The New Dynamic of Bioanalytical Chemistry in Pharmaceutical Companies

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Introduction

- JNJ pharmaceutical companies and our Credo
- Build a scientific career at J&J Bioanalysis
- Bioanalytical support in drug discovery and development
- Basic pharmacokinetics and pharmacodynamics
- Regulations applied in a BA laboratory and LC-MS assay validation
- Bioanalytical LC-MS workflow
- Examples of bioanalytical support
  - Drug candidates and their metabolites
  - Biomarkers
  - Novel scaffolds

Janssen Pharmaceutical Companies

- Cardiovascular and metabolic disorders
- Immunology
- Infectious diseases and vaccines
- Neuroscience
- Oncology

http://www.careers.jnj.com/ : career opportunities (include interns and co-ops)

https://www.jnj.com/ : J&J companies, products and history

Our Credo (Johnson & Johnson)

We believe our first responsibility is to the doctors, nurses and patients, the mothers and fathers and all others who use our products and services. In meeting their needs everything we do must be of high quality. We must constantly strive to reduce our costs in order to maintain reasonable prices. Customers' orders must be serviced promptly and accurately. Our suppliers and distributors must have an opportunity to make a fair profit.

We are responsible to our employees, the men and women who work with us throughout the world. Everyone must be considered as an individual. We must respect their dignity and recognize their merit. They must have a sense of security in their jobs. Compensation must be fair and adequate, and working conditions clean, orderly and safe. We must be mindful of ways to help our employees fulfill their family responsibilities. Employees must feel free to make suggestions and complaints. There must be equal opportunity for employment, development and advancement for those qualified. We must provide competent management, and their actions must be just and ethical.

We are responsible to the communities in which we live and work and to the world community as well. We must be good citizens—support good works and charities and bear our fair share of taxes. We must encourage civic improvements and better health and education. We must maintain the property we are privileged to use, protecting the environment and natural resources.

Our final responsibility is to our stockholders. Business must make a sound profit. We must experiment with new ideas. Research must be continued, innovative programs developed and mistakes paid for. New equipment must be purchased, new facilities provided and new products launched. Reserves must be created to provide for adverse times. When we operate according to these principles, the stockholders should realize a fair return.

Robert Wood Johnson, former chairman from 1932 to 1963 and a member of the Company's founding family, crafted Our Credo himself in 1943, just before Johnson & Johnson became a publicly traded company.

Build a Scientific Career at J&J Bioanalysis

- **Excel Your Work**
  - Keep innovation and publications
  - Volunteer high visibility projects and special assignments
  - Don’t ignore routine work
- **Develop Cross-function Knowledge**
  - Be a team player and build professional relationship
  - Find a mentor (could be external too)
  - Attend internal meetings and are actively engaged
- **Have a Healthy Work-life Balance**
  - Participate scientific community service
  - Stay positive and take care of family

Bioanalytical Support in Drug Discovery and Development

discovery	preclinical	clinical

• In vitro
  • Plasma protein binding
  • Transporter
  • Inhibition-induction
  • Metabolic stability
  • Plasma-blood distribution
 	
• In vivo
  • PK/PD
  • Salt form selection
  • Formulation
  • Dose range finding
  • Tissue distribution
	• Short term (2wk, 4 wk) TOX
	• Long Term (3mts +) TOX
	• Reproductive TOX
	• Carcinoma studies
	• Micronucleus studies
	• Animal ADME
	• Bioavailability (IV/ORAL)
		• SAD, MAD
		• Metabolite assessment (MIST)
		• Food effect
		• Drug-drug interaction
		• Comparator study
		• Human ADME
		• Population PK
		• Special population study (renal impaired, pediatric, etc)
		• Bioequivalence

drug-like?	known liabilities?	superior efficacy ?

The Change of Landscape of Bioanalysis

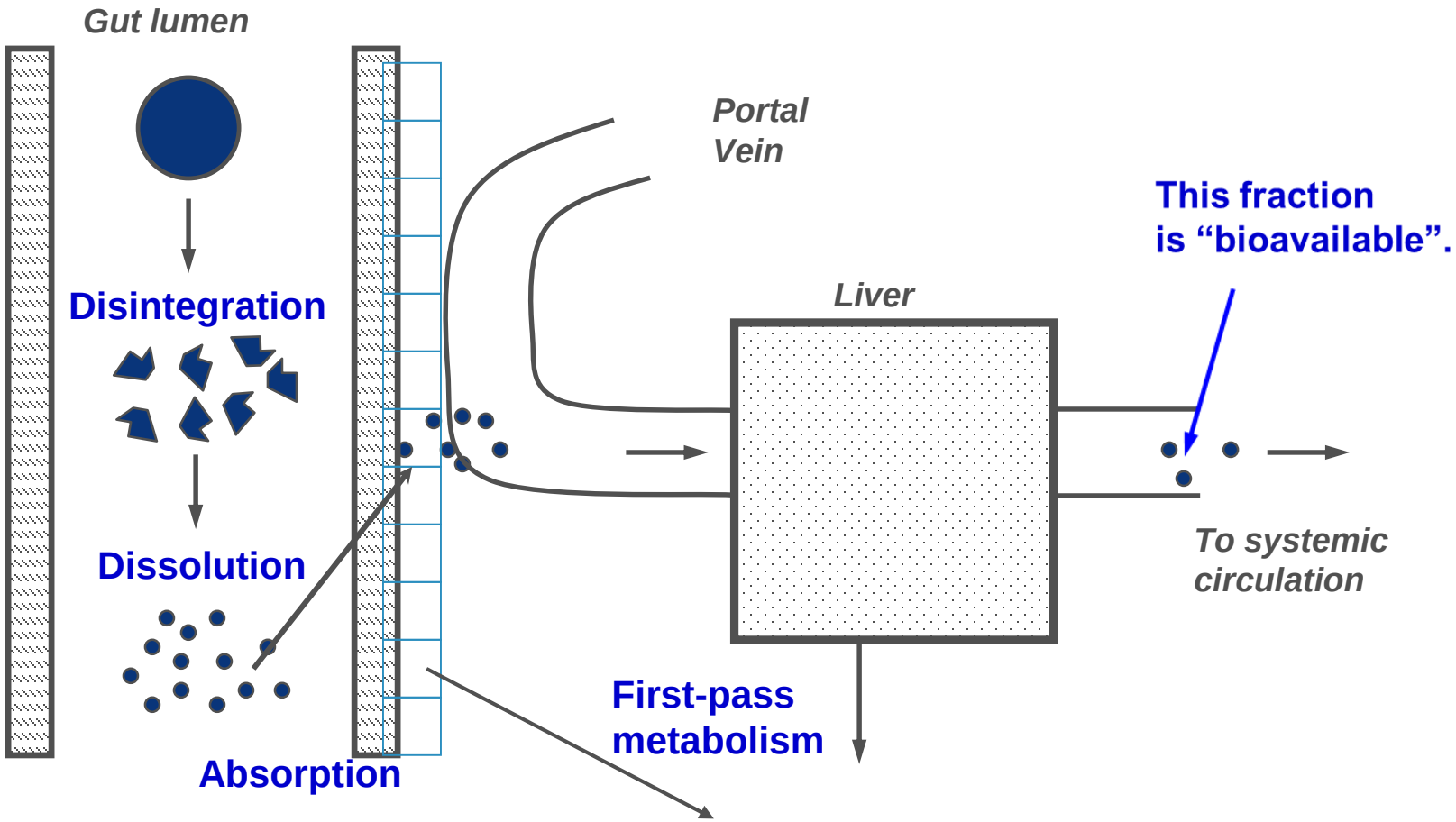
2009

- **GLP and clinical bioanalytical support internally**
- **Outsource some non-pivotal bioanalytical work**
- **Dedicated sample management team, method validation team, sample analysis team, and QC**
- **Address QA findings and prepare for FDA inspections**

2018

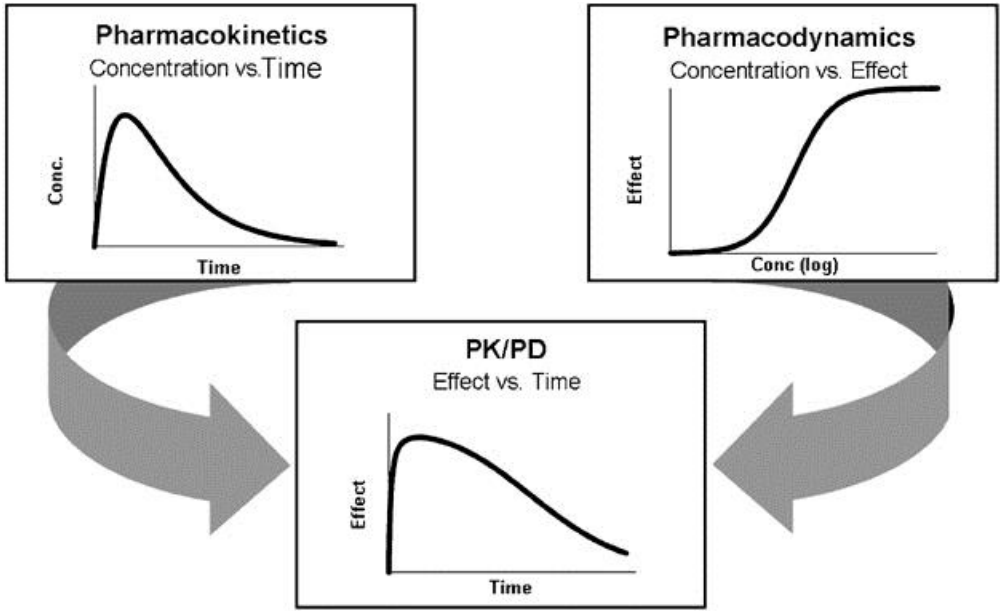
- **End-to-end bioanalytical support – discovery to clinical**
- **Support global discovery full package (in-life to PK report)**
- **Discovery and non-GLP pharmacokinetic (PK) support**
- **US center of excellence for regulated bioanalysis (via CROs)**
- **Capabilities of supporting capillary microsampling for tox studies**
- **Capabilities on LC-MS of large molecules including half-life extension platform**
- **Capabilities on LC-MS of biomarker quantitation**

Pharmacokinetics – Steps in Oral Bioavailability



Essential PK/PD Correlation for Any Drug

- **Pharmacokinetics (PK)**
  - Absorption, distribution, metabolism and elimination of drugs in the body
  - PK is a discipline that uses mathematical models to describe and predict the time course of a drug and its metabolite concentrations at various body sites over time.
- **Pharmacodynamics (PD)**
  - Effects of drugs/metabolites to the body and their mechanisms of actions
  - PD is the relationship between drug concentrations and pharmacological effects, and in turn, the relationship of these responses to clinical outcomes



What Regulations Applied in a BA Laboratory?

- **GLP (Good Laboratory Practice) studies**
  - **Toxicology study support (Toxicokinetics, TK)**
  - **Other drug safety related study support**
- **Non-GLP studies, but GLP principles are applied**
  - **Clinical study support**
    - ✓**Bioequivalence / bioavailability (21CFR320)**
    - ✓**New drug application (21CFR310)**
  - **Bioanalytical method validation**

******Evaluation of GLP compliance is an important tool for measuring a facility's fitness for use and assuring data integrity***

LC-MS/MS Assay Validation

- FDA issued guidance and a white paper for bioanalytical method validation to support pharmacokinetic (PK) studies with focus on LC-MS/MS methods:
  - US FDA (2001) Guidance for Industry: Bioanalytical validation
  - US FDA (2015) Guidance for Industry: Bioanalytical validation (Draft)
- Currently, there is no regulatory guidance governing biomarker assay validation and implementation.
- It is common for industry to adopt the guidance of PK study for small molecule biomarker assay.
- The consensus is to use a "fit-for-purpose" approach:
  - The intended use of biomarker data should be considered in assay development and validation.
  - The rigor of the validation increases as the biomarker data are used for increasingly advanced clinical or otherwise business-critical decision making.
  - Data should show the reliability (scientific confidence) of the assay for intended application.

Parameters for LC-MS/MS (PK) Assay Validation

- Accuracy and precision: intra-assay, inter-assay
- Linearity/curve range
- Lower limit of quantitation (LLOQ)/sensitivity
- Specificity/selectivity
- Dilution
- Matrix effect
- Recovery
- Carry-over
- Incurred sample reproducibility (ISR)
- Stability

  Stock solution stability: bench-top, long-term

  Stability during sample collection (e.g. whole blood stability)

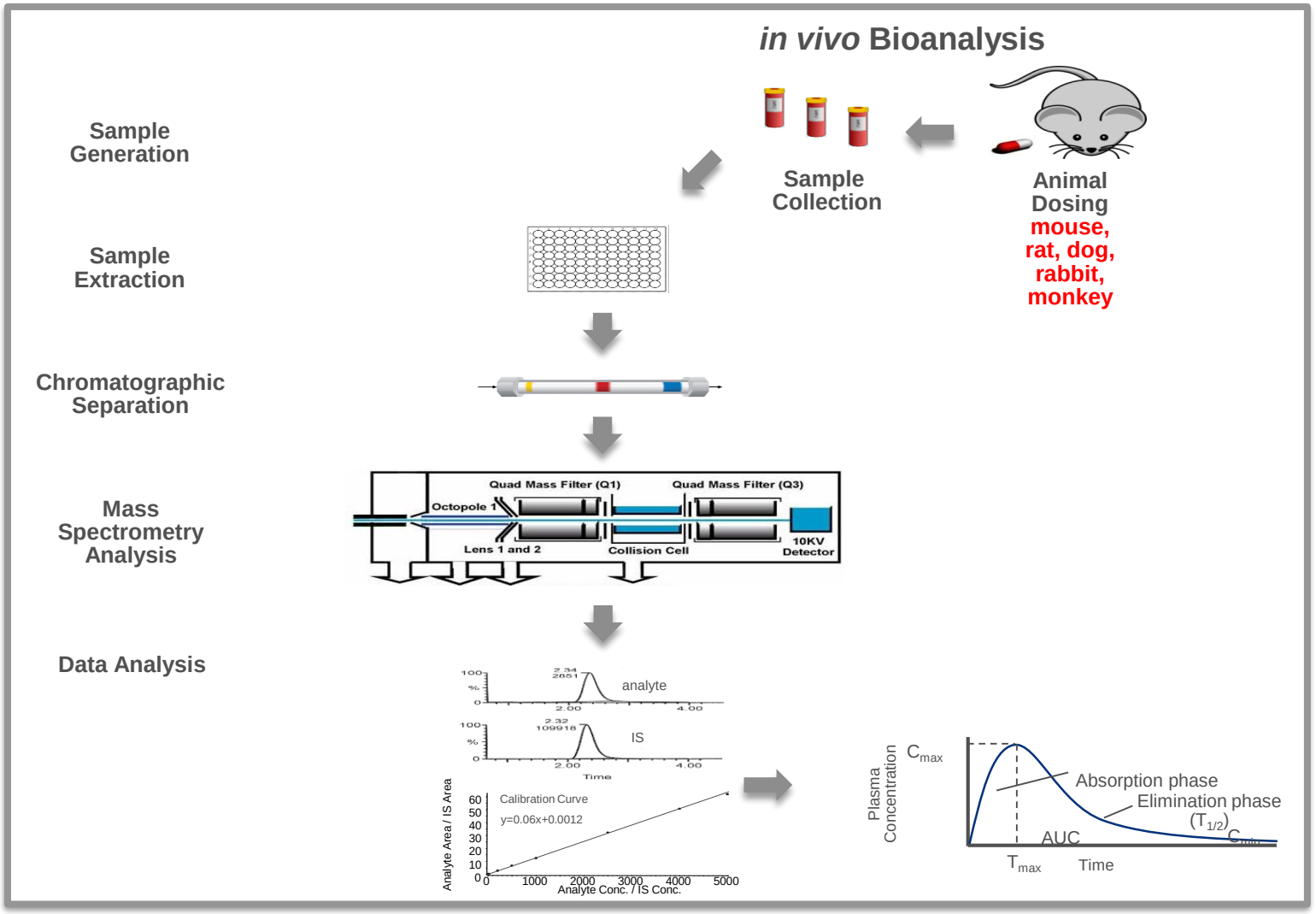
  Bench-top stability in matrix

  Freeze-thaw stability in matrix

  Long term frozen stability in matrix

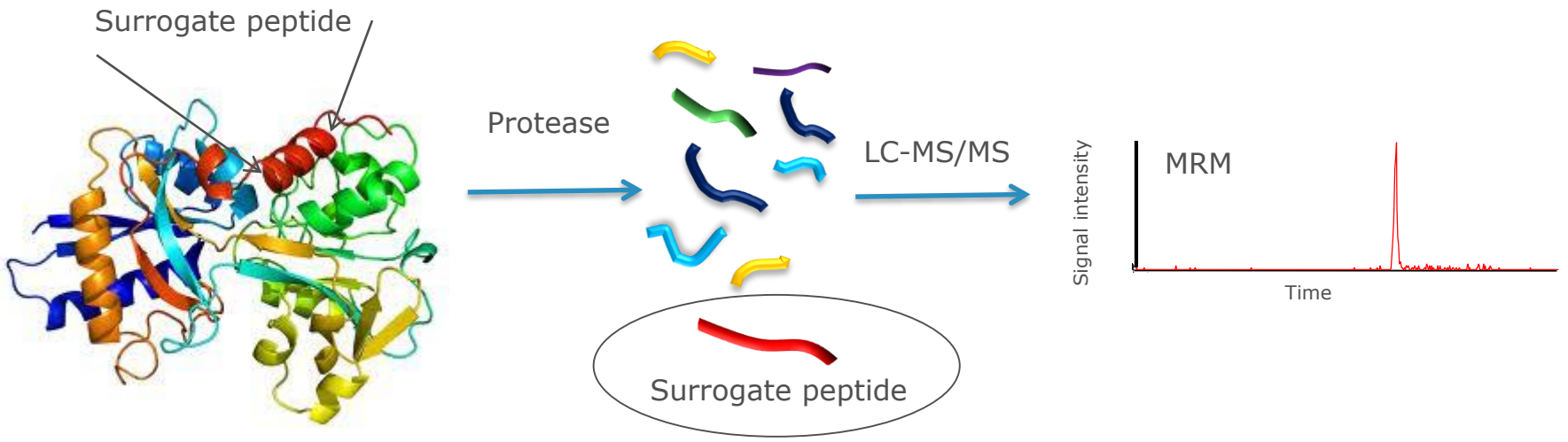
  Autosampler stability/re-injection reproducibility

Bioanalytical LC-MS Workflow for Small Molecules

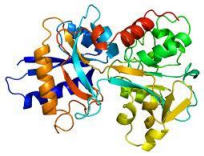


Bioanalytical LC-MS Workflow of Large Molecules

- Surrogate peptide，bottom-up



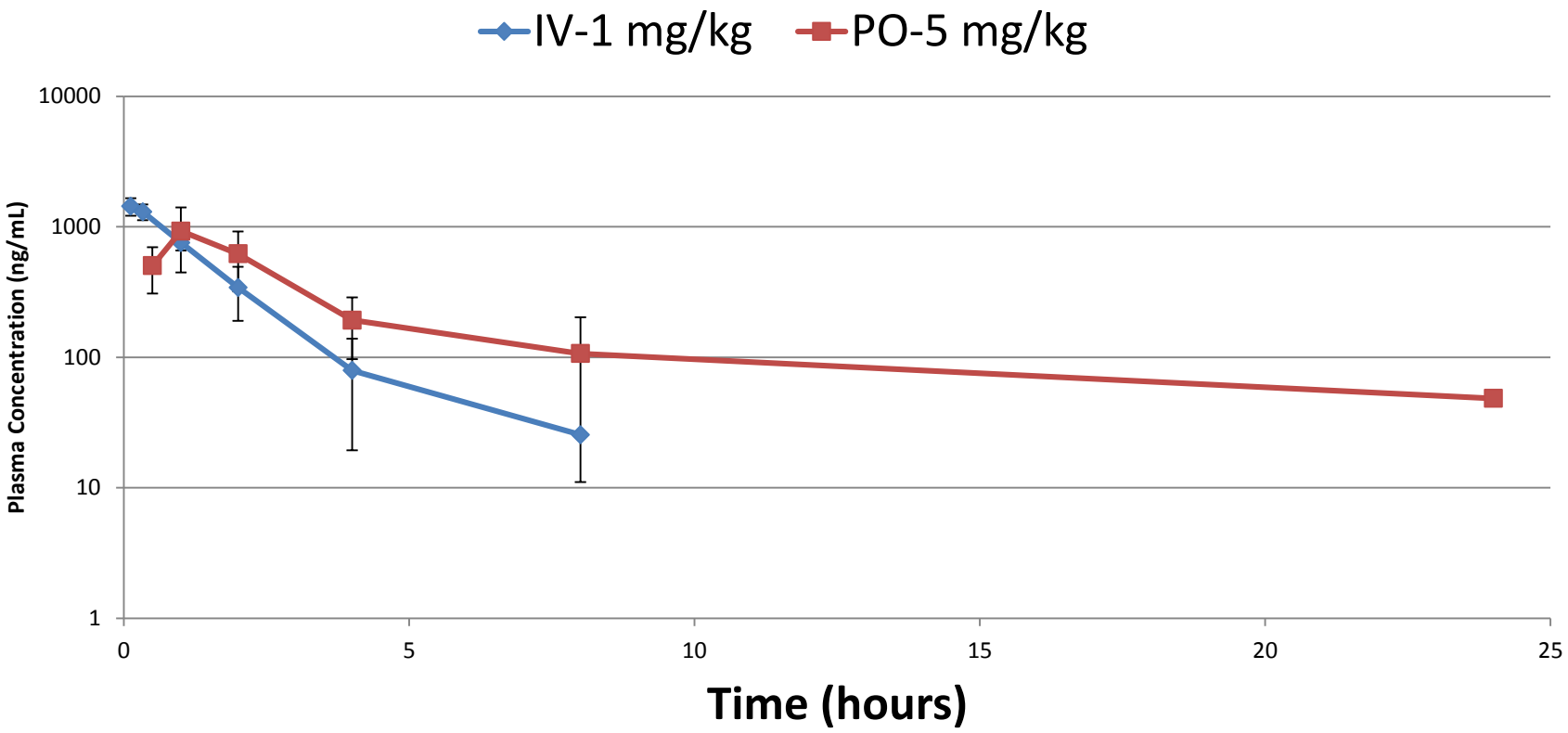
- Intact analysis，top-down Provide information of whole molecule
- Middle-down (new)



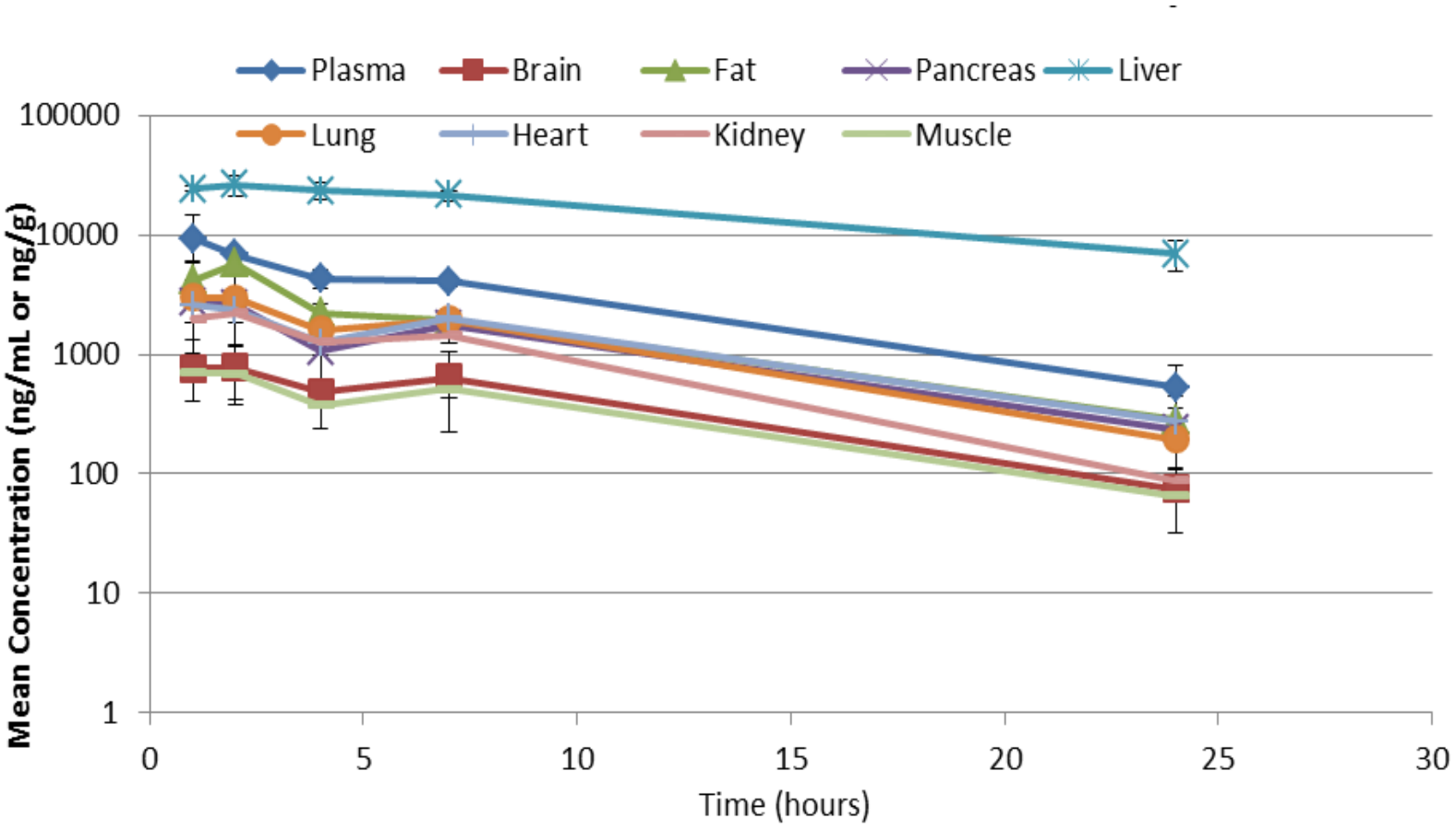
Examples of Bioanalytical Support

- Drug candidates and their metabolites
- Biomarkers
- Novel Scaffolds

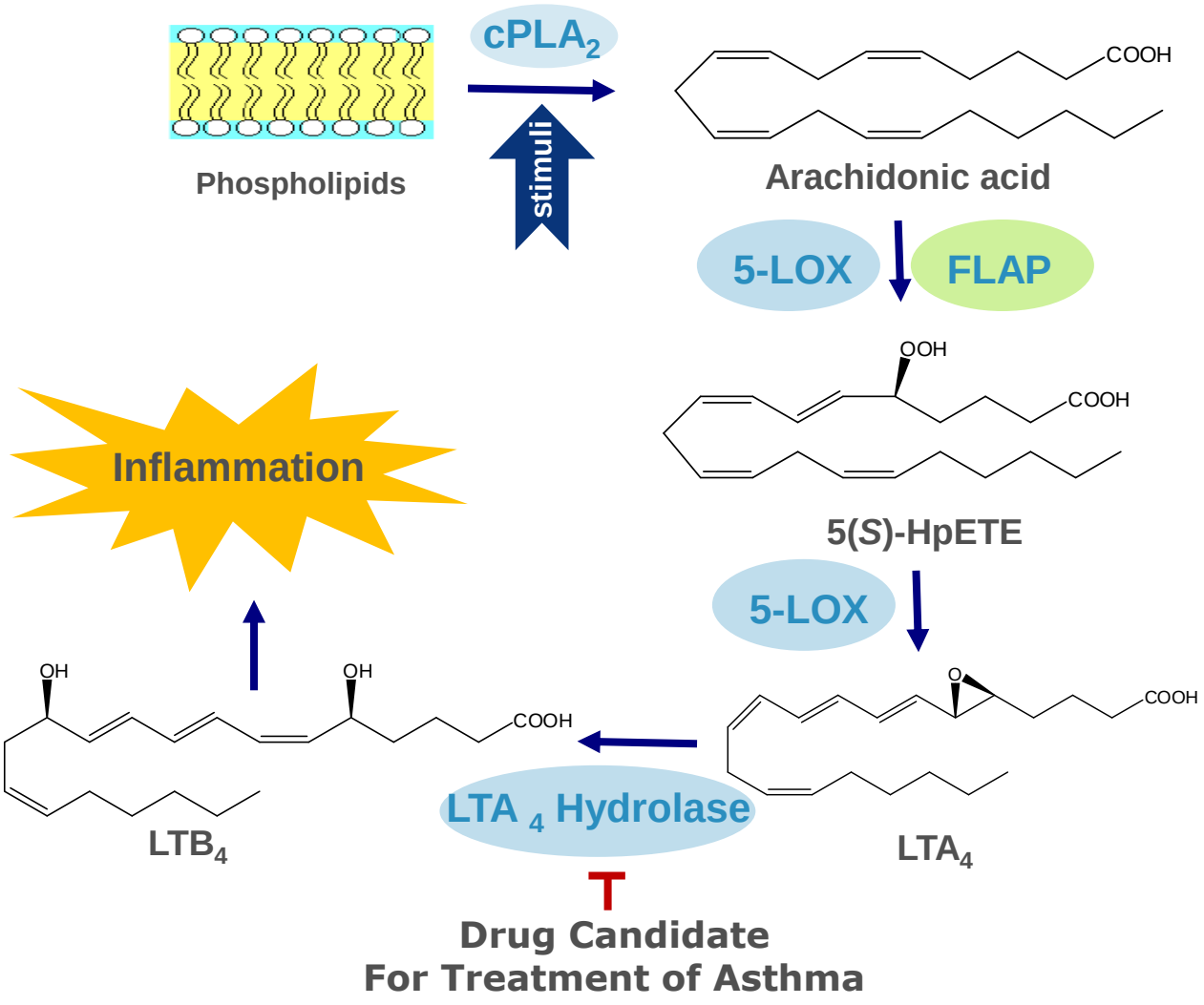
PK STUDY


IV-1 mg/kg
PO-5 mg/kg
Plasma Concentration (ng/mL)
10000
1000
100
10
1
0
5
10
15
20
25
Time (hours)

Tissue distribution study



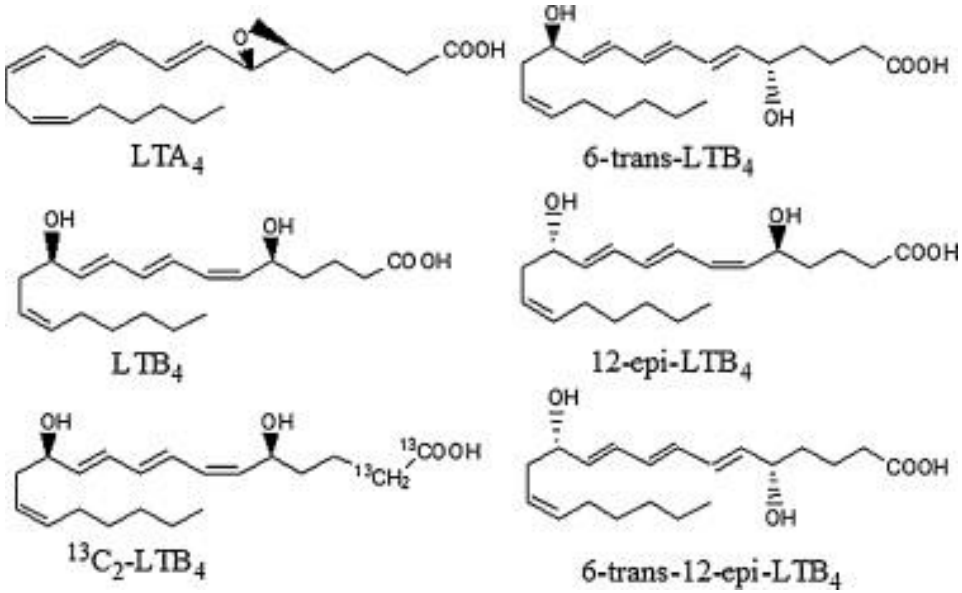
Efficacy Biomarker: Leukotriene B_4



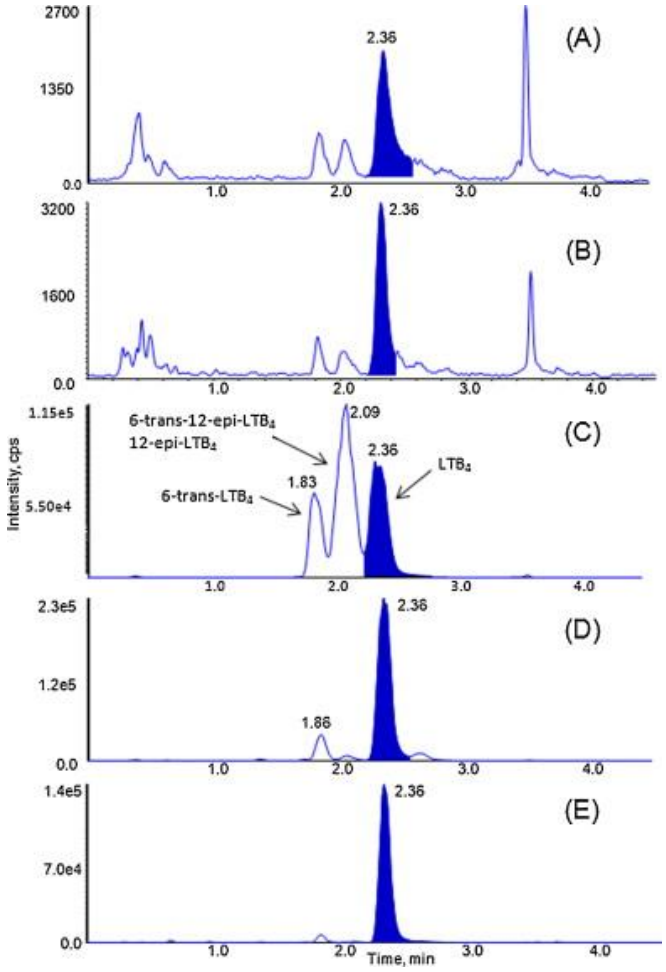
Journal of Chromatography B
Volume 932, 1 August 2013, Pages 59-65

Quantitation of leukotriene B_4 in human sputum as a biomarker using UPLC–MS/MS

Wenying Jian [a], Richard W. Edom [a], Xiaohua Xue [b], Mike-Qingtao Huang [a], Anne Fourie [b], Naidong Weng [a]



