Supplementary Material (ESI) for Chemical Communications

Novel polypeptide/thiol – SBA-15 hybrid materials synthesized *via* surface selective grafting

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I. Experimental Methods

Materials. Tetraethoxysilane (TEOS, Fluka, >99%), Pluronic P123 ($EO_{20}PO_{70}EO_{20}$, MW = 5800, BASF), ethanol (Sigma-Aldrich, 99.9%, ACS reagent), and HCl (Sigma-Aldrich, reagent grade, 37%) were used in the OMS synthesis as received. 3-aminopropyltrimethoxysilane (APTMS, Aldrich, 97%) and 3-mercaptopropyltrimethoxysilane (MPTMS, Aldrich, 97%) were used for post-synthetic grafting and distilled prior to use. Triphosgene (TCI America, 98%) and H-Lys(Z)-OH (Novabiochem, 98%) were used for *N*-carboxyanhydride synthesis. *n*-Hexane (Sigma-Aldrich, >95%, ACS reagent grade), tetrahydrofuran (THF, Sigma-Aldrich, >99.9%, Chromasolv HPLC) and toluene (Sigma-Aldrich >99.5%, ACS reagent grade) were used in the NCA synthesis and polymer grafting steps and were dried and deoxygenated using an MBRAUN MB SPS solvent purification system. *N,N*-Diisopropylethylamine (DIPEA, 98%) was purchased from Fluka. The NHS-Fluorescein (>90%) and TS-link BODIPY-TMR C5 thiosulfate were purchased from Pierce and Invitrogen, respectively.

SBA-15. [1] 4.0 g of Pluronic P123 were dissolved in 60 mL of 4 M HCl and 85 mL of deionized water by stirring for 5 h at room temperature. Then, 8.5 g of TEOS were added to that solution and stirred for 24 h at 35 °C. The mixture was then aged at 80°C for 24 h without stirring. After filtering, the solid product was dried at 40 °C until used.

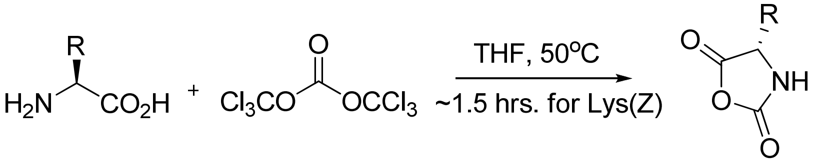
External Functionalization. 10 ml of the distilled APTMS or MPTMS was added to 1.2 g of as-made SBA-15 in a Teflon-capped 35ml glass tube. The slurry was stirred rapidly while irradiating with 300 W of 2.54 GHz microwaves for 15minutes in a CEM Discovery Microwave Reactor. A cooling fan was used in order to keep the temperature of the slurry around 50°C. The vessel was cooled and the powder filtered and rinsed in toluene and ethanol.

Extraction. The powder was then placed back in another clean glass tube with 20ml of 1:1 hexanes and ethanol. The slurry was stirred rapidly while irradiating with microwaves (100 W, 2.54 GHz) for three cycles of 2 minutes, with a cooling fan applied during irradiation and for 2 minutes between each cycle. The vessel was cooled and the powder filtered and rinsed in ethanol. This microwave-aided extraction procedure was repeated twice with 25ml ethanol.

Internal Functionalization. Internal functionalization was performed using a traditional post-synthetic grafting procedure. In a typical grafting, 0.5 g of OMS was dried under vacuum overnight (< 0.1 mbar). 25ml of dry toluene were added, followed by 0.25mmol, of distilled APTMS or MPTMS depending upon the desired organosilane. For APTMS, the reaction proceeded at RT for 24 hours. For MPTMS, 100 µl of water was added and the mixture was refluxed for 24 hours. The solid product was centrifuged, washed in toluene (1x), methanol (1x), methanol/water (1x), methanol (1x), and dried overnight in a 40°C oven.

