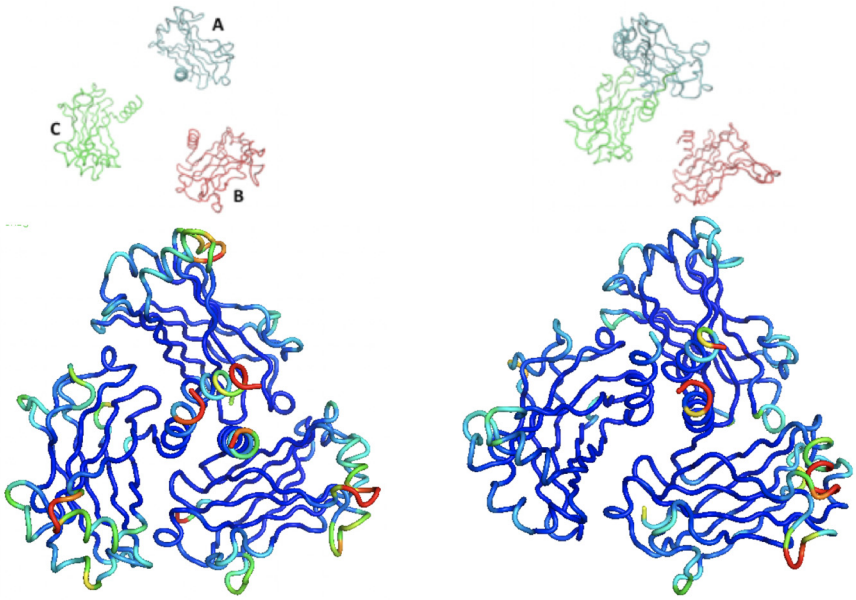

Figure 4. Upper panels: TRAF2 clusters identified by contact network analysis at time t = 0 (**left**) and t = 250 ns (**right**) of molecular dynamics simulation. The three subunits are identified by different colors, while the different association states have been artificially separated to enhance the clustering effect, using PISA software. Lower panels: Average mobility of the protein domains evaluated on the basis of the B-factors in the time intervals 0–50 ns (**left**) and 170–220 ns (**right**). The red-colored areas correspond to the highest average B-factor values, while those with progressively lower mobility are represented in orange, yellow, green, and blue, respectively.

3.3. Asymmetric and Correlated Motions in TRAF2-C Dynamics

The TRAF2-C dynamics upon relaxation from the initial crystal state are reported in Figure 5, for two independent MD simulations.

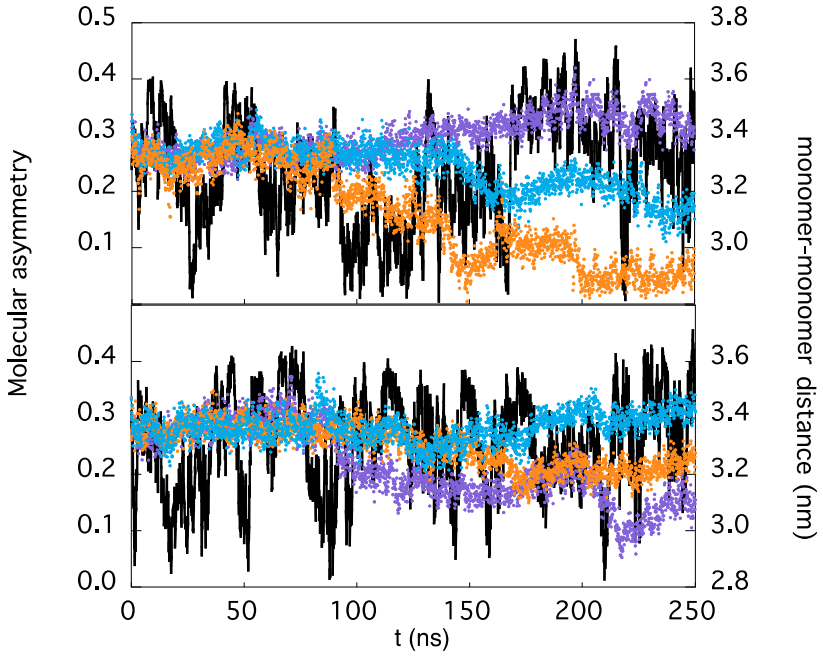

Figure 5. Monomer–monomer distances (A–B, purple; B–C, cyan; A–C, orange) and molecular asymmetry (black line) of TRAF2-C in two independent MD simulations.

