

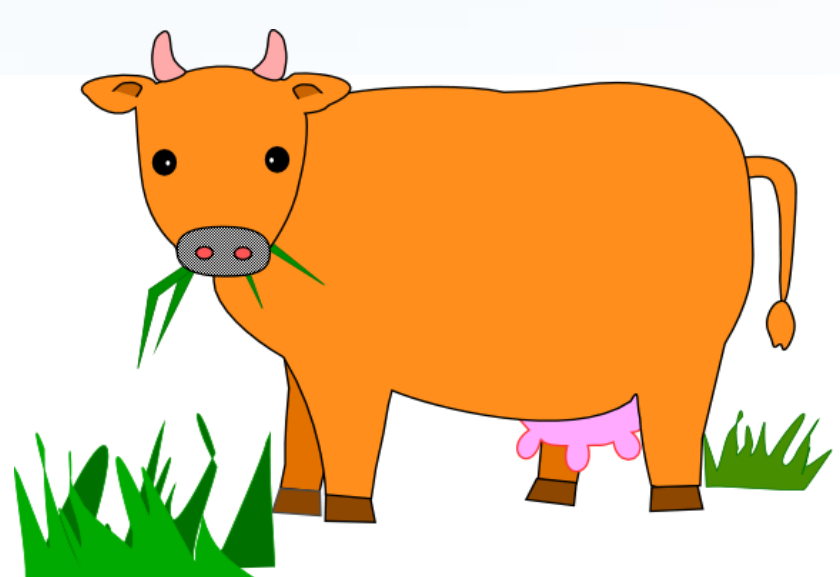
Surface Plasmon Resonance Biosensor for Detecting Luteinizing Hormone

