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4th Latin American Congress
2nd International Symposium on Lignocellulosic Materials
Biorefineries
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# Affibody functionalized bacterial cellulose tubes for bioseparation applications

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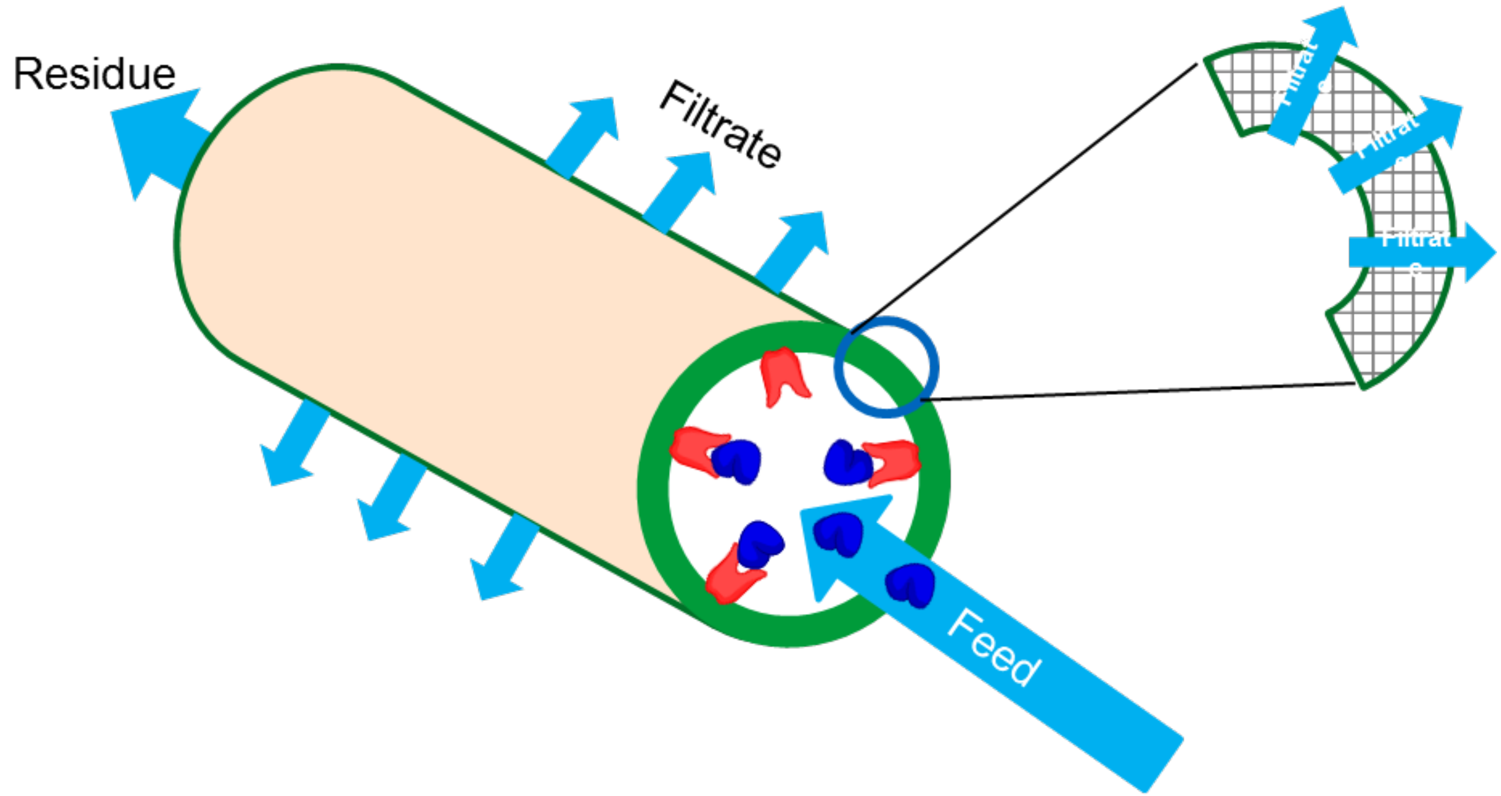
BiCMat
Bio-based Colloids & Materials

# Objective: Nanocellulosic porous filtration tube

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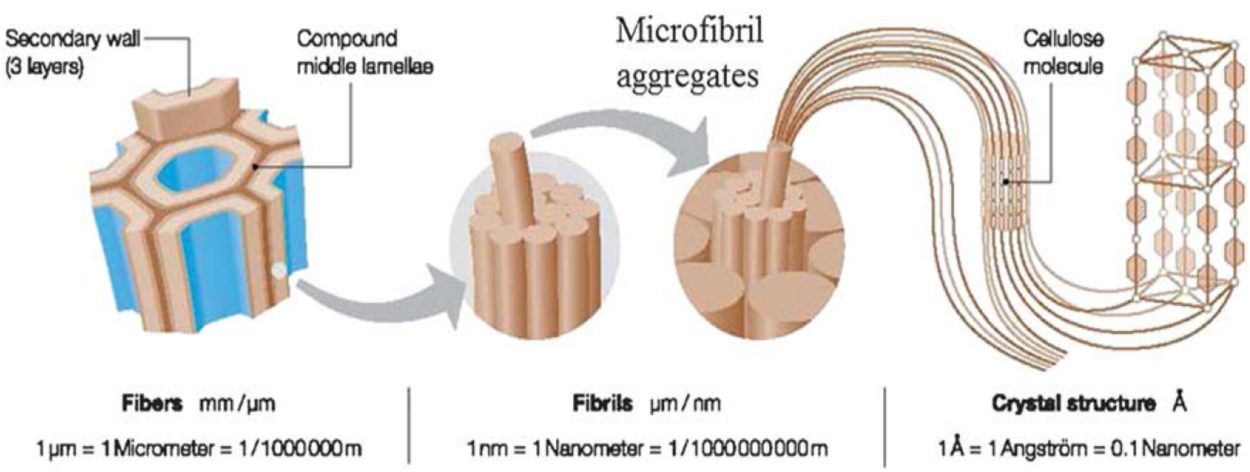
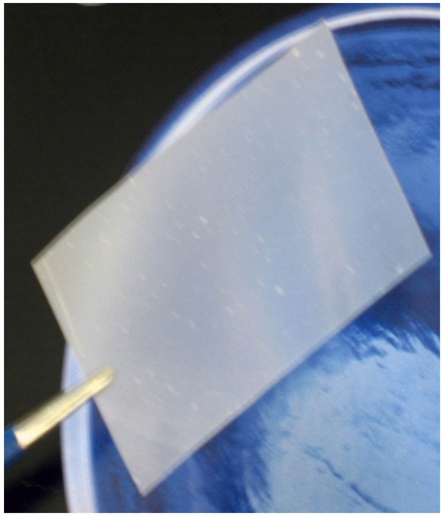
Orelma, H. et al. *RSC Adv.*, **2014**, 4, 51440-51450

