

Use of Animal-Origin–Free Recombinant Human Albumin For Enhanced Performance in Research and Diagnostic Applications



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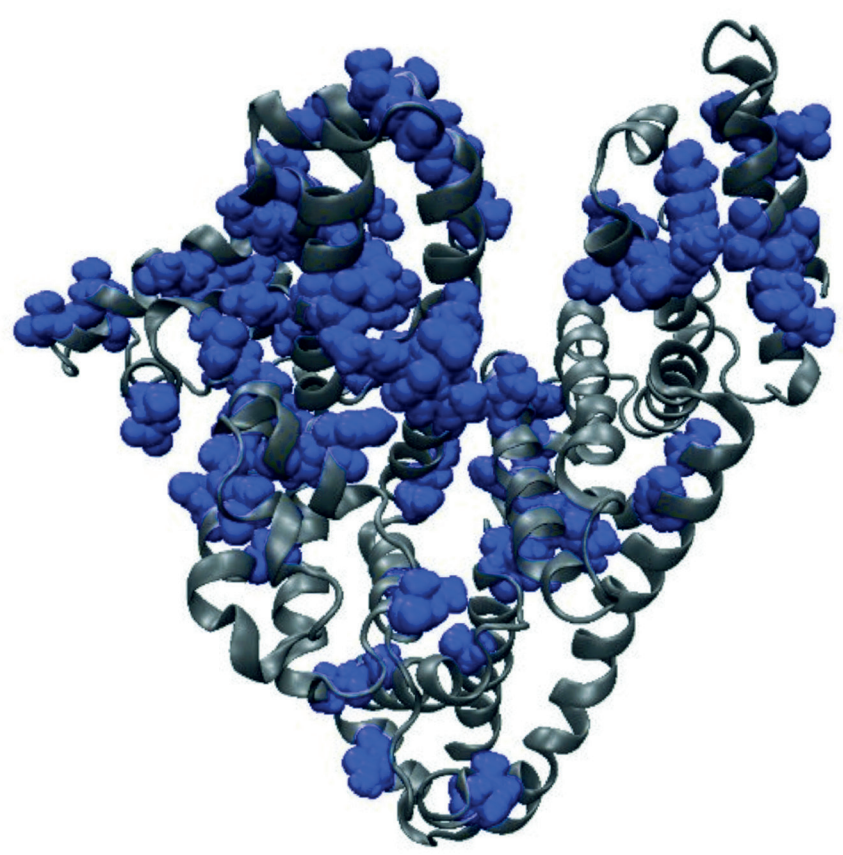
Advancing amplification performance with recombinant albumin

Albumins are the most abundant plasma proteins. Their functions include the reversible binding and transport of diverse ligands such as endogenous compounds and drugs. Albumins also contribute to blood buffering via abundant ionizable groups. Because of these features, traditional animal-derived albumins, such as bovine serum albumin (BSA), have long been used as stabilizers in molecular biology and medical diagnostics applications. However, animal-derived albumins pose significant challenges: risk of contamination by adventitious agents (RNases, viruses or prions), batch-to-batch variability and vulnerabilities in supply chains tied to animal sourcing. These issues can compromise sensitive PCR, qPCR and NGS library preparation, increase regulatory hurdles and raise ethical and sustainability concerns. QIAGEN® Recombinant Albumin, provides a reliable, animal-origin–free (AOF) alternative that eliminates these risks. Moreover, the use of a fully controllable expression system enables the use of in-silico, AI-based protein design. Such algorithms rationally engineer the albumin scaffold and generate variants with optimized stability and anti-inhibitory properties. In turn, this facilitates a new generation of tailor-made, high-performance molecular biology reagents.

Engineering albumin mutants with enhanced thermostability and aggregation resistance

The inherent instability of serum albumins such as human serum albumin (HSA) under elevated temperatures restricts its utilization in various biotechnological and industrial processes. Albumin variants bearing combinations of different mutations were generated using a computational workflow. This combined bioengineering tools (such as GROMACS, AMBER99SB, VMD, FoldX, MutateX and Scoop) in iterative rounds of design and selection, with the aim of obtaining variants with increased thermostability and/or resistance to aggregation. Other investigated characteristics included increased PCR anti-inhibitory properties, increased melting temperature and increased resistance to reducing agents.

