

Regulation of telomerase activity by macromolecular crowding

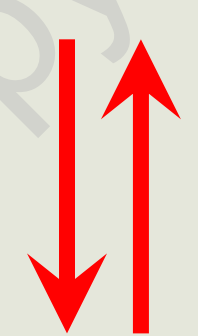
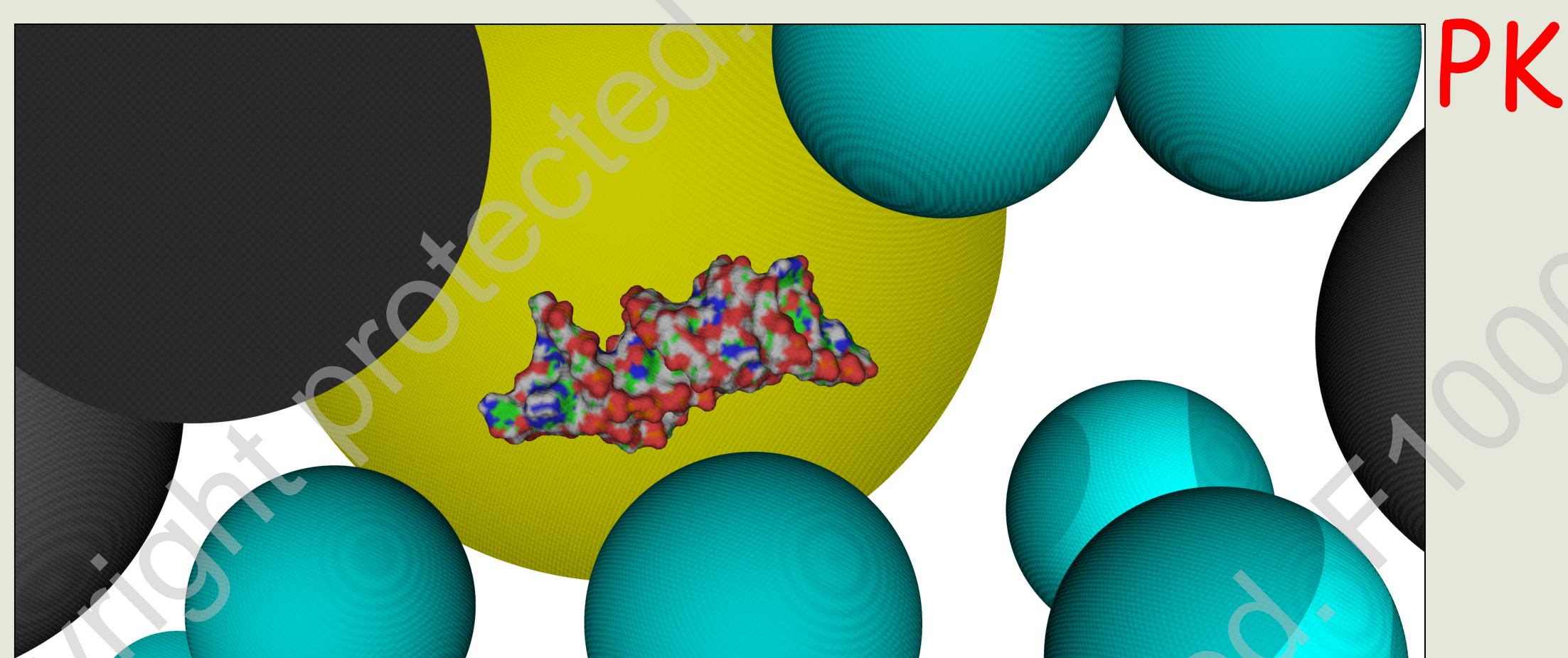
