

## **Supporting Information**

### **Natural and Modified Oligonucleotide Sequences Show Distinct Strand Displacement Kinetics and These Are Affected Further by Molecular Crowders**

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## 1. Kinetic plots of Cy5 fluorescence emission in PBS and PBS containing crowders

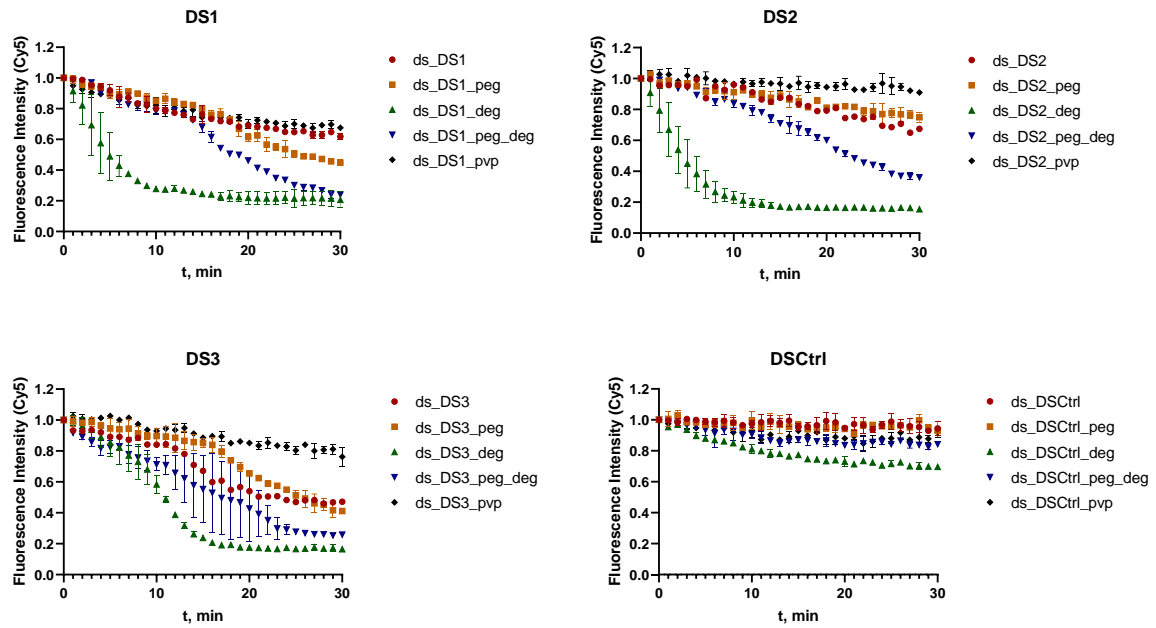


Figure S1. Fluorescence intensity Cy5 kinetic curves for duplex (Bmut-CoBmut) displaced with DS1, DS2, DS3, and DSCtrl in pure PBS buffer and buffers complexed with polyethylene glycol (peg), diethylene glycol dimethyl ether (deg), mixed peg-deg and polyvinylpyrrolidone (pvp) over time of minimum of 30 min at a temperature of 37 °C, as indicated. The displacement strands DS1, DS2, DS3, and DSCtrl are represented in Table 1. Error bars indicate  $\pm$  SD (n = 2)

## 2. Kinetic plots of Cy5 fluorescence emission in higher salt concentration PBS buffers

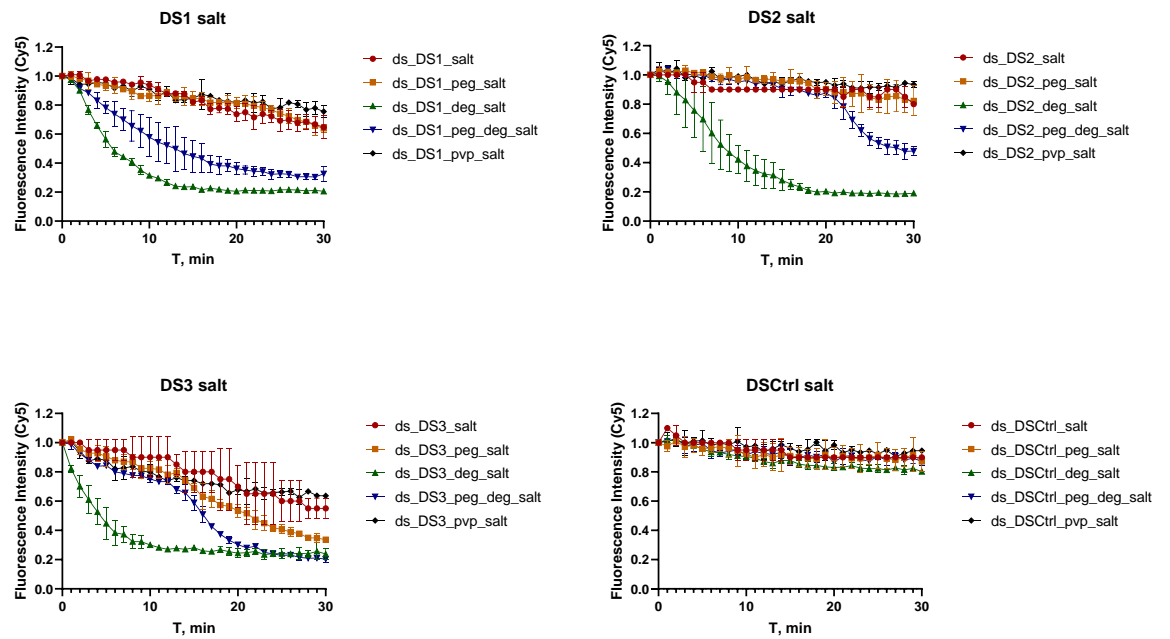


Figure S2. Fluorescence intensity Cy5 kinetic plots for duplex-ds (Bmut-CoBmut) with DS1, DS2, DS3, DSCtrl in all buffers (peg, deg, peg\_deg, pvp) with higher salt concentration (additional 150 mM NaCl) as indicated. Error bars indicate  $\pm$  SD (n = 2).

