# Computer-Aided Peptide Evolution for Virtual Drug Design

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**Abstract.** One of the goals of computational chemistry is the automated *de novo* design of bioactive molecules. Despite significant progress in computational approaches to ligand design and efficient evaluation of binding energy, novel procedures for ligand design are required. Evolutionary computation provides a new approach to this design issue. A reliable framework for obtaining ligands via evolutionary algorithms has been implemented. It provides an automatic tool for peptide *de novo* design, based on protein *surface patches* defined by user. A special emphasis has been given to the evaluation of the proposed peptides. Hence, we have devised two different evaluation heuristics to carry out this task. Then, we have tested the proposed framework in the design of ligands for the protein Prolyl oligopetidase, p53, and DNA Gyrase.

## 1 Introduction

Since one of the goals of computational chemistry—as well as general drug design—is the automated *de novo* design of bioactive molecules, significant progress in computational approaches to ligand design and efficient evaluation of binding energy have been done [1]. Nevertheless, novel procedures for ligand design are required. This is motivated by the always increasing number of protein targets for drug design, being functionally and structurally characterized. This situation is the result of major advances in both experimental methods for structure determination [2] and high-throughput modeling [3].

Peptides are emerging as promising future drugs for several illnesses. Passing from promising drugs to real drugs was difficult in the past because of their bad ADME (absorption, distribution, metabolism, excretion) properties. However, this handicap is soon to disappear thanks to the development of modern methods of drug delivery, and the use of derivatives—D amino acids—metabolically more stable.

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Nowadays, several research projects are trying to develop new methodologies for designing drug-oriented peptides. These methodologies can be classified in several ways. One of them is structure-based drug design, where the design process is seen as an engineering problem. Another well-known approach is combinatorial chemistry, where a huge number of compounds are screened against the target. Our approach hybridizes both methodologies. We proposed a new methodology to screen large quantities of drug candidates. However these peptides are designed following a semi-rational process, in our case, evolutionary computation. From now on, we will use *in silico* to refer to virtual structure-based drug design. In our approach ligands are built from scratch, which is usually termed as *de novo* design in the literature. The main advantage of this approach is that novel structures, not contained in any database, can be devised.

To achieve this goal, algorithms must address two main tasks. First, a competent search method must be provided to explore this high-dimensional chemical space. Second, the search space (the set of all algorithmically treatable molecules) must be structured into regions of higher and lower quality to allow the prediction of desired properties. In order to perform the search task, we implemented and tested four different evolutionary algorithms: Darwinist genetic algorithm (GA) [4], Lamarckian genetic algorithm (LGA) [5], population-based incremental learning (PBIL) [6], and Bayesian optimization algorithm (BOA) [7]. In this evaluation, we have also approached the second task, to structure the search space into regions of higher and lower quality, by implementing two different heuristics to calculate the fitness (binding energy) of each individual (peptide) proposed by the evolutionary algorithms. The first heuristics was built to be computationally affordable, whereas the second focused on achieving and accurate binding energy estimate—throughout referred as docking energy. It must be pointed out that we used AutoDock 3.0.5 [8] for the docking calculations required by the heuristics.

The main goal of this work is to design peptide drugs which serve as effective ligands to the target protein area defined by the user. This area is also known as surface patch. One application of such peptide drugs could be to act as inhibitors of some pathological functionalities of the target protein [9]. Finally, we tested the developed methodology in several specific cases. In particular the proteins prolyl oligopetidase, p53, and DNA gyrase (still under study and for this reason the results are not summarized in this paper.) The results obtained are encouraging. We have compared the proposed peptides with some others designed using a purely chemical-knowledge based approach by the peptide design experts from our research group. In all the tested cases, the peptides designed in silico present better docking energies than their counterparts designed by chemical experts. We are now currently synthesizing these peptides in order to do *in vitro* comparisons.

## 2 Related Work

Several approaches to *de novo* ligand design have been previously reported. However, only one of these approaches is quite similar to that implemented in this work. ADAPT [10] tries to design from scratch small organic molecules. The underlying mechanism uses docking as part of the fitness measure. The main difference between ADAPT and our approach is that our work is very specifically optimized for the synthesis of sequential compounds—peptides—whereas ADAPT is focused in small organic molecules. Therefore, ADAPT would have to be modified in order to design peptides to act as protein binders.