# Wood based cellulose nanofibrils



- **Non-toxicity against living organisms**
- **Strength and stability**
- **High surface area**
- **A high number of accessible OH-groups for chemical modification**

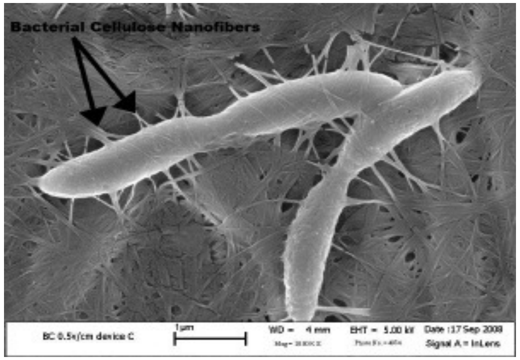
Tingaut et al. *J. Mater. Chem.* , 2012, **22**, 20105-20111

# Microbial cellulose / bacterial cellulose

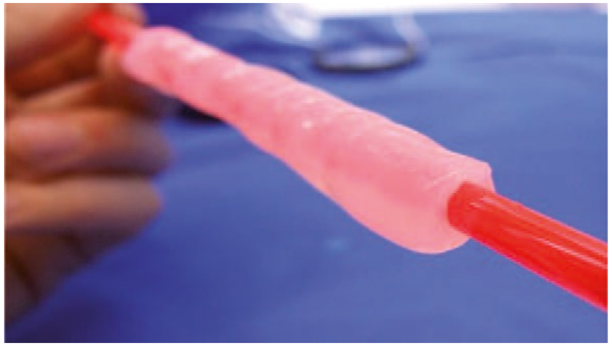
- *Acetobacter*, *Agrobacterium*, *Alcaligenes*, *Pseudomonas*, *Rhizobium* or *Sarcina* are able to extracellularly produce cellulose
  - *Acetobacter xylinum/Gluconacetobacter xylinus* is considered to be the most efficient strain
  - Continuous sources of air and carbon are required
  - Cellulose yield of 35-40% in relation to the applied glucose

- *Acetobacter* microfibrils usually have high crystal structure and thickness in the range of 6-10 nm

- Bacterial cellulose has a high degree of polymerization in the range of 4000-10000 anhydroglucose units
  - Contains more than 99 % water
  - Excellent wet strength




(Klemm et al. 2011, Angewandte Chemie International Edition, vol. 50, no. 24, pp. 5438-5466)



P. Gatenholm, D. Klemm, MRS Bull. 2010, 35, 208 – 213.

# Applications of bacterial cellulose

## Artificial blood vessels

Kowalska-Ludwicka, Karolina et al. Archives of Medical Science : AMS 9.3 (2013): 527–534. PMC. Web. 2 Mar. 2015.

## Scaffolds for tissue engineering


de Olyveira et al. Bacterial Nanocellulose for Medicine Regenerative. J. Nanotechnol. Eng. *Med.* 2(3), 034001 (2012)

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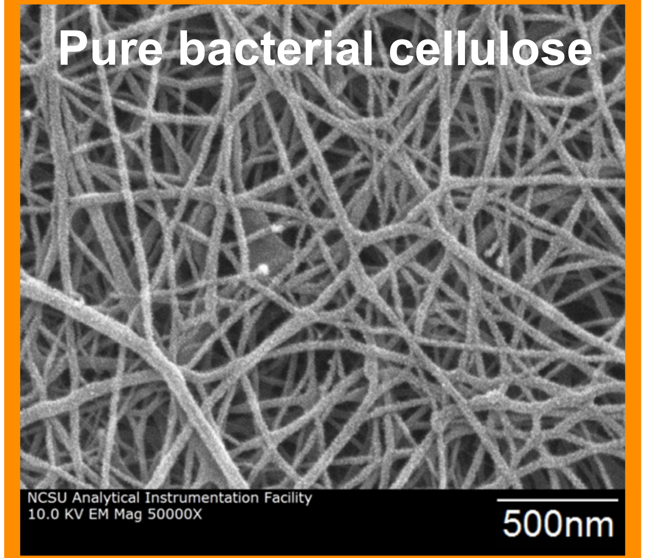



## Skin therapy


W. Czaja. et al. 2006 Biomaterials, 27 2

## Food


http://healthcarenutritionlifestyle.blogspot.fi/2012/11/be-slim-with-nata-de-coco.html **03/05/15**

## Nanopaper and paper additives

# Incubation conditions and modification strategies of BC

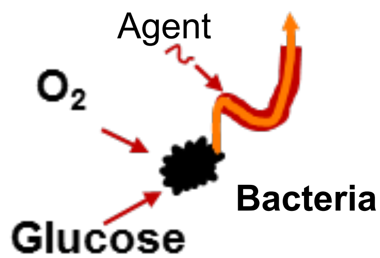
**Carbon source + Phosphate + Air + additives** → (**Bacteria**) → **Bacterial cellulose**

## Suitable carbon sources

- **Monosaccharides**
  - Glucose
  - Fructose
  - Galactose
  - Xylose
- **Disaccharides**
  - Sucrose
- **Polysaccharides**
  - Starch
- **Alcohols and other sources**
  - Glycerol
  - Ethanol
  - Arabitol
  - Coconut water
  - Fruit juices

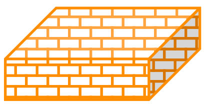
## Modification strategies

**In-situ**




-Carboxymethyl cellulose (CMC)
-Chitosan
-Xyloglucan (XG)
-Hydroxylethylcellulose (HEC)
-Polyvinyl alcohol (PVA)
-Polyethylene oxide (PEO)

