JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Communication

pubs.acs.org/JACS

# Reversible Control over the Valency of a Nanoparticle-Based Supramolecular System

Grégory Pieters, Cristian Pezzato, and Leonard J. Prins*

Department of Chemical Sciences, University of Padova, Via Marzolo 1, 35131 Padova, Italy

Supporting Information

**ABSTRACT:** The reversible "catch-and-release" of small molecules from the surface of monolayer-protected gold nanoparticles is described. The valency of the system (i.e., the number of molecules bound to the surface) can be controlled through the addition and removal of metal ions from the monolayer. Both the change in valency and the release rate of the molecules are strongly pH-dependent. The release rate can be regulated by altering the ratio of metal ions in the monolayer.

Multivalency has become a key concept in the fields of biorecognition,[1] catalysis,[2] supramolecular chemistry,[3] and nanotechnology.[4,5] Consequently, there is a strong interest in the functionalization of synthetic multivalent structures such as polymers,[6] dendrimers,[7] and nanoparticles.[8] In particular, monolayer-protected gold clusters (Au MPCs) have emerged as attractive scaffolds because of their straightforward preparation and functionalization. This, in combination with appealing intrinsic optical and electronic properties and high biocompatibility, has led toward numerous applications in nanomedicine[9] and diagnostics.[10] Nonetheless, a common feature of most systems, including Au MPCs, is that their valency is determined during synthesis. Systems that allow for "postsynthetic" control of the valency offer the important perspective of permitting active regulation of the strength of interaction with the target, including controlled dissociation if requested. Enormous progress has been made in the development of supramolecular aggregates (e.g., micelles, peptide amphiphiles, supramolecular polymers, etc.) that indeed show adaptive behavior of their valency to the target as a spontaneous process originating from the dynamic nature of these systems.[11−13] An attractive alternative approach relies on the modification of the monolayer surface on a nanoparticle through supramolecular interactions, which permits the valency of discrete assemblies to be tuned.[14−16] In this communication, we show that the valency of a discrete nanoparticle-based supramolecular system can be controlled in a reversible manner and that small molecules can be captured *and* released in a controlled manner from the surface of Au MPCs through the addition and removal of metal ions from the monolayer, respectively. This process is fully reversible, and both the change in valency and the release rate are pH-dependent.

We recently showed that Au MPCs **1**·$Zn^{2+}$ ($d_{Au}$ = 1.8 ± 0.4 nm) containing 1,4,7-triazacyclononane (TACN)·$Zn^{2+}$ head groups are attractive scaffolds for the formation of multivalent supramolecular structures (Figure 1).[17,18] It was found that oligoanions such as **ATP**, **ADP**, and Ac-**DDD**-OH have such high affinities for **1**·$Zn^{2+}$ that binding occurs under saturation conditions at low micromolar concentrations in water. Compared with Au MPC **1** (i.e., the same Au MPC but in the absence of $Zn^{2+}$), the presence of the $Zn^{2+}$ metal ions in the self-assembled monolayer (SAM) caused an increase in the surface saturation concentration of the oligoanions and thus the valency of the obtained structure. It was estimated that ∼18 molecules of 2-aminopurine riboside-5′-*O*-triphosphate (**ATP$_F$**), a fluorescent analogue of **ATP**,[19] were bound to the surface of **1**·$Zn^{2+}$ at saturation, in contrast to ∼5 for **1**. This was intriguing because the difference between the saturation concentrations of **1** and **1**·$Zn^{2+}$ suggested that $Zn^{2+}$ could potentially act as a regulatory element of the valency of the system. This hypothesis was verified by adding increasing amounts of $Zn(NO_3)_2$ to a solution of **1** ([TACN] = 10 $\mu$M[20]) and **ATP$_F$** (2 $\mu$M) at pH 7.0 (Figure 2a). In view of the surface saturation concentration of 0.8 $\mu$M for **ATP$_F$** at this pH (see below),[21] this implied that initially 1.2 $\mu$M **ATP$_F$** was free in solution. The addition of $Zn^{2+}$ resulted in the metalation of the TACN ligands and the corresponding "capture" of unbound **ATP$_F$** by **1**·$Zn^{2+}$. Complex formation was detected by fluorescence spectroscopy, taking advantage of the ability of Au nanoparticles to quench the fluorescence of surface-bound fluorophores.[22] The plot of the fluorescence intensity at 370 nm as a function of the amount of $Zn^{2+}$ added shows several features. First, the decrease in fluorescence intensity was a clear indication that the formation of TACN·$Zn^{2+}$

