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Molecular Docking on Recomposed versus Crystallographic Structures of Zn-Dependent Enzymes and their Natural Inhibitors

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***Abstract*—**Matrix metalloproteinases (MMP) are a class of structural and functional related enzymes involved in altering the natural elements of the extracellular matrix. Most of the MMP structures are cristalographycally determined and published in WorldWide ProteinDataBank, isolated, in full structure or bound to natural or synthetic inhibitors. This study proposes an algorithm to replace missing crystallographic structures in PDB database. We have compared the results of a chosen docking algorithm with a known crystallographic structure in order to validate enzyme sites reconstruction there where crystallographic data are missing.

***Keywords*—**matrix metalloproteinases, molecular docking, structure superposition, surface complementarity.

I. Introduction

MATRIX metalloproteinases (MMP) are a class of structural and functional related enzymes involved in altering the natural elements of the extracellular matrix [1,2]. MMP are dependent on Zn^{2+} and Ca^{2+} ions and are enzymes that are synthesized as zymogens in the cell and that can be inhibited by 4 classes of natural inhibitors called TIMPs (tissue inhibitor for matrix metalloproteinases) [3]. MMP plays important roles in physiological processes, while their overexpression plays also crucial roles in pathological processes as multiple sclerosis, arthritis, and mainly in cancer and metastasis [4-8].

According to the substrate specificity and primary sequence similarities, the members of this enzyme family can be grouped into five subfamilies: gelatinases (MMP-2, -9), which cleave denatured collagen, elastin, and type IV and V collagens; collagenases (MMP1,-8,-13), which cleave native collagen; stromelysins (MMP3,-10,-11), which may cleave proteoglycans; membrane-type MMPs (MMP14,-15,-16,-17), which are associated with activation of pro-MMPs [9].

From the structural point of view, MMP consist of four distinct domains: N-terminal pro-domain, catalytic domain, hinge region and C-terminal hemopexin-like domain. The latter may be responsible for the substrate recognition as well as for interaction with TIMPs. The first MMP structure in complex with a synthetic inhibitor was described by Lovejoy et al [10]. The MMP catalytic site is characterized by the presence of a Zn atom together with a conserved zinc binding motif, HExxHxxGxxH. In order for a molecule to become an effective inhibitor of the MMP it must show a functional group (e.g., hydroxamic acid, carboxylic acid, and sulfhydryl, etc.) capable of attaching to the catalytic zinc atom, at least one functional group which provides a hydrogen bond interaction with the enzyme backbone, and one or more side chains which undergo effective van der Waals interactions with the enzyme subsites. Gelatinase A (MMP2) is an unique member of metalloproteinase family while it is expressed by numerous cell types, shows an ubiquitous distribution and present an activation mechanism that differs from the other related enzyme family members. Together with progelatinase B (proMMP9) they are usually isolated as complexes with TIMP2 and TIMP1 respectively. Progelatinase A binds TIMP2 specifically but not TIMP1. The interaction between progelatinase and TIMP2 can mediate the gelatinase A activity at cell surface. MMP2 is involved, as most MMP, in regulating cell responses as proliferation, adhesivity or migration [11]. The purpose of this study was to compare the results for a chosen docking algorithm with a known crystallographic structure in order to validate enzyme sites reconstruction there where crystallographic data are missing.

We have chosen the Zn-dependent enzyme-inhibitor pairs due to the fact that there are extensive data regarding activation and inhibition of these enzymes, and also many already determined crystallographic structures of these enzymes in WorldWide ProteinDataBank (wwPDB) [12].

II. Material and Methods

A. Cristallographic structures

The chosen Zn dependent enzyme was the metalloproteinase 2 (gelatinase A), whose natural inhibitor is TIMP2 (tissue inhibitor for matrix metalloproteinase). For the docking algorithm we have chosen the complex proMMP2-TIMP2 in the 1GXD file in wwPDB. To compare and choose the aminoacids for MMP2 we have used the full MMP2 (gelatinase A) structure in 1CK7 file in wwPDB.

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B. Docking software

Docking software used in present study is part of the 3D-DOCK suite. This suite is able to dock large molecules, as protein-enzymes. FT-DOCK is able to perform a protein rigid docking. RPSCORE performs an empirical score systematization for the possible enzyme-inhibitor complexes, using potential pairs in aminoacid residues. FILTER scans the biological information in the chosen files for the plausible enzyme-inhibitor complexes. VMD software was used for the graphical representation of the maximum score variants. MULTIDOCK realizes energy minimization, a refinement of the enzyme-inhibitor complexes and calculates the binding energies.

