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MIRATE: MIPs RAtional dESign Science Gateway

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Abstract:

Molecularly imprinted polymers (MIPs) are high affinity robust synthetic receptors, which can be optimally synthesized and manufactured more economically than their biological equivalents (i.e. antibody). In MIPs production, rational design based on molecular modeling is a commonly employed technique. This mostly aids in (i) virtual screening of functional monomers (FMs), (ii) optimization of monomer-template ratio, and (iii) selectivity analysis. We present MIRATE, an integrated science gateway for the intelligent design of MIPs. By combining and adapting multiple state-of-the-art bioinformatics tools into automated and innovative pipelines, MIRATE guides the user through the entire process of MIPs' design. The platform allows the user to fully customize each stage involved in the MIPs' design, with the main goal to support the synthesis in the wet-laboratory. Availability: MIRATE is freely accessible with no login requirement at <http://mirate.di.univr.it/>. All major browsers are supported.

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1 Introduction

Molecularly imprinted polymers (MIPs) have been involved in a huge number of processes, boasting many hundreds of articles published annually in this area ([1] and references within). MIPs are frequently used in chromatography, sensors and assays, and they are considered as the cost-effective substitutes for biological macromolecules in several research areas and practical applications [2]. A molecularly imprinted polymer can be defined as the process of template-induced formation of specific recognition sites (binding or catalytic) in a material where the template directs the positioning and orientation of the material's structural components by a self-assembling mechanism. In a non-covalent molecular imprinting, for a template molecule (or target), appropriate functional monomers (FMs) are chosen and allowed to form a self-assembly construct. By co-polymerization with a cross-linking molecule, a polymer network is formed in which the self-assembly is set. Thereby, the position and the spatial conformation of the monomers are constructed according to the template. The embedded template can then be extracted and rebound to the molecularly imprinted polymer. The targets are usually small compounds or appropriate binding epitopes [2].

Recent improvements in the synthesis of molecularly imprinted nanoparticles [3] combined with their stability, low cost preparation of these imprinted materials [4] and the efficacy in antimicrobial, antiviral and anticancer therapy [5] have led the MIPs to be recognized as "plastic antibody" [6].

In recent years, computational techniques have been advanced and exploited in the optimization of polymer composition to rationally design high affinity synthetic receptors by using the molecular imprinting technique [7]. One of the most famous molecular mechanics/molecular dynamics protocol for MIPs' rational design, has been designed by Chianella and collaborators [8] and runs over the Sybyl modeling package of programs (Tripos Inc., St. Louis, MO, USA). Here, we present MIRATE, a new open-source science gateway as alternative to the previous cited patented protocol. MIRATE stems from the desire to find a free alternative computational method to the Chianella's protocol that relies on purchasing Sybyl. The combination of several state-of-the-art tools in a single publicly available science gateway is MIRATE's innovation. Nevertheless, MIRATE provides a unique environment to MIPs design and allows users to intervene in the process, preventing it from becoming a fully-automatic black-box.

