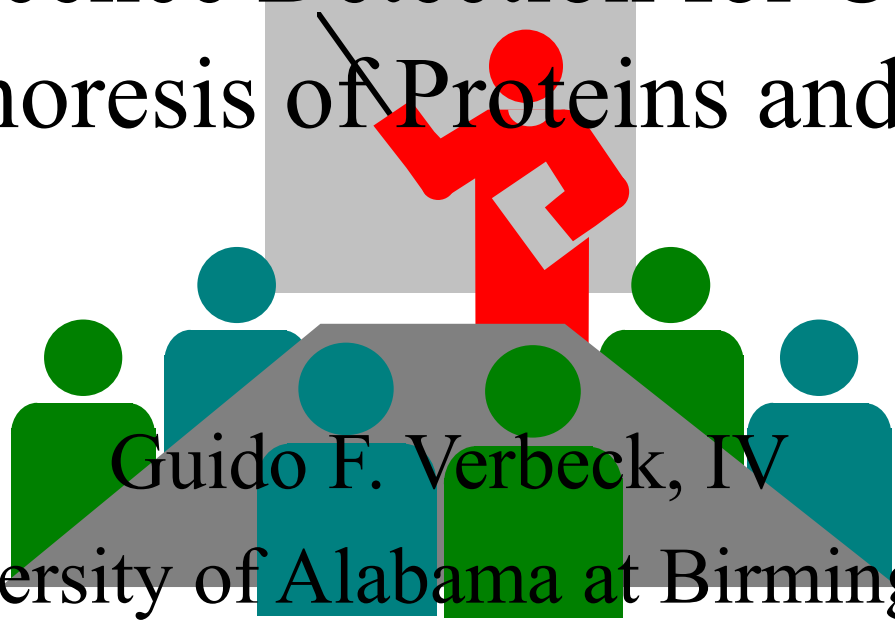


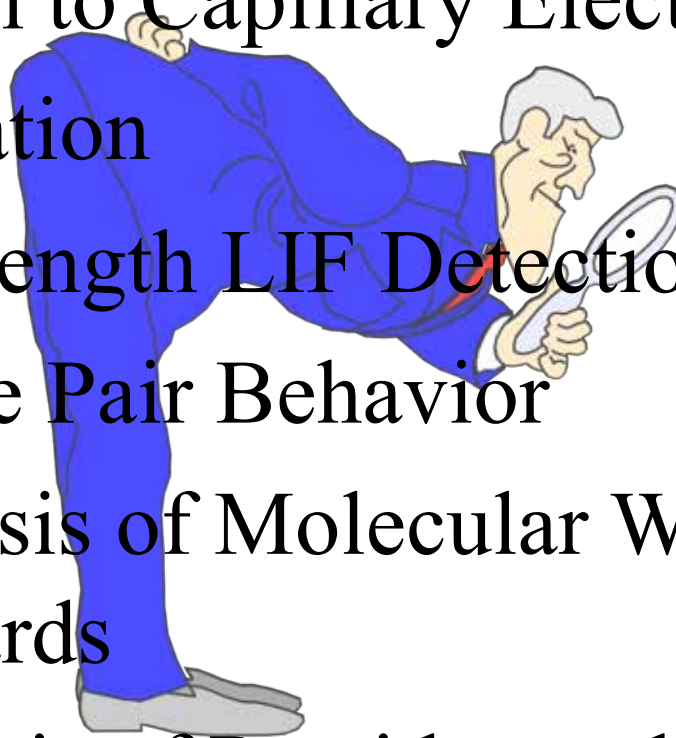
Two-Wavelength Laser-Induced Fluorescence Detection for Capillary Electrophoresis of Proteins and Peptides

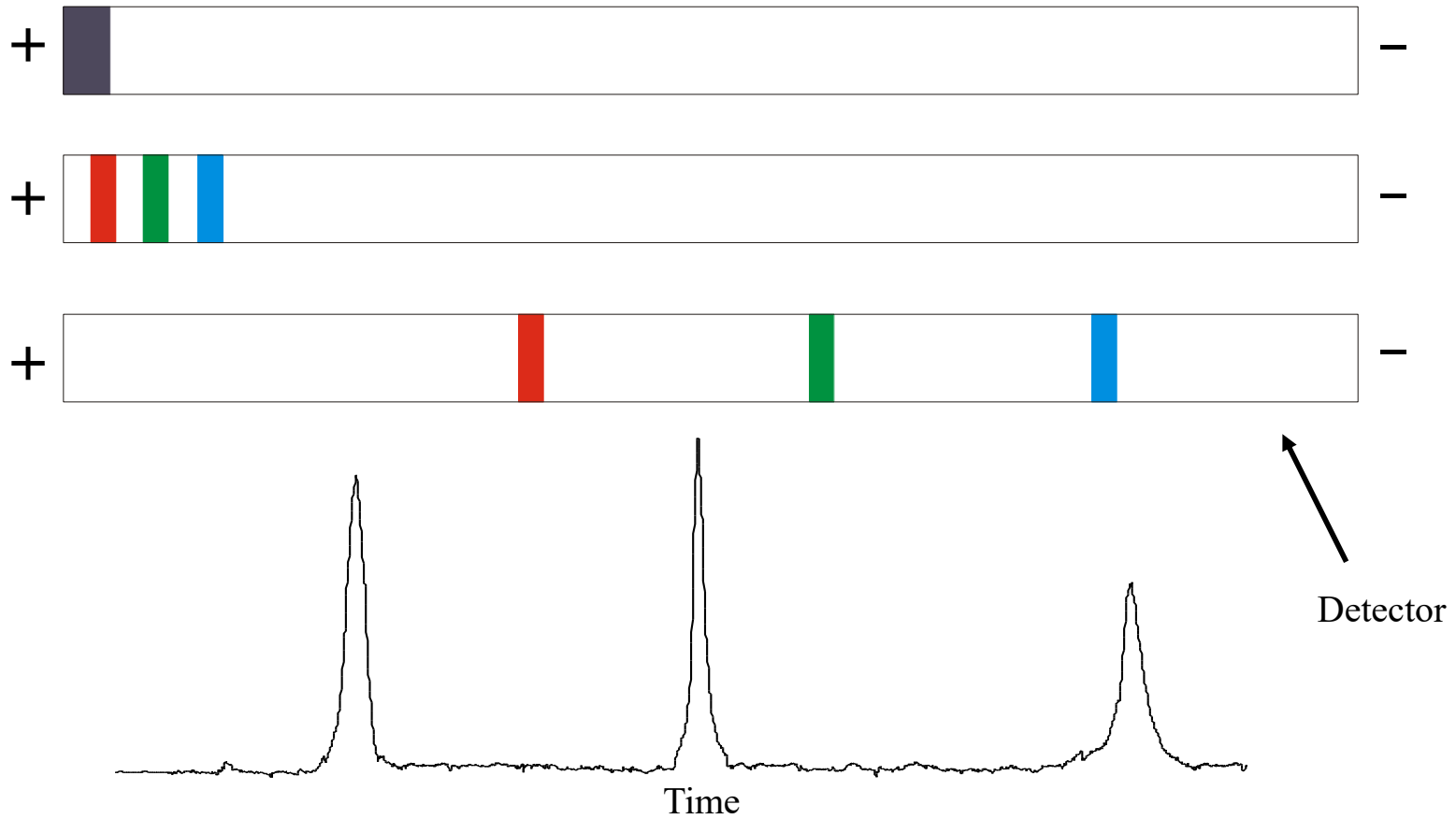
A stylized illustration featuring a red figure standing at a podium, pointing towards a gray rectangular screen. In front of the screen, several green and teal figures are seated, representing an audience. The entire scene is overlaid with a large, semi-transparent gray triangle pointing upwards.

Guido F. Verbeck, IV
University of Alabama at Birmingham
Birmingham, AL 35294

Outline

- Introduction to Capillary Electrophoresis
- Instrumentation
- Two-Wavelength LIF Detection
- Fluorophore Pair Behavior
- CGE Analysis of Molecular Weight using Size Standards
- cIEF Analysis of Peptides and Protein Digests





$V = \mu_e E$, where

V = ion velocity

E = applied electric field

μ_e = electrophoretic mobility

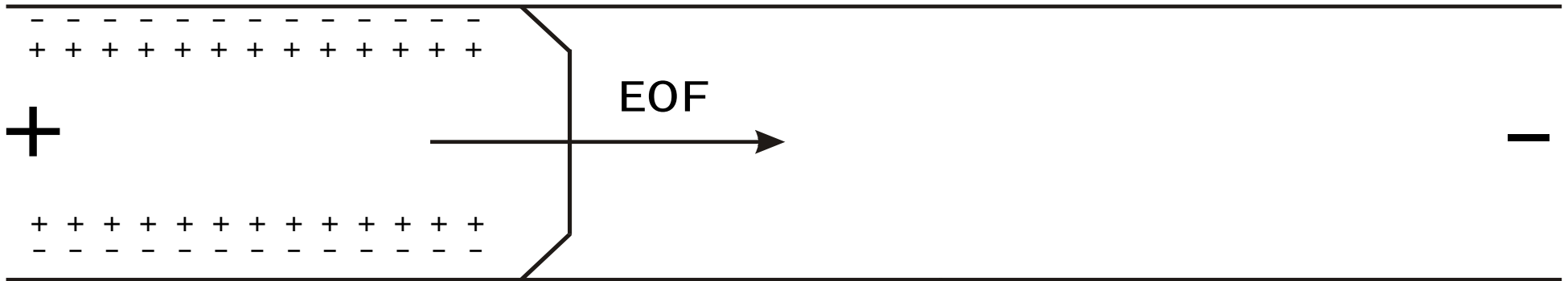
$\mu_e = q / (6\pi\eta r)$, where

q = ion charge

η = solution viscosity

r = ion radius

Electroosmotic Flow



$$V_{\text{EOF}} = \mu_{\text{EOF}} E, \text{ where}$$

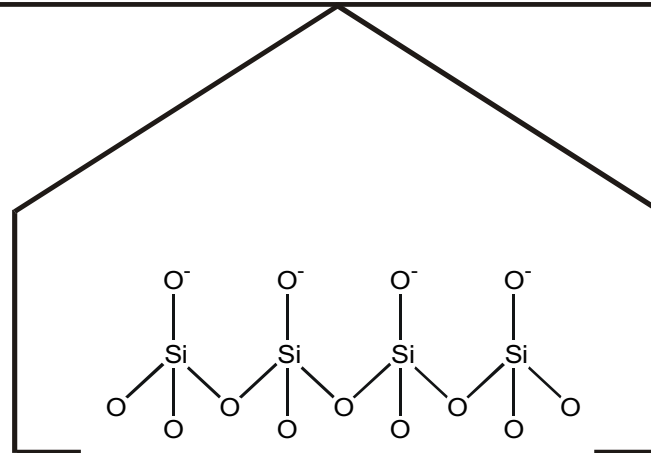
$$V_{\text{EOF}} = \text{EOF velocity}$$

