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Homology modeling and structural analysis of human N-acetyl-alpha-neuraminidase 3 (NEU3)

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ABSTRACT

Homology modeling and structural analysis of human N-acetyl-alpha-neuraminidase 3 (NEU3) were performed with a software package the Molecular Operating Environment. A human NEU2 (PDB code: 1SNT) was selected as a template for the 3D structure modeling of NEU3. The modeled NEU3 showed significant 2D and 3D similarities to NEU2. The contact energy profiles of the NEU3 model were in good agreement with those of the NEU2 structure. Ramachandran plots revealed that only 2.5% of the amino acid residues were in the disfavored region for NEU3. These results indicate that the NEU3 model was successfully modeled and analyzed. To the best of my knowledge, this is the first report of NEU3 model with detailed analyses, and the data verify that the model can be utilized for application to target NEU3 for the development of anticancer drugs.

Keywords: N-acetyl-alpha-neuraminidase 3 (NEU3), cancer, *in silico*

INTRODUCTION

Gangliosides consist in the outer leaflet of the plasma membrane and contain sialic acids [1,2]. Among them, GM3 ganglioside has been suggested to interact with microtubules and intermediate filaments and also bind to epidermal growth factor receptor (EGFR), regulating its activity [1-3]. Thus, gangliosides have been considered to play important roles in different biological events, such as cell-to-cell interactions and signal transduction. The location and pattern of membrane gangliosides is controlled by the balance between their synthesis and degradation. Sialidases are glycohydrolytic enzymes, involved in the degradation process of glycolipids and glycoproteins. They are widely distributed among species and degrade gangliosides by removing sialic acids [4]. Sialidases are also considered to be associated with various biological events such as antigenic expression, differentiation, infection, intercellular interactions and signal transduction [5]. Based on their cellular localizations, sialidases are classified into three categories: cytosolic, lysosomal and membrane-associated [6].

N-acetyl-alpha-neuraminidase 3 (NEU3, EC = 3.2.1.18) is a peripheral membrane protein and was identified as the membrane-associated member of the sialidase family [7]. It is often referred to as the “ganglioside sialidase” due to its high enzymatic specificity toward GM3 ganglioside [8-12]. NEU3 has been reported to play crucial roles in many cellular processes, including cell proliferation and differentiation [9]. Overexpression of NEU3 induced resistance to cell cycle withdrawing and accelerated the differentiation process in myoblasts [13]. NEU3 has also been reported to show positive participation in the formation, regulation and regeneration of neurons [14-16]. In pathological conditions, NEU3 has been attributed to activation of pro-survival pathways with concomitant suppression of apoptosis. Aberrant overexpression of NEU3 was observed in several neoplastic conditions, such as colon, ovarian, renal and prostate cancer, and is considered to be one of the key triggers of tumor growth and invasiveness [9,17]. Although over expression of NEU3 has been correlated to different tumors [8,9,17], little is known about the precise distribution of the enzyme in relation to membrane subcompartments, and its ability to modify and rearrange the ganglioside composition of these membrane compartments, thus critically regulating biological events. However, there has been a report

that suggested the equal distribution of NEU3 between the membrane subcompartments and degradation of NEU3 *via* the proteasomal pathway [18]. NEU3 also specifically modified the ganglioside composition of the membrane areas where it resides, and the enzyme trigged phosphorylation of AKT, even in the absence of exogenously administered EGF [18]. The report concluded that NEU3 was the enzyme responsible for the modifications of the ganglioside composition in the membrane and could be considered as a modulator of AKT phosphorylation, further supporting its role in tumorigenesis [18].

Finding new ways of triggering and enhancing the physiological cell responses to the modification of ganglioside composition may represent a new therapeutic approach for several cancers, and the structural analysis of human sialidases may not only give further insights into the molecular mechanisms of their substrate recognition but also provide important clues to understanding the catalytic mechanism of mammalian sialidases. Although a few NEU models have been publicized [19], no comprehensive structural analysis of NEU3 model has been reported to the best of my knowledge. Molecular modeling has found widespread utility in the field of drug development [20-22], and in the present study the homology modeling and structural analysis of human NEU3 by a highly sophisticated software package, the Molecular Operating Environment (MOE) 2013.08 (Chemical Computing Group Inc., Montreal, Canada) will be reported.