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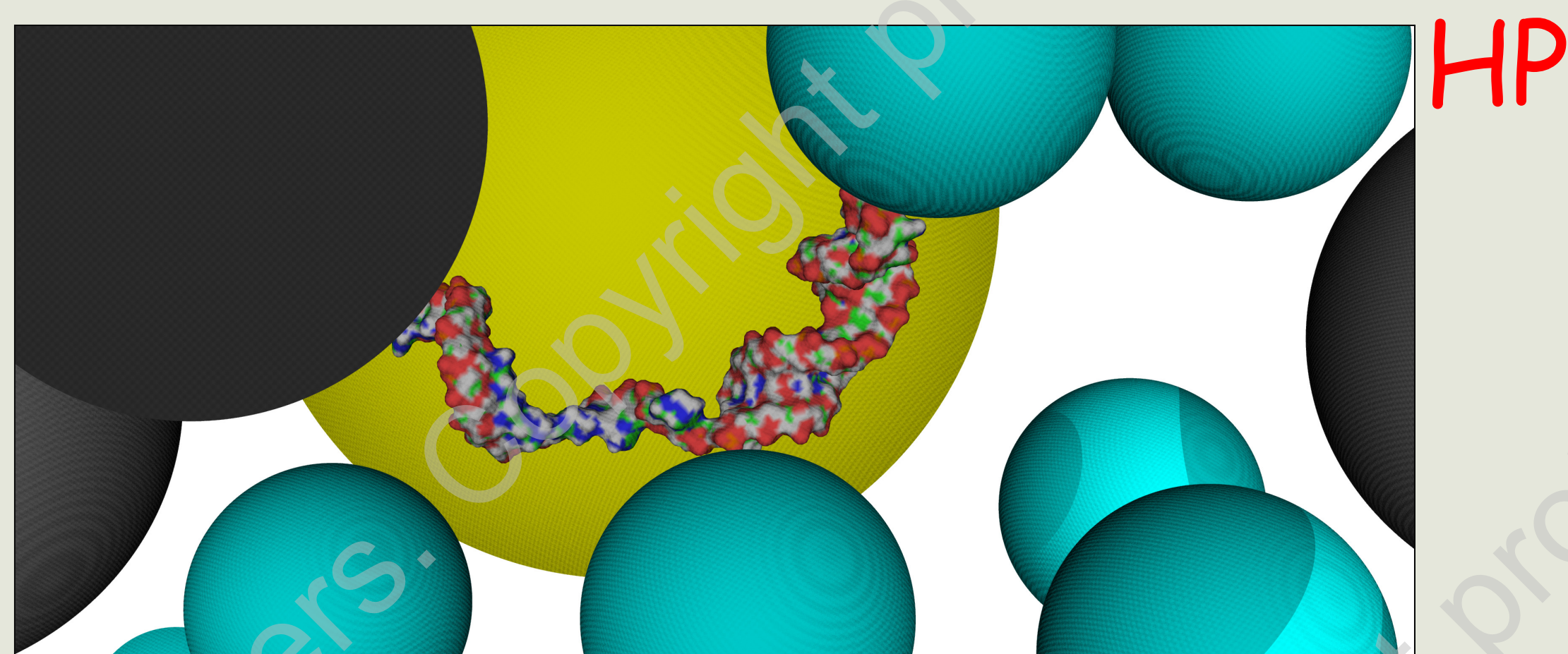
Telomerase activity is linked to a conformational switch

