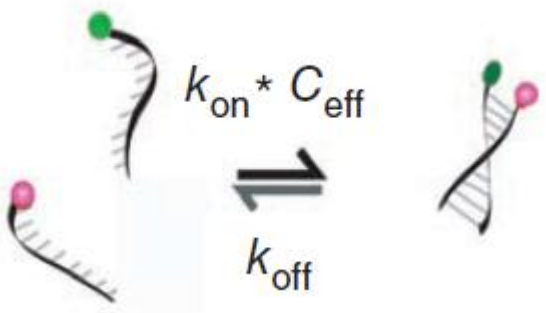

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A rule of seven in Watson-Crick base-pairing of mismatched sequences

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Sequence recognition through base-pairing is essential for DNA repair and gene regulation, but the basic rules governing this process remain elusive. In particular, the kinetics of annealing between two imperfectly matched strands is not well characterized, despite its potential importance in nucleic acid–based biotechnologies and gene silencing. Here we use single-molecule fluorescence to visualize the multiple annealing and melting reactions of two untethered strands inside a porous vesicle, allowing us to precisely quantify the annealing and melting rates. The data as a function of mismatch position suggest that seven contiguous base pairs are needed for rapid annealing of DNA and RNA. This phenomenological rule of seven may underlie the requirement for seven nucleotides of complementarity to seed gene silencing by small noncoding RNA and may help guide performance improvement in DNA- and RNA-based bio- and nanotechnologies, in which off-target effects can be detrimental.

Double-helix formation[1] of nucleic acids has been under investigation for more than 60 years. Thermodynamic parameters have been determined from compiled data of temperature-induced melting of DNA duplexes and theoretical analysis[2,3], allowing the prediction of melting temperatures (T_m) with 2 °C accuracy. The equilibrium constant (K_d), defined as the ratio of the rate of melting (k_{off}) to the rate of annealing (k_{on}), can also be determined. However, the determinants of these rates are still poorly understood, owing to the difficulty of directly observing the annealing and melting reactions.

k_{on} can be deduced from changes in diffusion times[4–6], from fluorescence resonance energy transfer (FRET) analysis in bulk[7,8], through relaxation analysis following electric shock[9] or temperature jumps[10–12] and by nuclear magnetic resonance (NMR)[13]. Recently, single-molecule techniques have enabled the determination of opening and closing rates for DNA and RNA hairpins[12,14,15] or oligonucleotides tethered inside membrane pore proteins[16] but not the observation of freely diffusing intermolecular reaction. Moreover, single-molecule mechanical studies have given extrapolated zero-force k_{off} values that were 100–10,000-fold different from the fluorescence-based estimate[17]. In none of the previous studies was the effect of base-pair mismatches on the annealing and melting kinetics examined, despite the likelihood that the kinetics have an important role in a variety of cellular processes in which two slightly mismatched oligonucleotides interact.

Here, we aimed to precisely quantify the effect of a single base-pair mismatch on k_{on} and k_{off} between two untethered DNA or RNA strands. We developed an assay based on single-molecule FRET[18] that can directly observe multiple rounds of melting and annealing reactions for a pair of DNA or RNA strands freely diffusing inside a porous vesicle. Confinement of the molecules by the vesicle[19–22] enables us to observe single-molecule reactions even when K_d is as high as 100 μM, which cannot be achieved by conventional methods. We observe that a single base-pair mismatch can cause a change in K_d of more than three orders of magnitude depending on the mismatch position. k_{off} increased gradually as the mismatch was placed closer to the middle of the sequence, whereas k_{on} showed a response more like a step function to the mismatch position, such that preventing the pairing of seven contiguous base pairs resulted in a reduction in k_{on} of up to two orders of magnitude. These results suggest that at least seven contiguous Watson-Crick base pairs are necessary for rapid duplex formation.

RESULTS

Kinetics of the full 9-base-pair DNA-DNA interaction

First, we designed two complementary 9-nucleotide (nt) DNA strands (**Fig. 1a**) that had a T_m near room temperature[2,3,23,24] and were devoid of secondary structures and dinucleotide repeats. The two DNA strands were fluorescently end-labeled with Cy3 (donor) and Cy5 (acceptor) so that their proximity could be detected with FRET. We used a vesicle encapsulation method described earlier, which allows the exchange of ions while keeping the nucleic acid oligomers within[21] (see Online Methods). Only those vesicles with fluorescence intensities and subsequent (single-step) photo-degradation consistent with one donor and one acceptor were included in the analysis.

