A versatile peptide-based toolbox for surface functionalization was established by a combination of a universal material binding peptide (LCI-anchor peptide) and sortase-mediated bioconjugation (sortagging). This toolbox facilitates surface functionalization either as a one- or a two-step strategy. In the case of the one-step strategy, the desired functionality was directly introduced to LCI. For the two-step strategy, LCI was modified with a reactive group, which can be further functionalized (e.g., employing "click" chemistry). Sortagging of LCI, employing sortase A from Staphylococcus aureus, was achieved with six different amine compounds: dibenzocyclooctyne amine, biotin-polyethylene glycol amine, Cyanine-3 amine, kanamycin, methoxypolyethylene glycol amine (Mn = 5000 Da), and 2,2,3,3,4,4,4-Heptafluorobutylamine. The purification of LCI-amine sortagging products was performed by a negative purification using Strep-tag II affinity chromatography, resulting in LCI-amine conjugates with purities >90%. For the two-step strategy, the LCI-dibenzocyclooctyne sortagging product was purified and enabled, through copper-free azide–alkyne “click” chemistry, universal surface functionalization of material surfaces such as polypropylene, polyethylene terephthalate, stainless steel, gold, and silicon. The click reaction was performed before or after surface binding of LCI-dibenzocyclooctyne. Finally, in the case of the one-step strategy, polypropylene was directly functionalized with Cyanine-3 and biotin-polyethylene glycol amine.

**KEYWORDS**
adhesion promoter, anchor peptide, click chemistry, sortase-mediated ligation, surface functionalization
1 | INTRODUCTION

Fabrication of functional materials through universally applicable and specific surface functionalization methodologies remains a challenge due to the vastly different surface properties and chemistries. Biological surface functionalization with material binding peptides (anchor peptides) is a simple and environmentally benign alternative to chemical and physical surface functionalization strategies (Care et al., 2015). Anchor peptides (APs) were applied as adhesion promoters to immobilize functional moieties such as enzymes (Zernia et al., 2016), bioactive peptides (Liu et al., 2016), antigens (de Juan-Franco et al., 2013), containers for drug delivery (Apitius et al., 2019), synthetic polymers (Dedisch et al., 2019; Meurer et al., 2017), and organometallic catalysts (Grimm et al., 2019) through noncovalent interactions. APs can be used in a resource- and energy-efficient manner for highly dense and specific surface functionalization at ambient temperatures in water and represent an attractive alternative to plasma treatment, UV irradiation, and employment of strong acids/bases (Dedisch et al., 2020; Goddard & Hotchkiss, 2007; Li et al., 2004; Rübsam et al., 2017; Vesel & Mazetic, 2017). The AP liquid chromatography peak I (LCI, 47 amino acids) from Bacillus subtilis was identified as a universal material binding peptide for functionalization of polymer surfaces (e.g., polypropylene [PP] and polyethylene terephthalate [PET]; Dedisch et al., 2020; Rübsam et al., 2017), metals (e.g., stainless steel and gold; Dedisch et al., 2020), silicon-based materials (Dedisch et al., 2020), and natural surfaces (e.g., teeth (Dedisch et al., 2019), hair (Dedisch et al., 2019), and plant leaves (Meurer et al., 2017)). LCI shows significantly superior binding strength to the latter surfaces when compared to proteins such as enhanced green fluorescent protein (EGFP), phytase, cellulase, and bovine serum albumin (Dedisch et al., 2019, 2020; Meurer et al., 2017; Rübsam et al., 2017). A dense and highly stable monolayer was reported for LCI coated gold surfaces (395 ng/cm²; Dedisch et al., 2019). A combination of the universal and dense surface coating capabilities of APs with sortase-mediated ligation (sortagging) and chemical conjugation chemistry (e.g., "click" chemistry) enables to introduce a plethora of functionalities to a broad array of chemically diverse materials and surfaces (e.g., polymers, metals, and silicon-based) and facilitates functionalization of materials that are challenging to functionalize by chemical or physical surface functionalization methodologies.

