様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　６ 年　 ５ 月 ２７ 日現在

機関番号：１５３０１
研究種目：若手研究
研究期間：2021～2023
課題番号：２１Ｋ１４１６９

研究課題名（和文）Computational design of recognition peptide based plasmonic biosensor for small molecules detection

研究課題名（英文）Computational design of recognition peptide based plasmonic biosensor for small molecules detection

研究代表者
ＷＡＮＧ　ＪＩＮ（WANG, JIN）

岡山大学・ヘルスシステム統合科学学域・准教授

研究者番号：１０８７０９７５

交付決定額（研究期間全体）：（直接経費）　　3,600,000円

研究成果の概要（和文）：SARS-CoV-2に関連するVOCを認識するために、オドラント結合タンパク質（OBP）由来の一連のペプチドが戦略的に設計されました。COVID-19感染中に呼気中に見られる代表的なVOCとして、エタノール、ノナナール、ベンズアルデヒド、酢酸、アセトンが選ばれました。これらのペプチドは、計算ドッキングおよび予測ツールを使用して特徴付けと分析が行われ、ドッキングモデル、結合親和性、配列特異性、構造折り畳みなどの要因が検討されました。さらに、DNAアプタマーやAPTESなどの有機受容体といったバイオレセプターも、小分子（コルチゾールや芳香族化合物など）の高感度検出に利用されました。

研究成果の学術的意義や社会的意義
The research on peptide-based plasmonic biosensors for detecting VOCs, biomarkers for diseases like COVID-19 and cancer, advances molecular detection technology. It enables early, non-invasive diagnosis, improving public health by enhancing early detection and monitoring capabilities.

研究成果の概要（英文）：A series of peptides derived from odorant-binding proteins (OBPs) were designed to recognize VOCs linked to SARS-CoV-2. Representative VOCs, including ethanol, nonanal, benzaldehyde, acetic acid, and acetone, which are found in exhaled breath during COVID-19 infection, were selected for this purpose. The peptides were characterized and analyzed using computational docking and prediction tools, assessing factors such as docking models, binding affinity, sequence specificity, and structural folding. In addition to OBP peptides, the study explored other bioreceptors like DNA aptamers and organic receptors such as APTES for their sensitivity in detecting small molecules, including cortisol and aromatic compounds. The interaction mechanisms between these receptors and their targets were elucidated using computational docking approaches.

研究分野：計測工学

キーワード：peptide　computational　design　plasmonic biosensor　small molecules

１．研究開始当初の背景

Sensing small molecules, especially the volatile organic compounds (VOCs) biomarkers, is an effective way to understand and uncover the situation of environmental issues, health, biological or chemical world. Compared with conventional recognition elements (large molecular antibody, protein, or cell) for small molecules sensing, the short peptide with low molecular weight has great promising properties such as easily synthesized, self-assembled on various materials, strong binding ability, long-term stability, low-cost and so on. However, the progress for developing the peptide-based biosensor has been hampered due to lack of efficient methods for obtaining or synthesizing the specific peptide and the binding mechanism is still not clear. Meanwhile, there is no applicable robust sensing devices for various small molecules detection.

２．研究の目的

Peptides are promising molecular-binding elements and have attracted great interest in novel biosensor development. In this study, a series of peptides derived from odorant-binding proteins (OBPs) were rationally designed for recognition of SARS-CoV-2-related volatile organic compounds (VOCs). Computational docking and prediction tools were utilized for OBPs peptide characterization and analysis. Multiple parameters, including the docking model, binding affinity, sequence specification, and structural folding, were investigated. The results demonstrated a rational, rapid, and efficient approach for designing breath-borne VOC-recognition peptides, which could further improve the biosensor performance for pioneering COVID-19 screening and many other applications.

Moreover, DNA aptamers and other organic receptors were also developed for highly sensitive and selective detection of small molecules by using terahertz biosensor and AuNPs based plasmonic biosensor.

