We have analyzed wild-type and three mutants of cN-II (R367Q, R238W, L375F) with varying protein concentration (0.25 to 2 mg/ml). Each protein variant was measured in the full-length and C-terminally truncated forms to evaluate a topology of the C-terminal acidic tail. In addition, we have assessed the effect of ATP on the wild-type and R367Q proteins.
Guinier analysis and calculation of P(r) functions reveal that the mutants and wild-type do not significantly differ in their size under varying protein concentrations. These data are consistent with the observations from size exclusion chromatography and Blue-Native PAGE. Taken together, our data showed that mutants possess identical overall shape as the with-type protein and the perturbations at the oligomeric interface (observed by another structural biology techniques) do not cause changes in the stability of tetramer assembly.
The comparison of the protein with the full-length and truncated variant revealed no significant changes in a radius of gyration showing that the C-terminal tail forms unfolded segment. In addition, no differences were also reported for the effect of ATP. These data demonstrate that activation of the cN-II enzymes is not accompanied with formation of higher oligomers as was proposed in previous studies.
Data will be used in publication describing structural properties of cN-II (manuscript under preparation).