# Novel polypeptide/thiol—SBA-15 hybrid materials synthesized *via* surface selective grafting†

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**Novel hybrid materials are synthesized through the surface selective grafting of poly-L-lysine and thiols from SBA-15.**

The continued interest in hybrid materials has led to intense efforts in developing new synthesis paradigms for making materials which advantageously combine the features of both soft and hard matter. In this vein, ordered mesoporous silica (OMS)–organic hybrids have attracted considerable attention.[1,2] Given the well-defined textural properties of OMS and the ability to functionalize silica using known silane chemistries, these appear to offer numerous opportunities for developing new hybrid materials.

We recently reported the synthesis of OMS–polypeptide hybrids *via* the use of surface-tethered amines as initiators for the ring-opening polymerization of *N*-carboxyanhydride (NCA) amino acids.[3] Building on that report, here we demonstrate the ability to selectively functionalize the outer or inner surface of SBA-15 with poly-L-lysine while grafting a different functional group, here thiols, to the opposite surface. We believe the ability to graft different organic architectures surface-selectively will extend the scope of OMS hybrids in a diverse field of scientific interests. In the current work, amines and thiols were chosen given their potential interest in catalysis[4–6] and molecular gating,[7] and their well-developed/orthogonal chemistries that permit subsequent reactions/use as chemical handles.

Recently, there has been increasing interest in developing such multifunctional materials. An array of different approaches have been reported in the literature. One method utilizes the OMS-templating agent as a barrier to selectively graft organics to the outer surface followed by template extraction.[7–10] A second approach uses sequential co-condensation in radially growing OMS nanoparticles to incorporate one or more functional groups.[11–13] Other methods employing diffusion-limited deprotection[14] and surface tension differences[15] have also been reported. Though likely less domain specific than "designer" methods used for OMS nanoparticles, the "barrier" approach yields high external organosilane concentrations and uses traditional OMS synthesis protocols applicable to a wide variety of common systems. For these reasons, it is the method of choice here.

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† Electronic supplementary information (ESI) available: Detailed synthesis procedures and analytical techniques; XPS and IR spectra; adsorption isotherms; SEM images; initial thiol grafting results; and results from polylysine-only hybrids prepared *via* traditional amine grafting. See DOI: 10.1039/b927487a

Though we found in initial investigations that amines graft easily to the external surface of SBA-15 through traditional reaction in toluene, thiols were much less reactive, requiring high temperatures, long reaction times, and the addition of water (ESI†). Additionally, we found that traditional ethanol extraction ineffectually removes the Pluronic template. Here, we present a modified approach adapted from the literature[16,17] using microwaves that rapidly and effectively grafts both silanes and extracts the Pluronic template.

SBA-15 was synthesized as described previously. External surface functionalization was achieved by reacting as-made SBA-15 in neat organosilane while stirring and irradiating with microwaves (300 W) for 15 minutes.‡ The Pluronic template was then removed by a rapid three step ethanol extraction which was assisted by microwaves (100 W). Extraction was followed by a traditional post-synthetic grafting of 0.5 mmol $g^{-1}$ functionalized $SiO_2$ (FS) of the other silane. In this manner, two samples were prepared: one with amines externally and thiols internally (**X**) and another with thiols externally and amines internally (**I**). Poly-*Z*-L-lysine (PZK) was grafted from the amine-functionalized surface, deprotected (PK) using HBr, and neutralized (Fig. 1). High and low monomer loading (10 and 5 mmol NCA $g^{-1}$ functionalized $SiO_2$) syntheses were performed to observe the effect of the polypeptide loading: **PZK-X-10** and **PZK-I-10** and **PZK-X-5** and **PZK-I-5**, for high and low, respectively.

XPS spectra taken after the second functionalization step show the effective incorporation of both silanes (Fig. 2C). As expected due to the surface sensitivity of XPS, the peaks at the characteristic binding energies of the externally grafted silane are more intense than those of the internal group. Additionally, NHS-fluorescein (amine selective) and BODIPY-TMR thiosulfate (thiol selective) dyes were used to visualize the degree of surface segregation of the amines and thiols by Confocal Microscopy. Despite limits in optical resolution, images of these samples show amine and thiol-selective dyes

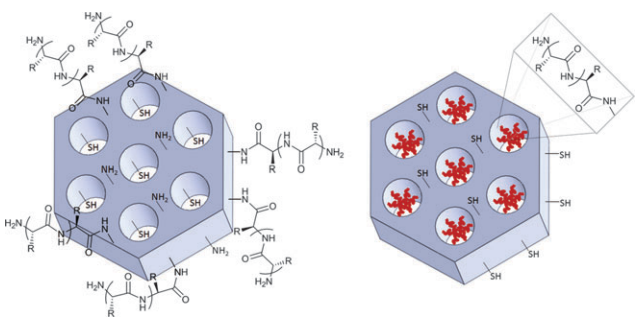

**Fig. 1** Idealized pictorial representations of **PK-X** (left) and **PK-I** (right); R = $–CH_2CH_2CH_2CH_2NH_2$.

 

