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Supplementary Information for "Massively parallel de novo protein design for targeted therapeutics"

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Figures

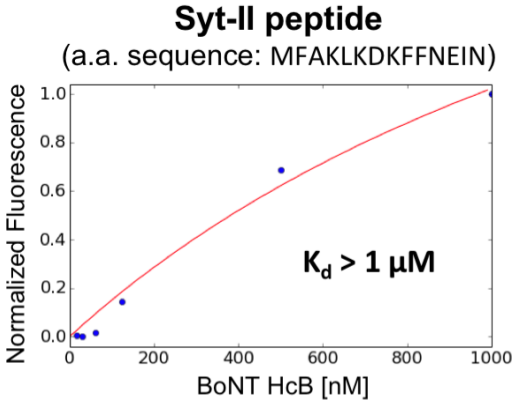


Figure 1. Yeast display titration of BoNT H_CB with a peptide fragment of Syt-II (Rattus norvegicus) on yeast display reveals low affinity for the 14 a.a. peptide fragment alone. Literature reported a Kd of ~40 nM for Syt-II towards H_CB; however, this is in presence of flanking regions (a.a. 1-61 and a GST-tag), which are known to be important for high affinity binding, but the details of its structural information is unknown. Without these flanking-elements present, we found that (yeast display system, see Methods) the affinity of Syt-II is drastically reduced to Kd >1 µM. A subset of the amino acids of this binding motif was used to guide the designs of the Bot binders.

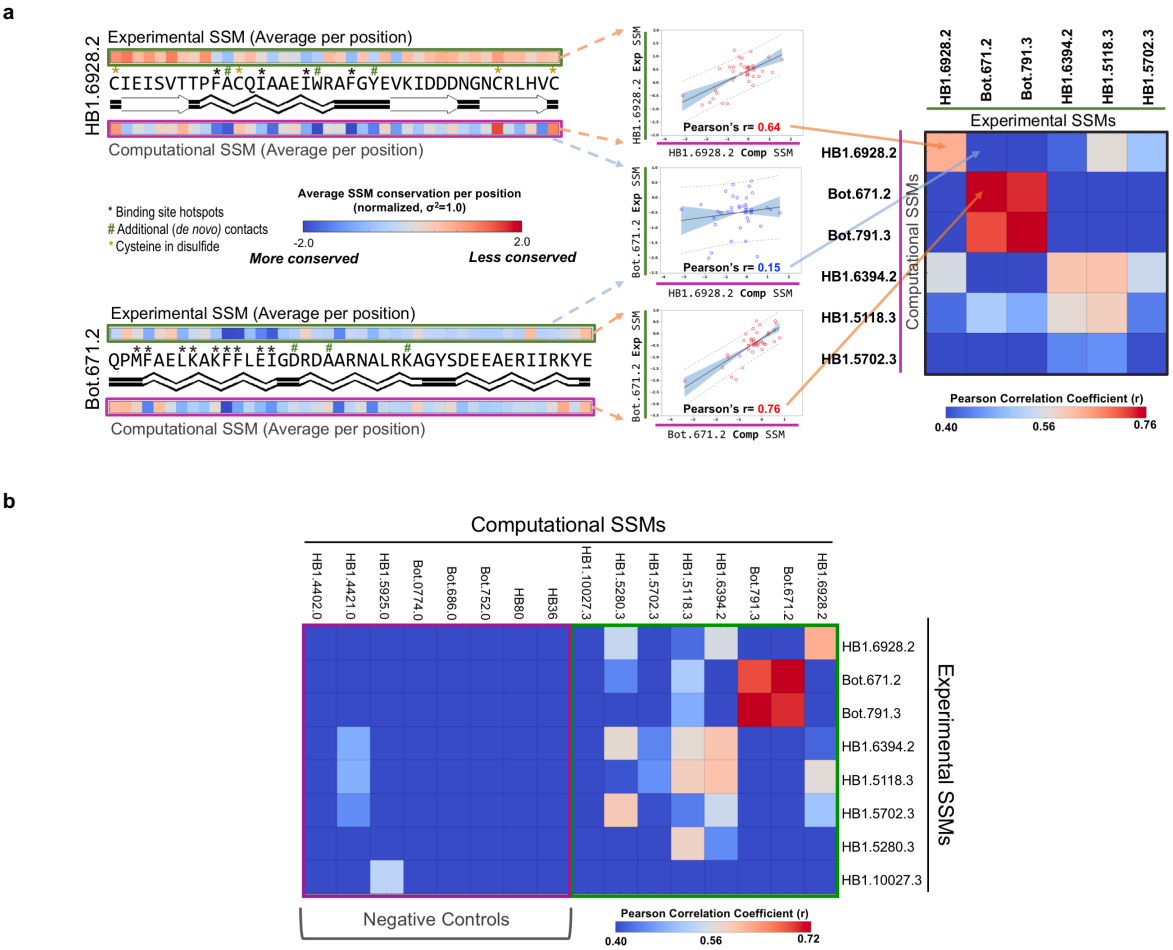


Figure 2. Correlation of experimental and Rosetta-predicted SSM calculations. a) The overall procedure used to calculate the experimental vs. computational (Rosetta) SSM correlation. For the experimental SSMs, each mutant was assigned to a binding category as in Extended Data Fig. 2, and a per-mutation score was calculated as the change in category compared with the designed (native) residue at that position (a positive score indicates a mutation improves binding). We then normalized these values for the whole dataset (σ^2=1) and calculated the average score of all mutations at each position. For the computational SSMs, we built computational models of each mutant and used them to calculate the change in monomeric and binding energy compared with the original design. We then normalized the monomeric and binding datasets separately (σ^2=1), summed them to compute an overall score for each mutation (for consistency, we made positive scores indicate improved binding), and again calculated the average of these scores at each position. Finally, we calculated the Pearson correlation between the per-position scores from the computational and experimental SSMs. When the size of the proteins differed, we just considered a 1-1 amino-acid correspondence starting from their first residues and ending at the length of the smallest protein in the pair. **b)** The results of the calculation described in "a" applied to 8 proteins included in the experiments and compared to all of its computational SSMs and 8 negative controls, including 2 natural proteins (previously reported HA binders HB36 and HB80) and 6 other designs chosen randomly. Most of the experimental data correlate better with the computational data for the same protein (i.e. HB1.6928.2, HB1.6394.2, HB1.5118.3 for HB1 designs, and Bot.671.2, Bot.791.3 for Bot designs) than with any other (unrelated) protein, and thus provides an indirect structural validation of the designed model.

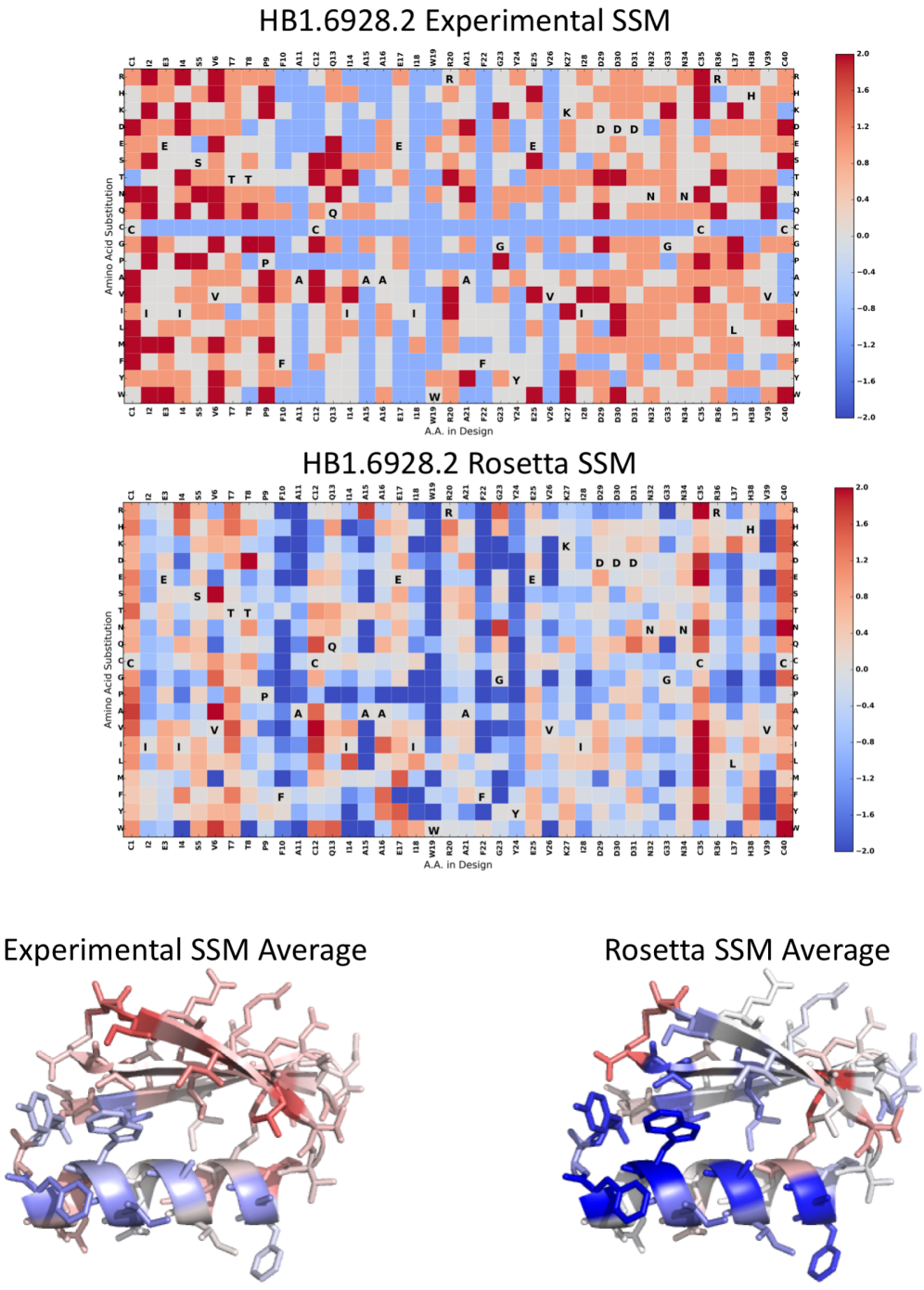


Figure 3a. Experimental and Rosetta-predicted SSM Heatmaps for HB1.6928.2, similar to Supplementary Information Figure 3a. Red indicates mutations that enhance binding, grey indicates mutations equivalent to the designed amino acid, and blue indicates mutations that reduce binding affinity. We show the average mutational effect for each position mapped onto the design model. Computational and experimental values were calculated as described in SI Figure 2. It can be observed there is a good agreement between the computational and experimental SSM, in particular in the core residues and hydrophobic hotspots in the binding helix.

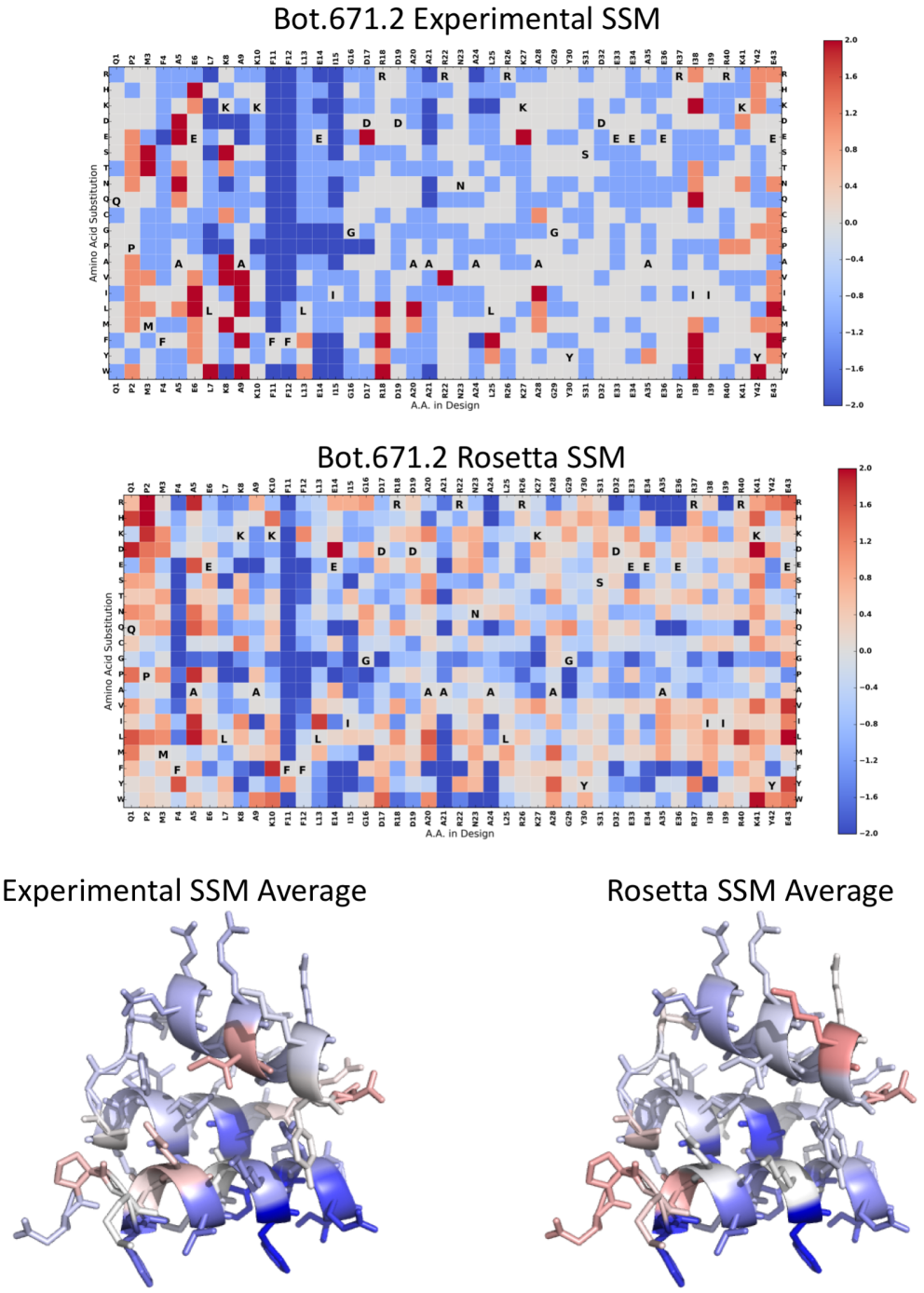


Figure 3b. Experimental and Rosetta-predicted SSM Heatmaps for Bot.671.2, similar to Supplementary Information Figure 3a. Red indicates mutations that enhance binding, grey indicates mutations equivalent to the designed amino acid, and blue indicates mutations that reduce binding affinity. We show the average mutational effect for each position mapped onto the design model. Computational and experimental values were calculated as described in SI Figure 2. It can be observed there is a good agreement between the computational and experimental SSM.

