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

Supporting Information for: Extending the essential dynamics analysis to investigate molecular properties: application to the redox potential of proteins

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1 Mut1 and Mut2

In Figure 1 the mean value along the MD trajectory of the single-residue electrostatic potential $\langle V_i \rangle$, reported in the main text for WT Az, is reported for Mut1 and Mut2 (in panels A and B, respectively). The comparison of Figure 1 with Figure 3 of the main text highlights that the residues that most contribute in determining the mean transition energy, highlighted in the Figure with coloured filled circles, do not vary upon mutation.

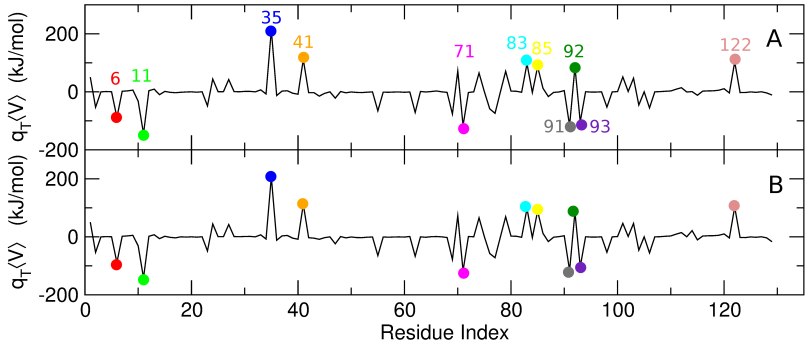

Fig. 1 Mean value along the trajectory of the single-residue electrostatic potential ($q_T\langle V_i \rangle$) obtained by averaging the results in the reduced and oxidised ensemble of Mut (panel A) and Mut2 (panel B). The residues showing an absolute contribution $\geq$ 85 kJ/mol (roughly corresponding to residues whose contribution exceeds a standard deviation from the average) are highlighted with coloured filled circles. The contribution of the solvent, treated as a further virtual residue, corresponds to the last plotted point.

The results of the weighting procedure reported in Figure 4 of the main text for WT Az are reported in Figures 2 and 3 for Mut1 and Mut2, respectively. In panel A the first eight eigenvalues are reported as provided by the diagonalization of the covariance matrix and in panel B the first eight weighted eigenvalues (see Eq. 11 in the main text) are shown. Note that in panel B for each weighted eigenvalue we show the corresponding eigenvector index (before the weighting) to highlight the effect of the procedure in exchanging the order of the modes.

The analysis of the single-residue electrostatic potential fluctuation modes that maximise the fluctuations of V, discussed in the main text for WT Az, is here reported for both Mut1 and Mut2 (using the simulations with both the reduced and the oxidised redox site) in order to observe possible changes of the fluctuation pattern. The results are shown in Figure 4 for Mut1 and 5 for Mut2. The main feature emerging from the analysis is that the residues that more relevantly contribute to the fluctuations of V remain unaltered upon mutation (see Figure 5 in the main text). In both mutants the residues that have been already identified as significant in WT Az are still present: residues 11, 13, 35, 44, 47 and 128 (see also Figure 6). In addition to these sites, residues 91 and 114 show up as relevant in Mut 1 and Mut 2, respectively.

Residue 91 is a negatively charged aminoacid (Glu) whose dynamics seems to vary in Mut1 with respect to WT Az. The analysis of its hydrogen-bond (HB) pattern shows that Glu91 is hydrogen-bonded to both His35 and Gly88. As a consequence of the N47S/M121L mutation, the HB between His35 and Glu91 is weakened while the one between Gly88 and Glu91 is strengthened in Mut1 with respect to WT Az. Due to its negative charge, the different fluctuation pattern of Glu91 in Mut1, determined by its different HB pattern, may have a significant role in determine E^0. As in the case of

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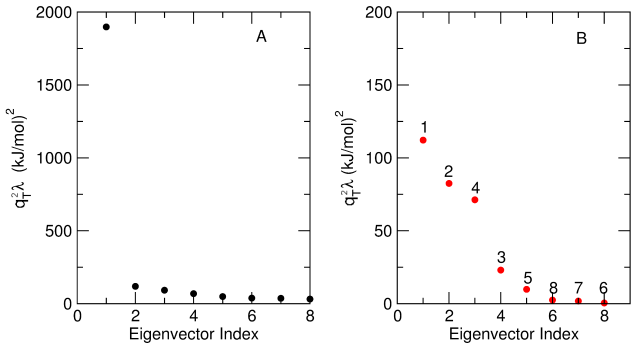

Fig. 2 Panel A: first eight eigenvalues of the covariance matrix of the single-residue electrostatic potentials as a function of the eigenvector index. Panel B: first eight weighted eigenvalues as a function of the eigenvector index. The numbers labelling the points in panel B correspond to the eigenvector index before the weighting procedure. The data are extracted from the MD simulation of MUT1 with the oxidised redox site.

