

Affinity and Selectivity of Cardiac Versus Skeletal Troponin I Towards Cardiac Troponin I Antibody: A Computational Study [†]

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Abstract: A computational study of the interaction between cardiac troponin I and its specific antibody is carried out. The aim of this study is to characterize the binding process by determining the binding sites, number of interactions and energies. Furthermore, a selectivity study of the binding efficiency of the cardiac troponin I antibody with the cardiac troponin I and with its principal interferon, the skeletal troponin I, is also performed to demonstrate that selectivity assays for sensing studies can be carried out computationally. Computational and simulation tools such as FTSite, FTMap, FTDock and pyDock were used to determine the binding sites and molecular docking performance, allowing us to obtain relevant information for a subsequent sensing system development.

Keywords: Cardiac Troponin I (cTnI); skeletal troponin I (sTnI); binding site; hot spot; FTDock; FTSite; molecular docking

1. Introduction

Cardiovascular diseases are the first cause of death worldwide [1] and heart failure is the end stage of almost all heart diseases. That heart failure is commonly produced by a damage of the heart myofilaments that can lead to systolic failure, resulting on a decrease of the blood circulation, not sufficing organism needs.

With a molecular mass of approximately 80 KDa, cardiac troponin (cTn) is a complex composed of three protein subunits having a different role in the myocardial contraction. Cardiac troponin T (cTnT) is a tropomyosin-binding subunit that regulates the interaction of the troponin complex with the thin filaments. Cardiac troponin I (cTnI) inhibits the interaction between myosin and actin, responsible of muscle contraction. Finally, cardiac troponin C (cTnC) is a Ca²⁺ binding subunit responsible of regulating the muscle contraction depending on the Ca²⁺ concentration. The binding of Ca²⁺ to cTnC produces a conformational change that reduces the inhibition of cTnI and thus leads to muscle contraction. That conformational change produced by cTnC is reversed when Ca²⁺ levels are restored, thus creating the contraction-relaxation cycle [2].

When a damage of the myofilaments of the cardiac muscle is produced, a breakage of the troponin complex takes place, leading to the release of the troponin subunits (cTnT, cTnI and cTnC) to the blood stream. Therefore, the levels of these cardiac troponin subunits in blood can be used as an indicator of myocardial damage. However, troponin is also found in the skeletal muscle, where it is also in charge of controlling the muscle contraction-relaxation cycle [3]. And as for the cTn,

skeletal troponin (sTn) subunits (sTnT, sTnI and sTnC) are also released to the blood stream when the myofilaments of the skeletal muscle are damaged [4]. Due to the similarity between cardiac and skeletal troponin, sTn subunits may interfere in the detection of cTn ones, thus limiting the performance of cTn detection-based analysis systems [5].

Previous works indicate that, from the three cTn subunits, the utility of cTnC for cardiac damage diagnosis is limited by the fact that it presents the same structure than sTnC, the only difference is the number of Ca²⁺ binding sites between them [6]. Regarding cTnT and cTnI, cTnT presents several specific cardiac isoforms whereas cTnI presents one. From here, their use in cardiac diagnostic and prognostic applications [7]. Several comparative studies between cTnT cardiac isoforms and cTnI specific cardiac isoform have been carried out, concluding that cTnI exhibits a higher specificity and accuracy than cTnT for the diagnosis of acute myocardial infarction at its early stage [8]. Therefore, cTnI has become the current gold-standard biomarker in clinical diagnosis for the identification of acute heart failures. However, it may presents a high cross reactivity with the sTnI regarding the interaction with cTnI antibody.

Within this context, in this work we report a computational study of the interaction of the cTnI antibody (α cTnI) capture probe with cTnI and sTnI. This study allows having a better understanding of the biochemical interactions between them and to computationally predict the binding performance and the selectivity of the α cTnI to cTnI versus sTnI. This information is very relevant for the development of analysis systems for myocardial failure diagnosis based on cTnI detection. First, FTSite and FTMap were used to determine and analyze the structure of the targets (cTnI and sTnI) and the antibody binding fragment (Fab). After the consideration of several possible conformations for cTnI and sTnI (more than 100), the most stable conformations (i.e., with the lowest total energy) were used to predict the binding sites. Next, FTDock and pyDock were used to study the molecular docking performance in order to select the most stable predicted conformations of the cTnI- α cTnI and sTnI- α cTnI complexes by their energies and coordinates. The 3D visualization of the molecules was done using PyMOL.

