Karlsruhe Institute of Technology

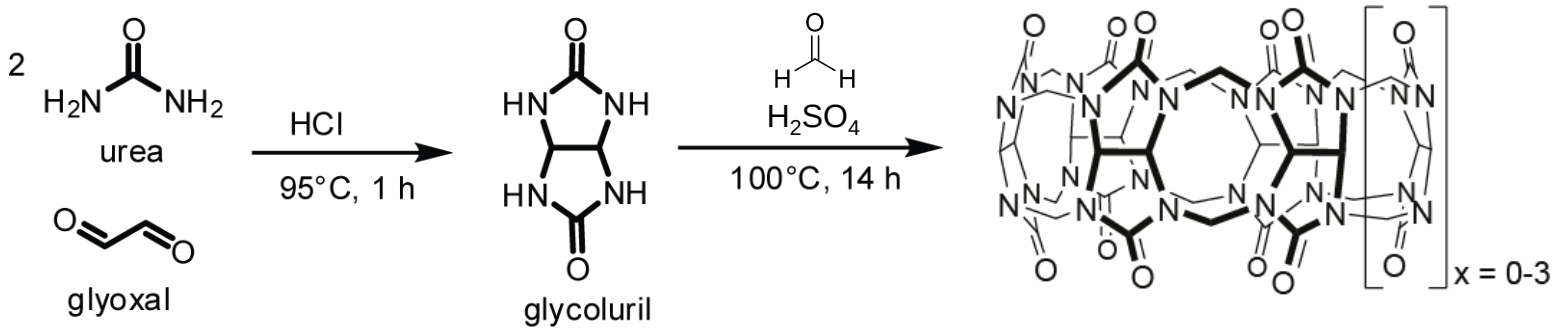
CSAC 2021

Cucurbit[*n*]uril-Immobilized Sensor Arrays for Indicator-Displacement Assays of Small Bioactive Metabolites

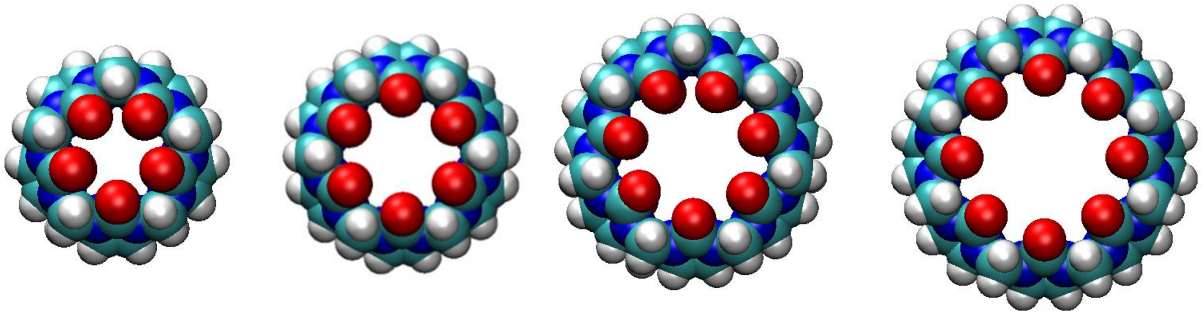
Chunting Zhong, 01-15 July 2021
Supervisors: Frank Biedermann and Michael Hirtz

Institute of Nanotechnology (INT)

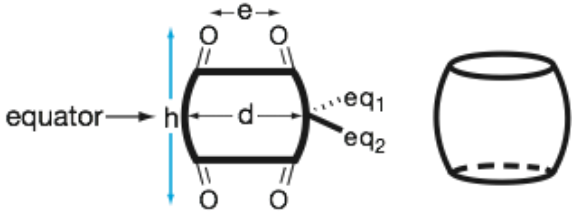
Cucurbit[*n*]urils – Barrel-shaped Hosts



Most prominent CB[*n*] homologues:



CB5 CB6 CB7 CB8



CB[n]	5	6	7	8
cavity, d (Å)	4.4	5.8	7.3	8.8
entry, e (Å)	2.4	3.9	5.4	6.9
height, h (Å)	9.1	9.1	9.1	9.1

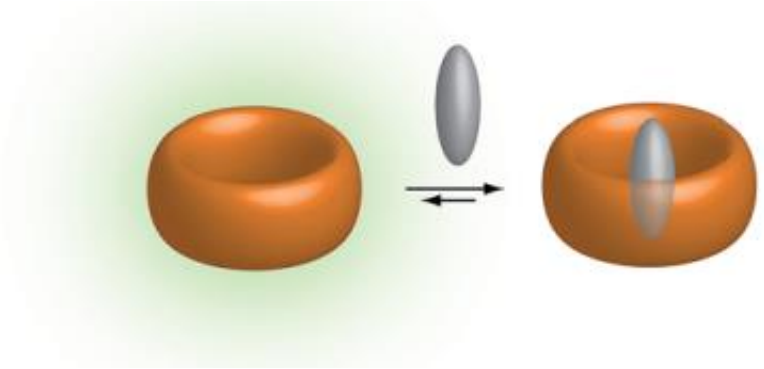

Kimoon Kim


Lyle Isaacs

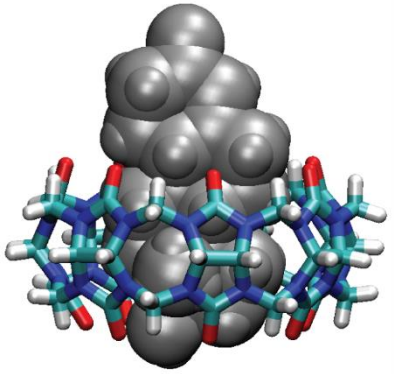

Werner Nau

R. Behrend et al., *Justus Liebig's Ann. Chem.* **1905**
W. L. Mock et al., *J. Org. Chem.* **1986**
K. Kim et al., *J. Am. Chem. Soc.*, **2000**
W. M. Nau et al., *Angew. Chem. Int. Ed.*, **2001**
L. Isaacs et al., *J. Am. Chem. Soc.*, **2002**

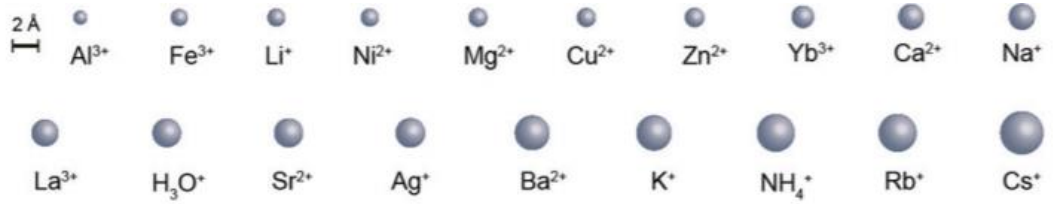
Application of CB*n*



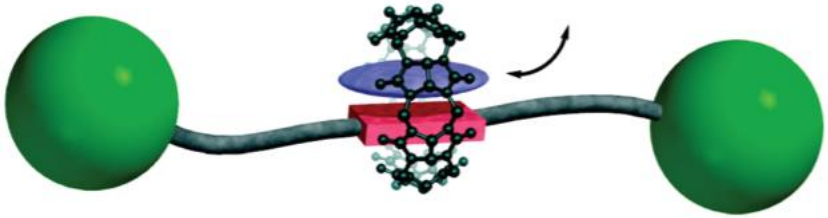
supramolecular host



drug delivery vehicles



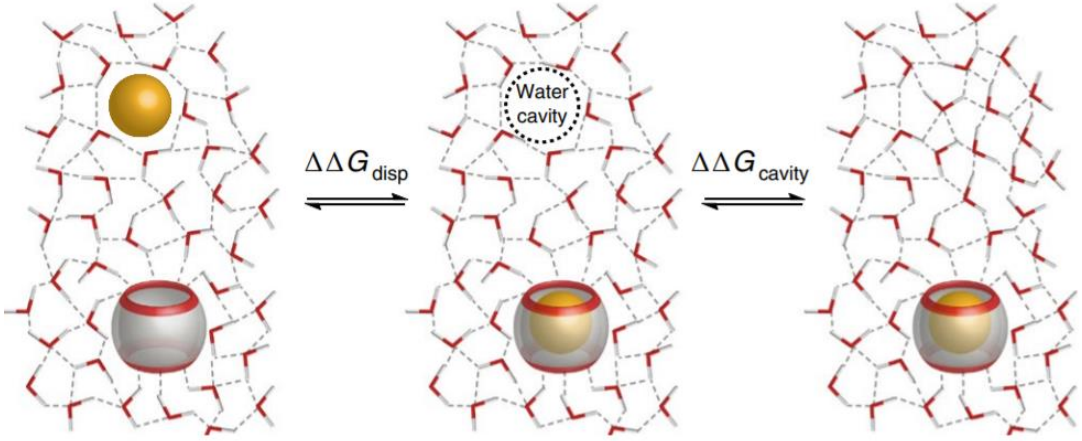
bind cations

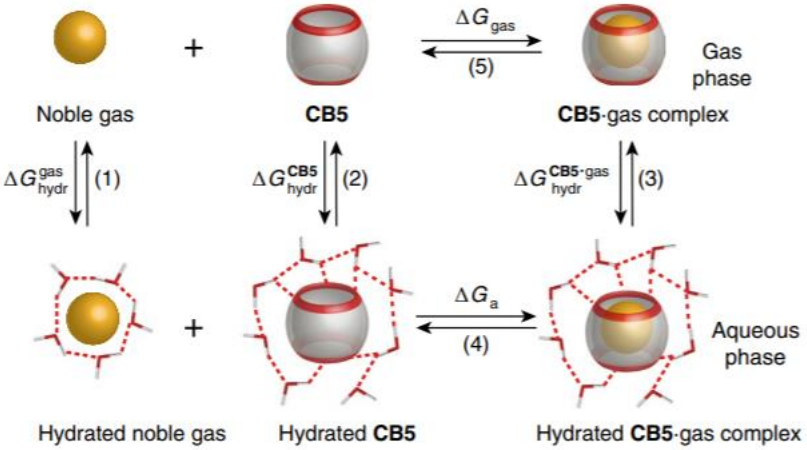


Rotaxane microcycles

W. M. Nau et al., *JACS,* **2016**
F. Biedermann et al.,*Chem. Sci.,* **2019**
F. Biedermann et al., *Chem. Commun.,* **2019**
A. Urbach et al., *Org. Lett.,* **2011**

Application of CB*n*





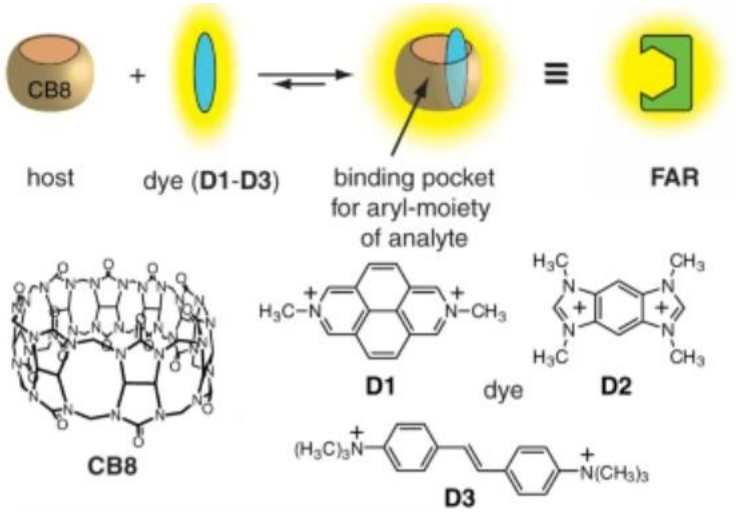
ARTICLES
https://doi.org/10.1038/s41557-018-0146-0
nature chemistry