Other groups are using different approaches, such as growing [11,12], linking [13,14], and physico-chemical properties [15]. The growing strategy is a clear example of a different approach. The building-up process start from a seed structure pre-placed in the surface patch. The user can assign certain growing sites on the seed structure and then the program will try to replace each growing site by a candidate fragment. The new structure will serve as the seed structure for the next growing cycle.

The building-up process in a linking strategy also starts from a pre-placed seed structure. However, the seed structure consists of several separate pieces that have been positioned to maximize the interactions with the target protein. The pieces grow simultaneously, while the linking program tries to link these pieces in an feasible way. This process continues until all the pieces are integrated into a single molecule.

Finally, in the physico-chemical approach, the main goal is to obtain an optimal set of physico-chemical properties that the peptide must exhibit in order to be a good ligand. Thus, a peptide is designed having in mind the obtained set of physico-chemical properties.

Previous approaches are aiming the design of new natural peptide ligands. Such peptides may present a poor bioavailability and, hence, become ineffective for their usage as drugs. Proteases catalyze the splitting of peptides into amino acids by a process known as proteolysis. Overcoming such constraints is another motivation of the methodology presented in this paper. Although we are currently working with natural amino acids (L amino acids), the framework is able to design new peptide ligands using D amino acids. The D amino acids are artificial amino acids which are the mirror image of the L amino acids. Therefore proteases do not recognize and split them [16], making them ideal candidates for drug design.

## 3 Computer-Aided Peptide Design

Evolutionary computation is being widely applied to different areas of bioinformatics [17]. Evolutionary computation techniques have recently been applied in many aspects related to drug and compound library design [14,18,19,20,21,22].

Evolutionary algorithms are perfect candidates for applications were deterministic or analytic methods fail, for instance, problems where the underlying

mathematical model is ill-defined or the search space is too big. In this work both aspects are present. The underlying mathematical model being optimized is given by AutoDock 3.0.5. However, is important to note that docking algorithms are not a perfect simulation of the reality yet [23]. Also, the search space is too big to be systematically explored and each evaluation using the fast heuristics developed takes more than 30 minutes of calculations on a Pentium IV 1.60 GHz. Hence, these circumstances lead us to use evolutionary algorithms to steer the search for *de novo* design. These assumptions have been previously used by other researchers [11,12,14].

### 3.1 The Problem

Our goal is to design good peptide ligands to a user-defined surface patch of a protein. To reach this goal we have implemented and tested several evolutionary algorithms and we have developed two different fitness functions—throughout called *heuristics*.

Each individual of the evolutionary algorithms (peptides) is defined by a 6 genes chromosome. Each gene can adopt 7 different amino acid values. We have only used 7 different amino acids among the 20 natural amino acids. We have only selected those amino acids representative of different physico-chemical properties. They are *alanine*, *arginine*, *glutamic acid*, *serine*, *isoleucine*, *triptophan* and *proline*.

The algorithms used to steer the search are four evolutionary algorithms. They are: Darwinist genetic algorithm [4], Lamarckian genetic algorithm [5] where for the local search we have used evolutionary strategies (1 + 1) [24], population-based incremental learning [6] and Bayesian Optimization Algorithm [7]. Details on how we have used these algorithms can be found in [25], as well as in the previous listed references.

### 3.2 Heuristics

The evolutionary algorithms need a *fitness function* to guide the search space into regions of higher and lower quality evaluating the peptides proposed. For this purpose, we developed two heuristics to dock the peptides to the surface patch defined by the user, giving us the docking energy. The first one is a quick one, whereas the second is an accurate one, hence computationally expensive. The user can choose the heuristics that better adapts to his necessities and constraints.

**Docking.** Docking algorithms are *in silico* tools that try to find the best mode of interaction between a small, possibly flexible, ligand and a large, usually rigid, macromolecular receptor. This is done by minimizing the energy of interaction, which is a complex function of variables describing the position, orientation, and conformation of the ligand. In our work, the small flexible ligands are the peptide proposed by the evolutionary algorithms, and the macromolecular receptor is the

surface patch of the protein defined by user, *i.e.:* a site on a protein to which a peptide ligand is desired.

To perform the docking experiments we have used the program AutoDock 3.0.5 [8]. At the beginning the heuristics prepare all the input files needed by AutoDock, and at the end they can process output files in order to extract the information needed. The AutoDock parameters are tuned as suggested in [8]. AutoDock uses a LGA as the algorithm for minimize the energy of interaction with 50 individuals per generation, 10000 generations, 300 steps of local optimization. And all this has been run ten times with different initializations for each docking experiment.

