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Excellence in Science

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In Barcelona, Spain

Validated bioanalysis for therapeutic antibodies by LC-MS: Fab-selective proteolysis nSMOL

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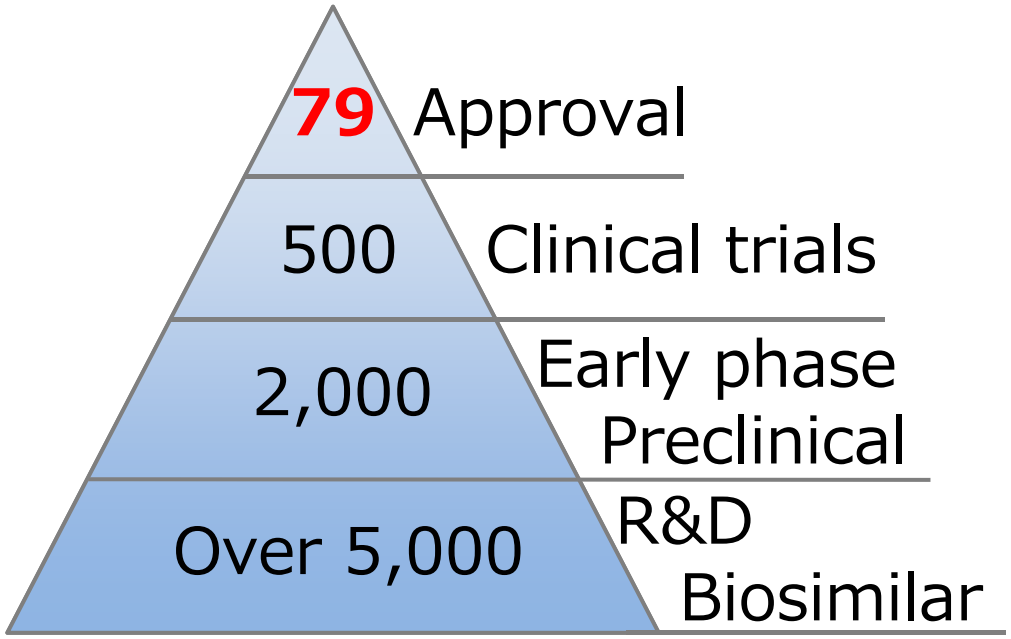
Market trend of antibody drugs

Market size (2017)	2025 forecast
US M$ 84,500	114,600
Immune checkpoint inhibitor US M$ 10,566	56,530



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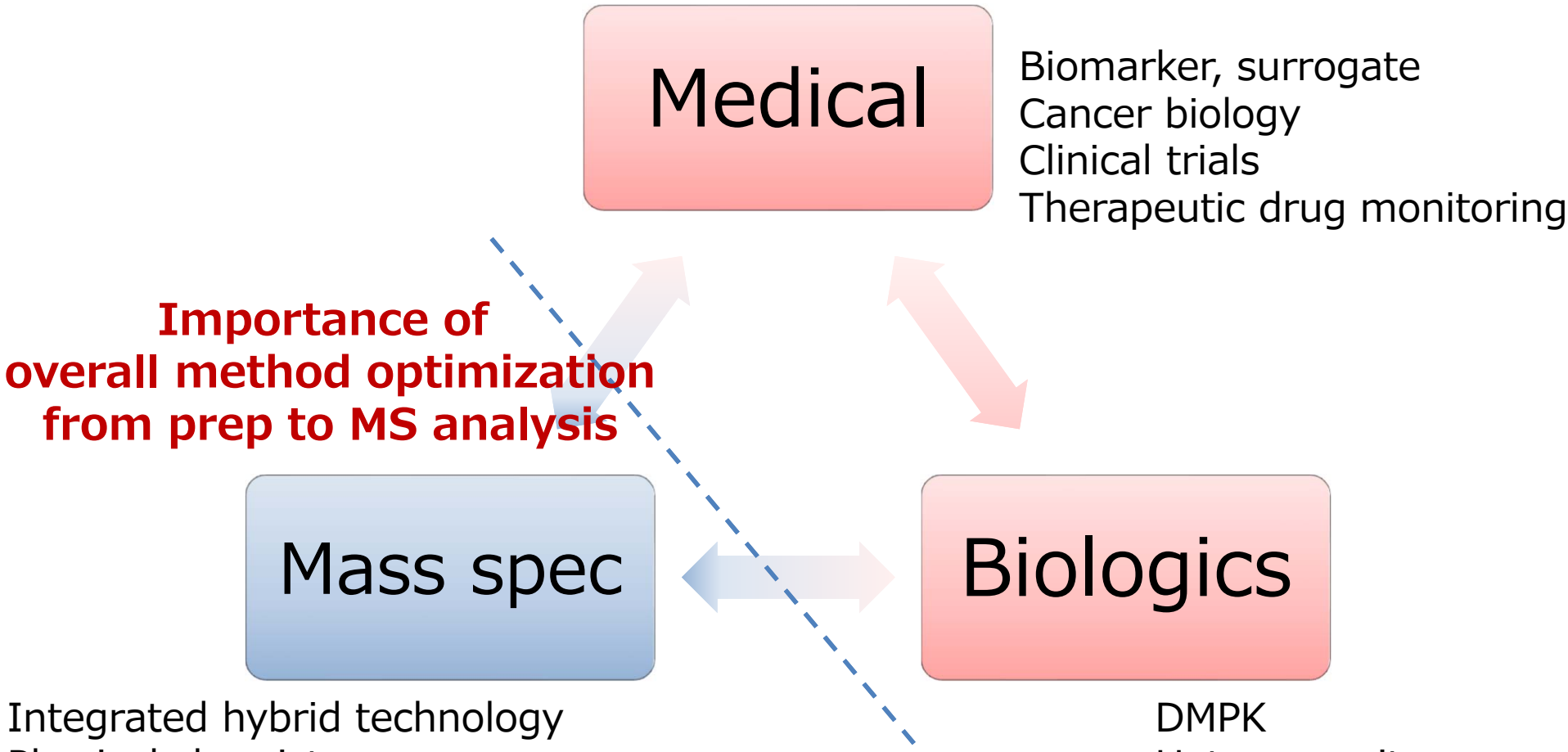
Strategy matching of MS technology to medicine and biologics