MATERIALS AND METHODS

Homology modeling of NEU3

The NEU3 (NCBI reference sequence: NM_006656) [23] and the crystal structure coordinates of NEU2 (PDB code: 1SNT) [19] were loaded into the MOE. The primary structures of NEU2 and NEU3 were aligned, carefully checked to avoid deletions or insertions in conserved regions and corrected wherever necessary. A series of the NEU3 model were independently constructed with the MOE using a Boltzmann-weighted randomized procedure [24] combined with specialized logic for the handling of sequence insertions and deletions [25]. There were no differences in the numbers and organizations of the secondary structural elements and no significant main chain deviations among the 10 models generated for NEU3. The model with the best packing quality function was selected in the study for full energy minimization and further inspection.

Assessment of the modeled structure

The overall geometric and stereochemical qualities of the final modeled structure of NEU3 were examined using Ramachandran plots generated within the MOE [26, 27]. The qualities of the protein folds of the NEU3 homology model were evaluated with the MOE by calculating the effective atomic contact energies, comprising the desolvation free energies required to transfer atoms from water to the interior of the protein [28]. Briefly, the contact desolvation energies were calculated for 18 different atom types of the 20 common amino acids that were resolved based on the clustering pattern of their properties. The contact potentials for each atom type were measured within a contact range of 6 Å by explicitly accounting for neighboring interactions

RESULTS AND DISCUSSION

Homology modeling of NEU3

The 1D sequence of NEU3 is shown in Fig. 1. The sequence revealed that the residues (Arg25, Asp50, Arg245, Arg340 and Tyr370) involved in the catalysis [19] were conserved in NEU3. The catalytic domain is often found in the crevice at the center of β-propeller [19], and this was also the case for NEU3. NEU2 (PDB code: 1SNT) was selected as a template (Fig. 2A) for the present 3D structure modeling because of its good crystal structure resolution (1.75 Å). For the construction of the NEU3 model, 10 independent models of the target proteins were built using a Boltzmann-weighted randomized modeling procedure in the MOE that is adapted from reports by Levitt [24] and Fechteler *et al*. [25]. The intermediate models were evaluated by a residue packing quality function, which is sensitive to the degrees to which non-polar side-chain groups are buried and hydrogen bonding opportunities are satisfied. The NEU3 model with the best packing quality function and full energy minimization is shown in Fig. 2B. It exhibited a similar 3D structure to the NEU2 model and was selected for further analyses.

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MEEVTTCSFNSPLFRQEDDRGITYRIPALLYIPPTHTFLAFAEKRSTRRDEDALHLVLRR  60
GLRIGQLVQWGPLKPLMEATLPGHRTMNPCPVWEQKSGCVFLFFICVRGHVTERQQIVSG 120
RNAARLCFIYSQDAGCSWSEVRDLTEEVIGSELKHWATFAVGPGHGIQLQSGRLVIPAYT 180
YYIPSWFFCFQLPCKTRPHSLMIYSDDLGVTWHHGRLIRPMVTVECEVAEVTGRAGHPVL 240
YCSARTPNRCRAEALSTDHGEGFQRLALSRQLCEPPHGCQGSVVSFRPLEIPHRCQDSSS 300
KDAPTIQQSSPGSSLRLEEEAGTPSESWLLYSHPTSRKQRVDLGIYLNQTPLEAACWSRP 360
WILHCGPCGYSDLAALEEEGLFGCLFECGTKQECEQIAFRLFTHREILSHLQGDCTSPGR 420
NPSQFKSN
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Figure 1. The 1D sequence of NEU3. The sequence reveals that the residues (Arg25, Asp50, Arg245, Arg340 and Tyr370) involved in the catalysis are conserved in NEU3. The catalytic domain is often found in the crevice at the center of β-propeller, and this is also the case for NEU3. The conserved residues are enclosed in rectangles.

