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Recombinant production and characterization of the integrin-binding LERGDT peptide

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Abstract

Chronic kidney disease (CKD) remains a global health burden, necessitating the development of scalable therapeutic interventions. While the LERGDT peptide has previously demonstrated integrin-binding and anti-fibrotic activity, its transition toward clinical applications is hindered by the high cost of large-scale chemical synthesis. This study presents a robust bioprocess for the recombinant production of a 20-repeat tandem LERGDT construct (PEP-1) using an *Escherichia coli* expression system. To ensure structural stability and protection against proteolytic degradation, PEP-1 was engineered as a fusion protein with N-terminal ketosteroid isomerase (KSI) and a C-terminal six-histidine tag. *In silico* analysis using AlphaFold and HADDOCK predicted high structural stability and specific binding affinity to integrins. The bioprocess system achieved a significant total protein yield of 52.94 mg.L^{-1}, with the fused protein purified via nickel-affinity chromatography and successfully cleaved into functional fragments (hPEP-1) using hydroxylamine hydrolysis. Product identity was confirmed by means of SDS-PAGE at ~35 kDa and validated by using tandem LC-MS. ELISA-based assays confirmed that both the fusion protein and released fragments maintained specific integrin-binding functionality. By establishing a high-yield, reproducible production protocol, this work provides a cost-effective alternative to synthetic methods, facilitating large-scale testing required for peptide-based CKD therapeutics. This scalable bioprocess accelerates the translation of LERGDT-based interventions from bench to bedside, enhancing the accessibility of affordable long-term treatments for CKD patients.

Keywords: Chronic kidney disease; LERGDT peptide; protein recombinant; bioinformatics; integrin

1. Introduction

Chronic kidney disease (CKD) is a prevalent global health issue characterized by a progressive loss of kidney function, triggering end-stage renal disease [1-2]. Currently, therapeutic strategies focus primarily on symptom management and delaying disease progression [3-4]. However, there remains a critical need for novel therapies that can effectively halt or reverse the progression of CKD [5]. Recent advances in biotechnology have highlighted the potential of recombinant peptides in therapeutic applications [6-7]. One of the most promising candidates is the LERGDT peptide. Hidayat et al. identified the LERGDT peptide, isolated from green pea protein hydrolysate, as a promising candidate for treating chronic kidney disease (CKD). Their study demonstrated its therapeutic potential in modulating key pathways involved in the disease's progression. This peptide functions by binding to integrin receptors, a key pathway involved in CKD pathogenesis, offering hope for new treatment methods [8-11]. While the therapeutic efficacy of LERGDT in CKD has been established via protein hydrolysis [8-11], the high-purity, large-scale production of this specific sequence remains a challenge. On one hand, chemical synthesis is a high-cost approach for long tandem repeats; on the other hand, hydrolysis often yields heterogeneous mixtures.

To resolve these issues, the production of recombinant peptides can be a solution. By integrating bioinformatic approaches and optimization of expression conditions, we aim to yield production of the soluble target active peptide. The peptide will be used for a future series of biochemical assays to verify the identity, purity, and bioactivity of the peptide

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[12-13].

In this study, we aim to express a recombinant anti-CKD peptide using *Escherichia coli* as an expression system. The *E. coli* system offers a cost-effective and efficient platform for the production of recombinant proteins. We address this gap by developing a robust bioprocess using a KSI-fusion system in *E. coli*. We focus on optimizing recombinant expression, chemical cleavage efficiency, and the confirmation of activity of the resulting peptide fragments against integrin. We expect it to contribute to the growing body of research on innovative CKD therapies, ultimately providing a foundation for future preclinical and clinical studies [5].

2. Materials and Methods

2.1. Bioinformatic analysis

The recombinant anti-CKD candidate, LERGDT, is designed in repeat-tandems. The gene contained 20 repetitions of LERGDT (PEP-1), with asparagine and glycine as chemical cleavage sites. The following is the amino acid sequence:

LERGDTNGLERGDTNGLERGDTNGLERGDTNGLERGDTNGL
ERGDTNGLERGDTNGLERGDTNGLERGDTNGLERGDTNGLE
RGDTNGLERGDTNGLERGDTNGLERGDTNGLERGDTNGLER
GDTNGLERGDTNGLERGDTNGLERGDTNGLERGDT

