

Supporting information

Repulsive soft-core potentials for efficient alchemical free energy calculations

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Utility of the GSC potential in the different alchemical FE transformations.

The GSC potential in eq. (10) can be flexibly used to perform the different types of alchemical FE simulation. This can be illustrated using the two-step annihilation protocol presented in **Figure 1b**. In Step 1, the non-bonded interactions of the solute are decoupled from the environment (i.e., bulk water), while the GSC potential is turned on between the solute and solvent molecules. In this process, the system with full interactions (i.e., “0” end state) has no GSC potential, while the other end state (i.e., “1” end state) has only the GSC potential. The simulation is performed by setting $\alpha_0 = 0$ for the “0” end state; i.e., $U_0^{nb-GSC} = U_0^{nb}$ in eq. (10a). For the “1” end state, $\alpha_1 = 5$ kcal/mol. Then, the total non-bonded interaction energy at a given λ value is

$$\begin{aligned}
 U^{nb}(\lambda) &= (1 - \lambda)U_0^{nb-GSC} + \lambda U_1^{nb-GSC} \\
 &= (1 - \lambda)U_0^{nb} + \lambda \left[U_1^{nb} + \sum'_{i,j} \alpha_1 e^{-\beta_1 \left(\frac{r_{ij}}{R_{1,ij}^{min}} \right)^x} \right] \quad (S1)
 \end{aligned}$$

In eq. (S1), U_1^{nb} of the “1” state does not include the electrostatic and van der Waals interactions for the solute-solvent pairs, because the charges and ϵ values of the solute are zero. In Step 2, the GSC potential is turned off between the solute and bulk water molecules. Namely, in the “0” state, the GSC potential is turned on ($\alpha_0 = 5$ kcal/mol) and in the “1” state, $\alpha_1 = 0$, i.e.,

$$U^{nb}(\lambda) = (1 - \lambda) \left[U_0^{nb} + \sum'_{i,j} \alpha_0 e^{-\beta_0 \left(\frac{r_{ij}}{R_{0,ij}^{min}} \right)^x} \right] + \lambda U_1^{nb} \quad (S2)$$

where in both states, the non-bonded interactions (i.e., U_0^{nb} and U_1^{nb}) do not include the solute-solvent electrostatic and van der Waals interactions.

The three-step annihilation (**Figure S1b**) can be performed in the similar manner. The first step is the same as the first step in the two-step annihilation, i.e., $\alpha_0 = 0$ for the

“0” end state and $\alpha_1 = 5$ kcal/mol for the “1” end state. In Step 2, the solute is mutated from the first side chain to the second side chain as presented in **Figure S1b**, in which both side chains have only the GSC potentials (i.e., α_0 and α_1 are 5 kcal/mol) and their charges and ϵ values are zero. Therefore, the total non-bonded interaction energy is

$$U^{nb}(\lambda) = (1 - \lambda)[U_0^{nb} + \sum'_{i,j} \alpha_0 e^{-\beta_0 \left(\frac{r_{ij}}{R_{0,ij}^{min}}\right)^x}] + \lambda[U_1^{nb} + \lambda \sum'_{i,j} \alpha_1 e^{-\beta_1 \left(\frac{r_{ij}}{R_{1,ij}^{min}}\right)^x}] \quad (S3)$$

where U_0^{nb} and U_1^{nb} do not contain the solute-solvent electrostatic and van der Waals interactions. This completes the annihilation of the first side chain. In Step 3, the electrostatic and van der Waals interactions of the second side chain are restored with the removal of the GSC repulsion. The simulation of this step is performed by setting the charges and ϵ values of the solute to zero and $\alpha_0 = 5$ kcal/mol for the “0” state, while full solute charges, ϵ values and $\alpha_1 = 0$ for the “1” end state.

Table S1. Annihilation free energies (in kcal/mol) of small molecule in water. For each molecule, the simulations were carried out by following the CSC and GSC protocols (**Figure 1**). Their free energies were determined by the TI method for the CSC protocol, and the TI, BAR, DW-FEP and IPDW-FEP methods for the GSC protocol, respectively. For the CSC protocol, the annihilation of solute was performed in vacuum to provide the value for the correction of the solute in vacuum.

ID Molecule	ΔF_{water}^{CSC} ^a	ΔF_{water}^{GSC} ^a				ΔF_{vacuum}^{CSC} ^c
	TI	TI	BAR	DW-FEP	IPDW-FEP	TI
Monoatomic ions						
1 K ⁺	71.41 ± 0.13	71.35 ± 0.12	71.38 ± 0.11	71.47 ± 0.14	71.43 ± 0.16	-
2 Cl ⁻	92.15 ± 0.13	91.96 ± 0.12	91.99 ± 0.13	91.98 ± 0.12	92.00 ± 0.14	-
3 Na ⁺	92.02 ± 0.14	92.14 ± 0.13	92.12 ± 0.12	92.10 ± 0.12	92.12 ± 0.15	-
4 Ca ²⁺	367.86 ± 0.25	367.77 ± 0.24	367.90 ± 0.13	367.77 ± 0.27	367.79 ± 0.22	-
Small molecules						
5 Glycine	-49.92 ± 0.11	-50.27 ± 0.09	-49.97 ± 0.06	-49.97 ± 0.06	-49.97 ± 0.07	-59.66
6 Ethanol	10.98 ± 0.09	10.74 ± 0.08	11.01 ± 0.04	11.02 ± 0.04	11.02 ± 0.05	5.29
7 Imidazole	14.64 ± 0.10	14.49 ± 0.08	14.76 ± 0.06	14.78 ± 0.06	14.78 ± 0.07	4.29
8 Imidazolium ⁻	13.31 ± 0.12	13.40 ± 0.11	13.69 ± 0.07	13.72 ± 0.10	13.72 ± 0.10	-38.08
9 Methylamine	-8.98 ± 0.08	-9.11 ± 0.06	-8.92 ± 0.04	-8.93 ± 0.05	-8.94 ± 0.05	-13.08
10 Methylammonium ⁻	41.75 ± 0.13	41.49 ± 0.12	41.66 ± 0.10	41.68 ± 0.11	41.67 ± 0.11	-19.14
11 Acetic acid	76.35 ± 0.10	76.58 ± 0.08	76.84 ± 0.07	76.82 ± 0.09	76.84 ± 0.10	72.54
12 Acetate ion ⁻	145.50 ± 0.14	145.48 ± 0.13	145.76 ± 0.11	145.82 ± 0.12	145.83 ± 0.11	47.44
13 Phospho-tyrosine ²⁻	214.29 ± 0.27	214.52 ± 0.26	214.88 ± 0.24	215.12 ± 0.29	215.07 ± 0.26	5.19
MSD ^b		-0.06	0.13	0.16	0.15	
MUD ^b		0.17	0.18	0.21	0.21	

^a In the two step annihilation, each step was accomplished by 19 λ simulations, i.e., a total of 38 λ simulations to complete the entire annihilation of an solute molecule.

