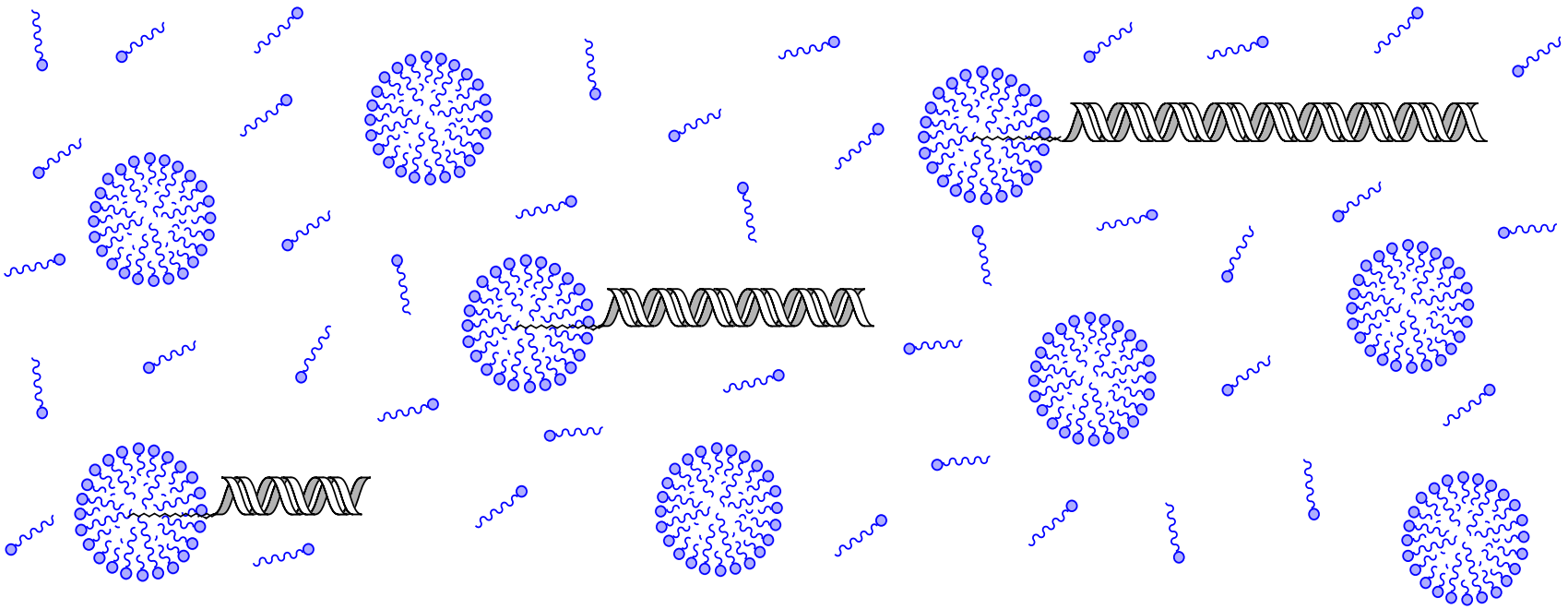
Micelle-tagging electrophoresis: Rapid, gel-free detection and separation of DNA



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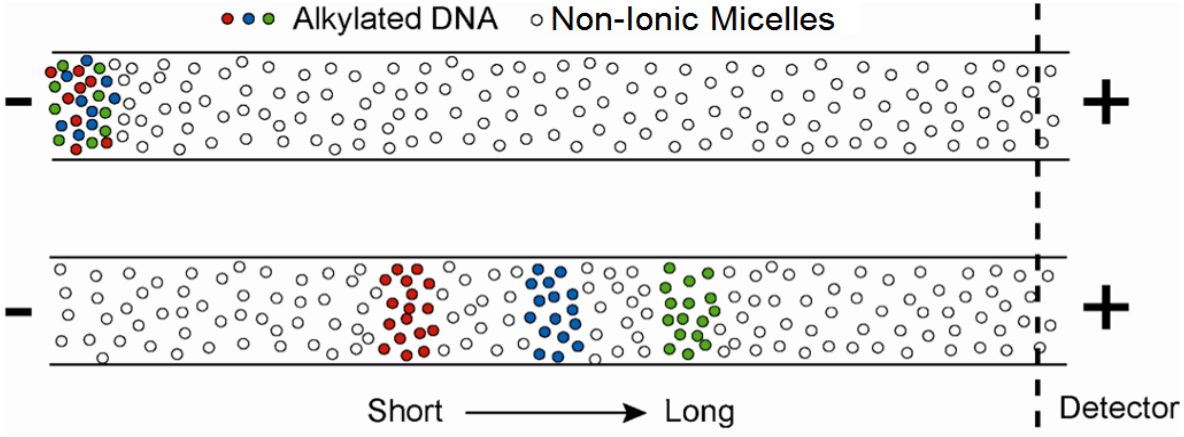
CBET-1605351

