

# Supporting Information

## Enhancing selectivity of protein biopharmaceuticals in ion exchange chromatography through addition of organic modifiers

Bastiaan L. Duivelshof <sup>1,2,†</sup>, Thomas Bouvarel <sup>1,2,†</sup>, Sebastian Pirner <sup>3</sup>, Vincent Larraillet <sup>3</sup>, Alexander Knaupp <sup>3</sup>, Hans Koll <sup>3</sup>, Valentina D'Atri <sup>1,2,\*</sup> and Davy Guillarme <sup>1,2</sup>

<sup>1</sup> School of Pharmaceutical Sciences, University of Geneva, CMU - Rue Michel Servet 1, 1211 Geneva, Switzerland

<sup>2</sup> Institute of Pharmaceutical Sciences of Western Switzerland, University of Geneva, CMU - Rue Michel Servet 1, 1211 Geneva, Switzerland

<sup>3</sup> Roche Diagnostics GmbH, Nonnenwald 2, 82377 Penzberg, Germany

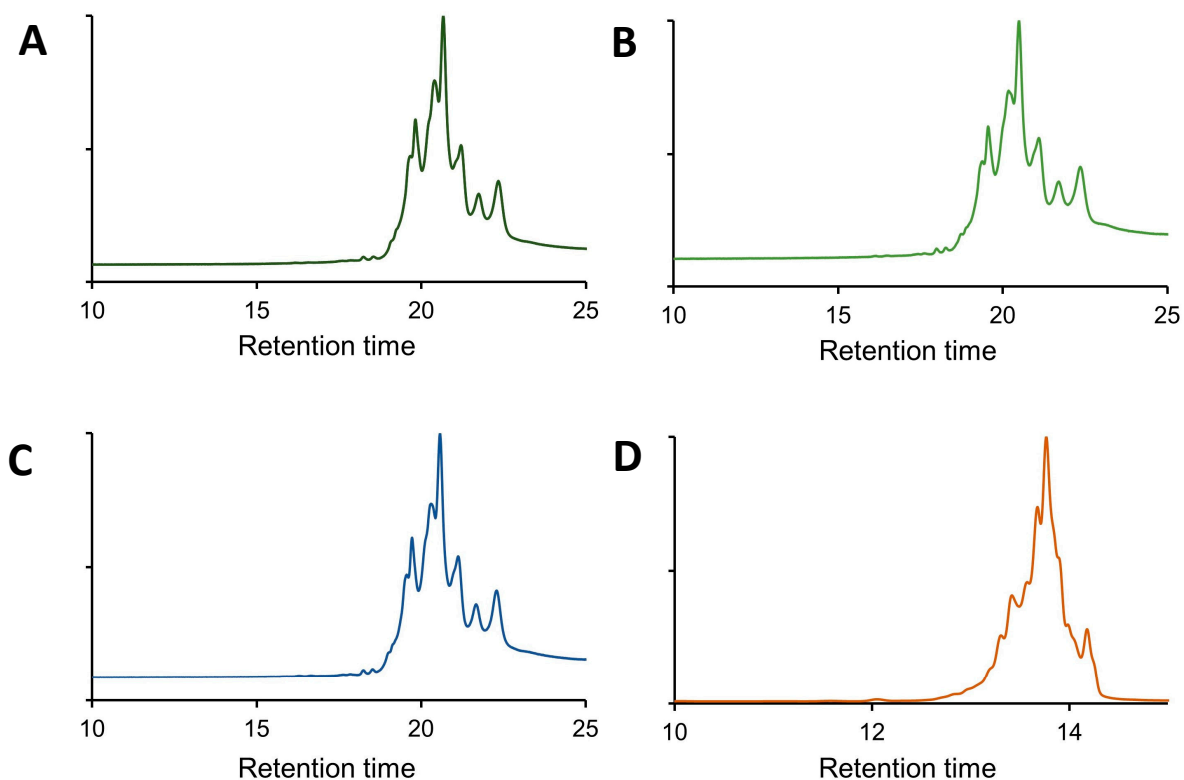
\* Correspondence: valentina.datri@unige.ch

† These authors contributed equally to this work.