An alternative to chemical methodologies is enzymatic bioconjugation which offers site-specific protein modification under mild reaction conditions (Zhang et al., 2018). In particular, sortagging has been used extensively for biomolecule functionalization due to its high selectivity, versatility, and ability to achieve biomolecule functionalization with high homogeneity (Dai et al., 2019). Transpeptidase sortase A (Sa-SrtA) from Staphylococcus aureus catalyzes the conjugation of two biomolecules equipped with a C-terminal sorting motif (LPXTG, X can be any amino acid, protein 1) to an N-terminal oligoglycine nucleophile (protein 2: Schmohl & Schwarzar, 2014). Furthermore, primary amine molecules (preferentially unbranched at the α-carbon) are also accepted as sortase nucleophiles (Glasgow et al., 2016). Key challenges of sortagging are the purity of the ligation products due to the reversibility of the conjugation reaction (the formed LPXTGGG sequence is a substrate for Sa-SrtA) and the low catalytic efficiency, which leads to a mixture of conjugated products and unconjugated substrates (Dai et al., 2019).

The azide–alkyne "click" reaction has taken a pivotal role in the fields of biomolecule functionalization and material science in the last decade (Nandivada et al., 2007). Click chemistry is defined by modular and orthogonal reactions with high reactivity under mild reaction conditions (Xi et al., 2014), which endows the application of click chemistry for functionalization of biomolecules and surfaces. Azide–alkyne click chemistry has been used for the surface functionalization of gold, silica, magnetic nanoparticles, carbon nanotubes, metal-organic frameworks, and semiconductor surfaces (Chen et al., 2019; Nebhani & Barner-Kowollik, 2009). Azide–alkyne click chemistry-based functionalization of polymers like PP or PET was reported through the incorporation of reactive groups during polymer synthesis or postpolymerization treatment (Yameen et al., 2010; Zhang et al., 2011). Functionalisation of unmodified PP or PET by azide–alkyne click chemistry has, to the best of our knowledge, not been reported yet. A combination of click chemistry and universal APs is a simple and versatile alternative to chemical and physical surface functionalization methodologies to modify the respective surfaces with the desired functionalities through orthogonal reactivities.

Following these notions, a versatile toolbox for surface functionalization of polymers, metals, and silicon-based materials was established through sortagging of LCI with various functional amine compounds (and subsequent purification of ligation products), such as reactive groups for click chemistry, biotin, fluorescent molecules, or antibiotics. LCI-modification with different functional groups was achieved through sortagging employing Sa-SrtA. Surface functionalization with modified LCI was either achieved by a one- or two-step strategy. In the case of the one-step method, the desired functionality was directly introduced to LCI. In contrast, for the two-step method, LCI was modified with a reactive group, which was employed for further functionalization (e.g., employing click chemistry).

2 | MATERIALS AND METHODS

2.1 | Chemicals and reagents

All chemicals were purchased from Sigma Aldrich, Carl Roth GmbH & Co KG, AppliChem GmbH, or Lumiprobe with analytical purity unless stated differently. Microtiter plates (MTPs) were purchased from Greiner Bio-One GmbH. All enzymes for polymerase chain reaction (PCR) were purchased from New England Biolabs (NEB). Primers for PCR were obtained from Eurofins Genomics. Materials for surface functionalization were purchased from Goodfellow Cambridge Ltd. (PET; PP), Plano GmbH (silicon wafer), Arnold Schröder Industrieöfen GmbH (stainless steel foil), and Sigma Aldrich (gold wafers).
2.2 | Generation of Sa-SrtA-Strep-tag II and His$_6$-EGFP-10ALA-TEV-LCI_KR2-LPETGK-Strep-tag II constructs

The N-terminal his$_6$-tag of Sa-SrtA (165Q/D186G/K196V; Zou et al., 2018) was replaced by Strep-tag II trough overlap extension PCR. The sequences of primers are listed in Table S1. The EGFP-LCI variant His$_6$-EGFP-10ALA-TEV-LCI_KR2 (EGFP-LCI; Y29R/G35R; Rübsam, Davari, et al., 2018) was C-terminally modified with a sortase recognition motif (LPETGK) and a Strep-tag II in two separate steps through overlap extension PCR. The sequences of primers are listed in Table S2. A detailed description of the generation of Sa-SrtA and EGFP-LCI constructs can be found in the Supporting Information.

