Supporting Information
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Structural Modulation of Chromic Response: Effects of Binding-Site Blocking in a Conjugated Calix[4]pyrrole Chromophore

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1. Binding models

1.1. Derivation of binding isotherms

Instead of using two distinct binding constants for protonation and binding of the acid’s anion we used a single constant because both phenomena are strongly correlated. A common binding constant definition can be used to model 1:

\[
K_i = \frac{[P_i^+ (A^-)^i]}{[P_i (A^-)^{i-1}][A]}
\]

for \(i = 1, ..., p\). \(P\) and \(A\) represent oxo porphyrinogen and acid, respectively.

Total concentration of oxo porphyrinogen and acid is expressed as:

\[
[P]_{\text{tot}} = [P] + \sum_{i=1}^{p} [P_i^+ (A^-)^i]
\]

\[
[A]_{\text{tot}} = [A] + \sum_{i=1}^{p} [P_i^+ (A^-)^i]
\]

A solution of this system of \(p+2\) equations (Equations S1 and S2) was carried out according to Hargrove and Anslyn. First, elimination of \([P_i^+ (A^-)^i]\) and \([P]\) leads to a polynomial equation of \((p+1)\)-th degree for \([A]\) which can be solved numerically (for numerical solution using Newton’s method see the Supplementary Information in Labuta). By this method, we obtain the function

\[
[A] = [A]( [P]_{\text{tot}}, [A]_{\text{tot}}, \{K_i\})
\]

Using Equations S1, S2 and S3 the expression

\[
[P] = [P]( [A]; [P]_{\text{tot}}, [A]_{\text{tot}}, \{K_i\})
\]

is obtained. Then from (S1)

\[
[P_i^+ (A^-)^i] = K_i [P_i (A^-)^{i-1}] [A] = K_i K_2 ... K_1 [P][A]^i
\]

After substitution of Equations S3 and S4 into Equation S5 we finally obtain expressions for concentrations of bound (protonated) species as a function of the binding constants and the total concentrations of oxoporphyrinogen and acid in the sample.

1.2. NMR binding isotherms

In the case of slow chemical exchange, the NMR signals of free and bound/protonated species are separated, hence intensities of particular peaks can be used to construct a binding isotherm as follows:

\[
\frac{\Sigma \text{intensity of bound species' signal/number of bound species' hydrogens}}{\Sigma \text{intensity of bound and free species' signal/number of bound and free species' hydrogens}} = \frac{\Sigma \text{[bound species]}}{\Sigma \text{[bound and free species]}}
\]
Fast exchange averages signals and chemical shifts are exploited for construction of binding isotherms using:

\[
\delta_{\text{observed}} = \delta_{\text{p}} \frac{[P]}{[P]_{\text{tot}}} + \sum_{i=1}^{p} \frac{[P^{\text{it}}]}{[P]_{\text{tot}}} \delta_{\text{p}^i}
\]

where \([P]\), \([P^{\text{it}}]\) and \([P]_{\text{tot}}\) denote free, \(i\)-times protonated and total oxoporphyrinogen concentration, respectively. \(\delta_{\text{p}}\) stands for the chemical shift of a given species and \(p\) is maximum protonation number.

2. **Singular value decomposition (SVD) and decomposition into the spectra of individual species (DSIS) analyses of UV-vis titration spectra**

2.1. **Description of singular value decomposition (SVD) used in this study**

Each absorption spectrum is a linear combination of spectra of individual absorbing species present in the sample (plus noise). Composition of the sample during the titration is determined by the corresponding binding constants. Singular value decomposition analysis (SVD, also known as factor analysis) can reveal how many absorbing species (absorbing in the measured wavelength range) are present in the sample and use of the correct binding model enables determination of the spectra of these individual species and their binding constants.

