Nanoparticles

International Edition: DOI: 10.1002/anie.201510337
German Edition: DOI: 10.1002/ange.201510337

Size-Controlled Formation of Noble-Metal Nanoparticles in Aqueous Solution with a Thiol-Free Tripeptide

*Stefano Corra, Urszula Lewandowska, Edmondo M. Benetti, and Helma Wennemers**

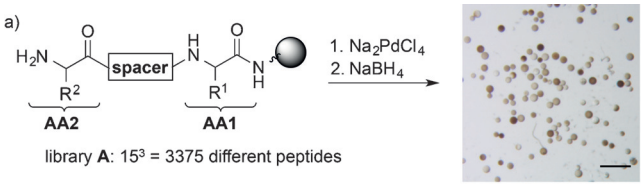
Abstract: *A combinatorial screening revealed the peptide H-His-D-Leu-D-Asp-NH_2 (**1**) as an additive for the generation of monodisperse, water-soluble palladium nanoparticles with average diameters of 3 nm and stabilities of over 9 months. The tripeptide proved to be also applicable for the size-controlled formation of other noble-metal nanoparticles (Pt and Au). Studies with close analogues of peptide **1** revealed a specific role of each of the three amino acids for the formation and stabilization of the nanoparticles. These data combined with microscopic and spectroscopic analyses provided insight into the structure of the self-assembled peptidic monolayer around the metal core. The results open interesting prospects for the development of functionalized metal nanoparticles.*

Biocompatible noble-metal nanoparticles (NPs) are increasingly important for applications in, for example, imaging and catalysis.[1–3] There is in particular a need for noble-metal NPs with sizes below 5 nm since larger NPs have shown toxicity.[4] A lot of research has focused on silver (Ag) and gold (Au) NPs whereas the formation of water-soluble palladium (Pd) and platinum (Pt) NPs has proven to be more challenging.[1–3,5] The preparation of water-compatible metal NPs is often carried out in organic solvents or biphasic solvent mixtures, which is unfavorable for biological applications and requires ligand exchange.[6,7] In the NP formation process, additives are necessary to control the nucleation, the growth, and the stability of the metal NPs.[6,7] Peptides are attractive biocompatible additives for all three stages of the NP formation since their high structural and functional modularity allows for tuning of the coordinating and self-assembly properties.[1,8–11] Thiol moieties (e.g. cysteine) typically serve as covalent anchors between the peptide ligand and the NP.[8] Yet, thiols can be unfavorable for applications in catalysis and biomedicine.[12] Also long peptides and proteins were used that can involve tedious syntheses.[1,13] Thus, alternative sulfur-free, easily accessible short-chain peptidic ligands that allow for the generation and stabilization of small metal NPs in aqueous environment are needed. Since the rational design of such thiol-free ligands is difficult, combinatorial approaches are attractive to identify suitable peptides for metal-NP formation.[10,11,14]

Recently, we introduced colorimetric on-bead screening of combinatorial split-and-mix libraries for the identification of peptides that control the formation of AgNPs.[11] Herein we used this method for the development of size-controlled PdNPs and present the tripeptide H-His-D-Leu-D-Asp-NH_2 (**1**) for the formation of stable and monodisperse PdNPs with an average diameter of approximately 3 nm in aqueous solution. We also present insight into the structure of the peptidic self-assembled monolayer (SAM) on the PdNP surface and show the versatility of the tripeptide for the preparation of other size-controlled noble metal NPs.

We started by preparing a one-bead-one-compound library (**A**) bearing structurally diverse D- and L-amino acids with functional groups, such as imidazole, hydroxy, amide, and carboxylic acid, that are binders of metal ions (Figure 1 a).[15] Cysteine was not included to bias the library towards non-thiol containing peptide ligands. The amino acids were connected by spacers with different degrees of rigidity. In each position 15 different building blocks were used resulting in a molecular diversity of maximally $15^3 = 3375$ different peptides. Unlike previous studies with AgNPs[11] the N-terminal amino groups were uncapped, which was envisioned to allow for the identification of new binding motives.

