J Mol Model (2013) 19:2971–2979
DOI 10.1007/s00894-013-1821-0

ORIGINAL PAPER

Retrospective molecular docking study of WY-25105 ligand to β-secretase and bias of the three-dimensional structure flexibility

Leo Ghemtio · Nicolas Muzet

Received: 4 December 2012 / Accepted: 10 March 2013 / Published online: 7 April 2013

Abstract β-Secretase (BACE) is a very promising target in the search for a treatment for Alzheimer's disease using a protein–ligand inhibition approach. Given the many published X-ray structures of BACE protein, structure-based drug design has been used extensively to support new inhibitor discovery programs. Due to the high flexibility and large catalytic site of this protein, sampling of the huge conformational space of the binding site is the big challenge to overcome and is the main limitation of the most widely used docking programs. Incorrect treatment of these pitfalls can introduce bias into ligand docking and could affect the results. This is especially the case with the WY-25105 compound reported by the Wyeth Corporation as a BACE ligand that did not fit into any of the known crystal structures. In the present retrospective study, a set of available X-ray enzyme structures was selected and molecular dynamics simulations were conducted to generate more diverse representative BACE protein conformations. These conformations were then used for a docking study of the WY-25105 compound. The results confirmed the need to use an ensemble of structures in protein–ligand docking for identification of new binding modes in structure-based drug design of BACE inhibitors.

Keywords Molecular dynamics · Protein-ligand docking · β-Secretase · Multiple receptor conformations · Protein flexibility · Ensemble docking

L. Ghemtio
Centre for Drug Research, Faculty of Pharmacy,
University of Helsinki, Helsinki, Finland

N. Muzet (✉)
R&D, Sanofi Aventis, 16 rue d'Ankara,
67080 Strasbourg, France
e-mail: nicolas.muzet@sanofi.com

Introduction

The membrane-associated aspartyl protease β-secretase (BACE) is involved in the early steps of amyloid precursor protein (APP) cleavage and is one of the most pharmacologically important and intensely investigated targets in the pathogenesis of Alzheimer's disease (AD) [1–8]. The cleavage of APP initiated by BACE generates the β-amyloid peptide of 40 or 42 amino acids. The aggregation of this peptide in the brain is believed to lead to the development of AD [9]. Inhibition of this enzyme by small molecules (BACE inhibitors) is expected to prevent the build up of β-amyloid plaques [2–6, 10]. The first inhibitors identified, e.g., OM99-2, were peptidomimetics imitating the peptide chain to be cleaved [11]. However, due mostly to poor metabolic stability, high molecular weight and production costs, peptide-based inhibitors were replaced by smaller heterocyclic molecules and low-molecular-weight core structures (fragments) that initially interact weakly with the target molecule but could be optimized into higher affinity ligands [4, 5, 12, 13]. With the advancement in high-throughput screening of large compound collections and the availability of X-ray structures of the protein, more and more structure-based studies have been conducted to find even more desirable small molecules that could pass the blood–brain barrier [14, 15]. Several X-ray structures containing compounds blocking the BACE active site have been published, all showing an interesting common feature: the presence of a flap closed down on the inhibitor in the protease [15–20]. These constitute a reliable source of data for a protein–ligand docking approach, a structure-based method used widely in drug discovery that has been successful with several protein families [21–24]. However, it is now clear that a flexible receptor is one of the most challenging problems for docking and scoring functions, besides others limitations like the presence of water, tautomers, and

protomer treatments. Thus, most popular protein-ligand docking programs available today do not correlate well with measured activities and do not predict the binding free energies of the correct binding poses [23–25].

Despite much effort in the research community to develop new improved and accurate scoring functions, the performance of existing functions depends strongly on energy function, free energy perturbation and, most importantly, on the target structure under investigation. The impact of the target structure is due mainly to the lack of incorporation of receptor flexibility and its rearrangements in the prediction of both ligand binding poses and binding scores [23, 25, 26]. Furthermore, the three-dimensional (3D) structure used to perform the protein–ligand docking study could introduce bias into the quality and accuracy of the results [27, 28]. It has also been demonstrated that the lack of the correct protein conformation greatly affects the accuracy of most popular scoring functions currently available in the literature [29]. This is why it is important to pay attention to the structural flexibility of the receptor when searching for new small molecular inhibitors of BACE. Such structural flexibility is especially suited to help address the challenges presented by its large catalytic site and low specificity for small molecules. The use of experimentally determined and computationally generated multiple receptor conformations may significantly improve performance of both docking poses and scoring [25, 27, 28, 30].

Initial structural studies on BACE showed that the overall structure was not very different from other aspartic proteases but that a large conformational change is seen upon substrate or inhibitor binding [31]. The presence of a flap (residues 68–74) closing down on the target peptide chain led to the idea that this could be exploited to create specificity for designed inhibitors [32–34]. Thus, the flexibility of this part of the active site became the subject of many modeling studies, examining flap behavior during molecular dynamic (MD) simulations, normal mode analyses or essential dynamic analysis [34–37]. In addition, the 10s-loop (residue 9–14) was also expected to exhibit some mobility and could be a second site for ligand binding, making BACE a challenging protein target.

Several other studies have used structure-based theoretical methods in order to develop BACE inhibitors [38–40]. In 2006, Wyeth reported their hit compound WY-25105 containing an acylguanidine moiety that can form an unusual binding mode involving the two catalytic Asp residues (Asp_{32} and ASP_{228}), with a flap fully open BACE1 active site (Fig. 1a) [41]. Such large opening of the flap had been observed previously in other protease X-ray structures like renin, but never in BACE. Surprisingly, the flap opening was very wide and not even the most open apo X-ray structure of the β-secretase protein (PDB code: 1SGZ) allows the acylguanidine compound to be docked [34, 41–43]. Binding of this inhibitor was also made possible due to rotation of the flap at Tyr71, accessing a conformation seen only in the BACE apo structure 1SGZ. This conformation was considered unstable or even an artifact of the crystal structure (F) [28, 34, 43, 44]. According to all these observations, the challenge detailed in this work was to use multiple receptor conformations of the BACE protein, generated by MD simulations, to find a conformation that allows docking of the acylguanidine WY-25105 compound in a specific way; similar to the binding mode as shown on the presented poster to guide design of new BACE inhibitors [41, 45, 46]. This study was carried out before the X-ray structure was published in the RCSB Protein Data Bank (PDB) with code 2QU2, but the outcome will be compared to it [47].