$$\mu_{\text{EOF}} = \text{EOF mobility}$$

$$\mu_{\text{EOF}} = \varepsilon \zeta / \eta, \text{ where}$$

ε = dielectric constant

ζ = zeta potential



$$\mu_{\text{A}} = \mu_{\text{e}} + \mu_{\text{EOF}}, \text{ where}$$

μ_{A} = apparent mobility

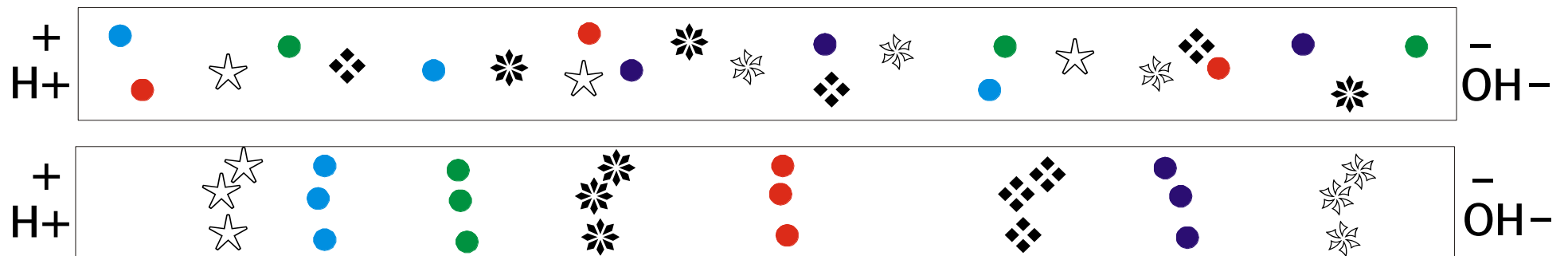
Capillary Zone Electrophoresis



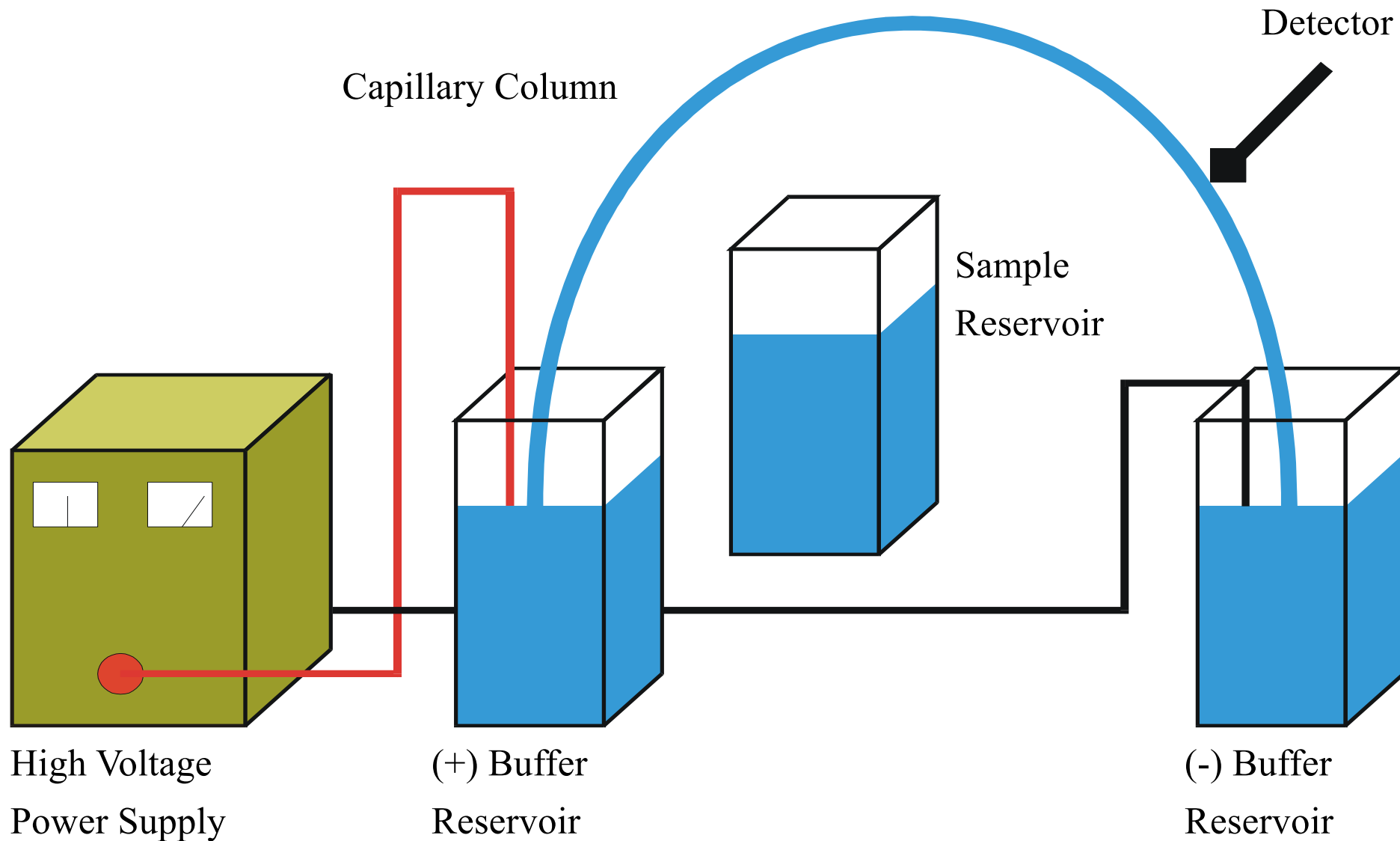
Capillary Gel Electrophoresis

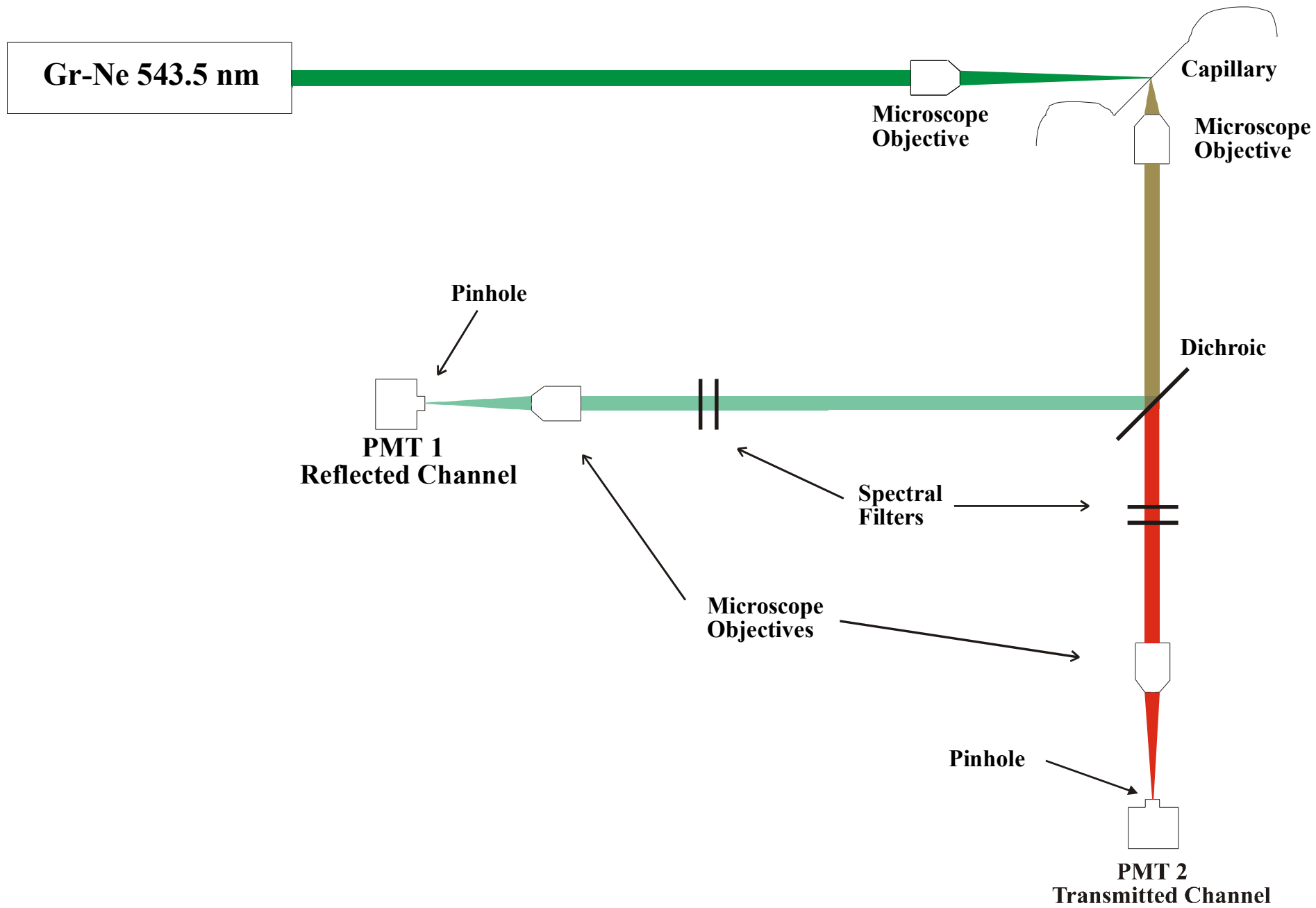


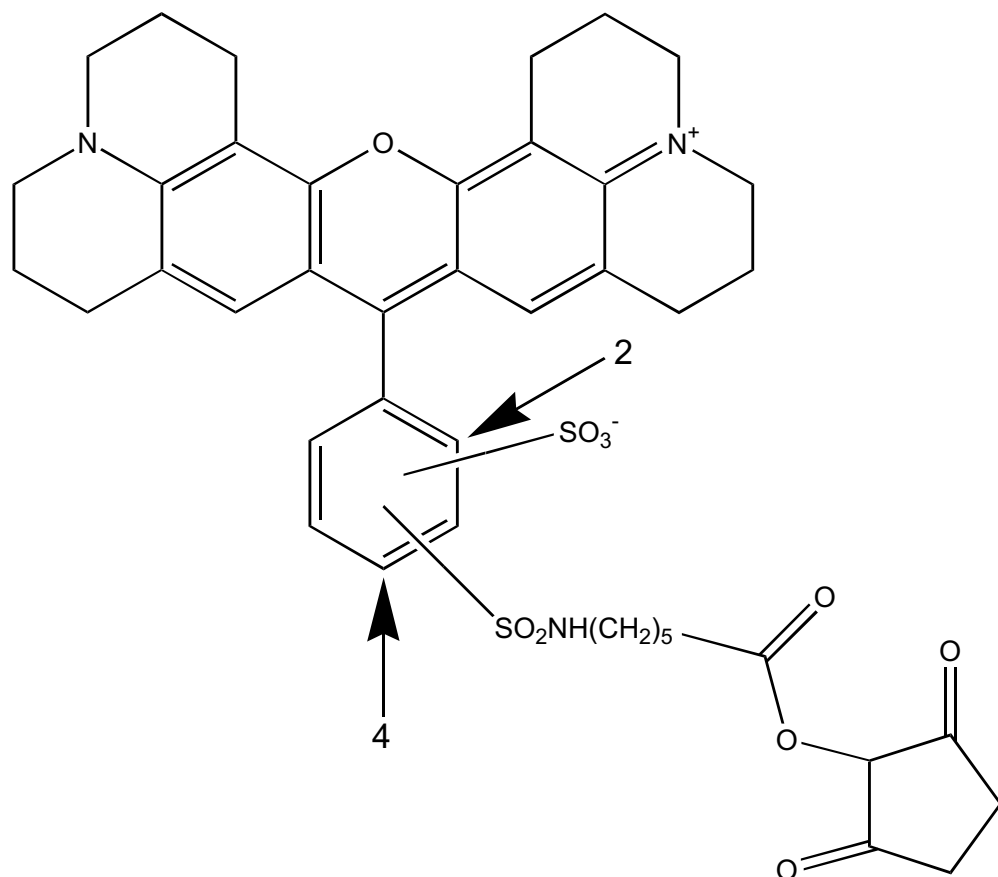
Capillary Isoelectric Focusing



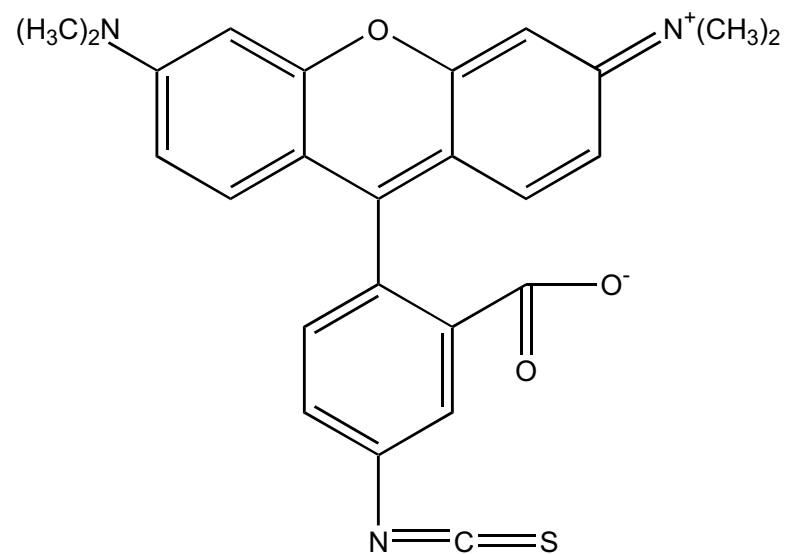
Basic Instrument Diagram



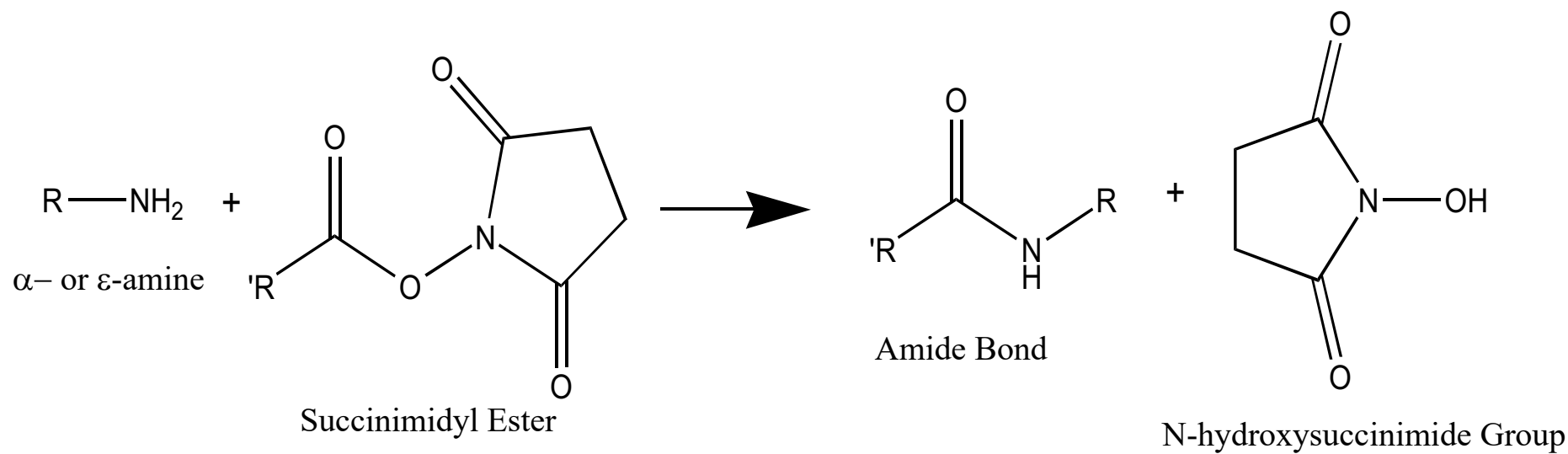
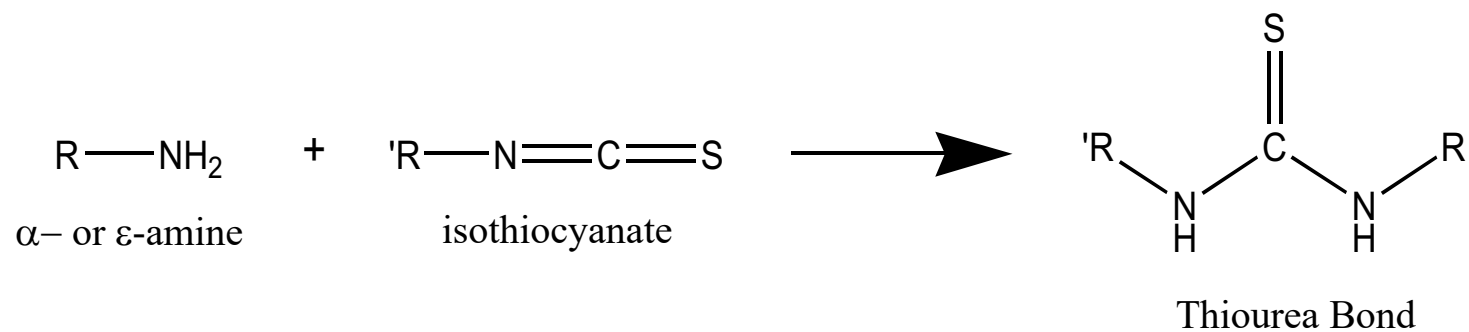


