Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information

Accurate calculation of absolute free energy of binding for allosteric inhibitors using free energy perturbation

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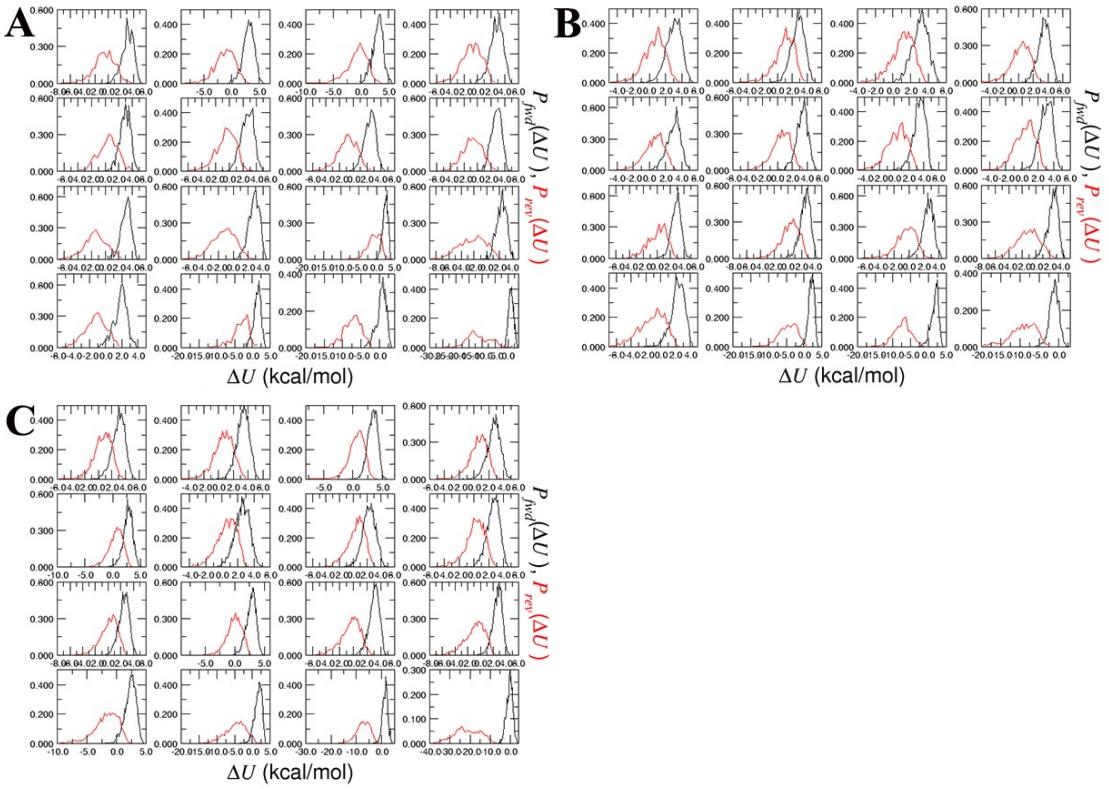

Figure S1. Probability distribution function underlying the binding of JAB3068 to SHP2. Shown in this Figureure are the distributions characterizing the forward, $P_{fwd}(\Delta U)$ (black solid lines) and the backward, $P_{rev}(\Delta U)$ (red solid lines) transformations for (**A**) all 16 intermediate states in a 16-window stratification and 16 ps for each window strategy, (**B**) all 16 intermediate states in a 16-window stratification and 32 ps for each window strategy and (**C**) all 16 intermediate states in a 16-window stratification and 64 ps for each window strategy.