The expression of the PEP-1 fused with KSI (KSI-PEP-1). The structure of KSI-PEP-1 was predicted and analyzed using the AlphaFold webserver (https://alphafold.ebi.ac.uk/) [14-15]. Further analysis using PROCHECK [16] to examine the Ramachandran plot [17], which is a 2D graph of the protein backbone torsion angles and amino acid conformations of the structure predicted using AlphaFold. Next, the solubility of KSI-PEP-1 was analyzed using Protein-Sol [18] (https://protein-sol.manchester.ac.uk/), a web server used to predict protein solubility based on amino acid sequences. Finally, the binding interactions of the fusion protein were modeled and analyzed using the HADDOCK 2.4 webserver [19] (https://wenmr.science.uu.nl/haddock2.4/).

2.2. Material

All chemicals used in this study were commercially obtained from Sigma-Aldrich or Merck, unless otherwise stated on the product. The study used *E. coli* BL21(DE3) as an expression host and *E. coli* TOP10F' as a host for subcloning and plasmid amplification. The expression plasmid pET-31b(+) was used, with the 20-repeat-tandem LERGDT coding sequence placed downstream of a ketosteroid isomerase (KSI)1 gene and upstream of a His-Tag sequence.

2.3. Methods

2.3.1. Purification of recombinant KSI-LERGDT peptide

The recombinant plasmid pET31b(+)-LERGDT was introduced into the *E. coli* strain BL21(DE3). A single colony was used to inoculate plates with LB medium supplemented with ampicillin (100 μg/ml) for expression. Plates were then cultured overnight at 37°C. Plated bacteria were used to inoculate 100 mL of antibiotic-treated LB medium to an λ600 nm ~0.1 and cultured at 37°C with vigorous shaking. When the OD600 reached 0.5, the expression of the fusion protein KSI-LERGDT-HisTag was induced by adding 1 mM IPTG and shaking for 5 hours. The cells were extracted by centrifugation (8000g, 4°C, 15 minutes) and kept at -80°C for further characterization.

Furthermore, 10 grams of frozen cells were loaded onto a nickel column and eluted using six column volumes of buffer. 2.5 ml fractions were collected and pooled for SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The concentration of the purified fusion protein was determined using the Bradford assay [20], and the purity of KSI-LERGDT-HisTag was analyzed using SDS-PAGE [21] in 10% of gel.

2.3.2. Peptide cleavage

KSI-PEP-1 was digested with hydroxylamine following the protocol established by Bornstein and Balian [22]. The resulting peptide fragments were subsequently analyzed and confirmed by commercial LC-MS analysis.

2.3.3. ELISA

A sandwich ELISA was conducted to quantify activity og PEP-1 against integrin (the ELK Biotechnologies ELISA kit, China). Briefly, 100 μL of standards or samples were added to pre-coated wells and incubated at 37 °C for 80 minutes. After four washes with PBS containing 0.05% Tween-20, 100 μL of primary antibody was added and incubated for 60 minutes at 37 °C. Following another wash cycle, 100 μL of horseradish peroxidase (HRP)-conjugated secondary antibody was applied and incubated under the same conditions. Wells were repeatedly washed, then 100 μL of 3,3',5,5'-tetramethylbenzidine (TMB) substrate was added and incubated in the dark for 15-20 minutes. The reaction was stopped with 100 μL of 2N H_2SO_4, and absorbance was measured at 450 nm.

3. Results and Discussion

3.1. Bioinformatics

The AlphaFold analysis indicates that peptide LERGDT is characterized by an extended β-sheet structure (Fig. 1(a)) with a spring-like configuration. This unique structural arrangement confirms that PEP-1 is sterically separated from KSI, allowing it to significantly enhance the solubility of KSI even when expressed as a fusion protein. Further validation based on the analysis of 118 high-resolution protein structures (with a resolution of at least 2.0 Å and an R-factor of no more than 20%) confirms that the AlphaFold model is of high quality. It is expected that more than 90% of the residues fall within the most favored regions. This is supported by the Ramachandran plot (Fig. 1(b)), which shows that 88.1% of the amino acid residues are located in the most favored regions, while the remaining 11.9% are in an additional favored region.