1. Human telomerase is a ribonucleoprotein responsible for maintaining telomeres
2. Up-regulated telomerase activity is associated with cancer
3. Down-regulated activity is related to dyskeratosis congenita (DKC) and aplastic anemia

A conserved pseudoknot domain of telomerase RNA (hTR) can exist in two alternative conformations, a pseudoknot (PK) and a hairpin (HP)

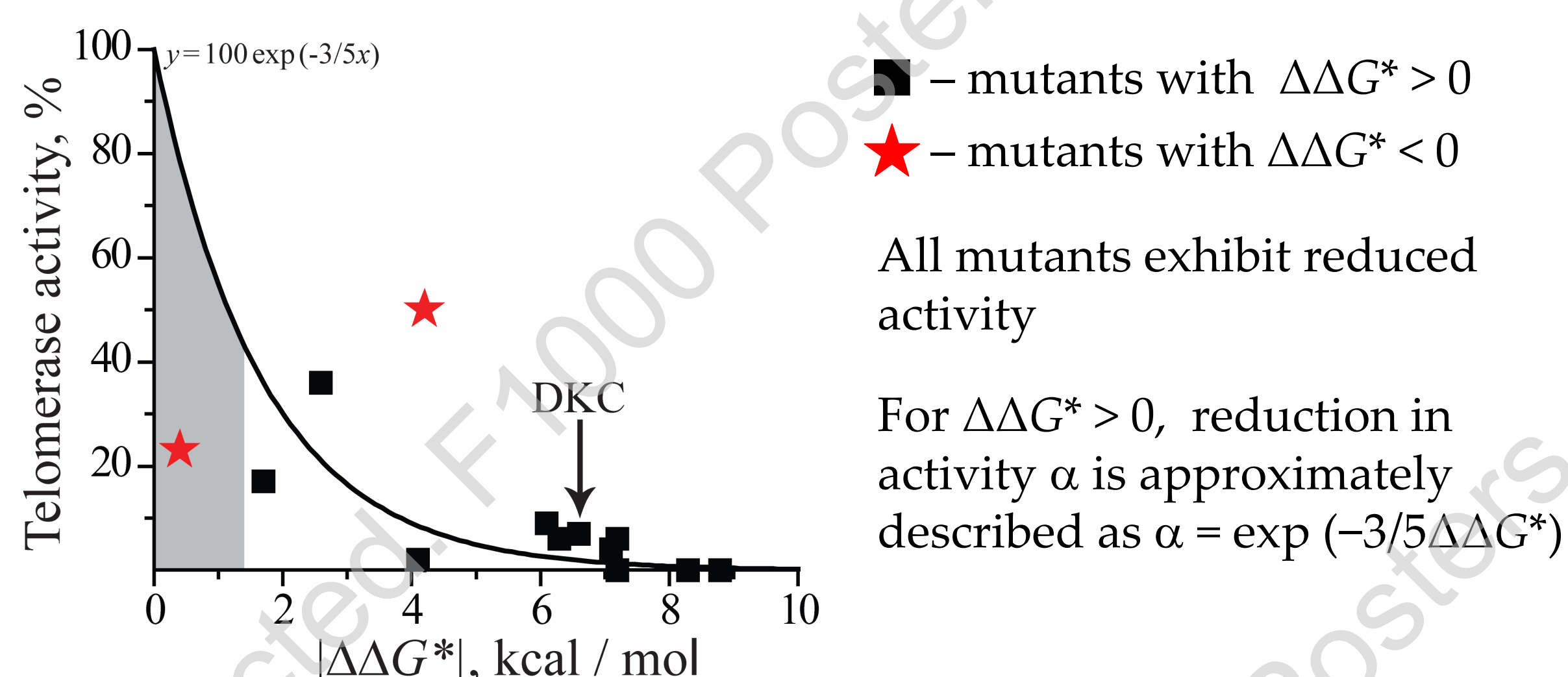


Interconversion between the PK and HP is linked to in vivo function of telomerase



Wild type (WT): ~ 95% of PK and 5% of HP
DKC mutant: ~ 50% of PK and 50% of HP

The figure below shows activity of mutant telomerase normalized to wild-type activity (100%), plotted vs. the absolute value of the stability difference $\Delta\Delta G^*$ between mutant and WT pseudoknots