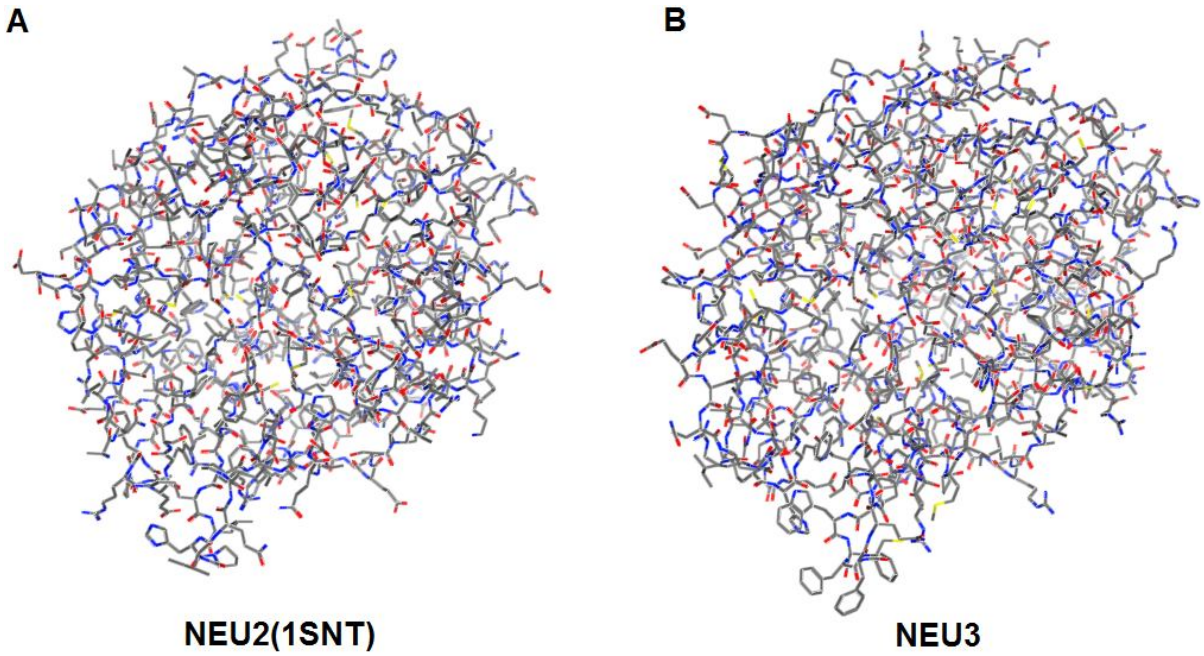

Figure 2. 3D structures of NEU2 (PDB code: 1SNT) and NEU3. (A) NEU2 (PDB code: 1SNT) is used as a template. The crystal structure resolution is 1.75 Å. (B) The constructed NEU3 model. The models exhibit similar 3D structures. *Blue*: nitrogen; *gray*: carbon; *red*: oxygen; *yellow*: sulfur.

Analysis of the contact energies for the NEU3 model

As reported by Zhang *et al*. [28], the effective atomic contact energies were calculated using the MOE for heavy atoms of standard amino acids within a contact range of 6 Å, assigning energy terms in kcal/mol for each contact pair. These energies were summed for each residue, and in general, a large negative value indicated that the residue was predominantly in contact with hydrophobic atoms and therefore expected to be in a buried protein environment. Conversely, residues with positive energy terms indicated contacts with predominantly hydrophilic atoms, and were expected to be in more solvent-exposed regions of the proteins. The contact energy profiles of the homology-modeled NEU3 (Fig. 3B) was compared with that of the X-ray structure of NEU2 (Fig. 3A), and the trends of the variation in the contact energy in most parts of the NEU3 model were in good agreement with that of the X-ray structure of NEU2.

