Mapping out the Aqueous Surface Chemistry of Metal Oxide Nanocrystals: Carboxylate, Phosphonate, and Catecholate Ligands

Loren Deblock, Eline Goossens, Rohan Pokratath, Klaartje De Buysse, and Jonathan De Roo*

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ABSTRACT: Iron oxide and hafnium oxide nanocrystals are two of the few successful examples of inorganic nanocrystals used in a clinical setting. Although crucial to their application, their aqueous surface chemistry is not fully understood. The literature contains conflicting reports regarding the optimum binding group. To alleviate these inconsistencies, we set out to systematically investigate the interaction of carboxylic acids, phosphonic acids, and catechols to metal oxide nanocrystals in polar media. Using nuclear magnetic resonance spectroscopy and dynamic light scattering, we map out the pH-dependent binding affinity of the ligands toward hafnium oxide nanocrystals (an NMR-compatible model system). Carboxylic acids easily desorb in water from the surface and only provide limited colloidal stability from pH 2 to pH 6. Phosphonic acids, on the other hand, provide colloidal stability over a broader pH range but also feature a pH-dependent desorption from the surface. They are most suited for acidic to neutral environments (pH <8). Finally, nitrocatechol derivatives provide a tightly bound ligand shell and colloidal stability at physiological and basic pH (pH 6−10). Whereas dynamically bound ligands (carboxylates and phosphonates) do not provide colloidal stability in phosphate-buffered saline, the tightly bound nitrocatechols provide long-term stability. We thus shed light on the complex ligand binding dynamics on metal oxide nanocrystals in aqueous environments. Finally, we provide a practical colloidal stability map, guiding researchers to rationally design ligands for their desired application.

KEYWORDS: nanoparticle, catechol, carboxylic acid, phosphonic acid, hafnium oxide

INTRODUCTION

Colloidal nanocrystals (NCs) have been considered for a multitude of biomedical applications, such as bioimaging, drug delivery, photothermal therapy, and radiotherapy enhancement. These NCs are typically hybrid objects, consisting of an inorganic core capped with organic ligands. Inorganic ligands are more useful for devices. Ligands determine the interaction between NC and solvent and the stability of the nanocolloid. In the case of biomedical applications, controlling the NC surface chemistry is key as it will play a role in particle agglomeration, cellular uptake, protein repelling or adsorption, cytotoxicity, circulation time, and targeted approaches. Although surface chemistry is important for all types of NCs (chalcogenides, pnictides, halides, and metal NCs), not one solution fits all. For example, thiolates and thiols have a strong binding affinity to Au and CdSe NCs but interact poorly with metal oxide NCs.

Metal oxide NCs have been particularly successful in nanomedicine. Three types of inorganic NCs have achieved clinical translation, and two of them are oxides: iron oxide and hafnium oxide. These particles are often first synthesized in nonpolar solvents and stabilized by surfactants (usually with a carboxylate or phosphonate headgroup and an aliphatic tail). Carboxylic acids (e.g., oleic acid) dissociate on the metal oxide surface, with carboxylates binding to surface metal sites and protons to surface oxygen atoms. This binding motif is written as NC(XX′) as both proton and carboxylate are X-type ligands. In nonpolar solvents, carboxylic acids are quantitatively exchanged by phosphonic acids in an X-for-X ligand exchange process. Indeed, phosphonic acids are very strong ligands for oxide surfaces. In contrast, catechol was found to be a rather weak ligand, only able to exchange a minor fraction of oleic acid. There is thus a clear order in binding strength in nonpolar solvents: catechol < carboxylic acid < phosphonic acid.

In aqueous (or other polar) environments, the order is less clear and further complicated by variable factors such as pH and salt concentration. For example, carboxylic acids are
frequently used to stabilize metal oxide NCs in water.\textsuperscript{30–34} They are able to provide colloidal stability in static systems with no competing ligands but not in phosphate-buffered saline (PBS) or cell culture media.\textsuperscript{34} The binding affinity is significantly increased for multidentate carboxylate ligands such as polymers.\textsuperscript{35,36} In general, literature reports agree that carboxylic acids are the weakest ligands in water, weaker than phosphonic acids or catechols.\textsuperscript{37} The literature is, however, inconclusive as to whether phosphonic acids or catechols are the best ligand. For example, Guénin et al. concluded from Fourier transform infrared spectroscopy and density functional theory calculations that caffeic acid (a catechol) has a higher affinity for 9 nm iron oxide NCs than a bisphosphonate at physiological pH.\textsuperscript{38} In contrast, Zeininger et al. determined that phosphonic acids bind more strongly than catechols to TiO\textsubscript{2} NCs in isopropyl alcohol at pH 7.\textsuperscript{39} Okada et al. came to the same conclusion on 9 nm iron oxide NCs by performing phase-transfer experiments using polar and nonpolar phosphonic acids and catechols.\textsuperscript{40} It is clear that, despite the common usage of phosphonic acids and catechols in metal oxide NC functionalization, there is no clear consensus on the relative binding affinity. Furthermore, a direct link between ligand binding equilibria and the final colloidal stability of the NCs is usually not made.

