Supplementary Information for:

Topological and enzymatic analysis of human Alg2 mannosyltransferase

Meng-Hai Xiang[1], Xin-Xin Xu[1], Chun-Di Wang[1], Shuai Chen[1], Si Xu[1], Xiang-Yang Xu[3], Neta Dean[2], Ning Wang[1,*] and Xiao-Dong Gao[1,*]

[1] Key Laboratory of Carbohydrate Chemistry and Biotechnology, Ministry of Education, School of Biotechnology, Jiangnan University, Wuxi 214122, China

[2] Department of Biochemistry and Cell Biology, Stony Brook University, Stony Brook, New York 11794-5215, USA

[3] Zaozhuang Jienuo enzyme co., Ltd, Zaozhuang 277100, China

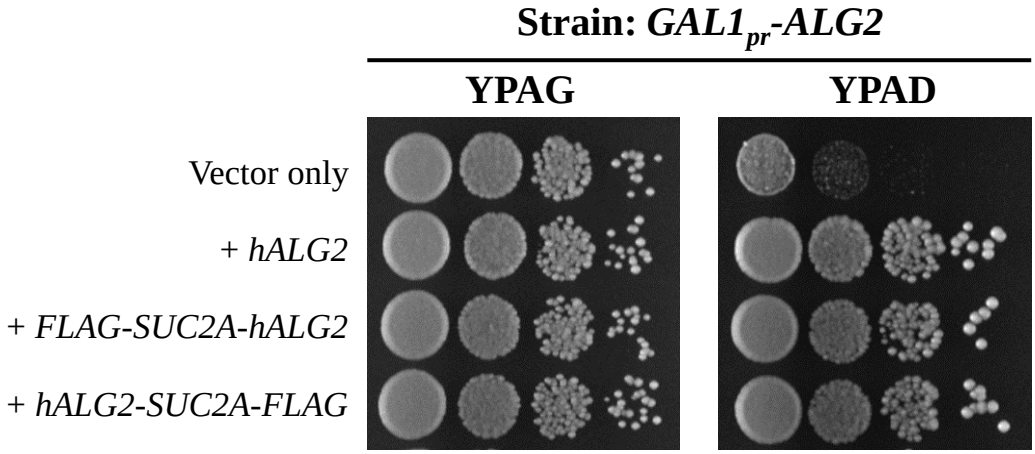
* To whom correspondence should be addressed:

Xiao-Dong Gao, E-mail: xdgao@jiangnan.edu.cn; Tel/Fax: +86-510-85197003

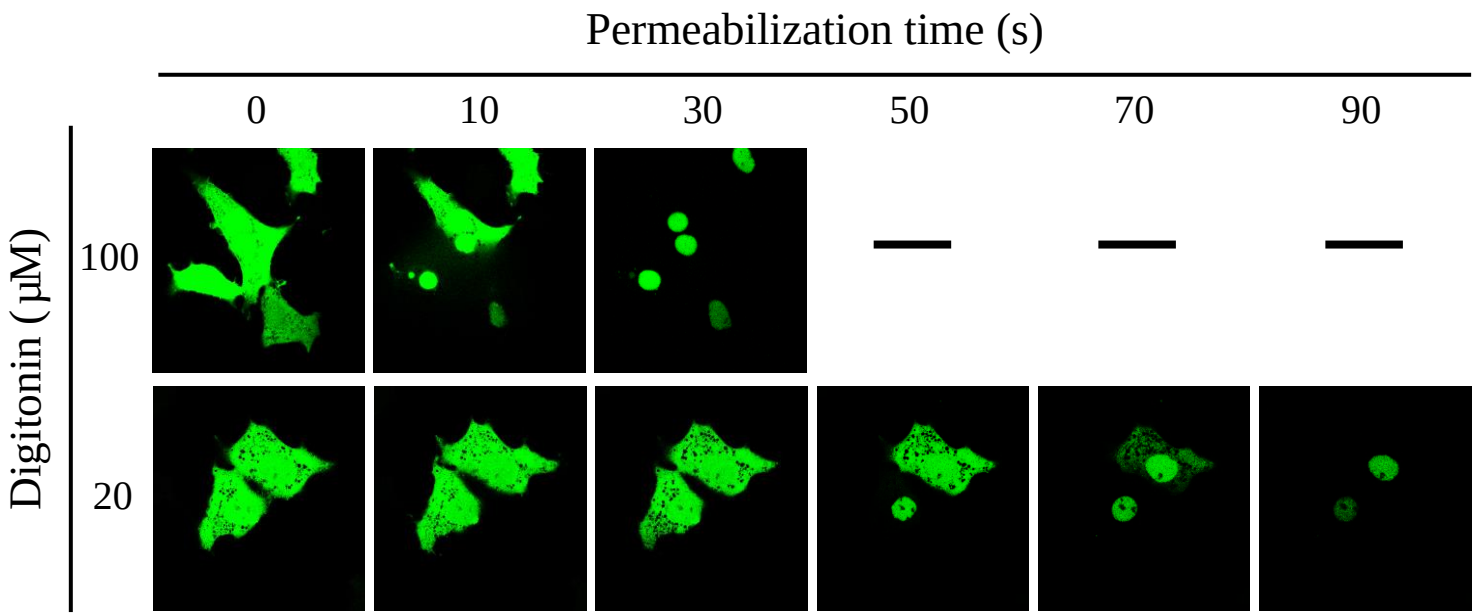
Ning Wang, E-mail: wangning@jiangnan.edu.cn

