dPEG® Conjugates Significantly Increase Hydrodynamic Volume of Proteins

INTRODUCTION

The covalent attachment of PEG to a protein is referred to as PEGylation.[1,2] Protein PEGylation can occur on a protein at a specific site using available free thiols or randomly on the protein using surface available groups such as lysine or arginine.[2,4] Either linear or branched PEGs with various functional groups can be attached through PEGylation. PEG molecules attached to proteins in this manner reduce protein aggregation, increase solubility in water, and reduce immunogenicity of the modified protein compared to the unmodified protein.[3,4]

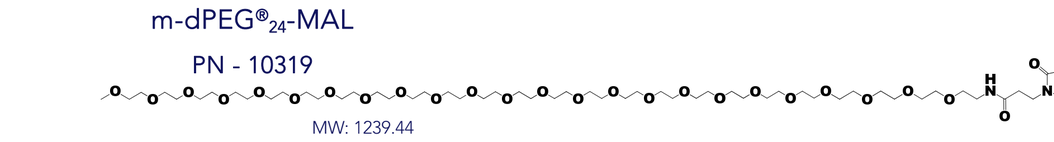
The term dPEG® is an acronym for "discrete polyethylene glycol" or "discrete PEG" and refers to a unique class of PEGs, having a unique, specific, single molecular weight, synthesized *de novo*, from pure, small units (*e.g.*, triethylene glycol or tetraethylene glycol). These dPEG® compounds differ dramatically from long chain PEG polymers, which are made through polymeric processes and have variable chain lengths and consequently, variable polydispersity values.

In the therapeutic/diagnostic field, the advantage of increased apparent molecular weight allows proteins to appear larger *in vivo*, thus prolonging the half-life of the protein in the circulatory system as well as preventing it from being cleared through the kidneys as quickly as smaller proteins. This results in reduced dosing frequencies. Other advantages of conjugating PEG to proteins are lower incidences of side effects and decreased chances for immunogenicity. An enormous range of benefits to protein-based medicine through the application of PEGylation is already in published studies, and it is not difficult to envision further extension of known benefits and development of new benefits through PEGylation.[5,7,8]

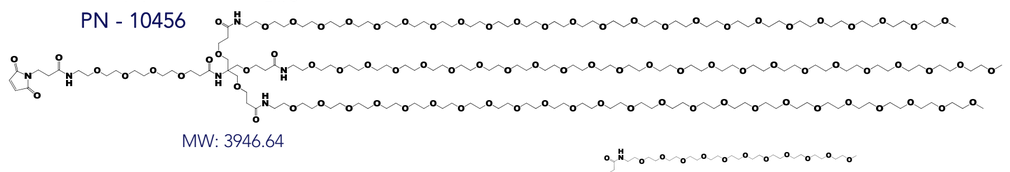
This series of experiments was designed to demonstrate that discrete PEGs, when conjugated to proteins, increase the apparent molecular weight of the protein (presumably through increased hydrodynamic volume of the PEGylated protein). Both linear and branched dPEG® constructs were used with purified BSA as a model protein to demonstrate this occurrence. Data obtained from size exclusion chromatography was compared to MALDI-MS determined molecular weights to demonstrate a trend of increasing dPEG® construct size yielding increasing hydrodynamic volumes.

Figure 1. dPEG® constructs used in the study

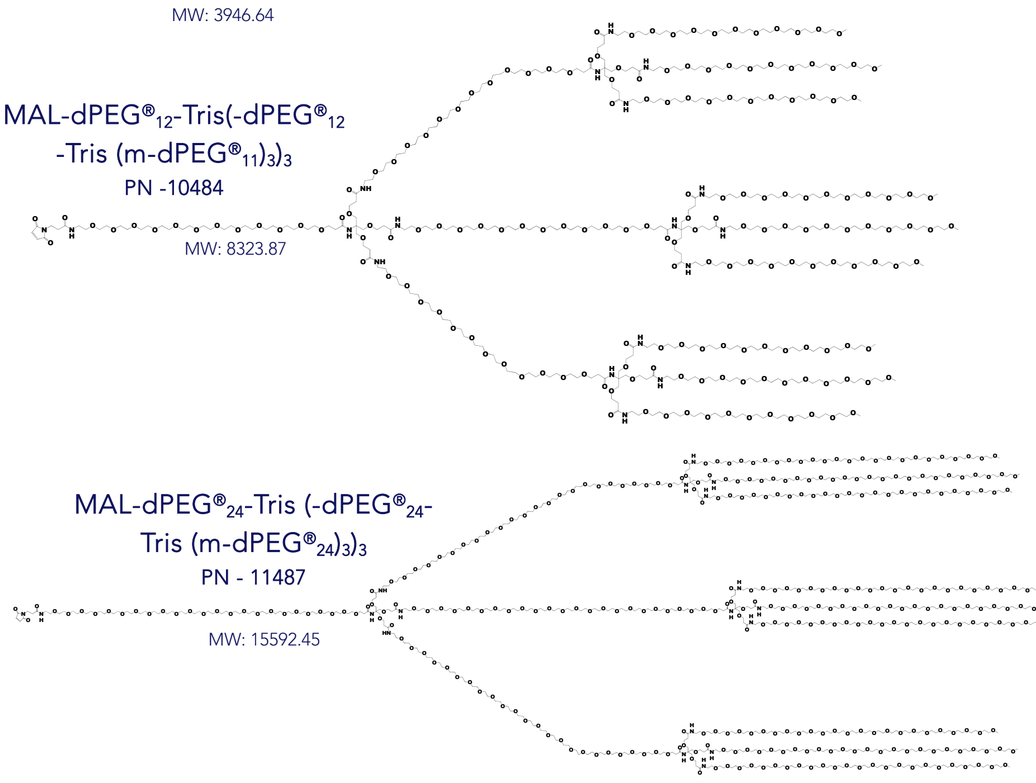
m-dPEG®$_{24}$-MAL
PN - 10319
MW: 1239.44



MAL-dPEG®$_4$-(m-dPEG®$_{24}$)$_3$
PN - 10456
MW: 3946.64



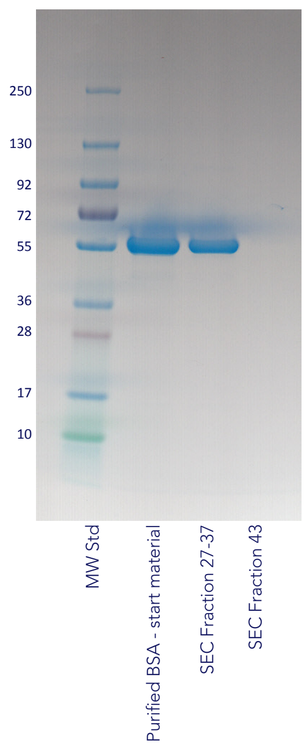
MAL-dPEG®$_{12}$-Tris(-dPEG®$_{12}$-Tris (m-dPEG®$_{11}$)$_3$)$_3$
PN -10484
MW: 8323.87



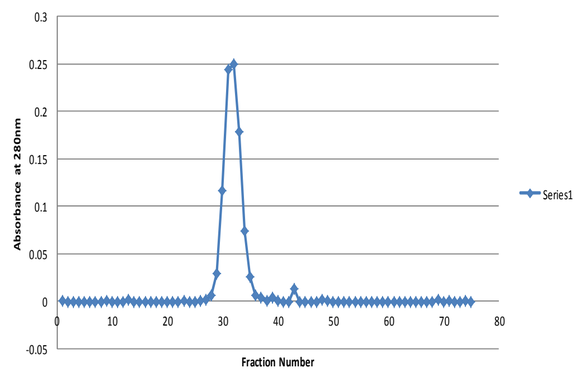
MAL-dPEG®$_{24}$-Tris (-dPEG®$_{24}$-Tris (m-dPEG®$_{24}$)$_3$)$_3$
PN - 11487
MW: 15592.45

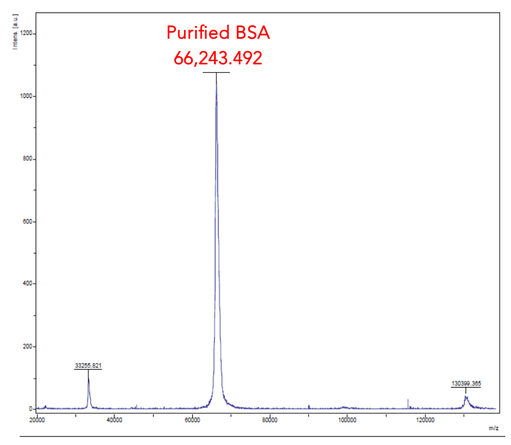
Figure 2. Purified BSA Data

SEC of purified BSA was performed to establish the apparent molecular weight of BSA on the Superdex 200 column. This is the starting material control.



Superdex 200 purification of purified BSA





MALDI TOF MS Purified BSA

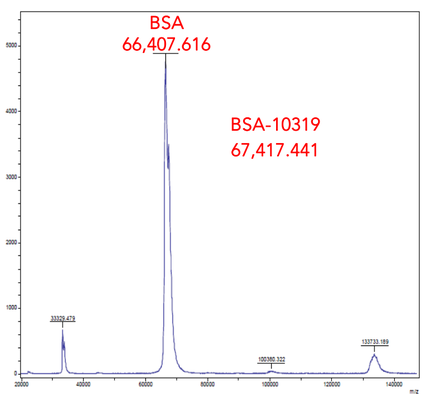
BSA Conjugate MW Determinations

Molecular weights determined by MALDI-MS were compared to molecular weights calculated from the SEC calibration curve. The results are tabulated above.

	Purified BSA	BSA-10319	BSA-10456	BSA-10484	BSA-11487
Estimated MW	66,000	67,239	69,947	74,324	81,592
MW by MALD-MS	66,243	67,417	70,245	74,598	81,803
MW by SEC		72,257	101,321	129,789	178,929
Apparent Mass Increase		3.8x	7.9x	6.6x	6.2x

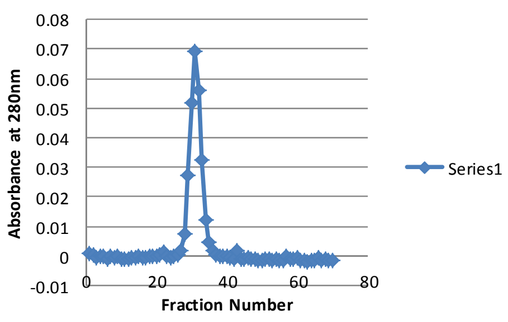
Figure 3. BSA-10319 Conjugate Data

Linear dPEG® construct m-dPEG®$_{24}$-Mal, molecular weight 1,239.45 Da, was conjugated to purified BSA. The data below represent steps of the conjugation procedure.



MALDI TOF MS BSA-10319

Superdex 200 Purification of BSA-10319



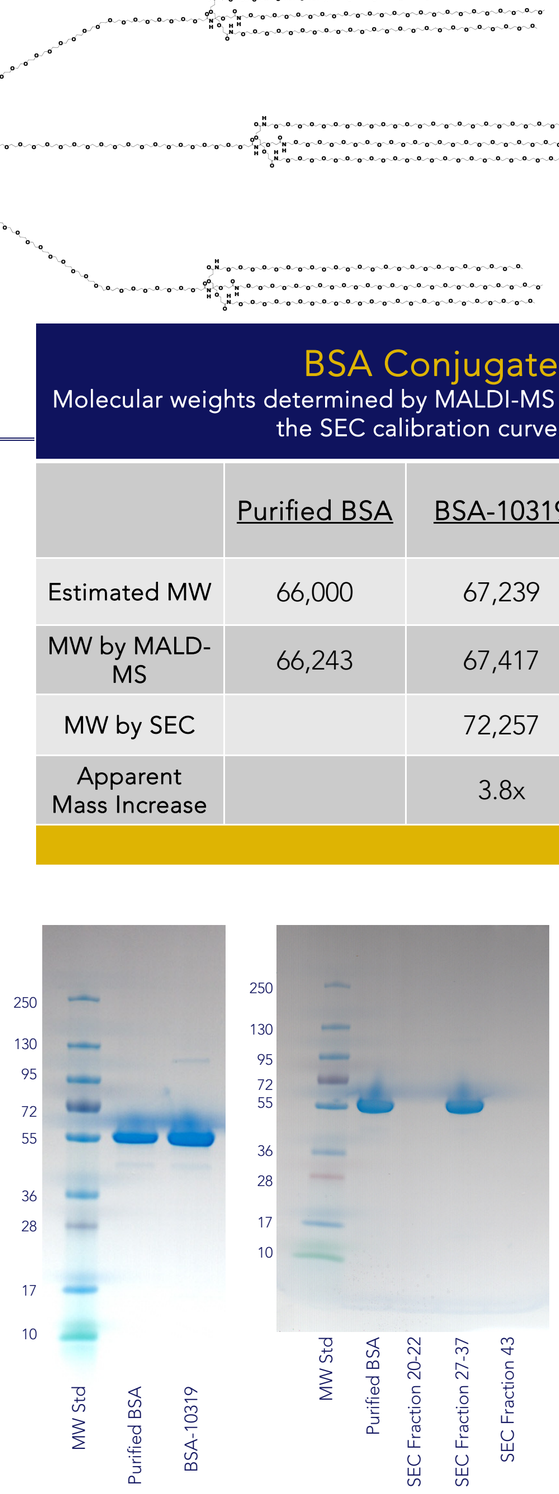
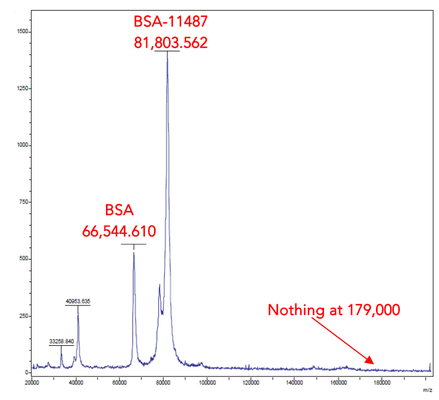
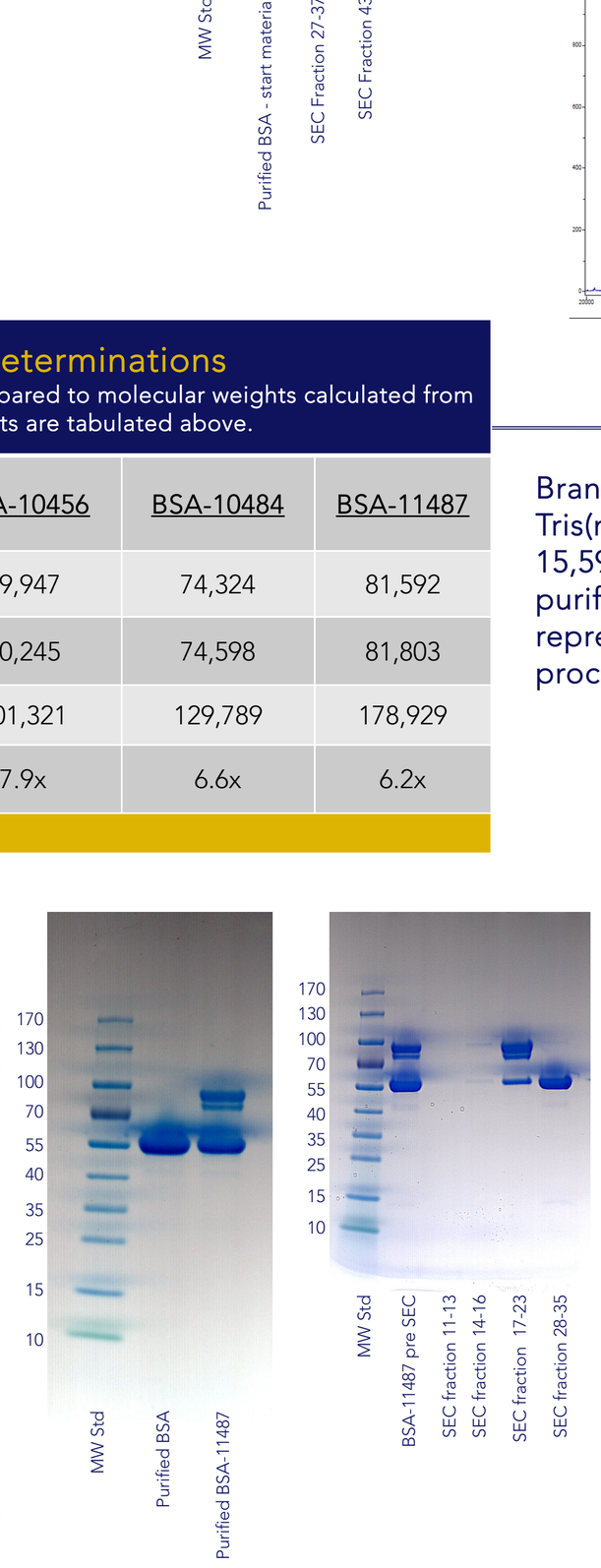


Figure 4. BSA-11487 Conjugate Data

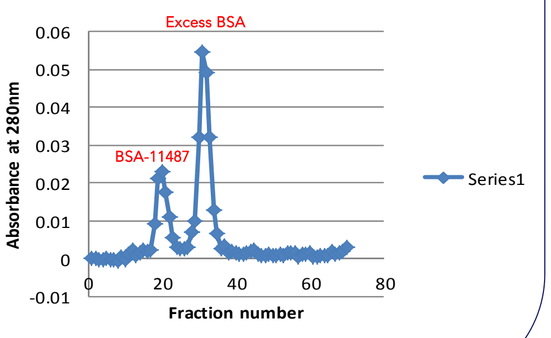
Branched Mal-dPEG®$_{24}$-Tris (dPEG®$_{24}$-Tris(m-dPEG®$_{24}$)$_3$)$_3$, molecular weight 15,592.61 Da, was conjugated to purified BSA. The data below represent steps of the conjugation procedure.



MALDI TOF MS BSA-11487



Superdex 200 purification of BSA-11487



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