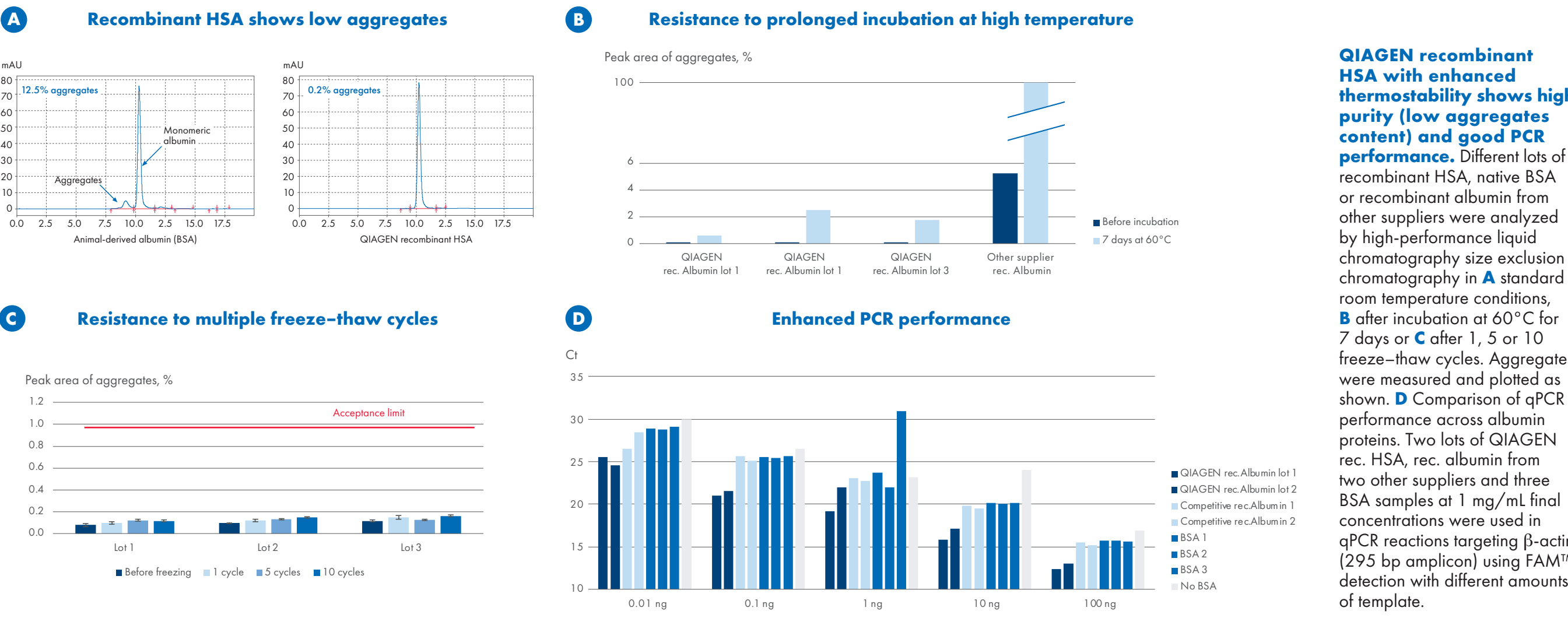
Computational workflow used to generate recombinant HSA sequence variants		
Step	Software	Results
1 Preparation of 3D structure model for WT of HSA (Uniprot: P02768 26-609), building solvation box (10 nm) filled with missing hydrogen atoms and ions and water (154 mM NaCl).	GROMACS package	HSA WT Pdb model in NTP conditions
2 Analysis of energy state ($\Delta\Delta G$) for each AA residue (585) based on pdb model for WT HSA, approx. 9000 substitutions/pdb models.	GROMACS package AMBER99SB Force Field Visual Molecular Dynamics (VMD)	Identified approx. 90 out of 585 AA residues for mutagenesis
3 Assembling approx. 100 variant mutants, calculation of $\Delta\Delta G$ values.	FoldX MutateX	100 mutant variants of rHSA
4 Analysis of thermodynamic properties of selected variant mutants, calculating changes in enthalpy and in heat capacity upon folding, the melting temperature and the folding free energy.	SCoP	20 variants with increased T_m , $\Delta\Delta G$



Representation of the model generated by the computational workflow. The model shows the structure of HSA protein (grey) with the residues (blue) in which mutations could affect the protein thermostability.

