

Ali Nabhani, William Chollett, Jackson Valencia and William Park
Biochemistry and Biophysics, Texas A&M University
College Station, Texas

e.g. Allen and Taatjes (2015) Nat Rev Mol Cell Biol 16:155-166.

Are specific structural features of mediator in *S. cerevisiae* responsible for enhanced ethanol tolerance?

Med7/10/19 "hook"

CTD binding gap

Med31 "knob"

Med8 CTD binding region

Med8₂₀₉₋₃₂₁

Med8₁₃₉₋₁₇₂ "neck region"

Med20

Med18

Med8₁₆₀₋₂₂₁

Med8_{18/20} tether

Not visible in structure

structure 500M
Schilbach et al. (2017)
Nature 551: 204-209

Diagram illustrating the "neck" region of Med8 protein variants. The diagram shows four horizontal bars representing protein sequences. Above the bars are labels: "CTD binding" (yellow), "Ethanol tolerance" (blue), "Med8/20 tether" (grey), and "TAP" (yellow). The bars show the following amino acid ranges: 107-121 (yellow), 139-172 (blue), 190-223 (grey), and TAP (yellow). The labels "Med8_{1-223-TAP}", "Med8_{1-189-TAP}", "Med8_{1-172-TAP}", and "Med8_{1-138-TAP}" are placed to the right of the bars.

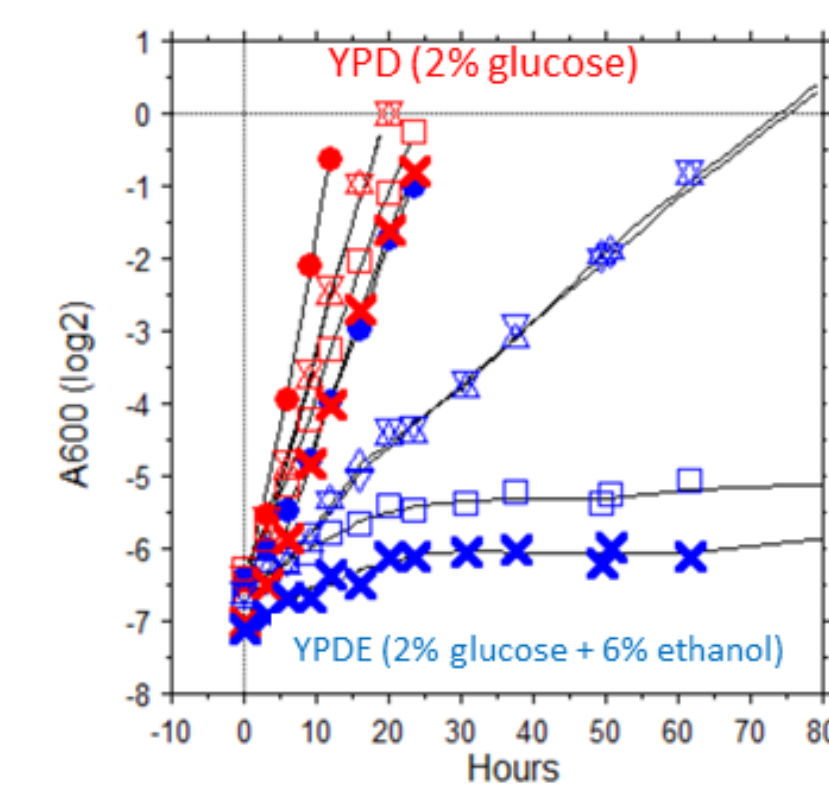


Figure 1 displays spot assays for three *Med8* strains: *Med8*_{1-223T}, *Med8*_{1-189T}, and *Med8*_{1-138T}. The strains are tested on various media and conditions, showing growth patterns across serial dilutions (10-fold dilutions from left to right).

