BECKMAN COULTER
Life Sciences

Next-generation Polymer Dyes— A Stellar New Way to See Dim Populations

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Introduction

Beckman Coulter Life Sciences has introduced two new tandem polymer dyes: SuperNova (SN) v605 and v786 (and associated conjugated antibodies). SN v605 and v786 have their maximum excitation at 414 nm, and emission maxima at 605 nm and 786 nm respectively. They are detected using the 610/20 and 780/60 nm bandpass filters of the flow cytometer.

These polymer dyes are brighter for their respective channel (allowing the assessment of dim populations by flow cytometry) and show minimal nonspecific staining (attributed to the proprietary formulation), ultimately providing flow cytometry laboratories with greater confidence in their results.

In addition to optimization of the individual analyte specific reagent (ASR) conjugated antibodies performance, the associated manufacturing processes are being enhanced and validated to meet Current Good Manufacturing Practices (cGMPs) requirements and to deliver conjugates with low variability.

In the study, SN v605 and v786 were tested for their compatibility with other fluorochromes.

Material & Methods

Three different lots of SuperNova conjugates were used to stain whole blood specimens. Median fluorescence intensity, % recruitment of positive cells, stain index and non-specific binding on negative populations (monocytes or granulocytes) were analyzed and compared against performance of commercially available polymer dye conjugates at their recommended dose.

SuperNova tandem dyes are synthesized by conjugating a highly fluorescent polymer backbone with desired acceptor dye, followed by reactions to activate and conjugate it to a respective antibody (Figure 1). Tandem conjugates are purified to remove impurity in order to attain consistent fluorophore-to-protein ratio, thus ensuring consistent flow performance.

