29th Symposium on Chemistry Postgraduate Research in Hong Kong

Photofunctional Cyclometalated Iridium(III) Polypyridine Complexes Bearing a Perfluorobiphenyl Moiety for Bioconjugation, Bioimaging, and Phototherapeutic Applications

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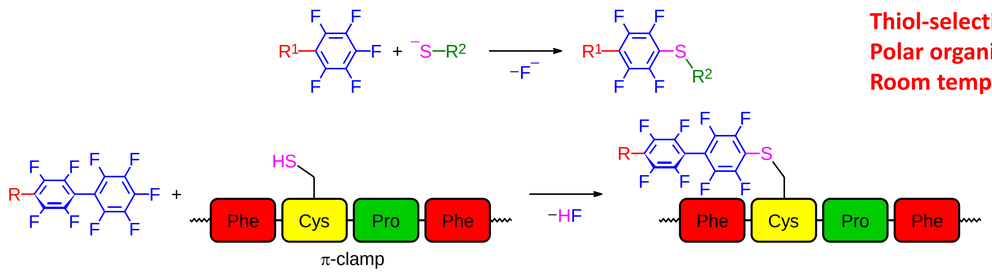
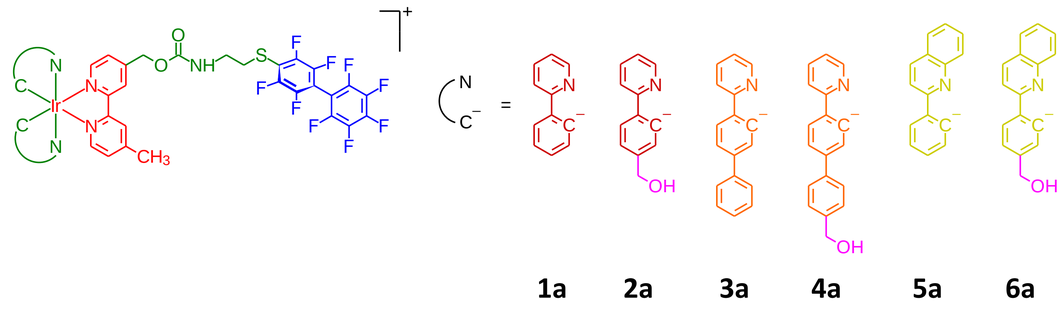
Introduction

Cysteine is a prominent target for the chemoselective modification of peptides and proteins, owing to the high nucleophilicity of its thiolate side chain and its low natural abundance. Recently, cysteine arylation with perfluoroaromatics has gained considerable attention.

