A Click Chemistry Approach to cis-Platinum(II)-TFO Targeted DNA Crosslinking

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Introduction

Therapies that target the downstream inhibition of gene expression are of great current research significance with considerable efforts now dedicated to the discovery of new antisense oligonucleotides (ASOs). The development of nucleic acid probes targeting DNA, such as triplex-forming oligonucleotides (TFOs),[1] offers an alternative strategy whereby gene expression is directly inhibited at the genomic level. Here, we present a click chemistry strategy for the generation of a new class of *cis*-platinum(II)-TFO hybrid biomaterial that possess DNA binding and crosslinking activity (**Figure 1**).[2]

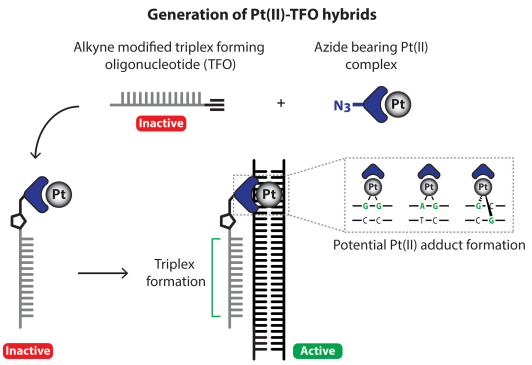


Figure 1. Overview of hybrid design and application. Development of clickable Pt(II) complex prior to utilisation of click chemistry to generate Pt(II)-TFO hybrid. Crosslink formation on either duplex strand is facilitated by the triplex formation.

cis-Platinum(II)-TFO Design and Generation

A family of *cis*-platinum(II) complexes with azide-modified handles—conceptually based on cisplatin, carboplatin and oxaliplatin—have been developed to allow for facile incorporation onto the TFO sequences **(Figure 2A-B)**. The use of the CuAAC and SPAAC 'click' chemistry strategies have afforded novel Pt(II)-TFO hybrids. TFO3 hybrids demonstrated efficient triplex formation and crosslinking activity **(Figure 2C).**

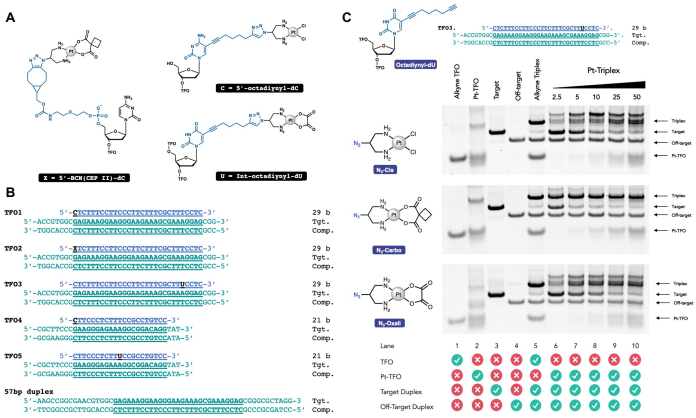
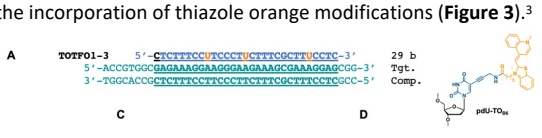


Figure 2. A. Alkyne-modified nucleobases. Click conjugation with each of the azide-functionalised platinum(II) complexes. **B.** TFO sequences with nucleobase modifications denoted and specific GFP gene recognition sites indicated. **C.** TFO3 hybrid triplex formation and crosslinking.

Triplex-Stability Studies

Triplex stability was identified using UV thermal melting analysis. Hybrid crosslinking actively destabilises the triplex, however this is remediated and enhanced by the incorporation of thiazole orange modifications (**Figure 3**).[3]



B

Oligo	T_M (°C)	ΔT_M (°C)
Alkyne-TFO3	52.0 ± 0.1	0
TFO3-Cis	42.6 ± 0.5	-9.4
TFO3-Oxali	44.1 ± 0.6	-7.9
TFO3-Carbo	45.5 ± 0.5	-6.5
TOTFO1-Carbo	57.2 ± 0.7	5.2
TOTFO2-Carbo	> 65	>13

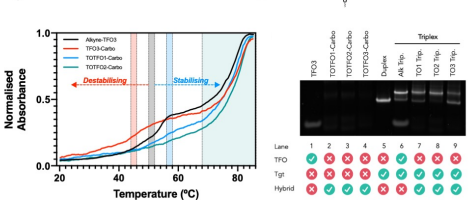


Figure 3. A. Modified TFO sequence with 1-3 TO incorporations. **B.** T_M values of triplex melting observed for TFO3 and TOTFO hybrids. **C.** Thermal melting curves showing destabilising and stabilising effects in comparison to alkyne-modified TFO3. **D.** Page Analysis of TOTFO sequences.

