Hépatinov

Inserm

Institut national
de la santé et de la recherche médicale

U 785

IDA Institut d'Alembert

ENS CACHAN

Study of interactions between dsDNA and anti-dsDNA antibodies in Autoimmune Hepatitis and Systemic Lupus Erythematosus using Surface Plasmon Resonance Imaging-based strategy

• **E. Ballot**
Praticien Hospitalier, Hôpital Saint-Antoine, Paris

•**J.C. Duclos-Vallée**
Professeur des Universités, Hôpital Paul Brousse, Villejuif

• **Eleonora De Martin**
Assistante hospitalo-universitaire , Hôpital Paul Brousse, Villejuif

• **Malcolm Buckle**
Directeur de Recherche, ENS Cachan

• **Hervé Leh**
Ingénieur de recherche, ENS Cachan

Background

- Anti-double strand-DNA (ds-DNA) antibodies are specific for systemic lupus erythematosus (SLE), but can also be detected in 30% of type 1 autoimmune hepatitis (AIH) patients.

Muratori, Am J Gastroenterol 2009

- Some AIH sera are ANA negative by IIF but positive for anti-dsDNA by Farr test.

Saint-Antoine Immunology laboratory
Unpublished results

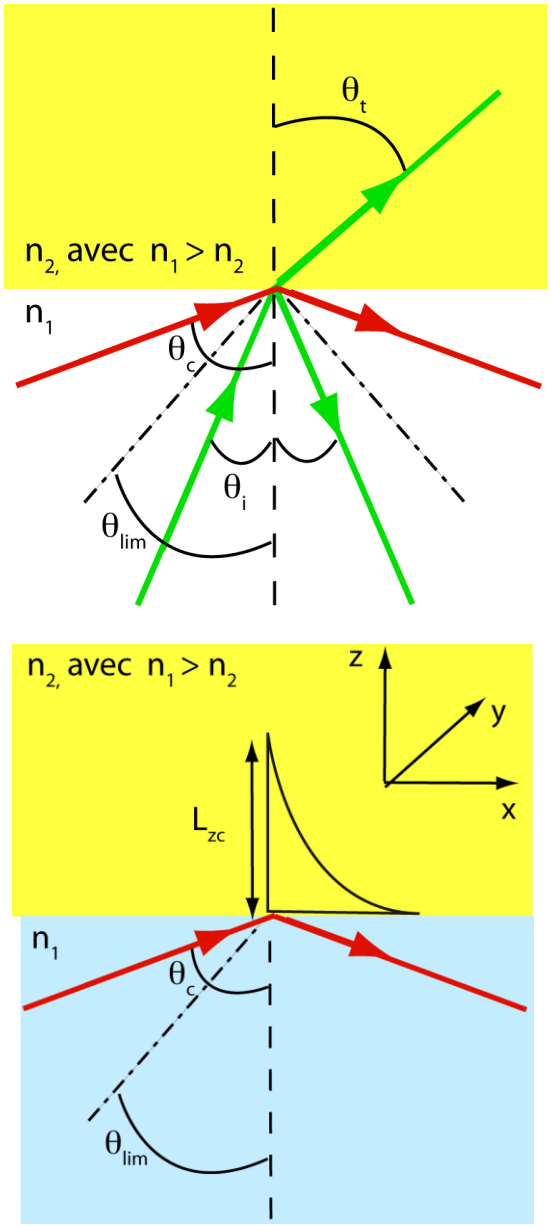
- Antibodies avidity varies with different antigenic dsDNAs, as demonstrated by kinetics binding variations.

Buhl, Biosens and Bioel 2009

SPR-based strategy

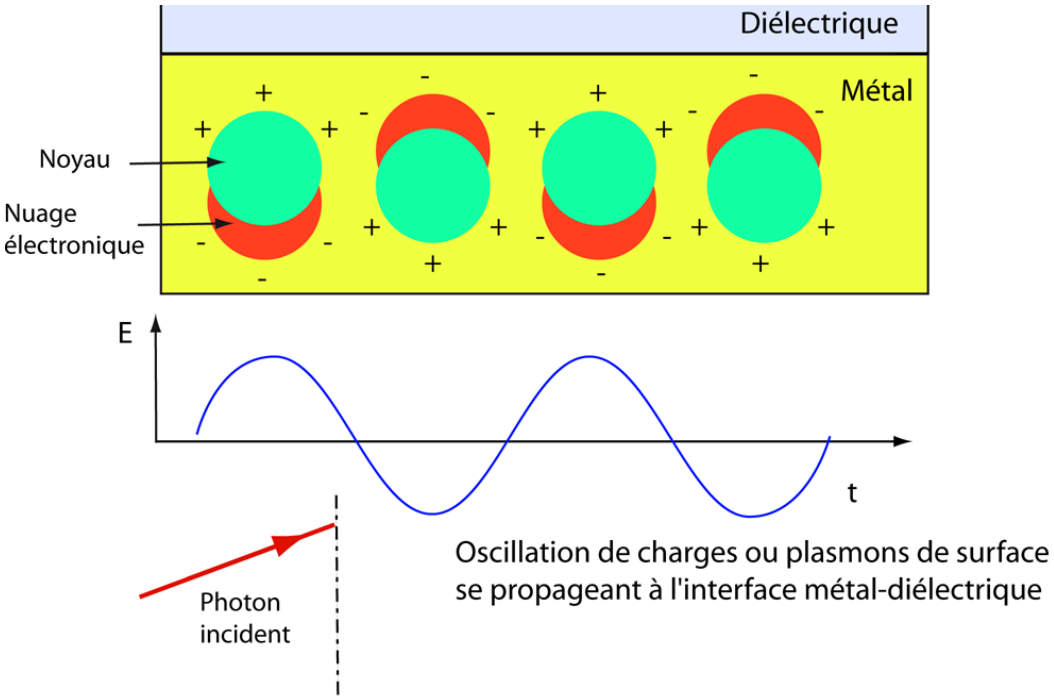
Surface Plasmon Resonance (SPR) is a real-time, label-free, optical detection method for studying the interaction of soluble analyte with immobilized ligand

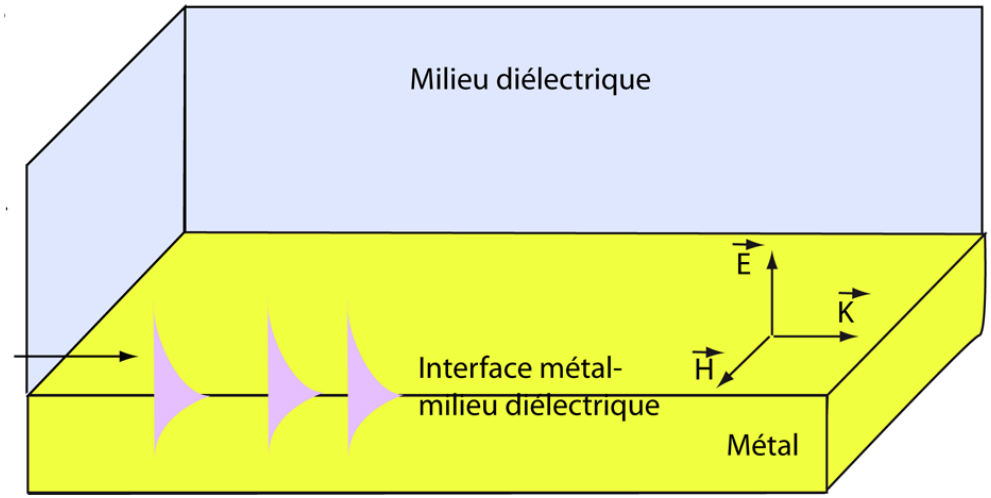
Résonance plasmonique de surface (SPR) (1)



- A l'interface de 2 matériaux ayant des indices de réfraction différents
- Réflexion totale interne : au-dessus d'un angle particulier, quand n1 > n2.
- En même temps, une onde électromagnétique évanescente se propage en s'éloignant de l'interface.

