Use of DNA thermodynamics for low-abundance mutation detection by DNA hybridization

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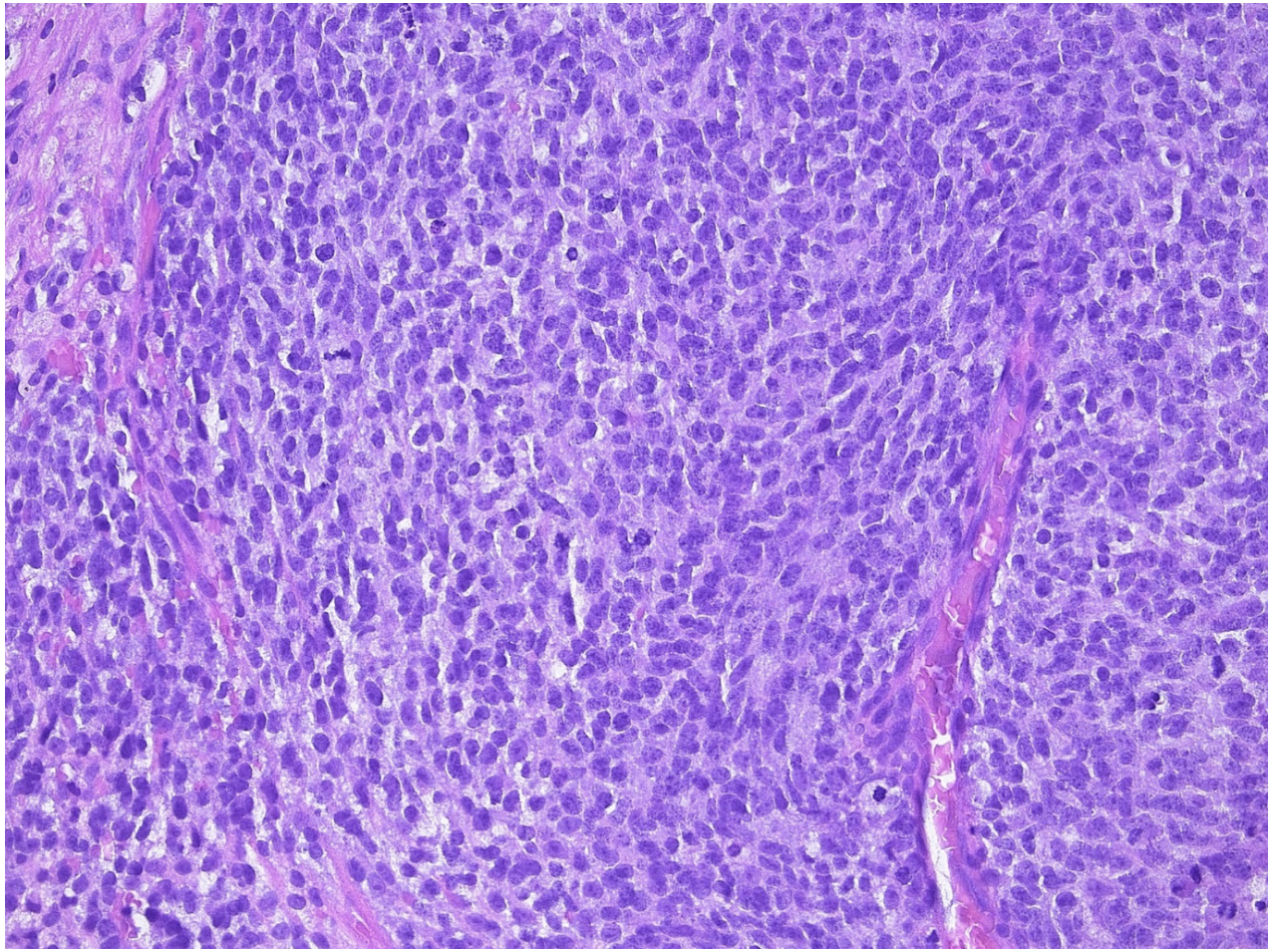
May 17, 2023

The why

Human cells are awesome, but...



H&E stain of sarcoma

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DNA as a two-state model

- Watson-Crick pairs
  - $A = T$
  - $G \equiv C$
- free energy difference ΔG

Example:

```
5'AAGGGCATGAGCTGCATGATGAG 3'
3'TTCCCGTACTCGACGTACTACTC 5'
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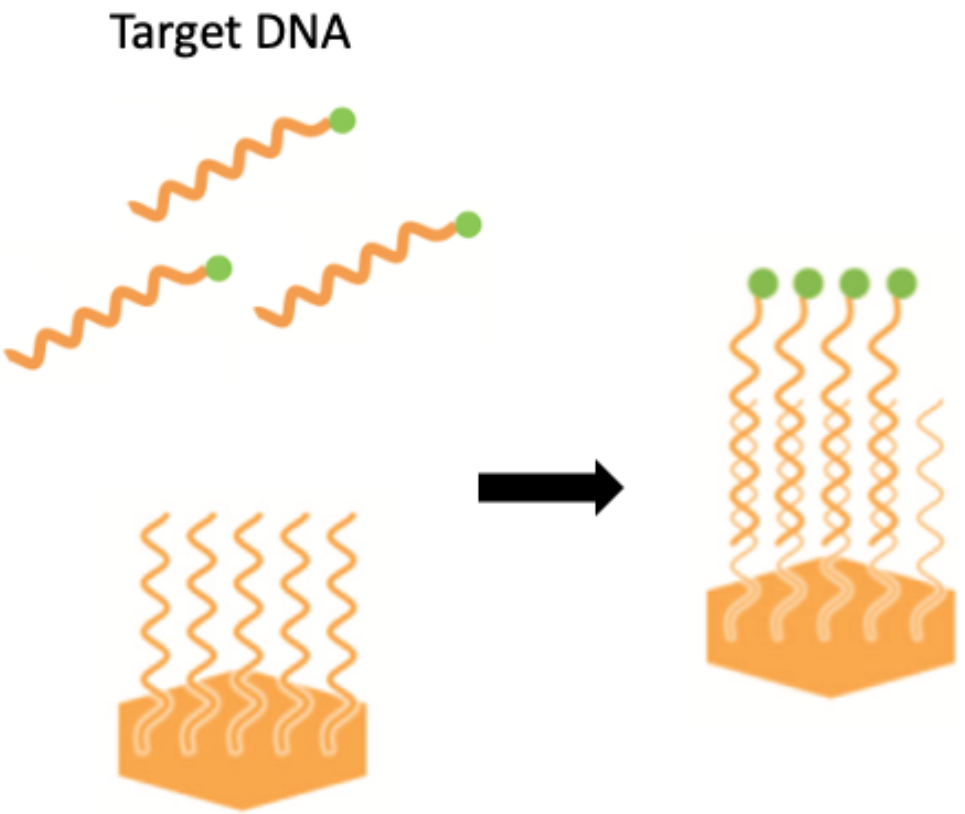
DNA Biosensor



Probe DNA: complement of target