### 3. Strand exchange rate constants in higher salt concentration PBS buffers

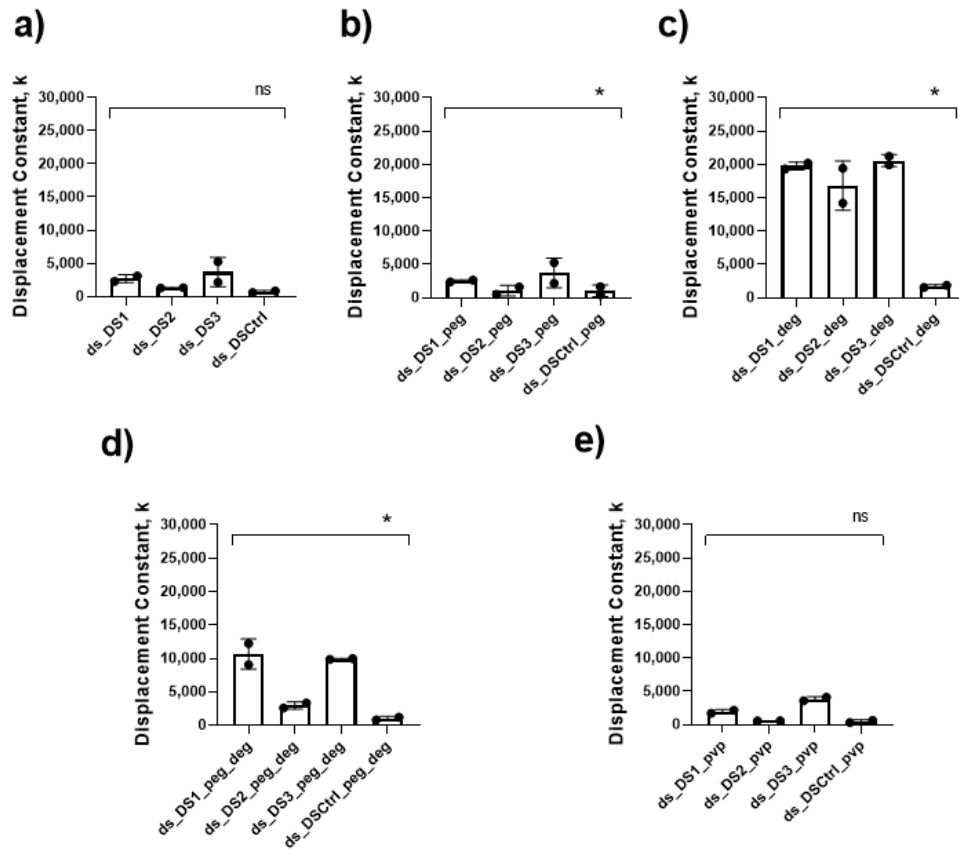


Figure S3. Displacement constant  $k$ , (L·min<sup>-1</sup>·mol<sup>-1</sup>) for duplex (Bmut-CoBmut) displaced with DS1, DS2, DS3, and DSCTRL in (a) PBS buffer with higher salt concentration ( addition of 150 mM NaCl) or PBS buffers with higher salt concentration enriched with (b) polyethylene glycol (peg), (c) diethylene glycol dimethyl ether (deg), (d) mixed peg-deg and (e) polyvinylpyrrolidone (pvp) as indicated. The displacement strands DS1, DS2, DS3 and DSCTRL are represented in Table 1. Error bars indicate  $\pm$  SD (n = 2). P values were calculated using one-way ANOVA. Abbreviations: “\*” indicates 95% confidence; “ns” indicates statistically not significant.

#### 4. Viscosity normalized strand exchange rate constants in PBS buffers

**Table S1.** Measured viscosity [Pa\*s] of the various PBS buffers

	<b>pbs</b>	<b>peg</b>	<b>deg</b>	<b>peg_deg</b>	<b>pvp</b>
Unit	[Pa*s]				
Measurement s	0.000806	0.005668	0.000932	0.004836	0.004251
	0.000839	0.005635	0.000999	0.004710	0.004113
	0.000693	0.005698	0.000911	0.004782	0.004264
	0.000660	0.005704	0.000901	0.004782	0.004242
	0.000678	0.005658	0.000882	0.004832	0.004328
	0.001032	0.005833	0.001307	0.005242	0.004751
	0.000809	0.005753	0.001109	0.004835	0.004386
	0.000855	0.005908	0.001008	0.004978	0.004387
	0.000630	0.005213	0.001011	0.004930	0.004414
	0.000779	0.006015	0.00105	0.004696	0.005117
Average	0.000778	0.005709	0.001011	0.004862	0.004425

