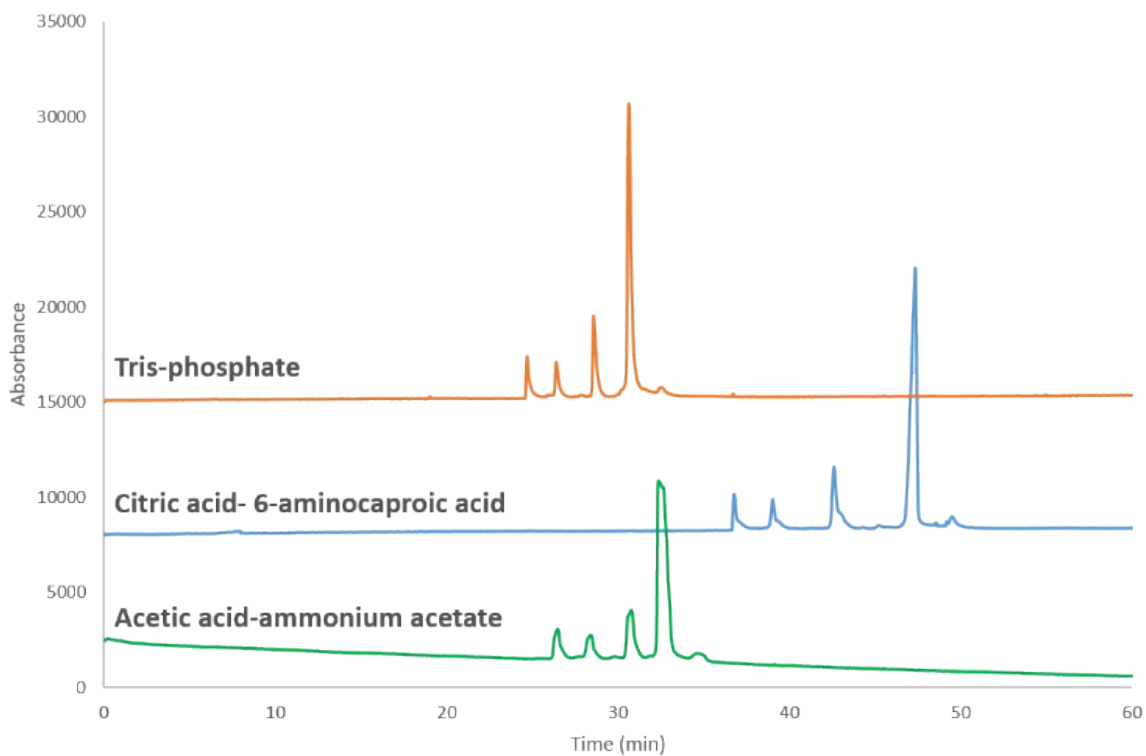


## Supplementary information

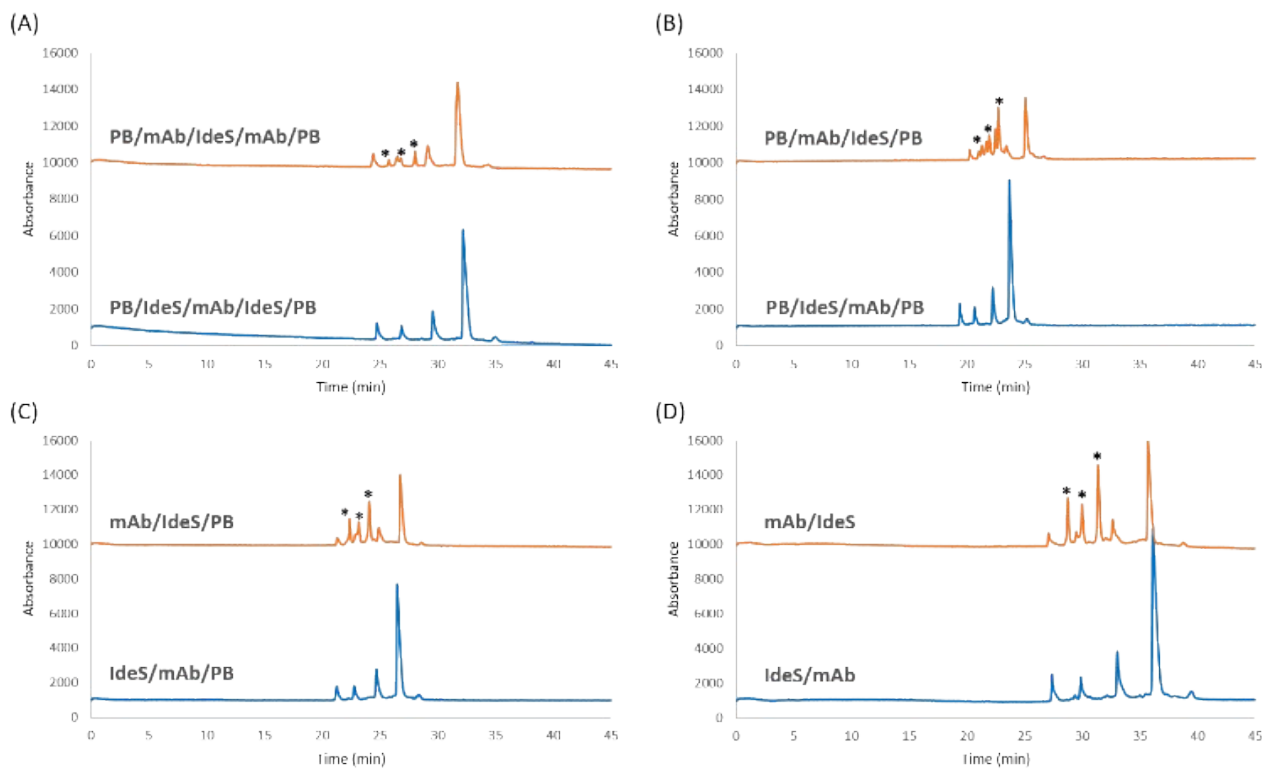
### **In-line enzymatic microreactor for the middle-up analysis of monoclonal antibodies by capillary electrophoresis**

Meriem Dadouch<sup>a</sup>, Yoann Ladner<sup>a</sup>, Claudia Bich<sup>a</sup>, Marion Larroque<sup>b</sup>, Christian Larroque<sup>b</sup>, Jacques Morel<sup>c</sup>, Pierre-Antoine Bonnet<sup>a</sup>, Catherine Perrin<sup>a\*</sup>

