# Investigating complex molecular interactions using novel SPR analysis approaches

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## Introduction

We describe how intricate interactions of the multiple component DNA mismatch repair system were studied through creative use of ***Dual***, ***ABA***, and ***Poly*** injection commands. Our results show the importance of binding order and dose dependencies of components in the sophisticated DNA mismatch repair process. The ***Poly*** command was used to further study interdependent interactions of the receptor binding domain (RBD) of the viral spike glycoprotein SARS-CoV-2 with ACE2 human receptor, monoclonal antibody against RBD, and a mouse anti-human IgG (Fc) antibody. A systematic manner of referencing varying injection sequences of components and blanks revealed convoluted aspects of the interactions.

## Biacore™ 1K+ SPR system

Biacore™ 1K+ is a one-needle SPR system equipped with six flow cells that can be addressed individually, in pairs, in quadruplets or together in sequence.

**Fig 1.** Biacore™ 1K+ SPR system.

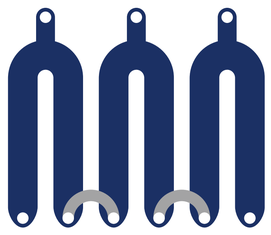


**Fig 2.** Flow cell configuration of Biacore™ 1K+ SPR system.

## New tools expand application space

When studying rapidly dissociating multi-component complexes it is crucial to avoid delays between the different components. Now available in Biacore™ 1 series:

### *Dual* command

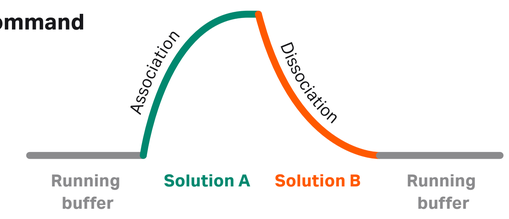



**Fig 3.** ***Dual*** command injects two different solutions in sequence with no time delay or liquid between in the order Solution A and Solution B.

### *ABA* command

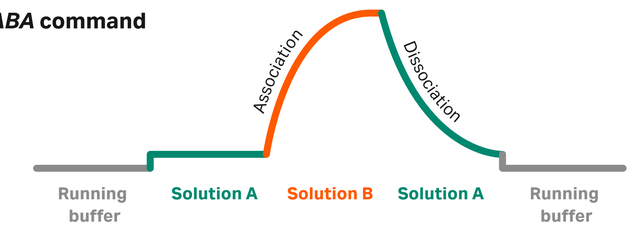



**Fig 4.** ***ABA*** command injects two different solutions in sequence with no time delay or liquid between in the order Solution A, Solution B, then Solution A.

### *Poly* command

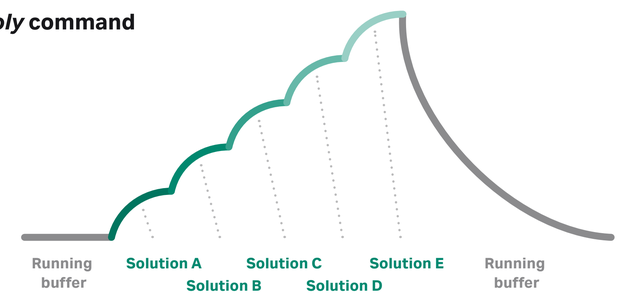
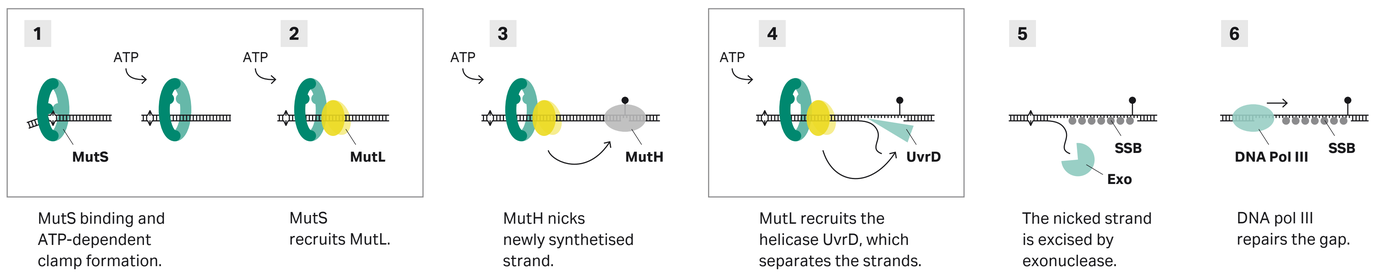



**Fig 5.** ***Poly*** command injects 3 to 5 different solutions in sequence with no time delay or liquid between in the order Solution A to E.