The media and conditions tested are:

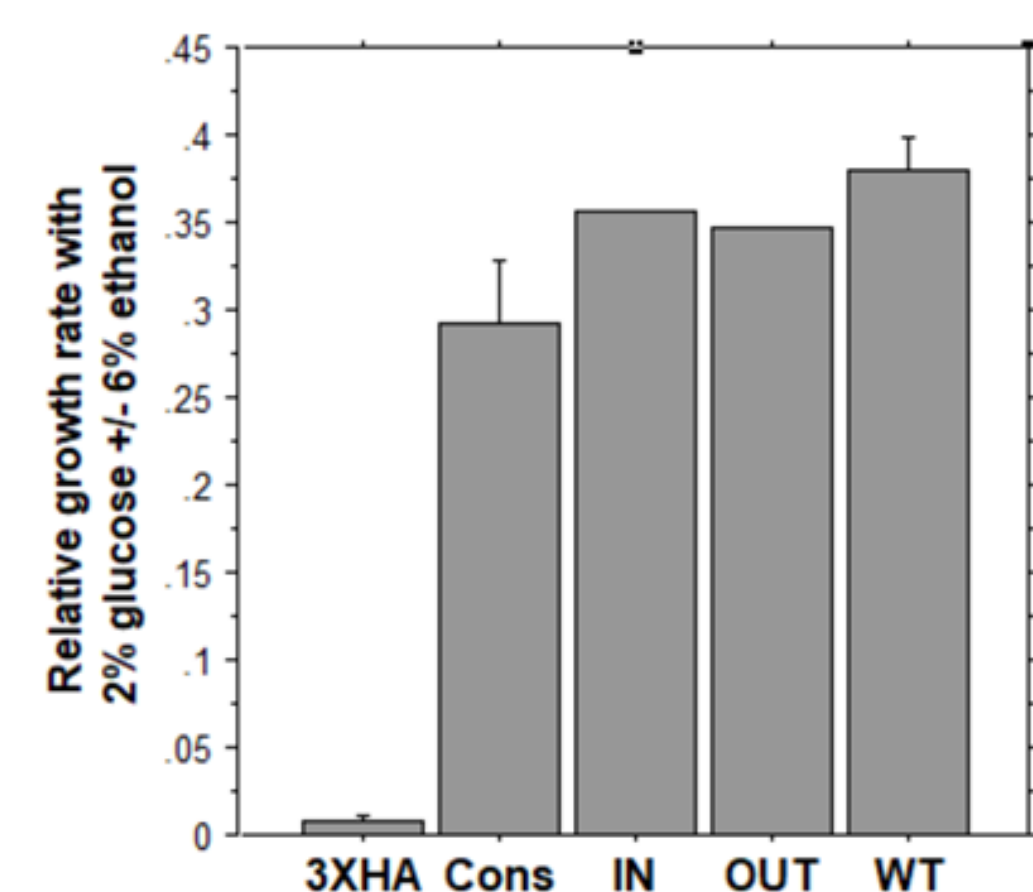
- YPD 30°C
- YPD 37°C
- YP galactose (2%) 30°C
- YPD + 1 M KCl 30°C
- YPD + 2 M Glycerol 30°C
- YPD + 0.05% MMS 30°C

The results show that *Med8*_{1-223T} grows on all media. *Med8*_{1-189T} shows growth on YPD 30°C, YPD 37°C, YP galactose (2%) 30°C, and YPD + 2 M Glycerol 30°C, but no growth on YPD + 1 M KCl 30°C and YPD + 0.05% MMS 30°C. *Med8*_{1-138T} grows on all media except YPD + 0.05% MMS 30°C.

[illegible]

Identical or very similar somewhat similar

Cons LLKDEAIEKLLQQAARIANAARTTFRNAYGKHDFK
IN LLKDDEIEKLLQQAARIANWARTTARNAYGAHAFK
OUT LLKDEAIEKLLAADRAITAAARTTFRNAYGKHDFK
3XHA AAVYPYDVPDYAGYPYDVPDYAGSYPYDVPDYAPK
WT Med8₁₃₉₋₁₇₂ LLKDEEIEKLLQQDRITNTWARTTFRNENKGHDFK



144/6
 151/6P
 161/6
 3XHA
 WT Med18¹³⁷⁻¹⁷³

TALLKDEP**I**PKLLQDREITN**W**ART**F**RNEYGKHDFK
 TALLKDEIEIKL**Q**PDREITN**W**ART**F**RNEYGKHDFK
 TALLKDEIEIKLLQDREITN**W**ART**F**RNEYGKHDFK
 TAAAYYPYDVPDYAGYFYDVPDYAGSYFYDVPDYAPK
 TALLKDEIEIKLLQDREITN**W**ART**F**RNEYGKHDFK

