

In silico identification and action mechanism of potential sleep-promoting peptides from egg proteins

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Abstract: Chronic insomnia can significantly impair cognitive function and emotional stability, and may even trigger or exacerbate systemic health issues such as cardiovascular disease and metabolic disorders. This study intended to identify novel potential sleep-promoting peptide candidates from egg proteins and elucidate their molecular mechanisms with γ -aminobutyric acid (GABA) type-A receptor (GABAAR), orexin 2 receptor (OX2R), and nociceptin/orphanin-FQ receptor (NOPR). Through *in silico* hydrolysis, water solubility and toxicity prediction, molecular docking, and molecular dynamics simulations, six novel potential peptide candidates were identified. Among these, QPVDNY exhibited the strongest binding affinities of -9.2 , -9.7 and -9.0 kcal/mol with GABAAR, OX2R and NOPR, respectively. Subsequently, peptide LKPIAAEVY showed affinities of -8.6 , -9.7 and -8.2 kcal/mol with the three receptors, respectively. Molecular docking results showed that peptides QPVDNY and LKPIAAEVY bound to residues Leu259, Thr256, His267, and Glu270 of GABAAR via π -alkyl and hydrogen bonds, formed π -alkyl bonds with residues Pro131, Cys107, Phe227, Phe346 and His350 of OX2R. In addition, these two peptides formed π -alkyl bonds and hydrogen bonds with residues Val283, Met134 and Tyr131 of NOPR, meanwhile, they bound to residue Asp130 of NOPR via hydrogen bonds and a salt bridge. Molecular dynamics simulations confirmed the good stability of the peptide-receptor complexes. This study identifies novel potential candidates for natural sleep-promoting foods and establishes an efficient approach for screening food-derived bioactive peptides.

Keywords: potential sleep-promoting peptides; molecular docking; molecular dynamics simulations; egg proteins

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1 Introduction

Sleep is an indispensable physiological process for maintaining human physical and mental health. Chronic sleep deprivation or poor sleep quality significantly raises the risk of various chronic diseases, including cardiovascular diseases^[1], metabolic syndrome^[2], cognitive decline^[3], and mental disorders^[4]. The global prevalence of chronic insomnia reaches 35%^[5]. Clinical interventions for insomnia primarily rely on benzodiazepines and non-benzodiazepine receptor agonists^[6]. While effective, these medications may cause side effects such as daytime somnolence, drug dependence, and cognitive impairment. Food-derived bioactive peptides have attracted considerable interest owing to their high safety, good bioavailability, and multiple physiological regulatory functions. Some research indicates that specific peptides released from food proteins via enzymatic digestion exhibit potential sedative, anxiolytic, and sleep-promoting properties. Two sleep-enhancing peptides have been identified from casein tryptic hydrolysate via virtual screening^[7].

Currently, the most common sleep receptors are the γ -aminobutyric acid (GABA) type-A receptor (GABAAR) and the orexin 2 receptor (OX2R). Activating the GABA receptor with two main subtypes has been the most popular mechanism of regulating sleep^[8]. As a ligand-gated chloride channel receptor, the GABAAR elicits rapid inhibitory post-synaptic potentials and is activated by either its agonists or positive allosteric modulators^[9–10]. Orexin neurons play an important role in sleep-state transitions^[11] through

potentially indirect effects on other arousal promoting brain regions^[12]. It has been demonstrated that OX2R exerts a greater influence on sleep-wake regulation than OX1R^[13–14]. The mechanism of some sleep-promoting peptides interacted with these two receptors was elucidated through molecular docking^[15]. Additionally, the nociceptin/orphanin-FQ receptor (NOPR) has been discovered in hypothalamic and brainstem regions, which are known to be involved in sleep/wake control. This system has been proposed as a unique receptor for sleep therapeutics^[16].

As one of the most widely consumed animal-derived protein sources, egg protein features a balanced amino acid composition and high digestibility, making it an excellent substrate for producing bioactive peptides. Current research on the functional properties of egg proteins primarily focuses on antibacterial^[17], antioxidant^[18], and antihypertensive effects^[19]. Traditional approaches require screening complex enzymatic hydrolysates for peptides with specific functions, which is time-consuming, costly, and inefficient. Recent advances in computational biology and bioinformatics have revolutionized the discovery of bioactive peptides, making it more efficient. Despite this progress, the potential of egg protein-derived peptides in promoting sleep remains unexplored.

This study aimed to identify novel potential sleep-promoting peptide candidates from egg proteins and elucidate their molecular mechanisms of action regarding key receptors. The research employed *in silico* hydrolysis followed by a comprehensive evaluation of water solubility and toxicity using bioinformatic tools.

Subsequently, molecular docking was performed to investigate the binding affinity of the peptides with GABAAR, OX2R, and NOPR, while molecular dynamics simulations were utilized to assess the dynamic stability of these interactions. These findings will provide an efficient strategy for discovering potential food-derived bioactive peptides.

2 Materials and methods

2.1 *In silico* protein hydrolysis

The amino acid sequences of ovalbumin (AAB59956.1), ovotransferrin (NP_990635.2), and ovomucin (BAD22545.1) were retrieved from the National Center for Biotechnology Information database (<https://www.ncbi.nlm.nih.gov/>, accessed on April 20, 2025). Subsequently, *in silico* enzymatic hydrolysis was performed using the ExPASy PeptideCutter tool (http://web.expasy.org/peptide_cutter/, accessed on April 20, 2025). Chymotrypsin (EC 3.4.21.1, high specificity), trypsin (EC 3.4.21.4), and pepsin (EC 3.4.23.1, pH 1.3) were selected for *in silico* hydrolysis because they represent the primary digestive enzymes in the human gastrointestinal tract^[20]. Simulating the cleavage patterns of these enzymes helps identify peptides that are more likely to be released during physiological digestion, thereby increasing the potential bioavailability of the candidate peptides identified in this study.

2.2 Water solubility and toxicity predictions of egg-derived peptides

The peptides obtained from egg protein hydrolysates were subjected to water solubility and toxicity prediction using the Peptide Properties Calculator (<http://www.innovagen.com/>, accessed on April 20, 2025) and the toxicity prediction tool ToxinPred (<https://webs.iitd.edu.in/raghava/toxinpred/index.html>, accessed on April 20, 2025), respectively. Egg-derived peptides with good water solubility and non-toxicity were selected for subsequent studies.

2.3 Molecular docking of peptides with GABAAR, OX2R, and NOPR

The 3D structures of the GABAAR, OX2R, and NOPR receptors were retrieved from the Protein Data Bank (RCSB PDB, <https://www.rcsb.org/>, accessed on April 21, 2025) with the PDB IDs of 6HUP, 4S0V, and 5DHG, respectively. Potential ligand-binding sites for each receptor were predicted via the PrankWeb platform (<https://prankweb.cz/>, accessed on August 11, 2025). Prior to molecular docking, all receptor proteins and peptides were preprocessed with AutoDock Tools (version 1.5.6). Removal of water molecules, addition of hydrogen atoms, and optimization of protonation states were performed during preprocessing. Docking calculations were performed using AutoDock Vina. The active site locations were defined based on the predicted binding sites, with grid box centers set as follows: GABAAR (center: $x = 134.767$, $y = 135.074$, $z = 161.275$), OX2R (center: $x = 53.057$, $y = 9.062$, $z = 55.189$); and NOPR (center: $x = -7.497$, $y = 30.350$, $z = 3.503$). For all receptors, the number of points dimensions (x , y , z) were 40, 40 and 40, respectively, with a grid point spacing of 1.0 Å. Subsequent visualization analysis was performed using PyMOL 3.1 (version 3.1.6.1) and LigPlot+ (version 2.3).

