Development of a customized quantification of engineered proteins with the Octet

Hervé Dubois, Sanofi R&D

SANOFI

Global Biotherapeutics

Contents

Personal presentation

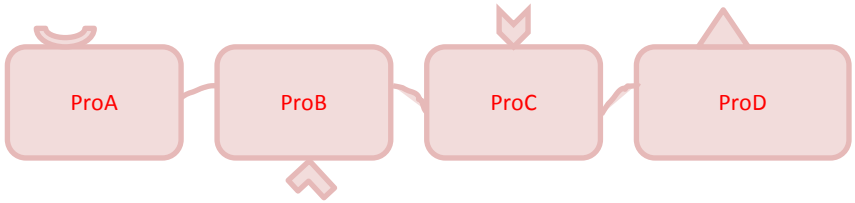
- Rhone-Poulenc Rorer R&D Vitry Site in 1992
  - **Technician in Solid Phase Peptide Synthesis and Combinatory Chemistry**
- Gencell/Centelion, Aventis Group
  - **Technician in Quality Control Department for release lot of Recombinant Adenovirus and Plasmidic DNA**
- Sanofi Industrial Affairs, Process Development Biochemistry
  - **Analyst of recombinant protein (IgG)**
- Sanofi R&D, Bio-Therapeutic
  - **Analyst of recombinant protein**

Introduction

- Bio-Process Analytics:
  - **Analytical support to Bioprocess Development: Cell Engineering, Up-Stream Processing at different production scales, Down Stream Processing**
  - **Analysis: Product titration at different stages of process, quality of product, contaminants titration, media composition during process…**

Context

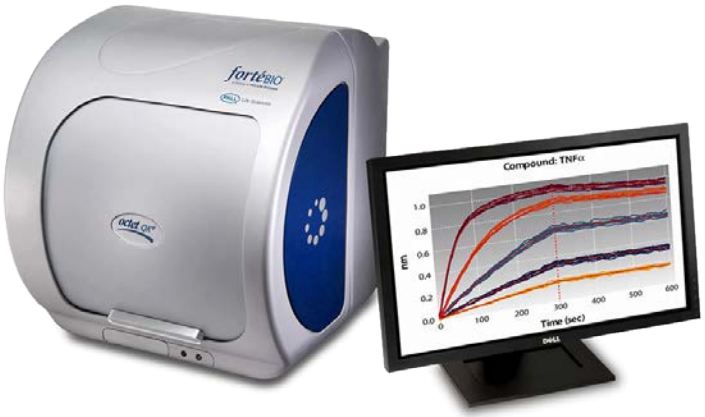
- Method to be developped for clone selection: High Throughput Method
- Molecule:



- Tetrameric protein where each monomere is specific for a target
- Monomeres are separated by a peptidic spacer
- No specificity :
  - **No tag (Like poly-Histidine or streptavidin…)**
  - **No fragment easy to detect (Fc fragment for example)**

