

# Structure, dynamics, and ligand recognition of human-specific CHRFA7A (Dup $\alpha$ 7) nicotinic receptor linked to neuropsychiatric disorders

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## Abstract

Cholinergic  $\alpha$ 7 nicotinic receptors encoded by the CHRNA7 gene are ligand-gated ion channels directly related to memory and immunomodulation. Exons 5-7 in CHRNA7 can be duplicated and fused to exons A-E of FAR7a, resulting in a hybrid gene known as CHRFA7A, unique to humans. Its product, denoted herein as Dup $\alpha$ 7, is a truncated subunit where the N-terminal 146 residues of the ligand binding domain of the  $\alpha$ 7 receptor have been replaced by 27 residues from FAM7. Dup $\alpha$ 7 has a negative effect on the functioning of  $\alpha$ 7 receptors associated with neurological disorders, including Alzheimer's diseases and schizophrenia. However, the stoichiometry for the  $\alpha$ 7 nicotinic receptor containing dup $\alpha$ 7 monomers remains unknown. In this work, we developed computational models of all possible combinations of wild-type  $\alpha$ 7 and dup $\alpha$ 7 pentamers and evaluated their stability via atomistic molecular dynamics and coarse-grain simulations. We assessed the effect of dup $\alpha$ 7 subunits on the Ca<sup>2+</sup> conductance using free energy calculations. We showed that receptors comprising of four or more dup $\alpha$ 7 subunits are not stable enough to constitute a functional ion channel. We also showed that models with dup $\alpha$ 7/ $\alpha$ 7 interfaces are more stable and are less detrimental for the ion conductance in comparison to dup $\alpha$ 7/ dup $\alpha$ 7 interfaces. Based on these models, we used protein-protein docking to evaluate how such interfaces would interact with an antagonist,  $\alpha$ -bungarotoxin, and amyloid A $\beta$ <sub>42</sub>. Our findings show that the optimal stoichiometry of dup $\alpha$ 7/ $\alpha$ 7 functional pentamers should be no more than three dup $\alpha$ 7 monomers, in favour of a dup $\alpha$ 7/ $\alpha$ 7 interface in comparison to a homodimer dup $\alpha$ 7/dup $\alpha$ 7 interface. We also showed that receptors bearing dup $\alpha$ 7 subunits are less sensitive to A $\beta$ <sub>42</sub> effects, which may shed light on the translational gap reported for strategies focused on nicotinic receptors in Alzheimer's Disease research.

## Introduction

Nicotinic  $\alpha$ 7 receptors, encoded by the CHRNA7 gene, are ligand-gated ion channels involved in cognition, memory, and immunomodulation, which have emerged as attractive targets for neuropsychiatric disorders neuroinflammation, neuropathic pain, and autoimmune diseases (Lew et al., 2018). The overall three-dimensional structure of nicotinic  $\alpha$ 7 receptors is well characterised (Hendrickson et al., 2013). Each receptor is composed of five identical  $\alpha$ 7 subunits that are symmetrically arranged around the central channel's axis. Each subunit contains a large agonist-binding extracellular

domain, a transmembrane region formed by four helices, and an intracellular domain-containing receptor modulation and protein-protein interactions (Maroli et al., 2019). The acetylcholine (ACh) binding sites are located at the interfaces of the extracellular domains of adjacent subunits. The  $\alpha 7$  receptor has five identical ACh binding sites; however, ACh binding to only one site is sufficient for the receptor activation (Andersen et al., 2013). ACh binding triggers the conformational changes that propagate from the binding site toward the interface between the extracellular and transmembrane domains, called the coupling region. These conformational changes contribute to the molecular mechanism of the receptor activation (Andersen et al., 2013). The secondary structure of each subunit comprises a long hydrophilic extracellular domain (EC domain) with an N-terminal  $\beta$ -sheet, three hydrophobic transmembrane regions (M1-M3) that are all  $\alpha$ -helical (TM domain), followed by an intracellular loop (IC domain), a fourth transmembrane helix (M4), and an extracellular C-terminus (Albuquerque et al., 2009; Cecchini and Changeux, 2015; Dani, 2015; Hendrickson et al., 2013). Upon agonist binding to the extracellular domain, all subunits undergo a large conformational change, which results in the opening of the transmembrane hydrophilic channel. Hallmarks of  $\alpha 7$  receptors include their high  $\text{Ca}^{2+}$  permeability and very rapid desensitisation (Andersen et al., 2013).

In humans, exons 5-10 in *CHRNA7* may be duplicated and fused to exons A-E of *FAM7A* (family with sequence similarity 7A), resulting in the hybrid gene denoted as *CHRFAM7A* (Jiang et al., 2019; Sinkus et al., 2015; Sinkus et al., 2009). Its product, denoted as Dup $\alpha 7$ , is a truncated subunit where the N-terminal 146 residues of the ligand binding domain of the  $\alpha 7$  receptor have been replaced by 27 residues from *FAM7* protein (Jiang et al., 2019; Sinkus et al., 2015; Sinkus et al., 2009); the remaining sequences of  $\alpha 7$  and Dup $\alpha 7$  are identical (Costantini et al., 2015; Lasala et al., 2018). Dup $\alpha 7$  is associated with neuropsychiatric disorders, including Alzheimer's disease (Lew et al., 2018; Szigeti et al., 2020), schizophrenia (Kalmady et al., 2018), and immunomodulation (Chan et al., 2020; Maroli et al., 2019), most likely through regulating the function of the  $\alpha 7$  receptor by direct interactions (Lasala et al., 2018). Besides, Kunii and coworkers have observed that *CHRFAM7A* was upregulated in the brains of patients with schizophrenia and bipolar disorder, and the ratio of *CHRFAM7A/CHRNA7* increased (Kunii et al., 2015). It is postulated that Dup $\alpha 7$  acts as a dominant-negative inhibitor of  $\alpha 7$  function, suggesting its role in human cognition and immune responses by perturbing normal  $\alpha 7$  activities (Costantini et al., 2019). However, the exact mechanism and specific contribution of Dup $\alpha 7$  to the biology of  $\alpha 7$  receptors remain highly elusive.

Even though the quaternary arrangements of both nicotinic receptors are known (Hendrickson et al., 2013), and reports on overall stoichiometries of heteromeric  $\alpha 7$  and dup $\alpha 7$  receptors have been published recently (Lasala et al., 2018), atomistic details controlling those assemblies are still missing. Unravelling the molecular mechanisms governing the formation of the most probable dup $\alpha 7/\alpha 7$  pentamers is of high clinical interest, crucial for structure-guided approaches to target those heteromeric receptors, which account for the translational gap in research focusing on nicotinic receptors as therapeutic targets for neurodegenerative diseases (Szigeti et al., 2020).

To describe the most likely  $\alpha 7$ /dup $\alpha 7$  stoichiometry at an atomistic level of detail and to elucidate the structural, energetic and functional effects of incorporation of dup $\alpha 7$

monomers into the  $\alpha 7$  channels, we combined molecular modelling, multiscale molecular dynamics (MD) simulations (all-atom and coarse-grain), and umbrella sampling (US) simulations of the whole receptors embedded in a DCCP membrane. We have also studied extracellular domains separately using all-atom MD simulations, focusing on their structure and intrinsic dynamics alongside the binding side dynamical behaviour. We have subsequently studied how the formation of  $\alpha 7$ /dup $\alpha 7$  heteromeric receptors affected the binding of two known ligands of  $\alpha 7$  nicotinic receptors: the  $\beta$ -amyloid ( $A\beta_{42}$ ) and  $\alpha$ -bungarotoxin ( $\alpha$ -BTX) using molecular docking methods. Our results explain several experimental observations previously published. Our data link the structure and dynamics to the function of human-specific dup $\alpha 7$  receptors and address the specific translational gap reported for cholinergic strategies in Alzheimer's Disease (Szigeti et al., 2020). The outcomes of our study may serve as the starting point for structure-guided development of potent and selective dup $\alpha 7$  modulators to be rendered as future therapeutics for neurological and immune disorders.

