

# S4MPLE – Sampler For Multiple Protein–Ligand Entities: Simultaneous Docking of Several Entities

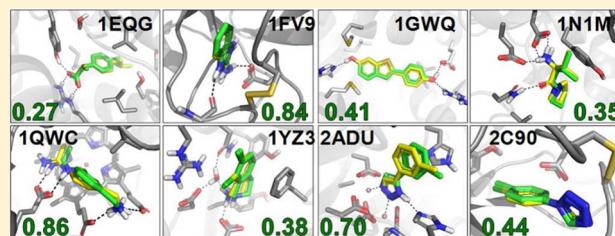
Laurent Hoffer<sup>‡,§</sup> and Dragos Horvath<sup>\*,‡</sup>

<sup>‡</sup>Université de Strasbourg, 1 rue B. Pascal, Strasbourg 67000, France

<sup>§</sup>Novalix, BioParc, bld Sébastien Brant, BP 30170, Illkirch 67405 Cedex, France

## Supporting Information

**ABSTRACT:** S4MPLE is a conformational sampling tool, based on a hybrid genetic algorithm, simulating one (conformer enumeration) or more molecules (docking). Energy calculations are based on the AMBER force field [Cornell et al. *J. Am. Chem. Soc.* 1995, 117, 5179.] for biological macromolecules and its generalized version GAFF [Wang et al. *J. Comput. Chem.* 2004, 25, 1157.] for ligands. This paper describes more advanced, specific applications of S4MPLE to problems more complex than classical redocking of drug-like compounds [Hoffer et al. *J. Mol. Graphics Modell.* 2012, submitted for publication.]. Here, simultaneous docking of multiple entities is addressed in two different important contexts. First, simultaneous docking of two fragment-like ligands was attempted, as such ternary complexes are the basis of fragment-based drug design by linkage of the independent binders. As a preliminary, the capacity of S4MPLE to dock fragment-like compounds has been assessed, since this class of small probes used in fragment-based drug design covers a different chemical space than drug-like molecules. Herein reported success rates from fragments redocking are as good as classical benchmarking results on drug-like compounds (Astex Diverse Set [Hartshorn et al. *J. Med. Chem.* 2007, 50, 726.]). Then, S4MPLE is successfully challenged to predict locations of fragments involved in ternary complexes by means of multientity docking. Second, the key problem of predicting water-mediated interaction is addressed by considering explicit water molecules as additional entities to be docked in the presence of the “main” ligand. Blind prediction of solvent molecule positions, reproducing relevant ligand-water-site mediated interactions, is achieved in 76% cases over saved poses. S4MPLE was also successful to predict crystallographic water displacement by a therefore tailored functional group in the optimized ligand. However, water localization is an extremely delicate issue in terms of weighing of electrostatic and desolvation terms and also introduces a significant increase of required sampling efforts. Yet, the herein reported results – not making use of massively parallel deployment of the software – are very encouraging.



## 1. INTRODUCTION

Docking can be defined as the structural prediction of a complex (e.g., ligand-protein) at the atomic resolution scale. In principle, this is just a particular instance of the more general problem of conformational sampling: docking consists in the sampling of ligand geometry, of the intermolecular rototranslational degrees of freedom (DoF), plus optionally of some internal DoF of the binding site. In practice, however, usual docking simulations make an explicit distinction between the two different molecules classes (binding site and ligands). A lot of methods, using various algorithms, have been developed to address the docking problem.<sup>5</sup> Most of them expect two distinct molecular input files – the binding site, in some biomolecule-compatible format (.pdb, .mol2, etc.) and a ligand, in a file explicitly providing bond order information (.mol2, .sdf, etc.). Exceptions to this classical scheme (protein–protein docking, self-assembly of small organic molecules) are usually not supported by main-stream docking programs and are often handled by dedicated soft.<sup>6,7</sup> Furthermore, classical docking tools all include two key steps: the (typically force-field driven) sampling of the DoF of the system and the scoring/ranking of

obtained poses. The latter step implicitly assumes the receptor to be a protein, binding one single ligand – typically drug-like for the scoring functions were trained under these assumptions. Therefore, such scoring functions are often misleading when applied to small, fragment-like molecules<sup>8</sup> or in docking scenarios conserving explicit site waters.

In fact, molecular recognition phenomena are never a two-body problem: involved partners are solvated. Solvent effects are known to be very complex – they are extremely hard to model – and have a major role in the free energy of binding.<sup>9</sup> Specific terms, accounting among other for the desolvation phenomenon of ligand and binding site, have been developed to enhance the accuracy of scoring functions.<sup>10,11</sup> While solvation is increasingly taken into account, the critical aspect of handling displaceable crystallographic water molecules in the binding site, putatively involved in water-mediated hydrogen bonds between the ligand and the target,<sup>12,13</sup> is not properly handled by classical docking tools, which cannot cope with

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multiple mobile entities in the protein site. It has been shown that conserving tightly bound waters in simulations significantly enhances the accuracy of cross-docking results<sup>14</sup> – yet, the nontrivial question is which waters to conserve. Huang et al. investigated the effect of including waters in massive virtual screening.<sup>15</sup> Different approaches, for example based on energy computations<sup>16</sup> or classifier algorithms,<sup>17</sup> tried to address this fundamental question by flagging waters as conserved or displaceable. Besides, some ligands may gain in binding energy by explicitly replacing poorly anchored crystallographic H<sub>2</sub>O in order to directly interact with the target.

Specific approaches typically deal with these issues by introducing dedicated DoF piloting the status of water molecules. Much effort has been invested to determine the preferred location of water probes in protein sites.<sup>18</sup> Alternatively, docking approaches incorporate crystallographic water molecules in the usual docking process where, at least the oxygen atom is considered rigid, but the water particles can be turned ON/OFF during the simulation.<sup>19,20</sup> As for example, FlexX is able to both rotate waters in order to optimize the hydrogen bonds network and turn them ON/OFF if necessary. However, an alternative approach allowing explicit particles, modeling water molecules, to be introduced during the incremental ligand reconstruction step has been published by FlexX authors,<sup>20</sup> but it was not implemented in the commercial releases of the program.

Recently, Lie et al. reported<sup>21</sup> an interesting approach, implemented in the Molegro Virtual Docker (MVD) program, involving waters in docking. The ligand is surrounded by waters at every moment, and the solvent molecules can be toggled on/off in order to make a favorable direct contact with the site. Thus, the number of active waters can change during the process, and a simple "entropic" penalty is added to somehow compensate for the "appearance" of activated water molecules (i.e., addition of their interaction terms from the force field/scoring function). By contrast to most of the previously described algorithms, waters are not included into the binding site, they are not static since they move with the ligand, and fair results were obtained using a data set composed of 12 PDB complexes.

The sampling and docking tool S4MPLE (Sampler For Multiple Protein–Ligand Entities) advocates a completely generalized approach, treating docking as a particular case of sampling of multiple entities. It may search for the lowest-energy arrangements of arbitrary molecular and supramolecular systems, starting from a single molecule (ligand geometry sampling/small peptide folding) to site-ligand complexes (classical docking), to ternary (or even higher-order) systems, addressing both inter- and (user-controlled) intramolecular DoF. S4MPLE<sup>3</sup> is a molecular modeling tool based on a hybrid "Lamarckian" Genetic Algorithm (GA), combining local optimizations in addition to classical evolutionary sampling strategies. Allowing full control of the considered DoF, S4MPLE is a completely general approach to explore the conformational space of the flexible parts of the studied system. By default, all atoms of the system are considered flexible. An explicit list of fixed atoms – for example, binding site atoms – needs to be provided.

The energy function is based on the AMBER/GAFF force-field (FF) formalism. AMBER<sup>22</sup> is a popular FF used to simulate biological macromolecules. Recently, an extension to handle small compounds such as ligands has been reported: General AMBER Force Field (GAFF).<sup>2</sup> In addition to the

classical *in vacuo* FF terms, S4MPLE includes a specific continuum solvation term of maximal simplicity, in order to minimize the computational overhead associated with it. The solvent model terms include simple functions (linearly distance-dependent relative dielectric constant and pair-based desolvation term) of interatomic distances of the same complexity as usual FF terms. Explicit solvent boxes,<sup>23</sup> widely used in molecular dynamics, are not compatible with evolutionary-based strategies involving large-scale random jumps in conformational space. Besides, a term rewarding favorable interactions such as hydrogen bonds and hydrophobic enclosure is included too. All these additional terms, their calibration/validation, and the genetic operators are described elsewhere.<sup>3</sup> This "Fit FF" leads to enhancement with respect to the "Core FF" in redocking experiments using the most popular docking data set (Astex Diverse Set<sup>4</sup>).

Occasional local optimization is mandatory for molecules during the evolutionary-based sampling procedure. This is due to the extreme ruggedness of the FF-based energy function. All genetic operators will include, as a last step, a small local optimization. A population diversity control mechanism, in the form of interaction fingerprints, is included in order to avoid the risk of premature convergence.

This work continues to explore the performances and putative applications of the single CPU workstation version of S4MPLE, focusing on multiple entity docking, and addressing two main key issues:

- the problem of water-mediated ligand binding. The implicit solvent terms model the shielding effect of water on electrostatics but not the water-mediated hydrogen bonds. Here, waters are treated as additional ligands competing for anchorage to the active site, and

- the issue of simultaneous docking of multiple fragment-like ligands in Fragment-Based Drug Discovery/Design (FBDD). FBDD emerged this past decade as a powerful way to identify fragment-hits which are subsequently evolved into high affinity lead compounds.<sup>24</sup> Any rigorous *in silico* FBDD strategy must include two main steps: prediction of fragment binding modes and their subsequent optimization/evolution into more potent ligands. Prior to simultaneous docking of multiple fragments, a single fragment redocking study was performed to estimate the ability of S4MPLE to cope with fragment-size ligands.

