Acrylamide-based Pd-Nanoparticle Carriers as Smart Catalyst for the Suzuki-Miyaura Cross-Coupling of Amino Acids

Viktor Sabadasch, Steffen Dachwitz, Yvonne Hannappel, Thomas Hellweg, Norbert Sewald.

Affiliations below.

DOI: 10.1055/a-1782-4224


Conflict of Interest: The authors declare that they have no conflict of interest.

This study was supported by Deutsche Forschungsgemeinschaft (http://dx.doi.org/10.13039/501100001659), SE 609/16-1

Abstract:
Polyacrylamide-based waterborne microgels were prepared with copolymerized carboxylic acid and tertiary amine moieties. The colloidal gels were loaded with palladium nanoparticles and utilized for the Suzuki-Miyaura cross-coupling of amino acids and peptides. The thermoresponsive properties of the prepared microgels were characterized by means of photon correlation spectroscopy (PCS) at solvent conditions of the catalytic reaction. The localization and morphology of the incorporated nanoparticles were characterized with transmission electron microscopy (TEM). Palladium-catalyzed Suzuki-Miyaura cross-coupling of Nα-Boc-4 iodophenylalanine and Nα-Boc-7-bromotryptophan with phenylboronic acid was carried out under ambient atmosphere in water at 20 °C, 37 °C and 60 °C, respectively. The properties of the thermoresponsive microgel showed a strong influence on the reactivity and selectivity towards the respective substrate. For the amine containing microgels a recyclability for up to four cycles without loss in activity could be realized. Furthermore, the systems showed good catalytic activity regarding Suzuki-Miyaura cross-coupling of halogenated amino acids in selected tri- and tetrapeptides.

Corresponding Author: Norbert Sewald, Universität Bielefeld, Department of Chemistry, Bielefeld, Germany, norbert.sewald@uni-bielefeld.de

Affiliations:
Viktor Sabadasch, Universität Bielefeld, Department of Chemistry, Bielefeld, Germany
Steffen Dachwitz, Universität Bielefeld, Department of Chemistry, Bielefeld, Germany
Yvonne Hannappel, Universität Bielefeld, Department of Chemistry, Bielefeld, Germany
Norbert Sewald, Universität Bielefeld, Department of Chemistry, Bielefeld, Germany
Acrylamide-based Pd-Nanoparticle Carriers as Smart Catalyst for the Suzuki-Miyaura Cross-Coupling of Amino Acids

Viktor Sabadasch
Steffen Dachwitz
Yvonne Hannappel
Thomas Hellweg
Norbert Sewald

Abstract Polyacrylamide-based waterborne microgels were prepared with copolymerized carboxylic acid and tertiary amine moieties. The colloidal gels were loaded with palladium nanoparticles and utilized for the Suzuki-Miyaura cross-coupling of amino acids and peptides. The thermoresponsive properties of the prepared microgels were characterized by means of photon correlation spectroscopy (PCS) at solvent conditions of the catalytic reaction. The localization and morphology of the incorporated nanoparticles were characterized with transmission electron microscopy (TEM). Palladium-catalyzed Suzuki-Miyaura cross-coupling of N-$\text{N}$-Boc-4-iodophenylalanine and N-$\text{N}$-Boc-7-bromotryptophan with phenylboronic acid was carried out under ambient atmosphere in water at 20 °C, 37 °C and 60 °C, respectively. The properties of the thermoresponsive microgels showed a strong influence on the reactivity and selectivity towards the respective substrate. For the amine containing microgels a recyclability for up to four cycles without loss in activity could be realized. Furthermore, the systems showed good catalytic activity regarding Suzuki-Miyaura cross-coupling of halogenated amino acids in selected tri- and tetrapeptides.

Key words Palladium catalysis, bioorganic chemistry, cross-coupling, amino acids, nanoparticles, polyacrylamide microgel, halogenated peptide, polymers

Bio-orthogonal catalysis under mild, aerobic conditions in water has become more and more important in search for more economical and ecologically friendly processes. In particular, the Suzuki-cross-coupling is suitable for this purpose, since the required bases and aryl boronic acids are highly water soluble and tolerate most functional groups. Hence, the Suzuki-Miyaura reaction is an excellent method for (bio-)orthogonal late-stage functionalization of halogenated amino acids as well as complex biological molecules like halogenated peptides or proteins. The arylation of a protein containing L-4-iodophenylalanine was reported by Chalker et al. using a Pd-pyrindine precatalyst under aqueous conditions. Deb Roy et al. reported a Suzuki-Miyaura reaction of unprotected halotryptophan in water and investigated the fluorescence properties of the obtained arylated tryptophans. However, unprotected halogenated amino acids required high temperatures. Willemse et al. investigated the influence of different amino acid side chains in halophenylalanine- and halotryptophan-containing dipeptides on the Suzuki-Miyaura reaction. The combination of biocatalytic halogenation and subsequent chemocatalytic arylation in a two-step one-pot reaction without isolation of the halogenated species was reported independently by Latham et al. and Fresse et al. Based on the fluorescence properties of aryalted tryptophans, Schnepel et al. designed a high-throughput fluorescence assay for screening halogenases in directed evolution assays. The first arylation of biologically active macromolecules containing halotryptophan were reported by Sharma et al., describing an in vivo Suzuki-Miyaura cross coupling on bromopacidamycin at 37 °C. Kemker et al. increased stability and selectivity of cyclic RGD peptides using a Suzuki-Miyaura cross-coupling for fluorescence labelling or side chain to side chain cyclization.

Catalysis by nanoparticles recently attracted attention, since their catalytic activity does not require inert gas atmosphere. The direct use of nanoparticles is difficult to realize because agglomeration becomes a major problem when bulk metals are scaled down to nanoparticle size. Exceptionally, we were able to use ligand free Pd-nanoparticles for the direct arylation of L-7-bromotryptophan and bromotryptophan containing peptides under ambient conditions. However, embedding nanoparticles in polymeric matrices may improve their colloidal stability and even their catalytic activity. Dumas et al. incorporated Pd nanoparticles into poly(d,l-lactide-glycolide)-block-poly(ethylene glycol) copolymer (PLGA-PEG-Pd-NPs). Using these assemblies as a catalyst in the Suzuki-Miyaura reaction of N-$\text{N}$-Boc-L-4-iodophenylalanine provided up to 98 % conversion in water at 37 °C after 18 h. Recently, Peramo et al. reported a self-assembling NHC-Pd-loaded calixarene as a catalyst for the arylation of Boc-protected L-4-iodophenylalanine reaching 40 % conversion after 3 h at 37 °C in water.

Thermoresponsive microgels became promising candidates for the incorporation of nanoparticles because of their broad range
of properties. Their characteristic feature is the responsiveness towards a change in temperature. When heated above the so-called volume phase transition temperature (VPTT) the highly hydrophilic polymer network collapses, shrinks in size, and expels water from its network. The mesh size of the polymer network decreases, and the gel becomes much more hydrophobic. One of the most studied microgel systems is based on poly-\(N\)-isopropylacrylamide (PNIPAM) which has a VPTT at about 32 °C.\(^{13,14}\) Due to their unique properties, microgels found application such as drug release\(^{15}\) or carriers for organo-catalysts\(^{16}\) or catalytic nanoparticles.\(^{20}\) In the latter example, it is of particular interest that the activity of the embedded catalyst can be tuned with the degree of swelling of the polymer network. For gold nanoparticles embedded in a PNIPAM-based yolk-shell system Wu et al. demonstrated that the reactivity of hydrophilic reactants decreases and that of hydrophobic reactants increases when the system was heated above the VPTT.\(^{21}\) Angioletti-Uberti et al. were able to prove that the change in solubility observed by Wu et al. is mainly attributed to the change of solution free-enthalpies at temperatures above the VPTT.\(^{22}\)

Copolymerization of pH-sensitive monomers such as amines or acids can greatly alter the thermoresponsive properties of microgels.\(^{23}\) In the case of acrylamide-based microgels copolymerized with methacrylic acid, the microgels show almost identical thermoresponsive properties in the protonated state of the acid as the system without methacrylic acid.\(^{24}\) Increasing the pH so that the methacrylic acid is completely deprotonated is accompanied by a large increase in particle size. This is due to the strong electrostatic repulsion of the carboxylate ions, which additionally impart a strong hydrophilic character to the network. The VPTT of the system was strongly broadened and shifted to much higher temperatures. Temperature-dependent catalysis of 4-nitrophenol on palladium nanoparticles embedded in that system showed that the catalytic activity strictly followed Arrhenius-like behavior and the microgels VPTT had no effect on the reactivity of the embedded nanoparticles.\(^{24}\) By applying an acid-free shell to the microgels, the switchable property of the acrylamide material was restored. In contrast, another core-shell architecture loaded with silver nanoparticles also enabled an activating effect of the shells VPTT towards hydrophilic substrates.\(^{25}\)

