Comparative Molecular Simulation Method for Ang2/Aptamers with *in vitro* Studies

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***Abstract*—Many researchers have reported about angiopoietin-2 (Ang2), which plays vital aspect of tumor Angiogenesis. Literature studies confirm that aptamers generated by *in vitro* selection strategy (systematic evolution of ligands by exponential enrichment, SELEX) has the ability to bind and inhibit Ang2 *in vitro*. A comparative simulation was performed between aptamers and Ang2 in computational environment using Discovery Studio 3.5. ZDOCK which uses Pairwise Shape Complementary as scoring function was performed for simulation, along with ZRANK which assess accurately reranking the rigid body docking results from ZDOCK. From this approach, our computational results showed consistent to in vitro results, thus providing ZDOCK with ZRANK as an alternative method for computational selection of best binding aptamers. This study also presents the binding interface between Ang2/aptamers which can be informative in the future study.**

***Index Terms*—Angiopoietin 2, aptamers, pairwise sequence complementary, molecular simulation**

I. Introduction

Angiogenesis is a physiological process in which new blood vessels form from pre-existing vessels and are regulated at multiple steps by interacting between pro- and anti-angiogenic factors [1]. The balance between neovessel formation and homeostasis of the resting vasculature is maintained by balance of proangiogenic and antiangiogenic mediators. The Tie/Angiopoietin receptor ligand system is significantly involved in maintaining vascular homeostasis and vessel maturation as well as vascular destabilization and remodeling [2], [3]. Four Tie2 ligands have been identified, Ang-1, Ang-2, Ang-3, and Ang-4. Among these angiopoietins, Ang-1 and Ang-2 are characterized in most detail as both bind the Tie2 receptor with similar affinity [4]. Numerous investigations have revealed the significance of Tie2 and the Angiopoietin in normal and pathological angiogenesis [5].

Researches has investigated the specific role of Ang2 in adult vasculature by iterative *in vitro* selection strategy called as SELEX (systematic evolution of ligands by exponential enrichment) and generated nuclease-resistant RNA ligands (aptamers) that bind Ang2 with high affinity [6]. Other studies have demonstrated that such aptamers can inhibit angiogenesis *in vivo* [7].

Many approaches are used in initial-stage docking, with respect to both searching and scoring. The FTDOCK, GRAMM, and ZDOCK are all algorithms carry out grid-based spatial searches by using Fast Fourier Transform (FFT). The scoring functions for initial stage docking utilize some items for scoring like desolvation, electrostatics, and a novel shape complementarity function. ZDOCK in Discovery Studio employs an FFT-based method using a Pairwise Shape Complementarity (PSC) function for identifying docked conformations and scores hits based on atomic contact energies, thus predicting interactions of novel targets rapidly and accurately with performing rigid body docking. The PSC method is optionally augmented with desolvation (DE) and electrostatic (ELEC) energy terms to rank the docked poses [8], [9]. The initial-stage docking program ZDOCK has been proven effective both against a docking benchmark7 and in several rounds of the CAPRI experiment [10], [11].

ZDOCK is a grid-based docking algorithm that performs a systematic search in 6D and typically can output 3600 or 54,000 predictions, depending on the sampling density in the rotational space of 15^{o} or 6^{o}. The development of ZRANK, a program that quickly and accurately reranks the rigid body docking results from ZDOCK. ZRANK can quickly process and rerank the 54,000 predictions produced by the ZDOCK 6^{o} sampling search. The ZRANK scoring function is a linear combination of van der Waals attractive and repulsive energies, short and long range repulsive and attractive energies, and desolvation [12]. Here we have performed ZRANK simulations for Ang2 and 15 aptamers to appraise the probable for the best aptamers and to compare their binding affinity.

II. Materials and Methods

The 15 RNA aptamers were collected from literatures were converted into 3D structures using RNAComposer-

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automated RNA structure 3D modeling server [13] (http://rnacomposer.cs.put.poznan.pl/).

All the computational experiments/simulations were performed in Inter(R) Xeon(R) CPU E31230 @ 3.20GHz supported by Windows Web Server 2008 R2.

