Supporting Information

Component-resolved diagnostic of cow's milk allergy by immunoaffinity capillary electrophoresis – matrix assisted laser desorption/ionization mass spectrometry

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SI-1: Chemicals and Materials

Estapor tosyl-activated superparamagnetic beads (MBs) of uniform size (1.29 μm diameter) were kindly offered by Merck Chimie (France). Blood serum from the patient with cow's milk allergy was purchased from Bioreclamation LLC (NY, USA). Control blood serum from the patient with no allergies was kindly offered by the Regional Blood Transfusion Service of canton Vaud, Switzerland. Human IgE control antibodies (Abs) and dimethyl suberimidate (DMS) were obtained from Pierce Biotechnology (IL, USA). Monoclonal anti-human IgE Abs (0100-0414) were purchased from AbD Serotec (Oxford, UK).

Bovine milk proteins like β-lactoglobulin (A+B), α-lactalbumin type I, serum albumin, lactoferrin, α-casein, β-casein and κ-casein were purchased from Sigma-Aldrich (Buchs, Switzerland). Hydroxypropylcellulose (HPC) was purchased from Acros (Basel, Switzerland). Acetic acid (99.5%), trifluoroacetic acid (TFA), sinapinic acid (≥ 99%), triethanolamine (≥ 99%) were purchased from Fluka (Buchs, Switzerland) and sodium borate, ammonium acetate from Merck (Darmstadt, Germany). Urea and Polyoxyethylene-sorbitan monolaurate (Tween 20) were purchased from Sigma-Aldrich (Buchs, Switzerland). All the buffers and sample solutions were prepared with water produced by an alpha Q-Millipore System (Zug, Switzerland). Commercial bovine skimmed milk powder (35 g protein/100 g) and ultra-high-temperature (UHT) treated liquid milk were purchased in a local supermarket.

The IACE experiments were carried out using fused silica capillaries (50 μm i.d., 375 μm o.d.) obtained from BGB analytik AG (Böckten, Switzerland). For MBs capturing two permanent cylindrical magnets (Nd-Fe-B, 4 mm diameter, 12 mm length) were purchased from Supermagnete, Zürich, Switzerland.

SI-2: IACE coupling with MALDI MS

One extremity of the HPC coated separation capillary (50 µm i.d., 41.5 cm effective length, 50 cm total length) was covered with silver ink (Ercon, Wareham, USA) over a length of about 10 cm (from the outlet) and cured at 80ºC during 60 min in the oven for the ink fixation. Then, the capillary was placed in a special CE-MS cassette and installed into the CE instrument with its painted extremity left outside the CE apparatus. This part of the capillary was fixed in a ceramic holder, which was an integrated part of the homemade robotic system. This system was capable to move the capillary along y- and z-axes above the MALDI plate (Anchor Chip MALDI target, Bruker, Bremen, Germany) positioned on the stage moving along x-axe. Capillary and MALDI plate stage movements, as well as the timescale of CE fractions delivery, were controlled via a Labview program (National Instruments, Austin, USA). For the performance of the CE separation the painted capillary extremity, served as a second electrode, was grounded and dipped into a droplet (4 µl) of separation buffer which was prespotted directly on the MALDI plate at a certain position. Delivery of the CE separated species in one position was followed by the capillary quick lifting and moving to the next spotting position. These movements were fast enough to avoid the interruption of the separation current. At the end of the separation all spotted CE fractions were dried at room temperature and 2 µl of matrix solution (2 mg/ml sinapinic acid in 70% acetonitrile, 29.9% water, 0.1% TFA) were deposited above each fraction spot and were allowed to dry. The MALDI-TOF-MS Microflex instrument (Bruker Daltonics, Bremen, Germany) was operated in a positive linear ion mode. The average spectra from 500 laser shots at different spot locations were collected for every CE fraction spot.