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Development of mAb bioanalysis

- **Clinical demands in antibody treatment**
  - ✓ Indicator of drug efficacy
  - ✓ Decision of dosing level
  - ✓ Drug distribution in plasma and tissue
- **Novel bioanalysis for clinical pharmacokinetics**
  - ✓ Structure similarity and sequence specificity for antibody CDR-targeting strategy
  - ✓ Independent of a variety of antibodies
  - ✓ Structure-indicated MS analysis
  - ✓ Clinical PK and discovery for antibody drugs
  - ✓ Regulated LCMS bioanalysis

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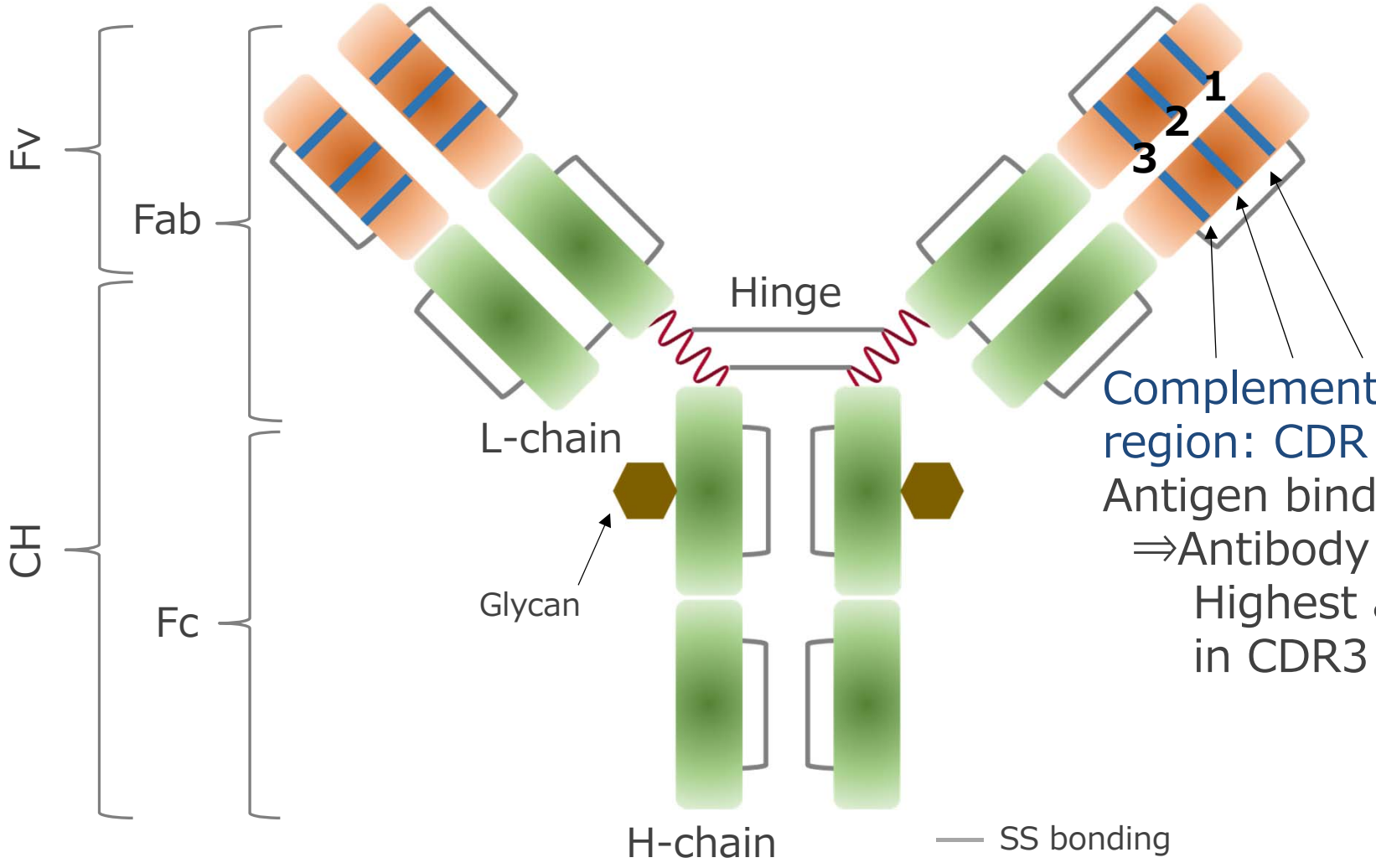
FDA Guidance Finalized on May 24, 2018

