Redox potentials in the decaheme cytochrome MtrF: Poisson-Boltzmann vs. molecular dynamics simulations

We previously computed the redox potentials for the ten hemes in the deca-heme cytochrome MtrF using Thermodynamic Integration (TI) in combination with all-atom, explicit solvent molecular dynamics (MD) simulation (1). In a recent study, Watanabe *et al.* recomputed these potentials using a Poisson-Boltzmann (PB) continuum approach (2). The potentials obtained from MD for the all-oxidized (all-ox) protein gave a nearly symmetrical free energy profile along the octa-heme chain with a small overall driving force of -48±66 meV from heme 10 to heme 5 and two symmetric free energy maxima of ~200 meV at heme 9 (domain IV) and heme 4 (domain II). PB gave a slightly larger overall driving force of -118 meV and predicted a free energy maximum in domain IV as well. However, by contrast to TI, a mostly downward slope through the rest of the chain was observed, i.e., no second maximum in domain II.

Watanabe *et al.* rationalized the asymmetry of their profile by noting that it is "*mainly caused by the acidic residues at Asp631, Asp518, Asp490 (in domain IV), […]. These acidic residues are not present in the corresponding regions of domain II."* This argument cannot be correct because the authors show that protonation of Asp631 (most important residue according to their Tables 2 and 3) leaves the qualitative features of the profile unchanged. Their apparent electron sink in domain II remains unexplained.

Watanabe *et al*. criticize our reported residue electrostatic contributions as being too high. However, this ignores the fact that in MD these are the bare electrostatic contributions, that when added up over all residues and the solvent give the full, thermally averaged electrostatic potential at the heme site. By contrast, in PB the residue contributions are screened by a simplistic dielectric medium used to approximate the protein environment. Therefore, it is only meaningful to compare the sign but not the magnitude of the single residue contributions.

Finally, Watanabe *et al.* attempted to reproduce our TI/MD redox potentials but none of their profiles matched ours concluding that this *"argues against the quality of their [Breuer et al.'s] calculated Em values."* However, close inspection of Watanabe's TI protocol raises serious concerns. "*TI simulations were conducted over 10 ns with an MD time step of 2.0 fs, namely* $\Delta\lambda = 2.0\times10^{-7}$*. [...] oxidized heme (*Fe^{3+}*) was gradually reduced (to* Fe^{2+}*) over 10 ns."* Apparently, in their approach the TI coupling parameter λ was erroneously changed *every* MD integration time step. This corresponds to a single configuration being used to define an ensemble average, which is nonsensical. This substantial flaw in their protocol seems to be a much more likely cause for the different TI-derived free energy profiles reported in their Fig. 5 than the supposed slow structural fluctuations in MtrF; for these fluctuations, the authors do not provide any evidence, nor do they seem plausible given the considerable stiffness of the deca-heme motif in MtrF.

References:

1 Breuer M, Zarzycki P, Blumberger J, Rosso KM (2012) Thermodynamics of electron flow in the bacterial deca-heme cytochrome MtrF. *J Am Chem Soc* 134(24):9868–9871.

2 Watanabe HC, Yamashita Y, Ishikita H (2017) Electron transfer pathways in a multiheme cytochrome MtrF. *Proc Natl Acad Sci USA* 114(11):2916-2921.