Visualize your research – SPR for protein complex formation studies

Cynthia Shuman[1], Alexander Fish[2], Elin Sivertsson[3], Linnea Nygren-Babol[3], Anna Moberg[3]

[1]Cytiva, Marlborough, MA, USA; [2]Netherlands Cancer Institute, Amsterdam, The Netherlands; [3]Cytiva, Uppsala, Sweden

Introduction

Most proteins operate as multimeric complexes, rather than as individual proteins. These protein complexes regulate numerous cellular processes. Advances in proteomic research led to the identification of thousands of protein complexes, and the number continues to grow.

Some protein complexes are stable, while others are more transient in nature. Here we present a novel SPR injection tool for protein complex formation studies where up to five sequential injections can be performed without any intermediate wash steps. This limits the delay, and thus time for dissociation, between injections of different interaction partners.

Biacore™ 1K SPR System

Biacore™ 1K is a one-needle SPR system equipped with six flow cells that can be addressed individually or in pairs. Biacore™ 1K generates high quality SPR data, provides ease of use, and shortens time to results through:

- Wide application and sample space
- Better utilization of the sensor chip with 50% more flow cells compared to previous one-needle Biacore™ systems
- Predefined run methods and flexible software tools
- Maximized efficiency with method queueing possibilities

Fig 1. Biacore™ 1K SPR System.

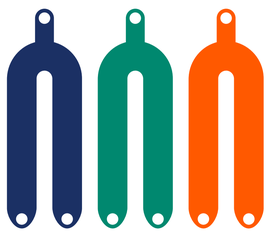


Fig 2. Flow cell configuration of Biacore™ 1K SPR System.

Poly command for analysis of protein complex formation

Biacore™ 1K comes with a novel injection tool that allows for a versatile assay design. The ***Poly*** command enables the injection of three to five solutions in immediate sequence with no intermediate washing steps which gives you new possibilities to study the formation of protein complexes.

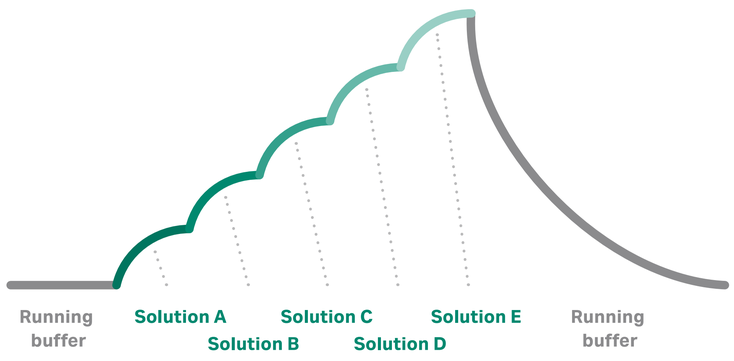


Fig 3. A schematic of the ***Poly*** command showing sequential injection of five components.

More info

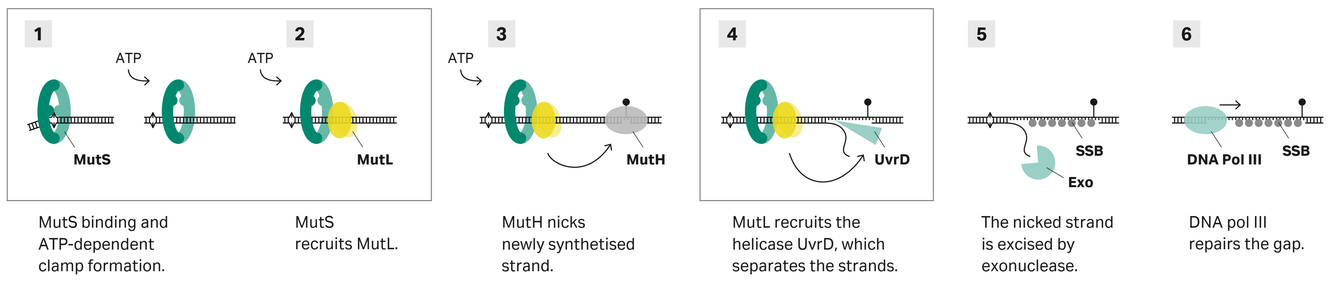
- Data file Biacore™ 1 series (CY29857)
- Data file Biacore™ Insight Evaluation Software (CY11720)

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

DNA mismatch repair complex formation

DNA mismatch repair (1) is an evolutionary conserved process for the detection and removal of DNA mismatches introduced during replication. A schematic overview is shown in Figure 4.



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 4. Schematic overview of DNA mismatch repair in *E. coli*.

Poly command was used to study three stages of DNA mismatch repair complex formation (boxed in Figure 4): MutS binding to a mismatch in an immobilized DNA ligand, followed by recruitment of MutL, and finally UvrD binding. The sensorgram in Figure 5 shows the step-wise formation of the protein complex, followed by complex dissociation.