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1. Introduction

Background

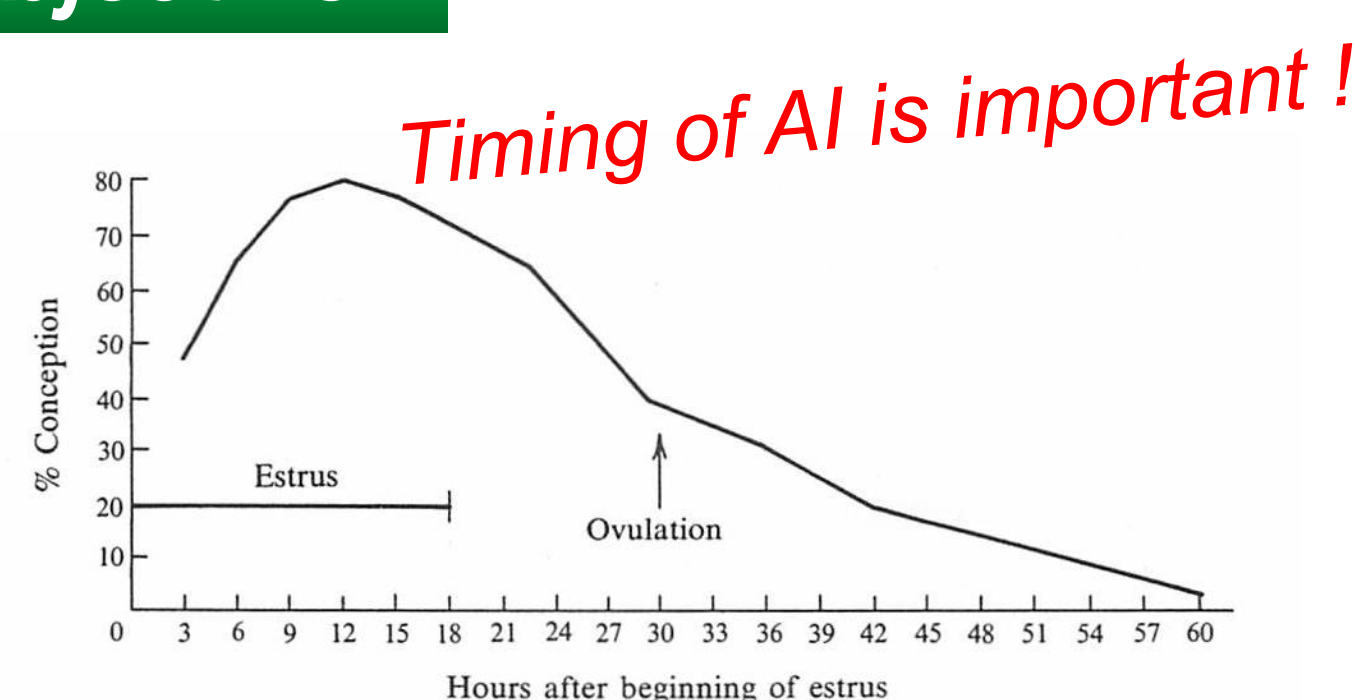


Livestock management depends on **Experience, Feeling, Luck**
⇒ Low productivity

Applying engineering technique
(Measurement of sexual hormones)

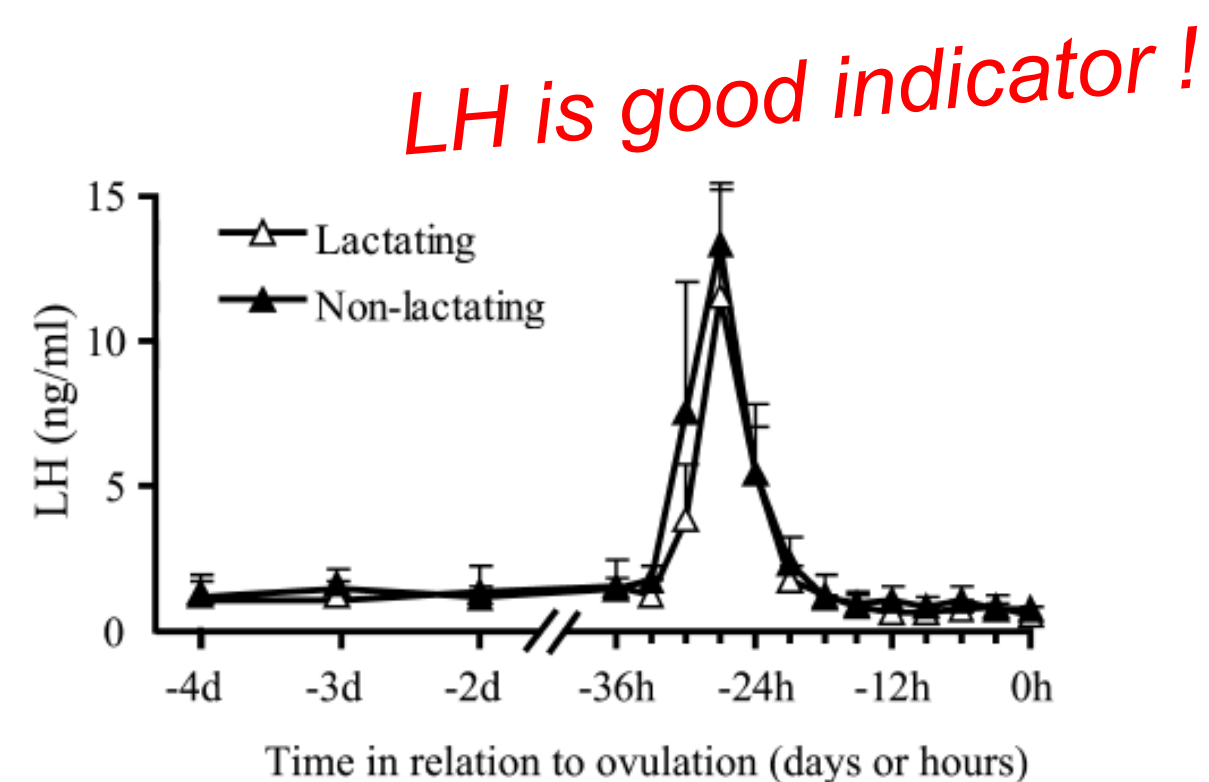
Highly efficient production

Objective



The effect of time of insemination in relation to estrus on conception rate

G.W. Salisbury et al., *Physiology of reproduction and artificial insemination of cattle*, 2nd ed., 565-576, 1978



