Supplemental Material

Tables S1, S2, S3, S4 Description

RMSD values are listed for individual test cases within each loop length, 6, 8, 10, and 12 residues within tables S1, S2, S3, and S4, respectively. RMSD's are backbone heavy atom (N C C O). Energy gaps (Egap) are in kcal/mol and are the difference between the predicted and native energies (see Methods). An "S" in the energy gap columns means sampling failure where no loops under 1 Å were sampled to provide a native energy. Col 1: PDB ID, Cols 2 and 3: the first and last loop residue numbers, Cols 4, 5 and 6: RMSD of predicted loop, RMSD of best sampled loop, and energy gap using old protocol on unperturbed crystal structures, Cols 7, 8, 9: RMSD of predicted loop, RMSD of best sampled loop, and energy gap using our new protocol on unperturbed crystal structures. Col 10: Backbone RMSD of loops in starting perturbed crystal structure, Cols 11, 12, 13: RMSD of predicted loop, RMSD of best sampled loop, and energy gap using old protocol on perturbed crystal structures, Cols 14, 15, 16: RMSD of predicted loop, RMSD of best sampled loop, and energy gap using new protocol on perturbed crystal structures.

S1

			Native crystal						Perturbed crystal						
			Old Protocol			New Protocol				Old Protocol			New Protocol		
6res	Loop start res num	Loop end res num	Predict RMSD	Best sampled RMSD	Egap (kcal/mol)	Predict RMSD	Best sampled RMSD	Egap (kcal/mol)	Start RMSD	Predict RMSD	Best sampled RMSD	Egap (kcal/mol)	Predict RMSD	Best sampled RMSD	Egap (kcal/mol)
1ads	_:149	_:154	0.3	0.2	0.0	0.2	0.2	0.0	6.0	0.8	0.2	0.0	0.2	0.2	0.0
1arp	_:282	_:287	0.2	0.2	0.0	0.2	0.2	0.0	4.8	0.8	0.6	0.0	0.2	0.2	0.0
1brt	_:174	_:179	0.5	0.2	0.0	0.4	0.2	0.0	6.5	3.8	2.9	S	0.5	0.2	0.0
1cbs	_:66	_:71	0.3	0.3	0.0	0.4	0.3	0.0	2.7	2.4	0.5	-0.9	0.6	0.5	0.0
1gca	_:100	_:105	0.2	0.2	0.0	0.3	0.3	0.0	2.6	0.3	0.2	0.0	0.3	0.2	0.0
1hbq	_:15	_:20	0.4	0.2	0.0	0.4	0.2	0.0	3.6	0.5	0.4	0.0	0.4	0.3	0.0
1mrp	_:215	_:220	0.8	0.3	0.0	0.3	0.3	0.0	1.9	1.7	0.8	-6.7	1.7	0.9	-6.9
1noa	_:57	_:62	1.1	0.3	-0.6	1.1	0.3	-0.2	2.3	1.1	0.3	0.0	1.1	0.3	0.0
1onc	_:12	_:17	0.3	0.2	0.0	0.9	0.2	0.0	5.0	3.4	0.7	-17.8	0.3	0.3	0.0
1ppn	_:144	_:149	0.3	0.2	0.0	0.3	0.2	0.0	3.0	0.5	0.5	0.0	0.4	0.2	0.0
1rie	_:126	_:131	0.3	0.1	0.0	0.4	0.2	0.0	2.8	1.4	0.6	-5.5	0.4	0.2	0.0
1tca	_:94	_:99	0.3	0.3	0.0	2.3	0.3	-6.1	3.2	0.3	0.3	0.0	2.4	0.2	-6.5
1tys	_:66	_:71	0.2	0.1	0.0	0.1	0.1	0.0	6.7	6.8	4.1	S	0.2	0.2	0.0
1xif	_:357	_:362	3.3	1.3	S	3.5	0.6	-9.2	4.0	1.2	1.3	S	0.3	0.2	0.0
2cba	_:82	_:87	0.5	0.5	0.0	0.8	0.5	0.0	2.3	1.1	1.1	0.0	0.8	0.5	0.0
2cpl	_:122	_:127	0.3	0.2	0.0	0.3	0.2	0.0	0.8	0.9	0.3	0.0	0.8	0.2	0.0
2pth	_:136	_:141	0.3	0.1	0.0	0.4	0.2	0.0	2.8	1.9	1.8	S	0.2	0.2	0.0
3tgl	_:82	_:87	3.1	0.3	-7.5	3.1	0.4	-6.9	3.0	2.9	0.5	-7.1	3.1	0.3	-3.1
5p21	_:104	_:109	0.5	0.4	0.0	0.5	0.4	0.0	2.3	1.2	1.2	S	0.5	0.4	0.0
7rsa	_:14	_:19	1.0	0.2	0.0	1.0	0.2	0.0	2.4	0.9	0.3	0.0	1.2	0.2	-0.8
Median			0.3	0.2		0.4	0.2		2.9	1.1	0.5		0.4	0.2	
Avg			0.7	0.3		0.8	0.3		3.4	1.7	0.9		0.8	0.3	

