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# Second-generation de novo design: a view from a medicinal chemist perspective

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**Abstract** For computational de novo design, a general retrospective validation work is a very challenging task. Here we propose a comprehensive workflow to de novo design driven by the needs of computational and medicinal chemists and, at the same time, we propose a general validation scheme for this technique. The study was conducted combining a suite of already published programs developed within the framework of the NovoBench project, which involved three different pharmaceutical companies and four groups of developers. Based on 188 PDB protein–ligand complexes with diverse functions, the study involved the ligand reconstruction by means of a fragment-based de-novo design approach. The structure-based de novo search engine FlexNovo showed in five out of eight total cases the ability to reconstruct native ligands and to rank them in four cases out of five within the first five candidates. The generated structures were ranked according to their synthetic accessibilities evaluated by the program SYLVIA. This investigation showed that the final candidate molecules have about the same synthetic complexity as the respective reference ligands. Furthermore, the plausibility of being true actives was assessed through literature searches.

**Keywords** Structure-based de novo design · NovoBench · Fragment space · Validation study · Synthetic accessibility · Feature Trees · FragView · FlexNovo · SYLVIA

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## Introduction

Fragment docking within a protein cavity has been long seen as a viable option for de novo design approaches [1]. Experimental fragment-based drug discovery (FBDD) showed that fragment binding, when properly combined with structure-based drug design (SBDD), leads to the successful identification of promising leads [2]. Recently, successful applications of fragment docking have been published [3, 4], and prompted us to validate a de novo design workflow developed during the past 4 years within the NovoBench project (BMBF Grant *313324A*) collaboration between medicinal and computational chemists and software developers. A major obstacle in the development of computational de novo design methods is their proper validation. In contrast to other computational methods like molecular docking, there is no simple quality measure. Since the proposed molecules are mostly new, only their

synthesis and experimental testing would be a valid strategy in theory. This is, however, extremely time- and cost-intensive. Here, we present the results of a retrospective validation study that, even though it does not disclose new algorithms, shows and collects solutions for the multiple problematic aspects of a two-decade-old discipline from its seminal definition [5].

Starting from the basic assumption that scoring functions are accurate enough to predict correct fragment positioning within protein cavities [1, 3], we investigated computational routes to generate fragments and recombine them without losing the chemical and geometric information of parent bond vectors. At the same time, we tried to integrate our current synthetic knowledge into the process. The final goal is, of course, to generate synthesis-amenable virtual compounds, which are meaningful for a medicinal chemist, as idea generators for lead discovery.

Structure-based de novo design still lacks a comprehensive validation study. Recently, Wang et al. [6] presented a study on the possible use of de novo approaches for isostere replacement of fragments. The lack of published positive (or negative) confirmatory data might have played a role in preventing formation of a thorough view of the state-of-the-art and reliability of this complex technique. Validation studies are conclusive when they offer experimental evidence of correct design prediction. Here, however, we would like to raise the confidence of the final user who will eventually validate the results using a reasonable testing scheme.

Within the NovoBench project, several tools addressing different aspects of de novo design were developed. They are all based on the concept of fragment spaces [1, 7]. Problems tackled are the creation of fragment spaces (Colibri), interactive design and browsing (FragView) as well as search problems like structure-based (FlexNovo [1]), ligand-based (Ftrees-FS [7]), and property-based (FragEnum [8]) design. All tools are combined with a novel approach to estimate synthetic accessibility of candidate compounds named SYLVIA [9, 10]. These tools cannot be validated individually in this study. Therefore, we focus on a sample workflow in which the structure-based de novo design program FlexNovo [1] is applied to a set of 188 PDB cavities selected from four different superfamilies of target proteins (Kinases, Nuclear Hormone Receptors (NHRs), Proteases and Phosphodiesterases) for a total of eight different protein families.

We investigated whether, and to what extent, FlexNovo was able to re-build known ligands of several protein complexes. Secondly, we analyzed, within the set of candidate ligands generated, those that were identical to the starting ligands in terms of their binding poses and energy ranks. This analysis gave us a sense of the accessibility of the candidate molecules and their likely fit in the protein cavities, i.e. of their design plausibility. Shredding the known ligands from the PDB complexes following retrosynthetically motivated rules created the chemical fragment spaces underlying the FlexNovo search. Another tool developed within the NovoBench project named FragEnum [8] was used to analyze the resulting chemical space coverage. FragEnum is able to enumerate all compounds from the fragment space within user-defined constraints of physicochemical properties. Furthermore, assessing the synthetic feasibilities of the candidates had to be a focal point for a comprehensive and reliable novel de novo approach. Therefore, we used the program SYLVIA [9, 10] as third component to assess the synthetic accessibility of all the candidate molecules. We provided an analysis on how accessible they were in comparison to their relative reference ligands and in comparison with actives that can be currently retrieved in a classical drug discovery database [11].

## Methods

Starting with 188 protein complexes from the PDB ,[1] the cavities were generated by a FlexX script, which selected all protein residues lying within a 9 Å sphere of the ligand in the structure. Co-crystallized water molecules were removed. We used a selection of CDK2, CHEK1, JNK3, PDE4b, PDE5a, BACE, ROCK1, and ESR1 protein complexes. Fragment spaces from ligands of the same protein class were generated using conversion to mol2 format with CORINA [12, 13] and shredding with CoLibri/Recore [14] with the help of the BRICS rule set [15].