## Results

### DNA mismatch repair (1) complex formation




MutS binding and ATP-dependent clamp formation.

MutS recruits MutL.

MutH nicks newly synthetised strand.

MutL recruits the helicase UvrD, which separates the strands.

The nicked strand is excised by exonuclease.

DNA pol III repairs the gap.

**Fig 6.** Schematic overview of DNA mismatch repair in *E. coli*.

### ***Dual*** command — Varying dissociation conditions

This experiment showed that the DNA binding protein dissociates faster in the presence of higher concentrations of nucleotide.

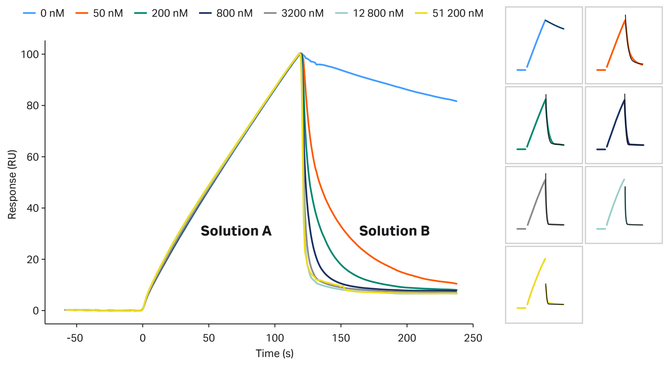



**Fig 7.** Series S Sensor Chip SA was immobilized with a DNA ligand. Injection of a DNA binding protein MutS (Solution A) immediately followed by running buffer containing increasing concentrations of ATP (Solution B) over the coupled DNA ligand. The 1:1 dissociation model was fitted to the dissociation phase.

### ***ABA*** command — Dose dependent binding of protein

MutL binds to the DNA-ligand and MutS complex. With the ***ABA*** injection command, binding of MutL could be explored before significant dissociation of MutS took place.

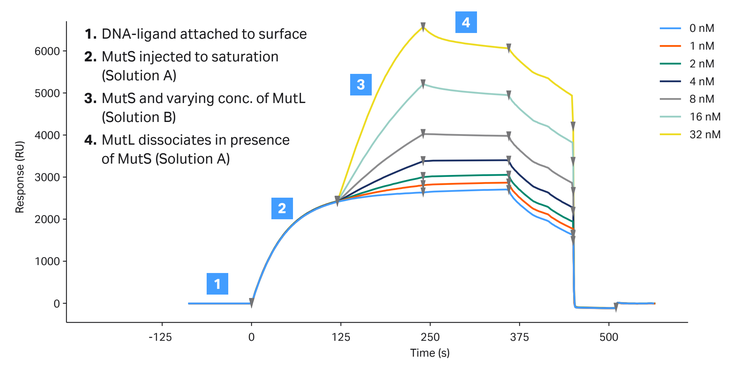



**Fig 8.** Series S Sensor Chip SA was immobilized with a DNA ligand. With 1 mM ATP present in the running buffer, MutS was injected to saturation (Solution A), followed by a mixture of MutS and varying concentrations of MutL (Solution B), before MutL dissociation still in the presence of MutL (Solution A).

### ***Poly*** command — Study of three stages of DNA mismatch repair (1) complex formation

This experiment showed a step-wise formation of the DNA mismatch repair complex, followed by complex dissociation, which was influenced by the amount of UvrD bound.

