The Abdus Salam
International Centre for Theoretical Physics

United Nations
Educational, Scientific and
Cultural Organization

IAEA
International Atomic Energy Agency

1932-8

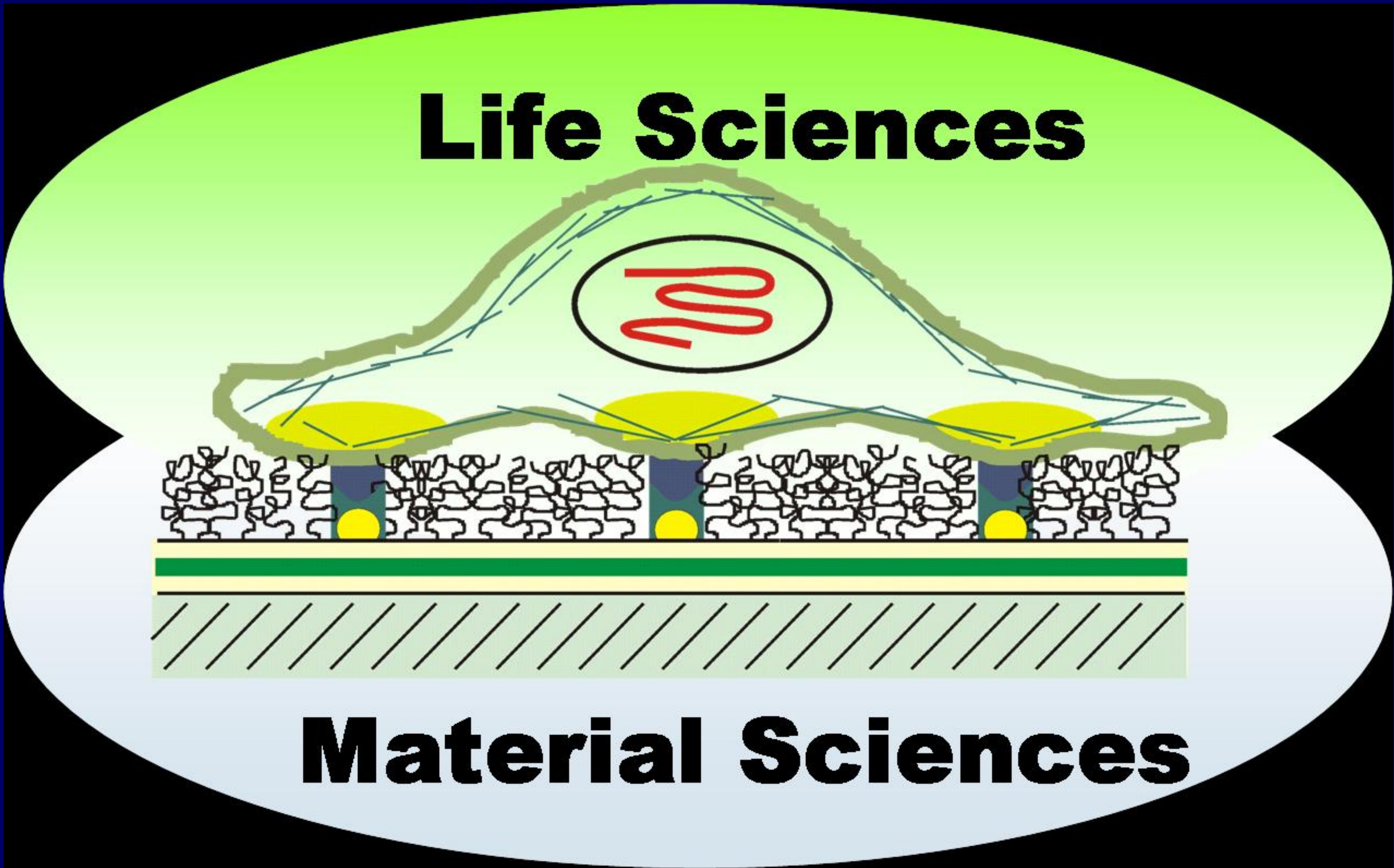
Winter College on Micro and Nano Photonics for Life Sciences

11 - 22 February 2008

Molecular Engineering of Cellular Environments:Cell Adhesion to Nano-Digital Surfaces (part I & II)

Joachim Spatz

Max-Planck-Institute for Metals Research
Department of New Materials and Biosystems
Stuttgart
Germany


Life Sciences
Material Sciences

The Extracellular Matrix

C. Ploetz et al. J. Struct. Biol., 106 (1991) 73-81

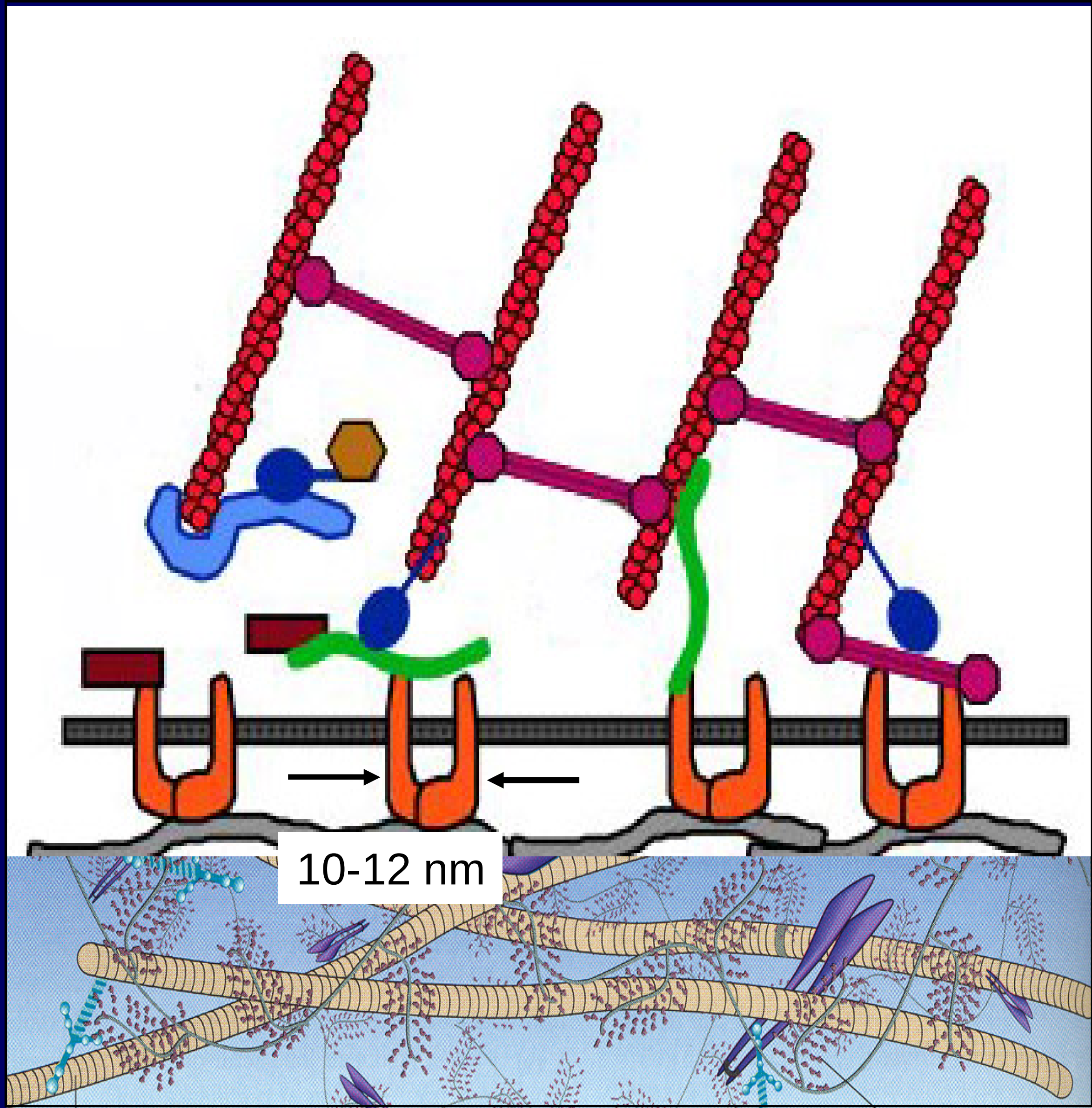
D.J. Müller et al. J. Struc. Biol. 2004, 148, 268

Matrix Information
- **Micro- and Nano- Topography**
- **Viscoelasticity**
- **Chemical Nano-Composition**

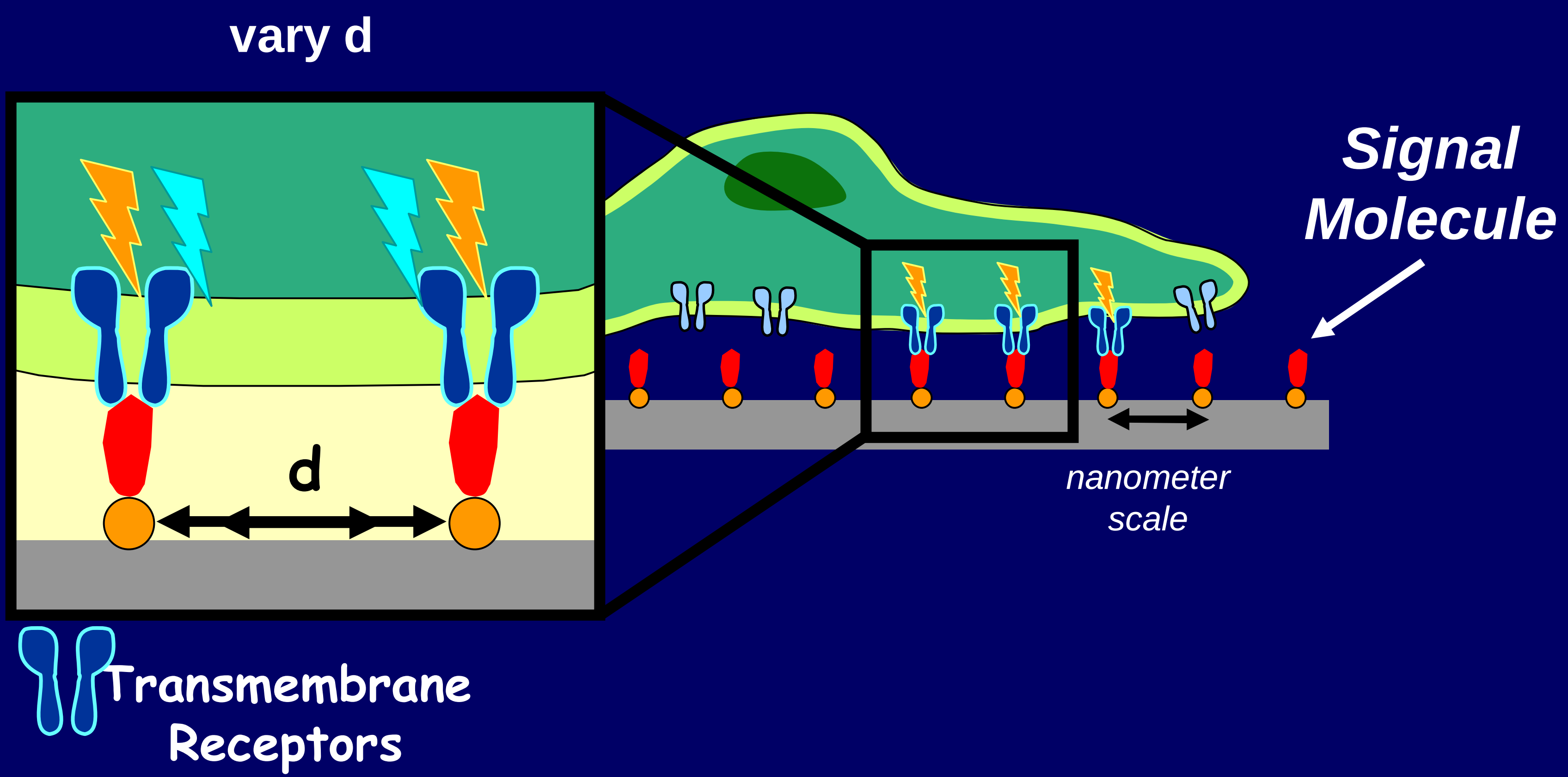
P. Fratzl: **Current Opinion in Colloid and Interface Science 8 (2003) 32–39**
K. Meller et al. Cell Tissue Res (1997) 288:111–118

Length Scales in Cell Sciences

PART I – Nanometer Length Scale
PART II – Micrometer Length Scale


10-12 nm

Controlled Clustering of Receptors Provides Functionality in Cell Biology

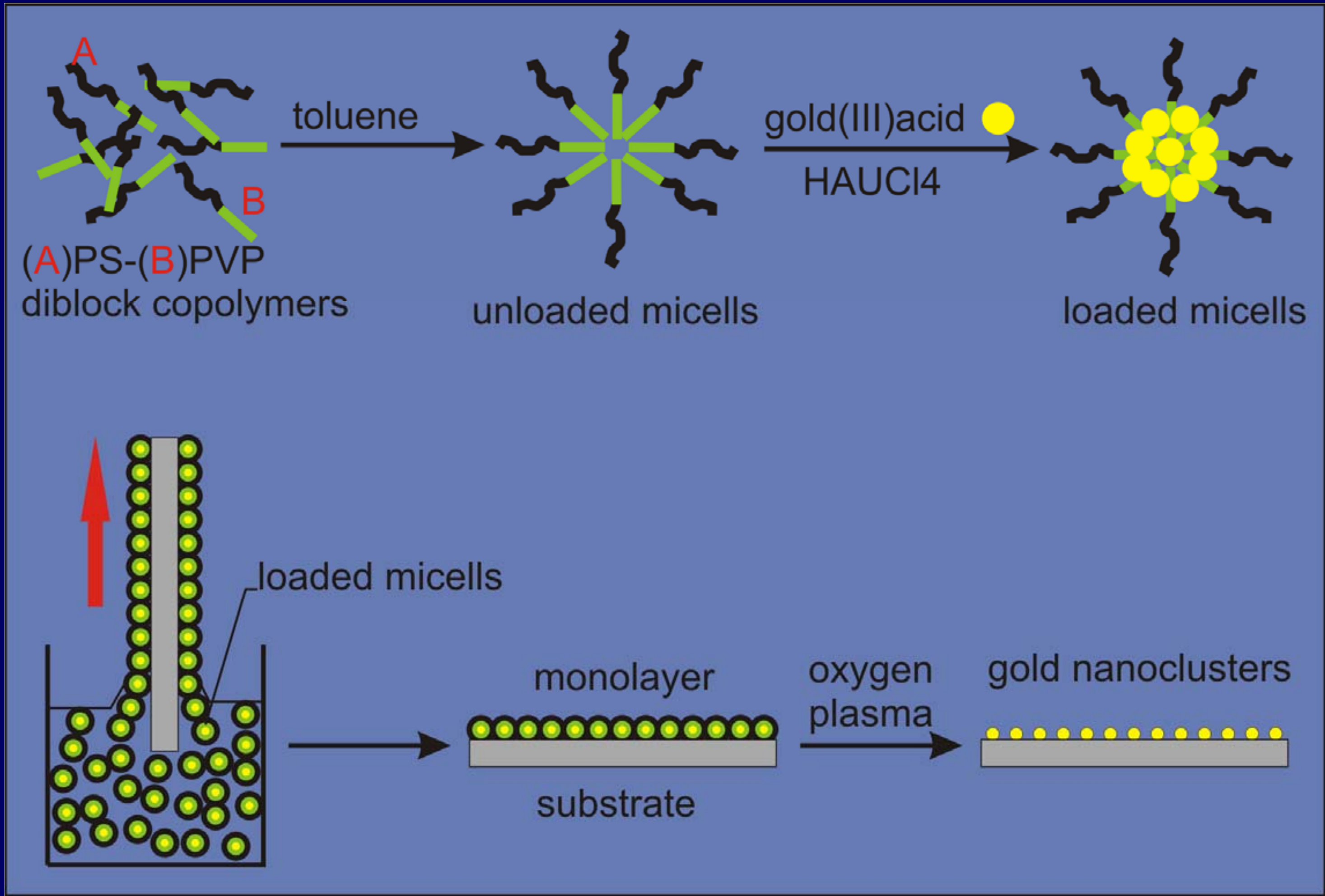


Nanolithography < 10 nm

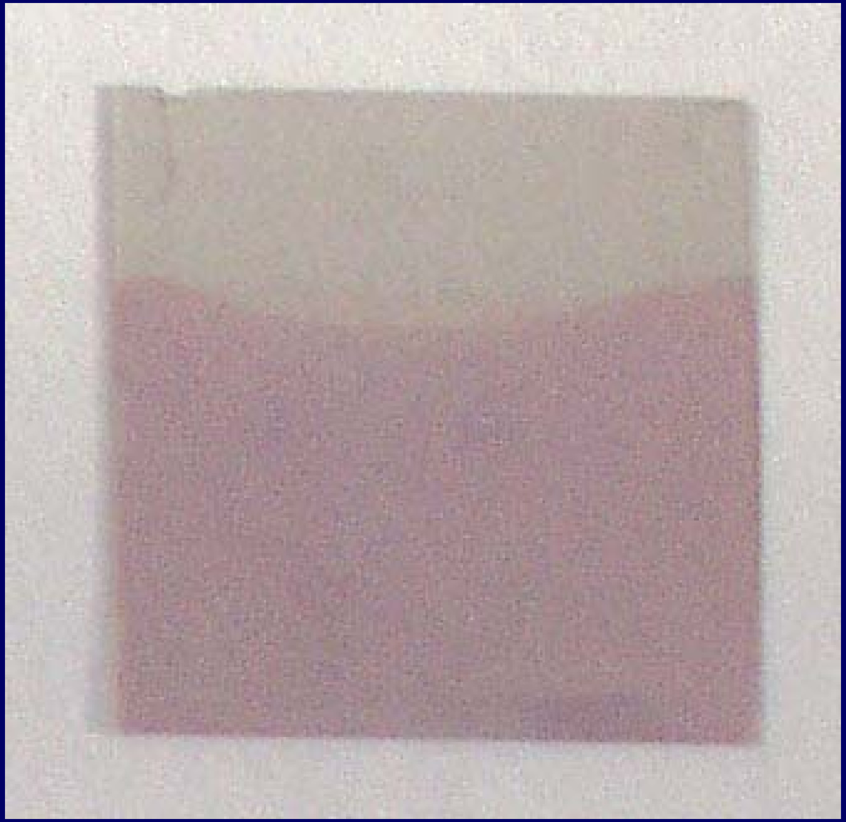
$\leftrightarrow$

Block Copolymer Micelle Nanolithography

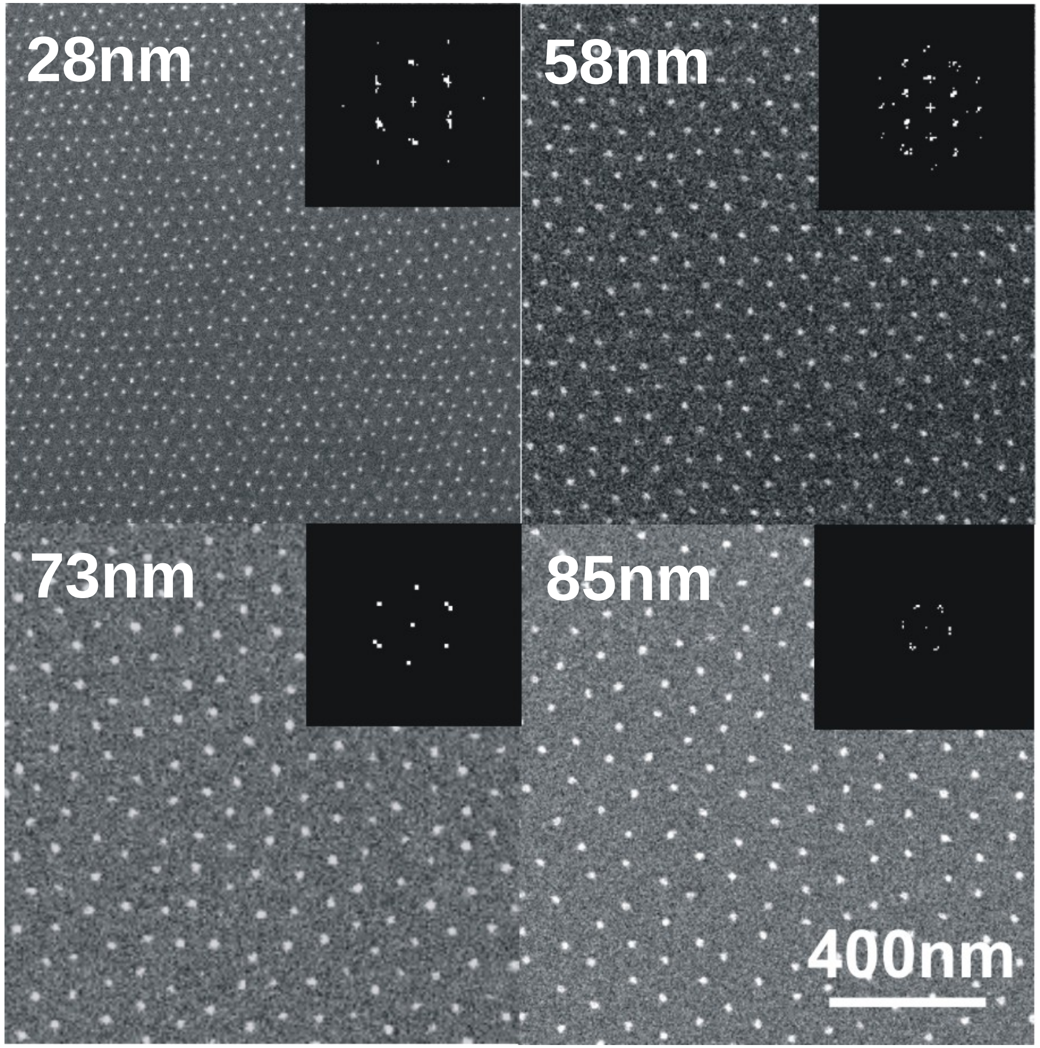
Block Copolymer Micelle Nanolithography

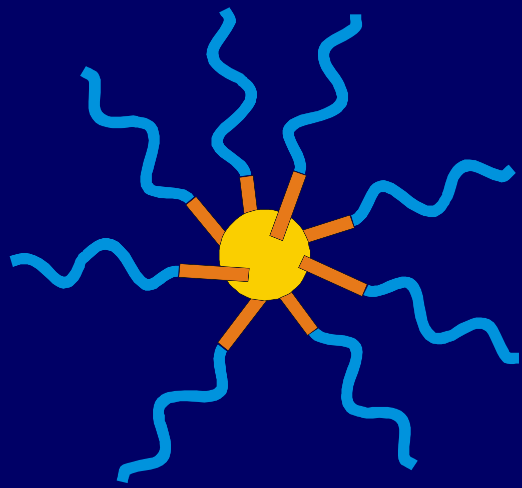


restricted to solid inorganic surfaces

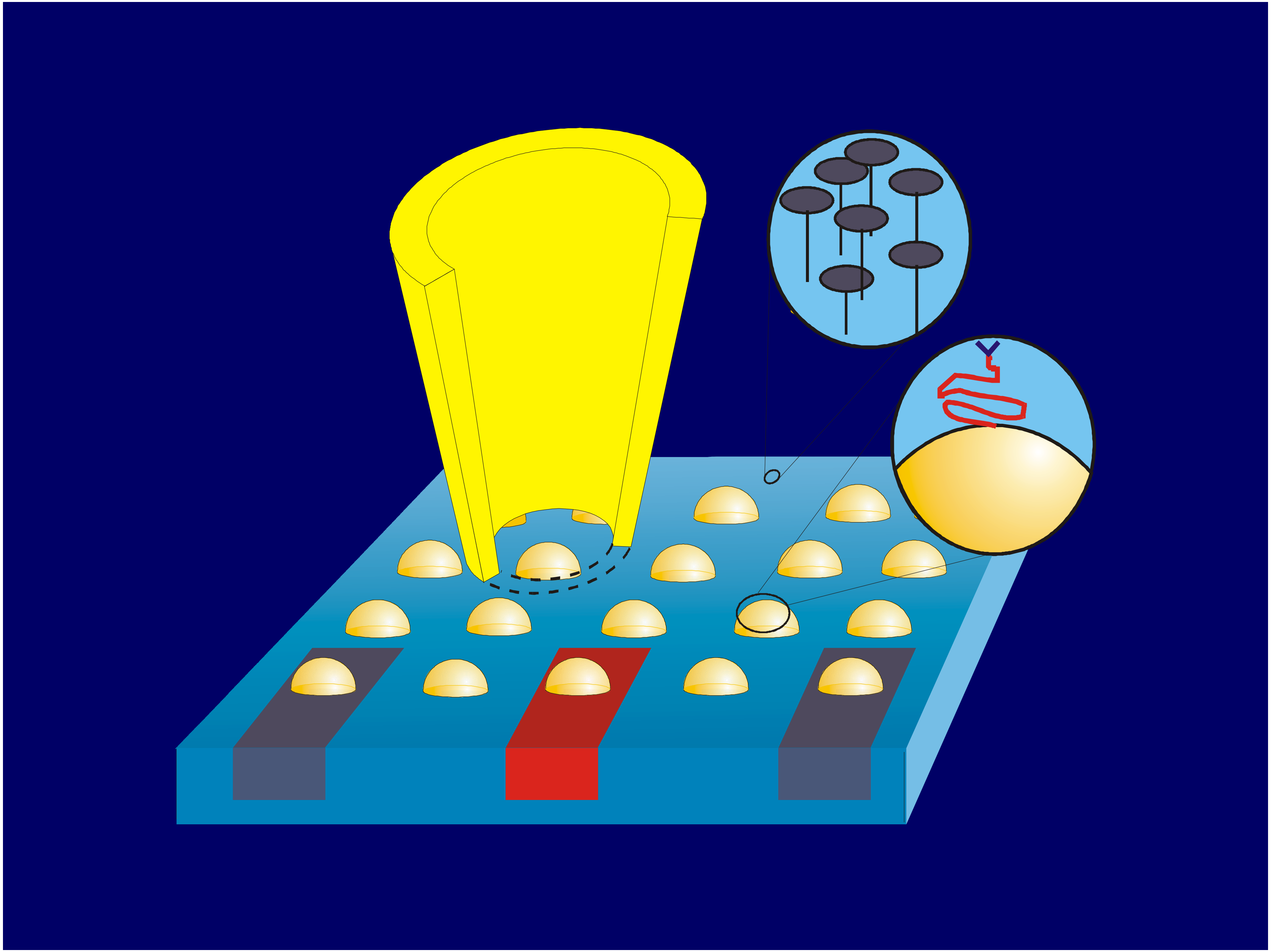


20 nm Au-dot pattern

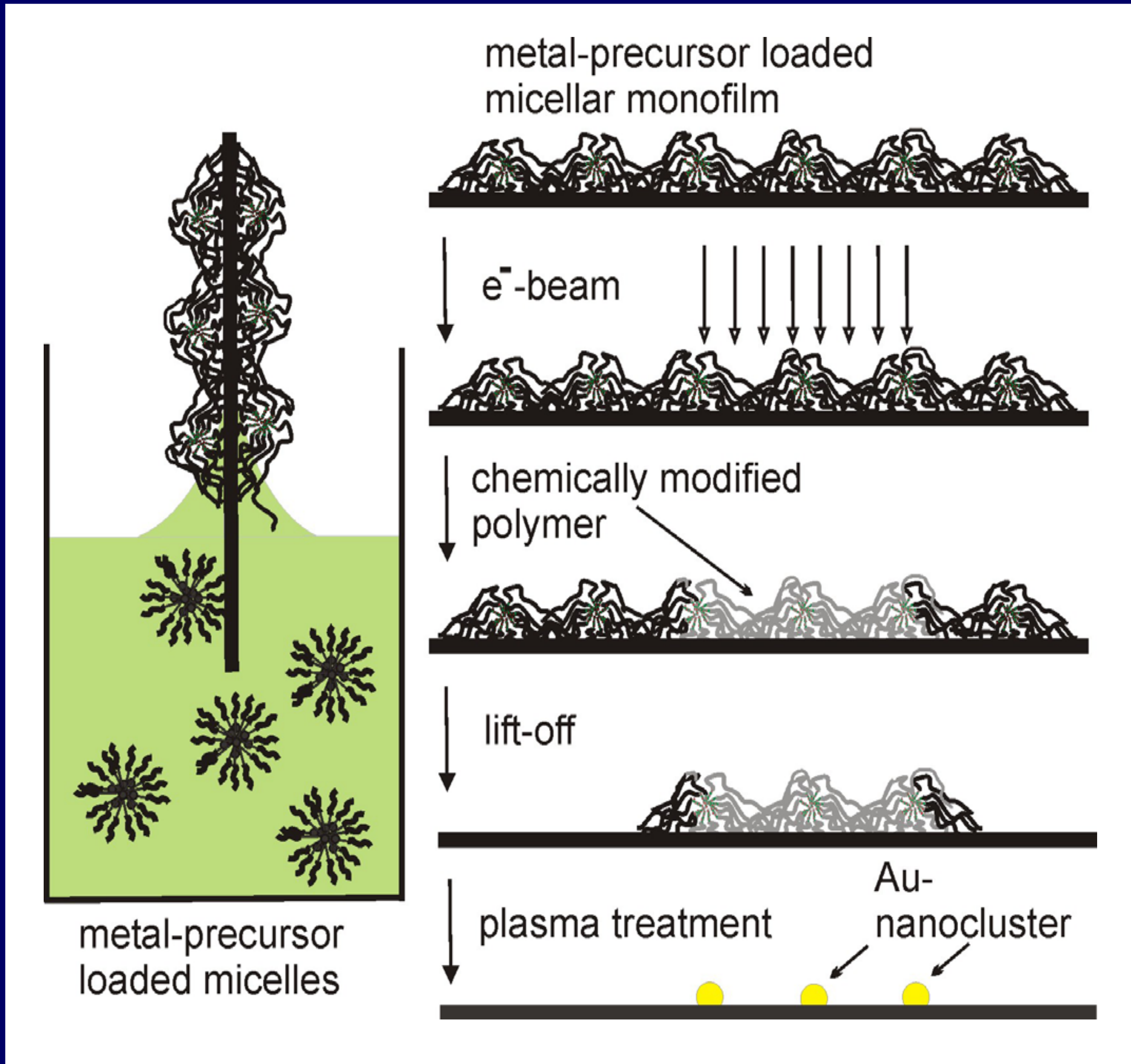




Macromolecules 1995
Advanced Materials 1995
Chemistry European J. 1995
Advanced Materials 1996
Langmuir 2000
Advanced Materials 2002
Advanced Funct. Mat. 2003



