# Cryoem Reveals the Mechanism of Mediator Driven Rad51 filament Formation in Homologous Recombination.

Dr Jaigeeth Deveryshetty[1], Dr Rahul Chadda[1], Ms Jenna Mattice[2], Mr Micheal Rau[3], Ms Simrithaa Karunakaran[1], Dr Nilisha Pokhrel[4], Mr Noah Englander[1], Prof James Fitzpatrick[3], Prof Brian Bothner[2], Prof Edwin Antony[1]

*[1]Saint Louis University, [2]Montana State University, [3]Washington University, [4]University, Marquette University*

***jaigeeth.deveryshetty.1@health.slu.edu***

Homologous recombination (HR) is a pathway for the accurate repair of double-stranded DNA breaks (DSBs). Unrepaired DSBs lead to gross chromosomal rearrangements and are a key driver of genomic instability. HR is a complex process orchestrated by over two dozen protein complexes, and mutations in HR-proteins (BRCA2, RAD51, and RAD52) are associated with genetic diseases and cancers. Cellular machinery on recognition, resects DSBs to yield long 3' single-strand DNA (ssDNA) overhangs. RAD51 is the recombinase, the enzymatic engine that drives the search for homology by forming an ATP dependent filament on ssDNA. Formation of the RAD51 filament is a critical step that commits the cell to HR. Thus, several mediator proteins (BRCA2, PALB2 and RAD52) regulate this step. Rad52 promotes HR by facilitating formation of Rad51 nucleoprotein filament on ssDNA. CryoEM structure shows that full length yeast Rad52 functions as a homodecamer. The N-terminal half of Rad52 is a well-ordered ring, while the C-terminal half is disordered. Apart from the N-terminal half, the disordered C-terminal half engages ssDNA and influences ssDNA binding function of Rad52. Based on HDX-MS, Crosslinking-MS and CryoEM analyses, the disordered C-terminal half appears to drive an intrinsic asymmetry within Rad52, where one or a few of the C-terminal halves interacts with the ordered N-terminal ring. Within the C- terminal half, we define two conserved positive and negative charged patches that harbor the Rad51 and RPA interacting motifs. Interactions between these two charged patches promote asymmetry and regulate a two-stage ssDNA binding mechanism. Interestingly, the intrinsic asymmetry allosterically drives Rad51 binding to a single position on the Rad52 ring. We propose the asymmetric C-terminal Rad52 catalyzed single-position nucleation model for the formation of pre-synaptic Rad51 filaments in HR.

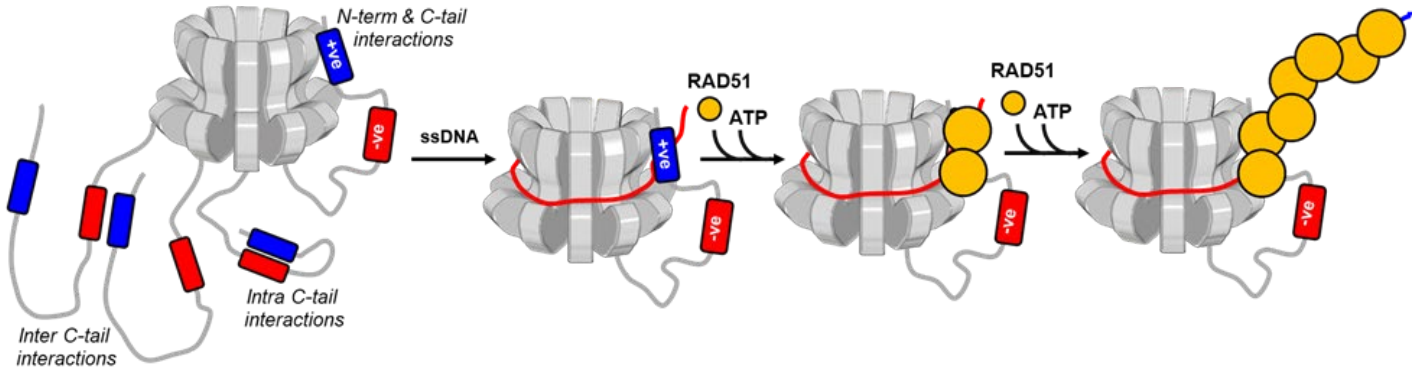


***Figure 1***