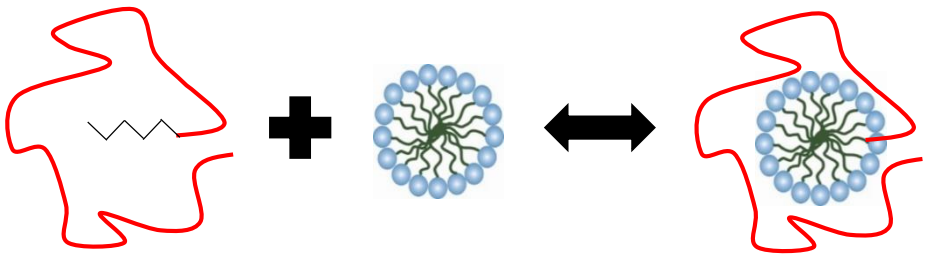
Micelle tagging electrophoresis (MTE)



Gel-free DNA electrophoresis method

- Provides mobility shifts using micelle drag-tag

Fast runtime



Standard MTE:

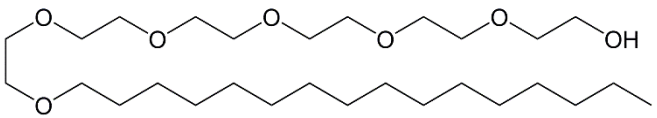
$$\mu = \mu_o \left(\frac{L}{L + \alpha} \right)$$

μ = mobility of tagged DNA
μ_0 = mobility of untagged DNA
L = length of DNA
α = micelle "size"

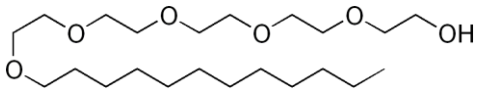
1. Slater *et. al*. *Anal Chem* (1994)

Long entangled worm-like micelles with C_iE_j surfactants

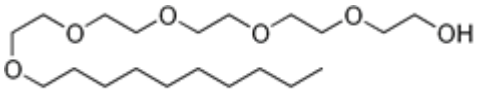
$C_{16}E_6$

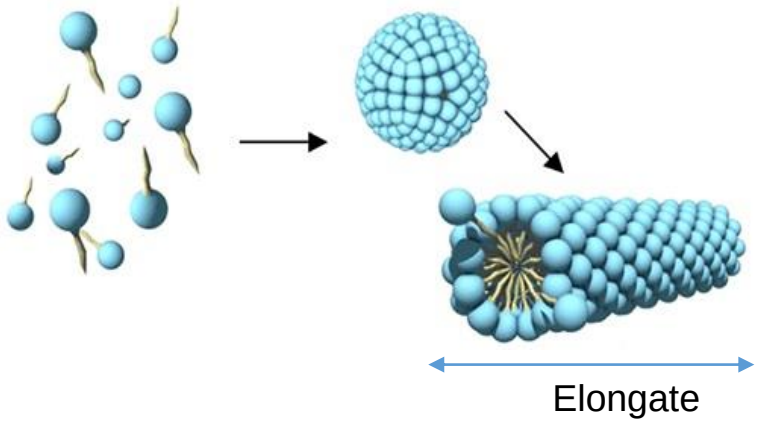


$C_{12}E_5$



$C_{10}E_5$





$$\bar{L} \approx \phi^{1/2} \exp[E_c(T) / 2k_BT]$$

$\bar{L}$: average micelle length
Φ: surfactant volume fraction
E_c: end-cap energy
k_BT: thermal (scaling) energy

