Tailorable Recognitive Biomaterials Synthesized via Free Radical and Living/Controlled Polymerization

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Statement of Purpose: This work highlights the rational design of recognitive polymer networks via polymerization reaction and characterization analysis. Recognitive networks, through non-covalent complexation interactions between "guest" molecules and functional monomers, have "macromolecular memory" resulting in networks that are highly selective to the template molecule due to the particular chemistry and orientation of the binding site.

Structural parameters such as the crosslinking agent size, crosslinking monomer incorporation, and the initiation scheme of acrylate and methacrylate-based templated systems were varied to study the effect on the template binding capacity, selectivity, affinity, diffusional transport, and network formation. Increasing the tailorability of recognitive biomaterials will yield promising materials for robust, inexpensive, future point-of- care diagnostic devices.

Methods: Methacrylic acid (MAA), chloroform, azobisisobutyronitrile (AIBN), ethylene glycol dimethacrylate (EGDMA) and the template molecules (Ethyladenine-9-acetate, EA9A, and testosterone) were purchased from Sigma Aldrich (Milwaukee, WI). Polyethylene glycol 200 dimethacrylate (PEG200DMA) was purchased from Polysciences Inc. (Warrington, PA). All chemicals were analytical grade and used as received except for MAA which had inhibitor removed by vacuum distillation prior to use. UV free-radical photo-polymerization produced each polymeric system at 52 mW/cm^2 for 17 minutes at 0^{o}C. Equilibrium rebinding and selectivity studies were conducted by introducing a known amount of dry polymer into various concentrations of the matching template solution. Absorbance reading of the equilibrium solution was performed via UV-Vis spectrophotometry. Permeation studies were conducted using side-by-side diffusional cells (Permegear, Bethlehem, PA) and taking samples every 15 minutes until equilibrium was reached.

Results and Discussion:

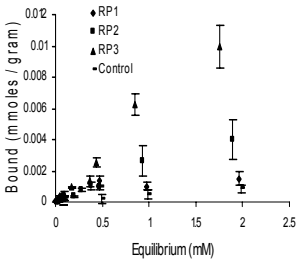

Figure 1: Binding Characteristics of Poly(MAA-co-EGDMA) Recognitive Networks for Ethyladenine (EA9A): Equilibrium Binding Isotherm. RP1 is the literature match recognitive polymer [1], RP2 has the same formulation composition except it contains 2.4 wt % initiator which demonstrated an increased double bond conversion of 48%, RP3 is recognitive polymer with iniferter, which leads to a significant increase in binding capacity. With RP2 a 37% increase in conversion above that of the original literature match (RP1) leads to a modest increase in the capacity of the recognitive polymer.

Table 1. Binding Characteristics for EA9A Recognitive Polymers

Polymer Network	Binding Affinity K_{avg} (mM^{-1})	Capacity, # Sites (µmole/g)
RP1	3.12+/-0.21	776+/-54
RP2	2.63+/-0.17	862+/-60
RP3	2.61+/-0.12	1421+/-64

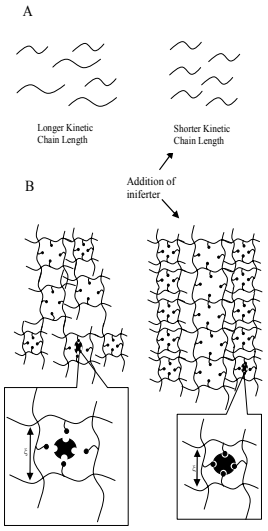

Figure 2: Controlled/Living Polymerization and the Effect on Imprinted Network Structure. **A**. In mono-vinyl polymerization, the use of iniferter yields a lower polydispersity of kinetic chains and decreased average chain length. **B.** Within crosslinked networks, addition of iniferter leads to a more uniform and higher population of appropriately sized imprinted macromolecular cavities for the template. An optimal mesh size, ξ, gives the binding site a better functional configuration which leads to enhanced binding properties (See Table 1).

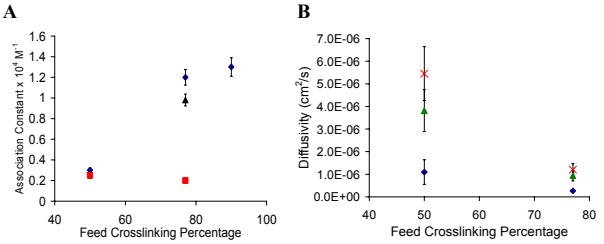

Figure 3: Testosterone Recognitive Poly (MAA-co-PEGnDMA) Networks: Affinity versus Diffusivity. A. Testosterone Binding Affinity Constants Versus Feed Crosslinking Percentage where (♦) represents EGDMA crosslinked polymer, (■) PEG(200)DMA crosslinked polymer and (▲) EGDMA crosslinked recognitive polymer binding progesterone. The higher the feed crosslinking percentage, the higher the association constant due to the increase formation and stability of binding sites during polymerization. **B. Testosterone Diffusivity for Varying Crosslinking Percentages and Crosslinking Agent**. (x) PEG200DMA control network, (▲)PEG200DMA recognitive network, and (♦) EGDMA recognitive network. As the length of the crosslinking agent is increased, the mesh size of the network increases allowing faster penetration of biomolecule. At higher crosslinking percentage, the polymer's architecture is more rigid with a decreased mesh size, limiting the rate of biomolecule diffusion.

Conclusions: Rational design, reaction analysis, and characterization of recognitive polymers has the potential to yield a greater understanding of the imprinting mechanism and associated binding parameters as related to the structural architecture. In this work, living polymerization techniques were used to produce molecularly imprinted biomaterials with a significant increase (63% increase) in binding capacity while retaining equivalent affinity for the template molecule. Also, structural considerations and rational biomaterials design can produce networks with favorable template diffusivity while maintaining adequate affinity. Additional work in this area will lead to optimized macromolecular structures for diagnostic systems.

[1] Umpleby II RJ. Macromolecules 2001; 34:8446-8452