^b The mean signed and mean unsigned deviations (MSD and MUD) relative to the CSC TI results.

^c The annihilation of solute in vacuum was carried out in two steps (**Figure 1a**). In Step 1, the charges of the solute were removed in vacuum, and in Step 2, the van der Waals interactions of the solute were removed in vacuum with the PSSP soft-core potential. The reported free energy values are the sum of the free energies determined in the two steps. For the monoatomic ions, the computed annihilation free energy in vacuum is zero by definition.

Table S2. Annihilation free energies (in kcal/mol) of 13 small molecules in water, determined by the TI method with the CSC and GSC potentials.^a

	ΔF_{TI}^{CSC}			ΔF_{TI}^{GSC}		
	Total	Step 1	2	Total	Step 1	2
Monoatomic ions						
1. K ⁺	71.41 ± 0.13	73.56 ± 0.12	-2.14 ± 0.05	71.35 ± 0.12	75.21 ± 0.12	-3.87 ± 0.03
2. Cl ⁻	92.15 ± 0.13	94.75 ± 0.12	-2.60 ± 0.06	91.96 ± 0.12	97.65 ± 0.12	-5.70 ± 0.04
3. Na ⁺	92.02 ± 0.14	93.67 ± 0.13	-1.66 ± 0.04	92.14 ± 0.13	94.78 ± 0.13	-2.65 ± 0.02
4. Ca ²⁺	367.86 ± 0.25	369.17 ± 0.25	-1.31 ± 0.04	367.77 ± 0.24	370.43 ± 0.24	-2.66 ± 0.02
Small molecules						
5. Glycine	-49.92 ± 0.11	-48.99 ± 0.06	-0.92 ± 0.09	-50.27 ± 0.09	-39.36 ± 0.06	-10.91 ± 0.07
6. Ethanol	10.98 ± 0.09	12.12 ± 0.04	-1.14 ± 0.08	10.74 ± 0.08	20.80 ± 0.04	-10.06 ± 0.06
7. imidazole	14.64 ± 0.10	14.85 ± 0.05	-0.20 ± 0.09	14.49 ± 0.08	24.20 ± 0.05	-9.71 ± 0.07
8. Imidazolium ⁻	13.31 ± 0.12	12.55 ± 0.09	0.77 ± 0.09	13.40 ± 0.11	21.94 ± 0.09	-8.54 ± 0.06
9. Methylamine	-8.98 ± 0.08	-8.12 ± 0.03	-0.87 ± 0.07	-9.11 ± 0.06	-1.99 ± 0.04	-7.13 ± 0.05
10. Methylammonium ⁻	41.75 ± 0.13	42.60 ± 0.10	-0.85 ± 0.07	41.49 ± 0.12	48.80 ± 0.10	-7.31 ± 0.06
11. Acetic acid	76.35 ± 0.10	77.40 ± 0.05	-1.05 ± 0.09	76.58 ± 0.08	86.43 ± 0.05	-9.85 ± 0.07
12. Acetate ion ⁻	145.50 ± 0.14	146.49 ± 0.12	-0.99 ± 0.08	145.48 ± 0.13	155.27 ± 0.12	-9.79 ± 0.06
13. Phospho-tyrosine ²⁻	214.29 ± 0.27	231.83 ± 0.23	-17.53 ± 0.15	214.52 ± 0.26	249.69 ± 0.23	-35.17 ± 0.11

^a Free energies are based on the two-step annihilation process (**Figure 1**): “Total” is for the entire process, “1” for Step 1, and “2” for Step 2 of the two-step annihilation process, respectively. Each annihilation step was accomplished by 19 λ simulations, thus a total of 38 λ simulations for the complete annihilation.

Table S3. Annihilation free energies (in kcal/mol) of 12 small molecules in water, determined by the GSC potentials at X=2 of eq. (10).^a Since that the FE values for Steps 1 and 2 are different for the different X values, only the total FE values are meaningful to compare between different X values. The results for X=4 are the same as **Table S2**.

	$\Delta F_{\text{TI}}^{\text{GSC}}$ at X=2			$\Delta F_{\text{TI}}^{\text{GSC}}$ at X=4 (Table S2)		
	Total	Step 1	2	Total	Step 1	2
Monoatomic ions						
1. K ⁺	71.25	80.80	-9.55	71.35	75.21	-3.87
2. Cl ⁻	91.94	105.65	-13.70	91.96	97.65	-5.70
3. Na ⁺	92.28	99.08	-6.80	92.14	94.78	-2.65
4. Ca ²⁺	367.19	374.05	-6.86	367.77	370.43	-2.66
Small molecules						
5. Glycine	-50.35	-23.57	-26.77	-50.27	-39.36	-10.91
6. Ethanol	10.43	37.56	-27.14	10.74	20.80	-10.06
7. imidazole	14.28	39.56	-25.28	14.49	24.20	-9.71
8. Imidazolium ⁻	12.90	34.29	-21.40	13.40	21.94	-8.54
9. Methylamine	-9.31	8.01	-17.32	-9.11	-1.99	-7.13
10. Methylammonium ⁻	41.29	58.68	-17.40	41.49	48.80	-7.31
11. Acetic acid	76.10	101.02	-24.92	76.58	86.43	-9.85
12. Acetate ion ⁻	145.19	169.91	-24.73	145.48	155.27	-9.79

^a Free energies are based on the two-step annihilation process (**Figure 1**): “Total” is for the entire process, “1” for Step 1, and “2” for Step 2 of the two-step annihilation process, respectively. Each annihilation step was accomplished by $N=19$ λ simulations, thus a total of 38 λ simulations for the complete transformation.

