

# Highly Multiplexed Label-Free Imaging Sensor for Accurate Quantification of Small-Molecule Binding Kinetics

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# Outline

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- The importance of **small molecule characterization**
- Our approach: the **Interferometric Reflectance Imaging Sensor (IRIS)**.
- Beating the sensitivity limit: **noise reduction methods** and proof of concept experiments (**Biotin**, MW = 244Da).
- Agro-biotech application: characterization of **fumonisin B1 toxin** (MW = 722 Da)
- Conclusions and future work



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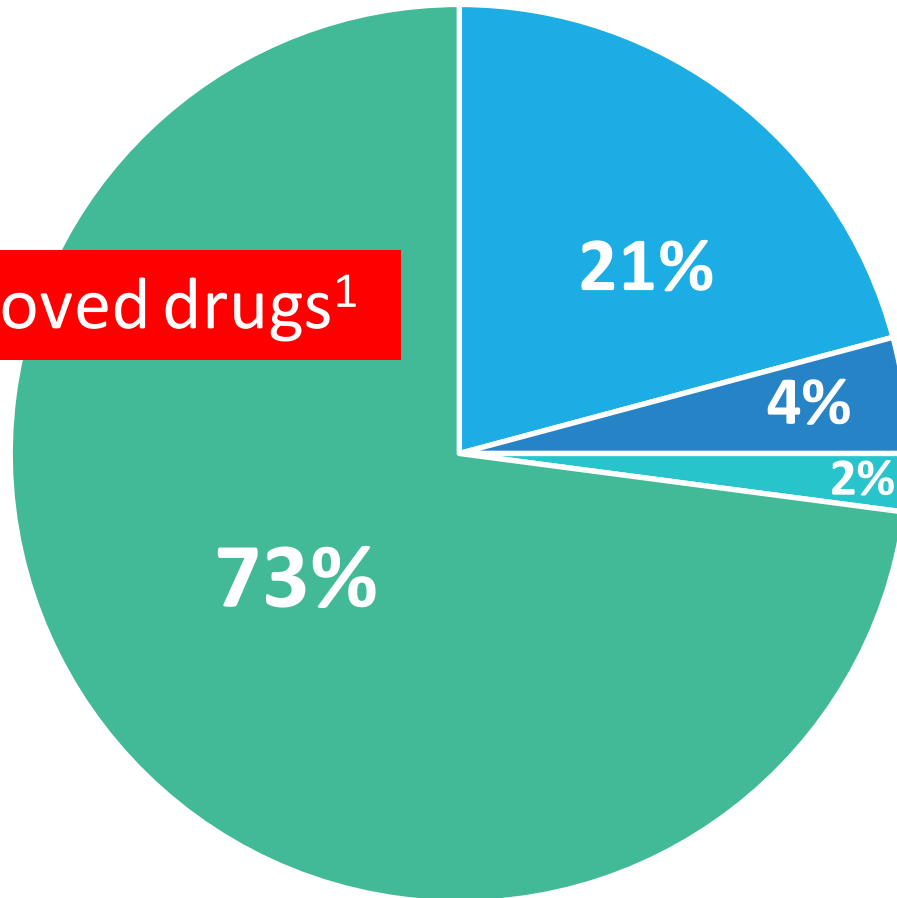


# Why small molecules?

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73% of 2019 FDA approved drugs<sup>1</sup>

99% of all toxins<sup>2</sup>



## FDA approvals (2019)

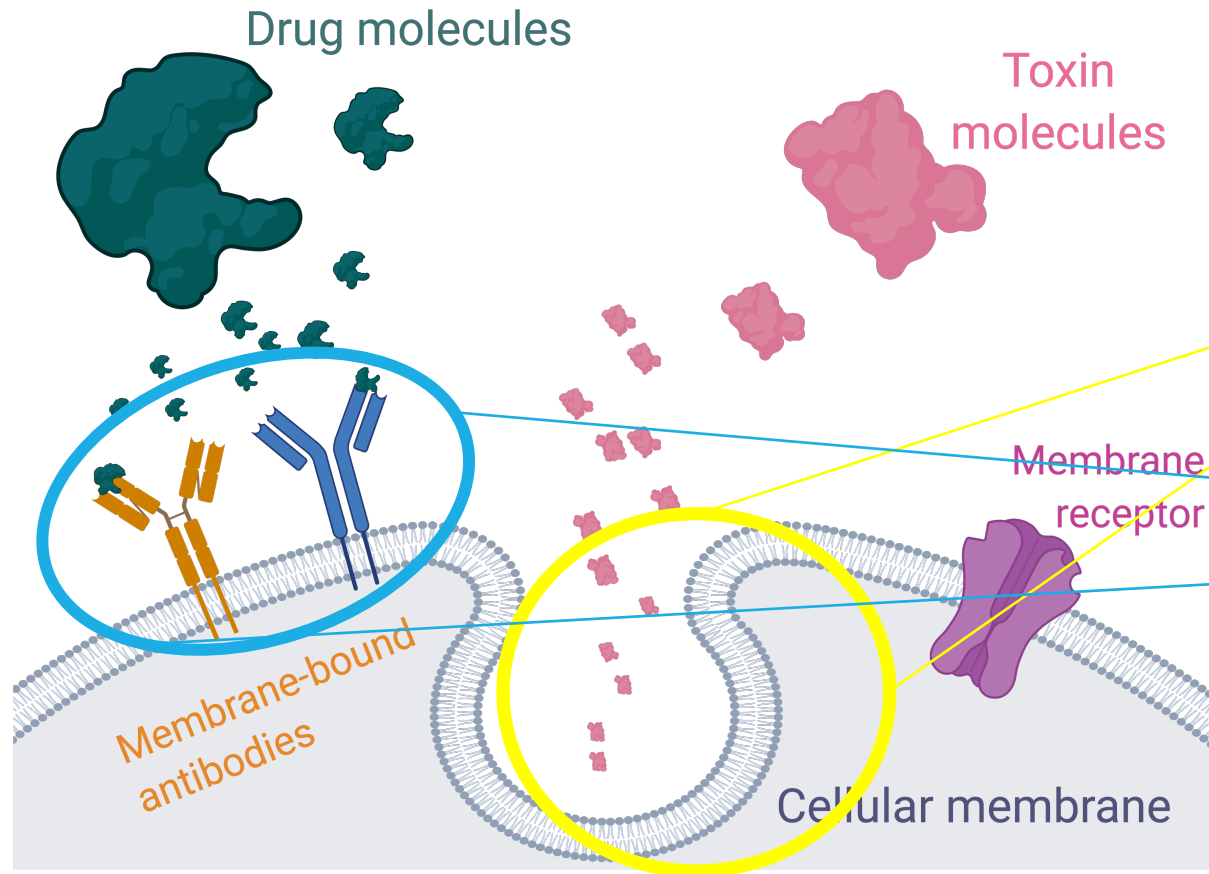
- Proteins
- Oligonucleotides
- Others
- Small molecules

[1] Mullard, *Nature Reviews Drug Discovery* **19**, 79-84 (2020)

[2] Wishard, D. et al., *T3DB: the toxic exposome database*. *Nucleic Acids Res.* (2015)

# Why small molecules?

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**A small size** implies...