３．研究の方法

(1) Computational design of small molecules binding peptide through molecular modelling, binding residue and sequence prediction, virtual analysis from the specific odorant binding protein, whose crystal structure could be obtained from the Protein Data Bank (PDB). Elucidate the binding pocket in the OBP in binding small molecules. To design the peptide sequence corresponding to the binding site on the OBP, short fragments (5-17 amino acids residues) near the binding pocket will be chosen and evaluated. To select peptide candidates, the affinity energy between peptides and small molecule will be calculated using docking simulation. The mechanism of binding reaction (hydrogen bond, π-interaction or electron donor-acceptor interaction) will be clarified through virtual analysis. After obtaining the appropriate binding amino acid sequence, the peptide will be customer synthesized and used for further plasmonic biosensing.

(2)Surface Plasmon Resonance (SPR) Evaluation and terahertz aptasensor

The designed binding peptides were evaluated using SPR plasmonic sensing and terahertz aptasensor. Optimization of VOC binding peptide immobilization, analysis of molecular binding affinity, and multiple binding peptide array evaluations were performed. The binding surface morphology was confirmed using atomic force microscopy and a charge-coupled device. A novel polypeptide architecture through thiol-coupling was employed to achieve high surface density, high affinity, and improved signal responses. Metrological parameters (including sensitivity, selectivity, and stability) of the plasmonic biosensor were presented.

(3)DNA Aptamers and AuNPs

For DNA aptamers, AuNPs were utilized to enhance sensitivity due to their high surface area and localized surface plasmon resonance, which amplify the signal and improve detection limits. This integration allows for more accurate and sensitive detection of target molecules, making it ideal for applications in biosensing and diagnostics.

４．研究成果

(1) peptides derived from odorant-binding proteins (OBPs) for VOCs detection

In this study, a series of peptides derived from odorant-binding proteins (OBPs) were rationally designed for recognition of SARS-CoV-2-related volatile organic compounds (VOCs). Ethanol, nonanal, benzaldehyde, acetic acid, and acetone were selected as representative VOCs in the exhaled breath during the COVID-19 infection. Computational docking and prediction tools were utilized for OBPs peptide characterization and

analysis. Multiple parameters, including the docking model, binding affinity, sequence specification, and structural folding, were investigated. The results demonstrated a rational, rapid, and efficient approach for designing breath-borne VOC-recognition peptides, which could further improve the biosensor performance for pioneering COVID-19 screening and many other applications. (Fig. 1-2) Furthermore, rational design of short peptide receptor derived from cortisol nanobody for stress hormone cortisol recognition was conducted. (Fig. 1-3)

