NANYANG TECHNOLOGICAL UNIVERSITY SINGAPORE
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SERS Detection of NAFLD via Multimodal Probe-Metabolite Interactions

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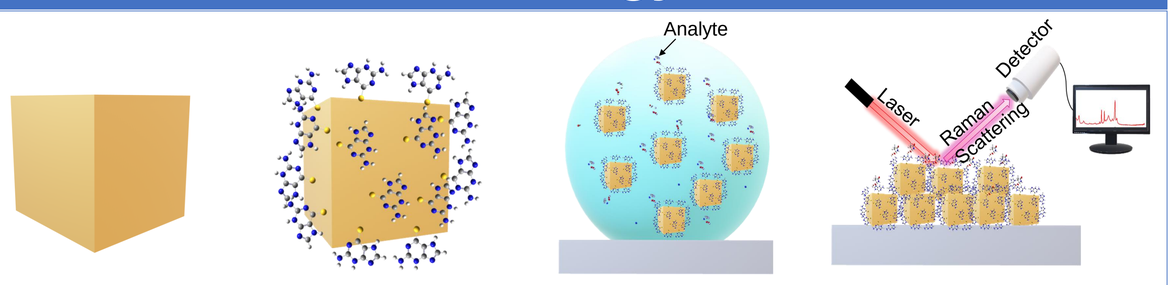
Introduction

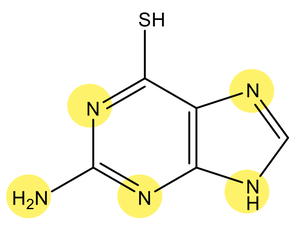
Non-alcoholic fatty liver disease (NAFLD) is a common cause of chronic liver disease and affects 15-30% of the general population. While liver biopsy remains the gold standard for early detection of NAFLD to terminate progression and avoid advanced complications, it is both invasive and expensive. This shows the need for novel, rapid and cost-effective detection methods. The current strategy in metabolomics revolving around NMR, GC-MS and LC-MS for identification and quantification of metabolites suffers from long screening time and large sample volumes. Thus, this research focuses on employing the use of Surface Enhanced Raman Spectroscopy (SERS), a powerful tool for detection of trace metabolites to detect target analytes at low concentrations. Thioguanine is used as the probe molecule to capture metabolites via multiple modes of interaction under different pH conditions.

Strategy



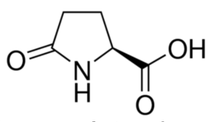
Synthesis of Ag Nanocubes | **Functionalization** | **Probe-analyte Interaction** | **SERS measurement**

Thioguanine as the probe molecule

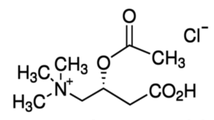


6-thioguanine

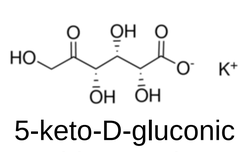
Formation of multi-valent hydrogen bonds with target analytes



L-pyroglutamic Acid (LP)

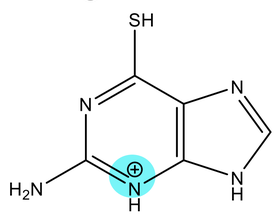


O-acetyl-L-carnitine hydrochloride (OAC)

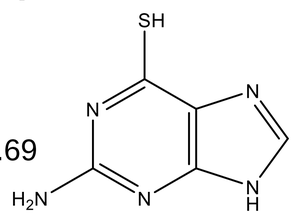


5-keto-D-gluconic acid potassium (5KD)

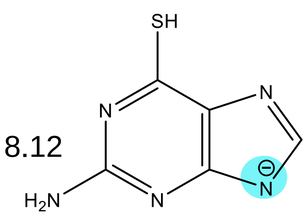
Thioguanine at different pH



pKa_1: 2.69



pKa_2: 8.12



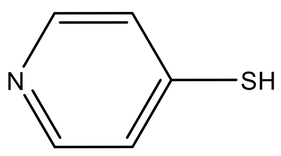
Different pH → Different configuration → Different Interaction

SERS phenomenon

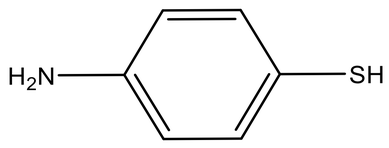
- Target analytes experience enhanced electromagnetic field
- Due to localized surface plasmon resonances of silver nanoparticles
- Enhanced signals, obtain fingerprints