Another important feature of applying pH-responsive moieties into microgel particles, is that these moieties introduce defined charges into the microgel. These changes can be used to incorporate metal ions into the microgel at the location of the copolymer. Using this method, a defined localization of metal nanoparticles within the microgel can be achieved.\(^{26}\)

In this work, four different palladium loaded microgel systems are presented, namely poly-\(N\)-\(n\)-propylacrylamide (PNNPAM) and PNIPAM systems, each copolymerized with either methacrylic acid (MAc) or \(N\)-[3-(dimethylamino)-propyl]-methacrylamide (DAPMA). The comonomers MAc and DAPMA incorporate a carboxylic acid or a tertiary amine, respectively, into the network (Figure 1). The microgels were prepared in a surfactant free precipitation polymerization approach.\(^{27}\) Potentiometric titration was used to determine the incorporation rates and apparent \(pK_a\) values of the comonomers. The thermoresponsive properties of the systems were investigated by means of photon correlation spectroscopy (PCS). Localization and size of the incorporated palladium nanoparticles were investigated with transmission electron microscopy (TEM). Catalytic activity towards interesting substrates was investigated by performing Suzuki-Miyaura cross-coupling on phenylboronic acid with \(N^\alpha\)-Boc-4-isodophenylalanine and \(N^\alpha\)-Boc-7-bromotryptophan. Regarding \(N^\alpha\)-Boc-4-isodophenylalanine, the performance was tested in several recycling cycles. We also investigated the catalytic activity with different peptides containing either 7-bromotryptophan or 4-iodophenylalanine.

![Figure 1: Structural formulae of the used acrylamide building blocks for PNNPAM and PNIPAM, the comonomers MAc and DAPMA and the crosslinker N'-methylene-bisacrylamide (BIS).](image)

The determined amount and apparent \(pK_a\) values of copolymerized MAc and DAPMA were analyzed by potentiometric titration as shown in Table 1

<table>
<thead>
<tr>
<th>System</th>
<th>(n_{co}/\text{mol/g})</th>
<th>(pK_a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNNPAM-co-MAc</td>
<td>0.99 ± 0.01</td>
<td>6.5 ± 0.1</td>
</tr>
<tr>
<td>PNNPAM-co-MAc</td>
<td>0.71 ± 0.02</td>
<td>6.6 ± 0.2</td>
</tr>
<tr>
<td>PNIPAM-co-DAPMA</td>
<td>0.67 ± 0.03</td>
<td>8.6 ± 0.2</td>
</tr>
<tr>
<td>PNIPAM-co-DAPMA</td>
<td>0.79 ± 0.02</td>
<td>8.9 ± 0.1</td>
</tr>
</tbody>
</table>

While free MAc and DAPMA monomers have \(pK_a\) values of 4.66 (acid)\(^{28}\) and 9.3 (ammonium)\(^{29}\) respectively, copolymerization into the polymer network shifts the \(pK_a\) of MAc to higher values of 6.6 and 6.5 and that of DAPMA to lower values of 8.6 and 8.9 in the respective PNNPAM and PNIPAM microgels. This shift is commonly observed in pH-responsive polymeric material and is attributed to the polyelectrolyte effect. Neighboring pH-responsive moieties influence each other upon deprotonation, shifting their \(pK_a\) towards weaker acids/bases with increasing degree of ionization.\(^{30}\) Comparing the incorporation rate of the comonomers, methacrylic acid is better incorporated in the slightly more hydrophilic acrylamide system PNNPAM, while the DAPMA system is better incorporated in the slightly more hydrophilic\(^{31}\) PNIPAM system.

We performed photon correlation spectroscopy (PCS) experiments to probe the temperature-dependent properties of the colloidal dispersed particles. The hydrodynamic radii of the PNNPAM and PNIPAM microgels copolymerized with methacrylic acid in a K\(_2\)PO\(_4\) solution at a pH value of 12 are plotted against the temperature in Figure 2. The chosen K\(_2\)PO\(_4\) solution and pH value corresponds to the requirements of the subsequent Suzuki-Miyaura cross-coupling reactions.
This article is protected by copyright. All rights reserved.

Since the apparent pH values of the methacrylic acid-containing systems are 6.6 and 6.5 respectively (Table 1), a pH value of 12 induces a complete deprotonation of the methacrylic acid leading to strong electrostatic repulsions of the carboxylates in the microgel. At a temperature of 12 °C both systems are in their completely swollen state and have a hydrodynamic radius of about 320 nm. When the temperature is increased, both systems show a steady decrease in size over the entire temperature range. The characteristic sharp VPTTs of homopolymer PNNPAM microgels at 22 °C and homopolymer PNIPAM microgels at 32 °C can no longer be clearly identified. The increased hydrophilicity and electrostatic repulsion, which result from the present carboxylates shift the VPTT of the systems to far higher temperatures. At 60 °C the PNNPAM and PNIPAM systems show hydrodynamic radii of about 250 nm and 235 nm, respectively. The PNIPAM system collapses to a greater extent than the PNNPAM system, which is attributed to the lower content of deprotonated hydrophilic carboxylates within the PNIPAM network (Table 1).

Temperature-dependent PCS measurements at a pH value of 12 were also performed for the PNNPAM-co-MAc and PNIPAM-co-MAc microgels (Figure 3). In contrast to MAC-DAPMA is an acrylamide-comonomer with a tertiary amine functionality. Its apparent pHc value is 8.6 or 8.9 in the PNNPAM-co-MAc or PNIPAM-co-DAPMA system, respectively. At a pH of 12 the amines are thus mostly deprotonated and no charges other than those of the initiator used, 2,2'-azobis-2-methylpropionamide, are present in the microgel. In the fully swollen state at a temperature of 12 °C, the PNNPAM system (270 nm) is about 30 nm larger than the PNIPAM system. Increasing the temperature leads to a VPTT of the PNNPAM system at about 26 °C and the PNIPAM system at about 32 °C. Within the volume phase transition, a strong increase in $R_h$ is observed. This increase is caused by strong interaction effects of the colloidal particles, which interfere the independent diffusion of the particles. The dynamic light scattering data above the VPTT can thus no longer be evaluated. This observation is characteristic for amine containing microgels. The effect of microgel interaction above the VPTT is very pronounced in the presented DAPMA microgels, indicating that the volume phase transition induces a strong shift of physicochemical properties of the particles.

The incorporation of palladium nanoparticles in the MAC and DAPMA microgels was performed with two different palladium complexes. While the MAC systems were mixed with a [Pd(NH$_3$)$_2$Cl$_2$] complex and the protonated DAPMA moieties as anchors for the anionic palladium chloride complexes at a low pH value. The aim was to use the deprotonated MAC as anchor points for the positive [Pd(NH$_3$)$_2$Cl$_2$] complex and the protonated DAPMA moieties as anchors for the anionic palladium chloride complexes. Subsequent addition of sodium borohydride reduced the metal salts to the catalytically active Pd nanoparticles. Using this method, we were already able to load methacrylic acid containing systems with palladium nanoparticles. Loading of DAPMA-containing systems was previously demonstrated for PNIPAM-based gel particles by Seto et al. TEM images of the prepared microgel/Pd-hybrid microgels are shown in Figure 4 (top). The microgels copolymerized with methacrylic acid show similar loadings with nanoparticles. While the PNNPAM-co-MAc system incorporated nanoparticles with a size of (8.7±0.8) nm the PNIPAM-co-MAc system contains nanoparticles with a size of (12.7±1.5) nm (ESI, Figure S1). In both cases, the localization seems to be in the center of the particle rather than in the outer part. Previous assumption of Hoare et al. state that MAC copolymerizes faster than NIPAM and is therefore more likely to be localized in the particle center. Although the images (Figure 4) are only two-dimensional views through completely dried microgels, localization in the particle center is likely.

