Supporting Information

Separation of Intact Proteins by Capillary Electrophoresis

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1. Molecular masses (MWs) and isoelectric points (pIs) of all analytes.

Table SI 1A. Molecular weights (MWs) and isoelectric points (pIs) for analytes used in the systematic evaluation.

	Molecular Weight [kDa]	Isoelectric point
β-Lactoglobulin (β-Lg)	18.3[1]	5.1[1]
Myoglobin (Mb)	17.6[2] (incl. heme group)	6.8-7.4[3]
Ribonuclease A (RNase)	13.7[4]	9.6[4]
Human Serum Albumin (HSA)	66.4[5]	4.7[5]
Transferrin (Tf)	79.5[9]	5.4[10]
Creatine Kinase (CK)	81.0[6]	7.2[6]
Sheep IgG Antibody (IgG)	150.0[7]	5.0-7.2[8]
Histidine (His)	0.155	7.6[11]

Table SI 1B. Molecular weights (MWs) and isoelectric points (pIs) for proteins identified in milk.

	Molecular Weight [kDa]	Isoelectric point
α-Lactalbumin A (α-Lac A)	14.1[12]	4.4[13]
β-Lactoglobulin A (β-Lg A)	18.4[12]	5.1[13]
β-Lactoglobulin B (β-Lg B)	18.3[12]	5.2[13]
α-Casein (α-CN)	22.1 - 23.7[12]	4.6[14]
A1 β-Casein (A1 β-CN)	24.0[12]	4.6[14]
A2 β-Casein (A2 β-CN)	24.0[12]	4.8 – 5.1[12]
Bovine Serum Albumin (BSA)	66.3[12]	4.7 – 4.9[12]

2. Coating time for CTAB-PDMS and SDS-PDMS capillary.

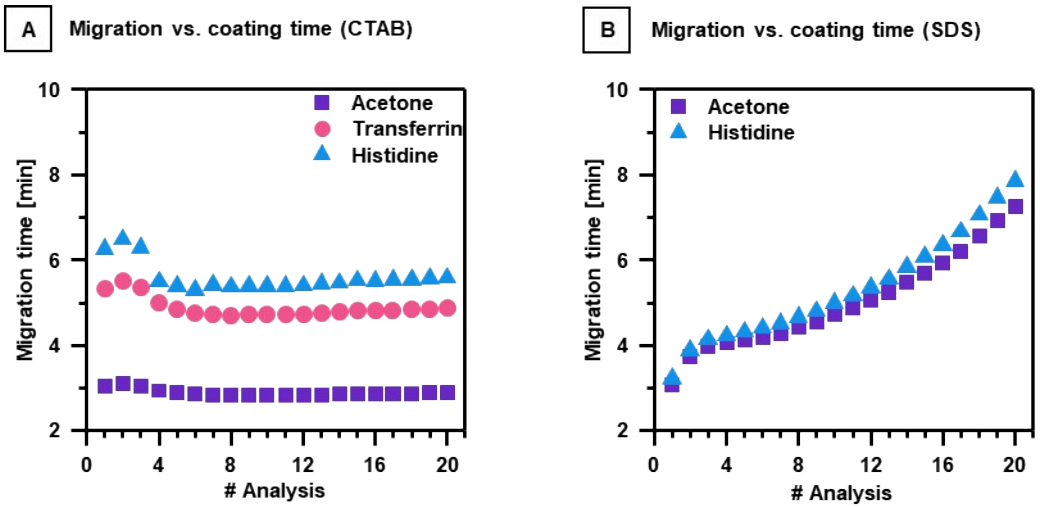

Figure SI 1: Influence of increased surfactant coating time on the migration time of the analytes. 0.1 mM CTAB was added to BGE I (**A**) and 0.01 mM SDS was added to BGE V (**B**). The capillary was flushed for 10 minutes followed by injection of an acetone, His and Tf standard. In scenario A, a peak for each analyte was observed and the migration time stabilized after 5 analyses. Scenario B only led to the detection of acetone and His, while Tf remained absent due to hydrophobic interactions with the capillary surface. Repeated coating and injection caused longer migration times for acetone and His, due to increasing Tf adsorption on the PDMS capillary. This issue may be prevented with a longer coating time before sample injection.