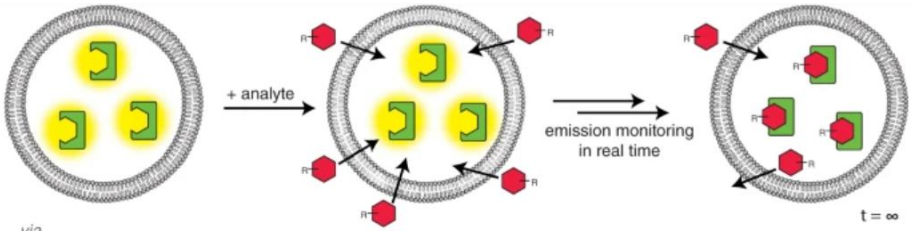
Cavitation energies can outperform dispersion interactions

Suhang He[1], Frank Biedermann[2*], Nina Vankova[1,3*], Lyuben Zhechkov[1,3], Thomas Heine[1,3,4*], Roy E. Hoffman[5], Alfonso De Simone[6], Timothy T. Duignan[7*] and Werner M. Nau[1*]

The accurate dissection of binding energies into their microscopic components is challenging, especially in solution. Here we study the binding of noble gases (He–Xe) with the macrocyclic receptor cucurbit[5]uril in water by displacement of methane and ethane as ^{1}H NMR probes. We dissect the hydration free energies of the noble gases into an attractive dispersive component and a repulsive one for formation of a cavity in water. This allows us to identify the contributions to host–guest binding and to conclude that the binding process is driven by differential cavitation energies rather than dispersion interactions. The free energy required to create a cavity to accept the noble gas inside the cucurbit[5]uril is much lower than that to create a similarly sized cavity in bulk water. The recovery of the latter cavitation energy drives the overall process, which has implications for the refinement of gas-storage materials and the understanding of biological receptors.

Application of CB*n*





chemosensor

COMMUNICATIONS BIOLOGY

ARTICLE

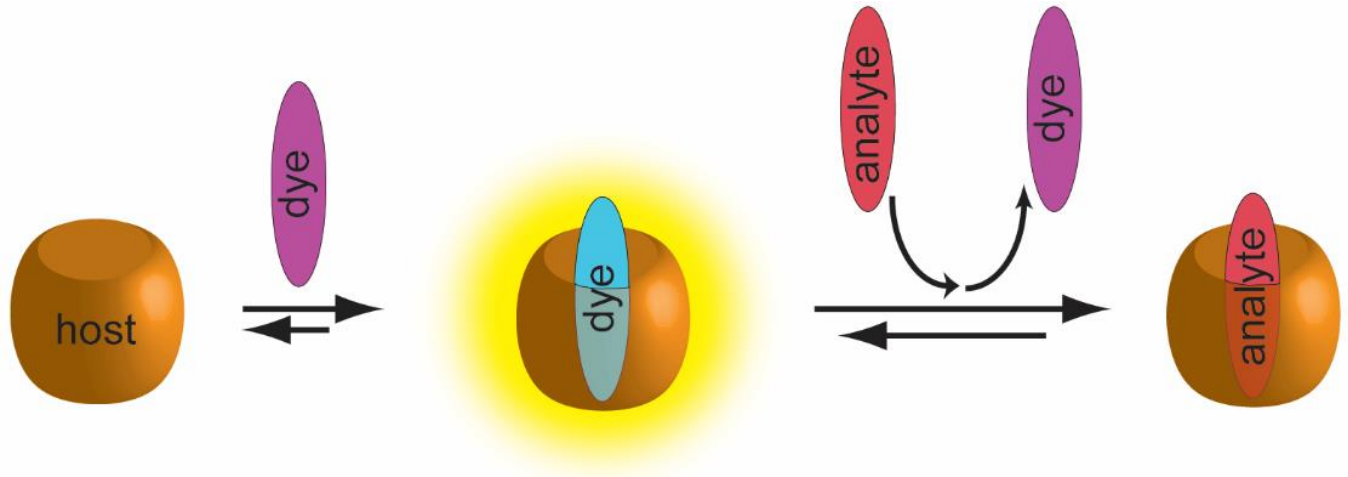
https://doi.org/10.1038/s42003-020-1108-9 OPEN

Fluorescent artificial receptor-based membrane assay (FARMA) for spatiotemporally resolved monitoring of biomembrane permeability

Frank Biedermann[1,2✉], Garima Ghale[2], Andreas Hennig[2] & Werner M. Nau[2✉]

The spatiotemporally resolved monitoring of membrane translocation, e.g., of drugs or toxins, has been a long-standing goal. Herein, we introduce the fluorescent artificial receptor-based membrane assay (FARMA), a facile, label-free method. With FARMA, the permeation of more than hundred organic compounds (drugs, toxins, pesticides, neurotransmitters, peptides, etc.) through vesicular phospholipid bilayer membranes has been monitored in real time (μs-h time scale) and with high sensitivity (nM-μM concentration), affording permeability coefficients across an exceptionally large range from 10^{-9}–10^{-3} cm s^{-1}. From a fundamental point of view, FARMA constitutes a powerful tool to assess structure-permeability relationships and to test biophysical models for membrane passage. From an applied perspective, FARMA can be extended to high-throughput screening by adaption of the microplate reader format, to spatial monitoring of membrane permeation by microscopy imaging, and to the compartmentalized monitoring of enzymatic activity.

Indicator displacement assays (IDA)

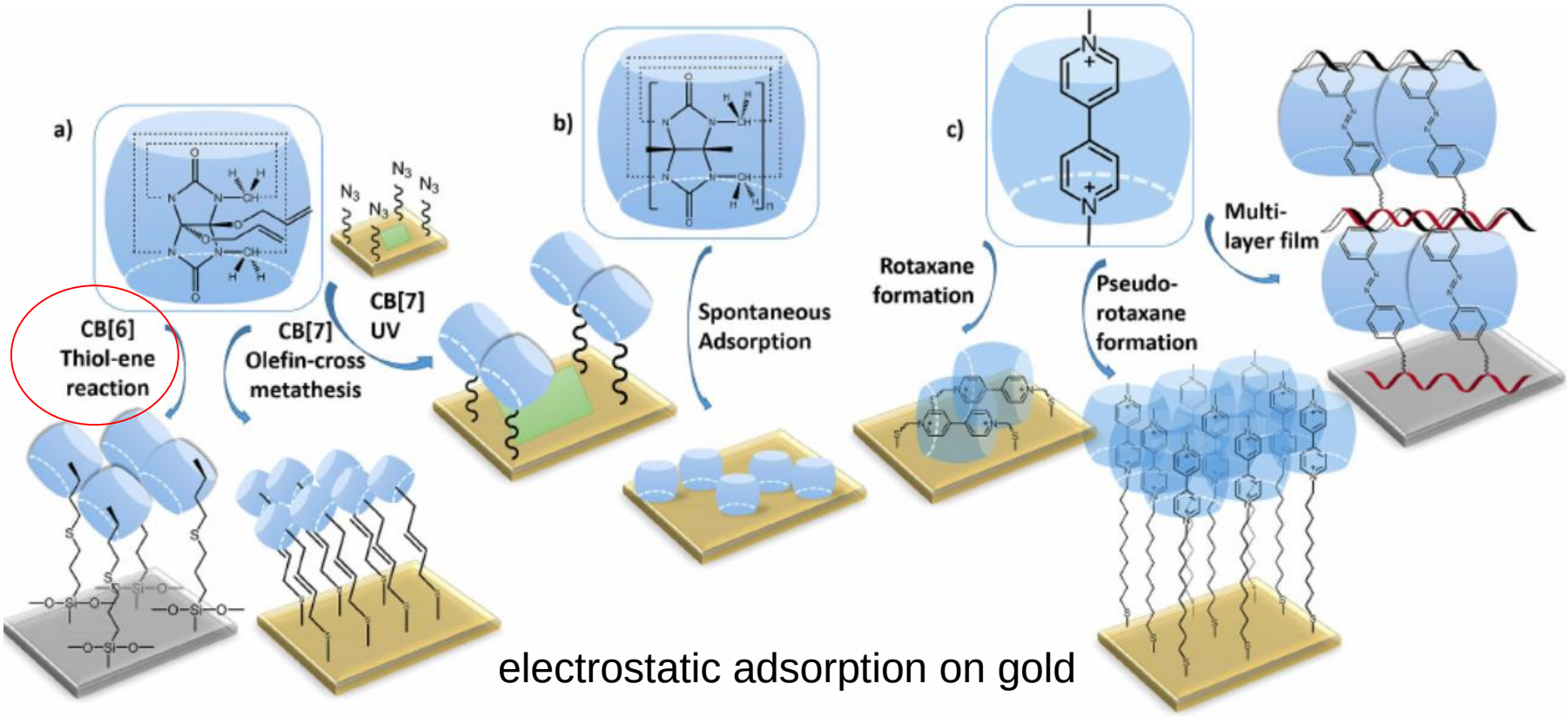


Pros:
fast sensing
high binding affinity
tunable selectivity

Limitation:
in solution
real practical application

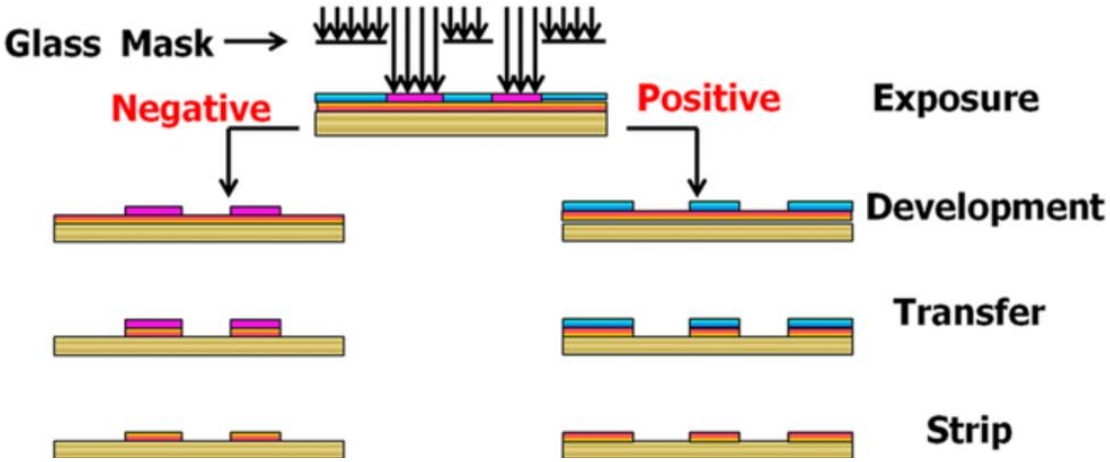
F. Biedermann et al., *Chem. Commun.*, **2020**
F. Biedermann et al., *Chem. Eur. J.*, **2020**