2.4 Molecular dynamics simulation

Molecular dynamics simulations validate and enhance the accuracy of molecular docking results by simulating biomolecular flexibility

and conformational changes at the atomic level. A 100 ns molecular dynamics simulation was performed on the complexes of each selected peptide with the sleep-related receptors using the GROMACS 2018 software package with the CHARMM36 force field^[21]. Initially, the protein and ligand files were preprocessed to generate topology files. Specifically, the CHARMM36 force field was applied to the proteins, while the ligand topologies were generated separately and subsequently merged. The complexes were then solvated in a cubic box using SPC water molecules. Ionic strength was maintained at 0.15 mol/L with Na⁺ and Cl⁻ ions to achieve system neutrality. Prior to the production run, energy minimization was performed to eliminate steric clashes, followed by NVT and NPT equilibration steps to stabilize the temperature and pressure. Finally, a 100 ns production MD simulation was performed using the GROMACS 2018 software package^[22]. Trajectory analysis was subsequently performed, thereby enabling assessment of the stability of the complexes. Root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF), radius of gyration (R_g), hydrogen bonds and solvent-accessible surface area (SASA) were evaluated by GROMACS 2018 analysis tools, with all figures created using Origin 2024 software^[23].

3 Results and discussion

3.1 *In silico* screening and activity prediction of egg-derived peptides

Bioinformatics is crucial for the rapid screening of bioactive peptides. Molecular simulation tools accelerate bioactive peptides screening, significantly reducing the preliminary experimental workload. In this study, 602 peptides were obtained through virtual enzymatic hydrolysis, primarily ranging from 3 to 15 amino acids in length. ToxinPred is widely used for predicting peptide toxicity. Good water solubility and non-toxic properties are key factors influencing the absorption of bioactive peptides. The Peptide Properties Calculator was used to assess physicochemical properties including water solubility. A total of 164 peptides with good water solubility and non-toxicity were identified. Although the enzymes used represent gastrointestinal proteases, theoretical cleavage predictions do not guarantee that the identified peptides are released during actual digestion. Future studies employing experimental hydrolysis followed by liquid chromatography-tandem mass spectrometry are needed to confirm the presence of these peptides in egg protein hydrolysates.

To better identify peptides with potential sleep-promoting activity, Autodock Vina was employed for docking simulations with GABAAR, OX2R, and NOPR. Peptides YFYPEL^[7] and DIQK^[15] were used as positive controls because they have been previously reported to exhibit sleep-promoting activities in non-addictive models, and their binding affinities provide a standard for evaluating our candidates. Binding affinity quantifies the strength of the interaction between a peptide and its receptor. The affinities of peptide YFYPEL with GABAAR, OX2R, and NOPR were -8.5, -11.0 and -9.9 kcal/mol, respectively. Similarly, peptide DIQK showed affinities of -7.0, -6.9 and -6.9 kcal/mol with the three receptors. Due to its role as the main inhibitory neurotransmitter receptor in the central nervous system, GABAAR is widely regarded as the primary target for sleep regulation^[23]. Based on the binding affinity of positive control peptide YFYPEL with GABAAR, we selected -8.5 kcal/mol as the threshold and identified six candidate peptides from 164 peptides (Table 1).

Table 1 The *in silico* water solubility, toxicity prediction and the docking affinity of egg-derived peptides with GABAAR, OX2R and NOPR.

Peptide sequence	Water solubility	Toxicity prediction	GABAAR (kcal/mol)	OX2R (kcal/mol)	NOPR (kcal/mol)
QPVDNY	Good	Non-toxin	-9.2	-9.7	-9.0
VEDIW	Good	Non-toxin	-8.7	-9.5	-8.2
LKPIAAEVY	Good	Non-toxin	-8.6	-9.7	-8.2
PTRPY	Good	Non-toxin	-8.6	-9.7	-8.1
DQLTPSPR	Good	Non-toxin	-8.6	-9.3	-7.9
NQITKPNDVYK	Good	Non-toxin	-8.5	-9.1	-7.5
YFYPEL (control)	Good	Non-toxin	-8.5	-11.0	-9.9
DIQK (control)	Good	Non-toxin	-7.0	-6.9	-6.9

Note: Six peptides were screened using the affinity of YFYPEL (positive control) with GABAAR as the threshold, and then ranked in ascending order based on the affinities of peptides with GABAAR.

A lower binding energy indicates a stronger interaction between a peptide and its receptor. The six peptides were ranked in the order of QPVDNY, VEDIW, LKPIAAEVY, PTRPY, DQLTPSPR, and NQITKPNDV based on their affinity with GABAAR. Peptide QPVDNY showed the strongest binding affinities with GABAAR, OX2R, and NOPR, with the scores of -9.2, -9.7 and -9.0 kcal/mol, respectively. Subsequently, peptides VEDIW and LKPIAAEVY exhibited binding affinities with GABAAR of -8.7 and -8.6 kcal/mol, respectively. Both displayed the same affinity with NOPR of -8.2 kcal/mol. However, their binding affinities with OX2R were -9.5 and -9.7 kcal/mol, respectively. Comparative analysis indicates that peptide LKPIAAEVY displays a stronger binding affinity than VEDIW. To further evaluate the potential of our peptides, they were compared with the highly active peptides FAWPQY and PVVPPF. The affinities of peptide FAWPQY with GABAAR and OX2R were -10.2 and -10.5 kcal/mol, respectively^[24]. Similarly, peptide PVVPPF showed affinities of -9.5 and -10.5 kcal/mol with the two receptors^[24]. Peptides QPVDNY and LKPIAAEVY exhibited binding affinities to GABAAR and OX2R comparable to peptides FAWPQY and PVVPPF. Therefore, these two peptides were selected for further docking analysis.

3.2 Molecular docking analysis of peptides with GABAAR

The interactions of the potential sleep-promoting peptides QPVDNY and LKPIAAEVY with the sleep-promoting targets GABAAR, OX2R and NOPR were explored, respectively. Peptide QPVDNY formed π -alkyl and π - σ bonds with residues Ala252, Leu259 and Val257 of GABAAR and bound to residues Thr256 and Thr278 of GABAAR via hydrogen bonds (Figure 1A). Peptide LKPIAAEVY formed a salt bridge with residue Arg269 of GABAAR and bound to residues Val257, Leu259, Leu264, Leu274, Ile271 and His267 via π -alkyl bonds. In addition, peptide LKPIAAEVY formed six hydrogen bonds with Thr261, Thr266, Thr268, His267, Glu270 and Asn275 of GABAAR and bound to residue Thr268 of GABAAR via π - σ bonds (Figure 1B).