The PNNPAM-co-MAc system shows a stronger microgel contrast than the PNIPAM system. This could be due to a higher content of crosslinkers in the microgel, resulting in a higher rigidity of the network, with the microgel drying in a more compact structure on the substrate. Nano-particle incorporation in the DAPMA containing systems shows significantly different results. In both cases (Figure 4 bottom) extremely small nanoparticles throughout the whole microgel were incorporated. In both DAPMA systems the nanoparticles with a size of < 3 nm are clearly separated, and no aggregation occurs. A quantitative size analysis of the palladium nanoparticles was not possible. Nevertheless, due to their very small size, the nanoparticles in the DAPMA systems yield a much larger surface-to-mass ratio than the nanoparticles incorporated in the MAC systems.
The thermoresponsive properties of the microgels are preserved after nanoparticle incorporation (ESI, S2 and S3). The catalytic activity of the embedded Pd-nanoparticles was investigated in a Suzuki-Miyaura cross-coupling of two different Nα-Boc-protected halogenated amino acids, namely 4-iodophenylalanine (1) and 7-bromotryptophan (2) with phenylboronic acid (3, 2 eq.). The four microgel Pd-nanoparticle hybrid systems were tested on a 10 mM scale of the amino acid with K2PO4 (5 eq.) as base under aerobic and aqueous conditions at room temperature, 37 °C and 60 °C, respectively. The reaction progress was monitored by reversed phase high performance liquid chromatography (RP-HPLC) (see Table 2 and 3). The palladium nanoparticles embedded in PNIPAM-co-MAc and PNIPAM-co-DAPMA microgels gave most promising results in the Suzuki-Miyaura coupling of Nα-Boc-4-iodophenylalanine giving full conversion at 37 °C after 24 h as well as reasonable conversions of 30 % and 46 % after 24 h at room temperature, respectively (Table 2, entry 2 & 4). Notably, the PNNPAM-co-MAc and PNIPAM-co-DAPMA systems show similar conversions at room temperature (Entry 2 & 3), but at 37 °C the PNNPAM-co-MAc encapsulated nanoparticles give full conversion after 24 h, while the PNIPAM-co-DAPMA system gives only 62 %. In the case of the PNNPAM-co-MAc system conversion was increased from 6 % at 20 °C up to 51 % at 37 °C, making the MAc containing microgels more favourable at elevated temperatures. All in all, for the Suzuki-Miyaura cross-coupling of iodophenylalanine (1) the cationic DAPMA systems show better catalytic activities at room temperature compared to the anionic MAc microgels, while the MAc embedded nanoparticle systems show better reactivities at 37 °C (for more detailed conversion as function of time see supporting information Figure S4-S6).

Due to the intense formation of microgel agglomerates of the DAPMA systems at 26 °C and 32 °C (see Figure 2), respectively, no reactions above 37 °C were performed. Reusability is a major advantage of microgel carried nanoparticle catalysts. Hence, the recyclability of the four microgel embedded Pd-nanoparticles was investigated by the reaction of iodophenylalanine (1) and phenylboronic acid (3) at 37 °C. Therefore, catalysis was performed over 48 h, afterwards the conversion was monitored by RP-HPLC at 220 nm and the reaction vessel was centrifuged at 10,000 rpm for 10 minutes. The supernatant solution was discarded, and the remaining catalyst was resuspended in a freshly prepared solution of all reactants. This procedure was repeated until a loss in reactivity could be observed (Figure 5).

<table>
<thead>
<tr>
<th>Entry</th>
<th>catalyst</th>
<th>20 °C</th>
<th>37 °C</th>
<th>60 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PNNPAM-co-MAc</td>
<td>6</td>
<td>51</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>PNIPAM-co-MAc</td>
<td>30</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>PNNPAM-co-DAPMA</td>
<td>31</td>
<td>62</td>
<td>n.d.</td>
</tr>
<tr>
<td>4</td>
<td>PNIPAM-co-DAPMA</td>
<td>46</td>
<td>100</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

[a] determined by HPLC (220 nm); [b] for detailed conversion as function of time see supporting information; [c] reaction was complete after two hours; [d] not determined.

The MAc copolymer microgels showed almost no recyclability giving 5 % and 19 % in the second cycle after 48 h, respectively. The DAPMA systems showed no observable loss in reactivity before the fifth cycle, still providing 39 % and 52 % conversion after 4 recyclings. In comparison the PNIPAM system seems to be...
slightly better to recycle than the PNNPAM system. The TEM images after the cycle in which reactivity is lost (cycle 2 for co-MAc and cycle 5 for co-DAPMA) show that the microgels are soaked with non-volatile material, which is presumably product, in the case of the MAC-containing systems (ESI, Figure S7). This most likely causes the microgel network to become clogged and no new reactant can reach the catalyst. The DAPMA systems, despite their now undefined outer region, appear to be unaffected by the catalytic cycles. As can be seen in the TEM micrographs shown in the SI, the nanoparticles generally retained their morphology and size after the use in the reaction (ESI, Fig S7).

Besides the very reactive Boc-iodophenylalanine $1$, the less reactive amino acid $N^\alpha$-Boc-7-bromotryptophan ($2$) was chosen as an additional starting material for Suzuki-Miyaura cross-coupling. Due to the lower reactivity of bromoarenes and the high electron density of the indole moiety of tryptophan, it represents a less reactive substrate in Suzuki-Miyaura couplings, leading to lower conversions compared to iodophenylalanine ($1$). However, both PNNPAM systems again gave higher $30\%$ and $29\%$ conversion to $N^\alpha$-Boc-7-phenyltryptophan ($5$) within $24\mathrm{\,h}$ at $37\,^\circ\mathrm{C}$ (Table 3, entries 2 and 4). In comparison, ligand free Pd-nanoparticles give full conversion after $2\,\mathrm{h}$ at $40\,^\circ\mathrm{C}$.

Table 3 Suzuki-Miyaura reaction of $N^\alpha$-Boc-7-bromotryptophan ($2$) with phenylboronic acid $3$ catalyzed by microgel Pd-nanoparticle hybrid systems at different temperatures.

<table>
<thead>
<tr>
<th>Entry</th>
<th>catalyst</th>
<th>conversion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$20,^\circ\mathrm{C}$</td>
</tr>
<tr>
<td>1</td>
<td>PNNPAM-co-MAc</td>
<td>n.d.</td>
</tr>
<tr>
<td>2</td>
<td>PNNPAM-co-MAc</td>
<td>traces</td>
</tr>
<tr>
<td>3</td>
<td>PNNPAM-co-DAPMA</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>PNNPAM-co-DAPMA</td>
<td>3</td>
</tr>
</tbody>
</table>

[a] determined by HPLC (220 nm); [b] for detailed conversion as function of time see supporting information; [c] not determined.

Notably, the PNNPAM-co-DAPMA microgel incorporated nanoparticles gave $45\%$ conversion after $24\,\mathrm{h}$ at $37\,^\circ\mathrm{C}$ (Table 3, entry 3) while PNNPAM-co-MAc nanoparticles only gave $5\%$ conversion (Table 3, entry 1). Comparing these results with the conversions of $N^\alpha$-Boc-4-iodophenylalanine ($1$) in which both mentioned catalysts showed almost the same reactivity giving $51\%$ (Table 2, entry 1) and $62\%$, respectively (Table 2, entry 3), this shows a major difference in the substrate selectivity of the anionic and cationic microgel systems. At room temperature, all catalytic microgels gave only low conversions of $N^\alpha$-Boc-7-bromotryptophan ($2$) with maximum of $5\%$ in $24\,\mathrm{h}$. Due to the intense formation of microgel agglomerates of the DAPMA systems at $26\,^\circ\mathrm{C}$ and $32\,^\circ\mathrm{C}$ (Figure 3), respectively, no reactions above $37\,^\circ\mathrm{C}$ were performed (for more detailed conversion as function of time diagrams see supporting information Figure S8-S10). During all reactions with the MAC microgels at $60\,^\circ\mathrm{C}$ byproducts like homo-coupled phenylboronic acid ($<5\%$) and cleavage of Boc protecting groups ($13\%$ in $24\,\mathrm{h}$) independently of substrate and catalyst were observed via RP-HPLC.

A remarkable shift in solubility of the reactants above the VPTT is observed for the PNNPAM systems. While the PNNPAM-co-MAc system showed only a conversion of $5\%$ at $37\,^\circ\mathrm{C}$, the PNNPAM-co-DAPMA system showed an increase from only $5\%$ conversion at $20\,^\circ\mathrm{C}$ to $45\%$ at $37\,^\circ\mathrm{C}$. This strong increase in reactivity can be attributed to the strong influence of the volume phase transition of the particle (see Fig. 3). Upon heating the PNNPAM-co-DAPMA system above its VPTT at $26\,^\circ\mathrm{C}$ it gets more hydrophobic and the likewise hydrophobic $N^\alpha$-Boc-7-bromotryptophan is more soluble in the network leading to an increased catalytic activity of the embedded palladium nanoparticles.

To finally prove the usability of microgel carried Pd-nanoparticles as catalysts for complex natural compounds such as peptides under environmental conditions, three different $4$-iodophenylalanine containing tripeptides were prepared as substrates for a Suzuki-Miyaura coupling. The cross-coupling was performed under ambient, aerobic conditions at $37\,^\circ\mathrm{C}$ in water to prevent degradation of the peptides (Table 4).

![Figure 5](image-url) Activity of microgel embedded Pd-nanoparticle catalysts for Suzuki-Miyaura cross-coupling of $N^\alpha$-Boc-4-iodophenylalanine $1$ with phenylboronic acid $3$ at $37\,^\circ\mathrm{C}$ in different cycles of $48\,\mathrm{h}$ each.
Table 4 Suzuki-Miyaura reaction of three different 4-iodophenylalanine containing tripeptides with phenylboronic acid 3 catalyzed by microgel Pd-nanoparticle hybrid systems.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>R = H</th>
<th>R = OH</th>
<th>R = Phenyl</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PNNPAM-co-MAc</td>
<td>traces</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>PNIPAM-co-MAc</td>
<td>traces</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>PNNPAM-co-DAPMA</td>
<td>3</td>
<td>61</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>PNIPAM-co-DAPMA</td>
<td>8</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

[a] determined by HPLC (220 nm); [b] full conversion after 48 h; [c] 79 % isolated yield after preparative HPLC; [d] 41 % conversion after 1 week; [e] 38 % isolated yield after 1 week of reaction and preparative HPLC.

