Binuclear Cu<sup>2+</sup> Complex Mediated L-Glutamate-L-Aspartate Discrimination in Water. Application for Electrochemical Sensing.

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## **SQWVs:**

Square wave voltammograms (SQWVs) were obtained in a three-electrode cell under an argon atmosphere with a glassy carbon working electrode (BAS MF 2012, geometrical area 0.017 cm<sup>2</sup>), a platinum wire auxiliary electrode and a AgCl (3 mol dm<sup>-3</sup> NaCl)/Ag reference electrode using BAS CV 50 W equipment. The potential of such a reference electrode was -35 mV vs. saturated calomel reference electrode (SCE).

Adsorbate-modified electrodes were prepared by immersion, for periods between 30 s and 300 s, of the bare carbon electrode [previously cleaned, polished and activated as reported in the literature (A. Doménech, E. García-España, P. Navarro and F. Reviriego, *Talanta*, 2000, **51**, 625)] into 2.0 mM CuSO<sub>4</sub>·5H<sub>2</sub>O plus 2.0 mM L·6HBr or 2.0 mM CuSO<sub>4</sub>·5H<sub>2</sub>O plus 1.0 mM L·6HBr aqueous solutions at pH values of 5.5, 6.5, 7.5, and 8.5. Monolayer electrodes were prepared by evaporation of a drop (50  $\mu$ L) of those CuSO<sub>4</sub>·5H<sub>2</sub>O plus 2.0 mM L·6HBr solutions over the surface of the carbon electrode at 85 °C.

Voltammograms were recorded after immersion of freshly prepared modified electrodes into 0.15 mol dm<sup>-3</sup> NaClO<sub>4</sub> plus HClO<sub>4</sub>/NaOH solutions in the pH range between 5.5 and 8.5. Similar experiments were performed in solutions of L-aspartic acid (Merck), L-glutamic acid (Merck), succinic acid (Merck) and glutaric acid (Merck) all in concentrations ranging from 0.2 to 2.0 mM.

System	Signal	δ(ppm)	Number of protons	Assignments	Temperature dependence	<i>T</i> <sub>1</sub> (ms)	$\Delta v_{1/2}$ (Hz)	$T_2 (\mathrm{ms})^{\mathrm{a}}$
Cu <sub>2</sub> L	а	9.0	8	$\beta CH_2$	anti-Curie	2.1	200	1.6
	b	3.1	3	H <sub>m,p</sub> -Py	anti-Curie	4.3	81	3.9
	c	-3.2			Curie	<1	1339	0.24
	d	-9.8	24	$\alpha CH_2$	Curie	<1	1339	0.24
Cu <sub>2</sub> L-Glu	a´	9.0	8	$\beta CH_2$	anti-Curie	2.4	204	1.6
	b´	5.3 <sup>b</sup>	$\sim 3 \times 2$	ββ'CH <sub>2</sub> -Glu		2.2	c	c
	c	3.5	3	H <sub>m,p</sub> -Py	anti-Curie	3.9	c	c
	ď	2.5	$\sim 2 \times 2$	γCH <sub>2</sub> -Glu	anti-Curie	4.5	144	2.2
	e	-0.92			Curie	<1	1143	0.28
	f	-9.8	24	$\alpha CH_2$		<1	1495	0.21
Cu <sub>2</sub> L-Asp	a''	8.9	8	$\beta CH_2$	anti-Curie	3.1	132	2.4
	b´´	3.6 <sup>d</sup>	3	H <sub>m,p</sub> -Py		2.5	c	c
	c´´	3.1			anti-Curie	3.3	192	1.7
	d´´	2.0	$\sim 3 \times 2$	$\beta\beta'CH_2$ -Asp	anti-Curie	2.9	c	c
	e´´	-1.8			Curie	<1	1231	0.26
	f	-9.4	24	$\alpha CH_2$	Curie	<1	1275	0.25

**Table S1.** <sup>1</sup>H NMR hyperfine-shifted resonances of Cu<sub>2</sub>L, Cu<sub>2</sub>L-Glu and Cu<sub>2</sub>L-Asp complexes in D<sub>2</sub>O at 40 °C and pH = 6.5, determined for 1:2molar ratio.

<sup>a</sup> Measured from the line width at half-height.<sup>b</sup> Measured at 288 K.<sup>c</sup> Overlap prevents measurement of this value.<sup>d</sup> Measured at 293 K.