Table S4. Accuracy of the BAR method for the different numbers of λ simulations for the Steps 1 and 2 of the GSC protocol in water (**Figure 1b**).^a The energy is in the unit of kcal/mol.

	$\Delta F_{\text{BAR}}^{\text{GSC}}$ (Step 1)					$\Delta F_{\text{BAR}}^{\text{GSC}}$ (Step 2)				
	$N=2$ ^a	3	6	11	19	$N=2$	3	6	11	19
1. K ⁺	-	75.73	75.10	75.14	75.23	-3.81	-3.85	-3.86	-3.87	-3.85
2. Cl ⁻	-	98.90	97.69	97.69	97.65	-5.75	-5.70	-5.75	-5.71	-5.66
3. Na ⁺	-	24.64	94.78	94.78	94.74	-2.68	-2.64	-2.63	-2.64	-2.63
4. Ca ²⁺	-	-	370.59	370.49	370.55	-2.69	-2.70	-2.68	-2.66	-2.65
5. Glycine	-38.92	-39.40	-39.32	-39.35	-39.37	-11.81	-10.70	-10.68	-10.67	-10.61
6. Ethanol	20.88	20.81	20.79	20.79	20.79	-10.83	-9.77	-9.77	-9.78	-9.78
7. imidazole	23.89	24.15	24.17	24.19	24.19	-10.18	-9.63	-9.68	-9.51	-9.43
8. Imidazolium ⁻	-	21.74	21.81	21.80	21.96	-9.43	-8.48	-8.23	-8.27	-8.28
9. Methylamine	-1.94	-2.00	-1.95	-1.98	-1.99	-7.03	-6.93	-7.00	-7.04	-6.93
10. Methylammonium ⁻	-	48.80	48.86	48.87	48.79	-7.37	-7.24	-7.15	-7.13	-7.13
11. Acetic acid	86.17	86.05	86.55	86.47	86.42	-11.74	-10.12	-9.60	-9.62	-9.58
12. Acetate ion ⁻	-	155.28	155.38	155.29	155.30	-9.43	-9.47	-9.63	-9.55	-9.54
13. Phospho-tyrosine ²⁻	-	-	250.41	249.70	249.51	-	-10.50	-34.82	-34.51	-34.64
MSD ^b	-	-	0.08	0.01		-	1.77	-0.06	-0.02	
MUD ^b	-	-	0.13	0.06		-	1.96	0.07	0.04	

^a The λ values in the the $N=2$, 3, 6 and 11-point λ simulation results are: for $N=2$, $\lambda=0$ and 1; for $N=3$, $\lambda=0$, 0.5 and 1; for $N=6$, $\lambda=0$, 0.2, 0.4, 0.6, 0.8 and 1; and for $N=11$, $\lambda=0$, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1, respectively.

^b MSD and MUD are relative to the 19-point simulation results for each step.

Table S5. Free energies of removing vdW terms using GSC in water. Before the alchemical simulation, the charges of each solute were removed. In each system, the λ values in the $N=2, 3, 6$ and 11 -point λ simulation results are: for $N=1, \lambda=0.95$; for $N=2, \lambda=0$ and 1 ; for $N=3, \lambda=0, 0.5$ and 1 ; for $N=6, \lambda=0, 0.2, 0.4, 0.6, 0.8$ and 1 ; and for $N=11, \lambda=0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9$ and 1 , respectively. The free energy based on the 19 - λ simulations are used as the reference for each system. For the GSC protocol, DW-FEP and TI were used to calculate each free energy value. For Step 2, the FE values are provided in **Tables 4** and **5**. The reference values of the total FE values (i.e., $N=38$ values) are from Step 2 of the CSC protocol (i.e., $N=19$ results in **Table 3**). All energies are in the unit of kcal/mol.

	$\Delta F_{\text{DW-FEP}}^{\text{vdW} \rightarrow \text{GSC}}$ (Step 1 of Figure 1c)					$\Delta F_{\text{TI}}^{\text{vdW} \rightarrow \text{GSC}}$ (Step 1 of Figure 1c)					$\Delta F_{\text{DW-FEP}}^{\text{vdW} \rightarrow \text{Null}}$ ^a	$\Delta F_{\text{TI}}^{\text{vdW} \rightarrow \text{Null}}$ ^a	
	$N=1$ ^b	2	3	6	11	19	$N=2$	3	6	11	19	38	38
1. K ⁺	1.73	1.71	1.72	1.71	1.72	1.71	0.52	1.17	1.52	1.63	1.71	-2.14 ± 0.03 ^c	-2.15 ± 0.03
2. Cl ⁻	3.13	3.12	3.12	3.11	3.12	3.13	1.86	2.56	2.92	3.04	3.11	-2.52 ± 0.03	-2.61 ± 0.05
3. Na ⁺	1.02	1.02	1.03	1.03	1.03	1.01	-0.57	0.25	0.73	0.89	1.01	-1.62 ± 0.02	-1.64 ± 0.02
4. Ca ²⁺	1.34	1.35	1.35	1.34	1.35	1.35	-0.29	0.57	1.06	1.21	1.33	-1.30 ± 0.02	-1.32 ± 0.02
5. Glycine	9.63	9.68	9.70	9.69	9.70	9.74	8.90	9.38	9.60	9.67	9.69	-0.86 ± 0.05	-1.17 ± 0.07
6. Ethanol	8.67	8.66	8.67	8.70	8.69	8.71	8.55	8.62	8.69	8.69	8.70	-1.07 ± 0.05	-1.35 ± 0.07
7. imidazole	9.40	9.35	9.36	9.33	9.35	9.36	9.26	9.32	9.32	9.35	9.36	-0.04 ± 0.07	-0.35 ± 0.07
8. Imidazolium ⁻	9.23	9.15	9.22	9.24	9.24	9.26	9.08	9.19	9.23	9.24	9.25	1.01 ± 0.04	0.72 ± 0.06
9. Methylamine	6.09	6.13	6.12	6.12	6.13	6.14	5.92	6.04	6.10	6.12	6.13	-0.80 ± 0.04	-0.99 ± 0.06
10. Methylammonium ⁻	6.27	6.28	6.26	6.24	6.24	6.23	6.12	6.20	6.22	6.23	6.23	-0.88 ± 0.03	-1.08 ± 0.06
11. Acetic acid	8.66	8.66	8.65	8.62	8.62	8.64	8.45	8.56	8.59	8.61	8.62	-0.96 ± 0.07	-1.21 ± 0.07
12. Acetate ion ⁻	8.57	8.53	8.57	8.52	8.53	8.54	8.35	8.49	8.49	8.52	8.54	-0.99 ± 0.08	-1.25 ± 0.06
13. Phospho-tyrosine ²⁻	16.58	17.03	17.16	17.20	17.19	17.25	15.09	16.40	16.99	17.11	17.17	-17.42 ± 0.07	-17.95 ± 0.12
MSD	-0.06	-0.03	-0.01	-0.02	-0.01		-0.74	-0.32	-0.11	-0.04		0.07	-0.14
MUD	0.08	0.04	0.02	0.02	0.02		0.74	0.32	0.11	0.04		0.07	0.15