Langmuir Theory:

$$I \sim c\, e^{\frac{\Delta G}{RT}}$$

Fluorescent signal
$\rightarrow$ presence of target DNA

Operational principle of:

- all hybridization-based sensors

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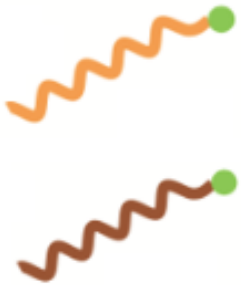
The Objective

Detect mutant DNA in mixture
in majority wild-type background

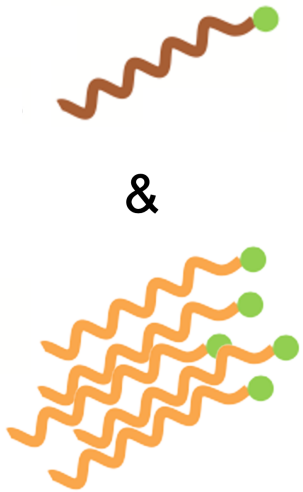
Mixture:

- Wild-Type DNA → EGFR wild-type
- Mutant DNA → T790M mutation



Sample
e.g. liquid biopsy

&

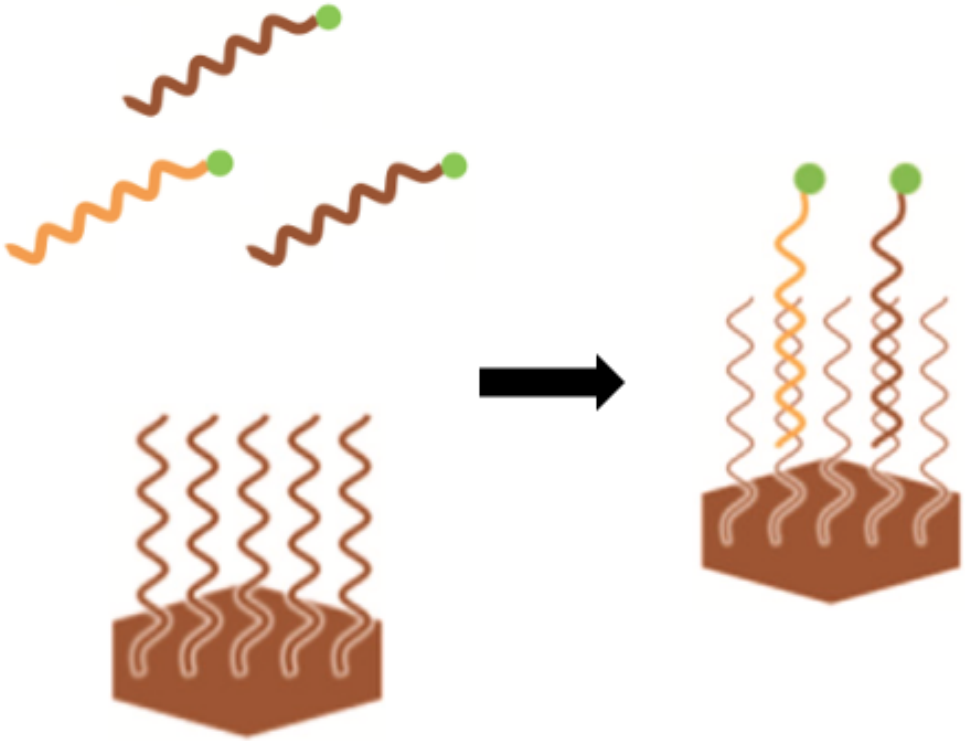


DNA Biosensor

Single-Nucleotide Variant of

Target mixture:

- **cross-hybridization**
- mismatch
- low specificity



