

Designing Artificial Photosynthesis: Production of a light activated metalloprotein

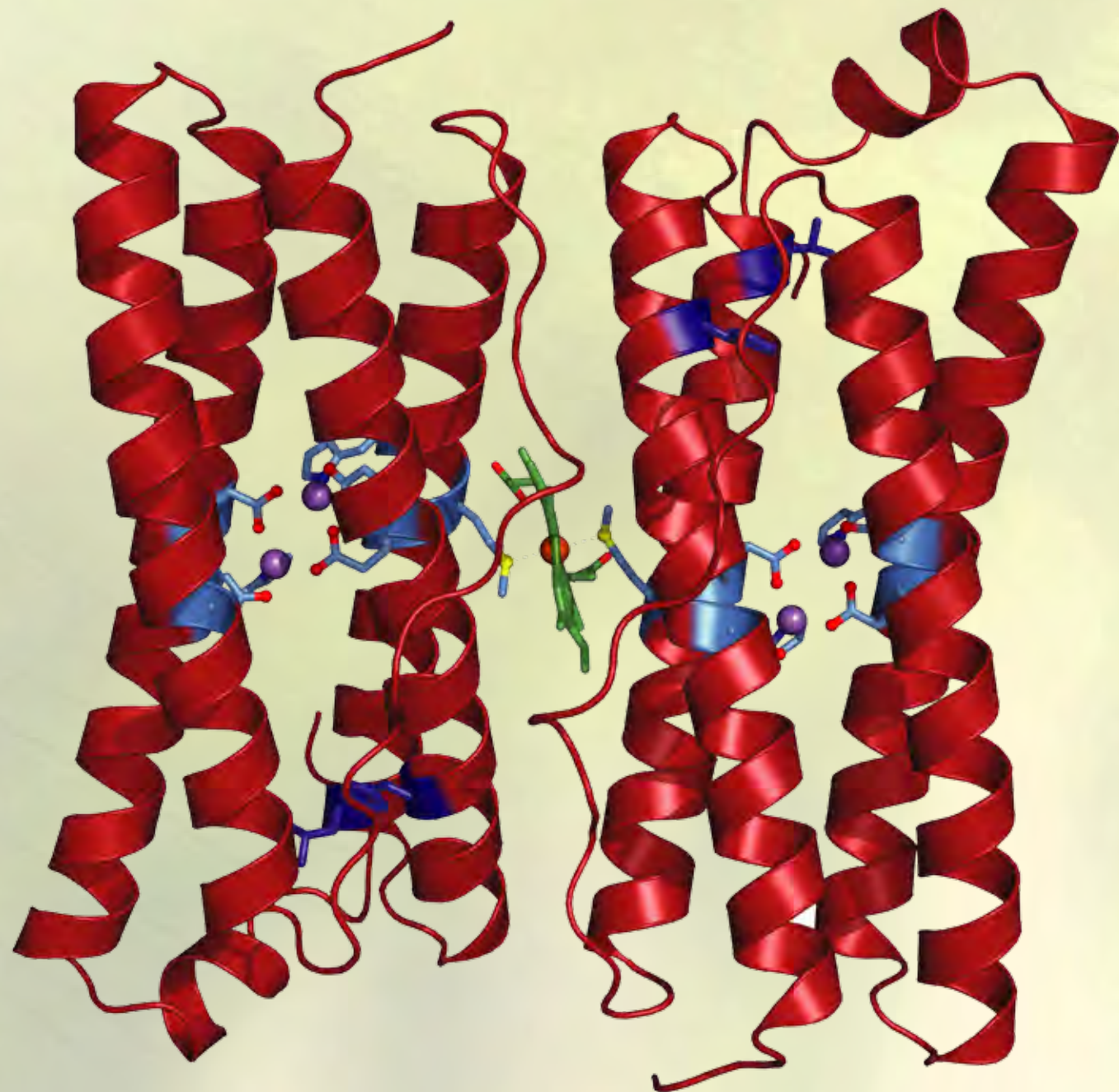
