



ランチヨンセミナー

1. 動的光散乱を用いた蛋白質解析

Avid Nano社 Ken Cunningham

2. MSによる蛋白質-蛋白質複合体解析

エーエムアール株式会社 板東泰彦

3. 質量分析法を用いた蛋白質の定量、及び翻訳後修飾プロファイリング同時解析

株式会社 バイオシス・テクノロジーズ 川上裕貴

第12回 日本蛋白質科学会年会

The 12th Annual Meeting of the Protein Science Society of Japan



Avid Nano
advancing dynamic light scattering

Presented by Ken Cunningham – Founder CEO

21st June 2012

Contents

- ◆ About Avid Nano
- ◆ Dynamic Light Scattering
- ◆ Products
- ◆ Accessories



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エーエムアール株式会社

About Avid Nano

- Established in July 2009

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- Based in UK. 50km West London.

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- Based in UK. 50km West London.
- Design & manufacture innovative DLS

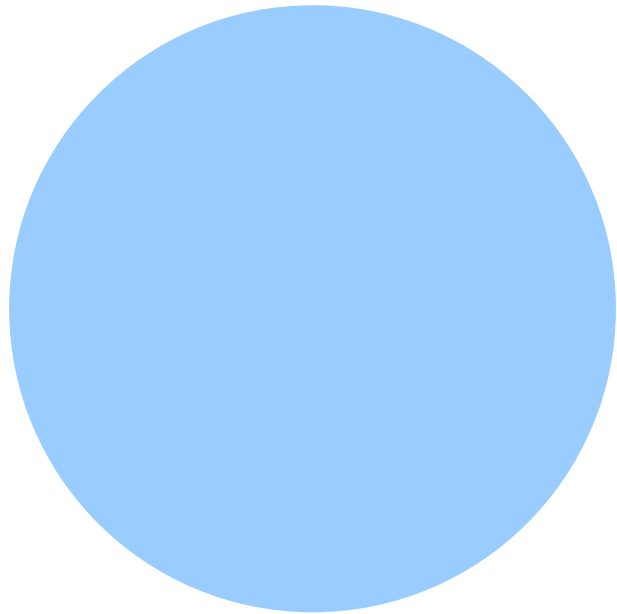


Avid Nano
advancing dynamic light scattering

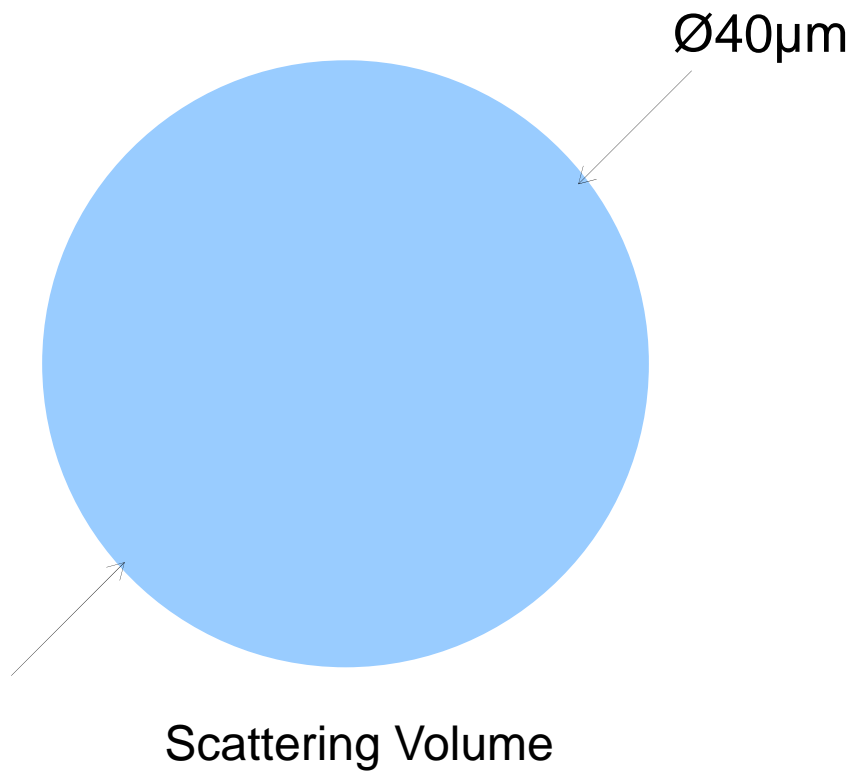
DYNAMIC LIGHT SCATTERING

Dynamic Light Scattering

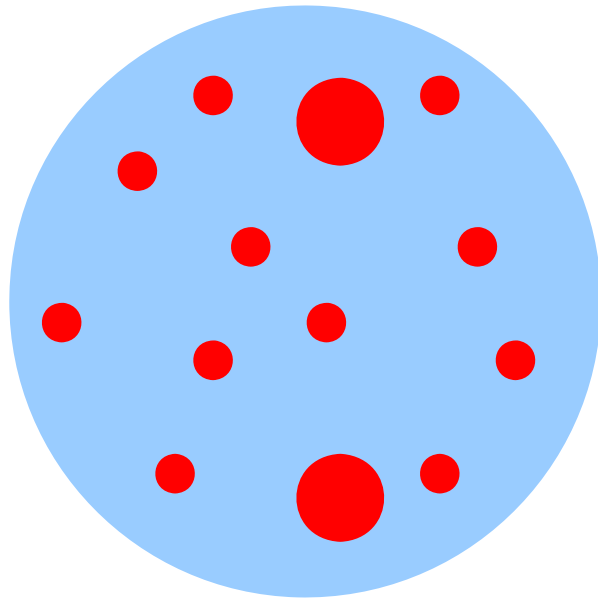
- Observe time dependent intensity fluctuations of light to directly measure...
 - Hydrodynamic radius of molecules / particles in solution / suspension
 - Intensity size distribution
 - Mass size distribution
 - Aggregation
 - Molecular weight can be estimated



Let's describe
this visually

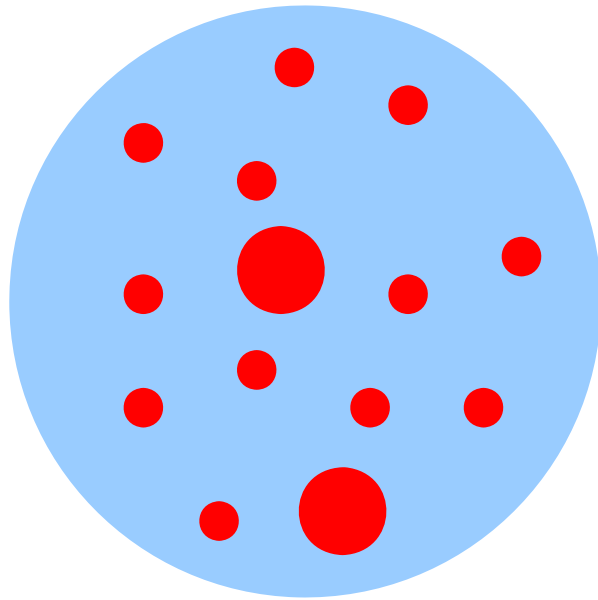


Let's describe
this visually

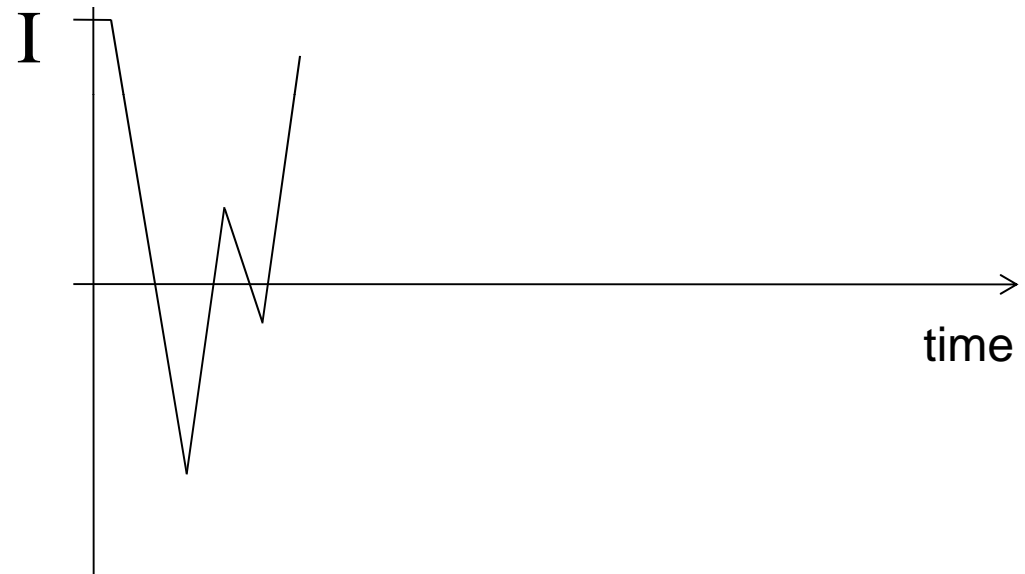


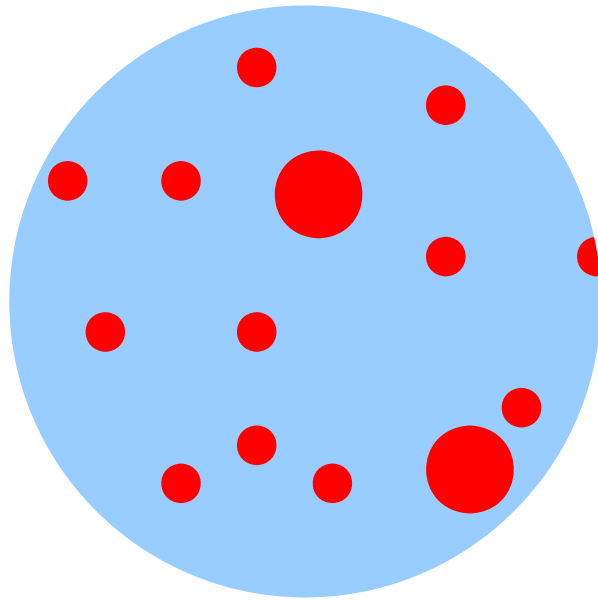
Scattering Volume



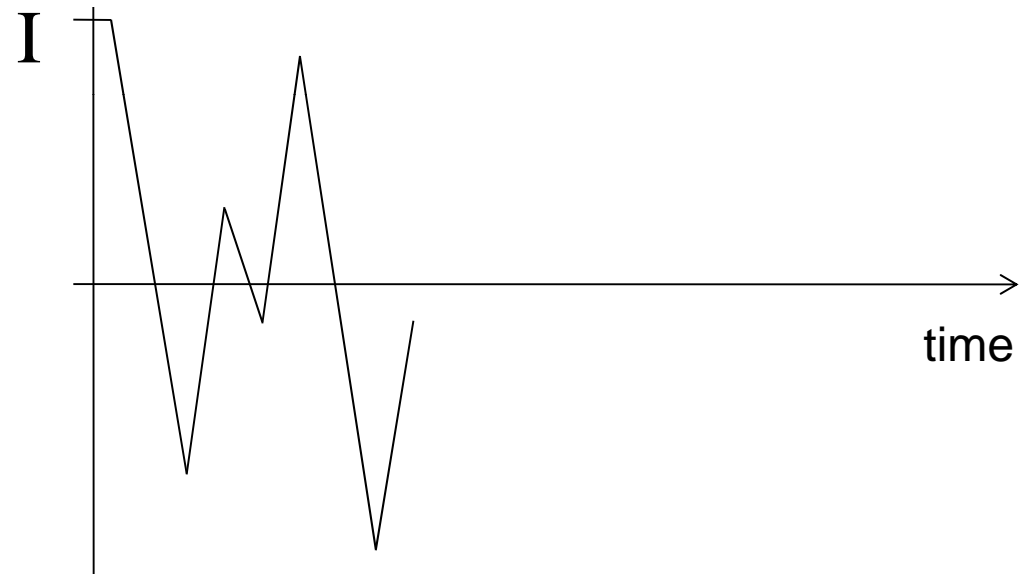


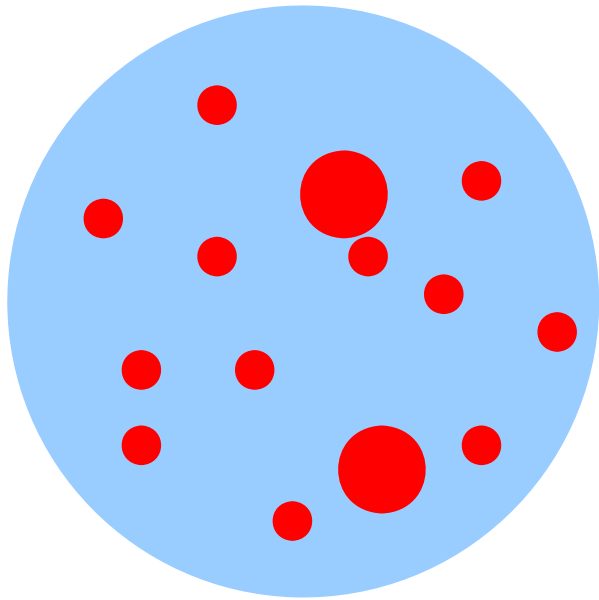
Scattering Volume



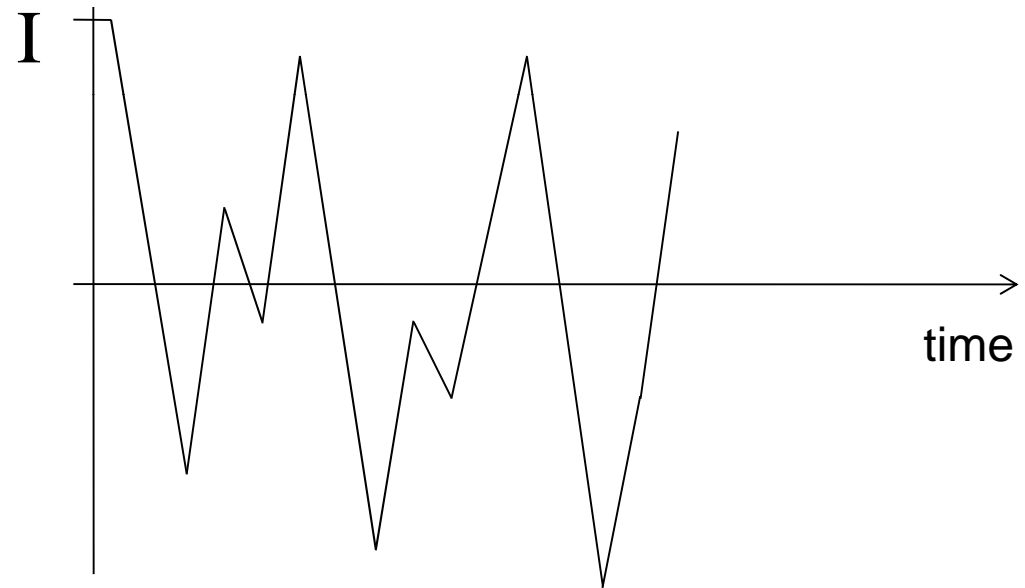


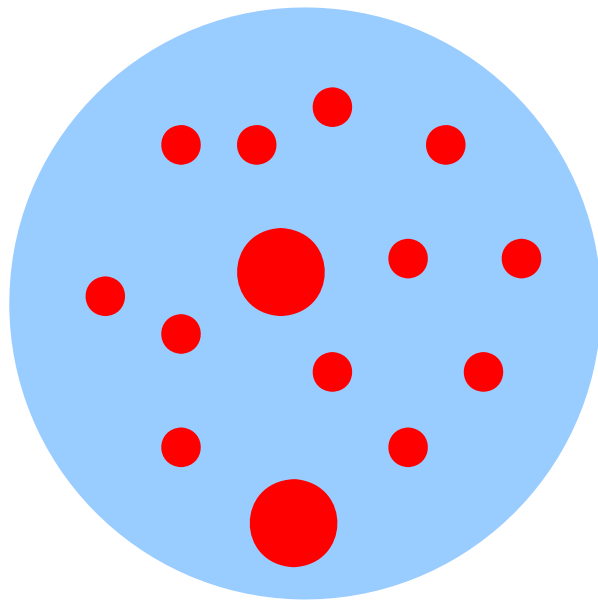
Scattering Volume



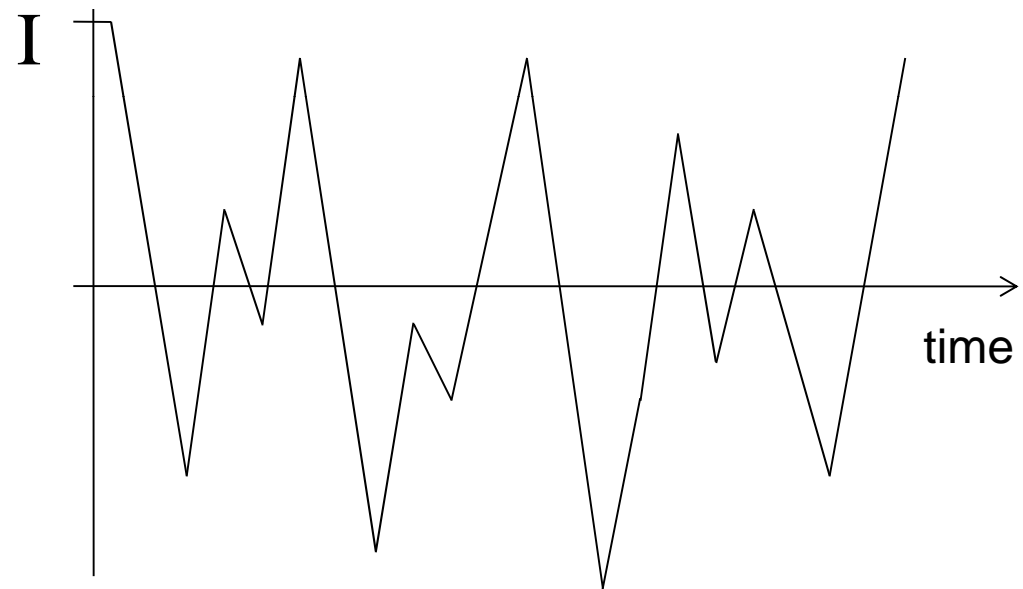


Scattering Volume

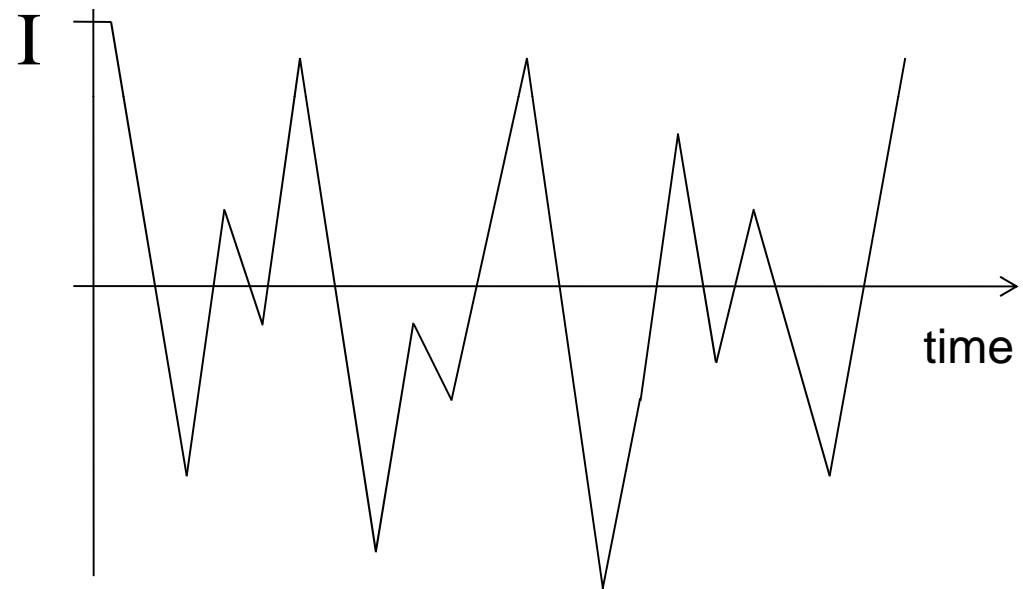




Scattering Volume

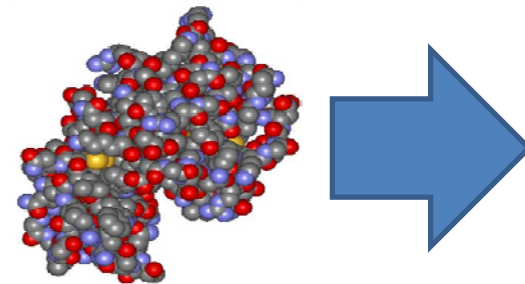
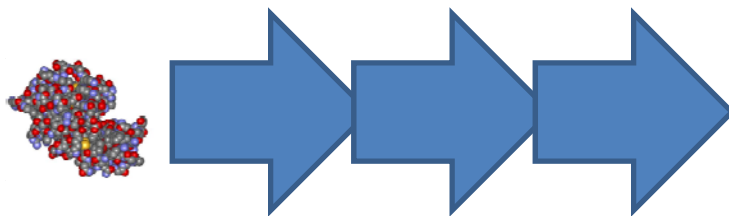
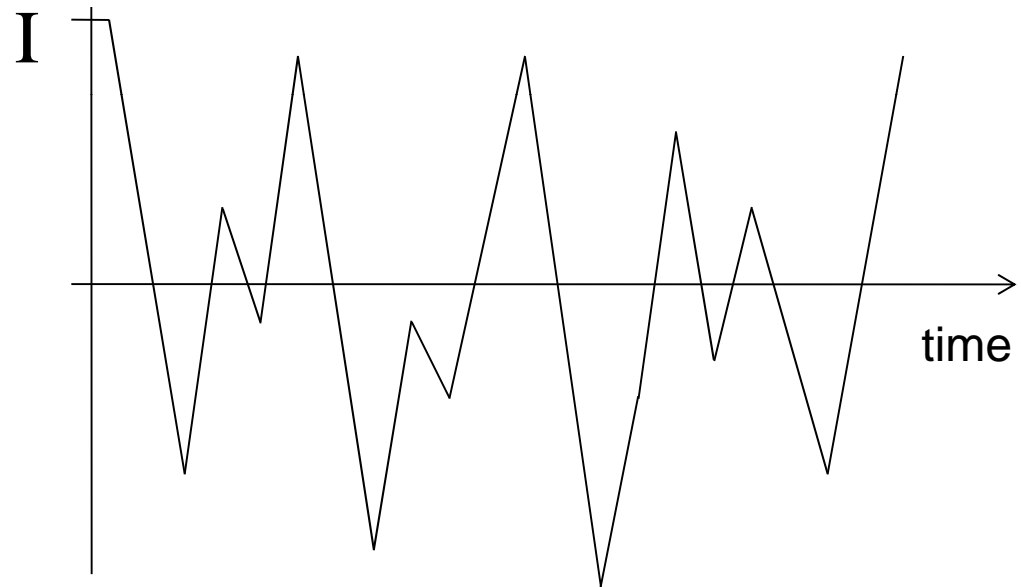


•Looks like random noise,
but...



•Looks like random noise,
but...

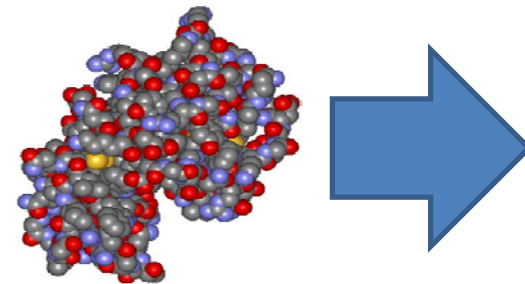
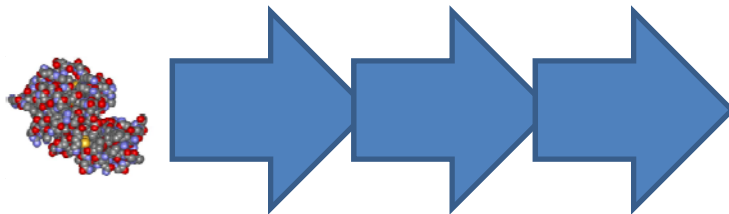
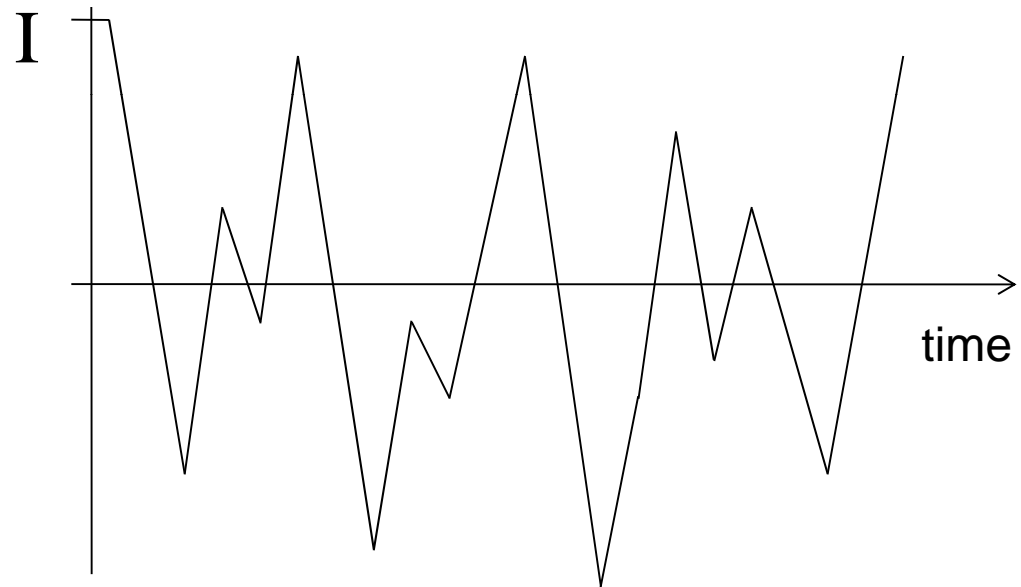
•...small sizes diffuse
more quickly than large
sizes, so...



- Looks like random noise, but...

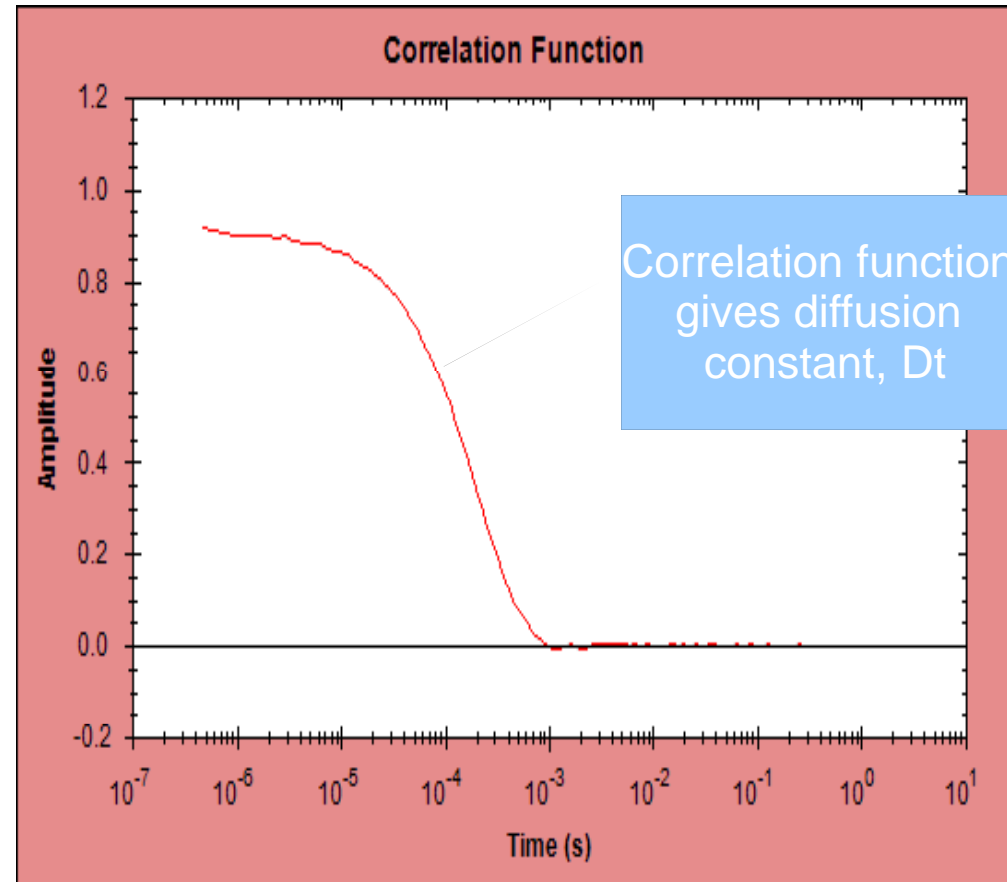
- ...small sizes diffuse more quickly than large sizes, so...