**Quick Heuristics.** The first heuristics developed is a quick one. We say that it is quick because it only has one docking experiment while the accurate heuristics has five. This heuristics has five stages: (1) three-dimensional reconstruction, (2) energetic minimization, (3) flexible angles definition, (4) docking, and (5) Boltzmann averaged binding energy.

*Three-dimensional reconstruction.* The internal representation of the peptides in the evolutionary algorithms is not a three-dimensional structure. It is a sequence of amino acids. But, the docking experiments need a structure, therefore we made a program using the NAB language [26] which takes at its input a sequence of letters representing amino acids, and gives us at the output a PDBQ file (PDB with charges) [27] with the extended structure of that peptide.

*Energetic minimization.* The next step is to do a short energetic minimization over the extended structure obtained in the previous step. To perform this, we have implemented the energetic minimization inside the program developed for the three-dimensional reconstruction [26]. We used a conjugate gradient minimization until the root-mean-square of the components of the gradient is less than 1.0.

*Flexible angles definition.* Before starting the docking calculations we redefined our ligand (peptide) to be flexible. We fixed the backbone but we give flexibility to the side chains. To perform this task we use AutoTors, which is an auxiliary script of the AutoDock.

*Docking.* In this stage we perform ten flexible docking experiments using the ligand built in the previous stages over the surface patch defined by the user.

*Boltzmann averaged binding energy.* Finally, we compute a Boltzmann averaged binding energy of each of the most stable structures found in each of the ten runs of the docking algorithm. The value obtained in this step is the fitness value for the peptide being evaluated in this moment.

**Accurate Heuristics.** The second heuristics developed implies a more accurate evaluation than the first heuristics. The main idea of this evaluation is to make five different docks, each one with a different probable peptide backbone structure. Once the docks have been carried out, we keep the more stable structure from those obtained. This heuristics has five steps: Secondary structure prediction, rotamers construction, flexible angles definition, docking, and Boltzmann averaged binding energy.

*Secondary structure prediction.* First of all we use the Chou-Fasman method for secondary structure prediction of peptides attending at their amino acid sequence. This method assigns at each conformation ($\alpha$-helix, $\beta$-strand or random coil) a number that inform us about the "probability" of adopting that conformation.

*Rotamers construction.* Attending to the number obtained using the Chou-Fasman prediction, we build five rotamers of the same peptide. In each rotamer, all the $\phi$ and $\psi$ angles of the peptide are chosen randomly from the area of Ramachandran map which represents the secondary structure being built. Angle $\omega$ is fixed to $180^o$.

*Flexible angles definition.* Flexibility is assigned to the five peptide structures in the side chains.

*Docking.* One flexible docking calculation is carried out for each rotamer built. Each of the docking calculations is carried out in the same manner than the docking calculation of the first heuristics.

*Boltzmann averaged binding energy.* Finally, we analyze which is the more stable rotamer docked, we keep it and we take as the individual fitness the Boltzmann averaged binding energy of each of the more stable structures found in each of the ten runs.

## 4 Results

We have tested the proposed methodology for peptide design using three proteins with high therapeutical interest. Making an extended comparison of the performance of each one of the evolutionary algorithms developed is not within of the scope of this paper. However, we explored the performance of the algorithms PBIL, LGA and BOA in three different problems. GA has not been used in these results because we decided to explore its extended version: LGA. No special criteria guided the choice of algorithms used in each domain.

The three systems where we have tested our *in silico* methodology are proteins: POP, p53 and DNA gyrase (since it is currently under study, the results are not reported in this paper.) These three proteins have been selected because each problem presents interesting features. The surface patch of the POP is inside a

large tunnel, the surface patch of the p53 is a large surface, and the surface patch of the DNA gyrase is an interaction area with DNA. For a proper comparison we have carried out two experiments for each of the studied systems. The first one is to make some docking experiments with the peptides designed by the design experts of our research group or some well-known natural ligands reported in the literature. The second followed the methodology reported in this paper. In this manner we compared rational- and evolutionary-designed peptides.

