Molecular profiling of Neprilysin expression and its interactions with SARS-CoV-2 spike proteins to develop evidence base pharmacological approaches for therapeutic intervention

Arun Kumar (arun.kumar@ucd.ie)
University College Dublin https://orcid.org/0000-0001-8422-0219

Research Article

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Abstract

Neprilysin due to its peptidase activity is involved in several physiological and pathological processes. Recently our group has reported the association of neprilysin with angiotensin-converting enzyme 2 (ACE2) network proteins which facilitate the entry of SARS-COV2 virus. The potential role of neprilysin beyond its peptidase activity is not known. Using the established sequence analysis and molecular docking tools, this study evaluated the molecular profile of neprilysin interaction with SARS-COV2 virus proteins. Human neprilysin protein showed a significant sequence similarity with SARS-COV2 spike protein, which was further confirmed by observation of considerable interaction in the molecular docking. Human neprilysin protein was also found to additionally interact with SARS-COV2 proteins facilitating virus replication. The potential of neprilysin inhibitors (Sacubitril and Sacubitrilat) to interfere with neprilysin and SARS-COV2 proteins interactions was assessed. The neprilysin inhibitors showed binding efficacy within therapeutically feasible concentration range (1 to 150 uM). This study while reporting a novel role of neprilysin as potential receptor for SARS-COV2 virus, highlights the merit in assessing clinical efficacy of neprilysin inhibitors for the management of SARS-COV2 infection.

Introduction

Neprilysin is a widely expressed peptidase located on the cell surface and has very diverse substrate specificity (Esser and Zraika, 2019; Karoor et al., 2013; Nalivaeva et al., 2020). Due to its diverse substrate specificity it is involved in regulating variety of physiological (electrolyte balance, blood pressure regulation, analgesia) and pathological (T cell lymphoma, Alzheimer's, diabetes, diarrhoea) process. The biochemical function of neprilysin is to hydrolyse peptides by cleaving them at their N-terminal side of hydrophobic amino acid residues (Acanfora et al., 2020; Esser and Zraika, 2019; Karoor et al., 2013; Mangiafico et al., 2013; Nalivaeva et al., 2020; Rice et al., 2004). The peptidase activity of the neprilysin is seen as its major physiological role which is achieved by modulating signalling of a variety of peptides in various organ systems (Mangiafico et al., 2013). Neprilysin is also reported to cleave several mitogenic peptides and hence can curtail development of tumours (Mangiafico et al., 2013; Nalivaeva et al., 2020). The major expression of neprilysin in mammals is reported in kidneys, lungs, GI tract and neuronal cells (Mangiafico et al., 2013; Nalivaeva et al., 2020; Pavo et al., 2019b; Shipp et al., 1991). Neprilysin is also located on the neutrophils and in lung, wherein respiratory irritants are reported to downregulate its expression and trigger inflammatory response (Borson et al., 1989; Dempsey et al., 2009). In contrast plasma neprilysin activity was reported to be increased in preclinical models associated with systemic inflammation, such as obesity and insulin resistance (Borson et al., 1989; Dempsey et al., 2009; Esser and Zraika, 2019; Karoor et al., 2013). Neprilysin is also expressed on common lymphoid progenitors, which give rise to cells (T, B and NK cells) having significant role in tissue inflammation and immune response (Pavo et al., 2019b; Rice et al., 2004; Shipp et al., 1991; Song et al., 2016). However it is not clear if changes in the activity of neprilysin is a cause or consequence of inflammation.

Neprilysin inhibitors are approved for clinical use in human patients with heart failure (Acanfora et al., 2020; El Tabaa and El Tabaa, 2020; Srivastava and Fonarow, 2019). In the past neprilysin inhibitors have

shown efficacy as therapeutics for several diseases associated with systemic inflammation (Acanfora et al., 2020; Esser and Zraika, 2019; Liczek et al., 2018). Although the role of neprilysin with airway inflammatory cascade is previously reported (Acanfora et al., 2020; El Tabaa and El Tabaa, 2020; Esser and Zraika, 2019; Liczek et al., 2018), its role in inflammatory cascade triggered by viruses in human patients is not clear. Our group recently reported the interaction of SARS-COV2 virus with several angiotensin-converting enzyme 2 (ACE2) network proteins (DPP4 and Meprin A alpha) with superior efficacy and neprilysin was observed as one of the ACE2 network proteins in this study (Goothy and Kumar, 2020). A low lymphocyte count among patients with SARS-COV2 infection is reported to be significantly associated with fatal outcome. Considering the expression of neprilysin on lymphocytes and its role in regulation of peptides interacting with ACE2, this study tested the hypothesis that SARS-COV2 virus can directly interact with neprilysin. Hence molecular interactions of neprilysin and its inhibitors with SARS-COV-2 proteins were assessed to establish their potential therapeutic merit.

Material And Methods

Protein network and sequence analysis: The neprilysin protein network was analysed using the STRING database (https://string-db.org/cgi/info.pl) (Sharma et al., 2015). The STRING database was searched using the neprilysin as protein name and Homo sapiens as organism. The Basic Local Alignment Search Tool (BLAST) was used to identify the regions of similarity between sequences of the neprilysin network proteins identified in the string database. Subsequent to initial alignment with all network proteins, similarities were further assessed between neprilysin, DPP4 and ACE2. To know the potential interaction of neprilysin with SARS-CoV-2 spike proteins the similarity between their sequences was compared (Goothy and Kumar, 2020; Hruz et al., 2008; Sharma et al., 2015).

Neprilysin expression analysis: Expression of neprilysin in various human tissues was analysed by comparing the gene, RNA and protein levels reported in following databases.(Hruz et al., 2008; Uhlen et al., 2015) (https://genevisible.com/tissues/HS/Gene%20Symbol/MME, http://biogps.org/#goto=welcome, and https://www.proteinatlas.org/ENSG00000196549-MME). Tissue specific protein expression was accessed on 14th August 2020 (https://www.proteinatlas.org/ENSG00000196549-MME/tissue).