Figure 1 shows the workflow of the studies performed. Table 1 reports the size of fragment spaces generated. We also tried to enumerate the fragment spaces applying the same physicochemical filtering thresholds (Lipinski "rule of five" [16] for all protein classes except for BACE where MW $<$ 850 Da and a maximum number of ten hydrogen acceptors and donors were allowed). This enumeration was

[1] PDB entries used in the study: 1a52 1ckp 1di8 1dm2 1e1v 1e1x 1e9h 1ere 1err 1fkn 1fvt 1fvv 1g50 1g5s 1gih 1gii 1gij 1gwq 1gwr 1gz8 1h00 1h01 1h06 1h07 1h08 1h0u 1h0v 1h0w 1h1p 1h1q 1h1r 1h1s 1jsv 1jvp 1ke5 1ke6 1ke7 1ke8 1ke9 1l2i 1ogu 1oi9 1oiq 1oir 1oit 1oiu 1oiy 1p2a 1p5e 1p8d 1pcg 1pf8 1pkd 1pq6 1pq9 1pqc 1pxi 1pxj 1pxk 1pxl 1pxm 1pxn 1pxp 1pye 1qkt 1qku 1r5k 1r78 1rkp 1ro6 1sj0 1tqf 1udt 1uom 1urw 1v1k 1vyw 1vyz 1w0x 1w51 1w8c 1wcc 1x7e 1x7r 1xlx 1xlz 1xm4 1xm6 1xmu 1xos 1xoz 1xp0 1xp1 1xp6 1xp9 1xpc 1xqc 1y2h 1y2j 1y8y 1y91 1yim 1yin 1ykr 1ym2 1ym4 1zky 1zlt 1zys 2a0c 2a4l 2ayp 2ayr 2b1v 2b1z 2b23 2b52 2b53 2b54 2b55 2b8l 2b8v 2bhe 2bhh 2bj4 2bkz 2bpm 2br1 2brb 2brg 2brh 2brm 2brn 2bro 2btr 2bts 2c3j 2c3k 2c3l 2c4g 2c5n 2c5o 2c5p 2c5t 2c5v 2c5x 2c5y 2c68 2c69 2c6i 2c6k 2c6l 2c6m 2c6o 2c6t 2cgu 2cgv 2cgw 2cgx 2chm 2clx 2duv 2erd 2ert 2esm 2etk 2eto 2etr 2exm 2f3e 2f3f 2fai 2fdp 2fvd 2g44 2g94 2g9x 2h44 2i0j 2i40 2irz 2is0 2iw6 2iw8 2iw9 2jf9 2jfa 3erd 3ert.

