

Understanding the Exacerbating Role of the Metalloproteinase Meprin during AKI, an *In Silico* Approach

Deepyan Chatterjee *

Centre for Systems Biology & Bioinformatics, UIEAST, Panjab University, Chandigarh, India

* Corresponding Author: 2deepayan@gmail.com

Keywords: AKI, Metalloproteinase, Meprin, Actinonin, Bioinformatics, *In silico* studies, Protein tertiary structure prediction, Interaction networks, Molecular docking.

ABSTRACT: Acute kidney injury (AKI) is a syndrome characterised by the rapid loss of the kidney's excretory function and is typically diagnosed by the accumulation of end products of nitrogen metabolism (urea and creatinine) or decreased urine output, or both. It is the clinical manifestation of several disorders that affect the kidney acutely. No specific therapies have yet emerged that can attenuate AKI or expedite recovery; thus, the only treatment is supportive therapies and intensive care. The present study was aimed to provide an insight into the importance of a metalloproteinase involved in the pathological conditions of AKI and potentially is a unique target for therapeutic intervention during the disease; Meprin. The data obtained using literature search from PubMed and interaction networks analysis software STRING strongly support the concept that meprin acts as a major matrix degrading enzyme in the kidney, and thus creating an environment that leads to impairment in cellular function rather than cellular stability in response to AKI. The present study discerns the structure of meprin alpha subunit using *in silico* tools SWISS-MODE, Phyre2 web server and identify the active site and critical amino acid residues in the active site using AADS (IIT Delhi), 3DLigandSite and DoGSiteScorer. Further it is documented that actinonin, a naturally occurring antibacterial agent as a pharmacologically active intervention for the metalloproteinase's α subunit by blocking its active sites from the environment which was validated using molecular docking algorithms of SWISS-DOCK and FlexX.

INTRODUCTION:

AKI is the new consensus term for acute renal failure (ARF) and is characterised by rapid, abrupt and sustained decline in glomerular filtration rate (GFR) and is the leading cause of nephrological disorder that is associated with high mortality rate. Clinically, AKI can be categorised into three primary aetiologies: 1) Prerenal: Caused by reduced blood flow to the kidney from renal artery. 2) Intrinsic: Caused by parenchymal damage to the internal structures of the kidney. 3) Postrenal: Caused by urinary tract obstruction. Continued production and release of chemokines and cytokines, degeneration of the extra cellular matrix (ECM) components leading to tubular and vascular injury, sustained necrosis events, continuum production of apoptotic bodies, death-signaling pathways initiation initiated by the binding of a FasL ligand to its receptor FAS resulting in the sequential recruitment of FADD and pro-caspase 8 represents an important additional component of AKI leading to the extension phase of injury. The disease burden of AKI results in an estimated \$50 billion in additional costs to the health care system across the world and is associated with a mortality of 45–70%. The diagnosis of AKI increases the risk of mortality 5.5 to 6.5-fold as compared to a similarly ill patient without AKI [1-4].

Meprin, a membrane-associated neutral metalloproteinase, belonging to astacin family of zinc endopeptidases that was first discovered as an azocasein and benzoyl-L-tyrosyl-*p*-aminobenzoic acid hydrolase, expressed in the brush-border membranes of proximal tubules and intestines of many mouse strains in the mid 1980s [5]. Further investigations showed that meprin is particularly abundant in mouse juxtamedullary nephrons and constitute ~5% of total protein in renal brush border membranes [6]. The structure and oligomeric assembly of meprins was performed by Bond and colleagues during 1995 after establishing the structural genes, MEP1A and MEP1B, for

the alpha and beta subunits of the metalloproteinase onto the human chromosomes 6p (near the histocompatibility complex) and 18q respectively [5,7]. Meprins are expressed as oligomeric or heteromeric forms of closely related meprin α and/or meprin β subunits but both meprin α and meprin β can also individually exist as a catalytically active entity. Both of them share 42% identity in amino acid sequences [7]. Kidney meprin A is a homooligomer of α subunits, or a heterooligomer of α and β subunits, whereas kidney meprin B is a homooligomer of β subunits [8]. Figure 1 shows that meprin subunits are bound together by disulfide bridges (horizontal black bars). Meprin A ($\alpha_2\beta_2$ or $\alpha_3\beta_1$) is bound to the brush-border membrane via the meprin β subunit and is called as the membrane bound meprin A. Meprin A that is not bound to the β subunit is secreted as a homooligomer and is called as the secreted meprin A [8].