In this paper, we aim to unambiguously establish the binding affinity order and provide the correct surface chemistry for optimal application in nanomedicine. We chose HfO\textsubscript{2} NCs as our model system for two reasons: (1) it is a relevant material in nanomedicine, and (2) it is compatible with solution nuclear magnetic resonance (NMR) spectroscopy. The latter has proven to be a very powerful tool to study nanocrystal surface chemistry,\textsuperscript{41–43} providing complementary information to solid-state NMR spectroscopy.\textsuperscript{33,48} Unfortunately, iron oxide NCs interfere with magnetic fields and cannot be studied in NMR. HfO\textsubscript{2} NCs are thus an ideal starting point also because their surface chemistry has already been extensively studied in nonpolar solvents using NMR spectroscopy.\textsuperscript{22,23} First, we evaluate the ligand exchange of the native carboxylic acid ligands for phosphonic acid and catechol ligands, using \textsuperscript{1}H and \textsuperscript{31}P NMR spectroscopy. Importantly, we use the same polyethylene glycol ligand chain for all three binding groups, thus ensuring that we can directly compare the binding groups to each other. Next, we assess the influence of solvent (methanol vs water) and the pH on ligand binding. Furthermore, we used NMR and dynamic light scattering (DLS) to determine the colloidal stability provided by the different ligand types in aqueous and buffer environments and directly correlate this to ligand binding dynamics. Finally, we constructed a colloidal stability map, showing which binding group provides colloidal stability as a function of pH. This practical guide will help researchers in designing future surface chemistries.

## RESULTS

**HfO\textsubscript{2}-MEEAA Model System**

We synthesized HfO\textsubscript{2} nanocrystals (NCs) from hafnium tert-butoxide and benzyl alcohol at 220 °C via an established solvothermal process (Figure 1A).\textsuperscript{47} The HfO\textsubscript{2} surface was functionalized with 2-[2-(2-methoxyethoxy)ethoxy]acetic acid (MEEAA; see Figure 1B) to stabilize the nanocrystals in toluene, and all unbound ligands were removed as described previously.\textsuperscript{48} The nanocrystals have a diameter of 2.64 ± 0.81 nm (μ ± σ) according to transmission electron microscopy (TEM) (Figure 1C) and possess the monoclinic (P2\textsubscript{1}/c) crystal structure according to X-ray total scattering and pair distribution function (PDF) analysis (Figure S1). The \textsuperscript{1}H NMR spectrum of the nanocrystal dispersion in toluene-d\textsubscript{8} only shows broadened resonances, assigned to bound ligands (Figure 1B). Indeed, spectral broadening is a typical attribute of bound ligands due to both homogeneous broadening (T\textsubscript{2} relaxation) and heterogeneous broadening (imperfect solvation of the ligand shell).\textsuperscript{45}

The nanocrystals can also be dispersed in ethanol, methanol, and water and are thus an ideal starting point for our investigation into ligand binding behavior in polar solvents. In methanol-d\textsubscript{4} (MeOD), the \textsuperscript{1}H NMR spectrum looks markedly different, with sharp signals superimposed on the broad resonances (Figure 1B). We assign the sharp signals to autodesorbed ligands, corroborated by the appearance of two sets of resonances in diffusion ordered spectroscopy (DOSY, Figure S2). DOSY allows one to separate (overlapping) NMR resonances according to their diffusion coefficient and thus separates the free ligands (quickly diffusing) from ligands bound to NCs (slowly diffusing).\textsuperscript{46} In Figure S2, the resonances with a diffusion coefficient D = 95 m\textsuperscript{2}/s represent the bound ligands, and the resonances with D = 450 m\textsuperscript{2}/s belong to the free ligands. We can selectively observe the bound ligands in a diffusion-filtered spectrum (Figure 1B), and upon close inspection, one observes that the bound MEEAA resonances in methanol-d\textsubscript{4} are slightly sharper than the ones in toluene-d\textsubscript{8}, indicating a better solvation of the ligand shell in methanol.\textsuperscript{48} Given that all ligands were bound in toluene, we infer that the solvent clearly plays a role in the adsorption–desorption equilibrium, for instance, by changing the solubility of the ligand.\textsuperscript{49} Indeed, even a higher fraction of MEEAA ligands desorbs in water, D\textsubscript{2}O (see Figure S3), but the nanocrystals remain visibly stable in the pH range of 2–6. The NCs quickly precipitate at pH >6, and therefore, MEEAA is
not a suitable ligand for many biomedical applications, which typically require stability at physiological conditions (pH 7.4).

Note that, aside from the main MEEAA signals, we also observed broad resonances with low intensity in the aromatic region of the $^1$H spectrum (Figure S4), assigned to benzoate ligands. Benzoic acid was previously identified as a side product of the nanocrystal synthesis and was found to be adsorbed on the nanocrystal surface.47 Functionalization of the surface with MEEAA after synthesis clearly did not remove all benzoate from the surface, and a small fraction remains present.

**Competitive Binding of Phosphonic Acids**

To evaluate the binding strength of phosphonic acids in methanol, we chose (2-(2-(2-hydroxyethoxy)ethoxy)ethyl)-phosphonic acid (PA-PEG) as a ligand with structure comparable to that of MEEAA (Figure 2A). In the $^1$H NMR spectrum, resonance 1 of PA-PEG has a chemical shift of 2.05 ppm, clearly separated from the MEEAA resonances, allowing us to selectively monitor the binding of PA-PEG (Figure 2B). As MEEAA has a methoxy group and PA-PEG does not, resonance $f$ is used to gain selective information about the binding of MEEAA. The other resonances (2–6 and b–e) overlap.