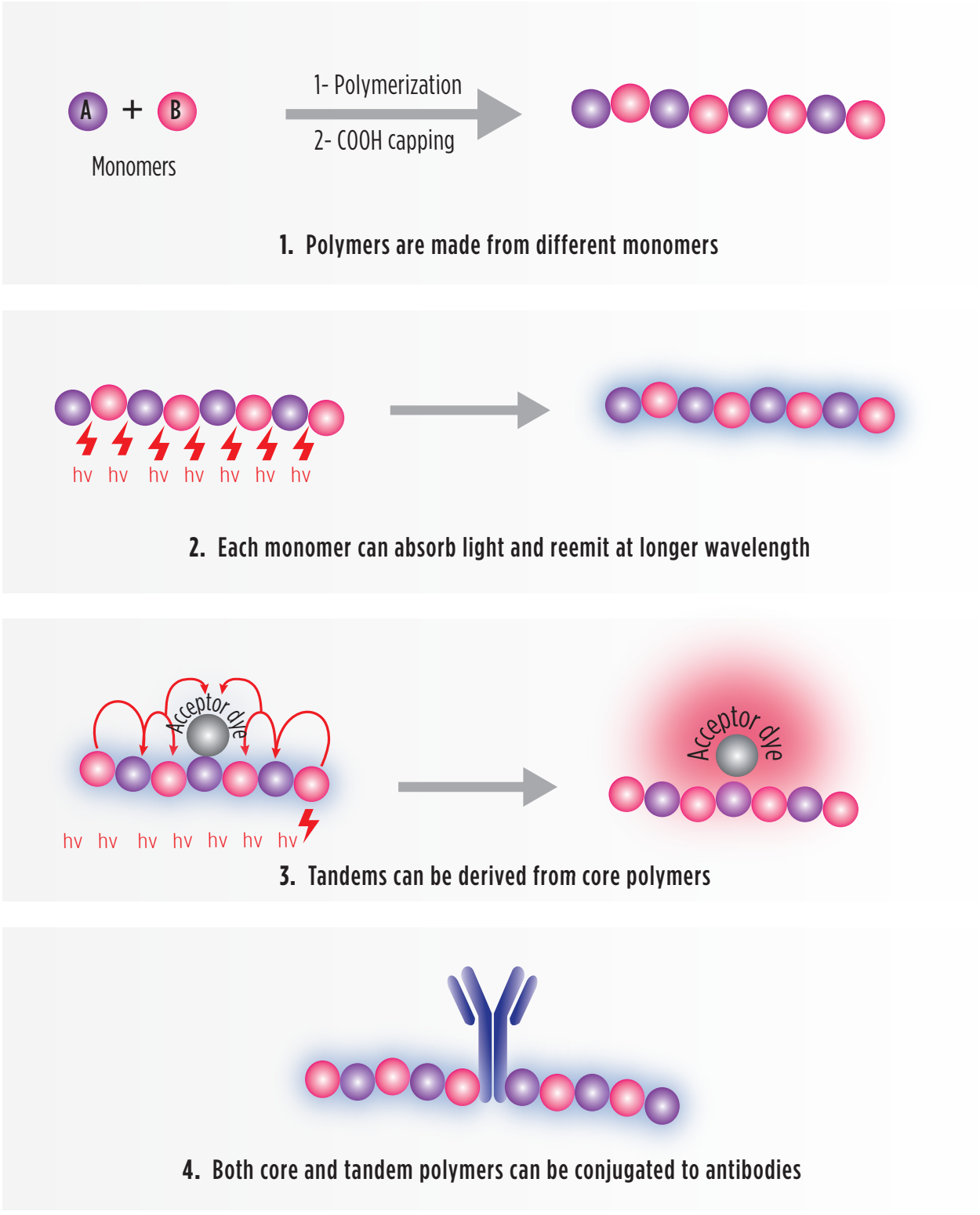

Figure 1. Pictorial representation of synthesis of SuperNova tandem conjugates.

The absorption and emission spectra for SN v605 and v786 was measured. Three lots of each SuperNova conjugates (Table 1) were manufactured and tested by staining the whole blood cells/cell lines (for CD103) on CytoFLEX / DxFLEX flow cytometers. Every lot, except for the SN v786-CD103 conjugate, was tested on five donors of whole blood specimens with three replicates.

Conjugates recruited as part of the study

SN v605	SN v786
CD25 SN v605	HLA-DR SN v786
CD38 SN v605	CD200 SN v786
CD20 SN v605	CD103 SN v786

For comparison, respective Brilliant Violet (BV) antibody conjugates were used

Table 1. Conjugates tested for comparative study.

For SN v786-CD103, only three replicates of cell line for each lot of conjugates were studied. To check the performance of SuperNova conjugate in a multi-color experiment, a 10 color panel (Table 2) with SN v605-CD20 and SN v786-CD200 was tested separately, without stain buffer.

Panel	FITC	PE	ECD	PC5.5	PC7	APC	AA700	AA750	PB	KrO	SN v605	SN v786
1	CD16	CD25	CD19	CD56	CD4	CD10	CD14	CD45	CD15	CD8	CD20	-
2	CD16	CD25	CD19	CD56	CD4	CD10	CD14	CD45	CD15	CD8	-	CD200
Control	CD16	CD25	CD19	CD56	CD4	CD10	CD14	CD45	CD15	CD8	-	-

Table 2. Multicolor panel with SuperNova conjugate.

For analysis, respective positive population (Table 3) was gated and stain index was calculated (stain index = MFIPos – MFINeg / 2 x Standard Deviation [SDNeg]). An average stain index for three replicates and five donors were calculated.

Conjugate	Specimen	Population of cells expressing the marker	Population considered for SI calculation
CD25 SN v605	Whole Blood	Lymphocytes	Lymphocytes
CD38 SN v605	Whole Blood	Lymphocytes, Monocytes, Granulocytes	Lymphocytes
CD20 SN v605	Whole Blood	Lymphocytes	Lymphocytes
CD20 SN v605	Whole Blood	Lymphocytes, Monocytes	Lymphocytes
CD200 SN v786	Whole Blood	Lymphocytes	Lymphocytes
CD103 SN v786	MOLT-16 cell line	MOLT-16 cells	MOLT-16 cells

Table 3. Summary of stain index calculation.

Results

The SuperNova conjugates had higher stain index with consistent lot-to-lot performance (denoted by the CV<4%) across the three lots tested for percentage recruitment, and reduced nonspecific staining on negative cells in comparison to the commercially available polymer dye conjugate. Equivalent performance was observed when SuperNova conjugates were tested as a single-color conjugate and in a multicolor panel consisting of conventional fluorochromes—such as FITC, PE, ECD, etc.—without affecting the characteristics of other fluorochromes in the panel.