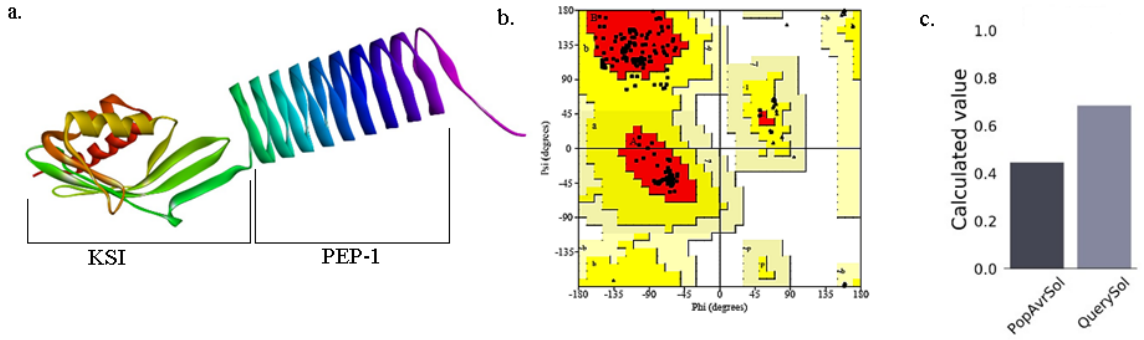

Fig. 1. (a) Predicted structure of protein fusion KSI-PEP-1 designed using AlphaFold, (b) Ramachandran plot of KSI-PEP1 analyzed with PROCHECK, and (c) Solubility analysis of KSI-PEP-1 compared to native soluble proteins from *E. coli* using Protein-Sol

Additionally, the solubility analysis indicates that the solubility value of the KSI-PEP-1 fusion protein is approximately 0.685, which is higher than the threshold of 0.45, indicating its water solubility (Fig. 1(c)). These findings suggest that KSI-PEP-1 displays high solubility, exceeding the average solubility of native *E. coli*-derived proteins, as reported in the dataset published by Niwa et al. (2009) [23]. Together, these results highlight the potential of KSI-peptide to improve the solubility of fusion proteins, providing a promising tool for protein engineering applications.

Structural analysis of the pre-designed PEP-1 sequences was conducted using HADDOCK 2.4. HADDOCK analysis was employed to evaluate molecular binding affinity and interaction characteristics, with the RGD-integrin complex (PDB ID: 4UM9) as a positive control. Key parameters: HADDOCK score, electrostatic and van der Waals energies, and RMSD, were used to assess binding strength and complex stability. Lower HADDOCK scores indicated stronger, more stable interactions.

Table 1. HADDOCK analysis of PEP-1 against integrin.
As a positive control used in this analysis was the RGD tripeptide

Parameter	Protein-Protein Complex	
	Integrin-LERGDT	Integrin-RGD
ΔG (K_{cal}.mol^{-1})	-7.6	-7.9
Kd (M at °C) × 10^{-6}	4.6	2.8
ICs charged-charged	8	10
ICs charged-polar	6	10
ICs charged-apolar	9	12
ICs polar-polar	5	0
ICs polar-apolar	9	4
ICs apolar-apolar	2	14
NIS charged	27.31	27.2
NIS apolar	39.17	39.42

HADDOCK analysis of PEP-1-integrin binding (Table 1) showed that PEP-1 exhibited a Gibbs free energy (ΔG) of -7.6 k_{cal}/mol and a dissociation constant (K_d) of 4.6 x 10^{-6} M, compared to -7.9 k_{cal}/mol and 2.8 x 10^{-6} M for the positive control. These values indicate strong and stable interactions at room temperature. Residue-level interaction analysis (IC) revealed that the control peptide formed more charge-charge (10 vs. 8), charge-polar (10 vs. 6), and apolar-apolar (12 vs. 9) contacts, enhancing complex stability. In contrast, PEP-1 showed fewer hydrophobic interactions (2 vs. 14), though both peptides had comparable non-interacting surface (NIS) areas, suggesting similar structural flexibility. Overall, PEP-1 is predicted to bind to integrin, supporting its potential as a therapeutic integrin-targeting ligand.

3.2. Expression, solubility assessment, and purification

Competent *E. coli* cells were prepared using a standard calcium chloride ($CaCl_2$) method, a widely adopted protocol that enhances cell membrane permeability for DNA uptake [24-25]. Transformation was carried out by introducing the recombinant plasmid pET31b(+)-*pep-1* into *E. coli* BL21(DE3) cells. Following the transformation, plasmid isolation was performed from selected colonies. The integrity and presence of the recombinant plasmid were verified by agarose gel electrophoresis. As shown in Fig. 2(a), the electrophoretic profiles confirmed successful incorporation of the pET31b(+)-*pep-1* construct in the transformed *E. coli* colonies.