2. Materials and methods

2.1. Materials

2.1.1. Molecules structures

The X-ray structures of the molecules used in the study were obtained from the Research Collaboratory for Structural Bioinformatics (RCSB) protein data bank (PDB). The IDs of the sTnI and the α cTnI structures are 1VDI and 4P48, respectively. For the case of the cTnI, it was extracted from the whole cardiac troponin complex provided with the ID 4Y99 by cutting the specific sequence from the whole structure. Before their use, those molecules were purified by removing those atoms not corresponding with the molecules of interest from the PDB files and that might be present in the downloaded models (water residues and ions).

2.1.2. FTSite and FTMap

The binding surface of proteins contains the so-called 'hot spots', specific regions that provide major contributions to the binding free energy. Such regions are more likely to bind small drug-like compounds with high affinity than the rest of the binding sites, and hence are the prime targets in several fields such as immunology or drugs design. On the other hand, screening libraries of fragment-sized compounds obtained by nuclear magnetic resonance (NMR) or X-ray crystallography demonstrate that such 'hot spots' regions can bind a large variety of molecules. Therefore, the challenge here is being able to determine those sites computationally rather than experimentally in order to predict their druggability [9].

In order to determine and analyze the binding regions of the cTnI, sTnI and α cTnI molecules, FTSite and FTMap computing tools were used. These tools perform a statistical search of the entire molecule surface using a Fast Fourier Transform (FFT) based correlation approach in order to

determine those regions prickly to bind other molecules [10]. For those binding regions, FTSite characterizes different properties such as volume, hydrophobicity, hydrogen bonding, energy potential, solvent accessibility, desolvation energy and residue propensity. On the other hand, FTMap allows representing the obtained energy expression on a grid, which includes attractive and repulsive van der Waals (VDW) terms, electrostatic interaction energies (Ele.), dissolvation energies (Dissolv.) based on Poisson–Boltzmann calculations, resulting in a very accurate way to identify low-energy spot clusters [11].

2.1.3. FTDock and pyDock

Molecular docking is a prediction methodology used to determine the preferred orientation of two molecules when bound to each other to form a stable complex. This preferred orientation can be used to predict their binding affinity, i.e., the association strength between those two molecules.

FTDock and pyDock were used to model the biomolecular docking for the cTnI- α TnI and sTnI- α TnI complexes. Both computational tools perform a rigid-body docking of two biomolecules in order to predict their specific binding geometries and their probability/stability, given by their lowest total energy [12, 13]. Predicted docked complexes were outputted in pdb files, which were displayed using PyMOL 3D viewer. FTDock and pyDock implement different molecular docking algorithms, what was used to confirm that the obtained results were valid. Finally, note that, besides providing the geometric description of the docked complex, pyDock also provides the best docking orientations as scored mainly by electrostatics and desolvation energy [14].

2.2. Methods

An initial mapping to determine the binding sites and hot spots was done using FTSite. Once the spots were determined, the corresponding binding sites for the proteins and the antibody were chosen based on the side chains around the binding sites for the flexibility and rotamers determination.

cTnI, sTnI and α TnI were mapped by FTMap and maximally binding sites were selected and subsequently uploaded to the FTDock server for the corresponding biomolecular docking calculations. 10.000 statistically best conformations were generated and the most stable prediction was chosen for the next steps. In parallel, the same process was done using pyDock for a comparative study of the docking results obtained with FTDock. 100 best conformations were generated in pdb files, only considering the most stable of each complex. The results found by the two docking methods were similar.

3. Results and discussion

First, the ligand binding sites and the ‘hot spots’ on the molecules surfaces were determined for the cTnI, sTnI and α TnI. There is often more than one potential binding site on the surface of each molecule. Even when the structure of a molecule is determined by crystallography in a complex with a ligand, a complete description of its binding sites with that ligand cannot be determined because complex structures may not fully exploit the overall properties of the binding site. Moreover, knowledge of the possible binding sites in the structure of a molecule also enables us to analyze and classify them through their binding pocket profiles. Figure 1 shows the chosen binding sites and pockets for cTnI, sTnI and α TnI determined using FTSite.