3. Longevity of the surfactant layer.

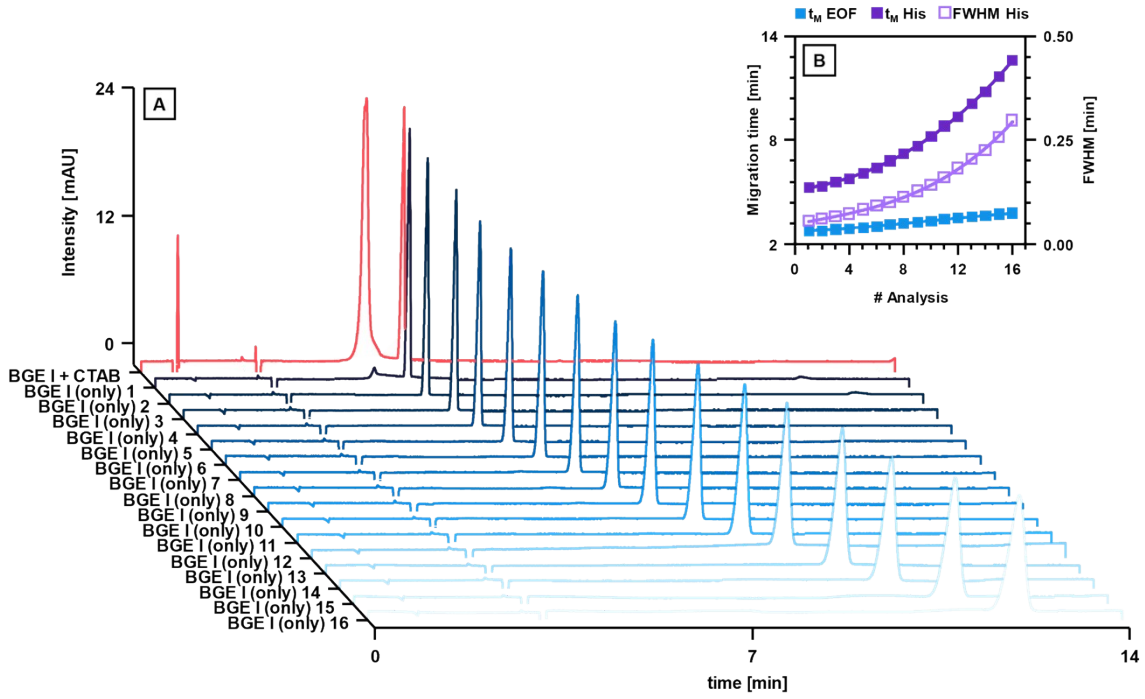

Figure SI 2: Longevity of the surfactant layer (**A**) and effect on the migration times and peak width (**B**). Successive analysis of the three analytes acetone, Tf and His after removal of CTAB from the BGE. The three analytes were observed as sharp peaks when CTAB was added to the BGE (red line). The subsequent analyses without CTAB resulted in protein absorption to the capillary surface and increased migration times (t_M) and larger peak width (FWHM) for acetone and His.

4. Electropherograms and effective mobilities for all analytes using the three different capillaries and six different BGEs.

