Molecular biosensors for antigen and antibody testing

Yie-Hwa Chang, Ph.D.
President & CEO
Mediomics, LLC

CDC Symposium:
Identification, screening and surveillance of HCV infection in the ERA of improved therapy for hepatitis C
December 1, 2011

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Mission

Mediomics focuses on developing

1. Fast, sensitive, robust and cost–effective biosensors for research and clinical diagnostics use;

2. New cost-effective biotherapeutics.

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Platform Technologies

- Bridge-It® Platform– research tools
  (Over 2000 targets)

- PINCER® Platform – research tools & diagnostics
  (Over 25,000 targets)

- PINCER® -based Aptamer Screening method– research tools & therapeutics.
  (Over 25,000 targets)

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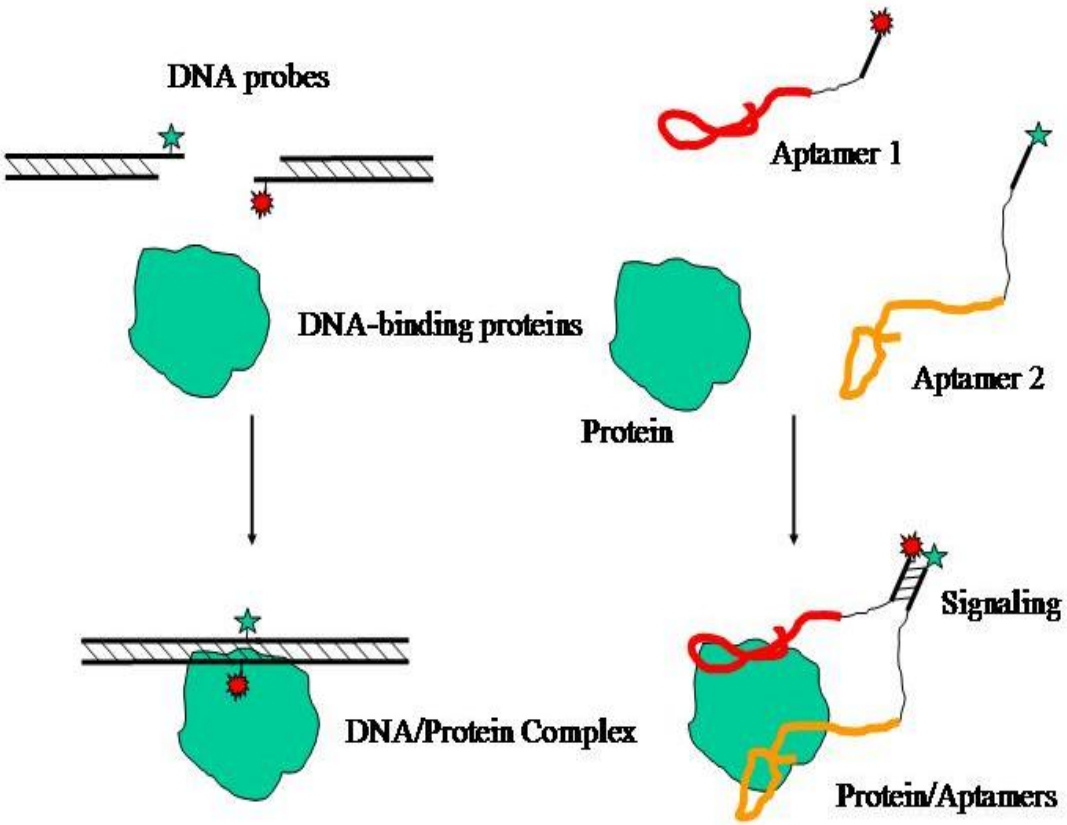
Bridge-It® and PINCER® Platforms



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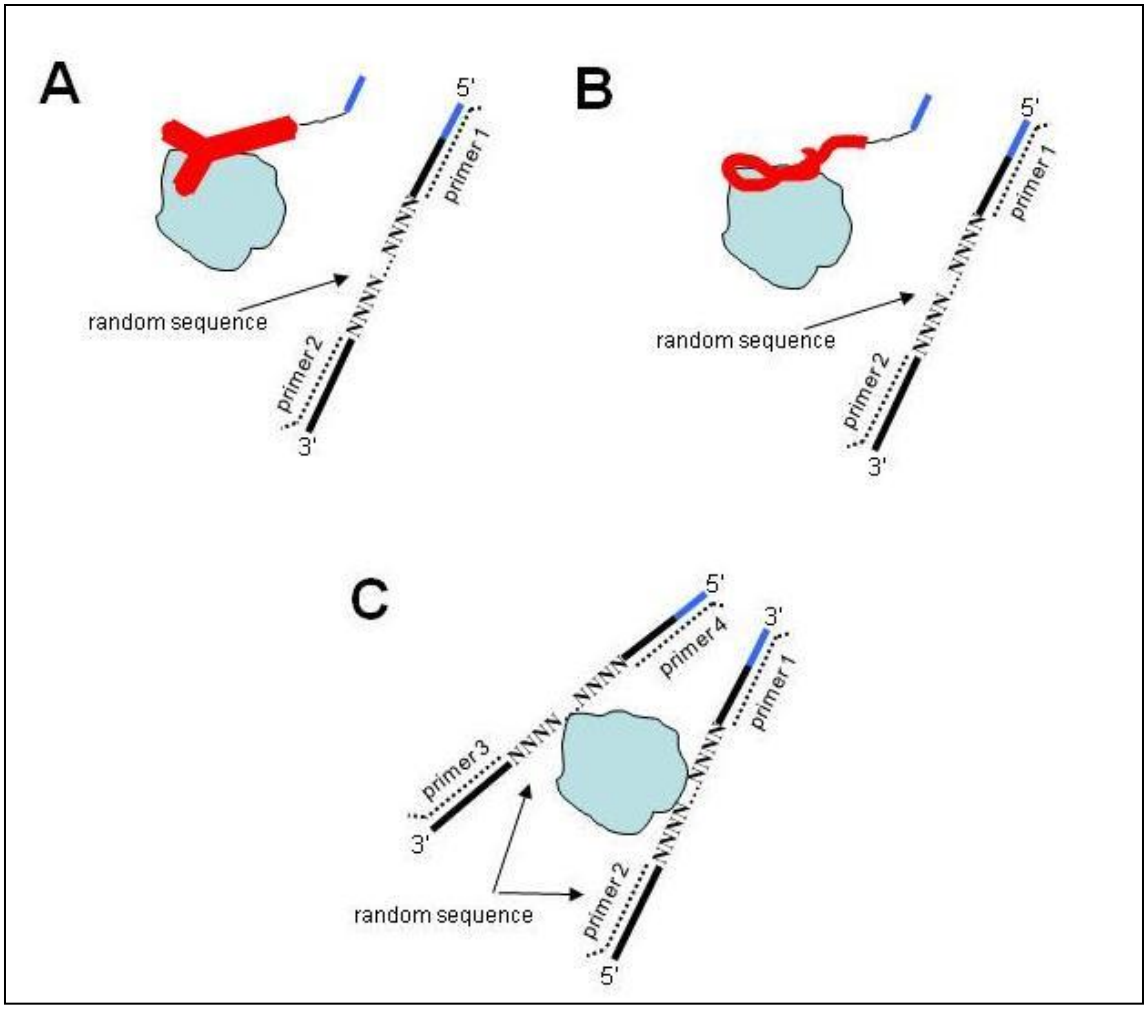
PINCER®-based Aptamer Screening



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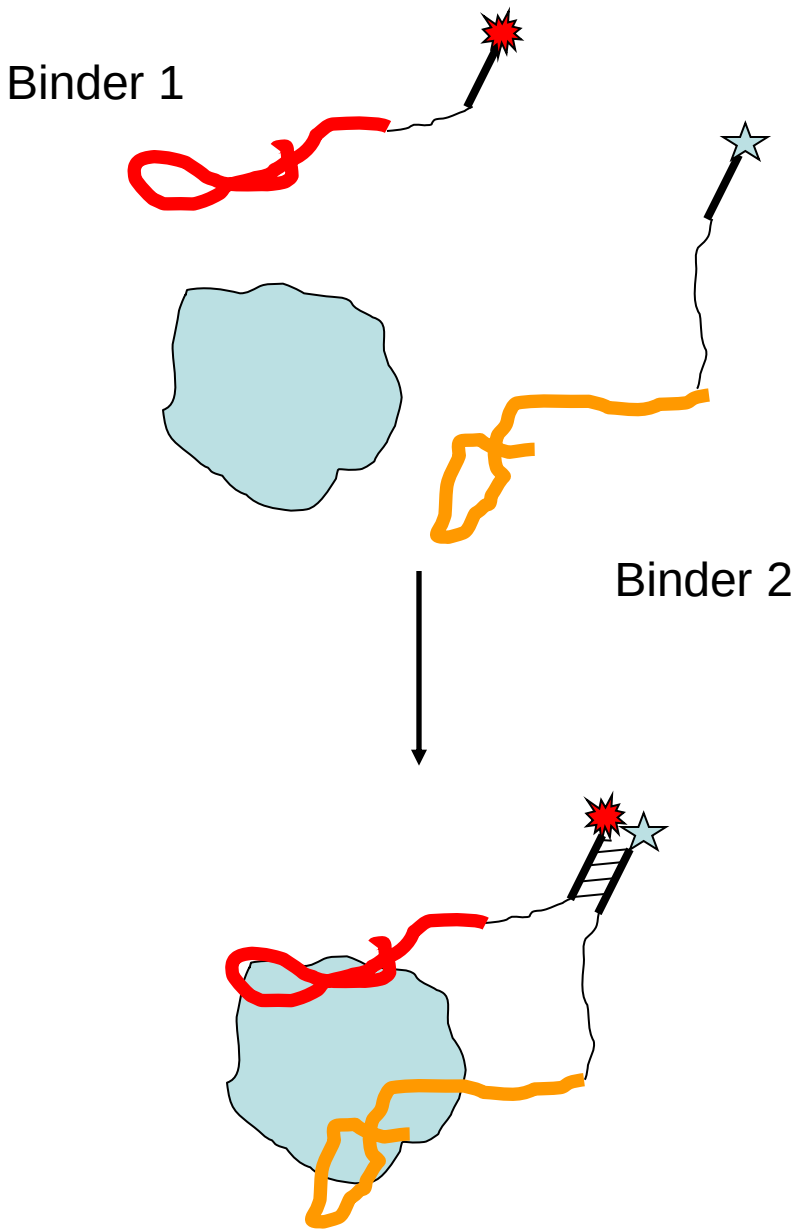
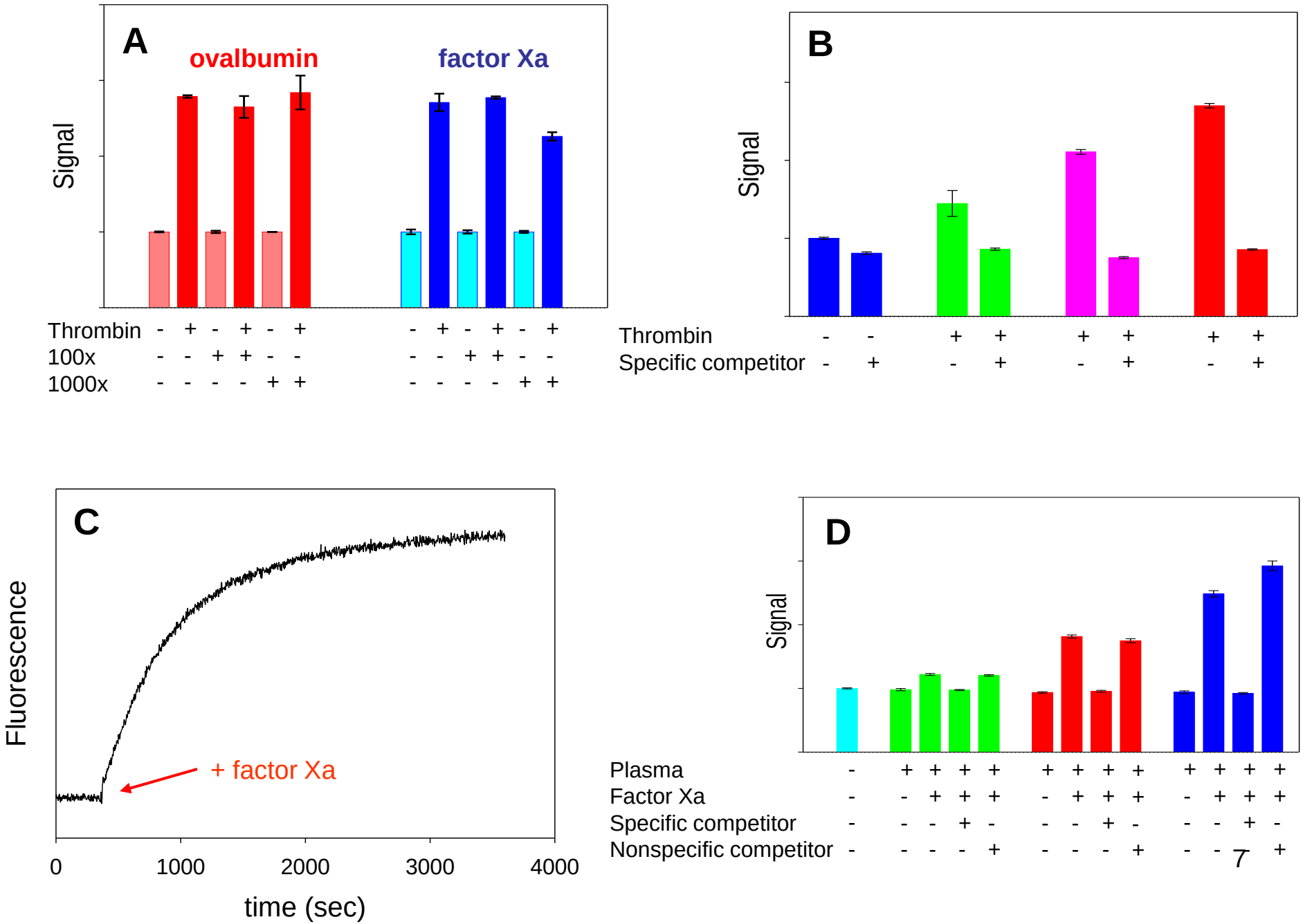
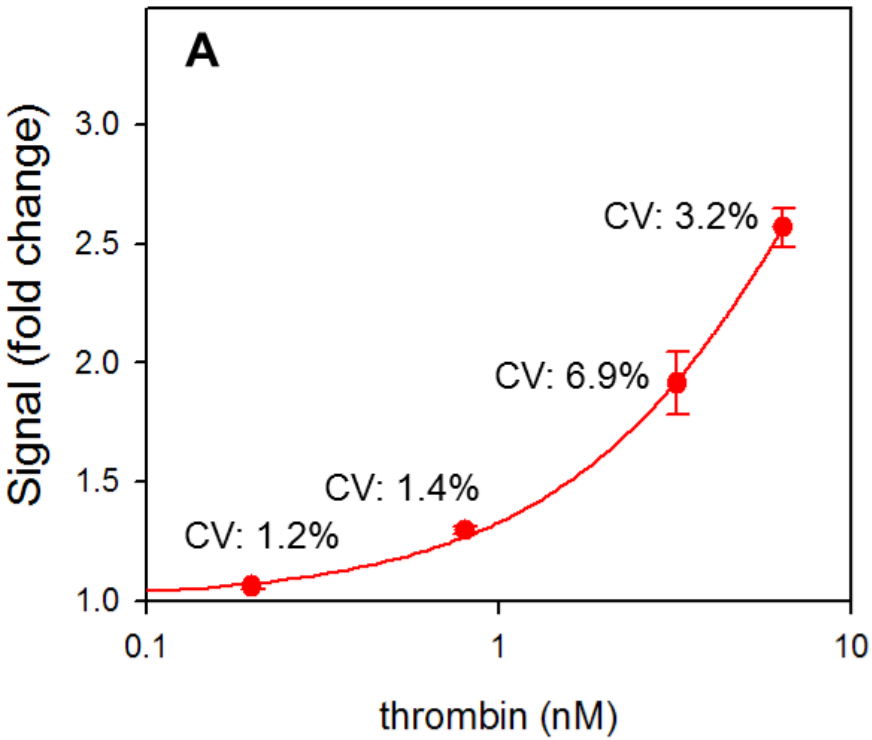
PINCER® Platform Technology