C. Chosen algorithm

FT-DOCK suite is able to consider two known protein structures that are forming a complex in an unbound configuration and offers a limited set of possible models for this complex (e.g. MMP2-TIMP2). From the 1GXD file that represents the proMMP2-TIMP2 complex we have extracted the MMP2 complete structure, compared it with the sequence in 1CK7 and saved it in a separate file; the same procedure was performed for the natural inhibitor TIMP2, which was saved in a separate file. FTDOCK performs a rigid docking for the detached molecules and also performs a global scanning for the potential translational and rotational positions of the selected molecules. The positions were chosen after applying a geometrical limitation (filter for surface complementarity) and an electrostatic filter. Finally, MULTIDOCK realizes the energy minimization, a refinement of the MMP2-TIMP2 complexes and calculates the binding energies.

III. RESULTS

Docking procedures for the protein complex formed by MMP2-TIMP2 determined the identification of 10000 possible positions. Resulted file contain information referring to the resulted complexes in the G_DATA line. The first sorting is unfiltered and assigned to specific IDs. Data sorting in this file is determined by the surface complementary score (SCscore) the first value being the most important. Electrostatic complementarity score (ESratio) may show variations of the favorable electrostatic states. This score represents a ratio, as opposed to the absolute values, in which zero value is the less favorable and the value “100” is the most favorable. Other data in the file refers to the translational coordinates, expressed as the displacements of the mobile molecule center (TIMP2) over the center of the fixed molecule (MMP). The latest values are represented by the rotation angles, expressed in degrees. These results (Fig. 1) were visualized by Visual Molecular Dynamics software [13].

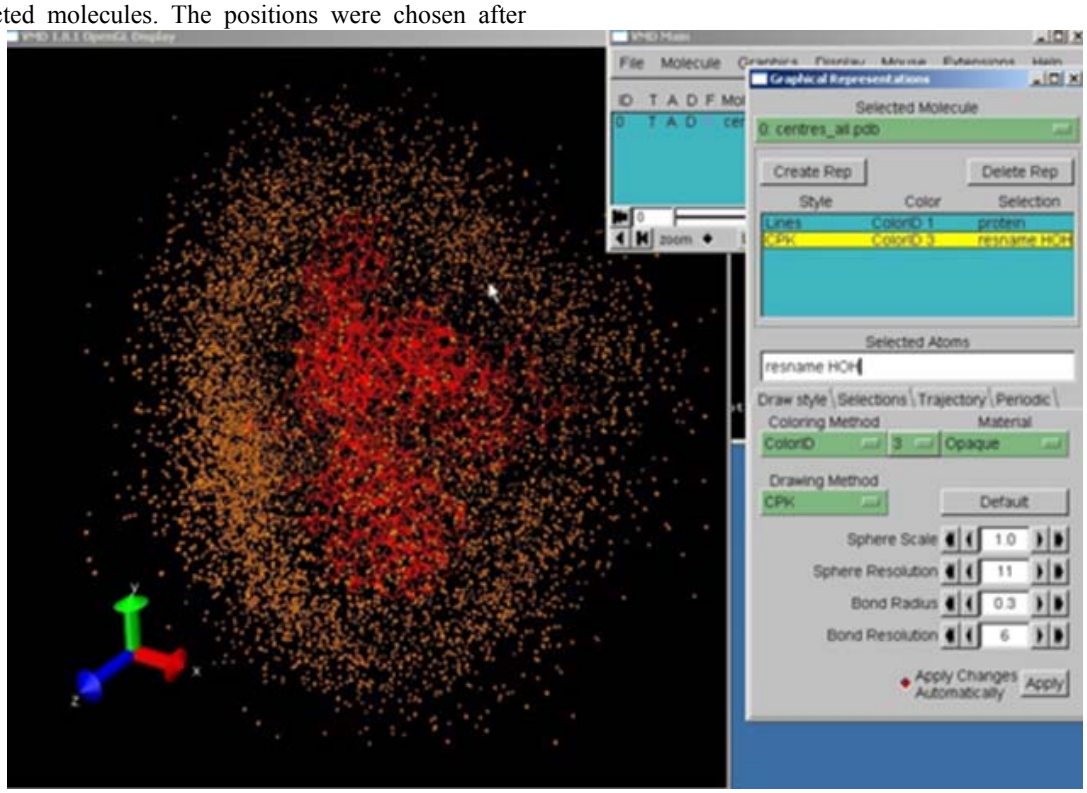

Fig. 1 Distribution of the possible docking positions for MMP2-TIMP2 complex. TIMP2 represented as dots around the central fixed structure of MMP2.