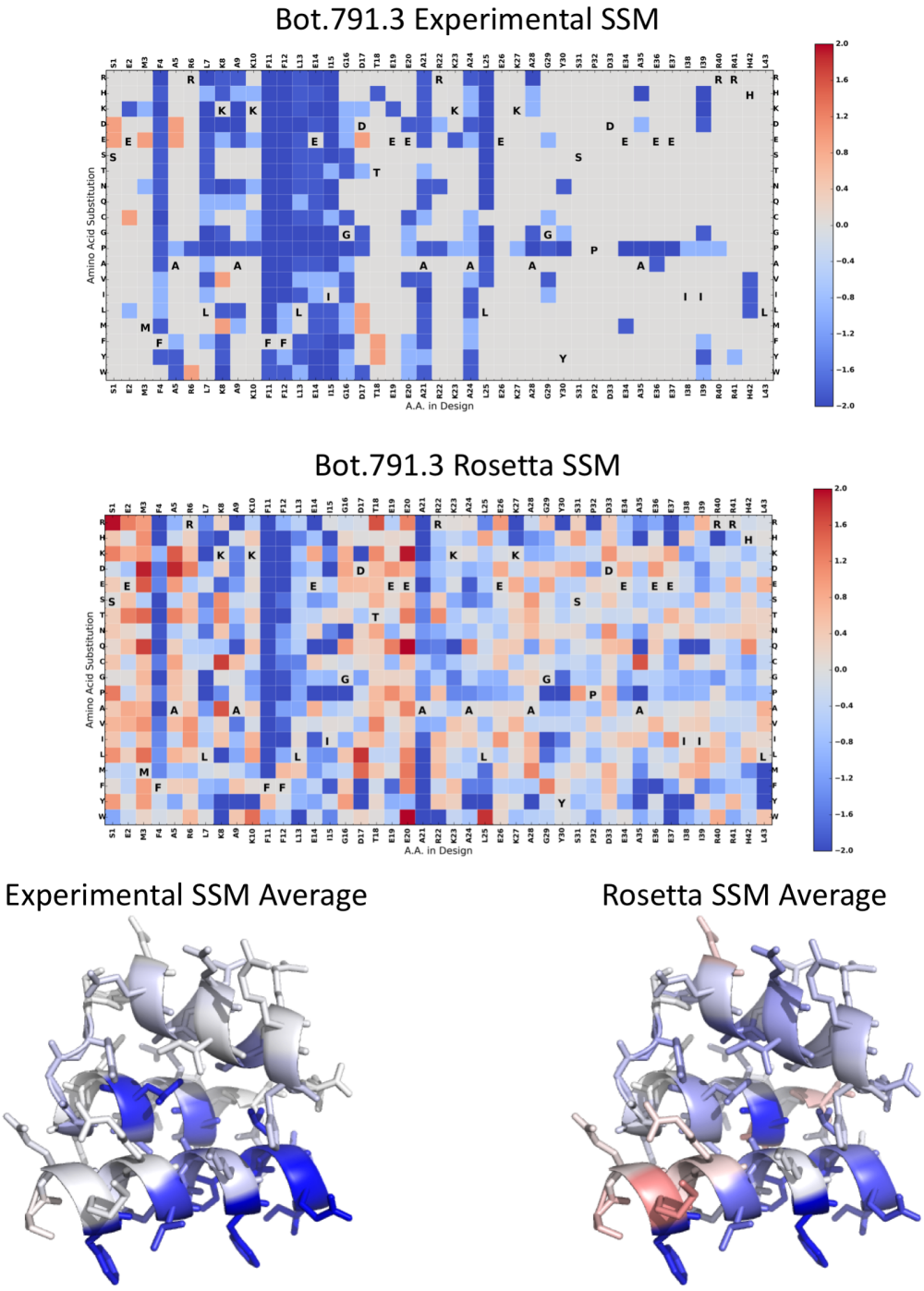
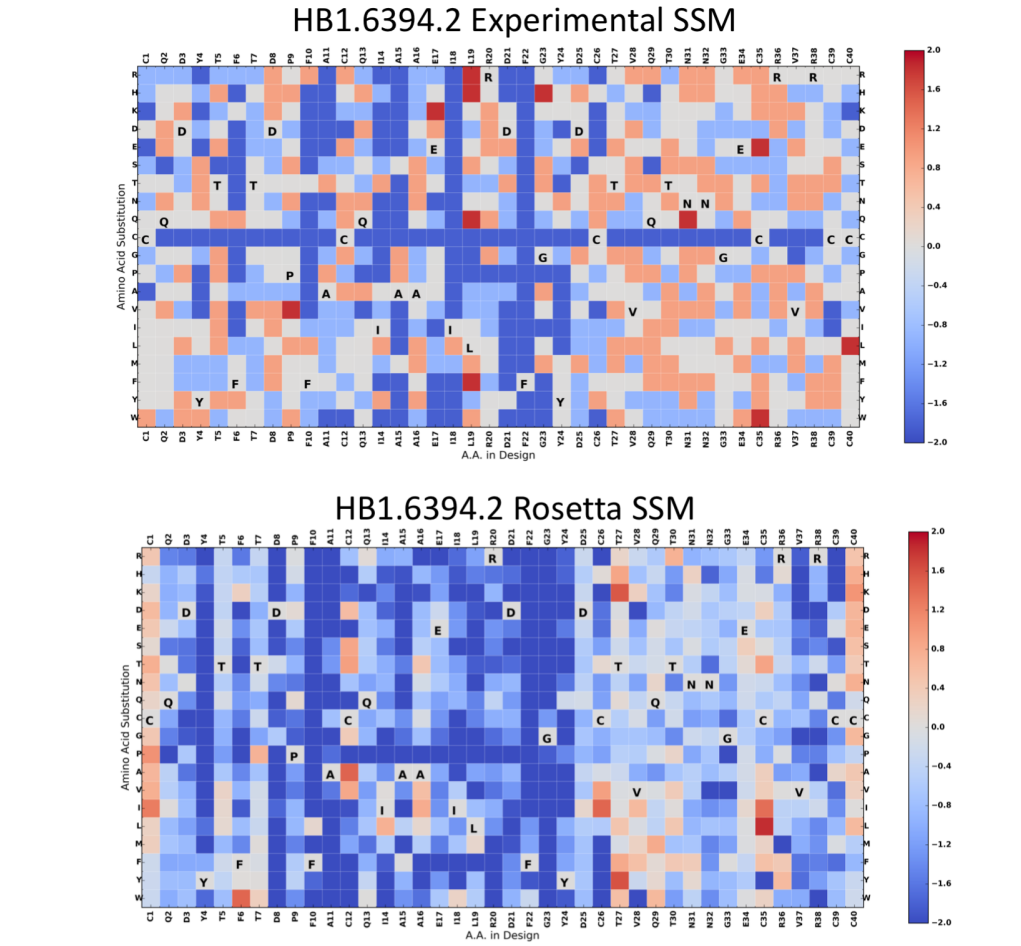


Figure 3c. Experimental and Rosetta-predicted SSM Heatmaps for Bot.791.3, the effect that each point mutation has on binding (experimental SSM) and sum of binding plus monomer stability (Rosetta SSM) is shown (for details see supplementary information Fig. 2). Red indicates mutations that enhance binding, grey indicates mutations equivalent to the designed amino acid, and blue indicates mutations that reduce binding affinity. We show the average mutational effect for each position mapped onto the design model. Computational and experimental values were calculated as described in SI Figure 2. It can be observed there is a good agreement between the computational and experimental SSM.



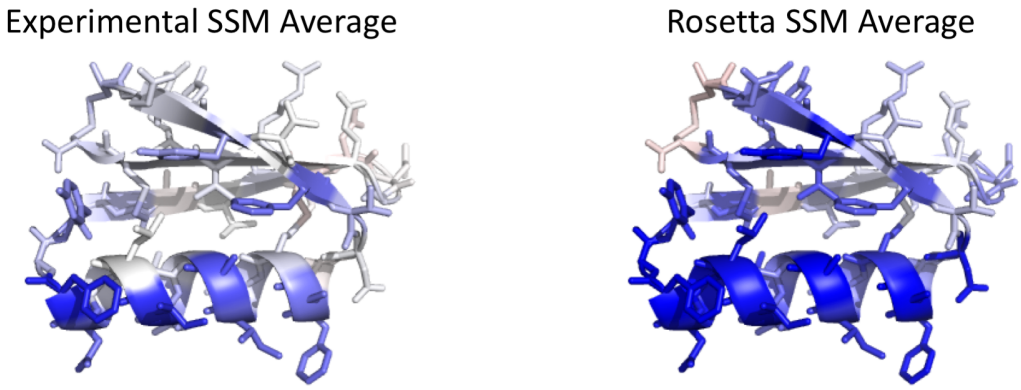
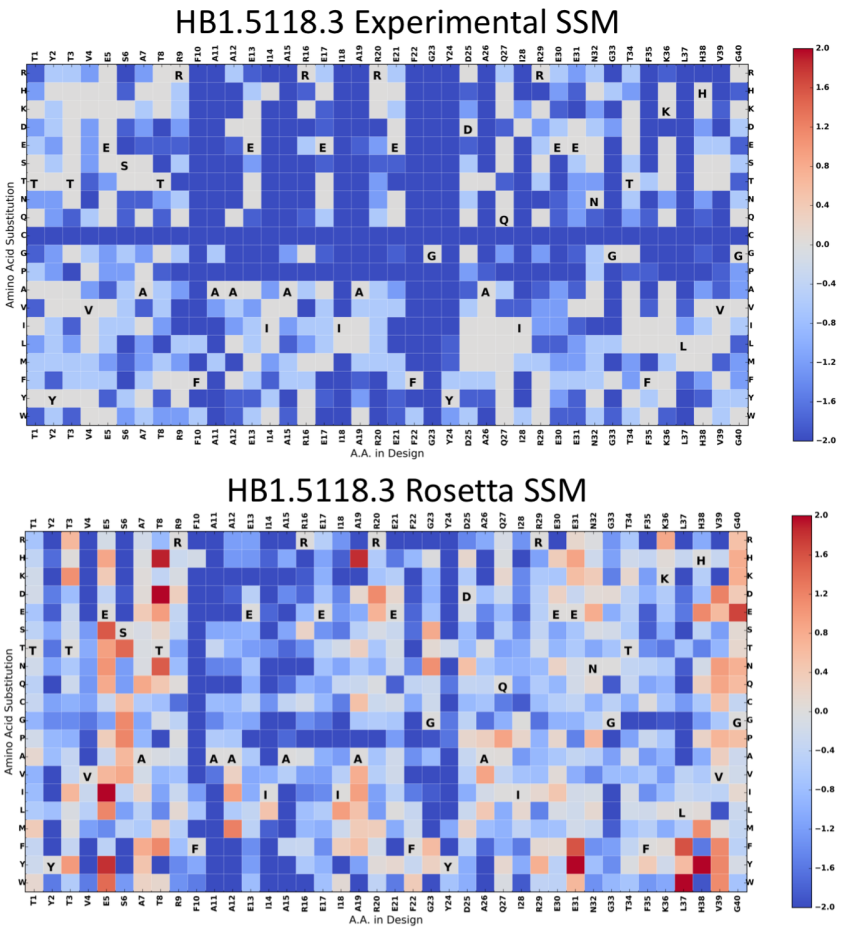


Figure 3d. Experimental and Rosetta-predicted SSM Heatmaps for HB1.6394.2, similar to Supplementary Information Figure 3a. Red indicates mutations that enhance binding, grey indicates mutations equivalent to the designed amino acid, and blue indicates mutations that reduce binding affinity. We show the average mutational effect for each position mapped onto the design model. Computational and experimental values were calculated as described in SI Figure 2. It can be observed there is a good agreement between the computational and experimental SSM.



