Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

On the Interaction of Copper(II) with Disulfiram

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General

Solvents were purchased from Sigma Aldrich and used without purification. Reagents and solvents were purchased from Sigma-Aldrich and Fisher respectively . HEPES buffer was purchased from Fisher. Deionised water was used in all studies.

Instrumentation

Mass spectrometry was performed using a Micromass Platform II instrument. High resolution accurate mass determination was performed using a Thermo-Finnegan MAT95XP mass spectrometer. EPR spectra were recorded on THF:water (95:5) glasses at *S*-band frequency (3.8 GHz) with a Bruker ELEXYS E580 EPR spectrometer equipped with an Oxford Instruments He cryostat. EPR simulations were performed using Bruker SimFonia software. Steady state UV-Vis experiments were performed using a Cary 5000 UV-Vis instrument. Initial rates measurements were performed using a Perkin Elmer 1050 WB instrument. Elemental analysis was performed by the University of Manchester analytical service.

Synthesis

CuL_2 was produced using the method of Jeliazkova *et al.* (*J. Photochem. Photobiol. A* **97**(1-2): 5-9). Anal. Calc for CuL_2 (%): C 33.4 H 5.6 N 7.8. found: C 33.3 H 5.7 N 7.6. HRMS (EI^+) calc for $C_{10}H_{20}N_2S_4Cu$: 358.9800. Found: 358.9793 (-1.9 ppm).

Mass Spectrometry.

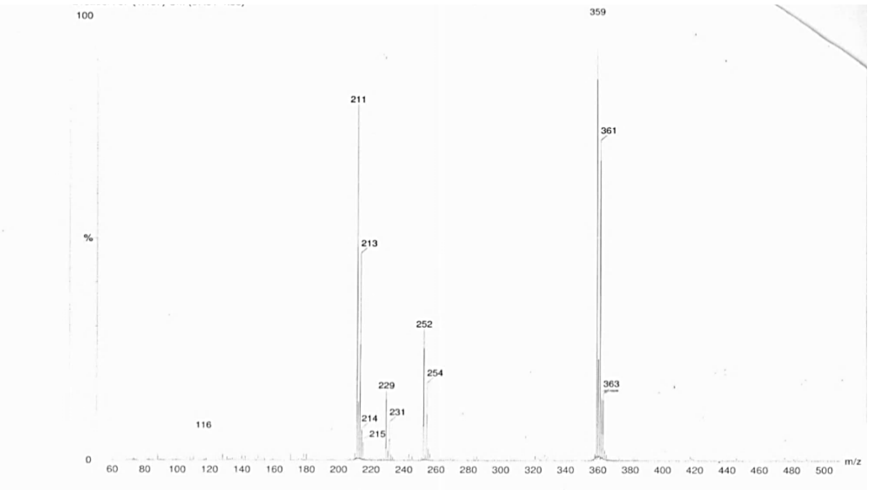
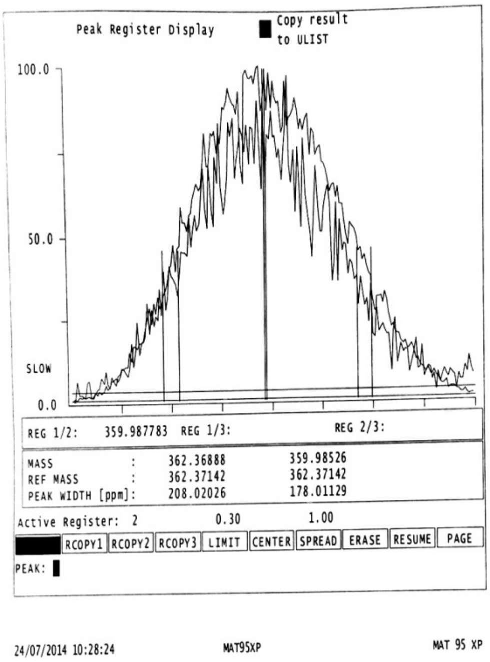


Figure S1: Full electrospray mass spectrum of a 1:1 mixture of Cu^{2+} and DSF. $[M+H]^+$ is observed at *m/z* 359.



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REG 1/2:    359.987783  REG 1/3:                REG 2/3:

MASS              :    362.36888        359.98526
REF MASS          :    362.37142        362.37142
PEAK WIDTH [ppm]:      208.02026        178.01129

Active Register:  2            0.30          1.00

PEAK:

24/07/2014 10:28:24            MAT95XP                  MAT 95 XP
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+ESI

Measured	Theoretical	Error [ppm]	Composition
359.9878	359.9878	-0.0	C_{10} H_{21} N_2 Cu_1 S_4

Figure S2: HRMS (ES^+) of the $[M+H]^+$ peak in a mixture of 1:1 Cu^{2+} and DSF. Calc. for $C_{10}H_{21}N_2S_4Cu$ ($[M+H]^+$): 359.9878. Found: 359.9878 (± 0 ppm) i.e. $[CuL_2 + H]^+$.