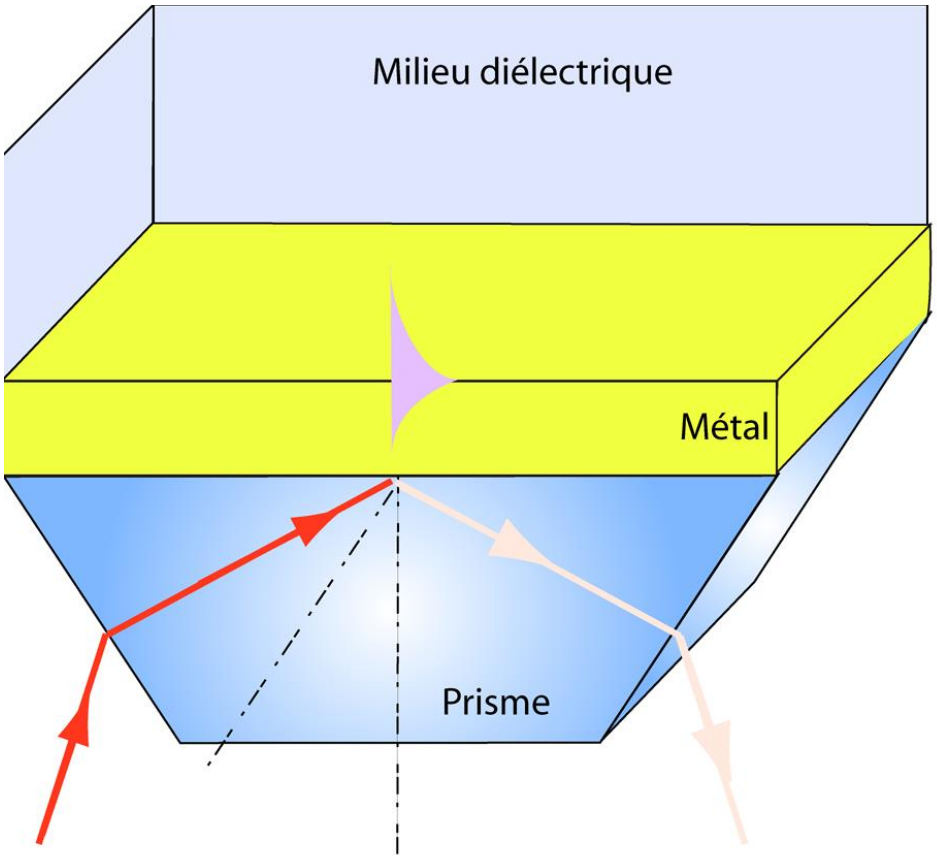
Résonance plasmonique de surface (SPR) (2)





- Mince film conducteur à l'interface
- L'interaction résonnante entre l'onde électromagnétique évanescente et les électrons libres du métal provoque une onde de densité d'électron appelé plasmon de surface et qui présente un caractère évanescent

Résonance plasmonique de surface (SPR) (3)



- Le champs évanescent créé par en condition de réflexion totale (par le prisme) permet de coupler un plasmon de surface.
- Une portion de la lumière incidente se complexe au plasmon.
- De l'énergie est absorbée par la résonance, l' intensité de la lumière réfléchie chute à l'angle de la SPR.

Composante longitudinale du vecteur d'onde du plasmon,

$$K_{sp} = \frac{\omega}{c}\sqrt{\frac{\varepsilon_m.\varepsilon_d}{\varepsilon_m + \varepsilon_d}}$$ avec $\varepsilon = n^2$

Résonance plasmonique de surface (SPR) (4)

