World Academy of Science, Engineering and Technology
International Journal of Biomedical and Biological Engineering
Vol:3, No:10, 2009

Computational Design of Inhibitory Agents of BMP-Noggin Interaction to Promote Osteogenesis

Shaila Ahmed[1], Raghu Prasad Rao Metpally[1], Sreedhara Sangadala[2] and Boojala Vijay B Reddy[1,*]

Abstract**—**Bone growth factors, such as Bone Morphogenic Protein-2 (BMP-2) have been approved by the FDA to replace grafting for some surgical interventions, but the high dose requirement limits its use in patients. Noggin, an extracellular protein, blocks the effect of BMP-2 by binding to BMP. Preventing the BMP-2/noggin interaction will help increase the free concentration of BMP-2 and therefore should enhance its efficacy to induce bone formation. The work presented here involves computational design of novel small molecule inhibitory agents of BMP-2/noggin interaction, based on our current understanding of BMP-2, and its known putative ligands (receptors and antagonists). A successful acquisition of such an inhibitory agent of BMP-2/noggin interaction would allow clinicians to reduce the dose required of BMP-2 protein in clinical applications to promote osteogenesis. The available crystal structures of the BMPs, its receptors, and the binding partner noggin were analyzed to identify the critical residues involved in their interaction. In presenting this study, LUDI de novo design method was utilized to perform virtual screening of a large number of compounds from a commercially available library against the binding sites of noggin to identify the lead chemical compounds that could potentially block BMP-noggin interaction with a high specificity.

Keywords**—**Transforming growth factor-beta, Bone morphogenic proteins, Noggin, LUDI *de novo* design method, CAP small molecules.

I. Introduction

THE members of the TGF-β (Transforming Growth Factor β) superfamily are structurally related secreted signaling proteins that such as cell-cycle progression, cell differentiation, development, motility, neuronal growth, bone morphogenesis, wound healing, and immune surveillance [1]. Bone morphogenetic proteins (BMPs) are signaling molecules that belong to the TGF-β superfamily. More than twenty different BMPs have currently been identified [2, 3]. BMPs have roles in the regulation of bone induction, maintenance and repair and induce osteoblast differentiation of various types of cells including undifferentiated mesenchymal cells, bone marrow stromal cells, and preosteoblasts [4]. BMP-2 and BMP-4 have been established to be the key factors in embryonic skeletal development [5, 6]. BMP receptors are the transmembrane receptors classified as type I or type II based on sequence homology and contain a Ser/Thr protein kinase domain [7]. BMP ligand binding to type I receptor (BMPRI) induces the association of BMPRI and BMPRII receptors, allowing the constitutively phosphorylated BMPRII to phosphorylate and activate the latent BMPRI [8]. After activation of BMPRI, the receptor regulated (R)-Smad1/5/8 is phosphorylated. The phosphorylation of R-Smad is then released from the receptor complex and associates with common Smad proteins (Co-Smad, Smad4). Subsequently, R-Smad/Co-Smad complex translocates into the nucleus and regulates the transcription of target genes by functioning in concert with other transcription factors (Figure 1) [8, 9].