- **Bioanalytical Method Validation Guidance for Industry have been finalized by FDA**

Summary

	Protein LBA	Small molecule LCMS	Protein LCMS
	Same as previously	Almost parameters are same as small molecules.	
Calibration curve		Blank, zero, and six calibration, including LLOQ in every run **±15%** of theoretical concentration, **±20%** of LLOQ **75%** and a minimum of six non-zero calibrator levels should meet the above criteria in each validation run.	
Quality Controls		**±15%** of theoretical concentrations, **±20%** at LLOQ for accuracy; and within **15%** CV, within **20%** CV for LLOQ	

Immunoglobulin structure



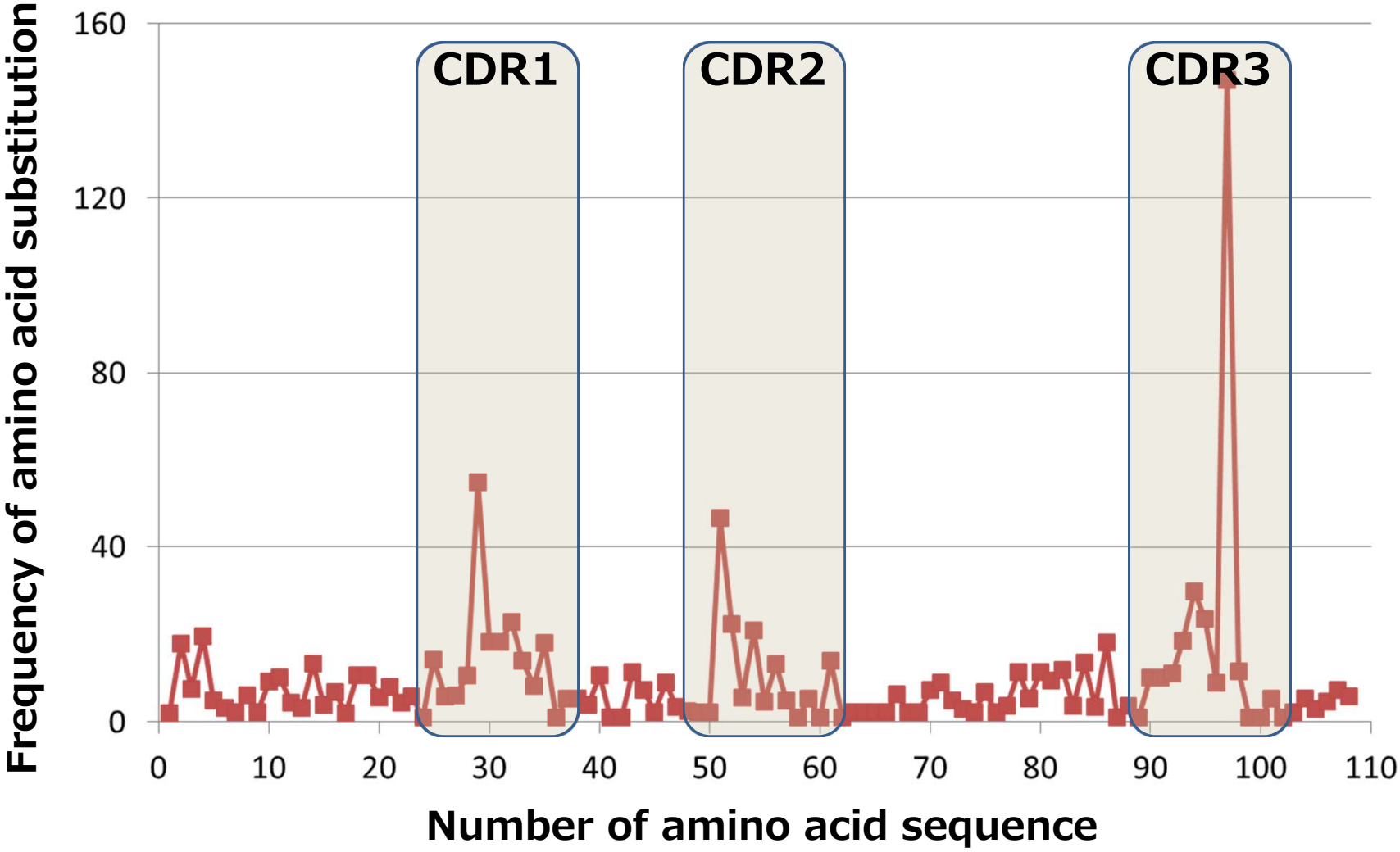
Complementarity-determining region: CDR

Antigen binding site

⇒Antibody specificity

Highest affinity to antigen in CDR3

Frequency of amino acid substitution in Fv region



TT Wu, et. al., J Exp Med. 1970, modified

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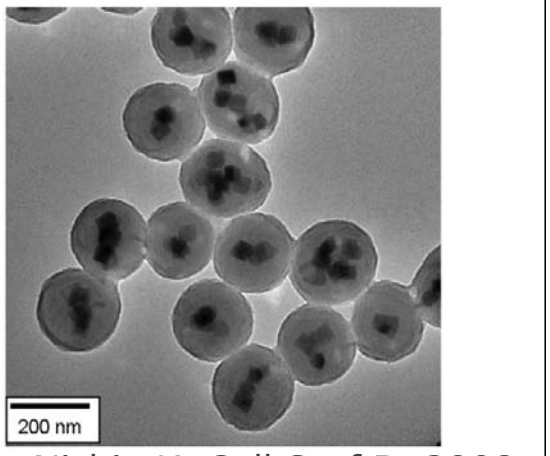
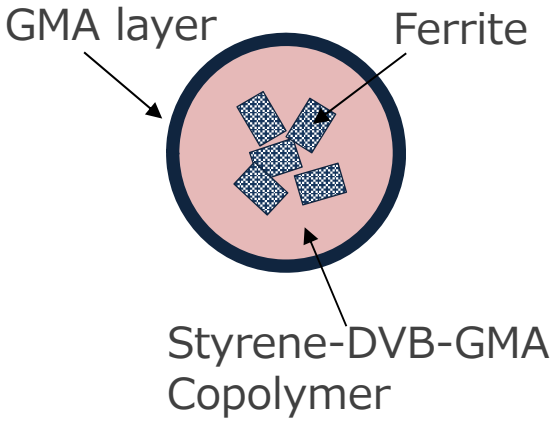
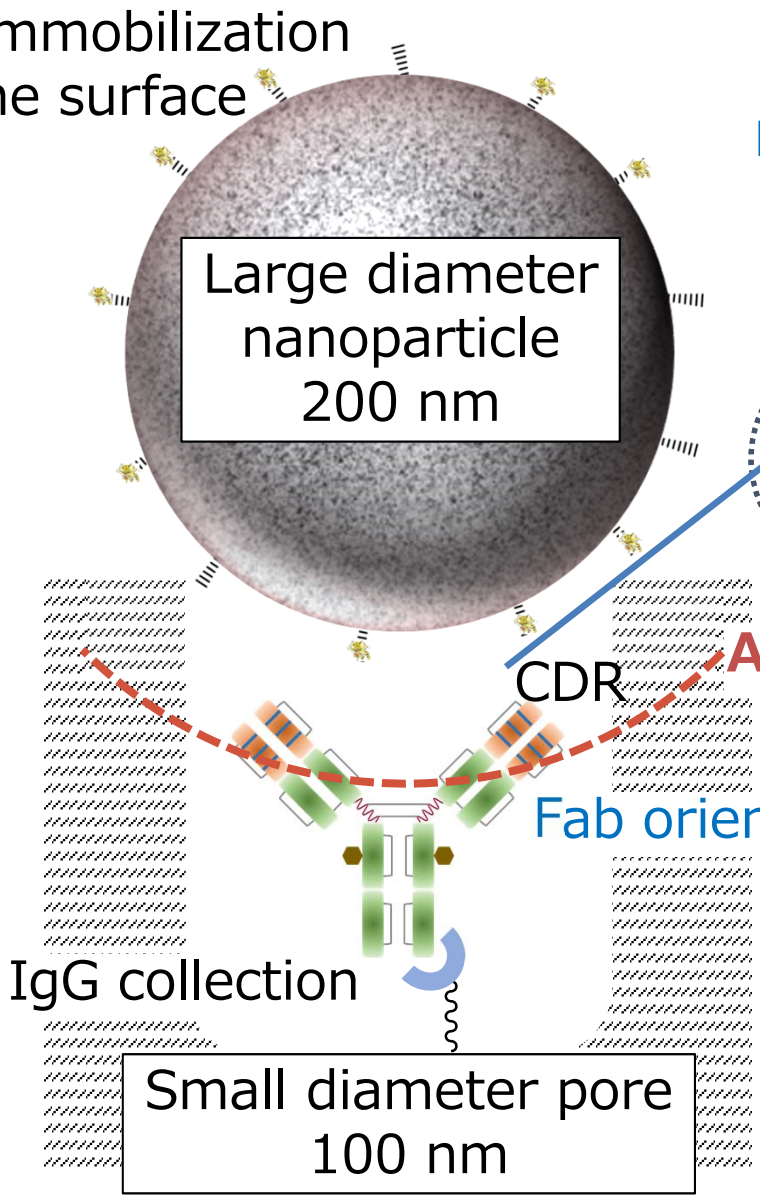
Concept representation of nSMOL