Fixation de matériel biologique

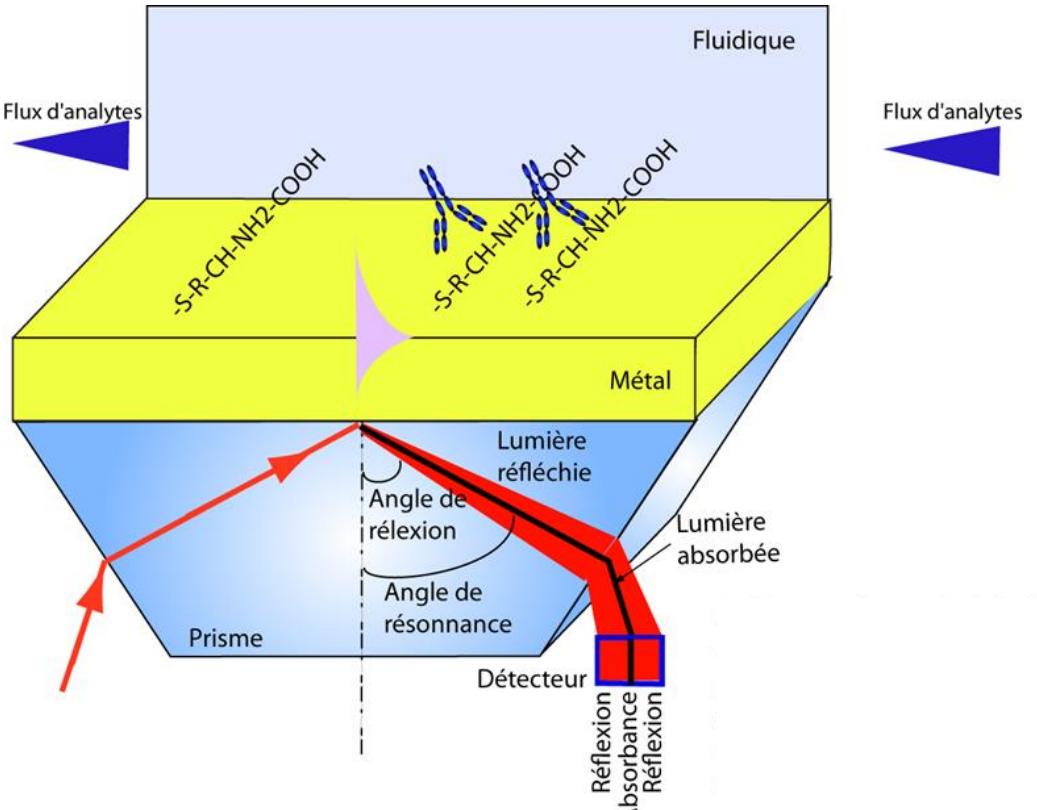
↓

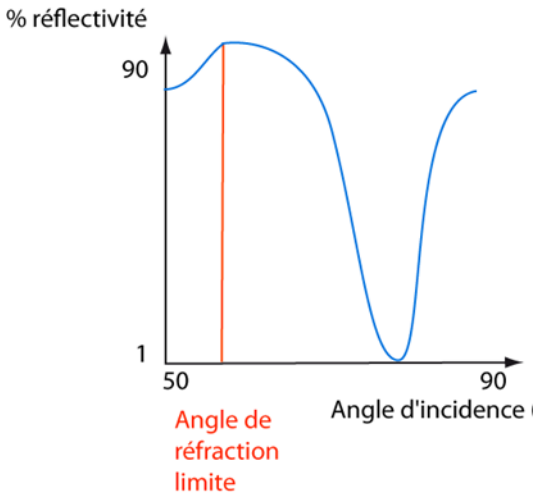
Perturbation de l'onde évanescente

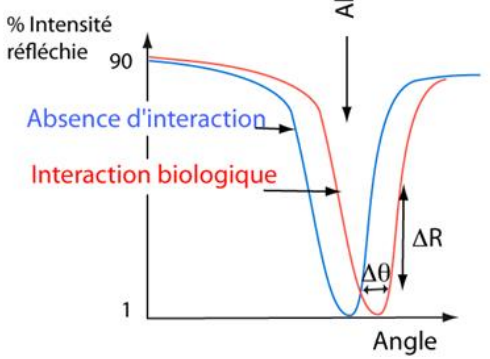
Modification de sa phase et amplitude

↓

Modification de l'angle de résonance

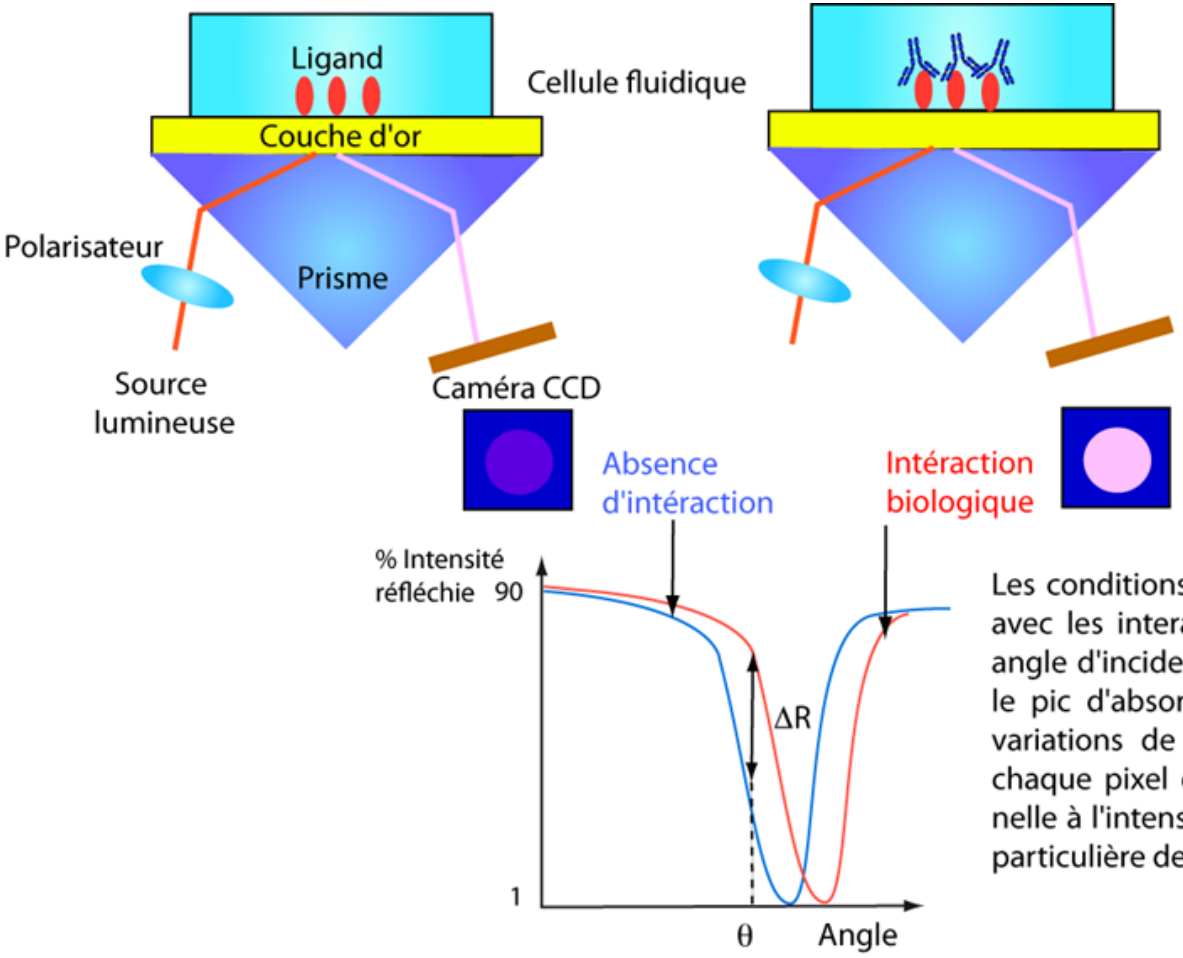






Résonance plasmonique de surface (SPR) (5)

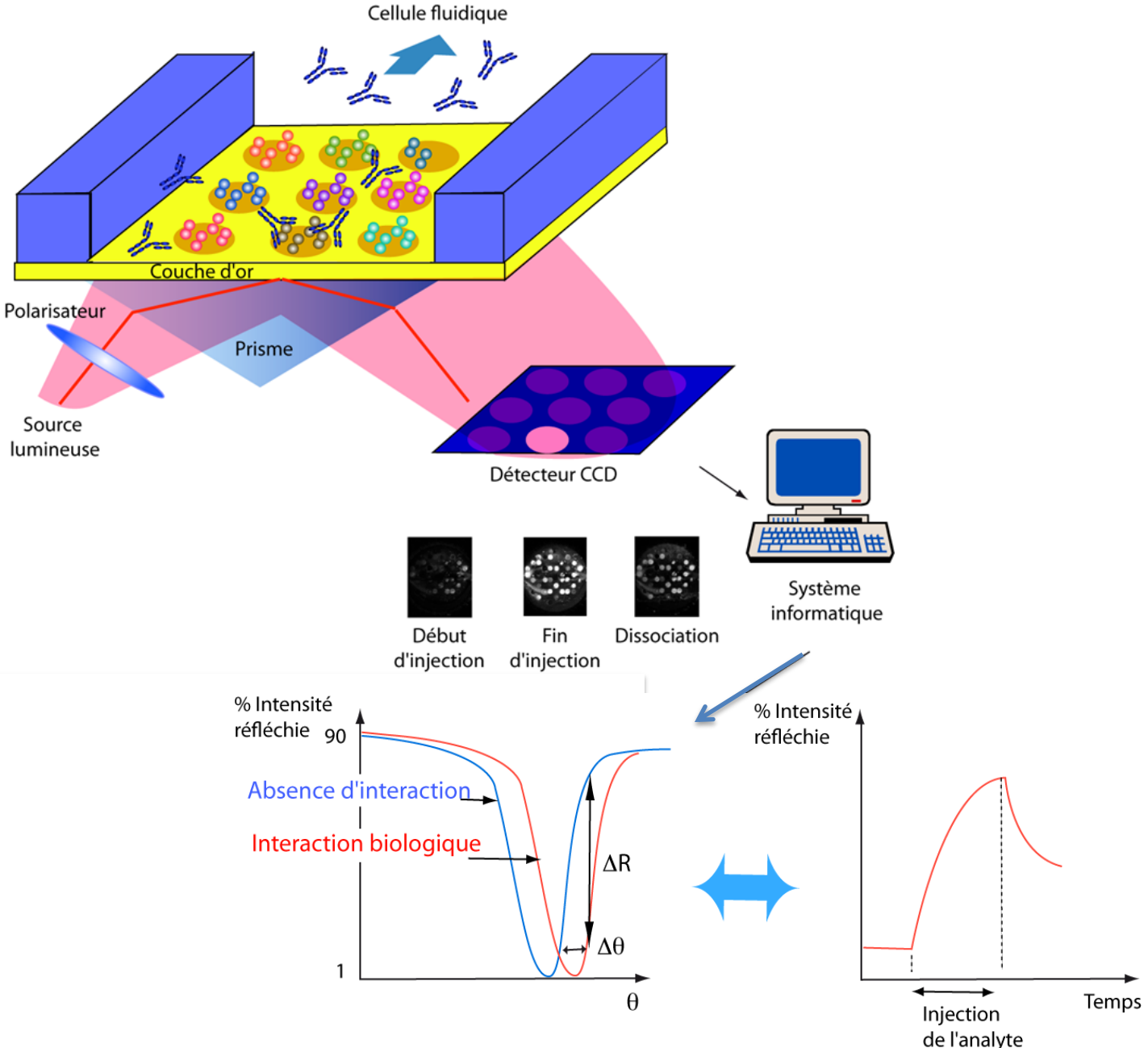
Imagerie par résonance plasmonique de surface (SPRi)