SI-3: Elimination of nonspecific adsorption

As mentioned in the main text of the article, to minimize the nonspecific adsorption in the system, a mixture of 10 mM PBS with Tween 20 (pH=7.4) was used as sample and washing buffers. The addition of detergent decreased the negative effect of nonspecific interactions, especially if the content of Tween 20 was the same in both sample and washing buffers, 0.1 or 0.05 % (Figure SI-3.a, blue and pink lines). The latter value of the detergent concentration showed the most effective suppression of nonspecific adsorption. Addition of the washing step with deionized water reduced protein adhesion on MBs and on the capillary walls even more (Figure SI-3.a, black line).

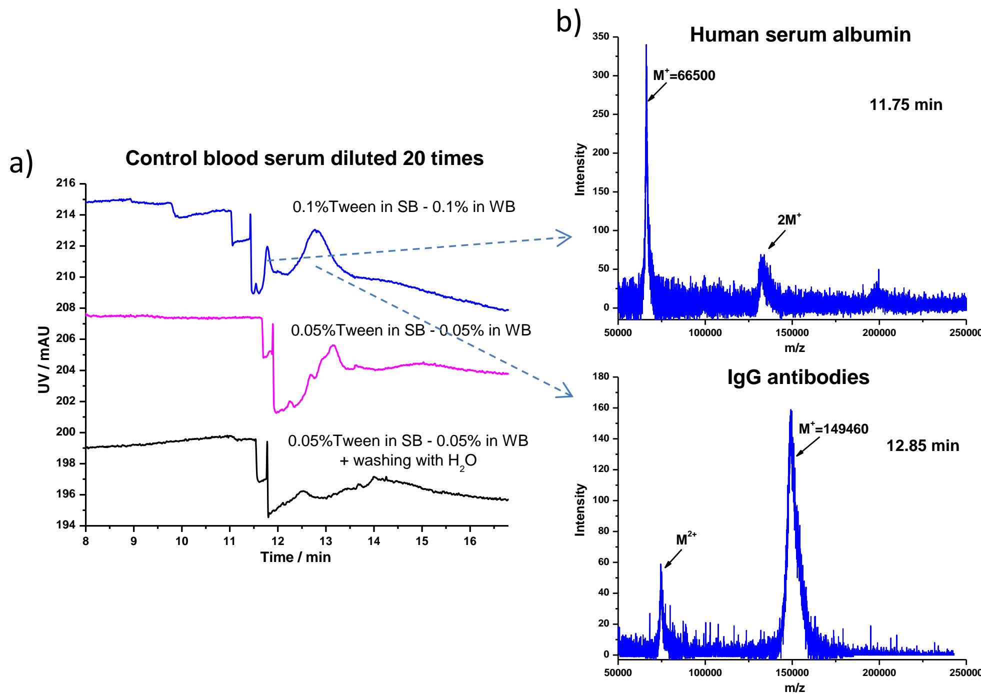

Figure SI-3. Nonspecific adsorption elimination on the example of control blood serum. a) Effect of additional washing step: 5 min washing with PBS/0.1% Tween 20 (blue line); 5 min washing with PBS/0.05% Tween 20 (pink line); 5 min washing with PBS/0.05% Tween 20 followed by 5 min washing with deionized water (black line). b) MALDI MS spectra collected during IACE-MALDI MS analysis of control blood serum using only 5 min washing step with PBS/0.1% Tween 20: human serum albumin eluted at 11.75 min and IgG antibodies eluted at 12.85 min of CE separation. Conditions: typical IACE protocol with 5 min of MBs (0.5 mg/ml) loading, 10 min sample injection, system washing, sample elution and application of 24 kV during 20 min for the CE separation; CE-UV electropherograms at 200 nm, HPC coated capillary, total/effective length 41.5/50 cm, 50 µm i.d., injection pressure of 40 mbar. PBS concentration: 10 mM. SB: sample buffer, WB: washing buffer.