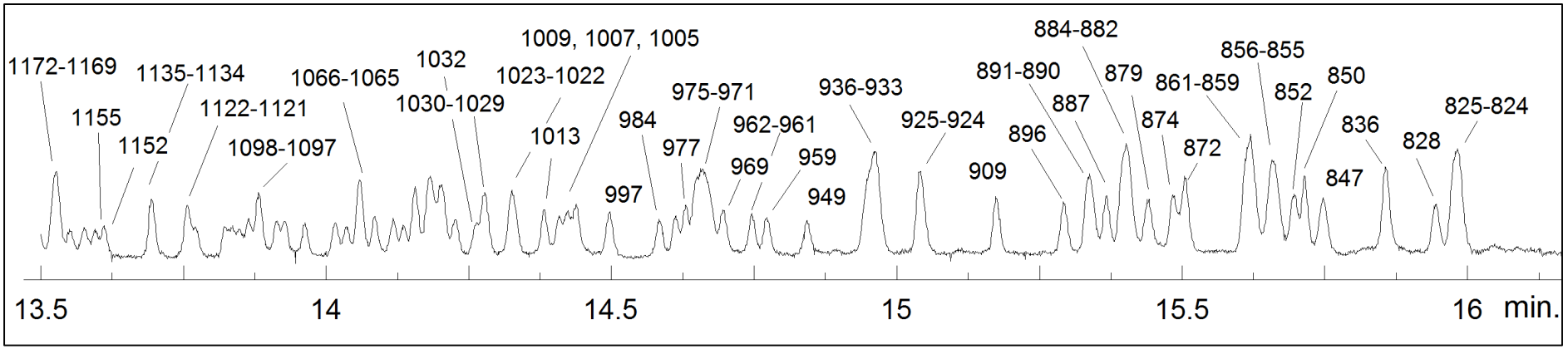
Micelle size can be precisely fine-tuned with:

- Buffer composition
- Separation temperature

1. Scott et al. *J. Phys. Chem.,* 1965
2. Imanish, et al. *J. Phys Chem.,* 2007
3. Ahmed, et al. *J. Colloid Interface Sci.,* 2008

High sequencing read length using MTE: greater than 1000 bases

Alkylated DNA fragments separated by 150mM $C_{12}E_5$ / 3M urea buffers (33°C)



EOF suppressant: 5% POP-6, ~3hr pre-conditioning

Length of capillary (l_c)/Length to detector: 43 cm/32 cm

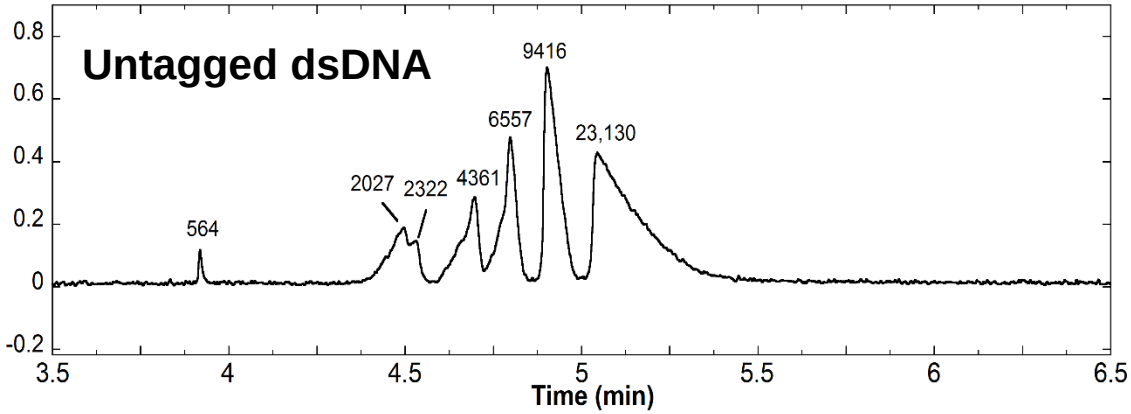
Injection: 2.5 kV, 15 seconds

Applied voltage (*V*): 15 kV

- Read length ~1,200 bases
- Highest read length with covalently attached drag tags is only 265 base*

1.*Barron et. al., Anal. Chem., vol 83, pp.509-515 (2011)
2. Yan et. al. *In Preparation*