SARS-CoV-2 surface proteins: The reported SARS-CoV-2 target proteins were searched in the protein data bank (https://www.rcsb.org/) and uniport database (https://www.uniprot.org/peptidesearch/) as reported previously.(Goothy and Kumar, 2020; Kumar, 2020) The following SARS-CoV-2 proteins were identified for binding analysis:

PDB/Protein ID	Brief Description
6W6Y	ADP ribose phosphatase of NSP3
6LXT	Post fusion core of S2 subunit
6Y2E	SARS-CoV-2 main protease
7JWB	SARS CoV2 Spike ectodomain
6ZB5	SARS CoV2 Spike protein open confirmation
6ZB4	SARS CoV2 Spike protein close confirmation
7DDN	SARS-Cov2 S protein at open state
7DDD	SARS-Cov2 S protein at closed state
7AD1	SARS CoV2 Spike
7DK3	SARS-CoV-2 S trimer, S-open
7A93	SARS-CoV-2 Spike Glycoprotein with 2 RBDs Erect
7KDI	SARS CoV2 Spike furin cleaved

Protein 3D structure and molecular docking: The 3D structure of SARS-CoV-2 targets listed above were processed for molecular docking as described previously. (Bordoli et al., 2009; Goothy and Kumar, 2020; Kumar, 2020; Yang et al., 2012). The structures of neprilysin inhibitors (Sacubitril and Sacubitrilat) were accessed from PubChem database and were processed for molecular docking as described previously using the Chimera software and AutoDock Vina (version 1.5.4) (Kumar, 2020; Seeliger and de Groot, 2010; Yang et al., 2012).

Simulation of dose response curves: Dose-response curves were modelled based on nonlinear regression analysis as reported before (Kumar, 2020; Sagar and Kumar, 2020).

Interaction analysis using Ligplot: The protein-protein or protein–ligand interactions was evaluated using the LigPlot software. The PDB output files from molecular docking were inputted into the LigPlot and the intermolecular interactions and their features (hydrogen bonds, hydrophobic contacts, and atom accessibilities) were assessed. Hydrogen bonds are represented by dashed lines. The amino acid residues of the protein involved in the molecular interactions are represented by an arc with spokes emerging towards the ligand atoms in contact (Bharatham et al., 2008; Mishra and Dey, 2019).

Results

The network analysis of human neprilysin protein in the string database showed 10 proteins in its primary network (Figure 1A). The Basic Local Alignment Search Tool (BLAST) analysis of the sequence of the neprilysin network proteins, showed a very weak identify (0.082%) (Table 1, Figure 1B). We and

others have reported the role of DPP4 and ACE2 proteins as cell surface receptors for entry of coronaviruses.(Goothy and Kumar, 2020; Stower, 2020) As both DPP4 and ACE2 were observed in the neprilysin protein work, BLAST was use to assess the similarities between these three proteins. Compared to the general neprilysin network proteins, both DPP4 and ACE2 showed significantly better identity (2.205%, 112 similar positions) in their sequence (Table 1, Figure 1B). Which suggested neprilysin may be a potential receptor for coronaviruses. Hence the SARS-COV2 Post fusion core of S2 subunit (PDB ID: 6LXT), which is reported to be an important component of spike protein interacting with ACE2 receptor was compared to neprilysin. The sequence similarities (235 similar positions) between neprilysin and 6LXT was significantly better (9.058 Vs 2.205%) than that between neprilysin and DPP4/ACE2 (Table 1, Figure 1B).

Table 1. Basic Local Alignment Search Tool (BLAST) analysis for sequence similarities

	Identical positions	Similar positions	Identity(%)
Neprilysin network proteins	1	2	0.082
Neprilysin, ACE2, DPP4	25	112	2.205
Neprilysin and 6LXT	126	235	9.058

The expression profile of receptors is essential to effectively correlate the associated pathology and as well for designing rationale therapeutic measures. Hence the relevant database were analysed to profile the gene, transcript and protein expression pattern of neprilysin in various human tissues (Figure 2). Differences between the relative expression of neprilysin RNA, gene and protein were observed across various human tissues/organs, which although not surprising was nevertheless interesting (Figure 2). Highest expression of neprilysin RNA was observed in adipocytes, whole blood, olfactory bulb and lymphoma (Figure 2A). In contrast highest expression of neprilysin gene was observed in glomerulus, jejunum and nephrons (Figure 2B). The RNA and gene expression pattern of neprilysin in human tissue differed from its protein expression pattern, although gene and protein expression pattern was similar (Figure 2B and C). Highest expression of neprilysin protein was observed in small intestines, followed by that in duodenum, colon, kidneys, and granulocytes (Figure 2C). Hence considering this expression pattern of neprilysin, it is likely that gastrointestinal, renal and immune physiology are predominantly influenced by factors interfering with neprilysin function. The symptoms of nausea, diarrhoea, generalised inflammation observed with SARS-COV2 infection does correlate with the expression pattern of neprilysin in human tissues. Hence to assess if SARS-COV2 virus proteins can interact with neprilysin, molecular docking of the selected combination of protein (Table 2) was performed. SARS-COV2 virus proteins (6LXT, 6Y2E, 6W6Y, 6ZB5) were observed to significantly interact with neprilysin through formation of hydrogen bonds in sufficient numbers for the interaction to be biochemically feasible (Figure 3A, table 2). The interaction of neprilysin with 6LXT was observed at Glu646, His587, His583, Asp950 regions with 44 hydrogen bonds, suggesting the possibility of neprilysin serving as a receptor for SARS-COV2 spike protein (Figure 3B, table 2). Of considerable interest was the superior interaction (more

number of hydrogen bonds) of neprilysin with SARS-COV2 proteins (6Y2E, 6W6Y) involved in its replication (Table 2).