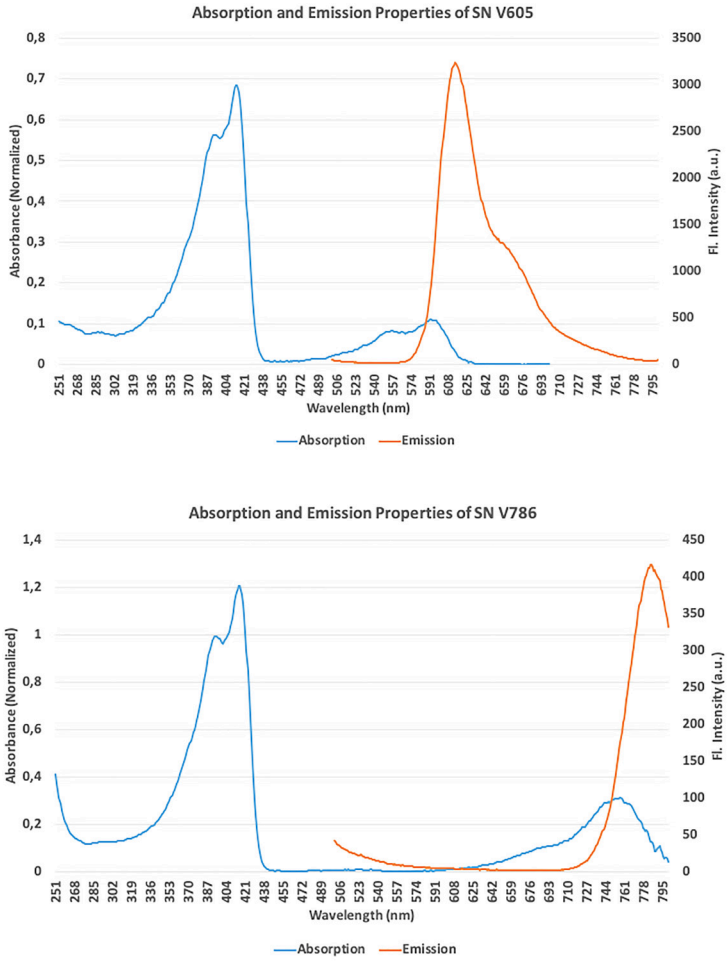

Figure 2. Absorption and emission spectra of SuperNova v605 and v786 in PbS.

The spectral graphs show the overlap of absorption and emission spectra of SuperNova tandem dyes. The optimized acceptor-to-donor ratio for the SuperNova tandem dyes enable >95% FRET efficiency and large Stokes shift to emit at 605 nm and 786 nm when excited by a 405 nm laser.

