Evidence for a Monomeric Intermediate in the Reversible Unfolding of F Factor TraM*

Dana L. Miller and Joel F. Schildbach†

Department of Biology, The Johns Hopkins University, Baltimore, Maryland 21218

Running Title: TraM Oligomerization Equilibrium

*This paper is based on work supported by National Science Foundation Grant MCB-9733655, American Cancer Society IRG 58-005-39-IRG, and National Institutes of Health Grant GM61017.

†To whom correspondence should be addressed.

Joel F. Schildbach

Phone: (410) 516-0176
Fax: (410) 516-5213
E-mail: joel@jhu.edu.
Summary

F-factor TraM is essential for efficient bacterial conjugation, but its molecular function is not clear. Because the physical properties of TraM may provide clues to its role in conjugation, we have characterized the TraM oligomerization equilibrium. We show that the reversible unfolding transition is non-two state, indicating the presence of at least one intermediate. Analytical ultracentrifugation experiments indicate that the first phase of unfolding involves dissociation of the tetramer into folded monomers, which are subsequently unfolded to the denatured state in the second phase. Furthermore, we show that a C-terminal domain isolated by limited proteolysis is tetrameric in solution, like the full-length protein, and that its loss of structure correlates with dissociation of the TraM tetramer. Unfolding of the individual domains indicate that the N- and C-terminal regions act cooperatively to stabilize the full-length protein. Together, these experiments suggest structural overlap of regions important for oligomerization and DNA binding. We propose that modulating the oligomerization equilibrium of TraM may regulate its essential activity in bacterial conjugation.
Introduction

Bacterial conjugation is a plasmid-mediated mode of lateral gene transfer. F factor was the first conjugative plasmid described (1). For successful F factor conjugation, the mating signal, which initiates DNA transfer upon formation of a stable mating pair, must be transmitted (2). TraM is a plasmid-encoded protein believed to be involved in mating signal transmission (3-5). TraM mutants form stable mating pairs and nick the plasmid at the origin of transfer (oriT)\(^1\), but DNA transfer does not occur (4). Although the identity of the mating signal is unknown, the signal is mediated by the conjugative pilus and requires cell-to-cell contact (6). Furthermore, signal transmission requires a step in addition to the interaction of the pilus with the recipient cell (2).

F factor TraM can interact with other F factor proteins and plasmid DNA, and its DNA binding activity correlates with its \textit{in vivo} function. TraM binds to three sites in \textit{oriT} (7-10). The two highest affinity sites, \textit{sbmA} and \textit{sbmB}, overlap with the two TraM promoters and negatively autoregulate the expression of TraM (11-13). Deleting the \textit{oriT} region that includes \textit{sbmA} and \textit{sbmB} from a mobilizable plasmid decreases its transfer efficiency by 100-fold (14). Further deletion of a region including \textit{sbmC}, the lowest affinity site, from this plasmid reduces the efficiency of mobilization by another 100-fold (14). In similar experiments with the \textit{oriT} of F-like plasmid R100, mutations in the site analogous to \textit{sbmC} inhibit transfer (15). These experiments indicate that TraM binding at \textit{sbmC} may be required for its essential role in conjugal DNA transfer. In addition to binding DNA, TraM associates with plasmid-encoded inner membrane protein TraD \textit{in vitro} (16). TraD is believed to be involved in transferring the single-stranded plasmid through the transfer pore to the recipient cell (5). The ability to interact with \textit{oriT} DNA and a membrane protein suggests that TraM may function to tether the plasmid near
the site of DNA transfer.

It is not clear how TraM’s putative tether function is important in transmitting the mating signal. The TraM tether may be formed in response to the mating signal, implying that the ability of TraM to interact with oriT or TraD is modulated by the mating signal. In vitro, TraM binds to DNA as a tetramer (17) and to the three oriT sites cooperatively (9). Therefore, small changes in the cellular concentration of tetrameric TraM in response to the mating signal could alter the occupancy of the lowest affinity sbmC binding site. It is unlikely that the concentration of TraM in the cell is increased transcriptionally, since expression is negatively autoregulated. Furthermore, conjugation efficiency is not affected by inhibitors of RNA or protein synthesis (4), suggesting that transcription and translation are not required for mating signal transmission.

We have performed a thermodynamic characterization of TraM oligomerization and stability, a necessary first step to understanding how TraM might respond to the mating signal. We report the first reversible equilibrium unfolding of TraM and, based on these and other data, we propose that TraM unfolds via a folded monomeric intermediate. Moreover, we show that the DNA binding and oligomerization activities of TraM can not be structurally separated. The thermodynamic characteristics of TraM and the interaction between structures that are important for DNA binding and oligomerization suggest a mechanism for regulating the activity of TraM in vivo.

**Experimental Procedures**

*Materials and Bacterial Strains.* Guanidine hydrochloride (Gnd-HCl, Mallinckrodt) solutions were prepared fresh and filtered (0.45 µm). Chromatography supplies and Gradifrac
system were from Amersham Pharmacia Biotech. Oligonucleotides were synthesized by Integrated DNA Technologies and used without further purification. DNA sequencing was performed by the DNA Analysis Facility, mass spectrometry by the AB-Mass Spectrometry Facility, and N-terminal peptide sequencing by the Synthesis and Sequencing Facility, all located at The Johns Hopkins University School of Medicine. Standard molecular biology procedures were performed as described (18).

Bacterial strains used were BL21(DE3) (19), BL21(DE3)/pLysS (Novagen), ER2738 (New England Biolabs), TB1 (New England Biolabs), JM109 (20) and DY330 (21). DY330 F′ ER was constructed by mating DY330 with F′ strain ER2738. The TraM open reading frame (ORF) of DY330 F′ ER was replaced with a kanamycin cassette by homologous recombination as described (21) to create DY330 F′ ER M::kan. The cassette was amplified by PCR from pET24a+ (Novagen).

Clones. The TraM ORF was amplified from JM109 genomic DNA by PCR with primers that incorporated appropriate restriction sites (22) and cloned into NdeI/XhoI-digested pET21a+ (Novagen). For pET-TraM the reverse PCR primer included a stop codon, but for pET-TraMH6 the primer lacked a stop codon, which allowed for expression with a C-terminal hexahistidine tag. The N-terminal peptide (pET-NTP) and C-terminal domain (pET-CTD) clones were constructed by removing unwanted sequence by PCR mutagenesis (22). This procedure resulted in the addition of an N-terminal methionine to the CTD ORF. Primer sequences are available upon request.

