Comparative Characterization of Highly Heterogeneous Drugs

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“Complex "Products

CONTEXT

Highly Heterogeneous Drugs

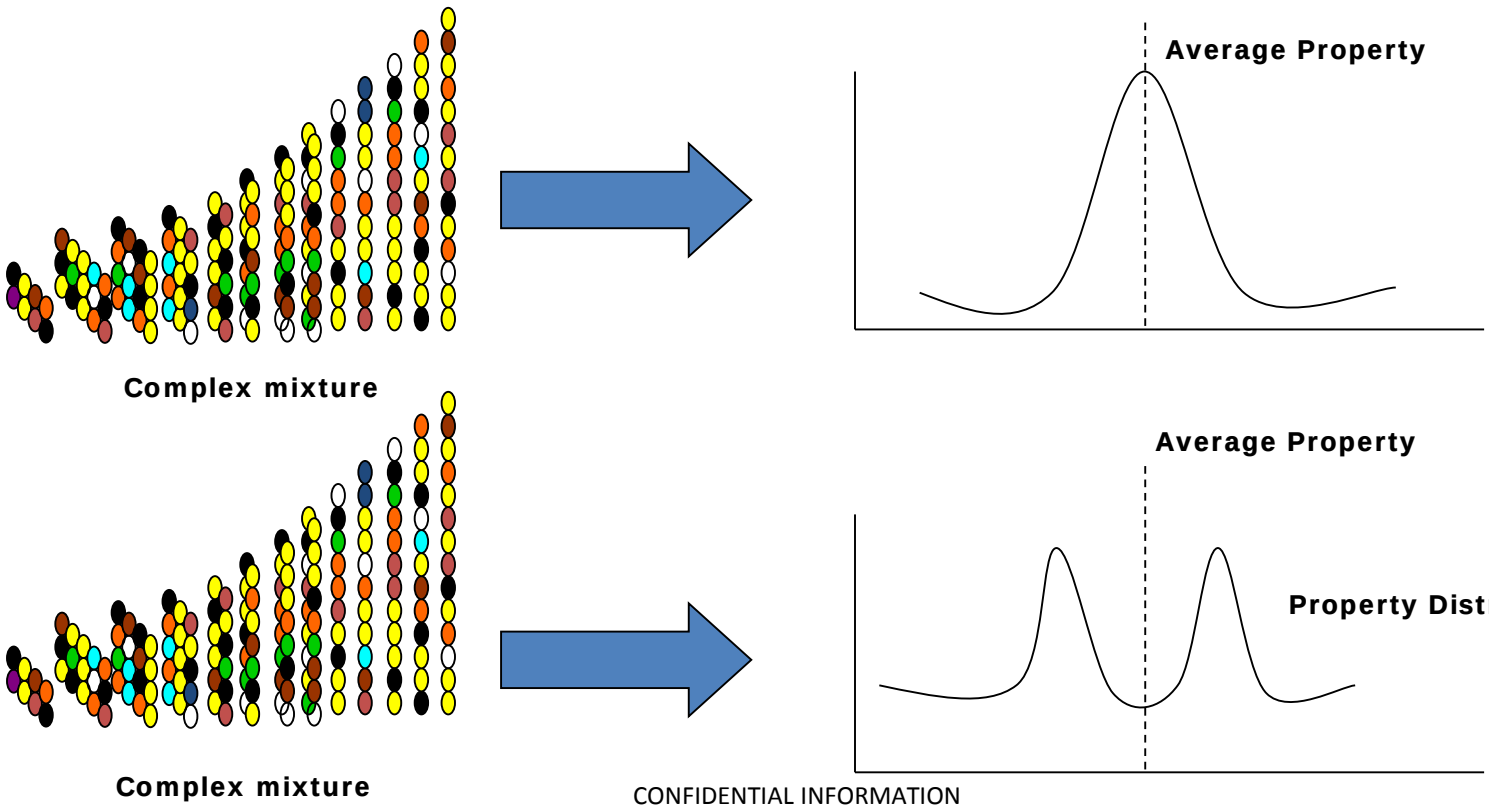
- Complex Drug Mixtures
  - Mixture of polypeptides: Glatiramer acetate
  - Mixture of complex sulfated sugars:
    - Animal source: Heparins, Low molecular weight heparins
    - Plant source: Pentosan polysulfate (Elmiron)
  - Complex formulations: Doxorubicin-Liposome
- Complex Biological Mixtures
  - Glycoprotein mixtures with complex glycosylation patterns: Erythropoetin, monoclonal antibodies, proteins, etc.
  - Vaccines

Approaches to Characterization of Complex Products

- Single and Discrete Analytical Methods
  - 1-D and 2-D NMR Spectroscopy: Quantitative relative abundance of key constituents, linkage position and specificity
  - Mass spectrometry: Comprehensive view of various components (and with some methods being able to do a quantification of components)
  - Analytical separation/characterization (ion exchange, size exclusion, electrophoresis): Obtaining a distribution of molecular weights and chain lengths and characterization of higher order components (microheterogeneity)
- Chemometric approach
  - Calibration of a given analytical method and signals they produce when applied to a complex sample using multivariate models
  - Clustering and pattern identification of signals across different complex samples
  - Quantifying performance metrics (rmsd, minimizing false positives or negatives) of a learning algorithm based on its ability to delineate samples
  - Applying these methods for modeling process and process-product relationship based on machine learning

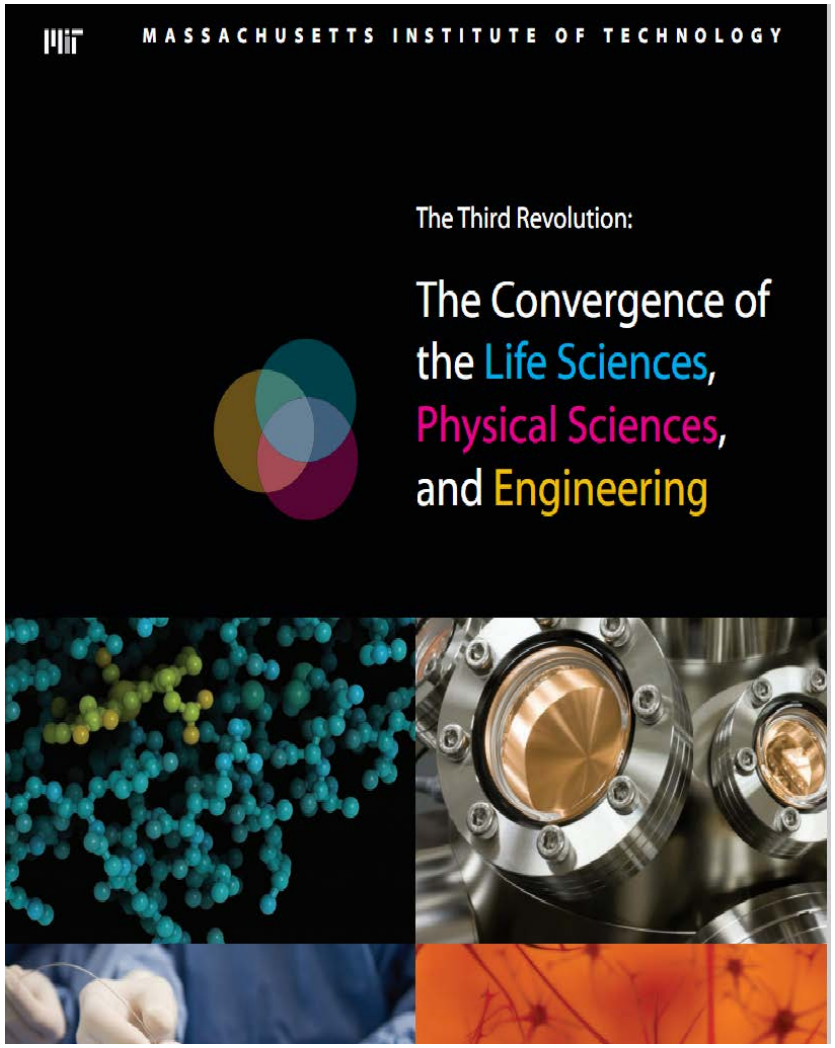
Defining ‘Structure’: Challenges with Complex drugs