Temporal relationships between plasma concentration of LH from four days before to the time of ovulation

Endo et al., *J. Reprod. Dev.*, 58, 685-690, 2012

Develop a measurement system of LH that is easy to use for farmers

➔ **SPR biosensor**

2. SPR biosensor

Apparatus

Sensor chip

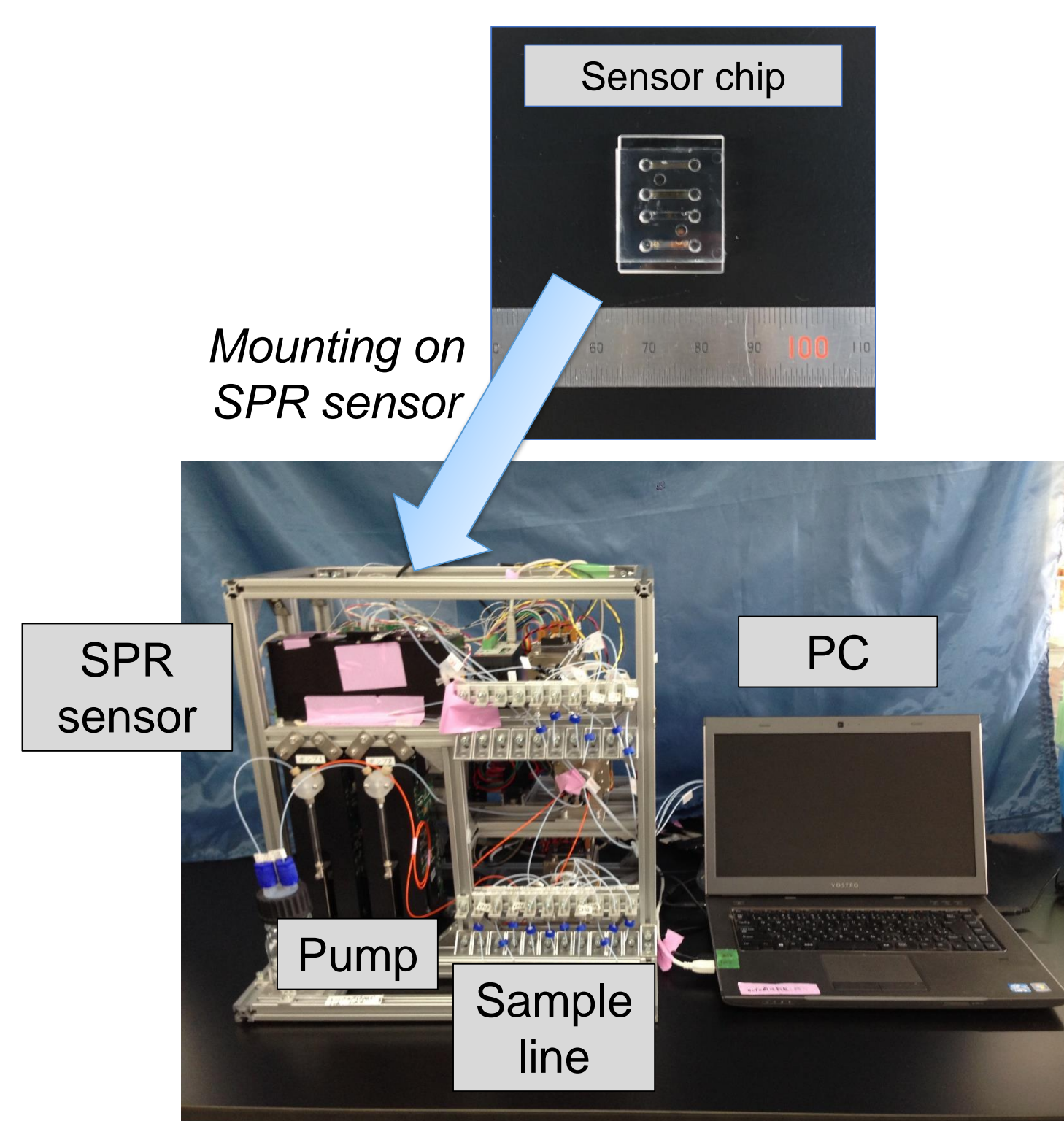
Sensor chip has 4 channels
2 of 4 channels are connected to the SPR system