They bind to the same two hydrophobic residues, Val257 and Leu259, through π -alkyl bonds. GABAAR is not a simple protein with a single active site, rather, it possesses binding pockets formed

by interfacial residues from different subunits^[23]. The M2 helix of the β -subunit contributes to forming the chloride ion channel pore together with the M2 helices of other subunits^[23]. The residues Leu259, Thr256, His267, and Glu270 in the binding sites of peptides QPVDNY and LKPIAAEVY with GABAAR are located within the M2 helix of the β_2 -subunit. Their binding may induce conformational changes that affect channel gating. Previous molecular dynamics studies have demonstrated that the β_2/α_1 interface of GABAAR undergoes opening due to outward shift of loop C upon ligand binding, and charged residues at the top of the channel pore can trap chloride ions to modulate pore properties^[25–26]. Based on these findings, we hypothesize that QPVDNY and LKPIAAEVY may act as positive allosteric modulators or agonists that promote chloride ion influx by stabilizing the open channel conformation. However, this hypothesis still requires further experimental verification.

3.3 Molecular docking analysis of peptides with OX2R

Peptide QPVDNY formed π -alkyl bonds with residues Pro131, Cys107 and Val209 of OX2R and bound to residues Asn324, His350, Tyr343 and Asp115 of OX2R via hydrogen bonds (Figure 2A). Peptide LKPIAAEVY formed carbon hydrogen bond with residue Pro131 of OX2R and bound to residues Thr231, Asn324, Arg328, Arg339 and Lys327 of OX2R via hydrogen bonds. In addition, peptide LKPIAAEVY formed three π - σ bonds with Phe227, Phe346 and His350 of OX2R and bound to residues Val209, Val138, Tyr343, Tyr354, Ile320 and Met191 of OX2R via π -alkyl bonds (Figure 2B).

Compared to the previous analysis of the two peptides with GABAAR, they exhibit significantly more shared interaction sites when binding to OX2R. Both peptides bind to Val209 and Asn324 of OX2R via π -alkyl bond and hydrogen bond, they also interact with Pro131, His350 and Tyr343 through different types of interactions. Residues Pro131, Phe227, His350, Gln134, Cys107, Leu26, Met28, Gly24, Ala22 and Thr27 are located within the classic ligand-binding pocket of OX2R. Among these residues, peptides QPVDNY and LKPIAAEVY interact with residues Pro131, Cys107, Phe227, Phe346 and His350. Importantly, Phe227 and Phe346 are known to play crucial hydrophobic roles in the binding of the antagonist Suvorexant with OX2R, demonstrating that our peptides target the correct binding pocket^[27]. Residues

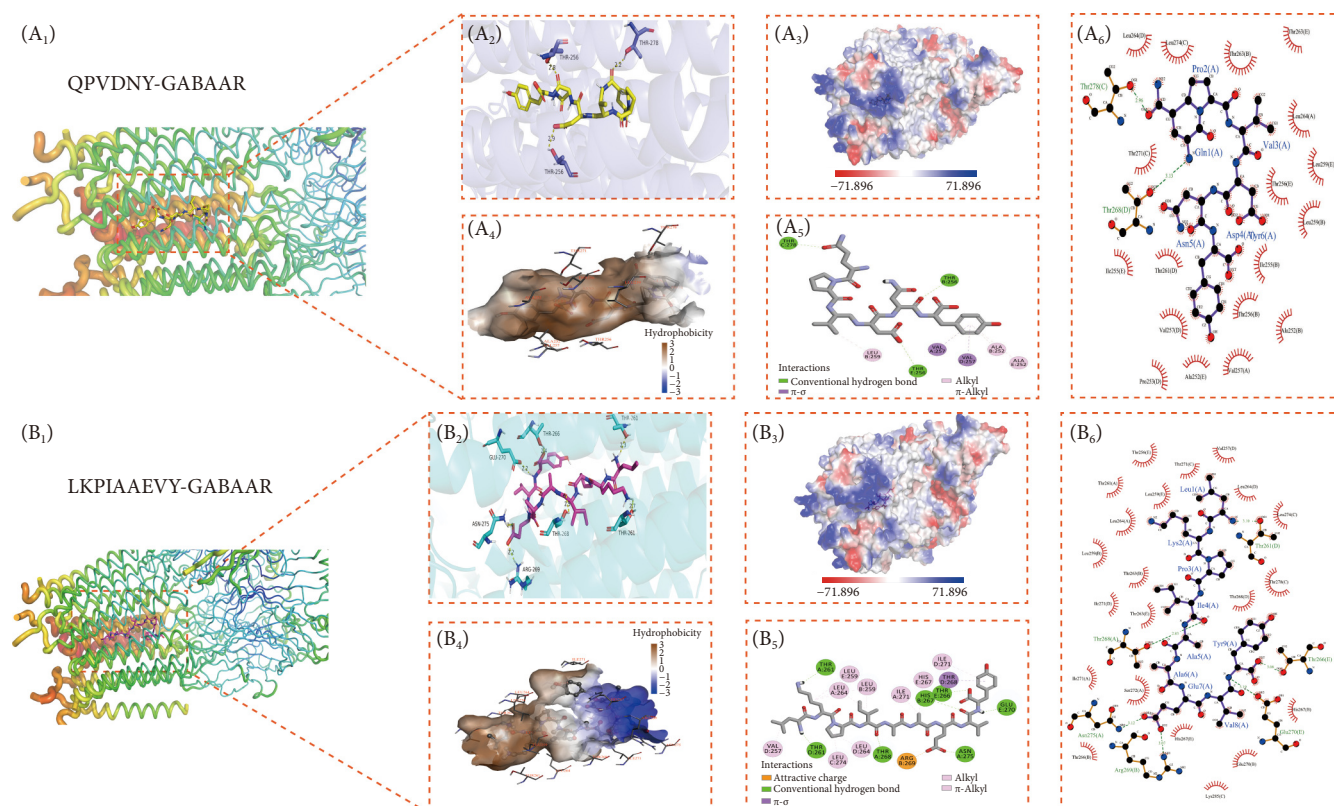


Figure 1 The docking interactions of (A) QPVDNY and (B) LKPIAAEVY with GABAAR. Subscript 1, the crystal structure of the two combined; subscript 2, the 3D diagram of their interaction, the yellow color represents hydrogen bond interaction; subscript 3, the electrostatic potential of GABAAR; subscript 4, the hydrophobic interfaces of the two combined; subscript 5, the 2D diagram of their interaction; subscript 6, the another 2D diagram form of the interaction.

