

Supporting Information

Highly specific binding on antifouling zwitterionic polymer-coated microbeads as measured by flow cytometry

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TABLE OF CONTENTS

Additional XPS Spectra	S-3
FT-IR Spectra	S-10
Additional Flow Cytometry Data	S-11
HRP quantification assay	S-18
Additional Confocal Microscopy Images	S-20
References	S-20

ADDITIONAL XPS SPECTRA

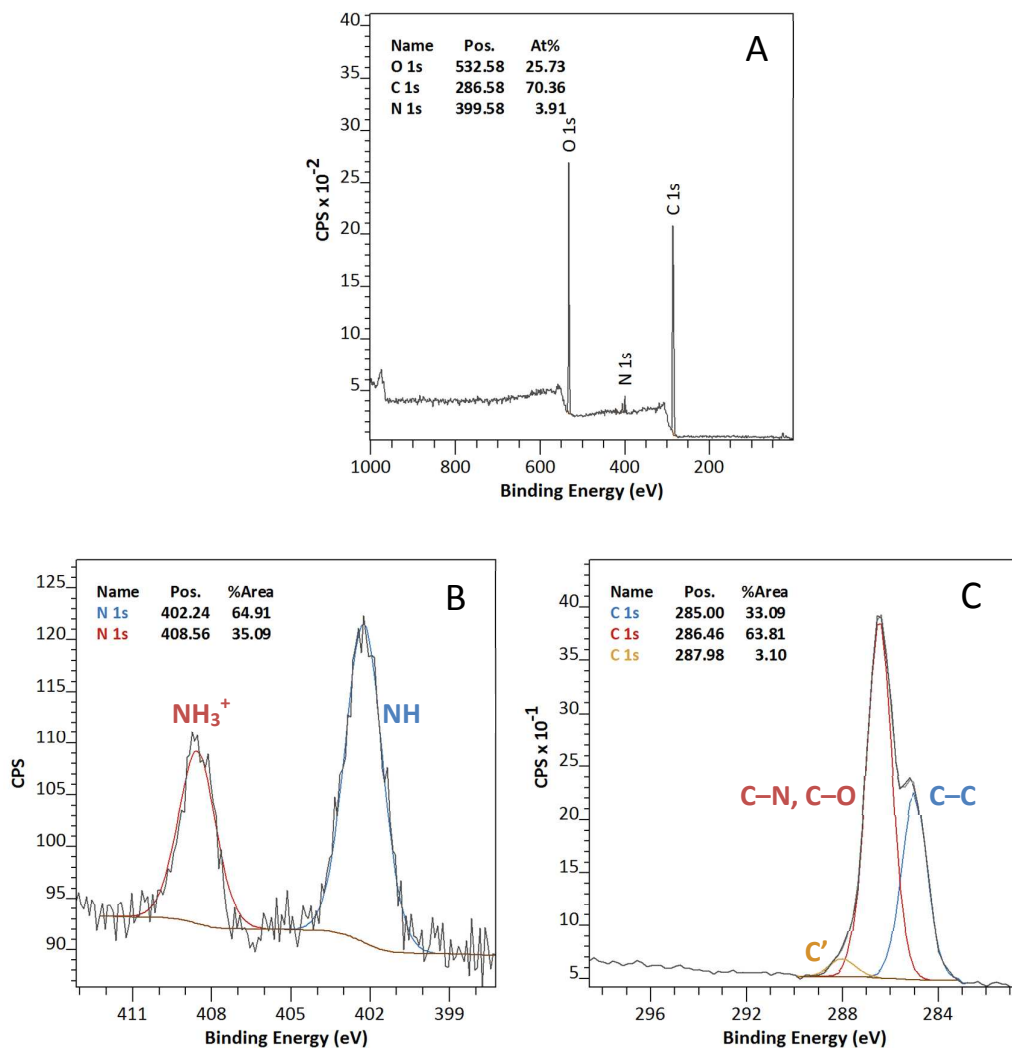


Figure S1. XPS spectra of non-modified Dynabeads: (A) wide-scan and narrow-scans of (B) N 1s and (C) C 1s. The peak labelled as C' is probably from a C–O or C–N species which originates from the bare Dynabead (as obtained from the supplier) and can also be found in beads modified with an ATRP bromo-initiator (Figure S2).

