Detection of Progesterone and Estradiol Using Poly (N-isopropylacrylamide) Microgel-Based Biosensors

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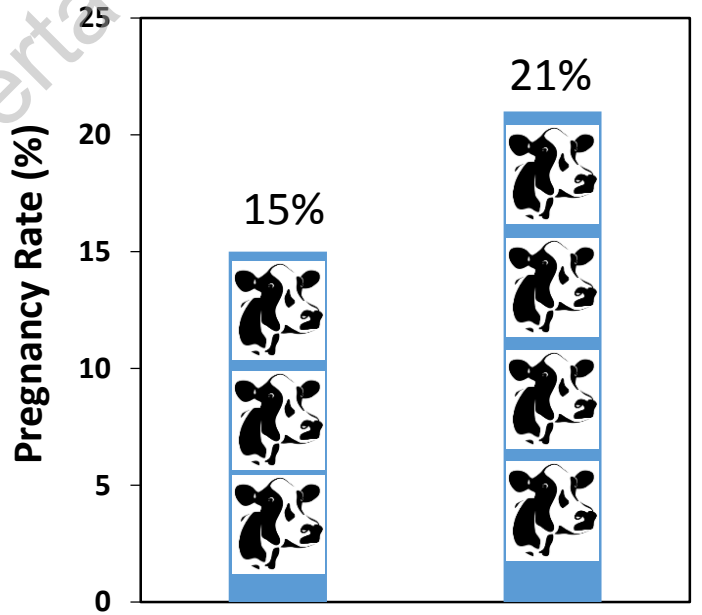
Alberta Agriculture and Forestry

Species of Interest to the Milk Industry

- Poor reproductive efficiency is a well-documented challenge facing the dairy industry.
- Undetected estrus is the main contributor to poor reproductive efficiency in dairy herds in Alberta, Canada.

✓Progesterone (P4)
✓17β-Estradiol (E2)

Predict estrus



6%

$60,720/year in an average Alberta farm of **138 cows**

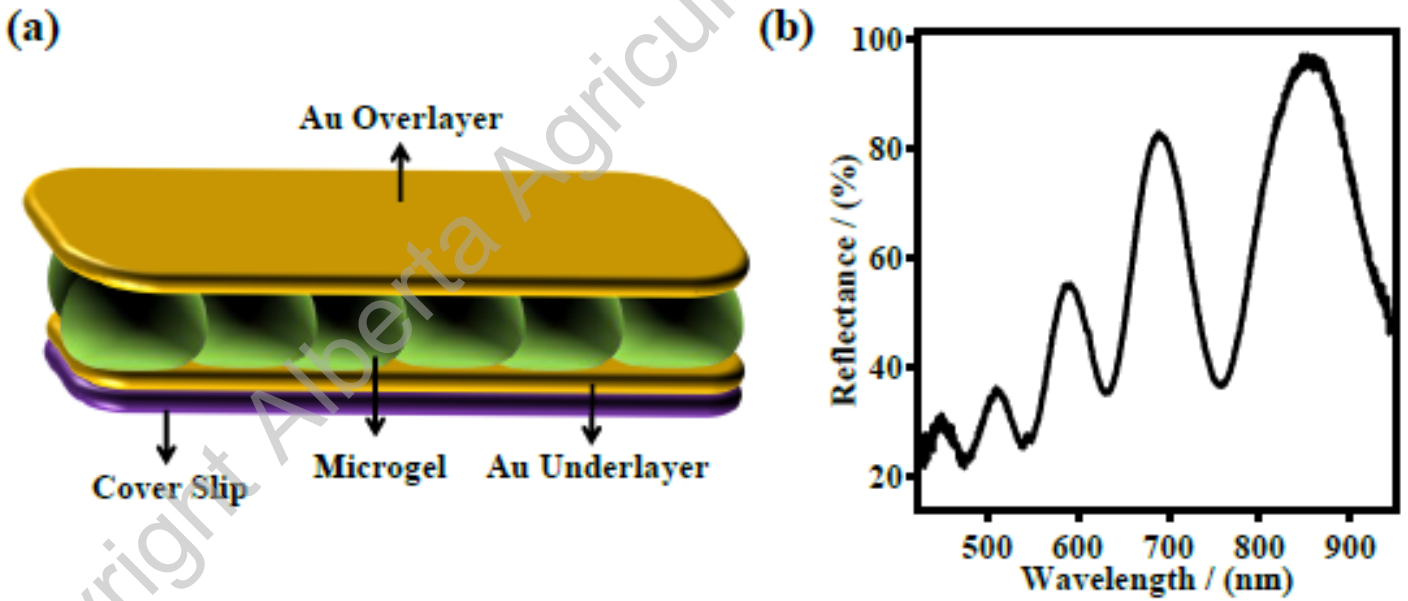
Available Methods for P4 and E2 Detection

- **Laboratory**: Radioimmunoassay (RIA); Enzyme linked immunosorbent assay (ELISA); Advanced instrumental methods such as high-performance liquid chromatography (HPLC), liquid chromatography/mass spectrometer (LC/MS) or gas chromatography/mass spectrometer (GC/MS)

- **"Cow-side" test**: There are not cow-side tests commercially available for E2; the available P4 tests are relative expensive and lack sensitivity.

Poly (N-isopropylacrylamide) (pNIPAM) Microgel Based Etalon Sensors

- High sensitivity
- Simplicity
- Low cost



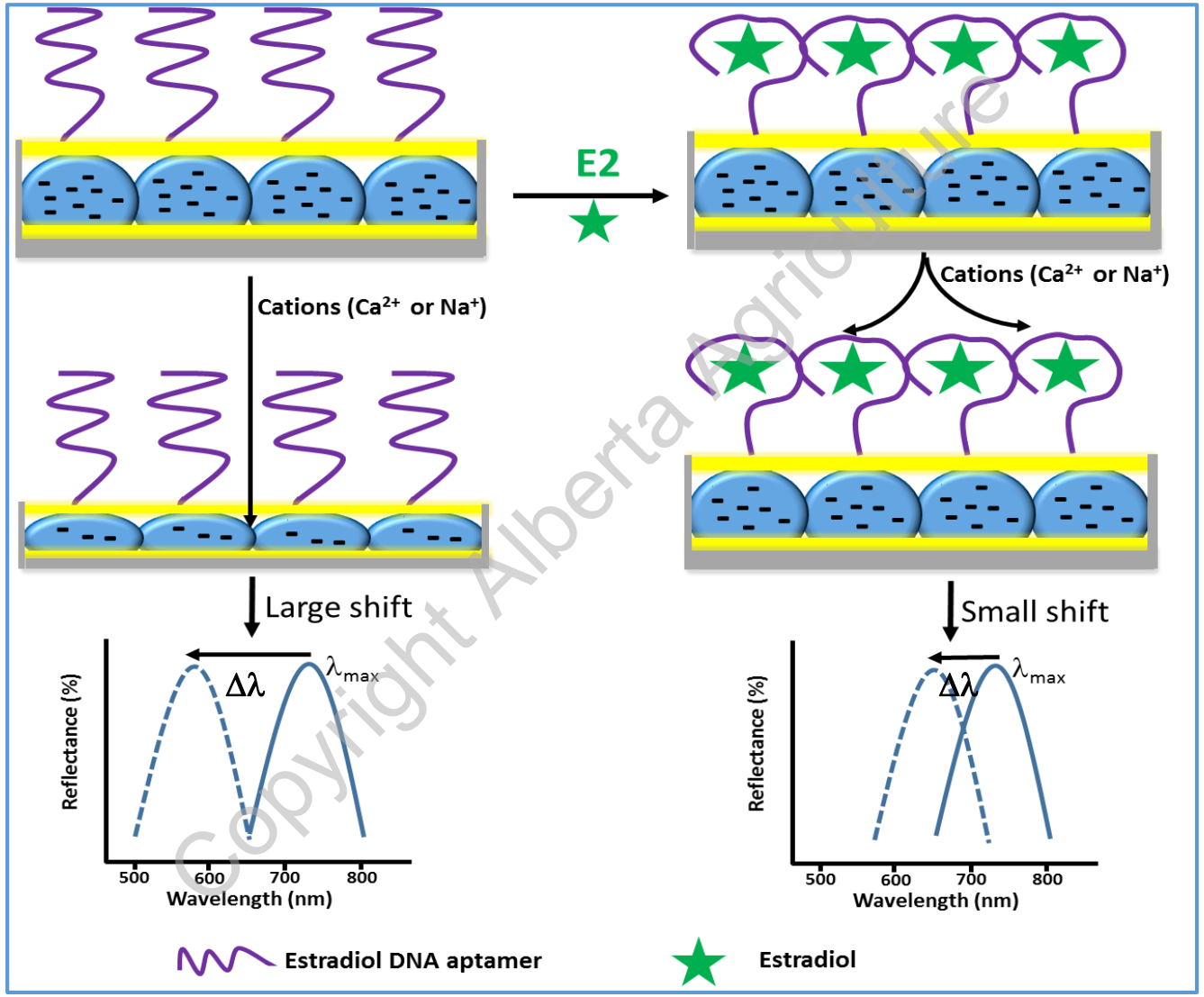
Islam and Serpe, *Biosensors and Bioelectronics*, 2013, 49, 133–138