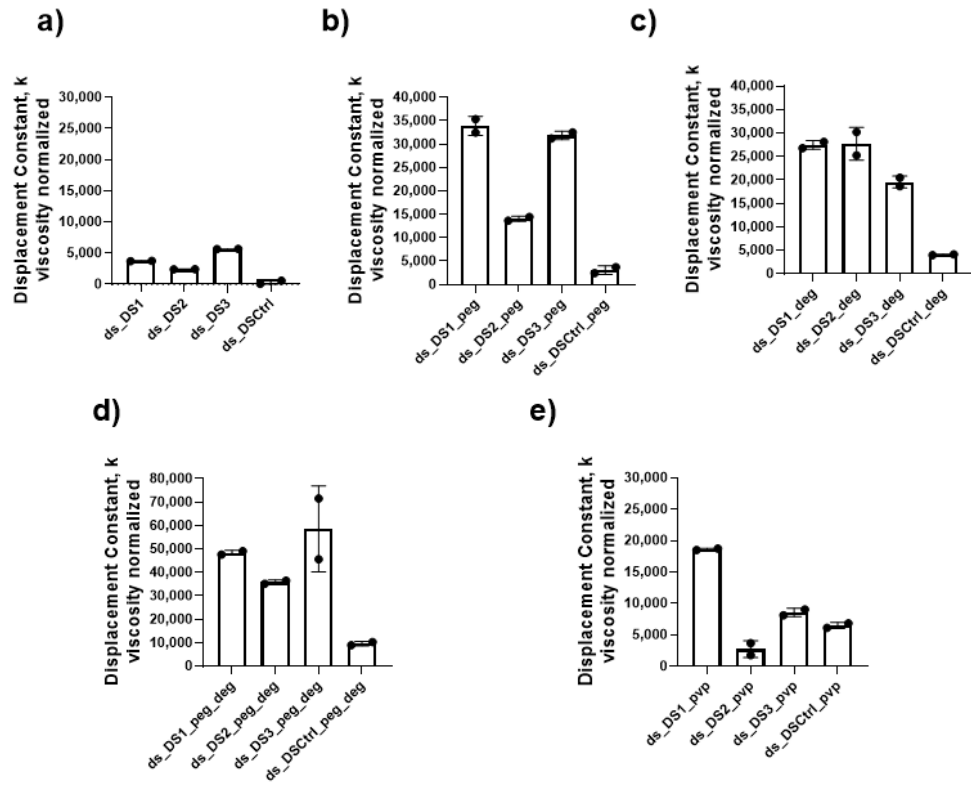
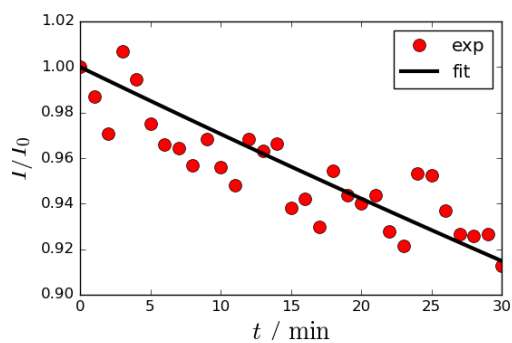


Figure S4. Normalized displacement constant  $k$ , ( $L \cdot \text{min}^{-1} \cdot \text{mol}^{-1}$ ) with the measured viscosity (Table S1) for duplex (Bmut-CoBmut) displaced with DS1, DS2, DS3, and DSCtrl in (a) PBS buffer or PBS buffers enriched with (b) polyethylene glycol (peg), (c) diethylene glycol dimethyl ether (deg), (d) mixed peg-deg and (e) polyvinylpyrrolidone (pvp) as indicated. The displacement strands DS1, DS2, DS3 and DSCtrl are represented in Table 1. Error bars indicate  $\pm$  SD ( $n = 2$ ).

## 5. Fit results

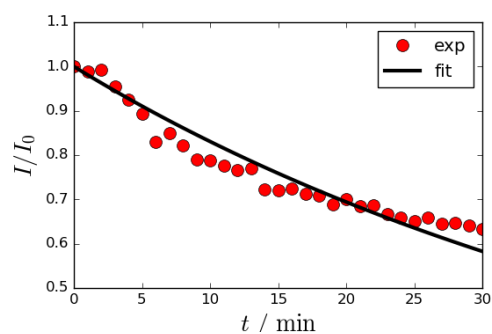
### Pure PBS buffer

ds\_DSctrl



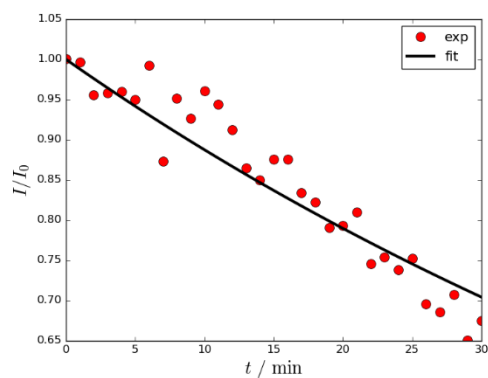
598	+/- 29 (4.9%)
95	+/- 46 (49.2%)

ds\_DS1



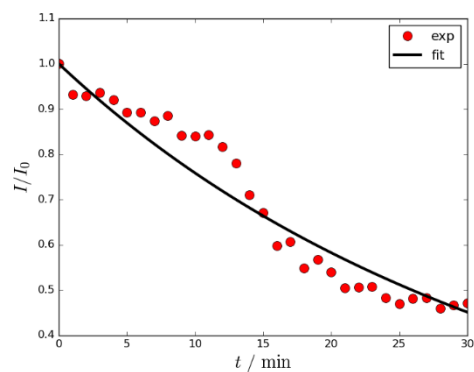
3773	+/- 105 (2.8%)
3715	+/- 75 (2.0%)

ds\_DS2



2429	+/- 96 (4.0%)
2412	+/- 97 (4.0%)

