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Research Article

# In-silico Analysis of Molecular Interaction Between Silk Proteins with BMP-2 Type IA and Type II Receptors

**Nandhinee Thiagaraj, Lekha Ashokkumar*, Ram Sabarish, Balaji SK**

## ABSTRACT

**Background**: Alveolar ridge defects are commonly associated with delayed tooth replacement. Natural biomaterial with enhanced regenerative potential is always sought after as a primary choice for ridge reconstruction. Silk, a biopolymer with its constituent proteins (fibroin and sericin) has recently demonstrated promising outcomes in vitro. However, the molecular mechanism by which this occurs remains to be elucidated. **Objective:** We assessed the molecular interactions between silk proteins bone morphogenetic protein (BMP)-2 type IA and type II receptors using molecular docking. **Methodology**: The N-terminal domain of silk proteins and structural complex of BMP-2 type IA and type II receptors were considered for protein–protein docking using the high ambiguity-driven protein–protein docking (HADDOCK) server. HADDOCK scores are a measure of the predicted stability of the protein–protein complex, and a lower score indicates a more stable complex and a higher affinity for binding. **Results**: The HADDOCK scores and root mean square deviation value for interaction between silk proteins with BMP-2 type IA and type II receptors were (−114.2 ± 25.0 and −143.1 ± 11.3) and (2.9 ± 0.4 and 1.9 ± 0.5), respectively, for fibroin and (−1.8 ± 15.6 and −9.7 ± 25.2) and (3.5 ±0.3 and 0.9 ± 0.6), respectively, for sericin. **Conclusion**: The interaction between fibroin and BMP-2 receptors was more stable with higher affinity.
**Keywords:** Fibroin, sericin, HADDOCK, RMSD, biopolymer.

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## INTRODUCTION

Periodontitis is an inflammatory disease that affects the periodontal tissues and is caused by multiple etiological factors. The primary characteristic of periodontal disease is alveolar bone loss, which can ultimately lead to tooth loss. Alveolar ridge defects are commonly attributed to delayed tooth replacement.[1] Augmentation of ridge defects requires a special barrier membrane to facilitate guided bone regeneration. Natural biomaterials have always been the primary choice for bone regeneration in defective areas. Recently, silk-based biomaterials have demonstrated excellent biocompatibility, biodegradability, immunocompatibility, antibacterial, anti-inflammatory, and other mechanical properties.[2]

Silk, derived from the silkworm species Bombyx mori (B.mori), is a natural polymer comprising two proteins: fibroin (75%) and sericin (25%).[2] Sericin is a soluble, glue-like, amorphous protein that functions as an adhesive binder and can be eliminated using a thermochemical technique called degumming. This process exposes the fibroin, which can be effectively used as a biomaterial. Fibroin is an insoluble semi-crystalline fibrous protein composed of H, L, and P25 linked via a single disulfide bond at a ratio of 6:6:1. Silk protein-based regenerative materials have demonstrated superior osteogenic properties and have been used in different forms such as hydrogels and films. These silk-based natural biomaterials have also been used as scaffolds for bone regeneration, drug delivery, wound dressing, and healing.[3]

Bone morphogenetic proteins (BMP) are multifunctional growth factors that belong to the transforming growth factor superfamily. BMPs are classified into three subfamilies: 1) type-I includes BMP-2,4 which comprises 80% of the amino acid sequence and induces osteoblast differentiation and apoptosis, 2) type-2 includes BMP-5,6,7 which comprises 78% of the amino acid sequence and plays an important role in chondrogenesis, osteoblast differentiation, and osteo-induction, and 3) type-3 includes BMP-3, which is the most abundant BMP present in the bone that inhibits osteogenesis and also helps in embryonic skeletogenesis.4 BMPs participate in signaling through canonical and non-canonical pathways. BMPs attach to cell surface receptors and initiate the signal transduction cascade to form a complex structure with two dimers, Type I and II serine/threonine kinase receptors, in the canonical signaling pathway, whereas BMPs communicate through the suppressor of mothers against Decapentaplegic and mitogen-activated protein kinase-independent signaling pathways in the non-canonical signalling pathway. In addition, BMPs enhance the formation of new connective tissues, cementum, periodontal ligaments, and alveolar bone.[4]

Bombyx mori-based silk proteins exhibit excellent biocompatibility and induce osteoblastic cell attachment and proliferation. The in vitro regenerative potentials of sericin and fibroin have been reported previously. However, the molecular mechanism by which it interacts with osteoblasts remains to be elucidated.[5,6] Hence, we assessed the molecular interactions between silk proteins

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and BMP-2 Type IA and II receptors using molecular docking and molecular dynamic simulations.