## **Materials and Methods**

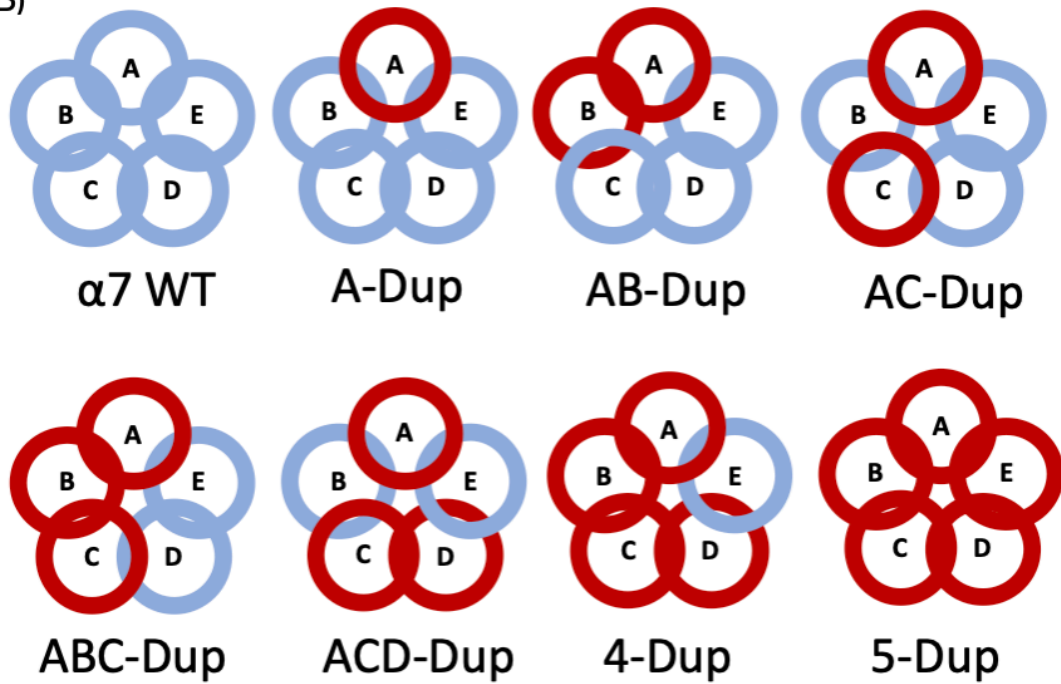
### **Molecular modelling of $\alpha 7$ and dup $\alpha 7$ receptors**

The initial models of pentameric  $\alpha 7$  homopentamers and partially duplicated dup $\alpha 7$  subunits were created using SWISS-MODEL, a fully automated protein structure homology-modelling server, accessible via the ExPASy web server (Bertoni et al., 2017; Waterhouse et al., 2018). The primary sequences of the human canonical  $\alpha 7$  and dup $\alpha 7$  were obtained from the UniProt repository (entries P36544 and Q494W8, respectively). Fifty models were generated and ranked according to their sequence similarity and QMEAN (Waterhouse et al., 2018) quality scores combined. After the visual inspection, the model based on the crystal structure of  $\alpha 7$ -AChBP in complex with lobeline (PDB code: 5AFH) combined with high-resolution (4.3 Å) cryo-EM structure of mouse 5-HT<sub>3</sub> serotonin receptor (PDB code: 6BE1) was chosen for both receptors, since the difference comes mainly from the N-terminal region (Figure 1A). Different stoichiometries were generated in UCSF Chimera molecular modelling and visualisation toolkit (Pettersen et al., 2004), resulting in eight receptor models in total (Fig. 1). A disulfide bridge at the conserved Cys-loop was ensured for each model. All models, arising from different stoichiometries of dup $\alpha 7$  and  $\alpha 7$  subunits, were quality checked by UCSF Chimera, having any missing loops modelled by MODELLER interface (Huang et al., 2014; Yang et al., 2012) within UCSF Chimera, and conformations of interfacial side chains checked for steric clashes.

A)

ACHA7_HUMAN	MRCSPGGVWLALAASLLHVSLQGEFQRKLYKELVKYNPLERPVANDSQPLTVYFSL	SLL				
CRFM7_HUMAN	-----	-----MQKYCIYQHF-----				
	70	80	90	100	110	120
ACHA7_HUMAN	QIMDVDEKNQVLTTNIWLQMSWTDHYLQWNVSEYPGVKTVRFPDQGIWKPDILL	YNSADE				
CRFM7_HUMAN	-----QFQLLIQHLEWIAAN-----	-----CDIADE				
	130	140	150	160	170	180
ACHA7_HUMAN	RFDATFHTNVLVNSSGHCQYLP	PGIFKSSCYIDVRWFP	FDVQHCKLKFGSWSYGGW	SLDL		
CRFM7_HUMAN	RFDATFHTNVLVNSSGHCQYLP	PGIFKSSCYIDVRWFP	FDVQHCKLKFGSWSYGGW	SLDL		

B)



**Figure 1:** A) Sequence alignment of  $\alpha 7$ /dup $\alpha 7$  extracellular (EC) domains (residues 1-180), performed by ClustalW. B) Schematic representation of all eight different model arrangements dup $\alpha 7$ - $\alpha 7$  pentamer, considered in this study: the canonical (WT)  $\alpha 7$  subunits are coloured blue; dup $\alpha 7$  subunits are coloured red.

### Coarse-grain molecular dynamics simulations

To equilibrate the modelled pentamers and understand the intrinsic dynamics in a time scale relevant to the ion channel conductance, coarse-grain simulations of all eight models were done. The atomistic models were translated to MARTINI beads (Kmieciak et al., 2016) and parametrised with the MARTINI 2.2 force field (Marrink et al., 2007). The principal protein axis was aligned to the Cartesian Z-axis. The membrane was built using the insane.py script. The membrane model was built using dipalmitoylphosphatidylcholine (DPPC), with 15 nm in the X and Y axis and 25 nm in the Z-axis, resulting in a box of 15x15x25 nm in dimensions. The system was then solvated,

using 90% of MARTINI water beads and 10% anti-freeze MARTINI water beads (Marrink et al., 2007). The solvated receptor-membrane systems were energy minimised using steepest descent algorithm and equilibrated. In the minimisation, the energy step size was set to 0.001 nm, and the maximum number of steps was set to 50,000. The minimisation was stopped when the maximum force fell below 1000 kJ/mol/nm using the Verlet cutoff scheme. Treatment of long-range electrostatic interactions and Van der Waals interactions were set to be shifted to 0 and 0.9 nm, respectively, beyond the cutoff of 1.5 nm. After the energy minimisation, heating to 300 K was performed for 10 ns with a time step of 20 fs. The temperature coupling was set between the protein and the non-protein entities using a Berendsen thermostat, with a time constant of 1 ps and the temperature set to reach 300 K with the pressure coupling. Pressure equilibration was run at 300 K with a semi-isotropic Berendsen barostat and set to 1 bar in an NPT ensemble. Both NVT and NPT had harmonic position restraints were applied to the backbone. The constraint algorithm used was LINCS. The production run was made using the same parameters as NPT, except the backbone position restraints were removed. The production run was made in triplicates of 1  $\mu$ s each, for each of the eight combinations of  $\alpha 7$ /dup $\alpha 7$ .

### **Atomistic molecular dynamics (MD) simulations**

Atomistic MD simulations have been carried out to generate ensembles to get a detailed insight into the stoichiometry of nicotinic  $\alpha 7$ /dup $\alpha 7$  receptors in atomistic resolution. The simulations were performed for  $\alpha 7$ /dup $\alpha 7$  pentamers with different stoichiometries for the models containing all three domains (EC-TM-IC) and only the EC domain to evaluate the effect of the TM-IC domain on the dynamics.

All simulations were performed using Gromacs 2016.3 (Van Der Spoel et al., 2005). The protein was parametrised using the AMBER99SB-ILDN force field, with the DPPC lipid bilayer and TIP3P water model (Lindorff-Larsen et al., 2010). The  $\alpha 7$  and dup $\alpha 7$  models were embedded in a DPPC bilayer lipid molecule, using the computational membrane builder tool in the CHARMM-GUI server ([www.charmm-gui.org](http://www.charmm-gui.org)) (Jo et al., 2007; Jo et al., 2008; Wu et al., 2014). Box distance was set to 1 nm, and periodic boundary conditions were applied. The box was solvated and Na<sup>+</sup> and Cl<sup>-</sup> ions were added to achieve a 0.1 M concentration and maintain charge neutrality of the unit. The solvated receptor-membrane systems were energy minimised and equilibrated. The minimisation ran using steepest descent for 1,000 cycles followed by the conjugate gradient. Energy step size was set to 0.001 nm, and the maximum number of steps was set to 50,000. The minimisation was stopped when the maximum force fell below 1000 kJ/mol/nm using the Verlet cutoff scheme. Treatment of long-range electrostatic interactions was set to Particle Mesh-Ewald (PME) (Darden et al., 1993), and the short-range electrostatic and van der Waals cutoff set to 1.0 nm. After the energy minimisation, heating to 300 K was performed for 20 ps with a time step of 2 fs and position restraints applied to the backbone in an NVT ensemble. The constraint algorithm used was LINCS, which was applied to all bonds and angles in the protein (Hess et al., 1997). The cutoff for non-bonded short-range interaction was set to 1.0 nm with the Verlet cutoff scheme. Long-range electrostatics were set to PME. The temperature coupling was set between the protein and the non-protein entities using a Berendsen thermostat, with a time constant of 0.1 ps and the temperature set to reach 300 K with the pressure coupling off. Pressure equilibration was run at 300 K with a Parrinello-Rahman barostat and set to 1 bar

(Parrinello and Rahman, 1981) in an NPT ensemble. The equilibration trajectories were set to 5 ns (discarded from the analysis), and the production MD simulations were performed for 100 ns. Each trajectory was run in triplicates.

