Anchoring of histidine-tagged proteins to molecular printboards: self-assembly, thermodynamic modeling and patterning


Dedicated to Professor David N. Reinhoudt on the occasion of his 65th birthday

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Modeling of the binding of His$_6$-MBP to the molecular printboard

The binding of His$_6$-MBP to βCD SAMs via Ni•4 can be monovalent, divalent, or trivalent. In Scheme 2 all possible equilibria are presented, assuming the Ni•4 complex forms completely, as discussed in the main text. At βCD SAMs, all His$_6$-MBP units complexed to one Ni•4 will behave as monovalent guests, binding to surface-confined βCD (βCD$_s$) in a similar fashion as to βCD in solution (βCD$_l$). For His$_6$-MBP units that are bound via two or three Ni•4 complexes, the binding to βCD SAMs is governed by an effective concentration term ($C_{ef}$), which is the driving force for the formation of multivalent complexes at βCD SAMs. It has to be noted, that the effective concentration represents the probability that an unused guest site finds a complementary host site and thus incorporates all entropic multivalency factors, including e.g. entropy changes due to conformational losses between the guest sites. For the system at hand, the effective concentration concept provides a rigorous quantitative description of the equilibria involved.$^{[1,2]}

Scheme 2. Equilibria for all species (solution and surface) for the attachment of His$_6$-MBP at the molecular printboard (charges are omitted for clarity). Subsequent complexation steps of Ni•4 to MBP are shown in red, and all surface species are given in green.
A general description is given for the multivalent binding of His$_6$-MBP•(Ni•4)$_x$ (x=1-3) to the molecular printboard. The stepwise adsorption of e.g. His$_6$-MBP•(Ni•4)$_3$ to the surface involves an intermolecular adsorption step and two intramolecular binding steps, the latter of which are both governed by $C_{\text{eff}}$. All solution and surface species of MBP are shown in Scheme 1. All intrinsic stability constants for $\beta$CD$_l$ and $\beta$CD$_s$ are assumed equal for all steps given in Scheme 2.$^{[1]}$

Similar to the binding studies described before,$^{[1,2]}$ SPR titrations performed for the binding of His$_6$-MBP to $\beta$CD SAMs in the presence of Ni•4 are fitted here, yielding $K_l$ values for the His$_6$ tag-Ni•4 interaction, while the intrinsic binding constant of an adamantyl guest to $\beta$CD in solution and the intrinsic binding constant of an adamantyl guest to a surface-confined $\beta$CD cavity, $K_{i,l}$ and $K_{i,s}$, respectively, are fixed to the values determined by ITC and SPR.

The statistical factors relating $K_2$ and $K_3$ for additional Ni•4 were determined by noting that: (i) Ni•4 binds to two neighboring histidines, (ii) binding Ni•4 to His$_6$-MBP is 5 times as likely as binding to a His$_2$ unit (which is the intrinsic interaction motif in this case), (iii) there are 2 or 3 His$_2$ sites free for interaction of a second Ni•4 to MBP•Ni•4 in 60% and 40% of the MBP•Ni•4 complexes, respectively, and (iv) only for 46.7% of the MBP•(Ni•4)$_2$ complexes there is an additional free His$_2$ site available for a third Ni•4 unit (See Scheme 3). This leads to the prefactors of $K_2 = \frac{6}{25} K_l$ and $K_3 = \frac{7}{225} K_l$ as given in Scheme 2.

\[ K_i = 5 K_l \]
\[ K_i = 1.2 K_i = (6/25)K_l \]
\[ K_i = (1.4/9)K_i = (7/225)K_l \]

\[ 2 k_i \]
\[ (0.4 \times 3 + 0.6 \times 2)k_i \]
\[ (0.2 + 0.4 \times 2/3)k_i \]

\[ 3 k_i \]

\[ \text{Scheme 3. The statistical factors relating } K_1, K_2 \text{ and } K_3 \text{ to } K_l. \]
Since all measurements have been performed at pH = 7.5, Ni•4 is always formed completely (see main text). The mass balances that can be constructed based on Scheme 2 are the following (charges are omitted for clarity):