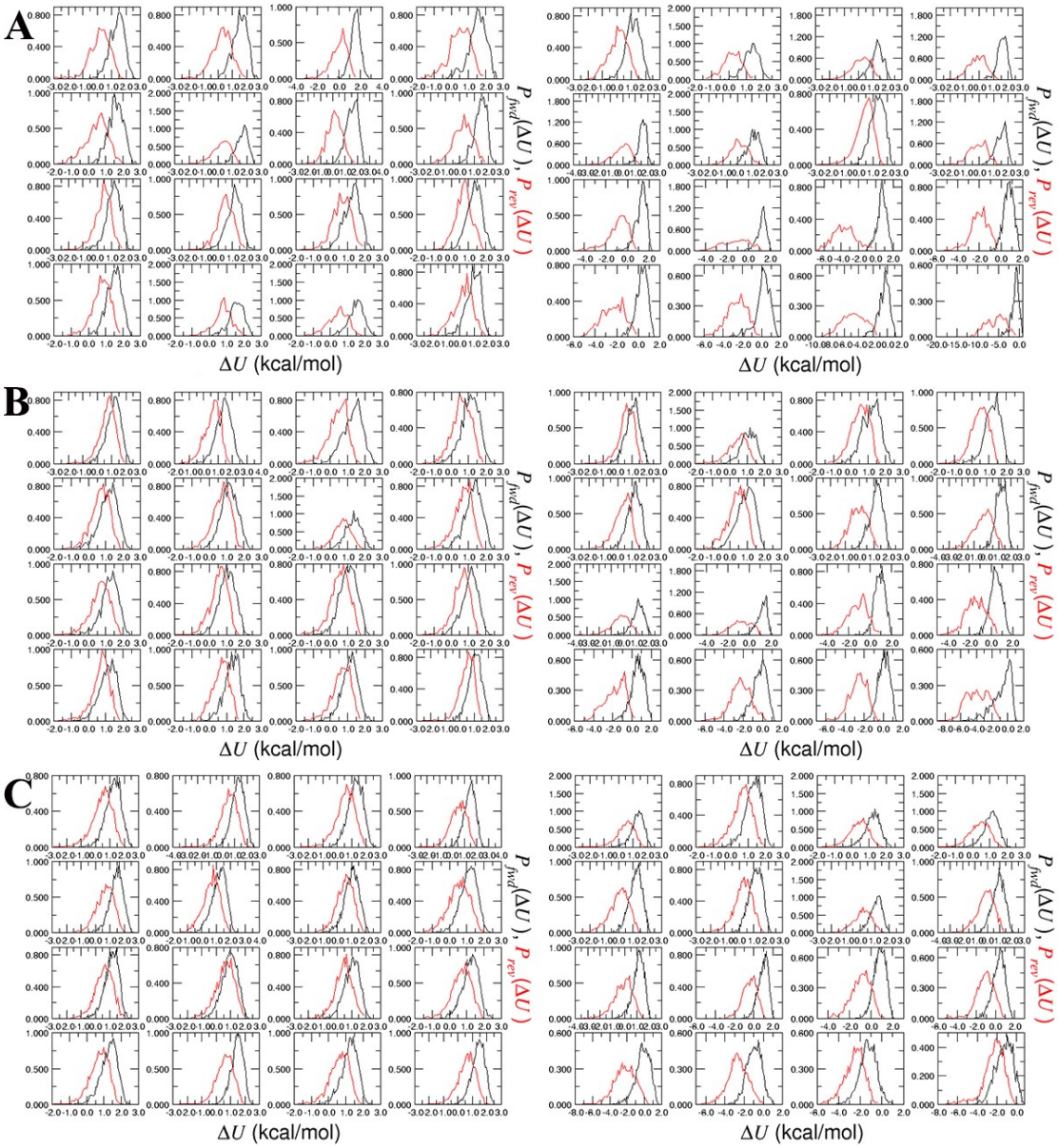

Figure S2. Probability distribution function underlying the binding of JAB3068 to SHP2. Shown in this Figureure are the distributions characterizing the forward, $P_{fwd}(\Delta U)$ (black solid lines) and the backward, $P_{rev}(\Delta U)$ (red solid lines) transformations for (**A**) all 32 intermediate states in a 32-window stratification and 16 ps for each window strategy, (**B**) all 32 intermediate states in a 32-window stratification and 32 ps for each window strategy and (**C**) all 32 intermediate states in a 32-window stratification and 64 ps for each window strategy.