Analysis of the trajectories was performed using GROMACS tools, including root-mean-square deviation (RMSD) to assess overall stability, per-residue root-mean-square fluctuation (RMSF) to assess the local flexibility, and calculating SASA for solvent-mapping.

### **Umbrella sampling (US) simulations**

Umbrella sampling (steered molecular dynamics) (Kästner, 2011) simulations were used to assess the influence of different stoichiometry of receptors on the pentamer's ion conductance. The energies of the  $\text{Ca}^{2+}$  ion pulled through the axis of the channel pore (Z-axis) were calculated using the Weighted Histogram Analysis Method (WHAM) method (Ferrenberg and Swendsen, 1989) to extract the potential of mean force (PMF). To prevent the channel from moving out of the membrane, the receptor subunits were position-restrained during the pulling simulations, using  $1000 \text{ kJ mol}^{-1}\text{nm}^{-2}$ . The ion has been placed above the top of the EC domain and pulled downward along the Z-axis towards the TM and IC domains over 5 ns at a rate of  $0.01 \text{ \AA.ps}^{-1}$ . A series of umbrella sampling windows were generated from the pulling trajectory to proceed with the umbrella sampling. The entire pathway covering the range of  $[-10, 10] \text{ \AA}$  was divided into  $0.7 \text{ \AA}$ , totalizing 40 windows.

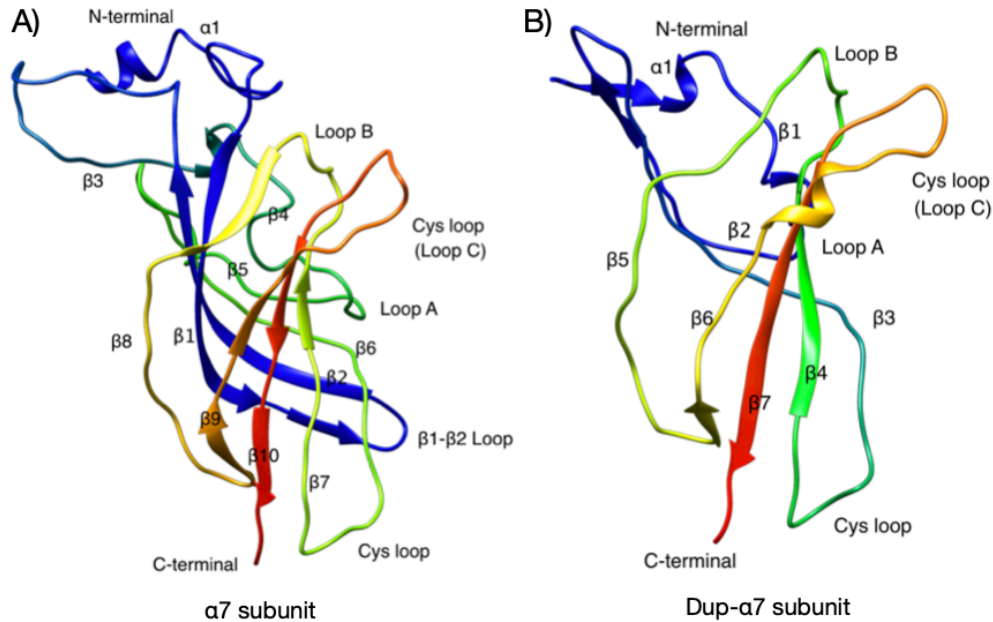
### **A $\beta$ 42 and $\alpha$ -bungarotoxin ( $\alpha$ -BTX) binding to $\alpha$ 7 pentamers**

The analysis of interactions between different  $\alpha$ 7/dup $\alpha$ 7 receptors and two established macromolecular ligands: A $\beta$  and  $\alpha$ -BTX, were performed by molecular docking. A $\beta$  (PDB code: 6RHY) and  $\alpha$ -BTX (PDB code:4HQP) were docked to all four possible combinations of  $\alpha$ 7 and dup $\alpha$ 7 interfaces using ClusPro web server (Desta et al., 2020; Kozakov et al., 2017). The top 10 lowest-energy complexes were selected to further analysis, and the binding affinities were calculated by SeeSAR ([www.biosolveit.de](http://www.biosolveit.de)) (Lee et al., 2016), using the HYDE scoring function (Reulecke et al., 2008).

## **Results**

### **Stoichiometry studies for different combinations of dup $\alpha$ 7/ $\alpha$ 7**

The three-dimensional molecular models of extracellular domains of the canonical  $\alpha$ 7 and dup $\alpha$ 7 are shown in Fig. 2. In dup $\alpha$ 7, the N-terminal segment (blue region in Fig. 2) of  $\alpha$ 7 has been replaced by a shorter  $\alpha$ -helix (Fig. 2B). Three  $\beta$ -sheet segments ( $\beta$ 1- $\beta$ 3 in Fig. 2) are missing, yet the following segment ( $\beta$ -sheets 4 to 10) resembles the canonical structure.

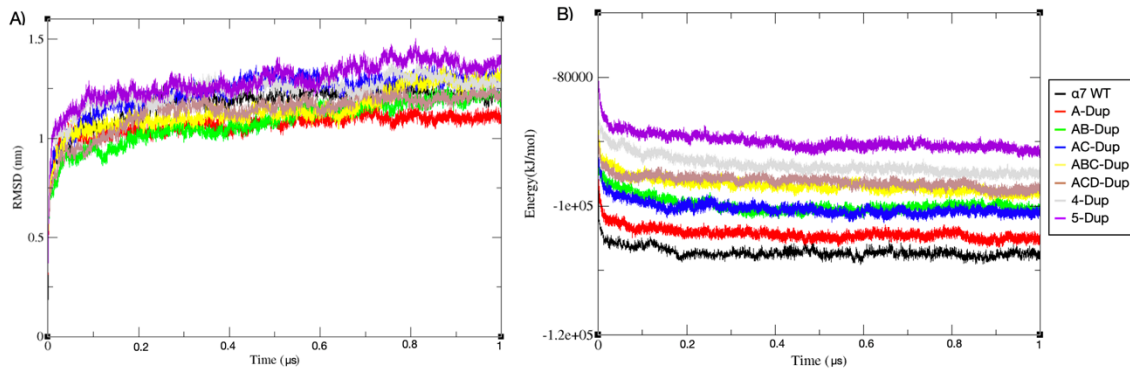


**Figure 2:** The extracellular EC domain conformation of  $\alpha 7$  subunit (residues 1-180). (A) and Dup $\alpha 7$  subunit (B). The structures are coloured by gradient, from blue (N-terminus) to red (C-terminus).

Given that the pentamer's overall structure containing Dup $\alpha 7$  subunits has not been experimentally solved, we built and investigated all possible models. These different combined pentamers totalise eight models, with seven containing various combinations of Dup $\alpha 7$  nicotinic receptors (Fig. 1B). The functional pentamer is assembled by the dimerisation through only two interfaces. Given this, four possible dimer combinations can be formed: WT-WT, dup $\alpha 7$ -WT, WT-dup $\alpha 7$ , dup $\alpha 7$ -dup $\alpha 7$ ). Those combinations are functionally relevant since the orthosteric binding site is located at the interface between two monomers. Thus, each particular dimer combination will affect the receptor's function.

The eight models were first simulated using a coarse-grain approach by translating the atomistic model to MARTINI beads (Supp. Fig. S.1). The RMSD and the total potential energy in the function of time are shown in Fig. 3. All eight pentamers showed a similar magnitude of deviation from their starting structures (Fig. 3A), with the average values fluctuating between 1 to 1.4 nm, which was achieved by most of the models after 50 ns of simulation.