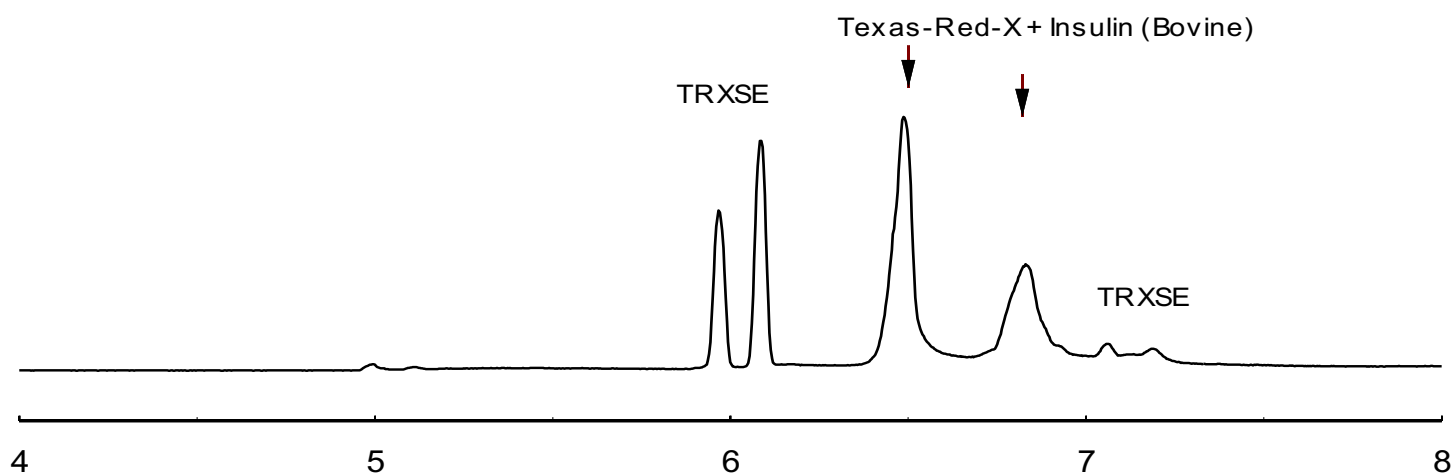
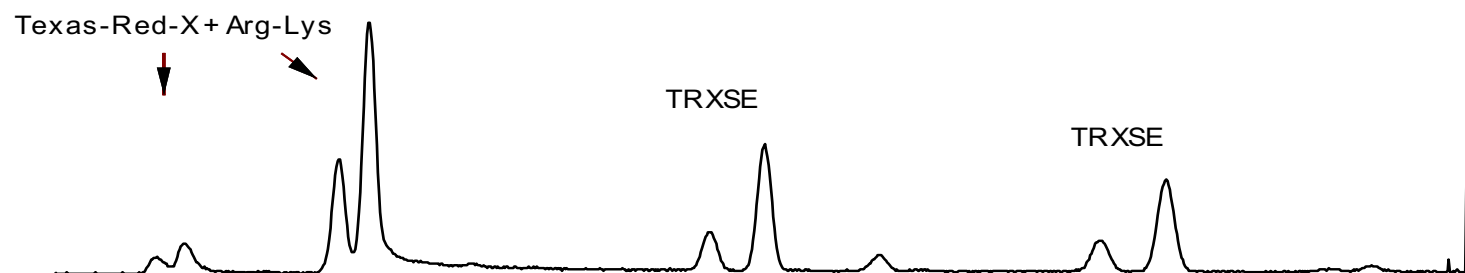