Les conditions de résonnance se modifiant avec les interactions de surface, on suit à angle d'incidence et longueur d'onde fixés, le pic d'absortion de la SPR à travers les variations de la réflectivité. La valeur de chaque pixel de la caméra est proportionnelle à l'intensité réfléchie issue d'une zone particulière de la surface.

Imagerie par résonance plasmonique de surface (SPRi)

Dispositif expérimental

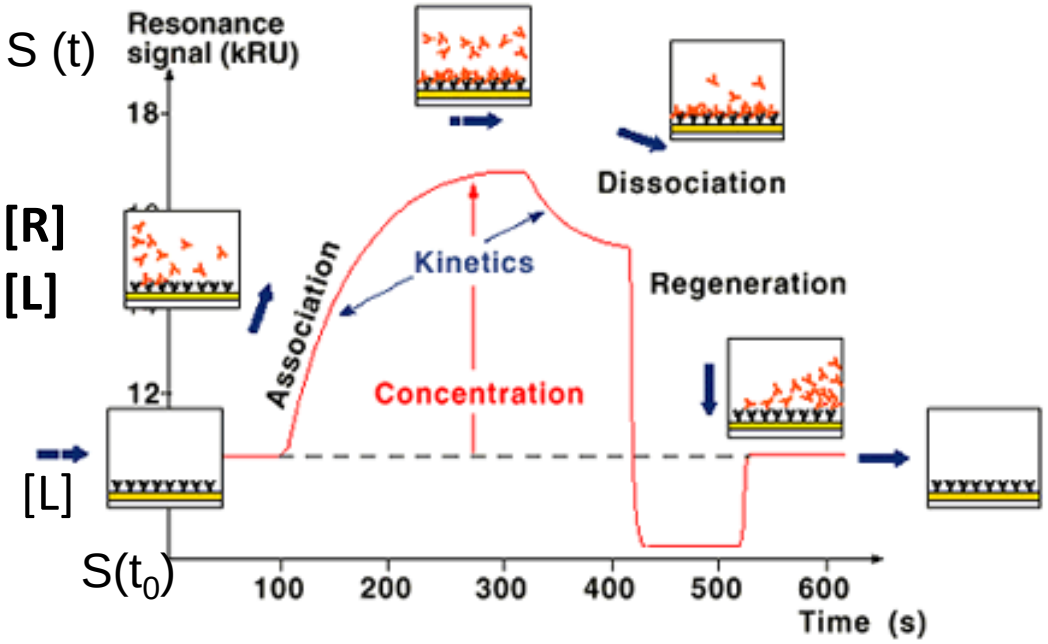


SPRi-based strategy Sensorgram analysis

$$[R] + [L] \xrightarrow{k_{on}} [RL] \xrightarrow{k_{off}} [R] + [L]$$

K_D, constante d'équilibre

$$K_D = \frac{k_{off}}{k_{on}}$$



Association rate

$$\frac{d[RL]}{dt} = k_{on}[R]\,[L]$$

$$St = \frac{[R].S\max}{[R] + K_D}\left(1 - e^{-(kon.[R]+koff).(t)}\right)$$

Dissociation rate

$$-\frac{d[RL]}{dt} = k_{off}\,[RL]$$

$$St = S0.e^{-koff(t-t0)}$$

Aim

To monitor the interactions between dsDNA and anti-dsDNA antibodies in autoimmune hepatitis and systemic lupus erythematosus, using surface plasmon resonance imagery (SPRi) focusing on Ag-Ab complex stability and dissociation constant rate k_{off} calculation.

Patients sera

SERA /IFI on Hep-2 cells, anti-dsDNA by Farr test, diagnosis confirmed			
AIH , ANA IIF +/dsDNA + n = 9	AIH, ANA IIF -/dsDNA + n = 5	SLE, ANA IFI +/dsDNA + n = 7	Negative Control n = 7

IgGs and IgMs purification

‘Protein G HP SpinTrap’ kit (GE Healthcare)

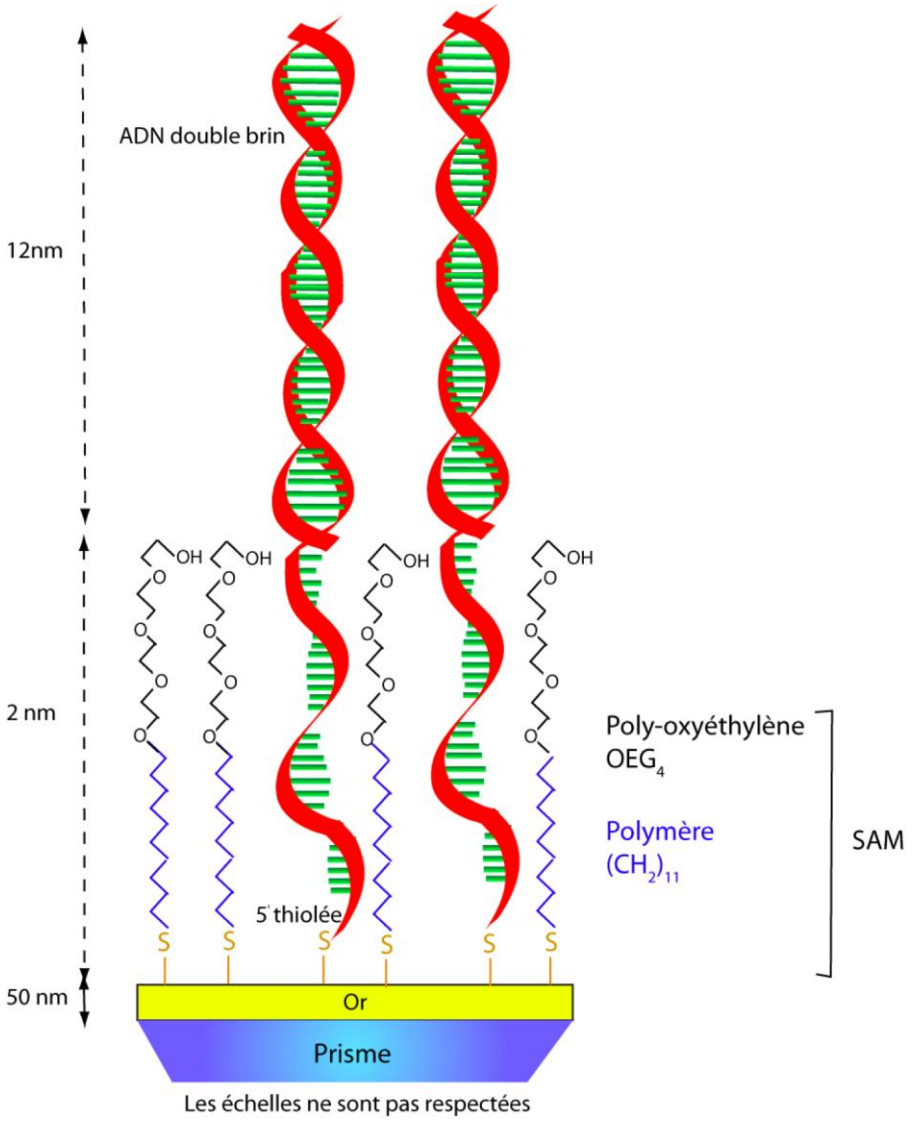
‘HiTrap IgM Purification HP’ kit (GE Healthcare)

