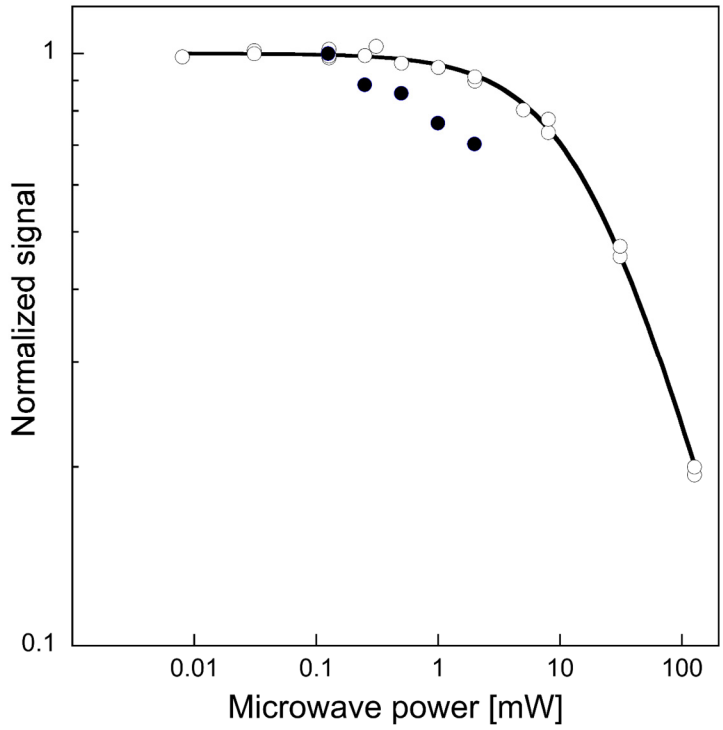
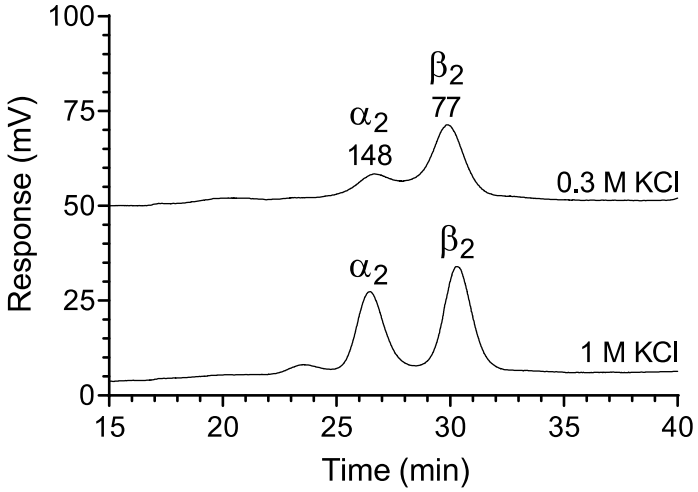
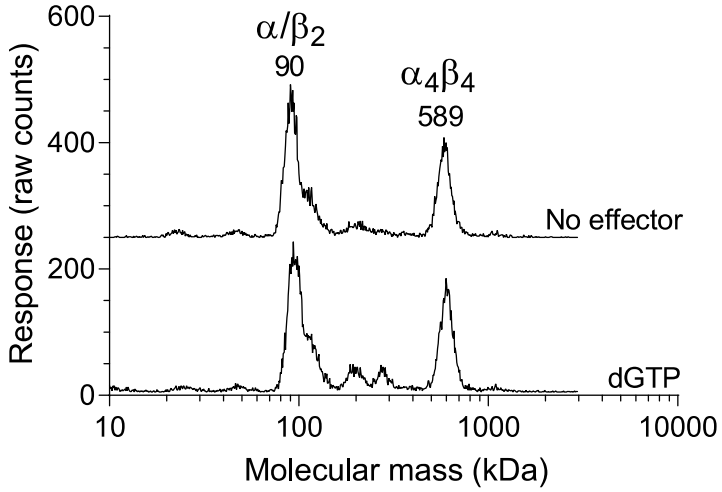
SUPPORTING INFORMATION



Supporting Figure S1. EPR saturation behavior of the tyrosyl radical in the *C. botulinum* R2 protein (●) compared to that of the *E. coli* R2 protein (NrdB) (○). Saturation curves at different temperatures determine the microwave power at half saturation $P_{1/2}$. The temperature dependence of $P_{1/2}$ gives information about possible relaxing transition metals in the metal center close to the radical (41). The values shown for the *C. botulinum* R2 protein are not for a full saturation curve. The purpose of the measurements was only to find conditions to give an undistorted EPR signal for the radical. Hence, the true value for the normalized signal amplitude of 1.0 is most likely at a slightly lower power. Nevertheless, it is clear that the $P_{1/2}$ for the *C. botulinum* R2 protein is at least one order of magnitude less than for the *E. coli* R2 protein as a result of weaker interaction with the dinuclear iron center.



Supporting Figure S2. Size exclusion chromatography analysis of *C. botulinum* R1-R2 complexes. The top trace shows the analysis of a mixture of 0.05 mg/ml R1 protein and 0.025 mg/ml of R2 protein in a mobile phase containing 100 μM dATP, 0.3 M KCl, 10 mM $MgCl_2$ and 50 mM Tris-HCl pH 7.6. The bottom trace shows a similar analysis but where the KCl concentration was increased to 1 M. R1-R2 complexes were not observed, possibly due inhibition of the interaction at high salt concentrations (lower trace). At the lower salt concentration (upper trace) it was difficult to draw any conclusions since the R1 protein tended to disappear, possibly due to protein instability or adherence to the column.



Supporting Figure S3. GEMMA analysis of the *C. botulinum* RNR. The R1 and R2 proteins were analyzed together in the absence or presence of 200 µM dGTP (and equimolar concentration of magnesium acetate). The experiments were performed with 0.05 mg/ml R1 protein (from preparation 1) and 0.025 mg/ml R2 protein in a solution containing 0.025 mM DTT, 0.005% /v/v) Tween-20 and 100 mM ammonium acetate pH 7.8.