# Self-assembly and ion transport by stimuli-responsive ruthenium(II) complexes

**Anatolii Zagorodnii**

**Supervised by: Dr Cally Haynes, Dr Kylie Yang, Dr Anna McConnell, Dr Jennifer Hiscock and Junyang Tan**

## Introduction

Cytoplasmic membrane transporters play a crucial role in the metabolic pathways, cell communication and proper functioning and cell maintenance in general. Malfunctions of the cellular transport can lead to disruptions of the ion gradients, lysosomal pH, the buildup of metabolites, and trigger programmed cell death. Consequently, the field of synthetic transporters' development has seen a tremendous rise in recent years. Particularly, ruthenium complexes attracted considerable research attention for their unique chemical and physical properties. For example, the luminescence they exhibit and their affinity towards nucleic acids was successfully used to detect RNA and DNA mismatch mutations, as demonstrated in numerous research papers (1,2). The other useful property is their general stability in biological environments and photodissociation that can be induced via irradiation. It allows for the switchable behaviour of such complexes, controlled cytotoxicity, and drug delivery applications since it can be manipulated to dissociate and release drugs at the desired microenvironments. Lastly, similar SSAs have been demonstrated to undergo self-assembly, form larger aggregates, exhibit antimicrobial activity and increase the efficacy of antibiotics if applied together (3). Therefore, the project's aim is to synthesise novel ruthenium complexes (Fig 1) and perform full characterisation together with a series of experiments to assess their antimicrobial potential.

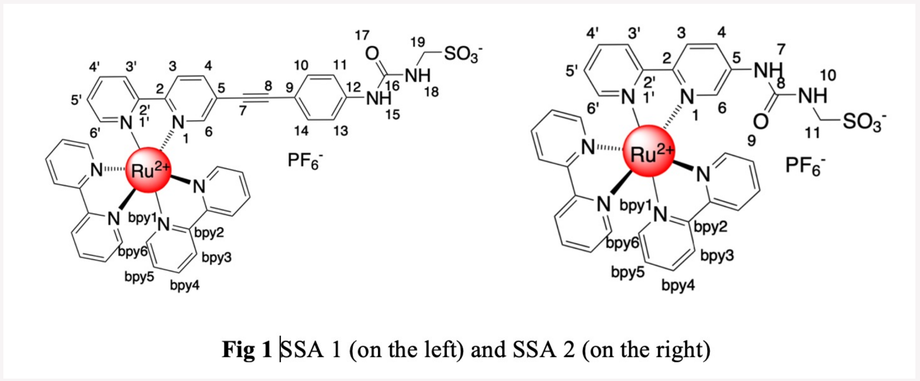


**Fig 1** SSA 1 (on the left) and SSA 2 (on the right)

## Synthesis

The standard procedure of the small-scale synthesis of SSA 1 and 2 (Fig 1) was followed:

- Dissolve [Ru(bipy)2]Cl2 together with corresponding tetrabutylammonium salt in ethanol
- Use bath sonicator and heat gun to ensure that reagents are completely dissolved
- Heat the mixture under reflux at 80 °C for 20 hours
- Leave it to cool down to the room temperature
- Add excess of ammonium hexafluorophosphate and place the flask in the fridge for 24 hours, so that precipitation can take place
- Perform a vacuum filtration and wash the product with 10 ml of deionised water to remove any unreacted ammonium salts
- Dry the product to obtain red crystals

## Fluorescent Spectroscopy

The aims of the fluorescent spectroscopy included:

- Figuring out absorption, excitation and emission wavelengths
- Adjusting filter excitation range for the subsequent fluorescent microscopy experiments with living cells.

| Name | Concentration | Absorption | Excitation | Emission |
|---|---|---|---|---|
| SSA 1 | 3.1 µg/mL | 1st peak at ~360nm, 2nd peak at ~456nm | ~459.2 nm | ~672 nm |
| SSA 2 | 3.1 µg/mL | 1 peak at ~448nm | ~457.2 nm | ~636 nm |