Control Experiments – Single Valency Probes

Affirm superiority of thioguanine with multiple bonding sites



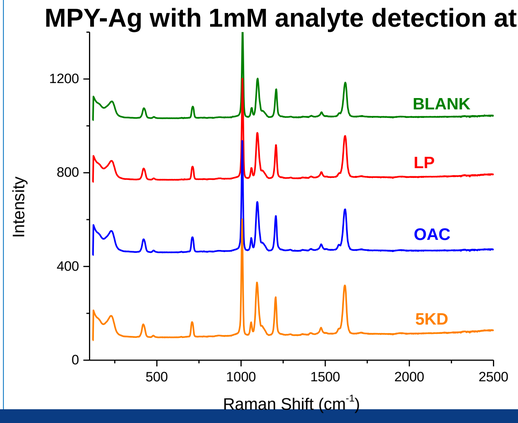
4-mercaptopyridine



4-aminothiophenol

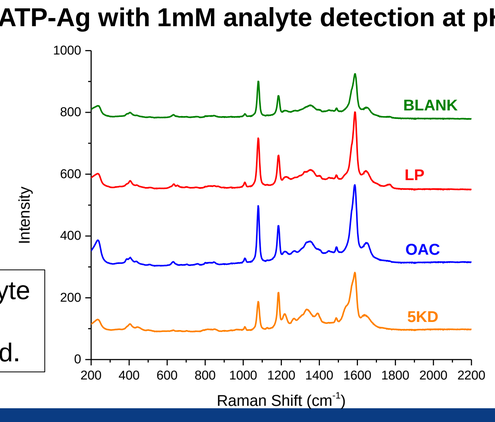
Results and Discussion

MPY-Ag with 1mM analyte detection at pH 2



No analyte peaks observed.

