Ligand Exchange

Deutsche Ausgabe: DOI: 10.1002/ange.201508252
Internationale Ausgabe: DOI: 10.1002/anie.201508252

Spectra Library: An Assumption-Free In Situ Method to Access the Kinetics of Catechols Binding to Colloidal ZnO Quantum Dots

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***Abstract:** Assumption-free and in situ resolving of the kinetics of ligand binding to colloidal nanoparticles (NPs) with high time resolution is still a challenge in NP research. A unique concept of using spectra library and stopped-flow together with a "search best-match" Matlab algorithm to access the kinetics of ligand binding in colloidal systems is reported. Instead of deconvoluting superimposed spectra using assumptions, species absorbance contributions (ligand@ZnO NPs and ligand in solution) are obtained by offline experiments. Therefrom, a library of well-defined targets with known ligand distribution between particle surface and solution is created. Finally, the evolution of bound ligand is derived by comparing in situ spectra recorded by stopped-flow and the library spectra with the algorithm. Our concept is a widely applicable strategy for kinetic studies of ligand adsorption to colloidal NPs and a big step towards deep understanding of surface functionalization kinetics.*

Surface functionalization of colloidal nanoparticles (NPs) by organic or inorganic ligands has been widely applied. However, resolving the interplay between particle surface and ligand is still highly challenging.[1] Although a variety of methods have been established to investigate the ligand adsorption, little is known about the kinetic aspect in colloidal NP systems.

There are some case studies on kinetics of ligand adsorption to colloidal particles, which include (1D, 2D, continuous) nuclear magnetic resonance (NMR),[2] electron paramagnetic resonance (EPR),[3] UV/Vis spectroscopy,[4] time-correlated single photon counting spectroscopy (TCSPC),[5] photoluminescence (PL),[6] resonance elastic light scattering (RELS),[7] and surface-enhanced Raman scattering (SERS),[8] as well as gas chromatography (GC) for the supernatant.[9] However, all these strategies suffer from one or more of the following disadvantages: 1) the required assumption that the extremum of the signal corresponds to full coverage is not necessarily correct, as adsorption sites might be blocked by steric hindrance or ligands remaining from synthesis; 2) low time resolution; 3) no defined target property of functionalized NPs that can be quantitatively evaluated (most of the approaches are based on colloidal gold particles which have as a kind of fortunate singularity only one well-defined surface plasmon resonance (SPR) peak); and 4) tedious sample preparation. Therefore, none of these methods is generally applicable for time-resolved kinetics in colloidal particle–ligand systems. Meanwhile, knowledge of adsorption kinetics is of vital importance regarding the generation of NPs with specific surface properties in applications. Therefore, assumption-free, direct access of bound and free ligand with high time resolution is urgently needed.

To address this important question, a widely applicable strategy to study the kinetics of ligand adsorption to colloidal surfaces was established by using spectra library. To show the capability of this approach, it is applied on catechol derivative (ethyl-3,4-dihydroxybenzoate (CAT), Figure 1-I inset) binding to ZnO quantum dots (QDs). ZnO is of high technical relevance and has not been studied quantitatively in terms of ligand adsorption kinetics. CAT was chosen as it is an excellent candidate anchor group in biomedical applications and dye-sensitized solar cells (DSSCs).[10] Moreover, the interaction between ZnO and CAT changes the extinction coefficient of both, ZnO QDs and bound CAT. As indicated by two black vertical lines in Figure 1-II, the two peaks of free CAT show a clear red-shift that is due to the electron delocalization over the aromatic ring that occurs when CAT molecules bind to the ZnO surface.[1a,c] Thus, on the one hand, CAT@ZnO can be seen as a promising model system of technical relevance, which on the other hand already represents a challenging scenario of high complexity that can still be handled by our approach. The advantages of our concept are: 1) the absolute amounts of bound and free CAT are directly obtained from inline measurements; 2) no predefined models are needed; and 3) high time resolution (12 ms).