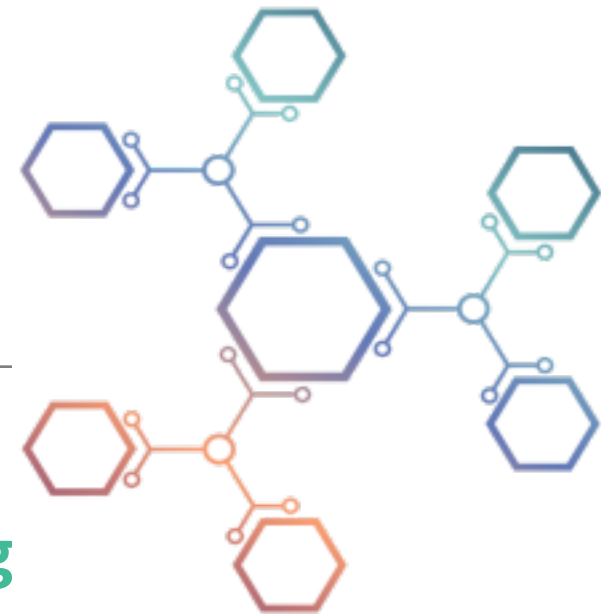
**Easy access to the cellular membrane**

**Easy antibody-based recognition**

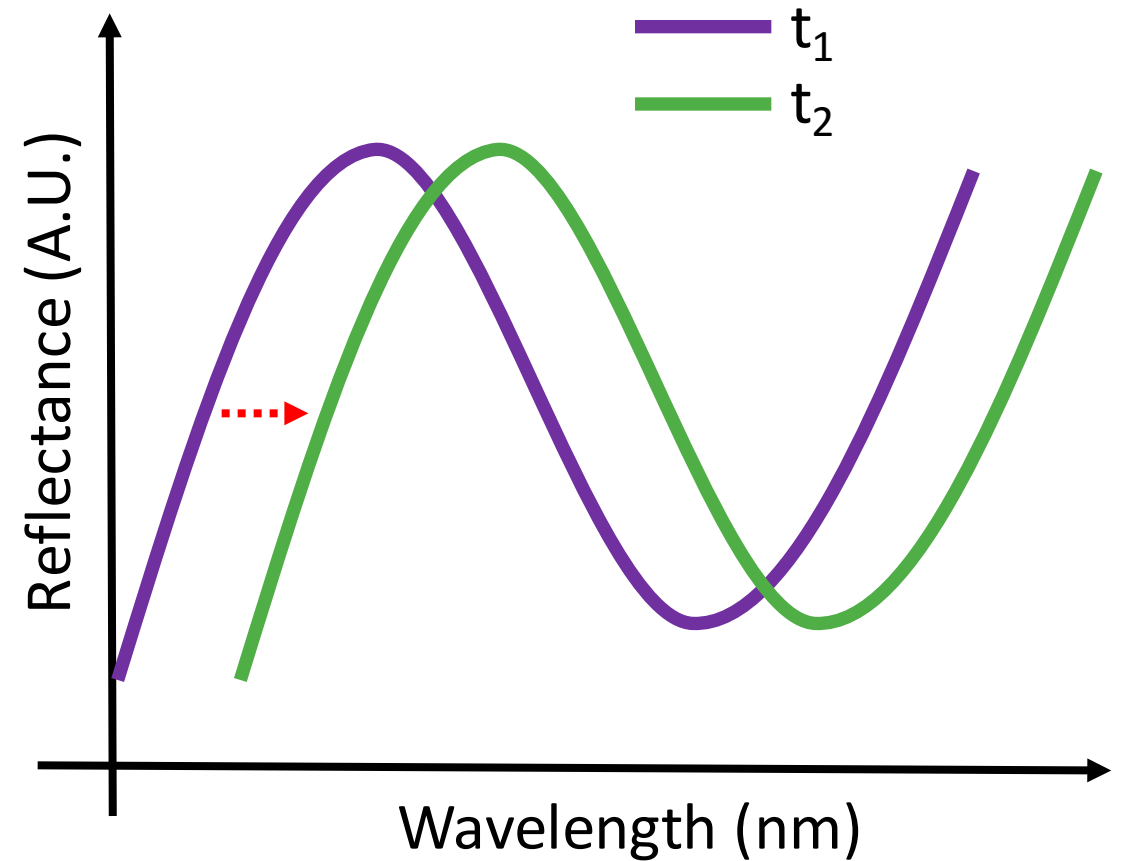
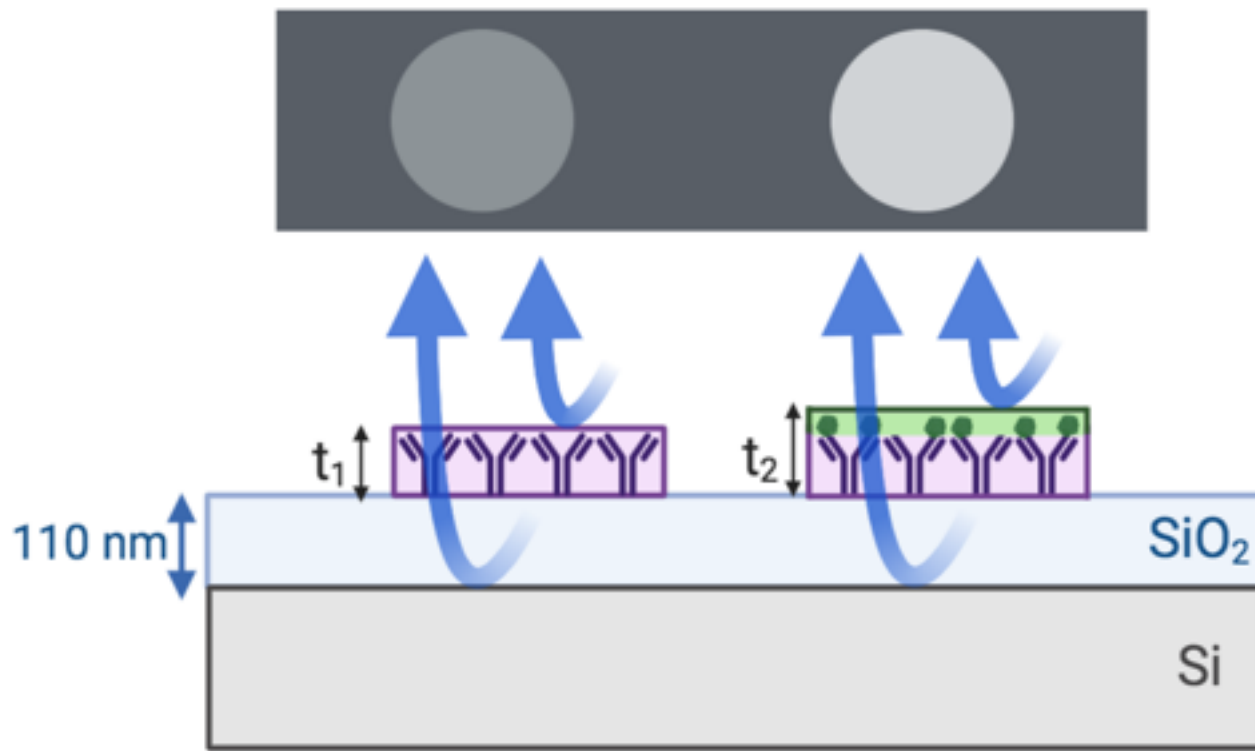