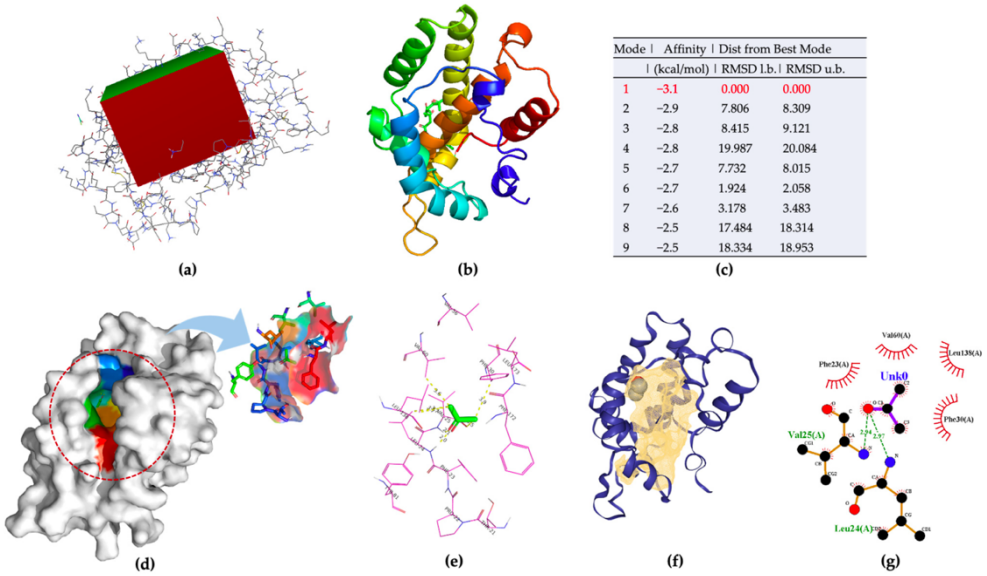

Fig. 1 Computational design of peptide for VOCs

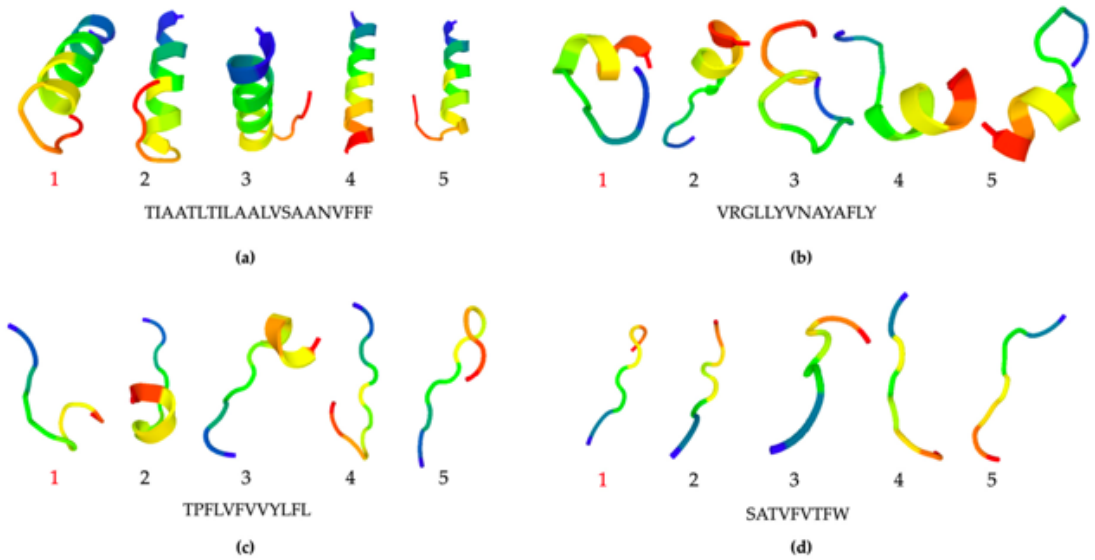

Figure 2. Best five models (representatives of five best clusters) of peptide structure prediction using PEP-FOLD according to the clustering reports: nonanal- grand-average-of-hydropathicity-binding peptide (a); benzaldehyde-binding peptide (b); acetone-binding peptide (c); ethanol-binding peptide (d)

References: Molecules 27 (12), 3917, 2022.

