Characterization of Protein Interactions with Molecularly Imprinted Hydrogels that Possess Engineered Affinity for High Isoelectric Point Biomarkers

John R. Clegg[1,3], Justin X. Zhong[2], Afshan S. Irani[1], Joann Gu[2], and Nicholas A. Peppas[1,2,3,4,5].
[1]Department of Biomedical Engineering, [2]McKetta Department of Chemical Engineering, [3]Institute for Biomaterials, Drug Delivery, and Regenerative Medicine, [4]College of Pharmacy, [5]Dell Medical School. The University of Texas at Austin.

Statement of Purpose: Recognitive polymer networks, synthesized by molecular imprinting, offer an environmentally-robust, cost-efficient alternative to antibodies for applications in biosensing and drug delivery. During molecular imprinting, nanocavities are formed with complementary supramolecular structure to molecular templates. One major pitfall of molecular imprinting strategies, however, is the necessity for template-destructive purification techniques to render void nanocavities. Consequently, despite many years of promising research, the majority of molecular imprinting studies are conducted on a few model templates[1]. To overcome the cost-limitation of disease-relevant protein biomarkers, we have synthesized molecularly imprinted polymers (MIPs) with inexpensive "analogue" protein templates which possess similar size, shape, and isoelectric point. We establish that imprinting increases the polymer's affinity for physically similar, non-template proteins.

Methods: Hydrogel microparticles containing poly(caprolactone) nanoparticles[2] were synthesized under dilute condition (45 mM) by free radical polymerization in the absence (NIP) or presence of trypsin (TMIP), lysozyme (LMIP), or cytochrome c (CMIP). The incorporation of functional monomers was validated by potentiometric titration and FTIR, while size, morphology, and effective surface charge were assessed with TEM, DLS, and zeta potential measurement. MIPs and NIPs were incubated with various proteins (Table 1), in non-competitive environments to quantify concentration-dependent adsorption. Subsequently, MIPs and NIPs were incubated simultaneously with lysozyme, cytochrome c, and trypsin to characterize competitive adsorption behavior. Protein concentrations were quantified throughout by direct absorption or a MicroBCA colorimetric assay.

Results: The synthesized poly(methacrylic acid-co-(diethylamino)ethyl methacrylate – co - acrylamide imprinted and non-imprinted networks possessed a z-average hydrodynamic diameter of 2.82±0.31 μm, were highly polydisperse (PDI=0.71±0.05) and were anionic (ζ=-45.7±8.0**).** In non-competitive adsorption assays at initial protein concentrations less than 5 times the polymer concentration (by mass), both MIPs and NIPs exhibited the greatest adsorption capacity for lysozyme, and significant but lesser adsorption capacities for trypsin, cytochrome c, and hemoglobin. Negligible quantities of BSA and trypsin inhibitor, relative to other tested proteins, adsorbed to all formulations at all initial concentrations **(Fig 1a)**. Molecular imprinting increased the polymer's adsorption capacity for trypsin, lysozyme, cytochrome c, and hemoglobin at initial protein concentrations less than twice the concentration of polymer (by mass) irrespective of the imprinting template identity. As the protein concentration was elevated to quantities greater than double the polymer concentration, imprinting either continued to improve the polymer's adsorption capacity (cytochrome c, hemoglobin), or provided negligible benefit (lysozyme, trypsin). Imprinted polymers recognized cytochrome c and lysozyme in competitive solutions containing up to 160 times excess (by mass) of competitive protein (albumin, gamma globulin, and fibrinogen). When incubated with trypsin, lysozyme, and cytochrome c simultaneously TMIPs, LMIPs and CMIPs bound significantly more cytochrome c than NIPs, and LMIPs and TMIPs adsorbed significantly more lysozyme **(Fig 1b)**. No formulation, imprinted or non-imprinted, bound detectable quantities of trypsin in the competitive condition.

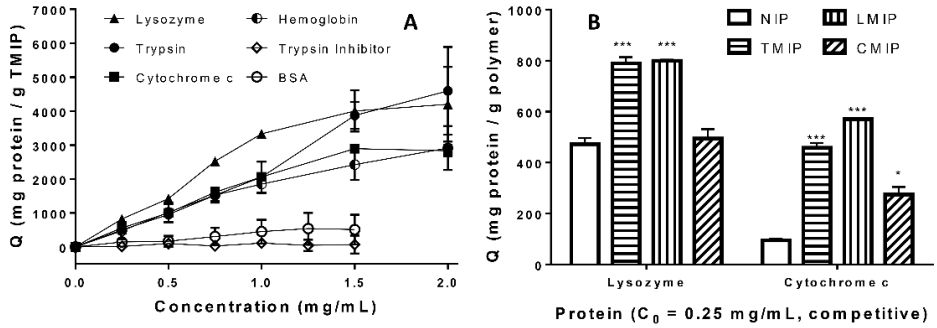

Figure 1: *(a) Non-competitive protein adsorption to TMIPs. (b) Competitive adsorption following incubation of MIPs or NIPs with lysozyme, cytochrome c, and trypsin. (n=3)(*p<.05, ***p<.001, vs. NIPs, 2-way ANOVA).*

Conclusions: Molecular imprinting increased the microparticles' affinity for lysozyme, trypsin, cytochrome c, and hemoglobin irrespective of the imprinting template. Polymer formulation, relative to protein physiochemical properties, had the greatest impact on adsorption capacity. Increased affinity and adsorption capacity for lysozyme and cytochrome c were imparted by molecular imprinting of trypsin, lysozyme, or cytochrome c, with most pronounced benefit arising in dilute protein solutions. These findings reveal that it is viable to synthesize recognitive MIPs with analogue templates that impart affinity for a desired protein without using expensive biomarker templates during polymer fabrication.

Protein	Lysozyme	Trypsin	Cytochrome c	Hemoglobin	Trypsin Inhibitor	BSA
MW (kDa)	14.3	24.0	12.4	60.2	28.0	66.5
pI	11.35	10.1	10.5	6.8	4.9	4.7

Table 1: *Physiochemical properties of proteins in adsorption studies.*

References: [1] Kryscio DR, Peppas NA. *Acta Biomateriala* 8.2 (2012) 461-473. [2] Culver HR, Steichen SD, Herrera-Alonso M, Peppas NA. *Langmir* (2016) 32: 5629-5636.