Upon incubating the library with an aqueous solution of Na_2PdCl_4 followed by washing with water to remove excess



AAn: (L or D) Pro, Arg, Lys, His, Trp, Asp, Glu, Asn, Gln, Ser, Thr, Tyr, Phe, Val, Ala

spacer: Pro, ProAib, ProGly, Gly, (1*R*,2*R*)Achc, (1*S*,2*S*)Achc, Leu, DLeu, Phe, DPhe, Val, DVal, Ala, Ahx,

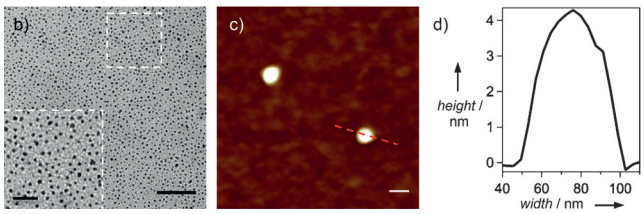


Figure 1. a) General structure of library **A** and combinatorial assay for PdNPs (scale bar 50 μm).[15] b) TEM image of PdNPs generated in the presence of **1** (scale bars 50 nm and 15 nm in expansion). c) AFM tapping mode micrograph of PdNP**@1** (scale bar 50 nm). d) Cross-section along the broken red line of the AFM micrograph in (c).

[*] S. Corra, Dr. U. Lewandowska, Prof. Dr. H. Wennemers
Laboratory of Organic Chemistry, D-CHAB
ETH Zürich
Vladimir-Prelog-Weg 3, 8093 Zürich (Switzerland)
E-mail: helma.wennemers@org.chem.ethz.ch
Dr. E. M. Benetti
Laboratory for Surface Science and Technology, D-MATL, ETH Zürich
Vladimir-Prelog-Weg 5, 8093 Zürich (Switzerland)

Supporting information for this article can be found under: http://dx.doi.org/10.1002/anie.201510337.

Pd^{II} and treatment with $NaBH_4$ several colored beads were observed (Figure 1a). The dark color is indicative of PdNPs and suggests that the peptides on the colored beads complex Pd^{II} and induce the formation of PdNPs upon chemical reduction. Isolation and analysis of several of the colored beads revealed His-D-Leu-D-Asp as a consensus sequence.[16]

Next we resynthesized H-His-D-Leu-D-Asp-NH_2 (**1**) and evaluated the ability of the peptide to generate and stabilize PdNPs in solution. Systematic variation of parameters such as the stoichiometry, the concentration, and the nature of the reducing agent, showed that adding tetrabutylammonium borohydride ($TBABH_4$) to a mixture of peptide **1** and Na_2PdCl_4 (10:1) in H_2O:CH_3CN (3:1) is optimal for the generation of stable, monodisperse PdNPs.[16] Transmission electron microscopy (TEM) revealed the formation of highly monodisperse PdNPs with a mean diameter of 2.8 ± 0.8 nm (Figure 1b). Noteworthy, this degree of monodispersity was obtained by simple mixing of the reaction partners without the need for further separation by, for example, size-exclusion chromatography. Energy-dispersive X-ray spectroscopy (EDX) verified the formation of PdNPs and atomic force microscopy (AFM) provided an average size of the nanoparticles of around 4 nm (Figure 1c,d). This difference of about 1 nm compared to the diameter observed by TEM suggests that the peptide forms a layer around the Pd core of the NP.[17] This finding was also supported by thermogravimetric analyses (TGA) of the particles, which revealed a surface coverage of six peptides per nm^2.[16] The generated PdNPs proved to be stable for more than 9 months as judged by repeated inspection by TEM. This is remarkable since a similar level of stability without aggregation has previously only been observed with thiol-containing ligands that form covalent bonds to the metal core.[8,18]

To understand the molecular basis for this unusual stability of the PdNPs formed in the presence of peptide **1**, analogues **1a**–**k** were prepared and evaluated for PdNP formation under the same conditions (variations from peptide **1** are highlighted in bold):