Sample line

10 samples can be connected to the SPR biosensor

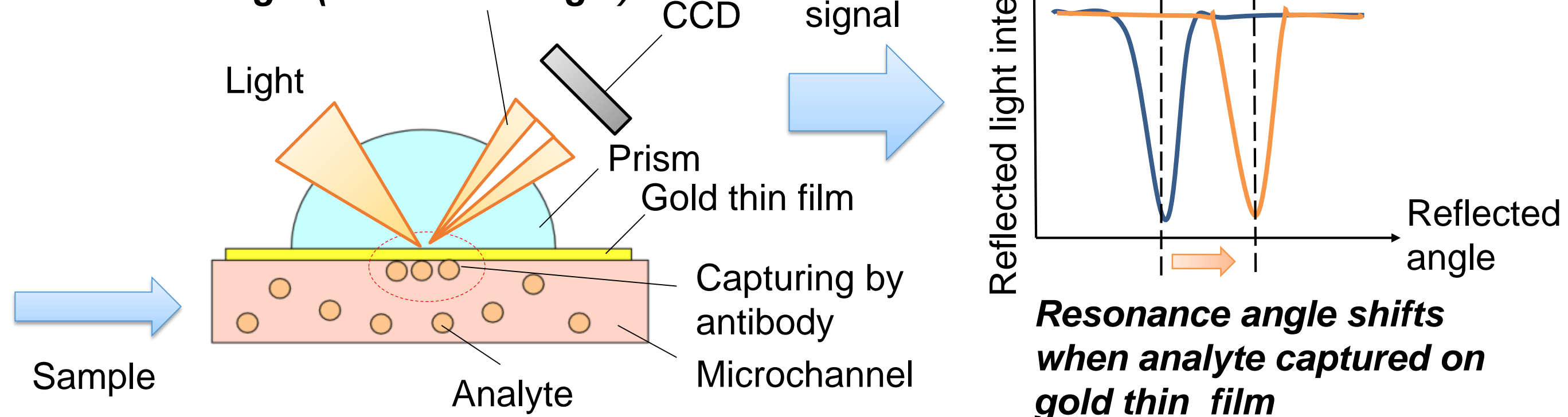
Operation

SPR biosensor is automated system
It is controlled with PC



Principle

Reduction of reflected light intensity at certain angle (resonance angle)



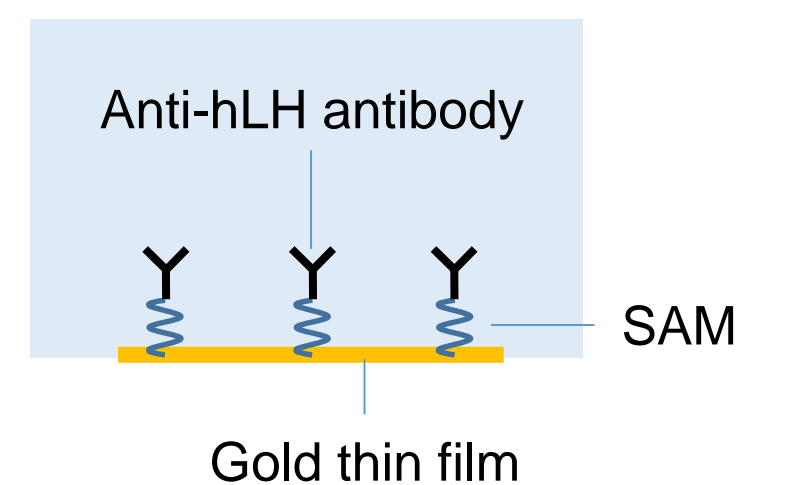
3. Measurement

Procedure

Sandwich immunoassay was applied to amplify SPR signal

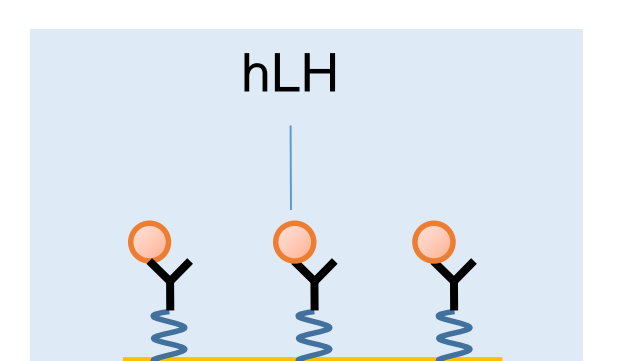
Step 1. Immobilization of primary antibody