SVD is the decomposition of a matrix to produce three different matrices with special properties. Let us assume we have made \(N_{\text{exp}}\) titration steps and each spectrum consists of \(N_{\text{exp}}\) discrete points. We create an \(N_{\text{tot}} \times N_{\text{exp}}\) matrix \(A\) whose columns are formed by the absorbance values. Then the SVD reads:

\[
A_{\lambda n} = \sum_{\mu=1}^{N_{\text{tot}}} \sum_{m=1}^{N_{\text{exp}}} U_{\lambda \mu} W_{\mu n} V_{mn}
\]

where \(\lambda = 1, 2, ..., N_{\text{tot}}\) and \(n = 1, 2, ..., N_{\text{exp}}\).

\(U\) is a square orthonormal matrix of dimensions \(N_{\text{tot}} \times N_{\text{tot}}\), its columns are the so called basis spectra (sometimes denoted as subspectra) from which the measured experimental spectra can be reconstructed. \(W\) is a rectangular diagonal matrix of dimensions \(N_{\text{tot}} \times N_{\text{exp}}\). These numbers are denoted as singular values and are sorted in descending order. \(V\) is a square orthonormal matrix of dimensions \(N_{\text{exp}} \times N_{\text{exp}}\). Its interpretation is clarified after obtaining the matrix product with \(W\). Operation \(WV\) multiplies \(i\)-th row of \(V\) with \(i\)-th singular value. Rows of the matrix \(WV\) are coordinates of the basis spectra from \(U\) reconstructing the original spectra \(A\). Since matrix \(V\) is normalised (orthonormal), the magnitude of the coordinate values depend largely on elements of \(W\). Assuming that there are \(N_{\text{spec}}\) absorbing species, only the first \(N_{\text{spec}}\) singular values will be significant (\(N_{\text{spec}}\) is often denoted as the factor dimension). Basis spectra corresponding to lower singular values originate from noise. Average standard error of the SVD approximation for given factor dimension from measured spectra is denoted as the residual:

\[
\text{residual}(N_{\text{spec}}) = \sqrt{\frac{\sum_{j=N_{\text{spec}}+1}^{N_{\text{exp}}} W_{ji}^2}{N_{\text{exp}}(N_{\text{exp}} - N_{\text{spec}})} / N_{\text{exp}}}
\]

Plot of singular values and dependence of residuals on factor dimension can be useful for determination of the number of absorbing species if there is not too much noise or background absorption in the experimental spectra.

In order to remove noise, we define reduced matrices \(U_{\text{red}}\) consisting of the first \(N_{\text{spec}}\) columns of \(U\) and \(W_{\text{red}}\) consisting of the first \(N_{\text{spec}}\) rows of \(W\). Then the absorption spectra can be reproduced as:

\[
A_{\lambda n} \approx \sum_{i=1}^{N_{\text{spec}}} \sum_{m=1}^{N_{\text{exp}}} U_{\text{red},i} W_{\text{red},m} V_{mn}
\]
where $\lambda = 1, 2 \ldots N_{\text{res}}$ and $n = 1, 2 \ldots N_{\text{exp}}$. The experimentally measured absorbance is a linear combination of the absorption spectra of species present:

$$A_{\lambda n} = \sum_{i=1}^{N_{\text{spec}}} Z_{\lambda i} f_{in}$$

(S10)

where $\lambda = 1, 2 \ldots N_{\text{res}}$ and $n = 1, 2 \ldots N_{\text{exp}}$. Rows of the $N_{\text{res}} \times N_{\text{spec}}$ matrix $Z$ are spectra of the absorbing species, the $i$-th row of $N_{\text{spec}} \times N_{\text{exp}}$ matrix $f$ describes relative fraction of the $i$-th absorbing species during the titration. The relative fraction matrix $f$ is a function of unknown binding constants, therefore, at this point a binding model is introduced into the analysis. Spectra of absorbing species $Z$ can be expressed in terms of basis spectra $U$ after transformation with $N_{\text{spec}} \times N_{\text{spec}}$ matrix $R$.

$$Z_{\lambda j} = \sum_{i=1}^{N_{\text{spec}}} U_{\text{red} \lambda i} R_{ij}$$

(S11)

where $\lambda = 1, 2 \ldots N_{\text{res}}$ and $j = 1, 2 \ldots N_{\text{spec}}$.

