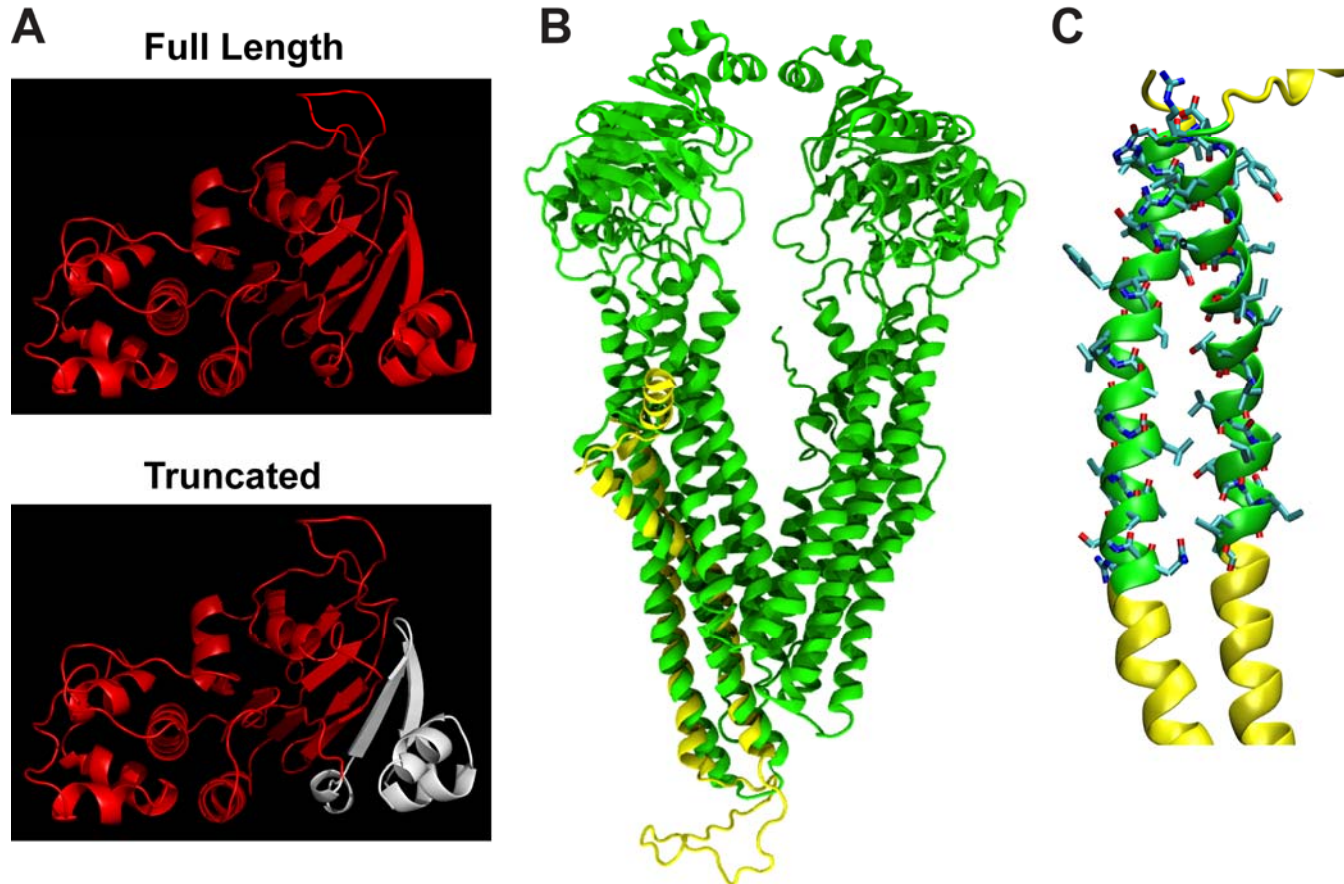


# Supplemental Materials

*Molecular Biology of the Cell*

Guerriero et al.

Supplemental Figure 1  
Guerriero et al.