Methods

MD simulations

To obtain the most diverse flap conformation ensemble, and to see whether different initial conformations could lead,

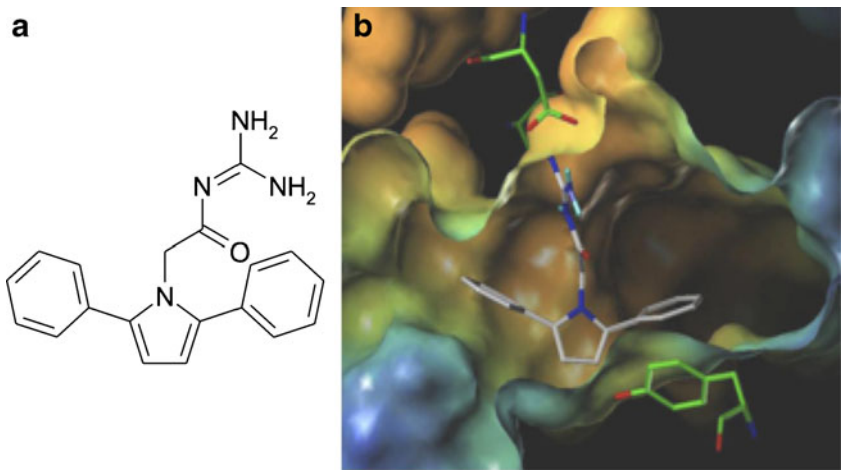

Fig. 1 **a** Wyeth compound WY-25105. Its binding mode between the catalytic aspartic acids Asp_{32} and Asp_{228} and the acylguanidine moiety in the X-ray structure was reported by Cole et al. [41]. **b** Results of WY-25105 docking in one of the conformations of 1SGZ generated by molecular dynamics (MD) simulations

through MD simulations, to a suitable flap opening for docking, two crystal structures (open and closed flap) were downloaded from the PDB database: the apo structure of BACE 1SGZ and a closed flap holo protein conformation 2B8L (Fig. 2) [18, 43]. From both structures, chain A was selected, crystallographic water molecules kept, and inhibitor removed. The protonation state of the proteins was prepared using Maestro Protein Prep [48]. Several studies have investigated the protonation state of catalytic aspartate (Asp32 and Aps 228) and shown that they could adopt both protonated or deprotonated configuration but this depended on the nature of the ligand involved [49, 50]. In our study, the aspartyl dyad (Asp32 and Asp228) was left deprotonated because Rajamani et al. [51, 52] suggested that the apo form of the protein is in the deprotonated state, and the acylguanidine compound, as shown in the published poster, clearly interacted with deprotonated Asp. VMD software was used for Charmm27 psf file generation of the apo proteins and system solvation. NAMD was used for all MD simulations and molecular mechanic calculations on a 24 processor SGI Origin 300 cluster [53–55].

In order to equilibrate the solvated proteins, while retaining the initial X-ray structure conformation as much as possible, we developed the following system preparation process: (1) Run a short 500 steps steepest descent minimization, all atoms free to move, in order to relax the crystallographic structure; (2) The system was then neutralized with Na^+ or Cl^- ions and solvated within a TIP3P water box of 12 Å around the protein followed by 6,000 steps of MD simulation to heat the box and 10,000 steps of volume equilibration, the protein being kept rigid; (3) Once all cavities of BACE seemed to be correctly solvated, in particular the active site, all TIP3P water molecules beyond 6 Å were removed and the whole remaining system was solvated again with an additional 11 Å box of water (final count of TIP3P residues about 15,500); (4) Four successive 4,000-step minimizations were run on the system, first with backbone fixed and side chains restraints, then with both decreasing restraints and eventually all protein atoms free; (5) Heating to 300 K with a small constraint on the backbone for 6,000 steps and final volume equilibration for 60,000 steps at 300 K. This entire preparation sequence was coded in our in-house program MolDys, which allows one to prepare any proteins within the force field values trying to disturb the initial X-ray conformation as little as possible. Simulation length for trajectories production was 10 ns for 2B8L and 6 ns for 1SGZ. For all MD simulations, the temperature was controlled with Langevin dynamic, pressure with the Nose-Hoover method, and long-range electrostatics were treated with a particle mesh Ewald grid with a timestep of 2 fs and multiple timestep parameters [56, 57].

Docking

Molecular docking to study binding of the WY-25105 molecule to the BACE conformations extracted from MD simulation was performed using version 3.1 of the Gold program [58]. The binding pocket for each conformation was defined around the catalytic site, between the two Asps and the flap. Docking was performed with default settings mode. For each conformation, a unique pose that had the highest Gold Score was selected for further analysis of binding mode.

Results

Simulations

All trajectories were checked by RMSD analysis and atomic fluctuations (Fig. 3). The overall conformation of the two initial structures was quite stable in the simulations. Most of the differences were observed between the flexible loops and the flap on the top of the active site. Atomic fluctuations showed that the behavior of the two proteins was relatively similar.

Two major parameters were checked carefully during the simulations: (1) the flap opening, by measuring the distance between Cα of Asp32 and Cα of Tyr71 (Fig. 4), and (2) the orientation of the Tyr71 with Chi1 vs Chi2 plots (Fig. 5).

