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Abstract

Intrinsic and extrinsic stimuli can cause DNA double strand breaks (DSBs), culminating in the assembly of a molecular machine that initiates the DNA Damage Response (DDR) pathway and maintains genome integrity. Mutations in DDR components lead to genomic instability, a hallmark of cancer. Homologous recombination (HR) provides complete and accurate repair of the DNA, and it starts by recruiting the PARP-1 molecule to the damaged site. Once bound, PARP-1 PARylates itself and other proteins, acting as a scaffold in the nucleus. Once repair has occurred, its activity is subsequently downregulated by the cytoplasmic endoglycohydrolase PARG. Currently, little is known about the downstream effect of PARP-1 recruitment during HR and the effect that compartmentalization has on PARylation of PARP-1 substrates. Using RuleBender, a general and a compartmental pharmacodynamic model were created to address these gaps. Preliminary data compared the concentrations of PARylated XRCC1, a substrate of PARylated PARP-1, over time in the two models. We conclude that when accounting for compartments, a delay in the rise and fall of the concentration is observed when running a simulation with a PARG turnover rate equal to 1 s^{-1} . Increasing the turnover rate for PARG seems to eliminate this delay and generate a distinct second peak in the concentration of PARylated XRCC1 in the compartmental model, possibly explained by the reversible translocation and inactivation of PARG. Once validated, new PARP-1 inhibitors can be placed into these models to be further analyzed and have their effects in DDR evaluated.

Introduction

DNA Damage: Types and Repair Pathways

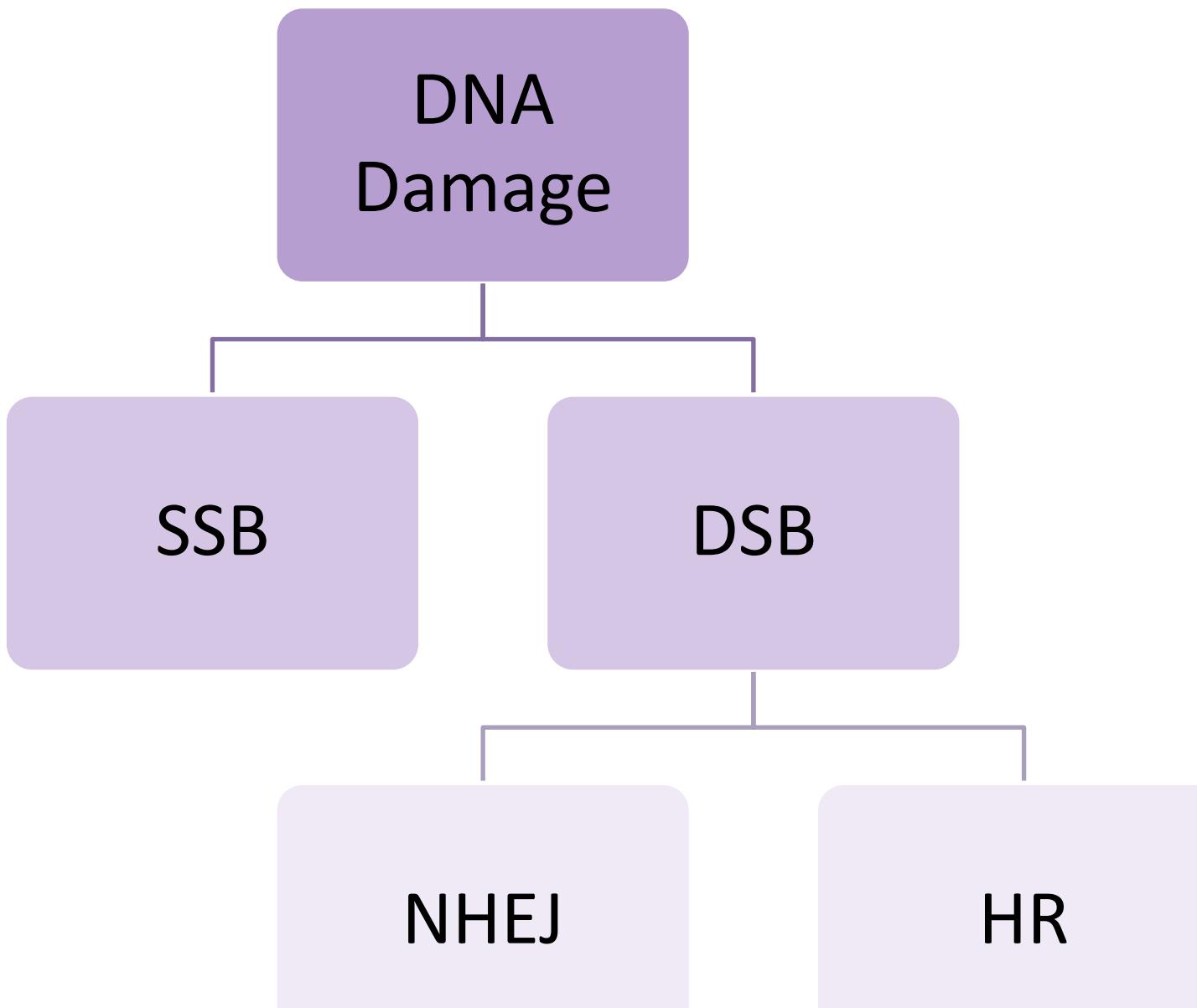


Figure 1. DNA damage can be split into two types: single and double strand breaks, and PARP-1 is known to be involved in the repair of both, though differences exist. The repair mechanisms of SSBs have been widely studied and are well understood. As for DSBs, repair via NHEJ (non-homologous end joining) or HR is possible, but while PARP-1 has been previously identified in both, its role has not been explained in as much detail for the latter.