Starting from MEEAA-stabilized HfO$_2$ nanocrystals in methanol-$d_4$, we add PA-PEG in steps while monitoring the $^1$H NMR spectrum; see Figure 2B (full range spectra and additional titration points in Figure S5). During the titration, we observe the gradual appearance of a broad resonance around 2.2 ppm, assigned to resonance $f$ of bound PA-PEG. Concomitantly, resonance $f$ becomes more narrow (Figure 2B), indicating the removal of MEEAA from the nanocrystal surface. Within the aromatic region, we also observe desorption of benzoic acid (Figure S5). We conclude that PA-PEG is effectively displacing MEEAA and benzoic acid. Given the absence of free phosphonic acid, the exchange is quantitative for most of the titration. After addition of 1.3 equiv of phosphonic acid, sharp signals appear in the same region, indicating that there is now free PA-PEG present. The same conclusions are drawn from the $^{31}$P spectra, as well (Figure 2C), where first a broad signal grows in intensity and a sharp $^{31}$P signal appears after adding more than 1 equiv. Interestingly, the bound PA-PEG resonance still increases slightly in intensity between 1.3 and 1.6 equiv. We infer that the last part of the exchange does not proceed quantitatively, and the remaining carboxylate ligands are more difficult to remove. This observation implies that the binding affinity

**Figure 2.** (A) Ligand exchange performed between MEEAA-functionalized NCs and PA-PEG. (B) $^1$H NMR reference spectra in MeOD of the free ligands as reference and the stepwise titration of MEEAA-functionalized NCs with PA-PEG, equivalents are with respect to the total amount of MEEAA present. (C) $^{31}$P NMR spectra (4096 scans) in MeOD for the stepwise titration of MEEAA-functionalized NCs with PA-PEG; broadened signals are indicative of NC binding. (D) Diffusion-filtered $^1$H NMR spectra of MEEAA-functionalized NCs in MeOD after addition of 1.3 equiv of PA-PEG. Signals arising from bound MEEAA are denoted in red, and signals arising from PA-PEG are denoted in striped blue. $C_{NC} = 1210 \, \mu$mol L$^{-1}$, corresponding to 34 mg NCs of this size in 0.5 mL of MeOD. Resonances denoted as * are unidentified impurities.
Unfortunately, the NMR spectra feature a lot of spectral overlap, with superimposed signals of free and bound ligands. At the end of the titration, the resonances of free ligands dominate the spectrum. To gain more insight into the composition of the ligand shell at that point, we turn to the diffusion-filtered spectrum (Figure 2D, full range in Figure S6). Whereas resonances 2–6 overlap with resonances b–e, the clear presence of resonance f indicates the presence of residual MEEAA on the surface. Based on the diffusion-filtered profile of bound MEEAA (Figure S4), we assigned the part of the spectrum belonging to MEEAA as the red shaded area. The blue striped area is assigned to PA-PEG. There is also still benzoate present on the surface according to the regular $^1$H spectrum, but the signal-to-noise of these resonances was too low in the diffusion-filtered spectrum due to their faster $T_2$ relaxation (because of rigidity and proximity to the surface). The above results thus confirm that the exchange between the carboxylate ligands and PA-PEG does not go to completion in methanol. This conclusion stands in contrast to the relative binding affinities of fatty acids and alkylphosphonic acids in nonpolar solvents, where phosphonic acids quantitatively replace carboxylic acids in a 1:1 stoichiometry.49

The autodesorption of MEEAA in methanol already indicated that the ligand solubility can change the binding affinity. Indeed, ligand binding is an equilibrium process, governed by the chemical potential of each species:

$$\text{NC}^*(L) + L \rightleftharpoons \text{NC}^*(L) \quad (1)$$

Therefore, this adsorption–desorption equilibrium is dependent on the chemical potential of the free ligand (and thus the solubility).50 To further explore this concept in practice, we designed a ligand shell architecture that would mimic a micelle. That PA-hex-PEG behaves as a micelle mimic, avoiding contact stability to iron oxide nanoparticles better than unsubstituted catechol because the nitro group decreases the uncharged affinity for the hydrogen bond acceptor for water molecules. PA-PEG, on the other hand, is highly water-soluble, and thus the affinity for the hydrogen bond acceptor is decreased upon increasing the water content.

**Synthesis and Binding of Catechols**

We synthesized $N$-(4,5-dihydroxy-2-nitrophenoxy)-2-(2-methoxyethoxy)ethoxy)acetamide (nitrodopamine-mPEG); see Scheme 1. We opted for a nitrocatechol instead of an unsubstituted catechol because the nitro group decreases the catechol $pK_a$ values and improves the oxidative stability of the catechol. Nitrodopamine was also shown to provide colloidal stability to iron oxide nanoparticles better than unsubstituted dopamine.52 Starting from dopamine hydrochloride, a one-step nitration reaction results in the formation of nitrodopamine hemisulfate. Separately, MEEAA was converted in an activated $N$-hydroxysuccinimide ester (MEEAA-NHS) using $N,N'$-dicyclohexylcarbodimide (DCC), $N$-hydroxysuccinimide (NHS), and 4-dimethylaminopyridine (DMAP). The coupling between nitrodopamine hemisulfate and MEEAA-NHS was performed using $N$-methylmorpholine (NMM) acting as a non-nucleophilic base. The final nitrodopamine-mPEG ligand...
was purified using preparative high-performance liquid chromatography (HPLC) and fully characterized with electrospray ionization/high-resolution mass spectrometry (ESI-HRMS) and NMR spectroscopy (see Supporting Information).

