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List of RGJ advisors 2023/2024

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Keywords: Carbapenem resistant *Enterobacterales*, Genosensor, Electrochemistry

Summary of research:

The global spread of Carbapenem-resistant *Enterobacterales* (CRE) inflicts a severe threat to human health, who are under long-term medical care from severe entities, such as those with major surgery/injury or urinary/intravascular/respiratory catheters. The CRE infections have resulted in an increased mortality rate in hospitals and other health-care settings. The most common species of CRE causing infections in the clinical settings are *Klebsiella pneumoniae* and *Escherichia coli*. The current gold standards for microbial detection are culture-based methods; however, these methods are time-consuming and laborious, making these methods unsuitable for rapid diagnosis. Although molecular testing such as commonly polymerase chain reaction (PCR)-based assays provides timely results, it remains resource-demanding, both in terms of time-consuming about 3-4 h to obtain results. Thus, the objectives of this work are to design primers and DNA probes to specifically amplify the target DNA sequences of carbapenem resistant *Enterobacterales* genes, to fabricate multiplex electrochemical genosensors (Lab-on-Chip) for rapid, sensitive, and simultaneous monitoring carbapenem resistant *Enterobacterales* genes, and to validate efficiency of genosensors against standard culture methods for detection of carbapenem resistant *Enterobacterales*. Therefore, development of novel sensitive and specific detection platforms is still required to detect Carbapenem-resistant *Enterobacterales* (CRE) in order to facilitate diagnosis and treatment in patients.

Curriculum vitae

Name: Assistant Professor Dr. Kamonrat Phopin

Position: Assistant Professor **Affiliation:** Faculty of Medical Technology, Mahidol University

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Education:

SUBJECTS OF STUDY	INSTITUTE	YEAR
Ph.D. (Molecular Biology)	Department of Biological Sciences, Faculty of Arts and Sciences, Lehigh University, USA	2012
M.Sc. (Medical Microbiology)	Department of Microbiology, Faculty of Medicine, Chulalongkorn University, Thailand	2003
B.Sc. (Medical Technology)	Faculty of Medical Technology, Mahidol University, Thailand	2000

Work Experiences:

2004-2006	Lecturer, Department of Medical Technology, Faculty of Allied Health Sciences, Thammasat University
2011	Research Assistant at Department of Biological Sciences, Lehigh University, PA, USA.
2012-2017	Lecture, Center for Research and Innovation, Mahidol University
2018-Present	Assistant Professor, Center for Research Innovation and Biomedical Informatics, Mahidol University

Research Interests: Cell and Molecular Biology, Toxicology, Biosensor, Medical Microbiology, and Neurobiology

H Index: 15

Funding Experience (Research project and funding sources):

1. Synthesis, bioactivity investigations, and advanced computational analysis for development of N-, S-heterocyclics and sulfonamides in medical applications supported by Thailand Science Research and Innovation (Fundamental Fund 2023)
2. Development of rapid DNA-based rapid test for *Enterobacteriaceae* and effect of benzyl isothiocyanate on controlling the spread of antimicrobial resistance plasmids supported by Thailand Science Research and Innovation (Fundamental Fund 2023)
3. Synthesis and neuroprotective effects of novel triazole derivatives under oxidative stress, pharmacokinetics, and in silico models supported by New Discovery and Frontier Research (Mahidol University, 2022)
4. Synthesis, investigation on neuroprotective effects of 8-aminoquinoline sulfonamide derivatives

on human neuronal cells and computational studies (QSAR and molecular docking analysis) supported by Thailand Science Research and Innovation (Fundamental Fund 2022)