2.3 | Expression and purification of Sa-SrtA-Strep-tag II (Sa-SrtA)

Shake flask expression of Sa-SrtA-Strep-tag II (Sa-SrtA) was performed as previously described (Guimaraes et al., 2013). Purification of Sa-SrtA was achieved through Strep-tag II affinity purification. The purification was conducted with a GE StrepTrap™ HP column (GE Healthcare; 5 ml) following the manufacturer’s instructions. The purity of the elution fraction was estimated by gel electrophoresis on a tricine sodium dodecyl sulfate-polyacrylamide gel (Tricine-SDS-PAGE; 10%). The concentration of Sa-SrtA was determined spectrophotometrically at 280 nm. The molecular weight (MW; 19.19 kDa) and extinction coefficient (19940 M$^{-1}$ cm$^{-1}$) of Sa-SrtA were calculated by using the ProtParam software (October 2020; https://web.expasy.org/protparam/; Gasteiger et al., 2005). A detailed description of Sa-SrtA expression and purification can be found in the Supporting Information.

2.4 | Expression and purification of His$_6$-EGFP-10ALA-TEV-LCI_KR2-LPETGK-Strep-tag II (EGFP-LCI)

Shake flask expression of His$_6$-EGFP-10ALA-TEV-LCI_KR2-LPETGK-Strep-tag II (EGFP-LCI) was performed as previously described (Rübsam, Davari, et al., 2018). Purification of LCI_KR2-LPETGK-Strep-tag II (LCI) was performed by Ni-NTA immobilized metal affinity chromatography (IMAC) purification of EGFP-LCI followed by a tobacco etch virus (TEV) protease cleavage step and a subsequent Ni-NTA IMAC purification (separation of cleaved LCI from EGFP and EGFP-LCI) as described previously (Rübsam et al., 2017). The purification was conducted with a GE HisTrap™ HP column (GE Healthcare; 5 ml) following the manufacturer’s instructions. The purity of the elution and wash fractions was estimated by gel electrophoresis on a Tricine-SDS-PAGE (10%). The concentration of EGFP-LCI and LCI was determined spectrophotometrically at 280 nm. The MW (EGFP-LCI: 38.06 kDa; LCI: 7.47 kDa) and extinction coefficient (EGFP-LCI: 49975 M$^{-1}$ cm$^{-1}$; LCI: 26470 M$^{-1}$ cm$^{-1}$) of EGFP-LCI and LCI were calculated by using the ProtParam software (October 2020; https://web.expasy.org/protparam/; Gasteiger et al., 2005). A detailed description of EGFP-LCI expression and purification and LCI purification can be found in the Supporting Information.

2.5 | Sortagging of LCI with primary amine compounds

Dibenzocyclooctyne (DBCO) amine, 2,2,3,4,4,4-Heptafluorobutyl (CF) amine, and Cyanine-3 (Cy-3) amine were dissolved in DMSO. Kanamycin sulfate, biotin-polyethylene glycol (biotin) amine, and methoxypolyethylene glycol (PEG; Mn = 5000 Da) amine were dissolved in ultrapure H$_2$O. Sortagging reactions (150 µl reaction volume) were performed with LCI (40 µM), Sa-SrtA (5 µM), NaCl (150 mM), and CaCl$_2$ (5 mM) in Tris–HCl buffer (pH = 8; 50 mM; Tris–HCl buffer ≥ 58% of total reaction volume). Concentrations of amine compounds in ligations are listed in Table S3. Ligations with DBCO, kanamycin sulfate, Cy-3, and biotin amine were conducted for 2 h (800 rpm; 20°C), while ligations with CF and PEG amine were performed for 18 h (800 rpm; 20°C). The success of ligation reactions was confirmed with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) with a Bruker ultraflexXtreme™ MALDI-TOF/TOF MS spectrometer (BRUKER DALTONIK GmbH). Therefore, samples were supplemented with TFA (0.1%), concentrated, and desalted with C18 Millipore® ZipTips (Merck Millipore Ltd.). Finally, the samples (2 µl) were crystallized with super-DHB (50 mg/ml) in TA50 (50% ultrapure H$_2$O; 50% acetonitrile; 0.1% TFA) on a ground steel target.