Overview of HR Pathway for DSB Repair

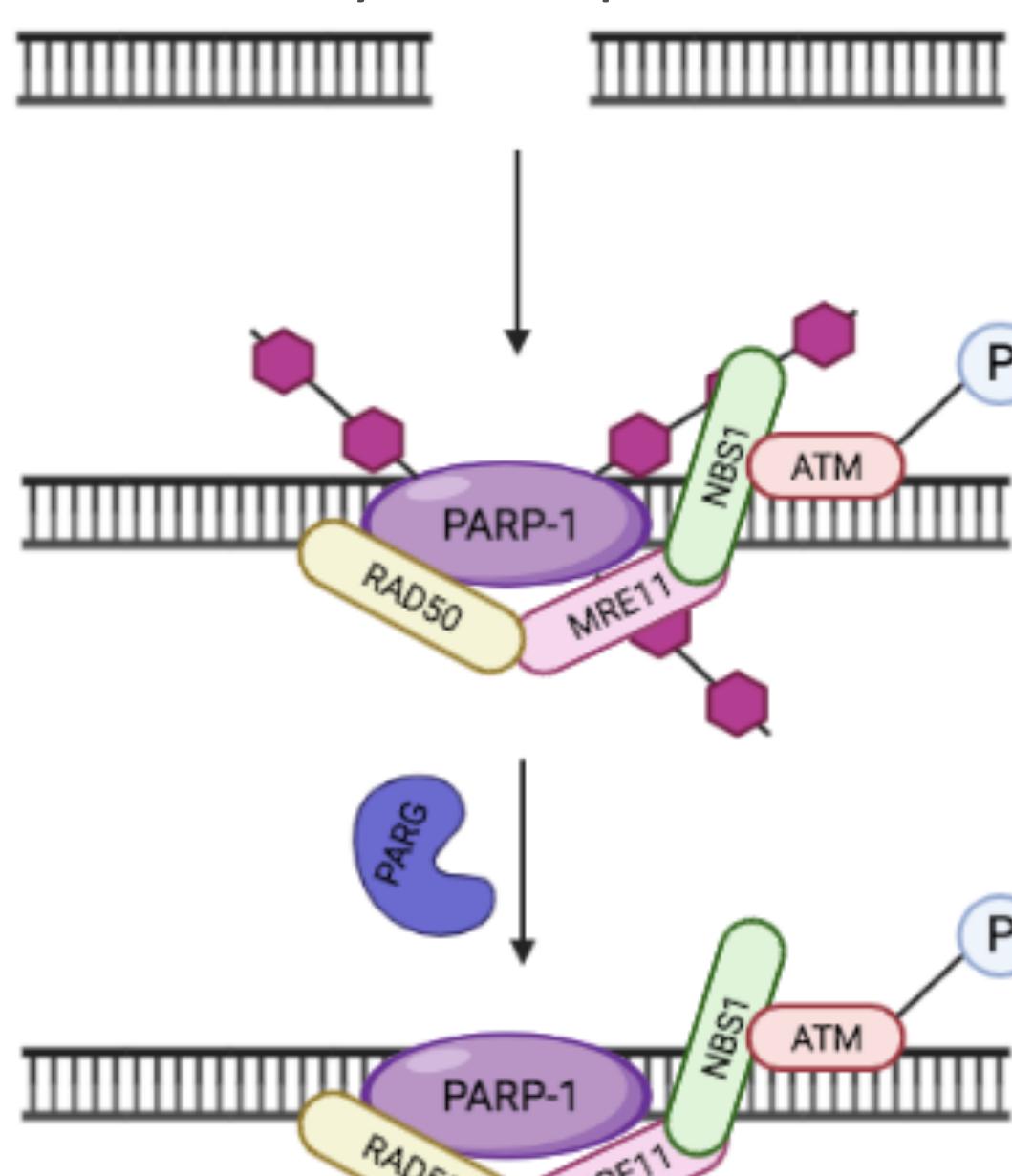


Figure 2. Once PARP-1 binds to the DSB of DNA, it initiates the HR repair pathway, PARylating itself and recruiting the MRN complex (MRE11, RAD50, and NBS1). The pathway results in complete and accurate repair of the DNA after PARG removes the ADP-ribose chains and downregulates the model.

