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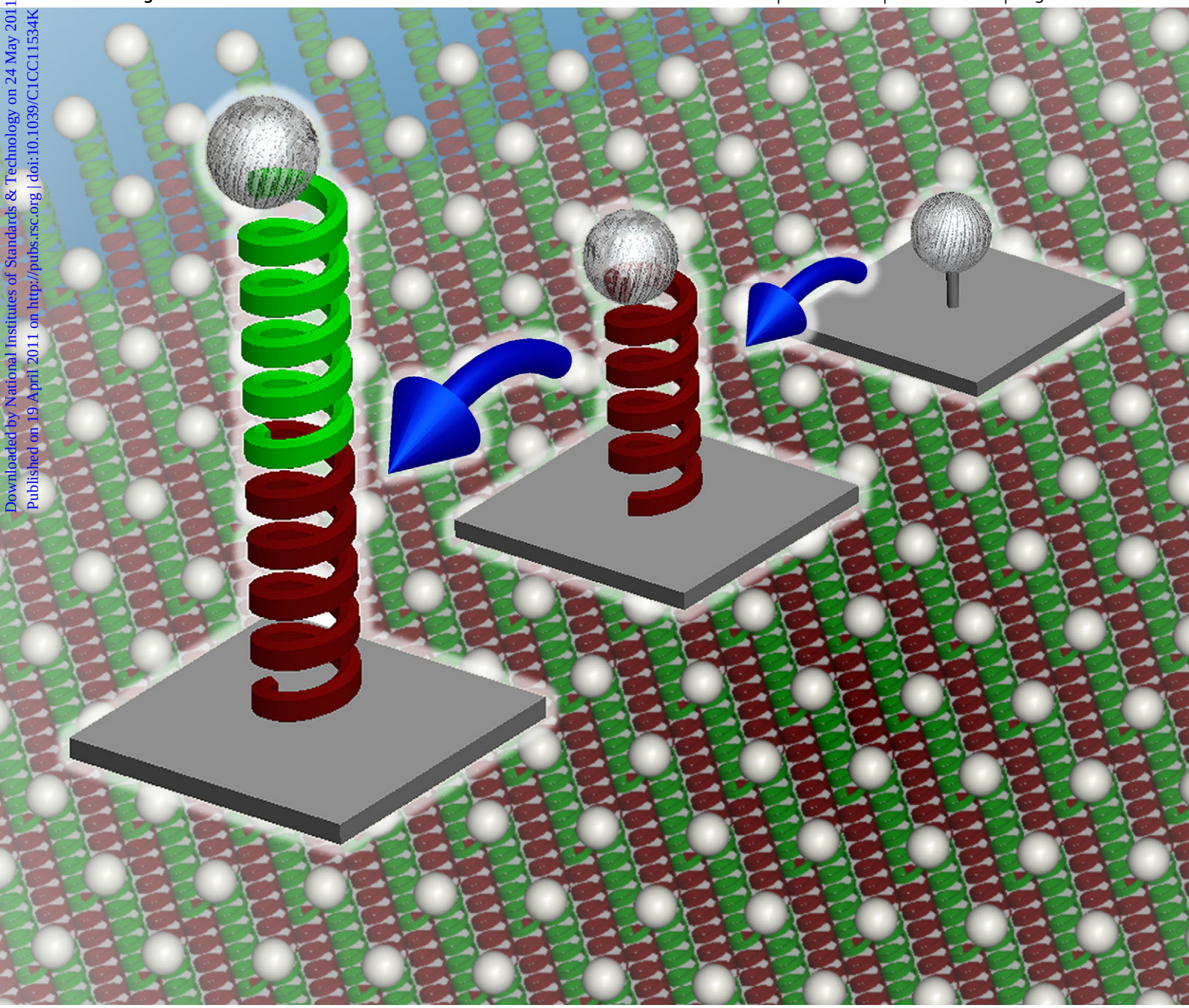

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COMMUNICATION

Synthesis of thiol-clickable and block copolypeptide brushes *via* nickel-mediated surface initiated polymerization of α-amino acid *N*-carboxyanhydrides (NCAs)†‡

Bradley J. Sparks,[a] Jacob G. Ray,[a] Daniel A. Savin,[a] Christopher M. Stafford[b] and Derek L. Patton*[a]

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We describe the synthesis of homo-, block, and clickable copolypeptide brushes from low surface area substrates using nickel-mediated surface-initiated polymerization of α-amino *N*-carboxyanhydrides.