Fluorescent Labeling. Labeling was performed as recommended by the suppliers. ~ 1 mg of each sample was dispersed in 1 ml 0.1M phosphate buffer (pH = 7.5) followed by the addition of 100µl of a 1 mg/ml solution TS-link BODIPY-TMR C5 thiosulfate in water. The samples were shaken for 3 hours. The powder was centrifuged and rinsed repeatedly in water and ethanol. The dyed powder was redispersed in a 0.2M sodium borate buffer (pH = 8.5) followed by the addition of 100µl of a 5 mg/ml solution of NHS-Fluorescein in DMF. The samples were again shaken for 3 hours. The solid was centrifuged and rinsed repeatedly in DMF and ethanol.

***N*-carboxyanhydride synthesis.** The N_ε-Z-L-Lysine (L-Lys(Z)) NCA was synthesized in dry THF using triphosgene as described by Daly and Poche [2] (Scheme 1). Standard Schlenk line techniques were employed in the NCA monomer synthesis and polymer grafting steps. Typically, 4.6g of L-Lys(Z) (16.4mmol) and 2.2g of triphosgene (7.4mmol) were dried under vacuum for a minimum of 20 minutes in separate Schlenk flasks. 75ml and 50ml of dry THF were added to the L-Lys(Z) and triphosgene, respectively, directly from the solvent drying system. The triphosgene solution was added via cannula transfer to a rapidly mixing L-Lys(Z) slurry. The reaction flask was capped and placed in a 50°C water bath. After ~1.5 hours of stirring at 50°C under argon, the solution became transparent indicating the completion of the reaction. The NCA was recrystallized in 400ml of dry n-hexane overnight in a standard freezer twice and, subsequently, filtered and dried under vacuum for immediate use (~5g or >95% yield).



Scheme 1S. *N*-Carboxyanhydride Synthesis: R= $–CH_2CH_2CH_2CH_2NHCbz$ for L-Lys(Z).

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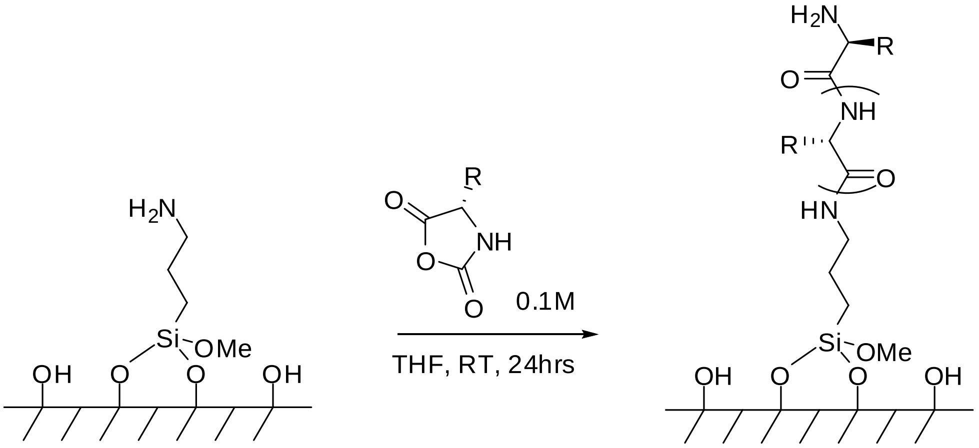
Polymerization. The NCA was polymerized in a *graft from* approach using the amines on the OMS surface as initiators (Scheme 2S). In a typical procedure, 0.25g of functionalized silica were dried overnight under vacuum. A 0.1M NCA solution was prepared in dry THF and 12.5ml (5mmol/g FS) or 25ml (10mmol/g FS) were transferred to the dried OMS via cannula. The powder and solution were vigorously stirred at room temperature for 24 hours. After completion the powder was centrifuged and rinsed in 50ml of THF (2x), DMF (2x) and ethanol (2x). The composite was dried in a 40°C oven and stored.



Scheme 2S. Peptide grafting to silica surface: R=–$CH_2CH_2CH_2CH_2NHCbz$. Note: The number of silane bonds to the surface is not necessarily as depicted.

Deprotection. The Cbz-protected peptides were deprotected using a 33wt% solution of HBr in acetic acid. For example, 200mg of composite material were stirred for 30 minutes in 2ml of HBr solution. The composite was filtered and rinsed multiple times with toluene and acetone. The sample was neutralized by stirring in a 40:1 solution of ethanol and DIPEA for ~2hrs, centrifuged, and rinsed with ethanol.