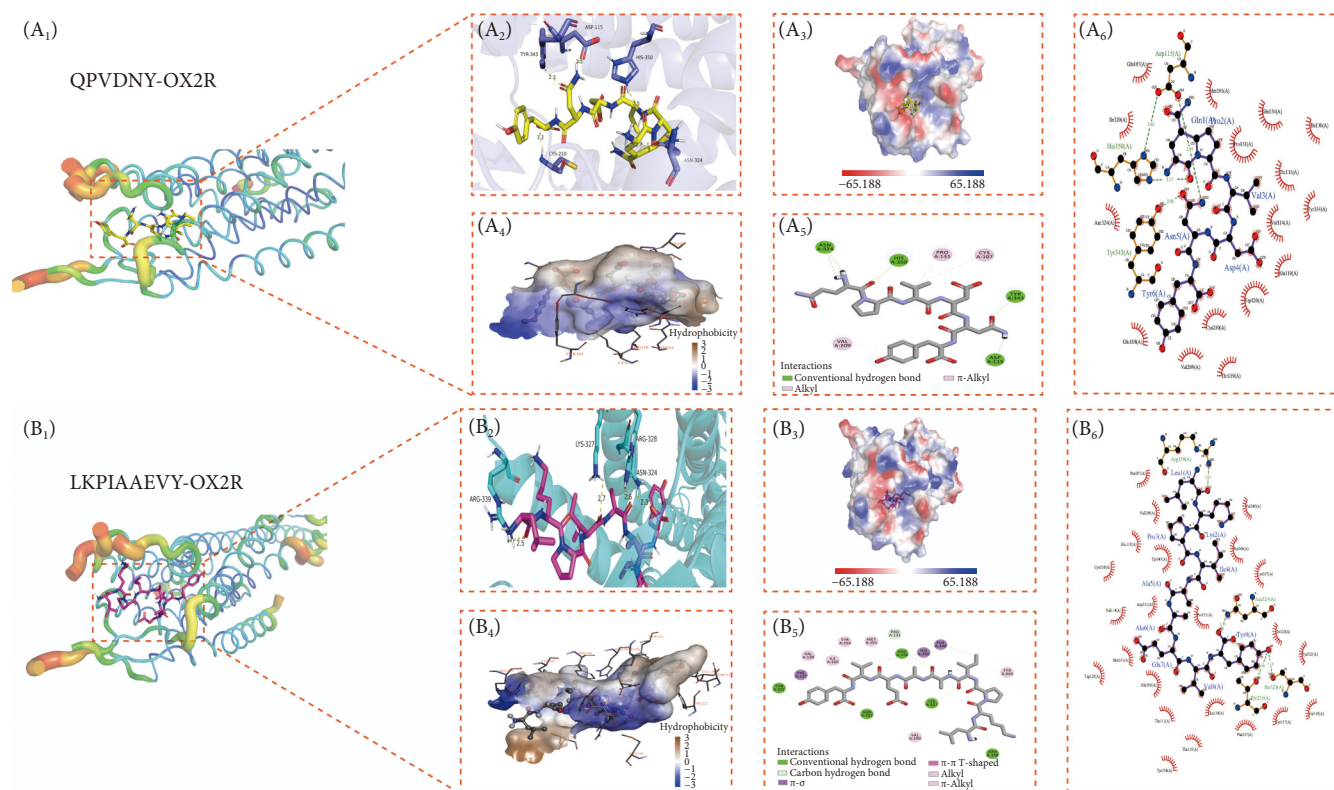


Figure 2 The docking interactions of (A) QPVDNY and (B) LKPIAAEVY with OX2R. Subscript 1, the crystal structure of the two combined; subscript 2, the 3D diagram of their interaction, the yellow color represents hydrogen bond interaction; subscript 3, the electrostatic potential of OX2R; subscript 4, the hydrophobic interfaces of the two combined; subscript 5, the 2D diagram of their interaction; subscript 6, the another 2D diagram form of the interaction.

Trp200 on TM3 and Tyr362 on TM7 are the two most conserved hydrogen-bond anchors in the pocket of OX2R^[28].

The observation that our peptides do not bind to residues Trp200 and Tyr362 suggests a mode of interaction distinct from classical antagonists such as suvorexant, which stabilizes an inactive receptor conformation by forming specific contacts with Phe227, Phe346, and other hydrophobic residues within the orthosteric pocket^[27–28]. Molecular dynamics simulations have shown that the horseshoe-shaped OX2R binding pocket undergoes conformational changes upon antagonist binding, with residues such as Asn324 playing critical roles in stabilizing ligand orientation^[28–29]. Our peptides bind to Asn324 via hydrogen bonds, which may induce pocket rearrangements leading to receptor inhibition. However, unlike suvorexant, they interact with only a subset of hydrophobic pocket residues and do not engage Trp200 or Tyr362. This binding result suggests that QPVDNY and LKPIAAEVY may function as negative allosteric modulators, inhibiting OX2R signaling through a distinct mechanism, potentially by stabilizing a partially inactive conformation without directly competing with orexin. Further functional assays using calcium mobilization or cAMP accumulation are needed to confirm this hypothesis.

3.4 Molecular docking analysis of peptides with NOPR

Peptide QPVDNY formed π -alkyl bonds with residues Ala216, Val283 and Met134 of NOPR and bound to residues Asp130 and Tyr131 of NOPR via hydrogen bonds (Figure 3A). Peptide LKPIAAEVY formed a salt bridge with residue Asp130 of NOPR and bound to residues Gln286, Tyr131, Arg302 and Val202 of

NOPR via hydrogen bonds. In addition, peptide LKPIAAEVY formed five π -alkyl bonds with Val283, Met134, Leu301, Ile219 and Ile204 of NOPR (Figure 3B).

When binding to NOPR, the two peptides both bound to key residues Val283, Met134 and Tyr131, forming π -alkyl bonds and hydrogen bonds. These residues have been clearly shown to constitute the conserved hydrophobic core of the pocket in NOPR^[30]. Additionally, both peptides target the residue Asp130 through hydrogen bonds and a salt bridge, which serves as the determinative residue for the recognition of nearly all ligands by NOPR, validating the rationality of our peptides binding mode at the atomic level.

The NOPR has emerged as a promising therapeutic target for sleep disorders, with selective NOP agonists demonstrating significant sleep-promoting effects in clinical trials^[31]. Binding to the conserved hydrophobic core residues Val283, Met134 and Tyr131 together with the determinative residue Asp130 is characteristic of both agonists and antagonists, indicating that these interactions alone do not define functional outcome. Previous structure-activity relationship studies have shown that different ligands for the NOPR can induce distinct signaling pathways, including biased agonism toward G protein versus β -arrestin pathways^[32–33]. Based on the binding mode observed for QPVDNY and LKPIAAEVY, it is possible that these peptides act as agonists that stabilize an active receptor conformation, potentially promoting sleep through $G_{i/o}$ -mediated inhibition of arousal-promoting neurons. However, exact classification requires experimental validation using receptor-specific functional assays.

