

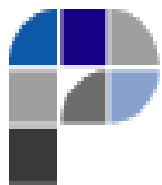


The  
University  
Of  
Sheffield.

# **Bacteria responsive poly(N-isopropyl acrylamide)s: The future of smart polymer biotechnology**

*Stephen Rimmer*

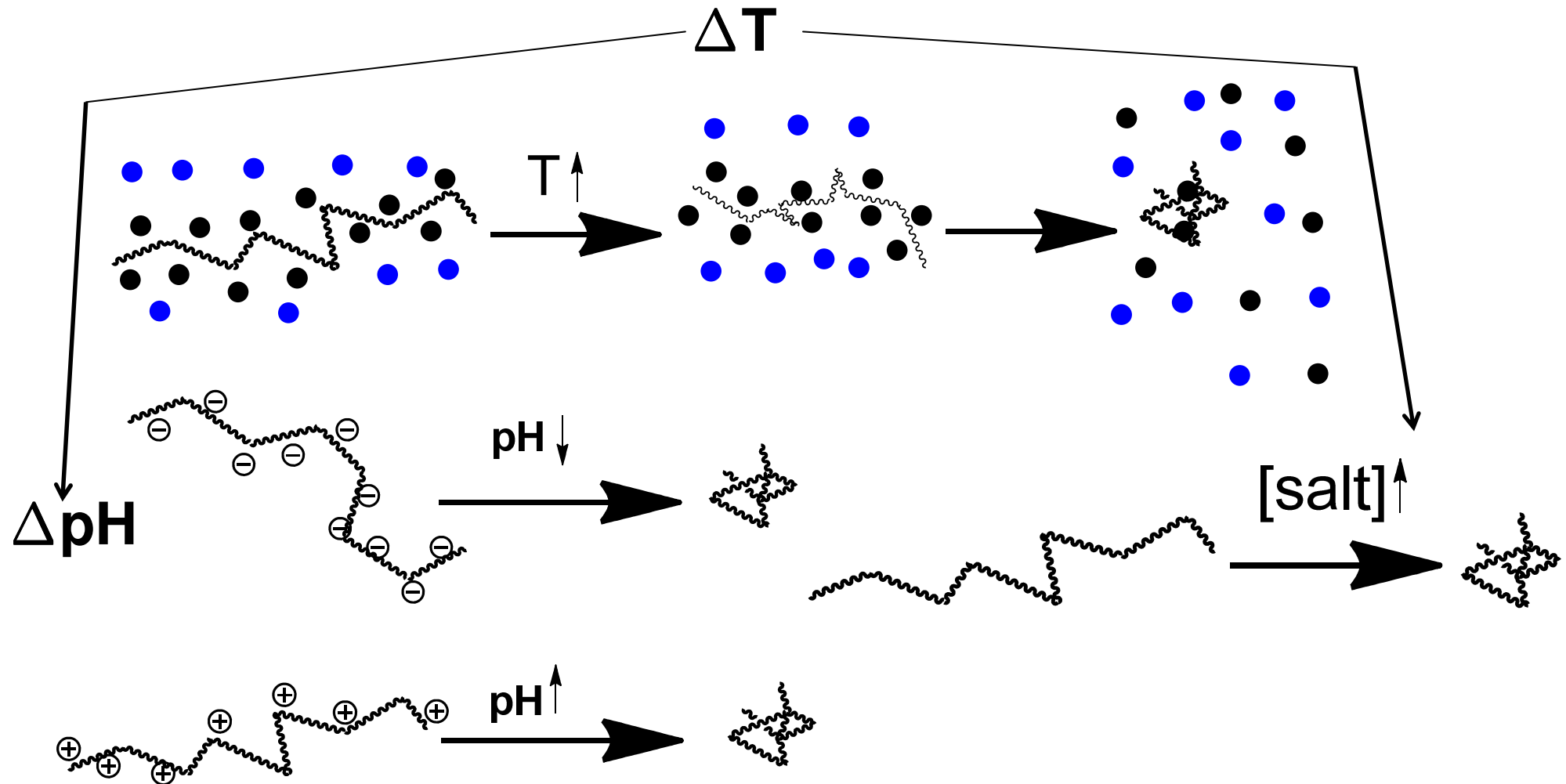
*Polymer and Biomaterials Chemistry Laboratories  
University of Sheffield, UK*



Polymer  
Centre



# Smart polymers and stimuli

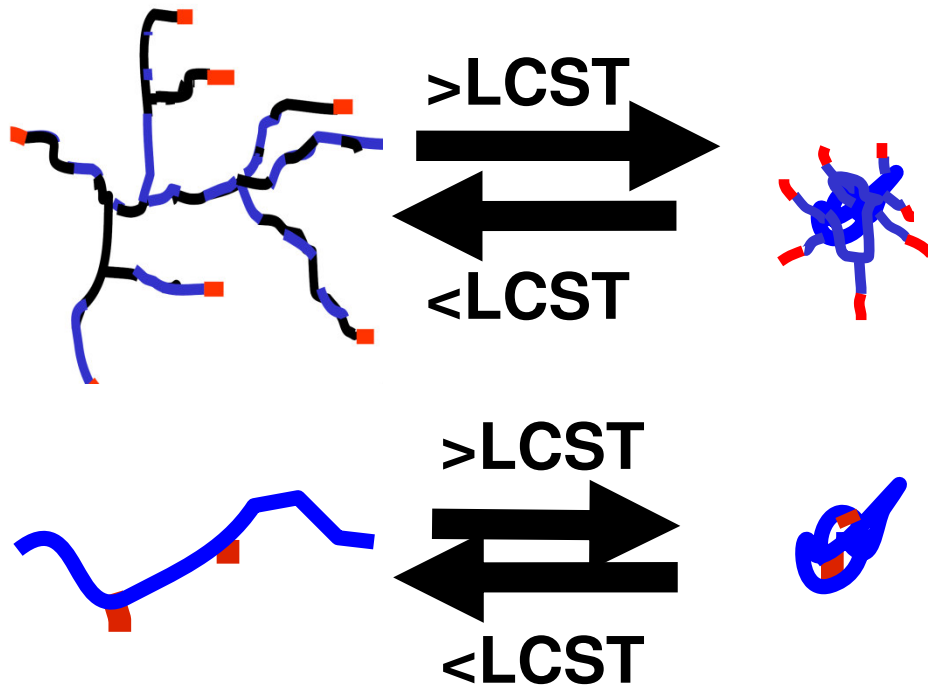


Can we mimic biological “smartness”

binding  $\rightarrow$  conformational (folding) change

## Some consequences of branching

chain ends do not penetrate the coil



Chain ends

expressed in outer domains  
available for binding to cells

shielded in inner domains  
not available for binding

So:

❖ *Branching effects end group availability.*

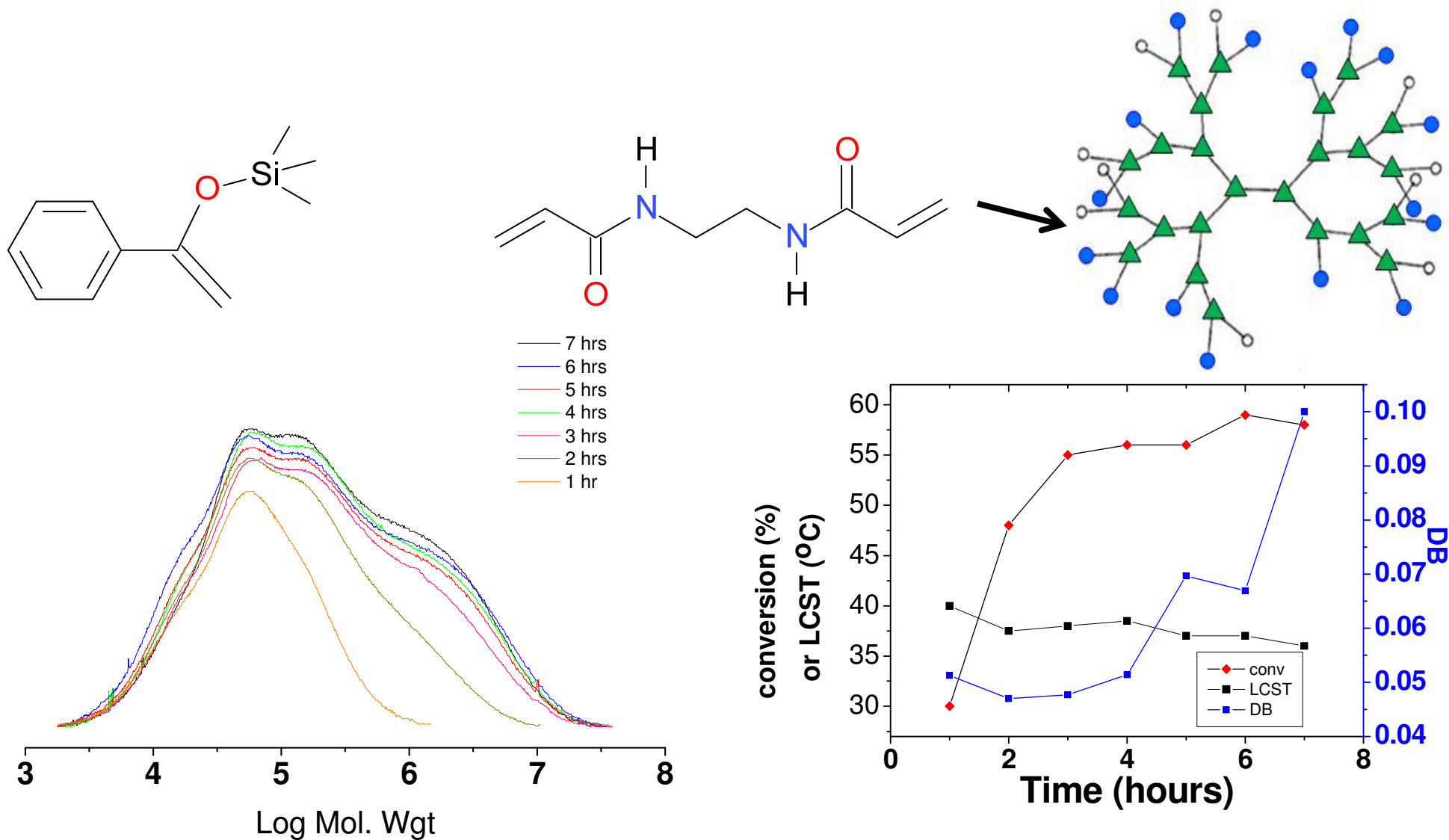
❖ *Currently we have studied highly branched*