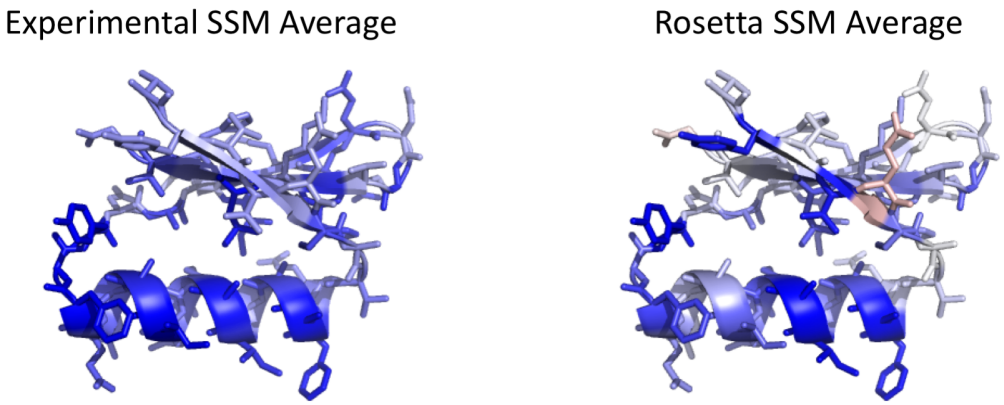


Figure 3e. Experimental and Rosetta-predicted SSM Heatmaps for HB1.5118.3, similar to Supplementary Information Figure 3a. Red indicates mutations that enhance binding, grey indicates mutations equivalent to the designed amino acid, and blue indicates mutations that reduce binding affinity. We show the average mutational effect for each position mapped onto the design model. Computational and experimental values were calculated as described in SI Figure 2. It can be observed there is a good agreement between the computational and experimental SSM. It can be observed there is a good agreement between the computational and experimental SSM, in particular in the core residues and hydrophobic hotspots in the binding helix.

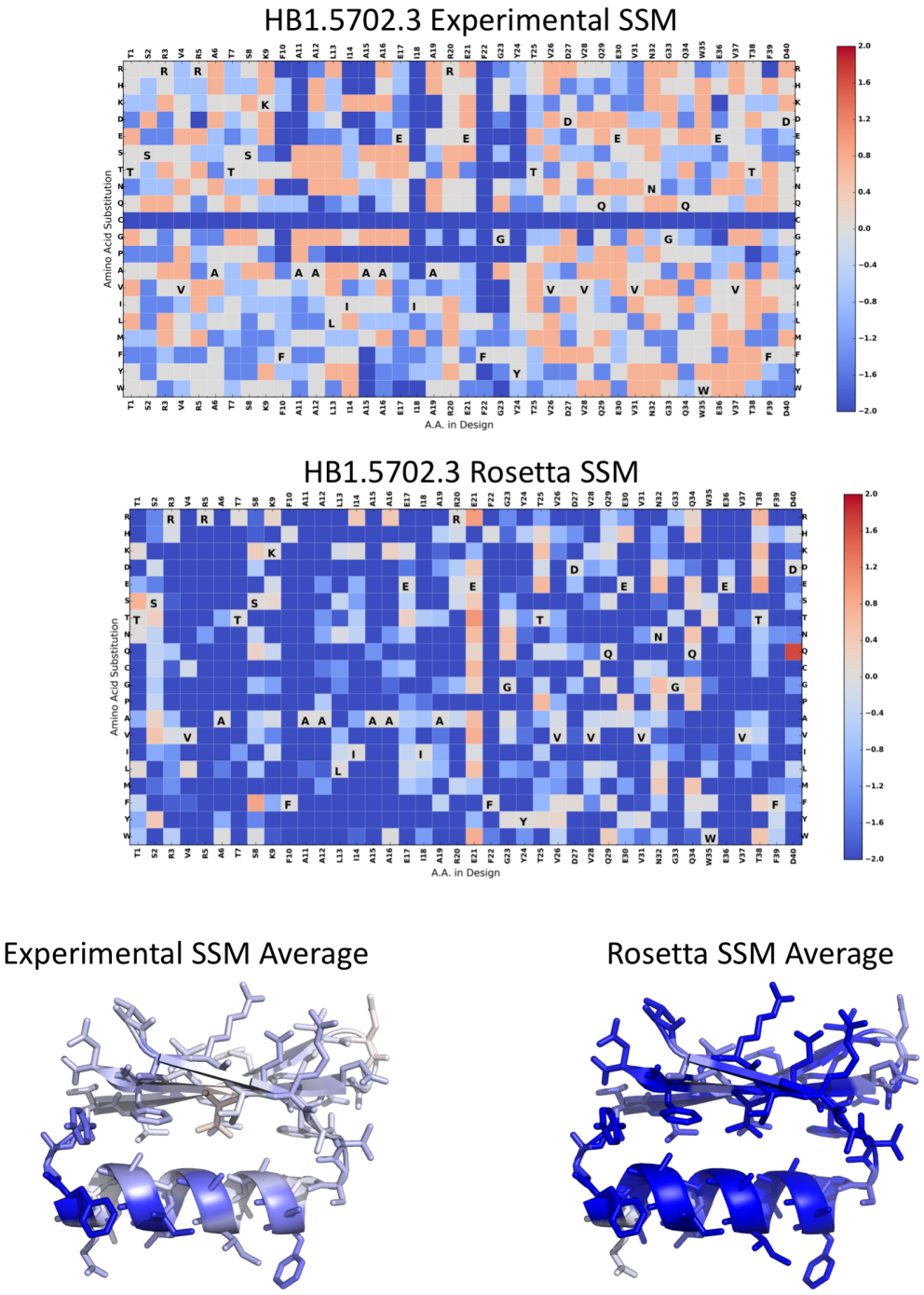


Figure 3f. Experimental and Rosetta-predicted SSM Heatmaps for HB1.5702.3, similar to Supplementary Information Figure 3a. Red indicates mutations that enhance binding, grey indicates mutations equivalent to the designed amino acid, and blue indicates mutations that reduce binding affinity. We show the average mutational effect for each position mapped onto the design model. Computational and experimental values were calculated as described in SI Figure 2.

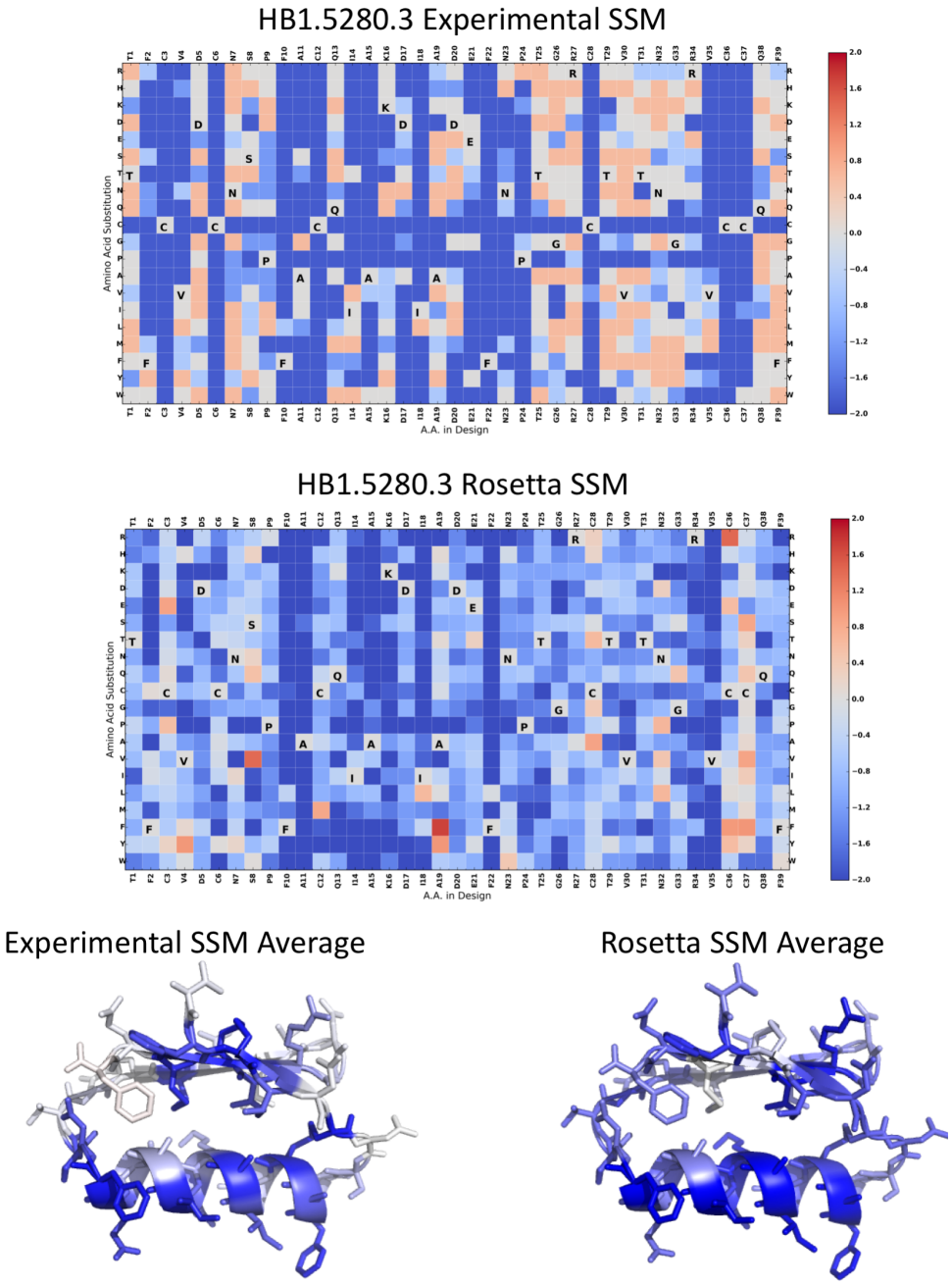


Figure 3g. Experimental and Rosetta-predicted SSM Heatmaps for HB1.5280.3, similar to Supplementary Information Figure 3a. Red indicates mutations that enhance binding, grey indicates mutations equivalent to the designed amino acid, and blue indicates mutations that reduce binding affinity. We show the average mutational effect for each position mapped onto the design model. Computational and experimental values were calculated as described in SI Figure 2. It can be observed there is a good agreement between the computational and experimental SSM. Consistent with the design model, substitutions in the binding and core positions disrupt function; Rosetta fails to capture the details of the disulfide bond which results in a poor agreement with the Rosetta predictions (Supplementary Information Figure 2).

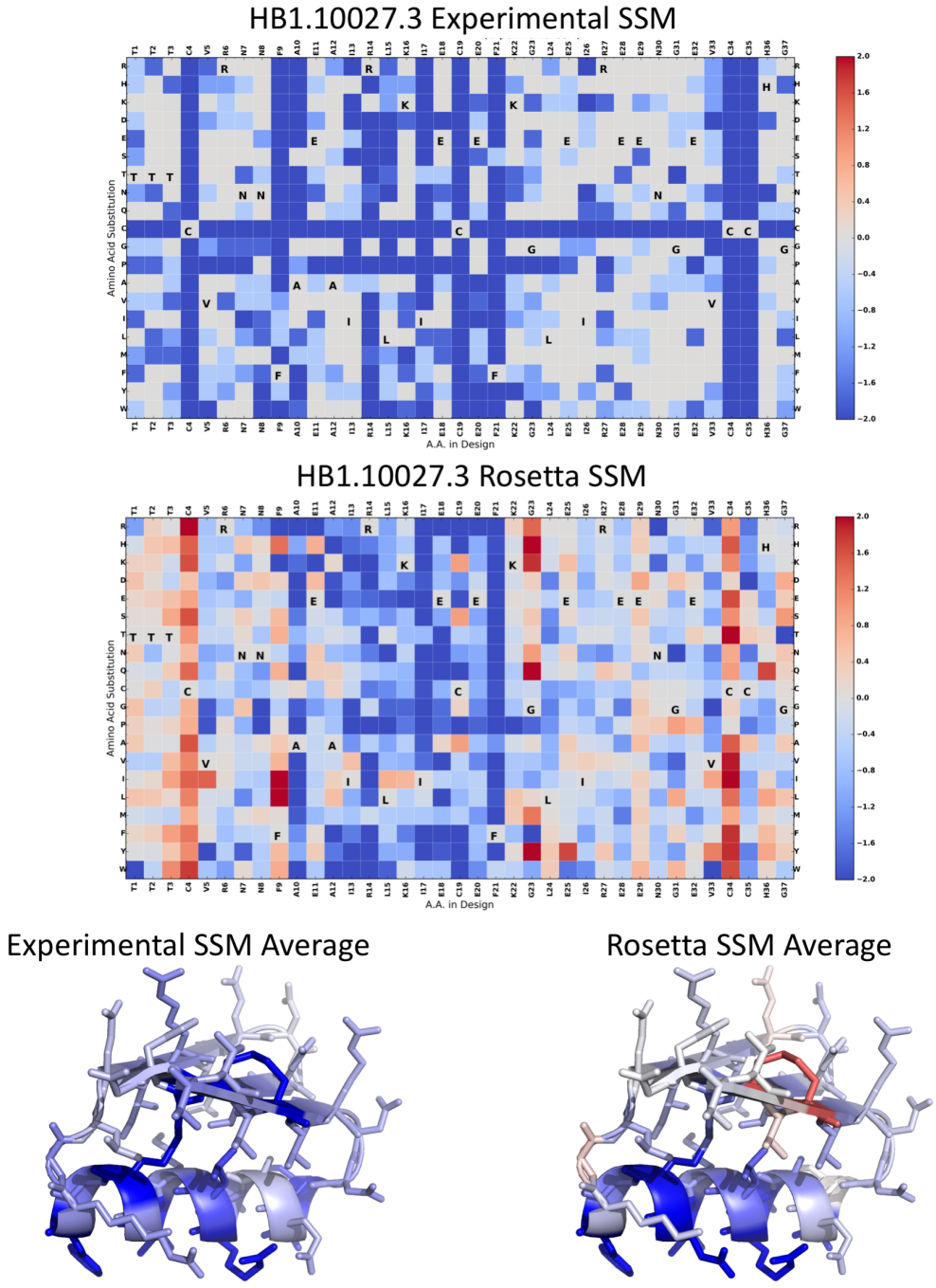


Figure 3h. Experimental and Rosetta-predicted SSM Heatmaps for HB1.10027.3, similar to Supplementary Information Figure 3a. Red indicates mutations that enhance binding, grey indicates mutations equivalent to the designed amino acid, and blue indicates mutations that reduce binding affinity. We show the average mutational effect for each position mapped onto the design model. Computational and experimental values were calculated as described in Supplementary Information Figure 2. Similar to Supplementary figure 3g, substitutions in the binding and core positions are consistent with the design model; the agreement with the Rosetta predictions is poor (Supplementary Information Figure 2) as Rosetta fails to capture the details of the disulfide bonds, and perhaps the experimental SSM lacks dynamic range.