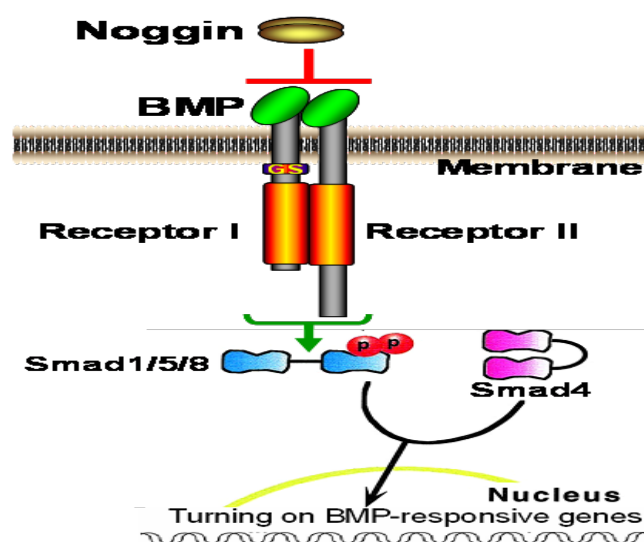

Fig. 1 BMP signaling pathway: BMP binds to BMP receptor type II that in turn activates BMPR-I. Activated BMPR-I phosphorylates receptor regulated Smads (Smad1/5/8) which form complexes with Smad4. The activated Smad complexes regulate gene expression of BMP-responsive genes. Noggin antagonizes signaling by binding to BMP.

BMP activity is tightly regulated prior to receptor binding by the presence of several structurally distinct extracellular BMP antagonists such as noggin, follistatin, sclerostatin, chordin, DCR, BMPMER, cerberus, gremlin, DAN, and many others [8, 10]. These are secreted proteins that bind to BMPs and reduce their bioavailability for interactions with the BMP receptors, effectively blocking BMP action. The effects of BMP-2 and BMP-4 are inhibited by a 60-kDa homodimeric protein called noggin [11]. Noggin binds with equal avidity to BMP-2 and BMP-4 and competitively inhibits their interaction with the BMP receptor type IA (BMPRIA) [12, 13]. The application of BMP-2 or an anti-noggin antibody reversed the effect of exogenous or endogenous noggin, respectively [10]

[1]Authors are with the Laboratory of Bioinformatics and *In Silico* Drug Design, Department of Computer Science, Queens College of City University of New York, 65-35 Kissena Blvd, Flushing, NY 11375, USA.

[2]Author is with Atlanta VA Medical Center and the Department of Orthopaedic Surgery Emory University School of Medicine, Atlanta, Georgia 30329, USA.

*Corresponding Author Boojala VB Reddy, Phone: 718-997-3487, Fax: 718-997-3513, e-mail: breddy@qc.cuny.edu.

Open Science Index, Biomedical and Biological Engineering Vol:3, No:10, 2009 publications.waset.org/1665.pdf

Open Science Index, Biomedical and Biological Engineering Vol:3, No:10, 2009 publications.waset.org/1665.pdf

and BMP-2 injection partially cured impaired osteoblast differentiation and bone loss in aged animals [14].

The structural data revealed that the surfaces of BMPs have two prominent hydrophobic patches, the convex type II receptor-binding interface (knuckle epitope) and the concave type I receptor-binding interface (wrist epitope) [15]. The residues determining binding affinity and specificity of BMP-2 and BMPRIA were identified by mutational analysis. This suggested that the elusive binding determinants of the wrist epitope for the BMP-2/BMPRIA interaction involved the main chain amide groups of amino acids L51 and D53 with minor contribution from the hydrophobic interactions. Two hydrogen bonds are formed between L51 (main chain amide and carbonyl) of BMP-2 and Q86 of BMPRIA [7]. The core structure of BMPRII shares the same pattern of disulfide connectivity as ActRII [2]. In an earlier analysis a hypothetical complex between BMPRII and BMP-2, created by superimposing BMPRII in the position of ActRII in a ternary complex between ActRII, BMP-2, and BMPRIA, suggested that BMPRII makes similar overall ligand binding contacts to BMP-2 as does the ActRII receptor. The residues Y67, W85, and F115 of BMPRII are in the main hydrophobic patch. The data also suggested that the polar residues K81, S86, E93, and Y113 are also important binding determinants. On the other hand, H87 and Y40 confer specificity in BMPRII ligand binding [2]. The previous mutational analysis has identified A34, H39, S88, L90, and L100 residues as the binding determinants of BMP-2 for BMPRII and ActRII [7].