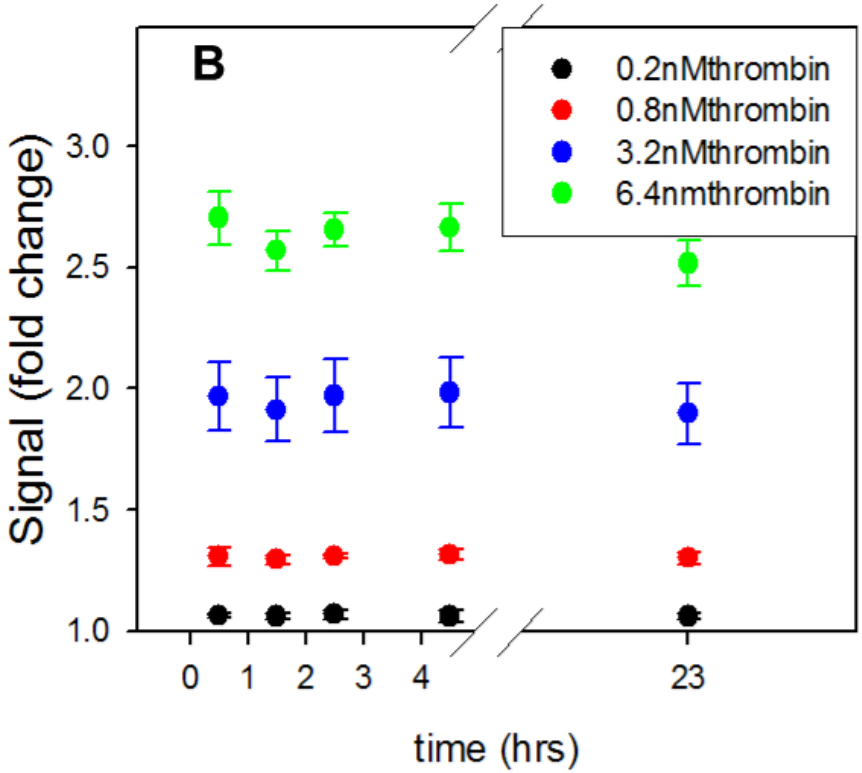


Detection of thrombin in complex mixtures

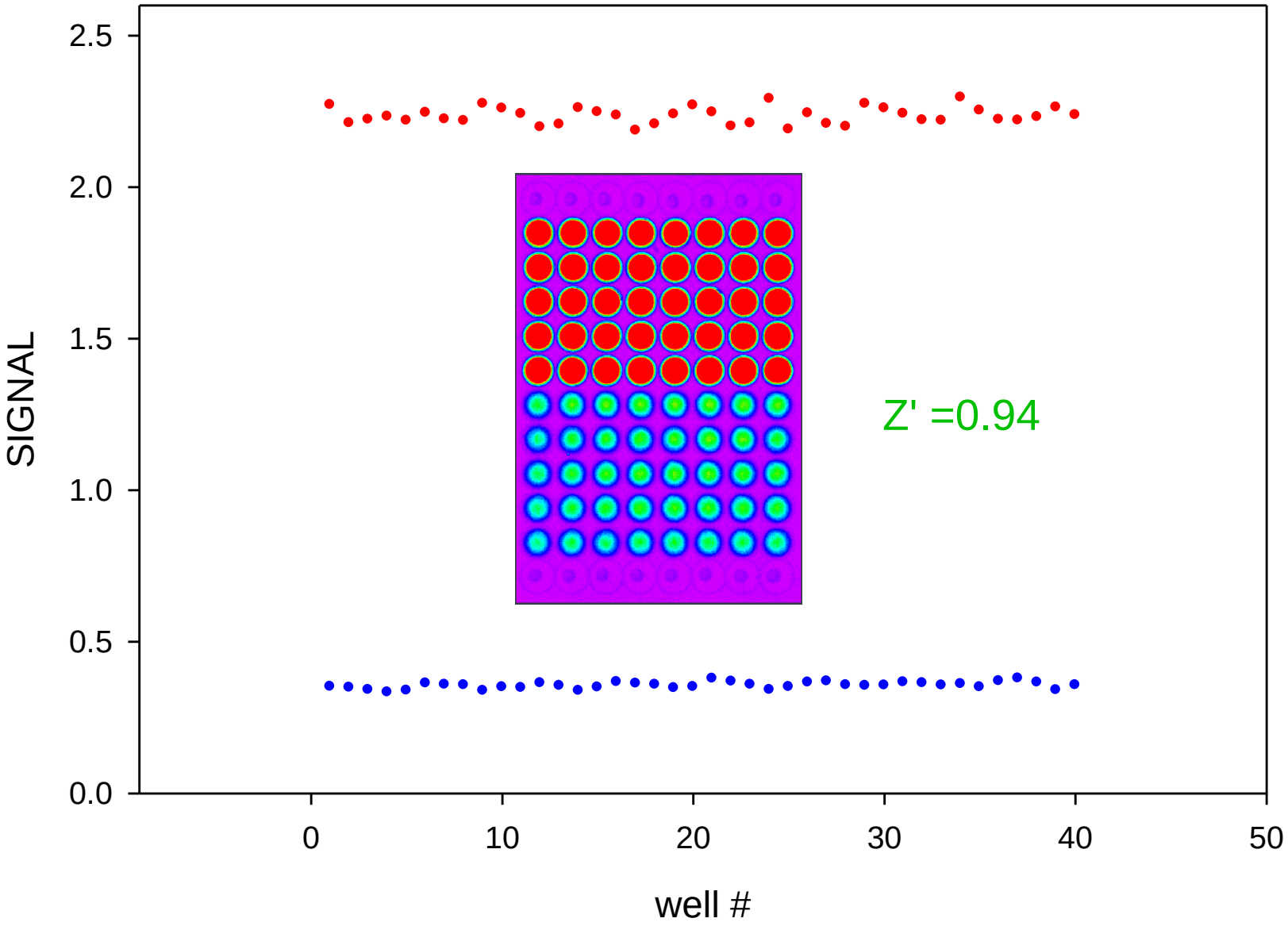


Reproducibility and stability

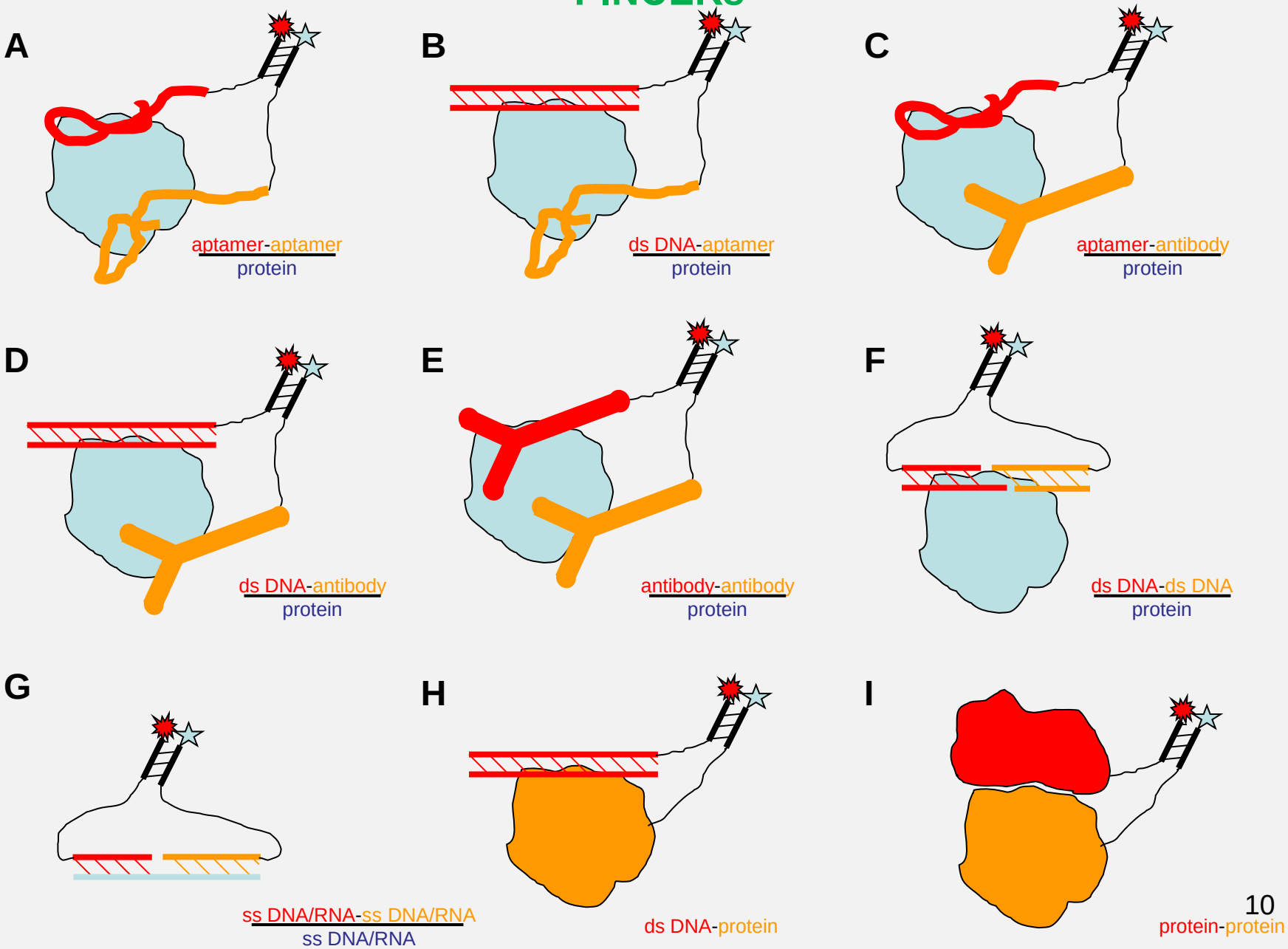


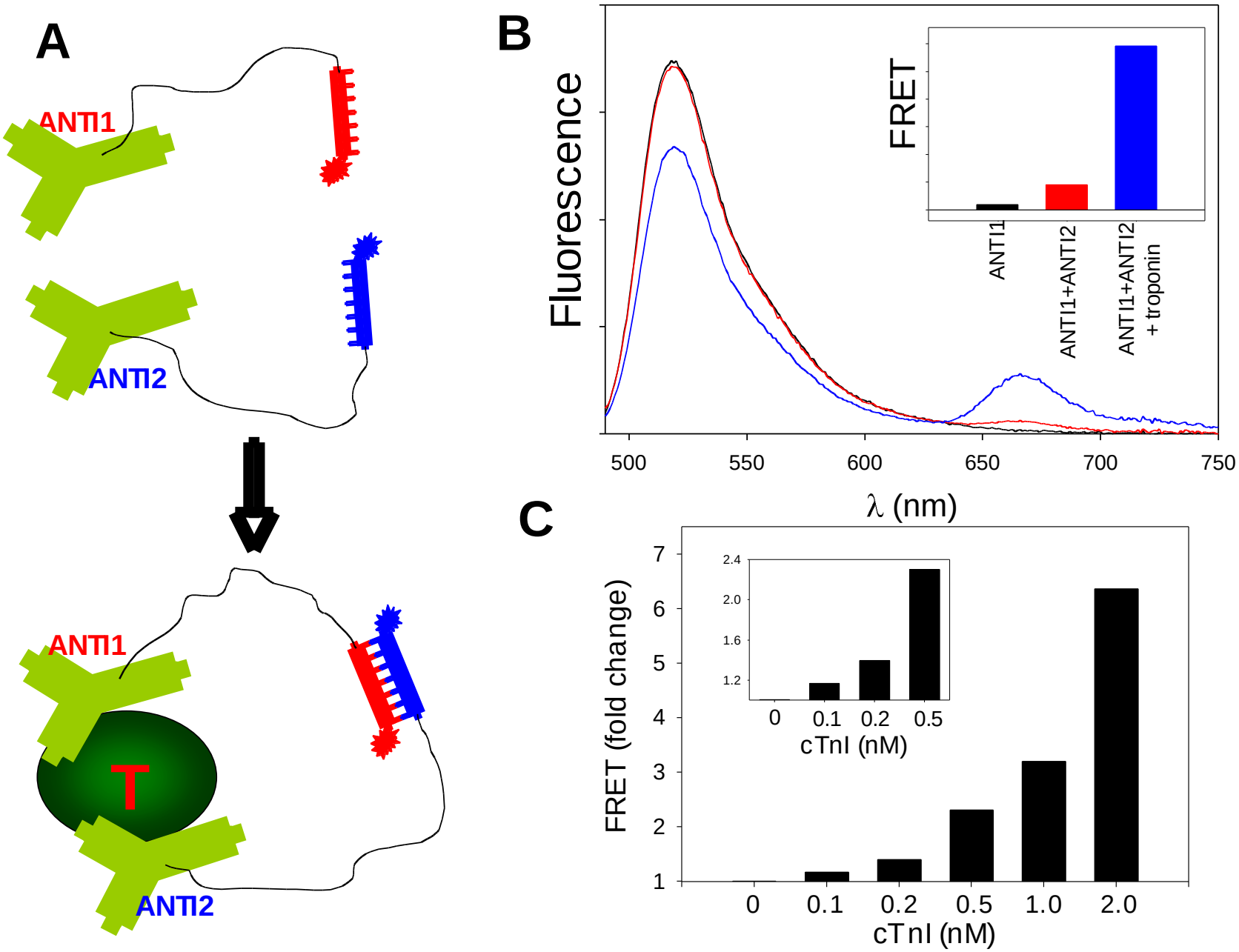


Compatibility with high-throughput screening

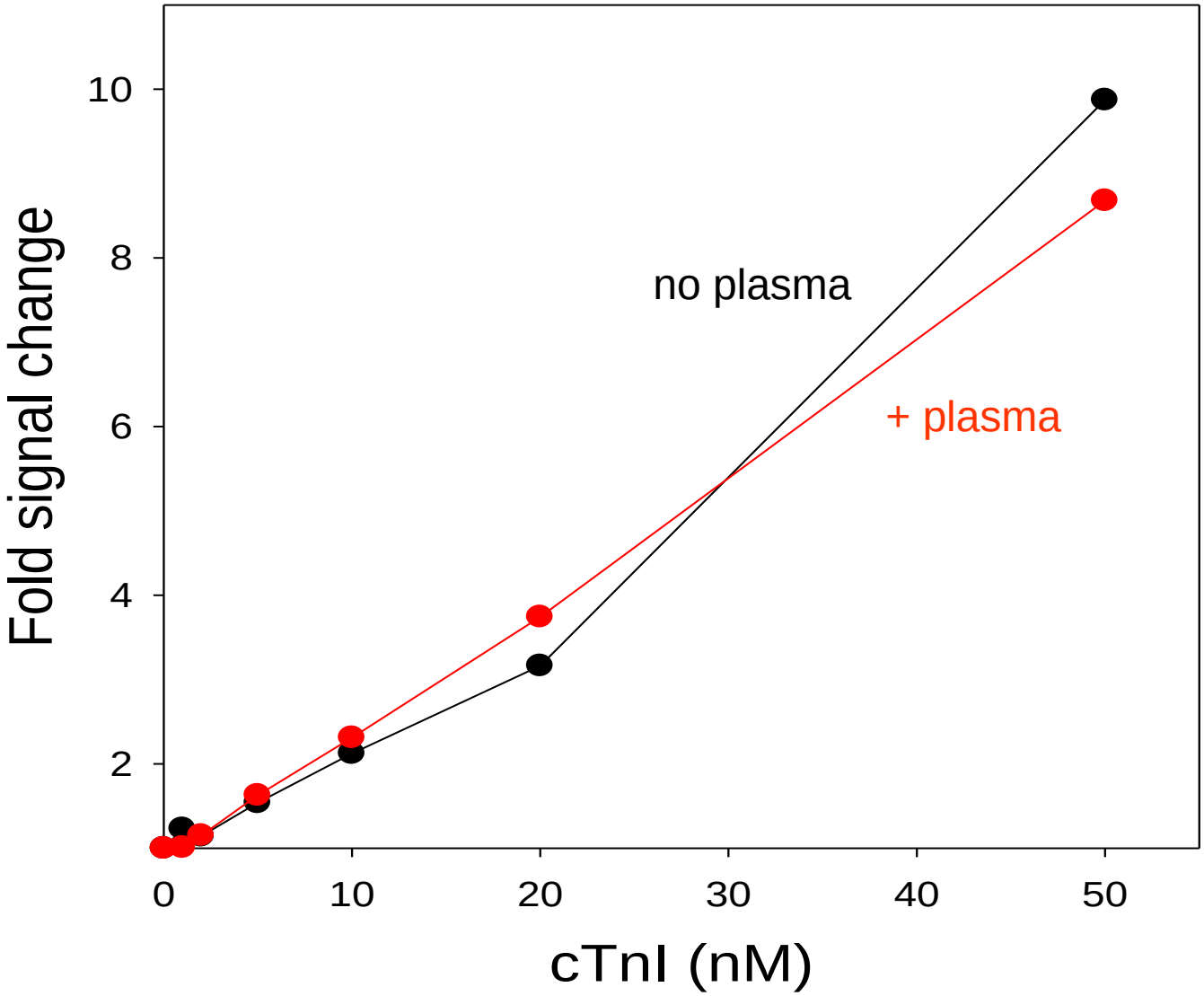