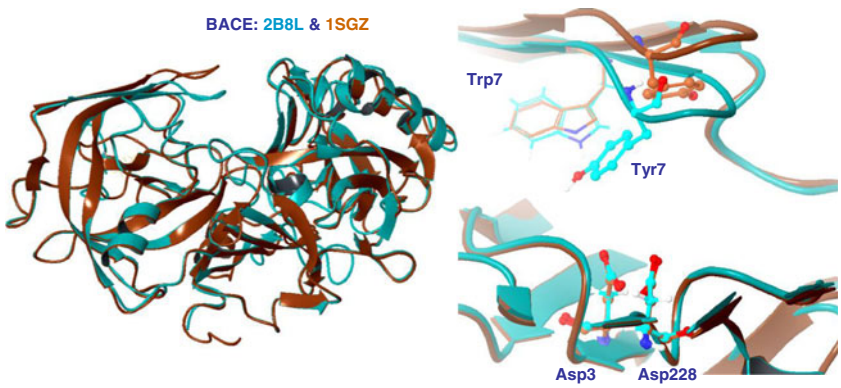

Fig. 2 *Left* Superimposition of β-secretase (BACE) 2B8L (*green*) and 1SGZ (*brown*) crystal structures. *Right* Comparison of the localization and orientation of key catalytic residues (Asp32, Asp228, Trp76 and Tyr 71)

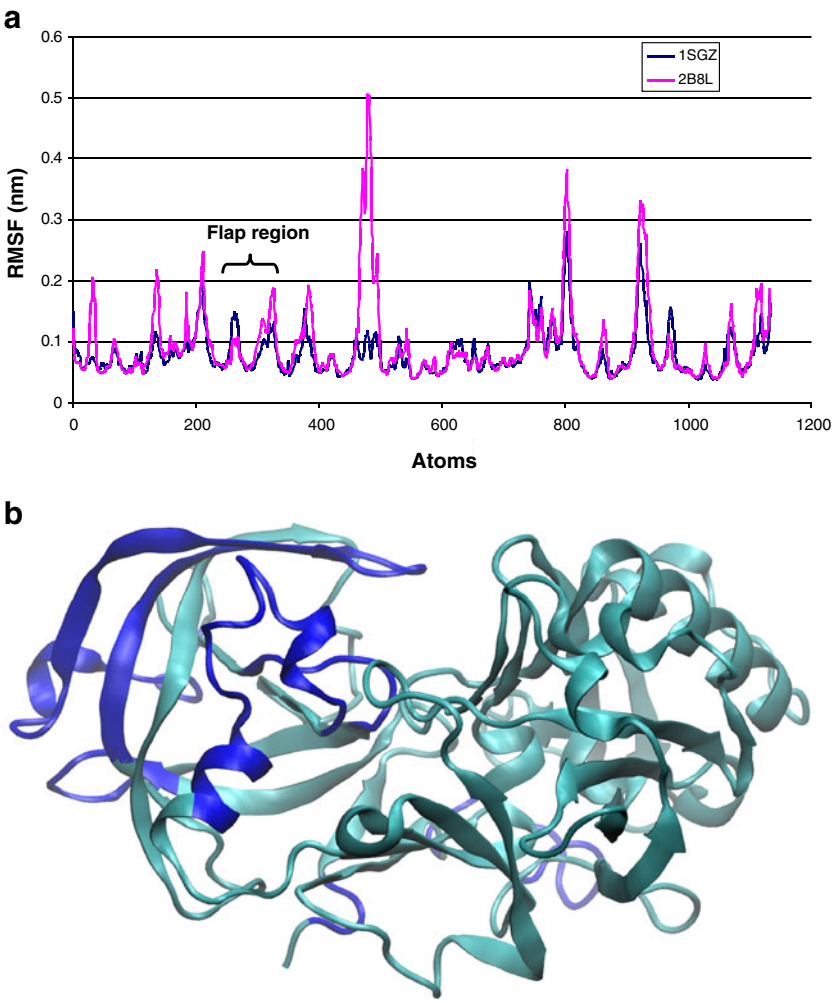

Fig. 3 **a** Atomic fluctuations [root mean square fluctuations (RMSF)] of BACE backbone atoms during full MD simulations. The flap region is noted. **b** X-ray structure 2B8L with residues with the largest fluctuations in *blue*, i.e., all amino acids surrounding the flap movement and additional loops on the surface of the protein

The 10s loop fluctuation was also measured but is not reported here as it is not involved directly in binding our ligand of interest. The trajectories have shown that, irrespective of the initial conformation, both structures have sampled a large conformational space of the flap over the active site in a reduced timescale, just as previously observed [34, 59]. For 2B8L, the flap adopted a swing motion, as already observed in other simulations, leading to a wider active site. The Tyr71–Asp32 Cα distance varies from 11 to 15 Å, corresponding to the breaking of the well-known Tyr71–Trp76 H-bond [34, 37].