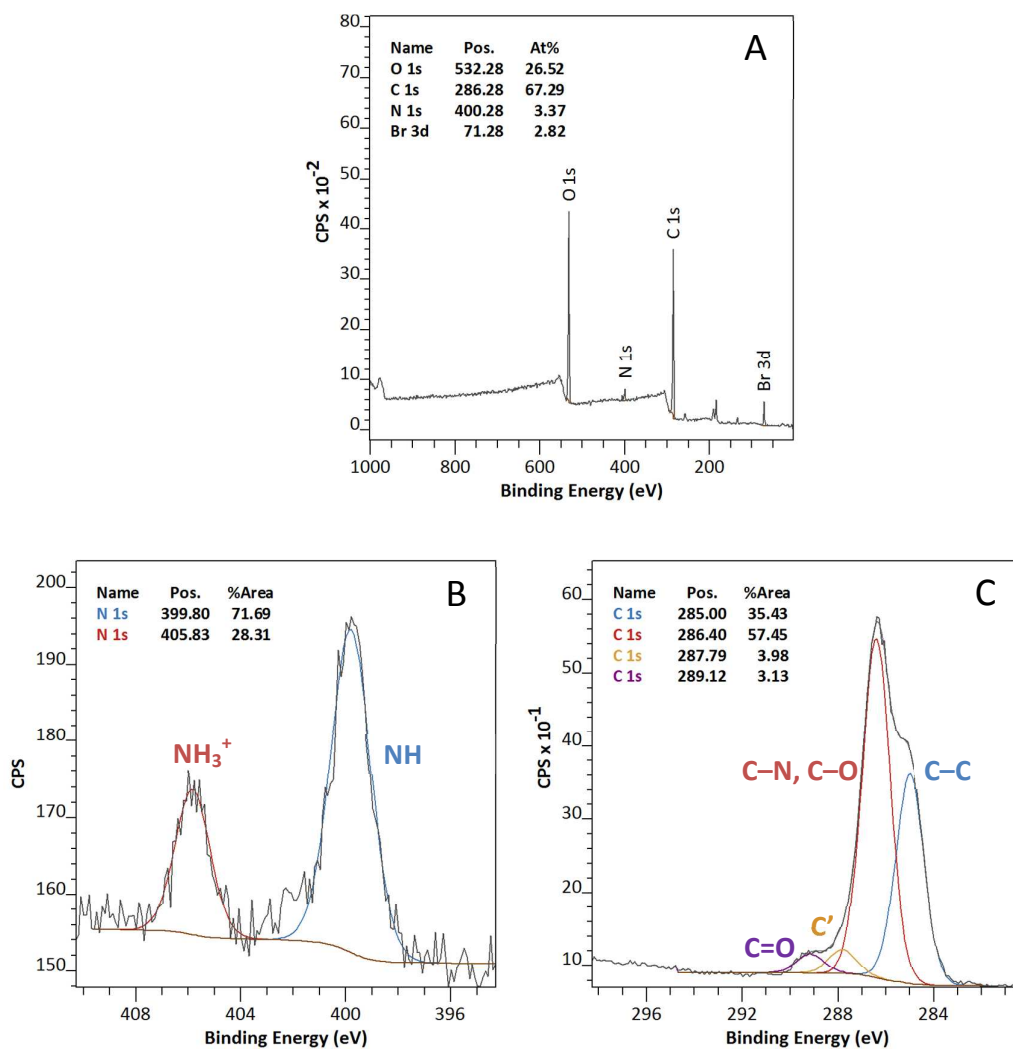


Figure S2. XPS spectra of Dynabeads functionalized with α -bromoisobutryl bromide: (A) wide-scan and narrow-scans of (B) N 1s and (C) C 1s. The peak labelled as C' originates from the bare Dynabead.

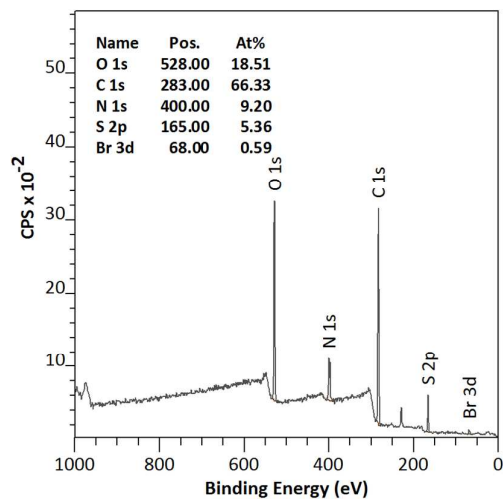


Figure S3. XPS wide-scan of pSB-coated beads.

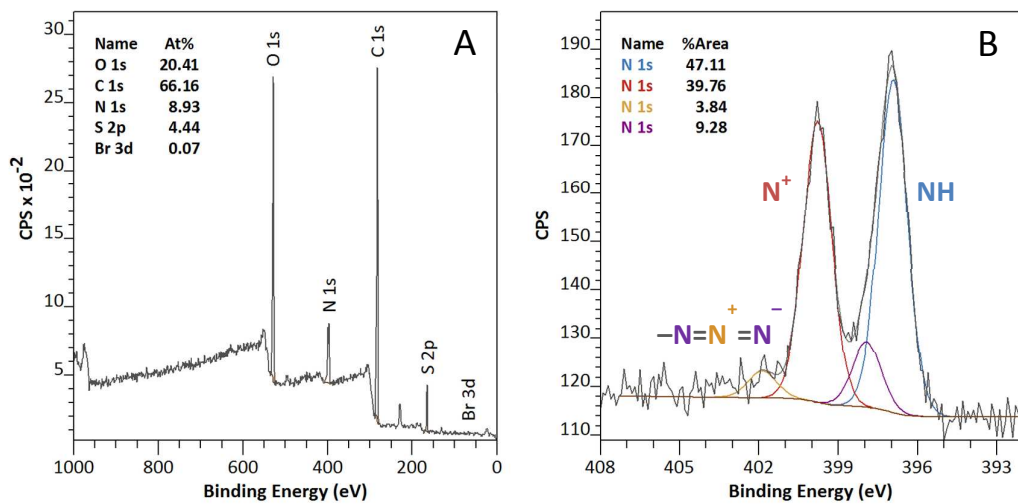


Figure S4. XPS spectra of pSB-azide beads: (A) wide-scan and (B) N 1s narrow-scan.

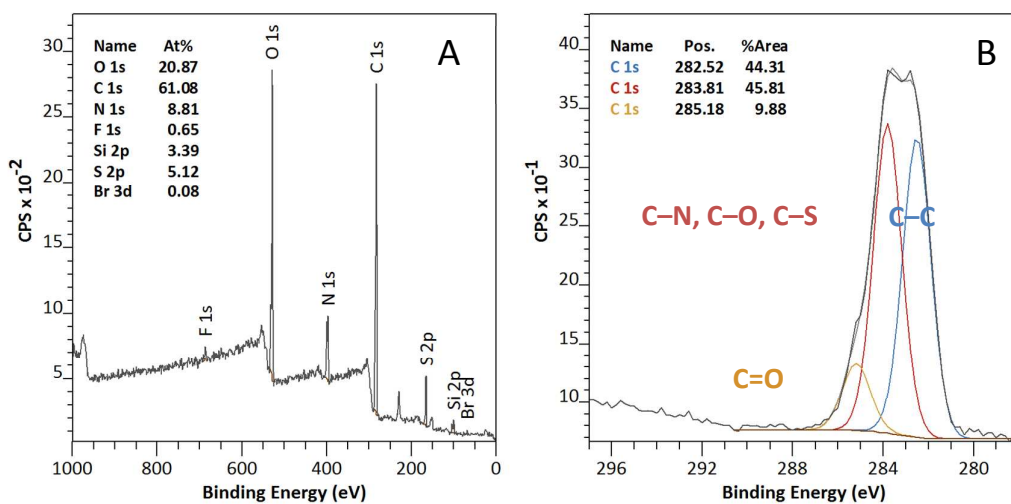


Figure S5. XPS spectra of *p*SB-F₇ beads, as obtained via the substitution reaction between heptafluorobutylamine and the terminal bromide of the *p*SB-coated beads: (A) wide-scan and (B) C 1s narrow-scan.