^a Free energies of the removal of the solute vdW terms by the GSC protocol (Step 1 and Step 2 in **Figure 1c**), in which each step was achieved with 19λ simulations. Thus, a total of 38λ simulations for the entire transformation. MSD and MUD were relative to the $N=19$ TI values of Step 2 of the CSC protocol (**Table 3**).

^b For the $N=1$ case, the free energy was calculated by original FEP, i.e., $\lambda=0.95$ to $\lambda=0$ and also to $\lambda=1$.

^c The error value was estimated by the bootstrapping method.

Table S6. Annihilation free energies (FEs) of the GSC protocol determined using the IPDW-FEP method in water. Step 1 of the annihilation is determined with 3 λ simulations at $\lambda=0.1, 0.5$ and 0.9 , and Step 2 with 2 λ simulations at $\lambda=0.5$ and 0.95 , respectively. For Step 2, the FE values determined with 1 λ simulation at $\lambda=0.95$ are also provided. The energy is in the unit of kcal/mol.

	$\Delta F_{\text{IPDW-FEP}}^{\text{GSC}}$ (Total)		$\Delta F_{\text{IPDW-FEP}}^{\text{GSC}}$ (Step 1)		$\Delta F_{\text{IPDW-FEP}}^{\text{GSC}}$ (Step 2, 2 points) ^a		$\Delta F_{\text{IPDW-FEP}}^{\text{GSC}}$ (Step 2, 1 point) ^b	
	FE	Error ^c	FE	Error ^c	FE	Error ^c	FE	Error ^c
1 K ⁺	71.57	0.14	75.44	0.16	-3.87	-0.02	-3.98	-0.13
2 Cl ⁻	92.32	0.32	98.02	0.37	-5.70	-0.04	-5.96	-0.30
3 Na ⁺	91.60	-0.52	94.28	-0.46	-2.67	-0.05	-2.70	-0.08
4 Ca ²⁺	364.87	-2.92	367.56	-2.88	-2.69	-0.04	-2.69	-0.04
5 Glycine	-50.15	-0.18	-39.47	0.10	-10.68	-0.08	-10.81	-0.21
6 Ethanol	10.81	-0.21	20.80	0.00	-9.99	-0.21	-10.13	-0.36
7 imidazole	14.97	0.19	24.19	0.00	-9.22	0.19	-9.20	0.21
8 Imidazolium ⁻	13.50	-0.22	21.78	-0.19	-8.28	-0.04	-7.99	0.25
9 Methylamine	-9.12	-0.18	-1.99	0.01	-7.12	-0.18	-7.29	-0.35
10 Methylammonium ⁻	41.51	-0.16	48.57	-0.21	-7.06	0.05	-7.12	-0.01
11 Acetic acid	76.27	-0.57	85.71	-0.73	-9.44	0.15	-9.80	-0.20
12 Acetate ion ⁻	145.81	-0.02	155.39	0.04	-9.59	-0.06	-8.84	0.69
13 Phospho-tyrosine ²⁻	212.82	-2.25	247.34	-2.36	-34.52	0.12	-38.36	-3.72
MSD		-0.51		-0.49		-0.02		-0.33
MUD		0.61		0.58		0.10		0.50

^a The annihilation free energy calculated by the IPDW-FEP method for Step 2 using 2 λ simulations at $\lambda=0.5$ and 0.95 .

^b The annihilation free energy calculated by the FEP method for Step 2 using the simulation at $\lambda=0.95$.

^c For the Total FE change, the errors are relative to the 19-point IPDW-FEP values presented in **Table S1**. For Steps 1 and 2, the reference values are provided in **Table S7**.

Table S7. Accuracy of the IPDW-FEP method for the different numbers of λ simulations for Steps 1 and 2 of the GSC protocol in water (**Figure 1b**).^a All free energies are in the unit of kcal/mol.

	$\Delta F_{\text{IPDW-FEP}}^{\text{GSC}}$ (Step 1)					$\Delta F_{\text{IPDW-FEP}}^{\text{GSC}}$ (Step 2)				
	<i>N</i> =2	3	6	11	19	<i>N</i> =2	3	6	11	19
1. K ⁺	75.04	75.70	75.09	75.14	75.28	-3.87	-3.85	-3.85	-3.86	-3.85
2. Cl ⁻	102.55	99.09	97.80	97.69	97.65	-5.98	-5.76	-5.77	-5.72	-5.66
3. Na ⁺	102.59	95.42	94.78	94.77	94.74	-2.73	-2.68	-2.63	-2.65	-2.62
4. Ca ²⁺	365.68	358.43	370.40	370.63	370.44	-2.72	-2.71	-2.68	-2.67	-2.65
5. Glycine	-39.22	-39.39	-39.33	-39.34	-39.37	-11.76	-10.92	-10.83	-10.80	-10.60
6. Ethanol	20.94	20.84	20.79	20.79	20.80	-11.83	-10.13	-9.90	-9.71	-9.78
7. imidazole	23.68	24.16	24.17	24.19	24.19	-11.01	-10.18	-9.97	-9.48	-9.41
8. Imidazolium ⁺	26.05	22.25	21.80	21.79	21.97	-11.16	-8.99	-8.33	-8.28	-8.24
9. Methylamine	-1.92	-1.99	-1.96	-1.98	-2.00	-7.03	-7.05	-7.00	-7.05	-6.94
10. Methylammonium ⁺	50.34	48.59	48.88	48.88	48.78	-7.88	-7.58	-7.38	-7.16	-7.11
11. Acetic acid	86.20	86.31	86.65	86.80	86.44	-12.12	-10.50	-9.68	-9.68	-9.59
12. Acetate ion ⁻	156.66	155.43	155.25	155.29	155.35	-10.37	-9.61	-9.60	-9.57	-9.53
13. Phospho-tyrosine ²⁻	227.05	243.73	249.57	249.81	249.70	-37.85	-36.51	-35.60	-34.66	-34.64
MSD ^b	-0.64	-1.19	-0.01	0.04		-1.21	-0.45	-0.20	-0.05	
MUD ^b	3.73	1.64	0.10	0.10		1.21	0.45	0.20	0.06	