Micellar Block Copolymer Resist Lithography



Adv. Func. Mat. 2003

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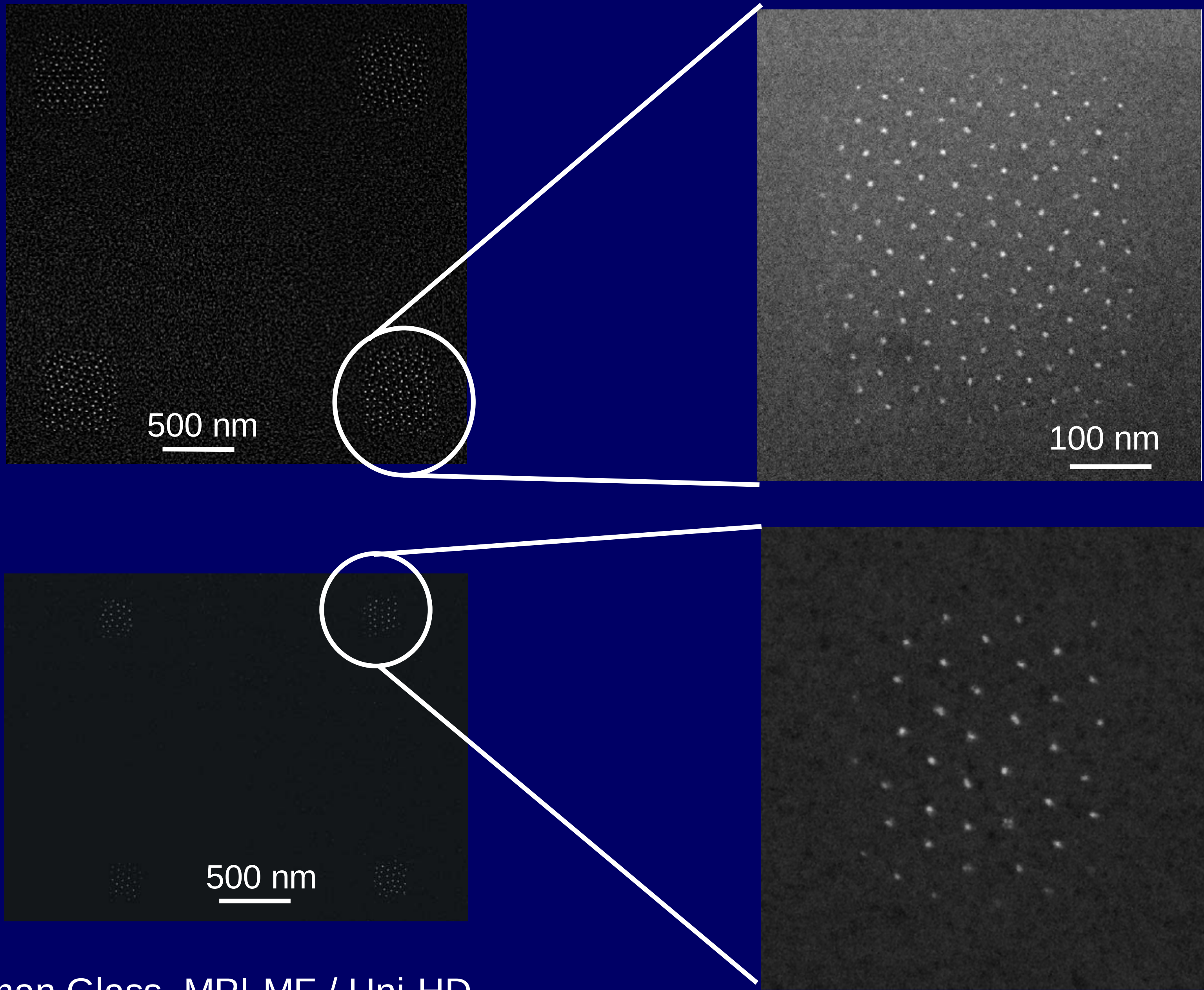
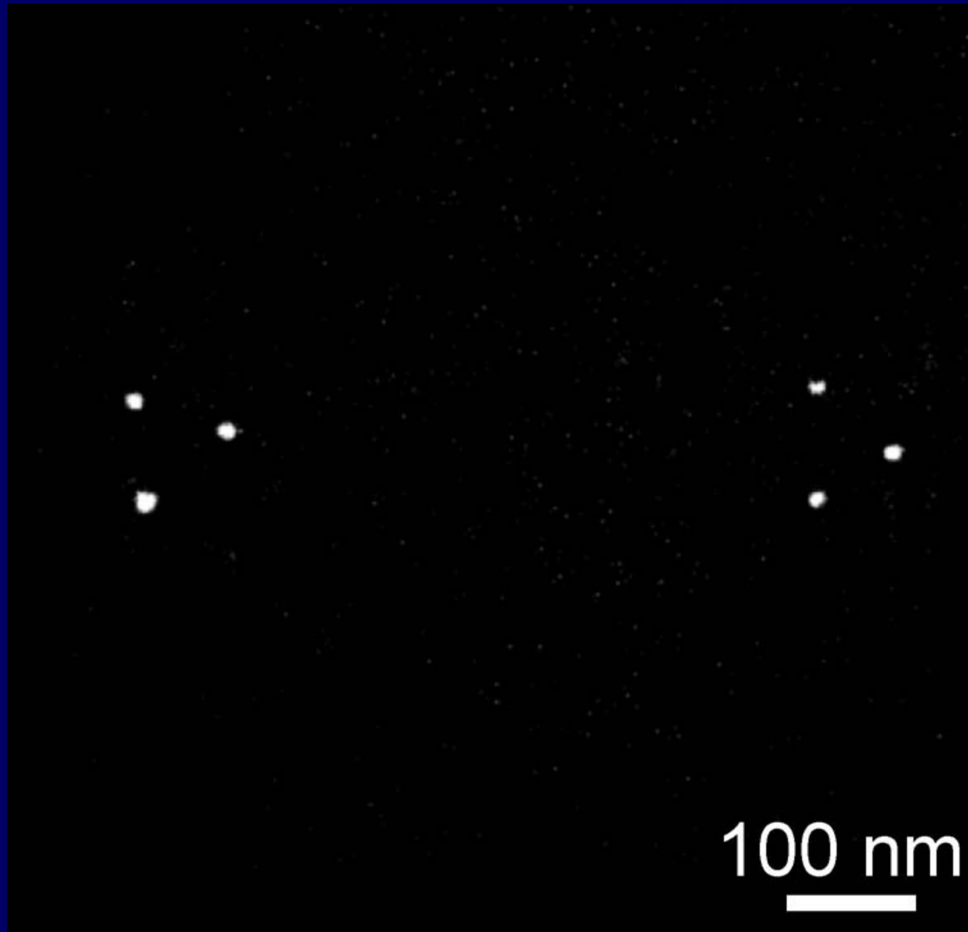
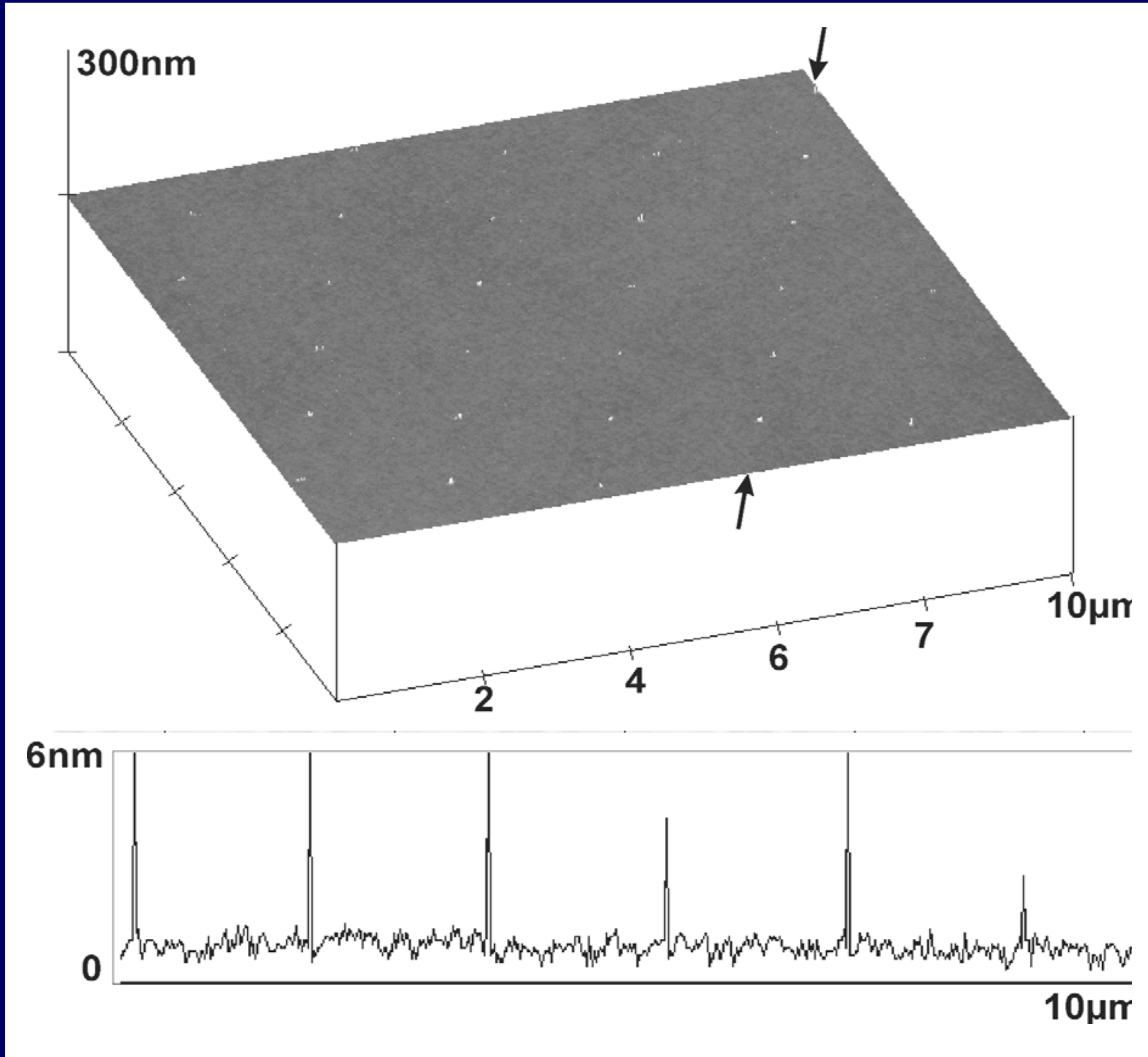
"Micro" Nanostructures



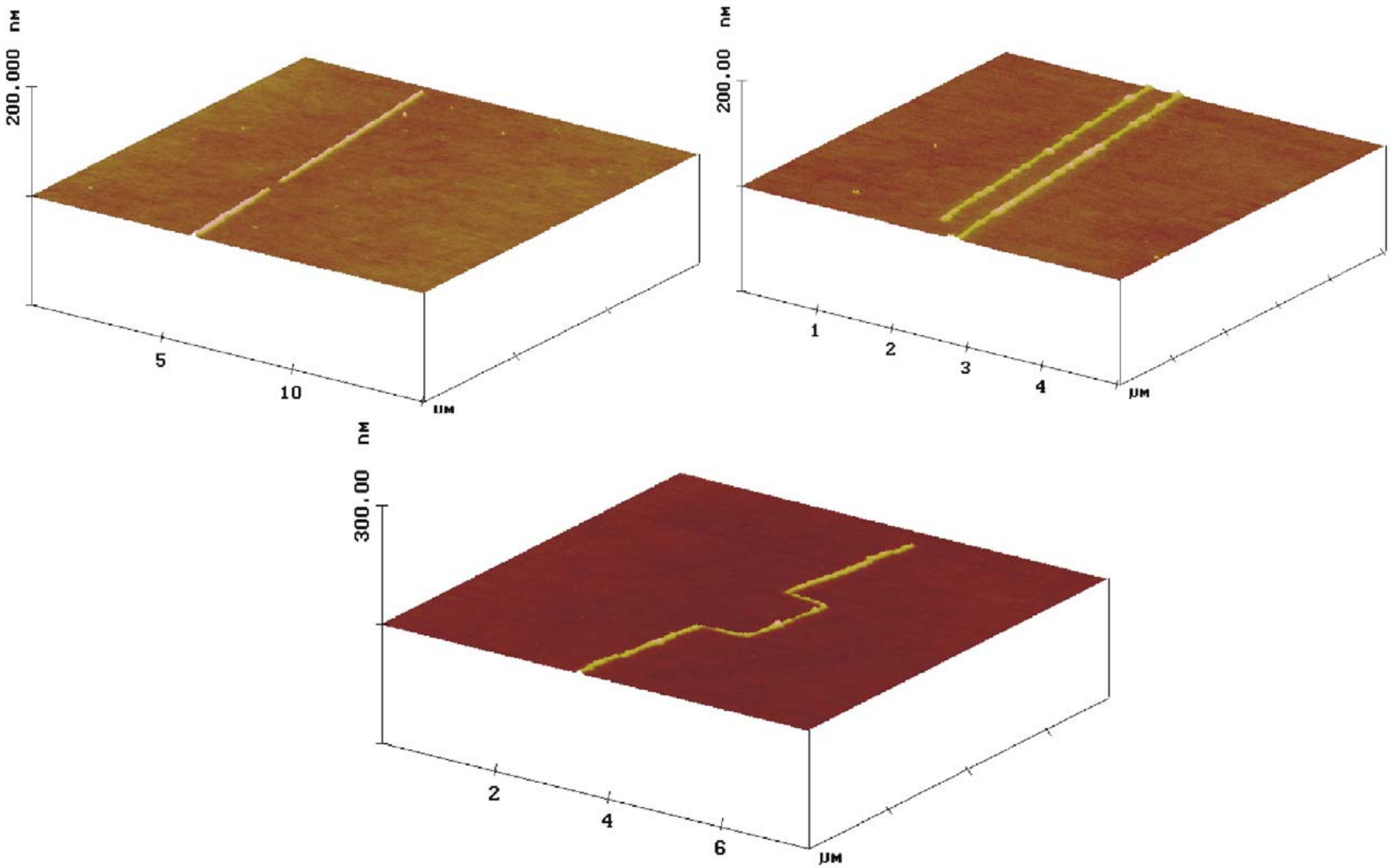
Dr. Roman Glass, MPI-MF / Uni-HD



Dr. Roman Glass, MPI-MF / Uni-HD



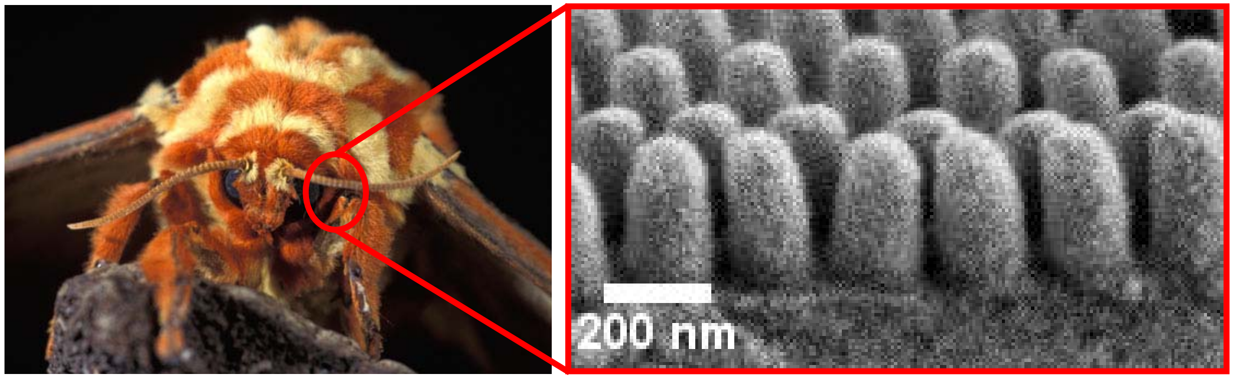
Dr. F.-M. Kamm, University of Ulm



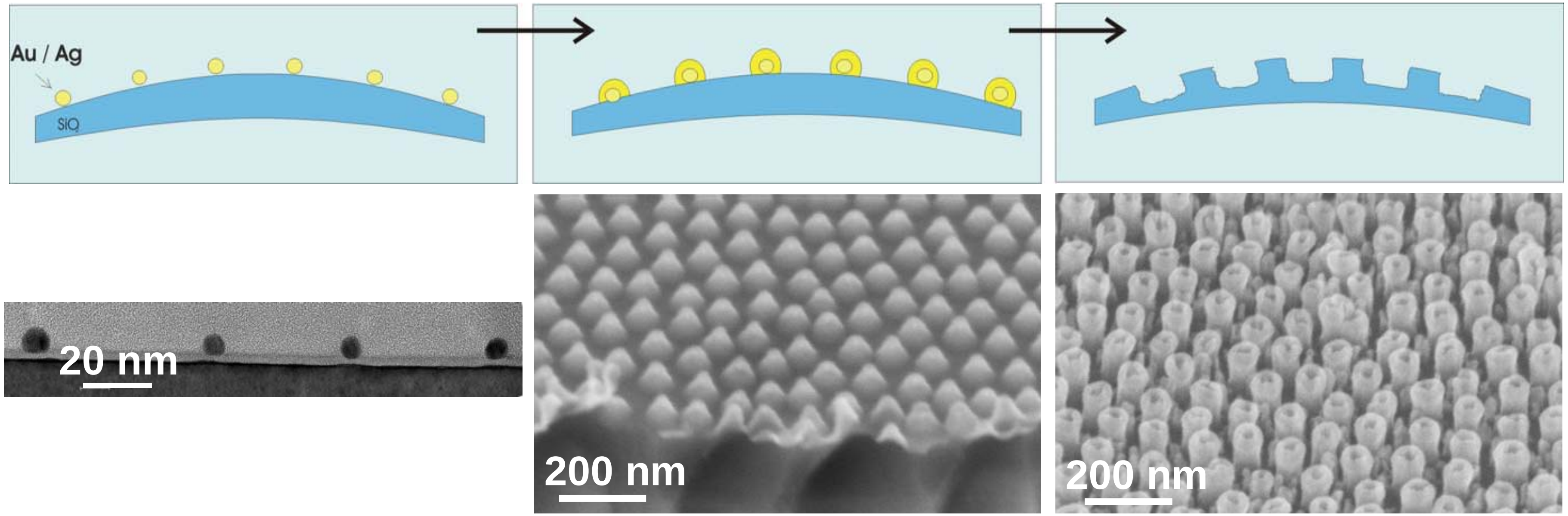
JACS 2001

Anti-Reflective Interfaces / Micro-Optics

Moth Eye



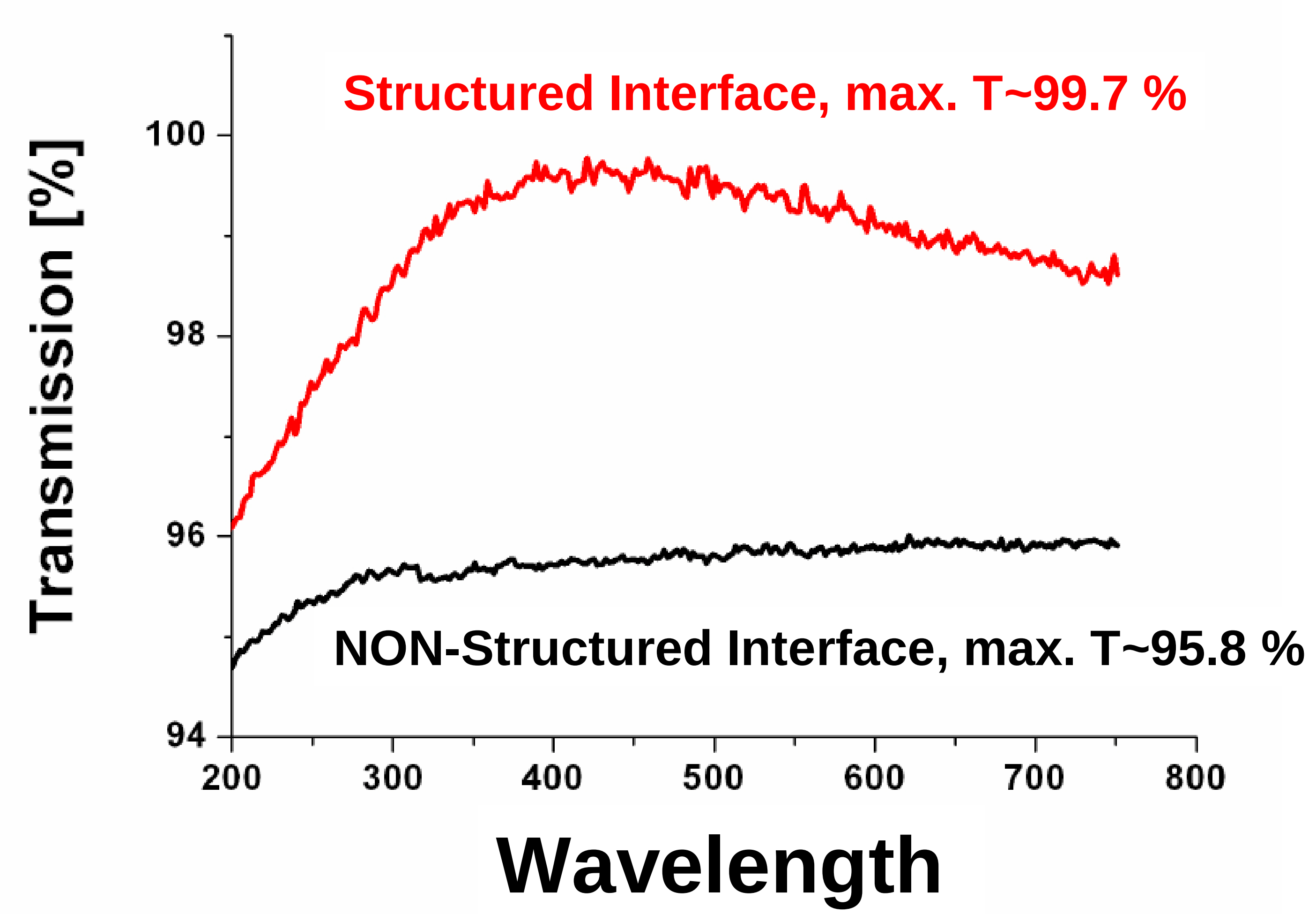
Optical Lense



Cooperation with ZEISS

Theo Lohmüller, MPI-MF & Uni-HD

Anti-Reflective Interfaces / Micro-Optics



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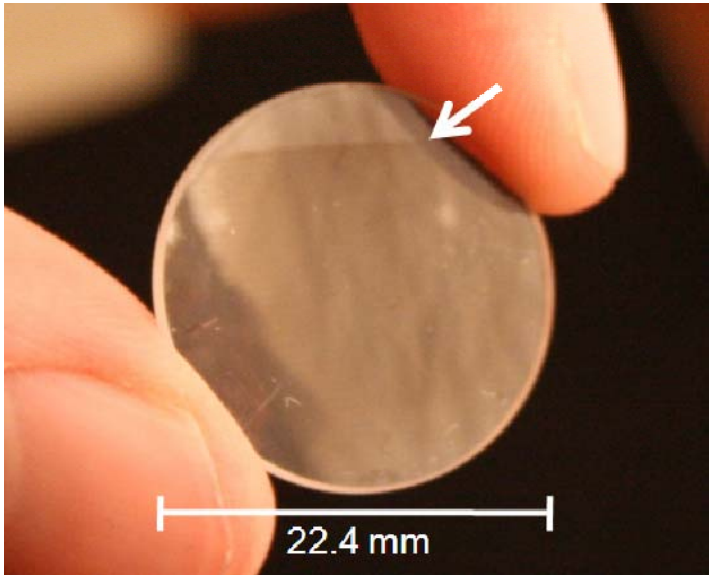
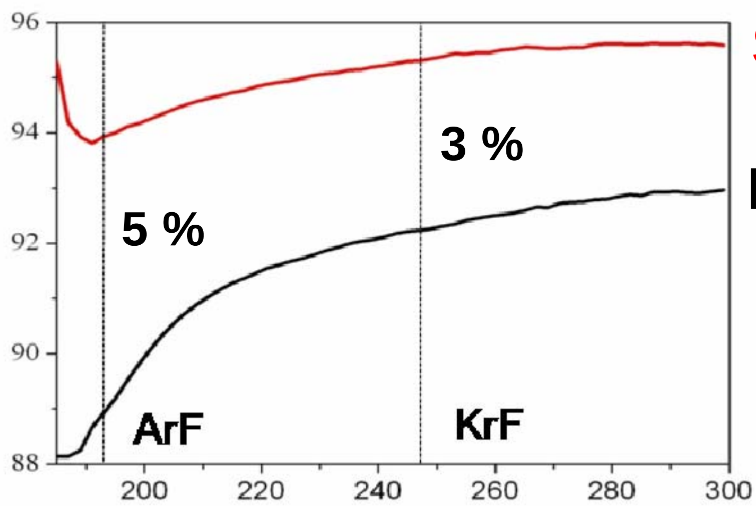
Anti-Reflective Interfaces / Micro-Optics