Tripeptide (8), which contains an additional phenylalanine to increase the lipophilicity of the tripeptide, showed no conversion with any catalyst at the chosen reaction conditions and only substrate was recovered. Using the MAc microgel embedded Pd-nanoparticles only traces of 9 could be observed by HPLC. Surprisingly DAPMA containing microgel systems showed contrary substrate selectivity. The PNNPAM-co-DAPMA embedded nanoparticles gave 3 % conversion of 9 after 24 h but a twenty-fold higher conversion (61 %) of the slightly more polar substrate 7 to give 10 (Table 4, entry 3). PNIPAM-co-DAPMA embedded nanoparticles showed a three-fold higher conversion (8 %) within 24 h of 6 to give 9 compared to 2 % conversion of 7 (entry 4).

Encouraged by these results, a 7-bromotryptophan containing tripeptide and tetrapeptide were synthesized as substrates for a Suzuki-Miyaura cross-coupling (Table 5). Since we recently observed a drastic acceleration effect by methionine in the peptide chain on the reactivity of bromotryptophan containing peptides in Pd-nanoparticle catalyzed Suzuki-Miyaura cross couplings, these two peptides were prepared with a methionine at the N-terminus.[7]

Table 5 Suzuki-Miyaura reaction of a 7-bromotryptophan containing tripeptide 12 or tetrapeptide 13 with phenylboronic acid 3 catalyzed by microgel Pd-nanoparticle hybrid systems.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>12</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PNNPAM-co-DAPMA</td>
<td>4</td>
<td>traces</td>
</tr>
<tr>
<td>2</td>
<td>PNIPAM-co-DAPMA</td>
<td>21</td>
<td>17</td>
</tr>
</tbody>
</table>

[a] determined by HPLC (220 nm).

To reach sufficient solubility of the tetrapeptide 13 in water, aspartic acid was also introduced into the peptide sequence. According to the results of the screenings before, only DAPMA copolymerized microgel systems were tested. In case of the bromotryptophan containing peptides PNNPAM-co-DAPMA embedded Pd-nanoparticles gave better conversions of 21 % of 12 and 17 % of 13 compared to the PNIPAM system which gave only 4 % maximum conversion. However, the tripeptide was the better accepted substrate by both systems.

In conclusion, polyacrylamide based microgels containing carboxylic acid and tertiary amine moieties loaded with Pd-nanoparticles have been identified as suitable catalysts for the bio-orthogonal Suzuki-Miyaura cross-coupling of halogenated amino acids under mild, aerobic, and aqueous conditions between ambient temperature and 60 °C. The thermoresponsive properties of the presented microgel systems were characterized by means of temperature-dependent PCS measurements at analogous conditions to the catalysis at elevated pH value. While the MAc containing systems showed a steady volume phase transition over the whole temperature range and full colloidal stability, a sharp change in properties was observed for the amine containing microgels. At the respective VPTT of the used acrylamide, strong aggregational effects were observed, indicating a strong shift in hydrophobicity within the microgel particle. The palladium loaded microgels were characterized via TEM, revealing that the MAc systems incorporate the nanoparticles with sizes between (8.7±0.8) nm and (12.7±1.5) nm, locate probably towards the particle center. The amine systems incorporated nanoparticles with only a few nanometers throughout the whole microgel spheres. Despite their very small size, no visible aggregation of the nanoparticles could be observed. The subsequent Suzuki-Miyaura cross-coupling of 4-iodophenylalanine and 7-bromotryptophan with phenylboronic acid revealed that hydrophilic MAc containing systems are more suitable for the catalysis of the coupling of 4-iodophenylalanine, DAPMA containing systems showed superior activity in the catalysis of 7-bromotryptophan coupling. The
PNPAM-co-DAPMA, with its pronounced volume phase transition, showed superior reactivity with respect to the conversion of the 7-bromotryptophan, when heated above the VPTT. This emphasizes that the thermoresponsivity adds an additional degree of freedom to tune the catalytic activity of embedded nanoparticles.

Regarding the catalysis of 4-iodophenylalanine on the DAPMA systems a recycling in up to 4 cycles without loss in activity was possible. The DAPMA containing systems were further used to catalyze the coupling of 4-iodophenylalanine or 7-bromotryptophan containing tripeptides and tetrapeptides showing that the chosen acrylamide is decisive for the selectivity.

Experimental

Materials: N-Isopropylacrylamide (NIPAM) was recrystallized from n-hexane. N-n-Propylacrylamide (NPNPAM) was prepared by a Schotten-Baumann synthesis as described by Hirano et al.36 Unless otherwise stated, the chemicals used were obtained from commercial suppliers with analytical grade.

Micromol synthesis: The microparticles were synthesized by precipitation polymerization. The respective acrylamide NIPAM or NPNPAM (38.51 mmol), BIS (1.92 mmol, 5 mol%), and respective comonomer Mac or DAPMA (3.85 mmol, 10 mol%) were dissolved in 490 mL water. The mixture was purged with nitrogen and heated up to 70 °C under constant stirring for 2 hours. The polymerization was initiated by the addition of ammonium persulfate (1.36 mmol, 3.5 mol%) for the Mac containing systems or 2,2'-azobis-2-methylpropionamidine (1.36 mmol, 3.5 mol%) for the DAPMA containing systems in 10 mL of purified water. The reaction proceeded for 4 h under constant stirring at 70 °C. Afterwards the reaction solution was cooled down to room temperature and stirred overnight. The microparticle was purified by five consecutive centrifugation, decantation and redispersion cycles with purified water. The PNNPAM-co-DAPMA system was synthesized in a reduced scale with 150 mL water.

Nanoparticle loading: For the loading of the PNNPAM-co-Mac and PNNPAM-co-Mac systems a reaction solution (45 mL) containing the respective microgel (100 mg dry mass of microgel) and a palladium salt were heated up to 70 °C under constant stirring for 2 hours. The palladium mass relative to microgel/nanoparticle systems or DAPMA (3.85 mmol, 10 mol%) were dissolved in 490 mL water. The mixture was purged with nitrogen and heated up to 70 °C under constant stirring for 2 hours. The palladium containing systems was dissolved in 10 mL of purified water. The reaction proceeded for 4 h under constant stirring at 70 °C. Afterwards the reaction solution was cooled down to room temperature and stirred overnight. The microparticles were purified by five consecutive centrifugation, decantation and redispersion cycles with purified water. The PNNPAM-co-DAPMA system was synthesized in a reduced scale with 150 mL water.

Nanoparticle characterization: The samples were prepared on carbon coated-copper grids (ECF200–Cu, 200 mesh, Science Services, Munich, Germany). The grids were pre-treated in a plasma cleaner (Diener Electronics, Ebhausen, Germany) with argon. The sample (3 μL) was applied onto the grids and after a sedimentation time of one minute, the excess suspension was removed with a filter paper. The prepared grids and after a sedimentation time of one minute, the excess suspension was redispersed in a solution of 5 % B to 95 % B in 3 min, total run time of 9 min) at a flow rate of 650 μL/min and column oven temperature of 40 °C. HPLC solvent A consisted of 99.9 % water with 0.1 % TFA, solvent B of 99.9 % acetonitrile with 0.1 % TFA.

Analytical HPLC: Analytical HPLC was performed on a Shimadzu Nexera XRS 20A System (Shimadzu, Kyoto, Japan) with autosampler, degasser, diode array detector and a Luna C18 column (Phenomenex, Torrance, CA, USA) (2.9 μm, 50 x 2.1 mm) with a gradient (in 5.5 min from 5 % B to 95 % B, 0.5 min 95 % B and back to 5 % B in 3 min, total run time 9 min) at a flow rate of 300 μL/min and column oven temperature of 40 °C. HPLC solvent A consisted of 99.9 % water with 0.1 % TFA, solvent B of 99.9 % acetonitrile with 0.1 % TFA.

NMR: NMR spectra were recorded on Bruker Avance III 500 HD (1H: 500 MHz, 13C: 126 MHz) or Avance 600 (1H: 600 MHz, 13C: 151 MHz). Chemical shifts δ [ppm] are reported relative to residual solvent signals (DMSO-d6, 1H: 2.50 ppm, 13C: 39.5 ppm). 2D spectra (COSY, HMQC, HMBC) spectra were used for signal assignment.