2.6 | MTP binding assay with LCI-Cy-3 and LCI-biotin

The surface functionalization of LCI-Cy-3 and LCI-biotin was investigated by PP an MTP binding assay reported by Rübsam, Davari, et al. (2018) that was modified for detection of Cy-3 and biotin. Therefore, ligation reactions were performed as described above. In deviation from the previous protocol, the reaction time was increased to 18 h to ensure maximum conversion of amine compounds. For Cy-3 amine, the surface-bound LCI-Cy-3 was directly detected fluorometrically in a Tecan Infinite M1000 Pro plate reader (Tecan Group; excitation: 545 nm; emission: 565 nm; gain: 100; 750 reads per well). In the case of LCI-biotin, surface-bound LCI-biotin was detected by incubation with Atto 425-Streptavidin (Sigma Aldrich; 10 µM; 100 µl). After incubation with Atto 425-Streptavidin, the fluorescence was detected in a Tecan Infinite M1000 Pro plate reader (Tecan Group; excitation: 545 nm; emission: 565 nm; gain: 100; 750 reads per well). A detailed description of the MTP binding assay with LCI-Cy-3 and LCI-biotin can be found in the Supporting Information.
2.7 Purification of LCI-DBCO

Separation of LCI-DBCO from unreacted DBCO and LCI as well as Sa-SrtA was achieved through Strep-tag II affinity purification. Ligation was performed as described above, except for adjusting the LCI concentration (20 µM) and the reaction volume (400 µl). The purification was conducted with a GE StrepTrap™ HP column (GE Healthcare; 1 ml) following the manufacturer's instructions. The purity of the flow-through (containing LCI-DBCO), wash, and elution fractions (containing LCI, DBCO, and Sa-SrtA) was monitored by high-performance liquid chromatography (HPLC) with a Nexera X2 HPLC system (Shimadzu Deutschland GmbH) and MALDI-TOF-MS with a Bruker ultraFleXtreme™ MALDI-TOF/TOF MS spectrometer (BRUKER DALTONIK GmbH). The concentration of purified LCI-DBCO was determined through an HPLC calibration curve. A detailed description of LCI-DBCO purification can be found in the Supporting Information.

2.8 Click chemistry and surface functionalization with purified LCI-DBCO

Copper-free azide–alkyne click chemistry functionalization of LCI-DBCO was performed before or after surface binding of LCI-DBCO. In the case of click chemistry functionalization before surface binding, purified LCI-DBCO (10.5 µM; 150 µl) was incubated with Cyanine-3 azide (Sigma Aldrich; Cy3 azide; 100 µM; 800 rpm, 18 h, 20°C), and the click reaction was confirmed with MALDI-TOF-MS as described previously. PP, PET, stainless steel, gold, and silicon were functionalized with LCI-DBCO-Cy3 by drop coating (50 µl; 10 min; RT). Subsequently, drops were removed, and samples were rinsed with ultrapure H2O (1 ml). After drying at RT, the fluorescence on the surface was analyzed by confocal laser scanning microscopy (TCS SP8, Leica Microsystems CMS GmbH). Quantification of LCI-DBCO-Cy3 surface binding was performed through a PP MTP binding assay as described previously. In the case of click chemistry functionalization after surface binding, purified LCI-DBCO (10.5 µM; 100 µl) was bound to a PP MTP as described above. Unbound LCI-DBCO was removed, and surface-bound LCI-DBCO was incubated with Cy3 azide (50 µM; 100 µl; 600 rpm; 18 h). Detection of surface-bound LCI-DBCO-Cy3 was performed as described above. A detailed description of LCI-DBCO click chemistry functionalization and surface binding can be found in the Supporting Information.

2.9 Purification of LCI-kanamycin

Separation of LCI-kanamycin from unreacted kanamycin and LCI as well as Sa-SrtA was achieved through Strep-tag II affinity purification. Ligation was performed as described above, except for adjusting the LCI concentration (50 µM) and the reaction volume (1000 µl). The purification was conducted with a GE StrepTrap™ HP column (GE Healthcare; 5 ml) following the manufacturer’s instructions. The purity of the flow-through (containing LCI-kanamycin), wash, and elution fractions (containing LCI, kanamycin, and Sa-SrtA) was monitored by HPLC with a Nexera X2 HPLC system (Shimadzu Deutschland GmbH) and MALDI-TOF-MS with Bruker ultraFleXtreme™ MALDI-TOF/TOF MS spectrometer (BRUKER DALTONIK GmbH). The concentration of LCI-kanamycin was determined spectrophotometrically at 280 nm. The MW (6.55 kDa) and extinction coefficient (20970 M⁻¹ cm⁻¹) of LCI-kanamycin were estimated by using the ProtParam software (October 2020; https://web.expasy.org/protparam; Gasteiger et al., 2005). A detailed description of the LCI-kanamycin purification can be found in the Supporting Information.