*poly(*N*-isopropyl acrylamide) (HB-PNIPAM)*

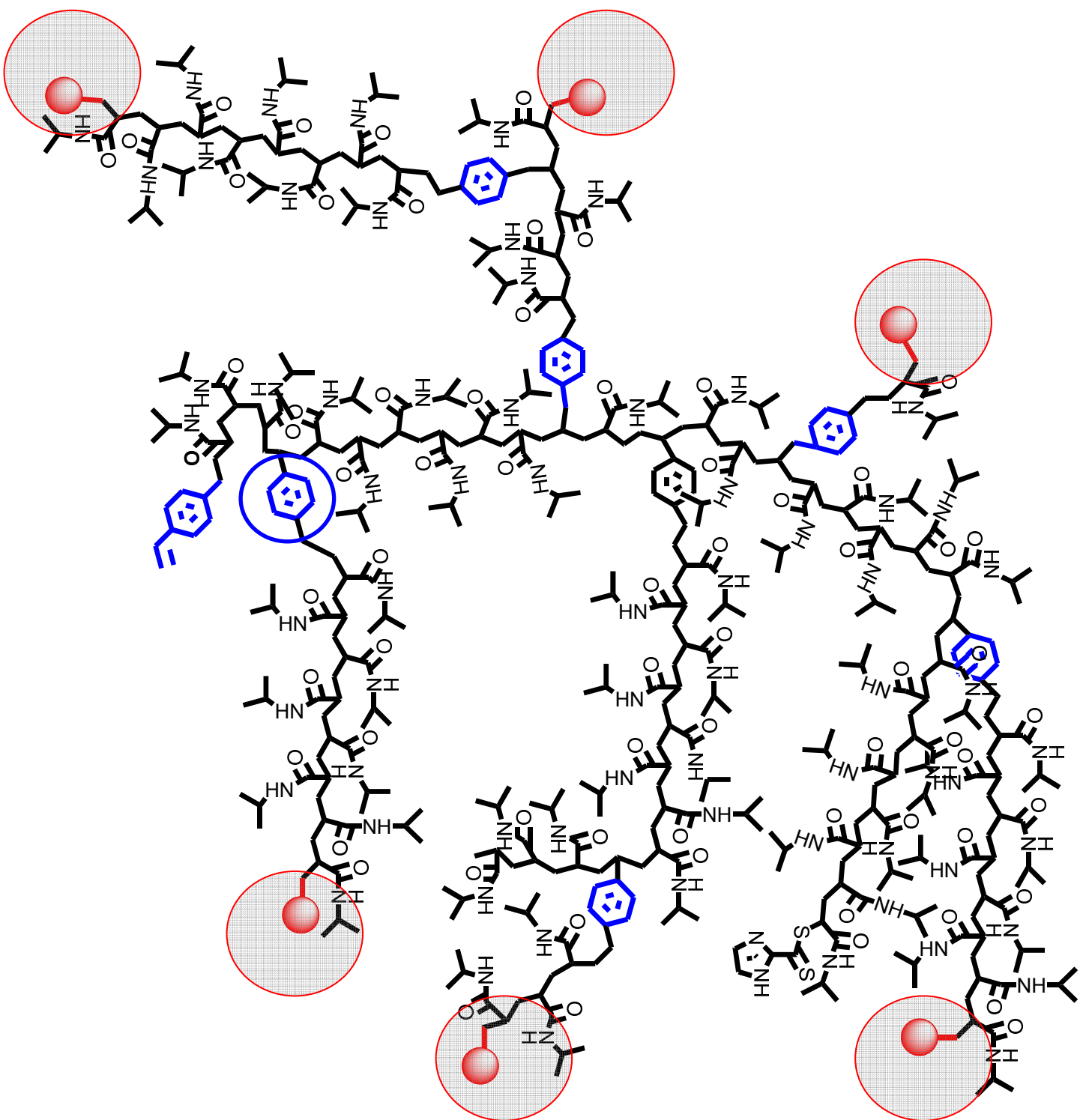


# Alternative route to HB-PNIPAM

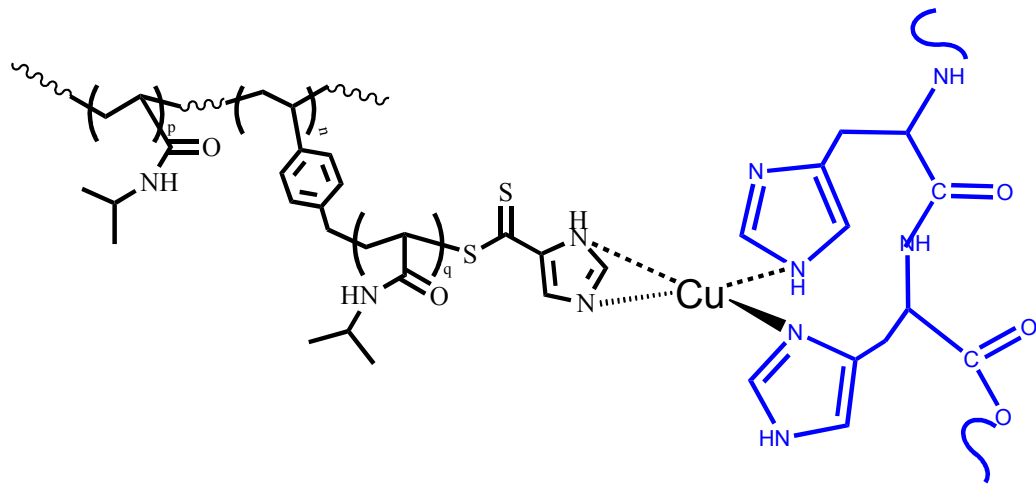
Use a variant of the cross-linking termination route



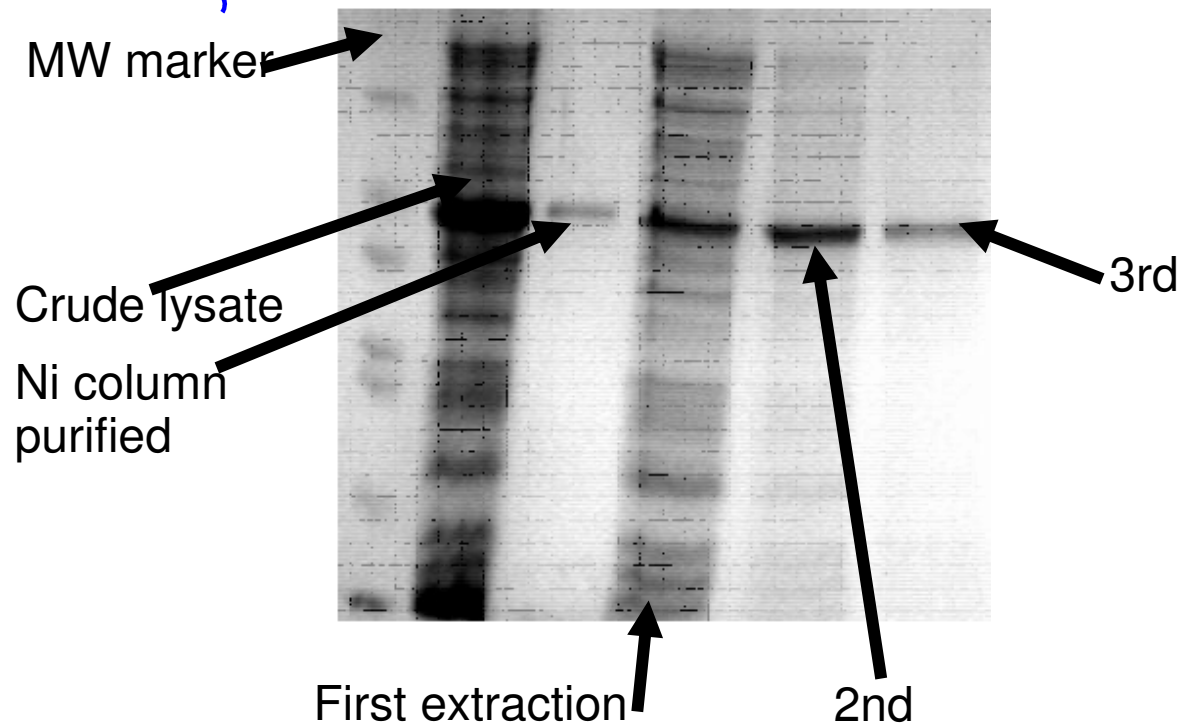
R.M. England, S. Rimmer *Chem. Commun.*, **46** 5767 (2010)



