Augmented Affinity in Antibody-Antigen Interactions; Impact on Design of Diagnostic Assays

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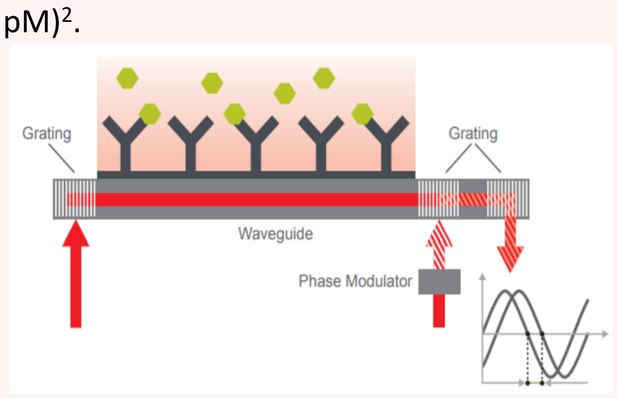
Summary

- The CARD HIV p24 lateral flow immunoassay is capable of an LOD by eye of 1 pg/ml (42 femtomolar) in whole blood without signal amplification.
- To investigate the binding dynamics of the antibody pair in this assay: mAb1 (sheep) and mAb2 (mouse), the binding kinetics were measured in the Creoptix Wavedelta system. Four reagent combinations were interrogated in real time either as p24 antigen to each immobilised antibody or a p24/antibody complex binding to the second antibody, both immobilised and free in solution.
- The results reveal a surprising enhancement in affinity due to a considerable on-rate shift when mAb1 recognises the mAb2 - p24 complex.

Method

- Anti-p24 antibody binding kinetics were measured using the Creoptix WAVEdelta system. The WAVEdelta is based on Grating-Coupled Interferometry (GCI)[1] applied to an integrated microfluidic chip enabling highly sensitive detection of label-free interactions. In contrast to SPR, GCI provides an evanescent field which penetrates less into the bulk and extends the light-to-sample interaction length (Fig 1) for improved sensitivity and detection within a broader affinity range (from mM to pM)[2].

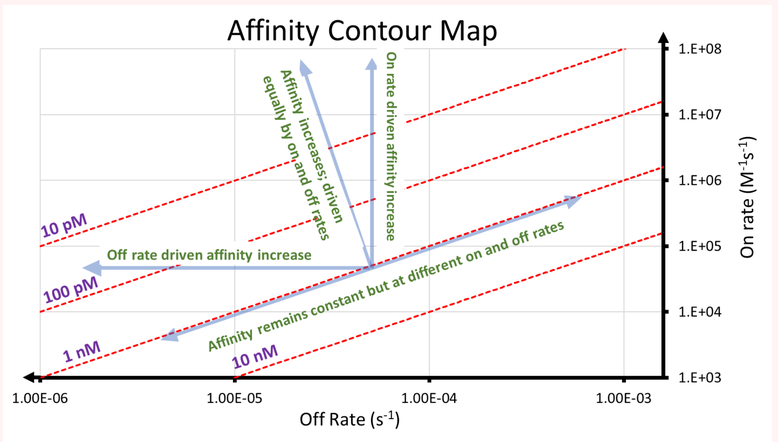


← **Fig 1:** Diagram of GCI (from Creoptix AG, used with permission).

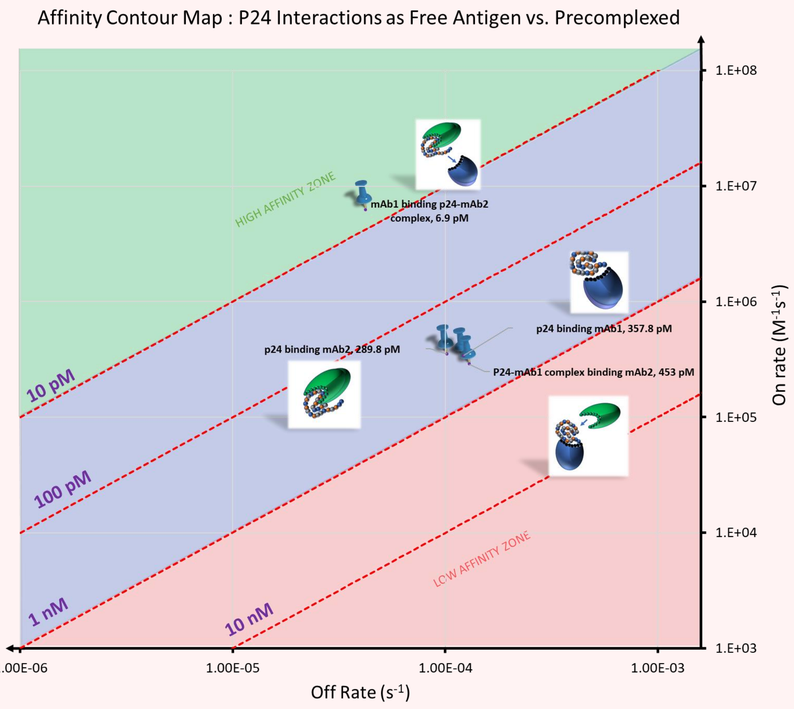
- In this study, four reagent combinations were interrogated in real time, either as p24 antigen (alone) binding to each immobilised antibody or a p24-antibody complex binding to the second antibody, both immobilised and free in solution.