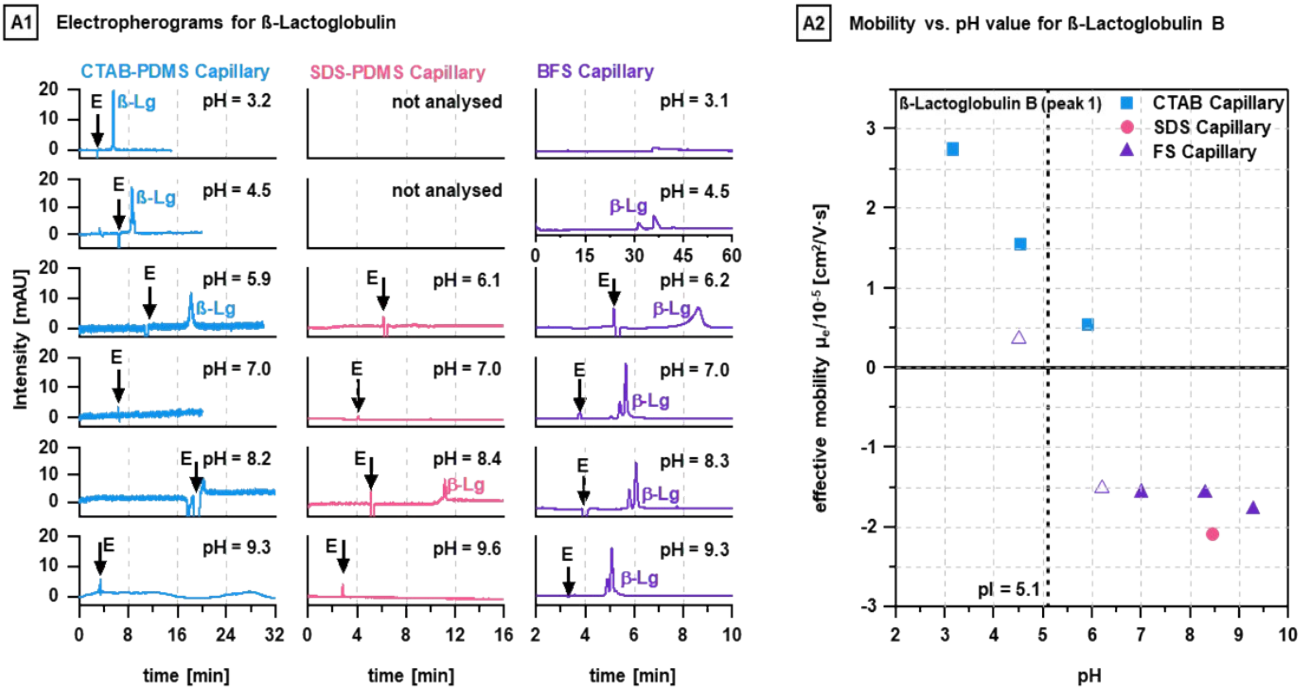

Figure SI 3A: Electropherograms (**A1**) and mobility vs. pH value plots (**A2**) for β-Lactoglobulin obtained with the CTAB-PDMS, SDS-PDMS and BFS capillary employing BGEs with pH values ranging from 3.0 to 9.6. The arrows in the electropherograms indicate the EOF (E) and system peaks are marked with an S.

