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Life Sciences

SuperNova v428: New Bright Polymer Dye for Flow Cytometry Applications

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IN THIS PAPER YOU WILL

- Discover the SuperNova fluorochromes, the latest generation of polymer dyes for flow cytometry applications
- Learn more about the unique properties of SuperNova dyes
- See example of flow cytometry staining data comparing SuperNova to other polymer dyes

Introduction to SuperNova v428

SuperNova v428 is a new violet laser (405 nm)-excitable water-soluble polymer dye which shows exceptional brightness. This is attributed to its high extinction coefficient (~3 million Lmol-1 cm-1) and fluorescence quantum yield (~0.6) in phosphate buffer. Excitation maximum of this polymer is measured as 414 nm and its emission maximum is at 428 nm (Figure 1) which can be detected using a 450/50 bandpass filter or equivalent in a flow cytometer.

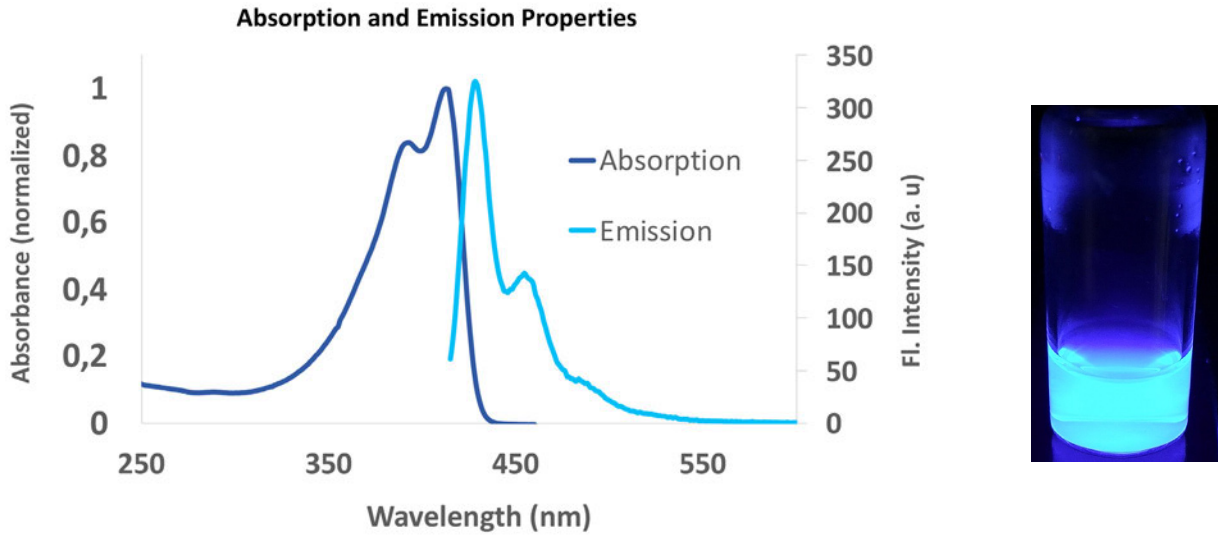

Figure 1: (Left) Absorption and emission spectra (λ_{exc} = 405 nm) of SuperNova v428 in PBS. (Right) SuperNova v428 solution in PBS upon illumination using a UV lamp at 365 nm.

Synthesis of SuperNova Polymer dyes and conjugation to Antibodies

SuperNova v428 dye is prepared by polymerization of water-soluble monomers as shown in Figure 2 which leads to formation of a highly conjugated fluorescent backbone. Capping is carried out on the polymer followed by activation using appropriate functionalities, resulting in polymer capable of conjugation to antibodies. These activated polymers conjugated to antibodies followed by purification using a standardized procedure.

