

## Appendix A. Supplementary data

### Broadband Laser-based Mid-Infrared Spectroscopy employing a Quantum Cascade Detector for Milk Protein Analysis

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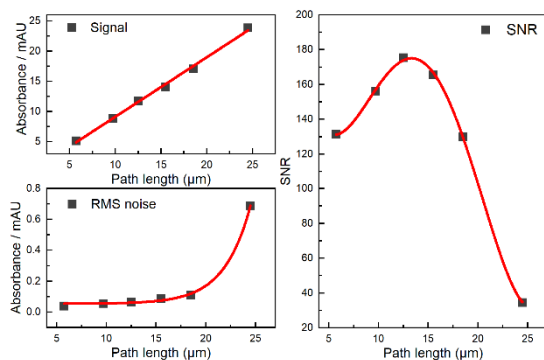
Figure S3. Calibration curves obtained for bovine milk proteins by the EC-QCL-QCD setup.

Equation S1. The degree of spectral overlap.

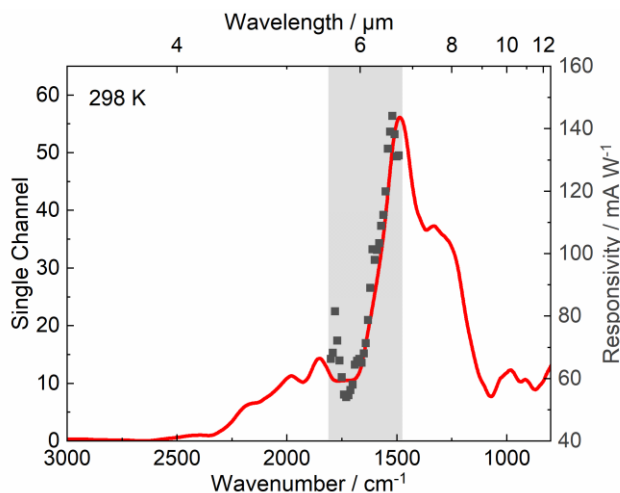
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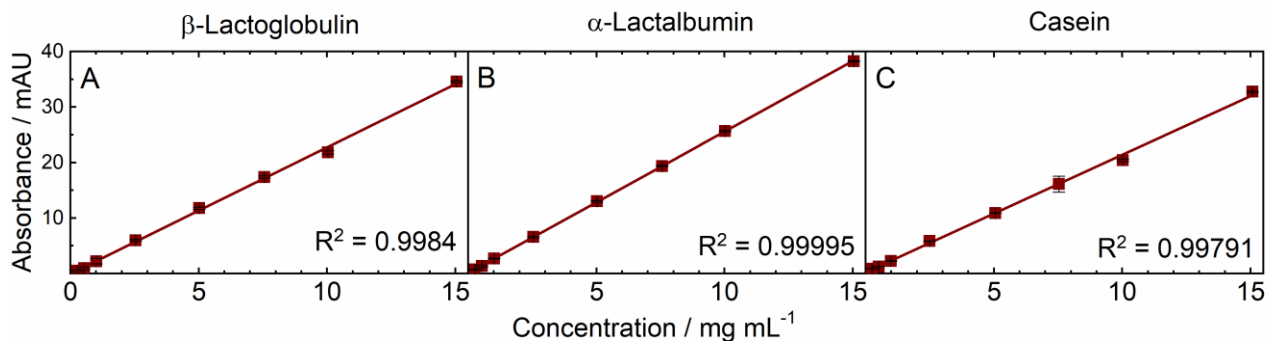
Figure S5. Nominal and predicted milk protein concentration values obtained by PLS.



**Figure S1.** Signal and noise levels of the EC-QCL – QCD setup at different transmission path lengths. Signal was defined as the absorbance at the  $1632\text{ cm}^{-1}$  (maximum of the amide I band for  $\beta$ -lactoglobulin) and the RMS noise level of the 100% lines of water between  $1700$  and  $1600\text{ cm}^{-1}$ . The measurements were performed at acquisition times of  $\sim 45\text{ s}$  (300 scans) and spectral resolution of  $2.6\text{ cm}^{-1}$ .