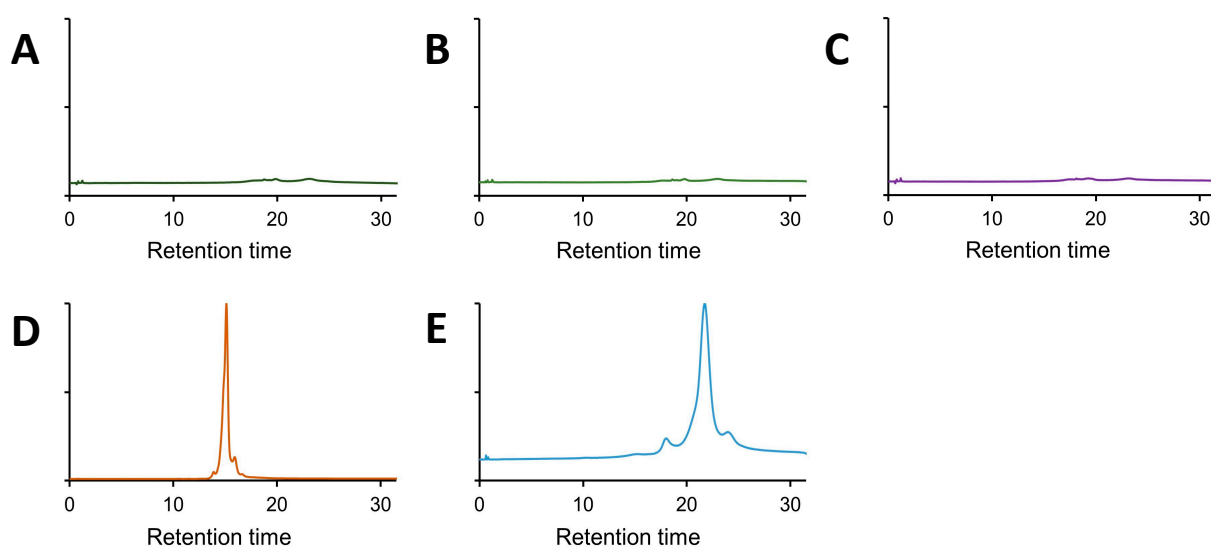
### Table of contents

<b>Figure S1</b>	Chromatograms of Infliximab: (A) 30% <i>v/v</i> IPA with 2 mM NH <sub>4</sub> F; (B) 30% <i>v/v</i> IPA with 20 mM TFE; (C) 30% <i>v/v</i> IPA with 50 mM HFIP; (D) 1% <i>v/v</i> DMSO with 50 mM HFIP.....	S1
<b>Figure S2</b>	Chromatograms of bsAb1: (A) 30% <i>v/v</i> IPA with 2 mM NH <sub>4</sub> F; (B) 30% <i>v/v</i> IPA with 20 mM TFE; (C) 30% <i>v/v</i> IPA with 50 mM HFIP; (D) 1% <i>v/v</i> DMSO with 50 mM HFIP; (E) 20% <i>v/v</i> IPA.....	S1
<b>Figure S3</b>	Chromatograms of bsAb2: (A) 30% <i>v/v</i> IPA with 2 mM NH <sub>4</sub> F; (B) 30% <i>v/v</i> IPA with 20 mM TFE; (C) 30% <i>v/v</i> IPA with 50 mM HFIP; (D) 1% <i>v/v</i> DMSO with 50 mM HFIP.....	S2
<b>Figure S4</b>	Chromatograms of bsAb3: (A) 30% <i>v/v</i> IPA with 2 mM NH <sub>4</sub> F; (B) 30% <i>v/v</i> IPA with 20 mM TFE; (C) 1% <i>v/v</i> DMSO alone; (D) 30% <i>v/v</i> IPA with 50 mM HFIP; (E) 1% <i>v/v</i> DMSO with 50 mM HFIP.....	S2
<b>Figure S5</b>	Evaluation of pH response with the MabPac 5 µm 2.1 x 50 mm column (green), the SEPAX SCX-NP5 Proteomix 5 µm 4.6 x 100 mm column (orange), the BioPro IEX SF 5 µm 4.6 x 100 mm column (blue), the BioResolve SCX 3 µm 4.6 x 50 mm column (red), the ProPac Elite WCX 5 µm 4 x 150 mm column (grey), and without column (yellow). pH was measured on-line on the H-Class Bio system equipped with an on-line pH meter when running a generic gradient from 0 %B to 100 %B in 30 minutes.....	S3

