Supplementary Information (SI) for ChemComm.

Electronic Supporting Information

Ionic gradients in flow to control transport of emissive ions

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Contents

S1 General Experimental

S1.1 General Experimental

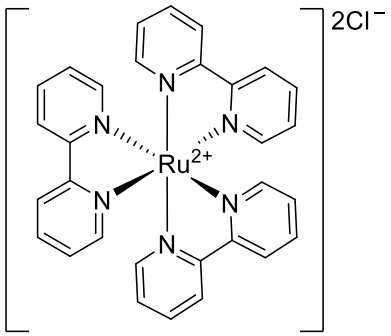
Reagents were purchased from Sigma-Aldrich, Merck, Chem Supply, Combi-Blocks or Alfa Aeser, and were used without purification. Experiments were completed in 50 mM 2-(N-morpholino)ethanesulfonic acid buffer (MES) buffered at pH 6 in MilliQ water.

Microfluidics components and tools were purchased from Darwin Microfluidics including 1 mm glass slides, silicon wafers, PTFE tubing, 0.5 mm hole punch and steel connectors.

Confocal microscopy experiments were performed with a Zeiss LSM 780 high-sensitivity laser scanning confocal microscope, equipped with a GaAsP photodetector. Our experiments used an air immersion Plan-Apochromat objective lens with 10x magnification and 0.45 numerical aperture. The available light sources included argon ion (458 nm, 488 nm, 514 nm) and DPSS (561 nm). The microscope was operated using *Zeiss ZEN black 2010* microscopy software.

Static fluorescence measurements were performed with a Horiba Scientific Duetta Fluorescence and Absorbance Spectrometer. Samples were contained in quartz cuvettes purchased from FireFlySci.

S2 Synthesis and characterization of $[Ru(bpy)_3]Cl_2$



Tris(2,2′-bipyridine)ruthenium(II) chloride $[Ru(bpy)_3]Cl_2$ was synthesized by literature procedures.[1]

S3 Modelling Fickian diffusion in 3-inlet channel

The model assumes simple Fickian diffusion of the ions in sodium chloride and sodium sulfate. It does not account for the effects of laminar flows or the electrostatic or dispersive interactions between species. The model is adapted from previously described.[2]

Time evolution of a species concentration (u) at position **r** is expressed as Equation 1:

$$\frac{\delta u}{\delta t}(\mathbf{r}) = D\nabla^2 u(\mathbf{r}).$$

where D is the diffusion coefficient and ∇ is the gradient operator. Equation 1 can be solved numerically with an explicit forward-time centered-space (FTCS) scheme:

$$u_j^{n+1} = u_j^n + D\frac{\Delta t}{(\delta x)^2}[u_{j+1}^n - 2u_j^n + u_{j-1}^n]$$

whereby j specifies the grid point and n specifies the time point.

The FTCS scheme requires initial conditions. For the case of species diffusion after being added to the central channel of a 3-inlet microfluidic device, the initial conditions can be given by the equation:

$$u_{t=0}^x = \frac{1}{2}u\{\tanh\left[\frac{\pi}{8}\left(\frac{L}{3} - x\right)\right] + 1\}.$$

where L is the length of the one-dimensional grid (the width of the channel, here L = 360 µm).

This ensures numeric stability with smoothing by hyperbolic tangent functions.[2] No flux boundary conditions are also employed.

Python code was used that numerically solves Equation 1 with the FTCS scheme discussed above. The code is provided at the end of this document (S10.1) and also at GitHub: https://github.com/lfillbrook/FickianModel.

Computed charge distributions were then converted to an electrical potential by numerical integration of charge density.

$$V(x_0) = \int_{-X}^{+X} \int_{-Y}^{+Y} \int_{-Z}^{+Z} \frac{\rho(x)}{4\pi\epsilon_0 r} dxdydz$$

Where r is the distance from a point on the x-axis to the charge element $\rho(x)dxdydx$ located at [x,y,z]. The charge density is determined from the difference in concentrations between cations, A, and anions, B.

$$\rho = F(Z_A[A] - Z_B[B])$$

Where F is the Faraday constant (96,485 C/mol) and Z represents the (absolute) ionic charge ($Z = -1$ for Cl^- and $Z = +1$ for Na^+). The limits of integration were taken from the physical dimensions of the apparatus (width = 360 µm and height = 50 µm) and a characteristic z-distance given by the product of flow velocity and sampling interval.

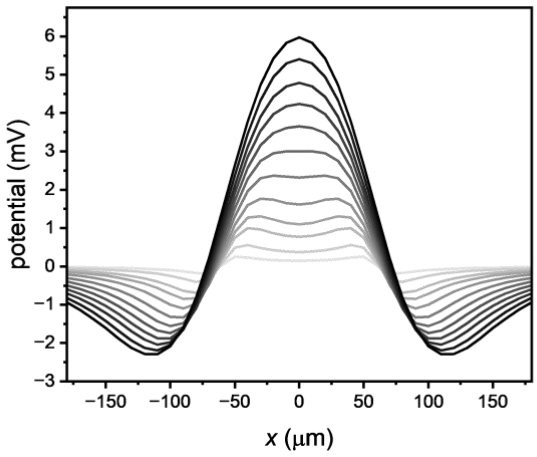


Figure S1 Modelling the formation of liquid junction potentials based on Fickian diffusion of sodium and chloride ions over time. Calculated electrical potential (mV) across the width of the channel is plotted over the same timescale as used for the Fickian model above.

S4 Static fluorescence and absorption measurements

A 50 µM stock solution of $Ru(bpy)_3Cl_2$ in MES buffer (50 mM, pH 6) was prepared. Using this stock solution, 50 μM solutions of $Ru(bpy)_3Cl_2$ were prepared with no salt (the control) or 1 M NaCl, or 1 M Na_2SO_4. The absorption spectrum of each sample was collected, shown in Figure S2, which confirms the visible absorption of the complex is unaffected by the presence of these salts. The change in absorbance at the MLCT (λ_{max} = 450 nm) was less than 1.5 % between the three samples.

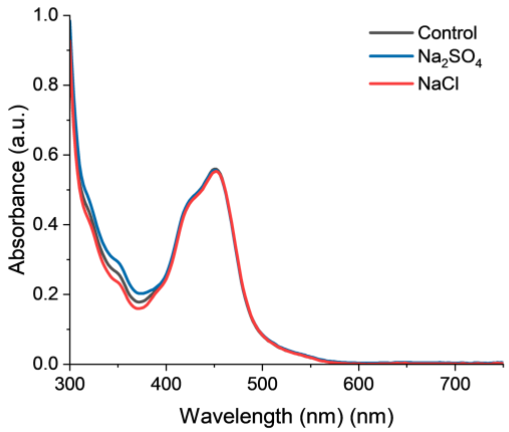


Figure S2. Absorption spectra for solutions of 50 μM $[Ru(bpy)_3]Cl_2$ in the absence (control) and presence of 1 M NaCl or 1 M Na_2SO_4, confirming the presence of salts do not affect the ^{1}MLCT absorption of the complex.

The emission spectra of the same samples were measured, with excitation at 450 nm (Figure S3). The emission intensity varied by less 12% between the samples, confirming minimal change in the emission quantum yield under these conditions.

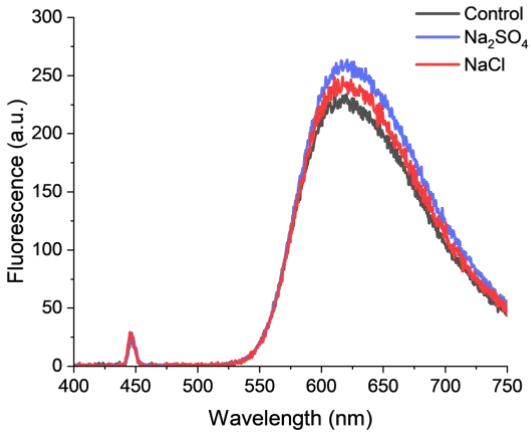


Figure S3 Emission spectra for solutions of 50 μM $[Ru(bpy)_3]Cl_2$ (λ_{ex} = 450 nm) in the absence (control) and presence of 1 M NaCl or 1 M Na_2SO_4.

A 50 µM stock solution of fluorescein in MES buffer (50 mM, pH 6.5) was prepared. Using this stock solution, 50 μM solutions of fluorescein were prepared with no salt (the control) or 1 M NaCl, or 1 M Na_2SO_4. The absorption spectrum of each sample was collected, shown in Figure S4, which confirms the visible absorption of the complex is unaffected by the presence of these salts. The absorption at λ_{max} = 490 nm increased by 18% and 21% in the presence of 1 M NaCl and 1 M Na_2SO_4 respectively.

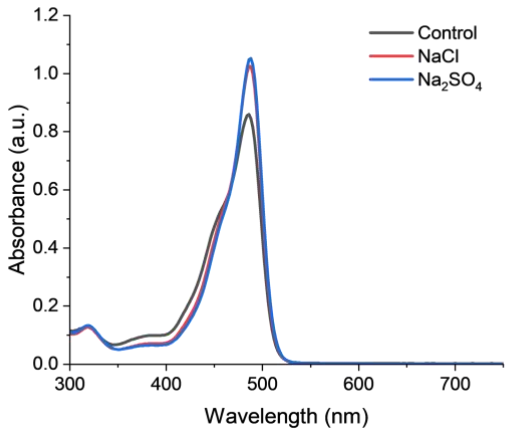


Figure S4 Absorption spectra for solutions of 50 μM Fluorescien in the absence (control) and presence of 1 M NaCl or 1 M Na_2SO_4, confirming the presence of salts do not drastically affect the absorption of the dye.

The emission spectra of the same samples were measured, with excitation at 490 nm (Figure S5). The emission intensity varied by less 5% between the samples, confirming minimal change in the emission quantum yield under these conditions.

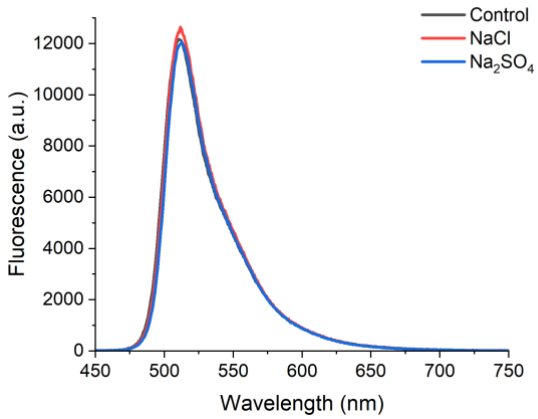


Figure S5 Emission spectra for solutions of 50 μM Fluorescein (λ_{ex} = 490 nm) in the absence (control) and presence of 1 M NaCl or 1 M Na_2SO_4.

A stock solution of $Ru(bpy)_3Cl_2$ (50 μM) and fluorescein (50 μM) in MES buffer (50 mM, pH 6.5) was prepared. Using this stock solution, solutions of $Ru(bpy)_3Cl_2$ (50 μM) and fluorescein (50 μM) were prepared with no salt (the control) or 1 M NaCl, or 1 M Na_2SO_4. The absorption spectrum of each sample was collected, shown in Figure S6, which confirms the visible absorption of the complex is minimally by the presence of these salts. The change in absorbance at the λ_{max} = 490 nm was less than 20 % between the three samples.

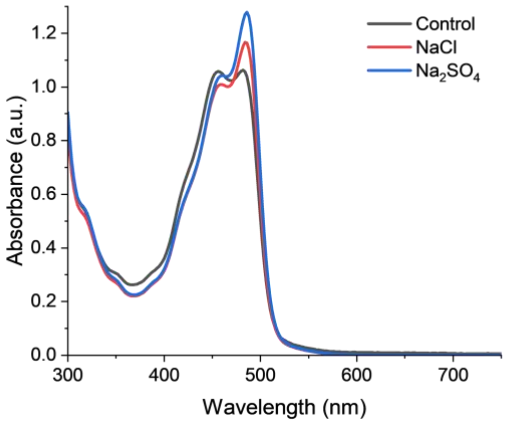


Figure S6 Absorption spectra for solutions of $Ru(bpy)_3Cl_2$ (50 μM) and fluorescein (50 μM) in the absence (control) and presence of 1 M NaCl or 1 M Na_2SO_4, confirming the presence of salts do not drastically affect the absorption of the dyes.

The emission spectra of the same samples were measured, with excitation at 490 nm and 450 nm (Figure S7). The emission intensity varied by less than 6% (for 490 nm excitation) and less than 20% (for 450 nm excitation) between the samples, confirming minimal changes in the emission quantum yields under these conditions.

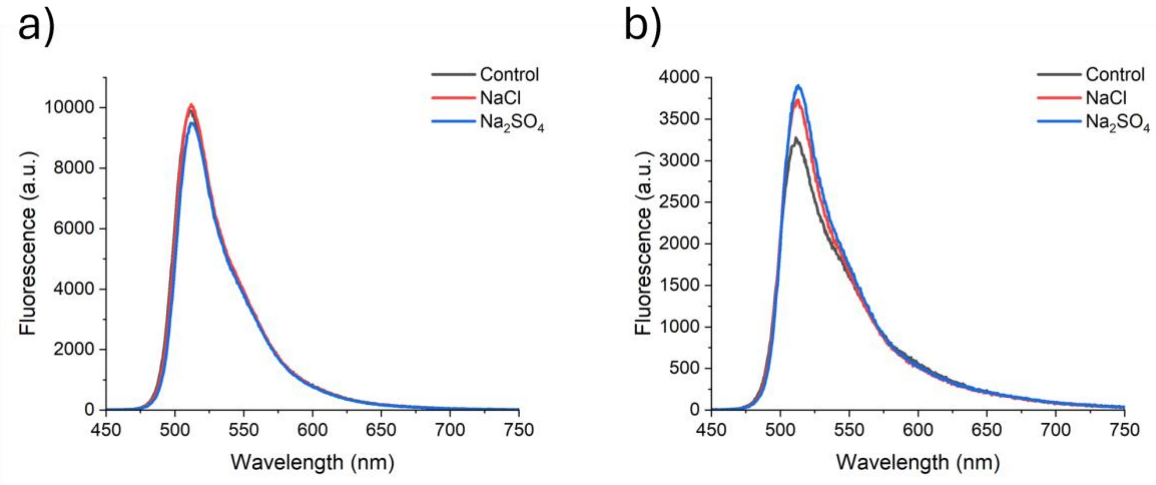


Figure S7 Emission spectra for solutions of $Ru(bpy)_3Cl_2$ (50 μM) and fluorescein (50 μM) at a) λ_{ex} = 490 nm and b) λ_{ex} = 450 nm in the absence (control) and presence of 1 M NaCl or 1 M Na_2SO_4.