nano-surface and molecular-orientation limited proteolysis



Nishio K. Coll Surf B, 2008





Minimizing sample complexity into LCMS analysis

CDR peptides

Accessible surface

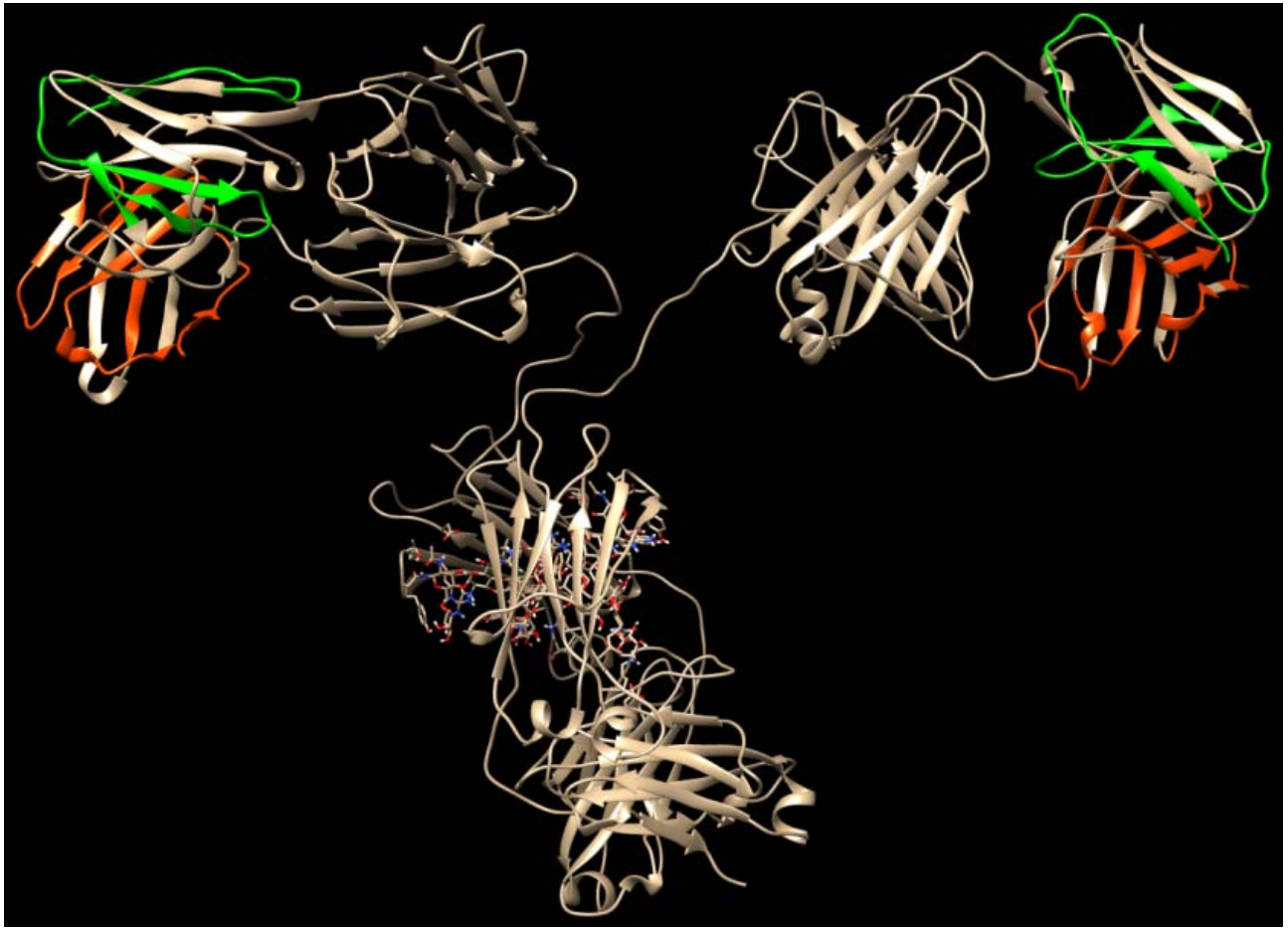
Fab orientation to the solution

HOT articles in Analyst

By Katherine Dunn, Publishing Assistant.

Iwamoto N. et.al. Analyst, 2014

Fv-selective detection by nSMOL



Peptide configuration in Nivolumab 3D structure
Detected signature peptides from **H-chain** and **L-chain**

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Benefit of nSMOL bioanalysis

		nSMOL	Affinity capture	ELISA
Method R&D	Collection or detection Ab	**No**	Individual 6-10 months	Individual 6-10 months
