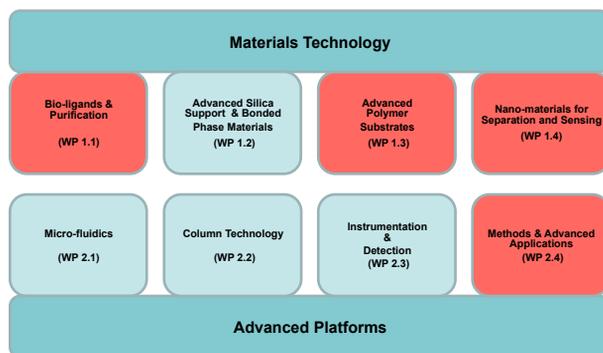


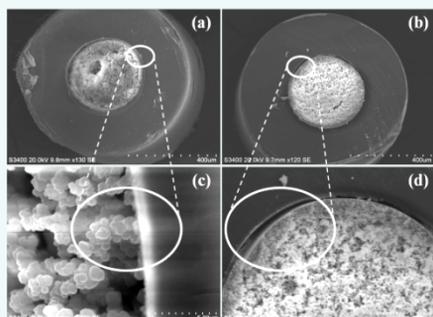
## AIMS:

- To *in situ* fabricate ethylene dimethacrylate porous polymer monoliths within the confines of a commercial 20  $\mu$ L polypropylene pipette tips.
- To enhance the monolith surface area by immobilising AuNPs and then functionalise the AuNPs with ECL lectin for selective extraction of galactosylated proteins from complex media.



## 1. Porous monolith fabrication in a pipette-tip

- Photo-grafted chains of poly(EDMA) were covalently attached to the polypropylene surface via hydrogen abstraction to facilitate subsequent covalent attachment of methacrylate monolith. (EDMA monolith formed *in situ* via photo-initiated polymerisation.



**Fig. 1:** SEM images of a monolith formed within a modified tip (a,c) and unmodified tip (b,d) prior to monolith polymerisation.

## 2. Monolith surface modification with AuNPs

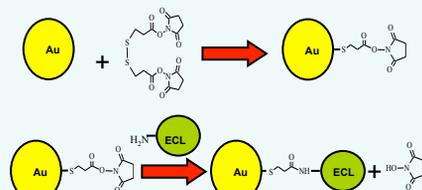
- Citrate-stabilised gold nanoparticles were immobilised upon the monolith after extensive amination of photo-grafted vinyl azlactone (amine reactive).



**Fig. 2:** (left): Image of PP tips incorporating AuNP-aggglomerated monolith at various stages of preparation. (right): Fe-SEM image of AuNP-aggglomerated polymer monolith (60,000x)

## 3. Immobilisation of *Erythrina cristagalli* lectin (ECL) on Au-modified monoliths

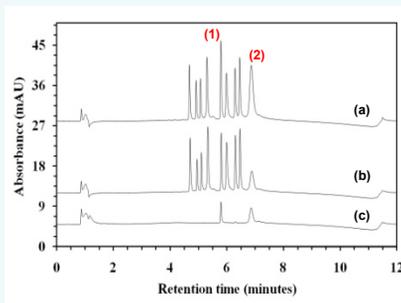
- ECL is a lectin with specificity for glycans with terminal galactose.
- Monolith was flushed with the amine-reactive bi-functional linker DTSP (3,3'-dithiodipropionic acid di(N-hydroxysuccinimide ester).
- The covalent attachment of the lectin on the Au surface was achieved due to native lysine residues on ECL reacting with pendant succinimidyl groups.



**Fig. 3:** Schematic diagram of the modification of the gold-immobilised monolith with ECL.

## 4. Bind-release and selectivity studies for selected glycoproteins

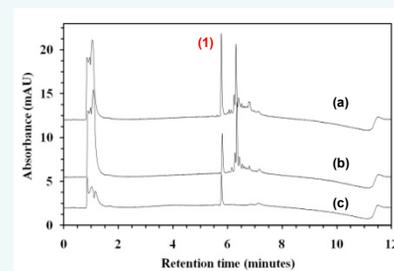
- Non-specific binding of protein was evaluated with a test mixture comprising selected non-glycosylated proteins as well as glycosylated proteins with and without terminal Gal residues.
- Only glycoproteins bearing terminal galactose were extracted due to comprehensive blocking of any remaining unreacted bare gold nanoparticles or unreacted succinimidyl groups (Step 3, above) with 1 M Tris.
- Fig 4 illustrates that ~35 % of a 20  $\mu$ g/mL standard of transferrin was extracted, with recovery (via galactose wash) exceeding 86 %.
- The %RSD for transferrin extraction (31 % average) was 14 % for six individually prepared extraction devices.



**Fig. 4 (left):** Test mix of proteins before (a) and (b) after passage through the affinity monolith. Chromatogram (c) is after a galactose wash. (Chromatographic conditions: Column: 100 cm x 100  $\mu$ m LaMA monolith, 1  $\mu$ L/min, TFA/ACN gradient. Peaks: (1): transferrin, (2): thyroglobulin.

## 5. Glycoprotein extraction from complex samples.

- Transferrin was spiked into an *E.coli* cell lysate sample at a level of 20  $\mu$ g/mL.



**Fig. 5:** Extraction of transferrin from a spiked *E. coli* cell lysate sample. Chromatograms are before extraction (a) and after extraction (b). Chromatogram (c) is after a galactose wash. Chromatographic conditions as in Fig 4.

## 3. Project outputs

### Publications:

- Pipette-tip selective extraction of glycoproteins with lectin modified gold nano-particles on a polymer monolithic phase, *The Analyst*, **Accepted**, 2011.

Authors: H. Alwael, D. Connolly, P. Clarke, R. Thompson, B. Twamley, B. O'Connor and B. Paull.

### Oral Presentations:

- Conference on Analytical Science Ireland (CASI), DCU, Ireland, Feb 2011.
- 22<sup>nd</sup> International Ion Chromatography Symposium, Cincinnati, USA, Sept 2010.

### Poster presentations

- 35<sup>th</sup> International Symposium on High Performance Liquid Phase Separations and Related Techniques, Boston, MA, USA. June 2010.
- Analytical Research Forum 2010, Loughborough University, UK, July 2010.
- 3<sup>rd</sup> Annual Meeting of GlycoScience Ireland, UCD, Ireland, Aug-Sep 2010.
- 28<sup>th</sup> International Symposium of Chromatography, Valencia, Spain, Sept 2010.