Steady state UV-Vis experiments.

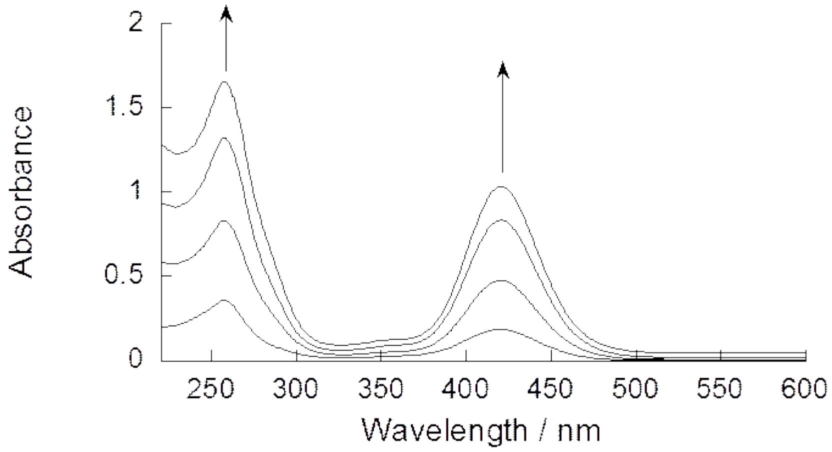

Figure S3: Steady-state UV-Visible absorbance titration of DSF into a solution of copper(II) chloride.

Full Error Analysis – Kinetics

Preamble: Combination of Errors

The following mathematical operations were used to propagate errors

Addition and Subtraction, $y = a + b - c$	$s_y = \sqrt{s_a^2 + s_b^2 + s_c^2}$
Multiplication and Division, $y = \frac{a \times b}{c}$	$\frac{s_y}{y} = \sqrt{\left(\frac{s_a}{a}\right)^2 + \left(\frac{s_b}{b}\right)^2 + \left(\frac{s_c}{c}\right)^2}$
Logarithm, $y = log_{10}a$	$s_y = 0.434\frac{s_a}{a}$

Where *a, b* and *c* are the values of experimental variables whose sample standard deviation or known absolute error are s_a, s_b and s_c respectively.

Systematic Error

Known Sources of Systematic Error are as follows for these conditions:

Volumetric flask, 100 mL	± 0.1 mL
Volumetric flask, 10 mL	± 0.025 mL
Eppendorf pipette, 10-100 uL	± 0.00005 mL
Eppendorf pipette,100 -1000 µL	± 0.0005 mL
Analytical balance	± 0.000005 g

Systematic Error in Concentrations of Reagents – Stock Solutions for Kinetics

To prepare the stock solutions for kinetics, *ca.* 0.01 mol dm^{-3} solutions of DSF in THF and ~0.01 mol dm^{-3} of $CuCl_2.2H_2O$ in water were prepared in 100 mL volumetric flasks. Both solutions were then diluted 10 × by pipetting 1 mL of either solution into 10 mL volumetric flasks which were filled to the line with the respective solvents to give *ca.* 0.001 mol dm^{-3} solutions of DSF in THF (C_1) and $CuCl_2.2H_2O$ in water (C_3).

Two more stock solutions of concentration *ca.* 0.1 mol dm^{-3} of DSF in THF (C_2) and *ca.* 0.1 mol dm^{-3} $CuCl_2.2H_2O$ in water (C_4) were prepared in 100 mL volumetric flasks.

Stocks were diluted for kinetics into either water or 1 M HEPES buffer. The water or buffer was added by 3 pipettings using a 100-1000 µL Eppendorf pipette.

DSF – solution C_1

Mass of disulfiram weighed	0.29837 ± 0.000005 g
RMM disulfiram	296.539 ± 0.005 g mol^{-1}
Dilution factor	0.1

Concentration

$$[C_1] = \frac{0.29837}{296.539} \times \frac{1000}{100} \times 0.1 = 0.001006\, mol\, dm^{-3}$$

Systematic error in dilution ± 0.1 mL, ± 0.025 mL (VFs) and ± 0.0005 mL (pipette)

Systematic error in concentration of stock C_1

$$s_{C_1} = 0.001006 \times \sqrt{\left(\frac{0.000005}{0.29837}\right)^2 + \left(\frac{0.005}{296.539}\right)^2 + \left(\frac{0.1}{100}\right)^2 + \left(\frac{0.025}{10}\right)^2 + \left(\frac{0.0005}{1}\right)^2} \approx \pm 0.000003$$

Error-adjusted concentration of stock C_1

$[C_1]$ = 0.001006 ± 0.000003 mol dm^{-3}

DSF – solution C_2

Mass of disulfiram weighed 2.95773 ± 0.000005 g

Concentration

$$[C_2] = \frac{2.95773}{296.539} \times \frac{1000}{100} = 0.09974\, mol\, dm^{-3}$$