When we performed a similar competitive binding experiment as before (addition of nitrodopamine-mPEG to MEEAA-capped HfO₂ nanocrystals in methanol-d₄), we found that 1 equiv of nitrodopamine-mPEG was unable to effectively compete for the nanocrystal surface; even at merely 0.4 equiv of catechol, freely diffusing nitrodopamine-mPEG signals could be observed (Figure S11). Such a low binding affinity was highly unexpected, given many reports of successful surface functionalization with catechols.40,53,56−60 However, in these reports, water or biological buffers were used as the solvent. Therefore, we designed a competitive binding experiment directly in water. Fortunately, MEEAA-stabilized HfO₂ nanocrystals are stable in D₂O, even though a significant portion of MEEAA is desorbed (Figure S3). The addition of 1 equiv nitrodopamine-mPEG, without adjusting the pH, results in a turbid suspension with pH at 2. When the pH is adjusted to pH 5, a stable suspension is obtained. To allow for a systematic, gradual addition of nitrodopamine-mPEG, we prepared a stock solution of nitrodopamine-mPEG with 2 equiv of NaOD to doubly deprotonate the catechol. Addition of the base changes the color of the solution from light yellow to a deep burgundy (Figure S12). We added this stock solution to a suspension of MEEAA-stabilized HfO₂ in steps of 0.5 equiv and recorded both standard ¹H NMR and diffusion-filtered NMR spectra. After addition of 0.5 equiv, we did not observe any sharp signals belonging to nitrodopamine-mPEG, whereas we did observe desorbed benzoic acid and desorbed MEEAA (Figure S13). In the diffusion-filtered spectrum, we also see a clear change. In the aromatic region, the broad signals of benzoate are replaced by the broad signals of nitrodopamine-mPEG. The peak shape of the region at 3−4 ppm is also altered (Figure S14). The exchange continues as more nitrodopamine-mPEG is added, and upon addition of 1.5 equiv, sharp (unbound) nitrodopamine-mPEG signals are detected (Figure S13). The suspension remains stable despite the pH being 10.3 at this point in the titration, providing further evidence that the ligand exchange was successful as MEEAA-stabilized nanocrystals would precipitate at pH >6. We conclude that nitrodopamine-mPEG can quantitatively displace MEEAA from the nanocrystal surface if the pH is >5.

The NCs were again purified using multiple cycles of spin filtration, using Milli-Q water as solvent, until the filtrate was nearly colorless (Figure S15). The concentrate was evaporated and redispersed in D₂O, and the pH was adjusted to 7.4. Figure 5B contains a regular ¹H NMR spectrum of the purified suspension; the purity of the sample is striking, and only broadened resonances pertaining to nitrodopamine-mPEG are observed. Based on a thermogravimetric analysis of mass loss of 23%, we calculated a nitrodopamine-mPEG ligand density of 2.14 nm⁻² at the nanocrystal surface. We conclude that nitrodopamine-mPEG forms a tightly bound ligand shell on the nanocrystals at physiological pH with no signs of desorption.

**Scheme 1.** (A) Synthesis of Nitrodopamine Hemisulfate, (B) Synthesis of MEEAA-NHS, and (C) Synthesis of Nitrodopamine-mPEG,"

Reagents and conditions: (i) NaNO₂, 20% H₂SO₄, H₂O, 0 °C to RT, 12 h, 50%; (ii) NHS, DCC, DMAP, dry THF, 0 °C to RT, 12 h, 83%; (iii) NMM, dry DMF, RT, 48 h, 75%.

As it is obvious that pH plays a crucial role in ligand binding in aqueous environments, we systematically varied the pH from 3 to 10 and used ¹H NMR, ³¹P NMR, and DLS to assess the ligand binding and colloidal stability (Figure 6). From NMR, we extract the bound ligand fraction for the phosphonic acid ligands via peak deconvolution of the ³¹P resonances and for nitrodopamine-mPEG via peak deconvolution of the aromatic ¹H resonances (Tables S3−S5). From DLS measurements, we obtained the Z-average value and the zeta-potential. The Z-average value is a single value describing the average particle size and is most sensitive to agglomeration. The zeta-potential indicates the degree of electrostatic repulsion between the

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**Figure 4.** (A,B) ³¹P NMR spectra of PA-PEG- and PA-hex-PEG-functionalized NCs at different D₂O volume %. (C) Free ligand fraction for PA-PEG and PA-hex-PEG at different D₂O volume %, determined by peak deconvolution.

**Figure 5.** (A) ³¹P NMR spectra of PA-PEG- and PA-hex-PEG-functionalized NCs at different D₂O volume %. (B) Free ligand fraction for PA-PEG- and PA-hex-PEG-functionalized NCs at different D₂O volume %, determined by peak deconvolution.
nanocrystals (zeta-potential values above +25 mV or below −25 mV indicate stable suspensions). From Figure 6, we clearly observe that the bound ligand fraction decreases with increasing pH for both PA-PEG and PA-hex-PEG. Taking the pK_a values of ethyl phosphonic acid (pK_a1 = 2.43; pK_a2 = 8.05) as a reference, it is striking that the bound ligand fraction decreases most steeply around pK_a2. We infer that the second deprotonation of the phosphonic acid causes ligand–ligand repulsion in the ligand shell and increases the solubility of the ligand in water. Both effects lead to a reduced bound ligand fraction. Not surprisingly, the loss of ligands has a detrimental effect on the colloidal stability, and the Z-average increases significantly for pH > pK_a2. Overall, PA-hex-PEG performs slightly better than PA-PEG, but the difference is small. Most likely, the double anionic phosphonate compensates for the short hydrophobic segment.

One might expect complete loss of colloidal stability upon ligand desorption. However, no visual turbidity was observed in the samples, and the Z-average remains below 100 nm. Note that the zeta-potential drops below −25 mV at pH >8. While steric stabilization is lost with progressing ligand desorption, electrostatic stabilization takes over, preventing the NCs from fully destabilizing. The negative charge could originate from residual-bound, double deprotonated phosphonates, or more likely from hydroxide adsorption on the nanocrystal surface.