Objectives

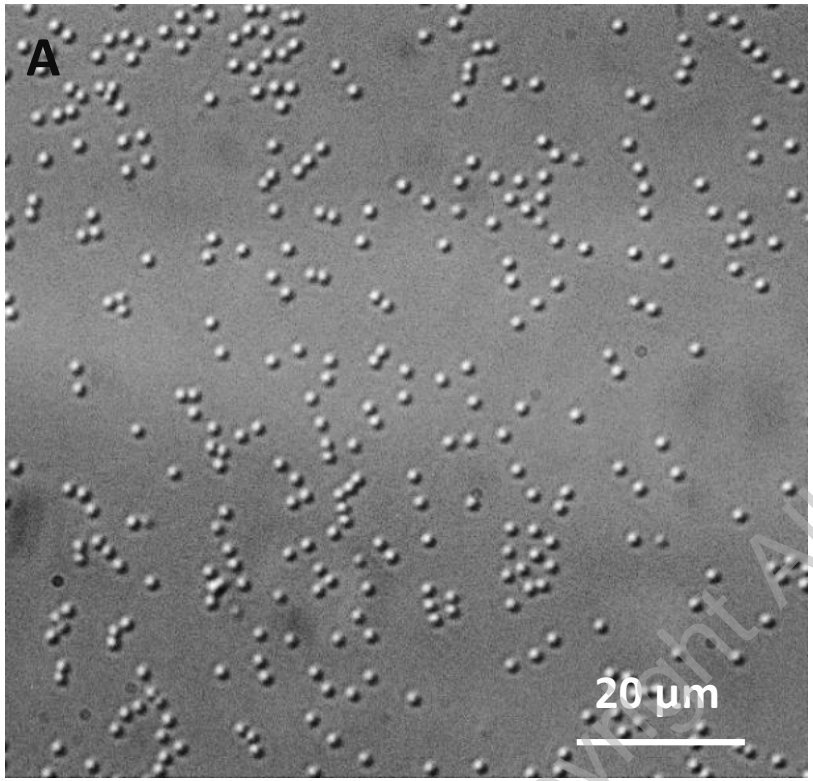
- Develop a pNIPAM microgel based sensor technology which is easier, more affordable, and more time effective for milk hormones detection;

- A sensor that can be used “cow-side” or in-line for real time P4 and E2 quantitation.