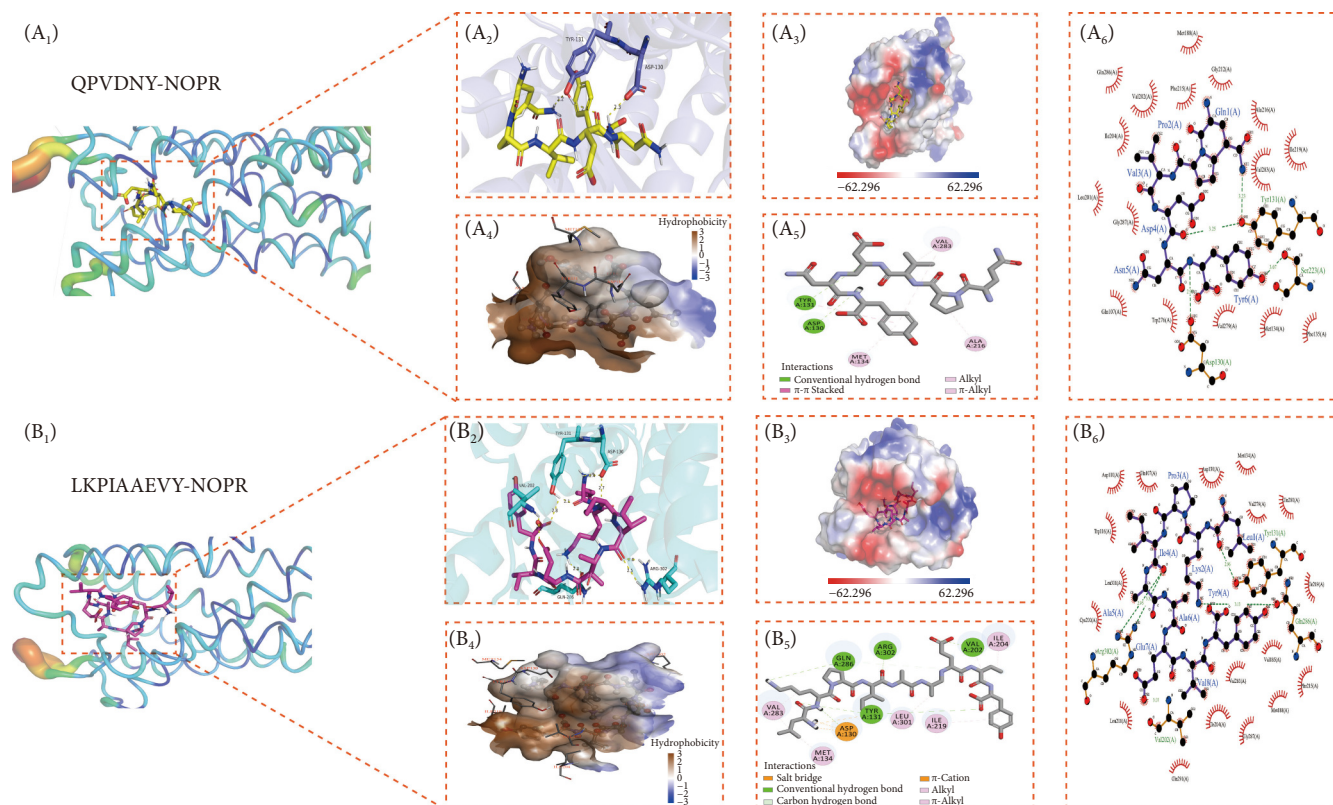


Figure 3 The docking interactions of (A) QPVDNY and (B) LKPIAAEVY with NOPR. Subscript 1, the crystal structure of the two combined; subscript 2, the 3D diagram of their interaction, the yellow color represents hydrogen bond interaction; subscript 3, the electrostatic potential of NOPR; subscript 4, the hydrophobic interfaces of the two combined; subscript 5, the 2D diagram of their interaction; subscript 6, the another 2D diagram form of the interaction.

3.5 Molecular dynamics simulation reveals dynamic binding stability

Molecular dynamics simulations were performed for the two peptides with their corresponding three receptor proteins. The RMSD of these complexes increased rapidly during the initial phase of the simulation and then entered a phase of stable fluctuation. Subsequently, all proteins and complexes exhibited only minor fluctuations within 70–100 ns, consistently maintaining relative stability and forming a distinct plateau phase (Figure 4A). This indicates that these complexes exhibited good overall structural stability under the simulation conditions.

RMSF analysis revealed distinct flexibility patterns across the protein structures. The majority of residues exhibited low fluctuations, indicating a stable protein core. However, several

localized regions displayed significantly higher flexibility, particularly in solvent-exposed loops, terminal regions, and functional sites such as ligand-binding pockets.

Since the GABAAR structure comprises six chains and the peptide binding sites identified by docking are predominantly located on chain A, RMSF analysis was performed specifically on chain A. As shown in Figure 4B₁, pronounced fluctuations were observed at residues Arg177, Glu183, and Trp317 in the unliganded GABAAR. In the GABAAR-QPVDNY complex, additional fluctuations appeared at Lys320, while the GABAAR-LKPAAEVY complex exhibited further fluctuations at Lys78 and Arg187. Flexible residues Arg177, Glu183, and Trp317 are located in the intracellular loop involved in GABAAR trafficking^[34], and their rigidification upon peptide binding suggests stabilization beyond the channel pore. Due to missing segments in the OX2R PDB