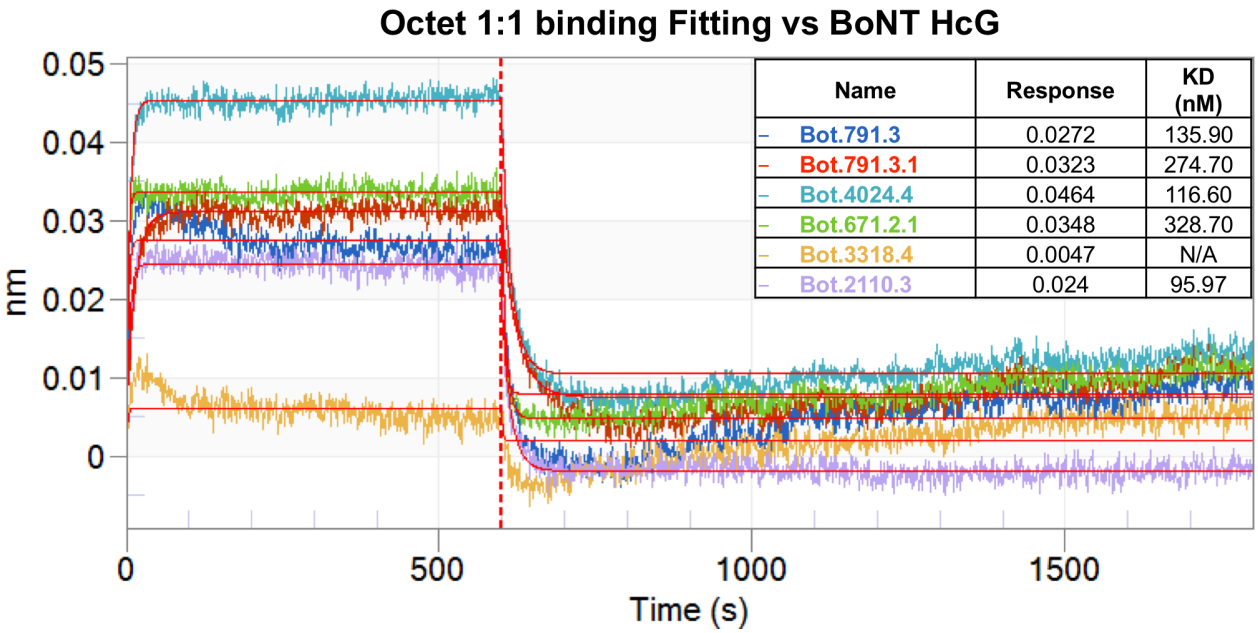


Figure 4. Cross-reactivity binding of Bot designs (BoNT H_CB binders) against BoNT/G (H_CG). The graph shows the results of a biolayer interferometry experiment (OCTET, 1:1 binding model, see Methods) to characterize the cross-reactivity binding of six functional designs against H_CG. The six binders presented can bind BoNT/B with high affinity (<~10 nM, see Main Text). Each line represents the binding and dissociation of an independent design, and the thin red line represents the adjustment of a 1:1 binding model. This demonstrates that 5 of the 6 designs exhibit cross-reactivity against HcG, but with ~10X lower affinity than for HcB (Extended Data Table 1).

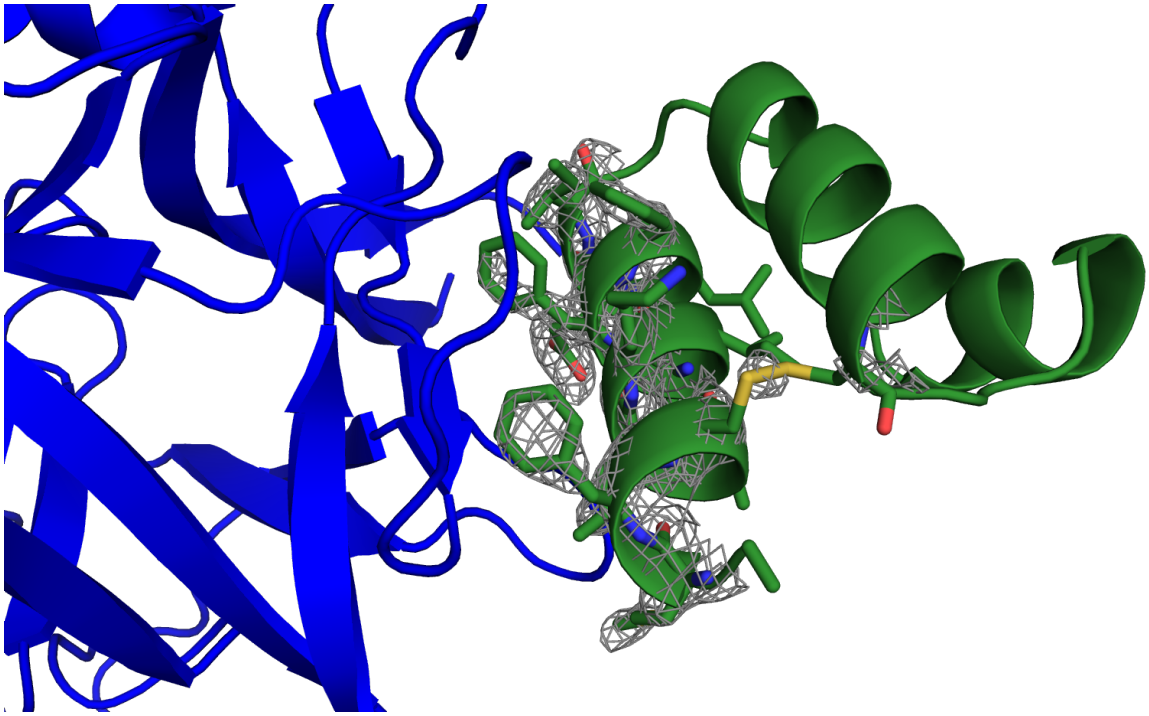


Figure 5. Co-crystal structure with interface side chain density for disulfide containing design Bot.2110.4 in complex with BoNT H_CB (PDB: 5VMR). $2F_O$-F_C map for Bot.2110.4 (green) residues 2-15 and disulfide bond (contoured at 1 sigma) shows density for side chains at the interface. The sulfur atoms of the disulfide bond show clear density. BoNT HcB is shown in blue.

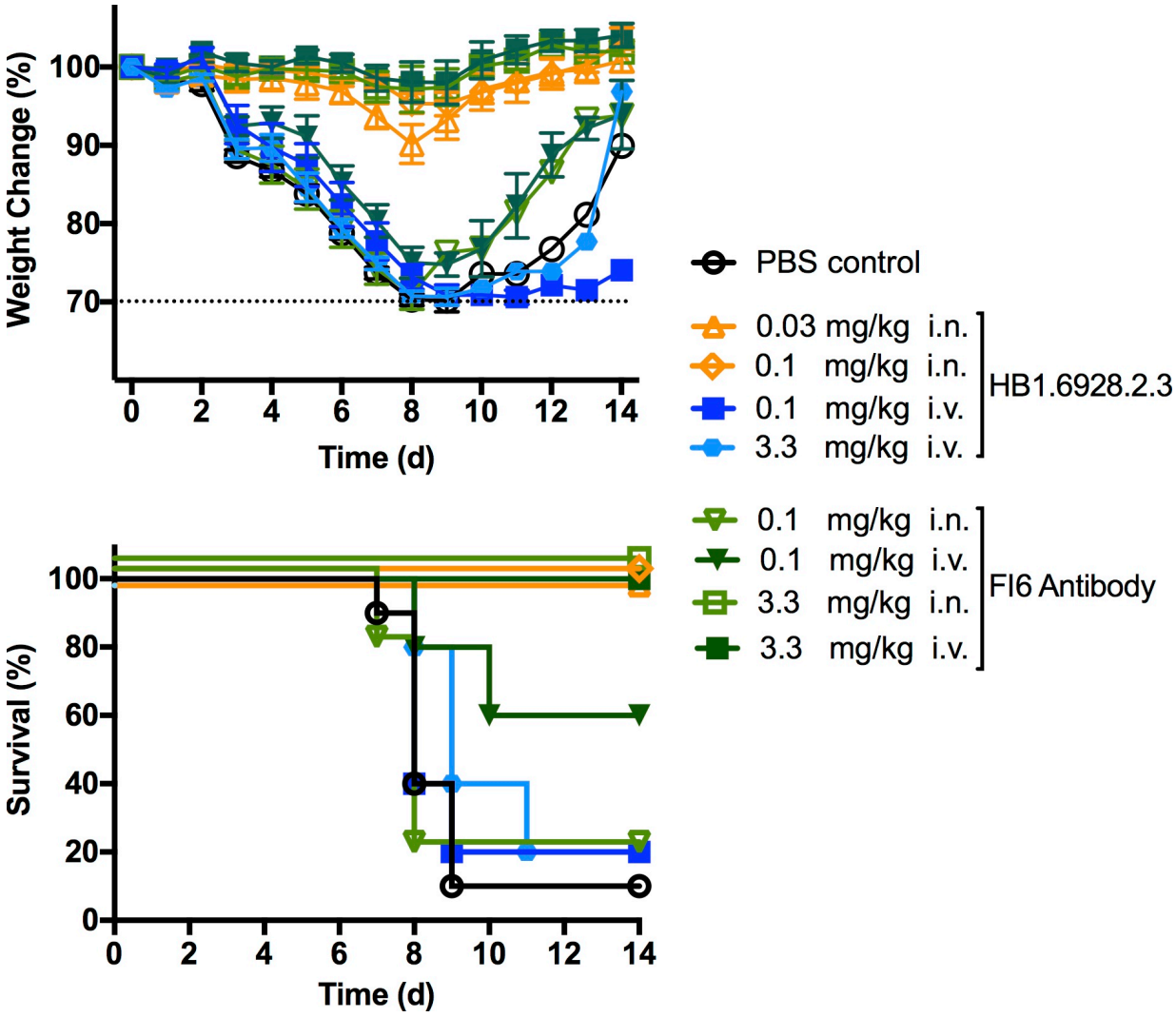


Figure 6. In vivo prophylactic efficiency of intranasal (i.v.) and intravenous (i.v) administration of the designed miniprotein HB1.6928.2.3 compared in a mass-to-mass basis to the FI6 antibody and a PBS control. Weight change (top) and survival (bottom) of BALB/c mice receiving different doses of HB1.6928.2.3 or the FI6v3 mAb, by the i.n. or i.v. routes, 24 hours prior to challenge with 2MLD50 of H1N1 CA09 virus (n=10, except 0.03mg/kg, n=5, see Main text methods). It is clear that in the i.n route HB1.6928.2.3 is much better neutralizing the virus than FI6 Antibody (mAb Fi6v3). However, HB1.6928.2.3 offers no protection in the i.v. route while FI6 does. It is likely that due to their small size, the mini-protein HB1.6928.2.3 is cleared from blood in a very short time, and is not available anymore to neutralize the virus at the time of challenge. In the top panel the whiskers show ±1 standard error of the mean (SEM).

Table 1. X-ray data collection and refinement statistics of co-crystal of design H_CB-Bot.671.2 and HCB-Bot.2110.4

	H_CB-Bot.0671.2 (PDB code: 5VID)	H_CB-Bot.2110.4 (PDB code: 5VMR)
Data collection		
Space group	P 1 21 1	P 1 21 1
Cell dimensions		
a, b, c (Å)	140.89, 87.31, 148.46	38.70, 246.48, 64.36
α, β, γ (°)	90, 114.53, 90	90, 102.90, 90
Resolution (Å)	87.31–2.75 (2.80–2.75)[a]	19.97–1.95 (1.99–1.95)[a]
R_{meas}	0.194 (1.099)	0.080 (0.513)
R_{merge}	0.143 (0.803)	0.058 (0.372)
I/σ(I)	8.1 (2.0)	7.7 (2.2)
CC1/2	0.986 (0.701)	0.997 (0.836)
Completeness (%)	100 (100)	99.6 (99.5)
Redundancy	3.8 (3.8)	3.5 (3.5)
No. unique reflections	85598 (4495)	84713 (4433)
Refinement		
Resolution (Å)	67.529–2.75	19.969–1.95
No. reflections	85,554	83967
R_{work} / R_{free}	0.219 / 0.232	0.181 / 0.206
No. atoms		
Protein	18,990	7,733
Ligand/ion	–	–
Water	74	575
Wilson B	45.72	33.14
B factors		
Protein	59.80	46.90
Ligand/ion	–	–
Water	46.30	47.60
R.m.s. deviations		
Bond lengths (Å)	0.01	0.008
Bond angles (°)	1.27	1.111
Ramachandran (%)		
Favored	96.5	96.5
Allowed	3.5	3.5
Outliers	0	0

[a] Values in parentheses are for highest-resolution shell.