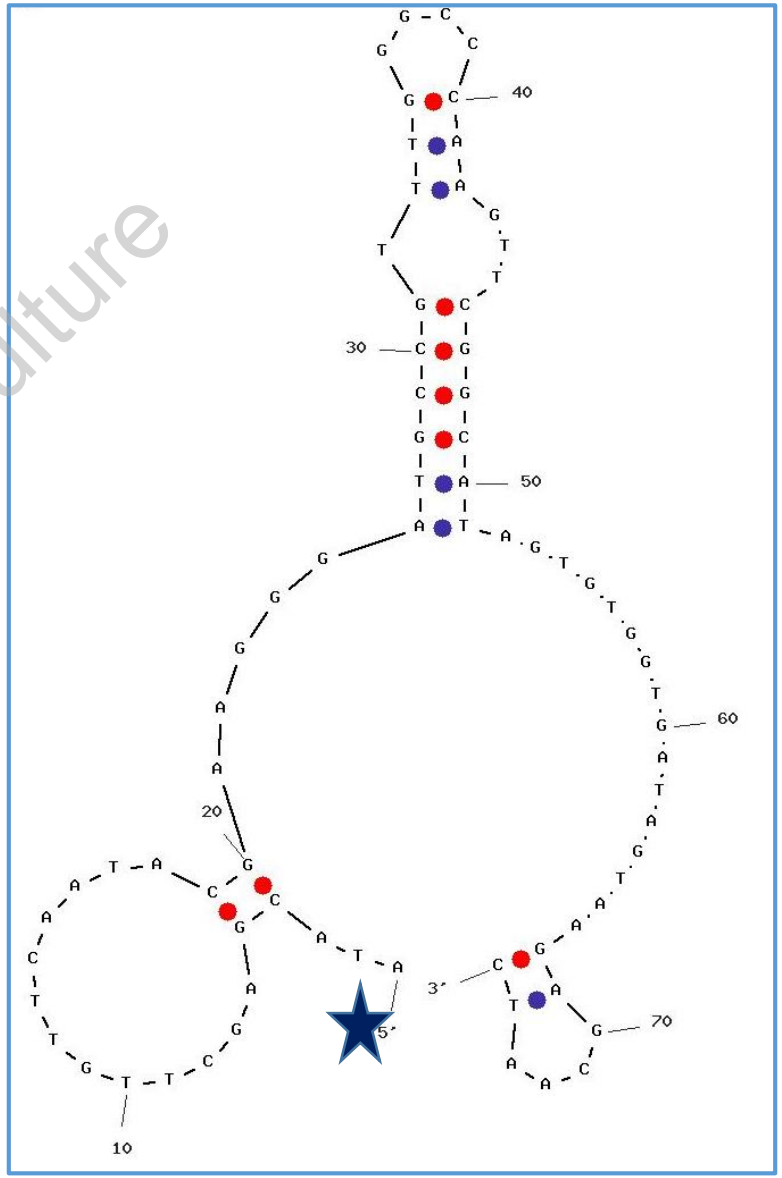
E2 Detection-Aptamer/Target Interaction



Microgel Characterization and Estradiol DNA Aptamer

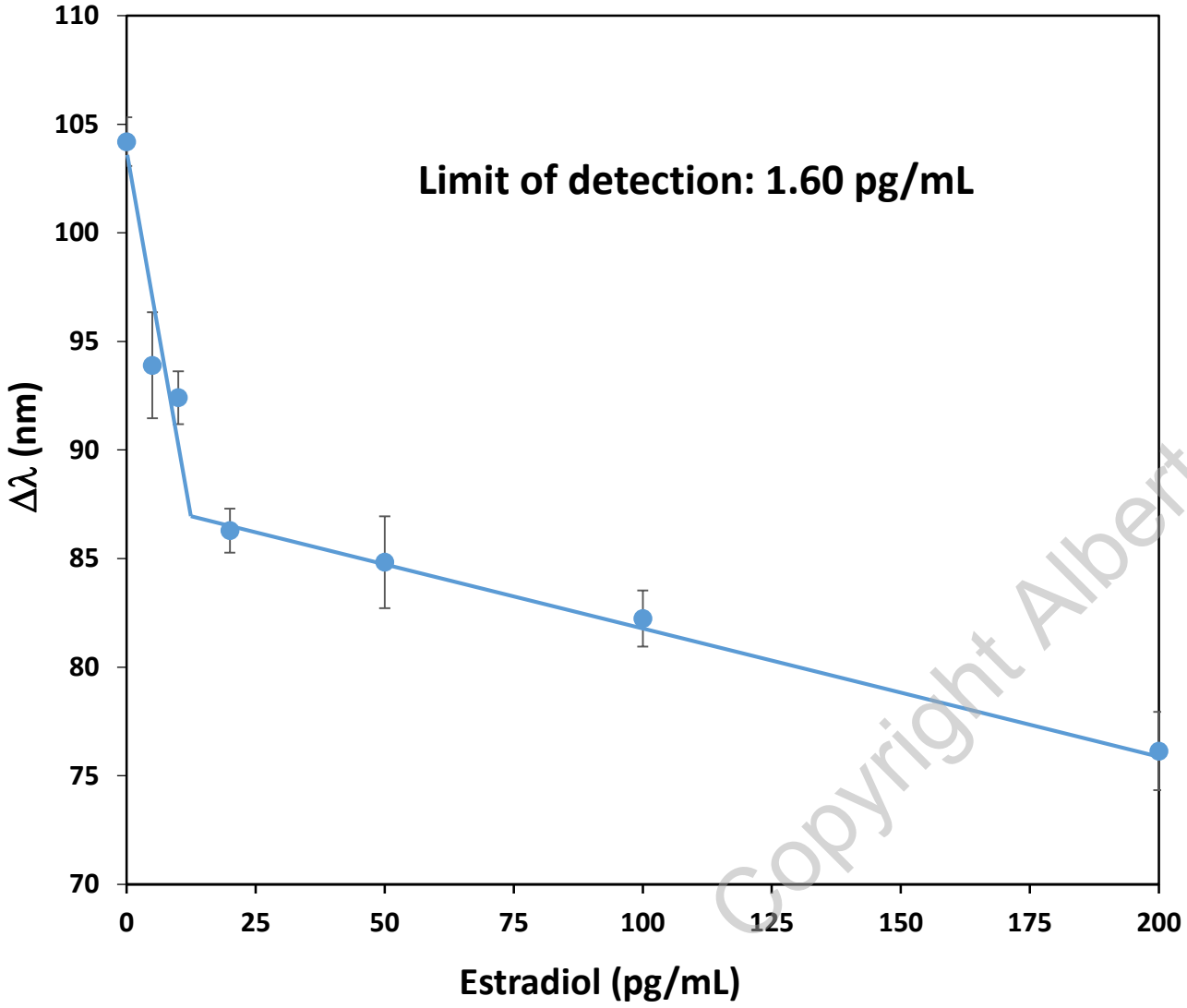


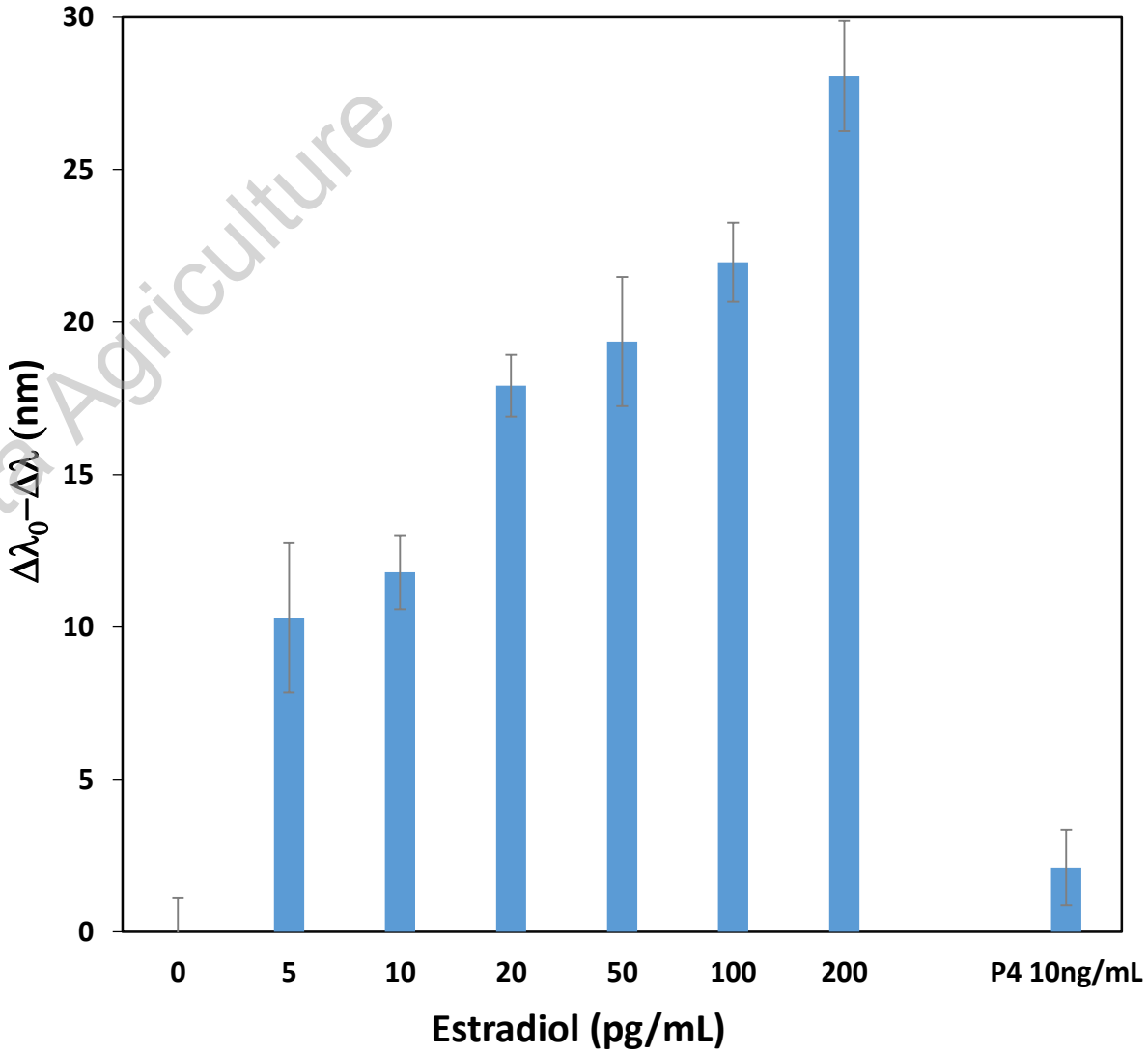
Size of Microgels: 1.1-1.2µm



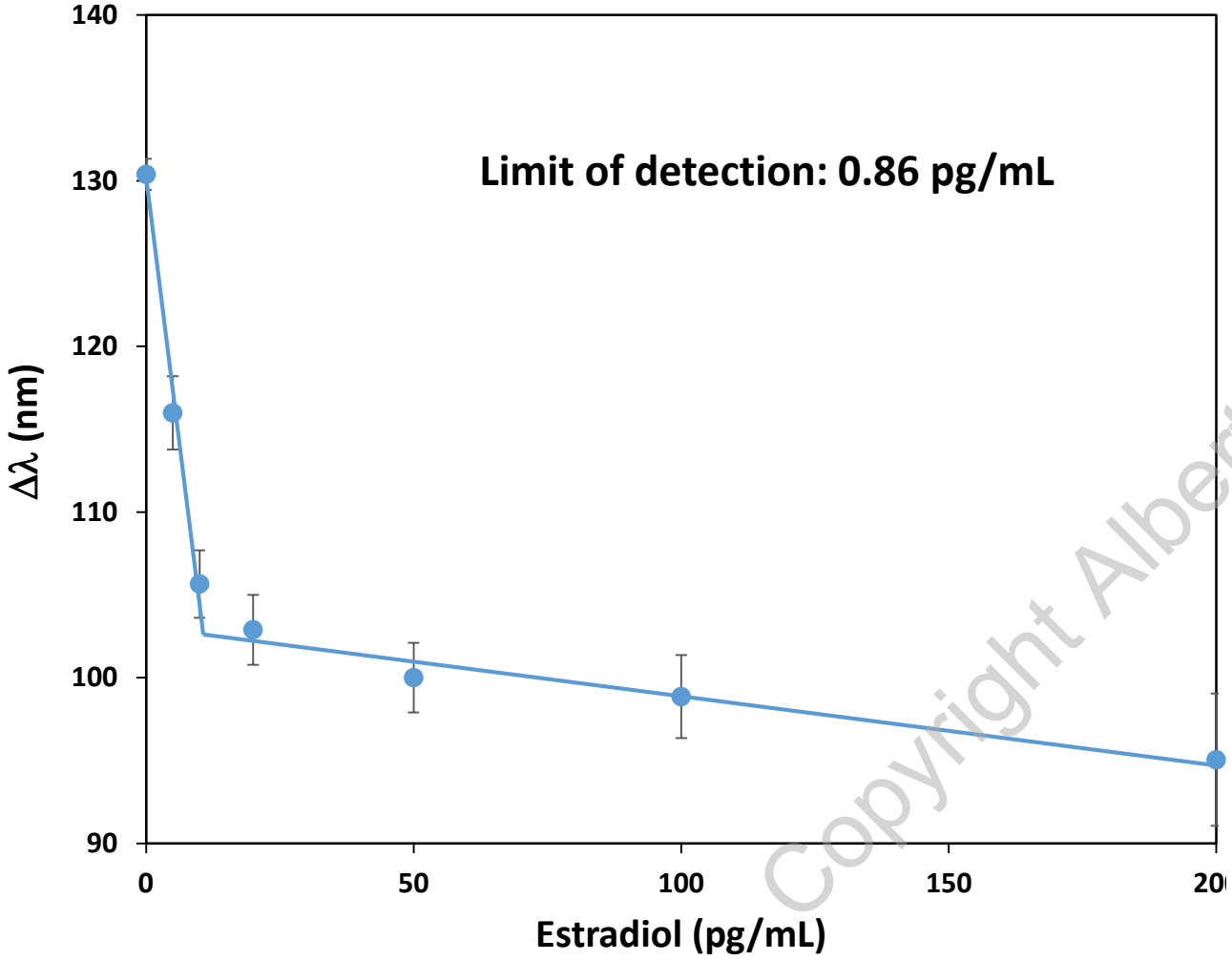
Alsager et al., *Anal Chem*. 2015, 87, 4201.