The superposition of the noggin/BMP-7 complex structure onto a model of the BMP signaling complex shows that noggin binding effectively masks both pairs of binding epitopes. Noggin contains an extended N-terminal 'clip' segment of about 20 residues [7]. The type I receptor-binding site is obstructed by a segment of the clip domain (Q28 to D39). The hydrophobic ring of P35 of noggin inserts into a hydrophobic pocket on BMP-7 formed by W52, W55, V87, Y128 and M131, which mimics a similar insertion of F85 from BMPRIA into the hydrophobic cleft on BMP-2. The type II receptor-binding site is masked extensively by the C-terminal half of the clip segment (N40 to E48) [7, 15]. Another mutational study revealed that noggin proteins with substitutions at positions L46D, E48K, I218E essentially abolished BMP-7 binding activity in some of the variants. Furthermore, a P35R substitution diminished the binding affinity for BMP-7 [16].

Together with the aforementioned structural knowledge about the binding determinants of BMPs, BMP receptors, and noggin obtained from previous mutational studies, the available structures of the BMPs, its receptors and noggin were initially analyzed and explored to identify the critical residues involved in their interaction. In the present study LUDI *de novo* design method was used to computationally screen large number of commercially available compounds against the binding sites of noggin to identify the lead chemical compounds that could potentially block only BMP-noggin interaction but not the BMP interaction with its receptors. Since the BMP binding regions of BMP receptors and the BMP binding regions of noggin are similar the small molecules that bind to noggin binding epitopes and that do not bind to the BMP receptor epitopes were identified. Here, an extensive computational analysis of result was described. The experimental validation of the computational result is in progress and will be discussed elsewhere.

II. Materials and Methods

The objective was to analyze BMP/noggin, BMP/BMP Receptors interactions from known structures of these complexes and use them to design small molecules that block the antagonist noggin binding to BMP. The employed methods included computational analysis of BMP, BMP receptors and noggin sequences, structures, and various docking procedures. The human sequences used from Swiss-Prot [17] database were: BMP-2 (P12643), BMP-7 (P18075), noggin (Q13253), BMPRIA (P36894) and BMPRII (Q13873). The structures used from Protein Data Bank (PDB) were: BMP-2 (1rew), BMP-7 (1m4u), noggin (1m4u), BMPRIA (1rew), ActRIIB (2h62) and BMPRII (2hlr).

A. Amino acid sequence alignment

The sequences of BMP, BMP receptors and noggin were retrieved from UniProt [18-20] sequence database. A multiple sequence alignment for these sequences was generated using ClastalW (default parameters) [21].

B. Solvent Accessible Contact Area Calculations

BMP, BMP Receptors and the antagonist noggin structures and their complexes were analyzed to identify various residues involved in their interaction by solvent accessible contact area calculation using the method of Richmond & Richards (1978) [22]. The method gives the surface area (Å^2) for each residue that can come into contact with a spherical probe of radius of 1.4 Å. The percentage difference in solvent accessible contact area ($pd\alpha_t$) of each residue in a protein on contact with another protein of the complex pair is then calculated as follows:

$$(pd\alpha_t) = (\alpha_i - \alpha_c) \times 100/\alpha_t$$

where α_t is total surface area of the residue in an open chain conformation of Gly-X-Gly form.

C. Graphical Viewing and Superposing the Protein Structures

Accelrys Inc. Discovery Studio tools were used for various computational needs such as to display, superpose or to generate molecular graphics figures. Rasmol [23, 24] was also used as molecular display tool. A web based tool called Superpose [25] was used to superimpose. The application modules in Discovery Studio 1.7 which provided many powerful tools that enabled docking and scoring ligands, performing *de novo* design of lead compounds, and analyzing the results.

D. LUDI de novo Receptor Mode

LUDI is a method for the *de novo* design of ligands for proteins [26, 27]. The DS LUDI was used extensively in this work which searches fragment libraries and identifies molecules that fit the requirements of the defined interaction sites. Ligand-receptor complexes may be evaluated using the empirical scoring functions available from the LUDI algorithm. The LUDI score is a sum of five contributions: from ideal hydrogen bonds, contributions from perturbed ionic interactions (ionic interaction is the interaction of

donor/acceptor in the receptor [e.g. COO-, or NH3+]), contributions from lipophilic interactions, contributions due to the freezing of internal degrees of freedom of the ligand, contributions due to the loss of translational and rotational entropy of the ligand.

