Detecting Cytosolic G4-binding proteins in Live Cells Using DBF-Conjugated AS1411

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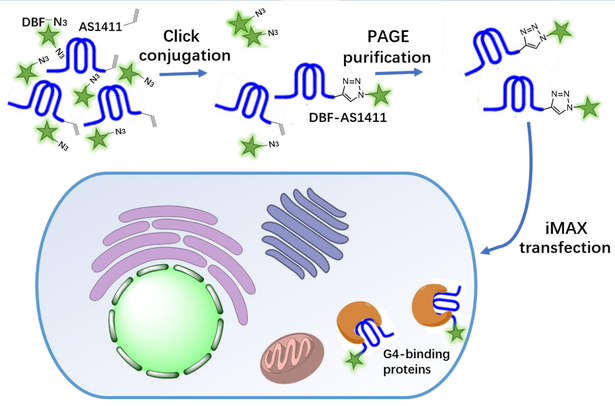
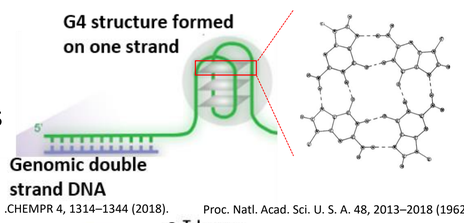
Abstract

G4/rG4 is a non-canonical DNA/RNA structure thought to be widely existed *in vivo.* Its stabilization lead to an anti-cancer effect, yet the explicit mechanism is still under investigation, calling for a deeper understanding of G4/rG4 interactomes. Here, we presented a proof-of-concept work that detects cytosolic G4-binding proteins by conjugating AS1411, a G4 forming oligo, with dibromofluorescein (DBF), a fluorescent photosensitizer. With optimized protocol, 5' Alkyne-AS1411 was clicked with DBF-Azide at a ~50% yield and purified to nearly pure after PAGE gel purification. The product was then transfected into HEK293T cells, with iMAX demonstrated to be the most efficient. The signal is generally constant but background increases over transfection time, probably due to degradation of AS1411 by cytosolic nucleases over time. In all, our findings suggest that DBF-conjugated AS1411 can be successfully prepared and transfected into live cells. We are looking into DBF-mediated photolabeling and enrichment to further detect cytosolic G4 binding proteins, laying solid foundation to identify novel G4 binding proteins and study their corresponding biological functions.



Introduction

- G4 is a non-canonical structure
- G4 involves in many biological processes
- Stabilization of G4 have anti-cancer effects
- Mechanism calls for interactome studies



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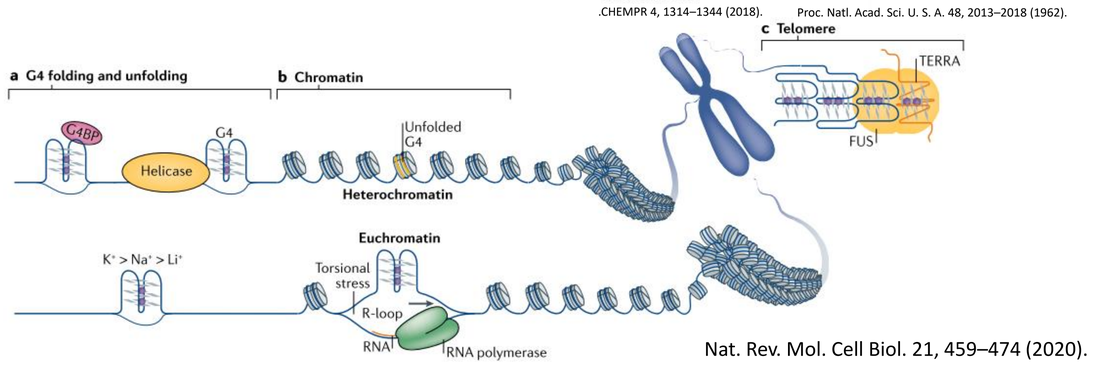
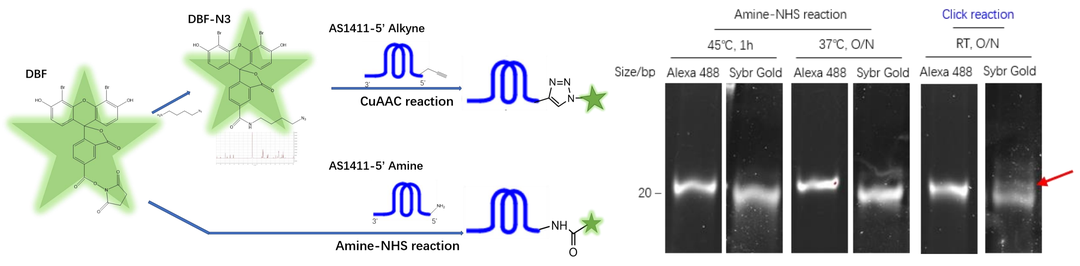