## MATERIALS AND METHODS

The protein structures of silk fibroin, silk sericin, BMP-2 type IA and II receptors were obtained from Uniprot (https://www.uniprot.org/uniprotkb/P07856/entry#structure; https://www.uniprot.org/uniprotkb/P07856/entry#structure) and the RCSB protein data bank (https://www.uniprot.org/uniprotkb/P07856/entry#structure; https://www.uniprot.org/uniprotkb/P07856/entry#structure) for in silico analysis. for performing the in-silico analysis. The interaction between N terminal domain of silk proteins and Complex BMP-2 Type 1A and II receptors were assessed using molecular docking and molecular dynamic simulation.

### Molecular docking

Molecular docking is a computerized technique in which the three-dimensional structures of proteins and ligands are docked to identify the binding sites and assess the stability of the biomolecular interaction. It helps predict the structure of the protein complex and calculate the potential binding energy of proteins with their binding affinity. This technique was designed to aid the development of therapeutic agents. Molecular docking was conducted between i) silk fibroin with BMP-2 type IA and II receptors and ii) sericin with BMP-2 type IA and II receptors using a high ambiguity-driven protein–protein docking (HADDOCK) server. This helps determine the interaction between the bound structure of two proteins formed from the coordinates of the unbound structure. The Z-score represents the standard deviation of the HADDOCK score of a given cluster from the mean of all clusters. A lower score indicates better interaction.[6]

### Molecular dynamic simulation

Molecular dynamic simulation is a computational simulation method used to analyze the physical movements of atoms and molecules. It predicts the movement of an atom in a protein over a fixed period. These simulations captured different biomolecular processes, ligand binding, and protein interactions. Molecular dynamics simulations were performed using the GROningen MAchine for Chemical Simulations (GROMACS) software, which is specifically designed to analyse the interactions between proteins, lipids, and nucleic acids, thereby assessing the stability of the interactions.[6]

### Root mean square deviation (RMSD)

The relative stability of a complex is represented as the RMSD, which is a useful metric for comparing the stability of protein complexes.

### Root mean square fluctuations (RMSF)

This determines which amino acids of the protein cause more vibrations, resulting in destabilization of the protein in the presence and absence of ligands. The RMSF values determined the vibration of the silk proteins, which were calculated against a simulation timescale of 0 to 100 ns.

**Radius of gyrus (Rg)**

Radius of gyration is the mass-weighted root mean square distance of atoms from their center of mass. The competence and shape folding of the overall structure at different time points during the trajectory can be observed in the Rg plot.

### Solvent accessible surface area (SASA)

This is the area of the protein sufficiently exposed to interact with neighboring molecules to measure the compactness of the hydrophobic core.

### Principle component analysis (PCA)

This is a component analysis in which a statistical technique is used to reduce the dimensionality of the data and extract important features or patterns. It is used in structural biology to analyze conformational changes in proteins and protein–ligand complexes.

### MM–PBSA binding free energy calculations

The Poisson–Boltzmann surface area of molecular mechanics was used to calculate the free energy of the relative binding strength of proteins.[7]

## RESULTS

### Protein–protein docking

Based on the results obtained from the HADDOCK server, the potential binding energy between the two proteins was calculated, and a stable confirmation that maximized the binding affinity was identified (Figure 1). Based on the statistics, the top 10 clusters were represented and considered the most reliable. Thus, the structure of silk sericin and fibroin interacting with BMP-2 type IA and II receptors, represented in clusters 4, 6, 6, and 7, respectively, was found to be better than that of other complexes, as it had the lowest Z-score (negative scores).

Examination of different clusters within the silk proteins with BMP-2 type-II and type-IA receptors through HADDOCK scores revealed distinct interaction patterns. Notably, cluster 4 of the silk–fibroin-BMP-2 type-II complex demonstrates a highly negative HADDOCK score (−143.1 +/− 11.3), indicative of strong and stable interactions, whereas cluster 6 of the silk–fibroin-BMP-2 type-IA complex displays a moderately negative score (−114.2 +/− 25.0), suggesting a relatively weaker interaction. Conversely, clusters 6 and 7 of the silk–sericin-BMP-2 complexes exhibit stronger HADDOCK scores, implying either weaker interactions or repulsive forces between the components (Table 1).

