

Instructions

Introduction:

SHNH is a bifunctional aromatic hydrazine linker used to incorporate HyNic (6-hydrazinonicotinamide) linkers onto biomolecules through their amino group via an activated ester (i.e. NHS; Figure 1). HyNic groups were developed to link Tc-99m to proteins for *in vivo* imaging.¹⁻⁴

The number of HyNic linkers incorporated on biomolecules can be quantified colorimetrically on reaction with 2-sulfobenzaldehyde (Solulink catalog# S-2005-100). The product yields a chromophore that absorbs at A350 with a molar extinction coefficient of 18000 (Figure 2).

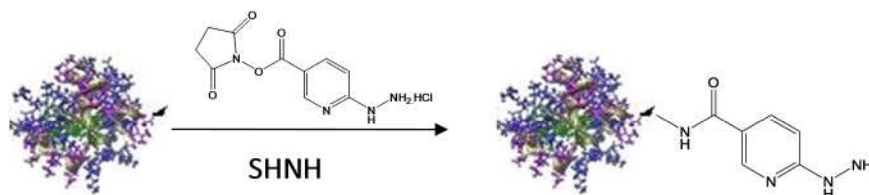


Figure 1: Scheme presenting the modification of a protein with SHNH.

Reagents

Desalting Spin columns (cat # S-4004-025)
 Modification Buffer (cat # S-4000-005)
 DMF (anhydrous) (cat # S-4001-005)

Equipment

Variable-speed bench-top microcentrifuge
 Spectrophotometer or Plate Reader
 1.5 mL microcentrifuge tubes

Note: This protocol and all documents linked below can be downloaded from the appropriate category in the Solulink Library at <http://www.solulink.com/library>.

Modification Procedure

Desalting procedure

- Desalt/buffer exchange the protein into Modification Buffer (100 mM sodium phosphate, 150 mM sodium chloride, pH 8.0); if needed, refer to the [desalting protocol](#).

Notes:

- It is necessary to remove all free amine-containing contaminants, e.g. tris, glycine, from the protein solution before modification.
- High-level buffering capacity, i.e. 100 mM phosphate, is necessary for successful modification.**
- For desalting Solulink recommends Pierce Zeba Desalt Spin columns (# 89882) for protein purification. Refer to desalting protocol for further instructions.

A. Determine the concentration of the protein

- Determine the concentration of the protein to be modified using a [Bradford assay](#) (BioRad, #500-0006) or the [BCA assay](#) (ThermoScientific, #23223). Alternatively the A280 can be used if the protein extinction coefficient is known (E1%).
- Adjust the concentration to 1-2.5 mg/mL in Modification Buffer pH 8.0, if necessary

B. Prepare the SHNH Solution

- Prepare a stock solution of SHNH in anhydrous DMF (or DMSO) by dissolving 2-4 mg of SHNH in 100 μ L anhydrous DMF.

Note: The SHNH/DMF stock solution must be used immediately.

C. Modification of a protein

- Using Table 1 as a guide, or the with the aid of the [Protein Modification With An NHS Ester Calculator](#), add the requisite volume of SHNH/DMF to the protein solution.

Notes:

- Note:** be sure to use the correct values for SHNH in the **Reagent Information** section of the calculator
 - Depending on the size of the protein and the desired level of modification, the number of equivalents should be adjusted.
- Allow reaction to incubate at room temperature for 1.5-2 hours.

IgG concentration (mg/mL)	SHNH mole equivalents added	Determined ratio of HyNic/protein
1.0	20	5.5
	30	8.2
4.0	15	4.7
	20	6.4
	25	7.8

Table 1: Recommended equivalents of SHNH linker to add to proteins at increasing concentrations to incorporate a specific linker substitution ratio.

D. Desalting procedure

1. Desalt/buffer exchange the protein or oligonucleotide into Conjugation Buffer (100 mM sodium phosphate, 150 mM sodium chloride, pH 6.0); if needed, refer to the [Protein Desalting Protocol](#) or [Oligonucleotide Desalting Protocol](#), if needed.

E. Quantifying modification level

1. The molar substitution ratio (MSR) can be determined using a colorimetric reaction with 2-sulfobenzaldehyde, as outlined in Figure 2. Addition of 2-sulfobenzaldehyde to a HyNic-modified biomolecule yields an aromatic molecule that absorbs at 350 nm. Refer to the [HyNic-Protein Colorimetric MSR Calculator](#) as well as the protocol that is appropriate for your lab equipment: [HyNic Colorimetric MSR Assay Protein Nanodrop Method Protocol](#), [HyNic Colorimetric MSR Assay Protein Spectrophotometer Method Protocol](#).

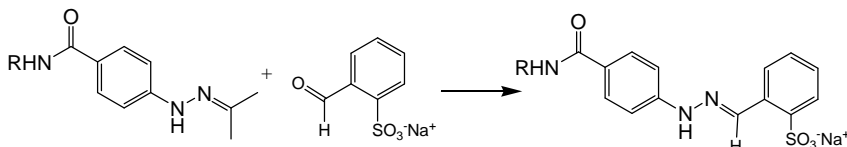


Figure 2: Colorimetric reaction used to quantify number of HyNic linkers on a biomolecule

2. The biomolecule is now SHNH-modified and ready for conjugation to Technetium-99M or 4FB-modified biomolecules and surfaces.

Note:

- a) HyNic-modified oligonucleotides are not stable and must be conjugated or immobilized immediately following desalting.

Troubleshooting

Problem	Possible Cause	Solution
Protein was not modified or poorly modified.	Protein has been contaminated with amine containing compounds	Desalt the protein more thoroughly with a new Zeba Spin column Increase the concentration of the protein to >2.0 mg/mL
	The concentration of the protein was too low	
SHNH was hydrolyzed	Wet or poor quality DMF/DMSO hydrolyzed the NHS ester	Use a good quality anhydrous DMF/DMSO to solubilize the SHNH molecule.

Stability

It is recommended to use the HyNic modified protein immediately. If long term storage is required it is recommended to store the modified protein <-20 °C and perform a time course stability study.

Related Solulink Products

S-4004	VivaSpin diafiltration column	S-2005	2-sulfo-benzaldehyde	S-2006	TurboLink Catalyst Buffer
S-1002	S-HyNic	S-4001	DMF anhydrous		
S-4000	Modification Buffer	S-4002	Conjugation Buffer		

References

1. Preparation and Preliminary Evaluation of 99mTc Labeled Fragment E1 for Thrombus Imaging, L.C. Knight, M.J. Abrams, D.A. Schwartz, M.M. Hauser, M. Kollman, F.E. Gaul, D.A. Rauh and A.H. Maurer, J. Nucl. Med. 33, 710 (1991).
2. Preparation of Hydrazino-Modified Proteins and Their Use for the Synthesis of 99mTc Protein Conjugates, D.A. Schwartz, M.J. Abrams, M.M. Hauser, F.E. Gaul, D. Rauh, J.A. Zubieta and S.K. Larsen, Bioconjugate Chem. 2, 333 (1991).
3. 11) 99mTc Human Polyclonal IgG Radiolabeled via the Hydrazino Nicotinamide Derivative for Imaging Focal Sites of Infection in Rats. M.J. Abrams, M. Juweid, C.I. tenKate, D. Schwartz, M.J. Hauser, F.E. Gaul, A.J. Fuccello, R.H. Rubin, H.W. Strauss, and A.J. Fischman, J. Nucl. Med. 31, 2022 (1990).
4. For an extensive list of references see PubMed or Google Scholar: keyword HYNIC

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