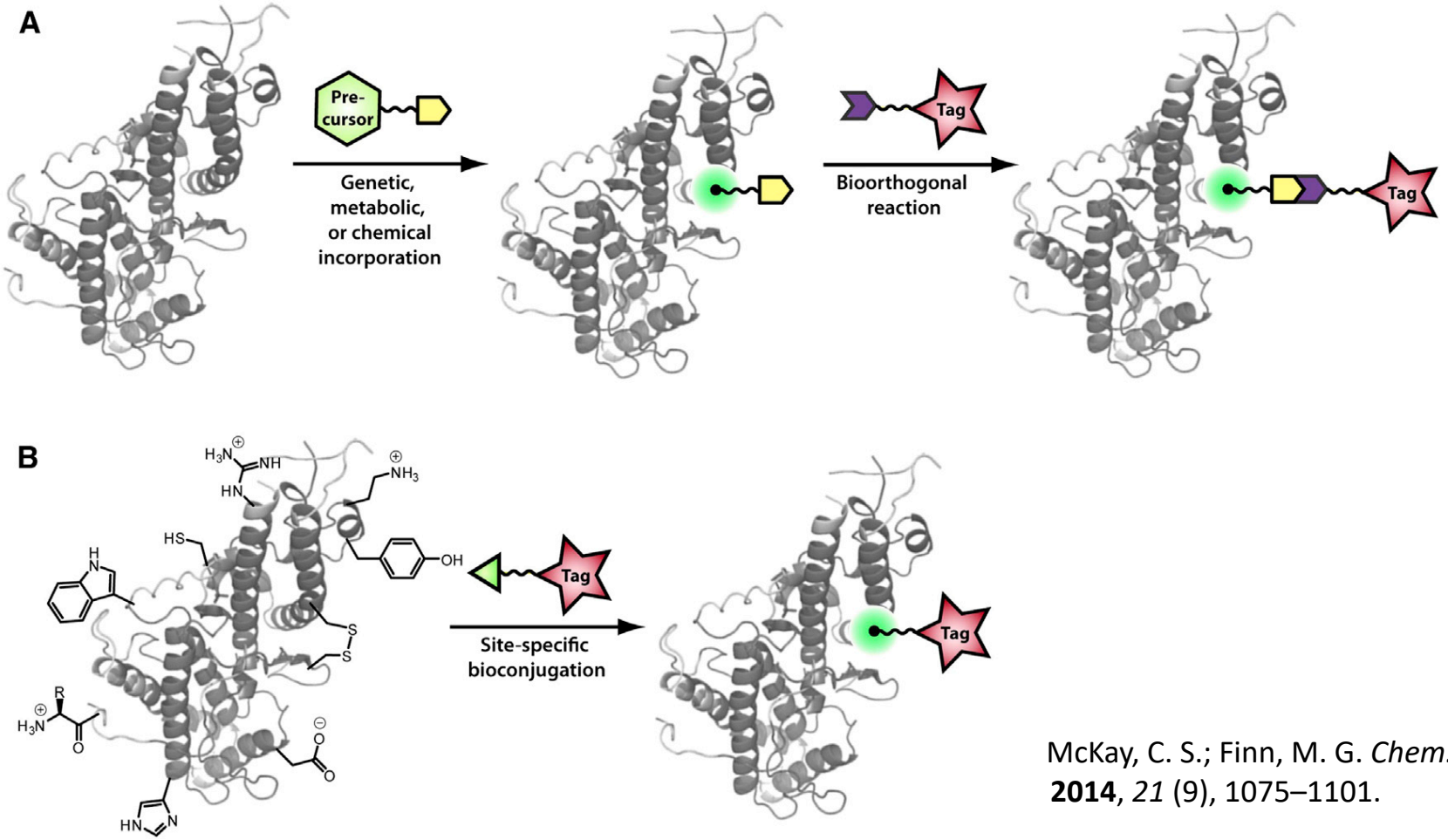
Host-guest chemistry based bioconjugation: progress toward *in cell* application

Literature seminar #1
B4 Yuki Yamanashi
2020/02/28(Fri)

Bioorthogonal molecular conjugation *in cells/ in vivo*



McKay, C. S.; Finn, M. G. *Chem. Biol.* **2014**, *21* (9), 1075–1101.

Tag

- visual tag (e.g., fluorescent molecular) →detection of target molecular
- immobilization agent (e.g., magnetic bead) →isolation of target molecular
- therapeutic agent (e.g., radioactive molecular) →pre-targeting therapeutics
- catalyst →selective-modification of target molecular

Current options: Host-guest chemistry has many advantages.