**Ex-situ**

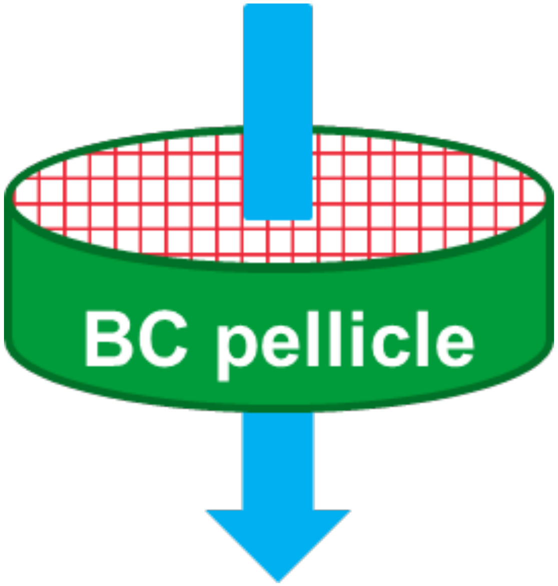


**Pellicle**

Traditional cellulose modification strategies can be employed

- TEMPO-mediated oxidation
- Carboxymethylation
- Esterification
- Amination

# Filtering properties of BC




## Physical properties of BC

### Molecular cut-off

- Never dried membrane up to 66 kDA (BSA)

Sokolnicki et al. 2006. J. Membr. Sci. 272. 15-27.

- Dried membrane 20 kDa (PEG)

Shibazaki et al. 1993. J. Appl. Pol. Sci. 50. 965-969.

### Pressure resistance

- Up to 880 mmHg (1.17 bar)

Bodin et al. 2006. Biotechnol. Bioeng. 97(2). 425-434.

### Chemistry

- No electrostatic interactions with proteins

- Highly stable due to high crystallinity degree

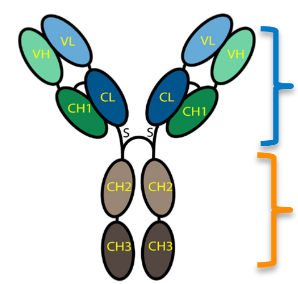
Vanderhart et al. 1984. Macromolecules. 17. 1465-1472.

# Anti-HSA affibodies

Affibody
14 kDa

**<u>Affibodies</u>:**

- engineered antibody mimics
- usable in therapeutic, diagnostic and biotechnological applications
- antigen binding site similar to native antibodies
- antigen affinity equal to native antibodies




Human IgG
150 kDa

**<u>Advantages:</u>**

- small size
- simple molecular structure
- robust physical properties
- ability to fold intracellularly



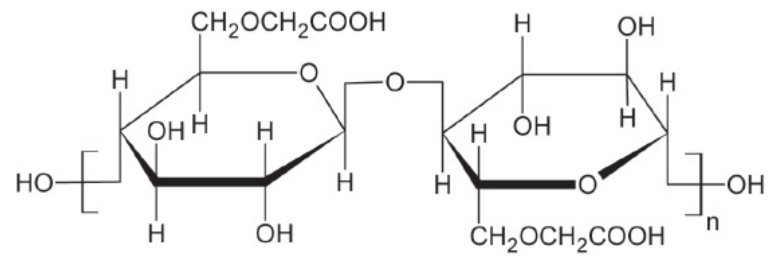
Images from Gremel et al. 2013. Frontiers in oncology. 271.
Review paper: Löfblom et al. 2010. FEBS letters. 584(12), 2670-2680.

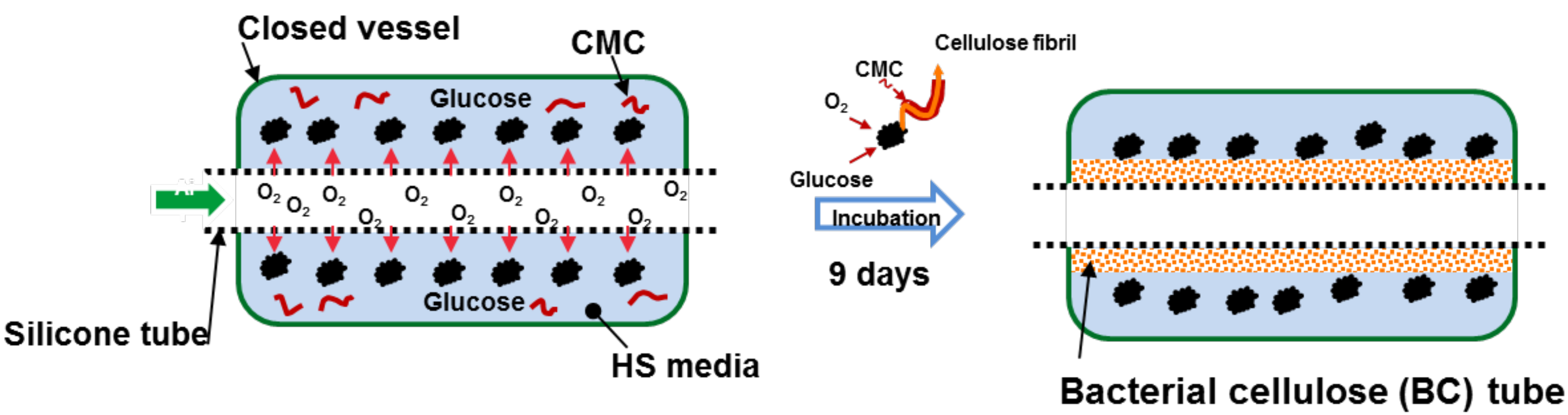
# Preparation of bacterial cellulose tubes