Mismatches happen

Example:

5'AAGGGCATGAGCTGCATGATGAG 3'
3'TTCCCGTACTCGACGCACTACTC 5'

Mismatch penalty:

$$\Delta\Delta G = \Delta G_{MM} - \Delta G$$
$$\approx 1\text{kcal/mol}$$

$$I \sim c_{\text{mut}}\, e^{\frac{\Delta G}{RT}} + c_{\text{wt}}\, e^{\frac{\Delta G_{\text{MM}}}{RT}}$$
$$\approx c_{\text{mut}}\, e^{\frac{\Delta G}{RT}} + c_{\text{wt}}\, e^{\frac{\Delta G}{RT}} / 100$$

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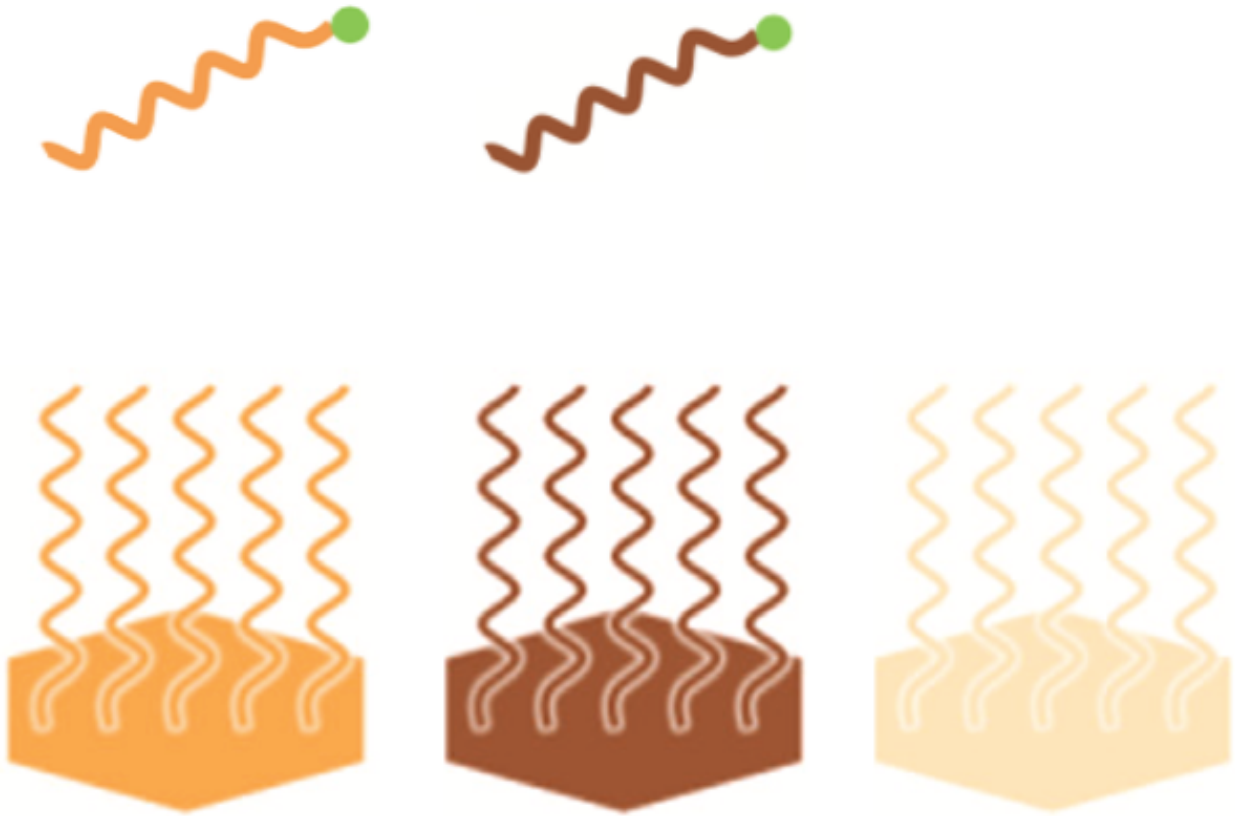
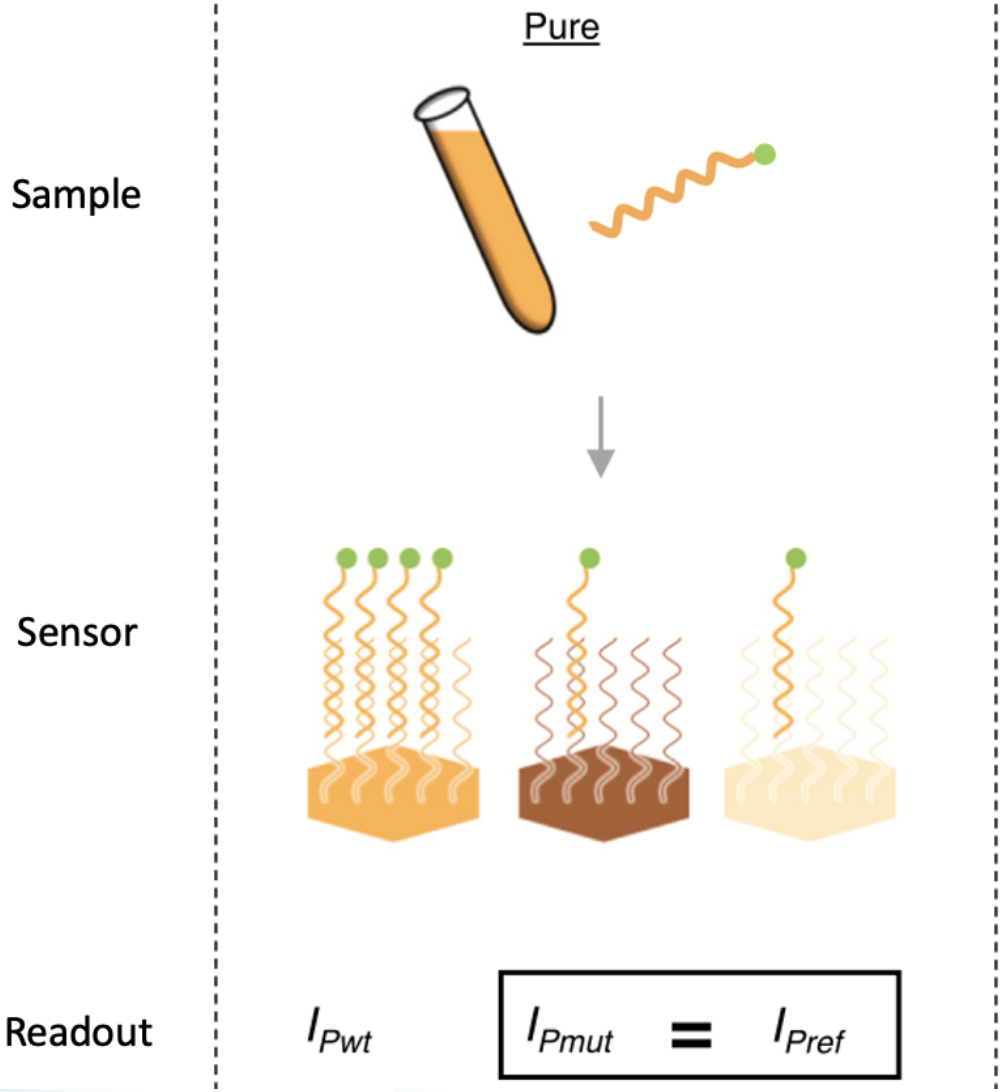
Probes

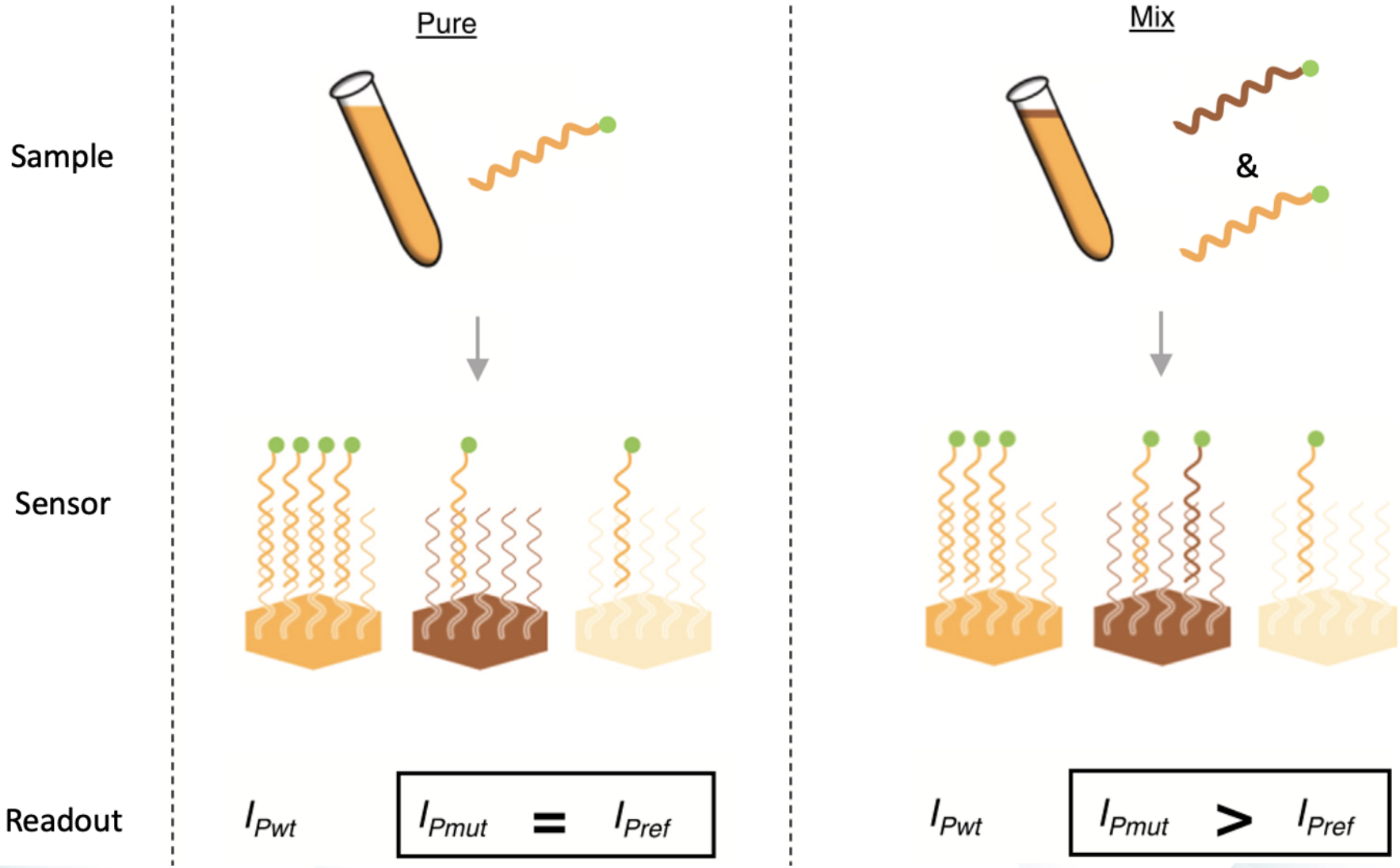


Wild-type Mutant Reference

Setup



Setup