Visible-Light Reflection



UV-Light Transmission



Structured interface

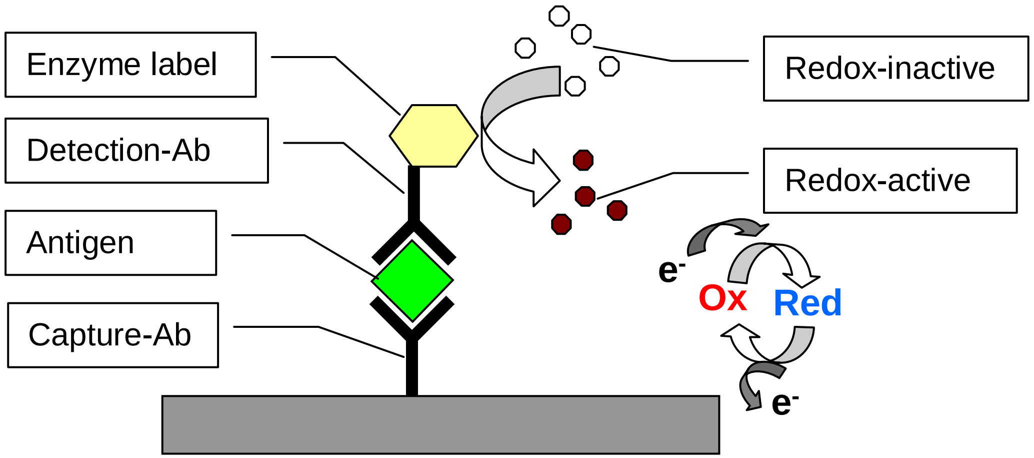
NON structured interface

Wavelength [nm]

Theo Lohmüller, MPI-MF & Uni-HD

Purpose

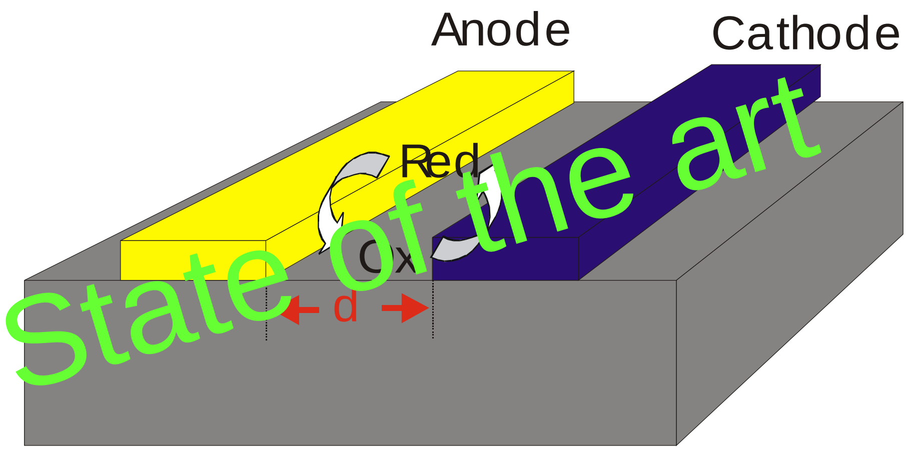
Assays with electrochemical detection



- Incubation with antigen (sample)
- wash
- incubation with enzyme labeled detection antibody
- wash
- add substrate
- measurement

- Enzyme converts redox-inactive molecule to redox active mediator
- Repeated oxidation / reduction will increase signal: ***redox - cycling***

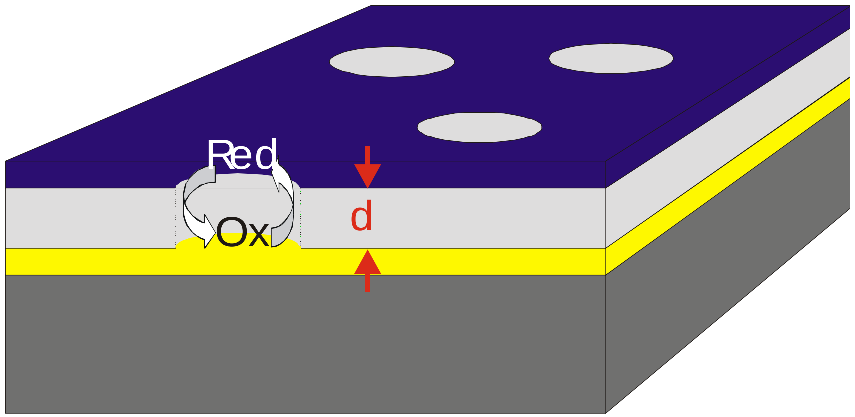
Redox cycling: why nano-electrodes?



- Requires small anode / cathode spacing <1µm
- State of the art: amplification factor of ≈10
- Difficult & expensive fabrication limits applicability in diagnostics

Technology

The NanoBioPore concept

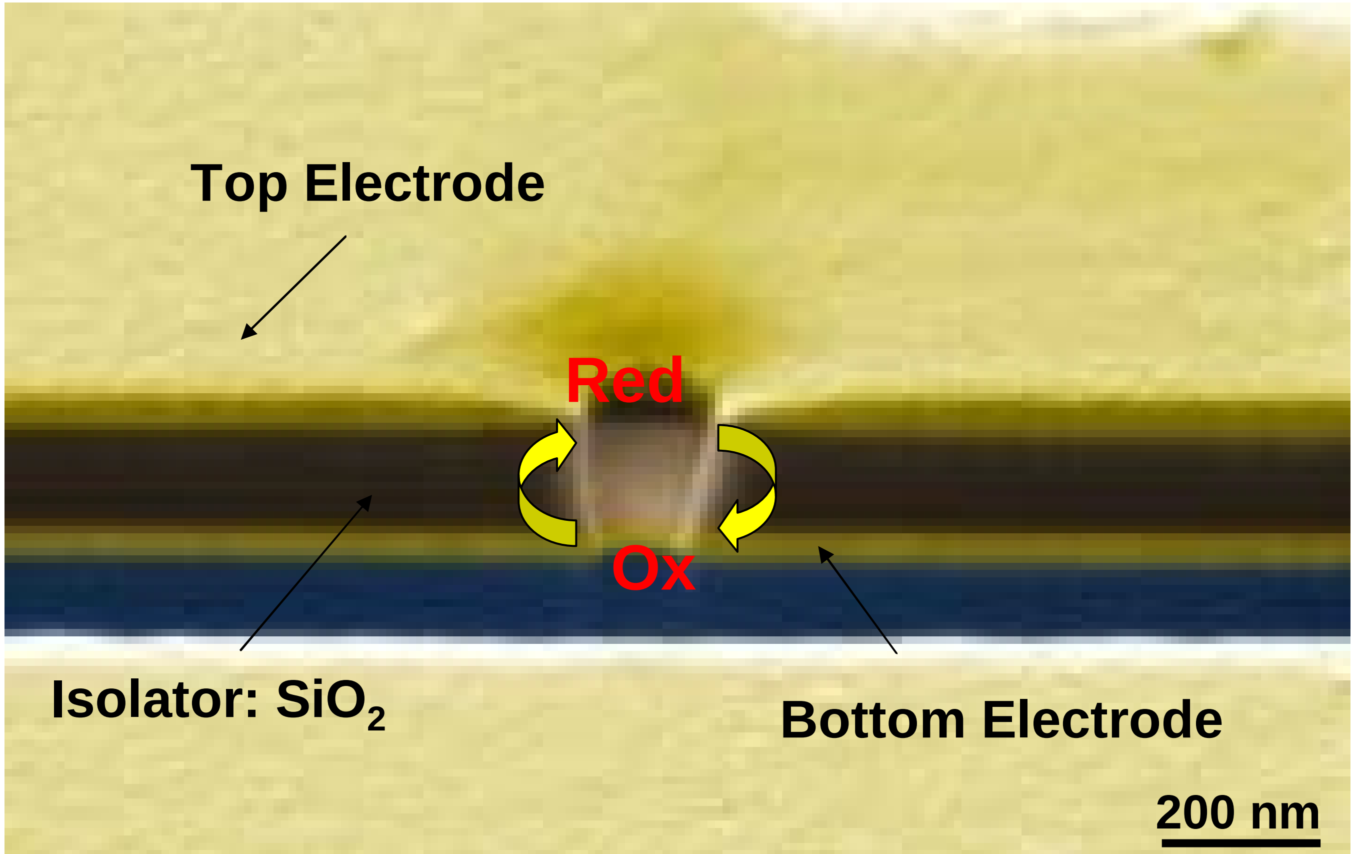


- Multiple micro- or nanopores, laterally connected
- Electrode spacing defined by insulator thickness

➡ Micro-structure technology based on ***self-assembly*** process

Martin Stelzle, NMI Reutlingen

REDOX-CYCLING IN PORES



in collaboration with M. Stelzle, **NMI Reutlingen**, W. Schuhmann, **Bielefeld**
S. Linke, **HL Planar**, C. Kottig, **EVOTEC**

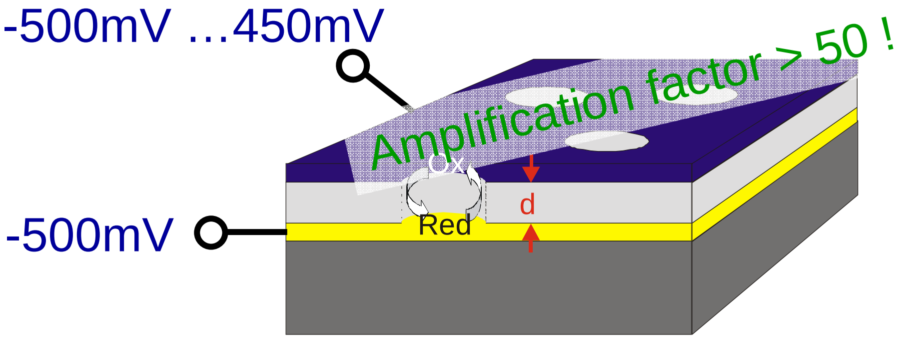
… also applied as filters with pore diameter smaller 30 nm

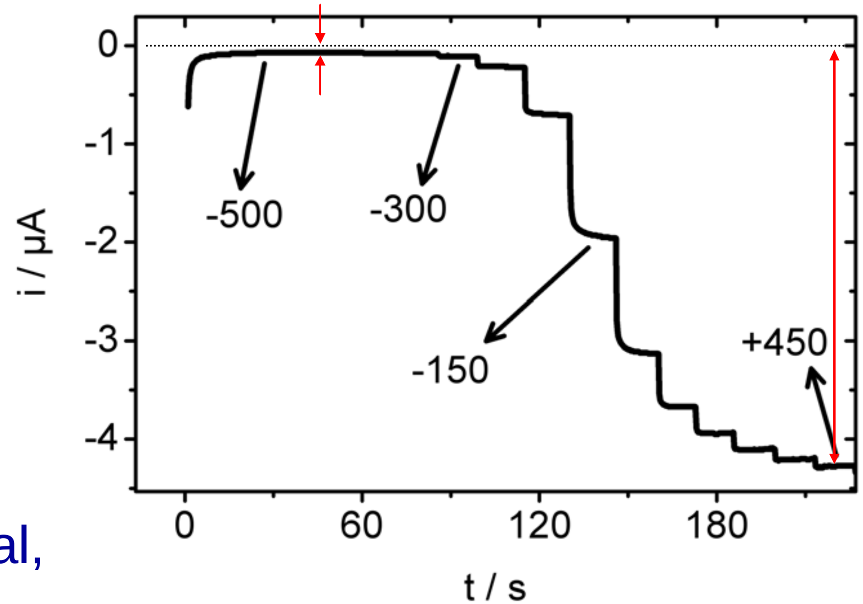
Theo Lohmüller

Redoxcycling in Nanopores

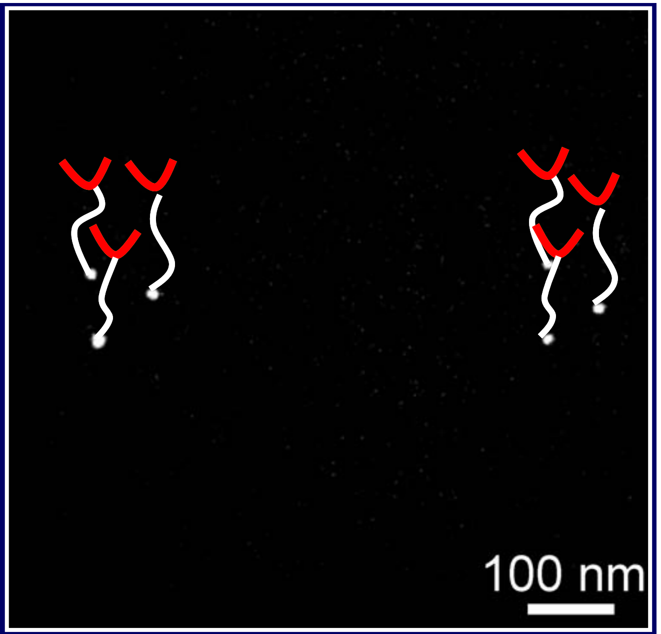
Signal amplification by redoxcycling in nanopores demonstrated!

Redox couple: $Ru[NH_3]_6Cl_3$



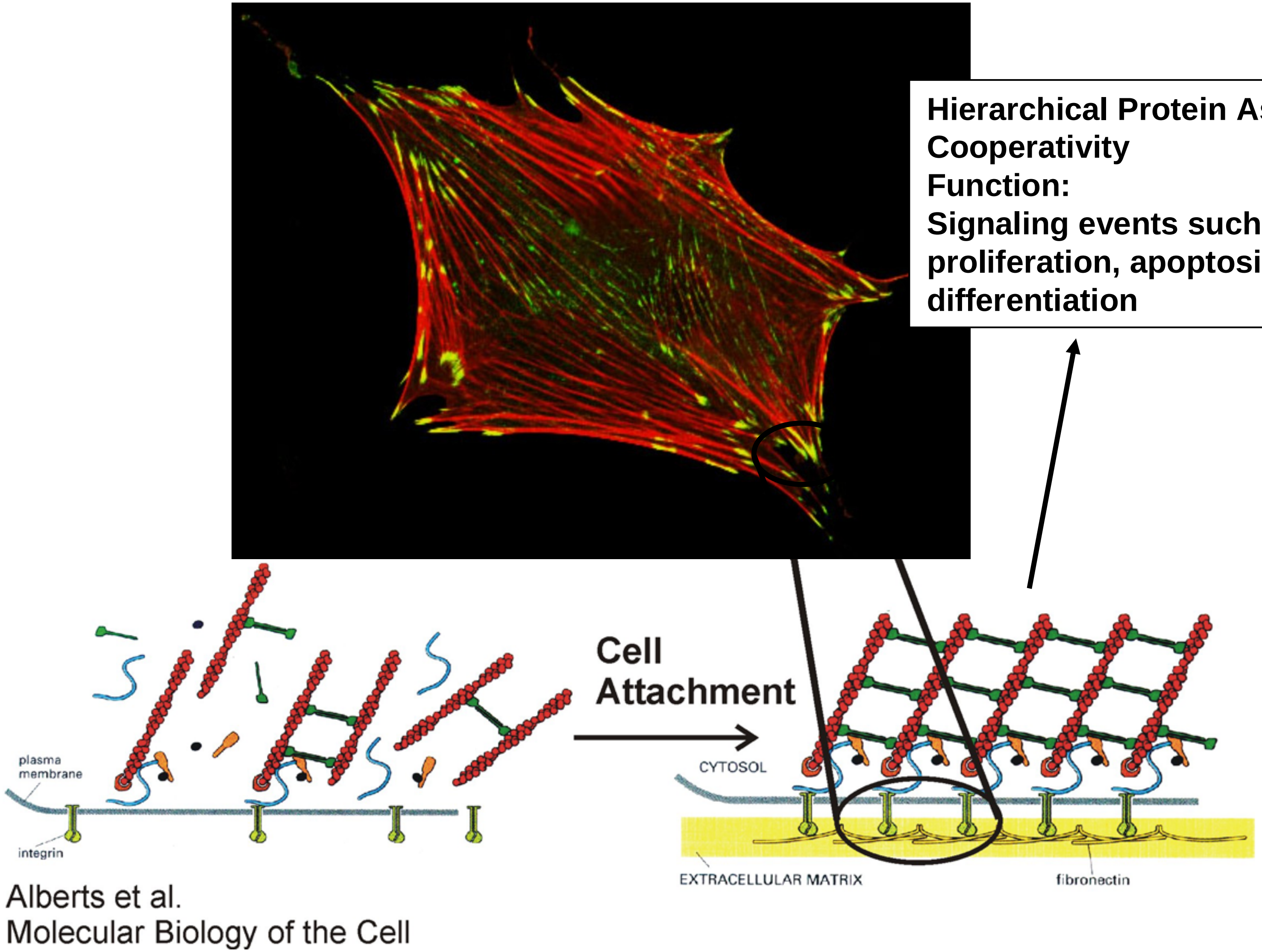


Bottom electrode at constant potential,
Variation of potential of top electrode



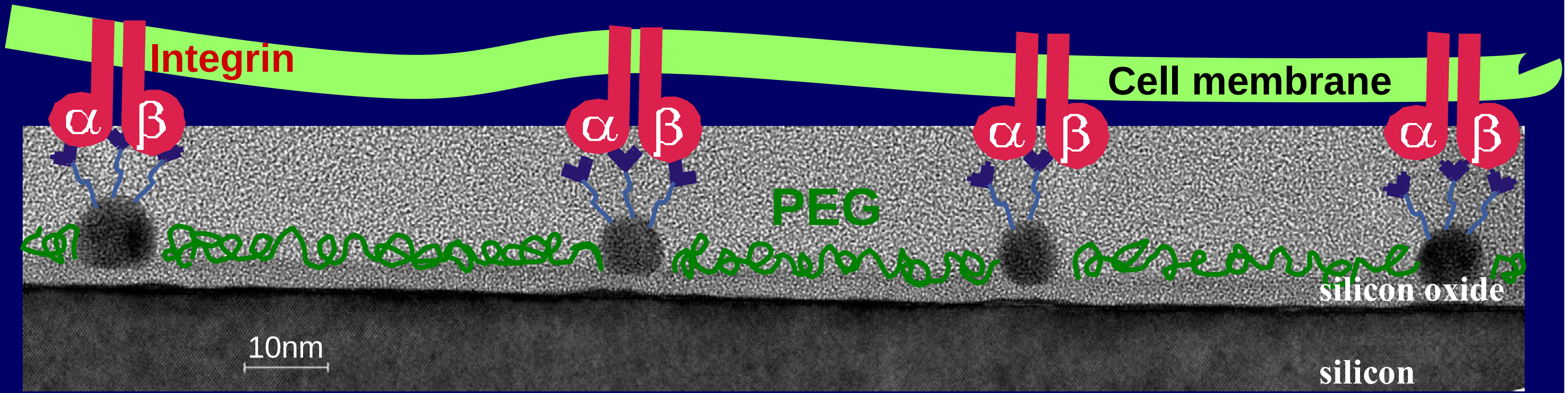
Challenge

to position and group single proteins by single chemical binding sites into different geometries on rigid platforms and to investigate their cooperative function.

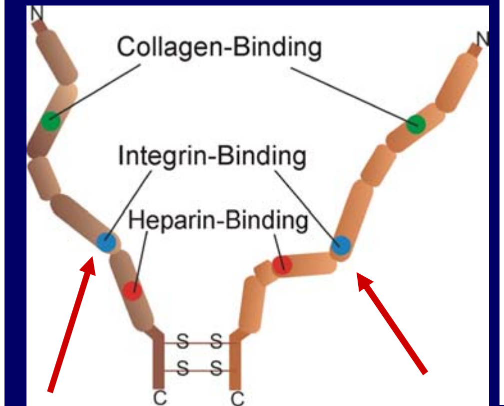


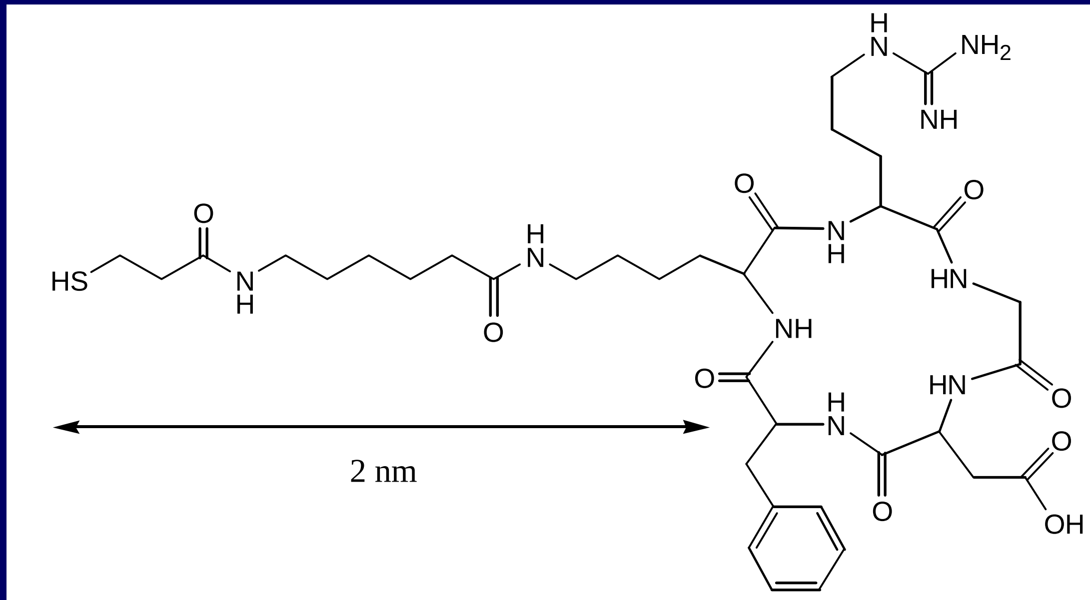
Hierarchical Protein Assembly
Cooperativity
Function:
Signaling events such as proliferation, apoptosis, differentiation

Bio-Functionalisation



cell adhesive motif





Arginine R

Glycine G

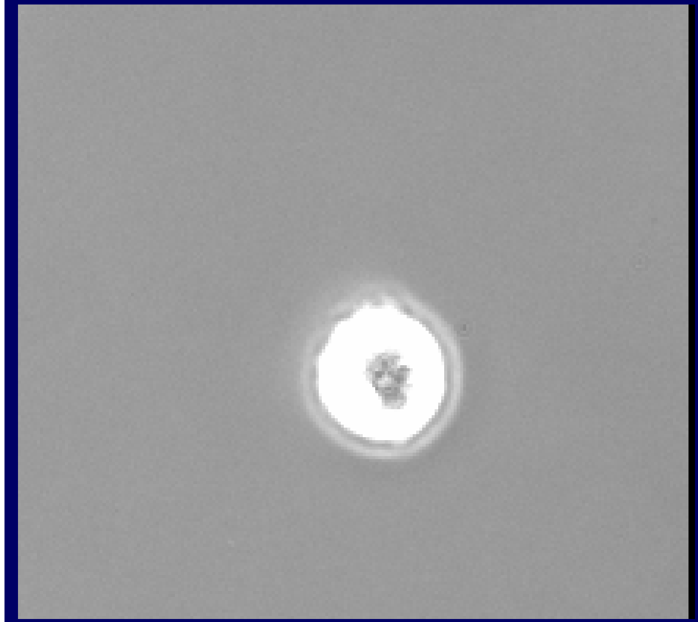
Aspartate D

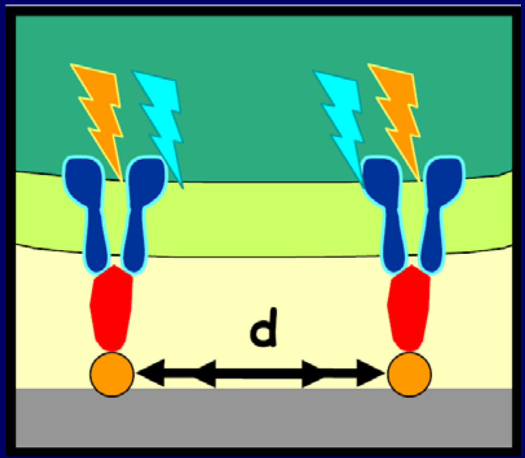
Prof. Kessler
TU Munich

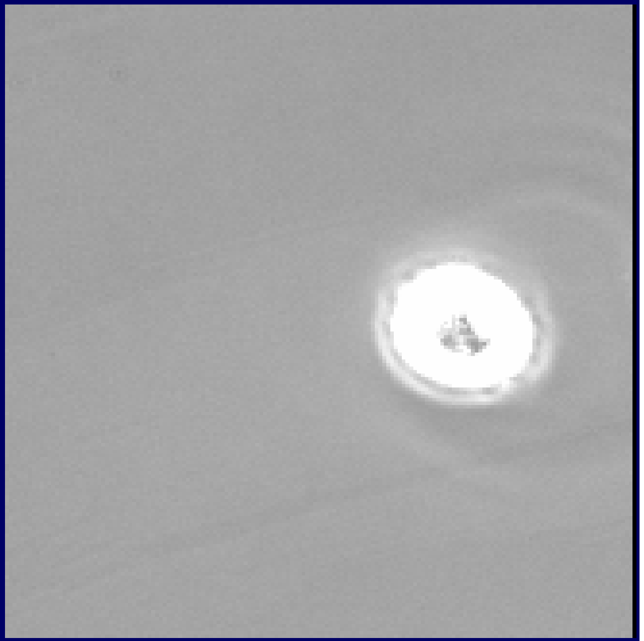
Dr. Jacques Blümmel, Dr. Marco Arnold, MPI-MF / Uni-HD

