Overview of protein-ligand interface design (ligand binding and enzdes)

RosettaCon 2012

Workflow for design of small molecule binders

Scaffold Library

Dock in pocket (PatchDock)
Design sequence

Select globally shape complementary poses

RosettaMatch/Design
Introduce "theosites"

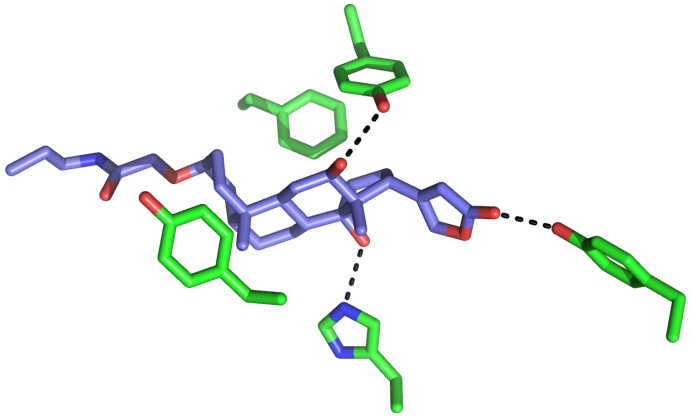
Select Designs for
Predicted Pre-organization

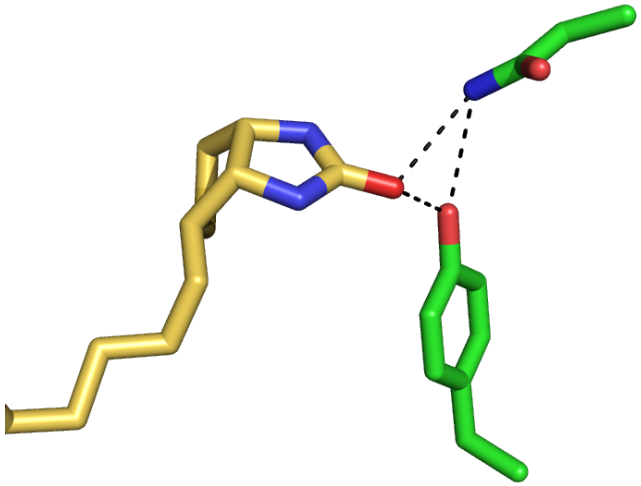
Structural
homologs of
scaffold (DALI)

Enrichment

Experimental characterization

Theosite





Scaffold Selection

- Start from a set of ligand binding proteins
- PDB files for protein, and ligand
- Make_patchdock.pl wrapper script.
- PatchDock details:

  ~/PatchDock/buildParams.pl $protpdb $ligpdb 2.0 drug;

  Make posfile to specify what area of protein to dock into

Add to params.txt:

receptorActiveSite 1a53.pos

Initial design on top25 PatchDock poses for each output

- Perturb-design-filter (xN)

<PredesignPerturbMover name=ppm constraints=0 trans_magnitude=1.0 rot_magnitude=5.0 dock_trials=100 task_operations=edto2,limchi2/>

<EnzRepackMinimize name=des design=1 scorefxn_repack=soft_rep minimize_rb=1 minimize_sc=1 minimize_lig=1 cycles=1 task_operations=edto,up,limchi2/>

<ParsedProtocol name=pert_des>
<Add mover_name=ppm/>
<Add mover_name=des/>
</ParsedProtocol>

<GenericMonteCarlo name=multides mover_name=pert_des filter_name=interfE trials=5 sample_type=low temperature=0.6 drift=0/>