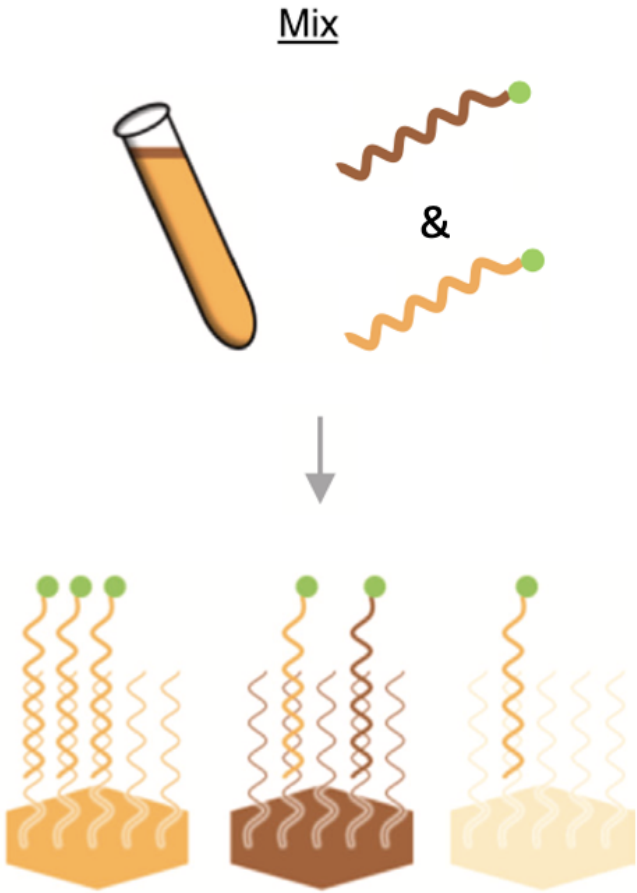

Pure
Mix
Sample
&
Sensor
Readout
I_{Pwt}
$I_{Pmut} = I_{Pref}$
I_{Pwt}
$I_{Pmut} > I_{Pref}$

Detection Signal

$$S \equiv \ln \frac{I_{\text{Pmut}}}{I_{\text{Pref}}}$$

Described by **Langmuir theory**

$$S = \ln\left(1 + \frac{c_{mut}}{c_{wt}}\, e^{\frac{\Delta\Delta G_{\text{Pmut}}}{RT}}\right)$$



I_{Pwt} $I_{Pmut} > I_{Pref}$

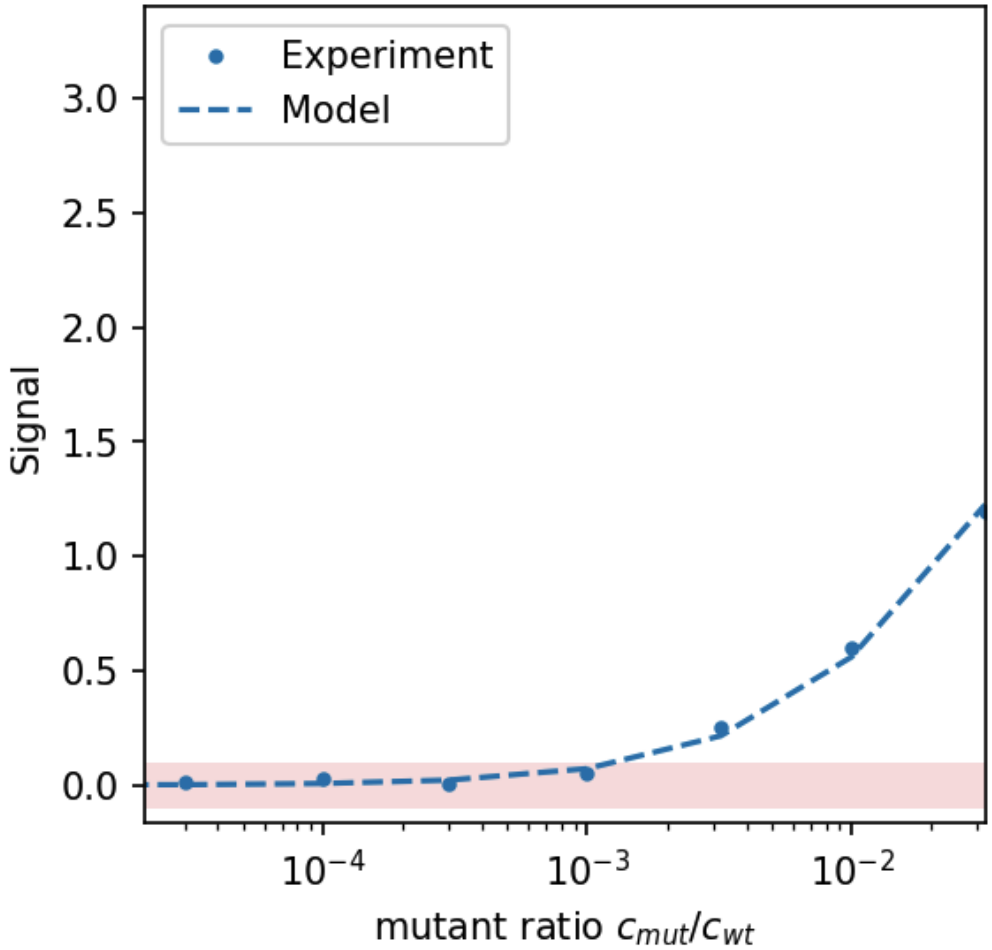
Detection Signal

$$S \equiv \ln \frac{I_{\text{Pmut}}}{I_{\text{Pref}}}$$

Described by **Langmuir theory**

$$S = \ln\left(1 + \frac{c_{mut}}{c_{wt}}\, e^{\frac{\Delta\Delta G_{\text{Pmut}}}{RT}}\right)$$

- Experimental agreement