**Strain and incubation conditions:**

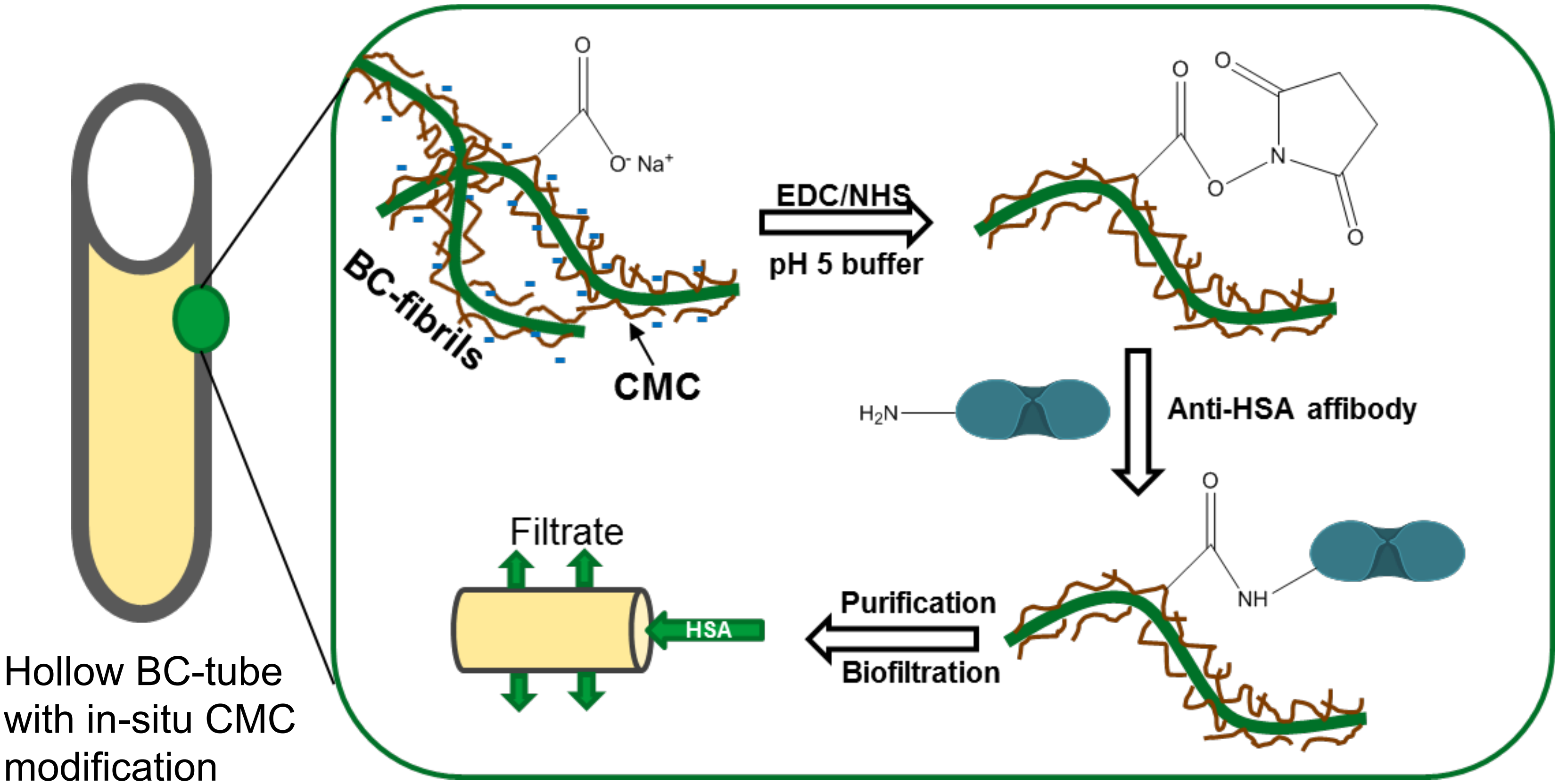
- Hestrin-Schramm (HS) medium at fixed pH of 4.5
- Gluconoacetobacter medellenisis *(G. medellinensis)*
- Closed vessel with permeable silicone tubes
- Statical incubation at 28 C for 9 days

**Carboxymethyl cellulose (CMC)**



# Conjugation of affibodies onto the CMC modified BC-tubes via EDC/NHS chemistry




- ❑ Conjugations of 0.1 mg/ml anti-HSA to CMC with 0.1 M EDC + 0.4 M NHS in 10 mM NaOAc buffer at pH 5

# Characterization methods

## Wet methods

- **Conductometric titration (SCAN-CM 65:02)**
  - Charge density of BC after CMC addition
- **Water retention value (SCAN-C 62:00)**
  - Bound water and irreversible structural changes of BC
- **Surface Plasmon Resonance (SPR)**
  - Interactions between cellulose, CMC and proteins

## Dry methods

- **Imaging with SEM**
  - Surface topography of BC
  - Lyophilized via liquid nitrogen
  - Surface and cross-section
- **Fluorescence imaging with Confocal Laser Scanning Microscopy (CLSM)**
  - Dansylated HSA
  - Tubes were lyophilized via liquid nitrogen

# Methods: Interaction analyses with Surface Plasmon Resonance (SPR)

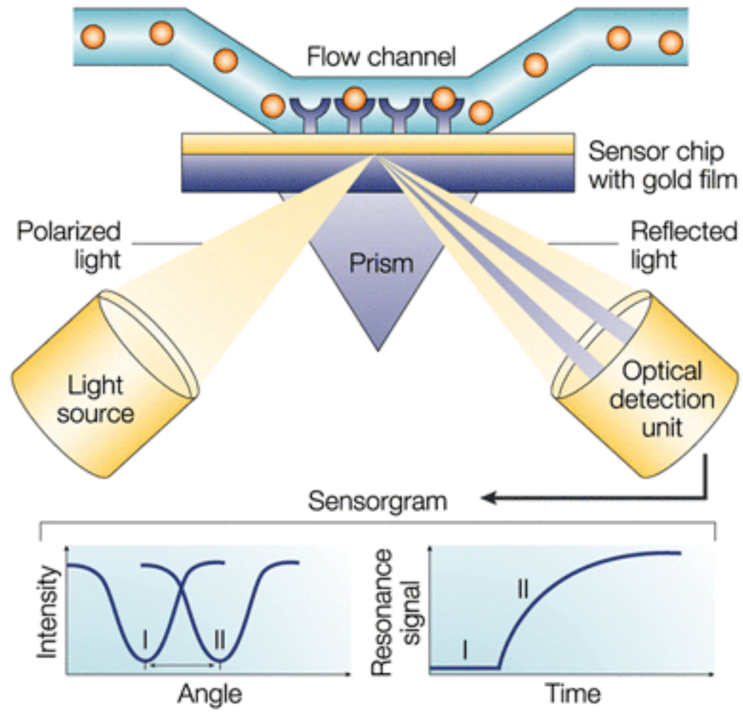
**SPR and cellulose model surfaces**

- Multimode SPR Navi 200, Oy Bionavis Ltd.
- Angular scan mode
- Langmuir-Schaefer deposited TMSC based cellulose surfaces
  - Cellulose II content up to 70%
- Thickness was modelled with a model

