

Supporting information: a method to measure protein unfolding at an air-liquid interface

Danielle L. Leiske^{1*}, Ian C. Shieh², Martha Lovato Tse¹

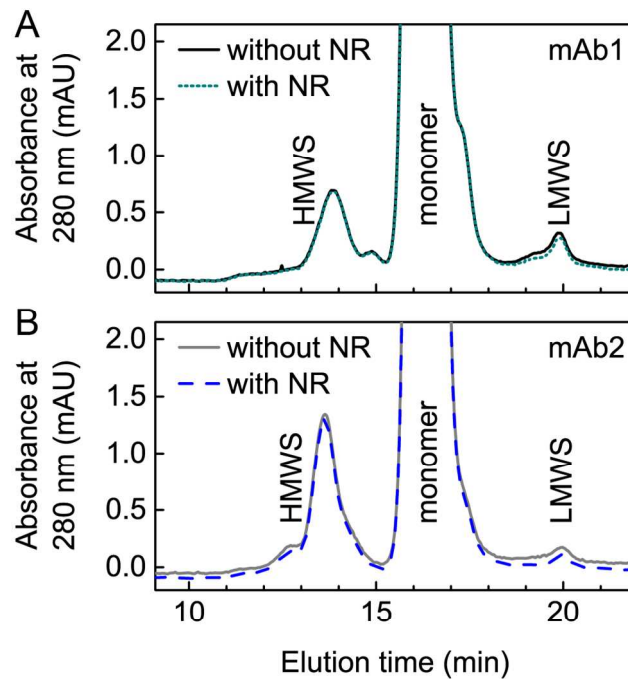
¹ Early Stage Pharmaceutical Development, ² Late Stage Pharmaceutical Development,
Genentech, South San Francisco, CA

* Corresponding author: leiske.danielle@gene.com

Table of contents

S1: Size exclusion chromatography of mAbs with and without Nile red	Page 2
---	--------

Supporting Information: Size exclusion chromatography of mAbs with and without Nile red



% relative area			
	HMWS	monomer	LMWS
mAb1	1.5	98.0	0.5
mAb1 + NR	1.5	98.1	0.4
mAb2	2.4	97.5	0.2
mAb2 + NR	2.4	97.4	0.2

Figure S1. Nile red did not induce aggregation of the antibodies in solution. Solutions of mAb1 and mAb2 at 0.2 mg/mL antibody were incubated with and without 0.2 μ M Nile red (NR) at room temperature for 4 h. These samples were then assayed using size exclusion chromatography to assess the impact of Nile red on antibody aggregation. The distribution of high molecular weight species (HMWS), monomer, and low molecular weight species (LMWS) was unaltered for both mAbs in the presence of Nile red. Sizing analysis was performed using an Agilent 1200 series HPLC equipped with a Tosoh TSKgel G3000SWxl column via an isocratic elution of 0.2 M K_3PO_4 and 0.25 M KCl (pH 6.2) at 0.5 mL/min for 30 min.