Supporting Information

Electrophoretic Deformation of Individual Transfer RNA Molecules Reveals Their Identity

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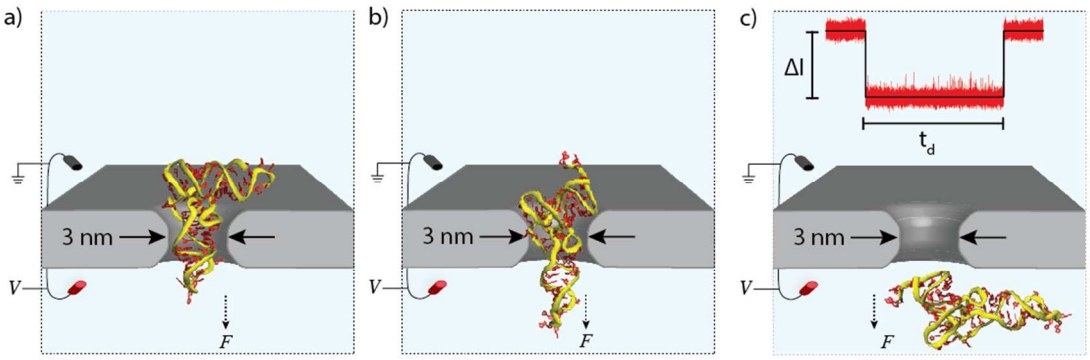

Figure S1. Cartoon of voltage-induced tRNA deformation/translocation through a 3 nm diameter nanopore. a) Trans-pore voltage induces a pore-localized electric field that drives a tRNA molecule into the pore constriction; b) as the elbow region reaches the pore, electrophoresis through the constriction induces tRNA deformation from its native conformation; c) Electro-deformed tRNA translocates through the pore. Sample experimental pulse is inset to c), with markers indicating the mean current blockade (ΔI) and dwell time (t_d).

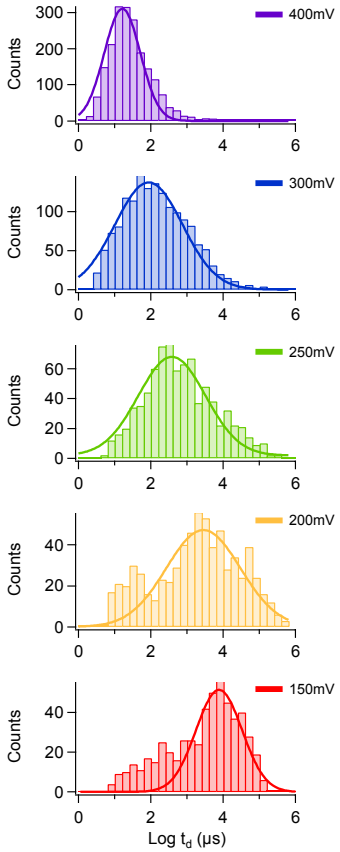

Figure S2: Histograms for the log of dwell times for $tRNA^{Arg}$ molecules at different voltages fit to log-normal distributions. This data and the fits we're used for the voltage vs. dwell time plot in Figure 2b. Location of peak values indicates most probable dwell times, where the standard deviation of the fit is propagated to find the error.

