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Deliverable Title:	D2.7.5 (D12.5 D57) - CDP6 Components Report for SGA1 M13-M24
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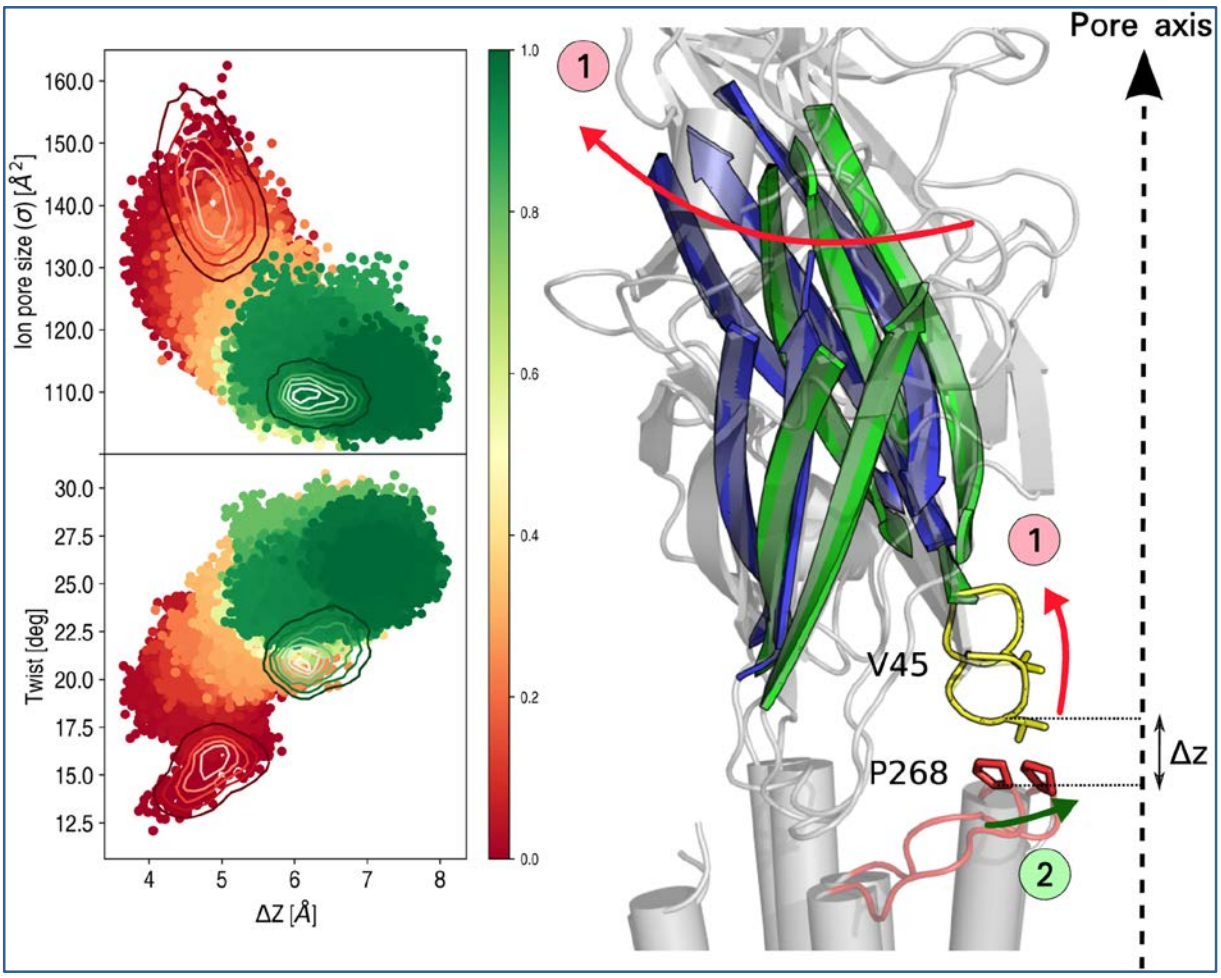
Abstract:	The main deliveries from April-2017 to March-2018 are: • identification of the connection between vibrational energy exchange and allosteric modulation in M2 GPCR • the first integral description of a pLGIC gating transition at the atomistic level in the microsecond timescale • identification of the open state of an ion channel at the atomistic level • development of a method to identify new binding sites on the nicotinic acetylcholine receptor • in addition to these *in silico* findings, an early involvement of nAChRs early in AD disease was found *in vivo* SP2 anatomical localisations and densities of 15 different receptors in visual areas have been established (MS2.2). With SP6 Subcellular and Molecular Modelling, CDP6 has greatly profited from the collaboration with SP7 to optimise use of HPC resources: the Brain Simulation Platform and Coordination and Community (WP6.1, WP6.4, WP6.5). With SP7, CDP6 has benefitted from the facilities of the Simulation Technology Data-Intensive Supercomputing (WP7.1, WP7.2) to drive the model development process and to validate tools. Lastly, ethical issues about drug design, in particular the Opiate epidemics, are being debated with SP12.
Keywords:	drug design, neurotransmitter receptors, ligand gated ion channels, GPCRs, allosteric interaction, conformational changes, molecular dynamics