Cell Spreading on 58 nm pattern

Cell Spreading on 73 nm pattern

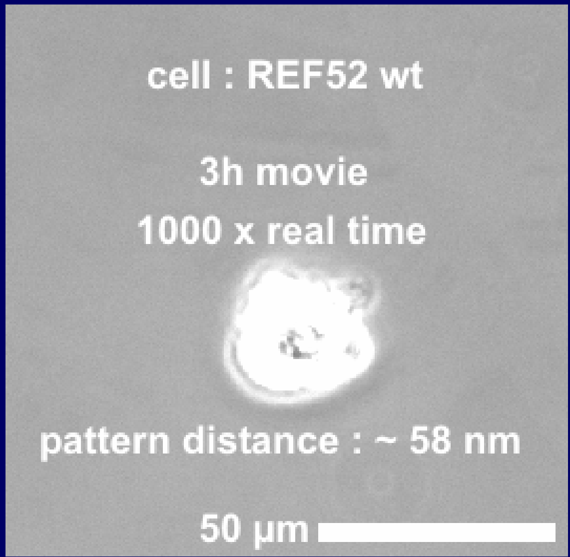




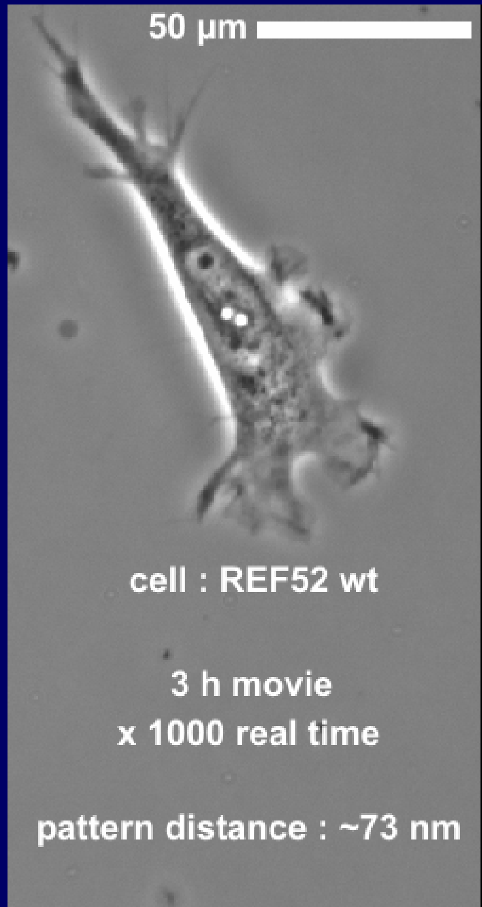


Dr. Ada Cavalcanti-Adam, Dr. Marco Arnold, MPI-MF / Uni-HD

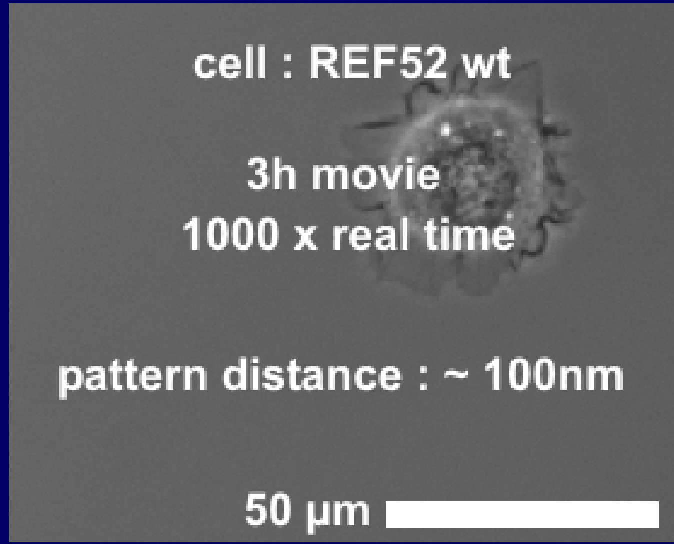
58 nm RGD pattern



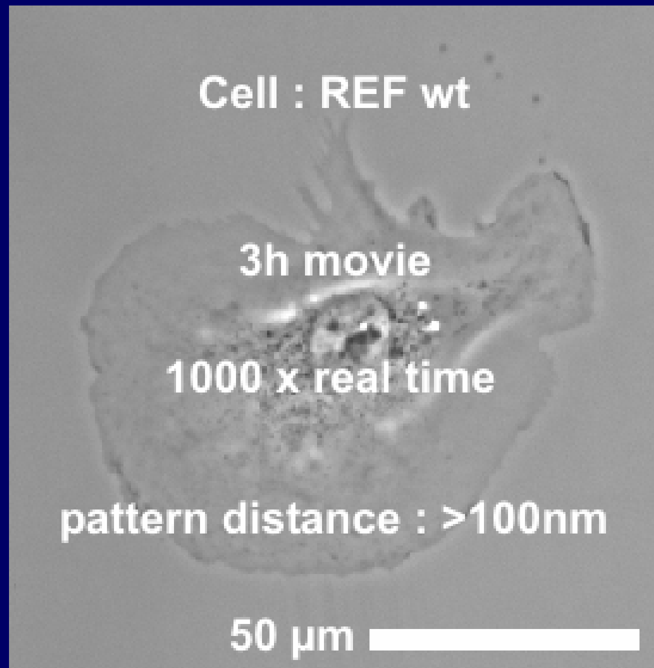
73 nm RGD pattern



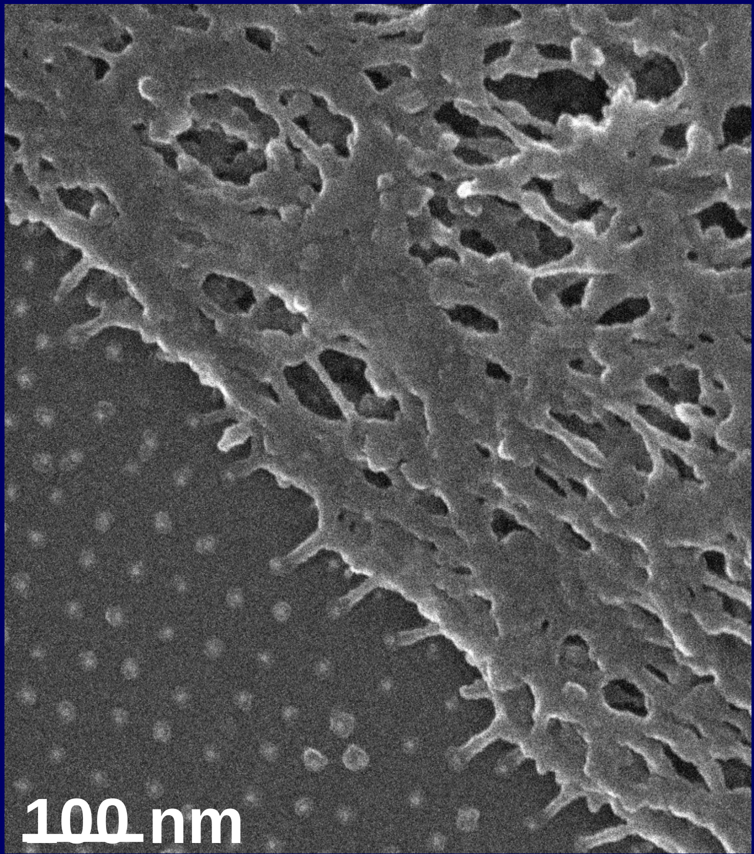
100 nm RGD pattern

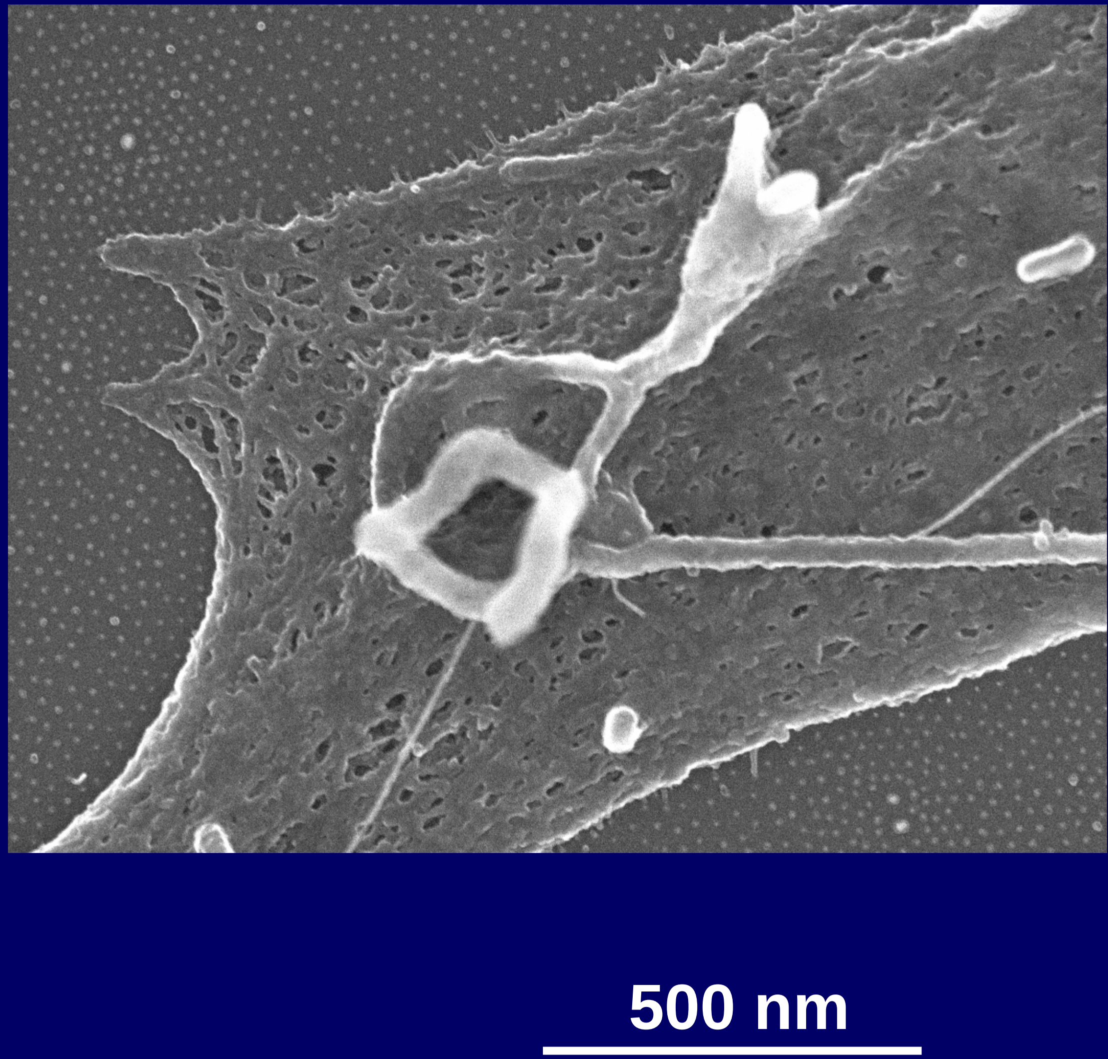


> 100 nm RGD pattern



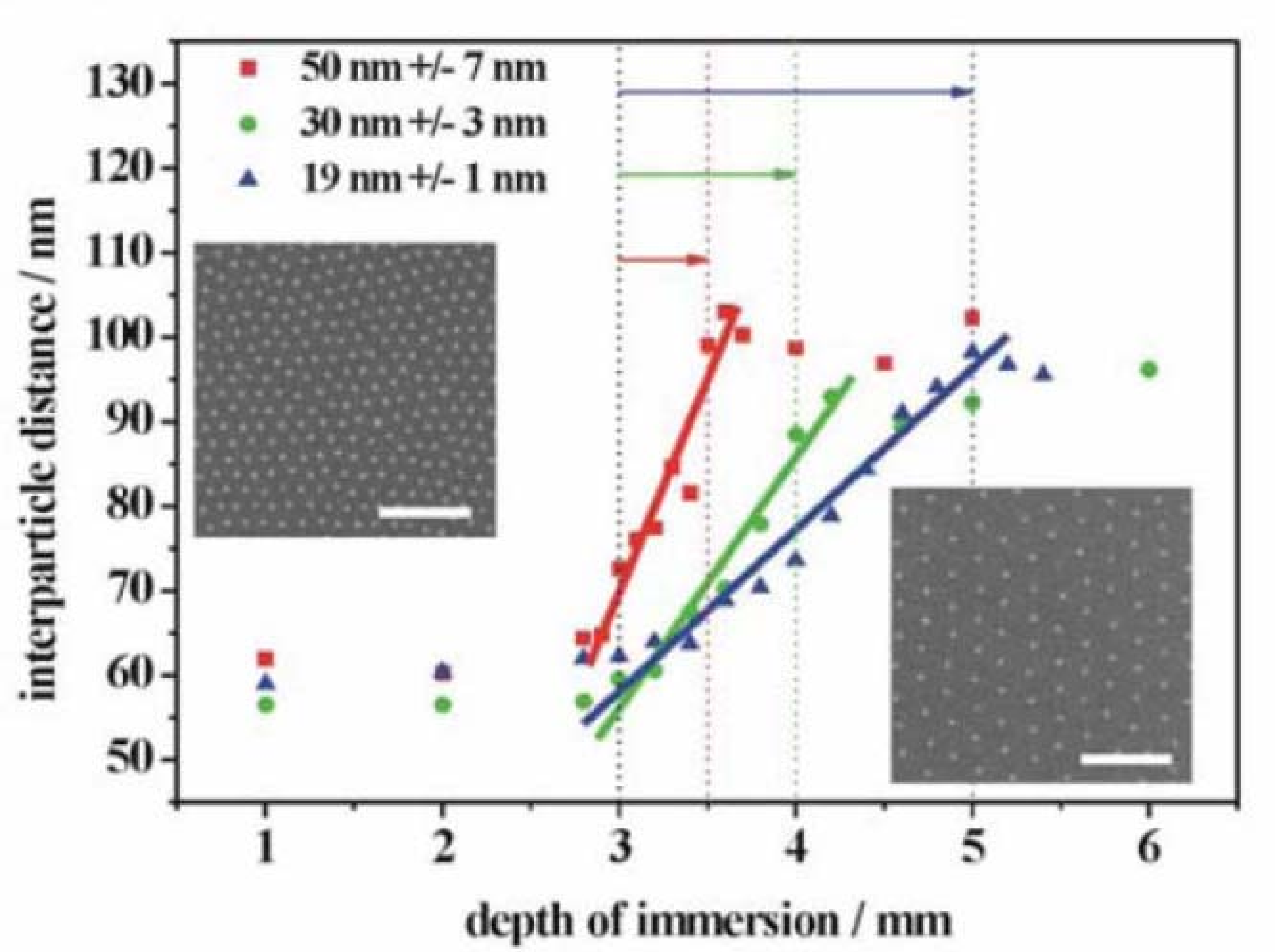
Dr. Ada Cavalcanti-Adam, Dr. Marco Arnold





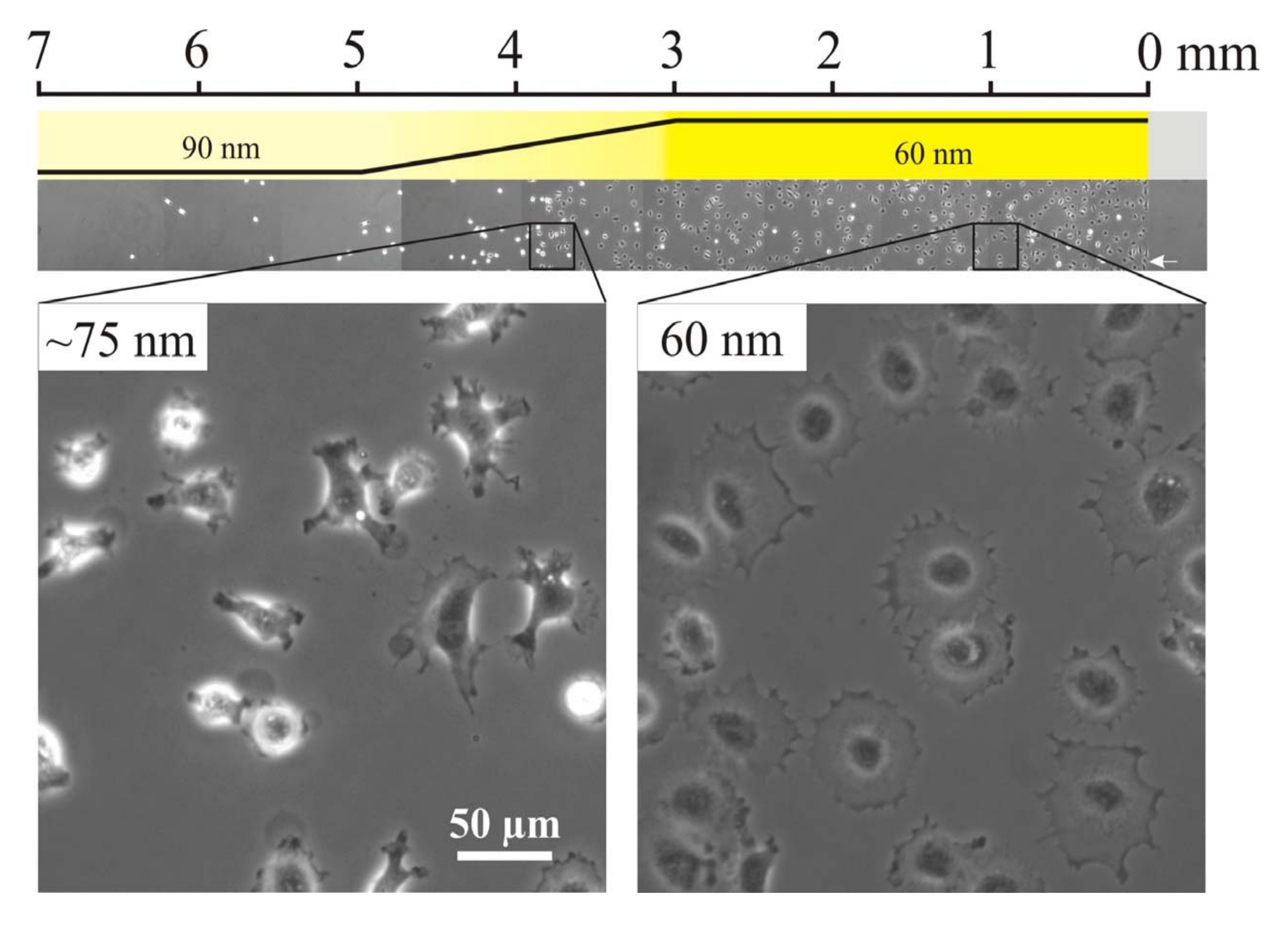
Dr. Marco Arnold, ,Dr. Ada Cavalcanti-Adam, MPI-MF / Uni-HD

Nanoparticle Distance Gradient

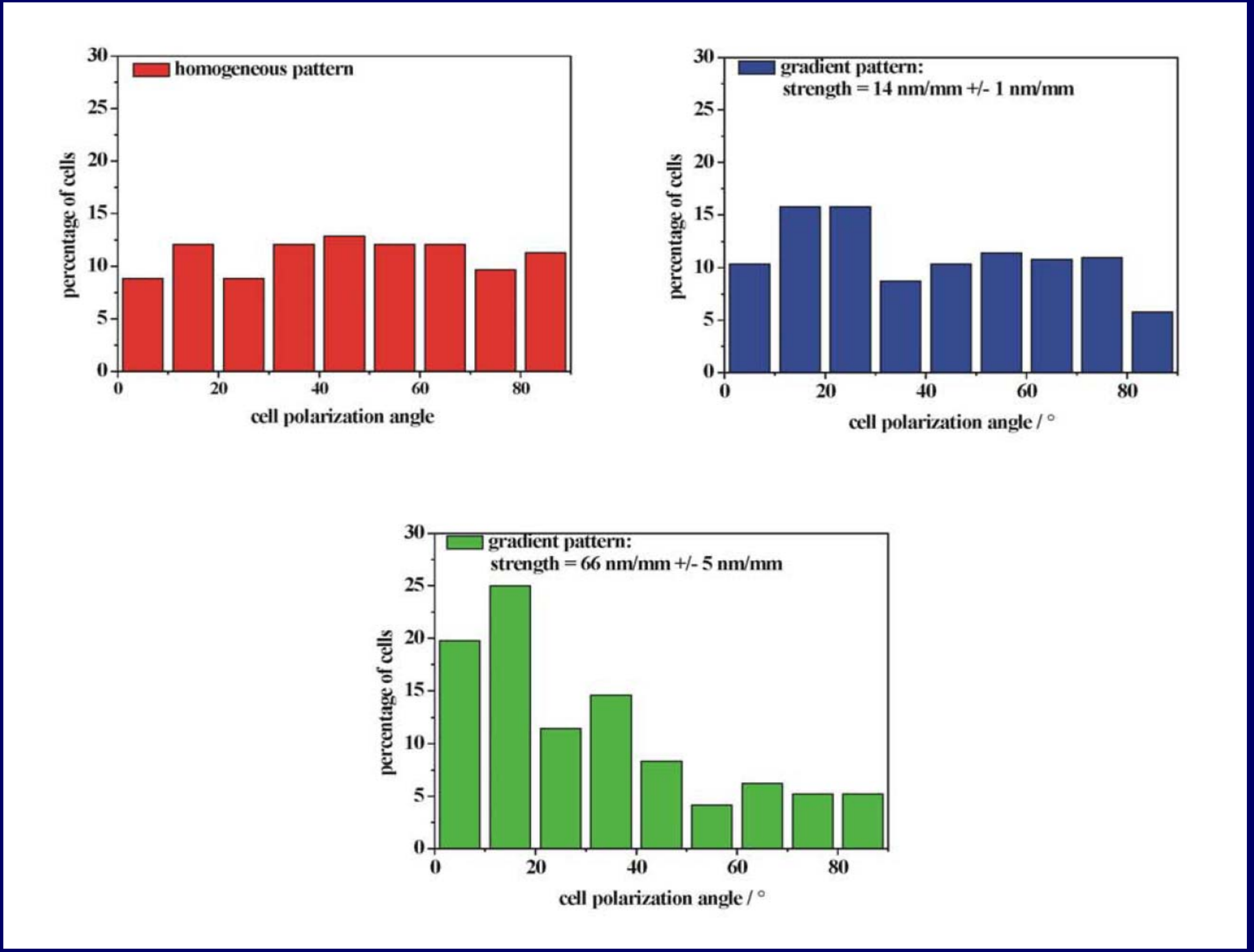


Vera Jakubick, Dr. Marco Arnold, MPI-MF / UNI HD

Molecularly Defined RGD-Peptide Gradient



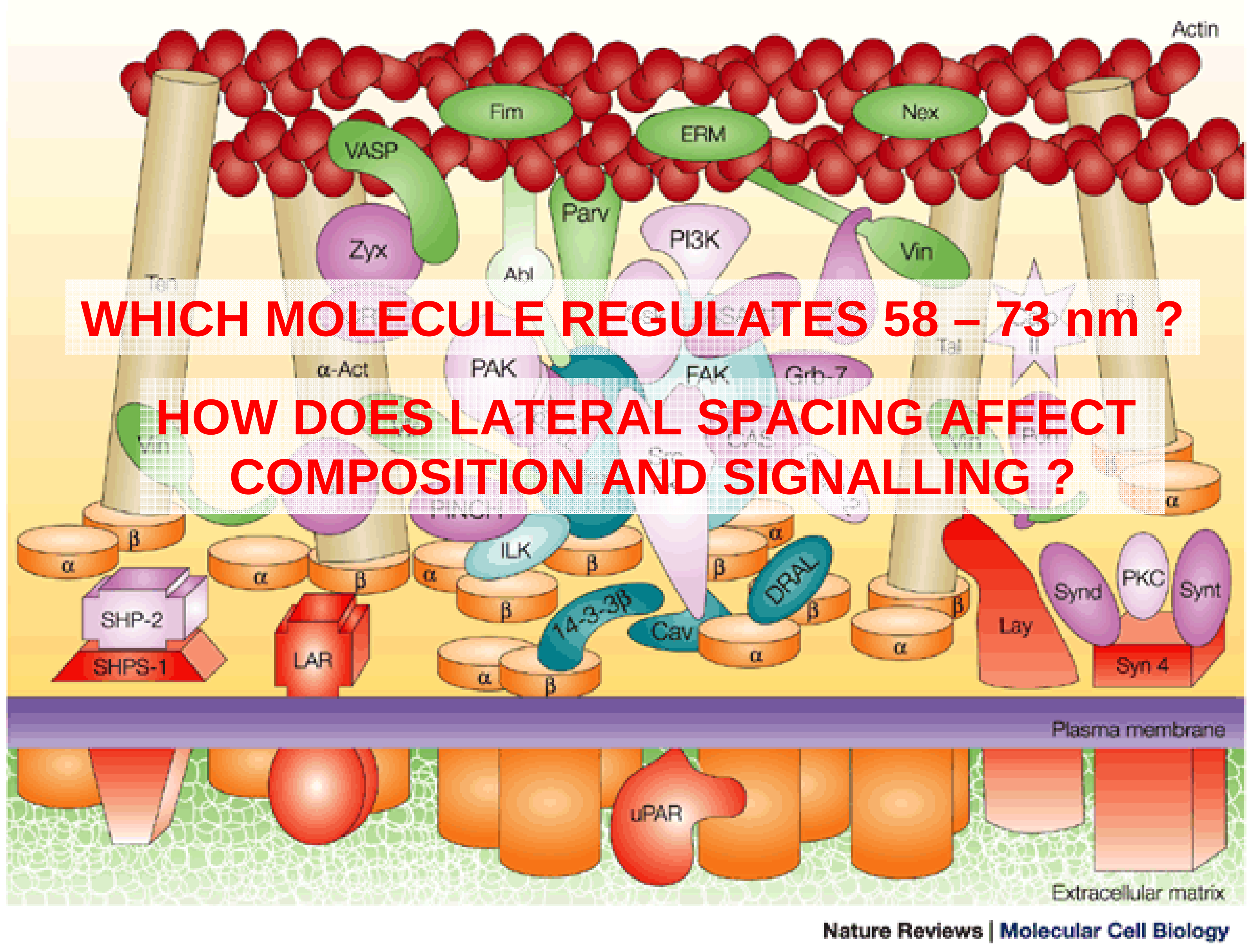
Dr. Marco Arnold, , Vera Jakubick, MPI-MF / UNI HD



~3 nm difference between cellular back and front upon signal integration for 24 hours

Vera Jakubick, Dr. Marco Arnold, MPI-MF / UNI HD

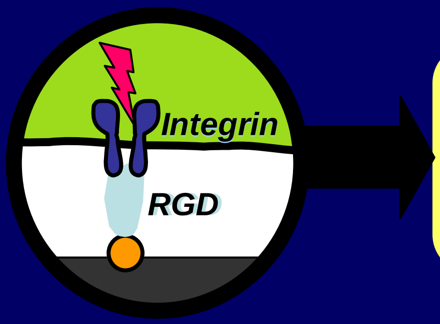
Programming Cell Function



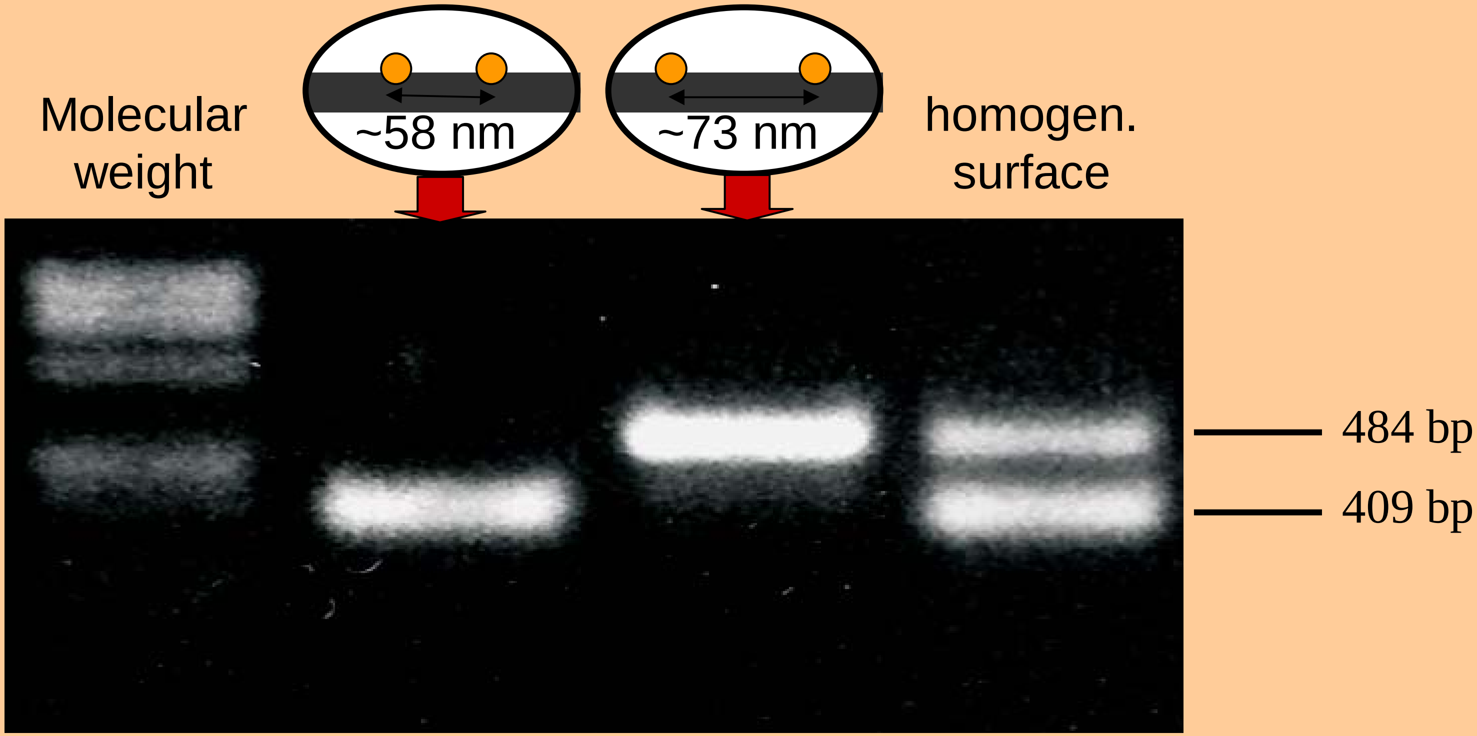
WHICH MOLECULE REGULATES 58 – 73 nm ?