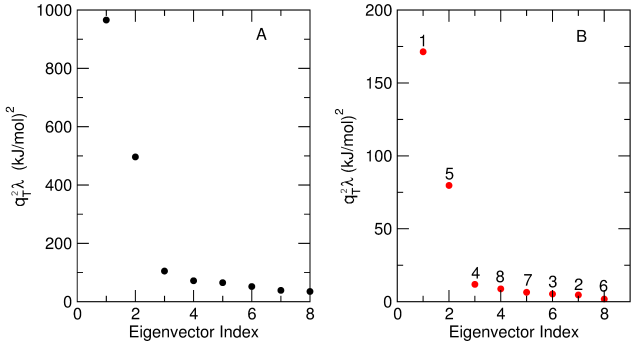

Fig. 3 Panel A: first eight eigenvalues of the covariance matrix of the single-residue electrostatic potentials as a function of the eigenvector index. Panel B: first eight weighted eigenvalues as a function of the eigenvector index. The numbers labelling the points in panel B correspond to the eigenvector index before the weighting procedure. The data are extracted from the MD simulation of MUT2 with the oxidised redox site.

Lys128, the role of this residue was not previously observed, *i.e.*, was not observed by analysing the mean single-residue potentials.

The contribution of residue 114 in Mut2 obviously arises from the F114N mutation. The dynamic behaviour of the mutated site was previously characterised,[1] showing that the formation and rupture of its HB to one of the copper ligands (Gly45) has relevant effects on E^0.

References

1 L. Zanetti-Polzi, C. A. Bortolotti, I. Daidone, M. Aschi, A. Amadei and S. Corni, *Organic & biomolecular chemistry*, 2015, **13**, 11003–11013.

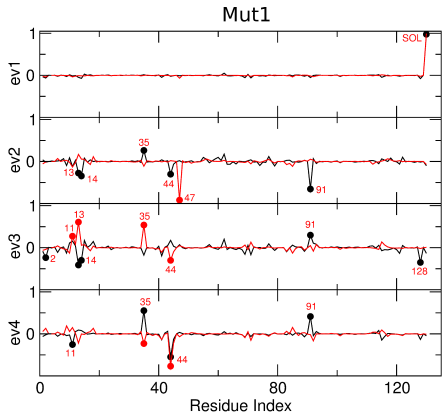

Fig. 4 Components of the first four eigenvectors obtained from the MD simulation of Mut1 (N47S/M121L) with the reduced (in red) and oxidised (in black) redox site. The components whose square equals or exceeds 0.05 are highlighted with dots

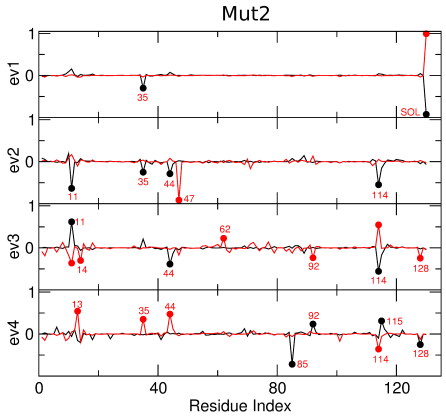

Fig. 5 Components of the first four eigenvectors obtained from the MD simulation of Mut2 (N47S/F114N/M121L) with the reduced (in red) and oxidised (in black) redox site. The components whose square equals or exceeds 0.05 are highlighted with dots.

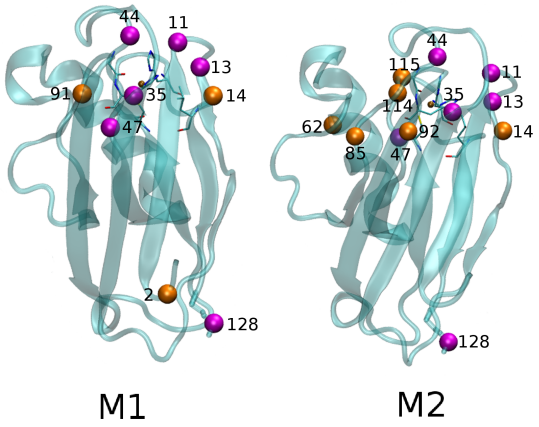

Fig. 6 Schematic representation of the position of the residues that most contribute to the fluctuations of V for Mut1 and Mut2. For the sake of clarity, the residues are represented only with coloured dots. The residues whose contribution is present in all the four species are coloured in magenta while the other relevant residues are coloured in orange. The redox site (the copper and its five ligands) is also shown in licorice.