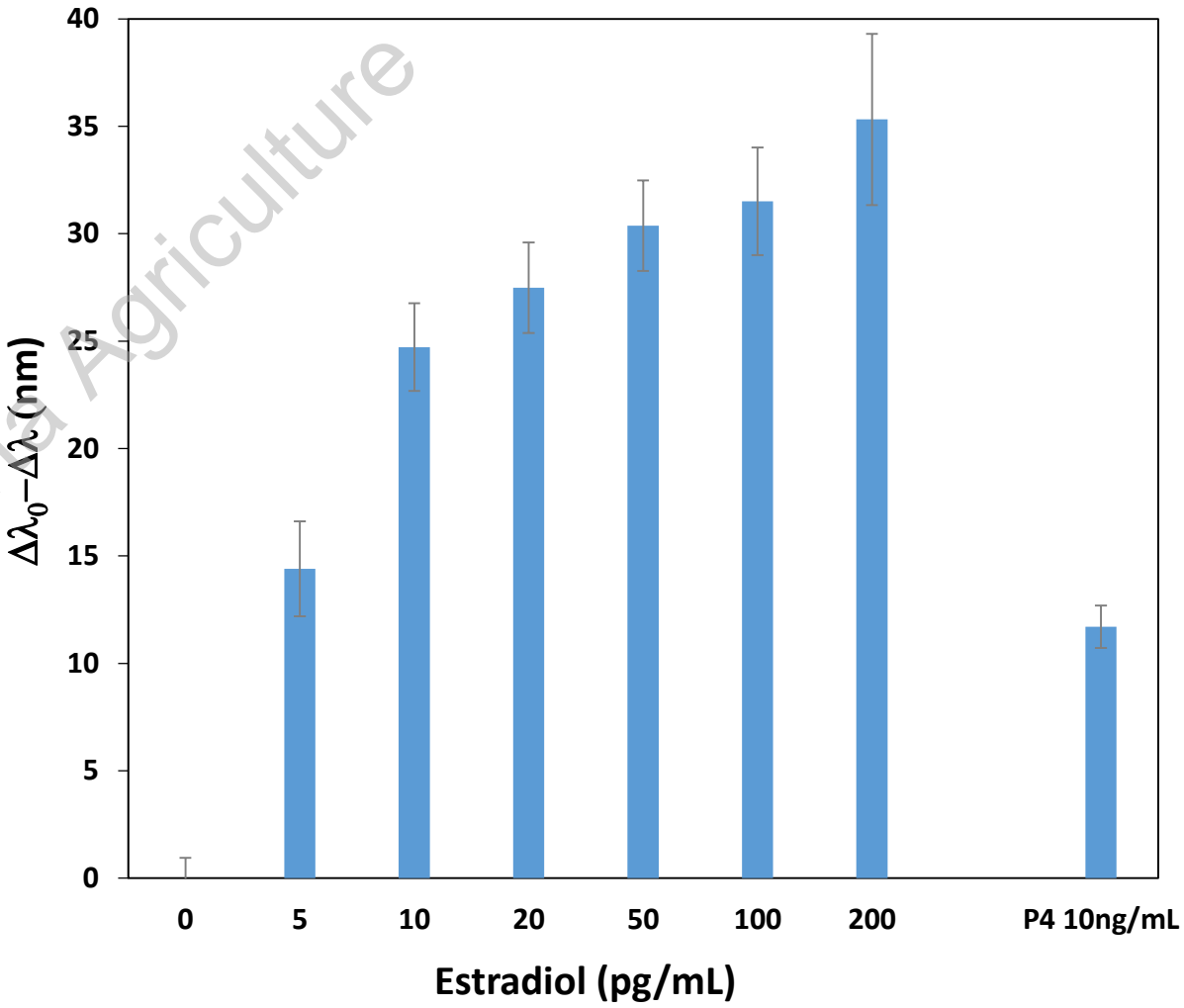
Detection of Estradiol Based on the NaCl Surface Blocking