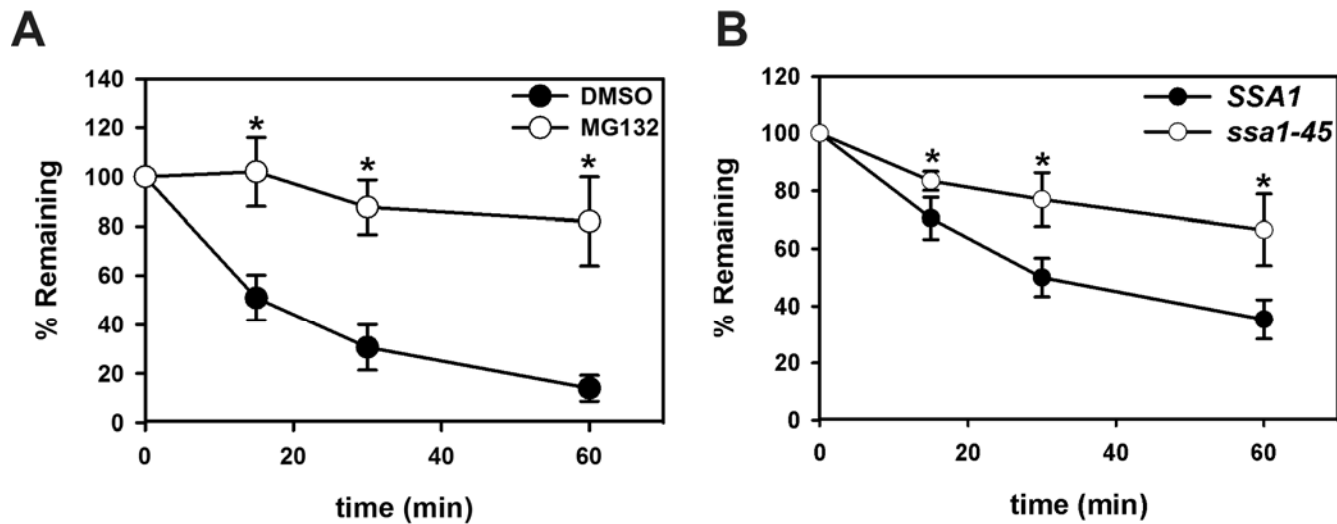


**Supplemental Figure 1. Homology models of Ste6p TMH1 and 2 and NBD2**

A) Homology model of Ste6p NBD2, a.a. 1047-1290, using mouse p-glycoprotein, NBD2, 4M1M. The 42 amino acid truncation, a.a. 1249-1290, is colored white for the truncated view (bottom). B) Superimposition of Ste6p TMH1 and 2 (highlighted yellow) on the structure of mouse p-glycoprotein (green), 3G5U, via a homology model. C) Magnified view of

Ste6p helix 1 and 2 (yellow), where TMH1, a.a. 21-48 and TMH2, 73-99 are highlighted in green, and these chains are displayed. The image was rendered using visual molecular dynamics (VMD).

Supplemental Figure 2  
Guerrero et al.



**Supplemental Figure 2. The Chimera A\* degradation requirements are similar regardless of high expression or low expression systems**

A) Yeast deleted for the drug efflux pump, Pdr5p, were transformed with a Chimera A\* expression vector in which protein production is under the control of the PGK promoter. Yeast were then subjected to a cycloheximide chase analysis following a 20 min preincubation with DMSO (closed circles) or 100  $\mu$ M MG132 (open circles) for the indicated times. Samples were processed as described in the Materials and Methods. B) Degradation of Chimera A\* under the control of the PGK promoter was examined in SSA1 and ssa1-45 yeast at 37 °C following a 20 min preincubation at that temperature. Data represent the means from at least 3 independent experiments  $\pm$  SE. \*,  $p < 0.04$  for *pdr5* $\Delta$  and  $p < 0.02$  for *ssa1-45*.