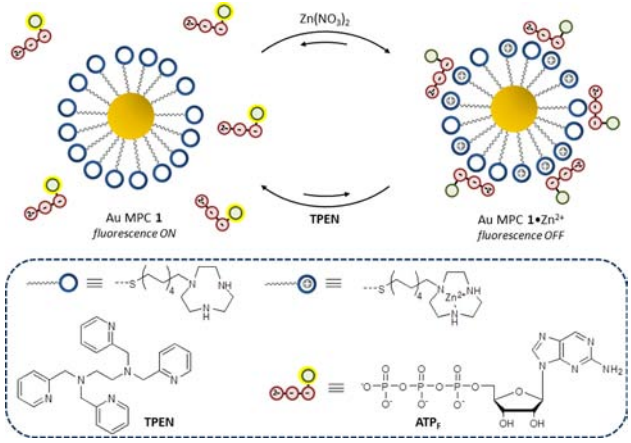


**Figure 1.** Schematic representation of metal-mediated control over the valency of the nanoparticle-based supramolecular system.

**Received:** August 2, 2012
**Published:** September 4, 2012

  

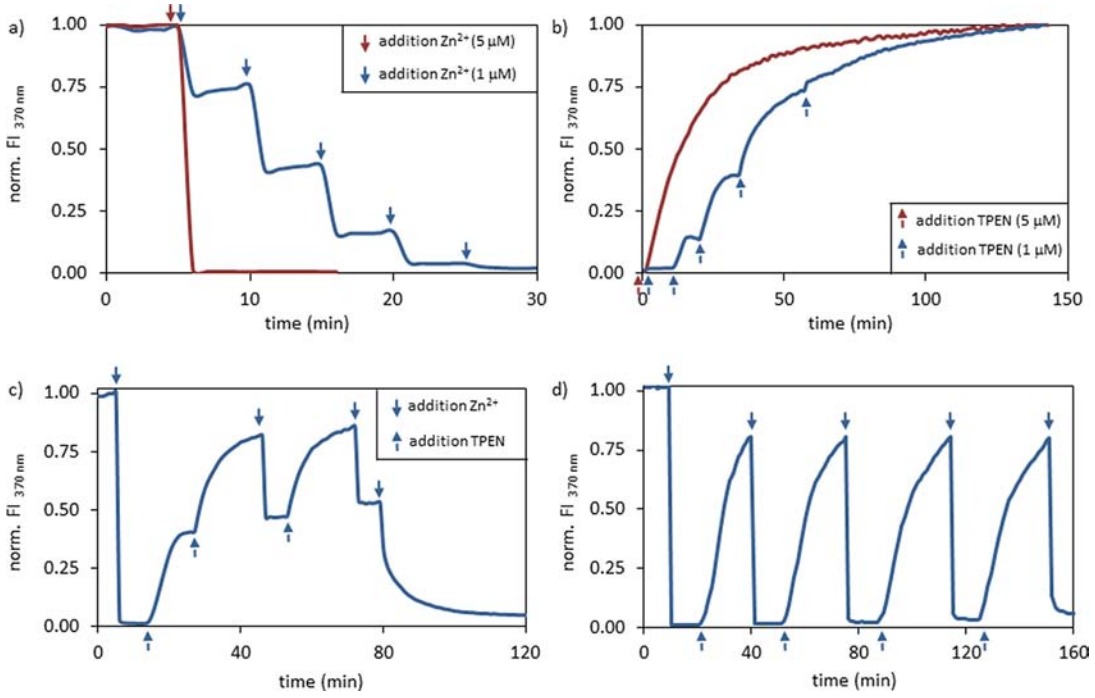


**Figure 2.** (a) Normalized fluorescence intensity at 370 nm as a function of the addition of aliquots of $Zn^{2+}$ (1 $\mu$M) to a solution of Au MPC **1**. (b) Normalized fluorescence intensity at 370 nm as a function of the addition of aliquots of TPEN (1 $\mu$M) to a solution of Au MPC **1**·$Zn^{2+}$. (c) Normalized fluorescence intensity at 370 nm upon alternating additions of aliquots of $Zn^{2+}$ (downward arrows; 5, 1, 1, and 3 $\mu$M, respectively) and TPEN (upward arrows; 3, 1, and 1 $\mu$M, respectively). (d) Normalized fluorescence intensity at 370 nm upon alternating additions of aliquots of $Zn^{2+}$ (5 $\mu$M; downward arrows) and TPEN (5 $\mu$M; upward arrows). Experimental conditions: [TACN] = 10 $\mu$M, [$\mathbf{ATP_F}$] = 2.0 $\mu$M, [HEPES] = 10 mM, pH 7.0, 37 °C.