Variants of general design of "PINCERs"

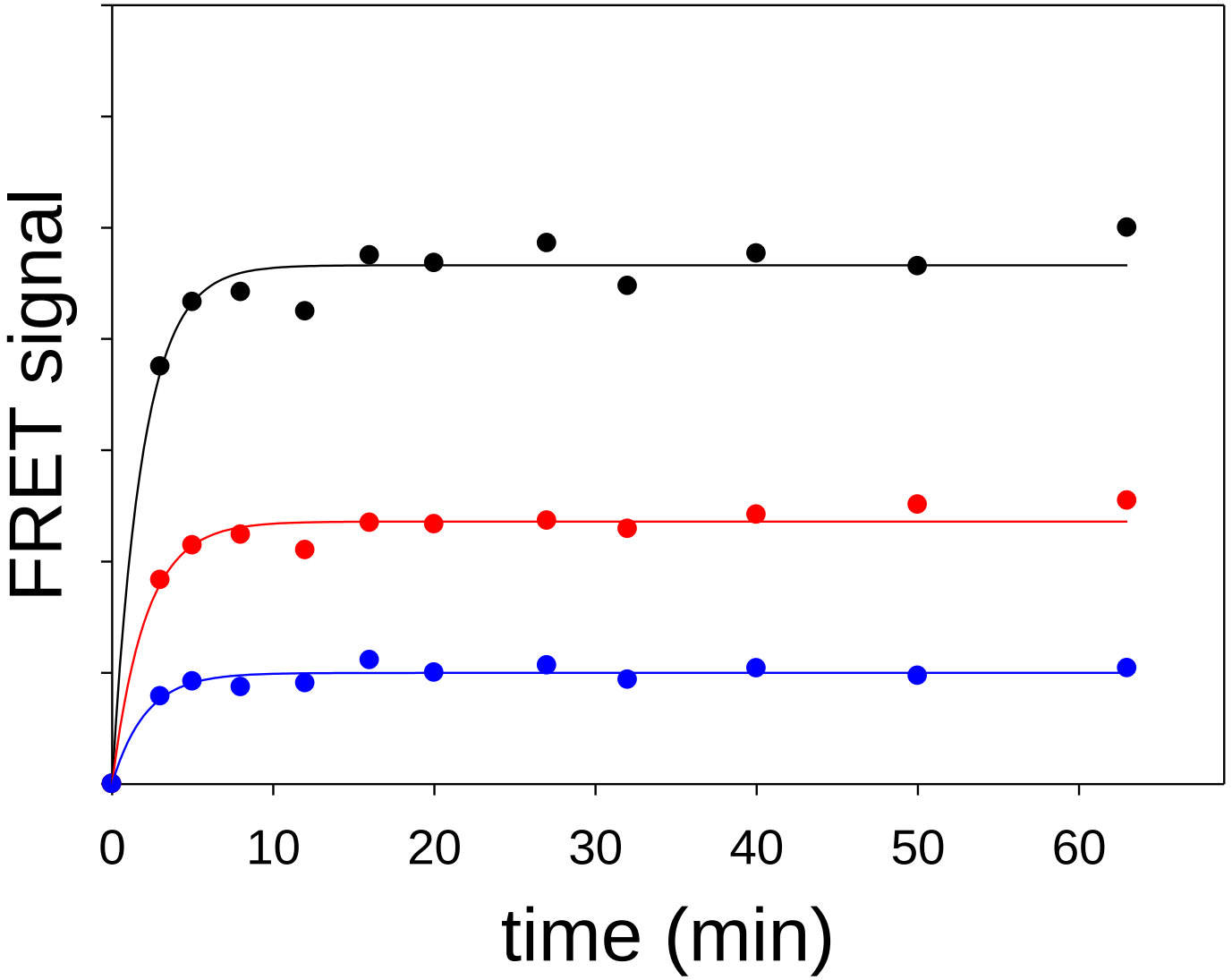



A
ANTI1
ANTI2
ANTI1
T
ANTI2
B
Fluorescence
FRET
ANTI1
ANTI1+ANTI2
ANTI1+ANTI2
+ troponin
500
550
600
650
700
750
λ (nm)
C
FRET (fold change)
7
6
5
4
3
2
1
0
0.1
0.2
0.5
1.0
2.0
cTnI (nM)
2.4
2.0
1.6
1.2
0
0.1
0.2
0.5
cTnI (nM)