S2

			Native crystal						Perturbed crystal						
			Old Protocol			New Protocol				Old Protocol			New Protocol		
8res	Loop start res num	Loop end res num	Predict RMSD	Best sampled RMSD	Egap (kcal/mol)	Predict RMSD	Best sampled RMSD	Egap (kcal/mol)	Start RMSD	Predict RMSD	Best sampled RMSD	Egap (kcal/mol)	Predict RMSD	Best sampled RMSD	Egap (kcal/mol)
135l	_:84	_:91	0.4	0.2	0.0	2.6	0.4	-17.4	5.2	2.1	0.9	-5.3	2.5	0.3	-13.3
1alc	_:34	_:41	5.9	2.5	S	4.5	1.0	-37.9	5.6	5.2	2.7	S	6.9	0.4	-6.4
1btl	_:50	_:57	0.3	0.3	0.0	1.6	0.2	-1.3	7.2	3.1	2.0	S	0.9	0.5	0.0
1cex	_:73	_:80	3.9	0.6	-6.8	3.7	0.6	-3.2	4.8	3.2	1.6	S	2.5	1.5	S
1clc	_:313	_:320	0.3	0.2	0.0	3.0	0.2	-1.4	4.8	4.3	2.7	S	0.4	0.2	0.0
1ddt	_:127	_:134	1.0	0.3	0.0	0.9	0.3	0.0	3.4	0.9	0.4	0.0	1.1	0.4	0.0
1ezm	_:92	_:99	0.4	0.2	0.0	0.5	0.3	0.0	2.7	3.2	1.8	S	0.5	0.3	0.0
1hfc	_:142	_:149	0.2	0.2	0.0	0.3	0.2	0.0	7.3	0.4	0.5	0.0	0.3	0.2	0.0
1iab	_:48	_:55	1.6	0.5	-0.3	0.8	0.4	0.0	2.4	2.6	0.7	-14.1	0.5	0.3	0.0
1ivd	_:413	_:420	0.9	0.7	0.0	1.1	0.6	-1.9	3.3	3.3	2.3	S	1.3	0.7	-2.9
1lst	_:101	_:108	0.6	0.2	0.0	0.7	0.3	0.0	7.0	0.9	0.5	0.0	0.7	0.3	0.0
1nar	_:192	_:199	0.6	0.4	0.0	1.5	0.4	-10.3	2.7	0.7	0.6	0.0	1.2	0.6	-4.0
1oyc	_:80	_:87	0.5	0.3	0.0	0.4	0.2	0.0	2.1	2.2	0.7	-4.1	0.6	0.3	0.0
1prn	_:150	_:157	1.7	1.1	S	2.4	0.3	-10.8	4.1	0.7	0.3	0.0	2.3	0.4	-9.6
1sbp	_:107	_:114	3.3	3.9	S	0.3	0.2	0.0	3.8	3.4	1.9	S	0.3	0.2	0.0
1tml	_:187	_:194	0.3	0.2	0.0	1.4	0.2	-14.5	2.1	1.2	1.0	-2.0	1.5	0.4	-19.3
2cmd	_:270	_:277	0.4	0.2	0.0	0.4	0.2	0.0	8.0	6.4	1.2	S	0.4	0.3	0.0
2exo	_:262	_:269	0.4	0.2	0.0	0.4	0.2	0.0	3.5	1.9	1.3	S	0.7	0.2	0.0
2sga	_:32	_:43	1.2	1.0	0.0	1.2	0.6	0.0	4.0	1.0	0.9	0.0	1.1	0.4	0.0
5p21	_:45	_:52	0.4	0.3	0.0	0.9	0.3	0.0	2.4	2.2	0.7	-3.5	0.8	0.3	0.0
Median			0.6	0.3		1.0	0.3		3.9	2.2	1.0		0.8	0.3	
Avg			1.2	0.7		1.4	0.4		4.3	2.4	1.2		1.3	0.4	

