Characterizing the Hot Spots Involved in RON-MSPβ Complex Formation Using In Silico Alanine Scanning Mutagenesis and Molecular Dynamics Simulation

Omid Zarei1,2,3, Maryam Hamzeh-Mivehrou3,4, Silvia Benvenuti5, Fulya Ustun-Alkan6, Siavoush Dastmalchi2,4

1 Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.
2 Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.
3 Students Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran.
4 Department of Medicinal Chemistry, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.
5 Molecular Therapeutics and Exploratory Research Laboratory, Candiolo Cancer Institute-FPO-IRCCS, Candiolo, Turin, Italy.
6 Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Istanbul University, Istanbul, Turkey.

Abstract
Purpose: Implication of protein-protein interactions (PPIs) in development of many diseases such as cancer makes them attractive for therapeutic intervention and rational drug design. RON (Recepteur d’Origine Nantais) tyrosine kinase receptor has gained considerable attention as promising target in cancer therapy. The activation of RON via its ligand, macrophage stimulation protein (MSP) is the most common mechanism of activation for this receptor. The aim of the current study was to perform in silico alanine scanning mutagenesis and to calculate binding energy for prediction of hot spots in protein-protein interface between RON and MSPβ chain (MSPβ).

Methods: In this work the residues at the interface of RON-MSPβ complex were mutated to alanine and then molecular dynamics simulation was used to calculate binding free energy.

Results: The results revealed that Gln52, Arg287, His286, Pro288, Glu289, and His284 residues from RON and Arg50, His528, Ser529, Glu530, and Arg463 from MSPβ may play important roles in protein-protein interaction between RON and MSP.

Conclusion: Identification of these RON hot spots is important in designing anti-RON drugs when the aim is to disrupt RON-MSP interaction. In the same way, the acquired information regarding the critical amino acids of MSPβ can be used in the process of rational drug design for developing MSP antagonizing agents, the development of novel MSP mimicking peptides where inhibition of RON activation is required, and the design of experimental site directed mutagenesis studies.

Introduction
Protein-protein interactions (PPIs) are involved in many biological processes as key regulatory steps1 and when aberrantly regulated are implicated in the development of many diseases such as cancer.2,4 These versatile roles make PPIs attractive for therapeutic intervention and rational drug design.5,7 Different classes of the approved therapeutic agents or those in development stages have been shown to interfere with PPIs in order to overcome the corresponding diseases.8-11

In PPI, the amino acids at the interaction interface have great importance in terms of starting point for the initiation of the biological and cellular functions.12 Usually several residues are exposed at the interface between the interacting proteins, but they do not contribute equally to the binding energy. The important key residues when mutated into alanine residue weaken the binding strength (increase of free energy of binding at least 2.0 kcal/mol) are called “hot spots”.13,14 Identification of the hot spots is critical in designing therapeutic agents which exert their effects by influencing PPIs. One of the experimental methods commonly used for identification of these hot spots is site-directed mutagenesis followed by comparative functional assays of the mutated and the wild type proteins; however, these experiments are time-consuming and expensive.15 Structural elucidation of the partner proteins within a complex by means of biophysical methods such as X-ray crystallography and NMR is also possible but again costly and demanding.16 In the era of modern drug discovery and development, the use of in silico methods shortens the rational drug design process in terms of both time and cost.17-21 In this regard, identifying hot spots is not an exception.22,23 Computational alanine scanning mutagenesis is a virtual
method which has been extensively used for the characterization and prediction of hot spots in protein-protein, protein-DNA and protein-small molecule complexes.\textsuperscript{52,24-29} Charged, polar, or bulky amino acids are virtually mutated to a neutral, small and non-polar amino acid such as alanine and then binding free energy is calculated for both wild type and mutant forms in order to estimate the contribution of the mutated residues to the binding energy.\textsuperscript{30,31} One of the most routinely used approaches for computational estimation of binding free energy is based on accessible surface area models of implicit solvation method where molecular mechanics data are treated by Generalized Born surface area (MM-GBSA) algorithm.\textsuperscript{52-40}

