

# WEB303 - Analytical Gefi with reduced refolded Protein

Benjamin Weigel

August 6, 2014, Halle

## 1 Description

- from WEB301 experiments with analytical gel filtration it seems as if SOMT elutes in tetrameric, as well as monomeric form
  - however those runs were under **non-reducing** conditions
  - ↔ re-run a sample eluted from the WEB301 GPC run under **reducing** conditions

## 2 Experimental

### 2.1 Protein sample

**Sample:** concentrated eluate from pooled fractions A2 and A3 of **WEB301.Run2**

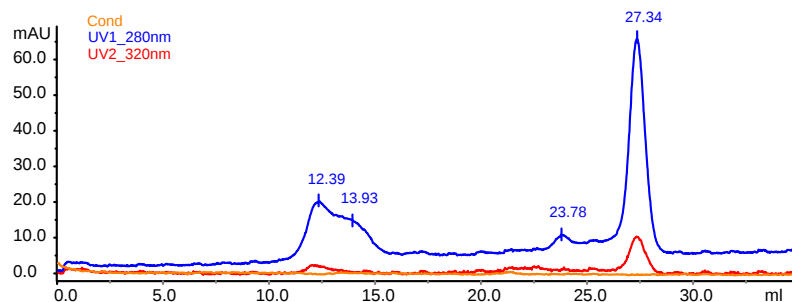
**Treatment:** added approximately 10 mM DTT to sample ↔ let incubate on ice for approx. 1h

### 2.2 ÄKTA Run conditions (Fig. 1a)

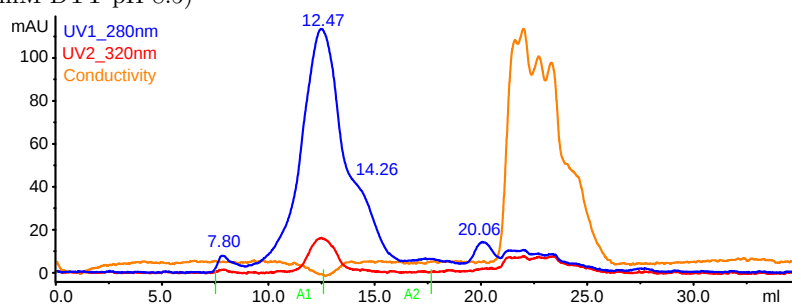
**ÄKTA column:** Superdex 200 10/300 GL

**Running buffer:** 0.1 M Tris/HCl, 150 mM NaCl, **1 mM DTT**, pH 8.5

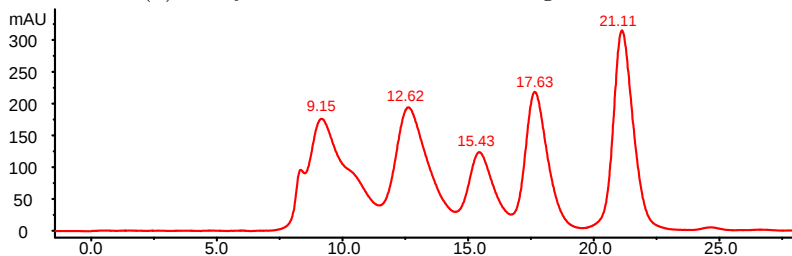
**Sample:** 100  $\mu$ l of the sample from **2.1** (xx mg/mL) [100  $\mu$ l loop]



(a) Analytical gel filtration run with protein that was subjected to gel filtration before (WEB301 Run2, see Fig. 1b). The protein was treated with 10 mM DTT prior to the run. Buffer: 0.1 M Tris/HCl, 150 mM NaCl, 1 mM DTT pH 8.5)



(b) Analytical Gefi run - non-reducing conditions



(c) Analytical standards

Figure 1: ÄKTA runs