CB*n* immobilization

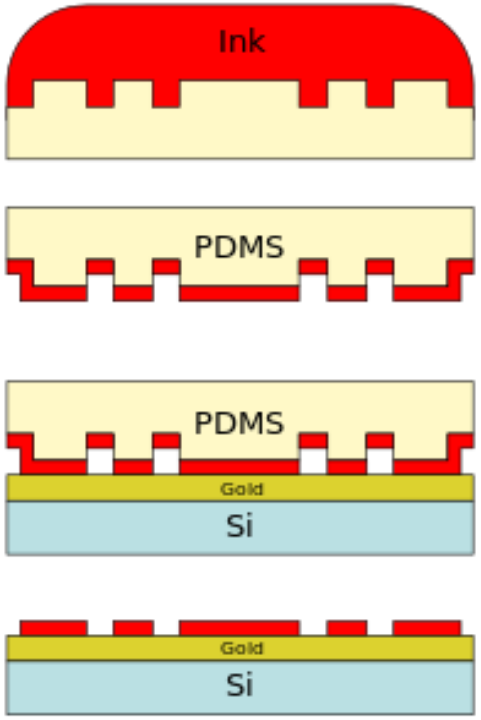


P. Jonkheijm et al., *Isr. J. Chem.* **2018**

Micro-patterning approaches



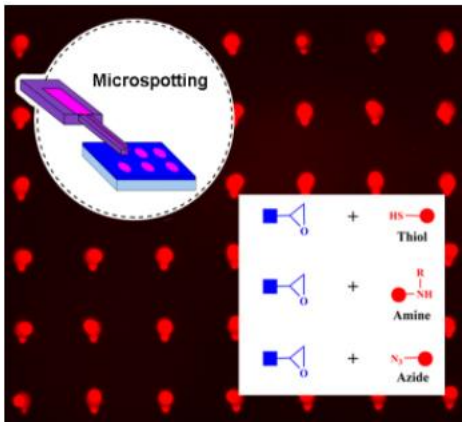
photolithography

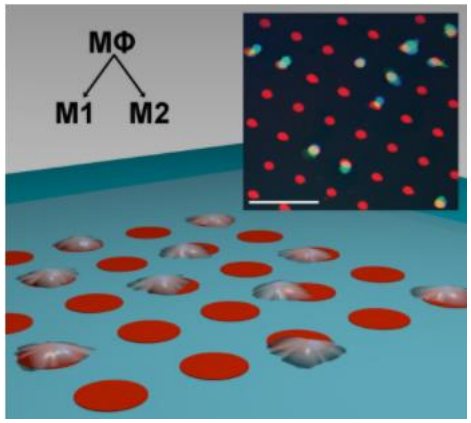


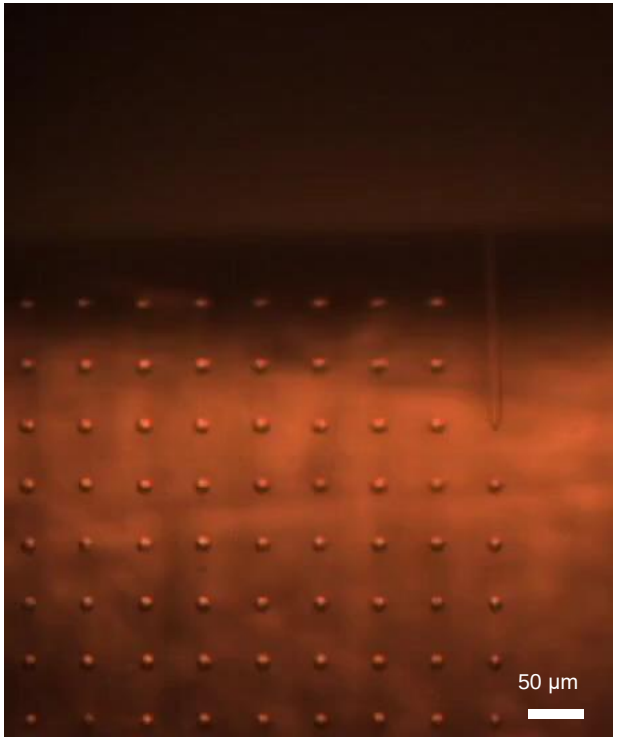
contact printing
(stamp)

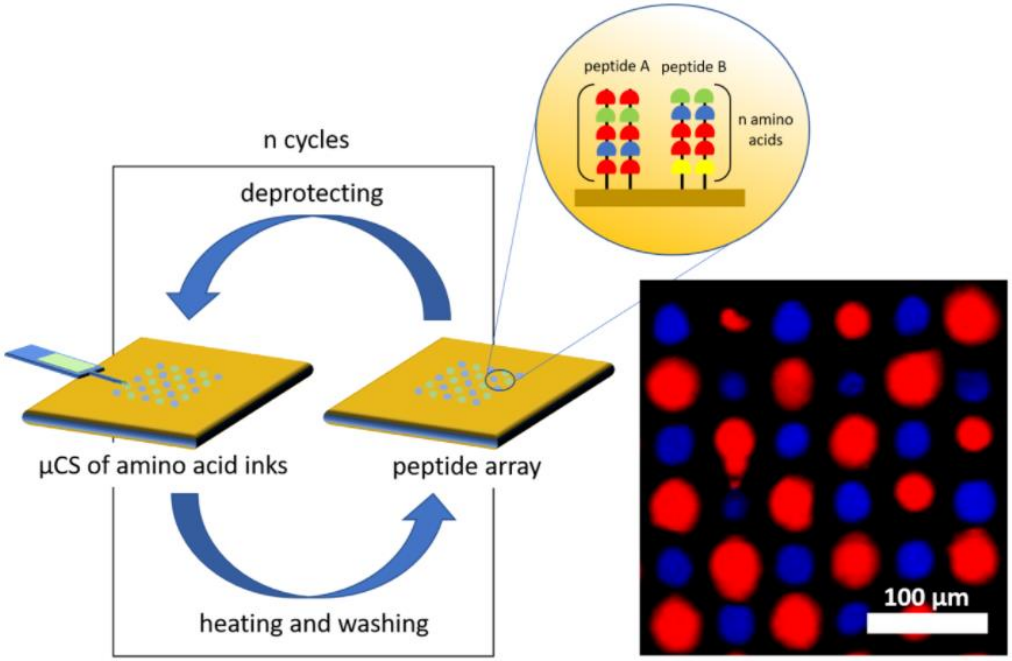
K. Lynn et al., *J. Vac. Sci. & Technol. B*, **2013**

Microchannel cantilever spotting (µCS)