- Limit of detection: 0.38%



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Depletion

Wild-type probes **selectively** reduce available targets

Enhanced mutant ratio

$$\frac{c^*_{mut}}{c^*_{wt}} = \frac{c_{mut}}{c_{wt}} e^{\frac{\Delta\Delta G_{Pwt}}{RT}} > \frac{c_{mut}}{c_{wt}}$$

$$S = \ln\left(1 + \frac{c_{mut}}{c_{wt}} e^{\frac{\Delta\Delta G_{Pmut} + \Delta\Delta G_{Pwt}}{RT}}\right)$$

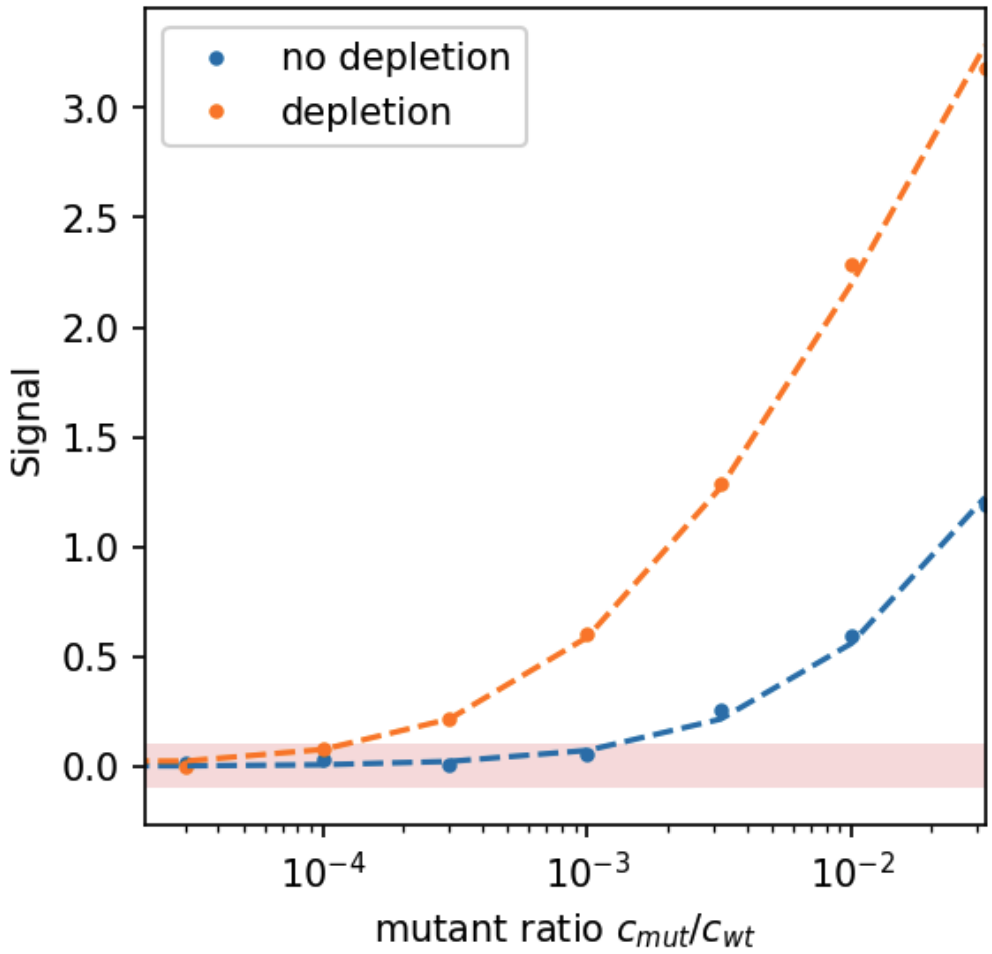
- Improves detection limit

Detection Signal

- Limit of detection: 0.05%
- ∼**10-fold** improvement
- comparable to current methods

Measure signal → determine c_{mut}

Accurate for clinical samples?