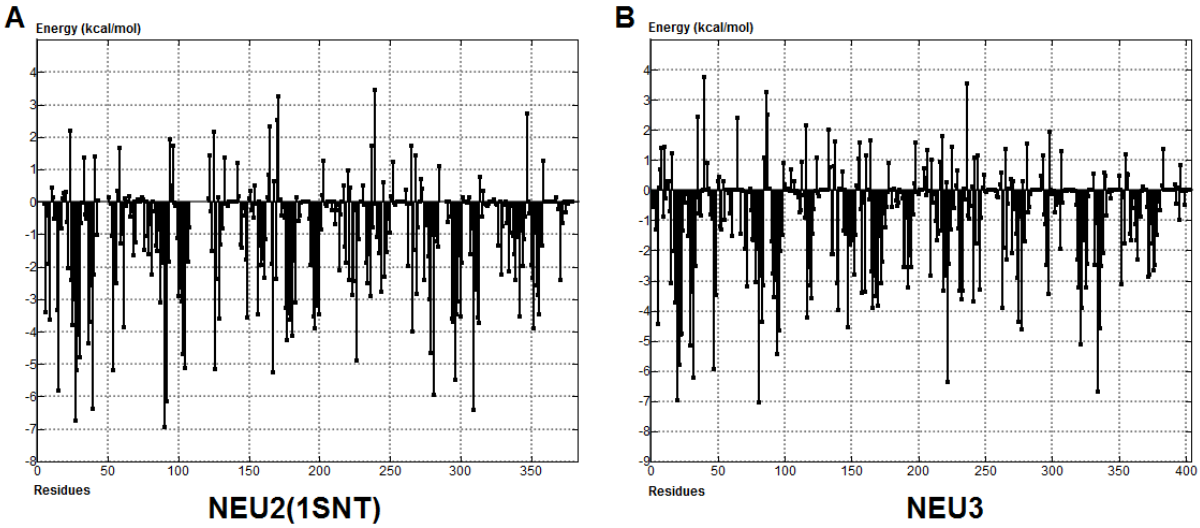

Figure 3. Contact energy profiles of NEU2 (PDB code: 1SNT) and NEU3. (A) NEU2 (PDB code: 1SNT). (B) The constructed NEU3 model. The positions of the amino acid residues are shown on the *x*-axis, while the contact energies are shown on the *y*-axis. The trends in the variation of the contact energy in most parts of the NEU3 model are in good agreement with that of the X-ray structure of NEU2.

Evaluation of the secondary structure of the NEU3 model

As shown in Fig. 4A, the generated model extended from residues Asn10 to Ala412 for NEU3. NEU3 contains a YRIP box (Tyr-Arg-Ile-Pro: Tyr24-Pro27) at the N-terminal region and three Asp boxes (Ser-X-Asp-X-Gly-X-X-Trp/Phe: Ser131-Trp138, Ser205-Trp212, Ser256-Phe263) in the amino acid sequence. These motifs are commonly found in sialidases of microorganisms and vertebrates. The YRIP motif is a

part of the active site and mutation of this motif leads to decreased enzymatic activity, and the Asp box is considered to play roles in the proper 3D structure formation of NEU3 [19,29,30]. The ligand-binding site (LBS) was characterized as the acidic crevice site at the center of the β-propeller that forms an activating core for the enzymatic catalysis, and the basic residues at the mouth of the crevice coordinate substrates (Fig. 4B) [29]. On the opposite side of the β-propeller, an acidic deep cleft extends very close to the back of the catalytic site. This cleft is also found in sialidases from other organisms [30]. These results are in agreement with the NEU2 model [19].