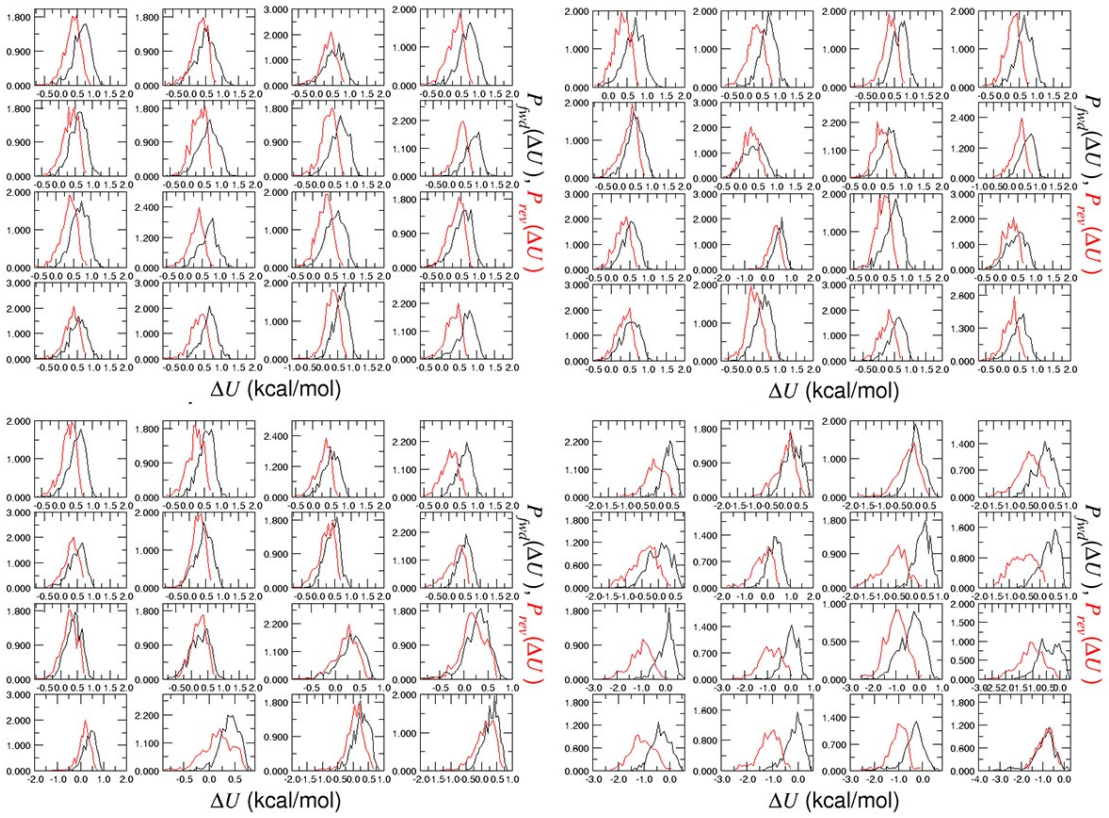

Figure S3. Probability distribution function underlying the binding of JAB3068 to SHP2. Shown in this Figureure are the distributions characterizing the forward, $P_{fwd}(\Delta U)$ (black solid lines) and the backward, $P_{rev}(\Delta U)$ (red solid lines) transformations for all 64 intermediate states in a 64-window stratification and 16 ps for each window strategy.