The score systematization process and filtering procedures for the localization of the active sites have generated separate files in which the structures that correspond to the selection criteria are listed according to their specific resulted score.

The filtration process was performed for the complexes realized by catalytic site (Fig. 2) and the hemopexinic site

(Fig. 3), each of them with TIMP2, those being the preferred sites for coupling this natural inhibitor. We have observed the preferential position reordering in a new list expressed by the new ID field, together with the position occupied before filtering (prvID – previous ID). Now, data systematization depend only on the potential scores at aminoacid residues level.

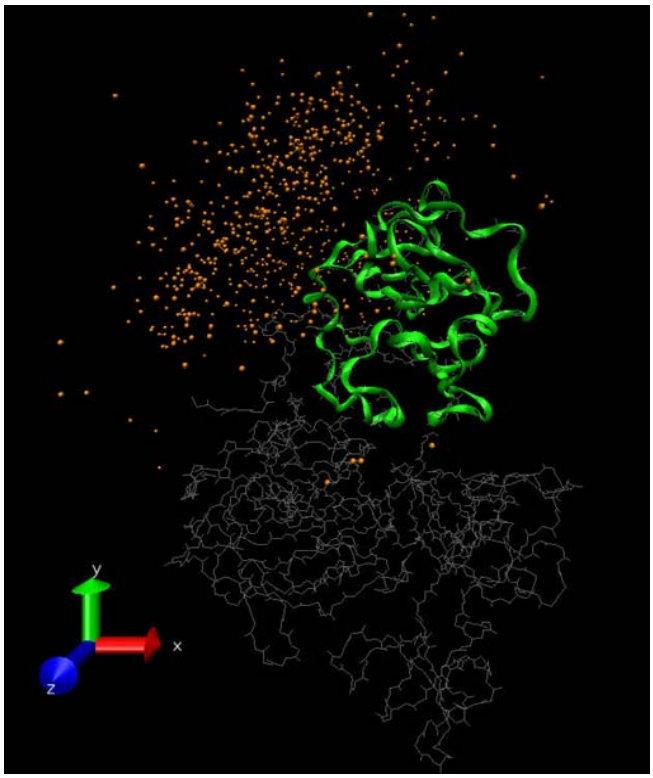

Fig. 2 Graphic representation for the distribution possibilities of the complex Cat_MMP2-TIMP2, following the filtering process (MMP2 – gray backbone; Cat_MMP2, catalytic domain of MMP2 – green ribbon, possible filtered positions of TIMP2 around MMP2 catalytic site – orange dots).

Comparing the two graphic representations in Fig.2 and Fig. 3 we can observe the abundance of the binding positions for the TIMP2 around the catalytic site of MMP2 against the available positions around hemopexinic site of the same enzyme.

The resulted structures, saved in separate files, were the superposed on the known crystallographic model (in the file 1GXD). Superposition was performed by the Swiss-PDB-Viewer application that allows also the calculation for the root mean square deviation (RMSD) that represents the distance between the initial coordinates for the atoms in the crystallographic structure and the final coordinates, partially altered by the remodeling procedure (fig.4). Swiss-PDB-Viewer application allows also the residue alignment for the original and the rebuilt structure, following the filtration process.