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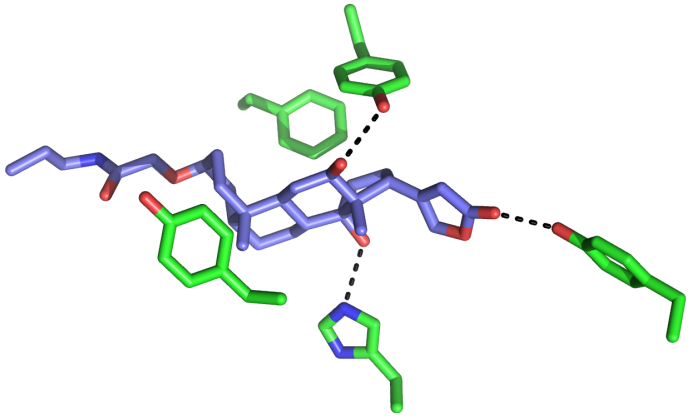
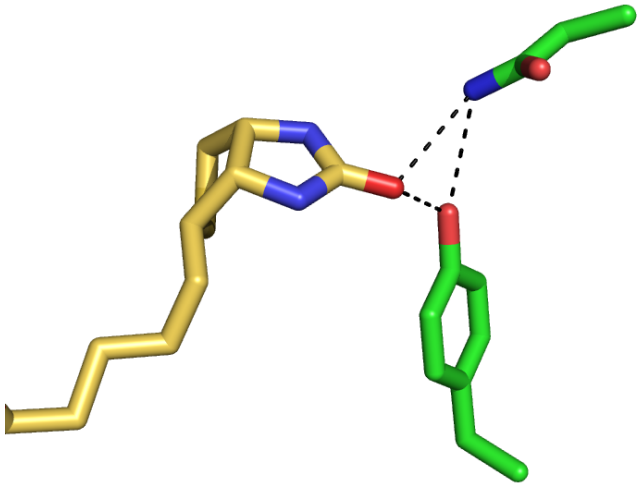




Match

Florian

Design using RosettaScripts

- TaskOperations:

<DetectProteinLigandInterface name=edto design=1 cut1=6.0 cut2=8.0 cut3=10.0 cut4=12.0
catres_interface=1 catres_only_interface=0 arg_sweep_interface=0/>

<RestrictConservedLowDdg name=ddg ddG_filename="ddg_predictions.out"
conservation_cutoff=0.6 ddG_cutoff=1.5 verbose=1/>

<SetCatalyticResPackBehavior name=fixcat fix_catalytic_aa=1/>

<ProteinLigandInterfaceUpweighter name=ligUp1 interface_weight = 1.0 catres_interface_weight
= 1/>

Protocol

Add constraints: <AddOrRemoveMatchCsts name=addcst cst_instruction=add_new />

Design-minimize

Buttress Binding Residues: <PackRotamersMoverPartGreedy name=prgm scorefxn_repack=soft_rep scorefxn_repack_greedy=hbup scorefxn_minimize=soft_rep target_cstids=1B,2B,3B distance_threshold=6.0 task_operations=LigInterface,catres,limchi2,init,includeCurrent/>

Design-minimize: <EnzRepackMinimize name=desmin design=1 repack_only=0 scorefxn_minimize=myscore scorefxn_repack=soft_rep minimize_rb=1 minimize_sc=1 minimize_bb=0 cycles=3 minimize_lig=1 min_in_stages=0 task_operations=edto,limchi2,ddg,up,init/>

(Filter – low stringency)

Remove Constraints: <AddOrRemoveMatchCsts name=addcst cst_instruction=remove/>

Repack

(Filter – higher stringency)

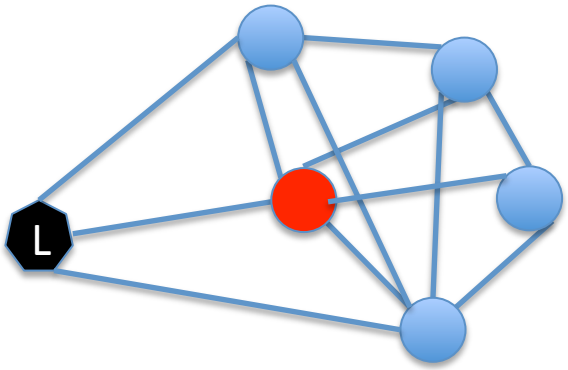
Make point mutations to optimize filters:

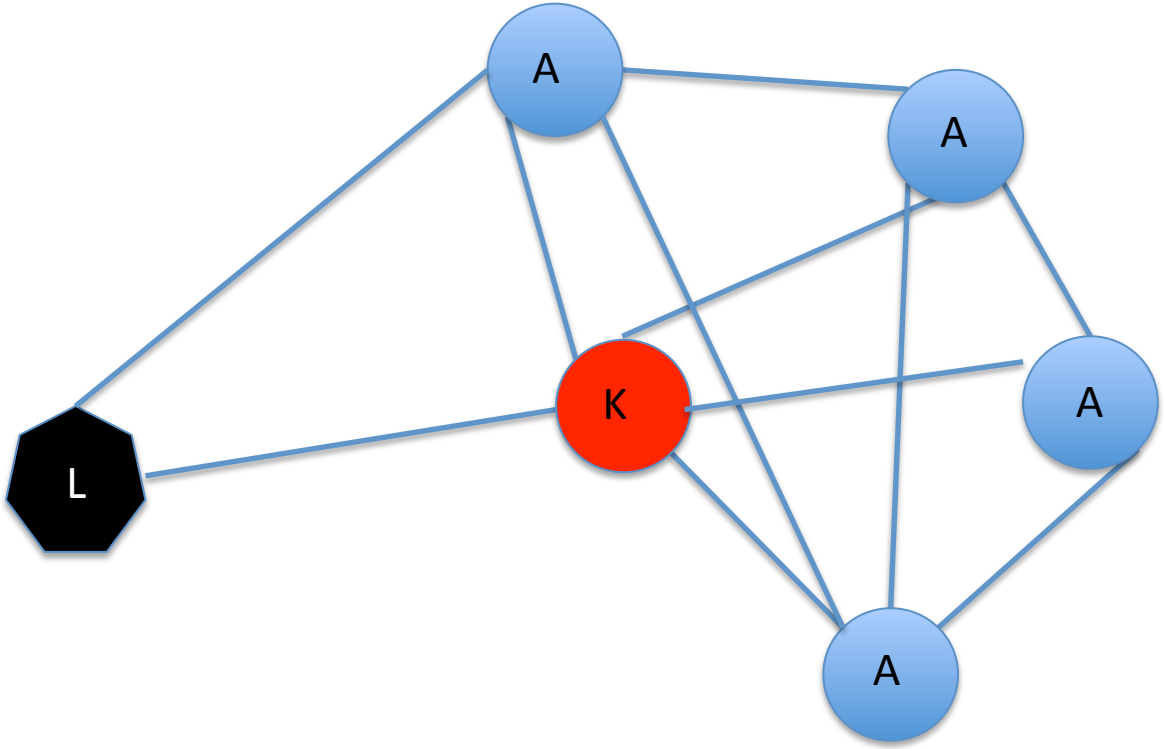
<GreedyOptMutationMover name=greedyscan **relax_mover**=repack_min **filter**=allFilter task_operations=LigInterface,fixcat scorefxn=soft_rep/>