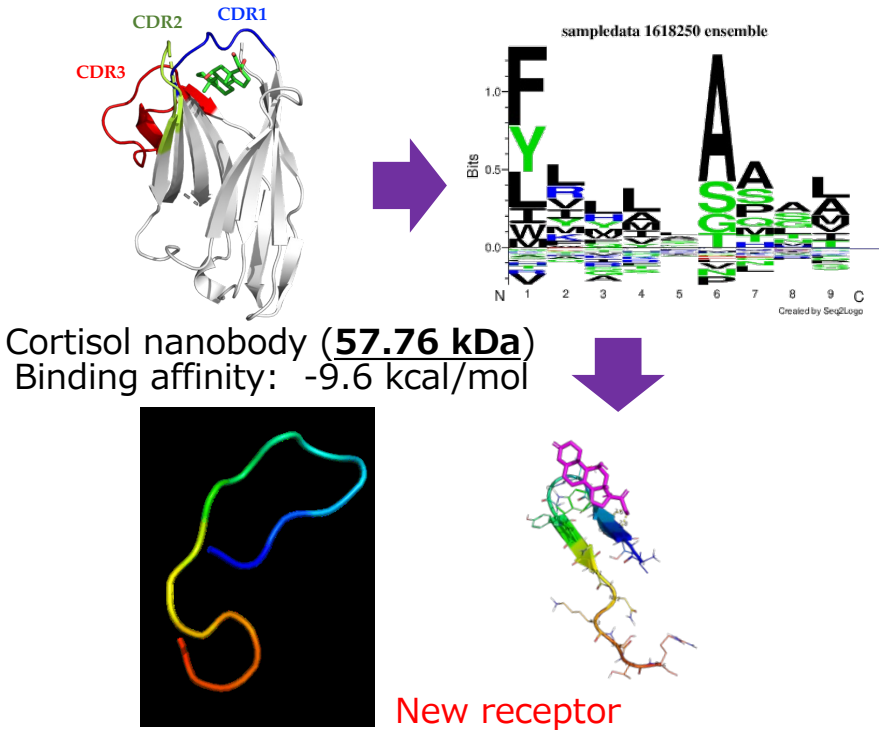

Fig. 3 rational design of short peptide receptor derived from cortisol nanobody for stress hormone cortisol recognition.

(2) DNA Aptamer-Based Terahertz Biosensor for Cortisol Sensing

In this study, we developed an innovative terahertz (THz) aptasensor for the detection of the small molecule cortisol. This sensor leverages the unique properties of DNA aptamers and terahertz technology to achieve highly sensitive and specific cortisol detection.

Molecular Docking and Simulation

2D/3D Molecular Docking: Using advanced 2D and 3D molecular docking techniques, we analyzed the binding interactions between DNA aptamers and cortisol. These simulations revealed that hydrogen bonds play a crucial role in the binding event, ensuring high specificity and strong affinity between the aptamer and the cortisol molecules.
Simulation Studies: Detailed simulation studies provided insights into the binding mechanisms, highlighting the key interaction sites and the stability of the aptamer-cortisol complex. These simulations were instrumental in optimizing the aptamer sequences for maximum binding efficiency.

Aptamer-Modified Terahertz Biosensor

Conformational Changes: The DNA aptamer underwent conformational changes upon binding to cortisol, which were effectively observed using the THz biosensor. These conformational changes are critical for the biosensor's functionality as they directly influence the THz signal.
Detection Mechanism: The THz aptasensor operates by detecting the changes in the terahertz signal when the aptamer binds to cortisol. This method provides a direct and real-time measurement of cortisol levels.
Performance and Sensitivity

High Sensitivity: The developed THz aptasensor demonstrated highly sensitive detection capabilities, capable of detecting cortisol levels in the parts-per-billion (ppb) range. This sensitivity is critical for applications in medical diagnostics where low levels of biomarkers need to be accurately measured.
Specificity and Accuracy: The specificity of the DNA aptamers towards cortisol ensured

minimal cross-reactivity with other molecules, providing accurate and reliable results. This high specificity is due to the precise binding interactions identified through molecular docking studies.