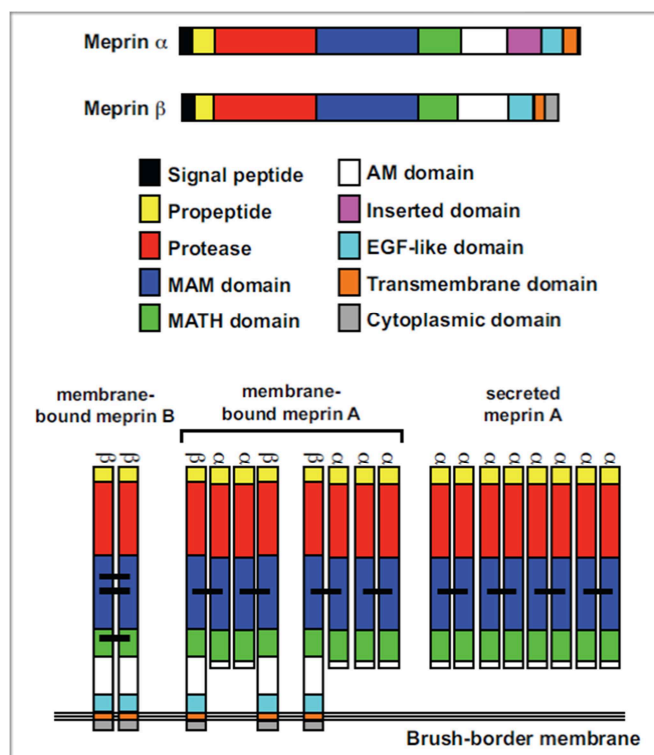


Figure 1: Structural domains and organization of meprin in brush border membrane of kidney, adopted from AJP- Renal physiol [8].

The present study did *in silico* protein tertiary structure prediction to decipher the α subunit of meprin and then identified its critical amino acids, cavities and active sites. Mice strains with low level of renal tubular meprin A expression developed less severe renal failure compared to those with normal meprin level after subjecting to ischemia-reperfusion (IR) [9], thus interaction networks analysis using STRING [10] was performed along with literature search to expose the role meprins undertake during the disease state and to establish that meprins can potentially be a unique target for therapeutic intervention during AKI.

Actinonin was initially identified as a peptide defromylase, essential in prokaryotes and absent in mammalian cells that blocks the biogenesis via transnational inhibition, making it an attractive and novel antibiotic that naturally occurs [11]. Further evidences have ascribed an important inhibitory activity; Inhibition of transmembrane protease aminopeptidase-N/CD13 (EC 3.4.11.2) and actinonin's effects against membrane bound MMP and astacin group of proteases [12,13]. The present study finally evaluated the therapeutic potential of actinonin as an inhibitor of meprin A and thus ameliorate AKI conditions via the *in silico* docking approach using SWISS-DOCK [14] and FlexX [15].

MATERIALS AND METHOD:

1) Data and information retrieval: Comprehensive analysis of the available information was performed using NCBI (Accession No: NP_005579 for meprin α and Accession No: NP_005916 for meprin β), UniProt (ID Q16819 - MEP1A_HUMAN and Q16820 - MEP1B_HUMAN). Crystal structure of meprin β was available at PDB with the ID 4GWN [25,26].

2) *In silico* protein tertiary structure prediction: Homology based fully automated modelling server SWISS-MODEL [16] along with the hidden markov model based Phyre2 [17] web server (Protein Homology/analogy Recognition Engine), both among the best ranked algorithms were used for generating a consensus tertiary structure for meprin α . UCSF Chimera [18] was used for visualization and highlighting the amino acid of the protein structures.

3) *In silico* protein cavity and active site prediction: AADS (Automated active site identification, docking and scoring from IIT Delhi) [19] an automated active site finder, 3DLigandSite [20] for predicting ligand binding sites and DoGSiteScorer [21] a server for automatic binding site prediction, analysis and druggability assessment were used.