. . . : : :
 . . . : : :

PSIPRED helix score

Position

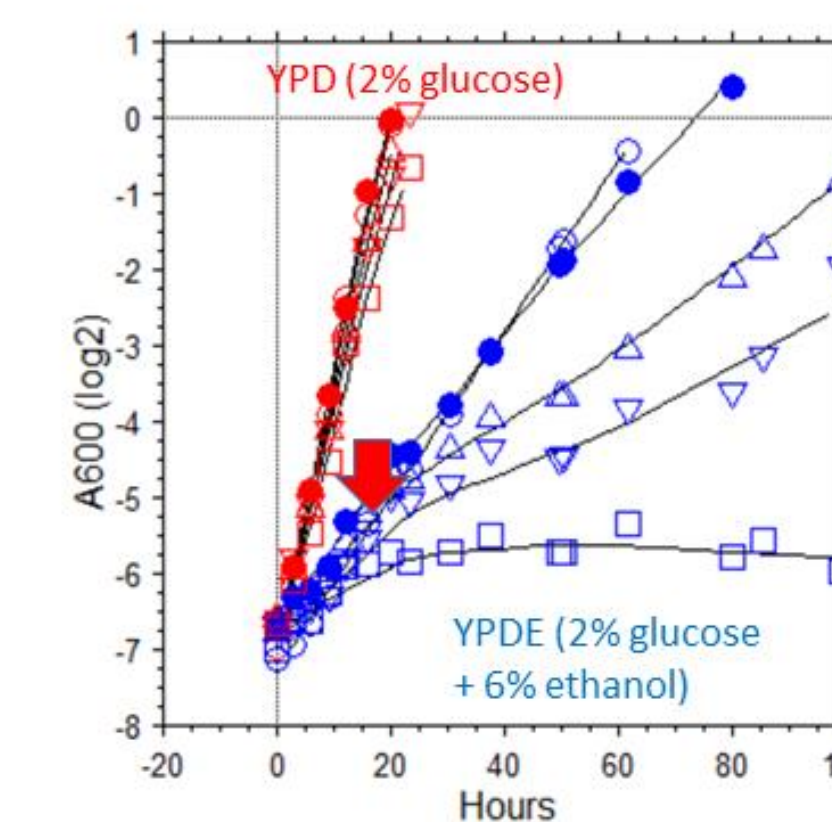
WT
 144/6
 151/6
 161/6
 SBP
 3XHA

Relative rate \pm 6% ethanol

144/6 151/6 161/6 3XHA SBP WT

HA1 137-145 **YFYPDVPDYA**EKKLLQQDREITNWARTTFRNEYGKHDF
 HA2 146-154 TALLKDEE**I**EKKLLQDREY**YFYPDVPDYA**TNWARITFRNEYGKHDF
 HA3 155-163 **HA3 155-163** TALLKDEE**I**EKKLLQDREY**YFYPDVPDYA**FRNEYGKHDF
 HA4 164-173 TALLKDEE**I**EKKLLQQDREITNWARTTF**YFYPDVPDYA**
 WT Med8₁₃₇₋₁₇₃ TALLKDEE**I**EKKLLQDREITNWARTTFRNEYGKHDF

.....:..*..