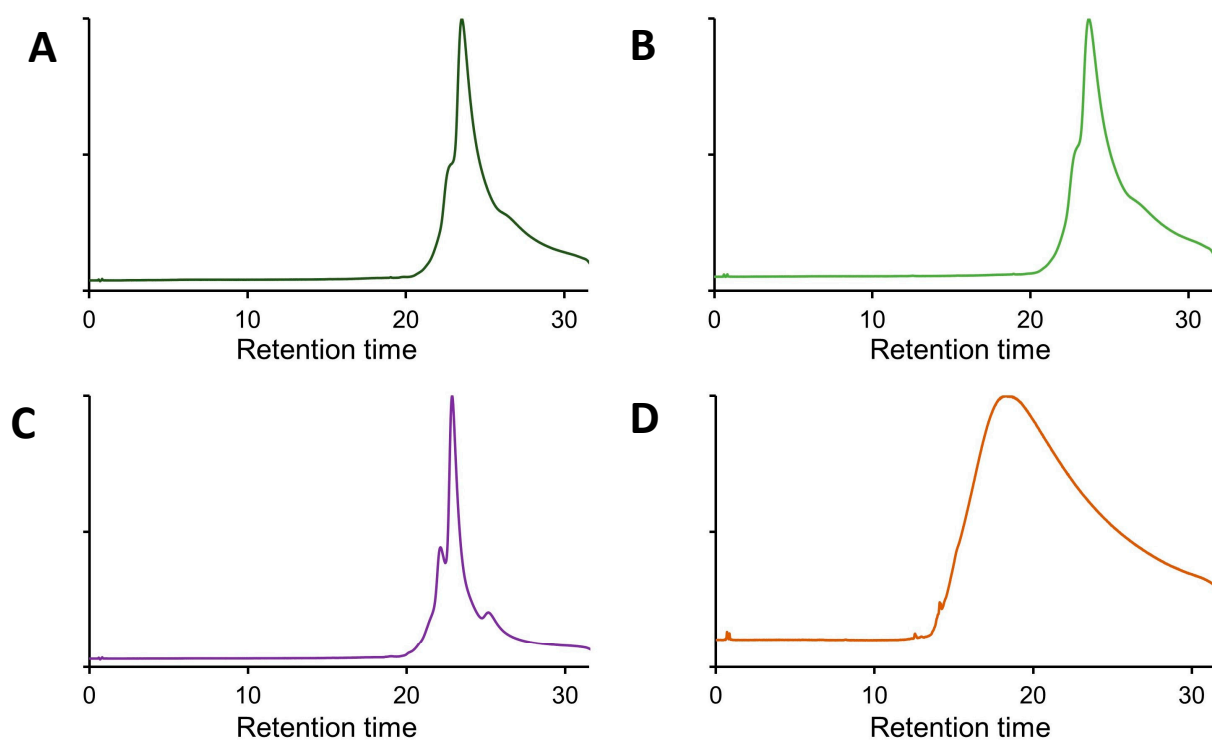
**Figure S1:** Chromatograms of Infliximab: (A) 30% v/v IPA with 2 mM  $\text{NH}_4\text{F}$ ; (B) 30% v/v IPA with 20 mM TFE; (C) 30% v/v IPA with 50 mM HFIP; (D) 1% v/v DMSO with 50 mM HFIP.