$$d = \frac{l_d}{2} \frac{\Delta_{SPR\ angle}}{m(n_a - n_0)}$$

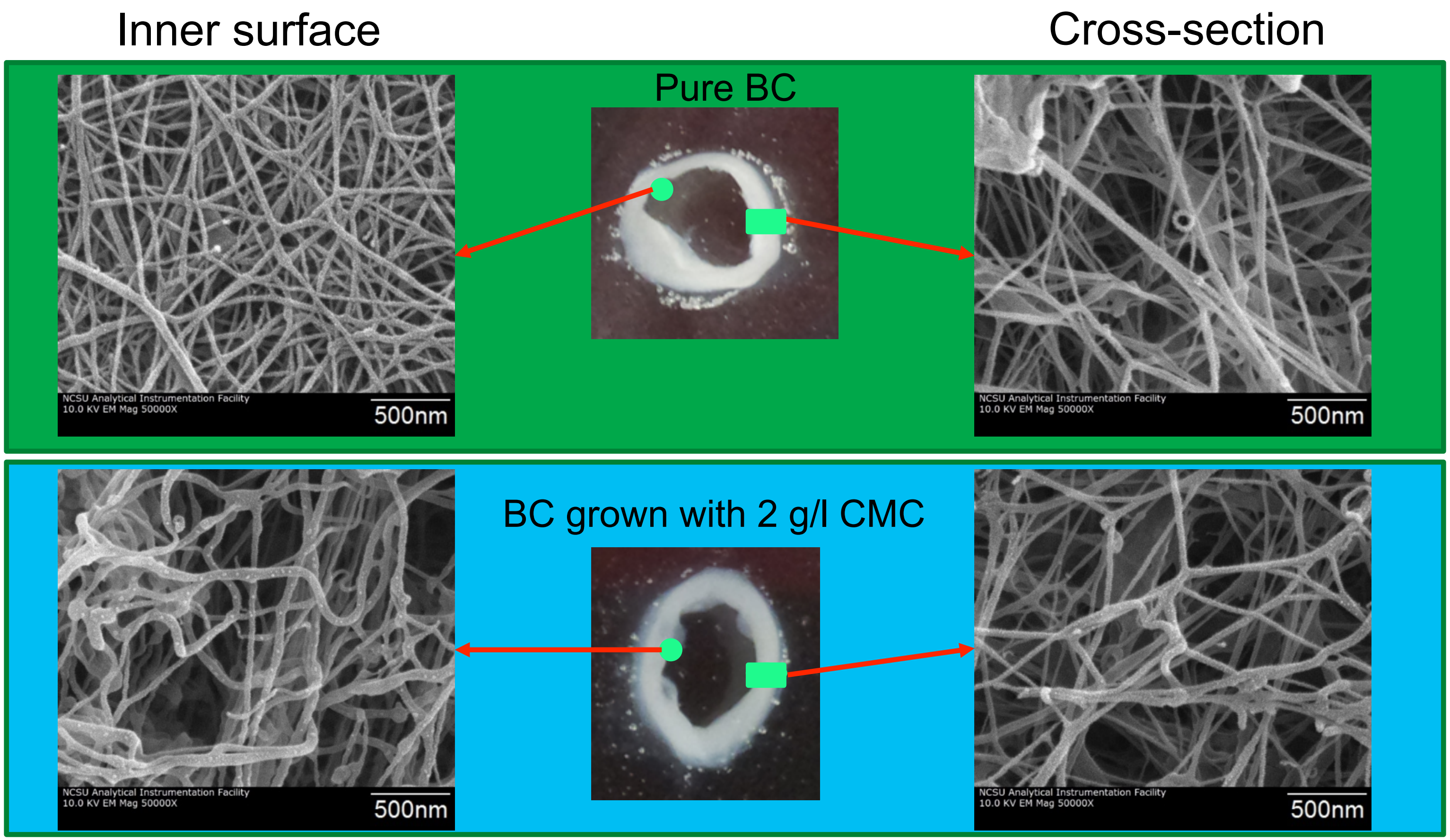
- Surface coverage was calculated with an equation

$$\Gamma = d * \rho$$




**Matthew A. Cooper, Nature Reviews Drug Discovery 1, 515-528 (2002)**
**SPR model from Jung et al. Langmuir 1998, 14, 5636-5648**