S5 Microfluidics Experiments

S5.1 Modelling the Design

The 2D design of the microfluidic device was created using AutoCAD (Figure S8). The channel dimensions are 4 cm × 360 μm (L × W; where the model is in 2-dimensions and the height is determined by the photoresist used, detailed in S5.2). Markers are included in the design to be able to easily identify how far along the channel you are observing under the microscope.

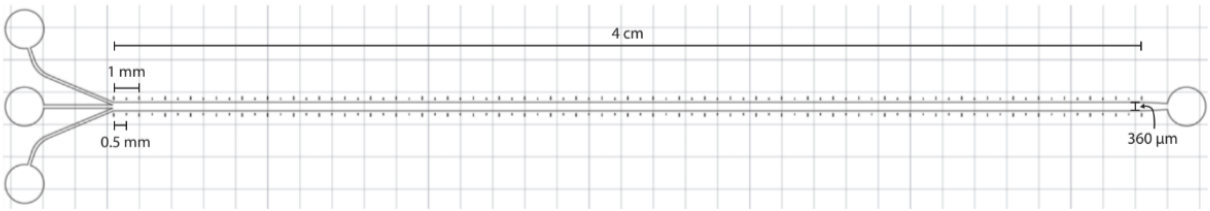


Figure S8 Design of microfluidic device used, modelled on AutoCAD with dimensions shown.

S5.2 Patterning the Silicon Wafer

The process involves depositing a photoresist on the silicon wafer in an even layer via spin coating. The negative photoresist SU-8 was used which can reliably generate the desired height of 50 μm. After evaporation of the photoresist solvent by baking on a hotplate at 70 °C, the wafer is irradiated with a photomask of the design made in the previous step. The areas that are not exposed to light can be washed away by placing the wafer in developer, leaving the channel pattern standing out from the silicon wafer base after drying.

To facilitate easy detachment of the PDMS from the surface of the silicon wafer base, silinization is completed. Wherein trichloro(1H, 1H, 2H, 2H-perfluorooctyl)silane (PFOCTS) is deposited as a hydrophobic monolayer on the wafer by leaving the chemical and the wafer in a desiccator under vacuum for 30 minutes.

PDMS Molding

To prepare the PDMS for molding, 50 g base (Sylgard 184 silicon Elastomer Base) and 5 g curing agent (Sylgard 184 Silicone Elastomer Curing Agent) were mixed for 2 minutes in a small plastic cup inside a clean room and left under vacuum for 1 hour to remove any bubbles formed.

The PDMS was then poured over the silanized silicon wafer, and the dish was placed back under vacuum for a further 30 minutes to remove any remaining bubbles. The dish was then placed flat in an oven at 70 °C to cure for 3 hours before allowing it to cool to room temperature overnight. Once the PDMS was cured and the sample was cool, a scalpel was used to pull the PDMS away from the silicon wafer.

A scalpel was used to cut out the individual sections corresponding to a single device (there were eight per silicon wafer). A hole puncher (diameter = 0.5 mm) was used to punch holes in the PDMS for the inlet and outlet ports.

Sealing PDMS to Glass Slide

For the plasma bonding stage, the PDMS was placed pattern side facing up, next to the glass slide on a glass tray covered in aluminum foil. The tray was placed inside a Denton Oxygen Asher, with an RF of 50 Watts, and an ashing rate of 10 nm/min for 45 seconds. The tray was then removed and the PDMS lifted and pressed onto the glass slide, with the pattern side touching the glass, and slight pressure applied to seal the two together. The device was sandwiched between two metal trays covered in aluminum foil and placed in the oven at 70 °C to cure for a further 2 hours. The finished device was removed and allowed to cool before testing.

S5.3 Testing microfluidic devices

The microfluidic devices were tested by flowing Milli-Q water through the three inlets and out of the outlet by hand. Syringes are connected to the ports via 23G steel connectors and 1/32" polytetrafluoroethylene (PTFE) tubing. Devices showing any signs of leakage or PDMS damage were discarded and not used.

Experiment Setup

Three components are required to monitor diffusion within the microfluidic devices: the microfluidic device, syringe pumps, and the confocal microscope. The microfluidic device is placed in the slide mount within the confocal microscope. The syringes are attached syringe pumps which are positioned on lab jacks adjacent to the microscope to minimize distance (Figure S9a). The syringes are attached to the microfluidic device via PTFE tubing and steel adaptors. The microfluidic devices can be used multiple times before showing signs of channel degradation. The setup of the other two components (syringe pumps and confocal microscope) is discussed in more detail below.

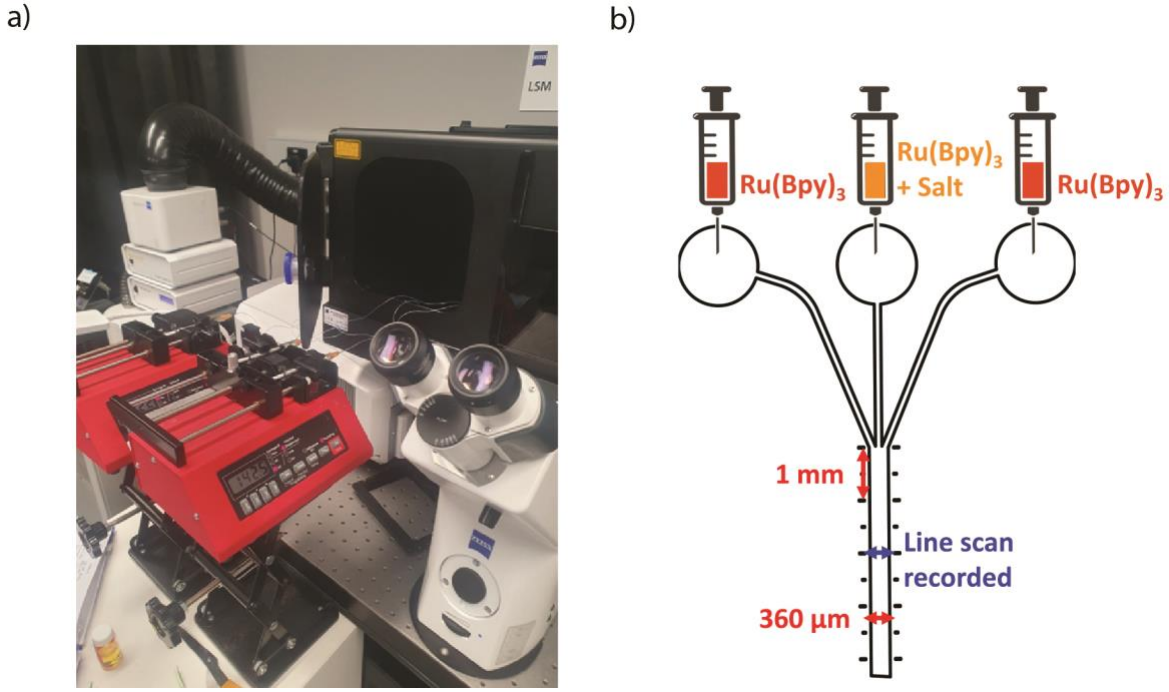


Figure S9 Illustration of the experimental set-up. A) Syringe pumps positioned on lab jacks adjacent to confocal microscope with PTFE tubing feeding into microfluidic device located in the confocal slide mount B) Schematic of experiment, showing the device with 50 µM $[Ru(bpy)_3]Cl_2$) in MES buffer flowing into three inlets and salt added to the central inlet. The channel is 360 µm in width, 50 µm height and measurements are recorded 2 mm from the channel entry point.

S5.4 Syringe Pumps

New Era syringe pumps: NE-1002X (single) and NE-4002X (dual), pumped simultaneously into all three inlets. The syringes were filled with the solutions, placed in the syringe pumps and then connected to the microfluidic device via PTFE tubing (outer diameter of 1/32" and inner diameter of 0.32 mm) and blunt steel connectors (23G). To prevent bubbles in the device the pumps were switched on at 40 µL min^{-1} and the tubing filled before connecting to the microfluidic device and beginning the experiments.

S5.5 Confocal Microscope

The microfluidic device was placed in the confocal microscope sample holder after the tubing had been connected (Figure S9a). We used a Zeiss LSM 780 laser scanning confocal microscope with a 10×/0.45 objective lens. Line images were recorded 2 mm from the start on the channel (Figure S9b) with the z axis being set at the intersection of the channel height. Given the continuous flow of

solution, laser power was set to 100%, gain to 650 and 12,000 images were taken for each flow rate and the data average (see S11). The syringe pumping is discontinuous, which requires averaging so many images for reliable intensity data.

S5.6 Image processing

Data from Zen 2021 software is produced as Carl Zeiss Image (CZI) files. Python was used as the processing tool. To read czi files in Python the czifile library must first be imported. The Python code used concerns only the third and sixth dimensions of the seven-dimension array produced by the measurement. This corresponds to the cycle of the time series and each pixel in the line scan.

For these experiments, three conditions are processed simultaneously as this provides more information than any one single FI profile. The script normalizes the data where salt has been added to the system with respect to the control experiment where there is no salt. With just $[Ru(bpy)_3]Cl_2$) being flowed through all three inlets, the fluorescence intensity (FI) profile should be flat across the width of the channel. A background correction is applied to the control experiment and the same correction is applied to the subsequent experiments with the addition of salt.

For ease of processing, the CZI files to be compared should be saved in the same directory and named so the control experiment appears first. The data can then be read and stored in Python using the script provided at the end of this document (S10.2) and also at GitHub: (https://github.com/lfillbrook/TimeSeriesProcess-CZI).

S6 Varying the salt concentration

A 50 µM stock solution of $Ru(bpy)_3Cl_2$ in MES buffer (50 mM, pH 6) was prepared. Using this stock solution, the salt solutions were prepared with either NaCl, or Na_2SO_4 at concentrations 0.1, 0.25, 0.5, 1.0 and 2.0 M.

The 50 µM stock solution of $Ru(bpy)_3Cl_2$ in MES buffer was flowed through the two external inlets and the salt solutions were flowed through the central inlet. The syringe pumps controlled the flow rates at 20, 15, 10, 6, 4, 3, 2, 1.5, 1.2, 1 µL min^{-1} which led to residence times from 0.108 and 2.160 s.

The data shown below was processed as described in S5.6. for concentrations of either NaCl or Na_2SO_4 (0.1, 0.25, 0.5 and 2 M).

S6.1 Change in normalised fluorescence intensity data for different sodium chloride or sodium sulfate concentrations

100 mM NaCl or Na_2SO_4

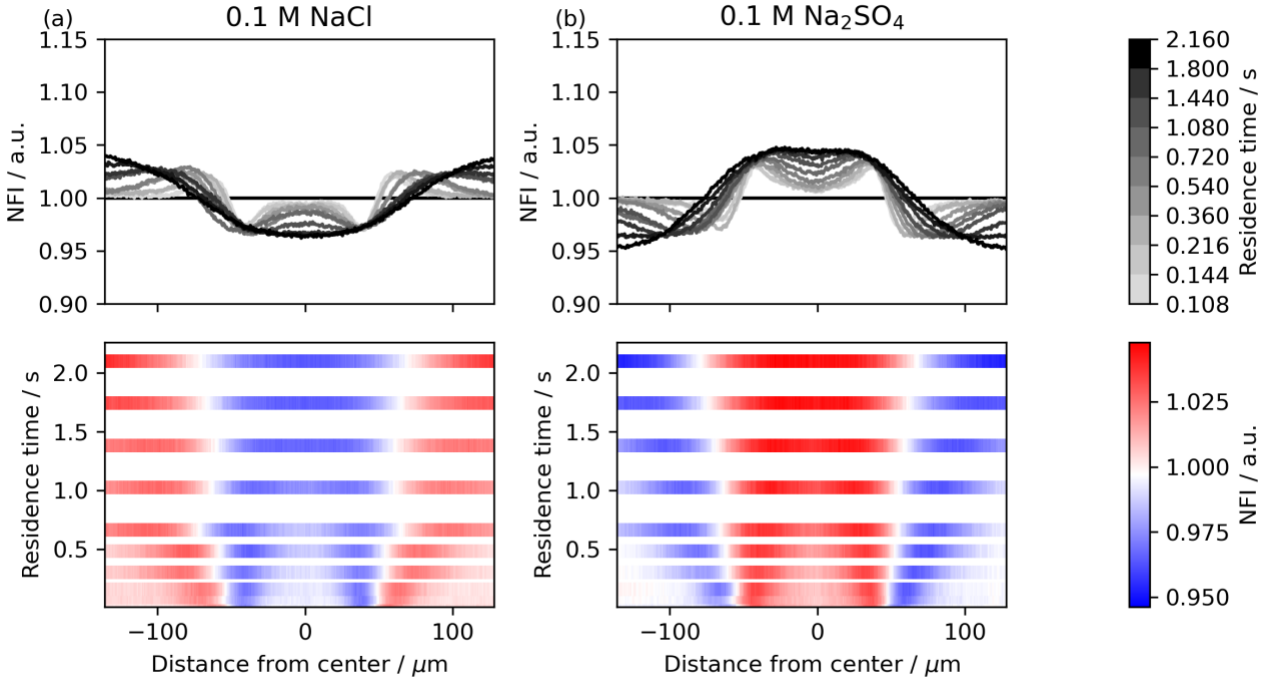


Figure S10. The distribution of $[Ru(bpy)_3]Cl_2$ in a microfluidic device can be controlled by adding 100 mM salt. The dye $[Ru(bpy)_3]Cl_2$ (50 μm in 50 mM MES buffer) is pumped through all three inlets. (a) 100 mM NaCl or (b) 100 mM Na_2SO_4 is added to the solution at the central inlet. Imaging occurs 2 mm from the start of the main chamber. The residence time is altered by using different flow rates, from 1 to 20 μL min^{-1}. The data in the top and bottom plots are the same, plotted either as the normalised fluorescence intensity (NFI) or the residence time to help with visualisation.