Poses are solely evaluated according to their AMBER/GAFF energies, without any additional scoring function based reranking (not straightforwardly applicable to multiple entity contexts, anyway). These multiple entity simulations proved to be more complex and time-consuming than the standard single-ligand benchmark previously reported. Nevertheless, S4MPLE successfully managed to predict both water-mediated interactions and multiple fragment binding modes, within the classical limitations due to FF accuracy.

## 2. METHODS

### 2.1. Brief Overview of S4MPLE.

As already mentioned, a detailed technical description of the GA-driven sampling procedure and benchmarking studies with respect to classical docking problems were submitted for publication elsewhere.<sup>3</sup> Here, the original features of S4MPLE will be briefly mentioned in order to allow a better understanding of the current results.

S4MPLE is a completely general approach to visit the conformational space of arbitrary molecules or complexes. As such, it may be equally well used for conformational sampling and docking – which is nothing but sampling of a ligand in the

presence of a binding site. The ‘site’ does not need to be a protein, which may eventually render S4MPLE useful for simulations of arbitrary molecule self-assembly processes. It was conceived in view of large-scale deployment on computer grids, but the current paper describes the workstation version of the tool. Its applicability is only limited by (a) the studied system size vs available computational resources and (b) the availability of force field parameters for the studied molecules. The program is written in object-Pascal and used in command-line mode.

**2.1.1. Force Field.** The empirical force field (FF) used by S4MPLE in the current work is an adapted AMBER<sup>22</sup>/GAFF<sup>2</sup> parameter set, including a continuum solvent model, based on simple functions of interatomic distances:

- A linearly distance-dependent relative dielectric constant is used in the Coulomb term, which *de facto* makes it a function of  $1/d^2$ .
- A pair-based desolvation term<sup>25</sup> in  $1/d^4$ , function of the squares of partial charges (with tunable proportionality constant).
- Contact terms,<sup>25</sup> rewarding favorable interactions such as hydrophobic contacts and hydrogen bonds.

Since these terms are not “native” to AMBER/GAFF, the additional parameters needed calibration, as described in ref 3. The resulting “Fit FF” was used in the present work. Benchmarking of fragment-like ligand docking was also performed, for comparative reasons, with the plain AMBER/GAFF formalism (without desolvation, without contact terms), herein referred to as “Core FF”.

**2.1.2. General, Chemically Meaningful Genetic Operators.** The ability of S4MPLE to indiscriminately handle intra- and intermolecular degrees of freedom is the key to sample ensembles including an arbitrary number of independent species and thus be applicable as classical conformational sampler, docking program, and multiple ligand docking tool. This ability is achieved through the appropriate design of the genetic operators, which in typical docking approaches focus on torsional angles to control intramolecular and on rototranslational DoF. In S4MPLE, a genetic operator works on some randomly picked molecular substructure, which may be either covalently connected or not. If connected, the operator will perform movements all while respecting the covalent constraints (bond length, valence angles). If not – the substructure in question being, for example, one of the ligands competing for a binding pocket of the active site – the guidance role of the absent covalent bond is taken over by a putatively favorable contact axis, randomly chosen as a pair of atoms (one in the substructure, one being an external partner) that shall be brought together in order to form a hydrophobic or hydrogen bonding contact. When constrained by covalent bonding distance, operators would basically perform some rotation around the covalent bond (directed mutation) or swapping of substructures between parents (crossing-overs). With nonbonded substructures, operators will follow the optimal contact distance, returning basically random (and yet as clash-free as possible) poses featuring this randomly chosen contact. Please refer to the technical article previously mentioned for detailed information.

**2.1.3. Population Diversity Control: Interaction Fingerprints.** S4MPLE adopts the postulate that two geometries may be considered redundant if they share a same set of contacts. This postulate is embodied by novel, fuzzy, and differentiable

Pairwise Interaction Fingerprints (PIF). Unlike classical ligand-protein IF used in docking,<sup>8,26,27</sup> PIFs are

- general, regrouping both intra- and intermolecular favorable contacts: hydrogen bonds and hydrophobic contacts
- symmetry-compliant, i.e. invariant to swapping of the contact status of topologically equivalent atoms.
- smooth and differentiable, rather than binary: contact status varies smoothly between “absent” (0) and “fully established” (1.0) as a function of contact distance.

A geometry is characterized by its interaction fingerprint, and fingerprint dissimilarity scores are used to assess geometry redundancy, by means of a user-defined dissimilarity threshold.

**2.2. Data Sets.** **2.2.1. Fragments Set.** All complexes featuring fragment-like ligands from the Astex Diverse set,<sup>4</sup> Astex/CCDC clean subset (no covalent ligand),<sup>28</sup> and from Congreve’s review<sup>29</sup> are merged into the fragment data set. A compound is defined as a fragment if its mass is below 300 Da. This mass threshold is used in the rules of three, a set of empirical rules to define fragment-like compounds.<sup>30</sup> The total number of complexes, after removing one PDB duplicate (1N1M), is 142. This complex is only included into the smaller set (Congreve subset) before computing the statistics for each complexes sources (subsets).

**2.2.2. Ligand-Water Set.** A subset of Astex Diverse set, composed of 16 complexes in which water-mediated interactions play a key role, is used to test the multientities docking ability of S4MPLE. In this run, the ligand plus one or several explicit water molecules (considered as additional ligands) are docked, in an attempt to correctly position both ligand and waters as the lowest-energy configuration. These examples include, unsurprisingly, many complexes for which standard water-free redocking failed<sup>3</sup> (RMSD of top-ranked pose > 2 Å). All investigated PDB complexes are listed in Table 1. Additionally, S4MPLE has been challenged to correctly predict the position of a crystallographic water in the poly(ADP-ribose) polymerase (PARP) structure, which is alternatively seen to either mediate a ligand-site interaction

**Table 1. 16 PDB Complexes Selected for the Simulations with Free Explicit Waters<sup>b</sup>**

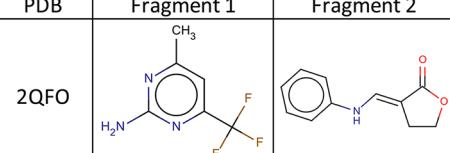
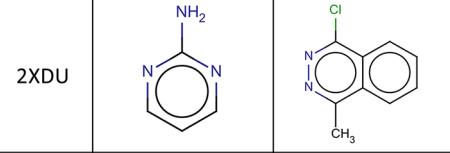
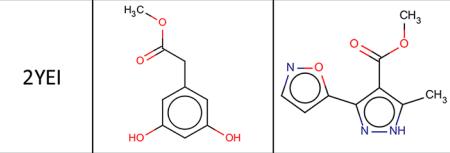
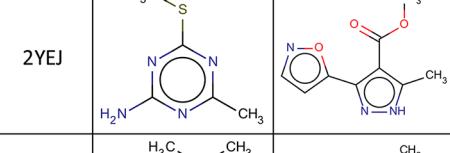
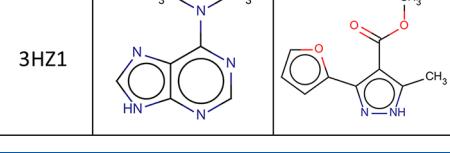
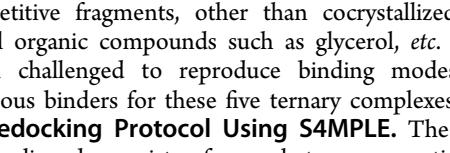
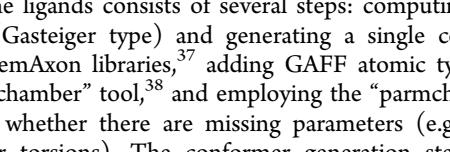
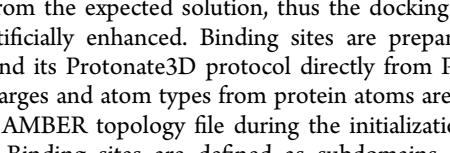
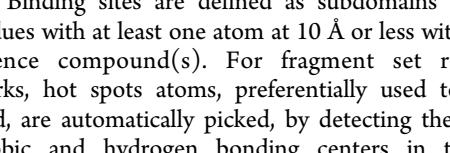
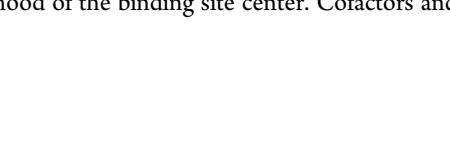
PDB	number of free waters	water IDs
<b>1G9V</b>	3	9596 9209 9269
<b>1GM8</b>	2	13899 13905
<b>1GPK</b>	1	9154
<b>1HVY</b>	6	20311 20320 20344 20383 20461 20689
<b>1LPZ</b>	1	5166
<b>1MEH</b>	1	5560
<b>1N2J</b>	2	8735 8978
<b>1N2V</b>	4	6547 6550 6556 6559
<b>1OPK</b>	2	7964 8009
<b>1P2Y</b>	1	6511
<b>1Q41</b>	1	11312
<b>1SQ5</b>	3	5012 5123 5126
<b>1T9B</b>	2	18426 21222
<b>1XM6</b>	1	5563 5488 <sup>a</sup>
<b>2BR1</b>	1	5381
<b>2BSM</b>	3	3687 4044 4047

<sup>a</sup>An additional water (fixed oxygen) is included since it coordinates a metal ion in the vicinity of the ligand. <sup>b</sup>Misdocked complexes in classical water-free redocking simulations are displayed in bold.

