

## Quantification of Proteins via $A_{280}$

--> *This method only works with proteins containing aromatic residues*

- start the Cary WinUV program “Simple Reads” (or “Scan”) and let the UV-lamp warm up
- dilute protein solution (if necessary) in ddH<sub>2</sub>O (or buffer) and mix gently (100μL total)
- blank UV spec. against ddH<sub>2</sub>O (or buffer)
- measure  $A_{280}$  (make sure that  $A_{280}$  reading is between 0.1 and 1.0!!!) (or scan 250-290nm)

$A_{280} \times (\text{dilution factor}) : \epsilon_{\text{protein}} = C_{\text{protein}} [\text{M}]$  in undiluted sample

### Absorption maxima of aromatic residues:

Tryptophane	(Trp, W)	280nm	$\epsilon_{280} = 5600 \text{ M}^{-1} \text{ cm}^{-1}$
Tyrosine	(Tyr, Y)	274nm	$\epsilon_{280} = 1400 \text{ M}^{-1} \text{ cm}^{-1}$
Phenylalanine	(Phe, F)	257nm	$\epsilon_{280} = 200 \text{ M}^{-1} \text{ cm}^{-1}$