Table 2. X-ray data collection and refinement statistics of co-crystal of design HB1.6928.2.3

	A/PuertoRico/8/1934 HA - HB1.6928.2.3 complex (PDB code: 5VLI)
Data collection	
Space group	R32
Cell dimensions	
a, b, c (Å)	110.7 110.7 327.4
α, β, γ (°)	90 90 120
Resolution (Å)	47.4-1.80 (1.86-1.80)[a]
Unique reflections	71,919
R_{sym}	8.9 (172.2)
R_{pim}	2.3 (47.0)
I/σ(I)	27.0 (1.9)
$CC_{1/2}$	0.973 (0.870)
Completeness (%)	100 (100)
Redundancy	16.1 (14.3)
Refinement	
Resolution (Å)	1.80
No. reflections	71,713
R_{work} / R_{free}	0.182 / 0.213
No. atoms	
Protein (HA)	3934
Glycan	81
Peptide (A13_r33)	308
Water	420
Wilson B	30
B-values	
Protein (HA)	43
Glycan	64
Peptide (A13_r33)	45
Water	49
R.m.s. deviations	
Bond lengths (Å)	0.018
Bond angles (°)	1.45
Ramachandran(%)	
Favored	97.4
Allowed	100
Outliers	0

[a] Values in parentheses are for highest-resolution shell.

Table 3. DNA sequences of gene pool primers and combination libraries

HB1.5702.2	GGGTCGGCTTCGCATATGACATCTVKAGTTAGARBGACGRBCAAATTT GCTGCGTTGATTGCTGCTGAAATTGCCAGASAATTTGGGTACACAGTT GACGTTCAARWSRHAAATGGTVWATGGDDSGTGRYATTTGATCTCGA GGGTGGAGGTTCC
HB1.10027.3	GGGTCGGCTTCGCATATGRCAACTDVGTGTGTTDNAAACAATTTTGCC GAGGCAATACGTTTGAAGATTGAGTGCGAGTTTAAAGGTTTGVWMAT CRDGGAAGAAAATGGTNMAGTTTGCTGCYHCNBGCTCGAGGGTGGA GGTTCC
HB1.6928.2.3	GGGTCGGCTTCGCATATGKGCMNAGAAMHAAGTBDCACTACTHYGTT CGCTTGTCAADYAGCCGCTGAAATCTGGCGTGCGTTCGGCTATRNAGT GAAAATTVHSGWCGATAATGGCAACTGCCGTTTGCACGTTTGTCTCG AGGGTGGAGGTTCC
HB1.6394.2.3	GGGTCGGCTTCGCATATGTGCCAARDSTATVVMTTTACTRACCCATTC GCTTGTCAAATCGCAVBCGAGATATTGAGAGATTTTGGCTACKVCTGC ACTGTCCAAACCMWMVMCGGANAMTGTAGGGTTAGATGCTGTCTCG AGGGTGGAGGTTCC
HB1.5118.3	GGGTCGGCTTCGCATATGVVATATACTGTTGAATCTKBGACCAGATTT GCTGCTGAGATCGCCAGAVWSATCVYAAGARAATTTGGGTACGACGC TCAAATARNAGAGGAAAATGGGACCTTTWHMTTGHWCGTTKGCCTC GAGGGTGGAGGTTCC
HB1.5380.3	GGGTCGGCTTCGCATATGANGTTTTGTGTTVRMTGCHACTCTCCATTT GCATGTCAGDKSGCCAAAGACATCBHCGAMGAGTTTAATCCARVAGG TCGTTGTACTGTAACCAACGGTAGAGTTTGTTGCSRANHCCTCGAGGG TGGAGGTTCC
Yeast_fwd_pool_primer	AGTGGTGGAGGAGGCTCTGGTGGAGGCGGTAGCGGAGGCGGAGGGT CGGCTTCGCATATG
Yeast_rev_pool_primer	GATCTCTATTACAAGTCCTCTTCAGAAATAAGCTTTTGTTCGGAACCT CCACCCTCGAG

Appendix A. RosettaScripts' XML used for designing mini-protein scaffolds

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        <Add mover_name="dump"/>

        </ParsedProtocol>
        <LoopOver name="lover1" mover_name="build_dssp1" iterations="500" drift="0"
ms_whenfail="FAIL_DO_NOT_RETRY" />
        <ParsedProtocol name="phase1" >
                <Add mover_name="set_ssene1" />
                <Add mover_name="lover1" />
        </ParsedProtocol>

</MOVERS>
```

```
<PROTOCOLS>
        <Add mover_name="phase1" />

</PROTOCOLS>
</dock_design>
```

Appendix B. RosettaScripts' XML used for design Influenza of Binders Generation 1/2 (HB1)

```
<dock_design>
    <TASKOPERATIONS>
        <InitializeFromCommandline name="init"/>
        <LimitAromaChi2 name="arochi2"/>
        <IncludeCurrent name="inclcur"/>
        <ExtraRotamersGeneric name="exrot" ex1="1" ex2="1" extrachi_cutoff="1"/>
        <DisallowIfNonnative name="only_native_GPH" disallow_aas="GPH"/>
        <RestrictIdentities name="dont_design_GPH" identities="PRO,HIS" prevent_repacking=0 />
        <OperateOnCertainResidues name="hotspot_norepack">
            <PreventRepackingRLT/>
            <ResiduePDBInfoHasLabel property="HOTSPOT"/>
        </OperateOnCertainResidues>
        <OperateOnCertainResidues name="scaffold_onlyrepack">
            <RestrictToRepackingRLT/>
            <ResiduePDBInfoHasLabel property="SCAFFOLD"/>
        </OperateOnCertainResidues>
        <OperateOnCertainResidues name="context_norepack">
            <PreventRepackingRLT/>
            <ResiduePDBInfoHasLabel property="CONTEXT"/>
        </OperateOnCertainResidues>
        <OperateOnCertainResidues name="context_onlyrepack">
            <RestrictToRepackingRLT/>
            <ResiduePDBInfoHasLabel property="CONTEXT"/>
        </OperateOnCertainResidues>
        <RestrictToInterface name="interface_design_long" jump="1" distance="12.0"/>
        <RestrictToInterface name="interface_design_short" jump="1" distance="8.0"/>
        <SelectBySASA  name="core_norepack"  mode="sc"  state="monomer"  probe_radius="2.2"  core_asa="20"
surface_asa="40" core="0" boundary="1" surface="1" verbose="1" />
        <RestrictIdentities name=no_glycan identities=AX1 prevent_repacking=1 />
        <RestrictIdentities name=no_CYS identities=CYS prevent_repacking=1 />
         <RetrieveStoredTask name=design_task task_name="design_task" />
    </TASKOPERATIONS>
    <SCOREFXNS>
        <talaris2013_resCons weights="talaris2013">
            <Reweight scoretype="res_type_constraint" weight="1.0"/>
        </talaris2013_resCons>
        <talaris2013_extra weights="talaris2013">
            <Reweight scoretype="fa_elec" weight="1.0"/>
            <Reweight scoretype="hpatch" weight="1.0"/>
            <Set fa_elec_dielectric="1.0" fa_elec_max_dis="15.0" fa_elec_min_dis="3.0"/>
        </talaris2013_extra>
        <talaris2013_cart weights="talaris2013">
            <Reweight scoretype="cart_bonded" weight="1.0"/>
            <Reweight scoretype="pro_close" weight="0.0"/>
         </talaris2013_cart>

    </SCOREFXNS>
    <FILTERS>
        <Geometry name="omega" omega="165" cart_bonded="35" start="491" confidence="0"/>
        <Ddg  name="ddg"  scorefxn="talaris2013"  jump="1"  repack="1"  repeats="5"  threshold="-18.0"
confidence="1"/>
        <ResInInterface name="resInInterface" residues="22" jump_number="1" confidence="0"/>
        <Sasa name="sasa" threshold="1200" confidence="1"/>
        <BuriedUnsatHbonds  name="buriedUnsatBonds"  scorefxn="talaris2013"  jump_number="1"  cutoff="9"
confidence="0"/>
```

```
        <ShapeComplementarity     name="shapeComplementarity"     jump="1"     verbose="1"     min_sc="0.6"
write_int_area="0" confidence="1"/>
        <InterfaceHoles name="interfaceHoles" jump="1" threshold="200" confidence="0"/>
        <AverageDegree         name="averageDegree"         threshold="8.3"         distance_threshold="8"
task_operations="init,interface_design_short,context_norepack" confidence="0"/>
        <PackStat name="packstat" repeats="5" threshold="0.59" confidence="0"/>
        <ScoreType    name="hpatch"    scorefxn="talaris2013_extra"    score_type="hpatch"    threshold="35"
confidence="0"/>
        <ScoreType   name="lr_elec"   scorefxn="talaris2013_extra"   score_type="fa_elec"   threshold="1200"
confidence="0"/>
        <ScoreType   name="total_score"   scorefxn="talaris2013"   score_type="total_score"   threshold="0"
confidence="0"/>
    </FILTERS>
    <MOVERS>
        <!-- Epigraft Mover -->
                <StoreTaskMover               name=store_design_task               task_name="design_task"
task_operations="init,hotspot_norepack,core_norepack,context_onlyrepack,arochi2,inclcur,exrot,only_native_
GPH,dont_design_GPH,no_glycan,no_CYS"/>

                <MotifGraft             name="motif_grafting"             context_structure="%%CONTEXT%%"
motif_structure="%%MOTIF%%"  RMSD_tolerance="1.0"  hotspots="%%HOTSPOTS%%"  NC_points_RMSD_tolerance="1.0"
clash_score_cutoff="10"           clash_test_residue="ALA"           combinatory_fragment_size_delta="0:0"
max_fragment_replacement_size_delta="-2:2"                                  full_motif_bb_alignment="1"
allow_independent_alignment_per_fragment="0"                          graft_only_hotspots_by_replacement="0"
only_allow_if_N_point_match_aa_identity="0"                     only_allow_if_C_point_match_aa_identity="0"
revert_graft_to_native_sequence="1" allow_repeat_same_graft_output="1"/>
        <!-- Design Movers -->
                <FavorSequenceProfile     name="favor_nativeSeqProfile"     scorefxns="talaris2013_resCons"
use_current="1"  chain="0" matrix=MATCH weight="0.2"/>
                <PackRotamersMover           name="design_graft1"           scorefxn="talaris2013_resCons"
task_operations="init,hotspot_norepack,core_norepack,context_onlyrepack,arochi2,inclcur,exrot,only_native_
GPH,dont_design_GPH,no_glycan,no_CYS,scaffold_onlyrepack"/>
                <PackRotamersMover         name="design_interface1"         scorefxn="talaris2013_resCons"
task_operations="init,hotspot_norepack,core_norepack,context_onlyrepack,arochi2,inclcur,exrot,only_native_
GPH,dont_design_GPH,no_glycan,no_CYS,interface_design_short"/>
        <!-- Minimization Movers -->
        <TaskAwareMinMover  name="chain2_kinematic_min_noBB"   scorefxn="talaris2013"       bb="0"  chi="1"
jump="1" task_operations="init,context_norepack,arochi2,inclcur,exrot"/>
        <TaskAwareMinMover  name="chain2_kinematic_min_wBB"   scorefxn="talaris2013"        bb="1"  chi="1"
jump="1" task_operations="init,context_norepack,arochi2,inclcur,exrot"/>
        <TaskAwareMinMover  name="chain2_cart_min"              scorefxn="talaris2013_cart"  bb="1"  chi="1"
jump="1" cartesian="1" task_operations="init,context_norepack"/>
    </MOVERS>
    <APPLY_TO_POSE>
    </APPLY_TO_POSE>
    <PROTOCOLS>

                <Add mover_name="store_design_task"/>
        <!-- Epigraft -->
                <Add mover_name="motif_grafting"/>
        <!-- Favor sequence profile -->
                Add mover="favor_nativeSeqProfile"/>
        <!-- First Design round -->
                <Add mover_name="design_graft1"/>
                <Add mover_name="design_interface1"/>
                Add mover_name="design_core1"/>
        <!-- Minimize (kine no BB) -->
                <Add mover_name="chain2_cart_min"/>
                <Add mover_name="chain2_kinematic_min_noBB"/>
                <Add mover_name="chain2_cart_min"/>
```

```
        <!-- Second Design round -->
                <Add mover_name="design_graft1"/>
                <Add mover_name="design_interface1"/>
                Add mover_name="design_core2"/>
        <!-- Minimize (all BB) -->
                <Add mover_name="chain2_cart_min"/>
                <Add mover_name="chain2_kinematic_min_wBB"/>
                <Add mover_name="chain2_cart_min"/>
        <!-- Filter by omega to ensure correct BB geometry and atomic distances -->
                <Add filter="omega"/>
        <!-- All the other Filters -->
                <Add filter="ddg"/>
                <Add filter="total_score"/>
                <Add filter="resInInterface"/>
                <Add filter="sasa"/>
                <Add filter="buriedUnsatBonds"/>
                <Add filter="shapeComplementarity"/>
                <Add filter="packstat"/>
                <Add filter="averageDegree"/>
                <Add filter="hpatch"/>
                <Add filter="lr_elec"/>
                <Add filter="interfaceHoles"/>
    </PROTOCOLS>
</dock_design>
```

Appendix C. RosettaScripts' XML used for design of BoNT/B Binders (Bot)

```
<ROSETTASCRIPTS>

        <SCOREFXNS>
                <sfxn_talaris2013 weights="talaris2013" />
        </SCOREFXNS>