Tyrosine kinase receptors (TKRs) involved in well characterized protein-protein interactions are among potential candidate targets for anticancer drug development.\textsuperscript{41-46} TKRs are cell surface receptors for different polypeptide ligands and have pivotal roles in regulation of many cellular functions and physiological events\textsuperscript{47,48} and when aberrantly expressed and activated play key functions in development and progression of different types of cancers.\textsuperscript{49-54} Ligand-mediated receptor dimerization is the main mechanism of activation triggered by ligand binding to the extracellular domain of its specific receptor.\textsuperscript{55-57} This protein-protein interaction causes receptor dimerization followed by autophosphorylation of tyrosine residues located within the intracellular tyrosine kinase domain (catalytic tyrosines) followed by phosphorylation of tyrosine residues located within the C tail (docking tyrosines) that become the docking site for adaptor/effector proteins responsible for transducing the downstream signaling pathways resulting in cellular proliferation, differentiation, metabolism, survival, migration, and cell cycle control.\textsuperscript{58} In principle, all PPIs mediated by TKRs (including the downstream PPIs) could be targeted for cancer therapy\textsuperscript{59,60} but generally therapeutic PPI inhibitors interfere with the binding of endogenous ligands to the receptor.\textsuperscript{61-67} Therefore, it is obvious that uncovering the details of PPIs between TKRs and their ligands can provide useful information applicable to design of new anticancer agents.

RON (Recepteur d'Origine Nantais) is a member of TKRs superfamily, its role in tumorigenesis has been established in different cancer types and numerous studies have suggested RON as a promising target for anticancer drug development.\textsuperscript{68,69} RON also known as MSTR1 (Macrophage Stimulating Receptor1) belongs to MET proto-oncogene family.\textsuperscript{70} and is usually expressed at low levels in normal tissues while it is highly expressed in cancer cells.\textsuperscript{71} Structurally, RON is a disulfide linked heterodimer protein made of two chains, an extracellular α-chain and a β-chain which consists extracellular, transmembrane, and intracellular regions. The extracellular domain comprises three distinct domains including Sema, Plexin-Semaphorin-Integrin (PSI), and three Immunoglobulin-Plexin-Transcription factor (IPT1-IPT3) domains.\textsuperscript{68} The natural ligand of RON is MSP (Macrophage Stimulating Protein),\textsuperscript{72} a member of plasminogen-related kringole protein family\textsuperscript{73} which is a heterodimeric protein made of an α-chain composed of four kringole domains and a β-chain containing a serine protease-like domain.\textsuperscript{74} The α- and β-chains of MSP show low and high affinities to RON Sema domain, respectively.\textsuperscript{75} Several monoclonal antibodies against RON extracellular domain have been developed (in preclinical phases) to specifically inhibit the protein-protein interactions between RON and MSP.\textsuperscript{69} Identifying the key residues working as hot spots responsible for receptor-ligand (RON-MSP) interaction is of great importance for drug design and development. The aim of the current study is to identify hot spots involved in RON-MSP$\beta$ interaction using \textit{in silico} alanine scanning mutagenesis by MM-GBSA method. The results can be used in anticancer drug designing where inhibition of RON is needed.

Materials and Methods

\textbf{Structure preparation and in silico alanine mutagenesis}

Experimental coordinates of RON complexed with MSP$\beta$ (PDB ID: 4QT8) determined at 3.0 Å resolution by X-ray crystallography\textsuperscript{76} was retrieved from the Protein Data Bank at the Research Collaboratory for Structural Bioinformatics (http://www.rcsb.org/pdb/home/home.do).\textsuperscript{77} Preparation of structures along with mutation of the residues were carried out using Swiss-Pdb Viewer (DeepView) version 4.01.\textsuperscript{78} Only one of the complexes in the reported crystal structure was used (chains B and D) for further analysis. The residues at the RON-MSP$\beta$ interface were inferred based on crystal structure reported by Chao and collaborators\textsuperscript{76} in both ligand and receptor were virtually mutated to alanine as listed in Table 1.