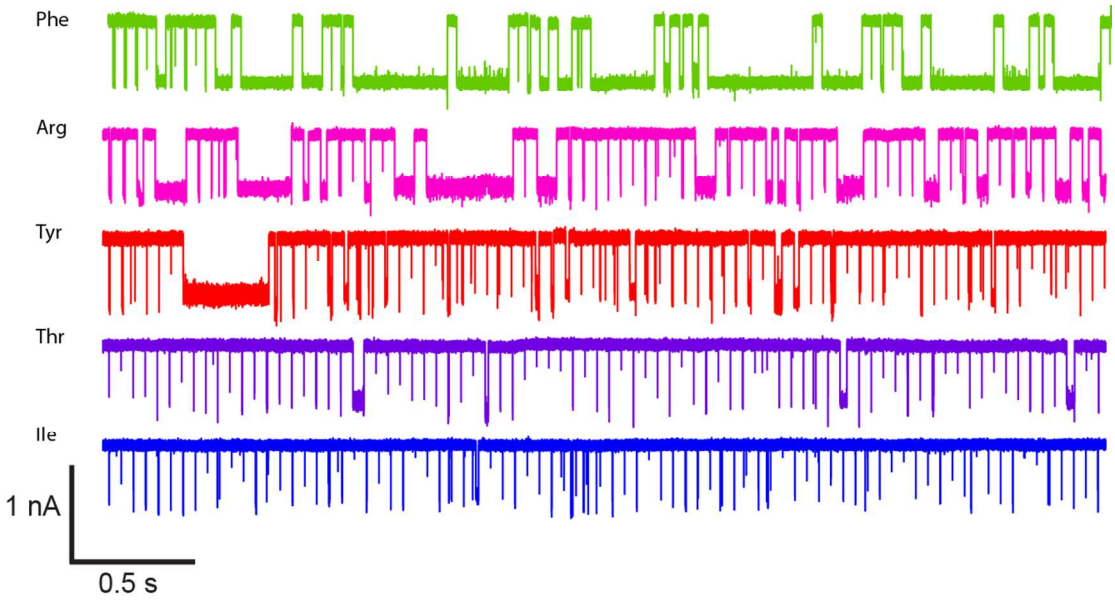

Figure S3: Traces showing concatenated events for each of the tRNA samples passing through a pore (~3nm diameter, 10nm thickness) with an applied voltage of 300mV. Data was sampled at 4.166 MHz and shown after low-pass filtering at 300kHz. Detected events are separated by 10,000 data points.

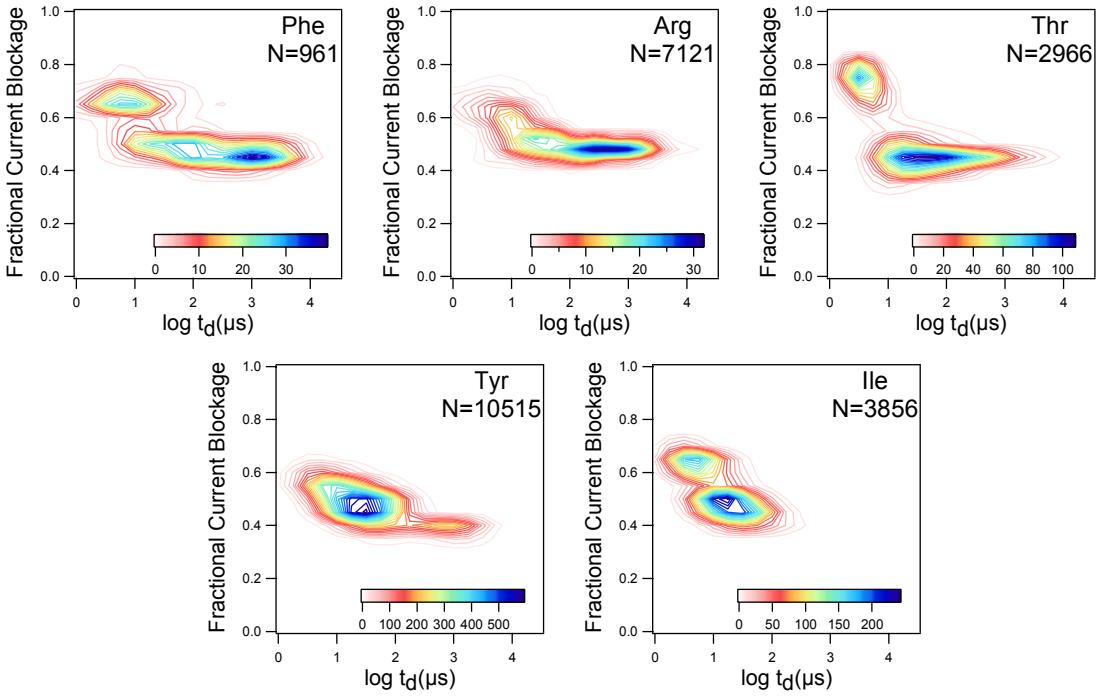

Figure S4: Contour plots depicting the fractional current blockage and dwell time of events for each of the tRNA samples passing through a pore (~3nm diameter, 5nm thickness) with an applied voltage of 500mV.