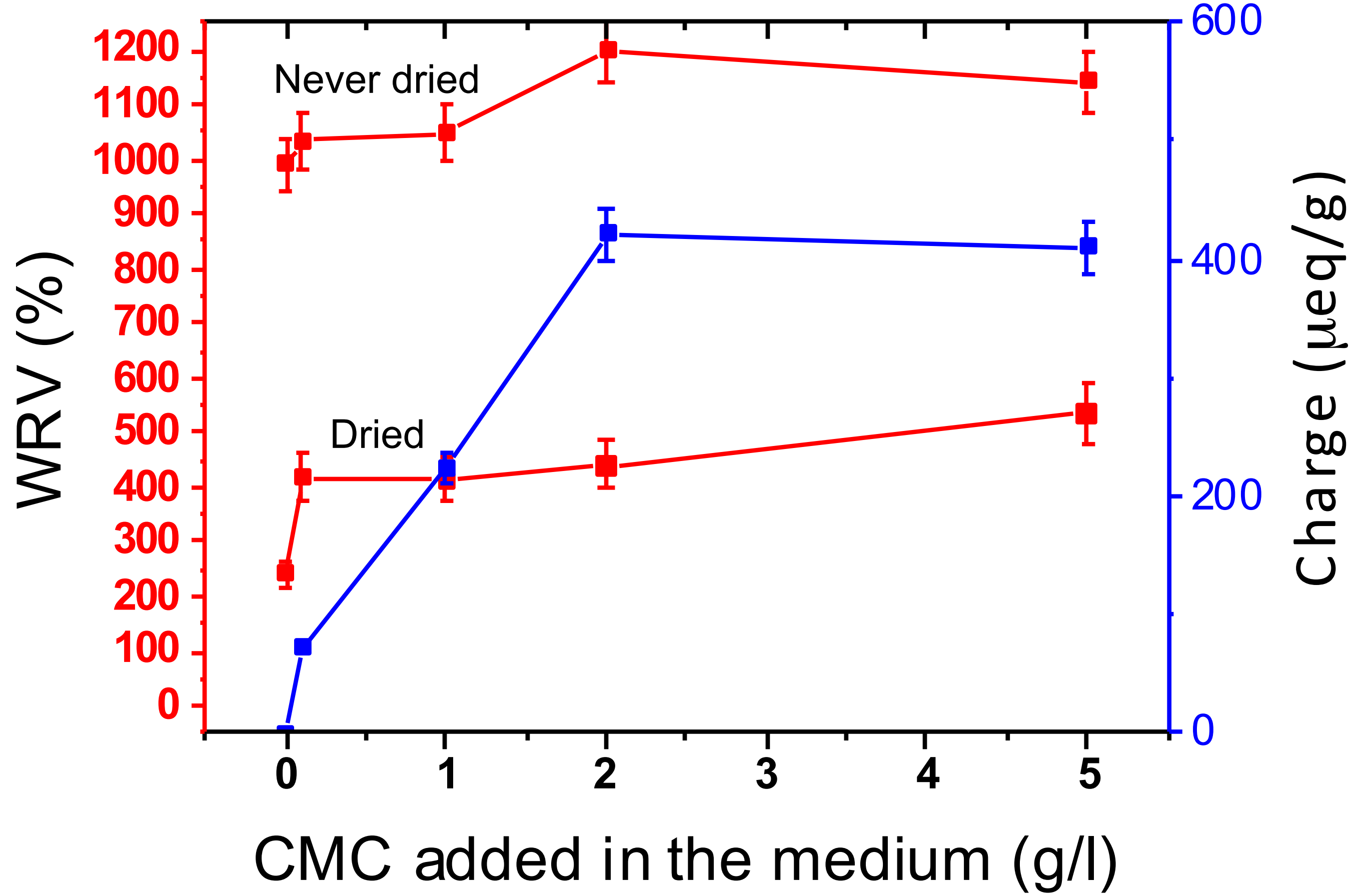
# Topography of BC tubes




**Physical properties of grown BC tubes**

- Length ~20 cm
- Diameter ~1 cm
- Wall thickness (wet) 1.8 ± 0.2 mm

# Water binding capacity of BC

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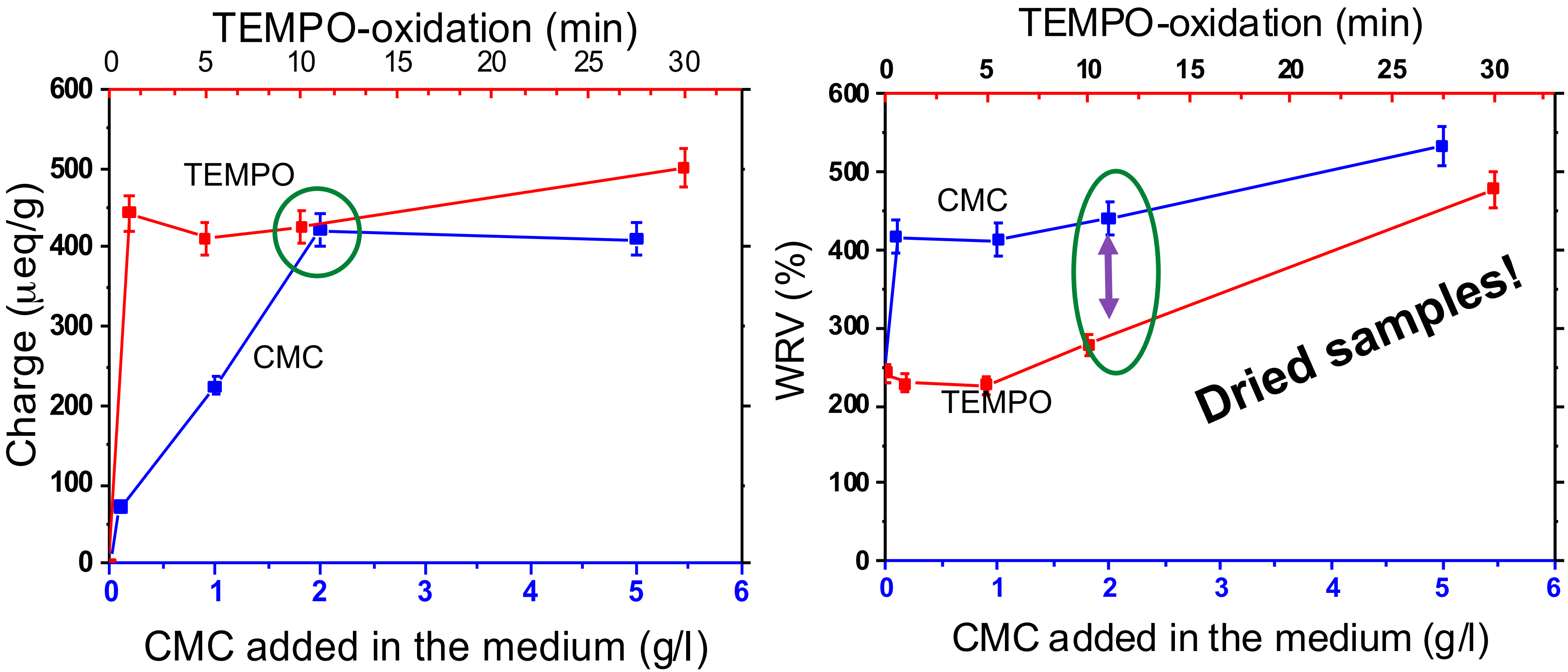
- CMC has a small effect on the WRV of never dried BC
- Considerable effect on water binding properties of dried BC
- Highest charge was obtained with CMC additions above 2 g/l

# Irreversible changes during drying




- WRV of air-dried TEMPO-oxidized BC sample is lower than that of air-dried CMC-BC samples
- Hornification occurs to a greater extent when the carboxyl groups are located only on the surface of BC-fibrils