        <FILTERS>
                <Geometry name="omega" omega="165" cart_bonded="20" start="115" confidence="0"/>
                <ShapeComplementarity   name=sc_filt   jump=1   verbose=1   min_sc=0.5   write_int_area=0
confidence=0 />
                <Ddg  name=ddG_filt  scorefxn=sfxn_talaris2013  jump=1  repack=1  repeats=3  threshold=0.0
confidence=1 />
                <BuriedUnsatHbonds name=buriedUnsatBonds scorefxn=sfxn_talaris2013 jump_number=1 cutoff=9
confidence=0/>
                <Sasa name=sasa threshold=700 hydrophobic=0 polar=0 jump=1 confidence=0 />
                <ScoreType name=total_score scorefxn=sfxn_talaris2013 score_type=total_score threshold=500
confidence=0/>
                <FragmentLookupFilter     name="faulty_fragments"     lookup_name="source_fragments_4_mer"
store_path="/work/dadriano/Shared/source_fragments_4_mer/"   lookup_mode="first"   chain="2"   threshold="0"
confidence="0" />
        </FILTERS>

        <MOVERS>
                //Here is the mover that generates multiple pose results
                <MotifGraft name="motif_grafting"
                        context_structure="./contextStructure.pdb" motif_structure="./motif_2NM1B.pdb"
                        RMSD_tolerance="1.0" NC_points_RMSD_tolerance="1.0"
                        clash_score_cutoff="5" clash_test_residue="GLY"
                        hotspots="2:9:10"                              combinatory_fragment_size_delta="0:0"
max_fragment_replacement_size_delta="0:0"
                        full_motif_bb_alignment="1"  allow_independent_alignment_per_fragment="0"
                        graft_only_hotspots_by_replacement="0"
only_allow_if_N_point_match_aa_identity="0"
                        only_allow_if_C_point_match_aa_identity="0"    revert_graft_to_native_sequence="1"
allow_repeat_same_graft_output="0" />
                //HERE You add  all the other design movers
                <MultiplePoseMover name="MPM_design" max_input_poses=10>
                <SELECT>
                </SELECT>
                        <ROSETTASCRIPTS>
                                <SCOREFXNS>
                                        <sfxn_talaris2013downAla weights="talaris2013_downAla" >
                                        </sfxn_talaris2013downAla>
                                        <sfxn_soft weights="soft_rep" >
                                        </sfxn_soft>
                                        <sfxn_soft_cst weights="soft_rep" >
                                                <Reweight  scoretype="coordinate_constraint"  weight="2.0"
/>
                                        </sfxn_soft_cst>
                                        <sfxn_soft_cart_cst weights="soft_rep" >
                                                <Reweight  scoretype="coordinate_constraint"  weight="1.0"
/>
                                                <Reweight scoretype="cart_bonded" weight="1.0"/>
                                                <Reweight scoretype="pro_close" weight="0.0"/>
                                        </sfxn_soft_cart_cst>
                                        <sfxn_talaris2013_cart_cst weights="talaris2013" >
```

```
                                        <Reweight  scoretype="coordinate_constraint"  weight="1.0"
/>
                                        <Reweight scoretype="cart_bonded" weight="1.0" />
                                        <Reweight scoretype="pro_close" weight="0.0" />
                                </sfxn_talaris2013_cart_cst>
                                <sfxn_talaris2013_cst weights="talaris2013" >
                                        <Reweight  scoretype="coordinate_constraint"  weight="1.0"
/>
                                </sfxn_talaris2013_cst>
                                </SCOREFXNS>
                        <TASKOPERATIONS>
                                <InitializeFromCommandline name="init"/>
                                <IncludeCurrent name="current" />
                                <ConsensusLoopDesign name="disallow_nonnative_loop_sequences"/>
                                <RestrictIdentities      name="notGPH"      identities="PRO,HIS"
prevent_repacking="0" />
                                <OperateOnCertainResidues name="hotspot_onlyrepack">
                                        <RestrictToRepackingRLT/>
                                        <ResiduePDBInfoHasLabel property="HOTSPOT"/>
                                </OperateOnCertainResidues>
                                <OperateOnCertainResidues name="scaffold_onlyrepack">
                                        <RestrictToRepackingRLT/>
                                        <ResiduePDBInfoHasLabel property="SCAFFOLD"/>
                                </OperateOnCertainResidues>
                                <OperateOnCertainResidues name="context_norepack">
                                        <PreventRepackingRLT/>
                                        <ResiduePDBInfoHasLabel property="CONTEXT"/>
                                </OperateOnCertainResidues>
                                <RestrictToInterface     name="interface_design_long"     jump="1"
distance="12.0"/>
                                <RestrictToInterface    name="interface_design_short"    jump="1"
distance="8.0"/>
                                SelectBySASA   name="core_norepack"   mode="sc"   state="monomer"
probe_radius="2.2" core_asa="20" surface_asa="40" core="0" boundary="1" surface="1" verbose="1" />
                                SelectBySASA    name="core_design"    mode="sc"    state="monomer"
probe_radius="2.2" core_asa="20" surface_asa="40" core="1" boundary="0" surface="0" verbose="1" />
                                <DisallowIfNonnative name="only_native_GPH" disallow_aas="GPH"/>
                                <LimitAromaChi2 name=limitaro />  #avoids extra rotamers
                                <RestrictIdentities name=noCYS identities=CYS prevent_repacking=1
/>
                                <ProteinInterfaceDesign  name=PID  repack_chain1=0  repack_chain2=1
design_chain1=0 design_chain2=1 interface_distance_cutoff=10/>
                                <LayerDesign    name="layer_design"    layer=core_boundary_surface
core_E=20 core_H=20 core_L=25 surface_E=50 surface_H=50 surface_L=26 make_pymol_script=0>
                                        <core>
                                                <all append="MACFILVWY" exclude="ERKHGQ"/> #PDNST
left normal
                                        </core>
                                        <boundary>
                                                <all append="ACDEIKLNQRVYWF" exclude="HMST"/> #PG
left alone
                                        </boundary>
                                        <surface>
                                                <all  append="ACDEHKNQRSTY"  exclude="MVILWF"  />
#PG left alone
                                        </surface>
                                </LayerDesign>
                        </TASKOPERATIONS>
                        <FILTERS>
                        </FILTERS>
```

```
                        <MOVERS>
                                <AddConstraintsToCurrentConformationMover        name=constrainCA
task_operations="current" CA_only=1 />
                                <ClearConstraintsMover name=clearConstraints />
                                <PackRotamersMover  name=design_interface_soft  scorefxn=sfxn_soft
task_operations="init,current,limitaro,disallow_nonnative_loop_sequences,noCYS,PID,layer_design,hotspot_on
lyrepack,scaffold_onlyrepack,context_norepack" />
                                <PackRotamersMover            name=design_interface_scaffold_soft
scorefxn=sfxn_soft
task_operations="init,current,limitaro,disallow_nonnative_loop_sequences,noCYS,PID,layer_design,hotspot_on
lyrepack,context_norepack" />
                                <PackRotamersMover                      name=design_interface_hard
scorefxn=sfxn_talaris2013downAla
task_operations="init,current,limitaro,disallow_nonnative_loop_sequences,noCYS,PID,limitaro,layer_design"
/>
                                <PackRotamersMover            name=design_interface_scaffold_hard
scorefxn=sfxn_talaris2013downAla
task_operations="init,current,disallow_nonnative_loop_sequences,limitaro,noCYS,PID,layer_design" />
                                <TaskAwareMinMover name=min_soft_cart scorefxn=sfxn_soft_cart_cst
bb=0 chi=1 jump=1 cartesian=1 task_operations="init,current,limitaro,hotspot_onlyrepack,context_norepack"
/>
                                <TaskAwareMinMover   name=min_soft   scorefxn=sfxn_soft_cst   bb=0
chi=1 jump=1 cartesian=0 task_operations="init,current,limitaro,hotspot_onlyrepack,context_norepack" />
                                <TaskAwareMinMover                                name=min_hard_cart
scorefxn=sfxn_talaris2013_cart_cst           bb=0           chi=1           jump=1           cartesian=1
task_operations="init,limitaro,current,hotspot_onlyrepack,context_norepack" />
                                <TaskAwareMinMover   name=min_hard   scorefxn=sfxn_talaris2013_cst
bb=0 chi=1 jump=1 cartesian=0 task_operations="init,current,limitaro,hotspot_onlyrepack,context_norepack"
/>
                        </MOVERS>
                        <APPLY_TO_POSE>
                        </APPLY_TO_POSE>
                        <PROTOCOLS>
                                **DESIGN STEP1>
                                <Add mover_name=constrainCA /> #Constraint CAs
                                <Add mover_name=design_interface_soft /> #design with soft_rep at
interface
                                <Add  mover_name=design_interface_scaffold_soft  />  #design  with
soft_rep at interface and the sorrounding scaffold
                                <Add mover_name=min_soft_cart /> #Min cartesian
                                <Add mover_name=min_soft /> #Min kinematic
                                <Add mover_name=min_hard_cart /> #Min cartesian with normal LJ
                                <Add mover_name=min_hard /> #Min kinematic with normal LJ
                                <Add mover_name=clearConstraints /> #Remove constraints to CAs
                                **DESIGN STEP2>
                                <Add  mover_name=design_interface_soft  /> #design  once  more  with
soft_rep at interface
                                <Add  mover_name=design_interface_scaffold_soft  />  #design  once
more with soft_rep at interface and the sorrounding scaffold
                                <Add mover_name=min_soft_cart /> #Min cartesian
                                <Add mover_name=min_soft /> #Min kinematic
                                <Add mover_name=min_hard_cart /> #Min cartesian with normal LJ
                                <Add mover_name=min_hard /> #Min kinematic with normal LJ
                        </PROTOCOLS>
                </ROSETTASCRIPTS>
        </MultiplePoseMover>
        //HERE You add filters
        <MultiplePoseMover name="MPM_filters" max_input_poses=10>
                <SELECT>
                        <AndSelector>
```

```
                                        <Filter filter=omega />
                                        <Filter filter=faulty_fragments/>
                                        <Filter filter=sc_filt />
                                        <Filter filter=ddG_filt />
                                        <Filter filter=buriedUnsatBonds />
                                        <Filter filter=sasa />
                                        <Filter filter=total_score />
                                    </AndSelector>
                                </SELECT>
                            </MultiplePoseMover>
                    </MOVERS>

                    <APPLY_TO_POSE>
                    </APPLY_TO_POSE>

                    <PROTOCOLS >
                    //HERE you combine everything together
                    <Add mover_name=motif_grafting />
                    <Add mover_name=MPM_design />
                    <Add mover_name=MPM_filters />
                    </PROTOCOLS>

</ROSETTASCRIPTS>
```

Appendix D. RosettaScripts' XML used for relaxing mutated models of mini-protein binders (in complex with target) in cases of: SSMs, core to valine, scrambled core, scrambled sequence, disulfided or loops to Gly-Ser

```
<ROSETTASCRIPTS>

        <SCOREFXNS>
                <SFXN_talaris2014_vanilla_cart weights="talaris2014_cart.wts" symmetric=0 />
                <SFXN_beta15_vanilla weights="beta_nov15.wts" symmetric=0 />
                <SFXN_beta15_vanilla_cart weights="beta_nov15_cart.wts" symmetric=0 />
        </SCOREFXNS>

        <TASKOPERATIONS>
                <InitializeFromCommandline name="init"/>
                <IncludeCurrent name="inclcur"/>
                <LimitAromaChi2 name="limitchi2" />
                <RestrictToRepacking name="onlyRepack" />
                <PreventChainFromRepacking  name="notChainA" chain=1/>
        </TASKOPERATIONS>

        <MOVERS>
                <SwitchChainOrder name="keep_only_chain_A" chain_order="1"/>
                <SwitchChainOrder name="keep_only_chain_B" chain_order="2"/>
        </MOVERS>

        <FILTERS>
                <ScoreType     name="score"     scorefxn="SFXN_beta15_vanilla"     score_type="total_score"
threshold=0.0 confidence="0" />
                <ResidueCount name="nres" confidence="0" />
                <CalculatorFilter name="score_res" equation="SCORE/NRES" threshold="-3.2" confidence="0">
                        <SCORE name="SCORE" filter_name="score" />
                        <NRES name="NRES" filter_name="nres" />
                </CalculatorFilter>
                <SSPrediction     name="sspred"     cmd="/work/dadriano/PROGRAMS/psipred/runpsipred_single"
use_probability="0" use_svm="0" threshold=0.80 confidence="0"/>
                <RmsdSimple     name="rmsd_ALL"     reference_name="reference_conformation"     align="1"
threshold="2.0" confidence="0" />
                <RmsdSimple      name="rmsd_A"      reference_name="reference_conformation"      align="1"
threshold="2.0" confidence="0" chain=1/>
                <RmsdSimple      name="rmsd_B"      reference_name="reference_conformation"      align="1"
threshold="2.0" confidence="0" chain=2/>
                <Ddg name="ddg"  scorefxn="SFXN_beta15_vanilla" jump=1 repack=1 repeats=3 threshold="0.0"
confidence="0" />
                <Sasa name="interface_sasa" jump="1" confidence=0/>
                <ShapeComplementarity  name="interface_sc"  verbose=0  min_sc=0.65  write_int_area=1  jump=1
confidence="0"/>
                <CalculatorFilter        name="ddg_100sasa"        equation="DDG*hundred/(SASA+small_val)"
threshold="0.0" confidence="0">
                        <DDG name="DDG" filter_name="ddg" />
                        <SASA name="SASA" filter_name="interface_sasa" />
                        <VARA name="hundred" value=100.0 />
                        <VARB name="small_val" value=0.0001 />
                </CalculatorFilter>
                <!--Chain A/B Filters-->
                <MoveBeforeFilter  name="score_res_chainB"  mover="keep_only_chain_B"  filter="score_res"
confidence="0"/>
                <MoveBeforeFilter     name="sspred_chainB"     mover="keep_only_chain_B"     filter="sspred"
confidence="0"/>
```