Supplemental Table 1 Computational values

<b>Parameter</b>	<b>Value</b>
force field	PARSE
counter ions	$\pm 1 e $ , 0.1 M, 2.0 Å
temperature	298.15 K
grid dimensions	129×129×129
coarse grid size	300×300×300 Å <sup>3</sup>
fine grid size	50×50×50 Å <sup>3</sup>
protein dielectric	2
membrane dielectric	2
headgroup dielectric	80
solvent dielectric	80
hydrophobic thickness	28.5 Å

headgroup thickness	8.0 Å
grid center	origin
solution method	npbe
boundary condition	zero
charge model	spl2
surface model	mol
spline width	0.3 Å
solvent probe radius	1.4 Å
surface sphere density	10 Å <sup>-2</sup>
nonpolar surface tension	23 cal/mol/Å <sup>2</sup>

Supplemental Table 2 Computed free energies for insertion for Chimera A\* variants

TMH1 sequence	% retrotranslocation	Phenomenological scale	Physics-based model	Physics-based model (zeroed)
RNDYRLLMIMIIGTVATGLVPAITSILT	35.6	0.31	-48.6	0
GGPGAAAALALALALALALAAAAAGGPG	36.4	-0.19	-46.1	2.5
GGPGNAAAALLLLLLLLLLLAAANGGPG	31.1	-1.07	-51.3	-2.7
RNDYRLLMIMIIALVALALVLAITSILT	21.9	-4.38	-65.4	-16.8
GGPGLLLLLLLLLLLLLLLLLLLGGPG	22.3	-7.15	-62.0	-13.4

\* All energies are reported as kcal/mol

**Supplemental Table 3 Yeast strains used in this study**

<b>Strain</b>	<b>Genotype</b>	<b>Reference/Source</b>
BY4742	<i>MAT<math>\alpha</math>, his3<math>\Delta</math>1, leu2<math>\Delta</math>0, lys2<math>\Delta</math>0 ura3<math>\Delta</math>3</i>	(Winzeler <i>et al.</i> , 1999)
<i>pdr5<math>\Delta</math></i>	<i>MAT<math>\alpha</math>, his3<math>\Delta</math>1, leu2<math>\Delta</math>0, ura3<math>\Delta</math>3 pdr5<math>\Delta</math>::KanMX</i>	(Winzeler <i>et al.</i> , 1999)
<i>pep4<math>\Delta</math></i>	<i>MAT<math>\alpha</math>, his3<math>\Delta</math>1, leu2<math>\Delta</math>0, ura3<math>\Delta</math>3 pep4<math>\Delta</math>::KanMX</i>	(Latterich <i>et al.</i> , 1995)
<i>pdr5<math>\Delta</math> pep4<math>\Delta</math></i>	<i>MAT<math>\alpha</math>, met15<math>\Delta</math>0, his3<math>\Delta</math>1, leu2<math>\Delta</math>0, ura3<math>\Delta</math>3 pdr5<math>\Delta</math>::KanMX, pep4<math>\Delta</math>::KanMX</i>	This lab
SSA1	<i>MAT<math>\alpha</math>, his3-11,15, leu2-3,112, ura3-52. trp1-<math>\Delta</math>1, lys2, ssa2-1(LEU2), ssa3-1(TRP1), ssa4-2(LYS2)</i>	(Becker <i>et al.</i> , 1996)
<i>ssa1-45</i>	<i>MAT<math>\alpha</math>, his3-11,15, leu2-3,112, ura3-52. trp1-<math>\Delta</math>1, lys2, ssa1-45, ssa2-1(LEU2), ssa3-1(TRP1), ssa4-2(LYS2)</i>	(Becker <i>et al.</i> , 1996)
<i>KAR2</i>	<i>MAT<math>\alpha</math> ura3-52, leu2-3,112, ade2-101</i>	(Sanders <i>et al.</i> , 1992)
<i>kar2-1</i>	<i>MAT<math>\alpha</math>, kar2-1, ura3-52, leu2-3,112, ade2-101</i>	(Brodsky <i>et al.</i> , 1999)
<i>HLJ1 YDJ1</i>	<i>MAT<math>\alpha</math>, ade2, his3, leu2, ura3, trp1</i>	(Youker <i>et al.</i> , 2004)
<i>hlj1<math>\Delta</math> ydj1-151</i>	<i>MAT<math>\alpha</math>, ade2, his3, leu2, ura3, trp1, can1-100, ydj1-2::HIS3 ydj1-151::LEU2 hlj1::TRP1</i>	(Youker <i>et al.</i> , 2004)
<i>DOA10HRD1</i>	<i>MAT<math>\alpha</math>, his3-<math>\Delta</math>200, leu2-3,112, ura3-52, lys2-801, trp1-1, gal2</i>	(Huyer <i>et al.</i> , 2004)
<i>doa10<math>\Delta</math></i>	<i>MAT<math>\alpha</math>, his3-<math>\Delta</math>200, leu2-3,112, ura3-52, lys2-801, trp1-1, gal2, doa10<math>\Delta</math>::HIS3</i>	(Huyer <i>et al.</i> , 2004)
<i>hrd1<math>\Delta</math></i>	<i>MAT<math>\alpha</math>, his3-<math>\Delta</math>200, leu2-3,112, ura3-52, lys2-801, trp1-1, gal2, hrd1<math>\Delta</math>::LEU2</i>	(Huyer <i>et al.</i> , 2004)
<i>doa10<math>\Delta</math> hrd1<math>\Delta</math></i>	<i>MAT<math>\alpha</math>, his3-<math>\Delta</math>200, leu2-3,112, ura3-52, lys2-801, trp1-1, gal2 doa10<math>\Delta</math>::HIS3, hrd1<math>\Delta</math>::LEU2</i>	(Huyer <i>et al.</i> , 2004)
<i>cdc48-2</i>	Back-crossed 3X to BY4742	(Moir <i>et al.</i> , 1982)