Primary antibody (MyBioSource Inc., #MBS592103) was immobilized on gold thin film of sensor chip by amine coupling with carboxyl acid self-assembled monolayer



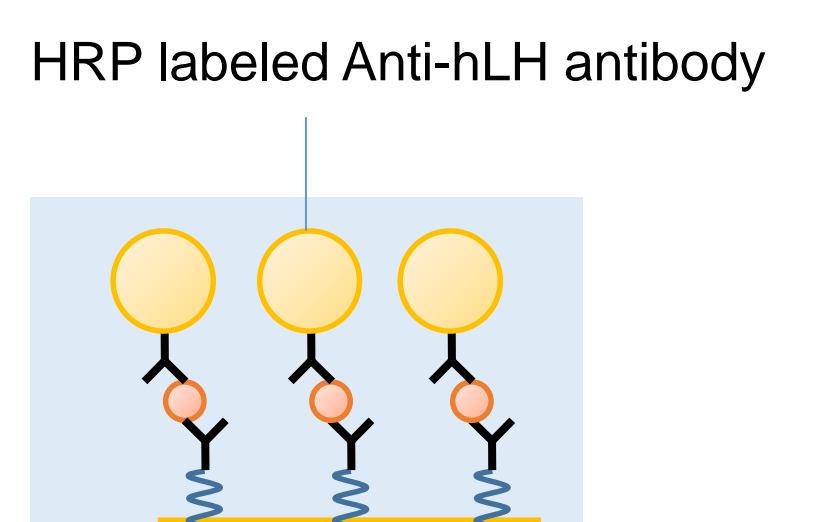
Step 2 Capturing luteinizing hormone (LH)

Human LH (Bio-Rad Laboratories, Inc., #PHP286) was used instead of bovine LH in this experiment



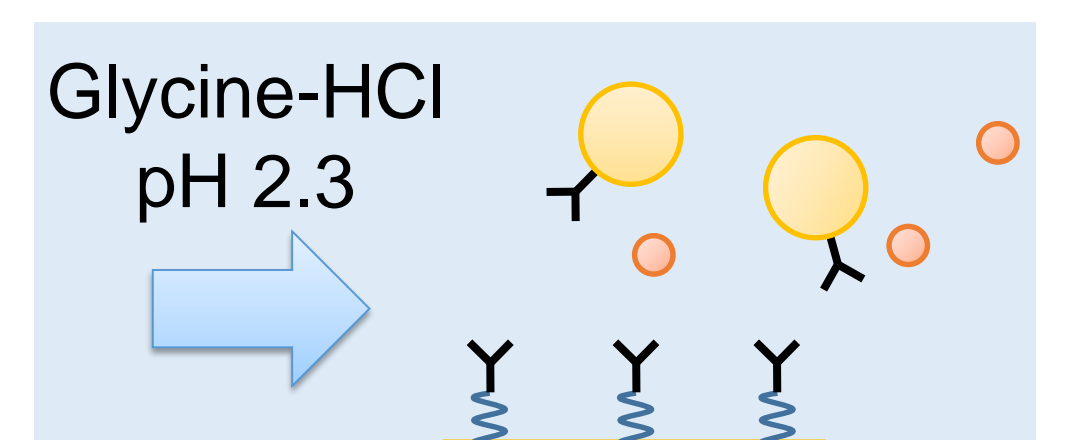
Step 3 Injection of HRP labeled secondary antibody

HRP was labeled to the antibody (Bio-Rad Laboratories, Inc., #MCA5806G) with HRP labeling kit (Dojindo Molecular Technologies, Inc., #LK11)