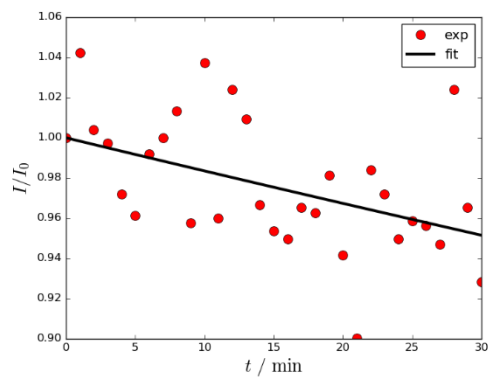
ds\_DS3



5660	+/- 202 (3.6%)
5679	+/- 202 (3.5%)

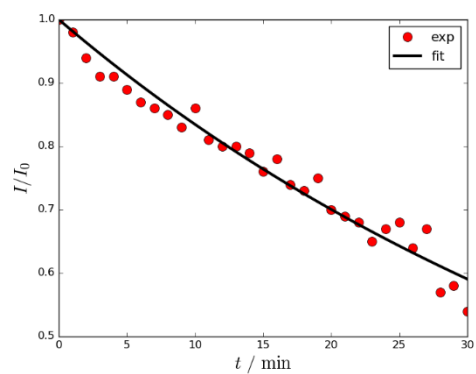
## Peg buffer

ds\_DSctrl\_peg



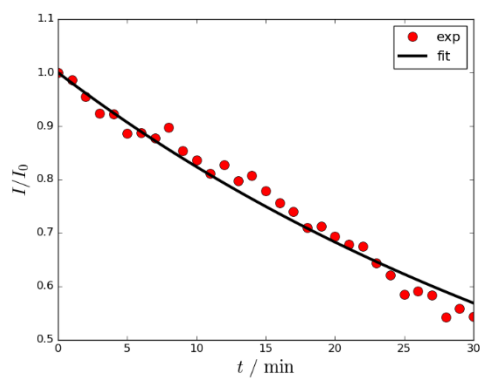
508	+/- 56 (11.1%)
332	+/- 60 (18.2%)

ds\_DS1\_peg



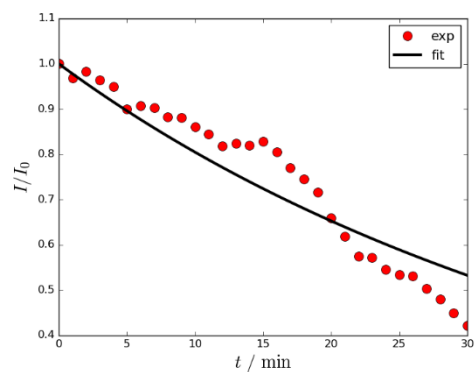
4818.501	+/- 213 (4.4%)
4424.28536	+/- 224 (5.1%)

ds\_DS2\_peg



1860	+/- 66 (3.6%)
1969	+/- 70 (3.6%)

ds\_DS3\_peg

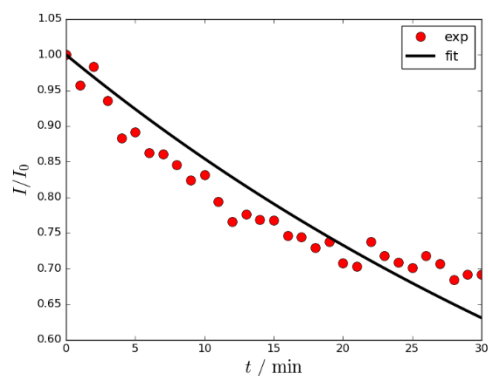


4430	+/- 211 (4.8%)
4254	+/- 380. (8.9%)



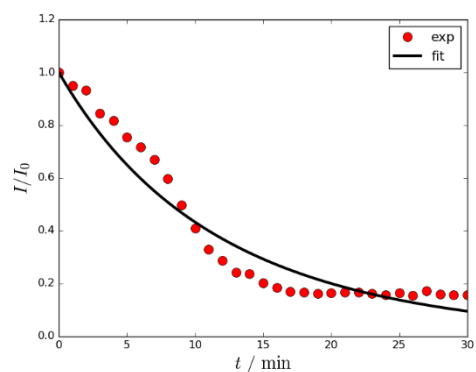
## Deg buffer

ds\_DSCTRL\_deg



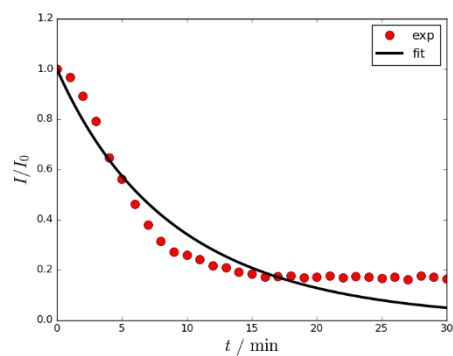
3066	+/- 114 (3.7%)
3198	+/- 110 (3.5%)

ds\_DS1\_deg



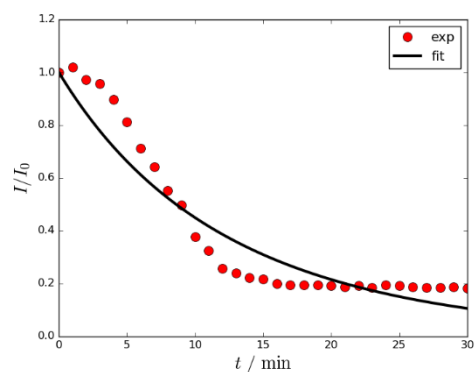
20616	+/- 1029 (5.0%)
21628	+/- 1857 (8.6%)

ds\_DS2\_deg



19427	+/- 1210 (6.2%)
23241	+/- 1191 (5.1%)