**Supplemental Table 4 Primers and Plasmids used in this study**

<b>Primer/Plas mid</b>	<b>Sequence/Genotype</b>	<b>Reference</b>
<i>Primer</i>		
oKN53	GCGCCGCGGTTATTCACTATGCGTTAT	This study
oKN54	GATCATCAAGGAAGT	This study
oKN57	GATGTGCATCCAACCTGTTAG	This study
oKN58	CCCGATATAAGTAGAGGCCAA	This study
oKN85	CATACCCGATATAAGTAGA	This study
oCG06	AGGCAAAATTTAAATAGA	This study
oCG07	TCTATTTTAATTTTGCCT	This study
oCG12	CCCCAACTAGTACAGAGGTCAGGTGGTCCAGGTGCTGCTGCTGCT TTGGCTTTGGCTTTGGCTTTGGCTTTGGCTTTGGCTGCTGCTGCTG GTCCAGGTGGTATGCACATCATACCCGATATA	This study
oCG13	TATATCGGGTATGATGTGCATACCACCTGGACCAGCAGCAGCAGC CAAAGCCAAAGCCAAAGCCAAAGCCAAAGCCAAAGCCAAAGCAGCAGCAGC ACCTGGACCACCTGACCTCTGTACTAGTTGGGG	This study
oCG15	CCCCAACTAGTACAGAGGTCAATGCACATCATACCCGATATA	This study
oCG16	TATATCGGGTATGATGTGCATTGACCTCTGTACTAGTTGGGG	This study
oCG26	ATGGTTATAACGCATAGTGAACAAATGATGAGGTCTTGTAACCTCGAT TGCAGTTCTTAAAGATGGTAAAGTGGTTGAGCGAGGTAACCTTCGAC ACTTTATATAATAATCGCGGGGAATTATTCCAAATTGTTTCCAACCA AAGCAGTTAACCGCGGTGGAGCTCCAAT	This study
oCG27	ATTGGAGCTCCACCGCGGTTAACTGCTTTGGTTGGAAACAATTTGG AATAATTCCTCGGATTATTATATAAAGTGTGCAAGTTACCTCGCTC AACCACCTTACCATCTTTAAGAAGTCAATCGAGTTACAAGACCTCA TCATTTGTTCACTATGCGTTATAACCAT	This study
oCG37	TACGTGAACATACGGAATGACTCTATCCTGACGGGCAGAGTG	This study
oCG38	CACTCTGCCCGTCAGGATAGAGTCATTCCGTATGTTACGTA	This study
oCG39	TACGTGAACATACGGAATGACGGTGGTCCAGGTGCTGCTGCTGCT TTGGCTTTGGCTTTGGCTTTGGCTTTGGCTTTGGCTGCTGCTGCTG	This study