Separation of LTB4 from Its Isomers



(A) Control human sputum

(B) LQC in human sputum

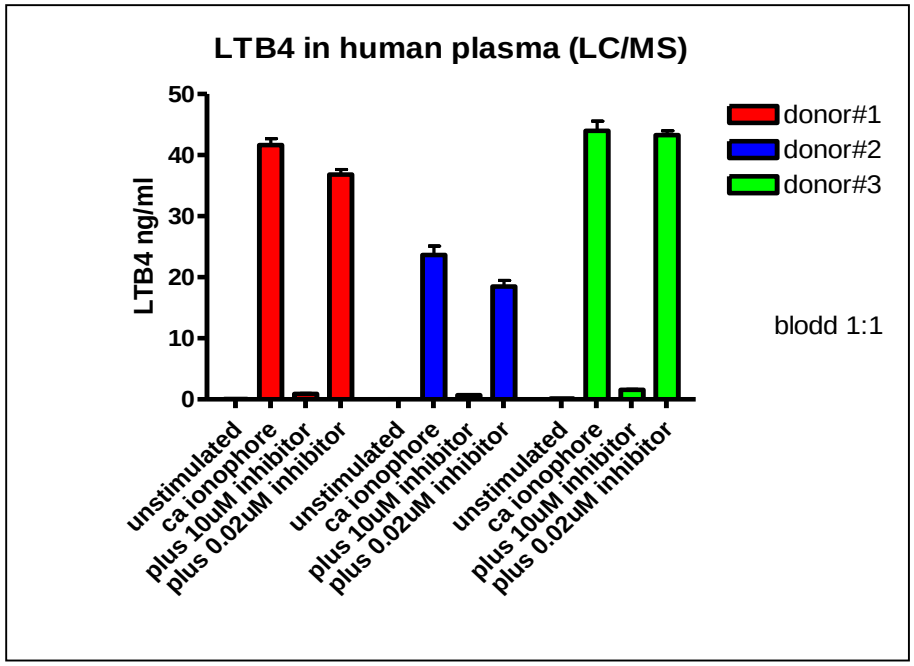
(C) neat solution of LTB4 and its isomers showing separation

(D) an incurred human sputum sample

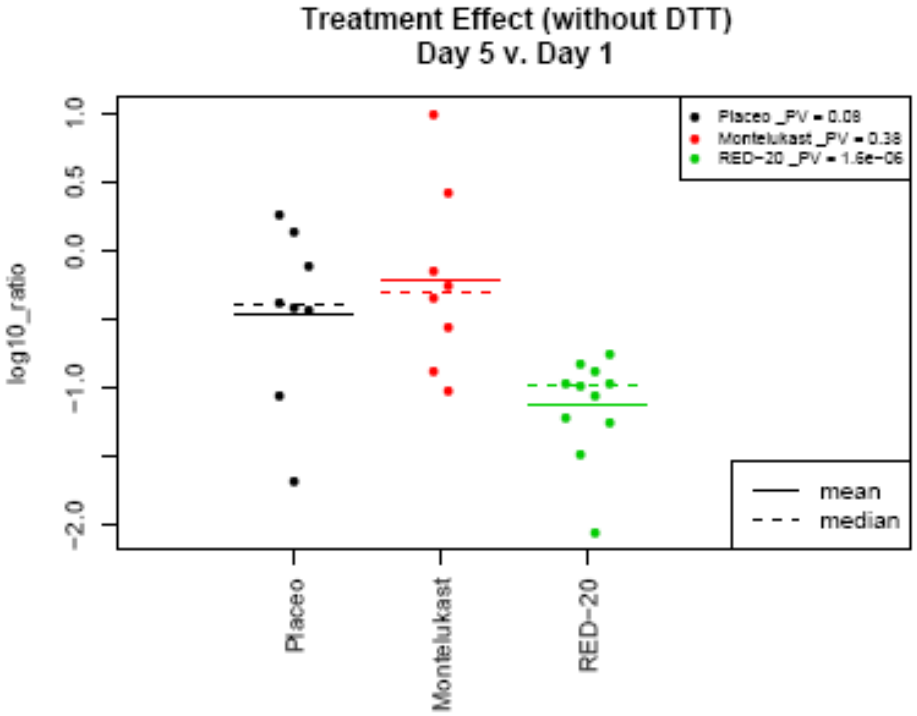
(E) internal standard in human sputum

LTB_4 is a Biomarker for LTA_4 Hydrolase Inhibition

LTB_4 in Blood



LTB_4 in sputum



Jian, et al. *Journal of Chromatogram B*, 2014. 932:59

Fate of Peptides/Proteins in the Body

Biotransformation/elimination of peptides/proteins *in vivo*

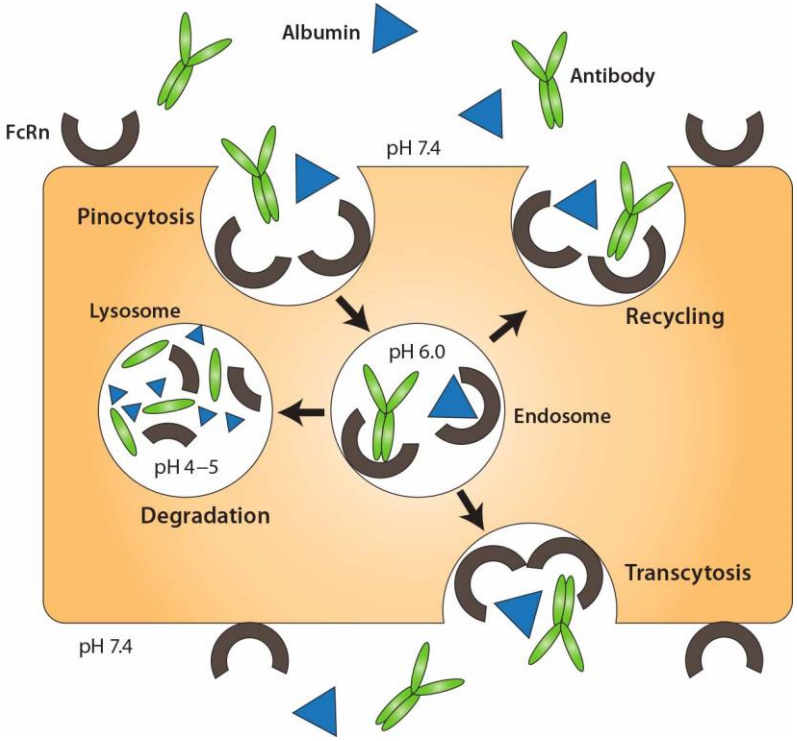
- **Proteolysis**: Unspecific proteolysis by proteases and peptidases is considered to be a major elimination pathway for peptides and proteins instead of the oxidative hepatic metabolism which is typical for the majority of small-molecule drugs.