Keywords

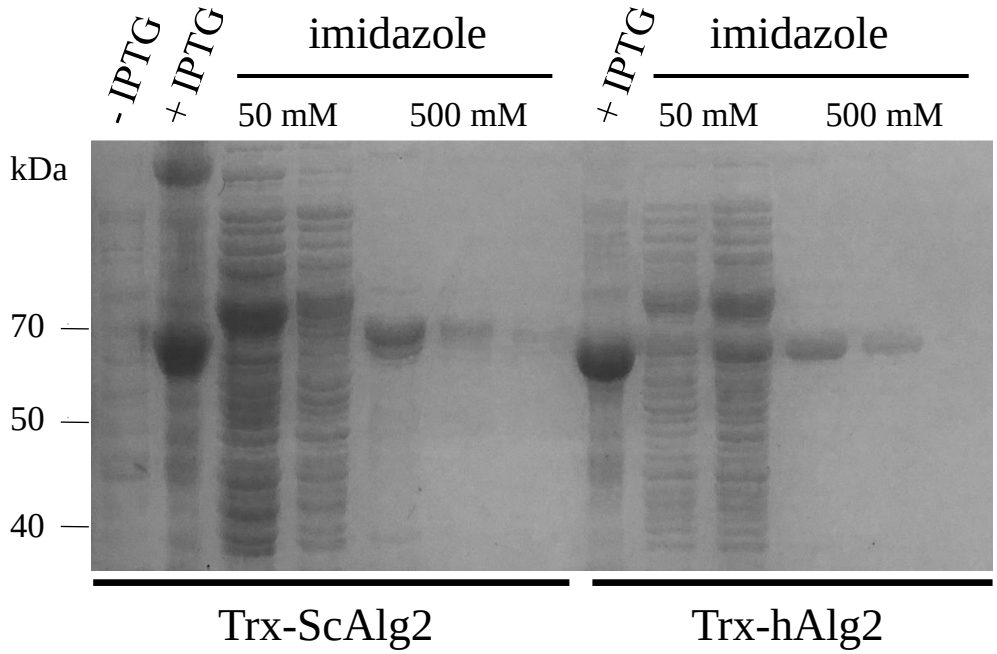
N-glycosylation, lipid-linked oligosaccharide, human Alg2, bifunctional mannosyltransferase, enzyme kinetics.