2.10 Resazurin microtiter plate assay with purified LCI-kanamycin

The resazurin microtiter plate assay (REMA) with Xanthomonas citri (strain 306 [IBSBF 1594]) was performed according to Silva et al. (2013). LCI-kanamycin (25 µM) and LCI (25 µM) were investigated for antimicrobial activity against X. citri. Kanamycin (25 µM) and Tris–HCl buffer (pH = 8; 50 mM) were employed as negative control and vehicle control, respectively. To investigate if the unreacted kanamycin was removed from the LCI-kanamycin sample, a kanamycin control reaction (4 mM kanamycin; dissolved in Tris–HCl buffer; pH = 8; 50 mM) was subjected to the same dialysis conditions used for LCI-kanamycin as described in the Supporting Information (purification of LCI-kanamycin).

3 RESULTS

The results section is divided into three parts. The first section summarizes the sortagging results of LCI with different primary amine compounds (Figure 1). The second section presents a purification strategy for LCI-amime conjugates (LCI-DBCO and LCI-kanamycin) based on Strep-tag II affinity purification. The concluding third section focuses on the functionalization of polymers (PP and PET), metals (stainless steel and gold), and silicon with amine-functionalized LCI. The latter was achieved either through a one-step (LCI-Cy3 and LCI-biotin) or a two-step (LCI-DBCO) surface functionalization strategy. In the case of the one-step method, the desired functionality was directly introduced to LCI. In contrast, for the two-step method, LCI was modified with a reactive group, which was employed for further functionalization (azide–alkyne click chemistry; Figure 1).

3.1 Sortagging of LCI with amine compounds

Sortagging of LCI was achieved with six different amine compounds: dibenzocyclooctyne amine (DBCO amine), biotin-polylethylene glycol amine (biotin amine), Cyanine-3 amine (Cy3 amine), kanamycin, methoxypolylethylene glycol amine (PEG amine; Mn = 5000 Da), and 2,2,3,3,4,4,4-Heptafluorobutylamine (CF amine).

The LCI variant Y29R/G35R (Rübsam, Rübsam, et al., 2018), which was engineered for enhanced PP binding, and the Sa-SrtA variant
D165Q/D186G/K196V (Zou et al., 2018), which was improved for resistance against dimethylsulfoxide (DMSO), were selected for the development of the universal surface functionalization toolbox. In detail, the latter Sa-SrtA variant was selected to enable modification of LCI with hydrophobic amine compounds, which require high concentrations of cosolvents like DMSO for solubilization. LCI was C-terminally modified with an LPETGK motif, which was required for sortagging with amine compounds, and with a Strep-tag II. The latter was employed for the purification of LCI-amine sortagging products (Figure 2). LCI was expressed and purified as previously reported (Figure S1; Rübsam et al., 2017). Six different amine compounds, DBCO, biotin, Cy-3, kanamycin, PEG (Mn = 5000 Da), and CF amine were employed for sortagging of LCI. Ligations with DBCO, kanamycin, Cy-3, and biotin amine were conducted for 2 h, while ligations with CF and PEG amine were performed for 18 h. MALDI-TOF-MS showed functionalization of LCI with all six amine compounds (Figure S2).

### 3.2 Purification of LCI-amine sortagging products

Purification of LCI-amine sortagging products was achieved through Strept-tag II affinity purification, and LCI-DBCO and LCI-kanamycin were obtained in high purity (>90%).

