Supporting Information: Detailed explanation considering the obtaining of the values of the difference in interaction energies of 1) mono- and dianionic arsenate in WT ArsC and 2) dianionic arsenate in WT and Arg16Ala ArsC.

1) The interaction energies of mono- and dianionic arsenate in WT ArsC are given by the energetic difference between the energy of the optimized complex and the optimized, isolated arsenate and non-complexed enzyme. These two interaction energies contain the same, unknown energy of the non-complexed enzyme, which cancels upon comparison.

$$\Delta\Delta E = \Delta E_m - \Delta E_d \quad (1)$$

with ΔE_m and ΔE_d the binding energy of mono- and dianionic arsenate in WT ArsC, which can be written as

$$\Delta E_m = E_{complex} - E_{monoanion} - E_{enzyme} \quad (2a)$$

$$\Delta E_d = E_{complex`} - E_{dianion} - E_{enzyme} \quad (2b)$$

with $E_{complex}$ and $E_{complex`}$ the energy of the mono- and dianionic enzyme-substrate complex, $E_{monoanion}$ and $E_{dianion}$ the energy of the free mono- and dianion in gas phase and E_{enzyme} the energy of the non-complexed enzyme.

Since E_{enzyme} is equal in Eq. (2a) and Eq. (2b), Eq. (1) can be written as:

$$\Delta\Delta E = E_{complex} - E_{monoanion} - (E_{complex`} - E_{dianion}) \quad (3)$$

Table 1: Numerical values calculated at the B3LYP/6-31+G** level. The values are given in a. u.

	$E_{complex}$	$E_{mono-/dianion}$	$E_{complex} - E_{mono-/dianion}$
Monoanion	-5568.468	-2536.006	-3032.462
Dianion	-5567.870	-2535.278	-3032.592

$$\Delta\Delta E = E_{complex} - E_{monoanion} - (E_{complex`} - E_{dianion}) = 0.130 \text{ a. u.} = 81 \text{ kcal/mol}$$

2) When comparing energetics between WT and Arg16Ala ArsC, ΔE_{enzyme} does not cancel out. However, interaction energies are calculated using the extracted geometry of the uncomplexed enzyme from the complexed one.

$$\Delta E_d = E_{complex} - E_{dianion} - E_{enzyme} \quad (4)$$

with $E_{complex}$ the energy of the dianionic enzyme-substrate complex, $E_{dianion}$ the energy of the free dianion in gas phase and E_{enzyme} the energy of the non-complexed enzyme with the geometry extracted from the optimized enzyme-substrate complex.

Table 2: Numerical values calculated at the B3LYP/6-31+G** level. The values are given in a. u.

	$E_{complex}$	$E_{substrate}$	E_{enzyme}	ΔE_d
WT	-5567.870	-2535.272	-3032.207	-0.391
R16Ala	-5284.400	-2535.265	-2748.940	-0.195

$\Delta\Delta E = \Delta E_{d(WT)} - \Delta E_{d(Arg16Ala)} = -0.196$ a. u. = -123 kcal/mol