\[
[\text{MBP}]_{\text{tot}} = [\text{MBP}]_{\text{free}} + [\text{MBP}\cdot\text{Ni•4}] + [\text{MBP}\cdot\text{Ni•4}\cdot\beta\text{CD}_1] + [\text{MBP}\cdot\text{Ni•4}\cdot\beta\text{CD}_2] + [\text{MBP}\cdot\text{Ni•4}_2\cdot\beta\text{CD}_1] + [\text{MBP}\cdot\text{Ni•4}_2\cdot\beta\text{CD}_2] + [\text{MBP}\cdot\text{Ni•4}_3\cdot\beta\text{CD}_1] + [\text{MBP}\cdot\text{Ni•4}_3\cdot\beta\text{CD}_2] + [\text{MBP}\cdot\text{Ni•4}_3\cdot\beta\text{CD}_3] + [\text{MBP}\cdot\text{Ni•4}_3\cdot\beta\text{CD}_3\beta\text{CD}_1] + [\text{MBP}\cdot\text{Ni•4}_3\cdot\beta\text{CD}_3\beta\text{CD}_2] + [\text{MBP}\cdot\text{Ni•4}_3\cdot\beta\text{CD}_3\beta\text{CD}_3] + [\text{MBP}\cdot\text{Ni•4}_3\cdot\beta\text{CD}_3\beta\text{CD}_2\beta\text{CD}_1] + [\text{MBP}\cdot\text{Ni•4}_3\cdot\beta\text{CD}_3\beta\text{CD}_2\beta\text{CD}_2] + [\text{MBP}\cdot\text{Ni•4}_3\cdot\beta\text{CD}_3\beta\text{CD}_3\beta\text{CD}_2] + [\text{MBP}\cdot\text{Ni•4}_3\cdot\beta\text{CD}_3\beta\text{CD}_3\beta\text{CD}_3] + [\text{MBP}\cdot\text{Ni•4}_3\cdot\beta\text{CD}_3\beta\text{CD}_3\beta\text{CD}_2\beta\text{CD}_1] + [\text{MBP}\cdot\text{Ni•4}_3\cdot\beta\text{CD}_3\beta\text{CD}_3\beta\text{CD}_2\beta\text{CD}_2] + [\text{MBP}\cdot\text{Ni•4}_3\cdot\beta\text{CD}_3\beta\text{CD}_3\beta\text{CD}_3\beta\text{CD}_2] + [\text{MBP}\cdot\text{Ni•4}_3\cdot\beta\text{CD}_3\beta\text{CD}_3\beta\text{CD}_3\beta\text{CD}_3] + [\text{MBP}\cdot\text{Ni•4}_3\cdot\beta\text{CD}_3\beta\text{CD}_3\beta\text{CD}_3\beta\text{CD}_2\beta\text{CD}_1] + [\text{MBP}\cdot\text{Ni•4}_3\cdot\beta\text{CD}_3\beta\text{CD}_3\beta\text{CD}_3\beta\text{CD}_2\beta\text{CD}_2] + [\text{MBP}\cdot\text{Ni•4}_3\cdot\beta\text{CD}_3\beta\text{CD}_3\beta\text{CD}_3\beta\text{CD}_3\beta\text{CD}_2] + [\text{MBP}\cdot\text{Ni•4}_3\cdot\beta\text{CD}_3\beta\text{CD}_3\beta\text{CD}_3\beta\text{CD}_3\beta\text{CD}_3]
\] (1)
Species involving βCDs are expressed in volume concentrations.\textsuperscript{[1]} The binding of the divalent MBP•(Ni•4\textsuperscript{2+}) and trivalent MBP•(Ni•4\textsuperscript{3+}) to βCD\textsubscript{d} involves statistical factors (Scheme 2) arising from the probabilities for binding relative to the monovalent species, in this case according to a normal 1:3 complexation sequence.
The binding constants for first intermolecular binding events of the divalent and trivalent species at the surface are:

\[
K = \frac{[\text{MBP} \cdot (\text{Ni} \cdot 4)_2 \cdot \beta \text{CD}_{s2}]}{[\text{MBP} \cdot (\text{Ni} \cdot 4)_2][\beta \text{CD}_s]} = 2K_{i,s}
\]  

(6)

\[
K = \frac{[\text{MBP} \cdot (\text{Ni} \cdot 4)_3 \cdot \beta \text{CD}_{s3}]}{[\text{MBP} \cdot (\text{Ni} \cdot 4)_3][\beta \text{CD}_s]} = 3K_{i,s}
\]  