Peptide-functionalized thin films are of great interest to the materials science community and have been explored for an array of applications including biomedical,[1] anti-fouling,[2] inorganic/peptide hybrids[3] and stimuli responsive materials.[4] Polypeptide brushes are a unique class of surface-grafted polymers in that they allow the incorporation of structural motifs typical of those found in proteins, such as α-helices, β-sheets, and random coils. Like their native counterparts in proteins, these surface-grafted motifs can respond dynamically to changes in their environment.[5] This feature presents an opportunity to control the brush structure, function, and response at a level difficult to achieve with conventional organic polymers, but requires the development of synthetic methods that enable the preparation of structurally complex peptide surfaces.

Polypeptide brushes have been synthesized using two different methods.[6] The "grafting to" approach involves a one-step reaction of reactive end-groups of pre-synthesized polypeptides with complementary reactive moieties on a surface. The adsorption and reaction of preformed polypeptides with surfaces quickly becomes diffusion-limited resulting in low grafting densities. The "grafting from", or surface-initiated approach, typically occurs *via* a ring-opening polymerization of α-amino acid *N*-carboxyanhydrides (NCAs) initiated from an amine-functionalized substrate in either the solution or vapor phase.[7,8] Since monomers can readily diffuse to the propagating chain end, the surface-initiated approach yields higher grafting densities as has been demonstrated for a broad range of homopolypeptide surfaces.[9] In contrast, relatively few examples of block copolypeptide (BCPP) surfaces have been reported in the literature. Wieringa *et al.*[10] first reported the synthesis of BCPPs from primary amine-functionalized substrates in solution. Wang and Chang[11] improved upon the synthesis of BCPPs under surface-initiated vapor polymerization, which significantly reduces the occurrence of side reactions yielding higher film thicknesses.

Transition metal-mediated polymerization of NCAs, as reported by Deming *et al.*,[12–15] allows the preparation of well-defined polypeptides including end-functionalized, sequenced and BCPPs. Witte and Menzel[16] utilized Ni(0) initiators to polymerize NCAs from high surface area polystyrene resins; however, no studies have been reported on Ni-mediated polymerization of polypeptides from low surface area substrates (*i.e.* silicon wafer). Due to the low concentration of initiator on the surface, polymerization behaviour can vastly differ from that of high surface area polymerizations. Furthermore, the flat substrate represents a model substrate configuration for biosensor platforms and other thin film applications.

In this communication, we describe the synthesis of homo, block, and clickable copolypeptides *via* nickel-mediated surface-initiated polymerization from low surface area substrates. As examples, we prepare blocked combinations of poly(*N*-carbobenzyloxy-L-lysine), poly(γ-benzyl-L-glutamate) and poly(*s*-*tert*-butylmercapto-L-cysteine) brushes from the respective NCA monomers. Additionally, we show that the cysteine-containing constituent lends itself well to a post-polymerization modification *via* an efficient thiol-Michael click reaction with maleimides resulting in a highly modular approach to functional polypeptide surfaces.