In both cases, the positions of the amino acids in the three chains oscillate, producing a regular approach and distancing of the three subunits, which induces a periodic change in the protein's overall symmetry (Figure 5). A topological analysis through a contact network approach revealed that the formation of well-defined molecular clusters takes place from the very initial steps [21]. It follows that the asymmetry and cluster formation are evidently correlated. It is worth mentioning that, in the case of oligomers, clusters and symmetry are influenced not only by the average distances between residues but also by the reciprocal orientation of the monomeric subunits, which is, in turn, dictated by the interface interactions between each pair of monomers [13]. The oscillation produced in the TRAF2-C symmetry (Figure 5) indicates that the intensity and topology of subunits interactions are time-dependent. We, therefore, studied the dynamic correlation among amino acids belonging to the same chain and/or different monomers of TRAF2-C building up the Dynamic Cross Correlation (*DCC*) matrix (cfr Materials and Methods). As an example, the graphical rendering of the DDC matrix is shown in Figure 6A for the first 90 ns of the first simulation of Figure 5 (upper panel).

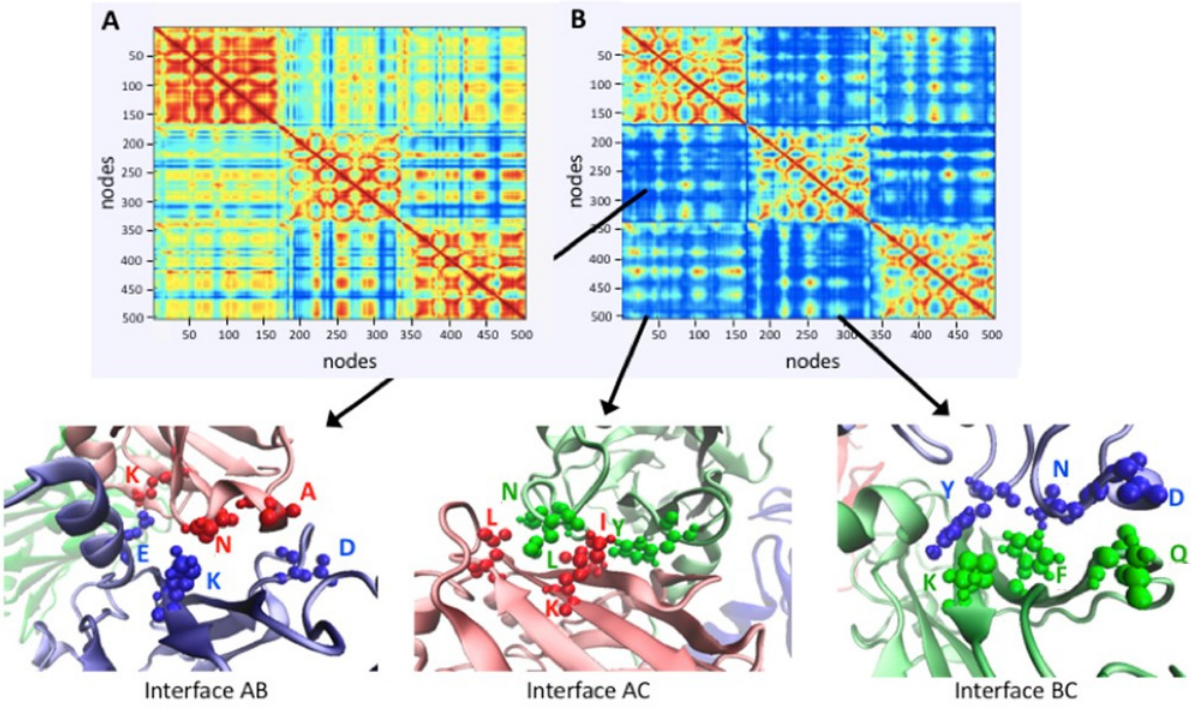

Figure 6. Graphic representation of the Dynamic Cross Correlation (*DCC*) matrix obtained for TRAF2-C MD in the time interval of 0–90 ns Panel (**A**) or 90–220 ns Panel (**B**). Red regions correspond to high positive Pearson correlation coefficients between residues trajectories, describing correlated motions. Values of the correlation coefficients close to zero are shown in dark blue, corresponding to uncorrelated motions. In the insets, the three inter-subunit sections with the highest values of correlation (red spots in Panel (**B**)) are reported. The pairs of residues at the interface, corresponding to the hottest points of Panel (**B**), are highlighted. Chains A, B, and C are drawn in red, blue, and green, respectively.

In this first phase, all three chains show strong internally correlated motions and good cross-correlation coefficients, as demonstrated by the reddish, honeycomb areas distributed along the principal diagonal (Figure 6A). Rectangles along the diagonal represent, in fact, the internal correlation maps for the three subunits (A, B, and C) and appear to be regularly ordered. The blue areas reported in Figure 6A represent low correlated motions of pairs of amino acids. Such areas are more present in the second and third rectangles along the principal diagonal, suggesting that several elements in both subunits B and C are more independent from each other. On the contrary, the more intense red coloring in the