### Molecular dynamic simulation

RMSD was calculated based on the analysis and results obtained using GROMACS.

### RMSD

The RMSD of silk fibroin interacting with BMP-2 type IA and II receptors for 0 to 100 ns were 0.62 +/− 0.03 nm, 0.41 +/− 0.02 nm, respectively. For sericin interacting with BMP-2 type IA and II receptors were 1.32 +/− 0.03 nm, 1.28 +/− 0.03 nm, respectively.

The complexes did not show any significant deviation from the unbound protein (Figure 2). Overall, the RMSD results indicated that all protein complexes considered were relatively stable throughout the simulation. The differences between the complexes were small, suggesting that they are very similar in terms of stability. This is likely because they contain similar structural components.

### RMSF

The RMSF for FIB-BMP-2 type IA and II receptors were 0.31 +/− 0.04 nm, 0.42 +/− 0.07 nm and for SER-BMP-2 type IA and II receptors were 1.68 +/− 0.08 nm, 1.61 +/− 0.09 nm, respectively. The results suggested no significant structural changes during the 100 ns simulation.

### Rg

The average (radius of gyrus) Rg values of Silk proteins interacting for 0 to 100 ns for FIB-BMP-2 type-II and type-IA receptors were 1.85 +/− 0.01 nm, 1.94 +/− 0.01 and those for SER-BMP-2 type-II and type-IA receptors were 3.91 +/− 0.01 nm, 3.53 +/− 0.01 nm, respectively. These values indicated that the protein complex was structurally stable throughout the simulation (Figure 3).

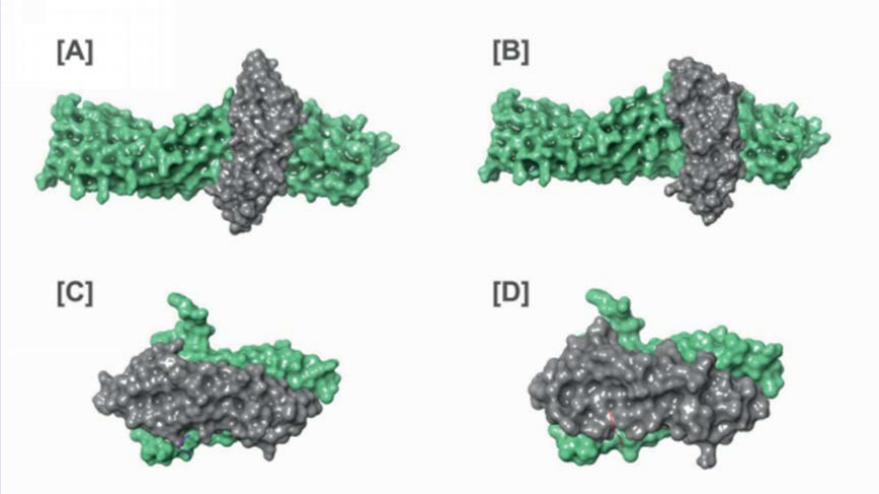


**Figure 1:** Protein–protein interaction of (A) silk sericin interacting with BMP-2 type IA, (B) silk sericin interacting with BMP-2 type II, (C) silk fibroin interacting with BMP-2 type IA, (D) silk fibroin interacting with BMP-2 type II.