### 4.1 Prolyl Oligopeptidase

**Introduction.** Prolyl oligopeptidase (POP, EC 3.4.21.26) is a cytosolic serine peptidase characterized by oligopeptidase activity. The three-dimensional structure of POP revealed a two-domain organization: (1) the catalytic one, and (2) the structural one. In the catalytic domain there are the residues Ser554, His 680 and Asp641 which form the catalytic triad of the protease. Those residues are essential for the catalytic activity of the enzyme and are located in a large cavity at the interface of the two domains [28]. Based on this information available from the crystal structure we have defined a surface patch around the residues of the catalytic center Ser554, His 680 and Asp641. The surface patch also comprises the cavity formed by the structural domain which corresponds to the interior of the $\beta$-propeller domain. Inside this cavity the substrate interacts with many residues of the enzyme and is directed to the active site. The box is big enough to accommodate peptides of six residues that are tested in this study.

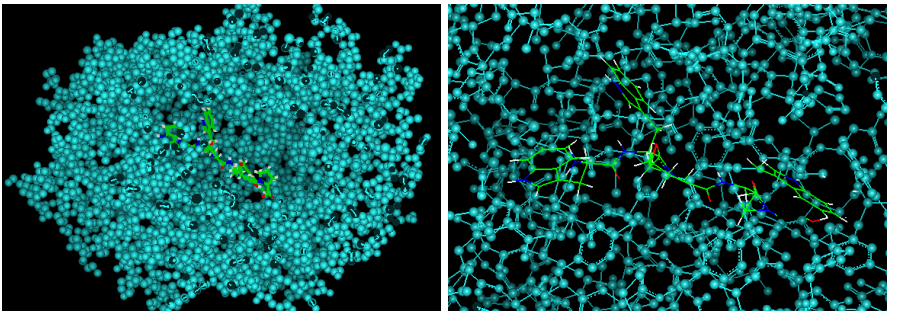
**Table 1.** Docking energy comparison for prolyl oligopeptidase peptides.

| Docking energy of fragments of POP inhibitors described at the literature. | | | | Docking energy of the best five individuals found by the algorithm | |
|---|---|---|---|---|---|
| *Peptide* | *Docking Energy* | *Peptide* | *Docking Energy* | *Peptide* | *Docking Energy* |
| GKPPIG | -7.186 | GKPPVG | -7.067 | WWPWPP | -13.737 |
| GVEIPE | -5.122 | GYPIPF | -5.843 | WWPSWA | -13.216 |
| HLPPPV | -8.542 | KPRRPY | -3.562 | WSPSWP | -13.032 |
| LLSPFW | -6.314 | LSPFWN | -9.538 | PWPEWA | -12.991 |
| MPPPLP | -8.862 | MTPPLP | -7.436 | WWPWSP | -12.936 |
| QNCPLG | -7.977 | QNCPRG | -8.874 | | |
| RPKPQQ | -7.546 | SPFWNI | -7.457 | | |
| TPPLPA | -7.913 | | | | |

POP is involved in the maturation and degradation of proline-containing neuropeptides related with learning and memory [29]. Physiological studies with human plasma have shown that POP activity is increased in mania, schizophrenia, post-traumatic stress and anxiety, while POP activity is decreased in depression, anorexia and bulimia nervosa [30]. Modulation of the POP activity with specific peptide inhibitors can be useful for the treatment of those diseases.

**Ligand Peptides Found in the Literature.** As POP hydrolyzes the peptide bond at the C-terminal side of prolyl residues, for the docking experiments we have chosen fragments of POP substrates and POP inhibitors containing 6 residues. The criterion followed to choose the fragments has been that at least one of those residues should be Proline. In table 1 are shown these peptides with their docking energies

***In Silico* Designed Peptides.** In this stage we have run a LGA. In table 1 are shown the energies of the 5 best individuals found by the algorithm. All of them are proline rich, the same as the natural ligands. In figure 1 there is a representation of the best individual found docked to the target protein.



(a) Docking position (b) Zoom

**Fig. 1.** Peptide proposed `WWPWPP` docked in the user-defined surface patch of the protein POP. High resolution color plates and 3D models of the figures displayed above can be found at `http://www-illigal.ge.uiuc.edu/~xllora/Pool/Papers/GECCO-2004-Figures/`.

### 4.2 p53

p53 protein is a transcription factor involved in different cellular functions, such as cell cycle control, programed cell death (or apoptosis) and differentiation. Actually the p53 gene was the first tumor-suppressor gene to be identified, and it was found that p53 protein does not function correctly in most of human cancer—around a 50% of cancer patients present patological mutations affecting the protein functionality.. The p53 can be divided in four domains: an N-terminus transactivation domain, a DNA-binding domain (DBD), a tetramerization domain (TD) and a C-terminus regulatory domain. The function of p53 is mediated by protein-DNA and by protein-protein interactions. The tetramerization domain has an essential role in its function, because only the tetrameric structure is active [31]. Peptides able to recognize this tetramerization domain and stabilize its native conformation could be of great interest in cancer research.