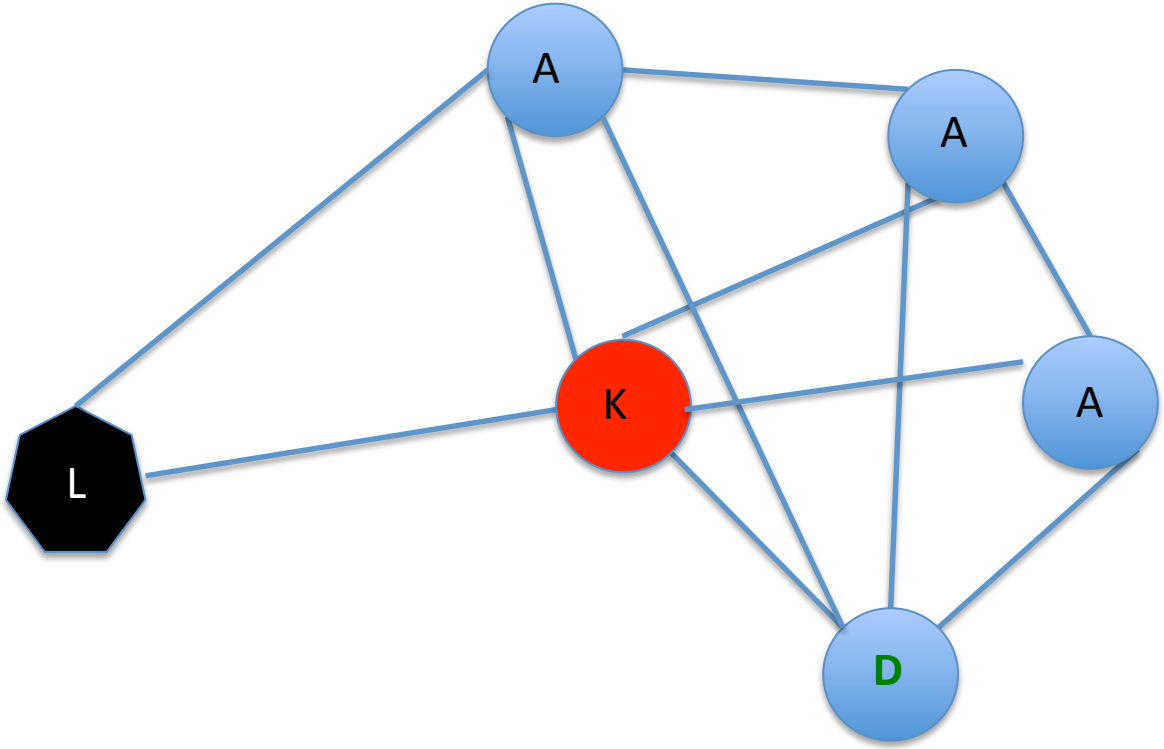
(Filter – highest stringency)