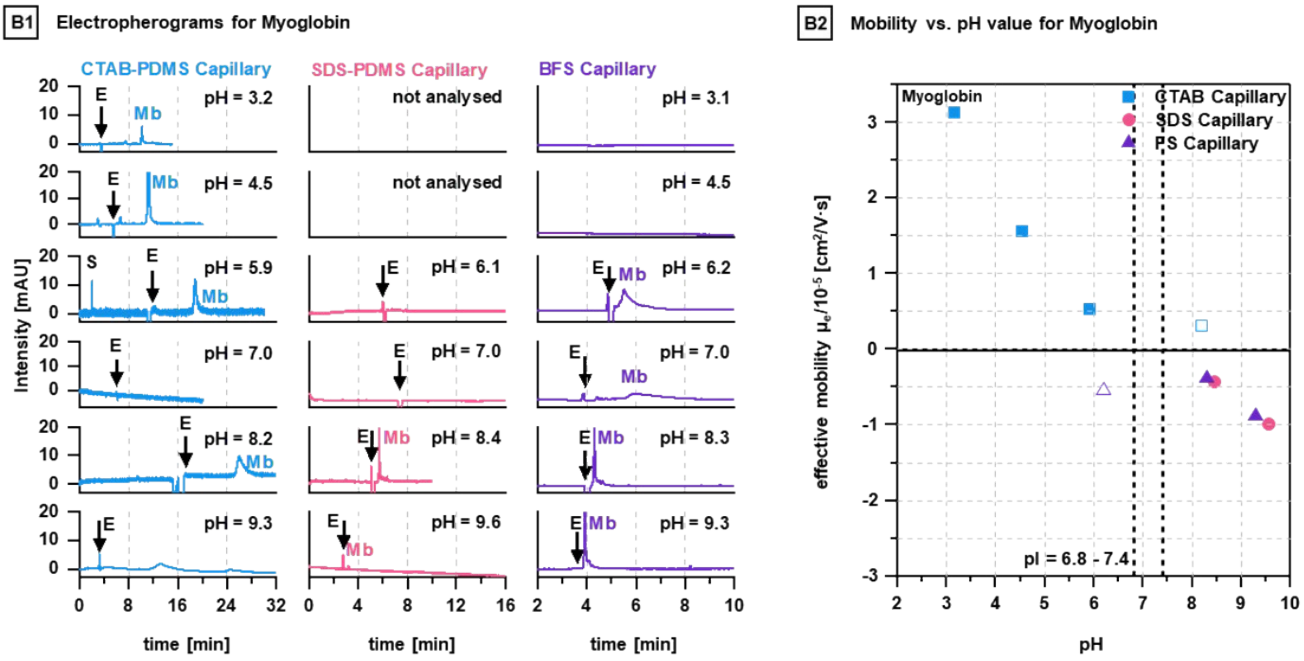

Figure SI 3B: Electropherograms (**B1**) and mobility vs. pH value plots (**B2**) for Myoglobin obtained with the CTAB-PDMS, SDS-PDMS and BFS capillary employing BGEs with pH values ranging from 3.0 to 9.6. The arrows in the electropherograms indicate the EOF (E) and system peaks are marked with an S.

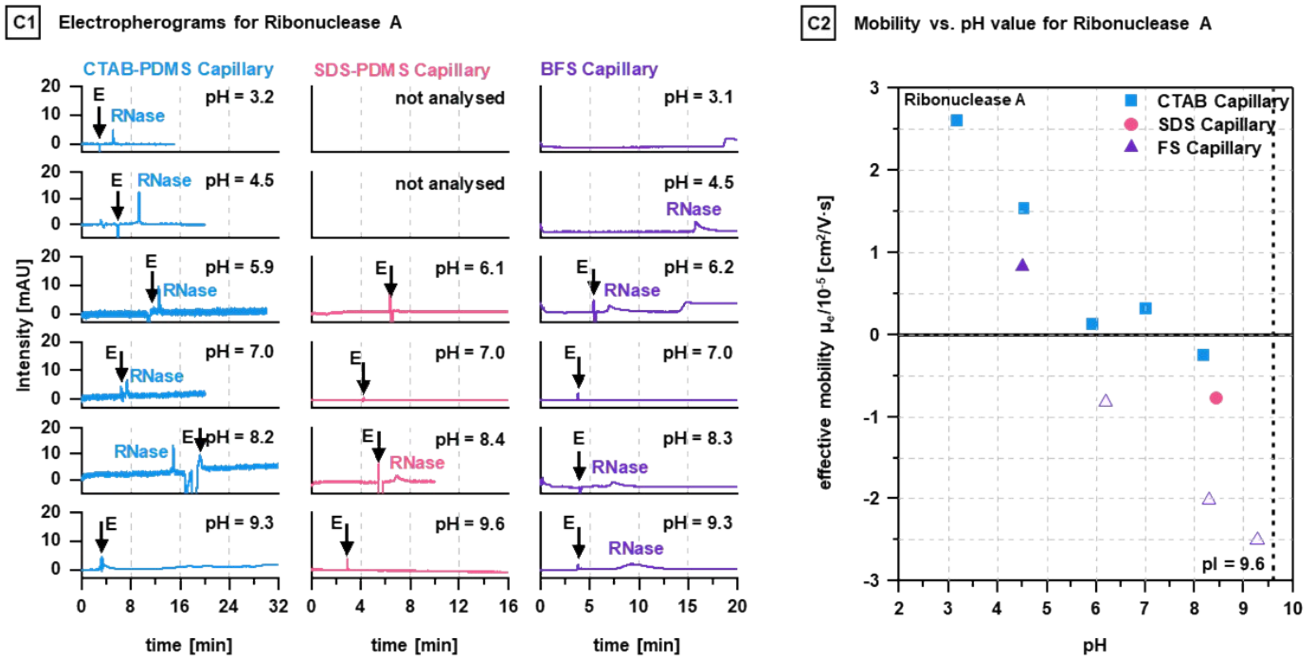

Figure S3C: Electropherograms (**C1**) and mobility vs. pH value plots (**C2**) for Ribonuclease A obtained with the CTAB-PDMS, SDS-PDMS and BFS capillary employing BGEs with pH values ranging from 3.0 to 9.6. The arrows in the electropherograms indicate the EOF (E) and system peaks are marked with an S.

