Supplementary materials

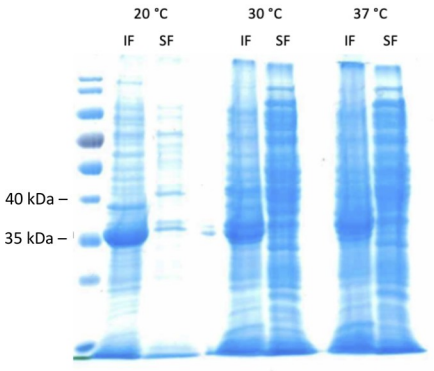

Figure S1. Optimization of the recombinant expression of *Tg*UGAE. After overnight incubation at various temperatures (20, 30 and 37 °C), the enzyme's expression level in both the insoluble fractions (IF) and soluble fractions (SF) was evaluated with SDS-PAGE. The enzyme's electrophoretic behavior corresponded well with its predicted molecular mass of about 37 kDa.

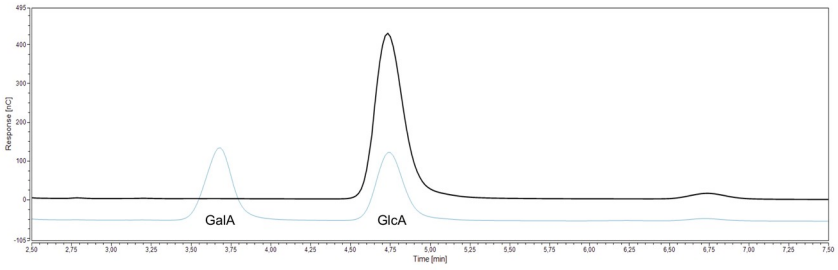

Figure S2. Analysis of potential UGA4E activity in the *E. coli* background. Chromatographic separation of reaction mixtures containing UDP-GlcA and *Tg*UGAE (blue) or UDP-GlcA and induced *E. coli* BL21(DE3) cells containing an empty pET21 vector (black). NDP-sugars were hydrolyzed to their respective monosaccharides prior to analysis.

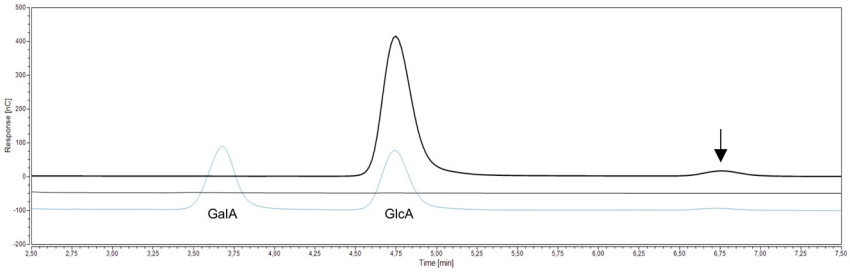

Figure S3. Chromatographic analysis of the reaction controls for *Tg*UGAE. Blue line: reaction mixture; upper black line: substrate control (10 mM UDP-GlcA in 100 mM MOPS pH 7.5); lower black line: enzyme control (100 µL cell free extract in 100 mM MOPS pH 7.5). The undefined extra peak is marked by an arrow. NDP-sugars were hydrolyzed to their respective monosaccharides prior to analysis.

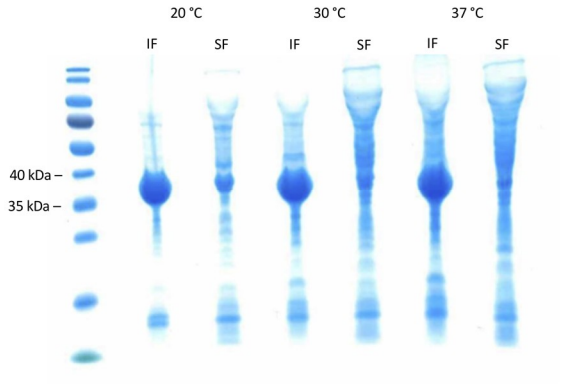

Figure S4. Optimization of *Ar*UGAE overexpression in *E. coli*. Various incubation temperatures (20, 30 and 37 °C) were evaluated overnight and the enzyme's expression levels were subsequently evaluated with SDS-PAGE. The apparent molecular mass is in agreement with the theoretical mass deduced for the amino acid sequence (37 kDa). IF: insoluble fraction; SF: soluble fraction.