As shown in Fig. 1, alloc-L-leucine-3-aminopropyltriethoxysilane, synthesized from the reaction of alloc-L-leucine-*N*-hydroxysuccinimide with 3-aminopropyltriethoxysilane (see ESI†), was used to covalently modify silicon oxide substrates

[a] *School of Polymers and High Performance Materials, The University of Southern Mississippi, 118 College Drive # 10076, Hattiesburg, MS 39406, USA. E-mail: derek.patton@usm.edu; Tel: +1 601-266-4229*
[b] *Polymers Division, National Institute of Standards and Technology, Gaithersburg, Maryland 20899, USA*

† Electronic supplementary information (ESI) available: Synthetic details and characterization parameters. See DOI: 10.1039/c1cc11534k

‡ Official contribution of the National Institute of Standards and Technology; not subject to copyright in the US. Equipment, instruments, and materials are identified in the paper in order to adequately specify the experimental details. Such identification does not imply recommendation by NIST, nor does it imply the materials are necessarily the best available for the purpose.

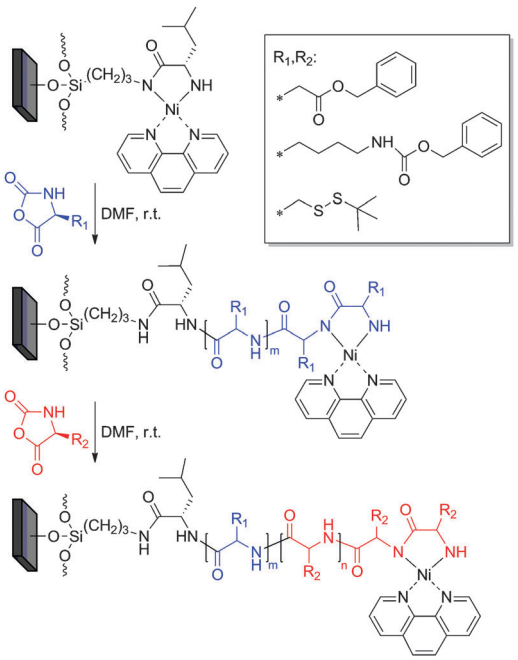

Fig. 1 Synthetic route for the preparation of homo- and block copolypeptide brushes *via* nickel-mediated surface-initiated polymerization of α-amino acid *N*-carboxyanhydrides (NCAs).

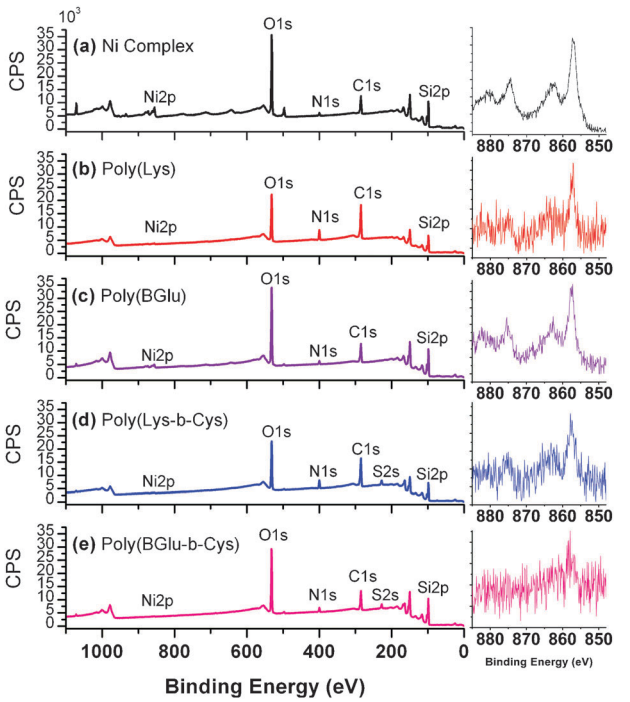

Fig. 2 XPS survey (left) and corresponding high resolution Ni2p spectra (right) for (a) tethered Ni-initiator complex, (b) poly(*N*-carbobenzyloxy-L-lysine), (c) poly(γ-benzyl-L-glutamate), (d) poly(*N*-carbobenzyloxy-L-lysine-*block*-*S*-*tert*-butylmercapto-L-cysteine), and (e) poly(γ-benzyl-L-glutamate-*block*-*S*-*tert*-butylmercapto-L-cysteine).