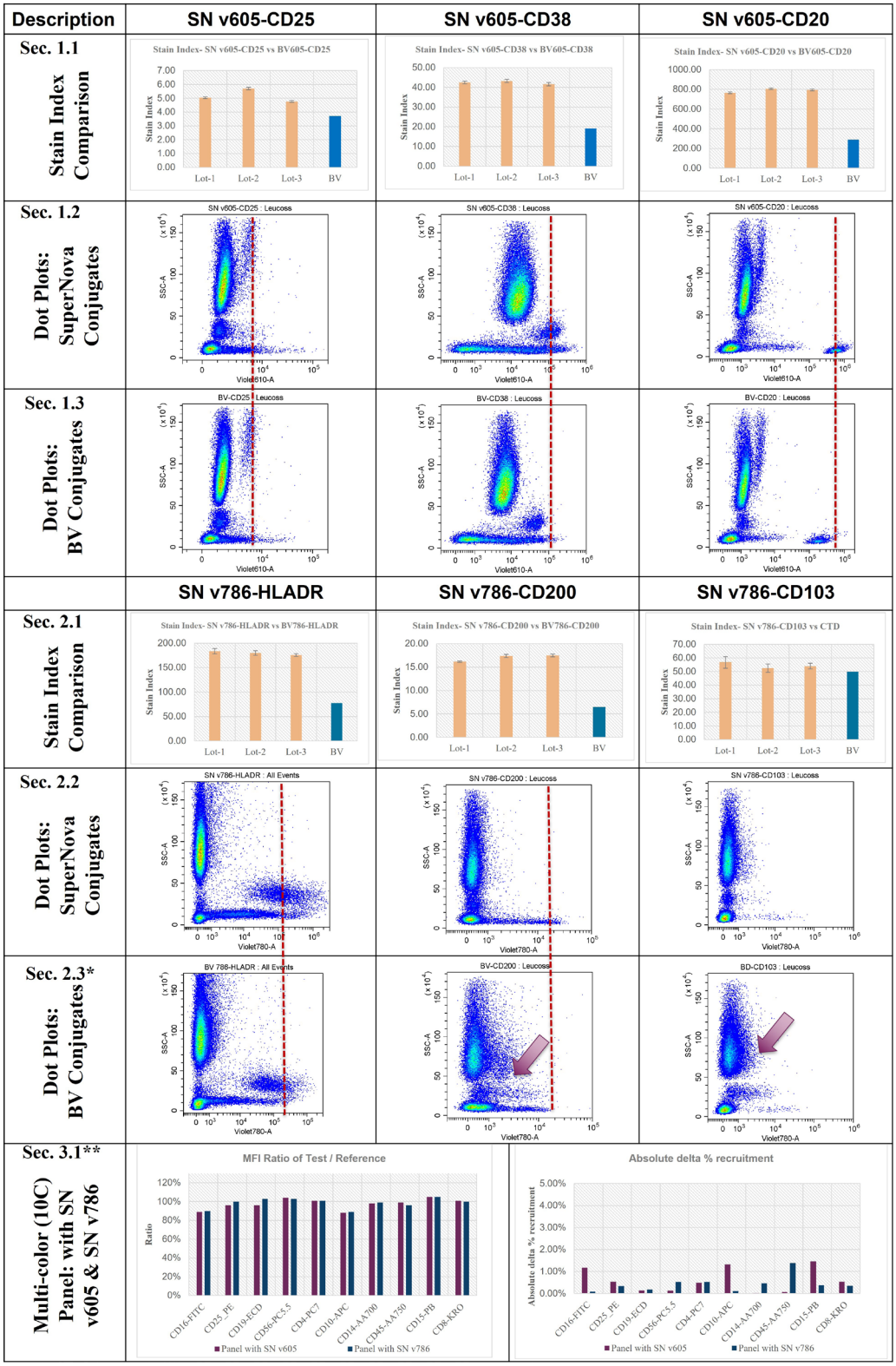

Figure 3. Comparison of stain index (Sec. 1.1 & 2.1), dot plots for SuperNova conjugates (Sec. 1.2 & 2.2) against Brilliant Violet (BV) conjugates (Sec. 1.3 & 2.3). The dotted (red colored) line represents the median of the positive population. The values of stain index represent the mean ± standard error of the samples (n=15). *In Sec. 2.3, the arrow mark shows non-specific pull out of undesired cells, whereas the same is not observed with SuperNova conjugates. **Sec. 3.1 depicts the ratio of MFI values for test (panel in presence of SN v605 or SN v786) and reference (panel in absence of SN v605 or SN v786). Absolute delta % recruitment is the difference of % recruitment between test and reference in the presence and absence of SN conjugates.

Conclusions

Overall, it has been demonstrated that SN v605 and SN v786 are brighter than their respective Brilliant Violet (BV) equivalent polymeric dye conjugates and that the proprietary formulation shows that SN conjugates results in minimal non-specific staining on negative cells.

Specificity	Stain Index		CV of % recruitment across 3 lots of SN conjugates
	SN	BV	
CD25	5.17	3.71	3.81%
CD38	42.42	19.02	0.77%
CD20	788.03	288.06	2.56%
HLADR	179.92	77.58	1.03%
CD200	17.0	6.44	1.67%
CD103	54.39	49.88	0.03%

In addition, the presence of SuperNova conjugates in a cocktail of conventional dyes—such as FITC, PE, ECD, etc.—did not change the properties in terms of MFI and % recruitment and an improved stain index of the SuperNova conjugates could be exploited for the detection of rare population in any multicolor panel.

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