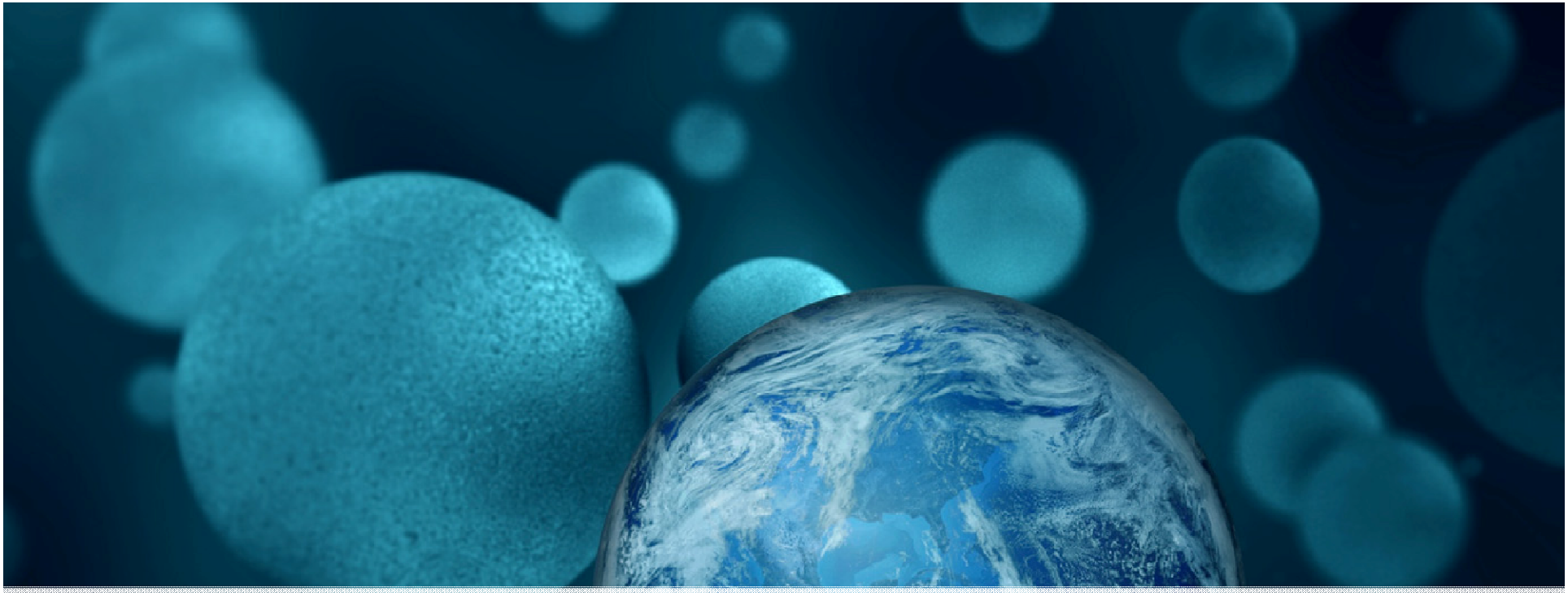

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High Resolution Anion Exchange Chromatography for Analysis and Purification of Oligonucleotides

5/24/2016

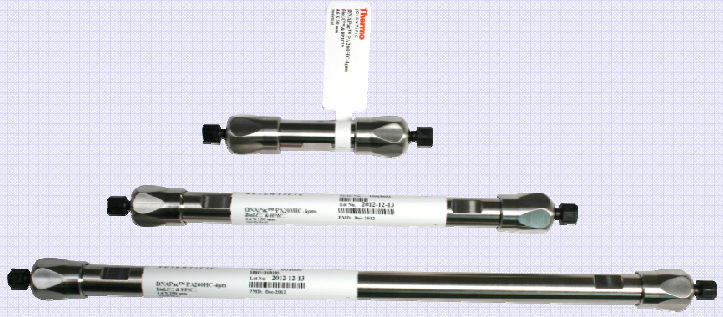
Julia Baek, Jim Thayer, Chris Pohl, Ilze Birznieks

The world leader in serving science

HPLC Analysis of Nucleic Acids

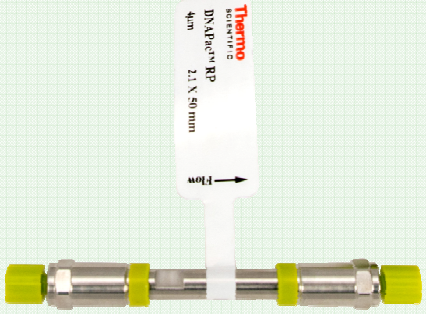
Anion Exchange Chromatography

DNAPac PA100
DNAPac PA200
DNAPac PA200 RS
DNASwift SAX-1S



Ion-Pair Reversed Phase Chromatography

DNAPac RP



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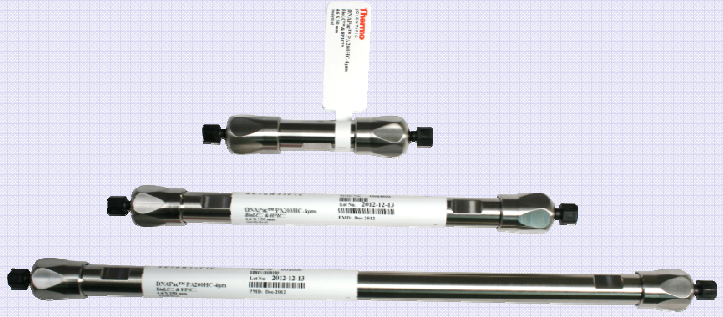
Anion Exchange Chromatography

Analytical Columns

DNAPac PA100
DNAPac PA200
DNAPac PA200 RS

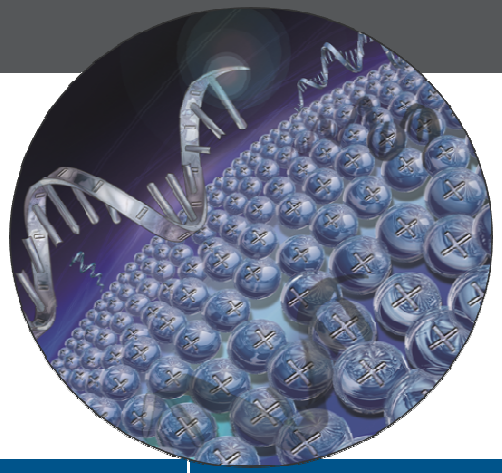


Semiprep Columns

DNAPac PA200
DNASwift SAX-1S

DNAPac PA Columns

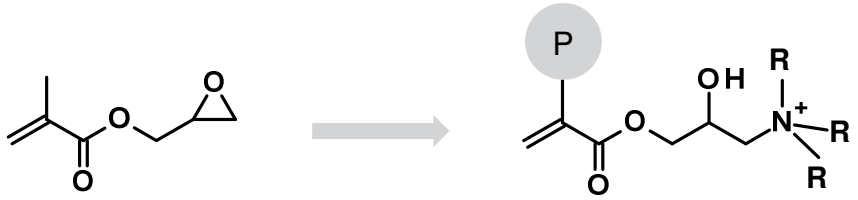
Pellicular Anion



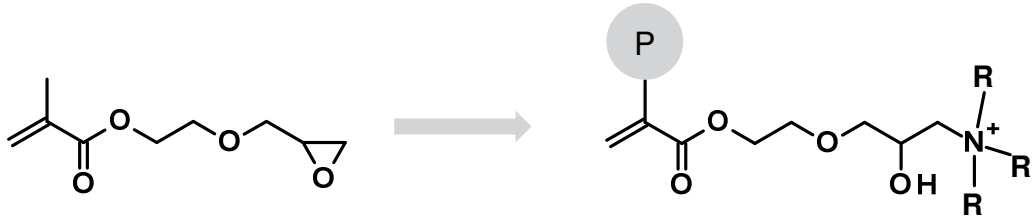
	DNAPac PA100	DNAPac PA200	DNAPac PA200 RS
Applications	ss/ds oligonucleotides	High resolution ss oligonucleotides	Ultra-high resolution ss oligonucleotides
Particle Size	13 µm	8 µm	4 µm
Column Chemistry	Non-porous polymer with quaternary amine functionalized latex beads		
pH Range	4-10 (up to 12.5 if [salt] > 5x [sodium hydroxide])		
Temperature Range	≤35 ºC at pH 8.5-12.5 ≤85 ºC at pH < 8.5		
Pressure Maximum	4,000 psi (276 bar)	4,000 psi (276 bar)	10,000 psi (690 bar)

DNAPac PA100 vs DNAPac PA200

DNAPac PA100, 13 µm



DNAPac PA200, 8 µm



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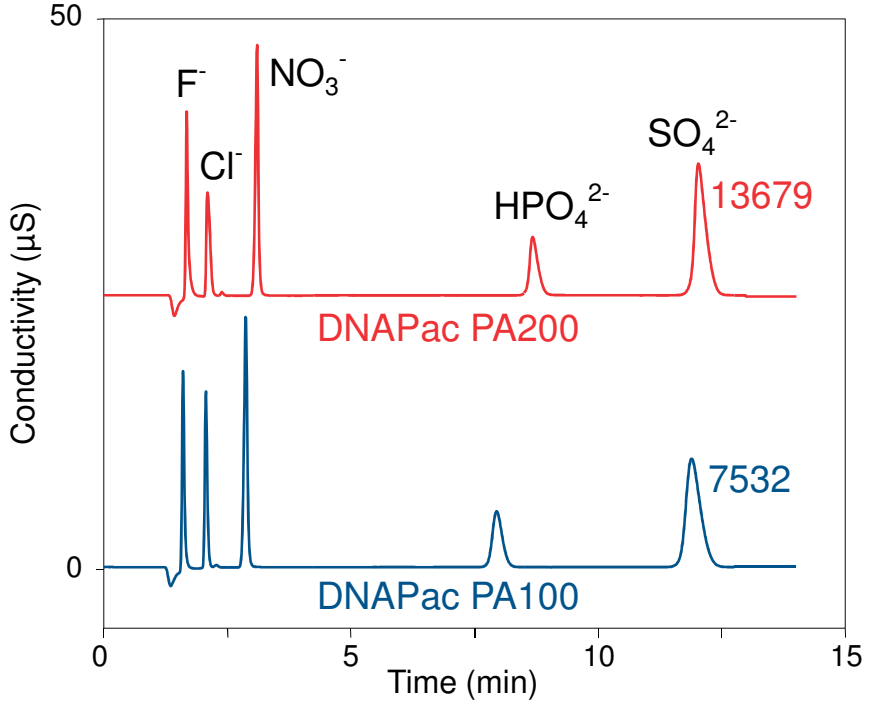
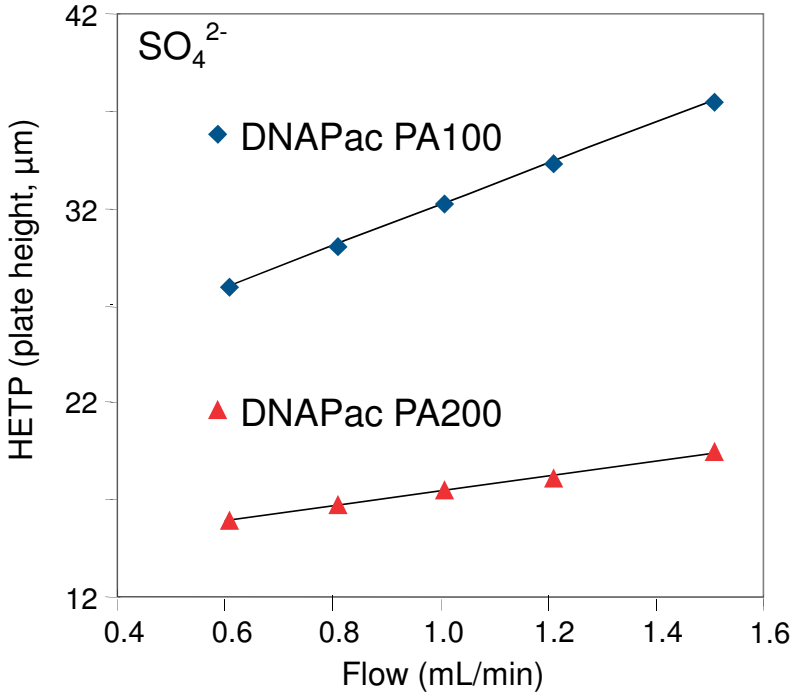
Chromatography Performance

Chromatography Performance



Relative Efficiency: HETP vs Flow



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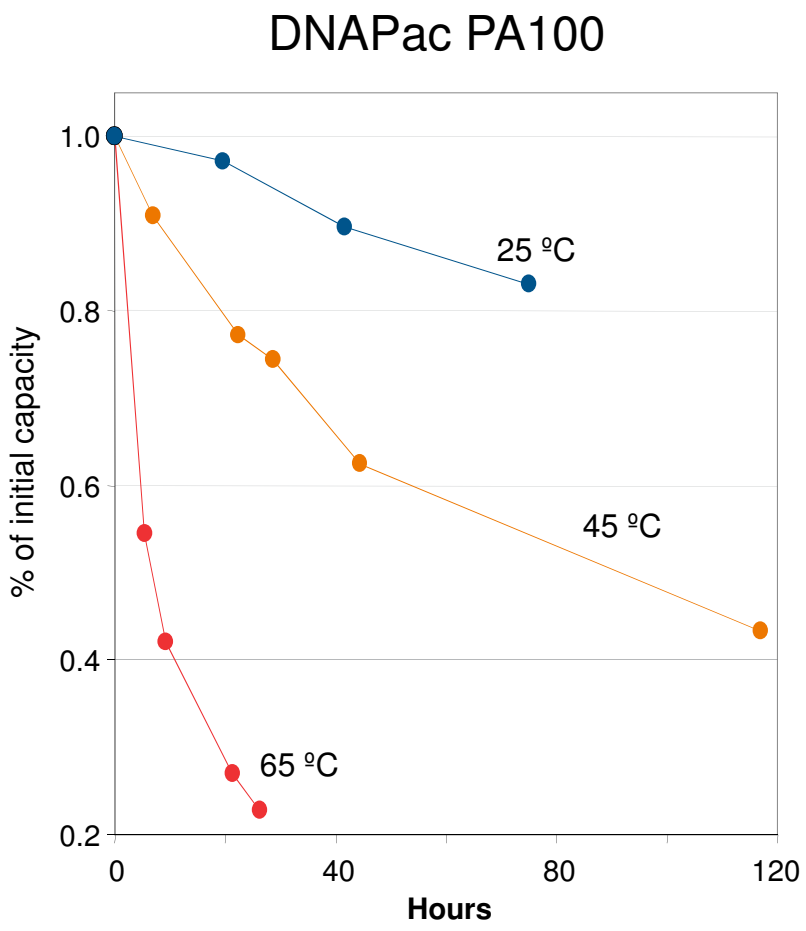
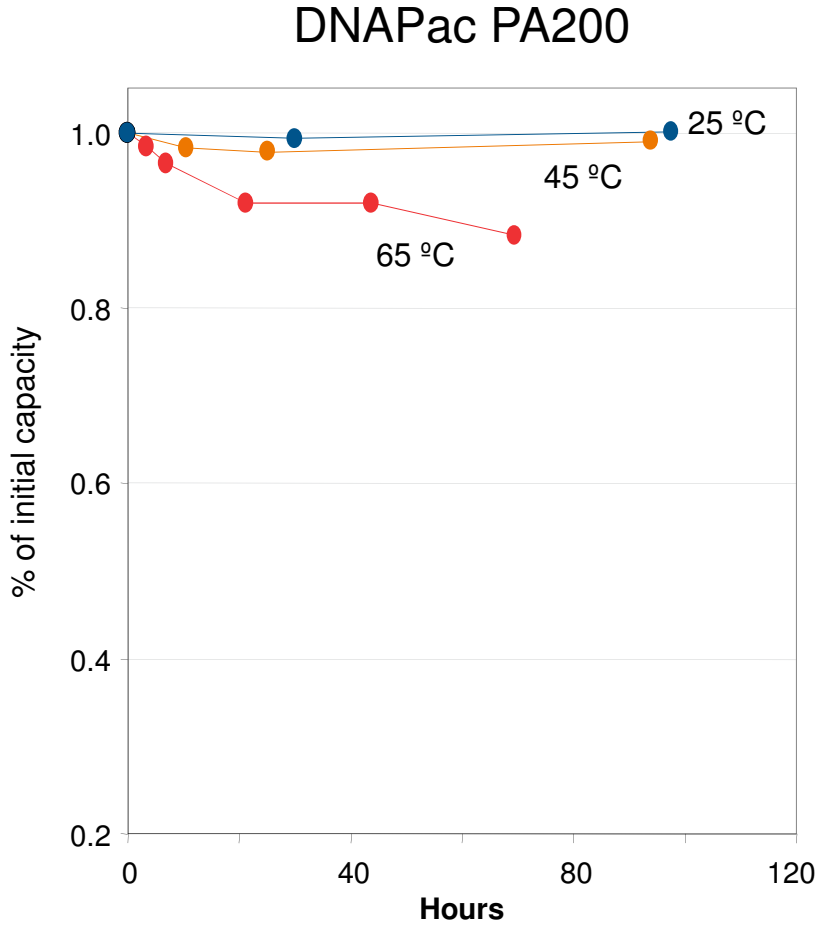
Phase Stability at pH 12.4





