REPLY TO BREUER ET AL.:

Molecular dynamics simulations do not provide functionally relevant values of redox potential in MtrF

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We previously reported that Asp631 decreased the redox potential (E_m) for heme 9 by 136 mV [1,362 mV according to Breuer et al. (1)] in native MtrF, whereas Asp631 protonation (corresponding to Asn631 mutation) did not significantly affect E_m for heme 9, because deprotonation of other residues compensated for the E_m shift (2). Breuer et al. (3) cannot observe the corresponding effect, since they fixed the protonation states of titratable residues (1).

According to Breuer et al. (1), residues make unusually large contributions to E_m; for example, Asp228 decreases E_m for heme 2 by −2,280 mV [−61 mV in our calculations (2)]. In their letter, Breuer et al. (3) state that "it is only meaningful to compare the sign but not the magnitude of the single-residue contributions"; however, in their original report, they focus on not only the sign but also the magnitude of the single-residue contributions, stating, for example, "we find that every charged residue in the environment of a cofactor contributes several tenths of volts" (1). The contribution of each residue to E_m must be comparable to E_m shifts experimentally measured upon mutations.

To verify E_m obtained using their molecular dynamic (MD) simulation-based thermodynamic integration (TI) approach, we employed the slow-growth TI approach (4), as widely used for free energy calculations (e.g., ref. 5), wherein the redox states gradually transit from Fe^{3+} to Fe^{2+} (2). Breuer et al. (3) argue against the time scale of our TI simulations (10 ns), but they justify the time scale of their TI simulations (11 ns) assuming the structural "stiffness of the decaheme motif" (however, see below). We emphasize that any MD-based approaches are not appropriate in this case, irrespective of simulation time due to the following reasons.

For the structural fluctuations (Fig. 1), we have pointed out that the side-chain orientations are incorrect in the β-barrel motif of domain I of the original MtrF crystal structure (e.g., hydrophobic residues are oriented toward the bulk solvent), resulting in remarkably high calculated B-factors (2). Nevertheless, Breuer et al. (1) have used the original side-chain orientations in the MtrF crystal structure. Heme 2 is closest to domain I (heme 7 to domain III). Notably, only E_m values of the heme 2 and heme 7 pair differ significantly (130 mV) in their perfectly symmetrical E_m profile (1). Moreover, even heme-binding domain IV is also unstable (Fig. 1). Since their simulations were still in the slow-decay process of domains I and IV, being far from equilibrium, E_m profiles strongly depend on the MD-starting structure (e.g., obtained after equilibration for 0, 100, and 1,000 ns) as demonstrated in our test MD calculations (2). From the statement, "the considerable stiffness of the decaheme motif" in Breuer et al.'s letter (3), they have missed this point while calculating E_m (1).

Finally, the E_m for hemes 2 (−57 mV) and 7 (74 mV) reported by Breuer et al. (1) seem unlikely to support a role of bound flavin [−150 mV (6)] serving as the terminal electron acceptor (7, 8). Their MD simulation-based TI approach, using their geometry and fixing the protonation states of titratable residues and heme-propionic groups, is unlikely to provide functionally relevant values of E_m in MtrF.

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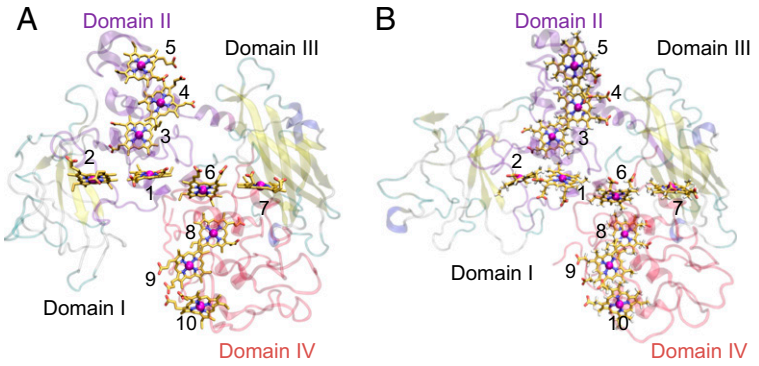

Fig. 1. (*A*) Crystal structure of MtrF (9). Hemes 2 and 7 have been proposed to be located near the flavin-binding site (9, 10). (*B*) MtrF structure obtained after equilibration for 1.2 μs. Domains I and IV show remarkably large structural deviations from the original MtrF crystal structure. For comparison with their calculation, we also used the original MtrF crystal structure in MD-based TI calculations.

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