HOW DOES LATERAL SPACING AFFECT COMPOSITION AND SIGNALLING ?

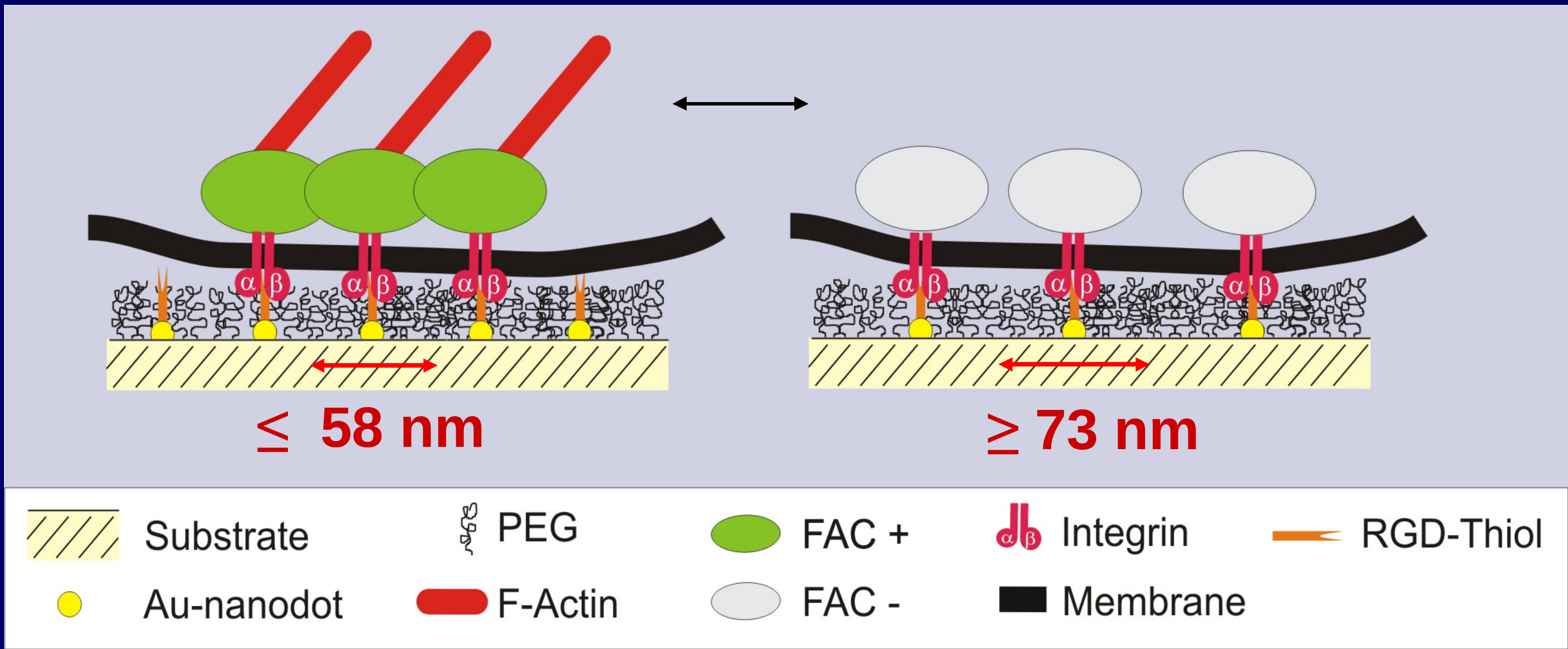
Benny Geiger, Weizmann Institute of Science



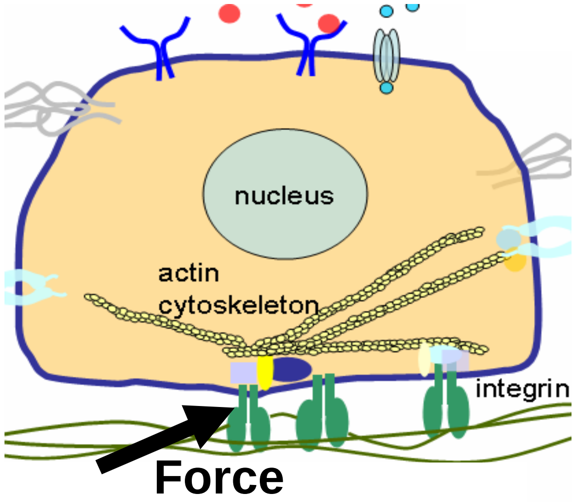
SWITCH ON / OFF the synthesis of different spliced variants of fibronectin



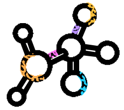
Integrin Cluster Activation Control by Nanopattern



MECHANOSENSITIVITY



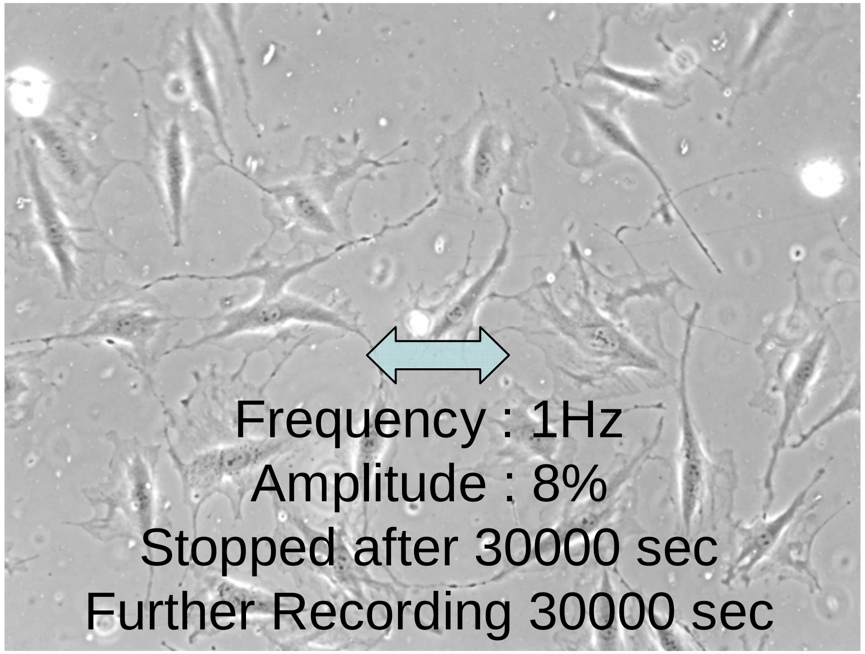
Force

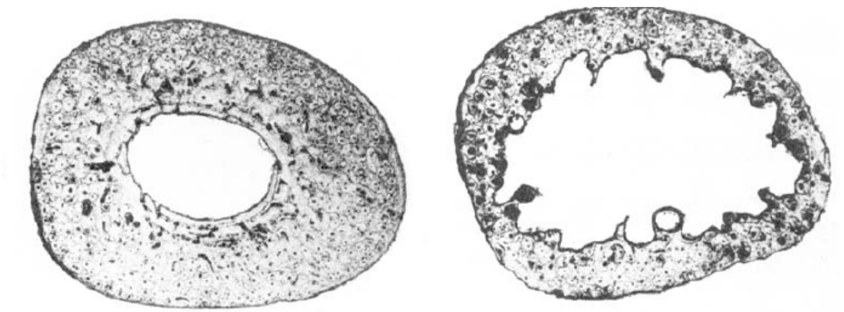


Biochemical Signal

Cellular Responses on Various Time Scales

Cells on periodically stretched substrate



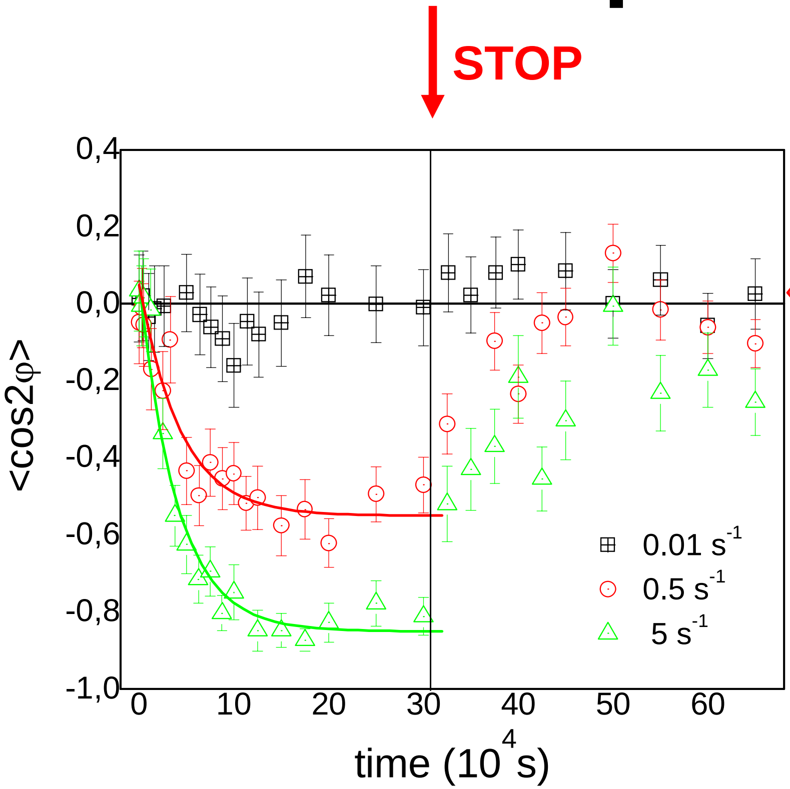


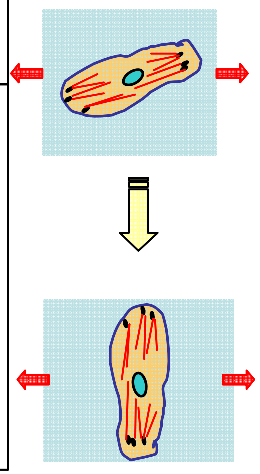
Uhthoff H.K. J. *Bone Joint Surg. Br.* 1978

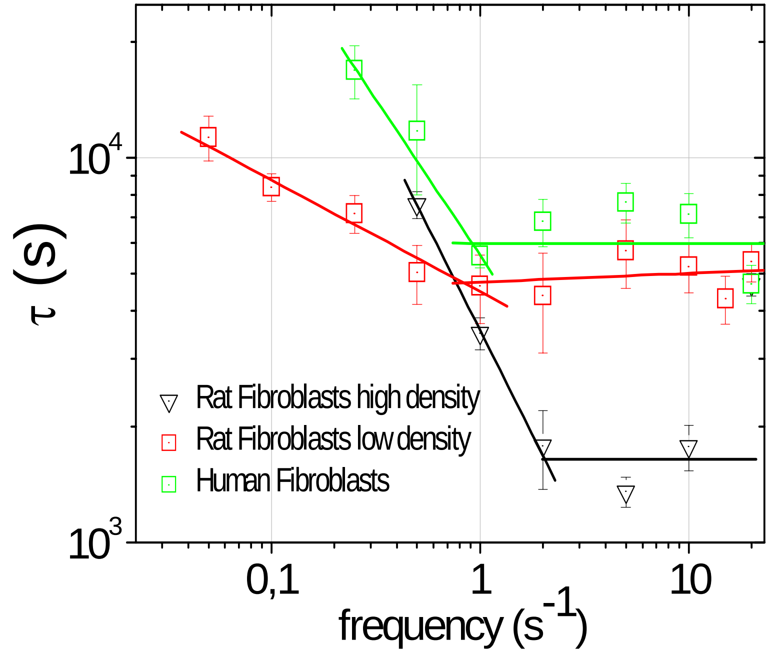
Dr. Ralf Kemkemer, Simon Jungbauer

MECHANOSENSITIVITY

Biphasic Time Response



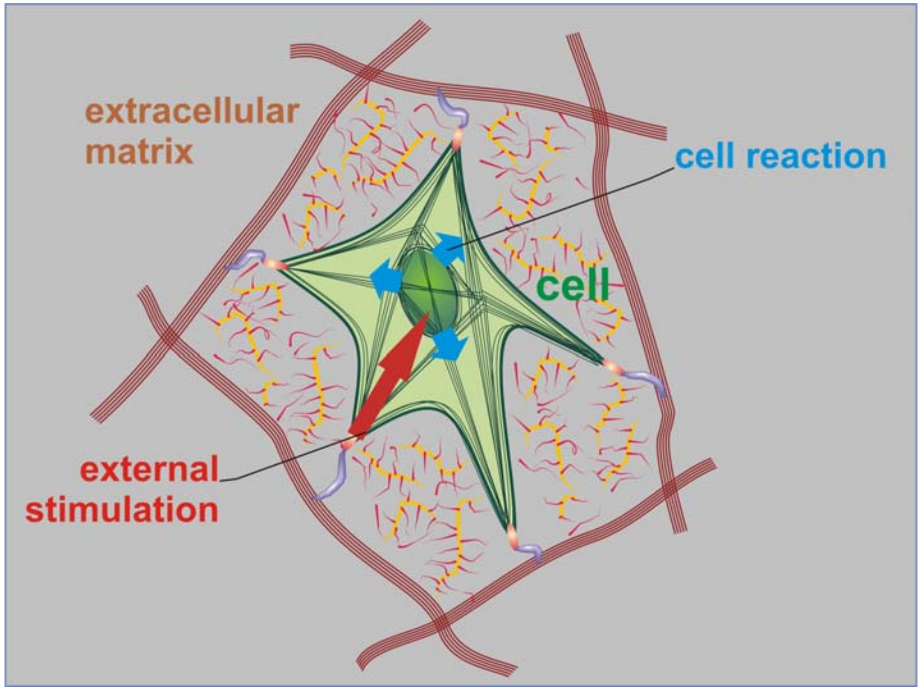


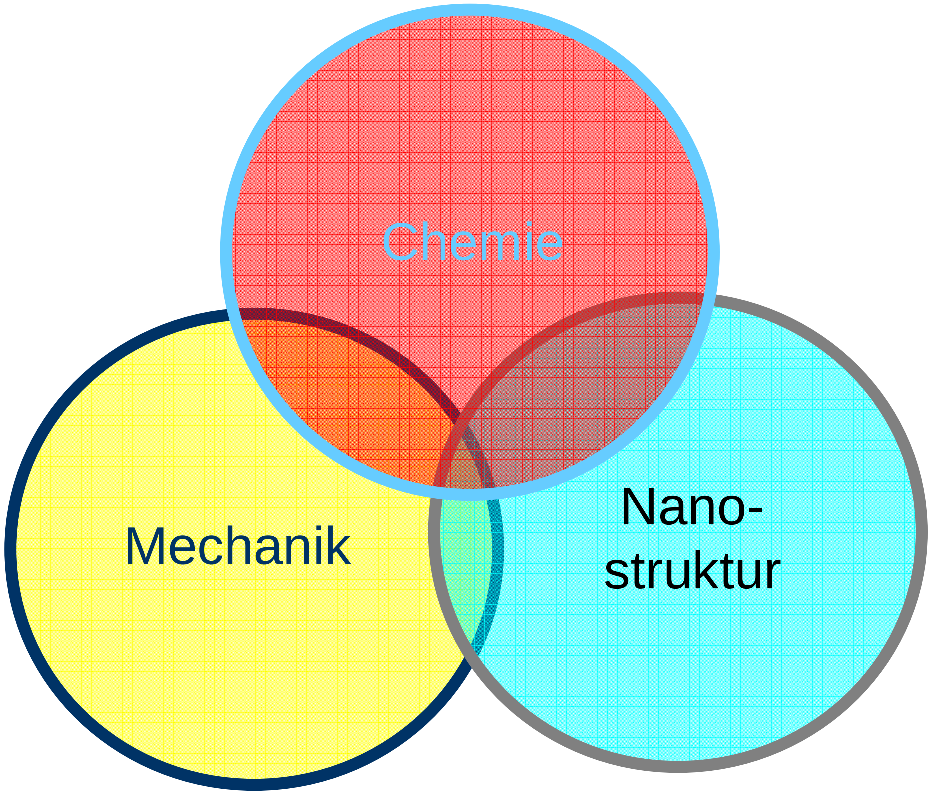


- Cell Type Dependent
- Density Dependent

Dr. Ralf Kemkemer, Simon Jungbauer

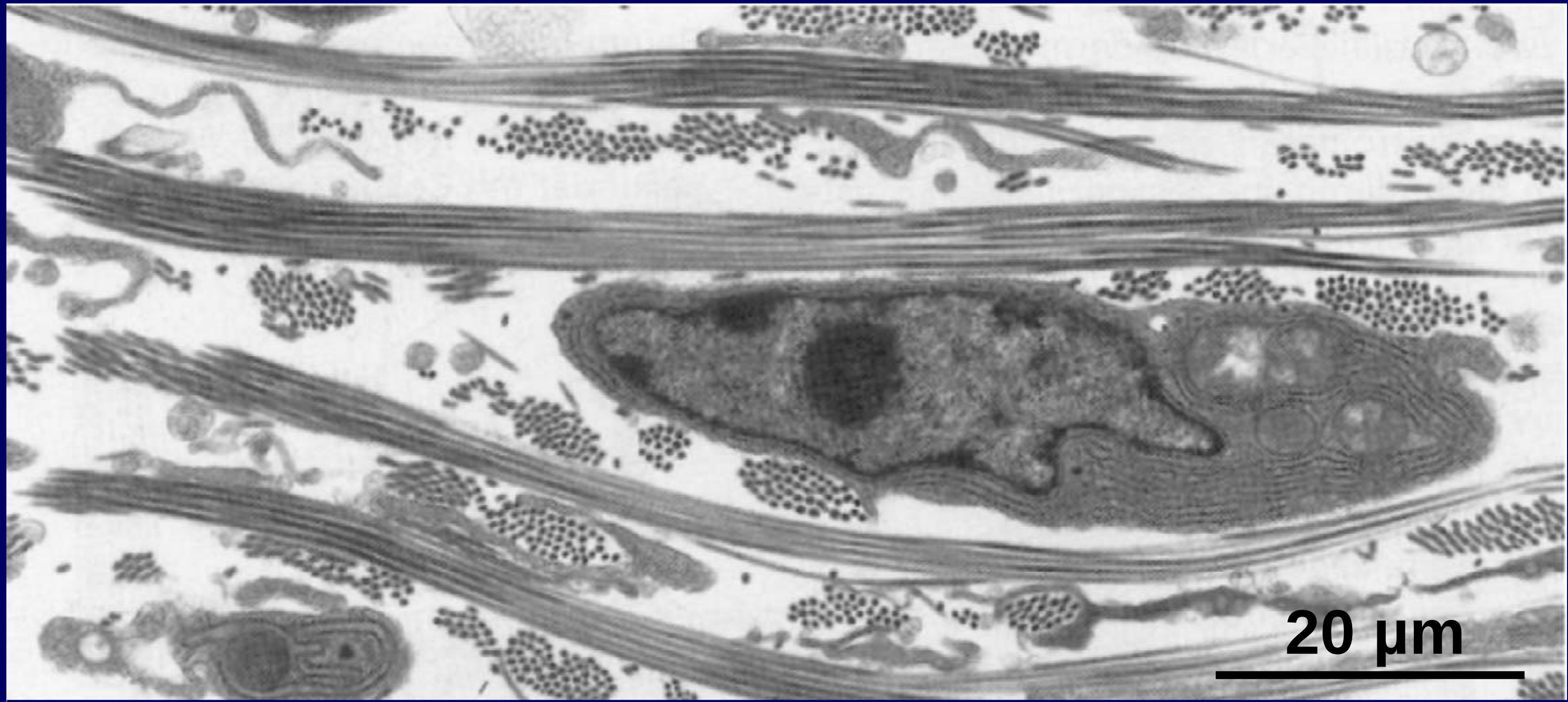
High-Throughput Screens for Identifying Cell Specific Material Parameters





PART II – Micrometer Length Scale

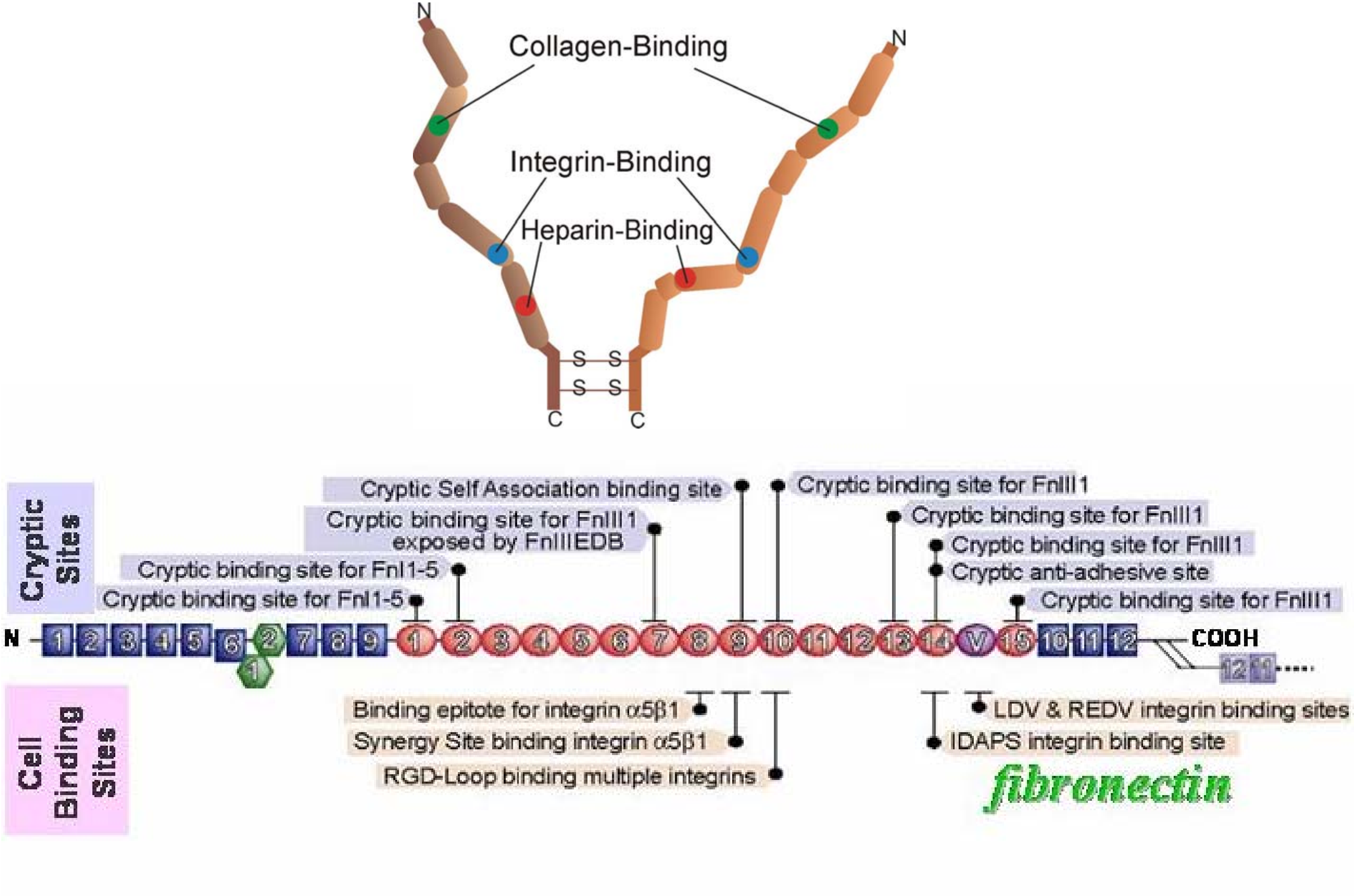
SKIN



Mimicking Protein Filament Networks *in vitro:*

Initiative of the VolkswagenStiftung
Viola Vogel (ETHZ), Mike Sheetz (CU),
Benny Geiger (WIS), Joachim Spatz (MPI-MF)