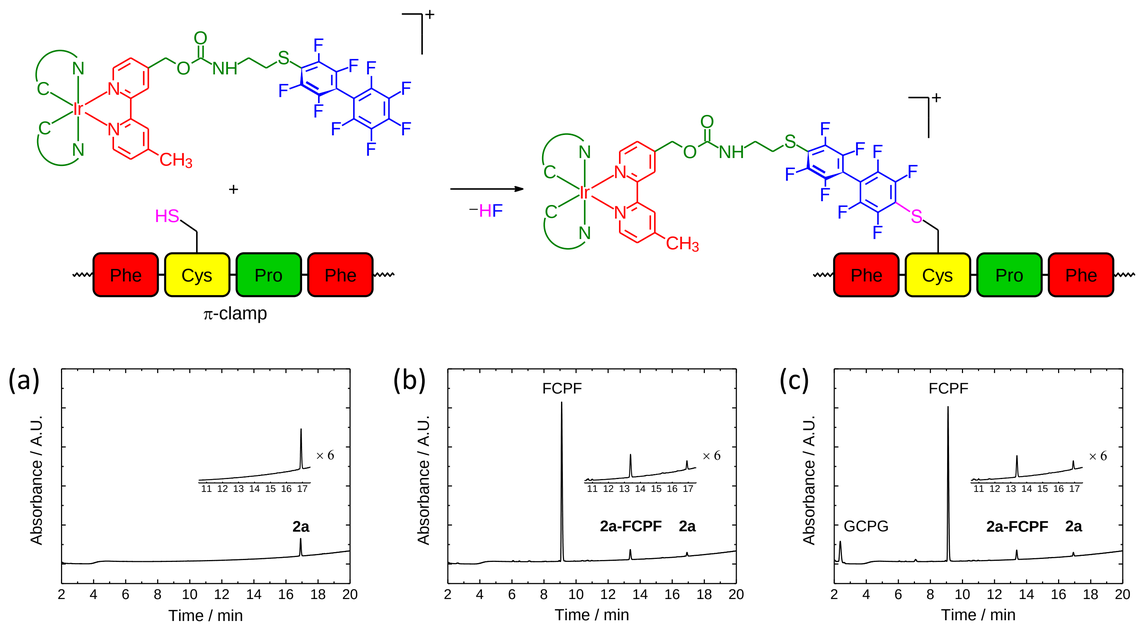


Structures of Complexes



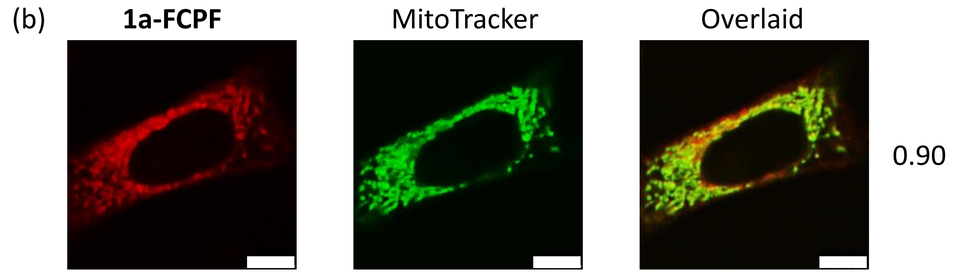
1. The perfluorobiphenyl (PFBP) moiety will specifically and selectively react with the cysteine residue of FCPF-containing peptides
2. The hydroxyl group will increase the water solubility of the complexes

Reaction with FCPF-containing Peptides



HPLC chromatograms of the reaction mixture of (a) complex **2a** (25 μM), (b) complex **2a** (25 μM) and FCPF (1.25 mM), (c) complex **2a** (25 μM), FCPF (1.25 mM), and GCPG (1.25 mM) in potassium phosphate buffer (200 mM, pH 8.0)/DMSO (9:1, *v*/*v*) containing TCEP (20 mM) after incubation at 37°C for 1 h. The absorbance was monitored at 220 nm.

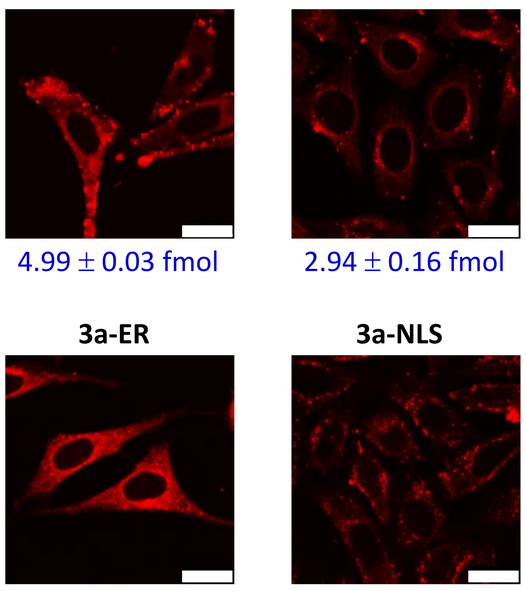
Cellular Localization of the PFBP Complexes and their FCPF Conjugates