Step 4 Regeneration of primary antibody

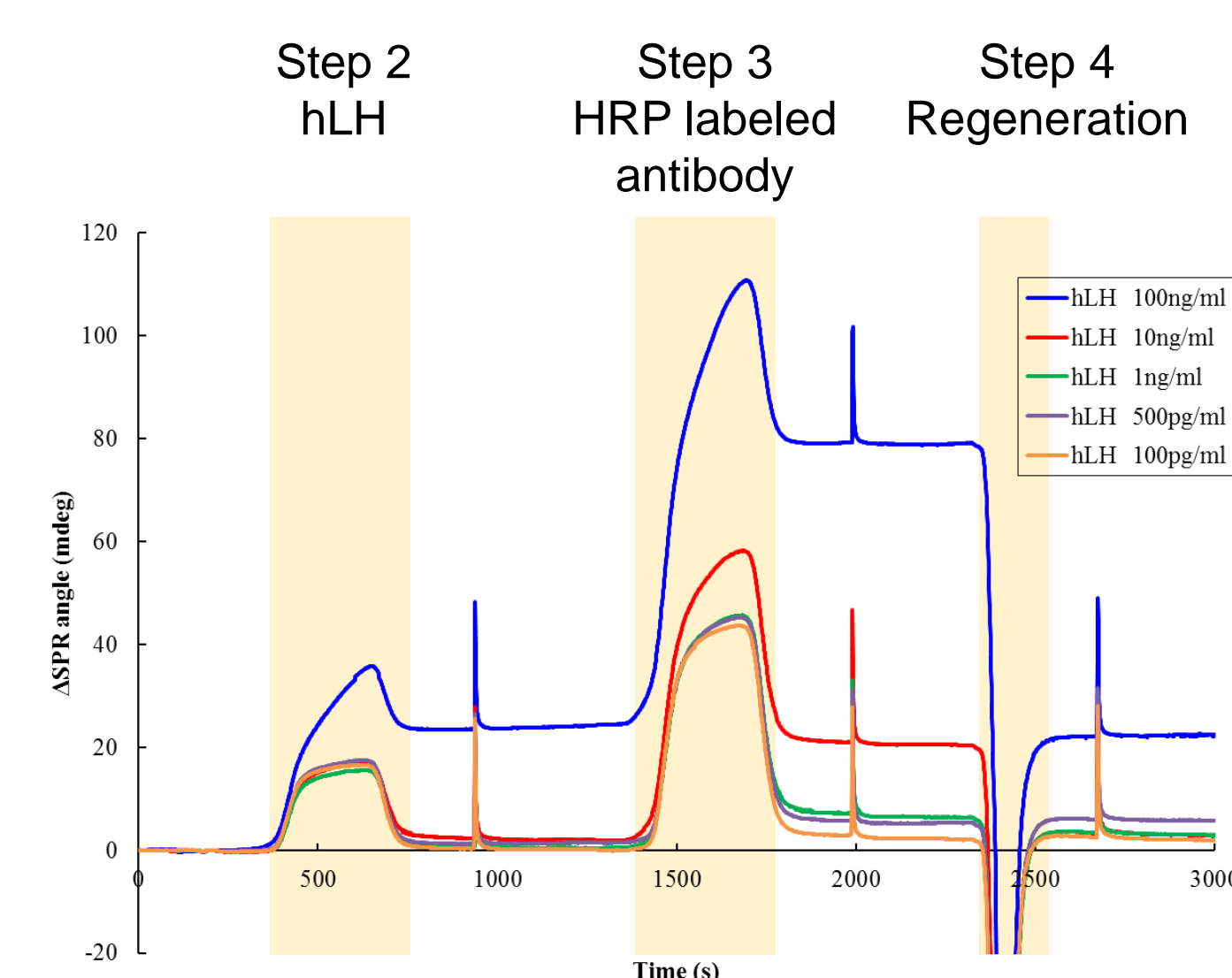
Glycine-HCl pH 2.3 was used for regeneration