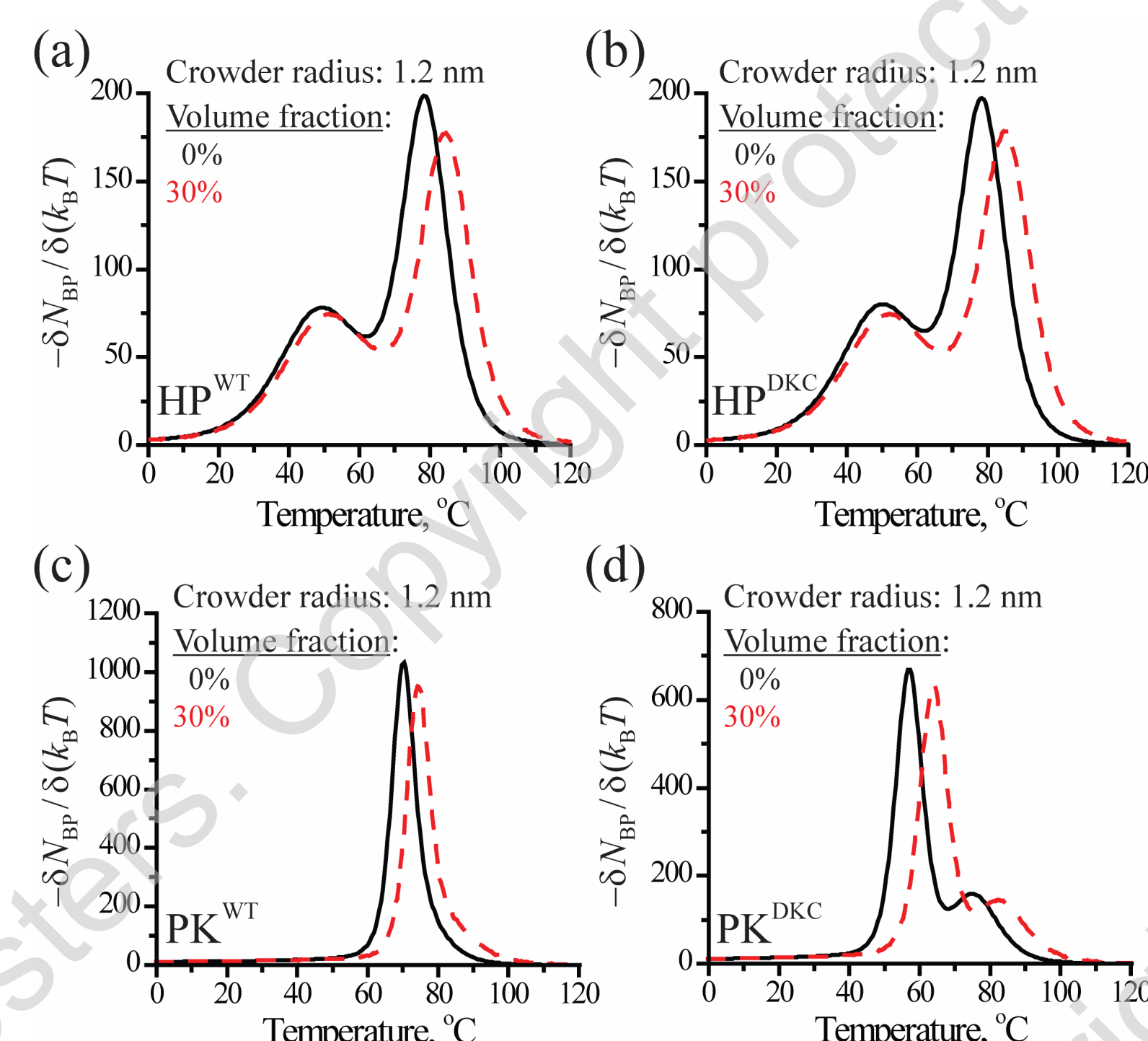


Macromolecular crowding stabilizes the pseudoknot and effectively reduces $\Delta\Delta G^*$ for mutants with $\Delta\Delta G^* > 0$

Grey area in the figure marks the range of potential mutants with $\Delta\Delta G^* > 0$ whose activity may be restored by crowding

Macromolecular crowding may affect telomerase activity!