^a The λ values in the *N*=2, 3, 6, 11 and 19-point λ simulation results are: for *N*=2, $\lambda=0$ and 1; for *N*=3, $\lambda=0$, 0.5 and 1; for *N*=6, $\lambda=0$, 0.2, 0.4, 0.6, 0.8 and 1; and for *N*=11, $\lambda=0$, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1, respectively. The common λ point from the forward and backward perturbations in DW-FEP was determined based on the predicted integrand difference between two neighboring λ simulations.

^b MSD and MUD are relative to the results from the 19-point simulation.

Table S8. Annihilation free energies (FEs) of the GSC protocol determined using the IPDW-FEP method in water. Step 1 of the annihilation is determined with 5 λ simulations at $\lambda=0.1, 0.3, 0.5, 0.7$ and 0.9 , and Step 2 with 2 λ simulations at $\lambda=0.5$ and 0.95 , respectively. The energy is in the unit of kcal/mol.

	$\Delta F_{\text{IPDW-FEP}}^{\text{GSC}} \text{ (Total)}$		$\Delta F_{\text{IPDW-FEP}}^{\text{GSC}} \text{ (Step 1)}$		$\Delta F_{\text{IPDW-FEP}}^{\text{GSC}} \text{ (Step 2)}$	
	FE	Error ^a	FE	Error ^a	FE	Error ^a
1. K ⁺	71.41	-0.02	75.28	0.00	-3.87	-0.02
2. Cl ⁻	91.91	-0.09	97.62	-0.03	-5.70	-0.04
3. Na ⁺	92.17	0.05	94.84	0.10	-2.67	-0.05
4. Ca ²⁺	367.79	0.00	370.49	0.05	-2.69	-0.04
5. Glycine	-50.03	-0.06	-39.35	0.02	-10.68	-0.08
6. Ethanol	10.77	-0.25	20.76	-0.04	-9.99	-0.21
7. imidazole	14.99	0.21	24.21	0.02	-9.22	0.19
8. Imidazolium ⁻	13.50	-0.22	21.78	-0.19	-8.28	-0.04
9. Methylamine	-9.12	-0.18	-1.99	0.01	-7.12	-0.18
10. Methylammonium ⁻	41.89	0.22	48.96	0.18	-7.06	0.05
11. Acetic acid	77.47	0.63	86.91	0.47	-9.44	0.15
12. Acetate ion ⁻	145.62	-0.21	155.21	-0.14	-9.59	-0.06
13. Phospho-tyrosine ²⁻	213.87	-1.20	248.39	-1.31	-34.52	0.12
MSD		-0.09		-0.07		-0.02
MUD		0.26		0.20		0.10

^a For the Total FE change, the errors are relative to the 19-point IPDW-FEP values presented in **Table S1**. For Steps 1 and 2, the reference values are provided in **Table S7**.

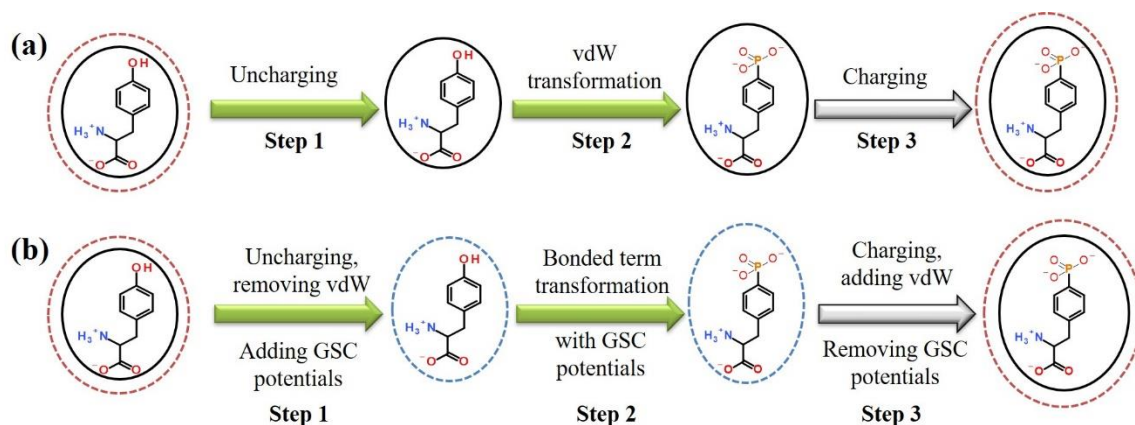


Figure S1. Three-step alchemical transformation of protein side chain. (a) In the CSC protocol, the first step (Step 1) removes the charge of the solute, the second step (Step 2) transforms the vdW parameters of the solute to those of the transformed solute with CSC potential and the third step (Step 3) introduces the charge of the transformed solute, respectively. (b) In the GSC protocol, the first step (Step 1) removes the charge and the vdW interactions of the solute with the introduction of the solute GSC potential, the second step (Step 2) transforms the solute GSC potential to the transformed solute's GSC potential and the third step (Step 3) introduces the charge and the vdW interactions of the transformed solute with the removal of the GSC potential, respectively.