Systematic error

$$s_{C_2} = 0.09974 \times \sqrt{\left(\frac{0.000005}{2.95773}\right)^2 + \left(\frac{0.005}{296.539}\right)^2 + \left(\frac{0.1}{100}\right)^2} \approx \pm 0.0001$$

Error-adjusted concentration of stock C_2

$[C_2]$ = 0.0997 ± 0.0001 mol dm^{-3}

$CuCl_2$ – solution C_3

Mass of $CuCl_2.2H_2O$ weighed	0.17552 ± 0.000005 g
RMM of $CuCl_2.2H_2O$	170.480 ± 0.005 g mol^{-1}
Dilution factor	0.1

Concentration

$$[C_3] = \frac{0.17552}{170.480} \times \frac{1000}{100} \times 0.1 = 0.001030\ mol\ dm^{-3}$$

Systematic error in dilution	± 0.1 mL, ± 0.025 mL (VFs) and ± 0.0005 mL (pipette)

Systematic error

$$s_{C_3} = 0.001030 \times \sqrt{\left(\frac{0.000005}{0.17552}\right)^2 + \left(\frac{0.005}{140.480}\right)^2 + \left(\frac{0.1}{100}\right)^2 + \left(\frac{0.025}{10}\right)^2 + \left(\frac{0.0005}{1}\right)^2} \approx \pm 0.000003$$

Error-adjusted concentration of stock C_3

$[C_3]$ = 0.001030 ± 0.000003 mol dm^{-3}

<u>$CuCl_2$ – solution C_4</u>

Mass of $CuCl_2.2H_2O$ weighed 1.72553 ± 0.000005 g

Concentration

$$[C_4] = \frac{1.72553}{170.480} \times \frac{1000}{100} = 0.1012\ mol\ dm^{-3}$$

Systematic error

$$s_{C_4} = 0.1012 \times \sqrt{\left(\frac{0.000005}{1.72553}\right)^2 + \left(\frac{0.005}{170.480}\right)^2 + \left(\frac{0.1}{100}\right)^2} \approx \pm 0.0001$$

Error-adjusted concentration of stock C_4

$[C_4]$ = 0.1012 ± 0.0001 mol dm^{-3}

Systematic Error in Initial Rate Measurements

Isolated Copper (Excess DSF)

Table S1: Summary of Systematic Error in log [Cu]

Mole Ratio, DSF: Cu	Vol DSF, C_2 (µL)	Vol CuCl$_2$, C_3 (µL)	Vol diluting solvent (per addition, total 3 additions, µL)	Error-adjusted final concentration in cuvette, [Cu^{2+}] (µM)	Error-adjusted log[Cu^{2+}] (log ([DSF]/mol dm^{-3}))
100:0.5	18.75 ± 0.05	9.40 ± 0.05	891 ± 0.1 × 3	3.59 ± 0.02	-5.445 ± 0.003
100:1	18.75 ± 0.05	18.75 ± 0.05	888 ± 0.1× 3	7.15 ± 0.03	-5.146 ± 0.002
100:2	18.75 ± 0.05	37.50 ± 0.05	881 ± 0.1× 3	14.31 ± 0.05	-4.845 ± 0.001
100:5	18.75 ± 0.05	93.80 ± 0.05	863 ± 0.1× 3	35.8 ± 0.1	-4.446 ± 0.001
100:10	18.75 ± 0.05	188 ± 0.5	831 ± 0.1× 3	71.7 ± 0.2	-4.144 ± 0.001
100:20	18.75 ± 0.05	374 ± 0.5	769 ± 0.1× 3	142.7 ± 0.4	-3.846 ± 0.001

Example Calculation: Systematic Error in 100:0.5 DSF:Cu

The additive error in the volume of the total solution in the cuvette is as follows:

$$S_{vol,\ cuvette} = 2700 \pm \sqrt{0.05^2 + 0.05^2 + 3 \cdot (0.1^2)} \approx 2700 \pm 0.2\ \mu\text{L}$$

Final concentrations of copper in the cuvette are calculated by:

$$C_{Cu,\,cuvette} = \frac{C_{stock}V_{stock}}{V_{cuvette}} = \frac{(0.001030) \times (9.40\ \times\ 10^{-6})}{2700\ \times 10^{-6}} = 3.4815\ \mu M$$

And thus the systematic error in concentration of copper in the cuvette were calculated by the following:

$$s_{Cu_{cuvette}} = 3.4815 \times \sqrt{\left(\frac{0.2}{2700}\right)^2 + \left(\frac{0.000003}{0.001030}\right)^2 + \left(\frac{0.05}{9.40}\right)^2} \approx \pm 0.0211$$

And the error-adjusted concentration of Cu^{2+} in the cuvette is :