Analytical. X-ray diffraction measurements were performed on SBA-15 and KIT-6 samples using a Bruker-AXS Rotating-Anode NANO-STAR Small Angle X-ray Scattering Instrument with Cu Kα radiation. Infrared spectroscopy was performed on the composites using a Nexus 670 FT-IR Spectrometer from Thermo Nicolet. Thermal gravimetric analyses (TGA) were performed using a TG 209C Iris Instrument from Netzsch over a temperature range of 100 to 900 °C using oxygen and nitrogen purges (1:1, 10ml/min) and a temperature ramping rate of 5°C min^{-1}. Nitrogen adsorption experiments were performed on a Micromeritics ASAP 2010 micropore system. The samples were degassed under vacuum at 100°C overnight prior to analysis. The surface areas and mesopore volumes were determined using the α_s-method. The mesopore size distributions were calculated from the adsorption branch of the isotherm using the Barret-Joyner-Halenda (BJH) method with a modified equation for the statistical film thickness.[3] X-ray photoelectron spectroscopy (XPS) was performed on a Kratos Axis Ultra Imaging XPS using a monochromatic Al Kα source. Confocal microscopy was performed using a Leica TCS SP5 microscope. The images were obtained using a pinhole diameter of 100nm, a 63X oil immersion objective, and Ar 488nm and HeNe 543nm lasers for excitation. SEM images were obtained with a JOEL JSM 7500F field emission scanning electron microscope. Samples were prepared by dispersing the powders in THF and depositing on a copper grid. A working distance of 6 mm was used and a 5.0kV voltage.

II. Additional Data

A. Initial Investigations

a. Selective grafting of Poly-L-lysine only (no thiols) using traditional methods.

Table 1S shows the adsorption, TGA and XPS results of some initial samples that were prepared with polylysine only. As explained in the main text, traditional grafting in toluene works well for amines. For the externally grafted sample (**X'**), 0.5 g vacuum-dried as-made SBA-15 was reacted with 1ml distilled APTMS in 25ml of toluene for 2 hours. Traditional extraction was used by refluxing 0.5g of product in 100ml ethanol for 6 hours. Polymerization, deprotection, and neutralization were carried out in the same manner as the samples in the main text (10mmol NCA/g FS). The internally grafted sample (**I'**) was prepared in a similar fashion with HMDS grafted to the outside, followed by extraction and functionalization with 0.5mmol APTMS/g. Qualitatively, the trends in organic content, porosity and external surface composition compare very well to the samples in the main text. Poor extraction efficiency [4] seemed to play a role in the lower porosity of **X'**. The same extraction approach for as-made SBA-15 yielded 13.2% organic content compared to 9.4% obtained using microwave extraction.

Table 1S Adsorption and TGA data of hybrids synthesized without thiols, by traditional amine grafting in toluene

Sample	$S(a_s)$ $[m^2/g]$	V_p [a] $[cm^3/g]$	d_p(BJH) [nm]	Organic [wt%]	C/Si by XPS	N/Si by XPS
X'	537	0.56	7.1	16.9		
I'	874	0.87	7.5	----[b]		
PZK-X'	----	n.p.	----	59.6	41.9	4.3
PK-X'	----	n.p.	----	53.8		
PZK-I'	----	n.p.	----	49.0	17.3	1.8
PK-I'	223	0.29	6.2	41.7		

[a] Recorded at $p/p_0 = 0.9$; n.p. = non-porous
[b] No sample was available for this measurement

b. Thiol (MPTMS) Grafting Methods

Figure 1S shows the XPS spectra of various MPTMS (thiol) grafting methods. Initially, we tried grafting the thiols to the outer surface in the same manner as the amines above, using 1ml MPTMS in 25ml toluene for 0.5 g as-made SBA-15 for 2 hour. This approach, however, yielded no observable S 2s or S 2p peaks by XPS (bottom spectra in Figure 1S). Increasing the reaction time to 24 hours also failed to graft observable thiol. By refluxing for 24 hours and adding 1ml of water, only a small amount of grafted thiol was observed (middle spectra in Figure 1S). The use of water, reflux conditions, and a long reaction time, however, is undersirable due to possible Pluronic extraction. Bifunctional peptide grafted samples using this approach when combined with extraction difficulties were messy and hard to interpret. The microwave approach yields a much higher degree of functionalization in only 15 minutes (top spectra in Figure 1S).