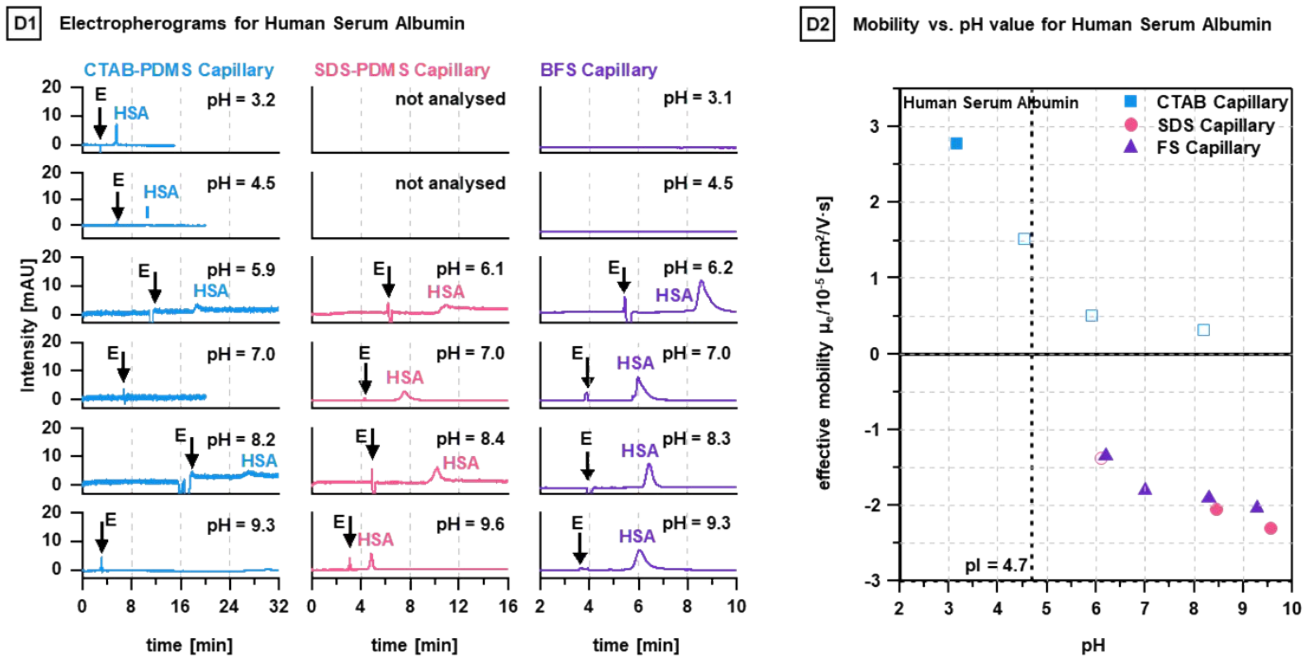

Figure SI 3D: Electropherograms (**D1**) and mobility vs. pH value plots (**D2**) for Human Serum Albumin obtained with the CTAB-PDMS, SDS-PDMS and BFS capillary employing BGEs with pH values ranging from 3.0 to 9.6. The arrows in the electropherograms indicate the EOF (E) and system peaks are marked with an S.

