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# LECTIN AFFINITY EXTRACTION OF GLYCOPROTEINS

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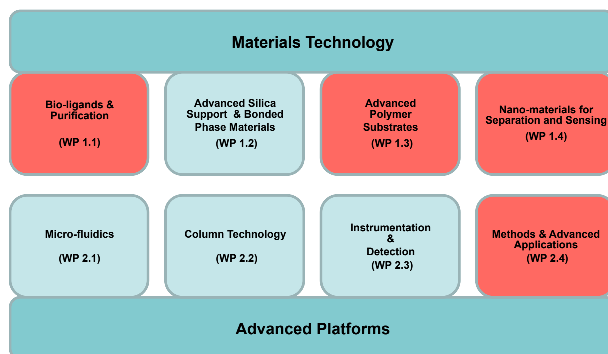
## AIMS:

- To *in situ* fabricate ethylene dimethacrylate porous polymer monoliths within the confines of a commercial 20 µL 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.

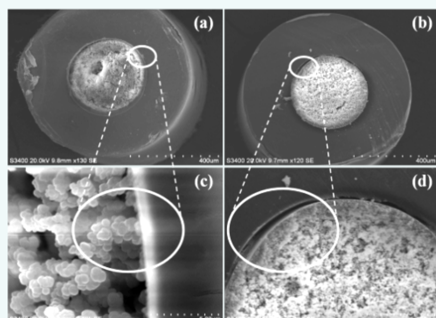



***Fig. 1:*** *SEM images of a monolith formed within a modified tip (a,c) and unmodified tip (b,d) prior to monolith polymerisation.*

## 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).

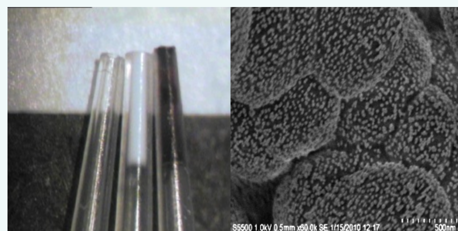


***Fig. 2:*** *(left): Image of PP tips incorporating AuNP-agglomerated monolith at various stages of preparation. (right): Fe-SEM image of AuNP-agglomerated polymer monolith (60,000x)*

## 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.

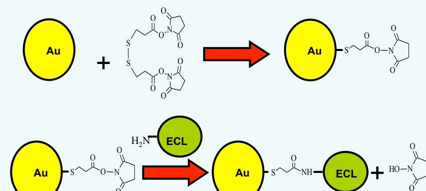



***Fig. 3:*** *Schematic diagram of the modification of the gold-immobilised monolith with ECL.*

## 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 µg/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.

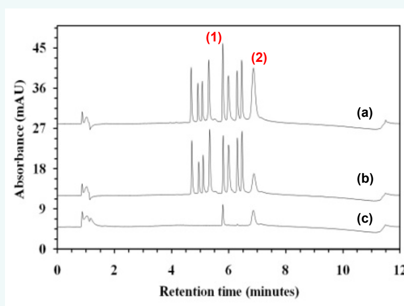



***Fig. 4 (left):*** *Test mix of proteins before (a) and (b) after passage through the affinity monolith. Chromatogram (c) is after a galactose wash. (Chromatographic conditions: Column: 100 cm x 100 µm LaMA monolith, 1 µL/min, TFA/ACN gradient. Peaks: (1): transferrin, (2): thyroglobulin.*

## 5. Glycoprotein extraction from complex samples.

- Transferrin was spiked into an *E.coli* cell lysate sample at a level of 20 ug/mL.
- Significant amounts of transferrin were extracted from this complex sample, with no detectable levels of interfering matrix proteins being co-extracted.

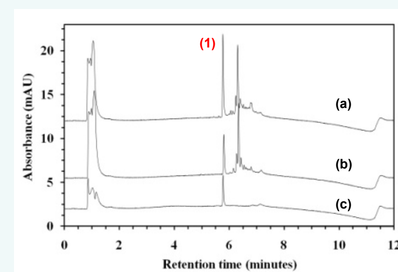



***Fig. 5:*** *Extraction of transferrin from a spiked E.coli cell lysate sample. Chromatograms are before extraction (a) and after extraction (b). Chromatogram (c) is after a galactose wash. Chromatographic conditions as in Fig 4.*

## 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'Conner and B. Paull.

**Oral Presentations:**

- **Conference on Analytical Science Ireland (CASi),** DCU, Ireland, Feb 2011.
- **22nd International Ion Chromatography Symposium, Cincinatti,** USA, Sept 2010.

**Poster presentations**

- **35th 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.
- **3rd Annual Meeting of GlycoScience Ireland,** UCD, Ireland, Aug-Sep 2010.
- **28th International Symposium of Chromatography,** Valencia, Spain, Sept 2010.

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