S3

			Native crystal						Perturbed crystal						
			Old Protocol			New Protocol				Old Protocol			New Protocol		
10res	Loop start res num	Loop end res num	Predict RMSD	Best sampled RMSD	Egap (kcal/mol)	Predict RMSD	Best sampled RMSD	Egap (kcal/mol)	Start RMSD	Predict RMSD	Best Sampled RMSD	Egap (kcal/mol)	Predict RMSD	Best sampled RMSD	Egap (kcal/mol)
1ads	_:170	_:179	0.8	0.4	0.0	2.3	0.8	-18.8	8.8	0.7	0.4	0.0	0.8	0.6	0.0
1arb	_:41	_:50	0.5	0.4	0.0	1.2	0.8	0.0	5.8	4.1	1.2	S	1.1	0.4	-3.1
1aru	_:128	_:137	0.2	0.2	0.0	0.3	0.2	0.0	1.8	1.8	0.7	0.0	2.0	0.2	-1.3
1dim	_:87	_:96	0.2	0.2	0.0	1.1	0.3	0.0	4.0	1.1	0.6	0.0	1.0	0.4	0.0
1edg	_:269	_:278	0.2	0.2	0.0	0.2	0.2	0.0	9.2	3.1	1.6	S	3.0	0.9	-42.9
1gvp	_:49	_:58	3.3	0.7	-11.7	2.7	0.8	-10.0	5.6	2.5	1.2	S	3.1	1.0	-19.0
1ixh	_:84	_:93	1.0	0.7	0.0	1.0	0.6	0.0	4.3	0.8	0.6	0.0	1.2	0.4	-0.1
1lst	_:10	_:19	0.6	0.2	0.0	0.7	0.3	0.0	4.7	2.0	1.3	S	0.5	0.5	0.0
1mrj	_:173	_:182	0.6	0.3	0.0	0.7	0.2	0.0	5.1	0.9	0.7	0.0	0.3	0.2	0.0
1pgs	_:68	_:77	0.4	0.3	0.0	1.8	0.7	-12.2	3.6	2.9	0.5	-1.5	3.1	1.0	-69.6
1plc	_:42	_:51	1.2	0.5	-3.9	1.1	0.9	-0.7	4.4	1.2	0.6	-1.0	1.2	0.5	0.0
1scs	_:65	_:74	0.4	0.3	0.0	2.2	0.5	-8.3	3.1	2.8	0.3	-7.3	2.1	0.6	-30.5
1tca	_:23	_:32	1.0	0.2	0.0	0.7	0.3	0.0	10.6	0.8	0.3	0.0	1.8	0.2	-0.9
2alp	_:90	_:105	0.2	0.2	0.0	0.4	0.2	0.0	3.7	0.4	0.3	0.0	0.3	0.2	0.0
2ayh	_:80	_:89	0.2	0.2	0.0	0.2	0.2	0.0	2.5	0.3	0.3	0.0	0.2	0.2	0.0
2cmd	_:57	_:66	0.4	0.3	0.0	0.7	0.4	0.0	3.7	2.0	1.0	S	1.0	0.4	0.0
2mnr	_:91	_:100	0.6	0.4	0.0	0.8	0.6	0.0	3.8	1.3	0.5	-9.9	4.6	0.7	-4.9
2sil	_:197	_:206	0.4	0.3	0.0	0.3	0.3	0.0	3.6	2.2	0.9	-24.9	0.4	0.3	0.0
3tgl	_:257	_:266	0.3	0.3	0.0	0.8	0.4	0.0	6.3	1.7	1.4	S	0.8	0.5	0.0
7rsa	_:33	_:42	0.4	0.4	0.0	1.0	0.5	0.0	3.6	1.2	0.4	0.0	1.0	0.5	0.0
Median			0.4	0.3		0.8	0.4		4.2	1.5	0.6		1.1	0.4	
Avg			0.6	0.3		1.0	0.5		4.9	1.7	0.7		1.5	0.5	