with a thin layer of alloc-amide groups. Successful modification of the surface was confirmed by ellipsometry and X-ray photoelectron spectroscopy (XPS). The mean thickness of the dry silane-modified layer was 1.7 nm ± 0.6 nm as measured by ellipsometry (std. dev. represents variability from repeat experiments, which is taken as the standard uncertainty of the measurement). The XPS survey scan of the alloc-amide functionalized surface indicated the expected chemical composition with observed bonding energies at 99 eV (Si2p), 285 eV (C1s), 399 eV (N1s), and 532 eV (O1s) (see ESI† for XPS spectrum). The alloc-amide substrates were then complexed with bis(cyclooctadiene) nickel(0) ($Ni(COD)_2$) and 1,10-phenanthroline in dry dimethylformamide (DMF) to create a surface tethered Ni(0) initiator complex from which surface-initiated polymerization (SIP) of NCAs could be facilitated. After washing the substrates extensively with DMF to remove excess/unbound Ni species, the Ni complexation on the surface was confirmed by XPS. As shown in Fig. 2a, the survey and high resolution Ni2p spectra indicate the presence of nickel with binding energy observed at 855 eV (Ni2p). The active Ni-initiator substrates were then submerged in a 0.26 mol L^{-1} NCA monomer solution in dry DMF for 24 h. After polymerization, Soxhlet extraction in THF was used to remove any physisorbed polymer from the polypeptide brush. For the purpose of this communication, optimization of experimental parameters such as monomer concentration, initiator grafting density, temperature, ligand, *etc*. were not explored. These parameters will be fully investigated in a forthcoming publication. After extraction, the poly(lysine) and poly(benzyl glutamate) peptide brushes yielded thicknesses of 4.2 nm ± 1.7 nm and 3.6 nm ± 1.8 nm, respectively. The low film thickness observed is likely a direct result of the low monomer concentrations employed in this study. The polypeptide surfaces were characterized by XPS where the retention of the Ni end-group would indicate a degree of controlled polymerization from the surface. As shown in high resolution Ni2p spectra in Fig. 2b and c, peaks corresponding to the Ni2p binding energies were observed albeit in reduced intensity compared to the Ni-initiator in Fig. 2a. This is expected as we are now probing the entire film thickness, as indicated by the increase in the C1s and N1s intensities in the survey spectra associated with the peptide backbone. An additional indication of a controlled polymerization process was demonstrated through the continued growth of the homopolypeptides and formation of block copolypeptides. BCPPs were synthesized by subsequently submerging the homopolymer substrates prepared as previously described in a 0.26 mol L^{-1} NCA–*S*-*tert*-butylmercapto-L-cysteine monomer solution. After 24 h reaction time and subsequent washing, the poly(cysteine) block segment thicknesses were measured at 4.4 nm ± 1.5 nm by ellipsometry. The equivalent thickness measured for each chain extension suggests similar end-group retention and blocking efficiency from both lower blocks. A more detailed XPS study will be necessary to quantify chain-end retention/blocking efficiency. Further supporting the presence of the poly(cysteine) block in the Lys-b-Cys and BGlu-b-Cys copolymers, XPS survey spectra (Fig. 2d and e) showed a peak corresponding to the S2s binding energy at 229 eV (high resolution S2p available in the ESI†). Notably, the Ni end-group is still observed in the Ni2p high resolution spectra suggesting the possibility of preparing short sequenced, multiblock polypeptide brushes using this approach.