**Expert Designed Peptides.** In our group, we have studied the recognition of p53 tetramerization domain using a tetraguanidinium compound [32]. Based on this molecule, we have designed and synthesized a peptide which is also able to interact with the domain. In order to improve the affinity, we designed a peptide library based on the substitution of the arginines for other residues. These substitutions are mainly based on modifications of the chain length and/or the functional group. In table 2 are shown the docking energies of the five best peptides found in the 47 peptide library. More details of the design and evaluation of those peptides can be found in [33].

**Table 2.** Docking energy comparison for p53 ligands.

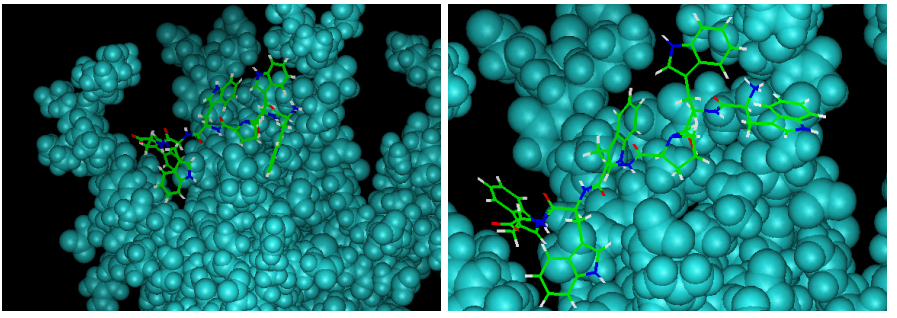
| Docking energy of the top-five peptides designed | | Docking energy of the best five individuals found by the algorithm | |
|---|---|---|---|
| *Peptide identification* | *Docking Energy* | *Peptide* | *Docking Energy* |
| Rpa3R_46 | -11.067 | WWPWWW | -13.294 |
| Rpa4_47 | -10.870 | WWWWWW | -13.260 |
| Oxaa_33 | -10.180 | AWWWWW | -13.182 |
| Rab4_51 | -9.707 | WWWWWA | -13.113 |
| Dapa3R_20 | -9.545 | AWRWWW | -12.827 |

***In Silico* Designed Peptides.** In this stage we have run a PBIL. In table 2 are shown the energies of the 5 best individuals found by the algorithm. In figure 2 there is a representation of the best individual found docked to the target protein.

## 5 Discussion

Recent advances in bioinformatics have provided a new *in silico* approach for the design of peptide ligands. This approach offers interesting complementary advantages to laboratory methods. The time invested in performing each run or experiment implies three issues: preparation, performance and evaluation. In each of them, *in silico* methods are faster than laboratory ones, although less accurate. However, such virtual screening can help to guide laboratory search. Another important issue is the cost, which is directly related, among other things, to the number of workers, equipment, time and reagents needed. Even if it does not seem obvious, the costs of performing an experiment might exceed enormously the expenses of executing a run. On the other hand, the available computational resources are the major constraint to *in silico* design accuracy. However, computational horsepower increases each month at the same time that it become cheaper.

We have observed that if the docking calculations were extremely good, the evolutionary algorithms developed would be a good tool for exploring the huge



(a) Docking possition (b) Zoom

**Fig. 2.** Peptide proposed `WWPWWW` docked in the user-defined surface patch of the protein p53. High resolution color plates and 3D models of the figures displayed above can be found at `http://www-illigal.ge.uiuc.edu/~xllora/Pool/Papers/GECCO-2004-Figures/`.

search space that this combinatorial search implies. Unfortunately, this assumption is not true. The scientific community is still working to improve docking techniques, as well as the experimental techniques in chemistry. Such cooperative endeavor may lead in the future to better virtual tools. However, methodologies such as the one proposed in this paper are ready to be used to recommend compounds to be synthesize, thus drug research can narrow the focus of their research to those regions suggested by the virtual screening tools.

Another conclusion of the work presented in this paper is that the peptides found by evolutionary algorithms have better docking energies. This fact has been shown comparing them to those designed using a rational chemistry approach in our research group, and those found in the related literature. Recently, we selected some evolved peptides, starting their synthesis *in vitro*. Once these new compounds become available, besides the comparison based on the docking energy, we will be be able to compare their behavior *in vitro*.

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