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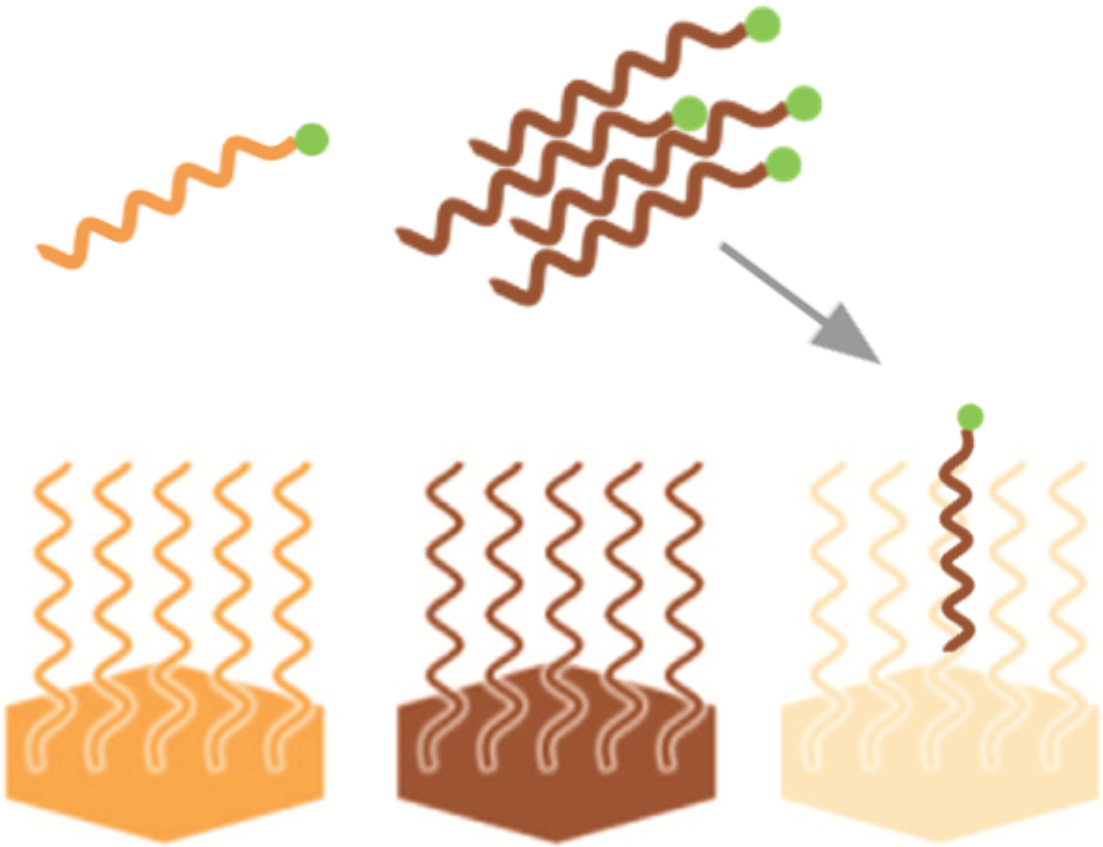
New Interaction @ high c_{mut}



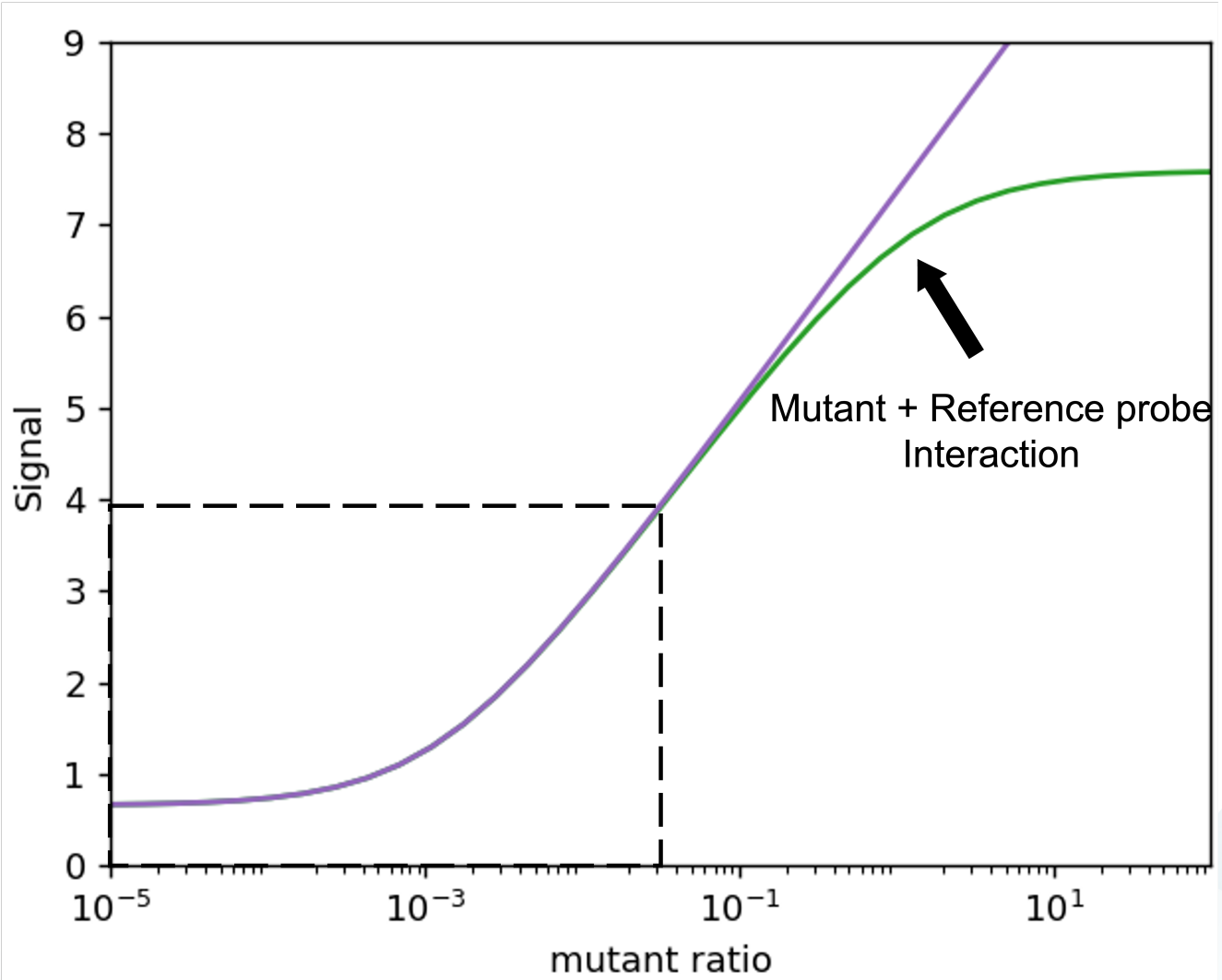
Wild-type Mutant Reference

$$S \equiv \ln \frac{I_{Pmut}}{I_{Pref}}$$

$$I_{Pref} \nearrow S \searrow$$

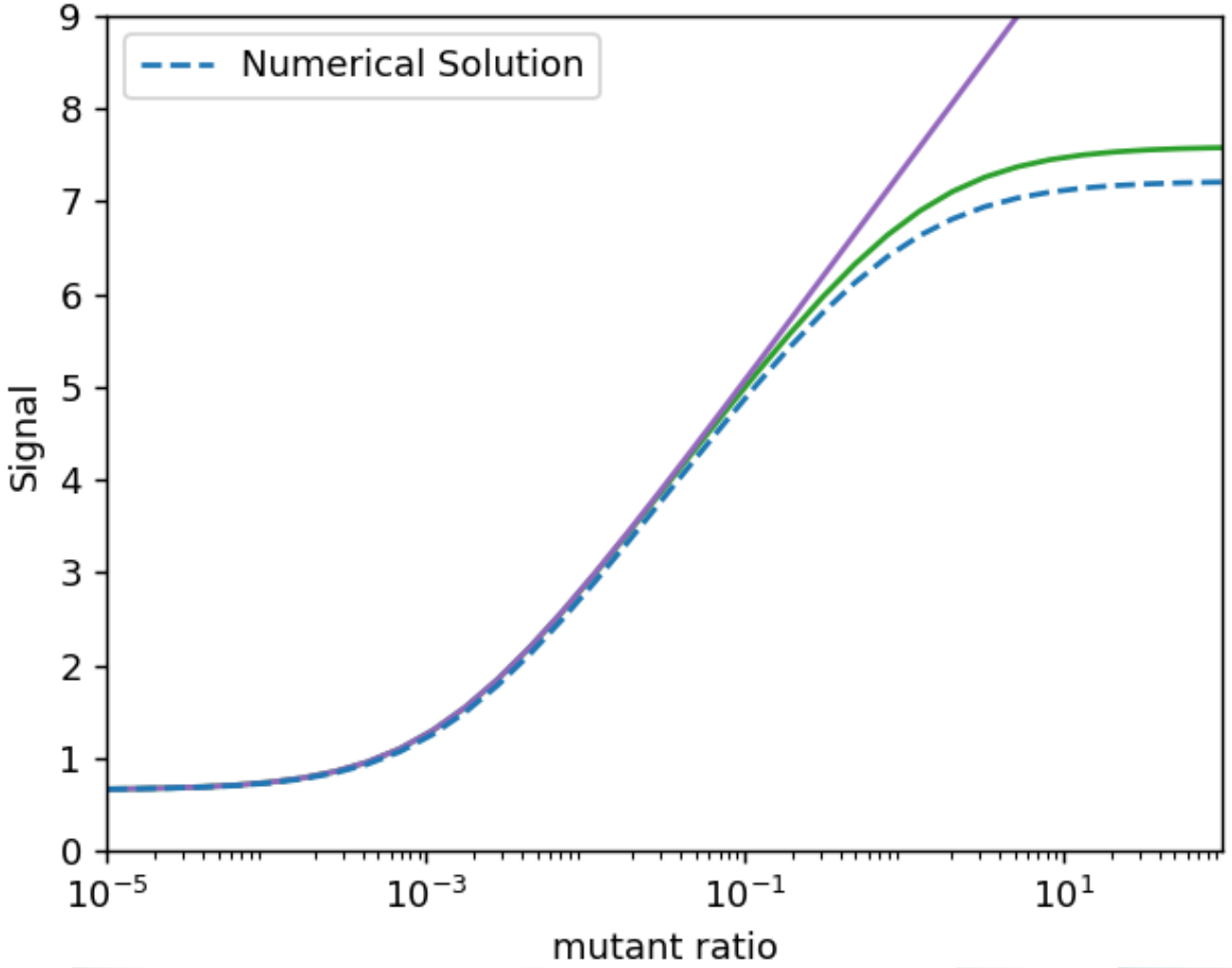
New Interaction

$$S_{\text{correction 1}} = -\ln\left(1 + \frac{c^*_{mut}}{c^*_{wt}}\, e^{\frac{\Delta\Delta G_{\text{Pref}}}{RT}}\right)$$



Numerical solution

$$S_{\text{correction 1}} = -\ln\left(1 + \frac{c^*_{mut}}{c^*_{wt}}\, e^{\frac{\Delta\Delta G_{\text{Pref}}}{RT}}\right)$$



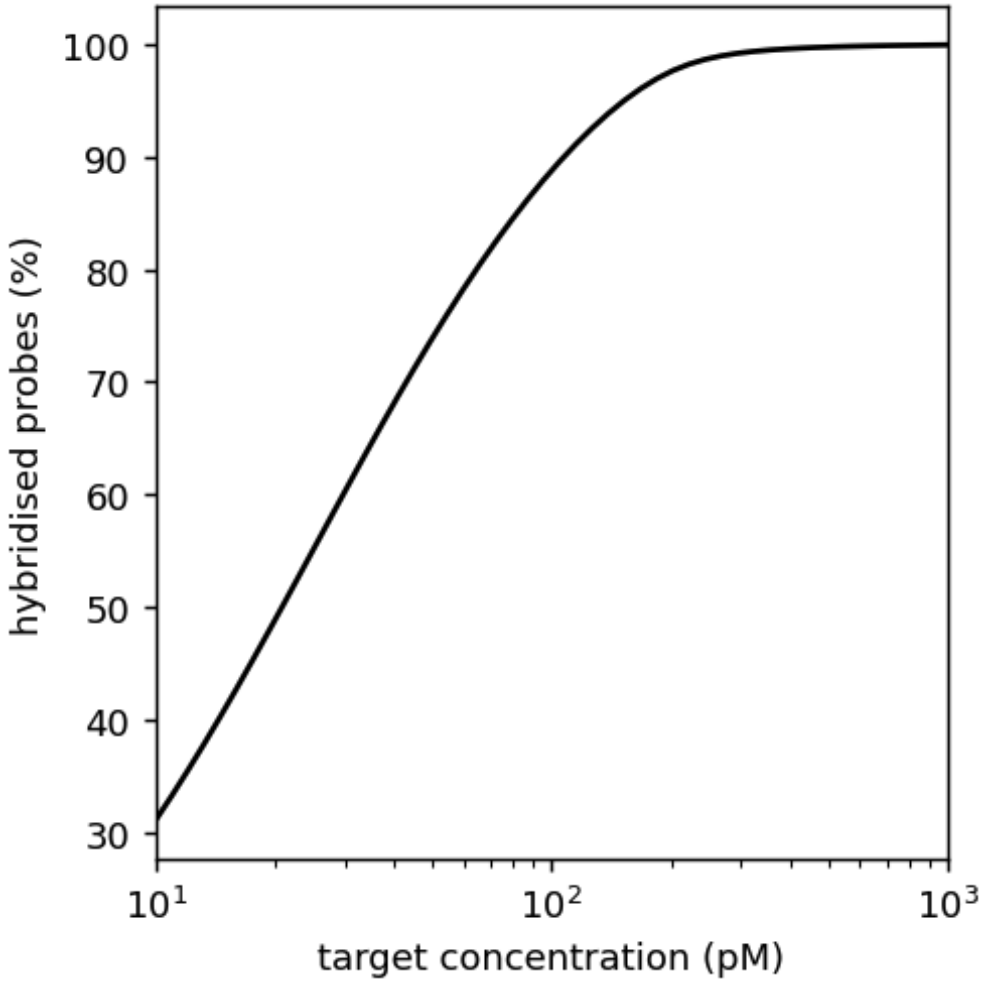