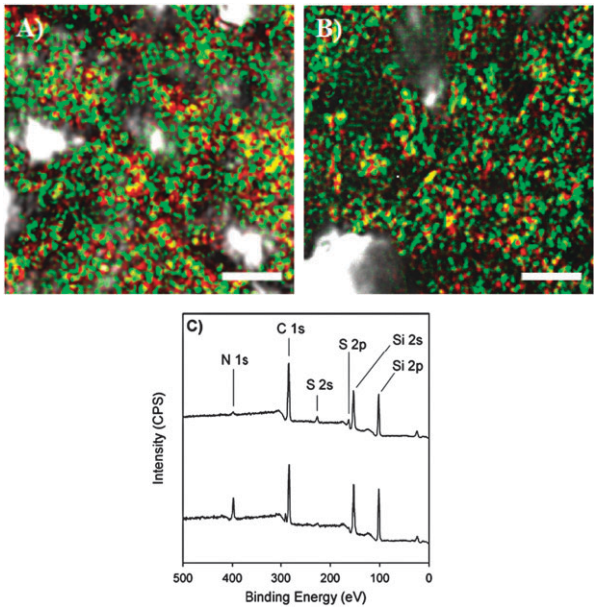


**Fig. 2** Confocal images of (A) **I** and (B) **X** dyed with NHS-fluorescein (green, amine selective) and BODIPY-TMR thiosulfate (red, thiol selective). The scale bar represents 5 μm. (C) XPS spectra of **I** (top) and **X** (bottom).

occupying separate domains (Fig. 2A and B), consistent with the XPS data and suggesting successful selective functionalization in both materials.

As in our previous work, we found that the ring-opening polymerization of *N*-carboxyanhydride amino acids proceeds from the surface of amine-functionalized SBA-15, demonstrating that the amines are accessible and the thiols do not inhibit the polymerization. Selectively-grafted samples with only amines show similar results (ESI†). IR (ESI†) indicates that significant amounts of polypeptide are formed in both sets of materials, evident from the amide peak intensities (~1650 $cm^{-1}$ and ~1550 $cm^{-1}$) relative to the Si–O–Si bending peak (~1100 $cm^{-1}$). It is similarly apparent that the polymer can be deprotected with ease in HBr, shown by the disappearance of the carbamate peak (~1700 $cm^{-1}$) (Fig. 5).

TGA shows that, with the exception of **PZK-I-5**, much larger amounts of peptide are formed compared to the ~3.5 mmol lysine per gram FS maximum achieved in our previous study (Table 1). For the externally grafted samples, this result may be explained by a lack of pore wall confinement on polymer growth. As might be expected taking this into consideration, the externally grafted peptide samples for both monomer loadings show larger amounts of polymer relative to their internally grafted counterparts. SEM show large amounts of polymer on the external surface of the elementary particles ($d$ ~ 0.5 μm) for these samples (ESI† and Fig. 3). For the internally grafted samples, polymer chains close to the pore mouth likely continue to grow outward without external interference leading to larger than expected polymer loadings; SEM, in support, shows polymer on the outer surface of **PZK-I-10** (ESI†). SEM images of **PZK-I-5** (Fig. 3), in contrast, show smaller and more defined particles indicative of much less or no polymer on the outer surface. Weight losses recorded for the deprotected samples are in excellent agreement with

**Table 1** Adsorption and TGA data

| Sample | $S(\alpha_s)$/ $m^2\ g^{-1}$ | $V_p{}^a$/ $cm^3\ g^{-1}$ | $d_p$(BJH)/ nm | Organic (wt%) | Lysine[b]/ mmol $g^{-1}$ FS |
|---|---|---|---|---|---|
| **SBA-15** | | | | 9.4 | — |
| **X** | 660 | 0.66 | 7.5 | 19.6 | — |
| **I** | 651 | 0.65 | 7.4 | 24.5 | — |
| **PZK-X-10** | — | n.p. | — | 68.9 | 6.04 |
| **PK-X-10** | 33 | 0.02 | 5.6 | 53.7 | 5.74 |
| **PK-X-10 H** | — | n.p. | — | | |
| **PZK-X-5** | — | n.p. | — | 63.8 | 4.66 |
| **PK-X-5** | 34 | 0.02 | 5.9 | 49.7 | 4.68 |
| **PZK-I-10** | — | n.p. | — | 68.0 | 5.18 |
| **PK-I-10** | 204 | 0.18 | 6.2 | 52.7 | 4.66 |
| **PK-I-10 H** | 60 | 0.03 | 6.2 | | |
| **PZK-I-5** | — | n.p. | — | 61.8 | 3.73 |
| **PK-I-5** | 256 | 0.23 | 6.6 | 45.8 | 3.07 |

[a] Determined at $p/p_0$ = 0.9; n.p. = non-porous. [b] Calculated from TGA data after subtracting organosilane and residual Pluronic contributions.

