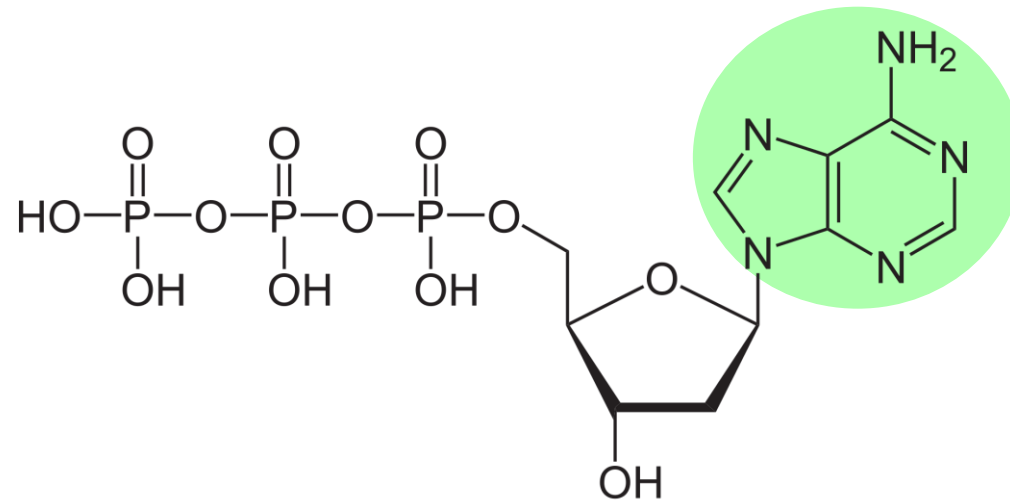
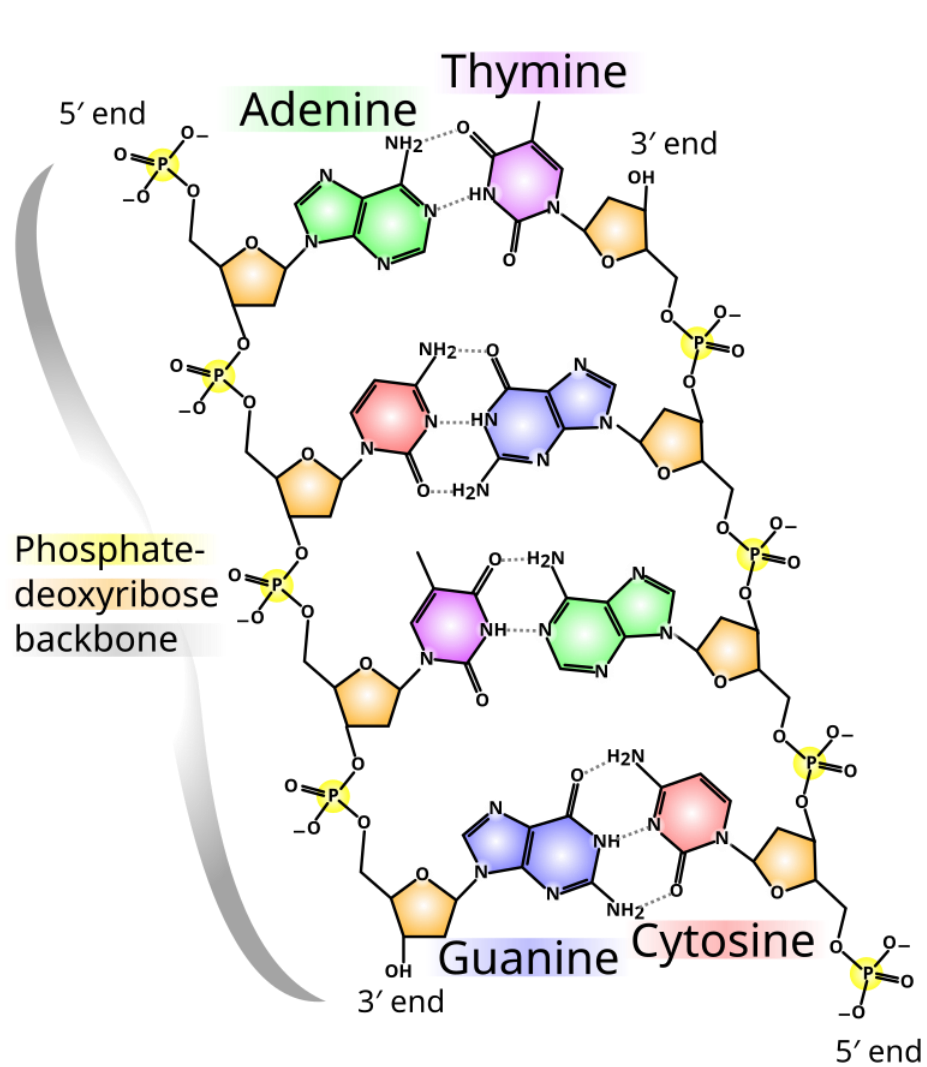


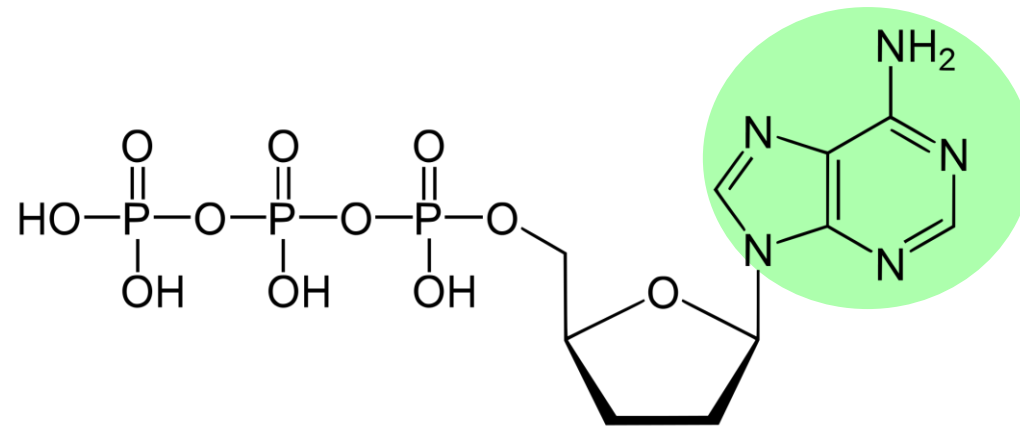
# Real-Time DNA Sequencing

Lena Eiwanger

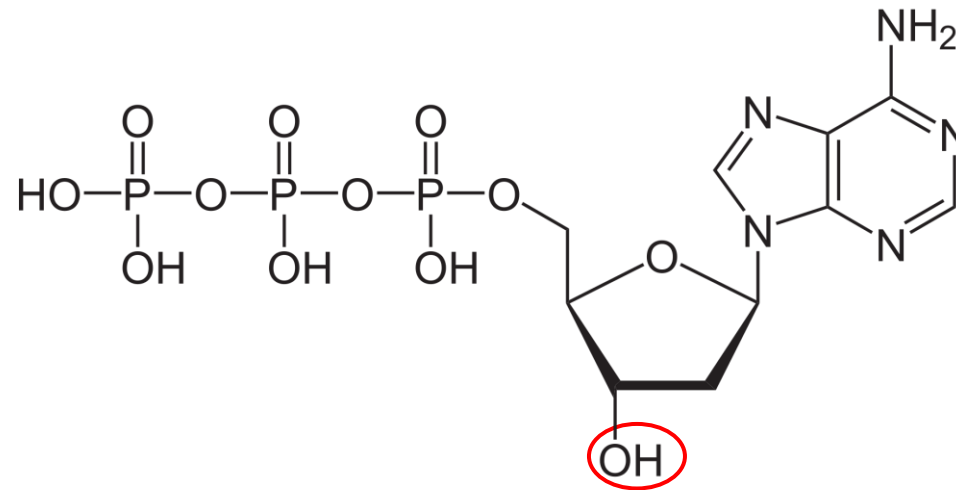
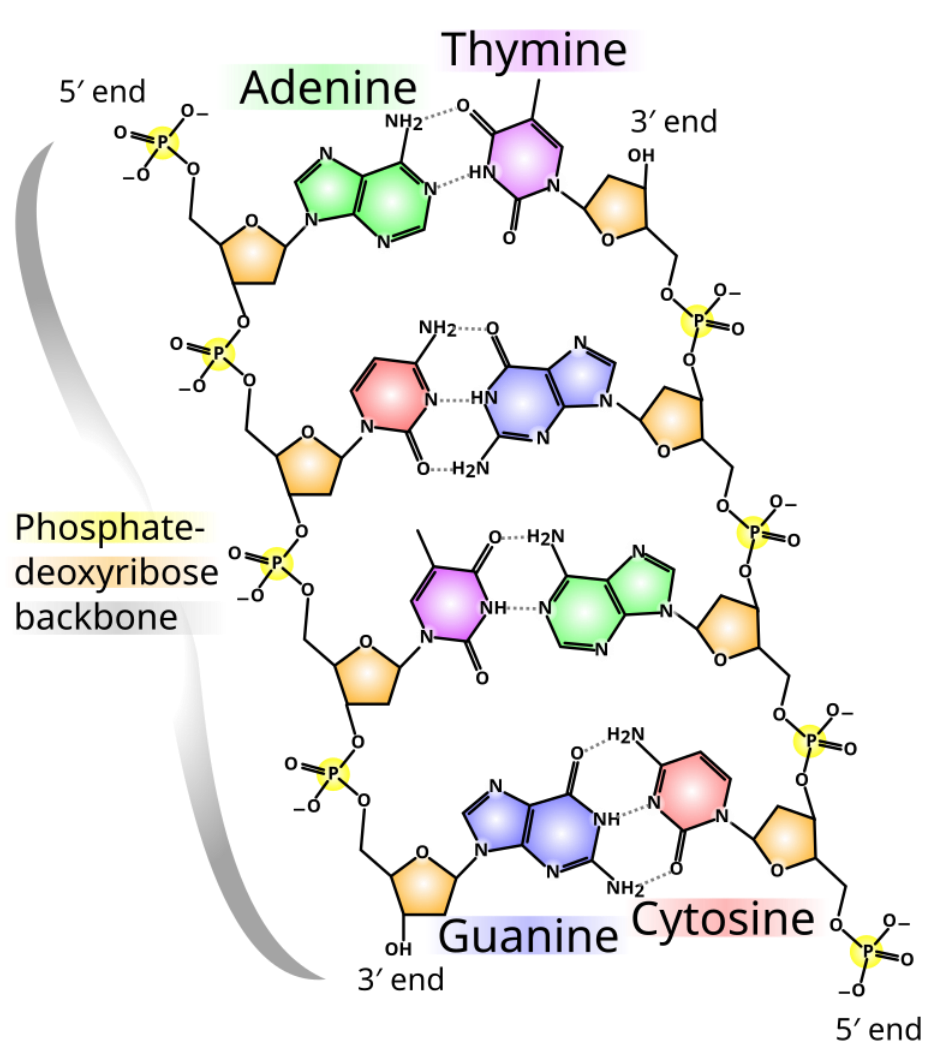
- Chemical Basics
- Sanger Sequencing Method
- Real-Time DNA Sequencing from Single Polymerase Molecules
- Summary