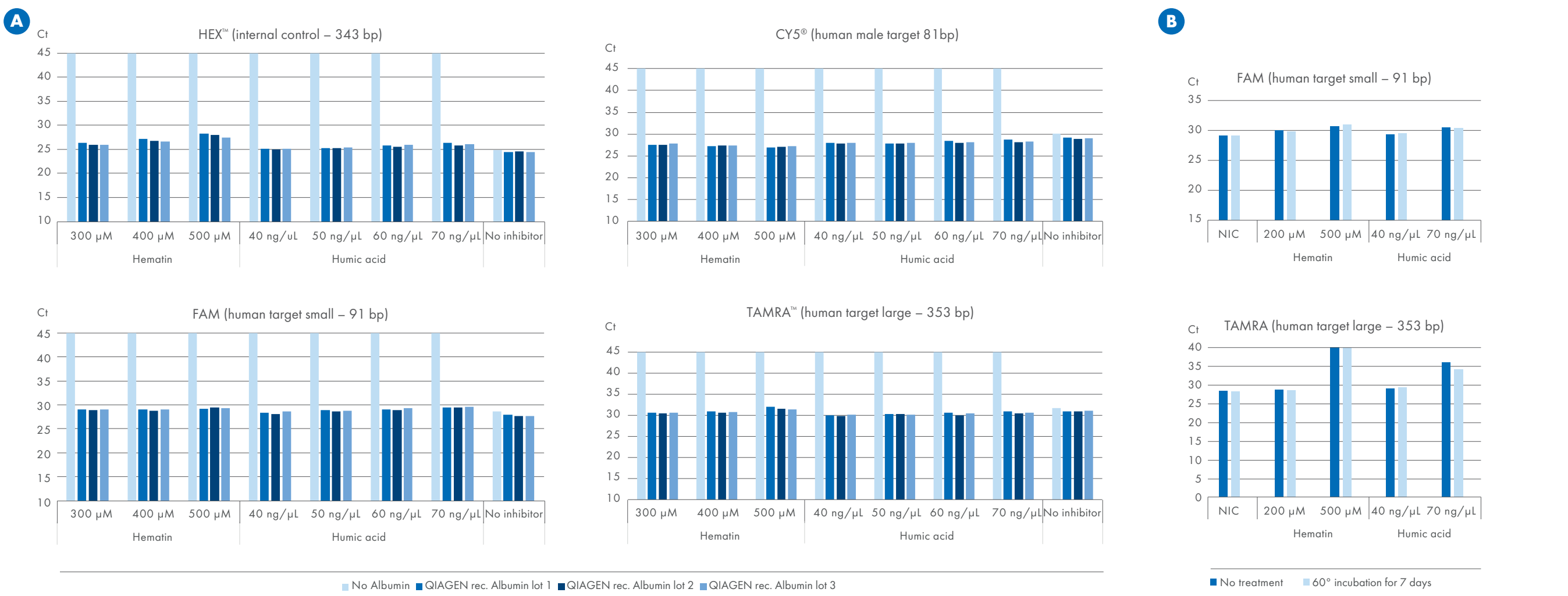
Enhanced albumin thermostability prevents aggregate formation and improves PCR performance

Despite the physiological stability of serum albumins under normal conditions, they undergo structural changes (e.g., unfolding) and aggregation at elevated temperatures. The increased thermostability of recombinant HSA variants prevents the formation of aggregates both in standard conditions and upon exposure to harsh temperatures. Albumins are widely used in molecular biological applications as stabilizers. BSA, purified from animal sources, is a well-established PCR additive and presents high batch-to-batch variability. Moreover, the low thermostability of native BSA makes it prone to aggregation and unfolding, which leads to precipitation in different PCR master mixes. Recombinant HSA eliminates the problem of batch-to-batch variability and the increased thermostability results in enhanced PCR performance.