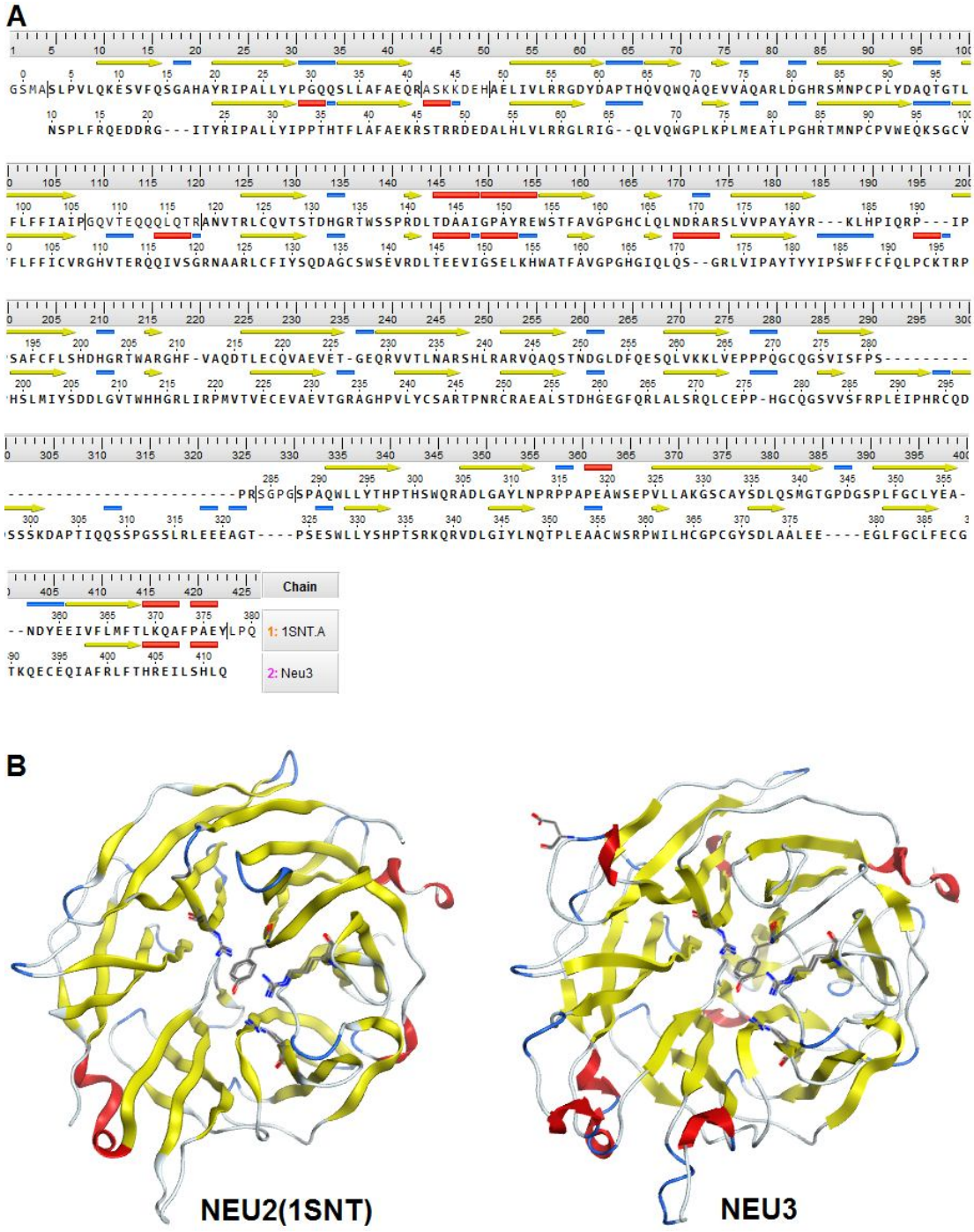

Figure 4. Evaluation of the secondary structures of the NEU3 model. (A) Homology-aligned sequences of NEU2 (PDB code: 1SNT) and NEU3. *Red line*: α-helix; *blue line*: turn; *yellow line*: β-sheet. (B) The LBS is characterized as the acidic crevice site at the center of the β-propeller that forms an activating core for the enzymatic catalysis, and the basic residues at the mouth of the crevice coordinate substrates. On the opposite side of the β-propeller, an acidic deep cleft extends very close to the back of the catalytic site. *Left panel*: NEU2 (PDB code: 1SNT); *right panel*: NEU3. *Red*: α-helix; *blue*: turn; *yellow*: β-sheet.

Evaluation of the stereochemical qualities of the NEU3 model

The phi and psi backbone dihedral angles for NEU3 were scored using 2D probability distributions calculated on a high-resolution collection of X-ray structures containing approximately 100,000 residues from 500 protein structures [31]. Each probability distribution was estimated with 2-degree spacing for

each of the phi and psi backbone dihedral angles with separate histograms for pre-proline, proline, glycine and general amino acids. The stereochemical qualities of the NEU3 model were assessed by Ramachandran plots (Fig. 5). For NEU3, 88% of the residues were in the favored region, 9.5% were in the allowed region and only 2.5% were in the disfavored region. The residues in the disfavored regions were located far away from the residues in the LBS. These results indicate that the phi and psi backbone dihedral angles in the NEU3 model are reasonably accurate.