- Advanced analytical techniques offer limited “projections” of the complex mixture
- Structural attributes captured as ensemble averages
- Comparability based on “point to point”

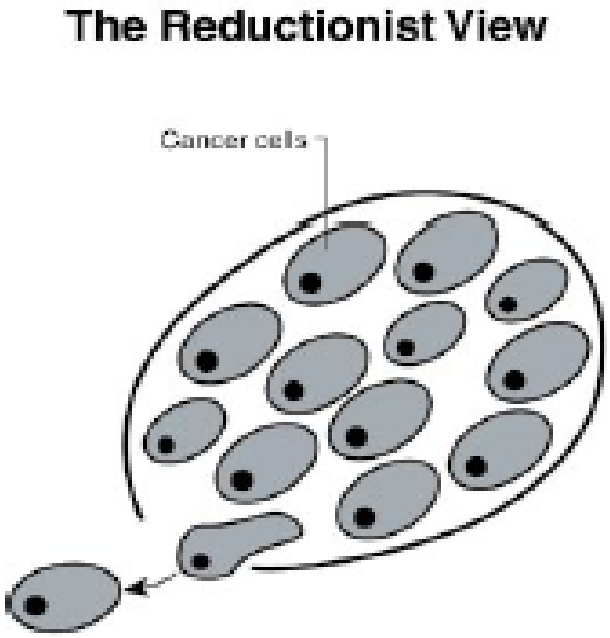


Historic Time: Convergence

- Advances of information technology, materials, imaging, nanotechnology, advanced optics, quantum physics and advanced computing, modeling and simulation, have already transformed Engineering.
  - They are now being brought to bear to transform life science.
- Past decades of advances in molecular biology and genomics have created important new knowledge bases
- The emerging 'convergence' of the life sciences with engineering sciences
- It is an interdisciplinary shift – an expansion of *how* research can be conducted – inclusive of a range of knowledge bases, from biology to computer science to engineering.
- It is not formed around a particular scientific advance but around a new thinking of 'integrated' approach.



Convergence is Transforming Life Sciences

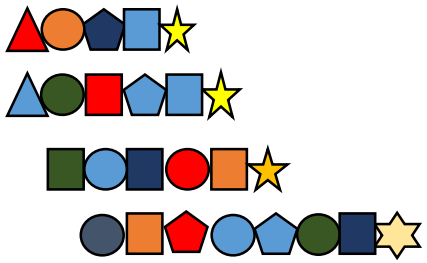


DNA-RNA-Proteins
Technology:
DNA and Protein sequencing
& Recombinant DNA

Complexity in Heterogeneous Molecules
Key Challenges

Starting Material

- Synthetic mixtures such as glatiramer – well defined
- Naturally derived mixtures such as polysaccharides – not well defined



Biological Readouts

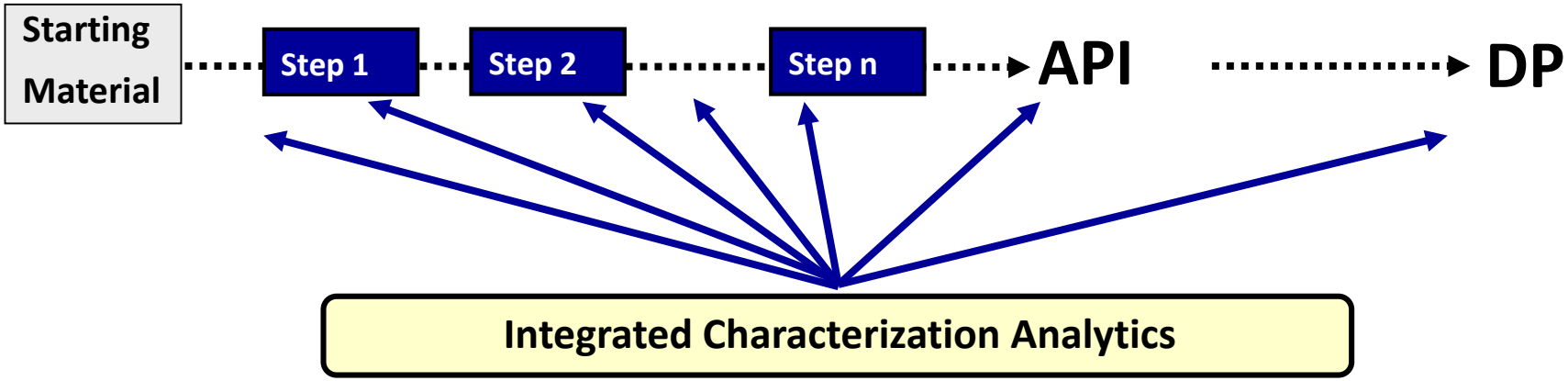
- Heparin/LMWH – structural signature associated with biological activity
- Glatiramer, PPS - Limited knowledge of mechanism of action and structure-activity relationships

Process Chemistry

- How to look at signatures associated with process chemistry on a starting material in intermediates and final product?

Addressing This Complexity

Perspective on defining heterogeneous drugs



Analytics

Orthogonal methods to characterize

- Starting Material
- Process Intermediates
- Product

Data Cross-correlation and Interpretation

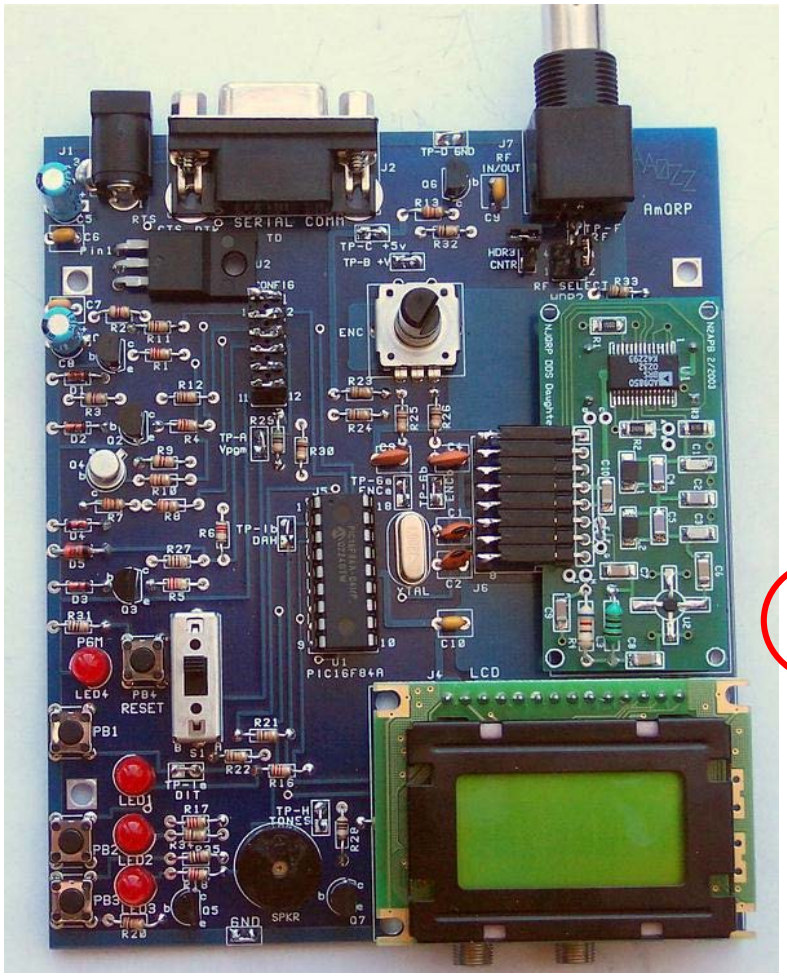
Robust constraints to define key signatures for

- Starting Material (Critical Material Attributes)
- Process Steps (Critical Process Parameters)
- Product (Critical Quality Attributes)