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2 Related Work

Computational design has become a common procedure in the manufacture of MIPs, and it has allowed important improvements in functional monomer selection, calculation of monomers-template ratio and selectivity analysis. As extensively presented by Cowen and collaborators [7], several studies used computational techniques for rational MIPs design (see Table 1, [7] and references within it for more details). Broadly speaking, the used computational techniques can be clustered based on the technique employed (e.g. monomer selection, study of the electronic structure of the template and monomers) and by the used algorithms: those span from molecular mechanics/molecular dynamics to quantum mechanics e.g. semi-empirical, *ab initio* (Hartree-Fock, Møller-Plesset, etc.) and Density functional theory [9]. The most employed quantum mechanics techniques for MIPs design are based on the use of density functional theory and the hybrid functional B3LYP [10]. Indeed, the combination of them bring together the accuracy of the quantum mechanical calculations with the effectiveness of *ab initio* method for describing the electronic structure under investigation. Thus, QM methods are commonly used for studying the monomer template interactions in order to investigate the appropriate pre-polymerisation mixture for high affinity and selectivity MIPs [11], [12], [13]. These kind of calculations, although extremely accurate, are very expensive from a computational point of view. In the other hand, molecular mechanics and molecular dynamics simulations are methods that are used for modeling multi-component systems and thus can model many interactions [14]. With these techniques, the screening of several FMs become a feasible task by minimization of energies and optimization of geometries [15]. One of the most used molecular mechanics and molecular dynamics simulations protocol for MIPs design, has been developed by some of us (S.P. polymer group) [8] and it includes the application of the LeapFrog program [16]. Although this protocol is widely used in both academic and industrial fields with successful results [17], a free open access protocol able to produce good predictions in a short period of time is needed. From the best of our knowledge, MIRATE is the first open-source science gateway in molecularly imprinted polymers field. In this context, our publicly accessible science gateway, MIRATE, was developed as a novel alternative for the rational design of MIPs. Our science gateway, offers and combines the predictions of the majority of the state-of-the-art software (such as HADDOCK, Autodock, Gromacs), which have been widely tested in several applications published in papers of high impact. This science gateway stems from the desire to find a free alternative computational method to the standard protocol that relies on purchasing Sybyl [8]. MIRATE, compared with the widely used and tested Sybyl method, has shown very good semblance and sometimes improved the Sybyl results (see Section 4). The predictive nature of MIRATE could result in a range of polymer materials with superior affinity, and selectivity of what is currently being used.

3 Workflow and Architecture

In the last few years we have been developing state-of-the-art, free, in silico molecular modeling techniques [18], [19], [20], [21] to optimize the polymer compositions and to design high affinity synthetic receptors based on molecular imprinting. The MIRATE science gateway aims at providing an efficient and user-friendly solution to apply and to combine all these approaches for the rational design of MIPs through a publicly accessible web-based platform.

Given the most used FMs and the molecular template, MIRATE suggests the putatively most affine FMs and the optimal stoichiometric ratio.

The science gateway provides three different workflows for MIPs' design (see Figure 1).

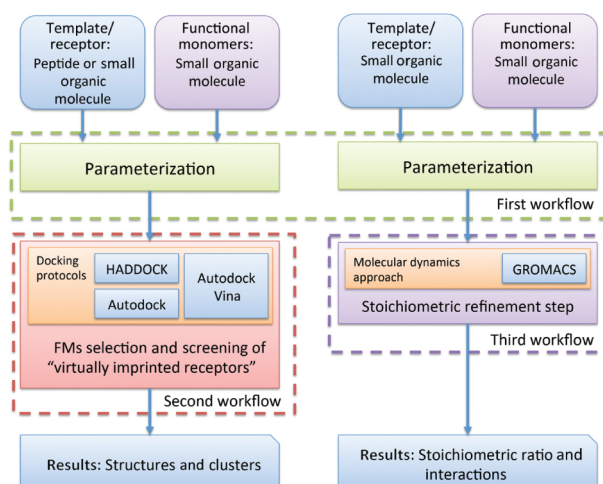


Figure 1: The MIRATE workflows.

1. The parametrization workflow. This workflow offers the possibility to parametrise the input molecules (template/“virtually imprinted receptor” and FMs) in several ways by using state-of-the-art approaches. Therefore, for instance, the users are driven by a simplified version of the ACPYPE software program [22], which offers several methods to assign charges to small organic molecules. Additionally, MIRATE gives the opportunity to expert users to upload partial charges already precalculated by another program/server such as R.E.D.-III [23] (see Supplementary Material S1.1 for more details about all parametrization protocols implemented in MIRATE).
2. The second workflow implements two tasks: (i) FMs selection and (ii) screening of “virtually imprinted receptors” for rebinding the molecular template [20], i.e. selectivity analysis. This workflow is based on three well-established docking protocols: (1) the knowledge-based HADDOCK [24], [25] provided through GOMoDo server [26], (2) the Autodock [27] and (3) the Autodock Vina [28] (see Supplementary Material S1.2). MIRATE also includes a virtual library containing the most commonly used parameterized FMs. It enables both, the FMs screening against a template and the “virtually imprinted receptors” screening against the molecular template. Then, the complexes are ranked by binding energy/docking score. Both the docking complexes and clusters are provided as well. The docking information is important for the wet-lab candidates preparation. Indeed, the success of the imprinting procedure is related to the formation of a strong monomer-template complex.
3. The third workflow relies on a stoichiometric refinement step, which aims at calculating the ideal template/-monomer stoichiometric ratio by using a novel molecular dynamics method based on GROMACS [29] (see Supplementary Material S1.3).