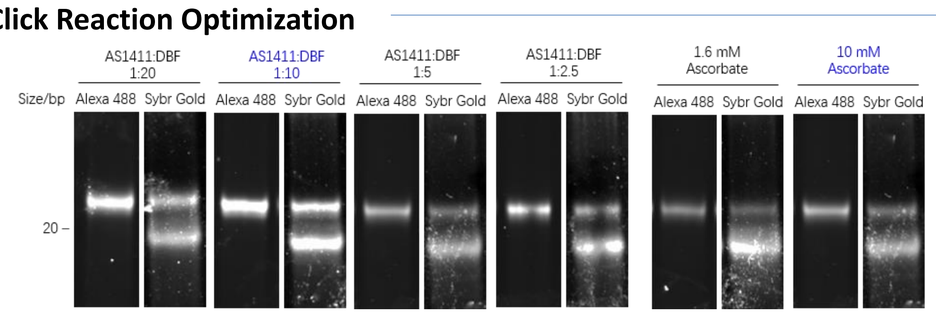
Nat. Rev. Mol. Cell Biol. 21, 459–474 (2020).

Oligo Conjugation Experiments

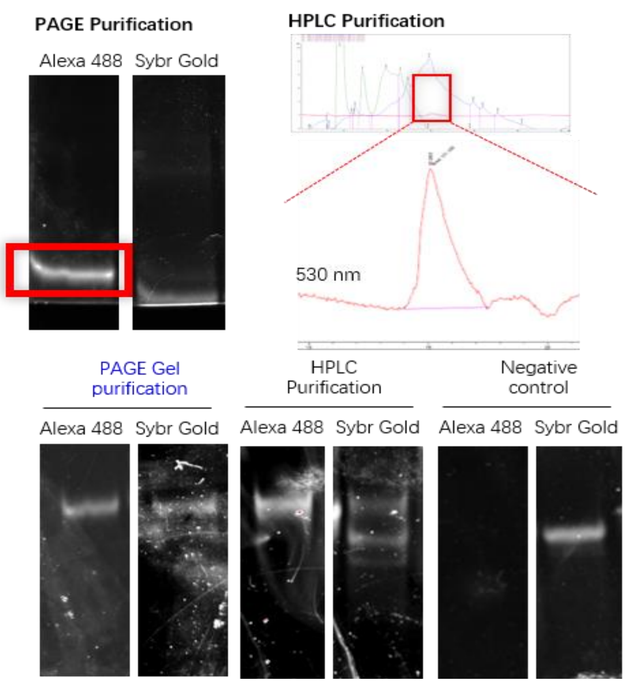
Click Reaction Yields Higher than Amine-NHS reaction



