Collaboration on protein measurement between AIST-NIST

Synthetic protein particles as measurement standards for subvisible aggregates: Progress 2014

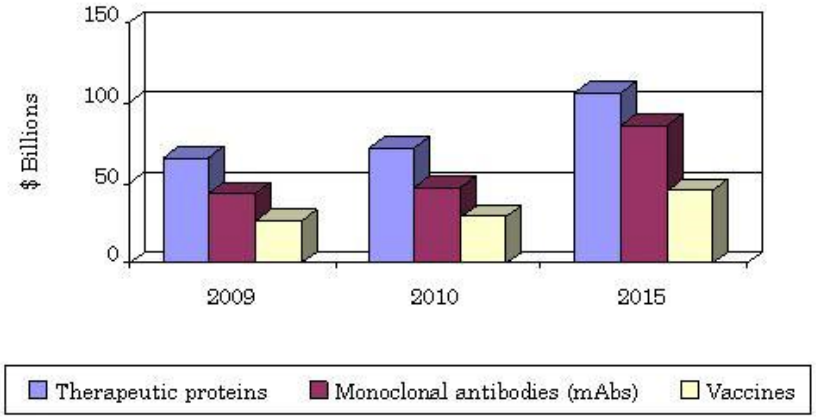
Shinya Honda

Molecular and Cellular Breeding Research Group

BioMedical RI, AIST, Japan

Growing market for pharmaceutical proteins

- The overall biologics market was **$135 billion** in 2009, and was expected to grow to **$149 billion** in 2010, a 9.4% growth rate.
- The total market is expected to grow to **$239 billion** by 2015, a compound annual growth rate of **9.9% since 2010**.



Source: BCC Research (http://bccresearch.wordpress.com/2012/09/25/)

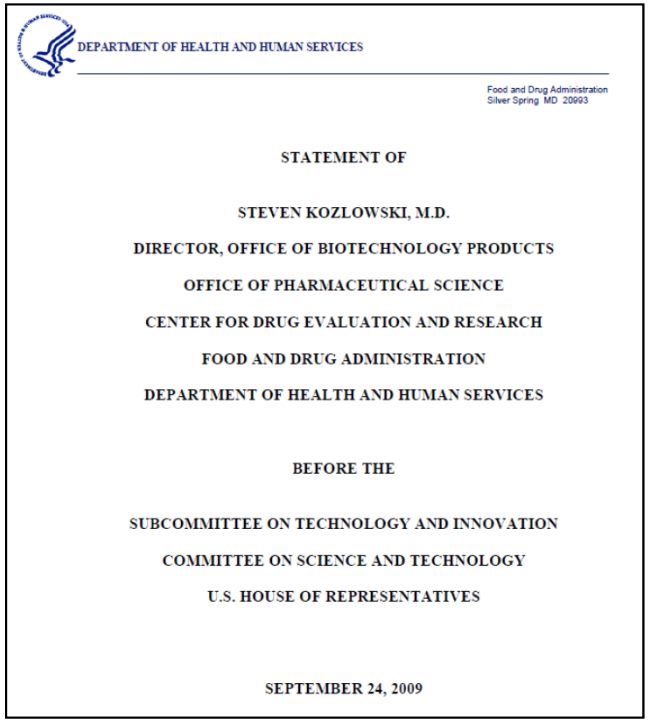
Three characteristics that should be analyzed

FDA statement before US House (2009)

- Advances in analytical tests during the last two decades have driven progress in biopharmaceutical manufacturing, but there is **still room for significant improvement**.
- FDA has identified **three properties** of therapeutic proteins that **cannot be sufficiently measured at this time** but that are very important for understanding the behavior of protein drugs.

Molecular properties on the frontier.

- **Post-translation Modifications**
- **Three-dimensional Structure**
- **Protein Aggregation**



DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration
Silver Spring MD 20993

STATEMENT OF

STEVEN KOZLOWSKI, M.D.
DIRECTOR, OFFICE OF BIOTECHNOLOGY PRODUCTS
OFFICE OF PHARMACEUTICAL SCIENCE
CENTER FOR DRUG EVALUATION AND RESEARCH
FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH AND HUMAN SERVICES

BEFORE THE

SUBCOMMITTEE ON TECHNOLOGY AND INNOVATION
COMMITTEE ON SCIENCE AND TECHNOLOGY
U.S. HOUSE OF REPRESENTATIVES

SEPTEMBER 24, 2009

Steven Kozlowski, M.D.
Director, Office of Biotechnology Products
Office of Pharmaceutical Science
Center for Drug Evaluation and Research
Food and Drug Administration

Inconsistency in measurements for protein aggregation

- Comparing the amount of aggregates from an antibody drug measured by three different methods
  - Gabrielson et al *J. Pharm. Sci.* 96, 268- (2007)

Table 1. Monomer and Soluble Aggregate Concentrations as a Mass Percentage of Total Protein for Unstressed and Acidified Antibody Samples

		% by Mass of Total Protein		
Aqueous Antibody Sample	Species	SV-AUC	SEC	AF4
Unstressed	Monomer	95.8 ± 0.5	99.6 ± 0.1	97.8 ± 1.1
	Total soluble aggregate	4.2 ± 0.5	0.4 ± 0.1	2.4 ± 1.1
Acidified	Monomer	84.6 ± 0.8	86.4 ± 1.5	80.3 ± 2.8
	Total soluble aggregate	15.4 ± 0.8	13.6 ± 1.5	19.7 ± 2.8

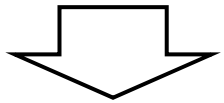
Reported values are mean ± one standard deviation ($n = 3$).

inconsistent

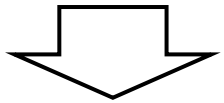
need for standardization

Aim and strategy

- Native proteins are generally unstable and smaller than aggregates.

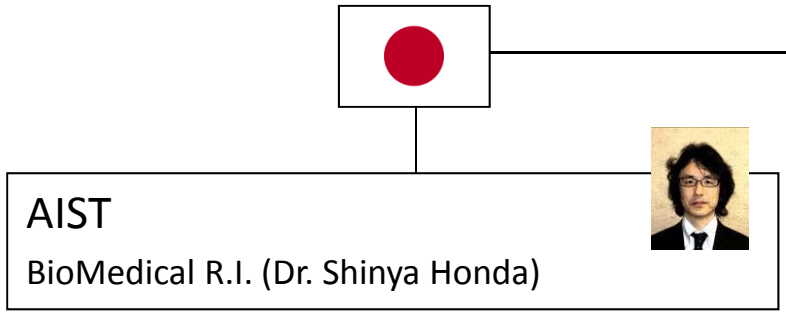


- We aim to synthesize **large protein particles** with enough stability by modifying native proteins using molecular design technique.



candidates for pre-authentication reference materials

Collaboration outline



AIST

BioMedical R.I. (Dr. Shinya Honda)

NIST

Material Measurement Lab.（Dr. Dean Ripple）

molecular design of artificial proteins

synthesis of proteins

measurements (size distribution, stability)

development of new analytical methods

synthesis of polymers

AIST molecules (below 1μm)

NIST substances (over 1μm)

comparative analysis

universities
biologics consortium
instrumental companies

pharmaceutical companies
regulation agencies
universities

Molecular candidates

type 1

Spherical

type 2

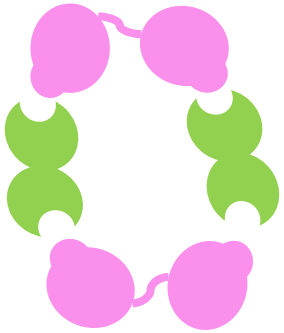
type 3

Cylindrical

type 4

Hollow

type 5



Complex: a pair of bivalent binders

Development flow

(Stage 1) Synthesis

- Gene synthesis
- Vector construction and cloning
- Bacterial expression
- Purification
- Identification
- Method optimization
- Large preparation
- Lyophilization

(Stage 2) Characterization

- Analytical size exclusion chromatography (SEC)
- MALDI-TOF-MS analysis
- Analytical ultracentrifuge
- Dynamic light scattering (DLS)
- FT-IR

(Stage 3) Homogeneity Assessment

- Analytical SEC
- DLS
- Circular dichroism
- Statistical analysis

(Stage 4) Stability Test

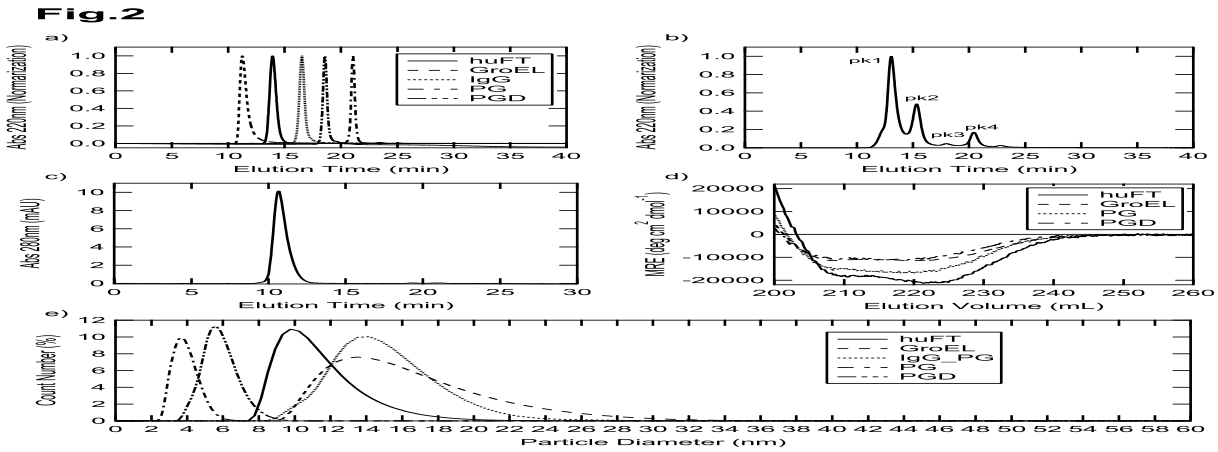
- Heat stress test
- Short-term storage stability
- Statistical analysis

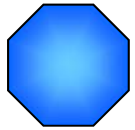
(Stage 5) Documentation

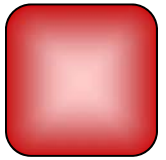
- Paper to journal
- Draft proposal to authority

Characterization: Type 1, 3, and 5

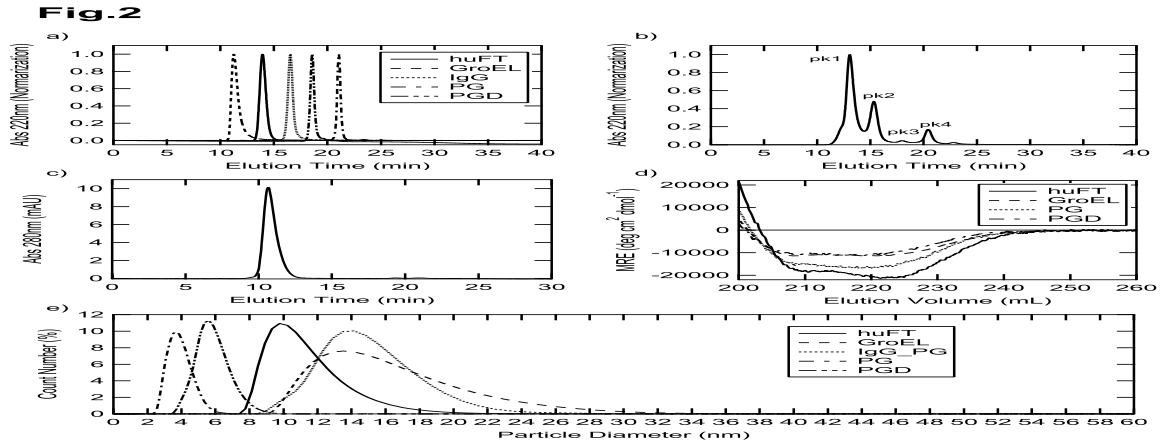
- SEC

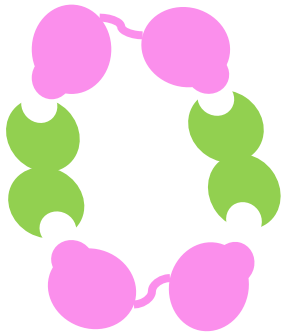






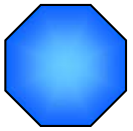
- DLS

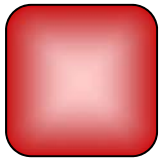




Homogeneity: Type 1 and 3

- Between-bottle

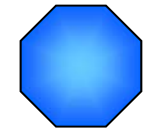




			Type 1	Type 3
SEC	Retention Time	within-bottle (min)	14.17 ± 0.004	12.57 ± 0.006
		between-bottle (min)	14.17 ± 0.011	12.56 ± 0.006
		F-test	No	Yes
		t-test	Yes	No
	Purity	within-bottle (%)	99.5 ± 0.03	99.9 ± 0.05
		between-bottle (%)	99.4 ± 0.09	99.9 ± 0.07
		F-test	Yes	Yes
		t-test	No	Yes
CD_{222nm}		within-bottle (MRE)	-20348 ± 355	-8222 ± 283
		between-bottle (MRE)	-20614 ± 668	-8712 ± 322
		F-test	Yes	Yes
		t-test	Yes	No
DLS		within-bottle (nm)	12.76 ± 0.32	17.26 ±0.14
		between-bottle (nm)	12.55 ± 0.17	17.37 ±0.11
		F-test	Yes	Yes
		t-test	Yes	Yes

Stability: Type 1 and 3

- Storage stability by SEC retention time
  - 40, 25, 4, -30℃; 75, 60% RH; 0-4 weeks



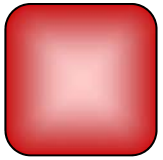
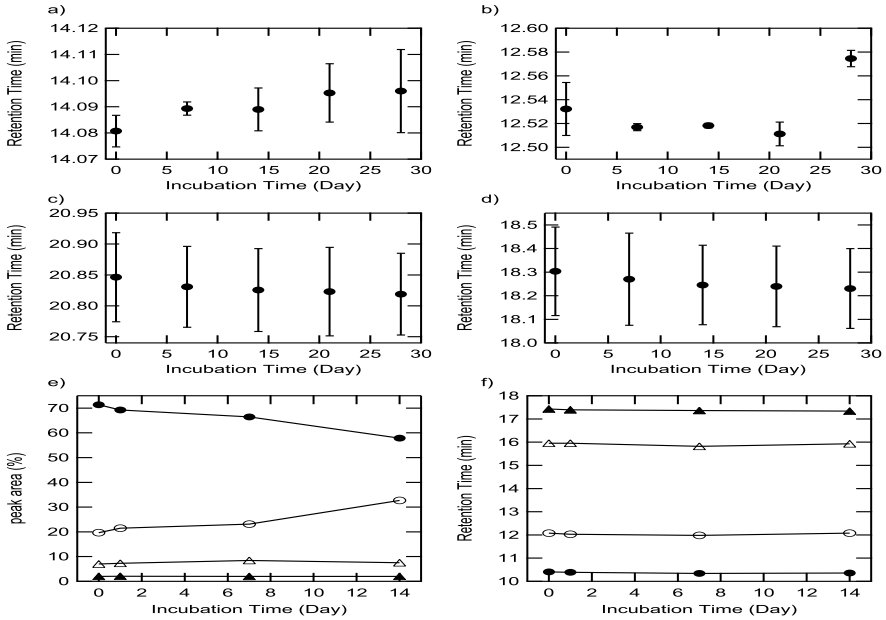
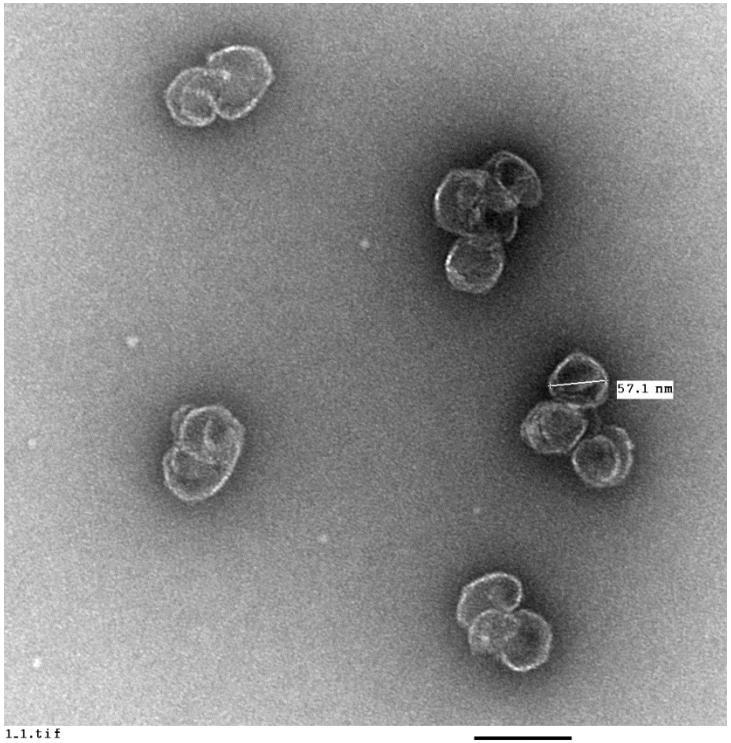


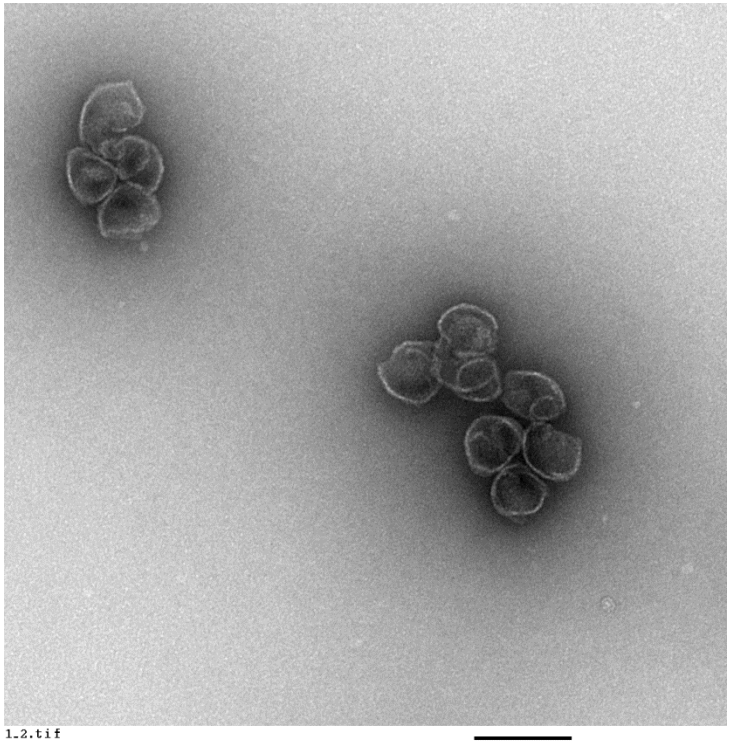
Fig. 4



Characterization: Type 4

- Electron microscope (negative staining)





Documentation

- Paper to journal
  - H Takahashi, Y Feng, A Ooishi, H Watanabe, and S Honda, “Towards the development of a reference material for protein particle”, *submitted*.
- Draft proposal to authority
  - “General requirements of the protein-related standard material to use for aggregation measurement analysis”, *in preparation*

2014 Summary and future plans

- Synthetic methods for all five types of candidates were established.
- Extensive characterizations for Type 1, 3, and 5 candidates were carried out.
- Homogeneity assessments and storage stability analyses for bottled samples (Type 1, 3, and 5 candidates) were performed.
- Purification method for Type 4 candidate was improved.
- Large amount of Type 1 and 3 samples was shipped to NIST.
- Paper and draft proposal were prepared.

- Storage stability analyses for Type 4 candidate will be conducted.
- Inter-laboratory validation study will be continued with NIST.

Acknowledgments

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  - H Watanabe, H Takahashi, Y Feng, A Ooishi in AIST
- Collaborators
  - Dean Ripple (NIST), Wyatt N. Vreeland (NIST)
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