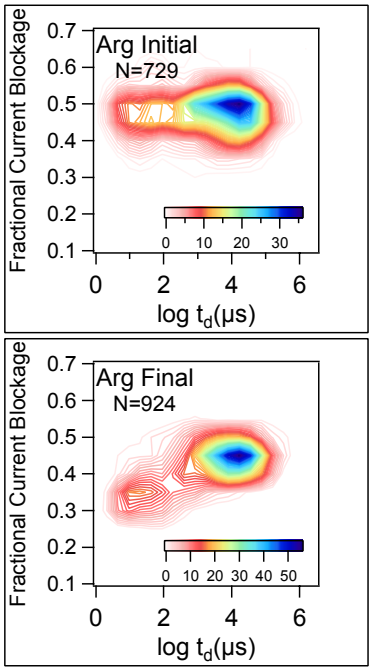

Figure S5: Contour plots depicting the fractional current blockage and dwell time of events for two different runs of the Arg tRNA on the same pore with the same conditions. Taken at the beginning and end of an experiment to show minimal pore expansion over the course of an experiment (~90 minutes).

Feature Name	Description	Unit
Max Amplitude	Greatest current blockade	nA
Spectrum Band 4	Power spectrum value at 833 kHz	nA
Log Spectrum Band 13	Power spectrum value at 47 kHz	Unitless
Log Spectrum Band 27	Power spectrum value at 275 kHz	Unitless
Log Spectrum Band 28	Power spectrum value at 301 kHz	Unitless
Dominant Frequency	Strongest noise frequency	kHz

Table S1: Full list of features used in SVM analysis for discrimination of $tRNA^{Phe}$, $tRNA^{Arg}$, $tRNA^{Tyr}$, $tRNA^{Ile}$, and $tRNA^{Tyr}$.

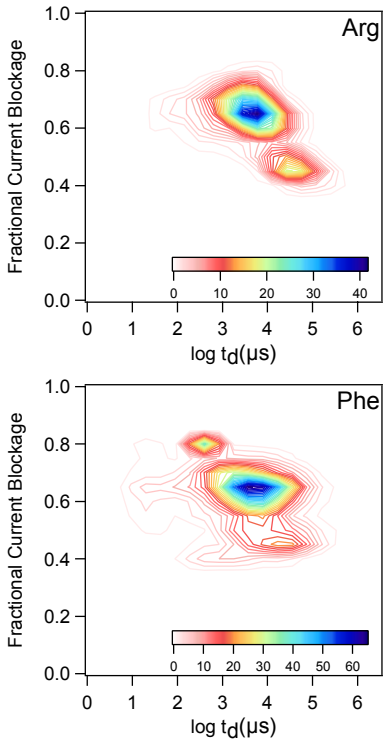

Figure S6: Comparison of $tRNA^{Arg}$ and $tRNA^{Phe}$ fractional current blockade and dwell time data used in mixture experiments. The two populations clearly display a very large degree of overlap.

Feature Name	Description	Unit
Peak Width	Full width half amplitude	ms
Spectrum Band 6	Power spectrum value at 1249 kHz	nA
Spectrum Band 8	Power spectrum value at 1666 kHz	nA
Spectrum Band 10	Power spectrum value at 2082 kHz	nA
OddEvenRatio	Even wavenumbers divided by odd wavenumbers	Unitless
Log Spectrum Band 5	Log of power spectrum value between 5.1 - 5.12 kHz	Unitless
Log Spectrum Band 6	Log of power spectrum value between 5.12 - 5.16 kHz	Unitless

Table S2: Full list of features used in SVM analysis for discrimination of $tRNA^{Phe}$ and $tRNA^{Arg}$ mixtures.

