

Supporting Information for the article entitled "Macroscopic  $pK_a$  calculations for fluorescein and its derivatives " by Marcin Król, Marta Wrona Christopher S. Page and Paul A. Bates

	Neutral	Monoanion	Dianion	$\Delta(\text{M-N})$	$\Delta(\text{D-M})$
UAKS_SP	-22.68	-68.39	-157.82	-45.71	-89.43
UAKS_OPT	-22.59	-68.40	-157.82	-45.81	-89.42
Pauling_SP	-30.49	-80.77	-173.27	-50.28	-92.50
Pauling_OPT	-32.42	-83.62	-176.06	-51.20	-92.44

S1. Comparison of solvation free energies [kcal/mol] calculated with UAKS and Pauling radii sets for geometries optimized with these sets (UAKS\_OPT and Pauling\_OPT) and for UAHF geometries (UAKS\_SP and Pauling\_SP). The last two columns show solvation free energy differences between the monoanion and neutral ( $\Delta(\text{M-N})$ ) and the di- and monoanion ( $\Delta(\text{D-M})$ ) forms, as used for  $\text{p}K_{\text{a}}$  calculations.

Fluorescein	Neutral	Monoanion	Dianion
V1	-717814.74	-717490.10	-717113.51
V2	-718105.18	-717780.06	-717404.24
V3	-718061.36	-717736.60	-717361.42
V4	-718047.73	-717721.20	-717345.93
S1	-20.67	-66.21	-156.08
S2	-21.19	-66.21	-156.08
S3	-29.01	-78.58	-171.52

S2. Solvation and vacuum free energies [kcal/mol] calculated with the methods **V1-V4** and **S1-S3** for fluorescein molecule. Solvation energies include relaxation energy.

DCF	Neutral	Monoanion	Dianion
V1	-1294066.633	-1293744.986	-1293373.862
V4	-1294700.052	-1294375.446	-1294007.371
S1	-13.57	-56.06	-145.18
S2	-14.08	-56.06	-145.23
S3	-28.00	-74.16	-164.08

S3. Solvation and vacuum free energies [kcal/mol] calculated with the methods **V1**, **V4** and **S1-S3** for DCF molecule. Solvation energies include relaxation energy.

DFF	Neutral	Monoanion	Dianion
V1	-842274.31	-841952.21	-841579.35
V2	-842596.64	-842274.07	-841901.76
V3	-842544.59	-842222.47	-841850.75
V4	-842531.64	-842205.92	-841835.68
S1	-17.33	-60.50	-150.91
S2	-17.87	-60.50	-150.91
S3	-30.96	-78.04	-170.03

S4. Solvation and vacuum free energies [kcal/mol] calculated with the methods **V1-V4** and **S1-S3** for DFF molecule. Solvation energies include relaxation energy.

	Neutral	Monoanion	Dianion	$\Delta(M-N)$	$\Delta(D-M)$	CPU time [s]
CBS- QB3	-598962.26	-598628.12	-598219.45	334.14	408.67	147249
V1	-599002.61	-598668.24	-598259.33	334.37	408.91	9312
V2	-599324.55	-598988.20	-598578.62	336.35	409.58	24923
V3	-599300.27	-598964.50	-598555.35	335.77	409.16	6247

S5. Comparison of vacuum free energies [kcal/mol] for the neutral, mono- and dianionic forms of 3-chloro-4-hydroxyphenol calculated with CBS-QB3 and the vacuum methods used in this paper. Free energy differences between the monoanionic and neutral forms, and between the di- and monoanions are also shown. The last column shows CPU execution time in seconds on a single Intel Itanium 2 1.5Ghz processor.

FLUORESCEIN		Neutral	Monoanion	Dianion
HF/6-31+G(d)	Vacuum	1.48	2.18	1.75
	UAHF	-1.30	-1.90	-1.72
	UAKS	-1.30	-1.90	-1.72
	Pauling	-1.36	-3.10	-3.13

S6.  $\Delta G_{\text{relax}}$  [kcal/mol] calculated for a fluorescein molecule in vacuum or in solvent using three different radii sets.

DCF		Neutral	Monoanion	Dianion
HF/6-31+G(d)	Vacuum	1.34	2.32	1.58
	UAHF	-1.15	-1.81	-1.58
	UAKS	-1.18	-1.81	-1.58
	Pauling	-1.23	-2.86	-2.81

S7.  $\Delta G_{\text{relax}}$  [kcal/mol] calculated for a DCF molecule in vacuum or in solvent using three different radii sets.

DFF		Neutral	Monoanion	Dianion
HF/6-31+G(d)	Vacuum	1.50	2.39	1.78
	UAHF	-1.25	-2.06	-1.74
	UAKS	-1.31	-2.06	-1.74
	Pauling	-1.36	-3.07	-3.04

S8.  $\Delta G_{\text{relax}}$  [kcal/mol] calculated for a DFF molecule in vacuum or in solvent using three different radii sets.

	fluorescein		DCF		DFF	
	UAHF	Pauling	UAHF	Pauling	UAHF	Pauling
Zwitterion	-718070.89	-718084.04	-1294711.01	-1294732.30	-842545.84	-842565.78
Quinoid	-718069.88	-718078.22	-1294715.96	-1294729.40	-842550.47	-842564.10
Lactone	-718075.13	-718079.26	-1294719.58	-1294730.84	-842553.37	-842562.90

S9. Total free energies [kcal/mol] of three tautomers of fluorescein and its derivatives calculated with UAHF and Pauling radii.