General procedure for Solid-Phase Peptide Synthesis: All peptides were synthesized on 2-chlorotrityl chloride resin using the Fmoc/Bu-strategy. In case of the 4-ido-phenylalanine containing tripeptides, the resin was loaded with Fmoc-Gly-OH (4 eq.) and DIEA (8 eq.) in DCM at room temperature, shaken for 2 hours and remaining binding sites were deprotected by addition of a mixture of amino acid (4 eq.), TBTU (4 eq.) and DIEA (8 eq.) in DMF to the loaded resin and shaking for 15 minutes at room temperature; this procedure was repeated once. After deprotection, the resin was washed with DMF (5 x 1 min). The obtained relaxation rates were used to calculate the translational diffusion coefficient Dₓ according to Eq. 1.

\[ D_x = \frac{\eta T}{k_B T} \] (1)

where q is the magnitude of the scattering vector. The hydrodynamic radius Rₕ can be calculated according to the Stokes-Einstein equation:

\[ R_h = \frac{k_B T}{6 \pi \eta q} \] (2)

with the Boltzmann constant k_B, the temperature T, and the solvent viscosity η.

Analytical HPLC: Analytical HPLC was performed on a Shimadzu Nexera XRS 20A System (Shimadzu, Kyoto, Japan) with autosampler, degasser, diode array detector and a Luna C18 column (Phenomenex, Torrance, CA, USA) (2.9 μm, 50 x 2.1 mm) with a gradient (in 5.5 min from 5 % B to 95 % B, 0.5 min 95 % B and back to 5 % B in 3 min, total run time 9 min) at a flow rate of 650 μL/min and column oven temperature of 40 °C. HPLC solvent A consisted of 99.9 % water with 0.1 % TFA, solvent B of 99.9 % acetonitrile with 0.1 % TFA.
at room temperature. After coupling, the resin was washed with DMF (5 x 1 min) and and MTBE (3 x 1 min) and dried in vacuo. Full conversion was verified by Kaiser test. Fmoc-iodo-l-phenylalanine and Fmoc-7-bromo-o-tryptophan (1.2 eq.) was coupled to the N-deprotected peptide on resin with HATU (1.2 eq.) and DIEA (2.4 eq.) in DMSO at room temperature for 6 hours. Full conversion was verified by a test cleavage and analytical LC-MS. Before final cleavage, the peptide was N-acetylated by addition of a solution of acetic anhydride (10 eq.) and pyridine (10 eq.) in DMF to the resin. Cleavage and side chain deprotection were performed by addition of a mixture of TFA/H₂O/TIS (95:25:2.5) to the resin (2 x 1.5 h) followed by peptide precipitation overnight in MTBE at -20 °C. In case of methionine containing peptides, a cleavage cocktail consisting of TFA/thioanisole/EDT/aniisol (90:5:3:2) was used for peptide cleavage (2 x 1.5 h) followed by peptide precipitation overnight in MTBE at -20 °C. This mixture was spun down (4000 rpm; 4 °C, 5 min), the MTBE layer discarded, the residue dried in water and freeze dried. All peptides were purified by RP-HPLC.

**General procedure for Suzuki-Miyaura cross coupling:** Cross-couplings were performed on benchtop. Aryl halide (5 µmol, 1.0 eq.) and boronic acid (2.0 eq.) were placed in a flask equipped with a stirring bar. K₂PO₃ (5.0 eq. dissolved in water (500mM, 50 µl) and water (200 µl)) were added giving a final aryl halide concentration of 10 mM. The vessel was heated to the desired reaction temperature and the microgel/Pd-nanoparticle hybrids dissolved in water (250 µl; 1 m; 5 mol%) were added. The reaction progress was monitored by RP-HPLC at 220 nM. After completion the Pd-nanoparticles were removed by centrifugation (10,000 rpm; 10 min) and the reaction mixture was directly purified by preparative RP-HPLC.

**Ac-Ala-Phe(4-I)-Gly-OH (6)** Synthesized by solid-phase peptide synthesis (SPPS) on a 2-chlorotrityl resin on a 0.1 mmol scale. The product 6 was isolated after reverse phase HPLC purification as a colorless solid (1.9 mg, 22 µmol, 22%).

**Ac-Ala-Phe(4-I)-Gly-OH (7)** Synthesized by solid-phase peptide synthesis (SPPS) on a 2-chlorotrityl resin on a 0.1 mmol scale. The product 7 was isolated after reverse phase HPLC purification as a colorless solid (2.4 mg, 51 µmol, 51%).
C6-H(CY-H)) 4.07 (s, 1H, CH); 8.09 (d, J=7.8 Hz, 1H, Cβ-H); 7.54 (d, J=7.8 Hz, 1H, C-H); 7.28 (dd, J=7.5 Hz, J=1.7 Hz, 1H, C6-H); 7.23 (d, J=2.6 Hz, 1H, C2-H); 6.94 (d, J=7.7 Hz, 1H, C4-H); 4.47 (dd, J=7.3 Hz, J=7.4 Hz, 1H, Cα-H); 4.36 (dd, J=7.3 Hz, J=4.9 Hz, 1H, Cβ-H); 4.29 (dd, J=8.9, J=6.5 Hz, 1H, C8-H); 3.21 (dd, J=14.9 Hz, J=7.6 Hz, 1CH3); 2.46 - 2.35 (m, 2H, Cy-H); 1.98 (s, 3H, CH3); 1.95 (dd, J=8.6 Hz, J=6.8 Hz, 1H, CH2); 1.89 - 1.81 (m, 4H, CH2); 1.73 (ddt, J=13.5 Hz, J=9.1 Hz, J=6.0 Hz, 1H, Cβ-H); 0.82 (d, J=6.8 Hz, 3H, Cy-H); 0.79 (d, J=6.8 Hz, 3H, Cy-H).

References


Biosketches

Thomas Hellweg currently works at the Faculty of Chemistry at Bielefeld University where he is head of the Dep. of Physical and Biophysical Chemistry. He studied chemistry and physics at Bielefeld University where he obtained a doctoral degree in physical chemistry in 1995. Afterwards he spent about two years as a postdoc with Dominique Langevin and Didier Roux at Centre de Recherche Paul Pascal (CNRS) at Bordeaux. In 1998 he moved to the Technical University of Chemnitz where he worked at the physics department in the group for materials research and liquids with Jens-Boie Sack. From Chemnitz he moved to TU Berlin where he was working in the group of Gerhard Findenegg and got his habilitation for physical chemistry in 2003. In 2007 he accepted a call to Bayreuth University. In 2010 he accepted a call to Bielefeld University.

He does research in Physical Chemistry and Materials Science with a focus on "Soft Matter". One of the current projects of the Hellweg group is 'Smart core-shell microgels as nanoparticle carriers for catalysis.' Another important current project is the study of saponins and their interaction with lipid bilayers. Moreover, at present confined microemulsions are also in the focus of the Hellweg group. The group uses mainly scattering experiments (light, X-rays, and neutrons) complemented by high resolution imaging techniques (cryogenic transmission electron microscopy (Cryo-TEM), scanning force microscopy, scanning electron microscopy (SEM), and different optical microscopy techniques).

Norbert Sewald was born in Munich in 1961 and studied chemistry at the Technical University of Munich. He obtained his PhD degree in Organic Chemistry ("New Strategies for the Synthesis of Trifluoromethyl Substituted Heterocycles, Amino Acids, and Hydroxy Acids") in the group of Prof. K. Burger. He was a postdoc in the group of Prof. J. E. Baldwin from 1991 to 1992 at the Dyson Perrins Laboratory, Oxford University (Studies towards the Biomimetic Synthesis of Penicillin). In 1998 he finished his habilitation at the University of Leipzig. Since 1999 he has been Full Professor of Organic and Bioorganic Chemistry at Bielefeld University.

Norbert Sewald is coordinator of the Bilateral Yaoundé-Bielefeld Graduate School YaBiNaPA (2016-2025) and the Marie Skłodowska-Curie Training Networks MAGICBULLET (2015-2018) and Magicbullet::reloaded (2020-2023). He currently is the President of the European Peptide Society.

His research interests comprise Biocatalysis (Enzymatic Halogenation), Bioconjugation (Drug Conjugates), Peptide Drugs, Late-Stage Diversification of Peptides, Peptidomimetics, and Natural Product Chemistry.

Yvonne Hannappel studied polymer and colloidal chemistry at the University of Bayreuth and received her diploma in 2007. Afterwards she started her PhD at the same university under the supervision of Prof. Thomas Hellweg, with her research focusing on the study of copolymer microgels and hydrogel nanoparticle hybrids. She successfully defended her thesis in 2011 and is currently working as a senior scientist in the laboratory of Prof. Hellweg at Bielefeld University.

Steffen Dachwitz received his B.Sc degree in Chemistry from the WWU Münster in 2015 and his M.Sc. degree in Chemistry from Bielefeld University in 2018 specialized in bioorganic synthesis. Since 2018 he is working on his PhD studies in the Organic and Bioorganic Chemistry research group under supervision of Prof. Dr. N. Sewald. He is particularly interested in transition-metal catalyzed late-stage diversifications of indole containing amino acids and peptides.
Viktor Sabadasch studied chemistry at Bielefeld University, where he received his bachelor's degree in 2015 and his master's degree in 2018 in the Physical and Biophysical Chemistry group under supervision of Prof. Thomas Helweg. In 2018 he started his doctoral studies in the same group with a research focus on the preparation and characterization of catalytically active metal-microgel hybrids.
Supporting Information

Acrylamide-based Pd-Nanoparticle Carriers as Smart Catalyst for the Suzuki-Miyaura Cross-Coupling of Amino Acids

Viktor Sabadasch[\textsuperscript{a}][\textsuperscript{[\textcopyright]}, Steffen Dachwitz[\textsuperscript{b}][\textsuperscript{[\textcopyright]}, Yvonne Hannappel[\textsuperscript{a}], Thomas Hellweg[\textsuperscript{a}] and Norbert Sewald[\textsuperscript{b}]

\textsuperscript{[\textcopyright]} These authors contributed equally.