250 mM NaCl or Na_2SO_4

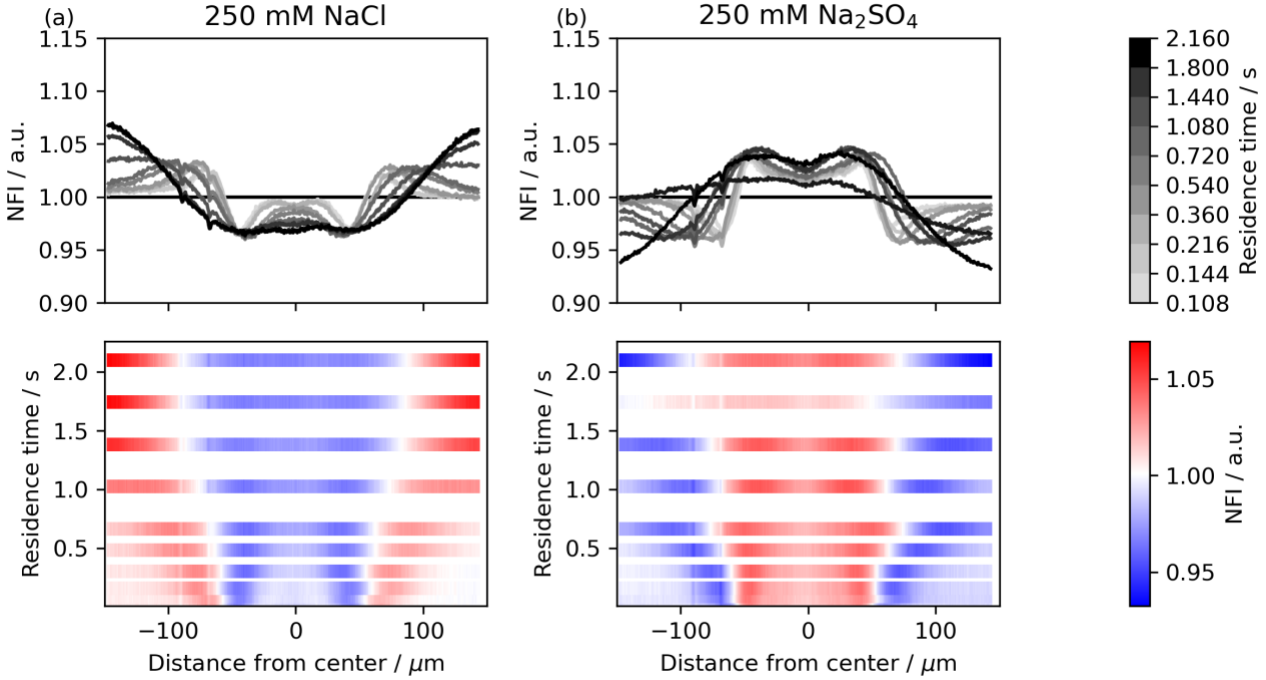


Figure S11 The distribution of $[Ru(bpy)_3]Cl_2$ in a microfluidic device can be controlled by adding 250 mM salt. The dye $[Ru(bpy)_3]Cl_2$ (50 μm in 50 mM MES buffer) is pumped through all three inlets. (a) 250 mM NaCl or (b) 250 mM Na_2SO_4 is added to the solution at the central inlet. Imaging occurs 2 mm from the start of the main chamber. The residence time is altered by using different flow rates, from 1 to 20 μL min^{-1}. The data in the top and bottom plots are the same, plotted as either the normalised fluorescence intensity (NFI) or the residence time to help with visualisation.

500 mM NaCl or Na_2SO_4

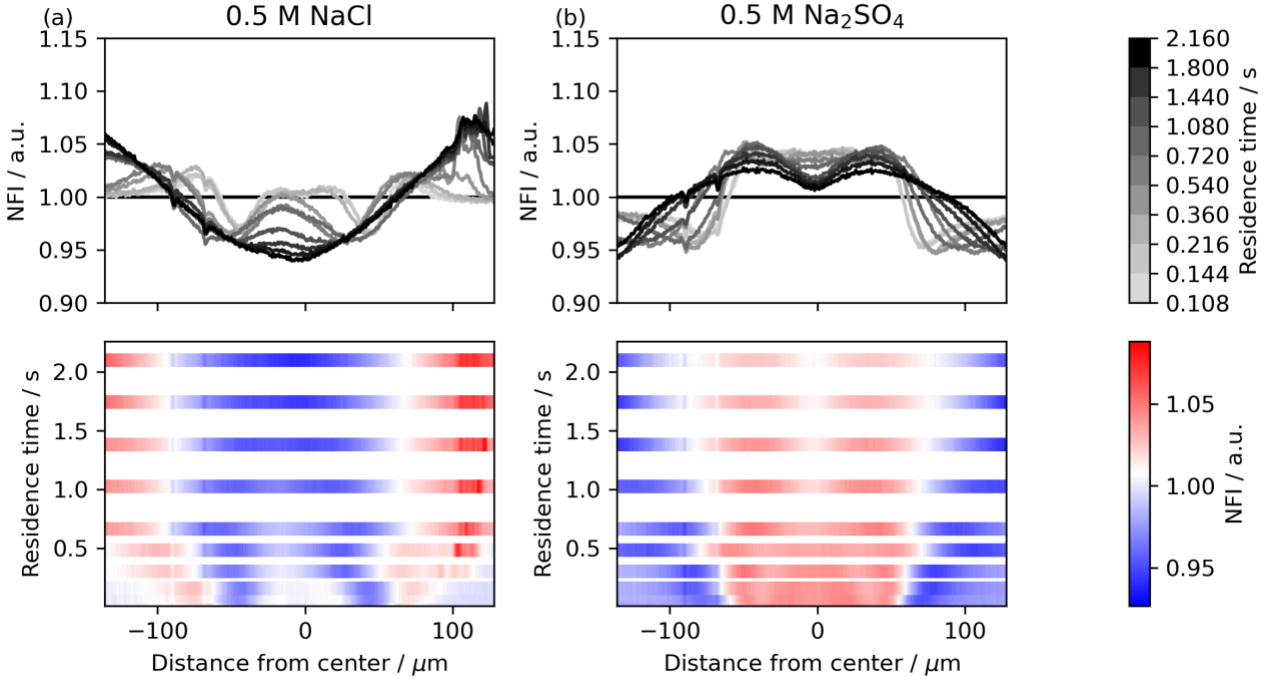


Figure S12 The distribution of $[Ru(bpy)_3]Cl_2$ in a microfluidic device can be controlled by adding 500 mM salt. The dye $[Ru(bpy)_3]Cl_2$ (50 μm in 50 mM MES buffer) is pumped through all three inlets. (a) 500 mM NaCl or (b) 500 mM Na_2SO_4 is added to the solution at the central inlet. Imaging occurs 2 mm from the start of the main chamber. The residence time is altered by using different flow rates, from 1 to 20 μL min^{-1}. The data in the top and bottom plots are the same, plotted either as the normalised fluorescence intensity (NFI) or the residence time to help with visualisation.

2 M NaCl or Na_2SO_4

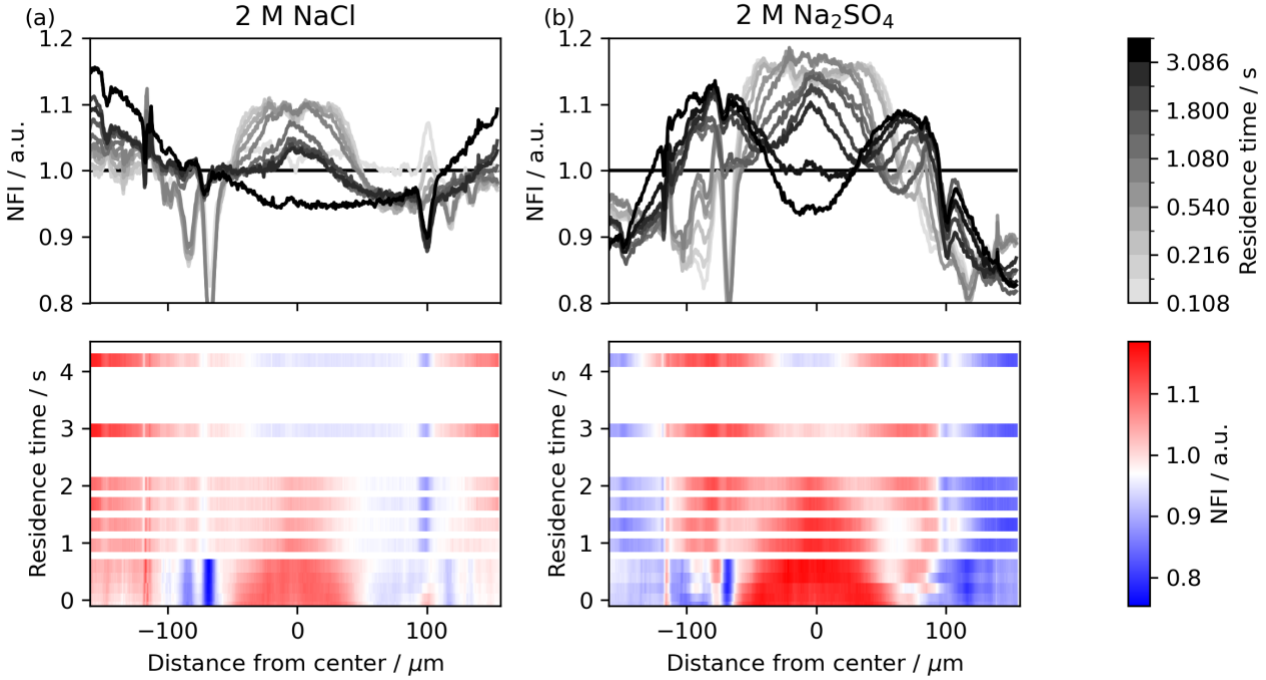


Figure S13 The distribution of $[Ru(bpy)_3]Cl_2$ in a microfluidic device can be controlled by adding 2 M salt. The dye $[Ru(bpy)_3]Cl_2$ (50 μm in 50 mM MES buffer) is pumped through all three inlets. (a) 2 M NaCl or (b) 2 M Na_2SO_4 is added to the solution at the central inlet. Imaging occurs 2 mm from the start of the main chamber. The residence time is altered by using different flow rates, from 1 to 20 μL min^{-1}. The data in the top and bottom plots are the same, plotted either as the normalised fluorescence intensity (NFI) or the residence time to help with visualisation.

S6.2 Collated change in normalised fluorescence intensity data for different sodium chloride concentrations

The difference in normalized fluorescence intensity (ΔNFI) measured between the edge and the centre of the channel at flow rate 1 µL min^{-1} and residence time of 2.16 s is shown in Figure S14, after the addition of 100 mM NaCl through the central inlet.

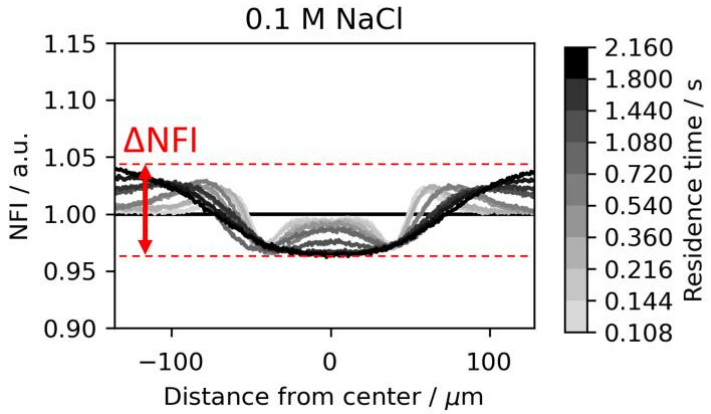


Figure S14 Illustration of calculation of ΔNFI from the 2.16 s fluorescence profile across the channel for 100 mM NaCl.

The change in the normalized fluorescence intensity (ΔNFI) resulting after the addition of sodium chloride (0.1 – 1 M, data shown in S6) via the central at flow rate 1 μL min^{-1} and residence time of 2.16 s is shown in Figure S15.

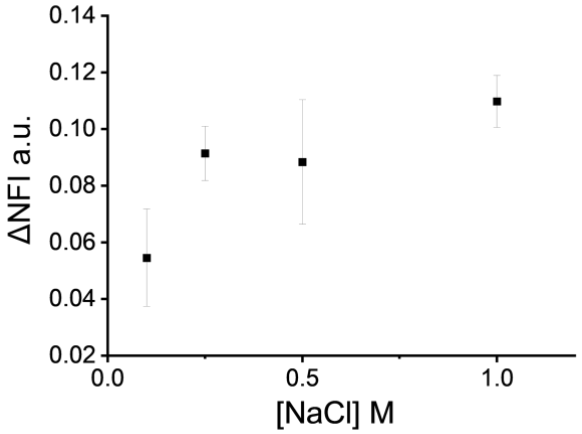


Figure S15 The difference in normalized fluorescence intensity (ΔNFI) of $[Ru(bpy)_3]Cl_2$ between the edge of the channel and the centre of the channel when NaCl (100 mM, 250 mM, 500 mM and 1 M) is added through the central inlet. The dye $[Ru(bpy)_3]Cl_2$ is pumped through all three inlets at 50 μm in 50 mM MES buffer, flow rate 1 μL min^{-1}. The error bars represent the calculated standard deviation after repeating in triplicate.

S7 Separating oppositely charged dyes

A stock solution was prepared of fluorescein (50 μM) and $Ru(bpy)_3Cl_2$ (50 μM) in MES buffer (50 mM at pH 6.5) and flowed through all three inlets of the microfluidic device and the fluorescence intensity measured across the width of the channel. Using this stock solution, solutions were prepared with either NaCl, or Na_2SO_4 to give a final concentration of 1.0 M. These were then flowed through the device via the central inlet. Images were acquired 2 mm from the start of the main channel, as previously described in S5.