\begin{table}[h]
\centering
\caption{List of residues mutated to alanine on RON and MSP}
\begin{tabular}{|c|c|}
\hline
RON & MSP \\
\hline
Glu$^{190}$ & Arg$^{522}$ \\
Glu$^{193}$ & Cys$^{247}$ \\
Ser$^{195}$ & His$^{528}$ \\
Arg$^{220}$ & Ser$^{555}$ \\
Glu$^{287}$ & Arg$^{639}$ \\
Pro$^{288}$ & Glu$^{644}$ \\
Glu$^{289}$ & Glu$^{658}$ \\
His$^{424}$ & Arg$^{683}$ \\
Glu$^{190}$/ Ser$^{195}$ & Arg$^{529}$/ Glu$^{644}$* \\
\hline
\end{tabular}
\end{table}

\textsuperscript{* Double mutation}

\textbf{Ligand-receptor binding free energy calculations using MM-GBSA method}

Energy minimization and binding free energy calculation were performed using the Assisted Model Building with Energy Refinement (AMBER) suite of programs (version
Results and Discussion

Binding free energies for the complexes of RON tyrosine kinase receptor and its ligand (i.e. MSP) as well as the mutants of either receptor or ligand (Table 1) were calculated by applying MM-GBSA method on molecular dynamic simulation data collected at different time. For this purpose, firstly the binding free energy was calculated for the RON-MSPβ wild type complex, then the residues involved in RON and MSPβ interaction were mutated to alanine followed by molecular dynamic simulation and re-calculation of the binding free energy for different time intervals ranging from 1 to 10 ns to estimate the contribution and the effect of individual residues in RON and MSPβ binding. The binding free energies (ΔGbind) for wild type and mutant forms were calculated as follows:

$$\Delta G_{\text{bind}} = G_{\text{water}}(\text{complex}) \cdot G_{\text{water}}(\text{receptor}) \cdot G_{\text{water}}(\text{ligand})$$

where $G_{\text{water}}$ (complex), $G_{\text{water}}$ (receptor), and $G_{\text{water}}$ (ligand) denote the free energies of the complex, receptor, and ligand, respectively. The free energy (ΔG) for each term is calculated using following equation:

$$G_{\text{molecule}} = E_{\text{gas}} + \Delta G_{\text{solvation}} + \Delta G_{\text{int}} = G_{\text{bind}}$$

where G is the calculated average free energy, $E_{\text{gas}}$ is the standard force-field energy, including internal energy ($E_{\text{int}}$) in the gas phase as well as non-covalent van der Waals ($E_{\text{vdw}}$) and electrostatic ($E_{\text{elec}}$) energies. $E_{\text{bond}}$, $E_{\text{angle}}$, and $E_{\text{tors}}$ demonstrate the contributions to the internal energy caused by the strain from the deviation of the bonds, angle, and torsion angle from their equilibrium values. $\Delta G_{\text{solvation}}$ is the solvation-free energy calculated with a numerical solution of the Poisson–Boltzmann equation and an estimate of the non-polar free energy using a surface area term.\(^{81,82}\)

Figure 1 shows the results of binding free energy calculations for the complex of wild type RON and MSPβ and their mutant forms using MM-GBSA method applied to molecular dynamic simulations ranging from 1 to 10 ns. These results have been also illustrated in Table 2. Results for $\Delta \Delta G$ binding ($\Delta G_{\text{binding-wild type}} - \Delta G_{\text{binding-mutant}}$) for RON and MSPβ are also available in Table 3. The details of all calculations for mutants and wild types of receptor and ligand are available in appendices 1 and 2.

Cancer is one of the most important causes of death in the world\(^{5,83}\) and several strategies including pharmacotherapy protocols are employed to control this devastating condition.\(^{84}\) Due to the importance of protein-protein interactions in cancer initiation and development, many efforts have been dedicated to target cancer cells by inhibition of those PPIs involved in cancer progression.\(^3,5,85,86\) RON a tyrosine kinase receptor has gained considerable attention as promising target in cancer therapy.\(^{58}\) Most of the therapeutic agents developed so far against RON interfere with RON and MSP binding highlighting the importance of PPIs.\(^{69}\) Therefore, the identification of hot spots involved in the interface of RON-MSP complex is of great importance in rational drug design.