Protein overexpression and purification. Plasmids were transformed into BL21(DE3) or BL21(DE3)/pLysS. To test for protein overexpression, several colonies were individually transferred to 1 mL Luria-Bertani medium (LB) with 60 µg/mL ampicillin (LB-amp) and grown
to OD$_{600} \sim 0.4$. The culture was split and protein expression was induced in one half for three hours by adding isopropyl-1-thio-β-D-galactopyranoside (IPTG) to 1 mM, while the other half was kept on ice. Protein expression was assayed by SDS-PAGE, and the reserved portion of the culture that demonstrated the best expression was diluted to 20 mL with LB-amp, regrown to OD$_{600} \sim 0.5$, and then used to inoculate 2 L of LB-amp. When this culture reached mid-log phase, IPTG was added to 1 mM. After three hours, cells were harvested by centrifugation and stored at –80 ºC. For protein purification, 500-mL cell pellets were resuspended in 25-50 mL 50 mM Tris-HCl pH 7.5, 0.1 mM ethylenediamine-tetraacetic acid (EDTA), 50 mM NaCl, 5 mM dithiothreitol (DTT), 1 mM phenylmethylsulfonyl fluoride (PMSF) and disrupted by sonication. The soluble fraction was cleared by centrifugation, and then ammonium sulfate (0.25 g/mL) was added and precipitated protein removed by centrifugation. The remaining soluble protein was precipitated by adding ammonium sulfate to saturation and collected by centrifugation. Protein was resuspended in 35 mL of PNE (25 mM NaPO$_4$ pH 7.5, 25 mM NaCl, 0.1 mM EDTA) with 1 mM DTT and dialyzed overnight against 4 L of the same buffer.

TraM and NTP were further purified by ion exchange chromatography. Buffer was generally kept on ice, especially for the purification of NTP, to minimize degradation of the protein. Dialyzed samples were centrifuged at 15,000 × g for 10 min. and loaded onto a 5-mL HiTrap Heparin column equilibrated in PNE with 5 mM DTT (PNED). A 100-mL linear gradient to 2 M NaCl was applied. Fractions enriched in the desired protein were pooled, diluted 1:5 with PNED, and applied to a 5-mL HiTrap Blue column equilibrated in PNED. A 75-mL linear gradient to 2 M NaCl was applied, and the purity of peak fractions was assessed by SDS-PAGE. When necessary, one or both of these two chromatographic steps were repeated. The protein in the final pooled fractions was concentrated by ammonium sulfate precipitation when

6
appropriate. Purified protein was dialyzed extensively against PIPES buffer (25 mM piperazine-
N-N_bis[2-ethanesulfonic acid] (PIPES) pH 7.0, 150 mM NaCl, 0.1 mM EDTA) or phosphate
buffer (25 mM NaPO_4 pH 7.5, 150 mM NaCl, 0.1 mM EDTA).

For purification of CTD, dialyzed cell extracts were cleared by filtering (0.45 μm) and
loaded onto a 5-mL HiTrap Q column equilibrated in PNED. Protein was eluted with a 100-mL
linear gradient to 2 M NaCl. Enriched fractions were combined and run over a 5-mL HiTrap
Heparin column followed in series with a 5-mL HiTrap Blue column. The flow-through was
collected and dialyzed overnight against 4 L PNED. The Q column step was repeated, and the
concentrated protein (3-5 mL) was loaded onto a HiPrep 16/60 Sephacryl S-200 size exclusion
column equilibrated in PIPES buffer at room temperature and eluted at 0.2 mL/min.

TraMH6 was purified by batch-loading the soluble fraction of sonicated cells onto Ni-
NTA agarose (Qiagen). The column was washed with 5 mM and then 20 mM imidizole in
phosphate buffer with 1 mM DTT, and protein was eluted with 600 mM imidizole in phosphate
buffer with 1 mM DTT. Imidizole was removed from the protein solution by dialysis. Protein
concentration was measured by absorbance at 280 nm, with extinction coefficients of 3960 M⁻¹
1cm⁻¹ for TraM and TraMH6, 2560 M⁻¹cm⁻¹ for NTP, and 1400 M⁻¹cm⁻¹ for CTD (23).

Guanidine Hydrochloride-Induced Unfolding. Stock buffer solutions were filtered (0.45
μm) before use. Concentrated protein solutions were used within two weeks of purification and
were centrifuged on the day of the experiment for one hour at 55,000 rpm in a Beckman Optima
TL Ultracentrifuge using a TLA-55 rotor at 4 ºC. Native and unfolded (in 4 M Gnd-HCl) protein
samples were diluted to the indicated concentration immediately prior to the titration. When
indicated, sbmA DNA (5′- CGCTAGGGGCGCTGCTAGCGGTGCGT-3′, annealed to its
complement; (3,9)) was included at an equal molar ratio to the TraM tetramer. All solutions
were allowed to equilibrate for five minutes before the first measurement was recorded. Protein and DNA concentration remained constant throughout the titration. All data were collected at 37 °C, and titrant was kept at 35 ± 2 °C during the experiment.

For titrations monitored by intrinsic tyrosine fluorescence, protein was in PIPES buffer to prevent potential interactions between phosphate and tyrosine (24,25). Titrations were monitored with a SLM Amico 48000 spectro fluorometer in the L-configuration equipped with a Neslab circulating water bath and magnetic stirrer. Polarizers were set to the magic angle. The sample was excited at 275 nm (4 nm excitation slit width) and emission monitored at 302 nm (16 nm emission slit width). Each fluorescence intensity measurement is the average of at least 200 data points acquired over 2-5 seconds, and each data point was averaged 5 times. The titration was performed manually as described (26). The sample was allowed to equilibrate with stirring for at least 2 minutes before each measurement.

Titrations monitored by circular dichroism (CD) were performed in PIPES or phosphate buffer with similar results. Data from unfolding of full length TraM were acquired on an Aviv 62A DS spectropolarimeter (Aviv Associates, Inc.) with a computer-controlled Hamilton Microlab 500 titrator with two 500 µL syringes (27,28). The samples were stirred for 90 seconds, and then data were averaged for 30 seconds. This was sufficient to achieve equilibrium, as indicated by the stability of the CD signal. Manual titrations were performed on a Jasco J-710 Spectropolarimeter equipped with a PTC-348WI thermostat. The procedure was as described for the fluorescence measurements, except that each measurement is the average of 600 data points acquired over 30 seconds. The standard deviation of each measurement is indicated by error bars on the graphs.