- ...rate of change tells us the mean particle size

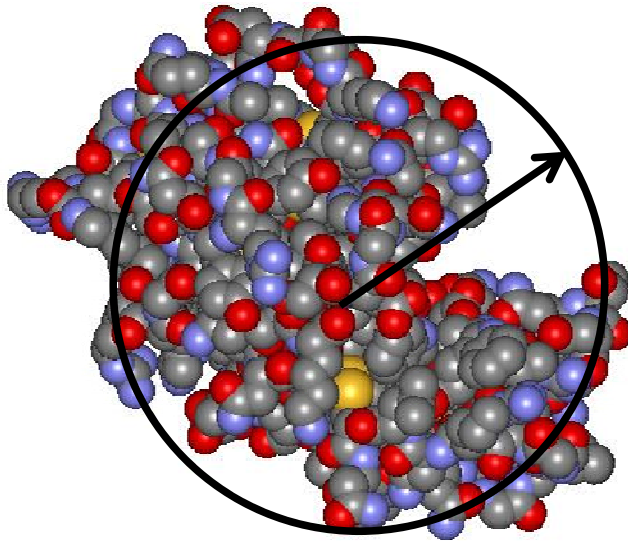


Dynamic Light Scattering

- Intensity pattern produces a correlation function and diffusion constant, Dt
- Calculate mean hydrodynamic radius, (R_h) and polydispersity index (Pdl)
- Typical measurement time, 10s



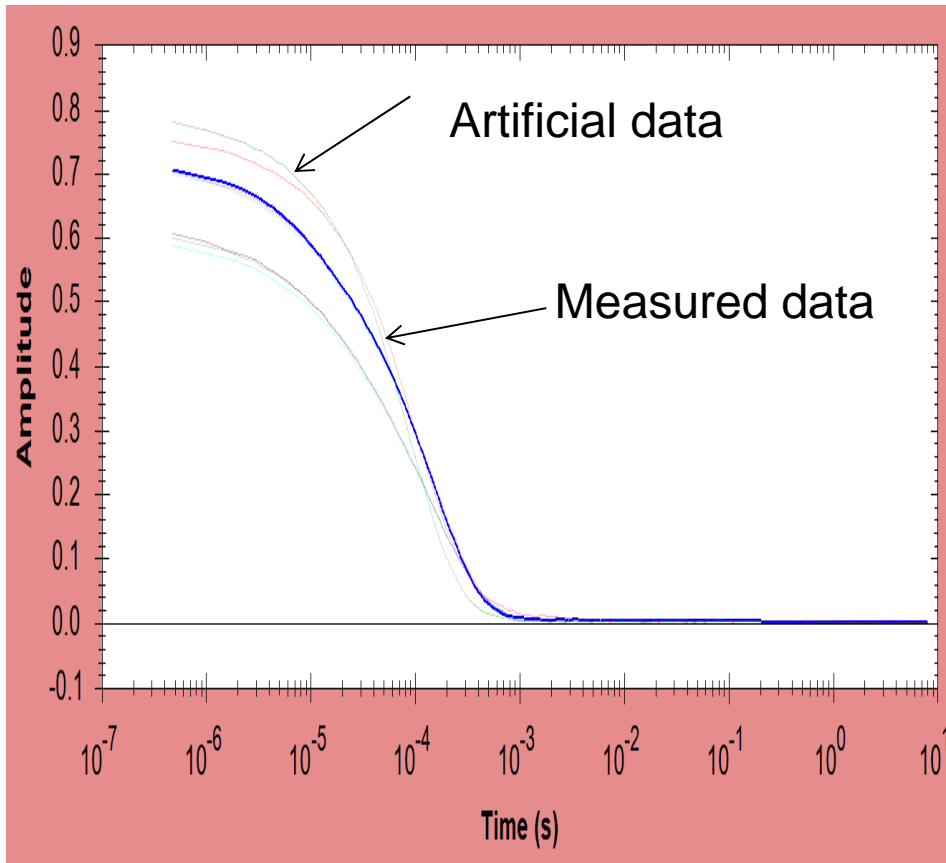
Dynamic Light Scattering



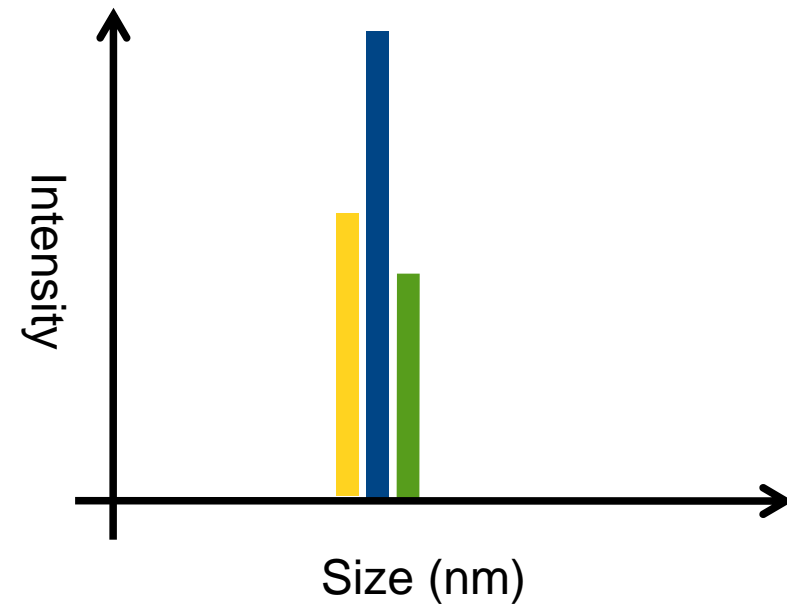
We can use the 'Stokes-Einstein' equation to easily calculate the average hydrodynamic radius, R_h

$$R_h = \frac{KT}{6\pi\eta Dt}$$

Size Distribution



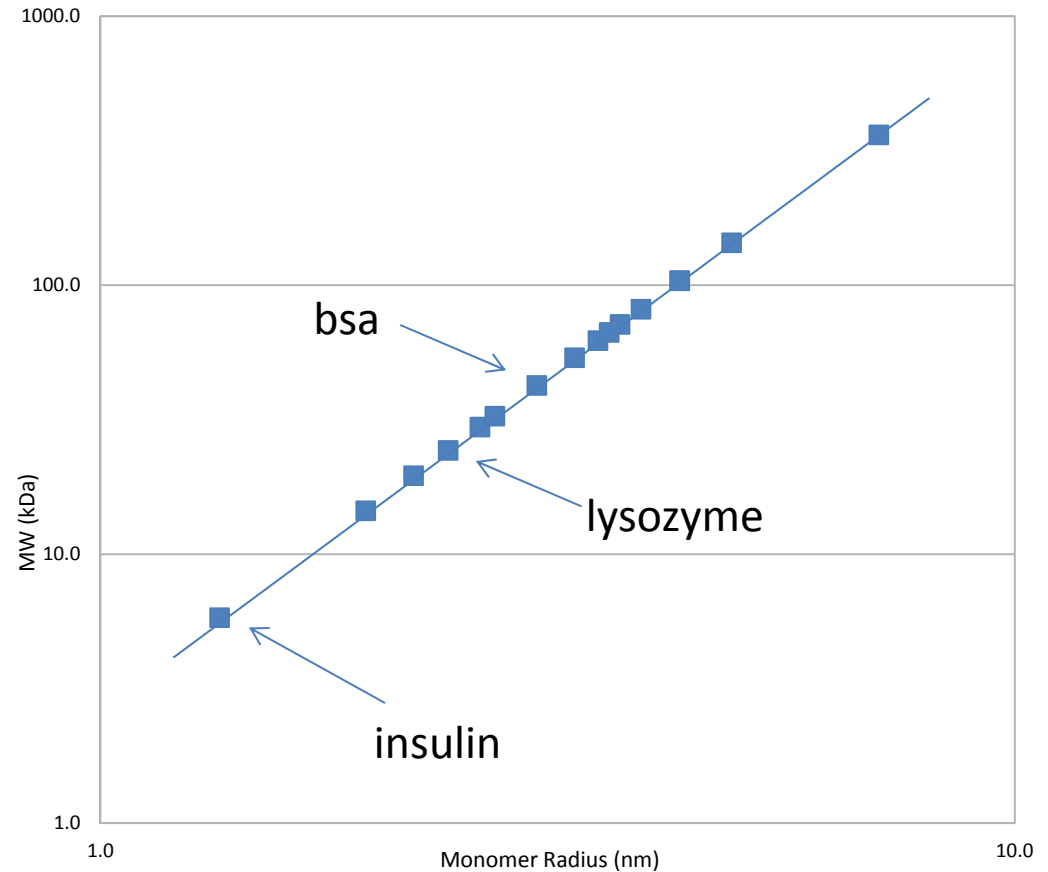
- By comparing the measured data to a series of artificial correlation data we can produce a size distribution



Molecular Weight Model

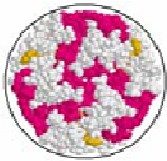
- We use a model curve of common proteins to estimate molecular weight

$$Mw \sim 2.75 Rh^{2.49}$$



Molecular Weight Model

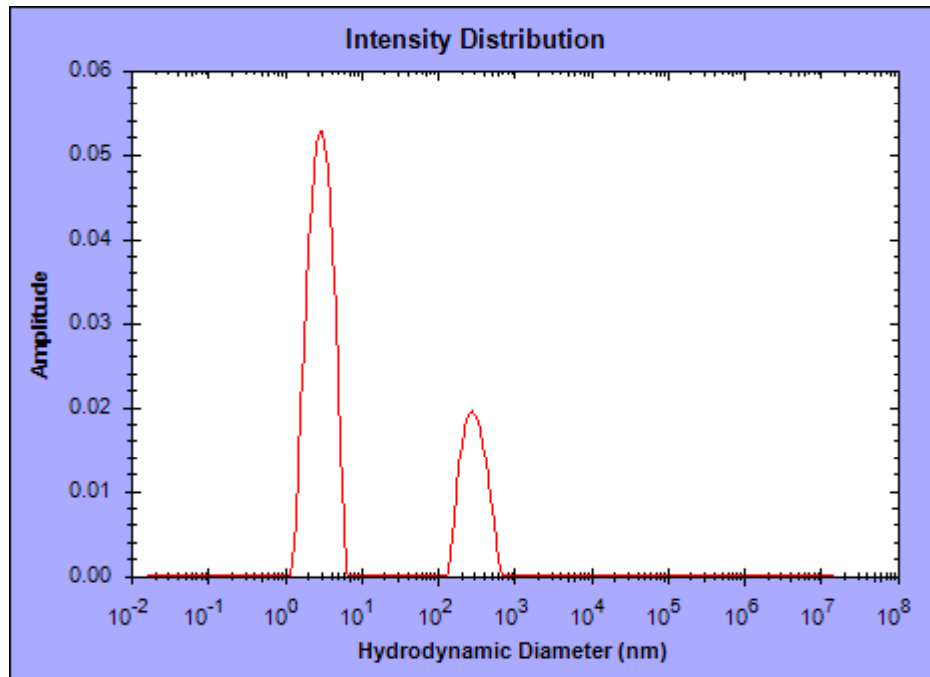
- The model works well for many proteins



Insulin - pH 7
 $M_W=34.2$ kDa
 $R_H=2.7$ nm

Molecule	Size (nm)	Est. MW (kDa)	MW (kDa)
Insulin (pH 2)	1.4	5.8	5.8
Lysozyme	2.0	14.5	14.7
Insulin (pH 7)	2.7	32.6	34.2
BSA	3.6	67.0	66.8
Hexokinase	4.3	104	102

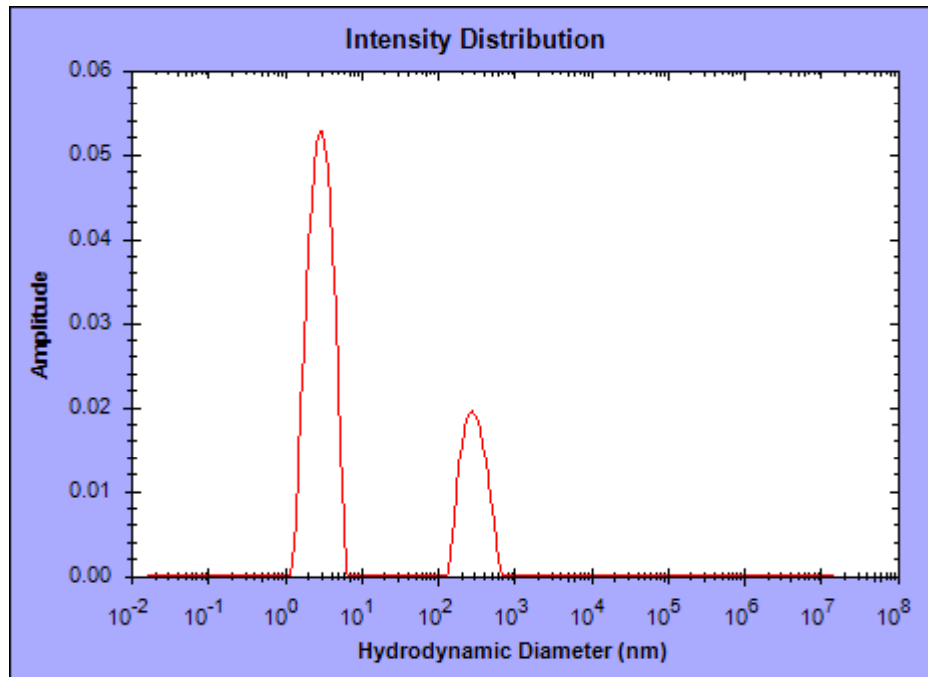
Distributions



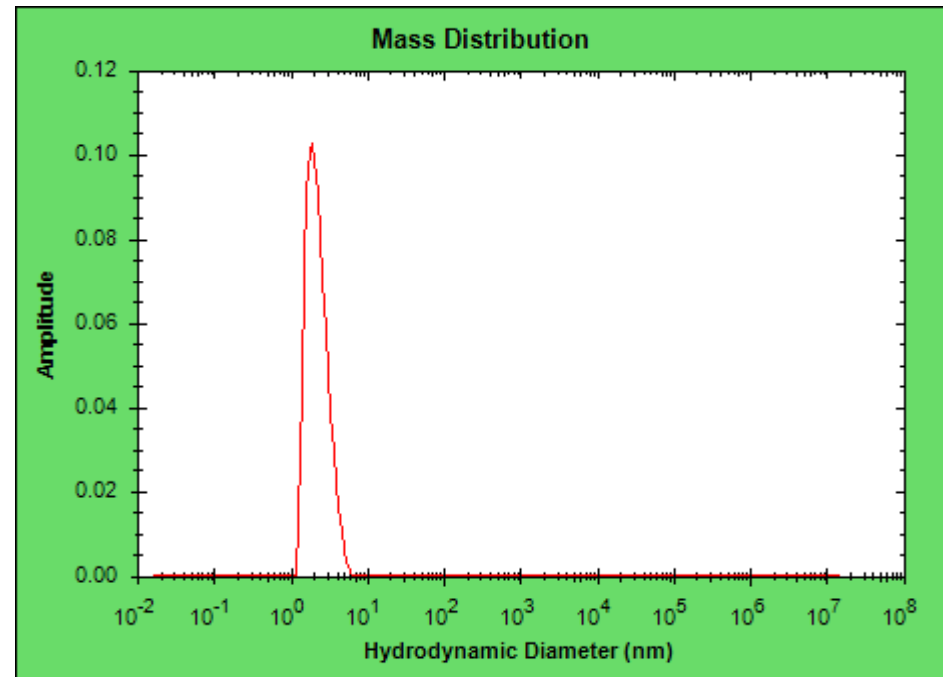
Secondary peak
often indicates
aggregate content

Intensity Distribution
Suited to aggregate detection

Distributions

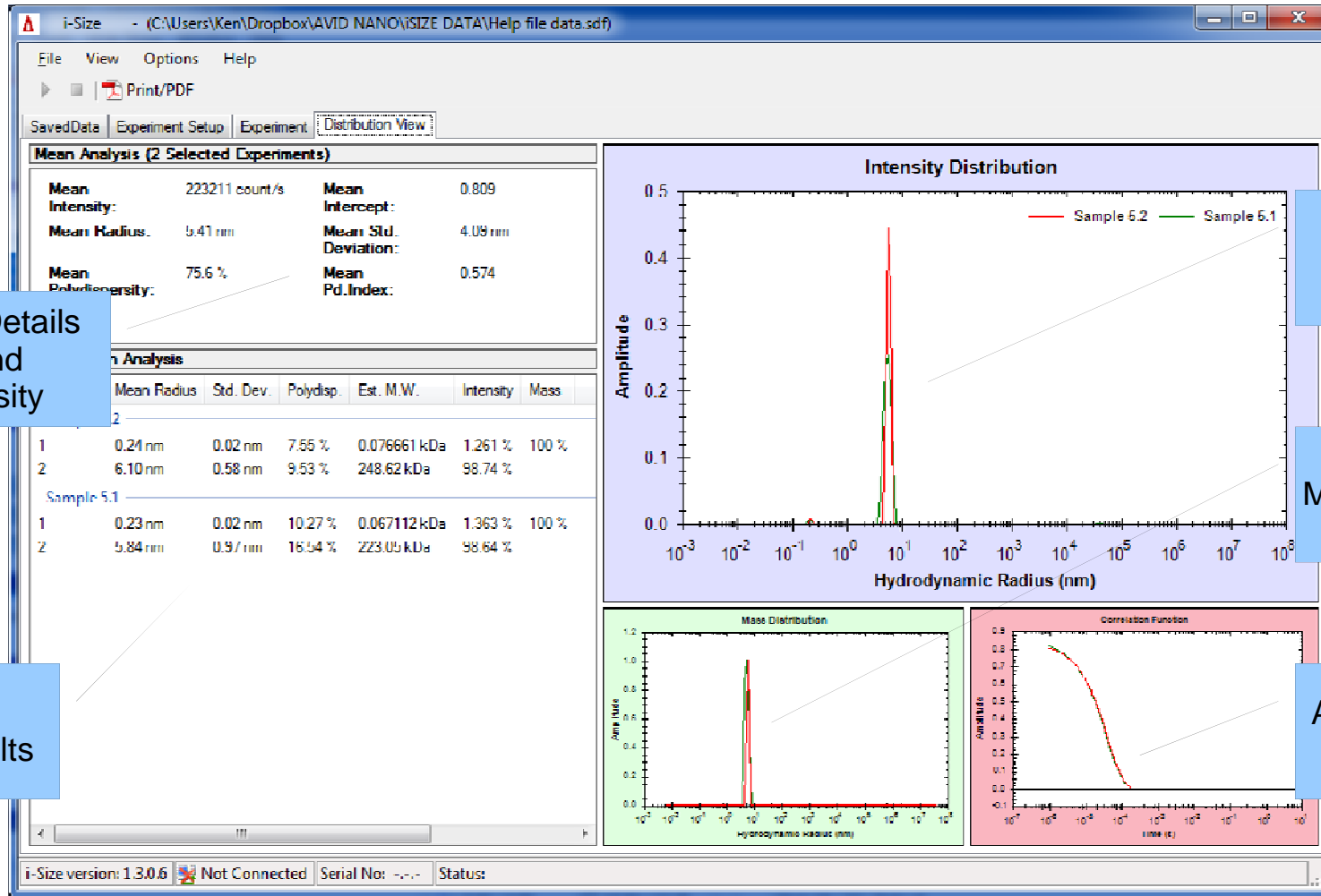


Intensity Distribution
Suited to aggregate detection



Mass Distribution
Assists with data interpretation

Typical Results



Experiment Details
Means and Polydispersity

Distribution Table of Results

Intensity Distribution

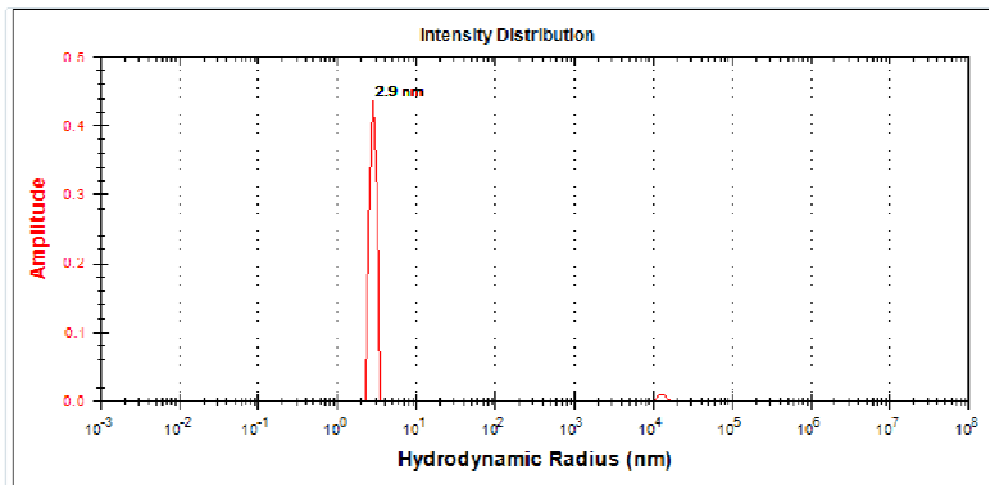
Mass Distribution

Ave. Correlation Function

Image from i-Size v1.3

Example 1 : Monodisperse Protein

Images taken from i-Size v1.2



Experiment Name: class 1-1

Distribution Intensity Data

Peak No.	% Area	Mn Rad (nm)	SD (nm)	Est MW (kDa)
1	97.7	3.05	0.34	44.264
2	2.3	14372.92	1606.66	

Cumulants Average

Parameter	Value
Mean Radius (nm)	3.11
Pd Index	0.159
SD (nm)	1.07
Intensity	367572.7

Highly monodisperse protein solution producing good quality crystals

Narrow intensity peak

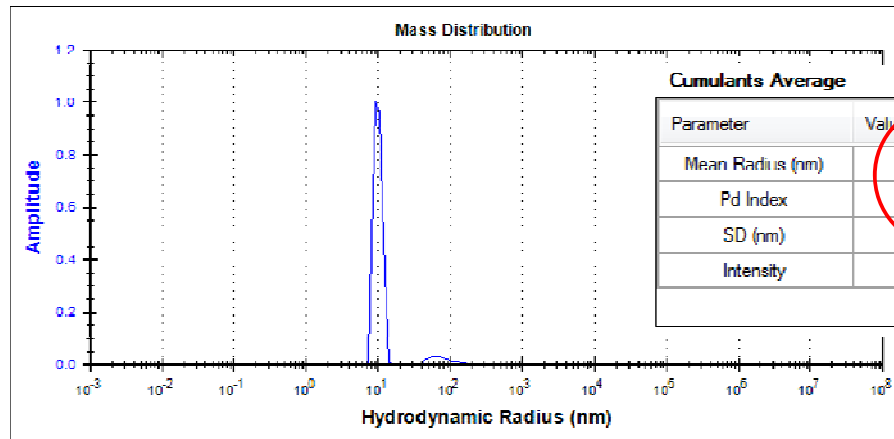
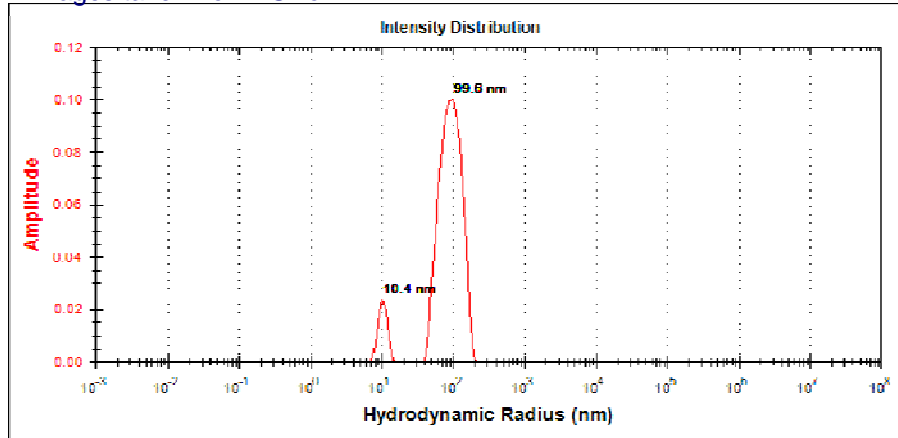
Low polydispersity index

Strong light scattering intensity from high concentration sample

Mean radius 3.1nm used for molecular weight estimate (42kDa actual)

Example 2 : Multi-Peak Distribution

Images taken from i-Size v1.2



Cumulants Average	
Parameter	Value
Mean Radius (nm)	77.64
Pd Index	1.287
SD (nm)	69.69
Intensity	294368

Mixture of certified standards

High Pdl indicates broad or multi-mode distribution

Intensity peaks confirm bi-modal distribution.

Light scatters proportionally to Rh^6

Mass distribution indicates the amount of 10nm material much greater than 100nm

Example 3 : Adding Surfactant

Mixing BSA and SDS

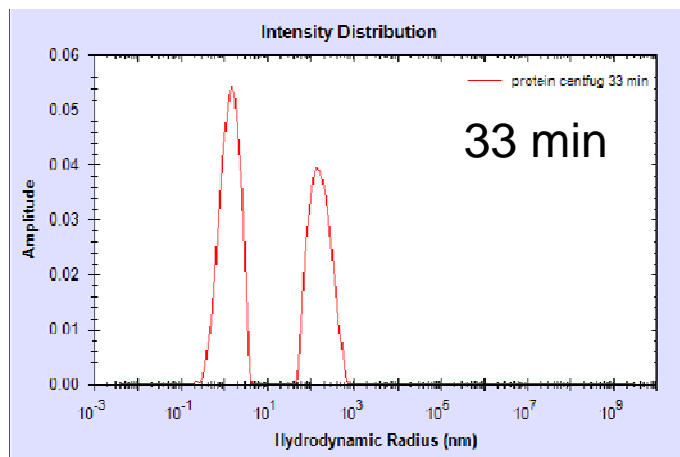
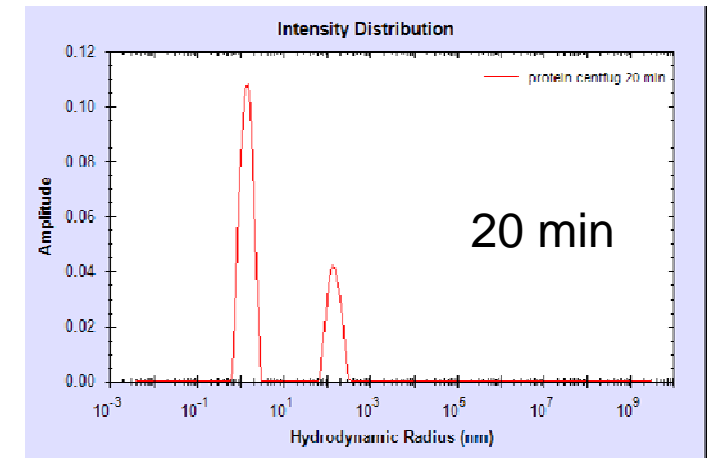
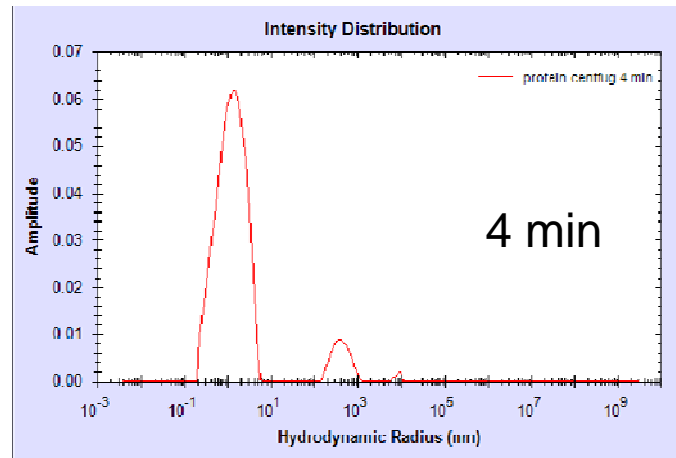
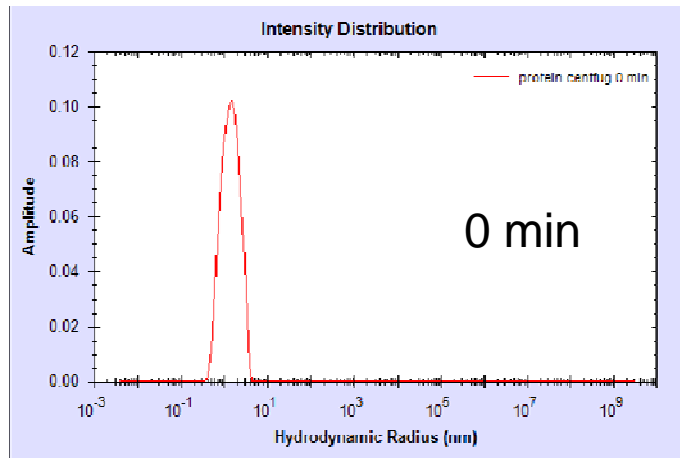
Experiment Name	Cuvette	Solvent	T (°C)	Int. (kcps)	Creation Date	Runs	Mean rad. (nm)	Pk.1 rad. (nm)	Pk.2 rad. (nm)
Directories									
...									
Single Mode									
BSA in PBS - 25C	Other	PBS	25.0	288,495	25/05/2012 14:32:51	10	4.17	4.01	13.11
BSA in PBS - 25C	Other	PBS	25.0	289,719	25/05/2012 14:35:41	10	4.26	4.27	16.89
BSA + SDS - PBS - 25C	Other	PBS	25.0	295,783	13/06/2012 14:51:31	5	6.75	6.54	-
BSA + SDS - PBS - 25C	Other	PBS	24.9	285,078	13/06/2012 14:55:49	5	6.69	5.96	31.10
BSA + SDS - PBS - 25C	Other	PBS	24.9	254,395	13/06/2012 15:04:12	5	6.79	6.05	23.48
BSA + SDS - PBS - 25C	Other	PBS	25.0	336,929	13/06/2012 15:26:14	5	6.84	6.34	34.73
SDS - PBS - 25C	Other	PBS	25.0	94,344	13/06/2012 15:56:30	10	3.61	3.08	35.17
SDS - PBS - 25C	Other	PBS	25.0	117,235	13/06/2012 15:58:43	10	4.04	2.98	38.15
SDS - PBS - 25C	Other	PBS	25.0	116,754	13/06/2012 16:02:37	10	3.78	2.86	39.87

3.8nm

4nm

6.8nm

Example 4 : Real Time Aggregation of peptide



Distribution Analysis

Peak No.	Mean Radius	Std. Dev.	Polydisp.	Est. M.W.	Intensity	Mass
protein centrifug 45 min						
1	1.57 nm	0.21 nm	13.67 %	8.4905 kDa	42.26 %	100 %
2	155.61 nm	24.16 nm	15.53 %	7.8972E+05 kDa	57.74 %	
protein centrifug 20 min						
1	1.58 nm	0.50 nm	31.67 %	8.6555 kDa	71.64 %	100 %
2	165.02 nm	54.31 nm	32.91 %	9.1414E+05 kDa	28.36 %	
protein centrifug 4 min						
1	1.61 nm	1.12 nm	69.62 %	8.9708 kDa	91.39 %	100 %
2	481.68 nm	221.80 nm	46.05 %	1.3165E+07 kDa	8.135 %	
3	9626.06 nm	1129.58 nm	11.73 %	2.281E+10 kDa	0.4762 %	
protein centrifug 0 min						
1	1.68 nm	0.79 nm	46.68 %	10.048 kDa	100 %	100 %

Dynamic Light Scattering

- Primary data

- Mean hydrodynamic radius
- Polydispersity index

- Secondary Data

- Intensity size distribution
- Mass size distribution
- Molecular weight estimate
- Calculate MW of proteins using static light scattering

Common Applications

- Protein purification
- Aggregation
- Micelle formation
- Thermal denaturing
- Colloids and nano-particles

Example Customers

- GSK (Stevenage, UK)
- Sanofi Aventis (Frankfurt, DE)
- Novo Nordisk (Copenhagen, DK)
- Novartis Pharma (Basel, CH) ~ 2 units
- Astex Pharmaceuticals (Cambridge, UK)
- MRC LMB (Cambridge, UK)
- University of Uppsala, BMC (Uppsala, SE)
- GIST (Gwangjun, Korea)
- Uni. Hohenheim (Stuttgart, DE) – **membrane proteins**

Andreas Kühn

Key Strengths

- Speed – especially with disposable cuvettes
- Incredible sensitivity to aggregation
- Requires little a priori knowledge
- Absolute measurement - no calibration
- Maintenance free

Products



W130i

- Designed for the protein specialist.
 - Unbeatable sensitivity (0.1mg/ml, 15kDa protein)
 - Low volume 5µl disposable cuvette
 - Temperature control (0-90°C)
 - Compatible with standard cuvettes