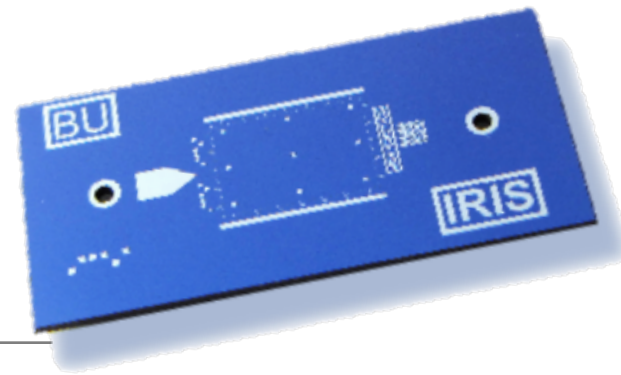
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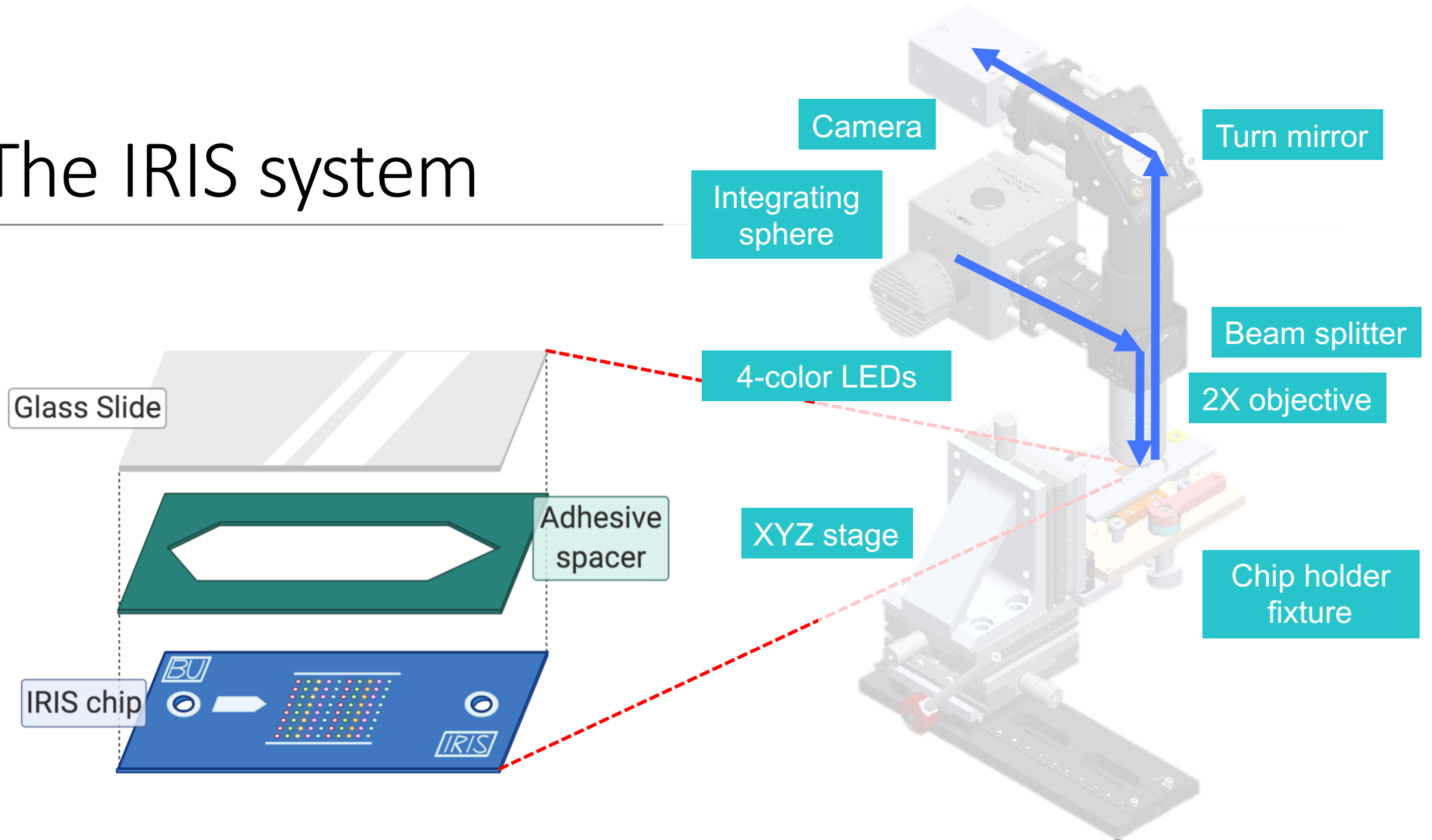
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# The IRIS system