- **Renal filtration**: Small proteins of < 50 kDa are eliminated through renal filtration.

- **Target-mediated disposition**: For larger proteins, elimination in other tissues and/or in target cells through target-mediated endocytosis followed by catabolism is more important than renal filtration.

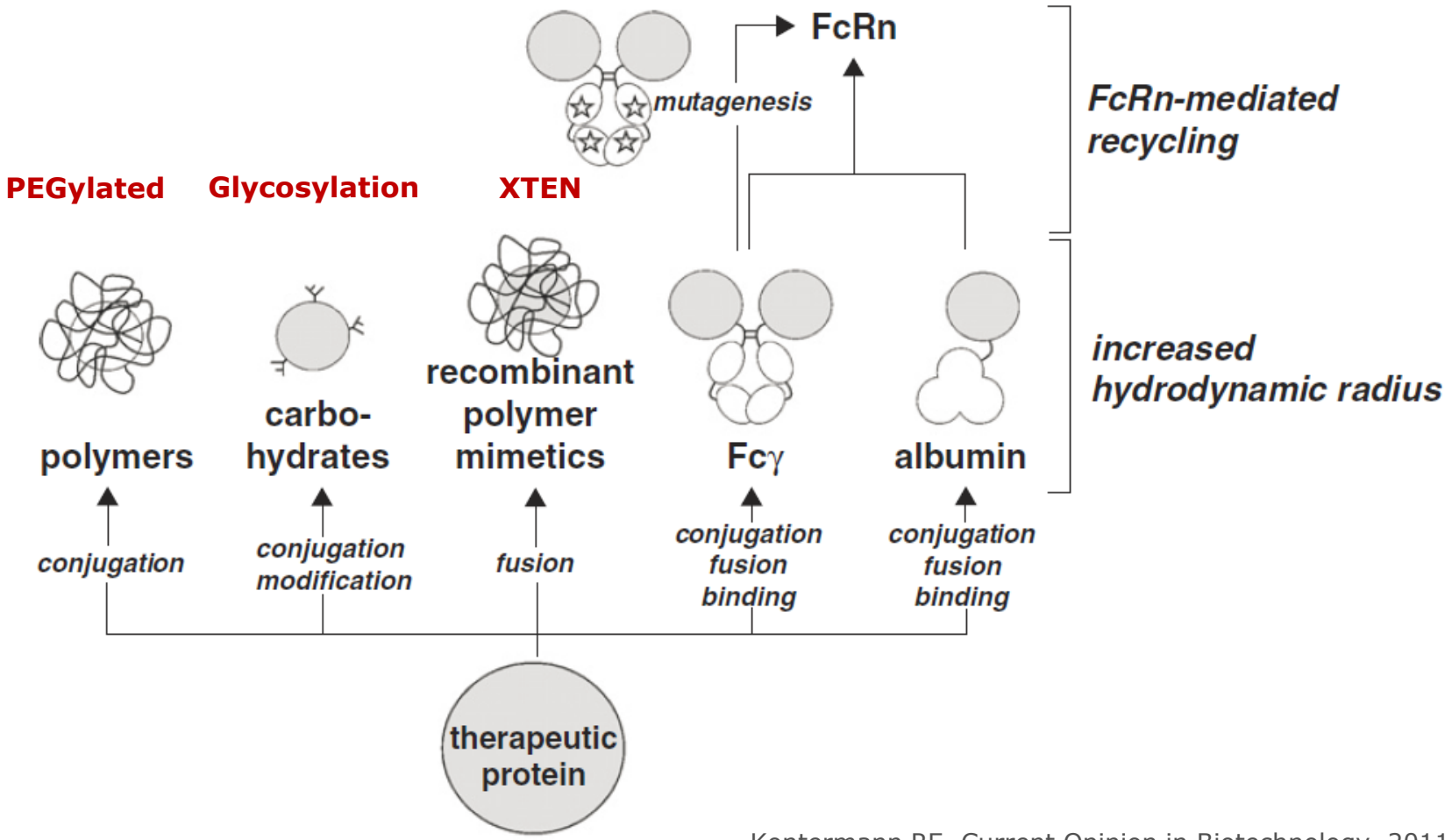
Protection of proteins *in vivo*

- **Recycling of antibody and albumin by neonatal Fc receptor (FcRn)**



Angela Linderholm and Steven M. Chamow
Http://www.bioprocessintl.com/manufacturing/antibody-non-antibody/immunoglobulin-fc-fusion-proteins-part-1-design-manufacture/

Strategies for Extending Serum Half-life of Protein Therapeutics



Kontermann RE. Current Opinion in Biotechnology, 2011, 868

Summary – The Landscape of Bioanalysis

- Support Drug Discovery/Development by providing pivotal PK/PD data for go/no-go decision
- Provide inputs to project team on optimization compound properties
- Apply new technology for better, quicker and more sensitive analysis
- Act as a good program manager and study monitor for work done externally
- Exercise the right vigor for balancing compliance and cost-effectiveness
- Demonstrate Credo behavior and be a good J&J ambassador to our scientific communities

Thank You!

Q&A?