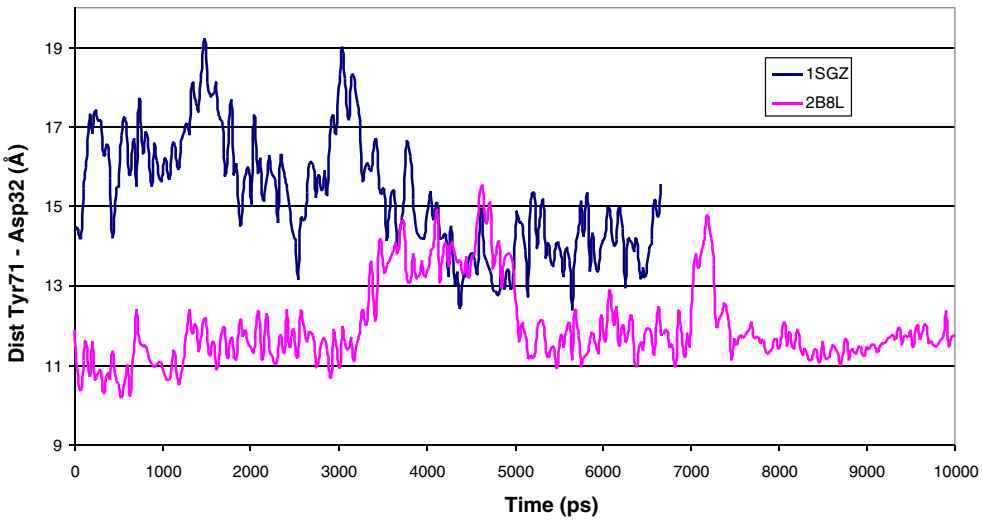

Fig. 4 Distance between Asp32 Cα and Tyr71 Cα during the entire MD simulation

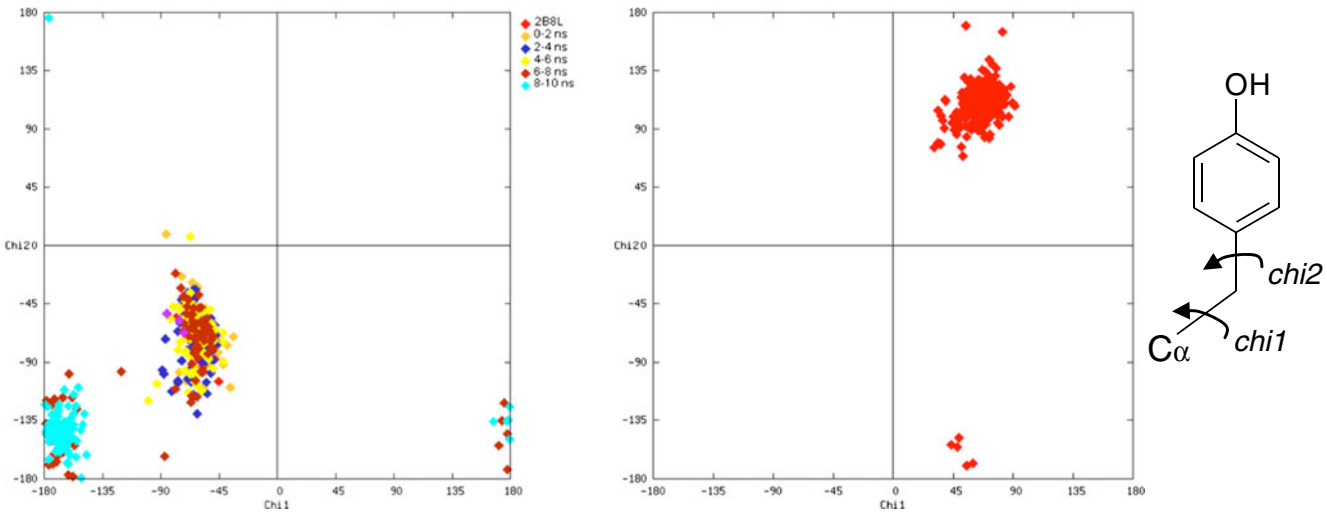

Fig. 5 Chi1/Chi2 representation of 2B8L (left) and 1SGZ (right) during the entire MD simulation. For 2B8L, color coding indicates evolution during the simulation

However, when this H-bond was broken, Tyr71 had more freedom to move, and kept a similar orientation exchanging between two major conformations (Fig. 5). For the 1SGZ simulation, the flap opened an even wider active site, starting from 14 Å to 19 Å during the first 6 ns. Tyr71 kept the initial conformation with very small variations. This supports the idea that this original orientation may not be a crystallization artifact, but may be linked to the widest opening of the active site [34,