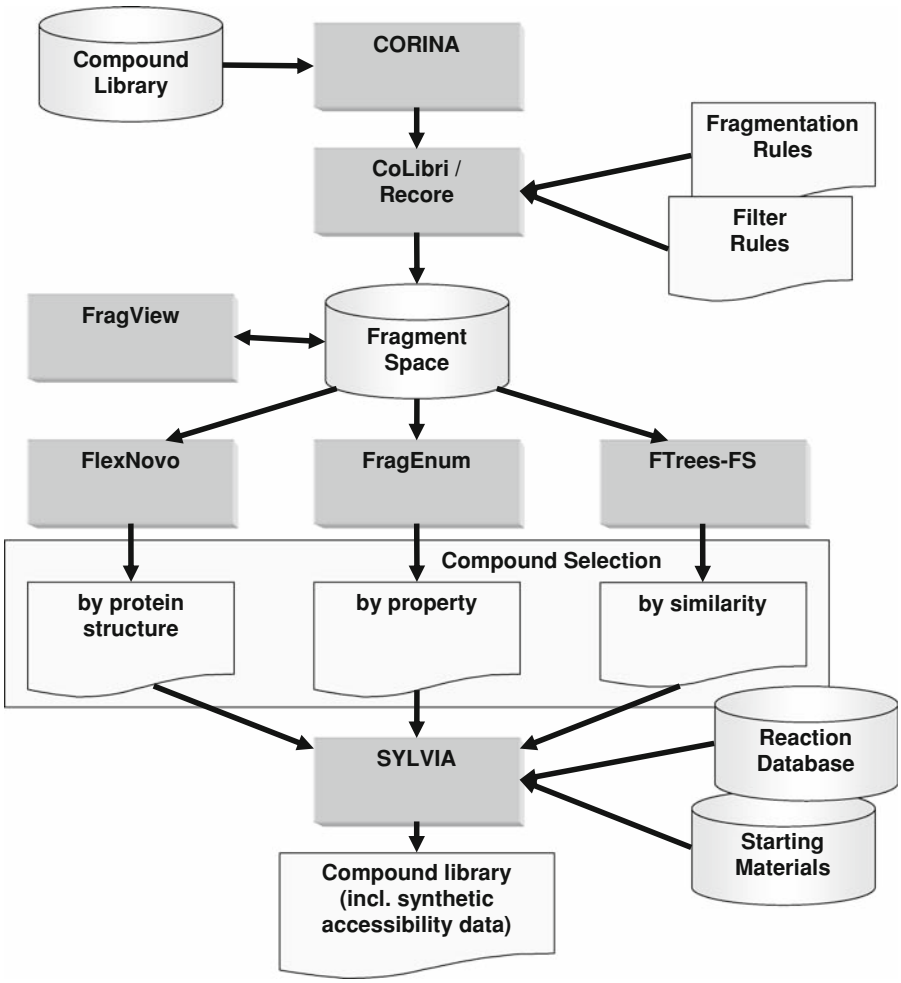



**Fig. 1** Schematic representation of information flow and suite of programs (*grey solid boxes*) in the NovoBench project

**Table 1** Protein cavities and fragment spaces used for validation of FlexNovo experiments

| Family | Protein | No. of Ligands within respective cavities[a] | No. of unique fragments | Enumerated results within filtering limits (see "Methods") |
|---|---|---|---|---|
| Kinases | CDK2 | 99 | 137 | NA[b] |
| | CHEK1 | 17 | 24 | 640 |
| | ROCK1 | 4 | 7 | 19 |
| | JNK3 | 7 | 21 | 7,771 |
| PDEs | PDE4b | 9 | 15 | 34 |
| | PDE5a | 6 | 14 | 42 |
| NHR | ESR1 | 24 | 32 | 604 |
| Proteases | BACE | 18 | 49 | 32,585 (5 cycles) |

[a] Only unique ligands have been used

[b] *NA* not assessed (>1Mio)

performed using FragEnum [8] and it was successful for all cases but CDK2.

For each protein family, FlexNovo runs were conducted in two steps. First, all the fragments from ligands bound to the protein family were placed in the corresponding active sites making use of at most two pharmacophoric constraints, one of which is essential. These geometrical constraints are classical distance constraints from residue atoms known to be important for the recognition of reference ligands. In case of kinase targets, for instance, FlexNovo preferentially positioned fragments interacting with the so-called backbone hinge region while avoiding

carboxylates or phenolic hydroxyls as interacting moieties. This type of constraints works as implemented in FlexX-Pharm [17]. Secondly, starting from the best-scored placements, a build-up generation loop was performed according to the FlexNovo strategy, which attaches new fragments to the first placed one. A maximum of 3 cycles of linking were performed before terminating the molecules and saving the first 100 top-ranked molecules. For ESR1, for which both smaller agonistic and larger antagonistic ligands were used as the fragment source, and for proteases (BACE), for which ligands with molecular weight exceeding 500 Da (short peptides and peptidomimetics) were utilized, a build-up process with 5 cycles was allowed. A total of 376 runs were performed. The resulting top-ranked 100 molecules from each run were analyzed individually and collectively within their corresponding family. After collecting the results for each family, the similarity with the source ligands was assessed through the Feature Tree descriptor [7]. We defined as "identical-to-ligand" a result molecule showing FTree (FT) similarity equal to 1.0 with one of the native ligands. This graph identity is theoretically necessary but not sufficient to define chemical identity [7]. We examined the chemical identity and the pose identity of the resulting molecule within the cavity by visual inspection. Figure 2 shows an example of the results obtained with the ROCK1 cavity (PDB: 2ETK). The top-ranked molecule, together with the top five and top ten compounds most similar to the reference ligand were used for the analysis. An internal diversity assessment was conducted within FlexNovo to discard intermediate and final fragment combinations having more than one identical fragment ID. This allowed us to attain the maximum possible diversity through the combination of fragments in each run. Figure 3 reviews some of the candidate molecules obtained as rank 1. In five of eight cases, the natural ligand could be rebuilt successfully.

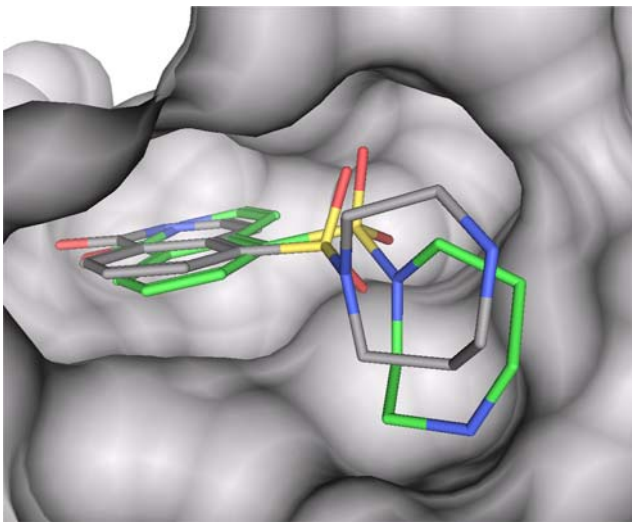


**Fig. 2** Example of rebuilding of ROCK1 ligand Y-27632 (*green* source ligand; *coloured by atom* FlexNovo solution) within the ATP binding pocket (PDB = 2ETK). Heavy-atom RMSD is 1.614 Å

It is known that de novo programs suffered from a severe drawback in the past: the proposed molecules were often not chemically stable, or only synthesizable by means of lengthy multi-step syntheses.

