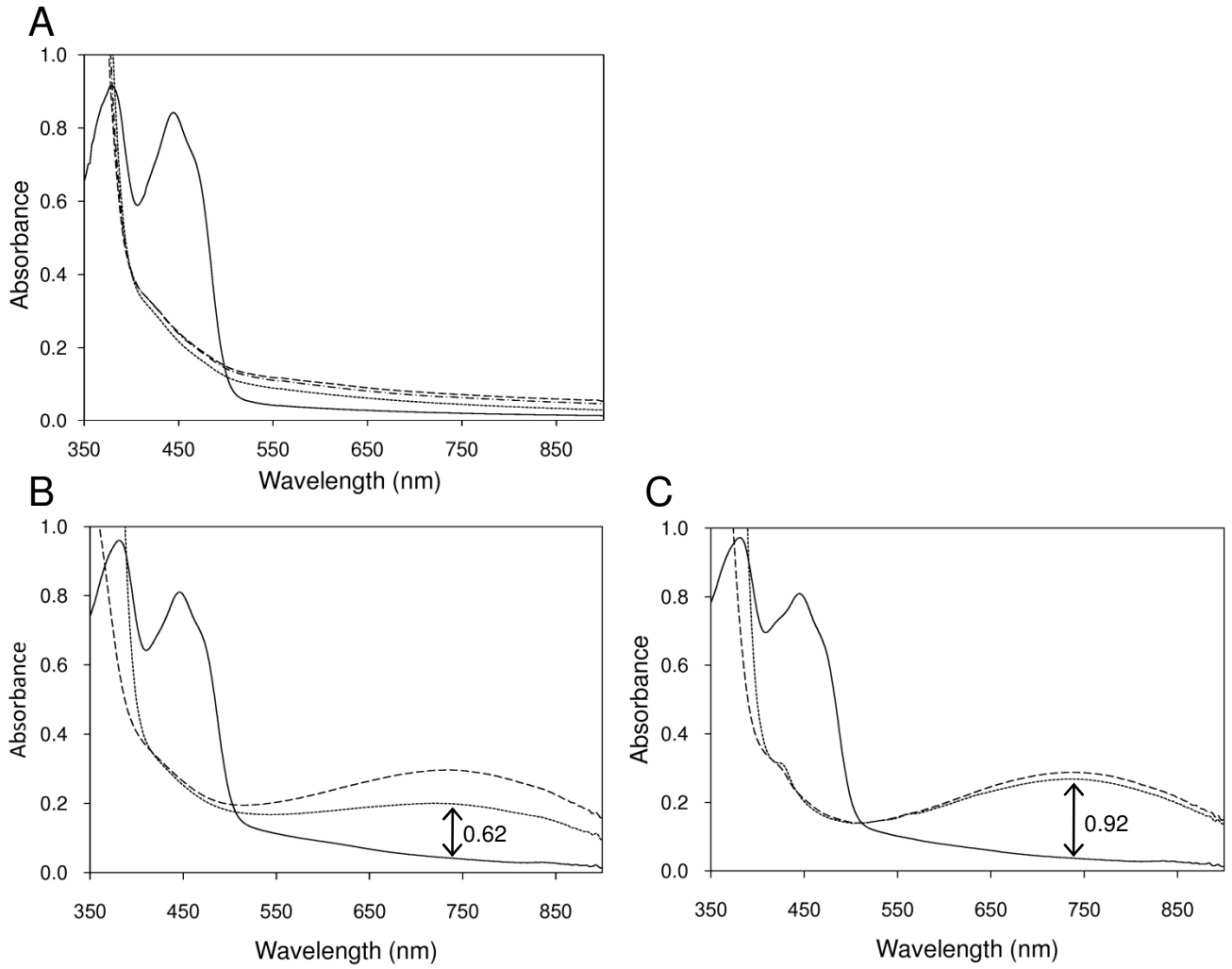

Supplemental Fig. 1. Effect of NADPH (*A*) and endogenous UQ (*B* and *C*) on the absorption spectrum of Ndi1. The absorption spectra of UQ-free (100 μM, *A* and *B*) and endogenous UQ_8-bound Ndi1 (0.32 mol of UQ_8/mol of enzyme: 100 μM, *C*) were measured at 25 °C in a buffer containing 50 mM Mops-KOH, pH 7.0, 0.1 mM EDTA, 10% glycerol, and 0.02% DM without 1 M NaCl (*A*) or with (*B* and *C*). The experiments were carried out using the anaerobic chamber (Coy Laboratory Products Inc. for *A*, and PLAS LABS, Inc. for *B* and *C*). *A*, UQ-free Ndi1 was reduced by the addition of 1 mM NADPH (dotted line), and 200 μM DBQ was added (dashed-dotted line). Then, the cuvette was opened under aerobic conditions (dashed lane: maximum height of the broad band). *B* and *C*, UQ-free and endogenous UQ_8-bound Ndi1 were also reduced by the addition of 1 mM NADH (dotted line). The peak height of the CT complex of UQ_8-bound Ndi1 (*C*) was higher than that of UQ-free enzyme (*B*). After the addition of NADH, the cuvette was opened under aerobic conditions (dashed lane: maximum height of the broad band). The reason why the peak heights of CT complex of both enzymes after addition of NADH (dotted line) were higher than that of UQ_6-bound enzyme (dotted line in Fig. 2*B*) may be caused by higher residual oxygen levels. The ratios of peak height in the presence of NADH (dotted line) per maximum height (dashed line) of the CT complex of UQ-free (*B*) and UQ_8-bound Ndi1 (*C*) were determined to be 0.62 and 0.92, respectively. The difference between these ratios (0.92 - 0.62 = 0.30) of UQ_8-bound and UQ-free Ndi1 was approximately the same as the UQ_8 contents of UQ_8-bound enzyme (0.32 mol of UQ_8/mol of Ndi1, Fig. 1*C*).