Un-gating and allosteric modulation of a pentameric ligand-gated ion channel captured by molecular dynamics.

An upward movement of the ß1-ß2 loop correlated with the twisting isomerisation couples orthosteric agonist unbinding to pore closing in glutamate-gated chloride channel (GluCl).

On the left, the correlation between the vertical separation of the ß1-ß2 loop from the M2-M3 loop (ΔZ) with the twisting angle (τ) and the cross section of the pore at the constriction point are shown. ΔZ is computed as the distance, projected on the Z axis, between the α-carbons of residues P268 (on M2-M3 loop) and V45 (on ß1-ß2 loop) averaged over the 5 subunits. The isocontour lines correspond to the simulations of GluCl active (red) and resting (green). The colour gradient from red to green illustrates the time evolution of GluCl active with IVM removed. On the right, the gating mechanism is illustrated using snapshots taken at the beginning (red) and the end (blue) of the MD relaxation. Upon L-Glu unbinding, global receptor twisting results in an upward movement of the ß1-ß2 loop that facilitates the passage of the bulky Proline 268 at the EC/TM interface to shut the pore at position 9′. Taken from https://doi.org/10.1371/journal.pcbi.1005784.

Targeted users/readers	Experts in computational biophysics
Contributing Work-Package(s):	SGA1 WP2.2, WP6.1, WP6.4, WP6.5, WP7.1, WP7.2 SP2, SP5, SP6, SP7, SP8, SP12
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1. Introduction

The original 2012 HBP flagship application included an important chapter on the use of computational methods for drug design. Yet, the original plans were not implemented until the Manchester meeting in 2017, which lead to the creation of a new CDP to foster a collaboration between SPs (SP2, SP5, SP6, SP7, SP8, SP12): CDP6. This initiative was taken by SP12, to deal with a major ethical responsibility of HBP: discover new drugs, treatments and care for the fight against brain diseases.

CDP6 started in SGA1, led by JP Changeux and P Carloni with G Rossetti as Scientific manager. Yet, **the program was financed for only the second year of SGA1, starting in principle at the 1st of April 2017 (though effectively only at the end of July 2017).**

The scientific goals of CDP6 are:

1) exploit recent developments in molecular dynamic simulations to target drug candidates to sites on neurotransmitter receptors.
2) adopt, instead of the classical concept of steric analogy between the pharmacological agent and the endogenous ligand (Daniel Bovet, James Black), the new paradigm of allosteric interaction (see Changeux 1961, Monod Wyman Changeux 1965). In contrast with the mechanism of competitive, steric, interaction between ligands for a common site, allosteric interactions take place between topographically distinct sites, they are mediated by a discrete and reversible conformational change of the protein. This concept applies to the signal transduction process mediated by neurotransmitter receptors and pharmacological effectors, referred to as allosteric modulators. It was discovered that allosteric modulators enhance or depress the transduction process when they bind to sites distinct from the orthosteric sites for the neurotransmitter, the G-proteins or the ion channel (reviewed by Changeux and Christopoulos, 2016).