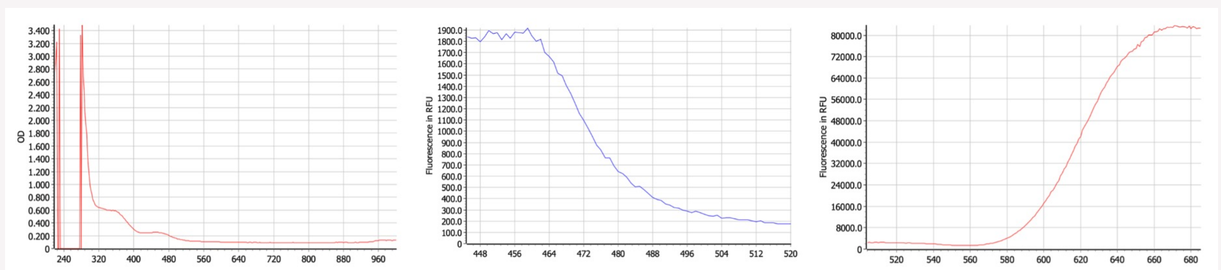


**Fig 2** SSA 1 Absorption (left), excitation (middle) and emission (right) spectra

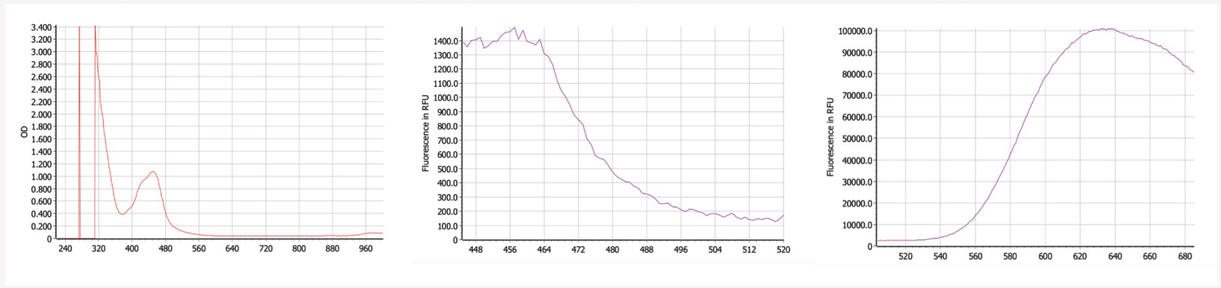


**Fig 3** SSA 2 Absorption (left), excitation (middle) and emission (right) spectra

During the experiments, common artefacts such as solvent peaks and Raman peaks were observed in the spectra. Therefore, additional processing and formatting were necessary to get concordant results and draw conclusions. Figs 2 and 3 demonstrate that both compounds have similar absorption and excitation wavelengths in the blue region of the visible light spectrum and more divergent emission wavelengths in the red region.

**Bibliography**

1. McConnell AJ, Lim MH, Olmon ED, Song H, Dervan EE, Barton JK. Luminescent Properties of Ruthenium(II) Complexes with Sterically Expansive Ligands Bound to DNA Defects. Inorganic Chemistry. 2012 Oct 31;51(22):12511–20.
2. McConnell AJ, Song H, Barton JK. Luminescence of $[Ru(bpy)_2(dppz)]^{2+}$ Bound to RNA Mismatches. Inorganic Chemistry. 2013 Aug 22;52(17):10131–6.
3. White LJ, Boles JE, Allen N, Alesbrook LS, Sutton JM, Hind CK, et al. Controllable hydrogen bonded self-association for the formation of multifunctional antimicrobial materials. Journal of Materials Chemistry B. 2020;8(21):4694–700.

## Quantitative NMR

qNMR was used quantify fraction of molecules that undergo self-association in the aqueous solution and form larger aggregates.