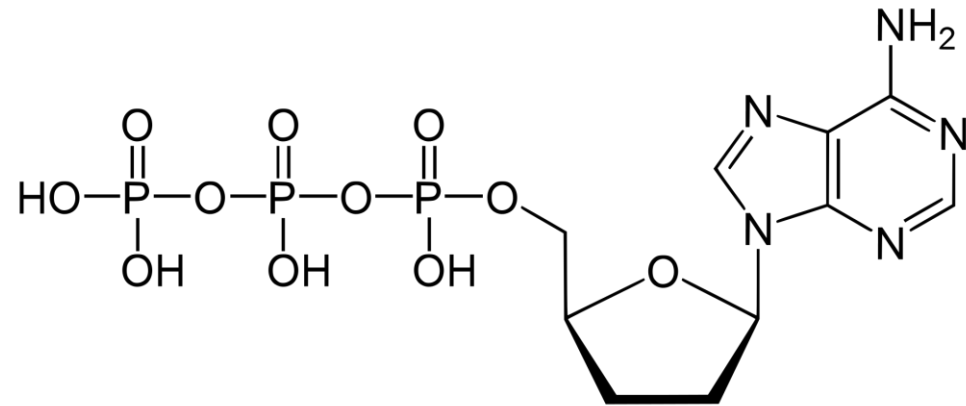
dATP



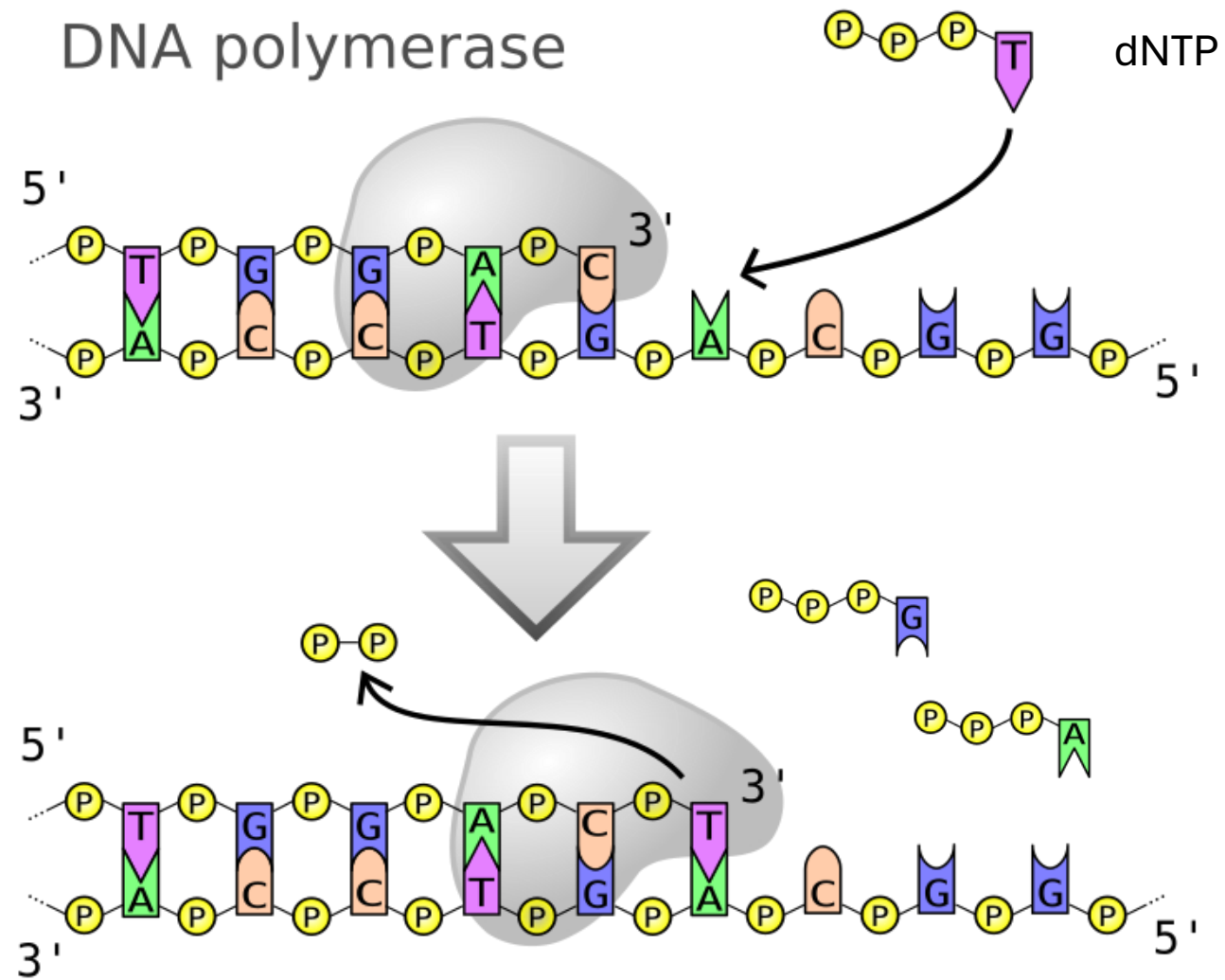
ddATP

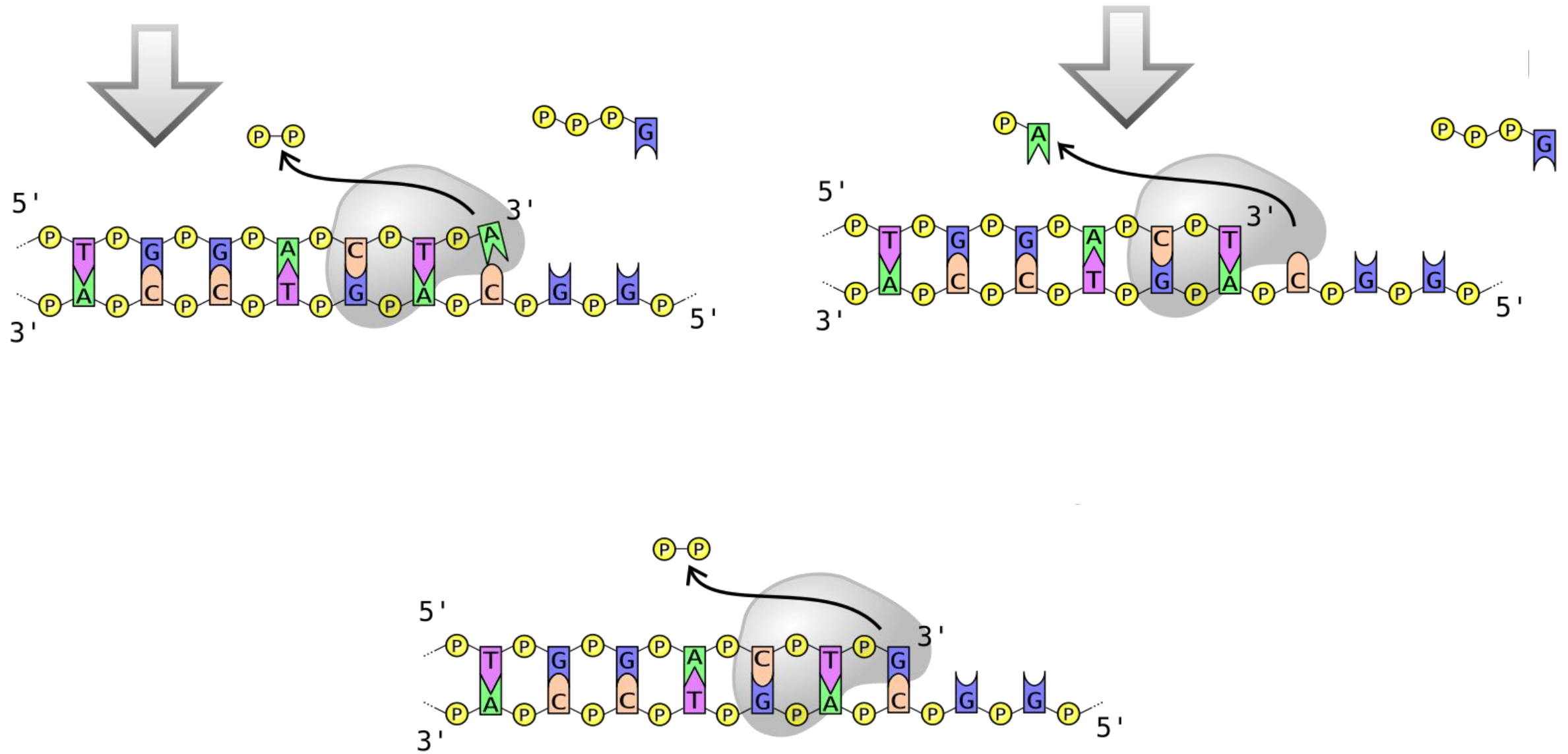


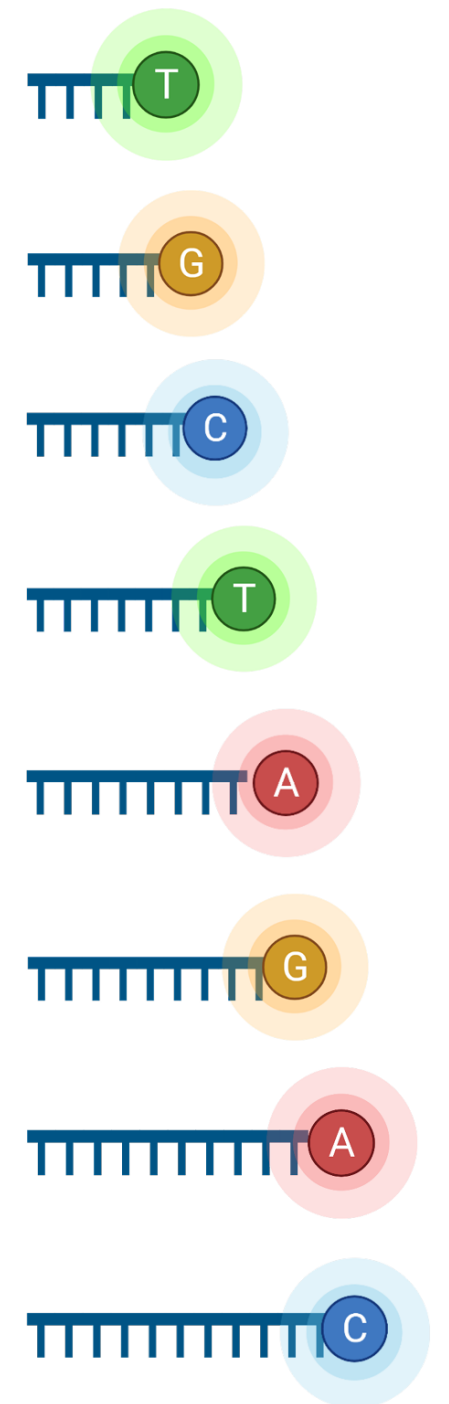
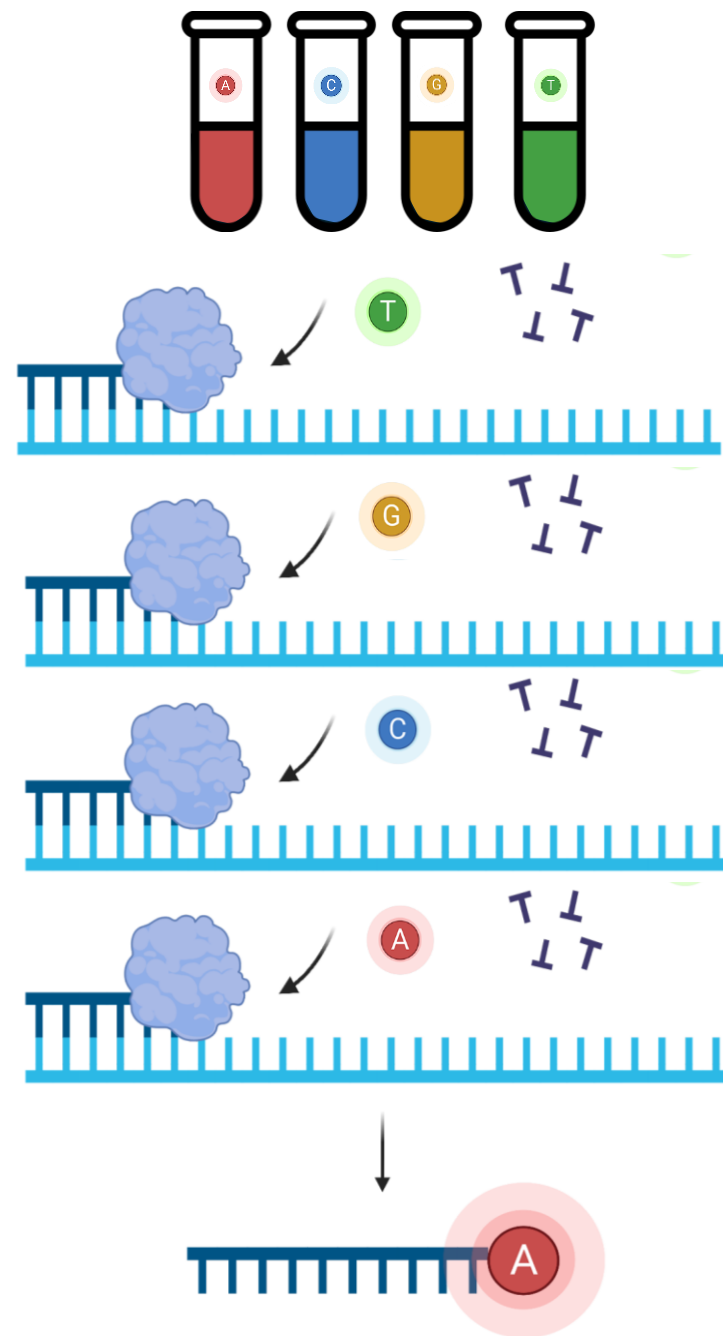