- "Click" reactions (azide-alkyne cycloaddition, Diels-Alder reaction, …)
  - ✓ Wide variety of reactions
  - ✗ Slow reaction rate (k_{on} ~ 10^1-10^4 $M^{-1}s^{-1}$)

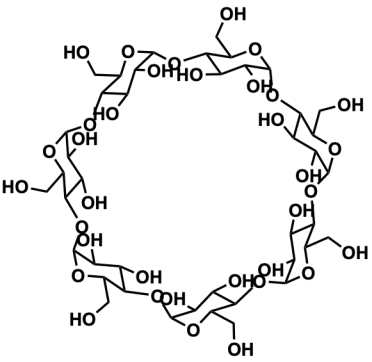
- Protein tagging (Halo-tag, CLIP-tag, Snap-tag, …)
  - ✓ High selectivity in mild conditions
  - ✗ Large size (~ 20-30 kDa) and instability of protein

- Biological host-guest pairs (biotin-avidin, eDHFR-TMP, aptamers, …)
  - ✓ Strong and rapid association (k_{on} ~ 10^9-10^{10} $M^{-1}s^{-1}$)
  - ✗ Large size (~ 10-50 kDa) and instability of protein/oligonucleotides

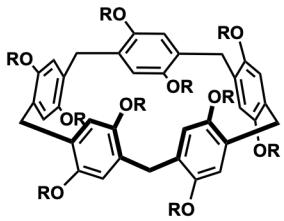
- Synthetic host-guest pairs (β-cyclodextrin, cucurbit[n]uril, …)
  - ✓ Strong and rapid association (k_{on} ~ 10^9-10^{10} $M^{-1}s^{-1}$)
  - ✓ Small(~1 kDa) and stable molecular

Schreiber, C. L.; Smith, B. D. *Nat. Rev. Chem.* **2019**, *3* (6), 393–400.
Sasmal, R.; Das Saha, N.; *et al*. *Anal. Chem.* **2018**, *90* (19), 11305–11314.

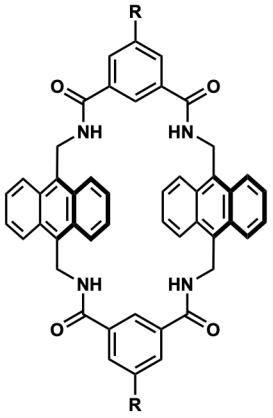
Synthetic host-guest pairs: Cucurbit[n]uril(CB[n]) family is suitable for bioconjugations.