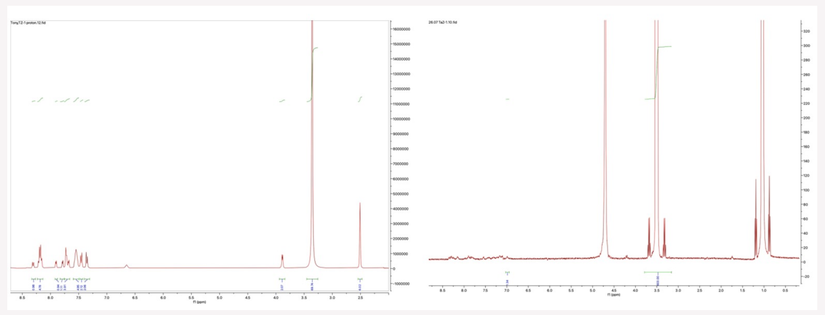

**Fig 4** SSA 1 in deuterated acetonitrile (left) and in 1:19 EtOH:H20 solution (right)

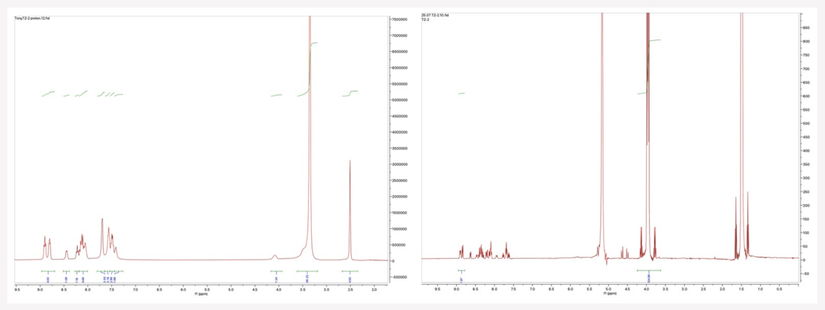

**Fig 5** SSA 2 in deuterated acetonitrile (left) and in 1:19 EtOH:H20 solution (right)

Fig 4 and 5 demonstrates that 33%  and 25% loss was observed for SSA 1 and 2 respectively.

## Vesicles screening experiments

The aim of the vesicle studies was to test whether SSAs would fuse with the phospholipid bilayer and facilitate ion transport across the membrane. The following procedure was followed:

- Phospholipid suspension preparation via standard protocol
- 9 freeze thaw cycles to
- Vesicles preparation using extruder with 200 nm polycarbonate membrane
- Purification using dialysis tubing
- Addition of SSA and voltage measurements using chloride selective electrode
- Addition of detergent after 5 min 30 sec to break open vesicles and calculate 100% chloride efflux

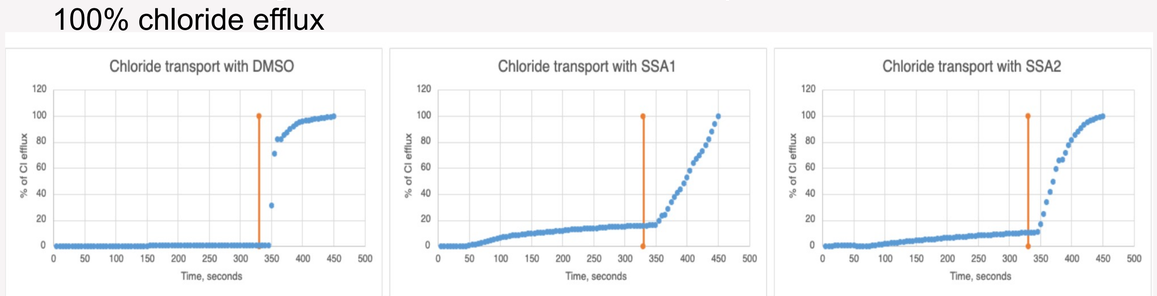


**Fig 6** results of vesicles screening experiments with DMSO (left), SSA1 (middle) and SSA 2 (right)

Fig 6 demonstrates that SSA 1 and SSA 2 produced up to 20% and 10% chloride efflux respectively.

## Conclusions

Results from fluorescent microscopy, qNMR and vesicle studies indicate that:

- Compounds crystallised instead of adhering to bacterial membranes
- Fraction of molecules that undergo self-association is quite low for both SSAs
- Ion transport observed for both compounds most likely won't generate significant therapeutic effects
- Prescence of additional hydrophobic moiety in SSA 1 improved compound's performance in both experiments by favoring self-association and fusion with the phospholipid bilayer

Therefore, further modifications to the chemical structure will be required to improve the compound's deliverability and get the desired antimicrobial effects.

**Acknowledgements**

I want to express special thanks to Dr Anna McConnell and Tjorge Neumann from Otto-Diels Institute, Dr Cally Haynes and Dr Kylie Yang, Hiral Kotak, Anamaria Leonescu and Junyang Tan from UCL ,Dr Jennifer Hiscock from the University of Kent, and the rest of the participants of this collaborative project for the provided training, supportive supervision, insightful feedback and valuable advice!

Contact details: zcqsaza@ucl.ac.uk