[Cu^{2+}] = 3.4815 ± 0.0211 µM

Hence the final systematic error in log$_{10}$[Cu^{2+}] for this measurement, as plotted on the initial rate graph is as follows:

$$s_{Cu} = 0.434\ \times \left(\frac{0.0211}{3.4815}\right) = \pm \mathbf{0.003}$$

Isolated DSF (Excess Copper)

Table S2: Summary of Systematic Error in log [DSF]

Mole Ratio, DSF:Cu	**Vol $CuCl_2$, C_4 (µL)**	**Vol DSF, C_2 (µL)**	**Vol diluting solvent (per addition, total 3 additions, µL)**	**Error-adjusted final concentration in cuvette, $[DSF^{2+}]$ (µM)**	**Error-adjusted log[DSF] (log ([DSF]/mol dm^{-3}))**
100:0.5	18.75 ± 0.05	9.40 ± 0.05	891 ± 0.1 × 3	3.50 ± 0.02	-5.455 ± 0.003
100:1	18.75 ± 0.05	18.75 ± 0.05	888 ± 0.1× 3	6.99 ± 0.03	-5.155 ± 0.002
100:2	18.75 ± 0.05	37.50 ± 0.05	881 ± 0.1× 3	13.97 ± 0.04	-4.855 ± 0.001
100:5	18.75 ± 0.05	93.80 ± 0.05	863 ± 0.1× 3	34.9 ± 0.1	-4.457 ± 0.001
100:10	18.75 ± 0.05	188 ± 0.5	831 ± 0.1× 3	70.0 ± 0.2	-4.155 ± 0.001
100:20	18.75 ± 0.05	374 ± 0.5	769 ± 0.1× 3	139.3 ± 0.4	-3.856 ± 0.001

Random Error in Kinetics Measurements

Table S3: Random error in measurement of $\log v_0$ *($t \leq 5$ min) for copper isolated (excess DSF), water*

Mole Ratio, DSF:Cu	$\bar{x}$, v_0 $t \leq 5$ min (mol dm^{-3} s^{-1})	*N*	**Sample Standard Deviation (mol dm^{-3} s^{-1})**	**Coefficient of variation (%)**	**log (v_0/ mol dm^{-3} s^{-1})**	**Random Error in log v_0**
100:0.5	3.881×10^{-8}	5	2.173×10^{-8}	56	-7.4	± 0.2
100:1	4.258×10^{-8}	5	1.551×10^{-8}	36	-7.4	± 0.2
100:2	5.935×10^{-8}	5	1.358×10^{-8}	23	-7.2	± 0.1
100:5	5.195×10^{-8}	5	1.956×10^{-8}	38	-7.3	± 0.2
100:10	4.918×10^{-8}	5	2.310×10^{-8}	50	-7.3	± 0.2
100:20	4.625×10^{-8}	5	2.078×10^{-8}	45	-7.3	± 0.2

Table S4: Random error in measurement of $\log v_0$ *($t \leq 5$ min) for DSF isolated (excess copper), water*

Mole Ratio, Cu:DSF	$\bar{x}$, v_0 $t \leq 5$ min (mol dm^{-3} s^{-1})	*N*	**Sample Standard Deviation (mol dm^{-3} s^{-1})**	**Coefficient of variation (%)**	**log (v_0/ mol dm^{-3} s^{-1})**	**Random Error in log v_0**
100:0.5	7.904×10^{-9}	5	2.692×10^{-9}	34	-8.1	± 0.1
100:1	1.683×10^{-8}	5	2.232×10^{-9}	13	-7.77	± 0.06
100:2	3.604×10^{-8}	5	2.252×10^{-9}	6	-7.44	± 0.03
100:5	1.061×10^{-7}	5	5.169×10^{-9}	5	-6.97	± 0.02
100:10	2.506×10^{-7}	5	1.773×10^{-8}	7	-6.60	± 0.03
100:20	5.611×10^{-7}	5	5.750×10^{-8}	10	-6.25	± 0.04

Table S5: Random error in measurement of $\log v_0$ *($t \leq 5$ min) for Copper isolated (excess DSF), HEPES*

Mole Ratio, DSF:Cu	$\bar{x}$, v_0 $t \leq 5$ min (mol dm^{-3} s^{-1})	*N*	**Sample Standard Deviation (mol dm^{-3} s^{-1})**	**Coefficient of variation (%)**	**log (v_0/ mol dm^{-3} s^{-1})**	**Random Error in log v_0**
100:0.5	7.254×10^{-9}	5	5.264×10^{-9}	73	-8.1	± 0.3
100:1	1.449×10^{-8}	5	6.977×10^{-9}	48	-7.8	± 0.2
100:2	2.254×10^{-8}	5	4.099×10^{-9}	18	-7.65	± 0.08
100:5	5.239×10^{-8}	5	3.083×10^{-8}	59	-7.3	± 0.3
100:10	6.572×10^{-8}	5	8.021×10^{-9}	12	-7.18	± 0.05
100:20	9.195×10^{-8}	5	1.688×10^{-8}	18	-7.04	± 0.08