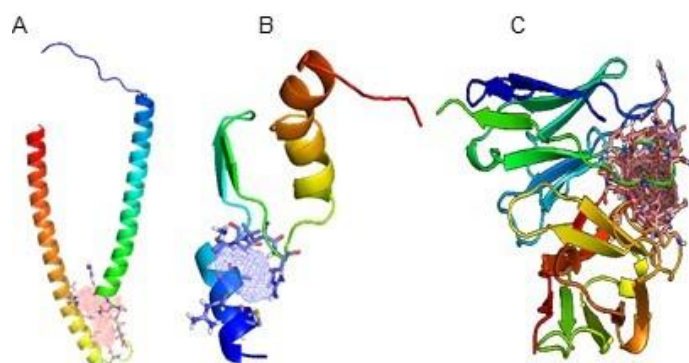


Figure 1. FTSite results showing the pockets (colored grid) and the binding sites (stick model) of (A) cTnI, (B) sTnI and (C) Fab region of the α TnI. The visualization has been done using PyMOL.

FTSite provides binding sites sequences. Specific binding aminoacids of those sequences of each protein interact with the antibody binding region forming the antibody-antigen complex. Table 1 shows all the aminoacid sequences that form the binding sites previously depicted in Figure 1. Here, FTMap represents in a grid the highest bonded/non-bonded H (grids not shown) and the highest H-bonded aminoacids forming the ‘hot spots’ from the whole binding sites.

Table 1. Complete description of the binding sites sequences given by FTSite for cTnI, sTnI and α TnI.

	cTnI	sTnI	αTnI
Binding sites sequences	RAQPPELLLLRVV	MRAKQGLR	GRYYKESADDNPQGTDSGDGYGGS

Table 2 shows the identified ‘hot spots’ hydrogen bond interactions for the cTnI- α TnI and the sTnI- α TnI complexes as defined by FTMap. Three H-bonds are identified for both complexes; however, while they are formed by three different aminoacids for the cTnI- α TnI complex, for the sTnI- α TnI complex two of them are formed by the same aminoacid GLY from the sTnI, what is translated into a weaker H-bonding than for cTnI- α TnI. This fact determines the higher affinity of α TnI towards cTnI than for sTnI.

Table 2. Identified hydrogen bonds for the cTnI- α TnI and the sTnI- α TnI complexes as defined by FTMap.

	cTnI-αTnI	sTnI-αTnI
H-Bonds	LEU-TYR	GLN-ASP
	GLN-ASP	GLY-THR
	GLU-SER	GLY-LYS

After binding sites identification and mapping, the next step is studying molecular docking for the cTnI and sTnI to α TnI binding. This process of docking provides the specific ‘hot spots’ aminoacids of the whole sequence that interact, taking into account spatial distribution, van der Waals interactions and rotational restrictions among others. For the docking analysis, FTDock and pyDock were used. FTDock generates several output pdb files with docking probabilities organized from the highest to the lowest probable one. On the other hand, pyDock generates several pdb files of the docked complexes being randomly organized, but results are accompanied by other generated files describing energies, positions and angles which can be used to order the predicted complexes. The ranking of molecular docking complexes obtained with both methods are the same, thus corroborating the simulation results. Figure 2 shows the docked complexes obtained for cTnI- α TnI and sTnI- α TnI, with highlighted binding zones.

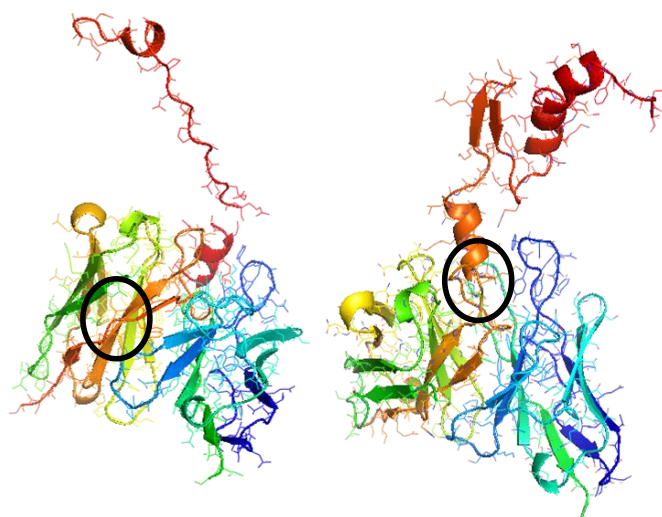


Figure 2. Most stable docked configurations of (left) cTnI- α TnI and (right) sTnI- α TnI complexes. Binding zones are black circled.