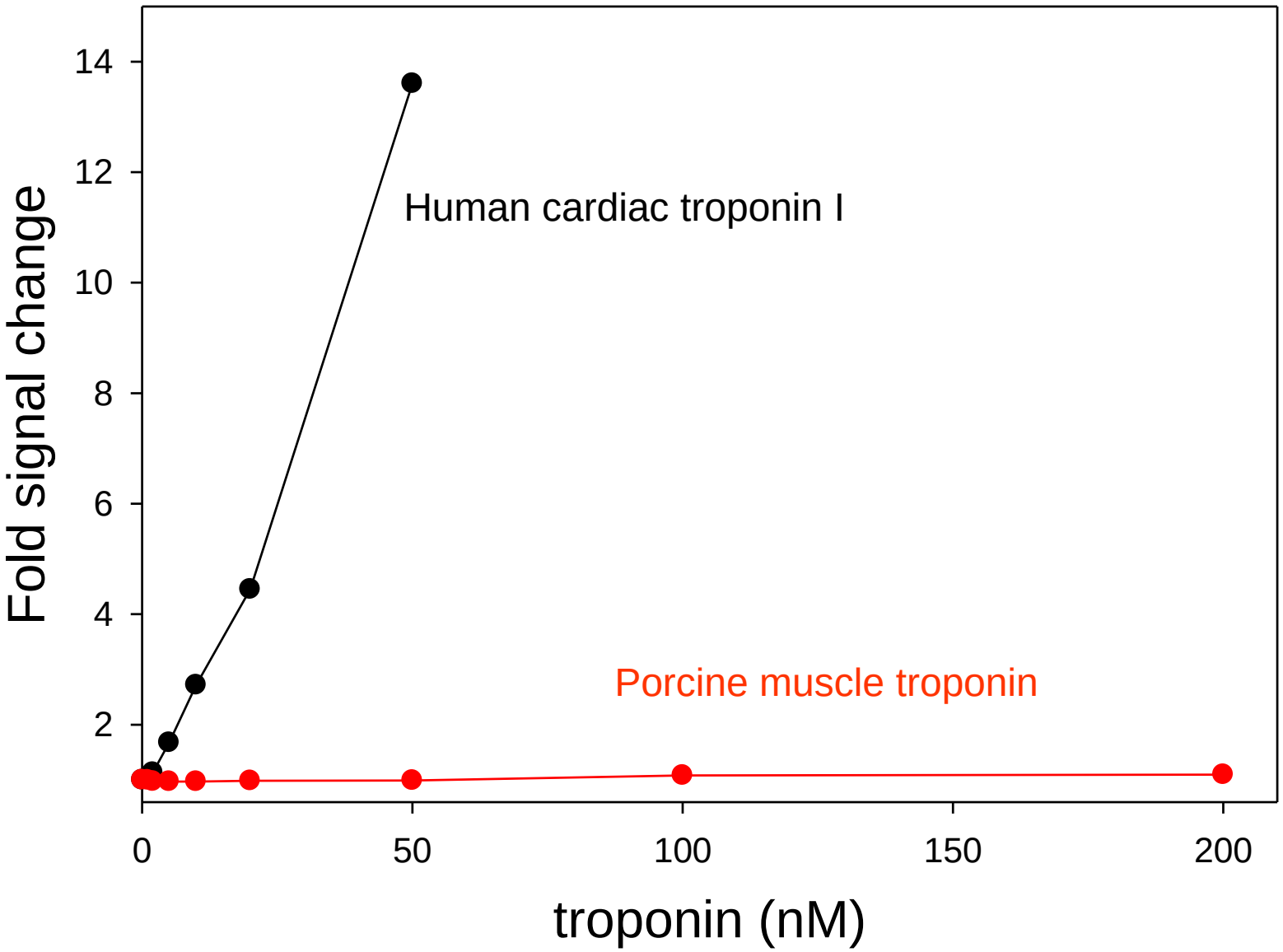
Performance in Plasma Samples



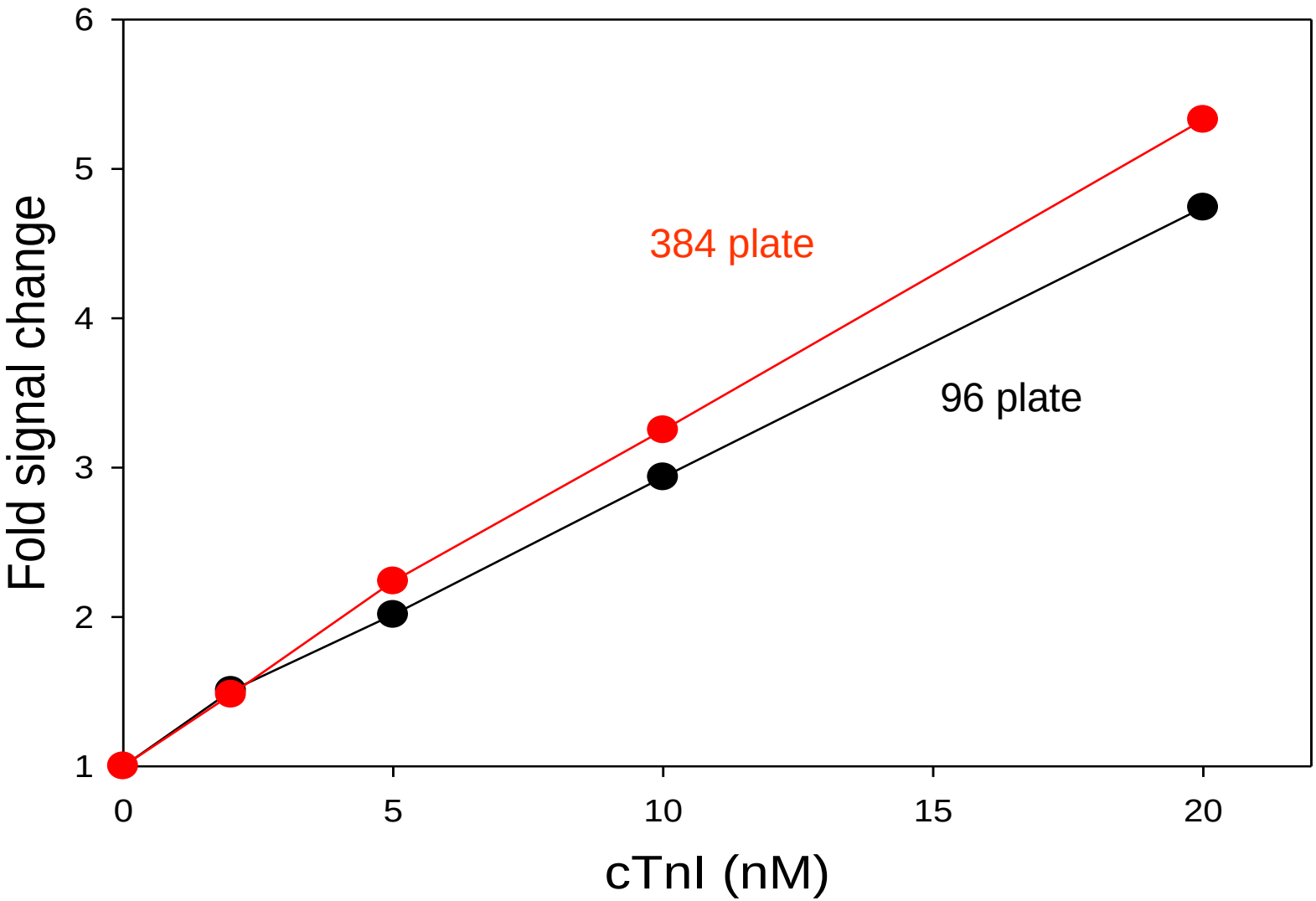
Assay Kinetics



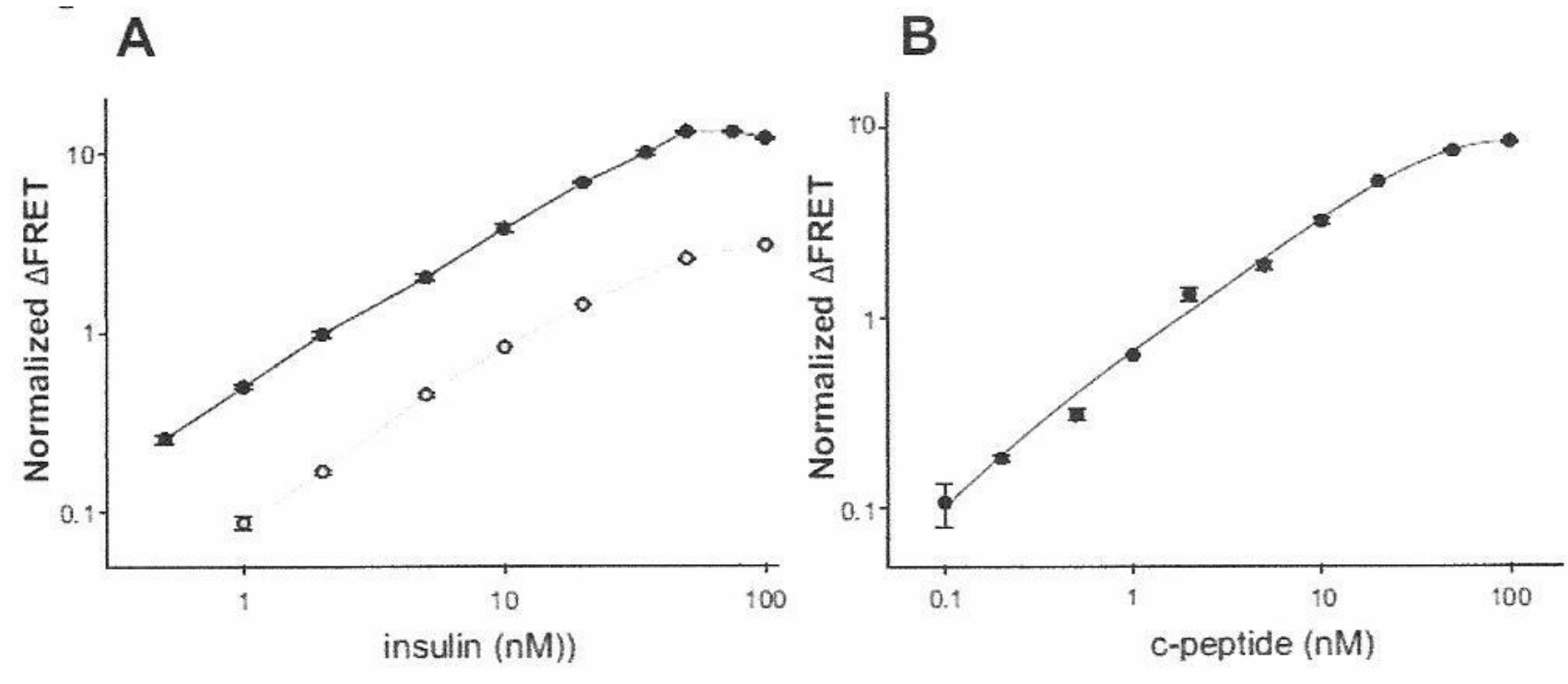
Specificity of the Assay



Throughput of the Assay

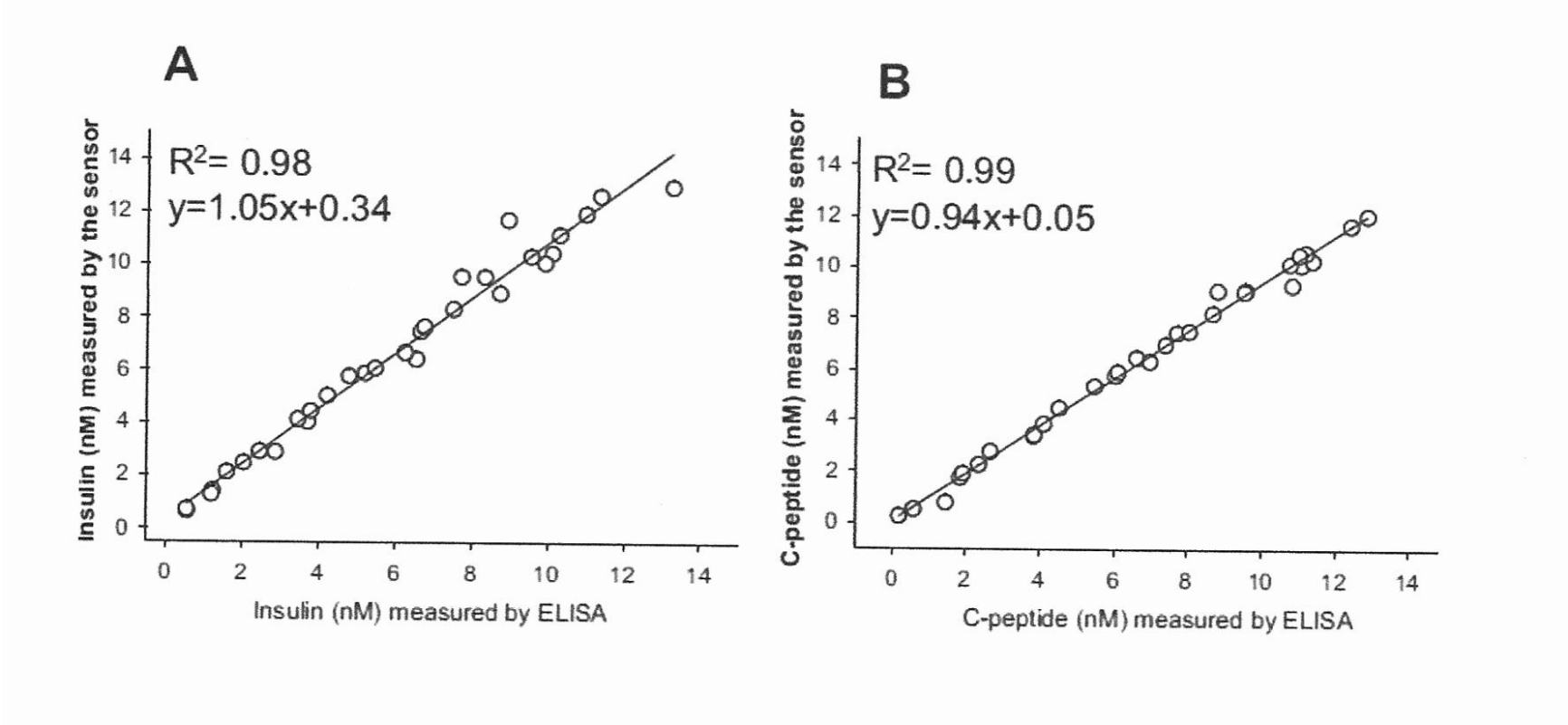


PINCER® assay for human insulin and C-peptide



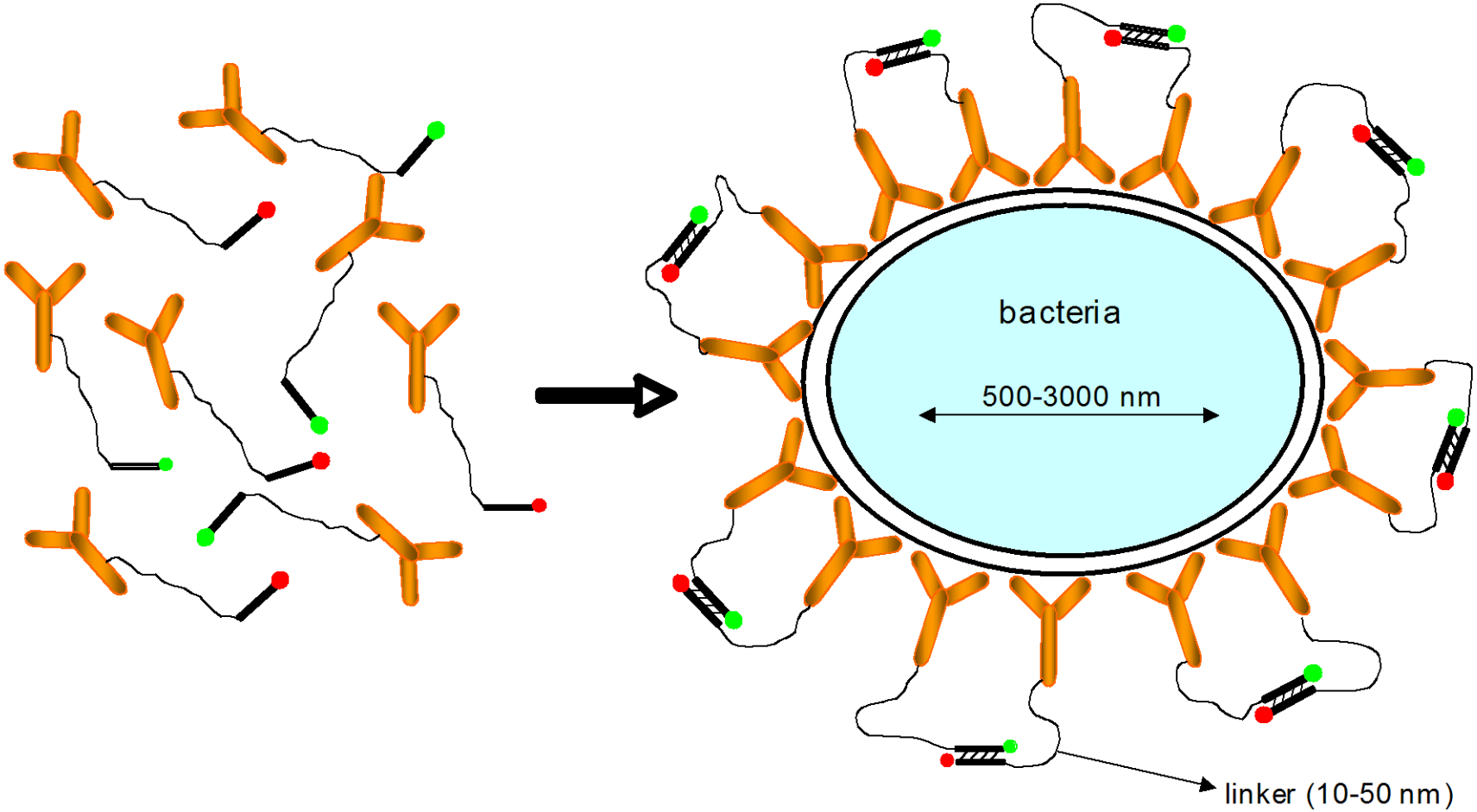
Heyduk T., Anal. Chem, 2010

PINCER ® Assay vs. ELISA-based Assay

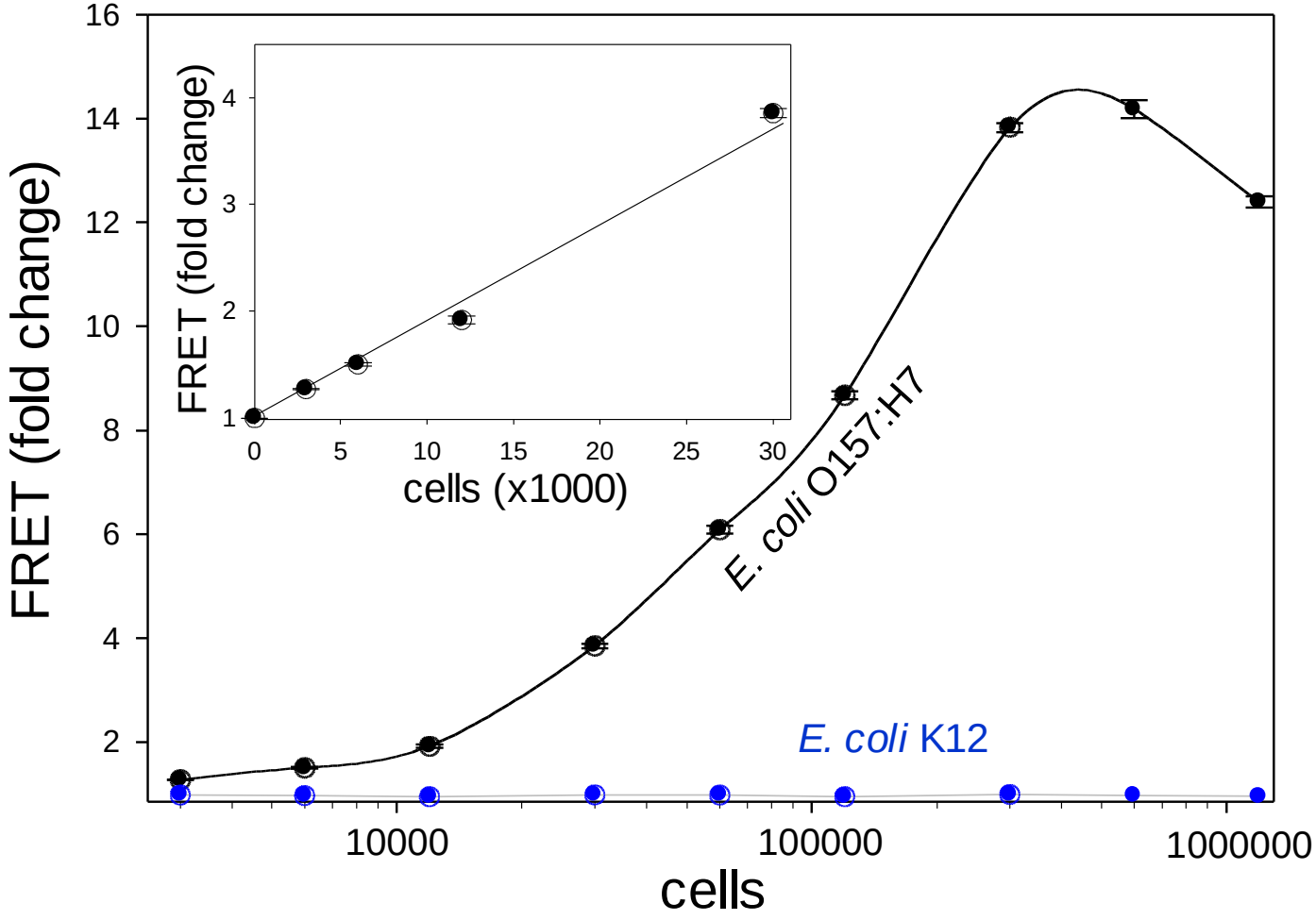


Heyduk T., Anal. Chem, 2010

Detection of pathogens



Detection of *E. coli* O157:H7



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Platform Technology Advantages

- Bioassays competitive advantages vs. ELISA-based assay
  - Simple – one step vs. multiple steps
  - Fast – 10-30 minutes vs. 2-4 hours
  - Cost-effective – manufacturing cost is 90% reduced
  - Reliable – easy to use, training need reduced
  - Flexible – use with beads, microarrays, different binders
- PINCER-based aptamer screening advantages vs. SELEX
  - Higher success rate
  - Higher throughput capability

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Hepatitis C facts

1. A global public health problem.

2. Early identification of acute HCV infection is important.