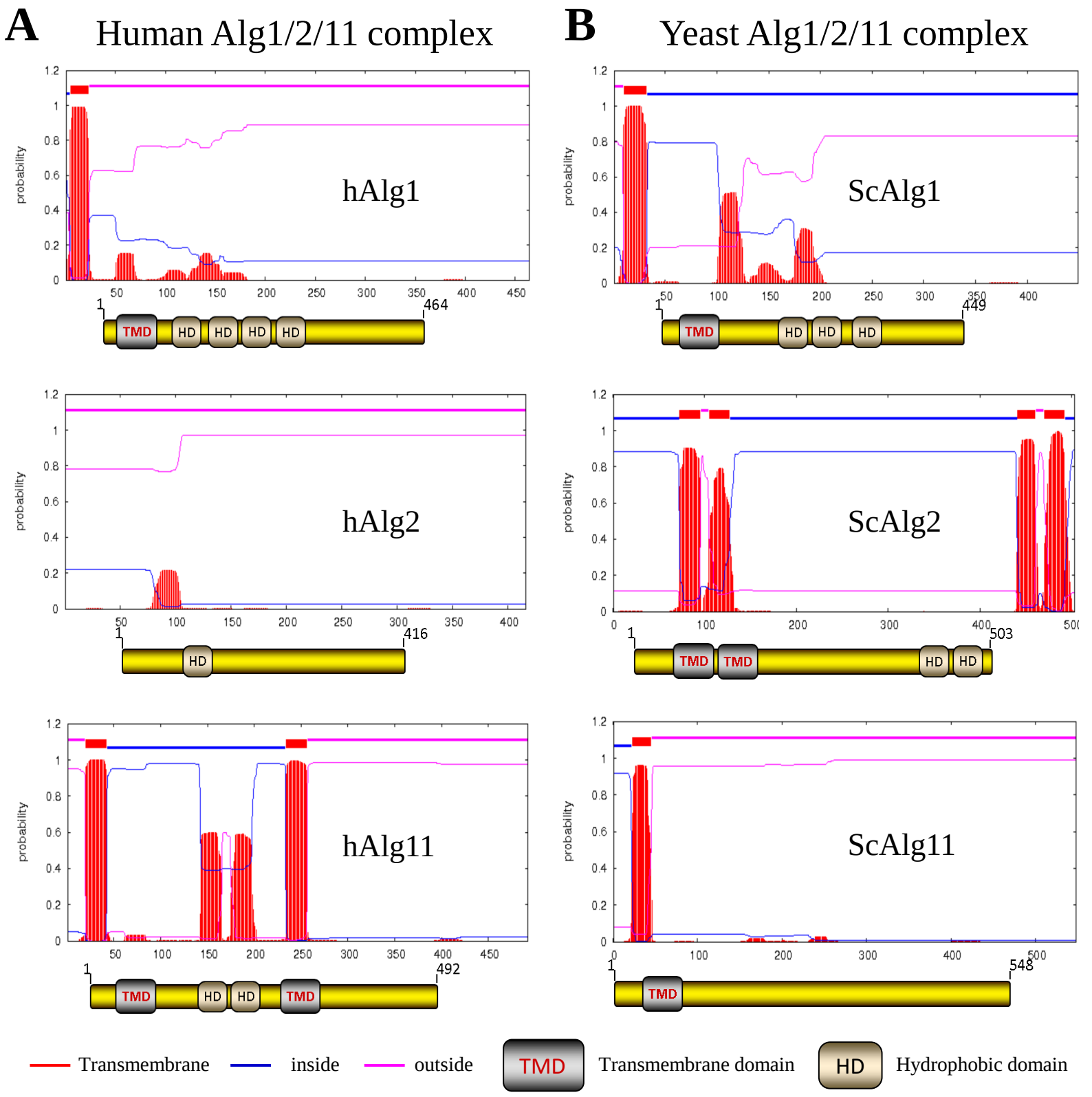
Supplementary Fig. 1: Complementation of yeast *alg2* with hAlg2 variants. Plasmids containing *hALG2*, *FLAG-SUC2A-hALG2* and *hALG2-SUC2A-FLAG* were transformed into yeast (*S. cerevisiae*) cells in which *ALG2* was under the control of the glucose-repressible *GAL1* promoter. Serial dilution of strains expressing these plasmids were spotted on YPA plates supplemented with galactose (YPAG, left panel) or glucose (YPAD, right panel) at 30 °C.



Supplementary Fig. 2: Optimizing efficiency of HEK293 permeabilization with digitonin. HEK293 cells expressing cytosolic GFP were used were treated with digitonin at 20 µM or 100 µM and GFP fluorescence was measured 10, 30, 50, 70 or 90 s. At a concentration of 20 µM, cytoplasmic fluorescence slowly disappeared over time until it disappeared completely after 90 s of digitonin treatment.



Supplementary Fig. 3: Analysis of recombinant Trx-ScAlg2 and Trx-hAlg2 proteins. The pET32 expression plasmid, containing Trx-ScAlg2 or Trx-hAlg2 gene was transformed into *E. coli* Rosetta (DE3) cells for expression of recombinant proteins with induction of IPTG. Recombinant Alg2 proteins were purified using a HisTrap affinity column with elution of imidazole. Samples were aliquoted at each stage of purification and analyzed by 10 % SDS-PAGE gel, followed by staining with Coomassie Brilliant Blue R-250. 50 mM, fractions eluted by 50 mM Tris-HCl, 150 mM NaCl and 50 mM imidazole (pH 8.0); 500 mM, fractions eluted by 50 mM Tris-HCl, 150 mM NaCl and 500 mM imidazole (pH 8.0).



Supplementary Fig. 4. Comparison of the membrane topology of yeast and human Alg1/2/11 MTase complex. The amino acid sequence of each Alg1, Alg2 and Alg11 proteins from human and yeast (*S. cerevisiae*) was applied to the TMHMM algorithm (http://www.cbs.dtu.dk/services/TMHMM/) for predicting the hydrophobicity respectively. (A) Schematic representations of predicted membrane topologies of human Alg1, Alg2 and Alg11. (B) Schematic representations of predicted membrane topologies of yeast Alg1, Alg2 and Alg11.