DVS Sciences' Reagents Roadmap: Near and Far

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in which I take credit for the brilliance of the R&D team

DVS Sciences

Discover more.
Imagine more.

MaxPar® Panel Kits: Key Benefits

- MaxPar Panel Kits are the first complete reagent solution for CyTOF® and CyTOF® 2 experiments

- Kit includes all the reagents require to conduct 16-17 marker assay

- Panel Kit Contents
  - MaxPar® Metal Conjugated Antibodies (MCA) 16-17 per kit
  - MaxPar® Cell Staining Buffer
  - MaxPar® Fix and Perm
  - MaxPar® Intercalator (Ir)
  - MaxPar® Water
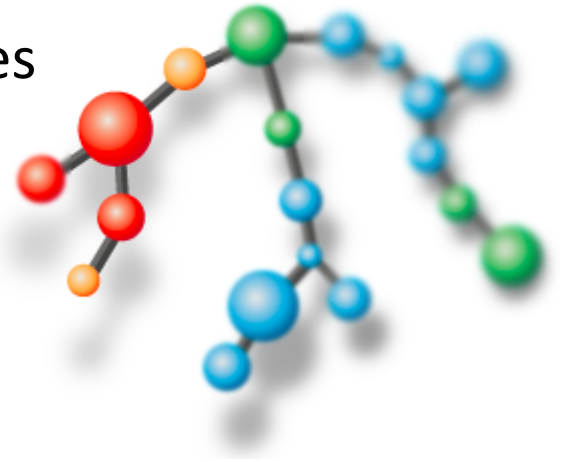
MAXPAR® Panel Kits

● **Human Kits**

  ● PB Phenotyping   17 marker

  ● T cell Phenotyping   16 marker

  ● PBV Basic Phenotyping   7 marker

● **Mouse Kits**

  ● Sp/LN Phenotyping   16 marker

  ● Sp/LN Basic Phenotyping   6 marker

● additional panels being developed for subsequent availability

sensitivity

Necessary characteristics for effective antibody tag
- stable, tightly bind, maintain ab avidity, …

Desirable characteristics
- maximum # of tag atoms, universal construct …

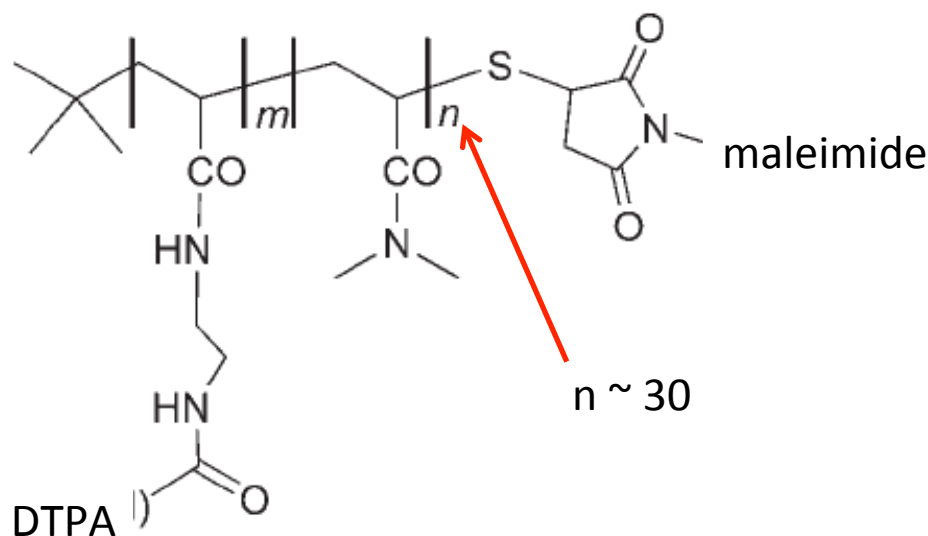How we approach those
- monodisperse metal-chelating polymer