Now there are two ways to express the matrix $A$:

$$A \approx U_{\text{red}} W_{\text{red}} V$$

and

$$A = Zf = U_{\text{red}} Rf$$

Comparing these two expressions we get the condition

$$W_{\text{red}} V \approx R f$$

(S12)

which can be achieved by fitting, e.g. using a least squares method minimizing the following function:

$$\text{sum of square errors} = \sum_{i=1}^{N_{\text{spec}}} \sum_{n=1}^{N_{\text{exp}}} [(W_{\text{red}} V)_{in} - (R f (K_1, K_2, \ldots ))_{in}]^2$$

(S13)

Fitting parameters are $N_{\text{spec}}^2$ elements of the transformation matrix $R$ and $N_{\text{spec}}$ binding constants $K_1, K_2, \ldots$.

2.2. Description of decomposition into the spectra of individual species (DSIS)

When consecutive spectral changes caused by the changing ratio of individual absorbing species occur during a titration experiment (i.e. every experimentally observed spectrum at a particular volume fraction of reagent $S_{\text{obs}}(\psi)$ is a combination of just two individual spectra, also implying the presence of isosbestic points) spectra of individual species $S_i$ can be easily obtained as experimentally observed spectra at the start and endpoint of the corresponding spectral changes. DSIS consists in obtaining relative fraction $f_i(\psi)$ of the particular individual species for which

$$S_{\text{obs}}(\psi) = \sum_{i} f_i(\psi) \times S_i$$

(S14)
Relative fractions must obey constraints \( \sum_i f_i(\phi) = 1 \) and \( f_i(\phi) > 0 \), which are used in the minimization of the following function:

\[
\text{sum of square errors} = \sum_{n=1}^{N_{\text{spec}}} \left[ S_{\text{obs}}(\phi_n) - \sum_{i=1}^{N_{\text{spec}}} f_i(\phi_n) \times S_i \right]^2
\]

where \( f_i(\phi_n) \) are fitting parameters.

**2.3. SVD and DSIS analyses of OxP titration with TFA**

In the DSIS decomposition of UV-vis data from titration of OxP with TFA, we found three spectra of absorbing species \( S_i \) at the start and endpoint of both spectral changes (Figure 2a–c in the main manuscript). We obtained vol. f. dependent relative fractions \( f_i(\phi) \) of particular absorbing species during the titration experiment (Figure 2d in the main manuscript, full circles) using:

\[
S_{\text{obs}}(\phi) = f_{\text{OxP}}(\phi) \times S_{\text{OxP}} + f_{\text{OxP}^2}(\phi) \times S_{\text{OxP}^2} + f_{\text{OxP}^4}(\phi) \times S_{\text{OxP}^4}
\]

SVD decomposition (Figure S1) confirms three absorbing species \( (N_{\text{spec}} = 3) \) via singular values and residuals plots (as indicated by red dotted lines in the Figure S1a, b). Further analysis (described in the Section 3.1) in combination with an OxP:TFA 1:4 binding model provides us with the corresponding binding constants. Due to the nature of the binding processes, it is impossible to evaluate independently \( K_1, K_2, K_3 \) and \( K_4 \) during the least squares fitting procedure. Therefore their products \( K_1K_2 \) and \( K_3K_4 \) describe the binding properties more reliably, which also reflects the cooperativities involved in these binding processes. In order to determine uncertainties of the fit (minima in the least squares fitting procedures were shallow), we explored the whole parameter space. Results are listed in Table 1 in the main manuscript. Determination of binding constants enables us to construct elements of the relative fraction matrix \( f \) (Figure 2d in the main manuscript, solid lines), that are equivalent to the \( f_i(\phi) \) coefficients of DSIS (Figure 2d in the main manuscript, solid circles). Elements of transformation matrix \( R \) were obtained by least squares fitting of absorption spectra \( S_i(\phi) \) from the DSIS using Equation S8. First four basis spectra (columns of the matrix \( U \)) and first four rows of the matrix \( W_{\text{red}} V \) (red line denotes fit by rows of the matrix \( R f \) using Equation S13) are shown in the Figure S1c, d. As can be seen in Figure S2 quality of both DSIS (red dashed line) and SVD analysis fit (green dashed line) is very good.
2.4 SVD and DSIS analyses of BzOxP titration with DFA