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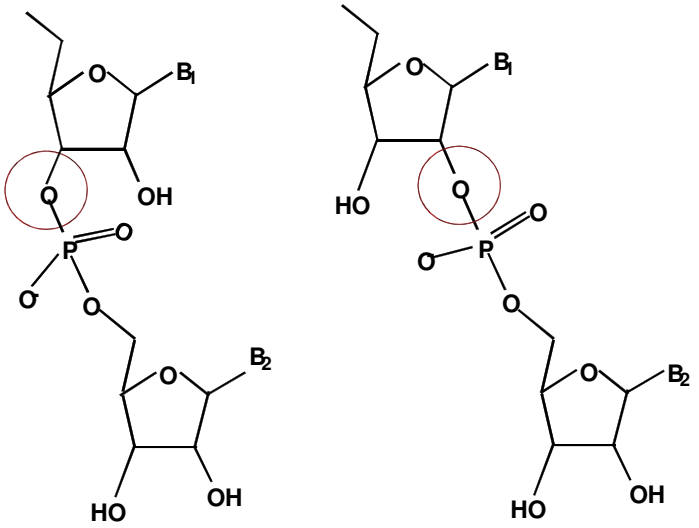
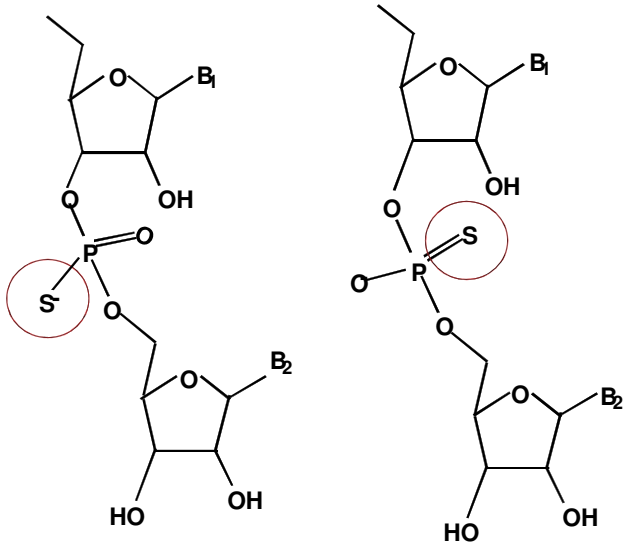
Applications
Comparison of DNAPac PA200 *RS* (4 µm) and DNAPac PA200 (8 µm)

- Resolution of dT samples
- Length based separations
- Separation of phosphorothioate diastereoisomers
- Separation of linkage isomers



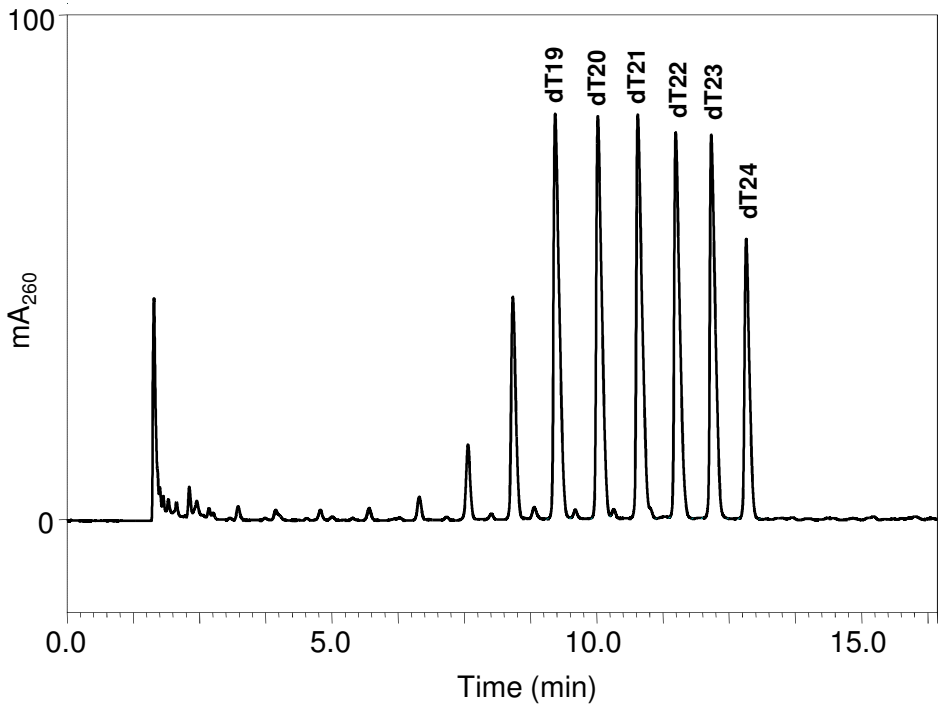
Phosphodiester Linkage Isomers



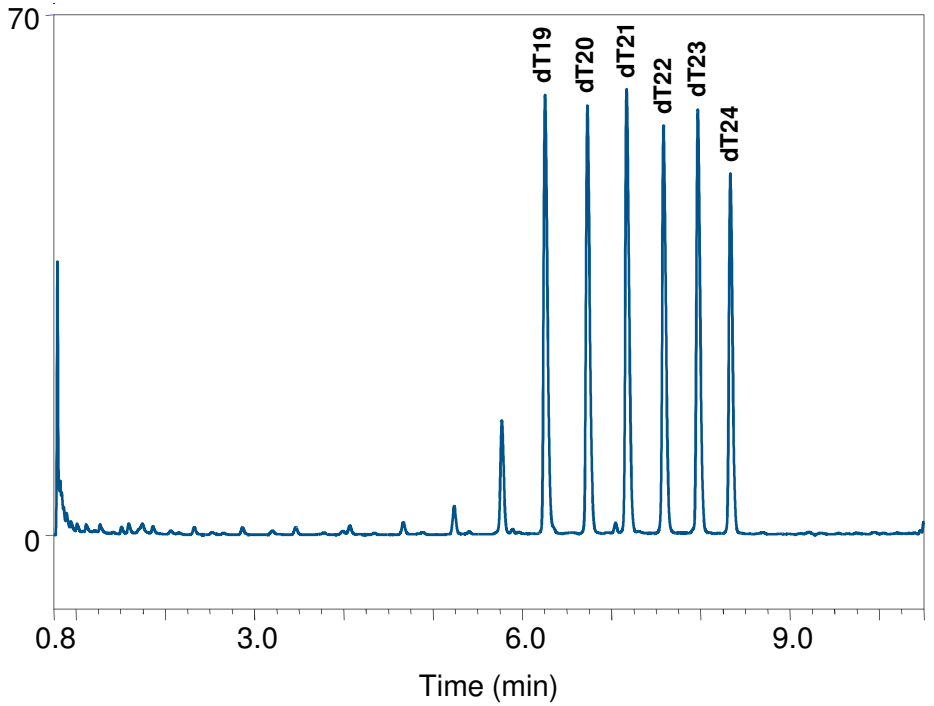
Phosphorothioate Diastereoisomers

Separation of dT19-24

DNAPac PA200 (4.0 × 250 mm)



DNAPac PA200 RS (4.6 × 150 mm)



Column	Flow (mL/min)	Pressure (psi)	RT (dT 23)	PW (5%)	Rs (19/20)	Rs (21/22)	Rs (22/23)	RT ratio (22/23)
DNAPac PA200	0.850	1,655	10.92	0.056	4.8	4.4	4.1	0.94
DNAPac PA200 RS	1.120	4,736	7.97	0.050	6.1	5.3	5.0	0.95

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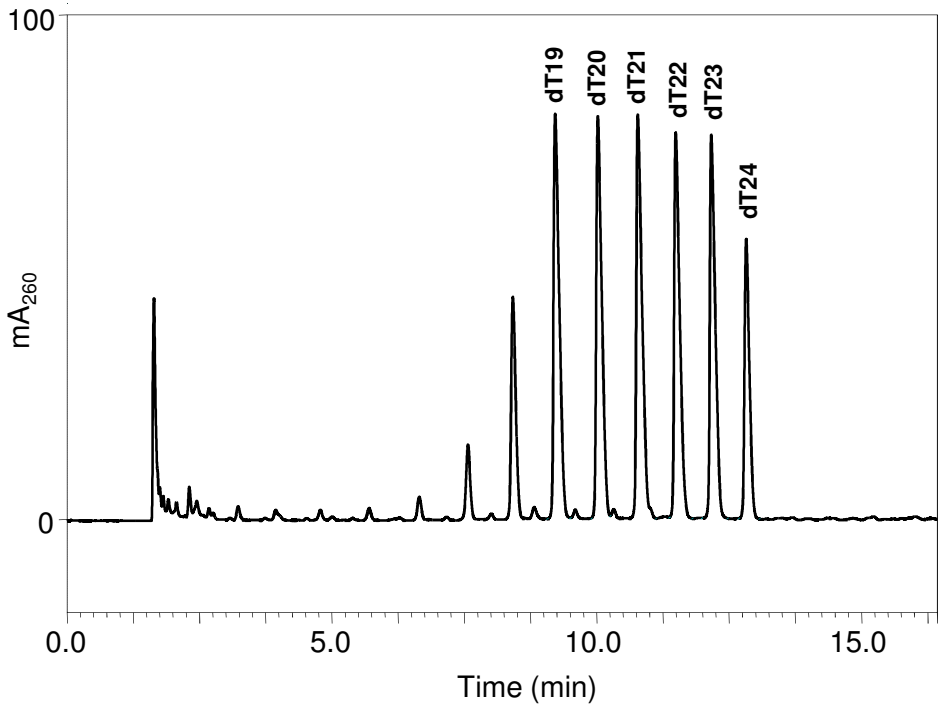
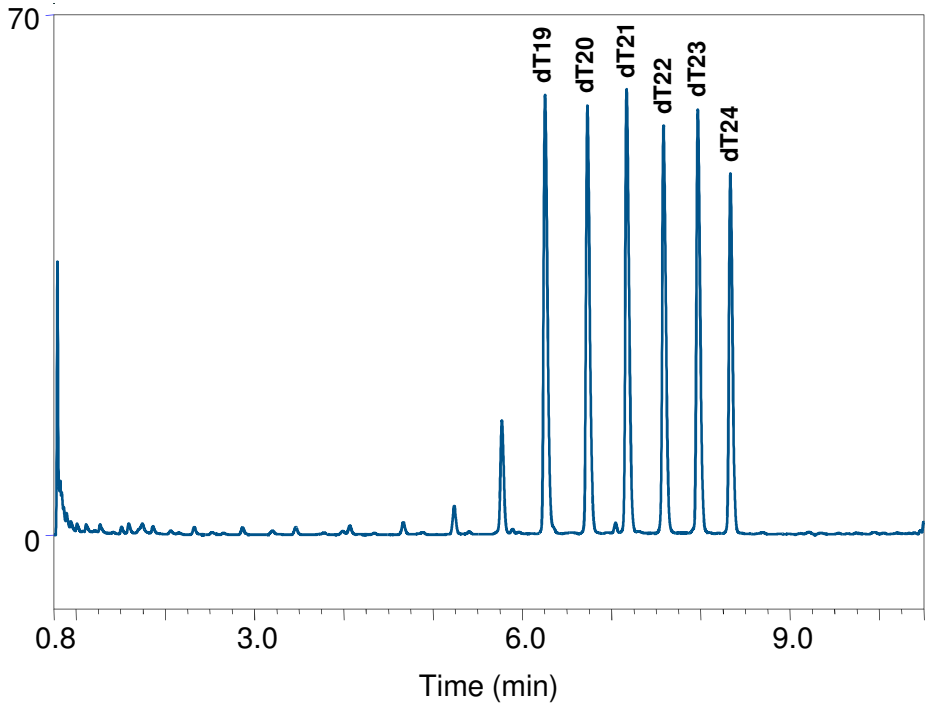
Separation of dT19-24

DNAPac PA200 (4.0 × 250 mm)



DNAPac PA200 RS (4.6 × 150 mm)



DNAPac PA200 RS with smaller particles shows:

- Improved Peak Width
- Improved Resolution
- Similar selectivity

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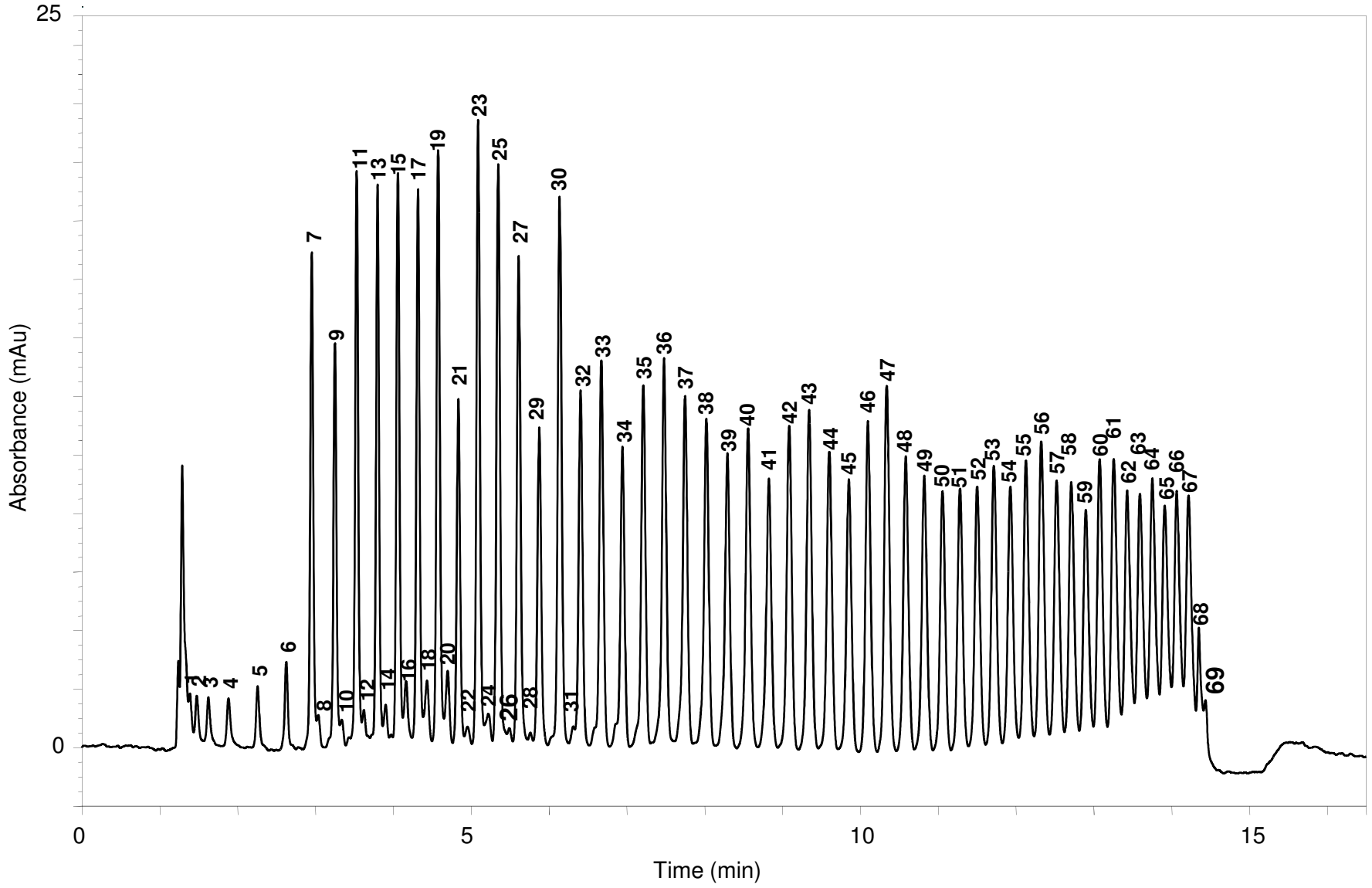
DNAPac PA200: Resolution of dT12-60