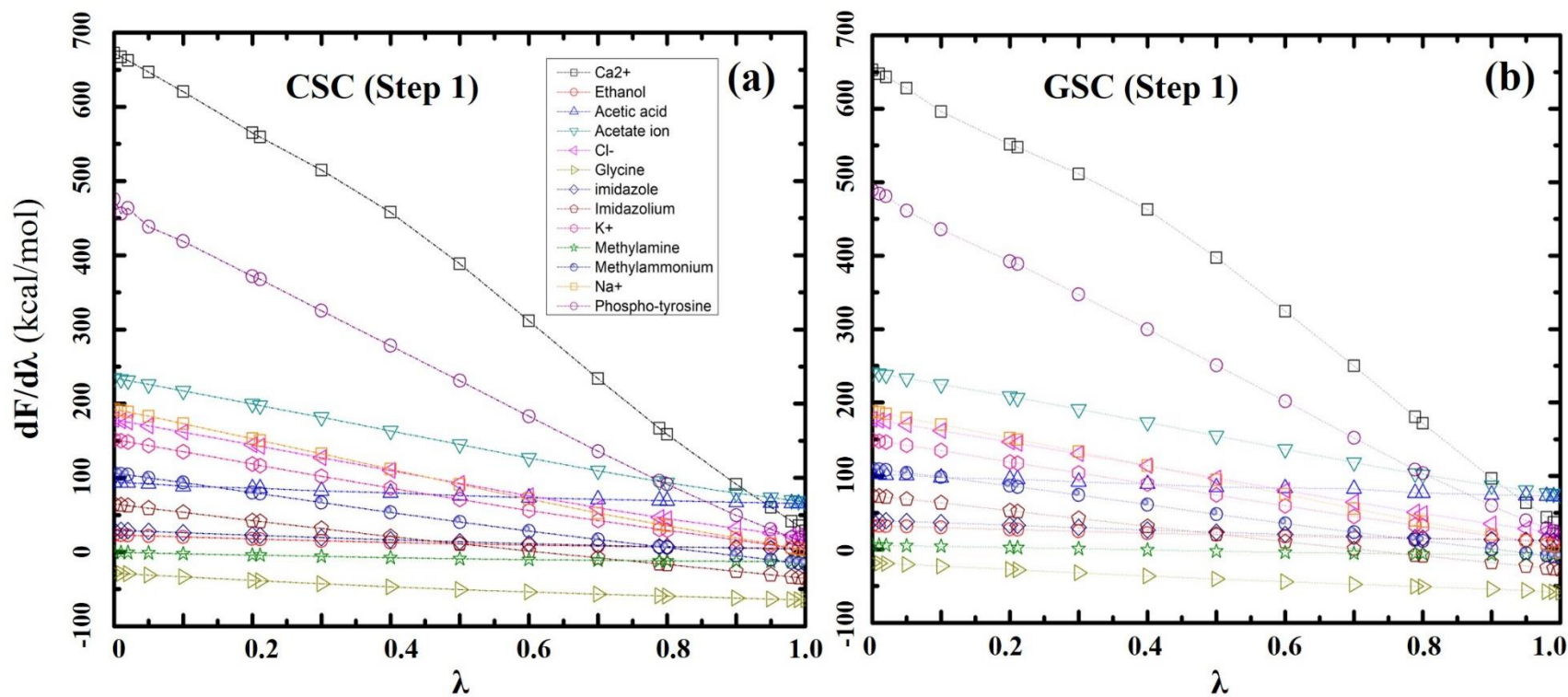
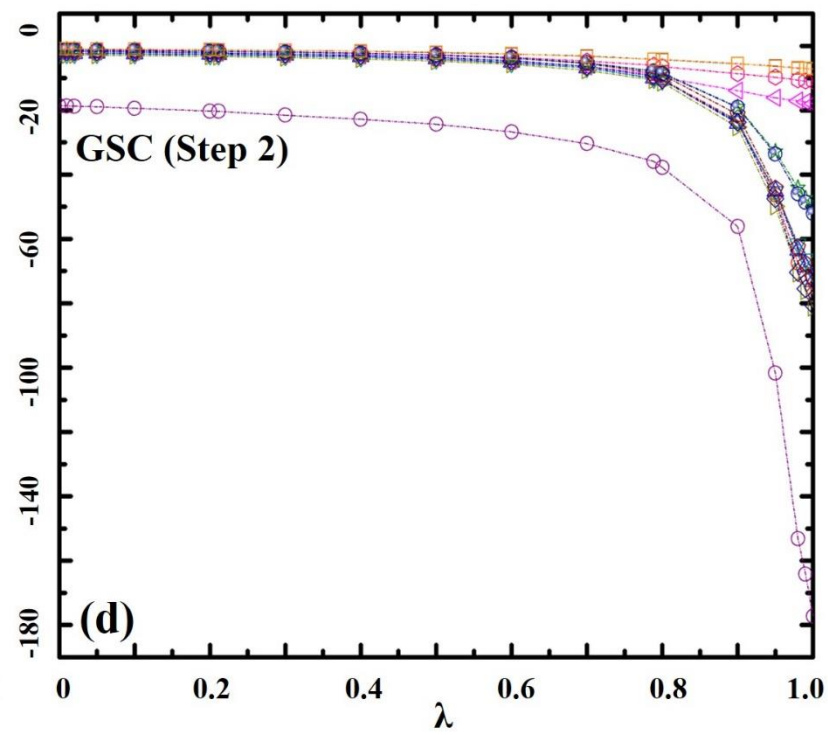
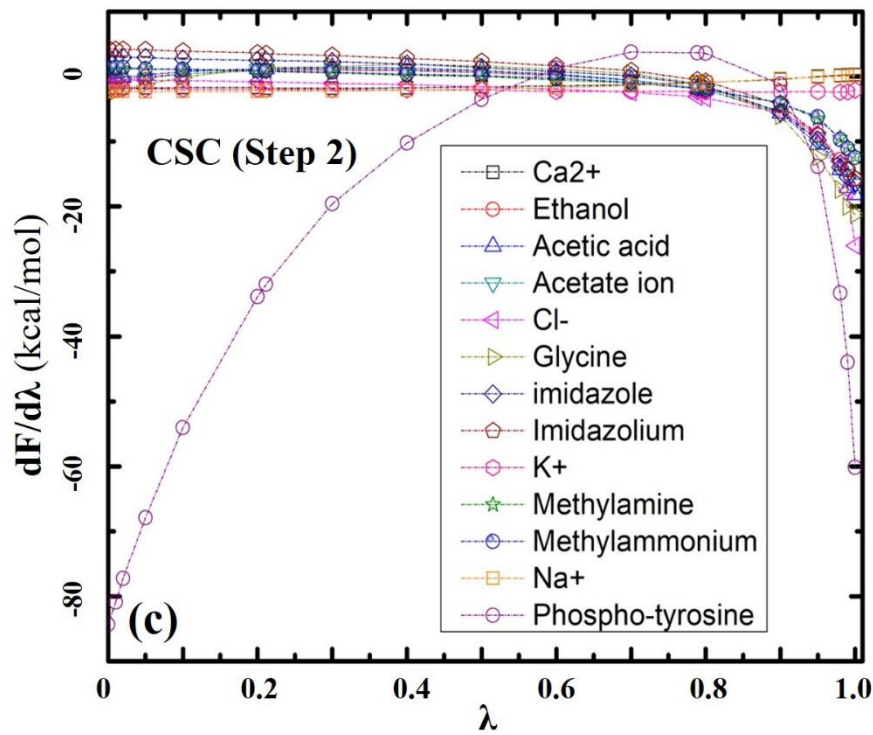
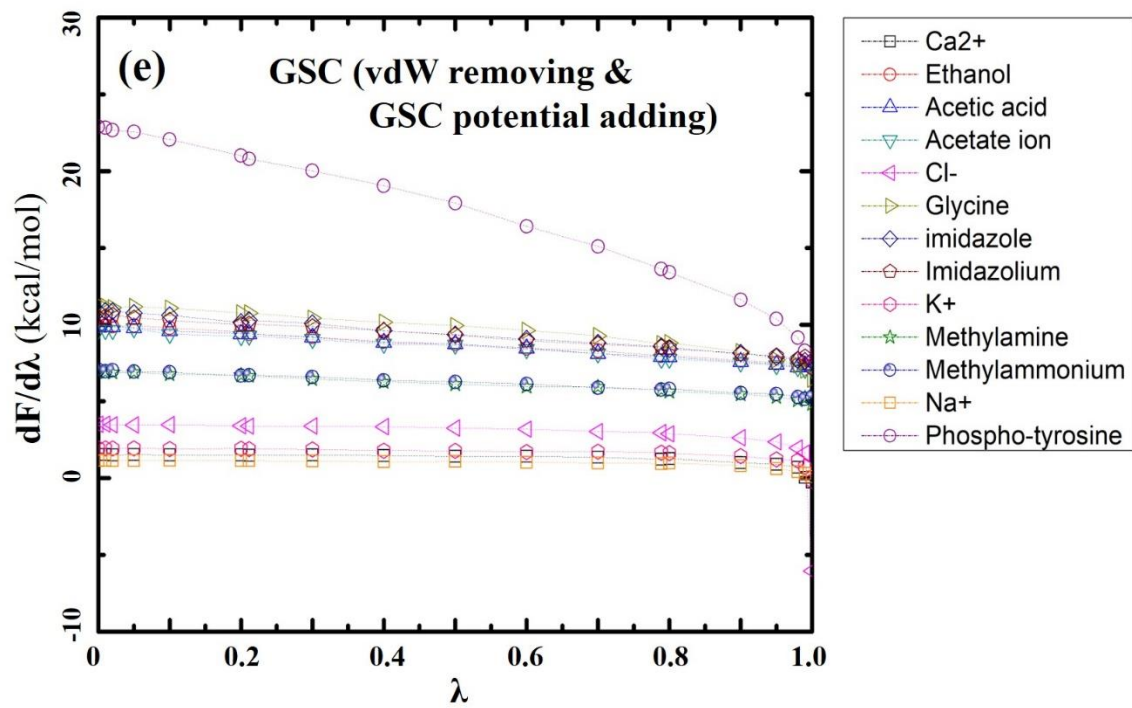