P-24 Antibody Pair: Affinity and Interaction

- Antibody interactions were investigated via an affinity contour map (Fig 4). Both antibodies to p24 mapped to close locations in affinity space, as did mAb2 when recognising p24 pre-bound to mAb1.
- The opposite order of pairing shows the surprise augmentation effect with an increase in on-rate by a factor of 21 (Fig 5).

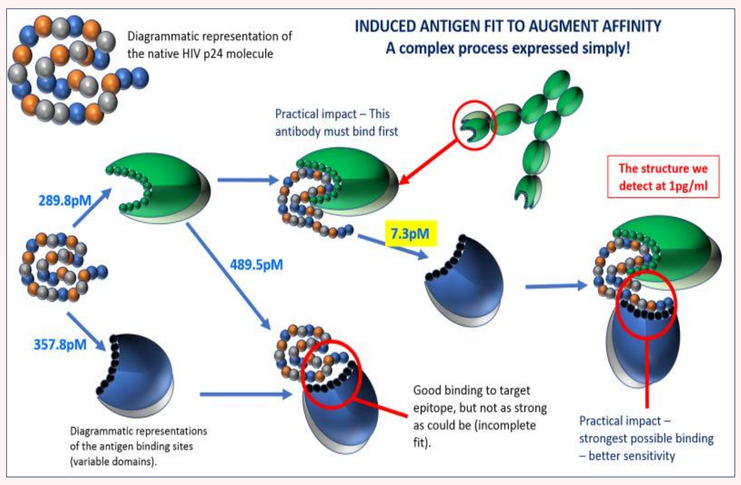


↑ **Fig 4:** A schematic of an affinity contour map, illustrating possible interactions.



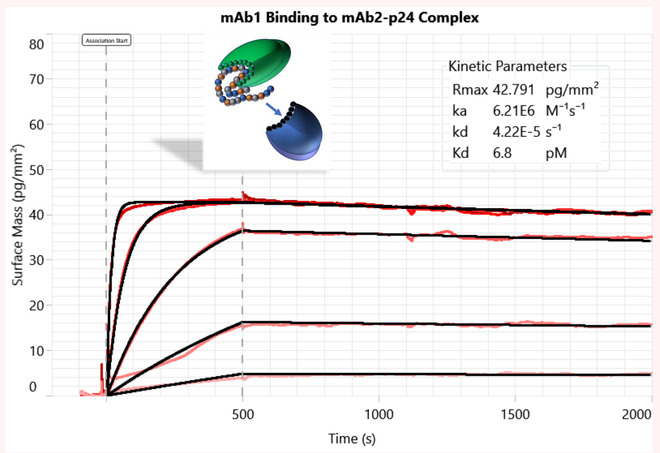
← **Fig 5:** Affinity Contour Map: Comparison of individual antigen recognition and o24 pairing interactions of mAb1 (blue) and mAb2 (green).

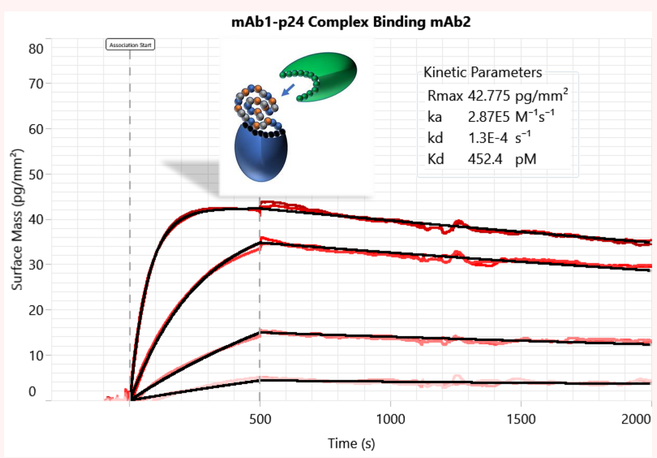
P-24 Antibody Pair Binding Kinetics



← **Fig 2:** Schematic of the induced-fit model for p24 binding to mAb2 (green) and mAb1 (blue).

