Site-specific pK_a determination of the carboxylate-binding subunit in artificial peptide receptors

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Supplementary Information

1. pH titration reference data

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1. pH titration reference data

For comparison with the UVRR/NMF methodology, conventional pH titrations of CBS-NH_2 (Fig. S1) and CBS-KKF (Fig. S2) were performed. Both substances were titrated twice: against 0.1 M NaOH and 0.1 M HCl (the mean value of these titrations was further used). This resulted in a pK_a of 6.4 for CBS-NH_2 and 6.3 for CBS-KKF.

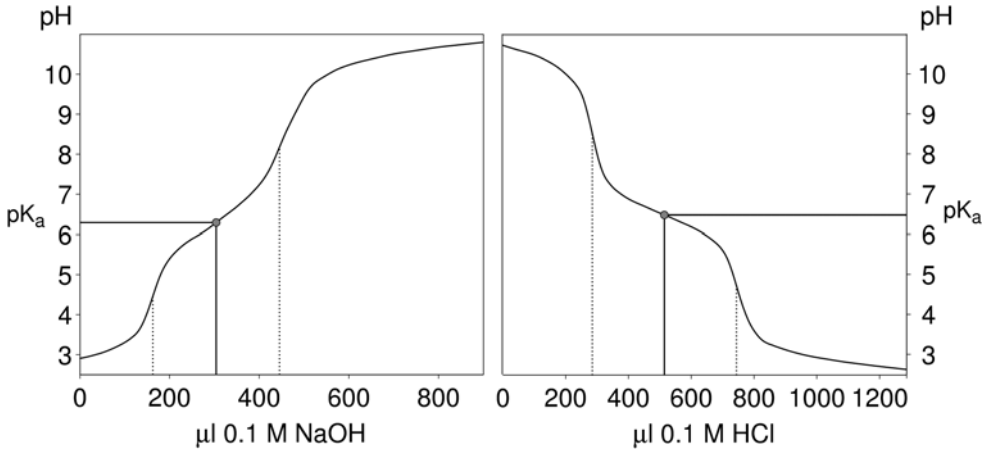

Fig. S1 Titration curves for CBS-NH_2 against 0.1 M NaOH (left) and 0.1 M HCl (right). The NaOH titration (left) resulted in a pK_a of 6.29, the HCl titration (right) in a pK_a of 6.49. For comparison with other methods, the mean of these two values was used (pK_a = 6.39).

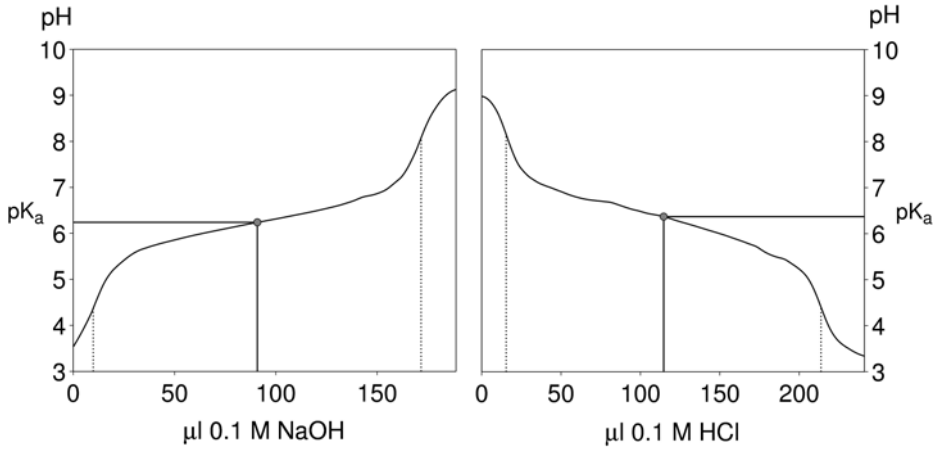

Fig. S2 Titration curves for CBS-KKF against 0.1 M NaOH (left) and 0.1 M HCl (right). The NaOH titration (left) resulted in a pK_a of 6.24, the HCl titration (right) in a pK_a of 6.37. For comparison with other methods, the mean of these two values was used (pK_a = 6.31).

2. UV RR/NMF data for the small model system CBS-NH_2

The model system CBS-NH_2 contains only the carboxylate binding site (CBS). Both CBS-NH_2 and the receptor CBS-KKF show similar behaviour under pH change as can be seen by comparing the spectra of CBS-NH_2 (Fig. S3) and CBS-KKF (Fig. 3) at three different pH values.

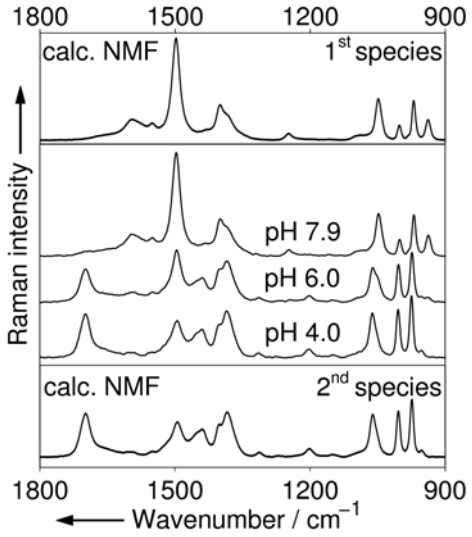

Fig. S3 UV resonance Raman spectra of CBS-NH_2 at three different pH values (center) and calculated component spectra (top and bottom). The first component spectrum (top) was assigned to the neutral, the second component spectrum (bottom) was assigned to the protonated CBS species.

