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FP7-HEALTH-2013-INNOVATION-1-602156-HeCaTos

Deliverable Report D1.1:

Report on predictions of nuclear receptor interaction with toxicants using GPU-MD

Work package 1

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RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

Contributions to deliverable - Internal review procedure

Deliverable produced by:	Date:
Ian R Gould - Partner ICL	November 2016
Matt Segall - Partner Optibrium	November 2016
Deliverable internally reviewed by:	Date:
Jos Kleinjans - Partner UM	November 2016

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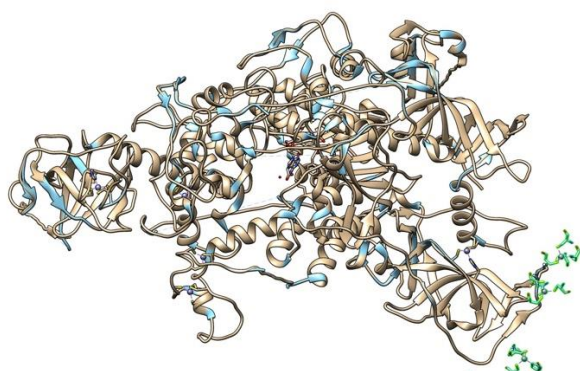
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PUBLISHABLE SUMMARY

We have not up until to this point in time performed any MD simulations of the Anthracyclines with a target receptor, due to there being no clearly identified target. In principle, we could begin simulations of the Anthracyclines with Cardiolipins, with the caveat that the TOCL we have parameterised is not found in human heart tissue, and we should parameterise TLCL if we are directed to pursue the cardiolipin question. We have a model for human cardiac troponin and could begin modelling the AC's interaction with this protein complex almost immediately if this is the preferred target.

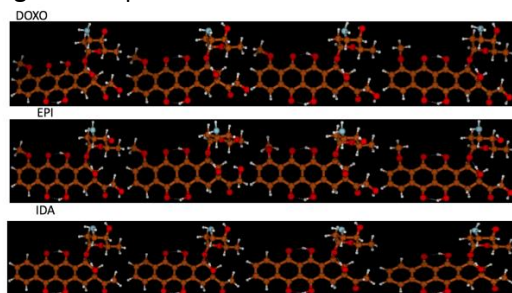
We have constructed a complete model of DNMT1 from the partial crystal structure, this has been subjected to preliminary MD study, as a holoenzyme and bound to its crystallographic inhibitor; Figure 1 shows the structure of DNMT1 with its inhibitor S-adenosyl-L-homocysteine bound.

Figure 1: Full length protein structure of DNMT1 with inhibitor bound.



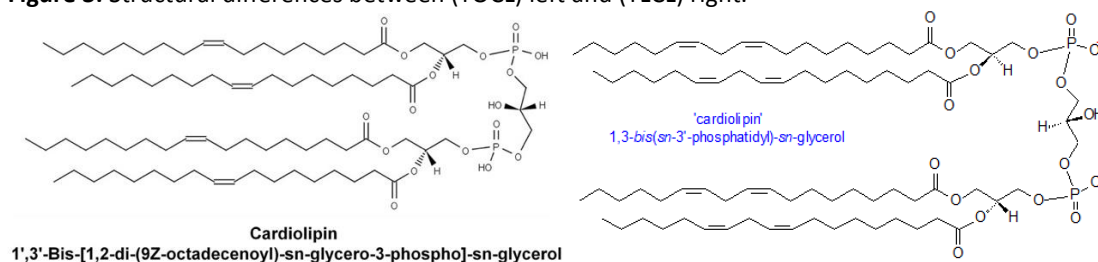
We have parameterized all four Anthracyclines identified in the HeCaTos MS.5 reference compound library, for use with the AMBER force field, to enable GPU-MD investigations of their binding to DNMT1, Cardiolipin and to Cardiac Troponin. Figure 2 illustrates the high number of possible conformers of the Anthracyclines which have been investigated to facilitate this parameterization.