In the UV-vis titration of BzOxP with DFA DSIS decomposition revealed four individual absorbing species and experimentally observed spectra were fitted using:

\[ S_{obs}(\varphi) = f_{BzOxP}(\varphi) \times S_{BzOxP} + f_{BzOxP^+}(\varphi) \times S_{BzOxP^+} + f_{BzOxP^{2+}}(\varphi) \times S_{BzOxP^{2+}} + f_{BzOxP^{3+}}(\varphi) \times S_{BzOxP^{3+}} \]

(S17)

Results of SVD analysis are listed in Figure S3. Singular values and residuals plots confirmed the presence of four individual species in the spectra (red dotted lines in Figures S3a, b). First spectral change was fitted with 1:1 binding model up to \( \varphi < 0.001 \), the other spectral changes remain unfitted because for higher vol. f. activity coefficients necessary for the fitting procedure are unknown (see Table 1 in the main manuscript). Elements of transformation matrix \( K \) were obtained by least squares fitting of absorption spectra \( S \) from the DSIS using Equation S8. The first four basis spectra (columns of the matrix.
and first four rows of the matrix $W_{\text{red V}}$ (red line denotes fit by the matrix $R_f$ using Equation S13) are shown in the Figure S3c, d. Quality of DSIS (red dashed line) as well as SVD 1:1 model fit is good (green dashed line) as can be seen in Figure S4.

**Figure S3**: SVD analysed titration of Bz2OxP with DFA (main manuscript, Figure 3a–c), (a) singular values, (b) residuals, (c) first four basis spectra and (d) first four rows of $W_{\text{red V}}$ matrix (full circles) fitted by 1:1 binding model for $q < 0.001$ (solid line).

**Figure S4**: Demonstration of fit quality of titration of Bz2OxP with DFA (Figure 3 in the main manuscript). Comparison of several experimentally measured spectra (black lines) and their DSIS decomposition (red dashed lines; also see Figure 3d for UV-vis spectra of individual species and Figure 3e, solid circles in the main manuscript for the fractions of individual species). Fitting using SVD and 1:1 binding model up to acid vol. f. $q = 0.001$ is shown as green dashed lines.
2.5. SVD and DSIS analyses of Bz₄OxP titration with DFA

The first spectral change in the UV-vis titration of Bz₄OxP with DFA consists of a solvatochromic shift which in principle cannot be decomposed using SVD or DSIS. Therefore, we have performed both these analyses only for the second and third spectral changes (i.e. $\varphi > 0.01$). DSIS decomposition is:

$$S_{\text{obs}}(\varphi) = f_{\text{Bz}_4\text{OxP-H-bond}}(\varphi) \times S_{\text{Bz}_4\text{OxP-H-bond}} + f_{\text{Bz}_4\text{OxP}^-}(\varphi) \times S_{\text{Bz}_4\text{OxP}^-} + f_{\text{Bz}_4\text{OxP}^{2+}}(\varphi) \times S_{\text{Bz}_4\text{OxP}^{2+}}$$  \hspace{1cm} (S18)

The spectrum of hydrogen bound oxoporphyrinogen species $S_{\text{Bz}_4\text{OxP-H-bond}}$ was extracted at $\varphi = 0.01$ just prior to commencement of the second spectral change. The DSIS matches the measured spectra very well, see Figure S6. Results of SVD analysis are listed in Figure S5. Singular values and residuals plots confirmed the presence of three individual species in the second and third spectral changes (red dotted lines in Figures S5a, b). In this case, no binding model was used because the prevailing high acid concentrations here require use of activity coefficients.