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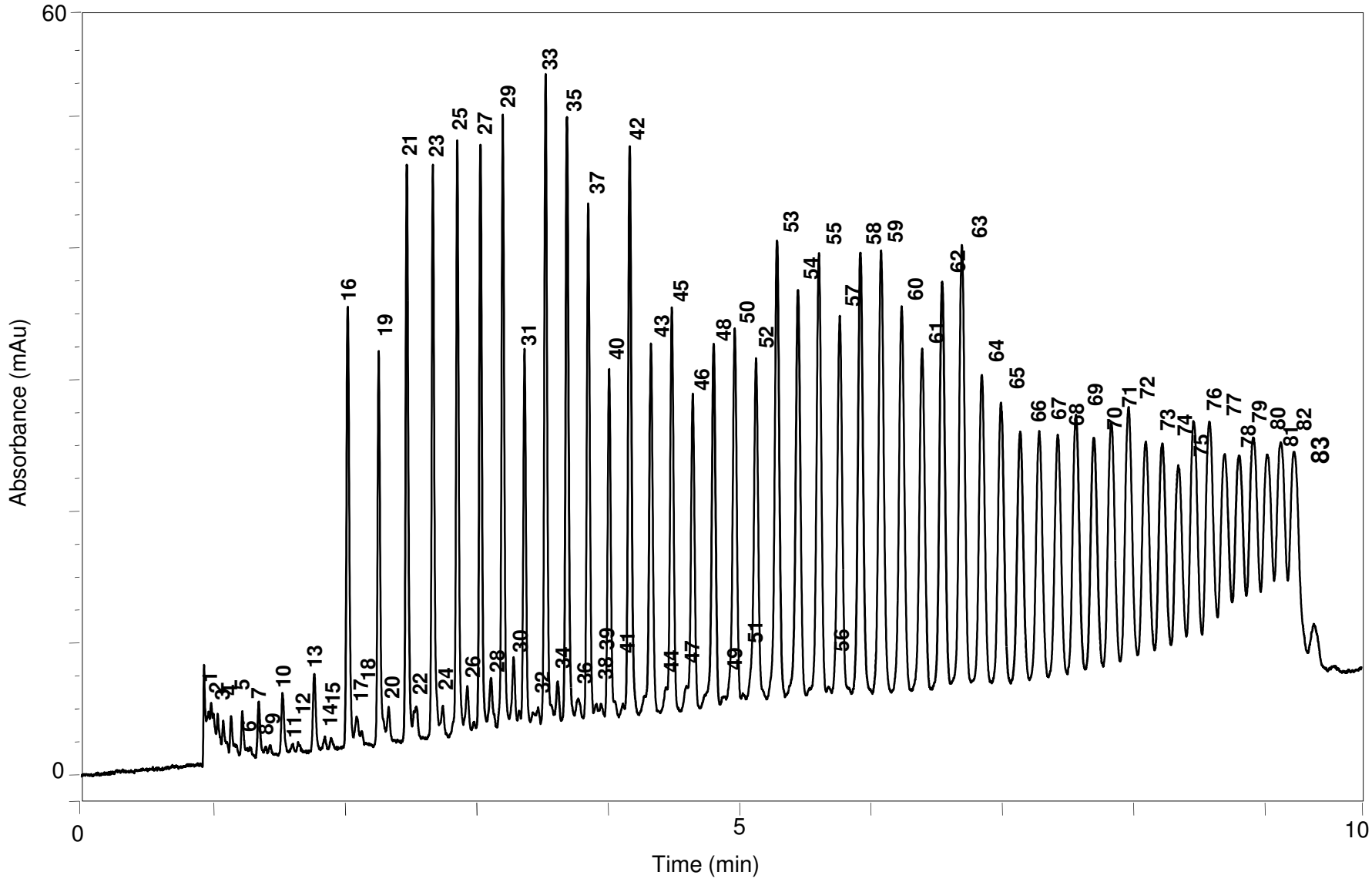
DNAPac PA200 **RS**: Resolution of dT12-60



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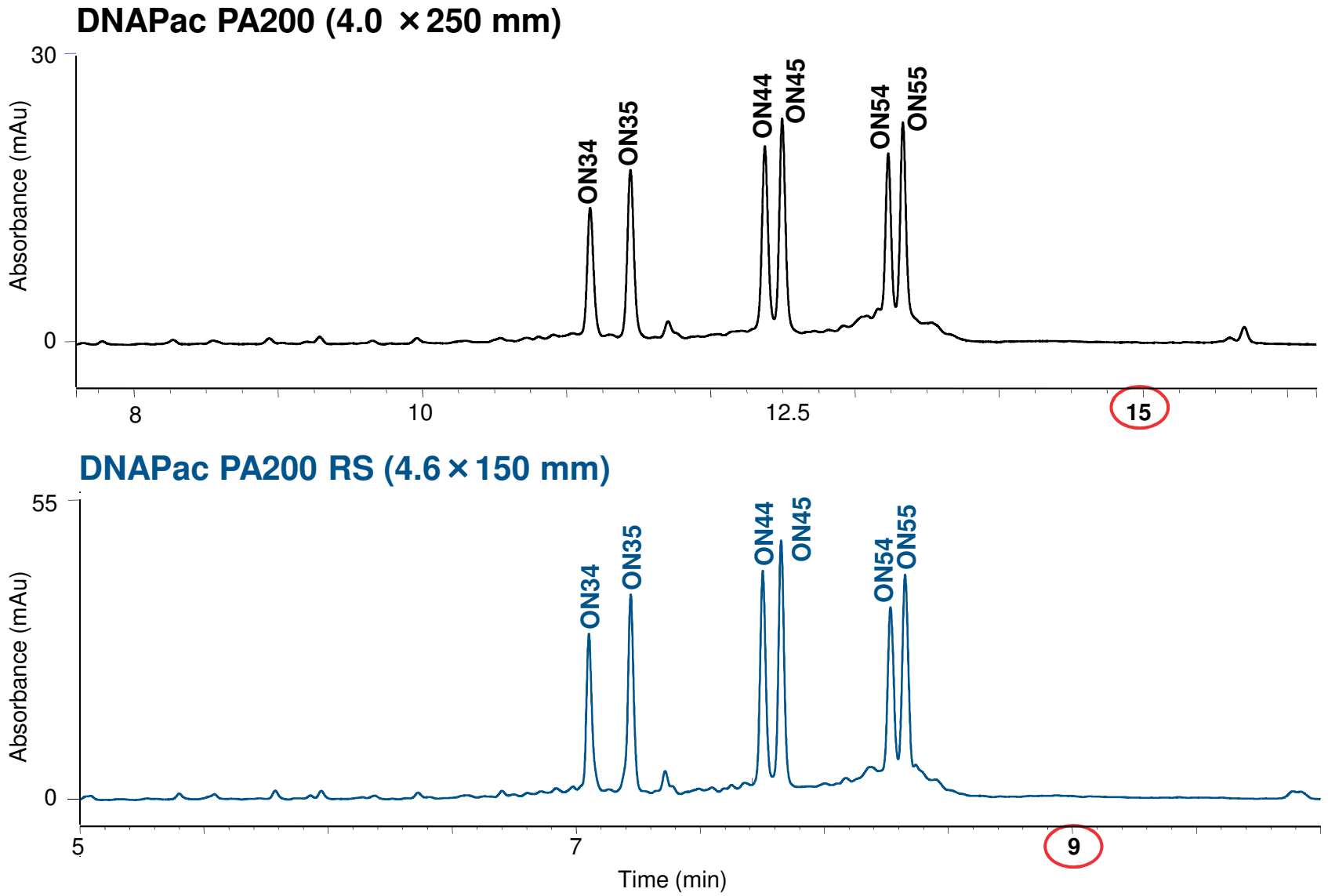
Resolution by Length: Mixed-Base ONs



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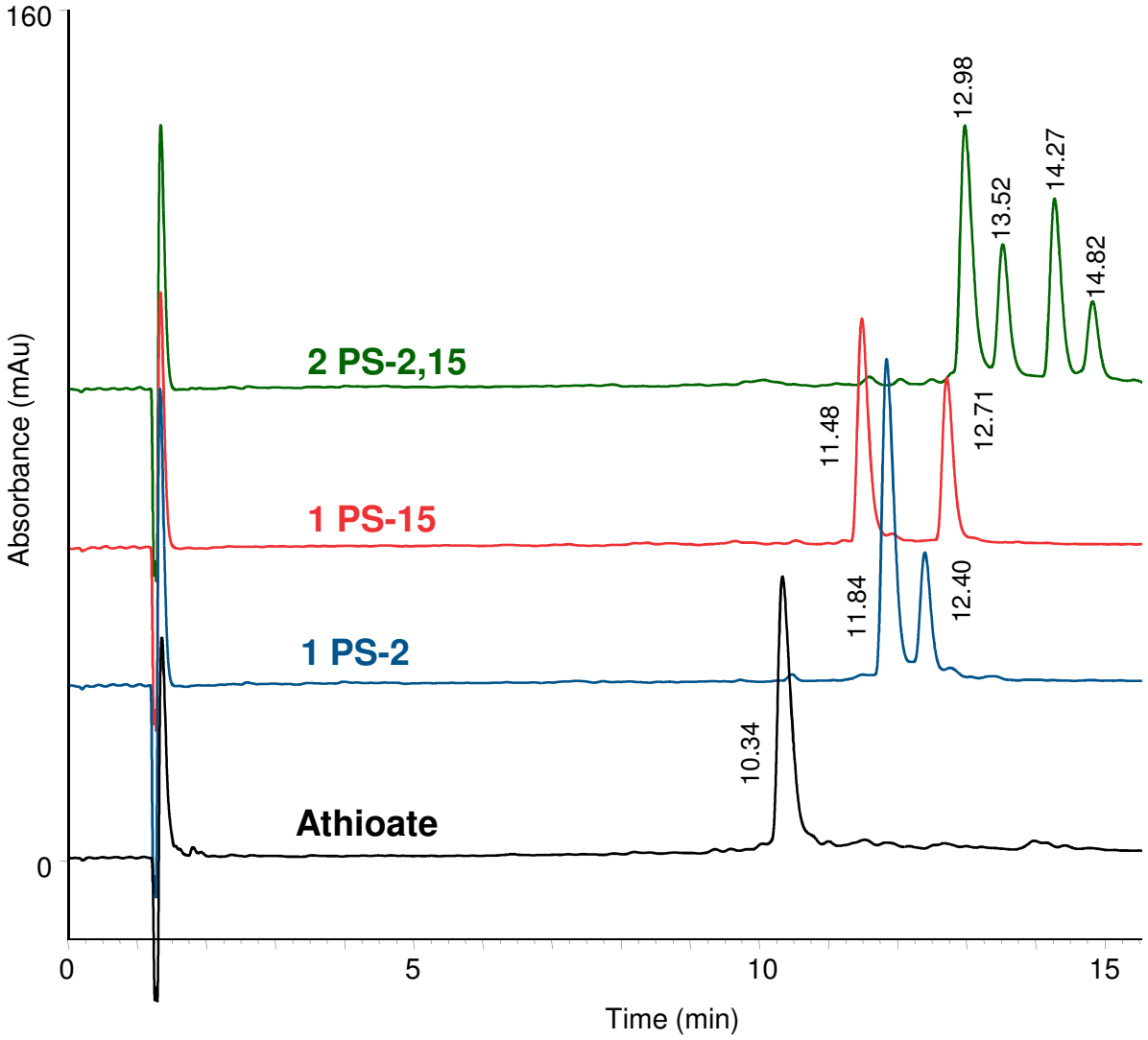
Differential Elution of PS RNAs



Column:	DNAPac PA200, 8 µm
Format:	4.6 × 250 mm
Mobile phase A:	40 mM Tris, pH 7.0
Mobile phase B:	40 mM Tris, 1.0 M NaCl
Gradient:	

Time (min)	%A	%B
-10.0	60.0	40.0
0.0	60.0	40.0
14.6	48.7	51.3
22.0	20.0	80.0

Flow rate:	1.0 mL/min
Temperature:	41 ºC
Detection:	UV (260 nm)
Sample:	37mer aptamer RNA (phosphorothioate at 2, 15)
Peak label:	Ret. Time

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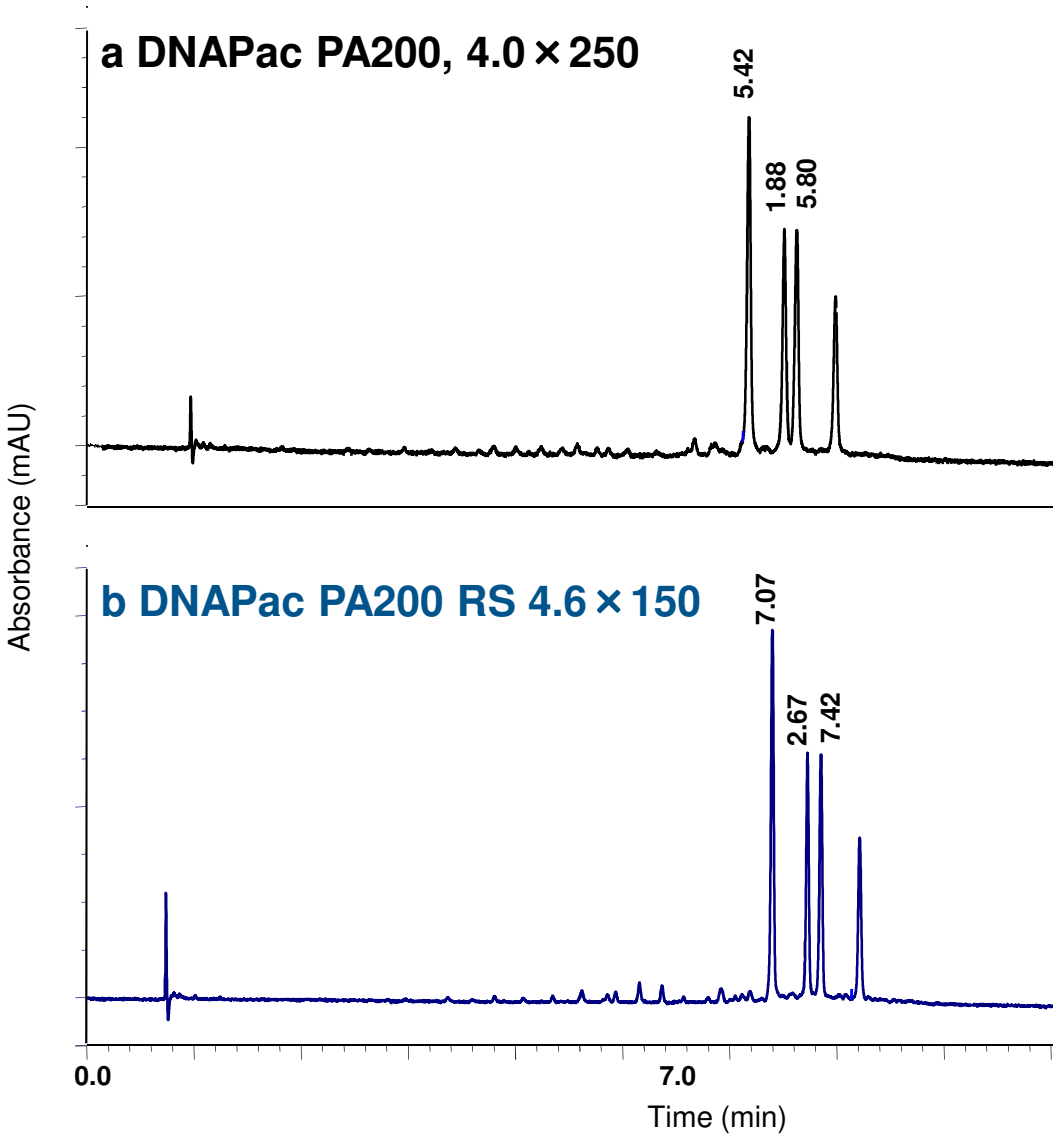
Comparison of RNA Diastereoisomer Resolution



Column:	a) DNAPac PA200, 8 µm b) DNAPac PA200 RS, 4 µm
Format:	a) 4.0 × 250 mm b) 4.6 × 150 mm
Mobile phase A:	40 mM Tris, pH 8.0
Mobile phase B:	40 mM Tris, 1.0 M NaCl

Gradient:

a)

Time (min)	%A	%B
-10.0	70.0	30.0
0.0	70.0	30.0
12.8	37.5	62.5
13.3	20.0	80.0

b)