first rectangle of Figure 6A indicates that the residues belonging to the A subunit are still characterized by very coherent movements. At variance with the central points of the *DCC* matrix, the elements outside the principal diagonal contain several asymmetric "blue channels", which are particularly evident in the case of the two rectangles representing the dynamic correlation between residues of subunits A and B and those of subunits B and C (Figure 6A). Greater coherence characterizes, instead, the movements of pairs of residues contained in subunits A and C, as revealed by the larger number of yellow spots present in the left bottom corner of the *DCC* matrix (Figure 6A). These findings are predictive of what occurs in the second part of both simulations, namely between 90 and 250 ns. As illustrated in Figure 5, this phase is more complex since two different phenomena overlap: The symmetry oscillations, produced by cluster formation and disassembly, and a dramatic change in the inter-subunit distances (Figure 5). This second effect produces an increase in the average value of TRAF2-C asymmetry in the longer time range (Figure 5), due to the approach of two subunits with respect to the third one. The formation of such a "prevalent" dimer has a random character: For instance, in the first simulation (Figure 5, upper panel), the closest distance at 250 ns is obtained for subunits A and C, while in the second simulation, a prevalent approach of monomers A and B is observed (Figure 5, lower panel).

The analysis of the correlation matrix performed in this second time interval (Figure 6B) shows a rather different scheme from that of the previous phase, and it can be summarized in two points: (i) All chains show a number of uncorrelated internal patches (blue zones along the central diagonal); (ii) the high number of cross-correlation regions belonging to different subunits (yellow areas in Figure 6A) has been substituted by a few highly correlated number of residues (dark red spots in the rectangles out of the central diagonal, Figure 6B). The first point suggests that within each subunit, the internal domains act more independently, with respect to the dynamics characterizing the first 90 ns of the simulation. The second point provides, instead, a new scenario at the level of the protein quaternary structure: The three chains move with increased autonomy but the trimeric structure is, however, guaranteed and controlled by a few contacts, represented by the red spots in the rectangles of the principal diagonal of the *DCC* matrix. Such pairs of highly correlated residues are located at the subunit interfaces, and the most representative (i.e., those which move more coherently) are reported in Figure 6B. Eleven among these eighteen residues are charged (7) or polar (4) amino acids; the remaining seven residues consist of four hydrophobic and three aromatic, non-polar side chains. The distribution of these residues is highly asymmetric, with the intersection between subunits A and C being characterized by the highest content of hydrophobic species (5), while interfaces AB and BC mainly consist of charged or polar amino acids: The hydrophilic nature of residues responsible for the interface asymmetry has been already demonstrated in allostery of enzymes [14].