**Figure S2.** Spectral FTIR characterization of the ridge QCD at room temperature. Gray-shaded area corresponds to the EC-QCL tunability region. Gray squares correspond to the responsivity of the QCD measured by an EC-QCL at different wavenumbers.



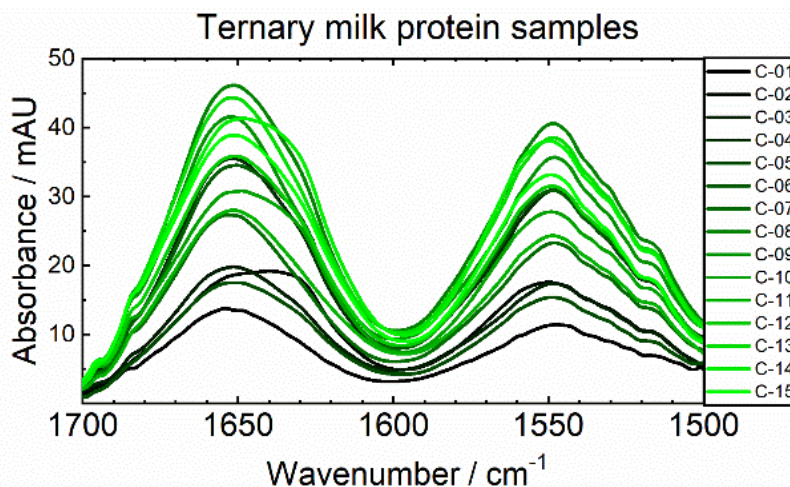
**Figure S3.** Calibration curves obtained for bovine milk proteins by the EC-QCL-QCD setup. Quantitative analysis was performed by evaluation of the height of the amide I band maxima at  $1632$ ,  $1654$  and  $1651\text{ cm}^{-1}$  for  $\beta$ -lactoglobulin,  $\alpha$ -lactalbumin and casein, respectively. Five measurements were averaged per concentration.

To quantitatively compare the agreement between the spectral components, the degree of spectral overlap ( $s_{12}$ ) is calculated:

$$s_{12} = \frac{\|s_1^T s_2\|}{\|s_1\| \|s_2\|} \quad (S1)$$

where  $s_1$  is the spectrum obtained by EC-QCL-QCD setup and  $s_2$  is the spectrum measured by an FTIR instrument. The value of  $s_{12}$  ranges from 0 to 1, where 0 indicates lack of overlap and 1 suggests complete spectral overlap [1].

- [1] M.J. Culzoni, H.C. Goicoechea, G.A. Ibañez, V.A. Lozano, N.R. Marsili, A.C. Olivieri, A.P. Pagani, Second-order advantage from kinetic-spectroscopic data matrices in the presence of extreme spectral overlapping. A multivariate curve resolution-Alternating least-squares approach, *Anal. Chim. Acta.* 614 (2008) 46–57. <https://doi.org/10.1016/j.aca.2008.03.013>.