Table S6: Random error in measurement of $\log v_0$ *($t \leq 5$ min) for DSF isolated (excess copper), HEPES*

Mole Ratio, Cu:DSF	$\bar{x}$, v_0 $t \leq 5$ min (mol dm^{-3} s^{-1})	*N*	**Sample Standard Deviation (mol dm^{-3} s^{-1})**	**Coefficient of variation (%)**	**log (v_0/ mol dm^{-3} s^{-1})**	**Random Error in log v_0**
100:0.5	2.667×10^{-8}	5	5.319×10^{-9}	20	-7.57	± 0.08
100:1	4.367×10^{-8}	5	3.367×10^{-9}	8	-7.35	± 0.03
100:2	5.390×10^{-8}	5	2.534×10^{-9}	5	-7.27	± 0.02
100:5	1.382×10^{-7}	5	2.371×10^{-8}	17	-6.86	± 0.07
100:10	1.853×10^{-7}	5	1.833×10^{-8}	10	-6.73	± 0.04
100:20	3.815×10^{-7}	5	9.896×10^{-8}	26	-6.4	± 0.1

Kinetic Experiments: Initial Rate Plots for DSF and Cu^{2+} in Unbuffered Solution including error analysis

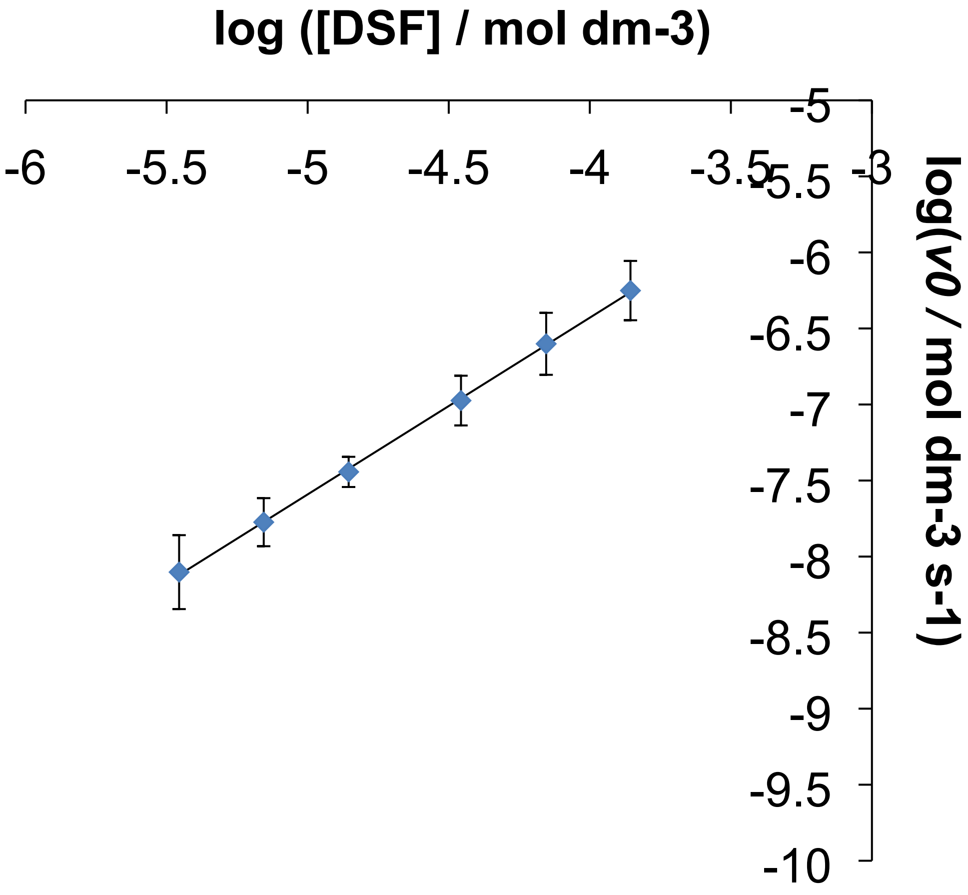

Figure S4: Initial rate plot showing the dependence of the initial reaction rate (v_0 ; t < 5 min) on DSF concentration in unbuffered solution. $[Cu^{2+}] = 6.9 \times 10^{-4}$ mol dm^{-3}. Variation of the DSF concentration gives a straight line with gradient equivalent to the reaction order for DSF in the rate law i.e. 1st order. Error bars in y (random error in measurement) correspond to ±1 sample standard deviation ($N = 5$). Systematic error in x is too small to be drawn.

