Rebuttal Letter

We thank the editors and reviewers for the detailed comments. Point-by-point responses to the comments are listed below. We have also revised the manuscript to reflect these changes.

Editorial Comments

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.
Response: We have thoroughly proofread the manuscript.

2. Please spell out each abbreviation the first time it is used.
Response: We have checked the manuscript to spell out all abbreviations the first time they appeared.

3. Please remove commercial language: Bioruptor.
Response: We removed Bioruptor (line 336).

4. Please add more details to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. Please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. Some examples:
1.1.2: What is the pore size of the filter?
Response: Please see line 119 "Filter the warm solution through filter paper (5-8 μm particle retention)".

1.2.3: Please how to confirm completion of the reaction with TLC.
Response: We decided to remove this line because in this step checking reaction progress via TLC is unnecessary, and it is also not used in the original publication (Ref 25, Chong et al., Macromolecules, 36 (7), 2256-2272, (2003)).

1.2.7: Please mention how to perform column chromatography and NMR. For instance, mention sample preparation, NMR solvent, parameters used, etc.
Response: Please see line 153 "1.2.7 Purify the product by column chromatography using 400 mL of silica gel (pore size 60 Å, 63-200 mesh particle size) and petroleum ether as eluent, yielding 5 g of benzyl dithiobenzoate (BDB) as red oil. Confirm product purity by ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02-7.99 (2H, m), 7.55-7.50 (1H, m), 7.41-7.29 (7H, m), 4.60 (2H, s)."

1.3.6: Please mention how to determine polymer molecular weight using GPC. Alternatively, references may be provided.
Response: Please see line 180 "1.3.7 To measure polymer molecular weight, gel permeation chromatography (GPC) is used. The GPC system uses THF as the mobile phase at 35 °C with a 1 mL/min flow rate, and the calibration curve is constructed using monodisperse polystyrene standards. To acquire GPC measurement, dissolve polymer in THF (1-2 mg/mL) and filter through 0.2 μm disposable polytetrafluoroethylene (PTFE) filter. Inject 100 μL of sample into the GPC instrument. Convert the measured sample retention time to molecular weight using the polystyrene calibration curve."

2.1.4, 2.1.5, 2.2.4: What volume of DMSO is used?
Response: The exact volume of DMSO used for each step has been added.

2.1.5: Please describe how DLS and XPS analyses are done.
Response: Please see line 210. The following paragraphs have been added.

"2.1.5 To check particle size distribution, DLS analysis is performed. Take one drop of the suspension prepared in step 2.1.4 and place into a disposable UV cuvette. Dilute the sample by fill the cuvette with fresh DMSO until it is 2/3 full. Insert the sample into the cell holder to begin data acquisition. For particle size measurement, the following setup parameters are used: Temperature: 25 °C; Equilibration Time: 120 s; Measurement Duration: Automatic.

2.1.6 To check surface composition, XPS analysis is performed. Dry a small sample from the suspension prepared in step 2.1.4 in vacuum oven at 40 °C overnight. Take the dried polymer and pack evenly onto a 0.5 cm x 0.5 cm sample holder. Load the sample into the high vacuum chamber (10^{-8} torr) and begin data acquisition. For the particular XPS instrument used, photoelectrons are generated using a monochromatic Al Kα X-ray operated at 15 kV and 6.7 mA, and they are collected using hybrid mode magnification with the analyzer at a 50 eV pass energy for high resolution spectra, and a 100 eV pass energy for elemental surveys."

5. 1.3.1, 1.3.3, 1.3.5, 1.3.6, 2.1.5, 2.2.5, 2.2.6, etc.: Please write the text in the imperative tense.
Response: The text has been changed to imperative tense.

6. After you have made all the recommended changes to your protocol (listed above), please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol.
7. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense.
8. Please include all relevant details that are required to perform the step in the highlighting. For example: If step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be highlighted.
Response to comments 6-7: Sections for filming have been indicated with highlight.

9. Figure 1: Please label structures a and b. Please include a space between the number and the time unit (i.e., 5 h, 1 h, etc.).
Response: Figure 1 has been updated to reflect the recommended changes.

10. Table 1: Please define PDI.
Response: Per reviewer #3's comment, Table 1 has been deleted, and its contents have been replaced by Figure 2. The phase PDI has been defined in the legend of Figure 2.

11. Discussion: As we are a methods journal, please also discuss critical steps within the protocol, any modifications and troubleshooting of the technique, and any limitations of the technique.
Response: Per editor's suggestion, following paragraphs have been added to the Discussion section.

Line 442: "Note when protein recovery efficiencies of different bead systems are compared,

the IP experiments as well as the subsequent western blotting analyses should be done simultaneously. Due to the inherent variations in performing these experiments, data obtained on separate trials should not be directly compared."

Line 464: "While surface hydrophilicity influences the amount of antibody attachment to the poly(PFPA) brushes, it also has a significant effect on IP background due to non-specific enrichment. In a typical IP experiment, many washing steps are performed to remove unbound proteins. When the beads are very hydrophobic, such as the ones with 0 % PEG substitution, they tend to form large aggregates that are difficult to break apart. In this case, non-specific proteins can be trapped inside the aggregate structures, and washing cannot sufficiently remove them, leading to an increase in background. Therefore, when performing IP, it is important to optimize the bead surface property, and attention should be paid to ensure the beads are reasonably dispersed."

12. References: Please do not abbreviate journal titles.
Response: Reference section has been updated to show full journal titles.

Reviewer #1 Comments:
Major Concerns: none.