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Reaction <sup>a</sup>	L-Glutamate	L-Aspartate
$H_3L + AH = H_4AL$	1.89(4)	-
$H_4L + AH = H_5AL$	2.27(3)	2.27(2)
$H_5L + AH = H_6AL$	2.26(2)	2.29(2)
$H_6L + AH = H_7AL$	2.82(2)	2.91(2)
$H_6L + AH_2 = H_8AL$	2.44(3)	2.58(2)

**Table S2** Logarithms of the stability constants of complexes L-L-Glutamate and L-L-<br/>Aspartate determined at 298.1  $\pm$  0.1 K in 0.15 mol dm<sup>-3</sup> NaClO<sub>4</sub>.

<sup>*a*</sup> Charges omitted for clarity.<sup>*b*</sup> Values in parentheses are standard deviations in the last significant figure

Reaction	L-Glutamate	L-Aspartate
ML + A = MLA	8.918(7)	-
MLH + A = MLAH	9.142(7)	-
ML + AH = MLAH	9.202(7)	-
$MLH + AH = MLAH_2$	8.735(6)	-
$MLH_2 + AH = MLAH_3$	7.84(4)	2.50(1)
$MLH_3 + AH = MLAH_4$	7.562(3)	3.54(1)
$MLH_2 + AH_2 = MLAH_4$	8.012(3)	4.37(1)
$MLH_4 + AH = MLAH_5$	5.938(6)	3.435(8)
$MLH_3 + AH_2 = MLAH_5$	6.438(6)	4.315(8)
$MLH_4 + AH_2 = MLAH_6$	-	3.06(2)
$M_2L + AH = M_2LAH$	8.409(5)	4.148(8)
$M_2LH + AH = M_2LAH_2$	9.037(5)	5.098(7)
$M_2L + AH_2 = M_2LAH_2$	9.217(5)	5.658(7)
$M_2LH + AH_2 = M_2LAH_3$	8.09(2)	5.30(2)
$M_2L + 2A = M_2LA_2$	14.042(2)	8.88(1)
$M_2LAH + A = M_2LA_2H$	5.771(7)	5.043(9)
$M_2LH + 2A = M_2LA_2H$	19.270(7)	14.461(8)
$M_2LA_2 + H_2O = M_2LA_2(OH) + H$	10.44(1)	-
$M_2L(OH) + 2A = M_2LA_2(OH)$	11.373(9)	-

 Table S3 Logarithms of stability constants for the formation of mixed complexes  $Cu^{2+}L-L$ -Glutamate and  $Cu^{2+}-L-L$ -Aspartate determined at 298.1 ± 0.1 K in 0.15 mol dm<sup>-3</sup> NaClO<sub>4</sub>.

<sup>a</sup> Charges omitted by clarity.<sup>b</sup> Values in parentheses are standard deviations in the last significant figure



**Fig S1.** - 400 MHz <sup>1</sup>H NMR spectra in D<sub>2</sub>O at 313 K of (A) Cu<sub>2</sub>L, (B) Cu<sub>2</sub>L-Glu, (C) Cu<sub>2</sub>L-Asp. The asterisks mark the residual solvent and impurity signals (\*H<sub>2</sub>O; \*\*HOD).

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Fig S2. - SQWVs at a GCE modified by a deposit obtained vu evaporation of 50 μL of a 2.0 × 10<sup>-3</sup> mol dm<sup>-3</sup> CuSO<sub>4</sub>·5H<sub>2</sub>O plus 1.0 × 10<sup>-3</sup> mol dm<sup>-3</sup> L·6HBr solution at pH 6.5 immersed into a) 0.15 mol dm<sup>-3</sup> NaClO<sup>4</sup>; b) id. plus 2.0 × 10<sup>-3</sup> mol dm<sup>-3</sup> aspartic acid; c) id. plus 2.0 × 10<sup>-3</sup> mol dm<sup>-3</sup> glutamic acid, all at pH 7.5. d) id. plus 0.5 × 10<sup>-3</sup> mol dm<sup>-3</sup> glutamic acid, all at pH 7.5. Potential step increment 4 mV; square wave amplitude 25 mV; frequency 5 Hz



Fig S3. - SQWVs at a GCE modified by a deposit obtained by evaporation of 50 uL of a 2.0 mM CuSO<sub>4</sub>·SH<sub>2</sub>O plus 1.0 mM L·6HBr solution at pH 6.0 immersed into 0.15 mol·dm<sup>-3</sup> NaClO<sub>4</sub> solutions of: a) 2.0 mM succinic acid; b) 2.0 mM glutaric acid, both at pH 10.5. Potential step increment 4 mV; square wave amplitude 25 mV; frequency 5 Hz.

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Fig S4. - ESI (positive mode) mass spectrum (A) Cu<sub>2</sub>L-Glu 1:1, (B) Cu<sub>2</sub>L -Glu 1:2, (C) Amplification of spectrum B.