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| RC: |
| a)Report backbone torsion angle changes in loop between ternary complex and intermediate complex |
| Then in intermediate complex models you have prepared starting from the ternary xtal structure (e.g., 2,4 above), set these angles to their values from the intermediate complex xtal structure, followed by side chain optimization and minimization. Report the MM-GBSA/PBSA energies and compare to the low energy loop conformations produced by ab initio loop prediction in prime. |
| Then set up another MD simulation starting from this structure |
| b) Do simulation 4) for Sir2Tm as well. (If computational resources permit, do a) for Sir2Tm as well; at least the first parts should be done) |
| c) Please indicate whether structures are available to run any of these simulations with Sir2Af2 or SIRT2. |
| Please indicate approximate CPU time / ns of simulation for the above MD simulations, when they are started, and also a target date (approximate is fine) for when the results from the non-MD tasks (including 3) will be updated. Based on this info we will decide whether purchase of another GPU node is warranted. |
| d) Meeting with AS asap. All points mentioned on wiki and at group meeting regarding his tasks should be discussed (including next steps w sysadmin including secure ftp server appropriate users should be given access and the folder may be changed if needed). Either AS or PL should post the minutes of the meeting and how the listed points were addressed and AS's schedule for the entire following week. |
| AS should have reviewed and tested the MM-GBSA scripts within the week. |
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| RC (10/29): |
| -dataset appears reasonable; need more tabular info on the RMSDs between pairs of structures (see also next point below), indication of which pairs of structures will be compared, and whether structure/ligand pose prediction will be independently tested or the docking protocol will directly be tested as priority |
| -regarding NAD+/intermediate complexes, if I understand correctly class 1) includes intermediate and 2) includes ternary complex. If so the document appears to indicate a qualitatively different loop conformation between the two. This is related to several other tasks that were posted over last few weeks on group meetings page. Please see those, which pertain to more detailed analysis of these structural differences. |
| -regarding MD, please start w more info on prior simulations of the the NAM+intermediate complex (from paper 1; see meetings page) as requested vis-a-vis the intermediate/NAD comparison above. next steps for simulations are also provided on the meetings page. |
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| RC: Looking at the ppt from the last group meeting, MD was briefly mentioned in one slide and it wasn't clear which of these simulations were set up anew, which were run previously, and how some of the new simulations were being set up. |
| Please provide that info. |
| Regarding the simulation above, does this include NAM? If so, how was NAM prepared? Please see the original comments regarding this simulation. Do you have a MD average from the simulation so far? When was it started? |
| Also as noted above some more detailed info is desirable regarding the comparison of NAD+/intermediate structures (in absence of MD). Perhaps a closeup of the superposition with comments on which residues are contribution most to the RMSD.  |
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| The original plan of work indicated that the prior binding affinity simulations of NAM during PLOS paper could be studied in more detail first to get an idea of the B factors of loop/NAM and how the fluctuations observed during this sampling compare to the distance between the loop and NAM in the intermediate vs the NAD+ complex structure. If more clarification is needed here please let me know. Also please remind me if all our previous MD simulations with NAM had NAD+ in the AB pocket. |
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| RC: Looking at the meetings page, I referred to simulation (B) starting after cleavage of NAM (intermediate + NAM). Did you remove NAM from the structure? |
| The method of intermediate preparation is fine; the question is how you prepared NAM. |
| As noted if there were questions I would address them were they posed promptly. |
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| -Comment on constrained loop sampling options in prime. |
| -Some papers claim loop conformational change after cleavage is important for next step of deacetylation and also for NAM release. Can we confirm this (at least former - see PNAS 2004 Sir2 xtalography paper) based on SIRT3 structures? |
| -Compare C pocket interactions of NAM in SIRT3:peptide:NAD+ to NAM in SIRT3:intermediate:NAM |
| -Multiple sequence alignment of sirtuin loop and C pockets |
| -As I noted in a recent email regarding the paper plans, we may be including some of our results on the computational prediction of ligand binding affinities and correlation with respect to experimental data. For this purpose, I would like to start by revisiting the existing C pocket binding affinity data for the series of C pocket binding ligands we studied starting with Eric's work. Please indicate where I can find the correlations with XG's experimental data and whether this included MM-GBSA,PBSA, and glidescore correlations. After reviewing these, I will provide further instructions on whether we need to continue this work with new binding affinity calculations and associated experiments for the purposes of the paper(s). |
| -Consider preparation of movies for visualization of MD sims |
| -Matlab license question of AS. Checking AS comments/questions and providing any required replies for next week's work. |
| schedule meeting to discuss next steps for MM-GBSA code dev after PL verifies that AS has run existing code and batch servers properly. as should also schedule next tasks for sysadmin (like secure ftp server administration |
| after some more of these done will schedule addition of gpu node to batch server |
| Consider capacity usage of GPU node for MD simulations and recommend whether a new gpu node should be purchased. If so, may involve AS in the planning/purchasing. |
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| 1) Computational tasks |