H-His-D-Leu-D-Asp-NH_2 (**1**)
Ac-His-D-Leu-D-Asp-NH_2 (**1a**)
H-D-Asp-D-Leu-His-NH_2 (**1b**)
H-**Ala**-D-Leu-D-Asp-NH_2 (**1c**)
H-**Arg**-D-Leu-D-Asp-NH_2 (**1d**)
H-**Lys**-D-Leu-D-Asp-NH_2 (**1e**)
H-His-D-**Ala**-D-Asp-NH_2 (**1f**)
H-His-D-Leu-D-**Ala**-NH_2 (**1g**)
H-His-D-Leu-D-**Ala-OH** (**1h**)
H-His-D-Leu-D-Asp-**OH** (**1i**)
H-His-D-Leu-NH_2 (**1j**)
H-His-D-Asp-NH_2 (**1k**)

N-terminally acetylated peptide **1a**, peptide **1b** with a reversed sequence of amino acids and peptide **1c** with alanine in place of histidine all failed to provide PdNPs and only aggregation was observed. This suggests that bidentate coordination by histidine via the N-terminal amino group and the imidazole moiety to Pd is critical for PdNP formation. ^{1}H NMR spectroscopic studies that monitored the complex formed between peptide **1** and Na_2PdCl_4 corroborated this hypothesis and revealed a 2:1 peptide–Pd^{II} complex.[16,19] Furthermore, the replacement of histidine with other bidentate amino acids at the N-terminus, arginine (**1d**) or lysine (**1e**), resulted in the formation of polydisperse or unstable PdNPs that aggregated within two days.[16] Also peptide **1f** bearing alanine in place of leucine did not allow for the formation of stable PdNPs, which demonstrates that a bulky hydrophobic substituent is critical in the middle position.[20,21] Peptide **1g**, with alanine in place of aspartic acid at the C-terminus provided monodisperse PdNPs with diameters of 2.5 ± 0.8 nm, however, they aggregated within a few hours. This result showed the importance of a carboxylic acid moiety at the C-terminal residue, which was further corroborated by the formation of stable PdNPs in the presence of peptide **1h**, an analogue of **1g** with a C-terminal CO_2H-group. Determination of the *Z*-potential of the PdNPs showed a switch from -17.8 mV for PdNP@**1** and PdNP@**1h** that contain CO_2H-group to $+16.2$ mV for PdNP@**1g** in which the carboxylate is absent. These findings suggest that the C-terminal amino acid points to the outside and prevents NP aggregation by electrostatic repulsion between the particles. Yet, peptide **1i** with two carboxylic acid residues at the C-terminal amino acid did not allow for PdNP formation, presumably due to the disruption of the peptidic SAM by too much electrostatic repulsion. Additional control experiments with histidine and the dipeptides **1j** and **1k** that lack the Asp and Leu residues, respectively, provided only unstable or larger, polydisperse PdNPs and further confirmed the fundamental role of each amino acid. Similar observations were made with PdNPs generated in the presence of all possible diastereoisomers of peptide **1** illustrating the importance of the absolute configuration of each residue within **1**.[16]

These structure activity studies indicate that the integrity of the peptidic SAM and the stability of the PdNPs rely on a subtle balance between attractive and repulsive interactions. Remarkably, this balance is achieved by a short tripeptide, where each of the three amino acids fulfills a specific role: a) histidine provides a bidendate coordination site to Pd, b) leucine enables the formation of a hydrophobic shell around the NP that likely prevents solvation and detachment of the peptide from the NP, and c) aspartic acid provides as an ionic amino acid for water solubility and prevents aggregation of the NPs by electrostatic repulsion of the negatively charged carboxylate moieties (Figure 2a).

Circular dichroism (CD) spectra of peptide **1** and PdNP@**1** differ from each other (Figure 2b), which suggests

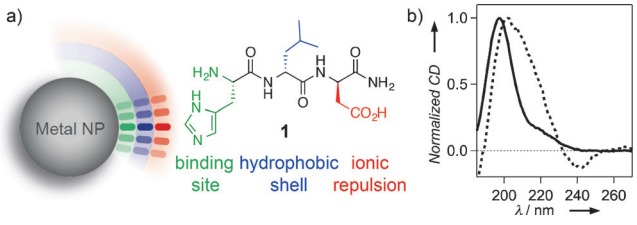


Figure 2. a) Proposed organization of peptide **1** in the SAM around the NPs. b) Normalized CD spectra of peptide **1** (solid line) and PdNP@**1** (dotted line).