Analogous to CBS-KKF, the pK_a of CBS-NH_2 was determined by a Henderson-Hasselbalch fit to be 6.59 (Fig. S4 top). For comparison, a wavenumber shift analysis based on a peak between and 970 and 975 cm^{-1} was performed. The fit routine resulted in a pK_a of 6.7.

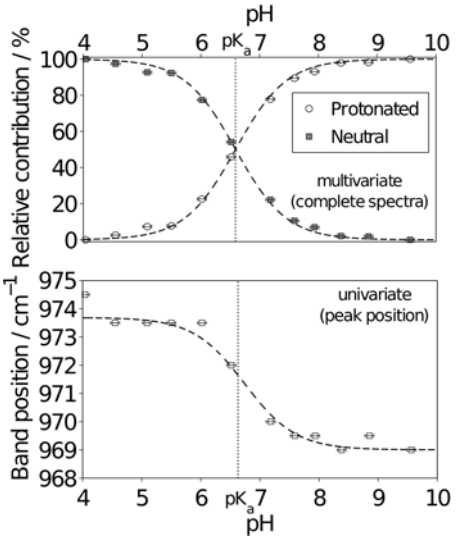

Fig. S4 Contributions of neutral (squares) and protonated CBS species (circles) in CBS-NH_2 derived from NMF analysis (top) and wavenumber shift analysis (bottom). Henderson-Hasselbalch fits, resulting in a pK_a of 6.59 (NMF) and 6.74 (wavenumber shift), are shown as dotted line. The mean deviations from the model are 2.9 % (NMF) and 10.4 % (wavenumber shift analysis).

3. Details on the NMF procedure and the Henderson-Hasselbalch fit

Conventional NMF for analyzing the pH dependent UV resonance Raman spectra[10] resulted in a contribution matrix which did not reliably reflect a pH titration curve due to a vertical offset (Fig. S5, dashed lines). When plotted against the pH value, the contributions of the protonated and neutral CBS species however should approach zero at high and low pH, respectively. Therefore we inplemented the assumption of a monotoneous decrease/increase within the column vectors of the contribution matrix during the the first iterations, followed by a conventional NMF run without these constraints (Fig. S5, solid lines). Adding the constraint in the first step forces the minimization in the right direction, in order to find the global minimum in the subsequent unconstrained NMF. This resulted in a contribution matrix that was chemically more reasonable compared to the results from conventional NMF. After the NMF procedure, the contribution matrix was normalized as mentioned in the main text.

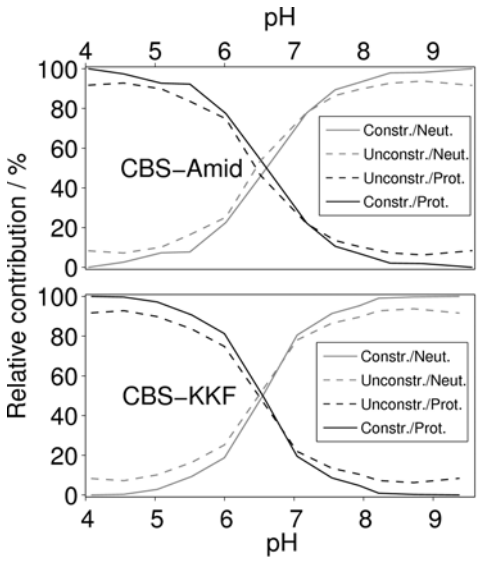

Fig. S5 Relative contributions (after normalization) of protonated (black) and neutral (grey) CBS species of CBS-Amid (top) and CBS-KKF (bottom) determined by constrained (dotted lines) and unconstrained (straight lines) NMF. The constrained NMF resulted in chemically more meaningful results without offset.

The overall amount of the neutral species (A) and the protonated CBS species (HA^+) is constant ($[HA^+]_0$ = 1 mM). Inserting this constraint into the Henderson-Hasselbalch equation results in the following expression: $\mathrm{pK}_a = \mathrm{pH} - \log\left(\frac{[\mathrm{HA}^+]_0}{[\mathrm{HA}^+]} - 1\right)$

This expression was used to fit the contribution data obtained by the NMF procedure. To fit the band position in case of the wavenumber analysis the analytical expression as described by Pieridou and Hayes[8] was used:

$$\nu_{obs} = \frac{\left(\nu_{HA^+} + \nu_A \cdot 10^{(pH - pK_a)}\right)}{\left(1 + 10^{(pH - pK_a)}\right)}$$

To compare both methods, the deviation from the model was calculated by the following equation:

$$\frac{\sum_{pH_j=4.5}^{9.5} \sum_{\tilde{\nu}_i=900\,cm^{-1}}^{1800\,cm^{-1}} \left| model(\tilde{\nu}_i, pH_j) - experiment(\tilde{\nu}_i, pH_j) \right|}{\sum_{pH=4.5}^{9.5} model(\tilde{\nu}_i, pH_j)}$$

The data from the wavenumber shift analysis was normalized prior to the determination of this deviation.