Spectroscopic data were fit using the non-linear least squares analysis curve fitting
function of Kaleidagraph 3.51. A signal-weighted two-state model was employed (26,29,30). A similar three-state model was derived to fit the observed spectroscopic signal \(Y_{\text{obs}}\) when appropriate. \(Y_{\text{obs}}\) is a sum of the fraction of each species (native, \(f_N\); intermediate, \(f_I\); unfolded, \(f_U\)) multiplied by its contribution to the signal \(Y_N, Y_I, Y_U\):

\[
Y_{\text{obs}} = f_N Y_N + f_I Y_I + f_U Y_U = \left( \frac{1}{1 + K_1 + K_2} \right) Y_N + \left( \frac{K_1}{1 + K_1 + K_2} \right) Y_I + \left( \frac{K_2}{1 + K_1 + K_2} \right) Y_U
\]

The spectroscopic signal associated with each species \(Y_N, Y_I, Y_U\) varied linearly with denaturant concentration. \(K_1\) and \(K_2\) are equilibrium constants for the first and second steps of the three-state equilibrium, respectively. According to the linear extrapolation model (29), each \(K\) can be expressed as a function of the standard state equilibrium constant, \(K^\circ\), and \(m\)-value.

The \(\Delta G^\circ\) was calculated from the fit \(K^\circ\) values. An unfolding curve was determined to exhibit hysteresis if the forward and reverse curves were reproducibly offset greater than the error in the measurement.

**DNA Dissociation Titration.** The dissociation of \(sbmA\) DNA (2.5 \(\mu\)M in PIPES buffer) upon addition of Gnd-HCl was monitored by absorbance at 260 nm with a Beckman DU-70 Spectrophotometer equipped with a temperature controlled cell holder. DNA was diluted into buffer at 37 °C and allowed to equilibrate for 5 min before each measurement.

**Analytical Ultracentrifugation.** Protein solutions in PIPES buffer with various concentrations of Gnd-HCl and matching buffer solutions were centrifuged at 55,000 rpm in a Beckman Optima TL Ultracentrifuge at 4 °C for at least one hour. The top half of each solution was removed for use in analytical ultracentrifugation experiments. Sedimentation velocity analytical ultracentrifugation (SV-AUC) experiments were performed in a Beckman XL-I Analytical Ultracentrifuge using absorbance optics and an An60Ti rotor. All runs were performed at 35 °C and 60 K rpm in cells with charcoal-filled epon 12 mm double-sectored
centerpieces and sapphire windows. The rotor and cells were pre-warmed. Radial absorbance
data were collected at 275 nm at a nominal point spacing of 0.003 cm with no averaging in
continuous scan mode.

Weight average sedimentation coefficients ($<s_{20,w}^*>$), extrapolated to $t=0$, were
calculated with the program DCDT+ version 1.14 (31,32). Solvent density, viscosity, and the
buoyant molecular weight of the protein species were calculated with the program SEDNTERP
version 1.06 (33). Data for CTD and NTP were analyzed by fitting to the modified Fujita-
Machosham function using the dc/dt mode of SVEDBERG version 6.39 (32,34,35), which is
better suited for small molecules. In all analyses, 14-20 scans were included in the fit. Goodness
of fit was evaluated by randomness in residuals, reduced chi-square, and visual examination of
the fits overlaid onto the experimental data. Regions of the data that showed signs of meniscus
effects or that indicated accumulation at the bottom of the cell were excluded from the fits,
although in the figures the fit values are extended over the entire $s_{20,w}$ range.

**DNA binding interference assay.** TraM, pre-incubated with NTP or CTD, was combined
with sbmA DNA. Electrophoretic mobility shift assays were performed essentially as described
(22) except that equilibration was at 37 °C.

**Mating assays.** DY330 $F_{ER}'$ and DY330 $F_{ER}''$ M::kan were transformed with pET21a+
(empty vector), pET-TraM, pET-NTP, or pET-CTD by electroporation. Overnight cultures from
single colonies were diluted 1:100 into media containing all appropriate antibiotics and regrown
to mid-log phase. Donor cells (150 µL) were spun down and resuspended in 100 µL warm LB,
and then 900 µL of mid-log phase TB1 recipient cells were added. Mating cultures were
incubated for 2 minutes at 37 °C without agitation. Conjugation was interrupted by vigorous
vortexing, and then cells were incubated on ice for 10 min. Serial dilutions in cold, sterile PBS
(18) were plated to select for donors and transconjugants. All mating experiments were repeated with at least three independent donor clones.

Limited proteolysis. TraM or TraMH6 was mixed with trypsin at a molar ratio of 75:1 and incubated at room temperature. Aliquots were removed at hour intervals and the reactions were stopped by addition of PMSF to 2 mM. The fragments generated were separated by electrophoresis on a 15% acrylamide SDS-PAGE gel and visualized by staining with Coomassie to monitor extent of digestion to ensure that both proteins generated the same proteolysis pattern. The C-terminal proteolytic fragments of TraMH6 were purified after four hours. The reaction was stopped with PMSF and mixed for 10 minutes at 4 °C with 25 µL of Ni-NTA agarose (Qiagen) equilibrated in phosphate buffer with 5 mM β-mercaptoethanol (BME). The Ni-NTA agarose was washed once and bound protein was eluted in phosphate buffer with 5 mM BME and 600 mM imidizole. Eluted protein was run on a SDS-PAGE gel, stained with Coomassie, and then transferred to Hybond-P membrane (Amersham Pharmacia Biotech) in 20 mM 3-[cyclohexylamino]-1-propanesulfonic acid (CAPS) pH 10, 1 mM DTT, 10% methanol at 300 V for 8 hours at 4 °C. Bands were excised from the membrane and subjected to N-terminal peptide sequencing.

Ni-NTA Coelution Assay. TraMH6 (5 µM) was combined with 15 µM TraM, NTP, or CTD in 50 µL reactions in PIPES buffer with 10 mM DTT and incubated for at least 30 minutes at 37 °C. Negative controls included all components except TraMH6. Each reaction was mixed with 25 µL Ni-NTA agarose (Qiagen) at room temperature for 5 minutes, the agarose was washed once with 0.5 mL PIPES buffer, and then bound protein was eluted in 50 µL PIPES buffer with 0.5 M imidizole. Protein that did not bind to Ni-NTA agarose (flow-through) was compared to retained species by SDS-PAGE. The gels were scanned and the band intensities
quantitated with the FluorChem Imaging System version 2.00 controlled by AlphaEase FC Software (Alpha Innotech Corporation). Background was subtracted from each band, and the coelution efficiency was calculated as the ratio of eluted test protein to TraMH6 in the same lane.