Time (min)	%A	%B
-10.0	65.0	35.0
0.0	65.0	35.0
10.0	32.5	67.5
10.4	20.0	80.0

Flow rate:	1.0 mL/min
Inj. volume:	12 µL (~1 µg)
Temperature:	30 ºC
Detection:	UV (260 nm)
Sample:	eGFP sense strand with two phosphorothioates (6,14)
Sequence:	AGC UGA$_s$ CCC UGA AG$_s$U UCA UdCdT
Peak label:	Resolution (ep)

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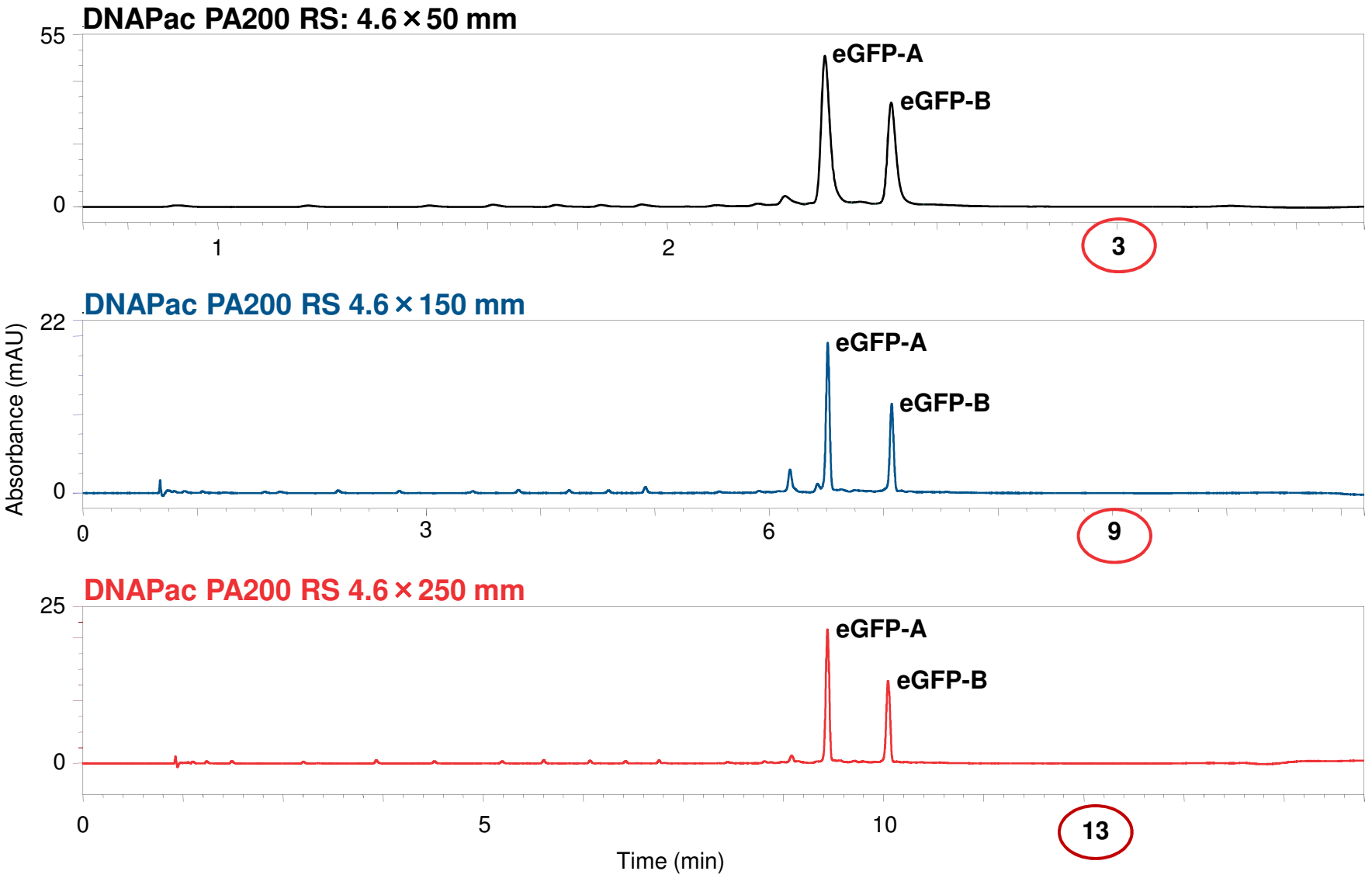
Separation of Phosphorothioate Diastereoisomers



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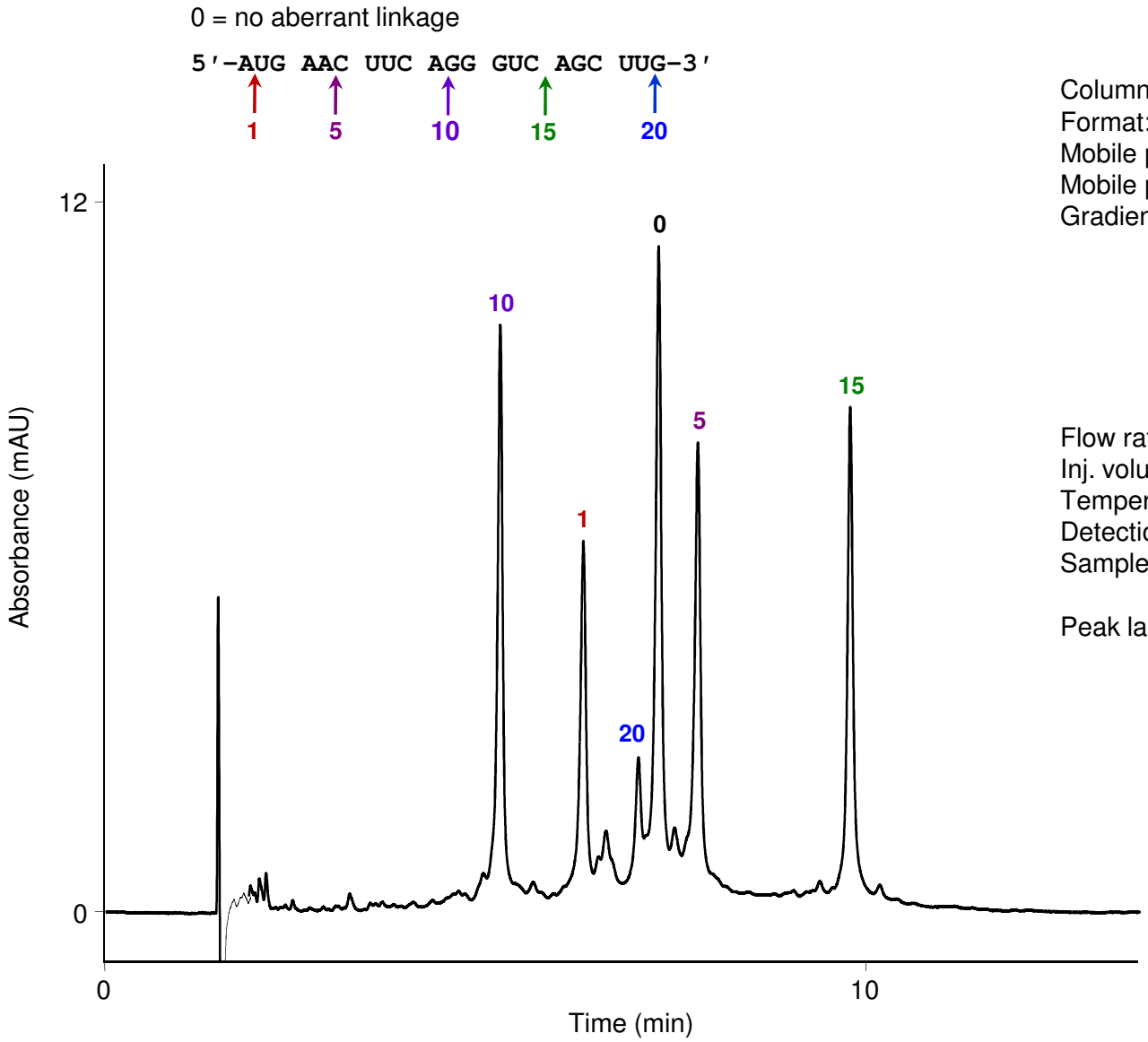
Separation of 2’,5’-Linkages at Different Positions



Column:	DNAPac PA200 RS, 4 µm
Format:	4.6 × 250 mm
Mobile phase A:	40 mM AMP, pH 9.5
Mobile phase B:	40 mM AMP, 1.25 M NaCl
Gradient:	

Time (min)	%A	%B
-10.0	51	49
0.0	51	49
19.5	30	70
20.5	20	80

Flow rate:	1.0 mL/min
Inj. volume:	8 µL
Temperature:	30 ºC
Detection:	UV (260 nm)
Sample:	21-nt RNA with 2’,5’-linkages at sites (50 µM each)
Peak label:	Location of 2’,5’-linkages

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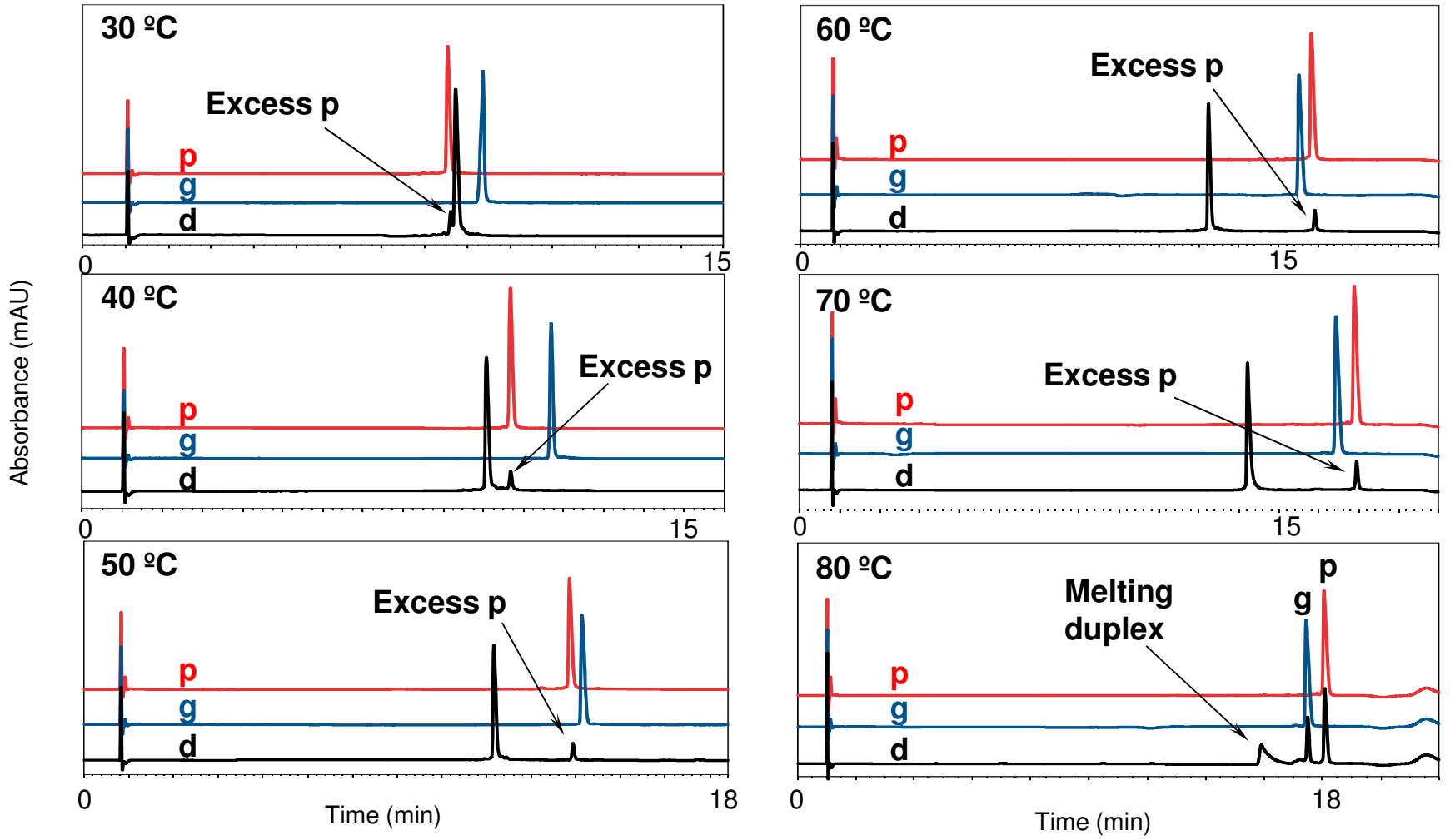
Analysis of Duplex, Guide and Passenger Strands of siRNA

DNAPac PA200, NaCl gradient, pH 7

eGFP passenger RNA (p) eGFP guide RNA (g) eGFP duplex RNA (d)



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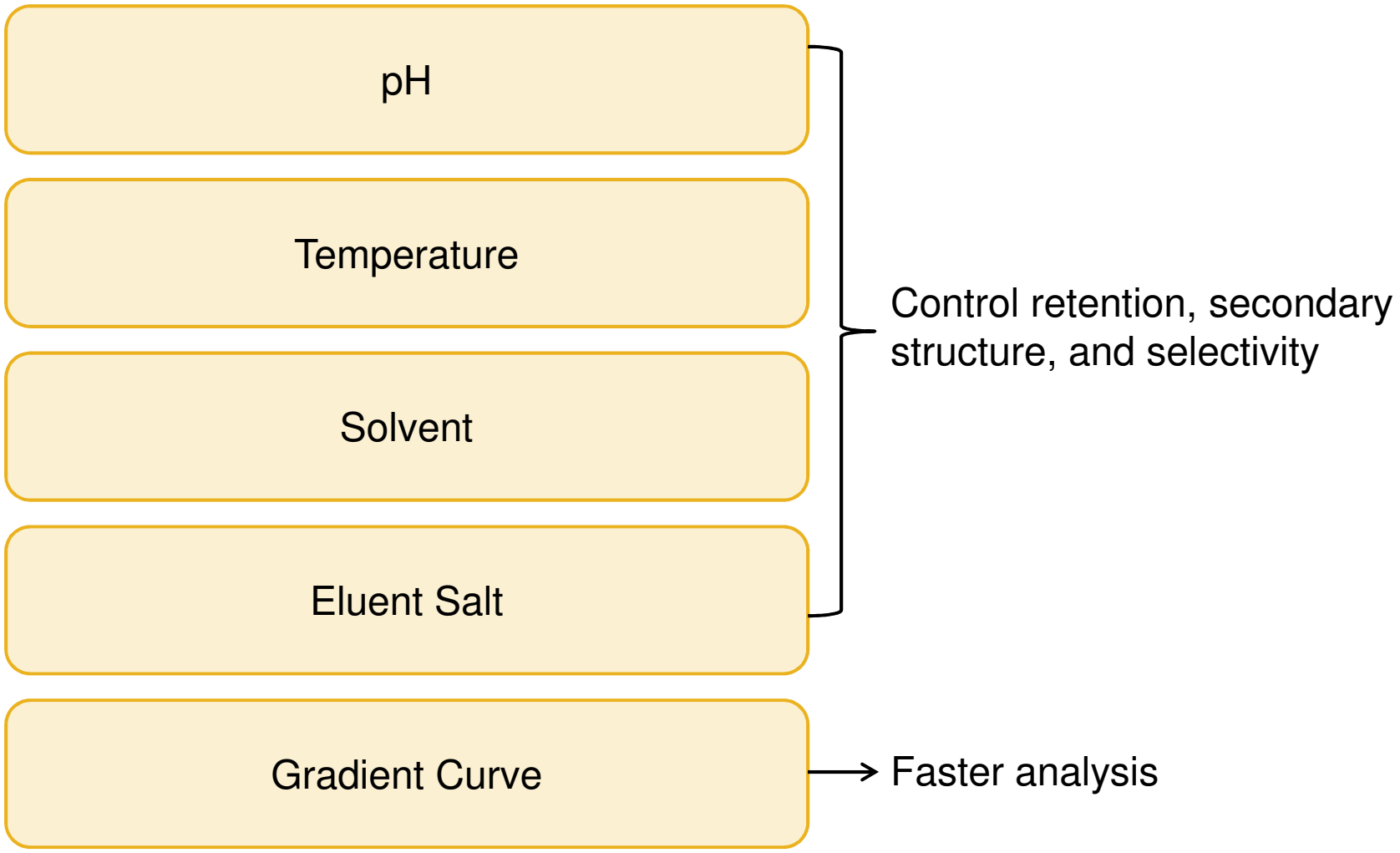
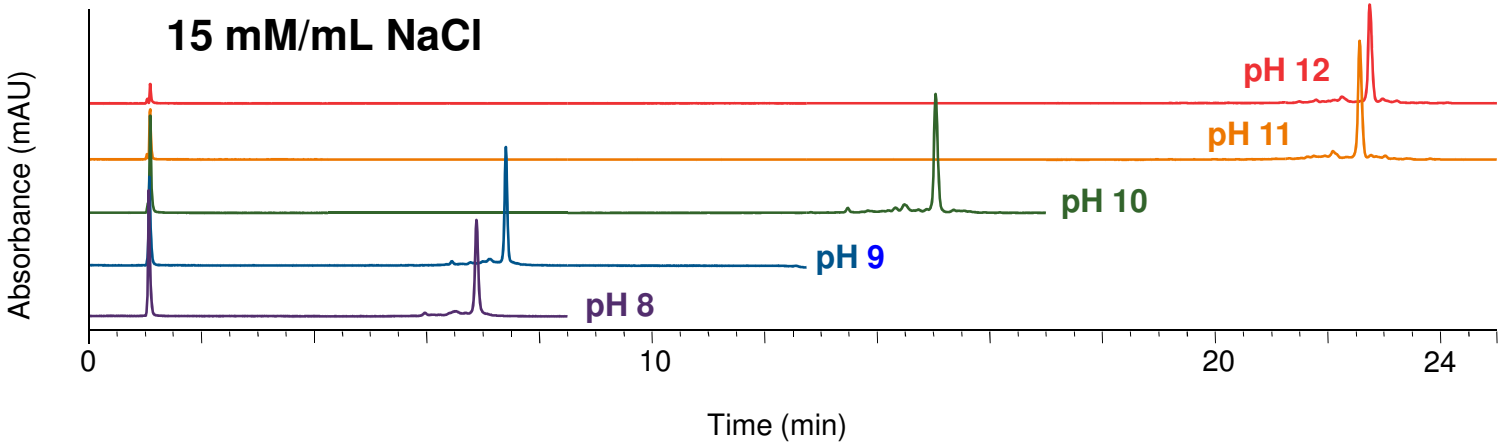
Levers for Control of Retention, Resolution, and Selectivity

