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Supplementary Material

Determination of pK_a values of carboxyl groups in the N-terminal domain of rat CD2: anomalous pK_a of a glutamate on the ligand binding surface

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Figure S1. Regions of the 2D H(C)CO spectra from the pH titration of rat CD2d1 showing glutamic acid and aspartic acid cross peaks at pH 8, pH 5 and pH 3. The spectra were recorded at 25°C at a protein concentration of 1.2 mM, in 20 mM phosphate buffer.

Figure S2. pH dependence of chemical shift of protons adjacent to the carboxyl groups (H^{γ} for Glu, H^{β} for Asp) in CD2d1. Both of the chemical shift profiles for the two methylene protons of selected carboxyl groups are shown. The chemical shift profiles were fit to a multi-component Henderson-Hasselbalch equation as described in the text.

Table S1. Acidity constants (pK_a values) determined from the pH titration of rat CD2d1 monitored by 2D [^{15}N,^{1}H]-HSQC experiments.

Residue (nucleus)[a]	Ionising group[b]	pK_a value[c]					
		No NaCl	100 mM NaCl	300 mM NaCl	Bis-tris	E41Q	E29Q
Asp2 (N)	Asp2	3.6	3.7	3.5	3.5	3.3	3.2
	α-NH_2	7.1	7.4	-[d]	7.0	6.9	7.2
His12 (N)	His12	6.8	6.9	6.9	6.7	6.5	6.8
	Glu99	4.3	4.1	4.1	4.4	4.3	3.6
Ile18 (H^N)	Asp62	4.4	4.3	4.3	4.2	4.1	4.0
Asp25 (N)	Asp25	3.7	3.6	3.5	3.6	3.4	3.3
Asp26 (N)	Asp26	3.7	3.7	3.5	3.6	3.5	3.1
Asp28 (H^N)	Asp28	3.4	3.4	3.3	3.3	3.5	3.2
	Glu41	7.1	6.9	6.9	7.0	-	5.0
Glu29 (H^N)	Glu41	6.6	6.6	6.6	6.8	-	5.0
Glu29 (N)	Glu41	6.6	5.8	5.2	6.4	-	4.9
	Asp28	3.4	3.1	3.3	3.5	3.1	2.9
Glu33 (N)	Glu33	4.3	4.2	4.1	4.1	4.3	3.6
Glu41 (H^N)	Glu41	6.9	6.8	6.7	6.8	-	5.1

	Glu29	4.9	4.9	4.6	4.8	4.6	-
Lys43 (N)	Glu41	6.7	6.6	6.8	6.7	-	5.1
	Glu29	4.9	4.8	4.7	4.5	4.4	-
Ser52 (N)	Glu41	6.8	6.7	6.7	6.7	-	-
Glu56 (H^N)	Glu56	4.0	4.0	4.0	3.9	3.7	3.8
Asn60 (H^N)	Asp62	4.4	4.3	4.0	4.2	4.1	4.0
Asp62 (H^N)	Asp62	4.2	4.2	4.0	4.1	4.0	4.0
Thr69 (H^N)	Asp72	4.3	4.3	4.3	4.1	4.4	3.6
Arg70 (N)	Asp71	3.1	3.3	2.9	3.4	3.0	3.1
Asp71 (H^N)	Asp72	4.5	4.5	4.5	4.3	4.2	4.2
Asp72 (N)	Asp72	4.5	4.5	4.6	4.2	4.2	4.1
Thr79 (N)	Glu41	6.7	6.6	6.6	6.7	-	-
Asp94 (N)	Asp94	4.1	4.0	4.0	4.0	3.8	3.7
Glu99 (N)	Glu99	4.6	4.9	5.0	4.7	4.4	4.4
	α-COOH	3.3	3.5	3.3	3.4	3.1	3.1

Notes

[a] Residue identification and nucleus (in brackets) giving rise to the resonance for which the chemical shift variation was monitored as a function of pH. Chemical shift pH profiles were fit to the Henderson-Hasselbalch equation as described in the text.

[b] The ionisable groups identified to be responsible for the pK_a values derived from fitting. If more than one pK_a is apparent in the chemical shift pH profile, only those

values which give rise to changes in chemical shift $\Delta\delta > 0.05$ ppm for H^N protons and $\Delta\delta > 0.5$ ppm for ^{15}N chemical shifts are listed.

[c] pK_a values were determined for wild-type CD2d1 at varying salt and buffer conditions as indicated, and separately for the E41Q and E29Q CD2d1 mutants at low ionic strength. All titrations were performed in 20 mM phosphate except for the experiment for wild-type CD2d1 in 10 mM Bis-tris buffer.

[d] pK_a value not determined due to increased amide proton exchange at higher salt concentration.