3. Presence of anti-HCV antibodies is an indirect evidence for HCV infection.

4. Acute phase vs chronic phase:

   A. anti-HCV-IgG reactivities to specific HCV antigens[1];

   B. anti-HCV IgM serum level[2].

1. Araujo AC, Astrakhantseva IV, Fields HA, Kamili S. 2011. *J Clin Microbiol*. 49, 54.
2. Coppola, et al. 2009. *J Clin Virol.*, 46, 222.

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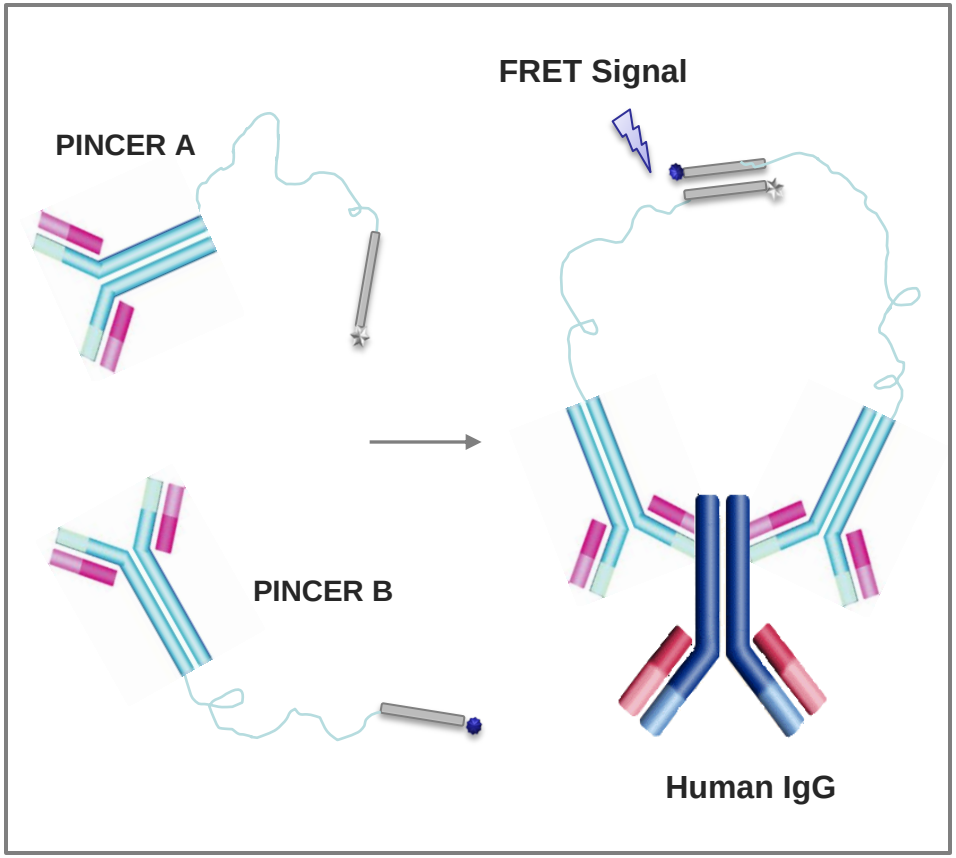
PINCER® Assay for Detection of Antibody

- PINCER pair: goat anti-human IgG antibodies modified with complimentary oligonucleotides.
- Presence of human IgG results in Fluorescent Resonance Energy Transfer (FRET).
- FRET signal is target concentration dependent.



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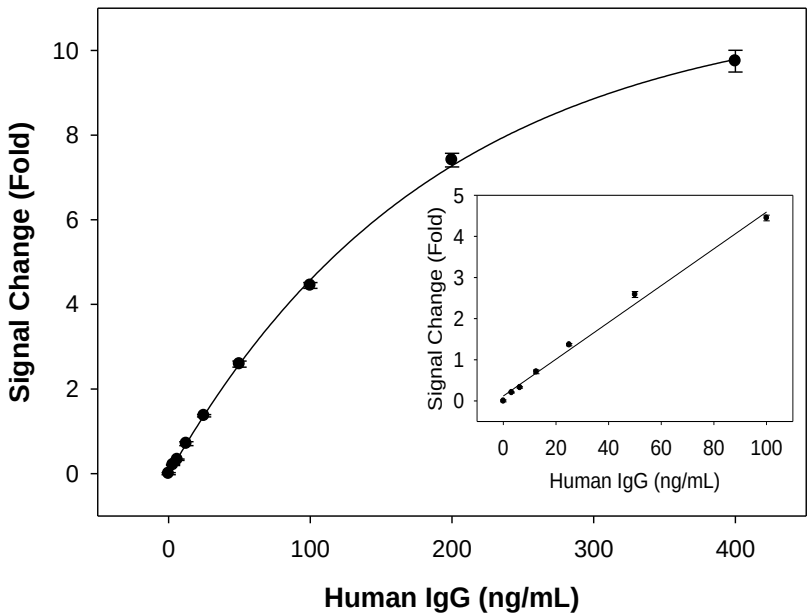
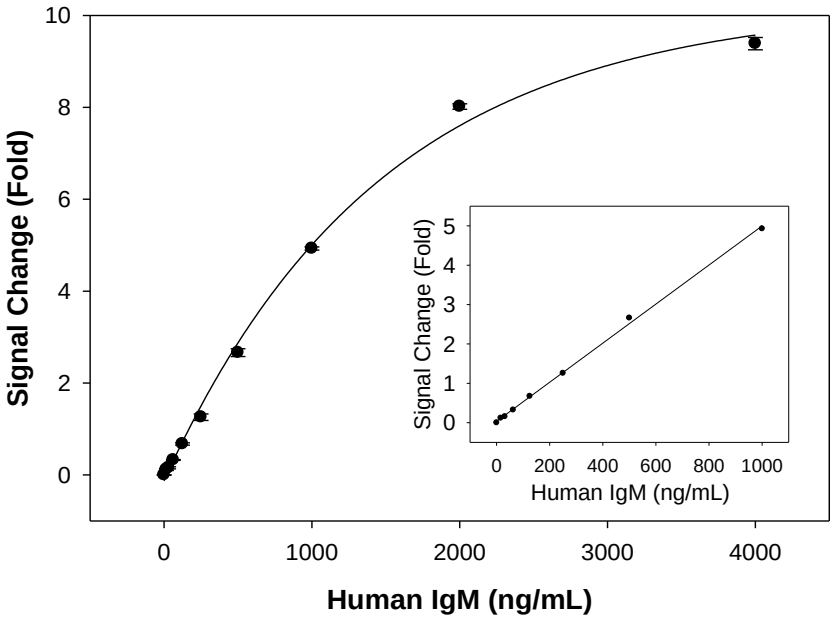
Optimization of PINCER® assays for detection of anti-HCV antibodies

Homogeneous Human IgG and IgM PINCER® assays.





LDL: 5 ng/ml (33 pM)
CV: < 3.4%
Linear dynamic range: 0-100 ng/ml

LDL: 15 ng/ml (16.5 pM)
CV: < 3.2%
Linear dynamic range: 0-1 μg/ml

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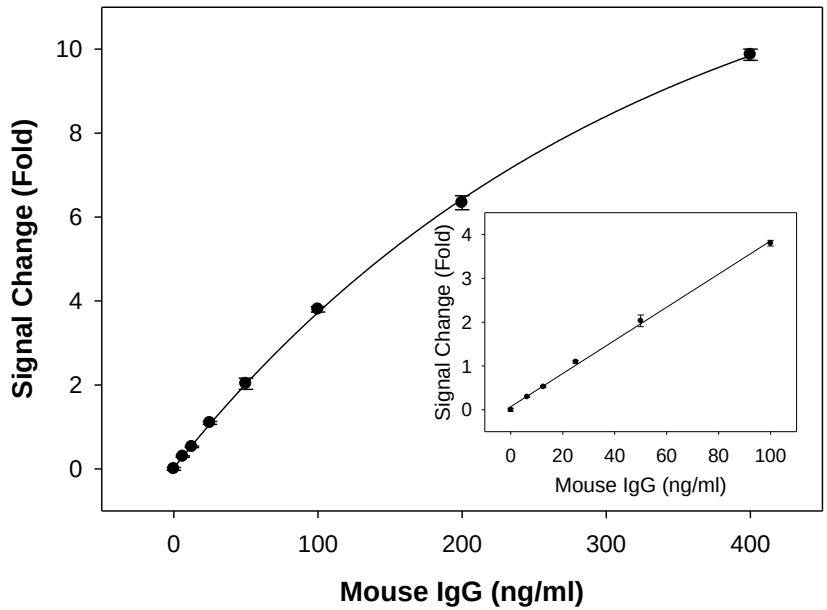
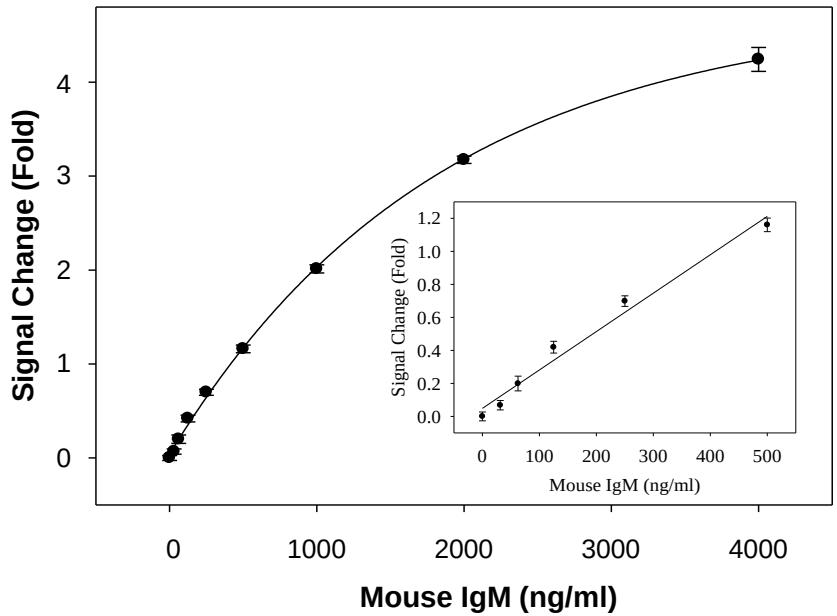
Optimization of PINCER® assays for detection of anti-HCV antibodies