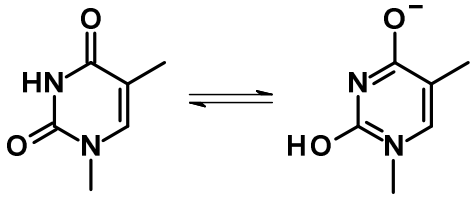


Effect of Eluent Salts on pH-Induced Retention



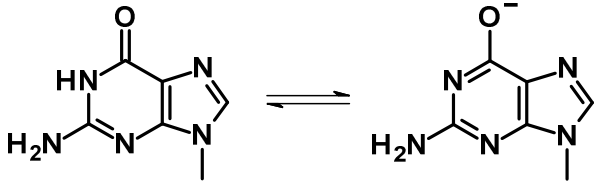
Sample: CTG ATT GTA GGT TCT CTA ACG CTG A (25mer)

Thymine



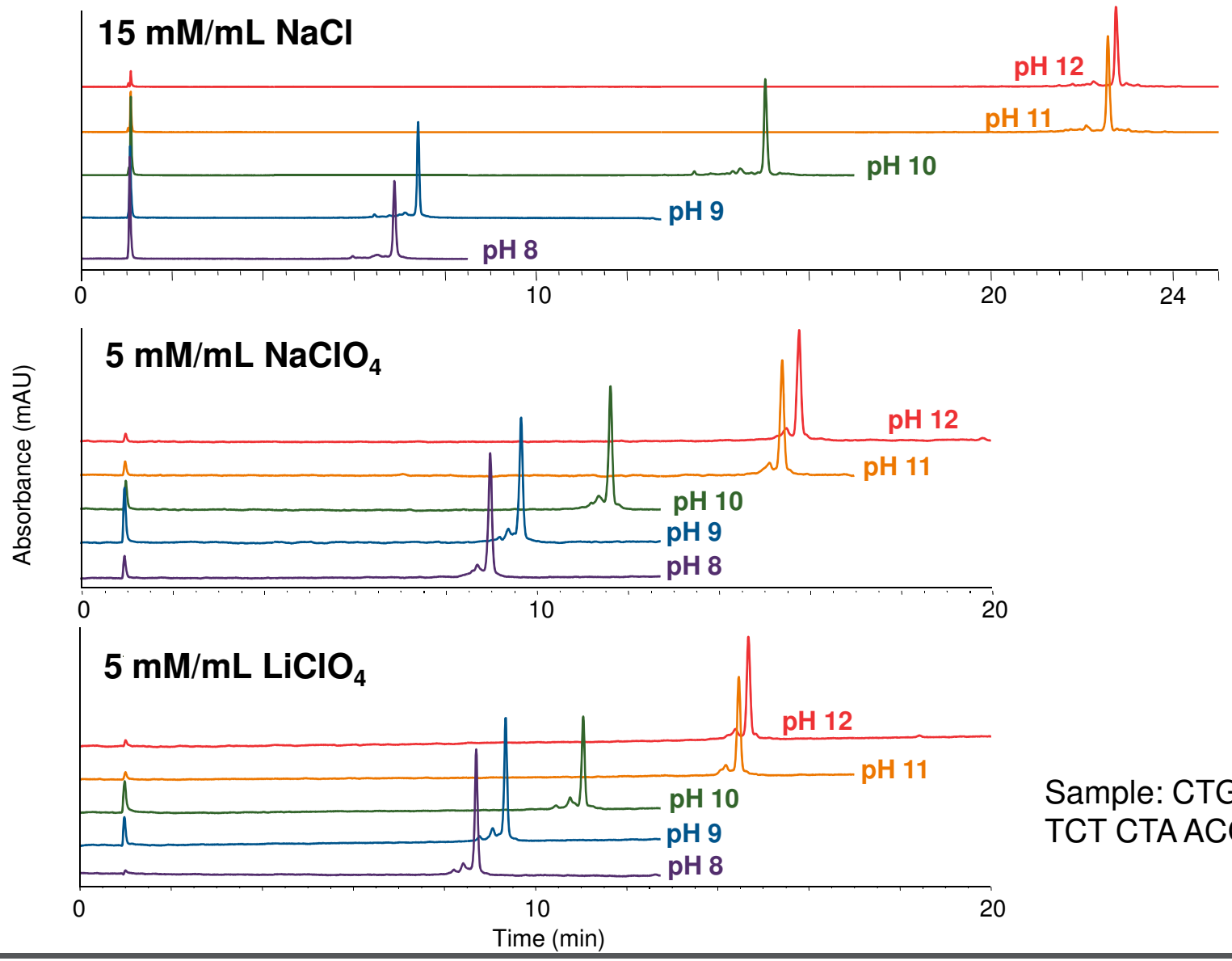
pH 7 pH 11

Guanine



pH 7 pH 11

Effect of Eluent Salts on pH-Induced Retention



Sample: CTG ATT GTA GGT TCT CTA ACG CTG A (25mer)

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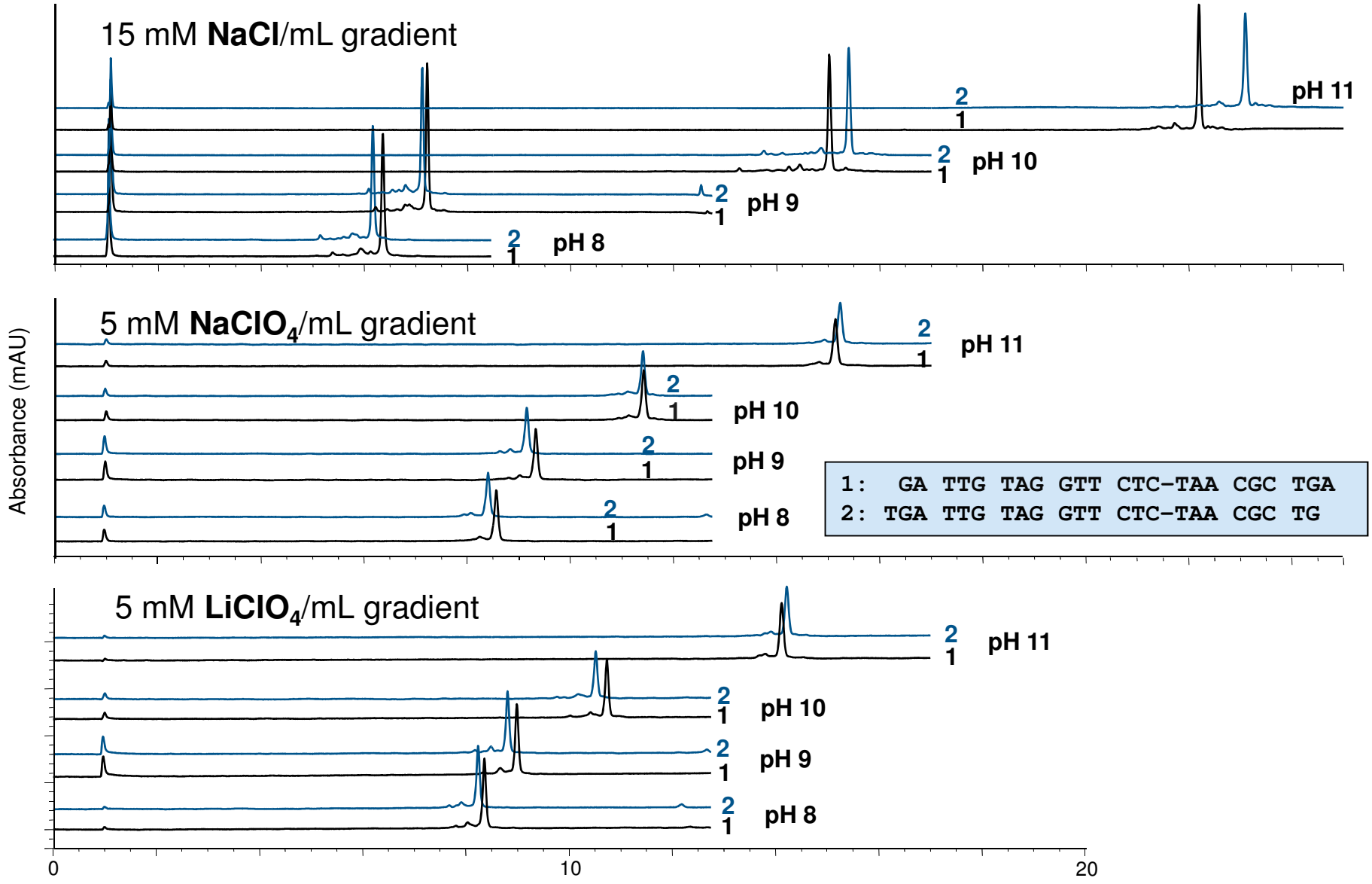
Effect of Eluent Salt on pH-Dependent Selectivity



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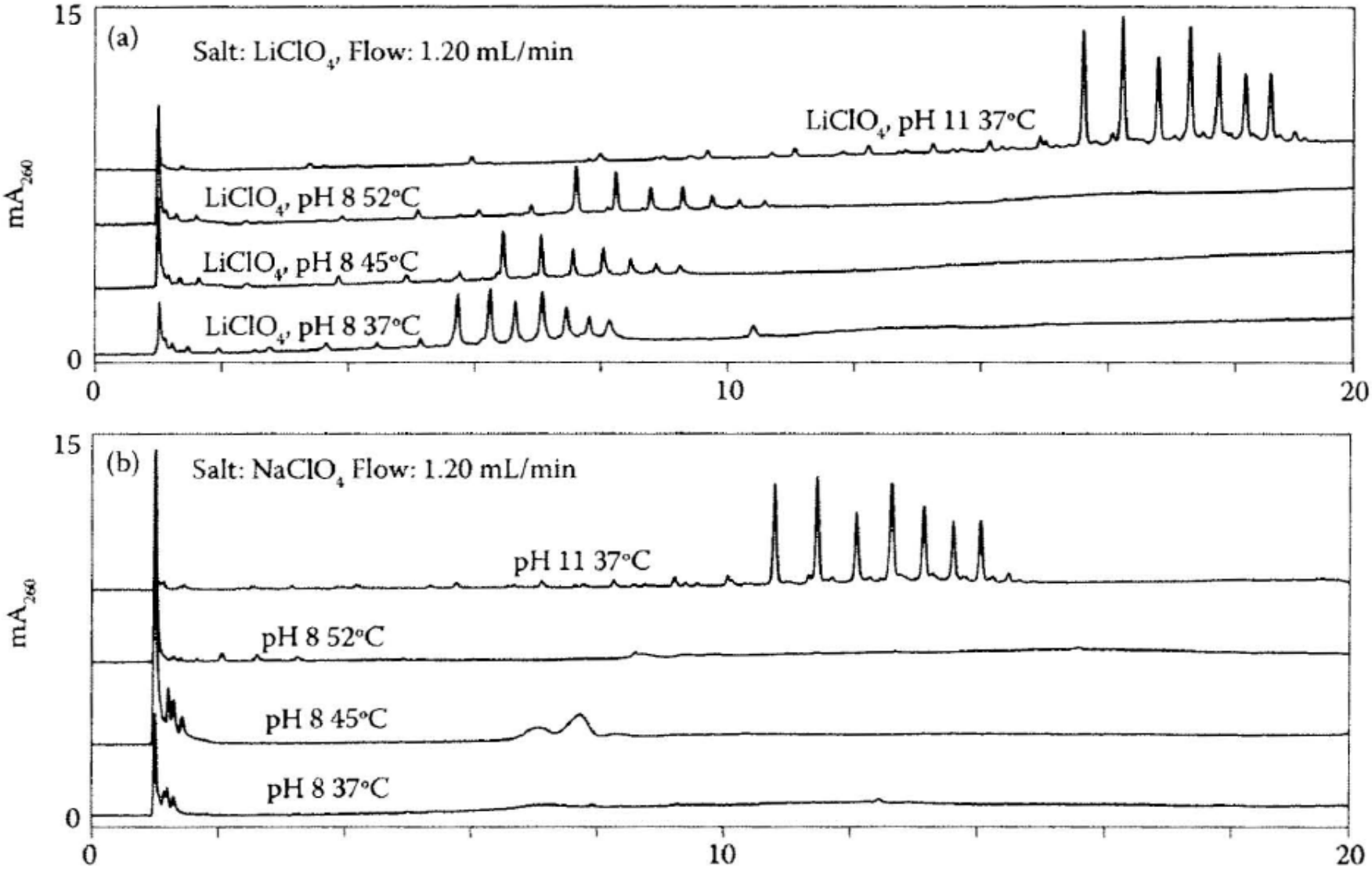
Effect of pH and Eluent Salt on Secondary Structure