6. Development of TripleVibrio DNA sensor for detection of most important Vibrio in seafood supported by Agricultural Research Development Agency (ARDA), 2020
8. Synthesis and neuroprotective effect of anthranilate sulfonamide and hydroxyquinoline derivatives supported by Srinakharinwirot University (2019)
9. Effect of *Spilanthes acemella* Murr. on chronic stress inducing cognitive impairment: Experimental and Computational studies supported by Mahidol University (2017-2018)
10. Study of Neuroprotective effects of 8-hydroxyquinoline and its derivatives on Japanese Encephalitis Virus (JEV)-infected neuronal cells. supported by Mahidol University (2017-2018)
11. Safety of Vegetables and Fruits for World Kitchen Supplies: Determination of Pesticide Residues in Vegetables and Fruits sold in Thailand supported by Agricultural Research Development Agency (ARDA)
12. Development of rapid screening tests for neurodegenerative diseases for self-reliance and sustainable development supported by Health Systems Research Institute (HSRI)

Publications:

1. Ruankham W, Frías IAM, **Phopin K**, Tantimongcolwat T, Bausells J, Zine N, Errachid A. One-step impedimetric NT-proBNP aptasensor targeting cardiac insufficiency in artificial saliva. Talanta. 2023;256:124280. (Impact Factor 2021 = 6.556, SJR Quartile = Q1)
2. Ruankham W, Tantimongcolwat T, **Phopin K**, Bausells J, Hangouët M, Martin M, Zine N, Errachid A. Split aptamers immobilized array microelectrodes for detection of chlorpyrifos pesticide using electrochemical impedance spectroscopy. Sens Actuators B Chem. 2022;372:132614; doi:10.1016/j.snb.2022.132614 (Impact Factor 2021 = 9.221, SJR Quartile = Q1)
3. Apiraksattayakul S, Pingaew R, Prachayasittikul V, Ruankham W, Jongwachirachai P, Songtawee N, Suwanjang W, Tantimongcolwat T, Prachayasittikul S, Prachayasittikul V, **Phopin K***. Neuroprotective properties of bis-sulfonamide derivatives against 6-OHDA-induced Parkinson's model via Sirtuin 1 activity and in silico pharmacokinetic properties. Front Mol Neurosci. 2022;15: 890838. (Impact Factor 2021 = 6.261, SJR Quartile = Q1) (Corresponding author)
4. **Phopin K**, Wanwimolruk S, Norkaew C, Buddhaprom J, Isarankura-Na-Ayudhya C. Boiling, Blanching, and Stir-Frying Markedly Reduce Pesticide Residues in Vegetables. Foods. 2022;11(10):1463. (Impact Factor 2021 = 5.561, SJR Quartile = Q1)
5. Ruankham W, Suwanjang W, **Phopin K**, Songtawee N, Prachayasittikul V, Prachayasittikul S. Modulatory Effects of Alpha-Mangostin Mediated by SIRT1/3-FOXO3a Pathway in Oxidative Stress-Induced Neuronal Cells. Front Nutr. 2022;28(8):714463. (Impact Factor 2021 = 6.590, SJR Quartile = Q1)
6. Ruankham W, Phopin K, Pingaew R, Prachayasittikul S, Prachayasittikul V. Tantimongcolwat T*. In silico and multi-spectroscopic analyses on the interaction of 5-amino-8-hydroxyquinoline and bovine serum albumin as a potential anticancer agent. Sci Rep. 2021;11:20187. (Impact Factor 2021 = 4.379, SJR Quartile = Q1)
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8. Ruankham W, Suwanjang W, Wongchitrat P, Prachayasittikul V, Prachayasittikul S, **Phopin K***. Sesamin and sesamol attenuate H2O2-induced oxidative stress on human neuronal cells via the

SIRT1-SIRT3-FOXO3a signaling pathway. Nutr Neurosci. 2021;24(2):90-101. (Corresponding author) (Impact Factor 2021 = 4.062, SJR Quartile = Q2) (Corresponding author)

9. Sooknual P, Pingaew R, **Phopin K***, Ruankham W, Prachayasittikul S, Ruchirawat S, Prachayasittikul V. Synthesis and neuroprotective effects of novel chalcone-triazole hybrids. Bioorg Chem. 2020;17(105):104384. (Impact Factor 2021 = 5.307, SJR Quartile = Q1) (Corresponding author)

10. **Phopin K**, Tantimongcolwat T. Pesticide Aptasensors: State of the Art and Perspective. Sensors 2020;20(23):6809; doi.org/10.3390/s20236809. Review. (Impact Factor 2021 = 3.847, SJR Quartile = Q1)

