**Self funded project**

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**Section 2 – *Project Information***

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| **Project Title** | Redefining DNA-double strand break repair in humans |
| **Project Summary**  |
| Numerous DNA damaging agents constantly cause damage to cellular DNA. These damaging agents jeopardize the integrity of the cellular genome, resulting in a range of developmental defects. A break across both strands of DNA is the most dangerous form of DNA damage and inaccurate or inefficient repair of these lesions can cause genomic instability and eventually cell death or cancer 1,2. Non-homologous end joining (NHEJ) is the main double-strand break (DSB) repair mechanism in humans 2,3. NHEJ is dependent on a few canonical proteins, however, there are also several NHEJ accessory factors that have overlapping and redundant functions 2,3. It has been shown that inhibiting NHEJ in combination with chemo- and radiotherapy is beneficial in cancer treatments. Therefore, understanding this critical repair mechanism is essential for the development of future therapies. The core NHEJ proteins includes the hetero-dimer Ku70/80, the large protein kinase DNA-PKcs, DNA ligase IV, X-ray repair cross-complementing protein 4 (XRCC4) and XRCC4-like factor (XLF) 2,3. Recently, using cryo-electron microscopy (cryo-EM) it has been shown that the DNA-PK holoenzyme complex (Ku70/80, DNA-PKcs, + DNA) can exist as a two alternate types of dimers 4-6. These two DNA-PK dimers represent long-range synaptic complexes, which hold the DNA at a long distance. These long-range complexes can then transition to a short-range assembly, to allow for DNA ligation, following the removal of DNA-PKcs through ATP phosphorylation events 6,7. However, it is unknown how these long-range complexes transition to the short-range complex following addition of ATP and the complete NHEJ mechanism remains unclear. Within this PhD proposal, biochemical, biophysical and structural biology, specifically cryo-EM will be used to understand the transition steps in the NHEJ mechanism. State-of-the-art cryo-EM facilities will be utilised, and training provided. Once structures are solved, the importance of key interacting protein residues within the structures will be determined using mutagenesis and biophysical assays. Furthermore, functional and *in vivo* experiments will be carried out with collaborators to determine the importance of these interactions for efficient DNA repair in the cell. Overall, this PhD proposal will aim to determine the detailed mechanism of NHEJ, which will have huge benefit for designing future therapeutics.  |
| **References** |
| 1 Aplan, P. D. *Trends Genet* **22**, 46-55, (2006).2 Zhao, B., Rothenberg, E., Ramsden, D. A. & Lieber, M. R. *Nat Rev Mol Cell Biol* **21**, 765-781, (2020).3 Stinson, B. M. & Loparo, J. J. *Annu Rev Biochem* **90**, 137-164, (2021).4 Chaplin, A. K., Hardwick, S. W., Liang, S., Kefala Stavridi, A., Hnizda, A., Cooper, L. R., De Oliveira, T. M., Chirgadze, D. Y. & Blundell, T. L. *Nat Struct Mol Biol* **28**, 13-19, (2020).5 Chaplin, A. K., Hardwick, S. W., Stavridi, A. K., Buehl, C. J., Goff, N. J., Ropars, V., Liang, S., De Oliveira, T. M., Chirgadze, D. Y., Meek, K. *et al.* *Mol Cell* **81**, 3400-3409 e3403, (2021).6 Chen, S., Lee, L., Naila, T., Fishbain, S., Wang, A., Tomkinson, A. E., Lees-Miller, S. P. & He, Y. *Nature* **593**, 294-298, (2021).7 Graham, T. G., Walter, J. C. & Loparo, J. J. *Mol Cell* **61**, 850-858, (2016). |