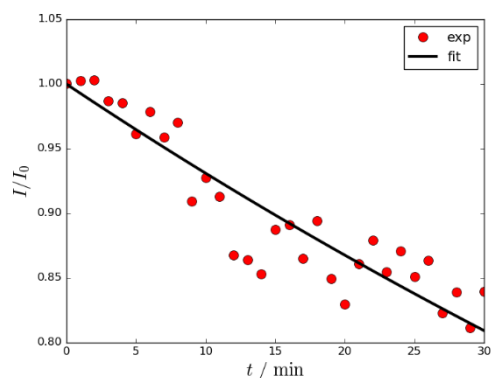
ds\_DS3\_deg



15764	+/- 720 (4.6%)
14385	+/- 1138 (7.9%)

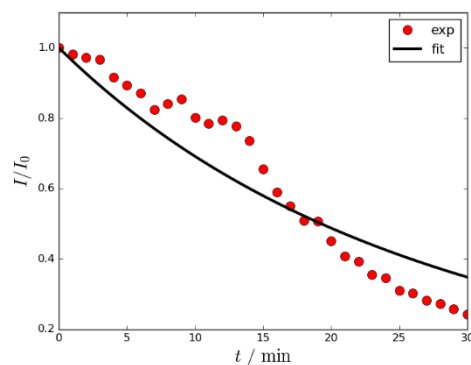
## Peg\_deg buffer

ds\_DSctrl\_peg\_deg



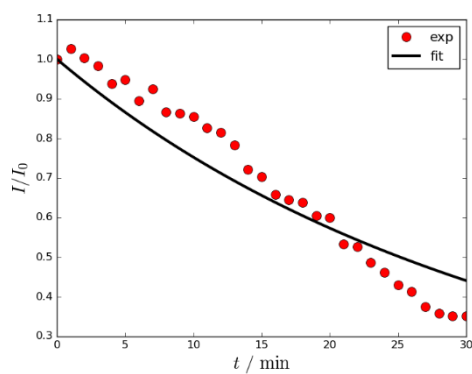
1439	+/- 58 (4.1%)
1644	+/- 103 (6.3%)

ds\_DS1\_peg\_deg



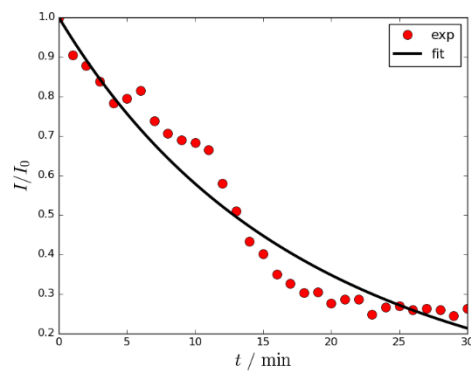
7622.631	+/- 434 (5.7%)
7857.06592	+/- 430 (5.5%)

ds\_DS2\_peg\_deg



5837	+/- 290 (5.0%)
5620	+/- 260 (4.6%)

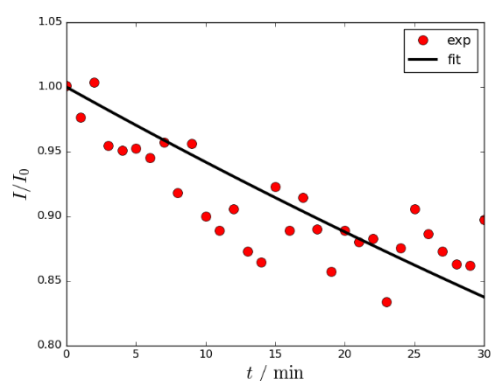
ds\_DS3\_peg\_deg



11447	+/- 391 (3.4%)
7304	+/- 438 (6.0%)

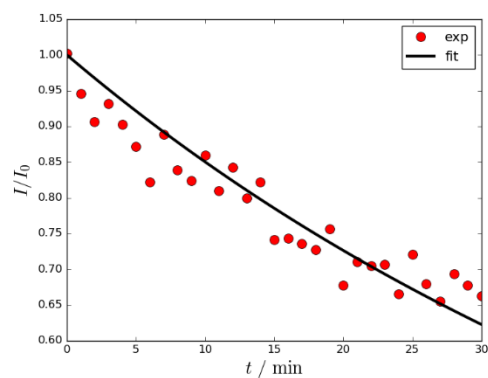
## Pvp buffer

ds\_DSctrl\_pvp



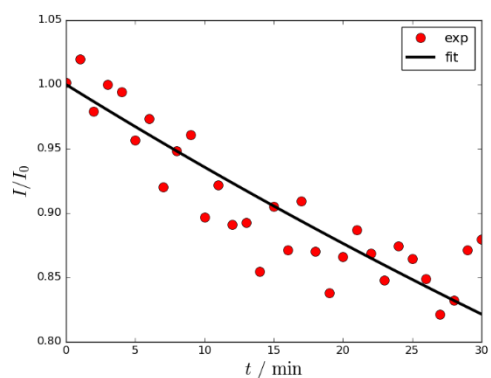
1076	+/- 102 (9.5%)
1202	+/- 70 (5.8%)