Faster than microfluidic devices?
Rapid dsDNA separation without lithography



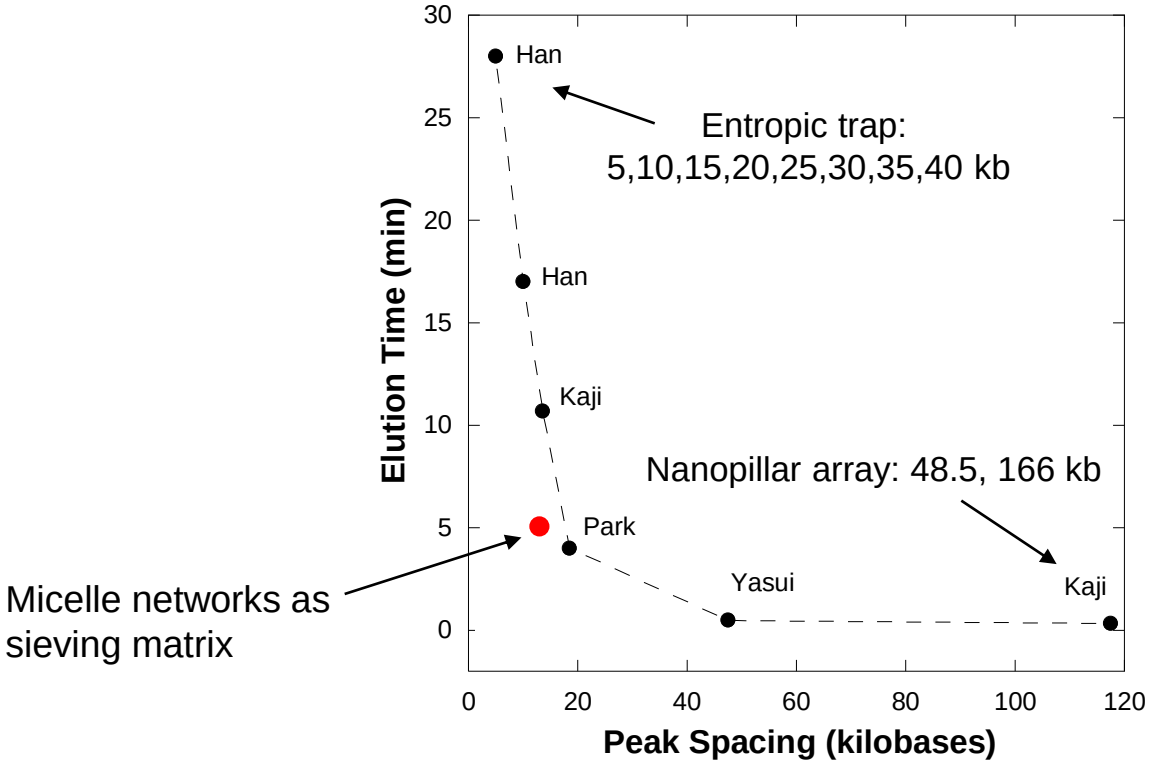
Sample: HindIII λDNA digest standard (untagged)