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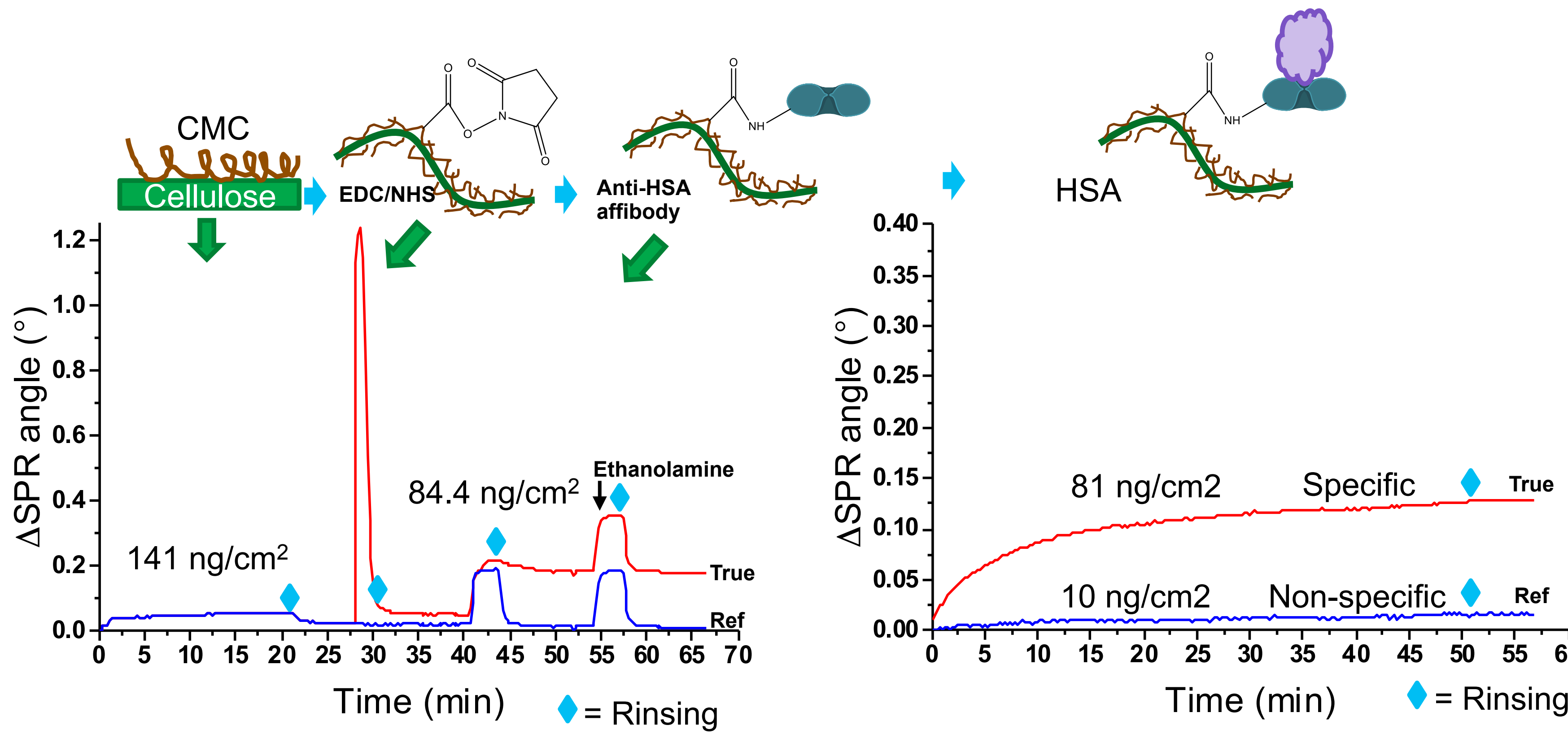
# Conjugation of anti-HSA affibodies onto cellulose monitored by SPR




- Specific binding of HSA on the prepared anti-HSA affibody biointerfaces was approximately eight-fold higher (~81 vs. 10 ng/cm2) when anti-HSA was conjugated onto cellulose via EDC/NHS chemistry

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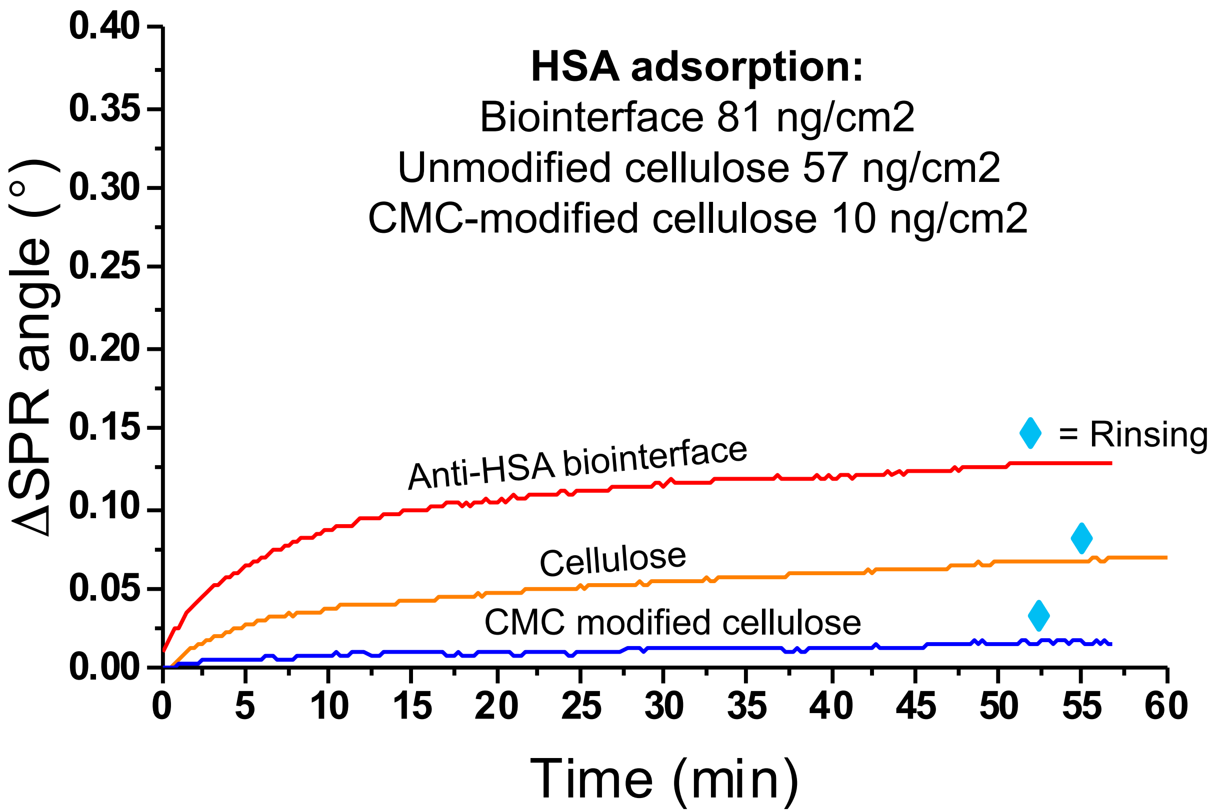
# Effect of CMC on the adsorption of HSA on cellulose