Our approach is initialized by a problem and based on an important finding. Regarding the former, when ZnO QDs are mixed with CAT solution, the individual absorbance contributions of ZnO QDs and bound CAT cannot be resolved due to the aforementioned interaction between them (Supporting Information, Figure S4A). Regarding the latter, we confirmed by means of multiwavelength analytical ultracentrifugation (AUC)[11] that the equilibrium dissociation of CAT between NP surface and solution does not depend on the absolute concentration of ligand that is provided. In fact, it is solely determined by the ratio of QD surface area and added monolayers (MLs) of CAT (see the Supporting Information, page 1-2 of Ref. [1c] for how MLs are calculated with taking the full particle size distribution (PSD) into consideration). This means that when the number of CAT MLs at the ZnO surface is fixed at a certain ratio of ligand to surface area, the absorbance of the whole, ZnO with bound CAT at the surface,

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Supporting information and ORCID(s) from the author(s) for this article are available on the WWW under http://dx.doi.org/10.1002/anie.201508252.

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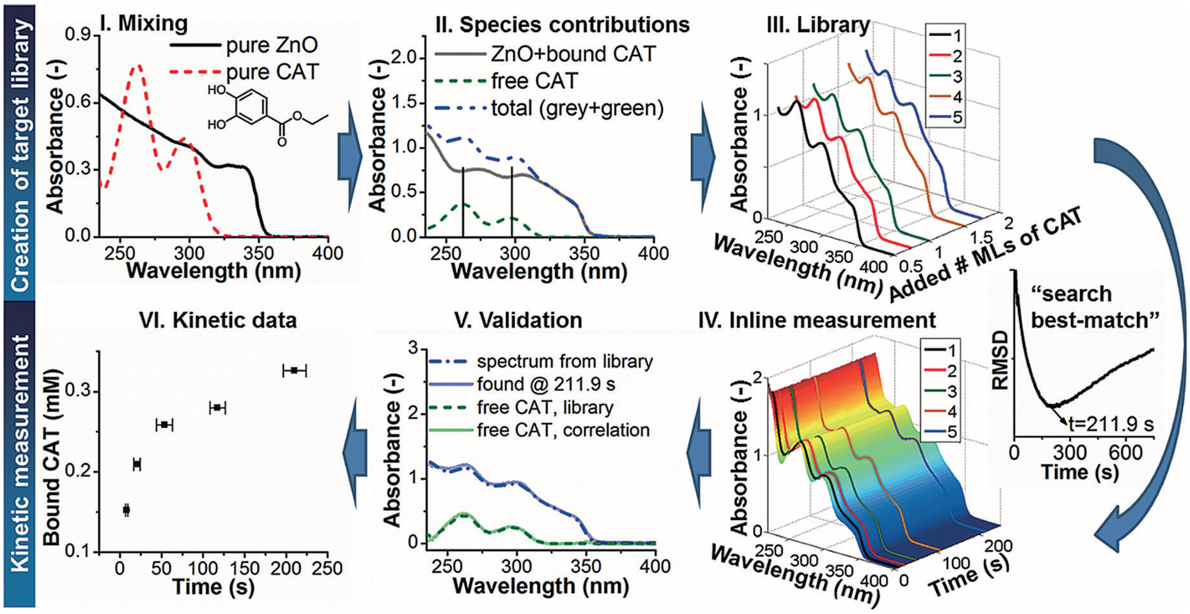