Sample: dG12-18



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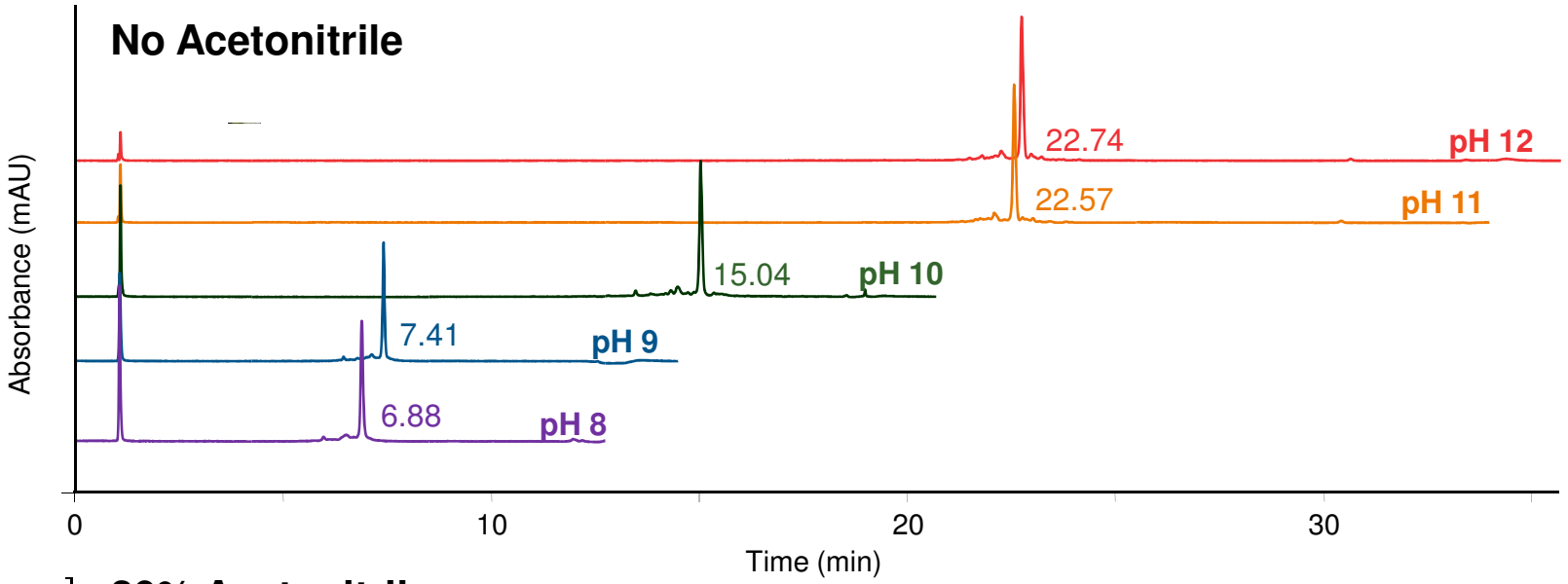
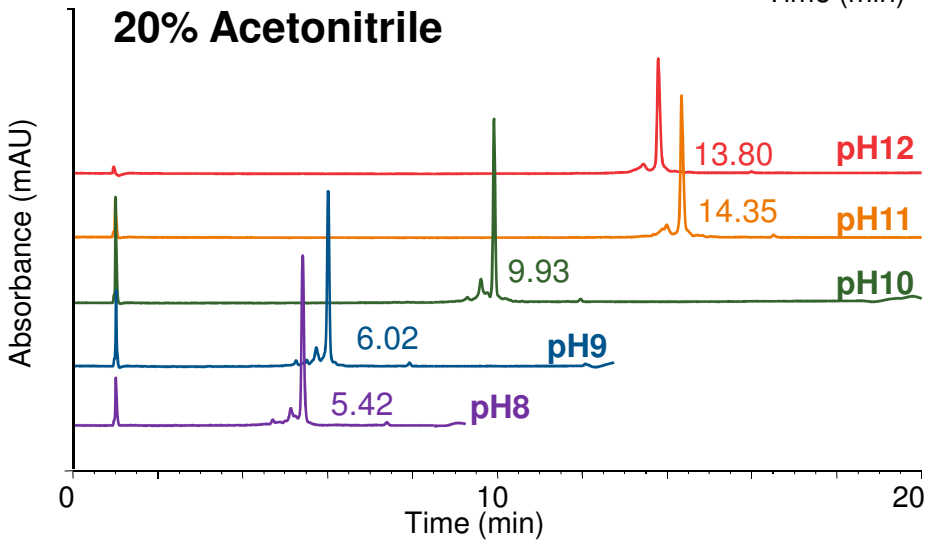
Effect of Solvent on Retention





Eluent Salt: NaCl
Sample: CTG ATT GTA GGT TCT CTA ACG CTG A (25mer)

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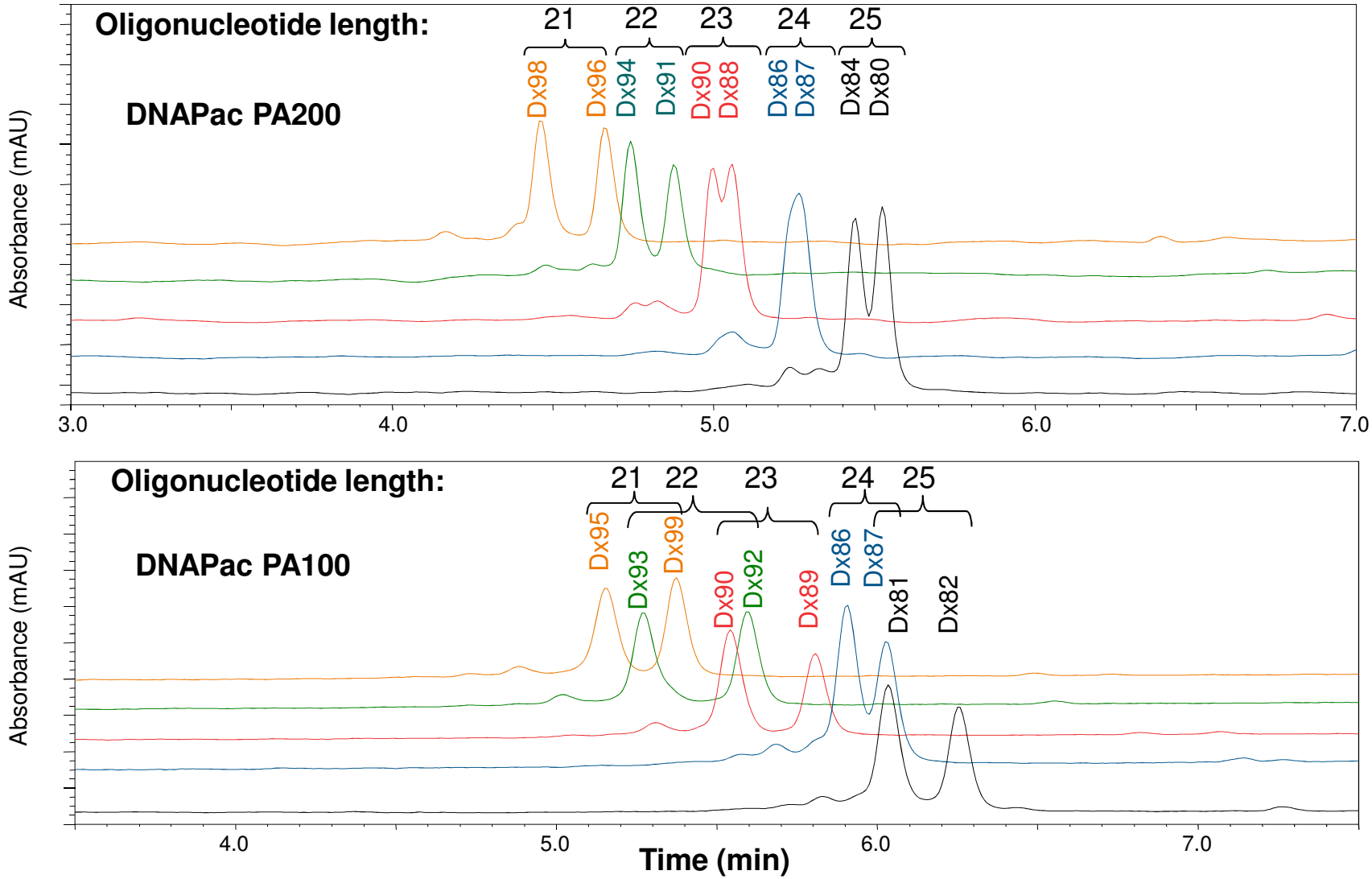
Oligonucleotide Elution Based Primarily on Length

Elution in **$NaClO_4$**, pH 6.5, 20% **Acetonitrile**, 30 ºC



J.Thayer et al. *Analytical Biochemistry* 338, 39-47.

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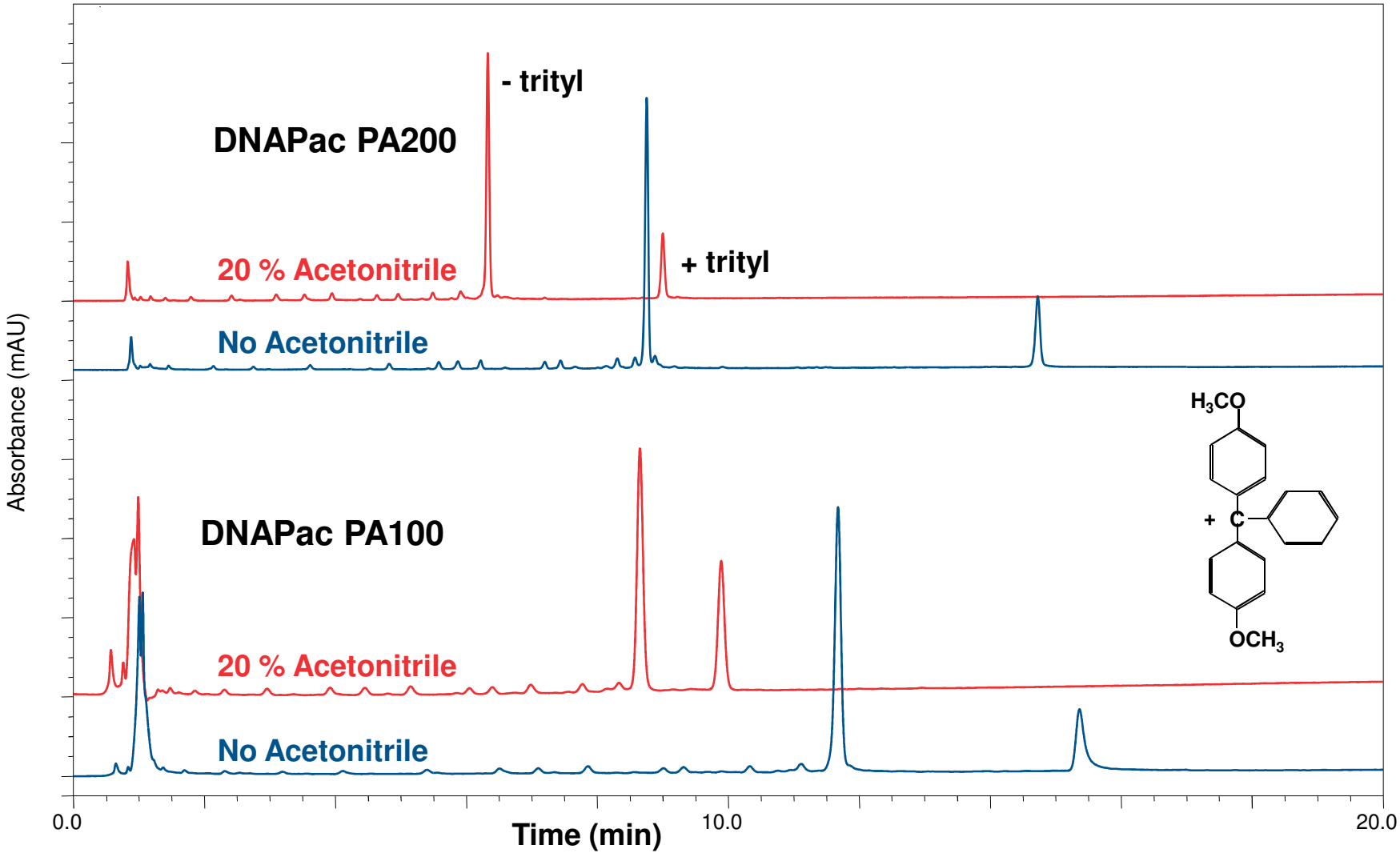
Effect of Solvent on Retention in DNAPac columns

Relative elution of 25mer ± Trityl-group, pH 8, 30 ºC, 15 mM NaCl /mL



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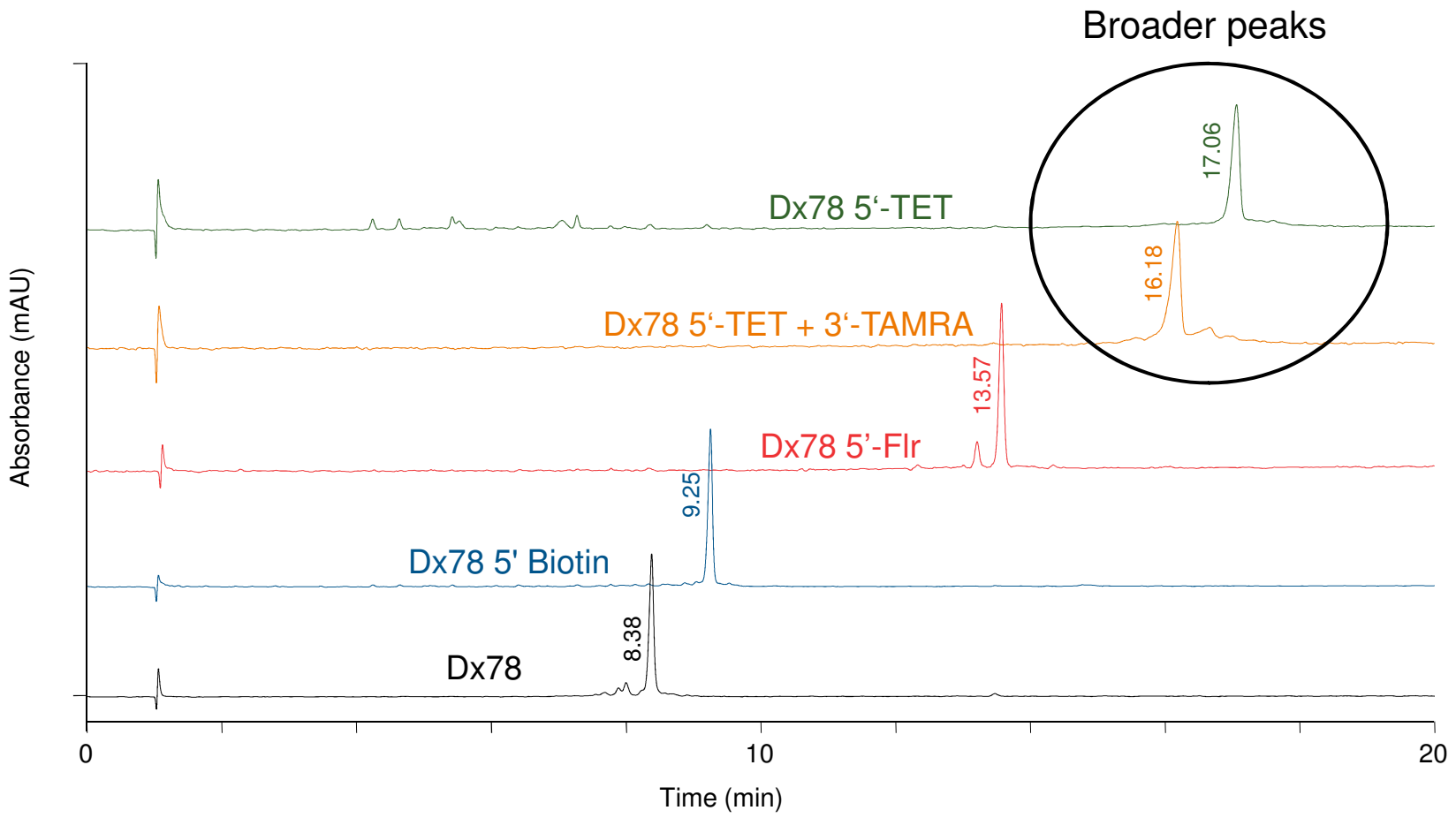
Elution of Derivatized Oligonucleotides