\textsuperscript{a} V. Sabadasch, Y. Hannappel, T. Hellweg
Department of Chemistry, Physical and Biophysical Chemistry
Bielefeld University
Universitätstraße 25, 33615 Bielefeld
E-mail: thomas.hellweg@uni-bielefeld.de

\textsuperscript{b} S. Dachwitz, N. Sewald
Department of Chemistry, Organic and Bioorganic Chemistry
Bielefeld University
Universitätstraße 25, 33615 Bielefeld
E-Mail: norbert.sewald@uni-bielefeld.de

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure_s1.png}
\caption{Histogram of palladium nanoparticle radii incorporated into PNNPAM-co-MAc and PNIPAM-co-MAc microgels. Both distributions were fitted with a gaussian function, indicated by the solid line in the respective color.}
\end{figure}
Figure S2: Swelling curves for the palladium loaded MAc systems investigated in a 50 mM K$_3$PO$_4$ solution at a pH value of 12. Despite slight size changes, the thermoresponsive properties of the microgels were maintained after loading with Pd nanoparticles.

Figure S3: Swelling curves for the PNPAM-co-DAPMA and PNIPAM-co-DAMPA microgels loaded with palladium nanoparticles. The samples were investigated in a 50 mM K$_3$PO$_4$ solution at a pH value of 12. The thermoresponsive properties of the microgels and the respective VPTTs at 26 °C and 32 °C are maintained after loading the systems with palladium nanoparticles. The change in size is more pronounced for the PNPAM systems than for the PNIPAM microgel.
Determination of Pd in solution

Determination of the concentration of dissolved Pd was done by the Mikroanalytisches Laboratorium Kolbe by atomic absorption spectroscopy (AAS). The determination was done with a suspension of the prepared microgel/palladium hybrid particles.

**Table S1**: Determination of Pd in ppm of mass.

<table>
<thead>
<tr>
<th></th>
<th>ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNNPAM-co-MAc</td>
<td>199</td>
</tr>
<tr>
<td>PNIPAM-co-MAc</td>
<td>257</td>
</tr>
<tr>
<td>PNNPAM-co-DAPMA</td>
<td>57</td>
</tr>
<tr>
<td>PNIPAM-co-DAPMA</td>
<td>83</td>
</tr>
</tbody>
</table>

Catalytic screening

**Figure S4** Suzuki-Miyaura reaction of Nα-Boc-4-iodophenylalanine and phenylboronic acid; conversion as function of time at room temperature. The dotted lines are an exponential growth fit as guide to the eyes.
Figure S5 Suzuki-Miyaura reaction of \(N\)-\(\alpha\)-Boc-4-iodophenylalanine and phenylboronic acid; conversion as function of time at 37°C. The dotted lines are an exponential growth fit as guide to the eyes.

Figure S6 Suzuki-Miyaura reaction of \(N\)-\(\alpha\)-Boc-4-iodophenylalanine and phenylboronic acid; conversion as function of time at 60°C. The dotted line is an exponential growth fit as guide to the eyes.
TEM imaging after catalysis

Figure S7: TEM images of selected microgel particles after catalysis of Nα-Boc-4 iodophenylalanine with phenylboronic acid. A: PNNPAM-co-MAc particle after one catalytic cycle. B: PNIPAM-co-MAc microgel after two catalytic cycles. C: PNNPAM-co-DAPMA particle and D: PNIPAM-co-DAPMA particle after five catalytic cycles. The systems in A and B seem to be trapped or soaked with material, indicated by a clear core-corona structure of the microgel particle, which was not present before reaction. A part of the originally (9.4±1.1) nm big nanoparticles in the PNNPAM-co-MAc system decreased significantly in size and appear to have bled out of the microgel. In contrast, the nanoparticles incorporated into the PNIPAM-co-MAc systems remained intact. In addition, a vast corona with slight contrast can be identified in this system. The nanoparticles embedded in the PNNPAM-co-DAPMA and PNIPAM-co-DAPMA system seemed to be intact after five consecutive cycles. However, a corona can also be observed for the PNNPAM-co-DAPMA system. In contrast to the MAc systems the nanoparticles are also located in the corona region of the PNNPAM-co-DAPMA gel.
Figure S8 Suzuki-Miyaura reaction of $N^\alpha$-Boc-7-bromotryptophan and phenylboronic acid; conversion as function of time at room temperature. The dotted lines are an exponential growth fit as guide to the eyes.

Figure S9 Suzuki-Miyaura reaction of $N^\alpha$-Boc-7-bromotryptophan and phenylboronic acid; conversion as function of time at 37°C. The dotted lines are an exponential growth fit as guide to the eyes.
Figure S10 Suzuki-Miyaura reaction of Nα-Boc-7-bromotryptophan and phenylboronic acid; conversion as function of time at 60°C. The dotted lines are an exponential growth fit as guide to the eyes.
Molecules

**GP1: General procedure for Suzuki-Miyaura cross coupling:**

Cross-couplings were performed on benchtop. Therefore, aryl halide (5 µmol, 1.0 eq.) and boronic acid (2.0 eq.) were placed in a flask equipped with a stirring bar. K_{3}PO_{4} (5.0 eq.) dissolved in water (500 mM, 50 µL) and water (200 µL) were added giving a final aryl halide concentration of 10 mM. The vessel was heated to the desired reaction temperature and the microgel carried Pd-nanoparticles dissolved in water (250 µL; 1 mM; 5 mol%) were added. The reaction progress was monitored by RP-HPLC at 220 nm. After completion the Pd-nanoparticles were removed by centrifugation (10.000 rpm; 10 min) and the reaction mixture was directly purified by preparative RP-HPLC.

**GP2: General procedure for Solid-Phase Peptide Synthesis:**

All peptides were synthesized on 2-chlorotrityl resin using the Fmoc/ tBu-strategy. In case of the 4-Iodo-phenylalanine containing triptides the resin was loaded with Fmoc-Gly-OH (4 eq.) and DIEA (8 eq.) in DCM at room temperature, shaken for 2 hours and remaining binding sites were capped by adding MeOH (15 eq.). The solution was filtrated and the resin was thoroughly washed with DCM (5 x 1min). Fmoc-deprotection was performed by addition of 20 % piperidine and 100 mM HOBt in DMF to the resin and shaking for 15 minutes at room temperature; this procedure was repeated once. After deprotection, the resin was washed with DMF (5 x 1 min). Natural Fmoc-protected amino acids were coupled to the Nα-deprotected peptide by addition of a mixture of amino acid (4 eq.), TBTU (4 eq.) and DIEA (8 eq.) in DMF to the resin and shaking for 2 hours at room temperature. After coupling, the resin was washed with DMF (5 x 1 min) and and MTBE (3 x 1 min) and dried in vacuo. Full conversion was verified by Kaiser-test. Fmoc-4-ido-L-phenylalanine or Fmoc-7-bromo-L-tryptophan (1.2 eq.) was coupled to the Nα-deprotected peptide on resin with HATU (1.2 eq.) and DIEA (2.4 eq.) in DMF at room temperature for 6 hours. Full conversion was verified by a test cleavage and analytical LC-MS. Before final cleavage, the peptide was N-acetylated by addition of acetic anhydride (10 eq.) and pyridine (10 eq.) in DMF to the resin. Cleavage and side chain deprotection were performed by addition of a mixture of TFA/H_{2}O/TIS (95:2.5:2.5) to the resin (2 x 1.5 h) followed by peptide precipitation overnight in MTBE at –20 °C. In case of Methionine containing peptides cleavage cocktail “reagent R” was used for peptide cleavage (2 x 1.5 h) followed by peptide precipitation overnight in MTBE at –20 °C. This mixture was spun down (4000 rpm; 4 °C; 5 min), the MTBE layer discarded, the residue dissolved in water and freeze dried. All peptides were purified by RP-HPLC.
L-7-Bromo tryptophan x TFA

L-7-bromo tryptophan was synthesized according to our previously reported procedure using RebH-PmF-RR-ADH combiCLEAs. The biocatalyst was produced using 8 g of lysed E. coli cells containing overexpressed tryptophan-7-halogenase RebH. The reaction buffer contained 1.25 mM L-tryptophan, 15 mM Na₂HPO₄, 30 mM NaBr, 0.1 mM NAD⁺, 1 µM FAD and 0.5 % (v/v) 2-propanol at pH = 7.4 in a total reaction volume of 1.250 L. Full conversion was usually observed after 4-7 days. The suspension was filtered and desalted. Therefore, the crude filtrate was concentrated up to a volume of about 100 mL and loaded on a 12 g RP-C₁₈-column and purified using an automated column chromatography using a Büchi Reveleris X2 with a binary pump and ELSD Detector. The gradient (4 min at 5 % B, up to 25 % B in 17 min, in 1 min up to 100 % B for 2 min and flushing with 80% B for 5 min, total run time 30 min) was used at a flow rate of 20 mL/min. Solvent A consisted of 99.9 % water and 0.1% TFA, solvent B of 99.9 % methanol and 0.1 % TFA. Freeze drying gave L-7-bromotryptophan x TFA as a colorless to yellow solid. If necessary, the product was purified by reversed-phase HPLC.