Figure 1 shows single-vesicle time traces and apparent FRET efficiency (E_{app}) histograms obtained under various NaCl concentrations, with each condition representing at least three different preparations of encapsulated samples. The lower-concentration salt conditions show

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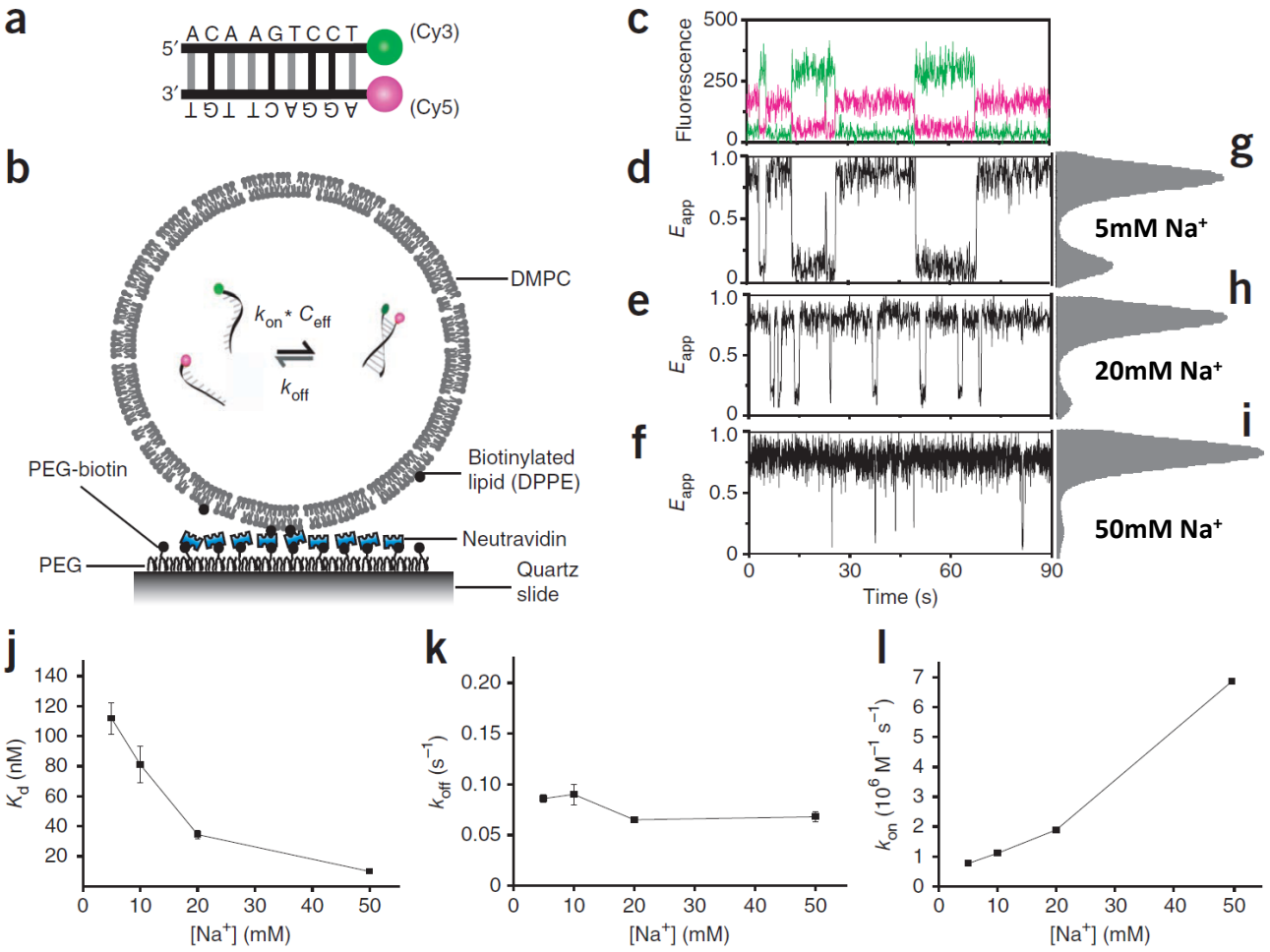
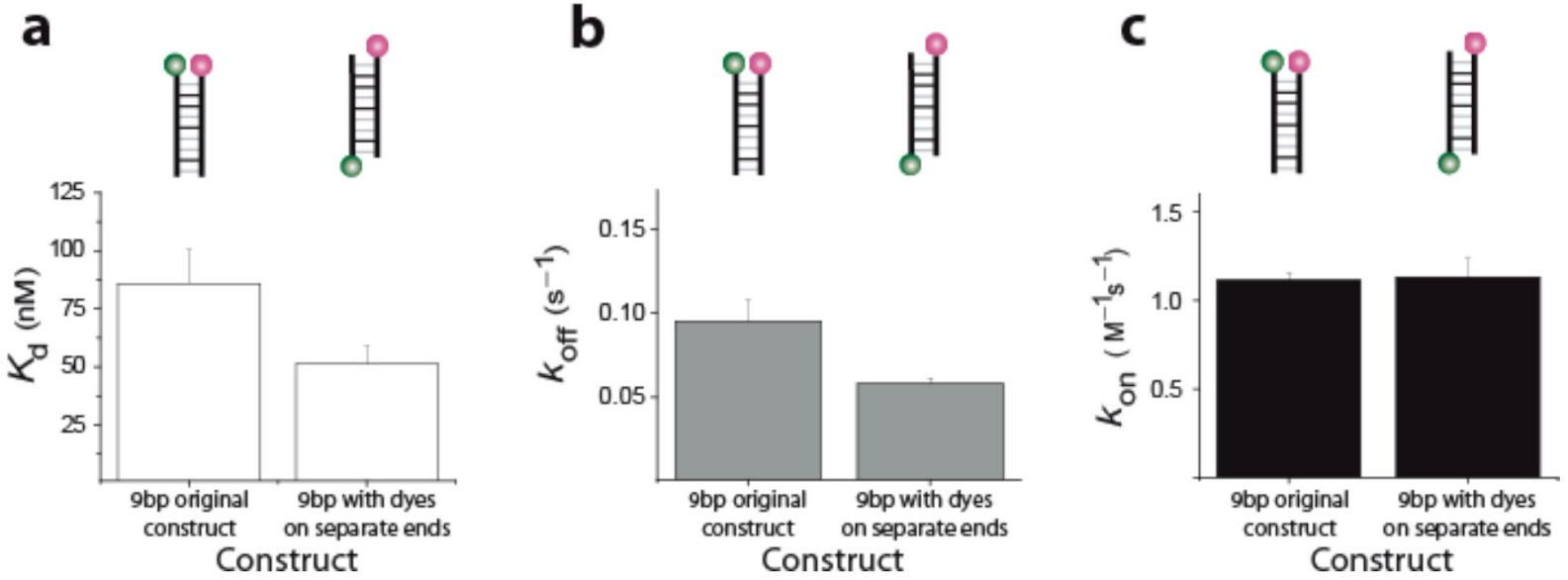
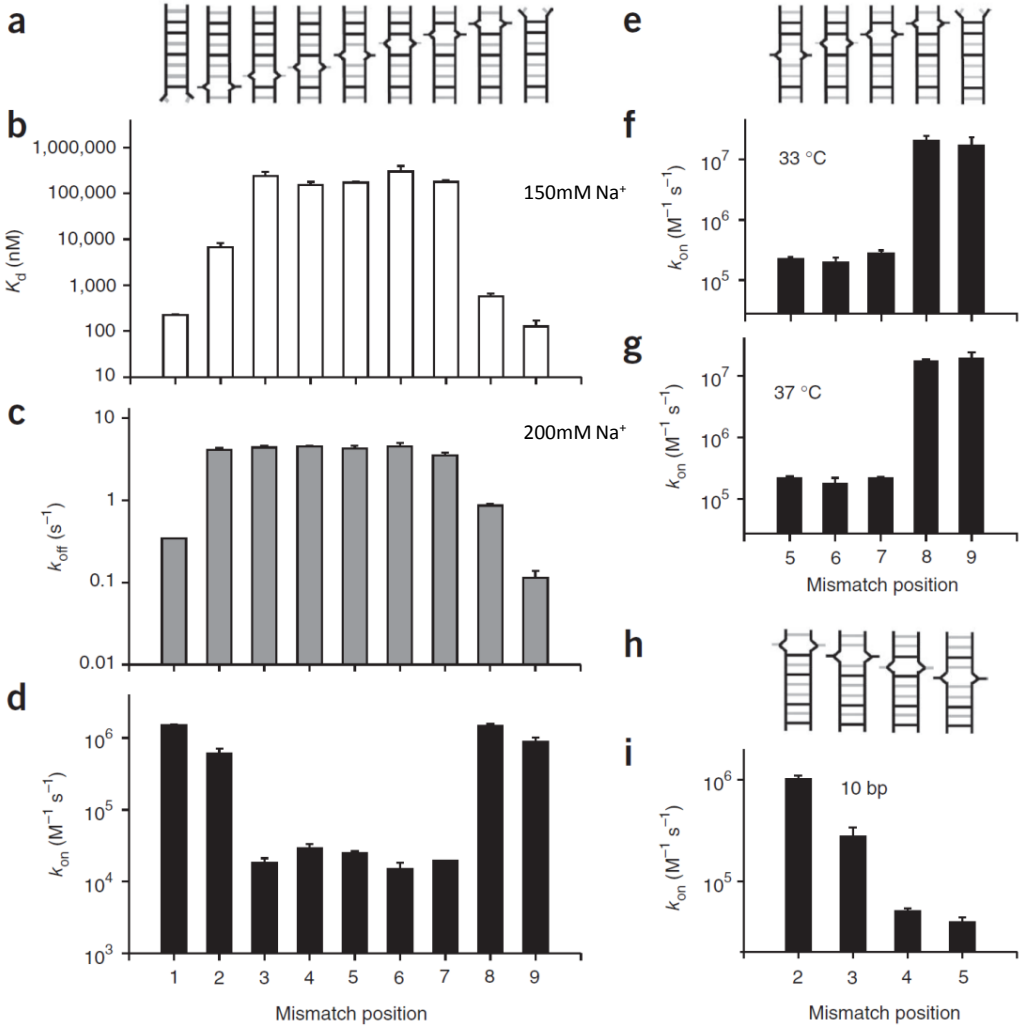