ds\_DS1\_pvp



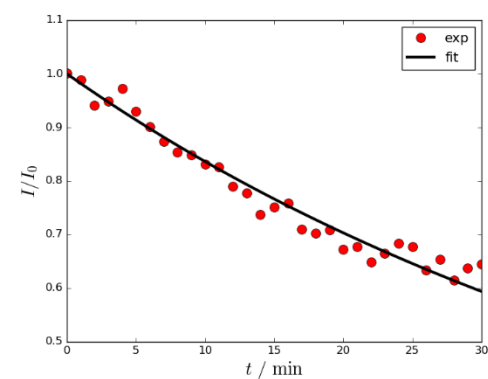
3292	+/- 110 (3.3%)
3252	+/- 133 (4.1%)

ds\_DS2\_pvp



643	+/- 19 (3.0%)
311	+/- 65 (20.9%)

ds\_DS3\_pvp

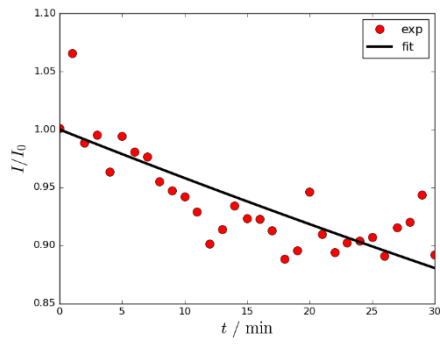


1590	+/- 89 (5.7%)
1425	+/- 69 (4.9%)

## 6. Fit results in higher salt

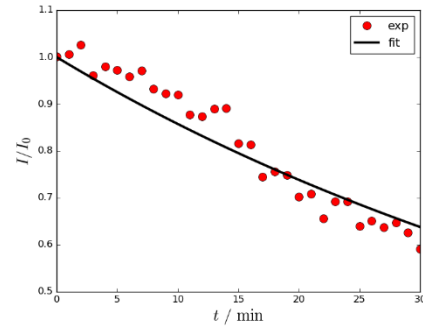
### Pure PBS\_higher salt

ds\_DSCTRL\_salt



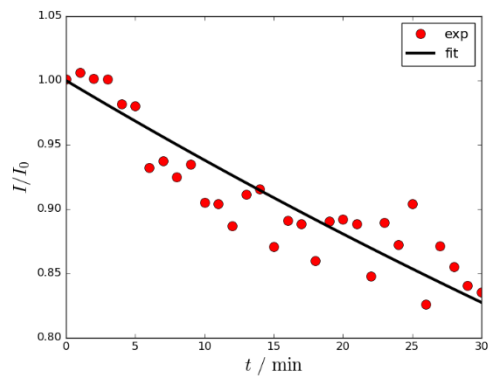
622	+/- 68 (10.9%)
8571	+/- 59 (6.9%)

ds\_DS1\_salt



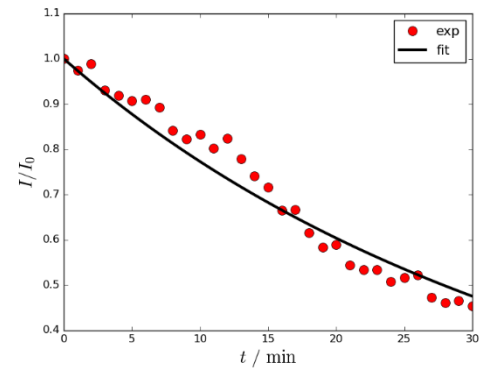
2283	+/- 79 (3.4%)
3117	+/- 127 (4.1%)

ds\_DS2\_salt



1285	+/- 54.6686840 (4.2%)
1329	+/- 61.9681403 (4.7%)

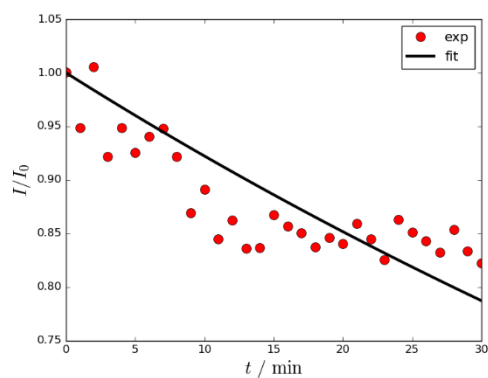
ds\_DS3\_salt



5271	+/- 148 (2.8%)
2157	+/- 174 (8.1%)

