

Confirming Dialysis Equilibrium

The effect of equilibration time during Donnan dialysis was tested for two solutions with 2 mg Cu L⁻¹, 1 mM Sr(NO₃)₂, 5 mM MES (pH=5), and either no ligand or 15.7 μM EDTA (to complex 50% of the Cu). Donnan dialysis was carried out as described in the manuscript, and 20 μL acceptor solution was removed at various times. The acceptor was diluted and analysed for Cu using ICP-OES. The results showed that there was little change in Cu concentration in the acceptor after 1 h equilibration, with the concentration in the acceptor close to the free Cu concentration of the donor (Figure S1).

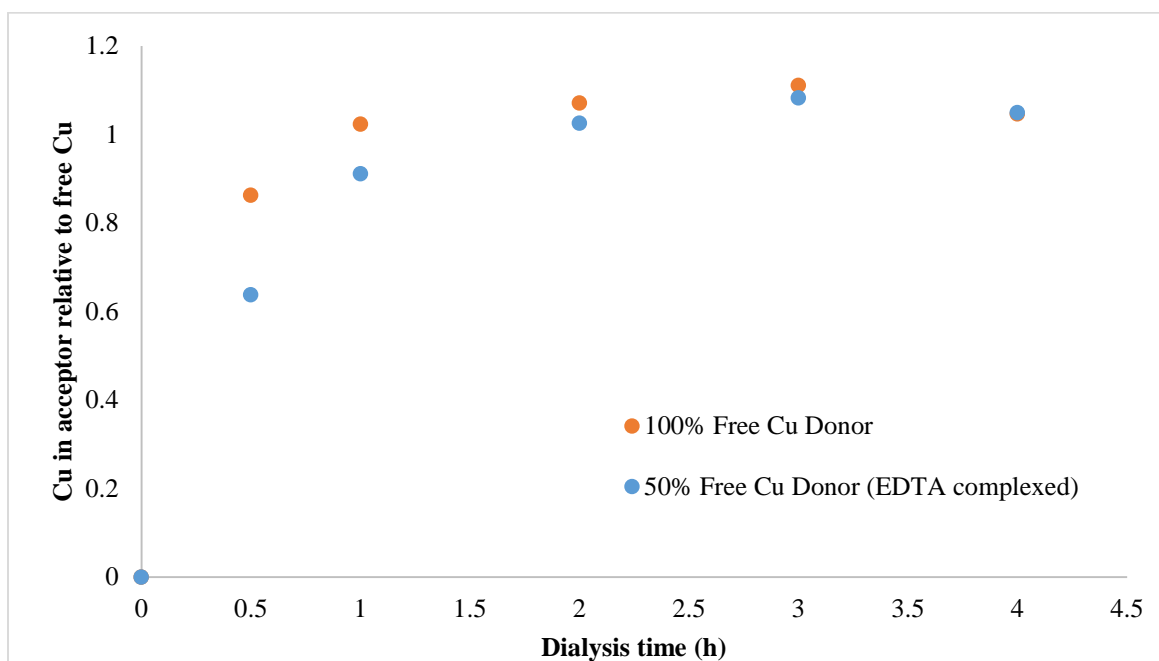


Figure S1. The Cu concentration in the acceptor solution (relative to the free Cu concentration in the donor solution) as function of the dialysis time, for a donor solution with only free Cu or for a donor solution with 50% free Cu and 50% Cu-EDTA.

Statistical Analysis

Given the variability in the isotope values measured regression analysis was performed to confirm the relationship between bond strength and $\Delta^{65}\text{Cu}_{\text{complex-free}}$ was significant. The significance of the relationship was assessed both with and without the inclusion of the fulvic acid data, and in both cases the relationship was significant (Table S1) (Figures S2 – S3).

Table S1. R^2 and significance of the slope (p-value) for the relationship between the fractionation factor ($\Delta^{65}\text{Cu}_{\text{complex-free}}$) and the logarithm of the stability constant, for all ligands (Figure S2) or when excluding fulvic acid (Figure S3).

With Fulvic Acid		Without Fulvic Acid	
R^2	0.77	R^2	0.42
p-value	1.27×10^{-7}	p-value	0.01