dsDNAs - Antigens

DNA	Bp	Modification	Methylation
o500	500	5'-thiol C3 spacer	No
o500c	500	5'-thiol C3 spacer	CpG (5Me-C)
o500g	500	5'-thiol C3 spacer	CpG (5Me-C)
o250	250	5'-thiol C3 spacer	No
o2/10	27	5'-thiol	No
o2/10c	27	5'-thiol	CpG (5Me-C)
o2/10	27	5'-thiol	EcoRI (6Me-A)
hp	22	5'-thiol and Hairpin with a 3 bases loop	No
THF	22	5'-thiol and Tetrahydrofurane (abasic site) in position 10	No
DL10	40	5'-thiol and ethenoA in position 10	No

Characteristics of oligonucleotides used as antigen (bp, bases pairs).
DNAs were spotted at 1µM

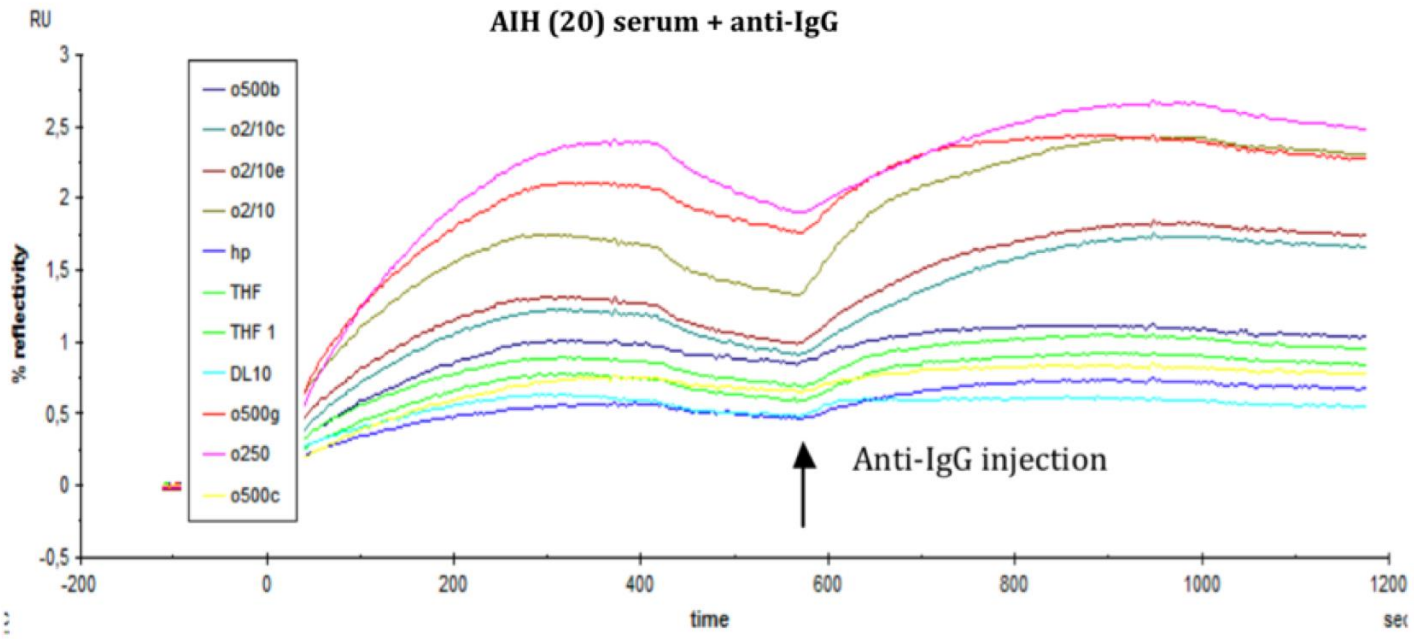
Chip surface design



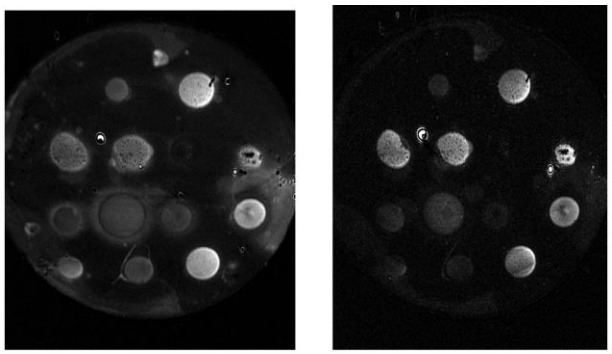
- Biochip is surmounted with a sparse self-assembled monolayer of ethylen glycol (SAM).

- The dsDNA with a thiol modification in 5’ end is adsorbed between the SAM.