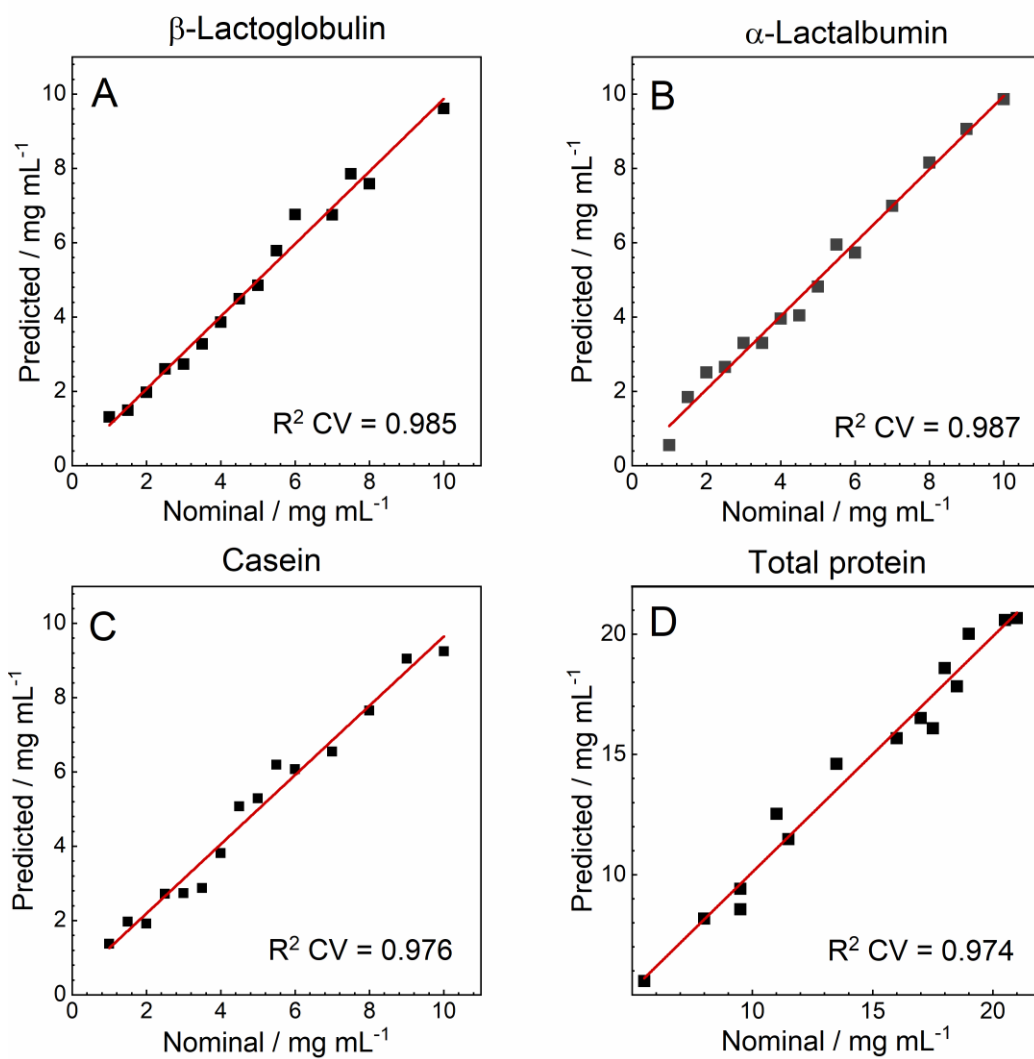


**Figure S4.** Absorption spectra of ternary milk proteins recorded with the EC-QCL-QCD setup for multivariate quantitation.

**Table S1.** Protein concentrations in calibration samples and figures of merit obtained by PLS.

Calibration sample	$\beta$ -LG (mg mL <sup>-1</sup> )		$\alpha$ -LA (mg mL <sup>-1</sup> )		Cas (mg mL <sup>-1</sup> )		Total protein (mg mL <sup>-1</sup> )	
	Nominal	Predicted	Nominal	Predicted	Nominal	Predicted	Nominal	Predicted
C-01	1.50	1.49	3.00	3.30	1.00	1.37	5.50	5.57
C-02	7.00	6.75	1.00	0.55	1.50	1.97	9.50	9.41
C-03	3.00	2.73	4.50	4.04	2.00	1.92	9.50	8.55
C-04	5.50	5.79	8.00	8.15	2.50	2.71	16.00	15.67
C-05	2.50	2.61	2.50	2.66	3.00	2.73	8.00	8.17
C-06	8.00	7.58	6.00	5.73	3.50	2.87	17.50	16.09
C-07	2.00	1.98	5.50	5.95	4.00	3.81	11.50	11.48
C-08	7.50	7.85	9.00	9.06	4.50	5.07	21.01	20.67
C-09	3.50	3.28	10.00	9.87	5.00	5.29	18.51	17.82
C-10	6.00	6.76	2.00	2.51	5.50	6.19	13.50	14.60
C-11	1.00	1.31	4.00	3.95	6.00	6.07	11.00	12.53

C-12	5.00	4.85	5.00	4.82	7.00	6.54	17.01	16.51
C-13	4.00	3.86	7.00	6.99	8.00	7.65	19.01	20.02
C-14	10.00	9.62	1.50	1.84	9.00	9.04	20.51	20.59
C-15	4.50	4.49	3.50	3.30	10.01	9.24	18.01	18.59



**Figure S5.** Nominal and predicted milk protein concentration values obtained by PLS.