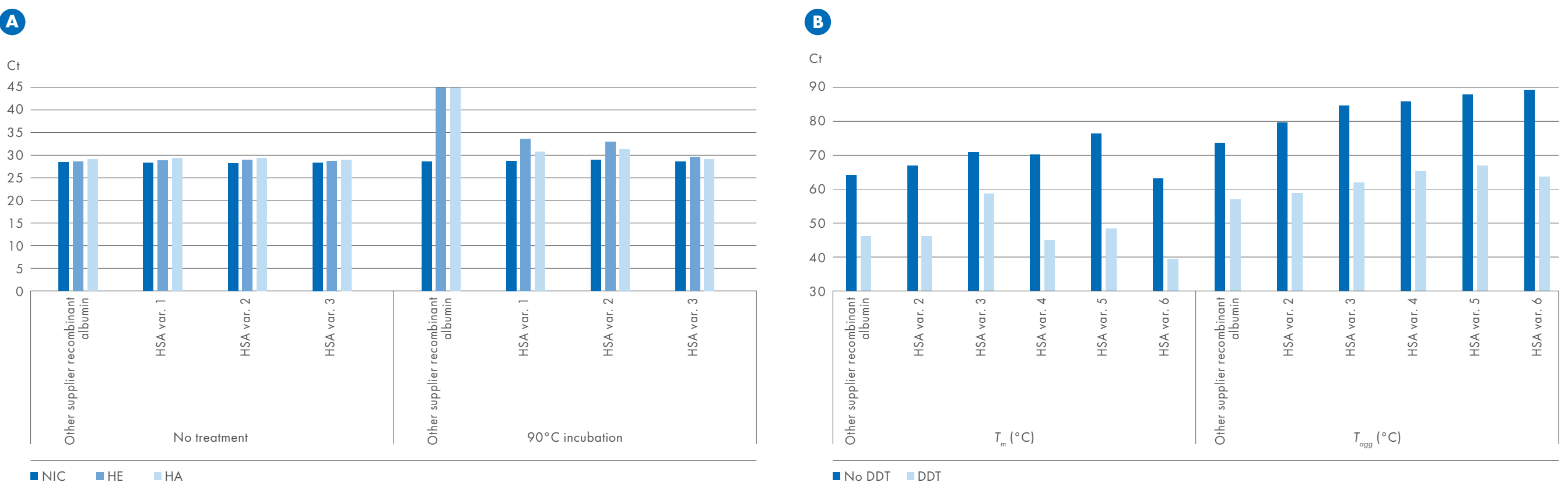
Superior inhibitor-tolerant properties of QIAGEN recombinant HSA enables robust PCR

Albumins are often used in PCR-based diagnostic methods, where they act as molecular sponges and bind PCR inhibitors commonly found in samples isolated from human bodies or collected from the environment (e.g., in forensic analysis). The qPCR performance of recombinant HSA in presence of common PCR inhibitors was evaluated. The use of recombinant HSA led to a reduction in qPCR inhibition at a range of temperatures, including incubation of HSA at high temperatures.



QIAGEN recombinant HSA variants as tailor-made molecular biology reagents

The computational workflow described above was used to design combination of mutations in the albumin scaffold with the aim of obtaining albumin variants with enhanced properties. The melting temperature (T_m) or aggregation temperature (T_{agg}) of different variants could be used to predict their stability and performance in different molecular biology applications (increased T_m and T_{agg} are predictors of better anti-inhibitory properties in PCR reactions). Similarly, the T_m and T_{agg} can be used as predictors of qPCR performance in the presence of problematic chemicals, such as reducing agents.



Conclusions

- Using an AI-driven, precision-design workflow, we have generated recombinant human serum albumin variants.
- These variants were engineered with defined amino acid modifications that significantly improve thermostability, resistance to aggregation and robustness under harsh conditions, compared to wild-type albumins.
 - These properties enable recombinant albumin that is highly pure and consistent batch-to-batch, with superior PCR anti-inhibitory performance.
 - This study opens the possibility of generating tailor-made albumin variants well suited for diagnostic and molecular biology applications where conventional animal-derived albumins fail.

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