DNAPac PA200, NaCl, mixed-base 25mers, pH 8, 45 ºC



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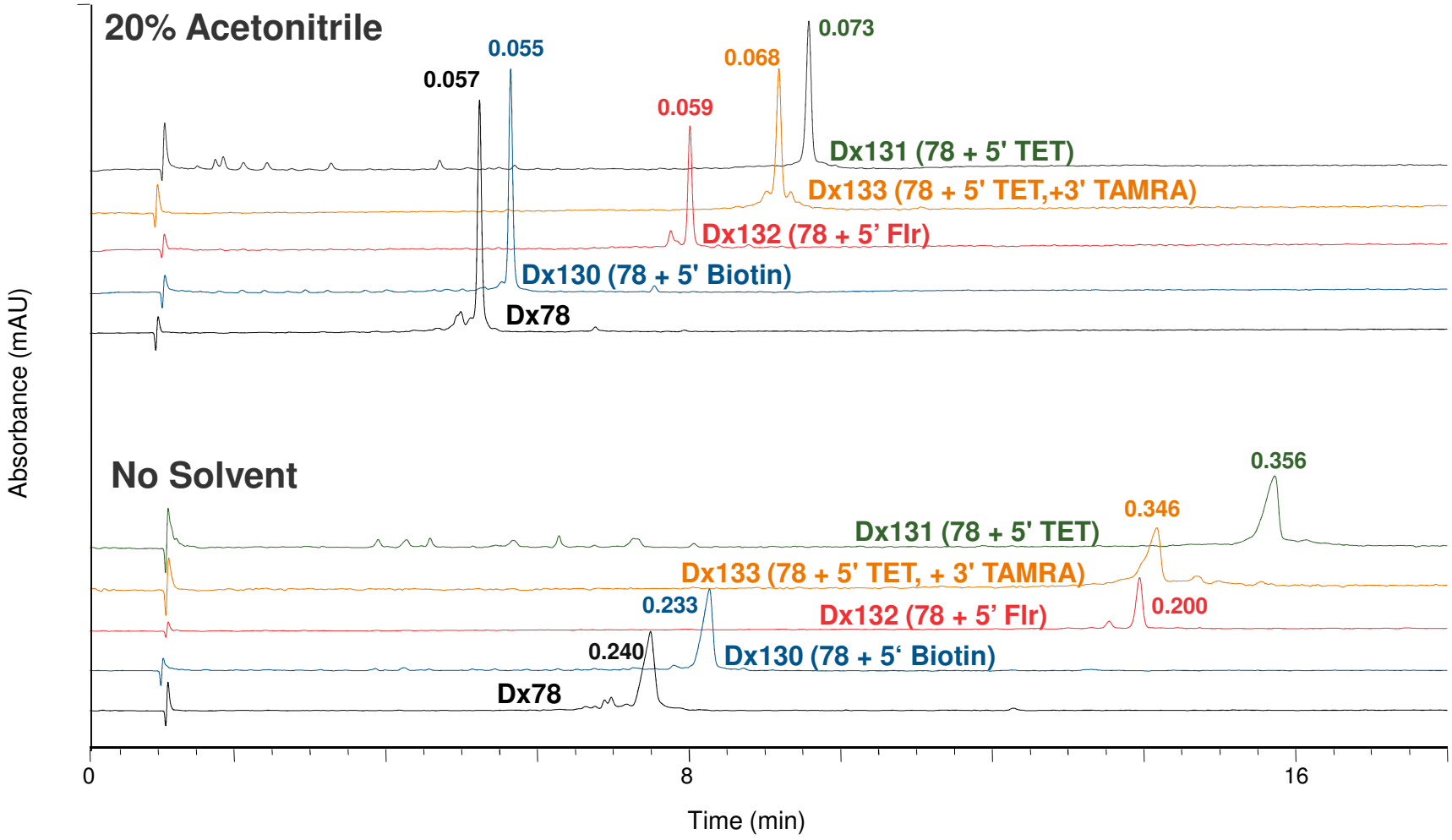
Effect of Solvent on Derivatized ON's Peak Width

DNAPac PA200, pH 8, 35 ºC, **NaCl**



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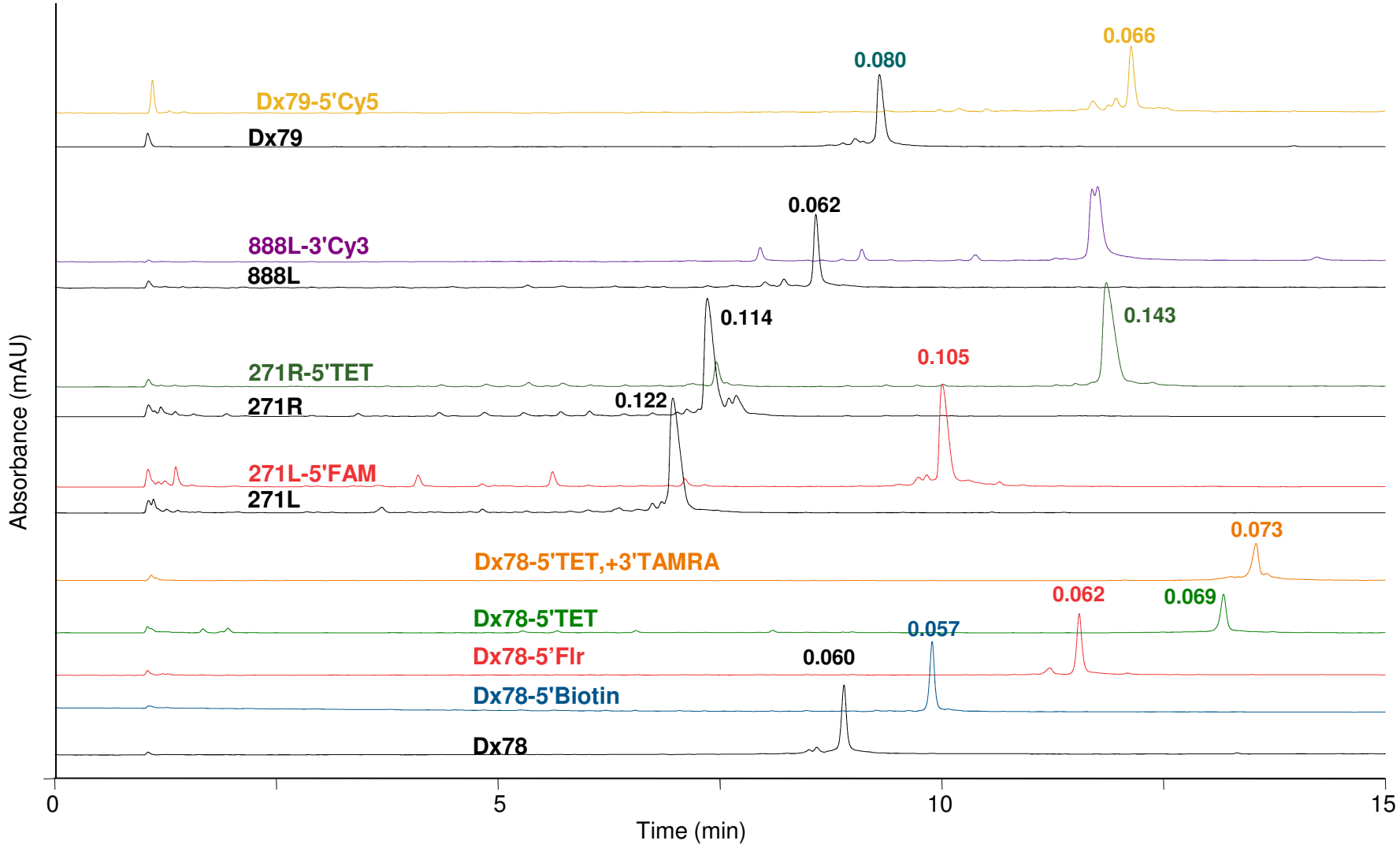
Effect of $NaClO_4$ on Derivatized ON's Peak Width

DNAPac PA200 pH 8, 35 ºC



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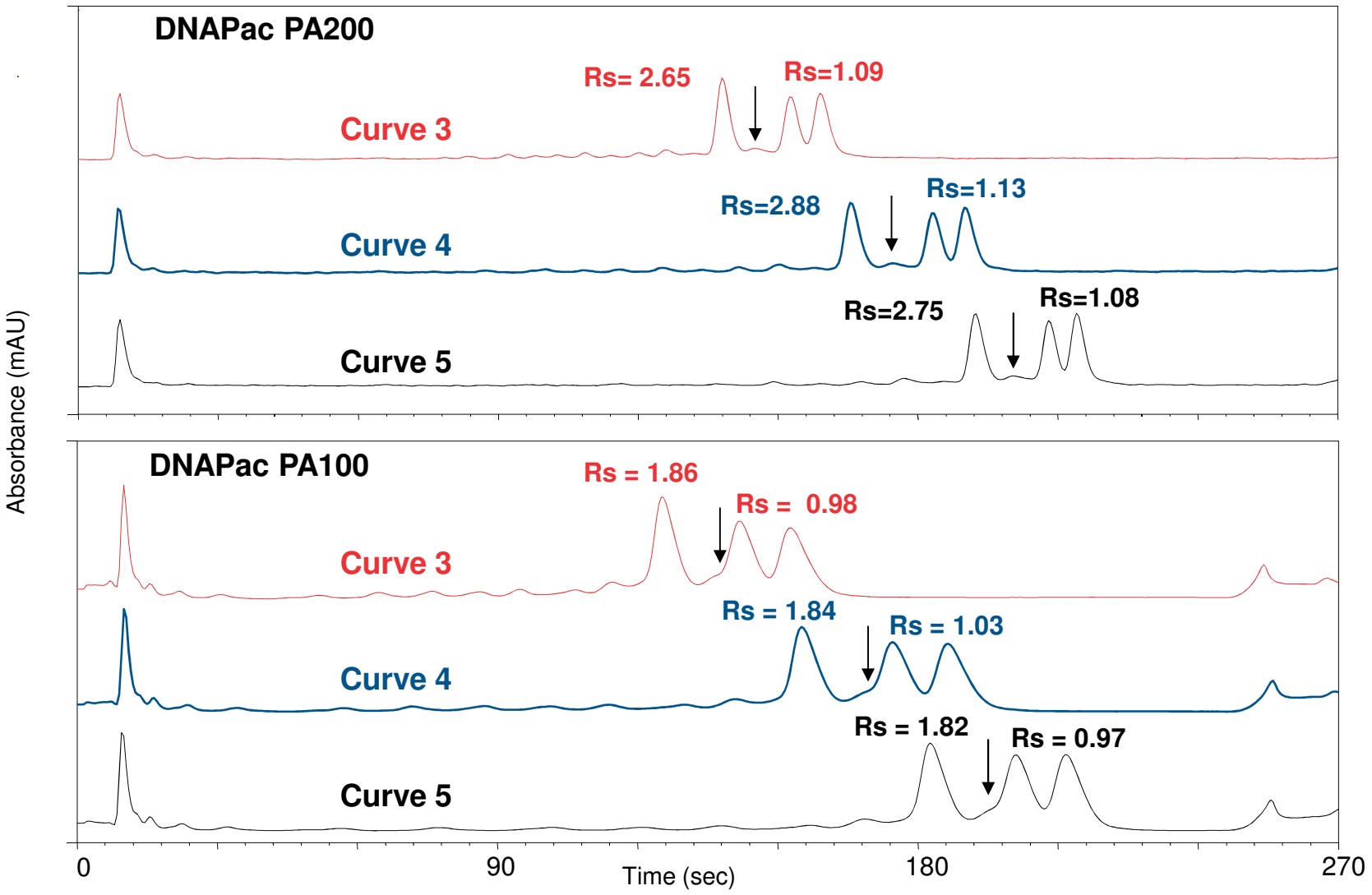
Adjustment of Gradient Curve for Faster Analysis

Separation of 21, 22 and 23mer ONs



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Summary

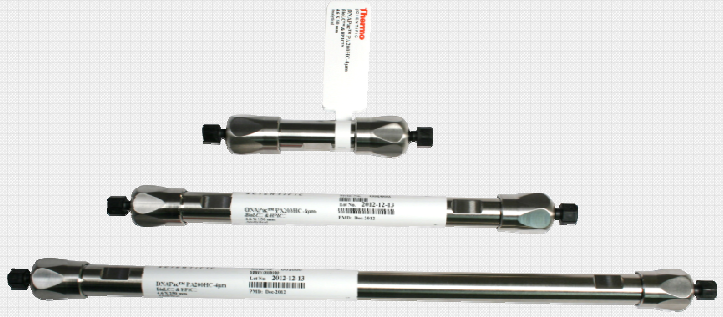
- DNAPac PA200 has higher efficiency and better phase stability at high pH and elevated temperatures compared to DNAPac PA100.
- High throughput oligonucleotide separation capability can be achieved using DNAPac PA200 RS column.
- DNAPac PA 200 column can separate phosphorothioate diastereoisomers and linkage isomers not resolved by MS.

- Oligonucleotide retention and secondary structure can be controlled by pH, eluent salt, temperature and solvent.
- Resolution of identical length oligonucleotides with different, but related base sequences can be achieved by tuning selectivity.
- $NaClO_4$ allows elution of oligonucleotides primarily in order of oligonucleotide length.
- Labeled oligonucleotides requires solvent or $NaClO_4$ for good peak shape and resolution.

Anion Exchange Chromatography

Analytical Columns

DNAPac PA100
DNAPac PA200
DNAPac PA200 RS



Semiprep Columns

DNAPac PA200
9 × 250 mm
22 × 250 mm

DNASwift SAX-1S
5 × 150 mm

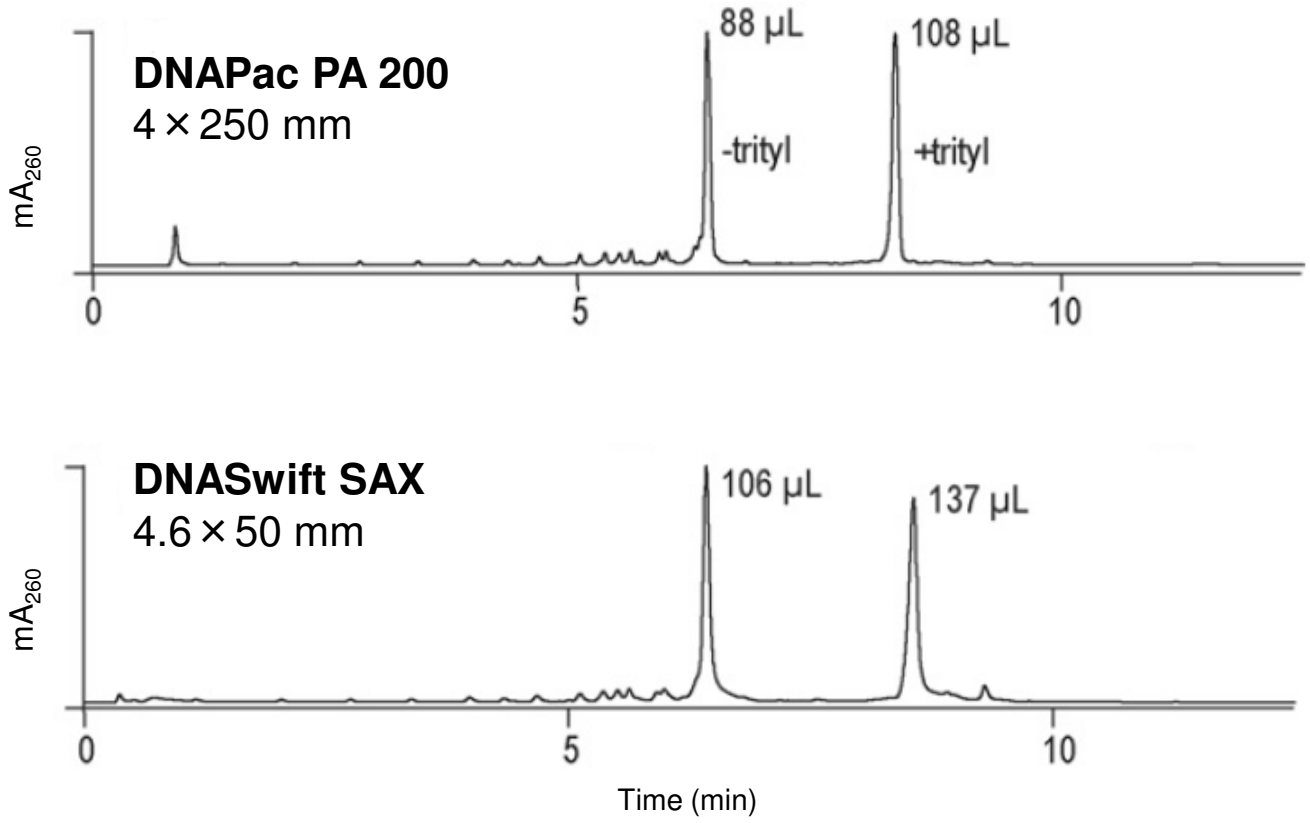
Semiprep Columns

	DNAPac PA200	DNASwift SAX-1S
Format	22×250 mm	5×150 mm
Capacity	~3 mg	~50 mg*
Particle Size	8 µm	Monolithic support
Column Chemistry	Non-porous polymer with quaternary amine functionalized latex beads	Monolithic support with quaternary amine functionalized latex beads
pH Range	4-10 (up to 12.5 if [salt] > 5x [sodium hydroxide])	6–10 (12.5 if [salt] >5× [sodium hydroxide]) 2–14 for cleaning *Note: Keep [salt] >5× [sodium hydroxide]*
Temperature Range	≤35 ºC at pH 8.5-12.5 ≤85 ºC at pH < 8.5	
Pressure Maximum	4,000 psi (276 bar)	1,500 psi (103 bar)

*Breakthrough capacity

Comparison of DNAPac PA200 and DNASwift

Sample: 20mer DNA with/without trityl group



J.R. Thayer et al. *J. Chromatogr. B* 878, 933-941.