For purification, the KSI-PEP-1 fusion protein, which was designed with a His-tag at its C-terminal, was subjected to nickel affinity chromatography. The chromatographic profile and SDS-polyacrylamide gel electrophoresis (SDS-PAGE) analysis, illustrated in the figure below, demonstrate that KSI-peptide binds effectively to the nickel matrix. During the elution steps at 60% and 100% buffer B (Fig. 2(b)), the KSI-peptide was successfully released from the matrix due to competition with the increased imidazole concentration, which disrupts the His-tag and nickel interactions. SDS-PAGE analysis of the eluted fractions confirmed that the KSI-peptide was obtained with high purity (Fig. 2(c)), demonstrating the efficiency of this purification strategy. The expression yielded a total protein concentration of 52.94 mg.L^{-1}.

The bioprocess yielded a concentration of 52.94 mg.L^{-1} representing the high efficiency of the KSI-fusion system in protecting the 20-repeat LERGDT tandem from proteolytic degradation. As a highly hydrophobic partner, KSI drives the targeted sequestration of the fusion protein into stable, pseudo-soluble inclusion bodies, effectively shielding the otherwise highly flexible and solvent-exposed tandem repeats from host proteases. This achievement represents a significant improvement over standard recombinant methods for short bioactive peptides [26-28]. The use of nickel-affinity chromatography confirmed a high-purity product (Fig. 2(c)), providing a scalable alternative to synthetic production.

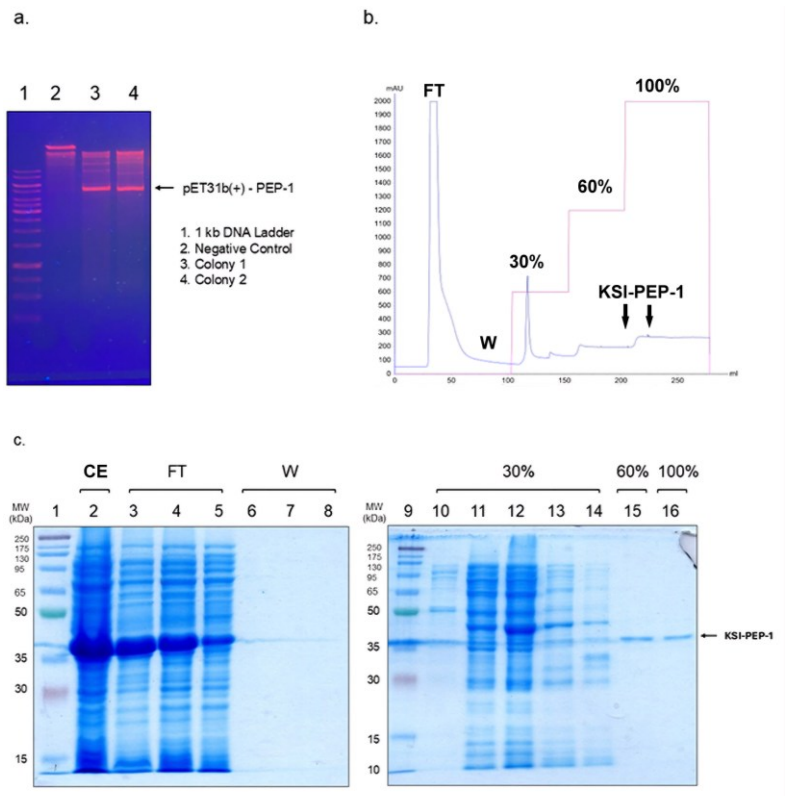

Fig. 2. (a) Analysis of pET31b(+)-pep-1 using agarose gel electrophoresis. The arrow indicates the presence of the pET31b(+)-*pep-1* plasmid. (b) The affinity chromatography profile of nickel Sepharose purification and (c) the corresponding 12% SDS-PAGE analysis of sample fractions. Purification was performed using a 5 mL column at 0.3 mPa and 1 mL/min flow rate, collecting 1.5 mL fractions. Buffer A (20 mM sodium hydrogen phosphate, 20 mM sodium dihydrogen phosphate, 300 mM NaCl) was used for equilibration and washing, while Buffer B (same composition with 500 mM imidazole) served as the eluent. The purification steps included flow-through (FT), wash (W), and elution with 30%, 60%, and 100% Buffer B. The KSI-peptide band is indicated by the arrow

3.3. Peptide cleavage

KSI-PEP-1 was cleaved using hydroxylamine and subsequently filtered by ultrafiltration with a 10 kDa cut-off to isolate the PEP-1. The cleavage efficiency and product identity were indicated by LC-MS. As shown in Fig. 3(a), the intact KSI-PEP-1 was undetectable following treatment, and Fig. 3(b) confirms the presence of PEP-1, indicating successful cleavage and recovery of the target peptide.