Texas Red-X SE

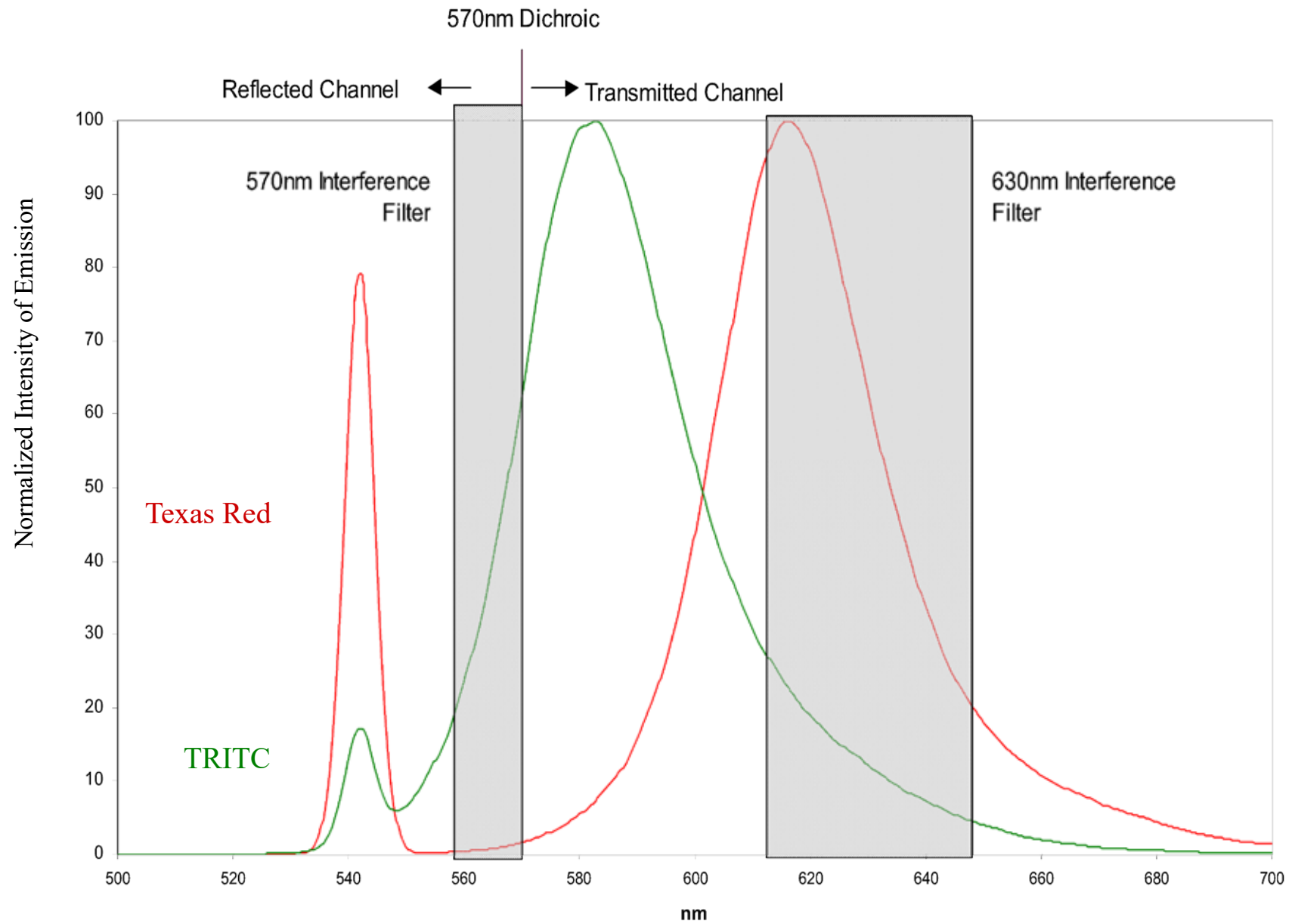


TRITC

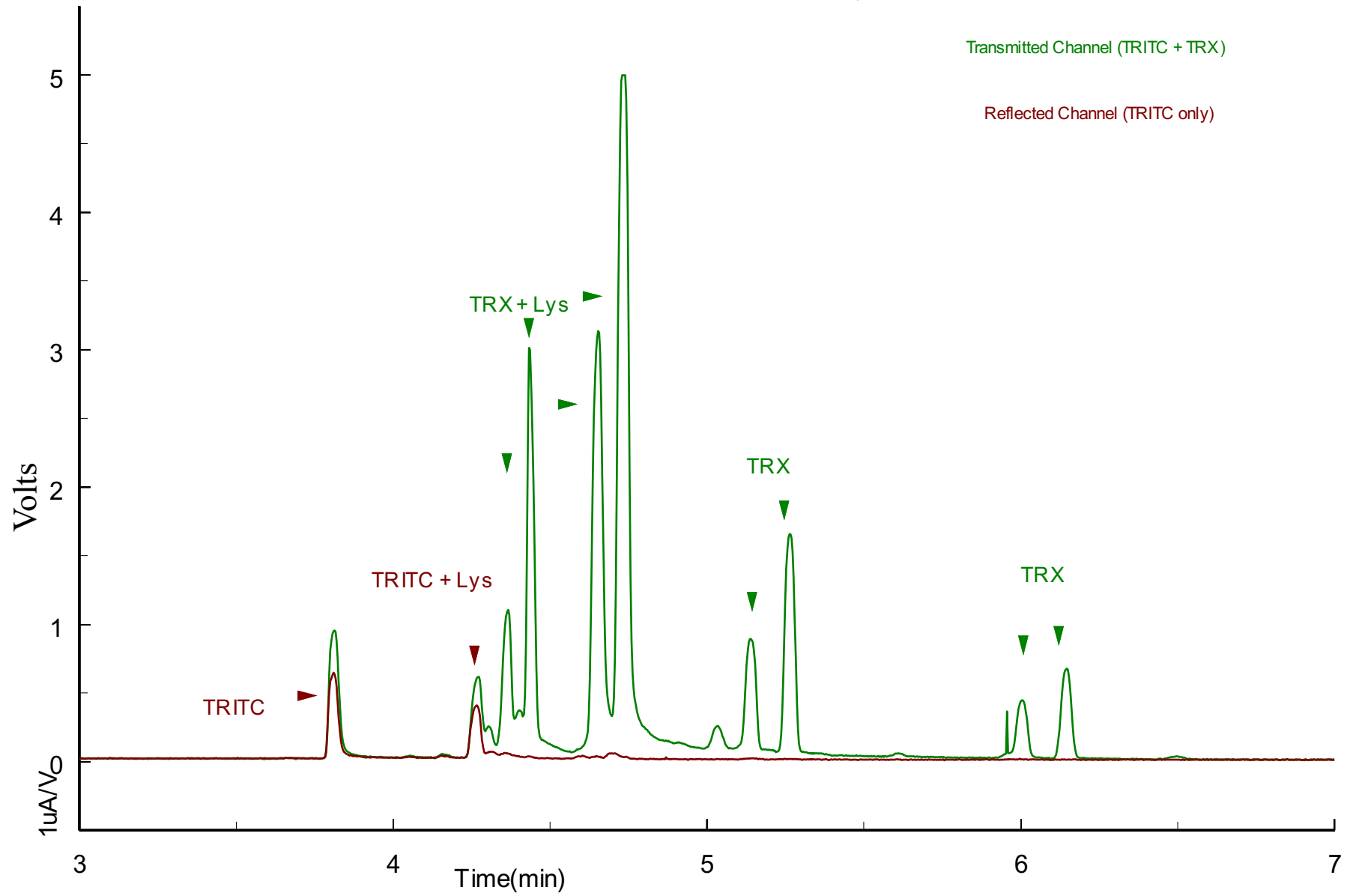


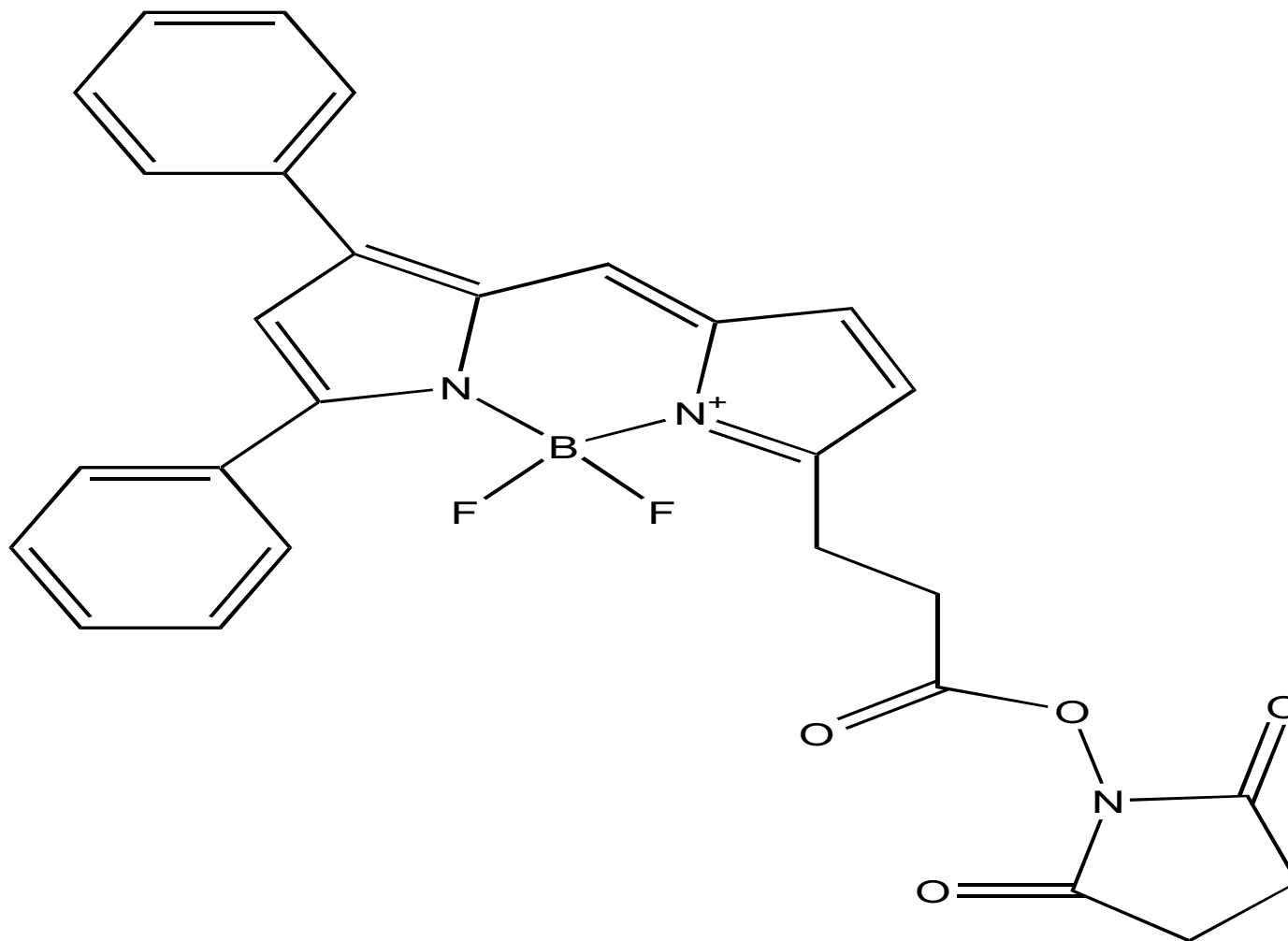


Emission Spectra, Excitation at 543.5nm



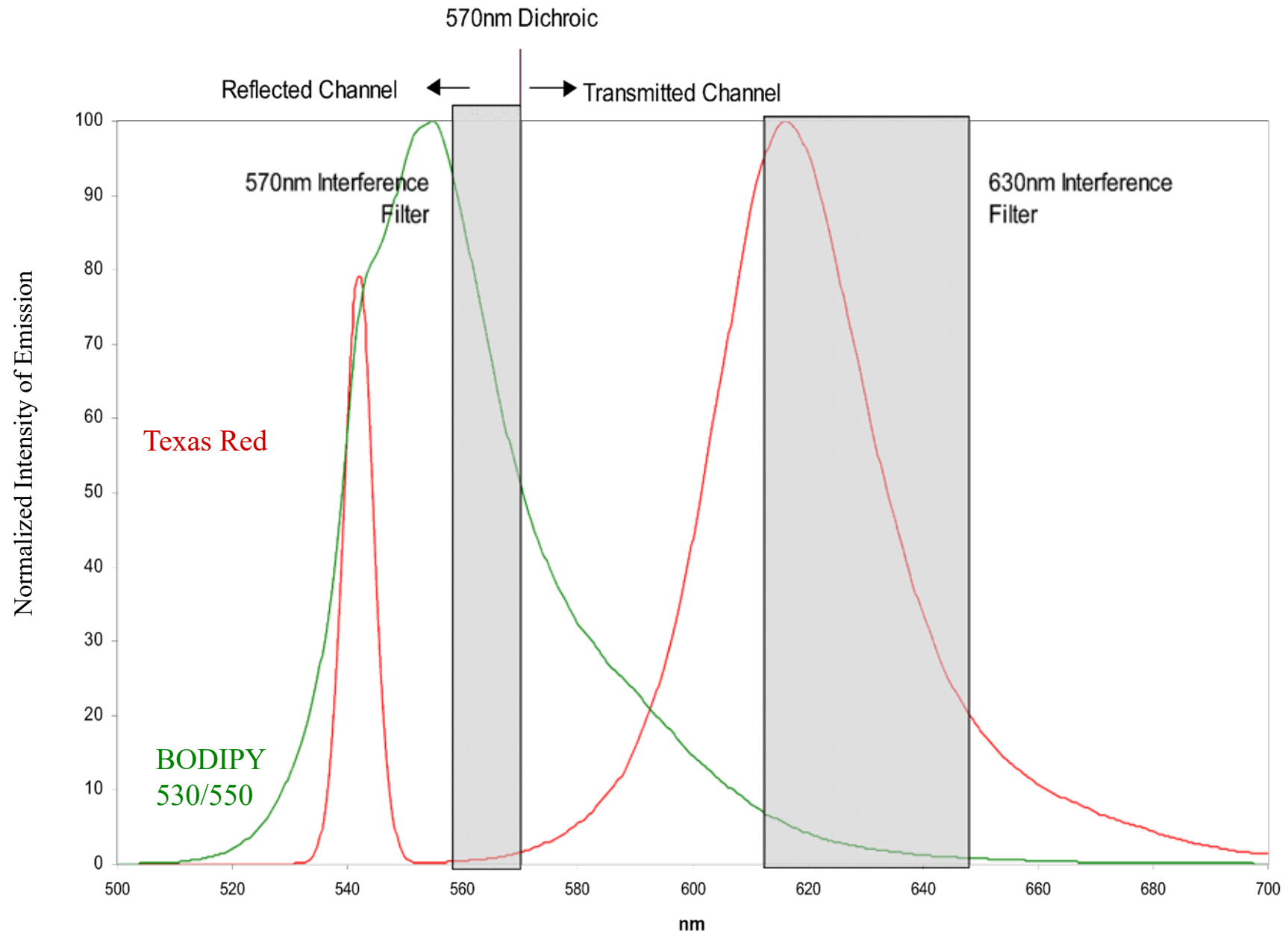
Texas Red-X and TRITC labeled Lysine (CZE)



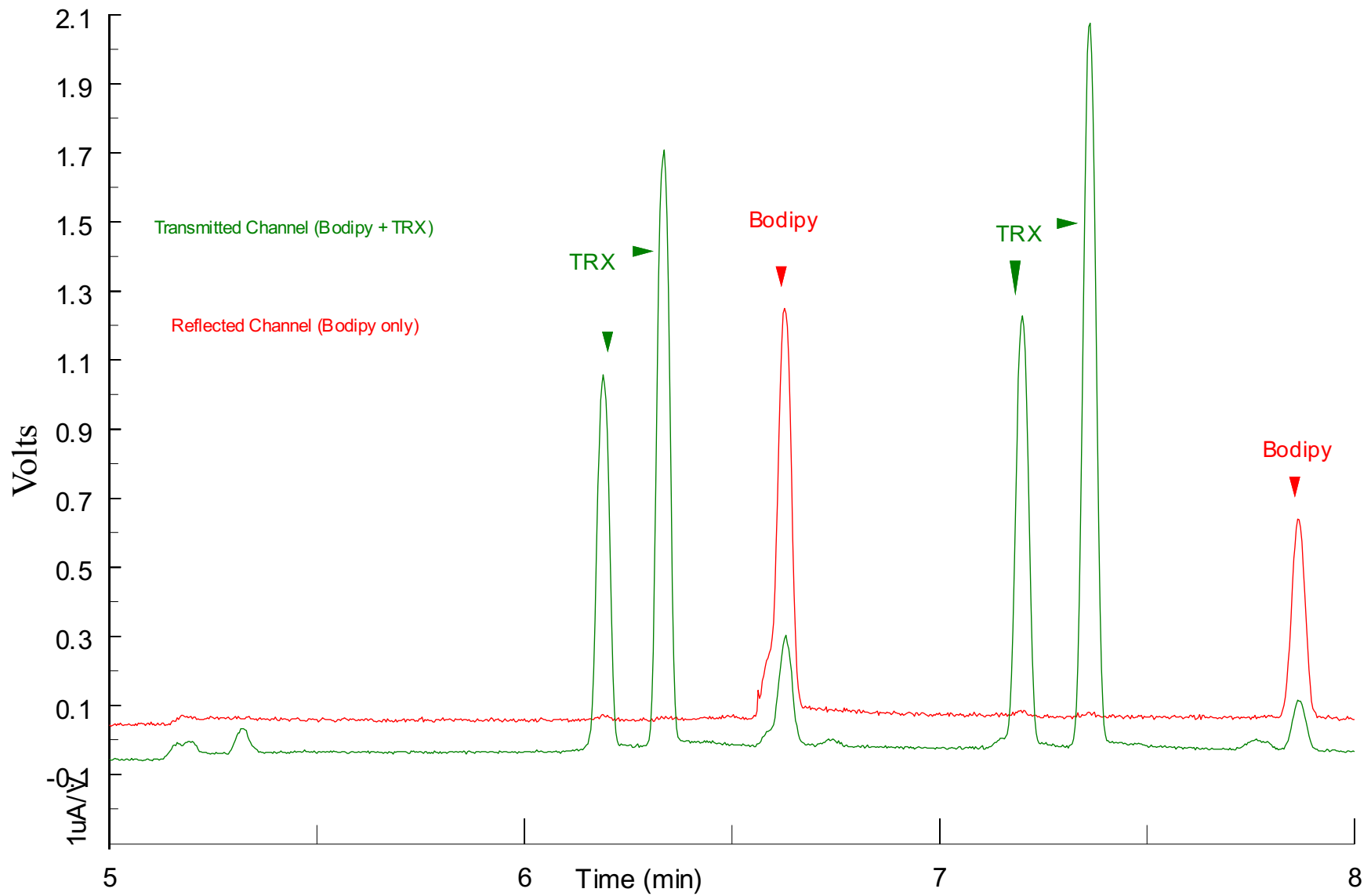


Bodipy 530/550 SE

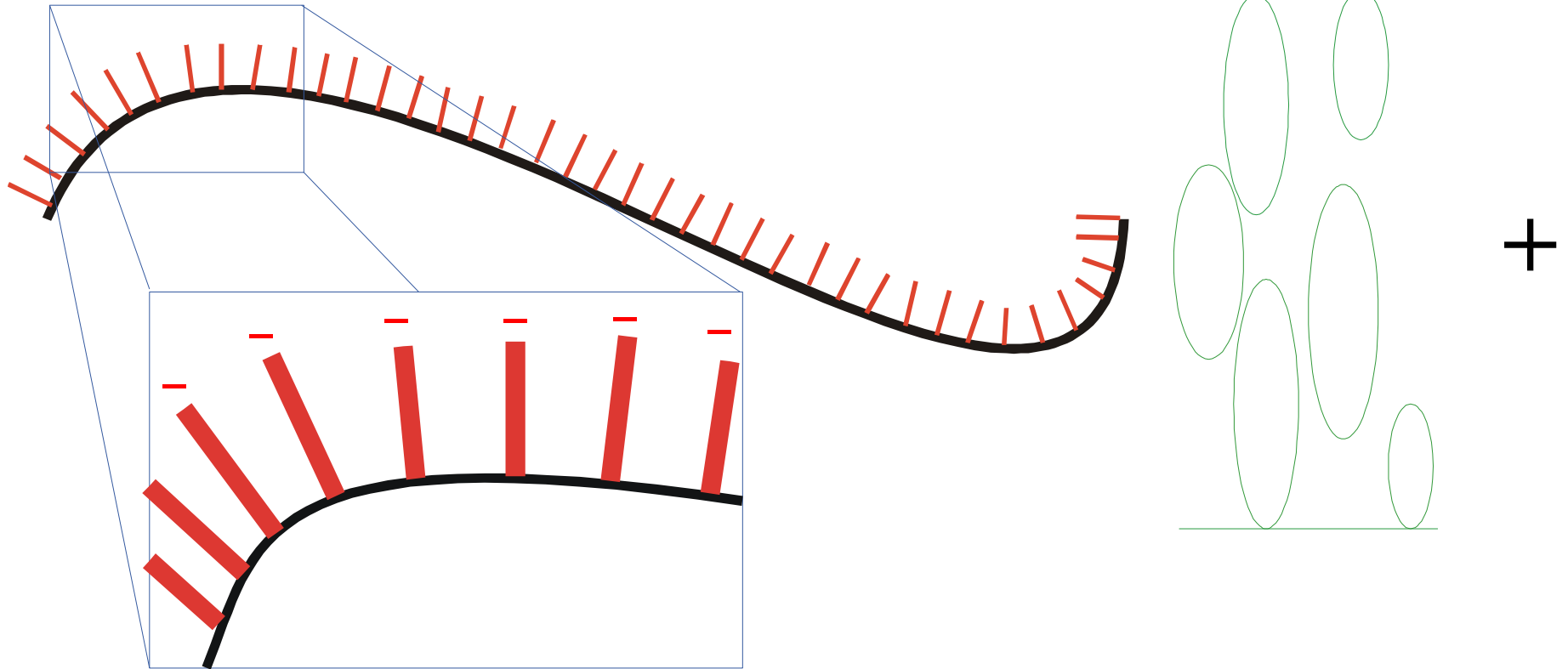
Emission Spectra, Excitation at 543.5nm



Texas Red-X and BODIPY 530/550 (CZE)