Currently offer two (effectively equivalent) polymers
- X8
- DN3

sensitivity

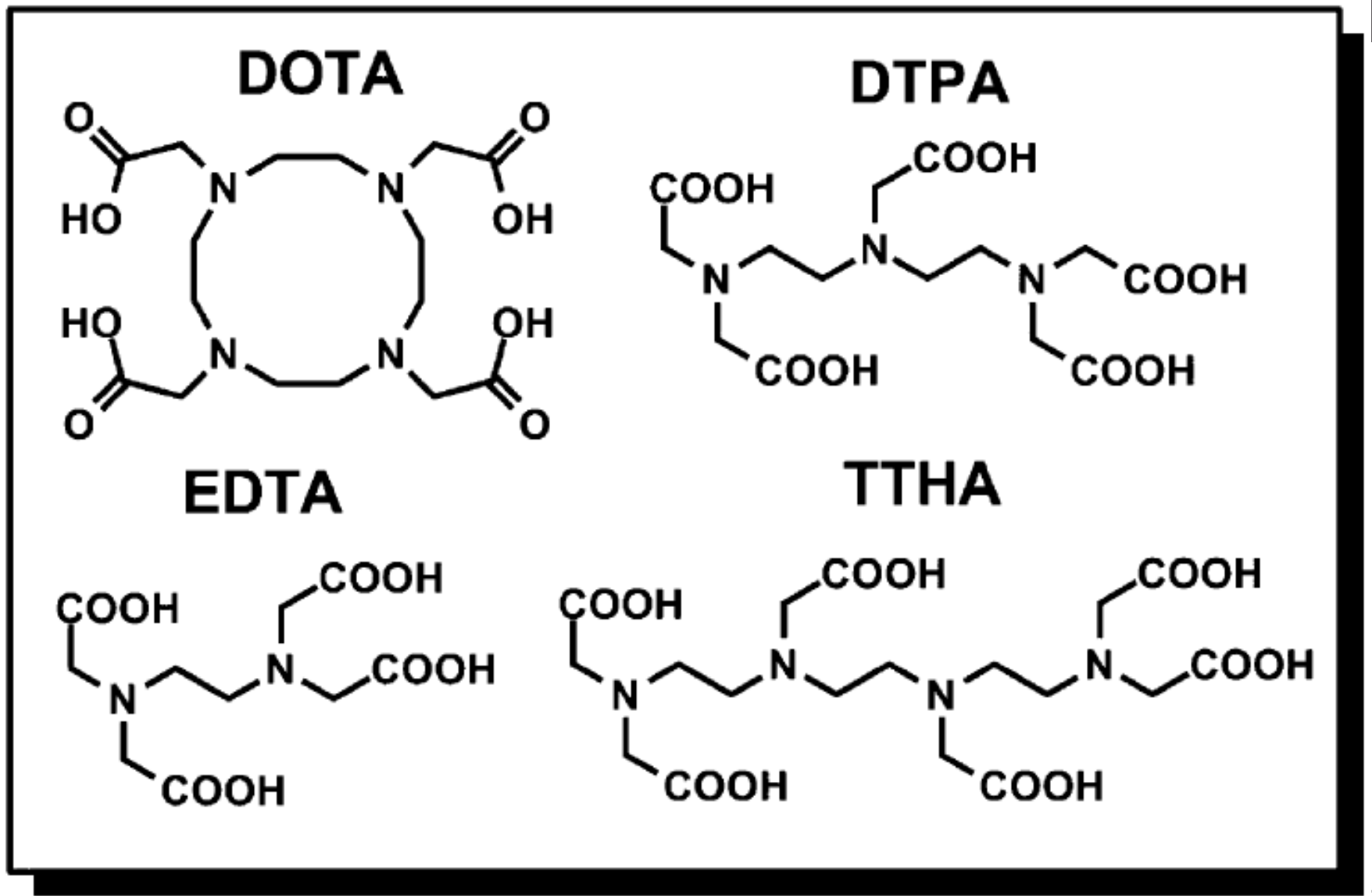Have never shown the structure of the X8 polymer

- but you may have seen a related structure published in 2007:

maleimide

n ~ 30

DTPA

Lou et al, Angewandt Chemie 46, 6111 (2007)

- X8: different backbone; n ~ 22

- DN3: based on a cystamine-core dendrimer; n ~ 16

chelators

DOTA **DTPA** **EDTA** **TTHA**

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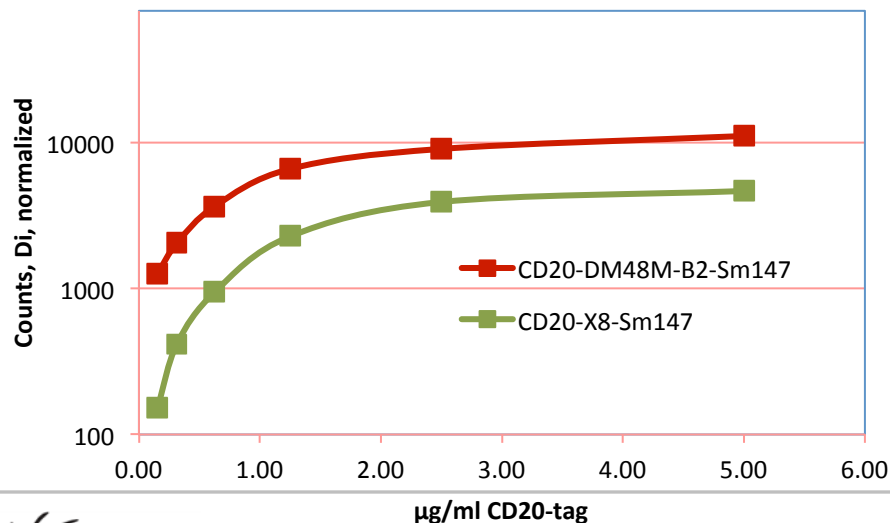
sensitivity