![SVD and DSIS analyses](image)

**Figure S5:** SVD analysed titration of Bz₄OxP with DFA for acid concentration $\varphi > 0.01$ (Figure 4a, b in the main manuscript), (a) singular values, (b) residuals, (c) first four basis spectra and (d) first four rows of $W_{\text{red}} V$ matrix (full circles).
2.6. SVD and DSIS analyses of OxP UV-vis spectra in CDCl₃/DMF binary mixture

OxP is soluble in the CDCl₃/DMF binary mixture up to about 0.2 DMF vol. f. All three spectral changes are pseudosaturated which enables DSIS. However, the first spectral change is a typical solvatochromic shift so that we have performed SVD and DSIS analyses for ϕ > 0.0015. SVD analysis is not conclusive in these cases, suggesting the presence of two absorbing species (although we know that three are present); a feature most likely due to the strong similarity between the spectra S_{OxP H-bond} and S_{OxP-DFA}, which could also be affected by ongoing slight solvatochromic shifts.

Figure S6: Demonstration of fit quality in DSIS of titration of Bz·OxP with DFA for ϕ > 0.01 (Figure 4 in the main manuscript). Comparison of several experimentally measured spectra (black lines) and their DSIS decomposition (red dashed lines; also see Figure 4c for UV-vis spectra of individual species and Figure 4d in the main manuscript for the fractions of individual species).

Figure S7: SVD and DSIS analyses of OxP UV-vis spectra in CDCl₃/DMF binary mixture for DMF vol. f. ϕ > 0.0015 (Figure 12a–c in the main manuscript). (a) DSIS (using spectra from Figure 12d in the main manuscript), SVD analysis: (b) singular values, (c) residuals, (d) first four basis spectra and (e) first four rows of \( W_{rel} V \) matrix.
Figure S8: Demonstration of fit quality in DSIS of titration of OxP with DFA for $\varphi > 0.0015$ (Figure 12a–c in the main manuscript). Comparison of several experimentally measured spectra (black lines) and their DSIS decomposition (red dashed lines; also see Figure 12d in the main manuscript for UV-vis spectra of individual species and Figure S7a for the fractions of individual species).

2.7. SVD analysis of BzOxP UV-vis spectra in CDCl$_3$/DMF binary mixture

BzOxP in a CDCl$_3$/DMF binary mixture exhibits two consecutive spectral changes. As in the case of OxP, the first spectral change is attributed to solvatochroism. We performed SVD and DSIS analyses for the second spectral change, i.e. for $\varphi > 0.0015$.

Figure S9: SVD and DSIS analyses of BzOxP UV-vis spectra in CDCl$_3$/DMF binary mixture for DMF vol. f. $\varphi > 0.0015$ (Figure 12e in the main manuscript). (a) DSIS (using spectra from Figure 12f in the main manuscript), SVD analysis: (b) singular values, (c) residuals, (d) first four basis spectra and (e) first four rows of $W_{rel} V$ matrix.
2.8. SVD analysis of BzOxP UV-vis spectra in CDCl$_3$/DMF binary mixture

The only spectral change of BzOxP in CDCl$_3$/DMF binary mixture is a bathochromic solvatochromic shift (see Figure 12g in the main manuscript and Figure S11a). Thus, DSIS decomposition cannot be performed. SVD analysis does not suggest any discrete number of individual absorbing species (Figure S11).

Figure S10: Demonstration of fit quality in DSIS of titration of BzOxP with DFA for $\varphi > 0.0015$ (Figure 12e in the main manuscript). Comparison of several experimentally measured spectra (black lines) and their DSIS decomposition (red dashed lines; also see Figure 12f in the main manuscript for UV-vis spectra of individual species and Figure S9a for the fractions of individual species).