SDS - PAGE



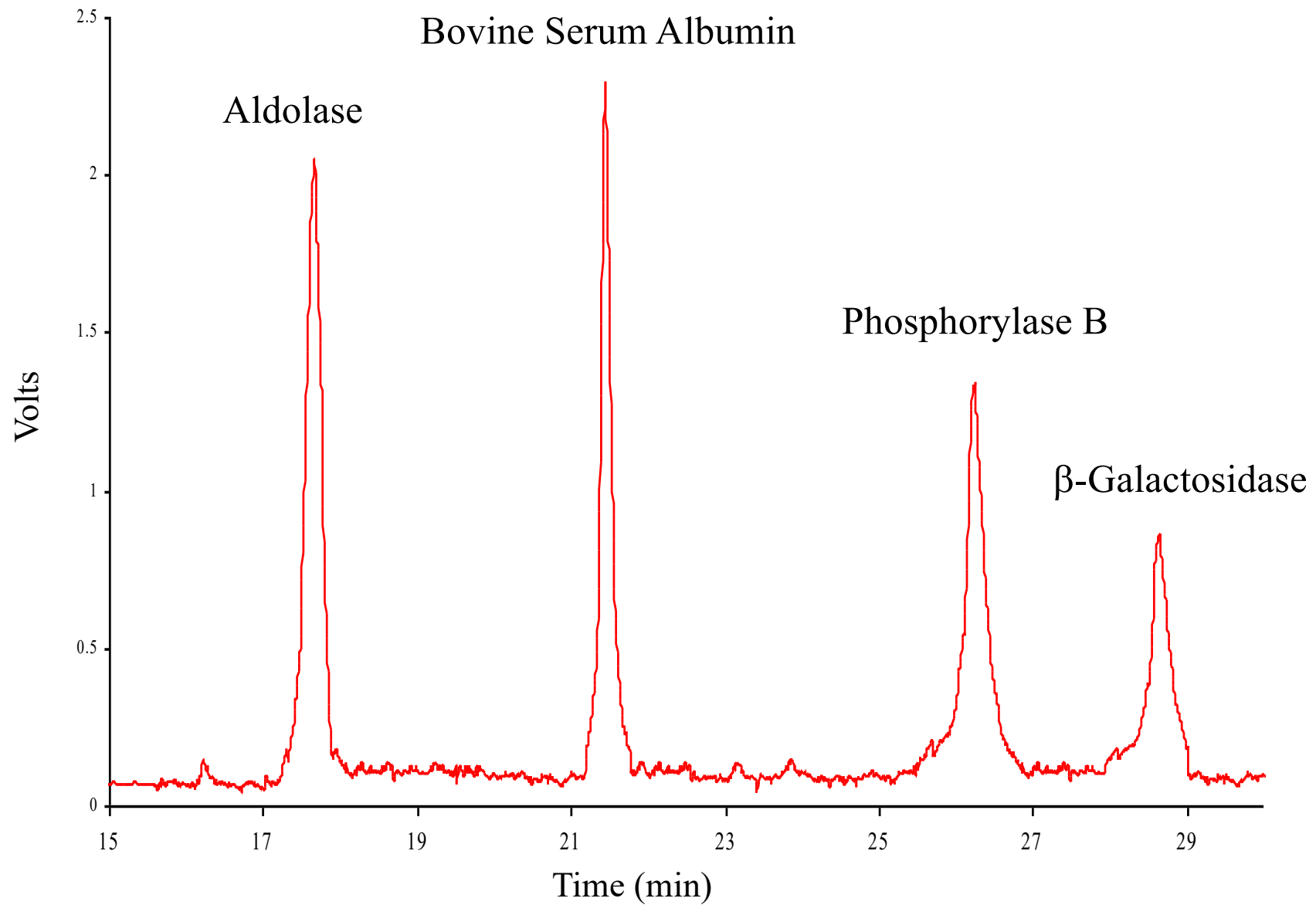
$$\mu = b - C \log(M_w)$$

μ = mobility

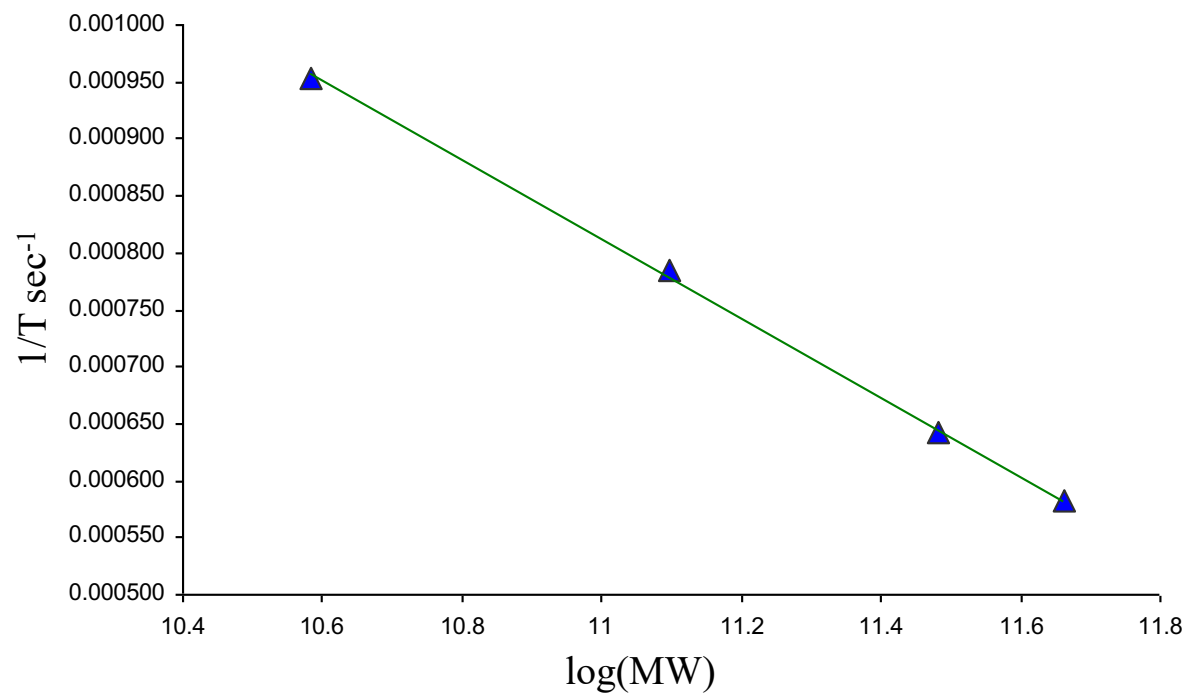
b = function of gel-free mobility

C = function of gel concentration

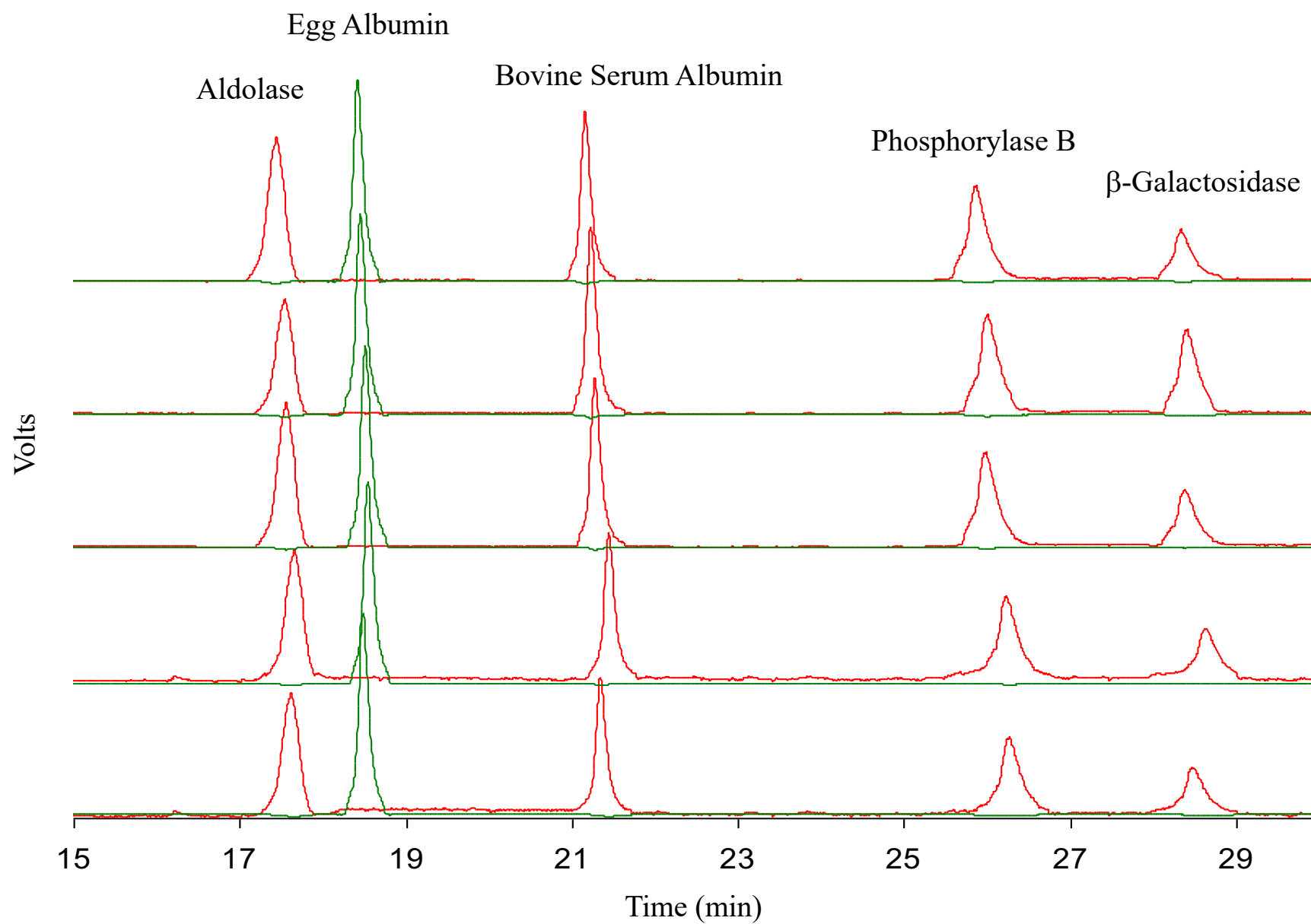
Denaturing CGE of SDS Proteins in 6%T Linear Polyacrylamide



	Aldolase	Bovine Serum Albumin	Phosphorylase B	β -Galactosidase
Molecular Weight	39500	66000	97000	116000
Time 1	1045.5	1269.0	1551.5	1699.5
Time 2	1045.0	1272.5	1556.0	1720.0
Time 3	1050.0	1275.5	1558.5	1721.0
Time 4	1052.5	1276.5	1559.0	1726.0
Time 5	1052.0	1277.0	1558.5	1730.5



Denaturing CGE of SDS Proteins in 6%T Linear Polyacrylamide



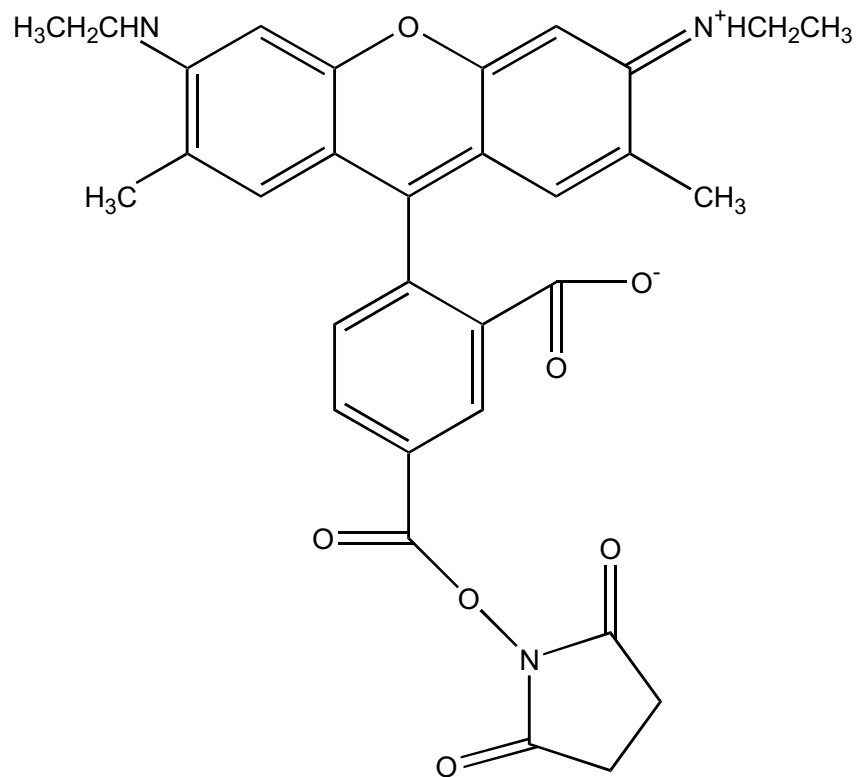
Bodipy + Egg Albumin - Run Independent from Standards

Run	Time	1/T	ln(MW)	MW
1	1094.0	0.00091408	10.7141	44985
2	1093.5	0.00091449	10.7131	44940
3	1095.5	0.00091283	10.7069	44661
4	1105.5	0.00090457	10.7259	45521
5	1115.0	0.00089686	10.7491	46588