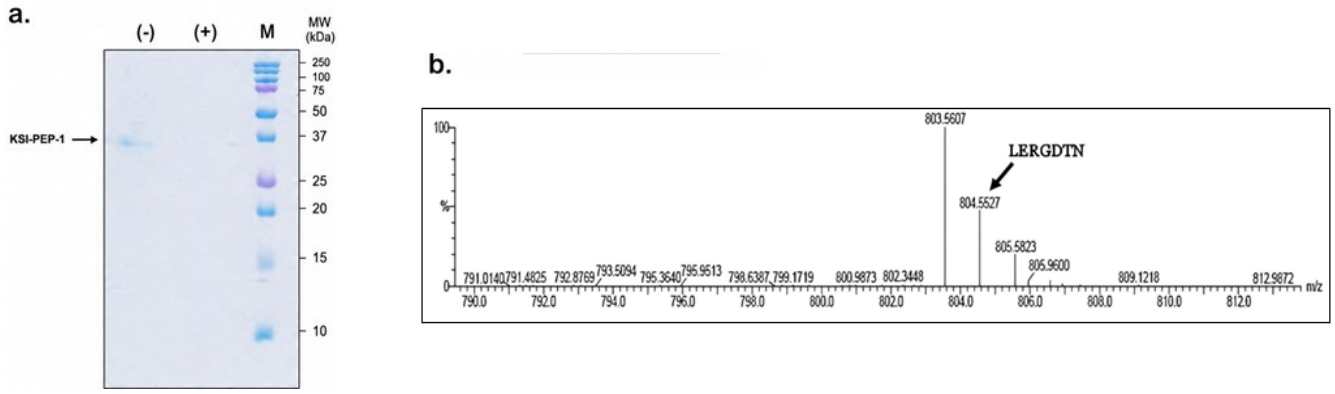

Fig. 3. (a) SDS-PAGE analysis of KSI-PEP-1 before and after hydroxylamine cleavage. Lane (–) shows the intact fusion protein; lane (+) shows the cleaved product, confirming specific cleavage at the Asn-Gly site. Lane M: molecular weight markers. (b) High-resolution mass spectrum of the cleaved 20× LERGDTN repeat peptide, with a peak corresponding to LERGDTN (m/z ~804.56)

3.4. ELISA

The purified samples were evaluated for their activity against integrin using the ELISA method. The overall results are presented in Fig. 4, with the corresponding standard curve shown in Fig. 4(a).

The calibration curve generated using serial dilutions of the standard showed a linear relationship between absorbance and peptide concentration over the tested range, with a high coefficient of determination, supporting the accuracy of subsequent quantitative analysis. Using this standard curve, KSI-PEP-1 and PEP-1 demonstrated a positive effect on integrin (Fig. 4(b)), even though with a low signal compared to the standard sLERGDT. The low signal suggested an insufficient epitope accessibility compared with the native integrin ligand mimic. However, from a bioprocess

perspective, the lower ELISA signal observed for hPEP-1 compared to the synthetic standard (sLERGDT) suggests that while the recombinant fragment is functional, its epitope accessibility may be influenced by the presence of the C-terminal His-tag or the specific cleavage conditions. This finding provides a baseline for further process optimization, such as varying the tandem-repeat length or utilizing alternative cleavage agents to enhance binding kinetics.