Results: AIH Serum – anti-IgG

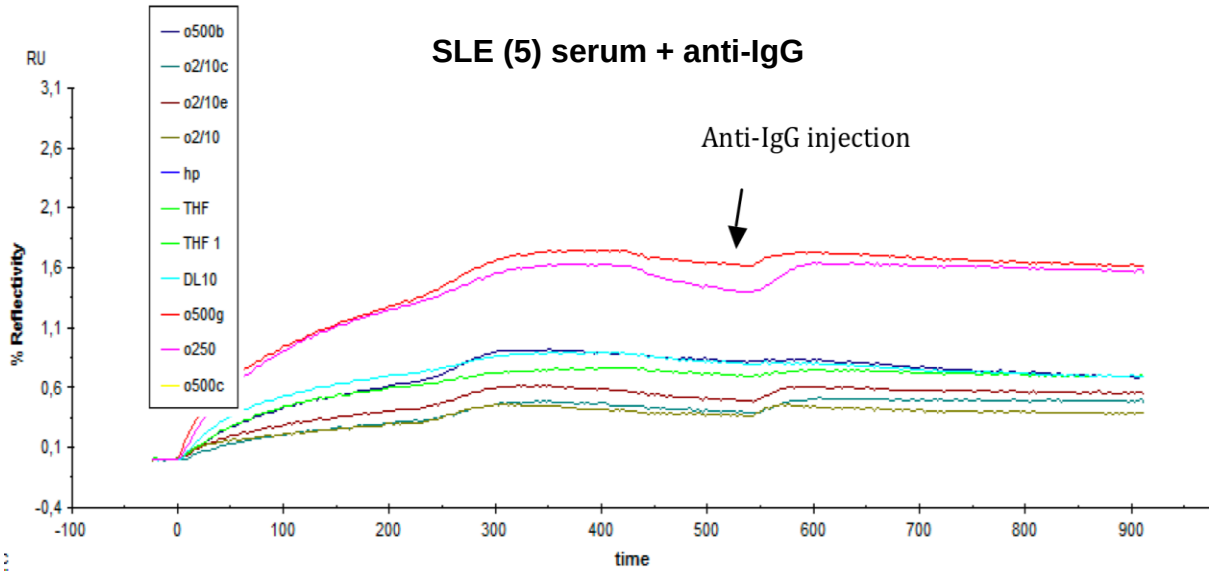


Sensograms AIH patient (800-fold dilution) and after anti-IgGs injection

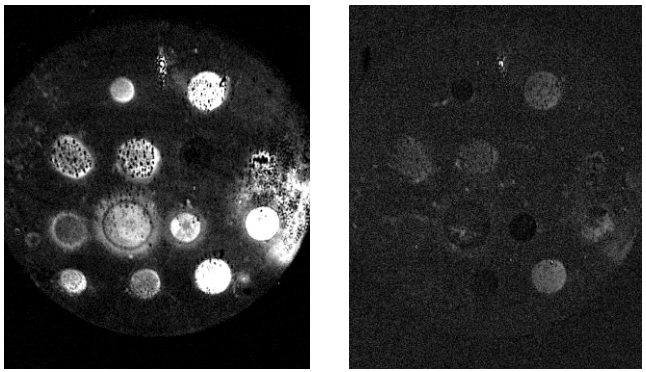


Differential images of chip surface at AIH sera injection (left) and anti-IgG injection (right)

Results: SLE Serum – anti-IgG

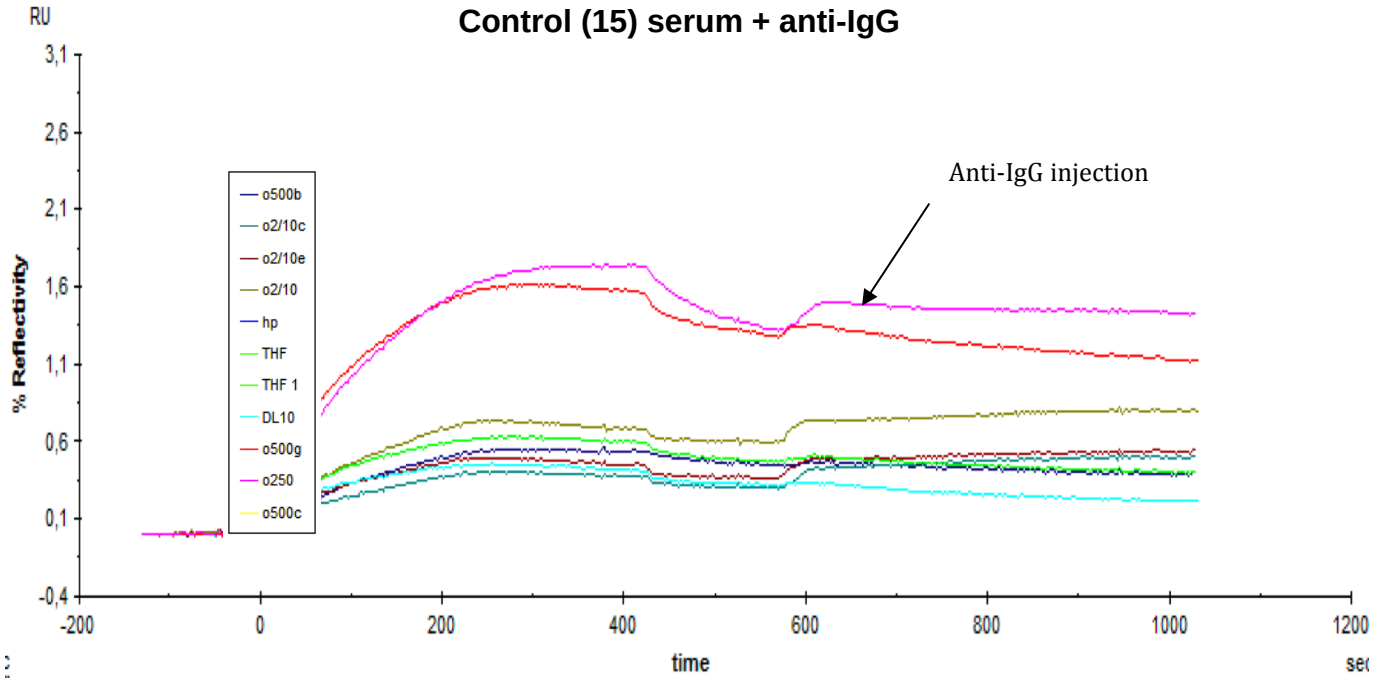


Sensograms SLE patient (800-fold dilution) and after anti-IgGs injection

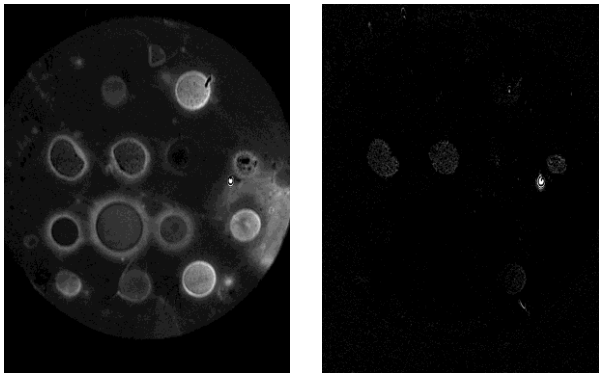


Differential images of chip surface at SLE sera injection (left) and anti-IgGs injection (right)

Results: control Serum– anti-IgG

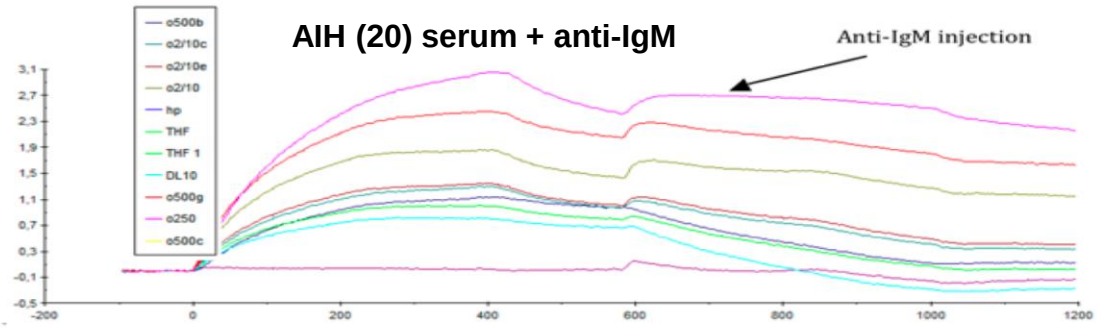


Sensograms control patient (800-fold dilution) and after anti-IgGs injection

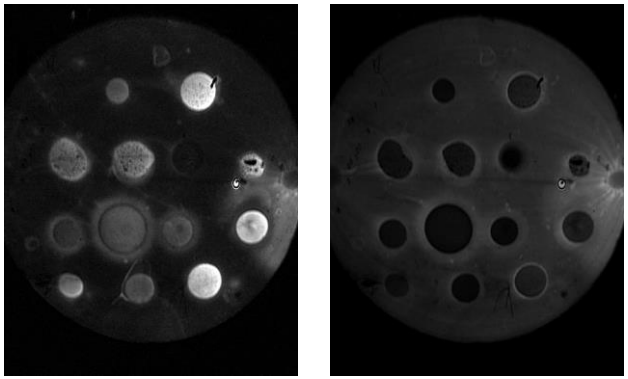


Differential images of chip surface at control serum injection (left) and anti-IgGs injection (right)