that the conformation of peptide **1** changes upon formation of a peptidic monolayer around the NPs. The structure activity relationship studies combined with the spectroscopic and microscopic data support an organization of the peptide around the NP where the N-terminal His residue is bound to the PdNP core and the C-terminal Asp residue points to the outside and is exposed to the solvent (Figure 2a). We therefore envisioned that the C-terminal Asp residue can be functionalized and still allow for the formation of PdNPs of the same size. Experiments with derivatives of **1** that bear aminohexanoic acid (Ahx) or tyrosine moieties at the C-terminus (H-His-D-Leu-D-Asp-Ahx-NH_2 (**2**) and H-His-D-Leu-D-Asp-Tyr-NH_2 (**3**)) provided indeed highly monodisperse PdNPs with average diameters of around 2.5 nm.[16] Furthermore, similarly monodisperse and stable PdNPs were formed when 10% of a C-terminally biotinylated analogue of **1** (H-His-D-Leu-D-Asp-spacer-biotin (**4**))[22] was used in combination with **1**. These findings open interesting prospects for functionalizing PdNPs.

Finally we probed the generality of peptide **1** for the size-controlled formation of noble metal NPs. Since the coordination geometry of Pt^{II} and Au^{III} complexes is similar to that of Pd^{II} complexes, we hypothesized that these noble metals should form similarly sized NPs.[23] NMR spectroscopic studies with peptide **1** and Pt^{II} and Au^{III} salts, respectively, supported this assumption and showed, as observed for Pd^{II}, complexes with a 2:1 ratio of peptide:metal ion and significant changes in the chemical shifts of the imidazole protons of peptide **1** upon addition of the metal salts.[16] Reassuringly, upon addition of the reducing agent $TBABH_4$ to solutions of **1** and K_2PtCl_4 and $HAuCl_4$, respectively, monodisperse PtNPs and AuNPs with average diameters of 2.6 ± 0.8 nm and 3.0 ± 0.9 nm, respectively, were obtained (Figure 3).

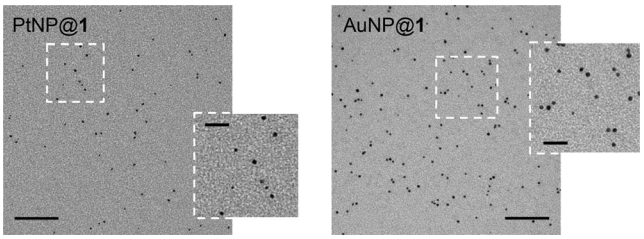


Figure 3. TEM images of PtNPs (left) and AuNPs (right) formed with peptide **1** (scale bar 50 nm and 15 nm in the expansion of the highlighted area in main image).

In analogy to the PdNPs, these PtNPs and AuNPs were stable for more than 9 months and have *Z*-potentials of −18.8 mV and −19.3 mV, respectively.[24] These findings support a comparable role of peptide **1** for nucleating and stabilizing all three different types of noble metal NPs.

In summary, we have introduced the peptide H-His-D-Leu-D-Asp-NH_2 as a thiol-free additive for the straightforward formation of highly monodisperse Pd, Pt, and Au nanoparticles that are stable for months in aqueous solution. Structure–activity relationship studies, in combination with microscopic and spectroscopic analyses, revealed the importance of each residue within the peptide for the NP formation and provided insight into the organization of the peptide around the metal core. These findings open intriguing prospects for the design of peptidic additives to develop size-controlled and functionalized metal NPs.

Experimental Section

*General procedure for PdNPs formation exemplified for peptide **1***: A stock solution of peptide **1** (20 mM in 500 μL of H_2O:CH_3CN 1:1) was diluted with H_2O (443 μL) followed by addition of an aqueous solution of Na_2PdCl_4 (30 mM, 33 μL). The mixture was stirred for 10 min and a freshly prepared solution of NBu_4BH_4 in CH_3CN (50 mM, 24 μL) was added dropwise under vigorous stirring. An immediate slightly brownish coloration of the previously colorless solution and the evolution of gas was observed. The NPs were characterized without further purification unless otherwise noted, see Supporting Information for details.