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12. **Phopin K**, Ruankham W, Prachayasittikul S, Prachayasittikul V, Tantimongcolwat T. Insight into the Molecular Interaction of Cloxyquin (5-chloro-8-hydroxyquinoline) with Bovine Serum Albumin: Biophysical Analysis and Computational Simulation. Int J Mol Sci. 2019;21(1):pii:E249. (Impact Factor 2021 = 6.208, SJR Quartile = Q1)

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Awards:

1. Winning the grand prize on the 8th Delta International Smart & Green Manufacture Contest (International contest) as a mentor (2022)
2. Howard Hughes scholarship for Research Assistant at Lehigh University, USA (2011)
3. NIH Trainee Award from American Society of Andrology (2011)
4. Full Scholarship from the Royal Thai Government for pursuing a Doctoral Degree in the United States of America (2006-2011)
5. ISHAM Trainee Award for International conference in USA (2003)
6. National Science and Technology Development Agency (NSTDA) Scholarship for Master degree study (2022)

การกรอกรายละเอียดในแบบฟอร์มนี้ ต้องดำเนินการให้ครบถ้วนตามความเป็นจริง หากตรวจสอบพบว่ามีการปกปิดหรือเป็นเท็จ วช. ขอสงวนสิทธิ์ที่จะไม่พิจารณาสนับสนุนและจะเป็นผู้ไม่มีสิทธิ์รับทุน วช. เป็นเวลา ๓ ปี

แบบเสนอโครงการวิจัย (Research Project)

ประกอบการเสนอขอทุนอุดหนุนการวิจัยของสำนักงานการวิจัยแห่งชาติ (วช.)

โครงการปริญญาเอกกาญจนาภิเษก (คปก.) ภายใต้ความร่วมมือไตรภาคีไทย-สวีเดน

ประจำปีงบประมาณ ๒๕๖๗

๑. ชื่อโครงการวิจัย

Development of smart genosensors for detection of carbapenem resistant *Enterobacterales* in clinical samples

๒. ชื่อ-สกุล อาจารย์ที่ปรึกษา ผศ.ดร.กมลรัตน์ โพธิ์ปิ่น (Asst. Prof. Dr. Kamonrat Phopin)

หน่วยงาน (สาขา/ภาควิชา คณะ และมหาวิทยาลัย) ศูนย์วิจัยพัฒนานวัตกรรม และชีวการแพทย์สารสนเทศ คณะเทคนิคการแพทย์ มหาวิทยาลัยมหิดล

สถานที่อยู่ที่ติดต่อได้สะดวก คณะเทคนิคการแพทย์ มหาวิทยาลัยมหิดล 999 ถนนพุทธมณฑลสาย 4

ต. ศาลายา อ. พุทธมณฑล จ. นครปฐม 73170

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๓. กลุ่มสาขาวิทยาศาสตร์พื้นฐานที่สมัคร (เลือกเพียง ๑ กลุ่ม)

☑ ชีววิทยา (Biology) ☐ เคมี (Chemistry)

☐ ฟิสิกส์ (Physics) ☐ คณิตศาสตร์ (Mathematics)

๔. ผู้ใช้ประโยชน์ (Research stakeholders) (กรณีมีความร่วมมือฯ) เช่น ความร่วมมือของหน่วยงานภาครัฐ (เช่น กระทรวง กรม)/เอกชนที่ร่วมสนับสนุนทุนวิจัย เช่น MOU เป็นต้น

☑ มี บริษัท อาฟเตอร์ แล็บ จำกัด

☐ ไม่มี

๕. คำสำคัญ (Keyword) ของโครงการ

Carbapenem resistant *Enterobacterales*, Genosensor, Electrochemistry

๖. ความสำคัญและที่มาของปัญหาที่ทำการวิจัย (Problem statement and significance of research)