The 'Smart Set-up' function of *Zeiss ZEN black 2010* (microscopy software) optimised the laser parameters for multiple dyes. Fluorescein was imaged using a 488 nm laser at 15 % power, emission wavelength = 545 nm, detection wavelengths range = 492 – 597 nm, gain was set to 400, DO = 0 and DG = 1.0. $Ru(bpy)_3Cl_2$ was imaged using a 458 nm laser at 100% power, emission wavelength = 678 nm, detection wavelengths range = 597 - 759 nm, gain set to 700, DO = 1 and DG = 1.1. At each flow rate the system was allowed to equilibrate, and then the fluorescein dye was imaged followed by $Ru(bpy)_3Cl_2$. Data was processed as described in S5.6.

S8 Monitoring the liquid junction potential using fluorescein

A 50 μM stock solution of fluorescein in MES buffer (50 mM, pH 6.5) was prepared. Using this stock solution, solutions were prepared with either NaCl, or Na_2SO_4 to give a final concentration of 1.0 M.

The 50 μM stock solution of fluorescein in MES buffer was flowed through the two external inlets and the salt solutions were flowed through the central inlet. The data was normalised using the fluorescence profiles generated when the 50 μM stock solution of fluorescein in MES buffer was flowed through all three external inlets. The syringe pumps controlled the flow rates at 20, 15, 10, 6, 4, 3, 2, 1.5, 1.2, 1 μL min^{-1} which led to residence times from 0.108 and 2.160 s. The data is shown in Figure S16 below.

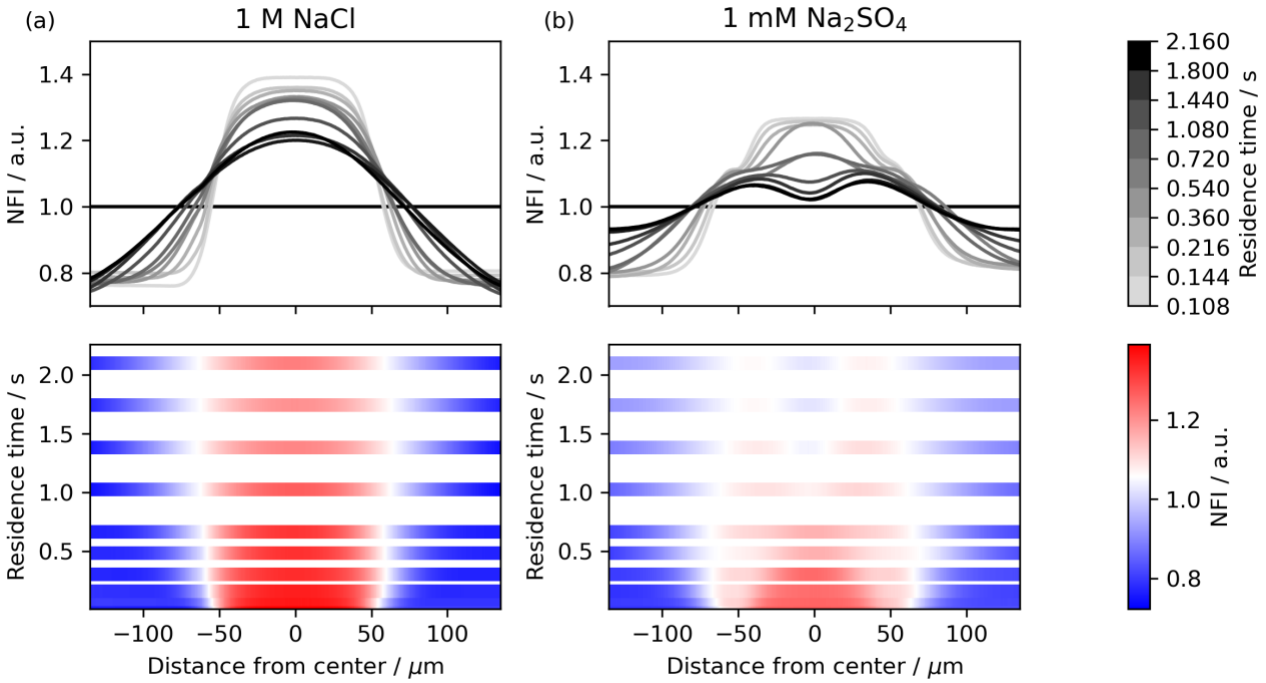


Figure S16 The distribution of fluorescein in a microfluidic device can be controlled by adding 1 M salt. The dye fluorescein (50 μm in 50 mM MES buffer) is pumped through all three inlets. (a) 100 mM NaCl or (b) 100 mM Na_2SO_4 is added to the solution at the central inlet. Imaging occurs 2 mm from the start of the main chamber. The residence time is altered by using different flow rates, from 1 to 20 μL min^{-1}. The data in the top and bottom plots are the same, plotted as either the normalised fluorescence intensity (NFI) or the residence time to help with visualisation.

S9 References

1. E. T. Luis, G. E. Ball, A. Gilbert, H. Iranmanesh, C. W. Newdick and J. E. Beves, *J. Coord. Chem.*, 2016, **69**, 1686-1694.
2. K. T. Krist, A. Sen and W. G. Noid, *J. Chem. Phys.*, 2021, **155**, 164902.

S10 Appendix

S10.1 Code for Fickian diffusion model:

```
"""
Modelling Fickian diffusion of species in solution in a microfluidic device.

Microfluidic device consists of 3 inlets, each with a channel that joins
a larger main channel at the same point. Solutions are pumped through all 3
inlets at the same flow rate.

This very simple model neglects parabolic velocity profile that forms in
laminar flows, and the electrostatic or dispersive interactions between
species on solution.

"""

import numpy as np
import matplotlib.pyplot as plt
from matplotlib.colors import Normalize, TwoSlopeNorm #for plotting
from matplotlib.cm import ScalarMappable, get_cmap #for plotting
import string


#%% MODELLING DIFFUSION

# Function to generate initial concentration distribution

# The 'ini' function sets the initial concentration distribution of the ions along the
channel using a
# mathematical formula involving hyperbolic tangent functions.
# This creates a high concentration in the middle and lower concentrations towards the
edges.
def ini(x, uu, L, sigma):
    rho = 0.5 * uu * (np.tanh(np.pi / sigma * (2 * L / 3 - x)) + 1) - 0.5 * uu *
(np.tanh(np.pi / sigma * (L / 3 - x)) + 1)
    rho[rho < 1e-15] = 0
    return rho


# Diffusivities of each ion (in μm²/s) - how fast each ion diffuses
DNa = 133.4
DCl = 203.2
DSO4 = 106.5

# Initial concentration (in μM)
uT = 1e6 #1 M is the same as the experimental data

# Channel width (in μm)
L = 360

# Maximum time (in s)
tmax = 9

# FTCS setup
# These are the channel and simulation parameters, where L is total length and tmax is
total time
x = np.linspace(-L/2, L/2, 1001) # This divides the length into discrete points where the
calculation can be performed, ie 1000 increments of 360 um
dx = np.abs(x[0]-x[1]) # this defines the step size, essentially just the gap between the
first two discrete x values, ie 0.36 um
time = np.linspace(0, tmax, int(tmax/(100*10**(-6)))) # This divides the tmax into
discrete points where the calculation can be performed, with a time step of 0.1 ms
dt = time[1] # here the first time point is 0.1 ms and thus this equals the time step

# Initial concentration distribution of salts
# The ini function is defined at the begining, here x + L/2 models the middle of the
channel, uT is the initial concentration value of 1 M, and 8 is the sigma parameter,
controlling the sharpness of the transition in the concentration profile
```

```
uNa = ini(x + L/2, uT, L, 8)
uCl = ini(x + L/2, uT, L, 8)
uSO4 = ini(x + L/2, uT, L, 8)

# Lists to store probability densities (ie concentration profiles at each timepoint)
probDensity_Na = []
probDensity_Cl = []
probDensity_SO4 = []

# Time points to run the alogrithm
# these time points correlate to the xth number in the time series, ie the 1000th entry
is 0.1 second and the 40000th entry is 4 seconds.
# this tells the formulation to only save the concentration profiles at these selected
time points
tpoints = [0, time[1000], time[2500], time[5000], time[7000], time[10000], time[14000],
time[18000], time[22000], time[26000], time[30000], time[35000], time[40000]]

# Run alogrithm for each ion
# the zip links the initial concentrations, diffusion coefficients and concentration
profiles at each time point
for uP, DP, probDensity in zip([uNa, uCl, uSO4], [DNa, DCl, DSO4], [probDensity_Na,
probDensity_Cl, probDensity_SO4]):
    for t in time:
        uP[0] = uP[-1] = 0
        if t in tpoints:
            probDensity.append(uP.copy())
        uP[1:-1] += DP * (dt / dx**2) * (uP[2:] - 2 * uP[1:-1] + uP[:-2])
```

S10.2 Code for Confocal (.czi files) data processing:

```
"""
Designed to process time series data collected on a Zeiss confocal microscope from
microfluidic flow experiments.

Data collection: A line scan is selected at one point along the microfluidics channel;
the line scan is repeated
to create a time series experiment. Time series experiments are repeated for a control
(in which the fluorescence
intensity profile should be flat), followed by 2 experiments in which the fluorescence
intensity profile is
expected to be perturbed. The experiments are obtained at different flow rates, to lead
to different residence
times at the same point along the microfluidics channel.

Data storage: Each flow rate has it's own directory, inside which the 3 time series
experiments (control, and two
others) are saved as CZI files. The control always appears at the top of the direction,
alphabetically, by
appropriate file naming (important for processing).

Using script: Run Cell 0 to set up processing for all flow rates (defining lists: dsets
and FR is important for
comparing across flow rates); only run once at the beginning. Import and check the
averaged data across each time
series in a single directory (flow rate) by running Cell 1, and normalizing with Cell 2.
If the data is acceptable,
use Cell 3 to store the data and flow rate (in dsets and FR, respectively). Repeat Cells
1-3 for each directory.
Once all of the directories of interest have been imported, checked, normalized and
stored (using Cells 1-3), run
Cell 4 to plot a comparison of the results across directories, which in this case
comprises of results at different
flow rates.
"""



# (0) Set up for processing across flow rates
```

```
# run only once at the beginning of processing experiments collected in same sitting
import os
import czifile as cz
import tkinter as tk
from tkinter import filedialog
import numpy as np
import matplotlib.pyplot as plt
import pandas as pd
import matplotlib as mpl
from matplotlib import cm
import string

#calculate the residence time at given flow rate (in uL/min) and disance from inlets (cm)
def resTime(flowrate,L,w=360,h=50):
    """
    Fuction to calculate residence time of solution in microfluidic channel,
    given the flow rate the solution is pumped at, and the dimensions
    of the microfluidic channel.

    Parameters
    ----------
    flowrate : float
        flow rate that solution is pumped into device at.
    L : float
        length along channel where images were collected (in cm).
    w : float, optional
        width of channel (in cm). The default is 360.
    h : float, optional
        height of channel (in cm). The default is 50.

    Returns
    -------
    resTime : float
        residence time of the solution in the channel at the point
        where the images are collected.

    """
    Lm = L*0.01 #(cm to m)
    wm = w*1e-6 #(um to m)
    hm = h*1e-6 #(um to m)
    volume = Lm*wm*hm
    vol_um = volume*1e9 #(m3 to uL)
    FR = flowrate/60 #(uL/min to uL/s)
    resTime = vol_um / FR #in seconds
    return resTime

# pixelsize should be noted from Zeiss software during measurement
pixelsize = input('pixel size (in um)? ')
PS = float(pixelsize)
# pixelsize should be noted from Zeiss software during measurement
L = input('distance from inlets (in cm)? ')
L = float(L)

# defining lists to collect data processed at multiple flow rates (in different folders)
dsets = []
FR = []

#%%
# (1) Inital data import and processing; plot
# opens file explorer to select directory containing data of interest
tk.Tk().withdraw()
path = filedialog.askdirectory()

# process all images in folder, assign them the same name as in the folder
stacknames = ()
for entry in os.scandir(path):
    stack = cz.imread(entry)
    # name each stack with filename (excluding .czi suffix)
    globals()[entry.name[:-4]] = stack
    stacknames += (entry.name[:-4],)
```

```
# average over each experiment in the time series
# plot the result compared to the raw data
for n,name in enumerate(stacknames):
    allx = np.zeros(len(globals()[name][0,0,0,0,0,:,0]))
    ns = globals()[name].shape[2] # shape[2] is number of cycles in time series
    npix = globals()[name].shape[5] # shape[5] is number of pixels in scan
    for i in range(ns):
        allx = np.add(allx,globals()[name][0,0,i,0,0,:,0])
    globals()[name + '_avg'] = allx/ns # save mean as name_avg


# comparison plot of averaged fluorescence intensity (FI) of every experiment in
directory
plt.figure(figsize=(5,3))
for n,name in enumerate(stacknames):
    globals()[name + '_avg'] = pd.DataFrame(globals()[name + '_avg'])
    plt.plot(globals()[name + '_avg'].index*PS,globals()[name + '_avg'],label=name)
plt.xlabel(r'Distance / $\mu$m')
plt.ylabel('FI / a.u.')
plt.xlim(0,PS*512)
plt.legend(frameon=False,prop={'size':9},ncol=2)
#plt.savefig('Example.pdf',bbox_inches='tight')


#%%
# (2) Select region unaffected by microfluidic channel walls, scale and replot
# ACTION REQUIRED: define distance to leave at either side to avoid edge effects
# this may differ for experiments taken on different days/positions
cutL = 30
cutR = 40

control = globals()[stacknames[0] + '_avg'].iloc[cutL:-cutR]
totFI = control.sum()

# normalise FI for every experiment in directory, collect and plot
control = globals()[stacknames[0] + '_avg'].iloc[cutL:-cutR] # define control experiment
(flat FI)
totFI = control.sum() # define total FI
ndata = [] # collect normalied dataset for each dataset
plt.figure(figsize=(5,3))
for n,name in enumerate(stacknames):
    data = globals()[name + '_avg'].iloc[cutL:-cutR]
    flattened = data/control # accounts for non-uniformity of laser
    scale = (data/control).sum() / (control/control).sum() # scale so each FI profile has
same integration
    ndata_i = flattened/scale
    ndata.append(pd.DataFrame(ndata_i.values, index=(data.index-512/2)*PS)) # adjust
index to plot distance from center
    plt.plot((ndata_i.index-512/2)*PS,ndata_i,label=name)
plt.xlabel(r'Distance from center / $\mu$m')
plt.ylabel('NFI / a.u.')
#plt.ylim(0.7,1.2)
plt.legend(frameon=False,prop={'size':9},ncol=2)
#plt.savefig('Example_norm.pdf',bbox_inches='tight')


#%%
# (3) Storing processed data, before repeating processing for other flow rates
# storing result from each flow rate, as calcuated in cell above
dset = input('name of dset? ')
globals()[dset] = ndata
dsets.append(dset)
# storing flow rate
flrate = input('flow rate? ')
flrate = float(flrate)
FR.append(flrate)

# Need to repeat cells 1-3 for each flow rate before moving to cell 4; do not run cell 0
again

#%%
# (4) Comparing results across flow rates
```

```
# PLOT 1
# set appropriate axis titles
title_L = '1 M NaCl'
title_R = r'1 M Na$_2$SO$_4$'
# plot of FI across channel, each flow rate represented by shades of grey
fig, ax = plt.subplots(1,2,figsize=(8,3))
for axs in ax:
    axs.plot(globals()[dsets[0]][0],'k') # same control on each (all normalised to y=1)
    axs.set_xlabel(r'Distance from center / $\mu$m')
    axs.set_ylabel('NFI / a.u.')
    axs.set_ylim(0.9,1.15)
cmap = plt.get_cmap('Greys')
cols = [cmap(i) for i in np.linspace(0, 1, len(dsets)+3)]
for i,col in zip(range(len(dsets)),cols[3:]):
    ax[0].plot(globals()[dsets[i]][1],color=col)
    ax[1].plot(globals()[dsets[i]][2],color=col)
# ACTION REQUIRED: set appropriate axis titles
ax[0].set_title(title_L)
ax[1].set_title(title_R)
plt.tight_layout()

# PLOT 2
# plot of FI across channel, each flow rate represented by shades of grey (top)
# plot of FI at each residence time, with FI represented on red-to-blue colourmap
(bottom)
fig, ax = plt.subplots(2,3,figsize=(8,4.5),gridspec_kw={'width_ratios':
[19,19,1]},sharex=('col'),)
ax = ax.flatten()
for axs in [ax[3],ax[4]]:
    axs.set_ylabel(r'Residence time / s')
    axs.set_xlabel(r'Distance from center / $\mu$m')
    axs.set_xlim(-136,128)
for n,axs in enumerate([ax[0],ax[1]]):
    axs.text(-0.16,1.05,'('+string.ascii_lowercase[n]+')',transform=axs.transAxes)
    axs.set_ylabel('NFI / a.u.')
    axs.plot(globals()[dsets[0]][0],'k') # can plot same control on each because all
normalised to y=1
    axs.set_ylim(0.9,1.14)
    axs.set_xlim(-136,128)
cmap = plt.get_cmap('Greys')
cols = [cmap(i) for i in np.linspace(0, 1, len(dsets)+3)]
cols = cols[3:]
RT = []
for i,col in zip(range(len(dsets)),cols):
    RT.append(resTime(FR[i],L=L))
    ax[0].plot(globals()[dsets[i]][1],color=col)
    ax[1].plot(globals()[dsets[i]][2],color=col)
ax[0].set_title(title_L)
ax[1].set_title(title_R)

# find overall max and min to scale the data appropraitely for colormapping
zmin = []
zmax = []
for i in range(len(dsets)):

zmin.append(min(min(globals()[dsets[i]][1].iloc[:,0]),min(globals()[dsets[i]][2].iloc[:,0
])))

zmax.append(max(max(globals()[dsets[i]][1].iloc[:,0]),max(globals()[dsets[i]][2].iloc[:,0
])))
for axs, n in zip([ax[3],ax[4]],[1,2]):
    for i in range(len(dsets)):
        x = globals()[dsets[i]][n].index
        y = resTime(FR[i],L=L)*np.ones_like(x)
        z = globals()[dsets[i]][n].iloc[:,0]
        # from: https://stackoverflow.com/questions/20165169/change-colour-of-curve-
according-to-its-y-value-in-matplotlib
        axs.scatter(x,y, c=cm.bwr((z-min(zmin))/(max(zmax)-min(zmin))), marker=3)

# set up colour bars:
#   for top plots
```

```
cmap = mpl.colors.ListedColormap(cols) # reassign colourmap as the section of colours
used (excluding the white bits cut off)
cb1 = mpl.colorbar.ColorbarBase(ax[2], cmap=cmap,
norm=mpl.colors.BoundaryNorm(boundaries=RT, ncolors=len(cmap.colors)))
cb1.set_label('Residence time / s')
#    for bottom plots
cb2 = mpl.colorbar.ColorbarBase(ax[5], cmap='bwr',
norm=mpl.colors.Normalize(vmin=min(zmin),vmax=max(zmax)))
cb2.set_label(r'NFI / a.u.')

plt.tight_layout()
#plt.savefig('500mM_salt_cbar.pdf',bbox_inches='tight')
```