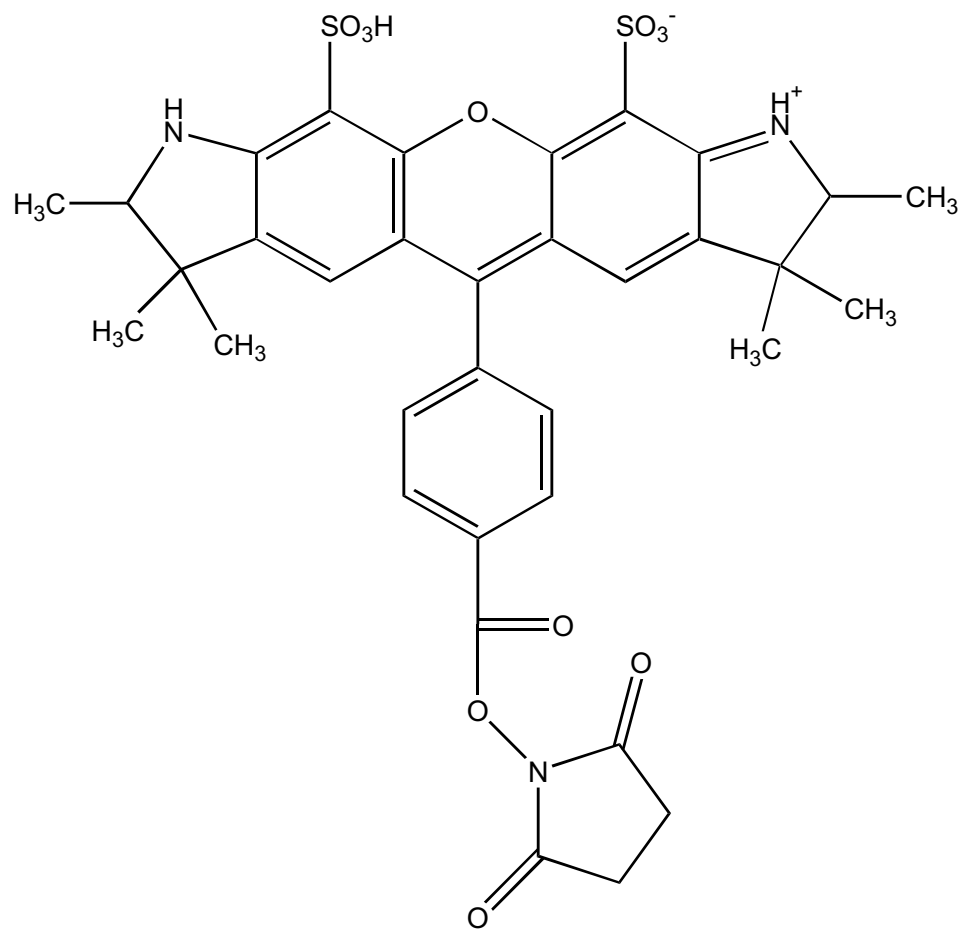
Texas-Red-X + Egg Albumin - Run Simultaneous with Standards

Run	Time	1/T	ln(MW)	MW
1	1104.0	0.00090580	10.738	46079
2	1105.0	0.00090498	10.740	46177
3	1110.0	0.00090090	10.741	46225
4	1109.5	0.00090131	10.735	45953
5	1110.5	0.00090050	10.739	46102

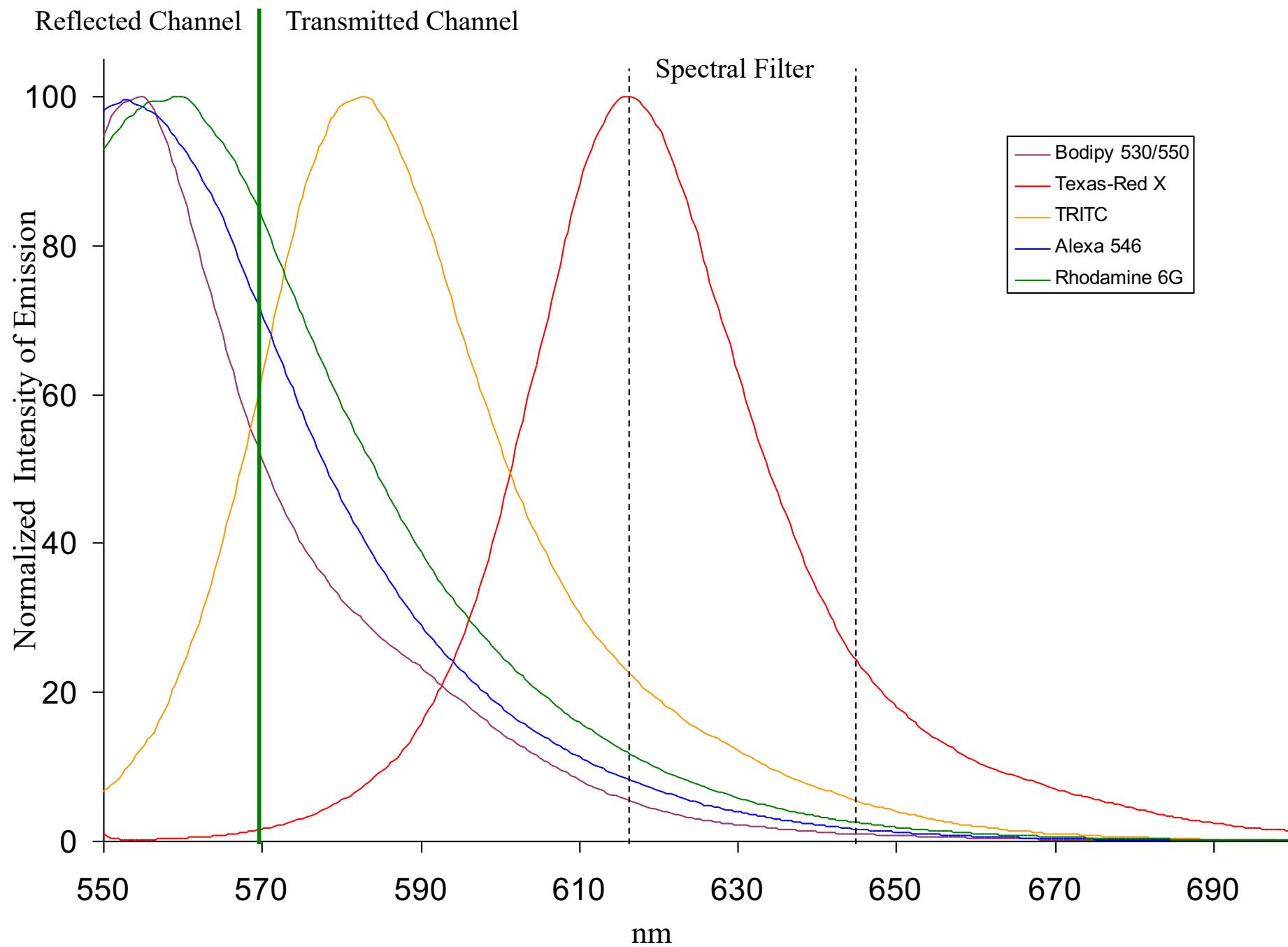
	Average	Standard Deviation
MW based on Combined Runs	45300	1000
MW based on Simultaneous Runs	46100	100



5-carboxyrhodamine SE



Alexa 532



Two Step cIEF

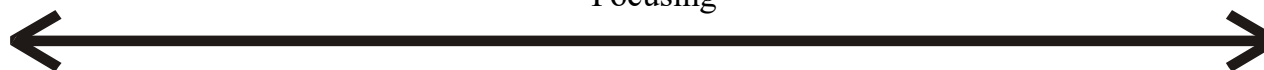
Column is
filled with the
sample and
ampholyte
mixture