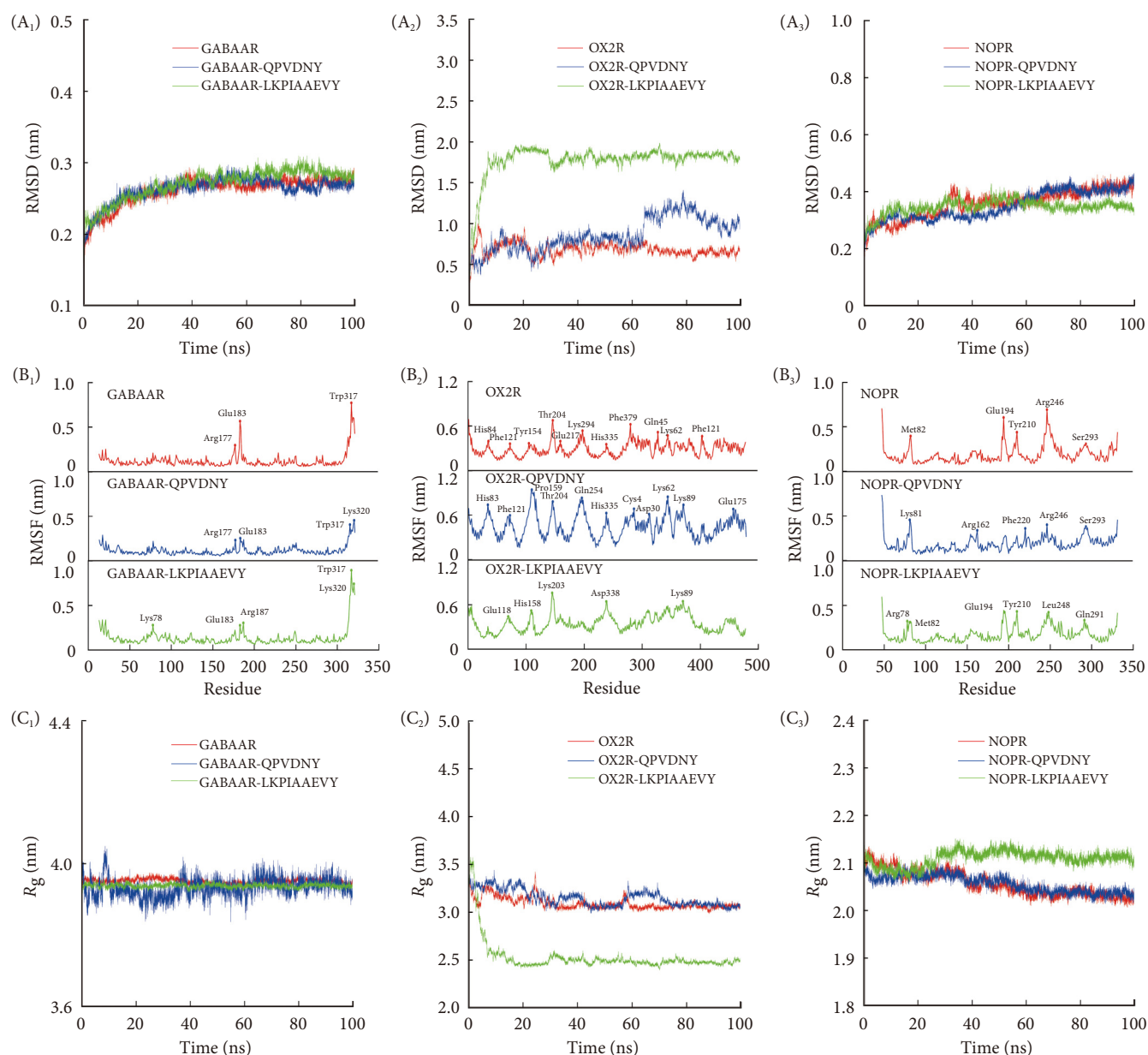


Figure 4 The results of molecular dynamics simulations. RMSD of (A₁) GABAAR, (A₂) OX2R and (A₃) NOPR with peptides, respectively. RMSF of (B₁) GABAAR, (B₂) OX2R and (B₃) NOPR with peptides, respectively. R_g of (C₁) GABAAR, (C₂) OX2R and (C₃) NOPR with peptides, respectively. Number of hydrogen bonds of (D₁–D₂) GABAAR, (D₃–D₄) OX2R and (D₅–D₆) NOPR with peptides, respectively. SASA of (E₁) GABAAR, (E₂) OX2R and (E₃) NOPR with peptides, respectively.

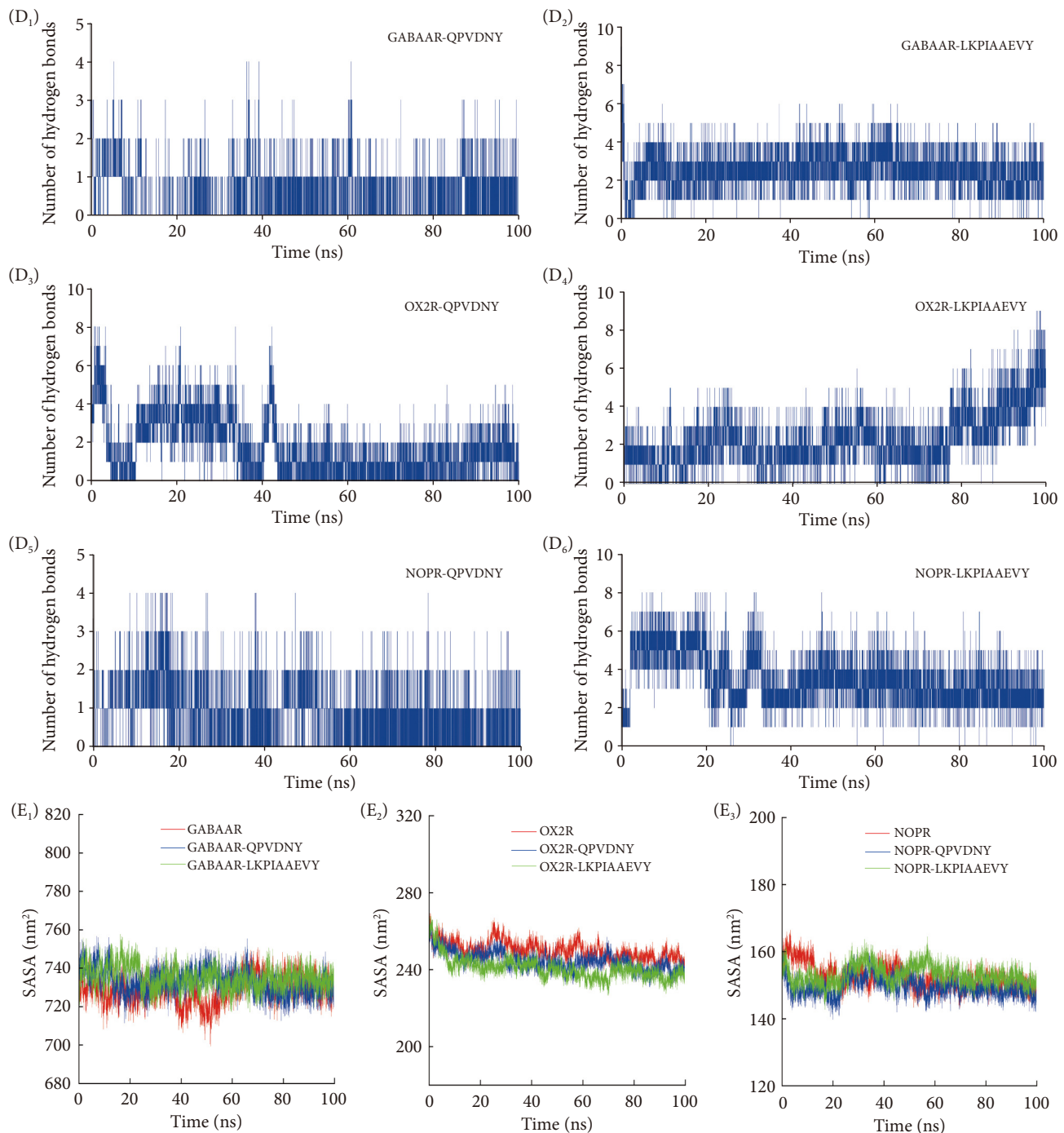


Figure 4 (Continued)

structure, residues were renumbered for analysis. Meanwhile, the reported fluctuating residues correspond to their original PDB numbering. Extensive fluctuations were observed in the OX2R-QPVDNY complex and OX2R-LKPIAAEVY complex, particularly at residues Phe121, Thr204, His335, Lys62, and Lys89 (Figure 4B₂). The retained flexibility in these extracellular loop residues after peptides binding, combined with the lack of engagement with Trp200 and Tyr362, supports a negative allosteric modulator mode^[35]. The NOPR-QPVDNY complex and the NOPR-LKPIAAEVY complex exhibited fewer flexible regions compared to OX2R, with significant fluctuations observed only at Met82, Glu194, Arg246, and Ser293 (Figure 4B₃). The low overall flexibility of NOPR and

the stabilization of its conserved hydrophobic core are consistent with the predicted agonist mode. These results indicate that peptide binding rigidifies the orthosteric pockets of all three receptors while preserving functionally relevant peripheral dynamics^[36], demonstrating that QPVDNY and LKPIAAEVY stabilize the spatial structure of the peptide-receptor complexes.