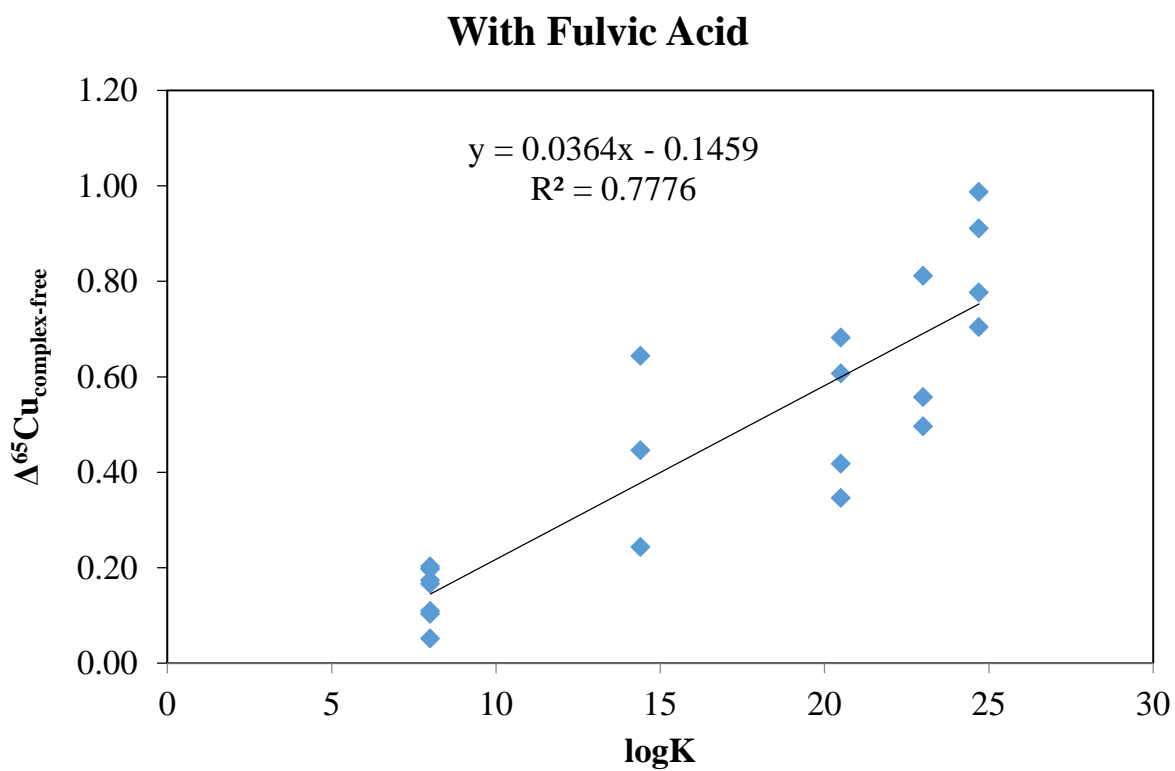


Figure S2. Relationship between the logarithm of the stability constant and the isotopic fractionation factor between complexed and free Cu (including complexes with fulvic acid).

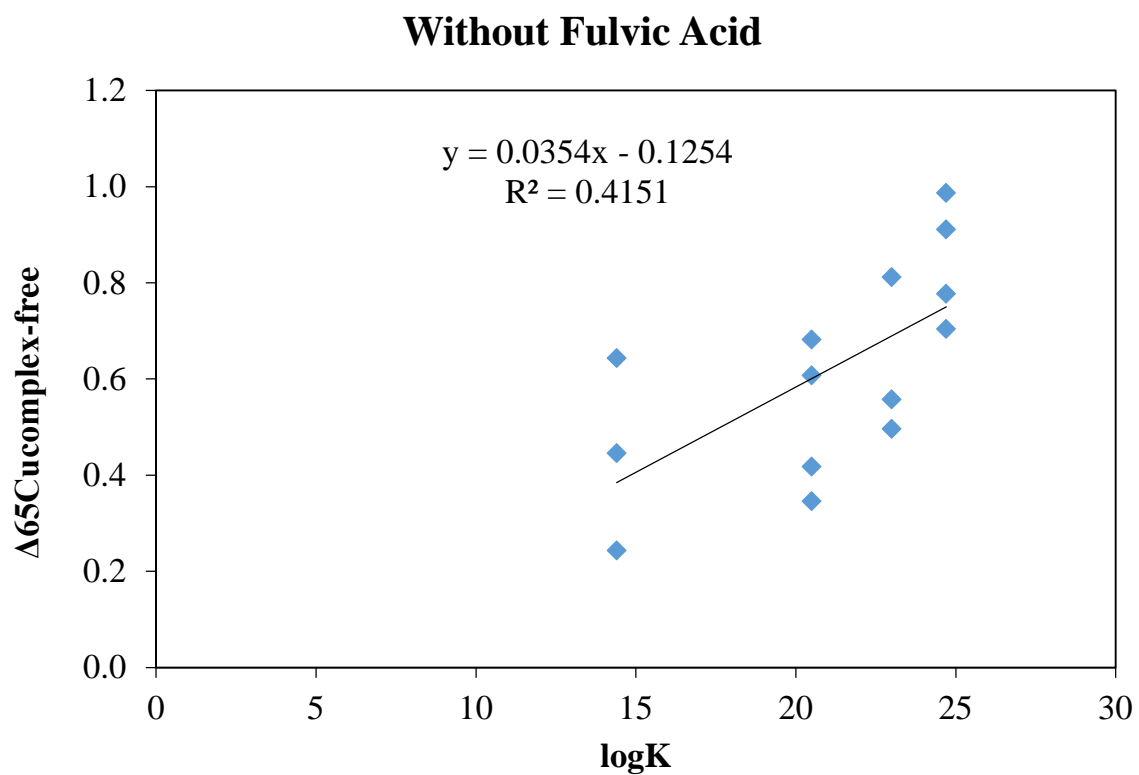


Figure S3. Relationship between the logarithm of the stability constant and the isotopic fractionation factor between complexed and free Cu (excluding complexes with fulvic acid).