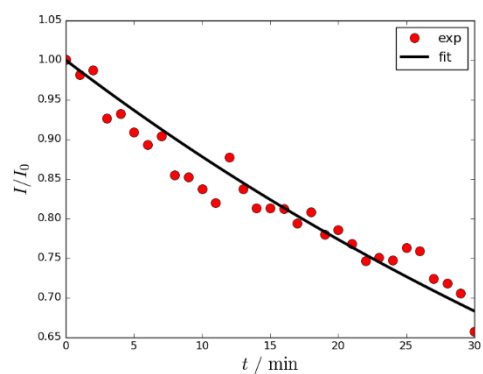
## Peg buffer\_higher salt

ds\_DSctrl\_peg\_salt



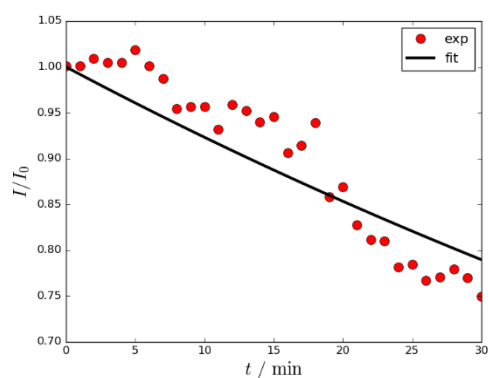
1629	+/- 91 (5.6%)
423	+/- 47 (11.1%)

ds\_DS1\_peg\_salt



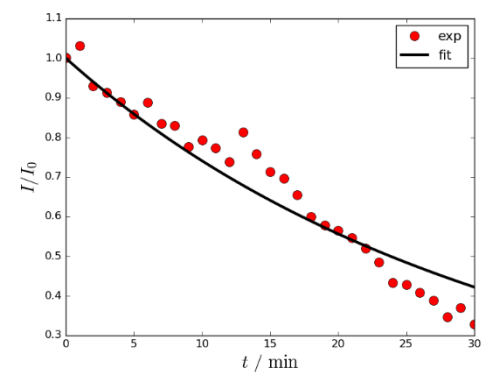
2628	+/- 69 (2.6%)
2352	+/- 129 (5.5%)

ds\_DS2\_peg\_salt



1609	+/- 95 (5.9%)
495	+/- 73 (14.7%)

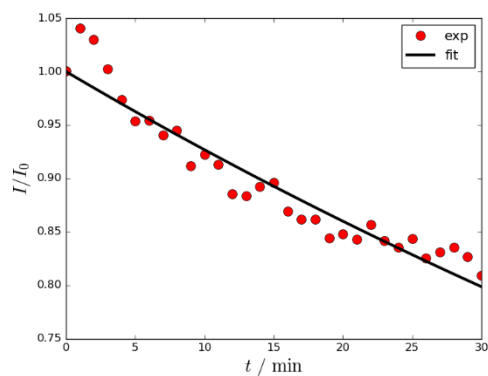
ds\_DS3\_peg\_salt



6165	+/- 242 (3.9%)
6558	+/- 368 (5.6%)

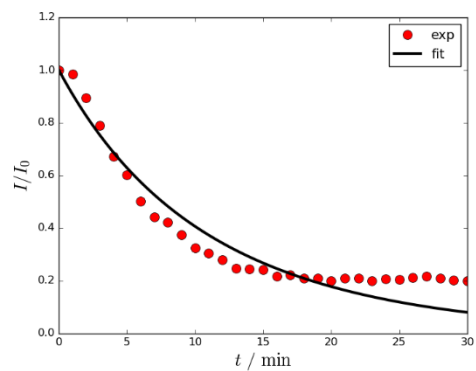
## Deg buffer\_higher salt

ds\_DSctrl\_deg\_salt



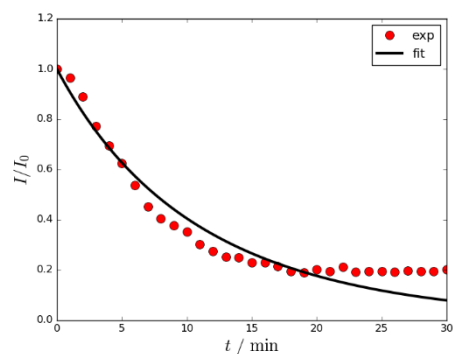
1868	+/- 65 (3.5%)
1529	+/- 46 (3.0%)

ds\_DS1\_deg\_salt



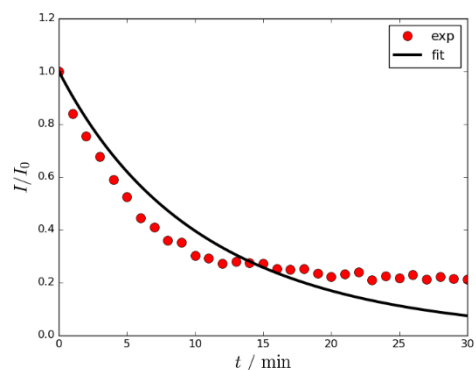
20183	+/- 1101 (5.5%)
19318	+/- 874 (4.5%)

ds\_DS2\_deg\_salt



14200	+/- 776 (5.5%)
19409	+/- 785 (4.0%)

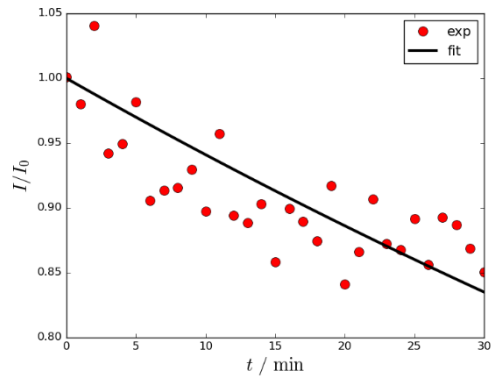
ds\_DS3\_deg\_salt



21203	+/- 2064 (9.7%)
19932	+/- 1151 (5.8%)