Domain Architecture and Compartments

Domain Architecture of PARP-1, MRN Complex, and ATM

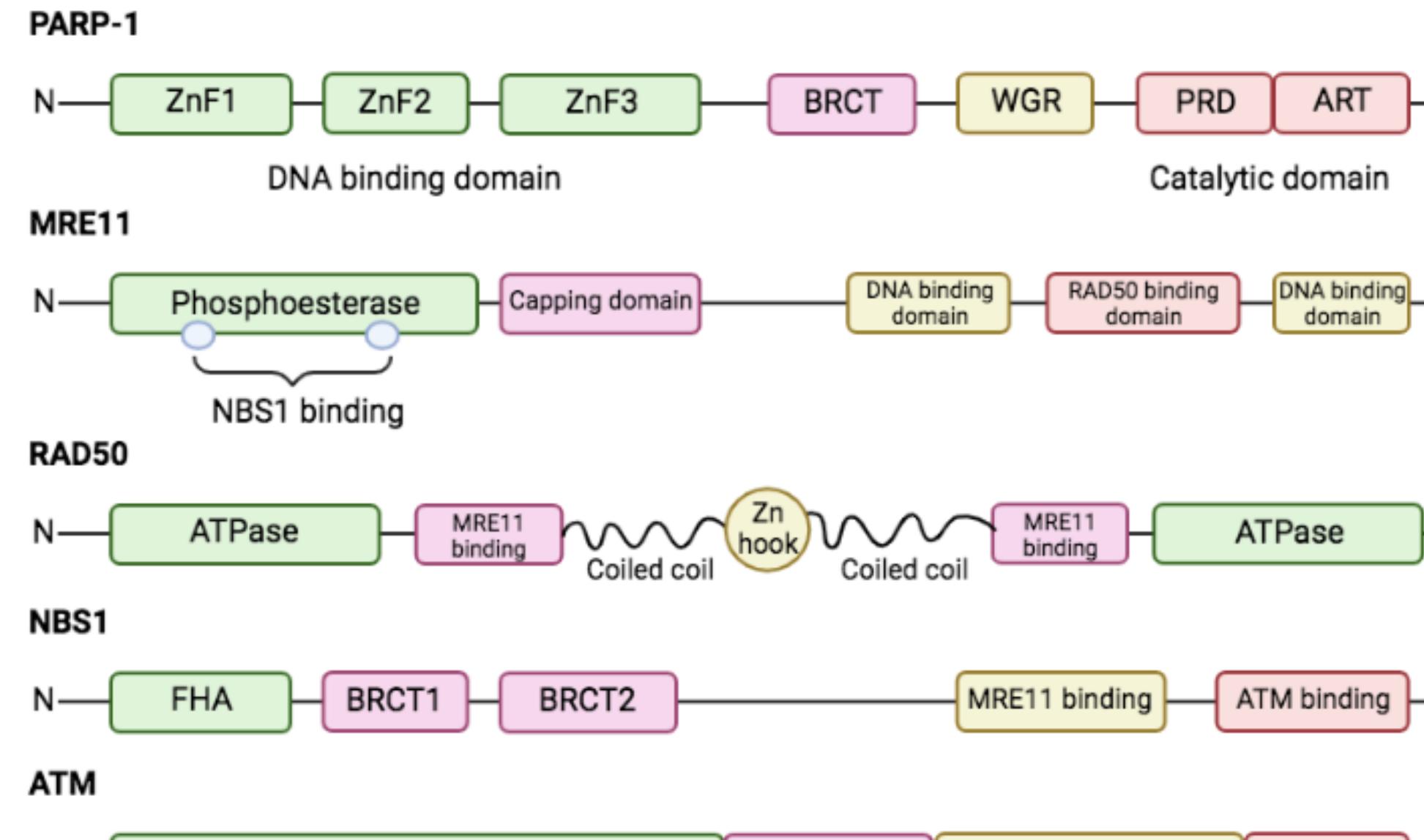


Figure 3. Domains relevant to our HR pathway model include: (1) DNA binding domain of PARP-1, (2) ATM binding domain of NBS1, and (3) Heat and kinase domains of ATM. Other domains are important, but are not included in the initial model.

Molecules and Their Compartments

Molecule	Compartment	Translocation?
DNA		
PARP-1	Nucleus	
XRCC1	Nucleus	
MRE11	Nucleus	
RAD50	Nucleus	
NBS1	Nucleus	
ATM	Nucleus	
PARG103	Cytoplasm	Cytoplasm \leftrightarrow Nucleus