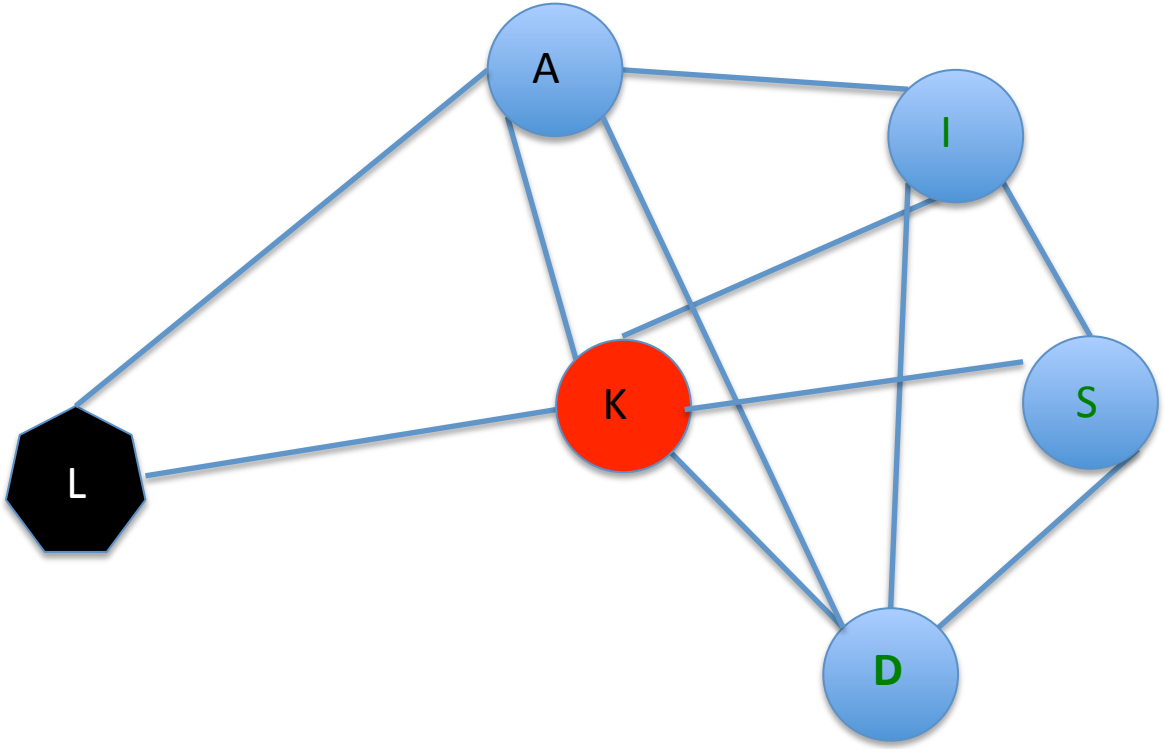
Buttressing binding residues

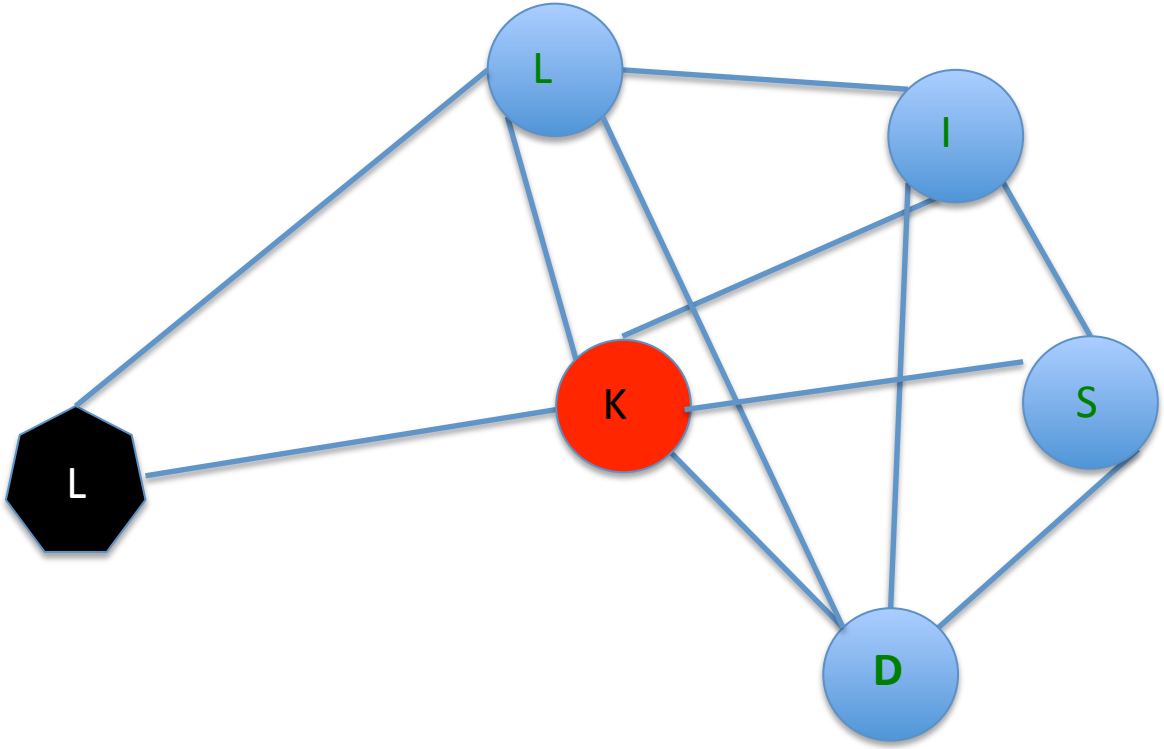
- Indirect: upweight interaction energies
- Direct: choose buttressing clusters




A
A
K
A
L
A


A
A
K
A
L
D


A
I
K
S
L
D


L
I
K
S
L
D

Filters

Scores etc:

<EnzScore name="allcst0" score_type=cstE scorefxn=sthyme_wts_minimal whole_pose=1
energy_cutoff=5 confidence=0.9/>

<HbondsToResidue name="hb1" partners=1 pdb_num=1X/>

<ShapeComplementarity name=SC min_sc=0.65 min_interface=0 verbose=0 quick=0 jump=1/>

<BuriedUnsatHbonds name=burUnsat jump_number=1 cutoff=99999
scorefxn=sthyme_wts_minimal/>

<Delta name=delta_burUnsat filter=burUnsat upper=1 lower=0/>

Ligand-specific:

<LigInterfaceEnergy name="interfE" scorefxn=sthyme_wts_minimal energy_cutoff= -10.0/>

<DiffAtomBurial name="BTLpointing" res1_res_num=0 atomname1="C12" res2_res_num=0
atomname2="O1" sample_type="less"/>

<RepackWithoutLigand name="rwl1" scorefxn=sthyme_wts_minimal target_res=all_repacked
rms_threshold=1.0/>

Combining Filters for greedy opt

#Combined filters with large cutoffs for use in greedy optimizer. Factor is the term's weight.

