Non-equilibrium free energy calculations accurately predict the molecular mechanism of a disease conferring mutation in proliferating cell nuclear antigen (PCNA)

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Abstract

Proliferating cell nuclear antigen (PCNA) is a key regulatory protein in human DNA metabolism [1,2]. PCNA forms a ring-like structure around the DNA that serves as a binding platform for a multitude of other proteins involved in DNA metabolism. Ataxia-telangiectasia-like disorder type 2 (ATLD2) is a neurodegenerative disease associated with impaired PCNA function. Recently, a mutation in the PCNA gene of ATLD2 patients was identified that encodes for the NM_002592.2(PCNA): c.443G > C(p.C148S) variant [3,4]. However, the molecular effect of this mutation was unclear. Here we used non-equilibrium (NEQ) alchemical free energy calculations to predict the effect of this single amino acid mutation on PCNA stability and on the interaction of PCNA with one of its binding partner's, p15 [5]. No change in binding affinity was predicted, while a significant decrease in the folding free energy was predicted for the $PCNA^{C148S}$ variant. These results were validated experimentally by differential scanning fluorimetry and a FRET-based PCNA-p15 interaction assay. The experiments confirmed a reduced folding free energy of the variant, while no direct influence of the mutation on the PCNA-p15 interaction is detected. However, the lower folding free energy of the variant caused a time-dependent denaturation of $PCNA^{C148S}$. This provides a possible molecular explanation for the association of the $PCNA^{C148S}$ variant with ATLD2. This study therefore underlines the effectiveness of NEQ alchemical free energy calculations in the analysis of disease conferring mutations.

Background

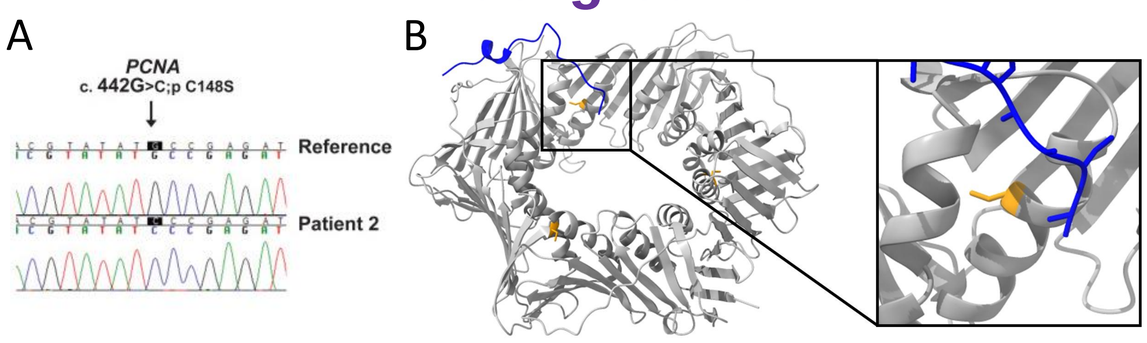
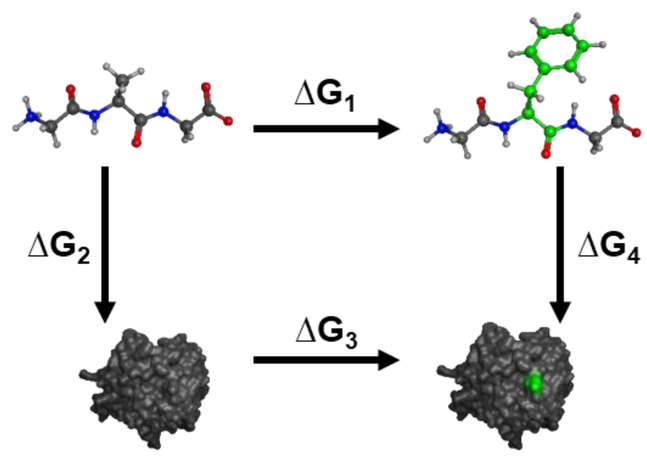


Fig. 1: (A) ATLD2, a neurodegenerative disease, is associate with the mutation $PCNA^{C148S}$, as revealed by Sanger sequencing of patients PCNA genes [4]. **(B)** Structure of PCNA (grey) in complex with $p15^{52-71}$ (blue). The position of C148 is shown in orange. C148 is located at the inner site of the PCNA ring, close to the interaction site of PCNA and p15.

Method



$$\Delta\Delta G_{folding} = \Delta G_2 - \Delta G_4 = \Delta G_1 - \Delta G_3$$

Fig. 2: Thermodynamic cycle adapted from [6] for the estimation of the change in folding/binding free energy (ΔΔG) upon amino acid mutation. The alchemical transitions between both end states were performed along the ΔG_1 arrow for the unfolded/bound and along the ΔG_2 arrow for the folded/unbound state.