Buffer: 24mM $C_{16}E_6$/ 8mM $C_{12}E_5$/ 3mM $C_{10}E_5$

Length of capillary/length to detector: 30/20 cm

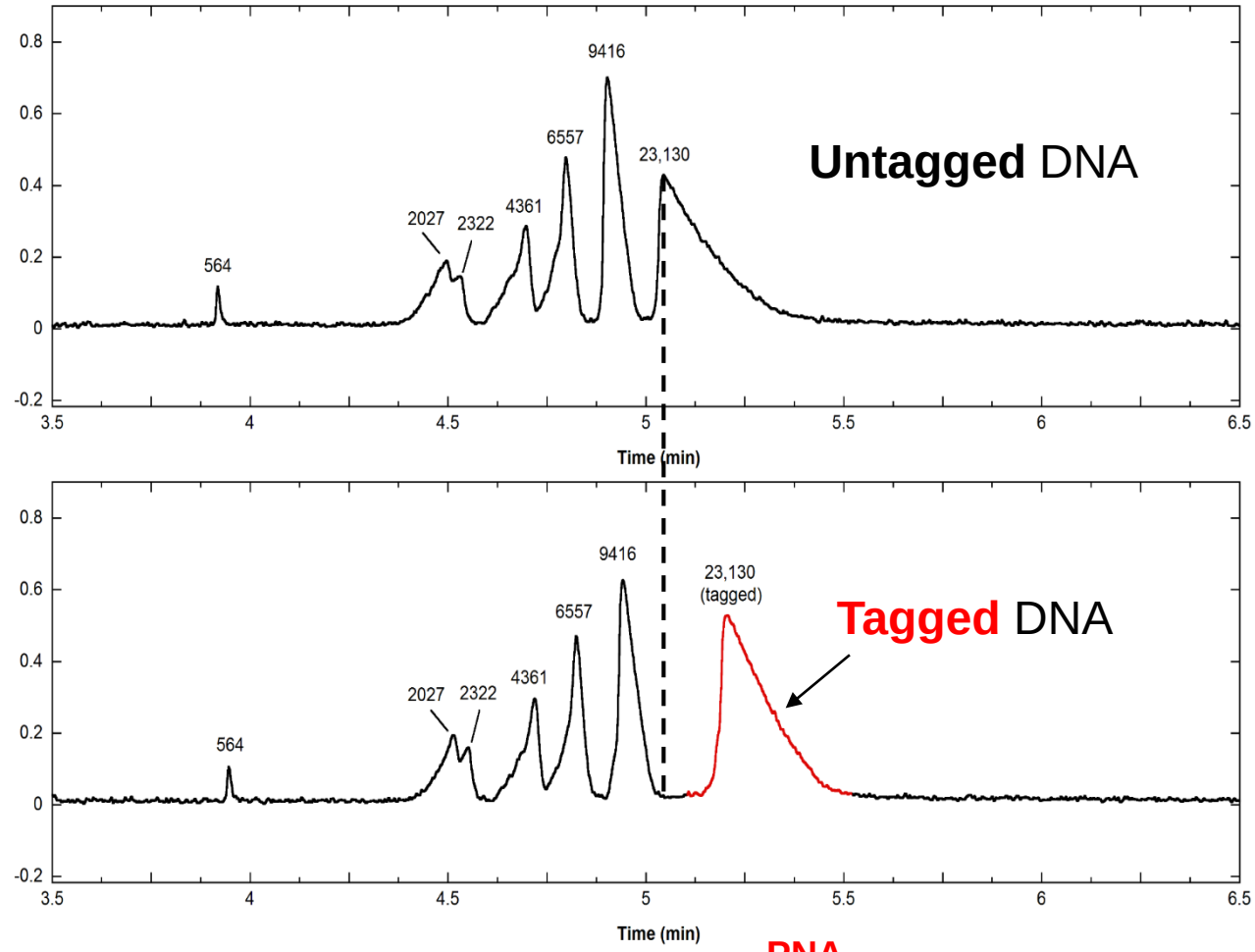
Applied voltage: 10kV

EOF suppressant: 10% POP-6, 10min rinse

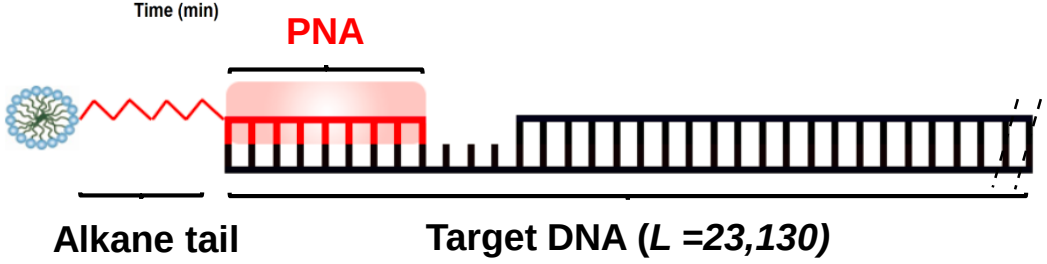


Kaji *et. al.*, *Analytical Chemistry* (2004).
Yasui *et. al*, *Analytical Chemistry* (2011)
Shi *et. al*, *Applied Physics Letters* (2007)
Park *et. al*,*Lab on a Chip* (2012)
Han *et. al*, *Science* (2000)

Simultaneous separation of untagged and tagged dsDNA



- Can **identity** and **separate** specific DNA (Virus/bacteria/RNA) in the presence of other DNA of similar size
- No apparent upper resolution limit for separating untagged vs tagged dsDNA