a
5′ ACA AGTCCT (Cy3)
3′ TGT TCAGGA (Cy5)
b
DMPC
k_{on}* C_{eff}
k_{off}
PEG-biotin
Biotinylated lipid (DPPE)
Neutravidin
PEG
Quartz slide
c
Fluorescence
500
250
0
d
e
f
E_{app}
1.0
0.5
0
0
30
60
90
Time (s)
g
5mM Na+
h
20mM Na+
i
50mM Na+
j
K_d (nM)
140
120
100
80
60
40
20
0
10
20
30
40
50
[Na+] (mM)
k
k_{off} (s^{-1})
0.20
0.15
0.10
0.05
0
[Na+] (mM)
l
k_{on} (10^6 M^{-1} s^{-1})
7
6
5
4
3
2
1
0
[Na+] (mM)

Dye effect

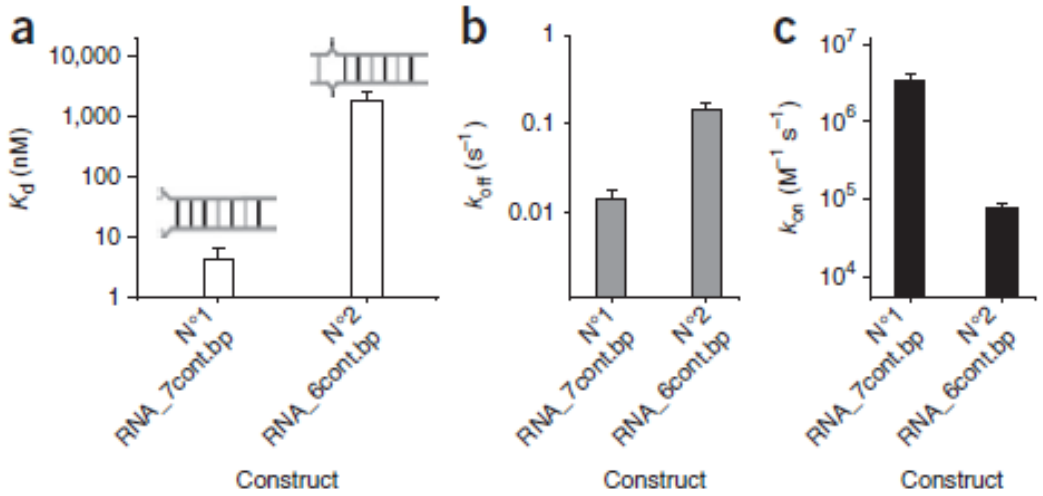

a
125
100
75
50
25
K_d (nM)
9bp original construct
9bp with dyes on separate ends
Construct
b
0.15
0.10
0.05
k_{off} (s^{-1})
9bp original construct
9bp with dyes on separate ends
Construct
c
1.5
1.0
0.5
k_{on} ($M^{-1}s^{-1}$)
9bp original construct
9bp with dyes on separate ends
Construct

Single mismatch in DNA



The RULE of SEVEN!!

Same for RNA?



yes!!