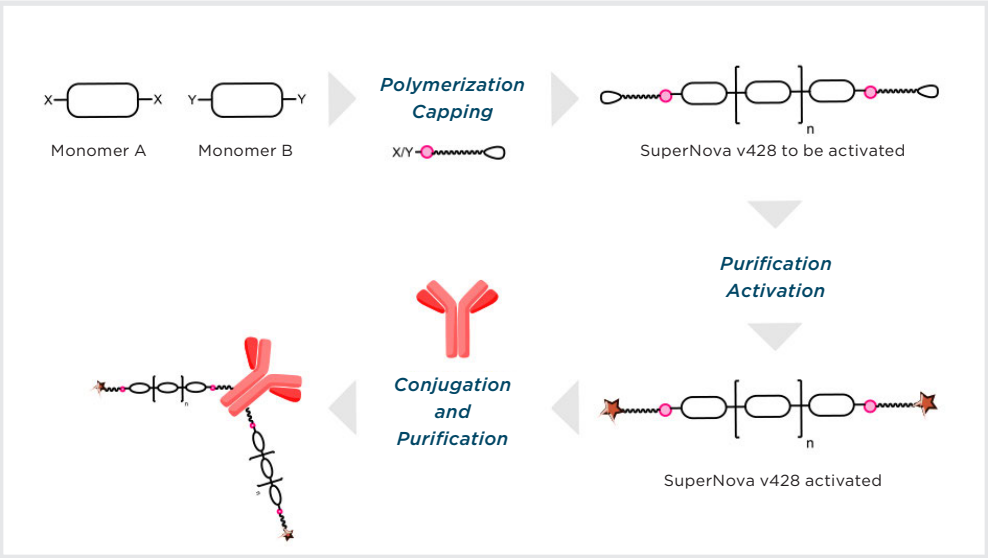

Figure 2: Schematic procedure for SuperNova v428 polymer synthesis, activation and conjugation process.

Polymer dye conjugates usually tend to generate significant non-specific staining, for instance by binding nonspecifically to cells expressing Fc receptors such as monocytes. A proprietary formulation has been identified and optimized to minimize this non-specific staining, allowing to further improve stain index of SuperNova v428.

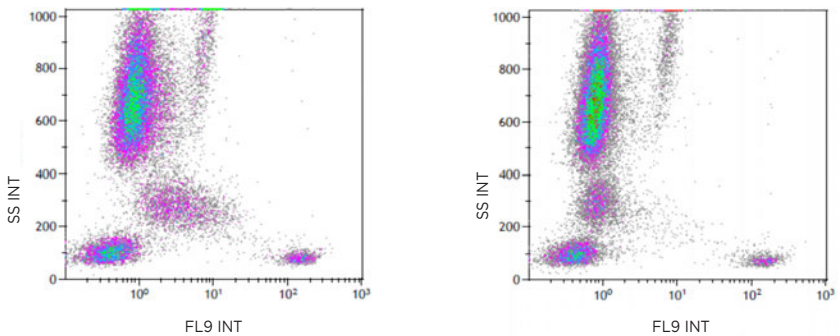

Figure 3: CD22-SNv428 whole blood staining before (left) and after (right) formulation with additives preventing non specific staining. Background on monocytes is significantly reduced.

Flow Cytometry results

SuperNova v428 was conjugated to different antibodies and compared with PE, Pacific Blue and Brilliant Violet polymer dye conjugates. Antibody-SuperNova v428 conjugates exhibited more than 10 times the brightness of Pacific Blue and also better brightness than PE and BV 421 in flow cytometry. Nonspecific staining was also significantly reduced compared to BV 421 conjugates.