complexes on the surface of **1** resulted in the capture of unbound $\mathbf{ATP_F}$. Second, complete quenching required the addition of only 5 $\mu$M $Zn(NO_3)_2$ instead of the 10 $\mu$M required to metalate all of the TACN ligands. An independent binding titration indeed confirmed that the surface saturation concentration of $\mathbf{ATP_F}$ on **1**·$Zn^{2+}$ did not change when 5 $\mu$M $Zn(NO_3)_2$ was present instead of 10 $\mu$M [see the Supporting Information (SI)]. Third, the capture of unbound $\mathbf{ATP_F}$ occurred almost instantaneously upon the addition of $Zn^{2+}$, in particular for the first additions. This suggested either that neighboring TACN ligands are selectively metalated to form bimetallic binding pockets or that the formation of binding sites composed of a single TACN·$Zn^{2+}$ complex in combination with a neighboring protonated TACN headgroup is sufficient to cause an increase in the surface saturation concentration.[23] Support in favor of the latter emerged from pH-dependent studies (see below), which showed that protonation of the TACN ligands also caused an increase in the surface saturation concentration. In any case, the addition of 5 $\mu$M $Zn(NO_3)_2$ in a single batch resulted in complete quenching of the fluorescence within 3 min (Figure 2a, red trace).

Since the addition of $Zn^{2+}$ to Au MPC **1** resulted in the capture of $\mathbf{ATP_F}$, we reasoned that removal of $Zn^{2+}$ from Au MPC **1**·$Zn^{2+}$ would cause the opposite effect, namely, the release of $\mathbf{ATP_F}$ from the surface. For that reason, the fluorescence intensity at 370 nm was measured upon the addition of *N*,*N*,*N*′,*N*′-tetrakis(2-pyridylmethyl)ethylenediamine (TPEN), a ligand with a much higher affinity for $Zn^{2+}$ compared with TACN (log $K_{TACN\cdot Zn^{2+}}$ = 11.5 vs log $K_{TPEN\cdot Zn^{2+}}$ = 15.4).[24] We were pleased to observe that the addition of 1 equiv of TPEN (relative to $Zn^{2+}$) indeed resulted in full recovery of the initial fluorescence intensity, indicating complete reversibility of the process (Figure 2b). Contrary to the capture of $\mathbf{ATP_F}$, the release process was much slower, with full release of $\mathbf{ATP_F}$ (1.2 $\mu$M) requiring nearly 2 h. Since previous studies had shown that $\mathbf{ATP_F}$ exchange on the surface of **1**·$Zn^{2+}$ is fast,[18] we postulate that the release rate is determined by the rate of dissociation of $Zn^{2+}$ from the TACN·$Zn^{2+}$ complex. This is supported by the observation that the release rate of $\mathbf{ATP_F}$ was independent of the concentration of TPEN added (see the SI) but dependent on the pH and type of metal ion used (see below). As before, the valency of the nanostructure could be controlled by the stepwise addition of TPEN. This experiment showed that the addition of small amounts of TPEN (1−2 $\mu$M) hardly induced the release of $\mathbf{ATP_F}$ from the surface, in line with the previously discussed results of the $Zn^{2+}$ titrations. The full control over the valency of the system was demonstrated by alternating additions of small aliquots of TPEN and $Zn^{2+}$ (Figure 2c). Finally, the complete reversibility of the process was demonstrated by performing four repetitive "capture-and-release" cycles through alternating additions of 5 $\mu$M $Zn^{2+}$ and TPEN (Figure 2d). To accelerate this experiment, $Zn^{2+}$ was added after an $\mathbf{ATP_F}$ release equal to 80% of the maximum value. In four cycles, no changes were observed apart from a slight decrease in the rate of $\mathbf{ATP_F}$ release. A control experiment showed that the surface saturation concentration of $\mathbf{ATP_F}$ on **1**·$Zn^{2+}$ was unaffected by the presence of up to 0.5 mM TPEN·$Zn^{2+}$ (see the SI). The nonlinear response of the system toward the addition of either $Zn^{2+}$ or TPEN points to a role of protonated TACN in the surface binding of $\mathbf{ATP_F}$. This is not surprising, considering that electrostatic interactions play an important role in complex formation. For that reason, it was decided to study the surface saturation concentration of $\mathbf{ATP_F}$ on **1** as a function of the pH. As before, saturation concentrations were determined by measuring the fluorescence intensity at 370 nm as a function of the amount of $\mathbf{ATP_F}$ added to a solution of **1** ([TACN] = 10 $\mu$M) at pH values ranging from 6.0 to 8.0. This study indeed confirmed a