<CombinedValue name=allFilter confidence=0>
<Add filter_name=ddg factor=0.5/> (interface energy)
<Add filter_name=hbond_sc factor=1.5/> (hbond)
<Add filter_name= fa_sol factor=1.0/>
<Add filter_name=rwl factor=2.0/> (Repack Without Ligand)
<Add filter_name=delta_total_score factor=0.25/>
<Add filter_name=sasa factor=-10.0/>
<Add filter_name=SC factor=-11.0/> (shape complementarity)
…
</CombinedValue>

<GreedyOptMutationMover name=greedyscan **relax_mover**=repack_min **filter**=allFilter task_operations=allaa,LigInterface,fixcat scorefxn=soft_rep/>

Local sequence structure compatibility

Looks at <RMS> of 9-mer fragments to WT backbone for designed and WT sequences.

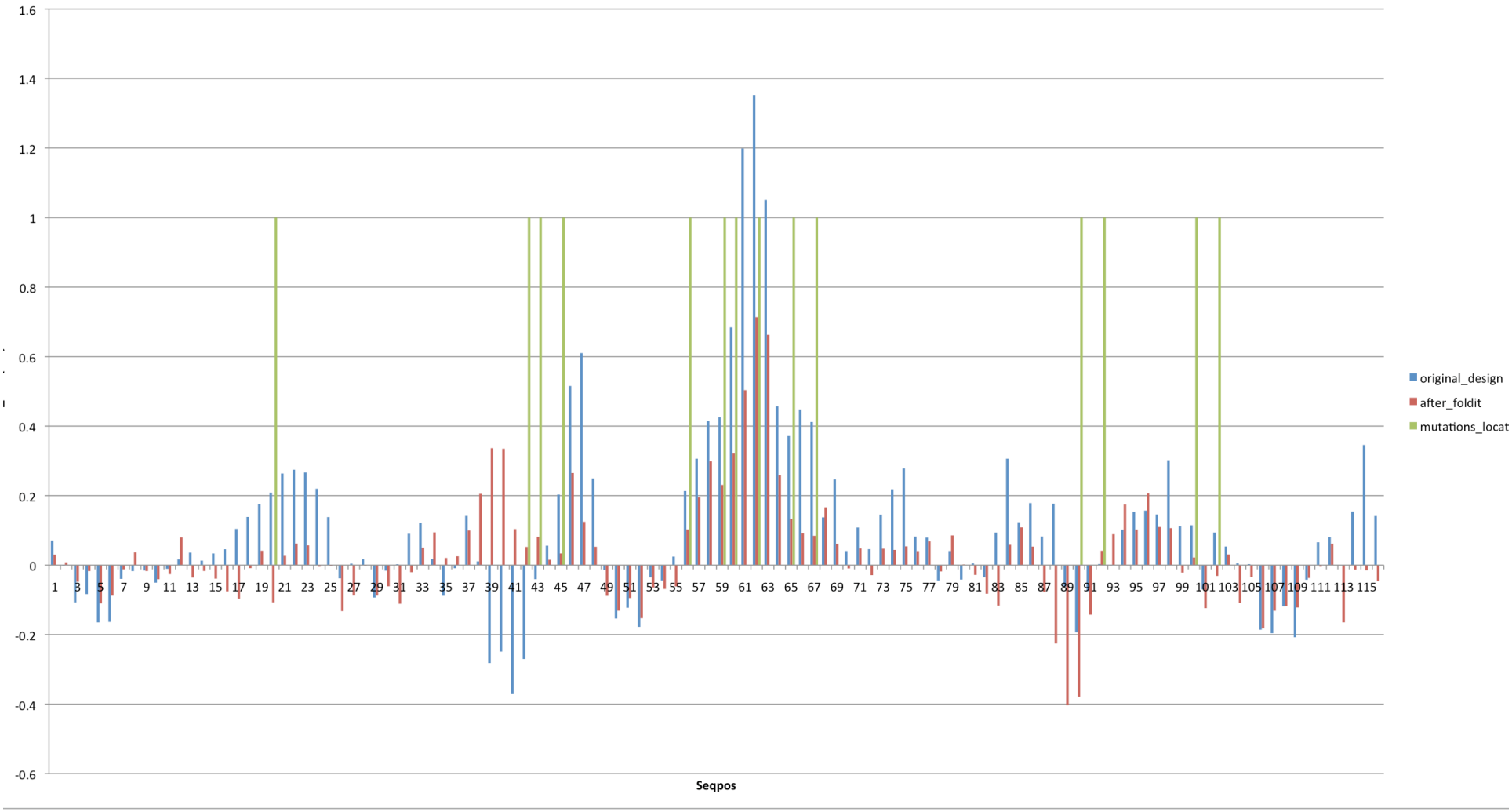
Differences of >0.8A (~s.d.) might indicate greater local disorder.