In receptor mode, LUDI searches a fragment library for complementary small molecules that best bind the defined interaction sites. During the search and fit computation LUDI also determines the energy estimates, or scores, for each conformation searched for the fragments in the library. The fragments are ranked by energy estimate, and the best are returned in the hit list. This hit list can then be inspected for the selection of candidate scaffolds. In this study, LUDI receptor mode was used against BMP-2, BMPRIA, BMPRII, and noggin which screened potential lead compounds from CAP small molecular weight compound databases that are predicted to block noggin epitopes that bind to BMP-2.

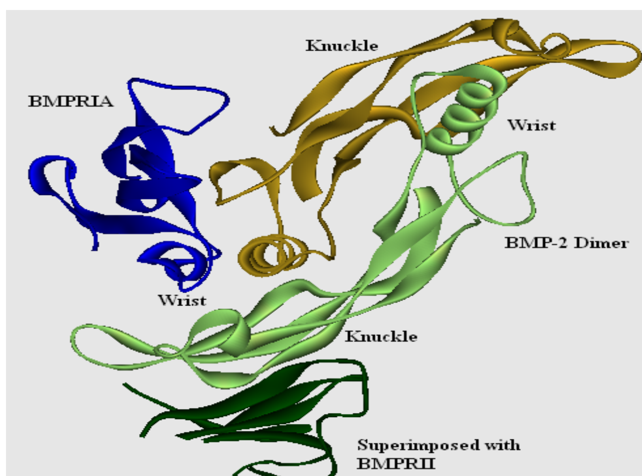

Fig. 2 Model of trimer structure of BMP-2/BMPRIA/BMPRII. BMPRII was modeled based on its homolog ActRIIB (2h62).

III. Results

The goal was to study the BMP/noggin interaction and computationally design small molecular weight inhibitors to block the key amino acid residues of noggin to prevent noggin from binding to BMP-2. The strategy undertaken was expected to result in designing noggin-blockers without affecting the natural binding and function of BMP and its receptors. Here, the outcome of the studies are presented that led to selection of manageable number high scoring noggin binding small molecules that may block its binding to BMP-2 for further experimental studies.

A. Identification of BMP-2/Noggin Binding Regions

Since the BMP-7, BMP-2, and the noggin are homologous structures ClustalW was used to identify the corresponding equivalent residues among these sequences. BMP-7 protein sequence is highly homologous to BMP-2 with 80% of sequence identity. Since BMP-2/noggin complex structure is not available the crystal structure of BMP-7 and noggin complex (1m4u) was used to identify equivalent residues of BMP-2 that interact with noggin and *vice versa*. To identify the noggin interacting regions with BMP-2 the biological unit of the BMP-2 dimer structure with a noggin monomer was used. From the calculation of percent solvent accessibility contact area (%SA) of this new complex in the presence or absence of the binding partners the interacting residues of noggin with BMP-2 were identified (Table 1).

Table I: The percentage of surface area of each amino acid of BMP receptors and the inhibitor Noggin involved in binding with BMP-2