Contact Map

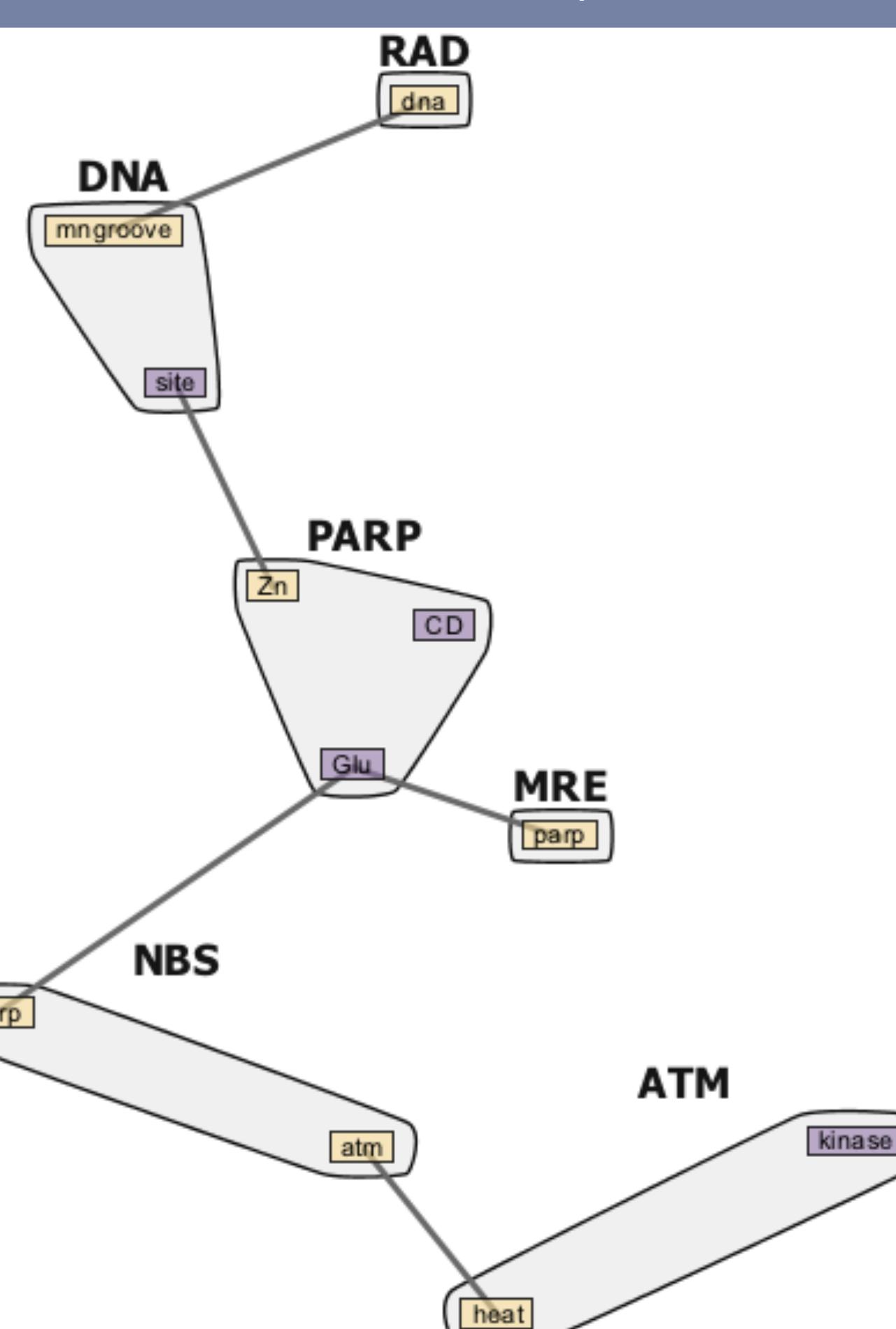


Figure 4. It is not until PARP-1 binds that the HR repair pathway initiates. PARP-1 binds to the DSB through its N-terminal zinc finger domains and auto-PARylates, becoming enzymatically activated, as the WGR domain moves and unblocks the catalytic site. Meanwhile, PARP-1 also PARylates other substrates, such as XRCC1. MRE11 binds to PARP-1 PAR chain, along with NBS1, while RAD50 binds to DNA through the minor groove. ATM binds to C-terminus of NBS1 and becomes activated, phosphorylating CHEK2, which in turn phosphorylates p53, preventing its degradation by MDM2. Active CKII activates PARG103, which translocates from the cytoplasm to the nucleus and removes PAR chains.