Indeed, in water, there are multiple adsorption–desorption equilibria present simultaneously.

\[
NC(\ast) + L^- \rightleftharpoons NC(L^-) \quad (2)
\]
\[
NC(\ast) + H^+ \rightleftharpoons NC(H)^+ \quad (3)
\]
\[
NC(\ast) + OH^- \rightleftharpoons NC(OH)^- \quad (4)
\]

In addition to these adsorption–desorption equilibria, there are also the acid–base equilibria, resulting in a highly complex pH dependence of the system. According to Figure 6, the phosphonic acids keep the nanocrystals stable between pH 3 and pH 8 in a static system. However, in a dynamic biological environment (e.g., a blood vessel), many competing ligands are present and desorbed ligands are quickly removed. The equilibrium will adjust and thus ligands will continuously desorb from the surface, eventually causing loss of colloidal stability. Despite an acceptable Z-average value at physiological pH, this dynamic behavior could explain why single phosphonate ligands are not necessarily successful at fully preventing aggregation in physiological media.

Interestingly, nitrodopamine-mPEG has almost the complete opposite behavior, unable to provide stable colloidal dispersions under acidic conditions with a very sharp transition around pH 5. This is evidenced by the steep increase in the Z-average (Figure 6). Between pH 5 and pH 10, all nitrodopamine-mPEG ligands remain bound, and only at pH 11, there is a slight decrease in the bound fraction from 100 to 97% (full

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**Figure 5.** (A) Ligand exchange performed between MEEAA-functionalized NCs and nitrodopamine-mPEG. (B) 1H NMR spectra before and after the ligand exchange titration performed in D_2O with nitrodopamine-mPEG. First, 1.5 equiv of nitrodopamine-mPEG was added, and the pH was kept above 5 at all times during addition. The purified nitrodopamine-functionalized NC spectrum was measured at pH 7.4. The reference spectrum of nitrodopamine-mPEG is also shown. C_{NC} = 128 μmol L^{-1}, corresponding to 14.4 mg NCs of this size in 2 mL of D_2O.

**Figure 6.** Effect of pH on ligand binding and stability in water for purified NCs functionalized with PA-PEG, PA-hex-PEG, and nitrodopamine-mPEG. (A) Bound ligand fraction in D_2O based on NMR peak deconvolution at different pH values. (B) Z-average value of NCs in DLS at different pH values. (C) Zeta-potential of the NCs at different pH values. All measurements were performed at constant ionic strength (0.01 mol L^{-1} NaCl) at 25 °C.
range {\(^1\)H NMR spectra in Figure S16}. This translates to an excellent colloidal stability in the pH range of 5–11. We conclude that for aqueous applications at physiological pH, nitrodopamine-mPEG outperforms both PA-PEG and PA-hex-PEG. For aqueous applications at acidic pH, on the other hand, the phosphonic acid ligands are better suited. To test if there would also be a temperature dependence on the binding dynamics of the functionalized NC systems, we performed variable-temperature NMR measurements in D\(_2\)O (pH 7.4) at 25, 37, and 60 °C (Figure S17). {\(^1\)H NMR spectra showed no change in ligand adsorption/desorption equilibria for both phosphonic acids and nitrodopamine-mPEG, leading us to tentatively conclude that the results from the pH titrations would also translate to physiological temperature.

To illustrate the complementary behavior of phosphonic acids and catechols, we performed a competitive exchange reaction on purified nitrodopamine-mPEG NCs in D\(_2\)O. One equivalent of PA-PEG was added, compared to the original amount added to functionalize the NCs with nitrodopamine-mPEG, and NMR measurements were performed at different pH values (Figure S18). Figure S18 clearly shows that, at acidic pH values, a partial exchange with PA-PEG occurs, evidenced by the appearance of sharp nitrodopamine-mPEG signals in the aromatic region and methylene triplet around 3 ppm. The nitrodopamine-mPEG NC suspension, which normally would fully destabilize below pH 5, remained colloidally stable at pH 2.22 due to the now mixed catechol–phosphonate ligand shell. The exchange equilibrium mostly shifts back toward nitrodopamine-mPEG as the pH moves toward neutral and basic values.

Stability in Phosphate-Buffered Saline

Finally, we assessed the stability of NCs functionalized with PA-PEG, PA-hex-PEG, and nitrodopamine-mPEG in PBS. Stability in PBS is an important prerequisite for biomedical applications, as many in vivo experiments inject the desired drug or contrast agent in saline solution or in PBS. A standard 1× PBS buffer contains 137 mmol L\(^{-1}\) NaCl, 2.7 mmol L\(^{-1}\) KCl, 10 mmol L\(^{-1}\) Na\(_2\)HPO\(_4\), and 1.8 mmol L\(^{-1}\) KH\(_2\)PO\(_4\). When the concentrations are half or double, these buffers are referred to as 0.5× PBS and 2× PBS, respectively. It is clear that PBS contains relatively high salt concentrations and phosphate ions (which will compete for the surface) and thus provides a real test for the stability of our functionalized nanocrystals. First, we varied the PBS concentration and immediately measured the Z-average by DLS (Figure 7A). Up to 1.5× PBS, all ligands keep the nanocrystals colloidal stable. As expected, PA-PEG is the weakest ligand and cannot prevent the start of nanocrystal aggregation in 2× PBS solutions. Second, the stability of all functionalized NCs was monitored over time in 2× PBS (Figure 7B). Clearly, PA-PEG-functionalized nanocrystals agglomerase quickly and visually precipitate after several hours. The colloidal stability of PA-hex-PEG-functionalized nanocrystals steadily decreases over 24 h, before completely agglomerating. Only nitrodopamine-mPEG-functionalized nanocrystals remain perfectly stable. There is no sign of aggregation over the course of 48 h, and the suspension also remains visually clear for at least 1 month.