	Cross reactivity	**No**	Yes	Yes
	Effect of ADAs	**No**	Yes	Yes
	Pre validation	**1-3 days**	1-3 days	2-3 weeks
Validation	Full validation	3-4 weeks	3-4 weeks	3-4 weeks
	Sample prep	**Dilution** 3-5 hours	Denature, Chemical modification 3-5 hours	Dilution 2-4 hours
Feature	Internal standard	**Universal**	Individual	Individual
	Dynamic range	Wide	Wide	Narrow
	Selectivity	High	High	Middle-High
	Multiplex assay	**Yes**	Additional collection Ab	Additional collection /detection Ab

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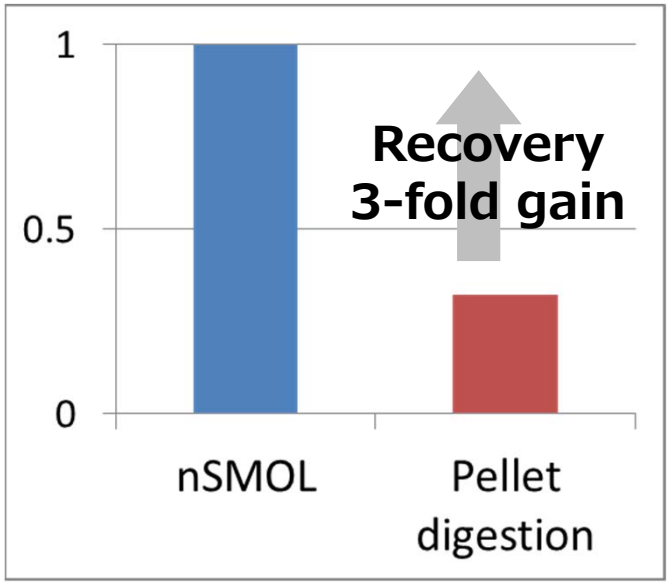
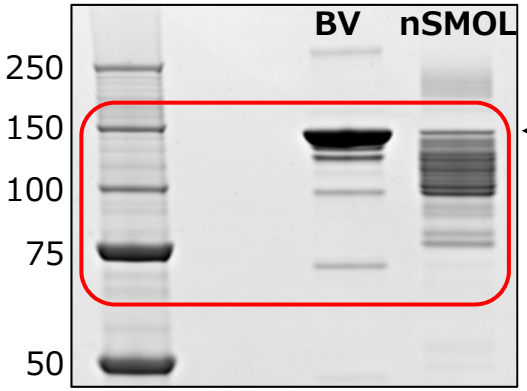
nSMOL advantage in LCMS bioanalysis