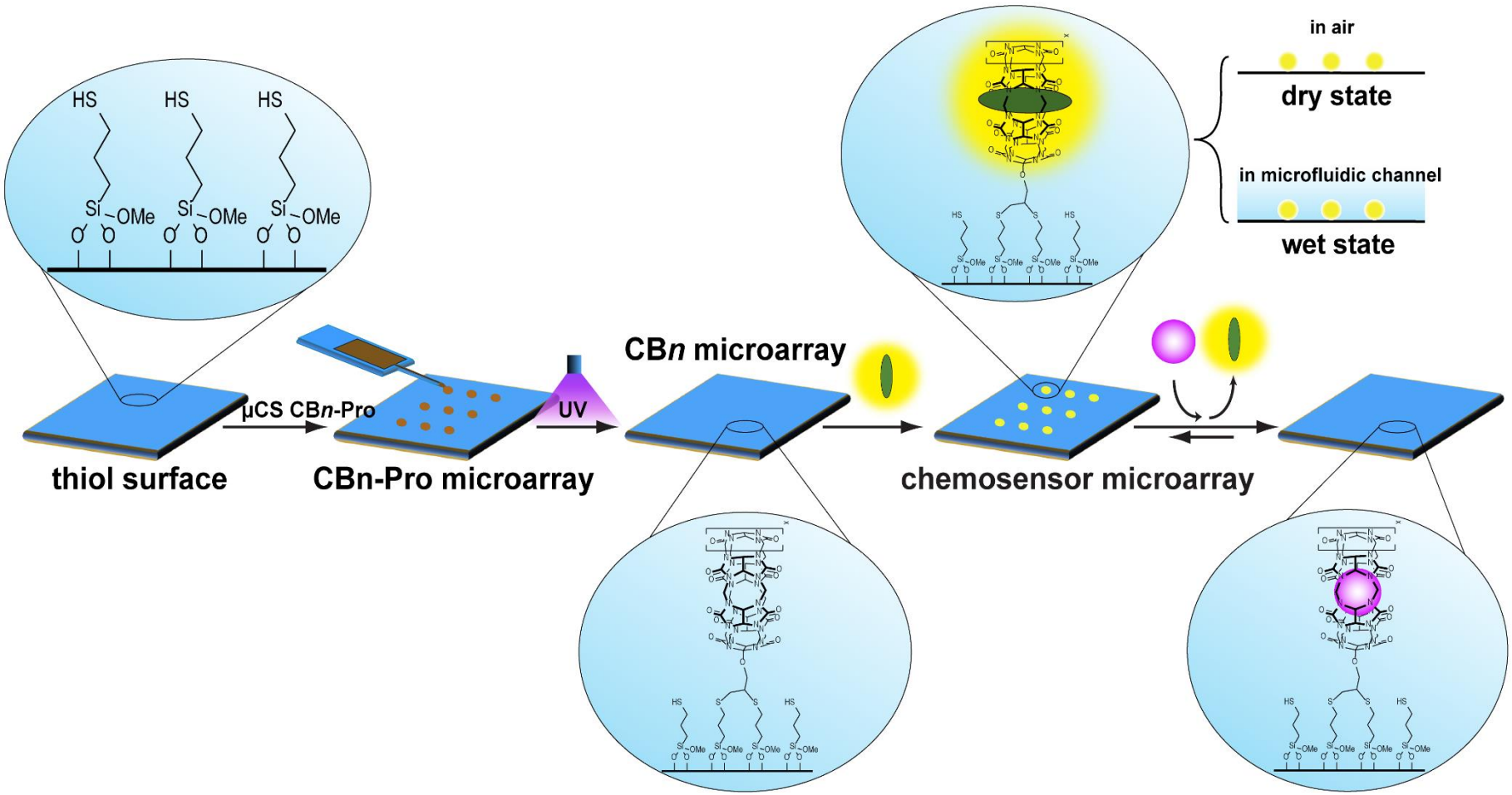
✓ flexible: directly writing

M. Hirtz et al., *Adv. Mater. Interfaces*, **2021**

M. Hirtz et al., *Adv. NanoBiomed Res*. **2021**

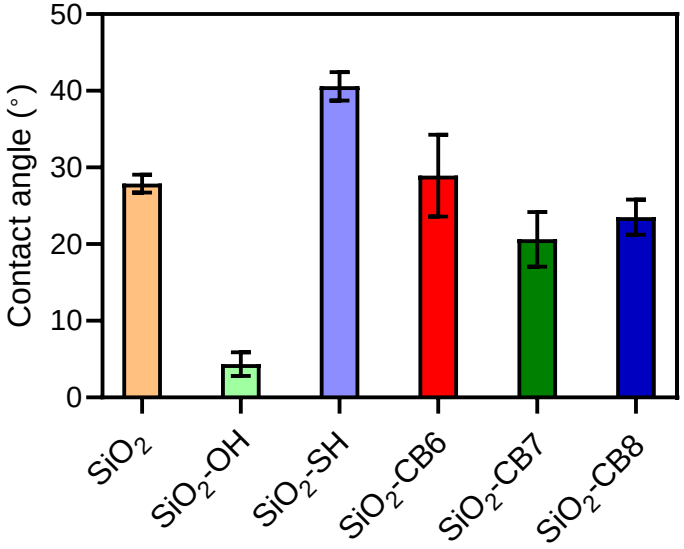
M. Hirtz et al., *Adv. Mater.* **2018**

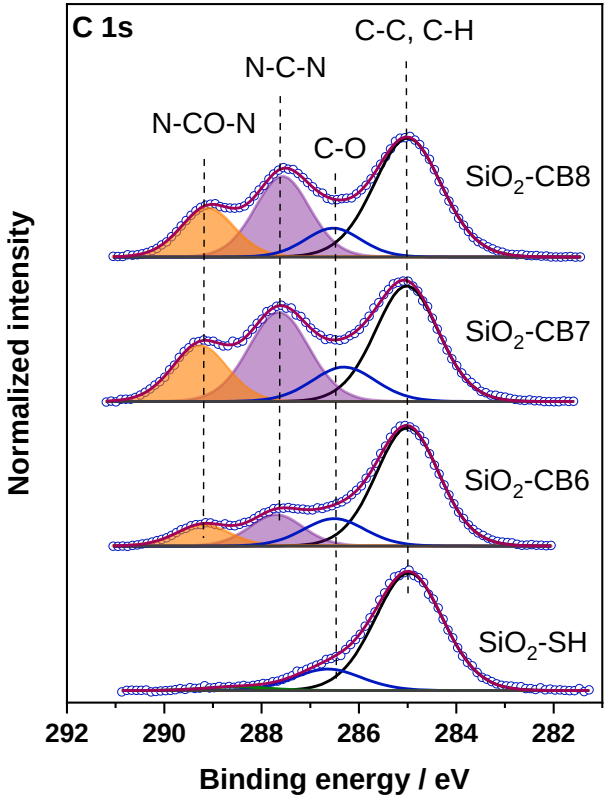
Strategy

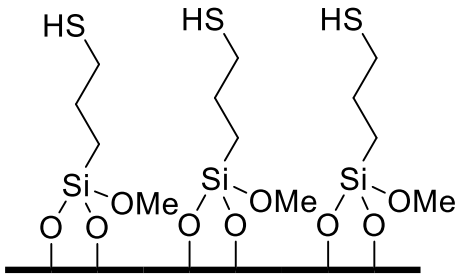


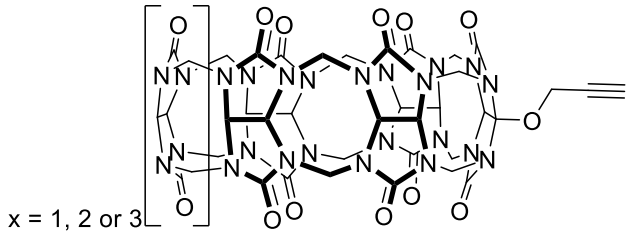
M. Hirtz et al., *ACS Appl. Nano Mater.* **2021**

