

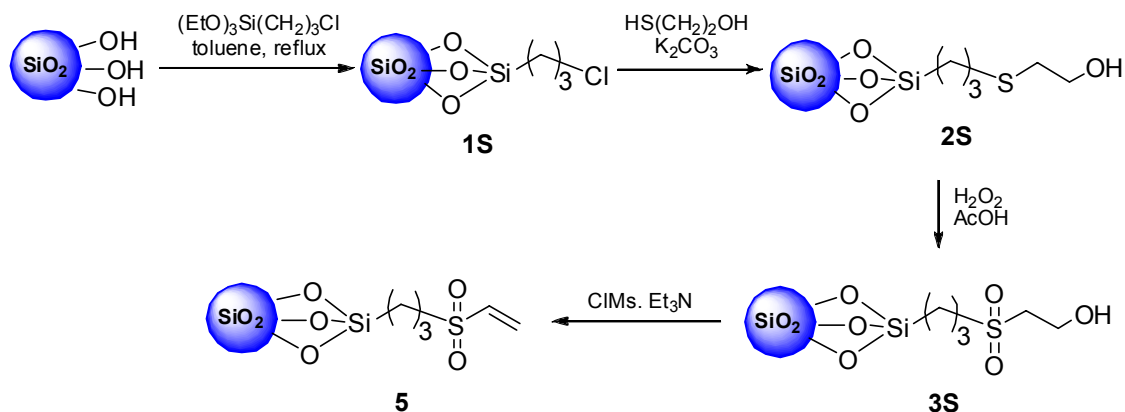
Supplementary Information

Vinyl sulfone functionalized silica: a “ready to use” pre-activated material for immobilization of biomolecules

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Synthesis of vinyl sulfone silica **5**



Preparation of chloro functionalized silica 1S. Activated silica gel (10 g) was suspended in dried toluene (50 mL) and then (3-chloropropyl)triethoxysilane (3.5 mL) was added. The magnetically stirred reaction mixture was heated under reflux for 2 h. Partial evaporation of the solvent to remove the formed ethanol was followed by reflux for an additional hour. The reaction mixture was filtered, and the white powder washed with toluene (2 x 30 mL) and acetone (2 x 30 mL), and dried under vacuum (1 mm Hg) at 50 °C for 16 h giving the chloro functionalized silica **1S** (11.1 g).

Preparation of mercaptoethanol functionalized silica 2S. Chloro functionalized silica **1S** (5.0 g) was suspended in acetonitrile (40 mL) and then 2-mercaptoethanol (3.5 mL) and potassium carbonate (6.9 g) were added. The magnetically stirred suspension was heated at 50 °C under an Ar atmosphere for 8 h. The reaction mixture was filtered and the white powder washed with hot water (2 x 20 mL) and finally with acetone (2 x 20 mL) and dried under vacuum (1mmHg) at 50°C for 16 h giving the mercaptoethanol functionalized silica **2S** (4.2 g).

Preparation of ethanol sulfone functionalized silica 3S. Mercaptoethanol functionalized silica **2S** (3.0 g) was suspended in acetic acid (6.0 mL) and then hydrogen peroxide 33% (15.0 mL) was added. The magnetically stirred reaction mixture was kept at room temperature for 24 h in the absence of light. After filtration, the white powder was washed with water (2 x 20 mL), methanol (2 x 20 mL) and finally acetone (2 x 20 mL), and dried under vacuum (1 mmHg) at 50 °C for 16 h giving the ethanol sulfone functionalized silica **3S** (2.9 g)

Preparation of vinyl sulfone functionalized silica 5. Ethanol sulfone functionalized silica **3S** (2.5 g) was suspended in anhydrous dichloromethane (40 mL). The magnetically stirred suspension was cooled at 0 °C by means of an ice bath. Methanesulfonyl chloride (0.9 ml) and triethylamine (3.5 ml) were then added. After the addition of the reagents, the reaction mixture was kept at room temperature for 7 h and then filtered. The white powder was washed with methanol (2 x 20 mL) and acetone (2 x 20 mL), and dried under vacuum (1mm Hg) at 50 °C for 16 h giving the vinyl sulfone functionalized silica **5** (2.4 g).

