Mass Spectrometry and Capillary Electrophoresis Frontal Analysis for Characterizing DNA-Small Molecule Interactions

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Gold statement
- Rapidly screen the small molecule candidates that target G-quadruplex DNA using ESI-MS
- Provide reliable binding constants and stoichiometries using pressure-assisted CE-FA
- Extract specific binding informations in the presence of nonspecific binding

Introduction
G-quadruplex (G4) induced in oncogenic G-rich promoter regions has certain impacts on their biological functions, such as transcription [1]. Thus, G4 DNA has become an attractive target for gene therapeutics ever since. However, the dynamic nature of G4 folding has made it difficult to study using methods that require immobilization or other types of modification before affinity evaluation. It is necessary to develop a systematic approach to screening for small molecule binding to the specific G4 DNA and providing more accurate biorelevant binding parameters [2,3].

Body
In this communication, we report a systematic approach to study noncovalent interactions between human oncogene promoter G4 DNA and small molecular ligands. The method utilizes ESI-MS to first rapidly screen for possible ligands and to provide the binding stoichiometry. Then a solution-based technique, pressure-assisted CE-FA, is used to confirm the solution state interaction and determine the accurate binding parameters. With the consideration of both specific and nonspecific bindings coexist in the G4-ligand interactions, a new equation is introduced to make this reference-free method based on CE-FA. Natural product drug candidates were tested for their ability to bind specifically the promoter G4 DNA with the reliable binding stoichiometry and affinity constants. Briefly, the combination of ESI-MS and CE-FA not only increases the speed of analysis but also improves the accuracy and specificity of the analysis compared to conventional binding approaches. In the future, we believe that the method described in this work can be used for other molecular interactions and drug candidate screening.

Conclusion
Using this reference-free method, jatrorrhizine and palmatine were demonstrated to bind specifically to the Bcl-2 promoter G4 DNA with stoichiometries of 4:1 and 3:1, respectively.

References
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