Surface modification

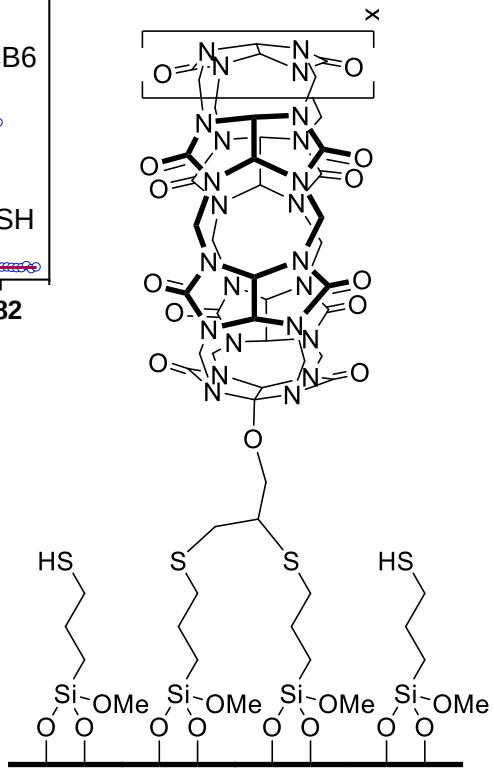




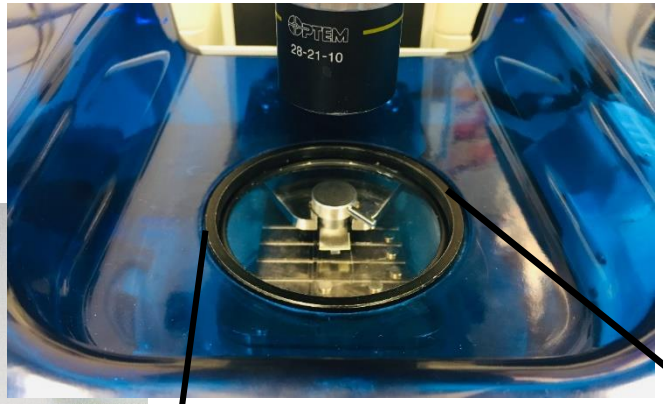


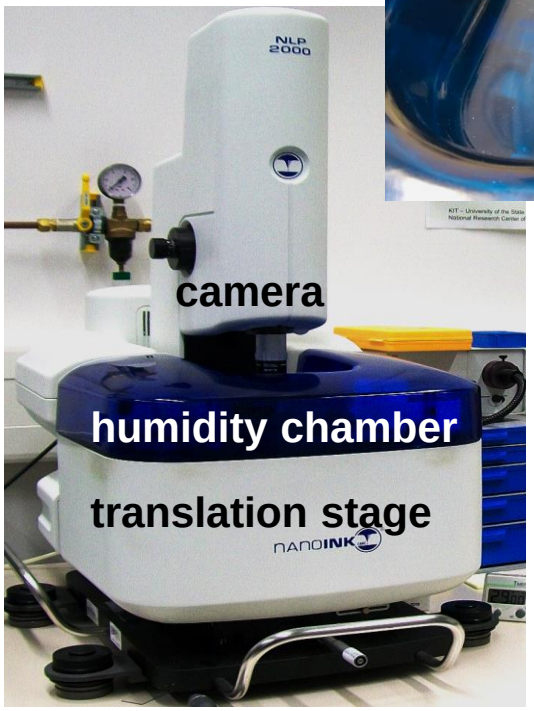


CB*n*-Pro

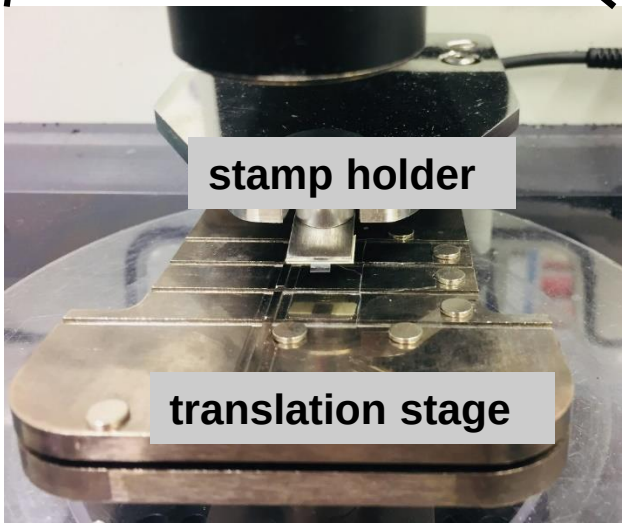


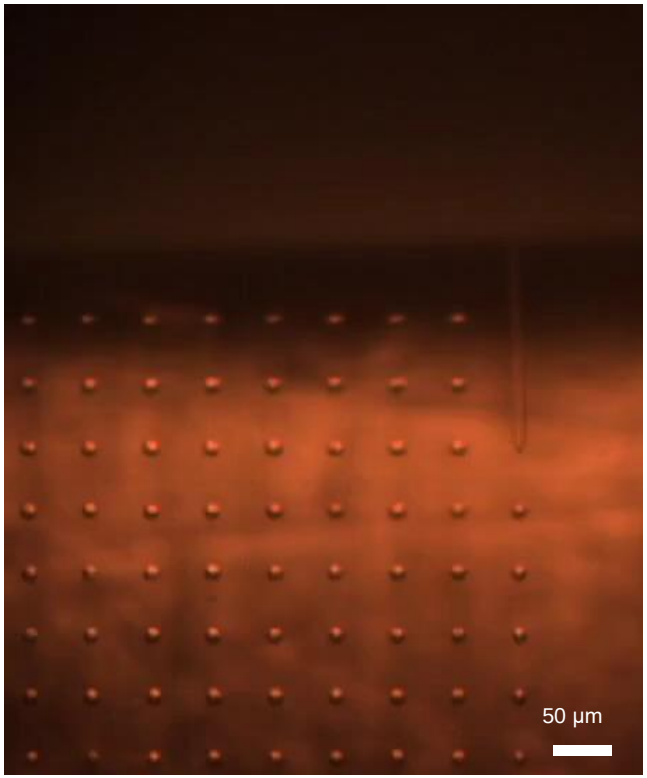
Microarray patterning



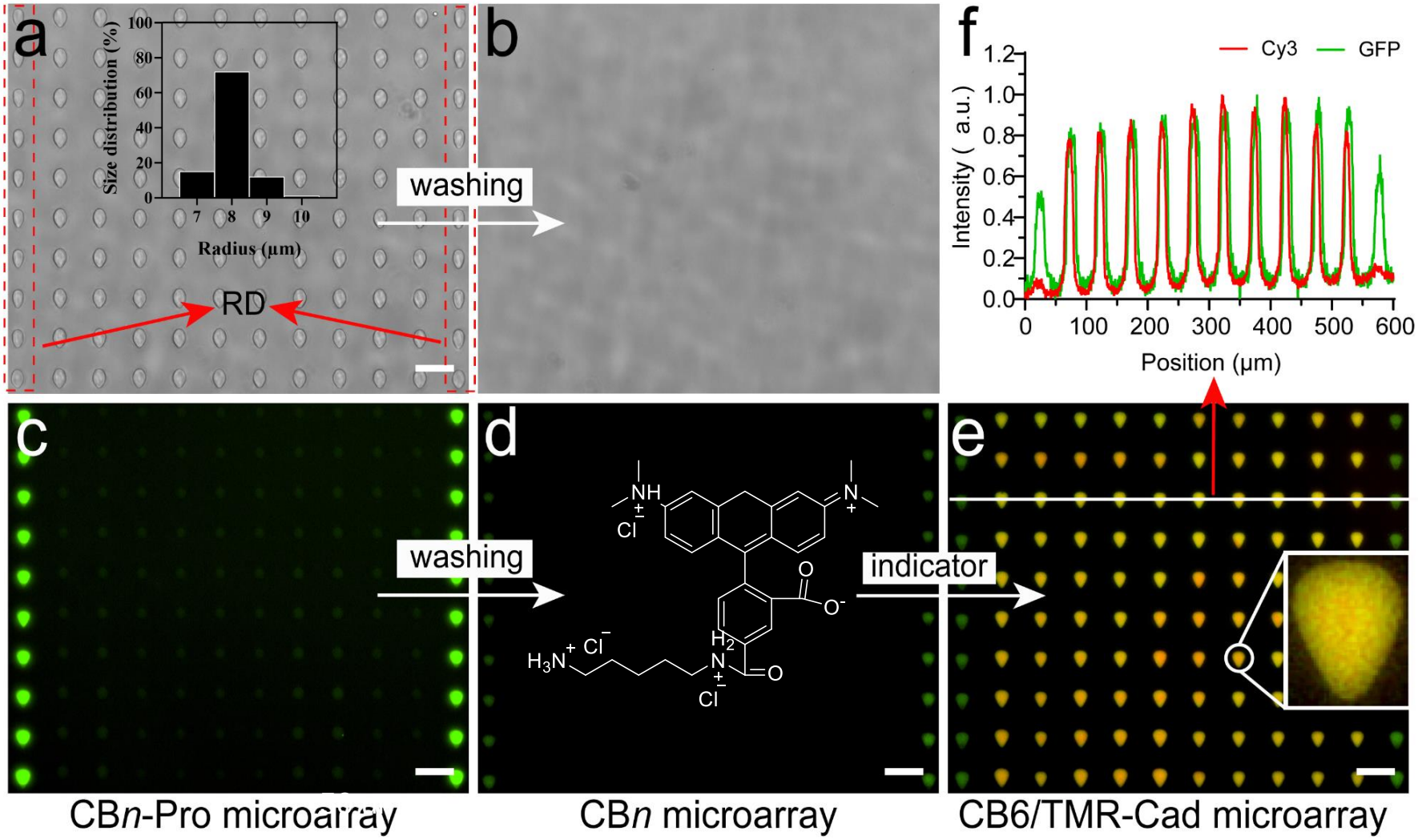


NLP 2000 System

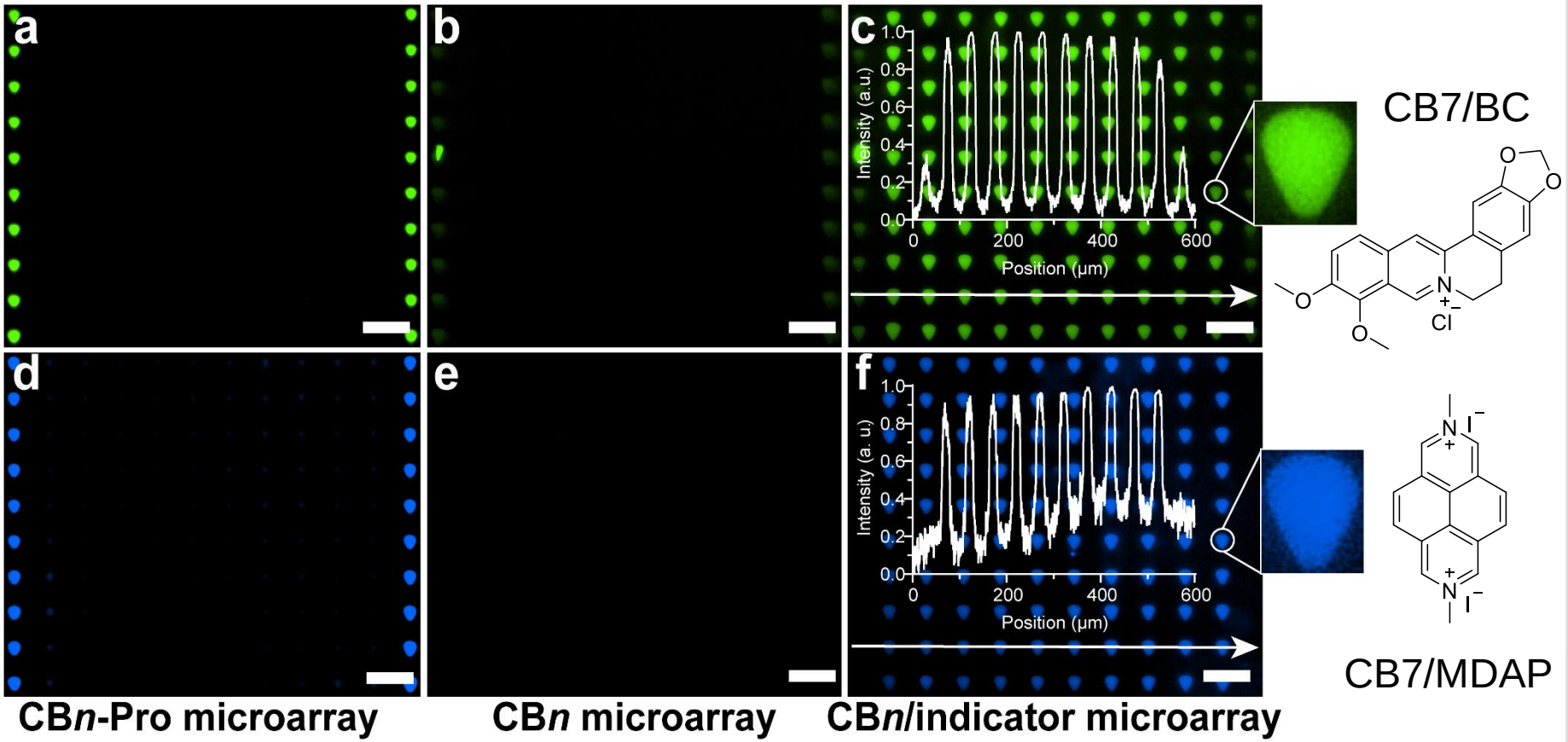




CB*n* microarray patterning and indicator loading

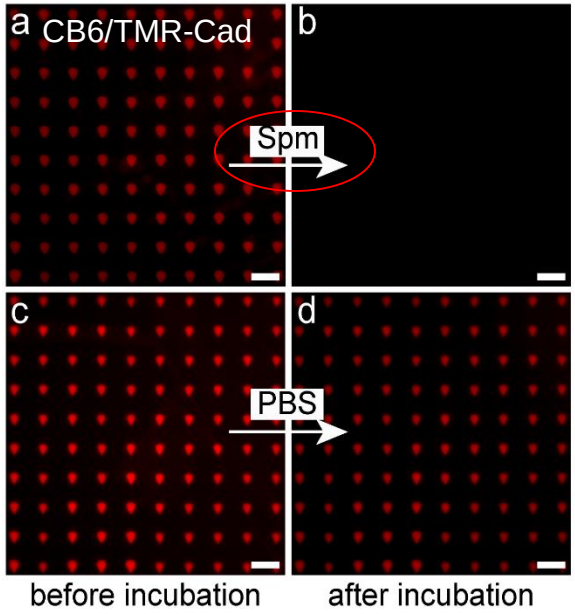


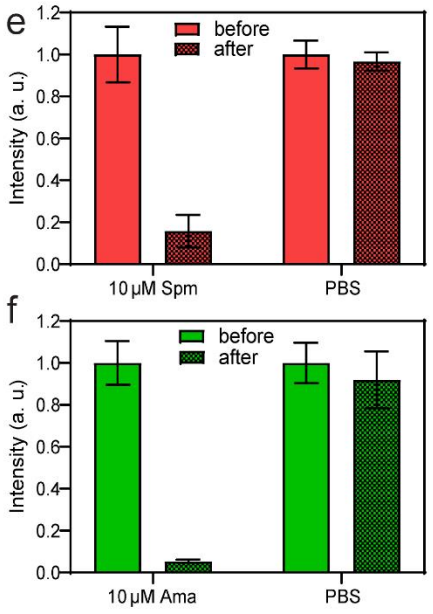
CB*n* microarray patterning and indicator loading