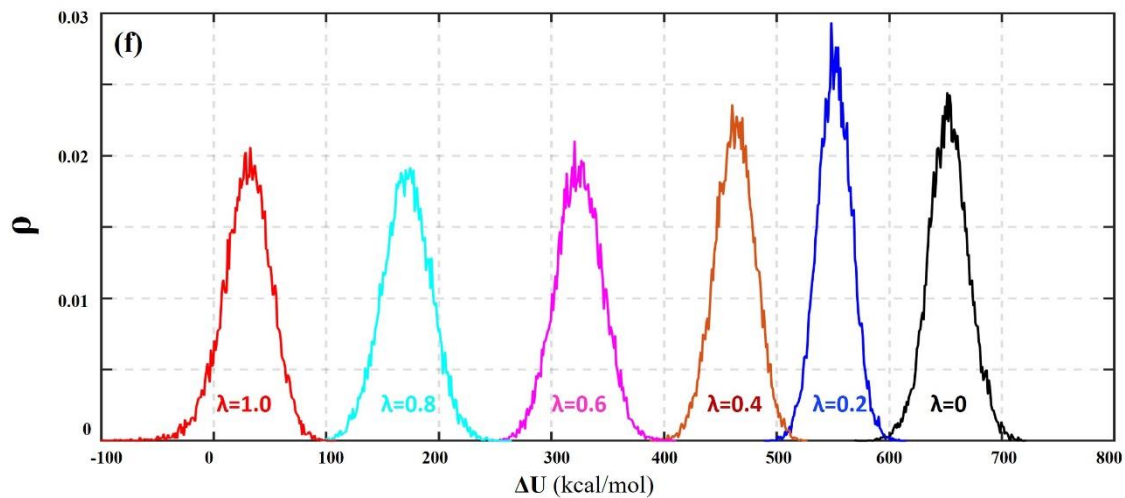
Figure S2. TI integrands of the 13 molecular systems: (a) and (c) are for the Steps 1 and 2 of solute annihilation with CSC (**Figure 1a**), respectively; (b) and (d) for the Steps 1 and 2 of the transformation with GSC, respectively (**Figure 1b**); (e) for the step of removing vdW terms and turning on the GSC potentials (i.e., Step 1 of **Figure 1c**). (f) The probability distributions of $\rho(\Delta U)$ for the first-step transformation of Ca²⁺ with the GSC potential.