S4

			Native crystal						Perturbed crystal						
			Old Protocol			New Protocol				Old Protocol			New Protocol		
12res	Loop start res num	Loop end res num	Predict RMSD	Best sampled RMSD	Egap (kcal/mol)	Predict RMSD	Best sampled RMSD	Egap (kcal/mol)	Start RMSD	Predict RMSD	Best sampled RMSD	Egap (kcal/mol)	Predict RMSD	Best sampled RMSD	Egap (kcal/mol)
1a8d	_:155	_:166	1.0	1.1	0.0	2.7	1.4	S	4.0	0.9	0.8	0.0	2.8	1.4	S
1arb	_:182	_:193	2.5	0.6	-2.4	1.0	0.5	0.0	4.8	2.2	0.6	-8.4	2.6	0.5	-3.3
1bhe	_:121	_:132	0.5	0.4	0.0	0.5	0.5	0.0	4.5	1.0	0.8	0.0	0.7	0.7	0.0
1bn8	A:298	A:309	1.5	0.4	-3.2	1.3	0.6	-24.7	5.0	8.3	2.4	S	2.6	0.5	-3.9
1c5e	A:82	A:93	0.4	0.3	0.0	0.4	0.3	0.0	5.1	1.8	0.8	-45.8	1.7	1.0	-51.3
1cb0	A:33	A:44	0.3	0.2	0.0	0.3	0.3	0.0	5.0	0.4	0.4	0.0	0.3	0.3	0.0
1cnv	_:188	_:199	2.2	0.6	-14.1	1.5	0.7	-9.9	4.5	3.2	0.9	-48.5	3.3	1.0	-45.0
1cs6	A:145	A:156	0.6	0.4	0.0	1.6	0.4	-7.7	5.1	3.4	1.4	S	3.5	1.4	S
1dqz	A:209	A:220	0.3	0.3	0.0	0.7	0.3	0.0	4.8	1.2	0.4	-3.8	0.6	0.3	0.0
1exm	A:291	A:302	0.7	0.4	0.0	4.5	1.2	S	4.6	4.0	1.2	S	0.5	0.5	0.0
1f46	A:64	A:75	0.3	0.2	0.0	0.5	0.4	0.0	5.1	3.8	0.3	-15.4	1.1	1.2	0.0
1i7p	A:63	_:74	0.3	0.2	0.0	0.3	0.2	0.0	5.0	0.5	0.5	0.0	0.3	0.2	0.0
1m3s	A:68	A:79	5.0	0.7	-34.5	5.1	1.4	S	5.0	5.3	1.3	S	5.6	0.6	-49.5
1ms9	A:529	A:540	1.9	0.6	-16.8	2.8	0.5	-31.7	2.9	2.9	0.5	-23.7	2.5	0.5	-31.5
1my7	A:254	A:265	0.5	0.4	0.0	0.9	0.6	0.0	5.1	1.4	0.8	-8.2	0.9	0.6	0.0
1oth	A:69	A:80	1.8	0.4	-0.8	0.5	0.5	0.0	4.1	2.3	2.1	S	0.7	0.7	0.0
1oyc	_:203	_:214	0.5	0.4	0.0	0.5	0.3	0.0	5.2	2.8	1.2	S	1.2	0.4	-7.5
1qlw	A:31	A:42	1.9	0.3	-22.3	2.0	1.6	S	4.1	1.5	0.8	-10.1	1.4	1.1	S
1t1d	A:127	A:138	0.5	0.4	0.0	0.8	0.5	0.0	3.3	0.6	0.5	0.0	1.0	0.6	0.0
2pia	_:30	_:41	0.6	0.4	0.0	0.5	0.4	0.0	4.6	3.7	1.0	-7.6	0.5	0.4	0.0
Median			0.6	0.4		0.9	0.5		4.8	2.3	0.8		1.2	0.6	
Avg			1.2	0.4		1.4	0.6		4.6	2.6	0.9		1.7	0.7	

Table S5 description

Comparison of loop predictions using previously published protocol (without simultaneous optimization of surrounding side chains) with and without inclusion of crystal symmetry chains on unperturbed crystal structures. Atoms from chains within the crystal are included if they are within 20 Å of any primary-chain atom. RMSD values are calculated for backbone heavy atoms for each prediction. Differences between these two predictions enable us to identify crystal packing effects on our predictions.

Table S5

With Crystal Symmetry?	Y	N		Y	N		Y	N		Y	N
6 residues			8 residues			10 residues			12 residues		
1ads	0.3	0.3	135l	3.6	0.4	1ads	0.8	0.8	1a8d	1.1	1.0
1arp	0.2	0.2	1alc	5.6	5.9	1arb	1.2	0.5	1arb	1.2	2.5
1brt	0.5	0.5	1btl	0.4	0.3	1aru	0.3	0.2	1bhe	0.9	0.5
1cbs	0.3	0.3	1cex	3.8	3.9	1dim	0.9	0.2	1bn8	1.5	1.5
1gca	0.3	0.2	1clc	0.3	0.3	1edg	0.2	0.2	1c5e	0.4	0.4
1hbq	0.3	0.4	1ddt	1.0	1.0	1gvp	1.9	3.3	1cb0	0.2	0.3
1mrp	0.3	0.8	1ezm	0.4	0.4	1ixh	1.0	1.0	1cnv	0.7	2.2
1noa	2.0	1.1	1hfc	0.2	0.2	1lst	0.6	0.6	1cs6	0.9	0.6
1onc	0.7	0.3	1iab	0.4	1.6	1mrj	0.2	0.6	1dqz	0.3	0.3
1ppn	0.2	0.3	1ivd	1.0	0.9	1pgs	0.6	0.4	1exm	1.0	0.7
1rie	0.3	0.3	1lst	0.6	0.6	1plc	0.6	1.2	1f46	0.5	0.3
1tca	0.4	0.3	1nar	0.5	0.6	1scs	0.3	0.4	1i7p	0.3	0.3
1tys	0.1	0.2	1oyc	0.5	0.5	1tca	0.5	1.0	1m3s	0.5	5.0
1xif	0.2	3.3	1prn	0.3	1.7	2alp	0.2	0.2	1ms9	1.9	1.9
2cba	1.0	0.5	1sbp	0.4	3.3	2ayh	0.2	0.2	1my7	0.5	0.5
2cpl	0.3	0.3	1tml	0.3	0.3	2cmd	0.4	0.4	1oth	0.5	1.8
2pth	0.5	0.3	2cmd	0.2	0.4	2mnr	0.4	0.6	1oyc	0.4	0.5
3tgl	0.7	3.1	2exo	0.2	0.4	2sil	0.5	0.4	1qlw	1.7	1.9
5p21	0.4	0.5	2sga	1.0	1.2	3tgl	0.5	0.3	1t1d	1.1	0.5
7rsa	0.8	1.0	5p21	0.2	0.4	7rsa	0.2	0.4	2pia	0.4	0.6
Median	0.3	0.3	Median	0.4	0.6	Median	0.5	0.4	Median	0.6	0.6
Avg	0.5	0.7	Avg	1.1	1.2	Avg	0.6	0.6	Avg	0.8	1.2

Table S6 Description

Overall median and average predicted backbone RMSD's are listed for the unperturbed (top) and perturbed (bottom) *filtered* test sets for both the old HLP and new HLP-SS methods. The filtered statistics are across all test cases but with the 9 cases removed that were determined to be adversely affected by crystal packing (see Results).

Table S6

	Crystal structures			
	Previous Method		New Method	
Loop Length	Median RMSD	Avg RMSD	Median RMSD	Avg RMSD
6 Residues	0.3	0.4	0.4	0.6
8 Residues	0.4	1.0	1.1	1.5
10 Residues	0.4	0.6	0.8	1.0
12 Residues	0.5	0.7	0.7	1.3

	Perturbed crystal structures			
	Previous Method		New Method	
Loop Length	Median RMSD	Avg RMSD	Median RMSD	Avg RMSD
6 Residues	1.1	1.7	0.4	0.7
8 Residues	2.2	2.5	0.9	1.4
10 Residues	1.5	1.7	1.1	1.5
12 Residues	1.6	2.4	1.1	1.3