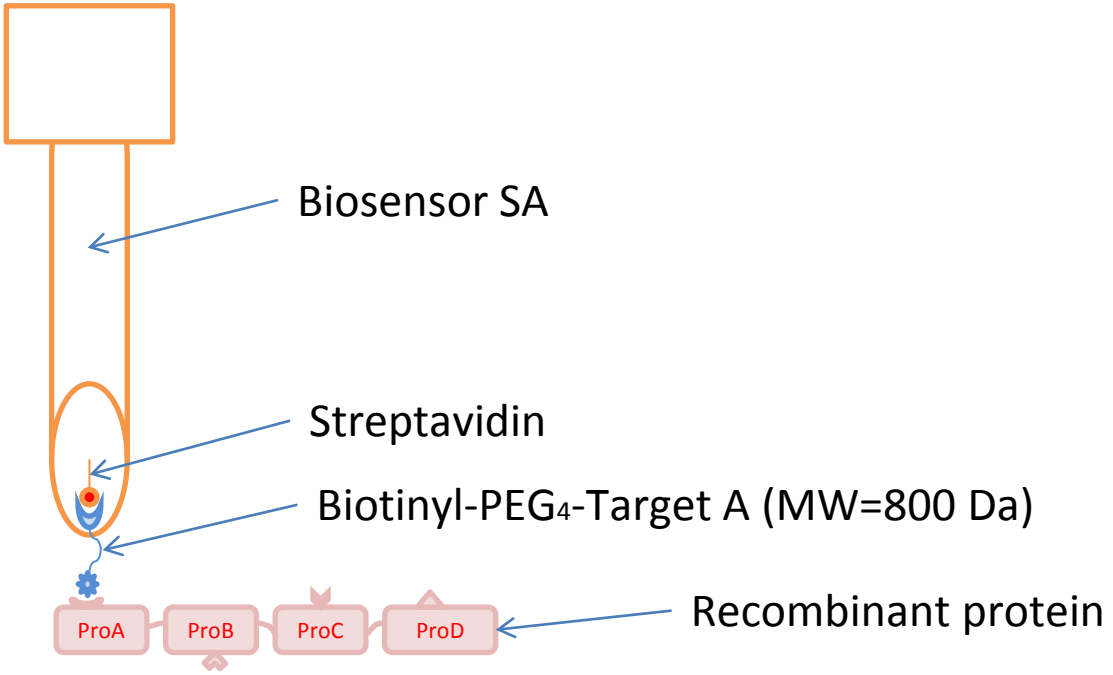
Requirement

- For first screening, protein assay needs to be:
  - **Rapid to develop** because of short delay
  - **Fast**: 1000 samples should be generated
  - **Fast**: Because of cell passage, results are needed in 2 days
  - **Easy**: Without sample preparation if possible
  - **Sensitive**: 1st screening is tested at Day 4 of culture; range between 0 to 100 µg/ml.
  - **Specific**: samples are assayed in supernatant. Some cell proteins are present
  - **Low cost**: Because of the large number of samples
  - **Simple** data reprocessing



Preliminary Tests

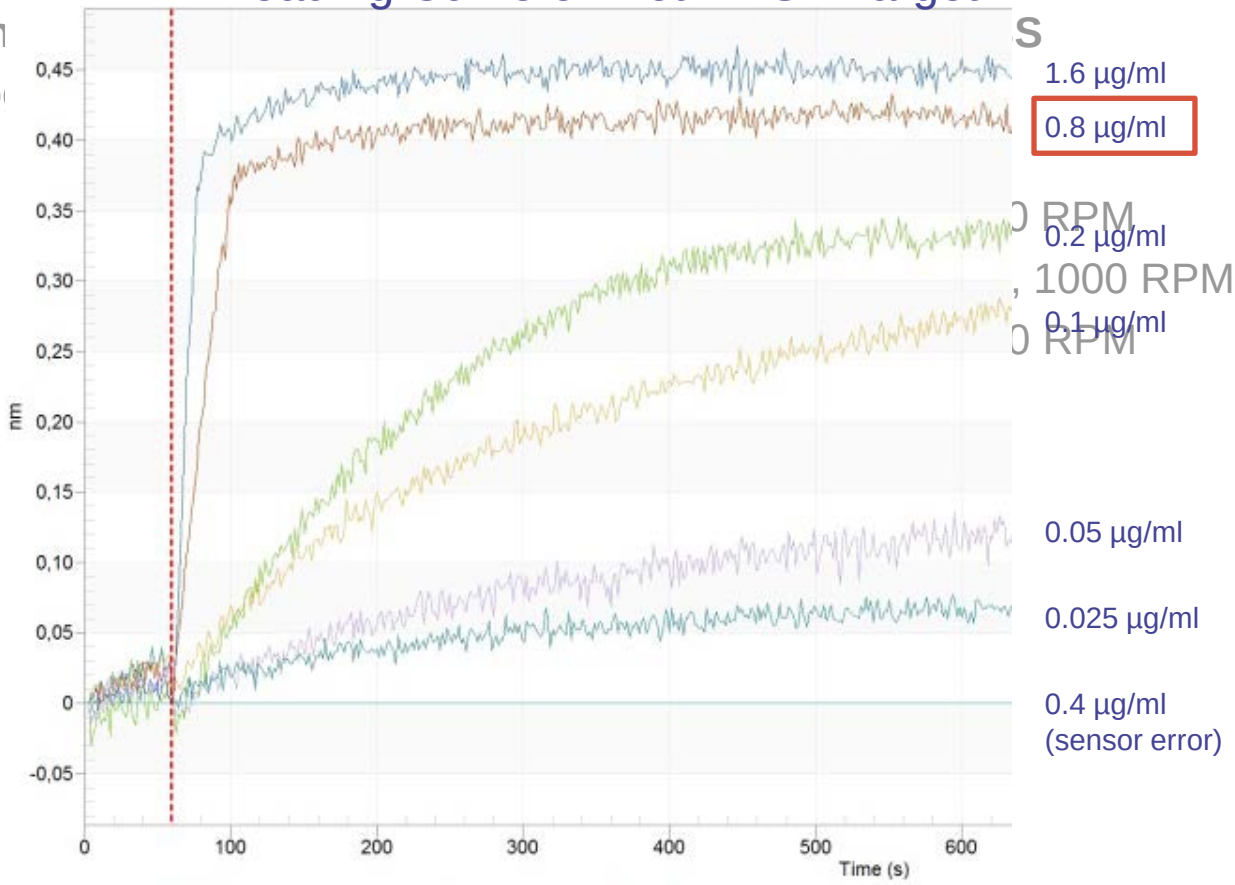
Preliminary tests and titrations were realized with BLItz system by Frankfurt Analytical Team of Bio-Innovation, Sanofi R&D