Anticipated impact

The knowledge of allosteric transitions of neurotransmitter receptors and ion channels at the atomistic level has a profound impact on our understanding of the operations carried out by the billions of nerve cells of our brain, reacting to chemical signals that mediate information processing in the brain from the molecular to the cognitive level. Therefore, for brain modelling, it is important to start at the molecular level, a level that is often under-evaluated or absent from artificial intelligence/neuromorphic modelling. It offers another view on neuronal plasticity, besides offering a broad range of new opportunities for drug design. Furthermore, the sensitivity and responsiveness of a given brain disease to a spectrum of drugs is a critical feature of disease classification and their so-called ontology. CDP6 therefore covers an important aspect of future medical informatics research: the establishment of causal links between human genome data and the origins of brain diseases.

An essential innovation of the Project is the design of allosteric modulatory drugs. Several allosteric drugs were, in the past, proven to be successful and they are available on the market (27 representative examples are listed in Changeux and Christopoulos, 2016). Also the ''allosteric database'' (Huang *et al.*, 2011) classifies nearly 72,000 substances as potential allosteric modulators (http://mdl.shsmu.edu.cn/ASD/), showing the ongoing growth and interest of the field.

In spite of the short funding period (less than one year) CDP6 made significant progress and established several collaborations within the HBP. These are summarised in the following sections.

2. Results

2.1 Allosteric modulation dynamics of GPCRs

Radio-ligands, used for brain imaging applications, need to be as specific as possible to obtain accurate measurements. Here, we aim to model more specific radio-ligands, targeting G protein-coupled Receptors (GPCRs). We exploit the fact that allosteric ligands, which bind to other protein regions than the orthosteric sites, are usually more specific than the traditional agonists/antagonists (Bartuzi *et al.* 2018 Methods in Molecular Biology). We focus on a receptor which is currently investigated by Prof. Zilles (SP2): the human **Muscarinic Receptor M2**. This receptor belongs to the family of Muscarinic Acetylcholine Receptors (mAchR), a sub-class of GPCRs, that is found abundantly throughout the central and peripheral nervous system. M2 is implicated in many physiological and pathological brain functions. Increasing our knowledge of the distribution of this receptor sub-class in different parts of the brain could be highly helpful for the diagnosis of brain diseases. In addition, CDP6 investigates the human MOP, which allows us to collaborate with a pharmaceutical company: Grunethal (Aachen, Germany) which is studying MOP.

Highlight 1. Simulation of micro-second long trajectories for the M2 in three different complexes: i) with the agonist (Iperoxo), ii) with the agonist plus a positive allosteric modulator (LY2119620), and iii) with the antagonist (QNB). The receptor has been embedded in a phospholipid membrane, mimicking a neuronal membrane. A quasi-harmonic approximation-based on the trajectory analysis allowed identification of the subregion of the protein responsible for the conformational rearrangements in the transition from the active to the inactive state (Maggi *et al. manuscript in preparation*).

Highlight 2. The same analysis is followed to **identify the M2** allosteric mechanisms, as well as of key residues involved in allosteric modulations.

2.1.1 Achieved impact

The results pf the GPCR team will eventually lead to identifications of new leads for painkillers and radioligands. The first will be tested by SP1 and SP2, impacting on receptor mapping strategies. The simulation protocols developed here may be integrated in the molecular dynamics working group of SP6.

Overall, these studies underline major similarities in the conformational transition and ligand binding between GPCRs and ligand gated ion channels.

These are meaningful steps towards a targeted impact for two pillars of HBP: accelerating neuroscience and empowering brain medicine, including the ethical dimension.

2.1.2 Component Dependencies

Component ID	Component Name	HBP Internal	Comment
2886	Atomistic models of the human μ-opioid receptor in a natural mimic-like environment	Yes	
2890	Identification of ligands acting as allosteric modulators	Yes	

2.2 The pentameric receptors linked ion channels & the nicotinic receptor family

Highlight 1. The first integral description of a pLGIC gating transition at the atomistic level in the microsecond timescale. Marco Cecchini, Jean-Pierre Changeux and colleagues provided the first atomic description, by micro-second Molecular Dynamics, of the functional isomerisation, from open to closed (un-gating), of the **glutamate-gated chloride channel (GluCl),** a pLGIC of the nicotinic receptor family, upon removal of the positive allosteric modulator ivermectin. The data support the conclusion that ion-channel deactivation is mediated by two quaternary transitions: a global receptor twisting followed by the radial expansion (or blooming) of the extracellular domain. A critical finding is that the same twisting isomerisation was shown to involve significant changes at both the orthosteric binding sites of the agonist (Glu) and the positive allosteric sites of ivermectin, paving the way for the design of conformation-specific modulators, targeting both sites (Cerdan, Cecchini, Changeux *in preparation* and Martin *et al.* 2017).