### DISCUSSION

The above results make clear that surface chemistry becomes much more complex when moving from nonpolar to polar (e.g., aqueous) solvents. In nonpolar solvents, charged ligands or nanocrystals are thermodynamically unstable, leading to a limited set of binding motifs and clear ligand exchange rules.\(^{25}\) For example, autodesorption of oleate (deprotonated oleic acid) does not occur in toluene. For the binding motif of PbS(PbX\(_2\)), removal of the entire Lewis acid PbX\(_2\), has been observed in chloroform or coordinating solvents like THF.\(^{46}\) Likewise, for HHO\(_2\)(H\(_2\)OOCR), desorption of oleic acid is possible by recombination of the carboxylate and proton.\(^{22}\) However, these restrictions disappear in polar solvents where charges are stabilized, and the proton and carboxylate have independent adsorption/desorption equilibria; see also eqs 2–4.

From the above data, we constructed a colloidal stability map, indicating which ligands provide colloidal stability in specific pH ranges (see Figure 8). It is interesting to correlate this stability map to the pK\(_a\) values of the ligands, which we experimentally determined via acid–base titrations (Figure S19). The pK\(_a\) values of PA-PEG are pK\(_{a1}\) = 2.65 ± 0.01 and pK\(_{a2}\) = 7.56 ± 0.01, and those of PA-hex-PEG are pK\(_{a1}\) = 2.99 ± 0.02 and pK\(_{a2}\) = 8.33 ± 0.01. At pH 3 (where the particles are stable), we calculate using eq 5 that approximately 70% of PA-PEG is monodeprotonated. One can assume that the phosphate will bind in this form to the nanocrystal surface.

\[
\text{pH} = \text{pK}_a + \log \left( \frac{[\text{base}]}{[\text{acid}]} \right) \quad (5)
\]

For pH >8.5 (above pK\(_{a2}\)), the bound ligand fraction decreases quickly and the particles start to aggregate (Figure 6). The double deprotonation of the ligand has two consequences; (1) the ligand becomes more soluble in water, and (2) electrostatic ligand–ligand repulsion appears, instead of the stabilizing hydrogen bonding. Both effects promote ligand desorption. In addition, the isoelectric point of HHO\(_2\) occurs at pH 8,\(^{67}\)
approximately 99% of the ligand is fully protonated and can (where the particles are unstable), we calculate that ± and we observe that NCs functionalized with MEEAA (pH. Finally, carboxylic acids can only be monodeprotonated in UV spectra of the free ligand (see Figure 9 and Figure S20). For 5

Figure 8. Colloidal stability map for metal oxide nanocrystals functionalized with carboxylic acids, phosphonic acids, and catechols. In this concrete example, we used HfO2 as the NC model system with its respective isoelectric point, MEEAA as the carboxylic acid, PA-PEG as the phosphonic acid, and nitrodopamine-mPEG as the catechol. The map colors should be interpreted as follows: green, excellent colloidal stability; striped green, high fraction of bound ligands but not perfect, use in a static system; orange, low bound ligand fraction, use in a static system without competition; red, nanocrystal aggregation/precipitation, do not use.

rendering the surface negatively charged at pH >8, thus further decreasing the binding affinity of the negatively charged phosphate ligand. Esarey et al. observed a similar phenomenon when studying the binding constant of a phosphonate-anchored rubidium complex on anatase TiO2 surfaces under varying pH conditions. A similar reasoning applies to the case of nitrodopamine-mPEG (pKa1 = 7.13 ± 0.01; pKa2 = 10.8 ± 0.01). At pH = 5 (where the particles are unstable), we calculate that approximately 99% of the ligand is fully protonated and can thus only interact with the surface via weak hydrogen bonds. With increasing pH, a progressively higher fraction of nitrodopamine-mPEG is monodeprotonated, and is able to coordinate to the surface metal sites. It also provides an additional hydrogen bond to further stabilize the bound state. This is confirmed by comparing the UV–vis spectra of nitrodopamine-mPEG bound to HfO2 NCs with the reference spectra of the free ligand (see Figure 9 and Figure S20). For S before fully precipitating above pH 6 (Figures S21 and S22). We hypothesize that they are less stable than phosphonates and catechols due to the lack of ligand–ligand hydrogen bonding.

The stability experiments in buffer solution point to an additional variable: competitive ligands. In phosphate-buffered saline, a high concentration of phosphate is present, which competes for the surface but does not provide steric stabilization. Since the phosphonic acids (PA-PEG and PA-hex-PEG) desorb at pH 7.4 (25% desorbed; see Figure 6), they are in equilibrium and can thus be slowly displaced over time. The nitrodopamine-mPEG is tightly bound (0% desorbed) and is not being displaced by phosphonic acid, judging from our competition experiments (Figure S18), hence the high stability of the particles capped with nitrodopamine-mPEG.