Assay developped by colleagues is functional but Biosensors aren't reusable

Ligand Loading

- Principle:
  - Protein concentration was constant: 100 µg/ml in Cell Culture media (CCM)
  - Ran S
  - Pro
    - 0 RPM
    - , 1000 RPM
    - 0 RPM



Regeneration Buffer Selection

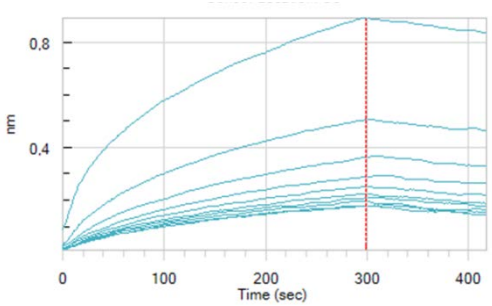
- Principle
  - **Test of a large number of buffers:**
    - Glycine 10 mM pH1.7,
    - Glycine 10 mM pH1.7, NaCl 5M
    - Glycine 10 mM pH1.7, Tween 20 0.5%
    - Glycine 10 mM pH1.7, Triton X100 0.5%
    - NaOH 0.01M
    - NaOH 0.05M
    - NaOH 0.1M
    - NaCl 5M
    - HCl 0.1M
    - H3PO4 0.5M
    - DPBS
    - DPBS, Triton X100 0.1%
    - DPBS, Triton X100 0.5%
    - DPBS, EDTA50mM
    - DPBS NaCl 5M
    - Acetic Acid 0.1%
    - Urea 8 M, pH=7
    - Urea 4M, pH=7
    - Urea 8M in Glycine buffer (pH=3)
    - Urea 6M in Glycine buffer (pH=2)
    - Urea 8M in NaOH 0.05M (pH=14)
    - Urea 6M in NaOH 0.05M (pH=14)
    - Urea 8M in Acetic Acid 0.1% (pH=5)
    - Urea 6M in Acetic Acid 0.1% (pH=4.5)
    - Guanidin 8 M, pH=7
    - Guanidin 6M, pH=7
    - Guanidin 8M in NaOH 0.05M (pH=14)
    - Guanidin 6M in NaOH 0.05M (pH=14)
    - Guanidin 8M in NaOH 0.1M (pH=14)
    - Guanidin 6M in NaOH 0.1M (pH=14)
    - Guanidin 8M in Acetic Acid 0.1% (pH=4)
    - Guanidin 6M in Acetic Acid 0.1% (pH=4)