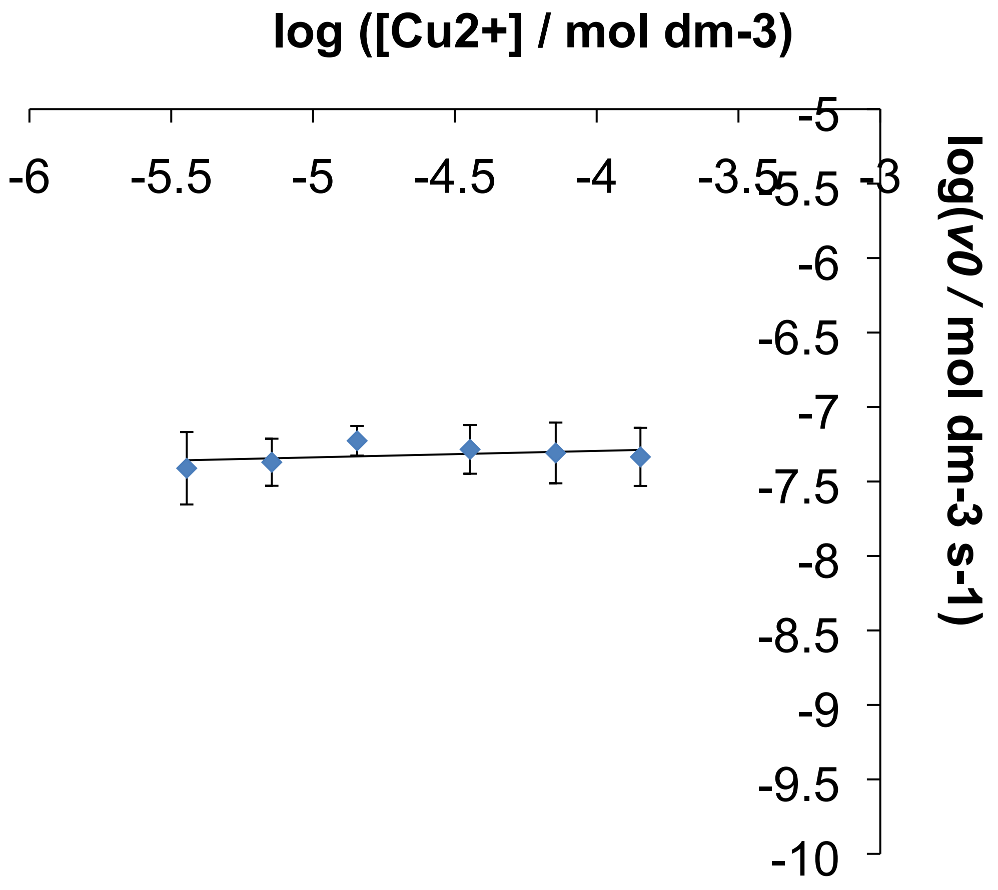

Figure S5: Initial rate plot showing the dependence of the initial reaction rate (v_0 ; t < 5 min) on Cu^{2+} concentration in unbuffered solution. [DSF] = 6.9×10^{-4} mol dm^{-3}. Variation of the Cu^{2+} concentration gives a straight line with gradient equivalent to the reaction order for Cu^{2+} in the rate law i.e. 0th order. Error bars in y (random error in measurement) correspond to ±1 sample standard deviation ($N = 5$). Systematic error in x is too small to be drawn.

Kinetic Experiments: Initial Rate Plots for DSF and Cu^{2+}in HEPES-Buffered Solution