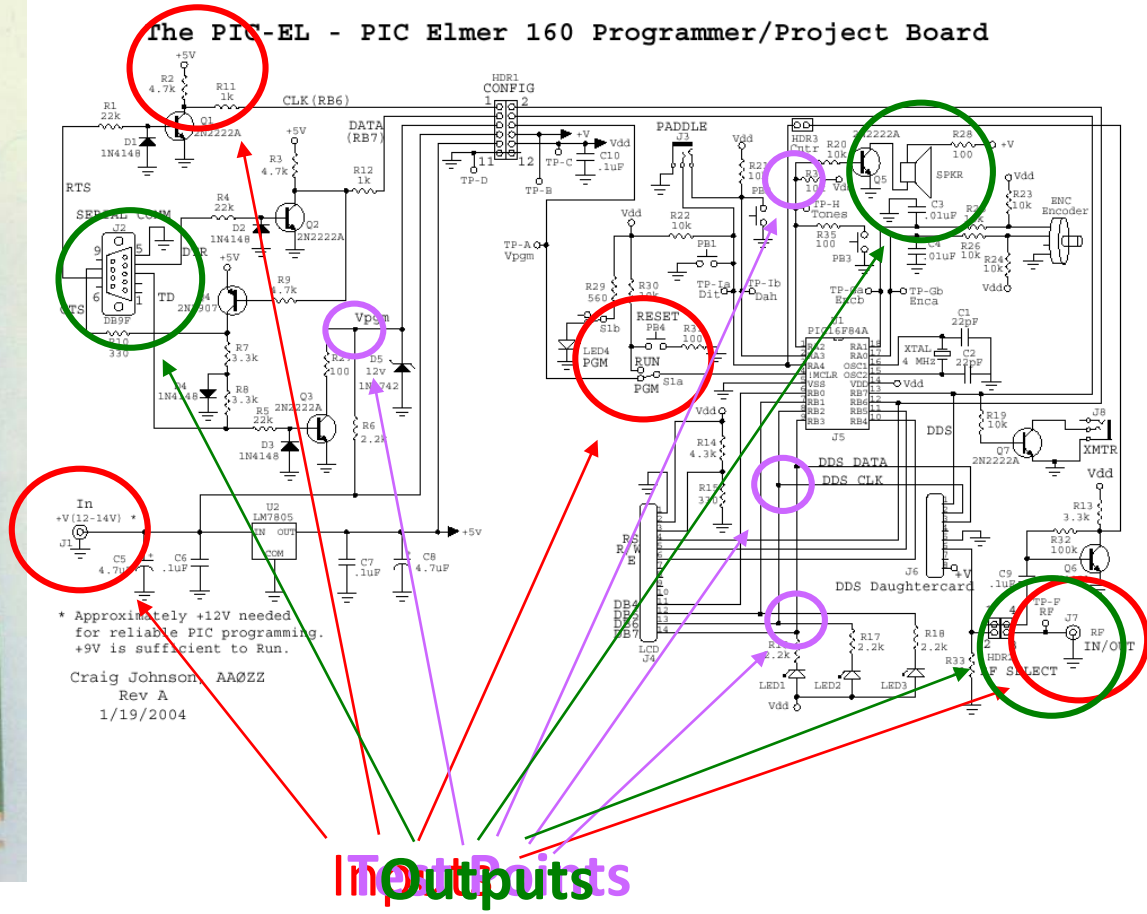
Integrated Process <-> Product Definition

Predictive framework based on design space to identify critical process test points and their impact on critical product attributes

Complex Molecule Process and a Circuit Board



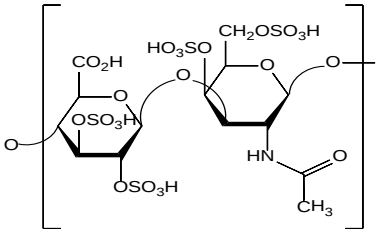
Courtesy Forest White



Product-Process Relationship

Heparin Contamination Story

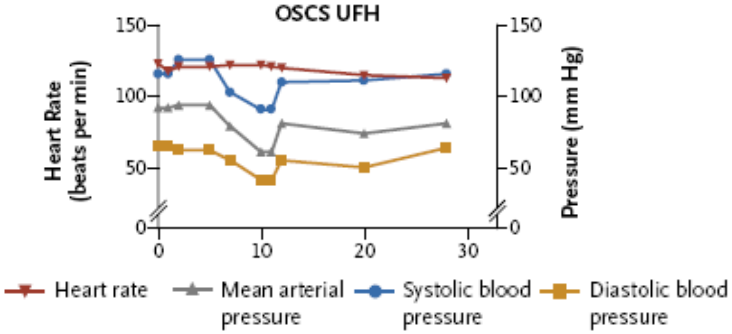
- **The major contaminant is OverSulfated Chondroitin Sulfate (OSCS)**



Oversulfated chondroitin sulfate is a contaminant in heparin associated with adverse clinical events

Marco Guerrini[1,7], Daniela Beccati[2,7], Zachary Shriver[2,3,7], Annamaria Naggi[1], Karthik Viswanathan[3], Antonella Bisio[1], Ishan Capila[2], Jonathan C Lansing[2], Sara Guglieri[1], Blair Fraser[4], Ali Al-Hakim[4], Nur Sibel Gunay[2], Zhenqing Zhang[5], Luke Robinson[3], Lucinda Buhse[4], Moheb Nasr[4], Janet Woodcock[4], Robert Langer[3,6], Ganesh Venkataraman[2,3], Robert J Linhardt[5], Benito Casu[1], Giangiacomo Torri[1] & Ram Sasisekharan[3]

- **Biological Mechanism: OSCS activates the contact system, cross-talk with the complement pathway**



Contaminated Heparin Associated with Adverse Clinical Events and Activation of the Contact System

Takashi Kei Kishimoto, Ph.D., Karthik Viswanathan, Ph.D., Tanmoy Ganguly, Ph.D., Subbiah Elankumaran, Ph.D., Sean Smith, B.S., Kevin Pelzer, Ph.D., Jonathan C. Lansing, Ph.D., Nammalwar Sriranganathan, Ph.D., Ganlin Zhao, M.D., Zoya Galcheva-Gargova, Ph.D., Ali Al-Hakim, Ph.D., Gregory Scott Bailey, B.S., Blair Fraser, Ph.D., Sucharita Roy, Ph.D., Thomas Rogers-Cotrone, M.S., Lucinda Buhse, Ph.D., Mark Whary, Ph.D., James Fox, Ph.D., Moheb Nasr, Ph.D., Gerald J. Dal Pan, M.D., Zachary Shriver, Ph.D., Robert S. Langer, Sc.D., Ganesh Venkataraman, Ph.D., K. Frank Austen, M.D., Janet Woodcock, M.D., and Ram Sasisekharan, Ph.D.

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Outbreak of Adverse Reactions Associated with Contaminated Heparin

David B. Blossom, M.D., Alexander J. Kallen, M.D., M.P.H., Priti R. Patel, M.D., M.P.H., Alexis Elward, M.D., M.P.H., Luke Robinson, B.S., Ganpan Gao, Ph.D., Robert Langer, Sc.D., Kiran M. Perkins, M.D., Jennifer L. Jaeger, M.D., Katie M. Kurkjian, D.V.M., M.P.H., Marilyn Jones, R.N., M.P.H., Sarah F. Schillie, M.D., M.P.H., Nadine Shehab, Pharm.D., Daniel Ketterer, M.D., Ganesh Venkataraman, Ph.D., Takashi Kei Kishimoto, Ph.D., Zachary Shriver, Ph.D., Ann W. McMahon, M.D., K. Frank Austen, M.D., Steven Kozlowski, M.D., Arjun Srinivasan, M.D., George Turabelidze, M.D., Ph.D., Carolyn V. Gould, M.D., Matthew J. Arduino, Dr.P.H., and Ram Sasisekharan, Ph.D.

