Enzymatic quantification and length determination of polyphosphate down to a chain length of two [1]

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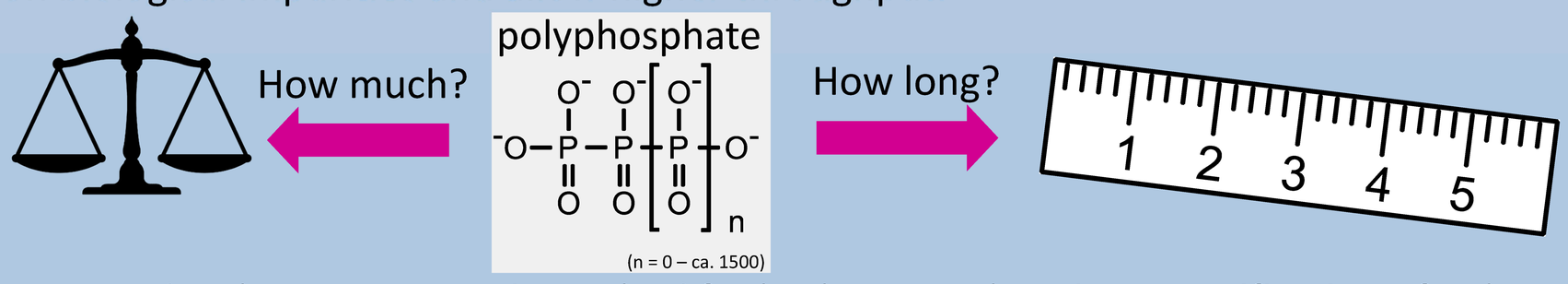
I Introduction and goal

Inorganic polyphosphate can be found as a linear polymer of orthophosphate (polyP) in all living organisms. To study polyP in organisms, analytical methods for both polyP quantification and length determination are required, which are insensitive to common biological impurities and allow higher throughput.



The aim of this study was to develop an enzymatic method for both comprehensive quantification of polyP and determination of the average polyP chain length from biological samples.

II Results

First, a colorimetric assay for orthophosphate detection was developed. Features of the assay:

- Addition of only one reagent to the sample
- Full color development in < 2 min
- Linear range: 5 to 200 µM orthophosphate
- Negligible hydrolysis of polyP during orthophosphate determination

III Results 2

Based on the colorimetric assay, a new assay for polyP quantification and chain length determination was developed. See Fig. 1 for the work flow and equation 1 for calculation of the average chain length.

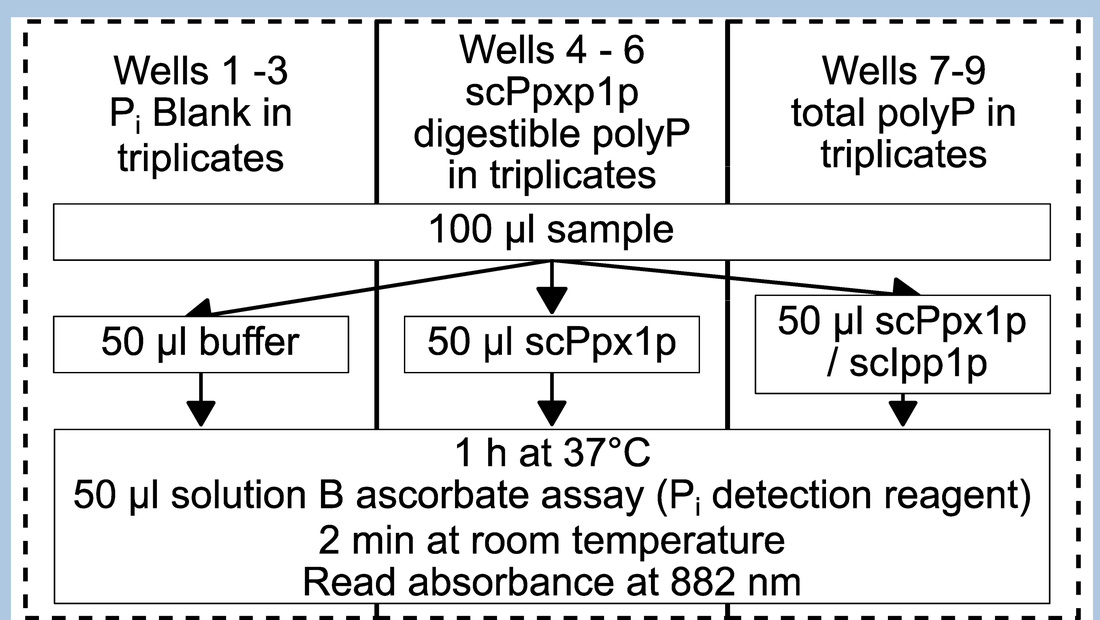


Fig. 1. Schematic workflow of the assay

$$\text{length}_{\text{polyP}} = \frac{2 * (\text{"scPpx1p digestible polyP"} - \text{"blank Pi"})}{\text{"total polyP"} - \text{"scPpx1p digestible polyP"}} + 2 \qquad \text{(Eq. 1)}$$

IV Results 3

Characteristics of the new assay for polyP quantification and length determination:

- Use of *Saccharomyces cerevisiae* exopolyphosphatase 1 and *S. cerevisiae* inorganic pyrophosphatase 1
- Comprehensive quantification of polyP of all chain lengths
- Average polyP chain length of short chain polyP can be quantitatively determined (Table 1)
- Common biological impurities (DNA, RNA, ATP etc.) do not interfere with the assay
- 2 h, 1.5 µg polyP and a plate reader required

Table 1. Comparision enzyme assay vs. gold standard (titration)

PolyP	Mean chain length ± SEM	
	Enzyme assay	Titration
Triphosphate	3.1 ± 0.0	n. d.
Budit 9	3.8 ± 0.0	4.1 ± 0.0
PolyP from Sigma	11.8 ± 0.1	10.8 ± 0.0
Budit 7	12.6 ± 0.3	11.9 ± 0.0
Budit 4	25.7 ± 2.3	21.2 ± 0.1
PolyP from Roth	27.9 ± 2.7	24.6 ± 0.1

V Conclusion

We present an enzymatic assay that allows for the first time both comprehensive polyP quantification, and length determination of short chain polyP.

1. Christ JJ, Blank LM (2018) Enzymatic quantification and length determination of polyphosphate down to a chain length of two, *Analytical Biochemistry*, 548 (82-90).