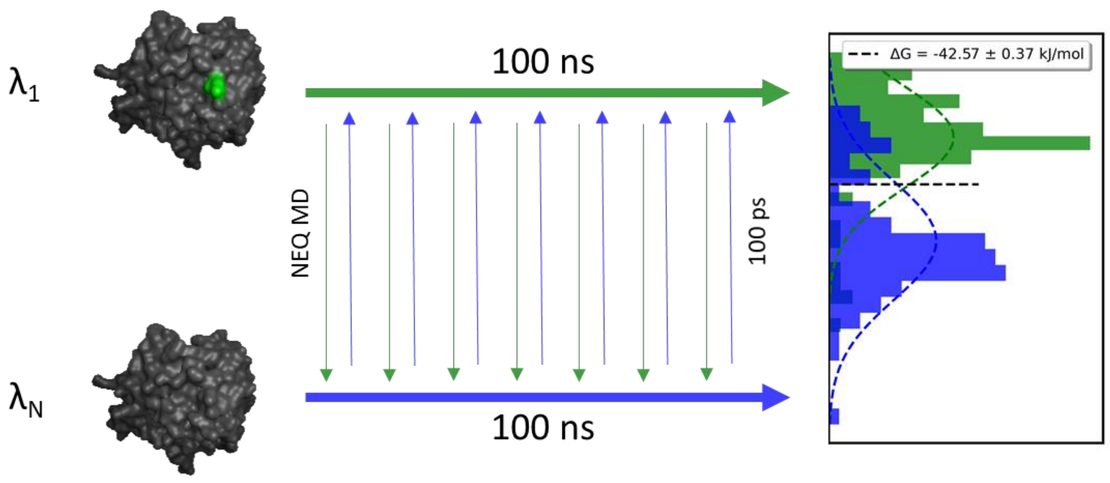


Fig. 3: Calculation of ΔG by NEQ alchemical free energy calculations. Equilibrium molecular dynamics (MD) simulations were performed for the physical end states (e.g. the WT and mutant protein). Many alchemical switching reactions were performed starting from conformations along the equilibrium simulation in both directions. The work distributions from these switching reactions are directly related to ΔG by the Crooks fluctuation theorem [7]. A comprehensive description of the *in silico* methods used can be found in [5].

Conclusion

In this study, we used NEQ alchemical free energy calculations to predict the functional consequences of the newly described PCNA mutation C148S, which is related to ATLD2. NEQ alchemical free energy calculations predicted that C148S does not effect the PCNA-p15-affinity, but the PCNA stability. Both could later be confirmed by experimental analysis of the melting point of PCNA and the determination of the binding constant of the PCNA-p15 interaction. We suspect that the disease is essentially driven by the reduced stability of $PCNA^{C148S}$, as this could lead to reduced levels of functional PCNA in ATLD2 patients. These results show the high accuracy of NEQ alchemical free energy calculations in the analysis of disease conferring mutations. They also provide new insights into the understanding of the ATLD2 disease and the development of new therapeutic approaches.

Results

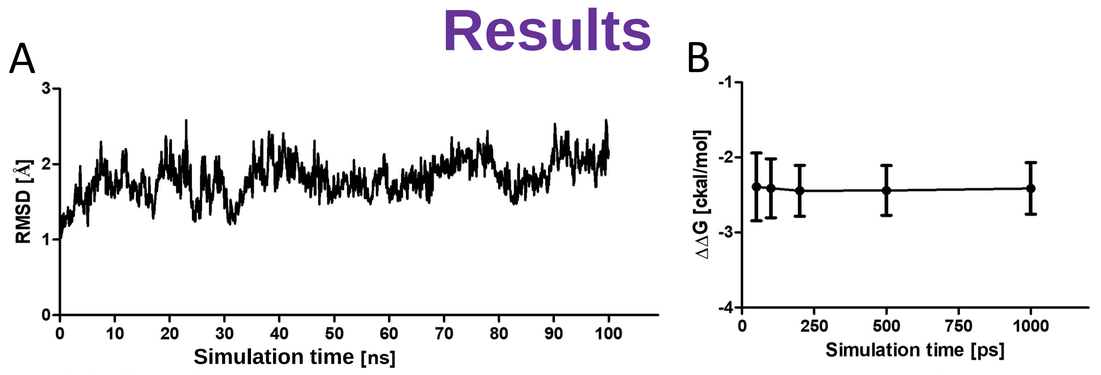


Fig. 4: (A) All simulations were performed with a single PCNA monomer. Prior to performing the NEQ alchemical free energy calculations stability of the monomer was verified by analysis of the backbone RMSD over 100 ns of MD simulation **(B)** Influence of simulation time of the NEQ MD on the calculated $\Delta\Delta G_{C148A}$. Based on this, a transition time of 100 ps (second data point) was deemed to be sufficient.