(7)

The second, intramolecular, binding event for the di- and trivalent species, and third, for the trivalent species (equations 9, 10, and 11) are governed by an effective concentration term, which is defined as given in equation 8.\(^{[1,2]}\) The effective concentration is given by multiplying the maximum effective concentration, \(C_{\text{eff, max}}\), which is the number of accessible host sites in the probing volume, with the fraction of free host sites at the surface.

\[
C_{\text{eff}} = C_{\text{eff, max}} \frac{[\beta \text{CD}_s]}{[\beta \text{CD}_s]_{\text{tot}}}
\]  

(8)

\[
K = \frac{[\text{MBP} \cdot (\text{Ni} \cdot 4)_2 \cdot (\beta \text{CD}_{s2})_2]}{[\text{MBP} \cdot (\text{Ni} \cdot 4)_2 \cdot \beta \text{CD}_{s2}][\beta \text{CD}_s]} = \frac{1}{2}C_{\text{eff}} K_{i,s}
\]  

(9)

\[
K = \frac{[\text{MBP} \cdot (\text{Ni} \cdot 4)_3 \cdot (\beta \text{CD}_{s3})_3]}{[\text{MBP} \cdot (\text{Ni} \cdot 4)_3 \cdot \beta \text{CD}_{s3}][\beta \text{CD}_s]} = C_{\text{eff}} K_{i,s}
\]  

(10)

\[
K = \frac{[\text{MBP} \cdot (\text{Ni} \cdot 4)_3 \cdot (\beta \text{CD}_{s3})_3]}{[\text{MBP} \cdot (\text{Ni} \cdot 4)_3 \cdot \beta \text{CD}_{s3}][\beta \text{CD}_s]} = \frac{1}{3}C_{\text{eff}} K_{i,s}
\]  

(11)

Since the SPR experiments were performed in a flow system, all solutions species concentrations can be calculated from simplified forms of equations 1-3 and 5. After numerical optimization of these equations, the values obtained for the solution species concentrations were used in the full equations 1-5 for calculations of the surface species.
Substitution of the equilibrium constant definitions into the mass balances for [MBP]\(_{\text{tot}}\), [βCD\(_s\)]\(_{\text{tot}}\), [βCD\(_l\)]\(_{\text{tot}}\), [3]\(_{\text{tot}}\), and [4]\(_{\text{tot}}\) (equations 1-5) provides a set of numerically solvable equations with [MBP], [βCD\(_s\)], [βCD\(_l\)], [3], and [4] as the variables.

Starting from an initial estimate for \(K_i\) (defined as: \(K_i = \frac{[\text{MBP} \cdot (\text{Ni} \cdot 4)]}{[\text{MBP}][\text{Ni} \cdot 4]}\) using fixed values for \(C_{\text{eff, max}}\) (0.1 M) and the other stability constants, this set of equations is solved numerically using a Simplex algorithm in a spreadsheet approach.\(^3\) When fitting SPR data, \(K_i\) is optimized in a least-squares optimization routine, assuming that the SPR response (intensity) is linearly dependent on the coverages of MBP, 3, and 4 adsorbed to the βCD SAM, regardless of the type of species. The maximum intensity (\(I_{\text{max}}\) of MBP) is then optimized as an independent fitting parameter as well while those of 3 and 4 were determined by independent SPR measurements.

Based on Scheme 1, the overall stability constant for His\(_6\)·MBP·(Ni·4)\(_s\)·(βCD)\(_3\) can be given by equation 12:

\[
K = (K_{i,s})^3 K_i K_2 K_3 C_{\text{eff}}^2 [\text{Ni} \cdot 4]^3
\]

Assuming \(K_{i,s} = 1.2 \times 10^4 \, \text{M}^{-1}\), \(K_i = 7.8 \times 10^3 \, \text{M}^{-1}\) (\(K_2 = \frac{6}{25} K_i\) and \(K_3 = \frac{7}{225} K_i\)), \(C_{\text{eff}} = C_{\text{eff, max}} = 0.1 \, \text{M}\) (at relatively low coverages), and \([\text{Ni} \cdot 4] = 1 \, \mu\text{M}\), an apparent conditional binding constant of ~ \(10^5 \, \text{M}^{-1}\) can be estimated.

References