Homogeneous Mouse IgG and IgM PINCER® assays





LDL: 5 ng/ml (33 pM)
CV: < 3.4%
Linear dynamic range: 0-100 ng/ml

LDL: 30 ng/ml (33 pM)
CV: < 3.6%
Linear dynamic range: 0-500 ng/ml

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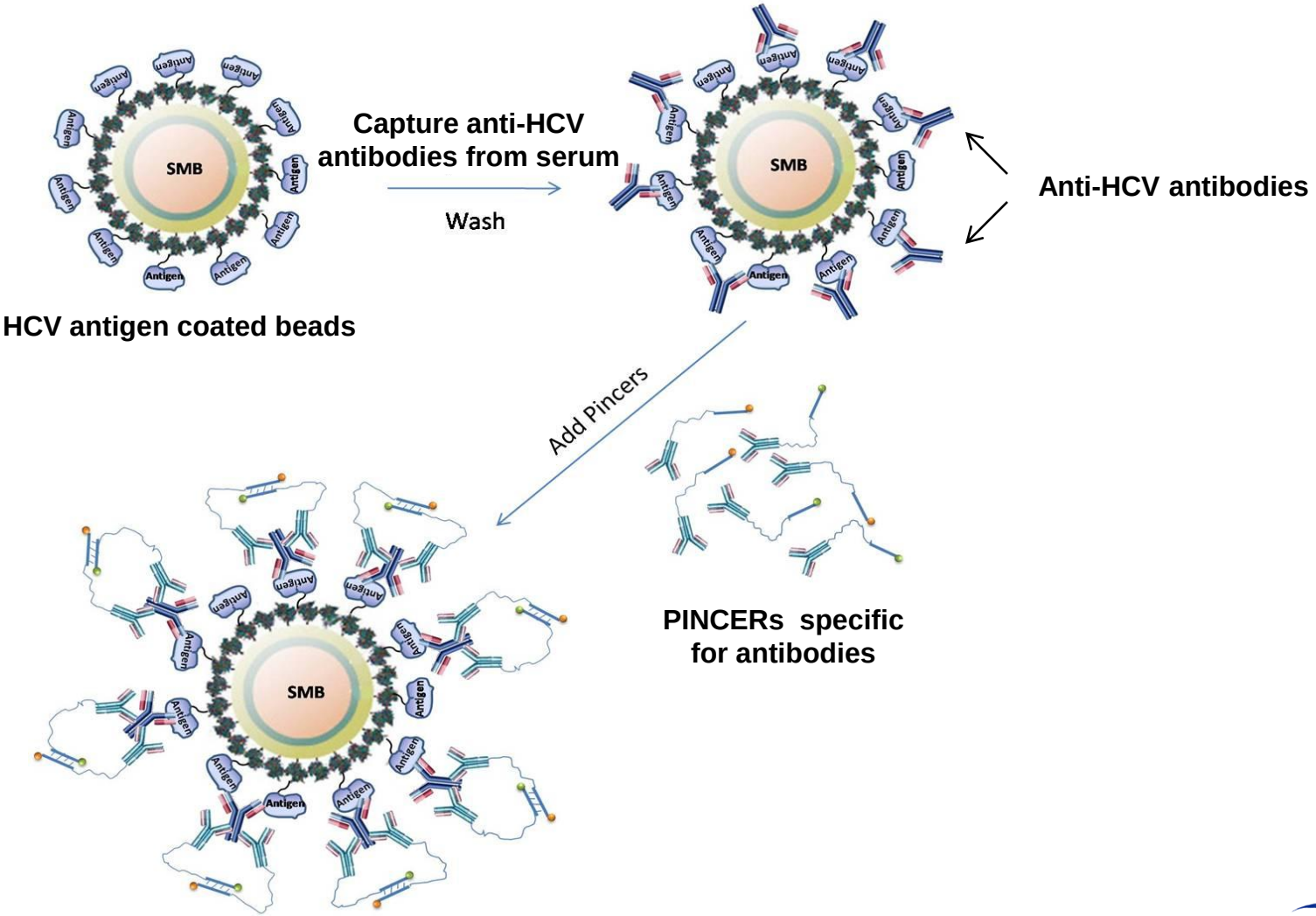
Detection of Anti-HCV Antibodies – How PINCER® Technology Is Applied?



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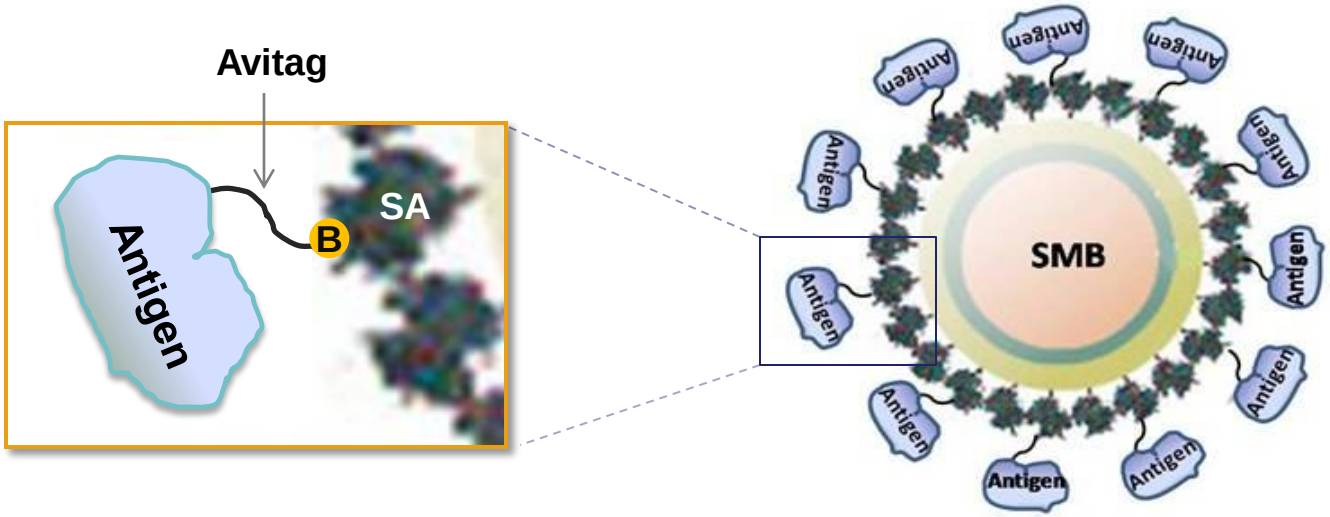
Immobilization of the recombinant HCV antigens on beads through Avitag.



Advantages to introduce an Avitag in the recombinant antigens:

1. Allows immobilization in a highly reproducible manner.
2. Allows all the epitopes on antigens to be exposed in solution and available for capture of anti-HCV antibodies.

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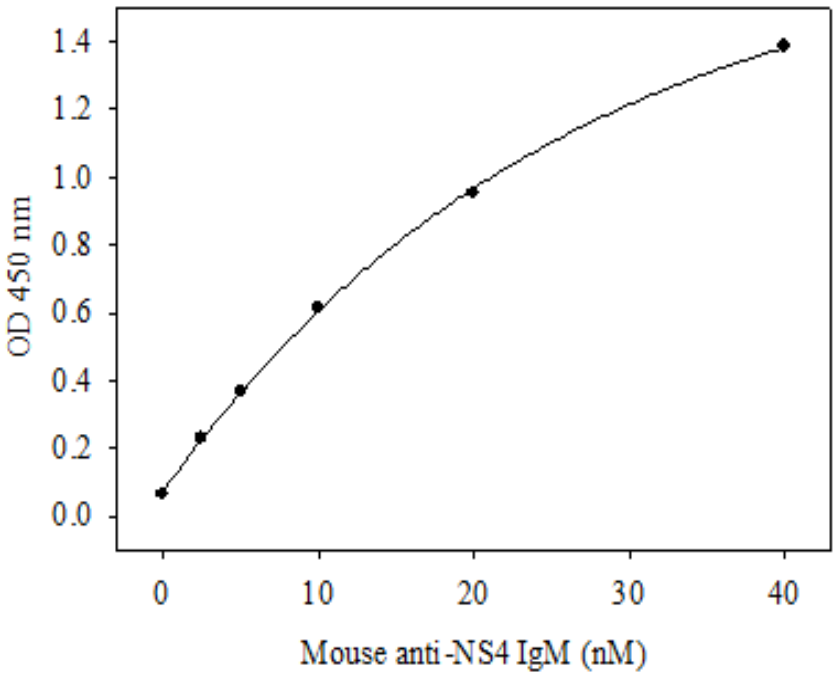
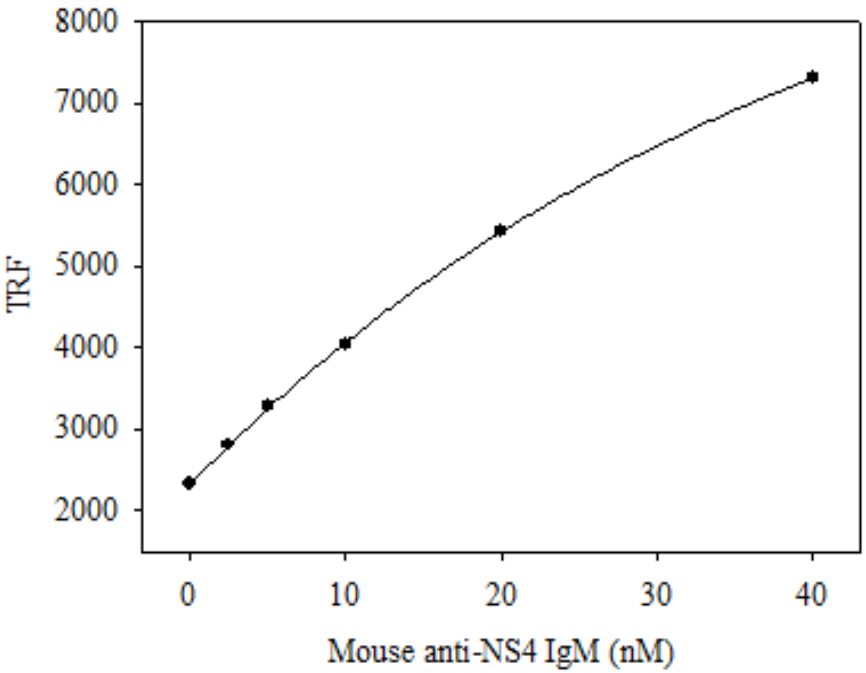
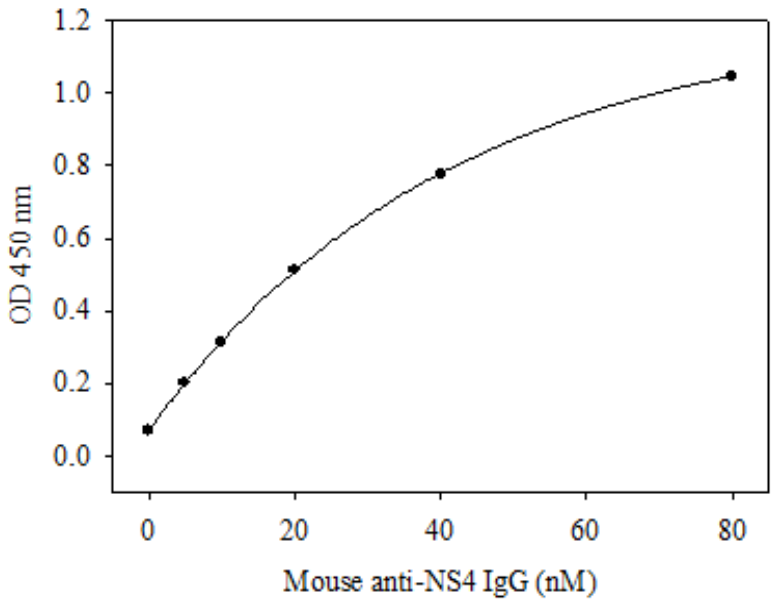
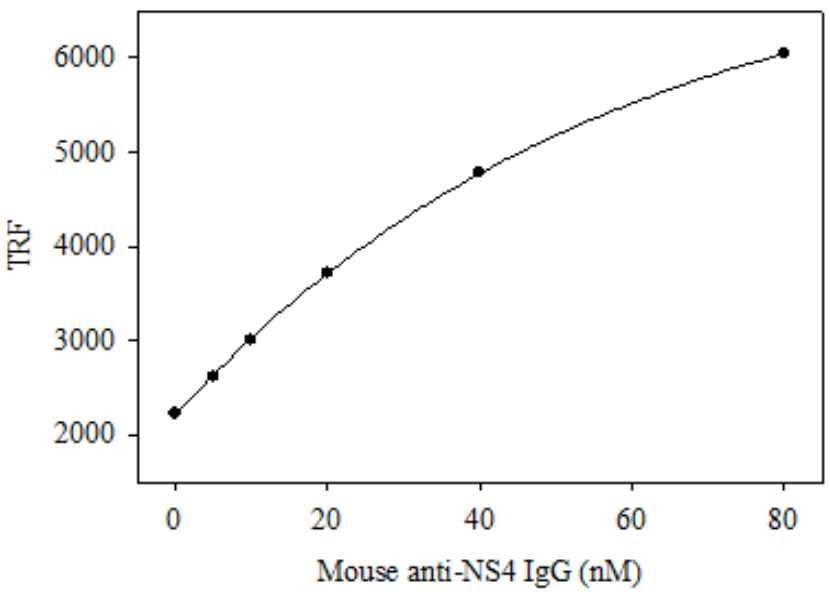
Detection of the mouse anti-NS4 IgM captured on beads using ELISA (left) or PINCER assay (right).