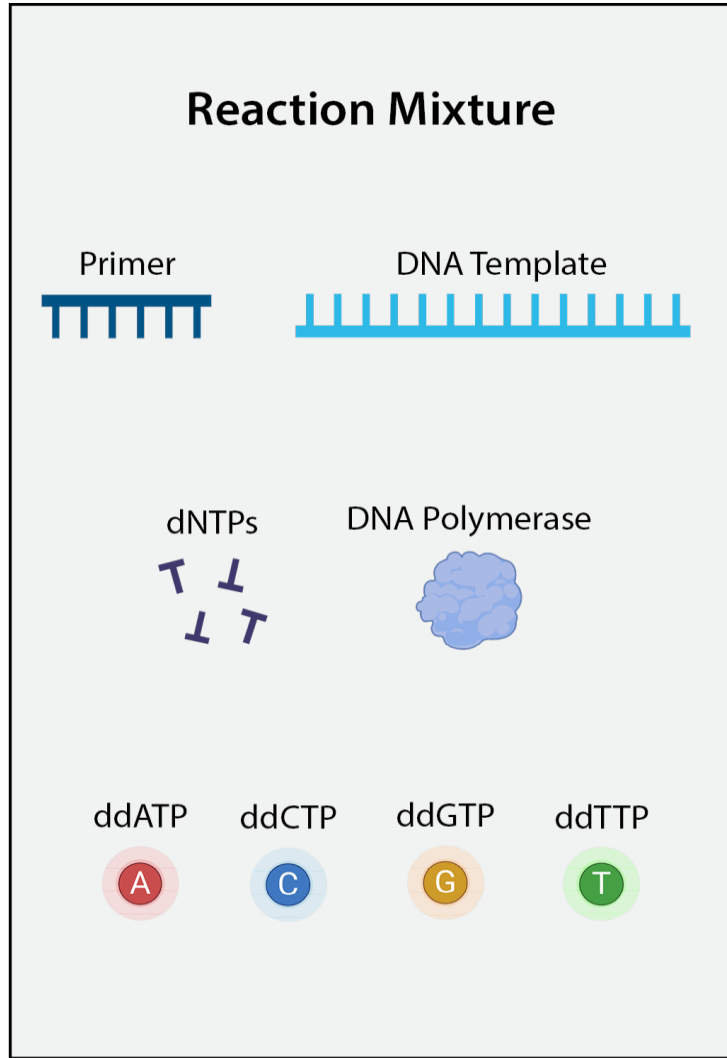
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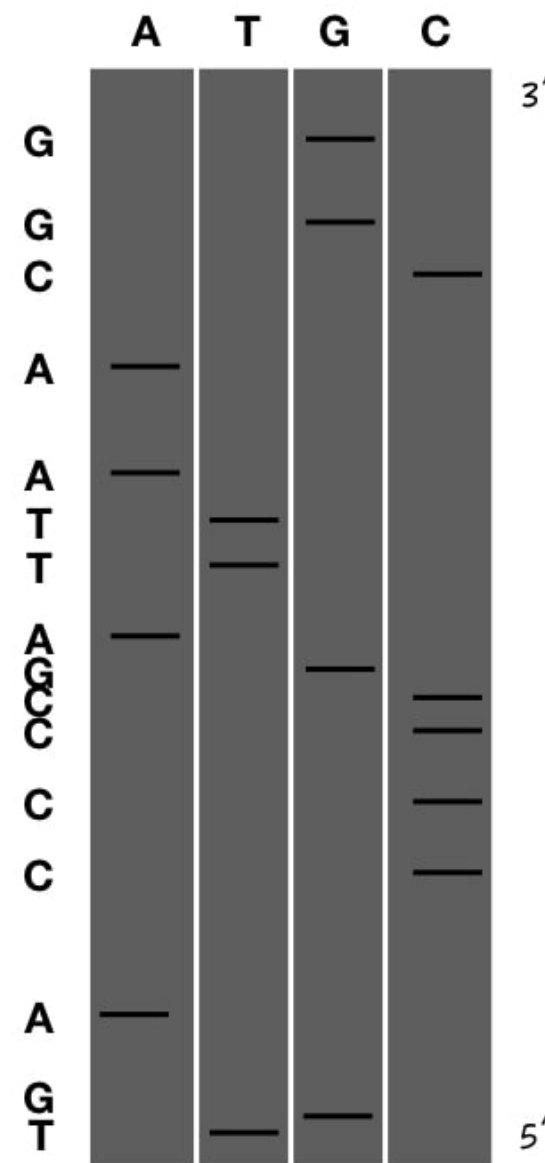
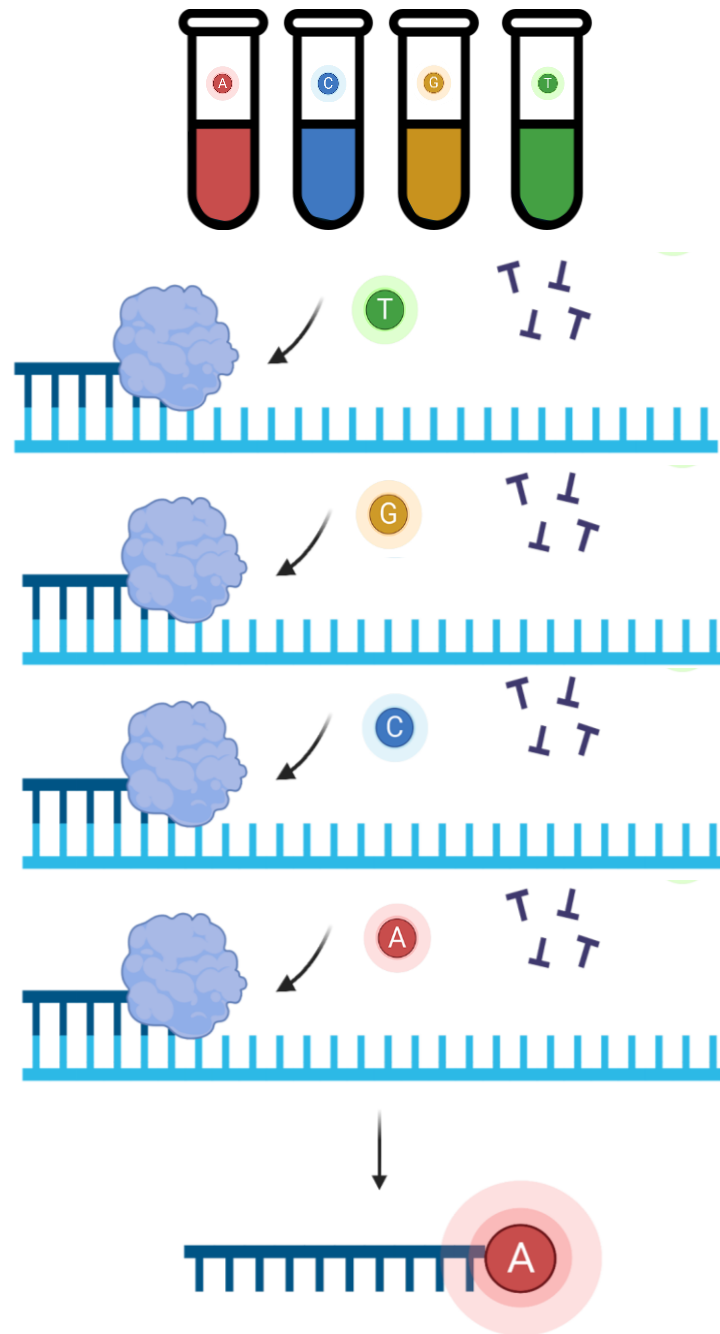
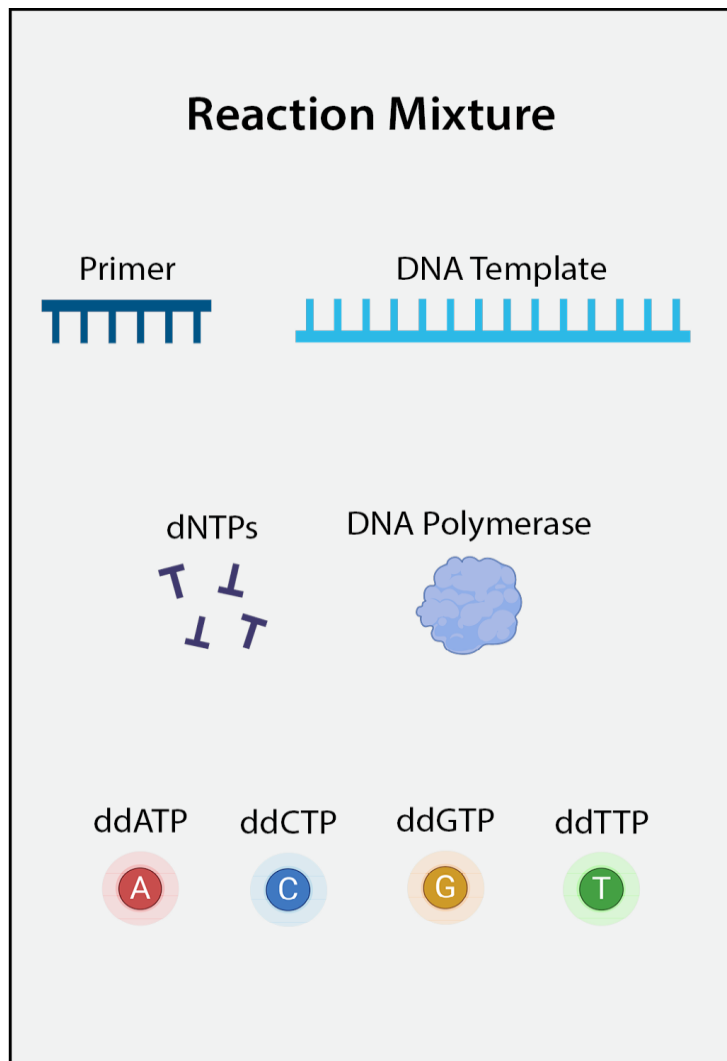
ddATP