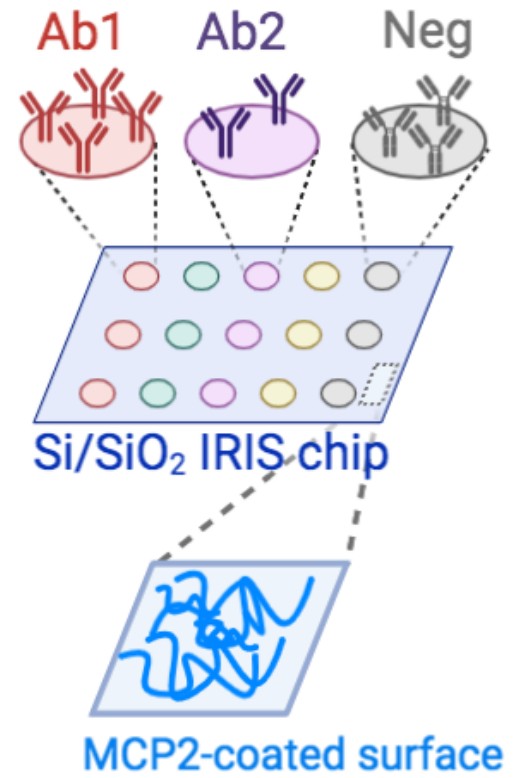
# The IRIS system



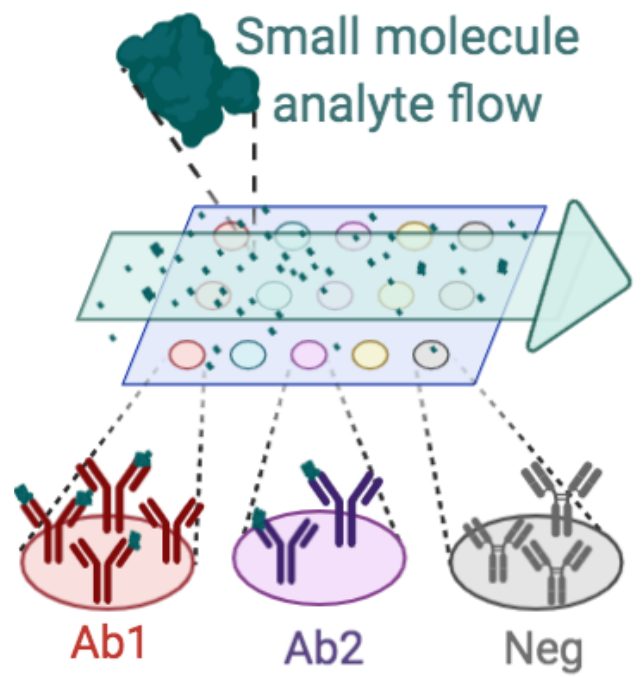


# Our approach: from printing to quantifying

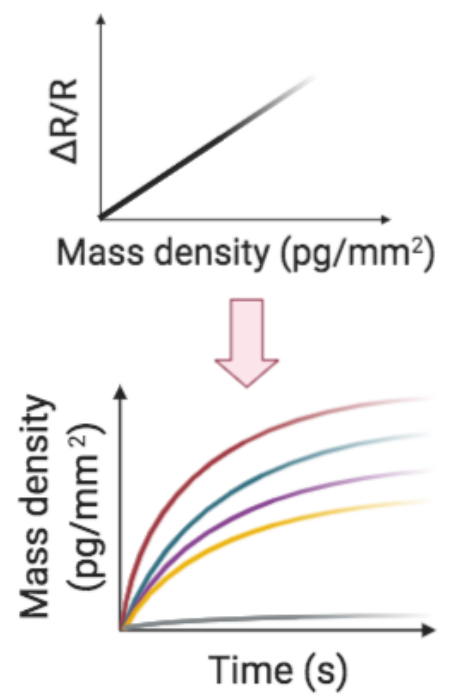
## 1 Microarray printing



## 2 Kinetic detection



## 3 Binding curves



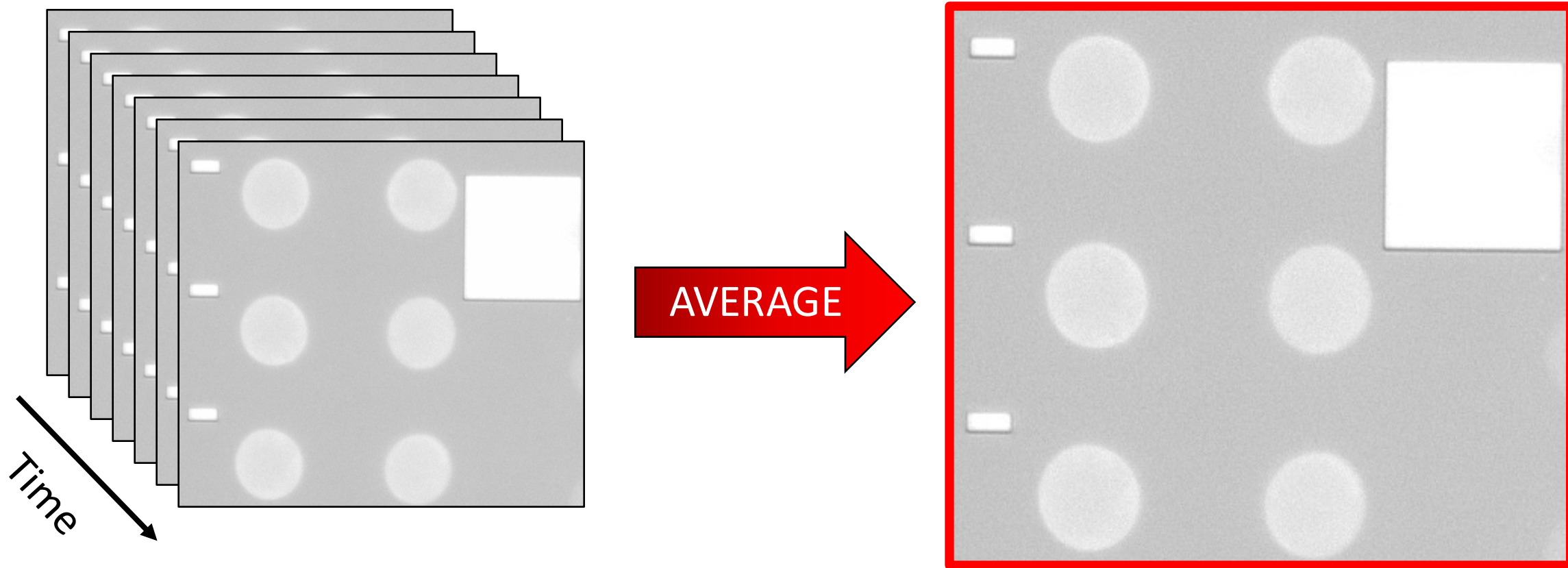