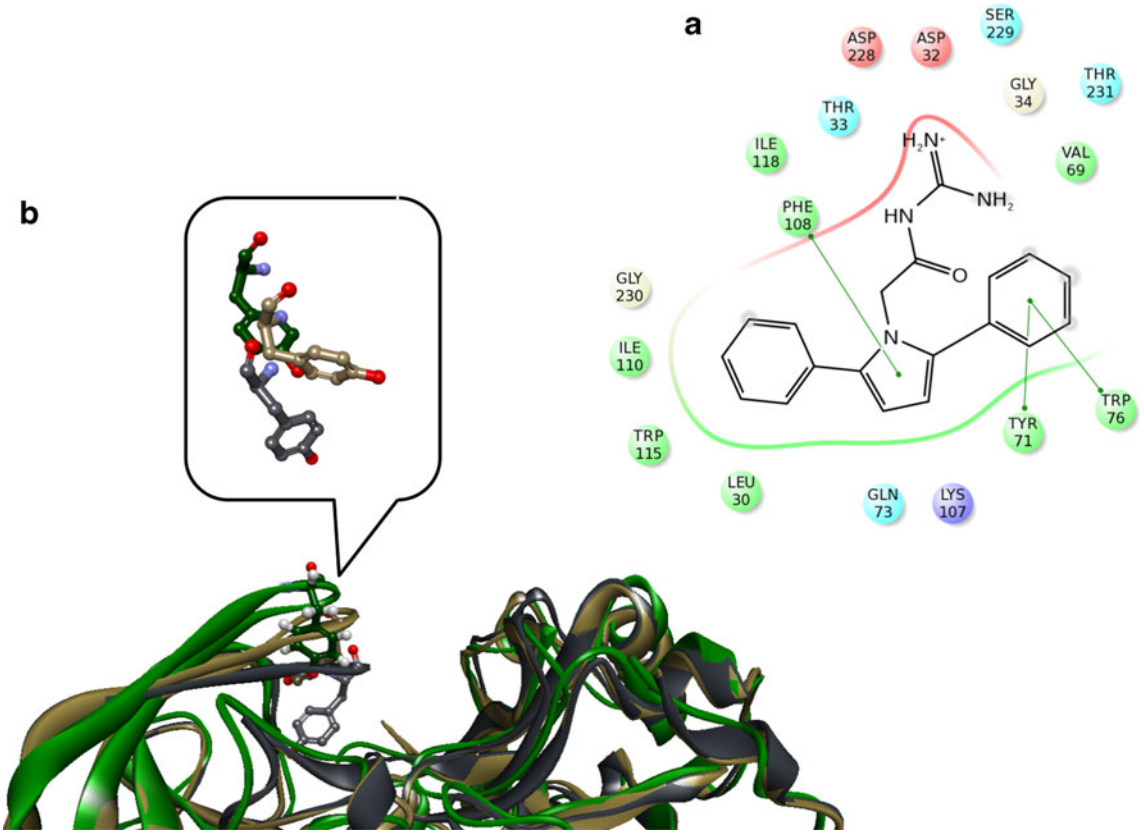

Fig. 6 **a** Interaction of compound WY-25105 with residues at 4 Å that constitute the binding site in the selected conformation #2775 from the MD simulation. **b** Superimposition of conformations taken directly from the docking (*green*) and the crystal structures 1SGZ (*brown*) and 2B8L (*gray*). The large opening of the flap in the simulation-extracted #2775 conformation and the orientation of Tyr 71 gave the best docking result and was found to agree well with the experimental X-ray structure

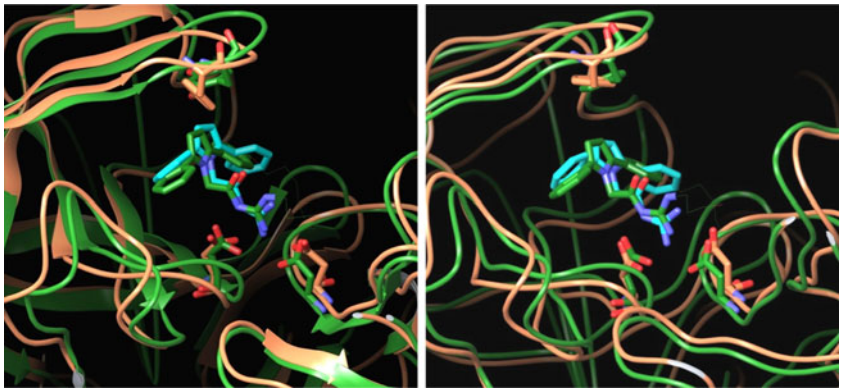

Fig. 7 Active site of BACE with the 2QU2 X-ray structure in *green*. The selected conformation #2775 from the MD simulation is in *orange* and the docked ligand is in *blue*. *Left* Conformation taken directly from the docking, *right* conformation of the MD-docked complex minimized with Macromodel

60]. Taken together, these two simulations (though quite short in time) suggest that the "H-bond lock" between Trp76 and Tyr71 needs to be broken to open the flap, and, when the flap is at its maximum, Tyr71 may change its orientation.

Docking

Flap opening graphs and Tyr71 orientation plots showed that many new conformations were available to search for a new structure capable of docking the acylguanidine WY-25105 compound. A selection of 400 conformational snapshots was made from 1SGZ simulation, as in the 2B8L simulation in which Tyr71 has not taken the expected orientation, but was quite close. As no clustering tool capable of managing such large structures was in our hands at that time, selection was made by selecting one conformation every 15 ps to be sure to catch rare events that could appear during the MD simulation.

Structures selected for docking were extracted from the simulation and converted to pdb format. All water molecules and ions were removed. Protonation states were kept as for the simulation; in particular, the aspartyl dyad was left deprotonated. Gold docking software with default parameters was used to dock the acylguanidine WY-25105 compound, asking for one unique solution for each protein structure [59]. In order to make an initial guess to compare the 400 docking pose results, the WY-25105 molecule was placed manually into the 1SGZ active site, without considering steric clashes, in order to reproduce as much as possible the observed pose in the published poster as the X-ray structure was not available at that time (Fig. 1b) [41, 59]. This virtual pose was then used to calculate the RMSD of the ligand with all docking results. Careful visual inspection of the 25 closest docked compounds was conducted and a final result was selected: protein conformation #2775 seemed to be in excellent agreement with the published results. This binding mode of compound WY-25105 is shown with residues located at maximum 4 Å in Fig. 6. Superimposition of the conformation taken directly from the docking and crystal structures 1SGZ and 2B8L helps to highlight the large opening of the flap in the simulation-extracted #2775 conformation, in particular the orientation of Tyr 71.

By the time this work was finished, the article corresponding to the poster of Cole et al. [41, 59] was published and the X-ray structure deposited in the PDB (2QU2). Interestingly, the overall RMSD between the X-ray structure and the simulation-extracted #2775 conformation was 1.63 Å on all heavy atoms. When considering only residues within a 10 Å radius around the inhibitor, this value went down to 1.46 Å (Fig. 7) showing a very similar active site conformation around WY-25105. The docked complex (simulation-extracted protein and WY-25105 compound) was further minimized using Macromodel, with implicit solvation and 2,500 conjugated gradient steps with the OPLS2001 force field. The 10 Å radius RMSD fell to 1.39 Å. Excellent agreement was found for most active site residues between this minimized #2775 conformation and the X-ray structure, from Tyr71, Asp32 and Asp228 and the residues constituting the S1 and S2' pockets (Fig. 7).

Conclusions

In the present work, we generated multiple receptor conformations from BACE crystal structures, then carried out flexible docking of compound WY-25105 for the most representative conformations. We have shown that this strategy allows sampling a larger conformational space than normally available, which improves docking as well as facilitating exploration of unusual binding modes. The following important prerequisites are necessary to conduct this work:

- Sufficient information concerning the final experimental structure is needed: in the present case the conformations of Tyr71, Asp32 and Asp228 and the inhibitor structure were used as restraints to generate the adapted conformation and select the final docking pose.
- While multiple receptor conformations may improve docking prediction, too many additional conformations

increase the chance of false positives in the case of docking of a large compound collection.

– The final important point is the selection of the starting structure used for the MD simulation. It should be representative of most of the binding site conformations and not an artifact of an incorrect pose prediction or false positive in virtual screening. In the case of BACE, several studies have shown that the overall structure of the protein does not change during MD simulations and also that the flap can open and close in a short timescale. Thus, careful choice of the initial X-ray structure is a major prerequisite, as strong residue reorientation cannot occur during a single simulation, as seen for 2B8L.