Figure 1. Summary of the method. I) A given amount of ZnO QDs (black ——) and pure CAT provided in solution (red -----) are mixed at a concentration equivalent to a defined number of monolayers (MLs). II) The absorbance contributions of ZnO + bound CAT (gray ——, the red-shift of two free CAT peaks are indicated by two vertical black lines) and free CAT (green -----) are obtained according to Equations (2) and (4). Then, total spectrum is generated by summation of them (blue –•–•). III) By applying step II to added 0.5 MLs (black), 0.75 MLs (red), 1 ML (green), 1.5 MLs (orange), 1.75 MLs (blue) of CAT samples, a library of spectra is created. IV) During the kinetic experiments, the UV/Vis spectra are monitored inline after mixing in stopped-flow. A "search best-match" algorithm (inset) was used to find the time of best match between all stopped-flow spectra and each library spectrum (for example, the closest spectrum found in inline measurement for spectrum number 5 from the library is at 211.9 s). V) Comparison between spectrum from library (blue –•–•) and spectrum found in inline measurement (light blue ——) as well as between free CAT, library derived from Equations (3) and (4) (green -----) and free CAT, correlation derived from Equation (6) (light green ——). VI) Evolution of bound CAT over time. The error bars are from a total of 9 repeated stopped-flow measurements at identical conditions.

correlates linearly with the concentration of ZnO (with respect to Zn^{2+}, the same hereafter). This holds true at least within the linear range of the Lambert–Beer law that is valid throughout our experiments. Thus, the total absorbance (scattering can be neglected owing to the small particle size with a balanced mean $x_{1,3}$ of 4.25 nm) in the region of chemisorption can be calculated as follows:

$$A_{\mathrm{tot}} = \varepsilon_{\mathrm{ZnO+bound\,CAT}}(\lambda, \mathrm{MLs})\, c_{\mathrm{ZnO}}\, d + \varepsilon_{\mathrm{pure\,CAT}}(\lambda)\, c_{\mathrm{free\,CAT}}\, d \tag{1}$$

where A_{tot} is the total absorbance of the mixture of ZnO colloid and CAT solution, d is the optical path length of the cuvette [mm], $\varepsilon_{\mathrm{ZnO+bound\,CAT}}(\lambda, \mathrm{MLs})$ is the extinction coefficient (with respect to the Zn^{2+} concentration) of ZnO QDs of given size with certain MLs of bound CAT at the surface [$\mathrm{L\,mol^{-1}\,mm^{-1}}$], $\varepsilon_{\mathrm{pure\,CAT}}(\lambda)$ is the extinction coefficient of pure CAT in EtOH [$\mathrm{L\,mol^{-1}\,mm^{-1}}$], and c_{ZnO} and $c_{\mathrm{free\,CAT}}$ are the concentrations of ZnO and free CAT [$\mathrm{mol\,L^{-1}}$]. The first term of Equation (1) will be referred to as $A_{\mathrm{ZnO+bound\,CAT,library}}$ whereas the second term of Equation (1) will be referred to as $A_{\mathrm{free\,CAT,\,library}}$.

In the following, we will describe how the unknown parameters of Equation (1) can be determined to make kinetics accessible. The procedure is sketched in Figure 1. Before doing any kinetic measurement, a library is created based on the chemisorption isotherm, which includes the extinction coefficients and thus absorbance spectra for numerous adsorption states (Figure 1 upper row).

First, samples with defined ZnO QDs of known surface area (Figure 1-I, black line) are mixed with dissolved CAT (Figure 1-I, red dashed line) equivalent to a defined number of MLs.

Second, after the equilibrium is reached, unbound CAT is removed by washing. The absolute amount of ZnO can be determined by titration with 2 $\mathrm{mol\,L^{-1}}$ HCl until all the absorbance features of ZnO are not detected anymore (Supporting Information, Section 4). Knowing the ZnO concentration, the extinction coefficient of ZnO with certain MLs of CAT at the surface ($\varepsilon_{\mathrm{ZnO+bound\,CAT}}(\lambda, \mathrm{MLs})$) is obtained (Supporting Information, Section 4). From this, the absorbance of ZnO with bound CAT [cf. Equation (1)] is calculated according to the Lambert–Beer law:

$$A_{\mathrm{ZnO+bound\,CAT,\,library}} = d\, \varepsilon_{\mathrm{ZnO+bound\,CAT}}(\lambda, \mathrm{MLs})\, \frac{1}{2}\, c_{\mathrm{ZnO,\,feed}} \tag{2}$$

where $A_{\mathrm{ZnO+bound\,CAT,\,library}}$ is the absorbance of ZnO with bound CAT (no CAT in solution) when the ZnO concentration is identical with half the feed concentration (due to mixing with CAT solution) during stopped-flow analysis (Figure 1-II, gray line), $c_{\mathrm{ZnO,\,feed}}$ is the feed concentration of ZnO (0.0109 $\mathrm{mol\,L^{-1}}$), and as mentioned, the factor 0.5 accounts for the volumetric mixing ratio of ZnO suspension and CAT solution of 1:1.

Moreover, after washing and dissolution of the particles with HCl, the absolute amount of bound CAT (released from the surface) can be derived. It is determined spectroscopically

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using the known extinction coefficient of pure CAT in ethanol as the dissolved Zn^{2+} does not affect the absorbance of CAT (Supporting Information, Figure S4). Thus, the ratio of ZnO to bound CAT for the library experiment is known. Owing to the fact that this ratio does not change within the applied absolute concentrations (confirmed by AUC; Supporting Information, Section 3), the bound CAT in terms of MLs remains constant. Hence, the concentration of free CAT at stopped-flow conditions is accessible:

$$c_{\text{free CAT, library}} = \frac{1}{2} c_{\text{CAT, feed}} \frac{N_{\text{MLs, feed}} - N_{\text{MLs, bound}}}{N_{\text{MLs, feed}}} \tag{3}$$

where $c_{\text{free CAT, library}}$ is the concentration of free CAT in solution at stopped-flow conditions, $c_{\text{CAT, feed}}$ is the concentration of CAT that is applied in the stopped-flow experiment ($1.45\ \text{mmol}\,\text{L}^{-1}$), $N_{\text{MLs, feed}}$ is the provided total number of CAT MLs in relation to the feed of ZnO that is applied in stopped-flow, $N_{\text{MLs, bound}}$ is the number of MLs that is bound at the ZnO surface, and the factor 0.5 accounts again for the volumetric mixing ratio of ZnO suspension to CAT solution of 1:1. With known concentration of free CAT at stopped-flow conditions, the absorbance of free CAT during the stopped-flow experiment ($A_{\text{free CAT, library}}$; Figure 1-II, green dashed line) can be calculated [cf. Equation (1)]:

$$A_{\text{free CAT, library}} = d\, \varepsilon_{\text{pure CAT}}(\lambda)\, c_{\text{free CAT, library}} \tag{4}$$

As the absorbance contributions of both ZnO with bound CAT ($A_{\text{ZnO+bound CAT, library}}$) and free CAT ($A_{\text{free CAT, library}}$) are now quantitatively known, the sum of them gives (according to Equation (1)) a so-called target spectrum with quantitatively known distribution of CAT@ZnO and free CAT in solution (Figure 1-II, blue dot–dash line). Thus, a correlation between the state of adsorption and its optical response is established.

This procedure is repeated multiple times with different MLs of CAT added to ZnO NPs (from 0.5 MLs to 1.75 MLs; Supporting Information, Table S1). This leads to a library of target spectra with known absolute concentrations of bound and free ligand (Figure 1-III).