# Used to bind to a His tagged protein for protein purification



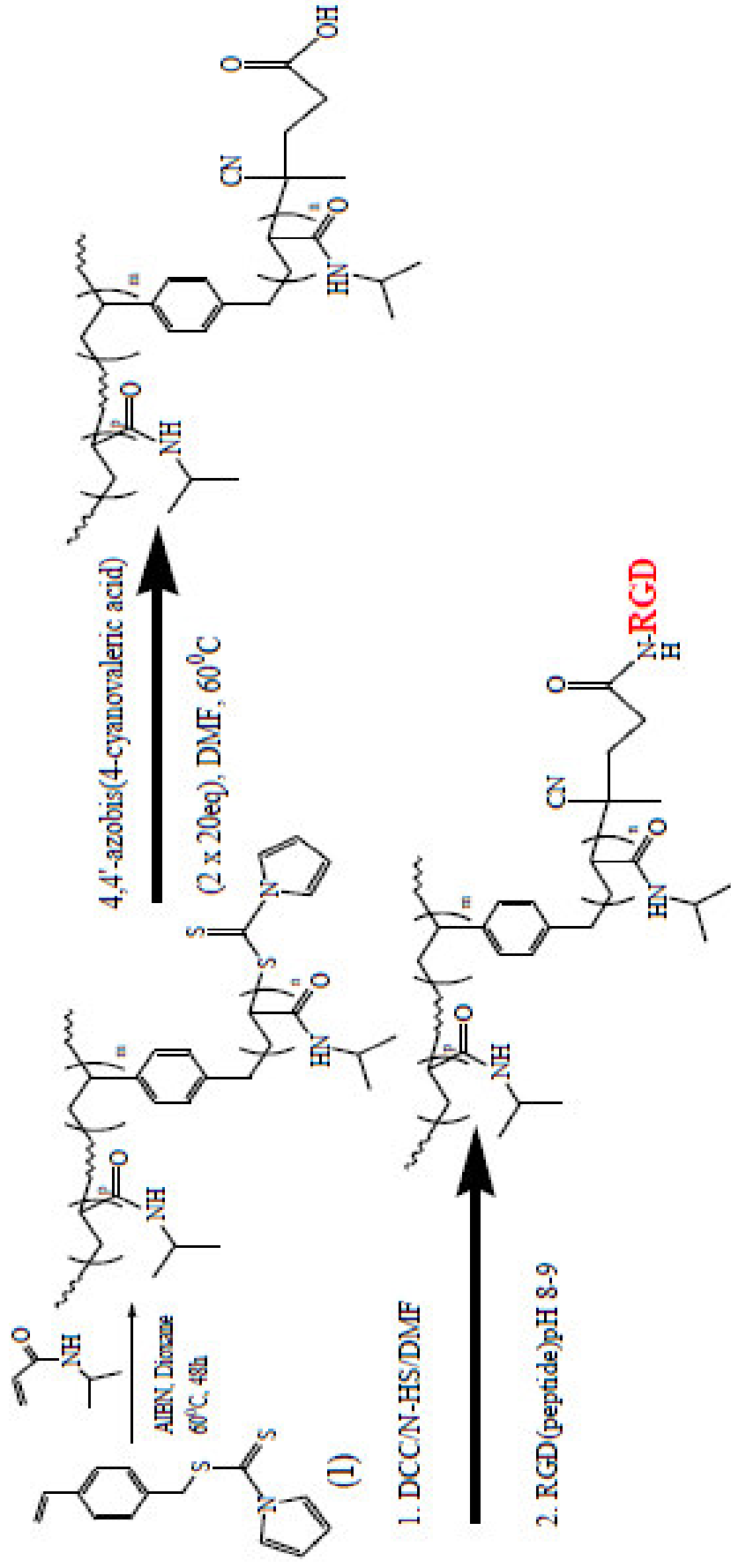
Purification of recombinant His-BRCA1



S. Carter, S. Rimmer, A. Sturdy, M. Webb, *Macromol. Biosci.* **5** 373 (2005)

S. Carter, S. Rimmer, R. Rutkaite, L. Swanson, A. Sturdy, M. Webb *Biomacromol.* **7** 1124 (2006)

# Peptide highly branched PNIPAM

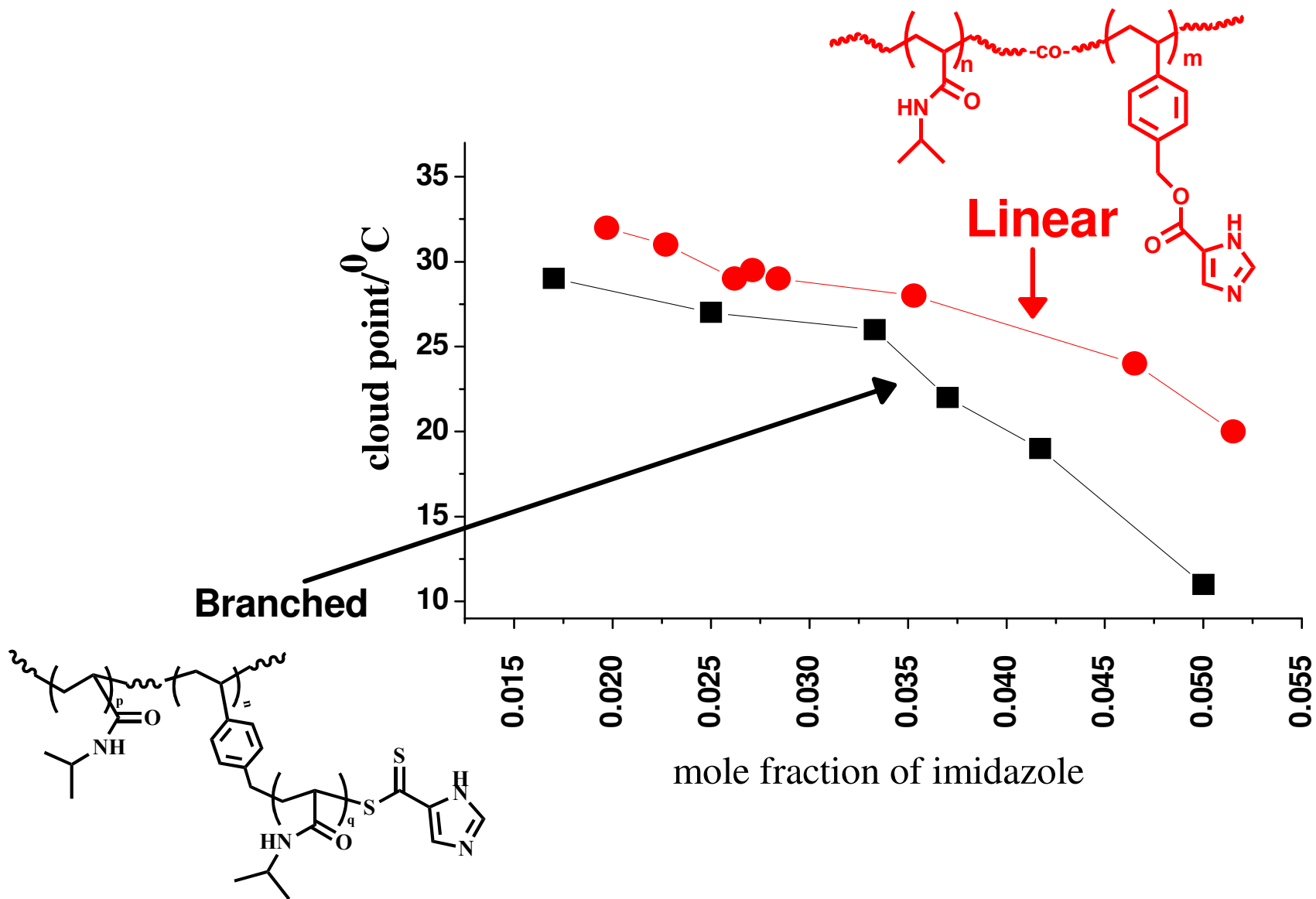


RGD=Arginine-Glycine-Aspartic acid

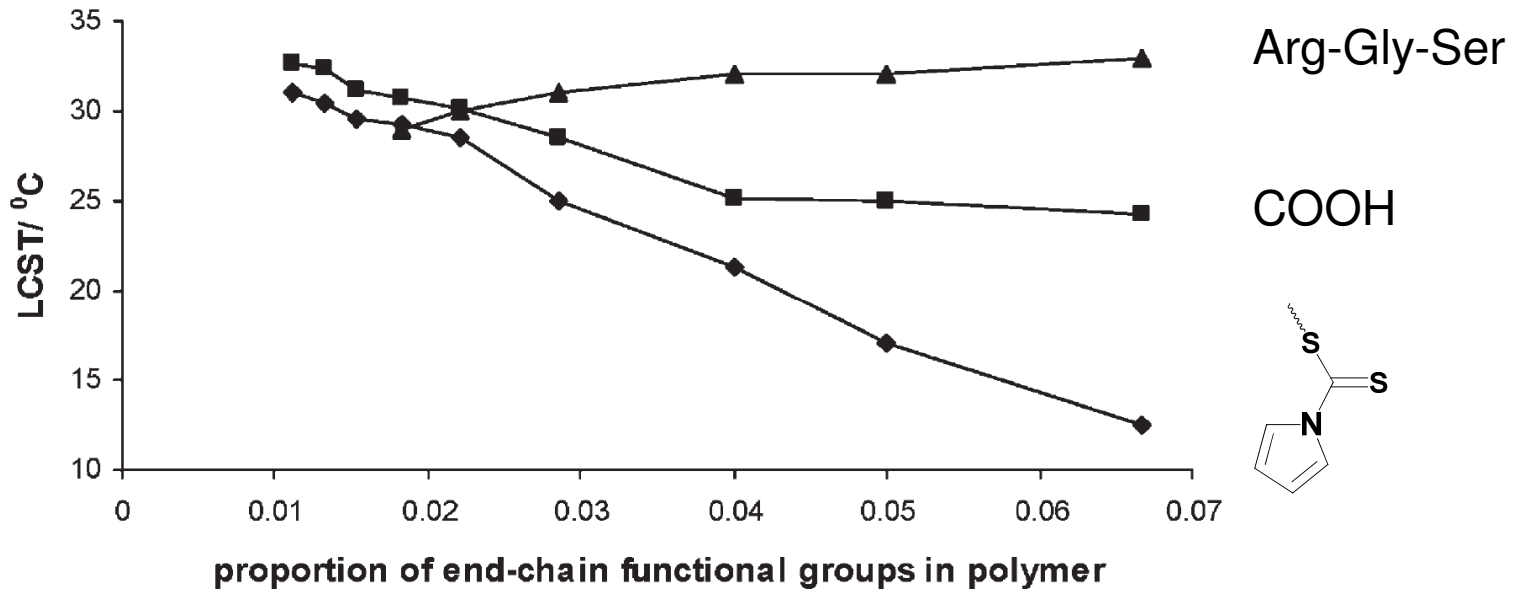
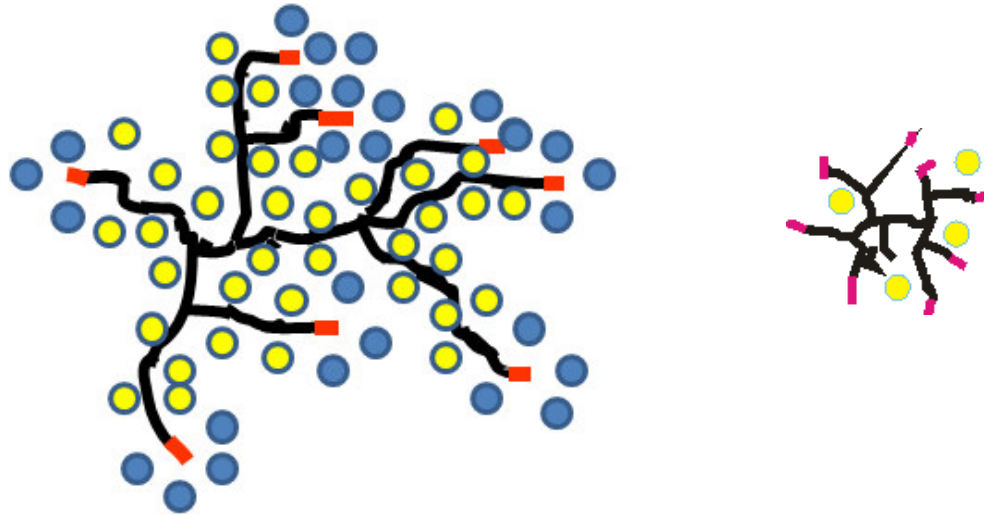
Rimmer, Carter, Rutkaite, Haycock, Swanson  
*Soft Matter* 3 971 (2007)



# Branching and the LCST

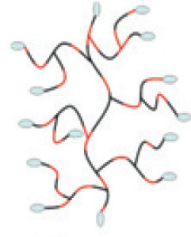


# End groups and the LCST

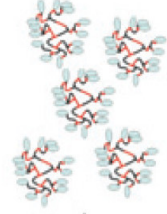
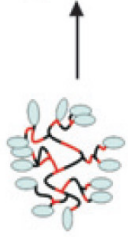


**GRGDS**

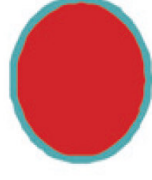
—binds to cell integrins



$\Delta T$



=

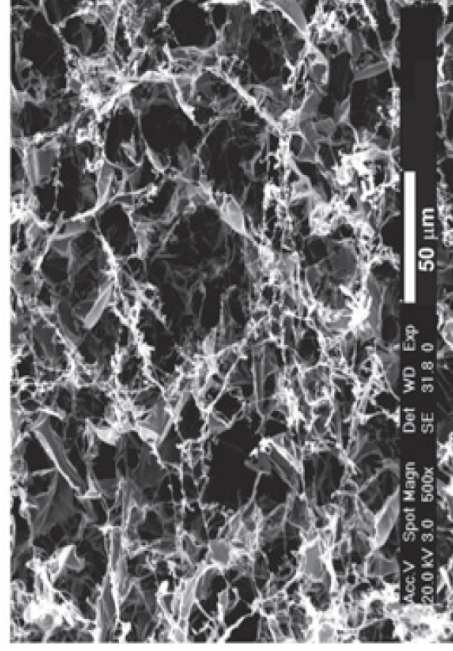


**Globules aggregate and particles form  
a topologically open microgel**

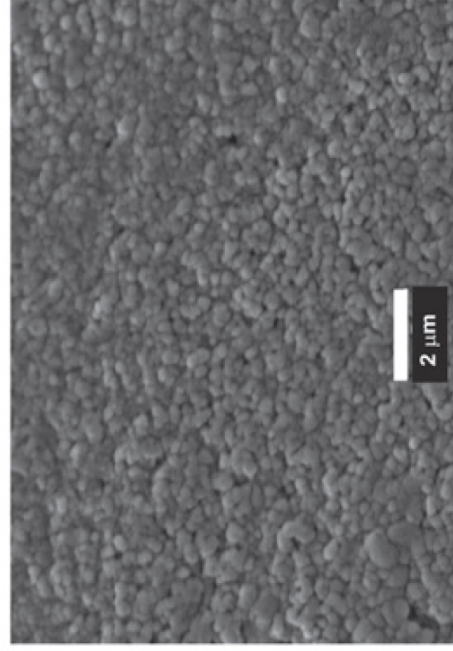
**Polymer in  
open chain  
conformation**