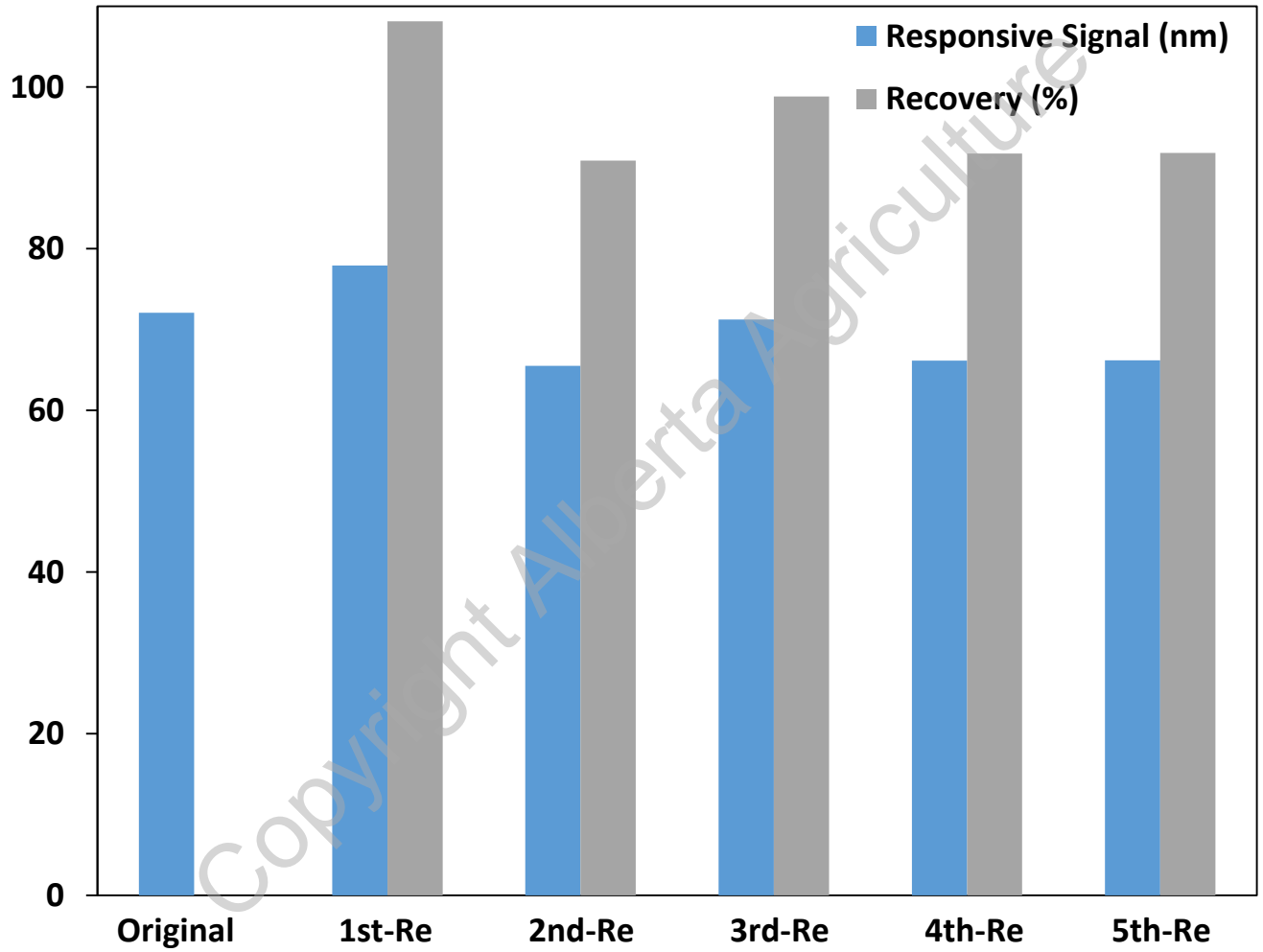


Detection of Estradiol Based on the $CaCl_2$ Surface Blocking





Regeneration of Estradiol Sensor



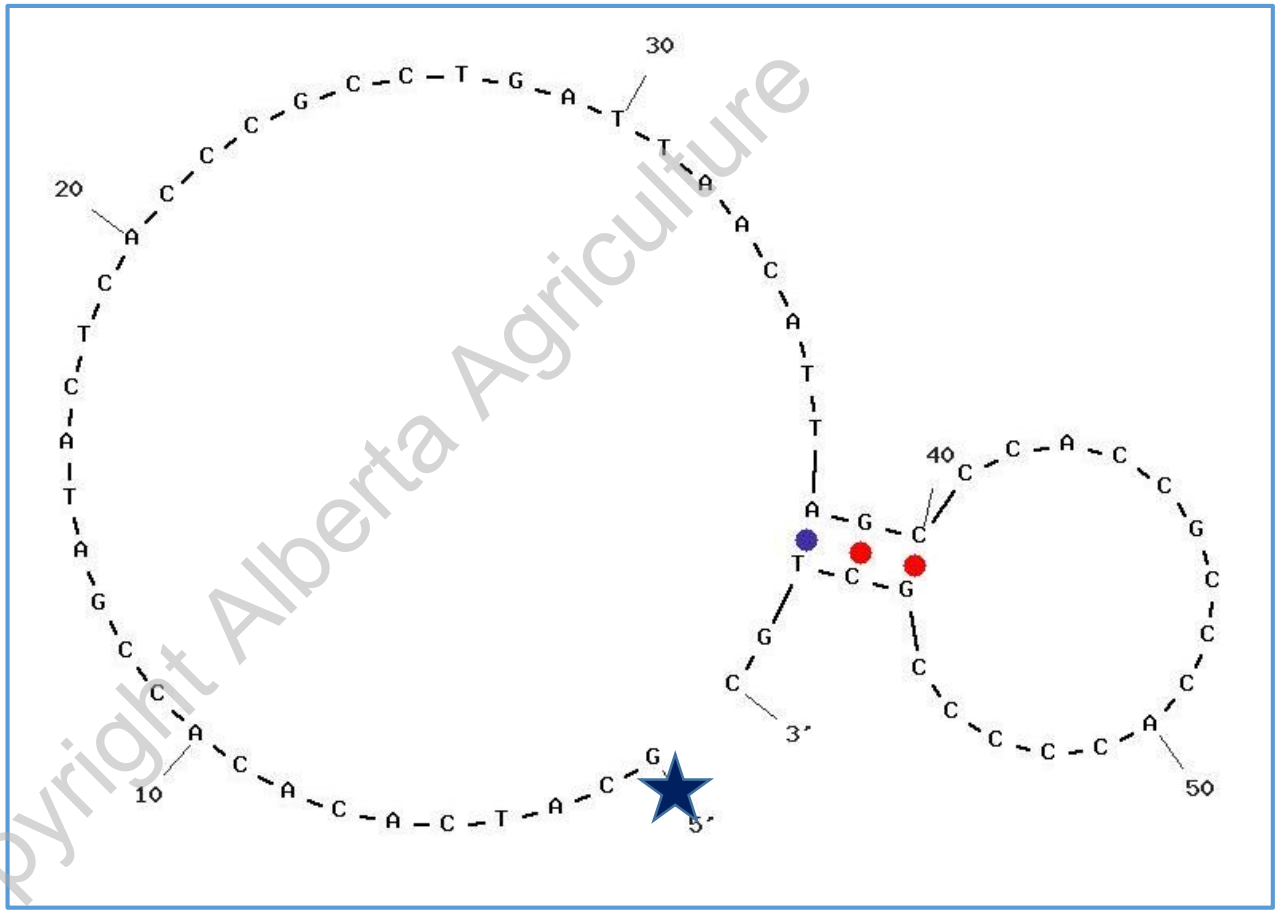
Estradiol Detection in Hormones Free Milk

Estradiol-Spiked (pg/mL)	Estradiol-Measured (pg/mL)				Recovery (%)			
	1st	2nd	3rd	Average	1st	2nd	3rd	Average
0	0.53	0.00	0.61	**0.38±0.27**	n/a	n/a	n/a	**n/a**
5	5.12	7.03	4.24	**5.46±1.16**	102.50	140.60	84.78	**109.29±23.29**
10	9.71	9.08	9.68	**9.49±0.29**	97.12	90.77	96.75	**94.88±2.91**
50	78.11	49.75	39.80	**55.89±16.23**	156.22	99.50	79.60	**111.77±32.46**

Estradiol Detection in Commercial and Farm Milk

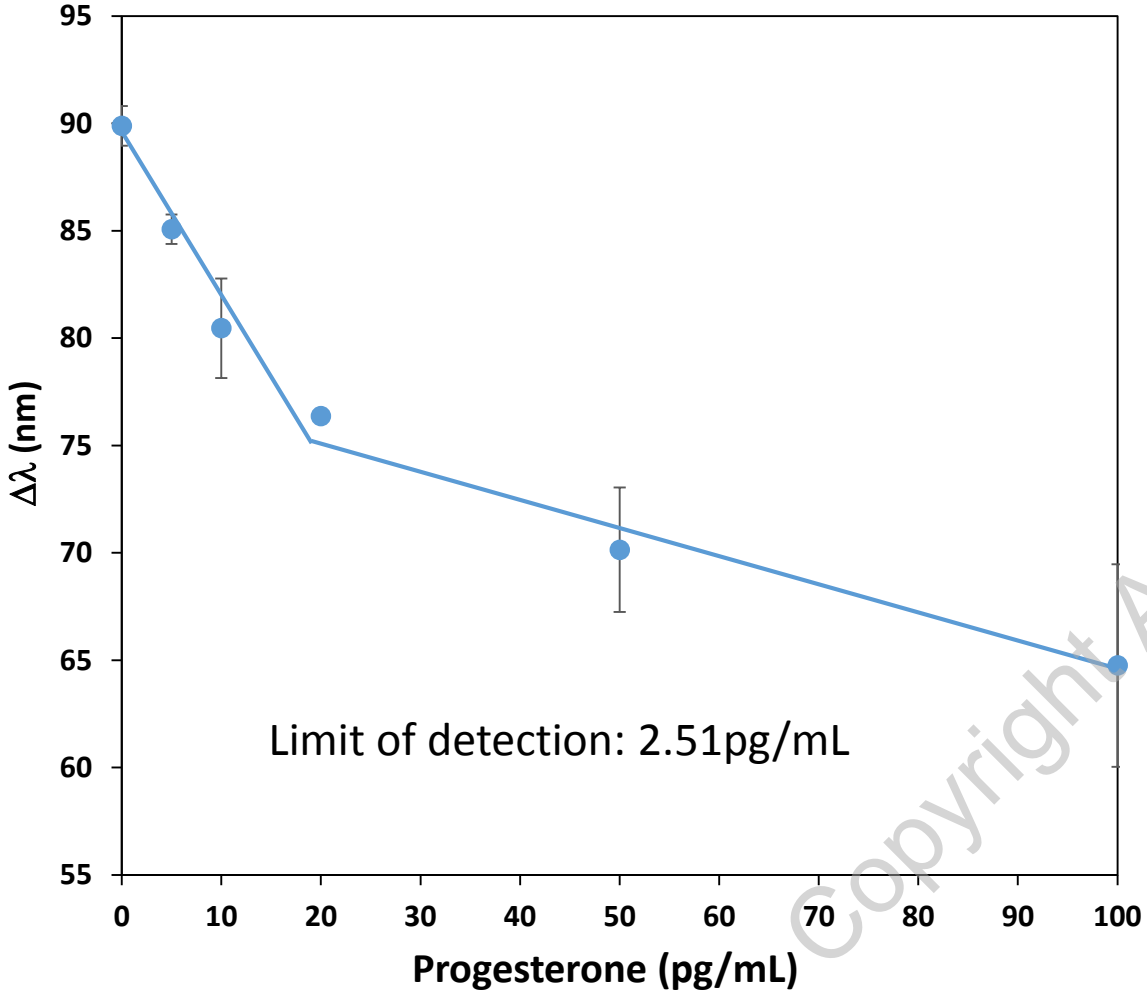
	Sample ID	Measured E2 (pg/mL)	Average measured E2 (pg/mL)
Commercial skim milk	#1	0	**0.89±1.25**
	#2	0	
	#3	2.66	
Commercial 2% milk	#1	7.09	**8.41±0.94**
	#2	9.13	
	#3	9.03	
Farm milk expected to have high concentration of E2	#3644	17.5	**9.10±4.32**
	#2258	5.25	
	#2301	7.16	
	#2521	8.5	
	#5088	7.11	
Farm milk expected to have low concentration of E2	#2267	4.60	**3.99±1.41**
	#2307	2.29	
	#5120	4.88	
	#2535	5.79	
	#5013	2.37	