Acknowledgements

We thank the Scientific Center for Optical and Electron Microscopy (ScopeM) and Prof. Nicholas D. Spencer for providing access to high-resolution TEM and AFM, respectively. Christian Mensing is acknowledged for TGA analyses. We thank the Volkswagen Foundation for financial support.

Keywords: combinatorial chemistry · nanoparticles · noble metals · peptides · self-assembled monolayer

How to cite: *Angew. Chem. Int. Ed.* **2016**, *55*, 8542–8545
Angew. Chem. **2016**, *128*, 8684–8687

[1] a) M. R. Knecht, T. R. Walsh in *Bio-Inspired Nanotechnology (From Surface Analysis to Applications)*, Springer, New York, **2014**; b) M. B. Dickerson, K. H. Sandhage, R. R. Naik, *Chem. Rev.* **2008**, *108*, 4935–4978; c) A. Care, P. L. Bergquist, A. Sunna, *Trends Biotechnol.* **2015**, *33*, 259–268; d) M. Sarikaya, C. Tamerler, A. K. Y. Jen, K. Schulten, F. Baneyx, *Nat. Mater.* **2003**, *2*, 577–585; e) B. D. Briggs, M. R. Knecht, *J. Phys. Chem. Lett.* **2012**, *3*, 405–418; f) J. M. Slocik, R. R. Naik, *Chem. Soc. Rev.* **2010**, *39*, 3454–3463.

[2] a) E. Boisselier, D. Astruc, *Chem. Soc. Rev.* **2009**, *38*, 1759–1782; b) S. Rana, A. Bajaj, R. Mout, V. M. Rotello, *Adv. Drug Delivery Rev.* **2012**, *64*, 200–216; c) S. Eckhardt, P. S. Brunetto, J. Gagnon, M. Priebe, B. Giese, K. M. Fromm, *Chem. Rev.* **2013**, *113*, 4708–4754.

[3] a) A. Balanta, C. Godard, C. Claver, *Chem. Soc. Rev.* **2011**, *40*, 4973–4985; b) D. Astruc in *Nanoparticles and Catalysis*, Wiley-VCH, Weinheim, **2008**.

[4] A. Elsaesser, C. V. Howard, *Adv. Drug Delivery Rev.* **2012**, *64*, 129–137.

[5] B. Lim, M. Jiang, J. Tao, P. H. C. Camargo, Y. Zhu, Y. Xia, *Adv. Funct. Mater.* **2009**, *19*, 189–200.

[6] a) M. Brust, M. Walker, D. Bethell, D. J. Schiffrin, R. Whyman, *J. Chem. Soc. Chem. Commun.* **1994**, 801–802; b) M. J. Hostetler, J. E. Wingate, C.-J. Zhong, J. E. Harris, R. W. Vachet, M. R. Clark, J. D. Londono, S. J. Green, J. J. Stokes, G. D. Wignall, G. L. Glish, M. D. Porter, N. D. Evans, R. W. Murray, *Langmuir* **1998**, *14*, 17–30; c) M. T. Reetz, S. A. Quaiser, *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2240–2241; *Angew. Chem.* **1995**, *107*, 2461–2463.

[7] a) M. J. Hostetler, A. C. Templeton, R. W. Murray, *Langmuir* **1999**, *15*, 3782 – 3789; b) F. Manea, C. Bindoli, S. Polizzi, L. Lay, P. Scrimin, *Langmuir* **2008**, *24*, 4120 – 4124.

[8] a) I. M. Rio-Echevarria, R. Tavano, V. Causin, E. Papini, F. Mancin, A. Moretto, *J. Am. Chem. Soc.* **2011**, *133*, 8 – 11; b) R. Lévy, N. T. K. Thanh, R. C. Doty, I. Hussain, R. J. Nichols, D. J. Schiffrin, M. Brust, D. G. Fernig, *J. Am. Chem. Soc.* **2004**, *126*, 10076 – 10084; c) M. E. Muroski, T. J. Morgan, C. W. Levenson, G. F. Strouse, *J. Am. Chem. Soc.* **2014**, *136*, 14763 – 14771; d) E. Longo, A. Orlandin, F. Mancin, P. Scrimin, A. Moretto, *ACS Nano* **2013**, *7*, 9933 – 9939; e) L. Fabris, S. Antonello, L. Armelao, R. L. Donkers, F. Polo, C. Toniolo, F. Maran, *J. Am. Chem. Soc.* **2006**, *128*, 326 – 336.