The RMSD plots did not show any statistical difference regarding relative motions regarding their starting configuration when a single WT  $\alpha 7$  is swapped for a dup $\alpha 7$ . However, there is a significant difference in total internal potential energy between the different stoichiometries. As expected, the WT  $\alpha 7$  has the lowest internal energy average value, closely followed by A-Dup $\alpha 7$ . Interestingly, both models with two Dup $\alpha 7$  (AB-Dup $\alpha 7$  and AC-Dup $\alpha 7$ ) have undistinguishable average values, which is also the case for both models with three Dup $\alpha 7$  (ABC. and ACD-Dup $\alpha 7$ ). This characteristic shows that CG models could discern the different stoichiometries' internal energy, but it does not have the resolution to discern which internal configuration is more stable. Therefore, we decided to simulate all eight models with an atomistic resolution.



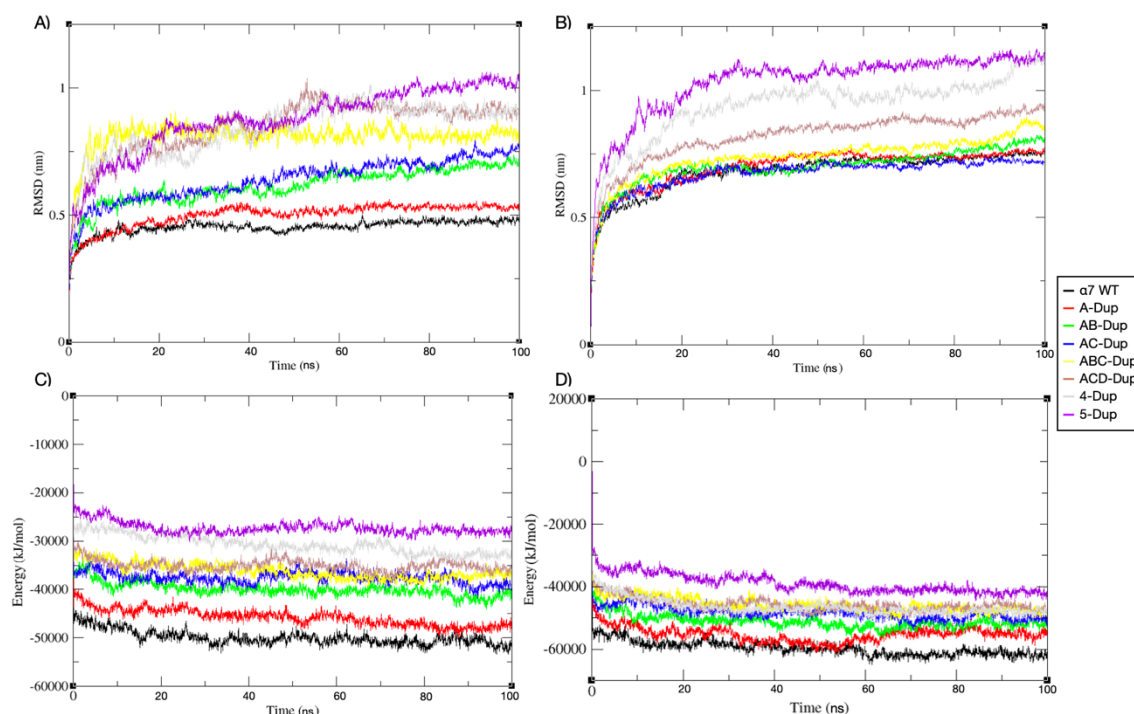
**Figure 3:** A) Root-mean-square deviation (RMSD) results of eight complete transmembrane model during 1  $\mu$ s CG MD simulation. B) Total potential energy vs time results of eight complete transmembrane model during 1  $\mu$ s MD simulation. The black line shows the data for  $\alpha 7$  WT model, red - A-Dup, green - AB-Dup, blue - AC-Dup, yellow - ABC-Dup, brown - ACD-Dup, grey - 4-Dup, and purple - 5-Dup. The schematic arrangements of all models are shown in Fig. 1B.

To unravel the most probable stoichiometry and the effect the interfacial interactions have on the system stability, all eight models of different combinations of Dup $\alpha 7$ / $\alpha 7$  were simulated for 100 ns (each replica, all simulations performed in triplicates) in an atomistic resolution. This step was made for both the whole model embedded in a DPPC membrane and its EC domain separately. The protein RMSD for the trajectories shows that for a higher ratio of Dup $\alpha 7$ / $\alpha 7$ , the overall average RMSD is higher compared to the starting structure. This characteristic is more pronounced for the EC domain simulations, as shown in Fig. 4A and Fig. 4B, respectively.

The pentamer simulations embedded in membrane show RMSDs quickly plateauing for most of the models containing less than three Dup $\alpha 7$  subunits. Nonetheless, the ACD-Dup $\alpha 7$  model shows a higher average RMSD than its ABC-Dup $\alpha 7$  counterpart, which may indicate that interfacial dup $\alpha 7$ -dup $\alpha 7$  interactions may be favourable for the molecular assemble.

The analysis of the pentamers' internal potential energy (Fig. 4C-D) shows that the average energy increases as the Dup $\alpha 7$ / $\alpha 7$  ratio increases. For the extracellular (EC) domain, the combination containing a single Dup $\alpha 7$  subunit has significantly lower energy than all the other models. However, the full-length receptor model simulations show that the A model's internal energy (Fig. 1B) fluctuates between similar values to the WT and AB model. The difference in energy between the EC domain and its counterpart containing all three domains may indicate that the transmembrane and intracellular (IC) domains may significantly stabilise the pentamers containing Dup $\alpha 7$  subunits.

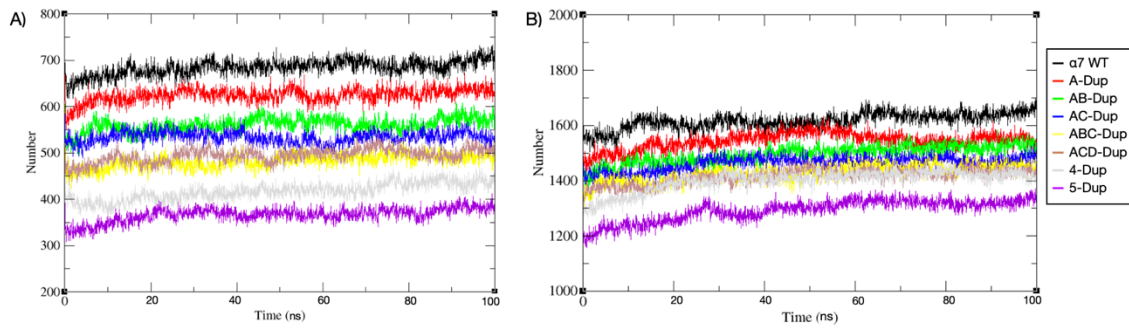




**Figure 4:** A) Root-mean-square deviation (RMSD) results of eight EC domain models during 100 ns MD simulation. B) Root-mean-square deviation (RMSD) of eight full-length receptor models during 100 ns MD simulation. C) Total potential energy vs time of eight EC domain during 100 ns MD simulation. D) Total potential energy vs time of eight complete transmembrane structure during 100 ns MD simulation. The canonical  $\alpha 7$  (WT) model is shown in black, A-Dup $\alpha 7$  - red, AB-Dup $\alpha 7$  - green, AC-Dup $\alpha 7$  - green, ABC-Dup $\alpha 7$  - yellow, ACD-Dup $\alpha 7$  - brown, 4-Dup $\alpha 7$  - grey, and 5-Dup $\alpha 7$  - purple. The schematic arrangements of all models are shown in Fig. 1B.

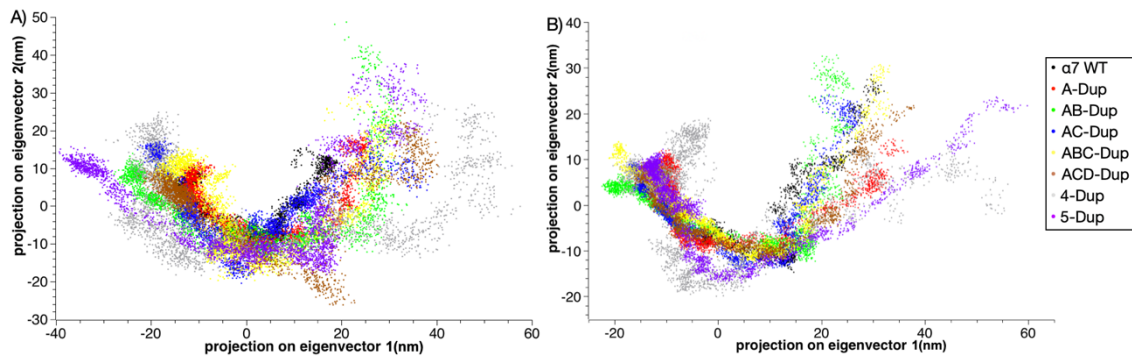
As expected, the total number of hydrogen bonds formed shows similar behaviour to the calculated potential energy. Specifically, the EC domain trajectories (Fig. 5A) show a higher number of H-Bonds for both WT and A-Dup than their counterparts. The pentamers containing a higher number of Dup $\alpha 7$  subunits show higher average internal energy and a lower average number of hydrogen bonds. Therefore, a clear correlation was observed between a higher Dup $\alpha 7$ /  $\alpha 7$  ratio and lower structural energy and fewer total hydrogen bonds. (Fig. 5B)

When observing the hydrogen bond formation between interfaces, several key residues were identified. All these sets of residues are listed in Suppl. Tables S.1-16. Investigating WT-WT dimer, we identified six residues that are often present on the interaction between two adjacent subunits (N69, N75, R101, P110, D111 and W172). In contrast to that, the number of interfacial hydrogen bond pairs for WT-Dup $\alpha 7$  interfaces varied between three to four. One of the main differences between the WT-WT interfaces and Dup $\alpha 7$  containing interfaces is that the latter does not have the hydrogen bond between R101 and the P110 of the sequential subunit residues are not present in the sequence of the Dup $\alpha 7$ .