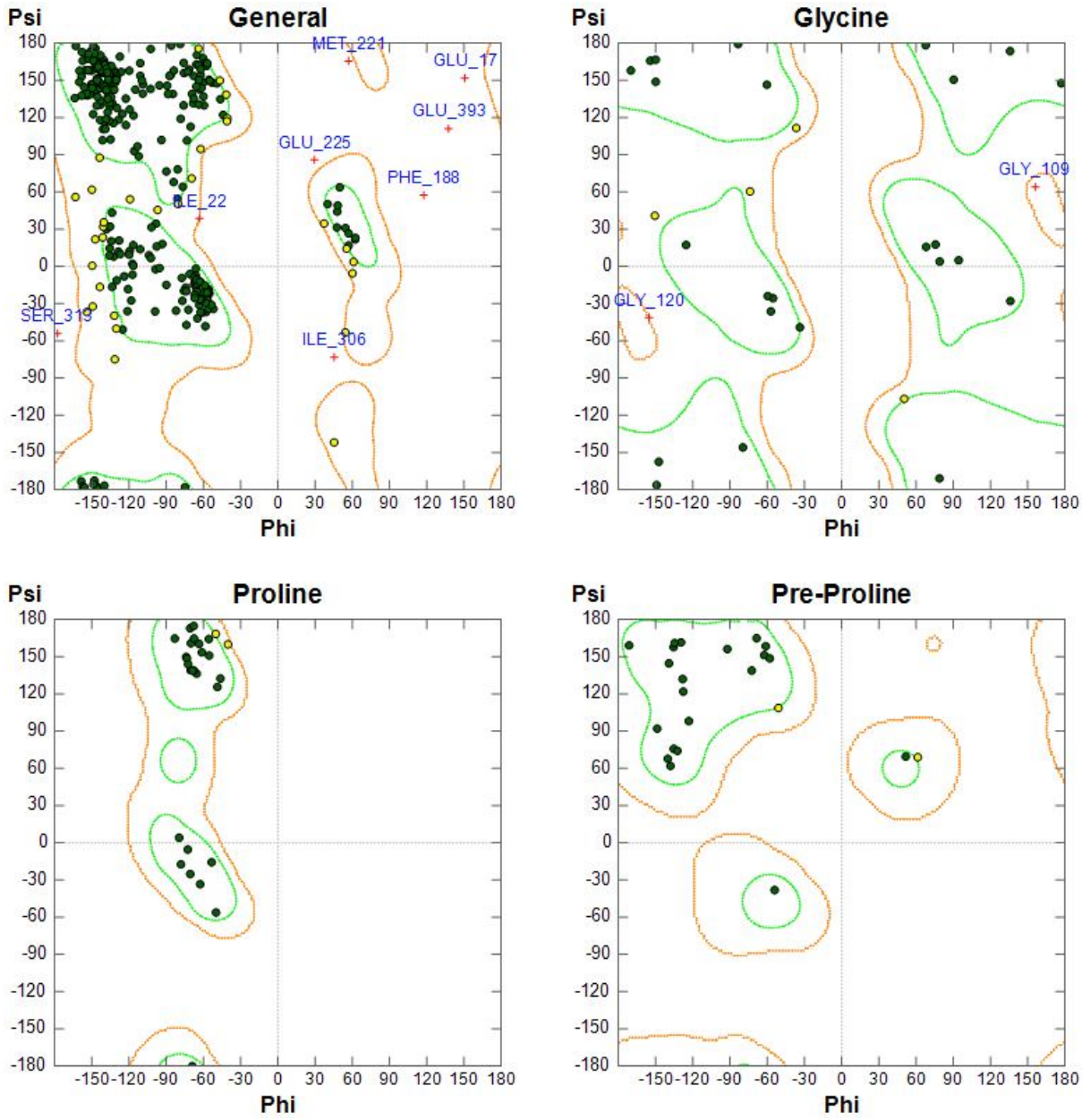

Figure 5. Ramachandran plots for the NEU3 model. For NEU3, 88% of the residues are in the favored region, 9.5% are in the allowed region and only 2.5% are in the disfavored region. The residues in the disfavored regions are located far away from the residues in the LBS. *Green*: favored region; *light-brown*: allowed region.

CONCLUSION

The 3D model of NEU3 was designed using the X-ray crystal structure of NEU2 (PDB code: 1SNT) as a template. The model was successfully evaluated and analyzed in terms of their folding and stereochemical properties. Consequently, it is proposed that the NEU3 model developed in the present study will be suitable for further *in silico* structure-based *de novo* drug designing. Such computer-based methodologies are now becoming an integral part of the drug discovery process, and may eventually lead to the development of potential inhibitors of NEU3 in the future. To the best of my knowledge, this is the first report of an NEU3 model by an accurate and sophisticated modeling system, and the present data verify that the model can be utilized for application to target NEU3 for the development of anticancer drugs.

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ABBREVIATIONS

EGFR, epidermal growth factor receptor; LBS, ligand-binding site; MOE, Molecular Operating Environment; NEU3, N-acetyl-alpha-neuraminidase 3.

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