Table 2. Molecular docking and Ligplot analysis

Protein	Ligand	Hydrophilic interaction	H-Bonds
Neprilysin	6LXT	Glu646, His587, His583, Asp950	44
Neprilysin	6ZB5	Didn't evaluate	168
Neprilysin	6Y2E	Didn't evaluate	87
Neprilysin	6W6Y	Didn't evaluate	356
6LXT	Sacubitril	Asp950, Gln949, Gln1180	26
Neprilysin	Sacubitril	His587, His 583, Glu646, Ser517	7

Sacubitril and Sacubitrilat are neprilysin inhibitors, which are approved for clinical use in humans. Hence the binding affinity of these two drugs against SARS-COV2 proteins was assessed. The binding affinity (Figure 4A) and the IC_{50} (Table 3) of Sacubitril and Sacubitrilat against the SARS-COV2 proteins (6LXT, 6Y2E and 6W6Y) were observed to be within therapeutically feasible concentration (Figure 4A, table 3). Based on the IC_{50} values, simulated dose response curves for Sacubitril and Sacubitrilat were generated for optimal estimation of therapeutic concentration range (1 to 150 uM) (Figure 4B). Sacubitril showed superior efficacy than sacubitrilat in interacting with SARS-COV2 targets (Figure 4B). Sacubitril was observed to form 26 hydrogen bonds with 6LXT (selectively at Asp950, Gln949 and Gln1180) suggesting significant binding affinity (Figure 4C, Table 2) and its potential to block interaction of neprilysin with the SARS-COV2 spike protein.

Table 3: Molecular docking

IC50 (uM)	Neprilysin	6Y2E (Protease)	6LXT	6W6Y
Sacubitril	9.33 ± 0.56	34.03 ± 0.34	0.91 ± 0.01	9.22 ± 0.08
Sacubitrilat	157.69 ± 5.41	91.98 ± 3.71	45.19 ± 0.76	133.17 ± 7.47

Discussion

Several cell surface receptors are known to facilitate the entry of viruses, which can be target for therapeutic intervention (Goothy and Kumar, 2020; Stower, 2020). This study reports an unexpected observation from network protein analysis, which resulted in identification of neprilysin as a potential receptor for the key proteins of recently reported SARS-COV2 virus. The molecular interaction of neprilysin was observed with SARS-COV2 virus post fusion core of S2 subunit (6LXT), spike protein open

confirmation (6ZB5), ADP ribose phosphatase of NSP3 (6W6Y) and its main protease (6Y2E) suggesting neprilysin may facilitate both viral attachment, entry and its replication. This novel role of neprilysin as a receptor for viruses is not reported before.

Viruses which spread at a pandemic scale are unlikely to depend on a single receptor type for it attachment and entry into the host cell. Dependency on multiple receptor types has been shown for many strains of coronaviruses reported previously (Goothy and Kumar, 2020; Stower, 2020). Most of these strains of coronaviruses have caused infections in large scale, both in humans and animals despite the endemic nature of the infections (Renu et al., 2020). SARS-COV2 is the recently reported strain of coronaviruses which has spread to a pandemic scale,(Goothy and Kumar, 2020; Renu et al., 2020; Stower, 2020) and paraphs it does utilize multiple receptor types at least in non-experimental settings for attachment and entry into the host cells. We have recently reported the role of ACE2 network proteins in facilitating SARS-COV2 virus attachment and entry into the host cells, with some of the network proteins (DPP4, Meprin A and XPNPEP2) showing superior molecular interactions with SARS-COV2 virus spike proteins compared to ACE2 (Goothy and Kumar, 2020). Coincidental observation of neprilysin association with the ACE2 network protein and its sequence similarities with the SARS-COV2 spike protein observed in this study suggest the possibility of neprilysin being a receptor for coronaviruses similar to DPP4 and ACE2 (Goothy and Kumar, 2020; Renu et al., 2020). Additionally superior molecular interaction of neprilysin with SARS-COV2 proteins (6W6Y, 6Y2E) regulating its replication was also observed. Although neprilysin is predominantly expressed on cell membrane, a few studies have reported its subcellular localisation,(Gregoriou et al., 2020; Nalivaeva et al., 2020) suggesting the potential role of neprilysin beyond its peptidase activity against natriuretic peptides. Facilitating attachment, entry and replication of virus in the host cell may be one alternative role of neprilysin, which merits further investigation.

Loss of smell and taste is reported to an early symptom of SARS-COV2 infection. While the exact mechanisms responsible for loss of this physiology is not known (Kilroy and Kumar, 2020), it is interesting to note that higher expression of neprilysin is observed in olfactory bulb. In contrast expression of neprilysin was least in the tongue. The differential loss of smell and taste sensation in SARS-COV2 infection are perhaps mediated by different pathways, with a potential role of neprilysin in regulating the sense of smell. The predominant expression of neprilysin in GI tract, kidneys and granulocytes, does correlate with the symptoms of nausea, diarrhoea, thrombosis and systemic inflammation observed in patients with SARS-COV2 infection (Kilroy and Kumar, 2020).