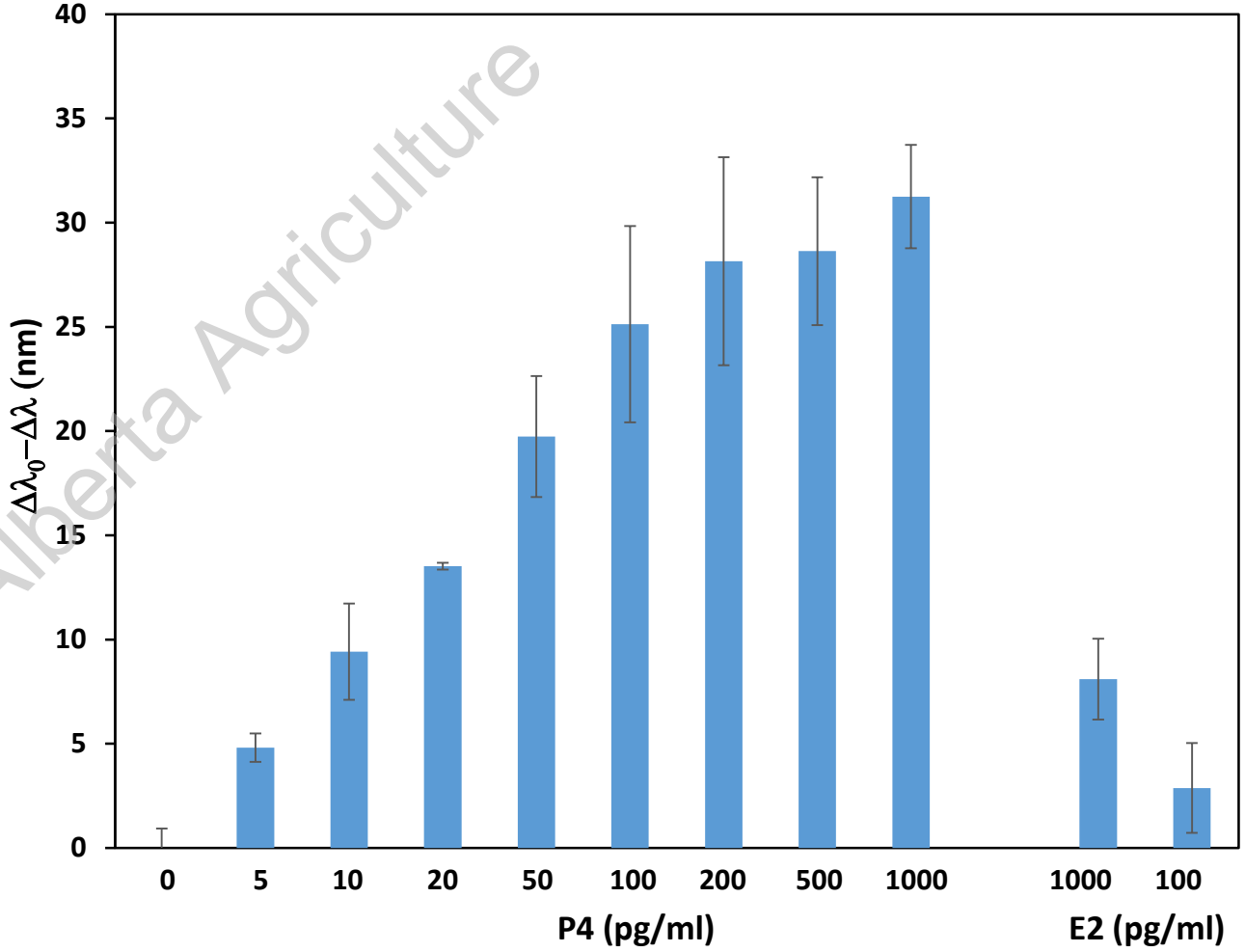
Progesterone DNA Aptamer



Jiménez et al., *Anal. Chem*. 2015, 87, 1075.

P4 Detection-Aptamer/Target Interaction-($CaCl_2$ Surface Blocking)