Results

Guanidine-Induced Unfolding of F factor TraM. The reversible unfolding reaction of TraM was followed by CD and intrinsic tyrosine fluorescence (Figure 1). CD is a measure of secondary structure, whereas tyrosine fluorescence can report on close tertiary interactions, hydrogen bonding of the fluorophore, and secondary structure. The Gnd-HCl-induced unfolding transition is biphasic with an apparent intermediate state populated near 1 M Gnd-HCl. The congruence of the curves when monitoring independent probes is consistent with each transition observed in the unfolding curve being two-state (36). This suggests that the full unfolding transition is three-state, involving only one intermediate. These experiments were performed at 37 °C because the unfolding transition at 20 °C exhibited pronounced hysteresis (not shown).

The equation describing a three-state model includes too many parameters relative to the number of data points to obtain reliable fits. Therefore, the slope and intercept of the baseline at high denaturant concentration were determined by fitting data from this region directly to a linear equation, and these values were fixed during subsequent fitting. It was not necessary to constrain the shorter native baseline in this manner. By fitting the constrained model to the CD data, we calculate that the overall stability of 10 µM TraM at 37 °C is 4.7 kcal/mol. The first transition (ΔG₁ = 2.7 kcal/mol, m₁ = 3.7 kcal/mol*M⁻¹Gnd-HCl) results in a greater decrease in free energy than the second transition (ΔG₂ = 2.0 kcal/mol, m₂ = 1.1 kcal/mol*M⁻¹Gnd-HCl). The fit of fluorescence data with the three-state model did not converge. This is likely due to a
lower signal-to-noise ratio in these data. However, when the ΔG and m-values derived from the CD fits were fixed, the fluorescence data were reasonably well fit by the three-state model (not shown).

Although the unfolding data indicate two transitions in TraM unfolding, we do not know how each transition reflects events of the unfolding pathway. To determine which phase of TraM unfolding involves a change in oligomerization, we performed the unfolding titrations of TraM at protein concentrations from 1-45 µM (Figure 2). 10 µM TraM has a greater molar ellipticity at 222 nm than 1 µM, showing that TraM is stabilized by increasing the protein concentration. While the offset between the two curves makes them difficult to compare, the first transition begins at a slightly lower concentration of denaturant at the lower protein concentration, suggesting that the first transition has shifted. Neither transition of the fluorescence-monitored unfolding is dramatically shifted when the concentration of TraM is raised from 10 µM to 45 µM. We also did not see a shift in either unfolding transition when sbmA, the highest affinity binding site, was included in the reaction. At this temperature and concentration, the midpoint of the Gnd-HCl-induced dissociation of sbmA is located at approximately 0.5 M (not shown).

**SV-AUC Analysis of TraM Oligomerization.** To directly evaluate how the oligomeric nature of TraM changes during unfolding, we performed SV-AUC at different concentrations of denaturant. The data from these experiments can be used to determine the molecular weight of the sedimenting species based on hydrodynamic models, and can also be used to monitor changes in oligomerization (37-40). We were not able to perform complementary equilibrium analytical ultracentrifugation because TraM aggregation occurred before the sample reached equilibrium. The data for SV-AUC experiments were collected under conditions similar to the
unfolding of 45 µM TraM shown in Figure 2. This is the lowest protein concentration that could be detected with the absorbance optics at 275 nm.

To determine if the first transition involves a change in oligomerization, we measured the sedimentation of TraM at 0.5 M, 0.75, and 1 M Gnd-HCl (Figure 3), denaturant concentrations that span the first unfolding transition. Visual examination of the curves suggests that, even at the lowest concentration of Gnd-HCl, the boundary shape is not symmetrical. This is consistent with the solution containing a heterogeneous mixture of species. As the concentration of Gnd-HCl is increased the distribution first becomes more asymmetric, and then the peak shifts to the left. This translation of the g(s*) distribution toward lower s* values is consistent with a change in oligomerization concurrent with the first phase in the unfolding. The g(s*) representation of the data shown are model-independent, and provide a qualitative comparison of TraM sedimentation as a function of unfolding (34).

We performed a more rigorous, quantitative analysis by determining the weight average sedimentation coefficient (＜s*₂₀,w＞) as a function of denaturant concentration, shown in Figure 3. The ＜s*₂₀,w＞ represents the equilibrium distribution of all species, regardless of the rate of interconversion, as long as the system is at equilibrium at the beginning of the run (41). In our experiments, the protein samples were incubated in the cell at 35 ºC for 20-45 min. before the experiment was initiated. When native TraM is diluted into 2 M Gnd-HCl at this protein concentration the fluorescence-monitored unfolding is complete within three minutes (not shown). The ＜s*₂₀,w＞ data indicate that the change in oligomerization that occurs upon unfolding of TraM is completed by 1.5 M Gnd-HCl under these conditions. We fit the two-state model to the ＜s*₂₀,w＞ data to derive the apparent tetramerization energy (ΔG_{app,tet}). From this fit, we determine ΔG_{app,tet} to be 2.9 kcal*mol⁻¹ and m_{app,tet} to be 3.1 kcal*mol⁻¹*M_{Gnd-HCl}⁻¹. These
values agree well with $\Delta G_1$ (2.7 kcal/mol) and $m_1$ (3.7 kcal/mol*1*M$_{\text{Gnd-HCl}}^{-1}$) from the chemical unfolding providing further evidence that the dissociation of the native tetramer is two-state. Together, these data indicate that the first phase in the unfolding of TraM involves dissociation of the native tetramer to a monomeric form.

*Isolating Structural Domains of TraM.* Using limited proteolysis, we generated a stable C-terminal fragment, beginning at TraM residue 58 (not shown). Expression vectors for this C-terminal domain (pET-CTD) and the remaining N-terminal peptide (pET-NTP) were constructed. Mass spectrometry of purified CTD matched the value predicted from the DNA sequence (8,100 Da; predicted molecular weight 8,103 Da). However, mass spectrometry and N-terminal peptide sequencing of purified NTP indicate that it does not include the initial methionine and the final two residues coded for in the genetic construct.