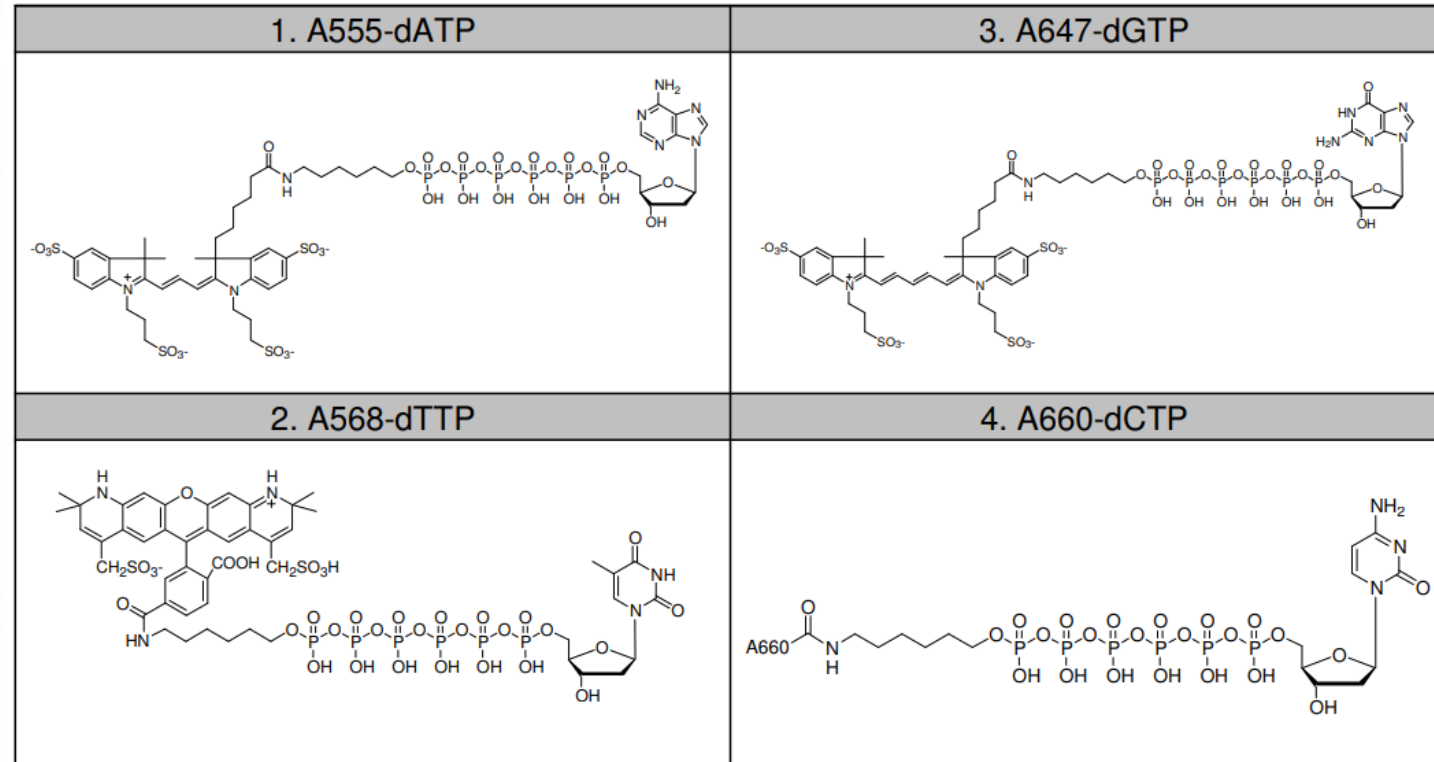
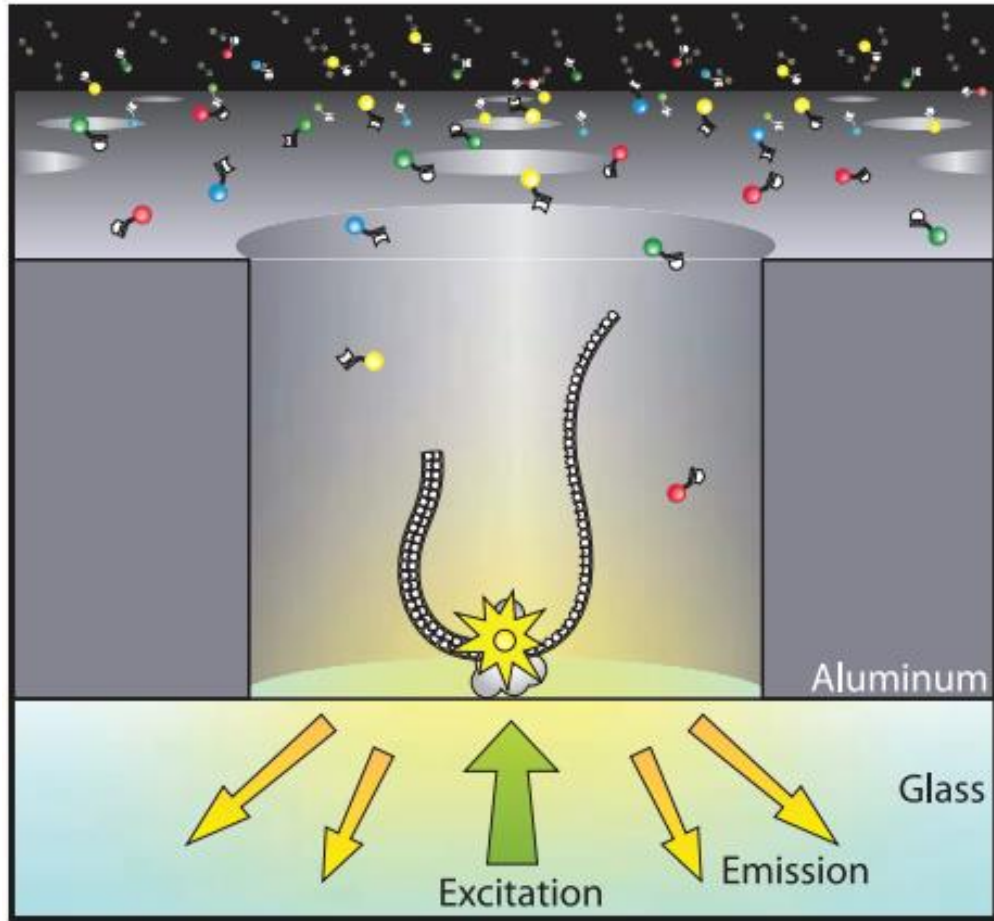