(3PAX complex) or is displaced by a larger ligand making a direct interaction with the site (2PAX complex).

**2.2.3. Multifragments Set.** The target Heat Shock Protein 90 (Hsp90) has been intensively studied within FBDD projects,<sup>31–36</sup> and a large number of fragment-protein complexes have been deposited in the PDB. Among them, there are 5 ternary complexes (2QFO,<sup>35</sup> 2XDU,<sup>32</sup> 2YEI/  
2YEJ,<sup>36</sup> and 3HZ1,<sup>31</sup> see Table 2) which include two

**Table 2. 5 Ternary Hsp90 Complexes Selected for the Multifragments Docking Simulations**

PDB	Fragment 1	Fragment 2
2QFO		
2XDU		
2YEI		
2YEJ		
3HZ1		

noncompetitive fragments, other than cocrystallized solvent and small organic compounds such as glycerol, etc. S4MPLE has been challenged to reproduce binding modes of the simultaneous binders for these five ternary complexes.

**2.3. Redocking Protocol Using S4MPLE.** The preparation of the ligands consists of several steps: computing partial charges (Gasteiger type) and generating a single conformer using ChemAxon libraries,<sup>37</sup> adding GAFF atomic types with the “antechamber” tool,<sup>38</sup> and employing the “parmchk” tool<sup>38</sup> to check whether there are missing parameters (e.g., bonds, angles, or torsions). The conformer generation step avoids starting from the expected solution, thus the docking accuracy is not artificially enhanced. Binding sites are prepared using MOE<sup>39</sup> and its Protonate3D protocol directly from PDB files. Partial charges and atom types from protein atoms are assigned from the AMBER topology file during the initialization of the program. Binding sites are defined as subdomains including only residues with at least one atom at 10 Å or less with respect to reference compound(s). For fragment set redocking benchmarks, hot spots atoms, preferentially used to anchor the ligand, are automatically picked, by detecting the putative hydrophobic and hydrogen bonding centers in the close neighborhood of the binding site center. Cofactors and ions are

included in the binding site, and all waters are removed. During the fragment set redocking benchmarks, all binding site atoms are fixed, by contrast to all other simulations, where polar hydrogens of hydroxyl groups are flexible in order to dynamically optimize the hydrogen bond network.

The redocking simulations of fragment set complexes consisted in 5-fold runs. Each run took 500 generations of 50 individuals, with default mutation and crossing-over probabilities, and the conformational redundancy parameter  $\text{minfpdiff} = 0.01$ . Each simulation can be described by two stages: the first consists in the docking process itself, where poses with the lowest potential energy are sought and saved. The second stage belongs to postprocessing category: there is an optimization cycle of selected poses (nonredundant configurations within an energy window +30 kcal/mol with respect to best one) from the previous stage, and the best poses are saved (maximum 30). Success rate (percentage of correctly predicted complex geometries, at given RMSD level) is then reported as the *average* of success rates of each independent run. Unless mentioned, this preparation workflow and the associated parameters are used for the other simulations too.

**2.4. Redocking Protocol Using FlexX.** FlexX is a very popular docking tool.<sup>40</sup> At the moment, it is included within the LeadIT (version 2.0.2) platform software developed by BioSolveIT. The redocking of the fragment set is performed with FlexX too, for benchmarking purposes. LeadIT is used to prepare a binding site from the PDB files: hydrogens are added, cofactors and metals located near fragment of interest are preserved, and all water molecules are removed. Residue protonation states and orientation of polar hydrogens are manually fixed if necessary. FlexX binding sites include all full residues with at least one atom within a distance of up to 6.5 Å with respect to the reference fragment.

Default values for the entire preparation workflow and docking process are used, except for fragment protonation states and metals where pharmacophoric restraints are disabled. Concerning the protonation states, those defined for S4MPLE runs are employed. For FlexX docking, success rates rely on RMSD listed within LeadIT.

**2.5. Multientity Docking Simulations.** Due to the completely general and unified treatment of the DoF, S4MPLE does not theoretically limit the number and the status of entities. This has several advantages and allows modeling of competitive or noncompetitive binders with respect to the same site. Unlike in multiple-copy approaches like MCSS,<sup>41</sup> each compound interacts with all others as in usual FF implementations. Technically, S4MPLE reads a multimolecule SDF file and produces relative arrangements of various conformers of all the herein present compounds. Stoichiometry may be controlled by explicitly duplicating concerned molecules in the input file. Since storage of a protein site in SDF format is cumbersome, alternative formats (MOL2 or CAR) are used for reading/writing biological macromolecules and their complexes.

Two main types of multiple entity simulations are performed:

- simultaneously docking of one ligand and one (or more) explicit waters

- simultaneously docking of two noncompetitive fragments

**2.5.1. Docking of One Ligand and Free Explicit Waters.** In the herein work, waters can be allowed to move freely, being modeled like ligands (but not appear or disappear). The only specific treatment reserved to H<sub>2</sub>O is the use of TIP3P charges

instead of the Gasteiger charges customarily employed with ligands. The challenge here consisted of checking whether addition of water molecules, in the absence of any information of their crystallographic positions, would allow it to predict the ligand-water-site interaction network, in correctly locating both ligand and at least some of the added water molecules. The explicit waters are also embedded into the continuum solvent model – their presence is necessary to highlight specific interactions that cannot be reduced to simple dielectric shielding. By default, S4MPLE treats all the DoF equally and pays no special attention to the “real” ligand vs accompanying water molecules. However, docking of a ligand plus few H<sub>2</sub>O molecules in an active site, which is completely stripped of crystallographic waters, may not necessarily find the water-mediated ligand-site interactions. The explicit waters might well be captured in significant site-water-site interactions. This makes it difficult to predict the number of explicit waters that must be added in order to solve the problem. Since the goal here is to try to reproduce experimentally observed binding modes, the experimental structure was used to answer this question. Various scenarios, featuring from 1 to 6 explicit waters, have been tested depending on the complex of interest. An addition of more water particles amounts to an increase in DoF – a significant one, if the site is frozen and the ligand itself is relatively small. Therefore, some general and some specific strategies have been developed for ligand+water docking:

- protocol A: usual settings, 2000 generations,
- protocol B: usual settings, 5000 generations,
- protocol C: protocol A, with a temporary bias toward water-ligand interactions,
- protocol D: protocol C, followed by a specific postprocessing refinement of water positions around the kept ligand poses.

The bias in protocol C is introduced in order to artificially enhance the chance of discovering poses with ligand-water-site interactions over site-water-site interactions, as hinted above. Recall that genetic operators will tentatively place waters in the neighborhood of randomly picked hydrogen bond donor and acceptor “hot spots”, which may belong either to the protein site or to the ligand. Site hot spots are automatically defined according to the same procedure used in the default redocking study,<sup>3</sup> such as to pick acceptors/donors situated close to the bottom of the site. Any putative donor/acceptor of the ligand automatically counts as “hot spot”. By default, hot spots are identically treated by the genetic operators, irrespectively of their origin. The bias consists in

- automatically removing putative site-water hydrogen bonds from the list of favorable contacts used by the crossover procedures, and
- specifically scaling up hydrogen bond interactions between waters and the actual ligand by a factor of 2 (with respect to default FF settings). After the sampling stage, this energy bias is removed, during a postprocessing stage reranking poses according to their unbiased Fit FF energy levels.

Protocol D includes a postprocessing step meant to “shake up” the suboptimally positioned waters at the docking step. Applied to each of the kept poses, this protocol allows the docked water configurations to be challenged by different water poses, evolved during 100 generations, from a population including the docked pose as an initial individual. During this procedure, the ligand atoms are assigned passive status, i.e. they are ignored by all the genetic operators except local gradient-based optimizations. As in default docking, all these protocols

(A–D) are followed by a final relaxation of all so-far kept binding modes, after the pruning of worst and redundant poses.

**2.5.2. Docking of One Ligand and Displacing a Water Molecule.** The analysis of X-ray complexes often highlights water-mediated hydrogen bonds between the ligand and the binding site. A subsequent ligand optimization can be a modification in order to displace the mediating water (especially if it appears poorly anchored), allowing a direct interaction between the ligand and the target. The ability of S4MPLE to predict this kind of effect is assessed, based on experimental data of this nature: two PDB complexes of the poly(ADP-ribose) polymerase, including distinct bound fragments, are used (2PAX/3PAX).<sup>42</sup> In the 3PAX structure, the methoxybenzamide compound makes three direct hydrogen bonds with the binding site (two with G863 and one with S904) and one water-mediated with E988. In the 2PAX structure, the amino-naphthalimide molecule adopts a similar binding mode but with a direct hydrogen bond with E988. The naphthalimide compound can be described as a slight optimization of the methoxybenzamide fragment in order to maintain the amide group into its optimal configuration through cyclization and to displace the water while maintaining the polar interaction with the E988 side chain.

In a first step, S4MPLE is challenged to predict the location of studied fragments into their own binding site (redocking) with one free water in both cases. In the ideal case, it will find the correct binding mode of fragments and the good location of the free water in the 3PAX structure. Since the water does not mediate the hydrogen-bond between the fragment and the target in the 2PAX structure, it should be docked in another area of the site (e.g., site-water-site interaction). Simulations were performed according to both above-mentioned protocols C and D, in order to verify whether the “aggressive” favoring of water-ligand interactions would not prevent the eviction of the water mediated interaction in 2PAX.