dx.doi.org/10.1021/ja307621d | *J. Am. Chem. Soc.* 2012, 134, 15289−15292

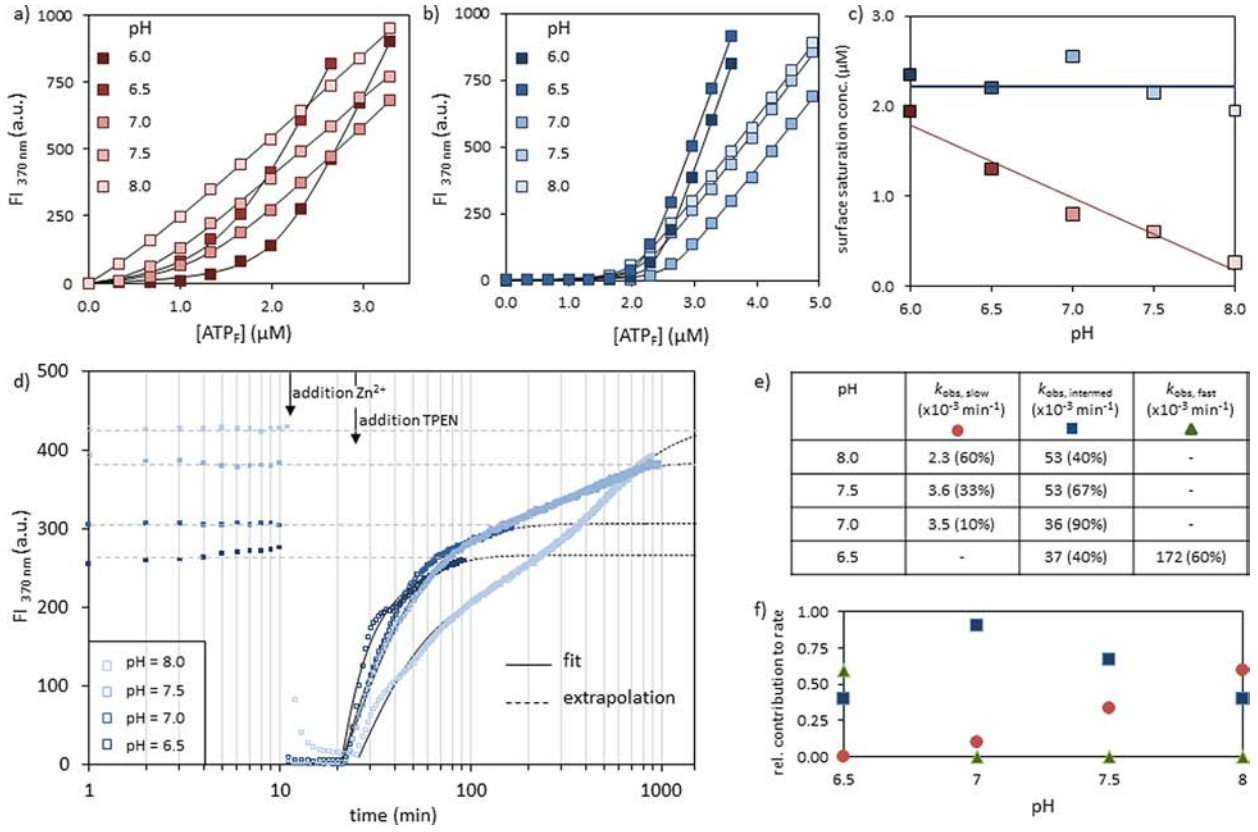


**Figure 3.** (a) Fluorescence intensity at 370 nm as a function of the amount of $\mathbf{ATP_F}$ added to Au MPC **1** at various pH. (b) Fluorescence intensity at 370 nm as a function of the amount of $\mathbf{ATP_F}$ added to Au MPC **1**·$Zn^{2+}$ at various pH. (c) Surface saturation concentrations of $\mathbf{ATP_F}$ on **1** (red) and **1**·$Zn^{2+}$ (blue) as functions of pH. (d) Fluorescence intensity at 370 nm as a function of time upon the respective additions of $Zn^{2+}$ and TPEN to **1**. The solid lines were generated by fitting the experimental data for the release of $\mathbf{ATP_F}$ to a kinetic model taking into account two first-order rate constants. (e) First-order rate constants determined at each pH. (f) Relative contributions to the overall rate by the slow (red ●), intermediate (blue ■), and fast (green ▲) processes at each pH. Experimental conditions: [TACN] = 10 μM, [$Zn(NO_3)_2$] = 5 μM, [HEPES] = 10 mM (for pH 7.0–8.0), [MES] = 10 mM (for pH 6.0 and 6.5), 37 °C. In (a) and (b), the difference in slope after saturation at pH 6.0 and 6.5 originated from the use of a different buffer system.

strong pH dependence of the saturation concentration of $\mathbf{ATP_F}$ on **1**, which decreased from 1.9 μM at pH 6.0 to 0.2 μM at pH 8.0 (Figure 3a; see the SI for determination of the saturation concentration). It is important to note that at pH 6.0 the surface saturation concentration almost reached that of **1**·$Zn^{2+}$. On the other hand, the same study performed on **1**·$Zn^{2+}$ showed a nearly constant surface saturation concentration of 2.3 ± 0.2 μM over the entire pH interval (Figure 3b). This is interesting because it implies that the regulatory effect of $Zn^{2+}$ is pH-dependent (Figure 3c). In particular, these data indicate that the change in valency of the system should be more pronounced at higher pH because of the reduced surface capacity of **1** for $\mathbf{ATP_F}$. This was indeed confirmed by performing catch-and-release cycles in the pH range 6.5 to 8.0. The increase in initial fluorescence intensity at higher pH values for the same concentrations of $\mathbf{ATP_F}$ (2 μM) and **1** ([TACN] = 10 μM) reflected the presence of larger amounts of unbound $\mathbf{ATP_F}$ (Figure 3d). In all cases, the addition of $Zn^{2+}$ (5 μM) resulted in rapid quenching of the fluorescence, indicating the quantitative capture of $\mathbf{ATP_F}$ by **1**·$Zn^{2+}$. At all pH values, the subsequent addition of TPEN (5 μM) caused complete release of $\mathbf{ATP_F}$ to restore the initial values, but with reduced rates at higher pH values. A detailed analysis showed that the release of $\mathbf{ATP_F}$ from the surface at each pH is described by (at least) two different kinetic components, which were quantified by fitting the release curves to a kinetic model (Figure 3e). A plot of the obtained first-order rate constants as a function of pH illustrates that at pH 7 the $\mathbf{ATP_F}$ release rate is dominated by a single (intermediate) value ($k_{obs,intermed} = 36 \times 10^{-3}$ min$^{-1}$). At lower pH, an additional second (faster) component is observed ($172 \times 10^{-3}$ min$^{-1}$), whereas at higher pH values, the second component of the rate is slower ($2.3 \times 10^{-3}$ min$^{-1}$). These observations indicate that different binding modes exist for the $\mathbf{ATP_F}$ probes on the surface, the relative ratios of which are presumably related to the different protonation states of the probe, TACN, and/or the TACN·$Zn^{2+}$ complex. In summary, these data show that the pH can indeed be used to modulate not only the amplitude of the $\mathbf{ATP_F}$ catch-and-release cycles but also the rate at which the probe molecules are released.

As the TACN ligand is well-known to complex several metals with different affinities, the controlled formation of a heterometalated surface would in principle allow regulation of the rate of $\mathbf{ATP_F}$ release from the surface through variation of the ratio of metal ions in the monolayer. To test this hypothesis, we verified that the addition of 5 μM $Cu(NO_3)_2$ to a solution of $\mathbf{ATP_F}$ (2 μM) and **1** ([TACN] = 10 μM) resulted in the quantitative capture of $\mathbf{ATP_F}$ (log $K_{TACN\cdot Cu^{2+}}$ = 15.4).[24] An independent titration indeed confirmed an $\mathbf{ATP_F}$ surface saturation concentration of 2.2 ± 0.1 μM on Au MPC **1**·$Cu^{2+}$ (see the SI). Interestingly, the addition of TPEN (log $K_{TPEN\cdot Cu^{2+}}$ = 20.2)[24] resulted in release of $\mathbf{ATP_F}$ as before, but at a roughly 20-fold lower rate compared with that observed for **1**·$Zn^{2+}$. Complete release of $\mathbf{ATP_F}$ required more than 24 h (Figure 4,