**Polymer in  
globular  
conformation**

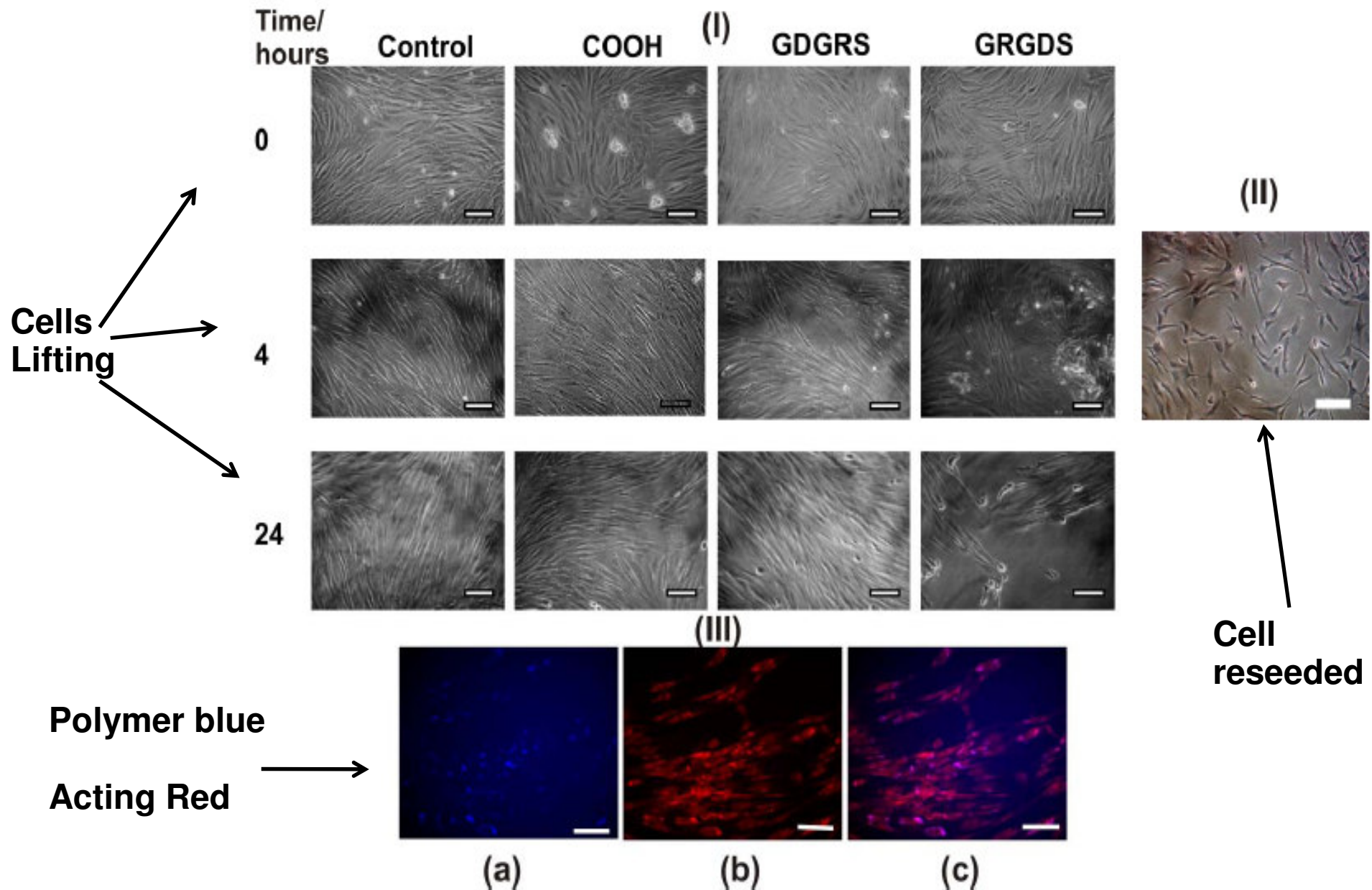
**(a) Below the LCST**



**(b) Above the LCST**



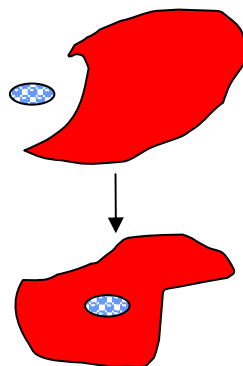
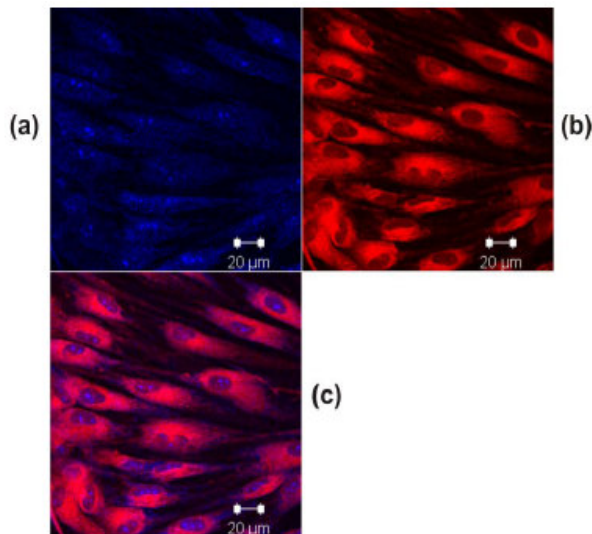
# Transfer of Human Dermal Fibroblasts Cells



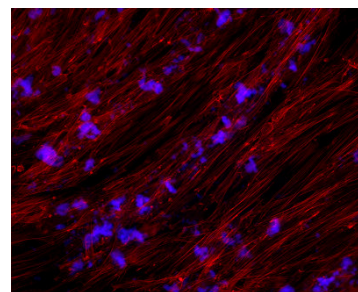
# COOH or imidazole end groups above LCST- particles enter cells

With HDFs

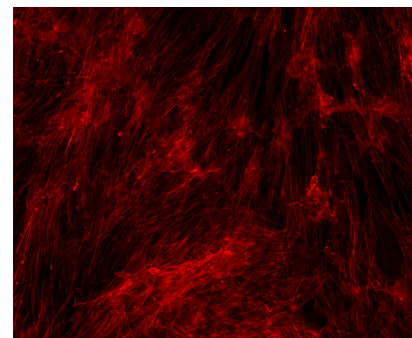
COOH at 37°C



Imidazole



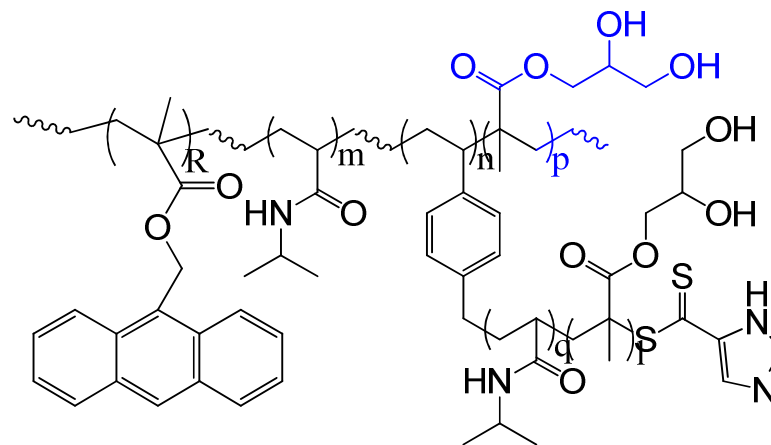
@ 37°C



@ 30°C

Polymer blue  
Actin Red

Imidazole polymer  
Needs GMA to form stable  
particles

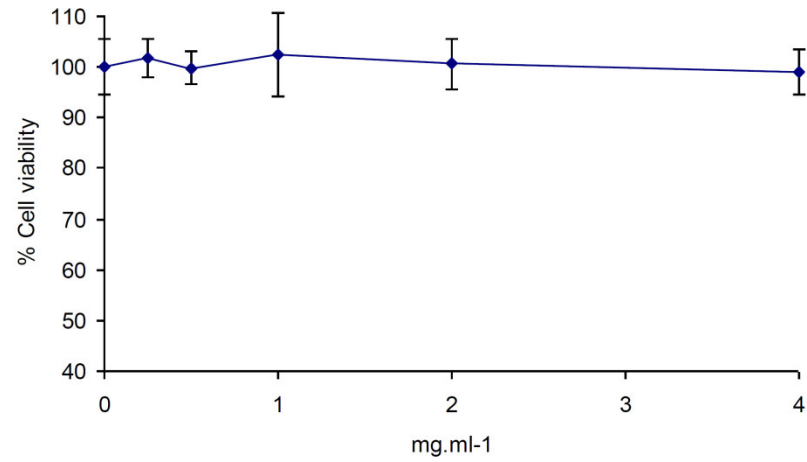


A clear alternative for delivery of charged therapeutics with function that does not require **challenging thermodynamics**.

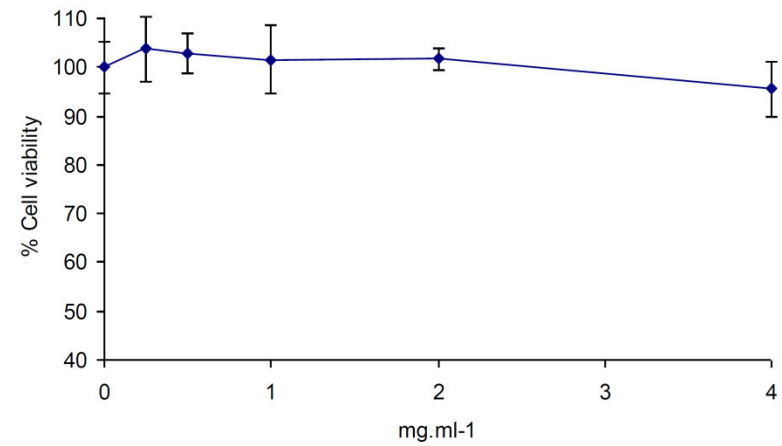
Hopkins et al *J. Mater. Chem.* 17 4022 (2007); *Soft Matter* 5, 4928, (2009)