ABSTRACT

- **Epidemiology: Adverse events are correlated with administration of contaminated heparin**

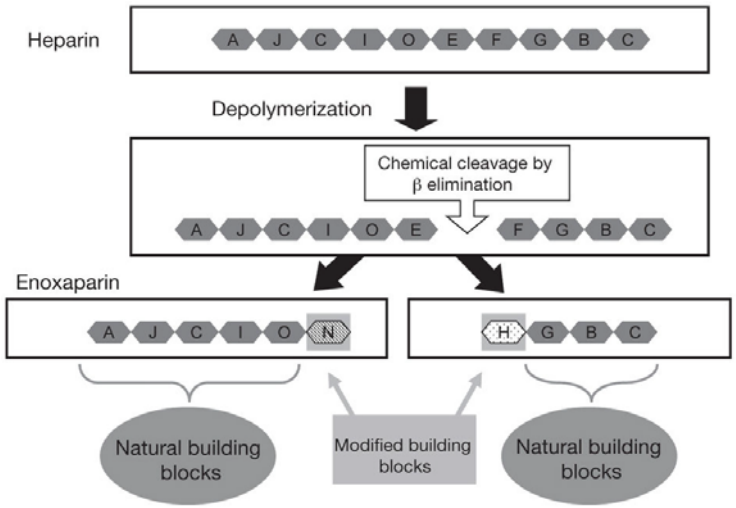
“Complex "Products

EXAMPLES

Defining Enoxaparin

Starting material USP Heparin:
- Mw, Mn, polydispersity, sulfation degree
- High relative abundance of trisulfated disaccharide – $I_{2S}H_{NS,6S}$
- Structural signature G-$H_{NS,3S,6S}$- associated with anticoagulant activity
- Linkage region oligomer distribution, etc.

Analytics:
- Mw, Mn, polydispersity
- Degree of sulfation
- Monosaccharide components
- Di/tri/tetrasaccharide components
- Higher order components (Microheterogeneity)



Data Cross correlation:
- GPC-TDA <-> Size fraction profile
- HSQC NMR <-> Enzyme digest CE building blocks, etc.

Process Chemistry:
- Chemical depolymerization
- Delta4,5 unsaturated linkage on non-red
- 1,6-andhydro linkage on reducing end
- Epoxide and Galacturonic acid formation
- Purification (final size or molecular weight distribution)

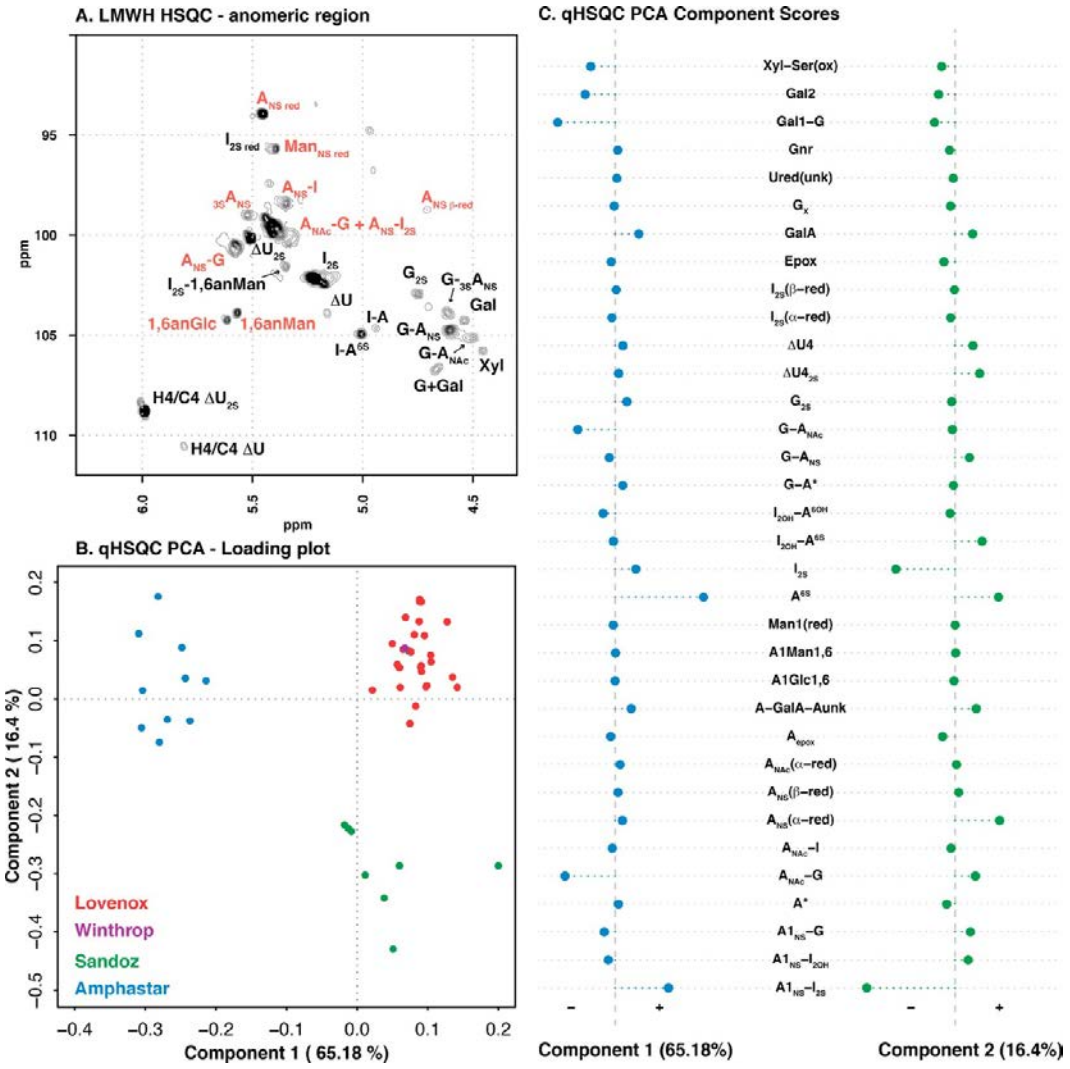
Test-points for Complex Mixture Definition

Signatures related to process:
- Distribution of non-red. and red. end sugars across size/affinity fractionated oligomers
- Oligomer size distribution

Signatures related to starting material:
- Distribution of $I_{2S}H_{NS,6S}$ and G-$H_{NS,3S,6S}$ across oligomers
- Linkage region oligomer (Gal-Gal-Xyl-O-Ser) distribution

Generic Enoxaparins

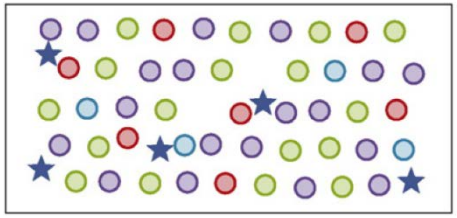
Chemometric differentiation using NMR



- **Possible drift in starting material (sulfation degree, linkage region sugars, etc.)**
- **Small changes in chemical environment during manufacturing (epoxide and Galacturonic acid, etc.)**
- **Small variations in process (sulfation degree, reducing, non-reducing end, chain size distribution, etc.)**

Characterization of Glatiramer Acetate

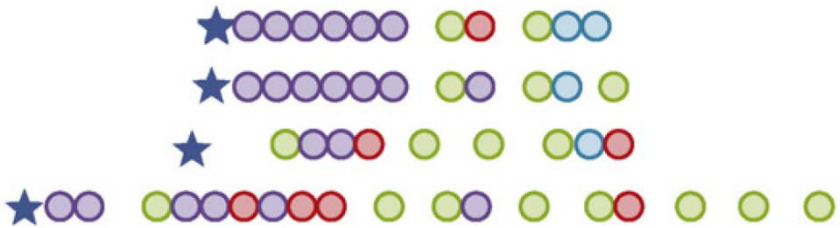
Starting Material



★ : Initiator **Diethylamine**

○○○○ : Monomers

E K T A – N-carboxyanhydrides



- **Mixture of several heterogeneous polypeptides where chain has very low relative abundance**
- **Cannot be defined the same way as natural product-derived Enoxaparin or PPS**

Analytics

- Molecular Weight
- N-terminal Sequencing
- Capped C-term, Uncapped C-term and ratios
- Spectroscopic fingerprinting