Historically, the initial focus of the de novo approach solutions concentrated on the geometrical optimization rather than the feasibility of the candidates. Within the NovoBench project, the program system SYLVIA [9, 10] was developed by Molecular Networks [18]. It allows for a rapid evaluation of the ease of synthesis of a given query compound. We took into account an assessment of the synthetic accessibility of the proposed ligands as a decisive factor for determining the acceptability of the results. The reference ligand sets and the corresponding resultant candidate molecules were scored against the MDDR database [11] as a reference for active molecules of high diversity. MDDR, representing a diverse set of actives, was filtered to discard molecules with MW greater than 850 Da.

## Results and discussion

Hundred and eighty-eight protein cavities belonging to 8 different protein families (CDK2, CHEK1, ROCK1, JNK3, PDE4b, PDE5a, BACE, ESR1) were used in this study. The 188 reference ligands (see "Methods") formed the basis for eight different fragment spaces by shredding, using the FlexNovo approach. As shown in Table 1, the size of each fragment space both in number of fragments as well as in number of molecules that can be theoretically enumerated substantially differs depending on the size, number, and retrosynthetic accessibility of the parent ligands. On average, if shredded, kinase inhibitors contributed with 1–1.5 unique fragments per ligand, while larger protease peptidomimetics inhibitors contribute with more fragments per ligand. This is not new and has been previously noted [8], though. Fragment space size and composition has also deep implications on the combinatorial complexity of the possible chemical space, which can be sampled by the building process within the cavities [8].

The theoretical number of molecules covered (and thus the probability of their diversity) in general depends on the number of fragments, on the number of their linkage positions and their respective chemical compatibility. We enumerated all possible fragment combinations using the program FragEnum [8] with the same physicochemical constraints used during the FlexNovo build-up process (Table 2). All examples studied produced a manageable number of molecules except for CDK2, where 137