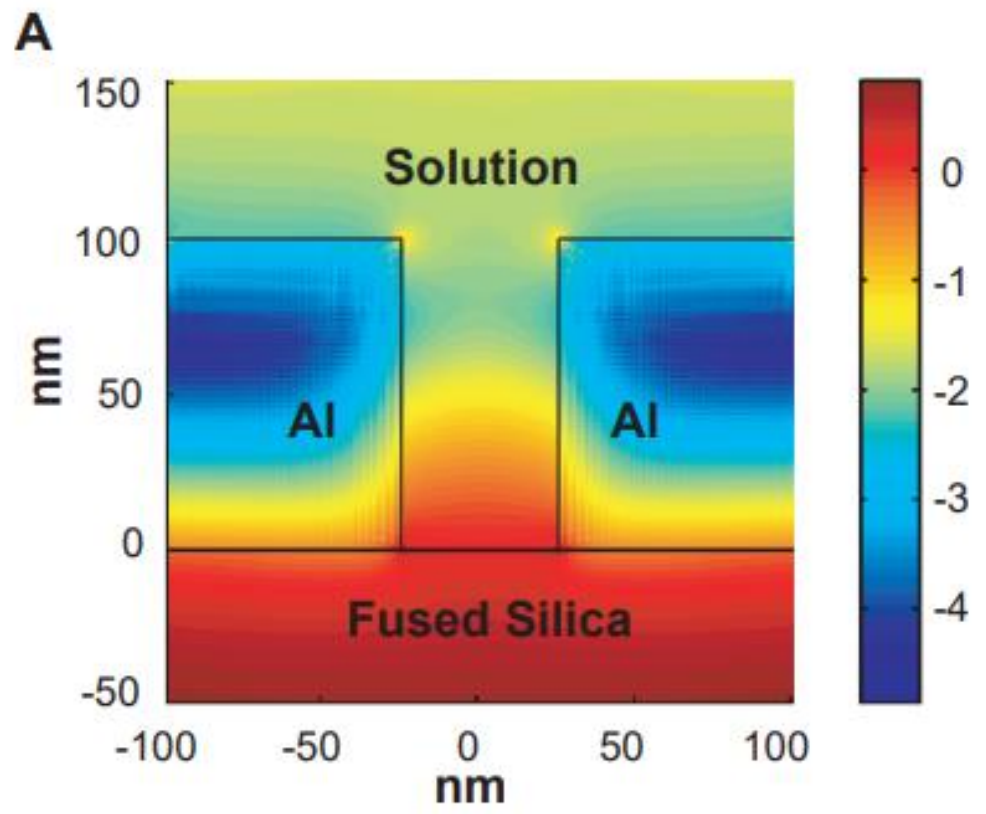
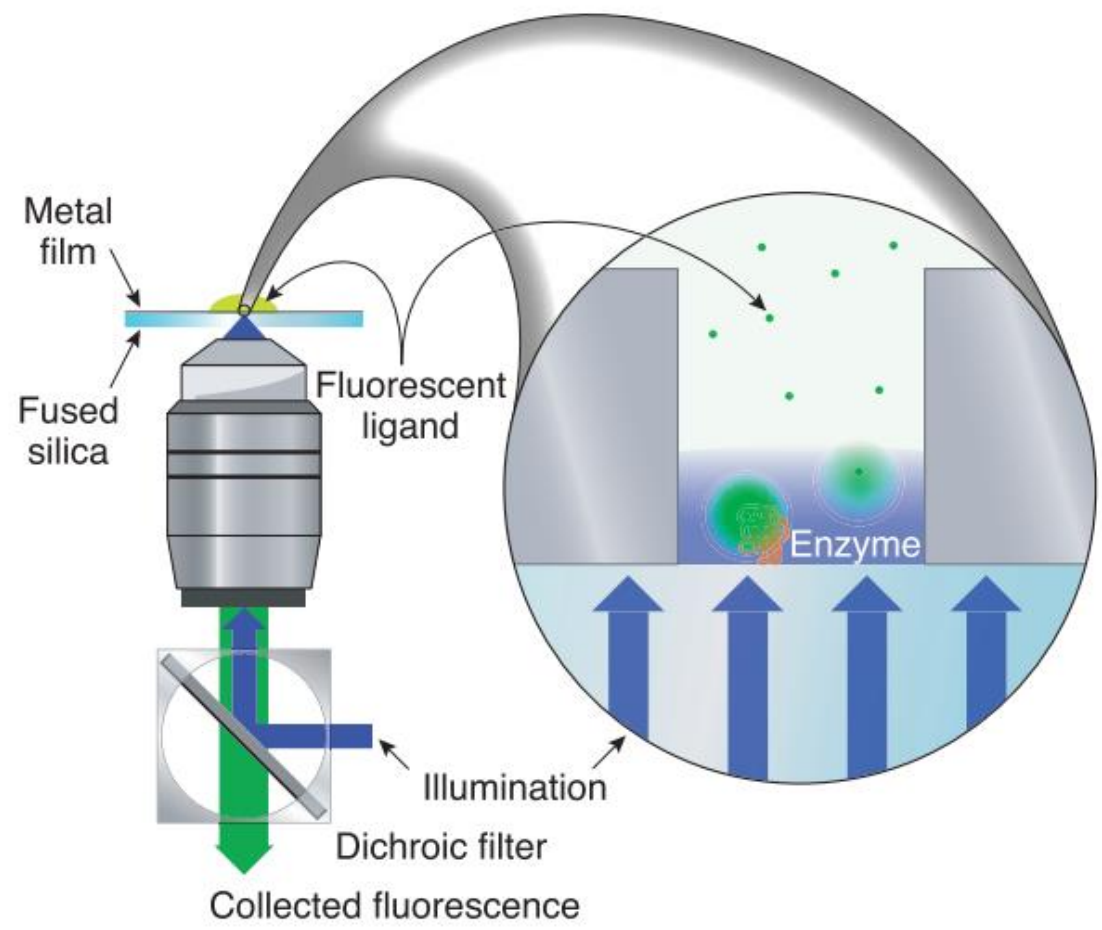