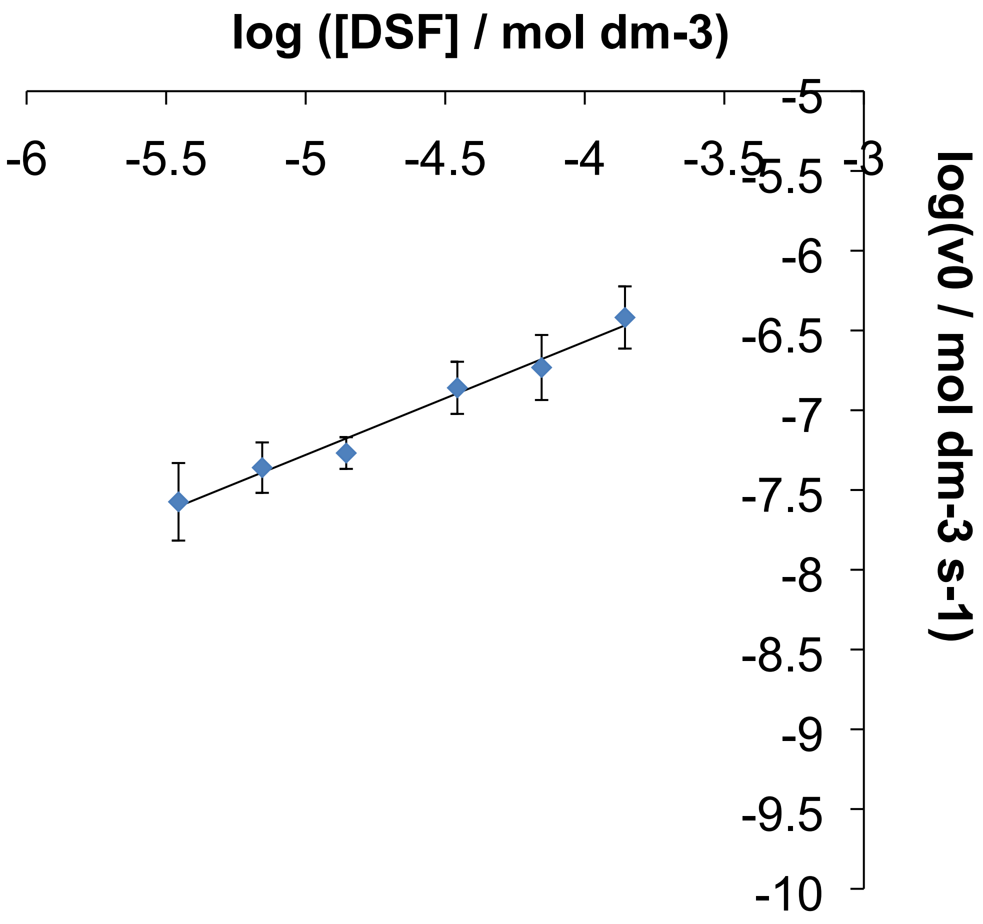

Figure S6: Initial rate plot showing the dependence of the initial reaction rate (v_0 ; t < 5 min) on DSF concentration in HEPES-buffered solution, pH 7.4. $[Cu^{2+}] = 6.9 \times 10^{-4}$ mol dm^{-3}. Variation of the DSF concentration gives a straight line with gradient equivalent to the reaction order for DSF in the rate law, i.e. 1st order. Error bars in y (random error in measurement) correspond to ±1 sample standard deviation ($N = 5$). Systematic error in x is too small to be drawn.

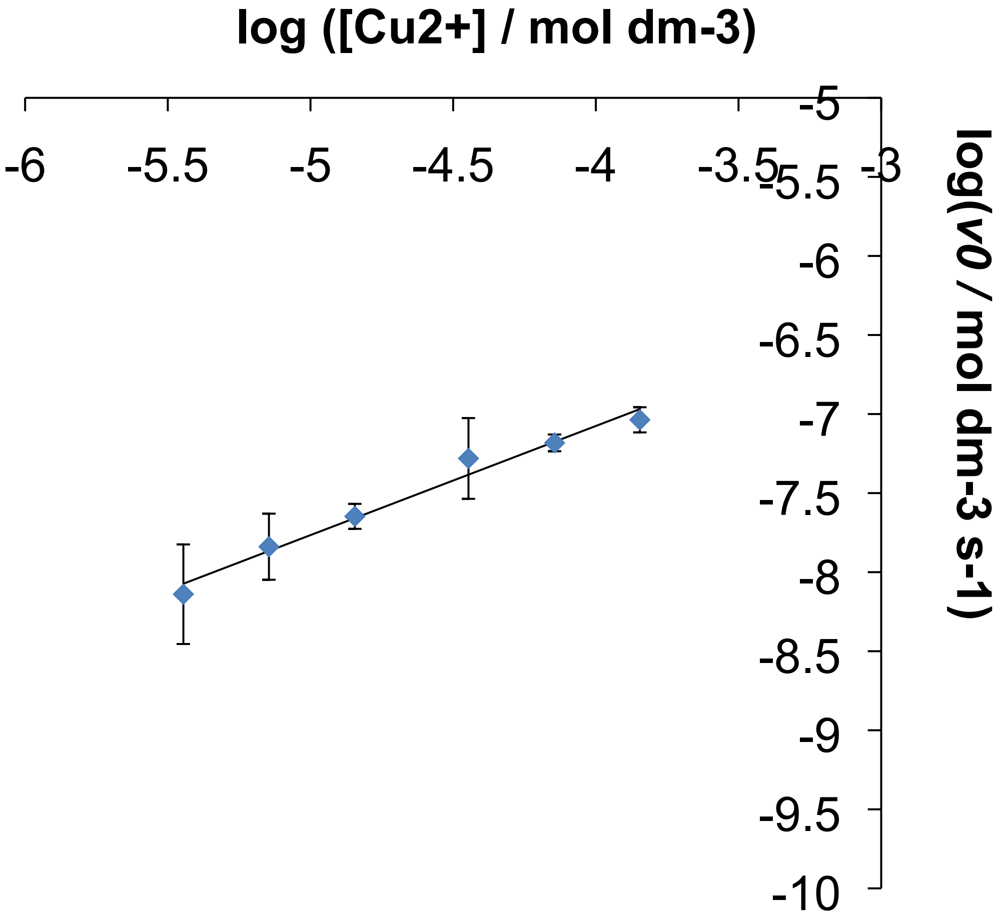

Figure S7: Initial rates plot for showing the dependence of the initial reaction rate (v_0 ; t < 5 min) on Cu^{2+} concentration in HEPES-buffered solution, pH 7.4. [DSF] = 6.9 × 10^{-4} mol dm^{-3}. Variation of the Cu^{2+} concentration gives a straight line with gradient equivalent to the reaction order for Cu^{2+} in the rate law i.e. 1st order. Error bars in y are ±1 sample standard deviation ($N = 5$). Systematic error in x is too small to be drawn.