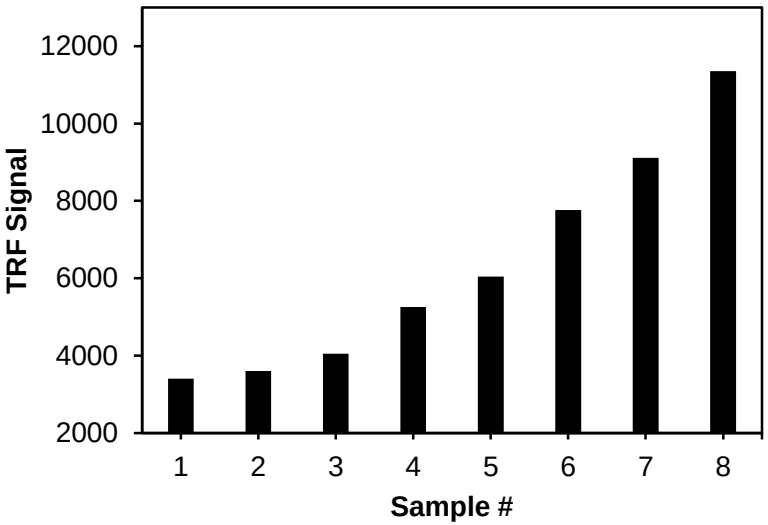


Detection of the mouse anti-NS4 IgG captured on beads using ELISA (left) or PINCER assay (right)

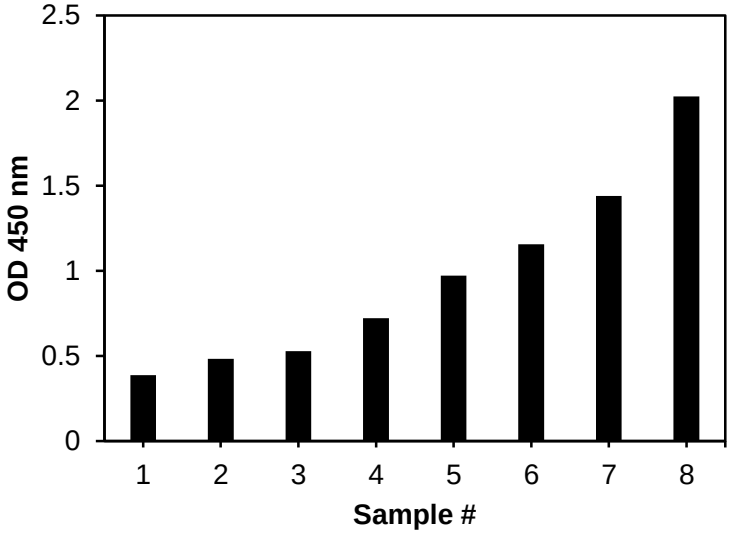




Evaluation of PINCER assay performance with patient serum samples

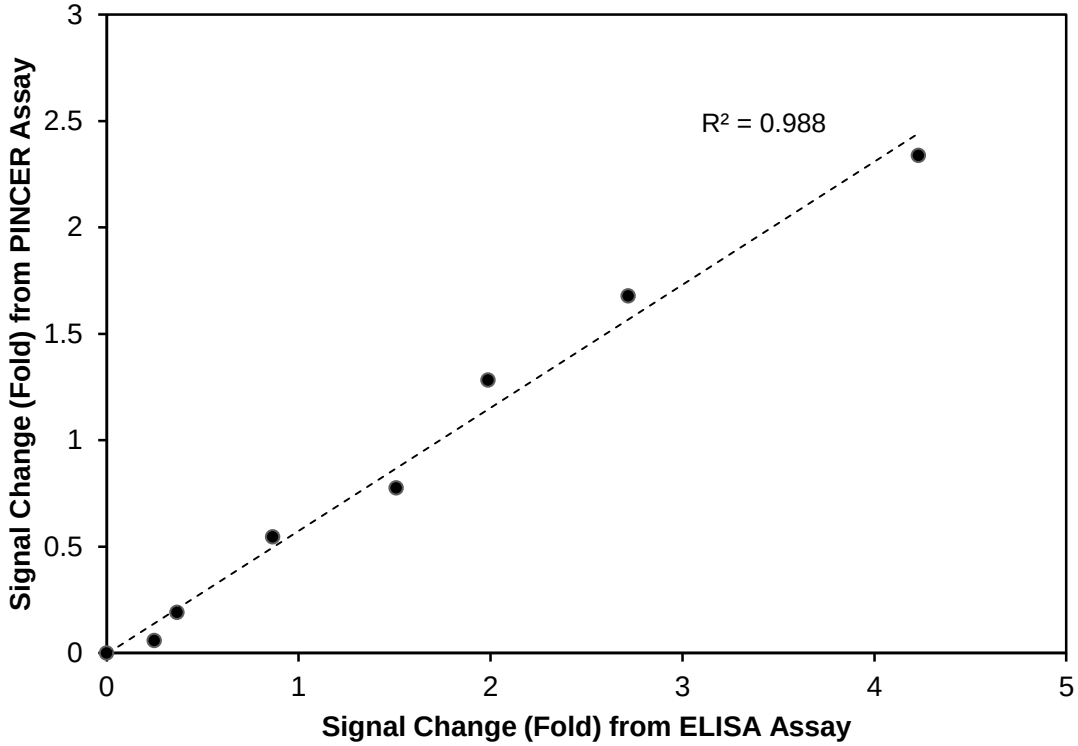


PINCER assay

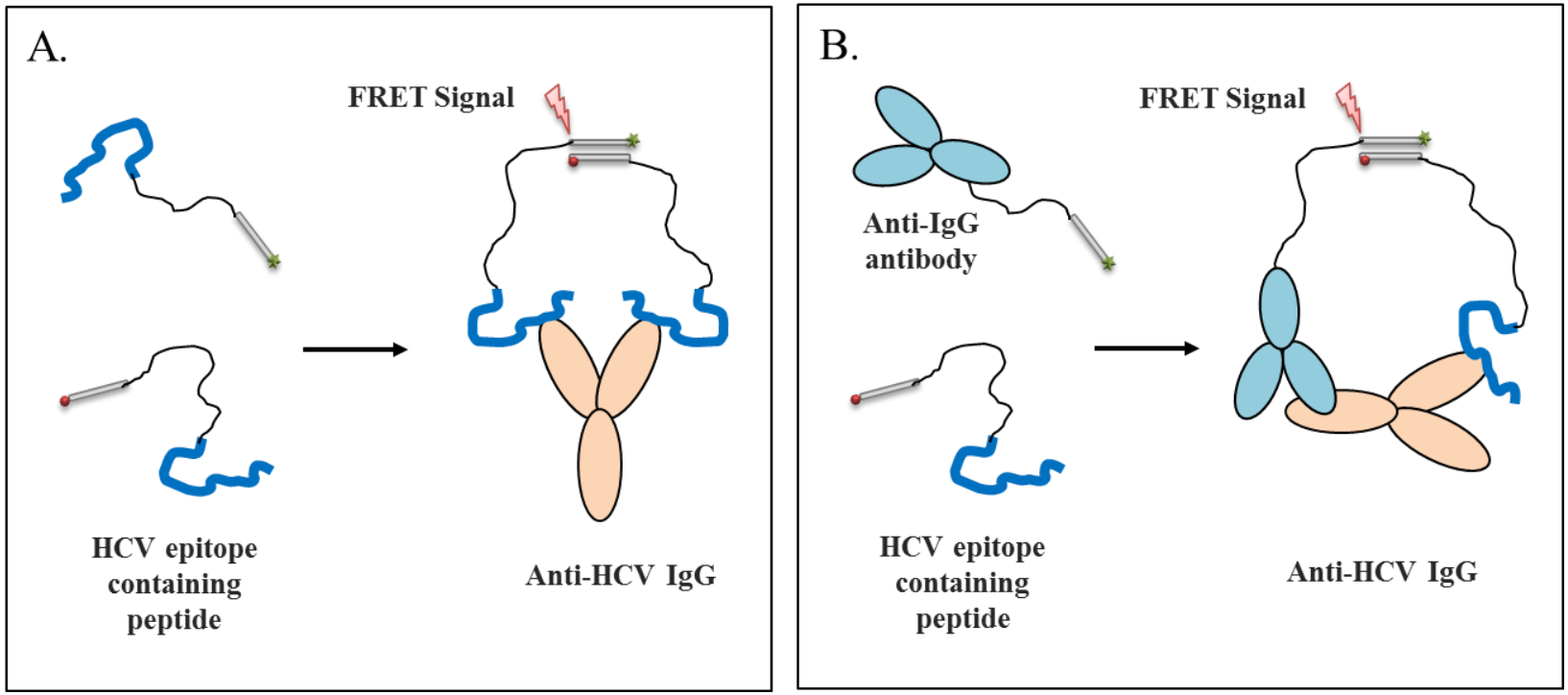


ELISA assay

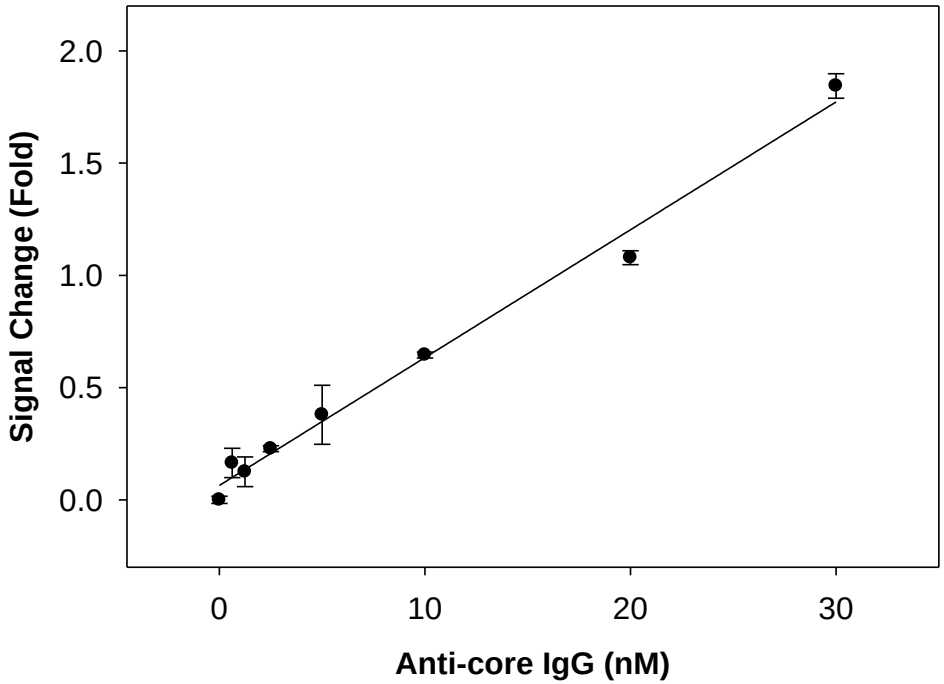
Correlation between data from PINCER and ELISA-based assays



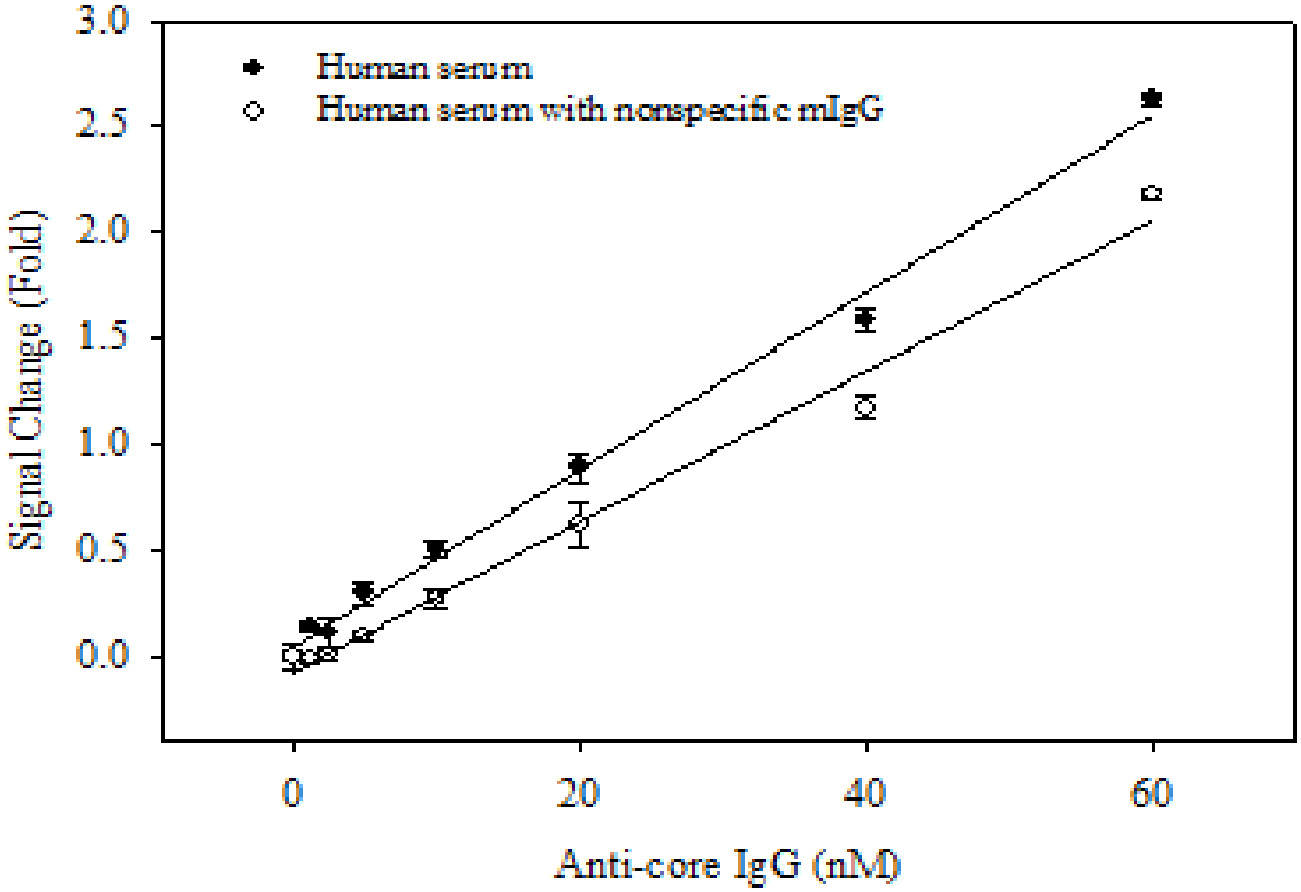
Designs of PINCER Assays for detection of anti-HCV antibodies