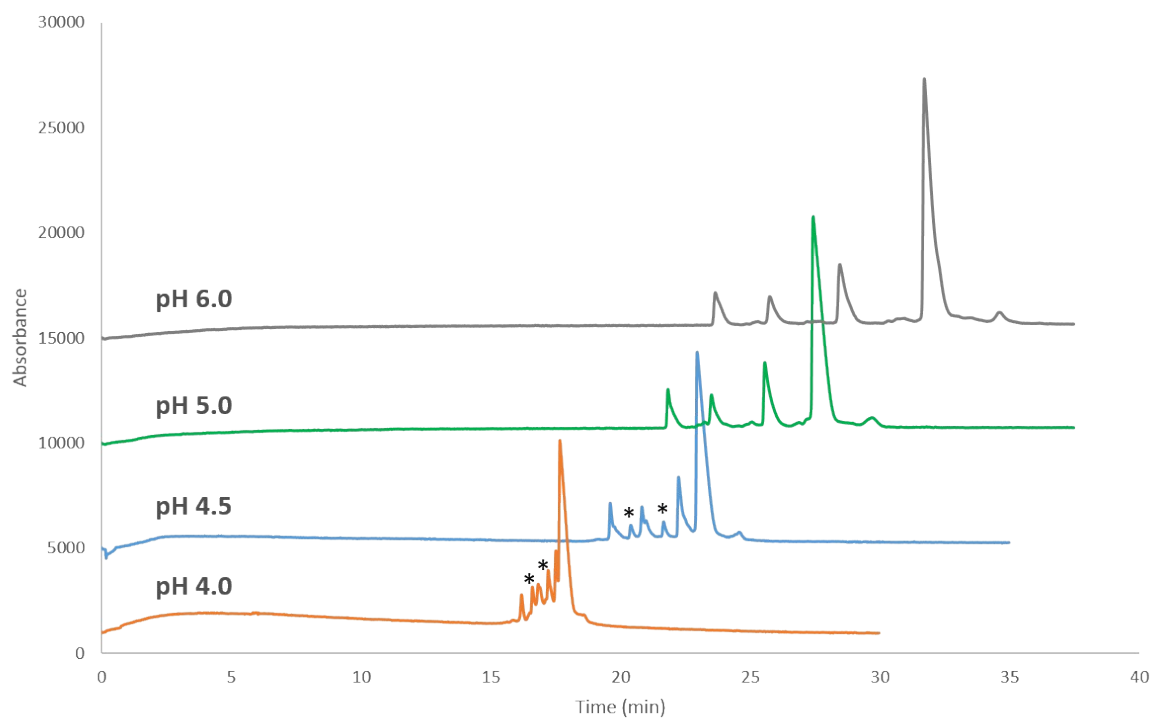
**Figure S1** Effect of the nature of the BGE on the separation of infliximab's subunits. Experimental conditions: PEO coated capillary (id = 30  $\mu\text{m}$ ,  $L_{\text{eff}}$  = 50 cm, L = 60 cm), off-line infliximab's digest (see section 2.4), injection: 5.13 nL, BGE: acetic acid-ammonium acetate, citric acid-6-aminocaproic acid and tris-phosphate IS = 50 mM pH 5.0, T = 35°C, Voltage = 15 kV.



**Figure S2** Effect of the injection scheme. Experimental conditions: PEO coated capillary ( $i_d = 30 \mu\text{m}$ ,  $L_{\text{eff}} = 50 \text{ cm}$ ,  $L = 60 \text{ cm}$ ), infliximab:  $1 \text{ g/L}$ , IdeS:  $0.2 \text{ g/L}$ , injection (see table 1), IdeS/mAb ratio:  $0.2/1 \text{ (w/w)}$ , BGE: acetic acid-ammonium acetate IS =  $50 \text{ mM}$  pH  $5.0$ ,  $T = 35^\circ\text{C}$ , Voltage =  $15 \text{ kV}$ , (\*) intact infliximab.



**Figure S3** Effect of BGE pH on the progress of in-line enzymatic reaction. Experimental conditions: PEO coated capillary (id = 30  $\mu\text{m}$ ,  $L_{\text{eff}}$  = 50 cm, L = 60 cm), injection scheme: IdeS/mAb, infliximab: 1g/L, IdeS: 0.2 g/L, IdeS/mAb ratio: 0.2/1 (w/w), BGE: acetic acid-ammonium acetate IS = 50 mM, T = 35°C, Voltage = 15 kV. (\*) Intact infliximab.



**Figure S4** Effect of IdeS/mAb ratio on in-line enzymatic reaction (A) Variation of the ratio by the variation of injection pressure of IdeS. (B) Variation of the ratio by the variation of injection time of IdeS. Experimental conditions: PEO coated capillary (id = 30  $\mu$ m,  $L_{eff}$  = 50 cm, L = 60 cm), injection scheme: IdeS/mAb, infliximab: 1g/L, IdeS: 0.2 g/L, BGE: acetic acid-ammonium acetate IS = 50 mM pH5.0, T = 37°C, Voltage = 15 kV.