Regeneration Buffer Selection

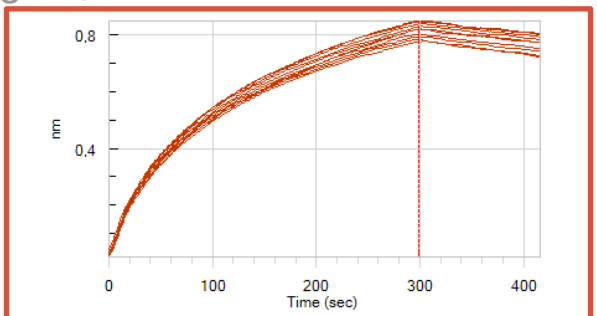
- Principle:
  - **Constant protein concentration : 100 µg/ml in CCM**
  - **Constant Biot-Target A: 0.8 µg/ml in PBS**
  - **Program in Basic Kinetic Mode:**
    - Baseline in PBS, 60 seconds, 1000 RPM
    - Loading in Biot-Target A in PBS, 120 seconds, 1000 RPM
    - Custom Baseline: Cell Culture media, 60 seconds, 1000 RPM
    - Regeneration: tested buffer, 5 seconds, 400 RPM
    - Neutratisation: Cell Culture Media, 5 seconds, 400 RPM
    - Association: Tetrameric protein solution 100µg/ml, 300 seconds 1000 RPM

X3

X10



Glycine buffer 10mM, pH=1.7



Guanidin 8M in Acetic Acid 0.1% (pH=4)

- Best result is obtained with Guanidin 8M with 0.1% of Acetic Acid

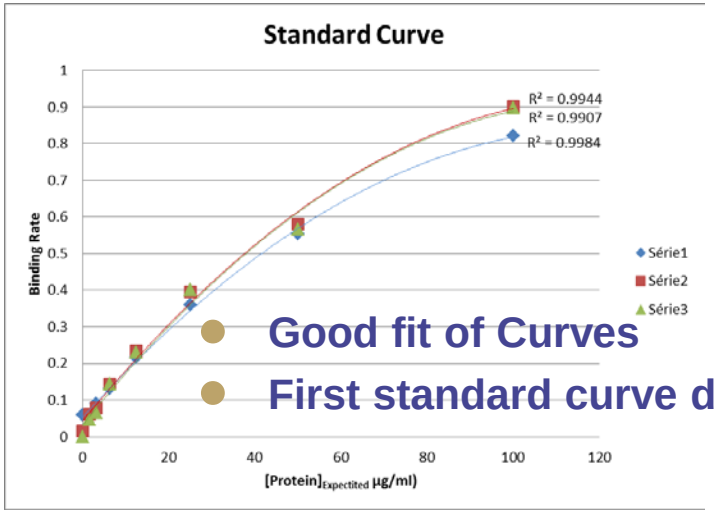
Assay Optimization

- Experiment:
  - Read 3 times this plate
  - Same program

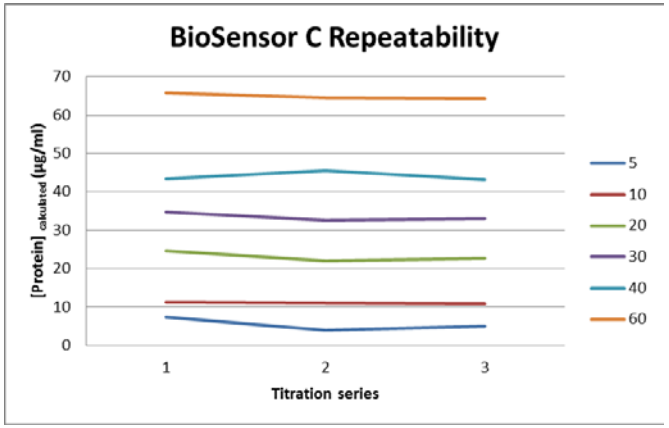
	Standard	Known concentration samples (µg/ml)									Regeneration	
	1	2	3	4	5	6	7	8	9	10	11	12
A	100.0	5	10	20	30	40	60				Regeneration: Guanidin 8M 0.1% Acetic Acid	Neutralisation: Cell Culture Media
B	50.0	5	10	20	30	40	60					
C	25.0	5	10	20	30	40	60					
H	12.5	5	10	20	30	40	60					
E	6.3	5	10	20	30	40	60					
F	3.1	5	10	20	30	40	60					
G	1.5	5	10	20	30	40	60					
H	0.0	5	10	20	30	40	60					