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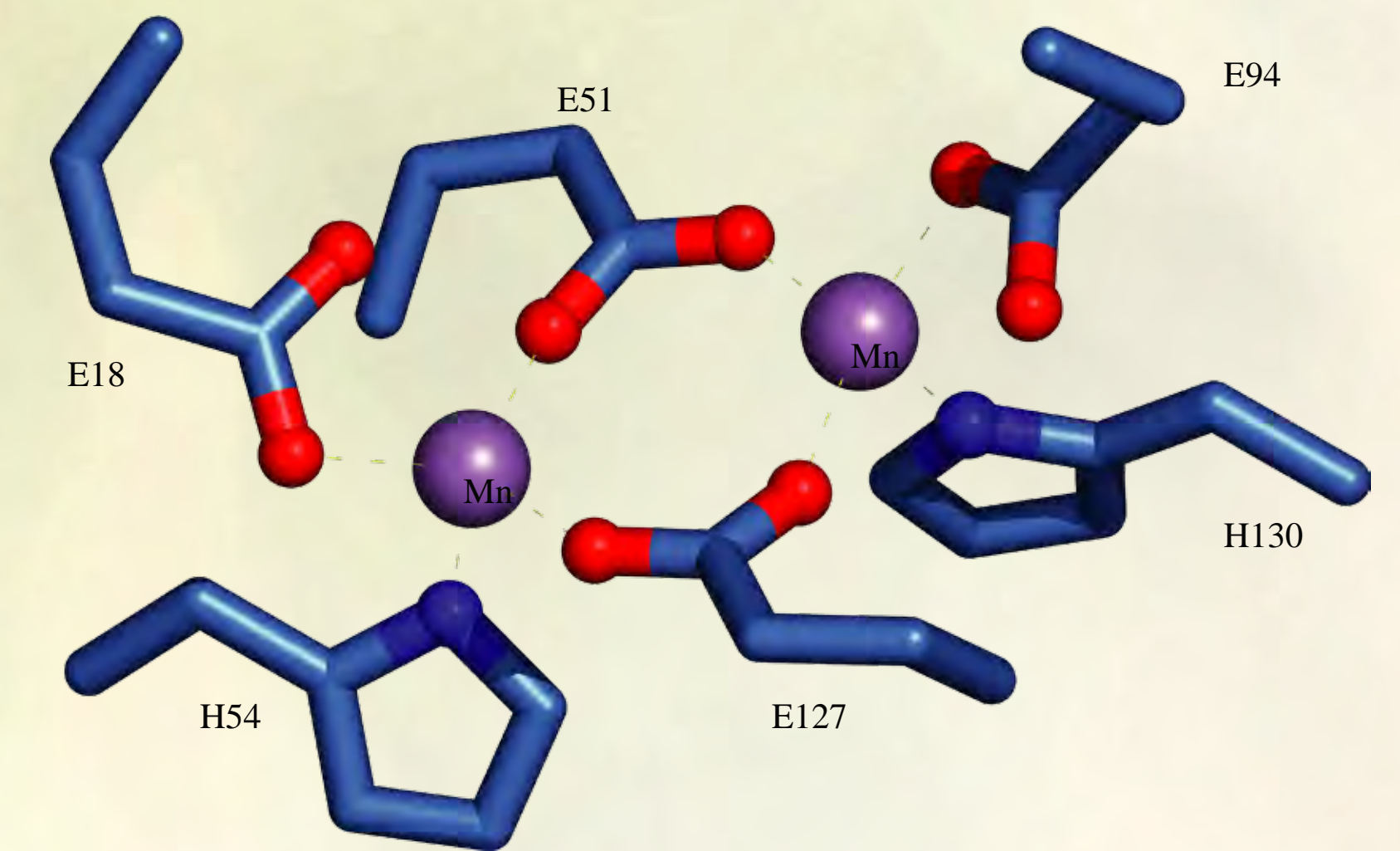
Introduction