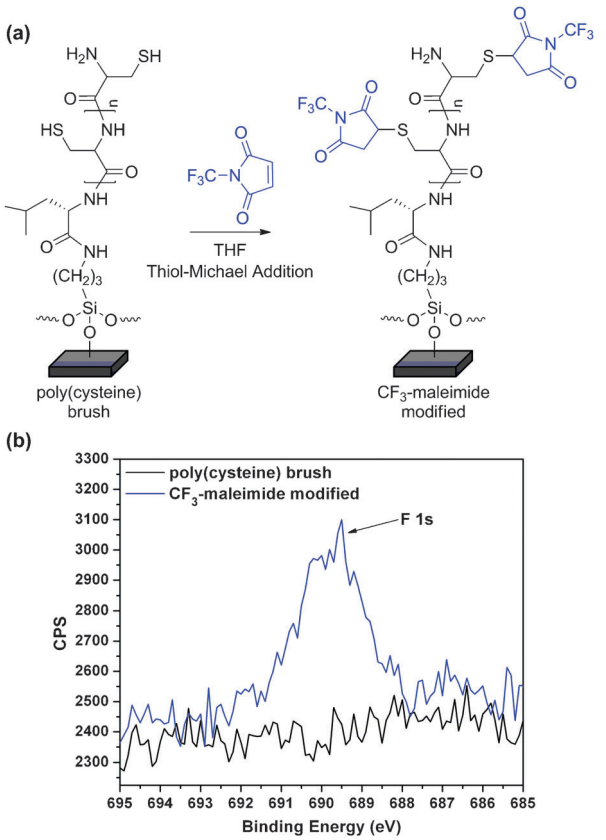

Fig. 3 (a) Synthetic scheme for thiol-Michael modification of a deprotected poly(cysteine) brush with a fluorine-labelled maleimide. (b) XPS data indicate a successful modification as shown by the observation of a peak at 689.5 eV in the high resolution F1s spectrum.

Recently, we and others have shown that click,[17,18] and particularly thiol-click reactions[19–21] are a powerful approach for engineering functional surfaces in a modular fashion. Thiol-ene reactions, more specifically the thiol-Michael addition, are of particular interest because of rapid kinetics, amiable reaction conditions and applicability in bioconjugations.[22] Pertinent to our current work, Habraken *et al.* showed that the thiol moiety of cysteine could be functionalized in solution utilizing the thiol-Michael addition of methacrylates to affix pendant groups.[23] Using a similar approach, we continue to show the versatility of thiol-click by preparing functional polypeptides using the pendant thiol of tethered poly(cysteine) brushes.

To demonstrate the concept, we prepared and characterized thiol-containing polypeptide brushes from NCA–*S*-*tert*-butyl-mercapto-L-cysteine as previously described. Subsequently, the *tert*-butyl mercapto moieties were deprotected with dithiothreitol in DMF at 60 °C to afford the brush–pendant thiols. These moieties were used as reactive handles for further functionalization. Fig. 3a shows the thiol-Michael reaction employed to functionalize the cysteine brush with a fluorine-labelled maleimide. The maleimide was dissolved in a dry THF solution and the deprotected peptide substrate was submerged in the maleimide solution for 24 h. The brush functionalization was characterized by XPS, which confirmed the attachment of the pendant maleimide as indicated by the peak at 689.5 eV in the high resolution F1s spectrum (Fig. 3b). A 3.2 nm increase in ellipsometric thickness was also observed consistent with an increase in molecular mass of the maleimide functionalized brush. Although the maleimide used in the current study was convenient for XPS analysis, the modification of the pendant thiol can easily be extended to other "enes" carrying appendages useful for a broad range of applications, *i.e.*, bioconjugation. This versatility allows for a simple and effective way to realize polypeptide surfaces that are otherwise unattainable by direct polymerization.

In summary, we have demonstrated a method to synthesize homo-, block, and clickable copolypeptides *via* nickel-mediated surface-initiated polymerization from low surface area substrates. XPS showed that the Ni species remains attached to the end-group of the brush, which leads us to believe that the Ni-complex continues to play a mediating role during chain extension to form block copolypeptides. Furthermore, we utilized the highly efficient thiol-Michael reaction to prepare functional polypeptide surfaces from thiol-containing poly(cysteine) brushes. Future work will focus on the kinetics of brush growth, initiation efficiency, and expanding the utility of thiol-clickable peptide surfaces.

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