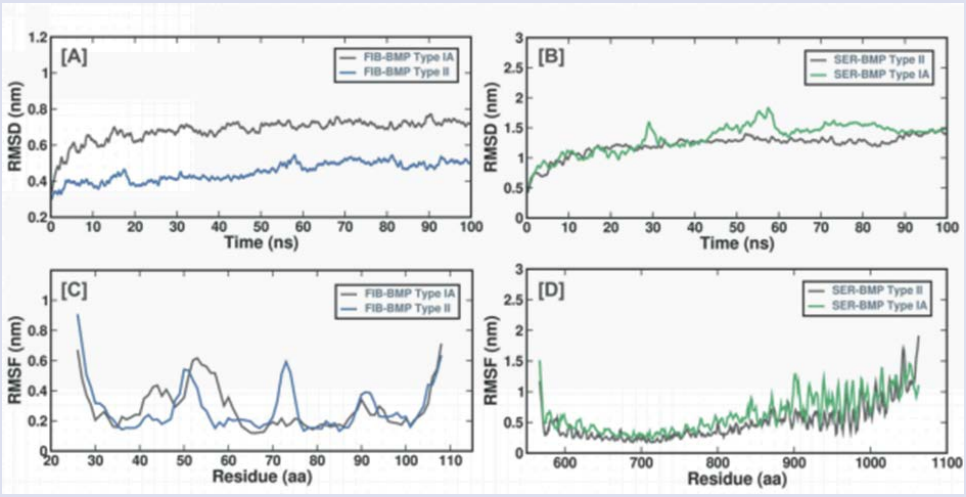


**Figure 2:** (A) Silk–fibroin-BMP-2 type-II, (B) silk–fibroin-BMP-2 type-IA, (C) silk–sericin-BMP-2 type-II and sericin-BMP-2 type-IA.