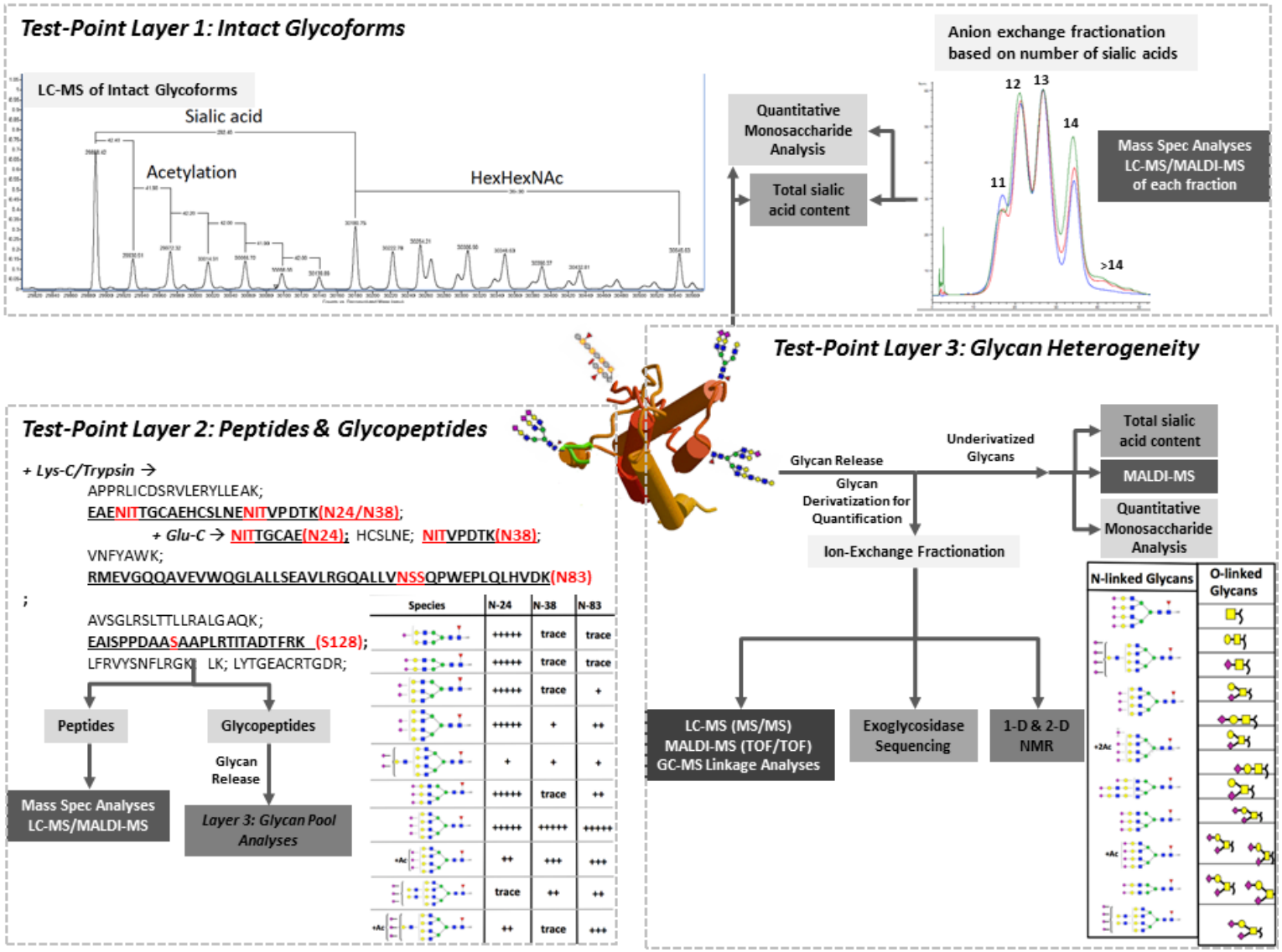
Process Chemistry:

1. Polymerization (Int-1)
2. Depolymerization /Deprotection (Int-2)
3. Deprotection (Int-3)
4. Purification (Final Product)

Test-Points for Complex Mixture Definition

- Model for process chemistry through defining kinetics of initiation, propagation and depolymerization
- Validating model test points by correlating modeled events with analytical readouts across process intermediate layers and final product

Characterization of Recombinant Human EPO API from Epogen®



Dataset-Cross Correlation

Sugars	Intact glycoform	Site spec glycans	N-glycan pool	O-Glycan Pool
Mannose	X	X	X	
Gal	X	X	X	X
GlcNAc	X	X	X	X
GalNAc	X			X
Fucose	X	X	X	
NeuAc	X	X	X	X

'X' indicates that the presence and relative abundance of the sugar is correlated across different measurements in each column

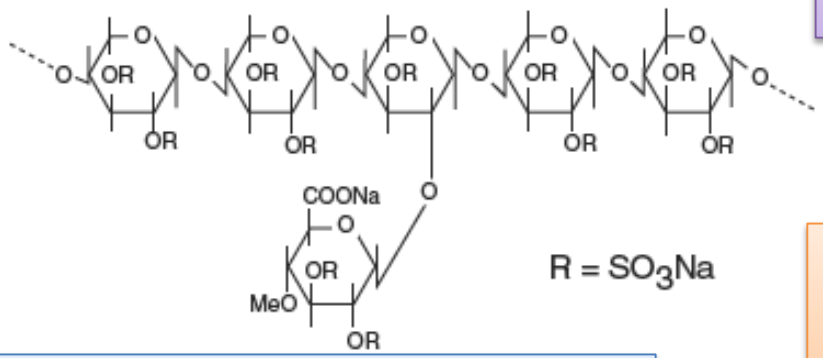
Data correlation between test-points permits

- Definition of key signatures to compare drift within RLD and between RLD and other manufacturers
- Validating upstream and downstream processes <-> product signatures
- Robust framework for integrated definition of the mixture

Extending Enoxaparin Definition to Other Polysaccharide Mixtures

Starting material:
- Beechwood Xylan
- Poorly Defined

Pentosan polysulfate

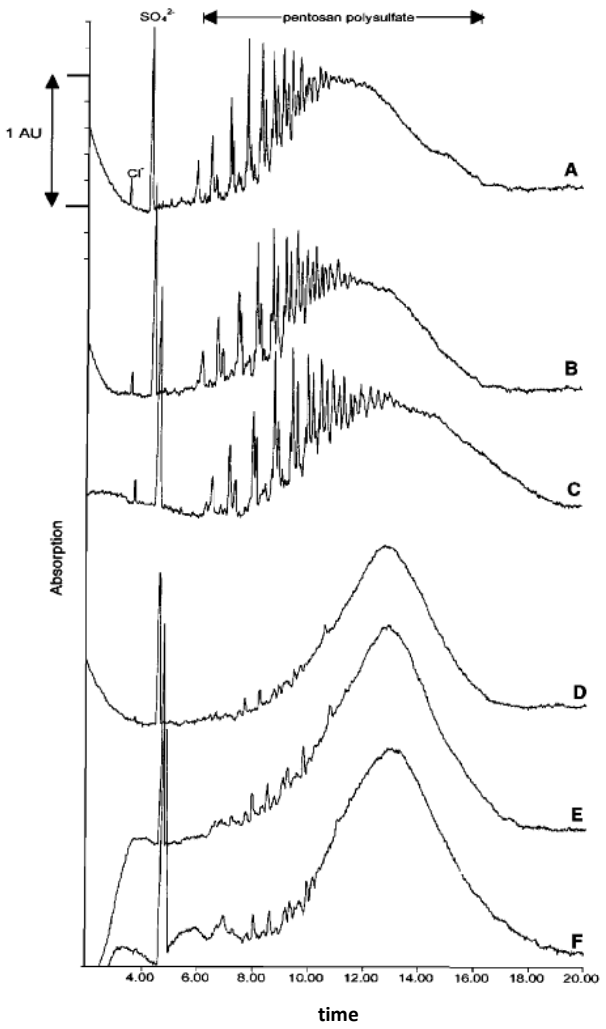


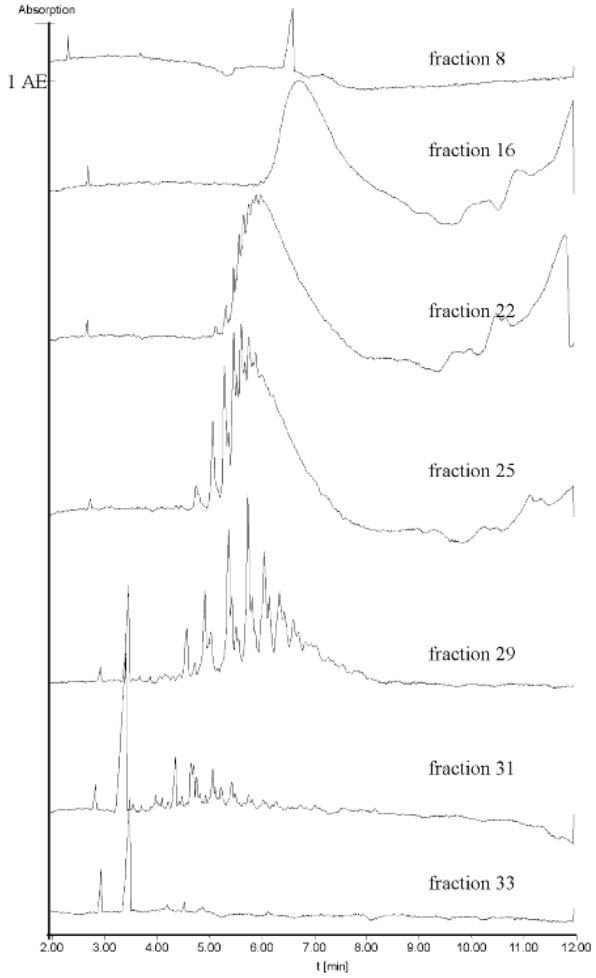
Analytics:
- Mw, Mn, polydispersity
- Sulfation degree
- Building Blocks
- Higher order components