- ALL SUPPLIED AS STANDARD



BladeCell Disposable Cuvette



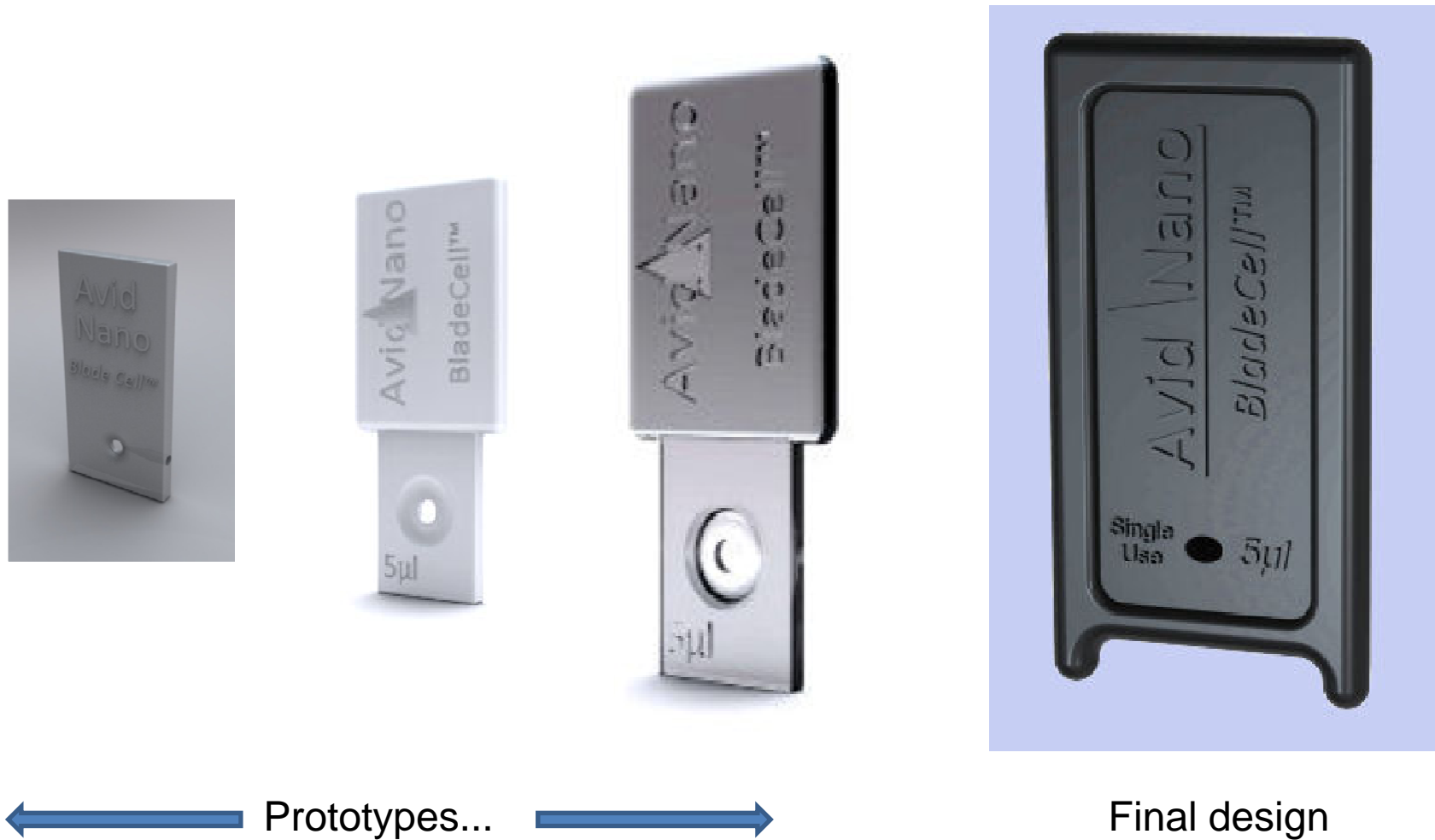
BladeCell Disposable Cuvette

- Traditional DLS means...
 - Expensive quartz cuvettes
 - Cuvette cleaning required
 - Cross-contamination issues

BladeCell Disposable Cuvette

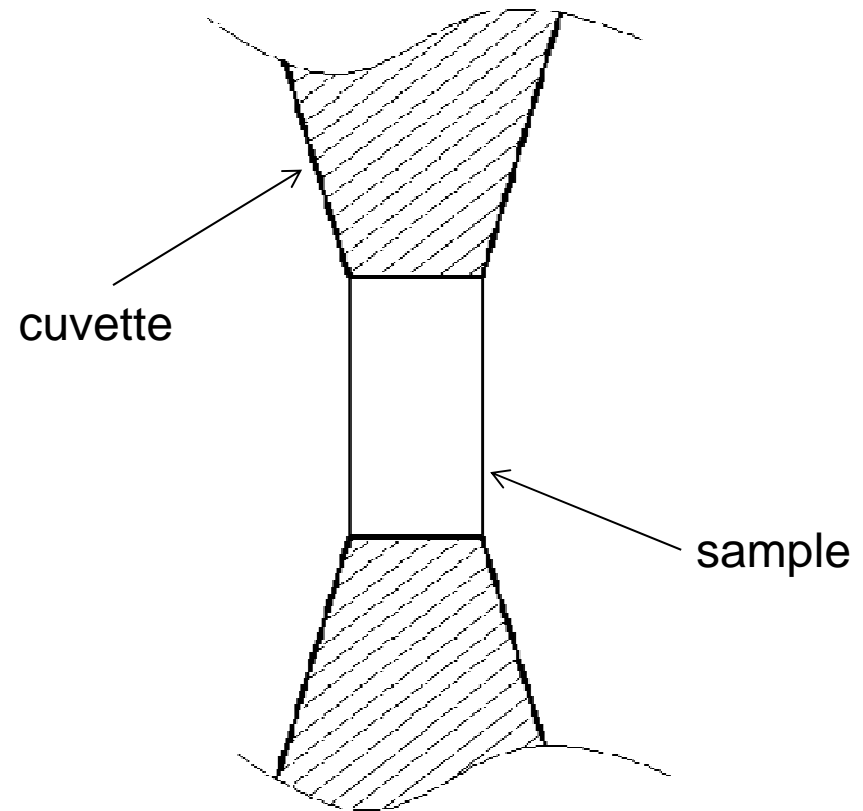
- BladeCell cuvette solves problem
 - Only 5 μ l
 - No cleaning or reference measurements
 - Much faster than quartz
 - Full sample recovery
 - Zero cross-contamination

Evolution of BladeCell



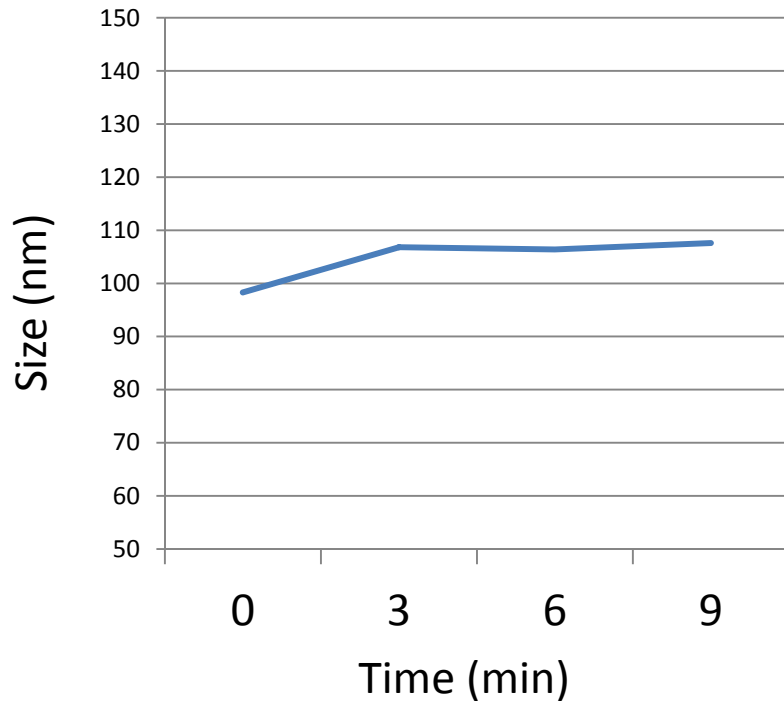
BladeCell Disposable Cuvette

- 'Liquid trap' design utilizes the force of surface tension to retain liquid within a defined shape and volume



BladeCell Disposable Cuvette

•Effect of evaporation on the measurement



5ul 100nm **0min** Intensity Distrib, (nm)
Cumulant Analysis
Mean: 49.13 (98.3 dia)

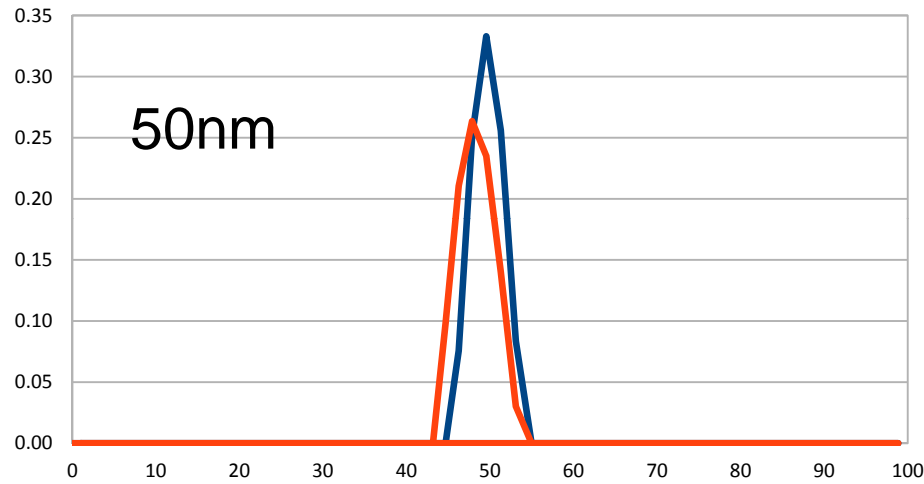
5ul 100nm **3min** Intensity Distrib, (nm)
Cumulant Analysis
Mean: 53.43 (106.8 dia)

5ul 100nm **6min** Intensity Distrib, (nm)
Cumulant Analysis
Mean: 53.18 (106.4 dia)

5ul 100nm **9min** Intensity Distrib, (nm)
Cumulant Analysis
Mean: 53.83 (107.6 dia)

100nm size standard.
Certified 97 – 106nm diameter +/- 2%
Min. Dia. 95.1nm
Max. Dia. 108.1

5 μ l BladeCell v. 2ml Macro Cuvette



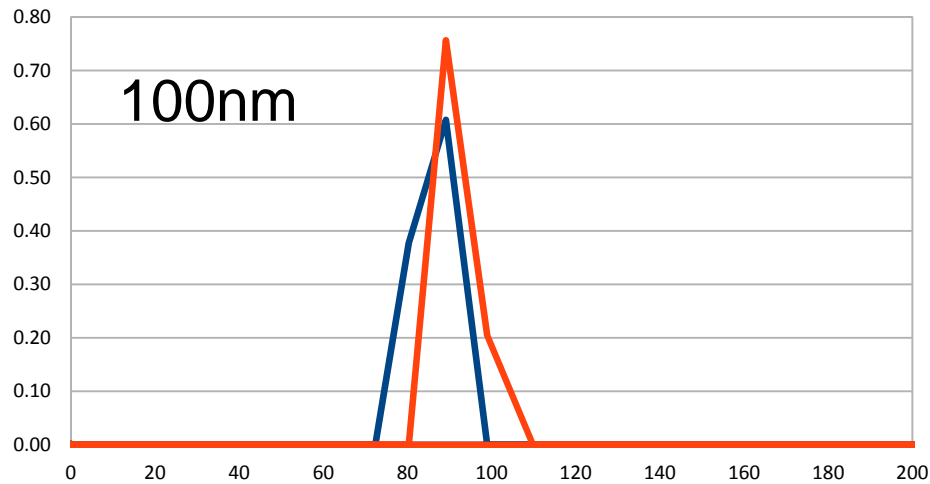
50nm SIZE STANDARD

Cumulant Analysis

Mean Radius : 51.01 nm
Standard deviation : 8.580 nm
Polydispersity : 0.028

Cumulant Analysis

Mean Radius : 49.53 nm
Standard deviation : 8.494 nm
Polydispersity : 0.029



100nm SIZE STANDARD

Cumulant Analysis

Mean Radius : 100.8 nm
Standard deviation : 3.656 nm
Polydispersity : 0.001

Cumulant Analysis

Mean Radius : 100.2 nm
Standard deviation : 12.56 nm
Polydispersity : 0.016

BladeCell Disposable Cuvette

- You have about 10 minutes to make a measurement before an (aqueous) sample evaporates

BladeCell Disposable Cuvette

- You have about 10 minutes to make a measurement before an (aqueous) sample evaporates
- Measurement quality as good as traditional cuvette

BladeCell Disposable Cuvette

- You have about 10 minutes to make a measurement before an (aqueous) sample evaporates
- Measurement quality as good as traditional cuvette
- Quick, convenient and inexpensive

BladeCell Disposable Cuvette

- You have about 10 minutes to make a measurement before an (aqueous) sample evaporates
- Measurement quality as good as traditional cuvette
- Quick, convenient and inexpensive
- No possibility of cross-contamination

Thank you for watching





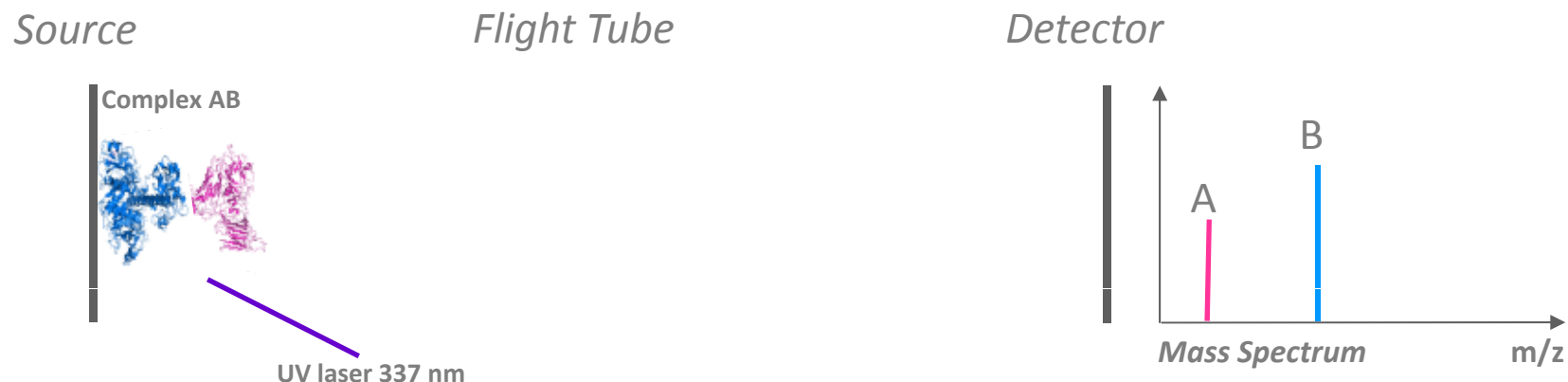
High-Mass MALDI ToF Mass Spectrometry for Protein Interactions and Macromolecular Analysis

CovalX AG
Ausstellungstrasse 36
CH-8003 Zürich
Switzerland
www.covalx.com

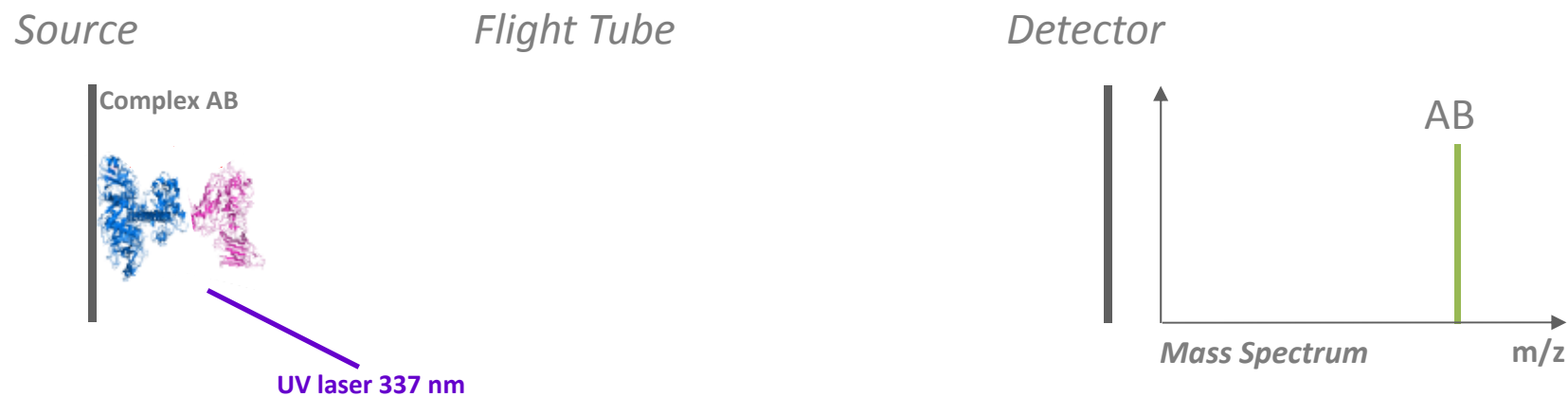


Two major issues

1) Dissociation Problem



Proposed Solution: Chemical Stabilization



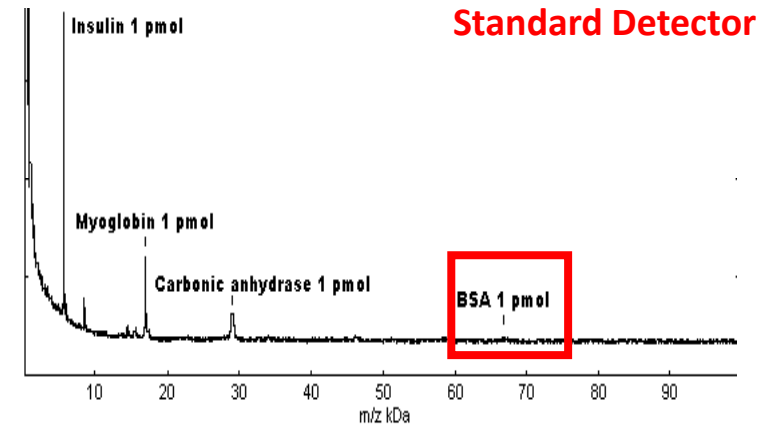
•Normally ions from a non-covalent complex are dissociated when doing a MALDI experiment

•Using chemical stabilization keeps the complex intact so that it can be detected

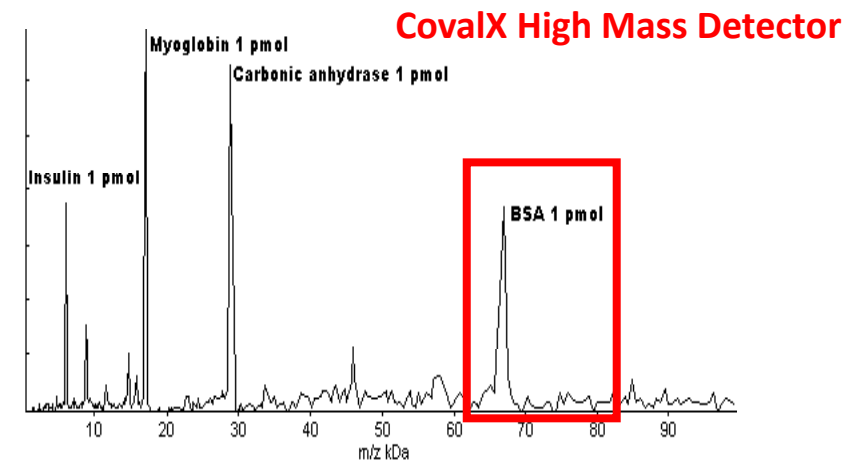
Two major issues

2) Detection Problem

- *Standard Linear Detectors (MCPs) have known **sensitivity** limitations in detecting higher mass (slower moving) ions.*
- *MCPs have difficulty detecting **saturation** with complex solutions where later arriving ions are undetected.*
- *MCPs have higher reaction times and therefore higher resolution when detecting lower mass ions (which require this speed).*



Proposed Solution



High-Mass Detection System

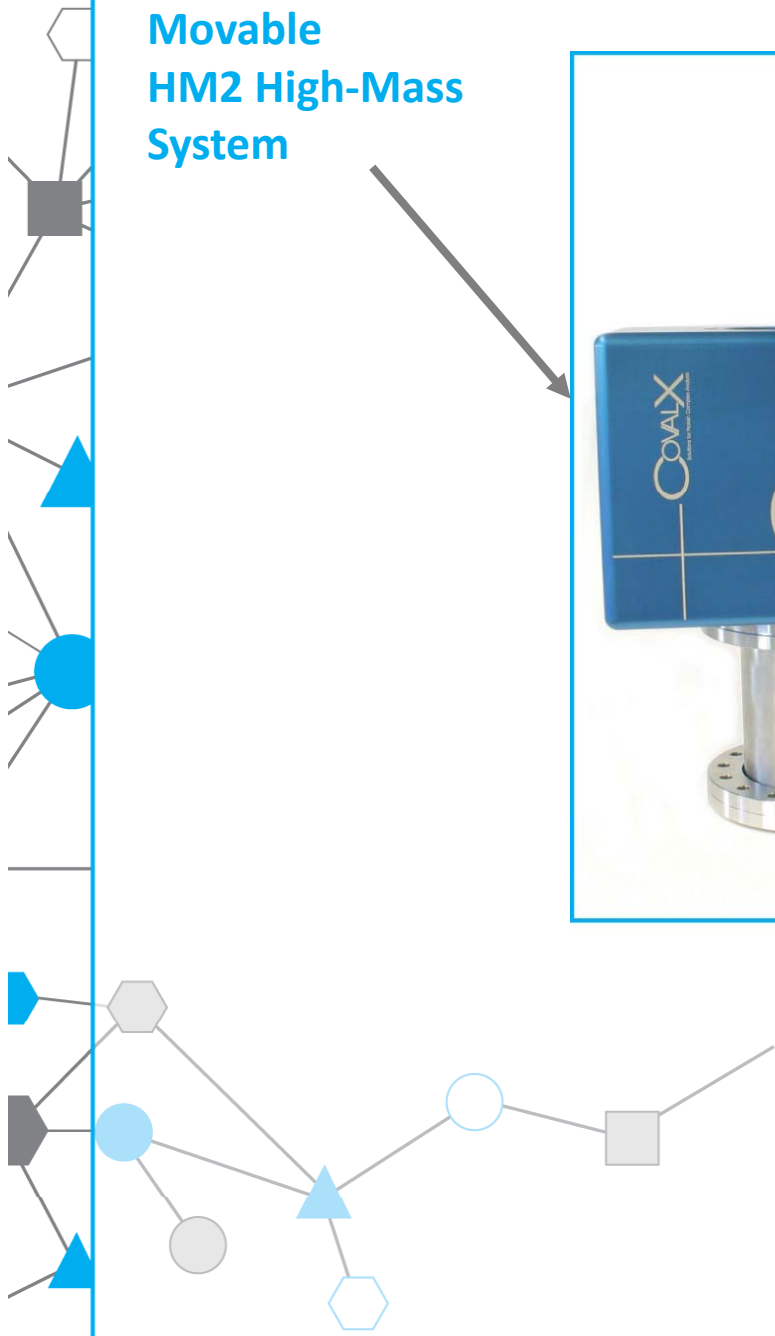
**Movable
HM2 High-Mass
System**



**Independant
Electronic and
High-Voltage
supply**

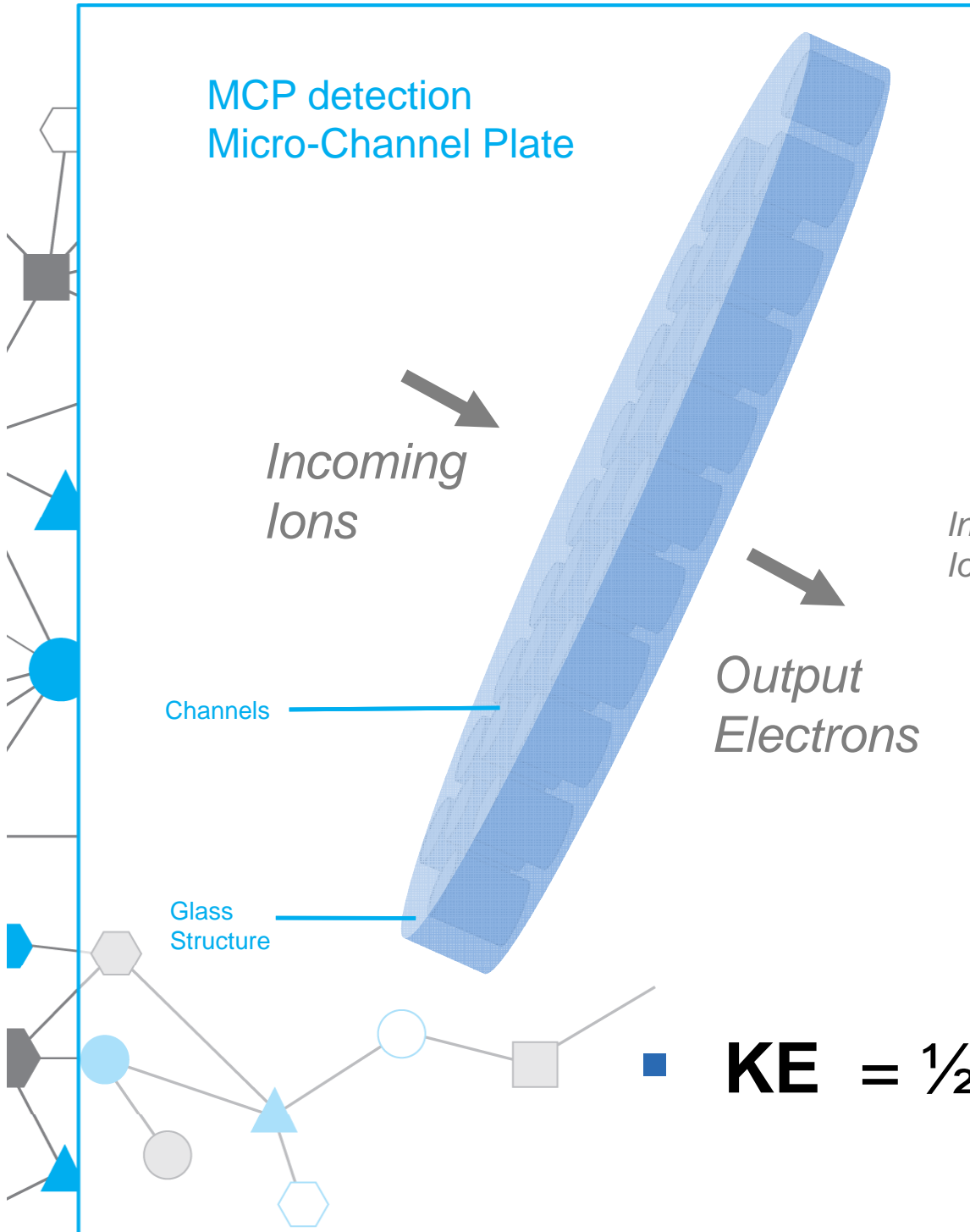
**Wireless Control
iPod Touch™
CovalX's application**

**Compatible with all commercially
available MALDI TOF equipment**

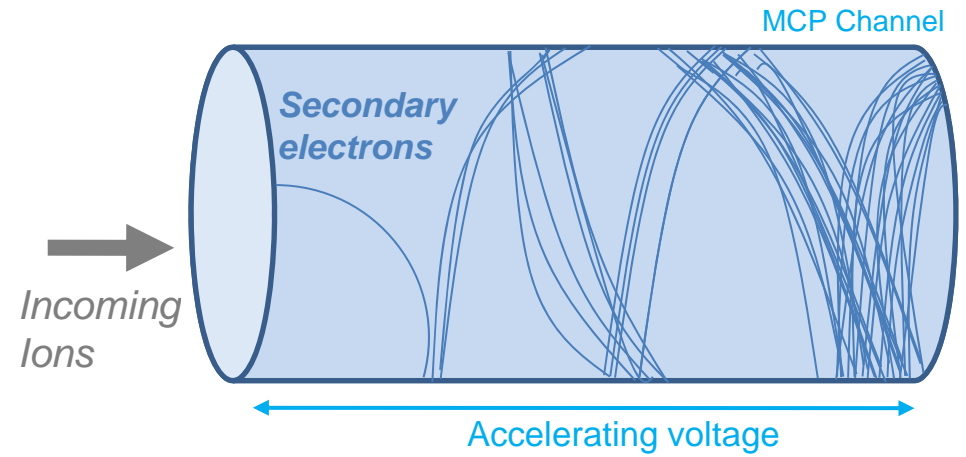


What is different?

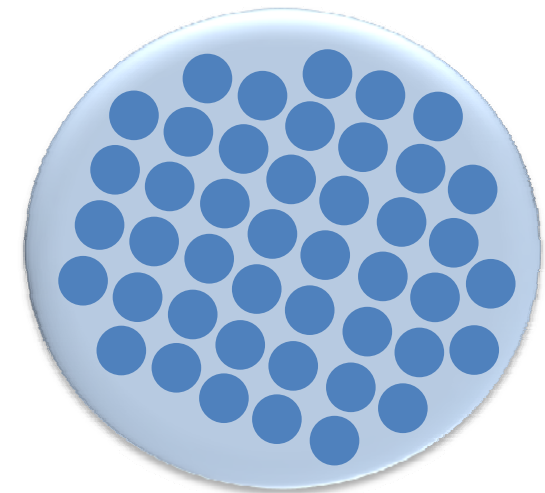
Principles of MCP detection



- Sensitivity issue



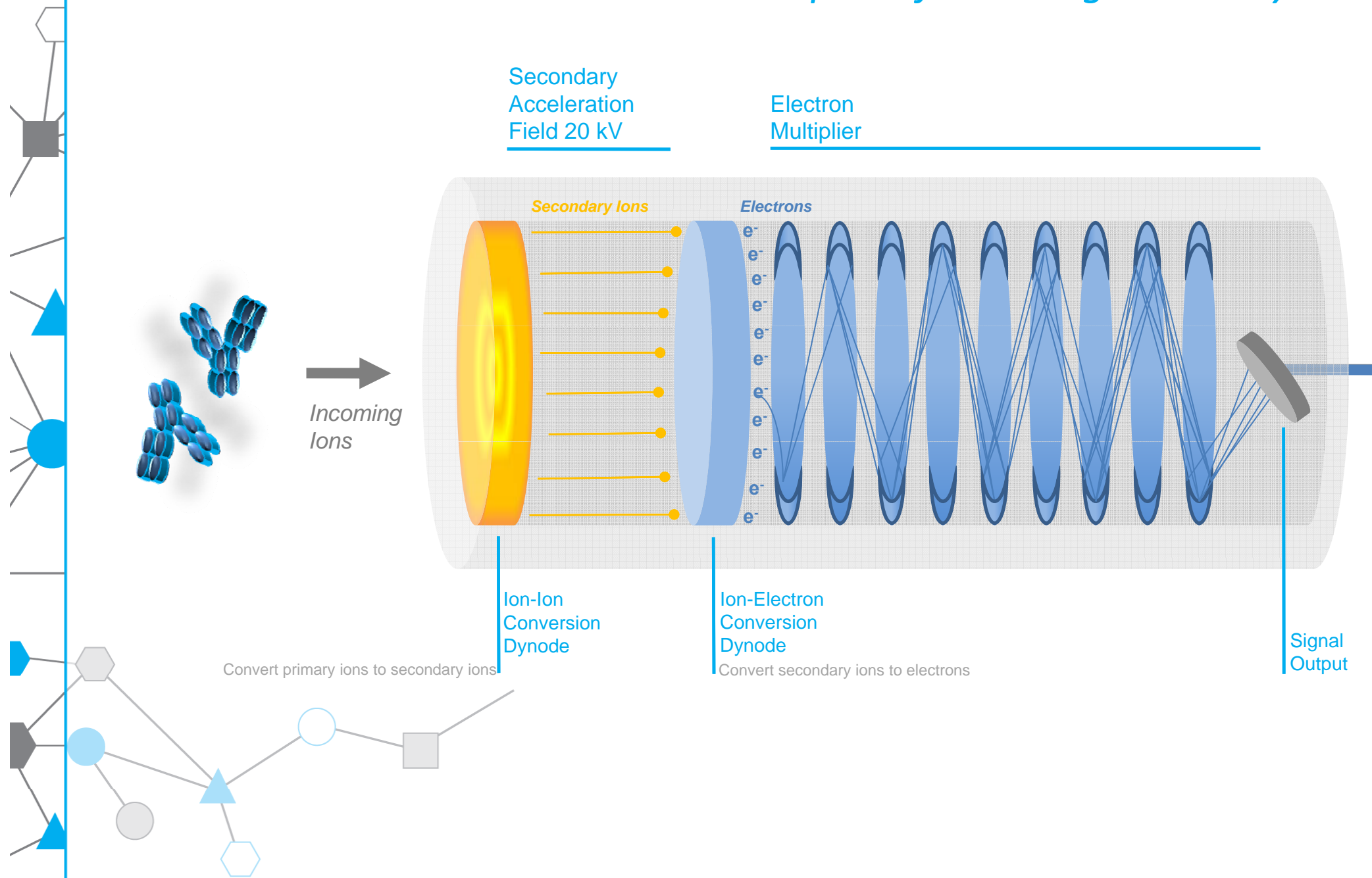
- Saturation issue



▪ $KE = \frac{1}{2} mv^2$

What is different?