New tagging polymer in development
- variants on "DM48M"

DM48M

Item	Sample	Copies of chelator	Atoms/Ab	Tags/Ab
1	DM48M_CD20-147Sm	100	365.89	3.7
2	DM48M_CD45-154Sm	100	382.40	3.8
3	DM48M_CD25-149Sm	100	330.67	3.3
4	DM48I_CD20-147Sm	100	418.38	4.2
5	DM48I_CD45-154Sm	100	260.32	2.6
6	DM48I_CD25-149Sm	100	318.43	3.2
7	X8_CD20-147Sm	22	95.32	4.3
8	X8_CD45-154Sm	22	84.60	3.8
9	X8_CD25-149Sm	22	68.56	3.1

X8

Titration for CD20-tag on Ramos

CD20-DM48M-B2-Sm147

CD20-X8-Sm147

Counts, Di, normalized

µg/ml CD20-tag

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sensitivity

long term goal

- quantum step in sensitivity using nanocrystal probes
    - e.g., a 10 nm dia La nanocrystal ~ 10,000 La atoms
    - poster #B193: Pengpeng et al
        - can make them
        - can functionalize them
        - need to deal with non-specific background issues

new metals

mid- to longer- time frame

- increased mass range facilitates use of ^{89}Y and ^{209}Bi as additional antibody probes
  - both work with current polymer tags
  - Y is particularly low mass = low sensitivity (for "bright" antigens
  - Bi loading requires special protocol = to be offered in conjugate ab's only
- ^{115}In loading works – expect to offer in conjugated ab's only
- continue to work on introducing new metals to expand the arsenal (prospects include Ru, Hf, Zr)
- isotopically-enriched cisplatin viability probe
- isotopically-enriched Pd isotopes for bar-coding

universality

linkers
- currently use maleimide
    - works well for most human and mouse ab's

for special cases, working on
- amine linkers (sulfo-SMCC)
- azide linkers

beads - passport

- 4-element "calibration" beads

  - Ce, Eu, Ho, Lu
    - ^{140}Ce (88.5%), ^{142}Ce (11.1%)
    - ^{151}Eu (47.8%) ^{153}Eu(52.2%)
    - ^{165}Ho (100%)
    - ^{175}Lu (97.4%)

  - Ce is unique identifier - easy to distinguish

  - Ce and Ho oxides inform on plasma temperature

  - together define mass response curve = quantification

- after tuning, confirm operational characteristics (=passport)

- added to sample, act as internal standard for quantification

- added to sample, provide basis for normalization

beads - magnetic

informational purposes

- we are acquiring magnetic beads from various manufacturers
  - will determine elemental composition
    - to advise which may be used without introducing lanthanide (or other) contamination
- we will share results so as to help advise which beads are most useful (and if appropriate advise on special precautions)

the evolution of mass cytometry
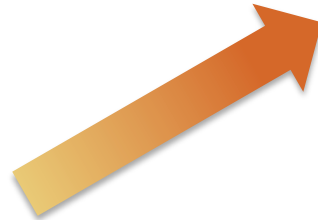
CyTOF® 2 upgrade path

Specification			CyTOF	CyTOF 2	Benefit	Upgrade Path
Sample	Type		Single cell suspension	Single cell suspension	No change	No change
	Introduction -	manual	Syringe, Single loop	Auto-charge syringe, Dual loop	Improves ease of use	**Yes**
		automatic	96 well autosampler	96 well autosampler	No change	No change
	Throughput -	events/sec	1000	1000	No change	No change
		parameters/sec	30,000	30,000	No change	No change
	replicate sample CV		< 3%	< 3%	No change	No change
Hardware	Channels		up to 91	up to 120	Increases possible parameters per cell	**Yes**
	Instrument Response (counts/pg Tb)		200,000	400,000	Improves detection of low abundance targets	**Yes**
	Dynamic Range (orders of magnitude)		4	4.5	Allows wider range of signal detection	**Yes**
	Tuning, Calibration and normalization		manual	automated	Improves data reproduciblity and ease of use	**Yes**