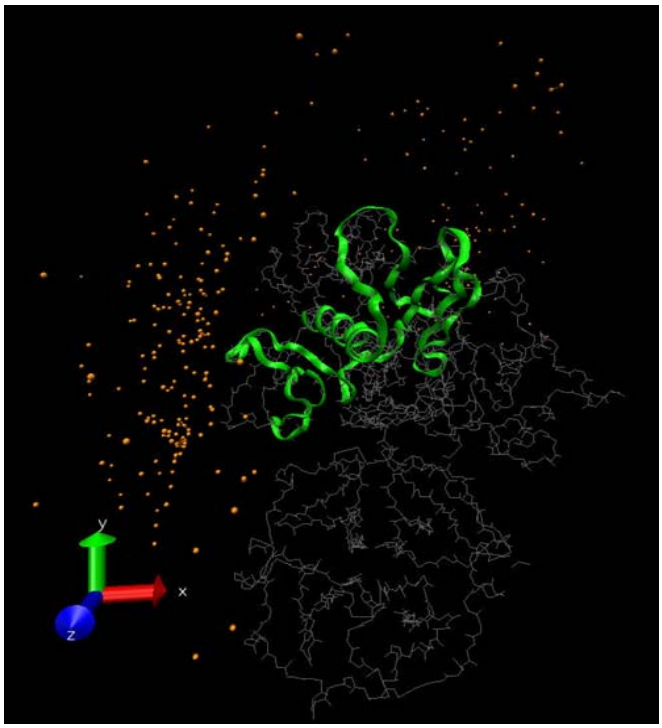

Fig. 3 Graphic representation for the distribution possibilities of the complex Hemopex_MMP2-TIMP2, following the filtering process (MMP2 – gray backbone; Hemopex_MMP2, hemopexinic domain of MMP2 – green ribbon, possible filtered positions of TIMP2 around MMP2 hemopexinic site – orange dots).

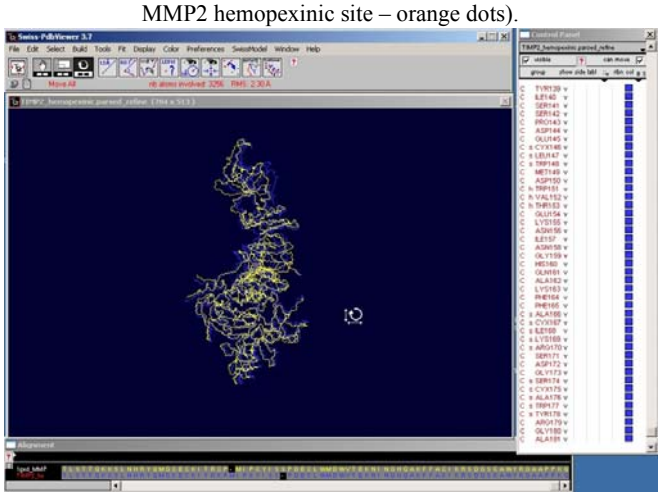

Fig. 4 Superposition of the crystallographic model of MMP2 (1GXD) and the complexes derived following modeling according to proposed algorithm, with RMSD calculation.

The complexes TIMP2-Cat_MMP2 and TIMP2-Hemopex_MMP2 with the maximal scores following filtration were submitted to energy minimization and steric conflict avoidance for the lateral chains between the two molecules, by the MULTIDOCK application. Determined binding energies were –2532 kcal/mol for TIMP2-Hemopex_MMP2 complex and –3286,7 kcal/mol for TIMP2-Cat_MMP2 complex. The affinity of TIMP2 is higher for the catalytic site (as demonstrated by experimental means).

RMSD values were 2,30Å that suggests an accurate

superposition and validates the proposed modeling algorithm.

The graphic representation for the maximal score TIMP2-Cat_MMP2 and TIMP2-Hemopex_MMP2 complexes is shown in Fig. 5 and Fig. 6.

Fig. 5 Final refined model of TIMP2-Hemopex_MMP2, in a position with the best score for favorable docking.

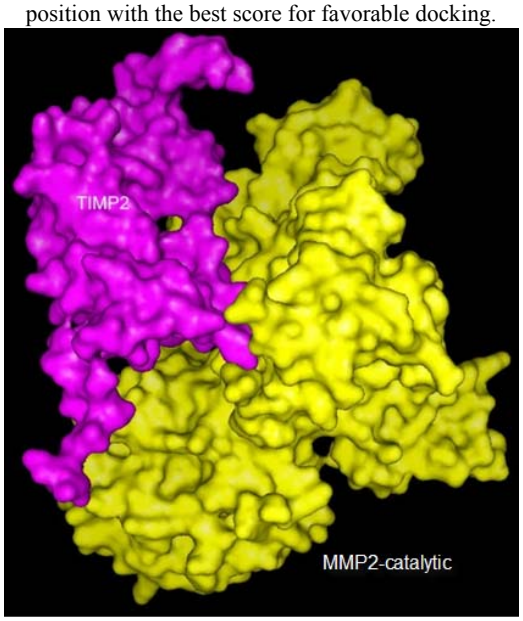

Fig. 6 Final refined model of TIMP2-Cat_MMP2, in a position with the best score for favorable docking.

IV. Conclusion

RMSD values obtained for the superposition between the detached enzyme-inhibitor pair and the crystallographic structure of the MMP2-TIMP2 complex (2,30Å) suggests an accurate superposition and validates the proposed modeling algorithm. Thus it may be useful to apply this algorithm when a enzyme-inhibitor structure in PDB is missing or is incomplete, while the crystallographic structure of its complex components are available.

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