Once the library has been created, kinetic measurements can be conducted and evaluated (Figure 1 lower row). Spectra of ZnO QDs during functionalization were recorded inline by highly dynamic stopped-flow UV/Vis spectroscopy (Figure 1-IV, time resolution of 12 ms). ZnO QDs used in inline measurements have the same surface properties as in the library preparation. A "search best-match" algorithm (Matlab) was used to find the exact points in time where a best match between all stopped-flow spectra and each target spectrum of the library (1–5) is identified according to:

$$t = \min_{t} \left(\sqrt{\frac{1}{n} \sum_{\lambda} \left[A_{\text{stopped-flow}}(t,\lambda) - A_{\text{library}}(\lambda) \right]^2} \right) \tag{5}$$

so for each target spectrum in the library ($A_{\text{library}}(\lambda)$), the time t is searched when the stopped-flow absorbance spectrum ($A_{\text{stopped-flow}}(t, \lambda)$) has minimum root-mean-square deviation (RMSD; the term in the brackets) from the corresponding library spectrum of n wavelengths recorded between 250 and 360 nm. This is exemplarily illustrated in the "search best-match" part of Figure 1 (inset, right).

For instance, if spectrum number 5 of the library (Figure 1-III, blue line) is used as target, the best match is found at 211.9 s (see the Supporting Information, Section 6 for others). Thus, every time found by the "search best-match" algorithm is a very well-defined data point that can be used for establishing a kinetic model. Being able to create a sufficiently large number of library spectra, kinetics of ligand chemisorption can be easily established without assuming any adsorption model.

The five spectra of the inline measurement showing a best match with the five targets are summarized in Figure 1-IV. In the future, much larger target datasets, for example, by high-throughput techniques, and evolutions of extinction coefficients $\varepsilon_{\text{ZnO+bound CAT}}(\lambda, \text{MLs})$ based on these might be realized to improve resolution and thus the identification of suitable kinetic models.

To validate the "search best-match" algorithm, the spectrum found in the inline measurement at 211.9 s (Figure 1-V, light blue line) is visually compared with the spectrum number 5 from the library (Figure 1-V, blue dot–dash line). Moreover, the spectrum of free CAT derived from Equations (3) and (4) (Figure 1-V, green-dashed line) and the free CAT derived from Equation (6) using the found stopped-flow spectrum (Figure 1-V, light green line) are also checked:

$$A_{\text{free CAT, correlation}} = A_{\text{found}} - A_{\text{ZnO+bound CAT, library}} \tag{6}$$

where $A_{\text{free CAT, correlation}}$ is the absorbance of free CAT and A_{found} is the spectrum found within the stopped-flow data to have a best match with the target spectrum of the library (current example is at 211.9 s). The agreement is excellent as the R^2 values for these 2 comparisons are 0.998 and 0.988, respectively (see the Supporting Information, Section 6 for the comparison of others with a minimum R^2 of 0.989 for the former and 0.964 for the latter, respectively). Of note, the peak positions of the free CAT derived from Equation (6) are all identical with pure CAT in EtOH.

Finally, a first kinetic dataset (the evolution of bound CAT over time) determined by our technique is shown in Figure 1-VI. Noteworthy, as the points show pure measurement data, no predefined kinetic models were necessary. To demonstrate the versatility and vital future role of this concept, a total of seven commonly used adsorption models were applied to analyze the data (see the Supporting Information, Section 10 for details and fitting equations as well as Section 11 for boundary conditions and generalization of our approach).[12]

In summary, the method of using well-established libraries of target spectra that are derived by means of careful offline analysis within large kinetic datasets can be used for any particle–ligand system, even if a strong interference between the particles and the ligand molecules does exist. This is of paramount importance as kinetic data are directly accessible in situ with much higher time resolution and much less experimental effort compared to previously reported techniques. We believe that our concept will affect fundamental research as well as various emerging fields where tailored liquid-borne particle surfaces are needed, for instance optoelectronic devices like LEDs or sensors, bioimaging, and energy conversion.

Acknowledgements

The authors acknowledge the funding of the Deutsche Forschungsgemeinschaft (DFG) through the Cluster of Excellence "Engineering of Advanced Materials" and research training group "Disperse Systems for Electronic Applications" GRK 1161 and DFG project PE427/28-1 as well as Dr. T. Thajudeen and Prof. D. Zahn for fruitful discussion.