Noggin (1m4u)			BMPR-IA (1rew)			BMP-2 (1rew)		
Pos	**AA**	**%SA**	**Pos**	**AA**	**%SA**	**Pos**	**AA**	**%SA**
27	MET	11.3	42	GLY	42.5	10	LEU	51.7
30	TYR	33	43	HIS	40.2	25	ASP	22.8
31	LEU	19.7	45	PRO	29.4	26	VAL	15.4
32	HIS	54.7	46	ASP	10.1	27	GLY	19.5
33	ILE	36.6	67	ASP	30	28	TRP	19.6
34	ARG	52.2	72	THR	11	30	ASP	11.5
35	PRO	88.7	77	CYS	23.1	31	TRP	25.9
36	ALA	30.2	79	LYS	41	33	VAL	31
37	PRO	54.2	81	GLU	16.2	34	ALA	36.7
38	SER	34.2	82	GLY	46	35	PRO	19.6
39	ASP	21.7	84	ASP	52.4	39	HIS	33.7
40	ASN	39.3	85	PHE	84.4	49	PHE	48.9
43	LEU	41.6	86	GLN	55.4	50	PRO	69
46	LEU	58.1	88	LYS	44.6	51	LEU	11.1
47	ILE	16.1	89	ASP	24.7	52	ALA	34.5
49	HIS	12.9	90	SER	52.1	53	ASP	91.4
199	HIS	20.4	91	PRO	11.3	54	HIS	49.8
204	ARG	15.4	92	LYS	42.6	57	SER	24.3
206	ARG	38.4	93	ALA	10.9	59	ASN	17.6
208	GLN	14.2	94	GLN	45.8	62	ILE	36.3
209	ARG	25.5	97	ARG	21.8	66	LEU	23.9
210	ARG	43	**BMPR-II (2hlr)**			69	SER	53.6
218	ILE	25.4	**Pos**	**AA**	**%SA**	70	VAL	40.4
219	PRO	31.2	40	TYR	37.5	72	SER	18.4
220	ILE	22.7	67	TYR	10.4	76	LYS	19.9
221	GLN	42.7	69	LEU	20.7	85	SER	21.6
223	PRO	12	81	LYS	24.8	86	ALA	23.6
			84	CYS	21	87	ILE	17.5
			85	TRP	27.7	88	SER	46.8
			86	SER	45.4	90	LEU	30.5
			87	HIS	34.3	96	GLU	18.8
			88	ILE	34.5	97	LYS	22.5
			89	GLY	51.4	98	VAL	45.1
			90	ASP	39.5	100	LYS	37.6
			93	GLU	16.3	101	LYS	18.7
			113	TYR	26.3	102	ASP	16
			115	PHE	21.4	103	TYR	16.1
						103	TYR	16.2
						107	VAL	10.1

B. Identification BMP-2 Binding Regions of the BMPRIA and BMPRII.

The crystal structure of BMPRII was superimposed on to the ActRIIB of ternary complex consisting of BMP-2 dimer / $BMPRIA_{EC}$ / ActRIIB using a web based tool Superpose [25]. Using these superposed structures a new hypothetical ternary complex consisting of BMP-2/$BMPRIA_{EC}$/BMPRII was modeled (Figure 2). The surface area ($Å^2$) for each residue and the percent solvent accessible contact area (%SA) of the model ternary complex of BMP-2/$BMPRIA_{EC}$/BMPRII and individual monomer structures were calculated, and the position (Pos) and name of the amino acid residues (AA) of both the receptors, BMPRIA and BMPRII that bind to BMP-2 were identified (Table 1).

Open Science Index, Biomedical and Biological Engineering Vol:3, No:10, 2009 publications.waset.org/1665.pdf

Open Science Index, Biomedical and Biological Engineering Vol:3, No:10, 2009 publications.waset.org/1665.pdf