[9] a) S. Si, T. K. Mandal, *Chem. Eur. J.* **2007**, *13*, 3160 – 3168; b) R. Coppage, J. M. Slocik, M. Sethi, D. B. Pacardo, R. R. Naik, M. R. Knecht, *Angew. Chem. Int. Ed.* **2010**, *49*, 3767 – 3770; *Angew. Chem.* **2010**, *122*, 3855 – 3858; c) G. Upert, F. Bouillère, H. Wennemers, *Angew. Chem. Int. Ed.* **2012**, *51*, 4231 – 4234; *Angew. Chem.* **2012**, *124*, 4307 – 4310; d) S. Papst, M. A. Brimble, C. W. Evans, D. J. Verdon, V. Feisst, P. R. Dunbar, R. D. Tilley, D. E. Williams, *Org. Biomol. Chem.* **2015**, *13*, 6567 – 6572; e) S. Kracht, M. Messerer, M. Lang, S. Eckhardt, M. Lauz, B. Grobéty, K. M. Fromm, B. Giese, *Angew. Chem. Int. Ed.* **2015**, *54*, 2912 – 2916; *Angew. Chem.* **2015**, *127*, 2954 – 2958.

[10] a) D. B. Pacardo, M. Sethi, S. E. Jones, R. R. Naik, M. R. Knecht, *ACS Nano* **2009**, *3*, 1288 – 1296; b) D. L. Feldheim, B. E. Eaton, *ACS Nano* **2007**, *1*, 154 – 159; c) J. M. Galloway, S. S. Staniland, *J. Mater. Chem.* **2012**, *22*, 12423 – 12434; d) C.-Y. Chiu, Y. Li, L. Ruan, X. Ye, C. B. Murray, Y. Huang, *Nat. Chem.* **2011**, *3*, 393 – 399.

[11] K. Belser, T. V. Slenters, C. Pfumbidzai, G. Upert, L. Mirolo, K. M. Fromm, H. Wennemers, *Angew. Chem. Int. Ed.* **2009**, *48*, 3661 – 3664; *Angew. Chem.* **2009**, *121*, 3715 – 3718.

[12] a) R. Munday, *Free Radical Biol. Med.* **1989**, *7*, 659 – 673; b) B. P. S. Chauhan, J. S. Rathore, T. Bandoo, *J. Am. Chem. Soc.* **2004**, *126*, 8493 – 8500; c) B. Panthi, A. Mukhopadhyay, L. Tibbitts, J. Saavedra, C. J. Pursell, R. M. Rioux, B. D. Chandler, *ACS Catal.* **2015**, *5*, 2232 – 2241.

[13] For examples, see: a) T. Ueno, M. Suzuki, T. Goto, T. Matsumoto, K. Nagayama, Y. Watanabe, *Angew. Chem. Int. Ed.* **2004**, *43*, 2527 – 2530; *Angew. Chem.* **2004**, *116*, 2581 – 2584; b) J. L. Burt, C. Gutiérrez-Wing, M. Miki-Yoshida, M. José-Yacamán, *Langmuir* **2004**, *20*, 11778 – 11783; c) J. Xie, J. Y. Lee, D. I. C. Wang, *J. Phys. Chem. C* **2007**, *111*, 10226 – 10232; d) M. Colombo, S. Mazzucchelli, V. Collico, S. Avvakumova, L. Pandolfi, F. Corsi, F. Porta, D. Prosperi, *Angew. Chem. Int. Ed.* **2012**, *51*, 9272 – 9275; *Angew. Chem.* **2012**, *124*, 9406 – 9409; e) K. C. Kwon, J. H. Ryu, J.-H. Lee, E. J. Lee, I. C. Kwon, K. Kim, J. Lee, *Adv. Mater.* **2014**, *26*, 6436 – 6441; f) R. R. Naik, S. J. Stringer, G. Agarwal, S. E. Jones, M. O. Stone, *Nat. Mater.* **2002**, *1*, 169 – 172; g) C. R. So, J. L. Kulp, E. E. Oren, H. Zareie, C. Tamerler, J. S. Evans, M. Sarikaya, *ACS Nano* **2009**, *3*, 1525 – 1531; h) J. Feng, J. M. Slocik, M. Sarikaya, R. R. Naik, B. L. Farmer, H. Heinz, *Small* **2012**, *8*, 1049 – 1059.