Figure S11: Analysis of BzOxP UV-vis spectra in CDCl$_3$/DMF binary mixture for the whole extent of DMF vol. f. (Figure 12g in the main manuscript). (a) Shift of absorbance maxima with DMF vol. f., SVD analysis: (b) singular values, (c) residuals, (d) first four basis spectra and (e) first four rows of $W_{\text{mix}} V$ matrix.
2.9. SVD analysis of Reichardt’s dye UV-vis spectra in CDCl$_3$/MeOH binary mixture

Spectral variation of Reichardt’s dye (i.e. shift of absorbance maximum) with increasing polarity of the solution (here due to addition of MeOH into a CDCl$_3$ solution, see Figure S12a, b) is induced purely by solvatochromic effect, i.e. all molecules have the same absorption spectrum for particular MeOH vol. f. Singular values and residuals plots in Figure S12c, d do not indicate a discrete number of absorbing species. An important property of the SVD method is the provision of the best possible $N_{\text{spec}}$-dimensional approximation of measured spectra (i.e. approximation as linear combination of $N_{\text{spec}}$ individual spectra). A two dimensional SVD approximation, which poorly describes the measured spectra, is shown in Figure S13. Hence, the spectral shifts cannot be considered as a consequence of variation of the relative fraction of two distinct species and, consequently, DSIS using two distinct individual spectra is impossible.

![Figure S12](image)

**Figure S12:** UV-vis spectra of Reichardt’s dye in CDCl$_3$/MeOH binary mixture for the whole extent of MeOH vol. f. (a), position of the long wavelength absorption band maxima (b) and SVD analysis: (c) singular values, (d) residuals, (e) first four basis spectra and (f) first four rows of $W_{\text{spec}} V$ matrix.

![Figure S13](image)

**Figure S13:** Two dimensional SVD approximation of Reichardt’s dye spectra in CDCl$_3$/MeOH binary mixture revealing the poor spectral matching.
2.10. Overview of DSIS, individual absorbing spectra and cuvette photos of all UV-vis experiments

- **a)** OxP + TFA in CHCl₃
  - DSIS decomposition

- **b)** Bz₂OxP + DFA in CDCl₃

- **c)** Bz₂OxP + DFA in CDCl₃

- **d)** OxP + DMF in CDCl₃

- **e)** Bz₂OxP + DMF in CDCl₃

- **f)** Bz₂OxP + DMF in CDCl₃

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**Figure S14**: DSIS relative fraction coefficients in linear scale, individual absorbing spectra and cuvette photos of investigated OxP derivatives in presence of (a–c) acid and (d–f) polar solvent. The initial solvatochromic shift in (c–e) is just schematically shown near zero vol. f.
3. NMR measurements

3.1. NMR spectra of investigated oxoporphyrinogens

Figure S15: Assigned NMR spectra of the investigated oxoporphyrinogens (in CDCl₃). For assignment of Bz₂OxP see Figure S21.

3.2. NMR titration of OxP with (R)-CSA

Figure S16: Titration of OxP with (R)-CSA (in CDCl₃, initial oxoporphyrinogen conc. 9 × 10⁻⁴ M was not held constant). Assignment symbols follow Figure S15a, free and protonated/complexed species are denoted by full and empty symbols, respectively. Red dotted arrows denote splitting of tert-butyl signal due to symmetry breaking after protonation and anion binding. Certain regions of spectra have been magnified for clarity (see factors above each region).
3.3. NMR titration of Bz₂OxP with (R)-CSA

Figure S17: Titration of Bz₂OxP with (R)-CSA (in CDCl₃, initial oxoporphyrinogen conc. 6 × 10⁻⁴ M was not held constant). Assignment symbols follow Figure S15b, free and protonated/complexed species are denoted by full and empty symbols, respectively. Red dotted arrows denote splitting of tert-butyl signal due to symmetry breaking after protonation and anion binding. Certain regions of spectra have been magnified for clarity (see factors above each region).