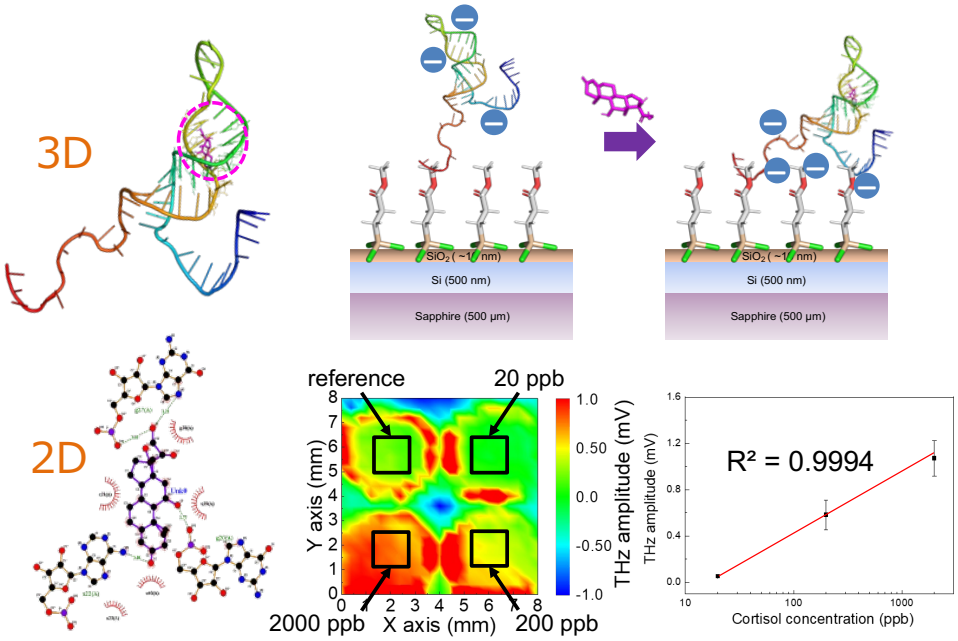

Fig. 4 Aptamer-Modified Terahertz Biosensor

References: Biosensors and Bioelectronics 220, 114901, 2023

(3) Drug molecule affinity evaluation

In this study, the binding affinity between small drug molecules and proteins was systematically evaluated. The research focused on identifying the specific protein that interacts with the drug molecule esculetin using both in silico simulations and in vitro measurements.

In Silico Simulation

Computational Docking: Advanced computational docking techniques were employed to predict the binding sites and affinity of esculetin to various target proteins. The simulations provided insights into the molecular interactions and conformational changes upon binding.

Molecular Dynamics: To further validate the docking results, molecular dynamics simulations were conducted. These simulations assessed the stability of the esculetin-protein complexes over time, offering a dynamic view of the interaction.

In Vitro Measurement

Binding Assays: terahertz chemical microscope was utilized to quantify the binding affinity and kinetics of esculetin to the target protein since it provided precise measurements of the binding constants especially for small molecules. The results showed the binding affinity Kd was 0.475 μM.

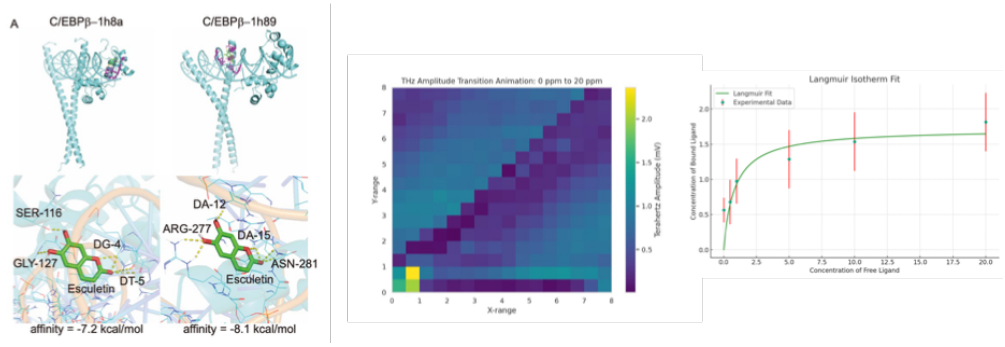

Fig. 5 Drug molecule affinity evaluation

References: Advanced Science, 2024, under review.