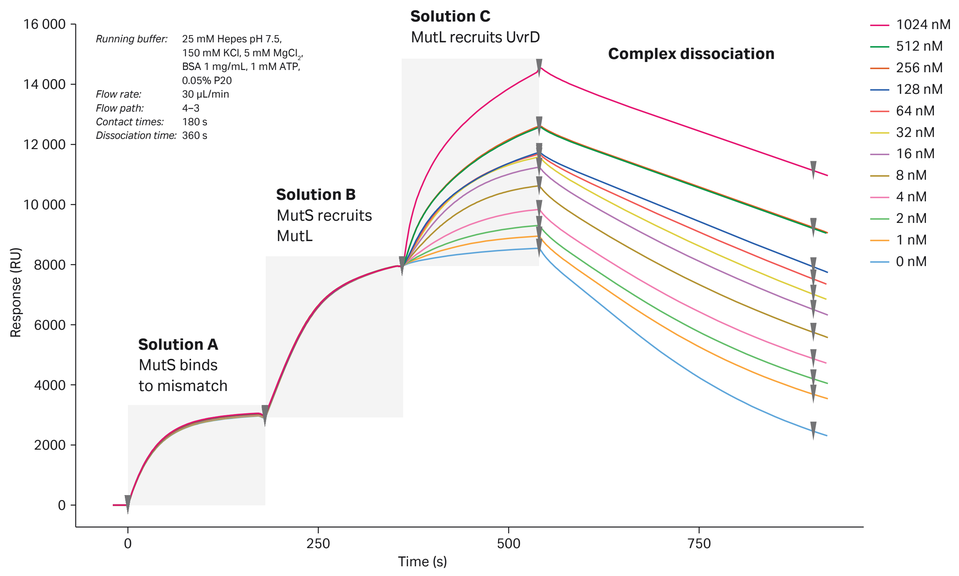


Fig 5. Complex formation of DNA mismatch repair proteins. Series S Sensor Chip SA was immobilized with a DNA ligand containing a single nucleotide mismatch and biotinylations on both ends. Using ***Poly*** command, MutS was injected to saturation (200 nM MutS, solution A), followed by a mixture of MutS 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).

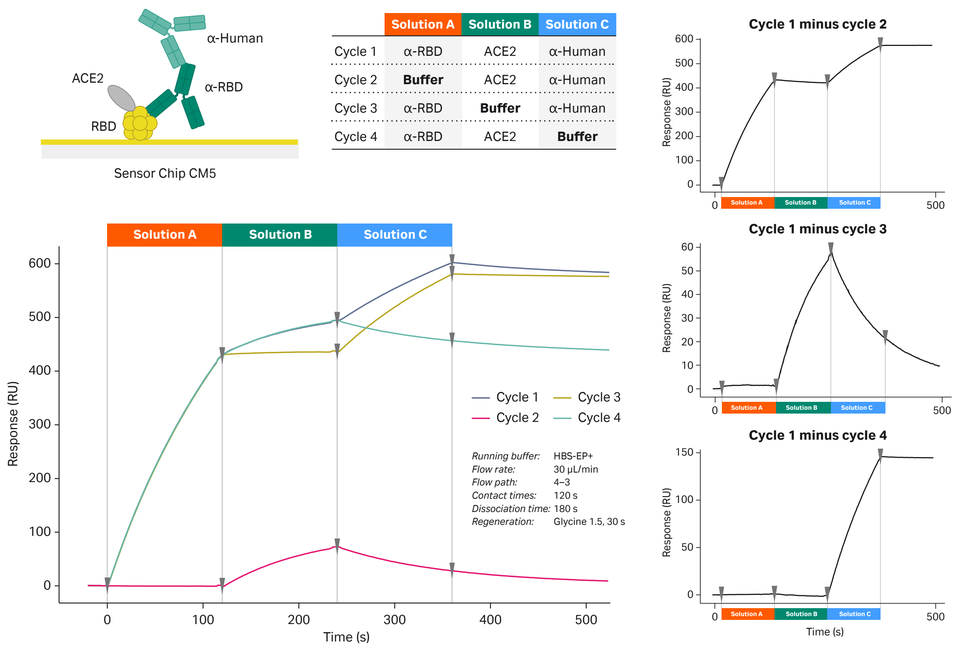
Binding dependencies in a SARS-CoV-2 protein complex

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 is a neutralizing antibody that targets a non-overlapping epitope on RBD (2).

We used ***Poly*** command to investigate the binding dependencies of RBD, CR3022 (α-RBD), ACE2, and an anti-human antibody (α-Human) (Fig 6). RBD was immobilized on Series S Sensor Chip CM5. In the first cycle, ***Poly*** command was used to inject all three potential binding partners. Next, three cycles where each protein in turn was replaced by buffer were performed.

During evaluation, cycles with buffer were subtracted one at a time from the cycle containing all proteins. The resulting sensorgrams are shown in Figure 6 (right panel) and reveal binding dependencies within the complex.

- **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.



	Solution A	Solution B	Solution C
Cycle 1	α-RBD	ACE2	α-Human
Cycle 2	**Buffer**	ACE2	α-Human
Cycle 3	α-RBD	**Buffer**	α-Human
Cycle 4	α-RBD	ACE2	**Buffer**

Fig 6. Binding dependencies of SARS-CoV-2 proteins. 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). By subtracting cycles containing buffer segments from the cycle containing all proteins, binding dependencies within the protein complex could be elucidated.

Conclusions

Poly command

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

cytiva.com/biacore

CY33705-21Dec22-PT

cytiva