S10.3 Data for Figure 3

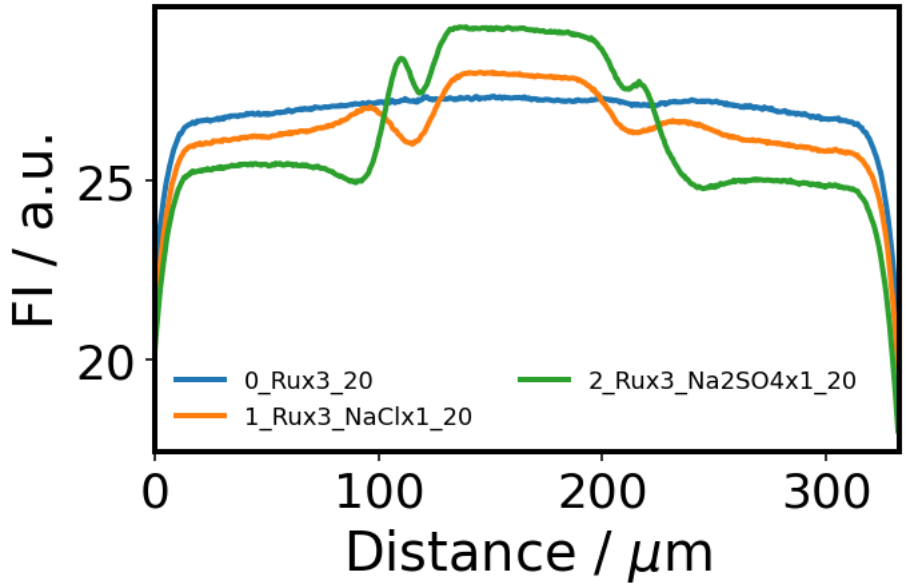


Figure S17 Averaged fluorescence intensity profile data (from the 12,000 images generated) across the width of the channel following the addition of no salt (blue), 1 M NaCl (orange) and 1 M Na_2SO_4 (green) via the central inlet and a solution of $Ru(bpy)_3Cl_2$ (50 µM) in MES buffer (50 mM at pH 6) flowed through all three inlets at a flow rate of 20 μL min^{-1}.

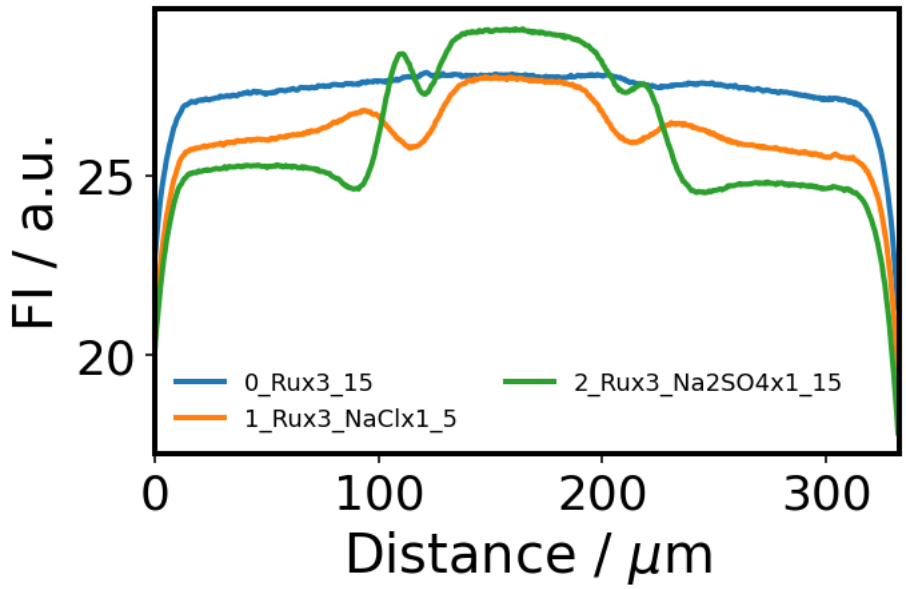


Figure S18 Averaged fluorescence intensity profile data (from the 12,000 images generated) across the width of the channel following the addition of no salt (blue), 1 M NaCl (orange) and 1 M Na_2SO_4 (green) via the central inlet and a solution of $Ru(bpy)_3Cl_2$ (50 μM) in MES buffer (50 mM at pH 6) flowed through all three inlets at a flow rate of 15 μL min^{-1}.

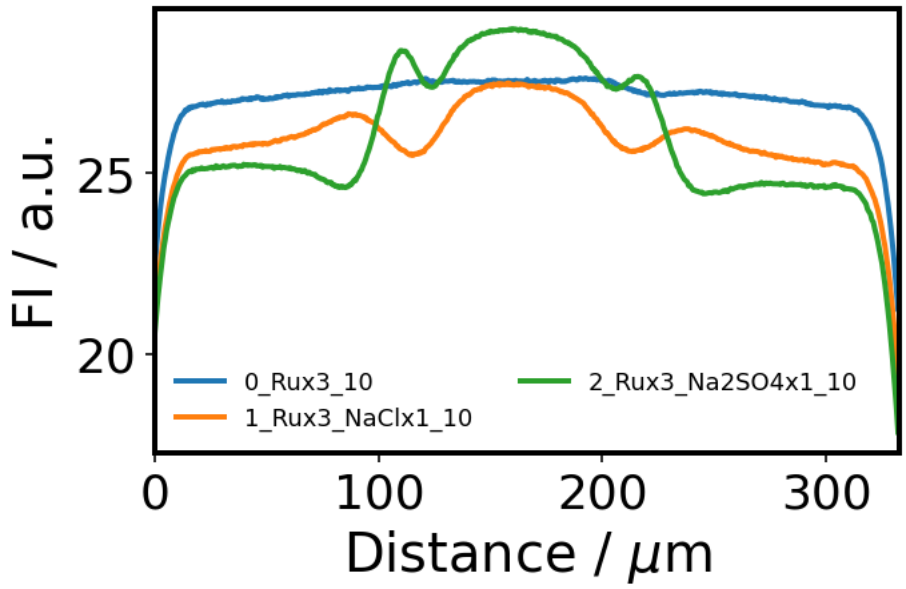


Figure S19 Averaged fluorescence intensity profile data (from the 12,000 images generated) across the width of the channel following the addition of no salt (blue), 1 M NaCl (orange) and 1 M Na_2SO_4 (green) via the central inlet and a solution of $Ru(bpy)_3Cl_2$ (50 μM) in MES buffer (50 mM at pH 6) flowed through all three inlets at a flow rate of 10 μL min^{-1}.

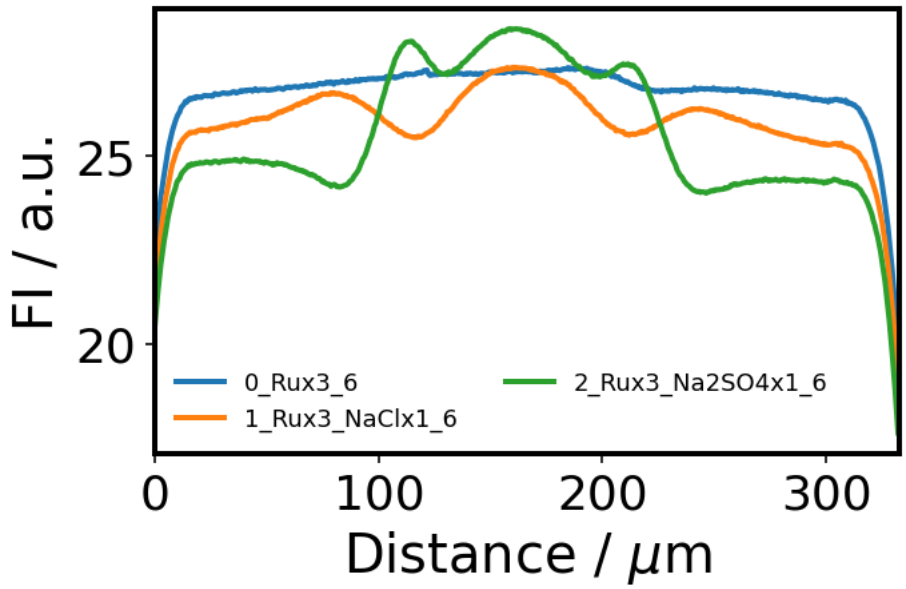


Figure S20 Averaged fluorescence intensity profile data (from the 12,000 images generated) across the width of the channel following the addition of no salt (blue), 1 M NaCl (orange) and 1 M Na_2SO_4 (green) via the central inlet and a solution of $Ru(bpy)_3Cl_2$ (50 μM) in MES buffer (50 mM at pH 6) flowed through all three inlets at a flow rate of 6 μL min^{-1}.