Having identified the most stable predicted structures, the formed complexes were visualized using PyMol to depict the specific interactions between the ‘hot spots’ sequences previously identified. Figure 3 details the binding points between the cTnI, sTnI and the α TnI as explained above. The obtained FTDock results were represented in the next figure, PyMOL was used to visually highlight the H-bonds between cTnI- α TnI and sTnI- α TnI.

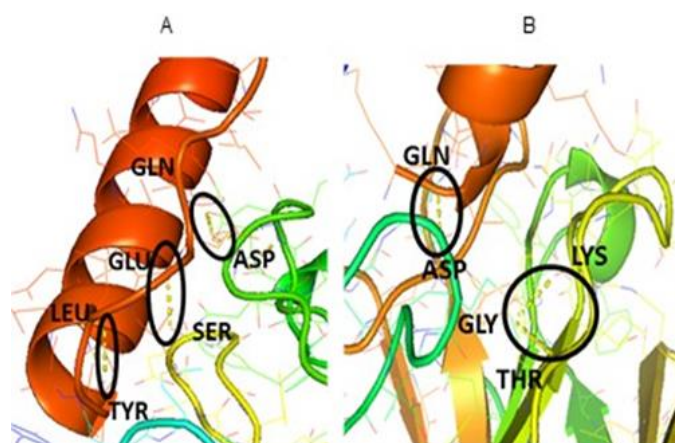


Figure 3 Detailed view of the (A) cTnI- α TnI and (B) sTnI- α TnI docked complexes. H-bonds between binding aminoacids are depicted with dash yellow line and highlighted with a black circle.

FTDock provides more than a thousand possible combinations for the docked proteins to α TnI. Different parallel data were also given to distinguish the most stable configurations as previously mentioned. Table 3 and Table 4 show the parameters describing the most stable docking configuration for the cTnI- α TnI and sTnI- α TnI complexes.

Table 3. Energy data (Kcal/mol) for the docked cTnI and sTnI with the α TnI for the most stable formed complexes configuration.

Complexes	Ele.	Desolv.	VDW	Total
sTnI- α TnI	-29.635	-3.683	72.809	-26.037
cTnI- α TnI	-11.866	-20.190	28.475	-29.208

Table 4. Positions data for the docked protein with the antibody fab for the most stable formed complexes configuration.

Complexes	SC score	ES ratio	Coordinates			Angles		
sTnI- α TnI	177	8.516	-5	16	-48	0	72	24
cTnI- α TnI	175	9.768	-23	63	1	168	72	204

From Table 3, the total energy of the cTnI- α TnI complex is considerably lower than for the sTnI- α TnI complex. Additionally, considering the previous results of the hydrogen bonding performance, the cTnI affinity towards α TnI is higher than for the sTnI.

This study reveals that the selected α TnI antibody is the adequate one for the experimental development of a cTnI-selective biosensing system in a subsequent stage. By performing this type of theoretical and computational analysis, we are able to determine the suitability of a certain capture antibody without the need of investing a large amount of resources on its experimental selection. This allows defining a better initial design of our biosensing experiments, with an enormous thrift on cost, effort and time.

4. Conclusions

In this work, we have demonstrated that a selectivity study can be performed computationally. As far as we know, this is the first time that a computational selectivity study is performed for biosensing purposes. The obtained affinity and selectivity results are highly important, since they compare the binding of cTnI and its principal interferon sTnI to bind the α TnI for a cross reactivity study, with the aim of a correct selection of a bioreceptor for the development of a cTnI biosensor for an effective, selective, fast and direct early detection of a myocardial failure.

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

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