

P19 – Rupture force of single small drug molecules binding a split aptamer

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Aptamers are specific oligonucleotides (DNA or RNA) which bind small inorganic or organic molecules, large proteins or cells [1]. In particular, the high affinity of aptamers is expected to lead to a new class of therapeutic reagents. Thus the detection and characterization of binding strength of small molecules is important for drug and medical research. Atomic force spectroscopy (AFS) with a force resolution in the piconewton range is a valuable tool for studying interactions on a single molecular level. The detection of very small target molecules - less than 500 Dalton is characterized by only a few hydrogen interactions between the aptamer and the target molecules. Thus tiny rupture forces well below 100 pN are predictable [2,3]. For AFS the target molecules as well as the aptamer probes are typically immobilized on the AFM-tip or sample surface, respectively. This concept suffers from the need to chemically manipulate or label the target analyte by binding sites that interact with surfaces. We solved this problem by using a split aptamer probe. Both components of the aptamer were immobilized on the SFM-tip and sample surface, respectively. During the AFS experiment the split aptamers form defined binding pockets for the free analyte. The concept of using a split aptamer allowed the detection of the binding of Adenosine mono-phosphate (AMP) molecules being label-free. We found an increase in rupture force of 11 pN in the presence of AMP molecules in both binding pockets. The route to use a split aptamer probes in AFS enables us to determine precisely the dissociation constant of the AMP-aptamer system ($3.7 \pm 1.5 \mu\text{M}$) by increasing the AMP concentration in solution [4]. The concept of a split aptamer binding single small target also worked for the cocaine and antibiotics molecules.

References

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Rupture Force of Single Small Drug Molecule Binding a Split Aptamer

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P19

Basic Idea of a Split Aptamer

Aptamers are specific oligonucleotides (DNA or RNA) which bind small inorganic or organic molecules, large proteins or cells. In particular, the high affinity of aptamers is expected to lead to a new class of therapeutic reagents. Thus, the detection and characterization of binding strength of small molecules is important for drug and medical research. Atomic force spectroscopy (AFS), the target molecules as well as the aptamer probes are typically immobilized on the AFM-tip or sample surface, respectively. This concept suffers from the need to chemically manipulate or label the target analyte by binding sites that interact with surfaces. We solved this problem by using a split aptamer probe. Both components of the aptamer were immobilized on the SFM-tip and sample surface, respectively. During the AFS experiment the ligand aptamers form defined binding pockets for the free analyte. The concept of using a split aptamer probe allowed the detection of the binding of Adenosine mono-phosphate (AMP) molecules being label-free. We found an increase in rupture force of ~11 pN in the presence of AMP molecules in both binding pockets. The route to use a split aptamer probes in AFS enables us to determine precisely the dissociation constant of the AMP-aptamer system (3.6 ± 1.5 μM).

The approach - a Split Aptamer

- (a) Tip far from surface
- (b) Tip closed to surface: hybridization & no hybridization (red rectangle)
- (c) 2 AMP molecules in 2 binding pockets
- (d) Rupture of tip from surface: rupture force is recorded.

The question

Question: T-G-A-C-C-T-G-C-G-G-G-A-T-C-T-A-C-G
A-C-T-G-G-A-A-G-C-G-C-G-A-T-G-C

- Adenosine mono-phosphate (AMP) - New H-bonds
- Aptamer: ssDNA or RNA bind to compounds (better than antibodies)
- 8 additional H-bonds → more stable hybridization.
- Detect rupture force with label-free?
- Possible to determine K_D ?

Selected small drug molecules

- Molecules with molecular weights less than 500 Daltons
- AMP, Cocaine, Antibiotics
- Formation of H-bond net with aptamer
- Bound energy is not yet clear.
- What minimum binding site can be detected?

The result 3: Probing Hydrogen Binding Sites

Reduction of binding group: AMP, CMA, AMP

reduction of binding group: rupture force reduced
 simplification of $S_{1/2}$ by increasing number of binding pocket
 obtaining single binding site of split aptamer by multiple binding pockets.

F-D curve by Force Spectroscopy

Without AMP: $S_{1/2} = 27.3$ pN
 With AMP: $S_{1/2} = 38.4$ pN

AMP bound aptamer: ~11 pN force increases
 → AMP binds in the binding pocket of the split aptamer

The result 1: Rupture force

Without AMP: $S_{1/2} = 21.93 \pm 5.6$ pN
 With AMP: $S_{1/2} = 32.8 \pm 5.4$ pN

no change in rupture force:
 $F_{\text{without}} = F_{\text{with}} = F_{\text{binding}}$

Split Aptamer Concept Works for...

Initial data showed that cocaine or antibiotic can be detected.

Amplification of Rupture Force

- 32 additional H-bonds
- clearer shift of rupture force
- more stable hybridization
- increase rupture force $S_{1/2} \sim 30$ pN

The result 2: Dissociation Constant

K_D of AMP binding aptamer: $3.6 \mu\text{M}$

- % bound AMP/aptamer from histogram distribution
- force saturates at $\geq 10 \mu\text{M}$
- low AMP concentration: few bound AMP/Aptamer
- $K_D = 3.6 \pm 1.5 \mu\text{M}$

Conclusions

The rupture force of a split aptamer that forms binding pockets for AMP was measured by atomic force spectroscopy. Change in the rupture force of ~11 pN was observed in the presence of AMP, while this effect was absent when mutant aptamers or inosine were used. The rupture force in absence of AMP is associated to the H-bonds formed by 12 Watson Crick base pairs and enabled us to determine binding constants on a single molecular level.

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