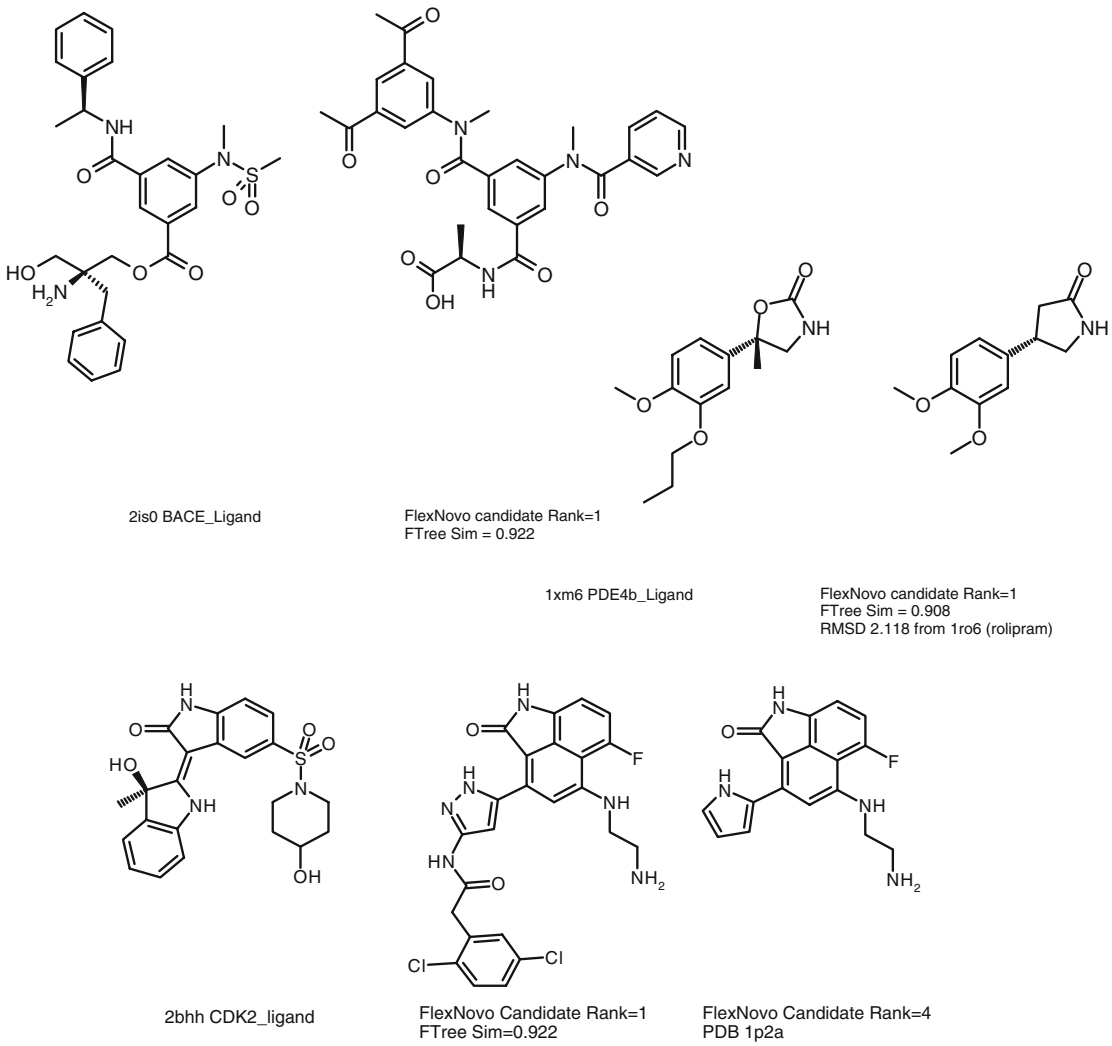



**Fig. 3** Four examples of top ranked molecules found by FlexNovo having high FT similarity. For three of them, literature examples have been found with interesting similar properties. The lack of experimental activity data did not allow us to confirm the plausibility of the results obtained, but similarity is striking

**Table 2** Maximum values of allowed chemical–physical constraints used during the theoretical enumerations and FlexNovo build-up processes

| Family | Protein | MW | No. Hacc | No. Hdon | Log *P* | No. ring | Rot. bond |
|---|---|---|---|---|---|---|---|
| Kinases | CDK2 | 550 | 8 | 5 | 6 | 5 | 8 |
| | CHEK1 | 550 | 8 | 5 | 6 | 5 | 8 |
| | ROCK1 | 550 | 8 | 5 | 6 | 5 | 8 |
| | JNK3 | 550 | 8 | 5 | 6 | 5 | 8 |
| PDE | PDE4b | 550 | 8 | 6 | 5 | 6 | 8 |
| | PDE5a | 550 | 8 | 6 | 5 | 6 | 8 |
| NHR | ESR1 | 550 | 6 | 4 | 6 | 5 | 11 |
| Protease | BACE | 850 | 10 | 10 | 6 | 20 | 9 |

fragments from 99 ligands delivered a number of molecules beyond a practical threshold for enumeration reasonable for this study (>1 million). JNK3 ligands, when compared to ESR1 or CHEK1, delivered a noticeable larger number of possible candidates due to the larger number of linkage atoms combined with their small size of JNK3 ligand fragments. Careful inspection and selection of fragments could preventively limit the combinatorial explosion typical for these kinds of assemblies.

## Re-building reference ligands

The choice of the ligands and cavities reflects the aim to examine maximal diversity in ligand size and flexibility, and to cover a range of protein cavities targeted by pharmaceutical discovery efforts. It is also relevant to note that kinase, PDE inhibitors and NHR modulators are mainly neutral compounds containing heterocycles, while BACE ligands are peptidomimetics scaffolds with higher molecular weight, flexibility, and overall polarity (see examples

in Fig. 3). Within each protein subtype (e.g. CDK2), more than one cavity structure was used to take into account some of protein flexibility. Thereby, the study considered structural variability both on a small detailed and on a larger scale. The X-ray structures included had resolutions ranging from 1.7 to 3.2 Å, allosteric-type inhibition as well as protein functional interaction states. For instance, for the estrogen receptor (ESR1), agonistic and antagonistic ligands were considered in order to see whether FlexNovo was able to deliver quality results on both functional situations of the receptor. It is known, in fact, that ESR1 antagonists possess higher volumes and flexibility due to the larger size of the cavity they induce [19, 20]. This fact should not be considered as a lack of consistency or control of the data set, but rather as a challenge for the FlexNovo approach that is typical for what a modeler would face for the target.

The use of physicochemical descriptors allowed FlexNovo to generate controlled lead-like candidates according to known rules [16, 21] (Table 2). In case of ESR1 antagonists and BACE inhibitors, higher values than typically considered drug-like in the literature were applied together with a larger number of build-up cycles to allow FlexNovo to build more diverse molecules.

Table 3 reports the number of solutions found by FlexNovo. It shows how many of these are "identical" to the reference ligands, and the highest rank of the identical-to-reference solutions. The assessment of identity and pose has been performed visually after ranking the candidates by FT similarity towards any of the reference ligands of the same protein family (see "Methods"). For five of the eight test cases (protein families), reference ligands could be rebuilt, while for JNK3, PDE5a and BACE this was not the case. It is evident that a large number of theoretical molecules does not statistically assure successful rebuilding of the references. As the docking score drives fragment ranking in the final and intermediate lists of candidates, particularly well-fitting individual fragments, or combinations thereof, sometimes dominate either the intermediate and/or the final list of results. Nevertheless, for four of the five cases where the reference ligand was rebuilt by the approach, the FlexX scoring function ranked the rebuilt reference ligands among the top five solutions.

