

Supplementary Material

Virtually Imprinted Polymers (VIPs): Understanding Molecularly Templated Materials via Molecular Dynamics Simulations

Stefan Zink^a, Francisco Alirio Moura^b, Pedro Alves da Silva Autreto^c, Douglas Soares Galvão^b, Boris Mizaikoff*

a Institute of Analytical and Bioanalytical Chemistry
Ulm University
Albert-Einstein-Allee 11
89081 Ulm, Germany

b Gleb Wataghin Physics Institute
State University of Campinas, Unicamp
CP 6165, 13083-970 Campinas, São Paulo, Brazil

c Center of Natural Human Science
Federal University of ABC (UFABC)
Santo Andre, SP, Brazil

* Institute of Analytical and Bioanalytical Chemistry
Ulm University
Albert-Einstein-Allee 11
89081 Ulm
boris.mizaikoff@uni-ulm.de
+49-731-50-22750

Table of Contents

1. FIGURE 1, EXTENDED: FROM THE FUNDAMENTAL CONCEPTS OF MOLECULARLY IMPRINTED POLYMERS TO VIRTUALLY IMPRINTED TUBES.	4
2. OBSERVATIONS DURING VIRTUALLY IMPRINTING 17-B-ESTRADIOL.....	7
3. COMPARISON OF IMPRINTED AND NON-IMPRINTED TUBE.....	8
4. MOVIE 1: VIRTUAL IMPRINTING OF MONOMER TUBES.....	9
5. MOVIE 2: VIRTUAL CHROMATOGRAPHY EXPERIMENTS WITH IMPRINTED AND NON-IMPRINTED TUBES.....	9

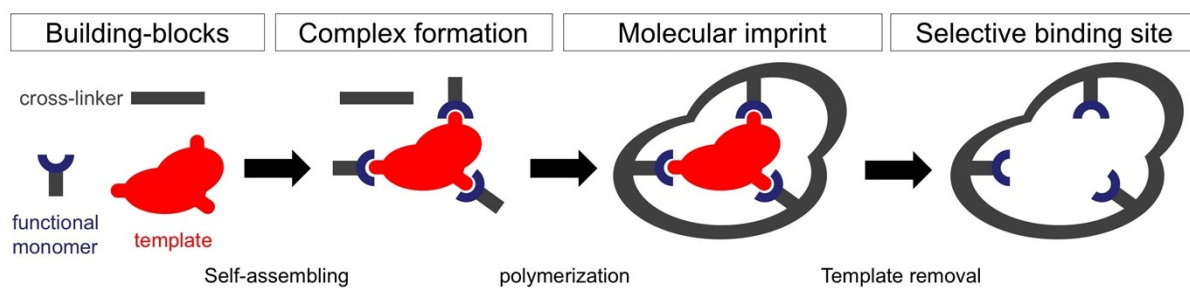
1. Figure 1, extended: From the fundamental concepts of molecularly imprinted polymers to virtually imprinted tubes.

- A) In this panel, the common concepts of molecular imprinting are described including the building blocks required for synthesizing a MIP (i.e., the template/target molecule, one or several different functional monomers, a cross-linker, and a porogenic solvent). In solution, template and functional monomers form a so-called pre-polymerization complex resulting from the interactions of their complementary functionalities. This complex is then fixed via polymerization with the cross-linker into a three-dimensional polymer network ideally encompassing selective binding sites for the template. Final extraction of the template exposes selective binding sites ready for rebinding the target.
- B) The second panel schematically illustrates the experimental approach to synthesize and characterize MIPs. Practically, all building blocks are dissolved in the porogenic solvent and polymerized, thereby usually forming a bulk imprinted polymer. Alternatively, direct generation of imprinted particles may be achieved by suspension, precipitation or miniemulsion polymerization strategies. Crushing and sieving the obtained co-polymer block yields porous imprinted polymer particles, which are then subject to solvent extraction for removing the template molecules. Frequently, MIPs are then packed as stationary phase into liquid chromatography (HPLC) or solid phase extraction (SPE) columns for further characterization. Usually, solutions of the template and structurally related constituents are injected for determining their