The aim of this work is to engineer an *in vitro* mimic of photosystem II (PSII). PSII utilises a special chlorophyll complex which upon excitation ejects an electron to external electron acceptors and then

oxidises a manganese containing metal centre to reclaim the lost electron (1). To mimic this reaction bacterioferritin (BFR) was engineered to produce a light activated, metal binding protein.

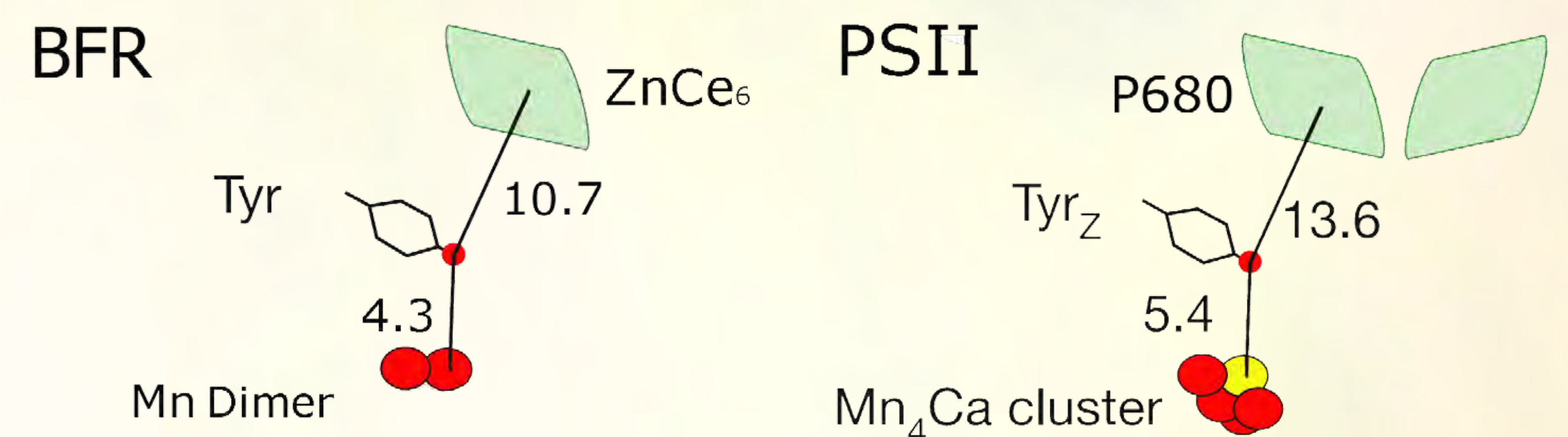


BFR contains a di-iron metal site and a heme *b* binding site (2). BFR is an 18.5kDa four helix bundle which forms a spherical shell from 12 homodimers (2-4). In the binuclear metal binding site each metal ion is ligated to one histidine and one glutamate and is bound to the neighbouring metal ion by di- μ -1,3 carboxylato bridges.



Engineering of the light activated protein

The *bfr* gene was cloned from *E.coli* and over-expressed. Modification of the wild type BFR was carried out using site-directed mutagenesis to produce BFR1 (H46R, H112R), which was used in all experiments. Manganese can be bound in the metal binding site and the heme group can be substituted with the photoactive zinc chlorin e_6 (ZnCe $_6$), which binds with a K_d of 83 μ M (5, 6). The similar distances between the cofactors in PSII and BFR1 (2, 8) inspired the use of this protein. The diagram on the right shows the distances between cofactors in Angstroms.



Manganese Binding

Two manganese ions bind per monomer of BFR1. The EPR six-line signal associated with Mn(II) hexa-aqua was used to measure bound and free Mn(II). Upon binding to the protein the six line signal is lost.