Minor Concerns:
1. The polymer concentration of poly(PFPA) for the SiO_2 attachment appear rather high and I wonder if the authors observed partial cross-linking of the solution. A comment on the choice of suitable concentrations would be helpful to the reader.
Response: We thank the reviewer for the comment. The following paragraph has been added

Line 229: "Note: In this study, a relatively low molecular weight poly(PFPA) (20 kg/mol) is used. Thus despite the high polymer concentration (10 mg/mL), no evidence of polymer crosslinking is observed. If a higher molecular weight polymer is used, then polymer solution concentration may need to be adjusted to avoid possible crosslinking."

2. Citation of PFPA literature is rather selective. Considering the enormous utilization in polymer science, the seminal work of the group of Theato in this area could be better stated.
Response: We added six new references (Ref 10, 12, 13, 14, 15, 16) to better reflect the works done by the Theato group in the area of poly(PFPA) synthesis and utilization.

Reviewer #2 Comments:
Major Concerns:
Protocol section 1.2 is vague in its description of the reactor setup. What is the reactor? A multi-neck round bottom with septa seems likely but should be described. How are things added; by syringe through septa, poured, etc?

Response: We thank the reviewer for the valuable comment. Section 1.2 has been rewritten to better describe the reaction setup.

"1.2.1 Prepare a 500 mL three-neck round-bottom flask equipped with magnetic stir bar, refluxing condenser, dropping funnel, and rubber septum. Connect the flask to nitrogen gas line through the refluxing condenser and flush out the inside air with nitrogen. Insert a

thermometer through the septum. Add 41 mL (0.041 mol) of 1 M solution of phenylmagnesium bromide in tetrahydrofuran (THF) via a syringe through the same septum.

1.2.2 Warm the phenylmagnesium bromide solution to 40 °C in an oil bath. Then add 3.1 g (0.041 mol) of carbon disulfide through the dropping funnel slowly, maintaining the solution temperature at 40 °C.

1.2.3 Add 7.1 g (0.042 mol) of benzyl bromide to the resultant mixture through the dropping funnel over 15 min. Increase the reaction temperature to 50 °C. Continue stirring at this temperature for 45 min.

1.2.4 Transfer the reaction mixture into a separatory funnel, and dilute with 15 mL of ice cold water. Extract the product by adding 15 mL of diethyl ether, and remove the lower water layer. Repeat the extraction with diethyl ether two more times.

1.2.5 Wash the combined organic phases with copious amount of water, then brine (solution of 50 % (w/v) NaCl in water), and dry the product over anhydrous magnesium sulfate.

1.2.6 Remove the solvent in vacuum at 40 °C using a rotary evaporator.

1.2.7 Purify the product by column chromatography using 400 mL of silica gel (pore size 60 Å, 63-200 mesh particle size) and petroleum ether as eluent, yielding 5 g of benzyl dithiobenzoate (BDB) as red oil. Confirm product purity by ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02-7.99 (2H, m), 7.55-7.50 (1H, m), 7.41-7.29 (7H, m), 4.60 (2H, s)."

Minor Concerns:
While mostly well written there are numerous issues with plurals, tense and article placement within the text. Many instances of 'poly(PFPA) brush' should read 'brushes' and 'antibody' 'antibodies'. The symbol for degrees changes in protocol #4. Protocol 1.2.7 (line 149) should list the 1H NMR spectroscopy chemical shifts used to confirm the product purity. Given values in % have inconsistent spacing (i.e X% vs X %) and do not always specify volume or mass or mole. For instance line 267 gives '0.1% (v/v)' while lines 492, 495, and 302 do not specify. These should be made more consistent. The XPS peak labeling in Figure 2 caption (line 370) should contain spaces to be consistent with the text (line 404); i.e. 'O 1s'. Line 268 reads ' Tween (PBST)' and should specify the Tween used; i.e. 'Tween-20 (PBST)'
Response: We have carefully proofread the manuscript and corrected all the mistakes and inconsistencies pointed out by the reviewer.

Reviewer #3 Comments:
Major Concerns: I do not have major concerns about this manuscript.

Minor Concerns:
1. Line 71-72 mentions "specially synthesized SI-RAFT chain transfer agent." The SI-CTA in the citation (reference 7) was actually previously synthesized, and was not made 'specially' for that particular work.
Response: We thank the reviewer for providing this information. The words "specially synthesized" have been removed.

2. Line 72: "silica-silane reaction" seems to more accurately describe the reaction than "silane reaction"
Response: Again, we thank the reviewer for the valuable comment. We changed “silane reaction” to “silica-silane reaction”.

3. Line 399: "diameter" here seems to be referring to "hydrodynamic diameter" as measured through DLS.
Response: The word “diameter” has been changed to “hydrodynamic diameter”.

4. Table 1: There seems to be a large size change from APTES-SiO2 to poly(PFPA)-SiO2. I wonder if this is from the formation of covalent aggregates induced by poly(PFPA) treatment (a single strand of poly(PFPA) may link multiple APTES-SiO2 beads). It would be nice to have the actual DLS profile of each of the three samples mentioned in the table. I do recognize that the formation of covalently-bound aggregates will not negatively influence the procedure described in the manuscript. However, having actual DLS curves will help users by providing a more visual comparison.
Response: Per reviewer’s suggestion, we replaced Table 1 with Figure 2, and the DLS curves for the differently functionalized beads are shown. For the poly(PFPA) functionalized beads, although the DLS curve only shows one nano-sized peak, the sample PDI is large (PDI=0.76). We suspect that there are indeed large aggregates in the suspension, but the amount may be small. We added the following explanation in the Discussion section.

Line 420 “It is important to point out that the polydispersity index (PDI) for the poly(PFPA) grafted beads is rather large (PDI = 0.76), which is indicative of poor quality sample containing large aggregates. Although the DLS curve only shows one nano-sized peak, small amount of aggregates may be present in the suspension.”