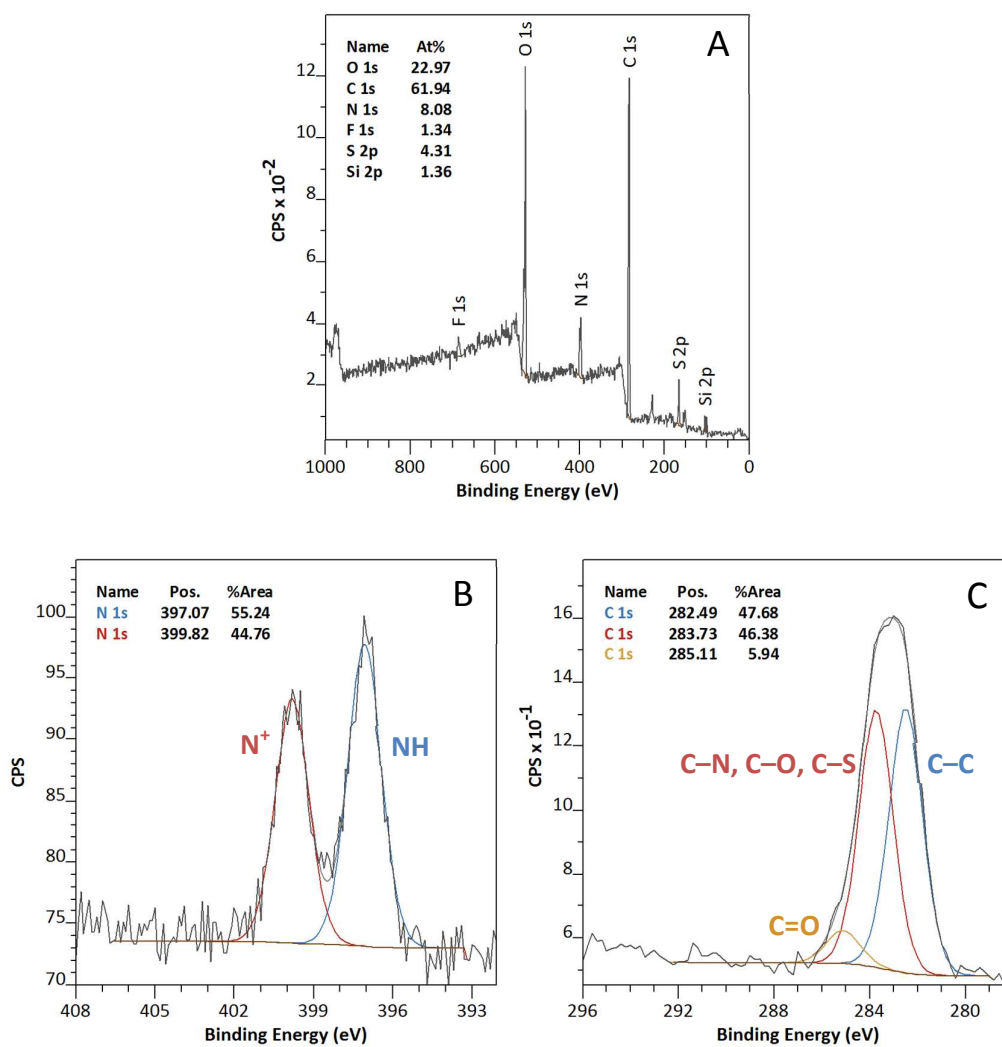


Figure S6. XPS spectra of *p*SB-CF₃ beads as obtained via a SPAAC reaction of BCN-CF₃ with *p*SB-azide beads: (A) wide-scan and narrow-scans of (B) N 1s and (C) C 1s.