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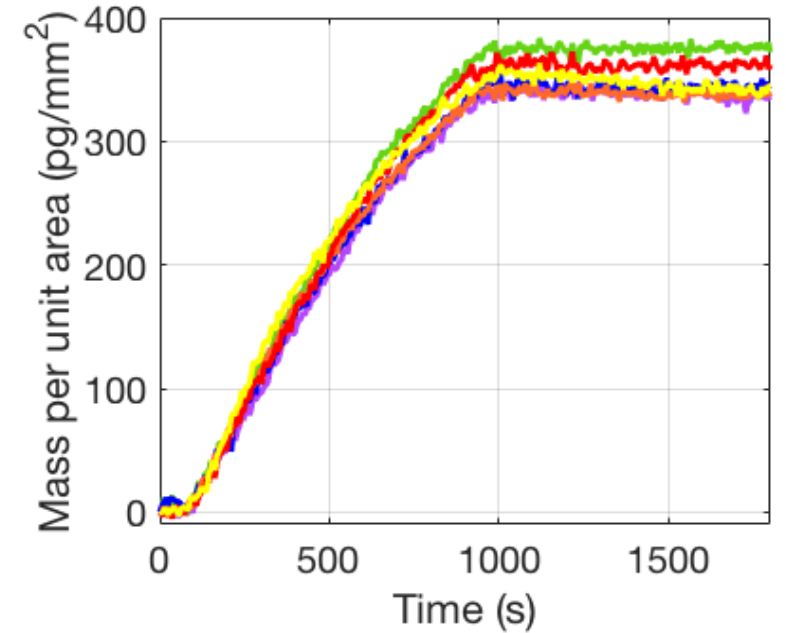
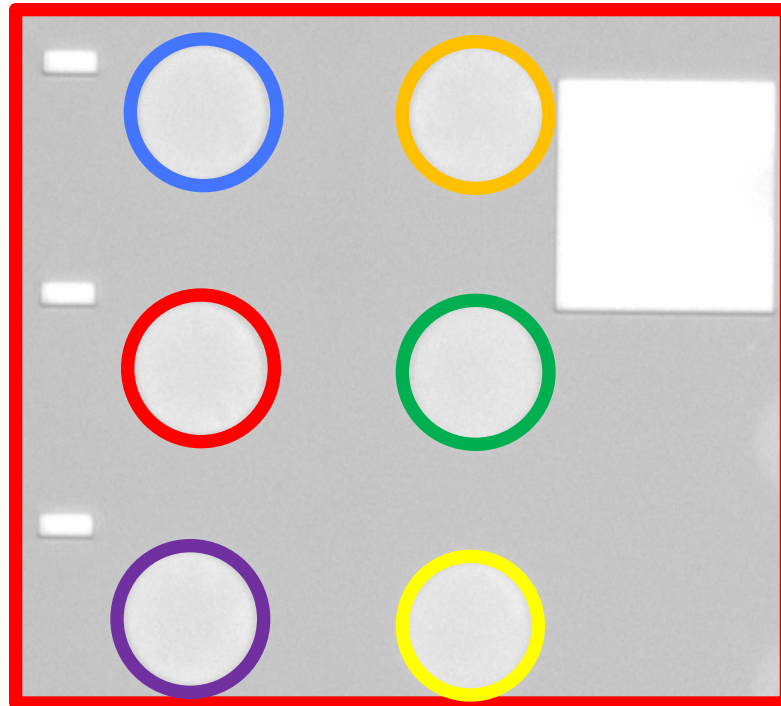
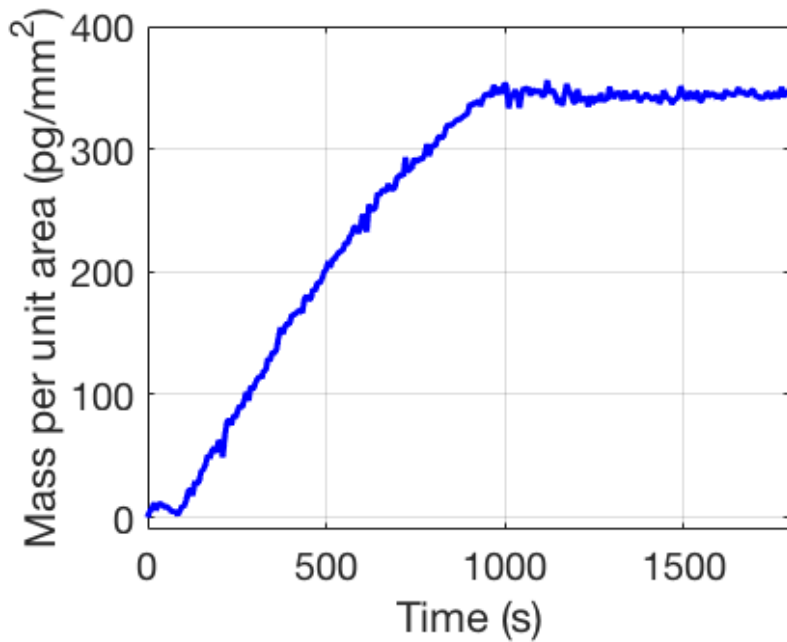
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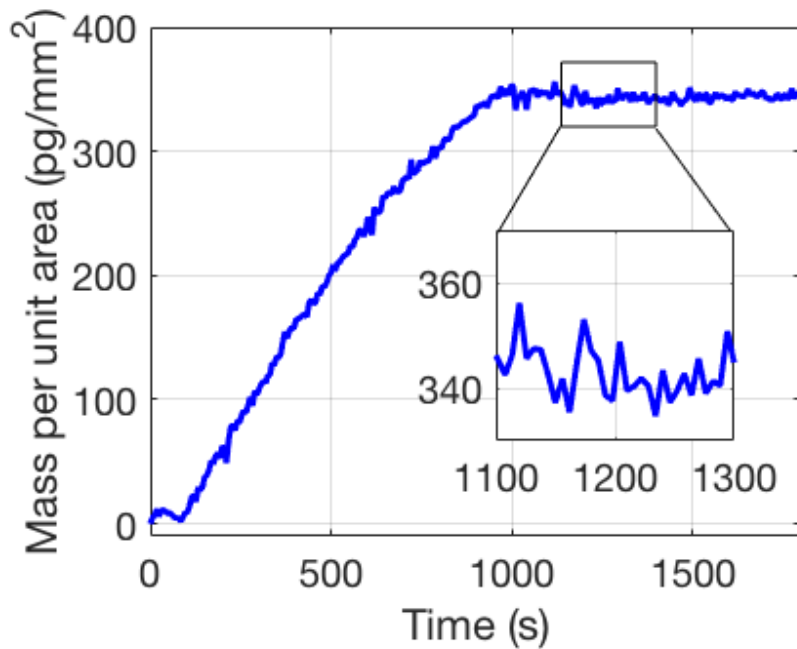
# Noise reduction: time and spatial averaging