Mechanotunable Fibronectin with Switchable Biological Activities

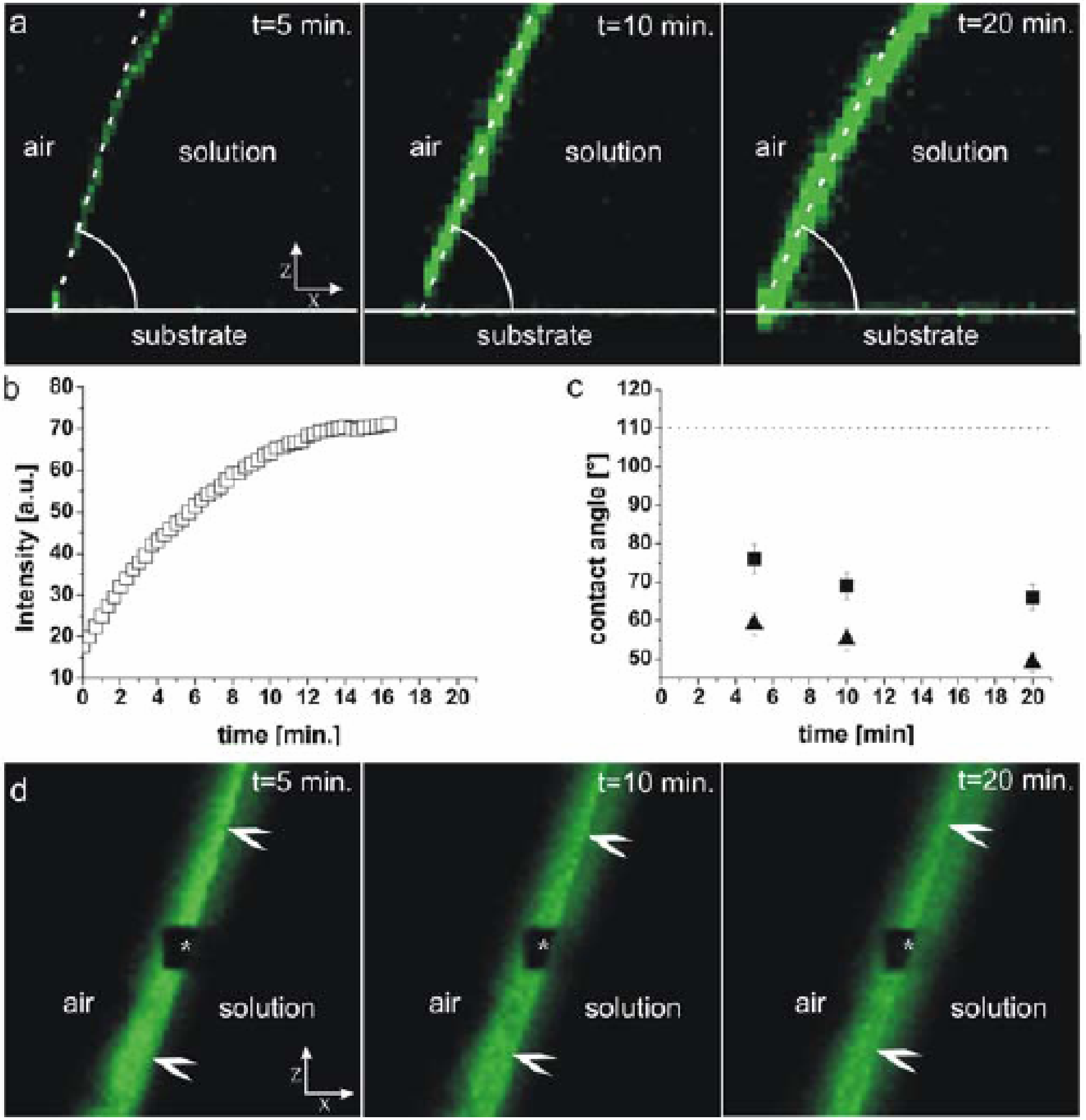


Viola Vogel (ETHZ) in, e.g. PNAS 2001

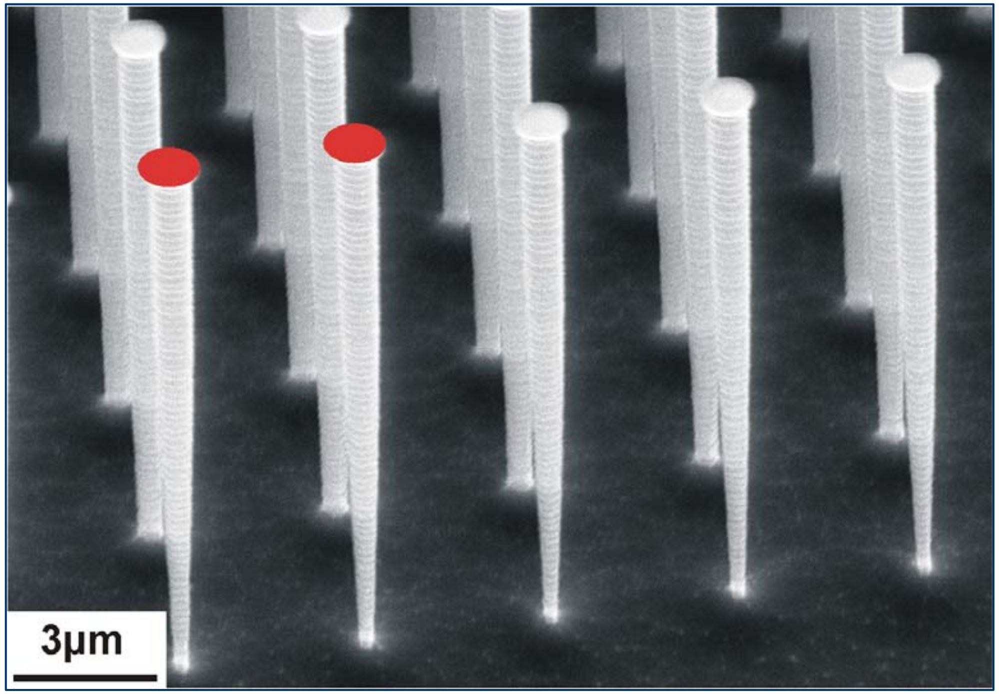
Harold Erickson (Duke University) in, e.g. J. Muscle Res. Cell Mobility 2002

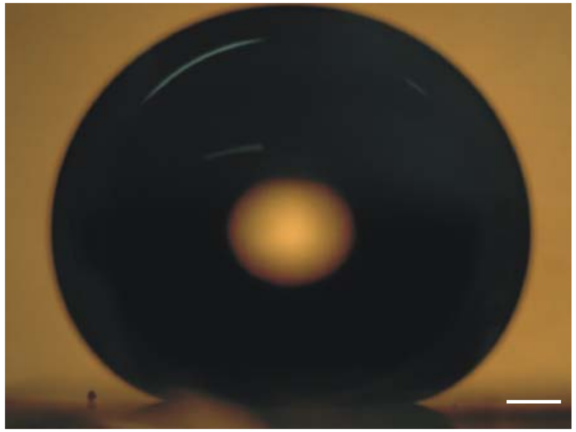
How to Form Fibronectin Matrix *in vitro*

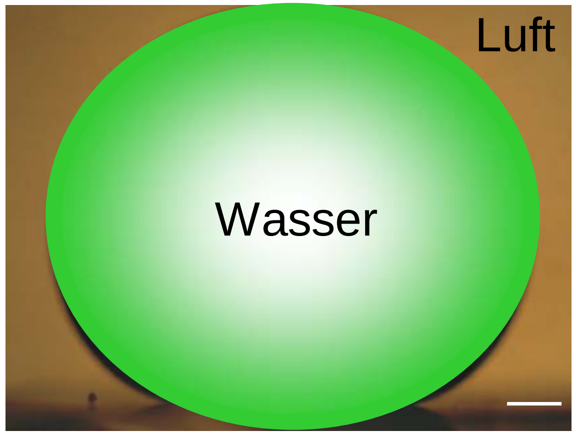
Aggregation of Fibronectin or Collagene at the Air-Water-Interface

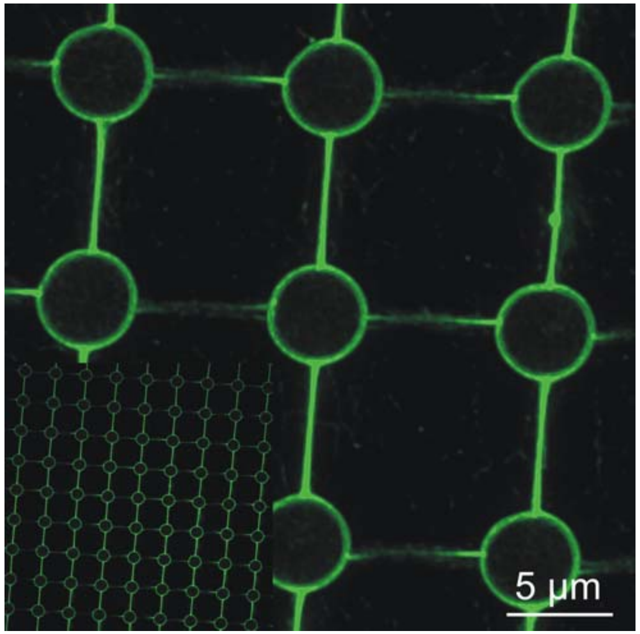


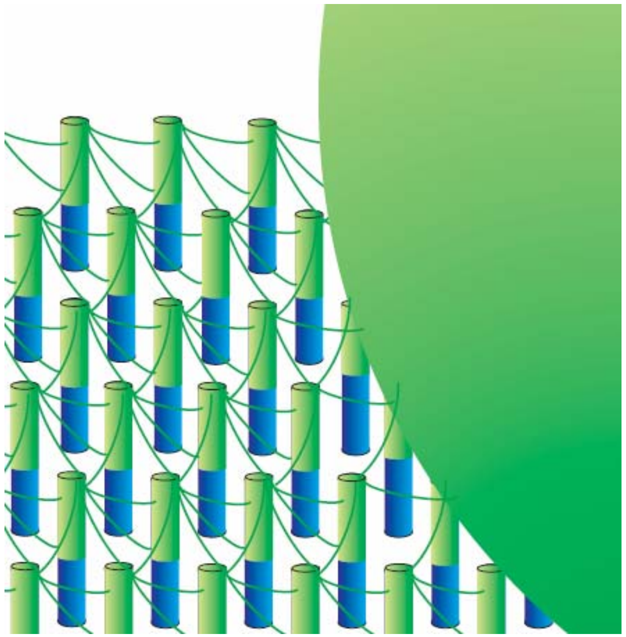
The Extracellular Matrix (FN) – *in vitro*

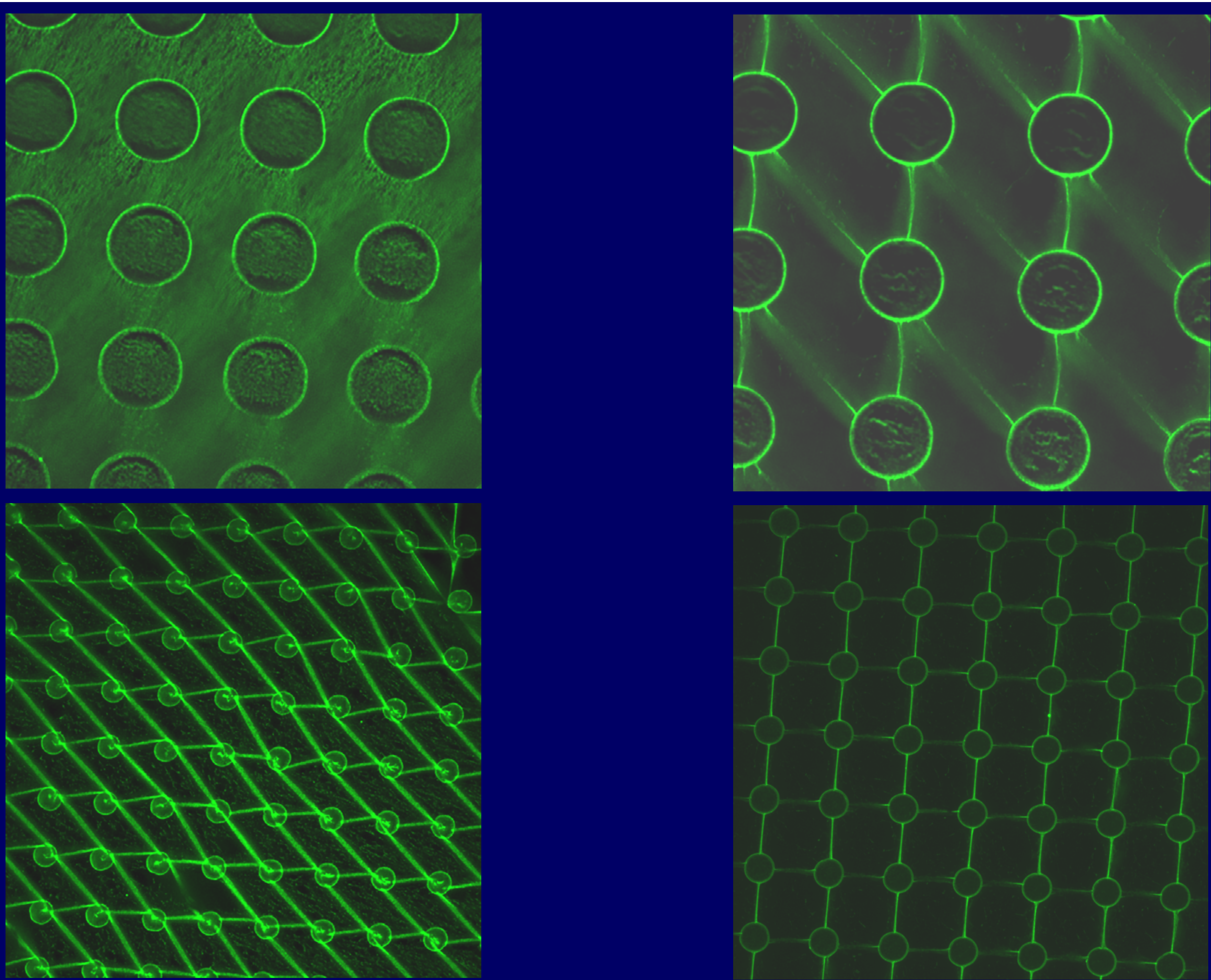


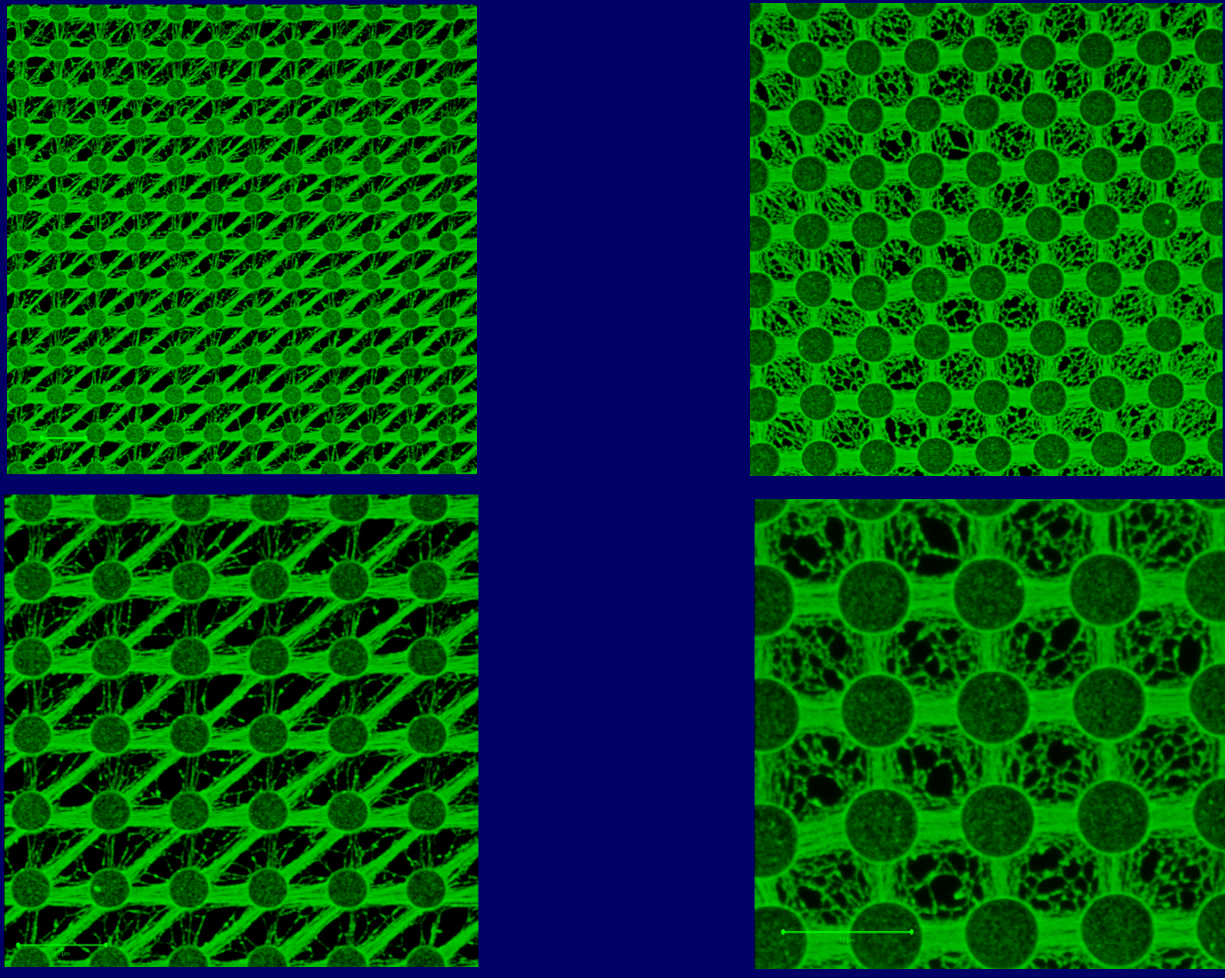




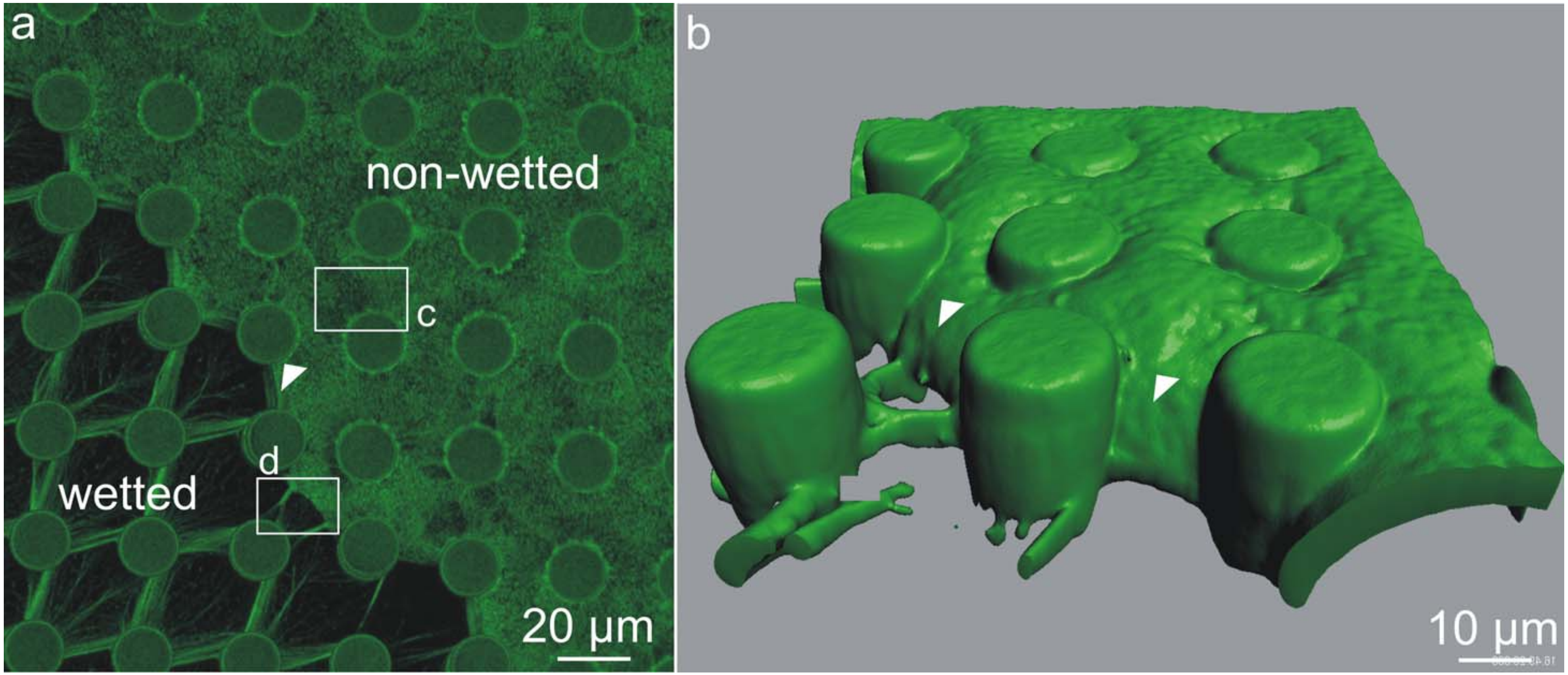




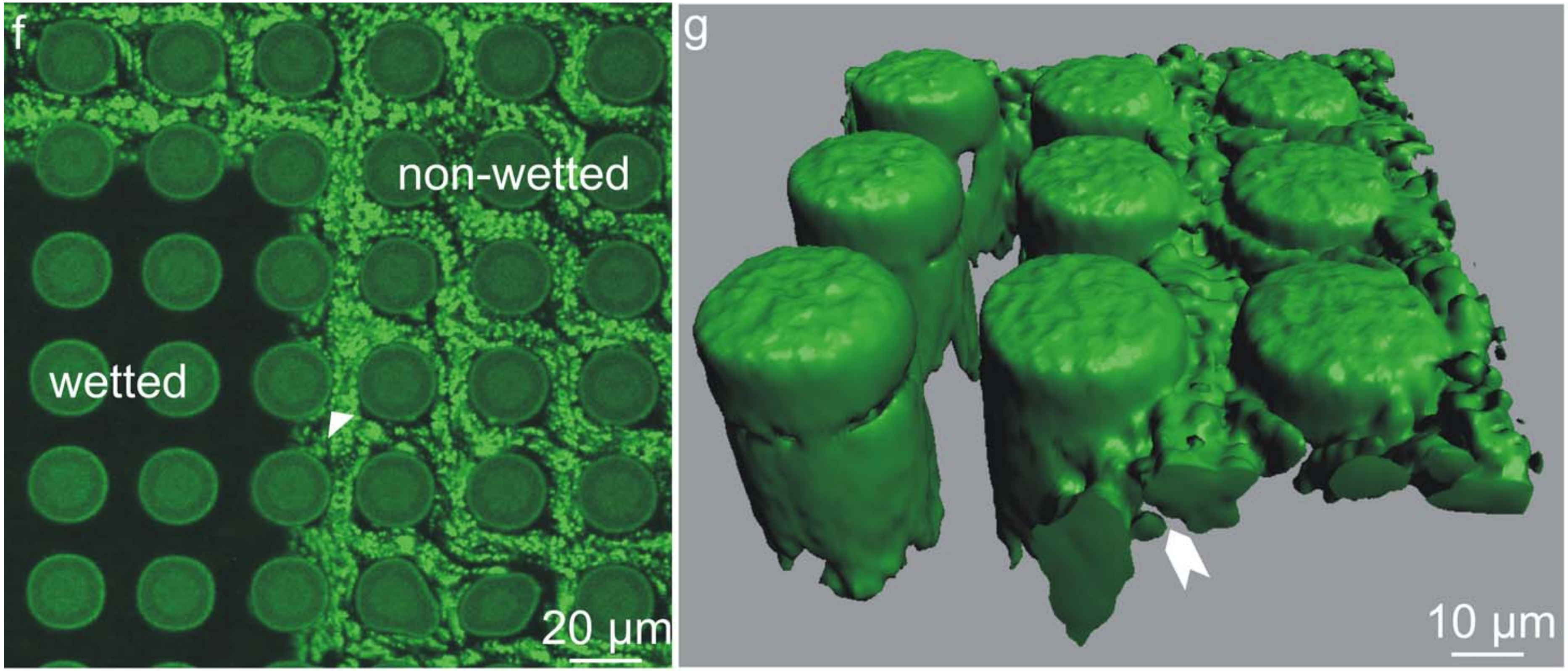




Suspended Fibronectin Films with different Structure

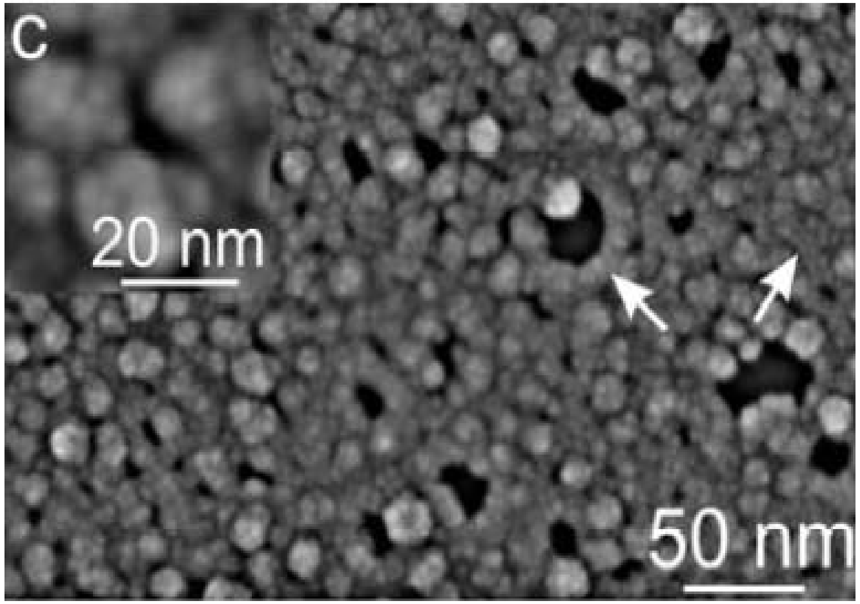


Suspended BSA Films with different Structure

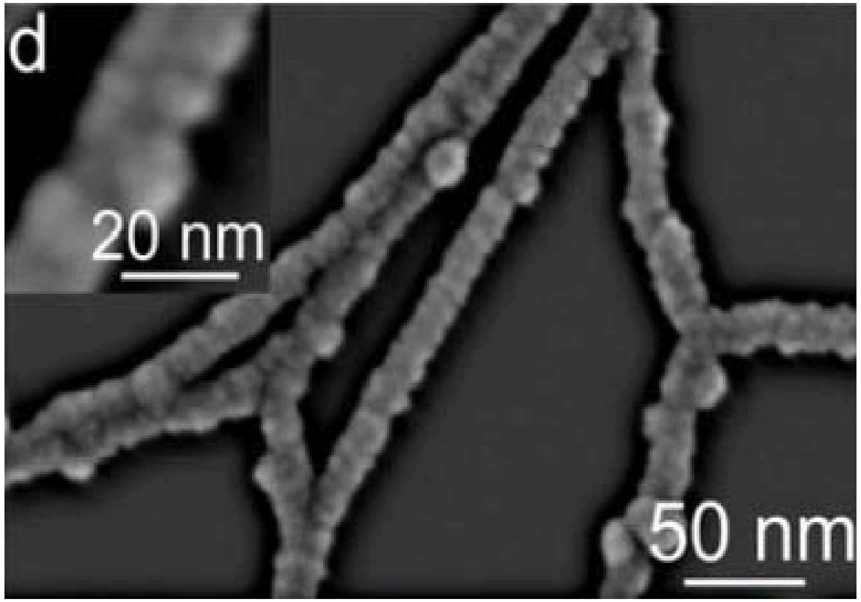


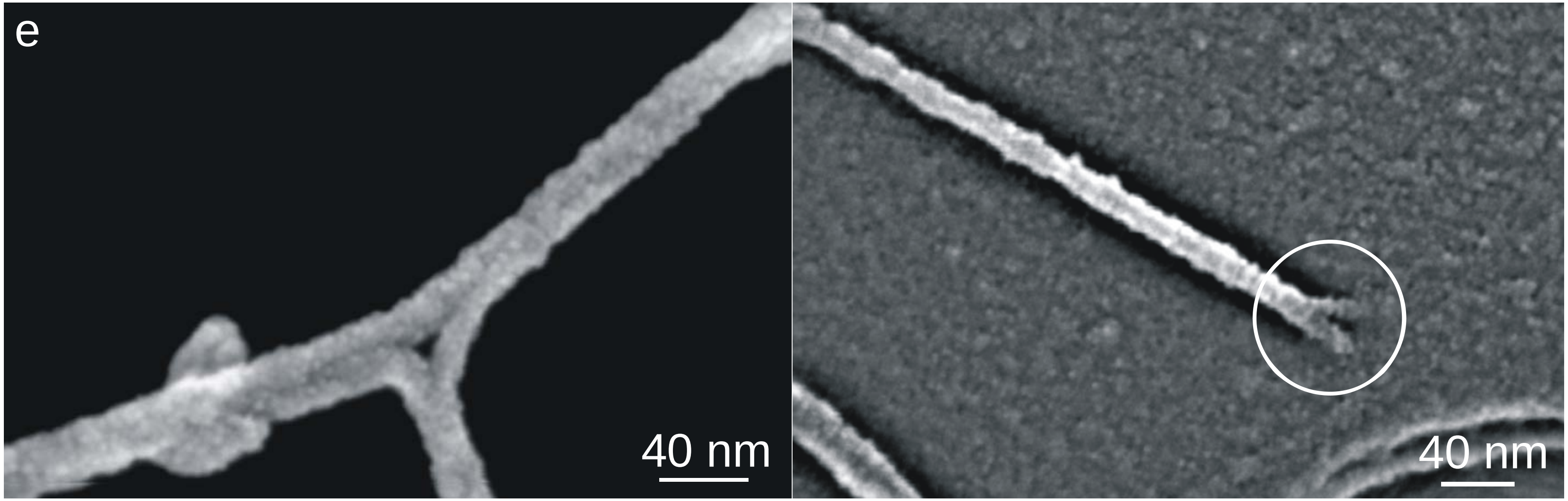
Scanning Electron Microscopy Displays the Formation of Fibronectin Fibers

