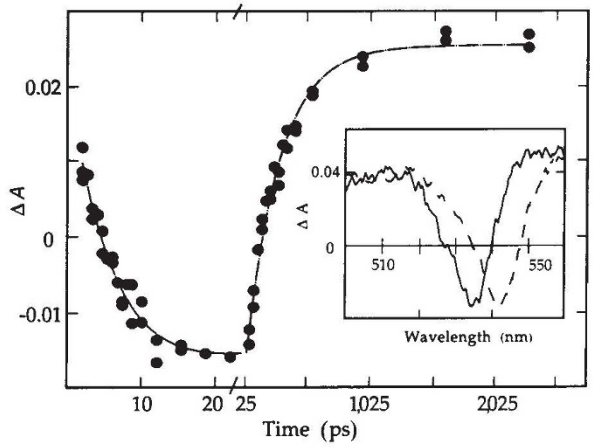

Fig. 3 Kinetics for the appearance and decay of bleaching in the 534 nm Q_X band of $Bphe_L$ observed after excitation of the $Glu^{L104} \rightarrow$ Leu RCs with a 350-fs flash at 582 nm. The solid line is a fit of the data to a constant plus two exponentials, yielding time constants of 6.1 ± 1.0 and 270 ± 30 ps. Similar results are obtained for the 536 nm bleaching in $Glu^{L104} \rightarrow$ Gln RCs (Table 1). The inset compares the position of the $Bphe_L$ bleaching observed at 12 ps in $Glu^{L104} \rightarrow$ Leu (solid) and the wild-type (dashed) reaction centres. By 2 ns, the bleaching has decayed completely to a positive absorbance associated with state $P^+Q_A^-$ in both of these RCs, as well as in $Glu^{L104} \rightarrow$ Gln (see data points at long times in the main figure for examples).

ponent in the decay kinetics of the 620 to 680 nm Bphe anion band, or of the Bphe Q_X band bleaching between 530 and 540 nm, or of the primary donor bleaching at 855 nm. The presence of a nanosecond component would be expected if an electron was transferred to $Bphe_M$, reflecting either the charge recombination of $P^+Bphe_M^-$ or a slow (because of the distance involved) electron transfer from $Bphe^-$ to Q_A (these RCs contain no secondary quinone, Q_B). A nanosecond or slower component would also be observed if the electron transfer from $Bphe_L^-$ to the primary quinone (Q_A) did not proceed with 100% yield. The absence of any such slow decay further demonstrates that these genetically modified RCs display primary photochemistry that is essentially the same as that found in wild-type RCs.

It is clear that there is an asymmetry in the environments of the two Bphes even after the substitution of leucine for glutamic acid, as demonstrated by the small red shift of the $Bphe_L$ Q_X band in the $Glu^{L104} \rightarrow$ Leu RC and the poor homology between the L and M subunits in the vicinity of the L104 residue[16]. In contrast, there is a high degree of homology between the D1 and D2 polypeptides in the vicinity of the analogous binding sites of the two pheophytins (Pheo) present in photosystem II RCs. The glutamic acid residue analogous to L104 is generally conserved in the D1 polypeptide of the photosystem II RC. Although the corresponding residue in the M subunit of the bacterial RC is a hydrophobic valine residue, the equivalent residue in the D2 polypeptide of the photosystem II RC is a glutamine[13]. Additionally, the increased environmental symmetry of the two Pheos is demonstrated in the absorption spectrum of photosystem II RCs, where the Q_X bands of the two Pheos are unresolved at 4 K (ref. 17). It seems reasonable that the principal source of the directionality of charge separation must be sought earlier in the electron transport chain, and/or in the combined effects of more than just a single pigment-protein interaction.

The glutamic acid residue at L104 in the RCs of *Rb. capsulatus* is principally responsible for the spectroscopic red shift of $Bphe_L$ compared with $Bphe_M$. Although mutations at this residue affect the electron transfer kinetics in the RC, Glu^{L104} is not the dominant contributor to the directionality of electron transfer, as the loss of the putative hydrogen-bonding interaction between $Bphe_L$ and Glu^{L104} has no significant effect on the ability of the RC to carry out the primary electron transfer reactions resulting in $P^+Q_A^-$ formation in high yield. Additional studies on $Glu^{L104} \rightarrow$ Leu and $Glu^{L104} \rightarrow$ Gln RCs and on other genetically modified RCs will provide a better understanding of the importance of pigment-protein interactions in the mechanisms of electron transfer in bacterial RCs. Our results show that an individual amino-acid residue can modulate the electronic properties of a chromophore, affecting both optical properties and electron transfer dynamics in pigment-protein complexes.

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Erratum

Activation *in vitro* of sequence-specific DNA binding by a human regulatory factor

Jeffrey S. Larson, Thomas J. Schuetz & Robert E. Kingston
Nature **335**, 372-375 (1988).

A correct version of Fig. 1 for this letter is shown below. In the original printing the image of the gels was laterally reversed.