Figure 2: Representative conformations of the three pharmaceutical derivatives of DaunoRubicin.



Initial parameterization of cardiolipin has been performed, this was performed on the most physico-chemically studied form, tetraoleoyl cardiolipin (TOCL). However, this is not the predominant form found in the human heart, which is tetra linoleoyl cardiolipin (TLCL), and we are in the process of parameterizing this more relevant cardiolipin. Figure 3 illustrates the structural differences between (TOCL) left and (TLCL) right respectively.

Figure 3: Structural differences between (TOCL) left and (TLCL) right.



We also have parameterized for AMBER simulations all of the reference compound library as detailed in report MS.5 and listed in Table 1.

Table 1: Reference compound library of ligands.

Hepatotoxic	Cardiotoxic	Non-toxic
isoniazid	Amiodarone HCl	penicillin G
methotrexate	Daunorubicin	aspirin
rifampin	Taxane	kanamycin
diclofenac	Docetaxel	Sibrafiban
erythromycin	Epirubicin	Acyclovir
azathioprine	Cyclophosphamide	Saquinavir
piroxicam	Doxorubicin HCl	Ambrisentan
valproic acid	Fluorouracil	Aliskiren
phenobarbital	Idarubicin HCl	Tiotropium
phenytoin	Mitoxantrone diHCl	Carisoprodol
pravastatin	celecoxib	Promethazine
simvastatin	lapatinib	
cyclosporine		

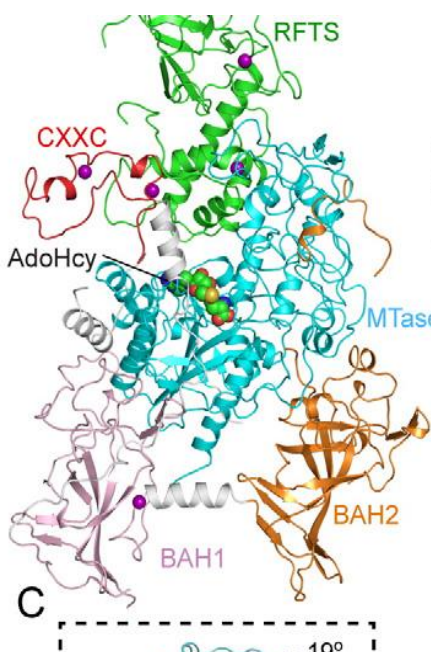
OBJECTIVES

The major objective was to investigate the interaction of the ligands (toxicants) interacting with a nuclear receptor, however, as a result of discussions which arose from the 1st and 2nd annual meetings it was clearly identified early in the timeline that the 'omics and pathway analyses had not been able to identify with significant clarity either relevant ligands (toxicants) or suitable receptors for investigation. At the 2nd annual meeting, results conveyed to the consortium by Prof. Ralf Herwig (Partner MPIMG) suggested that interaction of the Anthracyclines with the Human DNA1 Methyltransferase 1 (DNMT1) could be a potential system that could be investigated. As a result of this the research board concluded that this system was suitable for investigation with GPU-MD techniques. In April of 2016 Prof. Jos Kleinjans (Partner UM) embarked on a series of visits with the objective of achieving the integration of the models generated at different scales by WPs 1-4, as a result of this series of visits there was a proposed shift in objective to the study of the interaction of toxicants with Cardiolipin. As for DNMT1, the research board approved the change in objective away from DNMT1 to the investigation of Cardiolipin. In conjunction with D1.2 there is a need to parameterize the reference compound library to enable their integration with the AMBER force field to facilitated GPU-MD simulations of these ligands (toxicants) with identified targets, proteins or lipids.