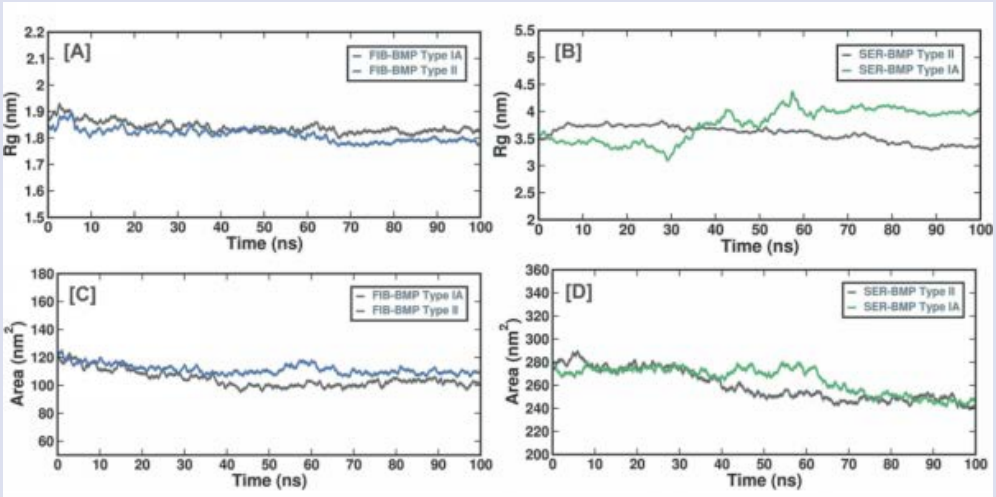


**Figure 3:** The radius of gyrus plot values for silk proteins.

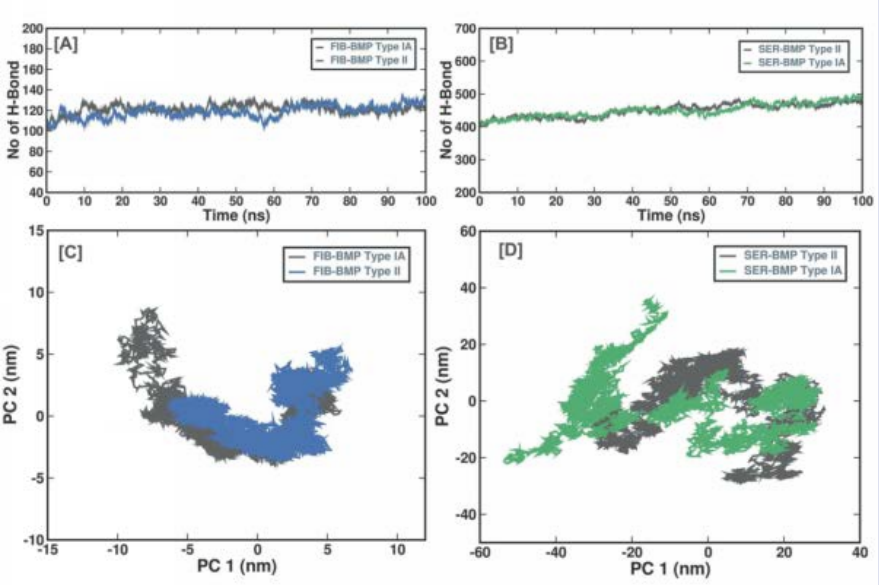


**Figure 4:** Hydrogen bond analysis between silk proteins.

**Table 1: Cluster statistics of the HADDOCK docking run calculated on the target protein of each cluster**

| | Silk-Fibroin-BMP 2-Type-II | Silk-Fibroin-BMP2-Type-IA | Silk-Sericin-BMP2-Type-II | Silk-Sericin-BMP-2Type-IA |
|---|---|---|---|---|
| HADDOCK score | -143.1 ± 11.3 | -114.2 ± 25.0 | -9.7 ± 25.2 | -1.8 ± 15.6 |
| Cluster size | 4 | 6 | 6 | 7 |
| RMSD | 1.9 ± 0.5 | 2.9 ± 0.4 | 0.9 ± 0.6 | 3.5 ± 0.3 |
| Van der Waals energy | -119.4 ± 13.4 | -112.9 ± 23.5 | -118.2 ± 4.1 | -90.2 ± 10.2 |
| Electrostatic energy | -602.9 ± 81.7 | -528.7 ± 124.9 | -247.5 ± 30.7 | -419.0 ± 36.0 |
| Desolvation energy | 1.9 ± 9.7 | -12.7 ± 5.1 | -34.0 ± 3.6 | -38.9 ± 2.3 |
| Restraints violation energy | 949.5 ± 91.1 | 1170.8 ± 182.8 | 1919.9 ± 155.1 | 2110.7 ± 130.9 |
| Buried Surface Area | 4538.1 ± 386.2 | 4219.5 ± 333.2 | 3487.3 ± 122.2 | 3063.0 ± 148.1 |
| Z-Score | -2.2 | -1.4 | -2.2 | -1.6 |

**Table 2: P-value < 0.05, statistically significant.**

| | BMP-2 Type II | | | BMP-2 Type I A | | |
|---|---|---|---|---|---|---|
| Variable | FIBROIN Mean/Std deviation | SERICIN Mean/Std deviation | P value- Type-II | FIBROIN Mean/Std deviation | SERICIN Mean/Std deviation | P value- TYPE- 1A |
| Van der Waal energy | - 590.328/27.645 | - 520.732/41 .811 | 0.007 | - 591.058/2 7.441 | - 411.897/27. 405 | 0.971 |
| Electrosta tic energy | - 2502.080/2 05.321 | - 1221.465/7 6.168 | <0.001 | - 693.177/1 15.082 | - 117.004/00 4 | <0.001* |
| SASA energy | - 88.300/4.27 0 | - 57.921/6.6 89 | 0.615 | - 70.240/5.6 29 | - 60.071/4.48 3 | 0.003 |
| Polar solvation energy | 2369.606/2 63.97 | 1168.444/2 07.356 | 0.914 | 1429.659/ 154.17 | 1154.968/1 | 0.002 |
| Binding energy | - 811.102/14 8.315 | - 631.674/10 0.564 | <0.001 | 75.183/77. 707 | 565.996/13 | <0.001* |

## SASA

The average SASA values of FIB-BMP-2 type-II and type-IA receptors for 0 to 100 ns were 112.04 +/− 3.14 nm2 and 118.5 +/− 3.57 nm2, while those for SER-BMP-2 type-II and type-IA receptors were 258.7 +/− 9.78 nm2 and 266.5 +/− 4.70 nm2, respectively. These values were attributed to minimal changes in the conformational states of the target protein.

## Hydrogen bond analysis

Both protein–protein complexes were stabilized by the formation of hydrogen bonds. In this study, the hydrogen bonds formed in the molecular docking analysis were confirmed by simulation analysis (Figure 4).

## PCA

PCA was used to analyse conformational changes in proteins or protein–ligand complexes. Structural differences between fibroin and sericin interacting with BMP-2 type IA and II receptors were analysed (Figure 4).

### MM–PBSA binding free energy calculations

The binding affinities of silk proteins interacting with BMP types IA and II were examined. The relative binding strength within the summer energy proteins was examined. Table 2 compares the binding strengths of fibroin and sericin with BMP-2 receptors for inhibitors computed using the MM–PBSA method. We calculated the residue-level contributions to the interaction energy across a stable simulation trajectory.