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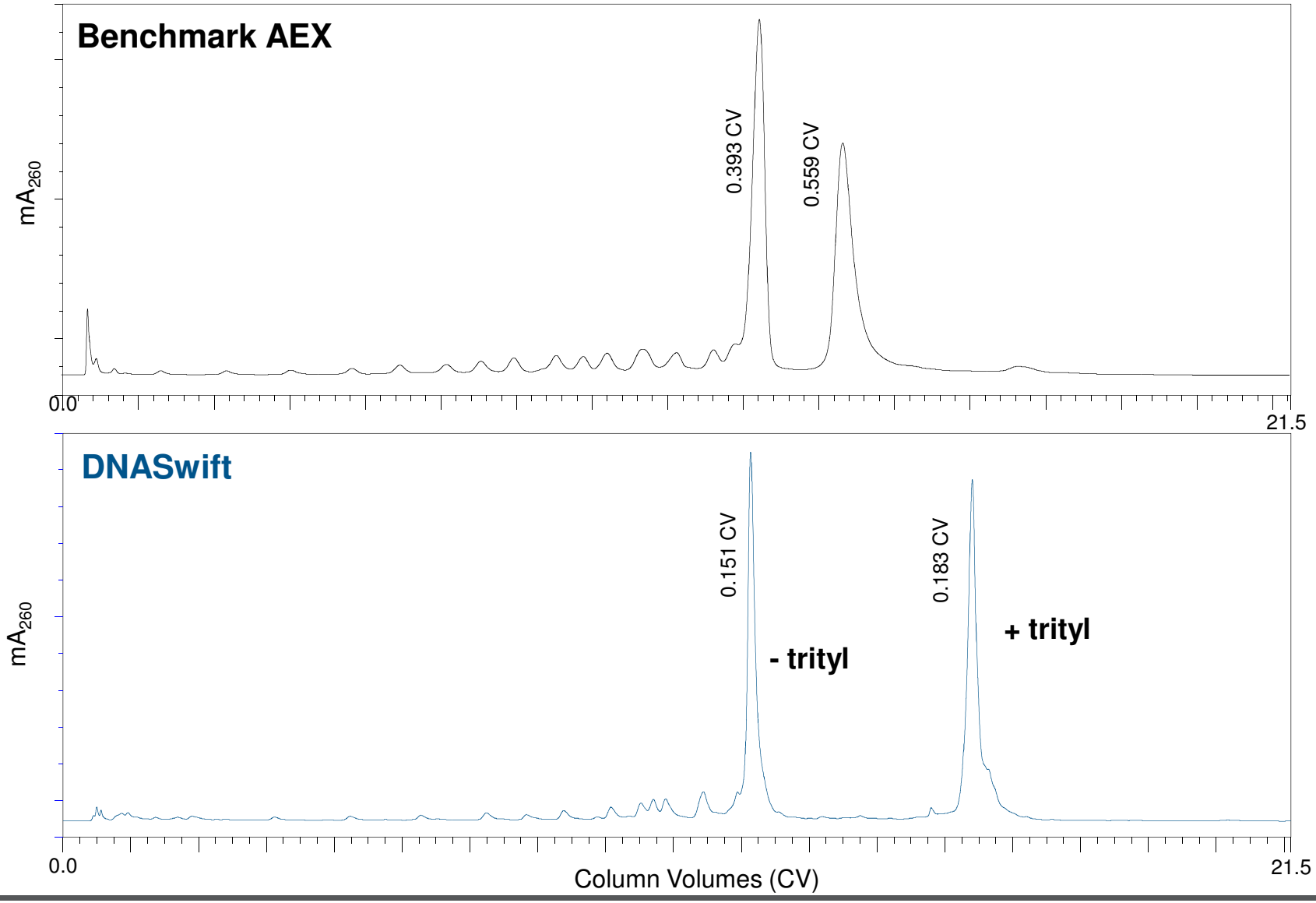
Comparison of Benchmark and DNASwift

20-mer, 100-800 mM in 21.5 Column Volumes (CV), Linear velocity 1.5mm/sec



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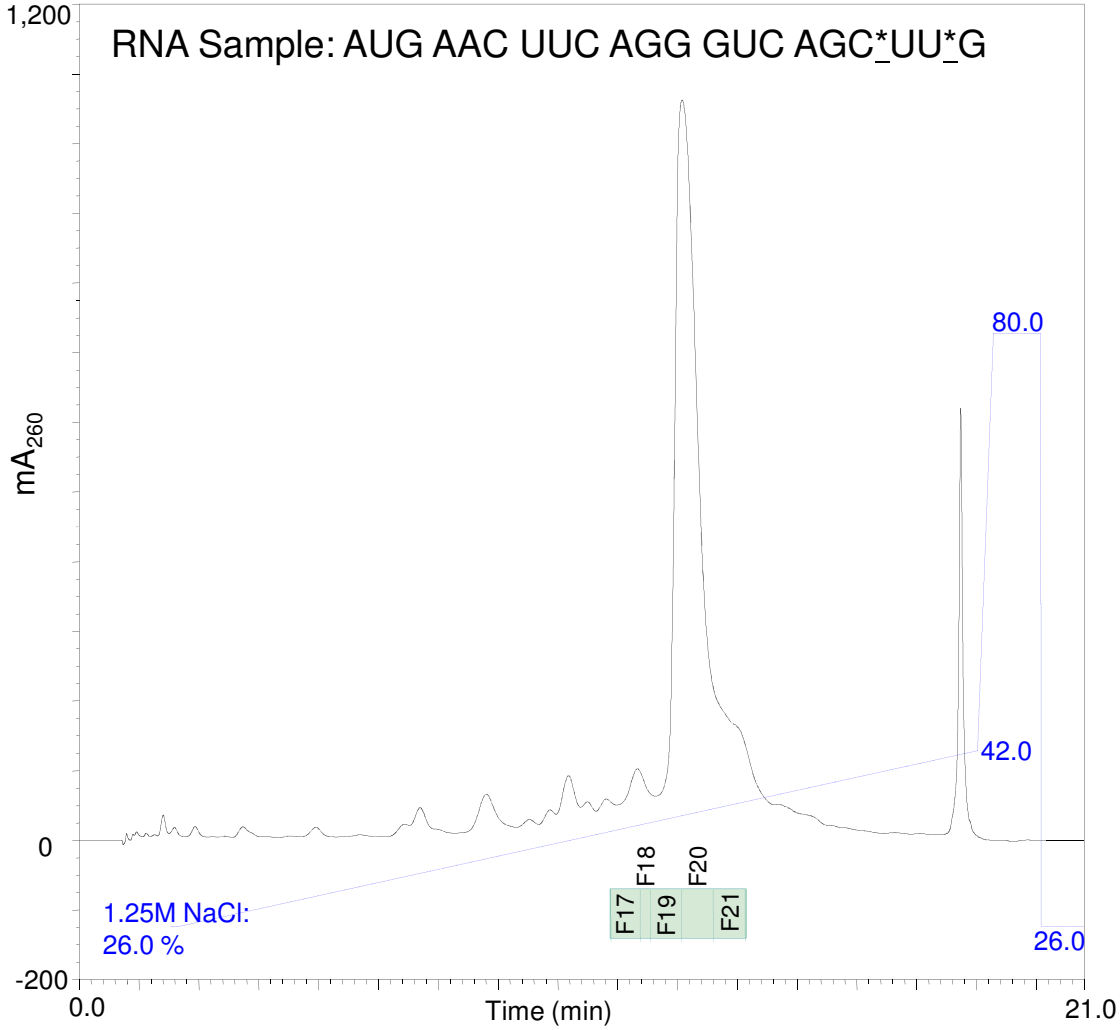
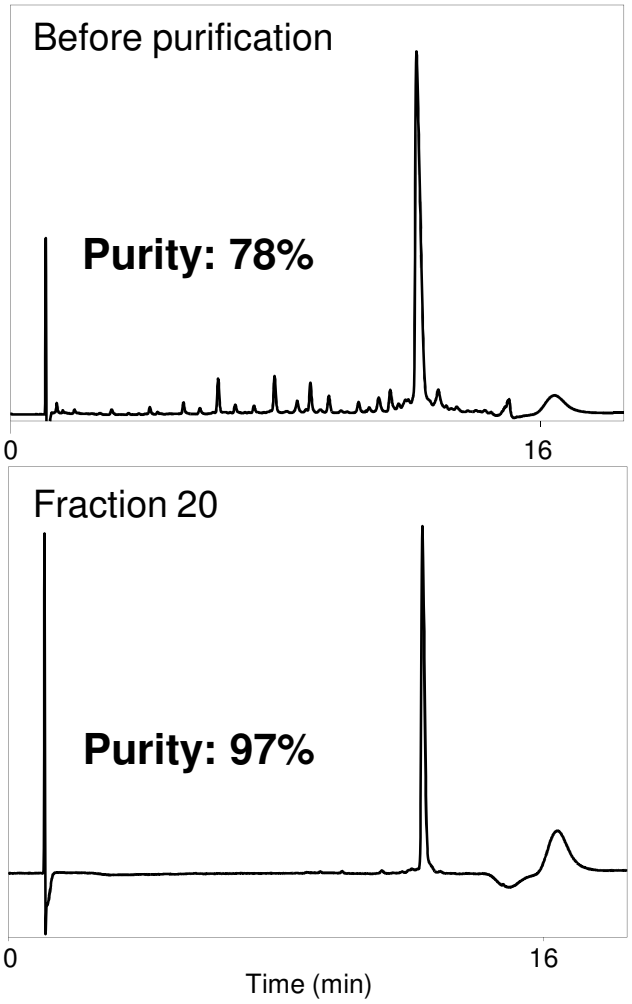
Purification of a 21mer RNA

325 – 525 mM NaCl in 10 CV, pH 7, 30 ºC 1.5 mL/min, 125 µg sample



DNAPac PA200 purity assessments



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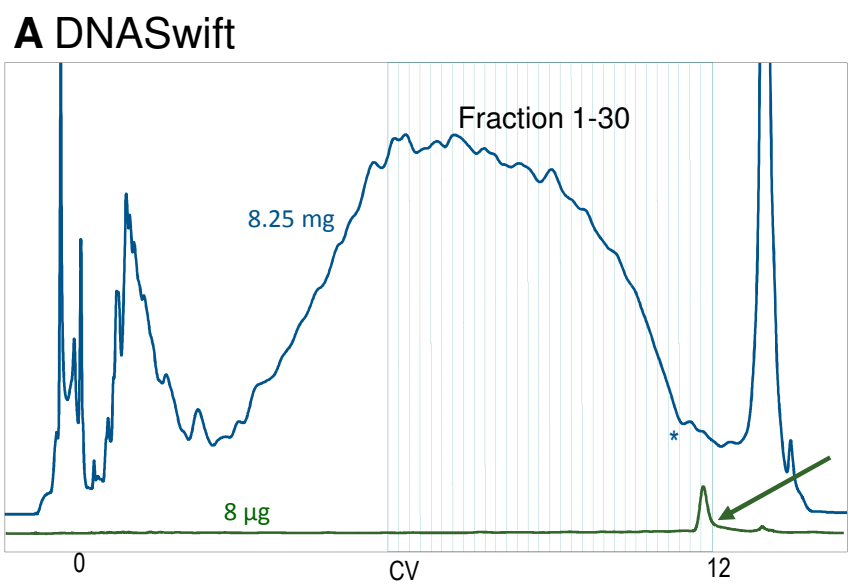
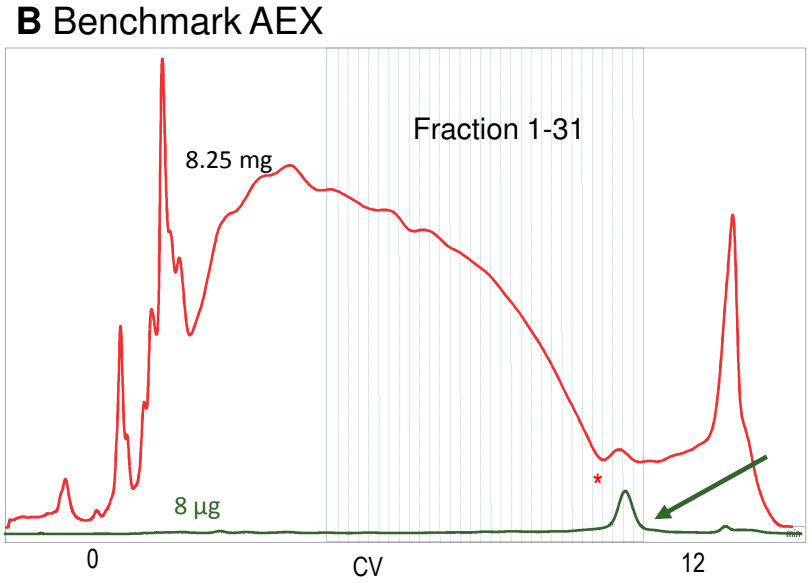
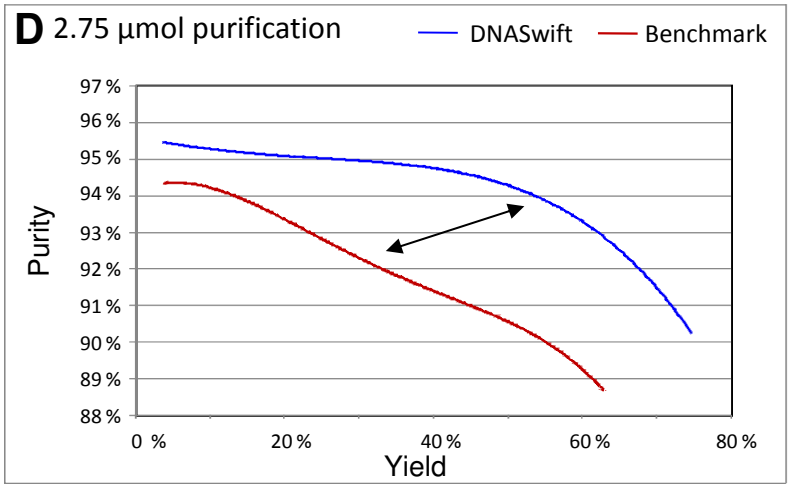
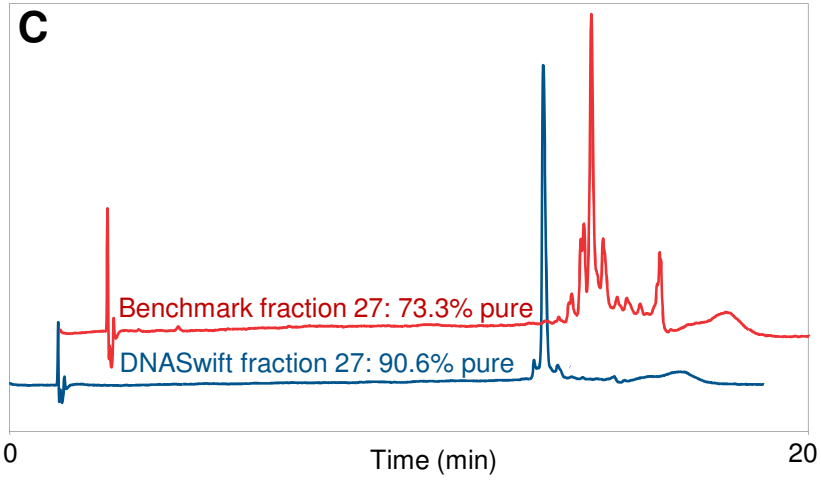
Yield Purity Curves for Sample Displacement at pH 12*: 8.25mg*









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Chromatography Conditions

A

Column:	Benchmark AEX
Format:	5 × 50 mm
Mobile phase A:	Water
Mobile phase B:	0.2 M NaOH
Mobile phase C:	1.25 M NaCl

Gradient:

Time (min)	%A	%B	%C
0.0	83.7	10	6.3
0.39	45.9	10	44.1
7.05	27	10	63.0
7.25	10	10	80.0
7.65	10	10	80.0
7.84	83.7	10	6.3

Flow rate:	1.77 mL/min
Inj. volume:	0.55 µL
Temperature:	30 ºC
Detection:	UV (260 nm)
Sample:	25mer DNA CTGATTGTAGGTTCTCTAACGCTGT

B

Column:	DNASwift
Format:	5 × 150 mm
Mobile phase A:	Water
Mobile phase B:	0.2 M NaOH
Mobile phase C:	1.25 M NaCl

Gradient:

Time (min)	%A	%B	%C
0.0	70	10	6.9
1.0	48	13	48.3
17.74	10	10	69.0
18.25	10	10	80
19.25	70	10	80.0
19.74	10.0	10	6.9

Flow rate:	1.77 mL/min
Inj. volume:	0.55 µL
Temperature:	30 ºC
Detection:	UV (260 nm)
Sample:	25mer DNA CTGATTGTAGGTTCTCTAACGCTGT

Thank You!

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