Supplementary Information

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Materials

All chemicals (unless otherwise specified) and DAUDI human cell line were purchased from Sigma—Aldrich (St. Louis, MO). Interferon-alpha 2a (IFN-α2a), Human was phurchased from Genescript (Piscataway, NJ). 10-kDa MW, 20-kDa MW and 40-kDa MW 2-arms branched MethoxyPEG COO NHS (NHS-mPEG) were purchased from NOF America Corporation (White Plains, NY). N-α-maleimidoacet-oxysuccinimide ester (AMAS) and Pierce Protein Concentrators were purchased from Thermo Fisher Scientific (Waltham, MA). Sprague Dawley rats were purchased from Charles River Laboratories (Burlington, MA). Daudi cells were purchased from American Type Culture Collection (ATCC). Human IFNα (multiple subtypes) bioluminescent ELISA kit was purchased from InvivoGen (San Diego, CA). Antibodies were purchased from Abcam (Cambridge, MA). 3,3',5,5'-Tetramethylbenzidine (TMB) substrate was purchased from Bethyl Laboratories (Montgomery, TX).

Methods

1. *Size-exclusion chromatography*

The native IFN-α2a and IFN-α2a conjugates were analyzed through a 1260 Infinity binary high performance liquid chromatography (HPLC) system equipped with a UV detector (Agilent Technologies, Santa Clara, CA), a miniDAWN TREOS light scattering (LS) detector, and a Optilab T-rEX differential refractive index (dRI) detector (Wyatt Technology, Santa Barbara, CA). The flow rate was set at 1 mL/min with the mobile phase PBS (pH 7.4). A Waters Ultrahydrogel 1000 column (7.8 mm x 300 mm, 12 μm particle size) was used for polymer-protein conjugates. All samples were filtered through 0.2 μm PTFE filters prior to the experiment. Molecular weight was determined by generating a standard curve with six PEG samples of known MW and PDI.

2. *Dynamic light scattering (DLS)*

The hydrodynamic sizes of the native IFN-α2a and its conjugate samples were measured by a DLS method using a Malvern Nano Zetasizer in PBS buffer at RT. Samples were treated with a filter of 0.2 μm pore size before analysis.

3. *Detection of anti-IFN and anti-polymer antibodies*

Indirect enzyme-linked immunosorbent assay (ELISA) experimental conditions were performed to detect antibodies specific for IFN-α2a, PEG, or pCB. The antigens used here consisted of native IFN-α2a (for detection of anti-IFN antibody), PEG-BSA conjbugate (for detection of anti-PEG antibody), and pCB-BSA conjugate (for detection of anti-pCB antibody). PEG-BSA conjugate and pCB-BSA conjugate were made following the same procedure as PEG-IFN and pCB-IFN samples. An antigen solution (10 μg/mL) in sodium carbonate buffer (0.1 M, pH 9.5) is first adsorbed onto the plate at 100 μL antigen solution (10 μg/mL) per well for 12 h at 4 °C. After moving antigen solutions, the plate was washed 5 times using PBS (pH 7.4) and then blocked with blocking buffer (1% BSA solution in 0.1 M Tris buffer, pH 8.0) for 1 h at 4 ◦C. After another 5× wash, Serial dilutions of rat sera in PBS were added to the plate (100 μL/well) and incubated for 2 h at 37 °C. The plate was then washed 5 times with PBS. Secondary antibody (goat anti-rat IgM or IgG conjugated to HRP) diluted 1:20,000 was then added and incubated at RT for 1 h followed by 5× wash with PBS. The HRP substrate 3,3',5,5'-tetramethylbenzidine (TMB) solution was then added and the plate was shaken for 15 min and quenched with 100 μL stop solution (0.2 M H_2SO_4). Absorbance at 450 nm with 570 nm values as background subtraction was recorded by a microplate reader. Pre-experiment blood samples were used as negative control for all ELISA detections. Anti-PEG IgM serum concentrations were quantified using commercial mono-clonal rat anti-PEG IgM, which is also the positive control for the polymer-specific assays. Polymer-specific antibody detection assays were further validated via competition with free pCB or PEG polymer.

Table S1. Dynamic light scattering (DLS) analysis of various polymer-IFN conjugates.

	IFN	pCB_{10K}-IFN	pCB_{20K}-IFN	PEG_{10K}-IFN	PEG_{20K}-IFN	PEG_{40K}-IFN
Hydrodynamic size (nm)	2.34 ± 0.23	3.74 ± 0.41	5.16 ± 0.35	4.94 ± 0.46	7.28 ± 0.61	10.47 ± 0.52
PDI	0.31 ± 0.04	0.17 ± 0.05	0.2 ± 0.06	0.14 ± 0.03	0.17 ± 0.04	0.19 ± 0.05

Table S2. Conversion and yield of various polymer-IFN conjugates.

	IFN + pCB_{10k}	IFN + pCB_{20k}	IFN + PEG_{10k}	IFN + PEG_{20k}	IFN + PEG_{40k}
IFN conversion	61.4% ± 9.2%	55.3% ± 7.2%	52.7% ± 6.5%	51.1% ± 5.7%	46.6% ± 8.3%
Yield	54.5% ± 5.9%	47.6% ± 8.4%	45.4% ± 4.9%	47.5% ± 6.3%	40.2% ± 4.7%

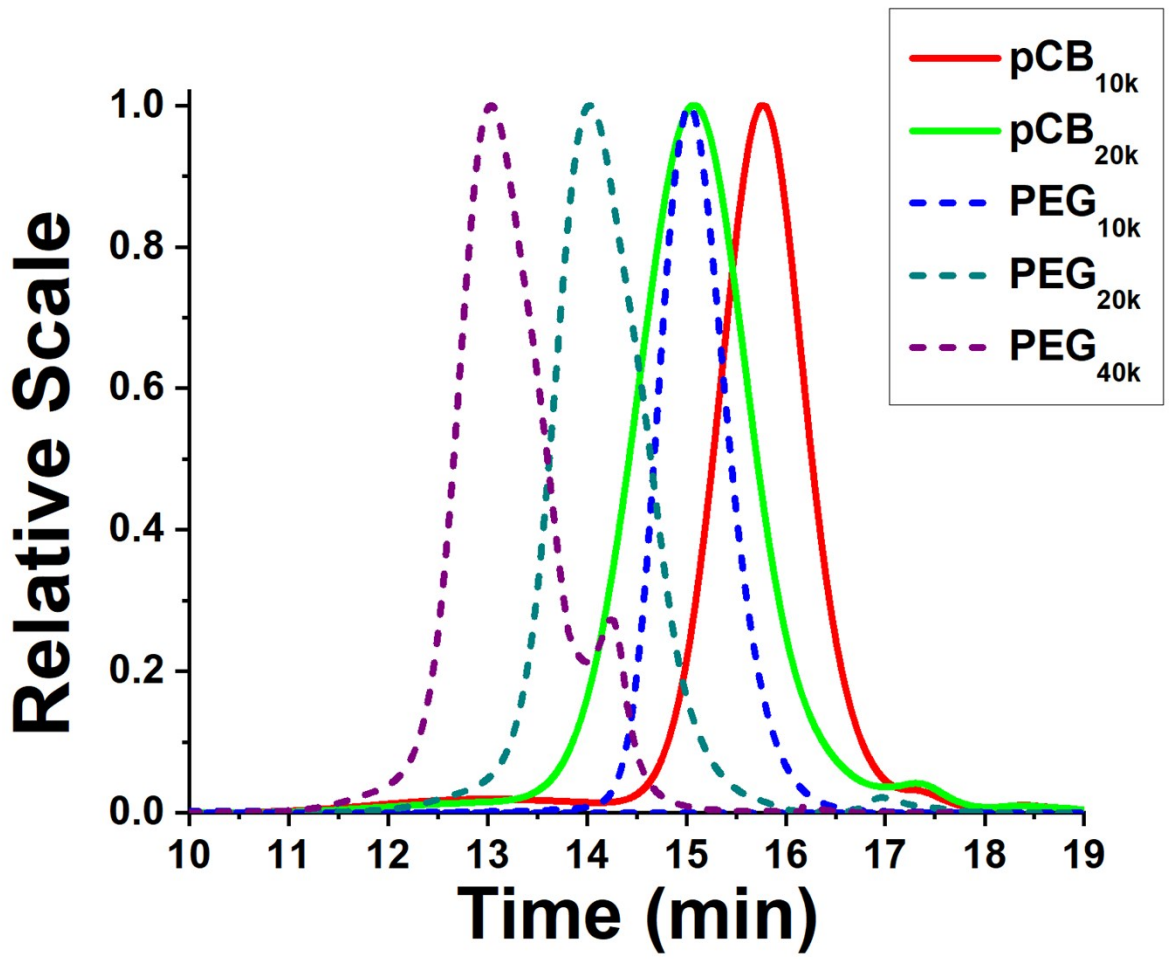

Figure S1. Representative size-exclusion chromatogram for different pCB and PEG polymers.

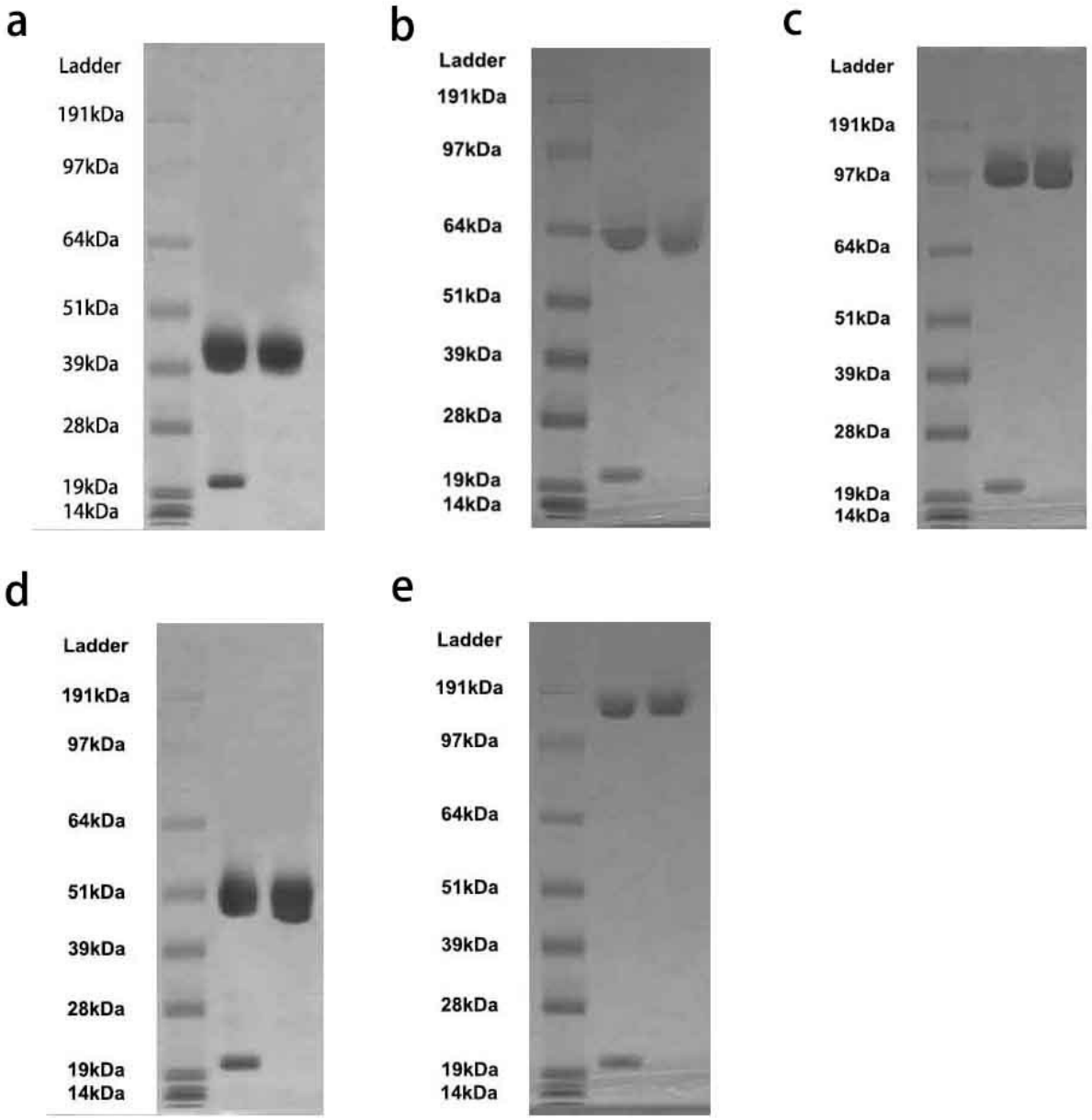

Figure S2. SDS-PAGE analysis of polymer conjugated IFN-α2a. a) PEG_{10k} conjugated IFN-α2a. Lane 1: Molecular weight marker proteins; Lane 2: Reaction mixture; Lane 3: purified PEG_{10k} -IFN. b) PEG_{20k} conjugated IFN-α2a. Lane 1: Molecular weight marker proteins; Lane 2: Reaction mixture; Lane 3: purified PEG_{20k} -IFN. C) PEG_{40k} conjugated IFN-α2a. Lane 1: Molecular weight marker proteins; Lane 2: Reaction mixture; Lane 3: purified PEG_{40k} -IFN. d) pCB_{10k} conjugated IFN-α2a. Lane 1: Molecular weight marker proteins; Lane 2: Reaction mixture; Lane 3: purified pCB_{10k} -IFN. e) pCB_{20k} conjugated IFN-α2a. Lane 1: Molecular weight marker proteins; Lane 2: Reaction mixture; Lane 3: purified pCB_{20k} -IFN.