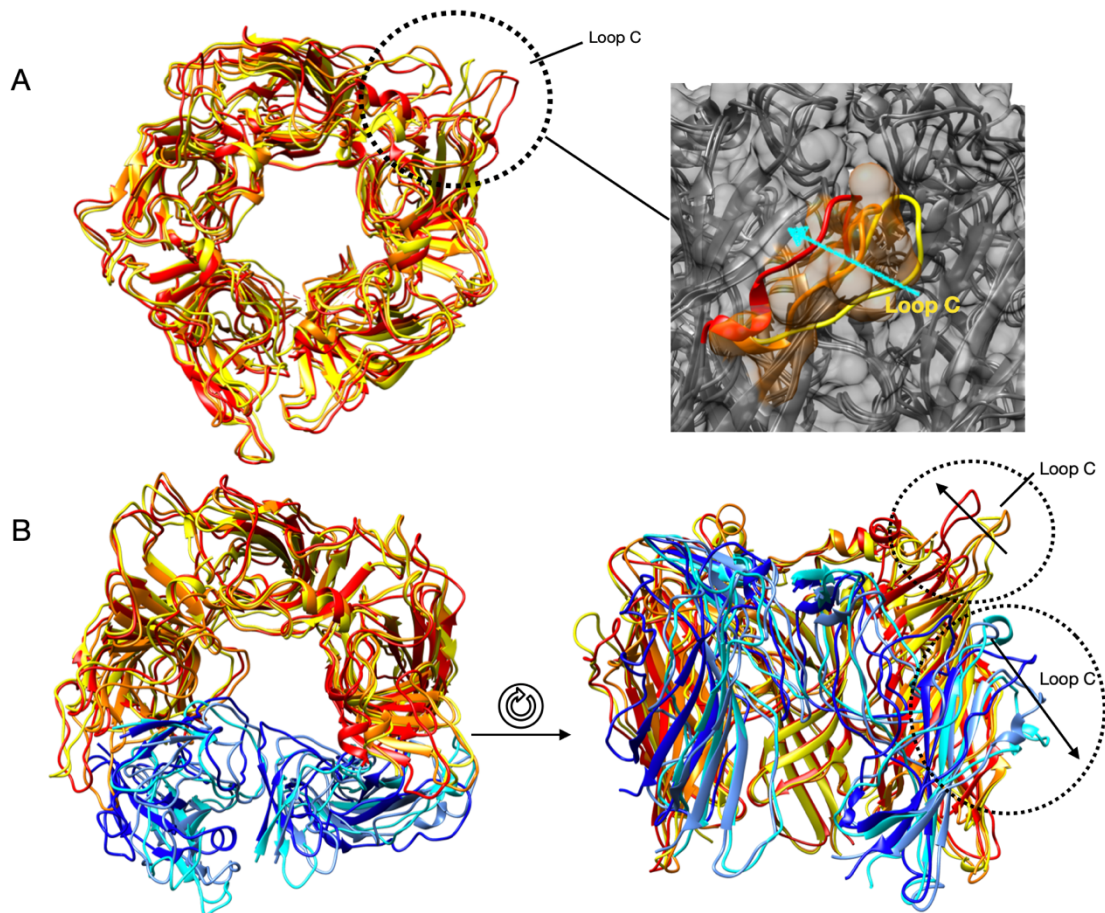
**Figure 5:** A) The total number of hydrogen bonds vs time of the eight EC domain combinations. B) The total number of hydrogen bonds vs time of eight fully models during 100 ns MD simulation. The black line shows  $\alpha 7$  WT model; red - A-Dup $\alpha 7$ , green - AB-Dup $\alpha 7$ , blue - AC-Dup $\alpha 7$ , yellow - ABC-Dup $\alpha 7$ , brown - ACD-Dup $\alpha 7$ , grey - 4-Dup $\alpha 7$ , and purple - 5-Dup $\alpha 7$ . The schematic arrangements of all models are shown in Fig. 1B.

A higher number of Dup $\alpha 7$  subunits directly changes the molecular dynamics. Fig. 6 shows that the trajectory projection on the two most significant principal components is affected by the dup $\alpha 7$ / $\alpha 7$  ratio. WT, A-Dup $\alpha 7$  and the complexes with two Dup $\alpha 7$  (AB and AC) subunits have similar distributions (Fig. 6A), especially for the transmembrane complexes (Fig. 6B). The most significant difference comes from models with three or higher number of dup $\alpha 7$  subunits. Given their higher energy, pentamers with four or five subunits resulted in higher magnitudes on their projection values, achieving a sparser distribution on the two-dimensional principal component space. These complexes show the same behaviour for their extracellular (EC) domain simulations, albeit less pronounced than the complexes containing a lower Dup $\alpha 7$ / $\alpha 7$  ratio when the EC domain is simulated by itself.



**Figure 6:** A) Principal component analysis, showing the 2D projection of eight different models of the extracellular (EC) domain. B) Principal component 2D projection of eight different fully models during 100 ns of the atomistic MD simulation. The data for  $\alpha 7$  WT model is coloured black; A-Dup $\alpha 7$  - red, AB-Dup $\alpha 7$  - green, AC-Dup $\alpha 7$  - blue, ABC-Dup $\alpha 7$  - yellow, ACD-Dup $\alpha 7$  - brown, 4-Dup $\alpha 7$  - grey, 5-Dup $\alpha 7$  - purple. The schematic arrangements of all models are shown in Fig. 1B.

These differences in the transmembrane model dynamics are directly related to the motions on its EC domain, mainly of loop C and the loops connecting the central  $\beta$  sheets (Fig. 7). The motion of Loop C changes depending on its neighbouring subunit for both simulated models, as shown in Fig. 7 and Supp. Fig. S.2. For WT-WT interfaces (Fig. 7A), the loop fluctuates between an open and closed conformation and the entire  $\alpha 7$  model remains stable overall (Supp. Fig. S.3). WT-Dup $\alpha 7$  and Dup $\alpha 7$ -Dup $\alpha 7$  interfaces (Fig. 7B and Fig. 7C) show a more open conformation and higher local flexibility.