Capillary wall is
coated with
polyacrylamide



Focusing



Anode

H_3PO_4



Cathode

NaOH

Anode

H_3PO_4

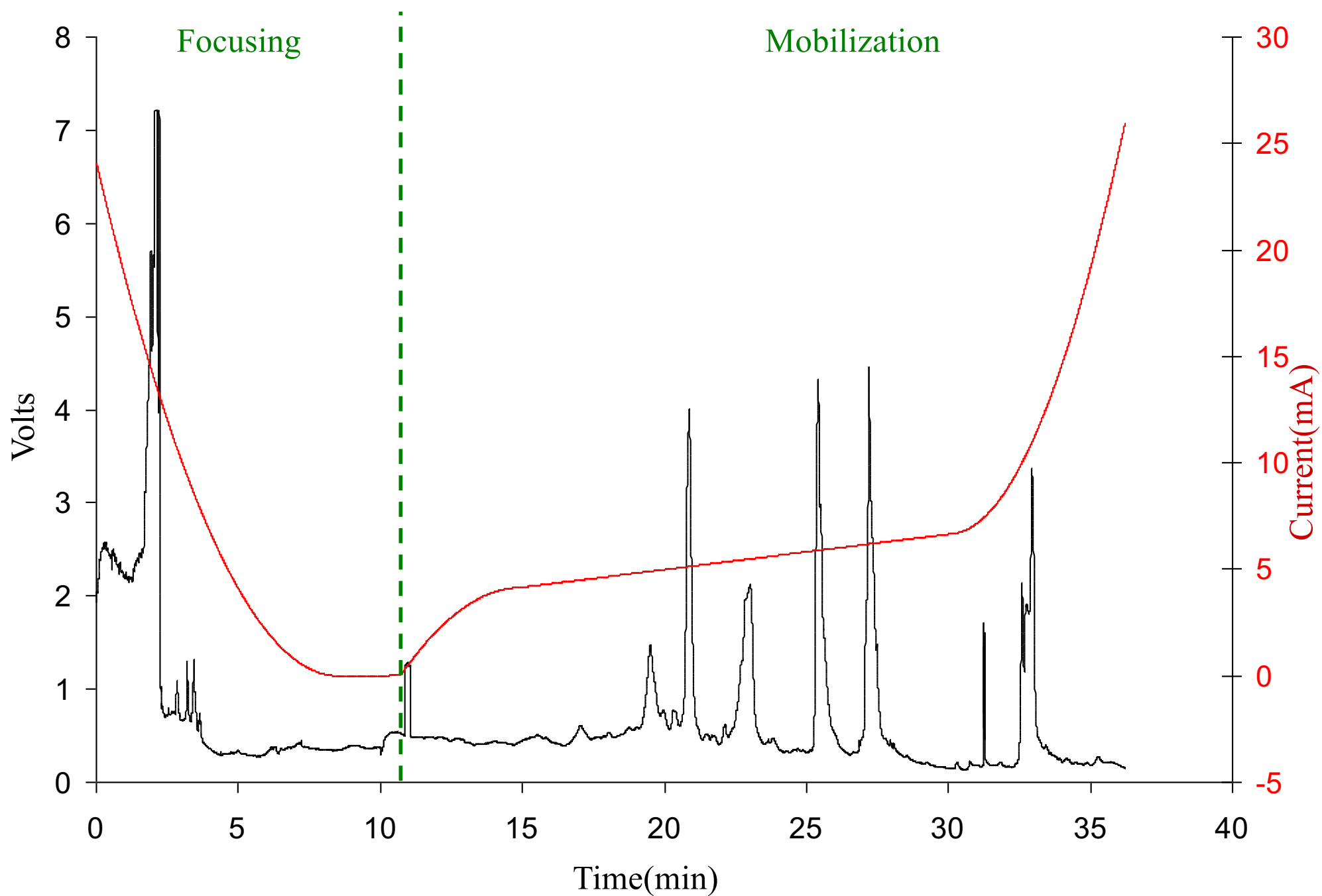


Cathode

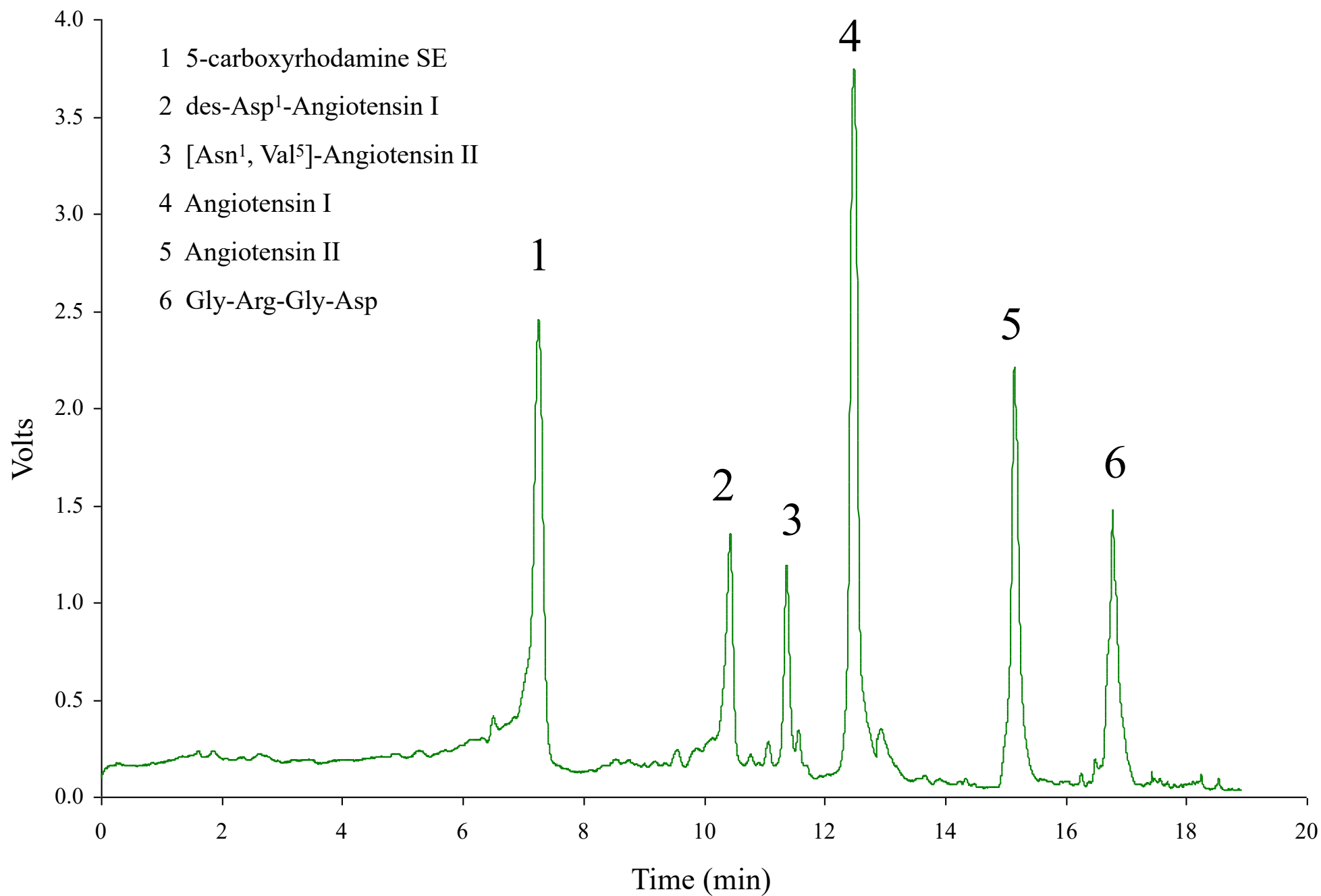
H_3PO_4

Detector

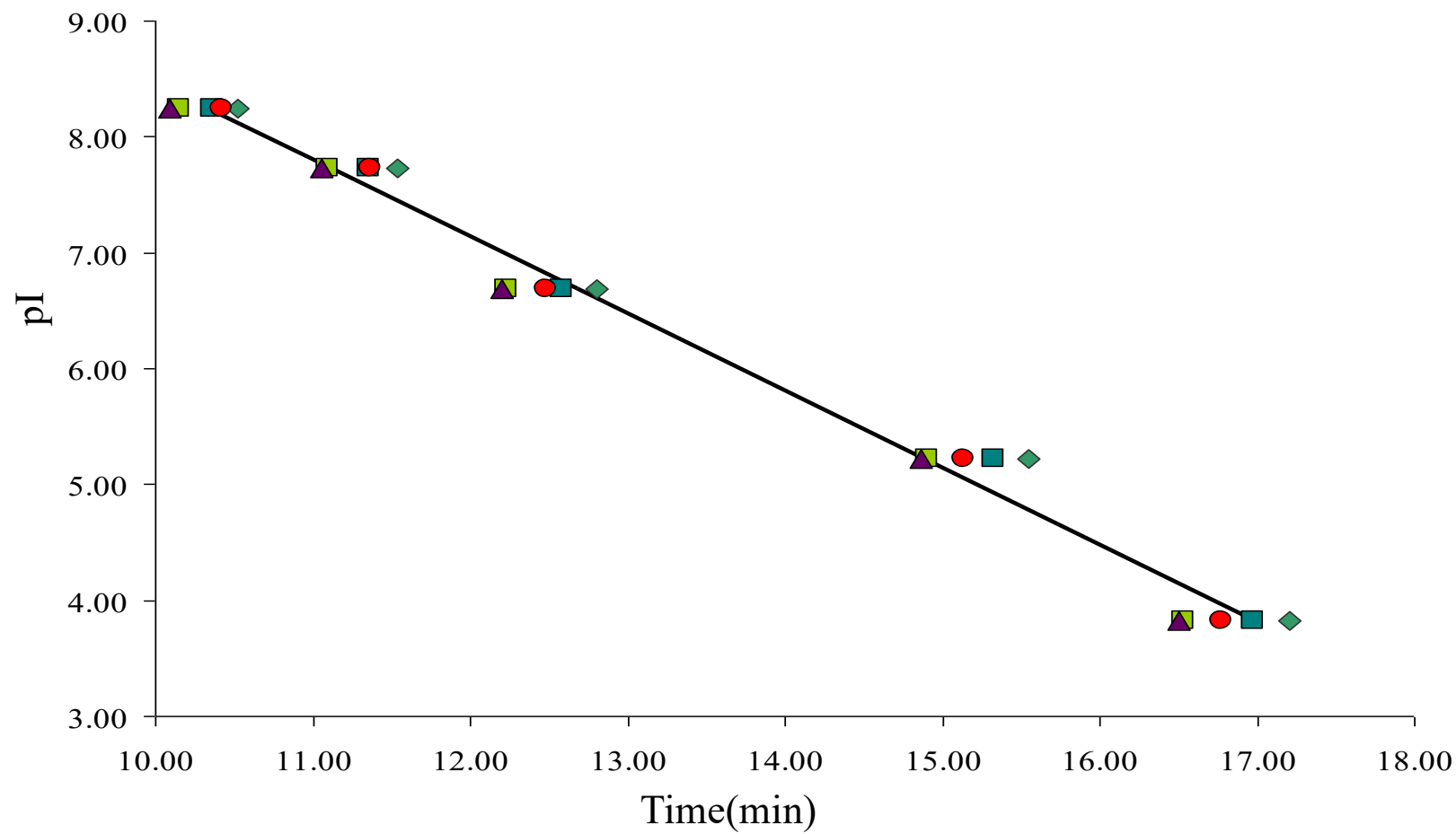




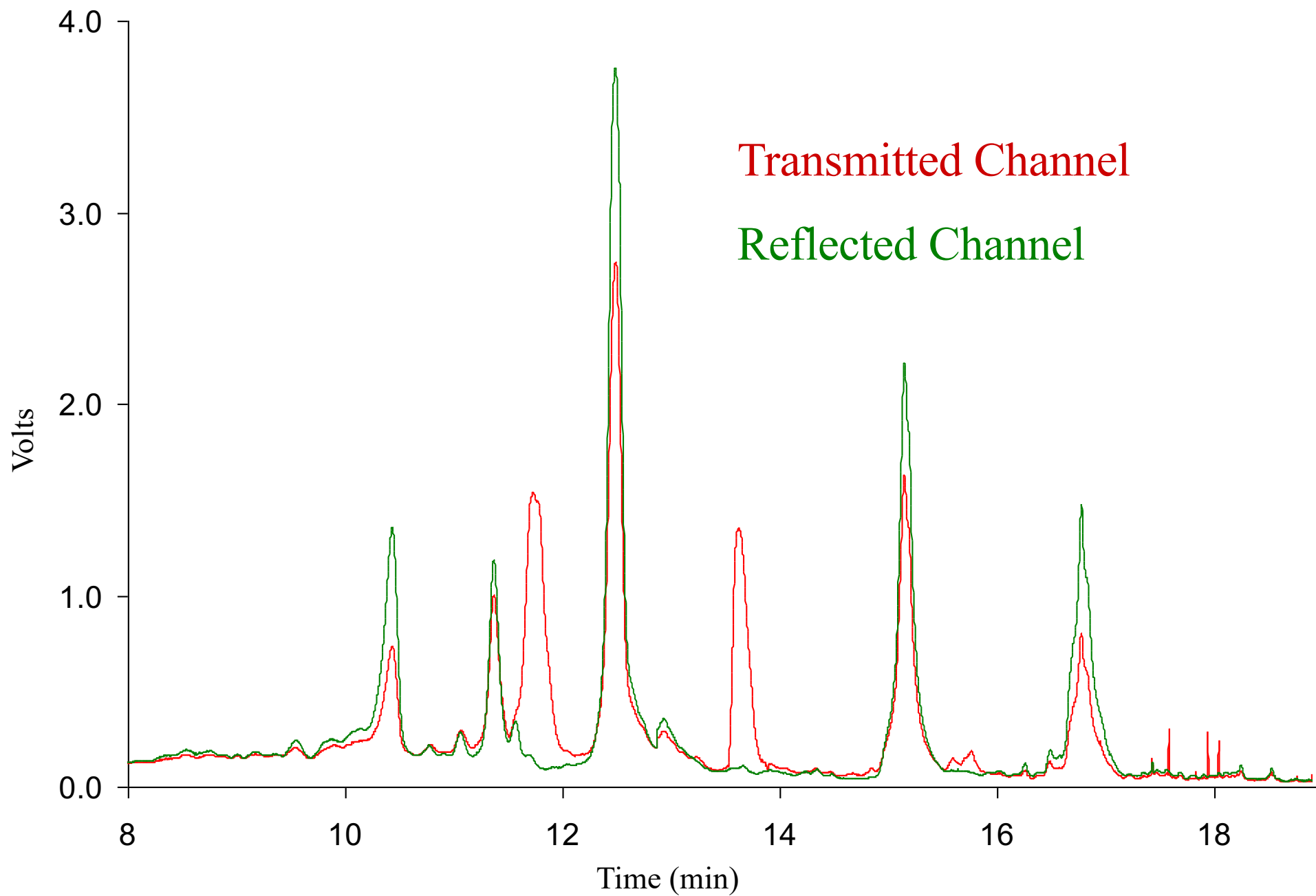
Mobilization Electropherogram of Focused pI Markers



Peptide	pI	Run 1	Run 2	Run 3	Run 4	Run 5
des-Asp1-angiotensin I	8.25	10.520	10.367	10.147	10.087	10.427
[Asn1-Val5]-angiotensin II	7.73	11.537	11.360	11.100	11.060	11.363
Angiotensin I	6.69	12.800	12.587	12.230	12.197	12.483
Angiotensin II	5.22	15.543	15.330	14.900	14.860	15.140
Gly-Arg-Gly-Asp	3.82	17.207	16.973	16.533	16.507	16.773

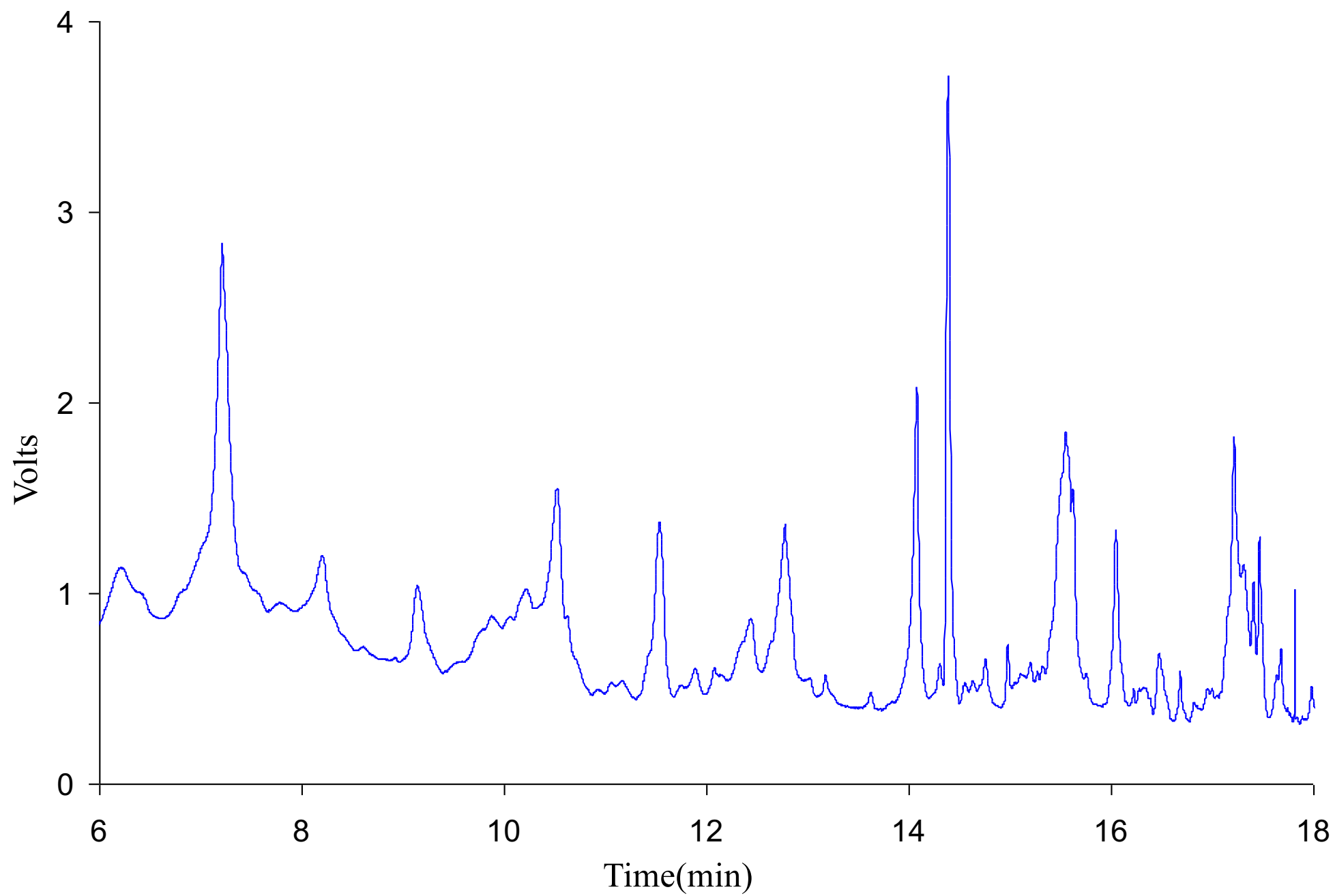


cIEF with 3/10 pH Ampholyte

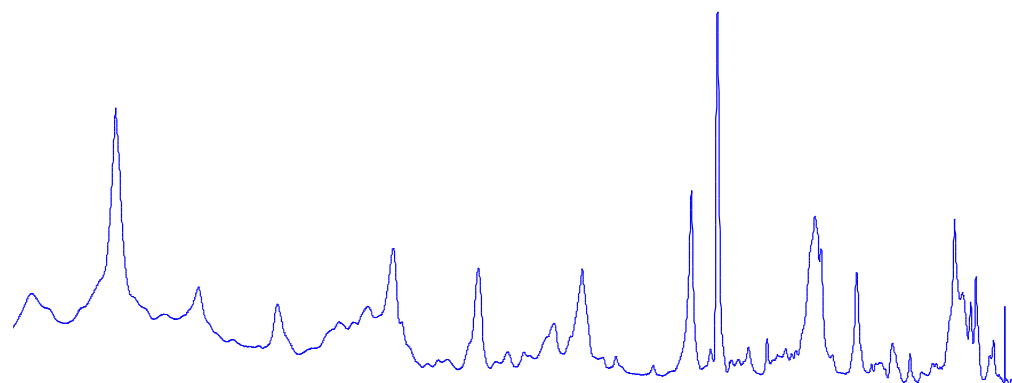


Run	External Calibration	Internal Calibration
1	7.23	7.42
2	7.29	7.37
3	7.46	7.34
4	7.54	7.39
5	7.34	7.39
Average	7.37	7.38
Standard Deviation	0.12	0.03