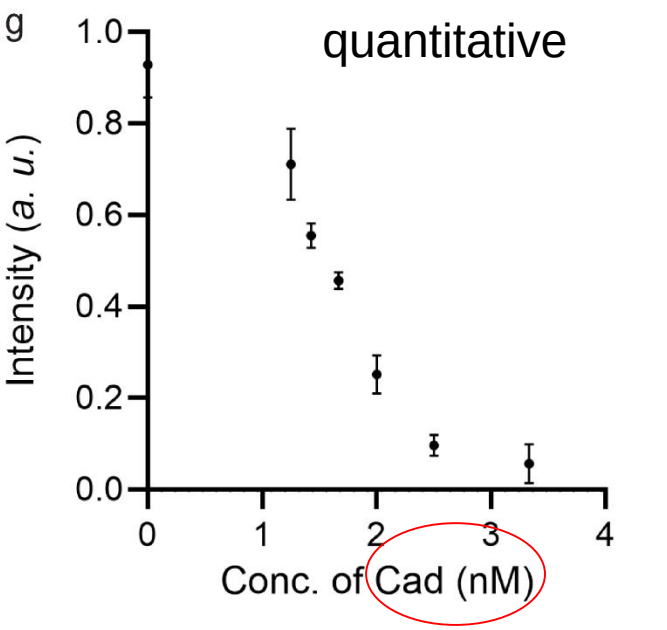


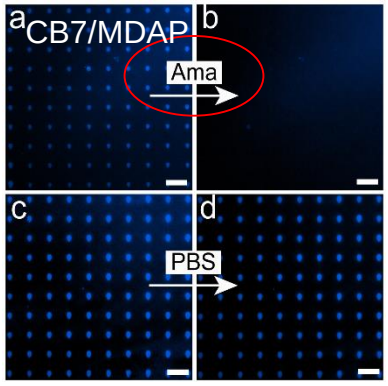
Analytes sensing

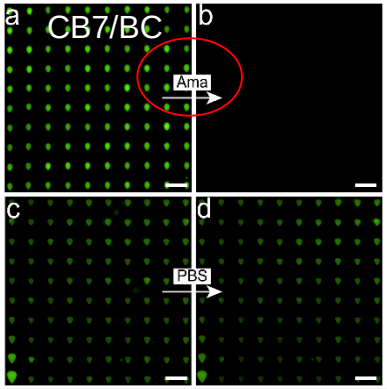
Mono-CB*n*/indicator Microarray

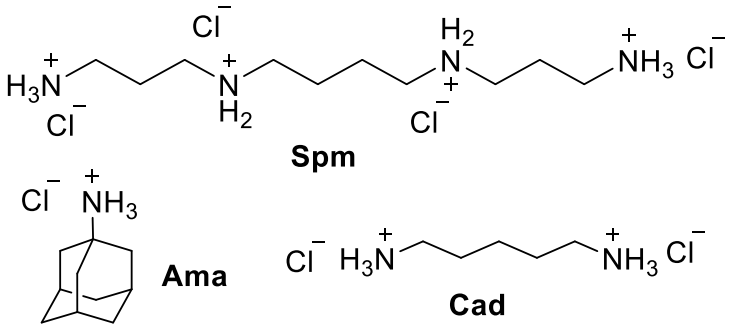




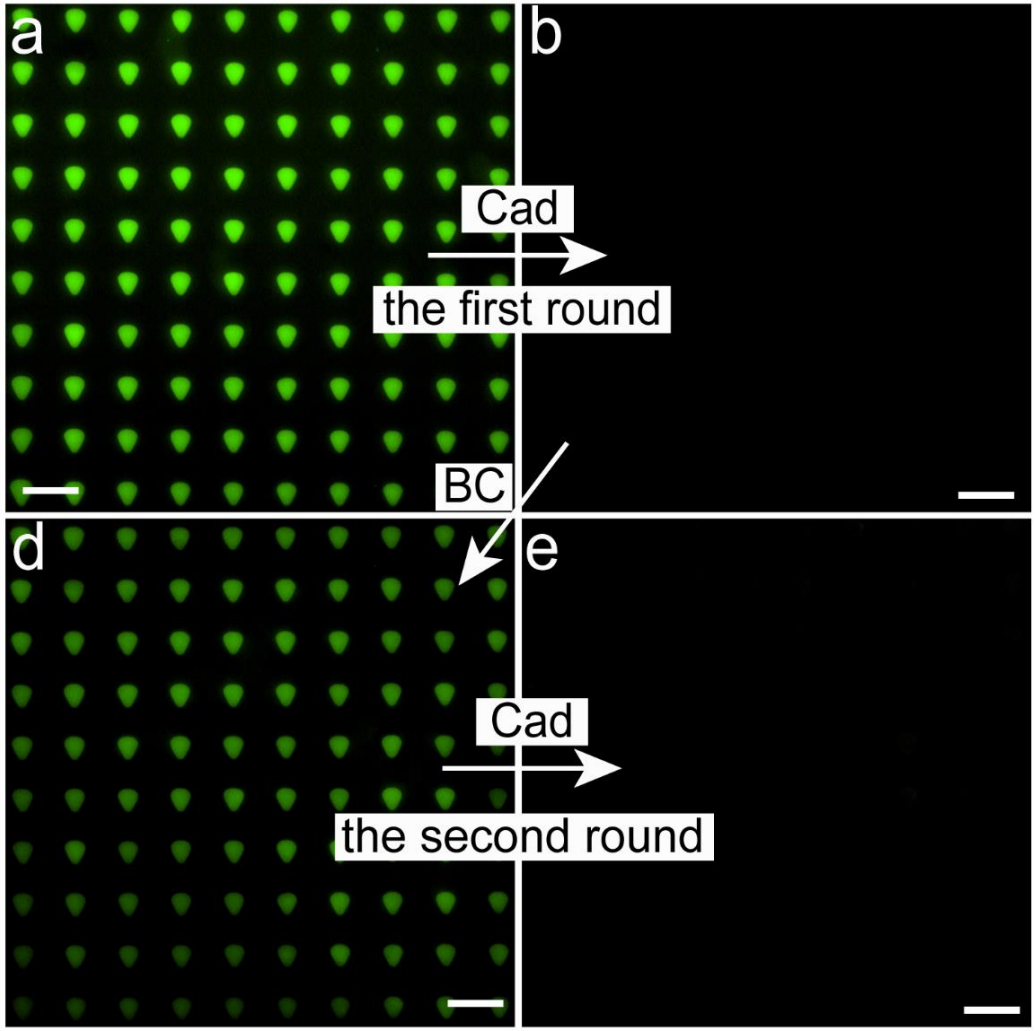


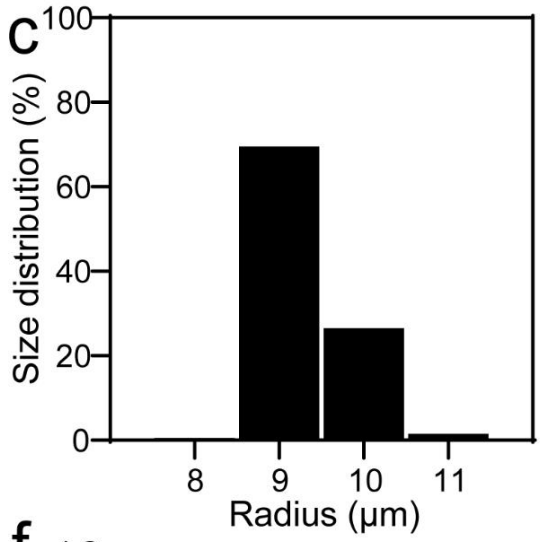


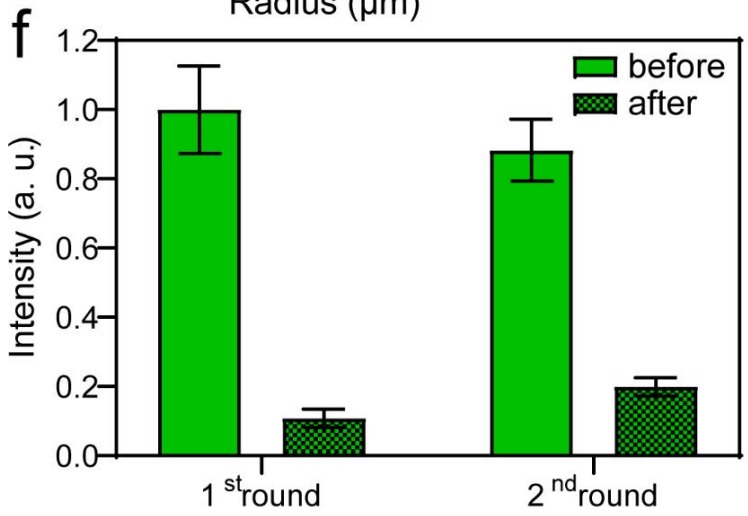




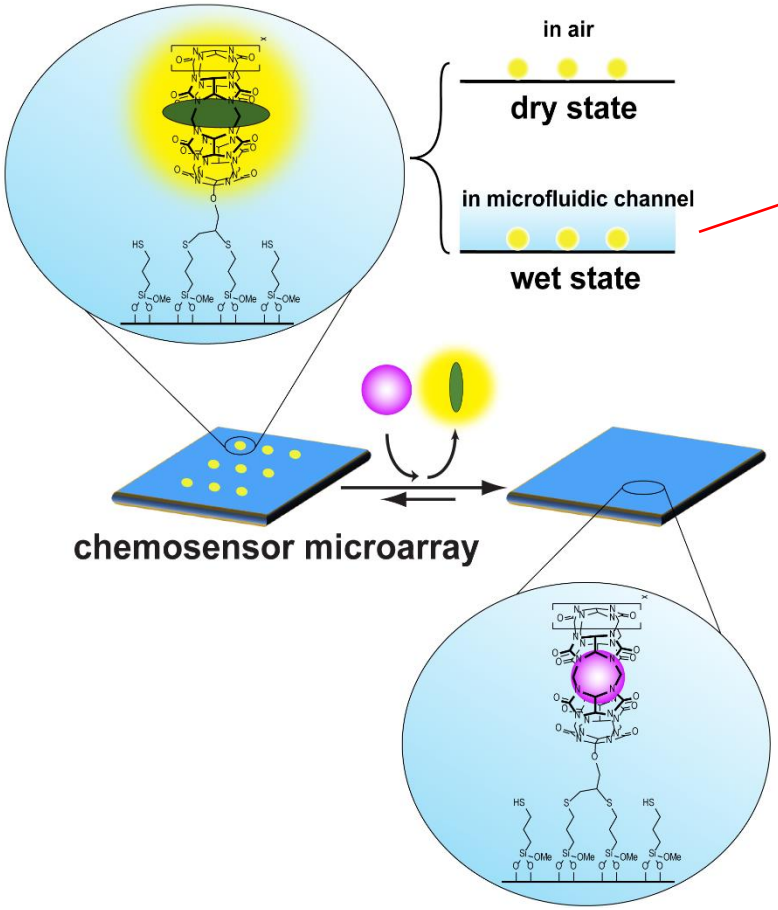
Regeneration

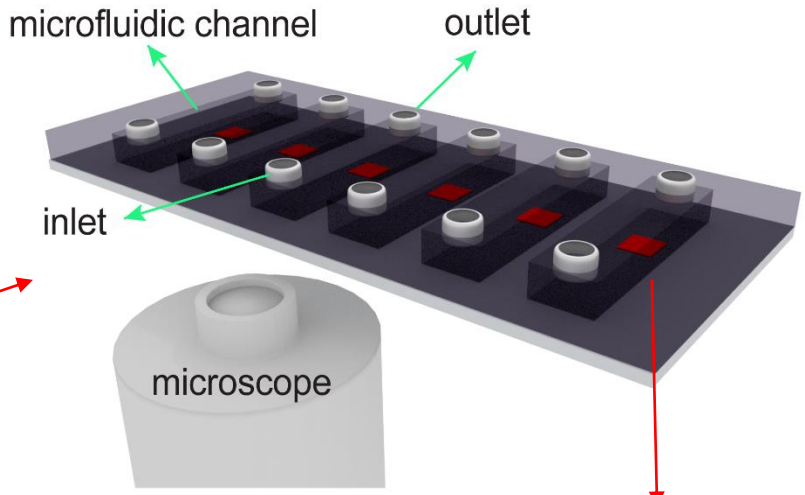


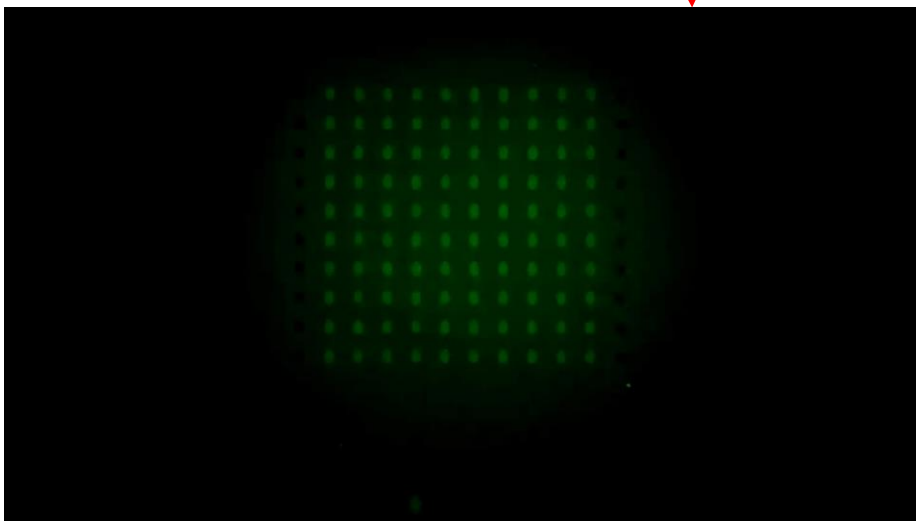




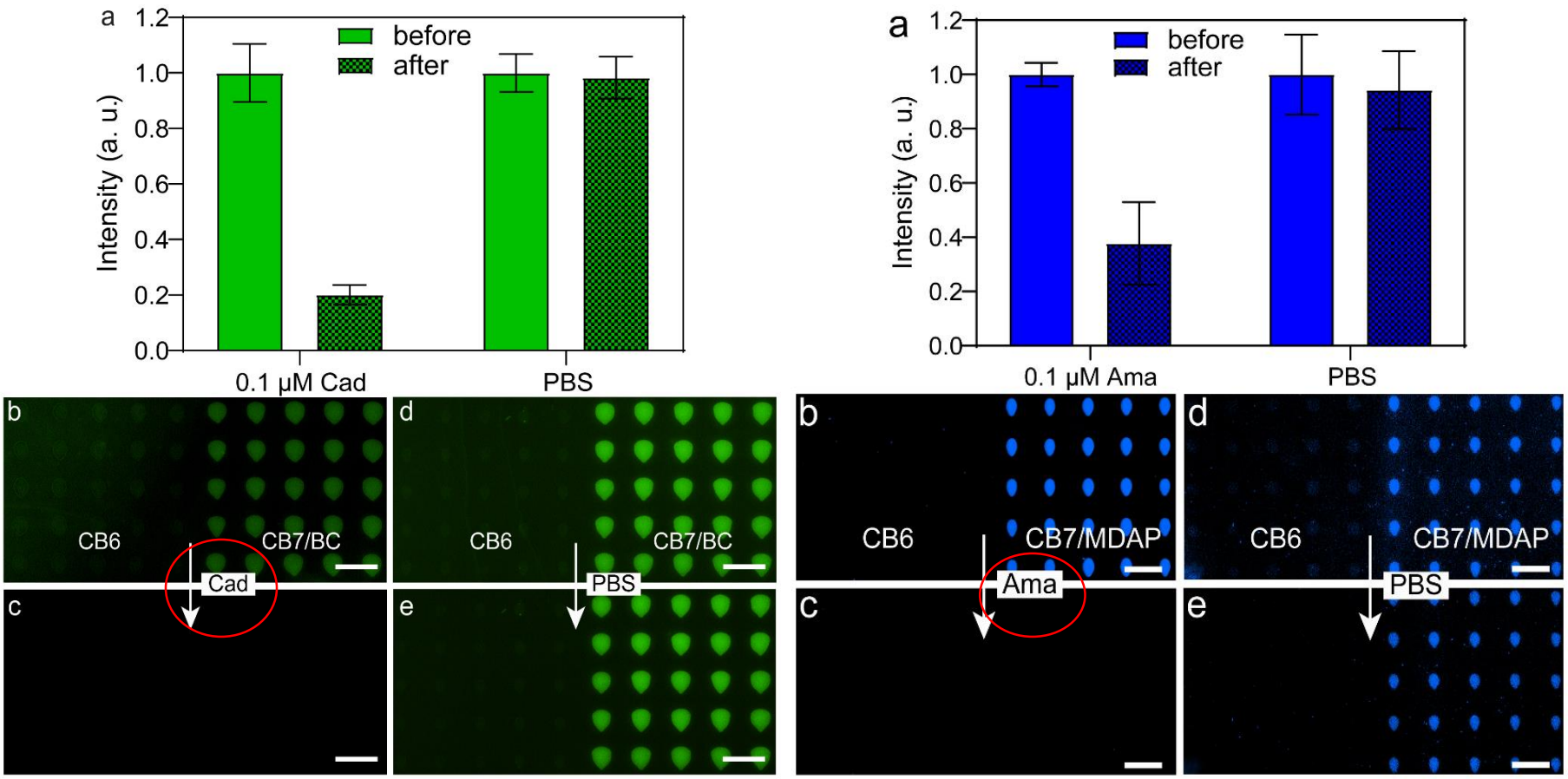
Real-time sensing in microfluidic channel