The above discussion shows that, in aqueous media, surface chemistry is a complex interplay between multiple factors. There is the pH-dependent charge of the surface, the pH-dependent deprotonation of the ligand, and competition by phosphate in buffer solution. Slight modifications to the ligand structure can alter the solubility of the ligands and thus the binding affinity. The binding group remains, however, the most important descriptor for the stability map, a conclusion supported by the relatively small differences between PA-PEG and PA-hex-PEG in their pH-dependent desorption from the surface. The colloidal stability map can thus be generalized to other carboxylate, phosphonate, and catecholate ligands. The pKa values of the ligands used here are quite close to reported pKa values for acetic acid (pKa = 4.75), ethyl-phosphonic acid (pKa1 = 2.43; pKa2 = 8.05), and nitrodopamine (pKa1 = 6.6; pKa2 = 11). For optimal accuracy, it is recommended to insert the actual pKa values for the ligands of interest, as the stable pH ranges will shift together with the pKa(s) of the ligand. For other oxide nanocrystals (e.g., TiO2 or Fe3O4), the corresponding isoelectric point needs to be used. Having thus the pKa(s) of the ligands available and the isoelectric point of the nanocrystals, one can now use the colloidal stability map (Figure 8) to start predicting which ligands will provide colloidal stability at a particular pH. A word of caution is warranted. Small structural variations in the ligand will have little influence on the predictions of the stability map, but significant structural variations, such as a high steric bulk, could have adverse effects on the binding affinity.

CONCLUSION

We have mapped out the binding affinity of the three most important ligands for metal oxide nanocrystal surface functionalization. By performing an in-depth surface chemistry and stability study, we found that ligand dynamics become much more complex when moving from nonpolar to polar solvents. In water, the pH influences the ligand solubility through deprotonation of the binding group. Carboxylic acids are weakly binding anchors in both nonpolar and polar environments and can be easily displaced by competitive ligands. Phosphonic acids are strongly bound ligands in MeOH but desorb in aqueous media, the extent of which is dependent on the pH. They are mostly suited to stabilize nanocrystals in acidic environments. Finally, nitrocatechol derivatives provide a tightly bound ligand shell and excellent stability at physiological and basic pH. They are superior at stabilizing metal oxide nanocrystals in phosphate-buffered saline solutions but cannot be used in acidic environments. We have

Figure 9. UV–vis spectra of purified nitrodopamine-mPEG-functiona-
lized NCs at different pH values in H2O.

< pH < 11, we find the monodeprotonated species. At pH >11, we see again the appearance of free ligands in NMR (Figure S16) and the double deprotonated species in UV–vis (Figure S20). For the reasons mentioned above, the double deprotonated species has a low binding affinity for the surface. In addition, the catechol becomes sensitive to oxidation at high pH. Finally, carboxylic acids can only be monodeprotonated and we observe that NCs functionalized with MEEAA (pKa = 3.61 ± 0.03) gradually lose colloidal stability above pH 3.5
summarized our results in a convenient colloidal stability map that can be generalized to other oxides and ligands, provided the isoelectric points and pKₐ values are available. This tool will allow researchers to rationally choose nanocrystal functionalization strategies for their desired applications in polar or aqueous environments.

### EXPERIMENTAL METHODS

**Materials**

(6·2·(2-Hydroxyethoxy)ethoxy)phosphonic acid and (2·(2-Hydroxyethoxy)ethoxy)phosphonic acid were purchased from SiKEMIA. N-Hydroxysuccinimide (≥98%) and dopamine hydrochloride (≥99%) were purchased from Acros Organics. 2·(2-Methoxyethoxy)ethoxy)acetic acid (≥95.0%) was purchased from TCI Chemicals. Hafnium(IV) tert-butoxide (99.99%), N,N′-dicyclohexylcarbodiimide (99%), 4-(dimethylamino)pyridine (≥99%), 4-methylmorpholine (99%), sodium nitrite (≥99.0%), and solvents used for synthesis were purchased from Sigma-Aldrich. All purchased chemicals were used without further purification. Further purified deuterated solvents were purchased from Sigma-Aldrich or Eurisotop.

**Synthesis of MEEAA-NHS**

This procedure was adapted from Meissler et al.:55 4 mmol (0.7128 g) 2·(2-methoxyethoxy)ethoxy)acetic acid and 4.2 mmol (0.482 g) N-Hydroxysuccinimide were dissolved in 8 mL of dry THF in a predried vial and cooled to 0 °C. Then 4.2 mmol (0.866 g) N,N′-dicyclohexylcarbodiimide was dissolved in a separate predried vial in 4 mL of THF and added dropwise to the first mixture by performing an air-free transfer. The mixture was stirred for 15 min at 0 °C, after which 0.2 mmol (0.024 g) catalytic 4-dimethylaminopyridine was added. The mixture was stirred overnight at room temperature, resulting in a white turbid mixture. The turbid solution was filtered over a por 4 fitted glass filter, and the white precipitate was washed once with 10 mL of THF to collect the remaining product. After solvent removal of the filtrate using a rotary evaporator, the viscous liquid was dissolved in a minimum amount of DCM and transferred to an extraction funnel via a 0.2 µm syringe filter, and the organic phase was then diluted to a volume of 12 mL using DCM. The organic phase was extracted four times with 12 mL of Milli-Q (MQ) water and two more times with a volume of 12 mL of brine. The organic phase was dried over MgSO₄ and dried using a rotary evaporator. Product was collected as a colorless to light yellow viscous liquid with 83% yield. ¹H NMR (500 MHz, CDCl₃): δ 4.5 (s, 2H), 3.8–3.76 (m, 2H), 3.7–3.66 (m, 2H), 3.65–3.56 (m, 2H), 3.55–3.51 (m, 2H), 3.36 (s, 3H), 2.82 (4H) ¹³C NMR (500 MHz, CDCl₃): δ 168.73 (s), 166.02 (s), 77.24 (s), 71.91 (s), 71.37 (s), 70.607 (s), 70.602 (s), 66.55 (s), 59.06 (s), 25.58 (s). HRMS 358.35 calc. for [M⁺], 357.35 [M⁺ + NH₄⁺]⁺ found.