4) Interaction networks analysis: A list of protein-protein interaction and substrates for meprin was obtained using STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) and literature studies via PubMed.

5) Molecular Docking Software's and Servers: Actinonin structure was retrieved from PubChem using CID: 443600 and saved in .mol2 format. It was then subjected to docking with SWISS-DOCK and further with FlexX to specifically dock actinonin within the vicinity of the cavity containing the critical amino acids of the active sites.

RESULTS AND DISCUSSION:

1) Data and information retrieved: The metal binding sites of meprin α and β revealed by UniProt is grouped at Table 1. The UniProt study also showed that meprin α subunit prefers small or aromatic amino acids flanking the scissile bond with the catalytic activity of hydrolysis of protein and peptide substrates preferentially on carboxyl side of hydrophobic residues, while meprin β subunit favour acidic residues proximal to the scissile bond with the catalytic activity of hydrolysis of 5-His-|-Leu-6, 6-Leu-|-Cys-7, 14-Ala-|-Leu-15 and 19-Cys-|-Gly-20 bonds in insulin B chain and exhibits a strong preference for acidic amino acids at the P1' position [22]. Molecular weights of meprin α was 84418 Da and meprin β was 79571 Da.

Table 1: Metal binding sites of meprin α and meprin β

Feature meprin α	Position	Length	Description	Feature meprin β	Position	Length	Description
Metal binding	58	1	Zinc binding	Metal binding	52	1	Zinc Binding
Metal binding	155	1	Zinc binding	Metal binding	152	1	Zinc Binding
Metal binding	159	1	Zinc binding	Metal binding	156	1	Zinc Binding
Metal binding	165	1	Zinc binding	Metal binding	162	1	Zinc Binding

2) *In silico* protein tertiary structure prediction results: According to SWISS-MODEL, 868 templates were found to match the target sequence, this list was filtered by a heuristic approach down to 50 and the top 4 templates were chosen for generating the model in SWISS-MODEL. Phyre2 web server reported a total of 120 hits, each having certain region in their template that have

similarities with the meprin α , but only top 20 were chosen for modelling meprin α just to ensure the confidence level does not go below 99.6%. The final consensus structure obtained is depicted in Figure 2.



Figure 2: Final structure of meprin α

3) *In silico* protein cavity and active site prediction results: A total of 32 cavities in the meprin α model were predicted by AADS (IIT Delhi) tool is depicted in Figure 3. The catalytic domain zinc-dependent metalloproteinase of the astacin family has the following unique signature pattern: HEXXHXXGFXHEXXRXDR [23]. To deduce on which cavity the signature sequence matched, all the 32 cavity sequences of the predicted results were subjected to pair wise sequence alignment using LALIGN [24] to look for local sequence similarities. The comparison of unique signature pattern of astacin family and the predicted cavity_4 sequence [HFSEPVKQDGWALYCTMNR] showed that the two sequences have 22.2% sequence identity (44.4% sequence similarity). The predicted ligand/substrate binding site of meprin α obtained using 3DLigandSite and the 18 binding pockets for meprin α as detected by DoGSiteScorer is shown in Figure 4 and Figure 5.