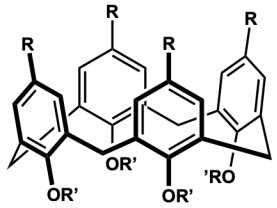
β-Cyclodextrin



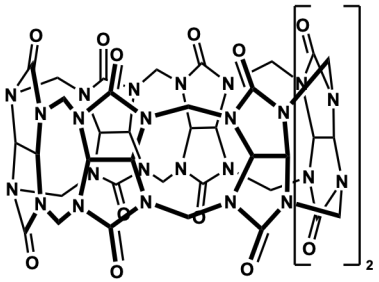
Pillar[5]arene



Tetralactam



Calix[4]arene

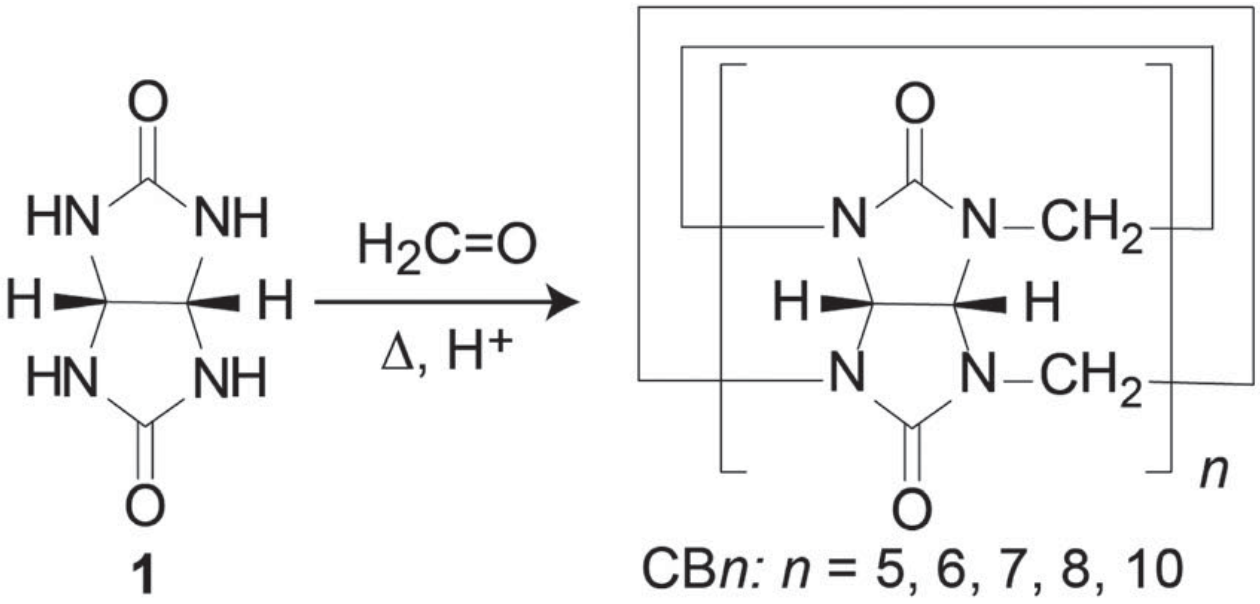


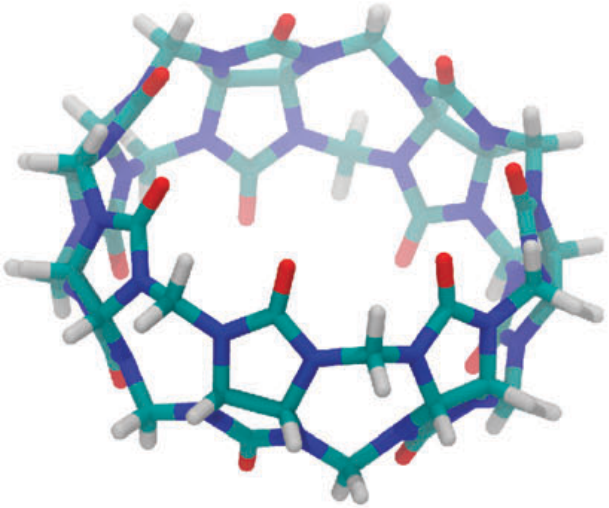
Cucurbit[7]uril

* (Strept)avidin-biotin: $K_d \sim 10^{-15}$

- β-Cyclodextrin – hydrophobic molecule
  - ✗ low affinity ($K_d \sim 10^{-6}$-10^{-3})
- Pillar[n]ene – hydrophobic molecule
  - ✗ low affinity ($K_d \sim 10^{-6}$-10^{-4})
- Tetralactam – squaraine
  - ✓ high affinity ($K_d \sim 10^{-11}$-10^{-7})
- Calixarene – hydrophobic cation
  - ✗ low affinity ($K_d \sim 10^{-6}$-10^{-4})
- Cucurbit[n]uril(CB[n]) – hydrophobic cation
  - ✓ very high affinity ($K_d \sim 10^{-15}$-10^{-9})
  - ✓ wide variety of guests

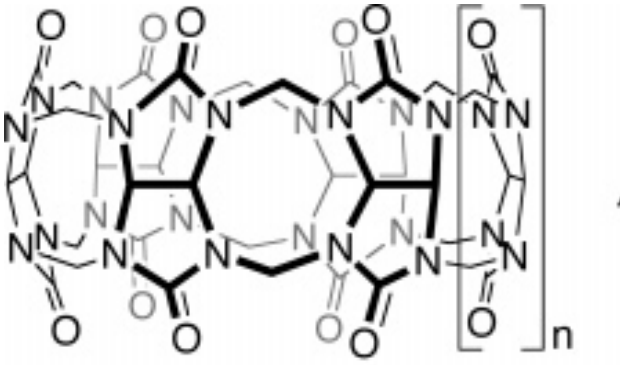
Schreiber, C. L.; Smith, B. D. *Nat. Rev. Chem.* **2019**, *3* (6), 393–400.