	GTCCAGGTGGTTCTATCCTGACGGGCAGAGTG	
oCG40	CACTCTGCCCGTCAGGATAGAACCACCTGGACCAGCAGCAGCAGC CAAAGCCAAAGCCAAAGCCAAAGCCAAAGCCAAAGCCAAAGCAGCAGCAGC ACCTGGACCACCGTCATTCCGTATGTTCCACGTA	This study
oCG77	TACGTGAACATACGGAATGACGGTGGTCCAGGTAATGCTGCTGCT GCTTTATTGTTATTGTTATTGTTATTGTTATTGGCTGCTGCTAATGGT CCAGGTGGTTCTATCCTGACGGGCAGAGTG	This study
oCG78	CACTCTGCCCGTCAGGATAGAACCACCTGGACCATTAGCAGCAGC CAATAACAATAACAATAACAATAACAATAAAGCAGCAGCAGCATTAC CTGGACCACCGTCATTCCGTATGTTCCACGTA	This study
oCG79	TACGTGAACATACGGAATGACTACAGGCTGTTAATGATAATGATAAT AGCTTTGGTGGCATTAGCTCTAGTGTTAGCAATTACTTCTATCCTGA CGGGCAGAGTG	This study
oCG80	CACTCTGCCCGTCAGGATAGAAGTAATTGCTAACACTAGAGCTAAT GCCACCAAAGCTATTATCATTATCATTAAACAGCCTGTAGTCATTCCG TATGTTCCACGTA	This study
oCG81	TACGTGAACATACGGAATGACGGTGGTCCAGGTTTATTGTTATTGTT ATTGTTATTGTTATTGTTATTGTTATTGTTATTGTTATTGTTAGGTCCA GGTGGTTCTATCCTGACGGGCAGAGTG	This study
oCG82	CACTCTGCCCGTCAGGATAGAACCACCTGGACCTAACAATAACAAT ACAATAACAATAACAATAACAATAACAATAACAATAACAATAAACCT GGACCACCGTCATTCCGTATGTTCCACGTA	This study
<b>Plasmid</b>		
pSM1911	2 $\mu$ <i>URA3 P-PGK ste6-166-3HA</i>	(Huyer <i>et al.</i> , 2004)
pKN05	2 $\mu$ <i>URA3 ste6-166tm1-2NBD2-3HA</i>	This study
pKN31	2 $\mu$ <i>HIS3 Pcup1-mycUb-Tcyc1</i>	(Nakatsukasa <i>et al.</i> , 2008)
pCG11	2 $\mu$ <i>URA3 ste6-166tm1-2(delete)NBD2-3HA</i>	This study
pCG12	2 $\mu$ <i>URA3 STE6tm1-2(<math>\Delta</math>G=0.31)NBD2-3HA</i>	This study
pCG19	2 $\mu$ <i>URA3 STE6tm1-2(<math>\Delta</math>G=0.31)NBD2-3HA</i>	This study
pCG27	CENADH <i>ste6-166tm1-2(<math>\Delta</math>G=0.31)NBD2-3HA</i>	This study
pCG29	CENADH <i>STE6tm1-2(<math>\Delta</math>G=0.31)NBD2-3HA</i>	This study



pCG32	2 $\mu$ URA3 <i>STE6tm1</i> (delete)-2( $\Delta$ G=0.31)NBD2-3HA	This study
pCG45	2 $\mu$ URA3 <i>STE6tm1</i> ( $\Delta$ G=-0.19)-2( $\Delta$ G=0.063)NBD2-3HA	This study
pCG78	2 $\mu$ URA3 <i>STE6tm1</i> ( $\Delta$ G=-1.07)-2( $\Delta$ G=0.063)NBD2-3HA	This study
pCG80	2 $\mu$ URA3 <i>STE6tm1</i> ( $\Delta$ G=-4.38)-2( $\Delta$ G=0.063)NBD2-3HA	This study
pCG82	2 $\mu$ URA3 <i>STE6tm1</i> ( $\Delta$ G=-7.15)-2( $\Delta$ G=0.063)NBD2-3HA	This study