Continued **Figure S2.**



Continued **Figure S2**.



Continued **Figure S2**.

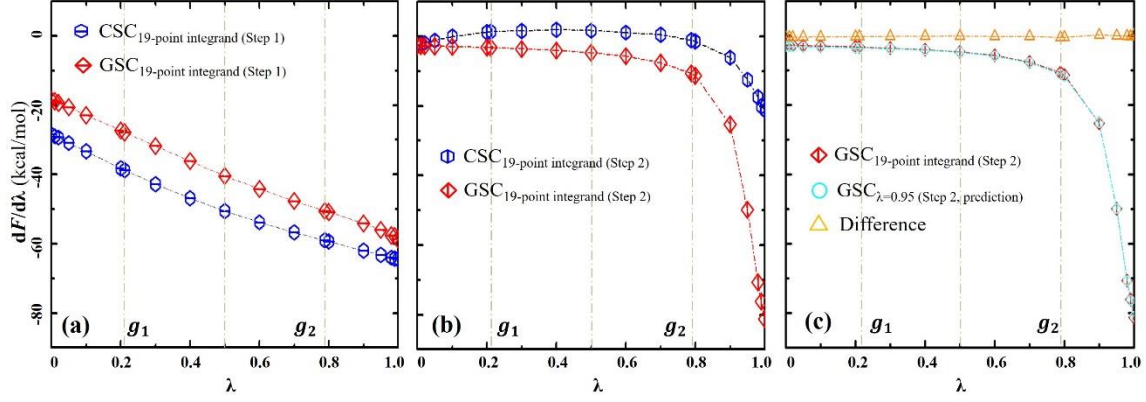


Figure S3. TI integrands for annihilation of glycine in water: (a) for Step 1 and (b) for Step 2 of the two-step annihilation (**Figure 1**). The blue and red symbols denote the transformation performed with the CSC and GSC potentials, respectively. Each transformation step is completed with 19-point λ simulations. (c) The predicted TI integrands based on the sampled configurations at $\lambda=0.95$ in Step 2 of glycine annihilation: the red symbol represents the TI integrands computed directly from each λ simulation and the cyan the predicted TI integrands based on eq. (12), respectively. The difference between the two TI integrand values, i.e., $\Delta = \langle \Delta U_{GSC}(\lambda) \rangle_{\lambda=0.95}^{predicted} - \langle \Delta U_{GSC} \rangle_{\lambda}^{direct}$, is shown in orange.

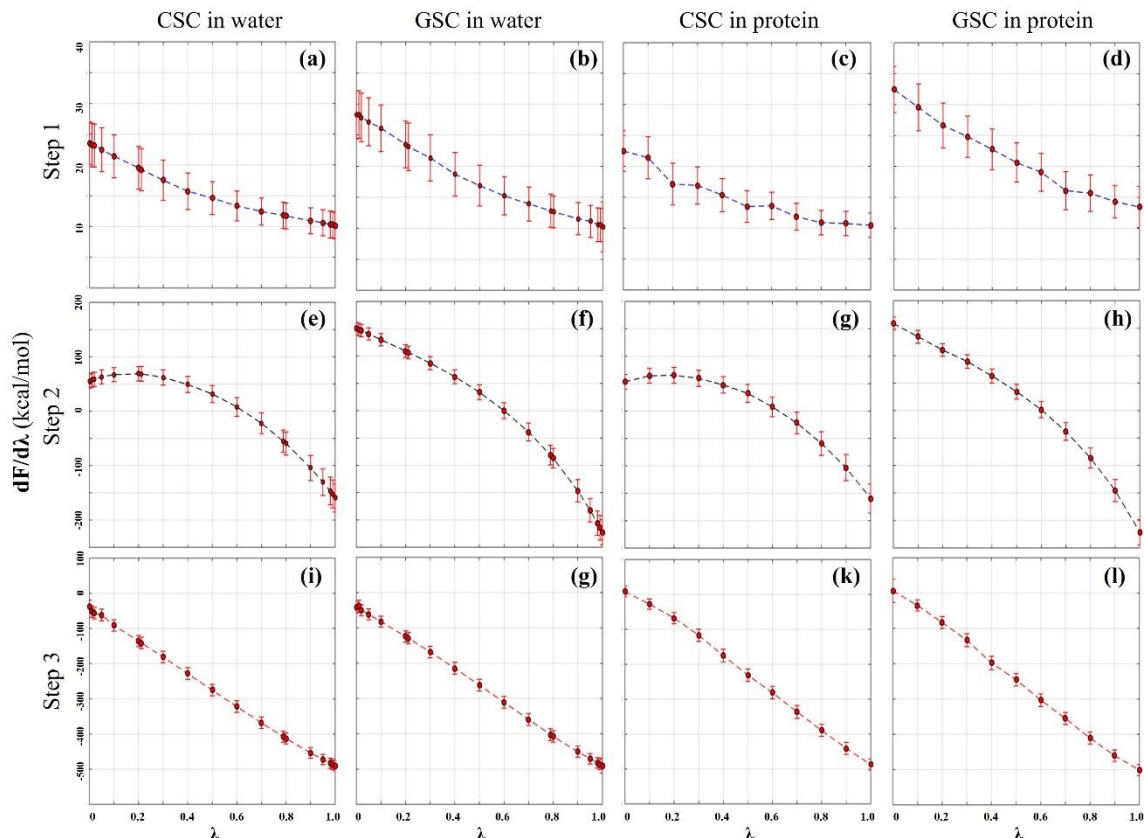


Figure S4. TI integrands for the Tyr-to-pTyr mutation in water and in IGF-1RK: (a), (e) and (i) are for Steps 1, 2 and 3 of the three-step alchemical mutation in water with CSC (**Figure S1a**); (b), (f) and (g) for the corresponding transformations in water with GSC (**Figure S1b**); (c), (g) and (k) for the mutation in protein with CSC; (d), (h) and (l) for the mutation in protein with GSC, respectively. The bar on each TI integrand represents the standard deviation of the $\partial U / \partial \lambda$ values.