

New Biomimetic Materials for Enzyme Inhibition and Recognition Obtained by Molecular Imprinting

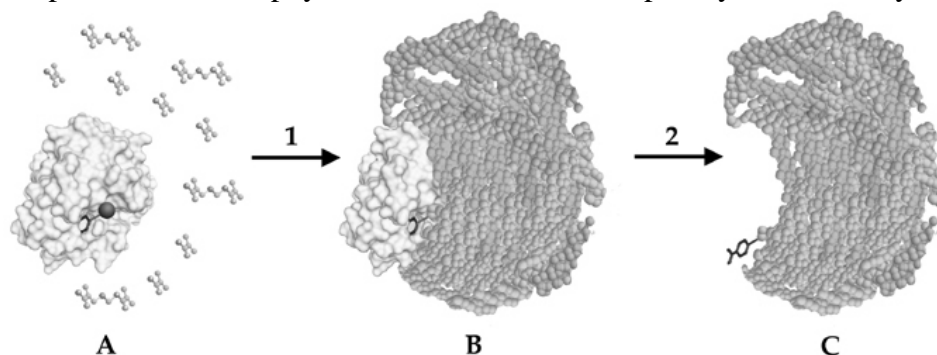
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Development of enzyme inhibitors with high specificity, selectivity and affinity is still a challenging endeavor. Multivalent inhibiting compounds (dendrimer-type) or modified polymeric carriers bearing inhibitor functionalities (chitosan or poly(acrylate) supports) have proven to be efficient approaches [1]. In this work, we describe enzyme inhibition and recognition properties of polymer nanogels obtained by molecular imprinting [2].

This technique allows the creation of specific recognition sites in synthetic polymers. These sites are tailor-made *in situ* by copolymerization of a cross-linker and functional monomers around the target molecule. Low molecular weight templates have been shown to yield high affinity and specificity molecularly imprinted polymers (MIPs). However, proteins are more difficult to imprint due to their physical size, functional complexity and tendency to denature.



Schematic presentation of enzyme molecular imprinting: The enzyme is put into contact with an anchoring monomer and co-monomers (A); polymerisation is conducted (1); a cross-linked polymer is molded around the substrate binding site in the form of a hydrosoluble polymer nanogel (B); the enzyme is extracted (2), revealing a specific recognition site with inhibitory properties (C).

Using trypsin as model target, we describe here a new strategy for the synthesis of nanosized hydrosoluble but cross-linked polymeric materials by direct molding on the enzyme's substrate binding site, thus generating a three-dimensional image of the protein surface. Polymerization is conducted with a specific inhibiting monomer carrying a benzamidine moiety, which provides a first anchoring point to the substrate binding site. The imprinted network is obtained by co-polymerization with acrylamide-type monomers.

After extraction of the target protein, the molecular imprinting effect of these resulting polymers was demonstrated by measuring trypsin activity after re-uptake of the enzyme. All imprinted networks exhibited a much higher recognition affinity towards the enzyme than non-imprinted control polymers (NIPs).

Inhibition constants K_i have been determined for MIPs and NIPs. A typical MIP showed stronger competitive inhibition ($K_i = 4.8 \pm 0.3 \mu\text{M}$) than the corresponding NIP ($K_i = 22.9 \pm 10.1 \mu\text{M}$) and free aminobenzamidine ($K_i = 18.9 \pm 1.2 \mu\text{M}$).

[1] Gestwicki, J.E. et al.. Influencing receptor-ligand binding mechanisms with multivalent ligand architecture J. Am. Chem. Soc. 2002, 124, 14922-14933

[2] Haupt, K. Imprinted polymers – The next generation Anal. Chem. 2003, 75, 376A-383A