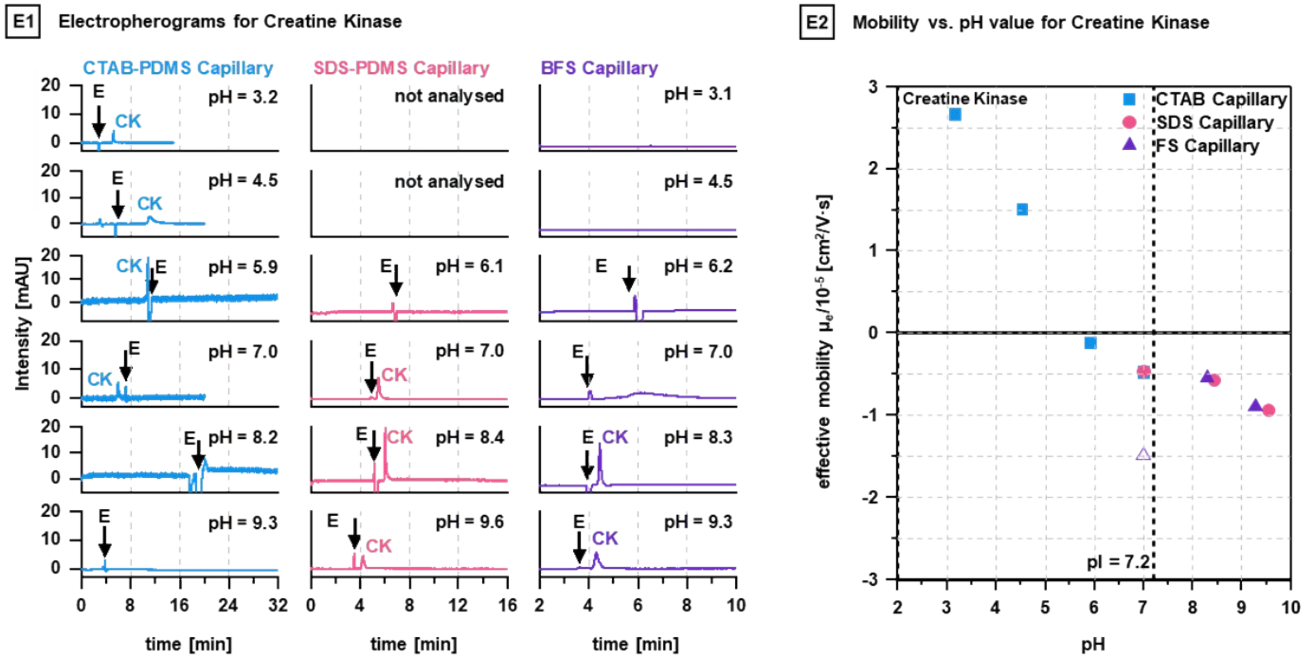

Figure SI 3E: Electropherograms (**E1**) and mobility vs. pH value plots (**E2**) for Creatine Kinase obtained with the CTAB-PDMS, SDS-PDMS and BFS capillary employing BGEs with pH values ranging from 3.0 to 9.6. The arrows in the electropherograms indicate the EOF (E) and system peaks are marked with an S.

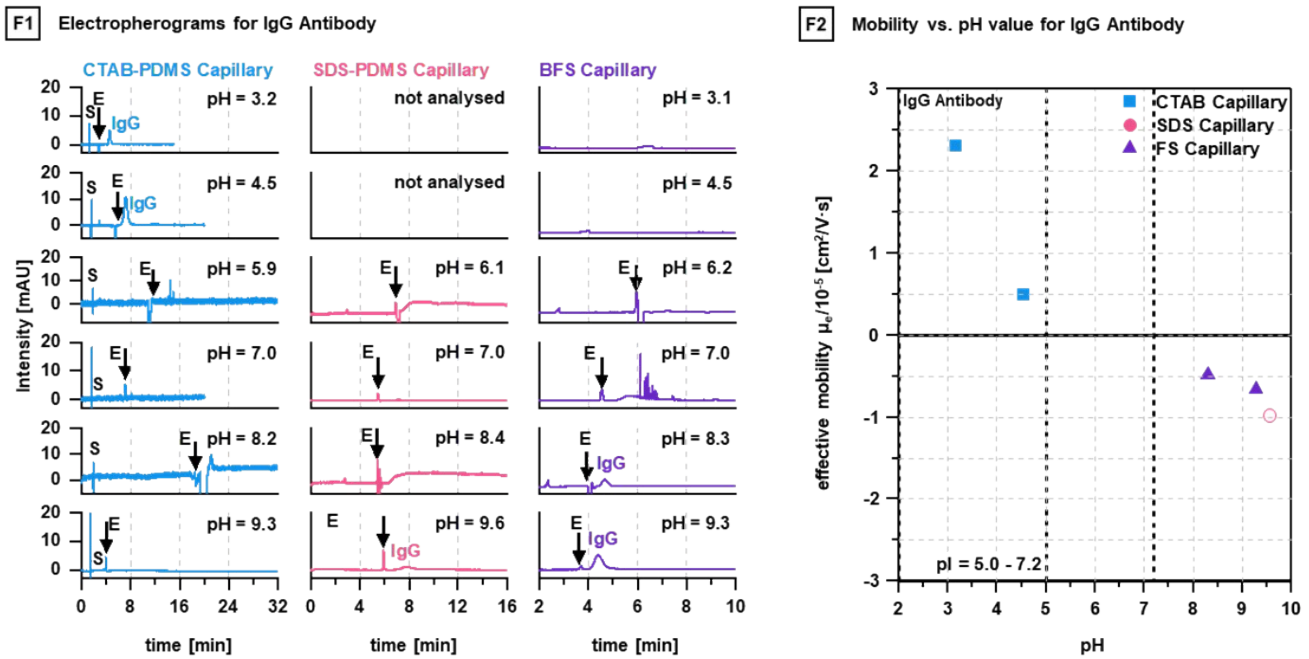

Figure SI 3F: Electropherograms (**F1**) and mobility vs. pH value plots (**F2**) for IgG Antibody obtained with the CTAB-PDMS, SDS-PDMS and BFS capillary employing BGEs with pH values ranging from 3.0 to 9.6. The arrows in the electropherograms indicate the EOF (E) and system peaks are marked with an S.