The global spread of Carbapenem-resistant *Enterobacterales* (CRE) inflicts a severe threat to human health, who are under long-term medical care from severe entities, such as those with major surgery/injury or urinary/intravascular/respiratory catheters. The CRE infections have resulted in an increased mortality rate in hospitals and other health-care settings. The most common species of CRE causing infections in the clinical settings are *Klebsiella pneumoniae* and *Escherichia coli*. Carbapenemase gene products, such as *K. pneumoniae* carbapenemase (KPC), metallo-β-lactamases, and oxacillinase (OXA-48), are often found in carbapenemase-producing *Enterobacterales*. Among them, the bla_{KPC} is the most common gene reported; however, prevalence of other genes, such as bla_{OXA}, bla_{VIM}, bla_{NDM}, and bla_{IMP}, are gradually increasing worldwide. It is therefore of great importance to identify CRE rapidly so that doctors can decide whether it

is necessary to resort to more toxic or novel treatments. The current gold standards for microbial detection are culture-based methods; however, these methods are time-consuming and laborious, making these methods unsuitable for rapid diagnosis. Although molecular testing such as commonly polymerase chain reaction (PCR)-based assays provides timely results, it remains resource-demanding, both in terms of time-consuming about 3-4 h to obtain results, specialized thermal cycler, and sophisticated skill level, for routine diagnostics. This can cause delays in clinical treatment and lead to inappropriate antibiotic uses. Therefore, development of novel sensitive and specific detection platforms is very important to detect Carbapenem-resistant *Enterobacterales* (CRE) in order to facilitate diagnosis and treatment.

๗. ทฤษฎี/สมมุติฐานของโครงการ (Hypothesis)

We hypothesized that multiplex electrochemical genosensors could detect carbapenem resistant *Enterobacterales* in clinical samples and would be sensitive and specific enough to show even small variations in electric output caused by changes in antibiotic effectiveness. To meet the demands of a rapid multiplex electrochemical genosensors, the specific probes onto the multiplex screen-printed electrode will be measured toward CRE targets. This novel genosensors may facilitate the diagnosed operation time and downstream clinical decision making. Besides potentially being able to guide personalized therapy for patients more rapidly if adapted to testing from clinical specimens.

๘. วัตถุประสงค์ของโครงการ (Objectives)

8.1 To design primers and DNA probes to specifically amplify the target DNA sequences of carbapenem resistant *Enterobacterales* genes

8.2 To fabricate multiplex electrochemical genosensors (Lab-on-Chip) for rapid, sensitive, and simultaneous monitoring carbapenem resistant *Enterobacterales* genes

8.3 To validate efficiency of genosensors against standard culture methods for detection of carbapenem resistant *Enterobacterales*

๙. การทบทวนวรรณกรรม/ผลงานวิจัยที่เกี่ยวข้อง (Literature Review)

Antimicrobial resistance is a global threat to human health and to treatment of bacterial infections. Carbapenem-resistant *Enterobacterales* (CRE) are gram-negative bacteria that have become pathogens of a major public health concern (1, 2). The global spread of CRE inflicts a severe threat to human health, who are under long-term medical care from severe entities, such as those with major surgery/injury or urinary/intravascular/respiratory catheters. The CRE infections have resulted in an increased mortality rate in hospitals and other health-care settings (3). The infections are difficult to treat as these bacteria have propensity to resist multiple antimicrobial drugs, particularly carbapenem, which is considered the last antibiotic resort for treatment of multidrug-resistant bacterial infections. The most common species of CRE causing infections in the clinical settings are *Klebsiella pneumoniae* and *Escherichia coli*. *K. pneumoniae* and related species are the most prominent carbapenem-resistant *Enterobacterales* (CRE) and cause an excess

hospital mortality of 27% in patients with septicemia and pneumonia (4). Carbapenemase gene products, such as *K. pneumoniae* carbapenemase (KPC), metallo-β-lactamases, and oxacillinase (OXA-48), are often found in carbapenemase-producing *Enterobacterales*. Among them, the bla_{KPC} is the most common gene reported; however, prevalence of other genes, such as bla_{OXA}, bla_{VIM}, bla_{NDM}, and bla_{IMP}, are gradually increasing worldwide. Therefore, fast detection of these resistance genes is crucial to better diagnose infections and administer suitable antimicrobial agents. It is of great importance to identify CRE rapidly so that doctors can decide whether it is necessary to resort to more toxic or novel treatments.