dx.doi.org/10.1021/ja307621d | J. Am. Chem. Soc. 2012, 134, 15289–15292

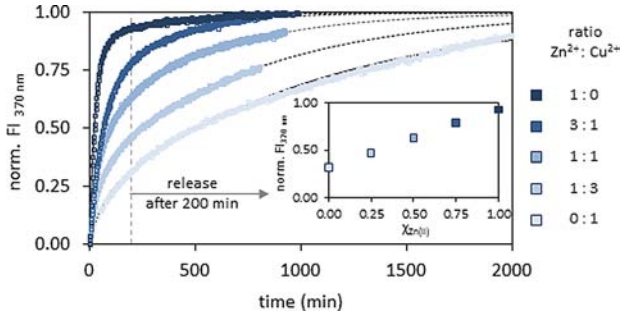


**Figure 4.** Normalized fluorescence intensity (FI) at 370 nm as a function of time upon the addition of TPEN (5 $\mu$M) to a solution of Au MPC **1**·$M^{2+}$ (M = $Zn^{2+}$/$Cu^{2+}$ as indicated at the right; [$M^{2+}$] = 5 $\mu$M). The inset shows the normalized FI after 200 min as a function of the mole fraction of $Zn^{2+}$ in the monolayer.

light-blue trace). The difference between $Zn^{2+}$ and $Cu^{2+}$ supports the hypothesis that dissociation of the metal ion from the TACN complex is indeed the rate-determining step for $\mathbf{ATP_F}$ release. Finally, a study of the release rate of $\mathbf{ATP_F}$ from Au MPC **1**·$M^{2+}$ (M = $Zn^{2+}$/$Cu^{2+}$) as a function of the $Zn^{2+}$/$Cu^{2+}$ ratio revealed that the release rate can be controlled rationally (Figure 4). In fact, a plot of the normalized fluorescence intensity at 200 min as a function of the $Zn^{2+}$/$Cu^{2+}$ ratio on the surface is nearly linear (Figure 4 inset). The control over the release rate is further reflected by the fact the rate of $\mathbf{ATP_F}$ release was composed of two (major) kinetic terms, the ratio of which corresponded nicely to the ratio of metal ions on the surface (see the SI). The deviation from the expected straight lines originates from the presence of additional kinetic components of the release rate due to the different binding modes of $\mathbf{ATP_F}$ on the surface (see above).

In conclusion, these studies show that metal ions can be used as regulatory elements for the reversible control of the valency of a supramolecular system composed of a monolayer-protected gold nanoparticle and small molecules. The pH of the solution plays an important role in determining both the amplitude and the release rate of the small molecules from the monolayer surface. At a single pH, the release rate can be tuned by varying the ratios of different metal ions. The current system offers the perspectives of regulating the multivalent interaction between nanoparticles and biotargets and controlling the release of small molecules from the nanoparticle surface into the bulk solution for sensing or signaling purposes or small-molecule delivery. For these purposes, it is of relevance to note that the monolayer surface can also be decorated with small peptide fragments.[17] Finally, the linear signal response as a function of the ratio of two metal ions illustrates the possibility of developing sensing systems that can detect combinations of metals.

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information

Experimental protocols, surface saturation concentrations in the presence of $Zn^{2+}$ or $Cu^{2+}$, release rates as a function of [TPEN], binding of $\mathbf{ATP_F}$ in the presence of TPEN·$Zn^{2+}$, and fitting of the $\mathbf{ATP_F}$ release rates from homo- and heterometalated surfaces. This material is available free of charge via the Internet at http://pubs.acs.org.

## ■ AUTHOR INFORMATION

### Corresponding Author

leonard.prins@unipd.it

### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

Financial support from the ERC (StG-239898) and COST (CM0703 and CM0905) is acknowledged.