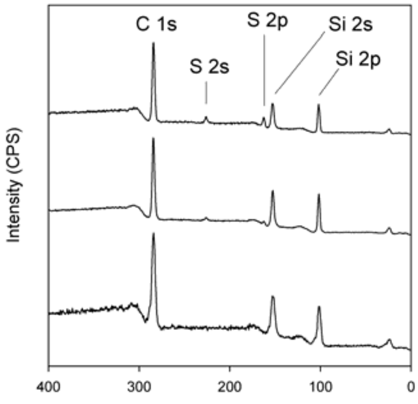


Figure 1S. XPS spectra of externally grafted MPTMS to SBA-15 by various method: (top) MW approach; (middle) 24 hours reflux w/ water; and (bottom) 2hours stirring at RT.

B. Additional Characterization of Principal Samples

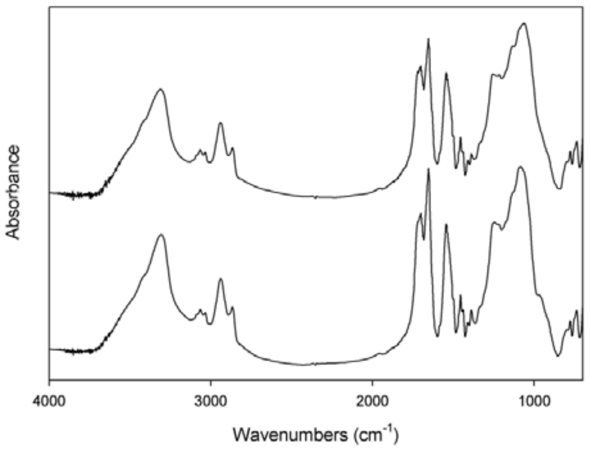


Figure 2S. IR spectra of **PZK-X-10** (top) and **PZK-I-10** (bottom).

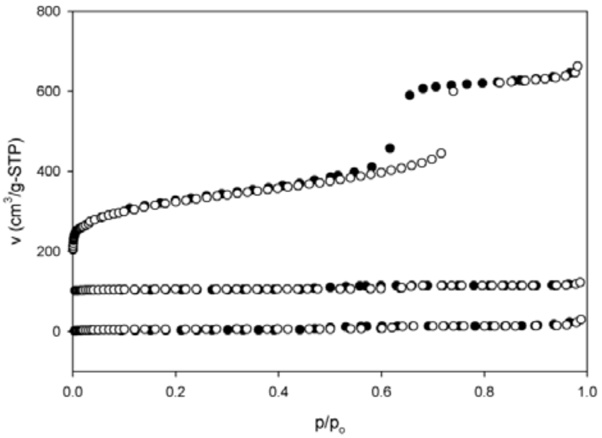


Figure 3S. Nitrogen adsorption isotherms of **X**, **PK-X-5**, and **PK-X-10** (from top to bottom)