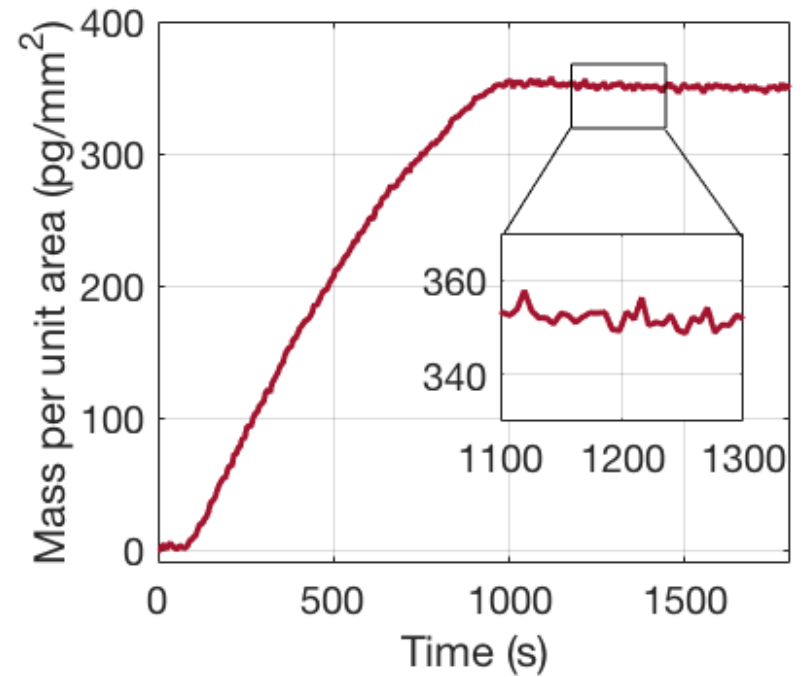
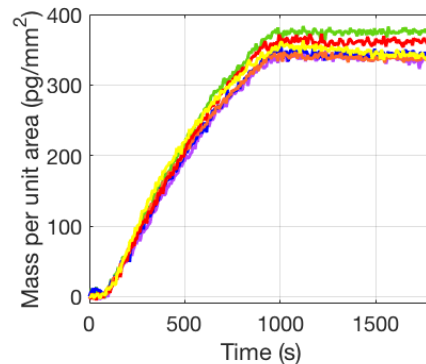
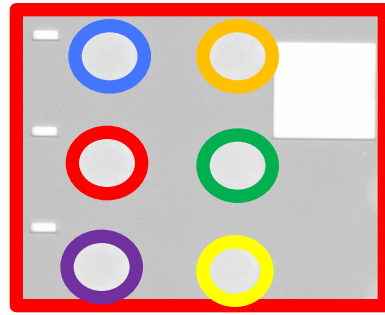
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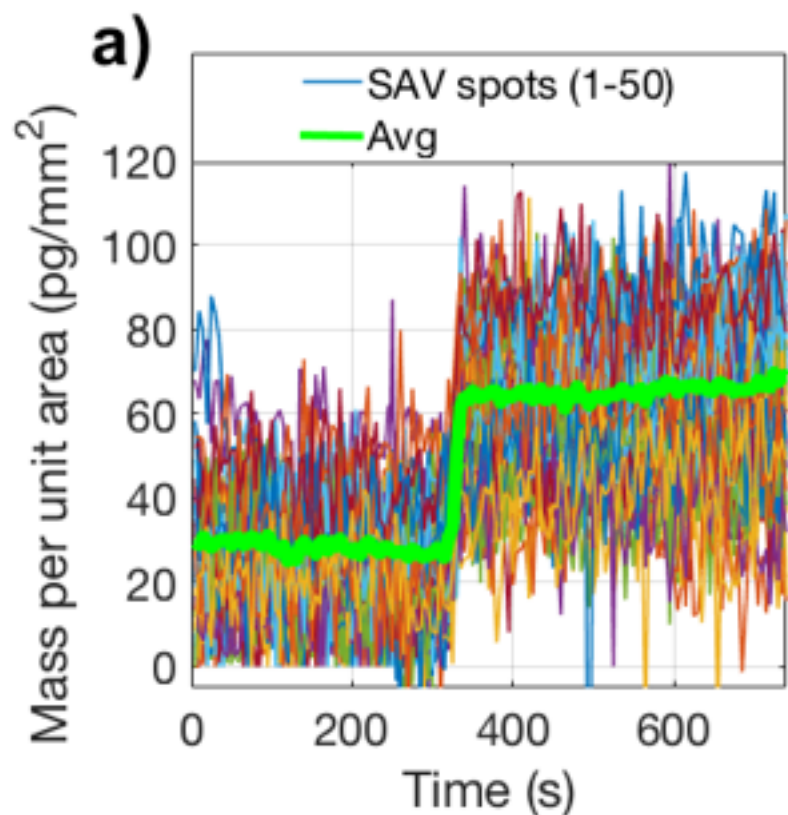


SNR = 30

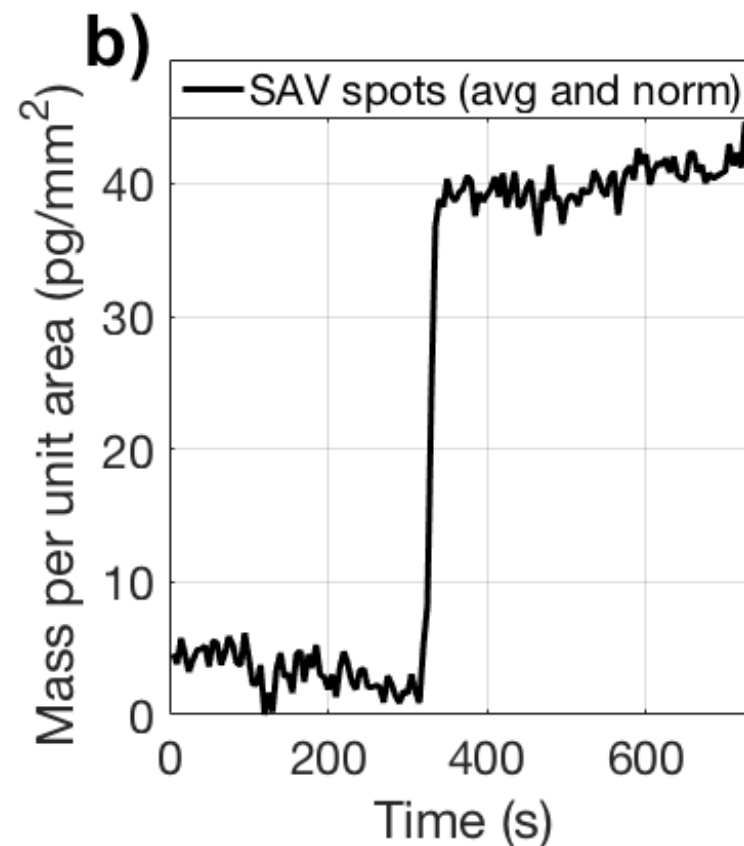
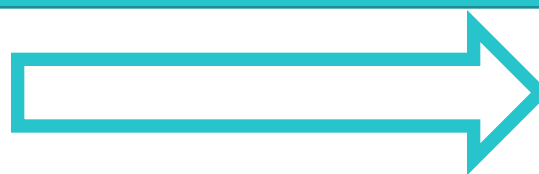