```
        </FILTERS>


        <MOVERS>
                <SavePoseMover                                          name="save_RMSDreference_conformation"
reference_name="reference_conformation"/>
                <AddConstraintsToCurrentConformationMover name="constrainCA"  cst_weight=0.5 CA_only=1 />
                <ClearConstraintsMover name="clearAllConstraints" />
                <TaskAwareMinMover  name="min_kine_sc_chainB"  scorefxn="SFXN_beta15_vanilla"  bb=1  chi=1
jump=0 cartesian=0 task_operations="init,inclcur,limitchi2,onlyRepack,notChainA" />
                <TaskAwareMinMover  name="min_kine_sc_chainAB"  scorefxn="SFXN_beta15_vanilla"  bb=0  chi=1
jump=0 cartesian=0 task_operations="init,inclcur,limitchi2,onlyRepack" />
                <TaskAwareMinMover  name="min_cart_chainB"  scorefxn="SFXN_beta15_vanilla_cart"  bb=1  chi=1
jump=1                    cartesian=1                   tolerance=0.001                    max_iter=1000
task_operations="init,inclcur,limitchi2,onlyRepack,notChainA" />
                <FastRelax             name="fast_relax_b_beta"             scorefxn="SFXN_beta15_vanilla"
task_operations="init,inclcur,limitchi2,onlyRepack,notChainA" >
                        <MoveMap name="mappyfd">
                                <Chain number=1 chi=0 bb=0/>
                                <Chain number=2 chi=1 bb=1/>
                                <Jump number=1 setting=1/>
                        </MoveMap>
                </FastRelax>
        </MOVERS>

        <PROTOCOLS>
                <Add mover_name="save_RMSDreference_conformation" />
                <Add mover_name="constrainCA" />
                        <Add mover_name="min_cart_chainB" />
                <Add mover_name="clearAllConstraints" />

                <Add mover_name="fast_relax_b_beta" />
                <Add mover_name="min_kine_sc_chainB" />
                <Add mover_name="min_kine_sc_chainAB" />
                <Add mover_name="constrainCA" />
                        <Add mover_name="min_cart_chainB" />
                        <Add mover_name="clearAllConstraints" />
                <Add mover_name="fast_relax_b_beta" />
                <Add  filter_name="rmsd_ALL" />
                <Add  filter_name="rmsd_A" />
                <Add  filter_name="rmsd_B" />
                <Add  filter_name="score_res_chainB" />
                <Add  filter_name="ddg" />
                <Add  filter_name="interface_sasa" />
                <Add  filter_name="ddg_100sasa" />
                <Add  filter_name="interface_sc" />
                <Add  filter_name="sspred_chainB" />
        </PROTOCOLS>

</ROSETTASCRIPTS>
```

Appendix E. RosettaScripts XML used for relaxing models of mini-protein binders (in complex with target) for Extended Data Table 1

```
<ROSETTASCRIPTS>

        <SCOREFXNS>
                <SFXN_talaris2014_vanilla_cart weights="talaris2014_cart.wts" symmetric=0 />
                <SFXN_beta15_vanilla weights="beta_nov15.wts" symmetric=0 />
                <SFXN_beta15_vanilla_cart weights="beta_nov15_cart.wts" symmetric=0 />
        </SCOREFXNS>

        <TASKOPERATIONS>
                <InitializeFromCommandline name="init"/>
                <IncludeCurrent name="inclcur"/>
               <LimitAromaChi2 name="limitchi2" />
                <RestrictToRepacking name="onlyRepack" />
                <PreventChainFromRepacking  name="notChainA" chain=1/>
        </TASKOPERATIONS>

        <MOVERS>
                <SwitchChainOrder name="keep_only_chain_A" chain_order="1"/>
                <SwitchChainOrder name="keep_only_chain_B" chain_order="2"/>
        </MOVERS>

        <FILTERS>
                <ScoreType     name="score"     scorefxn="SFXN_beta15_vanilla"     score_type="total_score"
threshold=0.0 confidence="0" />
                <ResidueCount name="nres" confidence="0" />
                <CalculatorFilter name="score_res" equation="SCORE/NRES" threshold="-3.2" confidence="0">
                        <SCORE name="SCORE" filter_name="score" />
                        <NRES name="NRES" filter_name="nres" />
                </CalculatorFilter>
                <SSPrediction     name="sspred"     cmd="/work/dadriano/PROGRAMS/psipred/runpsipred_single"
use_probability="0" use_svm="0" threshold=0.80 confidence="0"/>
                <RmsdSimple     name="rmsd_ALL"     reference_name="reference_conformation"     align="1"
threshold="2.0" confidence="0" />
                <RmsdSimple      name="rmsd_A"      reference_name="reference_conformation"      align="1"
threshold="2.0" confidence="0" chain=1/>
                <RmsdSimple      name="rmsd_B"      reference_name="reference_conformation"      align="1"
threshold="2.0" confidence="0" chain=2/>
                <Ddg name="ddg"  scorefxn="SFXN_beta15_vanilla" jump=1 repack=1 repeats=3 threshold="0.0"
confidence="0" />
                <Sasa name="interface_sasa" jump="1" confidence=0/>
                <ShapeComplementarity  name="interface_sc"  verbose=0  min_sc=0.65  write_int_area=1  jump=1
confidence="0"/>
                <CalculatorFilter        name="ddg_100sasa"        equation="DDG*hundred/(SASA+small_val)"
threshold="0.0" confidence="0">
                        <DDG name="DDG" filter_name="ddg" />
                        <SASA name="SASA" filter_name="interface_sasa" />
                        <VARA name="hundred" value=100.0 />
                        <VARB name="small_val" value=0.0001 />
                </CalculatorFilter>
                <!--Chain A/B Filters-->
                <MoveBeforeFilter   name="score_res_chainB"   mover="keep_only_chain_B"   filter="score_res"
confidence="0"/>
                <MoveBeforeFilter     name="sspred_chainB"     mover="keep_only_chain_B"     filter="sspred"
confidence="0"/>
        </FILTERS>
```

```
        <MOVERS>
                <SavePoseMover                                          name="save_RMSDreference_conformation"
reference_name="reference_conformation"/>
                <AddConstraintsToCurrentConformationMover name="constrainCA"  cst_weight=0.5 CA_only=1 />
                <ClearConstraintsMover name="clearAllConstraints" />
                <TaskAwareMinMover  name="min_kine_sc_chainB"  scorefxn="SFXN_beta15_vanilla"  bb=1  chi=1
jump=0 cartesian=0 task_operations="init,inclcur,limitchi2,onlyRepack,notChainA" />
                <TaskAwareMinMover  name="min_kine_sc_chainAB"  scorefxn="SFXN_beta15_vanilla"  bb=0  chi=1
jump=0 cartesian=0 task_operations="init,inclcur,limitchi2,onlyRepack" />
                <PackRotamersMover          name="pack_sc_chainAB"          scorefxn="SFXN_beta15_vanilla"
task_operations="init,inclcur,limitchi2,onlyRepack" />
                <TaskAwareMinMover  name="min_cart_chainB"  scorefxn="SFXN_beta15_vanilla_cart"  bb=1  chi=1
jump=1                    cartesian=1                   tolerance=0.001                    max_iter=1000
task_operations="init,inclcur,limitchi2,onlyRepack,notChainA" />
                <FastRelax             name="fast_relax_b_beta"             scorefxn="SFXN_beta15_vanilla"
task_operations="init,inclcur,limitchi2,onlyRepack,notChainA" >
                        <MoveMap name="mappyfd">
                                <Chain number=1 chi=0 bb=0/>
                                <Chain number=2 chi=1 bb=1/>
                                <Jump number=1 setting=1/>
                        </MoveMap>
                </FastRelax>
        </MOVERS>

        <PROTOCOLS>
                <Add mover_name="save_RMSDreference_conformation" />
                <Add mover_name="pack_sc_chainAB" />
                <Add mover_name="constrainCA" />
                        <Add mover_name="min_cart_chainB" />
                <Add mover_name="clearAllConstraints" />
                <Add mover_name="pack_sc_chainAB" />
                <Add mover_name="fast_relax_b_beta" />
                <Add mover_name="min_kine_sc_chainB" />
                <Add mover_name="min_kine_sc_chainAB" />
                <Add mover_name="constrainCA" />
                        <Add mover_name="min_cart_chainB" />
                <Add mover_name="clearAllConstraints" />
                <Add mover_name="fast_relax_b_beta" />
                <Add  filter_name="rmsd_ALL" />
                <Add  filter_name="rmsd_A" />
                <Add  filter_name="rmsd_B" />
                <Add  filter_name="score_res_chainB" />
                <Add  filter_name="ddg" />
                <Add  filter_name="interface_sasa" />
                <Add  filter_name="ddg_100sasa" />
                <Add  filter_name="interface_sc" />
                <Add  filter_name="sspred_chainB" />
        </PROTOCOLS>

</ROSETTASCRIPTS>
```

Appendix F. RosettaScripts XML used to analyze Rosetta Scores and Metrics over mini-protein Monomers

```
<ROSETTASCRIPTS>
        <SCOREFXNS>
                <SFXN7 weights="beta" >
                </SFXN7>
        </SCOREFXNS>
        <TASKOPERATIONS>
                <LayerDesign    name="layer_core_SCN"    layer="core"    pore_radius="2.0"    verbose="true"
use_sidechain_neighbors="True" core=4 />
                <LayerDesign     name="layer_core_SASA"     layer="core"     core="20"     pore_radius="2.0"
verbose="true" />
                <LayerDesign    name="layer_core_boundary_SCN"    layer="core_boundary"    pore_radius="2.0"
verbose="true" use_sidechain_neighbors="True" />

                <OperateOnCertainResidues name="no_ala_disulf" >
                        <RestrictToRepackingRLT />
                        <ResidueName3Is name3="ALA" />
                </OperateOnCertainResidues>

                <OperateOnCertainResidues name="no_repack_non-disulf" >
                        <PreventRepackingRLT/>
                        <ResidueName3Isnt name3="CYS" />
                </OperateOnCertainResidues>
        </TASKOPERATIONS>

        <MOVERS>
                <SwitchChainOrder name="keep_only_chain_B" chain_order="2"/>
        </MOVERS>

        <FILTERS>
                <AverageDegree   name="degree_core_SCN"   task_operations="layer_core_SCN"   confidence="0"
threshold="9.4" />
                <AverageDegree  name="degree_core_SASA"  task_operations="layer_core_SASA"  confidence="0"
threshold="9.4" />
                <AverageDegree name=degree confidence=0 threshold=9.5/>
                <ResidueCount name="res_count_all" max_residue_count="9999" confidence="0"/>
                <ResidueCount   name="res_count_core_SCN"   task_operations="layer_core_SCN,no_ala_disulf"
max_residue_count="9999" confidence="0"/>
                <ResidueCount  name="res_count_core_SASA"  task_operations="layer_core_SASA,no_ala_disulf"
max_residue_count="9999" confidence="0"/>
                <ResidueCount name="nres_cys" residue_types="CYS" confidence="0" />
                <ResidueCount      name="AlaCount"      residue_types="ALA"      max_residue_count="9999"
confidence="0"/>
                <TotalSasa     name="total_sasa"     threshold="1"     upper_threshold="1000000000000000"
report_per_residue_sasa="False" confidence="0" />
                <CalculatorFilter name="mean_sasa" equation="Tsasa / (rescount2 + 0.01)" threshold="-0.30"
confidence="0" >
                        <VAR name="Tsasa" filter="total_sasa"/>
                        <VAR name="rescount2" filter="res_count_all"/>
                </CalculatorFilter>
                <CalculatorFilter name="percent_core_SCN" equation="rescount_coreSCN / (rescount3 + 0.01)"
threshold="-0.35" confidence="0" >
                        <VAR name="rescount3" filter="res_count_all"/>
                        <VAR name="rescount_coreSCN" filter="res_count_core_SCN"/>
                </CalculatorFilter>
```

```
                <CalculatorFilter  name="percent_core_SASA"  equation="rescount_coreSASA  /  (rescount4  +
0.01)" threshold="-0.35" confidence="0" >
                        <VAR name="rescount4" filter="res_count_all"/>
                        <VAR name="rescount_coreSASA" filter="res_count_core_SASA"/>
                </CalculatorFilter>
                <AtomicContactCount name="contact_all" confidence="0" />
                <AtomicContactCount        name="contact_core_SCN"        task_operations="layer_core_SCN"
confidence="0" />
                <AtomicContactCount       name="contact_core_SASA"       task_operations="layer_core_SASA"
confidence="0" />
                <ResidueCount name="nres" confidence="0" />
                <ScoreType name="dslf_fa13" scorefxn=SFXN7 score_type=dslf_fa13 threshold=0/>
                <CalculatorFilter  name=mean_dslf  equation="dslf  /  (cyscount+0.01)"  threshold="-0.35"
confidence=0>
                  <VAR name="dslf" filter="dslf_fa13"/>
                  <VAR name="cyscount" filter="nres_cys"/>
                </CalculatorFilter>