Crowding promotes the compact PK



N_{BP} = base pairs formed

The figure at left shows that crowding increases the melting temperatures of both the pseudoknot and hairpin in the WT and in the DKC mutant

An increase in the melting temperature signifies an increase in the stability

For crowders with radius 1.2 nm and volume fraction 30%, the excess stabilities of the PK and HP due to crowding are -2.4 and -1.0 kcal/mol for the WT and -2.8 and -1.2 kcal/mol for the DKC mutant, at 37 °C

Crowders have a larger stabilizing effect on the pseudoknot

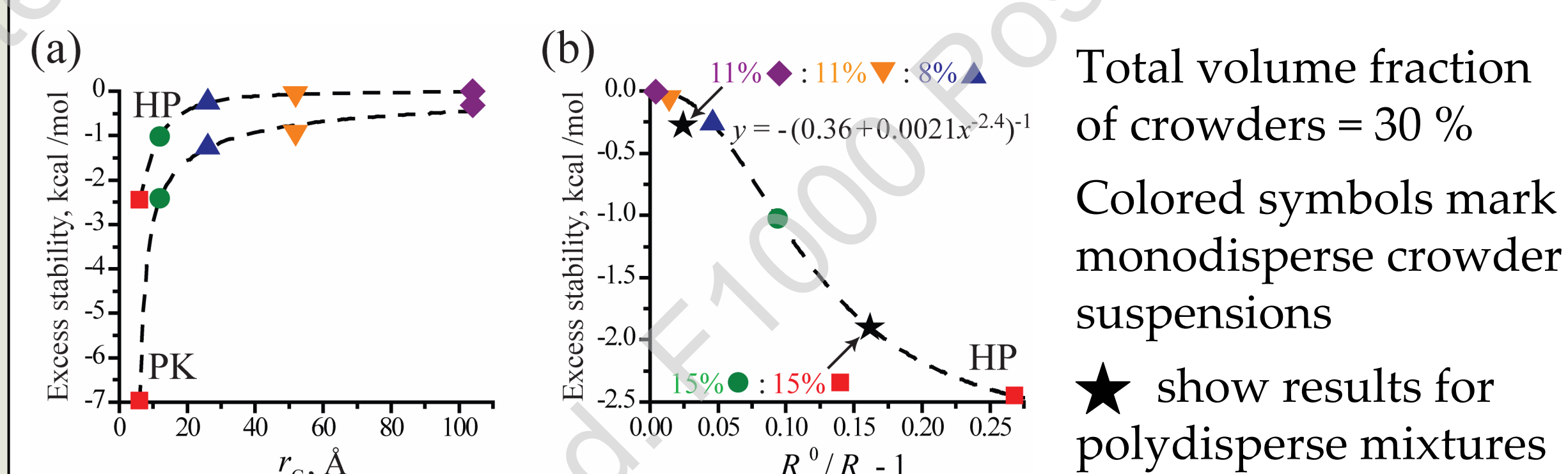
The excess stabilities due to crowding are similar for the WT and DKC

We conclude that crowding effect is sensitive to the RNA size, not sequence

Small crowders have the largest effect

In panel (a) below, we show the excess stability of RNA due to crowding as a function of the crowder radius r_C . The excess stability is small if $r_C > 20$ Å and increases sharply for $r_C < 20$ Å

The crossover value of 20 Å coincides with the radius of gyration of the unfolded RNA in the absence of crowders, R_G^0