# Cell Viability

## 33% branching-GMMA tips



## 28% branching-GMMA tips

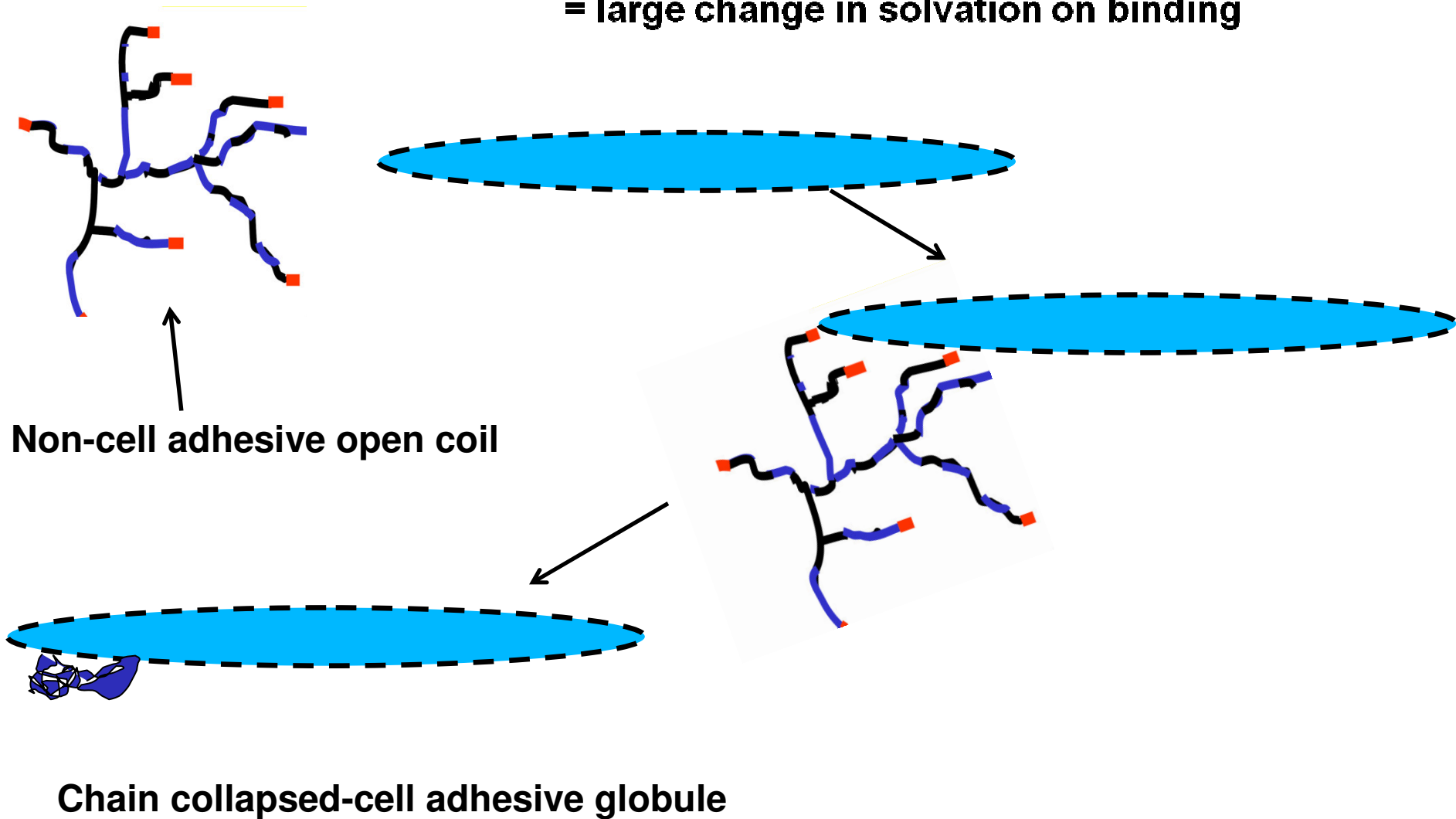


MTT cell viability after 48 hours

Human dermal fibroblasts

# Can we control the coil-to-globule transition with cells?

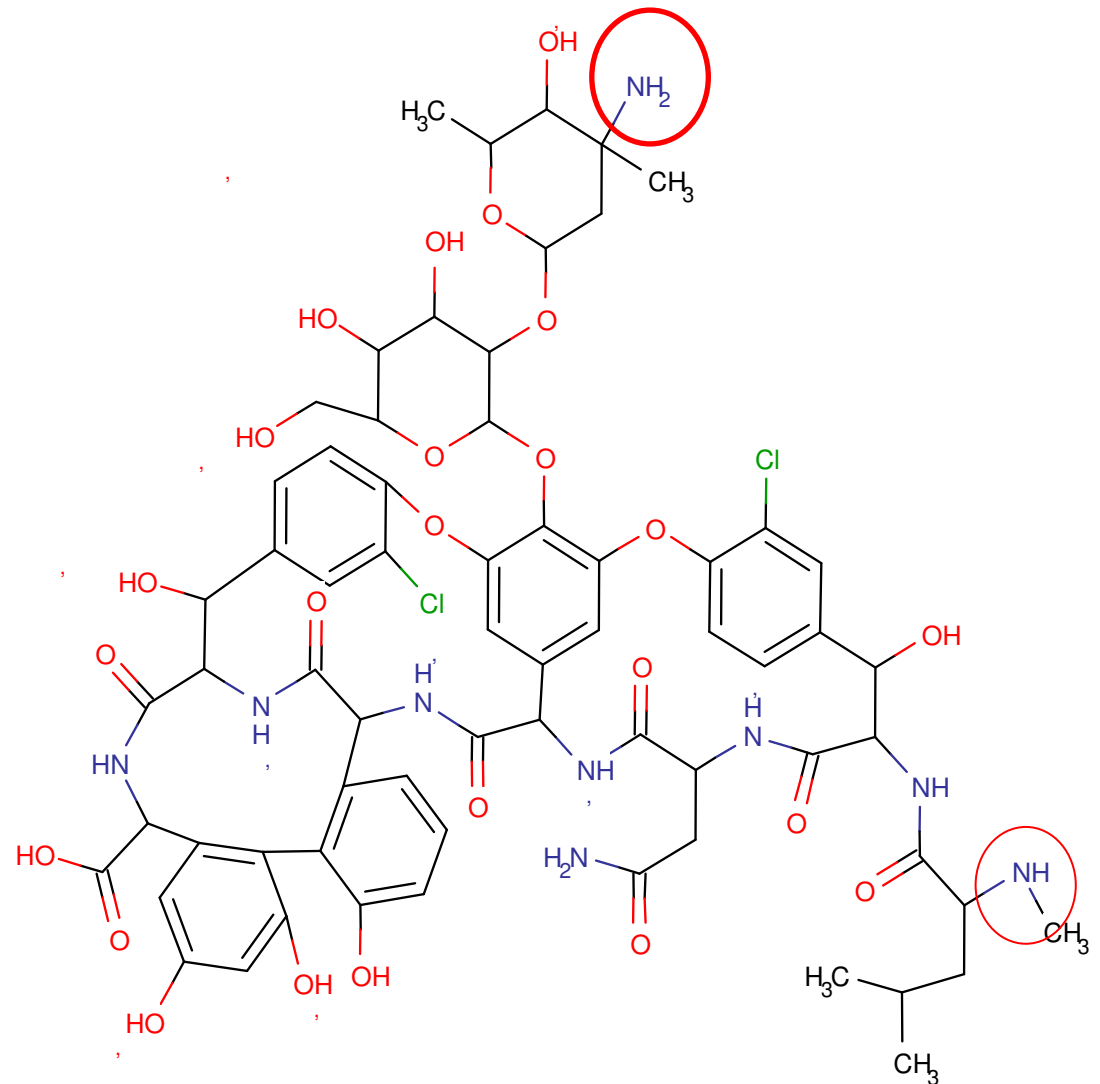
- Are the perturbations on binding end groups sufficient to induce collapse?
- Binding decreases the LCST
- In general ligand receptor interactions dominated by electrostatic interactions  
= large change in solvation on binding



# Bacteria-binding polymers

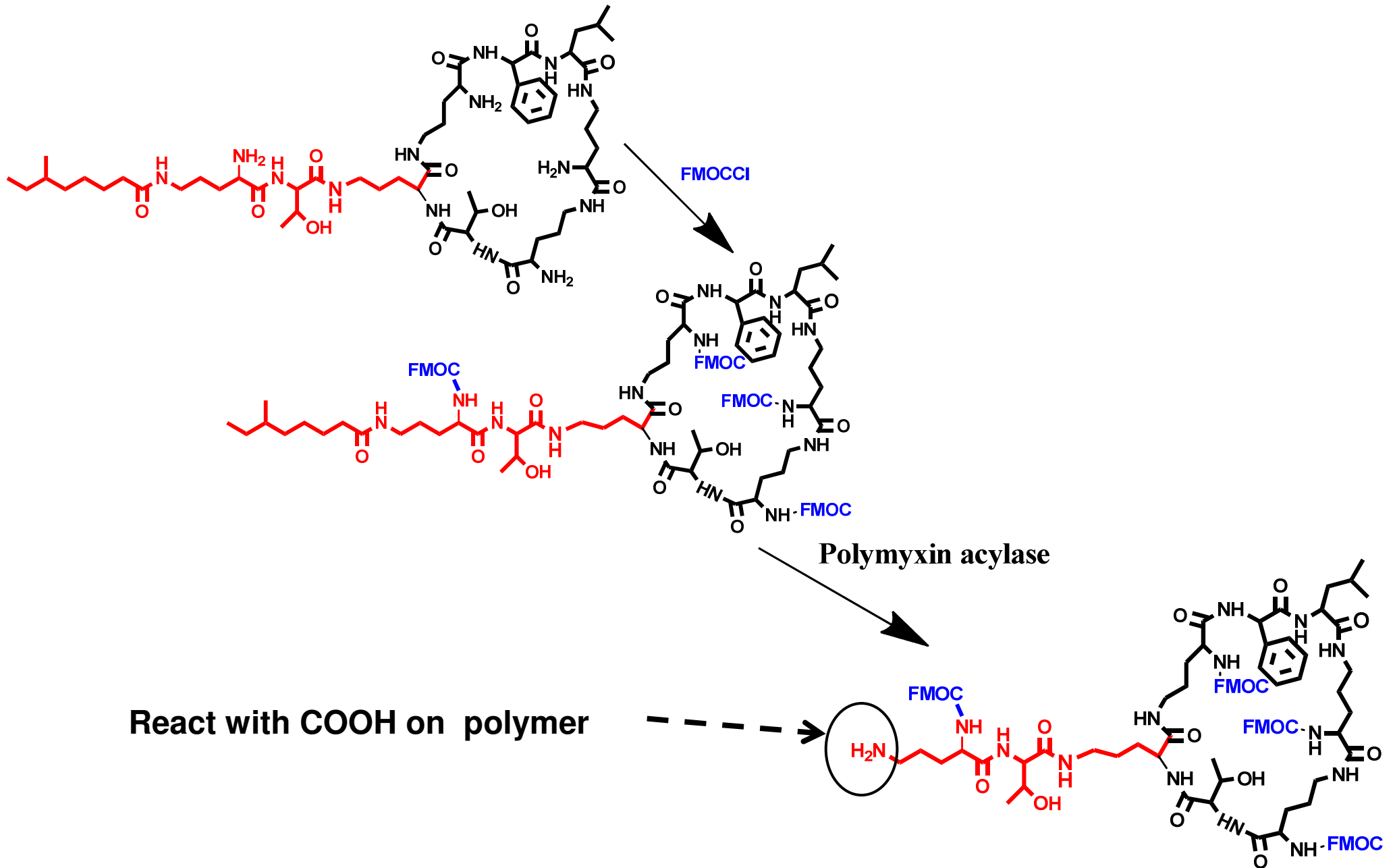
- **Branched poly(NIPAM) - antibiotics @ chain ends**

- e.g. **Vancomycin** – binds Gram+ve (*S.aureus*)



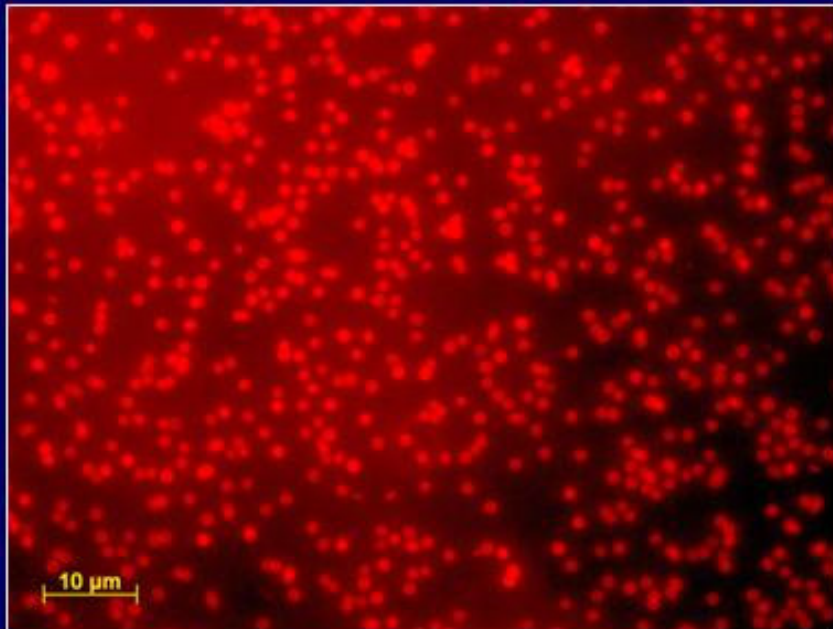


# Polymixin B binds Gram -ve (*P.aeruginosa*)

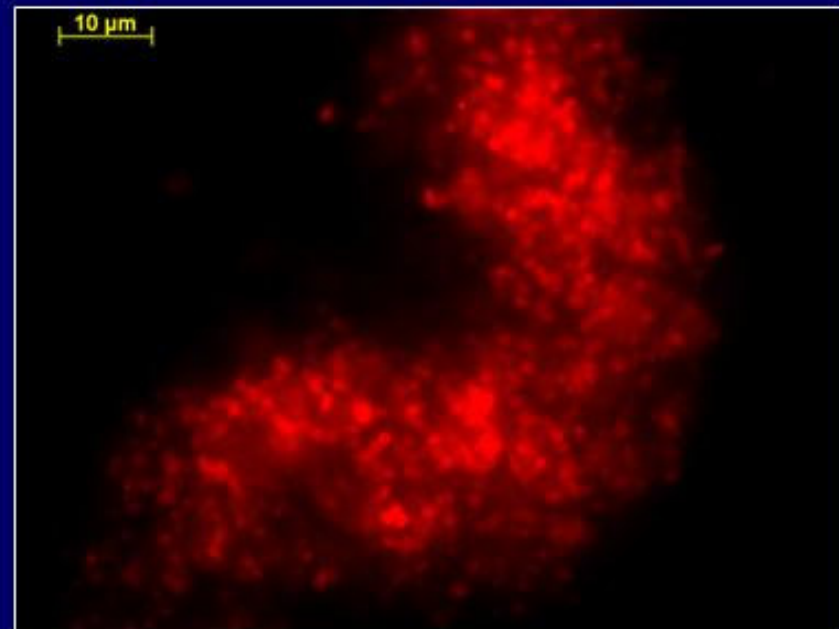


## Binding bacteria

- ❖ Red labelled bacteria
- ❖ Polymer binds then collapses and bacteria aggregate