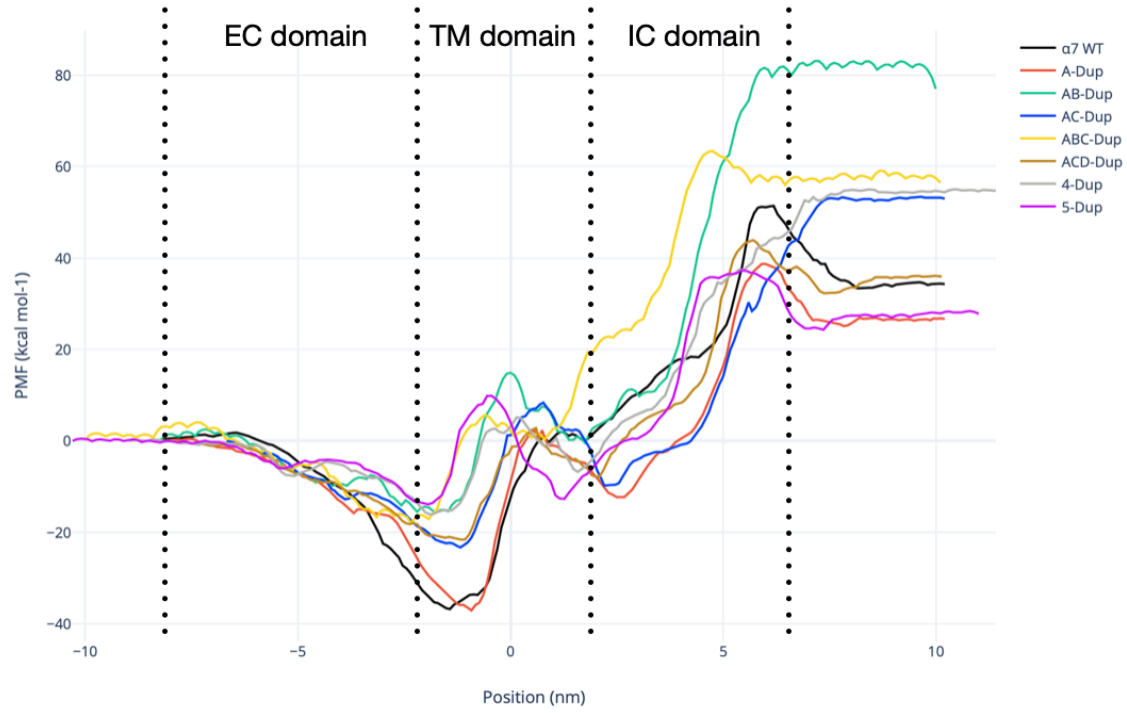
**Figure 7:** A) Left panel: The extracellular view of the three conformations of the  $\alpha 7$  model EC domain; Right panel: the Loop C motion. The representative configurations sampled around 15 ns, 50 ns and 85 ns are coloured yellow, orange and red, respectively; B) The extracellular view of the three conformations of the AB-Dup $\alpha 7$  model EC domain. The representative configurations for the starting conformation's state (around 15ns) are coloured yellow and cyan; the representative configurations for the state sampled around 50 ns are coloured orange and cornflower blue; the representative configurations for state sampled around 85 ns are coloured red and blue. The dashed lines represent the interfacial loops, which are the areas with the highest fluctuation.

### The effect of Dup $\alpha 7$ subunits on $\text{Ca}^{2+}$ conductance

To investigate the effect of different combinations of Dup $\alpha 7$ / $\alpha 7$  subunits on the  $\text{Ca}^{2+}$  passing, umbrella sampling simulations were carried out. As shown in Fig. 8, all eight potential mean force (PMF) curves show similar profiles. At -8 nm, the ion is at the tunnel entrance in the EC domain, transitioning to the transmembrane region (TM) around -1.5nm. The energetic differences between the eight combinations started to arise around -5 nm, near the TM region's start. At this region,  $\alpha 7$  and A-Dup $\alpha 7$  have the lowest energy (-37.5 kcal.mol<sup>-1</sup>). However, an energetic barrier emerges after the TM entrance, which is higher for AB, ABC, 4-Dup $\alpha 7$  and 5-Dup $\alpha 7$ . This energetic difference emerges from how the ions interact with the model, specifically with glutamate E254. The canonical  $\alpha 7$  (WT) structure can maintain the cohesion of the beginning of the  $\alpha$ -helix located in the TM entrance, where the E254 residue is located (Sequence: 250-LVAEIM-257). This behaviour allows a more favourable interaction with the E254, resulting in a higher number of contact points with  $\text{Ca}^{2+}$  (Supp. Fig. S.4 and Supp. Tables S.17-18)

The final 2nm – 6nm section represent the intracellular (IC) domain, which shows that the energy becomes significantly higher when the ion is distant from the TM domain.

The AB-Dup $\alpha$ 7 shows the highest energy (81.2 kcal.mol<sup>-1</sup>) at the bottom of the IC domain, with the lowest 5-Dup $\alpha$ 7 pentamer results (28.3 kcal.mol<sup>-1</sup>).



**Figure 8:** Potential of mean force (PMF) calculated for the position of the Ca<sup>2+</sup> moving through the pentamer axis. The black line shows the PMF obtained for the canonical  $\alpha$ 7 (WT) receptor, A-Dup $\alpha$ 7 - red, AB-Dup $\alpha$ 7 - green, AC-Dup $\alpha$ 7 - blue, ABC-Dup $\alpha$ 7 - yellow, ACD-Dup $\alpha$ 7 - brown, 4-Dup $\alpha$ 7 - grey, and 5-Dup $\alpha$ 7 - purple. The schematic arrangements of all models are shown in Fig. 1B.

The final 2nm – 6nm section represent the intracellular (IC) domain, which shows that the energy becomes significantly higher when the ion far away from the TM domain. The AB-Dup $\alpha$ 7 shows the highest energy (81.2 kcal.mol<sup>-1</sup>) at the bottom of the IC domain, with the lowest 5-Dup $\alpha$ 7 model results (28.3 kcal.mol<sup>-1</sup>).

*Table 1: Sampling results Ca<sup>2+</sup> permeability energetics*

Energy/Model (kcal. mol <sup>-1</sup> )	$\alpha$ 7	A-Dup	AB-Dup	AC-Dup	ABC-Dup	ACD-Dup	4-Dup	5-Dup
EC → TM (around -1.5 nm)	-37.5	-37.5	-15.7	-23.6	-16.4	-21.6	-15.9	-13.9
Transmembrane domain (around 0 nm)	0.2	1.8	14.6	7.7	4.1	2.3	4.8	9.84
TM → IC (around 2 nm)	-0.1	-12.5	-0.1	-10.4	1.1	-7.6	-6.6	-12.6
Exiting IC (around 6 nm)	51.1	37.6	81.2	52.3	63.1	43.7	52.8	37.3

## The effect of Dup $\alpha$ 7 subunits on macromolecular ligand binding

Alongside the effect of the Dup $\alpha$ 7/ $\alpha$ 7 ratio on the pentameric receptor dynamics, we also assessed the effects of Dup $\alpha$ 7/ $\alpha$ 7 ratio on the binding of two macromolecular ligands:  $\alpha$ -bungarotoxin ( $\alpha$ -BTX) and the amyloid  $\beta$  ( $A\beta_{42}$ ).  $\alpha$ -BTX is a well-established, 74 residues (8 kDa) neurotoxin that binds nicotinic acetylcholine receptors, including  $\alpha$ 7 subtypes, and acts as a competitive antagonist upon them. While the experimental structure of  $\alpha$ -BTX-  $\alpha$ 7 is known (PDB code: 4HQP), the structural information on interactions between receptors containing Dup $\alpha$ 7 subunits and  $\alpha$ -BTX is missing.  $A\beta_{42}$  and  $\alpha$ 7 interact with high affinity (Ihnatovych et al., 2019; Roberts et al., 2021). However, the details of those interactions remain elusive.

To gain insight into those interactions, we have performed molecular docking calculations. Molecular models of the  $\alpha$ -BTX binding sites were built on the dimerisation interface (orthosteric binding sites, inferred from the reported  $\alpha$ -BTX-  $\alpha$ 7 interactions). We evaluated the binding poses of  $\alpha$ -BTX and their binding affinities to all four combinations of receptors:  $\alpha$ 7/ $\alpha$ 7 (canonical),  $\alpha$ 7-Dup $\alpha$ 7, Dup $\alpha$ 7- $\alpha$ 7, and Dup $\alpha$ 7-Dup $\alpha$ 7.

To identify the binding site for  $A\beta_{42}$ , we performed the exhaustive scan of the whole extracellular receptor domain using ClusPro. The best-scoring poses (8/10) converged to the site, which partially overlaps with the reported  $\alpha$ -BTX binding sites, consistent with the reports on  $\alpha$ 7 receptor activation via orthosteric modality reversing the  $A\beta_{42}$  binding (Roberts et al., 2021). Therefore, we concluded that the amyloid  $\beta$  ( $A\beta_{42}$ ) binding site overlaps with that for  $\alpha$ -BTX.

The results are shown in Table 2.  $\alpha$ -BTX showed very low binding affinity to all the receptors containing Dup $\alpha$ 7 subunits (Table 2), suggesting that those receptors will be resistant to  $\alpha$ -BTX. This observation agrees with the published experimental data showing that *CHRFAM7A* decreased  $\alpha$ -BTX binding as detected by immunohistochemistry and flow cytometry and markedly decreased  $\alpha$ -BTX staining neuromuscular junction of *CHRFAM7A* transgenic mice (Chan et al., 2019).

For  $A\beta_{42}$  interactions, the ligand residues that interacted with the receptor binding sites were located in the C-terminal regions of the  $A\beta_{42}$  monomer (Table 2). The predicted binding affinity of the  $A\beta_{42}$  to the binding site comprising two  $\alpha$ 7 subunits (the canonical receptor) was in the low nM range, consistent with available experimental data (Roberts et al., 2021). As a comparison,  $\alpha$ -BTX was predicted to bind to the canonical  $\alpha$ 7 binding sites with one order of magnitude lower (high pM range) than  $A\beta$ . This difference, too, is consistent with the experimental data available.