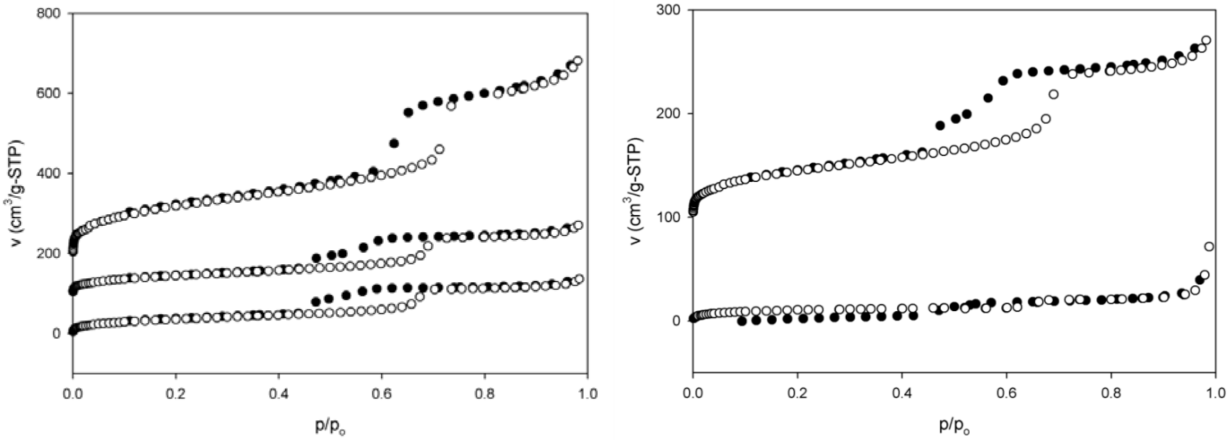


Figure 4S. (Left) Nitrogen adsorption isotherms of **X**, **PK-I-5**, and **PK-I-10** (from top to bottom); (Right) Nitrogen adsorption isotherms of the neutral **PK-I-10** (top) and protonated **PK-I-10 H** (bottom)

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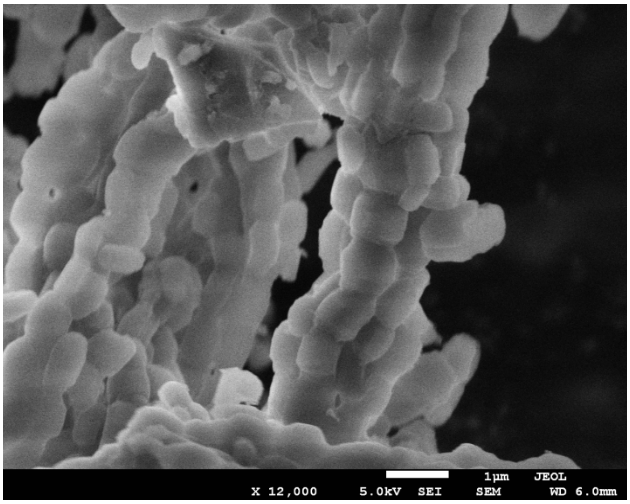

Figure 5S. SEM image of Organosilane Functionalized SBA-15 without polymer showing ~0.5μm elementary particles.

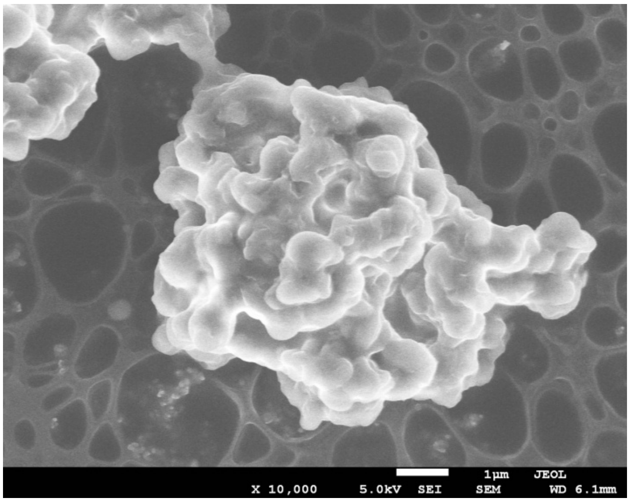

Figure 6S. SEM image of **PZK-I-10**.

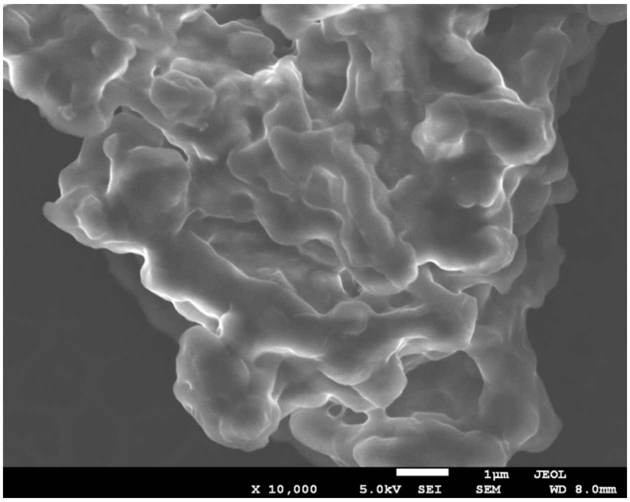

Figure 7S. SEM image of **PZK-X-10**.