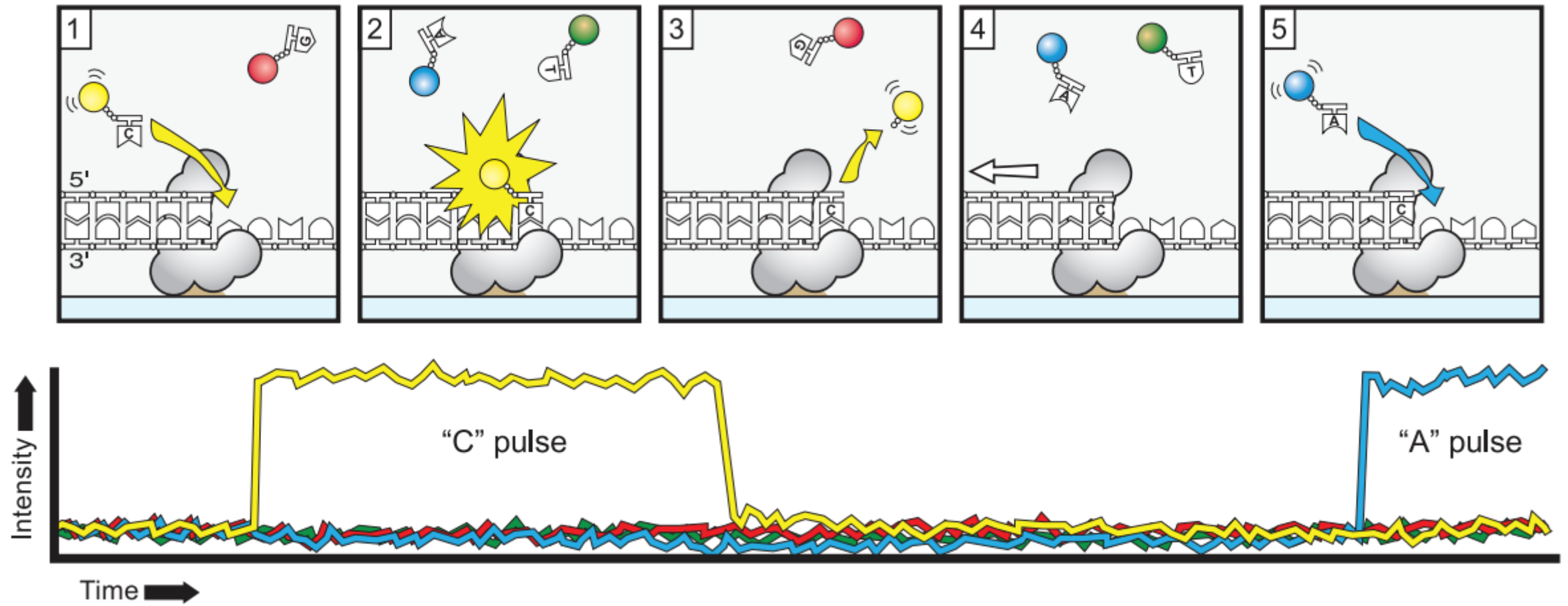
# Sanger Sequencing Method

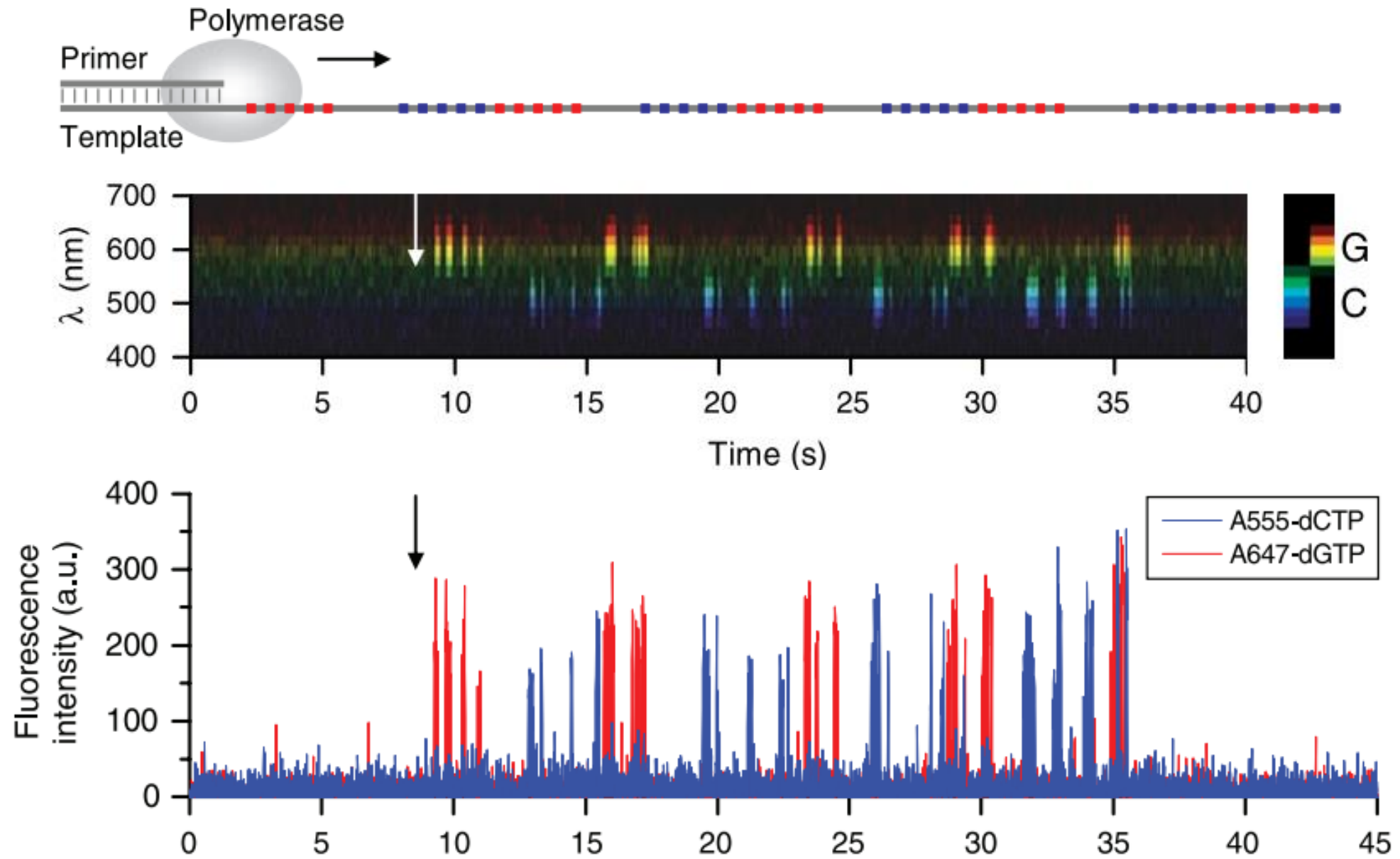


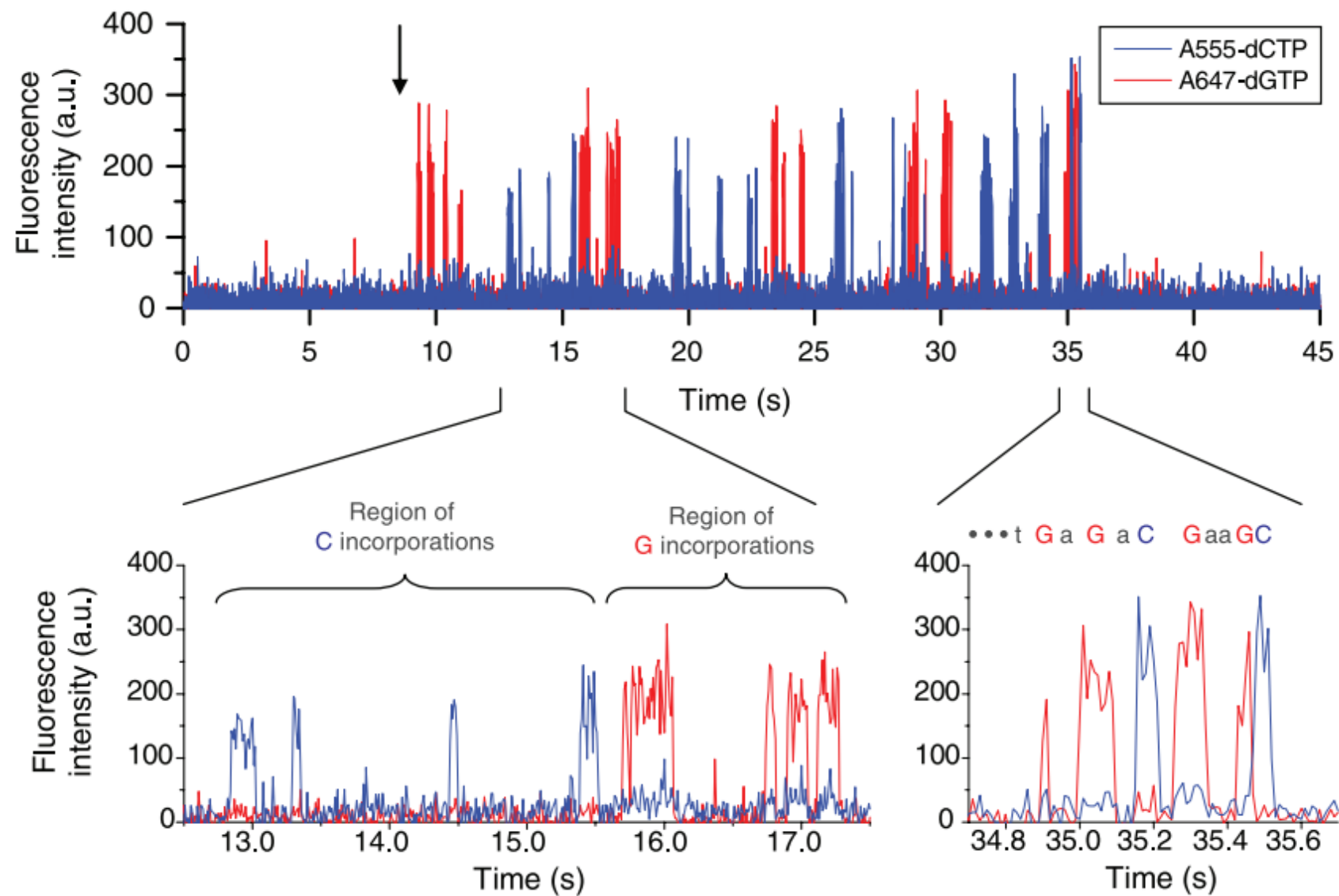








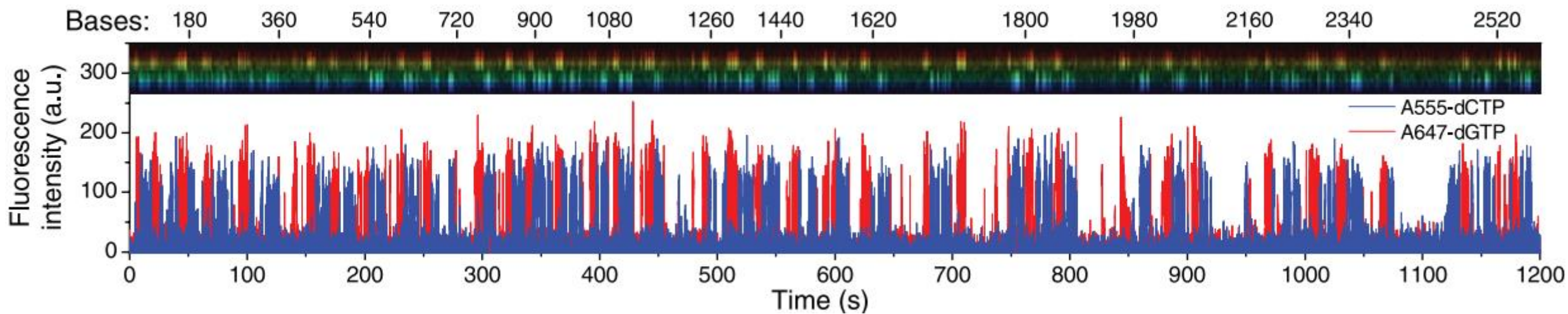
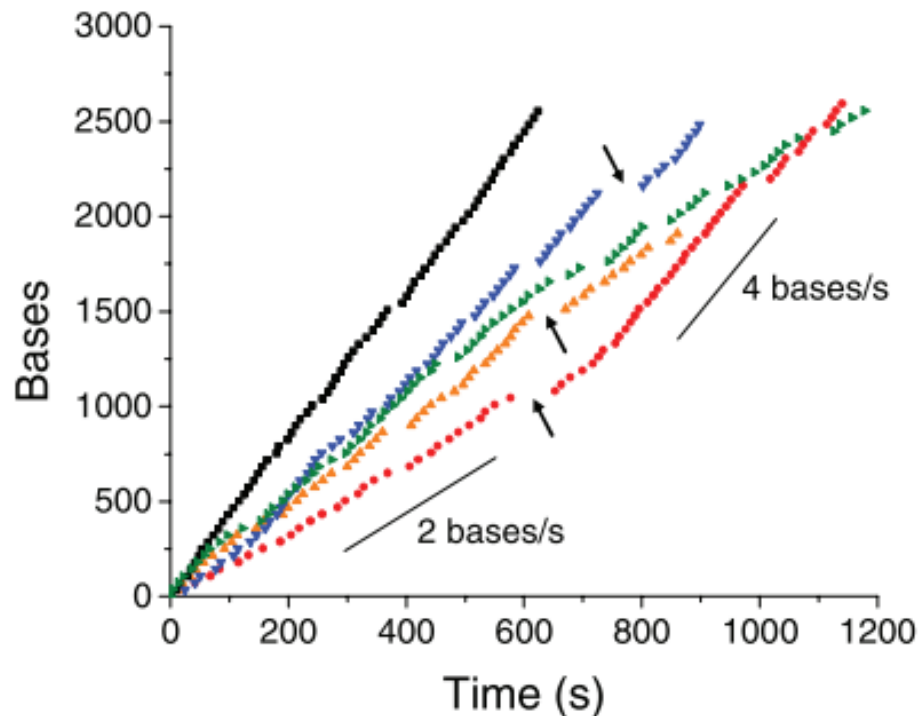
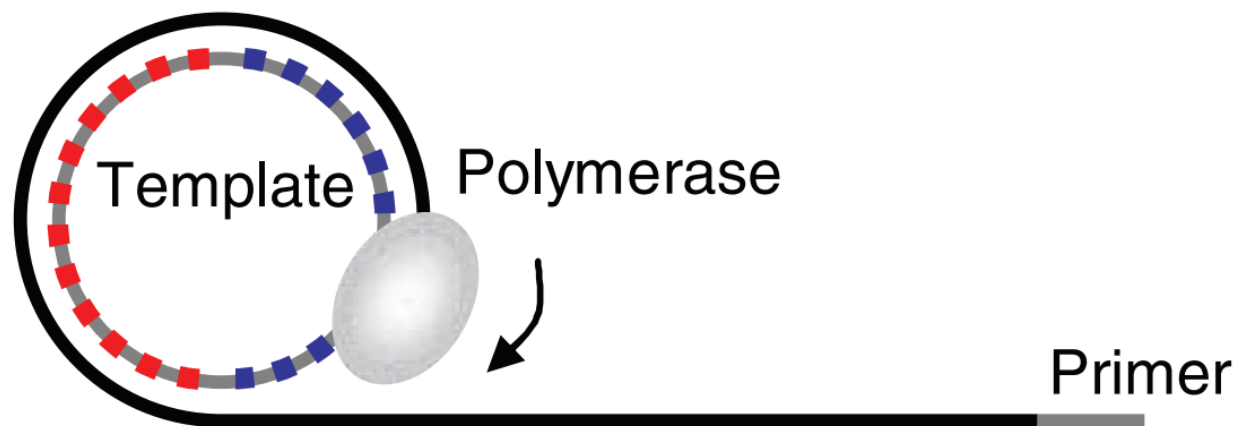




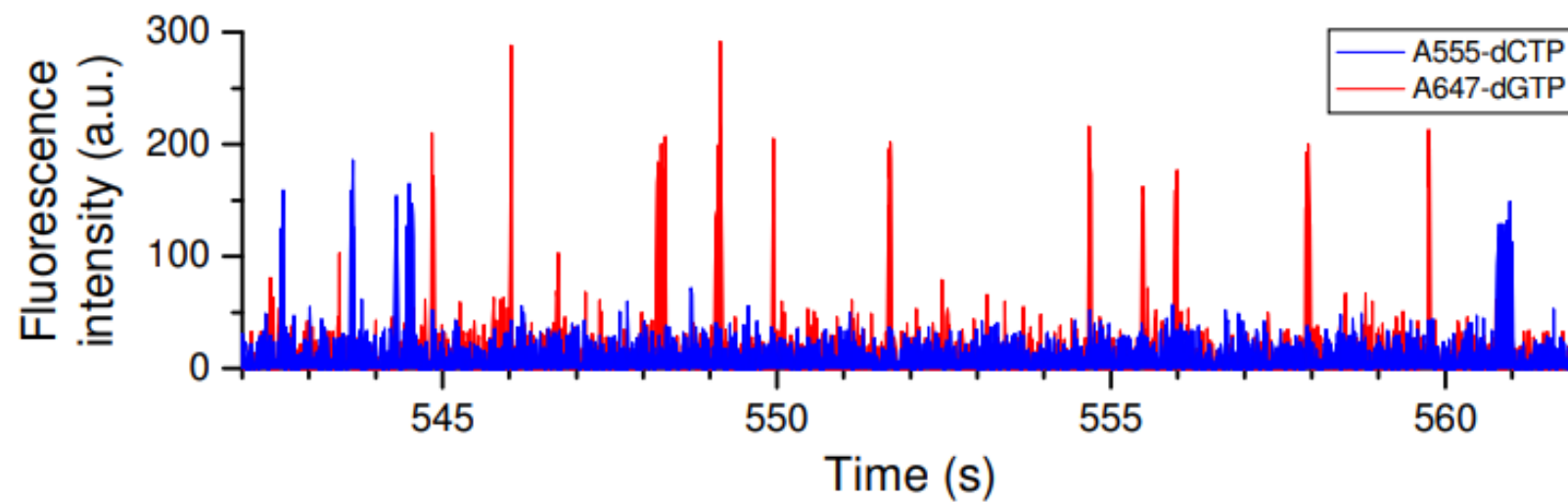
ZMW	A555-dCTP			A647-dGTP			Errors			Synthesis rate (bases/s)
	PW (ms)	Brightness (photons/s)	SNR	PW (ms)	Brightness (photons/s)	SNR	Mismatches	Insertions	Deletions	
1	60	4457	16	80	4274	14	2	2	1	2.9
2	60	8016	26	40	13361	30	2	3	0	4.7
3	65	8435	24	50	4937	14	1	2	2	4.5
4	80	9091	28	40	5678	12	2	3	0	2.5
5	65	6014	21	50	4176	12	1	3	0	3.4
6	35	9167	22	50	6042	17	0	1	3	5.1
7	55	4073	13	50	11258	24	3	2	0	4.0
8	50	6068	16	55	5445	16	2	2	2	3.0
9	40	7349	19	60	5728	17	0	0	4	5.7
10	90	7331	26	45	8512	21	2	1	0	5.5
<b>All (n=740)</b>	$77 \pm 30$	$7353 \pm 2970$	$24 \pm 10$	$70 \pm 27$	$8408 \pm 3381$	$25 \pm 10$	$0.6 \pm 0.5$	$1.3 \pm 0.8$	$2.1 \pm 1.0$	$4.7 \pm 1.7$