After the simulation reached 40 ns, the R_g of all proteins and complexes exhibited overall fluctuation of less than 0.3 nm, consistently remaining within a relatively stable range (Figure 4C). This indicates that the protein conformations are compact and stable. The R_g curve did not show significant directional changes, suggesting that the binding of peptides QPVDNY and

LKPIAAEVY did not induce large-scale conformational rearrangements in these proteins.

The complexes maintained a stable number of hydrogen bonds throughout the simulation. It is generally accepted that an average hydrogen bond count of 2 or more, with a relatively long occupancy, indicates stable and specific interactions between the ligand and receptor^[37]. Although the number of hydrogen bonds in this system exhibited some fluctuations, it remained within a stable range overall without prolonged disappearance (Figure 4D), signifying favorable binding stability of the ligand within the pocket.

The SASA of the proteins and complexes remained stable throughout the simulation trajectory, exhibiting only minor fluctuations around its mean value (Figure 4E). This indicates that the solvent exposure of the system remained essentially constant^[38]. The stable global SASA suggests the absence of major structural rearrangements, implying that the binding pocket likely maintained its architecture without drastic opening or closing, thereby supporting the overall stability of the complexes.

4 Conclusion

Peptides QPVDNY and LKPIAAEVY were identified through our work. Molecular docking revealed that these two peptides interact with crucial residues in the receptor binding pockets, suggesting potential modulation of receptor activity. The stability of the peptide-receptor complexes was confirmed by 100 ns molecular dynamics simulations, as evidenced by stable RMSD and RMSF fluctuation, consistent hydrogen bonding, and stable compactness (R_g), indicating robust binding under physiological conditions.

These findings indicate that egg proteins, particularly ovotransferrin and ovomucin, are promising sources of potential sleep-promoting peptides. The computational approach not only accelerates peptide discovery but also reveals mechanism at the molecular level. This work lays the foundation for egg-derived functional foods and natural sleep aids, contributing to the growing field of food-derived bioactive peptides for health promotion. To validate the computational predictions, further *in vitro/in vivo* experiments are necessary to determine whether these peptides act as agonists, antagonists, or modulators of GABAAR, OX2R, and NOPR. These predictions should be complemented by animal models to confirm their sleep-promoting effects and evaluate biosafety.

Conflicts of interest

The authors have declared no conflict of interest.

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Author contributions

Zexuan Ren: Data curation, formal analysis, writing-original draft. Xiaowang Wang: Methodology, validation. Wenzhu Zhao: Visualization, software. Zhipeng Yu: Conceptualization, supervision, funding acquisition, writing-review & editing.

Data availability

The datasets generated and/or analyzed during the current study are not publicly available due to privacy or ethical restrictions but are available from the corresponding author on reasonable request.

References

- [1] A. R. Tall, S. Jelic, How broken sleep promotes cardiovascular disease, *Nature* 566 (2019) 329–330. <https://doi.org/10.1038/d41586-019-00393-6>.
- [2] A. Smith, V. Raghavan, Statistical analysis of the effect of simulation time on the results of molecular dynamics studies of food proteins: a study of the Ara h 6 peanut protein, *Processes* 13 (2025) 581. <https://doi.org/10.3390/pr13020581>.
- [3] M. Zhou, S. Tang, Effect of a dual orexin receptor antagonist on Alzheimer's disease: sleep disorders and cognition, *Front. Med.* 9 (2023) 984227. <https://doi.org/10.3389/fmed.2022.984227>.
- [4] C. Baglioni, S. Nanovska, W. Regen, et al., Sleep and mental disorders: a meta-analysis of polysomnographic research, *Psychol. Bull.* 142 (2016) 969–990. <https://doi.org/10.1037/bul0000053>.
- [5] J. Shao, X. Zheng, L. Qu, et al., Ginsenoside Rg5/Rk1 ameliorated sleep via regulating the GABAergic/serotonergic signaling pathway in rodent model, *Food Funct.* 11 (2020) 1245–1257. <https://doi.org/10.1039/c9fo02248a>.
- [6] V. Navarrete-Anaya, I. Delgado-Enciso, G. A. Hernández-Fuentes, et al., Insomnia and benzodiazepine use as risk factors for erectile dysfunction: clinical evidence and *in silico* analysis of physicochemical properties, *J. Clin. Med.* 14 (2025) 6951. <https://doi.org/10.3390/jcm14196951>.
- [7] J. Qian, L. Zheng, G. Su, et al., Identification and screening of potential bioactive peptides with sleep-enhancing effects in bovine milk casein hydrolysate, *J. Agric. Food Chem.* 69 (2021) 11246–11258. <https://doi.org/10.1021/acs.jafc.1c03937>.
- [8] E. Sigel, M. E. Steinmann, Structure, function, and modulation of GABAA receptor, *J. Biol. Chem.* 287 (2012) 40224–40231. <https://doi.org/10.1074/jbc.R112.386664>.
- [9] J. W. Kim, C. S. Kim, Z. Hu, et al., Enhancement of pentobarbital-induced sleep by apigenin through chloride ion channel activation, *Arch. Pharm. Res.* 35 (2012) 367–373. <https://doi.org/10.1007/s12272-012-0218-4>.
- [10] R. W. Olsen, GABAA receptor: positive and negative allosteric modulators, *Neuropharmacology* 136 (2018) 10–22. <https://doi.org/10.1016/j.neuropharm.2018.01.036>.
- [11] C. B. Saper, P. M. Fuller, N. P. Pedersen, et al., Sleep state switching, *Neuron* 68 (2010) 1023–1042. <https://doi.org/10.1016/j.neuron.2010.11.032>.
- [12] M. E. Carter, A. Adamantidis, H. Ohtsu, et al., Sleep homeostasis modulates hypocretin-mediated sleep-to-wake transitions, *J. Neurosci.* 29 (2009) 10939–10949. <https://doi.org/10.1523/jneurosci.1205-09.2009>.
- [13] M. E. Carter, J. Brill, P. Bonnavion, et al., Mechanism for hypocretin-mediated sleep-to-wake transitions, *Proc. Natl. Acad. Sci. U. S. A.* 109 (2012) e2635–e2644. <https://doi.org/10.1073/pnas.1202526109>.
- [14] M. Michihiro, H. Emi, Y. Y. Kisanuki, et al., Differential roles of orexin receptor-1 and -2 in the regulation of non-REM and REM sleep, *J. Neurosci.* 71 (2011) 6518–6526. <https://doi.org/10.1523/JNEUROSCI.6506-10.2011>.
- [15] L. Mo, S. Wang, B. Wu, et al., Identification and exploration of sleep-enhancing peptides from goat milk casein by peptidomics and virtual screening, *Food Res. Int.* 221 (2025) 117333. <https://doi.org/10.1016/j.foodres.2025.117333>.