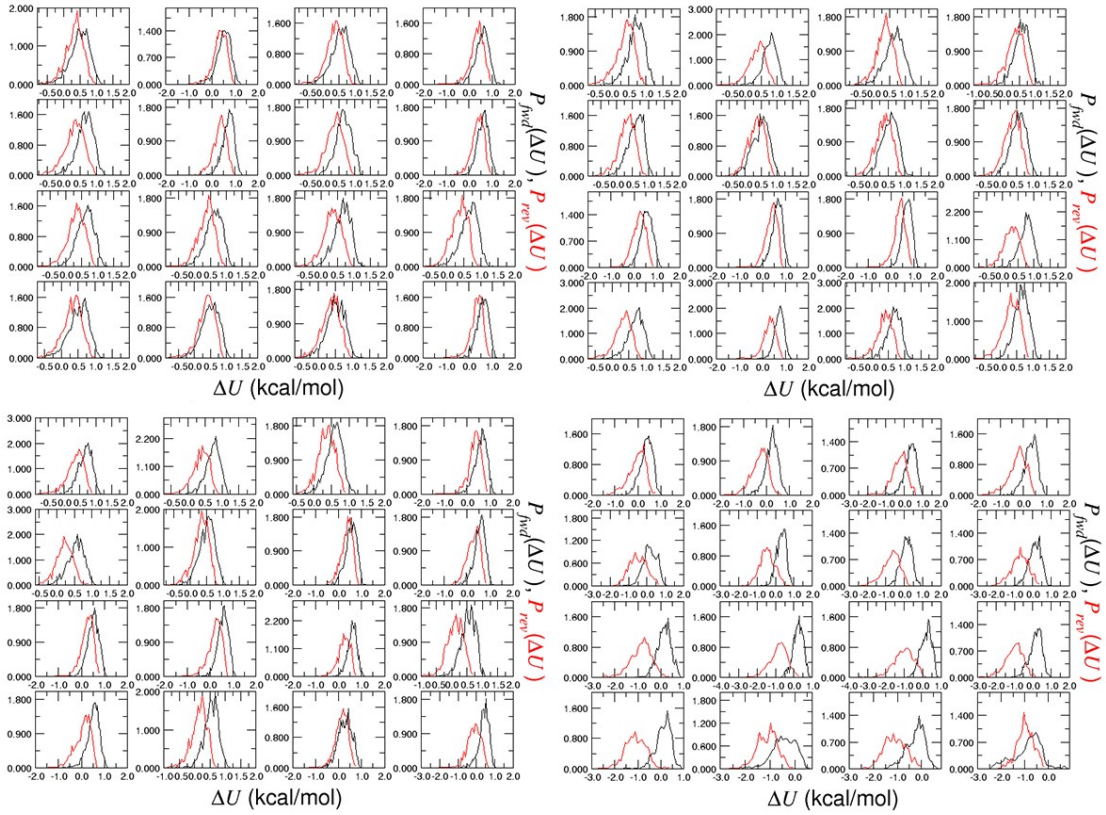

Figure S4. Probability distribution function underlying the binding of JAB3068 to SHP2. Shown in this Figureure are the distributions characterizing the forward, $P_{fwd}(\Delta U)$ (black solid lines) and the backward, $P_{rev}(\Delta U)$ (red solid lines) transformations for all 64 intermediate states in a 64-window stratification and 32 ps for each window strategy.