Respiratory irritants and pathogens (parainfluenza virus and rat coronavirus) are reported to interact with neprilysin on neutrophils and in lung epithelium to trigger inflammatory responses (Borson et al., 1989; Dempsey et al., 2009; Karoor et al., 2013). These prior studies are in concurrence with this study reporting the potential of neprilysin as a receptor for SARS-COV2 virus. Besides these a soluble circulating form of neprilysin is also reported in several body fluids (Pavo et al., 2019a), which together with high expression of neprilysin in whole blood may substantiate the systemic nature of inflammation observed in SARS COV2 infection. Neprilysin expression was also highest in the adipose tissue, which paraphs support the

incidence of higher mortality rate in obese patients with SARS COV2 infection (Nalivaeva et al., 2020; Shipp et al., 1991; Song et al., 2016). With fibrinogen as its substrate, neprilysin can regulate fibrin formation by thrombin (Burrell et al., 2016). Hence factors inhibiting neprilysin can enhance fibrinogen levels and lead to intravascular coagulation. The molecular interactions observed in this study does indicate the potential of SARS COV2 spike proteins to interact and inhibit neprilysin activity, weather this is the potential mechanism of disseminated intravascular coagulation observed in SARS COV2 infections remains to be validated. Several studies have associated neprilysin activity with negative remodelling of pulmonary and vascular structures, including increased microvascular permeability (Dempsey et al., 2009; Rice et al., 2004; Shipp et al., 1991; Steiner, 2009; Wick et al., 2011). The correlation of these features with symptoms observed in SARS COV2 infections together with the molecular interactions between SARS COV2 proteins and neprilysin reported in this study does support the notion for neprilysin being a potential receptor for interaction with SARS COV2 virus. Further the higher catalytic activity of neprilysin then ACE2 (El Tabaa and El Tabaa, 2020; Srivastava and Fonarow, 2019) may be more favourable for the enveloped virus to enter host cells.

Neprilysin inhibitors (Sacubitril and Sacubitrilat) are currently approved for clinical use and have shown efficacy in the treatment of acute diarrhoea and heart failure (El Tabaa and El Tabaa, 2020; Srivastava and Fonarow, 2019). The binding efficacy of both sacubitril and sacubitrilat against SARS-COV2 proteins (6LXT, 6W6Y and 6Y2E) were within therapeutically feasible range, indicating their potential in not only preventing virus attachment and entry into host cell but also the potential to prevent virus replication. This ability of neprilysin inhibitors to target full cycle of virus entry and replication can lead to synergistic outcomes and improved efficacy. Further the synergistic efficacy of neprilysin inhibitors could be a consequence of targeting both neprilysin as well as SARS-COV2 proteins independently. This dual targeting of both host cell and virus proteins in addition to curtailing the pathogenesis of the virus can also be helpful to harness the collateral benefits from neprilysin inhibition. Recent studies have supported the benefits from neprilysin inhibition by reducing the pro-inflammatory cytokines and neutrophil count in patients with SASR-COV2 infections (Acanfora et al., 2020; El Tabaa and El Tabaa, 2020; Srivastava and Fonarow, 2019). Sacubitril in combination with valsartan was reported to increase NO bioavailability and reduce high sensitivity C-reactive protein, which can be additionally beneficial by improving microvascular function and reducing systemic inflammation.

In conclusion the findings from this study provides evidence for the potential novel role of neprilysin as a receptor for SASR-COV2 virus, which can be effectively targeted by currently approved neprilysin inhibitors.

Declarations

Conflict of interest: none

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References

Acanfora D, Ciccone MM, Scicchitano P, Acanfora C and Casucci G (2020) Neprilysin inhibitor-angiotensin II receptor blocker combination (sacubitril/valsartan): rationale for adoption in SARS-CoV-2 patients. *Eur Heart J Cardiovasc Pharmacother* 6:135-136.

Bharatham K, Bharatham N, Park KH and Lee KW (2008) Binding mode analyses and pharmacophore model development for sulfonamide chalcone derivatives, a new class of alpha-glucosidase inhibitors. *J Mol Graph Model* 26:1202-1212.

Bordoli L, Kiefer F, Arnold K, Benkert P, Battey J and Schwede T (2009) Protein structure homology modeling using SWISS-MODEL workspace. *Nat Protoc* 4:1-13.

Borson DB, Brokaw JJ, Sekizawa K, McDonald DM and Nadel JA (1989) Neutral endopeptidase and neurogenic inflammation in rats with respiratory infections. *J Appl Physiol (1985)* 66:2653-2658.

Burrell M, Henderson SJ, Ravnefjord A, Schweikart F, Fowler SB, Witt S, Hansson KM and Webster CI (2016) Neprilysin Inhibits Coagulation through Proteolytic Inactivation of Fibrinogen. *PLoS One* 11:e0158114.

Dempsey EC, Wick MJ, Karoor V, Barr EJ, Tallman DW, Wehling CA, Walchak SJ, Laudi S, Le M, Oka M, Majka S, Cool CD, Fagan KA, Klemm DJ, Hersh LB, Gerard NP, Gerard C and Miller YE (2009) Neprilysin null mice develop exaggerated pulmonary vascular remodeling in response to chronic hypoxia. *Am J Pathol* 174:782-796.

El Tabaa MM and El Tabaa MM (2020) New putative insights into neprilysin (NEP)-dependent pharmacotherapeutic role of roflumilast in treating COVID-19. *Eur J Pharmacol* 889:173615.

Esser N and Zraika S (2019) Neprilysin inhibition: a new therapeutic option for type 2 diabetes? *Diabetologia* 62:1113-1122.

Goothy SSK and Kumar AHS (2020) Network Proteins of Angiotensin-converting Enzyme 2 but Not Angiotensin-converting Enzyme 2 itself are Host Cell Receptors for SARS-Coronavirus-2 Attachment. *BEMS Reports* 6:1-5.

Gregoriou GC, Patel SD, Winters BL and Bagley EE (2020) Neprilysin Controls the Synaptic Activity of Neuropeptides in the Intercalated Cells of the Amygdala. *Mol Pharmacol* 98:454-461.

Hruz T, Laule O, Szabo G, Wessendorp F, Bleuler S, Oertle L, Widmayer P, Gruissem W and Zimmermann P (2008) Genevestigator v3: a reference expression database for the meta-analysis of transcriptomes. *Adv Bioinformatics* 2008:420747.