One example of a correctly re-built reference ligand Y-27632 for the ROCK1 cavity (pdb code: 2ETK) is given in Fig. 2. For ESR1 (Fig. 4) and CHEK1 (Fig. 5), higher RMSDs are noted. In both cases a symmetry problem is the major reason for incorrect binding poses. In ESR1 (pdb code = 1R5K), for instance, ligand GW5638 does not make any hydrogen-bonds with the protein cavity, while in the energetically most-favored FlexNovo docked pose a carboxylate interaction with Arg384 was found (Fig. 4). For CHEK1, as in many kinases, in which the hydrogen-

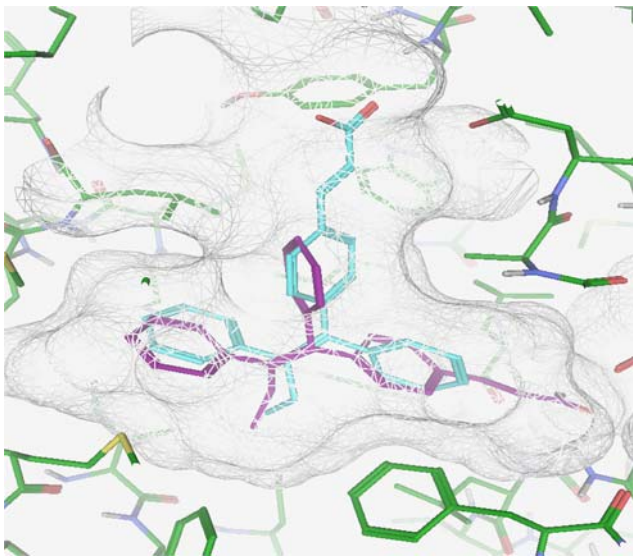


**Fig. 4** Example of rebuilding of ESR1 ligand GW5638 (*cyan* source ligand, *magenta* FlexNovo solution) within the estrogen binding pocket (PDB = 1R5K). Heavy-atom RMSD is 6.286 Å due to the trans isomer found at lower energy. Carboxylate interaction with Arg384 (*right*) is responsible for the incorrect assembly of two fragments around central styrenic double bond. Cavity entry is located on *upper site* of the picture

**Table 3** Analysis of FlexNovo solutions according to similarity to native ligand and solution rank

| Family | Protein class | Total no. of candidate ligands produced | No. of solutions identical to reference ligand | Highest rank of identical-to-ref. ligand solutions | Min. RMSD |
|---|---|---|---|---|---|
| Kinases | CDK2 | 5,702 | 6 | 4 | 2.172 |
| | CHEK1 | 1,224 | 4 | 21 | 6.922 |
| | ROCK1 | 32 | 6 | 1 | 1.614 |
| | JNK3 | 136 | 0 | – | – |
| PDEs | PDE4b | 148 | 11 | 2 | 2.118 |
| | PDE5a | 33 | 0 | – | – |
| NHR | ESR1 | 232 | 3 | 3 | 6.286 |
| Proteases | BACE | 1,531 | 0 | – | – |

bond interaction pattern direction in the hinge region can be reversed, an incorrect binding pose was obtained (see Fig. 5). The incorrect pose showed two instead of three interactions with the hinge region of the kinase. A larger number or more restrictive pharmacophoric constraints in general could solve this problem.

In addition to the above-discussed candidates, other candidate molecules were generated during the study. Some of them were found to be very similar to the corresponding reference ligands, others quite diverse. For obtaining a quantitative measure of the similarity of the candidate molecules, we ran 188 FT similarity searches