５．主な発表論文等

〔雑誌論文〕　計7件（うち査読付論文　7件／うち国際共著　0件／うちオープンアクセス　4件）

１．著者名 Jin Wang, Keiji Sakai and Toshihiko Kiwa	４．巻 27
２．論文標題 Rational Design of Peptides Derived from Odorant-Binding Proteins for SARS-CoV-2-Related Volatile Organic Compounds Recognition	５．発行年 2023年
３．雑誌名 Molecules	６．最初と最後の頁 3917
掲載論文のDOI（デジタルオブジェクト識別子） 10.3390/molecules27123917	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 －

１．著者名 jin Wang	４．巻 168
２．論文標題 Visualization of Charge-Transfer Complex for the Detection of 2,4,6-Trinitrotoluene Using Terahertz Chemical Microscope	５．発行年 2021年
３．雑誌名 Journal of the electrochemistry society	６．最初と最後の頁 117517
掲載論文のDOI（デジタルオブジェクト識別子） 10.1149/ 1945-7111/ac3a2c	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 －

１．著者名 Wang Jin、Ando Masaki、Nagata Hiroki、Yoshida Sota、Sakai Kenji、Kiwa Toshihiko	４．巻 220
２．論文標題 Multifunctional terahertz microscopy for biochemical and chemical imaging and sensing	５．発行年 2023年
３．雑誌名 Biosensors and Bioelectronics	６．最初と最後の頁 114901～114901
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.bios.2022.114901	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

１．著者名 Wang Jin、Sakai Kenji、Kiwa Toshihiko	４．巻 2
２．論文標題 All-in-one terahertz taste sensor: integrated electronic and bioelectronic tongues	５．発行年 2023年
３．雑誌名 Sensors & Diagnostics	６．最初と最後の頁 620～626
掲載論文のDOI（デジタルオブジェクト識別子） 10.1039/D3SD00038A	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 －

1．著者名 Wang Jin、Morita Kosei、Ando Masaki、Yoshida Sota、Nagata Hiroki、Sakai Kenji、Kiwa Toshihiko	4．巻 16
2．論文標題 Terahertz aptasensor for dopamine neurochemical detection	5．発行年 2023年
3．雑誌名 Applied Physics Express	6．最初と最後の頁 052002～052002
掲載論文のDOI（デジタルオブジェクト識別子） 10.35848/1882-0786/acd102	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 －

1．著者名 Yoshida Sota、Wang Jin、Sakai Kenji、Kiwa Toshihiko	4．巻 9
2．論文標題 Comparative Study of Terahertz Chemical Microscopy and Flexible ISFET Approaches for Calcium Ion Detection	5．発行年 2023年
3．雑誌名 IEEE Xplore	6．最初と最後の頁 1-2
掲載論文のDOI（デジタルオブジェクト識別子） 10.1109/IRMMW-THz57677.2023.10299237	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

1．著者名 Wang Jin、Yoshida Sota、Ando Masaki、Sakai Kenji、Kiwa Toshihiko	4．巻 9
2．論文標題 Terahertz Chemical Microscope for Calcium Ions and Stress Biomarker Sensing	5．発行年 2022年
3．雑誌名 IEEE Xplore	6．最初と最後の頁 1-2
掲載論文のDOI（デジタルオブジェクト識別子） 10.1109/IRMMW-THz50927.2022.9895468	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

〔学会発表〕　計2件（うち招待講演　0件／うち国際学会　2件）

1．発表者名 Jin Wang
2．発表標題 Comparative Study of Terahertz Chemical Microscopy and Flexible ISFET Approaches for Calcium Ion Detection
3．学会等名 2023 48th International Conference on Infrared, Millimeter, and Terahertz Waves (IRMMW-THz)（国際学会）
4．発表年 2023年

１．発表者名 Jin Wang
２．発表標題 Multifunctional Terahertz Chemical Microscope for Biomolecules Detection,
３．学会等名 11th international Conference on Materials for Advanced Technologies (ICMAT2023), (国際学会)
４．発表年 2023年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

―

６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関