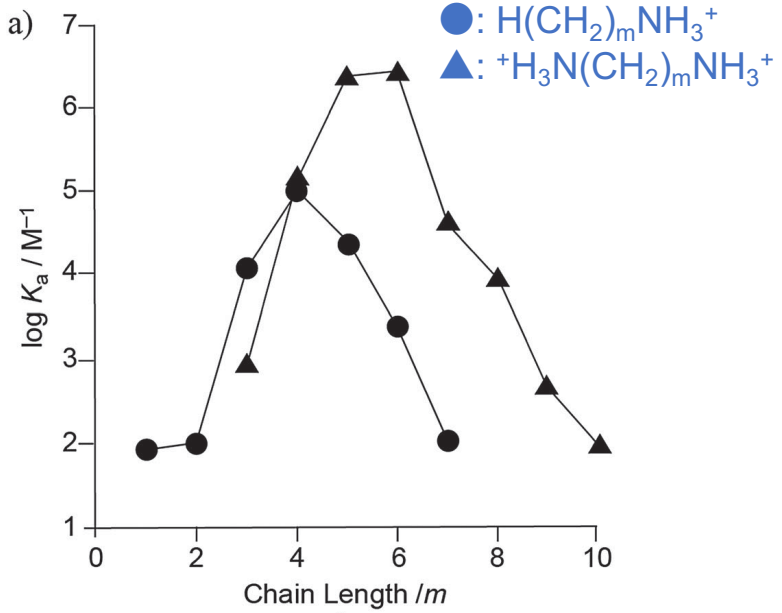


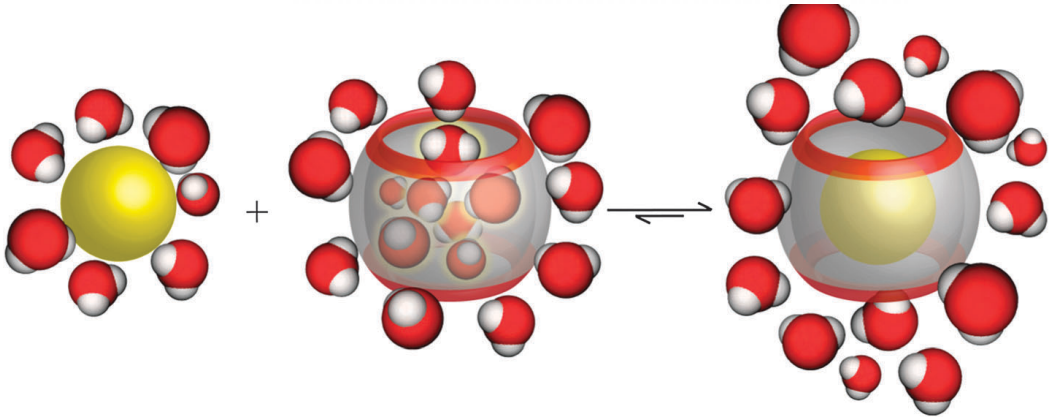
n	MW
5	830.7
6	996.8
7	1162.9
8	1329.1
10	1661.4

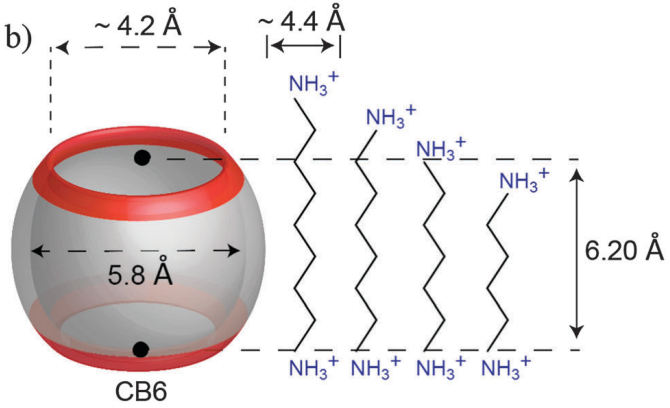
Assaf, K. I.; Nau, W. M. *Chem. Soc. Rev.* **2015**, *44* (2), 394–418.



CB[6] (n = 1)
CB[7] (n = 2)
CB[8] (n = 3)







Main driving forces:

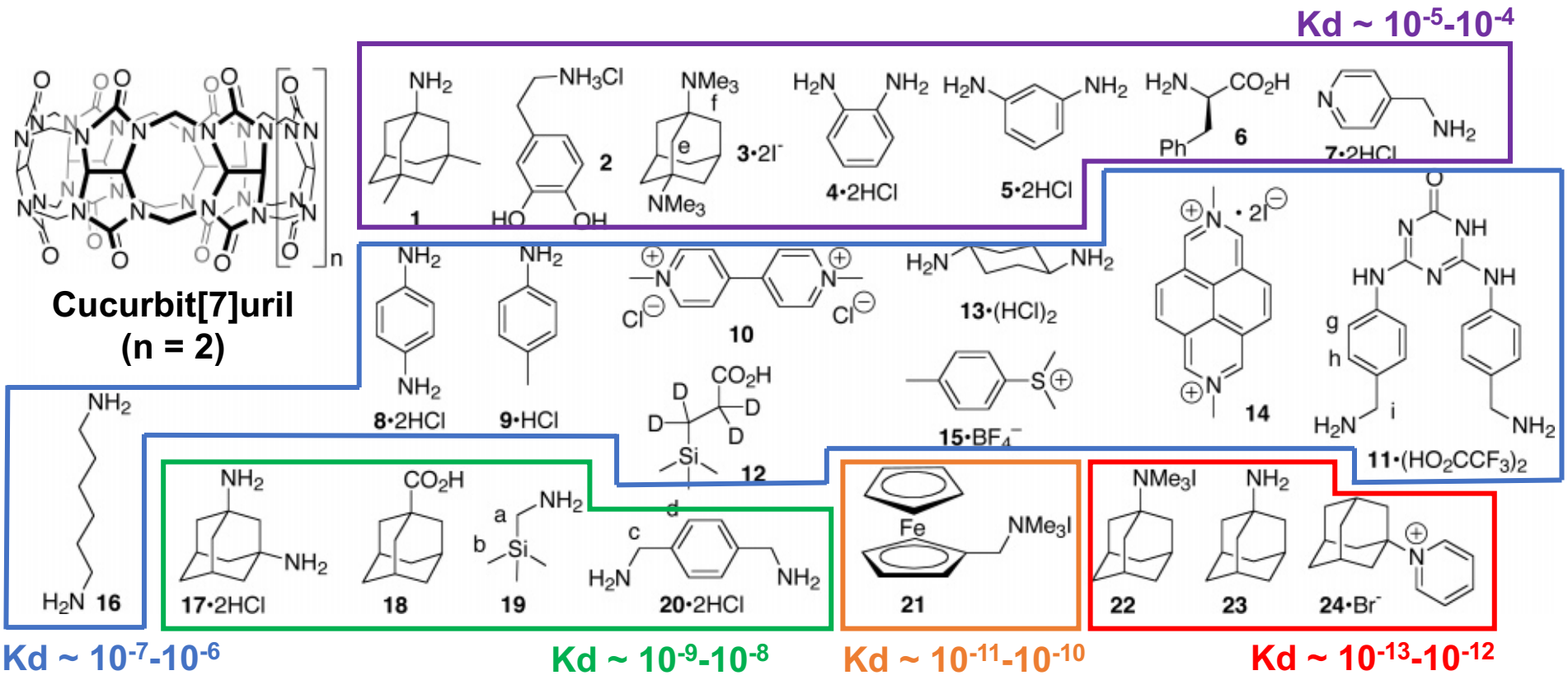
- ion(dipole)-dipole interaction
- hydrophobic effect

→largely depends on the cavity size

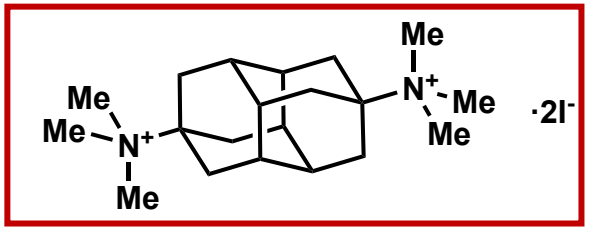
Assaf, K. I.; Nau, W. M. *Chem. Soc. Rev.* **2015**, *44* (2), 394–418.

CB[7] has wide range of affinity to various types of guests.

Affinity in 50 mM NaO_2CCD_3-buffered D_2O (pD=4.74)



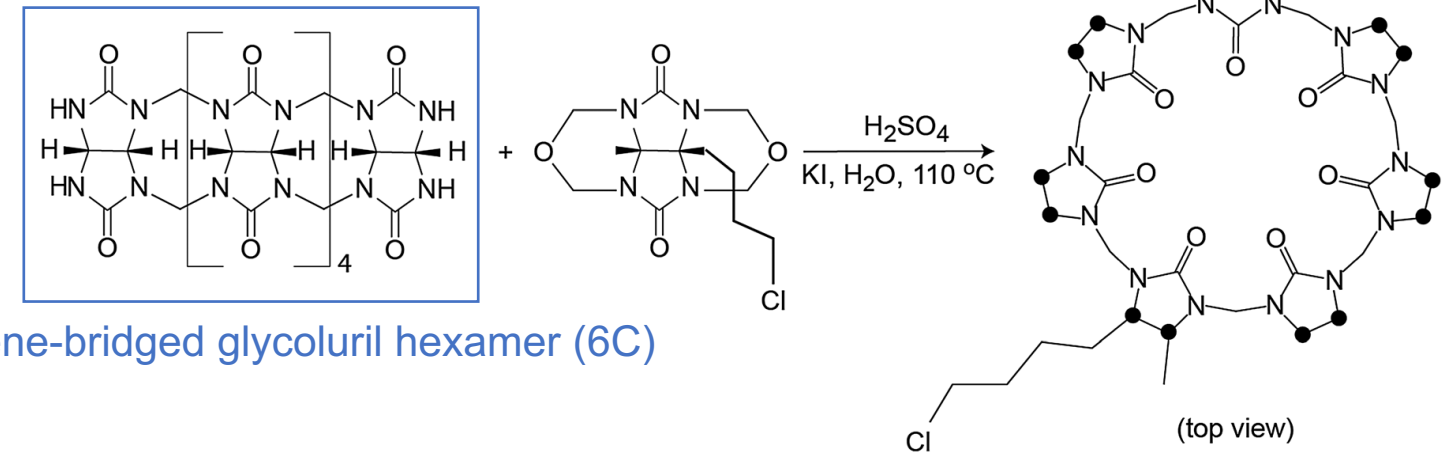
J. Am. Chem. Soc. **2005**, *127*, 15959–15967. (modified)



- K_d value ranges from 10^4 to 10^{15}.

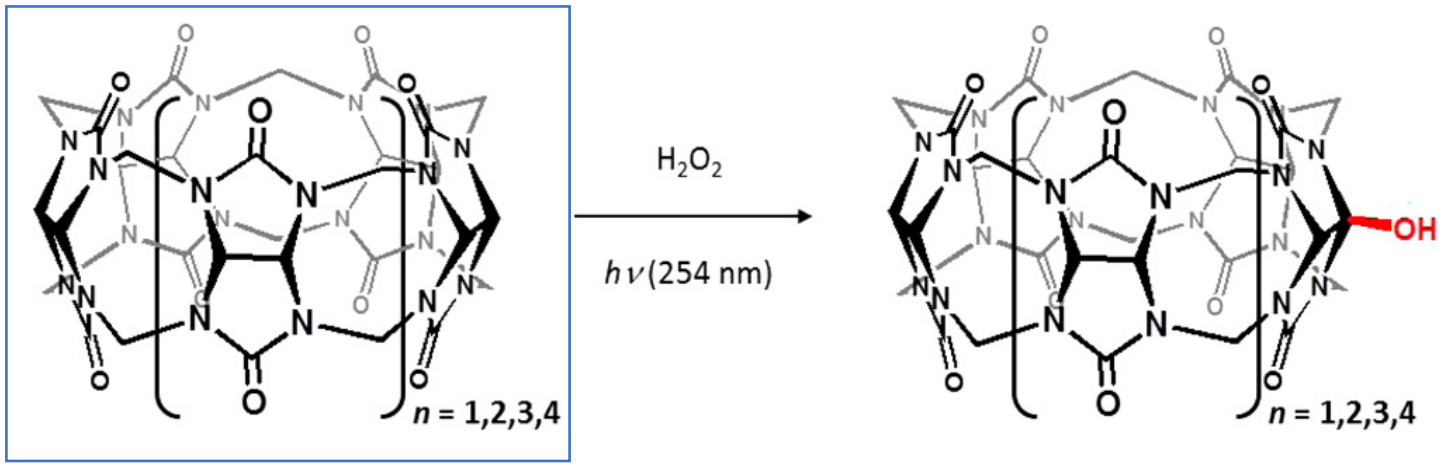
Cao, L.; Šekutor, M.; Zavalij, P. Y.; Mlinarič-Majerski, K.; Glaser, R.; Isaacs, L. *Angew. Chemie - Int. Ed.* **2014**, *53* (4), 988–993.
Liu, S.; Ruspic, C.; Mukhopadhyay, P.; Chakrabarti, S.; Zavalij, P. Y.; Isaacs, L. *J. Am. Chem. Soc.* **2005**, *127* (45), 15959–15967.

Monofunctionalized CB[n] derivatives are accessible.



Methylene-bridged glycoluril hexamer (6C)