Additionally, cross-docking simulations were also performed, in order to ensure that redocking success was not conditioned by the (very small) changes in active site geometries.

**2.5.3. Docking of Two Organic fragments.** The aim of this study is to evaluate the ability of S4MPLE to predict the simultaneous locations of two compounds within a given binding site. By contrast to previous multientities simulations involving one ligand and explicit solvent molecules, two “real” ligands are docked here. The investigated protein is the molecular chaperone Heat Shock Protein 90 through several ternary PDB complexes containing each two bound fragments. For convenient reasons, the first fragment of a given PDB code (XXXX) will be referred as XXXX\_1 and the second as XXXX\_2.

Two distinct multientity docking protocols (simultaneous and sequential) have been investigated. The simultaneous approach may be more rigorous than docking of individual known fragment binders, because it may implicitly account for any potential competition of different compounds for the same binding pocket and for favorable interfragment interactions. Nevertheless, the number of DoF quickly increases when simulating more than one compound; therefore, a higher number of generations must be allowed. In practice, 8 independent runs are performed and subsequently followed by the merging of all poses before the final relaxation stage. The number of generations is set to 2000. A maximum of 500 poses and a *minfpdiff* value of 0.004 are employed. All the other parameters use previously described values (see §2.3). Binding

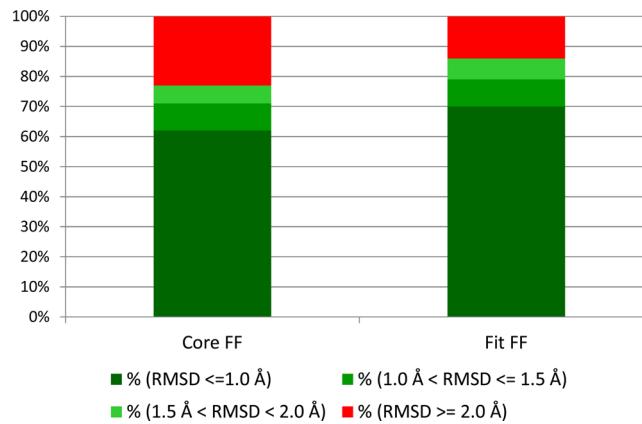
sites are prepared as usual, including all residues within a radius of 10 Å around the considered reference (here two distinct fragments). Conserved waters, over a large number of X-ray structures and mediating ligand-site interactions around the hot spot D93 (4 H<sub>2</sub>O in complexes 2QFO, 2XDU, 2YEJ, 3HZ1 and 3 H<sub>2</sub>O in 2YEI), are included into the binding site too.

Alternatively, sequential docking decouples the exploration of DoF of involved ligands, i.e. breaks the general simultaneous sampling down to two sequential analyses of each ligand alone, and then tries to find combinations of poses that are mutually compatible. In the first step of the sequential docking, fragments are individually docked into their binding site with the same parameters as in fragment redocking benchmark (see §2.3). In a second step, each kept pose of fragment A is automatically associated with anyone of fragment B in order to generate all the combinations. The last step consists of the usual poses filtering, with a larger energy window (*excess\_energy* = 20000 kcal/mol), allowing several slight overlaps – but not competitive poses for the same subpocket, in which both fragments significantly overlap. Selected combinations are eventually energy-minimized, putatively allowing the initially tolerated bad contacts to rearrange into favorable interactions between the binding fragments.

### 3. RESULTS AND DISCUSSION

**3.1. Redocking of Fragments.** The ability of S4MPLE to dock fragment-like compounds is evaluated using usual redocking simulations. It should be noted that these molecules can be very challenging since they can adopt a huge number of binding modes, because of their small size. Thus the scoring function must be able to highlight the good binding mode out of a lot of wrong poses, generally with scores/energies in a pretty small window. At the opposite, the selection of the correct binding mode of larger ligands is paradoxically easier: albeit the problem space volume may be much larger, the total number of clash-free poses within this volume is relatively small, as there are not so many ways to make the relatively large ligand fit into the defined binding site. As far as the issue of sampling these clash-free poses is solved, the scoring function must only be able to discriminate the correct binding mode among a smaller number of decoy conformations. However, a recent study<sup>43</sup> concluded that there is no significant difference in docking accuracies of “usual” ligands vs fragments, but authors highlighted that the observed failures stemmed from different causes as outlined above (scoring vs sampling issues). The authors also demonstrated that high Ligand-Efficiency (LE, defined as the affinity divided by the number of heavy atoms<sup>44</sup>) fragments are also docked with more accuracy – this can be understood intuitively, but a rigorous validation is still required. In S4MPLE, scoring is consistently performed in terms of the force field energy function used at the sampling stage, so artifacts due to fragment-like binders being outside the applicability domain of drug-like compound specific scoring functions are not expected.

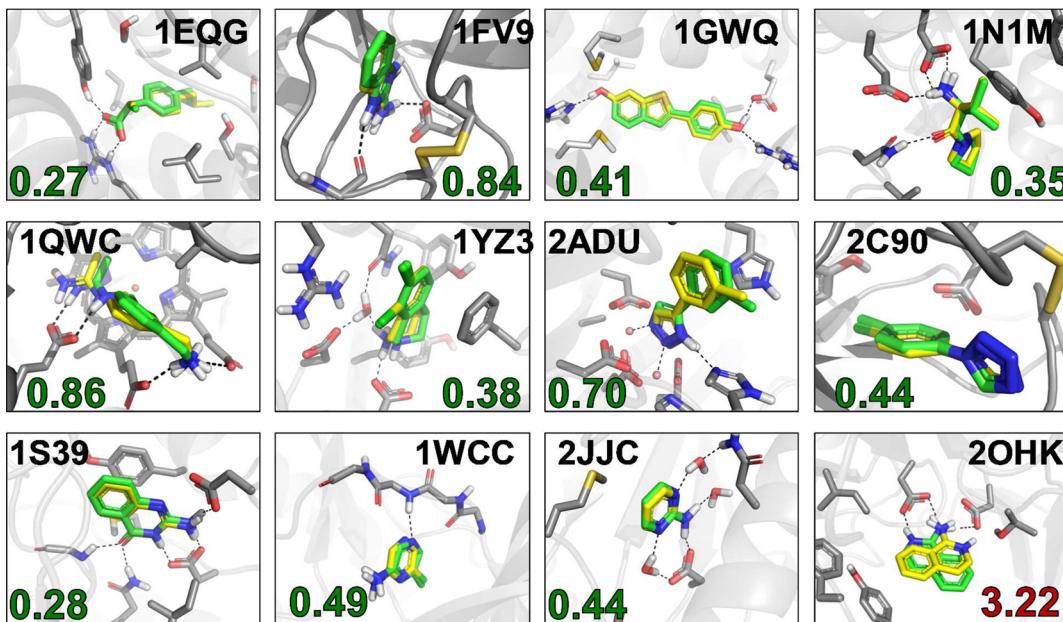
The fragments redocking results with S4MPLE are summarized in Figure 1, Figure 2, and Table 3. Full details about selected complexes and each run are also available in the Supporting Information. Success rates are quite high, and the Fit FF significantly outperforms the Core FF energy function (respectively 86% and 77% at 2 Å for the top-ranked pose), as in previous benchmark studies.<sup>3</sup> By contrast, the redocking using FlexX only led to 72% for the same criteria. However, by definition, these compounds are pretty small and do not make a



**Figure 1.** Docking performances (% complexes with RMSD of the top-ranked pose in a given range) over the full fragment set, for both Core FF and Fit FF energy schemes.

lot of interactions with the binding site compared to larger ligands. The usual RMSD threshold (2 Å) can be misleading: using an alternative metric<sup>8</sup> or more stringent thresholds (e.g., 1.5 Å or even 1 Å)<sup>43</sup> should be more appropriate to discard failures from successes. A noteworthy point is that the success rate only decreases of 7% by lowering the RMSD threshold from 2 Å to 1.5 Å for the Fit FF mode (79% accuracy). Besides, at the stringent 1 Å limit, the accuracy is still a quite high 70%. Expectedly, there are no sampling problems with these smaller compounds: the expected binding mode is quasi-systematically found among the saved poses (around 98%). Many (93) of these complexes (source: Astex/CCDC) were present in the calibration protocol of the Fit FF:<sup>3</sup> the consequence could be an artificially bias toward the experimental solution. However, the other complexes not used for FF fitting (Astex Diverse Set and complexes from the Congreve’s review) show equivalent or even better success rates. Indeed, most failure cases come from the Astex/CCDC-clean-subset itself for both Core FF and Fit FF.

Several papers focusing on fragments docking were published these last few years.<sup>8,43,45–47</sup> Among them, complexes from Congreve’s review<sup>29</sup> are often used as a reference data set.<sup>45,46</sup> Although small (12 PDB complexes – it was not designed for benchmarking), the latter covers diverse targets, a variety of interactions (purely van der Waals, hydrogen bonds, ionic bonds, or even metal coordination), and a wide affinity range (from sub-nM to high-mM). A comparative table (Table 4) summarizes the redocking results of S4MPLE and those found in the literature for these 12 complexes. 1QWC did not systematically converge toward the expected solution using Fit FF, hence the slightly lower average success rate. Figure 2 shows the superimposition of both experimental and predicted binding modes (using the Fit FF scoring scheme) and the polar interactions between fragments and the binding pocket. Haider et al. used two popular tools (GOLD<sup>48</sup> and MCSS<sup>41,49,50</sup>) to dock these fragments into their targets.<sup>46</sup> Besides, they investigated the impact of a rigorous rescoring scheme based on a Generalized Born Surface Area (GBSA) solvation model. Their results show a global success rate of 67% and 75% in the strategy based on GOLD, respectively without/with the GBSA rescoring scheme. At the opposite, Loving et al. described a perfect accuracy (100%) over this data set,<sup>45</sup> using Glide as docking engine.<sup>51</sup> However it should be noted that all waters around fragments are systematically included in the binding site



**Figure 2.** Top-ranked poses from redocking simulations of Congreve's data set, using the Fit FF energy function (RMSD with respect to X-ray coordinates are provided for each complex). Experimental and docked locations are represented in green and yellow, respectively. Main polar interactions are depicted as dash lines.