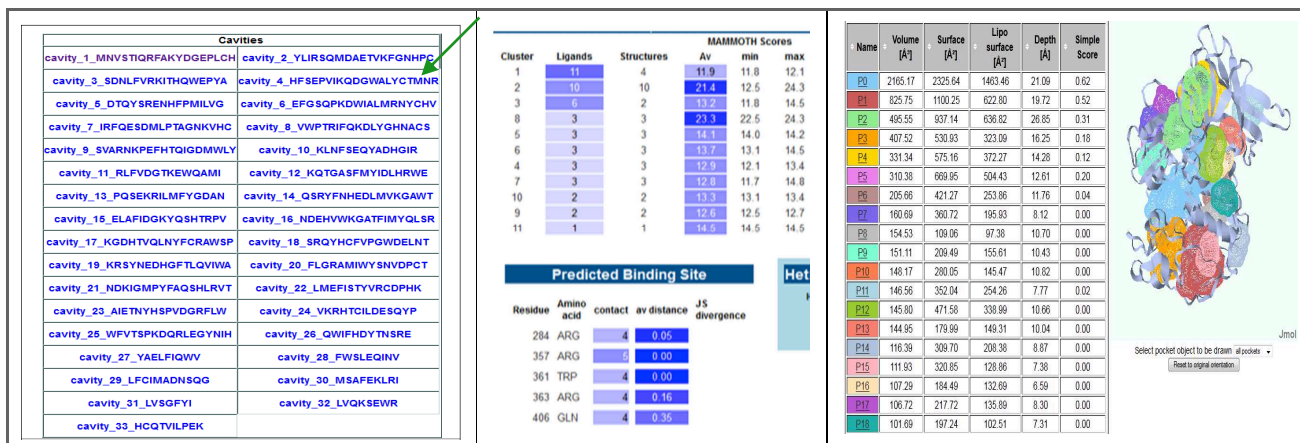


Figure 3: Cavities in meprin α model as predicted by AADS (IIT Delhi) tool.

Figure 4: The predicted ligand/substrate binding site of meprin α obtained using 3DLigandSite.

Figure 5: The 18 binding pockets for Meprin alpha as detected by DoGSiteScorer.

The critical amino acids in the active sites of Meprin alpha for substrate and peptide bond specificity, interpreted from the *in silico* protein cavity, active site prediction results and literature studies [8,22] were concluded as follows:

Amino acid residues	Asparagine (N)	Tyrosine (Y)	Tyrosine (Y)	Proline (P)	Histidine (H)
Residue numbers	72	175	194	226	268

4) Interaction networks analysis results: Meprin α and meprin β networks obtained using STRING is depicted in Figure 6 and the list of Substrates to Meprins, obtained via STRING and literature studies via PubMed is depicted in Table 2. It clearly indicates that the substrates to the enzymes are of a broad spectrum that also includes major cell cytoskeleton maintaining proteins.

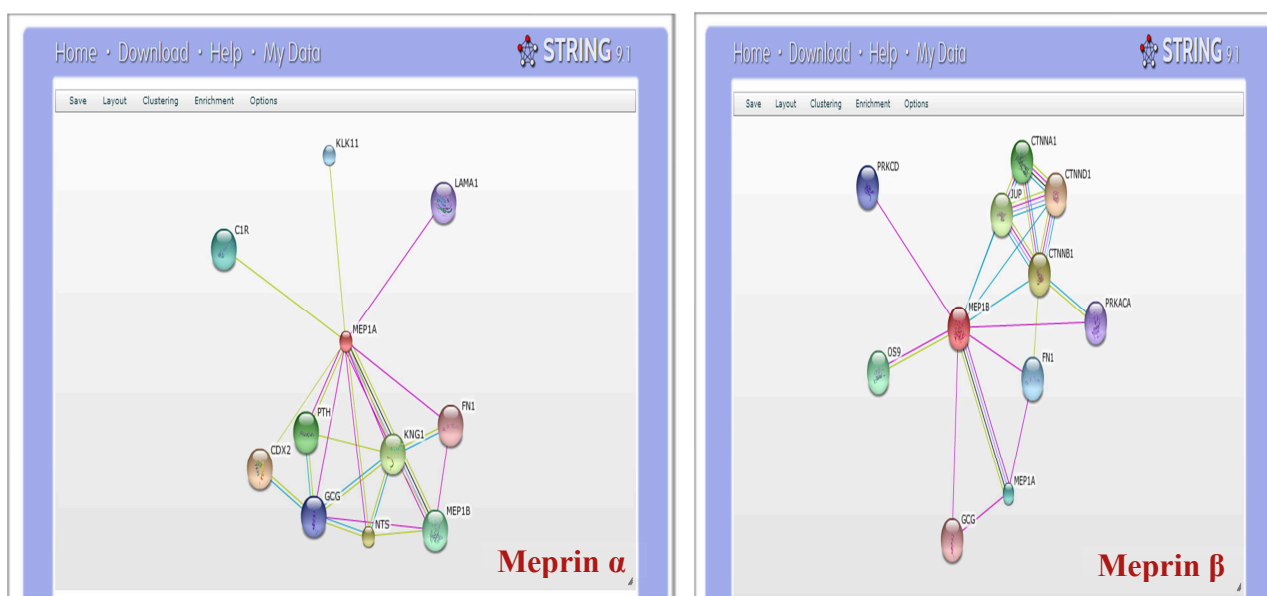


Figure 6: Meprin α and meprin β networks obtained using STRING.