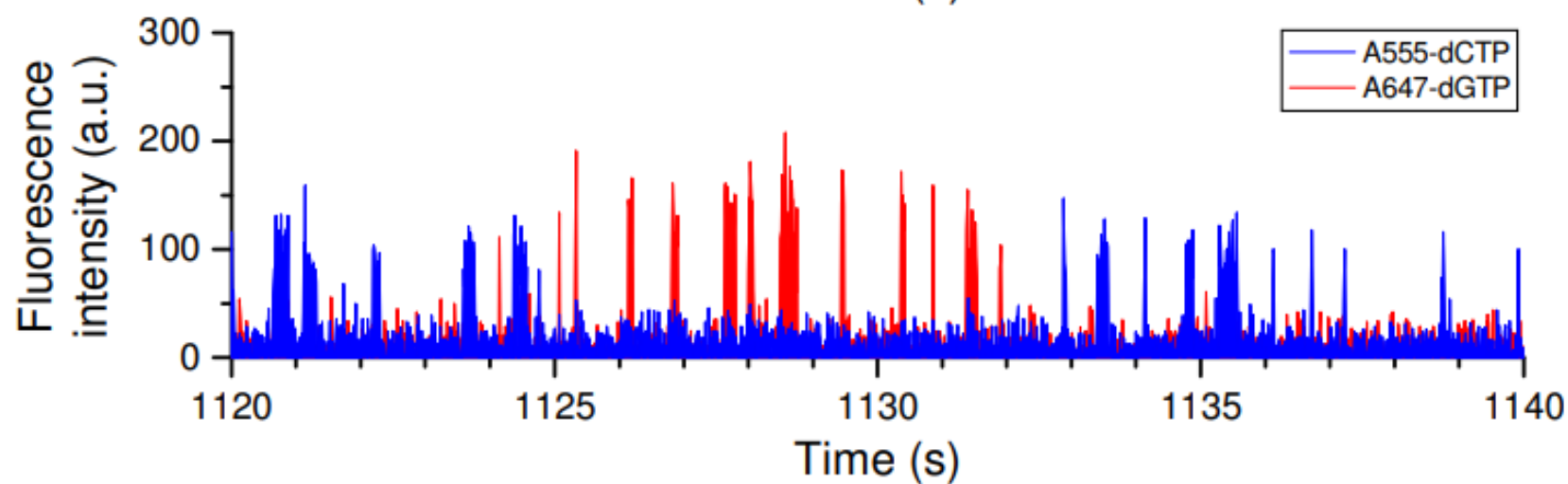
# Real-Time DNA Sequencing from Single Polymerase Molecules



~2 bases/s:

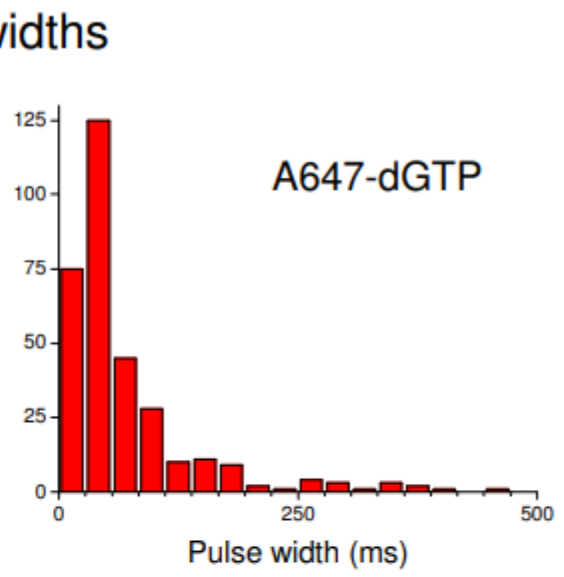
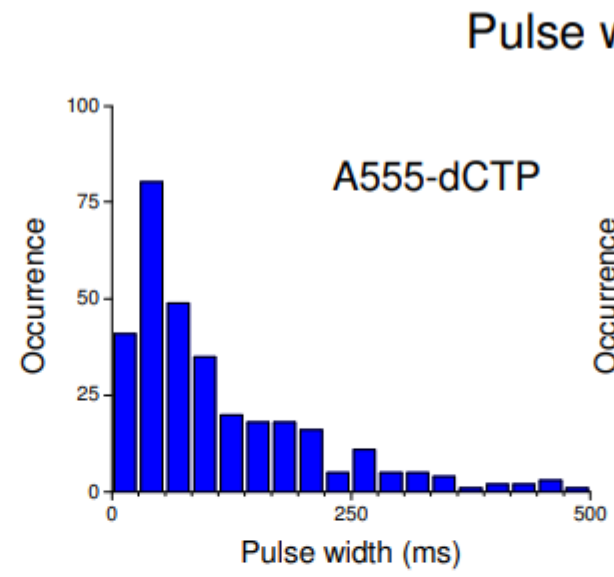
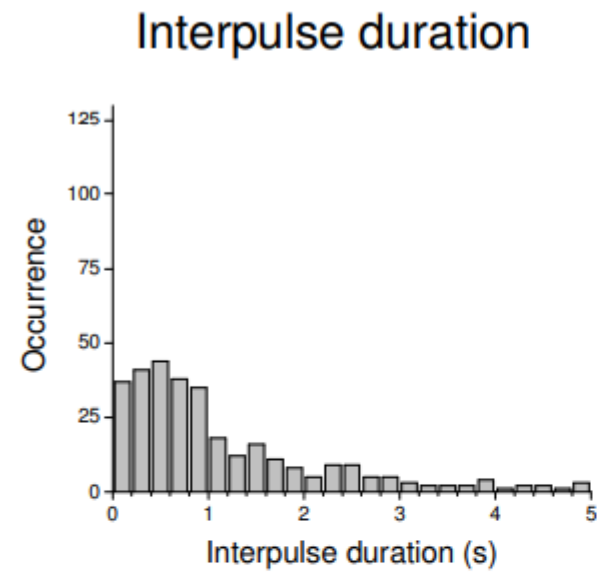


~4 bases/s:

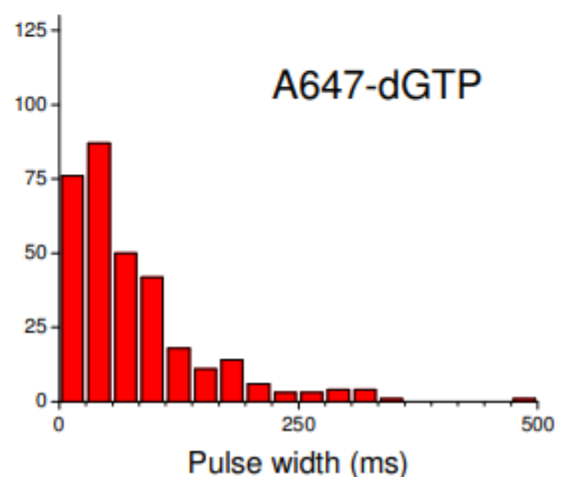
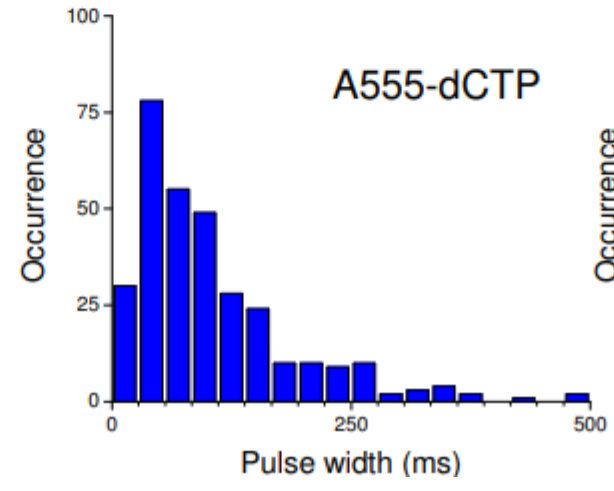
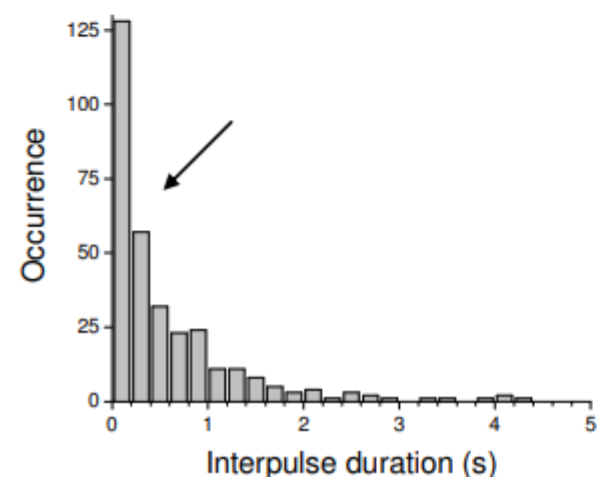