Highlight 2. The first *in silico* identification of the open state of an ion channel. The team of Marco Cecchini identified the ion-permeable state of the **Glycine Receptor (GlyR α1),** another pLGIC of the nicotinic receptor family, using jointly MD, computational electrophysiology and polyatomic anion permeation simulations. The new open-state model is stable and shows *in silico* permeability properties in qualitative agreement with patch-clamp physiological measurements. The critical finding is the proposal of a new functional annotation of the various allosteric states of pLGICs, which is decisive for the rational design of agonists, antagonists and allosteric modulators in this receptor family (Cerdan *et al, manuscript in preparation*).

Highlight 3. A method to identify new binding sites on the nicotinic acetylcholine receptor. Damien Monet, Arnaud Blondel and Pierre-Jean Corringer focused on the of the activation mechanism of the **α7-nicotinic Acetylcholine Receptors (α7-nAChR)**, using innovative computational methods. These methods assess, globally identify, and track cavities along the ion-channel activation to identify novel sites, presenting an allosteric lever on the channel gating (Monet *et al., manuscript in preparation*). A critical observation is that among the 68 cavities identified along the trajectory (which include twisting and blooming), 6 had a size and shape varying significantly with the conformational state of the protein (4 in the extracellular domain and 2 in the transmembrane domain). They include the orthosteric site, the previously described Ca++ and the Ivermectin (from GluCl) allosteric modulatory binding site. A differential *in silico* screening of known allosteric modulators is under development to probe the pharmacological and physiological relevance of these pockets.

2.2.1 Achieved Impact

The molecular dynamics of the gating transition - in the microsecond timescale - of several typical ligand-gated ion channels (GluCl, GlyR α1 and a7nAChR) was described at the atomistic level, revealing two distinct quaternary transitions: a global receptor twisting, resulting in pore opening/closing, followed by the radial expansion (or blooming) of the extracellular domain. The same isomerisation includes a reorganisation of the orthosteric binding site and the various positive and negative allosteric modulatory sites. This observation paves the way for a **fundamental innovation in drug design**. Pharmacological agents would not only target the diverse site categories (orthosteric and allosteric) of the receptor, but would also be **specific for a given receptor conformation**: active, resting, desensitised. Based on our results, new drugs might be designed: agonists, antagonists and desensitising drugs.

2.2.2 Component Dependencies

Component ID	Component Name	HBP Internal	Comment
2888	nAChR PAM and NAM against Schizophrenia, Alzheimer's disease and nicotine addiction	Yes	
2887	Pharmacological identification, *in silico* screening and *in vitro* testing of the new ligands	Yes	

2.3 Nicotinic acetylcholine receptors

Nicotinic acetylcholine receptors in Alzheimer's disease pathology. Amyloid-ß (Aß) peptides were identified as a hallmark in Alzheimer's disease (AD) pathogenesis and plausible allosteric interactions of Aß peptide with nAChRs have been suggested to take place. Fani Koukouli and her team investigated the mechanism (Rooy, Lamotte D'Incamps, Ascher, Gutkin, Changeux, Maskos) by multi-photon calcium imaging in the prefrontal cortex (PFC) of awake wild-type and mice that have genetically modified nAChRs subunits. They discovered that **Aß causes disruptions of the neuronal firing of pyramidal (PYR) cells through nAChRs**, present on three main classes of GABAergic inhibitory cells that differentially express nAChR subunits. A minimal computational model of the local PFC circuitry that incorporates specific nAChR subtype-mediated currents, predicts a differential involvement of nAChRs early in AD disease, suggesting a novel AD pharmacology (Koukouli *et al. manuscript in preparation*).