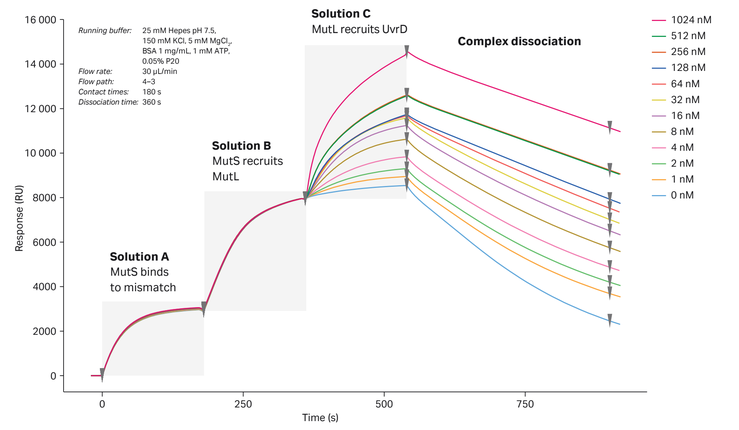



**Fig 9.** Series S Sensor Chip SA was immobilized with a DNA ligand containing a single nucleotide mismatch and biotinylations on both ends. Using ***Poly*** command, with 1 mM ATP in the running buffer, MutS was injected to saturation (200 nM MutS, solution A), followed by a mixture of MutS and MutL (200 nM each, solution B), and finally MutS, MutL, and varying concentrations of UvrD (200 nM MutS, 200nM MutL, and 0–1024 nM UvrD, solution C).

### ***Poly*** command — Binding relations in a SARS-CoV-2 protein complex

To gain entry to a host cell, the receptor binding domain (RBD) of the SARS-CoV-2 spike protein binds to angiotensin-converting enzyme 2 (ACE2) on the host cell surface. CR3022 (α-RBD Ab) is a non-neutralizing antibody that targets a non-overlapping epitope on RBD (2).

With RDB immobilized, in the first cycle all three potential binding partners, α-RBD Ab, ACE2, and α-Human Ab, were injected using ***Poly*** command. Next, three similar cycles where each protein in turn was replaced by buffer were performed.

The blank containing sensorgrams were subtracted from the sensorgram from Cycle 1 that had all three proteins.

- **Cycle 1 minus cycle 2** shows that solution A (α-RBD) and solution C (α-Human) bind to each other.
- **Cycle 1 minus cycle 3** shows binding of solution B (ACE2) only, as binding of solution A and solution C have been subtracted away.
- **Cycle 1 minus cycle 4** shows binding of solution C alone; note that this binding is not possible without preceding binding of solution A.

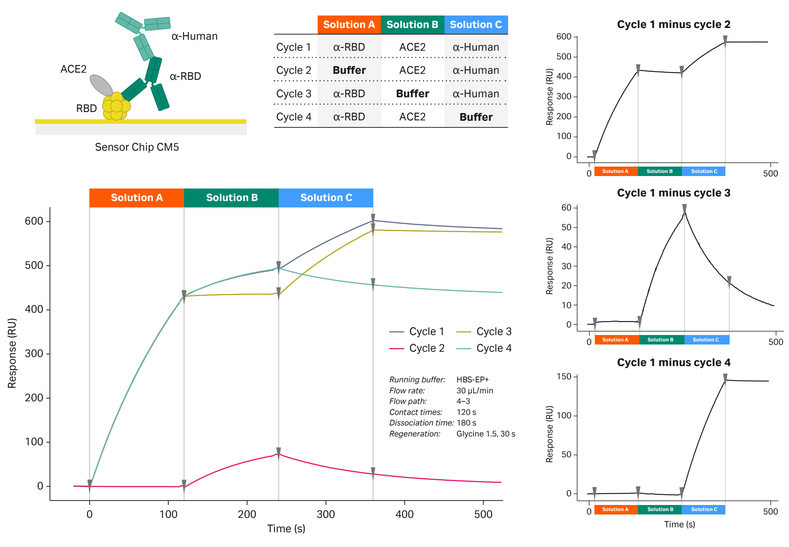



**Fig 10.** RBD was immobilized on Series S Sensor Chip CM5 (895 RU). ***Poly*** command was used to inject α-RBD (10 nM), ACE2 (750 nM) and α-Human (10 nM) (Cycle 1). Cycles where each protein in turn was replaced by buffer were also performed (Cycles 2, 3, and 4). Subtraction of each of the following cycles from Cycle 1 revealed elucidated binding relations.

## References

1. Fernandez-Leiro R, Bhairosing-Kok D, Kunetsky V, *et al.* The selection process of licensing a DNA mismatch for repair. Nat Struct Mol Biol. 2021;28(4):373-381. doi:10.1038/s41594-021-00577-7
2. Yuan M, Wu NC, Zhu X, *et al.* A highly conserved cryptic epitope in the receptor binding domains of SARS-CoV-2 and SARS-CoV. Science. 2020;368(6491):630-633. doi:10.1126/science.abb7269

## Conclusions

Novel SPR analysis approaches using ***Dual***, ***ABA***, and ***Poly*** commands

- Meets the need for more advanced analytical tools for characterization of protein complex formation
- Supports sequential injection of two to five components, minimizing dissociation time between injections
- Enables the study of binding dependencies using SPR

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