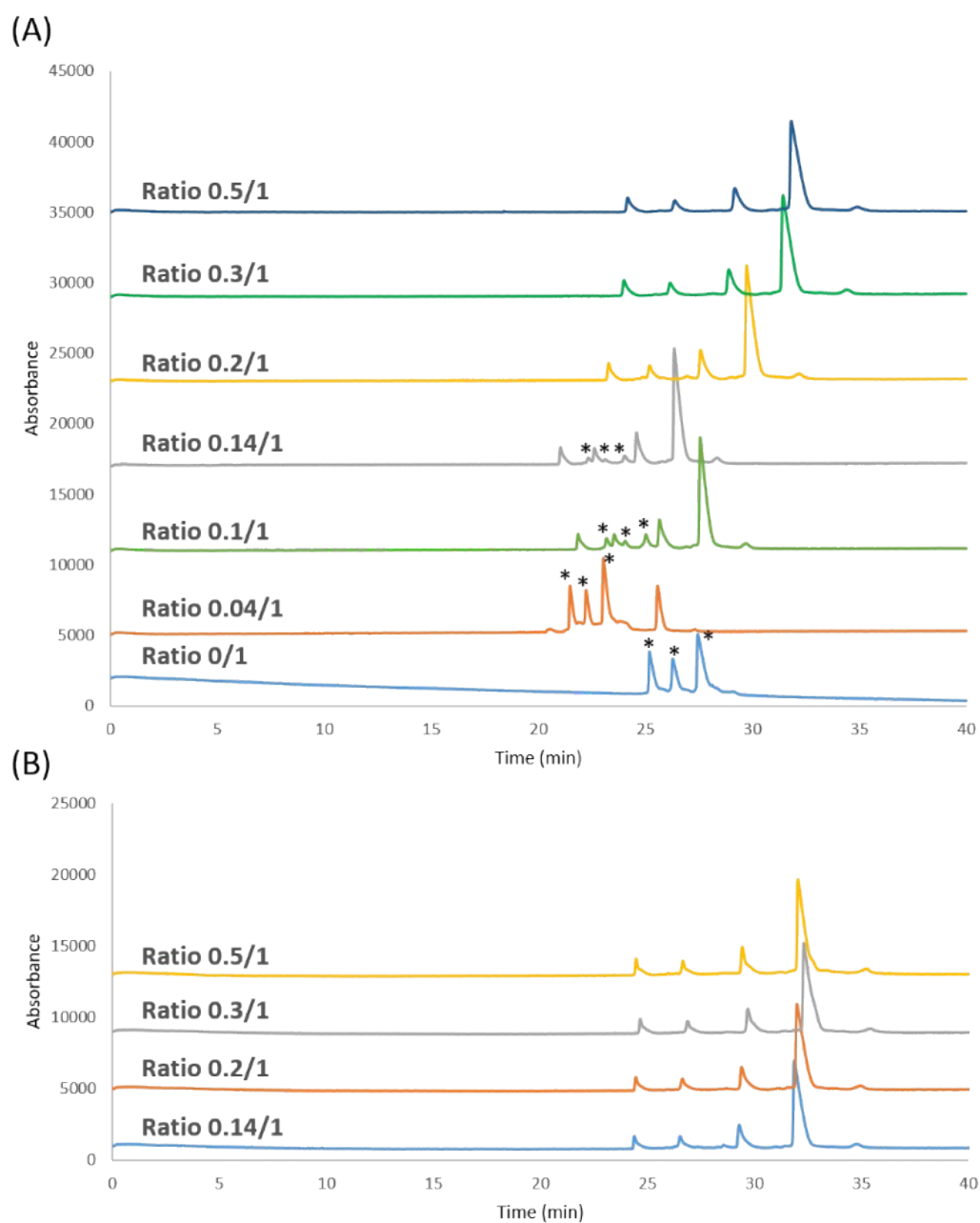
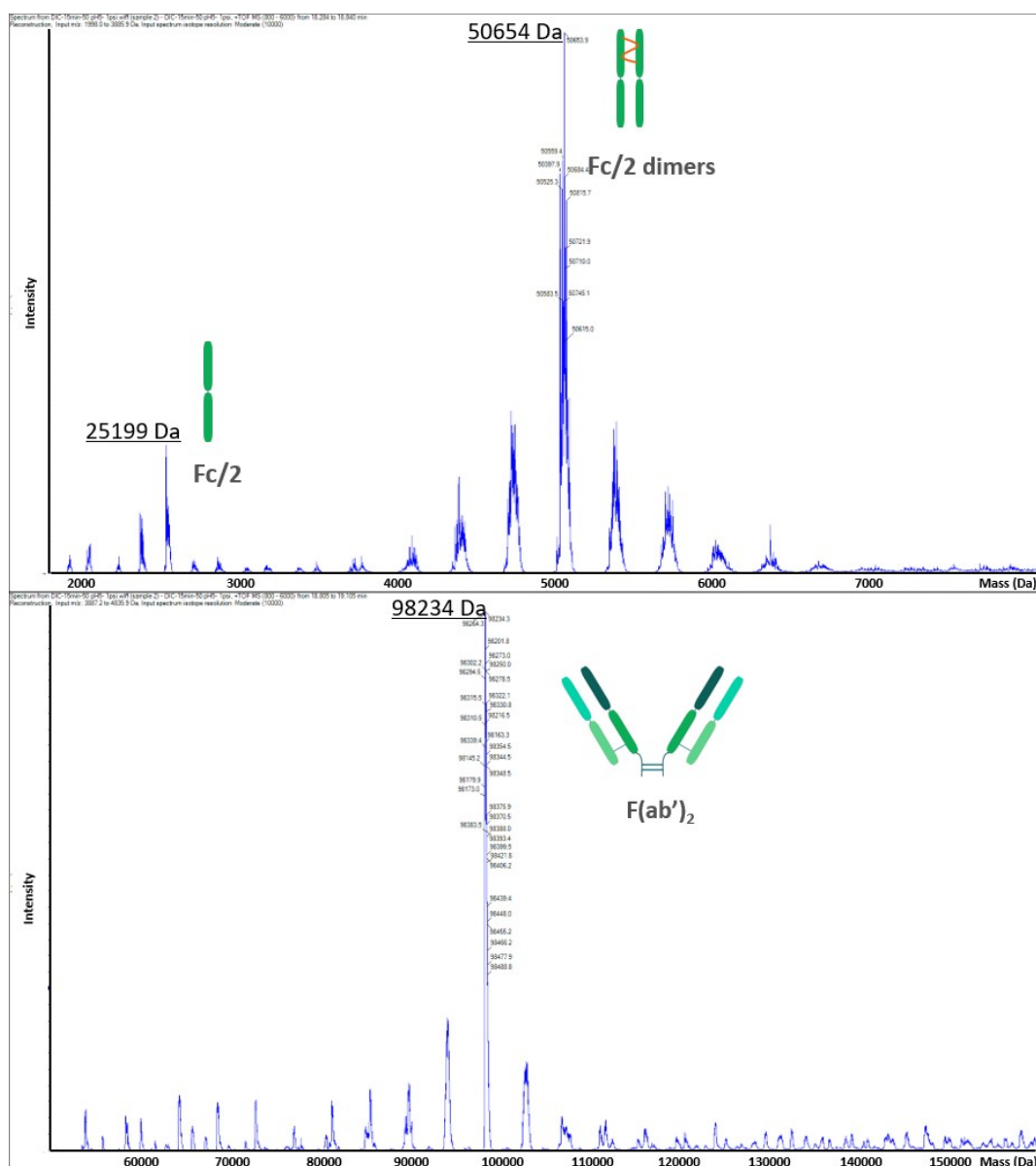


Figure S5 Deconvoluted MS spectra from 18.3 to 18.8 and from 18.8 to 19.1 min respectively



**Figure S6** (A) MS spectrum of undigested infliximab from 18.8 to 19.1 min, (B) deconvoluted MS spectrum of undigested infliximab ( $m/z$  from 4800 to 6000)