Modeling and Results

Proposed HR pathway

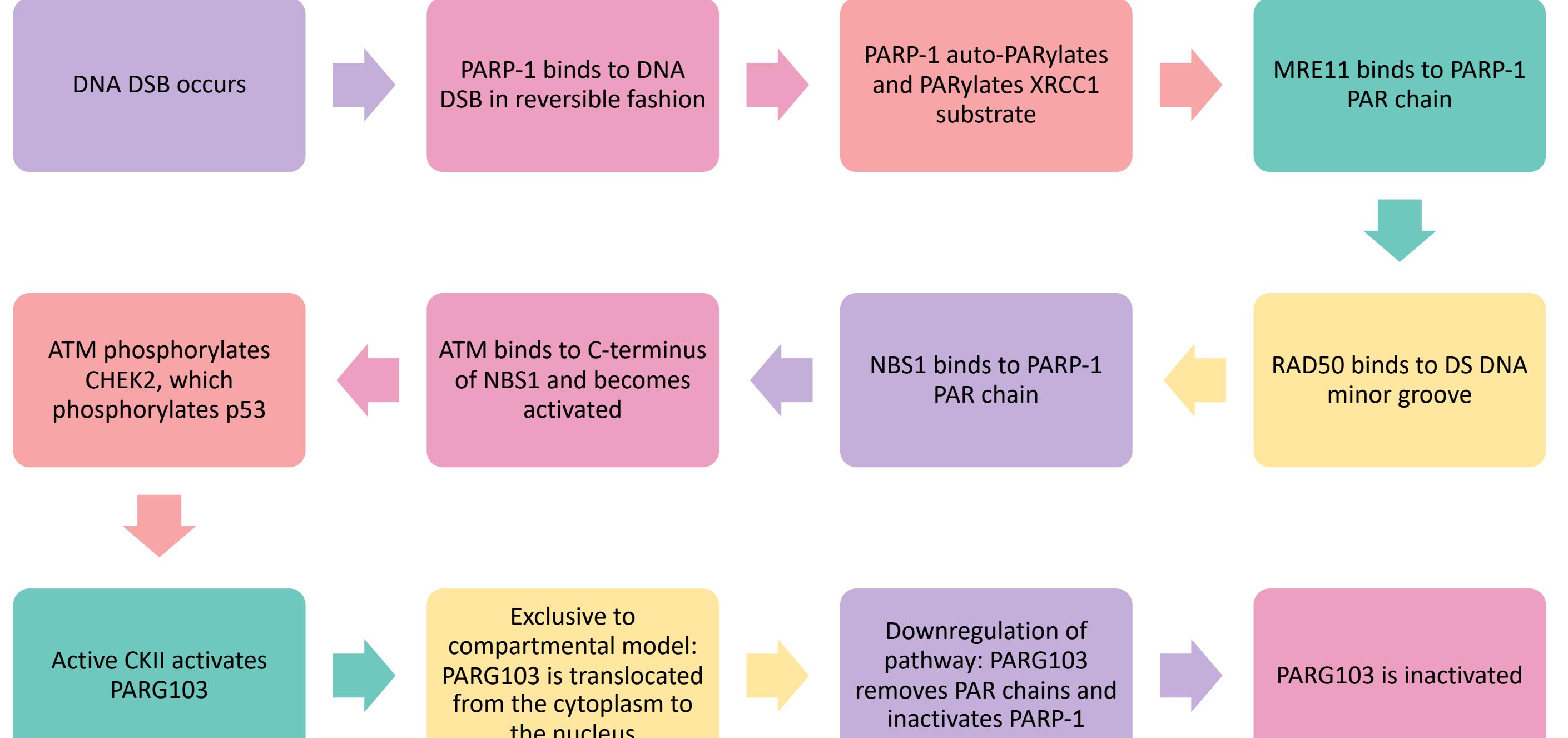
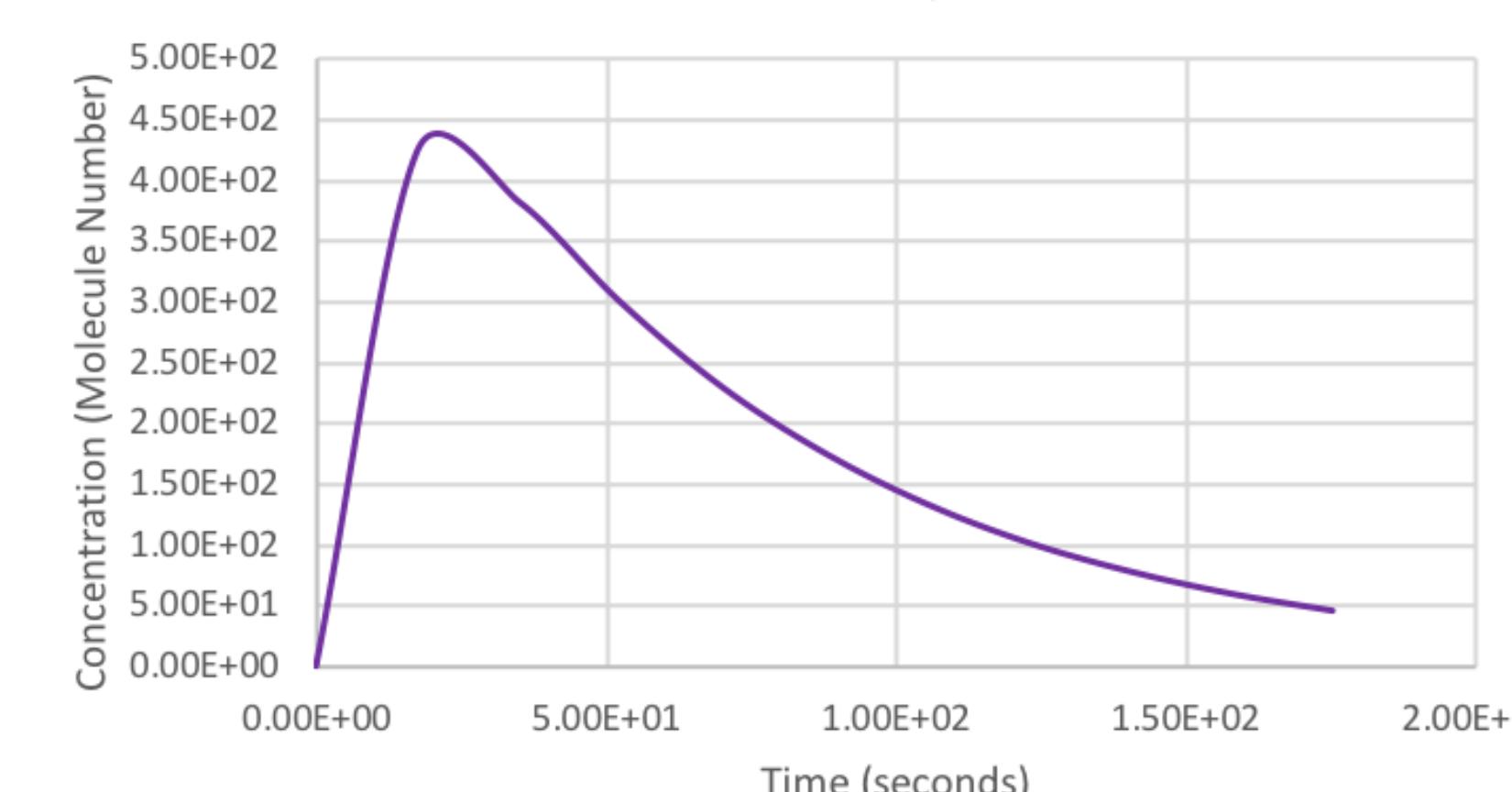