Therefore, LCI was C-terminally equipped with an LPETGK sorting motif and a Strept-tag II as described previously. In parallel, Sa-SrtA was equipped with an N-terminal Strept-tag II (Figure 2). The modification of LCI with the Strept-tag II, enabled the separation of functionalized LCI from nonfunctionalized LCI through Strept-tag II affinity chromatography. Within the sortagging reaction, Sa-SrtA cleaves the sorting motive, and GK-Strept-tag II is released. Subsequently, Sa-SrtA conjugates the amine compound to LCI-LPET. Therefore, the resulting sortagging product LCI-LPET-amine does not contain a Strept-tag II, and all unwanted side products within the reaction (unreacted LCI, Sa-SrtA, and GK-Strept-tag II) carry a Strept-tag II. The negative purification using Strept-tag II affinity chromatography removed all side products containing a Strept-tag II, and highly pure LCI-amine products were obtained (Figure 2). Removal of Sa-SrtA from the reaction stopped the sortagging reaction and prevented Sa-SrtA-mediated cleavage of the newly formed LCI-amine sortagging product (reversibility of the sortagging reaction). The purity of the LCI-amine sortagging products was monitored by HPLC, followed by MALDI-TOF-MS analysis to confirm the sortagging products in the purified fraction. In the case of sortagging with DBCO amine and kanamycin, highly pure LCI-DBCO and LCI-kanamycin were obtained (purity > 90%; Figures S3 and S4).

### 3.3 Surface functionalization with LCI-amine compounds: Two-step versus one-step strategy

#### 3.3.1 Surface functionalization using click chemistry: Two step-strategy

Surface functionalization with reactive groups for azide–alkyne click chemistry was performed for PP, PET, stainless steel, gold, and silicon.
The latter was achieved using the two-step surface functionalization strategy employing LCI-DBCO, and the the click reaction with the fluorescent dye Cyanine-3 azide (Cy-3 azide) was performed before surface functionalization. In the case of PP, the click reaction with Cy-3 azide was performed before and after surface binding of LCI-DBCO.

The two-step surface functionalization method was investigated through LCI-DBCO functionalization of PP, PET, stainless steel, gold, and silicon. DBCO is a reactive group that enables copper-free click chemistry with compounds containing an azide moiety (Pola et al., 2014). Modification of LCI with DBCO allowed two different strategies for click chemistry surface functionalization: LCI-DBCO could be functionalized with the respective azide moiety before or after surface binding of LCI. The fluorescent dye Cy-3 azide was selected to detect surface functionalization with LCI-DBCO. Before surface functionalization experiments, LCI-DBCO was purified as described above. The LCI-DBCO yield after the purification reaction was 24.8 µg (fraction of

**FIGURE 2** Purification strategy for LCI-amine sortagging products based on Strep-tag II affinity purification. During sortagging of LCI with amine compounds, a part of the sorting motif is cleaved off (amino acids GK) together with the Strep-tag II. The latter facilitates the separation of LCI-amine from nonfunctionalized LCI and cleaved sorting motif through affinity chromatography. Furthermore, Sa-SrtA is removed simultaneously, which stops the reaction (prevents Sa-SrtA-mediated cleavage of newly formed sortagging product) [Color figure can be viewed at wileyonlinelibrary.com]
functionalized LCI, which was initially employed in the sortagging reaction: 49%). To investigate click chemistry surface functionalization of LCI-DBCO before surface binding, purified LCI-DBCO was incubated with Cy-3 azide, and the click reaction product was identified by MALDI-TOF-MS analysis (Figures 3a and S5). Subsequently, LCI-DBCO-Cy-3 was employed for surface functionalization (drop coating) of PP, PET, stainless steel, gold, and silicon, followed by a washing step to remove nonbound LCI-DBCO-Cy-3 and Cy-3 azide. The functionalization of the different surfaces was visualized by confocal microscopy, and fluorescence was detected on all surfaces that were functionalized with LCI-DBCO-Cy-3, while the controls (only containing Cy-3 azide) exhibited no fluorescence (Figure 3b). In addition, a control containing non-functionalized LCI and Cy-3 azide (to exclude nonspecific interactions between LCI and Cy-3 azide) was performed for PP (Figure S6). The results show that all tested surfaces were functionalized by LCI-DBCO-Cy-3. Confocal microscopy revealed especially strong fluorescent intensities (corresponds to higher coating density) for the polymers PP and PET when compared with stainless steel, gold, and silicon. In addition, a PP 96-well MTP AP binding assay (Rübsam, Davari, et al., 2018) was modified to detect surface-bound LCI-DBCO-Cy-3. Therefore, LCI-DBCO-Cy-3 was bound to a PP MTP, followed by multiple washing steps (buffer and nonionic surfactant Triton X-100) to remove nonbound LCI-DBCO-Cy-3 and unreacted Cy-3 azide. Reactions containing Cy-3 azide, LCI, and DBCO were employed as controls. The results showed significantly higher fluorescence values (~5.2-fold) for wells coated with LCI-DBCO-Cy-3 (Figure 4a) compared with the controls. To investigate if LCI-DBCO was also accessible for click chemistry functionalization after surface binding, the aforementioned PP MTP binding assay was modified to detect click chemistry on the polymer surface. LCI-DBCO was bound to a PP MTP, followed by multiple washing steps to remove nonbound LCI-DBCO. Subsequently, surface-bound LCI-DBCO was incubated with Cy-3 azide. After multiple washing steps to remove unreacted Cy-3 azide (buffer and nonionic surfactant Triton X-100), the fluorescent signal was recorded. Reactions containing Cy-3 azide, LCI, and DBCO were employed as controls. The results showed significantly higher fluorescence values (~3.9-fold) for wells coated with LCI-DBCO (Figure 4b) compared to the controls. The latter shows that click chemistry can also be performed after surface binding of LCI-DBCO.