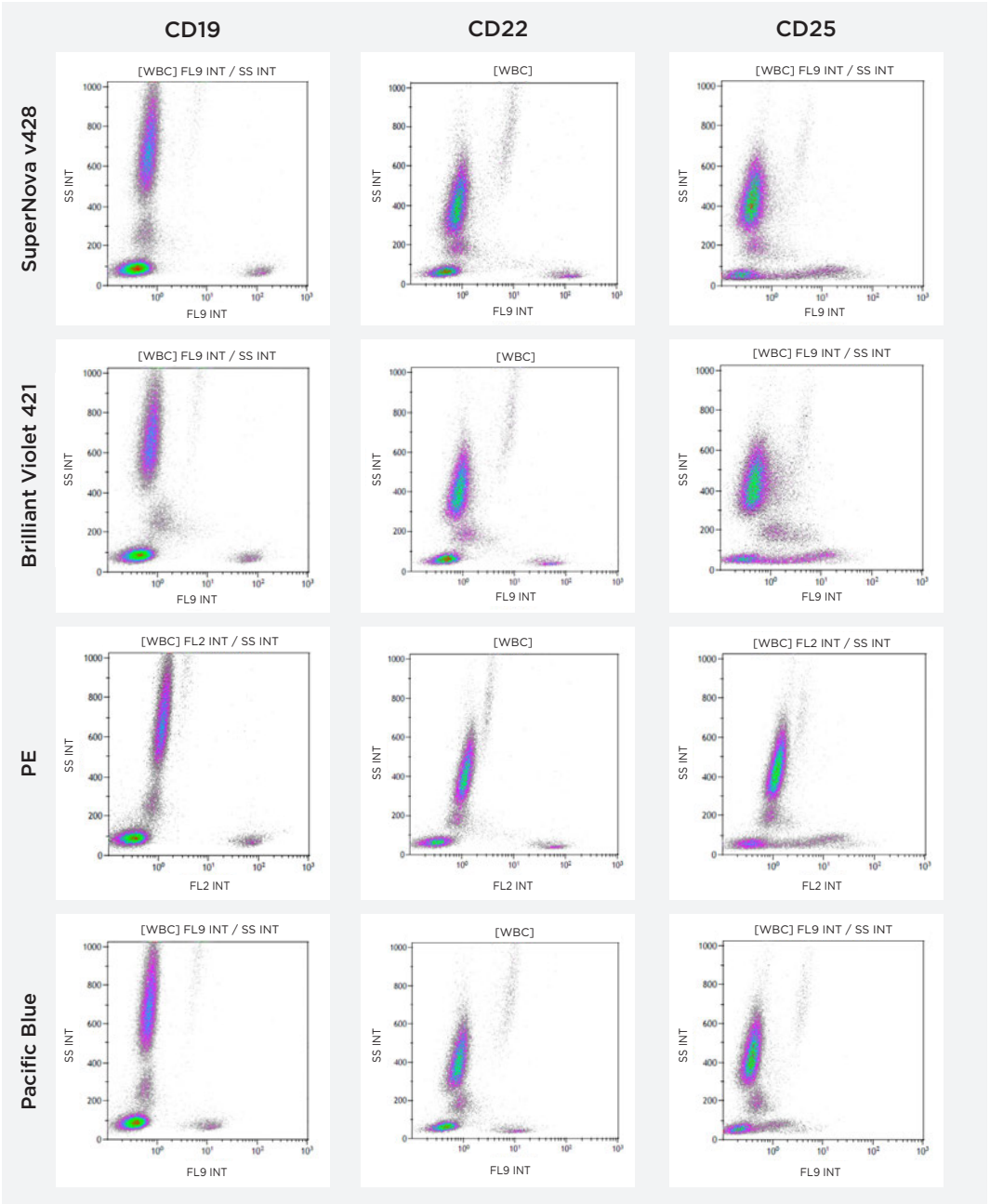

Figure 4: SuperNova v428 conjugates (with CD19, CD22, and CD25) compared against conjugates of BV421, PE and Pacific Blue in flow cytometry. All SuperNova v428 conjugates showed higher brightness to above mentioned commercially available conjugates. Nonspecific binding significantly reduced in the case of SuperNova v428 conjugates.

		Lymphocytes Stain Index	Monocytes MFI (background)
CD19	SN v428	281	0,688
	BV421	191	1,533
	PE	246	0,805
	Pacific Blue	48	0,600
CD22	SN v428	172	1,033
	BV421	123	1,476
	PE	136	0,716
	Pacific Blue	28	0,958
CD25	SN v428	36	0,731
	BV421	14	2,021
	PE	24	1,052
	Pacific Blue	6	0,522

Table 1: Lymphocytes staining index and monocytes MFI (background) obtained staining normal whole blood with lymphocytes markers conjugated to SN v428, BV421, PE and Pacific Blue

Conclusion

SuperNova polymer dyes are a new generation of polymer dyes for flow cytometry application. SuperNova v428 has unique photo-physical properties leading to extremely bright conjugates when conjugated to antibodies. A proprietary formulation leveraging unique additives allows to minimize non-specific staining for greater confidence in flow cytometry staining results and improved staining index. Therefore SuperNova v428 conjugates are particularly suited for the study of low abundance and dimly expressed markers.

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