In conclusion, the present retrospective study with short timescale simulations clearly demonstrates that MD and docking calculations are able to discriminate the selectivity of the investigated protein and provide significant contributions to underlying structural determinants of this selectivity. This gives an overview of how great a contribution the inclusion of receptor flexibility in MD could make to improving protein–ligand docking. However, even if sufficient to catch an adequate conformation for our docking purposes, this 10-ns simulation seems insufficient to fully open the flap of the 2B8L structure. It has been shown that the chi1/chi2 of Tyr 71 of 2B8L did not sample the space covered by the 1SGZ flap. Finally, the authors recommend the following multiple receptor conformation in protein–ligand docking calculations with recent advances in high-performance MD simulation, quantum mechanics simulation, mixed mode Monte Carlo/stochastic dynamics simulations and with the ability to generate very long continuous trajectories (microsecond to millisecond timescale) as a powerful tool to mimics receptor flexibility [61, 62]. This could be used alone, in parallel, or in combination with other computational modeling approaches (e.g., soft-receptor docking, induced-fit docking, grid-based protein-ligand docking) to attempt to incorporate different types of motions into the docking procedure [28, 46, 63].

Acknowledgments The authors gratefully acknowledge C. Chipot and A. Karlsson for their advice, useful discussions and for correcting the manuscript. L.G. gratefully acknowledges the support of the Drug Discovery and Chemical Biology Consortium of Finland, and Centre of Drug Research of University of Helsinki.

References

1. Cumming JN, Iserloh U, Kennedy ME (2004) Design and development of BACE-1 inhibitors. Curr Opin Drug Discov Dev 7(4):536–556
2. Evin G, Lessene G, Wilkins S (2011) BACE inhibitors as potential drugs for the treatment of Alzheimers disease: focus on bioactivity. Recent Patents CNS Drug Discov 6(2):91–106. doi:10.2174/157488911795933938
3. Evin G, Barakat A, Masters CL (2010) BACE: therapeutic target and potential biomarker for Alzheimer's disease. Int J Biochem Cell Biol 42(12):1923–1926. doi:10.1016/J.Biocel.2010.08.017
4. De Strooper B, Vassar R, Golde T (2010) The secretases: enzymes with therapeutic potential in Alzheimer disease. Nat Rev Neurol 6(2):99–107. doi:10.1038/nrneurol.2009.218
5. Ahmed RR, Holler CJ, Webb RL, Li F, Beckett TL, Murphy MP (2010) BACE1 and BACE2 enzymatic activities in Alzheimer's disease. J Neurochem 112(4):1045–1053. doi:10.1111/J.1471-4159.2009.06528.X
6. Vassar R, Kovacs DM, Yan RQ, Wong PC (2009) The beta-secretase enzyme BACE in health and Alzheimer's disease: regulation, cell biology, function, and therapeutic potential. J Neurosci 29(41):12787–12794. doi:10.1523/Jneurosci.3657-09.2009
7. Ghosh AK, Brindisi M, Tang J (2012) Developing beta-secretase inhibitors for treatment of Alzheimer's disease. J Neurochem 120(Suppl 1):71–83. doi:10.1111/j.1471-4159.2011.07476.x
8. Wolfe MS (2001) Secretase targets for Alzheimer's disease: identification and therapeutic potential. J Med Chem 44(13):2039–2060
9. Karran E, Mercken M, De Strooper B (2011) The amyloid cascade hypothesis for Alzheimer's disease: an appraisal for the development of therapeutics. Nat Rev Drug Discov 10(9):698–712. doi:10.1038/nrd3505
10. Baxter EW, Conway KA, Kennis L, Bischoff F, Mercken MH, Winter HL, Reynolds CH, Tounge BA, Luo C, Scott MK, Huang Y, Braeken M, Pieters SM, Berthelot DJ, Masure S, Bruinzeel WD, Jordan AD, Parker MH, Boyd RE, Qu J, Alexander RS, Brenneman DE, Reitz AB (2007) 2-Amino-3,4-dihydroquinazolines as inhibitors of BACE-1 (beta-site APP cleaving enzyme): use of structure based design to convert a micromolar hit into a nanomolar lead. J Med Chem 50(18):4261–4264. doi:10.1021/jm0705408
11. Ghosh AK, Bilcer G, Harwood C, Kawahama R, Shin D, Hussain KA, Hong L, Loy JA, Nguyen C, Koelsch G, Ermolieff J, Tang J (2001) Structure-based design: potent inhibitors of human brain memapsin 2 (beta-secretase). J Med Chem 44(18):2865–2868
12. Pellecchia M (2009) Fragment-based drug discovery takes a virtual turn. Nat Chem Biol 5(5):274–275. doi:10.1038/Nchembio0509-274
13. Hu B, Xiong B, Qiu BY, Li X, Yu HP, Xiao K, Wang X, Li J, Shen JK (2006) Construction of a small peptide library related to inhibitor OM99-2 and its structure-activity relationship to beta-secretase. Acta Pharmacol Sin 27(12):1586–1593. doi:10.1111/j.1745-7254.2006.00432.x
14. Hong L, Koelsch G, Lin X, Wu S, Terzyan S, Ghosh AK, Zhang XC, Tang J (2000) Structure of the protease domain of memapsin 2 (beta-secretase) complexed with inhibitor. Science 290(5489):150–153
15. Liu S, Zhou LH, Wang HQ, Yao ZB (2010) Superimposing the 27 crystal protein/inhibitor complexes of beta-secretase to calculate the binding affinities by the linear interaction energy method. Bioorg Med Chem Lett 20(22):6533–6537. doi:10.1016/j.bmcl.2010.09.050
16. Coburn CA, Stachel SJ, Li YM, Rush DM, Steele TG, Chen-Dodson E, Holloway MK, Xu M, Huang Q, Lai MT, DiMuzio J, Crouthamel MC, Shi XP, Sardana V, Chen Z, Munshi S, Kuo L, Makara GM, Annis DA, Tadikonda PK, Nash HM, Vacca JP, Wang T (2004) Identification of a small molecule nonpeptide active site beta-secretase inhibitor that displays a nontraditional binding mode for aspartyl proteases. J Med Chem 47(25):6117–6119. doi:10.1021/jm049388p
17. Patel S, Vuillard L, Cleasby A, Murray CW, Yon J (2004) Apo and inhibitor complex structures of BACE (beta-secretase). J Mol Biol 343(2):407–416. doi:10.1016/j.jmb.2004.08.018