Currently, the detection of CRE uses phenotypic, genotypic, and enzymatic activity-based methods. Phenotypic methods are common, but are usually rather limited by their low specificity and sensitivity as well as long turnaround time (2–3 days). PCR-based genotypic methods are the most mature, but suffer from high cost and an inability to detect unknown carbapenemase genes. MALDI-TOF MS, multiplex LAMP, and next-generation sequencing can greatly shorten assay times (from several days to hours) and enhance detection sensitivity (from 10^6 to 10^4 CFU/mL). Nevertheless, these methods are rarely implemented in most routine clinical microbiology laboratories because they require high skill levels and specialized equipment. Thus, development of simple and rapid methods for CRE detection is still an urgent requirement for modern clinical diagnosis (5, 6). Biosensors, analytical devices that translate biological responses into measurable signals, based on nucleic acid detection have the potential for providing high-specificity and allowing real-time POC detection. The key advantages of electrochemical sensors are their quick response (voltammetric measurements can be done in seconds), higher sensitivity when compared to colorimetric techniques, the possibility for use in real-time detection, and suitability for miniaturization (7-10).

Therefore, this project will focus on nucleic acid-based sensors using electrochemical techniques as a transducer where a biological response is converted into an electrical signal for detecting carbapenem resistant *Enterobacterales* genes including bla_{OXA}, bla_{KPC}, bla_{VIM}, and bla_{NDM}. Principally, the novel primer sets for each gene will be developed for discrete and multiplex amplifications of target DNA of those carbapenem resistant *Enterobacterales*. Concurrently, novel electrochemical transducers for specific recognition of target PCR products will be fabricated by functionalization of multiplex screen-printed carbon electrodes with specific probes for capturing target of interest. Finally, verification of the developed detection system efficiency toward clinical samples compared to standard culture methods will be determined. This approach is a promising new integrated Point-of-Care system for simple, sensitive, portable, and cost effective. It provides simultaneously quantitative and rapid outcomes at point of care, which is suitable for monitoring carbapenem resistant *Enterobacterales* and facilitating better patient management strategies during antibiotic therapy.

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2. Tunyong W, Arsheewa W, Santajit S, Kong-Ngoen T, Pumirat P, Sookrung N, et al. Antibiotic resistance genes among carbapenem-resistant Enterobacterales (CRE) isolates of Prapokklao Hospital, Chanthaburi Province, Thailand. Infection and drug resistance. 2021;14:3485-94.

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๑๐. ระเบียบวิธีวิจัย (Methodology)

10.1 The development of genosensors for detection of carbapenem resistant *Enterobacterales*

10.1.1 Search the target gene sequences that are unique and conserve among bacteria species (*Klebsiella pneumoniae*, *Escherichia coli*, and *Enterobacter* spp.) by using bioinformatic tools.

10.1.2 Design and synthesize primers and DNA probes to specifically amplify the target DNA sequences of all bacteria.

10.1.3 Optimize the PCR conditions of the designed primer sets for sensitive and specific amplification of the bacterial target genes, which include amplification temperatures, ionic strengths of the reaction, primer concentrations, sensing probes used for PCR product monitoring.

10.1.4 Determine the analytical sensitivity and specificity of the optimized primer sets and PCR condition toward various strains of bacteria and other gram-negative and gram-positive bacteria.

10.2 The design and fabrication of multiplex electrochemical genosensors (Lab-on-Chip) for monitoring bacterial PCR products

10.2.1 Immobilize the developed DNA probes by the covalent grafting of amine or thiol containing DNA probes on working electrodes of multiplex screen-printed carbon electrodes to produce a high affinity for the desired PCR products.

10.2.2 Characterize the immobilization of DNA probes at each modification step using CV and EIS analysis incorporation with Atomic Force Microscope (AFM), Fourier-Transform Infrared Spectroscopy (FTIR), X-ray Photoelectron Spectroscopy (XPS), and Scanning Electron Microscope (SEM).