To determine if the two phases observed in the full-length denaturation experiments correlate with sequential unfolding of these domains, we analyzed the unfolding of the individual domains (Figure 4). Although the midpoint of the NTP transition is similar to the second transition in the full-length protein, both $\Delta G_{\text{NTP}}$ (3.0 kcal/mol) and $m_{\text{NTP}}$ (2.0 kcal/mol*1*M$_{\text{Gnd-HCl}}^{-1}$) are greater than the corresponding $\Delta G_2$ (2.0 kcal/mol) and $m_2$ (1.1 kcal/mol*1*M$_{\text{Gnd-HCl}}^{-1}$). The unfolding of 45 $\mu$M NTP overlays the data collected with 10 $\mu$M protein, suggesting that this protein does not undergo a change in oligomerization upon unfolding. Furthermore, unlike TraM, the molar ellipticity of NTP does not change when the protein concentration is increased (not shown).

The unfolding transition of CTD is complete by 0.5 M Gnd-HCl. At this concentration of denaturant there is no observed loss of TraM structure. The native baseline in these unfolding curves is not well defined, which makes fitting these data difficult. The unfolding of 45 $\mu$M
CTD is shifted relative to 10 µM, consistent with it being oligomeric. When the two-state model is fit to the 45 µM data, ∆G\text{CTD,45}=2.0 \text{ kcal/mol}^{-1} and m\text{C,45}=3.6 \text{ kcal/mol}^{-1} \cdot \text{M}^{-1} \text{Gnd-HCl}. The \text{m}-value derived from these fits is very similar to \text{m}_{1} (3.7 \text{ kcal/mol}^{-1} \cdot \text{M}^{-1} \text{Gnd-HCl}) of the full-length protein. Since the \text{m}-value reflects the amount of surface area exposed upon unfolding, which should be the same at both protein concentrations, we fixed this parameter in fits of the two-state model to the data collected at 10 µM. In these fits, ∆G\text{CTD} (0.6-0.9 \text{ kcal/mol}^{-1}) is lower than ∆G_{1} (2.7 \text{ kcal/mol}^{-1}). These experiments suggest that loss of CTD structure coincides with dissociation of the tetramer.

We used SV-AUC to determine if CTD and NTP are oligomeric in solution (Figure 5). The concentration of protein in these experiments (125 µM) is higher than for the full-length protein (45 µM) to achieve sufficient signal for data collection. These data indicate that CTD is a tetramer in solution, consistent with the concentration-dependence of the unfolding curve. The SV-AUC data also suggest that NTP is dimeric in solution. These data are at odds with the observation that the NTP unfolding curve is not shifted when the initial protein concentration is changed. Together with the unfolding data, these experiments show that CTD structures are sufficient for tetramerization, although N-terminal residues may be involved in stabilizing the tetramer.

Functional Analysis of Domains. Our thermodynamic experiments indicate that CTD and NTP act cooperatively to stabilize the TraM protein. We proceeded next to see if both domains are required in vivo for the function of TraM. Indeed, only full length protein, and neither CTD nor NTP, are able to complement a \textit{traM} deletion mutant (Table 1). We also tested the effect of including each construct in trans to a wild-type F’ episome. The transfer efficiency of DY330 F’\text{ER} was slightly, but reproducibly, decreased in the presence of CTD, but not TraM.
or NTP. This observed dominant negative effect could indicate that CTD is included in
tetramers with the full-length protein *in vivo* and that these tetramers are not active.

To determine if the fragments interact with TraM *in vitro*, we monitored the coelution of
the untagged species with TraMH6 from Ni-NTA agarose (Figure 6). The concentration of
protein in these experiments was much lower than in the SV-AUC experiments. Both fragments
coelute with TraMH6, albeit at lower efficiency than TraM, although a genetic interaction was
seen only for CTD. We used a gel-retardation assay to address whether the observed interactions
affect the DNA binding activity of TraM (Figure 7). Although no *in vivo* affect of NTP
expression was observed, this fragment reduced the ability of TraM to bind DNA. Conversely,
the interaction of CTD with TraM does not significantly affect its DNA binding properties.

**Discussion**

We have established conditions for the first reported reversible unfolding of the TraM
protein, allowing us to describe its stability and oligomerization. Unfolding experiments were
performed at 37 °C, where the unfolding transition is relatively free of hysteresis, to allow for
application of thermodynamic models. Moreover, this is the physiological temperature at which
TraM functions. Although calorimetry experiments with R1 TraM (17) indicate that at 37 °C the
protein shows some structural alteration, this thermal unfolding reaction is irreversible. In our
equilibrium experiments, the Gnd-HCl-induced unfolding curve of TraM at 37 °C exhibits a
well-defined baseline at low concentrations of denaturant, which suggests that the protein is in a
native, folded state under these conditions. Our characterization of TraM in these experiments
defines a thermodynamic reference state that can be used in future studies to correlate observed
phenotypes with defects in stability or oligomerization. The more precise characterization of
TraM mutant phenotypes could help further elucidate the molecular role of TraM in mating signal transduction.

We have shown that monomeric and tetrameric forms of TraM are in equilibrium in solution. The existence of a folded monomeric species suggests that multiple forms of TraM may coexist inside a cell without being degraded. The three-state unfolding of F-factor TraM we report is consistent with the observation that the irreversible thermal unfolding of TraM from F-like plasmid R1 is non-two state (17). We calculate that at 10 µM TraM the energy difference between the monomeric and tetrameric states is on the order of 2.5 kcal*mol\(^{-1}\) at 37 ºC. This is only four times RT, the thermal energy at this temperature. The concentration of TraM in the cell is two to three orders of magnitude lower than in our experiments (12), which would decrease the energy difference between the monomer and tetramer. However, there are also factors \textit{in vivo} that could stabilize the tetramer, such as specific interaction with DNA or other proteins, or the cytoplasmic environment. The interplay of these factors will ultimately determine which oligomeric state is favored in the cell, although our experiments demonstrate it is feasible that both states are populated \textit{in vivo}. TraM was not apparently stabilized by the presence of its DNA ligand \textit{in vitro} (Figure 2). However, in the absence of DNA, the first unfolding transition does not begin until 0.5 M Gnd-HCl, which is near the midpoint of the \textit{sbmA} DNA dissociation transition. Furthermore, it is likely that Gnd-HCl can interfere with DNA binding by TraM. The effects of Gnd-HCl on either DNA dissociation or TraM-DNA interactions could prevent observation of TraM stabilization by a TraM-DNA complex.