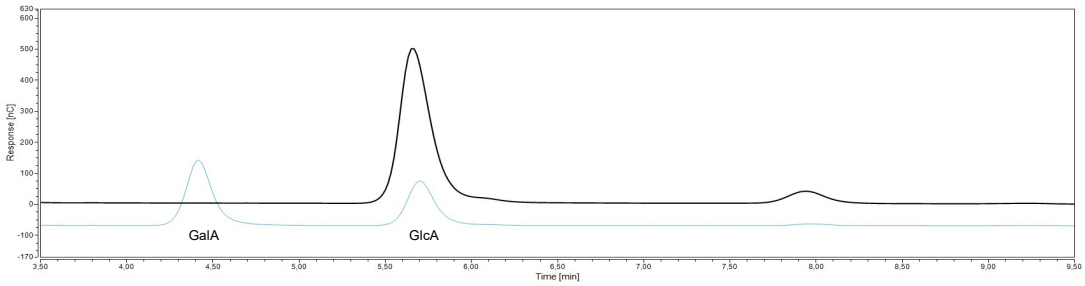

Figure S5. Exclusion of potential UGA4E activity in the *E. coli* background. Reaction mixtures containing *Ar*UGAE (blue) or induced *E. coli* BL21(DE3) cells containing an empty pET21 vector (black) were analyzed. NDP-sugars were hydrolyzed to their respective monosaccharides prior to analysis.

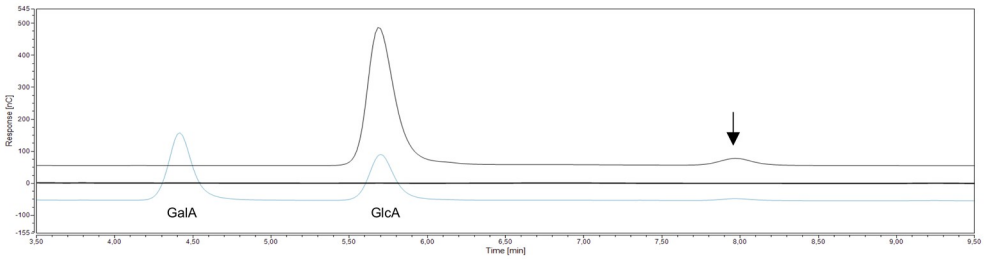

Figure S6. Chromatographic analysis of the reaction controls for *Ar*UGAE. Blue line: reaction mixture; upper black line: substrate control (10 mM UDP-GlcA in 100 mM MOPS pH 7.5); lower black line: enzyme control (100 µL cell free extract in 100 mM MOPS pH 7.5). The undefined extra peak is marked by an arrow. NDP-sugars were hydrolyzed to their respective monosaccharides prior to analysis.

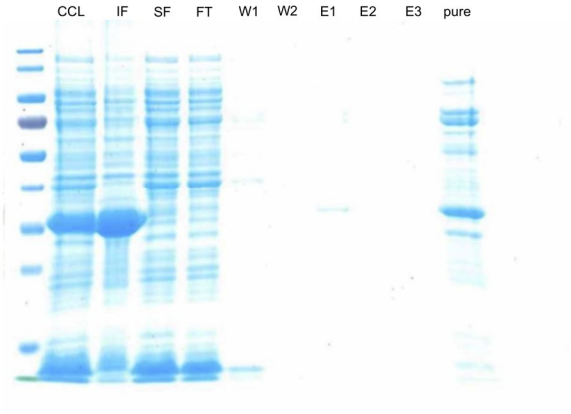

Figure S7. SDS-PAGE analysis of *Tg*UGAE purification. CCL: crude cell lysate; IF: insoluble fraction; SF: soluble fraction; FT: flow through; W: wash fraction; E: eluted fraction; pure: pure enzyme (after buffer exchange).

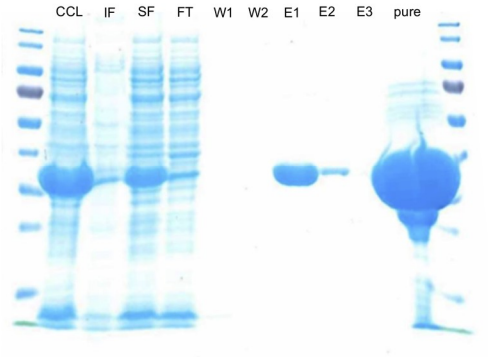

Figure S8. SDS-PAGE analysis of *Ar*UGAE purification. CCL: crude cell lysate; IF: insoluble fraction; SF: soluble fraction; FT: flow through; W: wash fraction; E: eluted fraction; pure: pure enzyme (after buffer exchange).