18. Stachel SJ, Coburn CA, Steele TG, Crouthamel MC, Pietrak BL, Lai MT, Holloway MK, Munshi SK, Graham SL, Vacca JP (2006) Conformationally biased P3 amide replacements of beta-secretase inhibitors. Bioorg Med Chem Lett 16(3):641–644. doi:10.1016/j.bmcl.2005.10.032
19. Turner RT 3rd, Hong L, Koelsch G, Ghosh AK, Tang J (2005) Structural locations and functional roles of new subsites S5, S6, and S7 in memapsin 2 (beta-secretase). Biochemistry 44(1):105–112. doi:10.1021/bi048106k
20. Yang W, Lu W, Lu Y, Zhong M, Sun J, Thomas AE, Wilkinson JM, Fucini RV, Lam M, Randal M, Shi XP, Jacobs JW, McDowell RS, Gordon EM, Ballinger MD (2006) Aminoethylenes: a tetrahedral intermediate isostere yielding potent inhibitors of the aspartyl protease BACE-1. J Med Chem 49(3):839–842. doi:10.1021/jm0509142
21. Rognan D (2010) Structure-based approaches to target fishing and ligand profiling. Mol Inform 29(3):176–187. doi:10.1002/Minf.200900081
22. Schapira M, Raaka BM, Das S, Fan L, Totrov M, Zhou ZG, Wilson SR, Abagyan R, Samuels HH (2003) Discovery of diverse thyroid hormone receptor antagonists by high-throughput docking. Proc Natl Acad Sci USA 100(12):7354–7359. doi:10.1073/Pnas.1131854100
23. Bottegoni G (2011) Protein-ligand docking. Front Biosci J Virtual Libr 17:2289–2306
24. Meng XY, Zhang HX, Mezei M, Cui M (2011) Molecular docking: a powerful approach for structure-based drug discovery. Curr Comput Aided Drug Des 7(2):146–157
25. Huang SY, Zou X (2010) Advances and challenges in protein-ligand docking. Int J Mol Sci 11(8):3016–3034. doi:10.3390/ijms11083016
26. Ferrara P, Gohlke H, Price DJ, Klebe G, Brooks CL (2004) Assessing scoring functions for protein-ligand interactions. J Med Chem 470(12):3032–3047. doi:10.1021/Jm030489h
27. Totrov M, Abagyan R (2008) Flexible ligand docking to multiple receptor conformations: a practical alternative. Curr Opin Struct Biol 18(2):178–184. doi:10.1016/J.Sbi.2008.01.004
28. Kokh DB, Wade RC, Wenzel W (2011) Receptor flexibility in small-molecule docking calculations. Wiley Interdiscip Rev Comput Mol Sci 1(2):298–314. doi:10.1002/wcms.29
29. Englebienne P, Moitessier N (2009) Docking ligands into flexible and solvated macromolecules. 5. Force-field-based prediction of binding affinities of ligands to proteins. J Chem Inf Model 49(11):2564–2571. doi:10.1021/Ci900251k
30. McGaughey G, Feuston B, Graham S, Holloway MK, Lindsley S, Nantermet P, Rajapakse H, Stauffer S (2006) BACE: accounting for flexibility in a scoring function. Abstr Pap Am Chem Soc 231
31. Xiong JP, Stehle T, Goodman SL, Arnaout MA (2004) A novel adaptation of the integrin PSI domain revealed from its crystal structure. J Biol Chem 279(39):40252–40254. doi:10.1074/jbc.C400362200
32. Bursavich MG, Rich DH (2002) Designing non-peptide peptidomimetics in the 21st century: inhibitors targeting conformational ensembles. J Med Chem 45(3):541–558. doi:10.1021/Jm010425b
33. Cascella M, Micheletti C, Rothlisberger U, Carloni P (2005) Evolutionarily conserved functional mechanics across pepsin-like and retroviral aspartic proteases. J Am Chem Soc 127(11):3734–3742. doi:10.1021/Ja044608+
34. Gorfe AA, Caflisch A (2005) Functional plasticity in the substrate binding site of beta-secretase. Structure 13(10):1487–1498. doi:10.1016/J.Str.2005.06.015
35. Holloway MK, Hunt P, McGaughey GB (2009) Structure and modeling in the design of β- and γ-secretase inhibitors. Drug Dev Res 70(2):70–93. doi:10.1002/ddr.20291
36. Neri, M, Cascella, Micheletti, C (2005) The influence of conformational fluctuations on enzymatic activity: modelling the functional motion of β-secretase. Institute of Physics, Bristol, UK
37. Xiong B, Huang XQ, Shen LL, Shen JH, Luo XM, Shen X, Jiang HL, Chen KX (2004) Conformational flexibility of beta-secretase: molecular dynamics simulation and essential dynamics analysis. Acta Pharmacol Sin 25(6):705–713
38. Rueeger H, Rondeau JM, McCarthy C, Mobitz H, Tintelnot-Blomley M, Neumann U, Desrayaud S (2011) Structure based design, synthesis and SAR of cyclic hydroxyethylamine (HEA) BACE-1 inhibitors. Bioorg Med Chem Lett 21(7):1942–1947. doi:10.1016/j.bmcl.2011.02.038
39. Hanessian S, Shao Z, Betschart C, Rondeau JM, Neumann U, Tintelnot-Blomley M (2010) Structure-based design and synthesis of novel P2/P3 modified, non-peptidic beta-secretase (BACE-1) inhibitors. Bioorg Med Chem Lett 20(6):1924–1927. doi:10.1016/j.bmcl.2010.01.139
40. Wang YS, Strickland C, Voigt JH, Kennedy ME, Beyer BM, Senior MM, Smith EM, Nechuta TL, Madison VS, Czarniecki M, McKittrick BA, Stamford AW, Parker EM, Hunter JC, Greenlee WJ, Wyss DF (2010) Application of fragment-based NMR screening, X-ray crystallography, structure-based design, and focused chemical library design to identify novel microM leads for the development of nM BACE-1 (beta-site APP cleaving enzyme 1) inhibitors. J Med Chem 53(3):942–950. doi:10.1021/jm901472u
41. Cole DC, Manas ES, Stock JR, Condon JS, Jennings LD, Aulabaugh A, Chopra R, Cowling R, Ellingboe JW, Fan KY, Harrison BL, Hu Y, Jacobsen S, Jin G, Lin L, Lovering FE, Malamas MS, Stahl ML, Strand J, Sukhdeo MN, Svenson K, Turner MJ, Wagner E, Wu J, Zhou P, Bard J (2006) Acylguanidines as small-molecule beta-secretase inhibitors. J Med Chem 49(21):6158–6161. doi:10.1021/jm0607451
42. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE (2000) The protein data bank. Nucleic Acids Res 28(1):235–242
43. Hong L, Tang J (2004) Flap position of free memapsin 2 (beta-secretase), a model for flap opening in aspartic protease catalysis. Biochemistry 43(16):4689–4695. doi:10.1021/Bi0498252
44. Spronk SA, Carlson HA (2011) The role of tyrosine 71 in modulating the flap conformations of BACE1. Proteins 79(7):2247–2259. doi:10.1002/prot.23050
45. Muzzioli E, Del Rio A, Rastelli G (2011) Assessing protein kinase selectivity with molecular dynamics and mm-pbsa binding free energy calculations. Chem Biol Drug Des 78(2):252–259. doi:10.1111/j.1747-0285.2011.01140.x
46. Lill MA (2011) Efficient incorporation of protein flexibility and dynamics into molecular docking simulations. Biochemistry 50(28):6157–6169. doi:10.1021/bi2004558
47. Fobare WF, Solvibile WR, Robichaud AJ, Malamas MS, Manas E, Turner J, Hu Y, Wagner E, Chopra R, Cowling R, Jin G, Bard J (2007) Thiophene substituted acylguanidines as BACE1 inhibitors. Bioorg Med Chem Lett 17(19):5353–5356. doi:10.1016/j.bmcl.2007.08.010
48. Schrödinger (2009) Schrödinger suite 2009 protein preparation wizard; impact version 5.5. New York, NY
49. Dominguez JL, Christopeit T, Villaverde MC, Gossas T, Otero JM, Nystrom S, Baraznenok V, Lindstrom E, Danielson UH, Sussman F (2010) Effect of the protonation state of the titratable residues on the inhibitor affinity to BACE-1. Biochemistry 49(34):7255–7263. doi:10.1021/bi100637n
50. Polgar T, Keseru GM (2005) Virtual screening for beta-secretase (BACE1) inhibitors reveals the importance of protonation states at Asp32 and Asp228. J Med Chem 48(11):3749–3755. doi:10.1021/jm049133b
51. Rajamani R, Reynolds CH (2004) Modeling the protonation states of the catalytic aspartates in beta-secretase. J Med Chem 47(21):5159–5166. doi:10.1021/Jm049817j
52. Friedman R, Caflisch A (2010) On the orientation of the catalytic dyad in aspartic proteases. Proteins 78(6):1575–1582. doi:10.1002/prot.22674

