ERASMUS UNIVERSITEIT ROTTERDAM

Erasmus University Rotterdam, the Netherlands
CSC PhD 2015 Project Description

School/Department:	ErasmusMC, Dept. of Genetics
Project Title:	Molecular switches for DNA mismatch repair: a biochemical and biophysical characterization of the activation mechanism of the MutL ATPase
Abstract:	DNA mismatch repair is essential for genome stability because it repairs replication errors and prevents illegitimate recombination. The basic pathway is conserved from prokaryotes to humans and loss of mismatch repair results in a mutator phenotype and an increased risk to develop cancer (HNPCC). Mismatch repair is initiated when MutS recognizes a mispaired base. MutS binds ATP and undergoes a conformational change that allows recruitment of MutL. These proteins also bind ATP, required to activate downstream activities that create a nick in the daughter strand containing the incorrect base, and that unwind and degrade the DNA. Over the years we have provided insights into mechanistic details of the mismatch-activated MutS molecular switch (see publication list references 2, 3, 8, 9). However, many aspects of the MutL molecular switch, such as complex formation with MutS, timing of events and consequences for downstream protein recruitment are not resolved. We are now able to address activation of the MutL molecular switch in more detail using new biochemical approaches developed in our laboratory and in collaboration with Peter Friedhoff in Giessen. We reconstitute partial mismatch repair reactions *in vitro* with purified proteins, to quantify enzymatic actions on DNA, to monitor conformational changes or to determine the structure of reaction intermediates. We are at the forefront of state-of-the-art integration of different techniques, combining kinetic parameters with identity and architectural organisation of intermediates. This is a unique combination of functional, conformational and structural approaches. **Our overall aim is to understand the molecular details of the activation of the MutL molecular switch and its consequences for initiation of mismatch repair and Lynch syndrome.** This will contribute to a new understanding of activation steps controlling DNA mismatch repair and problems that occur in Lynch syndrome patients.

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Requirements of candidate:	Background: The candidate should have an interest in the molecular mechanism of DNA repair or related processes. The candidate should have been educated in Biochemistry, Biophysics or Structural Biology. Techniques such as cloning, cell culture, protein purification, enzymatic assays, functional DNA modifying assays, surface plasmon resonance, spectofluorimetry and scanning force microscopy will be employed during the project. Master degree: Yes IELTS Grade: 7.0 *(minimal 6.0 per component)* *or* TOEFL: *100 (minimal 20 per component)*
Supervisor information:	Assistant Professor Dr. ir. JHG Lebbink Email address: j.lebbink@erasmusmc.nl Personal website: http://www.erasmusmc.nl/MScMM/faculty/CVs/lebbink_cv Recent publication list: 1. Wong TH, Chiu WZ, Breedveld GJ, Li KW, Hondius D, Hukema RK, Seelaar H, Frick P, Lammers G, Verkerk AJMH, Lebbink JHG, Kamphorst W, Rozemuller A, Netherlands Brain Bank, Bakker EB, The International Parkinsonism Genetics Network, Neumann M, Willemsen R, Bonifati V, Smit AB, van Swieten J. Mutation in the *PRKAR1B* gene associated with a new autosomal dominant neurodegenerative disorder with unique pathology. **BRAIN** 137, 1361-1373, 2014. 2. Tham KC, Hermans N, Winterwerp HHK, Cox MC, Wyman C, Kanaar R and Lebbink JHG. Mismatch Repair Inhibits Homeologous Recombination via Coordinated Directional Unwinding of Trapped DNA Structures. **Mol. Cell**. 51, 326-337, 2013. 3. Groothuizen FS, Fish A, Petoukhov MV, Reumer A, Manelyte L, Winterwerp HHK, Marinus MG, Lebbink JHG, Svergun DI, Friedhoff P and Sixma TK. Using stable MutS dimers and tetramers to quantitatively analyze DNA mismatch recognition and sliding clamp formation. **Nucleic Acids Res**. 14, 8166-8181, 2013. 4. Talens S, Lebbink JHG, Malfliet JJ, Demmers JA, Uitte de Willige S, Leebeek FW, Rijken D. Binding of carboxypeptidase N to fibrinogen and fibrin. **Biochem. Biophys. Res. Comm.** 427, 421-425,

	2012. 5. Laventie B, Rademaker HJ, Saleh M, de Boer E, Janssens R, Bourcier T, Subilia A, Marcellin L, van Haperen R, Lebbink JHG, Chen T, Prévost G, Grosveld F and Drabek D. Heavy Chain Only Antibodies and Tetravalent Bi-specific Antibody Neutralizing Staphylococcus aureus leucotoxins. **Proc. Nat. Acad. Sci**. 108, 16404-16409, 2011. 6. Xiao Y, Jung C, Marx AD, Winkler I, Wyman C, Lebbink JHG, Friedhoff P, Cristovao M. Generation of DNA nano-circles containing mismatched bases. **Biotechniques** 51, 259-265, 2011. 7. Wyman C, Lebbink JHG, Kanaar R. Mre11-Rad50 complex crystals suggest molecular calisthenics. **DNA Repair** 10, 1066-1070, 2011. 8. Monti MC, Cohen SX, Fish A, Winterwerp HHK, Barendregt A, Friedhoff P, Perrakis A, Heck AJR, Sixma TK, van den Heuvel RHH and Lebbink JHG. Native mass spectrometry provides direct evidence for DNA mismatch induced regulation of asymmetric nucleotide binding in mismatch repair protein MutS. **Nucleic Acids Res.** 39, 8052-8064, 2011. 9. Lebbink JHG*, Fish A, Reumer A, Natrajan G, Winterwerp HHK, Sixma TK. Magnesium coordination controls the molecular switch function of DNA mismatch repair protein MutS. **J Biol Chem** 285, 13131-13141, 2010. **Shared corresponding author.* 10. De Vlaminck I, Vidic I, van Loenhout M, Kanaar R, Lebbink JHG and Dekker C. Torsional regulation of hRPA-induced unwinding of double stranded DNA. **Nucleic Acids Res** 38, 4133-4142, 2010.