For the analysis of the results, the platform provides information regarding the monomers-template interactions (hydrogen bonds) and the number of FMs interacting with the template. The user can determine the type and quantity of FMs participating in the complex, and thus, extracting the ratio of the template and monomers for the polymer synthesis. As developed for the MIPs’ rational design, MIRATE supports molecular templates in input, such as simple short peptides or small organic molecules. Indeed, in the pipeline based on HADDOCK, MIRATE handles short peptides with plain standard aminoacids, free of auxiliary ions, ligands, and with up to one chain.

MIRATE carefully checks all the inputs uploaded by users (e.g. the input peptide in terms of aminoacids and atom type) in order to prevent any issue that could compromise the results.

MIRATE includes a complete documentation and step-by-step tutorials.

3.1 Overview of the MIRATE HW/SW Architecture

MIRATE provides a whole series of facilities to perform molecular docking and refinement, supporting both peptides and organic molecules. MIRATE does not require any specific library or adjustment to be used, thus allowing users to focus only on simulation and execution. The MIRATE user interface (UI) relies on the latest web technologies, such as HTML5, JQuery [30] and Twitter Bootstrap [31], aiming to increase user experience. The UI is fully responsive and interactive, adapting its content to be visualized through any desktop as well as mobile devices. In order to minimize data exchange between client and server, the platform relies on a custom

framework written in PHP language according to the model-view-controller design pattern [32]. The whole MIRATE science gateway is organized into two main blocks: a web interface designed to collect all submitted user jobs and a computational cluster designed for intense computation. The submitted jobs are collected in a dedicated batch queue and processed on a high-performance computer cluster (see Figure 2), using custom workers. A worker (job collector and submitter) is designed to create, and later submit, a new job according to a batch queue. A second worker (data retriever) is designed to retrieve, periodically, the job execution status, updating its information accordingly.

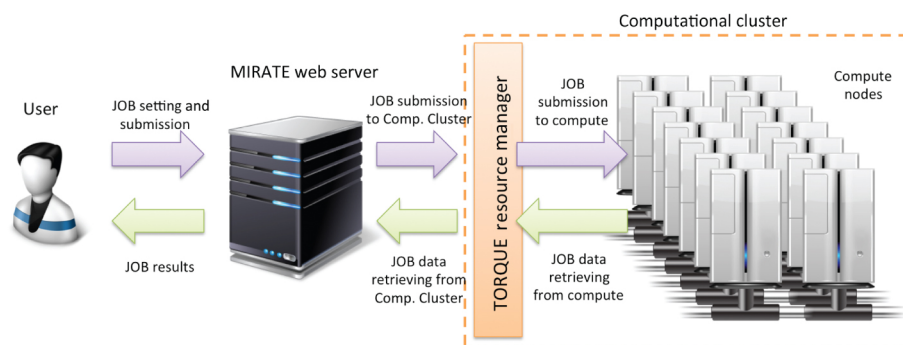


Figure 2: Overview of the MIRATE SW/HW architecture.

The local cluster is equipped with TORQUE/PBS Resource Manager (<http://www.adaptivecomputing.com/products/open-source/torque/>), used to distribute and control the batch jobs. TORQUE/PBS assigns each submitted job to each compute node (node of the cluster). In such a way, each computation is executed in a separate environment. In such a way, MIRATE is relieved from intensive computational tasks, providing a reactive system.