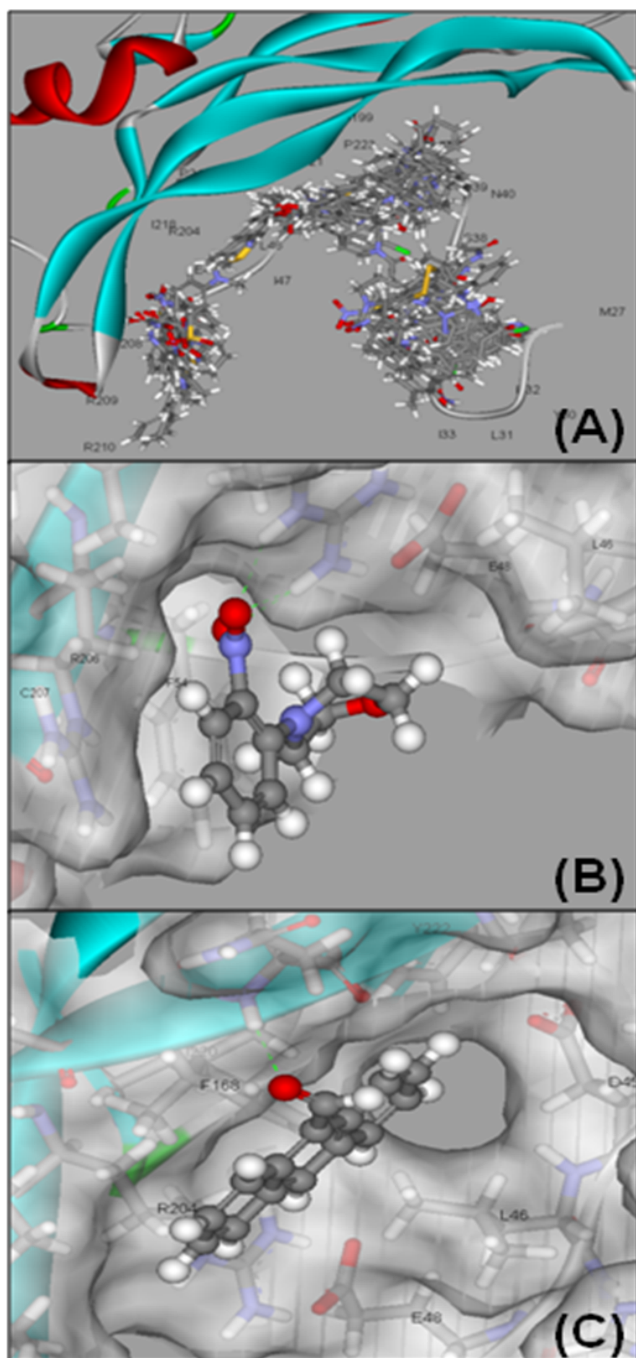

Fig. 3 Molecules identified by LUDI *de novo* design method: (A) Top scoring 50 molecules find three grooves on the binding site; (B) and (C) Poses of two top scoring molecules with Ludi3 scores 563 and 428, respectively.

C. Screening of CAP Small Molecular Database.

LUDI *de novo* design method of Accelrys Discovery Studios 1.7 was used against the noggin regions that bind to BMP. The BMP binding region of noggin was divided into four sub regions (with some overlapping residues) for LUDI runs. Region I consisted of amino acids at positions 27, 29, 30-37; region II consisted of amino acids at positions 37-46, 222-223; region III consisted amino acids at positions 47, 48, 168, 199, 204, 218-222; region IV consisted of amino acids at positions 54, 206-210. The binding site defined as a sphere of radius for each of 10.5, 10.5, 12, and 9 Å covering the amino acid in the regions I, II, III, and IV, respectively. The LUDI search against these four regions using the 70,000 small molecules in the CAP database yielded about 11,090 molecules with Ludi3 scores greater than 300. LUDI was used against the BMPRIA and BMPRII region that bind to BMP-2 and obtained 22,234 and 25,420 molecules, respectively. The small molecules that are common to either of the receptors were excluded from these molecules and the remaining 3717 molecules were unique to noggin binding. Using only these 3717 small molecules, a small library of compounds was generated and one LUDI run was performed on the entire BMP binding region of noggin using a 16 Å radius sphere. As a result, 1167 small molecules were found with Ludi3 binding scores greater than 300. A set of top 50 molecules with high scores that were distributed over the entire binding region are shown in Figure 3.

IV. Discussion

From previous site directed mutagenetic studies it was observed that the mutants L46D, E48K, I218E of noggin fail to bind to BMP. Therefore, few high scoring molecules were identified in the region surrounding these important residues that may block these residues. The Fig.3 gives a cluster of 50 top consensus scoring molecules over the entire binding region and Fig. 3 B and C shows binding poses of two high scoring molecules that may block L46, E48, and I218. Another set of LUDI runs were performed against BMP-2 residues that bind to BMPRIA and BMPRII. The idea was to exclude the molecules that are common between noggin and both the receptors.