| a) |
| i- All pending info on the dataset for IFD should be provided at group meeting and also on wiki |
| ii- All unanswered questions on tasks page should be answered on wiki before group meeting |
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| b) Provide the following at the group meeting: |
| i- First provide the residue-by-residue RMSD for each residue in loop and also NAM between the SIRT3:peptide:NAD+ and SIRT3:intermediate:NAM xtal structures (see group meeting minutes from ~ 1 month ago) |
| ii- It was indicated on tasks page that SIRT3:Intermediate:NAM MD simulations (starting from intermediate xtal structure) were previously run. Use ptraj in Amber to provide times series of RMSD with respect to the initial structure as well as the products of the NAM cleavage reaction (separately for both NAM and flexible loop). |
| iii- From the results of MD simulation starting from intermediate superimposed in the SIRT3:peptide:NAD+ xtal structure (currently running), provide time series plots of RMSD with respect to NAM and loop in intermediate complex, as well as analogous plots with respect to starting structure; also provide the time series plot of the associated energies.  |
| iv- Also provide residue-by-residue B factors and NAM B factors from these simulations |
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| c) The following simulation should be set up and ready for discussion at meeting (it was originally requested in place of b-iii above):  |
| i-MD simulations starting after NAM cleavage reaction initialized by energy minimization after bond cleavage, retaining NAM in the complex. Compare NAM pose to that prepared by docking to this same structure, prior to starting the simulation. |
| Related: |
| ii- compare C pocket interactions of NAM in SIRT3:peptide:NAD+ to NAM in SIRT3:intermediate:NAM |
| iii- provide single point energy for products of the NAM cleavage reaction (from c-i starting structure, after minimization)  |
| iv- provide ensemble average energies for intermediate:NAM complex (once available from c-i after equilibration) |
| (iii,iv should be provided in a table. Both MM-PBSA and MM-GBSA values can be provided) |
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| d) Multiple sequence alignment of all sirtuin C pockets with listing of residues interacting with NAM and how (latter from structural analysis) |
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| Sirtuin structure prediction validation: |
| - Dataset for systematic structure prediction methods development and testing should be provided prior to starting validation. This dataset can be provided on the paper 2 page as discussed. It should include a list of available sirtuin structures (esp SIRT3, but can also include other sirtuins if the SIRT3 dataset is too small), including ligand (drug) complexes wherein loop conformational shifts occur and for selected pairs of such structures, indicate the relevant RMSDs of active site side chains and relevant secondary structural motifs including the flexible loop. These can be used in testing. (Please indicate B factors from xtalography where relevant.) The superposition of structures shown e.g. in the latest group meeting ppt are difficult to interpret visually. See tasks page for more details on how to organize the dataset. |
| - For each such structure pair, one structure will be used as initial to predict second structure (this includes pairs where the structures are the same and one seeks to predict in the native environment). In each case where structure prediction accuracy is poor we are interested in determining if this was due to scoring function inaccuracy, inadequate sampling, or structural flexibility (energetic degeneracy). |
| - Eventually, for paper 2, it will be important to do (customized) IFD protocol testing on structurally characterized ligand complexes such as Ex-527 after above are completed to assess false positive rate. E.g., we should be able to show that preferred Ex-527 binding mode is not buried below the C pocket. For such scoring the IFD scoring function may need to be modified as discussed. |
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| Sirtuin structure prediction IFD scoring functions: |
| - After the dataset above is prepared and while some initial simulations are running, further information on the parameter estimation and testing protocols used to develop and validate induced fit methods in the literature should be summarized. In particular, when did Schrodinger test its advanced IFD protocol and with what dataset. |
| - A note: We have been planning to develop customized scoring functions for sirtuin structure prediction. In the present context, this can be used to improve structure prediction accuracy on the above testing dataset (need to first assess dataset size and break into training and testing subsamples). |
| - For later scaling of IFD calculations, note that we have PLOP (older gen prime) source code available and it is used in python protein design script. This can be run on an arbitrary number of nodes. Hence we can do extensive sampling of protein degrees of freedom in our protocols (moreso than ligand degrees of freedom for now) and may later modify IFD to use PLOP not prime as needed. AS may be involved in this. |
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| Conformational changes upon NAM cleavage (please indicate when you plan to schedule these tasks): |
| - Analysis of past MD simulation data on NAM in C pocket of intermediate complex should be provided as indicated below after our previous group meeting. Please provide analysis of B factors for loop and NAM and a comparison to NAD+ (ternary) complex structure (see below for more details). As noted below MD simulations of the conformational change between these complexes can follow after these results are reviewed and discussed as needed. These are relevant to paper 2. |
| - Differences in flexible loop between intermediate and ternary complex not yet clear. Is the comparison provided on 4BVG and 3GLS between intermediate and apo, from two different studies? How many intermediate structures are available and from which studies (this may be answered in context of dataset above)? Does the loop conformation differ so substantially in all structures if there are more than one? Please summarize the differences. Please also indicate whether a loop prediction is being run starting from ternary complex structure with NAM cleaved. |