2.3.1 Achieved Impact

In vivo analysis of the plausibly allosteric interaction of (Aß) peptides with nAChR subtype-mediated currents, predicts a differential involvement of nAChRs early in AD disease and a potential new AD pharmacology. This opens new strategies that are radically different from those currently practiced in the fight against AD.

2.3.2 Component Dependencies

Component ID	Component Name	HBP Internal	Comment
2888	nAChR PAM and NAM against Schizophrenia, Alzheimer's disease and nicotine addiction	Yes	
2887	Pharmacological identification, *in silico* screening and *in vitro* testing of the new ligands	Yes	

3. Component Details

The following is a list of the newly released internal Components for this deliverable.

3.1 Atomistic models of human μ-opioid receptor in natural mimic-like environment

Field Name	Field Content	Additional Information
ID	2886	
Component Type	Model	
Contact	CARLONI, Paolo	
Component Description	Microseconds atomistic simulations of human μ-opioid receptor in natural mimic-like environment in complex with three different ligands: agonist, antagonist and positive allosteric modulator.	
Latest Release	December 2018	See guidance below
TRL	NA	See guidance below
Location	data hosted by other HBP party	
Format	NA	
Curation Status	PLA registered	
Validation - QC	Unchecked	
Validation – Users	No	
Validation – Publications	No	Maggi *et al. in preparation*
Privacy Constraints	Human Research	
Sharing	partner	
License	NA	
Component Access URL	see Confidential Annex	
Technical documentation URL	see Confidential Annex	
Usage documentation URL	see Confidential Annex	
Component Dissemination Material URL	see Confidential Annex	

3.2 Identification of ligands acting as allosteric modulators of GPCRs

Field Name	Field Content	Additional Information
ID	2890	
Component Type	Data	
Contact	CARLONI, Paolo	
Component Description	Identification of ligands acting as allosteric modulators of M2 receptors	
Latest Release	December 2018	
TRL	NA	
Location	data hosted by other HBP party	
Format	molecular dynamic simulation data and chemical structures	
Curation Status	PLA registered	
Validation - QC	Unchecked	
Validation – Users	No	
Validation – Publications	No	Maggi *et al. in preparation*
Privacy Constraints	Human Research	
Sharing	partner	
License	NA	
Component Access URL	see Confidential Annex	
Technical documentation URL	see Confidential Annex	
Usage documentation URL	see Confidential Annex	
Component Dissemination Material URL	see Confidential Annex	

3.3 Pharmacological identification, *in silico* screening and *in vitro* testing of the new ligands for LGICs

Field Name	Field Content	Additional Information
ID	2887	
Component Type	Data	
Contact	CHANGEUX, Jean-Pierre	

Component Description	Developing allosteric modulators against Ligand-gated ion channels, in general, which are potentially involved in many neuro-psychiatric diseases including epilepsy, Alzheimer's & Parkinson's diseases, congenital and auto-immune myasthenia gravis.	
Latest Release	December 2018	
TRL	NA	
Location	data hosted by other HBP party	
Format	molecular dynamic simulation data and chemical structures	
Curation Status	PLA registered	
Validation - QC	Unchecked	
Validation – Users	No	
Validation – Publications	Yes	(Cerdan, Cecchini, Changeux *in preparation* and Martin *et al.* 2017)
Privacy Constraints	Human Research	
Sharing	partner	
License	NA	
Component Access URL	https://etuunistrafr-my.sharepoint.com/:f:/g/personal/adrien_cerdan_etu_unistra_fr/EopYaB5Lc5hFi0sy_wyueTIBT1Nyk8LNBXHOQJziowaFiw?e=hPWKmB and see Confidential Annex	
Technical documentation URL	https://etuunistrafr-my.sharepoint.com/:f:/g/personal/adrien_cerdan_etu_unistra_fr/EopYaB5Lc5hFi0sy_wyueTIBT1Nyk8LNBXHOQJziowaFiw?e=hPWKmB and see Confidential Annex	
Usage documentation URL	https://etuunistrafr-my.sharepoint.com/:f:/g/personal/adrien_cerdan_etu_unistra_fr/EopYaB5Lc5hFi0sy_wyueTIBT1Nyk8LNBXHOQJziowaFiw?e=hPWKmB and see Confidential Annex	
Component Dissemination Material URL	https://etuunistrafr-my.sharepoint.com/:f:/g/personal/adrien_cerdan_etu_unistra_fr/EopYaB5Lc5hFi0sy_wyueTIBT1Nyk8LNBXHOQJziowaFiw?e=hPWKmB and see Confidential Annex	