Suspended Air-Liquid Interface

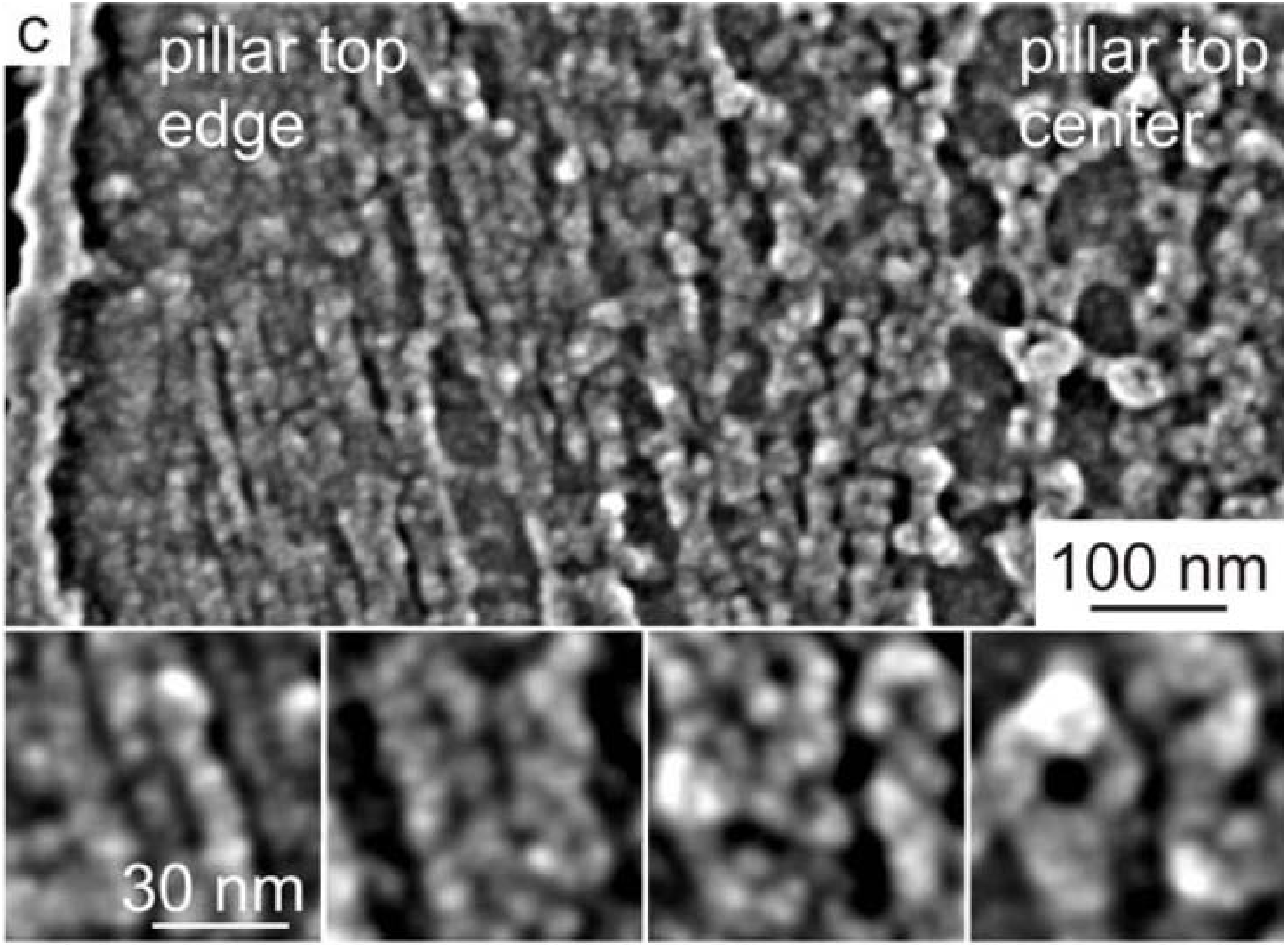


Fibres Bridging Pillar Plateaus



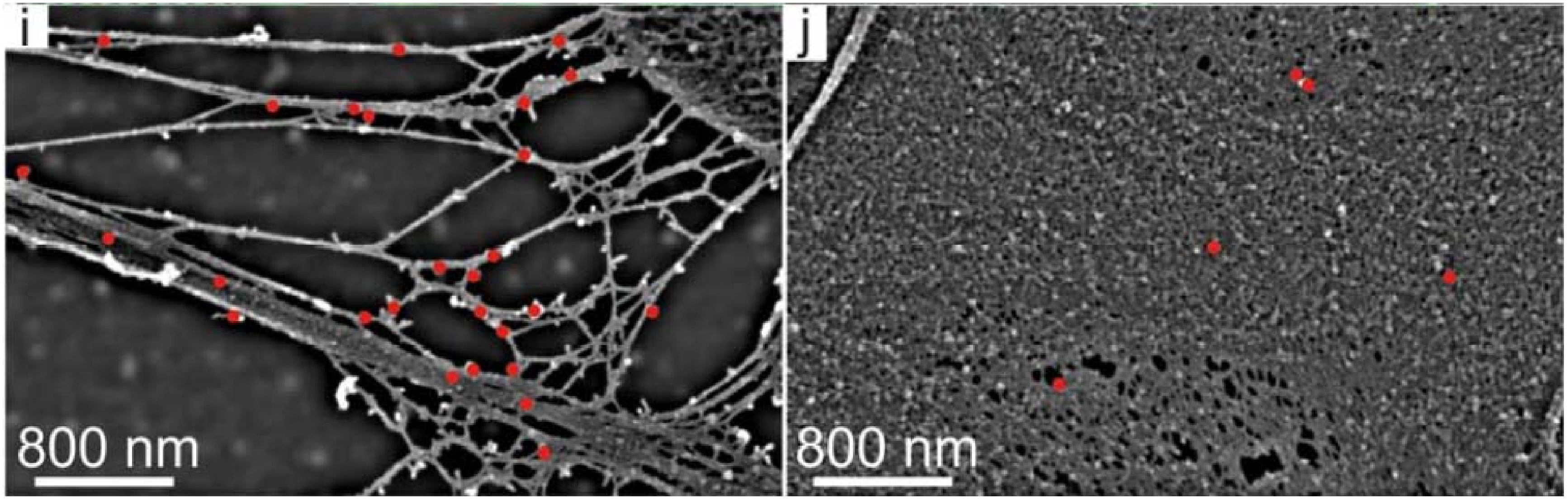

e
40 nm
40 nm

Scanning Electron Microscopy Displays the Formation of Fibronectin Fibers

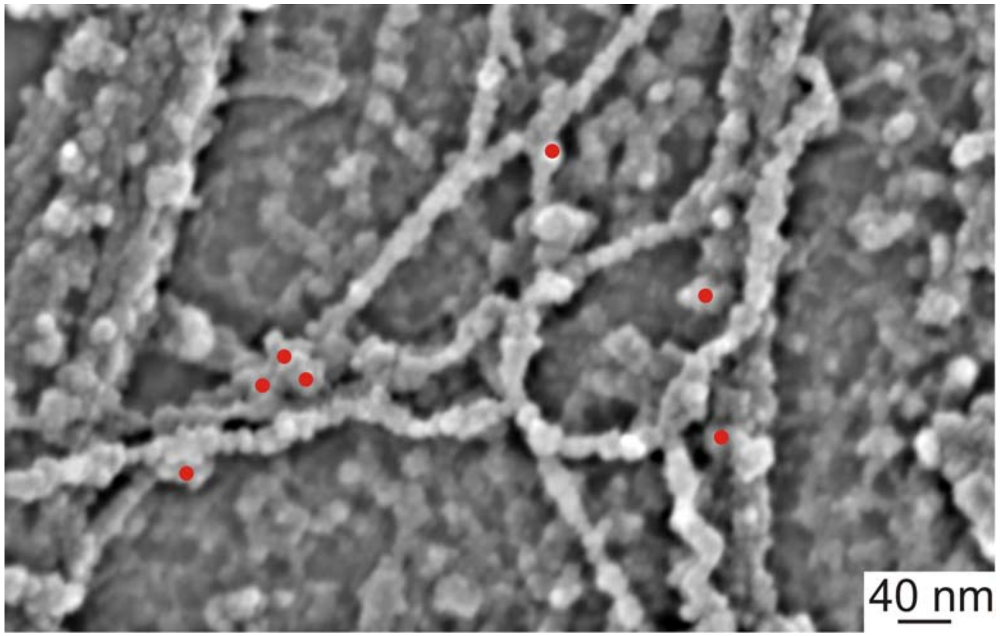


Different Fibronectin Fibre Conformation – Different Biological Activity

in vitro

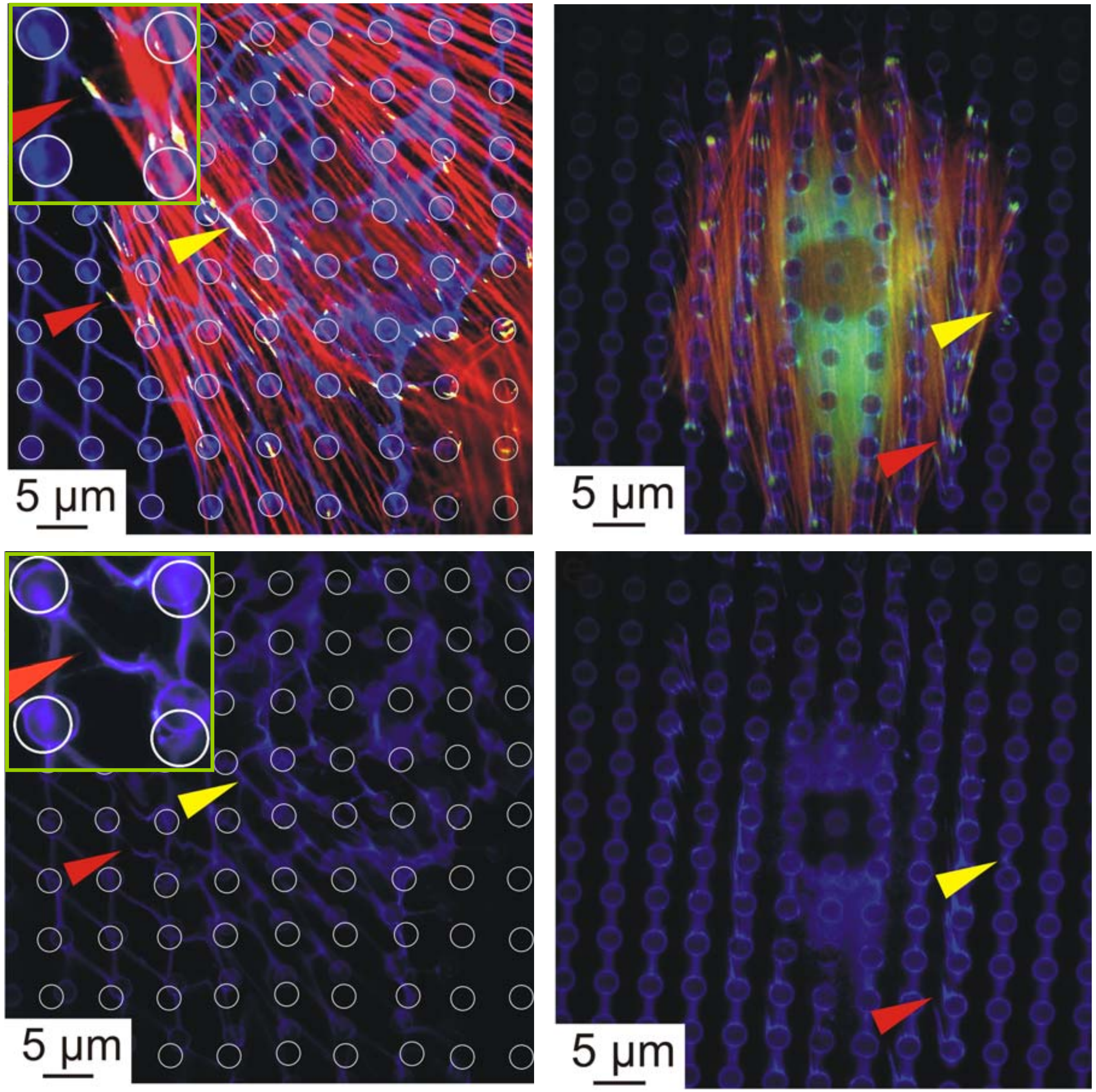


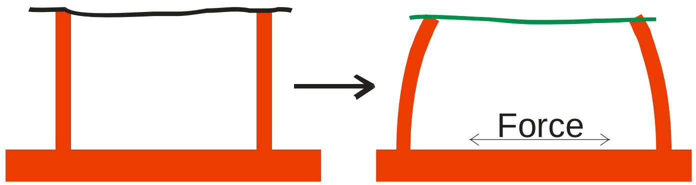
in vivo
Fibers produced
by cells



THE EXTRACELLULAR MATRIX

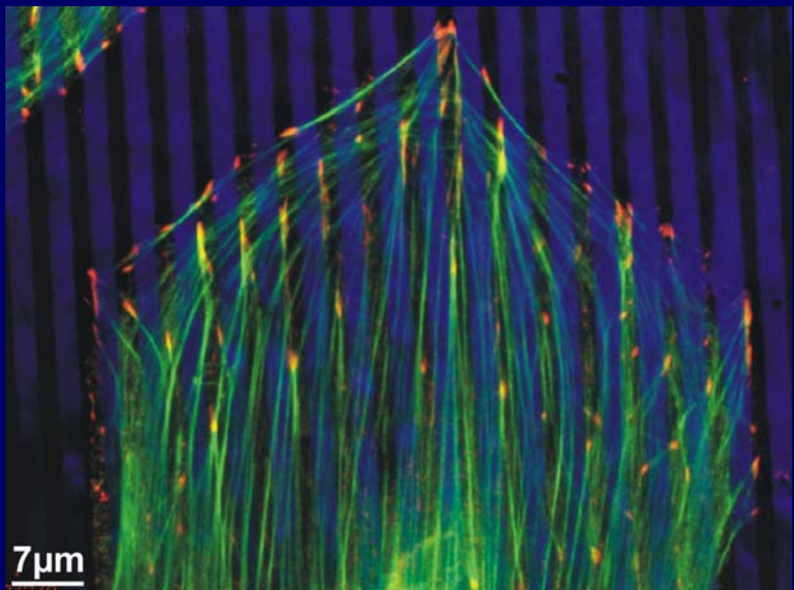
Mechanotunable Protein Networks with Switchable Biological Activities - FIBRONECTIN