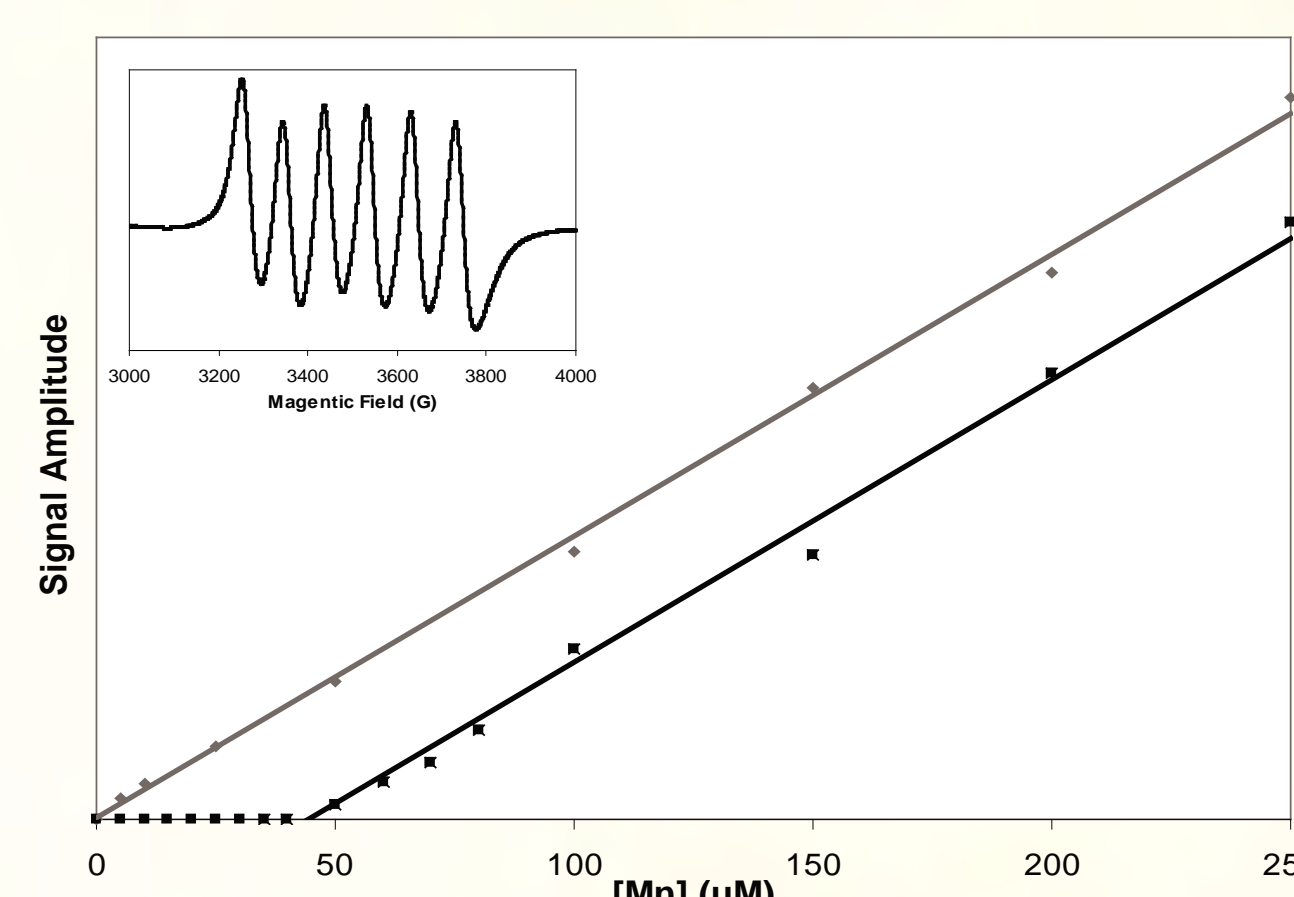


Figure 1 (left): Titration of Mn(II) into a 25 μ M solution of BFR1 (black) and buffer (grey), monitored using room temperature EPR. The inset shows a characteristic Mn hexa-aqua EPR spectra.

Light Induced Effects

EPR was used in order to determine if there is an oxidation state change in the manganese centre of BFR1 upon illumination (figure 2). The parallel mode low field signal from 300 Gauss to zero field was seen to increase upon illumination of the sample in the presence of hexachloroiridate (IV). This low field signal is attributed to a bound homodimeric Mn(III) complex (7). The structured signal from 470-1840

Gauss in perpendicular mode is attributed to monomeric Mn(II) bound within a structured environment.

A large radical signal is found at $g=2$ when BFR1 with ZnCe $_6$ bound is illuminated. The presence of manganese bound to BFR1 reduces the ZnCe $_6$ radical revealing a broad underlying radical signal which may originate from a tyrosine (figure 3). The loss of the ZnCe $_6$ radical signal is due to the oxidation of the metal centre and reduction of the ZnCe $_6$ radical species.

Figure 2 (right): EPR spectra of BFR1 with ZnCe $_6$ and Mn bound (black - perpendicular mode; grey - parallel mode). Microwave frequency 9.68 GHz, microwave power 50mW, modulation amplitude 10G, modulation frequency 100kHz, temperature 5K.