Karoor V, Oka M, Walchak SJ, Hersh LB, Miller YE and Dempsey EC (2013) Neprilysin regulates pulmonary artery smooth muscle cell phenotype through a platelet-derived growth factor receptor-dependent mechanism. *Hypertension* 61:921-930.

Kilroy D and Kumar AHS (2020) Anatomical perspective on the loss of smell and taste sensation in SARS-CoV-2 infection. *Anatomy* 14:145-149.

Kumar AHS (2020) Molecular Docking of Natural Compounds from Tulsi (Ocimum sanctum) and neem (Azadirachta indica) against SARS-CoV-2 Protein Targets. *BEMS Reports* 6:11-13.

Liczek M, Panek I, Damianski P, Jeczen O, Jazwiec J, Kuna P and Panek M (2018) Neprilysin inhibitors as a new approach in the treatment of right heart failure in the course of chronic obstructive pulmonary disease. Response to the letter of Siniorakis et al. *Adv Respir Med* 86:257-259.

Mangiafico S, Costello-Boerrigter LC, Andersen IA, Cataliotti A and Burnett JC, Jr. (2013) Neutral endopeptidase inhibition and the natriuretic peptide system: an evolving strategy in cardiovascular therapeutics. *Eur Heart J* 34:886-893c.

Mishra A and Dey S (2019) Molecular Docking Studies of a Cyclic Octapeptide-Cyclosaplin from Sandalwood. *Biomolecules* 9.

Nalivaeva NN, Zhuravin IA and Turner AJ (2020) Neprilysin expression and functions in development, ageing and disease. *Mech Ageing Dev* 192:111363.

Pavo N, Arfsten H, Cho A, Goliasch G, Bartko PE, Wurm R, Freitag C, Gisslinger H, Kornek G, Strunk G, Raderer M, Zielinski C and Hulsmann M (2019a) The circulating form of neprilysin is not a general biomarker for overall survival in treatment-naive cancer patients. *Sci Rep* 9:2554.

Pavo N, Gugerell A, Goliasch G, Bartko PE, Arfsten H, Novak JF, Gyongyosi M and Hulsmann M (2019b) Increased granulocyte membrane neprilysin (CD10) expression is associated with better prognosis in heart failure. *Eur J Heart Fail* 21:537-539.

Renu K, Prasanna PL and Valsala Gopalakrishnan A (2020) Coronaviruses pathogenesis, comorbidities and multi-organ damage - A review. *Life Sci* 255:117839.

Rice GI, Thomas DA, Grant PJ, Turner AJ and Hooper NM (2004) Evaluation of angiotensin-converting enzyme (ACE), its homologue ACE2 and neprilysin in angiotensin peptide metabolism. *Biochem J* 383:45-51.

Sagar VK and Kumar AHS (2020) Efficacy of Natural Compounds from Tinospora cordifolia against SARS-CoV-2 Protease, Surface Glycoprotein and RNA Polymerase. *BEMS Reports* 6:6-8.

Seeliger D and de Groot BL (2010) Ligand docking and binding site analysis with PyMOL and Autodock/Vina. *J Comput Aided Mol Des* 24:417-422.

Sharma A, Menche J, Huang CC, Ort T, Zhou X, Kitsak M, Sahni N, Thibault D, Voung L, Guo F, Ghiassian SD, Gulbahce N, Baribaud F, Tocker J, Dobrin R, Barnathan E, Liu H, Panettieri RA, Jr., Tantisira KG, Qiu W, Raby BA, Silverman EK, Vidal M, Weiss ST and Barabasi AL (2015) A disease module in the interactome explains disease heterogeneity, drug response and captures novel pathways and genes in asthma. *Hum Mol Genet* 24:3005-3020.

Shipp MA, Stefano GB, Switzer SN, Griffin JD and Reinherz EL (1991) CD10 (CALLA)/neutral endopeptidase 24.11 modulates inflammatory peptide-induced changes in neutrophil morphology, migration, and adhesion proteins and is itself regulated by neutrophil activation. *Blood* 78:1834-1841.

Song S, Zhang M, Yi Z, Zhang H, Shen T, Yu X, Zhang C, Zheng X, Yu L, Ma C, Liu Y and Zhu D (2016) The role of PDGF-B/TGF-beta1/neprilysin network in regulating endothelial-to-mesenchymal transition in pulmonary artery remodeling. *Cell Signal* 28:1489-1501.

Srivastava PK and Fonarow GC (2019) In-Hospital Initiation of Angiotensin Receptor-Neprilysin Inhibitors-The Time Is Now. *JAMA Cardiol* 4:195-196.

Steiner MK (2009) World Health Organization Class III COPD-associated pulmonary hypertension: are we there yet in understanding the pathobiology of the disease? *Chest* 136:658-659.

Stower H (2020) Virological assessment of SARS-CoV-2. *Nat Med* 26:465.

Uhlen M, Fagerberg L, Hallstrom BM, Lindskog C, Oksvold P, Mardinoglu A, Sivertsson A, Kampf C, Sjostedt E, Asplund A, Olsson I, Edlund K, Lundberg E, Navani S, Szigyarto CA, Odeberg J, Djureinovic D, Takanen JO, Hober S, Alm T, Edqvist PH, Berling H, Tegel H, Mulder J, Rockberg J, Nilsson P, Schwenk JM, Hamsten M, von Feilitzen K, Forsberg M, Persson L, Johansson F, Zwahlen M, von Heijne G, Nielsen J and Ponten F (2015) Proteomics. Tissue-based map of the human proteome. *Science* 347:1260419.

Wick MJ, Buesing EJ, Wehling CA, Loomis ZL, Cool CD, Zamora MR, Miller YE, Colgan SP, Hersh LB, Voelkel NF and Dempsey EC (2011) Decreased neprilysin and pulmonary vascular remodeling in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 183:330-340.

Yang Z, Lasker K, Schneidman-Duhovny D, Webb B, Huang CC, Pettersen EF, Goddard TD, Meng EC, Sali A and Ferrin TE (2012) UCSF Chimera, MODELLER, and IMP: an integrated modeling system. *J Struct Biol* 179:269-278.

Figures

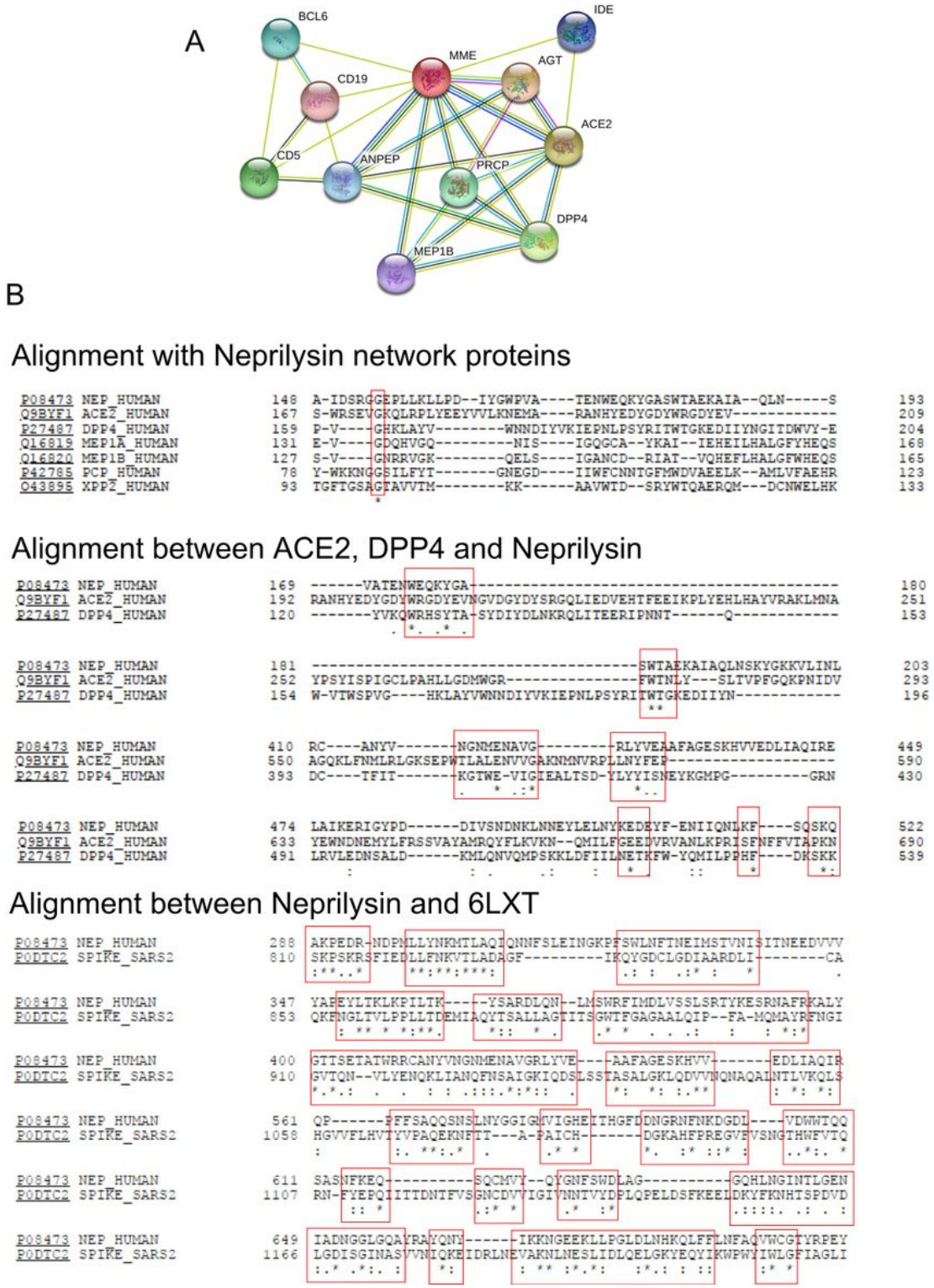

Figure 1

(A) Network protein analysis of human neprilysin protein (MME) in string database, showing 10 proteins in the primary network. The following proteins were identified: Insulin-degrading enzyme (IDE), Angiotensin-converting enzyme 2 (ACE2), Meprin A subunit beta (MEP1B), Lysosomal Pro-X carboxypeptidase (PRCP), Dipeptidyl peptidase 4 (DPP4), Angiotensinogen (AGT), Aminopeptidase N (ANPEP), T-cell surface glycoprotein (CD5), B-lymphocyte antigen (CD19), B-cell lymphoma 6 protein

(BCL6). (B) The similarities in the sequence (red box) of human neprilysin and its network proteins and SARS-COV2 Post fusion core of S2 subunit (spike protein) are shown.

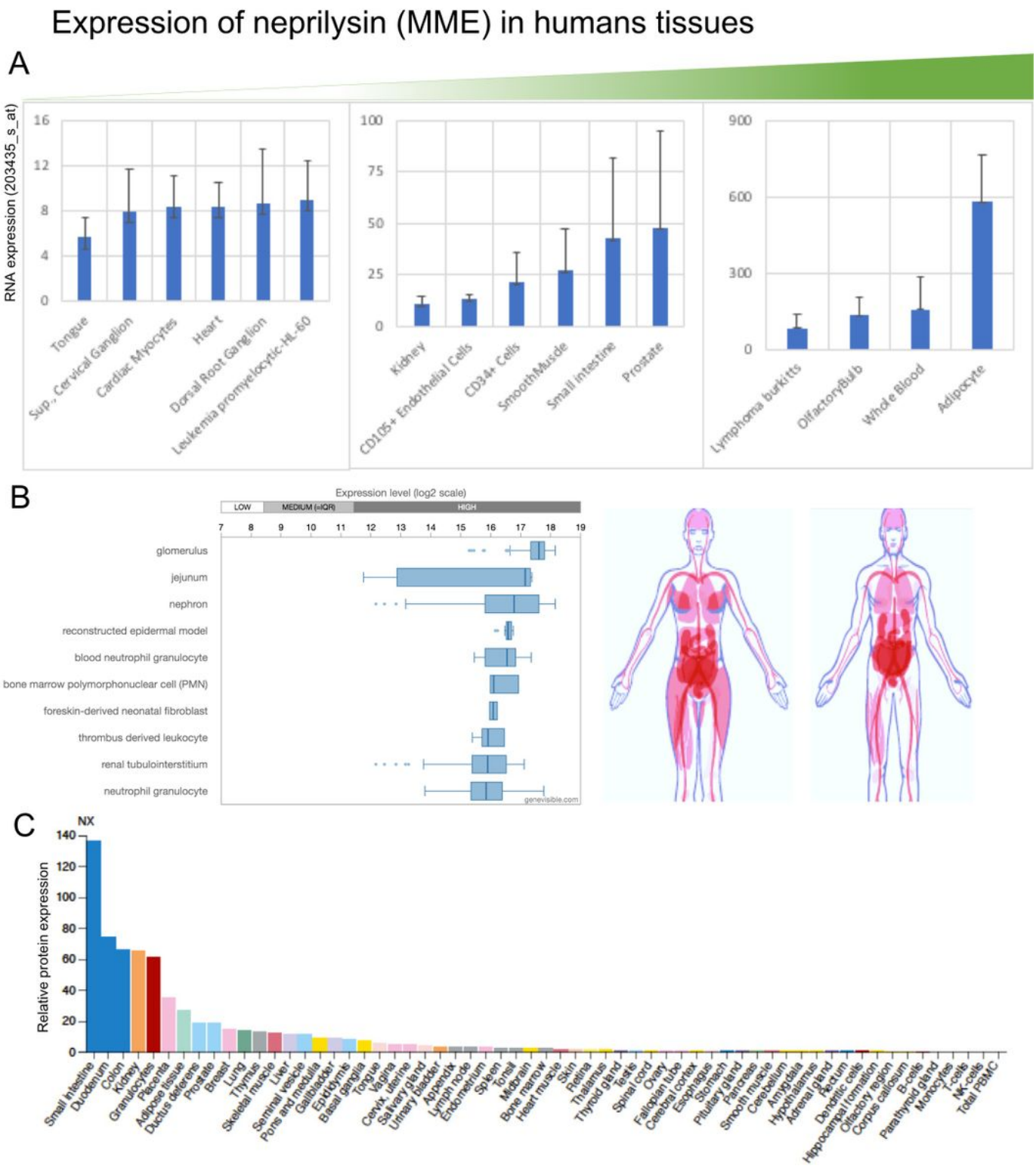

Figure 2

Expression profile of neprilysin RNA (A), gene (B) and protein (C) in various human tissues/organ. The green scale bar on the top indicates the degree of expression, with higher intensity of colour indicating

higher expression. The image corresponding to section B, indicates the various organs (in females and males) where neprilysin gene is expressed.

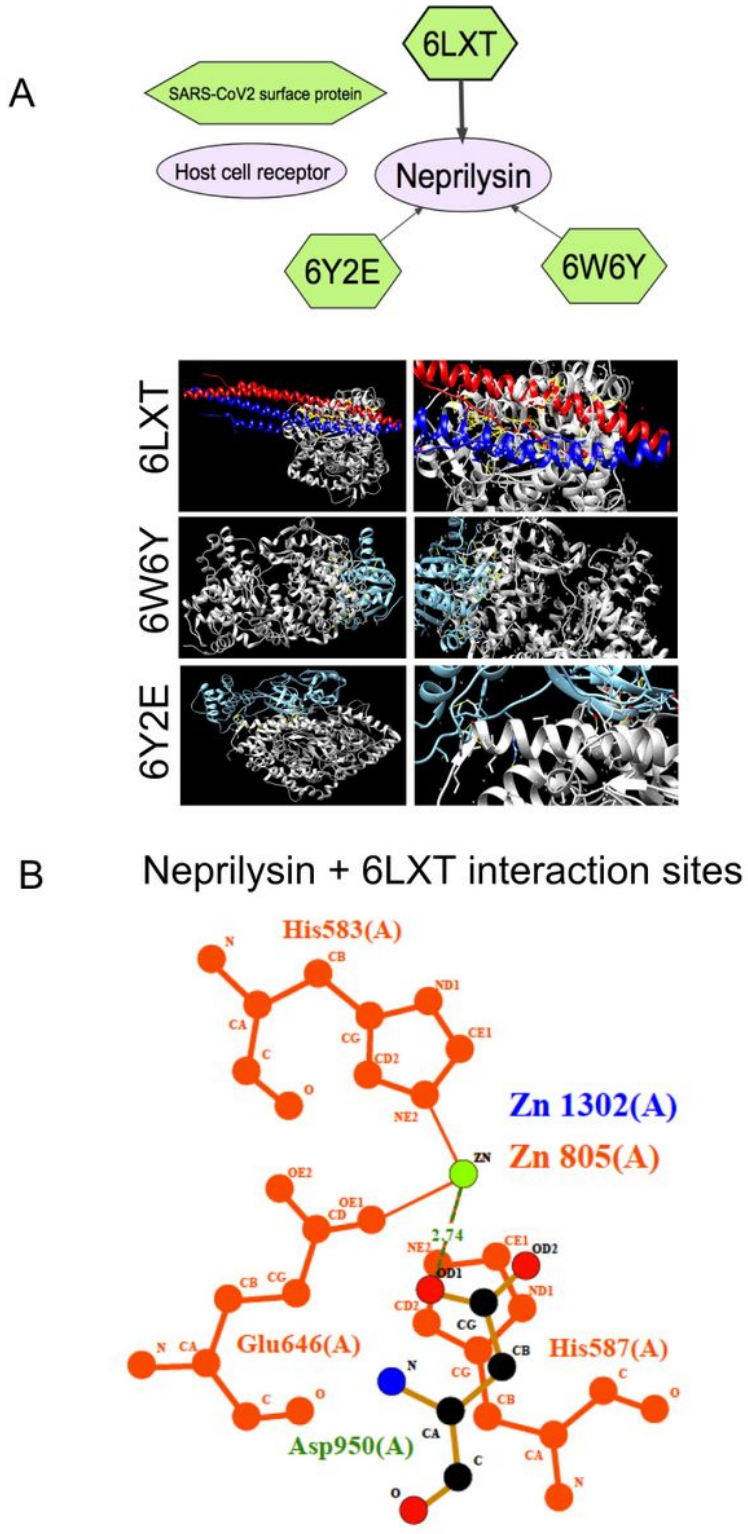

Figure 3

(A) Molecular docking of neprilysin with SARS-COV2 proteins (6LXT, 6W6Y, 6Y2E). Representative images of each of the interaction combinations with their respective magnified view (right) are shown. (B) Ligplot assessment of the molecular interacting sites between neprilysin and 6LXT is shown.

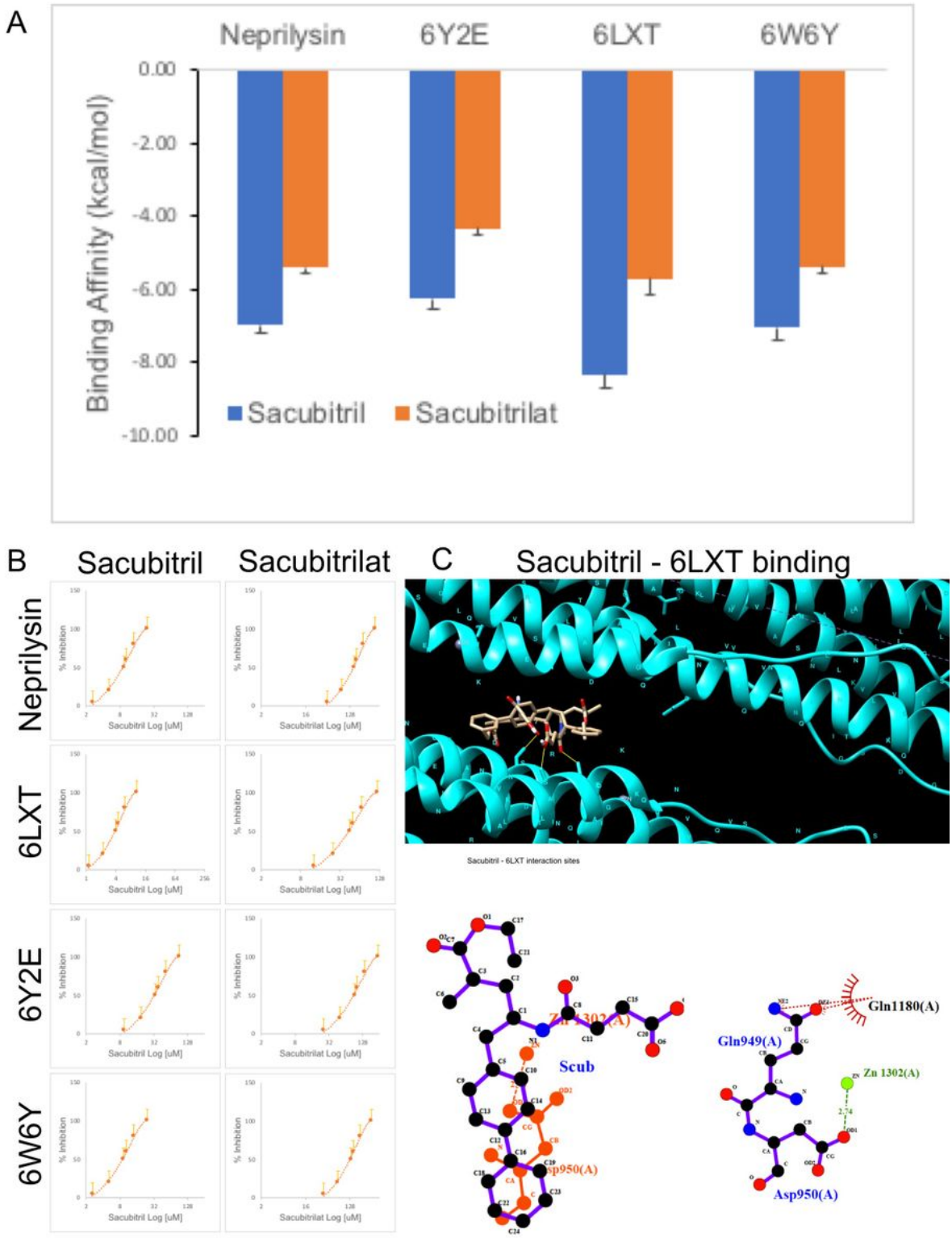

Figure 4

Molecular docking analysis of neprilysin inhibitors (Sacubitril and Sacubitrilat) with neprilysin and SARS-COV2 proteins (6LXT, 6W6Y, 6Y2E). (A) Binding affinity of neprilysin inhibitors with their targets is represented as bar graph. The data is presented as mean±SD of top nine interacting sites. (B) The simulated dose response curves of the neprilysin inhibitors with their targets is shown. The data is

presented as mean±SD of three sigma deviations from the mean IC50 value. (C) Ligplot assessment of the molecular interacting sites between Sacubitril and 6LXT is shown.