**FIGURE 3** Surface functionalization of PP, PET, stainless steel, gold, and silicon through LCI-DBCO. (a) Click chemistry functionalization of LCI-DBCO with Cy-3 azide. (b) Confocal microscopy analysis of surfaces functionalized with LCI-DBCO-Cy-3. Reactions containing Cy-3 azide were employed as controls. The white scale bars represent 250 µm for PP, PET, gold, and silicon, and 75 µm for stainless steel. The structure of LCI is based on the PDB entry 2B9K [Color figure can be viewed at wileyonlinelibrary.com]
3.3.2 | Direct surface functionalization: One-step strategy

Surface functionalization of PP with Cy-3 and biotin was performed through the one-step surface functionalization strategy (direct modification of LCI with the desired functionality).

As an alternative to the click chemistry surface functionalization approach (two-step strategy), the desired functionality can also be directly introduced to LCI (one-step strategy). This complementary approach can be used when the respective compound is not available with a reactive group designed for click chemistry (e.g., natural substances) or if its functionality could be affected by click chemistry (modification with reactive groups for click chemistry; click reaction product). Therefore, LCI was modified with Cy-3 and biotin amine through sortagging. In deviation from the previous protocol, the reaction time was increased to 18 h to ensure maximum conversion of amine compounds. Subsequently, the functionalization of PP by LCI-Cy-3 or LCI-biotin was investigated by the previously described PP MTP binding assay. LCI-Cy-3 and LCI-biotin were bound to PP MTP plates without further purification. After the initial binding step, nonbound LCI-Cy-3, LCI-biotin, Sa-SrtA, and unreacted Cy-3 and biotin amine were removed by multiple washing steps (nonionic surfactant Triton X-100 and/or buffer). Reactions containing Cy-3 or biotin amine, LCI, and Atto 425-Streptavidin were employed as controls. The surface functionalization with LCI-Cy-3 was directly detected fluorometrically. The results showed significantly higher fluorescence for LCI-Cy-3 compared to the controls (~4.4-fold), proving a successful surface functionalization with Cy-3 (Figure 5a). The detection of nonfluorescent LCI-biotin required a second incubation step with fluorescent Atto 425-Streptavidin after the initial binding step. After washing (buffer and nonionic surfactant Triton X-100) to remove nonbound Atto 425-Streptavidin, the reactions with LCI-biotin showed higher fluorescence values than the controls (~11.6-fold). The latter proves a surface functionalization with biotin (Figure 5b).

4 | DISCUSSION

Universal functionalization of a broad range of different materials and surfaces with a plethora of functionalities through a universally applicable surface functionalization technology or toolbox is of utmost importance for material science, biotechnological applications, and medical science. Universal APs, such as LCI, enable oriented immobilization on different surfaces (e.g., polymers, metals, silicon-based materials, and natural surfaces; Dedisch et al., 2019, 2020; Meurer et al., 2017; Rübsam et al., 2017). Sortagging enables bioconjugation with high selectivity and homogeneity (Dai et al., 2019). The combination of sortagging and LCI is a powerful toolbox technology to introduce a plethora of functionalities to a broad portfolio of different materials and surfaces by a single surface functionalization methodology. This toolbox allows for a one- or two-step surface functionalization strategy. In the case of the one-step method, the desired functionality can directly be introduced to LCI, while for the two-step method, LCI can be modified with a reactive group, which allows for further functionalization (e.g., employing click chemistry; Figure 1).
Sortagging of LCI was performed for six different amine compounds (Figure S2), and purification of LCI sortagging products was achieved for LCI-DBCO and LCI-kanamycin (Figures S3 and S4).