In the current study, the residues reported to be involved in RON-MSPβ interactions (Figure 2) were virtually mutated to alanine one at the time to determine the contribution of each residue using MM-GBSA approach. The binding free energy difference between the mutant and the wild type complexes was obtained as follows:

$$\Delta \Delta G_{\text{binding}} = \Delta G_{\text{binding-wild type}} - \Delta G_{\text{binding-mutant}}$$

In this expression, negative $\Delta \Delta G_{\text{binding}}$ value implies that the substitution of the corresponding amino acid with alanine is an unfavorable substitution whereas a positive value indicates a favorable substitution in terms of binding free energy compared to the wild type complex.\(^{87}\)
Figure 1. The plot of binding free energies (∆G) for the complexes of RON-MSPβ during different MD simulation time lengths (1–10 ns) using MM-GBSA calculation methods implemented in AMBER.

Table 2. Effects of alanine substitution on RON (A) and MSP (B) to contribution of binding energy (∆Gbind) for RON-MSP complex calculated using MM-GBSA method in a 1 to 10 ns molecular dynamic simulation.

|          | 1ns   | 2ns   | 3ns   | 4ns   | 5ns   | 6ns   | 7ns   | 8ns   | 9ns   | 10ns  |
|----------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| **A)**   |       |       |       |       |       |       |       |       |       |       |
| WT       | -77.17| -77.85| -78.58| -77.81| -77.78| -77.17| -78.33| -79.10| -80.36| -81.91|
| E190A    | -76.19| -78.62| -81.58| -85.02| -84.85| -85.41| -86.74| -88.76| -91.26| -94.65|
| Q193A    | -51.91| -55.39| -56.27| -58.29| -60.57| -62.00| -63.08| -64.22| -64.15| -63.56|
| S195A    | -94.36| -91.40| -89.31| -86.62| -86.64| -87.50| -89.57| -91.20| -91.76| -92.19|
| R287A    | -49.73| -50.15| -49.67| -50.17| -50.67| -51.18| -51.70| -51.52| -51.33| -52.06|
| E289A    | -69.70| -67.19| -67.55| -66.75| -65.81| -65.60| -65.24| -64.51| -64.20| -63.79|
| P199A    | -74.01| -62.30| -59.40| -59.00| -58.62| -58.67| -58.01| -58.14| -57.92| -58.12|
| E658A    | -59.17| -58.18| -59.67| -60.61| -61.96| -64.52| -64.92| -65.35| -67.40| -68.80|
| H424A    | -71.45| -65.70| -67.52| -67.99| -68.51| -70.10| -70.94| -71.88| -72.46| -73.37|
| E190A/S195A | -70.09| -68.40| -67.30| -65.73| -64.15| -64.02| -69.99| -65.81| -66.26| -67.10|
| **B)**   |       |       |       |       |       |       |       |       |       |       |
| WT       | -77.17| -77.85| -78.58| -77.81| -77.78| -77.17| -78.33| -79.10| -80.36| -81.91|
| R287A    | -69.23| -70.67| -69.80| -71.45| -70.41| -67.89| -66.31| -65.83| -65.14| -63.65|
| C527A    | -83.18| -79.98| -78.55| -80.10| -83.16| -82.49| -81.23| -79.92| -78.73| -77.63|
| H528A    | -72.37| -69.73| -68.66| -66.66| -66.17| -67.20| -68.09| -69.49| -70.27| -70.38|
| S639A    | -58.67| -60.44| -63.05| -64.01| -67.13| -67.90| -67.93| -68.80| -70.12| -71.47|
| E658A    | -79.74| -77.52| -82.47| -85.12| -87.36| -89.09| -91.37| -93.07| -94.06| -93.91|
| R644A    | -90.27| -89.90| -93.40| -95.66| -96.18| -95.22| -94.30| -93.31| -93.29| -93.42|
| R321A    | -52.77| -53.15| -54.63| -53.59| -51.00| -49.41| -49.05| -49.24| -49.31| -49.28|
| E658A    | -47.54| -48.21| -51.97| -54.61| -56.40| -57.25| -57.53| -57.68| -58.10| -58.24|
| R321A/E658A | -65.58| -61.56| -55.29| -53.06| -51.88| -52.91| -51.99| -54.55| -53.77| -55.28|
The involved hot spots in RON-MSPβ complex formation