Probe Saturation

$$I \sim c\, e^{\frac{\Delta G}{RT}} \rightarrow \frac{c\, e^{\frac{\Delta G}{RT}}}{1 + c\, e^{\frac{\Delta G}{RT}}}$$

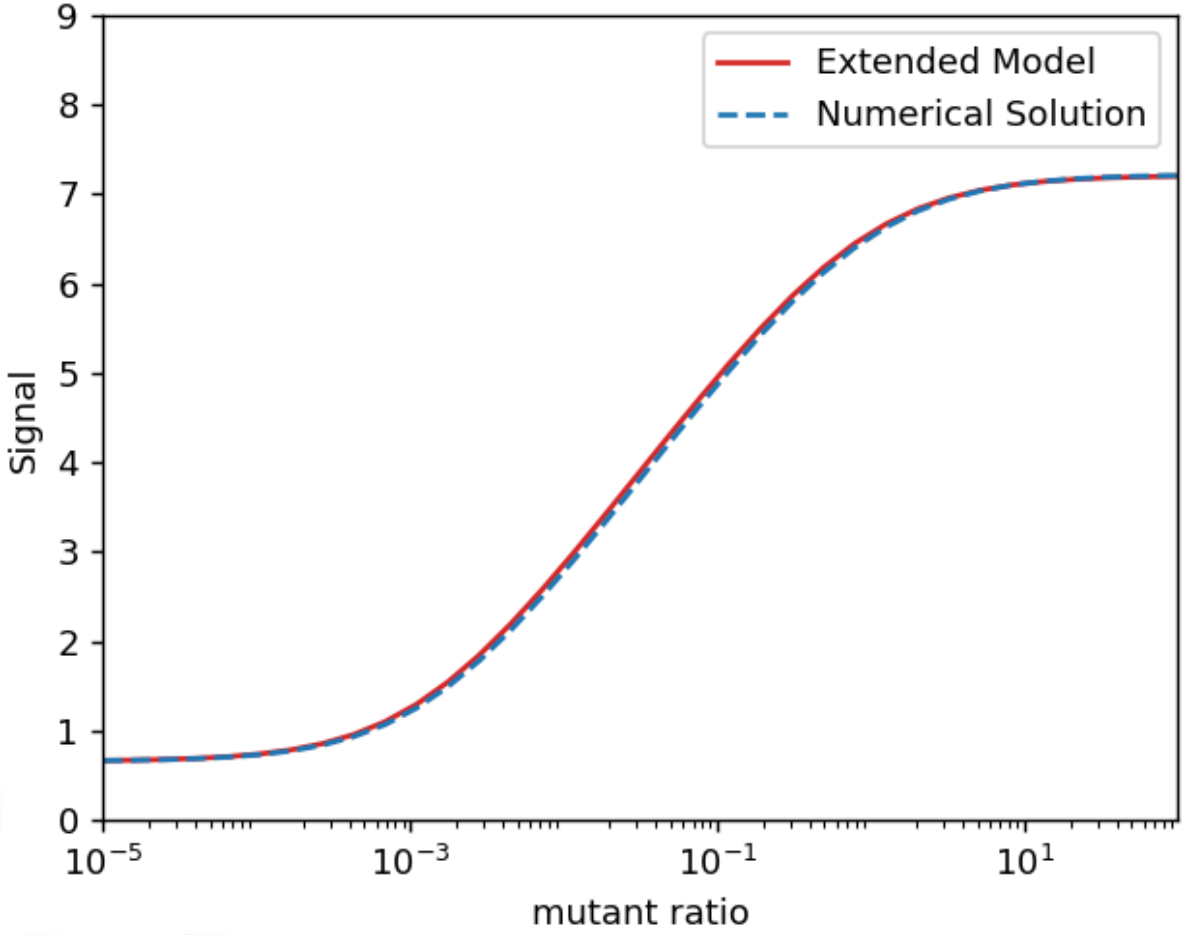
$$S_{\text{correction 2}} = -\ln\left(1 + c^*_{mut}\, e^{\frac{\Delta G_{\text{mut}}}{RT}}\right)$$

- More mutant target

$\Rightarrow$ Mutant probe saturation



New Model



$$S = \ln\left(1 + \frac{c^*_{mut}}{c^*_{wt}}\, e^{\frac{\Delta\Delta G_{\text{Pmut}}}{RT}}\right) - \ln\left(1 + \frac{c^*_{mut}}{c^*_{wt}}\, e^{\frac{\Delta\Delta G_{\text{Pref}}}{RT}}\right) - \ln\left(1 + c^*_{mut}\, e^{\frac{\Delta G_{\text{mut}}}{RT}}\right)$$