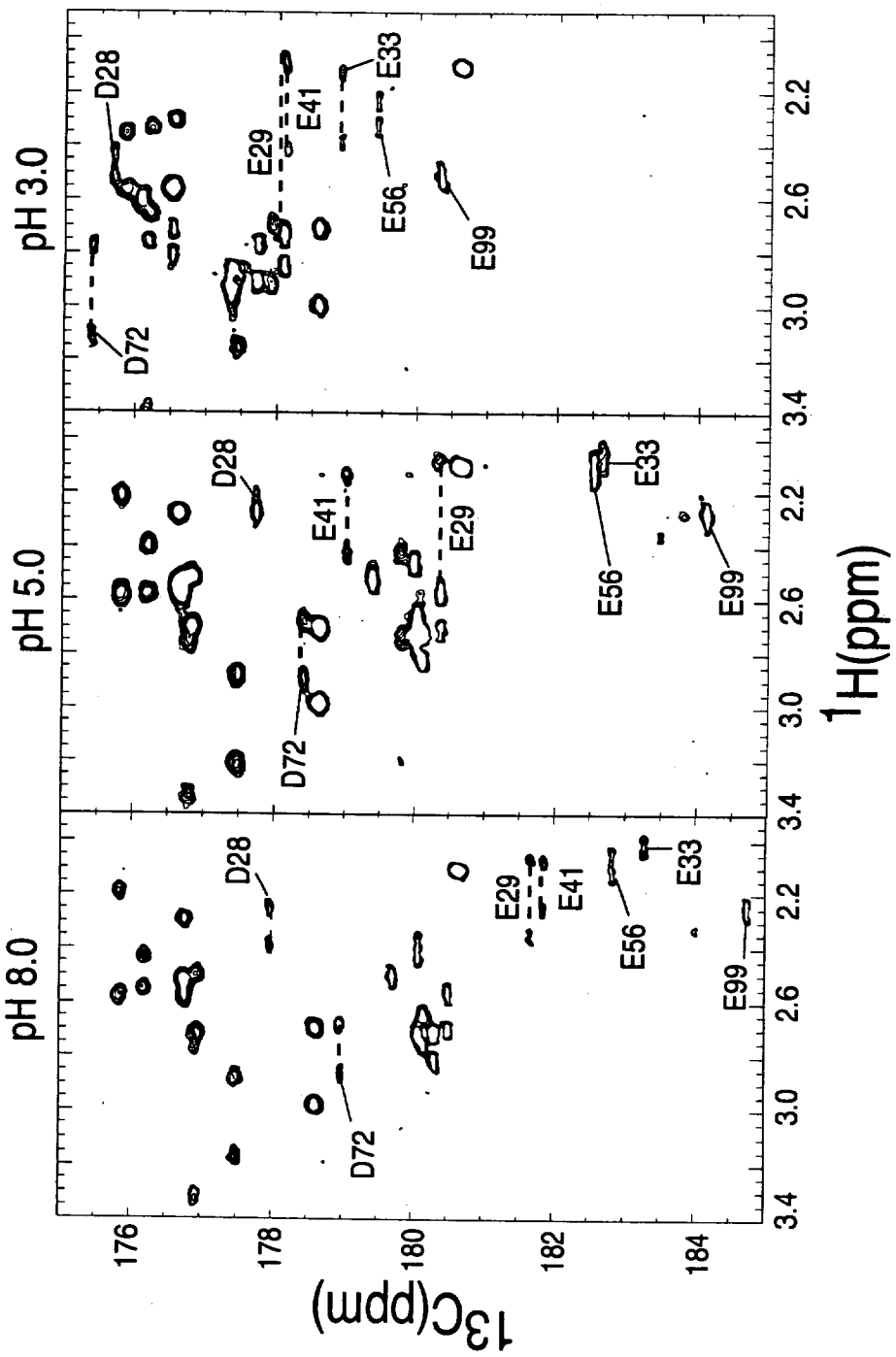

Figure S1

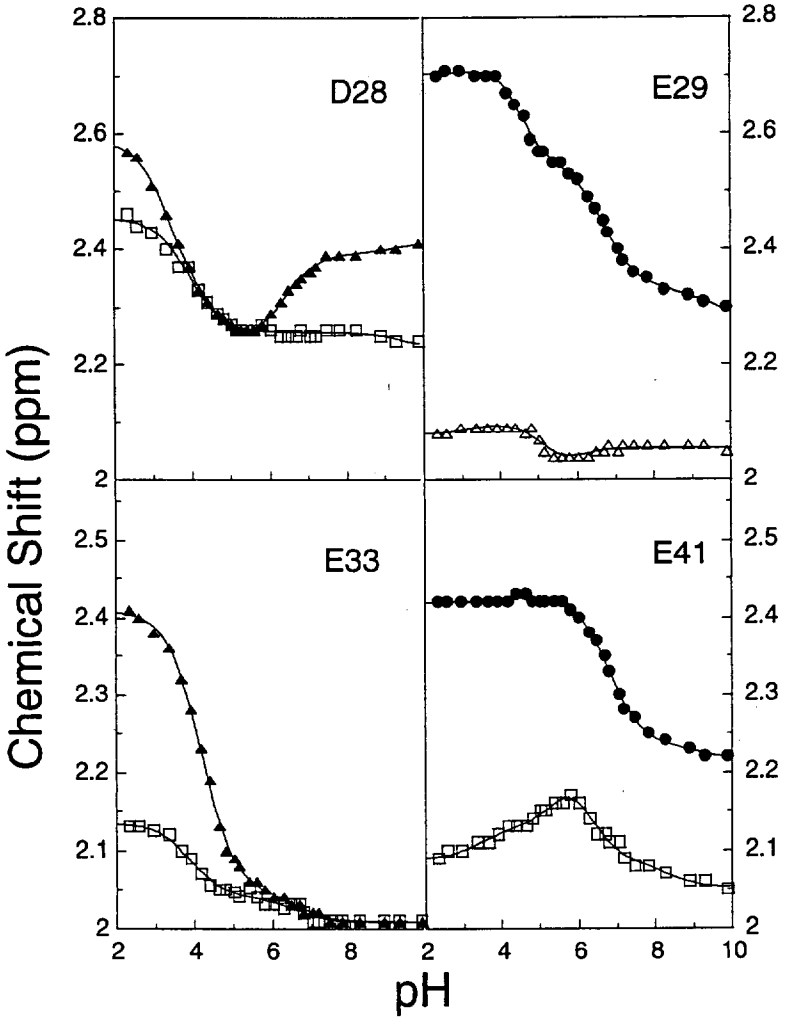

Figure S2