Table 2: A comprehensive list of Substrates of Meprins (both α and/or β subunit) [8]

Substrate	Meprin Source and Isoform
Insulin, bradykinin, angiotensin II	Mouse kidney meprin A
Luliberin or luteinizing-hormone-releasing hormone (LHRH) bradykinin, substance P	Rat kidney meprin A
Insulin B chain, oxytocin, substance P, bradykinin angiotensin I, and angiotensin II	Human small intestinal meprins
TGF α	Rat kidney meprin A
Parathyroid hormone (PTH)	Rat kidney meprin A
Collagen IV, laminin, fibronectin, gelatin, and nidogen	Rat kidney meprin A
Catalytic subunit of protein kinase A	Rat kidney meprin A
Laminin 1 and laminin 5	Homomeric human meprin A

Bombesin, neurotensin, LHRH, bradykinin, α -melanocyte stimulating hormone (α -MSH), substance P, PTH fragment, valosin, vasoactive intestinal peptide, and angiotensin I	Mouse meprin A
Gastrin 17, peptide YY, orcokinin, and kinetensin	Mouse meprin B
Cerulein, secretin, glucagon, Gastrin-releasing peptide (GRP), neuropeptide Y.	Both mouse meprin A and meprin B
Collagen IV, nidogen-1, and fibronectin	Human homomeric meprin A and human meprin B
Pro-IL-1 β	Rat meprin A, Human meprin B
GRP, glucagon, ghrelin, PTH, secretin, substance P, bradykinin, neurotensin, and α -MSH	Mouse meprin A, Human meprin A
Pro-IL-18, E-cadherin	Rat meprin B
Processing of procollagen III	Human meprin B
Pro-kallikrein-7 (Pro-KLK7)	Human meprin B
Villin and actin	Rat meprin B and mouse homomeric meprin A
α and γ -Epithelial Na ⁺ channel (ENaC) subunits	Rat meprin B
Amyloid precursor protein	Human meprin B
Pro-ADAM-10 (a disintegrin and metalloproteinase)	Human meprin B
Pro-MMP-9	Homomeric meprin A and meprin B

Meprins has the ability to degrade components of extracellular matrix (ECM) proteins, process pro inflammatory cytokines, adherens junction proteins and hormones. It indicates that the functional properties of the enzyme might be toxic during the pathological condition of AKI [8,9].

5) Molecular Docking Software's and Servers results: Molecular docking with actinonin (Figure 7A), a potential pharmacological inhibitor of astacin family of metalloproteinase predicted by SWISS-DOCK is depicting the element 0 in Figure 7B.



Figure 7A: 2D actinonin structure obtained from PubChem. Fig 7B: The most favourable docking site of Actinonin as predicted by SWISS-DOCK, also the 268 histidine is one of the 5 critical amino acid in the active site of meprin α .

FlexX was then used to specifically dock actinonin within the vicinity of the critical amino acids of the active sites. Figure 8 depicts the obtained result that suggests critical amino acids of meprin α certainly has strong interaction with actinonin.