Keywords: colloids · kinetics · ligand exchange · nanoparticles · surface analysis

How to cite: *Angew. Chem. Int. Ed.* **2016**, *55*, 932–935
Angew. Chem. **2016**, *128*, 944–947

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[1] a) R. Marczak, F. Werner, J. F. Gnichwitz, A. Hirsch, D. M. Guldi, W. Peukert, *J. Phys. Chem. C* **2009**, *113*, 4669–4678; b) V. Lesnyak, N. Gaponik, A. Eychmüller, *Chem. Soc. Rev.* **2013**, *42*, 2905–2929; c) W. Lin, J. Walter, A. Burger, H. Maid, A. Hirsch, W. Peukert, D. Segets, *Chem. Mater.* **2015**, *27*, 358–369; d) J. De Roo, Y. Justo, K. de Keukeleere, F. van den Broeck, J. C. Martins, I. van Driessche, Z. Hens, *Angew. Chem. Int. Ed.* **2015**, *54*, 6488–6491; *Angew. Chem.* **2015**, *127*, 6588–6591.

[2] a) B. Fritzinger, R. K. Capek, K. Lambert, J. C. Martins, Z. Hens, *J. Am. Chem. Soc.* **2010**, *132*, 10195–10201; b) Z. Hens, I. Moreels, J. C. Martins, *ChemPhysChem* **2005**, *6*, 2578–2584; c) R. Guo, Y. Song, G. Wang, R. W. Murray, *J. Am. Chem. Soc.* **2005**, *127*, 2752–2757.

[3] M. Zachary, V. Chechik, *Angew. Chem. Int. Ed.* **2007**, *46*, 3304–3307; *Angew. Chem.* **2007**, *119*, 3368–3371.

[4] a) K. Siriwardana, M. Gadogbe, S. M. Ansar, E. S. Vasquez, W. E. Collier, S. Zou, K. B. Walers, D. Zhang, *J. Phys. Chem. C* **2014**, *118*, 11111–11119; b) Y. Wang, O. Zeiri, A. Neyman, F. Stellacci, I. A. Weinstock, *ACS Nano* **2012**, *6*, 629–640.

[5] H. Xie, A. G. Tkachenko, W. R. Glomm, J. A. Ryan, M. K. Brennaman, J. M. Papanikolas, S. Franzen, D. L. Feldheim, *Anal. Chem.* **2003**, *75*, 5797–5805.

[6] X. Ji, D. Copenhaver, C. Sichmeller, X. Peng, *J. Am. Chem. Soc.* **2008**, *130*, 5726–5735.

[7] M. Stobiecka, K. Coopersmith, M. Hepel, *J. Colloid Interface Sci.* **2010**, *350*, 168–177.

[8] A. Orthaber, H. Löfås, E. Öberg, A. Grigoriev, A. Wallner, S. H. M. Jafri, M. P. Santoni, R. Ahuja, K. Leifer, H. Ottoson, S. Ott, *Angew. Chem. Int. Ed.* **2015**, *54*, 10634–10638; *Angew. Chem.* **2015**, *127*, 10780–10784.

[9] A. Kassam, G. Bremner, B. Clark, G. Ulibarri, R. B. Lennox, *J. Am. Chem. Soc.* **2006**, *128*, 3476–3477.

[10] J. F. Gnichwitz, R. Marczak, F. Werner, N. Lang, N. Jux, D. M. Guldi, W. Peukert, A. Hirsch, *J. Am. Chem. Soc.* **2010**, *132*, 17910–17920.

[11] J. Walter, K. Löhr, E. Karabudak, W. Reis, J. Mikhael, W. Peukert, W. Wohlleben, H. Cölfen, *ACS Nano* **2014**, *8*, 8871–8886.

[12] Y. Ho, *J. Hazard. Mater.* **2006**, *136*, 681–689.

Received: September 3, 2015
Revised: October 26, 2015
Published online: December 7, 2015

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