CHAPTER 5: CONCLUSIONS

The cellular uptake of transition metal complexes is only beginning to be explored, despite their potential utilization for biological applications. Here, we have demonstrated that luminescent dipyridophenazine (dppz) complexes of ruthenium(II) are well suited for studies of internalization and distribution in living cells. Using flow cytometry and confocal microscopy, we have systematically examined the effect of ancillary ligand variation on their cellular uptake. Lipophilicity is the strongest structural determinant for uptake efficiency, with the greatest internalization observed for $Ru(DIP)_2dppz^{2+}$, where DIP = 4,7-diphenyl-1,10-phenanthroline. This complex enters cells *via* passive diffusion, driven by the plasma membrane potential, as determined by a series of mechanistic studies. Furthermore, the dependence of cellular accumulation on lipophilicity is consistent with passive diffusion being the common mechanism of entry for the entire family. Surprisingly, although the nuclear pore complex is purported to be permeable to small molecules, nuclear accumulation of these complexes is relatively poor under conditions where they are present in the cytoplasm.

The dipyridophenazine complexes of ruthenium(II) furthermore serve as luminescent analogues of our 5,6-chrysenequinone diimine (chrysi) complexes of rhodium(III), which we are exploring as potential chemotherapeutic agents. These rhodium complexes target single base mismatches in DNA and selectively inhibit cellular proliferation in mismatch repair-deficient cell lines. Importantly, the biological activity of these complexes has been demonstrated to be a consequence of their DNA-binding,

suggesting that they reach the nucleus.[1,2] Naturally, we have a strong interest in understanding the uptake of these complexes, and optimizing their structure for the uptake and distribution properties that maximize therapeutic function. However, larger, more lipophilic complexes, although more readily internalized, interfere with DNA-binding, and hence biological activity.[2] As a result, we explored peptide conjugates, which we hypothesized should enter cells readily, without need of lipophilic bulk added near the metal.

Conjugation of D-octaarginine to $Ru(phen)(bpy')(dppz)^{2+}$ dramatically improves its rate of cellular uptake. At sufficient concentration of conjugate (~ 15 μM), the peptide also increases the nuclear localization; below this threshold concentration only cytoplasmic staining is observed. However, the uptake properties of the peptide are not independent of its payload. This is well demonstrated by the effects of tethering fluorescein to the metal-peptide conjugate. This doubly labeled peptide has a lower threshold concentration: the conjugate strongly stains the nucleus under conditions for which the construct without fluorescein is excluded. Furthermore, appending octaarginine to the rhodium complex increases the nonspecific affinity for DNA, decreasing the selectivity for mismatches.[3] We attempted to attenuate this effect by employing, shorter, less charged peptides, but found in each case that a much higher threshold concentration was required for nuclear entry. Hence, peptide conjugation as a strategy for nuclear delivery is subject to the same challenge as we demonstrated for the lipophilic complexes: structural variation for the optimization of uptake and distribution invariably affects functional properties, while the reverse is also true.

To avoid this complication, we are exploring strategies to allow optimization of the nuclear targeting moiety without affecting the activity of the complex. Current efforts involve separation of the uptake moiety from the payload with cleavable linkers. Ideally, these will release the active compound upon delivery to the target.

REFERENCES

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