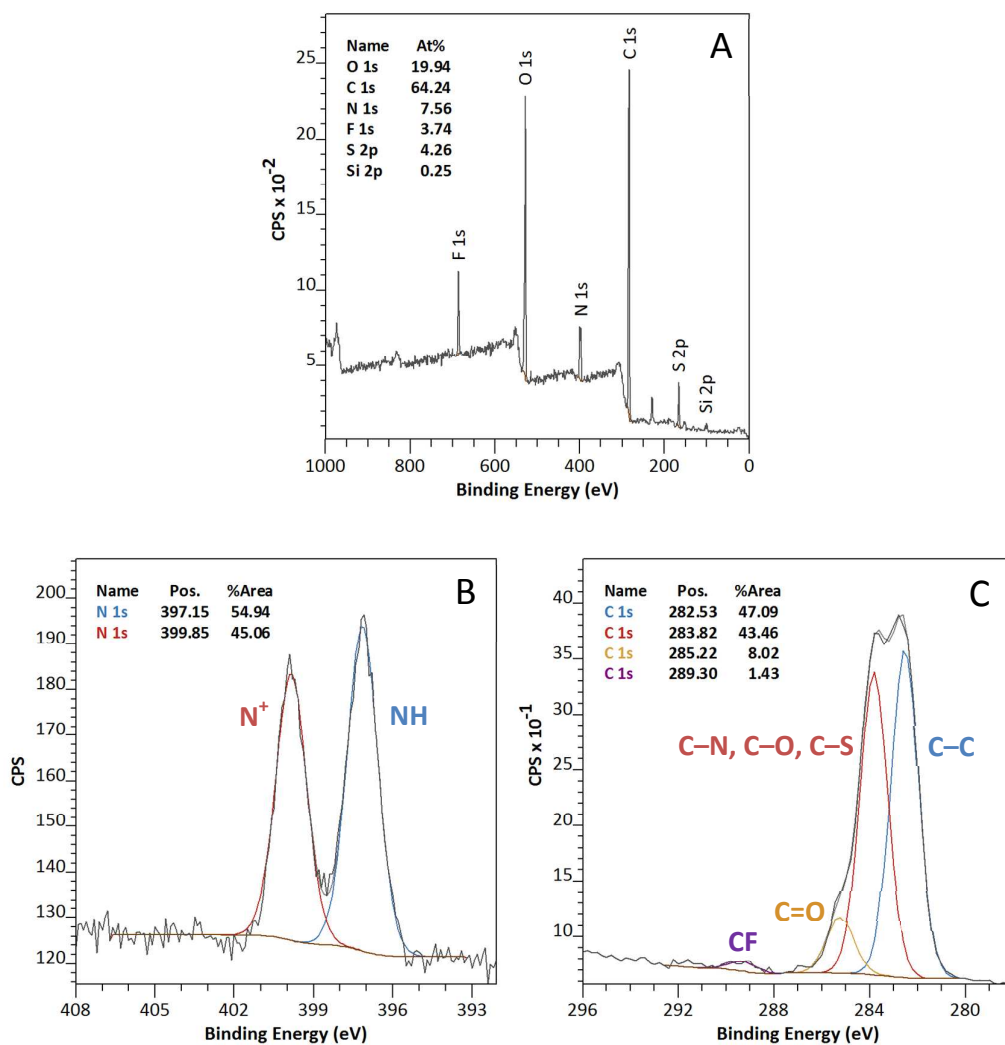


Figure S7. XPS spectra of *p*SB-F₉ beads as obtained via a CuAAC reaction of the F₉-alkyne with *p*SB-azide beads: (A) wide-scan and narrow-scans of (B) N 1s and (C) C 1s.

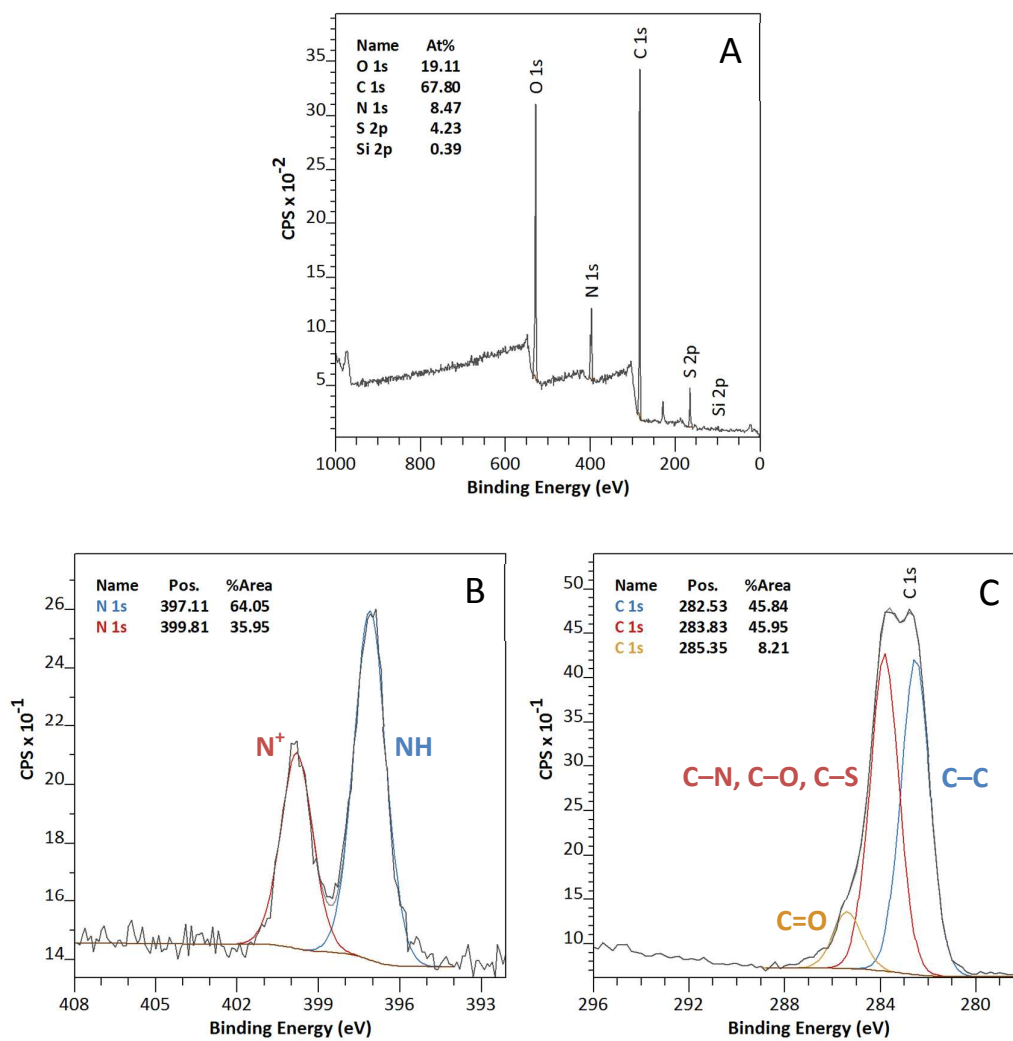


Figure S8. XPS spectra of *p*SB-biotin beads: (A) wide-scan and narrow-scans of (B) N 1s and (C) C 1s.

FT-IR SPECTRA

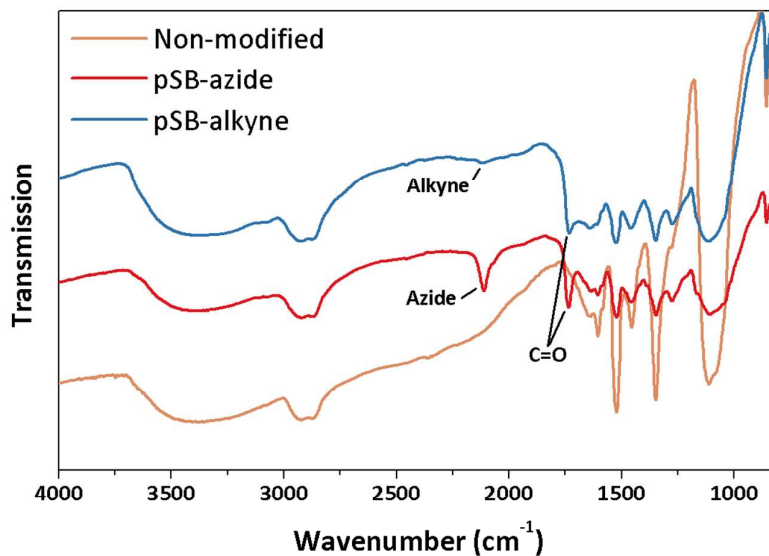


Figure S9. FT-IR spectra of non-modified, *pSB-azide* and *pSB-alkyne* beads as measured by a Bruker Hyperion 2000 IR-microscope.