Feature Name	Description	Unit
Spectrum Band 1	Power spectrum value at 17 kHz	nA
Spectrum Band 3	Power spectrum value at 33 kHz	nA
Spectrum Band 4	Power spectrum value at 39 kHz	nA
Spectrum Band 5	Power spectrum value at 49 kHz	nA
Peak Width	Full width at half amplitude	ms
Roughness	Standard deviation current peak	nA

Table S3: Full list of features used in SVM analysis for discrimination of $tRNA^{Ile}$ isoacceptors.

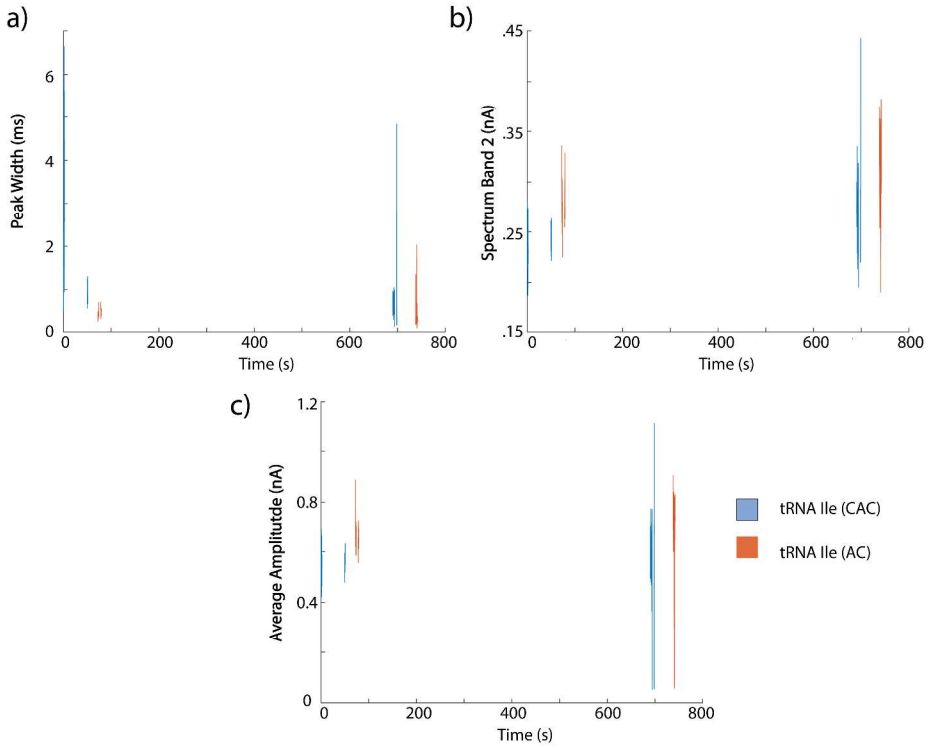

Figure S7: Time evolution of several SVM parameters during tRNA isoacceptor experiment. a) Peak width: the full width at half max of a translocation spike. b) Spectrum band 2: the power spectrum value at 18 kHz, measured during translocation. c) Average amplitude: average current blocked during translocation.