- The unusual binding kinetics behaviour of p24 to the antibody pair was thought to occur via an induced fit model (Fig 2).

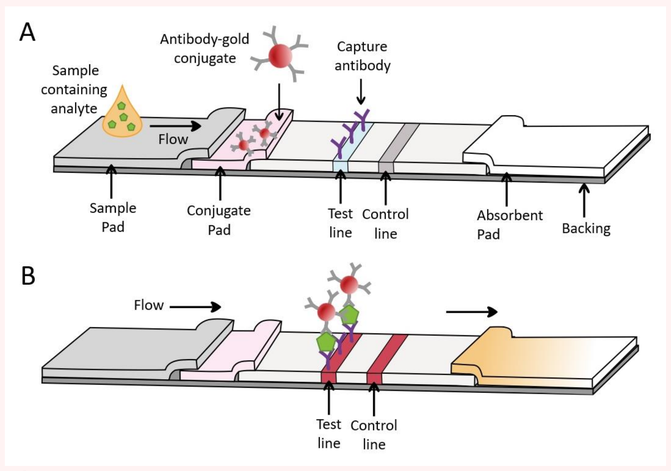




- Kinetics data obtained in this study supports an induced-fit model. The binding of mAb2 in complex with p24 results in a 21-fold increase in the mAb1 on-rate (Fig 3). In this situation, the p24 antigen-structure was evidently modified by its interaction with the mAb2 to improve mAb1 binding.

← **Fig 3:** Sensorgrams comparing two pairing interactions with p24 at dilutions of analyte ranging from 50 nM to 39 pM. When mAb2 (green) is bound to 24 first the recognition by mAb1 (blue) shows augmented affinity by a factor of 66; possibly due to a conformational epitope change.

Application in a Lateral Flow Assay

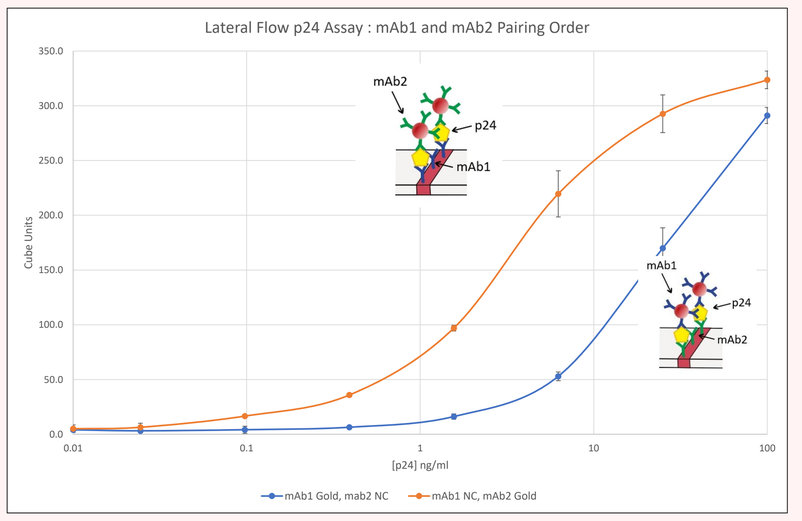


↑ **Fig 6:** A schematic of a lateral flow assay, before (A) and after use (B).

- The impact of augmented affinity was tested in a demonstrator lateral flow assay (Fig 6). There was substantial difference in the analytical sensitivity depending on which mAb was deployed on the gold NPs. With mAb2 on gold, the sensitivity was more than 10-fold better than for mAb1 (Fig 7). The difference was attributed to augmented affinity because the p24 antigen was inevitably exposed to the gold-bound mAb prior to capture on the test line.

→ **Fig 7:** Lateral flow dose response curves for the two different antibody arrangements.

Note: *This demonstrator lateral flow assay is not an example of the CARD supersensitive LFIA platform. It is used here to simply exemplify the isolated impact of augmented affinity on a standard assay with calibration standards in buffer*



Conclusion

The results of this study reveal a surprising enhancement in affinity due to a significant 21-fold increase in on-rate when anti-p24 mAb1 recognises the anti-p24 mAb2-p24 complex. In this situation, the p24 antigen-structure was evidently modified by its interaction with mAb2 in a manner such-that the mAb1-paratope was more efficiently bound than when the p24 was in free solution.

References

[1] Kozma (2011), Sens. Actuators B. Chem. 155:446-450. [2] Pitsawong (2018), e-Life 7:e-36656

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