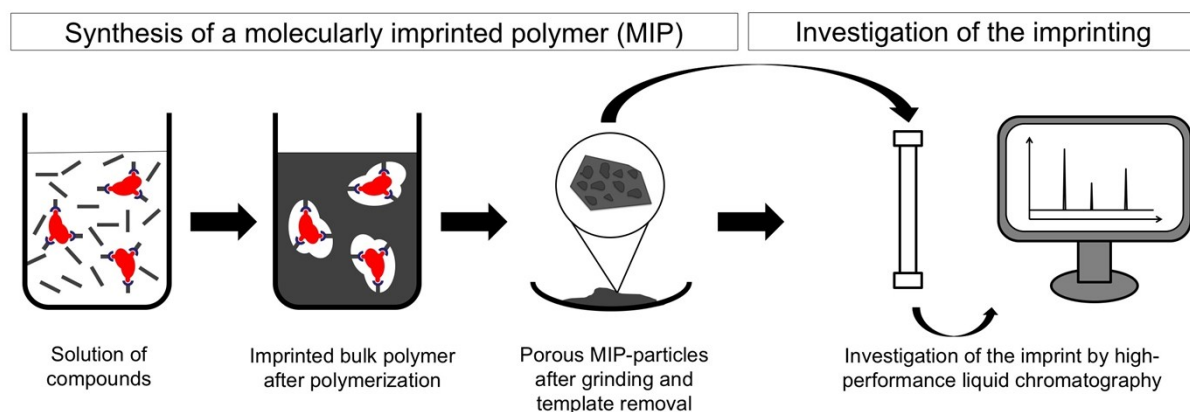
chromatographic retention behavior. Ideally, the MIP shows the highest affinity (i.e., most pronounced retention) for the template molecule. In addition, the obtained results are compared to a non-imprinted polymer (NIP) treated in the same fashion.

- C) This panel visualizes the concept behind transforming an experimentally imprinted polymer / polymer pore within a MIP particle into a theoretical model facilitating molecular dynamics simulations of such template materials. Herein, an imprinted polymer pore is transformed into a virtual tubular pore composed of initially deliberately arranged functional monomers, which serves as the potentially most simplified model of an imprinted polymer.
- D) The last panel briefly introduces the generation of the model, the virtual imprinting procedure, and the virtual chromatography experiments. A ring of hydrogenated functional monomers was created and multiplied along the Z-axis yielding in a regularly ordered molecular tube serving as a theoretical model for a non-imprinted virtual polymer (NVIP) pore. This NVIP tube is virtually imprinted during MD simulations allowing the functional monomers to change their position along the Z-axis during interactions with template molecules present within the tube. In analogy to the concepts shown in the first panel, a preferential arrangement of functional monomers is induced, thus creating 'imprints' of the template molecules at the tube wall (i.e., a virtually imprinted (VIP) tube). Finally, VIP and NVIP tubes are then used during virtual chromatographic experiments, which - comparable to the experimental procedures in the second panel - enable evaluating the performance of such imprints during MD simulations of their retention behavior.