>Chimera N\*

MNFLSFKTTKHYHIFRYVNIRNDYRLLMIMIIGTVATGLVPAITSIILTGRVFDLLSVFVANGSHQGLDLGRIFYPYDVPDYAGYPYDVP  
DYAGSYPYDVPDYAAQC GPD PQLVQRSMVMALGAASVPVMWLSLTSWMHIPDISRGQRAASWIYRILDEKHNTLEVENNNARTVGIAG  
HTYHGKEKKPIVSIQNLTFAYP SAPTAFVYKMNMFDMFCGQTLGIIGESGTGKSTLVLLLTCLYNCEVGKIKIDGTDVNDWNLTSLRKE  
ISVVEQKPLL FNGTIRDNLTYGLQDEILEIEMYDALKYVGIHDFVISSPQGLDTRIDTTLSSGGQAQRLCIARALLRKSILILDECTS  
ALDSVSSSIINEIVKKGPPALLTMVITHSE

>Chimera A\* $\Delta$ G=0.31

MNFLSFKTTKHYHIFRYVNIRNDYRLLMIMIIGTVATGLVPAITSIILTGRVFDLLSVFVANGSHQGLDLGRIFYPYDVPDYAGYPYDVP  
DYAGSYPYDVPDYAAQC GPD PQLVQRSGGPGAAAALALALALALALALAAAAGPGGMHIIPDISRGQRAASWIYRILDEKHNTLEVENNNA  
RTVGIAGHTYHGKEKKPIVSIQNLTFAYP SAPTAFVYKMNMFDMFCGQTLGIIGESGTGKSTLVLLLTCLYNCEVGKIKIDGTDVNDWN  
LTSLRKEISVVEQKPLL FNGTIRDNLTYGLQDEILEIEMYDALKYVGIHDFVISSPQGLDTRIDTTLSSGGQAQRLCIARALLRKSIL  
ILDECTSALDSVSSSIINEIVKKGPPALLTMVITHSE

>Chimera A\* $\Delta$ G=-0.19

MNFLSFKTTKHYHIFRYVNIRNDGGPGAAAALALALALALALALAAAAGPGGSILTGRVFDLLSVFVANGSHQGLDLGRIFYPYDVPDYAG  
YPYDVPDYAGSYPYDVPDYAAQC GPD PQLVQRSGGPGAAAALALALALALALALAAAAGPGGMHIIPDISRGQRAASWIYRILDEKHNTLE  
VENNNARTVGIAGHTYHGKEKKPIVSIQNLTFAYP SAPTAFVYKMNMFDMFCGQTLGIIGESGTGKSTLVLLLTCLYNCEVGKIKIDGT  
DVNDWNLTSLRKEISVVEQKPLL FNGTIRDNLTYGLQDEILEIEMYDALKYVGIHDFVISSPQGLDTRIDTTLSSGGQAQRLCIARALL  
RKSILILDECTSALDSVSSSIINEIVKKGPPALLTMVITHSE

>Chimera A\* $\Delta$ G=-1.07

MNFLSFKTTKHYHIFRYVNIRNDGGPGNAAAALLLLLLLLLLAAANGPGGSILTGRVFDLLSVFVANGSHQGLDLGRIFYPYDVPDYAG  
YPYDVPDYAGSYPYDVPDYAAQC GPD PQLVQRSGGPGAAAALALALALALALALAAAAGPGGMHIIPDISRGQRAASWIYRILDEKHNTLE  
VENNNARTVGIAGHTYHGKEKKPIVSIQNLTFA YPSAP TAFVYKMNMFDMFCGQTLGIIGESGTGKSTLVLLLTKLYNCEV GKIKIDGT  
DVNDWNLTSLRKEISVVEQKPLLFNGTIRDNLTYGLQDEILEIEMYDALKYVGIHDFVISSPQGLDTRIDTTLLSGGQAQRLCIARALL  
RKS KILILDECTSALDSVSSSIINEIVKKGPPALLTMVITHSE

>Chimera A\* $\Delta G=-4.38$

MNFLSFKTTKHYHIFRYVNIRNDYRLLMIMI IALVALALVLAITSILTGRVFDLLSVFVANGSHQGLDLGRIFYPYDVPDYAGYPYDVP  
DYAGSYPYDVPDYAAQC GPD PQLVQRSGGPGAAAALALALALALALALAAAAGPGGMHIIPDISRGQRAASWIYRILDEKHNTLEVENNNA  
RTVGIAGHTYHGKEKKPIVSIQNLTFA YPSAP TAFVYKMNMFDMFCGQTLGIIGESGTGKSTLVLLLTKLYNCEV GKIKIDGT  
DVNDWNLTSLRKEISVVEQKPLLFNGTIRDNLTYGLQDEILEIEMYDALKYVGIHDFVISSPQGLDTRIDTTLLSGGQAQRLCIARALLRKS  
KILILDECTSALDSVSSSIINEIVKKGPPALLTMVITHSE