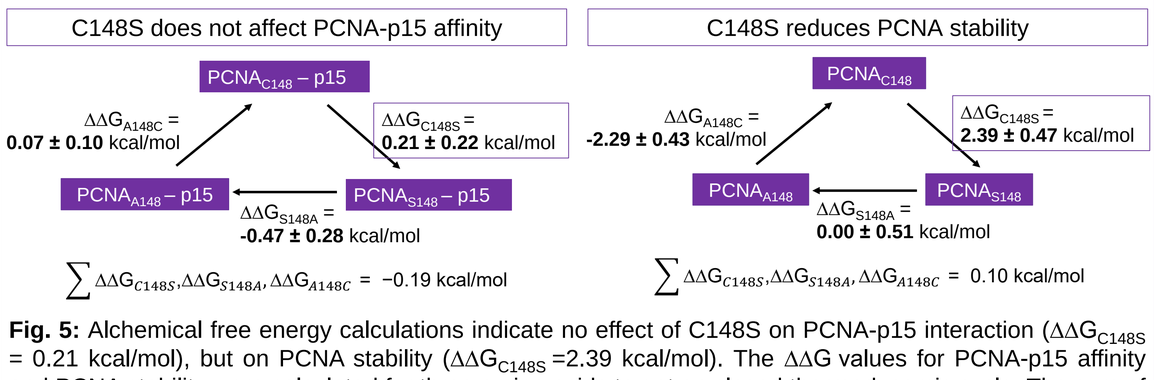
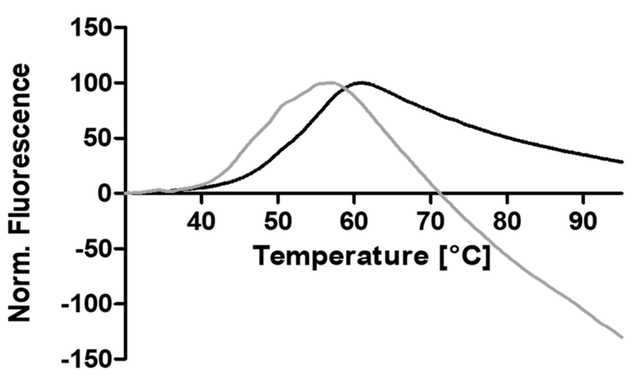


Fig. 5: Alchemical free energy calculations indicate no effect of C148S on PCNA-p15 interaction ($\Delta\Delta G_{C148S}$ = 0.21 kcal/mol), but on PCNA stability ($\Delta\Delta G_{C148S}$ =2.39 kcal/mol). The ΔΔG values for PCNA-p15 affinity and PCNA stability were calculated for three amino acids to get an closed thermodynamic cycle. The sum of all ΔΔG was 0.19 kcal/mol (affinity) and 0.10 kcal/mol (stability) demonstrating good convergence of the simulations.



	WT	C148S
T_m1 [°C]	49.5±0.10	n.d.
T_m2 [°C]	54.54±0.66	n.d.
ΔG [kcal/mol]	6.54±0.48	4.89 ±0.26

Fig. 6: Differential Scanning Fluorimetry measurements revealed a negative effect of C148S on the thermal stability of PCNA. The denaturation of $PCNA^{C148S}$ at lower temperatures as well as its lower ΔG value confirmed the destabilizing effect of C148S, predicted with NEQ alchemical free energy calculations.

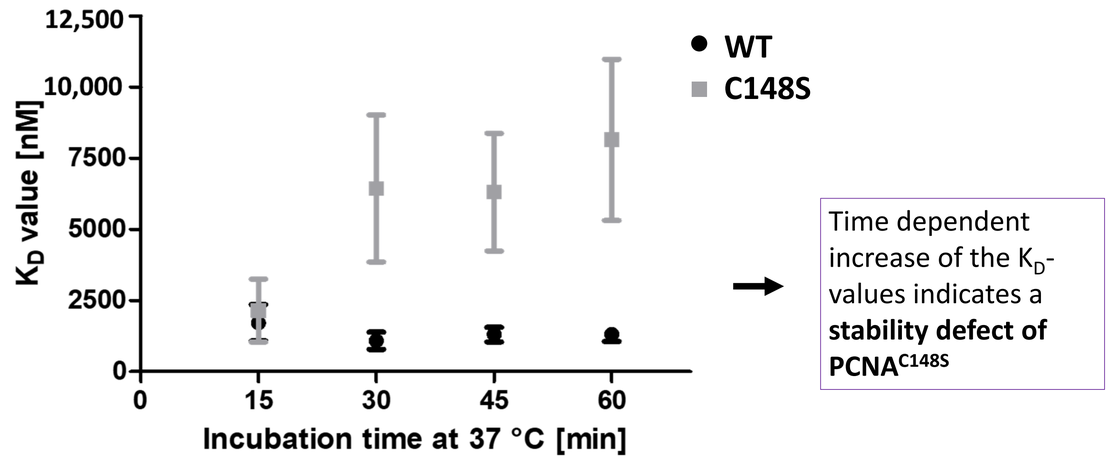


Fig. 7: K_D-values of the interaction between p15 and PCNA were determined after different incubation times with a FRET based interaction assay. The K_D-values of $PCNA^{WT}$ were constant, whereas the K_D-values of $PCNA^{C148S}$ increased with prolonged incubation at 37 °C . This confirms a stability defect of $PCNA^{C148S}$. It also confirms the prediction from NEQ alchemical free energy calculations that the mutation C148S has no direct influence of PCNA-p15 binding affinity.

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