CMC modification lowers the non-specific adsorption of HSA on cellulose

- Hydrogel like structure
- Anionic charge of CMC

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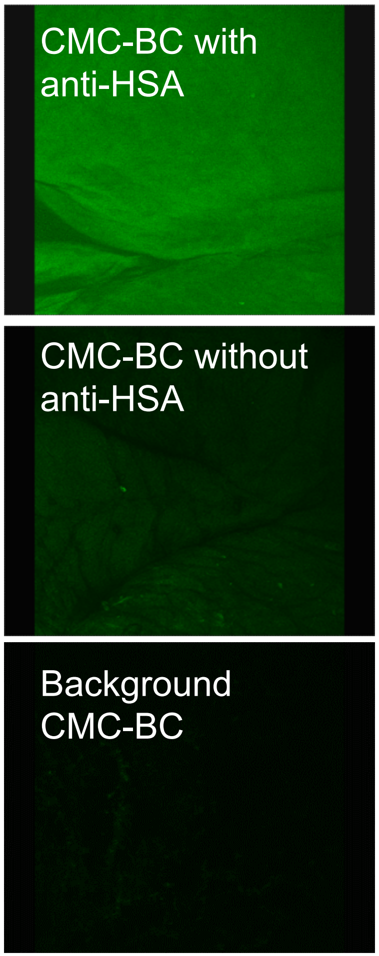
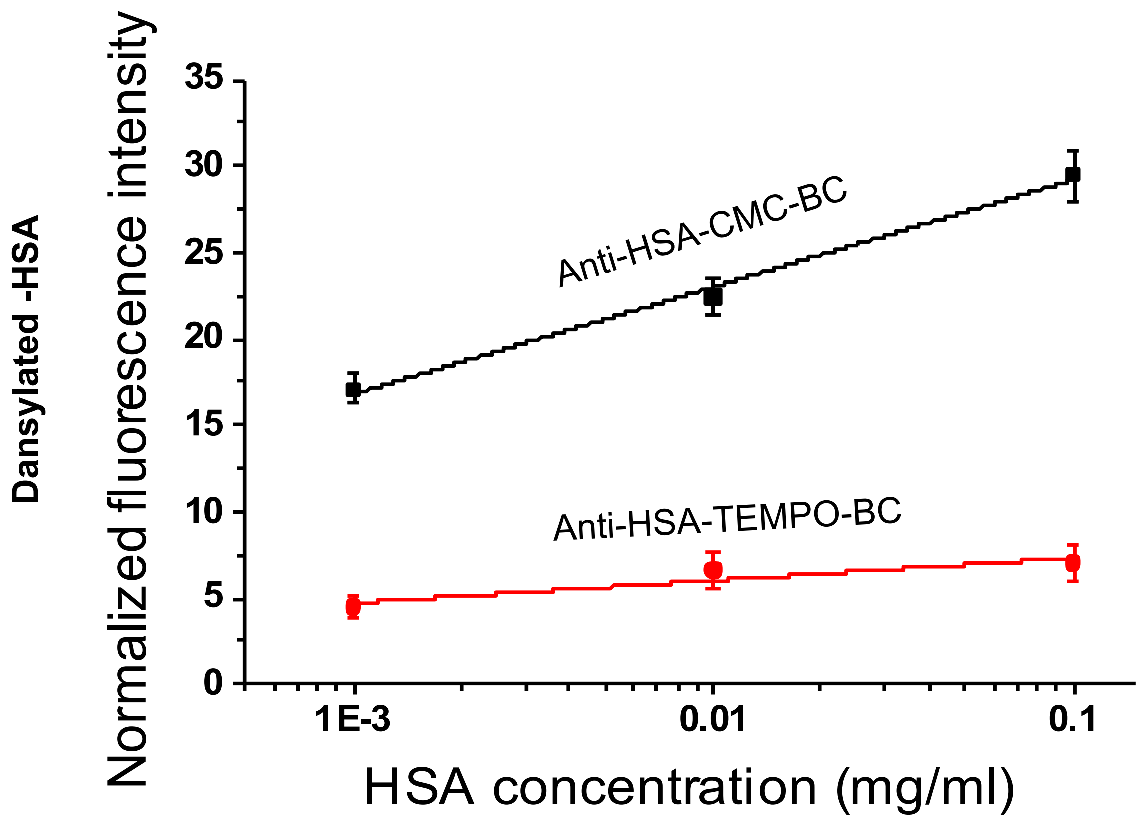
# Biofiltration of fluorescence stained proteins with functionalized BC-tubes




- Elevated fluorescence with conjugated anti-HSA
- CMC modification decreases the background noise (when compared to that of TEMPO-oxidized samples)

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# Concluding remarks

- Bacterial tubes were incubated with a presence of CMC
- CMC lowers permanent changes in BC within drying
- Affibodies were covalently conjugated to BC-tubes via EDC/NHS chemistry
- BC-tubes were utilized on the specific detection of HSA

# Acknowledgements


H Y B E R

Molecular Engineering of Biosynthetic Hybrid Materials Research

UPM

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FINNISH BIOECONOMY CLUSTER

Tekes

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