Principles of HM1 High-Mass System





Biflex

Shimadzu



Reflex



Autoflex



Ultraflex



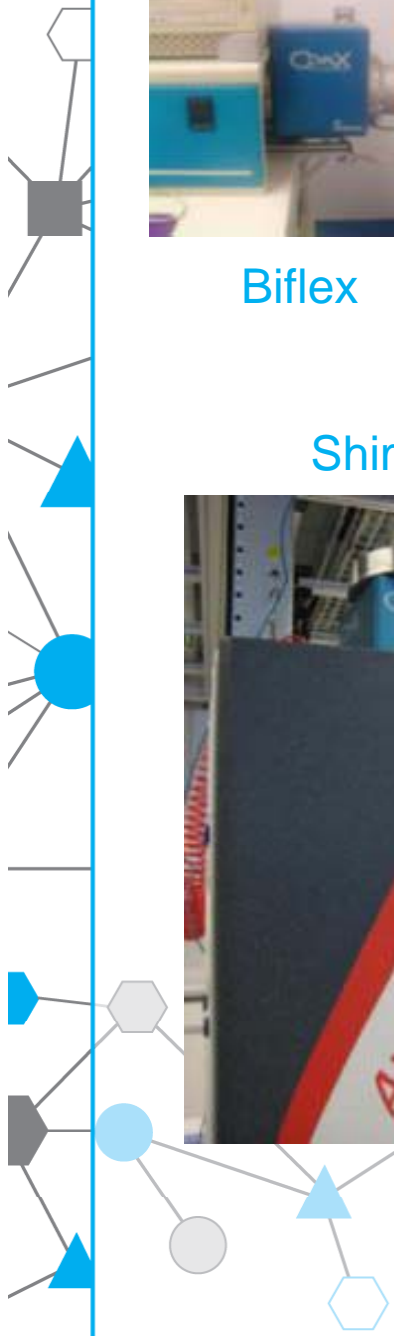
ABI STR



ABI DE Series



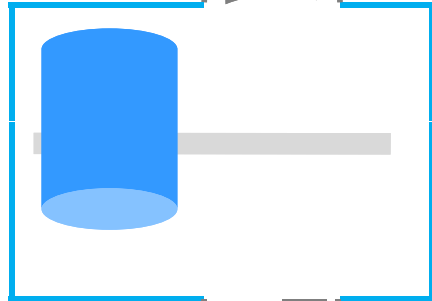
ABI 4800



High Mass Detector OFF
MCP detector ON

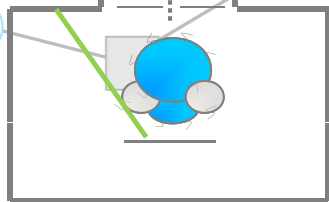
MCP detector

HM2 High-Mass Retrofit System



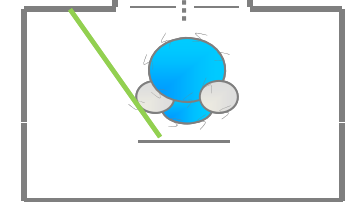
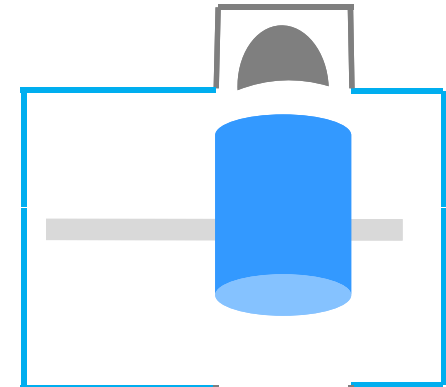
Fight Tube

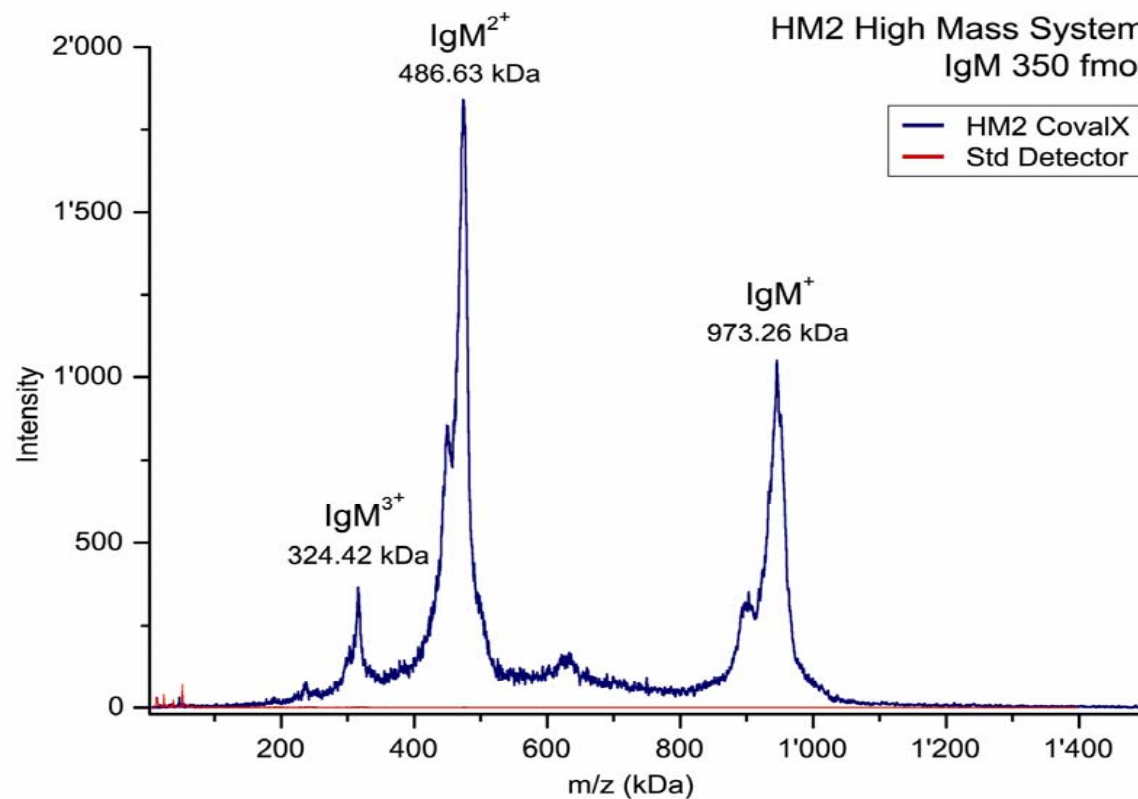
MALDI Source



High Mass Detector ON
MCP detector OFF

- MCP detector remains
- Reflectron & MS/MS remain unaffected
- Takes about 15 seconds to change positions
- Detection beyond 1.5MDa
- Low saturation for complex mixtures



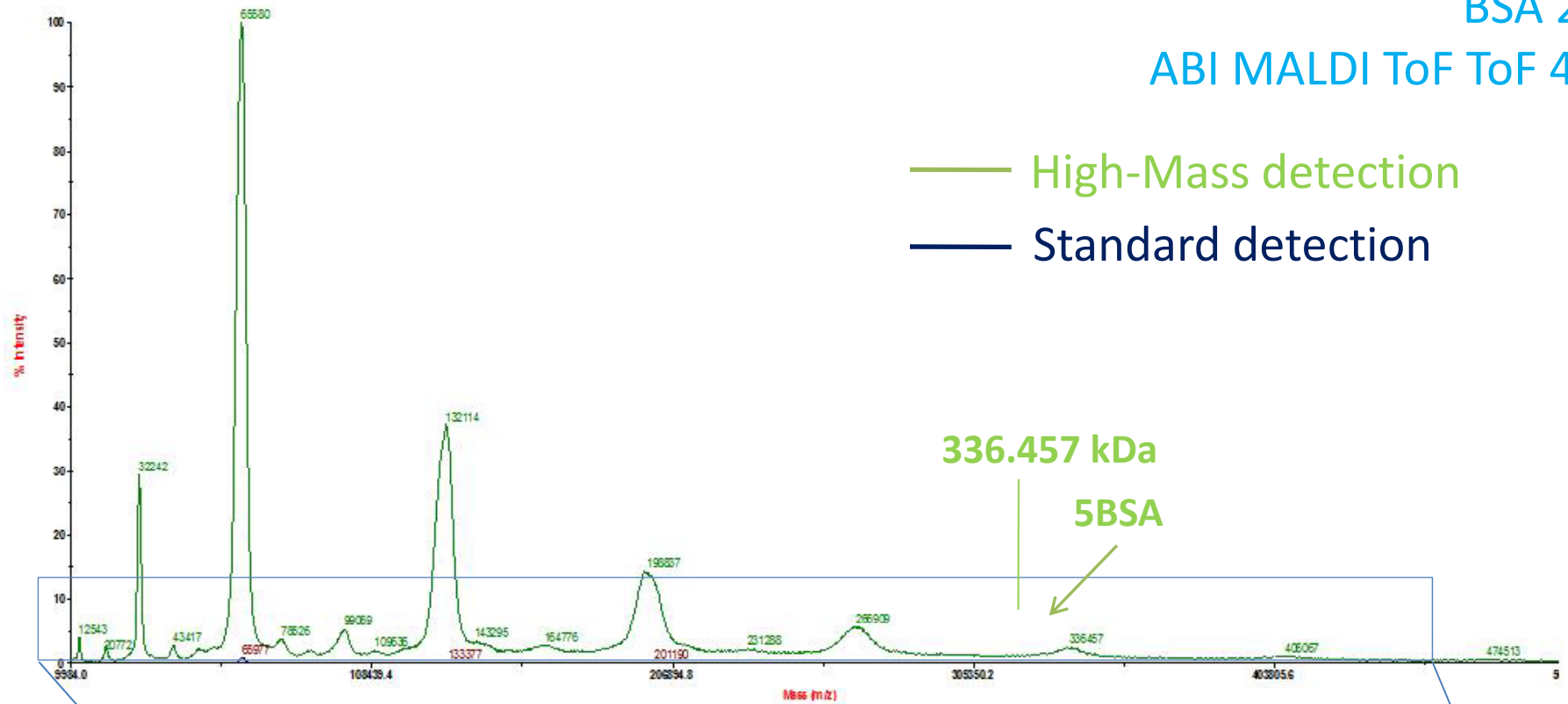


- **First and only fully integrated high mass MALDI instrument**
- **HM2 detector: outstanding sensitivity 10kDa – 1500 kDa**
- **Standard detector remains for lower masses 0-10kDa**
- **15 second changeover time**
- **Low saturation for complex mixtures**

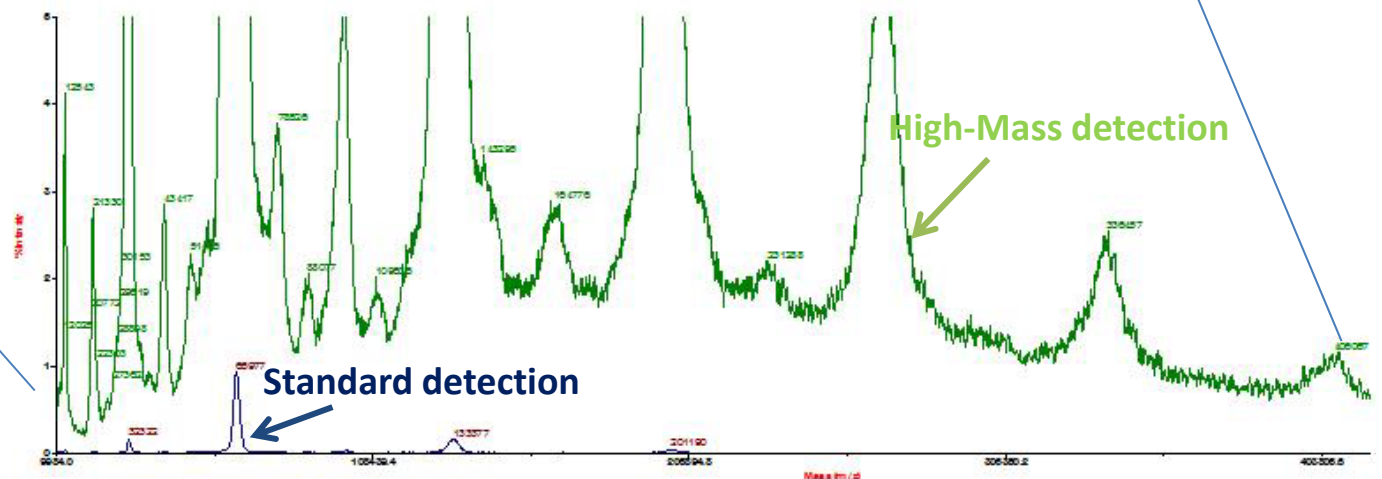
Comparison MCP/ High-Mass

BSA 2 μ M

ABI MALDI ToF ToF 4800



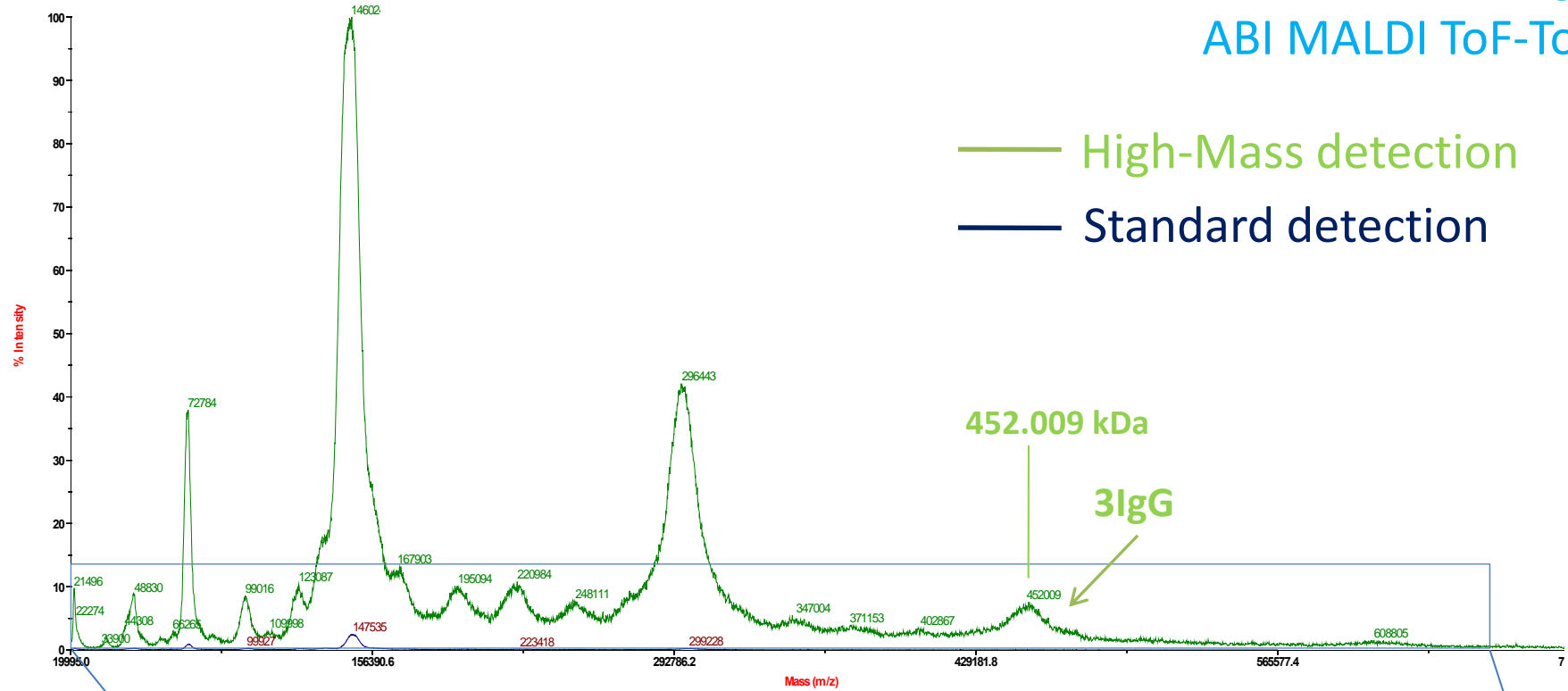
Zoom



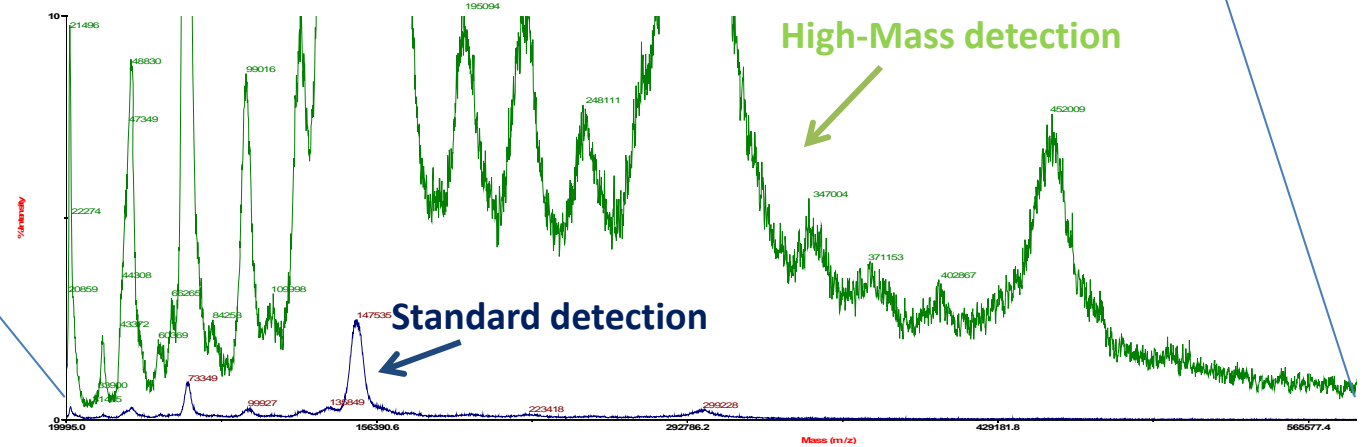
Comparison MCP/ High-Mass

IgG 4 μ M

ABI MALDI ToF-ToF 4800



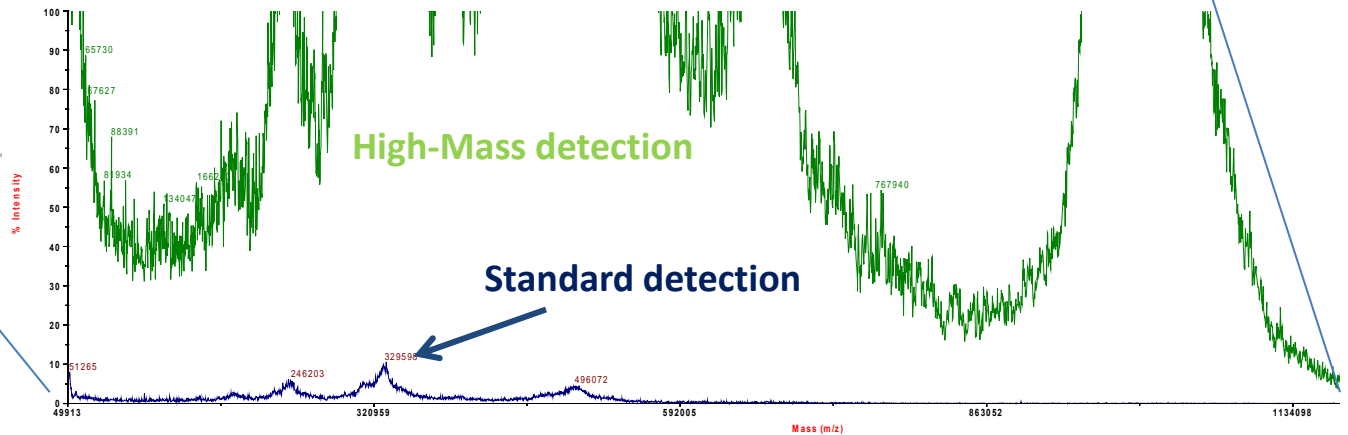
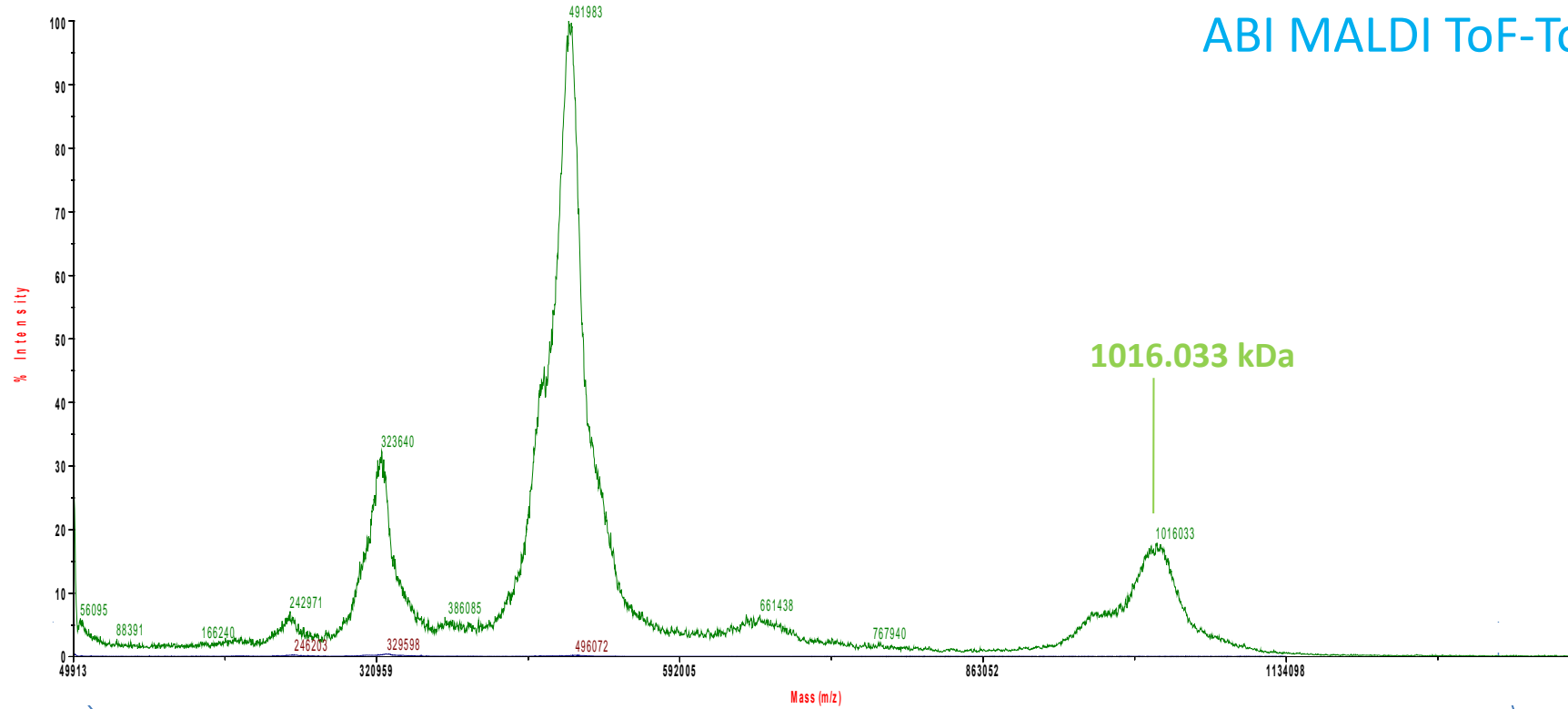
Zoom



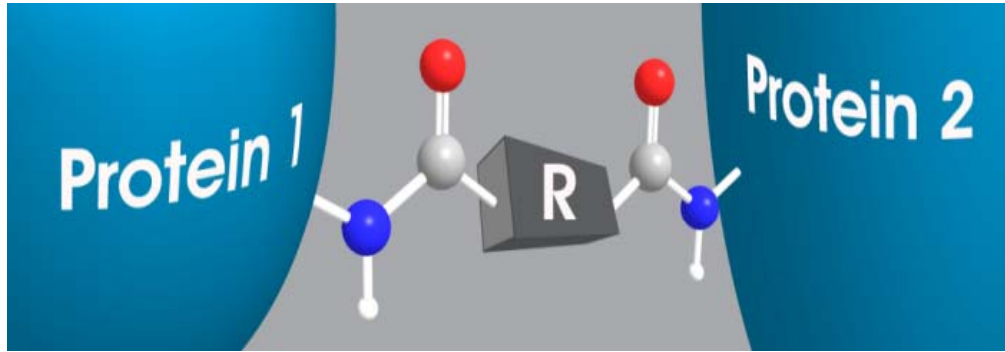
Comparison MCP/ High-Mass

IgM 1 μ M

ABI MALDI ToF-ToF 4800

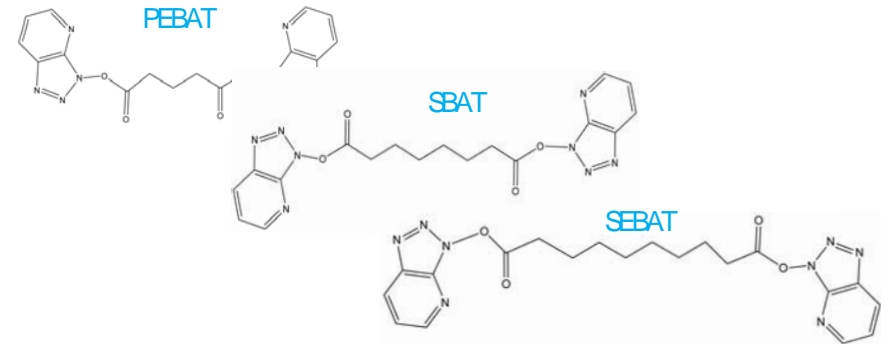


Zoom



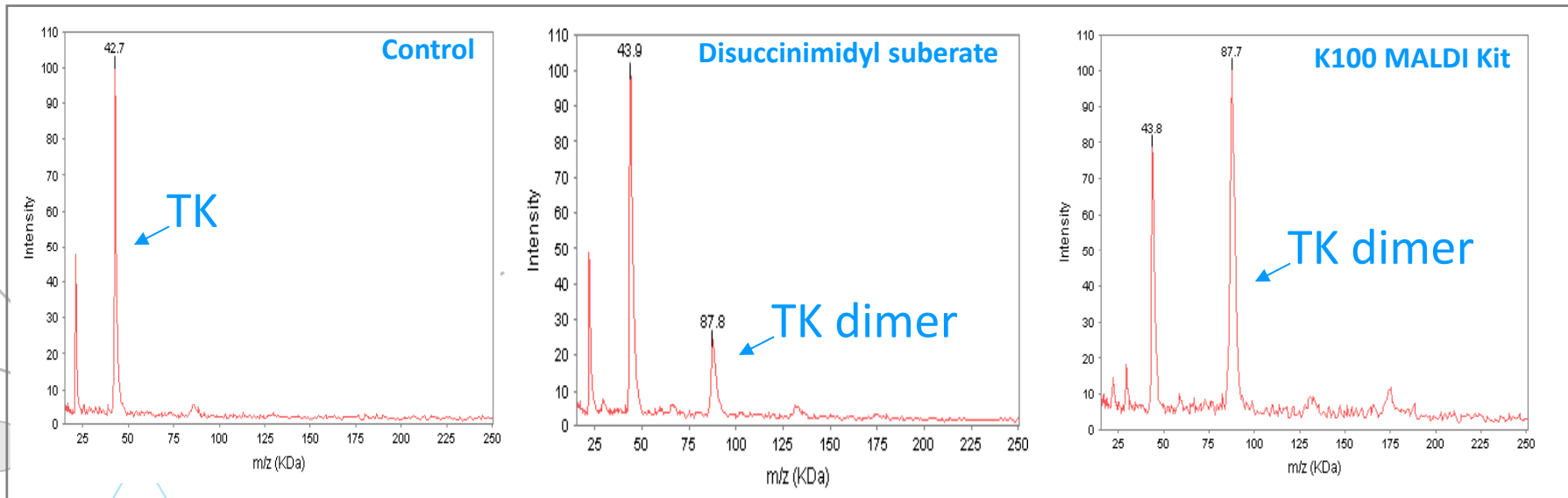
R: Spacer ranging from 6 Å to 16 Å

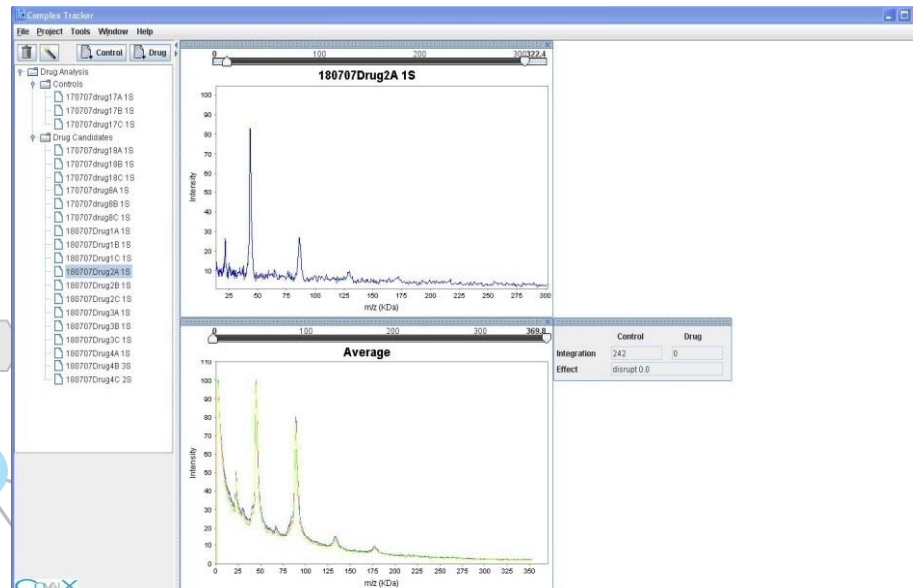
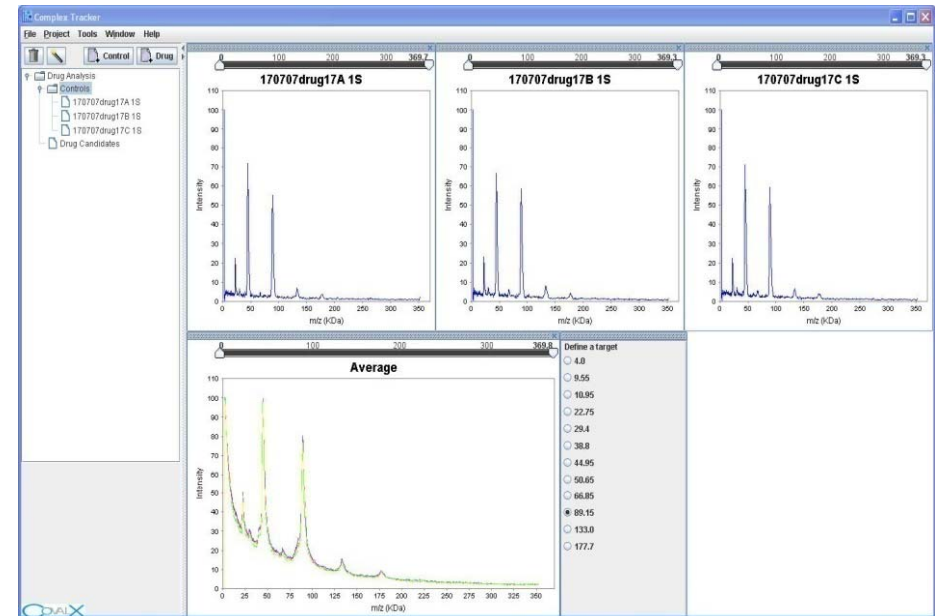
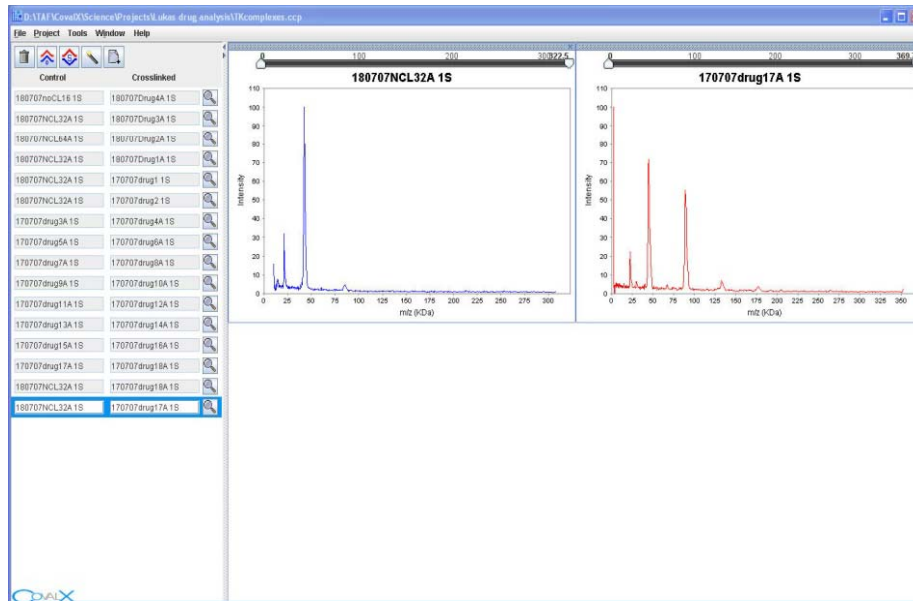
Cocktails of Cross-Linkers
To increase efficiency



Worldwide Patents Pending

Thymidine Kinase Dimer





Control Drug

Drug Analysis

- Controls
 - 170707drug17A 1S
 - 170707drug17B 1S
 - 170707drug17C 1S
- Drug Candidates
 - 170707drug18A 1S
 - 170707drug18B 1S
 - 170707drug18C 1S
 - 170707drug18A 1S
 - 170707drug18B 1S
 - 170707drug18C 1S
 - 170707drug18A 1S
 - 170707drug18B 1S
 - 170707drug18C 1S
 - 180707Drug1 1S
 - 180707Drug2 1S
 - 180707Drug3 1S
 - 180707Drug4 1S
 - 180707Drug5 1S
 - 180707Drug6 1S
 - 180707Drug7 1S
 - 180707Drug8 1S
 - 180707Drug9 1S
 - 180707Drug10 1S
 - 180707Drug11 1S
 - 180707Drug12 1S
 - 180707Drug13 1S
 - 180707Drug14 1S
 - 180707Drug15 1S
 - 180707Drug16 1S
 - 180707Drug17 1S
 - 180707Drug18 1S
 - 180707Drug19 1S
 - 180707Drug20 1S
 - 180707Drug21 1S
 - 180707Drug22 1S
 - 180707Drug23 1S
 - 180707Drug24 1S
 - 180707Drug25 1S
 - 180707Drug26 1S
 - 180707Drug27 1S
 - 180707Drug28 1S
 - 180707Drug29 1S
 - 180707Drug30 1S
 - 180707Drug31 1S
 - 180707Drug32 1S
 - 180707Drug33 1S
 - 180707Drug34 1S
 - 180707Drug35 1S
 - 180707Drug36 1S
 - 180707Drug37 1S
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 - 180707Drug67 1S
 - 180707Drug68 1S
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 - 180707Drug70 1S
 - 180707Drug71 1S
 - 180707Drug72 1S
 - 180707Drug73 1S
 - 180707Drug74 1S
 - 180707Drug75 1S
 - 180707Drug76 1S
 - 180707Drug77 1S
 - 180707Drug78 1S
 - 180707Drug79 1S
 - 180707Drug80 1S
 - 180707Drug81 1S
 - 180707Drug82 1S
 - 180707Drug83 1S
 - 180707Drug84 1S
 - 180707Drug85 1S
 - 180707Drug86 1S
 - 180707Drug87 1S
 - 180707Drug88 1S
 - 180707Drug89 1S
 - 180707Drug90 1S
 - 180707Drug91 1S
 - 180707Drug92 1S
 - 180707Drug93 1S
 - 180707Drug94 1S
 - 180707Drug95 1S
 - 180707Drug96 1S
 - 180707Drug97 1S
 - 180707Drug98 1S
 - 180707Drug99 1S
 - 180707Drug100 1S

Rank	Candidate	Target	Score	Effect
3	180707Drug1	88 15/0.0	disrupt	
3	180707Drug1	88 15/0.0	disrupt	
3	180707Drug2	88 15/0.0	disrupt	
3	180707Drug2	88 15/0.0	disrupt	
3	180707Drug3	88 15/0.0	disrupt	
3	180707Drug3	88 15/0.0	disrupt	
3	180707Drug1	88 15/0.37	disrupt	
3	180707Drug3	88 15/0.40	disrupt	
3	180707Drug3	88 15/0.47	disrupt	
3	170707drug1	88 15/0.53	disrupt	
3	170707drug1	88 15/0.53	disrupt	
3	170707drug1	88 15/0.53	disrupt	
3	170707drug1	88 15/0.58	disrupt	
3	170707drug1	88 15/0.58	disrupt	
3	170707drug1	88 15/0.58	disrupt	
3	170707drug1	88 15/0.61	disrupt	
3	180707Drug4	88 15/0.81	disrupt	
3	170707drug1	88 15/0.83	disrupt	
3	170707drug1	88 15/0.84	disrupt	
3	180707Drug10	88 15/0.86	disrupt	
3	180707Drug8	88 15/0.86	disrupt	
3	180707Drug4	88 15/0.86	disrupt	
3	170707drug1	88 15/0.72	disrupt	
3	180707Drug4	88 15/0.86	disrupt	
3	180707Drug3C 1S	88 15/1.31	associate	

CovalX's MALDI protein interaction analysis



1 **Cross-linking Kits:** To stabilize non-covalent protein complexes for MALDI analysis

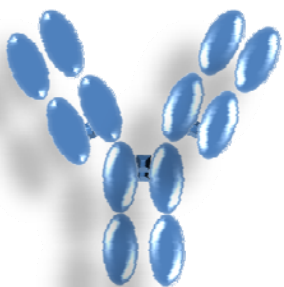


2 **High-Mass MALDI ToF System:** To detect intact protein complexes up to 1.5 MDa



3 **Complex Tracker Analysis Software:** To evaluate MS data generated

Antibody Characterization



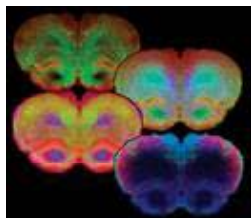
- Antibody-Antigen interactions
- Epitope mapping
- Sandwich assays



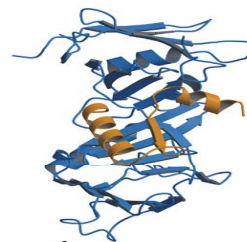
Inhibitors of Protein-protein interactions



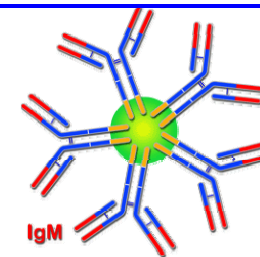
Protein Aggregates



High-Mass MALDI Imaging



Protein complexes characterization



Immunoglobulin M Characterization



PEG Protein Characterization



Plasma Screening

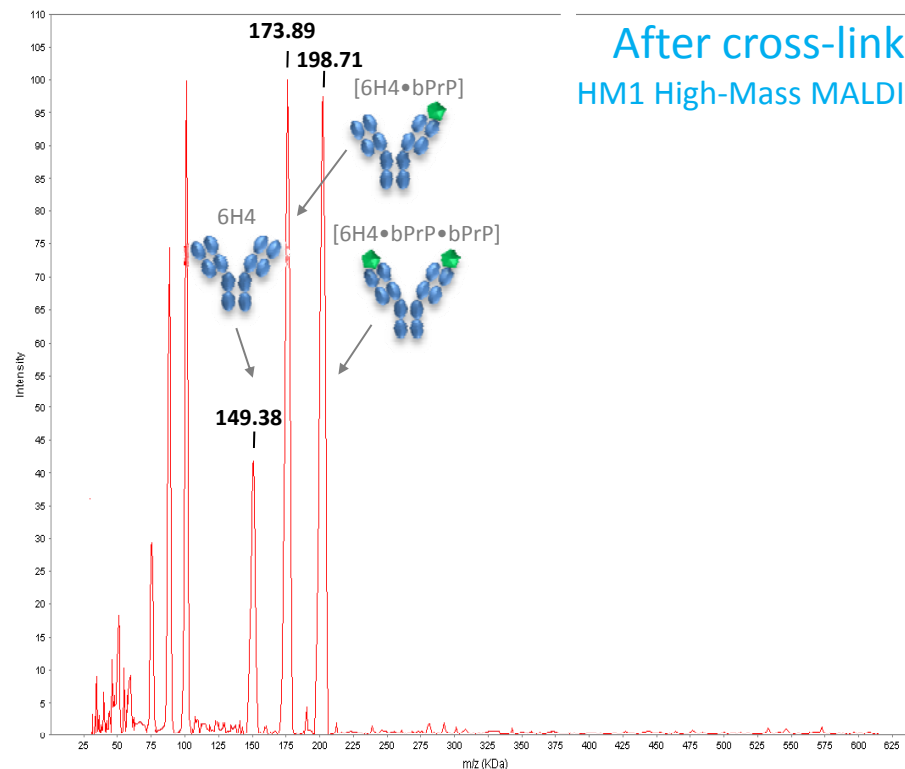
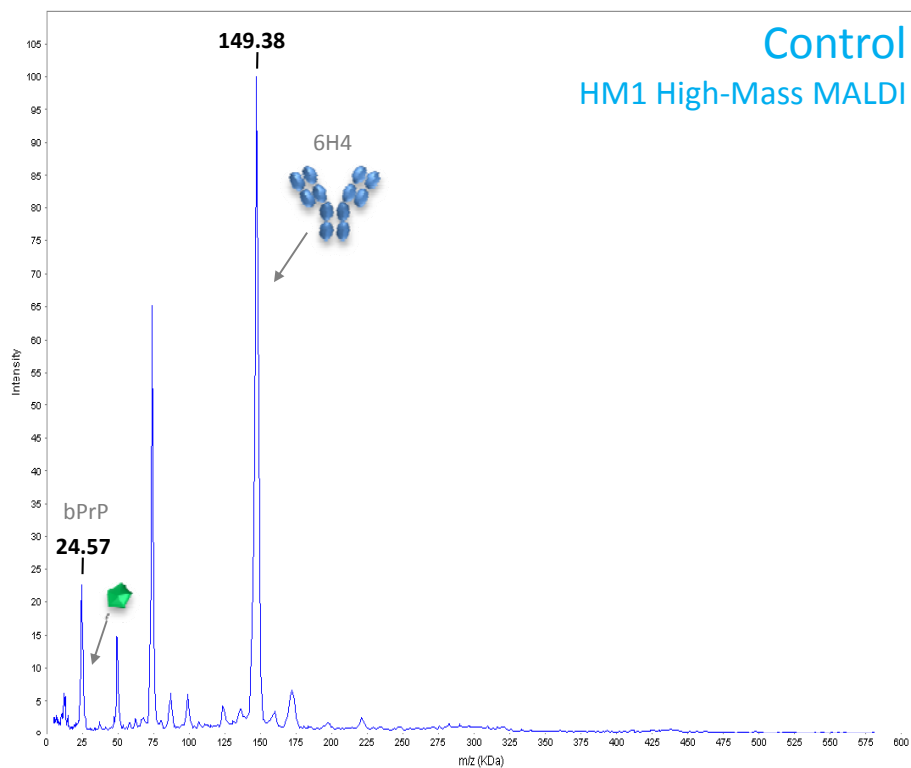
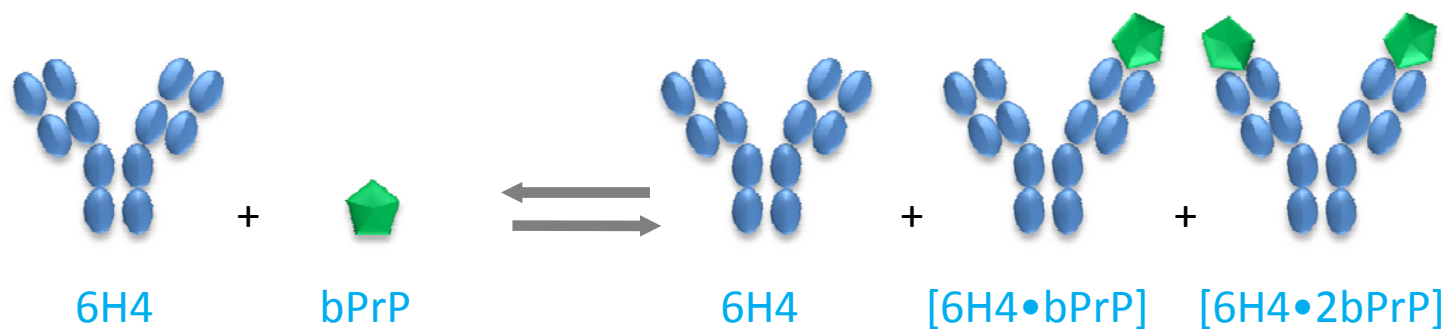


Polymer Analysis

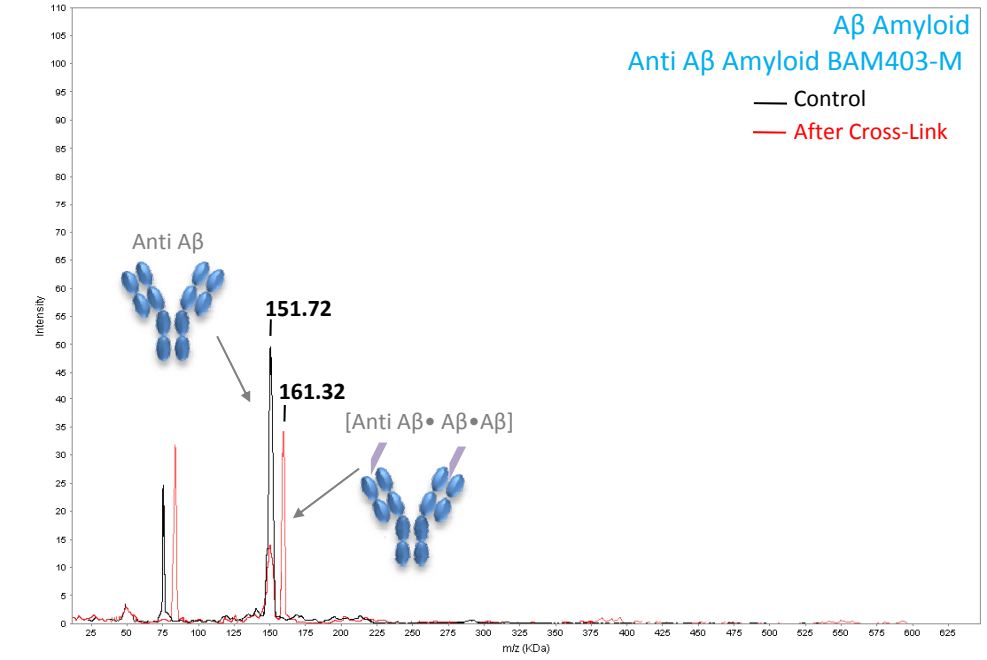
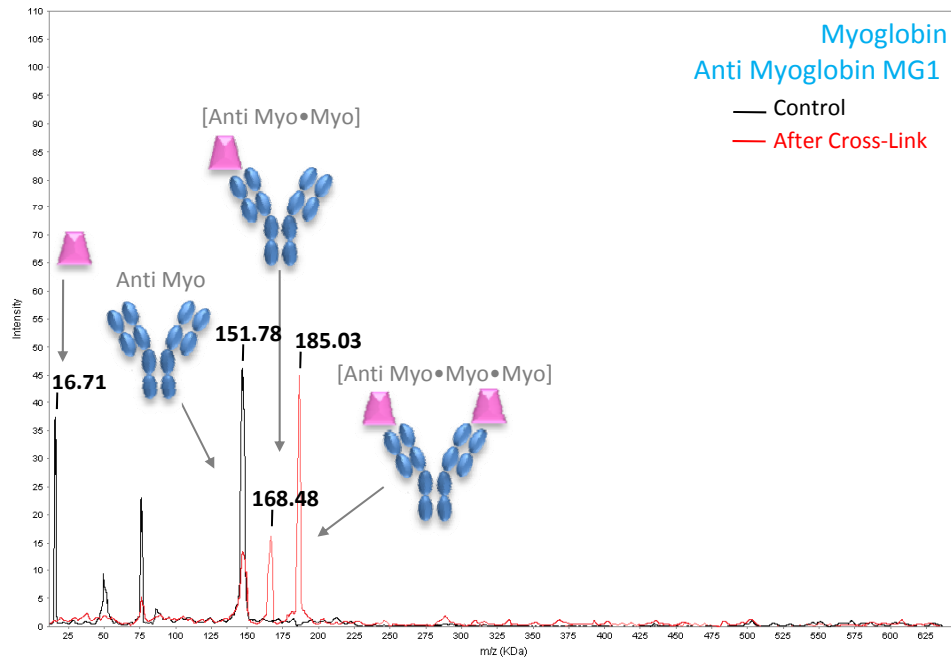
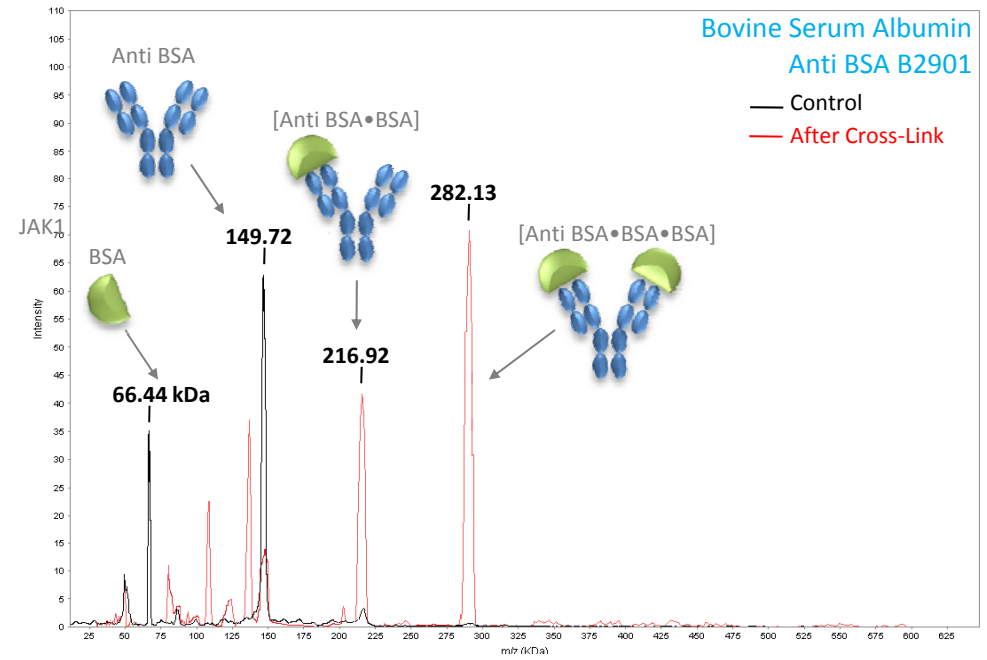
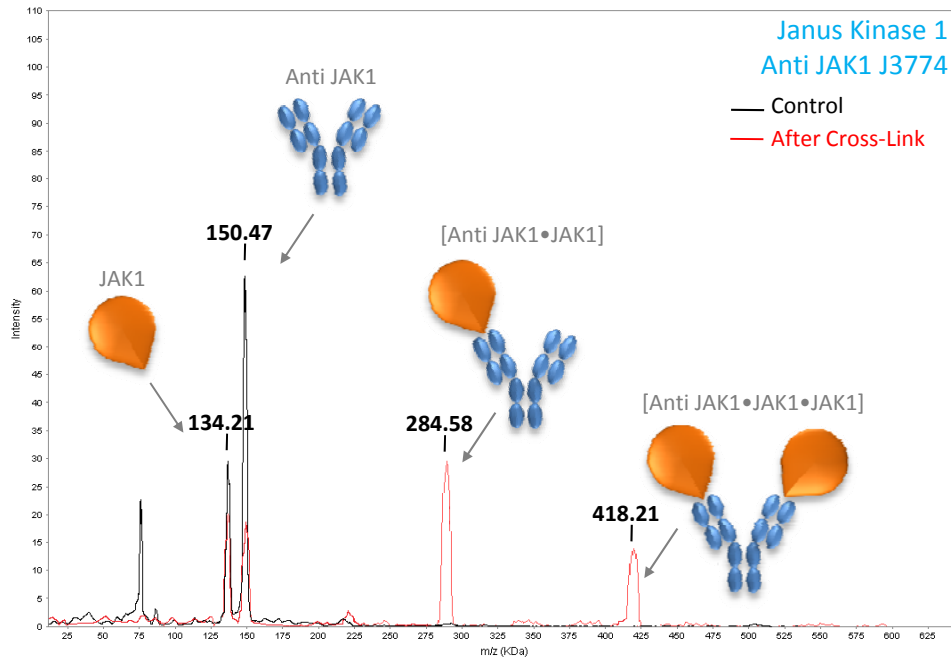
All CovalX applications available directly through our CRO services.

Antibody/Antigen interactions

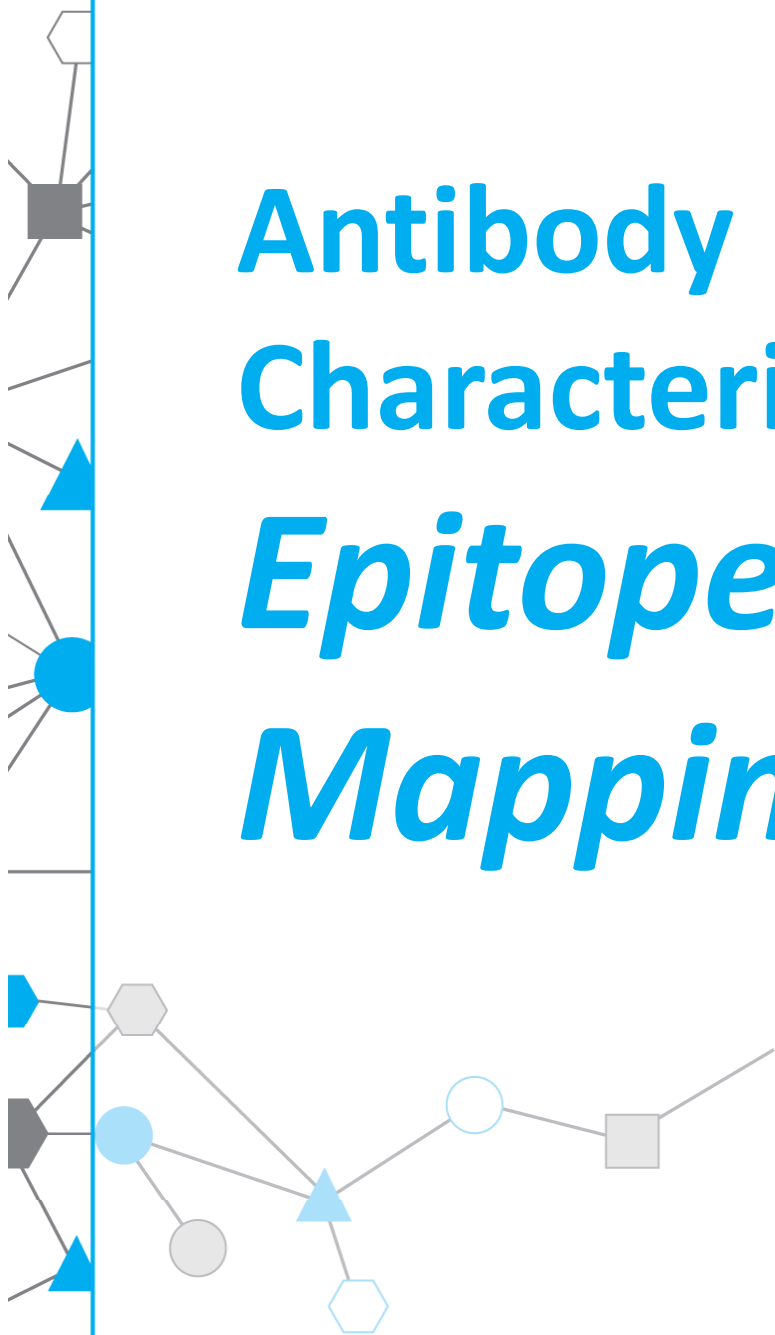
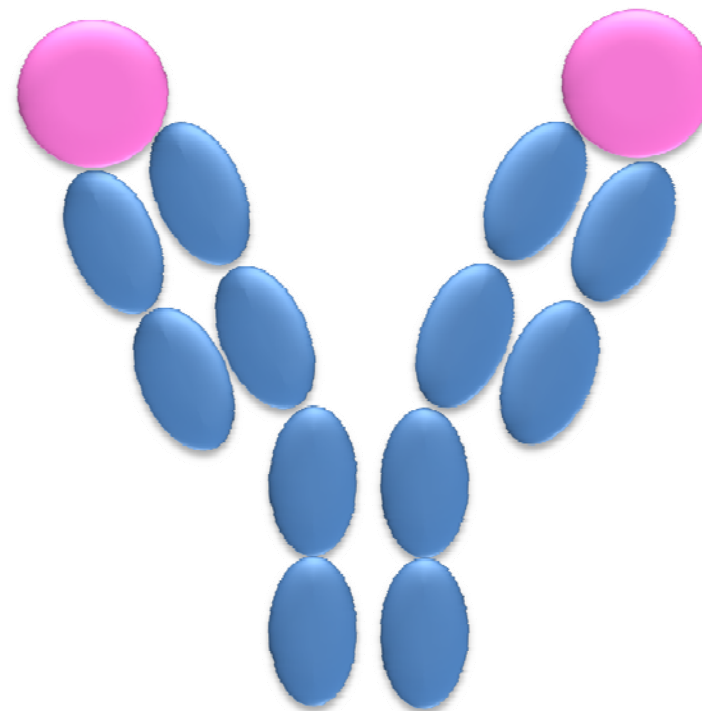
Immuno-complex analysis



Antibody/Antigen interactions



Antibody
Characterization
Epitope
Mapping



Epitope Mapping

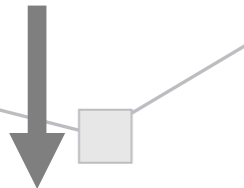
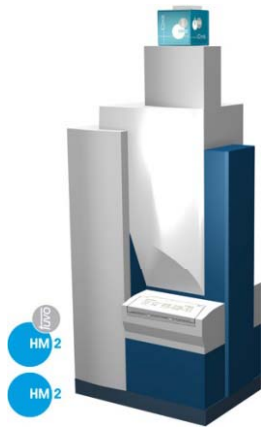
Linear or Conformational?

1

Linear Epitope



Peptide scanning
analyzed by
**High-Mass
MALDI Mass
Spectrometry**



Delivery of the results: 4 weeks

2

Conformational/Discontinue Epitope

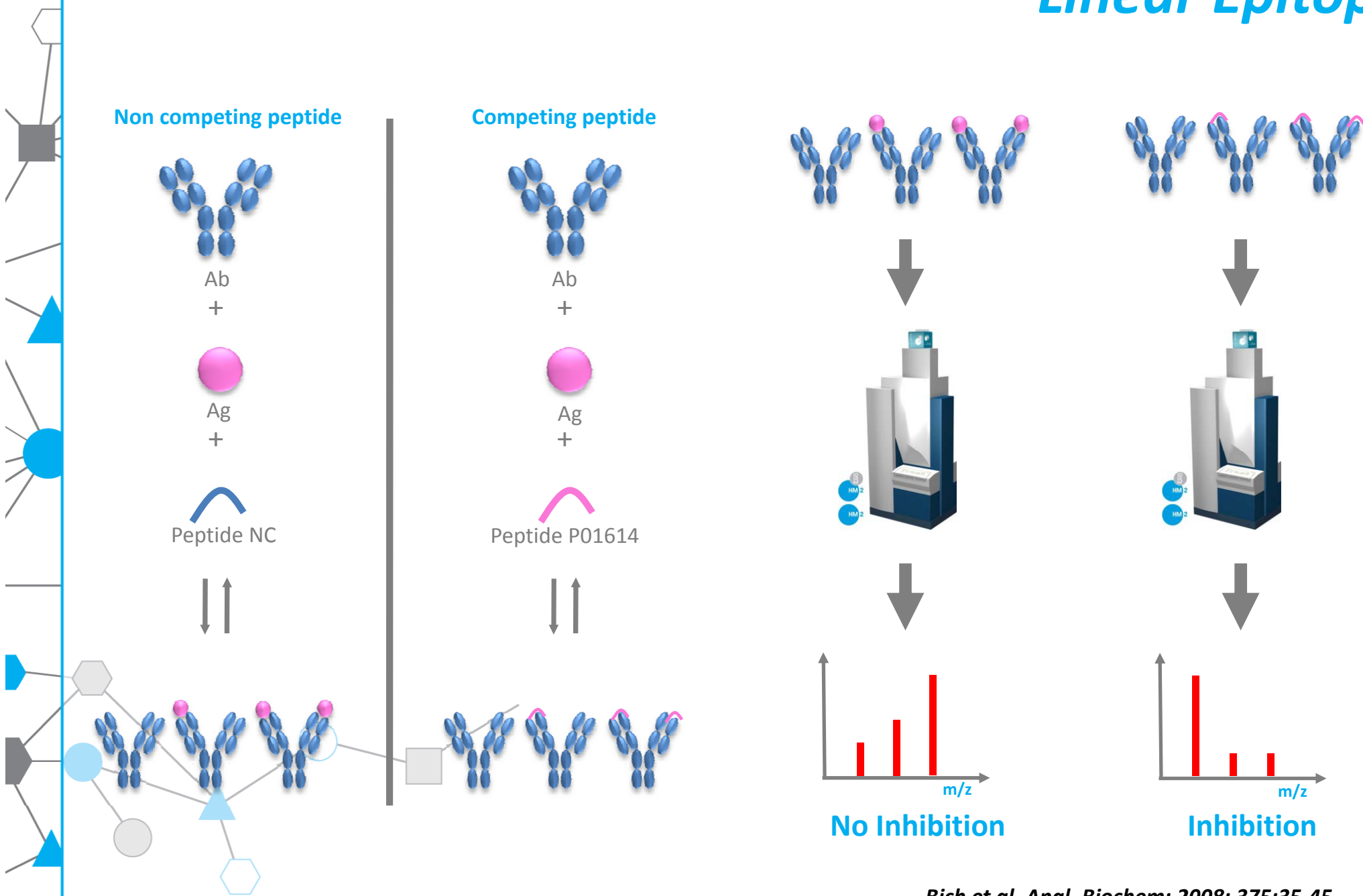


Differential cross-linked
peptide analysis
**High-Mass MALDI Mass
Spectrometry**
In combination with **LC
Orbitrap MS/MS**



Delivery of the results: 8 weeks

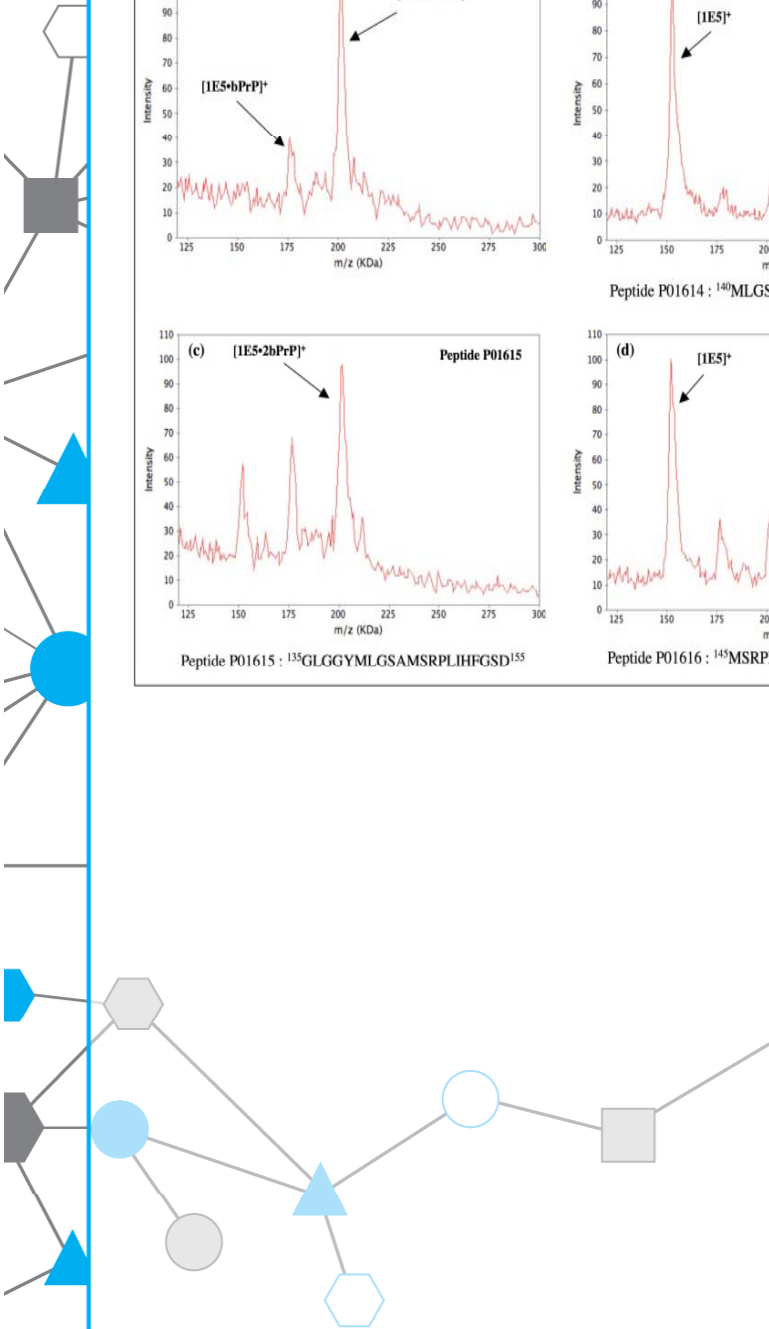
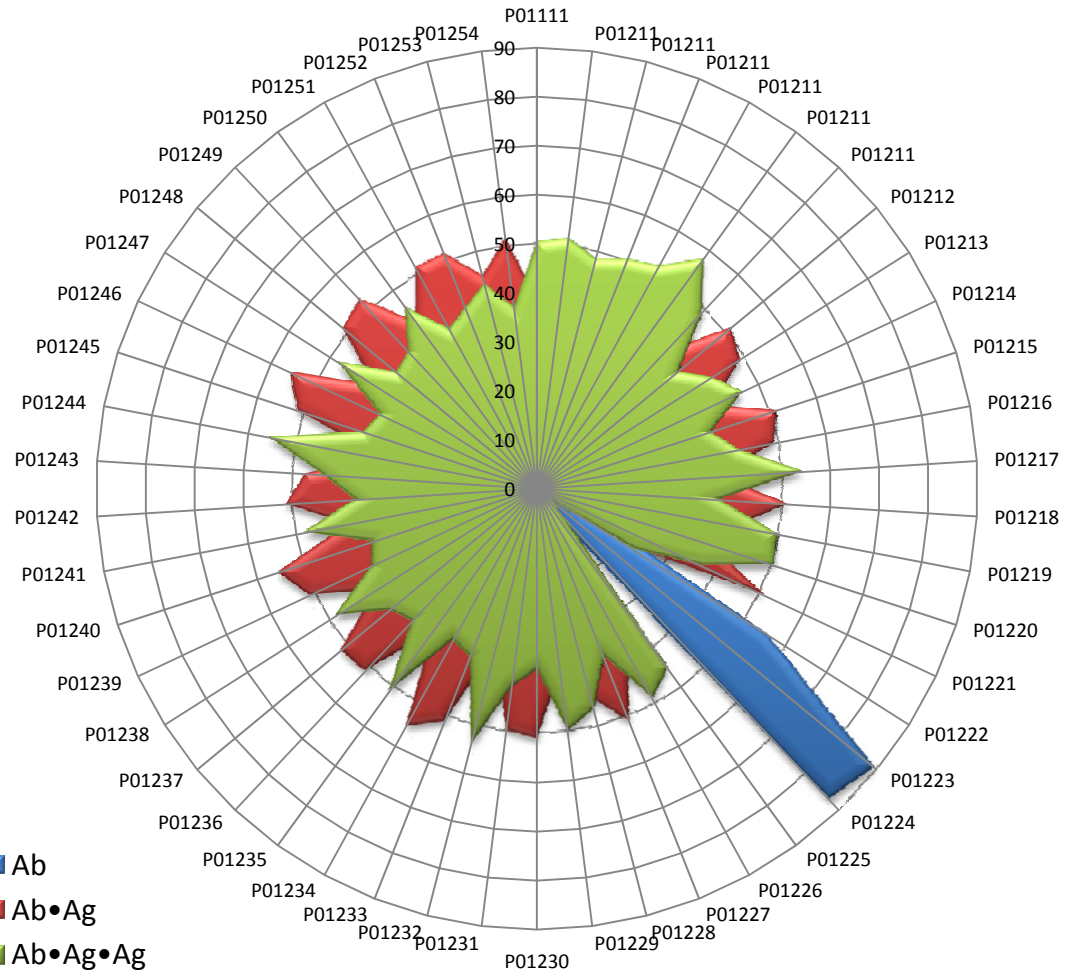
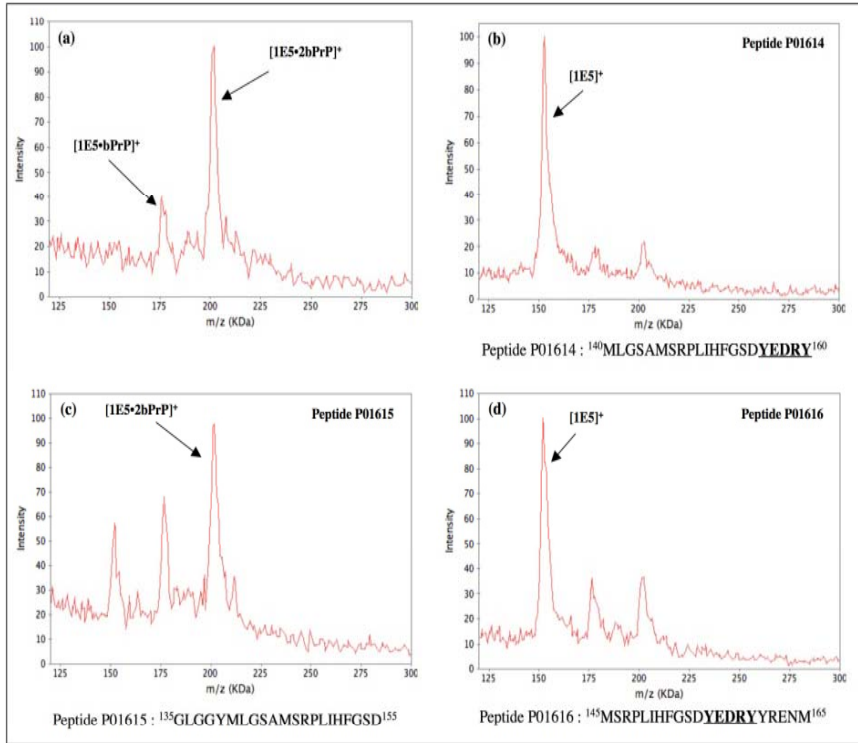
Epitope Mapping Linear Epitope



Antibody Characterization

Linear Epitope Mapping: Case study

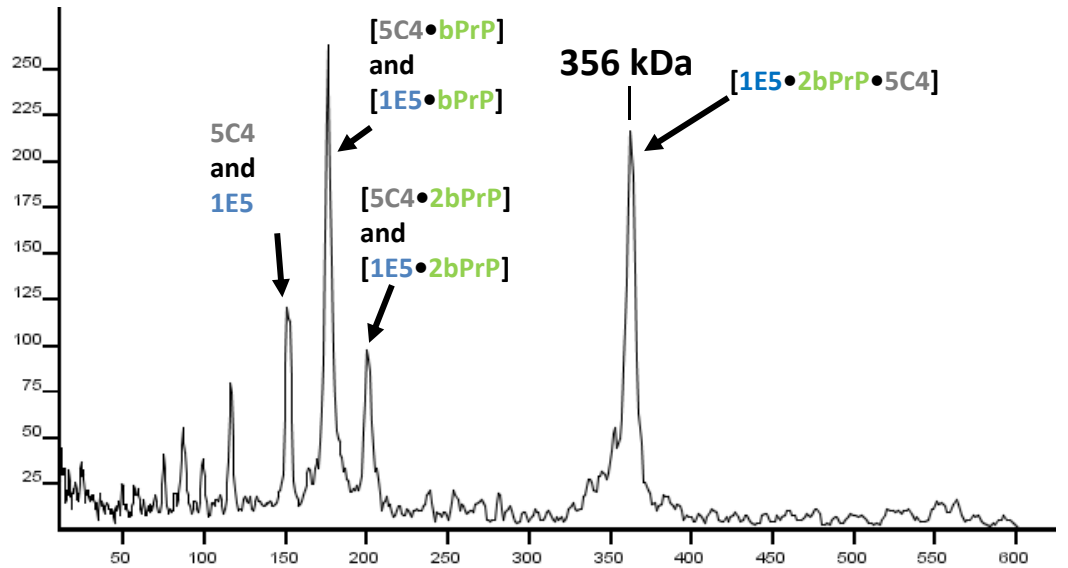
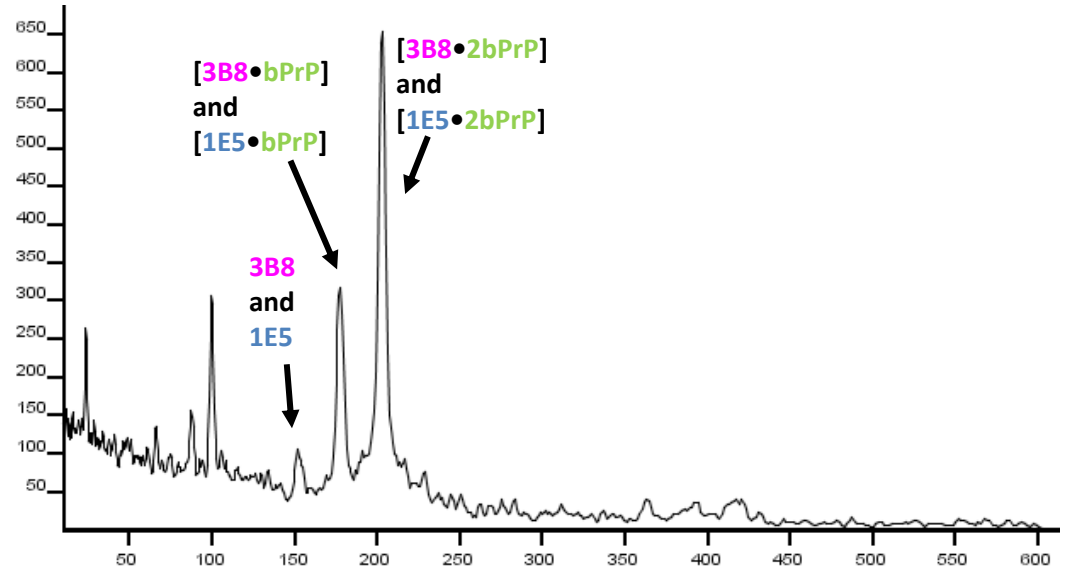
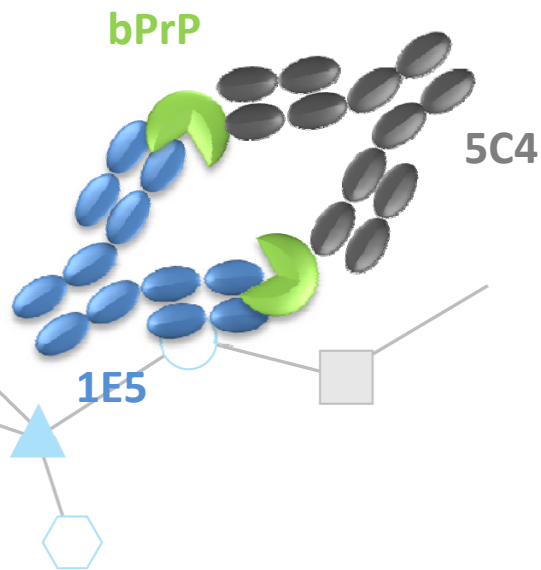
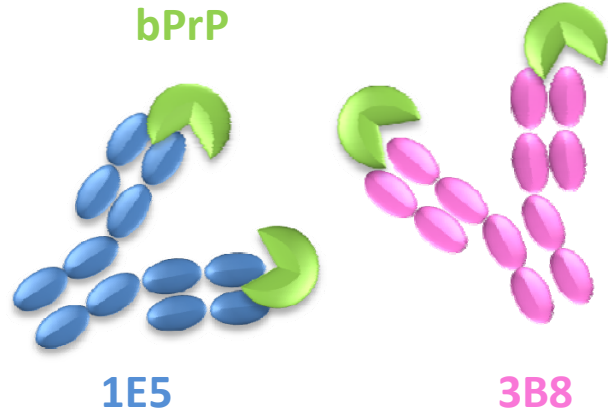
*Analysis of the epitope for the monoclonal antibody anti JAK1 HY011
For Sanofi Pasteur, France*



Antibody Characterization

Epitope Mapping: Sandwich Assay

Use of different monoclonal antibodies to evaluate the epitope region



Epitope Mapping

Conformational/discontinue epitopes



Monoclonal Antibody



Antigen

1 Test conformational/Linear epitope



+



+

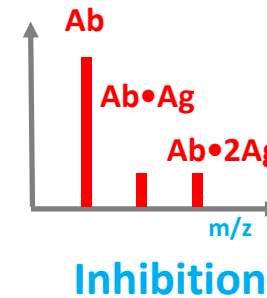


Ab

Ag

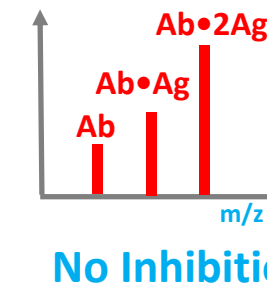
Pepsin

Digest of the Ag



Linear Epitope

Results 4 weeks

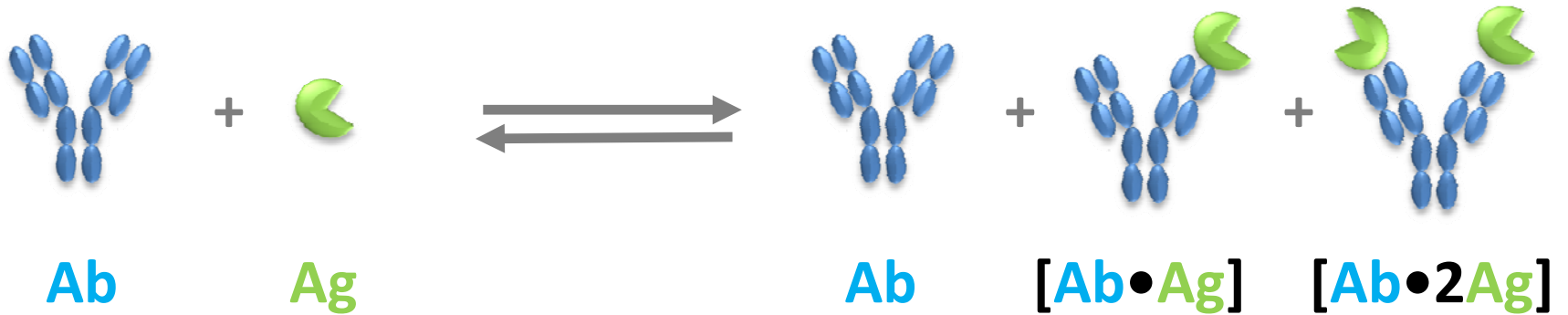


Conformational Epitope

Results 8 weeks

Conformational/discontinue epitopes

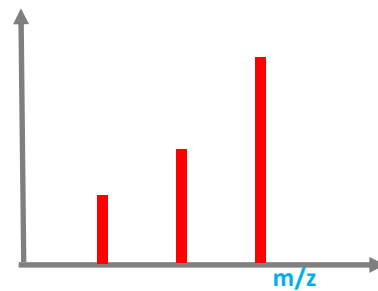
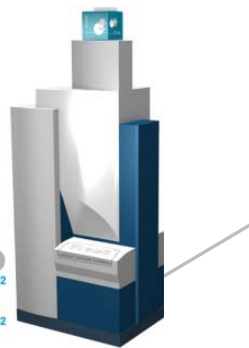
Purification of the cross-linked immuno-complex



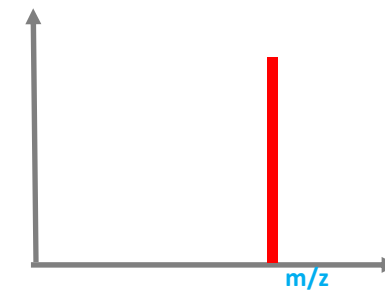
↓ Cross-link
Kd4



Optimisation
High-Mass
Maldi

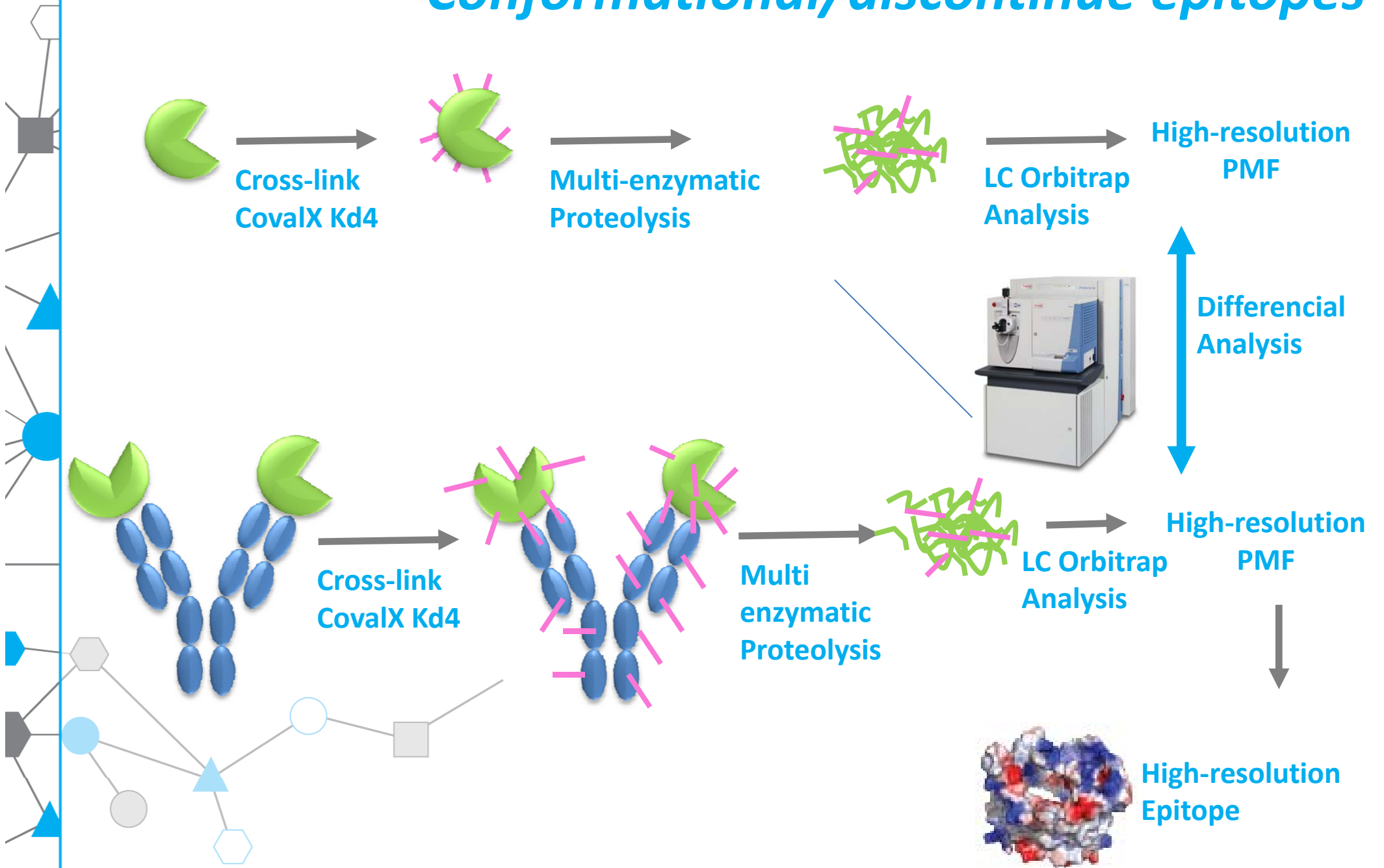


→ SEC



Epitope Mapping

Conformational/discontinuous epitopes



Therapeutic protein aggregates

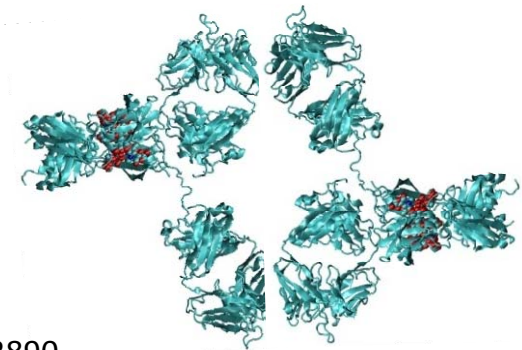


Aggregates formed with:

- Temperature stress
- pH Stress
- Check stress


>5% of aggregation can have major consequences for the patients treated with the therapeutic aggregates:

- Severe allergic reaction: immunogenicity
- Change in activity
- Regulatory guidance: A drug developer is responsible to provide a data-based justification for it's specification of soluble aggregates

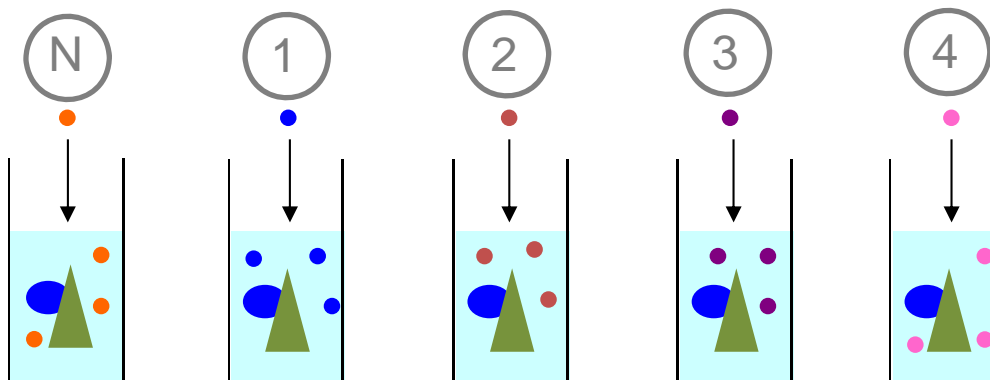


Screening
small molecules
targeting
protein complexes

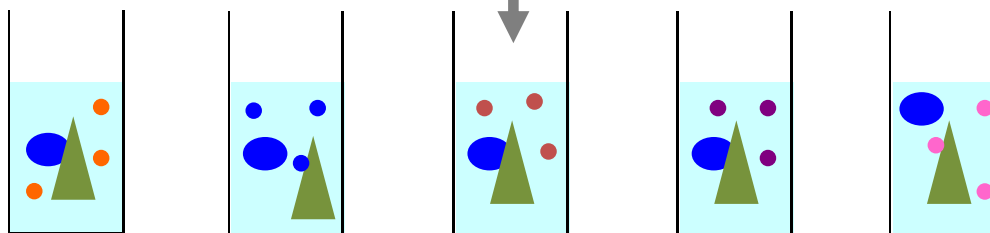
Target:
Protein complex



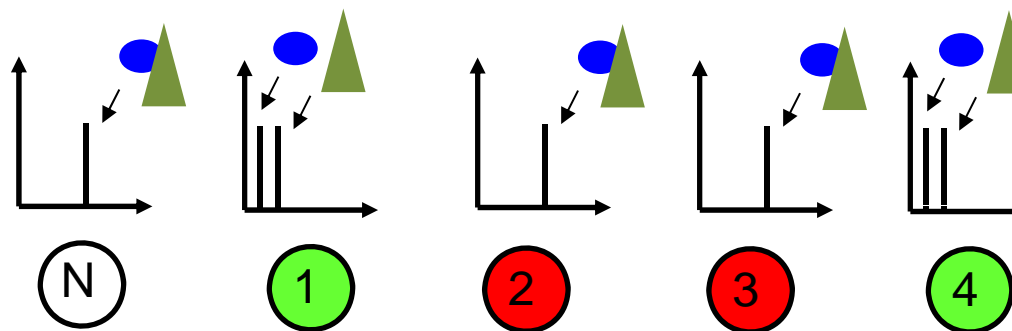
Candidate molecules



Incubation



Cross-linking Chemistry
+ high-mass MALDI MS



Negative control

1 **4** Drug 1&4 disrupt the target

2 **3** Drug 2&3 has no effect on the target

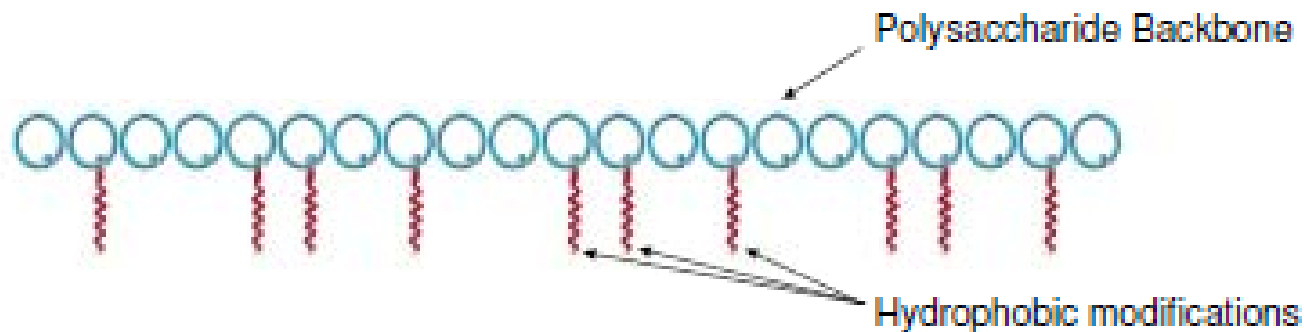
貫通型膜たんぱく質

GPCR

トランスポーター



NVoy Technology – What?

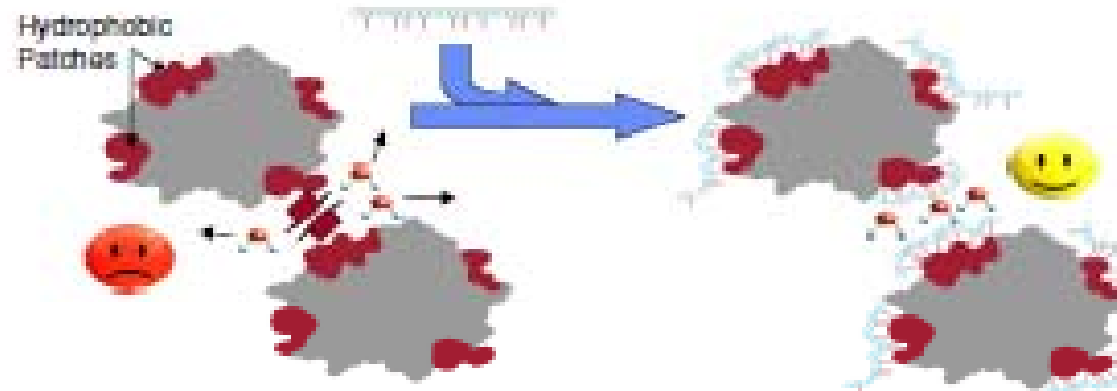


- **Specially chosen carbohydrate backbone with**
 - Linear
 - $M_w = 5\text{kDa}$ ($R_{\text{hyd}} \sim 18\text{kDa}$) \rightarrow Does not access binding sites
 - Multipoint, regio-specific modification \rightarrow Hydrophobic face
 - Uncharged, UV transparent and pH Stable (pH 2 to 11)
- **Dynamic interaction with protein**
 - $k_d = 28 \mu\text{M}$ (GFP) & $12 \mu\text{M}$ (Hexokinase)
 - is easy to remove from protein sample
 - interaction can be controlled

www.expedeon.com



NVoy Technology – How?



- Hydrophobic interaction: cited as the cause for protein aggregation in ~75% of cases (Key Note presentation at PEGS 2007 meeting)
- NVoy associates with surface exposed hydrophobicity and presents the hydrophobic backbone to the solvent.
- Prevents hydrophobic interaction between the target protein and
 - target proteins => prevents aggregation and promotes a heterogeneous sample
 - process surfaces (resin, membranes, etc.) => increasing yields
 - other proteins => increased purity & yield



NVoy Technology - Overview

Increased protein solubility

- Improved protein stability
- Reduced aggregation
- Process at high concentrations
- Retain protein structure and functionality

Strong

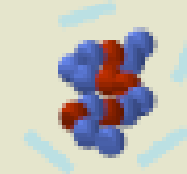


Controllable
Interaction



Controllable
protection

Weak



Protein Purification

- Reversible binding means polymer is removable
- Higher protein yields due to reduced non-specific binding
- Powerful tool for the removal of endotoxin

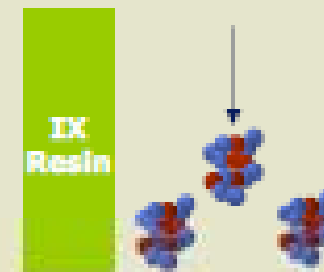
1 - Bind protein
to resin



2 - Wash resin



3 - Elute protein



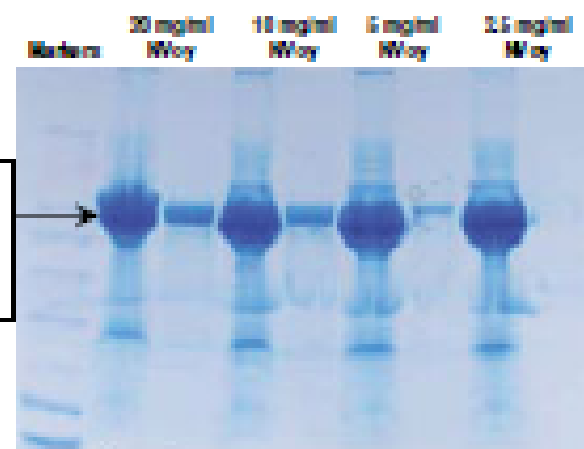


Membrane Proteins

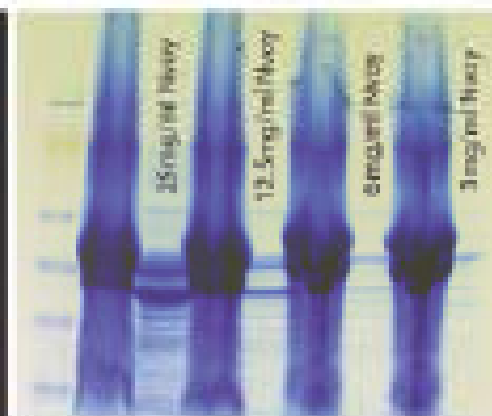
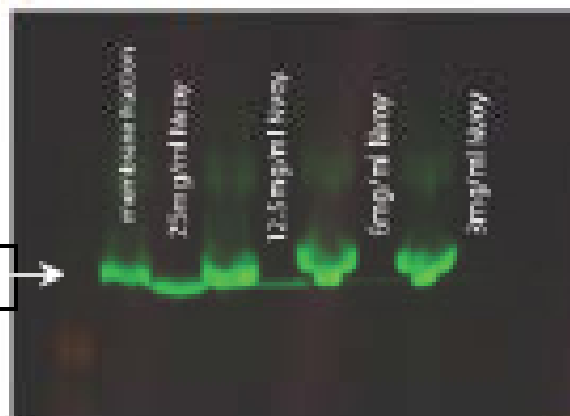
Replacing detergents

- Can be used to completely replace detergents in membrane protein preparation
- Optimal NVoy concentration for extraction is 15 mg/ml although up to 25 mg/ml has been used.

Histidine Kinase Receptor



E. coli IMP tagged with GFP





Protein Purification

Increase Purity & Stability

- Large membrane associated protein (>160kDa) that requires co-factors for activity

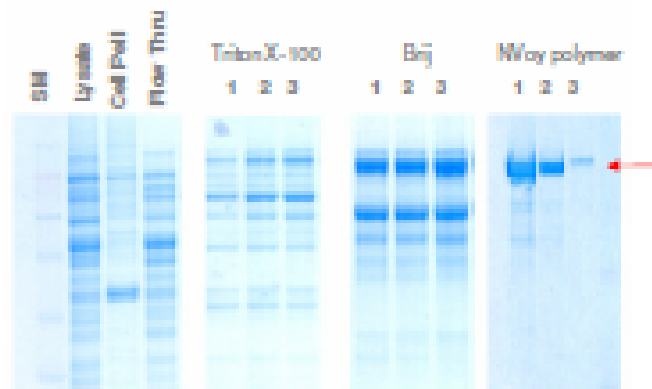
Using Detergents;

results in unstable enzyme preparations containing large number of contaminants

- At 4°C loss of activity within 24h
- At -80°C after one freeze/thaw cycle

Using NVoy Polymer;

- Cleaner protein preparations obtained
- Enzyme activity stable at 4°C for over 4 weeks
- Enzyme activity stable at -80°C for 4 freeze/thaw cycles



- Enzyme processed from *E. coli* lysates
- Cells cultured at 37°C, induced with IPTG and cultured for further 72h.
- Cells harvested then lysed with buffer containing co-factors and NVoy polymer / detergents Lysate clarified by centrifugation and purified on ADP Sepharose using NVoy polymer

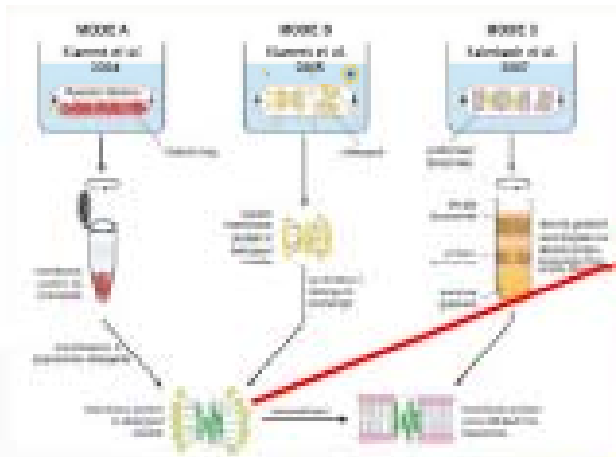
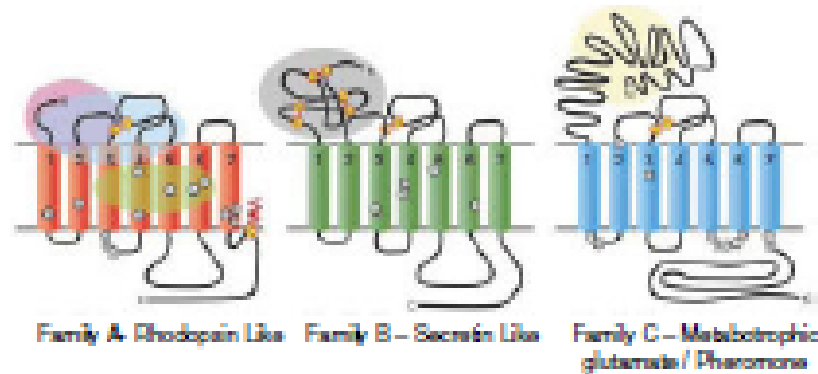
www.expedeon.com



Membrane Proteins

Cell Free Biosynthesis of GPCR's

- Family A and B GPCR's expressed CF Conventionally and with NVoy
- Found that NVoy works as in Mode B
- Concluded that NVoy does not interfere with CF expression
- NVoy enables soluble expression of active GPCR's (>10 nM ligand affinity)





Protein Purification

Increased Yields & Recovery

- Transcription Factor
- Batch Binding to magnetic beads
- Improved binding to the beads
 - less target in Flow Through
 - higher recovery & yields



Data by courtesy of Dr Ana Villegas-Mendez, HumProTher Laboratory, France

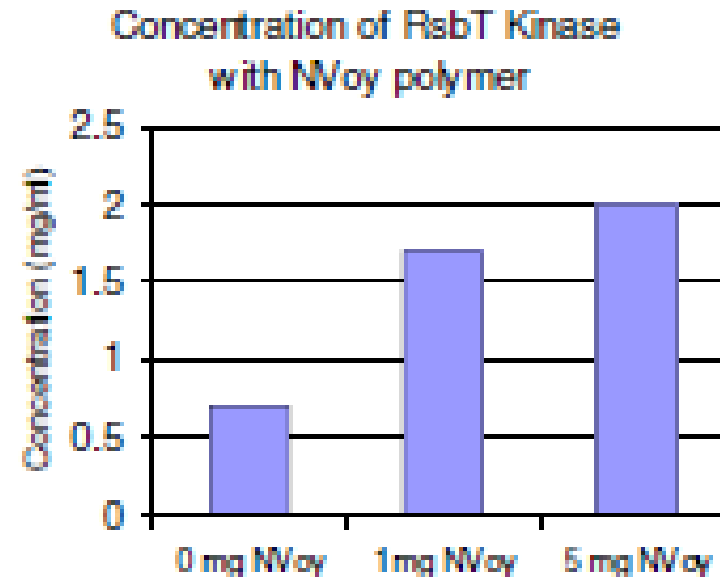
www.expedeon.com



Ultrafiltration

Improving protein recovery

- 4 kDa RsbT protein kinase
 - Purified 2 mg at 0.2 mg/ml
 - Wanted 10x concentrated (i.e. 10 ml -> 1 ml final)
 - Only soluble to 0.7 mg/ml
- + NVoy
 - Dissolved solid NVoy in protein solution
 - Achieved 10x Concentration
 - Effective at low concentration
 - Optimum ratio: 2:1 – 5:1



Data by courtesy of Institute for Cell and Molecular Biosciences Faculty of Medical Sciences Newcastle University Newcastle-upon-Tyne

質量分析法を用いた蛋白質定量、 及び翻訳後修飾プロファイリング解析

川上裕貴
Kawakami Hirotaka

株式会社バイオシス・テクノロジーズ



Resource

Full Dynamic Range Proteome Analysis of *S. cerevisiae* by Targeted Proteomics

Paola Picotti,¹ Bernd Bodenmiller,¹ Lukas N. Mueller,¹ Bruno Domon,¹ and Ruedi Aebersold^{1,2,3,4,*}

¹Institute of Molecular Systems Biology, ETH Zurich, Zurich CH 8093, Switzerland

²Competence Center for Systems Physiology and Metabolic Diseases, Zurich CH 8093, Switzerland

³Institute for Systems Biology, Seattle, WA 98103, USA

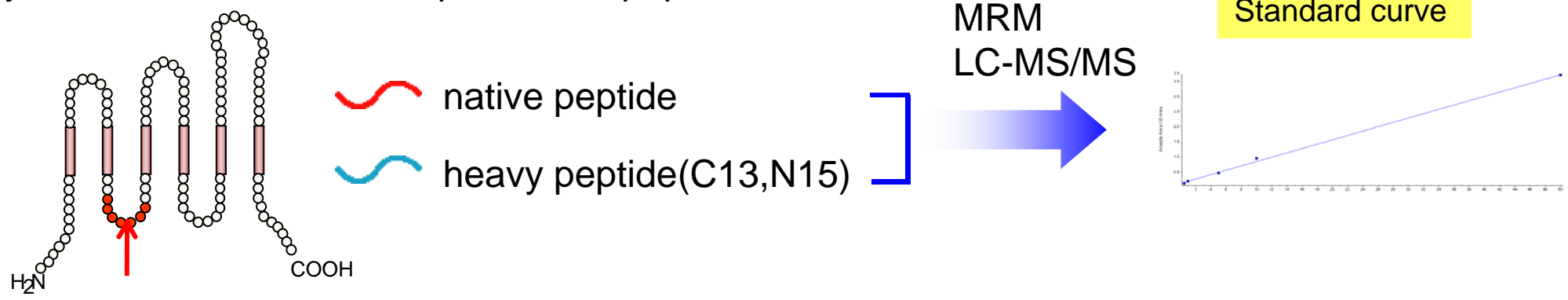
⁴Faculty of Science, University of Zurich, Zurich CH 8057, Switzerland

Target Proteomics

- ・検出器: 三連四重極型質量分析計
(Selected Reaction Monitoring: SRM)
- ・内部標準: 安定同位体標識ペプチド

安定同位体標識ペプチドによる定量分析

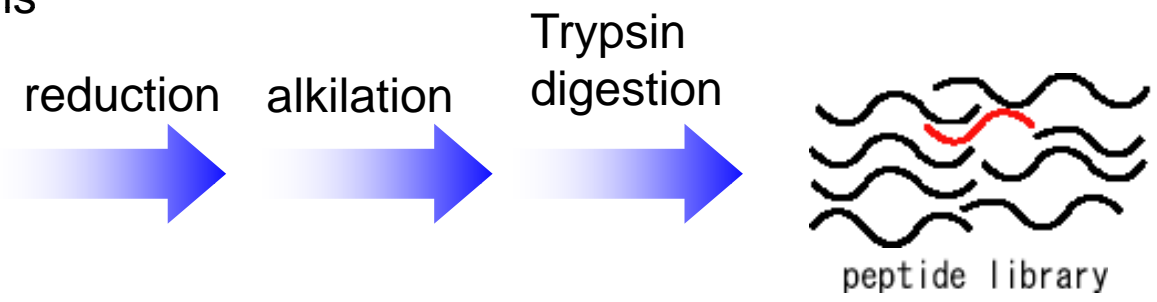
1) Synthesize native and isotope labeled peptide



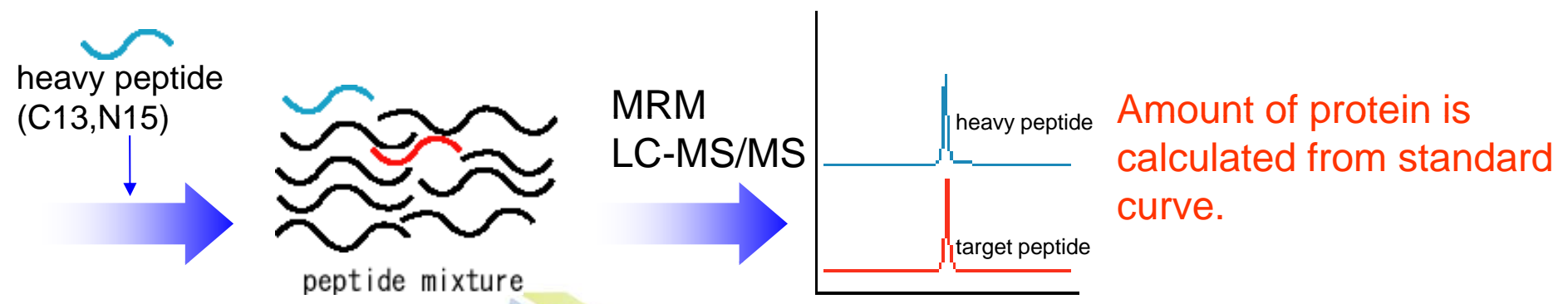
Target protein **selective** trypsin digested peptide

2) Sample preparations

- Materials
- tissues
 - cells



3) MRM LC-MS/MS analysis



標識ペプチドによる定量分析の失敗例

● 標識ペプチド購入前の挫折

選択条件

- ・化学修飾を受ける、Cys, Metを含まない
- ・対象タンパク質に対して特異的である。
- ・翻訳後修飾やSNPを含まない。
- ・Miss Cleavageの可能性のある部分を含まない
-

} 分析に適した
ペプチドが選択できない

● 標識ペプチド購入後の怒り 標識ペプチドの価格： ~20万円/1ペプチド

- ・報告例のない未知の翻訳後修飾を含んでいることが、後から発覚
- ・疎水性が極端に高い、もしくは低いため、LCのカラムから出てこない、すべる。
- ・感度が低く、検出できない。

● 分析後の不安

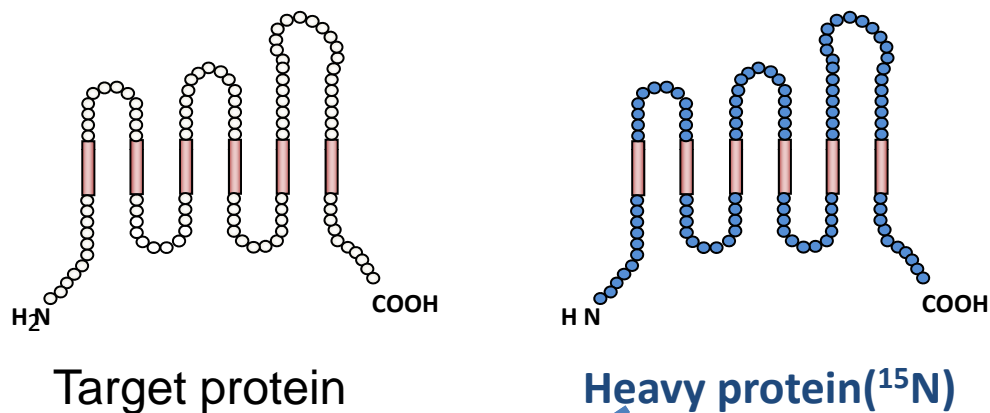
- ・前処理などの試料調製時の試料損失はない？
- ・酵素消化効率は100%？

} 定量値の過小評価

1タンパク質に対し、測定対象ペプチドはいくつ必要ですか？

安定同位体標識タンパク質による定量分析

1) Synthesize native and isotope labeled peptide



全てのアミノ酸を¹⁵N標識

→Cys, Metを含む部分も分析可能

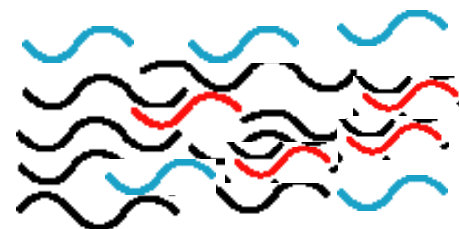
2) Sample preparations

Materials
• tissues
• cells

reduction

alkylation

Trypsin digestion



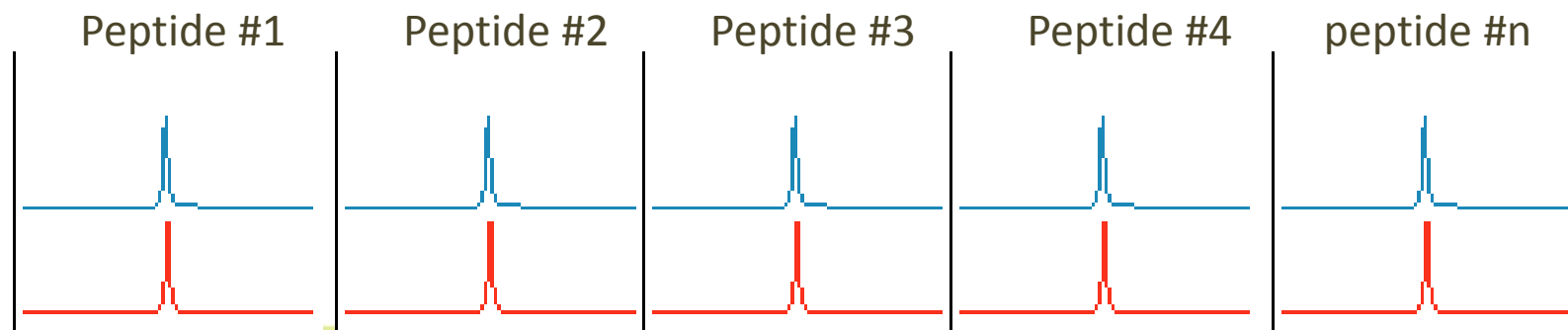
alkylation

前処理による試料損失、酵素消化効率の補正

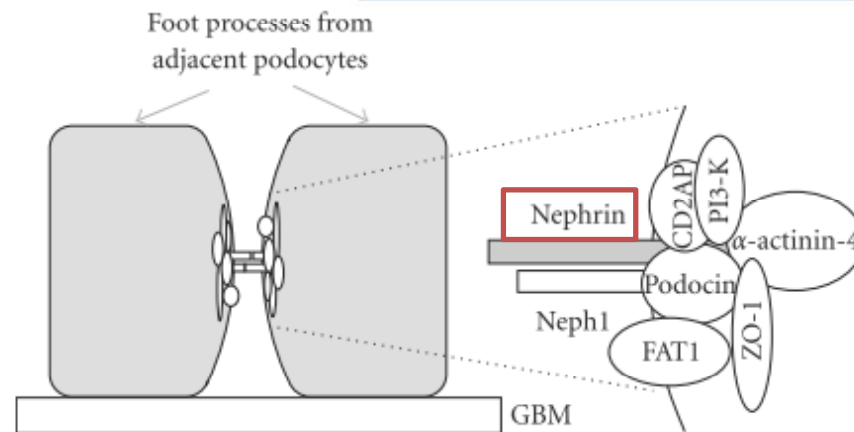
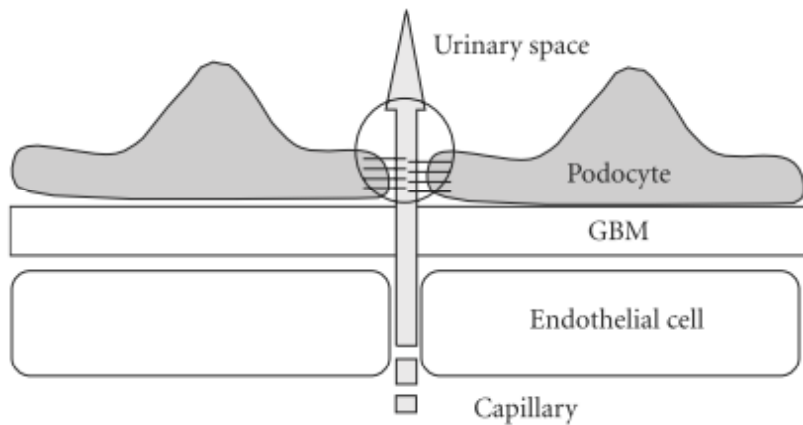
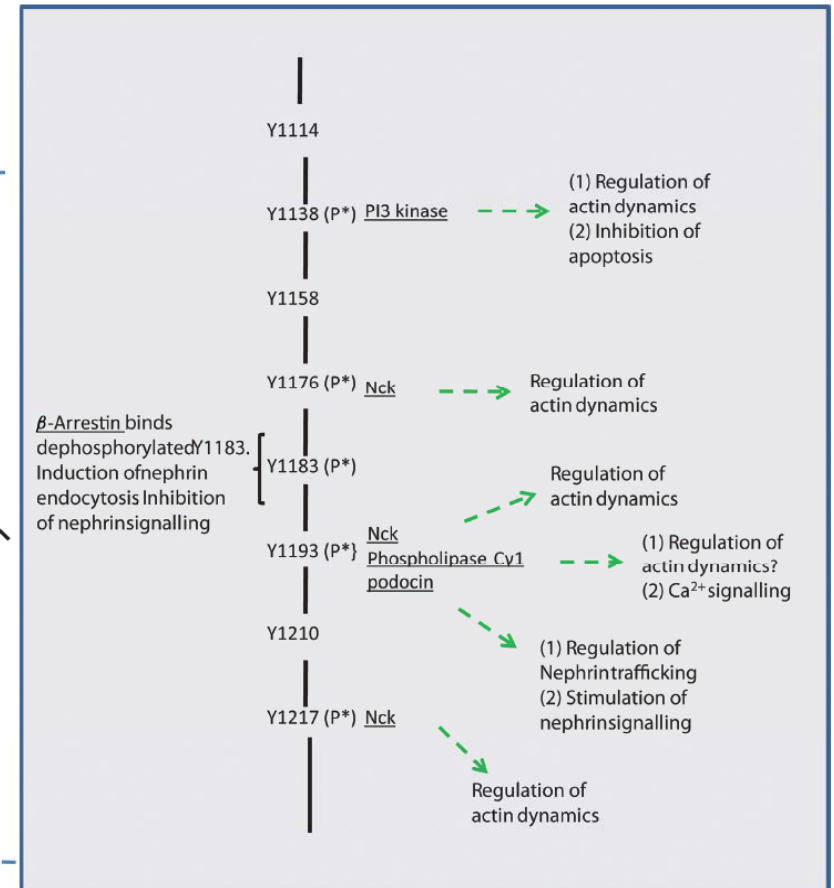
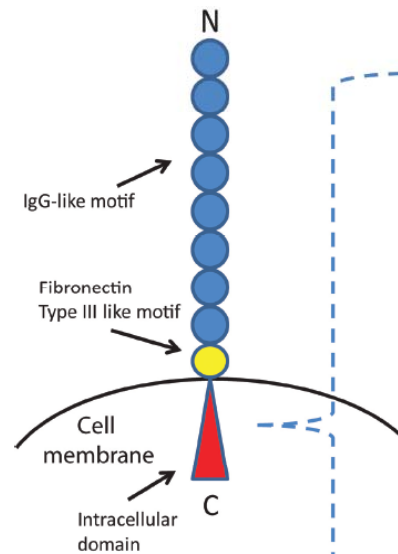
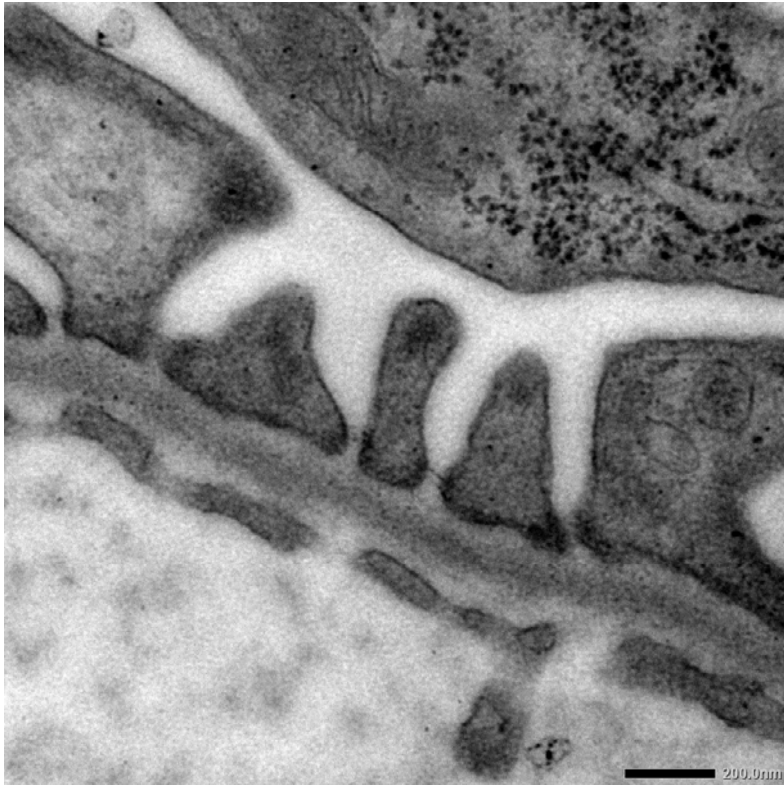
3) MRM LC-MS/MS analysis