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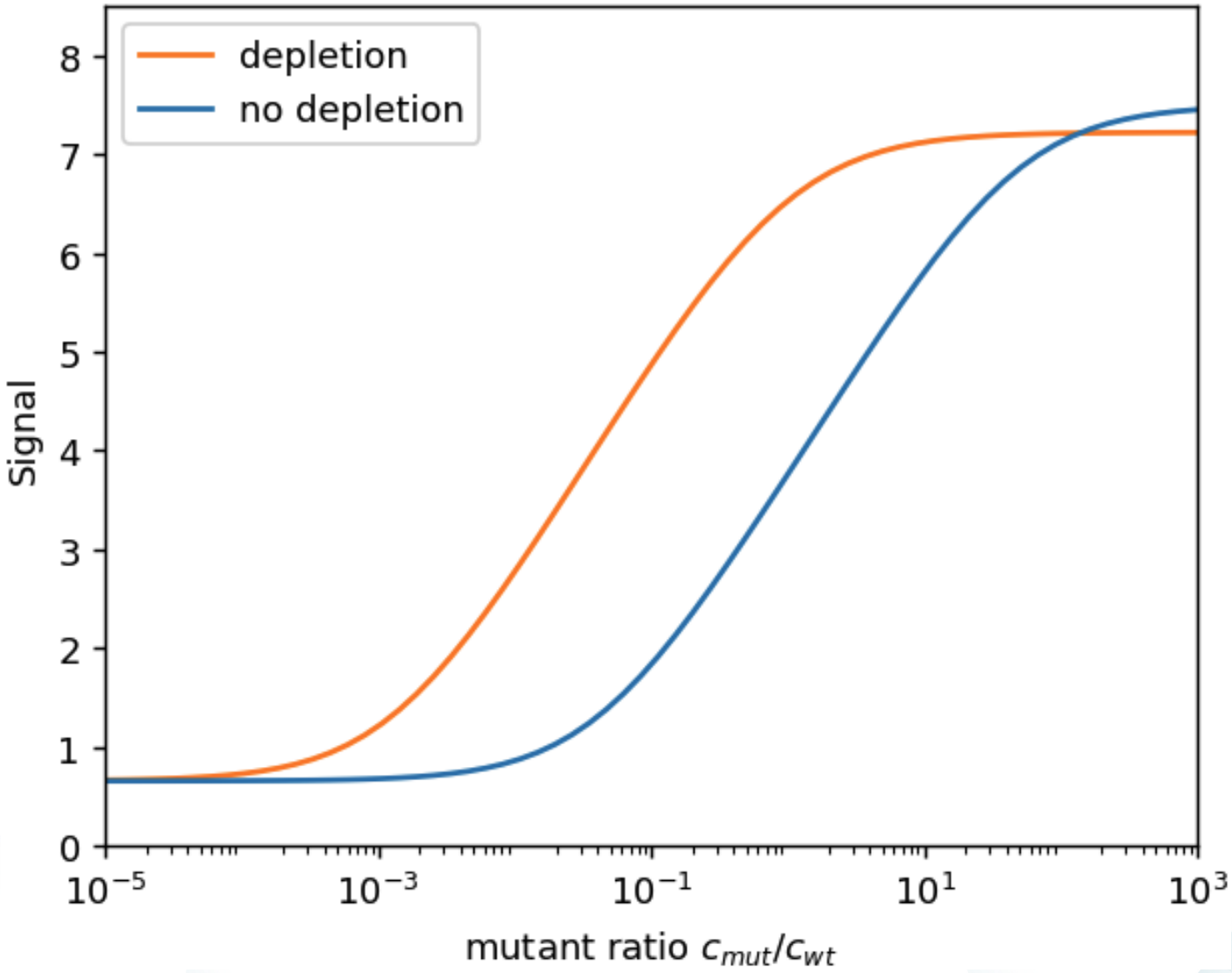
Numerical Solution

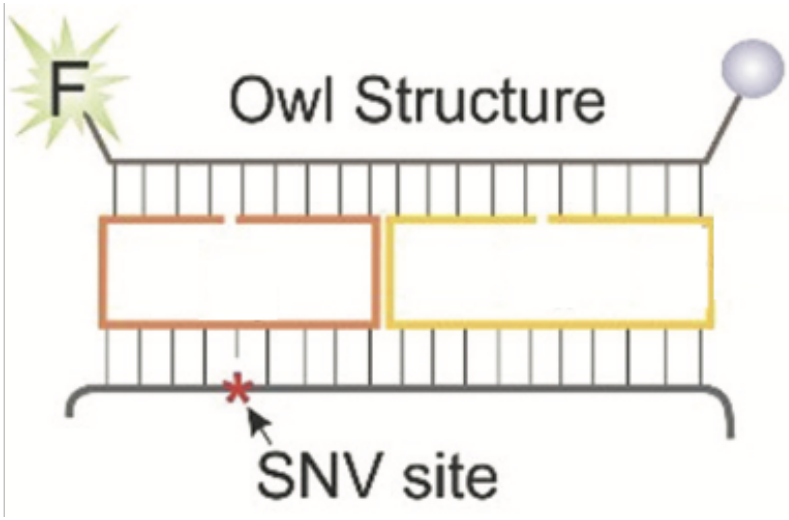


Accurate model in full mutant range

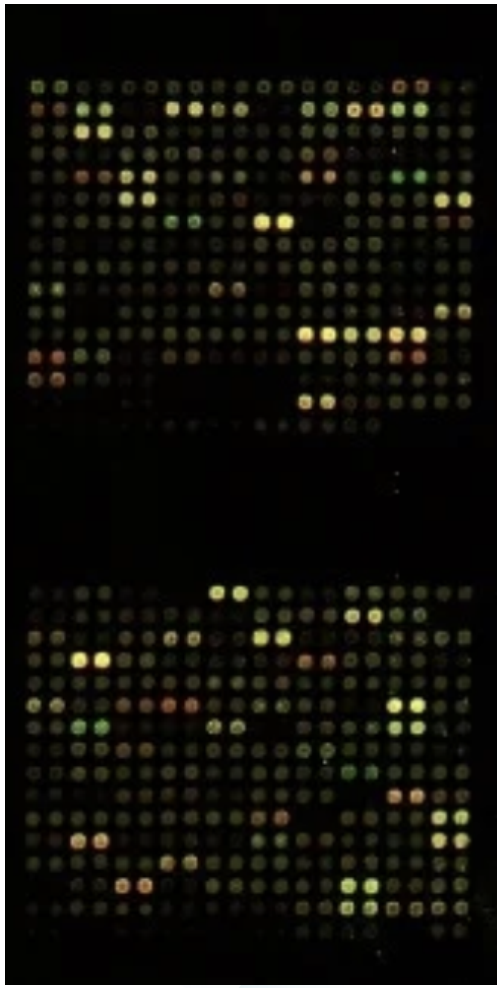
Outlook

Ongoing research:

- EGFR calibration experiment on microarrays
- Solution-based experiments
  - → cost-efficient sensors
  - → at room temperature



R.J. Karadeema et al., Nanoscale, 2018



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Thanks for your attention

Don't hesitate with questions:

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Research grant: BOF22OWB11 & BOF23BL05