                <DisulfideEntropy name="entropy" lower_bound="0" tightness="2" confidence="0"/>
                <TaskAwareScoreType    name="dslf_quality_check"    task_operations="no_repack_non-disulf"
score_type="dslf_fa13"  mode="individual" threshold="-0.27" confidence="0" />
                <ScoreType name="hbond_sfn" scorefxn=SFXN7 score_type=hbond_lr_bb threshold=0/>
                <SSShapeComplementarity name="ss_sc" verbose="0" confidence="0" />
                <SSShapeComplementarity  name="helix_sc"  verbose="1"  loops="0"  helices="1"  confidence="0"
/>
                <SSShapeComplementarity name="loop_sc" verbose="1" loops="1" helices="0" confidence="0" />
                <TotalSasa name="exposed_hydrophobics" confidence="0" hydrophobic=True />
                <TotalSasa name="exposed_total" confidence="0"/>
                <TotalSasa name="exposed_polars" confidence="0" polar=True />
                <CalculatorFilter  name="fxn_exposed_is_np"  equation="exposed  /  total"  threshold="1"
confidence=0>
                  <VAR name="total" filter="exposed_total"/>
                  <VAR name="exposed" filter="exposed_hydrophobics"/>
                </CalculatorFilter>
                <ScoreType name="total_hydrophobic" scorefxn=TotalHydrophobic threshold=0/>
                <CalculatorFilter name=buried_np equation="total - exposed" threshold="1" confidence=0>
                  <VAR name="total" filter="total_hydrophobic"/>
                  <VAR name="exposed" filter="exposed_hydrophobics"/>
                </CalculatorFilter>
                <CalculatorFilter    name=buried_over_exposed    equation="buried    /    (exposed+0.01)"
threshold="1" confidence=0>
                  <VAR name="buried" filter="buried_np"/>
                  <VAR name="exposed" filter="exposed_hydrophobics"/>
                </CalculatorFilter>
                <CalculatorFilter  name=buried_minus_exposed  equation="buried  -  exposed"  threshold="1"
confidence=0>
                  <VAR name="buried" filter="buried_np"/>
                  <VAR name="exposed" filter="exposed_hydrophobics"/>
                </CalculatorFilter>
                <CalculatorFilter   name=bb   equation="hbond   /   (rescount+0.01)"   threshold="-0.23"
confidence=0>
                  <VAR name="hbond" filter="hbond_sfn"/>
                  <VAR name="rescount" filter="nres"/>
                </CalculatorFilter>
                <CavityVolume name="cavity_volume" />
                <PackStat name=pack confidence=0 repeats="3" />
                <Holes name="holes" confidence=0/>
                <SSPrediction                  name="mismatch_probability"                  confidence="0"
cmd="/work/dadriano/PROGRAMS/psipred/runpsipred_single" use_probability="0" use_svm="0" />
                <BuriedUnsatHbonds name="unsat_hbond" confidence=0 jump_number=0/>
```

```
		<BuriedUnsatHbonds2 name="unsat_hbond2" confidence=0 jump_number=0/>
		<SecondaryStructureHasResidue	name="one_core_each"	secstruct_fraction_threshold="1.0"
res_check_task_operations="layer_core_SCN"	required_restypes="VILMFYW"	nres_required_per_secstruct="1"
filter_helix="1"	filter_sheet="1"	filter_loop="0"	min_helix_length="4"	min_sheet_length="3"
min_loop_length="1" confidence="0" />
		<SecondaryStructureHasResidue	name="two_core_each"	secstruct_fraction_threshold="1.0"
res_check_task_operations="layer_core_SCN"	required_restypes="VILMFYW"	nres_required_per_secstruct="2"
filter_helix="1"	filter_sheet="1"	filter_loop="0"	min_helix_length="4"	min_sheet_length="3"
min_loop_length="1" confidence="0" />
		<SecondaryStructureHasResidue	name="ss_contributes_core"
secstruct_fraction_threshold="1.0"	res_check_task_operations="layer_core_boundary_SCN"
required_restypes="VILMFYW"	nres_required_per_secstruct="1"	filter_helix="1"	filter_sheet="1"
filter_loop="0" min_helix_length="4" min_sheet_length="3" min_loop_length="1" confidence="0" />
		<FragmentLookupFilter	name="faulty_fragments_tolerant"
lookup_name="source_fragments_4_mer_tolerant"
store_path="/lab/databases/VALL_clustered/backbone_profiler_database_06032014"	lookup_mode="first"
chain="2" threshold="999999" confidence="0" />
		<FragmentLookupFilter	name="faulty_fragments"	lookup_name="source_fragments_4_mer"
store_path="/lab/databases/VALL_clustered/backbone_profiler_database_06032014"	lookup_mode="first"
chain="2" threshold="999999" confidence="0" />
	</FILTERS>

	<MOVERS>
	</MOVERS>

	<PROTOCOLS>
		<Add mover_name="keep_only_chain_B" />
		<Add filter_name="res_count_core_SCN" />
		<Add filter_name="res_count_core_SASA" />
		<Add filter_name="percent_core_SCN" />
		<Add filter_name="percent_core_SASA" />
		<Add filter_name="contact_all" />
		<Add filter_name="contact_core_SCN" />
		<Add filter_name="contact_core_SASA" />
		<Add filter_name="degree" />
		<Add filter_name="entropy" />
		<Add filter_name="dslf_quality_check" />
		<Add filter_name="mean_dslf" />
		<Add filter_name="cavity_volume" />
		<Add filter_name="ss_sc" />
		<Add filter_name="helix_sc" />
		<Add filter_name="loop_sc" />
		<Add filter_name="exposed_total" />
		<Add filter_name="exposed_hydrophobics" />
		<Add filter_name="exposed_polars" />
		<Add filter_name="fxn_exposed_is_np" />
		<Add filter_name="holes" />
		<Add filter_name="bb" />
		<Add filter_name="buried_np" />
		<Add filter_name="buried_over_exposed" />
		<Add filter_name="buried_minus_exposed" />
		<Add filter_name="pack" />
		<Add filter_name="mismatch_probability" />
		<Add filter_name="unsat_hbond" />
		<Add filter_name="unsat_hbond2" />
		<Add filter_name="one_core_each" />
		<Add filter_name="two_core_each" />
		<Add filter_name="ss_contributes_core" />
		<Add filter_name="AlaCount" />
	</PROTOCOLS>
```

```
</ROSETTASCRIPTS>
```

Appendix H. RosettaScripts XML used to analyze Rosetta Scores and Metrics of the complexes mini-protein<->Target

```
<ROSETTASCRIPTS>
        <SCOREFXNS>
                <NOV15 weights="beta_nov15.wts" />
                <VDW weights="empty" >
                        <Reweight scoretype="fa_atr" weight=1.0/>
                </VDW>
        </SCOREFXNS>

        <TASKOPERATIONS>
                <ProteinInterfaceDesign  name="pack_long"  design_chain1="0"  design_chain2="0"  jump="1"
interface_distance_cutoff="15"/>
                <ProteinInterfaceDesign   name="chain2_interface"   repack_chain1="0"   design_chain2="1"
jump="1" interface_distance_cutoff="8"/>
                <RestrictToInterfaceVector  name="restrict_to_interface"  jump="1"  CB_dist_cutoff="10.0"
nearby_atom_cutoff="5.5" vector_angle_cutoff="75.0" vector_dist_cutoff="9.0"/>
        </TASKOPERATIONS>

        <MOVERS>
                <TaskAwareMinMover name=min scorefxn=NOV15 bb=0 chi=1 task_operations=pack_long/>
                <SwitchChainOrder name=chain1only chain_order="1"/>
                <SwitchChainOrder name=chain2only chain_order="2"/>
        </MOVERS>

        <FILTERS>
                <Ddg  name="ddg"   threshold=-10  jump=1  repeats=5  repack=1  relax_mover=min  confidence=0
scorefxn=NOV15 />
                <Ddg   name="ddg_norepack"     threshold=-10   jump=1   repeats=1   repack=0   confidence=0
scorefxn=NOV15/>
                <Ddg   name="ddg_fa_atr"     threshold=-10   jump=1   repeats=5   repack=1   relax_mover=min
confidence=0 scorefxn=VDW />
                <Ddg  name="ddg_fa_atr_norepack"   threshold=-10  jump=1  repeats=1  repack=0  confidence=0
scorefxn=VDW/>
                <InterfaceHoles name="interface_holes" confidence=0 jump=1/>
                <Sasa name="interface_buried_sasa" confidence=0/>
                <Sasa name="interface_hydrophobic_sasa" confidence=0 hydrophobic=True/>
                <Sasa name="interface_polar_sasa" confidence=0 polar=True/>
                <BuriedUnsatHbonds name="interface_unsat_hbond" confidence=0 jump_number=1/>
                <BuriedUnsatHbonds2 name="interface_unsat_hbond2" confidence=0 jump_number=1/>
                <TotalSasa name="exposed_hydrophobics" confidence="0" hydrophobic=True />
                <MoveBeforeFilter name=exposed_np_chain1 mover=chain1only filter=exposed_hydrophobics/>
                <MoveBeforeFilter name=exposed_np_chain2 mover=chain2only filter=exposed_hydrophobics/>
                <CalculatorFilter  name="fxn_np_chain1_buried_approx"  equation="(sasa  /  2)  /  (np+0.01)"
threshold="1" confidence="0">
                        <VAR name="sasa" filter="interface_hydrophobic_sasa"/>
                        <VAR name="np" filter="exposed_np_chain1"/>
                </CalculatorFilter>
                <CalculatorFilter  name="fxn_np_chain2_buried_approx"  equation="(sasa  /  2)  /  (np+0.01)"
threshold="1" confidence="0">
                        <VAR name="sasa" filter="interface_hydrophobic_sasa"/>
                        <VAR name="np" filter="exposed_np_chain2"/>
                </CalculatorFilter>
                <CalculatorFilter name="np_chain1_exposed_approx" equation="np - (sasa / 2)" threshold="1"
confidence="0">
                        <VAR name="sasa" filter="interface_hydrophobic_sasa"/>
                        <VAR name="np" filter="exposed_np_chain1"/>
```

```
                </CalculatorFilter>
                <CalculatorFilter name="np_chain2_exposed_approx" equation="np - (sasa / 2)" threshold="1"
confidence="0">
                        <VAR name="sasa" filter="interface_hydrophobic_sasa"/>
                        <VAR name="np" filter="exposed_np_chain2"/>
                </CalculatorFilter>
                <CalculatorFilter   name="ddg_per_1000sasa"   equation="1000   *   ddg   /   (sasa+0.01)"
threshold="1" confidence="0">
                        <VAR name="ddg" filter="ddg"/>
                        <VAR name="sasa" filter="interface_buried_sasa"/>
                </CalculatorFilter>
                <CalculatorFilter  name="ddg_norepack_per_1000sasa"  equation="1000  *  ddg  /  (sasa+0.01)"
threshold="1" confidence="0">
                        <VAR name="ddg" filter="ddg_norepack"/>
                        <VAR name="sasa" filter="interface_buried_sasa"/>
                </CalculatorFilter>
                <CalculatorFilter  name="ddg_fa_atr_per_1000sasa"  equation="1000  *  ddg  /  (sasa+0.01)"
threshold="1" confidence="0">
                        <VAR name="ddg" filter="ddg_fa_atr"/>
                        <VAR name="sasa" filter="interface_buried_sasa"/>
                </CalculatorFilter>
                <CalculatorFilter   name="ddg_fa_atr_norepack_per_1000sasa"   equation="1000   *   ddg   /
(sasa+0.01)" threshold="1" confidence="0">
                        <VAR name="ddg" filter="ddg_fa_atr_norepack"/>
                        <VAR name="sasa" filter="interface_buried_sasa"/>
                </CalculatorFilter>
                <CalculatorFilter name="interface_fxn_hydrophobic" equation="hydrophobic / (sasa + 0.01)"
threshold="1" confidence="0">
                        <VAR name="hydrophobic" filter="interface_hydrophobic_sasa"/>
                        <VAR name="sasa" filter="interface_buried_sasa"/>
                </CalculatorFilter>
                <AverageDegree               name="interface_averageDegree"               threshold="8.3"
task_operations=chain2_interface confidence="0"/>
                <ShapeComplementarity  name="interface_sc"  verbose=0  min_sc=0.55  write_int_area=1  jump=1
confidence=0/>
                <AtomicContactCount  name="interface_contactcount"  task_operations="restrict_to_interface"
confidence="0" />
        </FILTERS>

        <MOVERS>
        </MOVERS>

        <PROTOCOLS>
                <Add filter_name="interface_holes"/>
                <Add filter_name="interface_unsat_hbond" />
                <Add filter_name="interface_unsat_hbond2" />
                <Add filter_name="interface_buried_sasa" />
                <Add filter_name="interface_hydrophobic_sasa" />
                <Add filter_name="interface_polar_sasa" />
                <Add filter_name="interface_fxn_hydrophobic" />
                <Add filter_name="ddg" />
                <Add filter_name="ddg_norepack" />
                <Add filter_name="ddg_per_1000sasa" />
                <Add filter_name="ddg_norepack_per_1000sasa" />
                <Add filter_name="ddg_fa_atr" />
                <Add filter_name="ddg_fa_atr_norepack" />
                <Add filter_name="ddg_fa_atr_per_1000sasa" />
                <Add filter_name="ddg_fa_atr_norepack_per_1000sasa" />
                <Add filter_name="fxn_np_chain1_buried_approx" />
                <Add filter_name="fxn_np_chain2_buried_approx" />
```

```
                <Add filter_name="np_chain1_exposed_approx" />
                <Add filter_name="np_chain2_exposed_approx" />
                <Add filter_name="interface_averageDegree" />
                <Add filter_name="interface_contactcount" />
                <Add filter_name="interface_sc" />
        </PROTOCOLS>

</ROSETTASCRIPTS>
```