5. Protein identification in milk sample.

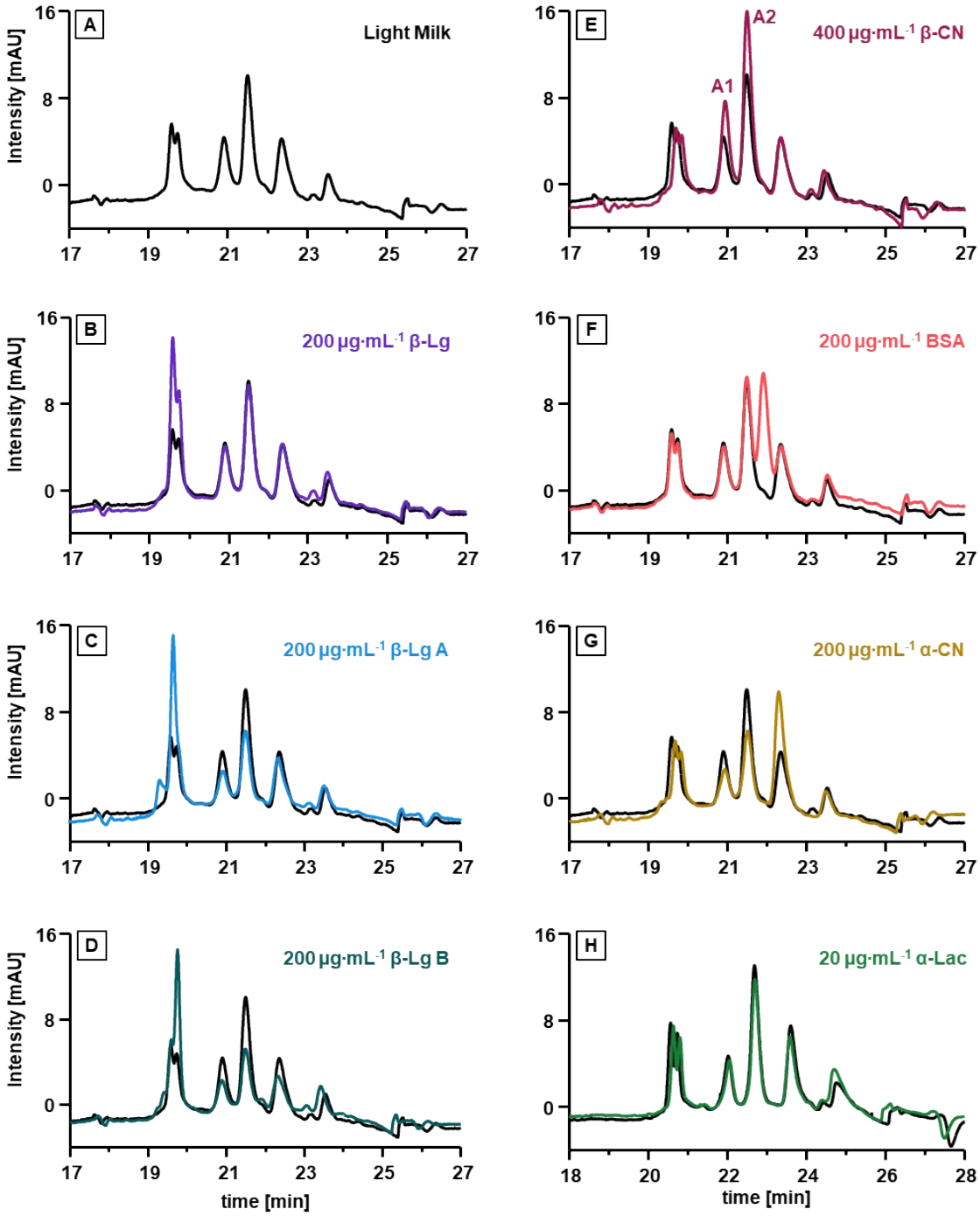

Figure SI 4: Protein identification in cow's milk. The milk samples were spiked with the individual protein standards **(A-H)**.

6. References

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