The equilibrium between tetramer and monomer is two-state, indicating that no other folding intermediates exist. These results are consistent with the studies of R1 TraM that show DNA binding only as a tetramer (17), but argue against a model where TraM initially binds to
DNA as a dimer (9). Interestingly, SV-AUC indicates that NTP is dimeric in solution although unfolding experiments suggest that it is not oligomeric. The discrepancy between the unfolding and SV-AUC experiments may indicate that dissociation of the NTP dimer does not perturb the structure in a manner that can be detected by either CD or intrinsic tyrosine fluorescence. An analogous fragment of R1 TraM, TraMM26, was also shown to be dimeric by gel-filtration (17), although it was monomeric under conditions used to solve the NMR structure (42). The observation that TraM does not form dimers suggests NTP structure may contribute to the stability of the TraM tetramer but are not sufficient to support oligomerization alone.

Our domain studies indicate that structures important for oligomerization and DNA binding overlap. Although CTD is able to act as a tetramerization domain, the TraM tetramer is more stable than the isolated CTD tetramer, consistent with the assertion that N-terminal structures influence the stability of the tetramer. Furthermore, it has been shown that NTP contains residues that are important for DNA binding (11,43,44), although neither this fragment nor CTD can reproduce the DNA binding activity of TraM. This suggests that the DNA binding domain of TraM includes structures that are involved in, or stabilized upon, oligomerization. Consistent with this hypothesis, the R1 TraM homologue is stabilized upon the addition of DNA (17).

We have shown that in vitro both NTP and CTD can interact with full-length TraM. Although we have not ruled out the possibility of other types of interactions, it is possible that the constructs are incorporated into tetramers with full-length protein. Our experiments demonstrate that in vitro CTD does not interfere with TraM DNA binding, although it does show a dominant negative interaction in mating experiments. We suggest that in vivo CTD interferes with an essential function of TraM that is separate from DNA binding. For example, it is
possible that the TraM-TraD interaction is disrupted in these cells. In contrast, NTP interferes with the ability of TraM to bind DNA although no genetic interaction was observed. This apparent contradiction can be explained if the interaction relieves the negative autoregulation of \textit{traM}. The increased expression of TraM would interfere with any dominant negative effect on conjugation.

We propose that the mating signal could modulate the oligomerization equilibrium of TraM. In this model, the negative autoregulation of TraM keeps the intracellular protein concentration at a level such that only \textit{sbmA} and \textit{sbmB}, the high affinity binding sites, are occupied by TraM tetramers. After successful mating pairs have been formed, the mating signal shifts the oligomerization equilibrium and the concentration of TraM tetramers is increased. This equilibrium shift results in occupation of the \textit{sbmC} binding site, thereby allowing for DNA transfer to begin. Our thermodynamic analysis of TraM oligomerization indicates that this model is consistent with the physical properties of TraM. Future studies will determine if the activity of TraM is regulated by its oligomerization \textit{in vivo}.

**References**

1. Lederberg, J., and Tatum, E. L. (1946) \textit{Nature} \textbf{158}, 558
2. Ou, J. T., and Reim, R. L. (1978) \textit{J. Bacteriol.} \textbf{133}, 442-445
3. Frost, L. S., Ippen-Ihler, K., and Skurray, R. A. (1994) \textit{Microbiol. Rev.} \textbf{58}, 162-210
4. Kingsman, A., and Willetts, N. (1978) \textit{J. Mol. Biol.} \textbf{122}, 287-300
5. Willetts, N., and Wilkens, B. (1984) \textit{Microbiol. Rev.} \textbf{48}, 24-41
6. Ou, J. T. (1975) \textit{Proc. Natl. Acad. Sci.} \textbf{72}, 3721-3725
7. Abo, T., Inamoto, S., and Ohtsubo, E. (1991) \textit{J. Bacteriol.} \textbf{173}, 6347-6354
8. Di Laurenzio, L., Frost, L. S., and Paranchych, W. (1992) *Mol. Microbiol.* **6**, 2951-2959
9. Fekete, R. A., and Frost, L. S. (2002) *J. Biol. Chem.* **277**, 16705-16711
10. Schwab, M., Gruber, H., and Hogenauer, G. (1991) *Mol. Microbiol.* **5**, 439-446
11. Schwab, M., Reisenzein, H., and Hogenauer, G. (1993) *Mol. Microbiol.* **7**, 795-803
12. Penfold, S. S., Simon, J., and Frost, L. S. (1996) *Mol. Microbiol.* **20**, 549-558
13. Abo, T., and Ohtsubo, E. (1993) *J. Bacteriol.* **175**, 4466-4474
14. Fu, F. Y.-H., Tsai, M.-M., Luo, Y., and Deonier, R. C. (1991) *J. Bacteriol.* **173**, 1012-1020
15. Abo, T., and Ohtsubo, E. (1995) *J. Bacteriol.* **177**, 4350-4355
16. Disque-Kochem, C., and Dreiseikelmann, B. (1997) *J. Bacteriol.* **179**, 6133-6137
17. Verdino, P., Keller, W., Strohmaier, H., Bischof, K., Lindner, H., and Koraimann, G. (1999) *J. Biol. Chem.* **274**, 37421-37428
18. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A LABORATORY MANUAL*, second Ed., Cold Spring Harbor Laboratory Press
19. Studier, F. W., and Moffatt, B. A. (1986) *J. Mol. Biol.* **189**, 113
20. Yanisch-Perron, C., Vieira, J., and Messing, J. (1985) *Gene* **33**, 103
21. Yu, D., Ellis, H. M., Lee, E.-C., Jenkins, N. A., Copeland, N. G., and Court, D. L. (2000) *Proc. Natl. Acad. Sci.* **97**, 5978-5983
22. Liang, P., and Schildbach, J. F. (1999) *J. Biol. Chem.* **274**, 1-5
23. Gill, S. C., and von Hippel, P. H. (1989) *Anal. Biochem.* **182**, 319-326
24. Cowgill, R. W. (1976) in *Biochemical Fluorescence: Concepts* (Chen, R. F., and Edelhoch, H., eds) Vol. 2, pp. 441-486, Marcel Dekker, Inc., New York
25. Lakowicz, J. R. (1999) *Principles of Fluorescence Spectroscopy*, second Ed., Kluwer Academic/Plenum Publishers, New York