Results: AIH Serum– anti-IgM

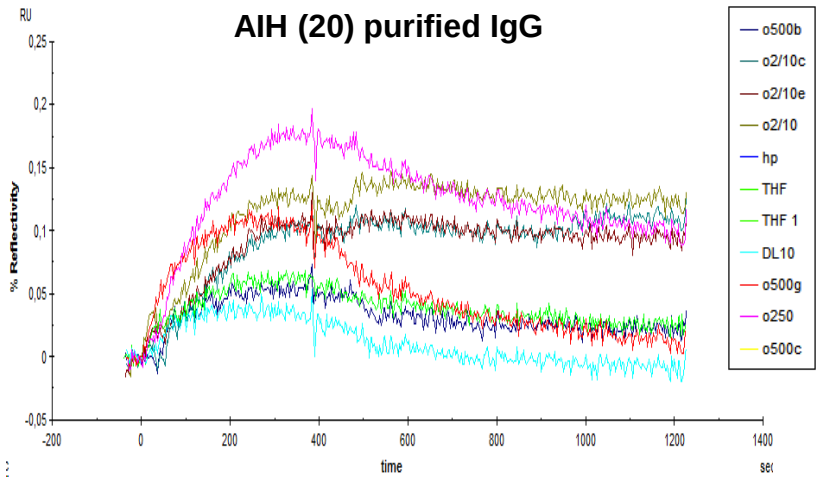


Sensograms AIH patient (800-fold dilution) and after anti-IgMs injection

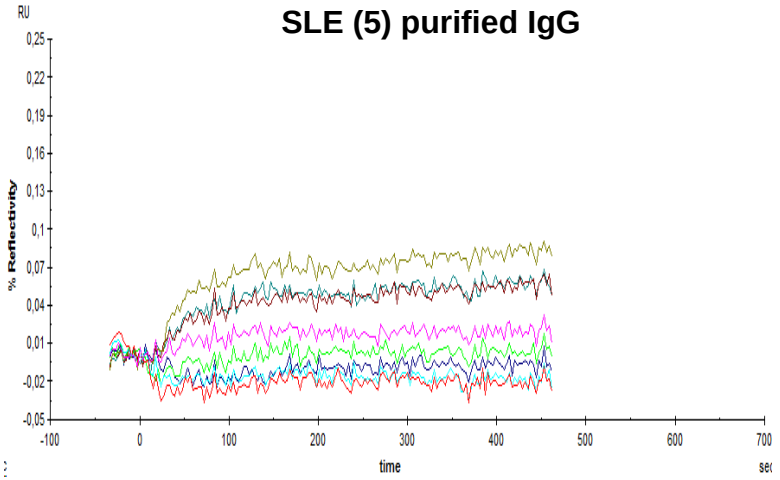


Differential images of chip surface at control serum injection (left) and anti-IgMs injection (right)