>Chimera A\* $\Delta G=-7.15$

MNFLSFKTTKHYHIFRYVNIRNDGGPGLLLLLLLLLLLLLLLLLLLLLGGGILTGRVFDLLSVFVANGSHQGLDLGRIFYPYDVPDYAGY  
PYDVPDYAGSYPYDVPDYAAQC GPD PQLVQRSGGPGAAAALALALALALALALAAAAGPGGMHIIPDISRGQRAASWIYRILDEKHNTLE  
VENNNARTVGIAGHTYHGKEKKPIVSIQNLTFA YPSAP TAFVYKMNMFDMFCGQTLGIIGESGTGKSTLVLLLTKLYNCEV GKIKIDGT  
DVNDWNLTSLRKEISVVEQKPLLFNGTIRDNLTYGLQDEILEIEMYDALKYVGIHDFVISSPQGLDTRIDTTLLSGGQAQRLCIARALLR  
KSKILILDECTSALDSVSSSIINEIVKKGPPALLTMVITHSE

>Chimera A $\Delta G=0.31$

MNFLSFKTTKHYHIFRYVNIRNDYRLLMIMI IGT VATGLVPAITSILTGRVFDLLSVFVANGSHQGLDLGRIFYPYDVPDYAGYPYDVP  
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RTVGIAGHTYHGKEKKPIVSIQNLTFA YPSAP TAFVYKMNMFDMFCGQTLGIIGESGTGKSTLVLLLTKLYNCEV GKIKIDGT  
DVNDWNLTSLRKEISVVEQKPLLFNGTIRDNLTYGLQDEILEIEMYDALKYVGIHDFVISSPQGLDTRIDTTLLSGGQAQRLCIARALLRKS  
KILILDECTSALDSVSSSIINEIVKKGPPALLTMVITHSEQMMRSCNSIAVLKDGKVVERGNFDTLYNNRGELFQIVSNQSS

>pSM1911 Ste6p\*

MNFLSFKTTKHYHIFRYVNIRNDYRLLMIMI IGT VATGLVPAITSILTGRVFDLLSVFVANGSHQGLDLGRIFYPYDVPDYAGYPYDVP  
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NRCVEELRSSAEASAITFQNLVAICALLGTSFYYSWSLTLIILCSSPIITFFAVVFSRMIVHYSEKENSETSKAAQLLTWSMNAQLV  
RLYCTQRLERKKFKEIILNCNTFFIKSCFFVAANAGILRFLTLTMFVQGFWFGSAMIKKGKLNINDVITCFHSCIMLGSTLNNTLHQIV

VLQKGGVAMEKIMTLLKDGSKRNPLNKTVAHQFPLDYATSDLTFANVSFSYPSRPSEAVLKNVSLNFSAGQFTFIVGKSGSGKSTLSNL  
LLRFYDGYNGSISINGHNIQTIDQKLLIENITVVEQRCTLFNDTLRKNILLGSTDSVRNADCSTNENRHLIKDACQMALLDRFILDLPD  
GLETLIGTGGVTLSSGGQQQRVAIARAFIRDTPILFLDEAVSALDIVHRNLLMKAIRHWRKGGKTTIILTHELSQIESDDYLYLMKEGEV  
ESGTQSELLADPTTTFSTWYHLQNDYSDAKTIVDTETEEKSIHTVESFNSQLETPKLGSCLSNLGYDETDQLSFYEAIIYQKRSNVRTRR  
VKVEEENIGYALKQQKNTESSSTGPQLLSIIQIIKRMIKSIRYKKILILGLLCSLIAGATNPVFSYTFSLLEGIVPSTDGKTGSSHYLA  
KWSLLVLGVAAADGIFNFAGKGFLLDCCSEYWMDLRNEVMEKLRKNMDWFSGENNKASEISALVLNDRDLRSLVSEFLSAMTSFVTV  
STIGLIWALVSGWKLSLVCISMFLPIIIIFSAIYGGILQKCE TDYKTSVAQLENCLYQIVTNIKTIKCLQAEFHFQLTYHDLKIKMQQIA  
SKRAIATGFGISMTNMIVMCIQAI IYYYGLKLVMIHEYTSKEMFTTFTLLLFTIMSC TSLVSQIPDISRGQRAASWIYRILDEKHNTLE  
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DVNDWNLTSLRKEISVVEQKPLLFNGTIRDNLTYGLQDEILEIEMYDALKYVGIHDFVISSPQGLDTRIDTTLLSGGQAQRLCIARALL  
RKSILILDECTSALDSVSSSIINEIVKKGPPALLTMVITHSE

