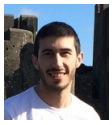
NOVEL STIMULI-RESPONSIVE POLYION COMPLEX (PIC) PARTICLES

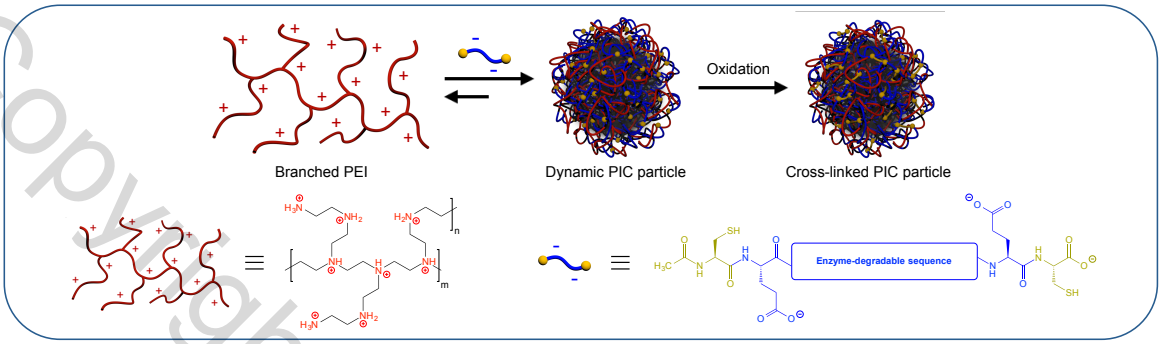


Ignacio Insua*, Nicolas Vergne, Maria Stroe, Anna F. A. Peacock and Francisco Fernandez-Trillo

School of Chemistry, University of Birmingham. B15 2TT, Birmingham (UK). *Email: ixi311@bham.ac.uk *Twitter:@InsuaNacho

INTRODUCTION

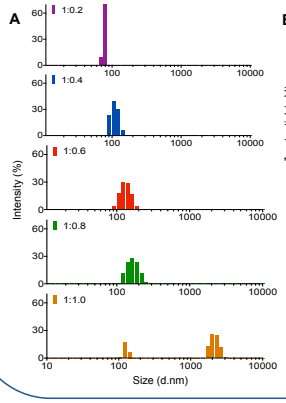
Polyethylenimine (PEI) is a cationic polymer widely studied in gene delivery, capable of forming discrete particles when mixed with nucleic acids.[1] The main force driving the self-assembly of these two macromolecules is the electrostatic attraction between their oppositely-charged groups, thus both components merge into polyion complex (PIC) particles.[2,3] The aggregation of polyions is known to be a dynamic system, where only the covalent bonding of electrolytes displaces the equilibrium to the formation of steady particles.[4] In this communication we present our preliminary studies on the synthesis and evaluation of cross-linked PIC nanoparticles, prepared from branched PEI and short acidic enzyme-degradable peptides (see Scheme 1).



Scheme 1: Reversible aggregation of PEI and small peptides into PIC particles and cross-linking of the assembly (top). Molecular structures of the polyions (bottom).

SYNTHESIS OF PIC PARTICLES

PEI and anionic peptides were mixed in different proportions -expressed as N:COOH, respectively- and the formation of PIC particles was studied by Dynamic Light Scattering (DLS) and ζ-potential. Likewise, increasing concentrations of polyions were assessed. Finally, the cross-linking kinetics was evaluated.



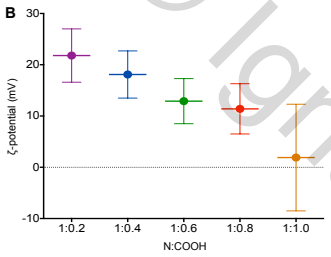


Figure 1. Size (A) and charge (B) of PIC particles at different N:COOH ratios. Equimolar mixtures of PEI and peptide formed neutral particles that came together into big aggregates.

