Structure Elucidation of the clCry4 C-terminal Domain and its Light-induced Conformational Dynamics by single molecule Fluorescence Resonance Energy Transfer (smFRET)

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BACKGROUND

Cryptochrome (CRY) is a uniquely distinctive member of the photosensitive flavoprotein family, performing diverse functions across different species. At the beginning of this century, it was boldly proposed as a biological magnetoreceptor protein involved in geomagnetic navigation. Subsequent experimental evidence has increasingly supported this prediction. Magnetoreception represents one of the most enigmatic senses in biology, providing migratory animals with directional navigation capabilities, yet its molecular mechanisms remain largely unresolved. As a candidate magnetoreceptor molecule, cryptochrome's working mechanism involves quantum effects of photoinduced radical pairs (Radical Pair Mechanism, RPM). In recent years, the radical pair-based magnetoreception hypothesis has gained theoretical and experimental support. However, crucial molecular-level evidence, particularly direct observation and validation of **how magnetic fields influence protein conformation**, remains lacking.

Single-Molecule FRET (smFRET)

Förster resonance energy transfer (FRET) is a mechanism describing energy transfer between two light-sensitive molecules. Measurements of FRET efficiency can be used to determine the distance between two fluorophores, if they are within a certain distance of each other.

The FRET efficiency (E) is the quantum yield of the energy-transfer transition.

$$E = \frac{1}{1 + (r/R_0)^6}$$

r : the donor-to-acceptor separation distance

R_0 : the Förster distance of this pair of donor and acceptor

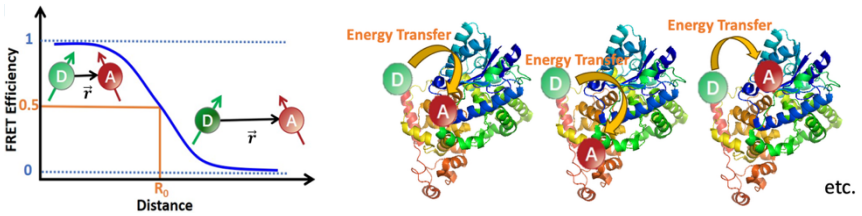


Figure 1. The principle of smFRET. Schematic illustration showing the inverse relationship between FRET efficiency and the donor-acceptor distance (r). As the distance increases, the energy transfer efficiency decreases. The molecular models on the right demonstrate by selecting donors and acceptors at different positions, we can model the three-dimensional conformational changes of the protein associated with energy transfer.

MICROSCOPY SETUP

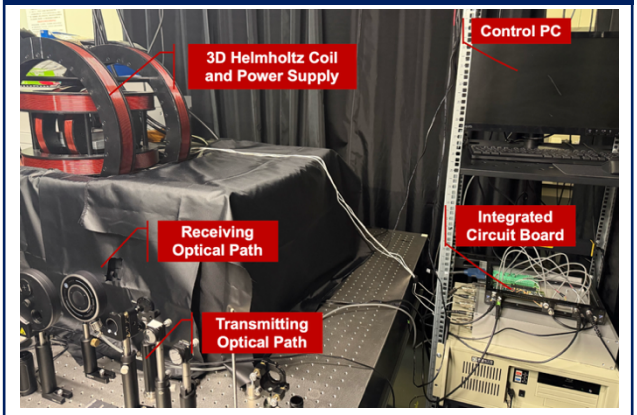
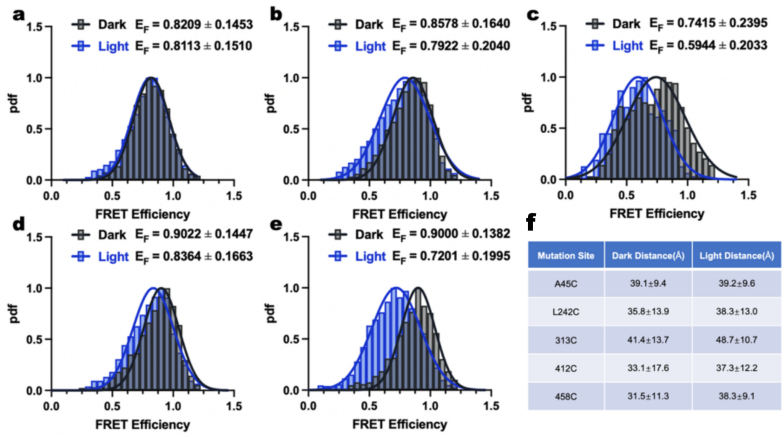


Figure 2. The custom-built magnetic field-compatible single-molecule fluorescence microscope. It can generate a uniform magnetic field that covers the intensity range of the Earth's magnetic field (25-65 μT) and accurately simulate the inclination of the Earth's magnetic field at different geographic latitudes worldwide. A program developed by our research group controls the Helmholtz coils and the data acquisition card respectively, thereby achieving synchronous magnetic field control and data acquisition.

RESULTS & CONCLUSION



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Mutation Site	Dark Distance(Å)	Light Distance(Å)
A45C	39.1±9.4	39.2±9.6
L242C	35.8±13.9	38.3±13.0
313C	41.4±13.7	48.7±10.7
412C	33.1±17.6	37.3±12.2
458C	31.5±11.3	38.3±9.1

Figure 3. The Single-molecule fluorescence resonance energy transfer (smFRET) quantitative data between five representative sites of the protein — (a) N-terminal (A45C), (b) middle-anterior region (L242C), (c) middle region (313C), (d) near C-terminal core (412C), (e) near C-terminal exterior (458C) — and the C-terminal terminal residue (525C). (f) Core Data Integration Table for Distances from Multiple Sites to the C-terminus.

Based on the experimentally measured distance data from five sites, the core characteristics of the light-induced conformational change at the C-terminus can be clearly summarized as follows: the direction of the light-induced displacement of the C-terminus is away from the PHR core, dissociating towards the exterior of the protein. Specifically, the near C-terminal core (412C), being fixed within the PHR framework, shows an increased distance to the C-terminus, which directly proves the outward displacement of the C-terminus. The near C-terminal exterior (458C) exhibits the maximum increase, verifying the extent of the dissociation on the outer side of the C-terminus. Furthermore, the observed 'distance increases' at the mid-anterior (L242C) and mid-section (313C) sites further rule out the possibility of internal contraction. All conclusions are derived from measured smFRET distance data and the spatial localization of the sites, without any additional hypothetical speculation, thereby providing a quantitative basis for investigating the mechanism of light-induced conformational changes at the C-terminus.

REFERENCE

[1] Helms V (2008). "Fluorescence Resonance Energy Transfer". Principles of Computational Cell Biology. Weinheim: Wiley-VCH. p. 202.

* The photo of the bird in the top right corner of this poster is from the cover of Science Volume 339, Issue 6123, 1 Mar 2013. The bird is a rock pigeon (Columba livia), and the protein studied in this project is derived from this species.