**Table 3. Docking Performance of Fragment-Like Compounds (Full Fragment Set and Its Subsets) Using S4MPLE<sup>a</sup>**

data set (size)	energy function	success rate (%) top-ranked pose	success rate (%) top 30 poses
full fragment set (142)	Core FF	77	98
	Fit FF	86	98
Congreve subset (12)	Core FF	92	100
	Fit FF	88	95
Astex Diverse subset (37)	Core FF	84	97
	Fit FF	90	99
CCDC clean subset (93)	Core FF	72	97
	Fit FF	84	98

<sup>a</sup>Success rates, defined as the percentage of correctly predicted geometries with RMSD < 2 Å for the top-ranked pose, are reported as the average success rates of the 5 independent runs.

**Table 4. Docking Performance of Several Docking Tools on the Congreve Data Set<sup>a</sup>**

docking program	inclusion of crystallographic waters	success rate for the top-ranked pose (%)
S4MPLE (Core FF)	2JJC	92
S4MPLE (Fit FF)	2JJC	88
GLIDE <sup>45</sup>	all sites	100
MCSS <sup>46</sup>	2JJC/1YZ3	67
MCSS (GBSA rescoring) <sup>46</sup>	2JJC/1YZ3	67
GOLD <sup>46</sup>	2JJC/1YZ3	67
GOLD (GBSA rescoring) <sup>46</sup>	2JJC/1YZ3	75

<sup>a</sup>SAMPLE success rates are reported as the average of success rates of the 5 independent runs, while other values are directly extracted from the literature. Binding sites in which crystallographic waters were not removed during the docking simulations are listed.

(distance threshold of 5 Å around the reference fragment) and certainly channel the ligand toward its expected position. Our results are good (one failure out of the twelve cases) using water-free binding sites, except for the Hsp90 2JJC complex, where waters around the D93 residue are known to be conserved over a huge number of PDB complexes and absolutely needed to explain the binding of a fragment mostly interacting with the site through water-mediated bridges. Surprisingly, the failure is not the same for both FF setup schemes: 1WCC for Core FF and 2OHK for Fit FF, see Figure 2. In the last case, the main interactions are globally conserved, but there is a flip of the isoquinolin cycle on itself.

As a final remark, it is interesting to point out that success rates obtained with fragment-like compounds are similar to those concerning a data set mainly composed of drug-like molecules,<sup>3</sup> as very recently described by Verdonk et al.<sup>43</sup>

### 3.2. Docking of One Ligand and Free Explicit Waters.

The results of these multiligands simulations, depicted in Table 5, are analyzed according to several criteria:

- “top-ranked pose” and “over saved poses”: ability to accurately reproduce the experimental binding mode, for the top-ranked pose and, respectively, within one of the saved ones (not necessarily the most stable) and
- “over all entities”: ability to visit at least once the expected location for every entity (ligand and water) over the whole set of saved poses

The heavy-atom RMSD is employed as metric to discard successes from failures, and two different thresholds are monitored (1 Å and 2 Å). By default, 2 Å is employed. However the more stringent value has its importance too, since a stricter limit should be more consistent with waters (only one heavy atom). Indeed a RMSD of 2 Å can be accepted for drug-like ligand poses, but not H<sub>2</sub>O poses, for it does not guarantee the preservation of hydrogen bonds.

The first two result columns “top-ranked pose” in Table 5 refer to the best-energy configuration retrieved by each protocol. Column “lig” reports the percentage of complexes

**Table 5.** Results for Each Protocol (A–D) from Ligand-Water Docking Simulations<sup>a</sup>

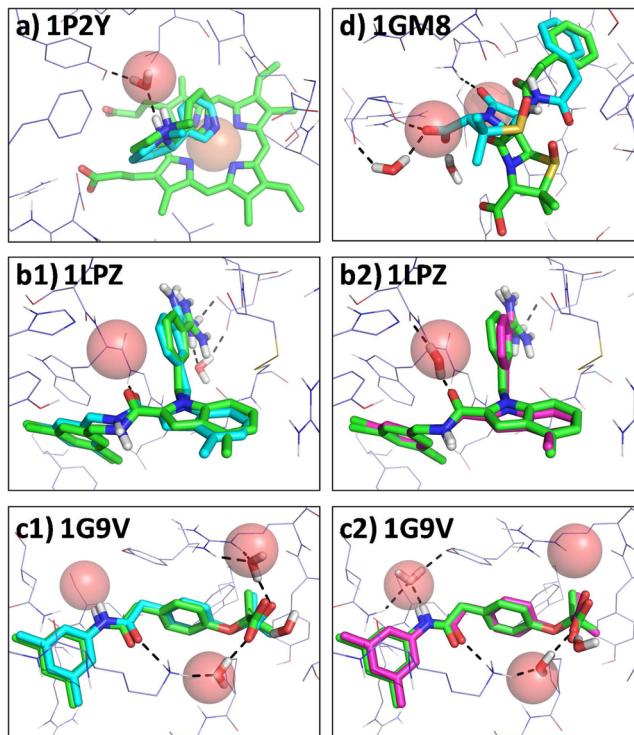
protocols	% (RMSD < 2 Å)					
	top-ranked pose		over saved poses		over all entities	
	lig	waters	lig	waters	lig	waters
protocol A	56	15	94	29	94	74
protocol B	56	9	100	32	100	74
protocol C	56	26	100	41	100	71
protocol D	69	41	94	76	94	94
protocols	% (RMSD < 1 Å)					
	top-ranked pose		over saved poses		over all entities	
	lig	waters	lig	waters	lig	waters
protocol A	31	9	81	18	88	56
protocol B	25	6	75	21	88	62
protocol C	38	15	81	32	81	62
protocol D	56	38	81	62	88	94

<sup>a</sup>For each considered criteria, values are divided into two subcategories (respectively for ligands and waters). Statistics for two different RMSD thresholds (1 and 2 Å) are compiled.

in which the ligand entity has been placed within the given RMSD threshold with respect to experiment: 56% means that in 9 complexes out of 16, protocol A (see §2.5.1) managed to place the ligand within 2 Å in the most stable pose. Column “waters” must be understood in the context of a variable number of added H<sub>2</sub>O entities in each complex. If in a complex with W water molecules only w are within 2 Å from expected position, this complex will count only as a partial success: the success counter is incremented by w/W. The reported percentage represents thus the sum of all w/W indices, over the 16 considered complexes and may be alternatively read as the percentage of correctly placed water molecules. The next two columns “over all entities” refer no longer to the top-ranked pose but to the geometrically best saved pose, featuring a correctly positioned ligand and as many well located waters as possible. This means that, for example, protocol A managed to visit at least one state with correctly positioned ligand for 15 out of 16 complexes (94%), at RMSD < 2 Å. However, over these states, only 29% of added water molecules also converged toward experimental positions. Eventually, the last two result columns “over all entities” refer to the overall ability to visit at least once each of the experimentally relevant positions of ligands and waters. Within the low-energy poses that were saved in protocol A, 74% of water molecules are at least once placed where they are expected – however, not simultaneously (some conformers witness a correct position of water 1, others of water 2, etc.).

It must be noted that this data set contains an important number of complexes which failed in water-free redocking simulations (7/16, see Table 1 and the associate study).<sup>3</sup> These failures should now be fixed, in as far as they were actually caused by the lack of water molecules. However, if different sources of errors affect those compounds (typical FF inaccuracies), then any errors in ligands placement will obviously prevent waters to be correctly located. Figure 3 shows the main scenarios which can be obtained in this kind of simulation (see Supplementary Information for details about all complexes):

a) successful placement of all entities (ligand and H<sub>2</sub>O) for the top-ranked pose,



**Figure 3.** Examples of scenarios obtained in multientities docking simulations with free waters using protocol D: a.) success in prediction of all locations of ligand and water(s) for top-ranked (1P2Y); b.) experimental geometry found among saved poses – other than first (1LPZ); c.) partial success (correct positioning of several entities, including ligand, and at least one water, 1G9V); and d.) no acceptable solution (1GM8). Experimental ligand conformer is in green, while expected water locations are red spheres. Predicted poses are represented in blue (if top-ranked) or purple (other rank) for ligand, and waters are represented as stick models. Main interactions are depicted as dash lines.

b) successful placement of all entities, but for a higher energy pose,

c) partial success: ligand and some but not all waters simultaneously and correctly located (e.g., over all poses),

d) full failure: there is no acceptable solution for the ligand and at least one water molecule over the whole set of poses.