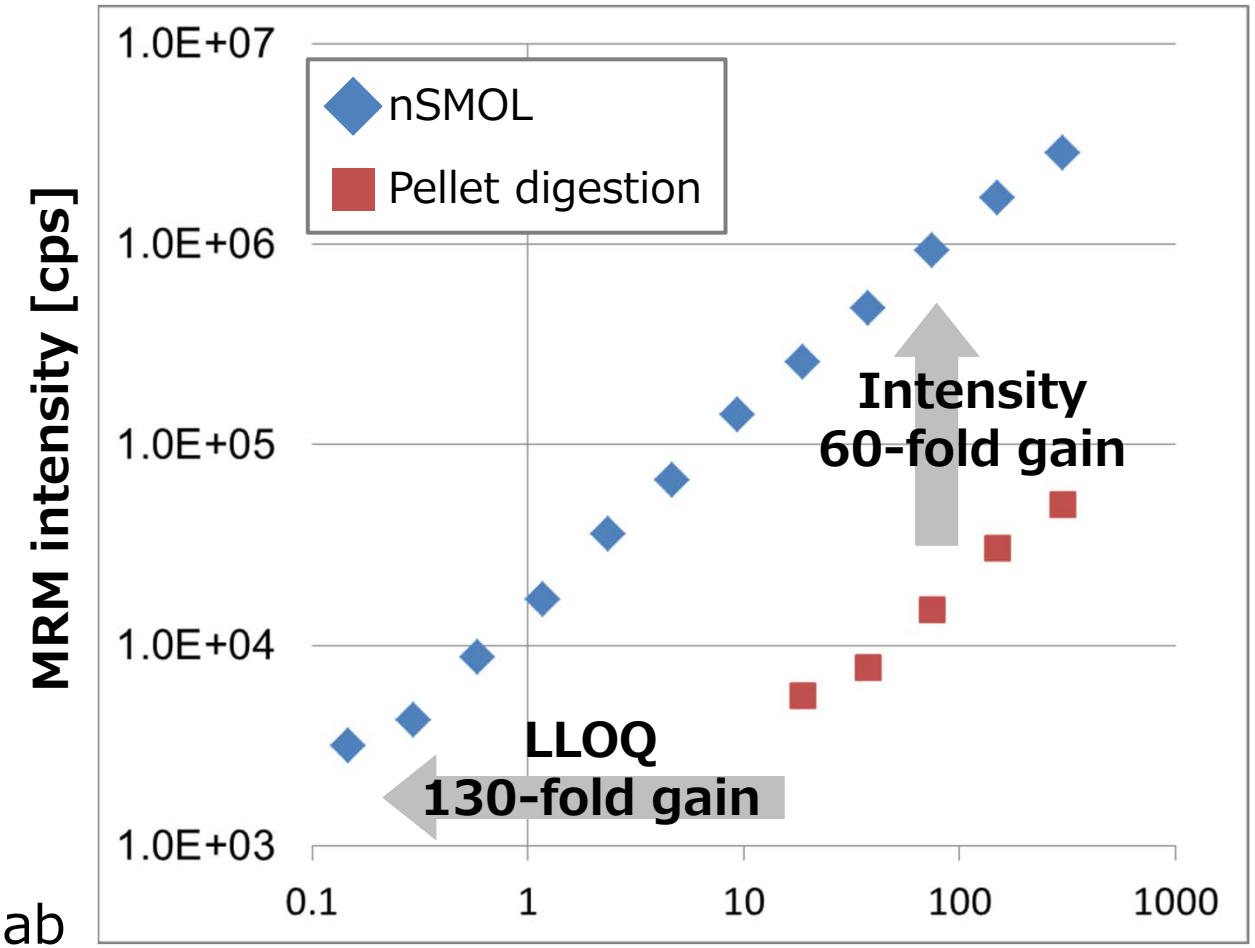
BV peptide recovery



BV proteolysis yield: 97%
(by SDS-PAGE, densitometry test)