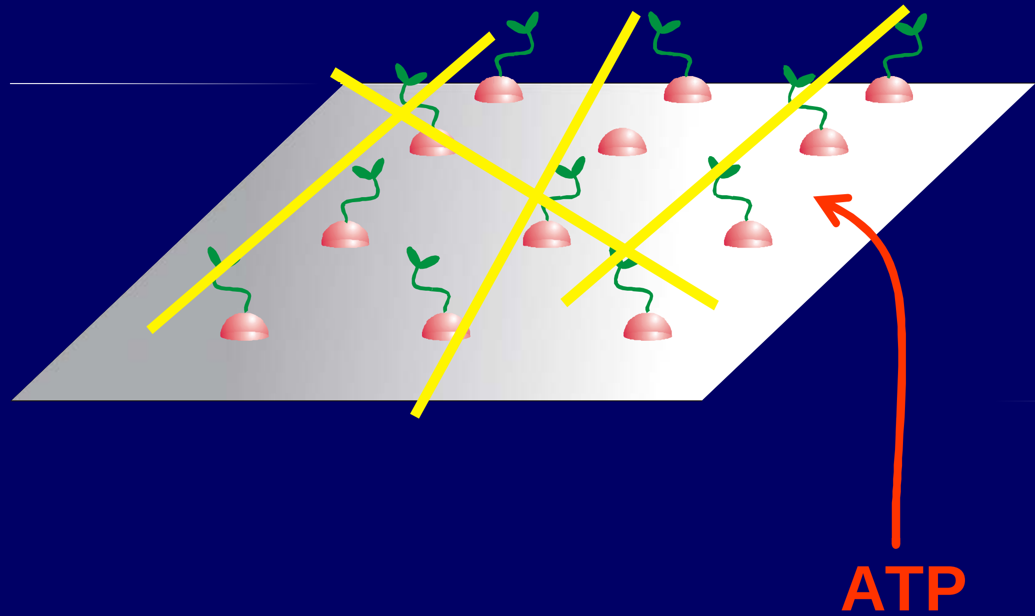


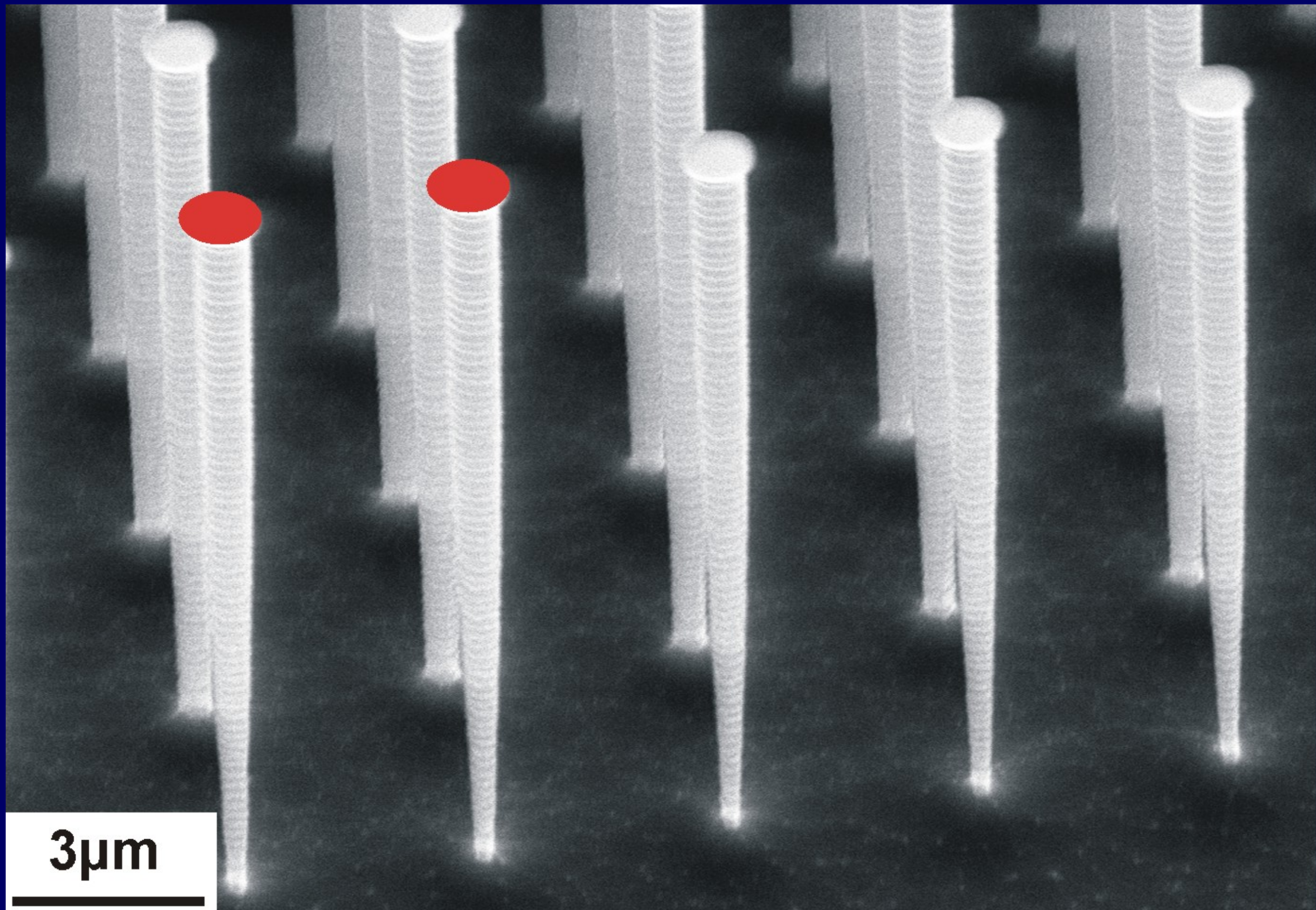


Mimicking the Actin-Filament System

cellular actin filament network ⟷ synthetic actin filament network

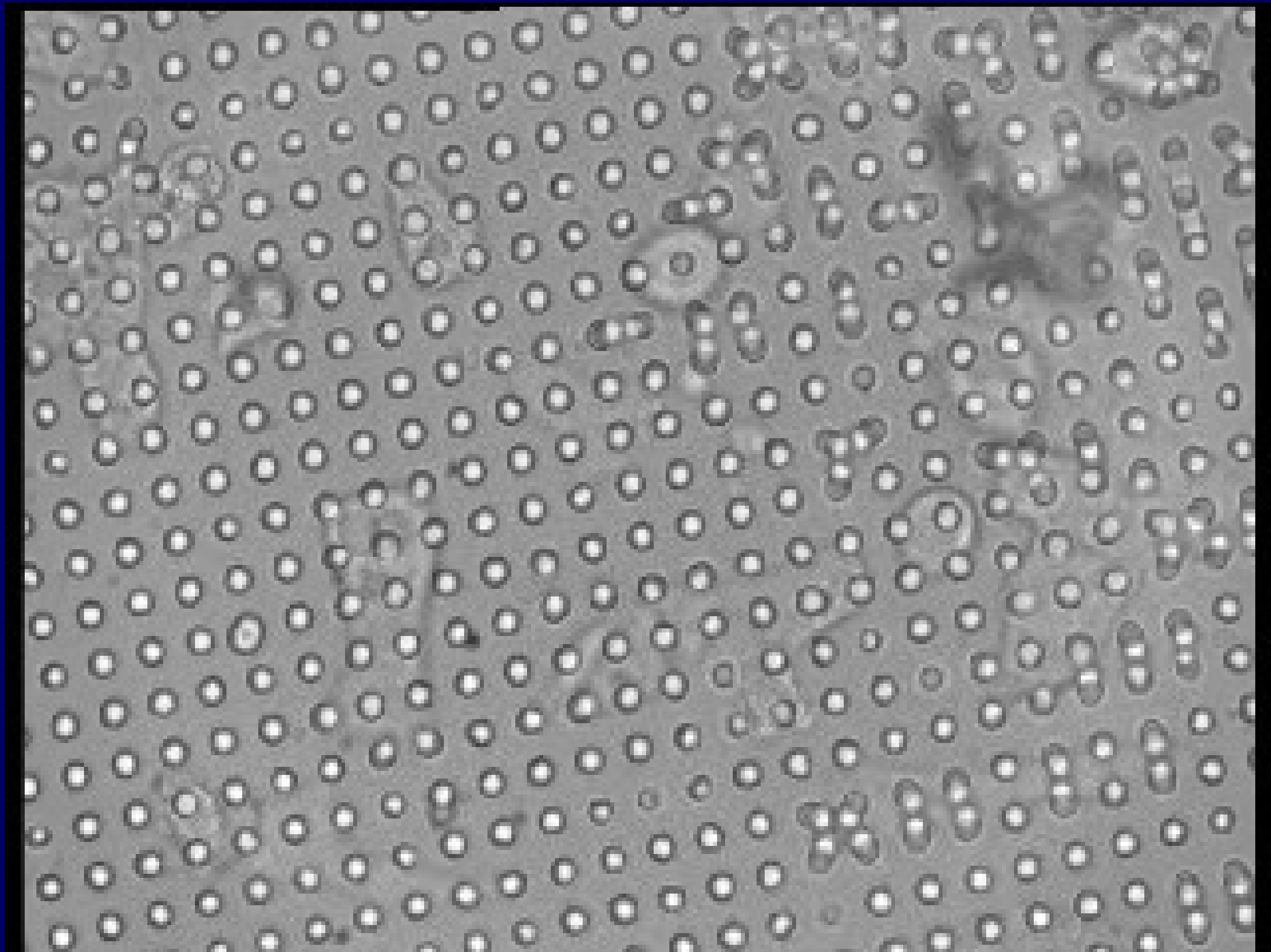




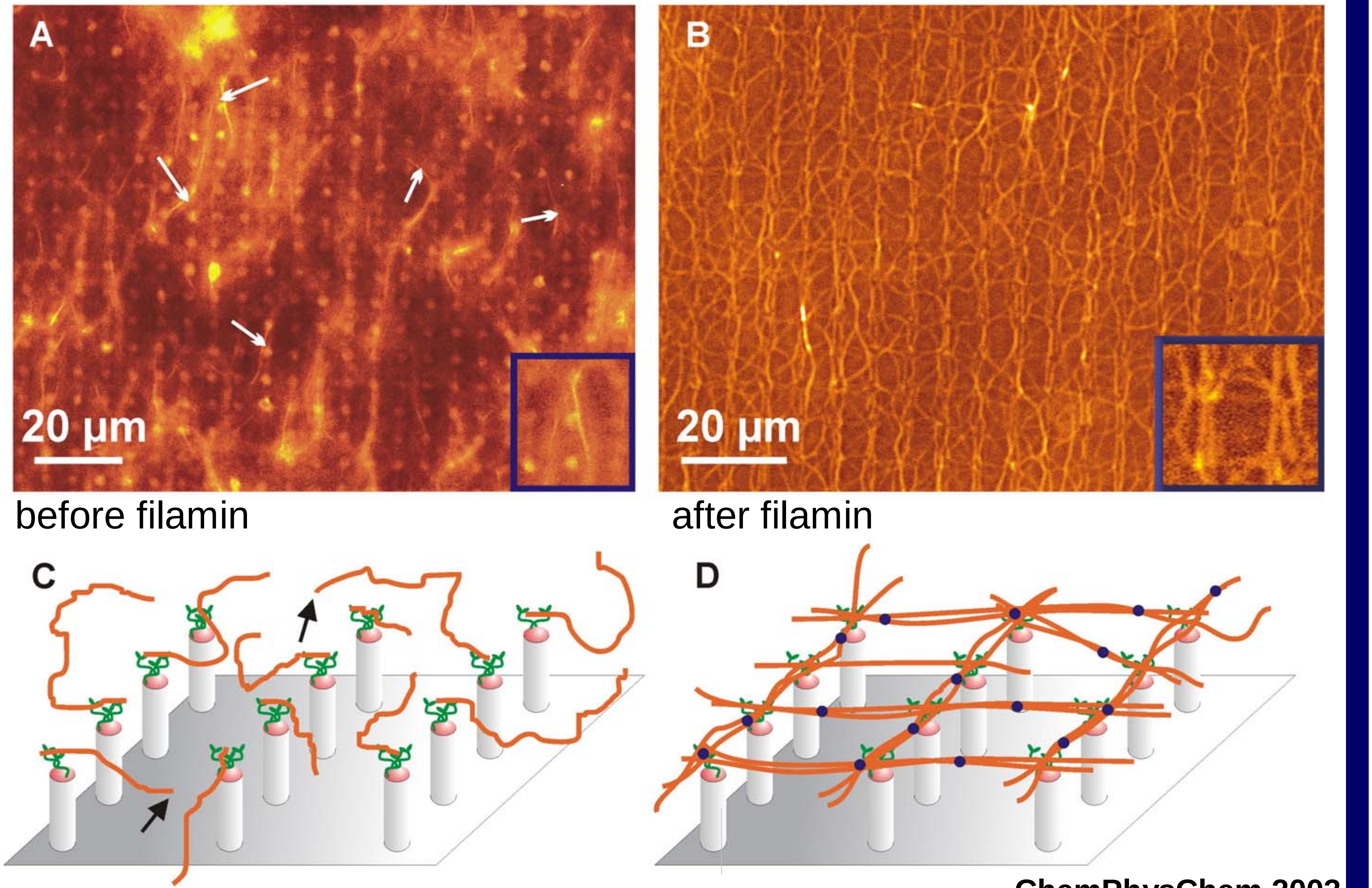


Si(100) or Polymer (PDMS)

Chicken Heart Cells

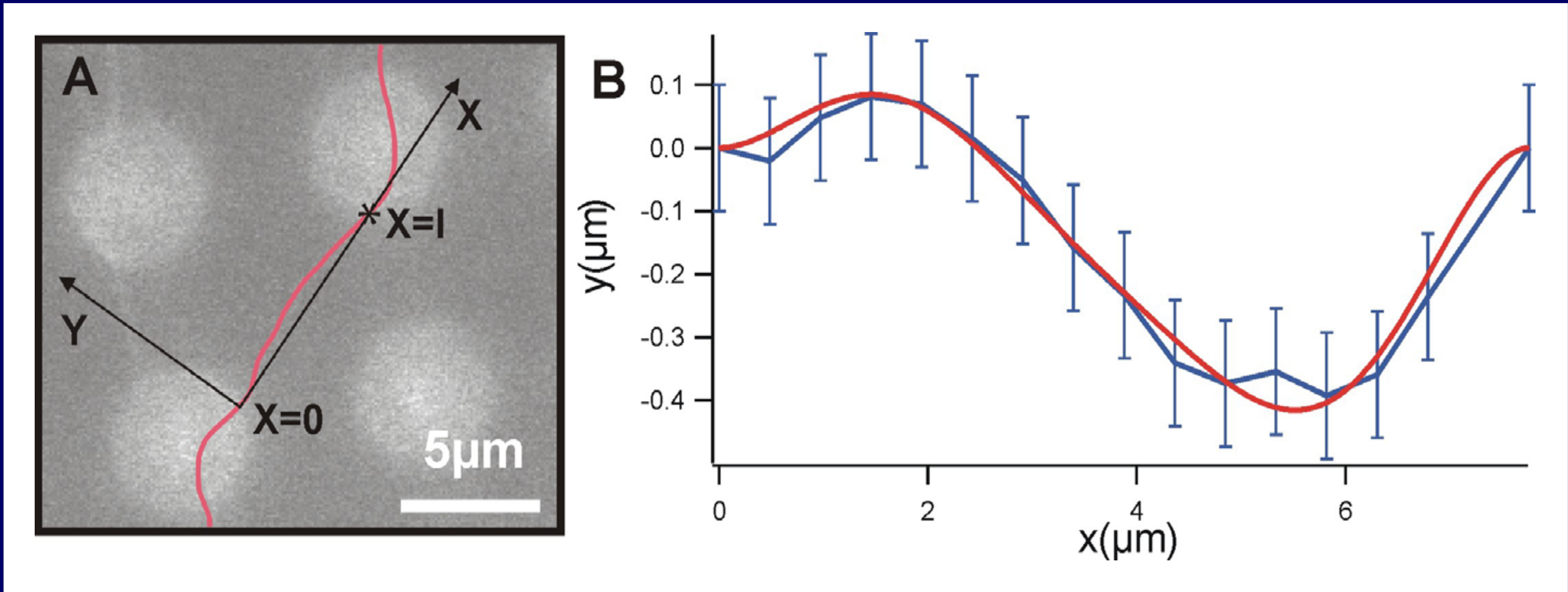


Biomimetics of the Actin Cortex



ChemPhysChem 2003

Filament Bending Modulus and Persistance Length of F-Actin in a 2-Dim Network



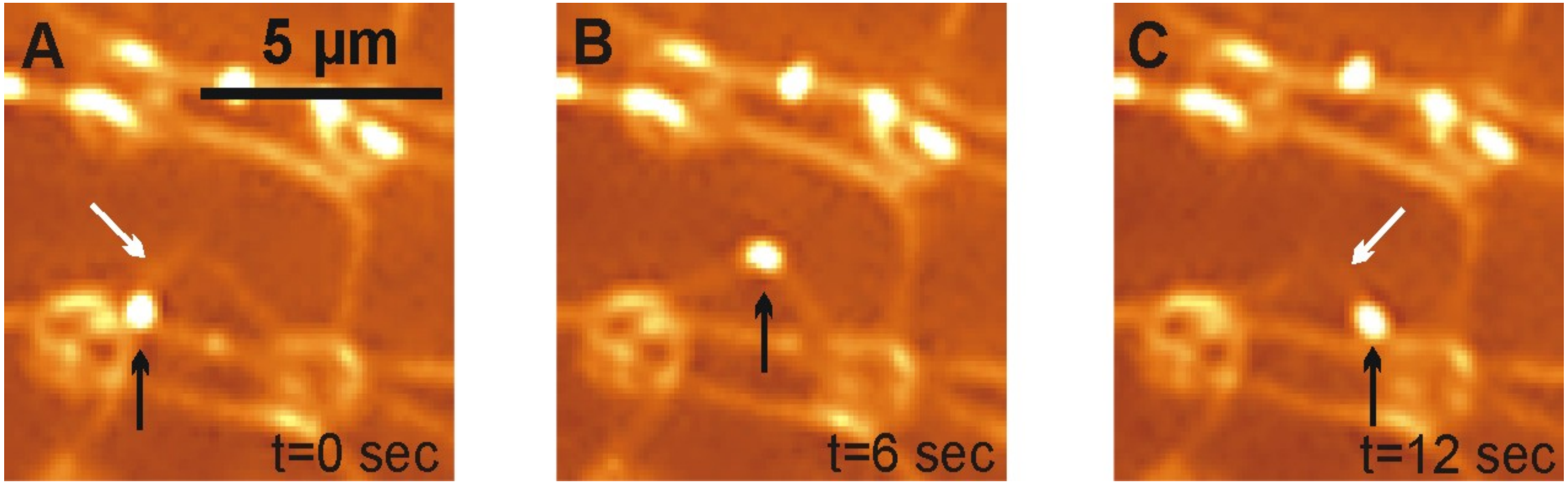
$$E_b = \frac{1}{2}\kappa \cdot \int_0^l \left(\frac{\partial^2 y}{\partial x^2}\right)^2 dx$$

For each mode this equals the thermal energy *kT*/2

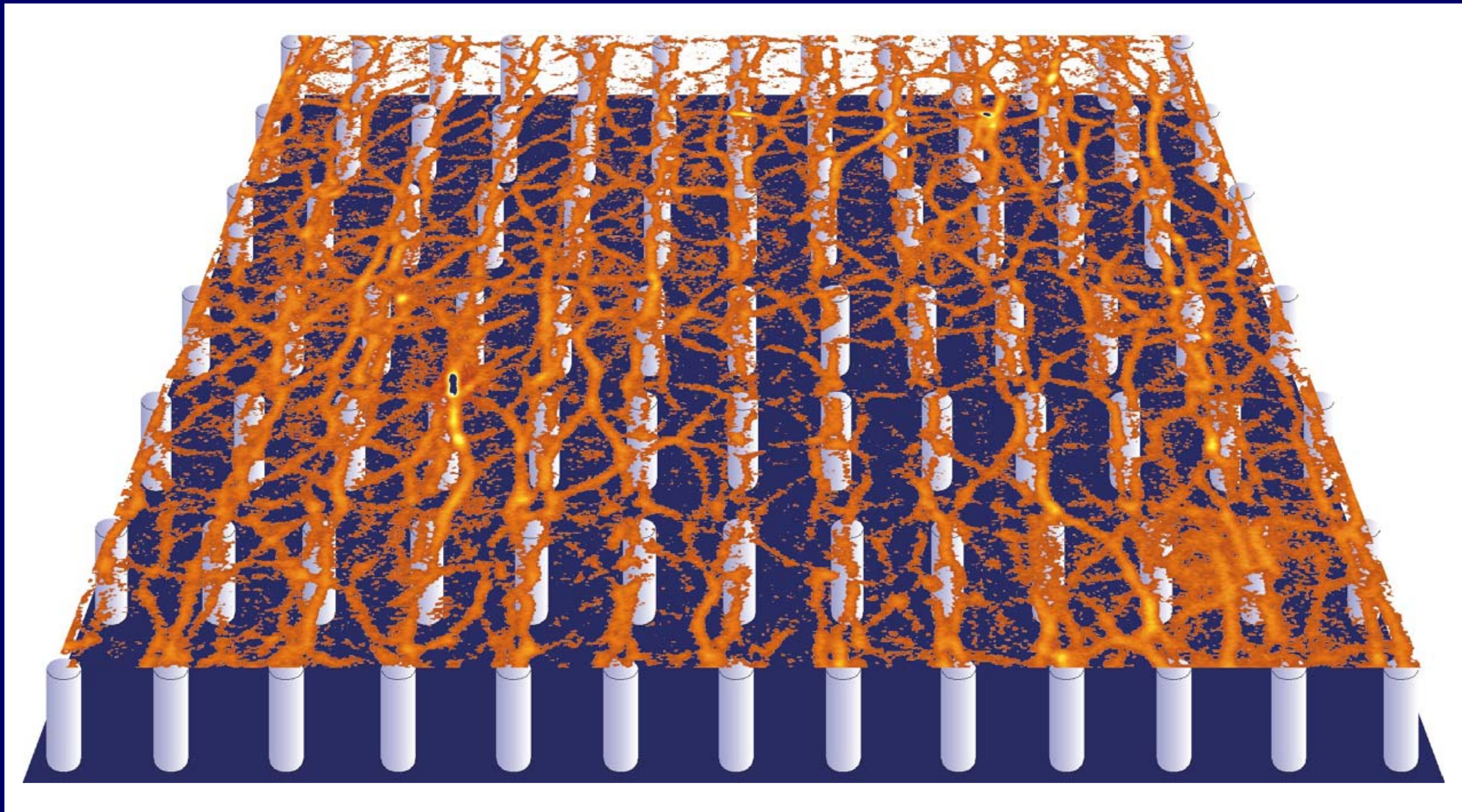
Persistance length $l_p = \kappa / kT = 15.3$ µm ($\kappa = 6.3 \ 10^{-26}$ Nm^2)

Transport in 2-dim. Actin Networks

Trajectory of Myosin V coated bead
V ~ 330 ± 50 nm/sec

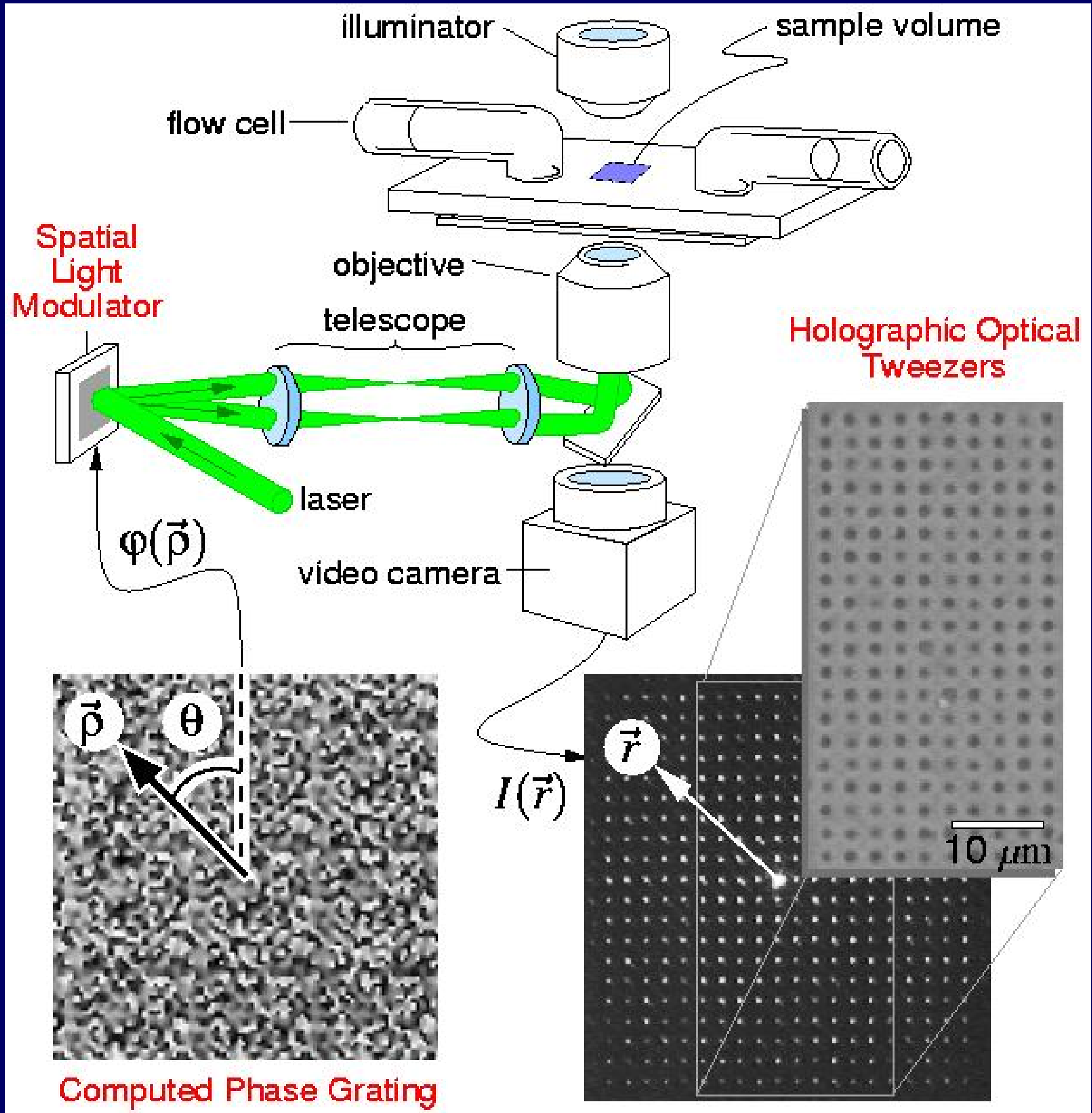


➢ **Active Diffusion**

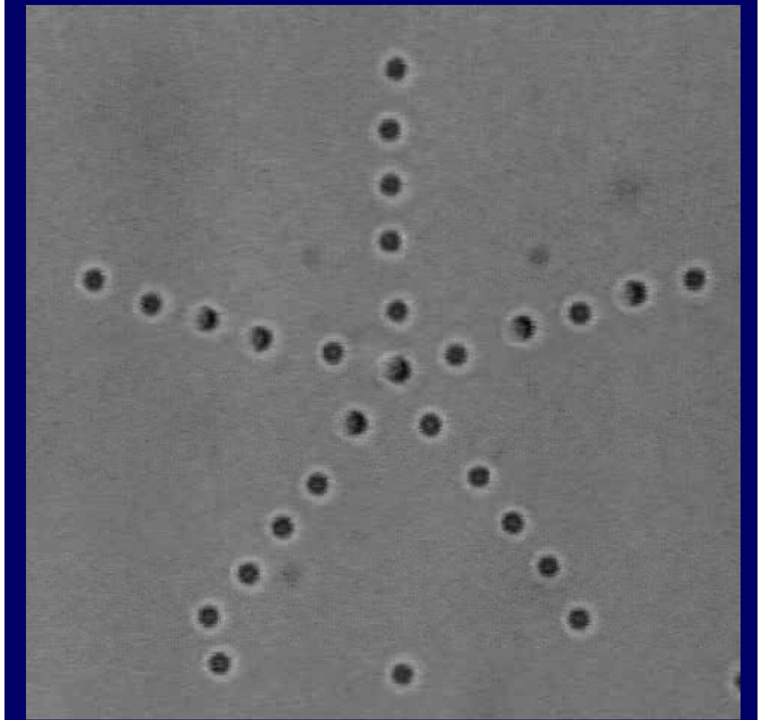


ΔForce > 100 pN

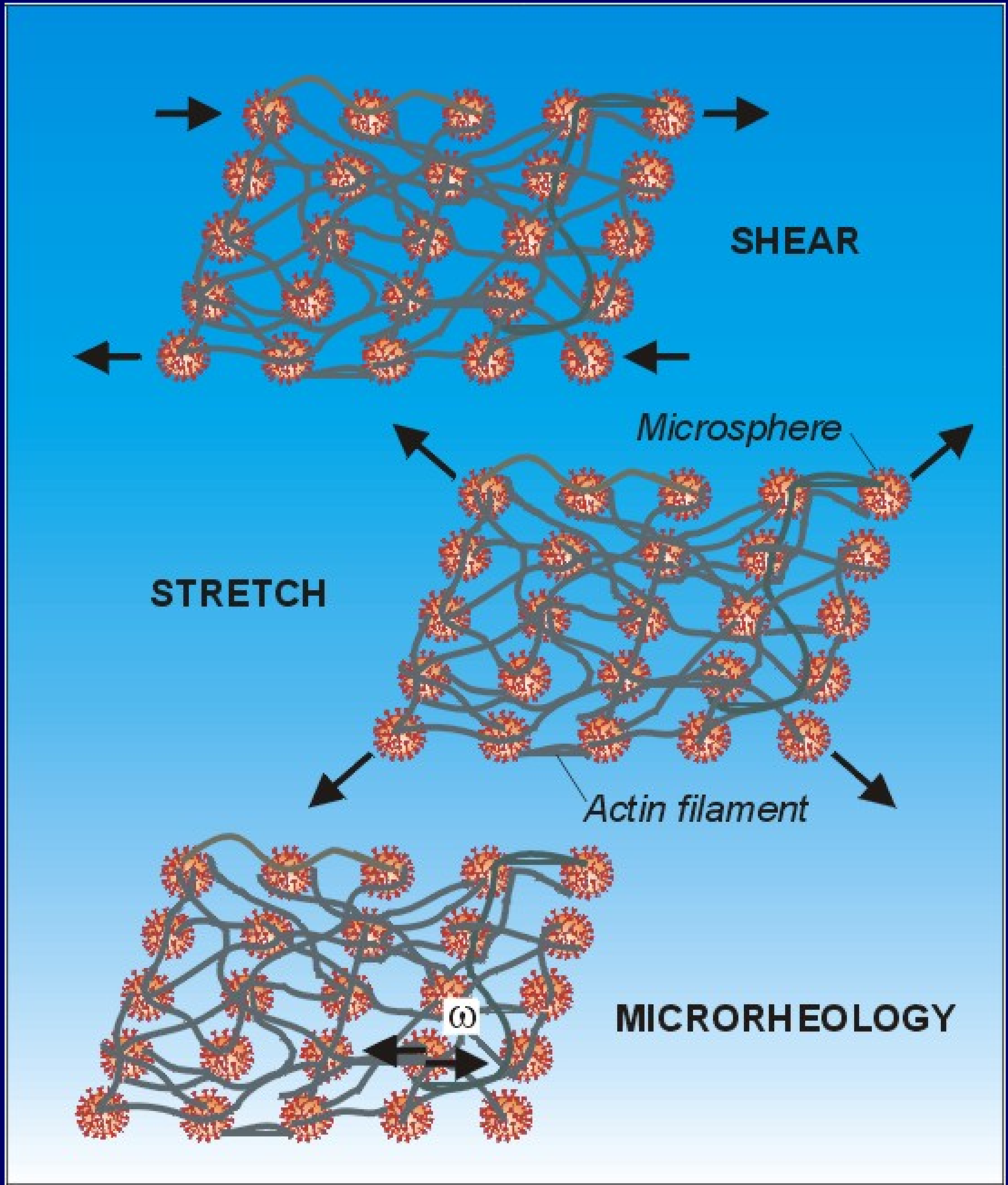
Dynamic Holographic Tweezers

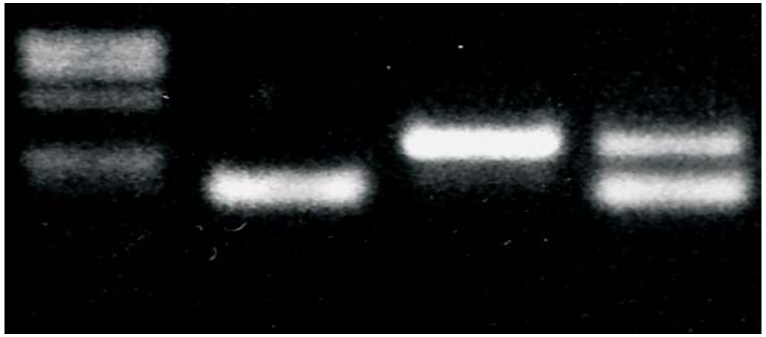


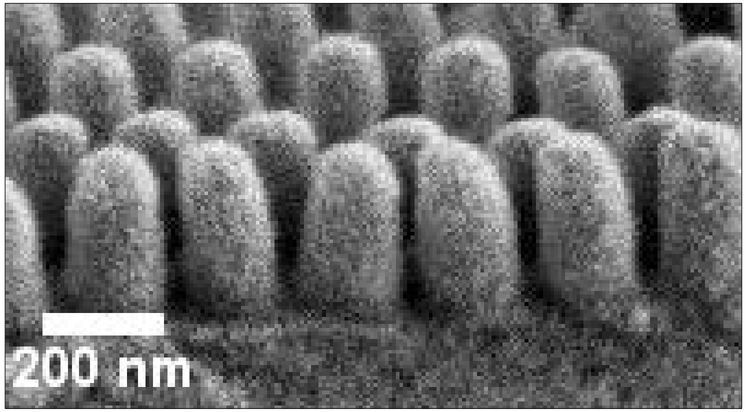
Many hands in an optical microscope



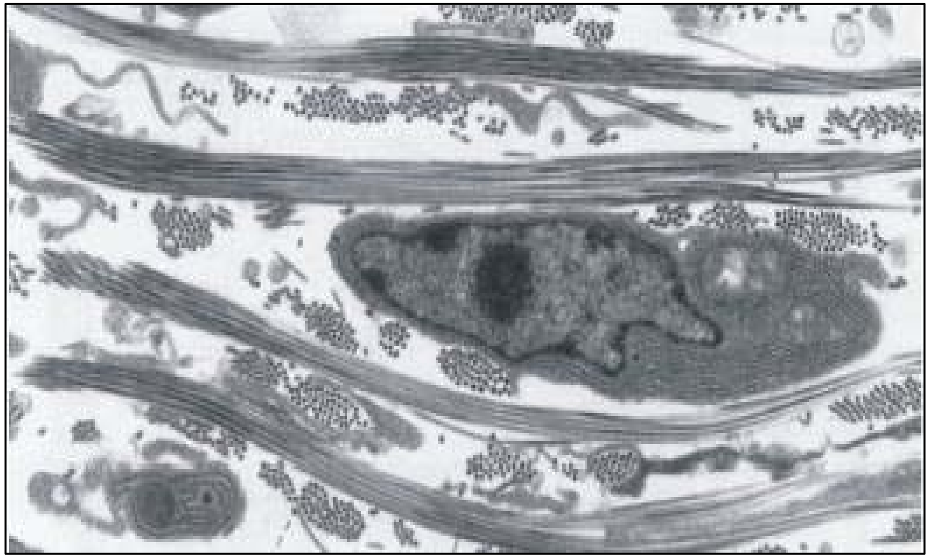
J. E. Curtis et al. Opt. Comm. **207**, 169 (2002).


SHEAR
Microsphere
STRETCH
Actin filament
ω
MICRORHEOLOGY





Bio Research ←→ Materials Research



Materials Research
&
Biosystem Research

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