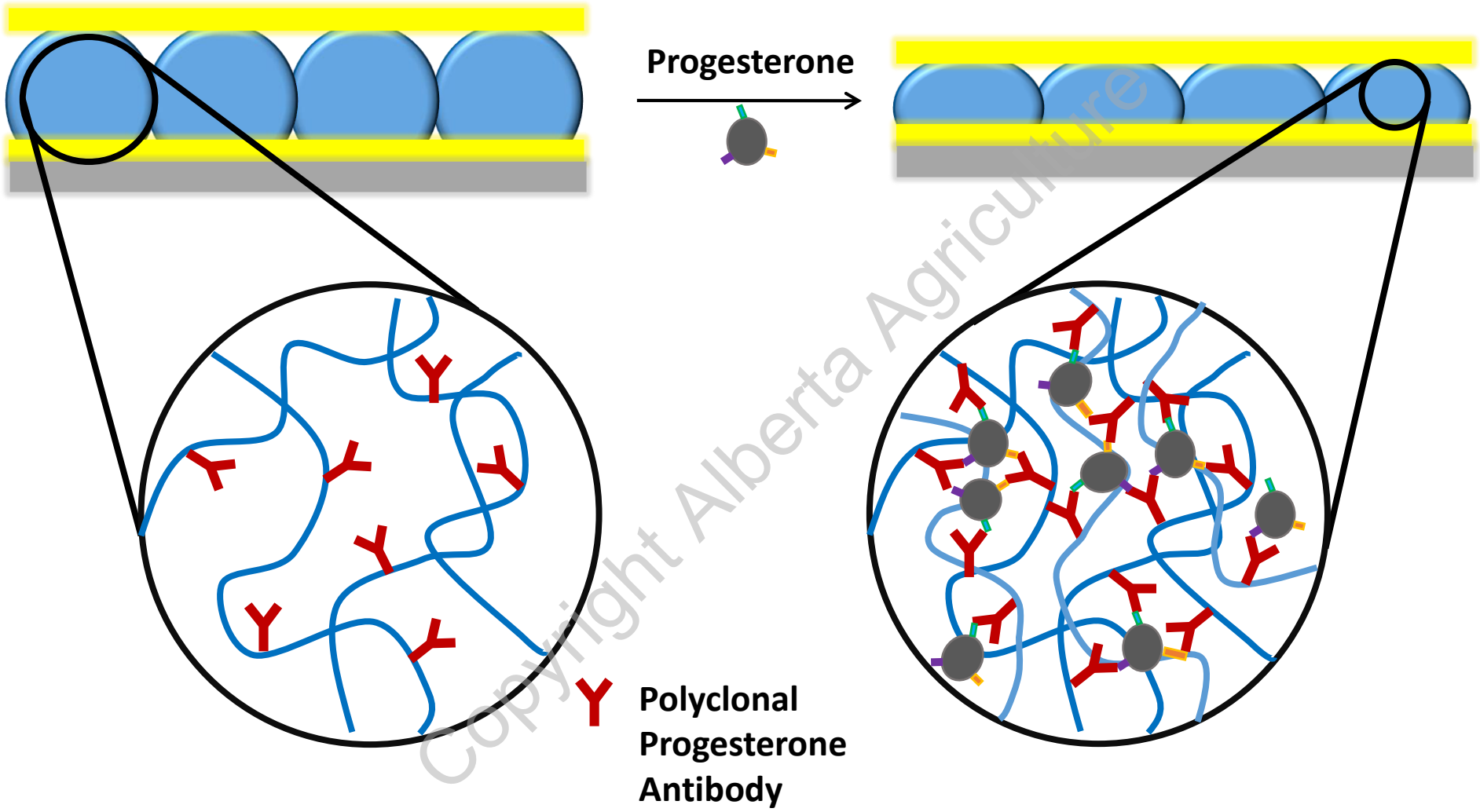




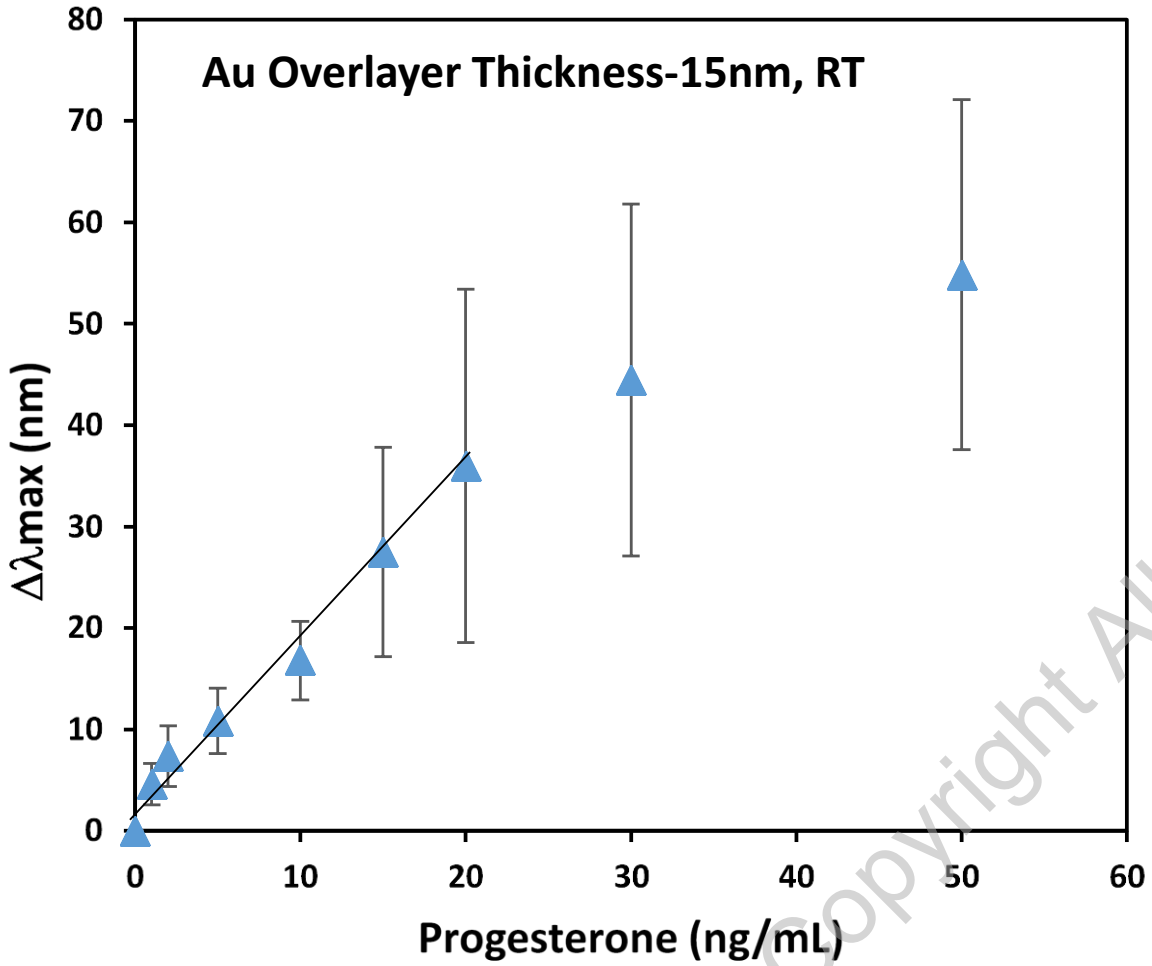
P4 Detection in Commercial and Farm Milk

	Sample ID	P4-Measured (pg/mL) Dilute 100X	P4-Calculated (ng/mL) Original sample	Average P4 (ng/mL)
Commercial skim milk	#1	4.6	0.46	**0.59±0.20**
	#2	8.7	0.87	
	#3	4.3	0.43	
Commercial 2% milk	#1	11.5	1.15	**1.82±0.55**
	#2	25.1	2.51	
	#3	18.2	1.82	
Farm milk expected to have low concentration of P4	#3644	14.8	1.48	**1.89±0.35**
	#2258	22.0	2.20	
	#2301	21.0	2.10	
	#2521	22.3	2.23	
	#5088	14.6	1.46	
Farm milk expected to have high concentration of P4	#2267	95.0	9.50	**8.27±1.00**
	#2307	70.3	7.03	
	#5120	80.9	8.09	
	#2535	93.4	9.34	
	#5013	74.1	7.41	

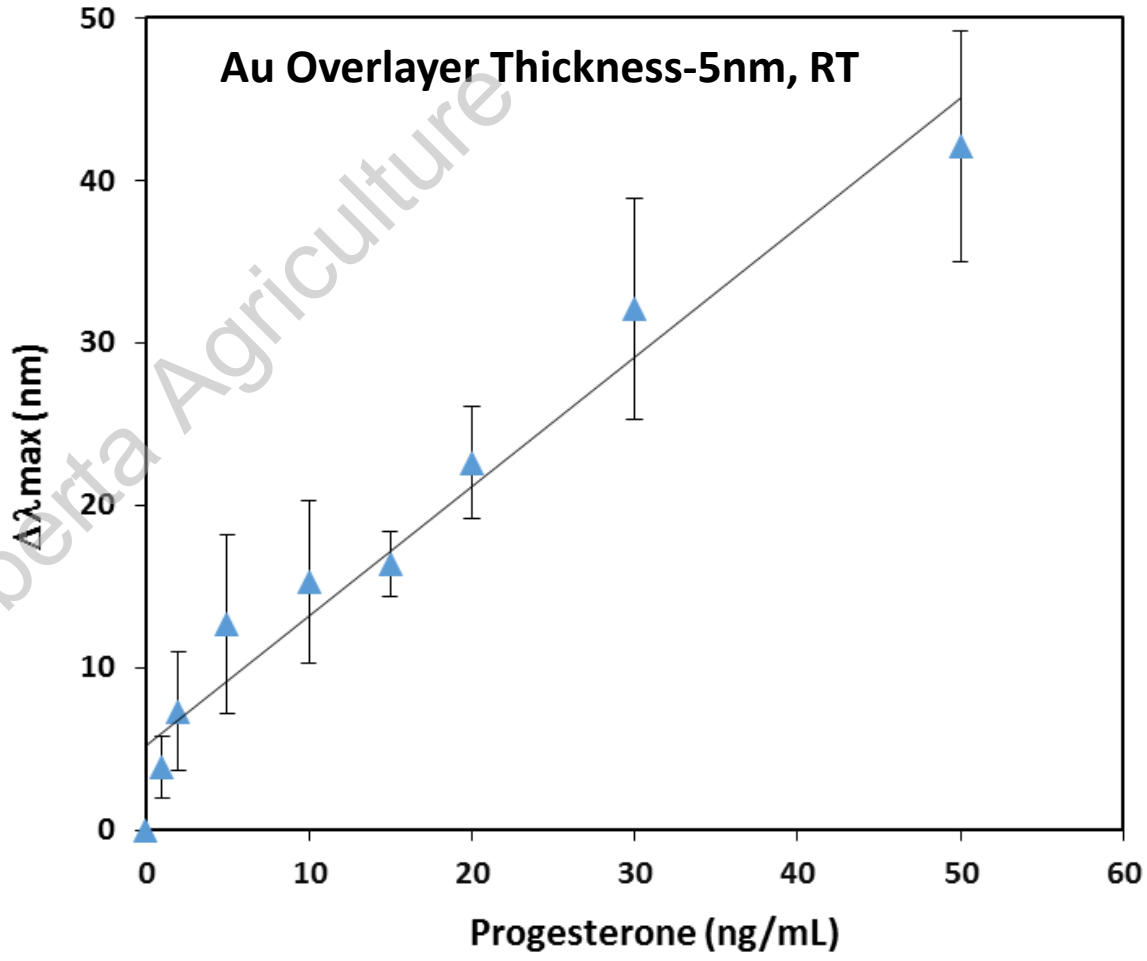
Progesterone Detection--Antibody/Antigen Interaction