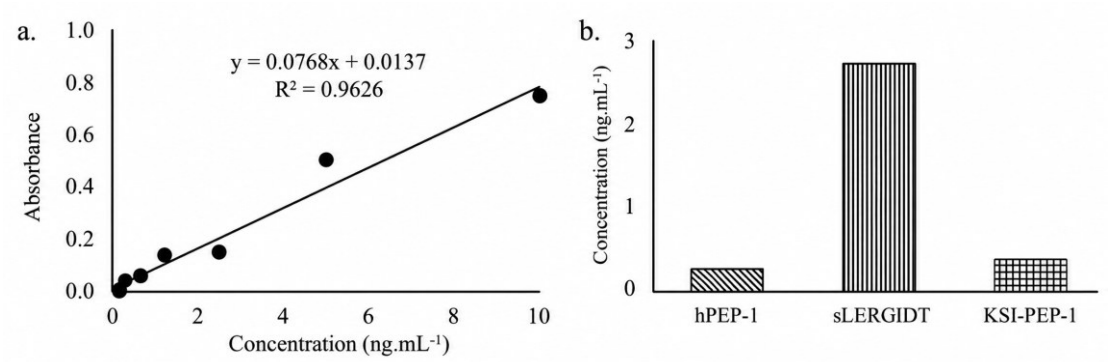

Fig. 4. ELISA analysis of KSI-PEP-1 and PEP-1 binding to integrin. Protein hydrolysate served as a carrier molecule to evaluate the activity of free peptides. (a) Standard curve, (b) quantitative binding data after subtraction of protein hydrolysate background activity. The hPEP-1, sLERGDT, and KSI-PEP-1 are indicated as cleavage fragment, standard synthetic PEP-1, and fused protein, respectively

3.5. Study limitations

This study focuses primarily on the feasibility and characterization of a recombinant production system. While the LERGDT sequence has previously shown anti-kidney fibrosis activity in cell-based models [8-11], the present work is limited to *in vitro* binding assays (ELISA). Future work will connect this gap by conducting comparative dose-response studies between recombinant hPEP-1 and synthetic standards using *in vivo* cell lines to validate the industrial potential of this bioprocess.

4. Conclusion

Taken together, this study demonstrates a successful bioprocess for the high-yield recombinant production of the LERGDT peptide. The 20-repeat tandem design fused with ketosteroid isomerase (KSI) protected the short bioactive sequence from proteolytic degradation in *E. coli*. Our results confirm that both the fused (KSI-PEP-1) and chemically cleaved (hPEP-1) products retain specific integrin-binding functionality, as predicted by our *in-silico* docking models. While the ELISA signal was lower than synthetic standards, suggesting that bioprocess variables such as epitope orientation or His-tag interference require further optimization. However, the protocol remains a cost-effective and scalable alternative to chemical synthesis. By providing a reproducible method to generate the LERGDT sequence, this work establishes a technical foundation for large-scale production. This bioprocess approach facilitates future translational research where high-purity recombinant peptides are required for extensive preclinical and signalling assays in renal pathophysiology.

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References

1. A.S. Levey and J. Coresh, *Chronic kidney disease*, Lancet. 379 (2012) 165-180.
2. V. Jha, G. Garcia-Garcia, K. Iseki, Z. Li, S. Naicker, B. Plattner, et al., *Chronic kidney disease: global dimension and perspectives*, Lancet. 382 (2013) 260-272.
3. KDIGO, *Clinical practice guideline for the evaluation and management of chronic kidney disease: Kidney disease: Improving global outcomes (KDIGO). CKD work group*, Kidney Int 105. 4S (2024) S117-S314.
4. G.L. Bakris and M.R.Weir, *Angiotensin-converting enzyme inhibitor-associated elevations in serum creatinine: is this a cause for concern?*, Arch. Intern. Med. 160 (2000) 685-693.
5. A.C. Webster, E. V. Nagler, R. L. Morton, and P. Masson, *Chronic kidney disease*, Lancet. 389 (2017) 1238-1252.
6. K.R. Tuttle, G. L. Bakris, R. W. Bilous, J. L. Chiang, I. H. de Boer, J. Goldstein-Fuchs, et al., *Diabetic kidney disease: a report from an ADA Consensus Conference*, Diabetes Care. 37 (2014) 2864-2883.
7. L. Wang, N. Wang, W. Zhang, X. Cheng, Z. Yan, G Shao, et al., *Therapeutic peptides: current applications and future directions*, Signal Transduct Target Ther. 7 (2022) 48.
8. M. Hidayat, S. Prahastuti, A. S. Rakasiwi, S. Prisilia, and K. Hasan, *Sub-chronic toxicity study of green peas protein hydrolysate in rats*, Toxicol Rep. 9 (2022) 735-742.
9. M. Hidayat, S. Prahastuti, E. Afifah, W. Widowati, M. Yusuf M, and K. Hasan, *The role of green peas protein hydrolysate in TGF/SMAD signaling to prevent renal fibrosis*, J. King Saud Univ Sci. 34 (2022) 101920.
10. M. Hidayat, R. Pangastuti, S. Prahastuti, and K. Hasan, *Protein hydrolysate of green peas bromelain attenuates kidney fibrosis in cisplatin-induced nephrotoxicity rats: emphasis on anti-inflammatory activities*, HAYATI J Biosci. 30 (2023) 347-357.
11. M. Hidayat, K. Hasan, M. Yusuf, S. Sriwidodo, C Panatarani, and I. M. Joni, *Nano delivery chitosan-protein/hydrolysate of green peas bromelain (PHGPB) synthesized by colloidal-spray drying method*, Polymers. 15 (2023) 2546.
12. G. Walsh, *Biopharmaceutical benchmarks*, *Nat. Biotechnol*. 36 (2018) 1136-1145.
13. G. L. Rosano and E. A. Ceccarelli, *Recombinant protein expression in Escherichia coli: advances and challenges*, Front. Microbiol. 5 (2014) 172.
14. J. Jumper, R. Evans, A. Pritzel, T. Green, M. Figurnov, O Ronneberger, et al., *Highly accurate protein structure prediction with AlphaFold*, Nature. 596 (2021) 583–589.
15. M. Varadi, D. Bertoni, P. Magana, U. Paramval, I. Pidruchna, M. Radhakrishnan, et al., *AlphaFold Protein Structure Database in 2024: providing structure coverage for over 214 million protein sequences*, Nucleic Acids Res. 52 (2024) D368-D375.
16. R. A. Laskowski, M. W. MacArthur, D. S. Moss and J. M. Thornton,