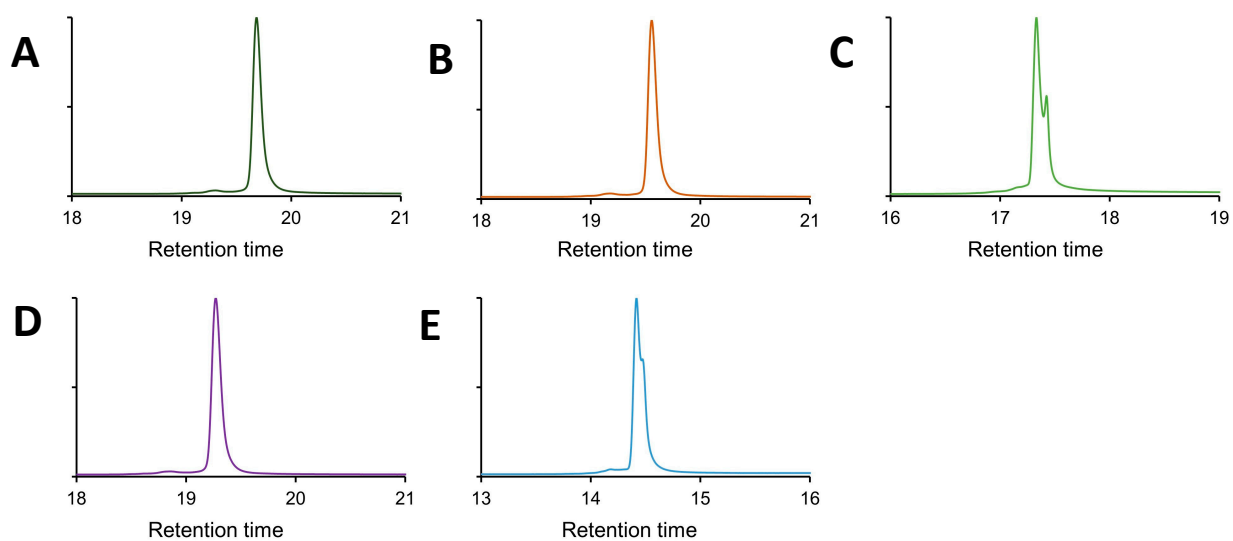
**Figure S2:** Chromatograms of bsAb1: (A) 30% v/v IPA with 2 mM  $\text{NH}_4\text{F}$ ; (B) 30% v/v IPA with 20 mM TFE; (C) 30% v/v IPA with 50 mM HFIP; (D) 1% v/v DMSO with 50 mM HFIP; (E) 20% v/v IPA.



**Figure S3:** Chromatograms of bsAb2: (A) 30% v/v IPA with 2 mM NH<sub>4</sub>F; (B) 30% v/v IPA with 20 mM TFE; (C) 30% v/v IPA with 50 mM HFIP; (D) 1% v/v DMSO with 50 mM HFIP.



**Figure S4:** Chromatograms of bsAb3: (A) 30% v/v IPA with 2 mM NH<sub>4</sub>F; (B) 30% v/v IPA with 20 mM TFE; (C) 1% v/v DMSO alone; (D) 30% v/v IPA with 50 mM HFIP; (E) 1% v/v DMSO with 50 mM HFIP.



**Figure S5:** Evaluation of pH response with the MabPac 5  $\mu$ m 2.1 x 50 mm column (green), the SEPAX SCX-NP5 Proteomix 5  $\mu$ m 4.6 x 100 mm column (orange), the BioPro IEX SF 5  $\mu$ m 4.6 x 100 mm column (blue), the BioResolve SCX 3  $\mu$ m 4.6 x 50 mm column (red), the ProPac Elite WCX 5  $\mu$ m 4 x 150 mm column (grey), and without column (yellow). pH was measured on-line on the H-Class Bio system equipped with an on-line pH meter when running a generic gradient from 0 to 100 %B in 30 minutes.