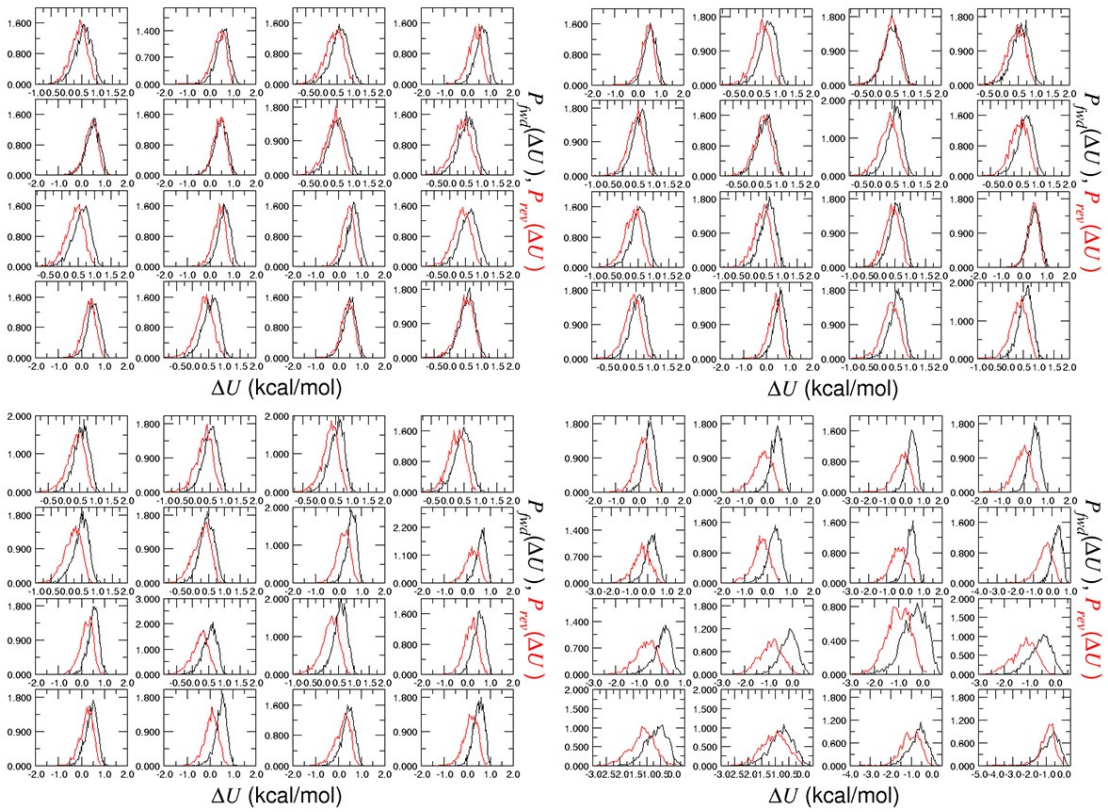

Figure S5. Probability distribution function underlying the binding of JAB3068 to SHP2. Shown in this Figureure are the distributions characterizing the forward, $P_{fwd}(\Delta U)$ (black solid lines) and the backward, $P_{rev}(\Delta U)$ (red solid lines) transformations for all 64 intermediate states in a 64-window stratification and 64 ps for each window strategy.

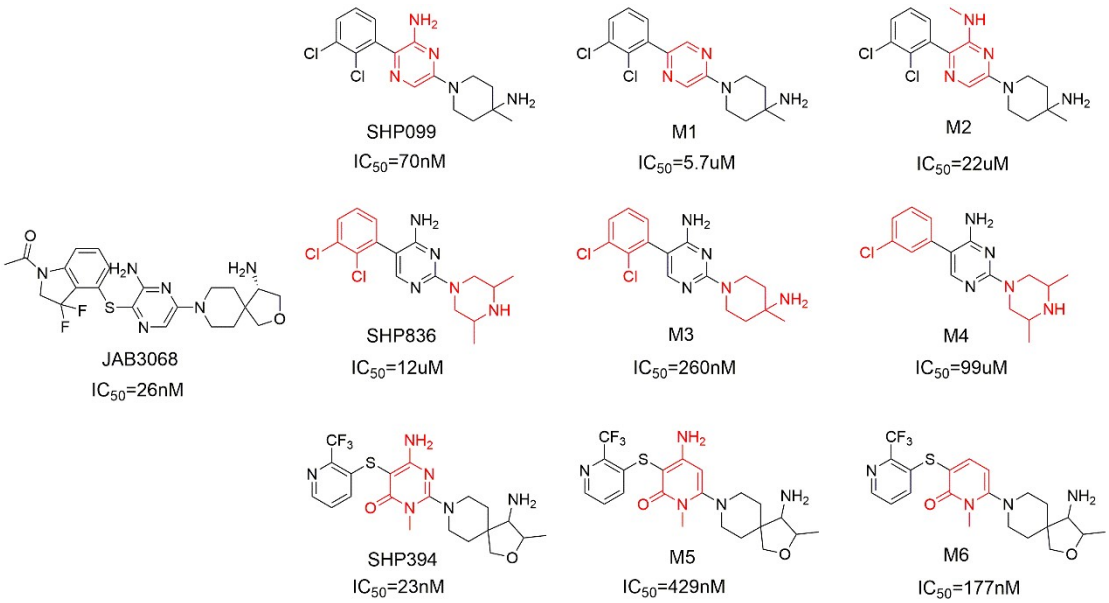

Figure S6. Structures and biological activity values of SHP2 allosteric inhibitors