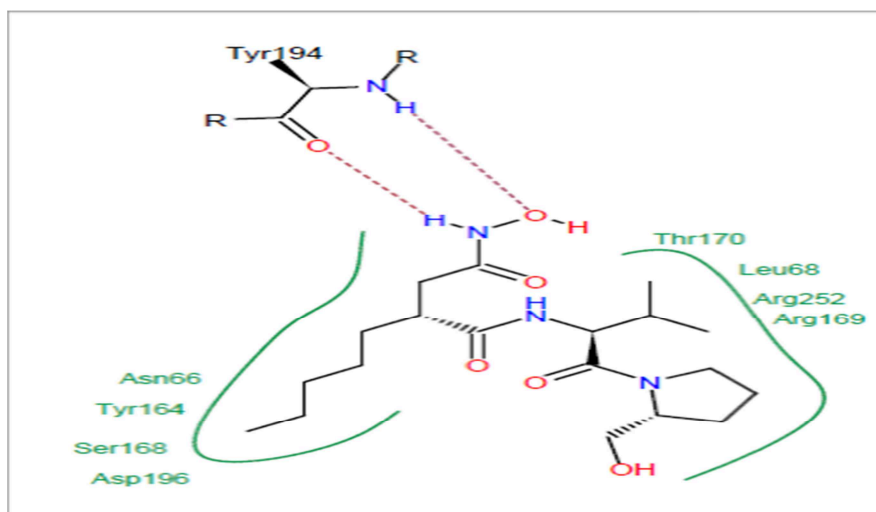


Figure 8: Interacting residues of meprin α docked with actinonin.

CONCLUSION:

The study provided an insight into the morbid roles meprins undertakes during the manifestation of the various pathological conditions of AKI, apart from understanding its biochemical features. It further established actinonin as a potent inhibitor of meprin and thus a potential pharmacological intervention against the AKI itself. The present study should be further taken to the next level of *in vivo* experiments on animal models not only to validate the above findings but also establish the drug's efficacy and the dose.

ACKNOWLEDGEMENT:

Author is thankful to Vice Chancellor of Panjab University, co-ordinator of centre for systems biology and bioinformatics for providing all the facilities to carry out this work. I would also like to acknowledge the support provided by online tools, servers, databases and softwares for successful accomplishment of the work.

REFERENCES:

- [1] Bellomo R, Ronco C, Kellum, Acute renal failure: definition, outcome, measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group, *Crit Care*, 8, (2004), 204–212.
- [2] Dissension AR, Acute renal failure: definition and pathogenesis, *Kidney int*, 66, (1988), 7-10.
- [3] David P, Melissa D, Anderson B, Timothy A, Pathophysiology of Acute kidney Injury, *Compr Physiol*, 2, (2012), 1303-1353.
- [4] Chertow GM, Burdick E, Honour M, Bonventre JV, Bates DW, Acute Kidney Injury, mortality, length of stay, and cost in hospitalisation, *Journal of the american society of nephrology*, 16, (2005), 80-91.
- [5] Bond JS, Beynon RJ, Reckelhoff JF, David CS, Mep-1 gene controlling a kidney metalloendopeptidase is linked to the major histocompatibility complex in mice, *Natural academy of science*, 81, (1984), 5542-5545.
- [6] Craig SS, Reckelhoff JF, Bond JS, Distribution of meprin in kidneys from mice with high and low meprin activity, *Am J Cell Physiol*, 253, (1987), 535-540.