Intergroup analysis of MM–PBSA showed a significant mean difference in the van der Waals energy, electrostatic energy, SASA energy, polar solvation energy, and binding energy between fibroin and sericin with BMP-2 receptors. Specifically, the binding energies of fibroin and sericin with BMP-2 type IA were (75.183/77.707) and (565.996/133.877) respectively, whereas those for BMP-2 type II were (−811.102/148.315) and (−631.674/100.564), respectively. Statistical analysis revealed a significant difference ($P < 0.001$) in the binding energy between fibroin and sericin with BMP-2 type IA and II receptors. The results suggested that compared to sericin, fibroin had a more stable interaction with both BMP-2 type IA and type II receptors.

## DISCUSSION

In silico analysis of silk fibroin and sericin revealed promising physical properties as biomaterials for bone regeneration. The results indicate a significant binding affinity between silk fibroin and BMP-2 type IA and II receptors.

Molecular docking was performed to determine the stability, interaction, and behaviour of protein binding with the ligand. Based on the results of our study, BMP activation may be a potential pathway for initiating osteoblastic differentiation. In molecular dynamic simulation, the physical movements of atoms and molecules were assessed when they were allowed to interact for a fixed period. GROMACS was designed for the simulation of proteins; hence, it was used in this study. The stability of the silk proteins and BMP receptors was confirmed by molecular dynamic simulation and molecular docking. RMSD values, gyrus radius, and other parameters exhibited greater stability of fibroin binding to BMP-2 receptors than sericin.

BMPs are pivotal in bone regeneration and repair. They were initially regarded as growth factors; however, they are now considered as differentiation factors and chemotactic agents. It stimulates the differentiation of stem cells into osteoblasts. BMPs have been proven to affect periodontal regeneration through mitogenic, chemotactic, and differentiating mechanisms.[8]

The signalling pathways of BMPs are important for osteoblast differentiation. Both type I and II receptors bind to ligands, and upon activation, phosphorylate downstream signalling molecules, leading to osteoblast differentiation and bone formation.[9] The results of in silico analyses were consistent with those of both docking and molecular dynamics simulations, confirming that both silk proteins (sericin and fibroin) have a strong binding affinity for BMP-2 type IA and II receptors.

Among the silk proteins studied, fibroin demonstrated stronger and more stable interactions with BMP-2 type IA and II receptors. Thus, it validates the previous literature that reports that fibroin-based scaffolds promote osteoblast attachment, proliferation, and differentiation. Silk fibroin has been successfully used as a scaffold for bone regeneration in one-walled periodontal defects in dogs.[10] When formulated as a scaffold, aqueous fibroin showed better biocompatibility and osteoconductivity than sericin.[11] In addition, silk fibroin exhibits wound-healing properties and has been proposed as a vehicle for drug delivery. When used in combination with a xenograft membrane, it resulted in good clinical improvement and regenerative potential for the treatment of Grade II furcation defects.[12] The biocompatibility of silk fibroin was proven when cultured with the MG63 osteoblastic cell line and has been suggested as an alternative material for guided bone regeneration.

In contrast, sericin with BMP-2 receptors exhibited weaker interactions. Previous literature by Lamboni et al. suggested that sericin-based scaffolds when checked for tissue engineering in gut repair, enhanced cell growth and cell differentiation remarkably upon the addition of silk sericin to the micro structured bacterial cellulose, which was subsequently used as a scaffold in prospective gut repair.[13] In 2020, Maria et al. discussed the important properties of sericin, such as its ability to promote cell growth, biocompatibility, and mitogenic effects. These characteristics make sericin a promising polymer for biomedical applications, particularly in tissue engineering and regeneration.[14]

Despite the positive insights gained from this molecular study, future in vitro studies must be performed to assess the osteoblastic potential of silk proteins for enhancing their application in bone regeneration.

## CONCLUSION

The interaction between fibroin and BMP-2 receptors was found to be more stable, with higher affinity. In accordance with the results of our study, BMP-2 activation may be a potential pathway through which silk fibroin initiates osteoblast differentiation. Hence, it can be considered a potential osteoinductive biomaterial for guided bone regeneration.

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## ABBREVATIONS

HADDOCK: High ambiguity-driven protein–protein docking

BMP: Bone morphogenetic protein

SASA: Solvent accessible surface area

Rg: Radius of gyrus

RMSF: Root mean square fluctuations

RMSD: Root mean square deviation

GROMACS: GROningen MAchine for Chemical Simulations

PCA: Principal component analysis

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