[14] For the use of split-and-mix libraries to discover metal–peptide complexes, see: a) M. B. Francis, N. S. Finney, E. N. Jacobsen, *J. Am. Chem. Soc.* **1996**, *118*, 8983 – 8984; b) A. S. Knight, E. Y. Zhou, J. G. Pelton, M. B. Francis, *J. Am. Chem. Soc.* **2013**, *135*, 17488 – 17493.

[15] Amino acids included in each position of the library: **AA1**: L-Pro, D-Arg, L-Lys, D-His, L-Trp, D-Asp, L-Glu, D-Asn, L-Gln, D-Ser, L-Thr, D-Tyr, L-Phe, D-Val, L-Ala; **Spacer**: L-Pro, L-Pro-Aib, 6-aminohexanoic acid, L-Pro-Gly, (1*R*,2*R*)-Achc (Achc = aminocyclohexanoic acid), (1*S*,2*S*)-Achc, no linker, Gly, L-Leu, D-Leu, L-Phe, D-Phe, L-Val, D-Val, L-Ala; **AA2**: D-Pro, L-Arg, D-Lys, L-His, D-Trp, L-Asp, D-Glu, L-Asn, D-Gln, L-Ser, D-Thr, L-Tyr, D-Phe, L-Val, D-Ala. For the concept of encoded one-bead-one-compound libraries, see: M. H. J. Ohlmeyer, R. N. Swanson, L. W. Dillard, J. C. Reader, G. Asouline, R. Kobayashi, M. H. Wigler, W. C. Still, *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 10922 – 10926.

[16] For further details, see the Supporting Information.

[17] Note that the AFM tip was too large to provide an accurate lateral profiling owing to tip convolution effects. Hence, only the height of the PdNP was reliably measured. See, for example, R. H. Terrill, T. A. Postlethwaite, C.-h. Chen, C.-D. Poon, A. Terzis, A. Chen, J. E. Hutchison, M. R. Clark, G. Wignall, J. D. Londono, R. Superfine, M. Falvo, C. S. Johnson, E. T. Samulski, R. W. Murray, *J. Am. Chem. Soc.* **1995**, *117*, 12537 – 12548.

[18] J.-W. Park, J. S. Shumaker-Parry, *ACS Nano* **2015**, *9*, 1665 – 1682.

[19] G. Pneumatikakis, C. Chassapis, A. Rontoyianni, *J. Inorg. Biochem.* **1993**, *49*, 83 – 96.

[20] For other examples in which Leu is critical for intermolecular interactions, see: a) P. W. Chun, *Int. J. Quantum Chem.* **2001**, *85*, 697 – 712; b) W. H. Landschulz, P. F. Johnson, S. L. McKnight, *Science* **1988**, *240*, 1759 – 1764.

[21] H-His-**D-Ser**-DAsp-NH_2 and H-His-**D-Asp**-DAsp-NH_2 bearing a hydrophilic residue in the middle position were also synthesized, but proved to be insoluble under the conditions required for NP formation.

[22] Spacer = Ahx_2-NH-PEO_4-NH, (PEO = polyethylene oxide).

[23] Only polydisperse Ag-nanoaggregates formed in the presence of peptide **1**. This finding corroborates the importance of the initially formed complex geometry since Ag^I forms linear complexes, see: L. Mirolo, T. Schmidt, S. Eckhardt, M. Meuwly, K. M. Fromm, *Chem. Eur. J.* **2013**, *19*, 1754 – 1761.

[24] For CD spectra and TGA analyses of PtNP@**1** and AuNP@**1**, see Supporting Information.

Received: November 6, 2015
Revised: March 23, 2016
Published online: April 21, 2016