Process Chemistry:
- Chemical sulfation of xylan
- Chemical Depolymerization of sulfated xylan and purification to achieve desired size (molecular weight) distribution

Defining Mixture: Considerations
- Branched vs. Linear
- Process-related vs. natural sulfation
- Need to establish key signatures

Characterization of PPS: Existing Methods

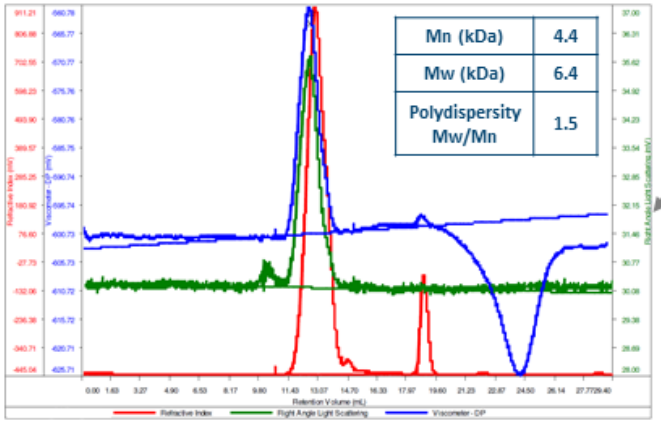




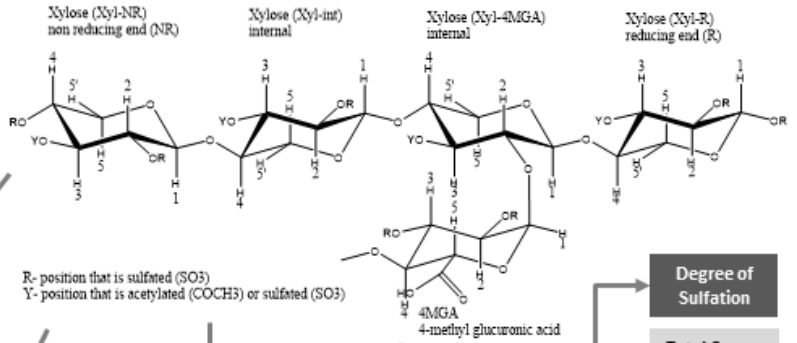
- Capillary Zone Electrophoresis separates species based on sulfation pattern
- There are differences in the CZE profiles of PPS from manufacturer 1 (A, B,C) and manufacturer 2 (D, E, F) but it is not know what these differences correspond to from the standpoint of the finer structural heterogeneity

Integrated Characterization of PPS

Test-Point Layer 1: Aggregate Properties



Mn (kDa)	4.4
Mw (kDa)	6.4
Polydispersity Mw/Mn	1.5



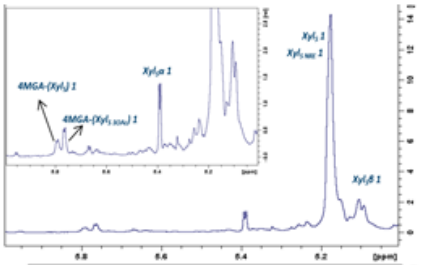
Sulfates/Xylose	2.2
Total sugar (%wt /wt)	40
4MGA (% wt/wt)	5

Degree of Sulfation

Total Sugar Content

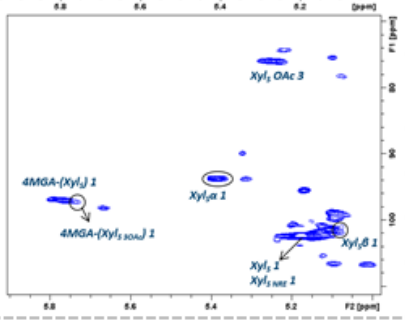
Uronic Acid Content

Test-Point Layer 2: Building Blocks



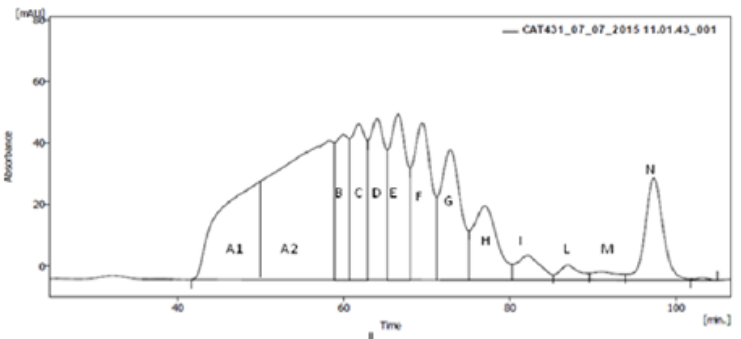
1-D Proton NMR

2-D HSQC NMR



Xyl_5-int + Xyl_5-NR	~ 81 (+/- 0.5)
Xyl_5-R	~11.5 (+/-0.2)
4MGA	~ 4 (+/- 0.3)
Xyl $_{5,3OAc}$	~3.6 (+/- 0.2)

Test-Point Layer 3: Oligosaccharide Microheterogeneity



LC-MS

Analyses of each fraction

1-D/2-D NMR

Representative oligomers:

Xyl[2S,3S,4S]-(Xyl[2S,3S])$_4$-Xyl[2S,3S]

Xyl[2S,3S,4S]-Xyl[2S,3S]-Xyl[2S,3S]-Py

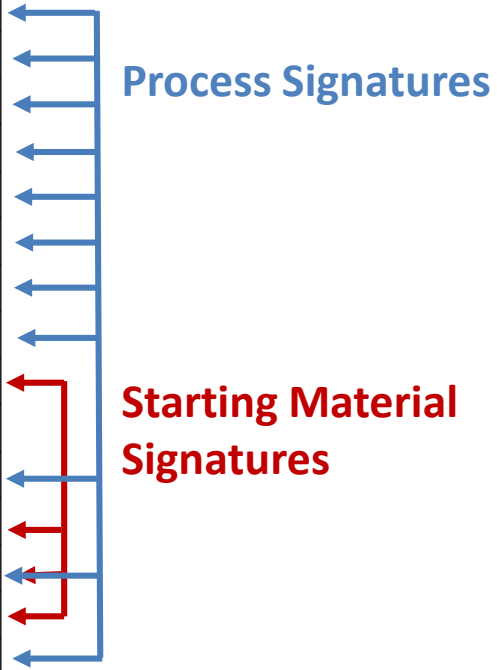
Xyl[2S,3S]-(Xyl[2S,3S])$_4$-Xyl[2S,3S]-H_2O