Table 3. The binding energy differences (ΔΔGw wartości - ΔGmutant) for wild type and mutant forms of RON-MSP complex. The mutations are performed on RON (A) and MSP (B) using in silico alanine substitution.

|       | 1ns  | 2ns  | 3ns  | 4ns  | 5ns  | 6ns  | 7ns  | 8ns  | 9ns  | 10ns |
|-------|------|------|------|------|------|------|------|------|------|------|
| ΔE<sup>206</sup>A | -0.98 | 0.78 | 3.01 | 7.21 | 7.07 | 8.24 | 8.41 | 9.66 | 10.90 | 12.74 |
| ΔQ<sup>123</sup>A | -25.26 | -22.45 | -22.30 | -19.52 | -17.22 | -15.17 | -15.25 | -14.88 | -16.21 | -18.35 |
| ΔS<sup>265</sup>A | 17.19 | 13.55 | 10.73 | 8.81 | 8.86 | 10.33 | 11.25 | 12.11 | 11.41 | 10.28 |
| ΔR<sup>220</sup>A | -27.44 | -27.70 | -28.90 | -27.64 | -27.12 | -25.99 | -26.63 | -27.58 | -29.03 | -29.85 |
| ΔE<sup>289</sup>A | -7.47 | -10.65 | -11.03 | -11.06 | -11.97 | -11.57 | -13.09 | -14.59 | -16.16 | -18.12 |
| ΔP<sup>388</sup>A | -3.16 | -15.54 | -19.18 | -18.81 | -19.17 | -18.50 | -20.32 | -20.96 | -22.44 | -23.79 |
| ΔE<sup>624</sup>A | -18.00 | -19.67 | -18.90 | -17.19 | -15.82 | -12.64 | -13.41 | -13.75 | -12.96 | -13.12 |
| ΔH<sup>E24</sup>A | -5.72 | -12.15 | -11.05 | -9.82 | -9.27 | -7.06 | -7.38 | -7.22 | -7.90 | -8.54 |
| ΔE<sup>190A</sup>ΔS<sup>195A</sup> | -7.08 | -9.44 | -11.28 | -12.08 | -13.63 | -13.15 | -8.33 | -13.28 | -14.10 | -14.81 |
| ΔR<sup>521A</sup> | -7.94 | -7.17 | -8.77 | -6.36 | -7.37 | -9.28 | -12.01 | -13.26 | -15.21 | -18.26 |
| ΔC<sup>221</sup>A | 6.01 | 2.14 | -0.03 | 2.29 | 5.37 | 5.32 | 2.90 | 0.82 | -1.62 | -4.28 |
| ΔH<sup>229</sup>A | -4.80 | -8.12 | -9.91 | -11.16 | -11.61 | -9.97 | -10.24 | -9.60 | -10.09 | -11.53 |
| ΔS<sup>655</sup>A | -18.50 | -17.40 | -15.53 | -13.80 | -10.66 | -9.27 | -10.40 | -10.30 | -10.24 | -10.44 |
| ΔR<sup>399</sup>A | 2.57 | -0.33 | 3.89 | 7.31 | 9.58 | 11.93 | 13.04 | 13.97 | 13.70 | 12.00 |
| ΔE<sup>414</sup>A | 13.10 | 12.06 | 14.82 | 17.85 | 18.40 | 18.06 | 15.98 | 14.21 | 12.94 | 11.51 |
| ΔE<sup>689</sup>A | -24.40 | -24.70 | -23.95 | -24.21 | -26.78 | -27.76 | -29.28 | -29.86 | -31.04 | -32.63 |
| ΔR<sup>683</sup>A | -29.63 | -29.64 | -26.61 | -23.19 | -21.38 | -19.92 | -20.79 | -21.42 | -22.26 | -23.68 |
| ΔE<sup>634</sup>AΔE<sup>689</sup>A | -11.59 | -16.29 | -23.29 | -24.75 | -25.91 | -24.26 | -26.33 | -24.55 | -26.59 | -26.63 |