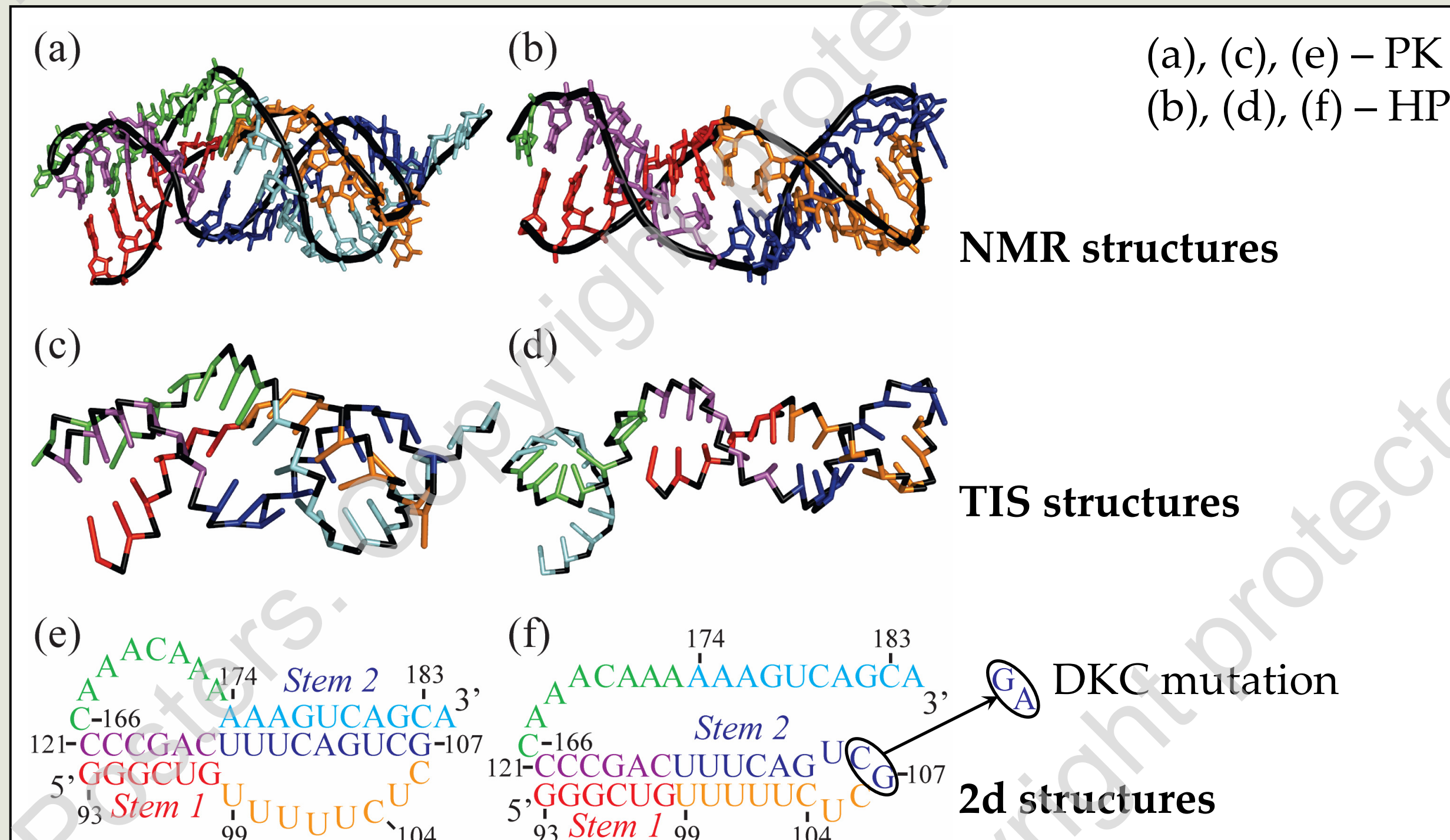


Panel (b) illustrates that the excess stability is related to conformational compaction of the unfolded state induced by crowders. R_G is the radius of gyration of the unfolded RNA reduced by crowders, $R_G < R_G^0$

This data shows that crowding reduces the conformational space accessible to the unfolded state by suppressing extended structures

The excess stability of RNA in polydisperse mixtures of crowders is similarly related to a decrease in R_G

METHODS



Presented results are obtained using 1) Langevin dynamics simulations of a coarse-grained three interaction site (TIS) model and 2) a novel force-field derived from experimental data

REFERENCES

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