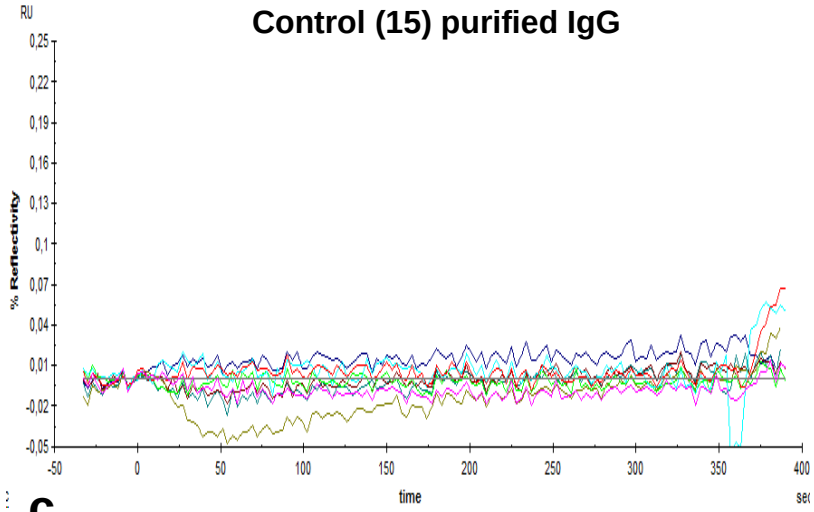
Purified IgGs



a



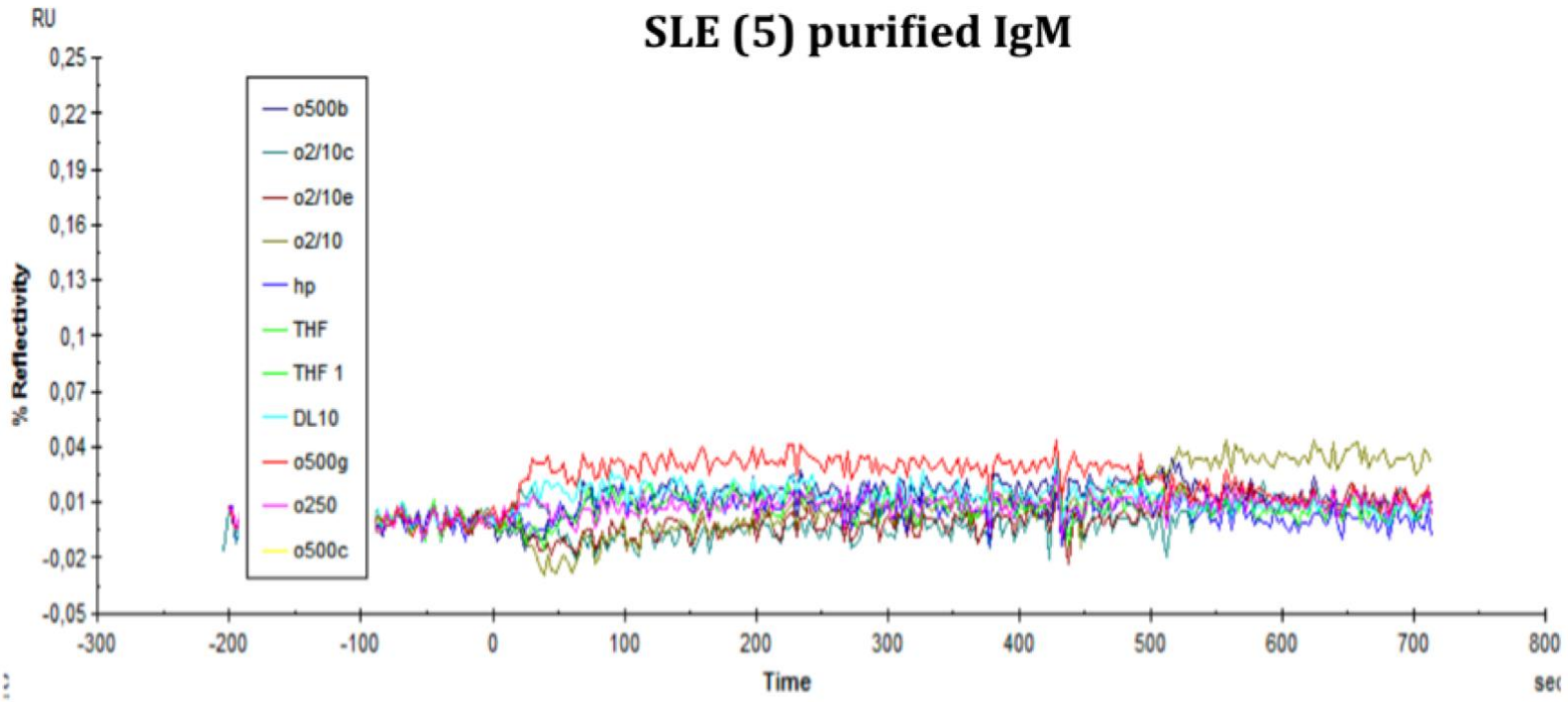
b



c

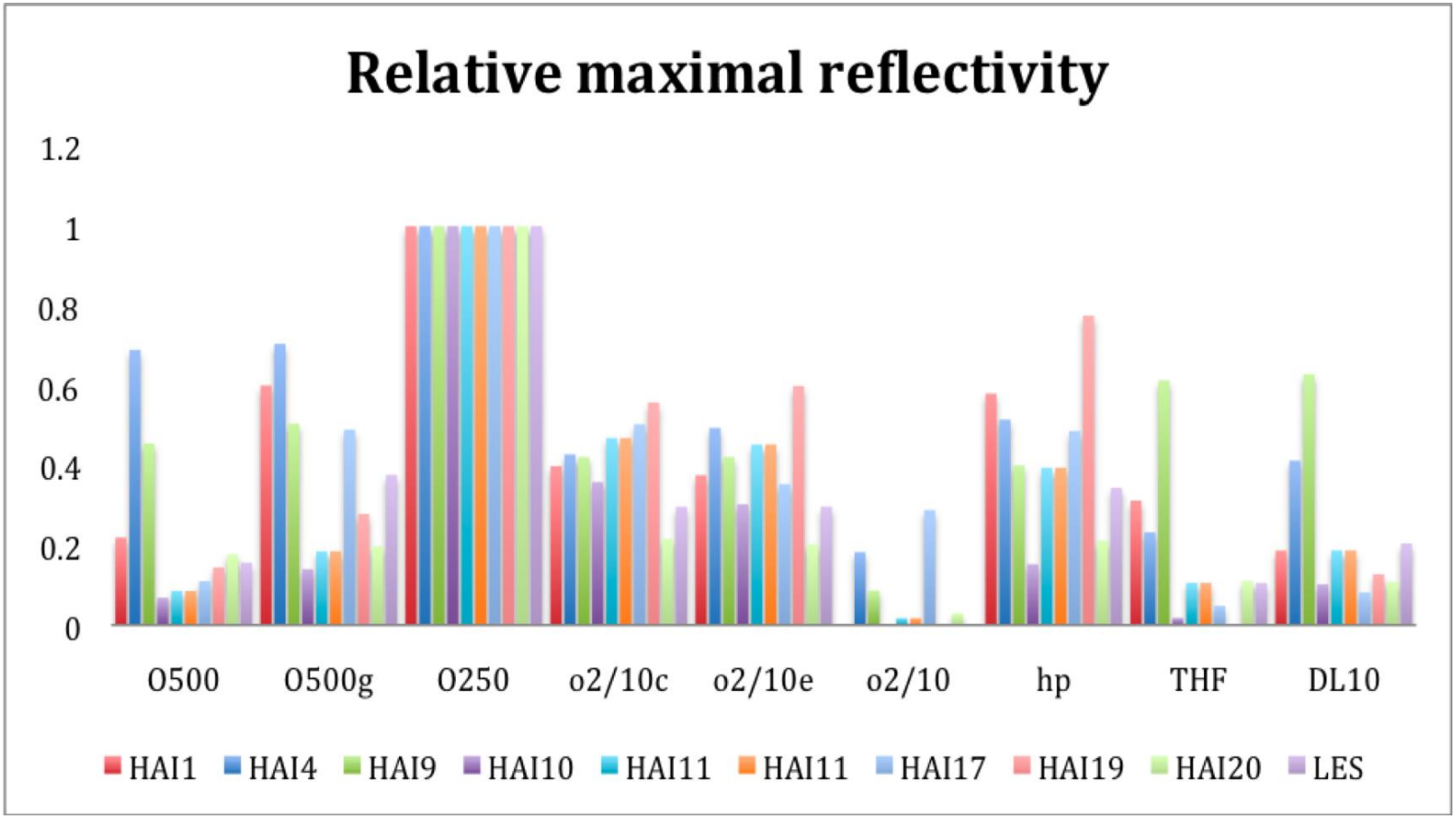
Representative SPRi sensograms obtained: **a.** AIH IgGs; **b.** SLE (5) IgGs; **c.** control IgGs Interacting with immobilized DNAs. SLE except number 5 have a similar reactivity than controls.

Reflectivity SLE purified IgMs



Representative surface plasmon resonance sensograms obtained after IgMs of SLE patient number 5, interacting with immobilized dsDNA

Reflectivity AIH purified IgGs according to oligonucleotides



The maximal reflectivity for each DNA is divided by the signal of o250 DNA

K_{off} AIH purified IgGs and dsDNAs

	HAI 1	HAI 4	HAI 9	HAI 10	HAI 11	HAI 17	HAI 18	HAI 19	HAI 20	SLE 5
250b	1.99 E-03	1.18 E-03	1.98 E-03	1.99 E-03	2.19 E-03	1.01 E-03	1.32 E-03	2.00 E-03	1.22 E-03	1.73 E-03
o500	ND	7.58 E-03	0.95 E-03	ND	2.51 E-03	4.92 E-03	4.92 E-03	ND	1.88 E-03	2.38 E-03
o2/10c	1.19 E-03	0.90 E-03	3.10 E-03	3.02 E-03	1.64 E-03	0.86 E-03	2.11 E-03	2.26 E-03	1.79 E-03	1.99 E-03
o2/10e	0.67 E-03	1.04 E-03	6.67 E-03	2.65 E-03	1.76 E-03	1.09 E-03	1.79 E-03	2.77 E-03	0.73 E-03	1.96 E-03
o2/10	ND	0.54 E-03	1.52 E-03	3.31 E-02	1.58 E-03	0.89 E-03	1.08 E-03	1.88 E-03	1.73 E-03	1.52 E-03

Mean k_{off} of interactions between purified IgGs from AIH patients and dsDNA on the chip surface. Mean k_{off} are expressed in sec-. ND: not determined, because of too low intensity

Conclusion

- SPRi strategy can detect variation among autoantibodies binding to dsDNA in different diseases

- Serum components from AIH, SLE and control sera bind dsDNAs, mostly IgGs for AIH patients

- AIH purified IgGs from sera bind dsDNAs compared to SLE and control IgGs

- SLE patients the immunocomplex formation might require a third partner that has to be identified, or a specific dsDNA structure

- IgMs tested do not bind tested dsDNAs

- Mean dissociation off rate for IgGs (K_{off}) were comparable among AIH patients

Future perspective

To study the third partner

1. **MALDI-TOF spectrometry** : after SPRi study the chip surface can be transferred into a spectrometer. Mass data analysis can help in the elucidation of the different compound between AIH and SLE.

2. **2D electrophoresis + mass spectrometry** : treatment of the chip surface with TCEP, 2D electrophoresis, treatment with trypsine and mass spectrometry.

to study a specificity conformation

1. **SPRi with inversion of analyte and ligand** : purified IgGs can spotted over the chip surface and dsDNAs injected in the flow chamber. dsDNAs with different conformation can be injected.