Anal. RP-HPLC: \( t_R = 3.3 \) min;

LC-MS: \( t_R = 5.2 \) min;

\(^1\)H NMR (500 MHz, DMSO-\( d_6 \)) \( \delta [ppm] \) = 13.96 (br s, 1H, COOH), 11.29 (d, \( ^3J = 2.7 \) Hz, 1H, indole-NH), 8.19 (brs, 3H, NH₃), 7.58 (d, \( ^3J = 7.9 \) Hz, 1H, C4-H), 7.33 (d, \( ^3J = 7.5 \) Hz, 1H, C6-H), 7.30 (d, \( ^3J = 2.7 \) Hz, 1H, C2-H), 6.98 (dd, \( ^3J = 7.8 \) Hz, \(^3J = 7.8 \) Hz, 1H, C5-H), 4.18 (dd, \( ^3J = 7.1 \) Hz, \(^3J = 6.2 \) Hz, 1H, Cα-H), 3.27 (dd, \( ^3J = 15.0 \) Hz, \(^3J = 5.7 \) Hz, 1H, Cβ-H), 3.23 (dd, \( ^2J = 14.8 \) Hz, \(^3J = 6.9 \) Hz, 1H, Cβ-H);

LC-MS (ESI); found [m/z] = 283.01 [M\(^{79}\)Br+H]\(^\ast\), 285.01 [M\(^{81}\)Br+H]\(^\ast\); calcd. [m/z] = 283.01 [M\(^{79}\)Br+H]\(^\ast\), 285.01 [M\(^{81}\)Br+H]\(^\ast\).
10-
α-Boc-L-4-iodo phenylalanine (1)

L-4-iodo phenylalanine (580.0 mg, 2.65 mmol) was dissolved in acetonitrile (50 mL) followed by addition of di-tert-butyl dicarbonate (1.011 g, 4.63 mmol, 1.7 eq.) and aqueous NaOH (1.0 M, 5.3 mL, 5.3 mmol, 2.0 eq.). The reaction progress was monitored by analytical HPLC. After complete conversion, the solvent was removed in vacuum and the crude residue was dissolved in water (50 mL) and adjusted to pH = 3 by addition of aqueous HCl (1.0 M), which led to precipitation of a colorless solid. The aqueous layer was extracted with EtOAc (3 x 50 mL) and the combined organic layers were dried over MgSO₄. The organic solvent was removed in vacuum and the crude product was purified by column chromatography (DCM/MeOH; 0.5 % -> 5.0 %) providing Nα-Boc-L-4-iodophenylalanine (1) as a colorless solid (816.8 mg, 2.08 mmol, 79 %).

Anal. RP-HPLC: \( t_R = 4.9 \) min;

LC-MS: \( t_R = 8.8 \) min;

\(^1\)H NMR (600 MHz, DMSO-\( d_6 \)) \( \delta \) [ppm] = 12.65 (br s, 1H, COOH), 7.63 (d, \( ^3J = 8.0 \) Hz, 2H, C3-H), 7.09 (d, \( ^3J = 8.5 \) Hz, 1H, OCONH), 7.06 (d, \( ^3J = 8.1 \) Hz, 2H, C2-H), 4.06 (ddd, \( ^3J = 10.1 \) Hz, \( ^3J = 8.5 \) Hz, \( ^3J = 4.6 \) Hz, 1H, Cα-H), 2.97 (dd, \( ^2J = 13.9 \) Hz, \( ^3J = 4.7 \) Hz, 1H, Cβ-H), 2.72 (dd, \( ^2J = 14.2 \) Hz, \( ^3J = 10.4 \) Hz, 1H, Cβ-H), 1.31 (s, 9H, C(CH₃)₃; cis/trans ratioBoc 8:1).

LC-MS (ESI): found \([m/z] = 414.02 \) [M+Na]\(^+\), 335.97 [M-tertButyl+H]+, 291.99 [M-Boc+H]\(^+\); calcd. \([m/z] = 414.02 \) [M+Na]\(^+\), 335.97 [M-tertButyl+H]+, 291.98 [M-Boc+H]\(^+\).
**Nα-Boc-L-7-bromo tryptophan (2)**

L-7-bromo tryptophan x TFA (192.0 mg, 500 µmol) was dissolved in acetonitrile (5 mL) followed by addition of di-tert-butyl dicarbonate (120.0 mg, 550 µmol, 1.1 eq.) and aqueous NaOH (1.0 M, 1.0 mL, 2.0 eq.). The reaction progress was monitored by analytical HPLC. After complete conversion, the solvent was removed in vacuum and the crude residue was dissolved in water (25 mL) and adjusted to pH = 3-4 by addition of aqueous HCl (1.0 M). The aqueous layer was extracted with EtOAc (3 x 50 mL) and the combined organic layers were dried over MgSO₄. The organic solvent was removed in vacuum and the crude product was purified by RP-HPLC providing Nα-Boc-L-7-bromotryptophan (2) as a colorless solid (152.8 mg, 399 µmol, 80%).

**Anal. RP-HPLC:** $t_R = 5.0$ min;

**LC-MS:** $t_R = 8.7$ min;

1H NMR (500 MHz, DMSO-d$_6$) $\delta$ [ppm] = 12.61 (br s, 1H, COOH), 11.08 (d, $^3$J = 2.6 Hz, indole-NH), 7.54 (d, $^3$J = 7.8 Hz, 1H, C4-H), 7.29 (d, $^3$J = 7.5 Hz, 1H, C6-H), 7.21 (d, $^3$J = 2.5 Hz, 1H, C2-H), 7.02 (d, $^3$J = 7.9 Hz, 1H, OCONH, cis/trans ratio$_{Boc}$ 7:1), 6.96 (dd, $^3$J = 7.7 Hz, $^3$J = 7.7 Hz, 1H, C5-H), 6.63 (d, $^3$J = 8.6 Hz, 1H, OCONH, cis/trans ratio$_{Boc}$ 7:1), 4.13 (ddd, $^3$J = 9.5 Hz, $^3$J = 8.0 Hz, $^3$J = 4.7 Hz, 1H, Cα-H), 3.11 (dd, $^3$J = 14.7, $^3$J = 4.7 Hz, 1H, Cβ-H), 2.97 (dd, $^3$J = 14.9 Hz, $^3$J = 9.5 Hz, 1H, Cβ-H), 1.33 (s, 9H, C(CH$_3$)$_3$; cis/trans ratio$_{Boc}$ 7:1);

**LC-MS (ESI):** found [m/z] = 381.05 [M($^{79}$Br)-H], 383.05 [M($^{81}$Br)-H]; calcd. [m/z] = 381.05 [M($^{79}$Br)-H], 383.05 [M($^{81}$Br)-H].
Nα-Fmoc-L-7-bromo tryptophan

L-7-bromo tryptophan x TFA (192.1 mg, 500 µmol) was dissolved in Acetonitrile (30 mL) followed by addition of Fmoc-OSu (205.1 mg, 600 µmol, 1.2 eq.) and aqueous NaOH (1.0 M, 1.0 mL, 1000 µmol, 2.0 eq.). The reaction progress was monitored by analytical HPLC. After completion, the solvent was removed in vacuum and the crude residue was dissolved in water (50 mL) and adjusted to pH = 3 by addition of aqueous HCl (1.0 M). The aqueous layer was extracted with EtOAc (3 x 100 mL) and the combined organic layers were dried over MgSO₄. The organic solvent was removed in vacuum giving a yellow oil, which was purified by RP-HPLC providing Nα-Fmoc-L-7-bromotryptophan as a colorless solid (205.1 mg, 405 µmol, 81%).