Results of each docking protocol (see §2.5.1) will be briefly analyzed in the following:

**3.2.1. Protocols A and B: Usual Docking Settings with 2000/5000 Generations.** Although the number of generations is critical (shorter simulations lead to expectedly bad results, data not shown), going for 5000 generations, instead of 2000, does not improve the poor success rates with respect to the three RMSD criteria. This is particularly true for the ability to simultaneously reproduce the expected binding mode of all entities within a same pose – preferentially the most stable one. The top-ranked statistics are equal to previous water-free redocking results for ligands (56% success rate), while the water molecules are poorly positioned (15%). Within the entire set of poses, the binding mode of ligands is well retrieved (94% and 100%), but the prediction rate of water-mediated interaction patterns remains low ( $\approx$ 30%). However, the actual hot spots involved in water-mediated interactions are indeed attractors in which water molecules are often found: about 3/4 of these locations are visited by waters for these two protocols. Logically, complexes with a lot of free waters (e.g., 1HVY,

1G9V, 1GM8) are, for pure probabilistic reasons, much more difficult to predict and lower the overall statistics, whereas simpler systems with a single additional water are well predicted (e.g., 1LPZ, 1P2Y). These results show that the sampling procedure is quite effective, in the sense that independent moieties do indeed visit their expected positions – however, the consensual event (all moieties being at their correct position at the same time) is too rare using default settings to reproducibly occur during a span of 5000 generations only.

In as far as the (energy-minimized) experimental pose is not the most stable in terms of its computed FF energy, failing to find it cannot be held against the sampling procedure – in this case, the expected geometry would be perceived as some arbitrary intermediately stable state. In this context, such failure may not necessarily be interpreted as a straightforward FF parametrization problem. It is not clear upfront that an explicit water molecule, in the presence of protein site, ligand and implicit solvent, would spontaneously favor the ligand–water–site mediator spot, for many other crystallographic waters have been removed during site preparations, and only a few have been reinstated during these simulations. The FF model relies on a fine balance between implicit desolvation and interactions with explicit water molecules. Since continuum desolvation is included in the energy function, the energy bonus due to water-mediated bridges is small, for it includes the desolvation penalty of the polar groups. Two water molecules embedded in a perfect solvent model should, on the average, not seek to make contact to each other, for the favorable Coulomb terms should be canceled out by the desolvation contribution. Unilateral site–water or ligand–water contacts may be only slightly beneficial, if the corresponding hydrogen bonding strength exceeds typical water–water interactions, or if water binding at a given hot spot is entropically favored. It is unclear to what extent FF calculations are accurate enough to reproduce the subtle balance of conflicting energy terms (desolvation penalty, Coulomb interaction, and hydrogen bond bonus) involved in the process.

On the opposite, if the expected configurations are the most stable ones, or energetically very close to the absolute minimum and were nevertheless not sampled, this means that the herein envisaged sampling problems were much harder than standard flexible ligand docking – and this, albeit the number of additional DoF does not significantly increase. It is the nature of the landscape, however, which may change. Water-mediated interaction contributions in the presence of an implicit desolvation term being small, the landscape may feature many possible local minima of rather equivalent depths, and not separated by any significant barriers – flat and shallow energy landscapes are notoriously difficult to sample. Furthermore, GAs are effective sampling algorithms due to their ability to inherit favorable “traits” of the ancestors. In flexible ligand docking, if a correct ligand–site anchoring point is found, this interaction is a favorable “trait” because it provides local stabilization. Individuals containing it will have, statistically speaking, larger chances of survival, and the well-formed contact will entropically enhance other hot spots, topologically close on the ligand, to approach their corresponding anchoring points in the site. Docking is locally cooperative – very much like folding of a protein  $\alpha$ -helix. By contrast, in multiple docking with several interacting species, it does not pay off to correctly position a water molecule unless the ligand is also in place. In other words, an energy bonus due to the site–water–ligand bridge will only emerge if both water and ligand are well

positioned. Otherwise, an individual featuring the correct site–water contact will not be preferentially selected with respect to other site–water contact featuring competitors, which cannot lead to the proper ternary configuration. Likewise,<sup>52</sup> folding of  $\beta$ -sheets is more difficult than folding of  $\alpha$ -helices: a state with one strand in  $\beta$ -sheet configuration is not energetically favored before the complementary half is formed and zipped into its final position. There is no local cooperativity in ligand–water–site docking either. Note that simultaneous docking of multiple fragments binding to different subpockets, i.e. not (significantly) interacting with each other in the lowest-energy poses would not suffer from such an entropic bottleneck (§3.4). The correct placement of one fragment in its subpocket does pay off, irrespectively whether the other fragments are well-located or not (in as far as they are not clashing).

In order to understand whether the poor success rates above are due to (a) suboptimal binding energies, inclusively due to FF inaccuracies or rather due to (b) difficult sampling cause by lacking local cooperativity, two additional docking protocols were envisaged:

**3.2.2. Protocol C: Protocol A + Energetic Bias in Favor of Water–Ligand Interactions.** In this strategy, a (temporary, sampling stage-only) bias toward water–ligand hydrogen bonds is automatically added in order to decrease the frequencies of site–water–site interactions, which are not interesting in the context of this study. This strategy improves water position predictions for the top-ranked pose (26% vs 15%) and over the saved poses (41% vs 29%). Note that the above “top-ranked” refers to the unbiased energy, after removal of the temporary strengthening of ligand–water hydrogen bonds. The improvement is thus mainly due to the sampling protocol spending more time in the relevant problem space, featuring compulsory ligand–water contacts. As in protocols A and B, low complexity systems are obviously more efficiently reproduced with respect to high complexity system. Even so, the final success rate is judged insufficient – yet, it suggests anyway that adapting the sampling strategy to the specific nature of this problem may pay off, hence the development of the strategy D with final water refinement.

**3.2.3. Protocol D: Protocol C + Water Refinements around Selected Poses.** This approach involves a refinement of all waters around each of the poses kept for the ligand. All new low-energy configurations are stored before the final relaxing step, where the considered DoF are minimized. Compared to previous protocols, there is a great improvement of results in all monitored categories. Thus, the accuracy of top-ranked pose increases from 56% to 69% for ligands and from 26% to 41% concerning waters. The improvement appears more even flagrant over the entire set of saved poses: regarding water location prediction, the accuracy jumps from 41% to 76%. Besides, most of these waters are particularly well predicted: 62% at 1 Å with respect to 76% at 2 Å. Regarding the ability to visit the expected location of all entities, there is an enhancement with respect to previous schemes, especially for waters (from 62% to 94% at 1 Å). These high accuracies reflect the ability to generate poses with both ligand and waters close to their expected locations, even with high complexity systems (e.g., 1G9V, 1HVY, 1N2V, 1SQ5, 2BSM which all contain at least 3 free waters).

Clearly, undertaking an exhaustive search of the optimal locations of water molecules around each of the putative ligand positions does pay off. This is consistent with the fact that the energy landscape is rather flat with respect to the DoF of

waters. Furthermore, the interaction fingerprint changes little with respect to water positions, since these are responsible for few (typically one or two) contacts — poses with identical ligand placements, differing only with respect to water positioning are at risk of being discarded as “redundant” during the evolutionary process. Default simulations would spend most of the effort in trying to explore alternative poses for the main ligand but do not spend enough time in trying to rearrange waters around each pose. If the latter aspect is artificially enhanced, like in the current protocol, significant improvement can be obtained: if the correct ligand pose is among the considered (true in 94% of the cases), the specific optimization of the explicit solvent surrounding it would eventually enumerate the wanted configuration as well. It is also clear — and not really surprising — that the desired water-mediated interaction configurations are not systematically the lowest energy configuration. This is partly an intrinsic weakness of the approach: if water-binding pockets of higher affinity than the ligand-water-site key points exist, they should be filled with explicit water before trying to position bridging water molecules, which are otherwise at risk of being captured by such energetically more rewarding sites. As for example, a water molecule is often located between two negatively charged side chains. The other part of responsibility pertains to the intrinsic FF inaccuracies. Unfortunately, it is very difficult to precisely delimit the role of each of these failure scenarios. In either case, these inaccuracies are however not large enough in order to completely disqualify the wanted configurations from the final list of kept poses.

The challenging complex 1GM8 is a major source of errors: the binding mode of the penicillin-G derivative involves several water-mediated interactions and the carboxylate group, totally solvent exposed, does not make any polar interaction (even mediated) with the binding site. The FF greatly favors (by a computed  $-24$  kcal/mol) a wrong binding mode involving this anionic group in both direct and water-mediated hydrogen bonds with the site. This example accounts for the 6% of cases in which a correct ligand pose never made it into the subset of saved geometries (“over saved poses” criterion decreasing from 100% to 94%). In previous protocols, the correct ligand pose was nevertheless saved — here, the aggressive optimization of water positions provides an additional stabilization for the wrong ligand pose, which eventually leads to discarding of the correct.

Like 1GM8, 1G9V is a recurrent failure in both classical (implicit solvent-only) and previous docking protocols. However, this protocol led to several poses, including the top-ranked one, very close to the expected configuration ( $\text{RMSD}_{\text{ligand}} < 0.5$  Å and 2 waters out of 3 make the crystallographic ligand-water-site interactions - see Figure 3.c1 and c2). Inclusion of waters allowed generating a good top-ranked solution for the 1G9V complex, when water-free simulations, whatever the considered energy scheme, failed to converge toward the experimental binding mode.