- [7] Bond, J. S., Rojas, K., Overhauser, J., Zoghbi, H. Y., Jiang, W. The structural genes, MEP1A and MEP1B, for the alpha and beta subunits of the metalloendopeptidase meprin map to human chromosomes 6p and 18q, respectively. *Genomics*, 25, (1995), 300-303.
- [8] Kaushal GP, Randy S, Christian H, Sudhir V, Meprin A metalloproteinase and its role in acute kidney injury, *Am J renal physiol*, 304, (2013), 1150-1158.
- [9] Carmago S, Shaw SV, Walker PD, Meprin, a brush-border enzyme, plays an important role in hypoxic/ischemic acute renal tubular injury in rat, *Kidney Int*, 61, (2002), 959-966.
- [10] Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, Simonovic M, Roth A, Santos A, Tsafou KP, Kuhn M, Bork P, Jensen LJ, von Mering C, STRING v10: protein-protein interaction networks, integrated over the tree of life, *Nucleic Acids Res*, 43, (2015), 47-52.
- [11] Dawn ZC, Dinesh VP, Corinne JH, Wen W, Geoffrey D, Dennis CY, Peter SM, Charlotte W, Joaquim T, Richard JW, Zhengyu Y, Actinonin a naturally occurring antibacterial agent, is a potent defromylase inhibitor, *Biochemistry*, 39, (2000) 1256-1262.
- [12] Bauvois B, Dauzonne D, Aminopeptidase-N/CD13 (EC 3.4.11.2) inhibitors: chemistry, biological evolution, and therapeutic prospects, *Med. Res*, 26, (2006), 88-130.
- [13] Asmaa S, Simon LD, Borhane A, Cell based evidence for aminoprotease N/CD 13 inhibitor actinonin targeting of MT1-MMP-mediated pro MMP 2 activation, *Elsevier*, 279, (2009), 171-176.
- [14] Aurelien Grosdidier, Vincent Zoete, Olivier Michielin, SwissDock, a protein-small molecule docking web service based on EADock DSS, *Nucleic Acids Res*, 39, (2011), 270-277.
- [15] Cross, Simon SJ, Improved FlexX docking using FlexS-determined base fragment placement, *Journal of chemical information and modeling*, 45, (2005), 993-1001.
- [16] Torsten Schwede, Jurgen Kopp, Nicolas Guex, Manuel C. Peitsch, SWISS-MODEL: an automated protein homology-modeling server, *Nucleic Acids Res*, 31 (13), (2003), 3381-3385.
- [17] Kelley LA, Mezulis S, Yates CM, Wass MN, Sternberg MJ, The Phyre2 web portal for protein modeling, prediction and analysis, *Nat Protoc*, 10 (6), (2015), 845-858.
- [18] Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE, UCSF Chimera--a visualization system for exploratory research and analysis, *J Comput Chem*, 25 (13), (2004), 1605-1612.
- [19] Singh T, Biswas D, Jayaram B, AADS-An automated active site identification, docking, and scoring protocol for protein targets based on physicochemical descriptors, *Journal of chemical information and modelling*, 51 (10), (2011), 2515-2527.
- [20] Mark NW, Lawrence AK, and Michael JE, 3DLigandSite: predicting ligand-binding sites using similar structures, *Nucleic Acids Res*, 38, (2010), 469-473.
- [21] Volkamer A, Kuhn D, Rippmann F, Rarey M, DoGSiteScorer: a web server for automatic binding site prediction, analysis and druggability assessment, *Bioinformatics*, 28 (15), (2012), 2074-2075.
- [22] Greg PB, Benjamin E, Turk J, Simon JH, Gail LM, John EB, jacqueline MC, Lewis C, Judith SB, Marked difference between metalloproteases Meprin A and B in substrate and peptide bond specificity, *JBC*, 276 (16), (2001), 248-255.
- [23] Stocker W, Bode W, Structural features of a superfamily of zinc- endopeptidases: the metzincins, *Curr Opin Struct Biol*, 5, (1995) 383-390.
- [24] LALIGN, Pairwise Sequence Alignment, EMBL-EBI, <http://www.ebi.ac.uk/Tools/psa/lalign/>, March 21, 2016.
- [25] Arolas JL, Broder C, Jefferson T, Guevara T, Sterchi EE, Bode W, Stocker W, Pauly C, Gomis Ruth F, Structural basis for the sheddase function of human meprin β metalloproteinase at the plasma membrane, *Proc Natl Acad Sci USA*, 109 (40), (2012), 16131-16136.
- [26] Protein Data Bank (PDB), An Information Portal for Biological Macromolecular Structures <http://www.rcsb.org/pdb/explore/explore.do?structureId=4GWN> March 15, 2016.

Volume 57

10.18052/www.scipress.com/ILNS.57

Understanding the Exacerbating Role of the Metalloproteinase Meprin during AKI, an *In Silico* Approach