ATP-Ag with 1mM analyte detection at pH 2

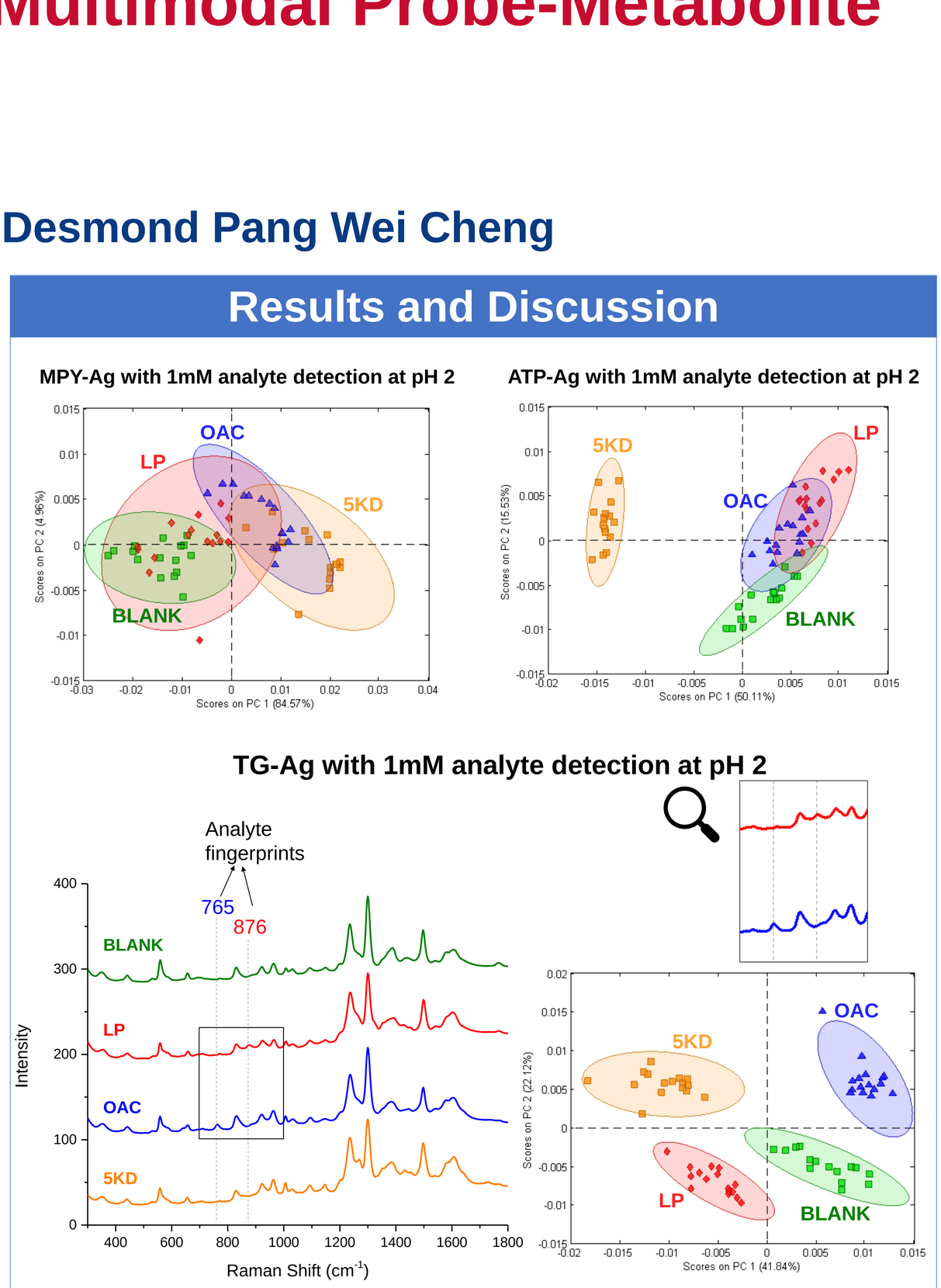


Results and Discussion

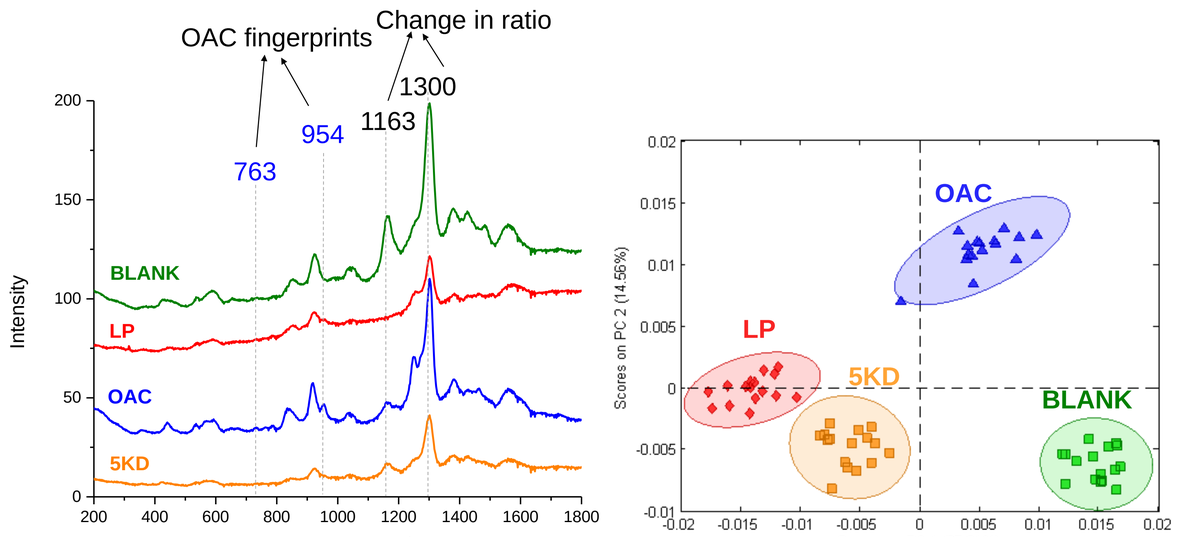
MPY-Ag with 1mM analyte detection at pH 2

ATP-Ag with 1mM analyte detection at pH 2

TG-Ag with 1mM analyte detection at pH 2



TG-Ag with 1mM analyte detection at pH 9



- PCA(Principal Component Analysis) separation shows that spectrum of different analytes can be distinguished due to analyte peaks and changes in TG peaks arising from interactions.

Conclusions and Future Work

- Successful functionalization of silver nanoparticles with thioguanine
- Able to observe analyte peaks for LP and OAC at 1mM at pH 2
- At pH 2, superiority of TG established compared to controls
- Able to distinguish between analytes with PCA plots at pH 2 and pH 9

- Detection of analytes at lower concentration (0.1mM)
- DFT simulation to determine configuration and orientation of TG

References

- Dong, S; Zhan, Z.Y.; Cao, H.Y.; Wu, C; Bian, Y.Q.; Li, J.Y.; Cheng, G.H.; Liu, P; Sun, M.Y. Urinary metabolomics analysis identifies key biomarkers of different stages of nonalcoholic fatty liver disease. World J Gastroenterol. 2017, Apr 21;23(15), 2771-2784.
- Kao, Y.C.; Han, X; Lee, Y.H.; Phan-Quang, G.C.; Lay, C.L.; Sim, H.Y.F.; Phua, V.J.X.; Ng, L.S.; Ku, C.W.; Tan, T.C.; Phang, I.Y.; Tan, N.S.; Ling, X.Y. Multiplex Surface-Enhanced Raman Scattering Identification and Quantification of Urine Metabolites in Patient Samples within 30 min. ACS Nano. 2020, 14, 2542-2552.