Progesterone Detection

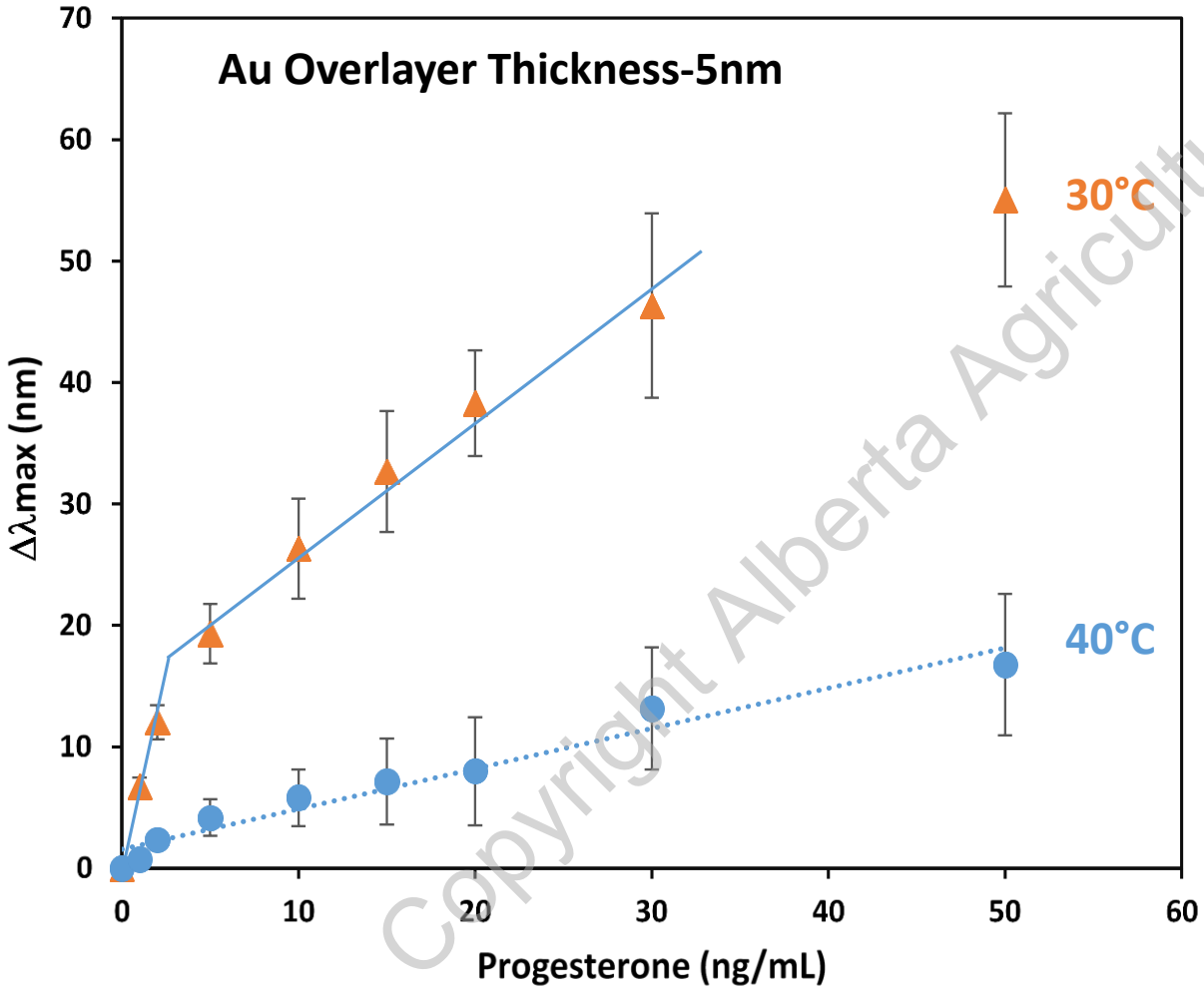


Limit of detection: 3.6 ng/mL



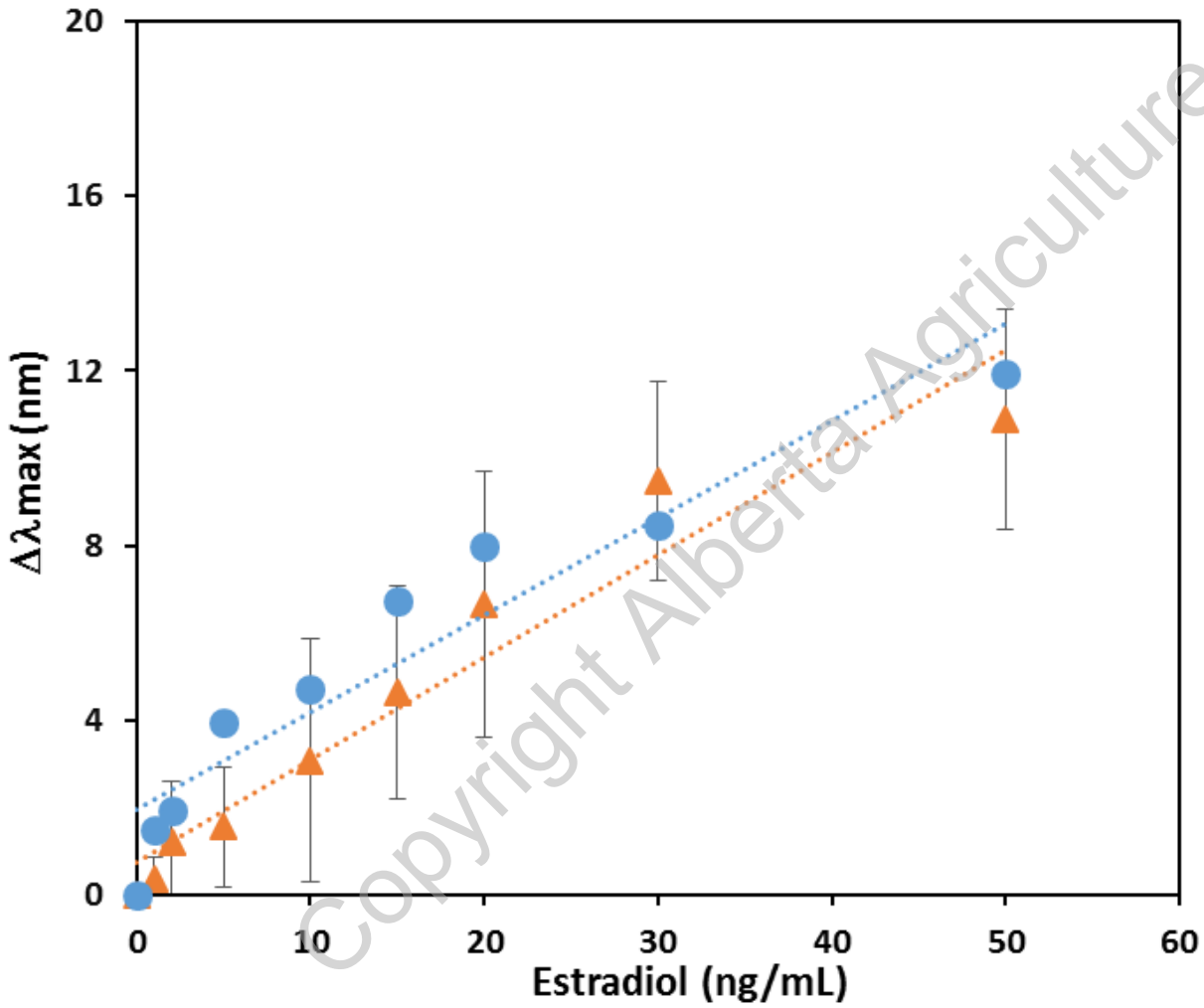
Limit of detection: 1.77 ng/mL

Effect of Temperature



Limit of detection: 0.25 ng/mL (30 °C)

Specificity of The P4 Sensor



30°C

Room Temperature (~21°C)

Summary of P4 Detection in aqueous samples

Thickness of Sensor's Overlayer (nm)	Sensing Temperature (°C)	Limit of Detection (ng/mL)	P4 Linear Range (ng/mL)
15	21	3.6	0-20
5	21	1.77	0-50
5	**30**	**0.25**	**0-30**

Conclusions

- P4 and E2 biosensors based on etalon device had been developed by sandwiching a pNIPAm-co-AAc microgels monolayer between two Au layers.

- E2 could be detected in aqueous samples with a linear range of E2 from 0 to 200 pg/mL and a detection limit of 0.86 pg/mL. The E2 sensor also could be regenerated 5 times, and had good performance to measure E2 in commercial and farm milk directly.

- P4 could be detected in aqueous samples with a linear range from 0 to 100 pg/mL and a detection limit of 2.51 pg/mL. The sensor showed good performance to detect P4 in diluted milk.

- Another immunosensor aims to detect P4 directly in milk also was developed with a linear range from 0 to 30 ng/mL and a calculated detection limit of 0.25 ng/mL at 30°C.

Acknowledgement

- The project was supported in part **by *Growing Forward 2*, a federal-provincial-territorial initiative.** The views and opinions expressed in this project/these findings are not necessarily those of Agriculture and Agri-Food Canada or Alberta Agriculture and Forestry.

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