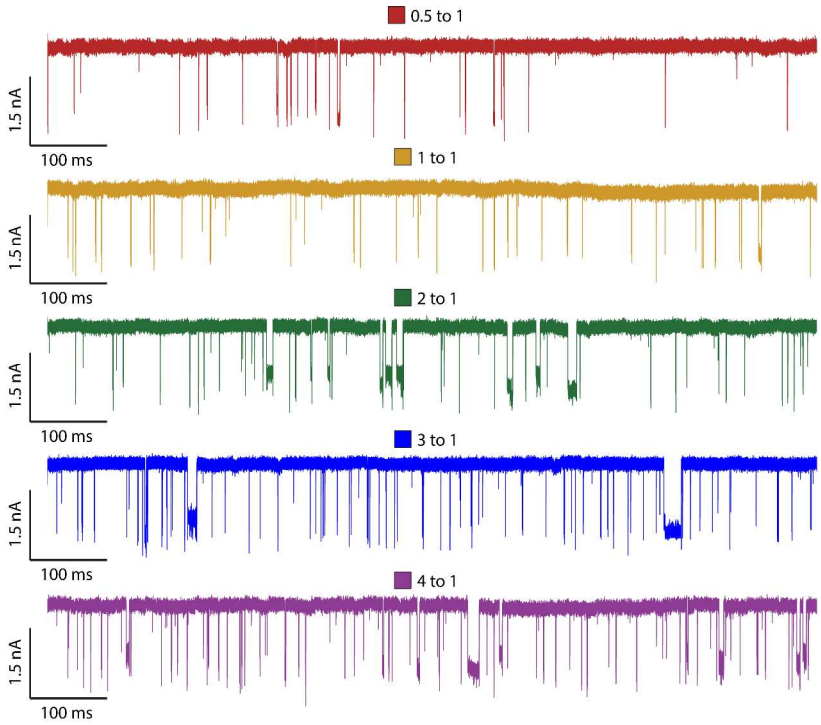

Figure S8: Raw current traces are shown from each of the mixture samples tested. Each mixture is labeled to show the ratio of Ile_{UAC} tRNA molecules to Ile_{CAC} tRNAs molecules. Data was collected at a sampling rate of 4.17MHz and shown after low pass filtering at 200kHz. Experimental buffer contains 400mM KCl, 10mM tris, and 1mM EDTA. A voltage bias of 500mV is applied.

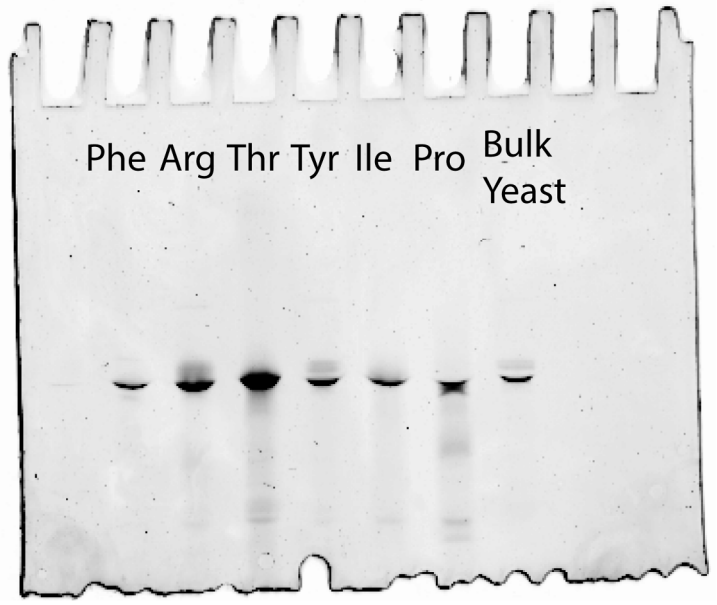

Figure S9: All tRNA samples used in these experiments shown on a 12% denaturing PAGE gel.

Pore #	Initial Size	Experiments used
1	Diameter ~ 3.2 nm Effective thickness ~5 nm	Voltage dependence (Fig. 1, SI2)
2	Diameter ~ 3 nm Effective thickness ~5 nm	Arg, Phe, Tyr, Thr, Ile (Fig. 2, 3, SI3, SI5) (Table 1, SI1)
3	Diameter ~ 3.2 nm Effective thickness ~9 nm	Arg & Phe mixture (Fig. 4, SI6) (Table SI2)
4	Diameter ~ 3.2 nm Effective thickness ~5 nm	Val isoacceptors (Fig. 5, SI7) (Table SI3)
5	Diameter ~ 3 nm Effective thickness ~10 nm	Arg, Phe, Tyr, Thr, Ile (Fig. SI4)

Table S4: Full list of nanopores used, their initial sizes, and the experiments they were used for. Pore sizes were estimated as described in the main text.