26. Zweifel, M. E., and Barrick, D. (2001) *Biochemistry* **40**, 14357-14367

27. Stites, W. E. (1995) *Anal. Biochem.* **227**, 112

28. Schwehm, J. M., and Stites, W. E. (1998) *Methods Enzymol.* **295**, 150

29. Pace, C. N. (1986) *Methods Enzymol.* **131**, 266-280

30. Greene, R. F., and Pace, C. N. (1974) *J. Biol. Chem.* **249**, 5388-5393

31. Stafford, W. F. (1992) *Anal. Biochem.* **203**, 295-301

32. Philo, J. S. (2000) *Anal. Biochem.* **279**, 151-163

33. Laue, T. M., Shah, B. D., Ridgeway, T. M., and Pelletier, S. L. (1992) in *Analytical Ultracentrifugation in Biochemistry and Polymer Science* (Harding, S. E., Rowe, A. J., and Horton, J. C., eds), pp. 90-125, Royal Society of Chemistry, Cambridge, U.K.

34. Philo, J. S. (1997) *Biophys. J.* **72**, 435-444

35. Lebowitz, J., Lewis, M. S., and Schuck, P. (2002) *Protein Sci* **11**, 2067-2079

36. Brandts, J. F., and Hunt, L. (1967) *J. Am. Chem. Soc.* **89**, 4826-4838

37. Rosenfeld, S. S., Rener, B., Correia, J. J., Mayo, M. S., and Cheung, H. C. (1996) *J. Biol. Chem.* **271**, 9473-9482

38. Qin, B. Y., Chacko, B. M., Lam, S. S., de Caestecker, M. P., Correia, J. J., and Lin, K. (2001) *Mol. Cell* **8**, 1303-1312

39. Herrera, J. E., Correia, J. J., Jones, A. E., and Olson, M. O. J. (1996) *Biochemistry* **35**, 2668-2673

40. Rivas, G., Stafford, W. F., and Minton, A. P. (1999) *Methods* **19**, 194-212

41. Stafford, W. F. (2000) *Methods Enzymol.* **323**, 302-325
42. Stockner, T., Plugariu, C., Koraimann, G., Hogenauer, G., Bermel, W., Prytulla, S., and Sterk, H. (2001) *Biochemistry* **40**, 3370-3377

43. Kupelwieser, G., Schwab, M., Hogenauer, G., Koraimann, G., and Zechner, E. L. (1998) *J. Mol. Biol.* **275**, 81-94

44. Polzleitner, E., Zechner, E. L., Renner, W., Fratte, R., Jauk, B., Hogenauer, G., and Koraimann, G. (1997) *Mol. Microbiol.* **25**, 495-507

45. Chen, L., Hodgson, K. O., and Doniach, S. (1996) *J. Mol. Biol.* **261**, 658-671

**Acknowledgments**

We thank Drs. Ludwig Brand for access to the fluorometer, Doug Barrick for discussions of thermodynamics and for use of the spectropolarimeter, and Beverly Wendland, Ernesto Freire, and Evangelos Moudrianakis for use of equipment. We are grateful to Dr. Michael E. Rodgers for guidance in analytical ultracentrifugation data collection and discussion of data, and to Olivia Doyle and members of the Schildbach lab for comments on the manuscript.

**Footnotes**

1Abbreviations: oriT, plasmid origin of transfer; Gnd-HCl, guanidine hydrochloride; CTD, C-terminal domain; NTP, N-terminal peptide; LB, Luria-Burtani broth; IPTG, isopropyl-1-thio-β-D-galactopyranoside; EDTA, ethylenediamine-tetraacetic acid; DTT, dithiothreitol; PMSF, phenylmethylsulfonyl fluoride; ORF, open reading frame; SV-AUC, sedimentation velocity analytical ultracentrifugation; BME, β-mercaptoethanol; CD, circular dichroism
Figure Legends

Figure 1: TraM (10 µM) was unfolded by titration with Gnd-HCl at 37 °C. The transition was monitored by CD (◊, top) or intrinsic tyrosine fluorescence (o, middle). The unfolding reaction is represented by open symbols, and refolding by filled symbols. The line through the CD data shows the constrained three-state model fit to the unfolding data. From this fit, $\Delta G_1 = 2.7 \pm 0.2$ kcal*mol$^{-1}$, $m_1 = 3.7 \pm 0.4$ kcal*mol$^{-1}$*M$^{-1}$Gnd-HCl, $\Delta G_2 = 2.0 \pm 0.3$ kcal*mol$^{-1}$, and $m_2 = 1.1 \pm 0.1$ kcal*mol$^{-1}$*M$^{-1}$Gnd-HCl. The bottom panel shows normalized data (45) plotted on the same axes (CD, ◊; fluorescence, •), demonstrating that both probes used to monitor the unfolding report very similar curves, with an intermediate populated near 1 M Gnd-HCl. Error bars in the top two panels, and other figures, are the standard deviation of the data points averaged for each measurement. In the top panel, the error bars are hidden by the data markers. Linear correlation coefficient (R) values from the fit are indicated on the graph.

Figure 2: Different initial concentrations of TraM were unfolded as in Figure 1. The top panel shows 1 µM (•) and 10 µM (◊) unfolding monitored by CD. The more negative starting value at higher protein concentration shows that TraM is stabilized at increased concentrations. The middle panel shows the fluorescence-monitored unfolding of 10 µM (◊) and 45 µM (▲) protein. The fluorescence data were normalized so that both titrations had the same starting value. At all concentrations tested, the shape of the curve is the same and neither transition midpoint varies dramatically. In the bottom panel, 10 µM TraM was unfolded in the absence (◊) or presence (▲) of 2.5 µM sbmA.

Figure 3: SV-AUC of TraM as a function of Gnd-HCl concentration. Top: $g(s^*)$ distribution
from sedimentation of 45 µM TraM in 0.5 M (•), 0.75 M (+), and 1.0 M (▲) Gnd-HCl are shown. Each g(s*) distribution was derived from 14-16 radial absorbance scans at approximately the same ω²t and was area normalized (37). As the concentration of Gnd-HCl is increased the peak in the g(s*) distribution becomes more asymmetric and shifts to lower s* values. The beginning and end points of the shift are marked with vertical lines. Bottom: The weight average sedimentation coefficient is plotted as a function of Gnd-HCl concentration (♦). The two-state model was fit to these data to derive ΔGtet,app of 2.9 ± 0.8 kcal*mol⁻¹ and mtet,app of 3.1 ± 2.3 kcal*mol⁻¹*M⁻¹ Gnd-HCl.