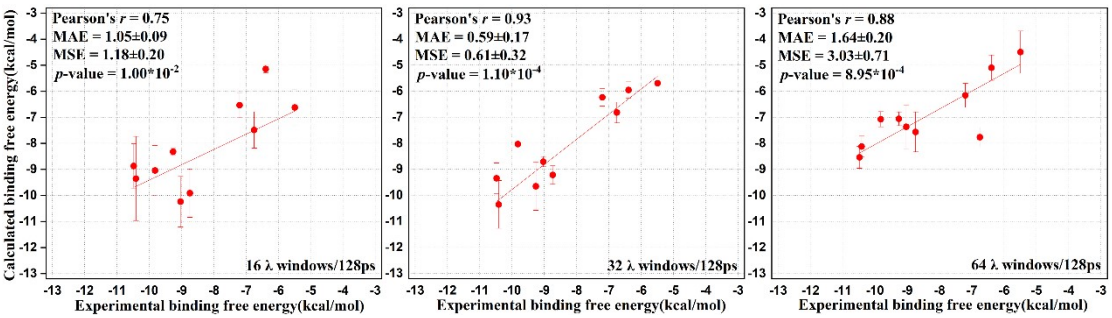

Figure S7 Correlation and p-value between experimental and calculated average affinities by FEP method under 16 λ windows/128ps, 32 λ windows/128ps and 64 λ windows/128ps sampling strategies. A smaller p-value means that correlation between experimental and calculated values is more significant. MAE (mean absolute error) and MSE (mean square error) represent the abbreviations for mean absolute error and mean square error, respectively. MAE and MSE are in kcal mol^{-1}. Error bars represent the standard error of mean across the five repeated calculations.

Table S1 RMSD values (Å) obtained by cross-docking study

PDB / Ligands	5EHP	5EHR	6MD7	6MD9	6MDA	6MDB	6MDC	6MDD
5EHP	0.21	7	1.31	1.85	1.77	0.8	0.66	2.66
5EHR	0.45	0.27	1.76	1.18	1.41	0.83	0.32	5.36
6MD7	2.26	12.51	0.41	1.39	3.13	1.23	2.23	6.83
6MD9	1.66	1.79	1.37	0.58	1.71	1.72	1.34	5.55
6MDA	1.04	2.68	1.31	0.61	0.24	0.86	1.3	8.63
6MDB	0.61	1.91	1.9	1.73	0.86	0.29	1.11	5.45
6MDC	1.27	8.45	6.49	2.3	5.42	2.73	1.12	11.59
6MDD	2.56	2.48	2.09	2.16	0.83	1.11	7.38	0.24

Table S2 Binding free energy of MM/PBSA calculation (kcal mol^{-1}).

Inhibitors	Experimental*	MM/PBSA
JAB3068	-10.41	-18.81±6.61
SHP099	-9.82	-13.38±4.01
SHP836	-6.75	-7.70±4.73
SHP394	-10.48	-27.31±7.17
M1	-7.2	-10.31±3.78
M2	-6.39	-10.36±4.60
M3	-9.03	-10.17±6.31
M4	-5.49	-6.16±6.72
M5	-8.74	-12.64±6.65
M6	-9.26	-12.41±7.34

The asterisk (*) denotes the following: the experimental binding free energy was estimated using $\Delta G = (RT\ln IC50)/4285.8518$ with T = 300 K.

Table S3 Pearson correlation (Pearson's *r*) for five single FEP calculations under different sampling strategies.

Windows/time	FEP1	FEP2	FEP3	FEP4	FEP5
16/16	0.06	-0.02	0.79	0.33	0.04
16/32	0.18	0.67	0.86	0.07	0.53
16/64	0.59	0.57	0.56	0.73	0.47
32/16	0.52	0.43	0.60	0.40	0.35
32/32	0.86	0.73	0.16	0.81	0.71
32/64	0.91	0.78	0.80	0.78	0.85
64/16	0.70	0.49	0.83	0.78	0.67
64/32	0.73	0.64	0.68	0.76	0.80
64/64	0.55	0.77	0.73	0.69	0.79

Table S4 *p*-value of independent samples t-test on Pearson correlation.

Windows/time	16/16	16/32	16/64	32/16	32/32	32/64	64/16	64/32	64/64
16/16		0.323	0.083	0.198	0.068	**0.017**	**0.023**	**0.031**	**0.017**
16/32	0.323		0.467	0.990	0.353	0.070	0.203	0.155	0.179
16/64	0.083	0.467		0.077	0.613	**0.001**	0.164	**0.026**	0.076
32/16	0.198	0.990	0.077		0.186	**0.000**	**0.013**	**0.001**	**0.004**
32/32	0.068	0.353	0.613	0.186		0.224	0.781	0.614	0.707
32/64	**0.017**	0.070	**0.001**	**0.000**	0.224		0.075	**0.027**	**0.044**
64/16	**0.023**	0.203	0.164	**0.013**	0.781	0.075		0.677	0.872
64/32	**0.031**	0.155	**0.026**	**0.001**	0.614	**0.027**	0.677		0.763
64/64	**0.017**	0.179	0.076	**0.004**	0.707	**0.044**	0.872	0.763	