To our knowledge, S4MPLE is the only tool not dealing with crystallographic waters in terms of on/off toggles but as actual physical entities. Empirical simulations with variable numbers of atoms are notoriously difficult to interpret, as the (free) energy functions piloting these were designed to account for conformational changes, not for variations of the included interaction lists. The “entropic” penalty should be rather understood as an empirical compensation for the varying number of terms in the scoring function. This is a weak point of

all attempts to deal with solvent by inserting or deleting waters, and does not concern S4MPLE, where explicit waters are displaced to alternative locations, not deleted. The weakness of our water treatment strategy is that the initial number of explicit waters was chosen as an empirical parameter - in perspective, it might be interesting to determine this number by “saturating” all the hydrogen bonding options of the ligand, like in the MVD strategy.<sup>21</sup>

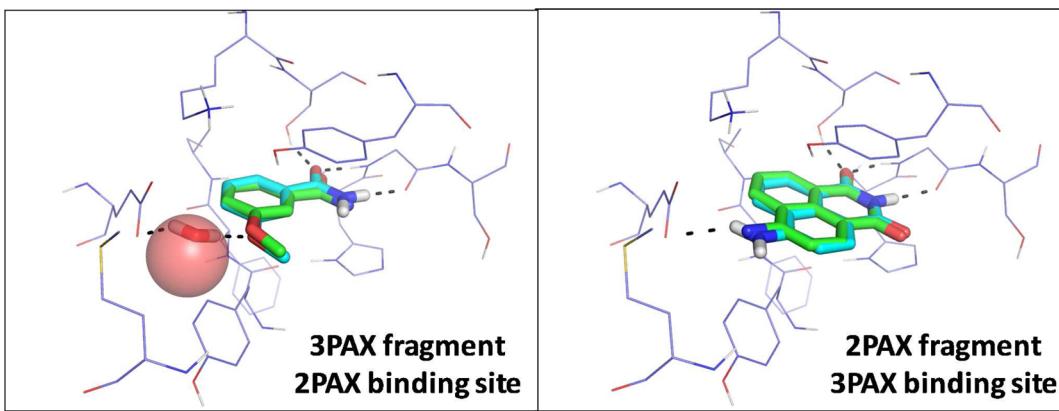
Albeit the so-far reported results are encouraging, the coexistence of explicit water molecules and implicit solvent may eventually require some dedicated fine-tuning of force field parameters. For example, the hydrophobic term, implicitly accounted for by the number of hydrophobic contacts, stands for the entropy gain associated with water molecules leaving the geometrically constrained orientations at the interface and rejoining the bulk solvent. It is unclear how the behavior of explicit waters (the “entropy” of which could, at least in principle, be illustrated by the ensemble of visited stable poses) would interplay with the implicit solvent terms.

**3.3. Docking of One Ligand and Displacing a Water Molecule.** This simulation could be classified as a special case of the previous runs involving one ligand and explicit water molecules, and the results are summarized in Table 6.

**Table 6. RMSD from Cross-Docking Experiments on the PARP Target (2PAX/3PAX Complexes) from the Displacing Water Study**

		binding site 2PAX	binding site 3PAX
entities 2PAX	fragment 2PAX	0.19	0.29
	water (no bridged HB)	10.67	10.43
entities 3PAX	fragment 3PAX	0.31	0.30 (#1) 0.42 (#2)
	water (bridged HB)	0.80	2.78 (#1) 0.82 (#2)

Concerning the 3PAX complex, both first and second poses from redocking contain the correct binding mode for the ligand (RMSD of 0.30 Å and 0.42 Å respectively). While preserving the expected water-mediated hydrogen bond in both geometries, only the second one correctly locates the water from a RMSD point of view (0.82 Å vs 2.78 Å). Indeed, the water is slightly translated in the top-ranked pose in order to make an additional (non-native) interaction with a tyrosine (Y907 - flexible hydroxyl group considered) in the vicinity of the ligand. The second pose, from an energetically point of view, perfectly reproduces the experimental data. Redocking of 2PAX leads to the expected solution with a very low RMSD (0.19 Å) for the ligand, which is larger than 3PAX and features no water-mediated hydrogen bond with the E988 side chain: the water molecule, as expected, moves to some arbitrary hydrophilic subpocket of the site, not far (~1.5 Å) from the location of an original crystallographic water (HOH 1018 in 3PAX). The amine group of the 2PAX ligand takes the place of water in 3PAX. The cross-docking runs converged toward the experimental binding modes for both ligands (RMSD of 0.29 Å and 0.31 Å) too, and the location of water is correctly predicted when needed (mediated interaction with the 3PAX fragment, but no bridged interaction with the larger 2PAX fragment). Figure 4 displays the obtained binding modes in the cross-docking runs. This last example, in addition to previous simulations on the ligand-water set, highlights the ability of



**Figure 4.** Structures from cross-docking run for the PARP target. Experimental ligand conformer is in green, while expected water locations are red spheres. Top-ranked poses are represented in blue for ligand and waters as stick. Main interactions are depicted as dash lines.

S4MPLE to deal with explicit water particles. Besides, the locations of entities, when correctly found, are generally reported with high accuracy. This holds irrespectively of the employed water docking protocol C or D, showing that statistically favoring ligand-water contacts did not spuriously lead to a failure to evince the water molecule.

**3.4. Docking of Two Organic Ligands.** Prediction of simultaneous binding modes of fragment-like compounds is an excellent benchmark for testing the multiligand sampling capacity of S4MPLE. This ability is of particular interest in the FBDD field.<sup>24</sup> Indeed, the probability of simultaneous binding in the same active site is higher than for larger molecules, and predicted binding modes are of direct interest, as this constitutes the starting point for drug design by fragment linking. Fragment screening based on native mass spectrometry<sup>53</sup> is able to detect a multibinding event. Although useful, this information is insufficient for a rational optimization since the binding modes remains unknown. In theory, X-ray crystallography can easily solve this problem, but it can be long and expensive in practice. Therefore, docking algorithms, able to deal with several entities, can be used to predict the geometry of these ternary complexes “fragment1+fragment2+protein”. The docking results, if judged credible (e.g., interactions with known hot spots), enable the subsequent use of SBDD for a more rational optimization or evolution by means of a linking strategy. Moreover, it has been experimentally demonstrated that fragments can adopt different binding modes when bound alone or simultaneously with another compound (see PDB codes 3HYY, 3HYZ, and 3HZ1 and the associated article<sup>31</sup>). Thus, it appears more consistent and reliable to try to get access to the geometry of the ternary complex itself. Table 7 sums up all the results relative to the multifragments docking performed on selected Hsp90 complexes.

**3.4.1. Simultaneous Protocol.** A successful docking is achieved for the complex 2QFO, in terms of top-ranked pose and accuracy ( $\text{RMSD} \approx 0.5 \text{ \AA}$  for both compounds). It should be noted that large conformational differences of the site are observed between 2QFO and other structures. In the latter, an additional subpocket, systematically occupied by one fragment, emerges as a result of a restructuration as  $\alpha$ -helix of the sequence K100 to E120. The consequence is that the 2QFO binding site is half as large as in other investigated structures (note that, given the definitions of active sites as sets of residues within a 10  $\text{\AA}$  sphere around any atom of the ligand, current

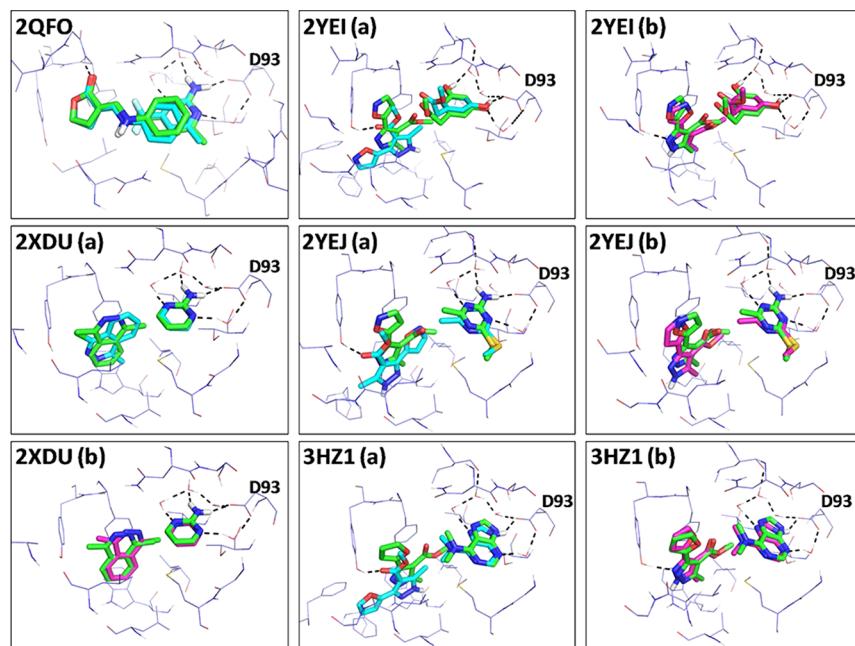
**Table 7. Results from Multifragments Simulations for Both Investigated Protocols (Simultaneous and Sequential)<sup>a</sup>**

PDB	simultaneous docking					
	top-ranked pose		all saved poses			
	rank	RMSD fragment1	RMSD fragment2	rank	RMSD fragment1	RMSD fragment2
2QFO	1	0.40	0.61	1	0.40	0.61
2XDU	1	13.47	0.62	12	1.17	0.69
2YEI	1	3.05	4.36	187	1.47	1.21
2YEJ	1	0.47	4.12	5	0.34	1.26
3HZ1	1	0.37	4.37	2	0.29	0.68
PDB	sequential docking					
	top-ranked pose		all saved poses			
	rank	RMSD fragment1	RMSD fragment2	rank	RMSD fragment1	RMSD fragment2
2QFO	1	0.26	0.46	1	0.26	0.46
2XDU	1	0.34	4.23	5	0.37	0.71
2YEI	1	0.98	4.31	3	0.98	1.25
2YEJ	1	0.59	4.24	10	0.53	1.34
3HZ1	1	0.66	4.44	4	0.54	0.51

<sup>a</sup>RMSD of individual fragments and corresponding ranks for the best pose and the closest native-like configuration (rank >1) are provided.

simulations include about 70 residues in 2QFO). As in the X-ray structure, 2QFO\_1 interacts with the hot spot D93 (directly and through close waters), while 2QFO\_2 makes one hydrogen-bond with N51 and an interfragment  $\Pi$ -stacking interaction.