INTRODUCTION

In the original grant proposal, it was envisioned that there would be clear identification of nuclear receptor targets which could be investigated through the application of GPU accelerated MD simulations to study the interactions with the ligands identified in report MS.5. However, in the first two years of the programme it became clear that the 'omics and pathway analyses had not been able to identify with significant clarity either relevant ligands (toxicants) or suitable receptors for investigation. At the 2nd annual meeting of the HeCaTos consortium there was evidence presented by Prof. Ralf Herwig (Partner MPIMG) that a potential receptor for investigation of Anthracycline toxicity would be Human DNA Methyltransferase 1 (DNMT1). DNMT1 is responsible for propagating the DNA methylation patterns during DNA replication. DNMT1 contains, in addition to a C-terminal methyltransferase domain, a large N-terminal regulatory region that is composed of an RFTS (replication foci targeting sequence) domain, a CXXC zinc finger domain and a pair of BAH (bromo adjacent homology) domains. The regulatory domains of DNMT1 mediate a network of protein–protein and protein-DNA interactions to control the recruitment and enzymatic activity of DNMT1. To facilitate GPU-MD investigations of ligand - receptor interactions it is necessary to have 3-D structures of both the ligand and the receptor, fortunately a partial crystal structure of DNMT1 was published in July 2015 [1], with all the structural domains (hDNMT1, residues 351-1600) in complex with S-adenosyl-L-homocysteine at 2.62 Å resolution. However, there were 86 residues in the structure which were unresolved, that is the 3-D positions of those residues were not clearly identified in the crystal structure. Figure 4A illustrates the homodimer of DNMT1 in complex with S-adenosyl-L-homocysteine (AdoHcy). Therefore, to enable GPU-MD investigations of DNMT1 it was necessary to model in the missing 86 residues and perform MD simulations of the holoenzyme, with the inhibitor S-adenosyl-L-homocysteine removed from the structure, and for the DNMT1 bound to the inhibitor. The Gould group has an international reputation for the construction of full length models of proteins from partial crystal structures which is demonstrated in the recent publications [2,3].

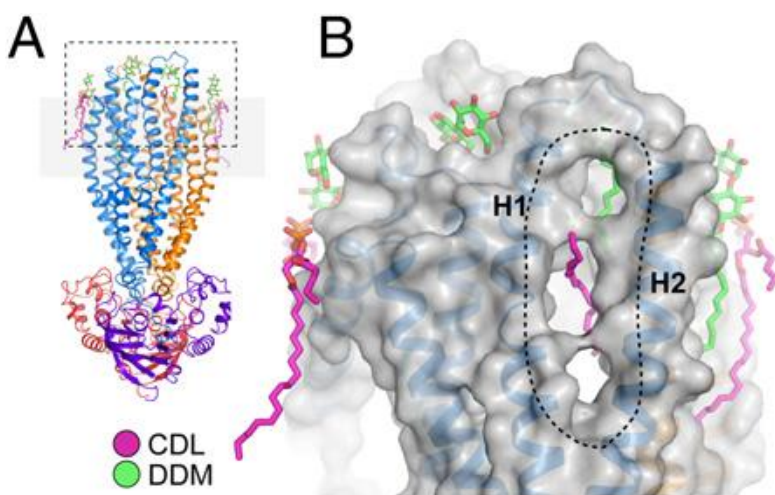
Figure 4: Homodimer of DNMT1 in complex with S-adenosyl-L-homocysteine.



In parallel with the development of the structural model for DNMT1, it was also necessary to produce the correctly parameterized ligands for the study, in this case the 4 Anthracyclines, Doxo-, Epi-, Ida- and DaunoRubicin. The parameterization of the 4 Anthracyclines for GPU-MD with the AMBER force field has a very well defined pipeline and so this has been performed and the quality of the parameterization can be clearly assessed with respect to experimental data. This parameterization has significant overlap with D1.2 report and further information on the methodology and validation of the lipid force field developed and extended in D1.2 should be consulted for further information and relevant references.

