Supporting Information

Molecular Imprinting of Maltose Binding Protein: Tuning Protein Recognition at the Molecular Level

Maya Zayats,[1] Manu Kanwar,[2] Marc Ostermeier,[2,3] and Peter C. Searson[1,3]

[1] Department of Materials Science and Engineering, Johns Hopkins University, Baltimore, Maryland 21218

[2]Department of Chemical and Biomolecular Engineering, Johns Hopkins University, Baltimore, Maryland 21218

[3] Institute for NanoBioTechnology, Johns Hopkins University, Baltimore, Maryland 21218

MBP Expression and Purification. MBP was produced from a modified version of the plasmid pMAL-c5X (NEB). pMAL-c5X typically is used for cytoplasmic expression of fusion proteins between a protein of interest and MBP separated by a protease factor Xa site. In addition, the version of MBP encoded by this plasmid contains three amino acid substitutions that improve MBP binding to an amylose column. We removed the fragment between the BglII and EagI sites of pMAL-c5X (containing the three mutations and the protease factor Xa site) and replaced it with the analogous fragment from the wildtype *malE* gene with an intact stop codon. The *malE* gene encoding maltose binding protein (MBP) was PCR-amplified from the vector pDIMC8-MBP[28] using primers that contained appropriately-placed BglII and EagI . The PCR product was digested with BglII and EagI. The vector pMAL-c5X was digested using the same enzymes and the larger DNA fragment was gel purified. The gel-purified fragment was ligated with the digested PCR product using T4 DNA ligase (NEB) to create pMAL-MBP. The construct was confirmed by DNA sequencing. Vector pMAL-MBP allows high cytoplasmic expression of wildtype MBP under an isopropyl β-D-1-thiogalactopyranosid (IPTG) inducible *tac* promoter.

A single colony of K12 TB1 *E.coli* (NEB) harboring pMAL-MBP was used to inoculate 10 mL of Lysogeny broth (LB) containing glucose (1% final) and ampicillin (100 μg mL^{-1}). The inoculated tube was incubated overnight at 37 ˚C. LB (1 L) was inoculated with 2 % overnight culture, glucose (1 %) and ampicillin (100 μg mL^{-1}) in a 2 L beveled flask and the culture was incubated at 37 ˚C and 200 rpm. The cells were allowed to grow until the optical density at 600 nm reached 0.6. MBP expression was induced by the addition of IPTG to 0.3 mM and the culture shaken at 25 ˚C for another 8 hours. The cells were pelleted by centrifugation (20 minutes at 4500xg) and resuspended in 25 mL TBS_{150} (20 mM Tris-HCl,

150 mM NaCl, pH 7.0) buffer containing 50 µL of protease inhibitor cocktail (Sigma). The cells were lysed using French Press (20,000 psi) and the lysate collected on ice. From this point onwards the samples were kept at 4 ˚C. The cell lysate was clarified by centrifugation (3 times for 20 minutes each at 14000x g) and the supernatant was recovered. The supernatant was filtered using 0.45 μm filters (Corning) and the filtered supernatant passed over a TBS_{150} equilibrated amylose resin (NEB) gravity column. The protein-loaded column was washed with TBS_{150} and MBP eluted with 10 mM maltose (Sigma) in TBS_{150}. The elution fraction was confirmed to contain MBP by western-blot using anti-MBP antibody (Chemicon International). The MBP (41 kD) elution fraction was dialyzed at 4 ˚C against 300 volumes of 50 mM HEPES buffer (pH 7.0) for two hours and then twice against 1000 volumes of the same buffer for 12 hours each using 3.5 kD dialysis cassette (Pierce). The dialyzed protein was judged by Coomassie staining of SDS-PAGE gel (Invitrogen) to be > 95 % pure. The yield was approximately 70 mg L^{-1} culture volume, and the protein was stored in aliquots at -20 ˚C. The protein concentration was obtained from the molar extinction coefficient at 280 nm, which was determined to be 64,720 M^{-1} cm^{-1}.

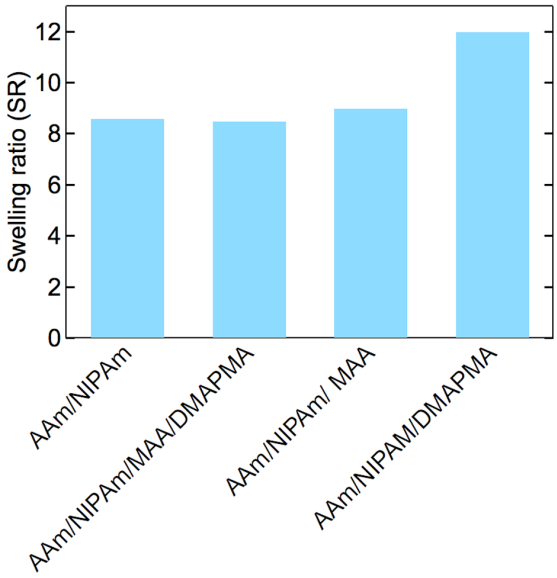

Figure S1. Degree of hydration of acrylamide-based non-imprinted hydrogels. Histogram showing swelling ratios of: AAm/NIPAm (49%:49%), AAm/NIPAm/DMAPMA/MAA hydrogel (48%:48%:1%:1%), AAm/NIPAm/MAA hydrogel (48.5%:48.5%:1%) and AAm/NIPAm/DMAPMA hydrogel (48.5%:48.5%:1%).