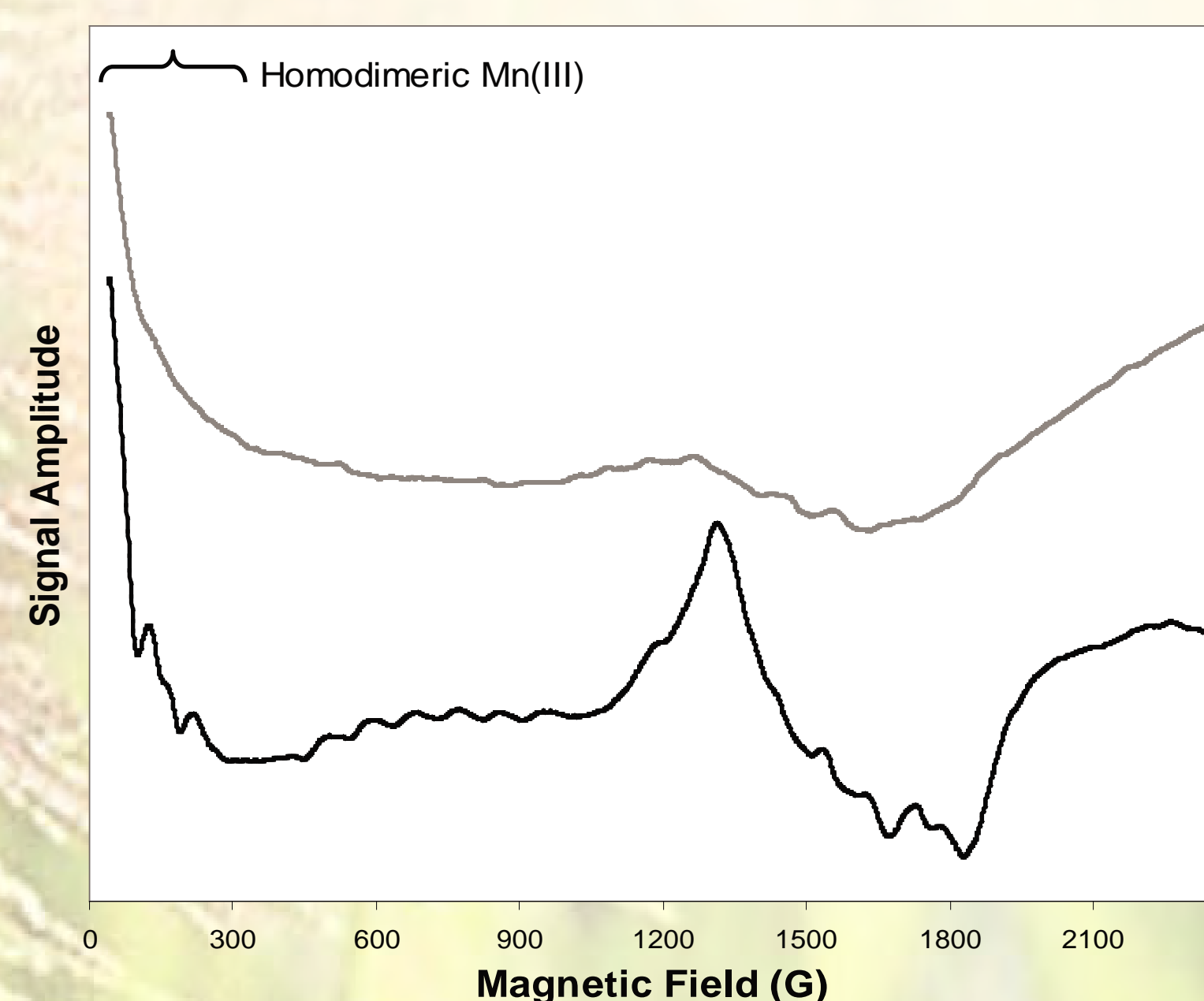
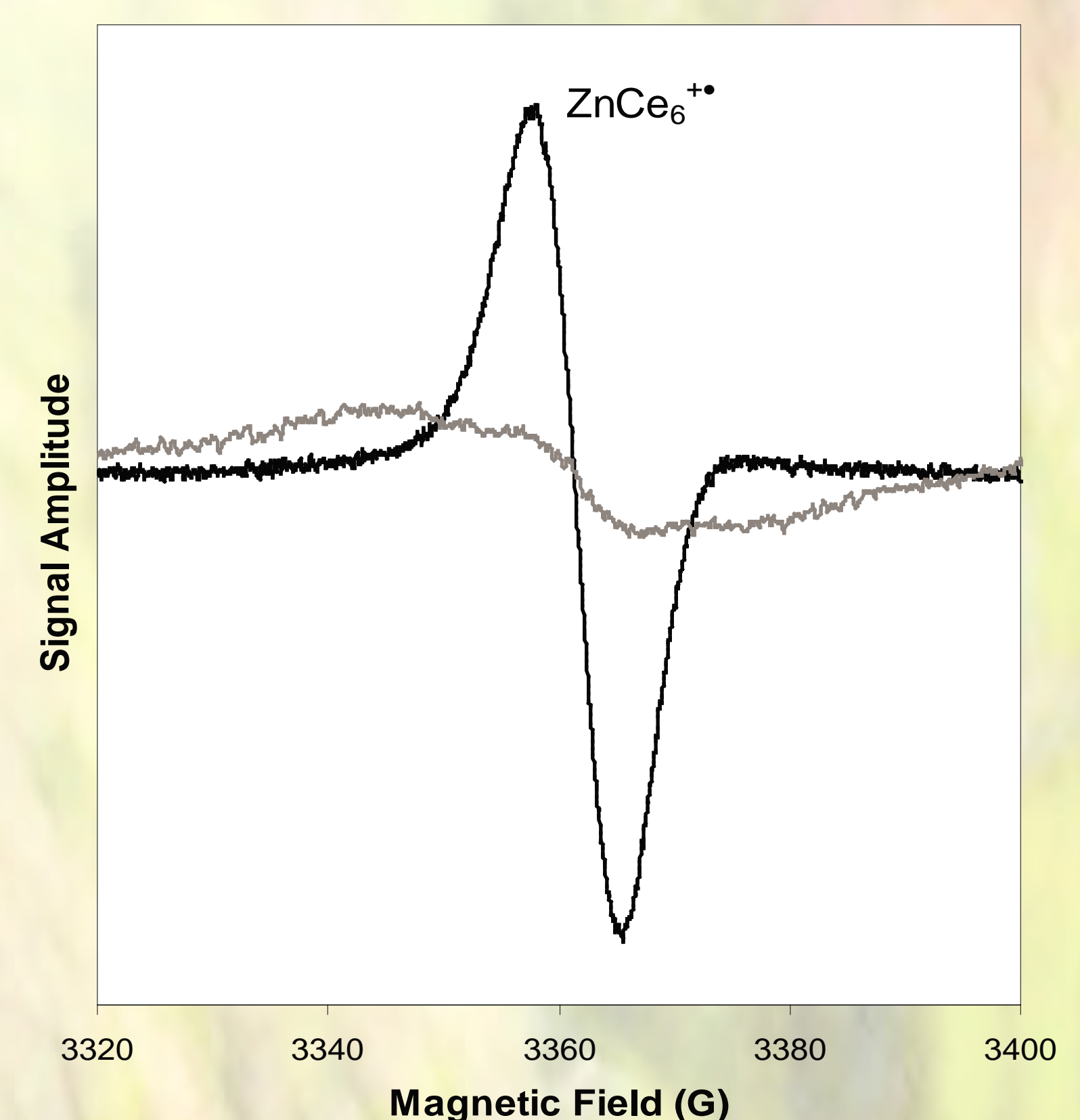


Figure 3 (right): Light minus dark EPR spectra of BFR1 with ZnCe $_6$ (black) and BFR1, ZnCe $_6$ and Mn (grey). Microwave frequency 9.68 GHz, microwave power 1 μ W, modulation amplitude 3G, modulation frequency 100kHz, temperature 5K.



Conclusions

Using EPR we have been able to show that manganese can be bound to the di-iron binding site in a redox active form and upon light activation of the pigment the manganese centre is oxidised.

References

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