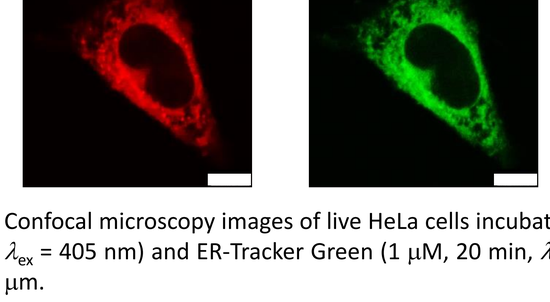
Confocal microscopy images of live HeLa cells incubated with (a) complex **1a** (5 μM, 2 h, λ_{ex} = 405 nm) or (b) conjugate **1a-FCPF** (25 μM, 2 h, λ_{ex} = 405 nm) and then MitoTracker Deep Red FM (100 nM, 20 min, λ_{ex} = 635 nm) at 37°C. Scale bar = 10 μm.

Cellular Uptake, Localization, and Photocytotoxicity of Organelle-targeting Peptide Conjugates

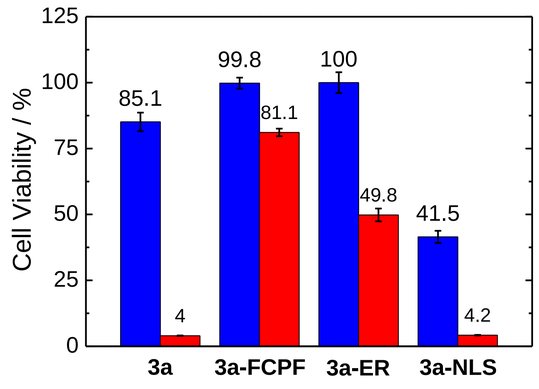
ER: Ac-FCPFKDEL; **NLS**: Ac-FCPFPKKKRKV-NH_2



Confocal microscopy images of live HeLa cells incubated with complex **3a** and its peptide conjugates **3a-FCPF**, **3a-ER**, and **3a-NLS** (25 μM) at 37°C for 2 h. Scale bar = 25 μm. The values indicate the amount of iridium associated with an average HeLa cell upon incubation with complex **3a** or its peptide conjugates (25 μM) at 37°C for 2 h, as determined by ICP-MS.



Confocal microscopy images of live HeLa cells incubated with conjugate **3a-ER** (25 μM, 2 h, λ_{ex} = 405 nm) and ER-Tracker Green (1 μM, 20 min, λ_{ex} = 488 nm) at 37°C. Scale bar = 10 μm.



Compound	Φ_Δ
3a	0.52
3a-FCPF	0.55
3a-ER	0.53
3a-NLS	0.53

Viability of HeLa cells incubated with complex **3a** and its peptide conjugates **3a-FCPF**, **3a-ER**, and **3a-NLS** (25 μM) at 37°C for 2 h, followed by incubation in the dark (blue) or irradiation at λ = 365 nm (red) for 30 min (light dose = 5.7 J cm^{-2}), and then further incubated with blank medium for 24 h.

Conclusion

Luminescent cyclometalated iridium(III) polypyridine complexes containing a PFBP moiety were synthesized, and their photophysical and photochemical properties and cellular behavior were studied. The modification of peptides with the PFBP complexes through the π-clamp-mediated cysteine conjugation afforded peptide conjugates that displayed interesting photophysical and photochemical characteristics, and different cellular uptake, localization properties, and (photo)cytotoxic activity. The results of this work will offer new insights into the development of luminescent cyclometalated iridium(III) polypyridine complexes as bioconjugation reagents.

Acknowledgements

We thank the Hong Kong Research Grants Council (Project No. CityU 11300019, CityU 11300318, CityU 11300017, CityU 11302116, and T42-103/16-N) for financial support.

Reference

Lee, L. C.-C.; Tsang, A. W.-Y.; Liu, H.-W.; Lo, K. K.-W. *Inorg. Chem.* **2020**, *59*, 14796 – 14806.