The two-step surface functionalization strategy was investigated through click chemistry surface functionalization (LCI-DBCO) of PP, PET, stainless steel, gold, and silicon. The results showed that all tested surfaces were functionalized by LCI-DBCO-Cy-3, but especially strong fluorescent intensities (corresponds to higher coating density) were detected for the polymers PP and PET when compared with stainless steel, gold, and silicon. The engineered LCI variant (Y29R/G35R) was evolved for strong binding to polymers (Rübsam, Davari, et al., 2018), which is supported by these results. Furthermore, the LCI-DBCO-Cy-3 functionalization of PP, PET, stainless steel, gold, and silicon was in good agreement with previously reported results for LCI binding (Dedisch et al., 2020). If the adhesion of LCI to gold, stainless steel, or silicon should be promoted, the binding strength and selectivity of LCI can be tailored by methodologies of directed evolution (e.g., KnowVolution or PePev; Rübsam, Davari, et al., 2018; Rübsam, Weber, et al., 2018). The click reaction with Cy-3 azide was performed efficiently before or after surface binding of LCI-DBCO, which shows that LCI is suitable to direct surface functionalization with DBCO in an oriented manner. In this study, Cy-3 azide was employed as a fluorescent probe, which can readily be replaced by other molecules containing an azide moiety or LCI can be functionalized with an azide moiety to facilitate click reactions with alkyne compounds and extend the substrate scope.

In the case of the one-step surface functionalization strategy, PP was successfully functionalized with Cy-3 and biotin. LCI was also functionalized with CF amine, PEG amine, and kanamycin. Antibacterial surface coatings play a pivotal role in introducing antifouling and bactericidal properties to materials applied in the medicine and food industry (Hasan et al., 2013). Functionalisation of LCI with antibiotic molecules such as kanamycin could open new ways to introduce antibiotic functionalities to a broad range of materials. Before the investigation of the antimicrobial activity, LCI-kanamycin was purified as described previously. The LCI-kanamycin yield after the purification reaction was 108.1 µg (fraction of functionalized LCI, which was initially employed in the sortagging reaction: 32%). Investigations of the antimicrobial activity against X. citri showed that the modification of LCI with kanamycin increases the antimicrobial activity of LCI in solution (Figure S7). Investigations of the application potential for antibacterial surfaces yet remain to be performed in the future. Furthermore, surface functionalization with CF or PEG amine could allow to tune the surface hydrophilicity (PEG amine) and hydrophobicity (CF amine). Surface PEGylation is a universally applicable technique for increasing the hydrophilicity and biocompatibility of different materials (Alcantar et al., 2000) while fluorine-based surface coatings are reported to promote surface hydrophobicity (Favia et al., 2003).

5 | CONCLUSION

In essence, we have shown that the one- or two-step strategy for AP-mediated surface modification, enables a universal functionalization of a broad range of chemically different materials and surfaces with a plethora of functionalities under mild reaction conditions. Sortagging makes the combination further attractive due to its high selectivity and orthogonality. We developed a broadly applicable coating
platform as a toolbox for the functionalization of chemically non-modified PP and PET and likely other substrates (e.g., metals and silicon-based); in addition, the authors are convinced that the toolbox character of both strategies will pave the way to new coatings with unique properties and will therefore find broad applications.

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CONFLICT OF INTERESTS
The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS
Maximilian Nöth, Zhi Zou, Felix Jakob, and Ulrich Schwaneberg conceived and designed the study. Maximilian Nöth, Zhi Zou, and Leticia C. de Lencastre Novaes performed the experimental work. Guilherme Dilarri and Henrique Ferreira contributed to antimicrobial assay design. Maximilian Nöth and Zhi Zou wrote the manuscript. Islam El-Awaad, Mehdi D. Davari, Felix Jakob, and Ulrich Schwaneberg contributed to the experimental design and reviewed the final manuscript. All authors approved the final manuscript.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author on reasonable request.

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