A. Building 3D structures for Aptamers

Fifteen aptamers taken from literatures [6], [7] were converted into dot-bracket notation with help of online tool CentroidFold (http://www.ncrna.org/centroidfold) [14]. Table I shows the dot-bracket notation of each aptamer, and this information can be used to generate three-dimensional models using RNAComposer-Automated RNA Structure 3D Modeling Server.

TABLE I. DOT-BRACKET NOTATIONS OF 15 APTAMERS WERE GENERATED FROM CENTROID FOLD WEB SERVER

No	Sequence	Dot-Bracket Notation
1	ACUAGCCUCAUCAGCUCAUGU GCCCCUCCGCCUGGAUCAC	....((.......))..... .....
2	UUAACCAUCAGCUCAUGGCCCC UGCCCUCUCAAGGACCAC	.....((..... .....))..
3	CACCAGACCGACAUCAGCUUAU GGCCCCUCACCCACACCG	....
4	CCACCGAUCGCAUCAGCUCAUG GCCCCUCCCGACCCGCCA	.....(((..... .....))).
5	CCAGACGUUCUCGCCCCGCCGA UCAUCAGCGCUGGCCCUAU	.....(((.(((.....)))..))).....
6	CACUACCACGCCAUAUCAGCUA AUGGCCCCUCCCUACGCA	.....(((((.....))))). .....
7	ACUCACCAGUCACCAUCAGCUC AUGCGCCCCUCCCCCGAC	....
8	UGACCAAGCCUCACGUUGAACC UGCCAGUAGACCCCGCCCA	....
9	GGAGCGCAAUUCGCCUCGCAA GUUGAACUCCGCUGGCGG	.(((...((.....))...)))((....)).
10	UAAGCUCUUUGGCUUAGCCCG ACACGUUGAACUCCAGAGU	((((((....))))))((.....)).....
11	CACGGUACCACCAAGUCACACG UUGAACUCCAUGCAGCUG	....
12	CAUGUCUACAACAAUCUCGCCC GUUGAGUCUCGUCGAAUU	....
13	CACUCAGCGCCCUGCGAAACGU UGCCGCCUCCCAACGUCU	.....((((((..... ...))))))..
14	CUCUUUUUGUCCCCGCACGUUG AACUCCUGUCCCUCUACU	....
15	GAGGACGAUGCGGACUAGCCU CAUCAGCUCAUGUGCCCCUC	((((..((((.((.....)).)))). .....))))

B. Aptamers-Ang2 Simulation

Using Discovery Studio version 3.5 as platform, Ang-2 was separated from Tie2 crystal structure 2GY7 obtained from PDB. The 2GY7 contained angiopoietin-2/Tie2 complex crystal structure with chain A and B (shown in Fig. 1(a)), and Ang2 can be isolated from the complex crystal structure (shown in Fig. 1(b)).

Ang2 contained 216 amino acids. For an intact structure of Ang2, this molecule is consisted of 496 amino acids. Therefore, the crystal structure of Ang2 presented in this file is just a part of structure of Ang2. In running the ZDOCK, the angular step size for rotational sampling of ligand orientations was kept to 6 degrees which performs finer conformational sampling and thus typically results in more accurate predictions. The van der Waals and short range electrostatics energies are calculated based on the parameter of the CHARMm 19 polar hydrogen potential.

