

Smart Polymer Preconcentration to Enhance the Sensitivity of *Chlamydia trachomatis* Immunoassays

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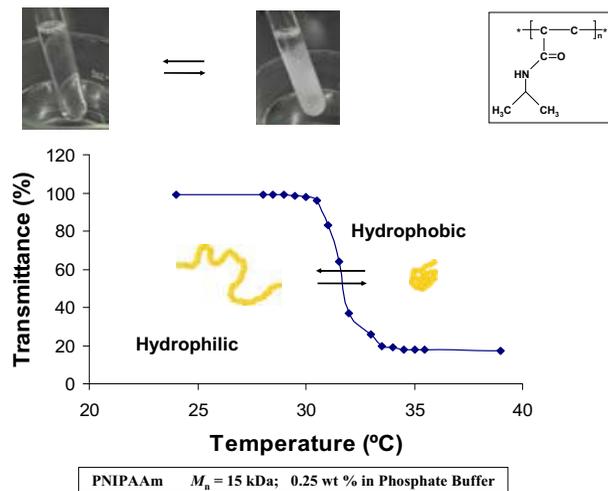
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SMART POLYMERS

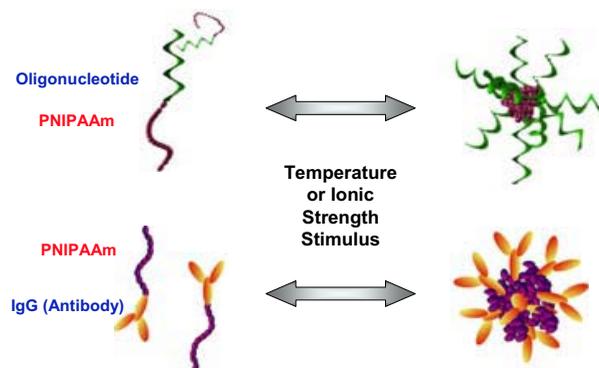
- Smart polymers are polymers that undergo a SHARP and REVERSIBLE hydrophilic to hydrophobic transition on application of stimuli such as temperature and ionic strength.



- Poly(N-isopropylacrylamide) (PNIPAAm) is an ionic strength and temperature sensitive smart polymer.

SMART NANOPARTICLES

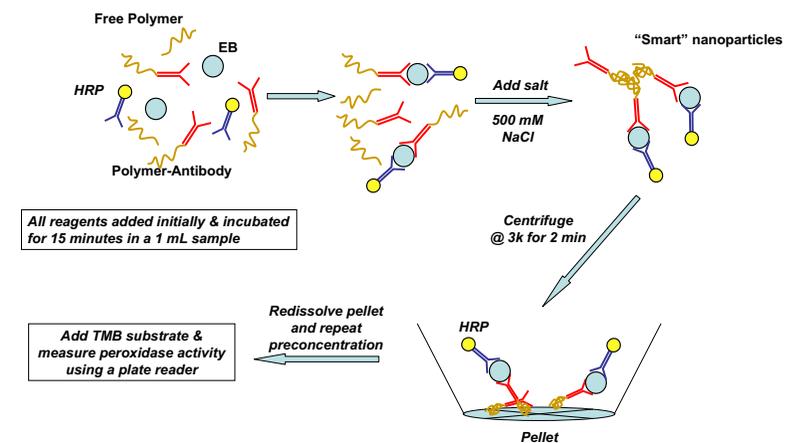
- At temperatures above 31°C or ionic strengths above 500 mM at room temperature, the PNIPAAm becomes hydrophobic and drives assembly of conjugates into uniform particles.



- These "Smart Nanoparticles" can be rapidly formed and dissolved and their size controlled in the range of ca. 100–900 nm by varying concentration, molecular weight, and ionic strength (Kulkarni et al, *Bioconjugate Chemistry*, 15, 747-753, 2004).

CAPTURE OF CHLAMYDIA EBs

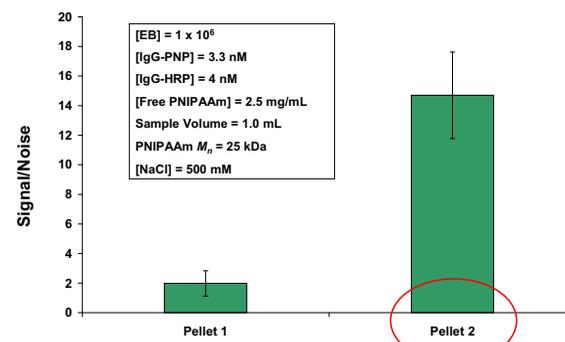
- There is a strong need to improve the sensitivity of rapid tests for *Chlamydia trachomatis*, especially in developing countries.



- Antibody against the MOMP antigen on the Chlamydia elementary bodies was conjugated to PNIPAAm and used in the capture.
- Pellet can be formed either by heating the sample or by adding 500 mM NaCl; here, salt is used for the precipitation.

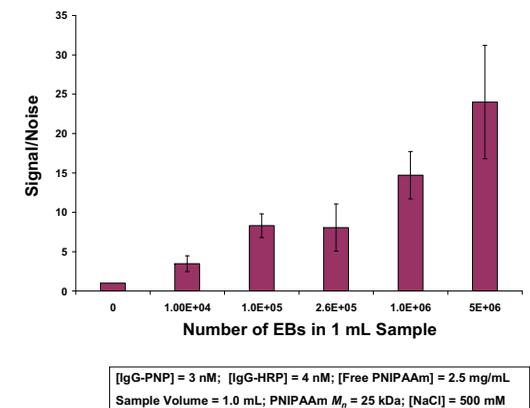
HIGH SIGNAL TO NOISE RATIO

- Signal/noise measured as ratio of absorbances of sample with Chlamydia EBs versus control sample without EBs.



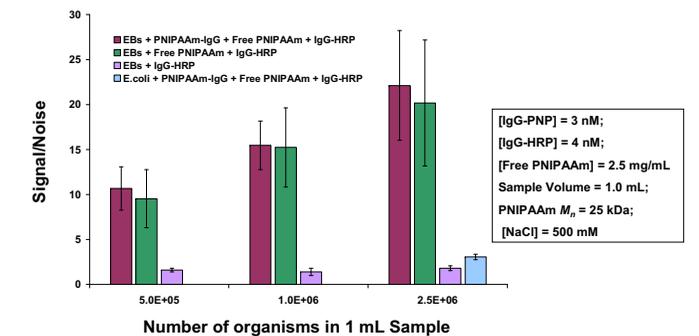
- A second round of salt precipitation was found to increase the signal/noise ratio to >10 fold.

DYNAMIC RANGE OF THE ASSAY



- The capture and detection of Chlamydia EBs is dependent on the concentration of EBs in solution, and the assay can be used to detect EBs in the concentration range of 10^4 /mL to 10^9 /mL reliably.

SPECIFICITY OF CAPTURE



- Binding of PNIPAAm to Chlamydia EBs is presumably due to hydrophobic- or hydrogen-bonding interactions.

CONCLUSIONS

- Preconcentration and sensitive detection of Chlamydia EBs (limit of detection = 10^4 EBs/mL) was demonstrated using the PNIPAAm-based system.
- The preconcentration system can be used as a stand-alone assay or as a sample preparation step for rapid tests and ELISAs.
- Platform can also be used to develop DNA-based lateral flow tests.

ACKNOWLEDGEMENTS

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