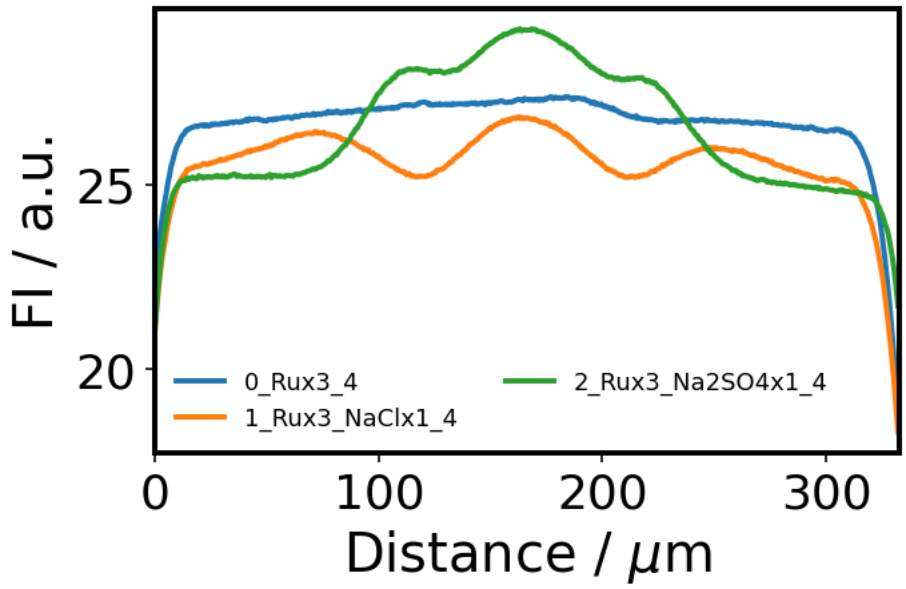


Figure S21 Averaged fluorescence intensity profile data (from the 12,000 images generated) across the width of the channel following the addition of no salt (blue), 1 M NaCl (orange) and 1 M Na_2SO_4 (green) via the central inlet and a solution of $Ru(bpy)_3Cl_2$ (50 μM) in MES buffer (50 mM at pH 6) flowed through all three inlets at a flow rate of 4 μL min^{-1}.

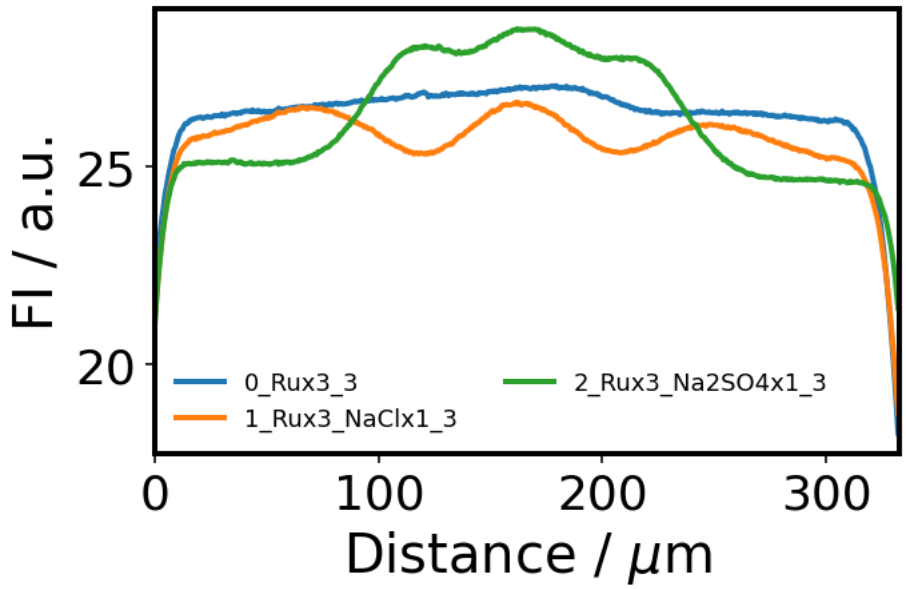


Figure S22 Averaged fluorescence intensity profile data (from the 12,000 images generated) across the width of the channel following the addition of no salt (blue), 1 M NaCl (orange) and 1 M Na_2SO_4 (green) via the central inlet and a solution of $Ru(bpy)_3Cl_2$ (50 μM) in MES buffer (50 mM at pH 6) flowed through all three inlets at a flow rate of 3 μL min^{-1}.

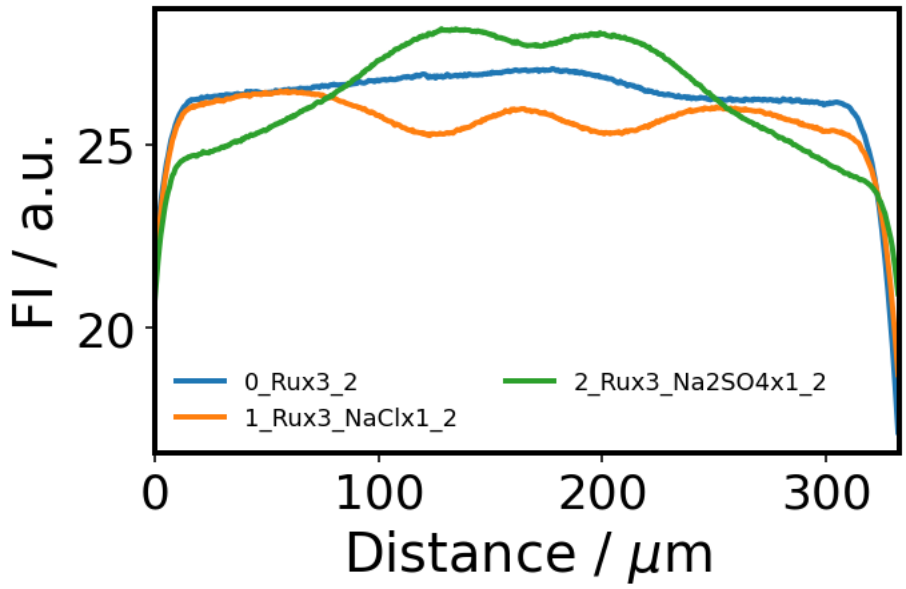


Figure S23 Averaged fluorescence intensity profile data (from the 12,000 images generated) across the width of the channel following the addition of no salt (blue), 1 M NaCl (orange) and 1 M Na_2SO_4 (green) via the central inlet and a solution of $Ru(bpy)_3Cl_2$ (50 μM) in MES buffer (50 mM at pH 6) flowed through all three inlets at a flow rate of 2 μL min^{-1}.

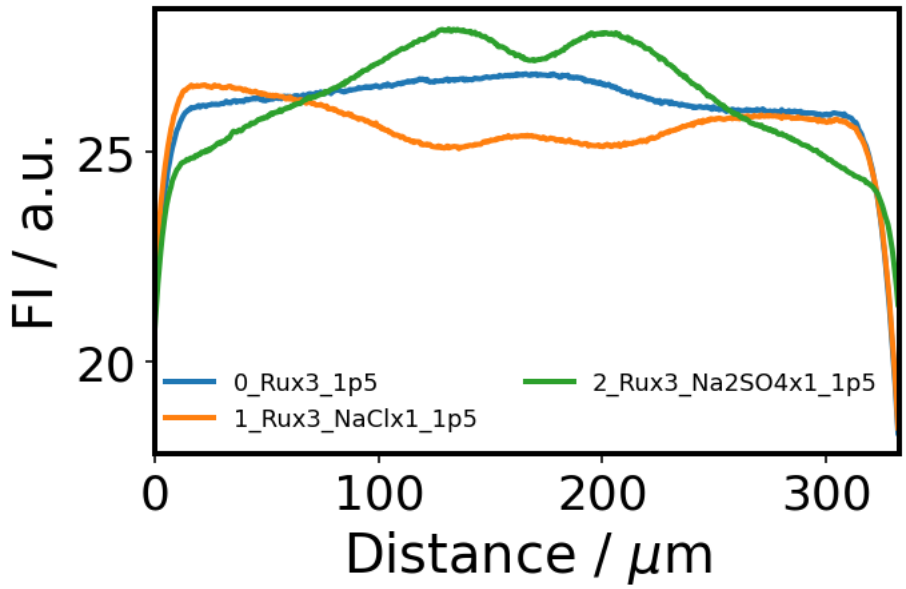


Figure S24 Averaged fluorescence intensity profile data (from the 12,000 images generated) across the width of the channel following the addition of no salt (blue), 1 M NaCl (orange) and 1 M Na_2SO_4 (green) via the central inlet and a solution of $Ru(bpy)_3Cl_2$ (50 μM) in MES buffer (50 mM at pH 6) flowed through all three inlets at a flow rate of 1.5 μL min^{-1}.

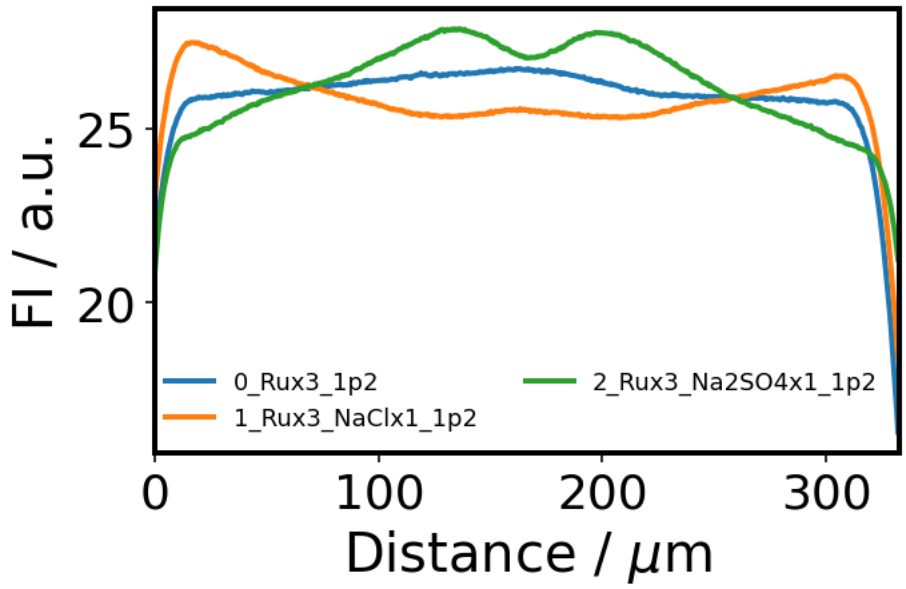


Figure S25 Averaged fluorescence intensity profile data (from the 12,000 images generated) across the width of the channel following the addition of no salt (blue), 1 M NaCl (orange) and 1 M Na_2SO_4 (green) via the central inlet and a solution of $Ru(bpy)_3Cl_2$ (50 μM) in MES buffer (50 mM at pH 6) flowed through all three inlets at a flow rate of 1.2 μL min^{-1}..

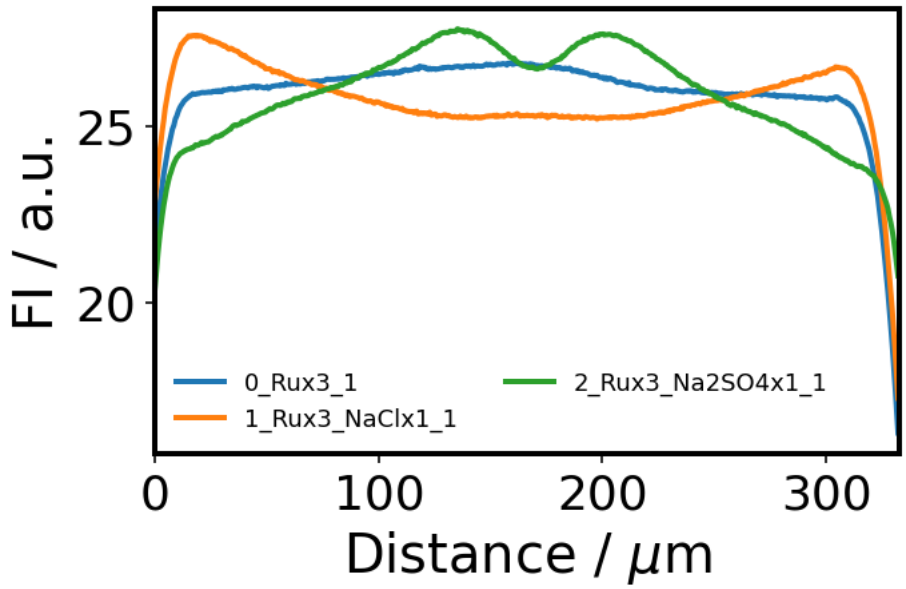


Figure S26 Averaged fluorescence intensity profile data (from the 12,000 images generated) across the width of the channel following the addition of no salt (blue), 1 M NaCl (orange) and 1 M Na_2SO_4 (green) via the central inlet and a solution of $Ru(bpy)_3Cl_2$ (50 μM) in MES buffer (50 mM at pH 6) flowed through all three inlets at a flow rate of 1 μL min^{-1}.

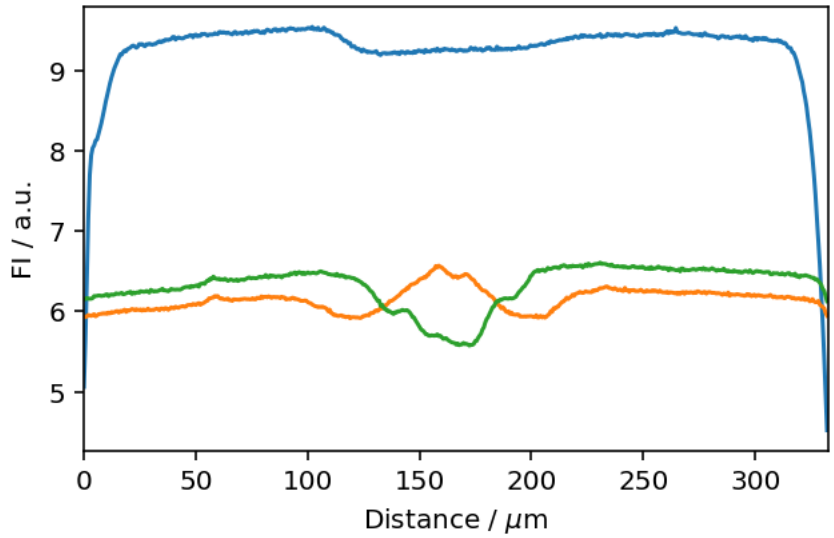


Figure S27 Averaged fluorescence intensity profile data (from the 12,000 images generated) across the width of the channel following the addition of no salt (blue), 1 M NaCl (orange) and 1 M Na_2SO_4 (green) via the external inlets and a solution of $Ru(bpy)_3Cl_2$ (50 μM) in MES buffer (50 mM at pH 6) flowed through all three inlets at a flow rate of 20 μL min^{-1}.