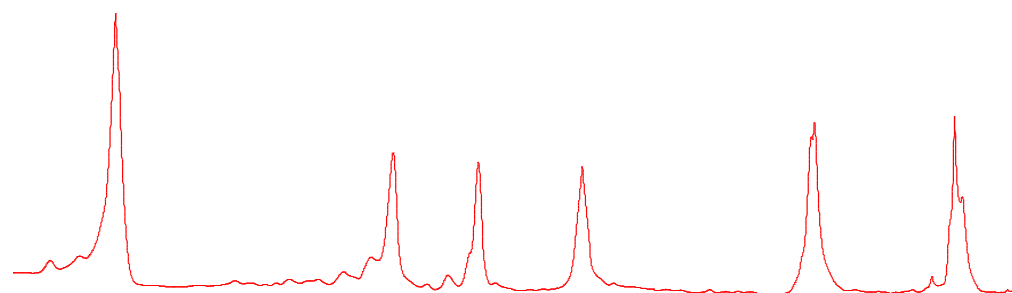
cIEF of digested β -Casein co-injected with pI standards



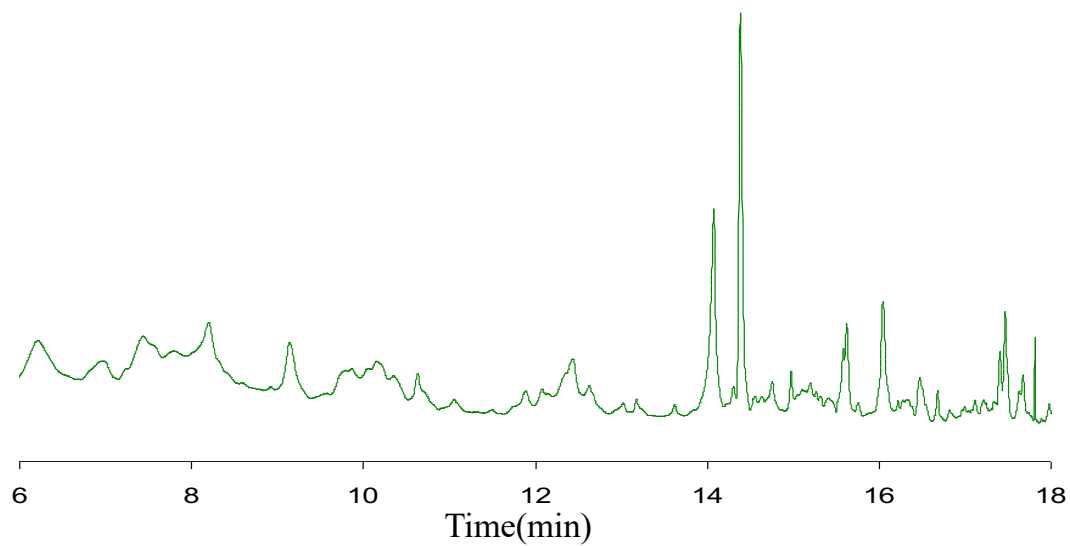
Transmitted Channel



Reflected Channel

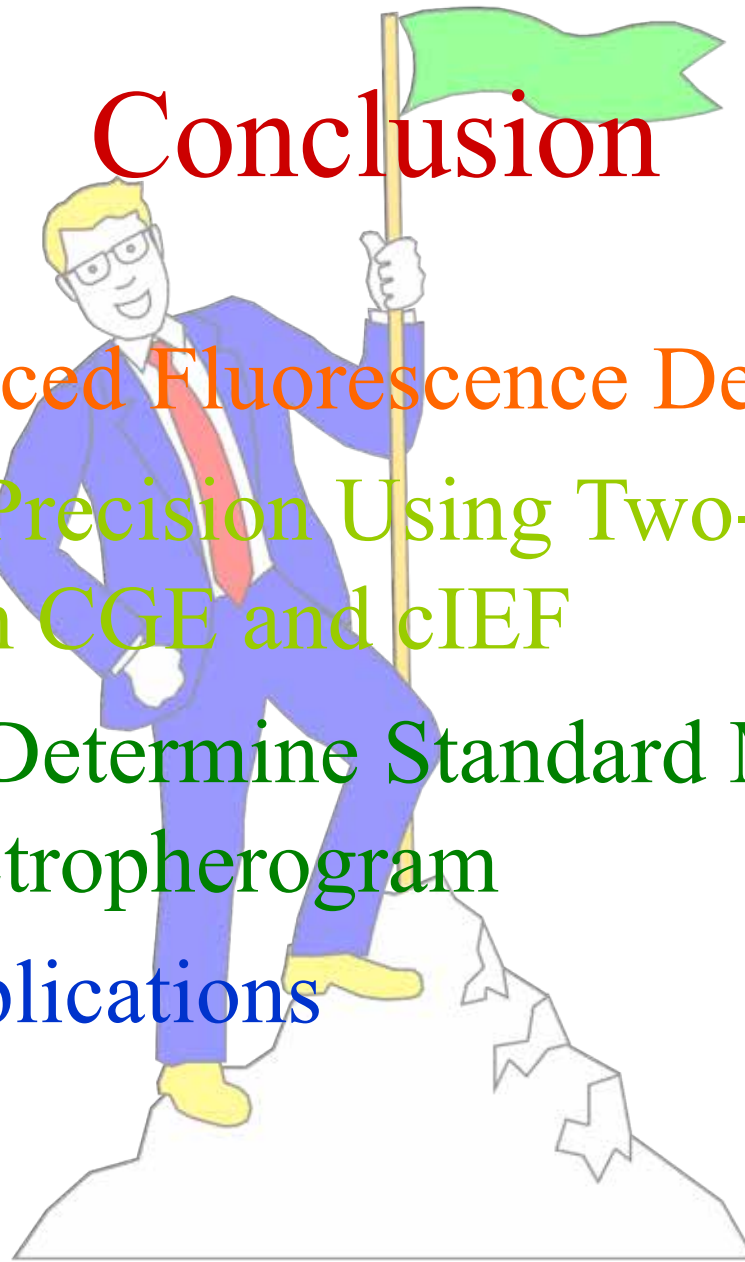


Subtracted



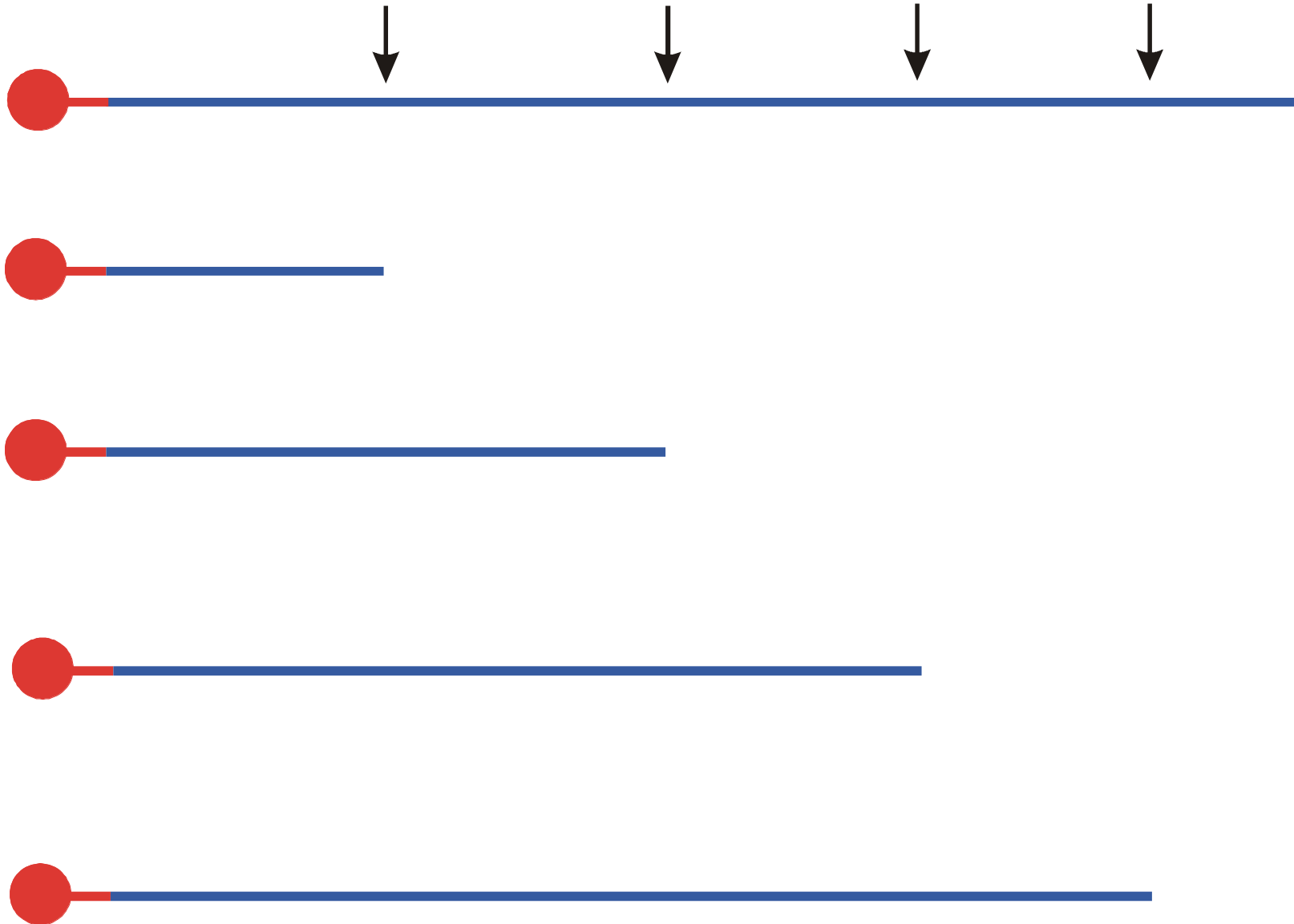
Conclusion

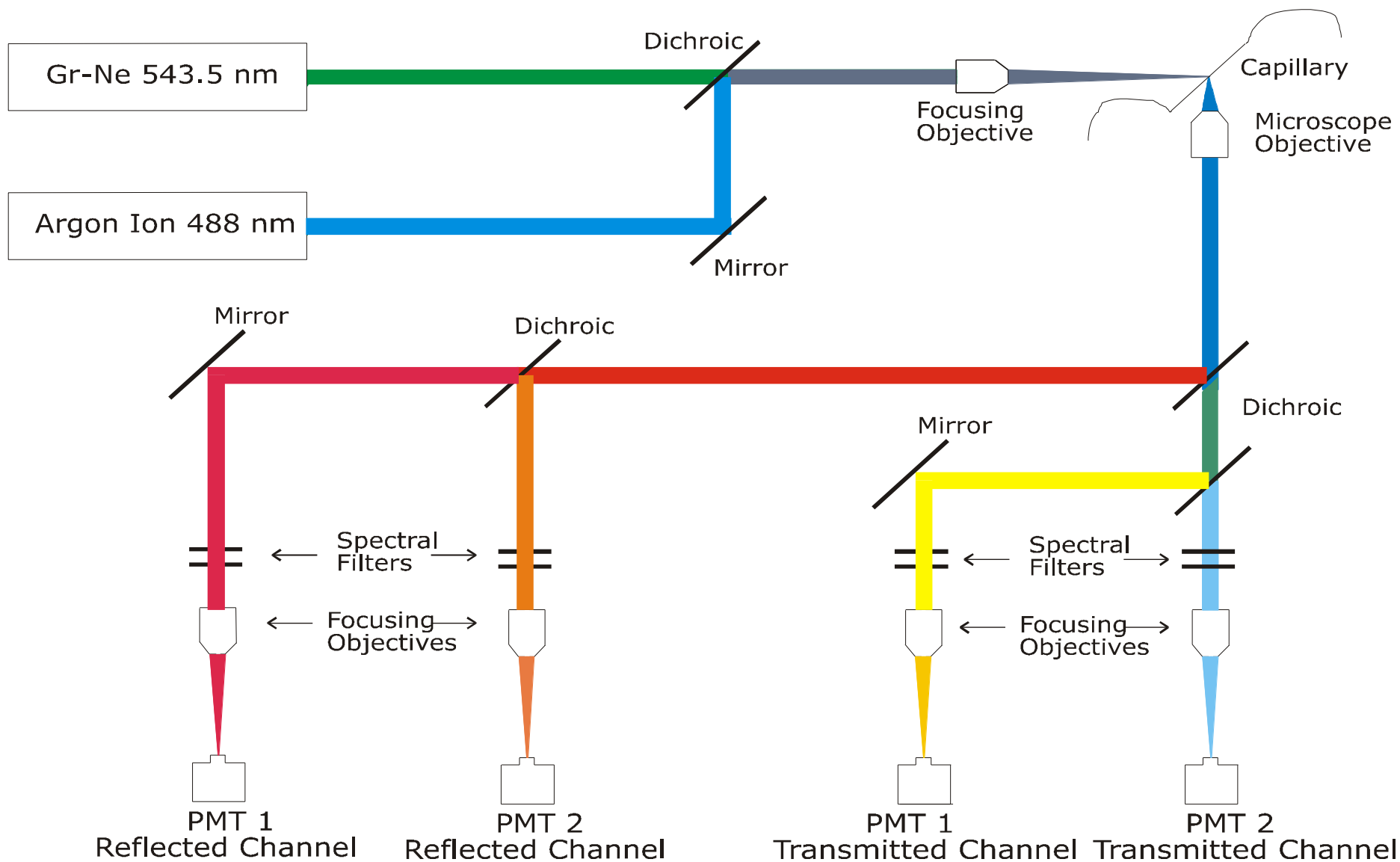
- Laser-Induced Fluorescence Detection
- Increased Precision Using Two-Wavelength Analysis in CGE and cIEF
- Ability to Determine Standard Markers in a Noisy Electropherogram
- Future Applications



Partial Protein Digest

Cleaving sites





Acknowledgements

- Dr. Stephen C. Beale
- Sam Wang
- Wendy Yang
- Sara Jane Osborne
- Dr. William K. Nonidez
- Dr. Donald D. Muccio
- Dr. Christopher M. Lawson
- Dr. Steven R. Harville
- Helen Ji
- Jerry Sewell
- Mentors and Friends at UAB
- NSF CHE-9624884
- UAB Department of Chemistry
- UAB Graduate School