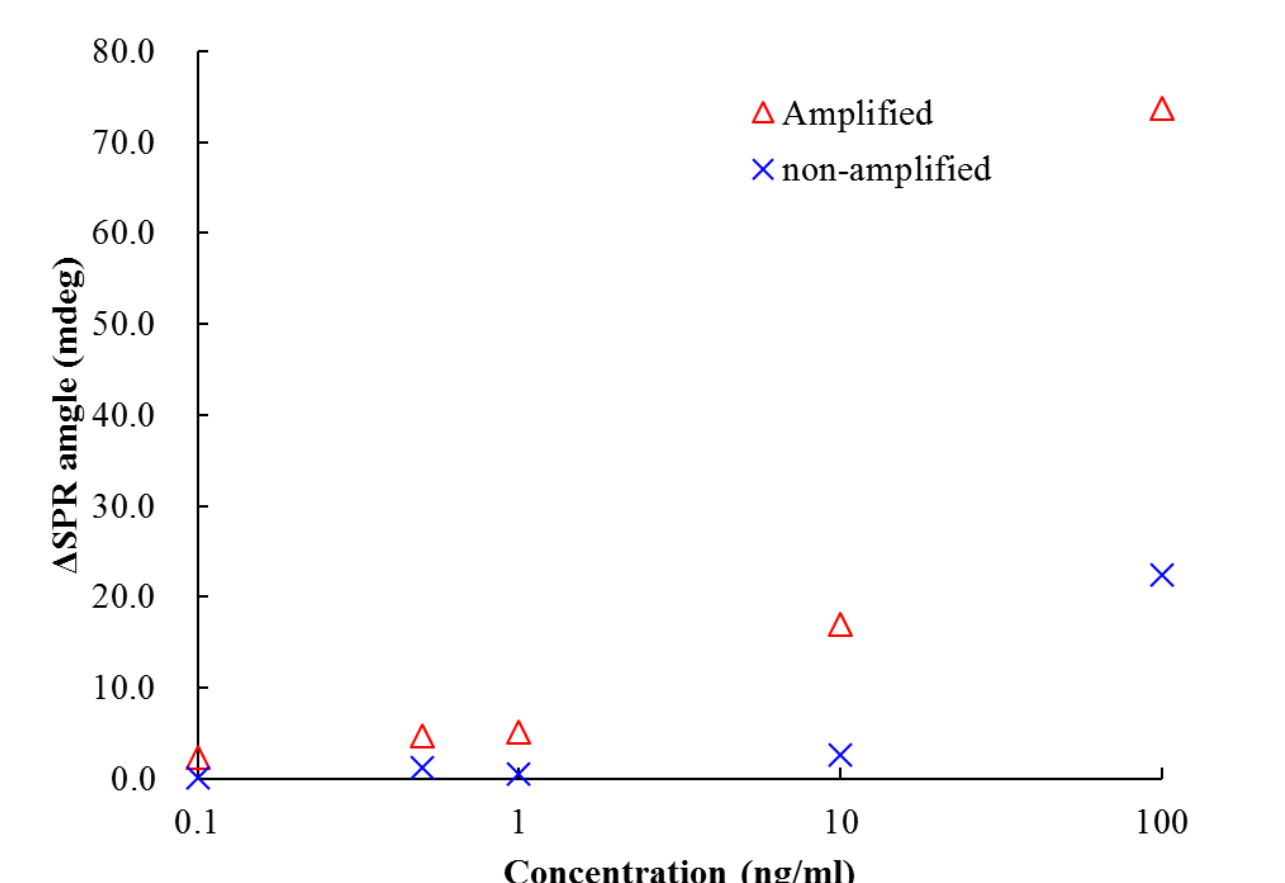
Step 5 Repetition

Step 2 ~ 4 were repeated to measure different concentration

4. Results



SPR signal at each concentration



Shift of SPR angle of amplified (Red triangle) and non-amplified (Blue cross) at each concentration of LH (n = 2)

SPR signal was amplified after HRP labeled antibody was injected
Shift of SPR angle of 500 pg/ml hLH can be detected by amplification

5. Conclusion

- Developed SPR biosensor
- Succeeded detecting 500 pg/ml hLH by amplifying with HRP labeled secondary antibody