Figure S9 shows a clear carbonyl peak at 1736 cm^{-1} for *pSB-alkyne* and *pSB-azide* coated beads that originates from the methacrylamide monomer, while for *pSB-azide* beads an additional azide peak at 2112 cm^{-1} is clearly visible. The alkyne absorption band of *pSB-alkyne* beads could not be convincingly observed (at 2119 cm^{-1} a very small peak could be seen), most likely due to the intrinsically weak signal of the $\text{C}\equiv\text{C}$ stretch vibration. Moreover, the $\text{C}\equiv\text{C}-\text{H}$ stretch vibration that should appear around $3330-3270\text{ cm}^{-1}$, overlaps with other signals and could therefore also not be visualized. However, subsequent successful CuAAC reactions on the *pSB-alkyne* beads, indicated successful instalment of the alkynes.

ADDITIONAL FLOW CYTOMETRY DATA

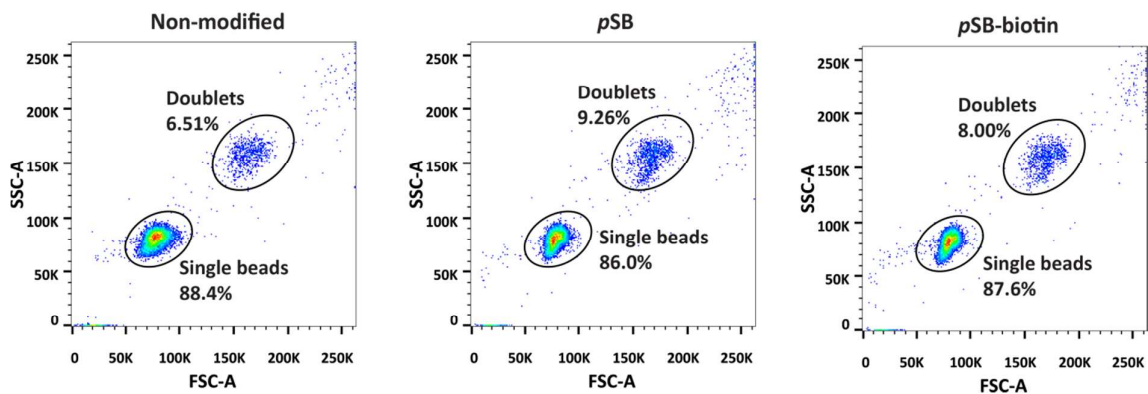


Figure S10. Representative gating on non-modified, *pSB* and *pSB*-biotin beads after being measured by flow cytometry. 10,000 single beads were measured per sample.

Figure S10 shows the dot plots of beads measured by flow cytometry, with on the *x*-axis the forward scatter (FSC) and on the *y*-axis the side scatter (SSC). The forward scatter is a measure for the size of the measured particle, while the side scatter is a measure for the granularity of the particle. Each dot represents one measuring event of a bead or a cluster of beads. A population of beads (beads with similar properties, found roughly within the same area of the dot plot) can be selected by setting the gate (represented here as black circles). For further reading about flow cytometry, the reader is referred to the excellent textbook by Shapiro.^{S1}

Figure S10 shows that more than 86% of the total events are single beads, demonstrating that the measured beads are well dispersed. The percentages of single beads were also very similar for non-modified beads, *pSB*-coated and *pSB*-biotin beads. For each measurement 10,000 single beads were measured, and only these single beads were used for further analysis, i.e. each histogram as shown below and in the main text represents single beads only.

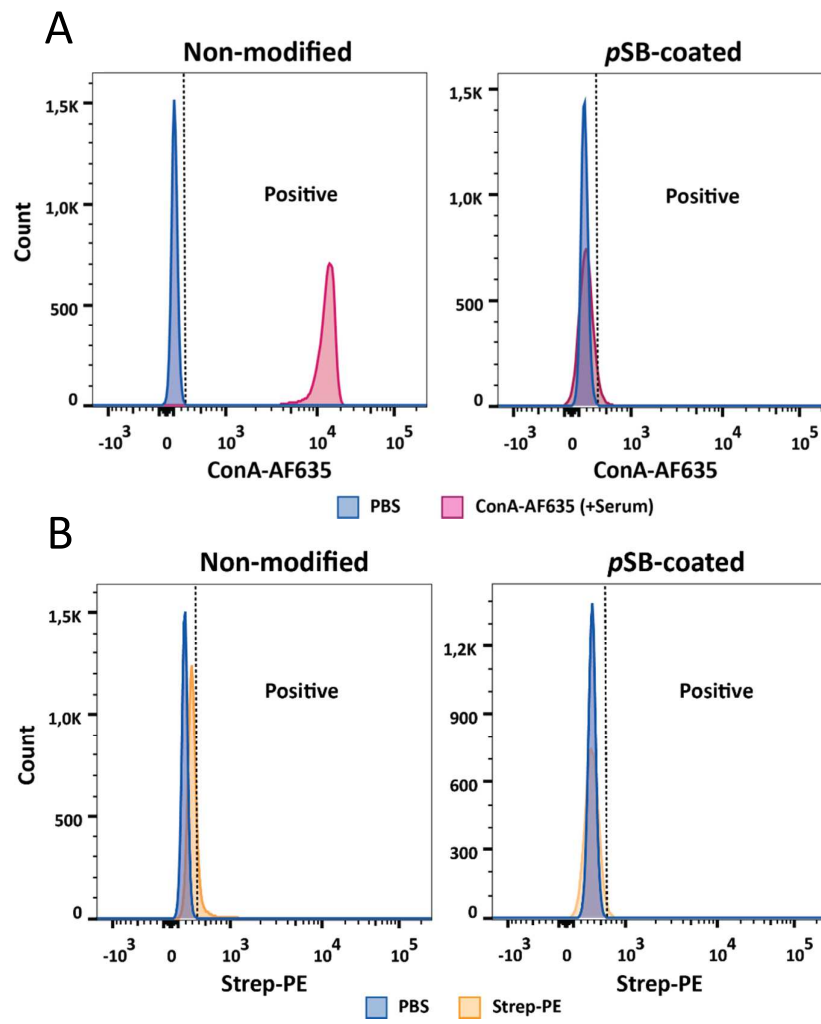


Figure S11. Representative flow cytometry data of non-modified and *pSB*-coated beads incubated with (A) PBS or ConA-AF635 and (B) PBS or Strep-PE.