- Calcul model: R-Equilibrium
  - Initial slope model is not suitable, association too slow
- Integration Time: 150 seconds is a good compromise
  - 100 Sec: Too much variation (VC> 10%) for concentrations <20µg/ml
  - 300 Sec: Increase analysis time
- Range between 1.5 to 100 µg/ml:

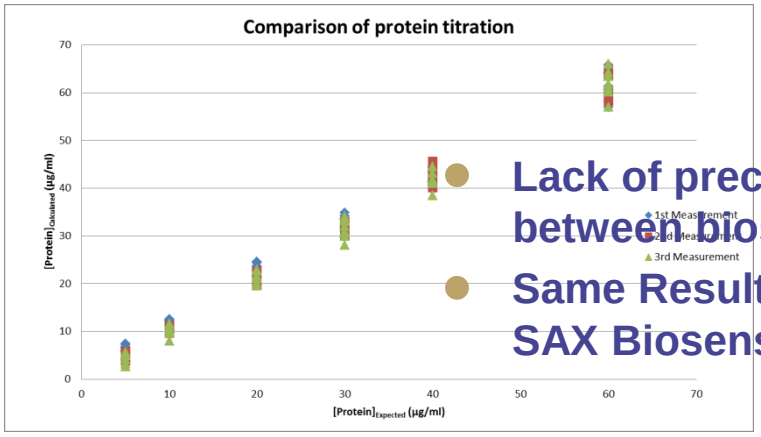
Result observations



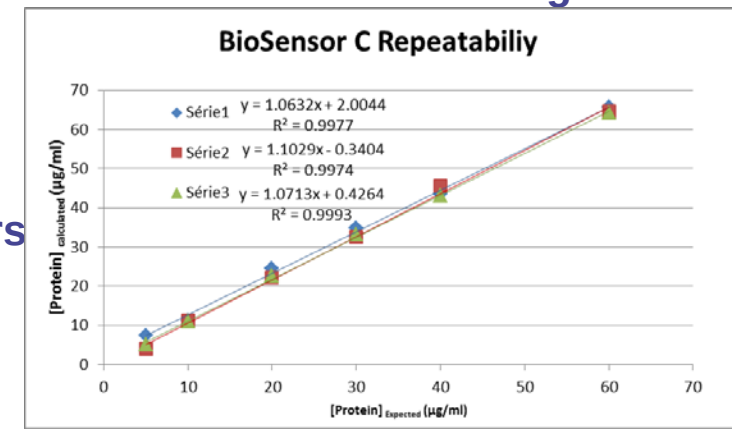
- Good fit of Curves
- First standard curve different



- Good repeatability of Biosensors
- No-decrease between regeneration

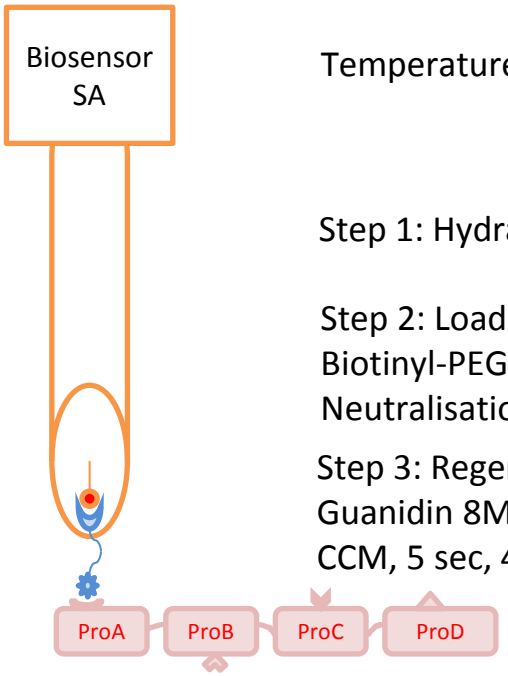


- Lack of precision between biosensors
- Same Results with SAX Biosensors



Conclusions

Final assay conditions



Temperature: 30°C

Step 1: Hydratation, 5 min in DPBS

Step 2: Loading Target
Biotinyl-PEG4-Target A: 0.8µg/ml in DPBS, 180 sec, 1000RPM
Neutralisation: CCM, 120 sec, 1000 RPM