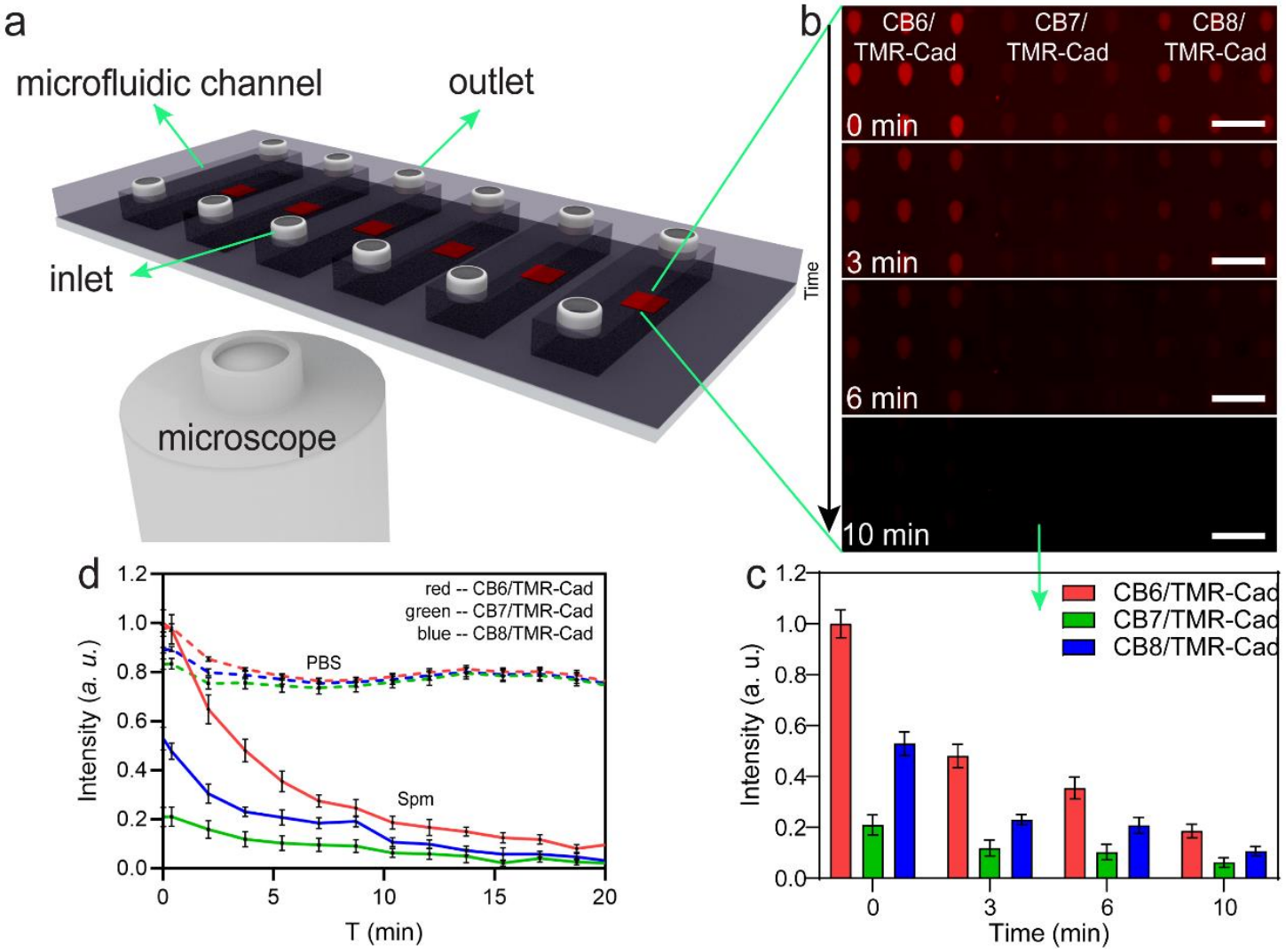




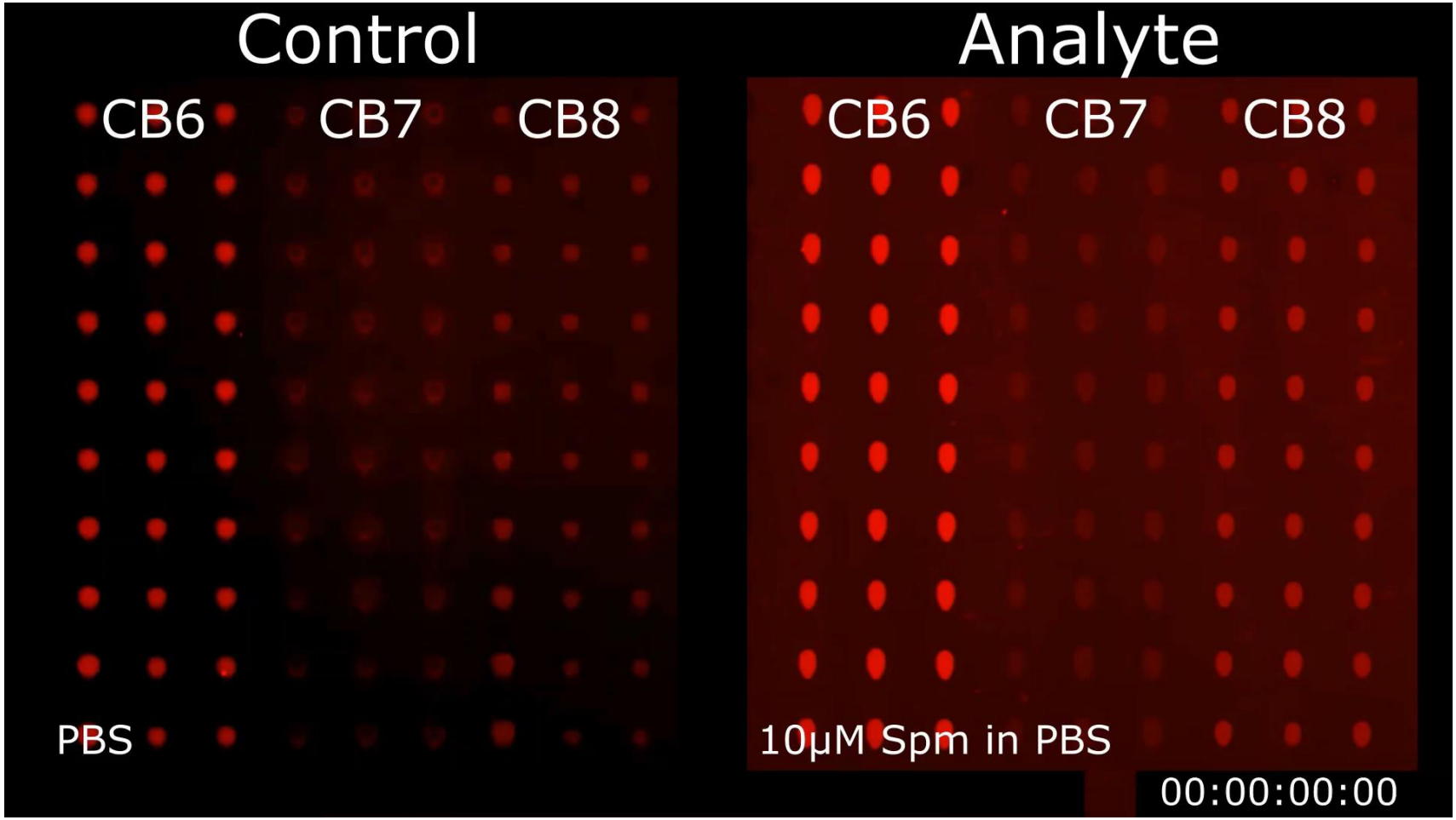
Di-CB*n*/mono-indicator Microarray



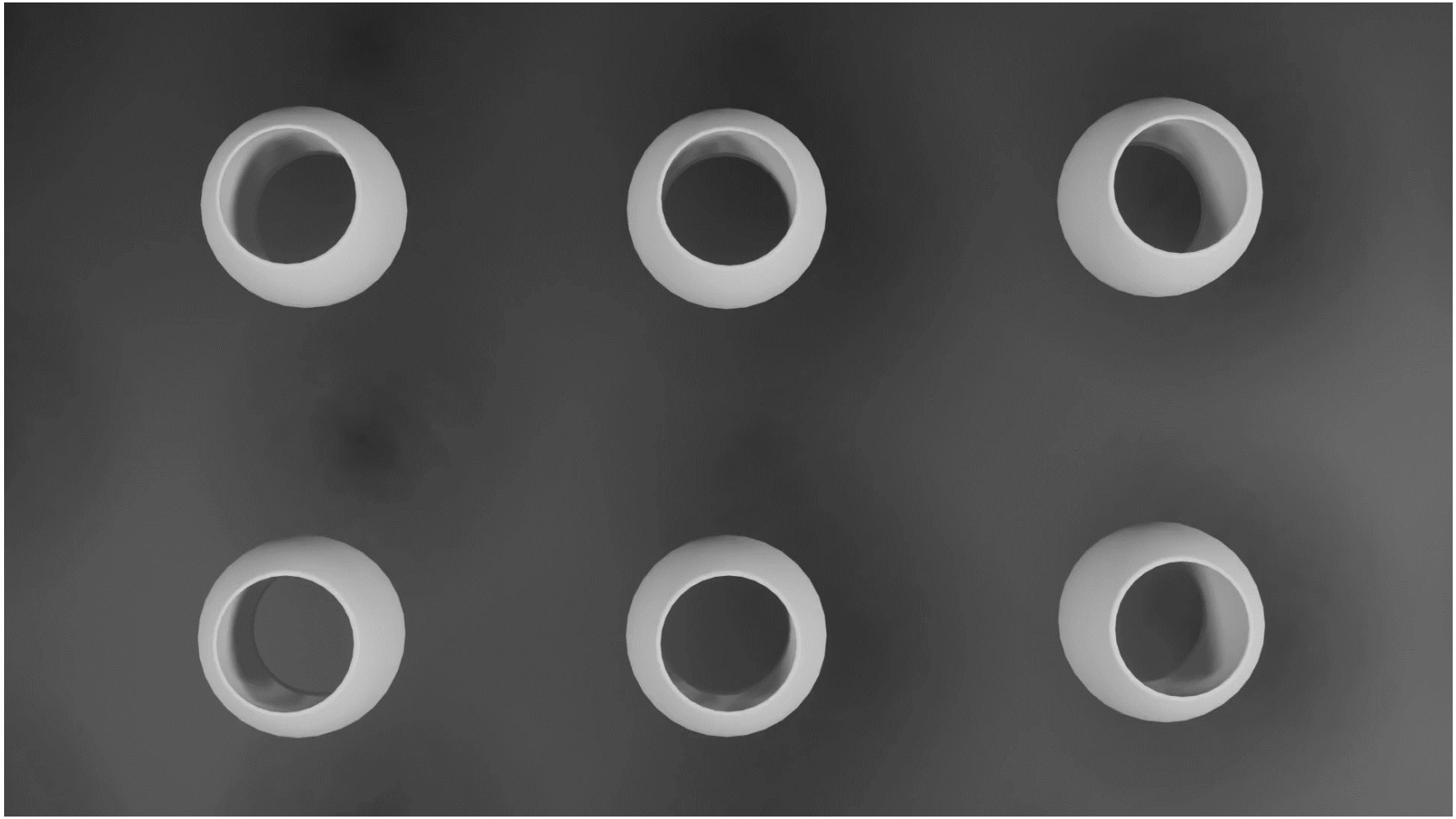
Multi-CB*n*/mono-indicator Microarray



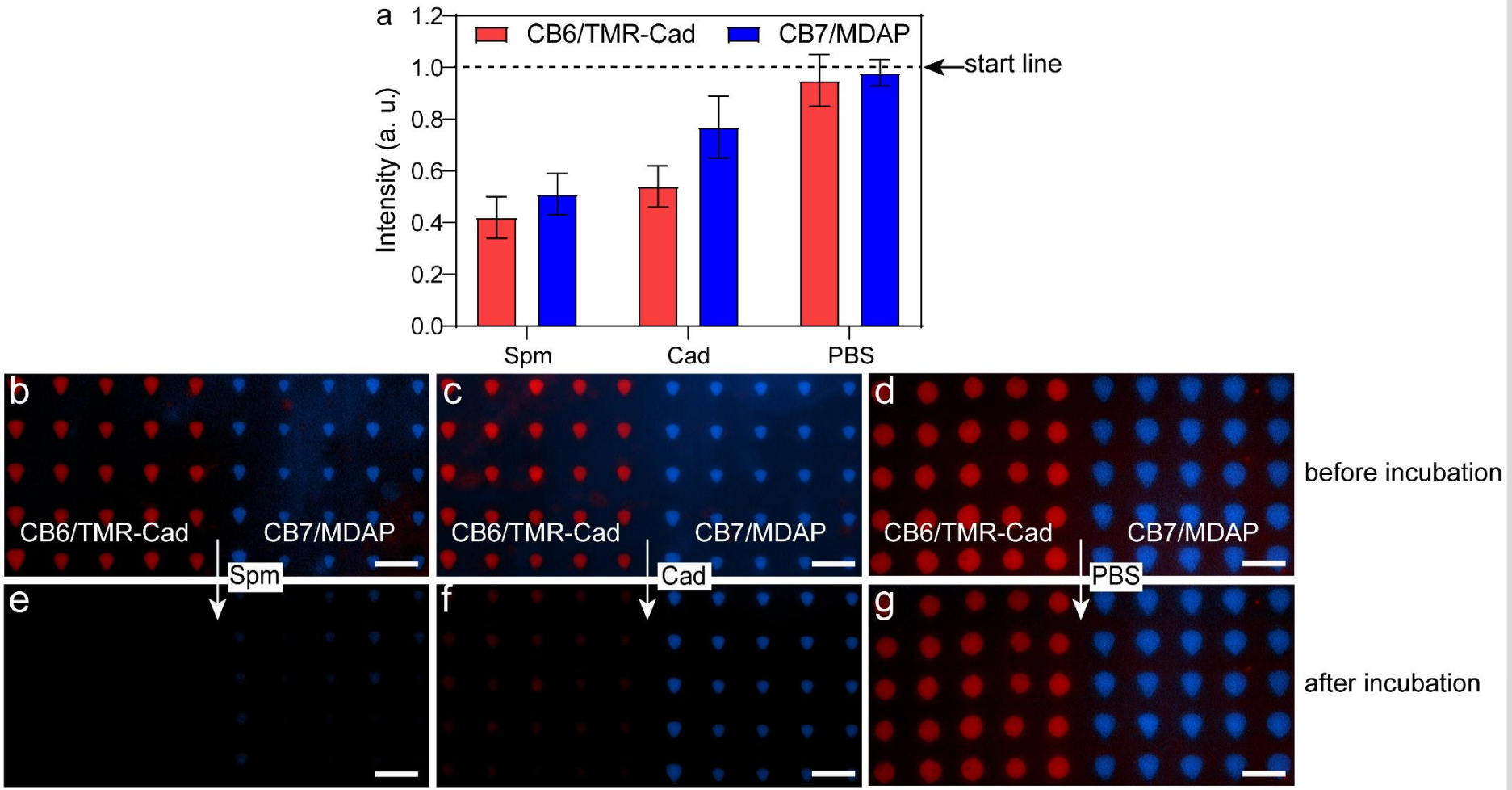
Multi-CB*n*/mono-indicator Microarray



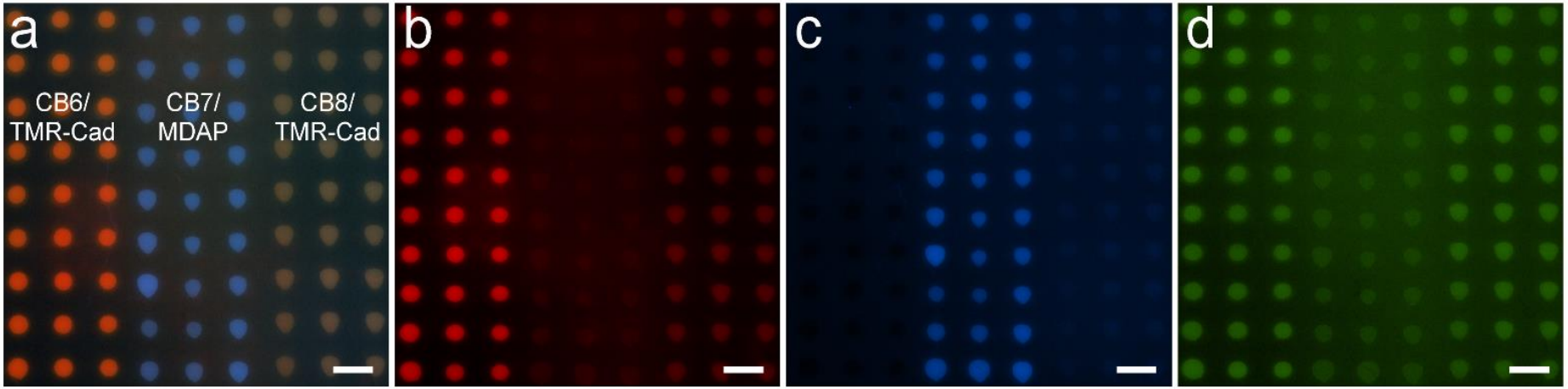
Di-CB*n*/di-indicator Microarray

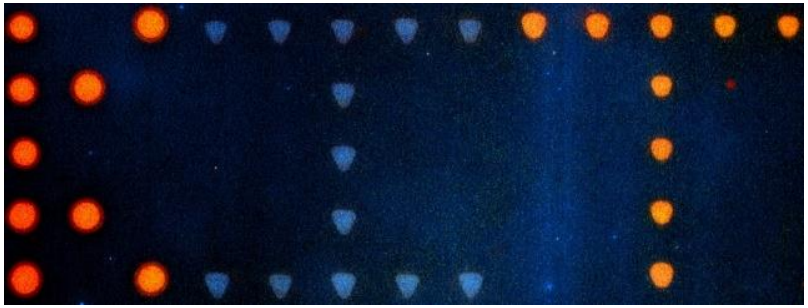


Di-CB*n*/di-indicator Microarray



Multi-CB*n*/di-indicator Microarray





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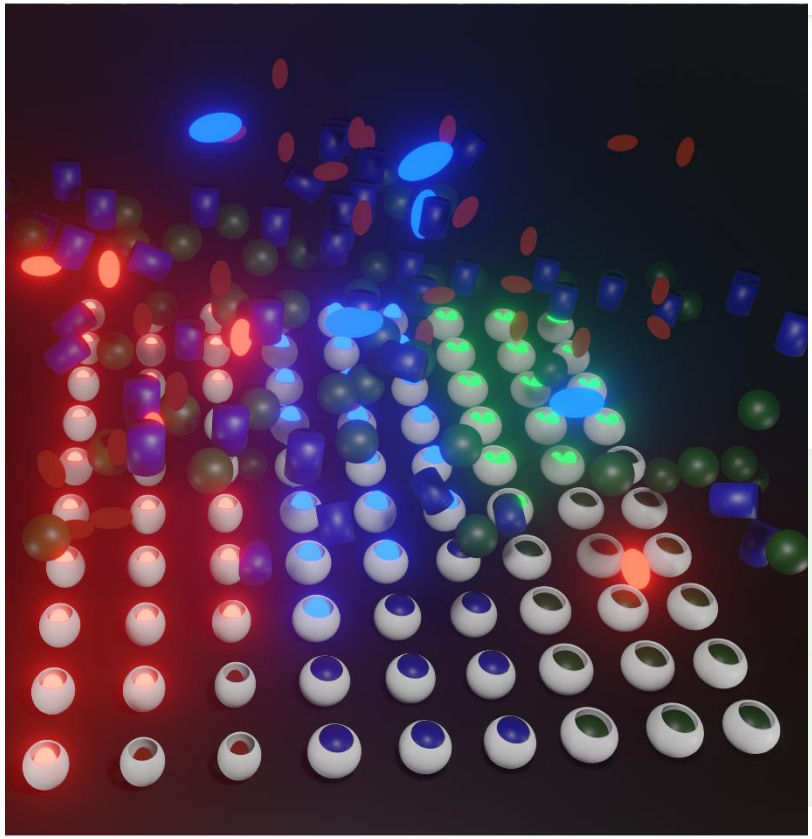

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Article

Cucurbit[*n*]uril-Immobilized Sensor Arrays for Indicator-Displacement Assays of Small Bioactive Metabolites

Chunting Zhong, Changming Hu, Ravi Kumar, Vanessa Trouillet, Frank Biedermann,* and Michael Hirtz*

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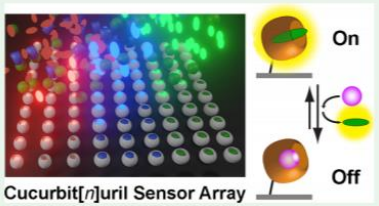
ABSTRACT: The patterned immobilization of chemosensors into nano/microarrays has often boosted utilization in diagnostics and environmental sensing applications. While this is a standard approach for biosensors, e.g., with antibodies, other proteins, and DNA, arraying is not yet adopted widely for supramolecular chemosensors which are still predominantly used in solution systems. Here we introduce the patterned immobilization of cucurbit[*n*]urils (CB*n*) into multiplexed microarrays and elucidate their prospects for the advancement of surface-bound indicator-displacement assays to detect small molecule analytes. The microarrays were generated by microchannel cantilever spotting of functionalized CB*n* and subsequent self-assembly of the corresponding indicator dyes from solution. Enhanced sensitivity of surface-bound microarrays was established in demonstrations with small bioactive metabolites (spermine, amantadine, and cadaverine) compared to bulk assays. Furthermore, the integration of the CB*n*/indicator microarrays into microfluidic channels provides an efficient route for real-time monitoring of the sensing process, allows easier handling, and reduces need for analyte volume. The concept was further extended to differential sensing of analytes on diplex or multiplex CB*n*/indicator microarrays, opening up a route for multicomponent sensing of small molecule analytes in complex liquids.



Michael Hirtz
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Frank Biedermann
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