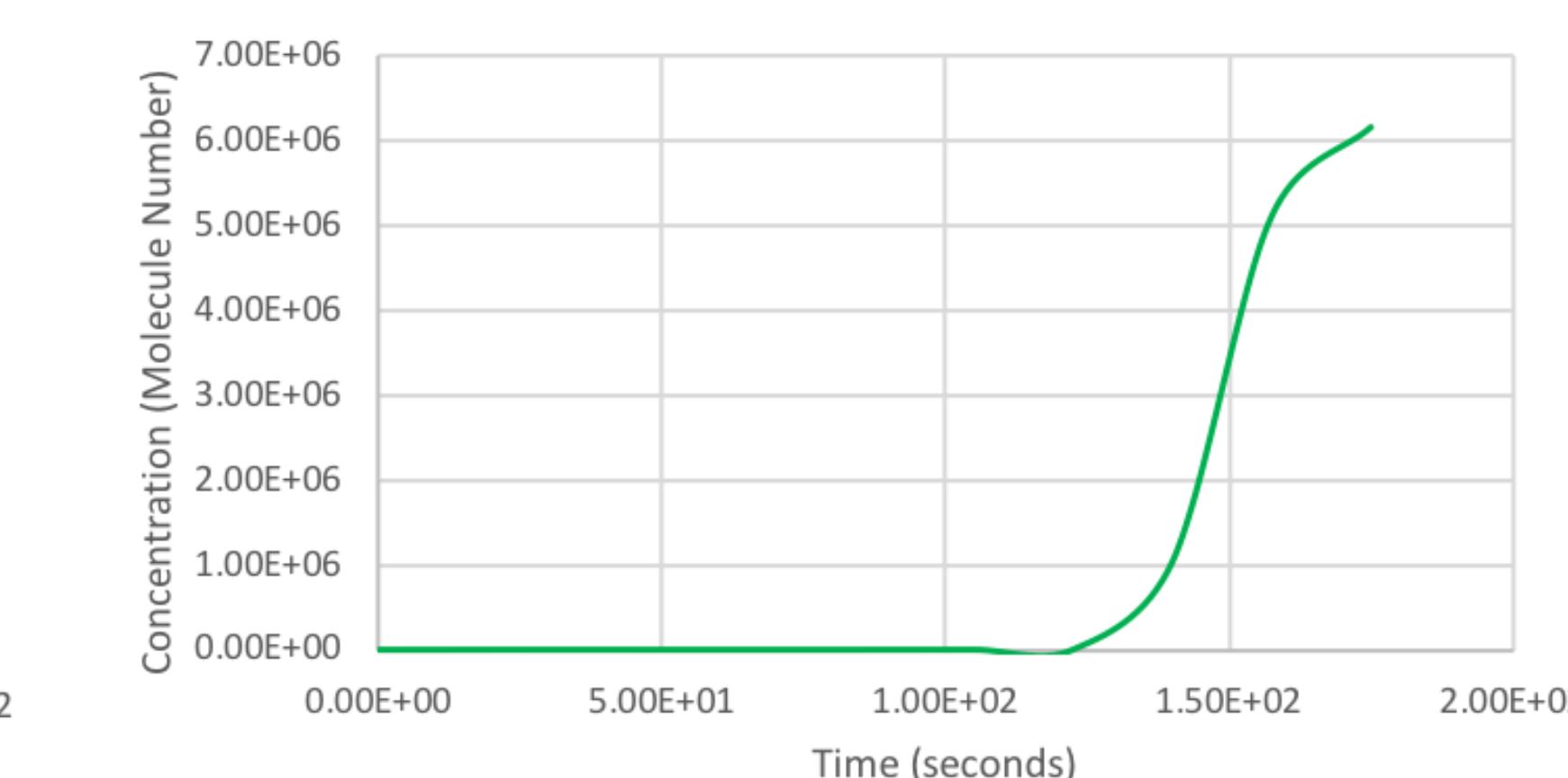
Figure 5. Step-by-step designed to write the reactions and build the models. Note that the translocation step of PARG103 is exclusive to the compartmental model and was not included in the general one.