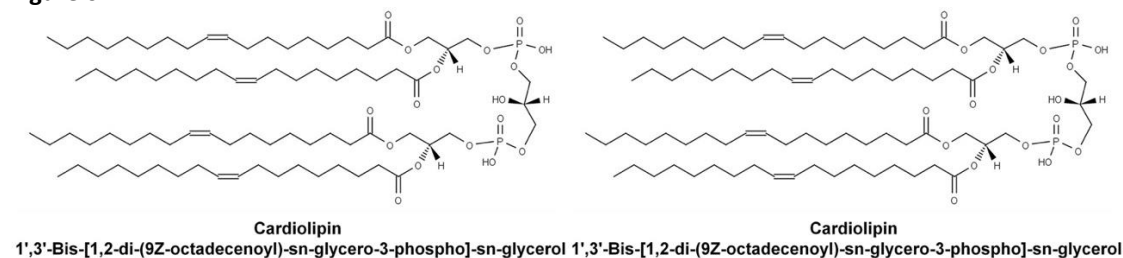
As documented in the objectives section in April 2016 as a result of Prof. Jos Kleinjans (Partner UM) visits to the associated WP contributors it was highlighted that the continued investigation of DNMT1 was not appropriate and that cardiolipin had been identified as a potential alternative target for study of its interactions with the Anthracyclines. Cardiolipin is not a protein receptor but is a class of unique lipids which have been identified as being bound to ABCB10 transporter in mitochondria [4] and to other transmembrane proteins. ABCB10 has cardiolipin and detergent bound to the transmembrane helices and a portal between helices TMH1 and TMH2, which is open in the rod crystal form and closed in the plate-form crystals. Figure 5 below to right (A) Overview of the ABCB10 structure showing the location of lipid (magenta) and detergent (green) binding sites. (B) Molecular surface representation of the TMD in rod form A crystals, with lipid and detergent molecules shown in magenta and green and the portal between TMH1 and TMH2 indicated with a dotted line. In the rod- form structures TMH1 and TMH2 are loosely packed revealing a 7 Å wide × 30 Å long portal connecting the central cavity of the TMDs with the membrane environment. The portal is occupied by a CDL alkyl chain (magenta).

Figure 5: Overview of the ABCB10 structure



Structural and mechanical properties of cardiolipin lipid bilayers have been determined using neutron spin echo, small angle neutron and X-ray scattering, and molecular dynamics simulations [5]. All MD simulations and parameterizations to date have been performed on tetraoleoyl cardiolipin (TOCL) which has the following unsaturated tail structure sn-1-sn-2 sn-2-sn1 18:1-18:1 18:1-18:1 (Figure 6 left)

Figure 6:



This is not the predominant form found in the Human Heart: which is tetra linoleoyl cardiolipin (no standard abbreviation) however we have adopted (TLCL) it is 18:2-18:2 18:2-18:2 (figure 6 right). We illustrate in Table 2 [6] the composition of Human cardiolipins in healthy and diseased patients and for rat liver.

Table 2: composition of Human cardiolipins

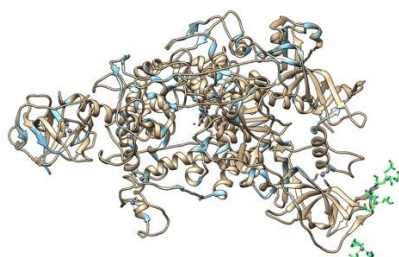
Molecular species of cardiolipin					
Source of cardiolipin	Peak	RT ^a (min)	Abundance ^b (%)	Cardiolipin species	
				<i>sn</i> -1- <i>sn</i> -2	<i>sn</i> -2- <i>sn</i> -1
Human heart (control)	1 ^c	21.3	80 ± 2	18:2-18:2	18:2-18:2
	2	25.7	12 ± 2	18:2-18:1	18:2-18:2
				18:1-18:2	18:2-18:2
Human heart (BTHS)	1	22.4	21 ± 1	Multiple species	
	2	26.9	26 ± 2	Multiple species	
	3	31.3	14 ± 2	Multiple species	
	4	35.5	13 ± 1	Multiple species	
Human lymphoblast (control)	1	26.7	14 ± 2	Multiple species	
	2	31.2	35 ± 3	16:1-18:1	18:1-18:1 ^d
	3 ^c	35.8	32 ± 3	18:1-18:1	18:1-18:1 ^d
	4	39.6	13 ± 3	Multiple species	
Human lymphoblast (BTHS)	1	35.4	31 ± 3	16:0-18:1	18:1-18:1 ^d
	2	40.0	43 ± 5	Multiple species	
Rat liver	1 ^c	21.6	55 ± 1	18:2-18:2	18:2-18:2
	2	25.8	30 ± 1	18:2-18:1	18:2-18:2
	3 ^c	29.9	9 ± 1	18:2-18:1	18:1-18:2 ^d

RESULTS

DNMT1

We have successfully accomplished the implementation of a full length human DNMT1, residues 351–1600, protein structure in both the holoenzyme form and with its crystallographically identified S-adenosyl-L-homocysteine inhibitor. Figure 7 is an illustrative snapshot from a 100 nanosecond GPU-MD simulation of the DNMT1 model with its inhibitor. Validation of the model from a comparison of the Root Mean Squared (RMS) deviation of the Carbon alpha backbone with the crystal structure yields a value of 1.8 Angstroms which indicates the model is stable and representative of the protein.

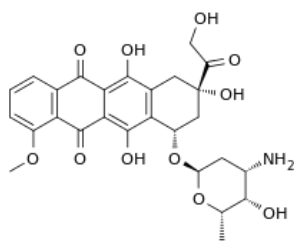
Figure 7: Snapshot from 100 nanosecond simulation



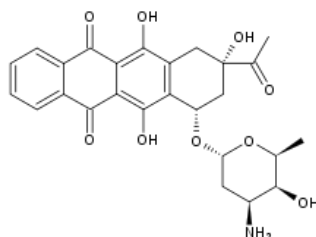
The DNMT1 GPU-MD simulations with the Anthracyclines has not been pursued as this part of the project has been put on hold as described in both the objectives and introduction sections of this document. In principle the investigation of Anthracyclines with DNMT1 can be restarted at any time.

Anthracycline Parameterization and Validation

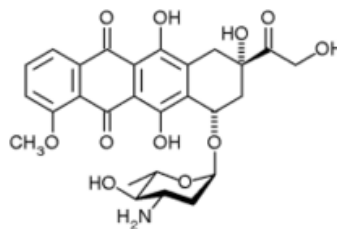
We have performed extensive parameterization work on the Anthracyclines: Doxo-, Epi-, Ida- and Dauno-rubicin. This has required significant application of high level quantum mechanical calculations. A result is that we now have a set of validated GAFF parameters [7] to facilitate the modelling of the Anthracyclines in lipid bilayers and/or with proteins. We will be able to publish a paper on this process and the level of agreement between our QM and MM results in particular in respect of Raman Spectra.



Doxorubicin



Idarubicin



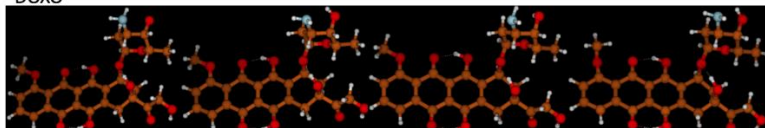
Epirubicin

Method: M06/cc-pvdz

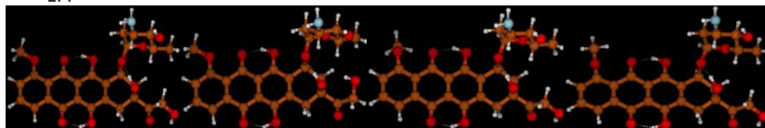
Conformers

Number of Conformers	DOXO	EPI	IDA	DNR
	39	43	11	31

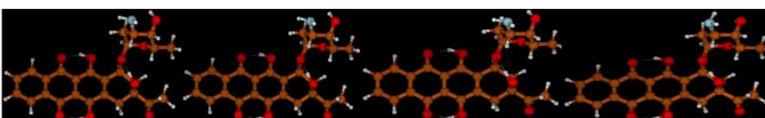
DOXO

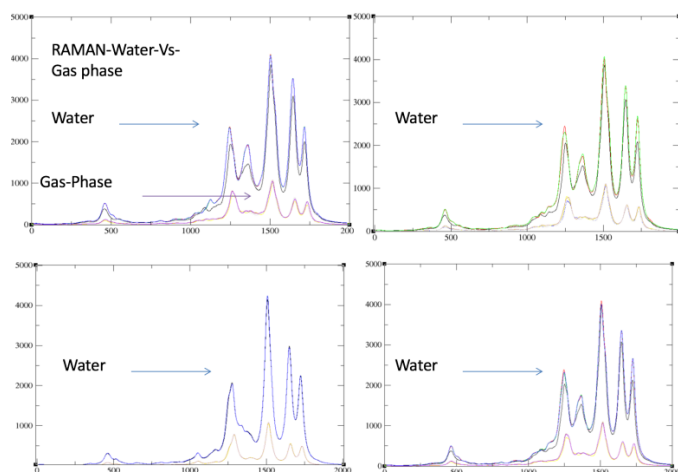


EPI



IDA





Cardiolipins

We have parameterized the very unusual phosphate head group which is constant for all of the cardiolipins described in Table 2 and shown in Figure 8. The tetraoleoyl tails of TOCL were already parameterized in Lipid 14 [8] and we have successfully constructed TOCL and subjected it to GPU-MD simulation Figure 9. We are currently parameterizing the more relevant tetralinoleoyl tails.

Figure 8: Double headed phosphate group of all cardiolipins

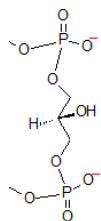
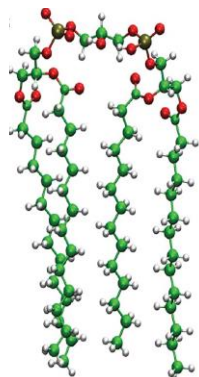


Figure 9: Complete structure of TOCL



Reference Compounds

We have also parameterized for AMBER simulations all of the reference compound library as detailed in report MS.5 and listed in Table 1. All of these ligands are available for GPU-MD simulations with clearly identified targets, proteins or lipids.

Table 1: Reference compound library of ligands.

Hepatotoxic	Cardiotoxic	Non-toxic
isoniazid	Amiodarone HCl	penicillin G
methotrexate	Daunorubicin	aspirin
rifampin	Taxane	kanamycin
diclofenac	Docetaxel	Sibrafiban
erythromycin	Epirubicin	Acyclovir
azathioprine	Cyclophosphamide	Saquinavir
piroxicam	Doxorubicin HCl	Ambrisentan
valproic acid	Fluorouracil	Aliskiren
phenobarbital	Idarubicin HCl	Tiotropium
phenytoin	Mitoxantrone diHCl	Carisoprodol
pravastatin	celecoxib	Promethazine
simvastatin	lapatinib	
cyclosporine		

DIFFICULTIES

There has been significant debate within the consortium as to what protein targets should be investigated. Therefore, as a result of discussions with the HeCaToS Executive Board this has significantly affected progress in this sub-project.

The yearlong gap between Dr. Dickson and Dr. Toroz starting on the project has meant that we are currently 6 to 12 months behind on this component of the project.

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