Table S1. Representative flow cytometry data summarizing MFI values of non-modified and *p*SB-coated beads incubated with PBS, BSA-AF488 (0.5 mg/mL), Serum-HLF488 (10%, ~6 mg/mL), Strep-PE (50 μ g/mL) or ConA-AF635 (50 μ g/mL). Samples using the same gate are clustered.

	solutions	MFI BSA-AF488 Serum-HLF488	MFI Strep-PE ConA-AF635
Non-modified	PBS	455	
	BSA-AF488	34038	
	Serum-HLF488	2285	
	PBS		343
	Strep-PE		454
	ConA-AF635		14053
<i>p</i>SB	PBS	791	
	BSA-AF488	796	
	Serum-HLF488	1063	
	PBS		409
	Strep-PE		413
	ConA-AF635		450

Inherent to the used method, the MFI of different fluorescently labelled protein solutions cannot be directly compared with respect to the amount of bound protein. For example, there is a significant difference in MFI observed between BSA-AF488 (MFI =34038) and Serum-HLF488 proteins (MFI = 2285) and this can most likely be attributed to the different fluorescent properties of the used fluorophores (Alexa Fluor 488 is known to be a highly fluorescent dye) and/or by the degree of fluorophores per protein.

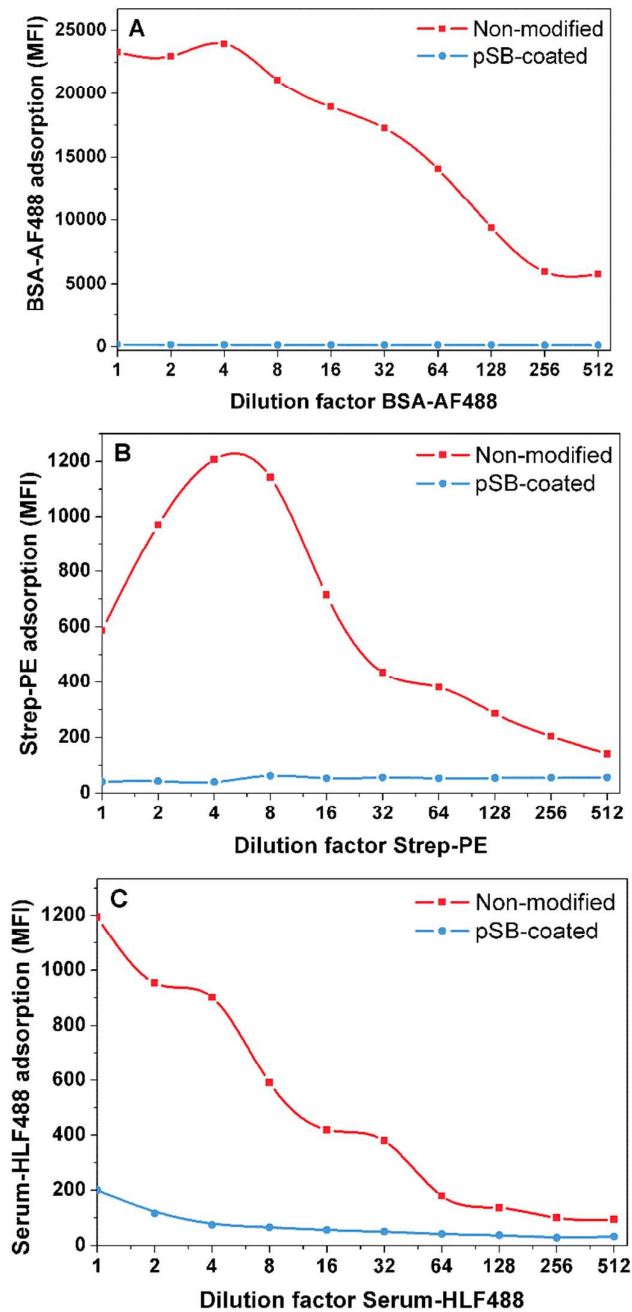


Figure S12. Flow cytometry data of *p*SB and non-modified beads, incubated with (A) BSA-AF488 (highest concentration: 1 mg/mL), (B) Strep-PE (highest concentration 100 μ g/mL) or (C) Serum-HLF488 (highest concentration: 10% serum, \sim 6 mg/mL).

Figure S12 shows the non-specific adsorption of fluorescently labelled proteins on non-modified and *p*SB-coated beads. Dilution series were prepared from the protein solutions, to each dilution the same amount of beads was added. The mean fluorescent intensity (MFI) of the beads with non-specifically bound proteins is plotted (*y*-axis) against the dilution factor of the protein solutions (*x*-axis, ²log scale). Incubating the beads in single-protein solutions (BSA-AF488 plot A, and Strep-PE plot B) resulted in evident protein adsorption on non-modified beads (in a concentration-dependent manner) while no distinct adsorption could be detected on *p*SB-coated beads. A direct comparison between the amount of adsorbed proteins on non-modified versus *p*SB beads is therefore not feasible. However, it does show the excellent antifouling performance of the *p*SB coating. Interestingly, Strep-PE shows an optimum in adsorption on non-modified beads around a concentration of 12.5-25 µg/mL whereas no such optimum was observed for BSA-AF488 or Serum-HLF488.