### Supplemental References

Becker, J., Walter, W., Yan, W., and Craig, E.A. (1996). Functional interaction of cytosolic hsp70 and a DnaJ-related protein, Ydj1p, in protein translocation in vivo. *Mol Cell Biol* 16, 4378-4386.

Brodsky, J.L., Werner, E.D., Dubas, M.E., Goekeler, J.L., Kruse, K.B., and McCracken, A.A. (1999). The requirement for molecular chaperones during endoplasmic reticulum-associated protein degradation demonstrates that protein export and import are mechanistically distinct. *Journal of Biological Chemistry* 274, 3453-3460.

Huyer, G., Piluek, W.F., Fansler, Z., Kreft, S.G., Hochstrasser, M., Brodsky, J.L., and Michaelis, S. (2004). Distinct Machinery Is Required in *Saccharomyces cerevisiae* for the Endoplasmic Reticulum-associated Degradation of a Multispanning Membrane Protein and a Soluble Luminal Protein. *Journal of Biological Chemistry* 279, 38369-38378.

Latterich, M., Frohlich, K.U., and Schekman, R. (1995). Membrane fusion and the cell cycle: Cdc48p participates in the fusion of ER membranes. *Cell* 82, 885-893.

Moir, D., Stewart, S.E., Osmond, B.C., and Botstein, D. (1982). COLD-SENSITIVE CELL-DIVISION-CYCLE MUTANTS OF YEAST: ISOLATION, PROPERTIES, AND PSEUDOREVERSION STUDIES. *Genetics* 100, 547-563.

Nakatsukasa, K., Huyer, G., Michaelis, S., and Brodsky, J.L. (2008). Dissecting the ER-associated degradation of a misfolded polytopic membrane protein. *Cell* 132, 101-112.

Sanders, S.L., Whitfield, K.M., Vogel, J.P., Rose, M.D., and Schekman, R.W. (1992). Sec61p and BiP directly facilitate polypeptide translocation into the ER. *Cell* 69, 353-365.

Winzeler, E.A., Shoemaker, D.D., Astromoff, A., Liang, H., Anderson, K., Andre, B., Bangham, R., Benito, R., Boeke, J.D., Bussey, H., Chu, A.M., Connelly, C., Davis, K., Dietrich, F., Dow, S.W., El Bakkoury, M., Foury, F., Friend, S.H., Gentalen, E., Giaever, G., Hegemann, J.H., Jones, T., Laub, M., Liao, H., Liebundguth, N., Lockhart, D.J., Lucau-Danila, A., Lussier, M., M'Rabet, N., Menard, P., Mittmann, M., Pai, C., Rebischung, C., Revuelta, J.L., Riles, L., Roberts, C.J., Ross-MacDonald, P., Scherens, B., Snyder, M., Sookhai-Mahadeo, S., Storms, R.K., Veronneau, S., Voet, M., Volckaert, G., Ward, T.R., Wysocki, R., Yen, G.S., Yu, K., Zimmermann, K., Philippsen, P., Johnston, M., and Davis, R.W. (1999). Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. *Science* 285, 901-906.

Youker, R.T., Walsh, P., Beilharz, T., Lithgow, T., and Brodsky, J.L. (2004). Distinct roles for the Hsp40 and Hsp90 molecular chaperones during cystic fibrosis transmembrane conductance regulator degradation in yeast. *Mol Biol Cell* 15, 4787-4797.