To check the efficiency of the measures against nonspecific interactions, IACE-MALDI MS analysis was performed for the following cases: for IACE protocol with 0.1% Tween 20 in sample and washing buffers, but without a water washing step, and for IACE protocol with 0.05 % Tween 20 in sample and washing buffers along with an additional water washing step (Figure SI-3.a, blue and black lines). As shown on the collected MALDI MS spectra (Figure SI-3.b) for the first case, the peak with the migration time of ~11.75 min in electropherogram corresponded to human serum albumin, while the peak with the migration time of ~12.85 min corresponded to IgG Abs. The peak with the migration time of ~11.50 min was a systematic peak and did not give any MS signal. In the second case no peaks were observed for the mass range of 50 kDa to 300 kDa (data not shown). These results indicate that the elimination of the nonspecific adsorption using buffers with 0.05% Tween 20 and additional water washing step was efficient. However, no MALDI MS signal for IgE Abs was registered for the peak presented in Figure 1.b (black line) with the migration time ~14.00 min, due to low IgE Abs concentration and poor sensitivity of typical MALDI MS instrument towards such large molecules. Therefore, for total IgE quantification by IACE analysis only UV detection was used, providing sufficient detection sensitivity.

SI-4: Defining the number of IgE antibodies extraction cycles

To demonstrate that the number of IgE Abs extraction experiments, as well as the number of serum injection/system washing cycles within one extraction should be always adapted in accordance with the initial IgE Abs concentration in the blood serum, the theoretical amount of IgE Abs trapped in one extraction experiment, can be calculated.

If it is assumed that during MBs solution injection, all the injected MBs are trapped by the magnets, 5 min of injection should result in 0.23 µg of MBs plug. As was assessed by BCA protein test, anti-human IgE Abs binding efficiency was 42 µg per 1 mg of MBs, providing 9.6 µg (64 pmol) of Abs per 0.23 µg of trapped MBs. Assuming that in the ideal steric conditions one anti-human IgE antibody molecule binds two IgE molecules, the theoretical binding capacity of MBs plug is 23 µg (128 pmol) of IgE Abs.

At the same time, the injection of MBs plug provokes the pressure drop across the capillary. Based on our previous experience (Anal. Bioanal. Chem., 2011, 401:3239–3248), 5 min of MBs injection (0.5 mg/ml solution) lead to the pressure drop around 1.1 times. Within 10 min of IgE Abs extraction step 840 nl of diluted blood serum can be percolated through the immunosupport. According to the results of performed IACE-UV analysis , such volume of diluted serum used in present work should contain only around 82 pg (450 amol) of IgE antibodies, which is much lower, than the binding capacity of the MBs plug.

If the concentration of IgE Abs in the blood serum is around the diagnostic threshold, i.e. 10 times lower, than in the presented work, only 8.2 pg (45 amol) of IgE Abs would be percolated through the immunosupport. Therefore, it would be reasonable to increase the amount of injected IgE Abs during the extraction step. For insuring good sensitivity of the CRD step, it is proposed to already increase the quantity of injected sample, when the detected total IgE Abs concentration in the blood serum is below 400 ng/ml (167 IU/ml). This value is 5 times smaller

than the one for the blood serum used in the present work, and corresponds to the 16.4 pg (90 amol) of IgE Abs percolated through the immunosupport during one standard extraction cycle.

As was demonstrated above, simple increase of the serum injection time leads to the increase of nonspecific interactions as well. To avoid this negative effect, the serum injection/system washing cycle can be repeated twice with the same MBs plug, *i.e.* using the following protocol:

- 5 min of MBs injection, followed by 4 min of the washing solution injection;
- 10 min of diluted serum injection;
- 5 min of the washing solution injection, followed by 5 min of deionized water injection;
- 10 min of diluted serum injection;
- 5 min of the washing solution injection, followed by 5 min of deionized water injection;
- 10 min of leading electrolyte injection;
- MBs plug elution by high pressure.

If it is necessary, the number of serum injection/system washing cycles can be further increased, as well as the number of extraction experiments.

SI-5: CRD of cow's milk allergy using UHT milk fractions

CRD experiments by IACE with UV and MALDI MS were also performed using UHT milk fractions. Obtained results are presented below on Figure SI-5.