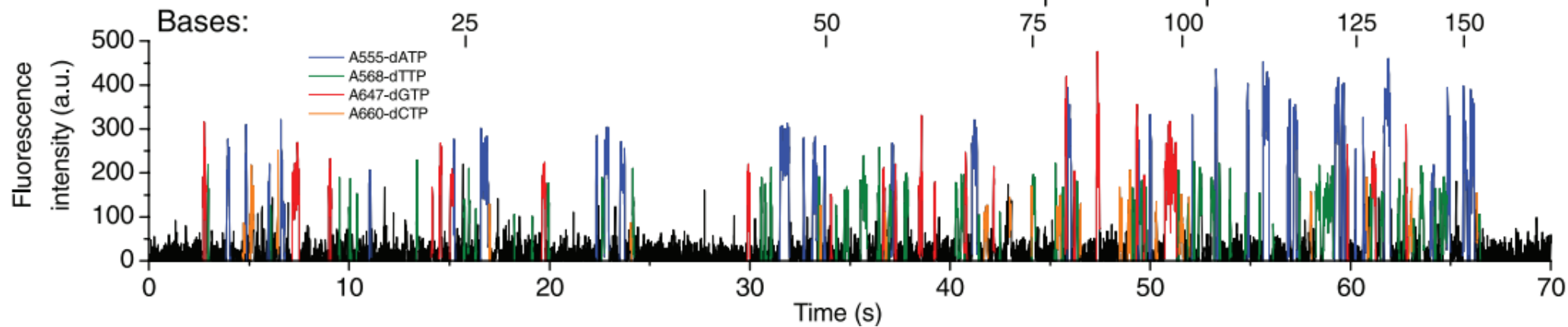
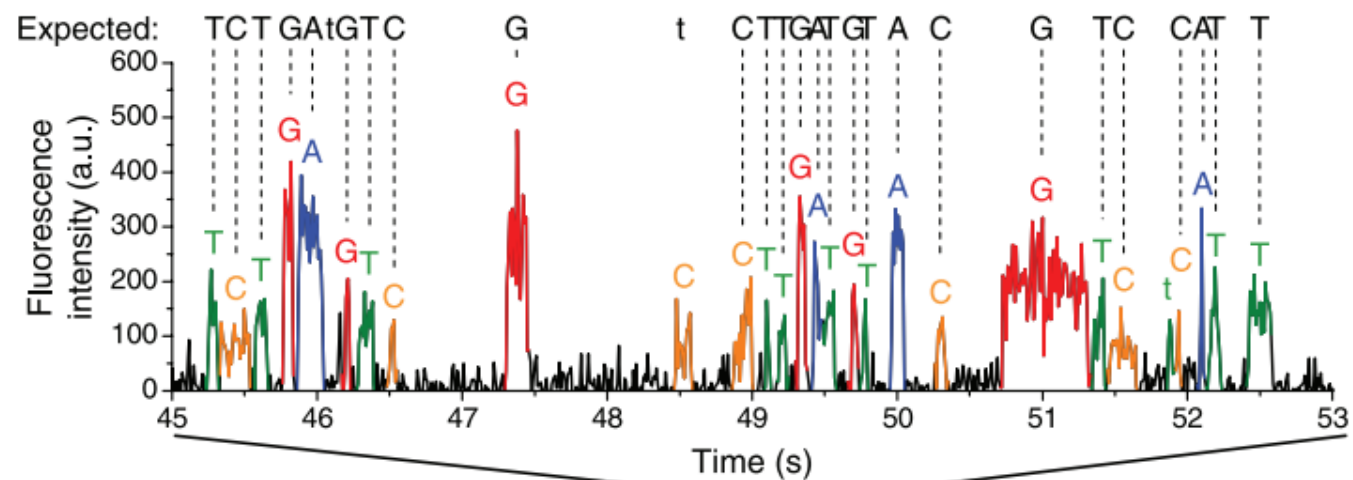
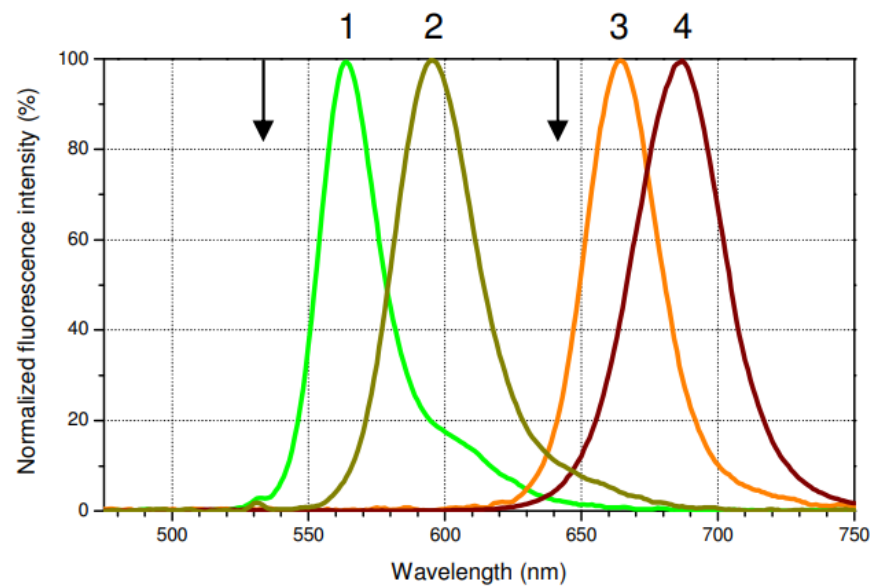
DNA  
synthesis  
rate  
  
~2 bases/s



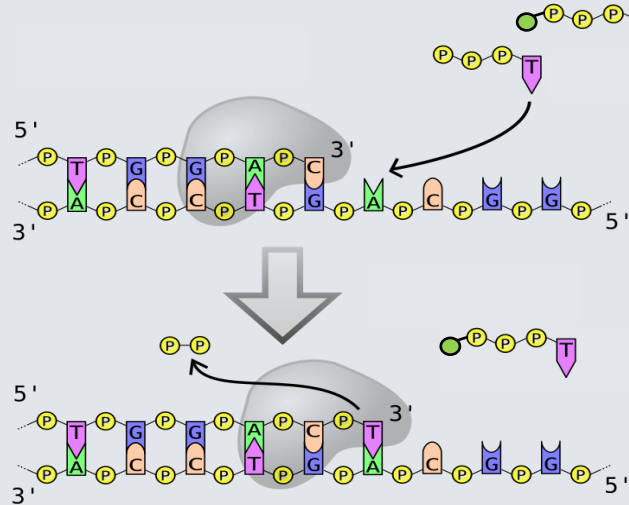
~4 bases/s



# Real-Time DNA Sequencing from Single Polymerase Molecules

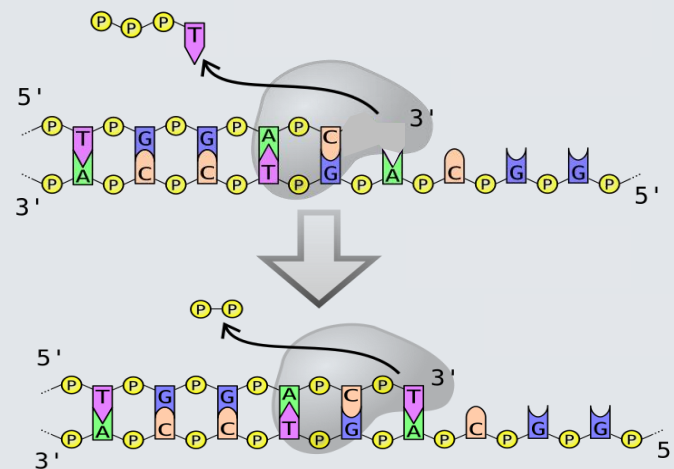


## Deletion



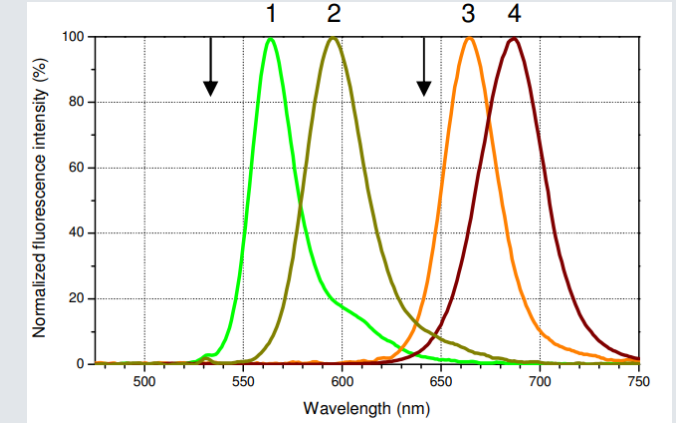
- Increasing fluorophore brightness
- Improving efficiency of light collection

## Insertion



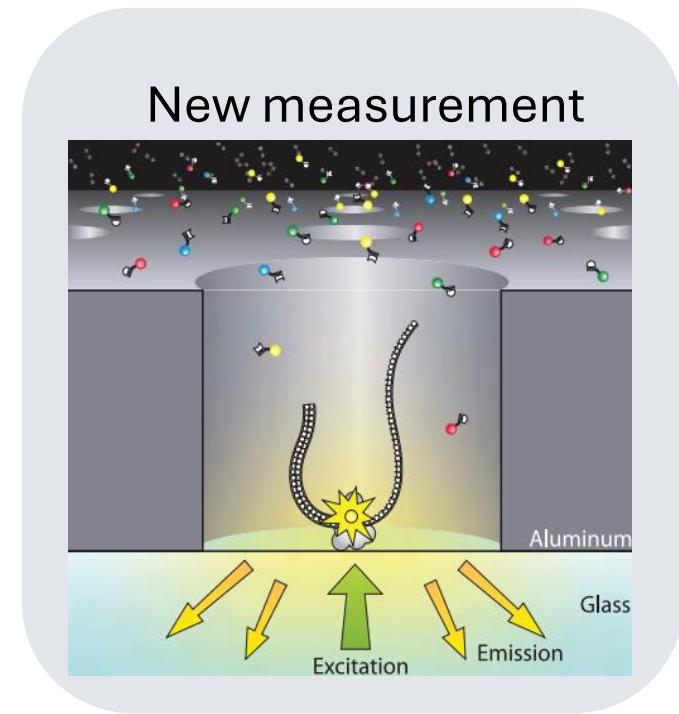
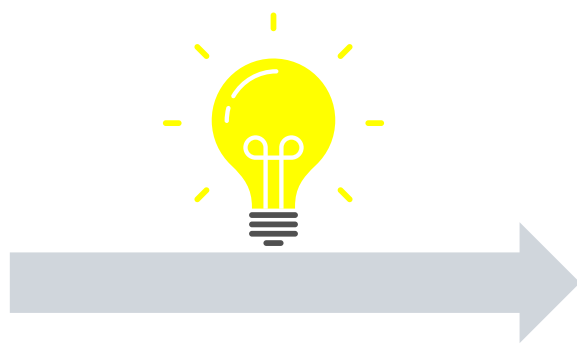
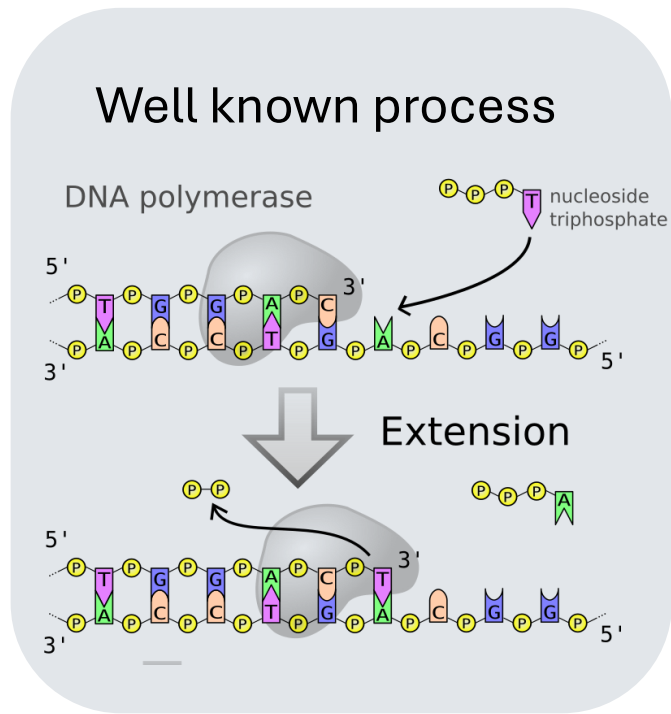
- Modifying the enzyme

## Mismatch



- Finding dye sets with larger spectral separations

99,3% median accuracy



Meaningful research does not always have to be complicated!