3.4 nAChR PAM and NAM against Schizophrenia, Alzheimer's disease and nicotine addiction

Field Name	Field Content	Additional Information
ID	2888	
Component Type	Data	See guidance below
Contact	CHANGEUX, Jean-Pierre	
Component Description	Developing allosteric modulators specifically against nAChR, which are potentially involved in neuro-psychiatric diseases including epilepsy, Alzheimer's & Parkinson's diseases, congenital and auto-immune myasthenia gravis.	
Latest Release	December 2018	
TRL	NA	
Location	data hosted by other HBP party	
Format	molecular dynamic simulation data and chemical structures	
Curation Status	PLA registered	
Validation - QC	Unchecked	
Validation – Users	No	
Validation – Publications	Yes	(Monet *et al. in preparation*)
Privacy Constraints	Human Research	
Sharing	partner	
License	NA	
Component Access URL	https://gitlab.pasteur.fr/dmonet/CDP6-Deliverable https://gitlab.pasteur.fr/fkoukoul/CDP6-deliverable.git and see Confidential Annex	
Technical documentation URL	https://gitlab.pasteur.fr/dmonet/CDP6-Deliverable https://gitlab.pasteur.fr/fkoukoul/CDP6-deliverable.git and see Confidential Annex	
Usage documentation URL	https://gitlab.pasteur.fr/dmonet/CDP6-Deliverable https://gitlab.pasteur.fr/fkoukoul/CDP6-deliverable.git and see Confidential Annex	
Component Dissemination Material URL	https://gitlab.pasteur.fr/dmonet/CDP6-Deliverable https://gitlab.pasteur.fr/fkoukoul/CDP6-deliverable.git and see Confidential Annex	

4. Conclusion and Outlook

During one year of funding (April 2017 to April 2018), with the application of molecular simulations, CDP6 was able to rationalise key molecular mechanisms regulating the functioning of two main classes of receptors for neurotransmitters, which are critically involved in signal transduction and brain plasticity:

1) the ligand-gated ion channels (LGIC), which include the various oligomers of the excitatory nicotinic acetylcholine and 5HT3 receptors as well as the inhibitory GABAA and glycine receptor
2) G-protein-linked receptors such as the muscarinic acetylcholine M2 and the mu opioid receptor

Namely, the connection between vibrational energy exchange and allosteric modulation in M2 GPCR was identified (section 2.1). The first integral description of a pLGIC gating transition at the atomistic level in the microsecond timescale was achieved (section 2.2); The open state of an ion channel at atomistic level was identified *in silico* (section 2.2); A method to identify new binding sites on the nicotinic acetylcholine receptor was proposed (section 2.2).

In addition to these *in silico* findings, an early involvement of nAChRs early in AD disease was found *in vivo* (section 2.3).

CDP6 work has settled the conceptual and practical informatics frame for the design of new orthosteric and allosteric drugs targeting neurotransmitter receptors – LGIC and GPCRs. Collaborations were continued with SP2, SP6, SP12 and new relationships established with SP7 for Simulation Technology Data-Intensive Supercomputing. Also, discussions are currently ongoing with SP8 to develop and strengthen relationships with Medical Informatics.

We will build on this during the next phase in SGA2 is to further develop *in silico* programmes for the rapid screening of conformation-specific drugs targeting the allosteric modulatory site carried by the diverse ligand-gated ion channel and GPCR present in the human brain. Closer collaborations will be established with the various SPs concerned, in particular medical informatics (SP8) to relate these molecular pharmacological studies to brain diseases actually investigated in SP8.

Moreover, we will try to identify allosteric modulators that bind M2, which will be experimentally tested by Prof. Zilles' Lab.