| - Summaries of MD results, as they become available, should also be organized systematically in a section of wiki page for 2nd paper. |
| - MD simulation results can also be used to prepare movies suitable for viewing by AS and other group members for purpose of visualizing MD results. |
| - As indicated below results of MD simulations of loops (when they are done) should be compared to results of highest ranked results from ab initio loop prediction to assess adequacy of loop sampling of the long loop (see below for more details). |
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| As a general principle, we need to start using multiple available structures for validation of computational structure prediction methods (starting w validation of known mechanism-based inhibitors). The dataset is of considerable size and as many structures as possible could be used for validation. Esp with IFD, there is a concern regarding false positives. In general, such problems may be broken down into issues with a) sampling; b) accuracy of the energy function. Both will be assessed in the context of sirtuin structure prediction.  |
| Current focus is on rank ordering of structures; later binding affinity datasets may be used.  |
| 1) Review MD simulation study in PLOS ONE\_ loop motions after NAM docked? |
| -check what kinds of loop motion we have sampled in our MD simulations of NAM in C pocket (summary statistics); what were starting structures? what were timescales over which significant loop motions were observed?  |
| Visualization of trajectories (lower priority) |
| -Summarize the structural differences (NAM and loop) between NAD+ and intermediate from literature and xtals  |
| -Related (lower priority), check Wolberger's "NAM flipping" conjecture vis-a-vis this analysis - compare flipped conformation to that we observe and indicate what side chains are involved in stabilizing the flipped conformation |
| 2) Energetic issues, induced fit calculation |
| -When ranking protein structures obtained by rotamer sampling or ligand poses only, same degrees of freedom are sampled for each ranked structure. We need to revise IFD protocol to be able to properly incorporate protein reorganization energy in scores used to rank order binding modes. We could, e.g., start by modifying script to keep track of exactly what residues are being sampled and then sample the same ones for different ligand poses. Same number of docking/protein structure prediction iterations should be used for each ranked binding mode. MD could then be used on structures prepared in this way for binding affinity estimation (receptor preparation also needed for reporting absolute binding affinities). See also (3) below regarding the energy function. |
| -Before moving on to new IFD calculations, we need to test such a protocol on pairs of sirtuin structures wherein protein conformation changes occur (to be selected). E.g., could test ability to predict Ex-527 conformation in co-product complex starting from a different receptor structure with important differences in protein conformation. In any such test, include scoring of the crystallographic structure to determine whether a test failed because of inadequate sampling or an energy function error.  |
| -After further discussion, relevant programming tasks will be assigned to AS |
| 3) formula of IFD score |
| MM-GBSA score may be used in place of docking score. |
| 4) Revisit Ex-527 PNAS paper: loop open/close conformation |
| 5) Thinking about possible MD simulations to exam (A)EX527:coproduct issue; (B) start from intermediate after the cleavage of NAM. Former is for purposes of computational methods validation; latter is a step of the sirtuin catalytic cycle that has not been studied computationally. |
| Results of 1,4) will be useful in advance. |
| -For (A), check if loop prediction can confirm that for Ex-527/coproduct complex, the drug stabilizes the closed conformation of the loop. Check if ab initio loop prediction ranks highly those loop conformations that appear most frequently in MD simulations. |
| -For (B), set up the MD simulation for at least as long as the timescale in 1). Further discussion will be possible after an update is provided on the results from 1). Provide summary statistics on loop and NAM conformations, comparing to intermediate xtal, and possibly visualize trajectory. Prepare initial structure by breaking/making the bonds manually, setting appropriate charges, and optimizing geometry.  |
| Confirm the length of the flexible loop. Assuming the intermediate complex structure differs significantly from that of NAD+ complex, try to predict the former from the latter using loop prediction, or at least whether the xtal loop structure is one of highly ranked ab initio structures. Energy minimize the xtal loop conformations and compare them to the ranked loop energies to determine effect of sampling efficiency vs energy function accuracy. Again compare the results to those from MD.  |
| If successful, MD or ab initio loop sampling may be useful in customized IFD protocols (to be subsequently automated) using a limited number of pre-generated loop conformations to reduce backbone sampling space. Constraints may also be applied later to allow limited sampling in this regard. Even if not successful, similar customized sampling algorithms may be developed.  |
| (Possible issues with experimental prep protocols for xtals and loop disorder will be discussed later.) |
| Some of this analysis may be relevant to paper 2. Other tasks related to continued outlining of subsections of this paper will be posted later. |
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| Prepare Arabinda's tasks and/or software development wiki pages on PMC-AT wiki. Note that there were many notes regarding prior software dev on the academic wiki (webdesign-ror). AS will be invited to that as well, and may copy immediately relevant info to this wiki. |
| PL can post the tasks for AS and AS management listed by RC last week to one of these pages. |
| After PL has decided whether to test the modified IFD script with license requests only and has updated on this, RC can review cluster NFS/NIS configuration and then RC/PL can expand upon/finalize AS tasks vis-a-vis above points as needed. Meeting schedule with AS will be decided at this time as well. |