**Synthesis of Nitrodopamine Hemisulfate**

The procedure was adapted from Napolitano et al.:54 8.753 mmol, 2.58 g, 2.21 mL) and anhydrous benzyl alcohol (45.6 mL) according to Lauria et al.47 After the synthesis, the nanocrystals were collected by adding diethyl ether (19 mL) to the reaction mixture and centrifugation (5000 rcf, 5 min) in plastic centrifuge tubes. The sediment was washed three times with diethyl ether (19 mL). For functionalization with 2·(2-methoxyethoxy)ethoxy)acetic acid, the sediment was first dispersed in 19 mL of toluene, resulting in a milky white turbid liquid. Then 381 µL of 2·(2-methoxyethoxy)ethoxy)acetic acid (0.328 g; 1.82 mmol) was added followed by 30 min of sonication, resulting in a transparent suspension with a few insolubles present. The insolubles were removed by centrifugation (5000 rcf, 5 min), and the clear top layer was transferred to new plastic centrifuge tubes. NCs were precipitated by addition of 1:2 volume of hexane (mixture of isomers); after centrifugation (5000 rcf, 5 min), the organic top phase was removed, and the NCs were resuspended in toluene. The dispersion in toluene can be dispersed and dried in ethanol. From ethanol, the dispersion can be dried again and resuspended in MeOH, and from MeOH, the dispersion can be dried again and resuspended in H₂O.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacsau.1c00565.

Additional experimental methods, NMR spectra of titrations and synthesized compounds, PDF analysis, peak deconvolutions, UV–vis spectra of nitrodopamine-mPEG, DLS data of carbamate-capped nanocrystals; acid-base titrations for all ligands (PDF)

**AUTHOR INFORMATION**

**Corresponding Author**

Jonathan De Roo — Department of Chemistry, University of Basel, 4058 Basel, Switzerland; orcid.org/0000-0002-1264-9312; Email: jonathan.deroo@unibas.ch

The procedure was inspired by Anstadt et al.:53 1.784 mmol (491 mg) MEEAA-NHS and 2.854 mmol (846 mg) nitrodopamine hemisulfate were dissolved in 25 mL of dry DMF in a predried flask, resulting in a dark orange solution. The flask was sealed, flushed with argon, and cooled in an ice bath. Then 785 µL of N-methylmorpholine was added dropwise to the mixture using air-free technique; after approximately 15 min of stirring, the solution became turbid. The mixture was allowed to stir 48 h at room temperature, followed by evaporation overnight under vacuum at 40 °C to remove DMF, yielding a dark brown liquid. Next, 40 mL of 1 M HCl was added to the crude and extracted three times with 40 mL of CHCl₃; a dark brown solid is formed during the process at the liquid interface, and care was taken to not allow this to enter the organic phase. The organic phase was extracted with 50 mL of brine, dried with Na₂SO₄, and evaporated using rotary evaporation. The resulting solid was purified with preparative HPLC using a gradient from solvent A (MQ water containing 0.1% TFA) to solvent B (ACN containing 0.1% TFA), and after being freeze-dried, the final product was isolated as a fluffy white-yellow solid with 75% yield. ¹H NMR (500 MHz, MeOD): δ 7.54 (s, 1H), 6.7 (s, 1H), 3.93 (s, 2H), 3.63–3.58 (m, 2H), 3.57–3.5 (m, 4H), 3.35 (s, 3H), 3.07 (t, 2H, J = 6.68 Hz). ¹³C NMR (500 MHz, MeOD): δ 173.05 (s), 152.4 (s), 145.53 (s), 141.06 (s), 129.59 (s), 119.61 (s), 113.56 (s), 72.97 (s), 72.07 (s), 71.4 (s), 71.39 (s), 71.24 (s), 59.21 (s), 40.47 (s), 34.14 (s). HRMS 358.35 calc. for [M⁺], 357.35 [M⁺ – H⁻]⁻ found.

**Synthesis of Hafnium Oxide Nanocrystals**

The NCs were synthesized from hafnium(IV) tert-butoxide (5.47 mmol, 2.58 g, 2.21 mL) and anhydrous benzyl alcohol (45.6 mL) according to Lauria et al.47 After the synthesis, the nanocrystals were collected by adding diethyl ether (19 mL) to the reaction mixture and centrifugation (5000 rcf, 5 min) in plastic centrifuge tubes. The sediment was washed three times with diethyl ether (19 mL). For functionalization with 2·(2-methoxyethoxy)ethoxy)acetic acid, the sediment was first dispersed in 19 mL of toluene, resulting in a milky white turbid liquid. Then 381 µL of 2·(2-methoxyethoxy)ethoxy)acetic acid (0.328 g; 1.82 mmol) was added followed by 30 min of sonication, resulting in a transparent suspension with a few insolubles present. The insolubles were removed by centrifugation (5000 rcf, 5 min), and the clear top layer was transferred to new plastic centrifuge tubes. NCs were precipitated by addition of 1:2 volume of hexane (mixture of isomers); after centrifugation (5000 rcf, 5 min), the organic top phase was removed, and the NCs were resuspended in toluene. This purification step was repeated three more times before final resuspension in toluene, and the final NC yield was 70%. The purified NC suspension in toluene remains stable for at least 1 year. The dispersion in toluene can be dispersed and dried in ethanol. From ethanol, the dispersion can be dried again and resuspended in MeOH, and from MeOH, the dispersion can be dried again and resuspended in H₂O.
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