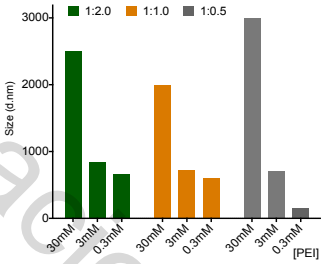


Figure 2. Size of PIC particles prepared at different concentrations. For all three N:COOH ratios studied it was seen that the lower the concentration, the smaller the particles.

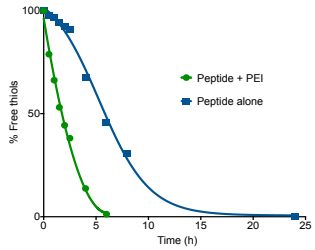


Figure 3. Peptide oxidation kinetics monitored by Ellman's assay[5]. Faster oxidation occurs in presence of PEI, where peptide molecules come together increasing the local thiol concentration (Scheme 1).

STIMULI-RESPONSIVENESS STUDIES

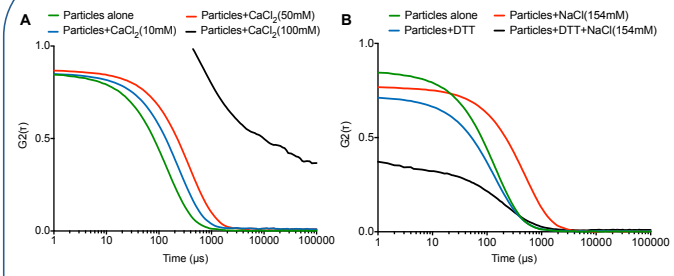


Figure 4. DLS correlation curves of PIC particles in presence of salts and reducing agent 1,4-dithiothreitol (DTT): Particles swelled with increasing amounts of $CaCl_2$ (A), eventually bursting at high salt concentration (black line). Reduction of cross-linking disulphide bonds does not affect the stability of the particles, but it spoils their salt tolerance (B).

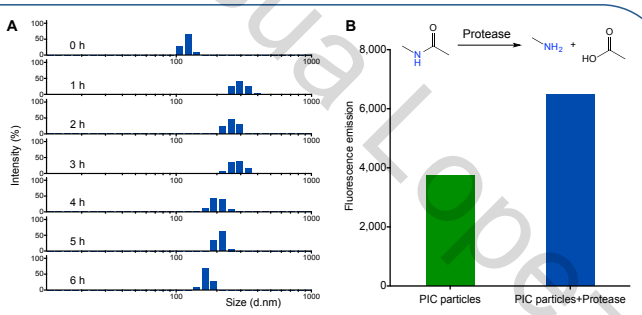


Figure 5. Enzymatic degradation of PIC particles: DLS data (A) suggests particles incubated with protease in buffer initially swell, to be then gradually degraded over time. After 6 hours, primary amines were quantified by fluorescence (B); higher emissions were seen in presence of protease. Both results indicate particles are enzyme-degradable.

CONCLUSIONS

- **A method for preparing cross-linked PIC nanoparticles with tunable sizes and ζ-potentials was developed.**
- **These particles respond to salt concentration and reductive agents, displaying also promising results in preliminary enzyme-triggered disassembly studies.**
- **Stimuli-responsive PIC particles arise as key tools for biomedical applications and chemical probing given their versatile responsiveness and unlimited design possibilities.**

REFERENCES

[1] W. T. Godbey, K. K. Wu, and A. G. Mikos, *J. Control. Release*, 1999, **60**, 149–160. [2] A. Harada and K. Kataoka, *Macromolecules*, 1995, **28**, 5294–5299. [3] S. Katayose and K. Kataoka, *Bioconjugate Chem.*, 1997, **8**, 702–707. [4] T. Blessing, J. S. Remy, and J. P. Behr, *Proc. Natl. Acad. Sci. U.S.A.*, 1998, **95**, 1427–1431. [5] P. W. Riddles, R. L. Blakeley, B. Zerner, *Anal. Biochem.* **1979**, *94*, 75–81.

ACKNOWLEDGEMENTS

We would like to thank the University of Birmingham and Mr. John Evans (John Evans Fellowship in Nanotechnology) for funding. Also, Dr. Marie-Christine Jones and Dr. Hanene Ali-Boucetta (UoB) for access to nanoparticle characterisation facilities.