SNR = 92.5

# Proof of concept: biotin detection (244 Da)



Time avg: 100 frames  
Spatial avg: 50 spots



# Outline

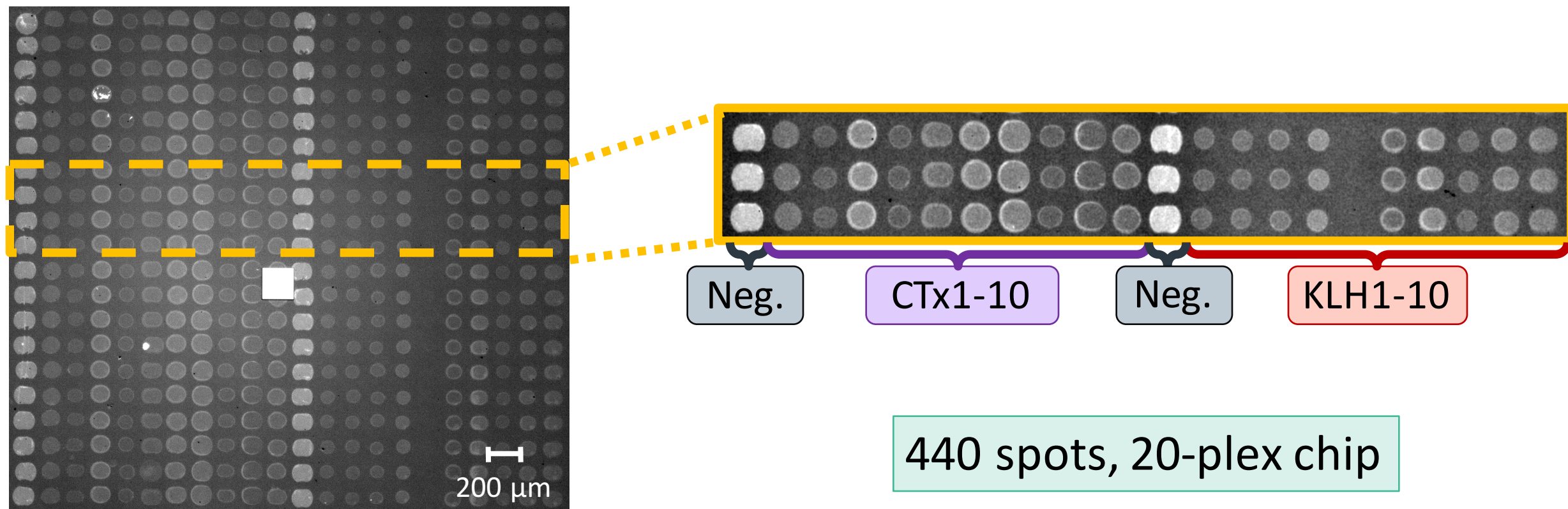
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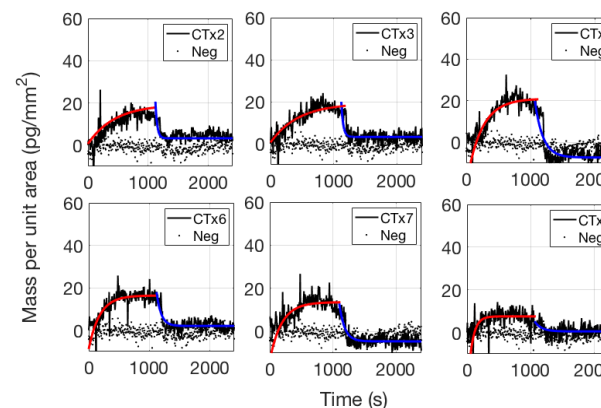
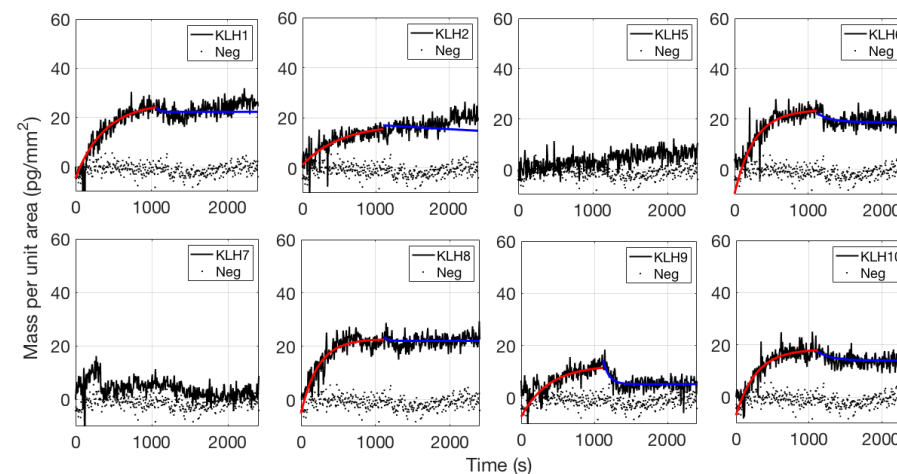
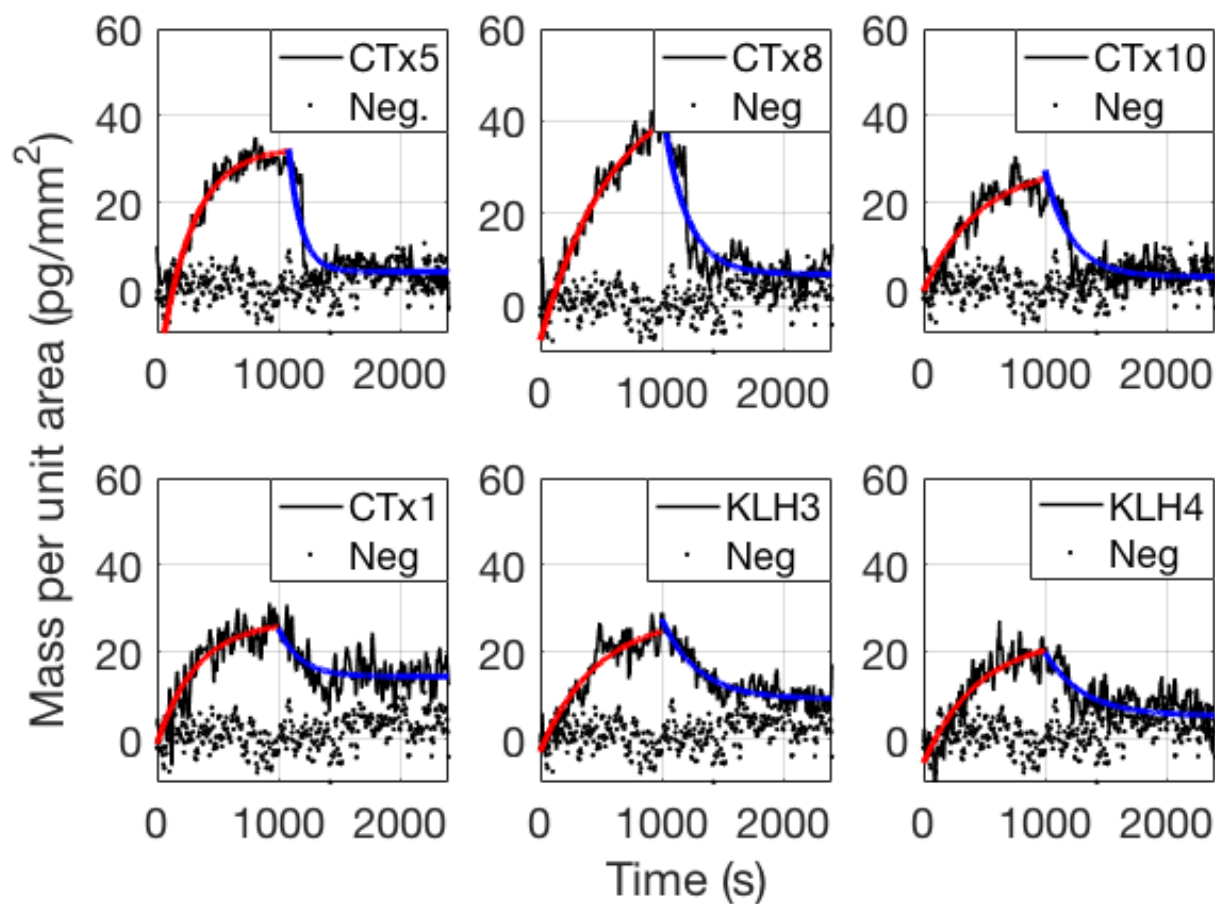


