Eco-Friendly Synthesis of Graphene Intercalation Material for Highly Sensitive MALDI-MS Bioanalysis

Yao-Tsung Hsu [a,b], Shih-Min Wang [c], **Fu-Der Mai [a,b*]**

[a] Graduate Institute of Medical Sciences, College of Medicine, Taipei Medical University, Taipei 11031, Taiwan.

[b] Department of Biochemistry and Molecular Cell Biology, School of Medicine, College of Medicine, Taipei Medical University, Taipei 11031, Taiwan.

[c] National Atomic Research Institute, Taoyuan 32527, Taiwan

Corresponding Author:

Fu-Der Mai,

Taipei Medical University, Taipei 11031, Taiwan. *E-mail: fordmai@tmu.edu.tw

Introduction: Developing highly sensitive and environmentally benign materials for biomolecular analysis remains a critical challenge. Matrix-Assisted Laser Desorption/Ionization-Mass Spectrometry (MALDI-MS) is a powerful tool in proteomics, but its sensitivity is often limited by the co-crystallization matrix. We propose a novel, **eco-friendly synthesized** intercalation material designed to function as an "amphiphile attractor" to significantly boost analytical performance.

Methods: Our methodology begins with the **sonication-induced scission** of few-layer precursory graphene, leading to the **asymmetric cleavage** and production of nanoscale **Asymmetrically Cleaved Graphene (ACG)**, with an average dimension of 41.58 nm. The ACG exhibits high surface energy, making it intrinsically **"amphiphile-attractive."** Subsequently, ACG is self-assembled and wrapped by amphiphiles into a **hemimicelle structure**, allowing it to **intercalate** into bulk graphite to form the final **Asymmetrically Cleaved Graphene Intercalated Material (ACGIM)**.

Results and Discussion: The unique structure of ACGIM is highly promising for bioanalysis. The attracted amphiphiles within the ACGIM effectively **stabilize biomolecules**, which is crucial for signal integrity. To validate its analytical potential, we conducted a signal enhancement experiment using ACGIM as a novel matrix substitute for **peptide detection** via MALDI-MS. The results demonstrated a remarkable **22-fold enhancement** in the detection signal for the target peptide compared to conventional methods.

Conclusion: The **ACGIM** represents a new class of **amphiphile-attractive** intercalation materials synthesized under green conditions. Its superior ability to stabilize biomolecules and significantly enhance signal intensity in MALDI-MS offers a robust platform for highly sensitive **bioanalysis**, particularly in peptide and protein research. Further exploration into its application for diverse biomolecule types is warranted.