Xyl[2S,3S,4S]-Xyl[2S,3S]-Xyl[2S,3S]-Me

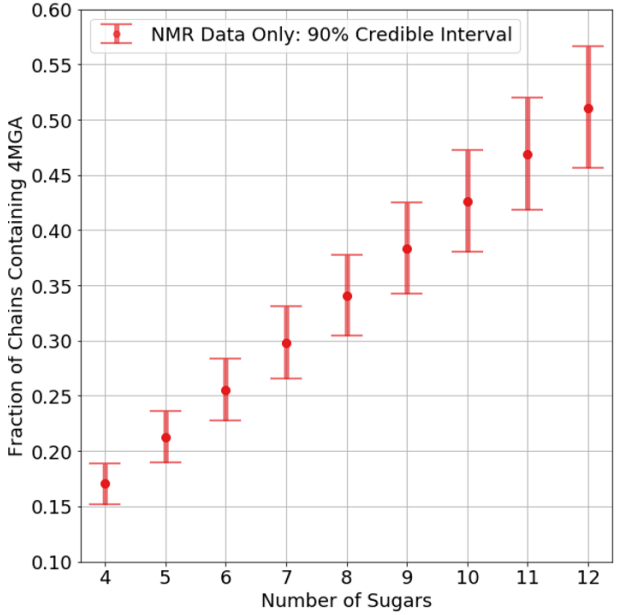
Xyl[2S,3S,4S]-{4MGA[2S,3S]-Xyl[3Ac]}-(Xyl[2S,3S])$_2$-Xyl[2S,3S]

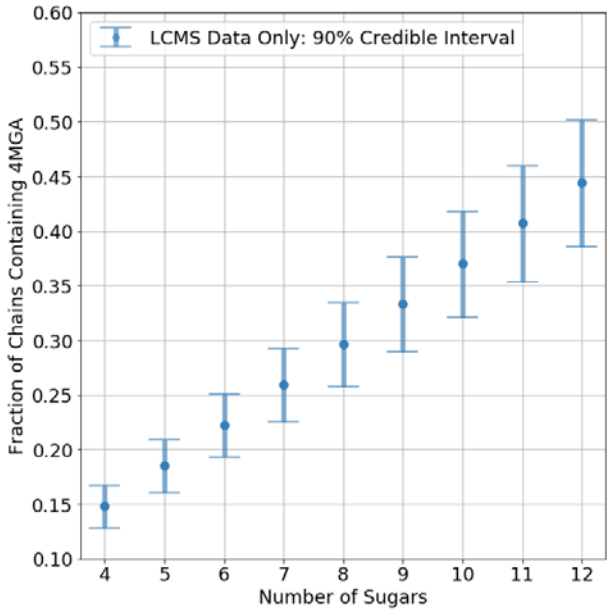
Framework for Data Integration Across Test Points

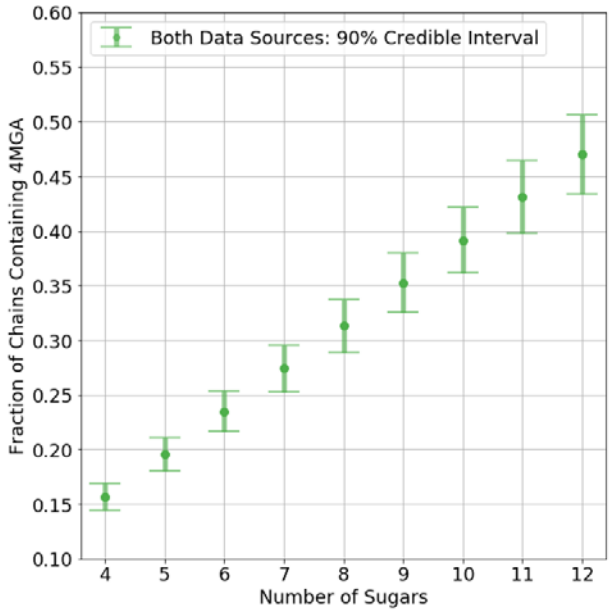
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
S4-X	0.08	0.93	1.83	2.73	3.54	3.69	3.45	3.02	2.47	1.91	1.33	0.92	0.63	0.44	0.31	0.22	0.16	0.10
S4-X-1DH	0.01	0.10	0.19	0.29	0.37	0.39	0.36	0.32	0.26	0.20	0.14	0.10	0.07	0.05	0.03	0.02	0.02	0.01
S4-X-1M	0.01	0.15	0.29	0.44	0.57	0.59	0.56	0.49	0.40	0.31	0.21	0.15	0.10	0.07	0.05	0.03	0.03	0.02
S4-X-1P	0.01	0.14	0.28	0.42	0.55	0.57	0.53	0.46	0.38	0.29	0.20	0.14	0.10	0.07	0.05	0.03	0.02	0.02
S4-X-1S	0.01	0.07	0.14	0.20	0.26	0.28	0.26	0.23	0.18	0.14	0.10	0.07	0.05	0.03	0.02	0.02	0.01	0.01
S4-X-G	0.00	0.08	0.24	0.49	0.84	1.11	1.29	1.38	1.38	1.29	1.09	0.92	0.77	0.66	0.58	0.53	0.51	0.49
S4-X-G-1DH	0.00	0.01	0.04	0.09	0.15	0.20	0.23	0.24	0.24	0.23	0.19	0.16	0.14	0.12	0.10	0.09	0.09	0.09
S4-X-G-1S	0.00	0.03	0.06	0.08	0.11	0.11	0.10	0.09	0.07	0.06	0.04	0.03	0.02	0.01	0.01	0.01	0.00	0.00
S4-X-GA	0.01	0.10	0.28	0.56	0.93	1.20	1.38	1.47	1.45	1.34	1.12	0.94	0.79	0.67	0.59	0.53	0.51	0.50
X	0.01	0.09	0.17	0.26	0.33	0.35	0.32	0.28	0.23	0.18	0.12	0.09	0.06	0.04	0.03	0.02	0.01	0.01
X-1DH	0.02	0.18	0.35	0.52	0.68	0.70	0.66	0.58	0.47	0.36	0.25	0.18	0.12	0.08	0.06	0.04	0.03	0.02
X-GA	0.00	0.01	0.03	0.05	0.09	0.11	0.13	0.14	0.14	0.13	0.11	0.09	0.08	0.06	0.06	0.05	0.05	0.05
X-GA-1DH	0.00	0.01	0.03	0.05	0.09	0.11	0.13	0.14	0.14	0.13	0.11	0.09	0.08	0.06	0.06	0.05	0.05	0.05
X-GS	0.00	0.01	0.03	0.05	0.08	0.11	0.12	0.13	0.13	0.12	0.10	0.08	0.07	0.06	0.05	0.05	0.05	0.04
X-GS-1DH	0.00	0.01	0.04	0.08	0.12	0.16	0.19	0.20	0.19	0.18	0.15	0.13	0.11	0.09	0.08	0.07	0.07	0.07



Significance of Test Point Data Integration



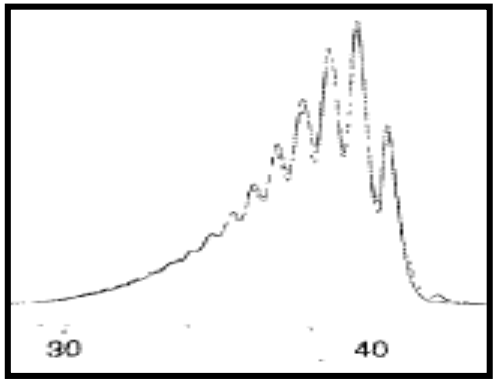


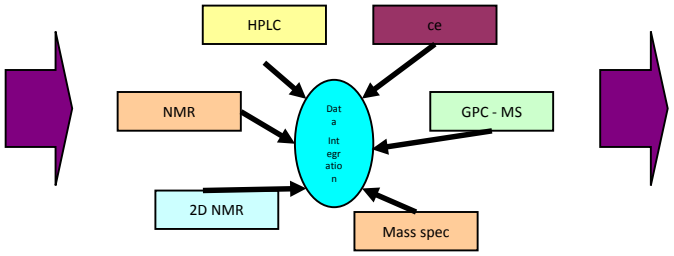


The Need for Data Integration

Summary of Data Integration Method

- Integration of unique, yet complementary data sets (derived from multiple analytical methods) enables the structural identification of complete mixture
- Analogous to image reconstruction
- Provides a rationale for methods (data sets) that are both necessary and sufficient to demonstrate thorough characterization as well as equivalence