10.3 The determination of detection efficiency of multiplex electrochemical genosensors toward clinical samples.

10.3.1 Collect clinical samples, such as blood and urine from the hospital.

10.3.2 Determine sensitivity, specificity, predictive values, and accuracy of the newly developed genosensors for the detection of carbapenem resistant *Enterobacterales* gene by standard additional method in comparison with standard methods.

๑๑. ขอบเขตของการวิจัย (Scope of the study)

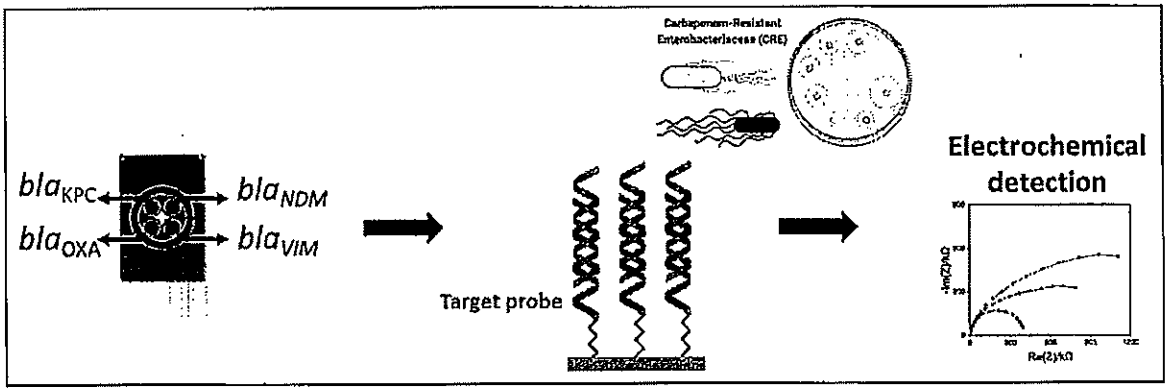

Figure 1. Schematic illustration of rapid multiplex Lab-on-Chip for carbapenem resistant *Enterobacterales*

๑๒. ผลผลิต (Output) ผลลัพธ์ (Outcome) และ ผลกระทบ (Impact) ที่คาดว่าจะได้จากการวิจัย

Outputs/outcomes	Who will benefit from its utilisation?	
	Organisation	Stakeholder
Developing 1-2 prototypes of novel impedimetric DNA sensors for detecting carbapenem resistant *Enterobacterales* genes	Public and private hospital Ministry of Public Health Import/export industry Academy and institute	Medical personnel Patient Business owner Researcher Student
Producing at least 2 papers published in international journals with peer reviews	Public and private hospital Ministry of Public Health Import/export industry Academy and institute	Medical personnel Business owner Researcher Student
Presenting the results in the conference both inside and outside the country at least once a year	Public and private hospital Ministry of Public Health Import/export industry Academy and institute	Medical personnel Business owner Researcher Student
Patents of novel impedimetric DNA sensors for detecting carbapenem resistant *Enterobacterales* genes	Public and private hospital Ministry of Public Health Import/export industry Academy and institute	Medical personnel Business owner Researcher Student
Producing excellent researchers and young scientists	Public and private hospital Ministry of Public Health Import/export industry Academy and institute	Medical personnel Business owner Researcher Student

ผลกระทบ (Impact)

- Smart genosensors can provide rapid and early detection of CRE in clinical samples, enabling timely intervention and improved patient outcomes and help guide antibiotic therapy by identifying the

specific resistance mechanisms present in CRE strains. This information can assist clinicians in choosing appropriate antibiotics and optimizing treatment regimens.

- The data collected from genosensor-based detection can contribute to surveillance efforts, helping public health authorities monitor the prevalence and spread of CRE strains in different regions.
- Early detection and appropriate management of CRE infections can lead to shorter hospital stays, fewer complications, and reduced healthcare costs associated with prolonged treatment and patient care.