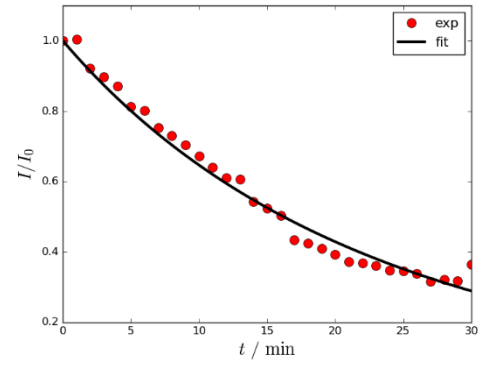
## Peg\_deg buffer\_higher salt

ds\_DSctrl\_peg\_deg\_salt



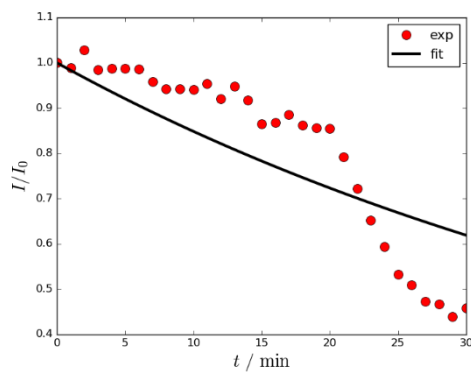
1222	+/- 77 (6.3%)
808	+/- 51 (6.3%)

ds\_DS1\_peg\_deg\_salt



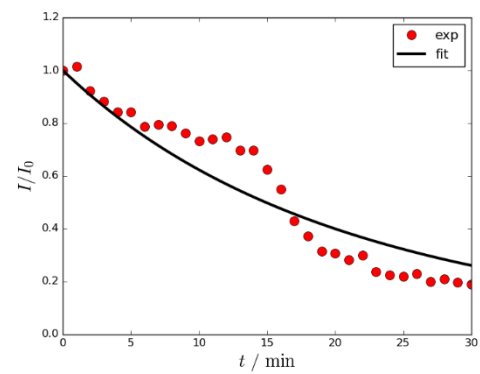
9057	+/- 166 (1.8%)
12243	+/- 388 (3.2%)

ds\_DS2\_peg\_deg\_salt



2581	+/- 318 (12.3%)
3335	+/- 341 (10.2%)

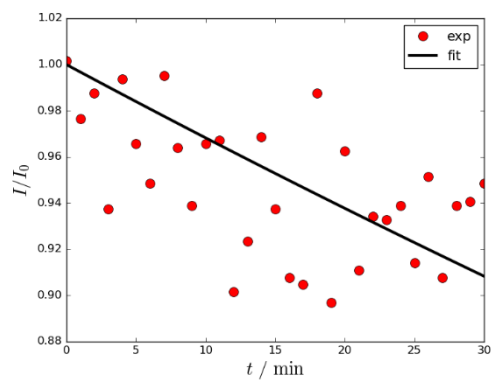
ds\_DS3\_peg\_deg\_salt



9976	+/- 504 (5.0%)
9855	+/- 592 (6.0%)

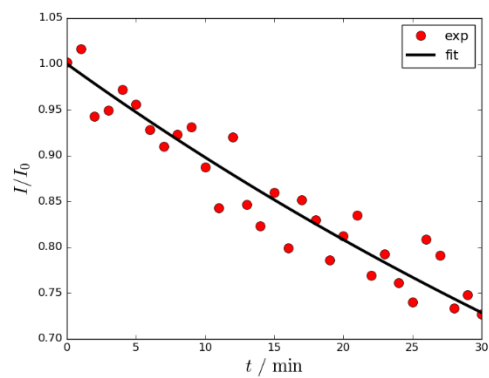
## Pvp buffer\_higher salt

ds\_DSctrl\_pvp\_salt



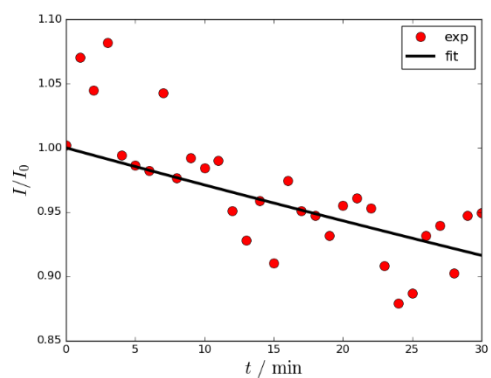
647	+/- 64 (9.9%)
324	+/- 76 (23.5%)

ds\_DS1\_pvp\_salt



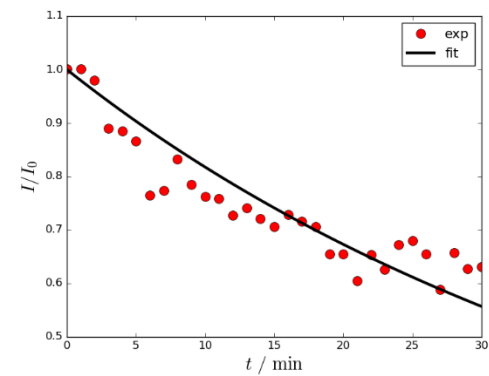
1712	+/- 86 (5.0%)
2172	+/- 69 (3.2%)

ds\_DS2\_pvp\_salt



587	+/- 75 (12.8%)
564	+/- 46 (8.2%)

ds\_DS3\_pvp\_salt



3591	+/- 119 (3.3%)
4103	+/- 169 (4.1%)