# Fumonisin: characterization of a corn toxin





# Fumonisin: characterization of a corn toxin



# Fumonisin: characterization of a corn toxin

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Antibody	$k_{\text{ON}}$ ( $\text{M}^{-1}\text{s}^{-1}$ )	$k_{\text{OFF}}$ ( $10^{-4}\text{s}^{-1}$ )	$K_{\text{D}}$ ( $\mu\text{M}$ )
CT1	$15.6 \pm 2.2$	$6.82 \pm 3.2$	$44 \pm 22$
CT2	$11.0 \pm 2.2$	$8.77 \pm 4.4$	$80 \pm 43$
CT5	$30.8 \pm 2.7$	$5.13 \pm 1.9$	$17 \pm 6$
CT8	$20.0 \pm 2.1$	$5.3 \pm 2.1$	$27 \pm 11$
CT10	$13.6 \pm 2.7$	$3.9 \pm 3.2$	$28 \pm 24$
KLH1	$8.2 \pm 1.5$	$17.9 \pm 4.7$	$219 \pm 70$
KLH2	$4.0 \pm 0.7$	$15.2 \pm 3.3$	$377 \pm 103$
KLH3	$9.3 \pm 1.1$	$14.1 \pm 3.0$	$151 \pm 37$
KLH4	$31.9 \pm 5.7$	$21.5 \pm 5.3$	$67 \pm 22$

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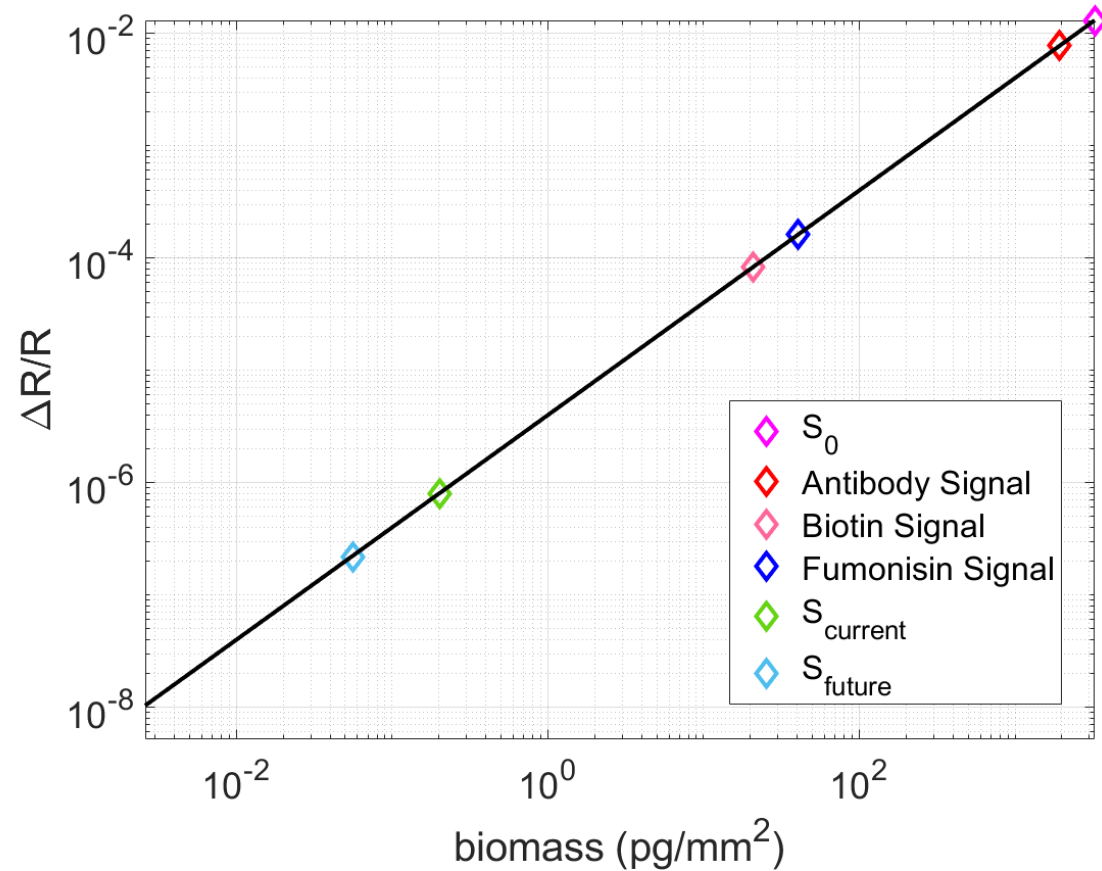
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- The IRIS approach to small molecule characterization is...
  - Highly **sensitive**
  - Versatile and **multiplexable**
  - **Label-free**
- Sensitivity can improve from current 1pg/mm<sup>2</sup> to **0.2pg/mm<sup>2</sup>** thanks to a novel camera sensor
- **Bulk effect reduction** methods: further reduction of noise



# Conclusions and future work



# Thank you!

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**Prof. Selim Ünlü**

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David AnKrapp (Neogen, Corp.)

John Rejman (Neogen, Corp.)

Iris Celebi

Negin Zaraee

Celalettin Yurdakul





# Any questions?

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