Anal. RP-HPLC: tᵣ = 5.7 min;

LC-MS: tᵣ = 10.2 min;

¹H NMR (500 MHz, DMSO-d₆) δ [ppm] = 12.70 (br s, 1H, COOH), 11.12 (d, ³J = 2.7 Hz, 1H, indole-NH), 7.89 (d, ³J = 7.6 Hz, 2H, C5'-H), 7.74 (d, ³J = 8.4 Hz, 1H, OCONH), 7.68 – 7.58 (m, 3H, C2'-H/C4'-H), 7.41 (dd, ³J = 7.1 Hz, 5J = 7.1 Hz, 2H, C4'-H), 7.34 - 7.25 (m, 4H, C3'-H/C2-H/C6-H), 6.96 (dd, ³J = 7.9 Hz, 5J = 7.9 Hz, 1H, C5-H), 4.24 (ddd, ³J = 9.9 Hz, 5J = 4.8 Hz, 1H, Ca-H), 4.21 - 4.16 (m, 3H, C1''-H/C2''-H), 3.20 (dd, ³J = 14.6 Hz, 5J = 4.6 Hz, 1H, Cß-H), 3.03 (dd, ²J = 14.6 Hz, 3J = 9.8 Hz, 1H, Cß-H);

LC-MS (ESI); found [m/z] = 505.08 [M(79Br)+H]+, 507.07 [M(81Br)+H]+; calcd. [m/z] = 505.08 [M(79Br)+H]+, 507.07 [M(81Br)+H]+.
L-4-iodophenylalanine (1.45 g, 5.0 mmol) was dissolved in acetonitrile (150 mL) followed by addition of Fmoc-OSu (2.02 g, 6.0 µmol, 1.2 eq.) and aqueous NaOH (1.0 M, 10.0 mL, 10.0 mmol, 2.0 eq.). The reaction progress was monitored by analytical HPLC. After complete conversion, the solvent was removed in vacuum and the crude residue was dissolved in water (150 mL) and adjusted to pH = 3 by addition of aqueous HCl (1.0 M), which led to precipitation of a colorless solid. The aqueous layer was extracted with EtOAc (3 x 100 mL) and the combined organic layers were dried over MgSO₄. The organic solvent was removed in vacuum giving a yellow solid, which was purified by column chromatography (5 %MeOH in DCM) providing Nα-Fmoc-L-4-iodophenylalanine as a colorless solid (1.69 g, 3.3 mmol, 66%).

Anal. RP-HPLC: tᵣ = 5.6 min;

LC-MS: tᵣ = 10.1 min;

¹H NMR (500 MHz, DMSO-d₆) δ [ppm] = 12.80 (br s, 1H, COOH), 7.88 (d, 3J = 7.6 Hz, 2H, C5'-H), 7.72 (d, 3J = 8.5 Hz, 1H, OCONH), 7.68 - 7.60 (m, 4H, C2'-H/C3'-H), 7.41 (dd, 3J = 7.5, 3J = 3.7 Hz, 2H, C4'-H), 7.30 (ddd, 3J = 15.0, 3J = 7.5, 4J = 1.1 Hz, 2H, C3'-H), 7.08 (d, 3J = 8.1 Hz, 2H, C2-H), 4.21 (d, 3J = 6.1 Hz, 1H, C2''-H), 4.23 - 4.12 (m, 3H, C1''-H/Cα-H), 3.04 (dd, 3J = 13.7, 3J = 4.4 Hz, 1H, Cβ-H), 2.82 (dd, 3J = 13.8, 3J = 10.6 Hz, 1H, Cβ-H).

LC-MS (ESI): found [m/z] = 514.05 [M+H]+, calcd. [m/z] = 514.05 [M+H]+.
Nα-Boc-L-4-phenyl phenylalanine (4) was synthesized according to GP1. Therefore, Nα-Boc-L-4-phenyl phenylalanine (1) (3.9 mg, 10 µmol), phenylboronic acid (2.4 mg, 20 µmol, 2 eq.) and K3PO4 aq. (500 mM, 100 µL, 5 eq) were dissolved in MPW (0.4 mL) and heated to 37 °C followed by addition of microgel carried Pd-nanoparticles (1 mM, 500 µL, 5.0 mol%). After full conversion, the Pd-nanoparticles were removed by centrifugation (10000 rpm, 10 min) and the reaction mixture was purified by RP-HPLC, giving Nα-Boc-L-4-phenyl phenylalanine (4) as a colorless solid (2.5 mg, 7.3 µmol, 73 %).

Anal. RP-HPLC: \( t_R = 5.3 \text{ min} \);

LC-MS: \( t_R = 9.0 \text{ min} \);

\(^1\)H NMR (600 MHz, DMSO-\(d_6\)) \( \delta \) [ppm] = 12.62 (s, 1H, COOH), 7.64 (d, \( ^3J = 7.6 \text{ Hz}, 2H, C3-H \)),
7.58 (d, \( ^3J = 8.2 \text{ Hz}, 2H, C2'-H \)), 7.45 (dd, \( ^3J = 7.7 \text{ Hz}, 3J = 7.7 \text{ Hz}, 2H, C3'-H \)), 7.34 (m, 2H, C2-H/C4'-H), 7.12 (d, \( ^3J = 8.4 \text{ Hz}, 1H, CONH \)),
4.12 (ddd, \( ^3J = 10.9 \text{ Hz}, ^3J = 8.3 \text{ Hz}, \)
\( ^3J = 4.5 \text{ Hz}, 1H, C\alpha-H \)),
3.06 (dd, \( ^3J = 13.8 \text{ Hz}, ^3J = 4.5 \text{ Hz}, 1H, C\beta-H \)), 2.87 (dd, \( ^3J = 13.9 \text{ Hz}, \)
\( ^3J = 10.3 \text{ Hz}, 1H, C\beta'-H \)), 1.32 (s, 9H, C(C\( \text{H}_3 \))\( _3 \)); cis/trans ratio\( _{\text{Boc}} \) 5:1.

\(^{13}\)C NMR (151 MHz, DMSO-\(d_6\)) \( \delta \) [ppm] = 174.0 (COOH), 155.9 (NH=C=O), 140.5 (C4), 138.7 (C1),
137.8 (C1'), 130.2 (C3'), 129.4 (C2), 127.7 (C4'),
127.0 (C3/C2'),
78.5 (C(CH\( \text{H}_3 \))\( _3 \)), 55.6 (Ca),
36.5 (C\( \beta \)),
28.6 (C(CH\( \text{H}_3 \))).

Nβ-Boc-L-7-phenyl tryptophan (5) was synthesized according to GP1. Therefore, Nβ-Boc-L-7-bromotryptophan (1) (3.8 mg, 10 µmol), phenylboronic acid (2.4 mg, 20 µmol, 2 eq.) and K3PO4 aq. (500 mM, 100 µL, 5 eq) were dissolved in MPW (0.4 mL) and heated to 37 °C followed by addition of microgel carried Pd-nanoparticles (1 mM, 500 µL, 5.0 mol%). After full conversion, the Pd-nanoparticles were removed by centrifugation (10000 rpm, 10 min) and the reaction mixture was purified by RP-HPLC, giving Nβ-Boc-L-7-phenyl tryptophan (5) as a colorless solid (3.0 mg, 7.7 µmol, 77 %).

Anal. RP-HPLC: tR = 5.4 min;

LC-MS: tR = 9.3 min;

1H NMR (500 MHz, DMSO-d6) δ [ppm] = 12.60 (br s, 1H. COOH), 10.77 (d, 3J = 2.3 Hz, 1H, indole-NH), 7.65 (d, 3J = 7.6 Hz, 2H, C2'-H), 7.55 (d, 3J = 5.2 Hz, 1H, C4'-H), 7.54 (dd, 3J = 7.7 Hz, 3J = 7.7 Hz, 2H, C3'-H), 7.43 (dd, 3J = 7.4 Hz, 1H, C4'-H), 7.18 (d, 3J = 2.7 Hz, 1H, C2-H), 7.15 - 7.10 (m, 2H, C5-H/C6-H), 7.04 (d, 3J = 8.1 Hz, 1H, OCONH), 4.19 (ddd, 3J = 7.1 Hz, 3J = 4.6 Hz, 1H, Co-H), 3.18 (dd, 3J = 14.6 Hz, 3J = 4.7 Hz, 1H, Cβ-H), 3.04 (dd, 3J = 14.6 Hz, 3J = 9.5 Hz, 1H, Cβ-H), 1.35 (s, 9H C(CH3)3; cis/trans ratioBoc 4:1).

13C NMR (126 MHz, DMSO-d6) δ [ppm] = 174.3 (COOH), 155.8 (NHC=O), 136.7 (C1'), 136.3 (C4'), 133.6 (C7a), 129.8 (C3'), 128.5 (C2'), 128.4 (C3a), 125.5 (C7), 124.9 (C2), 121.3 (C6), 119.4 (C4), 117.6 (C5), 110.9 (C3), 78.4 (C(CH3)3), 54.8 (Ca), 28.5 (C(CH3)), 27.1 (Cβ).
Ac-Ala-Phe(4-I)-Gly-OH (6)
Ac-Ser-Phe(4-I)-Gly-OH (7)
Ac-Phe-Phe(4-I)-Gly-OH (8)
Ac-Met-Val-Trp(7-Br)-OH (12)
Ac-Met-Asp-Gly-Trp(7-Br)-OH (13)
Ac-Ala-Phe(4-Phe)-Gly-OH (9)
Ac-Ser-Phe(4-Phe)-Gly-OH (10)