*S. aureus* & PBS (control)

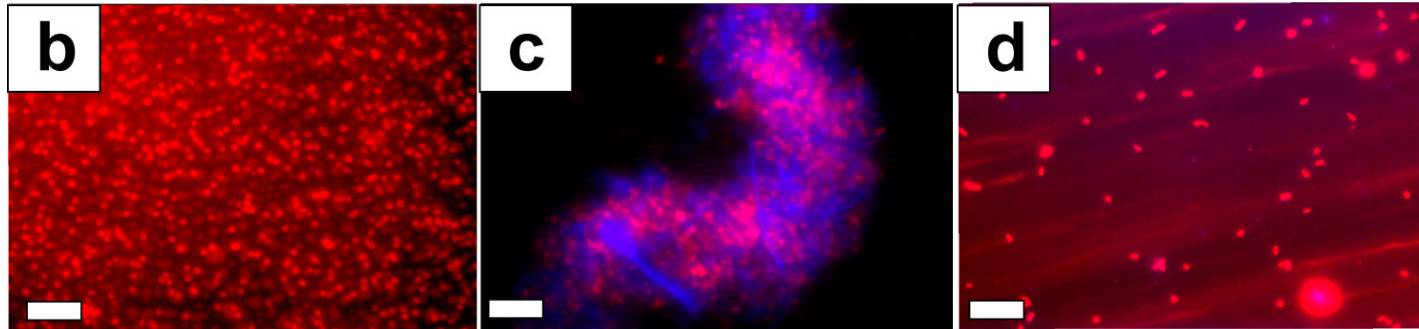
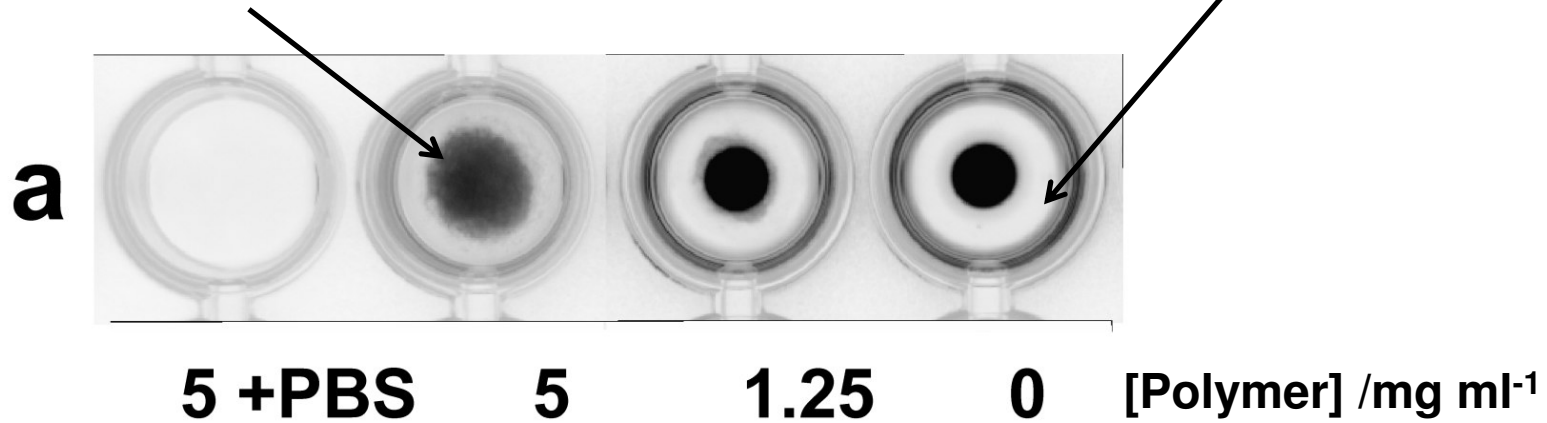


*S. aureus* & VAN-polymer

# Is the effect temperature responsive?

With polymer forms a mat

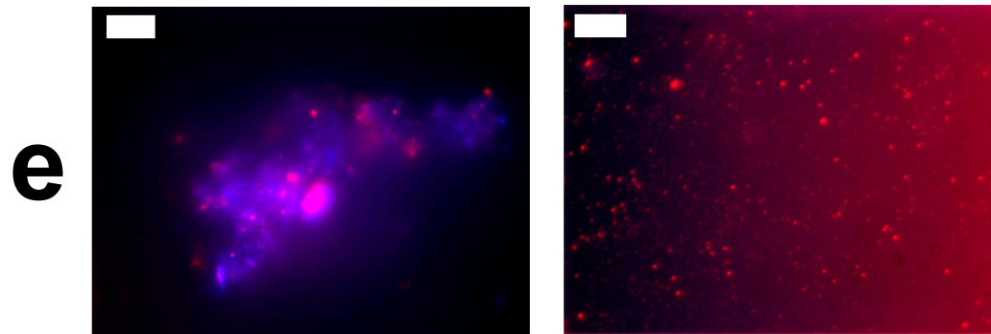
No polymer forms a button



*S. aureus*

HB-PNIPAM + *S. aureus*

HB-PNIPAM + *P. aeruginosa*



*S. aureus*

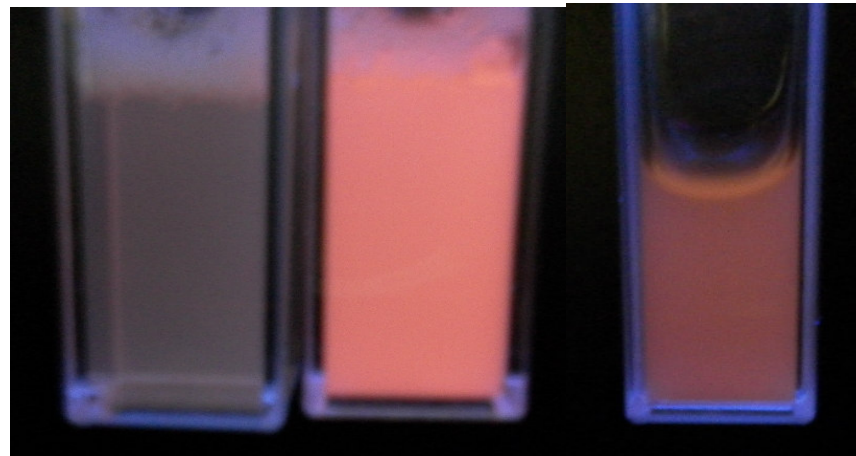
37 °C

4 °C

## Preliminary detection of coil collapse

A quick test for chain collapse uses Ethidium Bromide

-responds to incorporation in desolvated PNIPAM



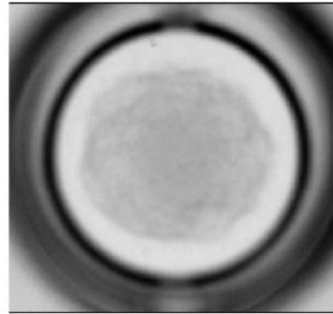
UV light

PNIPAM-  
Van in  
PBS

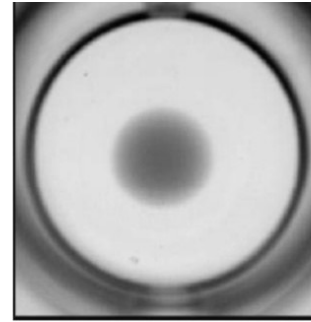
PNIPAM-  
Van in PBS  
with  
S.aureus

S.Aureus  
in PBS

# HB-PNIPAM-pmx with *P. Aeruginosa*: Gram-ve



37°C

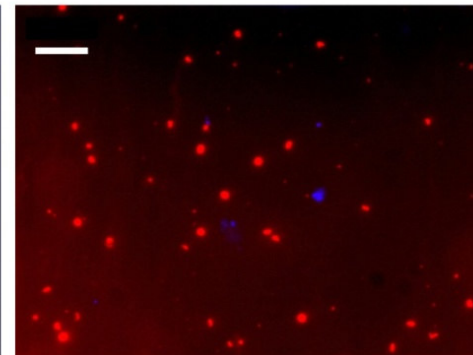
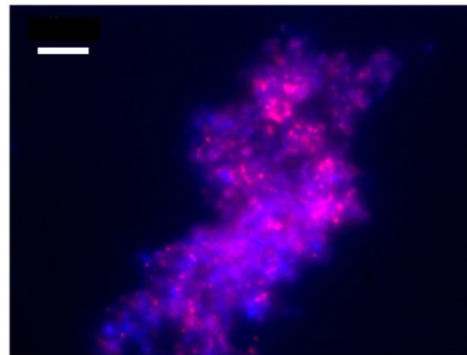