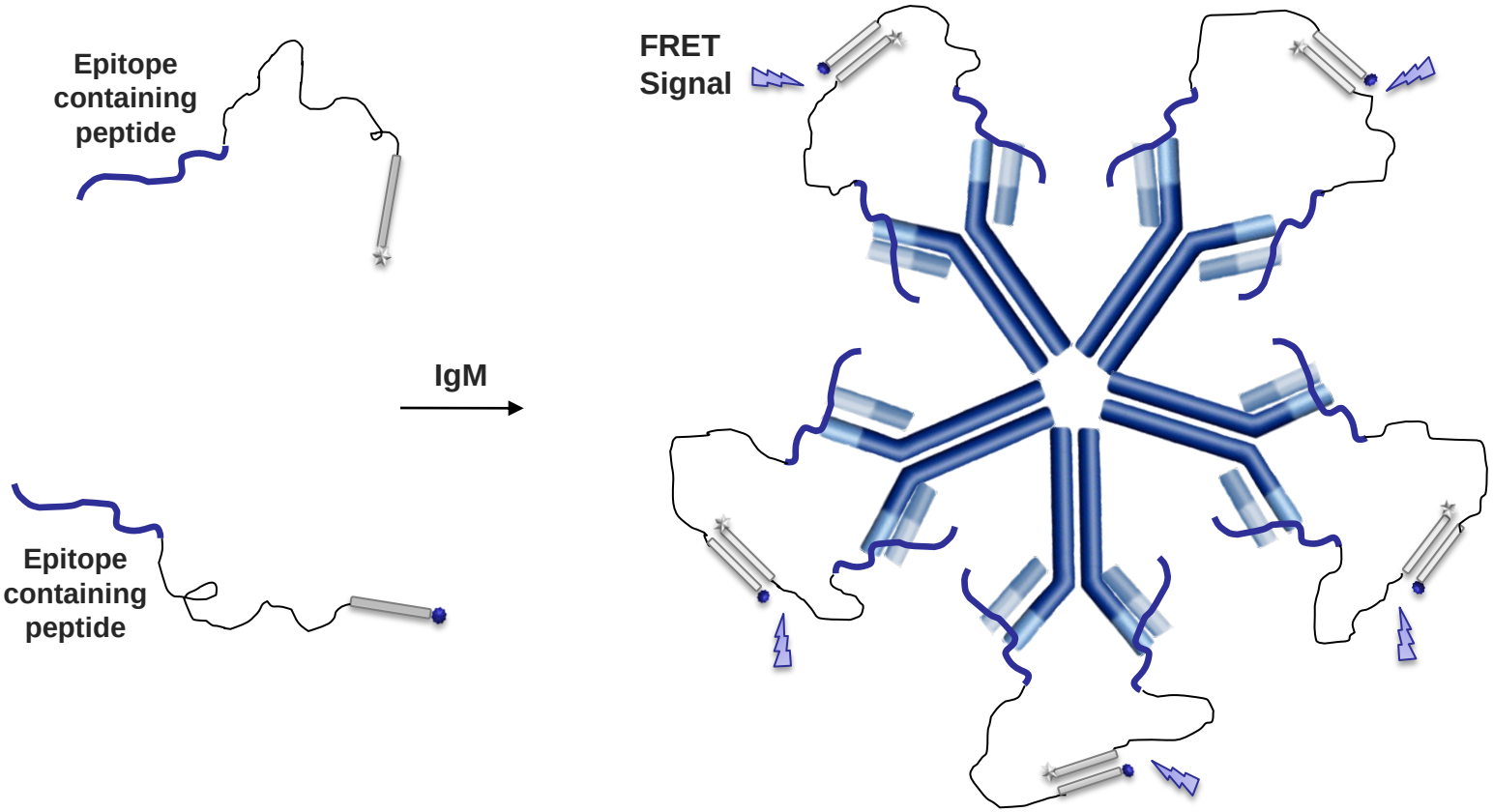
Evaluate the performance of the PINCER assays for anti-HCV core IgG



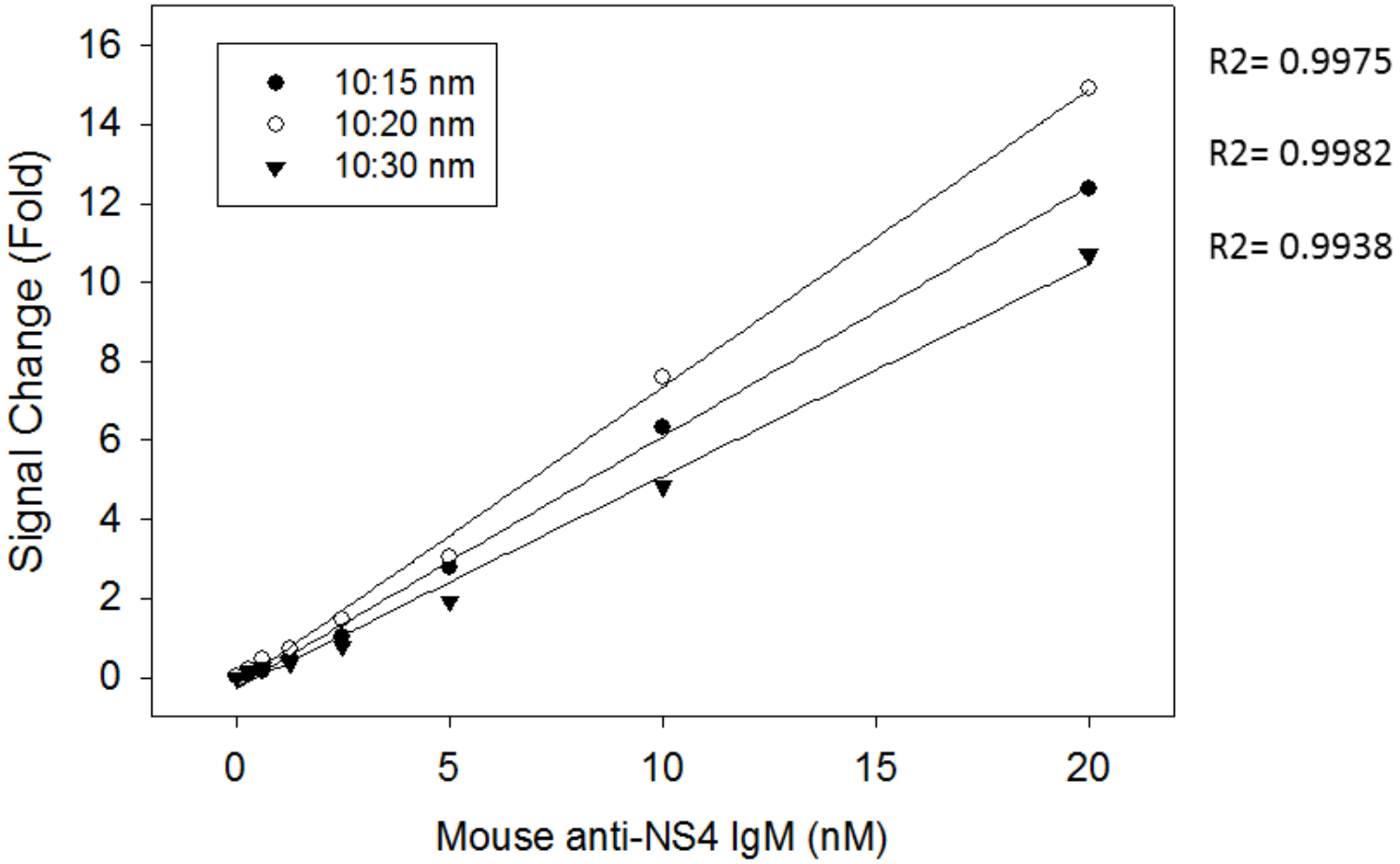
Evaluate the performance of the PINCER assays for anti-core IgG



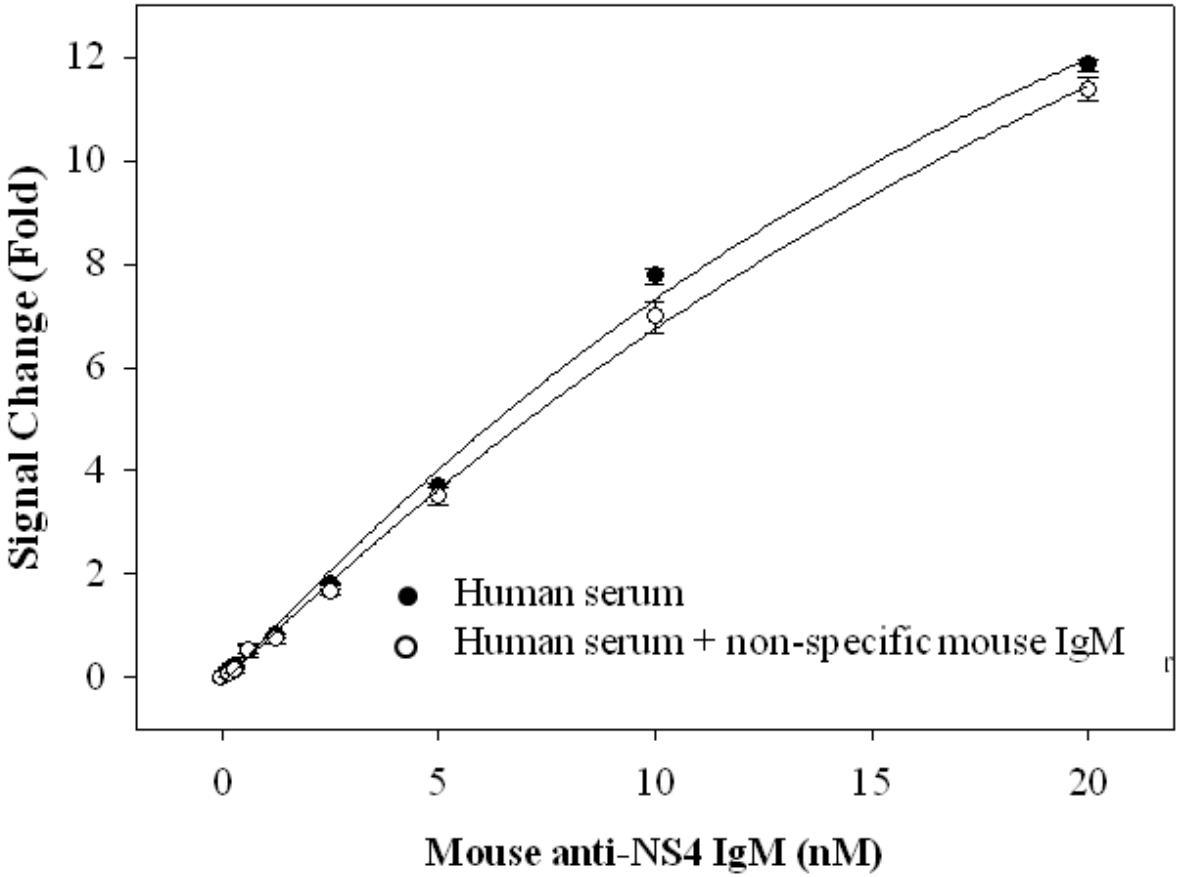
PINCER assay for detection of antigen-specific IgM



Evaluate the performance of the PINCER assays for anti-HCV NS4 IgM



Evaluate the performance of the PINCER assays for anti-NS4 IgM in spiked human serum samples



Summary

1. Homogeneous PINCER assays for anti-IgG and anti-IgM were developed.

2. Novel method to detect anti-HCV antibodies with the magnetic beads conjugated with HCV antigens coupled with quick PINCER assays were developed.

3. Homogeneous PINCER assay for direct detection of anti-HCV antibodies were developed.

Future Directions

1. Validate the Mosaic antigen-based PINCER assays with additional patient samples.

2. Optimize the PINCER-based homogeneous assays using the Mosaic antigen.

3. Validate the PINCER-based homogeneous assays to detect anti-HCV antibodies in patient samples.

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Acknowledgements

Mediomics, LLC

Ling Tian, Ph.D. (Project leader)
Ross Wang, Ph.D,.

Saint Louis University Doisy Research Center

Tomasz Heyduk, Ph.D.

Loyola University Medical Center

Claus Fimmel, M.D.

CDC

Aufra Araujo, Ph.D.
Saleem Kamili, Ph.D.

Funding:

CDC SBIR Phase I Contract

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Mediomics, LLC

Contact: Dr. Yie-Hwa Chang
yiechang@mediomics.com
314-971-3026

Dr. Ling Tian
Tianl@mediomics.com

Website: www.mediomics.com

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