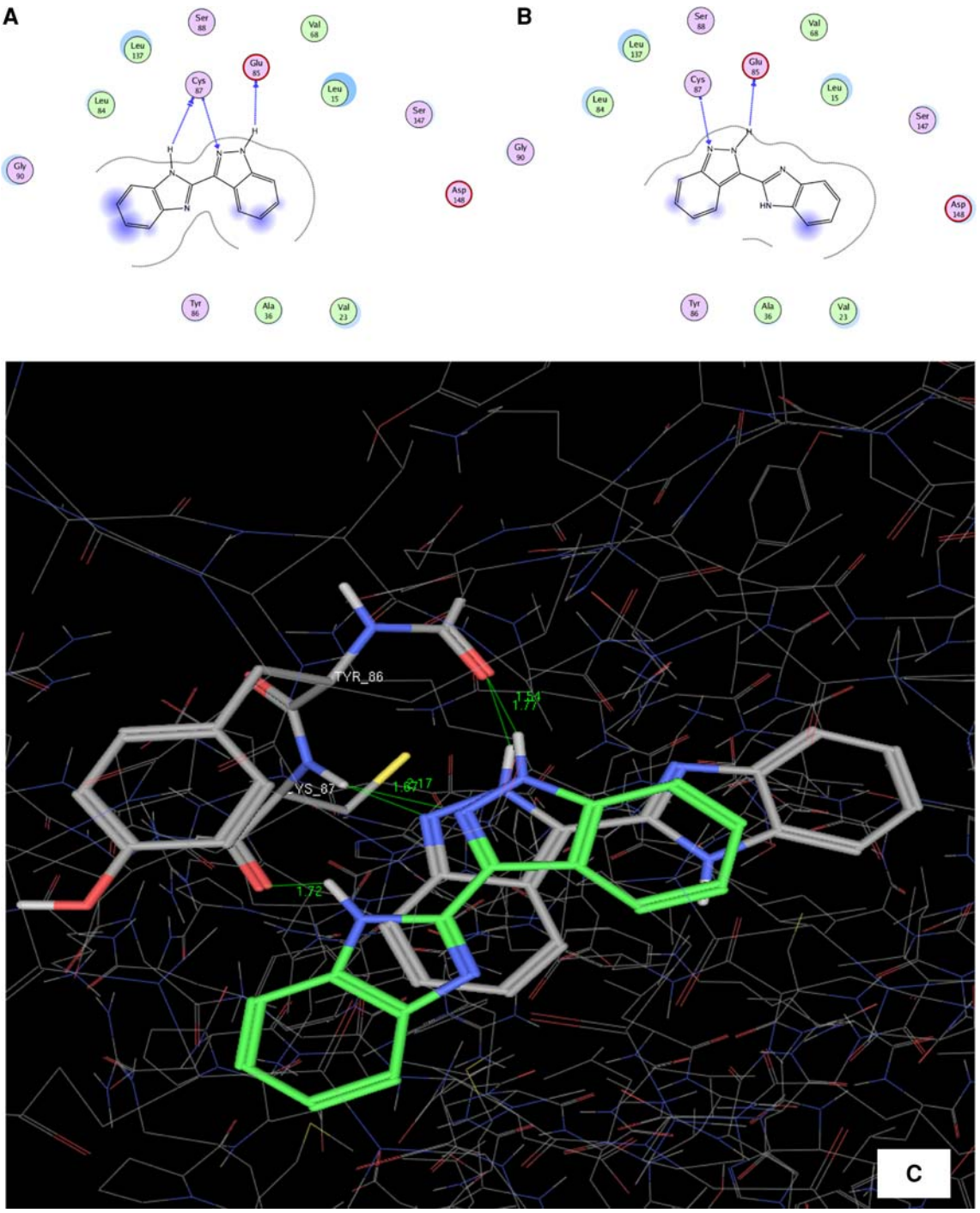
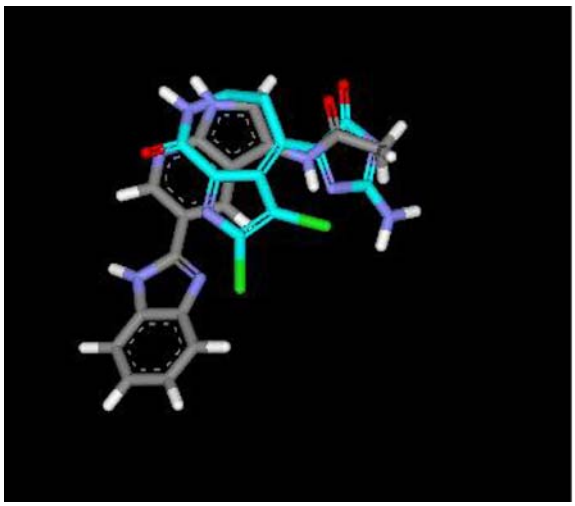
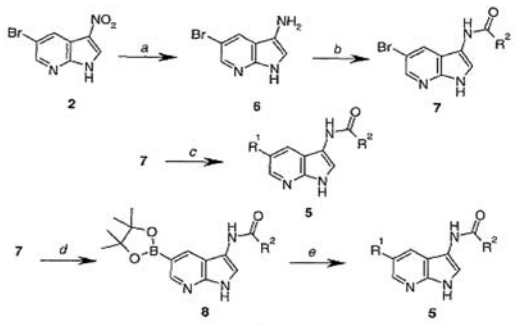



**Fig. 5** Example of rebuilding of CHEK1 indazole ligand (*green* source ligand, atom-type = FlexNovo solution) within the ATP binding pocket (PDB = 2C3L). Interactions map **a** depicts reference ligand and map **b** FlexNovo candidate. (**c**) Illustrates the symmetrical inverted pose found by FlexNovo with RMSD of 6.922 Å for the same candidate



Scheme II



(*a*) $SnCl_2$, HOAc, 12M HCl, 80 °C. (*b*) $R^2COCl$, pyr, rt; or $R^2CNO$, pyr, rt; or $(R^2CO)_2O$, pyr, rt; or $R^2CO_2H$, BOP, $Et_3N$, DMF, rt. (*c*) $R^1B(OH)_2$, $(PPh_3)_4Pd$, DMF/EtOH/aq. 2M $K_2CO_3$, 100 °C. (*d*) bis(pinacolato)diboron, $Pd(dppf)Cl_2$, KOAc, DMSO, 100 °C. (*e*) $R^1Br$, $(PPh_3)_4Pd$, DMF/EtOH/aq. 2M $K_2CO_3$, 100 °C.

**Fig. 6** On *left side*, 1st ranked molecule (*grey*) compared with native CHEK1 kinase inhibitor (*cyan*; PDB = 1ZLT). In this case the validation did not provide the expected results but the scaffold proposed (i.e. pyridopyrrole-benzimidazole) has no precedent in Scifinder searched as of May 2009, even if Stavenger RA et al. report a synthetic scheme for pyridopyrrole fragment in their patent application WO2003028724 A1

using as queries the set of reference ligands from each of the protein families against the first 100 candidates produced by FlexNovo in each cavity. In order not to miss any possibly active candidate, compounds showing an FT similarity score higher than 0.85 were considered highly similar. No synthetic or biochemical profiling of these molecules was performed. Figures 2 and 6 exemplify few of the most similar candidates. These results are only suggestive and have to be considered as starting points for brainstorming rather than as benchmarks for validation (see, for example, 110 candidate molecules generated for PDE4b cavity in Supplementary Material).