4°C

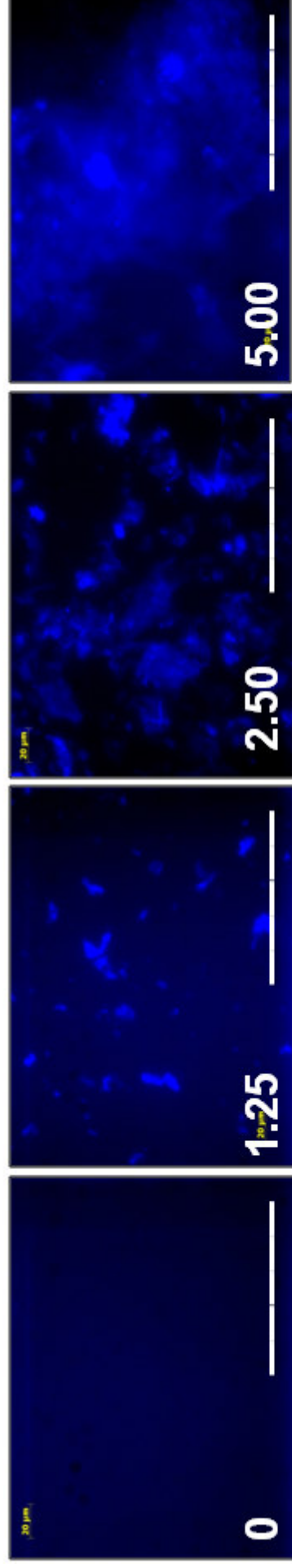
37°C / 1h

4°C/18h

HB-PNIPAM-pmx &  
*P.aeruginosa*

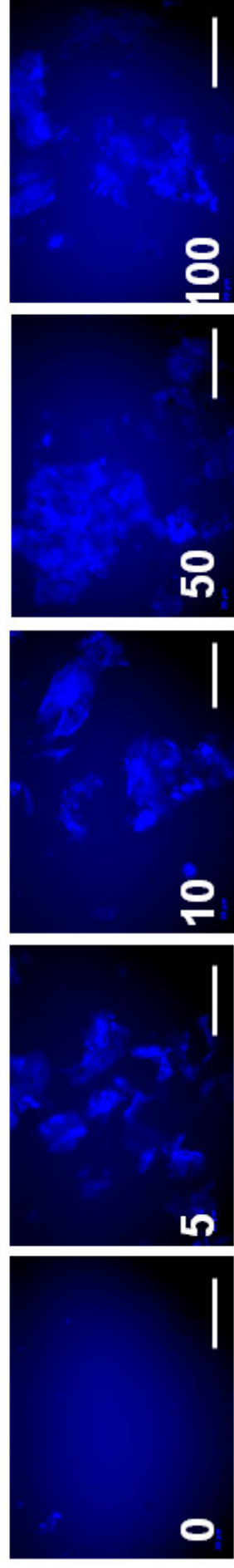


**a**



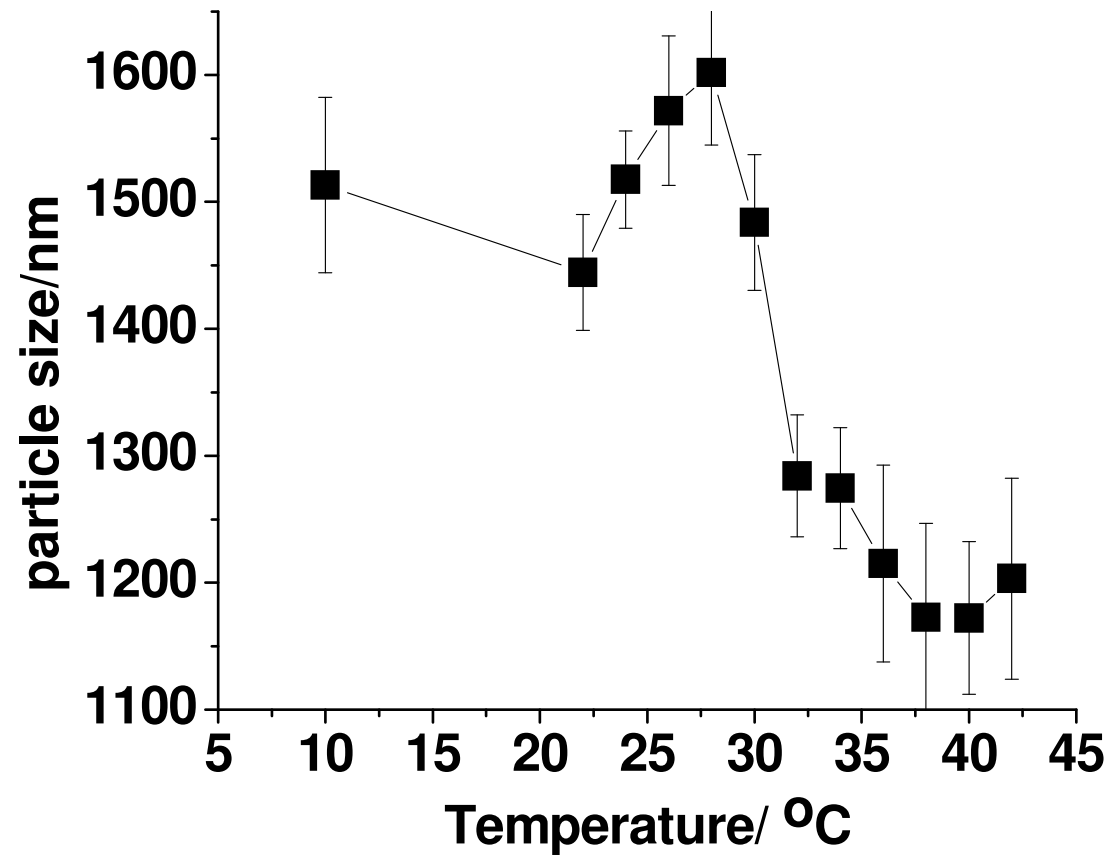
Increasing polymer concentration

**b**



Increasing LPS concentration

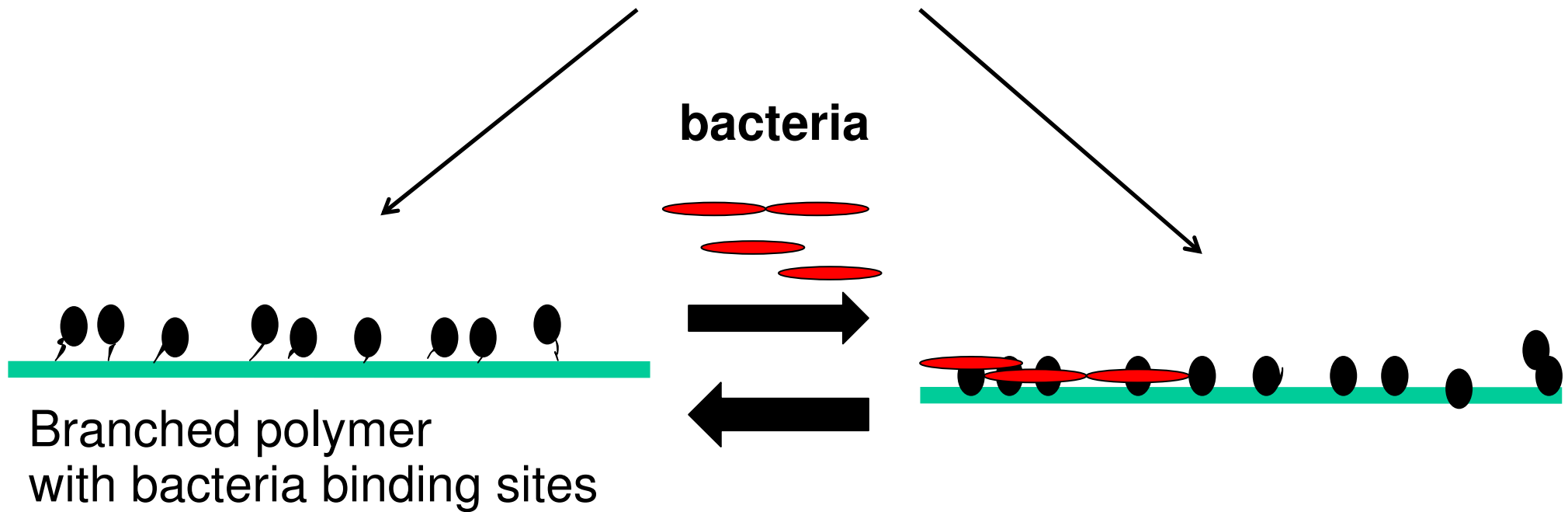
## Change in particle size with temperature



## Immobilisation on a hydrogel

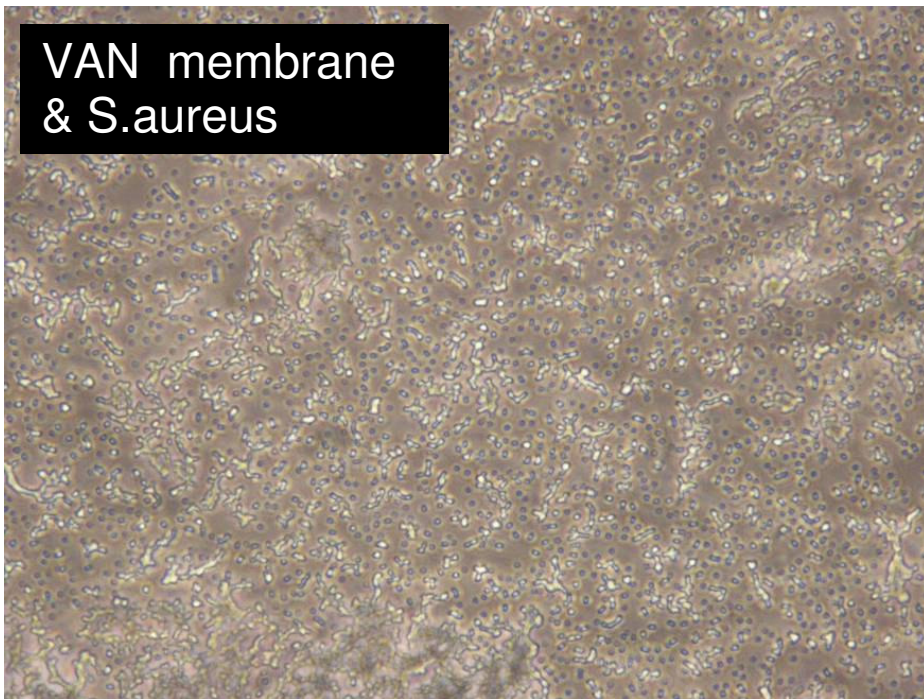
As a coating on packaging or a dressing etc.