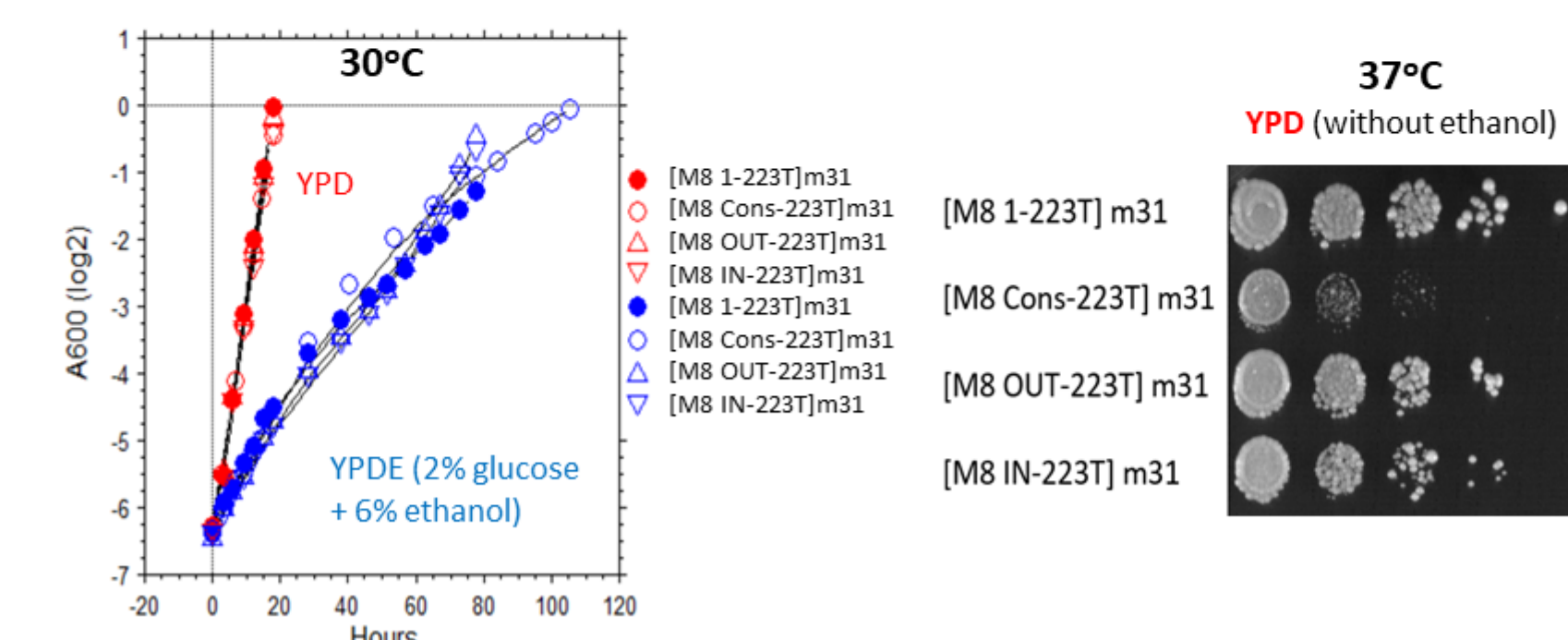
Note: These constructs do not contain the Med18/20 tether

Bar chart showing the relative rate of growth for various Med8 1-223 TAP mutations in the presence of 6% ethanol. The y-axis is 'Relative rate +/- 6% ethanol' (0 to 6). The x-axis shows mutations: 144P/6P, 151P/6P, 161P/6P, 3XHA, Cons, SBP, and WT. For each mutation, four bars are shown: white (no growth), red (growth in 6% ethanol), blue (growth in 12% ethanol), and green (growth in 18% ethanol). Error bars represent ± 1 standard deviation. The WT shows the highest growth rate in 18% ethanol (~4.8).

Mutation	White (No growth)	Red (6% ethanol)	Blue (12% ethanol)	Green (18% ethanol)
144P/6P	0	~2.8	0	~4.9
151P/6P	0	~2.3	0	~4.7
161P/6P	0	~2.5	0	~4.7
3XHA	0	~0.1	0	~4.5
Cons	0	~2.9	0	~4.8
SBP	0	~0.2	0	~4.6
WT	~0.2	~3.6	~3.1	~4.8

[illegible]

Cons TALLKDEAIEKLLQQARAIAAARTTFRNAYGKHDF
IN TALLKDEEIEKLLQQAREIANWARTTARNAYGAAHAF
OUT TALLKDEAIEALLAADRAITAAARTTFRNEYGKHDFK
WT Med8₁₃₈₉₋₁₇₂ TALLKDEEIEKLLQQDREITNWARTTFRNENGKHDFK



- Ethanol and heat tolerance are related, but not all Med8 “neck” mutants that block growth at 37°C also significantly reduce ethanol tolerance.