53. Feller SE (2007) Molecular dynamics simulations as a complement to nuclear magnetic resonance and X-ray diffraction measurements. Methods Mol Biol 400:89–102
54. Humphrey W, Dalke A, Schulten K (1996) VMD: visual molecular dynamics. J Mol Graph 14(1):33
55. Phillips JC, Braun R, Wang W, Gumbart J, Tajkhorshid E, Villa E, Chipot C, Skeel RD, Kale L, Schulten K (2005) Scalable molecular dynamics with NAMD. J Comput Chem 26(16):1781–1802. doi:10.1002/jcc.20289
56. Essmann U, Perera L, Berkowitz M, Darden T, Lee H, Pedersen L (1995) A smooth particle mesh Ewald method. J Chem Phys 103(19):8577–8593
57. Paterlini MG, Ferguson DM (1998) Constant temperature simulations using the Langevin equation with velocity Verlet integration. Chem Phys 236(1–3):243–252
58. Jones G, Willett P, Glen RC, Leach AR, Taylor R (1997) Development and validation of a genetic algorithm for flexible docking. J Mol Biol 267(3):727–748
59. Jones G, Willett P, Glen RC (1995) Molecular recognition of receptor sites using a genetic algorithm with a description of desolvation. J Mol Biol 245(1):43–53
60. McGaughey GB, Holloway MK (2007) Structure-guided design of beta-secretase (BACE-1) inhibitors. Expert Opin Drug Dis 2(8):1129–1138. doi:10.1517/17460441.2.8.1129
61. Roy J, Laughton CA (2010) Long-timescale molecular-dynamics simulations of the major urinary protein provide atomistic interpretations of the unusual thermodynamics of ligand binding. Biophys J 99(1):218–226. doi:10.1016/j.bpj.2010.03.055
62. Klepeis JL, Lindorff-Larsen K, Dror RO, Shaw DE (2009) Long-timescale molecular dynamics simulations of protein structure and function. Curr Opin Struct Biol 19(2):120–127. doi:10.1016/j.sbi.2009.03.004
63. Leis S, Zacharias M (2011) Efficient inclusion of receptor flexibility in grid-based protein-ligand docking*. J Comput Chem 32(16):3433–3439. doi:10.1002/jcc.21923