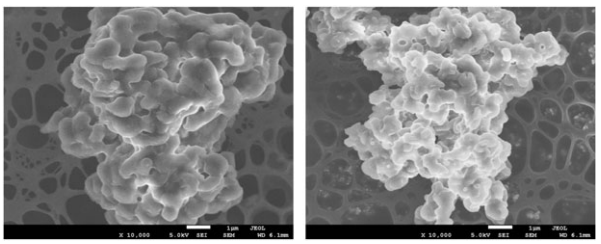

**Fig. 3** SEM images of **PZK-X-5** (left) and **PZK-I-5** (right). The white scale bar represents 1 micron.

estimated values for complete deprotection and neutralization as noted by the calculated mmol lysine per gram FS in Table 1.

Complementary to SEM, XPS shows a dramatic decreasing trend in the C/Si and N/Si atomic ratios from **PZK-X-10** to **PZK-I-5** (Fig. 4A). **PZK-X-10** has C/Si and N/Si ratios of 425 and 28, respectively, whereas, **PZK-I-5** has ratios of 60 and 6. The much lower contribution of N and C in the **PZK-I-5** spectra provides strong evidence for more internally confined polypeptide. **PZK-X-5** and **PZK-I-10** show similar ratios, again suggesting that excess peptide is exiting the pores in **PZK-I-10**. Another notable difference is observed in the spectra of the deprotected samples: the S 2s and S 2p peaks are visible for the internally grafted samples as opposed to the externally grafted samples where none are observed (Fig. 4B).

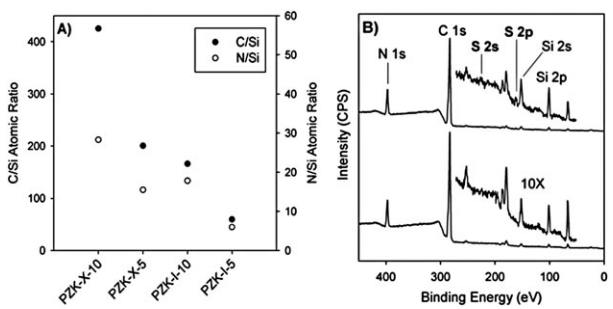


**Fig. 4** (A) Plot of N/Si and C/Si atomic ratios for poly-*Z*-L-lysine grafted samples; (B) XPS spectra of **PK-I-5** (top) and **PK-X-5** (bottom).

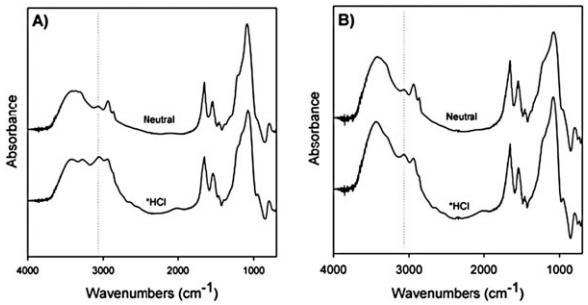


**Fig. 5** IR of neutral and protonated (A) **PK-X-10** and (B) **PK-I-10**. The dashed lines mark the amine absorption band at $\sim$3060 cm$^{-1}$.

As there are numerous studies on polyelectrolyte brush swelling,[18] we were interested in looking at the effects of side chain protonation on the deprotected samples. **PK-I-10** and **PK-X-10**, neutralized after deprotection, were treated with 0.1 M HCl and sonicated for 10 minutes. Nitrogen adsorption of **PK-I-10 H** shows a drastic decrease in porosity (Table 1). Similarly, though there was very little initial porosity, **PK-X-10 H** shows absolutely no porosity (Table 1).

There is also a chemical change for both of these samples evidenced by IR.§ As noted in the literature, when protonated, the amine absorption band at $\sim$3030 cm$^{-1}$ intensifies.[19] Here, this intensity increases significantly for **PK-X-10 H** (Fig. 5A), signifying a high degree of protonation, and much less for **PK-I-10 H**, signifying a lesser degree of protonation (Fig. 5B). The higher degree of protonation in the externally grafted sample is reasonable as the polymer layer has far more space to expand. The fact that the pores essentially close off in the internally grafted sample with limited protonation is evidence of a high sensitivity to pH. This feature may have very interesting implications for molecular gating. The ability to protonate the externally grafted sample easily suggests that a further decrease in the monomer loading may yield a similar effect. Continuing work is investigating whether the thiol groups of the externally grafted samples are accessible to chemical probes in solution under various conditions.

In summary, it has been demonstrated that amines and thiols can be selectively grafted to the exterior surface and within the pores of SBA-15 using a post-synthetic approach. Furthermore, the amines can be used as initiators in the grafting of large amounts of poly-*Z*-L-lysine, creating novel hybrid materials. The poly-*Z*-L-lysine layer is selective to the amine-functionalized surface as supported by a number of techniques. Porosity of the hybrids and the chemical state of the polymer layer are dependent on the protonation state of side chain amines.

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## Notes and references

‡ A comparison of the as-made functionalized samples with as-made SBA-15 indicates some Pluronic is likely extracted into the silane phase during functionalization; however, it does not seem to have a large effect on the resulting samples and its impact is probably limited to pore mouth functionalization. Polylysine-only hybrids prepared using traditional grafting in toluene lead to qualitatively similar porosity and surface composition trends (ESI†).
§ Protonation of the side chain amines is reversible as they were initially charged following deprotection, then neutralized.

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