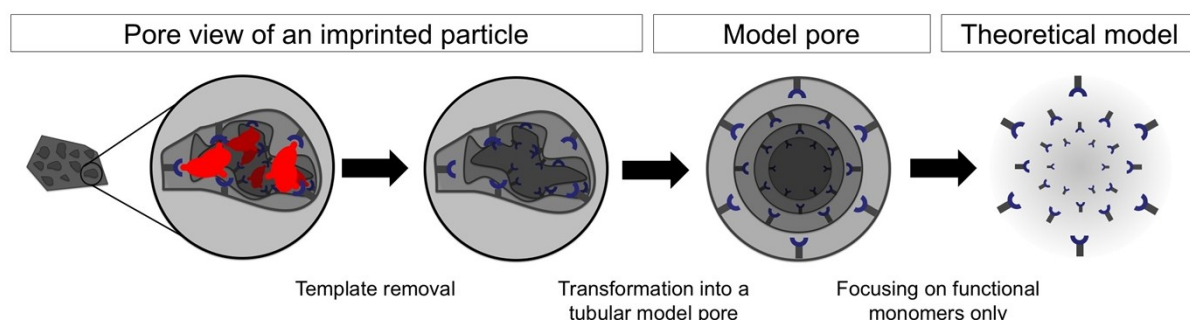
A. Molecular imprinting



B. Experimental imprinting



C. Towards the theoretical model



D. Virtual Imprinting

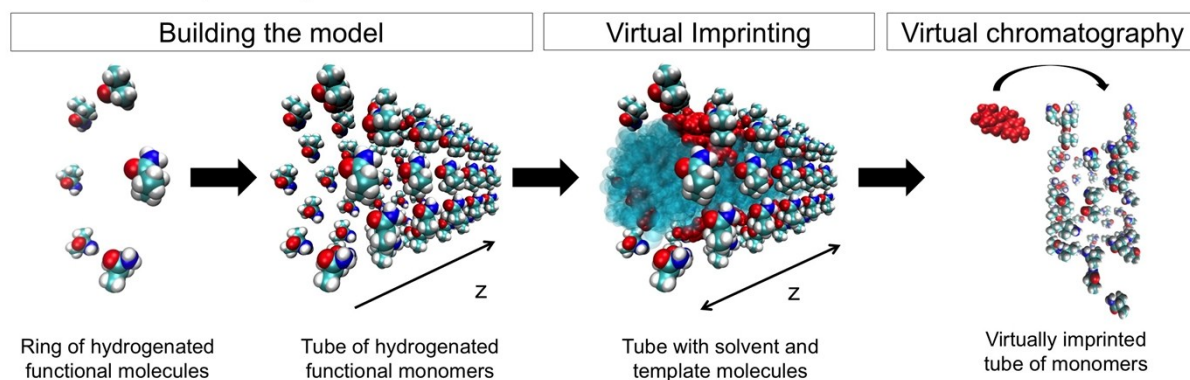


Figure 1. From the fundamental concepts of molecularly imprinted polymers to virtually imprinted tubes.

2. Observations during virtually imprinting 17- β -estradiol

During the imprinting simulations, as anticipated, the hMAAM monomers rearranged due to the prevalent thermodynamic motion, interactions with themselves, and according to their hydrogen bonding interactions with the target molecules simulated within the tube (movie 1, supplementary information). It was observed that the initially randomly distributed 17- β -estradiol molecules in fact migrate toward the inner tube wall driven by their interactions with hMAAM, thereby aligning themselves either radially or parallel to the tube wall. (movie 1, supplementary information). During this arrangement, either one or two hydrogen bonds are formed between the hydroxyl groups of the target and the functional monomers, which in fact confirmed the expected governing interactions during non-covalent imprinting procedures of 17- β -estradiol. Furthermore, the initial length of the tube slightly decreased due to annealing of the internal interactions between the hydrogenated monomers. Figure 4 (main article) shows the results of the hydrogen bond analysis for the 17- β -estradiol imprinting simulation evidencing that the number of hydrogen bonds stabilizes at an average of 45 after approximately 15 ps (i.e., 15,000 time steps of 1 fs duration each), and achieves a maximum of 57 hydrogen bonds in frame #666. Consequently, the spatial arrangement of the functional monomers in frame #666 was saved as the VIP tube (figure 2 B).

For the statistical evaluation of the virtual imprinting effect, the length of the tube was increased from 40 to 160 rings of monomers to achieve a more pronounced chromatographic effect when using the VIP and NVIP in the VCE simulations. Virtual imprinting of the larger tube achieved a maximum of 240 hydrogen bonds at a

In this movie, the virtual imprinting procedure of the functional monomer tube is demonstrated. The simulation comprises a tube of hydrogenated methacrylamide molecules (colored molecules), which is filled with a solution of 17- β -estradiol (red) in acetonitrile (cyan). It is evident that the hydrogenated methacrylamide monomers were fixed along the X- and Y-axis, yet free to move along the Z-axis. Thereby, the monomers may rearrange themselves according to their interaction with template molecules (i.e., 17- β -estradiol, red) freely moving inside the tube. During the MD simulation, it is clearly evident that arrangement of the monomers changes, and that temporary complexes are formed between monomer and template molecules giving rise to the final virtually imprinted tube structure.

5. Movie 2: Virtual chromatography experiments with imprinted and non-imprinted tubes.

This movie illustrates the combined results of virtual chromatography experiments with a 17- β -estradiol imprinted VIP tube (top), and a non-imprinted NVIP tube (bottom). For better visibility, the solvent molecules are not shown, the functional monomers were rendered transparent, and only the molecules constituting the VIP or NVIP tube along with the injected sample molecules were colored. For the simulations, infinite simulation boxes were used looping any molecule that has eluted from the VIP or NVIP column back to the beginning of the tube for another injection. During the VCE experiments, three sample molecules - 17- β -estradiol (red), 17- α -estradiol (yellow), and 17- α -ethinylestradiol (blue) - were 'injected' onto both columns (VIP and NVIP). The obtained results clearly demonstrate that the imprinted tube

shows pronounced affinity for the template molecule 17- β -estradiol used for virtually imprinting the tube, and that structural analogous molecules eluate more rapidly from the VIP tube. Furthermore, using the non-imprinted NVIP tube it is confirmed that no distinct selectivity for any of the three target molecules was obtained, as they show similar percolation, retainment, and elution behavior.