## Are all generated molecules synthetically accessible?

A well-known problem with de novo design approaches is the synthetic accessibility of the proposed compounds. Recently, reports [22, 23] have appeared proposing solutions to drive/measure the synthetic feasibility of the automatically generated structures. In this context, an automated scoring function quantifying structural complexity and synthetic accessibility of recognized intermediates towards commercial catalogues of reagents was found particularly useful [9, 10].

We have tried to address the problem by integrating a chemical feasibility score into the de novo process. We used SYLVIA that scores molecules from 1 (easy to synthesize) to 10 (difficult to synthesize). A variety of criteria were considered in this scoring function such as different structural complexity measures, different measures of similarity to available starting material and retrosynthetic reaction fitness. In our studies 65,000 structures of the MDDR database were taken as available starting materials (see "Methods"). Table 4 shows some basic statistics of SYLVIA scores for the candidate molecules generated by FlexNovo and for the reference ligand sets. As previously discussed, some BACE ligands are outliers in terms of higher complexity so they had poor (high) mean SYLVIA scores. However, all FlexNovo-generated molecules, with the exception of ROCK1 compounds, compared favorably in terms of synthetic accessibility of their reference molecules. In general, they generated candidates that tended to score better than the corresponding reference ligands. This fact could explain why the BACE reference ligands could not be successfully re-built, as the majority of candidates generated for BACE scored on average about 3.82 while the more complex references resulted with an average score of 6.01. It is interesting to note how effectively the applied physicochemical constraints (Table 2) worked in the selection/building process, selecting candidates within a certain range of synthetic feasibility score. Although physicochemical constraints should not a priori be able to drive structural complexity, they retained only such molecules possessing final SYLVIA scores with lower than average values compared to the reference ligands in seven out of eight cases. Finally, a comparison between the synthetic scores of candidates and those derived from a large selection (ca. 133,000 compounds with a maximum of MW = 850) of MDDR molecules showed that FlexNovo could generate molecules with an average synthetic accessibility score lower than the one of MDDR (5.29) by one unit. While a score cannot, per se, determine the real synthetic feasibility of a molecule, it could help prioritize,

**Table 4** Sylvia score [9, 10] for references and solutions

| Family | Protein | Mean of reference ligands | SD | Mean of candidate solutions | SD |
|---|---|---|---|---|---|
| Kinases | CDK2 | 4.22 | 0.83 | 4.21 | 0.70 |
| | CHEK1 | 4.42 | 0.50 | 4.24 | 0.75 |
| | ROCK1 | 4.11 | 0.12 | 4.49 | 0.18 |
| | JNK3 | 4.86 | 0.44 | 3.74 | 0.65 |
| PDE | PDE4b | 4.14 | 0.59 | 3.73 | 0.49 |
| | PDE5a | 5.23 | 0.86 | 3.88 | 0.49 |
| NHR | ESR1 | 4.85 | 0.58 | 4.15 | 0.63 |
| Protease | BACE | 6.01 | 1.20 | 3.82 | 0.72 |

together with the docking score, the final candidate in cases of multiple choices of candidates.

## Conclusion

We have shown that the NovoBench project delivered a suite of integrated programs (Fig. 1), which can be used for more efficient de novo design approaches. After fragment spaces were created by shredding with CoLibri, the fragments can be filtered, enumerated, and edited with FragView and FragEnum. Then, they can be linked to generate molecules within the protein cavity of choice with FlexNovo. In addition, FTrees-FS allows a selection of compounds based on the similarity to a reference ligand. Candidate compounds can then be scored for synthetic feasibility with SYLVIA. This novel de novo approach has been successfully validated through re-building reference ligands within several cavities of the same protein family. Notwithstanding the unproven assumption that the highest-ranked fragment placements should always lead to a re-build of the parent ligand, FlexNovo demonstrated the ability to rebuild reference ligands with native binding modes and conformations similar to X-ray structures.

Moreover, due to the built-in physicochemical filtering options, the overall objective of NovoBench to deliver candidate structures acceptable for medicinal chemists was achieved. This is evident by the average lower scores in synthetic accessibility for all the generated molecules, when compared to the scores of the reference ligands and/or active drugs contained in MDDR. Even if this finding does not assure an experimentally proven chemical feasibility and stability, we have shown that FlexNovo reached the validation bar we initially set. The scheme presented here is expected to be useful for future de novo design studies and retrospective validations. During the validation process, it also became clear that highly flexible ligands like peptidomimetics and extended protein cavities with different subcavities are still very challenging for de novo programs. Finally, it is also clear that unbiased structure-based de novo design without geometric constraints or a limited number of starting fragments can still be difficult to validate theoretically. When constraining the search space in multiple ways and including synthetic accessibility scoring, de novo design based on fragment spaces is a useful component in the computational/medicinal chemist's toolbox.

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