Error in linear regression and calculated mole fraction, Job plot (Fig 3, main article)

The standard deviation of the slope (*s_m*) of the fitted lines on either half of the Job plot were calculated separately for each linear fit using the following expression:

$$s_m = \sqrt{\frac{s_r^2}{S_{xx}}}$$

The standard deviation about regression (*s_r*) used in both of these calculations was calculated separately for each linear fit using the following expression:

$$s_r = \sqrt{\frac{\left(S_{yy} - m^2 S_{xx}\right)}{N - 2}}$$

Where *N* is the number of data points fitted, *m* is the gradient of the fitted line.

S_{yy} and *S_{xx}* are defined as:

$$S_{xx} = \sum (x_i - \bar{x})^2$$

$$S_{yy} = \sum (y_i - \bar{y})^2$$

Where x/y_i are the individual data values and $\bar{x}$, $\bar{y}$ the mean values of *x* and *y*.

The standard deviation of the *y*-intercept (*s_c*) was calculated separately for each linear fit using the following expression:

$$s_c = \sqrt{\frac{\sum x_i^2}{N\sum x_i^2 - \left(\sum x_i\right)^2}}$$

The following tables (Table S7A and S7B) present the calculated results to obtain s_m and s_c for both of the linear regions of the Job plot.

Table S7A: Calculated Random Error in Job plot fit
x = 0.6 - 1.0

x	***y***	***x***	***y***	$(x_i - \bar{x})^2$	$(y_i - \bar{y})^2$	S_{xx}	S_{yy}	s_r	s_m	x^2	y^2	s_c
1	0.00097	0.8	0.13072	0.04	0.01684	0.1	0.03993	0.02215	0.07004	1	9.324E-07	0.05690
0.9	0.07181	"	"	0.01	0.00347	"	"	"	"	0.81	0.0051561	"
0.8	0.12199	"	"	0	7.6E-05	"	"	"	"	0.64	0.0148816	"
0.7	0.22379	"	"	0.01	0.00866	"	"	"	"	0.49	0.0500820	"
0.6	0.23504	"	"	0.04	0.01088	"	"	"	"	0.36	0.0552438	"

Table S7B: Calculated Random Error in Job plot fit
x = 0 - 0.6

x	***y***	***x***	***y***	$(x_i - \bar{x})^2$	$(y_i - \bar{y})^2$	S_{xx}	S_{yy}	s_r	s_m	x^2	y^2	s_c
0.6	0.23504	0.3	0.16469	0.04	0.00495	0.23	0.02279	0.03227	0.06729	0.36	0.0552438	0.02199
0.5	0.21019	"	"	0.04	0.00207	"	"	"	"	0.25	0.0441798	"
0.4	0.21298	"	"	0.01	0.00233	"	"	"	"	0.16	0.0453605	"
0.3	0.17707	"	"	0	0.00015	"	"	"	"	0.09	0.0313538	"
0.2	0.14934	"	"	0.01	0.00024	"	"	"	"	0.04	0.0223024	"
0.1	0.0897	"	"	0.04	0.00562	"	"	"	"	0.01	0.0080461	"
0	0.07853	"	"	0.09	0.00742	"	"	"	"	0	0.0061662	"

Calculation of linear intersection and random error in calculated mole fraction

The error in the calculated value of *x* for equivalent values of *y* (i.e. the intersection of the two linear fits in Fig 3) was obtained by propagation of the standard deviations for slope and intercept, as calculated in tables S7A and S7B.

Calculation of where the two lines in Fig. 3 proceeds as follows:

$$0.62686\ \pm 0.05690 - 0.62013 \pm 0.07004x = 0.081746 \pm 0.02199 + 0.27648 \pm 0.06729x$$

Which is rearranged to give

$$0.62686\ \pm 0.05690 - \ 0.081746 \pm 0.02199 = 0.27648 \pm 0.06729x + 0.62013 \pm 0.07004x$$

The error on both left hand side and right hand side can be propagated separately with:

$$s_y\ =\ \sqrt{s_a^2 +\ s_b^2}$$

To give:

$$0.54511\ \pm 0.06100\ \ = 0.89661 \pm 0.09713x$$

Thus the calculated mole fraction, incorporating random error is:

$$x = \frac{0.54511 \pm 0.06100}{0.89661 \pm 0.09713} = 0.6083 \pm 0.0659$$