HOW TO:

To get fragments, nice ~rvernon/nnmake_new/make_fragments.9and3.pl -verbose -nocleanup 1a53d.fasta -id 1a53d -xx aa -nohoms&
this will generate a fragment file called aa*. make a list of these aa* files called list
then run/work/rvernon/rosetta_mini/mini_rfrag/bin/r_frag_quality.linuxgccrelease -database /work/rvernon/rosetta_mini/minirosetta_database/ -frags list -in:file:native ../1a53_11_0001.pdb
it will generate a file called aa*.rms
then run/work/khare/scripts/getfragrmsave.pl <your-rms-file-name> > frags.stats

format of the .stats file is column 1: sequence position column2: average fragment RMS column 3: std dev column4: min frag RMS

Local seq-structure compatibility



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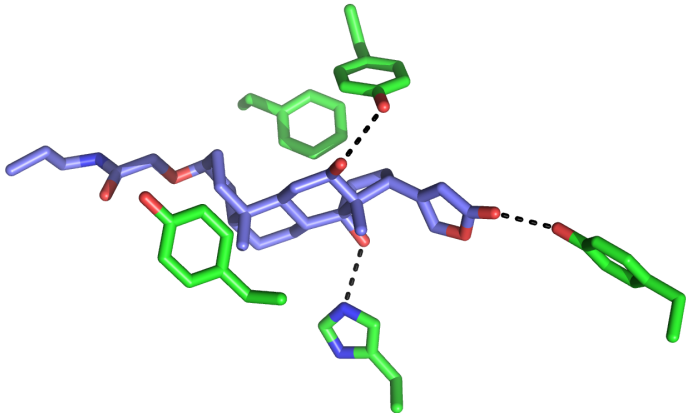

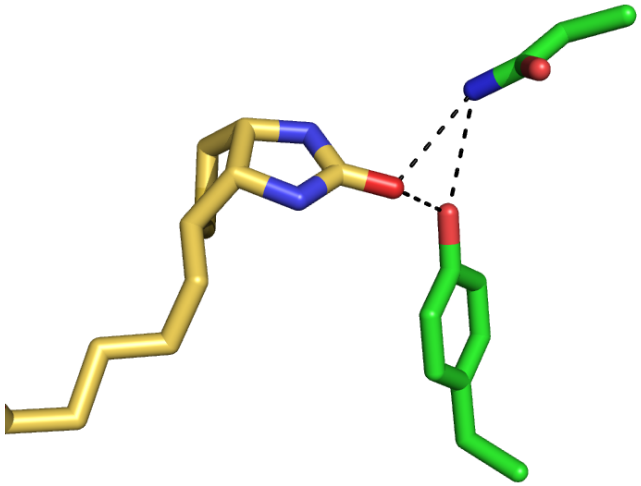

Strategies for global binding mode sampling: enriching homologous scaffolds

- DALI -> choose Z-score cutoff of 8
- Align homolog on design
- Scripts to match in a similar orientation (choose grid and positions to match based on alignment)