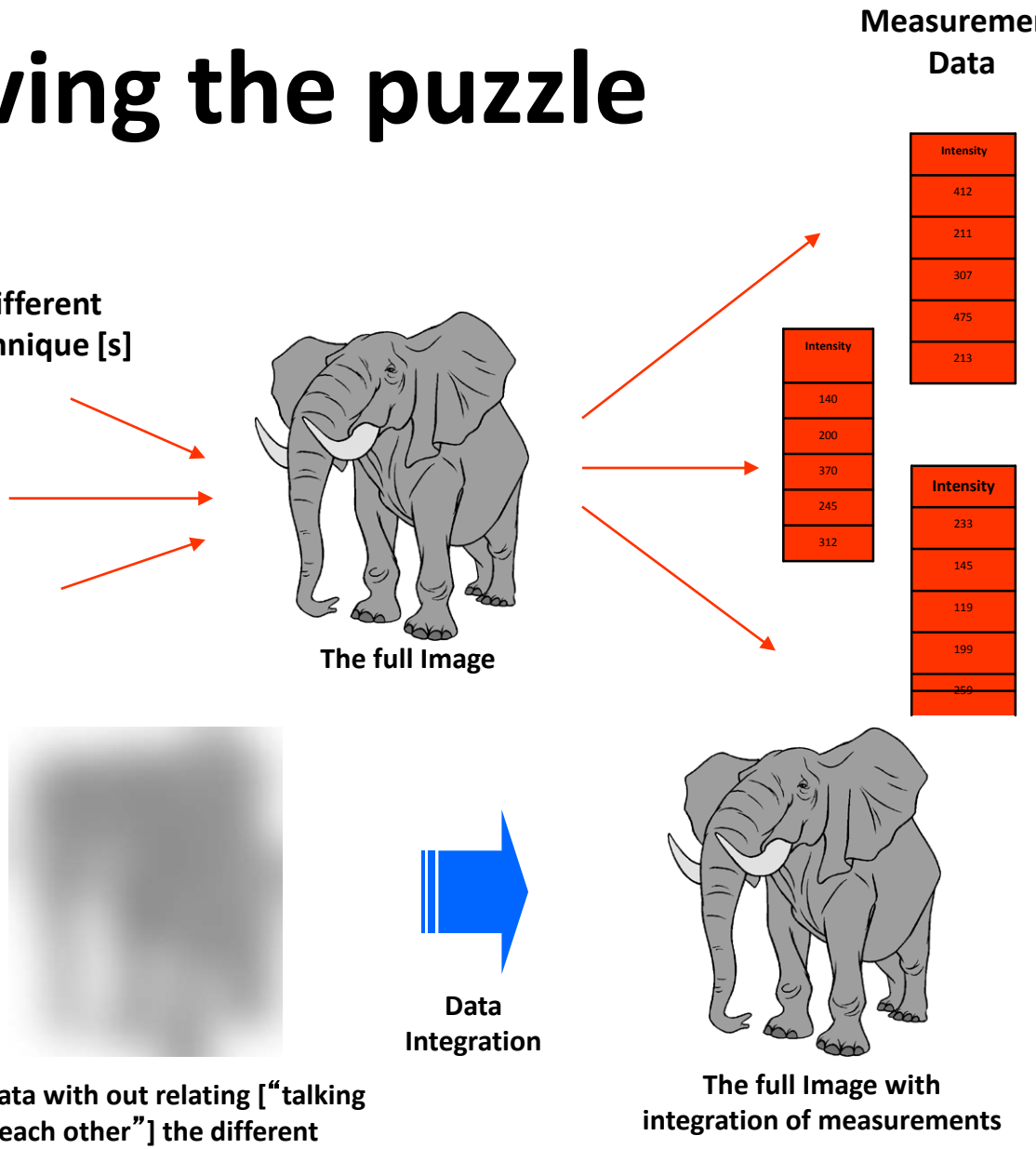
Sequence	Prevalence
SBN135NY89T6J7	57
GGH13HF68L4N5	63
SDFP	231
23HJKLO99	4
45THQCX8547	71
48DSMX	123
…..	
…….	

Earlier Approaches

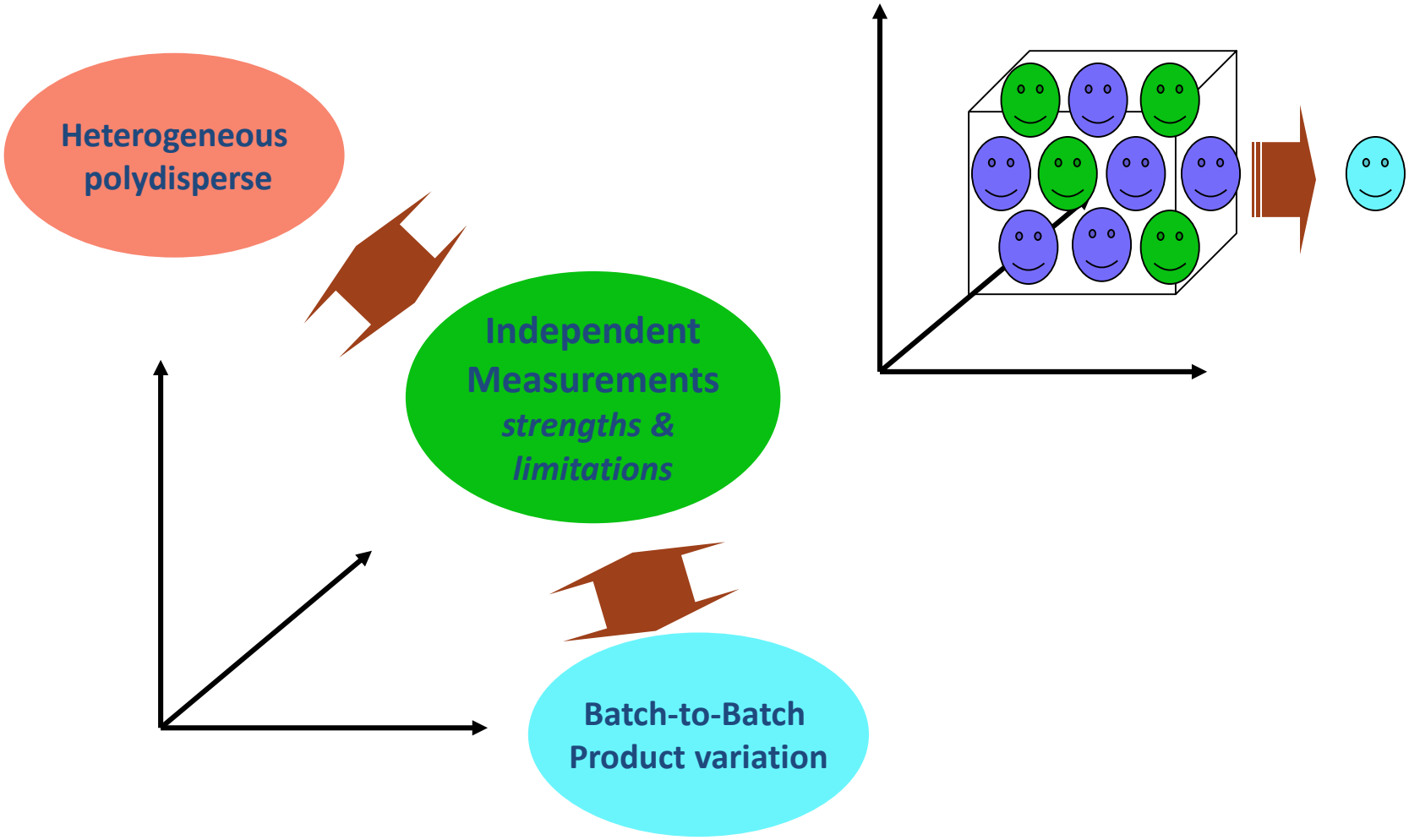
Multiple State-of-the-Art Characterization Technologies

Data Integration

Solving the puzzle



Multivarient Equivalence Windows for Complex Biologics Needs to be Established



Summary

- Current analytical techniques provide only selected "perspectives" or "projections" and thorough characterization of entire product is critical for assuring sameness and is now possible.

- For complex molecules there is a need for multiple measurements from orthogonal analytical techniques that provide different "perspectives"; these techniques can be integrated in a multidimensional "equivalence window" for demonstration of sameness

- Important to develop Product-Process relationship – this is analogous to a circuit board – network and connectivity parameters become important in defining the product