Calibration curve in BV bioanalysis



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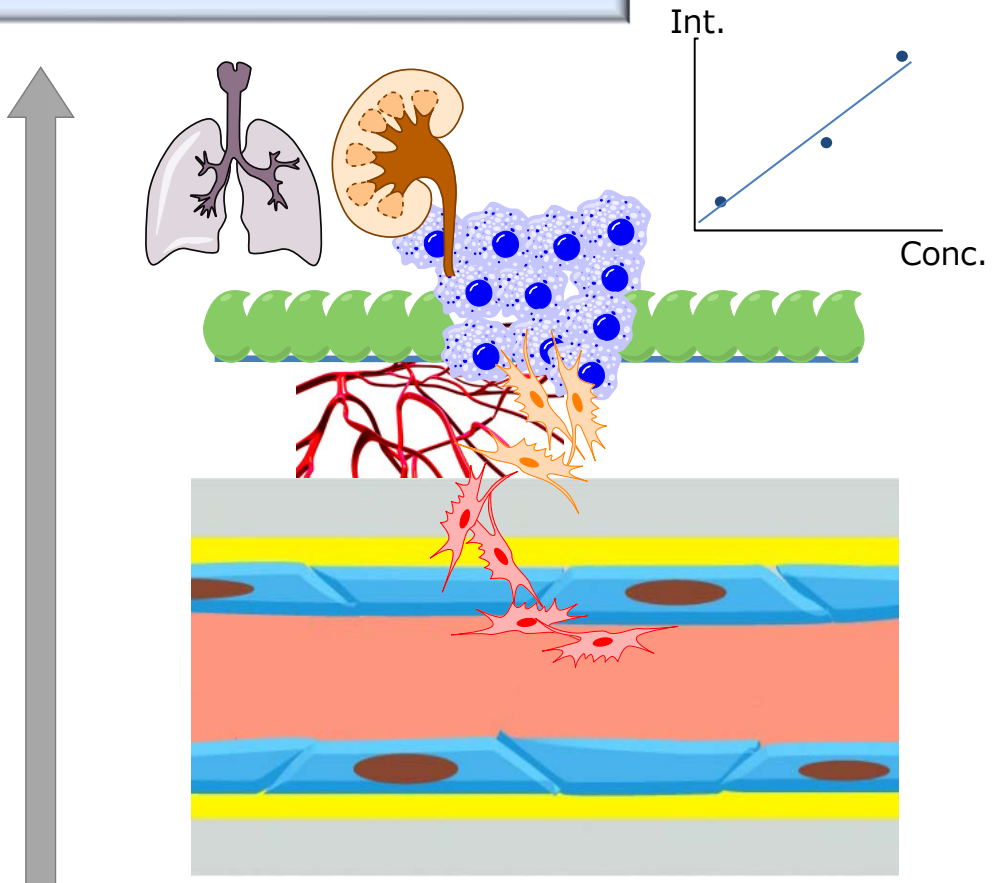
Progress of nSMOL project and clinical trials

Method setting	Full validation	Paper in progress	Clinical trials
25	20	Accepted: 12 In submission: 2	In submission: 3 In trials: 11

Antibodies	Antibodies
Trastuzumab, T-DM1 (0.06-250 µg/ml, Anal Methods, J Pharm Biomed Anal)	Infliximab (0.29-300 µg/ml, Curr Pharm Biotechnol)
Bevacizumab (0.15-300 µg/ml, Drug Metab Pharmacokinet)	Biosimilar of Infliximab (0.29-300 µg/ml, Curr Pharm Biotechnol)
Cetuximab (0.58-300 µg/ml, Bioanalysis)	Etanercept (0.20-100 µg/ml, Pharmacol Res Perspect)
Nivolumab (0.15-250 µg/ml, J Chromatogr B)	Abatacept (0.40-100 µg/ml, Pharmacol Res Perspect)
Rituximab (0.58-300 µg/ml, Biol Pharm Bull)	Tocilizumab (0.78-200 µg/ml, J Pharm Biomed Anal)
Brentuximab vedotin, multiplex assay (0.58-300 µg/ml, Clin Pharma Biopharma)	Coexistence with anti-drug antibodies (Anal Biochem)

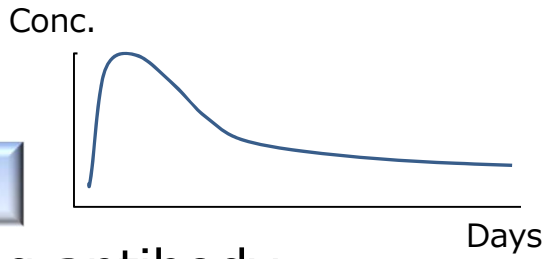
Overall antibody PK for efficacy biomarker

Drug level in disease tissue



Comparative PK
Dosing strategy

Blood monitoring



Effect of anti-drug antibody
Cytokine quantitation
Immune cell profiling

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