A pose close to the experimental solution is also retrieved for the 4 other cases but unfortunately not as the lowest FF-based energy configuration of the system. In practice, several best poses (e.g., top 30) are checked with thoroughness in prospective docking. Accordingly, correct predictions were generated for 4 out of the 5 investigated ternary complexes (2QFO, 2XDU, 2YEJ, and 3HZ1) within the 12 best poses. The expected locations of the two fragments are not simultaneously retrieved as the top-ranked pose. Among them, there are 3 complexes with one good fragment prediction in the top-ranked pose (2XDU\_2, 2YEJ\_1, 3HZ1\_1). Only the 2YEI docking appears as a complete failure, since the best pose is totally wrong (two large RMSDs for the considered fragments). Besides, the best-ranked couple of acceptable poses for 2YEI\_1 and 2YEI\_2 lags behind (#187).



**Figure 5.** Multifragment simulations (sequential protocol). Experimental locations are displayed in green for fragments. Predicted poses are represented in blue (top-ranked) or purple (other rank) for ligand. Main polar interactions are depicted as dash lines. The hot spot D93 is indicated.

Since the native conformations are quite accurately sampled with this “brute-force” approach, but not ranked as #1, the classical FF failure hypothesis is the preferred explanation. It should be noted that the scaffolds of misdocked fragments are highly similar: a methyl-pyrazole linked to a heterocycle (a furan for 2YEI\_2 and 2YEJ\_2 or an isoxazole for 3HZ1\_2) with a masked acidic group. The latter is enclosed by several hydrophobic side chains such as F138, L107, M98, and V150; therefore, it does not make any polar interaction with the site (this “quite useless” chemical function was later removed in the 3HZ1 fragment optimization using a linking strategy<sup>31</sup>). Finally, both 2YEI fragments contain an ester function which poorly interacts with the target, which seems to give more weight to the hypothesis of a less than optimal FF parametrization of esters.

**3.4.2. Sequential Protocol.** This alternative strategy, less greedy in terms of computing resources (e.g., smaller number of generations), has been performed in parallel to the simultaneous protocol. Although the fragments are docked separately in a first step, the final relaxation is performed over reasonable putative configurations of the system, including both investigated compounds within the binding site. Therefore, putative interfragment contacts are taken into account during this final stage. Figure 5 shows, for all investigated structures, the closest pose to X-ray data in addition to the top-ranked pose, each superimposed to the experimental one. As before, the 2QFO geometry is perfectly reproduced, even at the first rank, while pyrazole derivatives adopt wrong binding modes. By contrast to the simultaneous strategy, the top-ranked pose of the 2YEI complex contains one successfully predicted fragment (2YEI\_1). This time, the correctly predicted 2XDU fragment from the lowest-energy pose is not the same: using the sequential scheme, the potential energy is slightly better, and the location of the amino-pyrimidine fragment (2XDU\_1) is perfectly reproduced in the vicinity of D93 (RMSD < 0.5 Å) to the detriment of the less well anchored phthalazine fragment (2XDU\_2).

Generally speaking, the same trend emerges from the ability to reproduce the structure of these ternary complexes using this sequential strategy, with a slight improvement toward lower RMSD and with better acceptable ranks (when the top-ranked pose does not match the experimental data). This is coherent with the nature of this docking problem, where fragments are not strong competitors (they would not target a same subpocket). Interfragment interactions do exist, but they are less decisive with respect to much stronger site-fragment anchoring contributions (unlike in explicit water placement, which is highly dependent on the ligand position). Actually, the 2QFO system which displays the strongest interfragment contact (a π-stacking of respective phenyl rings) also happens to be the best predicted.

The analysis of the whole set unsurprisingly highlights that fragments with most polar groups involved in hydrogen bonds (e.g., 2QFO\_1, 2QFO\_2, 2XDU\_1, 2YEI\_1, 2YEJ\_1, 3HZ1\_1) are more often successfully docked, by contrast to others (2XDU\_2, 2YEI\_2, 2YEJ\_2, 3HZ1\_2). Besides, fragments from the 2QFO structure exhibit pretty high affinity with respect to their small size ( $K_{d,2QFO\_1} = 20 \mu\text{M}$ ,  $K_{d,2QFO\_2} = 150 \mu\text{M}$ , see ref 35), compared to some of the failed ones (e.g.,  $K_{d,2XDU\_2} > 1 \text{ mM}$ ,  $\text{IC}_{50,3HZ1\_2} = 1 \text{ mM}$ , see refs 32 and 31 respectively). This goes in the same direction as one main conclusion from the study of Verdonk et al. about fragment docking, in which they demonstrated that an important factor affecting docking accuracy of fragment-like compounds is ligand efficiency (LE).

Finally, it should be noted that top-ranked poses from docking simulations are systematically lower or equal in energy than geometries obtained by simple energy minimization of X-ray poses, accrediting the efficiency of the sampling engine. Although not perfect, these results, in addition to previous ones relative to the fragment set, demonstrate that S4MPLE is able to reproduce expected binding modes for fragment-like compounds, and even in the tricky multifragments case.

Therefore, a usage of S4MPLE within FBDD projects becomes possible and promising.

#### 4. CONCLUSION

S4MPLE, a conformational sampling tool based on a hybrid evolutionary algorithm, has been implemented to allow extensive atom flexibility, all the while being general and transcending the classical “one site, one ligand” docking scheme. It includes an original population diversity check relying on pairwise interaction fingerprints and generic operators acting in 3D Cartesian space on user-defined and general DoF. As a consequence, S4MPLE provides an equivalent treatment of intra- and intermolecular DoF, making no distinction between conformational sampling of a single compound or docking. Thus, the theoretical applicability range of S4MPLE is only limited by the set of available FF parameters and accessible computing time. This program is meant to address more difficult sampling and docking problems by contrast to high throughput docking at few seconds/ligand. While designed for deployment on grids, the current work relies on the few-CPU “workstation” version of S4MPLE. This second paper addressed in more details the following main issues:

- Docking of fragment-like compounds, in order to assess the applicability of the AMBER/GAFF force field to this class of organic ligands,
- Simultaneous docking of several entities, including two noncompetitive fragments, or one ligand and explicit free waters.

These results demonstrate the ability of S4MPLE to deal with several entities: experimental configurations are often generated but not systematically top-ranked. AMBER/GAFF displayed, for docking of single fragment-like ligands, success rates comparable to state-of-the-art drug-like molecule docking benchmarks. The previously reported<sup>3</sup> fitting of additional FF terms (continuum solvent model, contact bonus terms) proved beneficial for fragment-like docking as well. So far, we do not see any systematic FF bias due to lower ligand sizes although specific FF deficiencies remain – for example, a tendency to force ester groups into fake contacts with the protein site.

Beyond such “classical” FF problems, docking of explicit waters within a continuum solvent, in attempts to predict ligand-water-site interactions, is a very difficult problem because the targeted configurations are not necessarily the energetically most rewarding ones (the mobile explicit water may well be trapped into site subpockets binding crystallographic waters even tighter). Therefore, if the experimental ligand-water-site-featuring configurations were not ranked as first among sampled states, this is not (necessarily) a FF failure. The ability to enumerate such states within the typical top 30-saved geometries should count as success. Success rates are, expectedly, higher in simulations with fewer explicit waters. In general, adding explicit waters does not trigger a huge increase of the number of DoF but alters the energy landscape of the problem. The additional DoF create a quite flat energy profile (explicit water Coulomb interactions being counterbalanced by the continuum desolvation term), or the difficulty of a sampling problem is known to increase significantly in such cases (flat landscapes, with many shallow local minima). Alternative strategies enhancing the sampling of the intermolecular DoF of waters were introduced in order to deal with this problem.

Eventually, multiple docking of fragment-like ligands successfully managed to return experimental structures of ternary complexes, albeit – again – not always at the top of saved conformer lists. This is due to the above-cited FF problems.

The strength of S4MPLE is its flexible control of DoF and its adaptability to the computational resources one possesses: on a workstation, extensive fixing of estimated low-mobility moieties is mandatory. The key plus of S4MPLE is that, with extensive computer power, it is possible to easily unlock more DoF and go for in-depth sampling using massively parallel deployment. At the moment, S4MPLE is still a prototype. However, having successfully passed these tests about fragments docking, interesting perspectives should be reached in the FBDD, once a strategy relative to the *in silico* optimization (growing and linking) of fragments will be developed. Future work will focus in that direction.

#### ■ ASSOCIATED CONTENT

##### **S Supporting Information**

Detailed docking results with free water molecules, for each employed protocol (spreadsheets in ci300495r\_si\_001.xlsx) and detailed fragment redocking results, per individual simulation (ci300495r\_si\_002.xlsx). This material is available free of charge via the Internet at <http://pubs.acs.org>. The following are available for download on <http://infochim.unistra.fr>, DOWNLOADS section (tar.gz): x86\_64 executable of S4MPLE; User guide; various ligand preparation tools and force field parameter distributions; and a spreadsheet with the list of the training set compounds used to calibrate Fit FF and detailed docking results for each of the repeated docking runs. Docked poses are available upon request (PyMol .pse format).

#### ■ AUTHOR INFORMATION

##### **Corresponding Author**

\*E-mail: dhorvath@unistra.fr.

##### **Notes**

The authors declare no competing financial interest.

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