Each of the computational technique operates by assigning varying approaches. Priorities for particular approach can limit the expectations in different techniques. A very good criteria specific for one technique could be a constraint for the others. Different computational studies are therefore desirable to acquire comparatively matching outcome. Additional computational techniques such as SCORING, GOLDDOCK, GLIDE, COMBI-GLIDE, SIMBIOSYS are planned to authenticate the results obtained with LUDI to select a manageable number of high scoring compounds for experimental verification.

V. Conclusion

The crystal structure of the BMP-7/noggin complex, the BMP-2/BMPRIA ectodomain complex and the extracellular domain of BMPRII monomer are known. From the analysis of structures of these complexes the key amino acids residues present in the BMP interacting region of noggin were identified. Bioinformatics and *in silico* drug design methods were followed to screen BMP binding region of noggin to identify small molecular weight compounds from the CAP database. The LUDI *de novo* design method was used for screening the potential compounds that could block noggin from interacting with BMP-2. The high scoring potential noggin binding ligands were identified along with their theoretical binding scores. Subsequently, a manageable

Open Science Index, Biomedical and Biological Engineering Vol:3, No:10, 2009 publications.waset.org/1665.pdf

number of these molecules was selected for future experimental *in vitro* binding assays with the purified recombinant protein. If successful, these molecules will be taken for subsequent cellular studies on BMP-induced bone formation.

ACKNOWLEDGMENT

S. A. author is supported by Biochemistry Graduate Teaching Fellowship, CUNY Graduate Center, New York, and CUNY Tuition Fellowship and. R.P.R.M. author is supported by Howard Hughes Medical Institute Grant to Queens College, CUNY. We acknowledge Dormitory Authority – State of New York.

REFERENCES

[1] Lin SJ, Lerch TF, Cook RW, Jardetzky TS, Woodruff TK: The structural basis of TGF-beta, bone morphogenetic protein, and activin ligand binding. *Reproduction* 2006, 132(2):179-190.

[2] Mace PD, Cutfield JF, Cutfield SM: High resolution structures of the bone morphogenetic protein type II receptor in two crystal forms: implications for ligand binding. *Biochem Biophys Res Commun* 2006, 351(4):831-838.

[3] Gazzerro E, Minetti C: Potential drug targets within bone morphogenetic protein signaling pathways. *Curr Opin Pharmacol* 2007, 7(3):325-333.

[4] Kuo PL, Huang YT, Chang CH, Chang JK: Bone morphogenetic protein-2 and -4 (BMP-2 and -4) mediates fraxetin-induced maturation and differentiation in human osteoblast-like cell lines. *Biol Pharm Bull* 2006, 29(1):119-124.

[5] Rosen V, Cox K, Hattersley G: Bone morphogenetic proteins. *In Principles of Bone Biology* 1996: 661-671.

[6] Wozney JM. Bone morphogenetic proteins and their gene expression. *In Cellular and molecular biology of bone* 1993:131-167.

[7] Sebald W, Nickel J, Zhang JL, Mueller TD: Molecular recognition in bone morphogenetic protein (BMP)/receptor interaction. *Biol Chem* 2004, 385(8):697-710.

[8] Rosen V: BMP and BMP inhibitors in bone. *Ann N Y Acad Sci* 2006, 1068:19-25.

[9] Lin Y, Martin J, Gruendler C, Farley J, Meng X, Li BY, Lechleider R, Huff C, Kim RH, Grasser WA *et al*: A novel link between the proteasome pathway and the signal transduction pathway of the bone morphogenetic proteins (BMPs). *BMC Cell Biol* 2002, 3:15.

[10] Larrain J, Bachiller D, Lu B, Agius E, Piccolo S, De Robertis EM: BMP-binding modules in chordin: a model for signalling regulation in the extracellular space. *Development* 2000, 127(4):821-830.