Comparison of compartmental and general (no compartments) models

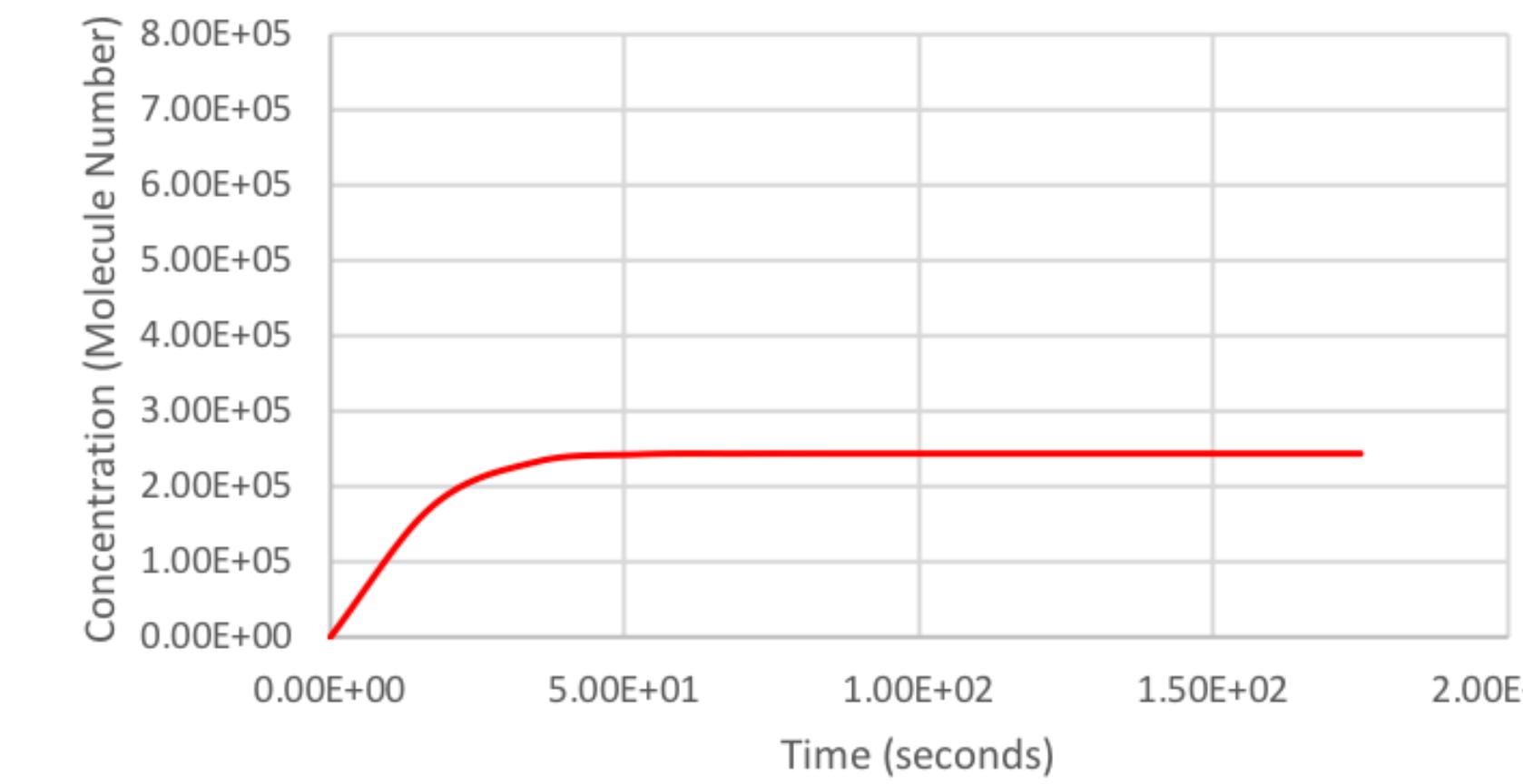
1) General Model: PARylated XRCC1



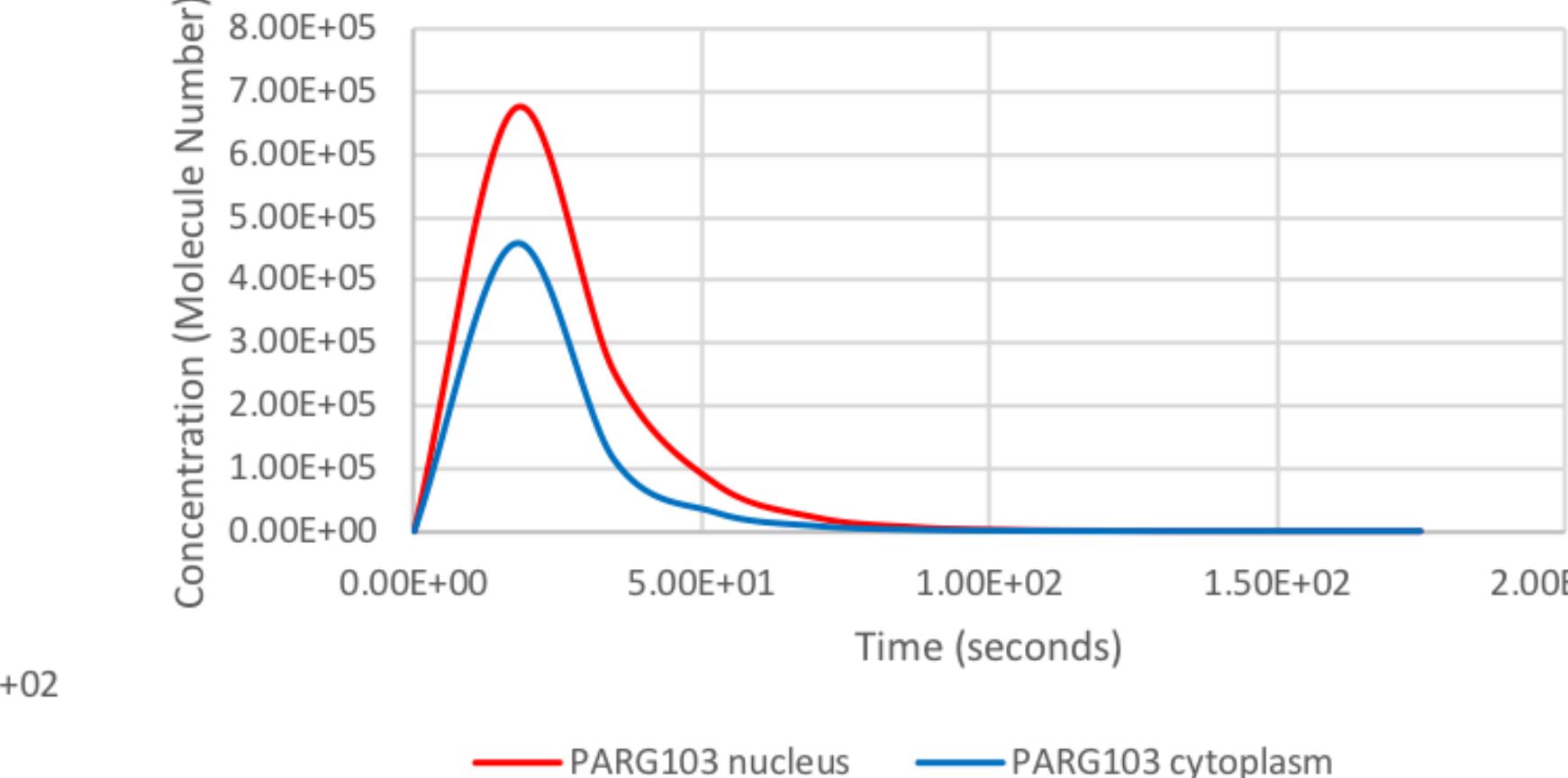
2) Compartmental Model: PARylated XRCC1



3a) General Model: Active PARG103



3b) Compartmental Model: Active PARG103



4) General + Compartmental Model: PARylated XRCC1 ($k_{cat5} = 5.0$)