Figure 4: Unfolding of NTP and CTD. A. The CTD unfolding transition was monitored by fluorescence (left panel: 10 µM, ◊; 45 µM, ◊) and CD (right panel: 10 µM, □). The unfolding curve is shifted to the right at higher protein concentrations, suggesting that this domain is oligomeric. At 45 µM, ΔGCTD,F = 2.2 ± 0.1 kcal*mol⁻¹ and mCTD,F = 3.6 ± 0.8 kcal*mol⁻¹*M⁻¹ Gnd-HCl. The m-value derived from the fit to the data at 45 µM was fixed in the fits of the data at 10 µM to compensate for the short native baseline in these unfolding curves. For 10 µM CTD, The fit of the CD data (right panel) indicates that ΔGCTD,CD is 0.9 ± 0.4 kcal*mol⁻¹, and the fluorescence data (left panel) yields ΔGCTD,F of 0.6 ± 0.4 kcal*mol⁻¹. B. NTP unfolding was also monitored by fluorescence (left panel: 10 µM, ◆; 45 µM, ◆) and CD (right panel: 10 µM, △). The unfolding curve of NTP is not sensitive to the initial protein concentration. At 10 µM NTP, the fit to the fluorescence data indicate that ΔGNTP,F is 3.0 ± 0.2 kcal*mol⁻¹ and mNTP,F is 2.0 ± 0.1 kcal*mol⁻¹*M⁻¹ Gnd-HCl. The fit to the CD data, shown on the right, is very similar (ΔGNTP,CD is 3.0 ± 0.6 kcal*mol⁻¹ and mNTP,CD is 2.1 ± 0.3 kcal*mol⁻¹*M⁻¹ Gnd-HCl). For all panels, the unfolding reaction is open symbols and the refolding is closed symbols. Lines through the data
points are the two-state model fit to the unfolding data.

Figure 5: SV-AUC of isolated domains (125 µM) were fit using the dc/dt mode of SVEDBERG. Each panel shows the one-species model fit (solid lines) overlaid on one data set (the difference between two scans), however, the fit was generated starting with 12-14 scans after the boundary had moved almost halfway down the cell. CTD (o, top) is tetrameric in this fit, and NTP (◊, bottom) is dimeric.

Figure 6: Ni-NTA coelution assay shows that both NTP and CTD can interact with the full-length protein. The indicated protein (15 µM) was combined with TraMH6 (5 µM) and equilibrated at 37 ºC. The mixtures were then added to Ni-NTA agarose, which retains TraMH6. Arrows indicate the extent of migration for TraMH6, TraM (M), CTD (C), and NTP (N). The label above each lane indicates which protein was equilibrated with TraMH6. The first three lanes (ELUTE) show proteins retained on the Ni-NTA agarose, and this coelution is quantitated in the graph. Both CTD and NTP are retained with TraMH6, suggesting that they can interact with full-length protein. The last three lanes (FT) show protein that was not bound to the Ni-NTA agarose.

Figure 7. The ability of TraM (50 nM) to bind DNA was measured after it was equilibrated with either CTD (from left, third through fifth lanes) or NTP (seventh through ninth lanes). The TraM:fragment molar ratio is indicated above each lane. TraM alone (50 nM, TraM lane) but neither NTP nor CTD (500 nM; NTP and CTD lane) can bind DNA. The appearance of free DNA (arrow, first lane) in the lanes with TraM indicates that an interaction with the fragment
inhibits DNA binding by TraM. Equilibration of TraM with NTP severely affects DNA binding, especially at a ratio of 1:10 (seventh lane, arrow).
Table 1: Mating Assay Data

| Donor Strain | plasmid   | Efficiency |
|--------------|-----------|------------|
| DY330 F'_ER M::kan | pET-21 | < 1.0×10^-7 |
|              | pET-TraM | 1.4×10^-5  |
|              | pET-CTD  | < 1.0×10^-7 |
|              | pET-NTP  | < 1.0×10^-7 |
| DY330 F'_ER | pET-21   | 1.2×10^-2  |
|              | pET-TraM | 1.7×10^-2  |
|              | pET-CTD  | 6.8×10^-3  |
|              | pET-NTP  | 1.2×10^-2  |

^a The indicated plasmid was transformed into the donor strain by electroporation, and the resulting transformants were used as donors in the mating assay. ^b The reported transfer efficiency is the average of three independent donors. When no transconjugants were observed (values preceded by <), the sensitivity of the assay is indicated.
Figure 1

![Graph showing the relationship between [Gnd-HCl] (M) and the fraction of signal lost, fluorescence intensity, and [\(\Theta\)]_{222nm} (deg cm\(^2\) dmol\(^{-1}\) res\(^{-1}\) / 1000). The correlation coefficient R is 0.99981.](http://www.jbc.org)
Figure 2

Fluorescence Intensity $\left[\Theta\right]_{222\text{nm}}$ (deg*cm$^2$*dmol res$^{-1}$)/1000

Fluorescence Intensity

$\left[\Theta\right]_{235\text{nm}}$ (deg*cm$^2$*dmol res$^{-1}$)/1000

[Gnd-HCl] (M)

1 µM

10 µM

45 µM
Figure 3

![Graph showing the relationship between $s^*_{20,w}$ [Svedbergs] and [Gnd-HCl] (M). The graph includes a line with the equation $R=0.99525$.](image)

- $g(s^*)$ [AU/Svedberg]
- $s^*_{20,w}$ [Svedbergs]
- $\langle s^*_{(20,w)} \rangle$
- [Gnd-HCl] (M)
Figure 4

A. CTD

B. NTP
Figure 5

CTD

NTP

Absorbance vs. radius (cm) for CTD and NTP samples.
Figure 6

[Diagram showing a gel with bands labeled ELUTE and FT, and a bar graph showing coelution efficiency for TraM, NTP, and CTD]
Evidence for a monomeric intermediate in the reversible unfolding of F factor TraM
Dana L. Miller and Joel F. Schildbach

J. Biol. Chem. published online January 15, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M212502200

Alerts:
  • When this article is cited
  • When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts