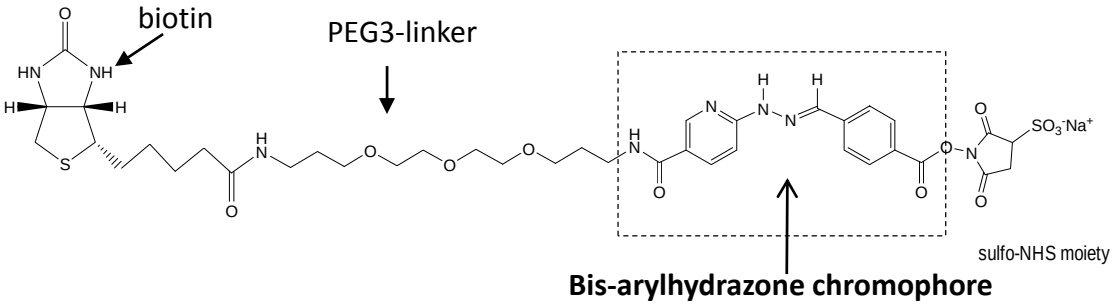
BIOL 193 - Direct spectrophotometric quantification of incorporated biotin on proteins.

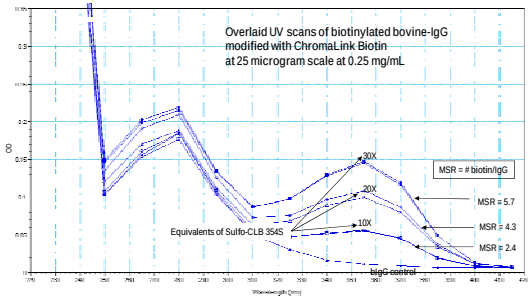
David Schwartz, Ph.D.*, Candice Miller, Jim Williams, Jamie McDonald, and Leopoldo Mendoza,
Solulink Biosciences, 9853 Pacific Heights Blvd., Ste H, San Diego, CA 92121.

Abstract: Biotin is an important reporter molecule used in microarrays, ELISA assays, flow cytometry and immunohistochemistry. Quantifying the number of biotins incorporated on proteins such as antibodies is important for assay reproducibility since over-modification can compromise binding affinity with the target antigen. Solulink has developed a new biotinylation reagent called ChromaLink Biotin (CLB) that embeds a chromophore (a bis-arylhydrazone) in the linker arm separating the biotin reporter from the reactive linking moiety (e.g. NHS or maleimide). CLB allows spectrophotometric quantification of incorporated biotin and permits direct and simple determination of biotin molar substitution ratios (MSR). The bis-arylhydrazone chromophore has an absorbance maximum @ 354 nm with a molar extinction coefficient of 29,000. These spectral properties allow biotin molar substitution ratios to be accurately determined at protein (e.g. antibody) concentrations as low as 250 ug/mL In contrast, classical HABA assays ((2-4'-hydroxyazobenzene-2-carboxylic acid)) that also measure biotin use a competitive binding assay/standard curve which is less sensitive, more time-consuming and not readily automated. Comparisons between HABA assays and the CLB spectral method confirm that HABA underestimates the true molar substitution ratio on antibodies by nearly 3-fold.

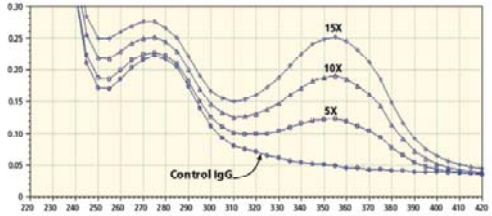


	0.25 mg/ml	0.5 mg/ml	1 mg/ml	2.5 mg/ml	5 mg/ml	10 mg/ml
	Biotin MSR	Biotin MSR	Biotin MSR	Biotin MSR	Biotin MSR	Biotin MSR
2X	n/m	n/m	1.7	1.6	1.7	1.7
3X	n/m	n/m	1.7	2.2	2.3	2.6
4X	n/m	n/m	1.7	2.0	3.0	3.4
5X	1.7	2.0	2.2	3.3	3.7	4.0
10X	2.4	2.9	3.5	6.1	6.7	6.5
20X	4.3	4.7	7.4	9.7	10.6	11.3
30X	5.7	6.0	10.7	13.3	13.9	13.9

Table presenting results of number of biotins incorporated on bIgG depending on equivalents of ChromaLink Biotin added with respect to bIgG concentrations. Modification buffer composition: 100 mM phosphate, 150 mM NaCl, pH 7.4

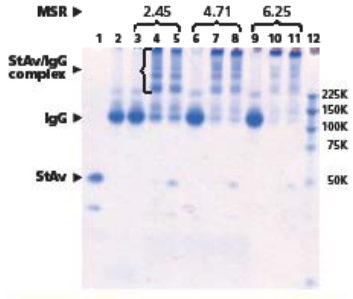


Three reactions (duplicates) were set-up using 10, 20, or 30 equivalents of Sulfo-ChromaLink Biotin 354S. All reaction contained 25 ug bovine IgG in 100 ul 1x Modification Buffer (pH 7.4). After 2 hours at room temperature, reactions were desalted using 0.5 ml Zeba™ spin columns and scanned on a Molecular Devices 96-Well plate reader

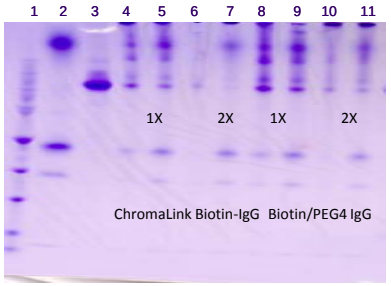


	Biotin/IgG HABA	Biotin/IgG A_{354}
5 x	1.03	2.45
10 x	1.60	4.71
15 x	2.22	6.25

Solutions of bovine IgG (bIgG) at 5 mg/mL in 100 mM phosphate, 150 mM NaCl, pH 7.4 were treated with 5, 10 and 15 equivalents of ChromaLink Biotin for 1.5 h and desalted. Overlaid spectra of ChromaLink Biotin-modified IgGs are illustrated. Absorbance at 280 and 354 nm were recorded and antibody concentrations determined. These were used to quantify the biotin molar substitution ratio.



Gel experiment correlating the number of bitoins/IgG and its binding to streptavidin (StAv). IgG modified @ 2.45, 4.71 and 6.25 biotins were treated with 1 equiv. (lanes 4, 7 and 10) and 2 equiv. (lanes 5, 8 and 11) of StAv. As can be seen, there was efficient binding of StAv with as few as 2.45 biotins/StAv.



Experiment to evaluate chromophore's deleteriously affects on StAv binding. ChromaLink Biotin-modified IgG and with Biotin/PEG4 (Quanta Biodesign) modified IgG at two levels were reacted with 1X and 2X StAv. Result indicate identical binding results with either reagent.