## ■ REFERENCES

(1) Kiessling, L. L.; Gestwicki, J. E.; Strong, L. E. *Curr. Opin. Chem. Biol.* **2000**, *4*, 696.
(2) Dahan, A.; Portnoy, M. *J. Polym. Sci., Part A: Polym. Chem.* **2005**, *43*, 235.
(3) Badjic, J. D.; Nelson, A.; Cantrill, S. J.; Turnbull, W. B.; Stoddart, J. F. *Acc. Chem. Res.* **2005**, *38*, 723.
(4) Mulder, A.; Huskens, J.; Reinhoudt, D. N. *Org. Biomol. Chem.* **2004**, *2*, 3409.
(5) Rybtchinski, B. *ACS Nano* **2011**, *5*, 6791.
(6) Gestwicki, J. E.; Cairo, C. W.; Strong, L. E.; Oetjen, K. A.; Kiessling, L. L. *J. Am. Chem. Soc.* **2002**, *124*, 14922.
(7) Gillies, E. R.; Fréchet, J. M. J. *Drug Discovery Today* **2005**, *10*, 35.
(8) Daniel, M. C.; Astruc, D. *Chem. Rev.* **2004**, *104*, 293.
(9) Giljohann, D. A.; Seferos, D. S.; Daniel, W. L.; Massich, M. D.; Patel, P. C.; Mirkin, C. A. *Angew. Chem., Int. Ed.* **2010**, *49*, 3280.
(10) Saha, K.; Agasti, S. S.; Kim, C.; Li, X. N.; Rotello, V. M. *Chem. Rev.* **2012**, *112*, 2739.
(11) Palmer, L. C.; Stupp, S. I. *Acc. Chem. Res.* **2008**, *41*, 1674.
(12) Lim, Y. B.; Moon, K. S.; Lee, M. *Chem. Soc. Rev.* **2009**, *38*, 925.
(13) Uhlenheuer, D. A.; Petkau, K.; Brunsveld, L. *Chem. Soc. Rev.* **2010**, *39*, 2817.
(14) Fitzmaurice, D.; Rao, S. N.; Preece, J. A.; Stoddart, J. F.; Wenger, S.; Zaccheroni, N. *Angew. Chem., Int. Ed.* **1999**, *38*, 1147.
(15) Ling, X. Y.; Reinhoudt, D. N.; Huskens, J. *Chem. Mater.* **2008**, *20*, 3574.
(16) Klajn, R.; Fang, L.; Coskun, A.; Olson, M. A.; Wesson, P. J.; Stoddart, J. F.; Grzybowski, B. A. *J. Am. Chem. Soc.* **2009**, *131*, 4233.
(17) Bonomi, R.; Cazzolaro, A.; Sansone, A.; Scrimin, P.; Prins, L. J. *Angew. Chem., Int. Ed.* **2011**, *50*, 2307.
(18) Pieters, G.; Cazzolaro, A.; Bonomi, R.; Prins, L. J. *Chem. Commun.* **2012**, *48*, 1916.
(19) McClure, W. R.; Scheit, K.-H. *FEBS Lett.* **1973**, *32*, 267.
(20) The concentration of TACN head groups was determined from kinetic titrations using either $Zn(NO_3)_2$ or $Cu(NO_3)_2$, as reported previously.[17] The accuracy of the obtained concentration was 10 ± 1 $\mu$M.
(21) This value is slightly higher than reported previously (0.5 $\mu$M).[18] We observed that the surface saturation concentration of $\mathbf{ATP_F}$ on **1** showed slight variations between different nanoparticle batches.
(22) Dulkeith, E.; Morteani, A. C.; Niedereichholz, T.; Klar, T. A.; Feldmann, J.; Levi, S. A.; van Veggel, F.; Reinhoudt, D. N.; Moller, M.; Gittins, D. I. *Phys. Rev. Lett.* **2002**, *89*, No. 203002.
(23) At this point, we do not have conclusive evidence on the precise nature of the type of complexes formed on the surface. On the basis of the observation that 5 $\mu$M $Zn^{2+}$ was needed to reach the $\mathbf{ATP_F}$ surface saturation concentration of 2.3 ± 0.2 $\mu$M (especially at pH 8.0, where **1** does not bind) and the absence of binding when TACN was deprotonated, we assume that at least two charged TACN head groups (either metalated or protonated) contemporaneously interact with a single $\mathbf{ATP_F}$ probe.
(24) Smith, R. M.; Martell, A. E.; Motekaitis, R. J. *NIST Critically Selected Stability Constants of Metal Complexes*, version 8; NIST Standard Reference Database *46*; National Institute of Standards and Technology: Gaithersburg, MD, 2004.