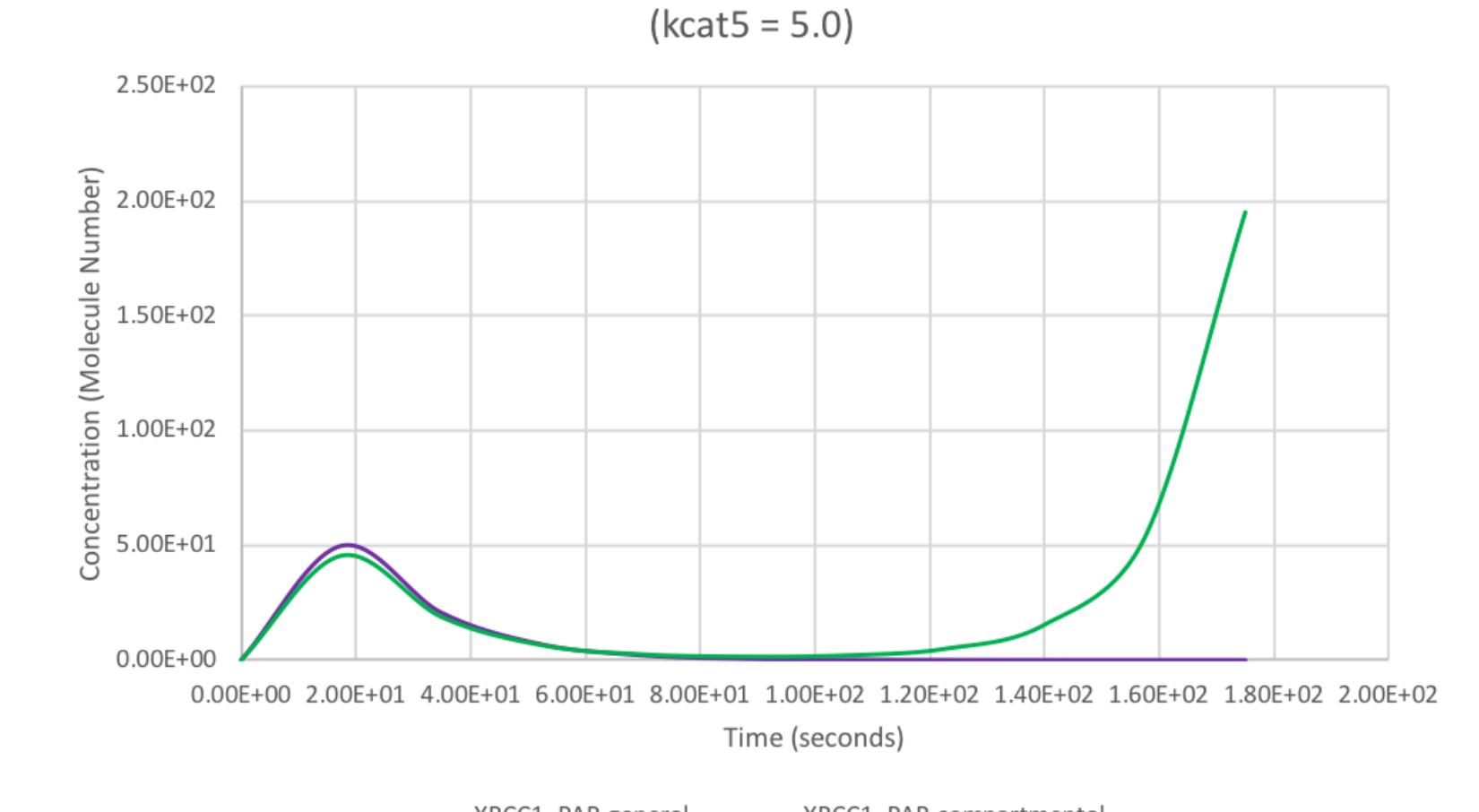


Figure 6. Comparison of compartmental and general (no compartments) models. Graphs 1 and 2 show the concentration of PARylated XRCC1 over time, a substrate that is PARylated by PARP-1 after it auto-PARylates itself. Graph 3a shows the change in concentration of active PARG103 in the general model, while Graph 3b shows the same but in the compartmental model (red and blue for nuclear and cytoplasmic compartments, respectively, indicating the translocation). Graphs 4-5 represent a simulation ran using $k_{cat5} = 1.0 \text{ s}^{-1}$ (turnover rate for PARG molecule). Graphs 4 and 5 show a compilation of PARylated XRCC1 in both models using $k_{cat5} = 5.0 \text{ s}^{-1}$ and 10.0 s^{-1} , respectively.

Conclusion

Preliminary data were analyzed and the concentrations of PARylated XRCC1 over time were compared between the two models. In the general model, the concentration of the PARylated substrate of PARP-1 rises and falls as expected, as the concentration of active PARG increases over time. When accounting for compartments, a delay in rise and fall of PARylated XRCC1 concentration was expected, but not initially observed. To explain the absence of the delay, a limited parameter scan was conducted where the turnover rate of PARG was varied. The delay in PARylated XRCC1 was then observed at 1 s^{-1} and then found to be absent at 5 and 10 s^{-1} , where no delay was observed. Further, the timing of the initial peak at 5 and 10 s^{-1} was similar to that found for the general model. Increasing the turnover rate for the PARG molecule also generated a distinct second peak in the concentration of PARylated XRCC1 in the compartmental model, possibly explained by the reversible translocation, as well as inactivation, of PARG. Once PARG returns to the cytoplasm or is inactivated, the remaining PARylated PARP-1 molecules can continue to PARylate their substrate.

Future Aims

Going forward, both models will need to be adjusted so that new PARP-1 inhibitor molecules can be placed in them to be further analyzed and have their effects in DDR evaluated. It is important to keep in mind that the models only include basic steps of the HR pathway, and not all of them. In the future, other important steps, such as the phosphorylation and recruitment of H2AX, 53BP1, BRCA1, and CtIP, may be included. In addition, reaction rates, and possibly simulation's end time, will need to be adjusted so that the generated graphs indicate (1) the decrease in concentration of PARylated XRCC1 in the compartmental model, and (2) the decrease in concentration of active PARG103 in the general model.

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