Advantages & applications of MTE in dynamic micelle networks

Advantages

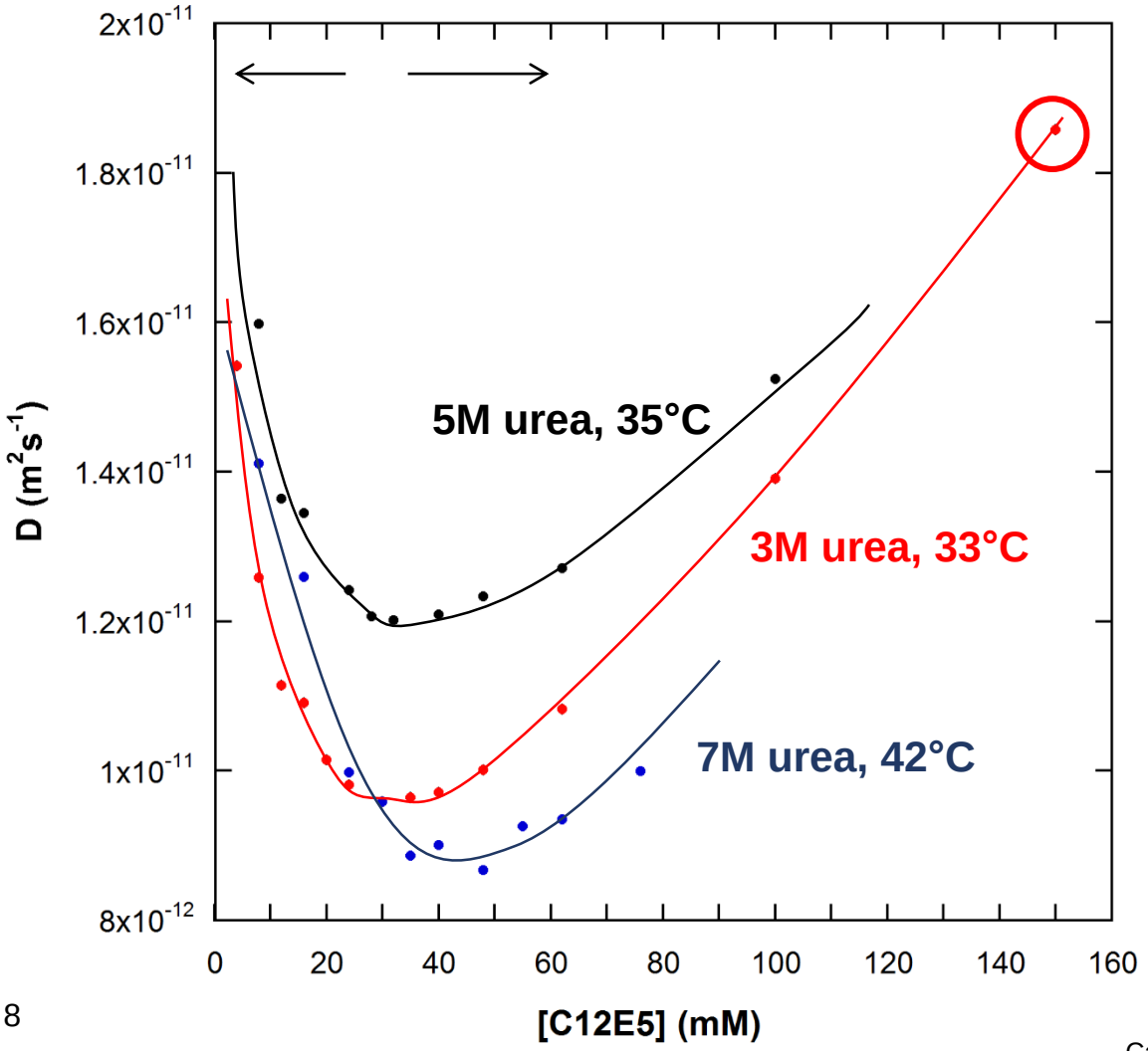
- Multiple ways to attach alkyl group to DNA of interest
- Easily implemented on any benchtop CE, even microchips
- Micelle size can be precisely controlled, even during an run
- High sensitivity using long DNA as fluorophore probe to detect sub-fM samples
- Unaffected by presence of serum contaminants