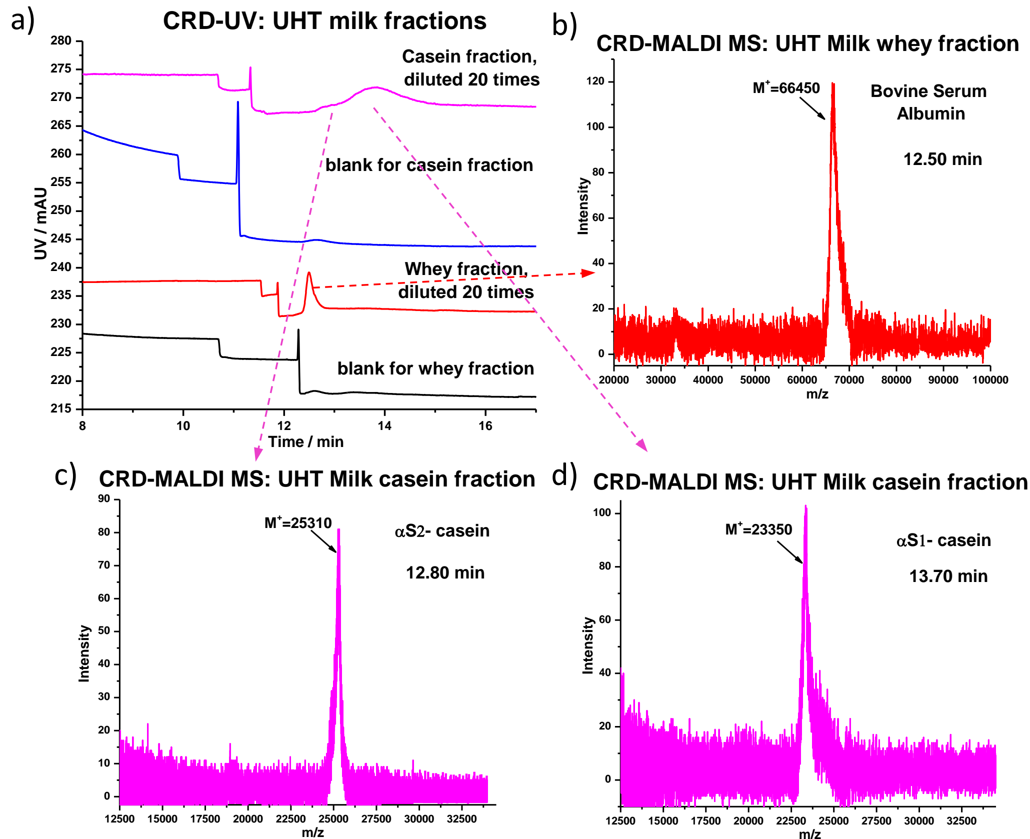

Figure SI-5. CRD of cow's milk allergy using UHT milk fractions. a) Electropherograms obtained during CRD by IACE-UV analysis of the milk fractions: blank for whey fraction (black line), whey fraction (diluted 20 times, red line), blank for casein fraction (blue line), casein fraction (diluted 20 times, pink line). Blank samples contained only sample buffer. b) MALDI MS spectra collected during CRD by IACE-MALDI MS analysis of the milk whey fraction: bovine serum albumin (eluted at 12.50 min). c), d) MALDI MS spectra collected during CRD by IACE-MALDI MS analysis of the milk casein fraction: αS2-casein (eluted at 12.80 min) and αS1-casein (eluted at 13.70 min), respectively. Conditions: 402 s of MBs loading (0.135 mg/ml, with extracted and cross-linked IgE Abs). For caseins analysis 0.1 mM urea was added to the elution and separation buffers. Other experimental conditions were the same as in Figure SI-3.

During the CRD using whey fraction of UHT milk the peak at 12.50 min in the IACE-UV electropherogram (Figure SI-5.a, red line) was detected as bovine serum albumin during IACE-MALDI MS analysis (Figure SI-5.b). For the CRD using casein fraction the large peak on the IACE-UV electropherogram (Figure SI-5.a, pink line) was defined by IACE with MALDI

MS detection as αS2-casein (Figure SI-5.c) and αS1-casein (Figure SI-5.d) eluted around 12.80 min and 13.70 min, respectively. These results are the same as for the ones obtained for the CRD using skimmed milk fractions, because both types of bovine milk have the similar origin and composition. Moreover, the manufacturing processes for UHT milk and skimmed milk powder are also rather similar.