

Supplementary Material (ESI) for Chemical Communications
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Supporting Information

A Furanosyl-Carbonate Autoinducer in
Cell-to-Cell Communication of *V. harveyi*

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General

Boron-free water was prepared by treating distilled water with Amberlite™ 743 resin. Autoinducer bioassay (AB) medium was prepared as previously described (Greenberg *et al.*) using either distilled water or boron-free water. *V. harveyi* strains MM30 (LuxS) and BB170 (LuxN) were propagated twice in boron-free AB medium (BFAB) and frozen stocks were prepared in BFAB containing 15% glycerol. The following compounds were obtained from Aldrich unless otherwise noted: FeCl₃, MgCl₂, Cs₂CO₃, CsCl, Fe(SO₄)₂·7H₂O (Sigma), CaCl₂·6H₂O (Acros), MnCl₂·4H₂O (Acros). 1M Stock solutions were prepared in boron-free water. DPD was prepared as previously described by Meijler *et al.*

V. harveyi Bioluminescence Assay

The assays were performed following the protocol reported by Schauder *et al.* In short, *V. harveyi* strain BB170 or MM30 were grown for 18 h at 30°C in AB or BFAB medium and diluted 1:500 in fresh BFAB medium. A 96-well microtiter plate was prepared with wells containing test compounds (120 µL/well) and 30 µL/well of the diluted cells. The plate was then allowed to incubate at 30°C and luminescence generally started to increase after four hours and was measured every 20 minutes, using a Microplate Luminometer LB96V (EG&G Berthold). Measurements were performed in triplicate.

Initial 1 mM Assay for Light Induction

The metals listed above were diluted to a concentration of 1.25 mM in BFAB medium and 120 µL aliquots were placed in a 96-well microtiter plate. 30 µL of either *V. harveyi* strain BB170 or MM30 cells (diluted 1:500 in BFAB) were added to each well and the plate was incubated at 30°C. Luminescence generally started to increase after 4 hours and was measured every hour. Cells were also monitored for growth at OD₆₀₀ (no effect was observed) and the pH was measured to be neutral.

Counter-ion Exchange Assays

A 1 M stock solution of silver triflate was prepared in boron-free water. A working solution of 100 mM was then prepared by diluting 1:10 in boron-free water. Equimolar amounts of metal chloride and silver triflate were mixed and the insoluble AgCl was filtered off.

Equimolar amounts of cesium carbonate and sodium chloride (in boron-free water) were mixed and diluted to a concentration of 625 µM in boron-free water.

The compounds were then tested for their ability to induce light (as described above) in *V. harveyi* strain BB170 at a starting concentration of 500 µM.

Decreasing [Borate] or [carbonate]

Boric acid, cesium carbonate, potassium carbonate, sodium carbonate and ammonium carbonate were diluted to a concentration of 2.5 mM in BFAB and serially diluted 1:1 in BFAB for a final volume of 120 µL. Subsequently, 30 µL of *V. harveyi* strain BB170 (diluted 1:500 in BFAB) was added to each well and the plate was incubated at 30°C. Luminescence generally started to increase after 4 hours and was measured every 20 minutes.

Decreasing [DPD]

DPD (initial concentration of 10 µM) was serially diluted 1:1 in BFAB for a final volume of 90 µL. 30 µL of 2.5 mM borate or carbonate was then added to each well. Subsequently, 30 µL of *V. harveyi* strain MM30 (diluted 1:500 in

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BFAB) was added to each well and the plate was incubated at 30°C. Luminescence generally started to increase after 4 hours and was measured every 20 minutes.