3.4. NMR titration of Bz₂OxP with racemic CSA

Figure S18: Titration of Bz₂OxP with racemic CSA (in CDCl₃, initial oxoporphyrinogen conc. 6 × 10⁻⁴ M was not held constant). Assignment symbols follow Figure S15b, free and protonated/complexed species are denoted by full and empty symbols, respectively. Red dotted arrows denote splitting of tert-butyl signal due to symmetry breaking after protonation and anion binding. Certain regions of spectra have been magnified for clarity (see factors above each region).
3.5. NMR shifts and corresponding binding models

Figure S19: Relative chemical shifts of peaks of investigated oxoporphyrinogens during titration experiments with DFA. Data were obtained by fitting Lorentz curves to spectra in the Figures 7, 9 and 10 in the main manuscript. Assignment symbols for oxoporphyrinogen peaks follow Figure S15, for two distinct tert-butyl peaks central chemical shift is displayed as they shift simultaneously. Non-exchanging DFA signal (triplet) is denoted by the diamond symbol (▲), CHCl₃ is denoted by asterisk (*). Solid lines stand for fits, possible range of corresponding binding constants are listed in Table 1 in the main manuscript. Geometric mean of minimum and maximum possible values of K₄ from Table 1 were used for construction of particular binding isotherms in this figure.

NMR shifts of the oxoporphyrinogens investigated are influenced by protonation, acid binding but also by polarity of the solvent. Except for the case of OxP, individual free and bound/protonated species undergo fast chemical exchange so that the resulting chemical shift is a weighted average of a few individual chemical shifts. Thus, to obtain the binding constants, we used the binding isotherms S6 for OxP and S7 for all oxoporphyrinogens. We obtained chemical shifts of all oxoporphyrinogen peaks from titration with DFA, as shown in Figure S19 (for spectra see Figures 7, 9 and 10 in the main manuscript). Lorentz curves were used to extract the peak positions from measured spectra. Application of binding models was possible basically up to vol. f. 0.001 (in the case of Bz₂OxP up to vol. f. 0.015). For higher acid content chemical shifts are influenced by high DFA polarity, even residual solvent peak (denoted as asterisk (*)) is shifting relative to TMS.

Due to the nature of the binding processes of OxP and DFA/CSA it is impossible to evaluate independently K₁, K₂, K₃ and K₄ during the least squares fitting procedure. Therefore, their products K₃K₄ and K₂K₃ describe the binding properties more reliably, which also reflects the cooperativities involved in these binding processes. In order to determine uncertainties of the fit (minima in the least squares fitting procedures were shallow), we explored the whole parameter space for each oxoporphyrinogen. Results are listed in Table 1 in the main manuscript.

DFA non-exchanging (triplet) peak reflects acid anion binding rather than protonation of oxoporphyrinogen. During titration of OxP all acid molecules are bound to the oxoporphyrinogen up to 2 equiv., see Figure S20. For Bz₂OxP, anion binding is not as strong as for OxP so that, even at the beginning of the titration, the corresponding chemical shift is not constant. In the case of Bz₂OxP, chemical shift of the acid is the same as if no oxoporphyrinogen is present. It confirms the lack of anion binding ability for Bz₂OxP.

S16
3.6. HMBC of Bz₄OxP – assignment of β- and hemiquinonoid proton signal

HMBC measurement in Figure S21 shows the correlation between tert-butyl carbon and hemiquinonoid proton (red circle). Also, there is a correlation between CH₂ carbon and inner benzyl proton (blue circle). This information enables assignment of the whole Bz₄OxP ¹H NMR spectrum.
3.7. Confirmation of presence of phenolic OH peak – addition of D₂O

Figure S22: Confirmation of presence of exchanging phenolic OH peak (a) bottom: OxP + 2 equiv. of DFA (25 °C), top: after addition of D₂O, (b) bottom: BzOxP + 0.5 equiv. of CSA (0 °C), top: after addition of D₂O. The OH peak denoted by red arrow disappears in both cases.

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