TFO-hybrid Crosslinking Activity

TFO-hybrid crosslinking activity was investigated through the use of sodium cyanide treatment experiments. Duplex sequences with integrated fluorescent tags provide visual distinction between standard triplex formation and platinum-assisted triplex formation. Comparison between control TFOs and platinum TFO-hybrids demonstrates crosslink reversal **(Figure 4)**.

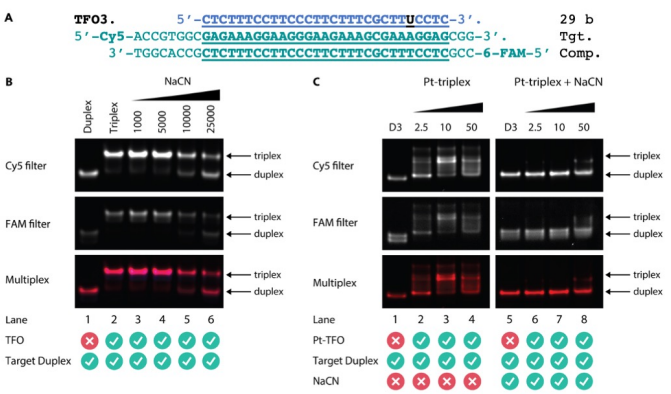


Figure 4. A. TFO3 and fluorescently-tagged duplex target sequence. **B.** Treatment of alkyne-TFO3 control shows retention of triplex formation at 25,000 eq. **C.** Treatment of TFO3-Carbo triplex with sodium cyanide at similar concentrations as the control demonstrates crosslink identity and reversal.

Crosslink Targeting Analysis

A shorter series of alkyne-modified TFO hybrids were developed to investigate the precise targeting of the GFP gene targets. TFO hybrids incorporating the carboplatin analogue were developed and treated against fluorescently-tagged short GFP gene duplex sequences. Denaturing PAGE analysis (**Figure 5**) was used to determine that the purine target was the location of the crosslink lesion upon triplex formation.

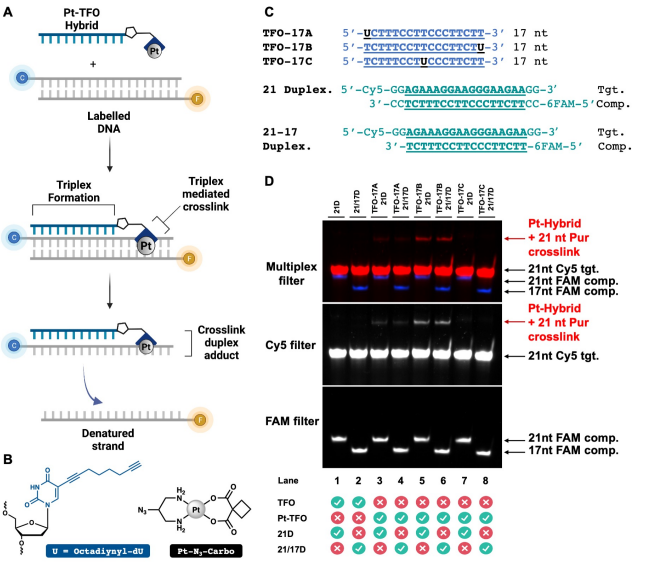


Figure 5. A. Schematically representation of denaturing PAGE experiment. **B.** Nature of nucleobase modification and platinum(II) complex. **C.** TFO-Carbo hybrids generated and GFP gene duplex targets. **D.** Denaturing PAGE analysis. Duplex structure consisting of the TFO-Carbo hybrid and the purine target sequence was identified using Cy5-labelled modification.

Conclusions

This work has shown the generation of a novel class of Pt(II)-TFO hybrids utilising click chemistry strategies with antigene applications. The ability for specific targeting of nucleic acid sequences has been demonstrated alongside the potential to discreetly deliver a platinum agent to a precise target. Future work consists of improving the thermal stability of the Pt(II)-TFO triplex motif using modified nucleobases and further thiazole orange derivatisation. Nucleic acid sequencing will be utilised to identify the location and nature of Pt(II)-TFO crosslinking.

References

1. Fox, K. R.; Brown, T.; Rusling, D. A. DNA Recognition by Parallel Triplex Formation, In *DNA targeting Molecules as Therapeutic Agents*, **2018**, 1-32.
2. **Hennessy, J; McGorman, B; Kellett, A.** ***et al. Angew. Chem. Int. Ed.*** **2022, 61, e202110455.**
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