Applications

- Sequencing
- STR analysis
- miRNA detection
- At-line detection of viral/bacterial contaminants
- Plasmid purification

DLS: Surfactant buffers form well-entangled network

$$D_M = \frac{kT}{6\pi\eta R_H} \qquad D_c = \frac{kT}{6\pi\eta\xi}$$



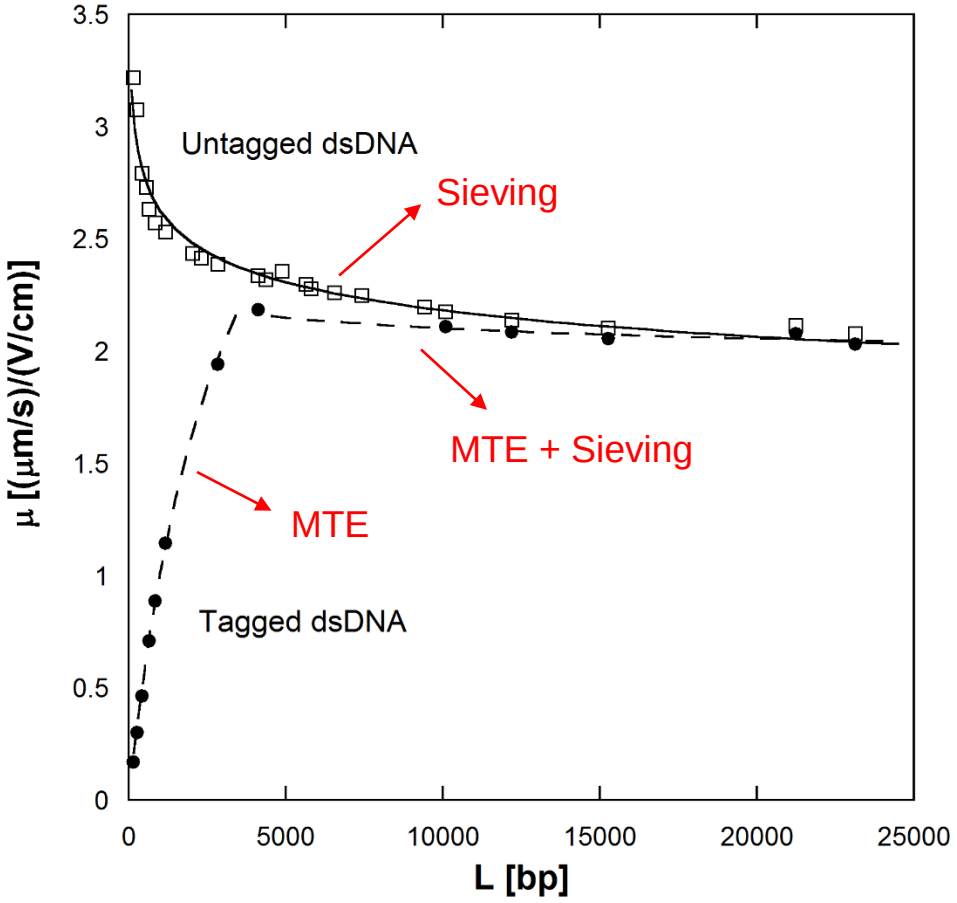
Buffer: 150mM $C_{12}E_5$/3M urea

- 6 wt%
- 5x overlap concentration (C*)
- 10-11 cP

- Overlapping micelles do not hinder micelle ELFSE separation

Cates, M.E. and S.J. Candau. *J Phys Condens Matter* (1990)

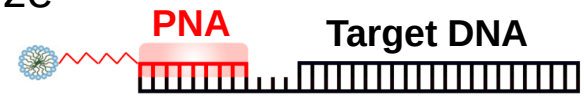
Multiple separation mechanisms



1. Untagged vs untagged
   - Micelle networks as dynamic sieving matrix

2. Tagged vs tagged
   - Short DNA: MTE
   - Long DNA: MTE + sieving

3. Tag vs untagged
   - Can identity and separate specific DNA (Virus/bacteria/RNA) in the presence of other DNA of similar size



No apparent upper resolution limit for separating untagged vs tagged dsDNA

1. *Yan et. al., *In Preparation*