10.18052/www.scipress.com/ILNS.57.18

DOI References

- [1] Bellomo R, Ronco C, Kellum, Acute renal failure: definition, outcome, measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group, *Crit Care*, 8, (2004).
10.3410/f.1047455.497408
- [3] David P, Melissa D, Anderson B, Timothy A, Pathophysiology of Acute kidney Injury, *Compr Physiol*, 2, (2012), 1303-1353.
10.1002/cphy.c110041
- [5] Bond JS, Beynon RJ, Reckelhoff JF, David CS, Mep-1 gene controlling a kidney metalloendopeptidase is linked to the major histocompatibility complex in mice, *Natural academy of science*, 81, (1984), 5542-5545.
10.1073/pnas.81.17.5542
- [7] Bond, J. S., Rojas, K., Overhauser, J., Zoghbi, H. Y., Jiang, W. The structural genes, MEP1A and MEP1B, for the alpha and beta subunits of the metalloendopeptidase meprin map to human chromosomes 6p and 18q, respectively. *Genomics*, 25, (1995).
10.1016/0888-7543(95)80142-9
- [9] Carmago S, Shaw SV, Walker PD, Meprin, a brush-border enzyme, plays an important role in hypoxic/ischemic acute renal tubular injury in rat, *Kidney Int*, 61, (2002), 959-966.
10.1046/j.1523-1755.2002.00209.x
- [10] Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, Simonovic M, Roth A, Santos A, Tsafou KP, Kuhn M, Bork P, Jensen LJ, von Mering C, STRING v10: proteinprotein interaction networks, integrated over the tree of life, *Nucleic Acids Res*, 43, (2015).
10.1093/nar/gku1003
- [11] Dawn ZC, Dinesh VP, Corinne JH, Wen W, Geoffrey D, Dennis CY, Peter SM, Charlotte W, Joaquim T, Richard JW, Zhengyu Y, Actinonin a naturally occurring antibacterial agent, is a potent defromylase inhibitor, *Biochemistry*, 39, (2000) 1256-1262.
10.1021/bi992245y
- [12] Bauvois B, Dauzonne D, Aminopeptidase-N/CD13 (EC 3. 4. 11. 2) inhibitors: chemistry, biological evolution, and therapeutic prospects, *Med. Res*, 26, (2006), 88-130.
10.1002/med.20044
- [13] Asmaa S, Simon LD, Borhane A, Cell based evidence for aminoprotease N/CD 13 inhibitor actinonin targeting of MT1-MMP-meiated pro MMP 2 activation, *Elsevier*, 279, (2009), 171-176.
10.1016/j.canlet.2009.01.032
- [14] Aurelien Grosdidier, Vincent Zoete, Olivier Michielin, SwissDock, a protein-small molecule docking web service based on EADock DSS, *Nucleic Acids Res*, 39, (2011), 270-277.
10.1093/nar/gkr366
- [15] Cross, Simon SJ, Improved FlexX docking using FlexS-determined base fragment placement, *Journal of chemical information and modeling*, 45, (2005), 993-1001.
10.1021/ci050026f

- [16] Torsten Schwede, Jurgen Kopp, Nicolas Guex, Manuel C. Peitsch, SWISS-MODEL: an automated protein homology-modeling server, *Nucleic Acids Res*, 31 (13), (2003), 3381-3385.
10.1093/nar/gkg520
- [17] Kelley LA, Mezulis S, Yates CM, Wass MN, Sternberg MJ, The Phyre2 web portal for protein modeling, prediction and analysis, *Nat Protoc*, 10 (6), (2015), 845-858.
10.1038/nprot.2015.053
- [18] Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE, UCSF Chimera-a visualization system for exploratory research and analysis, *J Comput Chem*, 25 (13), (2004), 1605-1612.
10.1002/jcc.20084
- [19] Singh T, Biswas D, Jayaram B, AADS-An automated active site identification, docking, and scoring protocol for protein targets based on physicochemical descriptors, *Journal of chemical information and modelling*, 51 (10), (2011), 2515-2527.
10.1021/ci200193z
- [20] Mark NW, Lawrence AK, and Michael JE, 3DLigandSite: predicting ligand-binding sites using similar structures, *Nucleic Acids Res*, 38, (2010), 469-473.
10.1093/nar/gkq406
- [21] Volkamer A, Kuhn D, Rippmann F, Rarey M, DoGSiteScorer: a web server for automatic binding site prediction, analysis and druggability assessment, *Bioinformatics*, 28 (15), (2012), 2074-(2075).
10.1093/bioinformatics/bts310
- [23] Stocker W, Bode W, Structural features of a superfamily of zinc- endopeptidases: the metzincins, *Curr Opin Struct Biol*, 5, (1995) 383-390.
10.1016/0959-440x(95)80101-4
- [25] Arolas JL, Broder C, Jefferson T, Guevara T, Sterchi EE, Bode W, Stocker W, Pauly C, Gomis Ruth F, Structural basis for the sheddase function of human meprin β metalloproteinase at the plasma membrane, *Proc Natl Acad Sci USA*, 109 (40), (2012).
10.1073/pnas.1211076109