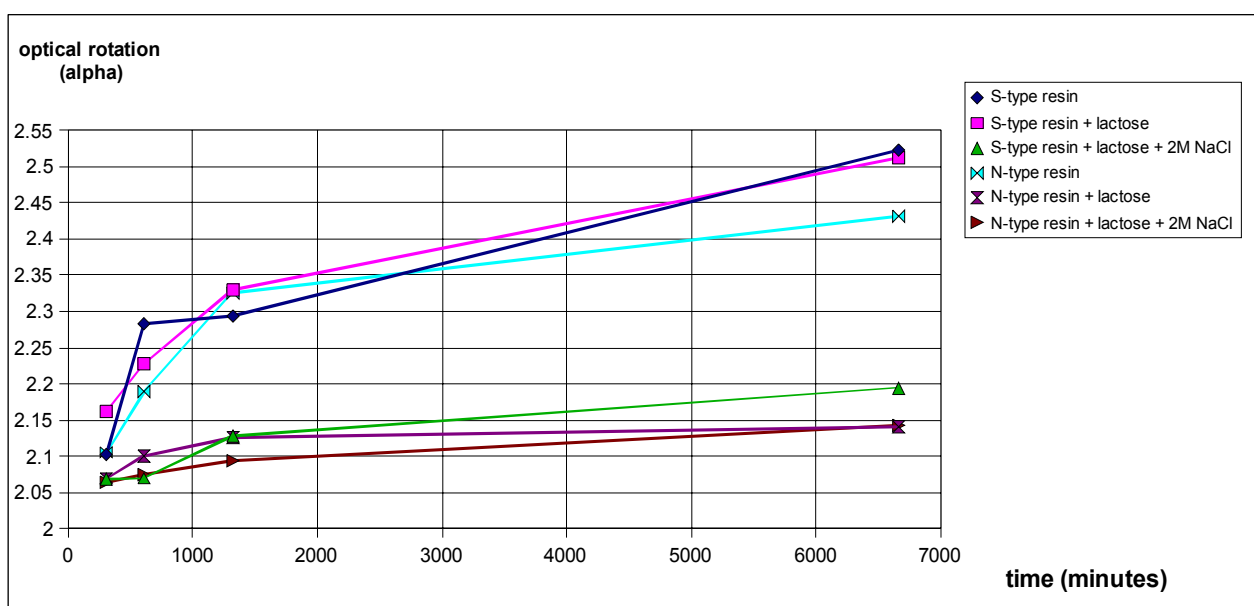


Figure 1S: Hydrolysis of lactose into glucose and galactose by lactase immobilized on N-type (3) and S-type (4) silicas with the active site non protected/protected and in presence/absence of ionic strength to minimize electrostatic interactions.

Proteins isolated by the PsTrxh2-vinyl sulfone silica 3 identified by mass spectrometry. Columns show the protein, source, accession code, probability-based score, protein score confidence interval, peptide count (mass fingerprint), identified peptides (mass/mass search) for those matches whose calculated molecular weight and estimated isoelectric point compatible with that from the 2D electrophoresis. Since the genome sequence of *Pisum sativum* is not available, one protein was identified by homology. Probability-based score is a parameter output by MASCOT (D. N. Perkins, D. J. C. Pappin, D. M. Creasy and J. S. Cottrell, *Electrophoresis* 1999, **20**, 3551-3567) to estimate the probability that the observed match is a chance event: the higher the score the lower the probability of a chance event. The confidence parameters (protein score confidence interval) describes the confidence of the identification: a C.I.% above 95% is typically considered significant.

Proteins eluted from the PsTrxh2-vinyl sulfone silica 3 conjugate by ionic strength

Protein	Source	Acc. Code	Score	C.I.%	Peptide count	Fragmentation
Superoxide dismutase (Mn)	<i>Pisum sativum</i>	P27084	204	100	8	HHQTYITNYNK, LVVETTANQDPLVTK
2-Cys peroxiredoxin	<i>Pisum sativum</i>	Q93X25	66	100	4	GLFIIDKEGVIQHSSTINNLGIGR
2-Cys peroxiredoxin-like (fragment)	<i>Hyacinthus orientalis</i>	Q676X3	63	99.4	3	SYGVLIPDQGIALR, SGGLGDLNYPLVSDVTK

Proteins eluted from the PsTrxh2-vinyl sulfone silica 3 conjugate by ionic strength and DTT

Protein	Source	Acc. Code	Score	C.I.%	Peptide count	Fragmentation
Superoxide dismutase (Mn)	<i>Pisum sativum</i>	P27084	232	100	8	HHQTYITNYNK, LVVETTANQDPLVTK
2-Cys peroxiredoxin	<i>Pisum sativum</i>	Q93X25	107	100	6	GLFIIDKEGVIQHSSTINNLGIGR
2-Cys peroxiredoxin-like (fragment)	<i>Hyacinthus orientalis</i>	Q676X3	212	100	5	SYGVLIPDQGIALR, SGGLGDLNYPLVSDVTK