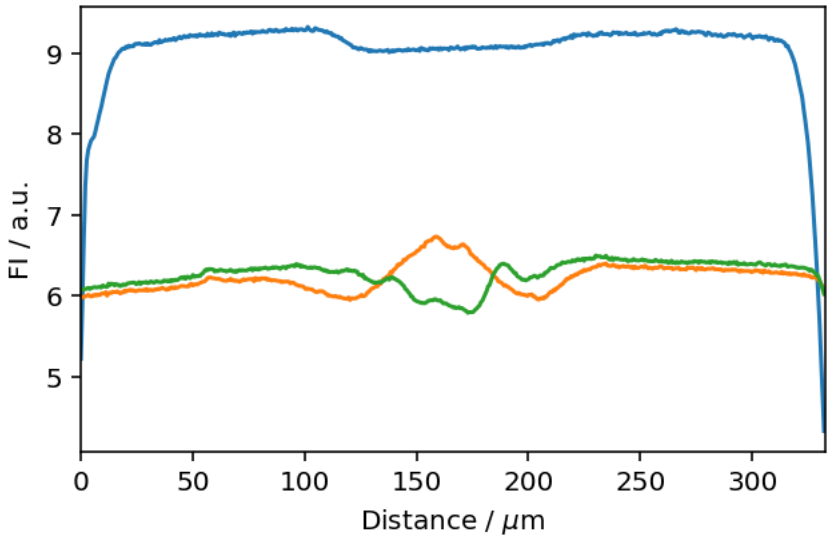


Figure S28 Averaged fluorescence intensity profile data (from the 12,000 images generated) across the width of the channel following the addition of no salt (blue), 1 M NaCl (orange) and 1 M Na_2SO_4 (green) via the external inlets and a solution of $Ru(bpy)_3Cl_2$ (50 μM) in MES buffer (50 mM at pH 6) flowed through all three inlets at a flow rate of 15 μL min^{-1}.

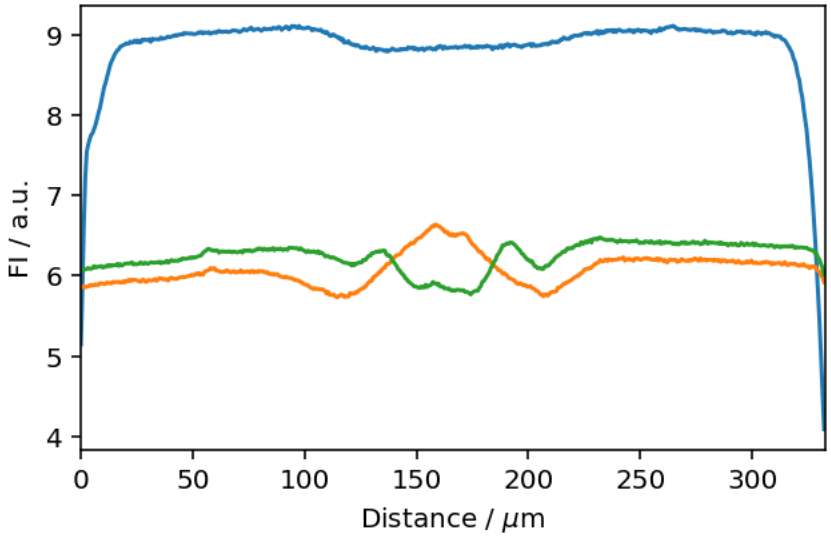


Figure S29 Averaged fluorescence intensity profile data (from the 12,000 images generated) across the width of the channel following the addition of no salt (blue), 1 M NaCl (orange) and 1 M Na_2SO_4 (green) via the external inlets and a solution of $Ru(bpy)_3Cl_2$ (50 μM) in MES buffer (50 mM at pH 6) flowed through all three inlets at a flow rate of 10 μL min^{-1}.

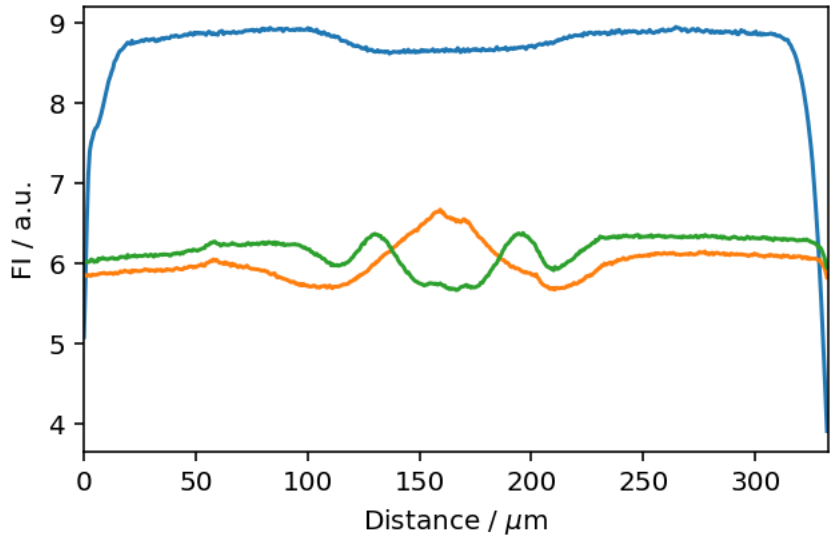


Figure S30 Averaged fluorescence intensity profile data (from the 12,000 images generated) across the width of the channel following the addition of no salt (blue), 1 M NaCl (orange) and 1 M Na_2SO_4 (green) via the external inlets and a solution of $Ru(bpy)_3Cl_2$ (50 μM) in MES buffer (50 mM at pH 6) flowed through all three inlets at a flow rate of 6 μL min^{-1}.

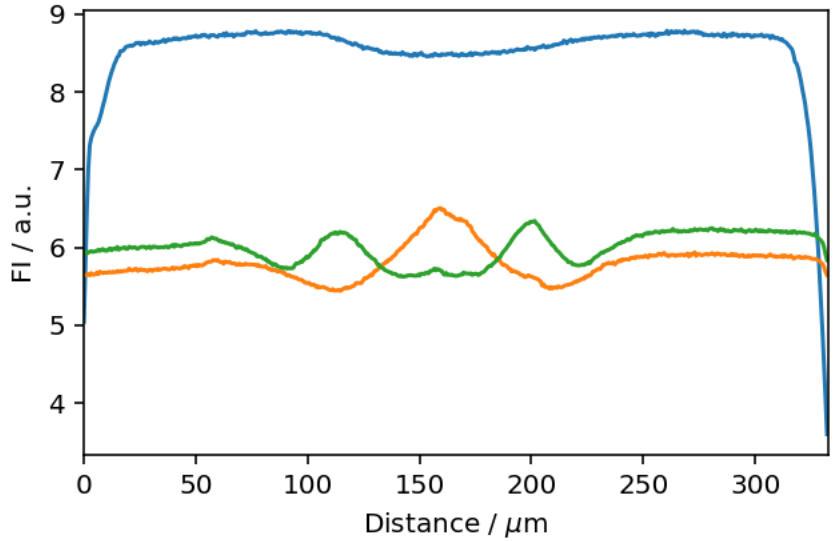


Figure S31 Averaged fluorescence intensity profile data (from the 12,000 images generated) across the width of the channel following the addition of no salt (blue), 1 M NaCl (orange) and 1 M Na_2SO_4 (green) via the external inlets and a solution of $Ru(bpy)_3Cl_2$ (50 μM) in MES buffer (50 mM at pH 6) flowed through all three inlets at a flow rate of 4 μL min^{-1}.

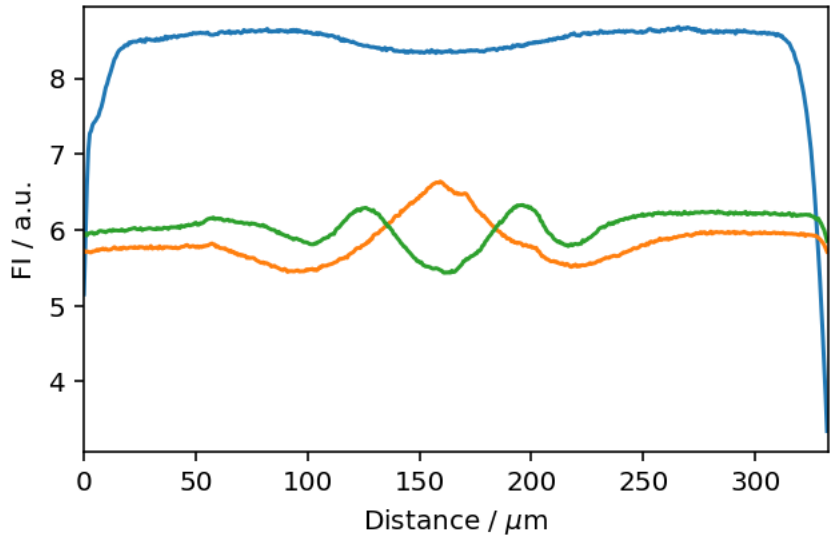


Figure S32 Averaged fluorescence intensity profile data (from the 12,000 images generated) across the width of the channel following the addition of no salt (blue), 1 M NaCl (orange) and 1 M Na_2SO_4 (green) via the external inlets and a solution of $Ru(bpy)_3Cl_2$ (50 μM) in MES buffer (50 mM at pH 6) flowed through all three inlets at a flow rate of 3 μL min^{-1}.

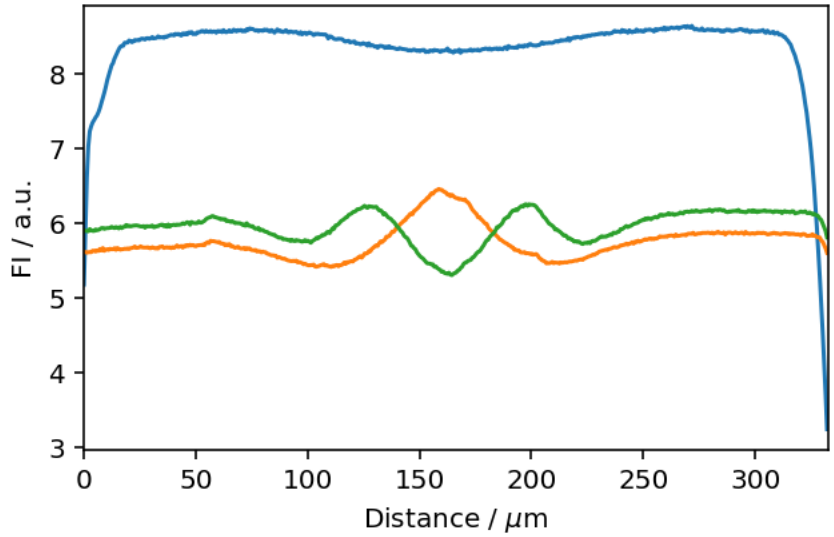


Figure S33 Averaged fluorescence intensity profile data (from the 12,000 images generated) the width of the channel following the addition of no salt (blue), 1 M NaCl (orange) and 1 M Na_2SO_4 (green) via the external inlets and a solution of $Ru(bpy)_3Cl_2$ (50 μM) in MES buffer (50 mM at pH 6) flowed through all three inlets at a flow rate of 2 μL min^{-1}.

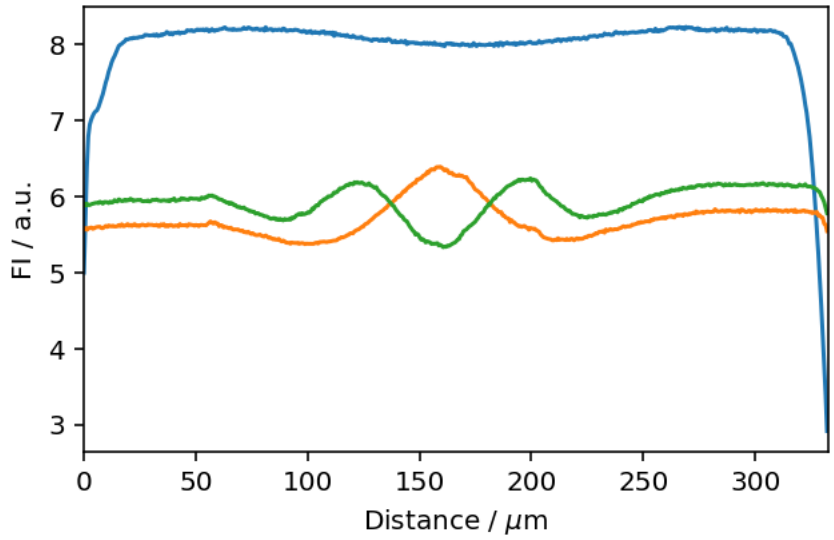


Figure S34 Averaged fluorescence intensity profile data (from the 12,000 images generated) across the width of the channel following the addition of no salt (blue), 1 M NaCl (orange) and 1 M Na_2SO_4 (green) via the external inlets and a solution of $Ru(bpy)_3Cl_2$ (50 µM) in MES buffer (50 mM at pH 6) flowed through all three inlets at a flow rate of 1.5 μL min^{-1}.

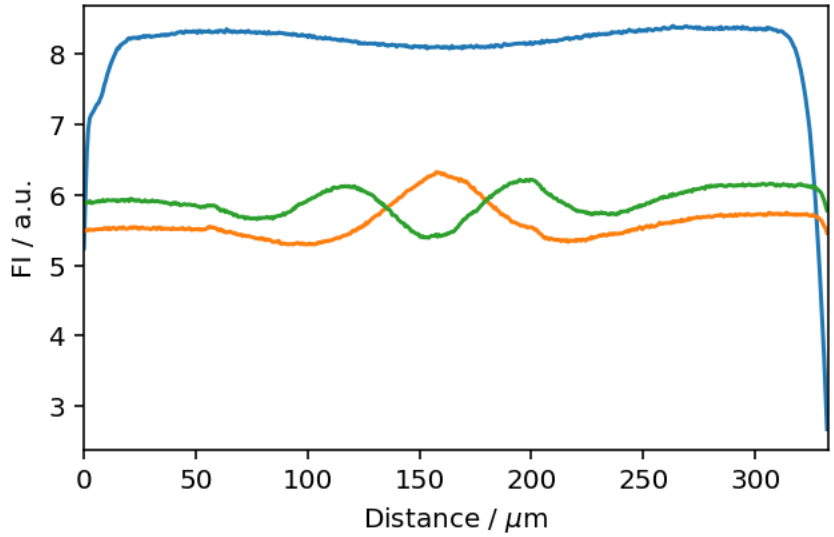


Figure S35 Averaged fluorescence intensity profile data (from the 12,000 images generated) across the width of the channel following the addition of no salt (blue), 1 M NaCl (orange) and 1 M Na_2SO_4 (green) via the external inlets and a solution of $Ru(bpy)_3Cl_2$ (50 µM) in MES buffer (50 mM at pH 6) flowed through all three inlets at a flow rate of 1.2 μL min^{-1}.