The  $\alpha$ 7 receptor residues crucial for maintaining the binding pose include R208, F209, and E211. All those residues are conserved in both  $\alpha$ 7 and Dup $\alpha$ 7 isoforms, albeit their dynamics are different in Dup $\alpha$ 7. In particular, R208 and E211 were involved in favourable, stabilising electrostatic interactions with the ligand. F209 is involved in the network of aromatic residues, including two tryptophan residues: W171 and W77. The latter is located at the different subunit, as showed in Fig. 9, and, despite variation in the N-terminal region of the receptor, it is also conserved in primary sequence alignment between  $\alpha$ 7 and Dup $\alpha$ 7 (Fig. 1A).

When we analysed the effects of Dup $\alpha$ 7 on the binding affinities, we observed a substantial increase of the predicted binding affinities for  $A\beta$  by both  $\alpha$ 7-Dup $\alpha$ 7 and

Dup $\alpha$ 7-Dup $\alpha$ 7 (predicted to be within low-mM range), compared to the canonical  $\alpha$ 7- $\alpha$ 7 binding site (affinities in low-nM range, Table 2). Intriguingly, calculated binding affinity for A $\beta$  to Dup $\alpha$ 7- $\alpha$ 7 improved (high nM to low  $\mu$ M range). This difference indicates that the residues involved in the interactions with A $\beta$  and are not conserved between Dup $\alpha$ 7 and  $\alpha$ 7 (S56, S58, L60, and Q61) may be necessary complex stabilisation. Also, it indicates that the  $\alpha$ 7 secondary structure architecture in this region is critical for the binding of A $\beta$ <sub>42</sub>. This characteristic suggests that receptors containing Dup $\alpha$ 7 subunits will be more resistant to A $\beta$ <sub>42</sub>-related toxicity, supported by recent experimental data (Szigeti et al., 2020).

Table2: Binding affinity ranges of A $\beta$ <sub>42</sub> or  $\alpha$ -BTX to orthosteric binding sites. All affinity ranges were calculated by SeeSAR.

<i>Binding site interface</i>	<i>A<math>\beta</math><sub>42</sub> affinity (K<sub>i</sub> calc)</i>	<i><math>\alpha</math>-BTX affinity (K<sub>i</sub> calc)</i>
<i><math>\alpha</math>7 – <math>\alpha</math>7</i>	Low nM	High pM
<i><math>\alpha</math>7-Dup<math>\alpha</math>7</i>	mM	>mM
<i>Dup<math>\alpha</math>7-<math>\alpha</math>7</i>	High nM to Low $\mu$ M	>mM
<i>Dup<math>\alpha</math>7-Dup<math>\alpha</math>7</i>	mM	>mM

## Discussion

### Dup $\alpha$ 7/ $\alpha$ 7 ratio directly affects the structural cohesion of the receptor pentamer

In this work, we aimed to understand the assembly and stability of different combinations of functional  $\alpha$ 7 nicotinic acetylcholine receptors (nAChR), bearing a partial duplicate Dup $\alpha$ 7nAChR.

Lasala and coworkers reported that WT  $\alpha$ 7 nicotinic receptors could form functional pentamers incorporating Dup $\alpha$ 7 subunits. However, for this to be the case, the minimum number of WT  $\alpha$ 7 had to be two (Lasala et al., 2018). Nonetheless, neither the effect of different stoichiometry on  $\alpha$ 7 nAChR receptor function nor the structural stability of pentamers containing Dup $\alpha$ 7 subunits is known.

Our results showed that the overall arrangement of the extracellular domain for both  $\alpha$ 7 and Dup $\alpha$ 7 subunits is similar, except for the N-terminal portion of the EC domain. This characteristic agrees with the models published shown by Lasala and coworkers (Lasala et al., 2018). Their models showed that changes in the  $\alpha$ 1 loop and differences in the configuration of the  $\beta$ -sheets in the EC domain core could be observed between the canonical and Dup $\alpha$ 7-including receptors. Our data confirmed that these modifications affected the heteropentamers' stability and showed and how the interfaces interact.



We showed how structural equilibration of the receptors with different stoichiometries occurs through time. For the pentamers with higher Dup $\alpha$ 7 content, the RMSD curves take longer to plateau, and they reach higher average values than their starting structure, which reflects their decreased stability and weaker cohesion. These characteristics are found in both resolutions used: coarse-grain and atomistic MD as well. Also, similar behaviour when comparing the internal structural energy can be found: the pentamers' average structural energy steadily increases with the Dup $\alpha$ 7/ $\alpha$ 7 ratio. This comes from several hydrogen bonds' disassemble and favourable interactions, both at the interfaces and hydrophobic core. One of the critical differences comes from the absence of the interaction with the R101 in the receptors containing Dup $\alpha$ 7 subunits. This residue interacts with several other residues located in the subsequential subunit, and the lack of these interactions directly affects the structural cohesion of the interface.

The truncation directly affects the orthosteric binding site configuration. As we showed, the loop dynamics that works as a gatekeeper entirely depends on the Dup $\alpha$ 7/ $\alpha$ 7 interactions.

The canonical  $\alpha$ 7 (WT) pentamer simulations showed that loop C moves from an open conformation to a closed conformation. At the WT-Dup $\alpha$ 7 interfaces, very different dynamic signatures are found. As discussed in previous works (Andersen et al., 2011; Andersen et al., 2013), the requirement for a functional  $\alpha$ 7 pentamer is a single functional orthosteric binding site, albeit this is less sensitive in comparison to a fully functional pentamer with up to five orthosteric sites. Given the destabilisation effect that dup $\alpha$ 7 subunit causes on its dimerisation interface and in the orthosteric binding site, the dup $\alpha$ 7 interface should be less sensitive to the orthosteric ligand binding. This effect is evidenced by how the loop C on the dup $\alpha$ 7 subunit moves away from the pentamer. This movement shows that the dup $\alpha$ 7 binding site might remain open, not stabilising the orthosteric ligand in the binding cavity.

The partial duplication and truncation that leads to the dup $\alpha$ 7 protein are located in the extracellular domain (Fig. 1A). Hence, this is the region that shows the highest difference between WT and dup $\alpha$ 7 dynamics. However, the transmembrane domain reduces the system's overall energy by creating a higher number of hydrogen bonds, resulting in a lower average RMSD and RMSD standard deviation compared to the EC domain by itself. Nonetheless, pentamers containing Dup $\alpha$ 7 subunits go through structural changes within the TM domain, mainly on the entrance region (residues 250-257). The energetic landscape referring to the extracellular to intracellular calcium intake showed that this region plays a vital role in this process.

Residues 250-257 go from an  $\alpha$ -helical conformation in the WT pentamer to a short  $\pi$ -helix in most dup $\alpha$ 7 models. This conformation transition destabilises the favourable interactions between the calcium and the residue E254 and its neighbouring residues, which was found in the WT simulation. This also is an effect that is directly related by the Dup $\alpha$ 7/ $\alpha$ 7 ratio: Both WT and A-Dup $\alpha$ 7 models show similar profiles, indicating that a single Dup $\alpha$ 7 subunit does not affect the calcium intake substantially. Nonetheless, another Dup $\alpha$ 7 subunit increases the system energy substantially for the TM domain entrance region. This energy difference suggests that pentamers bearing two consecutive Dup $\alpha$ 7 subunits are unlikely to occur.

These effects are also position-dependent: AB-dup $\alpha$ 7 model and ACD-dup $\alpha$ 7 model showed similar energetic profiles within the calcium intake TM. These profiles had lower free energy values than the AC-dup  $\alpha$ 7 model in the TM region. This indicates that Dup $\alpha$ 7 interfaces have a less pronounced effect on the function of the Dup $\alpha$ 7- $\alpha$ 7 interface for models with more than one Dup $\alpha$ 7 subunit. For models with four or five subunits, the 250-257 loop interactions and the calcium are significantly reduced. A higher barrier is found in comparison to the other models. Hence, this shows that pentamers with four or five dup $\alpha$ 7 subunits are not functionally viable.