- [16] S. R. Morairty, Y. Sun, L. Toll, et al., Activation of the nociceptin/orphanin-FQ receptor promotes NREM sleep and EEG slow wave activity, *Proc. Natl. Acad. Sci. USA* 120 (2023) e2214171120. <https://doi.org/10.1073/pnas.2214171120>.
- [17] Z. Wei, S. Wu, J. Xia, et al., Enhanced antibacterial activity of hen egg-white lysozyme against *Staphylococcus aureus* and *Escherichia coli* due to protein fibrillation, *Biomacromolecules* 22 (2021) 890–897. <https://doi.org/10.1021/acs.biomac.0c01599>.
- [18] J. Wang, W. Liao, C. Nimalaratne, et al., Purification and characterization of antioxidant peptides from cooked eggs using a dynamic *in vitro* gastrointestinal model in vascular smooth muscle A7r5 cells, *NPJ Sci. Food* 2 (2018) 7. <https://doi.org/10.1038/s41538-018-0015-7>.
- [19] Z. Yu, W. Xue, M. Zhou, et al., Potential antihypertensive mechanisms of the egg white-derived peptide QIGLF in spontaneously hypertensive rats revealed using untargeted serum metabolomics, *J. Agric. Food Chem.* 69 (2021) 12063–12071. <https://doi.org/10.1021/acs.jafc.1c05599>.
- [20] A. B. S. Luz, A. F. de Medeiros, G. C. B. S. de Medeiros, et al., Experimental protocols used to mimic gastrointestinal protein digestion: a systematic review, *Nutrients* 16 (2024) 2398. <https://doi.org/10.3390/nu16152398>.
- [21] S. Sinha, B. Tam, S. M. Wang, Applications of molecular dynamics simulation in protein study, *Membranes* 12 (2022) 844. <https://doi.org/10.3390/membranes12090844>.
- [22] Z. Yu, R. Kan, H. Ji, et al., Identification of tuna protein-derived peptides as potent SARS-CoV-2 inhibitors via molecular docking and molecular dynamic simulation, *Food Chem.* 342 (2020) 128366. <https://doi.org/10.1016/j.foodchem.2020.128366>.
- [23] C. Sun, H. Zhu, S. Clark, et al., Cryo-EM structures reveal native GABAA receptor assemblies and pharmacology, *Nature* 622 (2023) 195–201. <https://doi.org/10.1038/s41586-023-06556-w>.
- [24] W. Liu, S. Hou, Q. Zhang, et al., Food-derived bioactive peptides for anxiety and sleep management: mechanistic insights, bidirectional interactions, structure-activity relationship, and targeted preparation, *Trends Food Sci. Technol.* 168 (2025) 105468. <https://doi.org/10.1016/j.tifs.2025.105468>.
- [25] M. A. Michałowski, S. Kraszewski, J. W. Mozrzymas, Binding site opening by loop C shift and chloride ion-pore interaction in the GABAA receptor model, *Phys. Chem. Chem. Phys.* 19 (2017) 13664–13678. <https://doi.org/10.1039/C7CP00582B>.
- [26] A. Garifulina, T. Friesacher, M. Stadler, et al., β Subunits of GABA_A receptors form proton-gated chloride channels: insights into the molecular basis, *Commun. Biol.* 5 (2022) 784. <https://doi.org/10.1038/s42003-022-03720-2>.
- [27] C. Hong, N. J. Byrne, B. Zamlynny, et al., Structures of active-state orexin receptor 2 rationalize peptide and small-molecule agonist recognition and receptor activation, *Nat. Commun.* 12 (2021) 815. <https://doi.org/10.1038/s41467-021-21087-6>.
- [28] J. Yin, K. Babaoglu, C. Brautigam, et al., Structure and ligand-binding mechanism of the human OX1 and OX2 orexin receptors, *Nat. Struct. Mol. Biol.* 23 (2016) 293–299. <https://doi.org/10.1038/nsmb.3183>.
- [29] Q. Bai, H. Pérez-Sánchez, Z. Shi, et al., Computational studies on horseshoe shape pocket of human orexin receptor type 2 and boat conformation of suvorexant by molecular dynamics simulations, *Chem. Biol. Drug Des.* 92 (2018) 1221–1231. <https://doi.org/10.1111/cbdd.13181>.
- [30] A. Thompson, W. Liu, E. Chun, et al., Structure of the nociceptin/orphanin FQ receptor in complex with a peptide mimetic, *Nature* 485 (2012) 395–399. <https://doi.org/10.1038/nature11085>.
- [31] C. Mustazza, S. Pieretti, F. Marzoli, Nociceptin/orphanin FQ peptide (NOP) receptor modulators: an update in structure-activity relationships, *Curr. Med. Chem.* 25 (2018) 2353–2384. <https://doi.org/10.2174/0929867325666180111095458>.
- [32] S. Pacifico, F. Ferrari, V. Ambrosio, et al., Biased agonism at nociceptin/orphanin FQ receptors: a structure activity study on N/OFQ(1-13)-NH₂, *J. Med. Chem.* 63 (2020) 10782–10795. <https://doi.org/10.1021/acs.jmedchem.9b02057>.
- [33] D. Malfacini, C. Ambrosio, M. C. Gro', et al., Pharmacological profile of nociceptin/orphanin FQ receptors interacting with G-proteins and β -arrestins 2, *PLoS ONE* 10 (2015) e0132865. <https://doi.org/10.1371/journal.pone.0132865>.
- [34] T. C. Jacobson, GABA type A receptor trafficking and the architecture of synaptic inhibition, *Dev. Neurobiol.* 78 (2018) 238–270. <https://doi.org/10.1002/dneu.22536>.
- [35] M. C. Peeters, G. J. P. van Westen, Q. Li, et al., Importance of the extracellular loops in G protein-coupled receptors for ligand recognition and receptor activation, *Trends Pharmacol. Sci.* 32 (2011) 35–42. <https://doi.org/10.1016/j.tips.2010.10.001>.
- [36] X. Deupi, B. K. Kobilka, Structural insights into agonist-induced activation of G-protein-coupled receptors, *Curr. Opin. Struct. Biol.* 21 (2011) 541–551. <https://doi.org/10.1016/j.sbi.2011.06.002>.
- [37] S. Gorelov, A. Titov, O. Tolicheva, et al., Determination of hydrogen bonds in GROMACS: a new implementation to overcome memory limitation, *J. Chem. Inf. Model.* 64 (2024) 6241–6246. <https://doi.org/10.1021/acs.jcim.3c02087>.
- [38] Z. S. Chen, Y. D. Wu, J. H. Hao, et al., Molecular dynamic simulation of the porcine pancreatic lipase in non-aqueous organic solvents, *Front. Bioeng. Biotechnol.* 8 (2020) 676. <https://doi.org/10.3389/fbioe.2020.00676>.