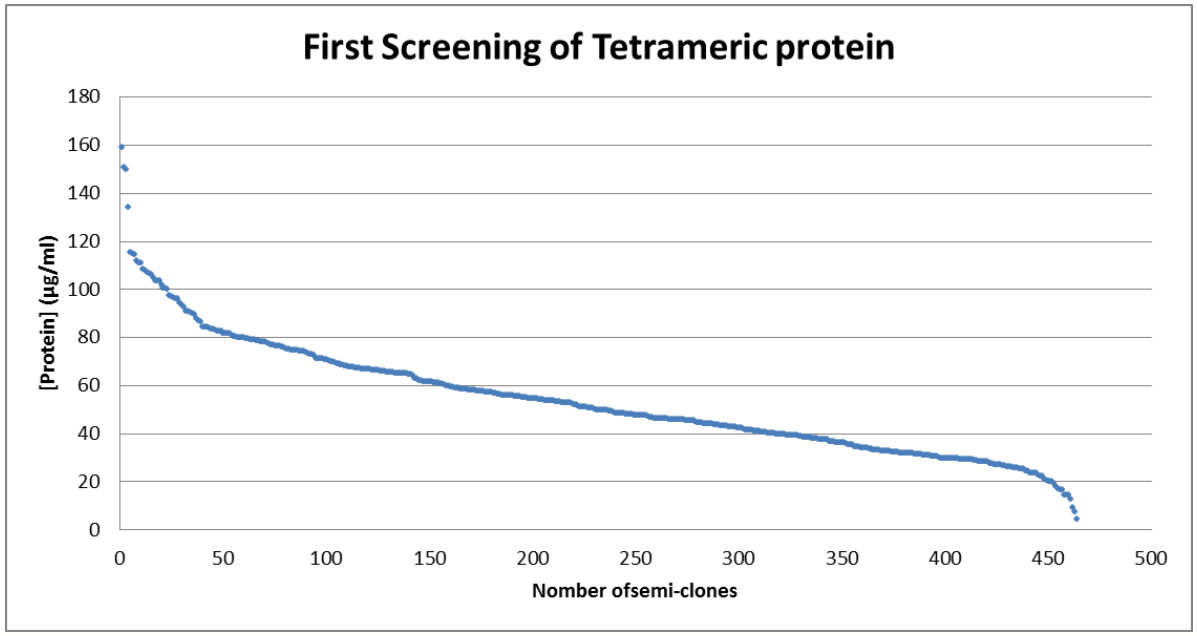
Step 3: Regeneration/neutralization
Guanidin 8M + Acetic Acid 0.1%, 5 sec, 400 RPM
CCM, 5 sec, 400 RPM
} 3 times

Step 4: Association
Titration: 0-100µg/ml of protein in CCM, Integration time 150 sec, 1000 RPM
} 10 times

- Limitations of assay:
  - Lake of precision
  - Repeatability
- Advantages of assay
  - Easy to use
  - Fast

Conclusions

- Optimization and validation of method have been beneficial
- Method was efficient to carry out the first screening of the project:
  - **Productivity of 450 clones measured at D4 of culture in a day**
  - **45 best producers selected for the next step**
  - **Final selection of cell line is in progess**



ACKNOWLEDGEMENT

- Cell Line Engineering (Vitry):
  - Corinne Gillard
  - Séverine Durant
  - Jennifer Di Carlo
  - Laetitia Maçon
- Up Stream Processing (Vitry)
  - Valérie Besset
- Chemist (LGCR, Vitry)
  - Nathalie Karst
- Frankfurt R&D Team
  - Christian Lange
  - Carsten Corvey
  - Ingo Focken
- Pall-FortéBio
  - Arnaud Vonarburg