複数ペプチドを定量対象とすることが可能

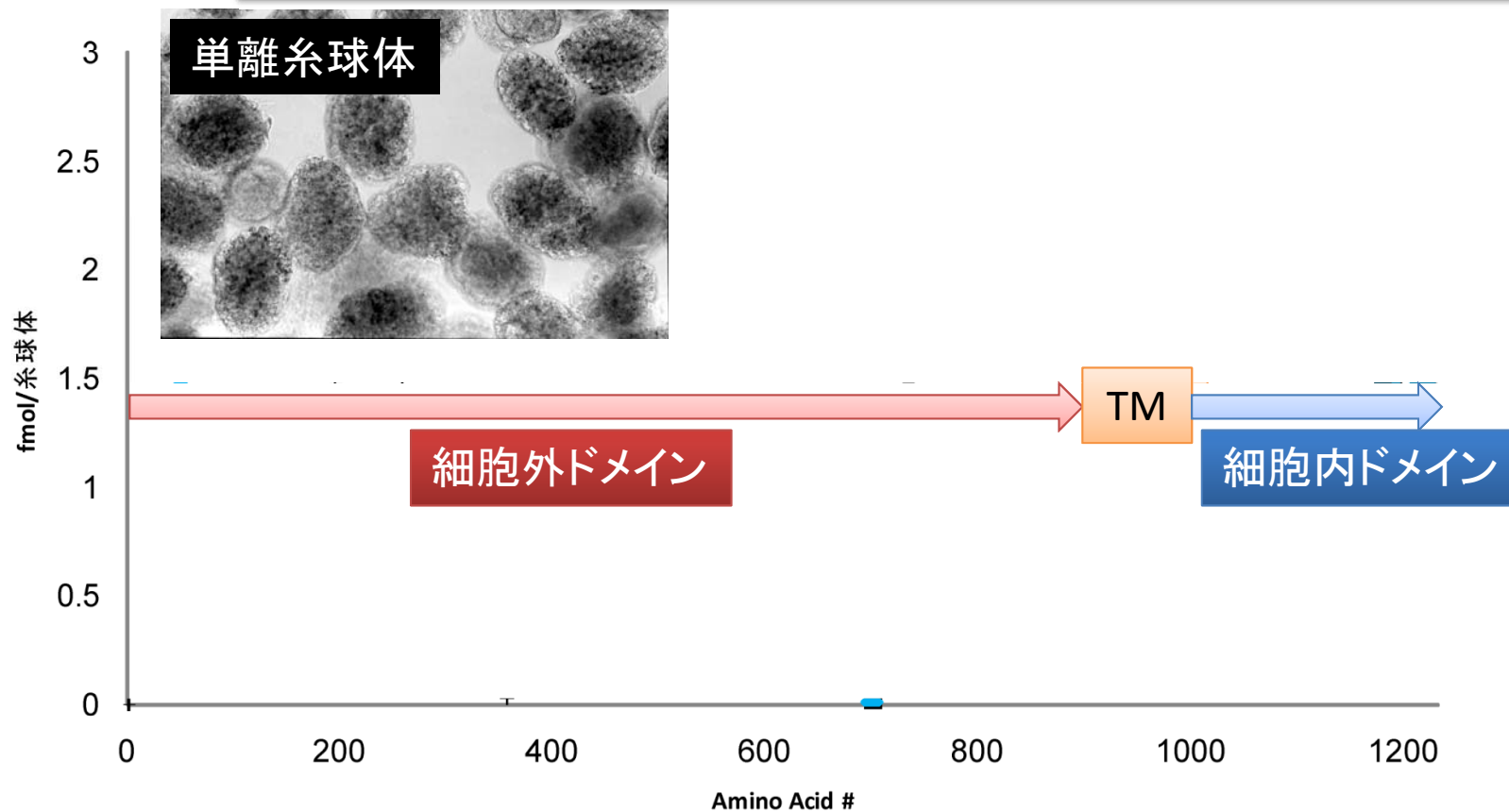
SRM
LC-MS/MS



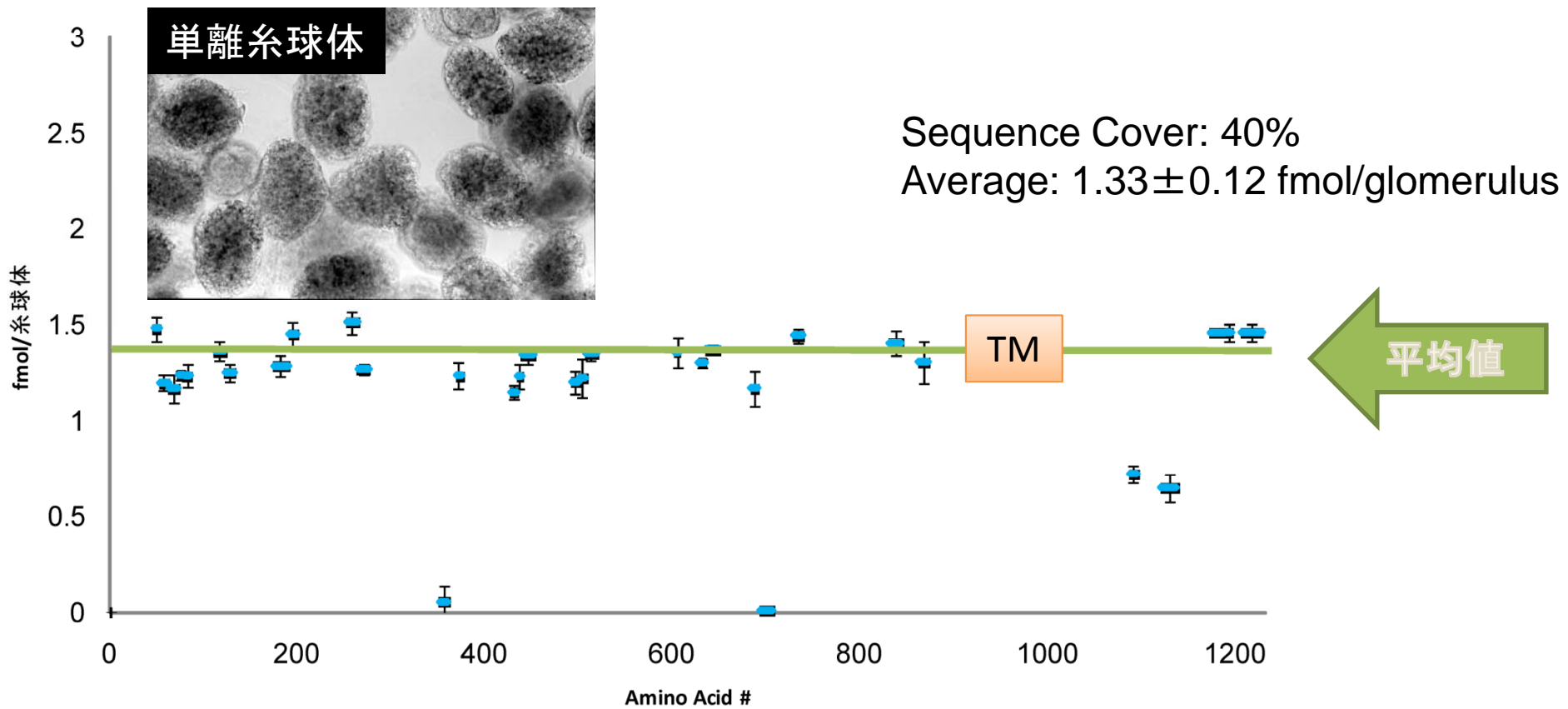
ラット腎臓糸球体足細胞障害モデルへの応用



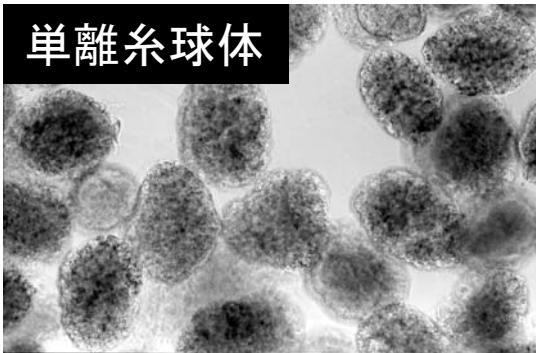
ラット糸球体タンパク質 Nephrinの定量



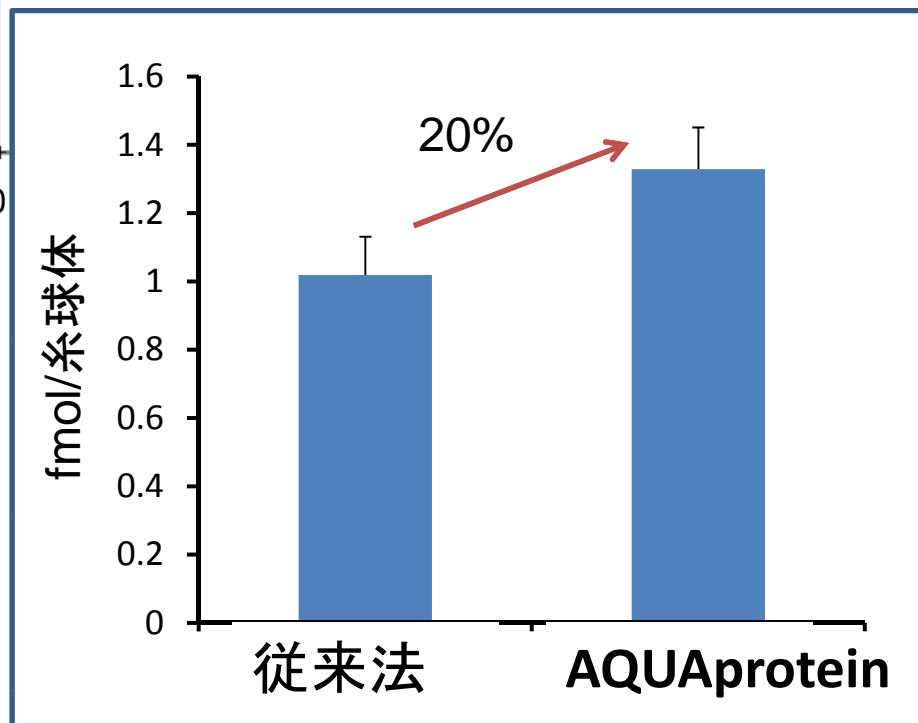
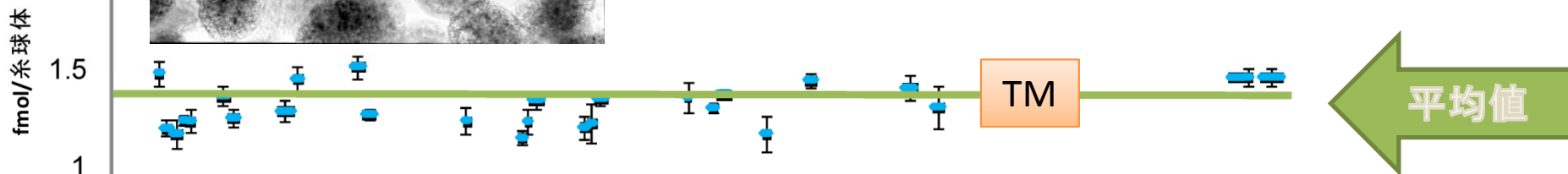
ラット糸球体タンパク質 Nephrinの定量



ラット糸球体タンパク質 Nephrinの定量

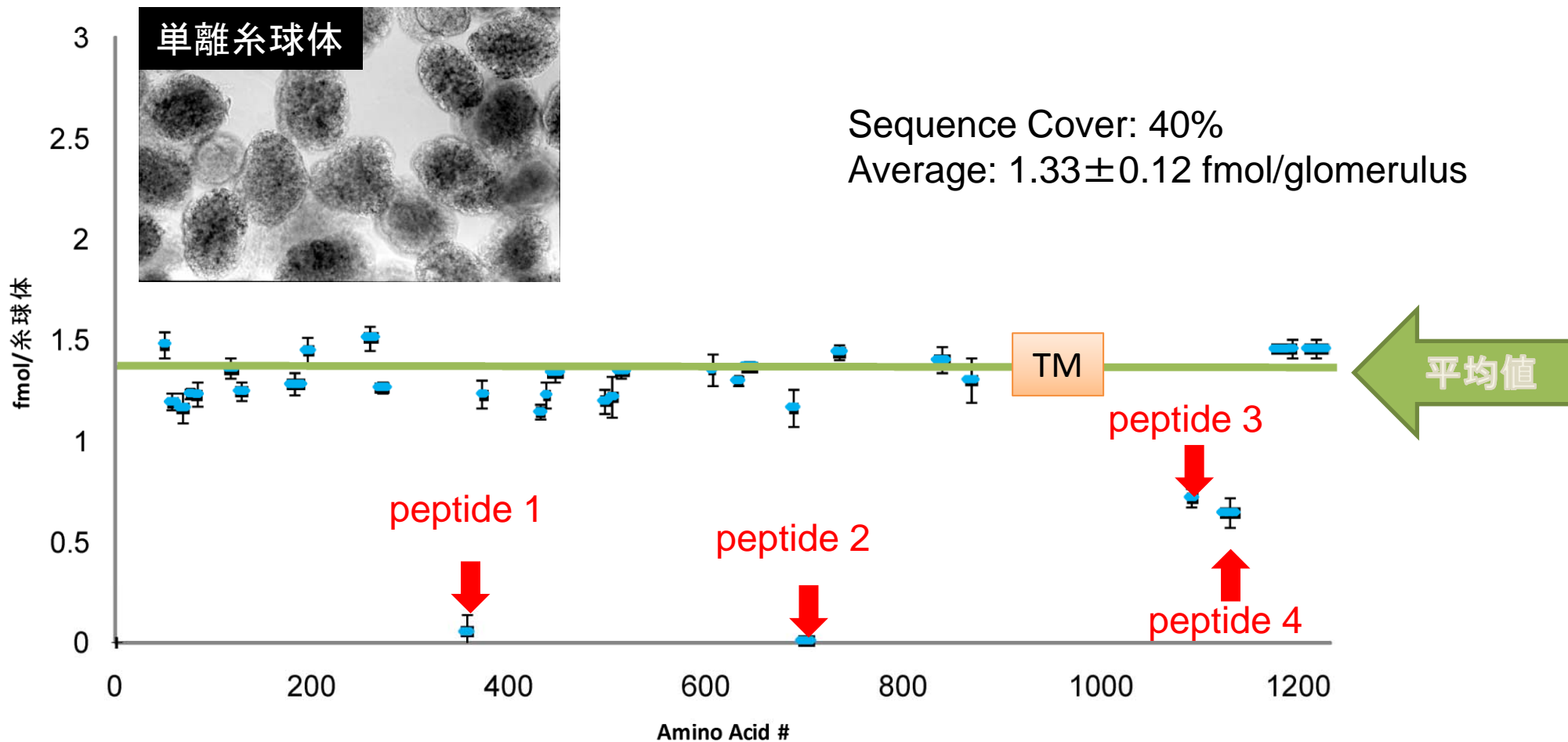


Sequence Cover: 40%
Average: 1.33 ± 0.12 fmol/glomerulus

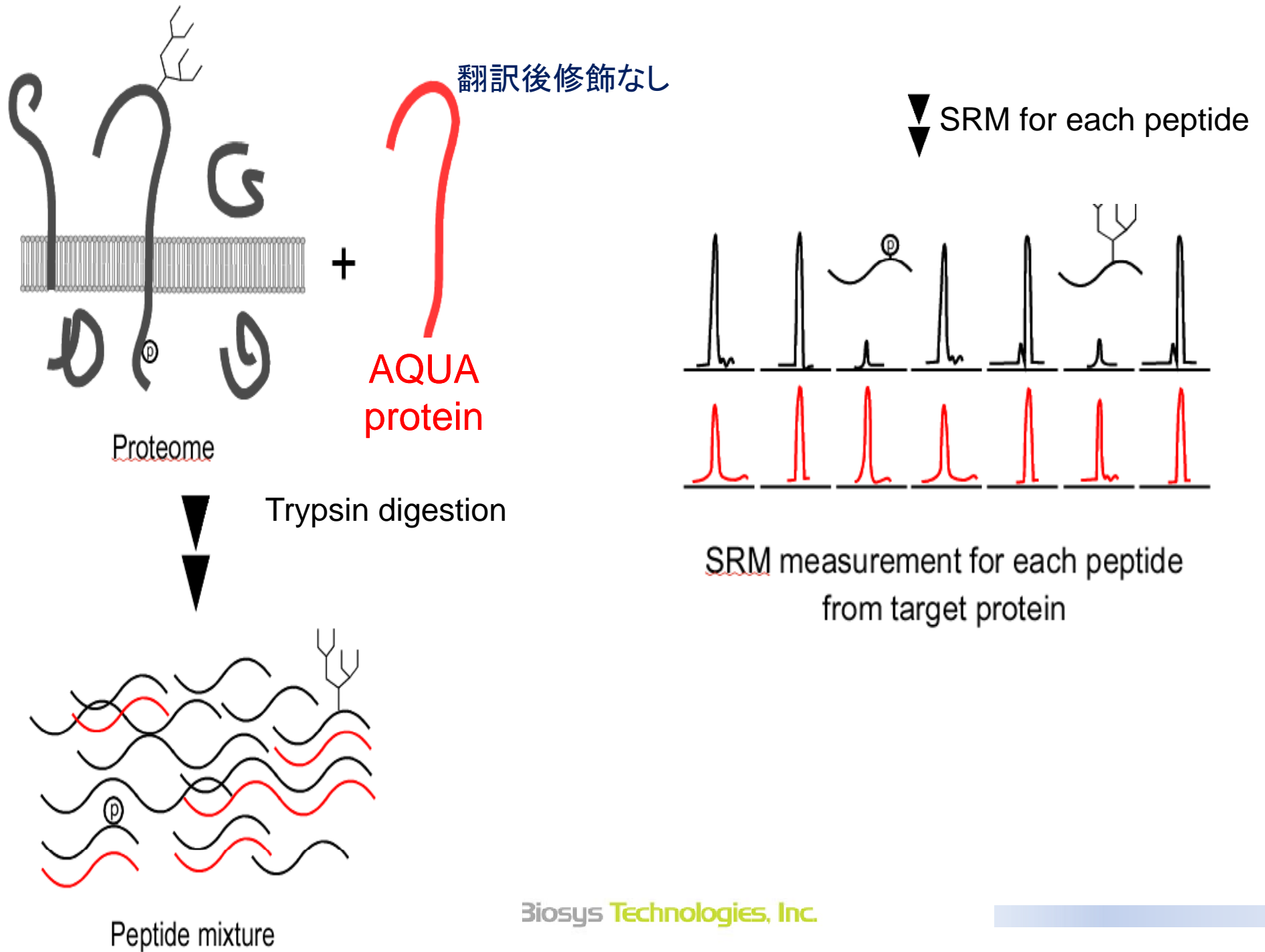


1000 1200

ラット糸球体タンパク質 Nephrinの定量



翻訳後修飾の解析



翻訳後修飾率の解析

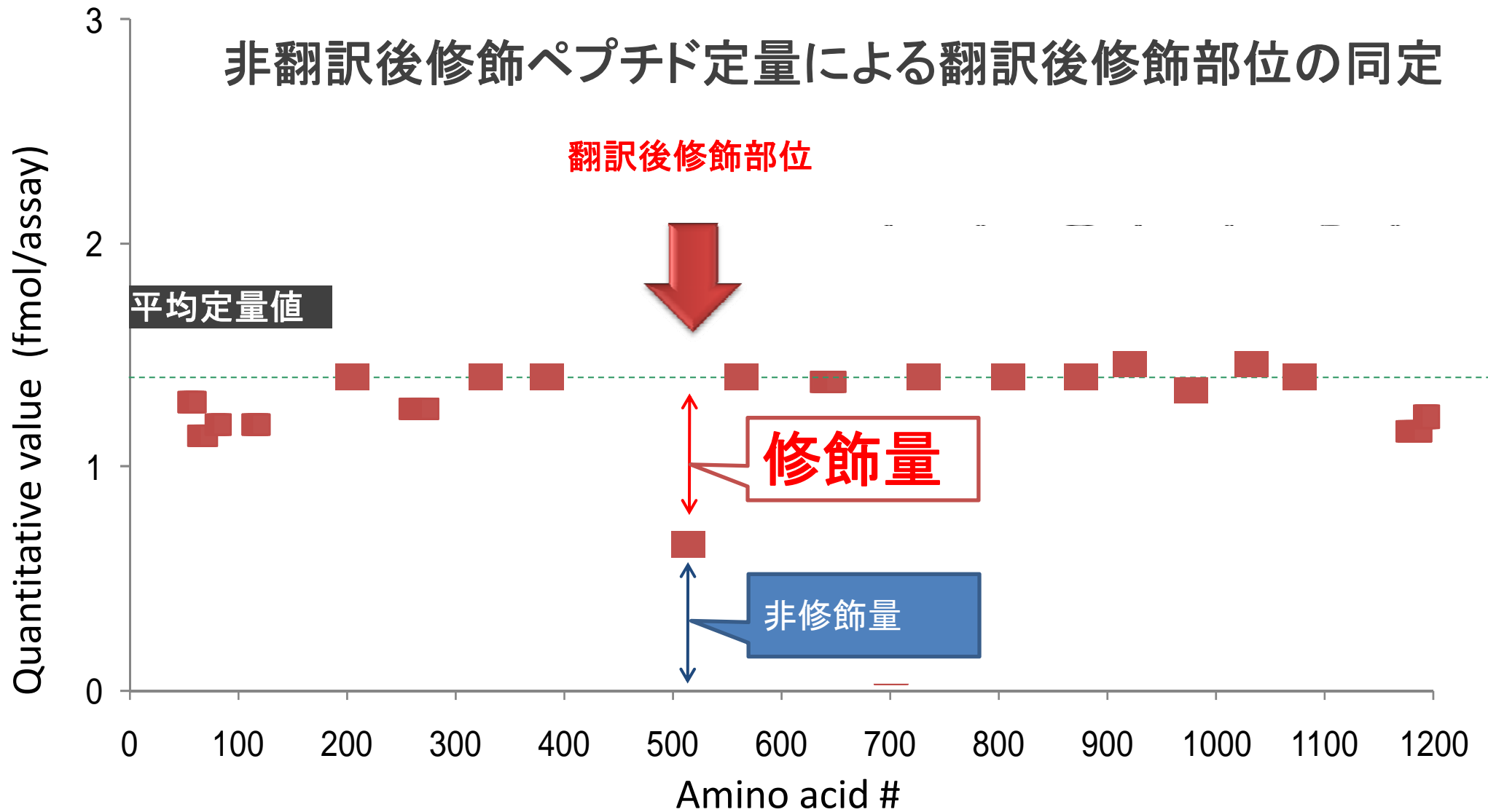
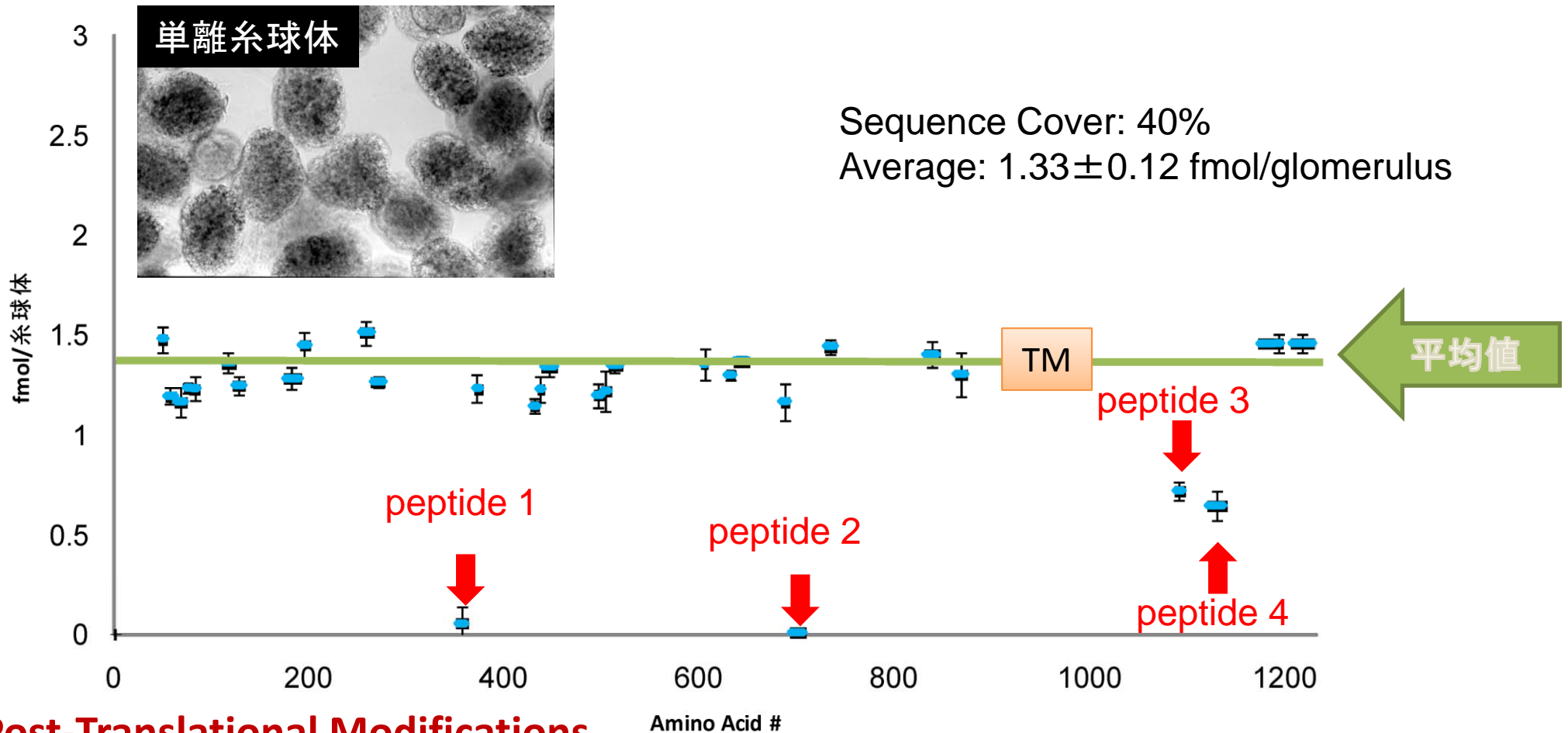


図3. 翻訳後修飾定量の原理



ラット糸球体タンパク質 Nephrinの定量



Post-Translational Modifications

	Type (Swiss-prot)	Modification%	location
peptide1	Glycosylation	96%	Extracellular
peptide2	Glycosylation	99%	Extracellular
peptide3	unknown	46%	Intracellular
peptide4	Phosphorylation	51%	Intracellular



リン酸化修飾の確認

アルカリフォスファターゼを用いた脱リン酸化処理

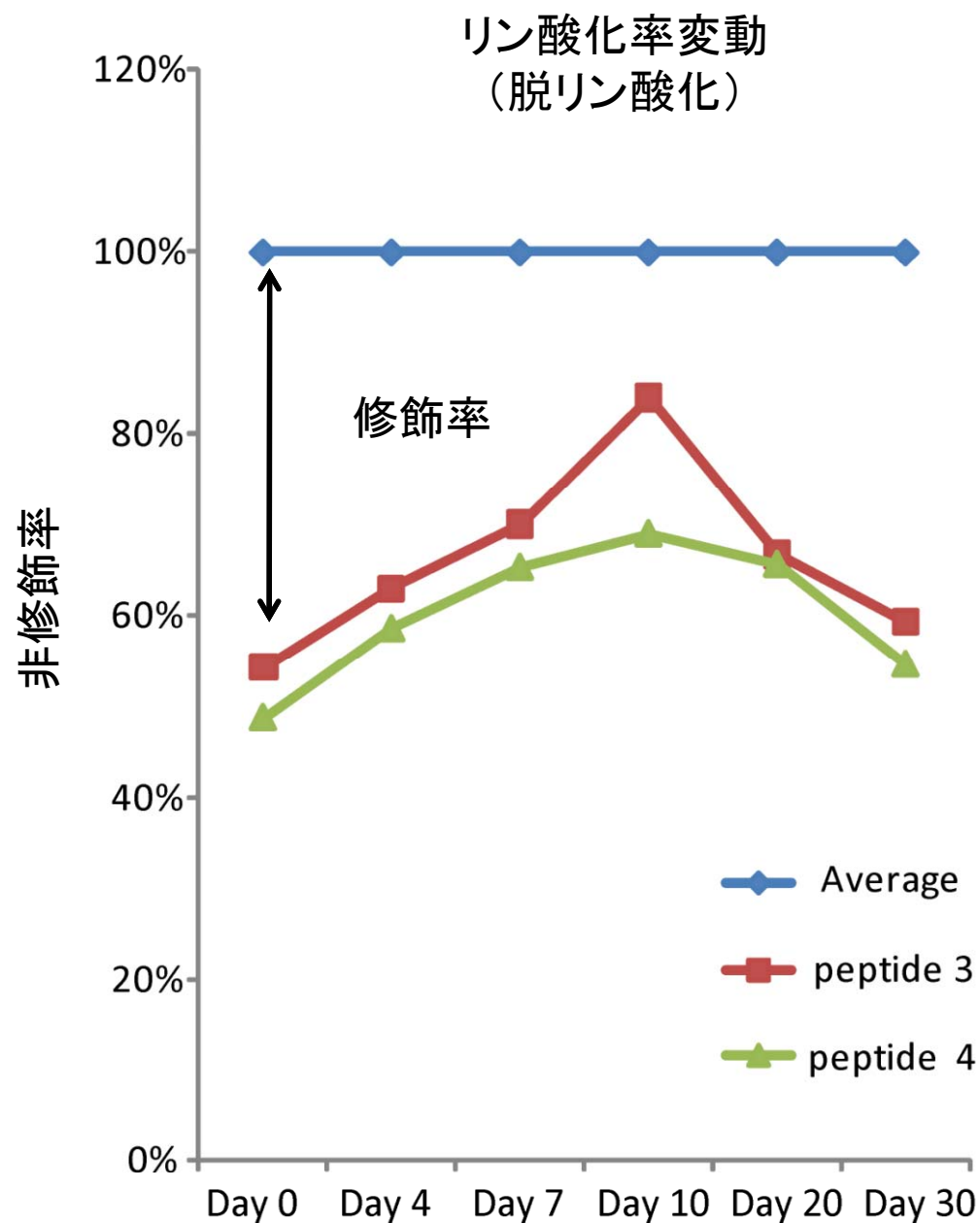
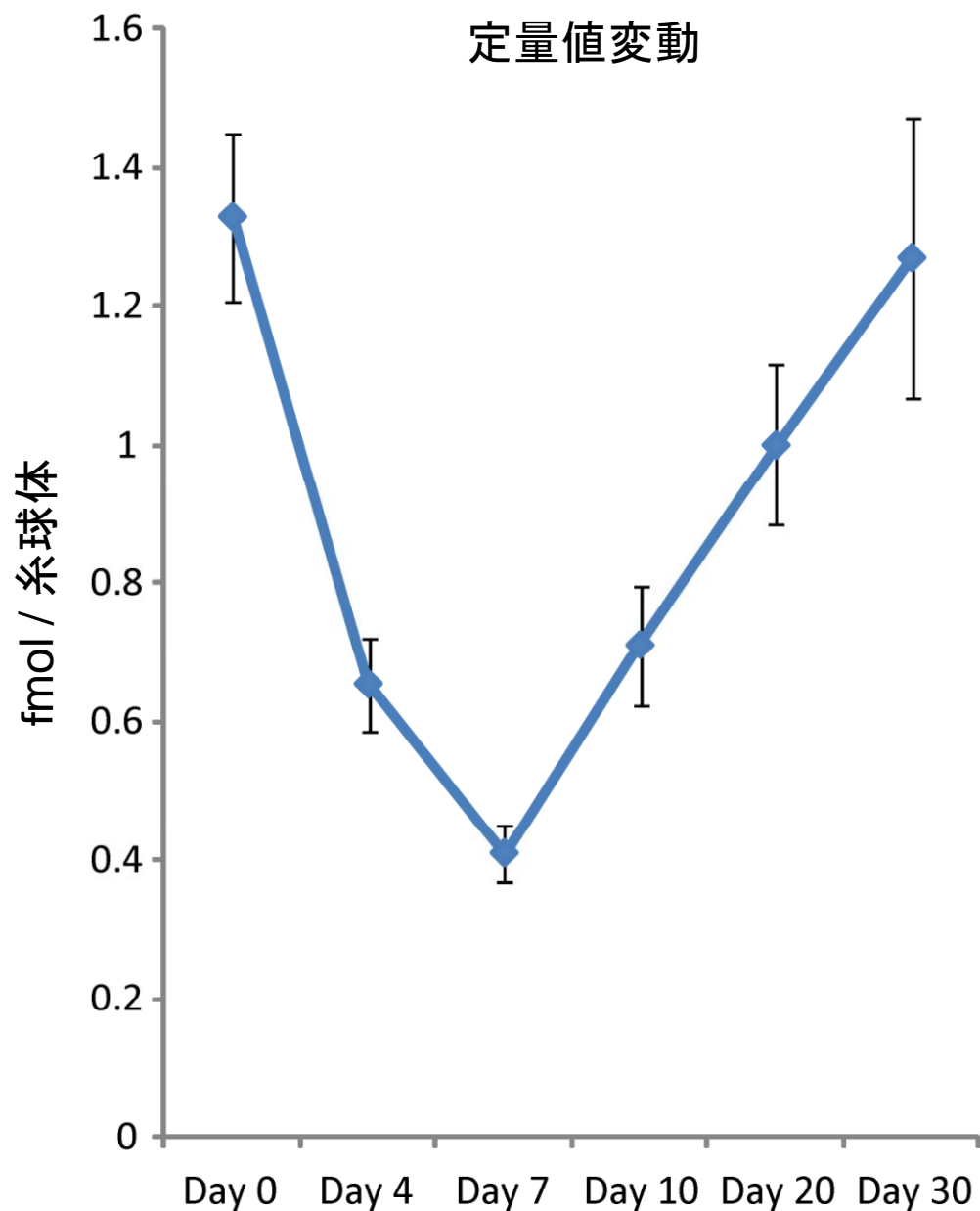
Tryptic peptide	modification	Control	CAIP
		Mean \pm SD	Mean \pm CV
Average	-	1.29 \pm 0.17	1.20 \pm 0.13
Peptide 1	glycosylation	0.01 \pm 0.00	0.01 \pm 0.01
Peptide 3	unknown	0.72 \pm 0.02	1.40 \pm 0.07
Peptide 4	phosphorylation	0.65 \pm 0.05	1.36 \pm 0.06



Peptide 3の新規リン酸化を確認



病態ラットの変動解析



- ・高精度タンパク質定量
- ・全種類の翻訳後修飾部位の同定
- ・翻訳後修飾率の算出

タンパク質機能に直結する情報を提供します。