3.4. Dynamic Clusters Analysis of TRAF2-C

The evident, strong differences that appear in the *DCC* evaluated at different time intervals of the MD simulation (Figure 6) strongly suggest that the concept of molecular clusters, as already introduced for TRAF2-C [13], must also be partly re-considered. In fact, rather than evaluating the clusters' association at a specific time, a more dynamic definition should be more appropriate, averaged on time intervals. Depending on the number of contacts between nearest-neighbor amino acids, clusters do not necessarily correspond to the single protein subunits of an oligomer, a particular condition that, for TRAF2-C, is obtained only at time t = 0, that is for the crystal structure. The results of the dynamic clustering analysis mediated on three different time intervals of the MD simulation are reported in Figure 7. The boundaries of the clusters do not exactly match the profile of the three subunits, and part of a monomer belongs to a cluster formed by residues of the other subunits, depending on the degree of correlation in the movements of pairs of interacting amino acids. Such dimeric coupling of TRAF2-C subunits is a function of time and depends

on a few specific residues at the subunit interfaces, as suggested by the *DCC* matrix in the longer time range.

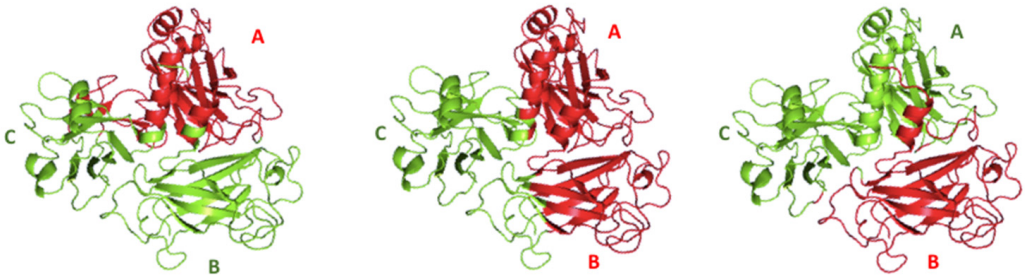

Figure 7. Map of the dynamic clusters (green or red) obtained between 10 and 60 ns (**left**), 80 and 130 ns (**central**), and 180 and 250 ns (**right** panel). For the sake of comparison, the chains are oriented and shown in the same configuration.

4. Conclusions

In summary, the ordered crystallographic symmetry of trimeric TRAF2-C might be partly compromised in water (Figures 5 and 6). In fact, the analysis of MD simulation demonstrates that dynamic clusters of two subunits are probably formed in water upon relaxation from the initial anhydrous structure, with the third subunit moving more independently from the other two chains. Independent MD simulations have demonstrated that such a phenomenon involves different pairs of monomers each time. Such a specific, tighter monomer–monomer association is characterized by a high correlation in the motion of hydrophobic residues located at the dimeric interface of the subunits forming the molecular cluster (AC in the case of Figure 6B). This is in line with pressure-induced dissociation measurements of TRAF2-C that demonstrate that the hydrophobic effect plays a relevant role in stabilizing the protein quaternary structure. The dimeric cluster formed at the end of the simulation is the one with the largest interface area and the greatest number of buried residues between the two subunits (Figure 2). Analysis of the molecular dynamics shows that the more independent subunit is characterized by enhanced fluctuations in the residue position (Figure 3) and higher B-factor values, with respect to the rest of the protein (Figure 4), thus counterbalancing the entropic cost of the more-ordered and cooler dimeric cluster. Analysis of the *DCC* matrix provides new, interesting insights into the role played by TRAF2-C interfaces. In fact, despite hydrophobicity being the main stabilizing force, the trimer structure at equilibrium is likely also regulated at the level of a few key residues that are mainly polar and hydrophilic, in the case of the more independent subunit. Such asymmetry in the topology of the more correlated pair of residues at the three interfaces might be important in regulating both the mechanical stress across the overall protein structure upon binding to other molecules (for instance, membrane receptors) and/or modulating the subunits' dissociation process. As a monomer, TRAF2-C is prone to binding synthetic [11] and biological membranes inducing vesiculation [12]. Moreover, docking simulation with receptor peptides [13] suggests that the tight association of two subunits might change the protein affinity for the receptor, influencing its biological function. The hypothesis suggested by in silico simulation that the balancing between two different kinds of interactions (hydrophobic and electrostatic) at the monomer–monomer interface might influence the TRAF2-C behavior in solution deserves more detailed experimental evidence, which is currently under development in our lab.

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Conflicts of Interest: The authors declare no conflict of interest.

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