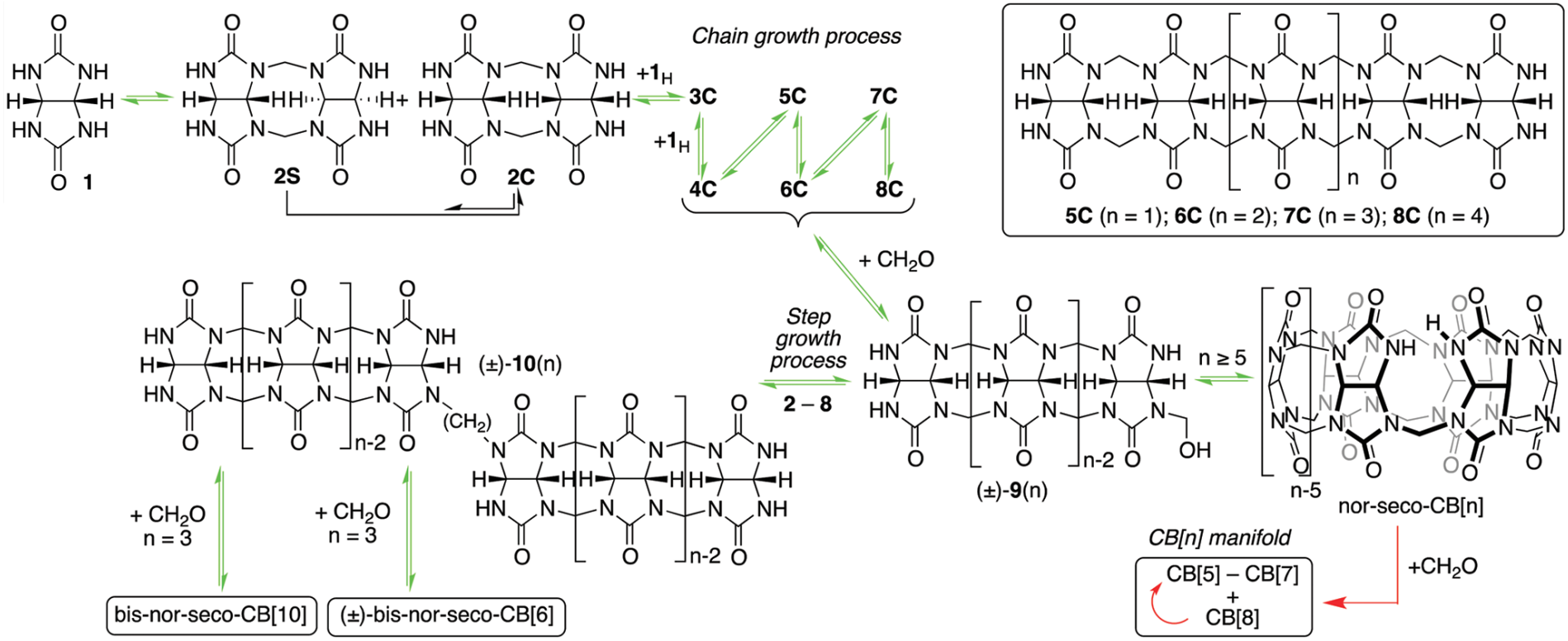
Vinciguerra, B.; Cao, L.; *et al*. *J. Am. Chem. Soc.* **2012**, *134* (31), 13133–13140.



Cucurbit[n]uril (n = 5, 6, 7, 8)

Ayhan, M. M.; Karoui, H.; *et al*. *J. Am. Chem. Soc.* **2015**, *137* (32), 10238–10245.

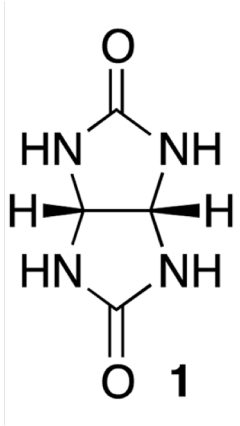
Synthesis of 6C and CB[n] (1): Mechanism of CB[n] formation



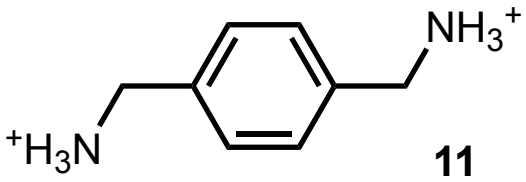
- CB[n] (n = 5, 6, 7, 8, 10) is synthesized by the condensation reaction of glycoluril **1** and formaldehyde under strongly acidic conditions.
- Each CB[n] was purified by fractional crystallization and dissolution.

Lucas, D.; Minami, T.; Iannuzzi, G.; Cao, L.; Wittenberg, J. B.; Anzenbacher, P.; Isaacs, L. *J. Am. Chem. Soc.* **2011**, *133* (44), 17966–17976.

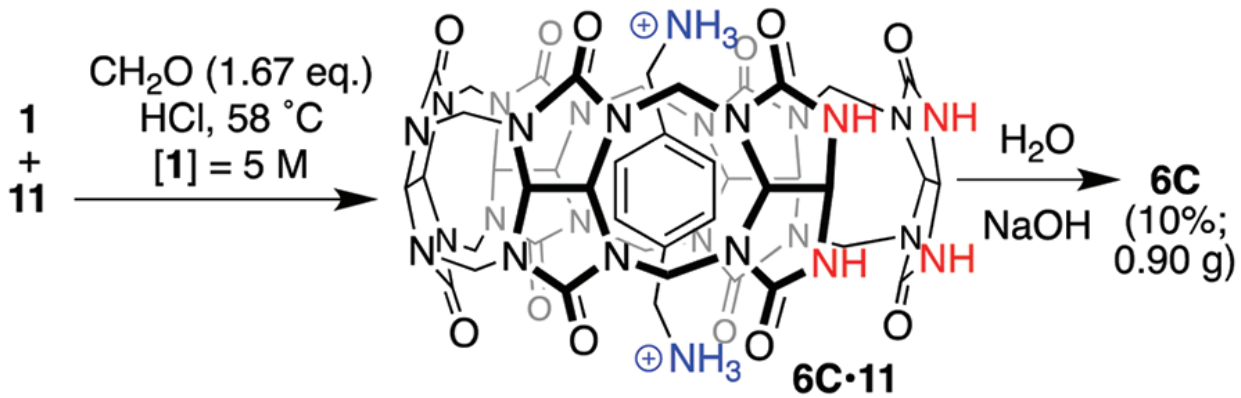
Synthesis of 6C and CB[n] (2): Template synthesis of 6C