Click Reaction Optimization

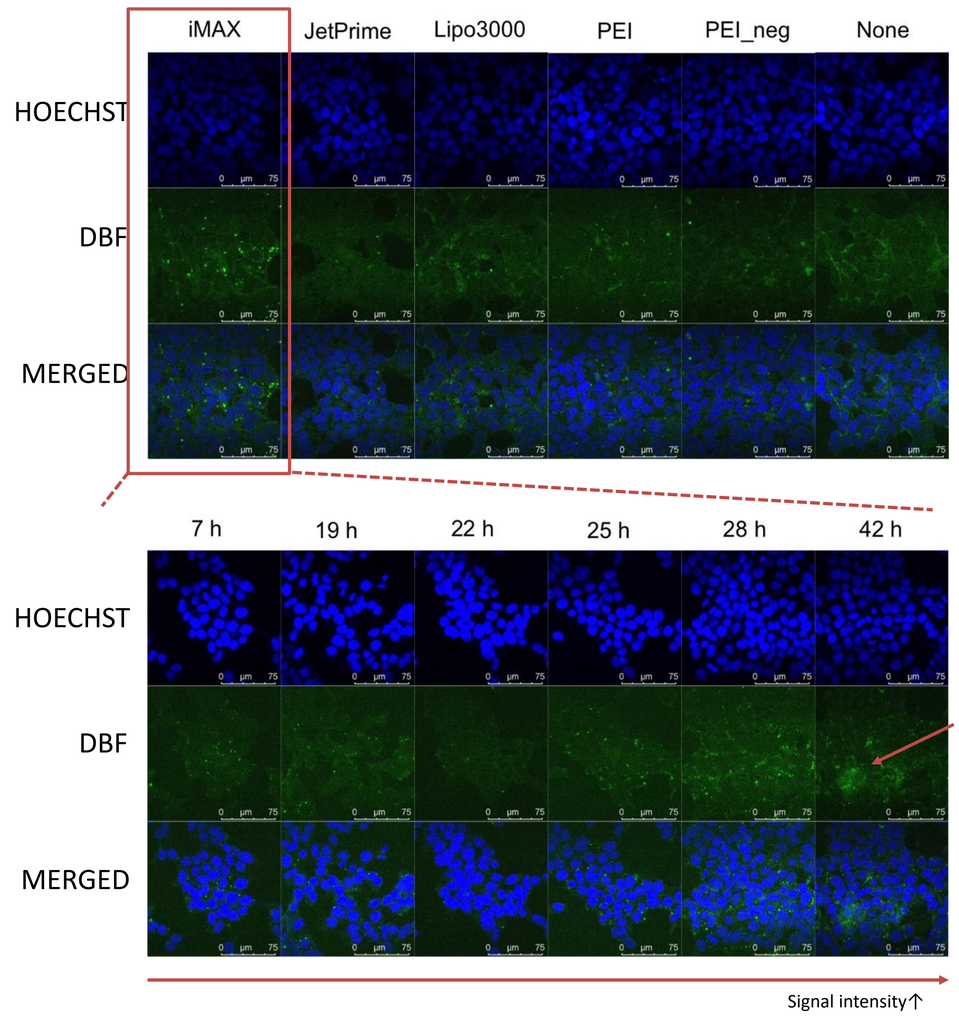


Purification



Transfection Experiments

Transfection and Visualization of DBF-Conjugated AS1411



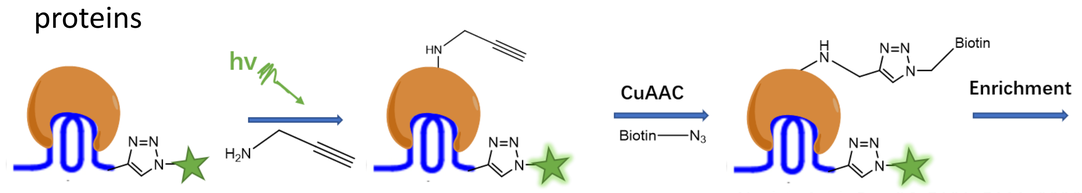
Discussion

1. Established protocols to conjugate DBF to AS1411 oligos
2. Optimized the approach to transfect cells with AS1411-DBF

In the future:
1) Calibration curve for more accurate measurement of oligo conjugation yield
2) CD spectrometry for better understanding the effect of conjugation on G4 formation

Conclusion and Outlook

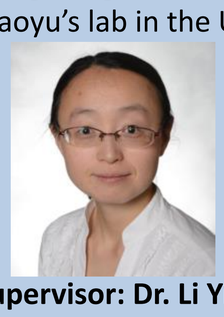
- ✓ Optimized click conjugation and PAGE purification for construction and purification of DBF-AS1411
- ✓ DBF-AS1411 can be transfected into HEK293T cells and formed dot-like signals
- ➢ Enrich DBF-AS1411 interactomes and confirm its binding towards G4-binding proteins



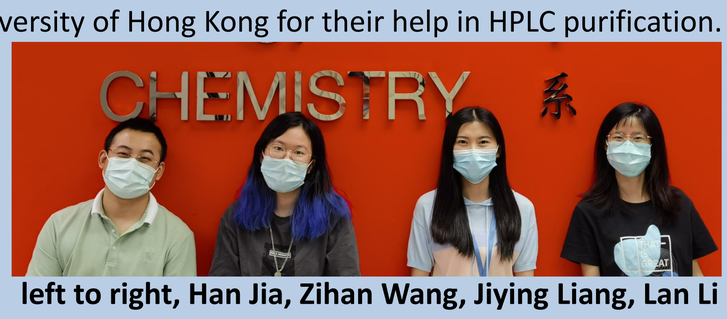
Nucleic Acids Res. **34**, 5101–5111 (2006)

Acknowledgement

The Author would like to thank Dr. Chun Kit Kwok's lab in the City University of Hong Kong for their suggestion in click reaction and PAGE purification, and Dr. Li Xiaoyu's lab in the University of Hong Kong for their help in HPLC purification.



Supervisor: Dr. Li Ying



left to right, Han Jia, Zihan Wang, Jiying Liang, Lan Li

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