4 Method Validation

In order to determine the accuracy and reliability of MIRATE predictions we compared MIRATE with the only one established protocol which employs a Sybyl-based screening model and is already extensively tested and applied in the literature. As the monomers used for the tests possess different physicochemical properties, therefore our virtual library contains acidic monomers: acrylamido-2-methyl-1-propanesulfonic acid (AMPSA), acrylic acid (AA), itaconic acid (IA), methacrylic acid (MAA) and trifluoromethylacrylic acid (TF-MAA); basic monomers: allylamine, 1-vinylimidazole (VI), 2-vinylpyridine (2-VP), 4-vinylpyridine (4-VP) and N,N-diethylamino ethyl methacrylate (DEAEM) and neutral monomers: acrolein, acrylamide, acrylonitrile, m-divinylbenzene, p-divinylbenzene, ethylene glycol dimethacrylate (EGDMA), 2-hydroxyethyl methacrylate (HEM), N,N-methylenebisacrylamide (MBAA) and styrene. MIRATE was found to match that of the Sybyl model with a 28.8% error in ranking from a sample size of 114 combinations (see Tables 2.1–2.3 in Supplementary Material). MIRATE shows very good semblance with Sybyl and for two (NK11 and AK9) out of three templates predicted itaconic acid as the best monomer in agreement with the experimental data while Sybyl predicted itaconic acid only as the second-best monomer. We have analyzed also the performance of the docking programs that showed a high agreement with experimental results (see Supplementary Material S2.2 for Autodock/Vina's explanation).

5 Application Cases

We applied MIRATE to evaluate the efficiency and the automation of the computational workflows for several MIPs application cases [18], [19], [20], [21]. We exploited the first and the second workflow in two of them, by which we synthesized high-affinity synthetic receptors [18], [19]. These included a high-affinity receptor for the iron-regulating hormone Heparin-25 [18], which is a significant marker for cardiovascular risk, and three receptors for the cardiac-troponin-I peptides (NITEIADLTQK, NIDALGMEGR and AYATEPHAK), which are important markers for cardiovascular risk [19].

We also applied the first and second workflow to evaluate the MIPs' rebinding ability (i.e. selectivity analysis), by taking into account the cumulative steric effect of multiple FMs within the receptor site [20]. Indeed, the

methodology, here, fully implemented in MIRATE, has been successfully applied in three cases, either confirming the experimental data available and aiding the synthesis of more selective MIPs. The application cases of this methodology were: (1) the detection of tetrahydrocannabinol using a Catechin-hydrate MIP with acrylamide as monomers; (2) the detection of Bisphenol A and Bisphenol F using a Bisphenol A MIP with 4-vinylpyridine as the monomer and (3) the detection of hypoxanthine by a selection of theophylline MIPs.

By using the first and third workflows, computational results also allowed us to synthesize and validate in a wet-lab an efficient MIP for melamine, a heterocyclic triazine that is very important in the “food safety” area [21].

6 Discussion

This unique publicly free available science gateway implements a novel protocol for the rational design of MIPs for practical applications. The science gateway allows a strong user intervention at different stages offering the user, the choice between the parameter for MIPs modeling and default parameters. However, the expert user can always modify the default parameters along the pipeline and introduce experimental information within the server limitations. Although we recommend preferential routes to be followed along the web server, according to some points e.g. the type molecules uploaded, the users’ goals and our case-studies; we always give maximum freedom to the user. Indeed, the protocol has been shown in several cases [18], [19], [20], [21] to be useful to analyse mainly the monomer-template and template-receptor interactions in a physiological water solvent, and to offer a reliable (within the limitations of the methods) prediction, not only on the monomers to be used but also on the concentration ratio. MIRATE is thus the first publicly available computational protocol aimed at the rational design of MIPs. This new free science gateway offers the possibility of performing fast screening of adsorbents and it is suitable for resolving particular separation/diagnostic problems. The main beneficiaries of the present work will be the scientific community as well as pharmaceutical and fine chemicals companies. Indeed, MIRATE, will pave the way for many experimental labs, using brute-force trial/error approaches, leading them to use this innovative computer-assisted way for synthetic antibodies design.

Conflict of interest statement: Authors state no conflict of interest. All authors have read the journal’s Publication ethics and publication malpractice statement available at the journal’s website and hereby confirm that they comply with all its parts applicable to the present scientific work.

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