In addition to the previously published works, the results shown in this work shed light on the plausible stoichiometry of Dup $\alpha$ 7/ $\alpha$ 7 subunits. We evaluated stability and functional differences emerging from the positioning of the Dup $\alpha$ 7 subunits within the pentamer. Albeit with a weaker interface interaction, the AC-dup $\alpha$ 7 model showed a lower effect on the calcium transition than the AB-dup $\alpha$ 7 model. A similar effect was observed when the pentamer had three dup $\alpha$ 7 subunits. The simulations of models with four or five Dup $\alpha$ 7 are both too unstable, with an unfavourable effect on the Ach binding site organisation compared to the crystal structure. Also, it disrupts the calcium transmission by the pentamer.

In summary, a number higher than three Dup $\alpha$ 7 subunits is unlikely to be naturally occurring and to be functional. The most stable stoichiometry is the 1:Dup $\alpha$ 7-4:WT  $\alpha$ 7, which is also one of the combinations with the least negative effect on calcium transmission. With two subunits, we expect to see Dup $\alpha$ 7 interacting with WT subunits, given it has a lower effect on its function. Naturally occurring pentamers with three subunits should be less likely than with two, but should also be more favourable the interaction between Dup $\alpha$ 7/ $\alpha$ 7 interfaces, which we would expect the predominance of an ACD dup $\alpha$ 7 organisation.

### **Dup $\alpha$ 7/ $\alpha$ 7 ratio affects the ligand binding and may be linked to the nicotinic translational gap**

The  $\alpha$ 7 receptor has been a promising target for diseases affecting cognition. However, the results gathered in animal studies failed to translate into human clinical trials identifying a translational gap. Being human-specific, CHRFAM7A, which encodes dup $\alpha$ 7, was not included in those preclinical studies, and effects arising from its distinct structural and dynamic features were not accounted for. Since the CHRFAM7A gene is present in different copy number variations in the human genome with high frequency (Ihnatovych et al., 2019), understanding distinct features of dup $\alpha$ 7 may offer novel insights when exploring the human  $\alpha$ 7 receptor as a drug target.

Recent reports have shown the direct interactions between  $\alpha$ 7 receptors and A $\beta$ <sub>42</sub> (Goldwasser et al., 2020; Hassan et al., 2019; Sun et al., 2019). These studies strongly suggest that the  $\alpha$ 7 receptor can contribute to synaptic dysfunction in Alzheimer's Disease (AD) since A $\beta$  oligomers can alter neuronal signalling through interactions with nicotinic receptors, particularly with  $\alpha$ 7. However, how exactly A $\beta$  interacts with  $\alpha$ 7 receptor, and whether human-specific dup $\alpha$ 7 increases or decreases those interactions, has not been fully understood.



Regarding interactions with disease-linked macromolecular ligands, our results indicate that in mixed functional receptors (i.e. comprising of both  $\alpha 7$  and dup $\alpha 7$  subunits), dup $\alpha 7/\alpha 7$  interfaces can bind  $A\beta_{42}$  with a higher affinity than  $\alpha 7/\text{dup}\alpha 7$  and dup $\alpha 7/\text{dup}\alpha 7$ , albeit impaired compared to canonical  $\alpha 7/\alpha 7$  sites. Receptors bearing Dup $\alpha 7$  subunits are shown to be insensitive to  $\alpha$ -BTX. These results collectively suggest that the receptors bearing Dup $\alpha 7$  subunits may be less sensitive to effects exerted by neurotoxin or  $A\beta_{42}$ . This data is in agreement with the recent study, which focused on the function of *CHRFAM7A* alleles *in vitro* in two disease-relevant phenotypic readouts: electrophysiology and  $A\beta$  uptake, and in the double-blind pharmacogenetic analysis on the effect of therapy using acetylcholine esterase inhibitors (AChEIs), based on *CHRFAM7A* carrier status (Szigeti et al., 2020).

Mechanistic insights arising from our work suggest competitive binding between  $\alpha$ -BTX (an orthosteric ligand) and  $A\beta_{1-42}$  to the  $\alpha 7$  receptors. The earlier studies support this characteristic, showing that both orthosteric agonists and antagonists mitigate  $A\beta$  uptake (Szigeti et al., 2020). Uptake of  $A\beta_{1-42}$  via  $\alpha 7$  receptors binding induces apoptosis, and orthosteric  $\alpha 7$  agonists mitigate the  $A\beta$ -induced apoptosis in animal models (Szigeti et al., 2020 and references therein). The results of our study highlight the mechanistic link between receptor structure and  $A\beta$  binding, indicating key differences between  $\alpha 7$  receptors and receptors bearing Dup $\alpha 7$  subunits, which may be translated to the clinic. Moreover, our results suggest that in receptors containing dup $\alpha 7$  subunits,  $A\beta_{42}$  might be competitive to  $\alpha$ -BTX, albeit its binding affinity is low, hence the significance of this potential competing is challenging to estimate. Further follow-up studies are needed to validate these findings.

At the time of translation to the clinical trials, virtually all drugs that were effective in animals have demonstrated a lack of efficacy in humans, showing a robust translational gap. Dup $\alpha 7$  functional studies are sparse and are lacking in the clinical context. Clinical efforts need to be continued with a trial design incorporating Dup $\alpha 7$  distinct structural biology, pharmacology and pharmacogenetics. Dup $\alpha 7$  non-carriers account for 25% of the Alzheimer's Disease (AD) population, which is significant, considering an increasing number of AD patients. Our results, which match neuronal toxicity data published (Szigeti et al., 2020), suggest that Dup $\alpha 7$  carriers should be protected against  $A\beta$  effects to some extent, and Dup $\alpha 7$  non-carriers should be more acutely affected by  $A\beta$  effects. Therefore, therapeutics that reduce amyloid burden could be effective in non-carriers. Considering the number of AD patients worldwide and AD being essentially an unmet clinical need, these findings pave the way to bring new AD therapeutics into the clinic.

## Conclusions

In this work, we performed a systematic study on all possible combinations of Dup $\alpha 7/\alpha 7$  nicotinic receptors, focusing on their structural stability and stoichiometry, to find the most probable functional pentamers bearing Dup $\alpha 7$  subunits. This understanding was essential since Dup $\alpha 7$  has been regarded as a dominant-negative regulator of  $\alpha 7$  receptors. However, reports of functional pentamers bearing Dup $\alpha 7$  subunits have been

published. To address the conflicting evidence from published studies, we modelled all possible combinations and evaluated them using structure-based multiscale computational methods. We showed that higher content of Dup $\alpha$ 7 subunits resulted in less cohesive pentamers, and dup $\alpha$ 7/dup $\alpha$ 7 interfaces, corresponding to the orthosteric binding sites, were markedly less stable than Dup $\alpha$ 7/ $\alpha$ 7 interfaces. These indicate that the most likely combinations were pentamers bearing one Dup $\alpha$ 7 subunit (A-dup $\alpha$ 7 model) or pentamers containing two non-consecutive subunits (AC-dup $\alpha$ 7 model). Pentamers bearing three subunits with the lowest dup $\alpha$ 7/dup $\alpha$ 7 interfaces (ACD-dup $\alpha$ 7 model) were also suggested to be functional. Our work has also shown that dup $\alpha$ 7 interfaces are insensitive to  $\alpha$ -bungarotoxin ( $\alpha$ -BTX), but not to A $\beta$ <sub>42</sub>, even though their A $\beta$ <sub>42</sub> binding is impaired compared to the canonical  $\alpha$ 7 receptors. This impairment indicates that receptors containing dup $\alpha$ 7 subunits are less sensitive to A $\beta$ <sub>42</sub> effects and that dup $\alpha$ 7 subunits, despite their impaired agonist binding, may offer protection against detrimental A $\beta$ <sub>42</sub> effects. We expect that our work will contribute to the elucidation of the biological roles of Dup $\alpha$ 7 subunits, generating models that can be used for a rational drug design. Future research aiming to characterise the function of Dup $\alpha$ 7 in the clinical context may result in novel pathways for AD treatment based on early-stage preclinical data. As  $\alpha$ 7 receptors are implicated in a broad range of diseases, including cognition, memory, schizophrenia, chronic pain and inflammation, mechanistic insights into receptors containing Dup $\alpha$ 7 subunits will impact all of these therapeutic areas, including those conditions which currently represent an unmet clinical need.

**Author Contributions:** D.L., J.V.S., and A.K.B. designed the study. D.L. carried out the equilibrium atomistic and coarse-grain molecular dynamics simulations. D.L. and J.V.S. carried out umbrella sampling simulations. J.V.S. wrote scripts for data analysis. A.A., D.L. and J.V.S. extracted and analysed data from the simulations. A.A., D.L., and A.K.B. performed molecular docking calculations. A.K.B supervised the study. D.L. and J.V.S produced the figures, plots, and supplementary data. D.L. and A.K.B. wrote the first draft of the manuscript. All authors reviewed the manuscript.

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