[11] Zimmerman LB, De Jesus-Escobar JM, Harland RM: The Spemann organizer signal noggin binds and inactivates bone morphogenetic protein 4. *Cell* 1996, 86(4):599-606.

[12] Valenzuela DM, Economides AN, Rojas E, Lamb TM, Nunez L, Jones P, Lp NY, Espinosa R, 3rd, Brannan CI, Gilbert DJ *et al*: Identification of mammalian noggin and its expression in the adult nervous system. *J Neurosci* 1995, 15(9):6077-6084.

[13] Re'em-Kalma Y, Lamb T, Frank D: Competition between noggin and bone morphogenetic protein 4 activities may regulate dorsalization during Xenopus development. *Proc Natl Acad Sci U S A* 1995, 92(26):12141-12145.

[14] Wu XB, Li Y, Schneider A, Yu W, Rajendren G, Iqbal J, Yamamoto M, Alam M, Brunet LJ, Blair HC *et al*: Impaired osteoblastic differentiation, reduced bone formation, and severe osteoporosis in noggin-overexpressing mice. *J Clin Invest* 2003, 112(6):924-934.

[15] Groppe J, Greenwald J, Wiater E, Rodriguez-Leon J, Economides AN, Kwiatkowski W, Affolter M, Vale WW, Belmonte JC, Choe S: Structural basis of BMP signalling inhibition by the cystine knot protein Noggin. *Nature* 2002, 420(6916):636-642.

[16] Groppe J, Greenwald J, Wiater E, Rodriguez-Leon J, Economides AN, Kwiatkowski W, Baban K, Affolter M, Vale WW, Belmonte JC *et al*: Structural basis of BMP signaling inhibition by Noggin, a novel twelve-membered cystine knot protein. *J Bone Joint Surg Am* 2003, 85-A Suppl 3:52-58.

[17] Gasteiger E, Jung E, Bairoch A: SWISS-PROT: connecting biomolecular knowledge via a protein database. *Curr Issues Mol Biol* 2001, 3(3):47-55.

[18] Apweiler R, Bairoch A, Wu CH, Barker WC, Boeckmann B, Ferro S, Gasteiger E, Huang H, Lopez R, Magrane M *et al*: UniProt: the Universal Protein knowledgebase. *Nucleic Acids Res* 2004, 32(Database issue):D115-119.

[19] Bairoch A, Apweiler R, Wu CH, Barker WC, Boeckmann B, Ferro S, Gasteiger E, Huang H, Lopez R, Magrane M *et al*: The Universal Protein Resource (UniProt). *Nucleic Acids Res* 2005, 33(Database issue):D154-159.

[20] Wu CH, Apweiler R, Bairoch A, Natale DA, Barker WC, Boeckmann B, Ferro S, Gasteiger E, Huang H, Lopez R *et al*: The Universal Protein Resource (UniProt): an expanding universe of protein information. *Nucleic Acids Res* 2006, 34(Database issue):D187-191.

[21] Thompson JD, Higgins DG, Gibson TJ: CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 1994, 22(22):4673-4680.

[22] Richmond TJ, Richards FM: Packing of a-helices: geometric constraints, and contact areas. *J Mol Biol* 1978, 119:537-555.

[23] Sayle RA, Milner-White EJ: RASMOL: biomolecular graphics for all. *Trends Biochem Sci* 1995, 20(9):374.

[24] Bernstein HJ: Recent changes to RasMol, recombining the variants. *Trends Biochem Sci* 2000, 25(9):453-455.

[25] Maiti R, Van Domselaar GH, Zhang H, Wishart DS: SuperPose: a simple server for sophisticated structural superposition. *Nucleic Acids Res* 2004, 32(Web Server issue):W590-594.

[26] Bohm HJ: The computer program LUDI: a new method for the de novo design of enzyme inhibitors. *J Comput Aided Mol Des* 1992, 6(1):61-78.

[27] Bohm HJ: On the use of LUDI to search the Fine Chemicals Directory for ligands of proteins of known three-dimensional structure. *J Comput Aided Mol Des* 1994, 8(5):623-632.