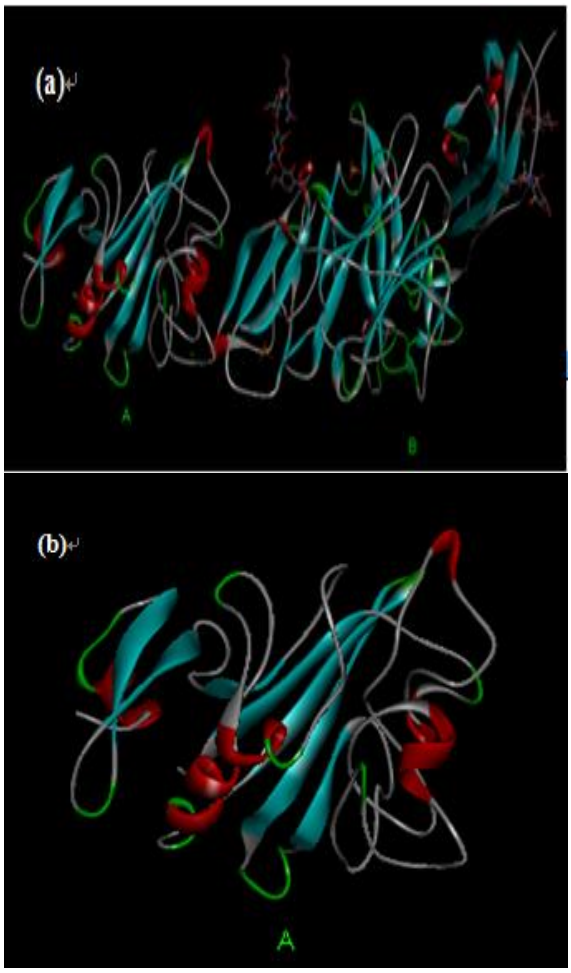

Figure 1. (a) Angiopoietin-2/Tie2 complex crystal structure (PDB code: 2GY7), chain A is Ang2 and Chain B represents Tie2; (b) Structure of Ang2.

III. RESULTS AND DISCUSSION

The computational time for each Ang2/aptamer simulation took around 24 hours. The top poses are reported with the poses clustered into groups according to their spatial proximity and poses are ranked according to the ZRANK scores. Top poses are determined by the linear combination of van der Waals attractive and repulsive energies, short and long range repulsive and attractive energies, and desolvation. The ZRANK scores for 15 aptamers were between -62.02 to -93.85 and all these scores of 15 aptamers were mentioned in Table II.

TABLE II. ZRANK SCORE FOR THE SIMULATION BETWEEN EACH APTAMER AND ANG2

Name of Aptamer	ZRANK Score
Sequence-1	-93.855
Sequence-2	-82.722
Sequence-3	-73.128
Sequence-4	-72.227
Sequence-5	-69.183
Sequence-6	-73.305
Sequence-7	-63.518
Sequence-8	-70.795
Sequence-9	-74.153
Sequence-10	-65.73
Sequence-11	-62.02
Sequence-12	-68.159
Sequence-13	-74.432
Sequence-14	-73.895
Sequence-15	-80.325

An *in vitro* study [6] reported that sequence-1 has a more binding affinity to Ang2. Accordingly, the ZRANK simulation results show best score for Sequence-1 and the second best score for Sequence-2. Sequence-15 which previously reported as it has the ability to inhibit tumor angiogenesis [7] has got third best score out of all. Our results are consistent with the findings from previous reports, suggesting this method has the potential in the refinement of binding affinity of the RNA aptamer produced from SELEX process.

However, there was no any study reporting actual binding site of aptamers/Ang2. With the ZRANK, we can see the binding interfaces between the Ang2/aptamers in which amino acids are involved in binding (Fig. 2).

IV. CONCLUSIONS

With the above simulation results, *in silico* selection of RNA aptamers for the inhibition of Ang2 seems a possible way. As the results are consistent with invitro studies in finding best binding aptamers, our study reveals that *in silico* selection RNA aptamer is a useful and practicable approach for evaluating the binding affinity of aptamer with the target protein. *In silico* selection of aptamers is efficient in saving both time and money. The information about amino acids involved in binding with aptamers may be helpful to realize the binding situation between Ang2 and aptamers for clarifying which amino acids are important to the interaction.