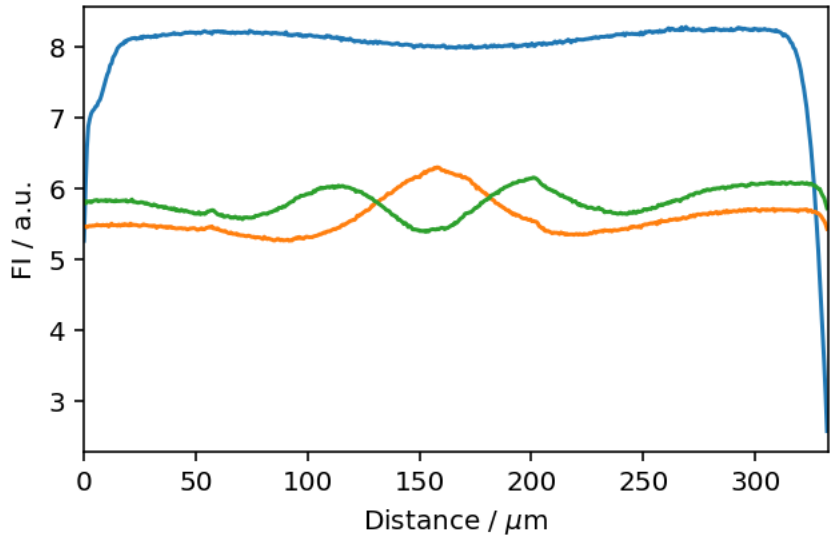


Figure S36 Averaged fluorescence intensity profile data (from the 12,000 images generated) across the width of the channel following the addition of no salt (blue), 1 M NaCl (orange) and 1 M Na_2SO_4 (green) via the external inlets and a solution of $Ru(bpy)_3Cl_2$ (50 μM) in MES buffer (50 mM at pH 6) flowed through all three inlets at a flow rate of 1 μL min^{-1}.

S10.4 Data for Figure 5

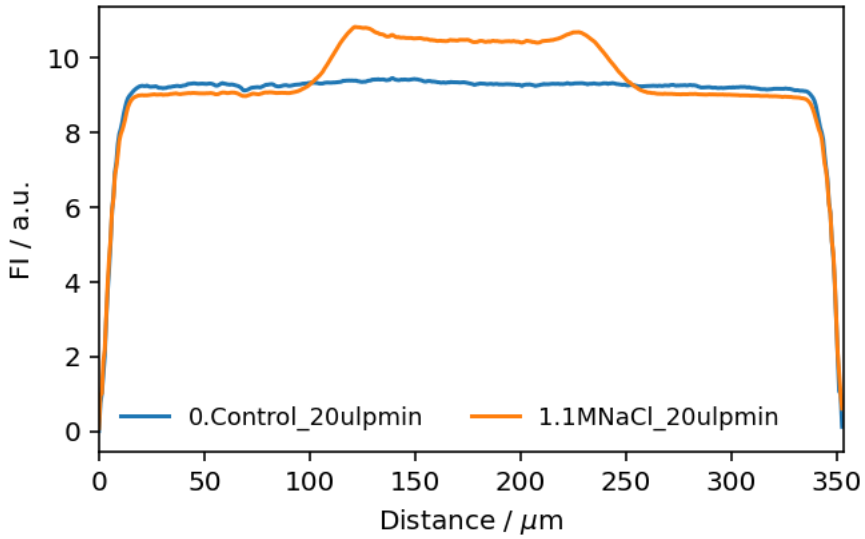


Figure S37 Averaged fluorescence intensity profile data (from the 12,000 images generated) after excitation with 488 nm across the width of the channel following the addition of no salt (blue) and 1 M NaCl (orange) via the central inlet and a solution of fluorescein (50 μM) and $Ru(bpy)_3Cl_2$ (50 μM) in MES buffer (50 mM at pH 6.5) flowed through all three inlets at a flow rate of 20 μL min^{-1}.

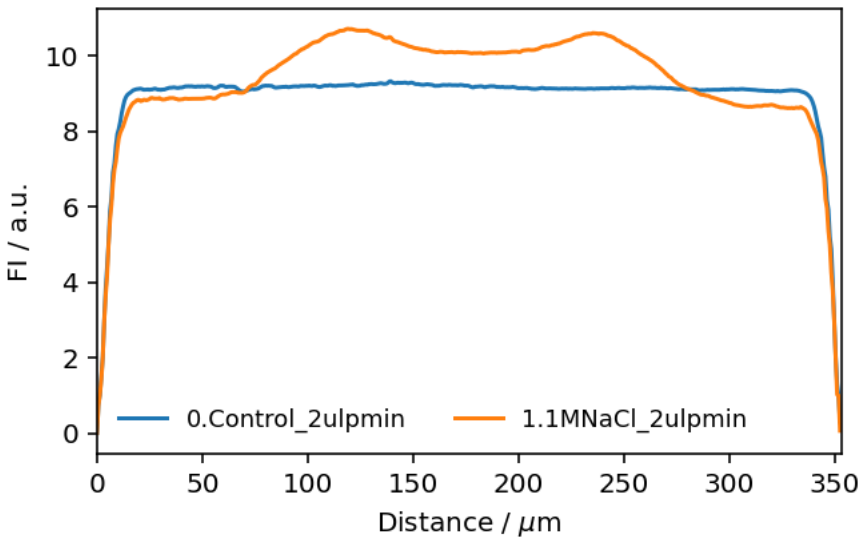


Figure S38 Averaged fluorescence intensity profile data (from the 12,000 images generated) after excitation with 488 nm across the width of the channel following the addition of no salt (blue) and 1 M NaCl (orange) via the central inlet and a solution of fluorescein (50 µM) and $Ru(bpy)_3Cl_2$ (50 µM) in MES buffer (50 mM at pH 6.5) flowed through all three inlets at a flow rate of 2 μL min^{-1}.

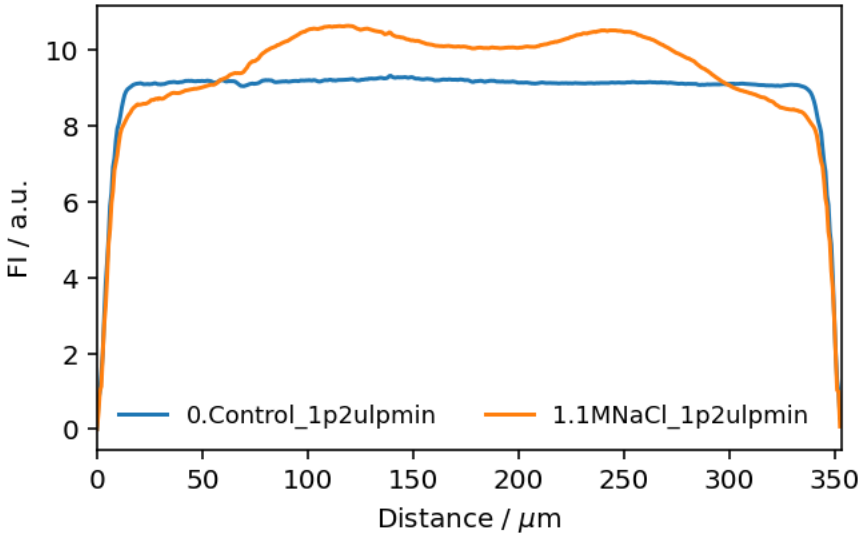


Figure S39 Averaged fluorescence intensity profile data (from the 12,000 images generated) after excitation with 488 nm across the width of the channel following the addition of no salt (blue) and 1 M NaCl (orange) via the central inlet and a solution of fluorescein (50 µM) and $Ru(bpy)_3Cl_2$ (50 µM) in MES buffer (50 mM at pH 6.5) flowed through all three inlets at a flow rate of 1.2 μL min^{-1}.

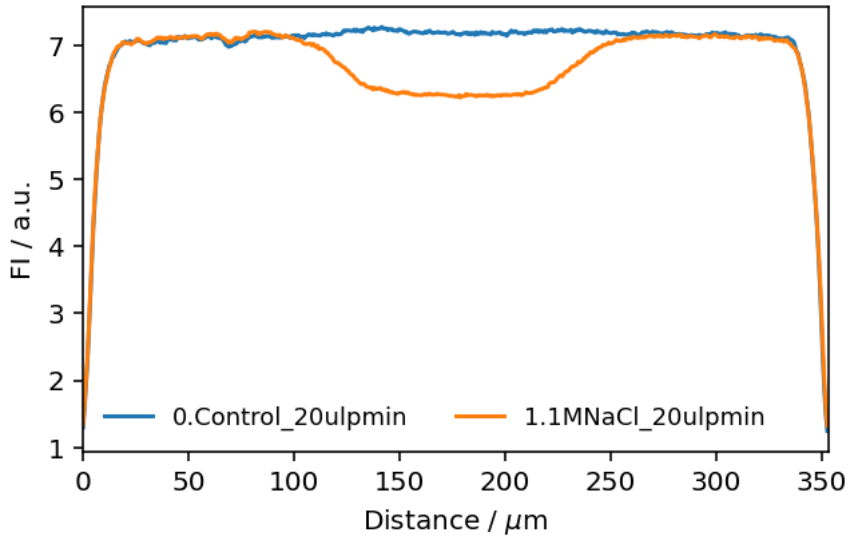


Figure S40 Averaged fluorescence intensity profile data (from the 12,000 images generated) after excitation with 458 nm for $Ru(bpy)_3Cl_2$ across the width of the channel following the addition of no salt (blue) and 1 M NaCl (orange) via the central inlet and a solution of fluorescein (50 µM) and $Ru(bpy)_3Cl_2$ (50 µM) in MES buffer (50 mM at pH 6.5) flowed through all three inlets at a flow rate of 20 μL min^{-1}.

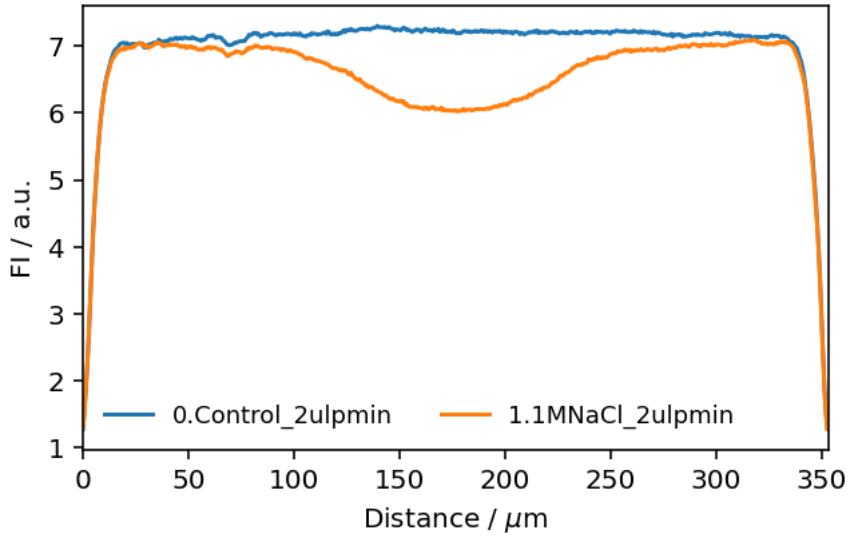


Figure S41 Averaged fluorescence intensity profile data (from the 12,000 images generated) after excitation with 458 nm across the width of the channel following the addition of no salt (blue) and 1 M NaCl (orange) via the central inlet and a solution of fluorescein (50 µM) and $Ru(bpy)_3Cl_2$ (50 µM) in MES buffer (50 mM at pH 6.5) flowed through all three inlets at a flow rate of 2 μL min^{-1}.

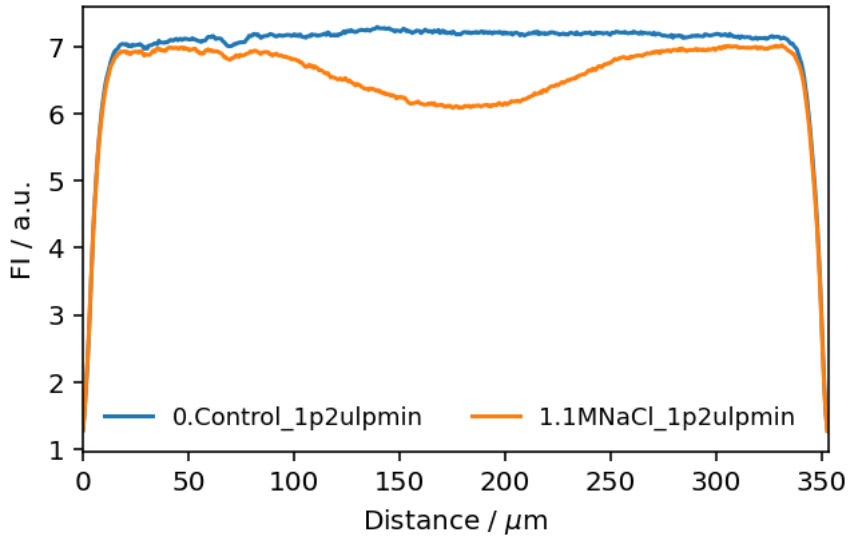


Figure S42 Averaged fluorescence intensity profile data (from the 12,000 images generated) after excitation with 458 nm across the width of the channel following the addition of no salt (blue) and 1 M NaCl (orange) via the central inlet and a solution of fluorescein (50 µM) and $Ru(bpy)_3Cl_2$ (50 µM) in MES buffer (50 mM at pH 6.5) flowed through all three inlets at a flow rate of 1.2 μL min^{-1}.