PROCHECK: a program to check the stereochemical quality of protein structures, J. Appl. Crystallogr. 26 (1993) 283-291.
17. G. N. Ramachandran, C. Ramakrishnan and V. Sasisekharan, *Stereochemistry of polypeptide chain configurations*. J. Mol. Biol. 7 (1963) 95-99.
18. M. Hebditch, M. A. Carballo-Amador, S. Charonis, R. Curtis and J. Warwicker, *Protein–Sol: a web tool for predicting protein solubility from sequence*, Bioinformatics. 33 (2017) 3098-3100.
19. R.V. Honorato, M.E. Trellet, B. Jiménez-García, J.J. Schaarschmidt, M. Giulini, V. Reys, et al., *The HADDOCK2.4 web server: A leap forward n integrative modelling of biomolecular complexes*, Nature Prot., 19 (2024) 3219-3241.
20. M. M. Bradford, *A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding*, Anal. Biochem. 72 (1976) 248-254.
21. U. K. Laemmli, *Cleavage of structural proteins during the assembly of the head of bacteriophage T4*, Nature. 227 (1970) 680-685.
22. P. Bornstein and G. Balian, *Cleavage at Asn-Gly bonds with hydroxylamine*, Methods Enzymol. 47 (1977) 132-45
23. T. Niwa, B. W. Ying, K. Saito, W. Jin, S. Takada, T. Ueda, et al., *Bimodal protein solubility distribution revealed by an aggregation analysis of the entire ensemble of Escherichia coli proteins*. Proc. Natl. Acad. Sci. USA. 106 (2009) 4201-4206.
24. M. Mandel and A. Higa, *Calcium-dependent bacteriophage DNA infection*, *J. Mol. Biol.* 53 (1970) 159-162.
25. A. Azka, H. Mohsin, R. Tanvir, and Y. Rehman, *Revisiting the mechanisms involved in calcium chloride induced bacterial transformation*, Front.Microbiol. 8 (2017) 2169.
26. L. Zheng, F. Yang, C. Wang, M. Zafir, Z. Gao, P. Liu, et al., *High-level biosynthesis and purification of the antimicrobial peptide Kiadin based on non-chromatographic purification and acid cleavage methods*. Biotechnol. Biof. Biop. 18 (2025) 5.
27. V. Rodríguez, J. A. Asenjo and B. A. Andrews, *Design and implementation of a high yield production system for recombinant expression of peptides*. Microb. Cell Fact. 13 (2014) 65.
28. X. Lu, Y. Sun, Z. Wang, X Jin, J. Zhang, F. Lu, et al., *Recombinant expression of antimicrobial peptide AMP-PD as tandem octamer and its antibacterial activity in vitro*. Protein Expr. Purif. 238 (2025)106839.