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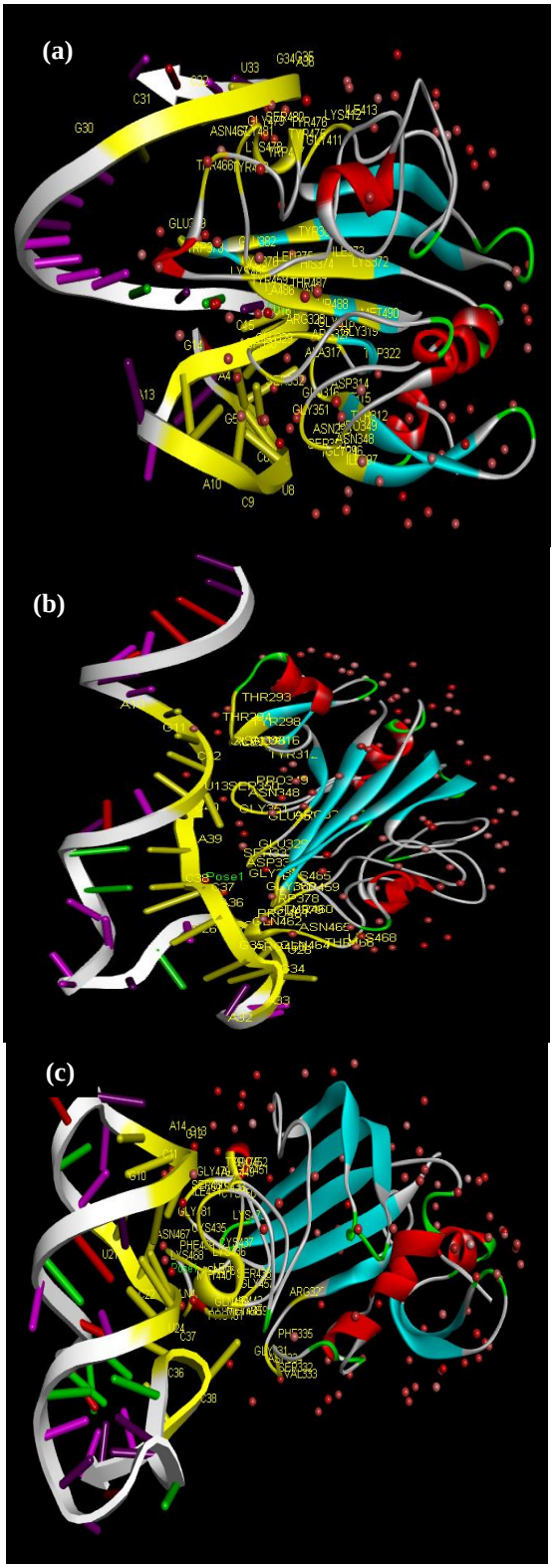

Figure 2. Results of docking simulations between aptamers and Ang2. (a)Sequence-1/Ang2 (b) Sequence-2/Ang2 (c) Sequence-15/Ang2. The red dots around the protein were poses. Yellow color is the binding interface between Ang2/aptamers. Amino acids involved in binding with 3 best Aptamers are mentioned below with their Amino acid and position number.

Sequence1:T294,N295,G296,I297,Y312,D314,M315,E316,A317,G318, G319,W322,R327,R328,E329,D330,S332,N348,P349,S350, G351,K372,I373,H374,L375,K376,W378,E379,E382,Y384, G411,K412,I413,Y459,T466,N467,Y475,Y476,W477,K478, G479,S480,G481,Y482,K485,A486,T487,T488,M490

Sequence2:N295,I297,Y312,D314,E316,A317,R327,R328,E329,D330, G331,S332,V333,D334,F335,R337,G347,N348,P349,S350, G351,E352,H374,K376,N381,E382,A383,Y384,G408,T409, A410,G411,K412,I413,G457,M458,Y459,Y460,P461,Y475, Y476,W477,K47

Sequence15:R328,G331,S332,V333,D334,F335,C433,I434,C435,K436, C437,S438,Q439,M440,L441,T442,A449,C450,G451,P452, G457,M458,Y459,Y460,P461,Q464,N467,K468,F469,N470, K473,Y475,G479,S480,G481

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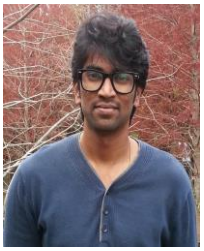

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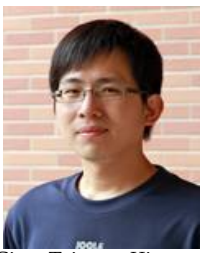

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