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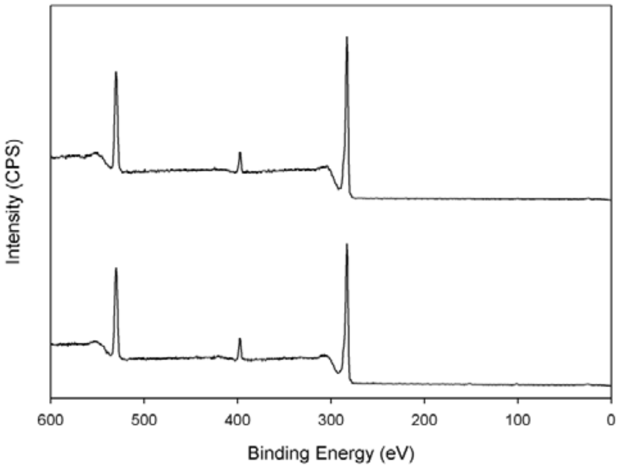

Figure 8S. XPS spectra of **PZK-X-10** (top) and **PZK-X-5** (bottom).

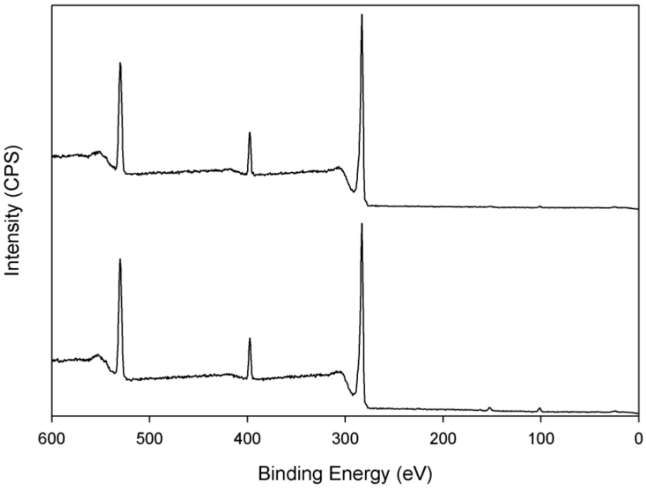

Figure 9S. XPS spectra of **PZK-I-10** (top) and **PZK-I-5** (bottom).

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C. Silane Extraction Control Experiment

A control experiment was performed in order to ascertain whether or not the Pluronic OMS template is extracted into the silane phase during the initial functionalization. As-made SBA-15 was functionalized with APTMS as described above, followed by filtering and rinsing in toluene (NH_2-SBA-15 as-made). TGA and adsorption were taken on this sample and compared with as-made and extracted SBA-15. Table 2S summarizes these results. Though it does appear that some Pluronic is extracted during functionalization, part of this may be due to Pluronic removal during the toluene rinsing step. Additionally, the overall porosity following functionalization does not increase significantly. The substanctial increase of pore volume and decrease in organic content of **X** relative to the NH_2-SBA-15 as-made sample , indicates there is still significant Pluronic remaining in the pre-extracted sample to promote selective functionalization. Removing the thiol contribution would further increase the porosity and organic content differences. However, as a result of some Pluronic extraction, it is likely that there is functionalization within the pore mouth and nor purely on the external surface.

Table 2S Adsorption and TGA data of as-made and extracted samples

Sample	V_p [a] [cm^3/g]	Organic [wt%]
SBA-15 as-made	0.24	47.8
SBA-15 extracted	0.90	9.4
NH_2-SBA-15 as-made	0.35	28.8
NH_2(X)-SH(I)-SBA-15[b]	0.66	19.6

[a] Recorded at $p/p_0 = 0.9$
[b] **X** from main article

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