Figure 2. Cartoon and stick representation of RON-MSPβ complex generated in PyMol (version 1.5.0.3).

The results of molecular dynamic simulation of RON indicated that all receptor (except for Glu<sup>190</sup> and Ser<sup>195</sup>) and ligand (except for Arg<sup>639</sup> and Glu<sup>644</sup>) mutants have low affinity compared to the wild type as deduced from the negative ΔΔG values shown in Figure 2 and Table 3. One of the crucial residues at the interface of RON-MSPβ complex is RON Gln<sup>191</sup>; its side chain NH group makes two ionic interactions with carboxylate group of MSP Glu<sup>644</sup> and carbonyl group of Arg<sup>639</sup>. In addition, Arg<sup>639</sup> of MSP is involved in another interaction with...
RON Glu$^{190}$ which will be discussed later. The MM-GBSA based binding energy calculations on the wild type and Q$^{193}$A mutant showed that this amino acid is important in the binding (also confirmed by Chao and coworkers) while the calculations did not support the importance of its partners MSP, i.e. Arg$^{639}$ and Glu$^{644}$. To shed more light on this issue, an in silico R$^{639}$/A/E$^{644}$/A double mutation was introduced on MSP and then the binding energy calculated. Surprisingly, results showed that the double mutation caused unfavorable effect on binding energy for RON-MSBP complex formation highlighting the importance of simultaneous interaction established between both Arg$^{639}$ and Glu$^{644}$ with Gin$^{193}$. The RON Arg$^{220}$ is another key residue involved in charge-charge interaction with Glu$^{558}$ of MSP. The $\Delta$AG values calculated for R$^{220}$/A mutant during 1 to 10 ns molecular dynamic simulation range from ~ -25 to -30 Kcal/mol, which are the highest negative values obtained for all RON mutants. This observation implies the great importance of this residue as a hot spot in the interaction between RON and MSBP. Interestingly, the $\Delta$AG values for E$^{644}$/A mutant has also high negative value (Table 2 and 3). This is in agreement with experimental observation reported previously.