Polymer collapses: get opacity or maybe a colour change

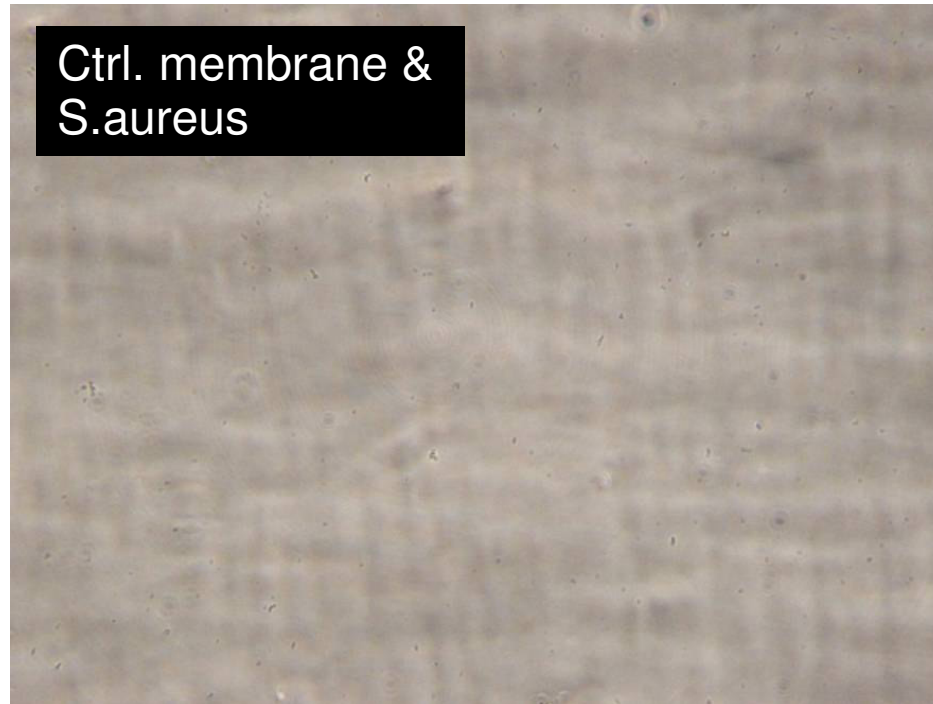




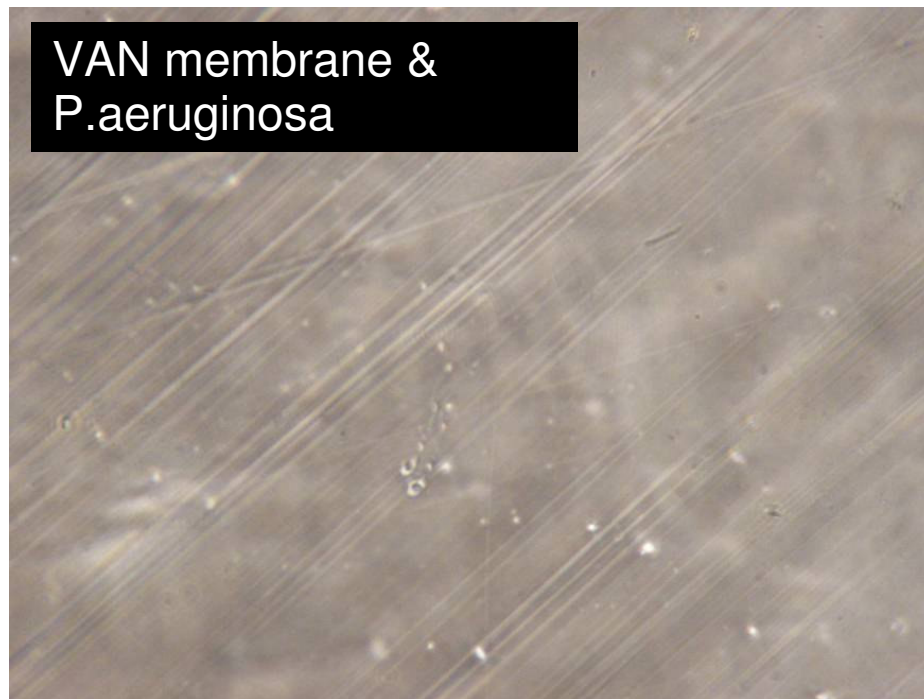
VAN membrane  
& S.aureus



Ctrl. membrane &  
S.aureus



VAN membrane &  
P.aeruginosa



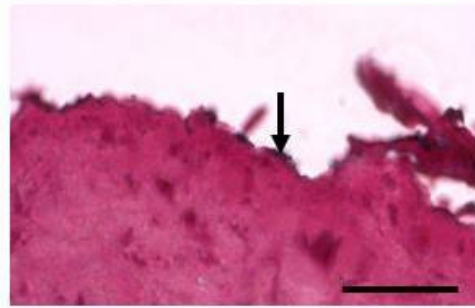
Ctrl. Membrane &  
P.aeruginosa



**Tissue engineered skin infected with *S. Aureus* and treated with HB-PNIPAM attached to hydrogel membrane**

2 x 1 hour applications of van-membrane

(a) Infection for 45 mins



a

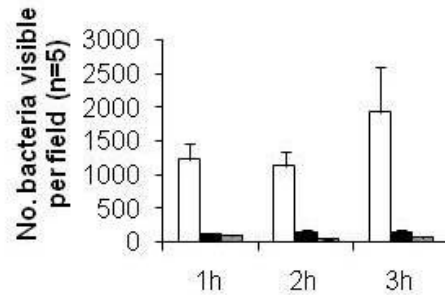


No treatment

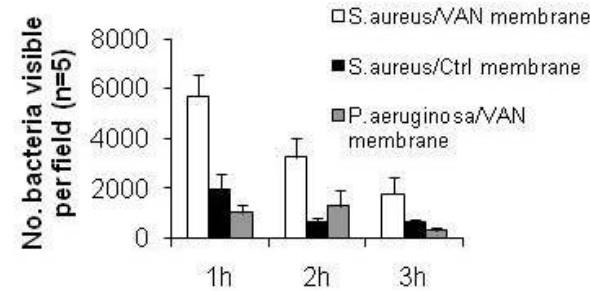
(b) Infection 45mins

b

(c) 45 mins  
(d) 24 hours



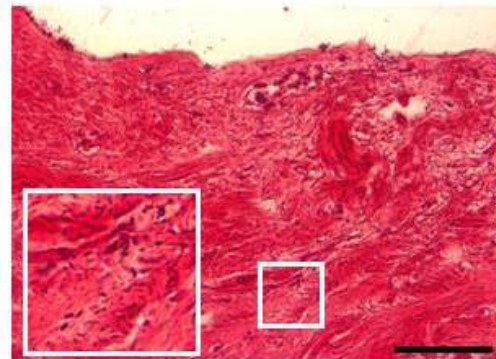
c



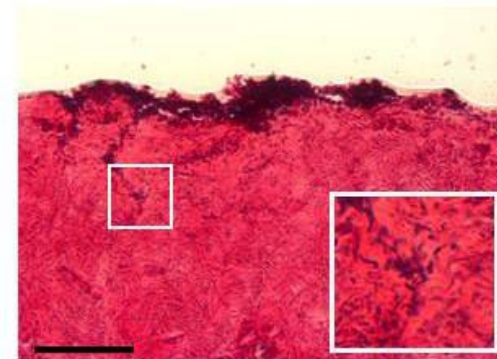
d

Numbers of bac on membrane

(e) Infection for 24 hours



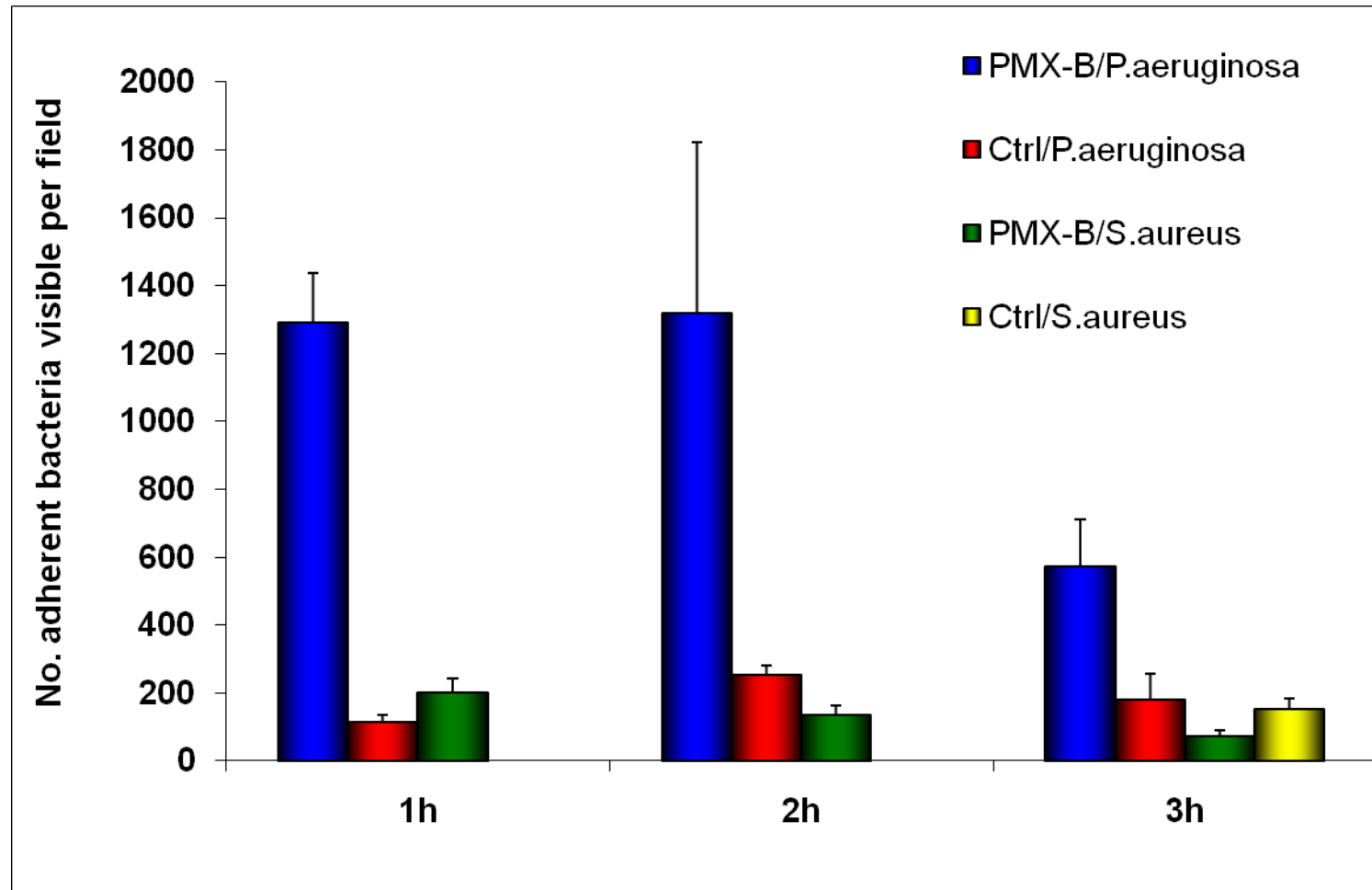
e



f

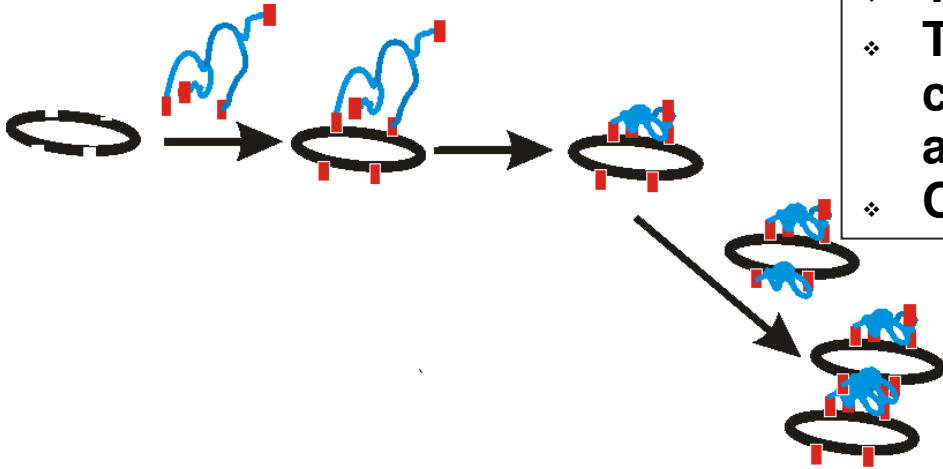
(f) Infection for 24 hours

# Counting bacteria on the membranes: PMX-*P. aeruginosa*

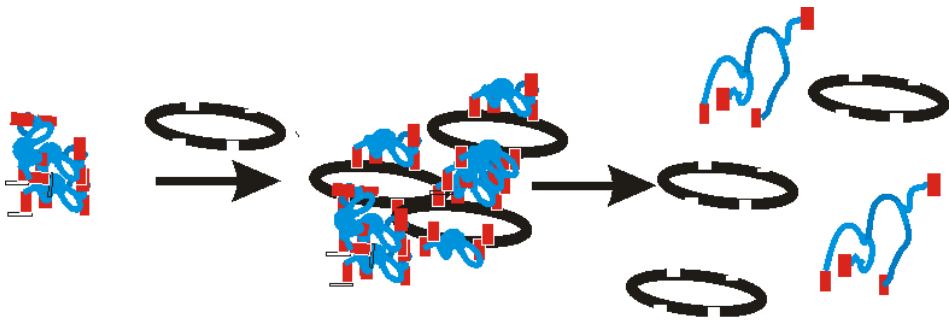


## Summary-we have several modes

- ❖ Polymers desolvate on binding
- ❖ The desolvated chain is more adhesive to cells
- ❖ The cells aggregate because the physico-chemical properties are now suitable to support adhesion
- ❖ Cooling release the cells



- ❖ Polymers desolvated on application
- ❖ Cells bind to particulate aggregates
- ❖ Cooling release the cells



- ❖ Polymers desolvated on application
- ❖ Phagocytosis of particulate aggregates



# Acknowledgements

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