*p*SB-coated beads that are incubated with Serum-HLF488 (Figure S12, plot C) perform still clearly better than non-modified beads, however, a small but significant amount of non-specifically bound serum proteins is also seen on *p*SB beads. Across the four highest measured concentrations (dilution factor 1, 2, 4 and 8, respectively), non-modified beads show a 6-10 times higher MFI, and hence serum adsorption, than *p*SB beads. *p*SB beads perform thus 6-10 times better in serum than non-modified beads under the measured conditions. Another way of evaluating the data is to look at the dilution factor of the protein solution that is needed to obtain the same amount of fouling on non-modified beads as found on *p*SB beads at the highest concentration measured. In this case, Serum-HLF488 has to be approximately 64 times diluted to obtain a similar amount of fouling on *p*SB-coated beads that were incubated with the highest measured concentration.

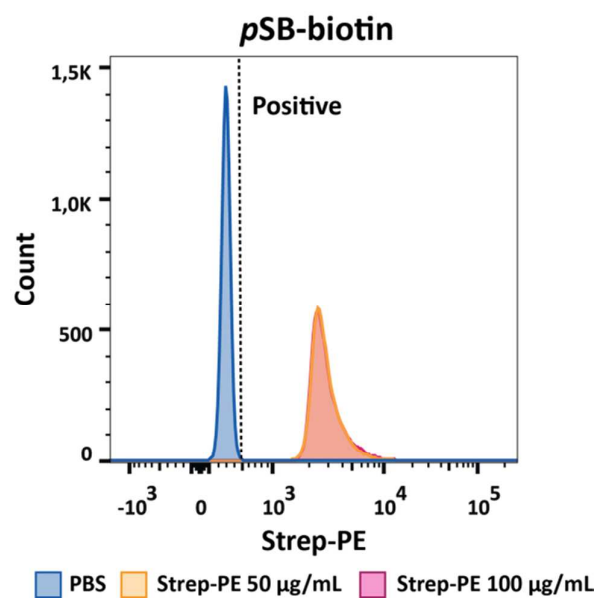


Figure S13. Flow cytometry data of *pSB-biotin* beads incubated with either 50 µg/mL or 100 µg/mL Strep-PE. The orange and magenta peaks completely overlap, demonstrating that there is no difference in the amount of Strep-PE binding, despite the difference in concentration.

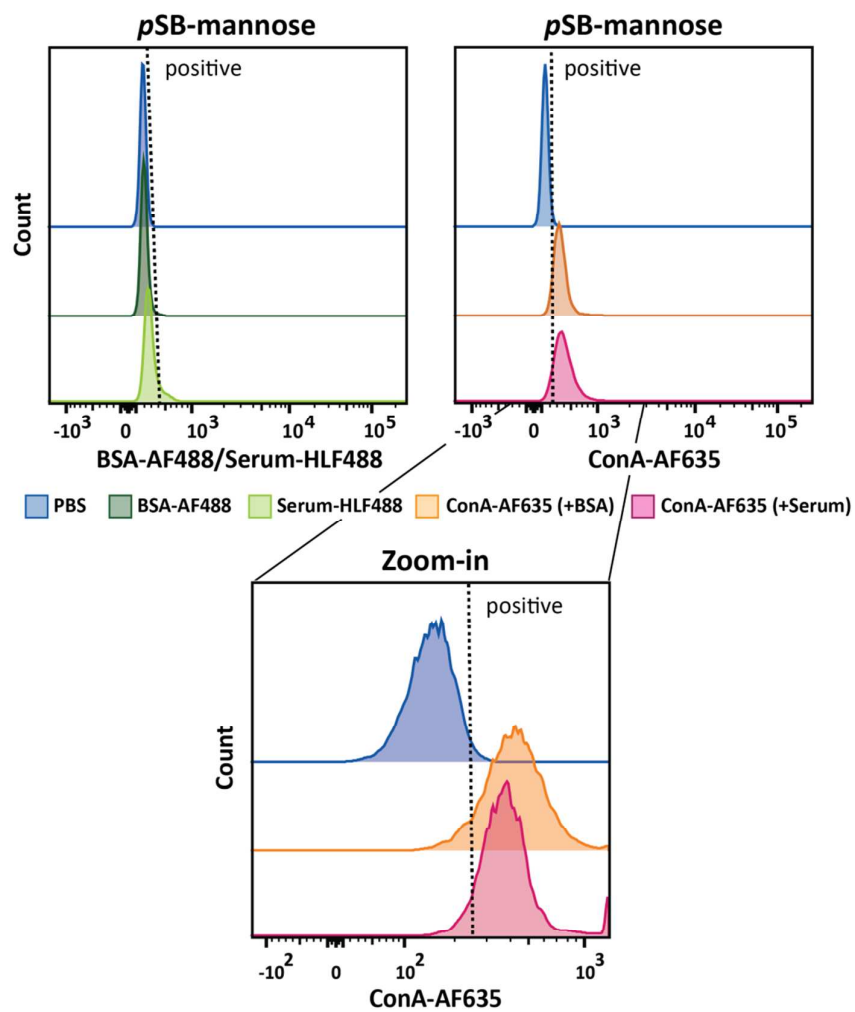


Figure S14. Representative flow cytometry data of *pSB-mannose* beads incubated with PBS, a mixture of ConA-AF635 (50 $\mu\text{g}/\text{mL}$) with either BSA-AF488 (0.5 mg/mL) or Serum-HLF488 (10%, $\sim 6 \text{ mg}/\text{mL}$).