According to the study of Chao et al, MSP Arg$^{521}$ simultaneously interacts with three residues of RON namely Glu$^{287}$, Pro$^{288}$ and Glu$^{289}$. Additionally, RON Glu$^{287}$ forms a hydrogen bond interaction with the hydroxyl group of MSP Ser$^{565}$ whereas Glu$^{289}$ of RON establishes an ionic interaction with MSP His$^{528}$ as well as interaction with the backbone NH group of MSP Cys$^{527}$. Moreover, MSP His$^{528}$ located in proximity of RON Glu$^{289}$ is engaged in aromatic interaction with His$^{424}$. The results of computational alanine scanning reported here revealed that Glu$^{287}$, Pro$^{288}$, Glu$^{289}$, and His$^{424}$ of RON located at the interface of RON-MSBP complex are crucial residues for its binding to MSBP. According to $\Delta$AG values, Pro$^{288}$ Glu$^{287}$, and Glu$^{289}$ of RON are the next most important amino acids after Arg$^{220}$ (see Table 3). It seems that the importance of these residues is related to their interactions with more than one residues on MSP (except for Pro$^{288}$). In the case of Pro$^{288}$ it interacts only with MSP Arg$^{521}$ which in turn is highly important due to its participation in multiple interactions with RON Glu$^{287}$, and Glu$^{289}$. Based on binding $\Delta$AG values, His$^{424}$ seems to be less important in comparison to other RON residues at the interface. However, this residue can also be considered as a hot spot on RON (Table 2 and 3). Additionally, MSP His$^{528}$ and Ser$^{655}$ are suggested to be important residues for RON binding despite the fact that their $\Delta$AG values are not as significant as those mentioned above (Table 2 and 3). The $\Delta$AG binding calculated for MSP Cys$^{527}$ using different molecular dynamic simulation intervals includes both positive (1 to 8 ns) and negative (9 to 10 ns) values, making it difficult to extrapolate its importance in the binding. It seems that interaction via Cys$^{527}$ switches on and off during molecular dynamic simulation. However, the $\Delta$AG values toward end of simulation reach -4 kcal/mol which indicates positive contribution of this residue in RON-MSP binding. E$^{644}$/A and S$^{105}$/A mutations can be considered exception as the binding affinities toward ligand were improved after mutation to alanine. The crystallography studies on RON-MSBP complex showed that RON Glu$^{190}$ is involved in two salt bridges via its carboxylate group with guanidinium group of Arg$^{639}$ and Arg$^{683}$. However, our results do not attribute positive contribution for this residue as inferred from its positive $\Delta$AG values in MM-GBSA calculations upon mutation to alanine (See Table 3). Such disagreement between the reported experimental results and our in silico estimates may be due to the fact that Glu$^{190}$ interacts with two different MSP residues (i.e. Arg$^{639}$ and Arg$^{683}$), which are already interacting with other RON residues. Therefore, lack of their interactions with Glu$^{644}$ may not contribute favorably in the overall binding energy. RON Ser$^{565}$ is shown to be involved in a charge-charge interaction with MSP Arg$^{683}$, however, our results did not identify this amino acid as an important residue in RON-MSBP complex (Table 3). Again the disagreement between our in silico estimates and the crystallographic data may be due to the formation of another interaction by Arg$^{583}$ with RON via Glu$^{190}$ which renders the interaction between Ser$^{565}$ and Arg$^{683}$ less important. The only previous experimental site directed mutagenesis studies on the residues at the interface of RON-MSP complex was carried out for Arg$^{683}$ and results obtained are in agreement with the ones discussed below. This amino acid is an important residue in the interaction of RON-MSBP complex based on in silico calculation despite the results obtained for its partners on MSP (i.e Glu$^{190}$ and Ser$^{565}$). In order to gain more information regarding these residues an in silico double mutation (E$^{190}$/A/S$^{105}$/A) study was performed. This double mutation lead to a positive $\Delta$AG value indicative of their harmonic interplay in the interaction with Arg$^{683}$.

Conclusion

In modern drug design and discovery process, computational approaches have streamlined a promising perspective by supplying useful and supportive information. In this context, identification of hot spots in biomolecules’ interactions through the estimating the binding affinity of molecules towards targets of interest can provide valuable information where protein-protein interactions are important initiators in cancer pathogenesis. Virtual alanine scanning mutagenesis is one of the tools that are commonly employed for this purpose. Therefore, in the current investigation, amino acids reported to be at the interface of RON-MSBP complex were evaluated using the MM-GBSA method and some of them were assigned as hot spots in the interaction. Taken together, in silico alanine scanning mutagenesis results revealed that Gin$^{193}$, Arg$^{220}$, Glu$^{287}$, Pro$^{288}$, Glu$^{289}$ and His$^{424}$ residues from RON and Arg$^{521}$, His$^{528}$, Ser$^{655}$, Glu$^{644}$, and Arg$^{683}$ form MSBP may play important roles in protein-protein interaction between
RON and MSP. Identification of these RON hot spots is important in designing anti-RON drugs when the aim is disruption of RON-MSP interaction. In the same way, the acquired information regarding the critical amino acids of MSPβ can be used in the process of rational drug design for developing MSP antagonizing agents, the development of novel MSP mimicking peptides where inhibition of RON activation is required, and the design of experimental site directed mutagenesis studies.

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Ethical Issues
Not applicable.

Conflict of Interest
The authors declare no conflict of interests.

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