HRP QUANTIFICATION ASSAY

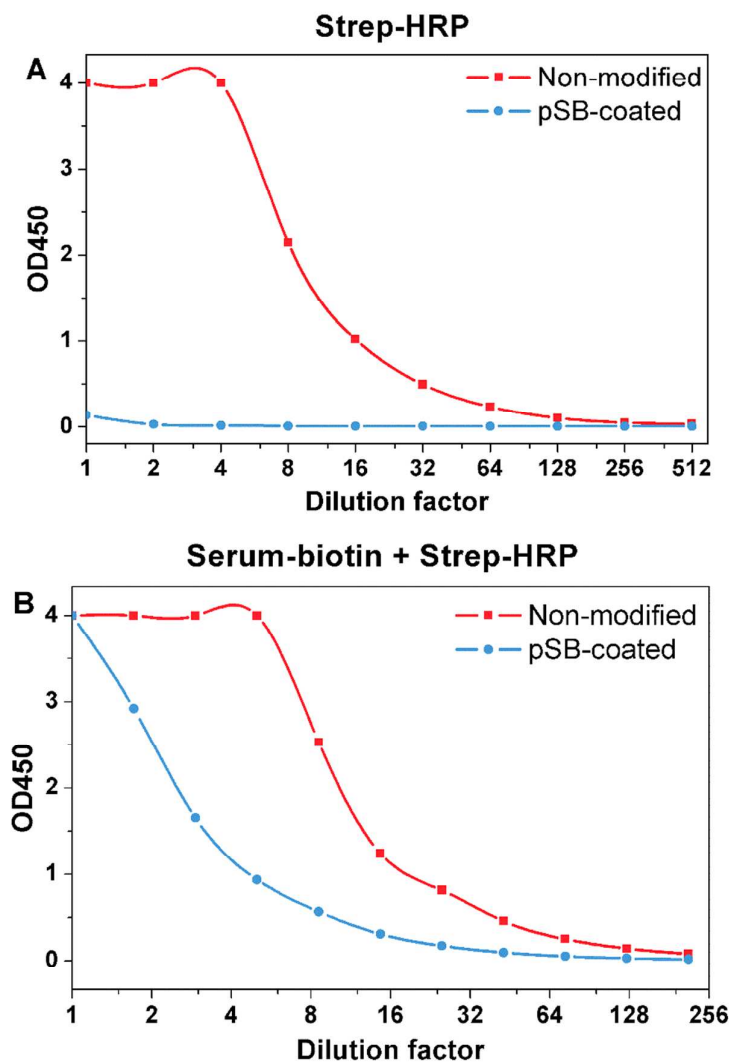


Figure S15. ELISA-like quantification assay on non-modified beads and *p*SB-coated beads, incubated with (A) Strep-HRP (1:100 dilution) and (B) with 10% biotinylated serum followed by incubation with Strep-HRP. 10×10^6 beads were incubated with protein solutions, serial dilutions were prepared from these samples before TMB (substrate for HRP) was added and the OD450 (optical density/absorbance) was determined.

In order to quantify the amount of fouling on the beads via a method independent of a fluorescence readout, protein quantifications were obtained based on a horseradish peroxidase (HRP) ELISA-like assay. Non-modified and *p*SB-coated beads were incubated with either Strep-HRP, or first with biotinylated Strep-HRP followed by Strep-HRP. The amount of directly or indirectly (via biotinylated serum proteins) bound HRP onto the beads can be spectrophotometrically quantified by measuring the conversion, facilitated by the HRP enzyme, of a colorless substrate (3,3',5,5'-tetramethylbenzidine, TMB) into its colored product. The enzymatic reaction can be stopped by adding a 4% HCl dilution; note that it is crucial to have the same incubation time for every sample to ensure a fair comparison between them.

Within this quantification experiment (Figure S15), 10 million non-modified and *p*SB-coated beads were incubated with a HRP containing solution, followed by extensive washing. Serial dilutions were made of the obtained beads, to these dilutions the TMB substrate was added which initiated the enzymatic reaction. In this way, titration curves could be generated that covered the most sensitive range of the assay; which was set at half of the maximum measured optical density (OD₄₅₀, absorbance at 450 nm). *p*SB beads that were incubated with only Strep-HRP, showed such low absorbance that it could not be reliably compared to non-modified beads that were incubated with Strep-HRP. When incubated with the more fouling biotinylated serum followed by Strep-PE, some fouling was seen on *p*SB beads and thus a direct comparison could be made with the non-modified beads. A factor 5-6 more *p*SB beads were needed to obtain a similar signal as with non-modified beads. This indicates that under these conditions, the *p*SB-coated beads contain 5-6 times less serum proteins than the non-modified beads. The quantification by ELISA corresponds therefore well with the MFI ratio around 6 as obtained by flow cytometry for the same serum dilution (10%), illustrating that flow cytometry can be used to evaluate fouling among different type of samples. It should be noted though, that a one to one comparison between the ELISA-like results and flow cytometry data (Figure S12) cannot be made as with flow cytometry fluorescently labelled serum is used while with the ELISA-like test the beads were first incubated with biotinylated serum, followed by an additional Strep-HRP incubation.

ADDITIONAL CONFOCAL MICROSCOPY IMAGES

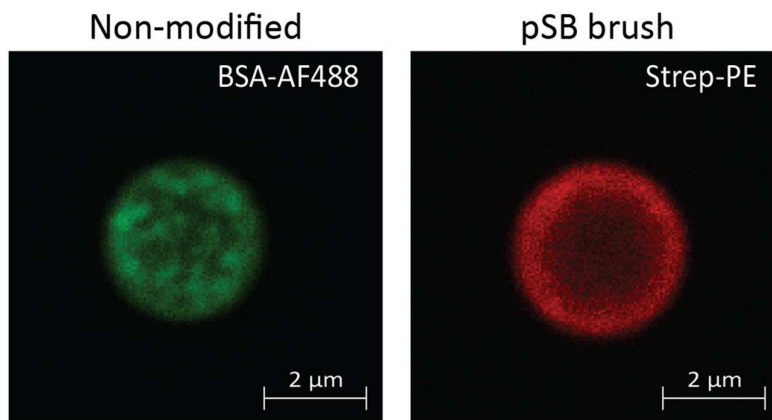


Figure S16. Confocal images of non-modified beads incubated with BSA-AF488 and *p*SB-biotin beads incubated with Strep-PE, both images were taken in such a way that the whole bead is visualized rather than a cross-section of the beads.

Figure S14 shows the patchy non-specific adsorption of BSA-AF488 proteins on non-modified beads and the homogeneous binding of Strep-PE to *p*SB-biotin beads.

REFERENCES

- S1. Shapiro, H. M., *Practical Flow Cytometry* 4th ed.; WILEY-LISS: Hoboken, New Jersey, 2003.