1



11

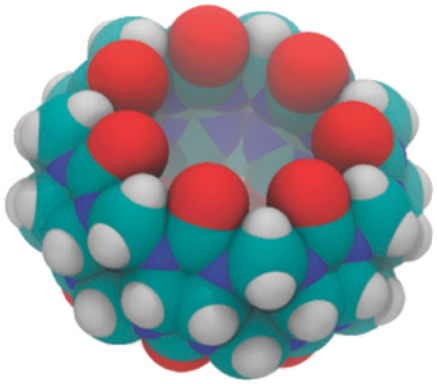


K_d(**6C**·**11**) = 4.5 × 10^{-8} M

K_d(CB[6]·**11**) = 1.8 × 10^{-3} M

- Diammmonium ion **11** acts as template.
- **11** slows down transformation of **6C** and paraformaldehyde into CB[6] by forming stable **6C**·**11** complex.

Lucas, D.; Minami, T.; Iannuzzi, G.; Cao, L.; Wittenberg, J. B.; Anzenbacher, P.; Isaacs, L. *J. Am. Chem. Soc.* **2011**, *133* (44), 17966–17976.



Cucurbit[n]uril

- Wide range of affinity
- Monofunctionalized derivative
- High water solubility
- High stability
- Low toxicity
- Bio-orthogonal binding

catalysis

molecular machine

material fabrication

bio-sensing

protein immobilization

protein modification

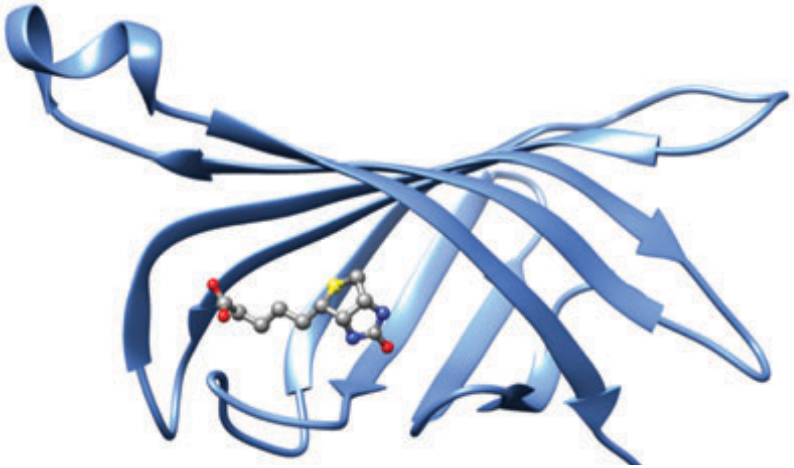
gas purification

pre-targeting therapy

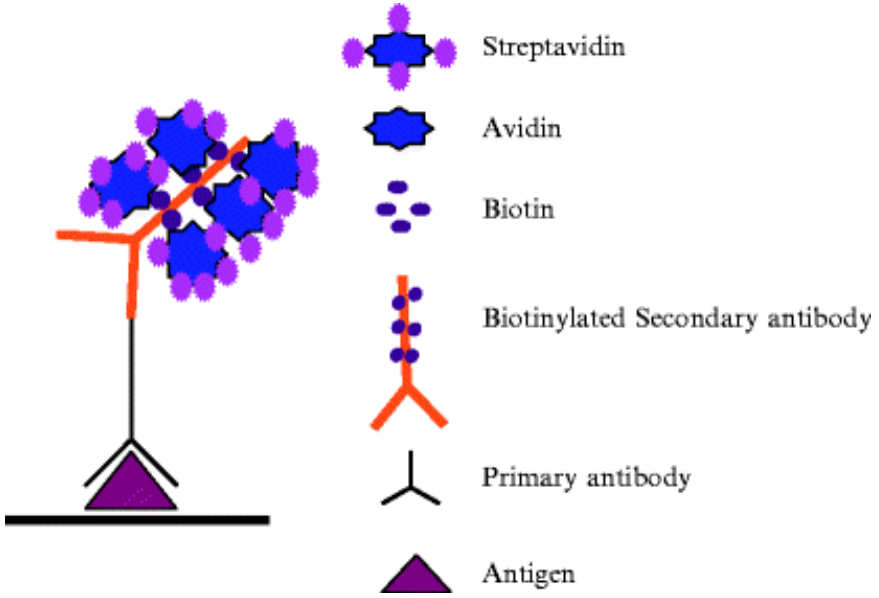
imaging

drug delivery

(Strept)avidin-biotin based bioimaging



Streptavidin: 53 kDa(tetramer)

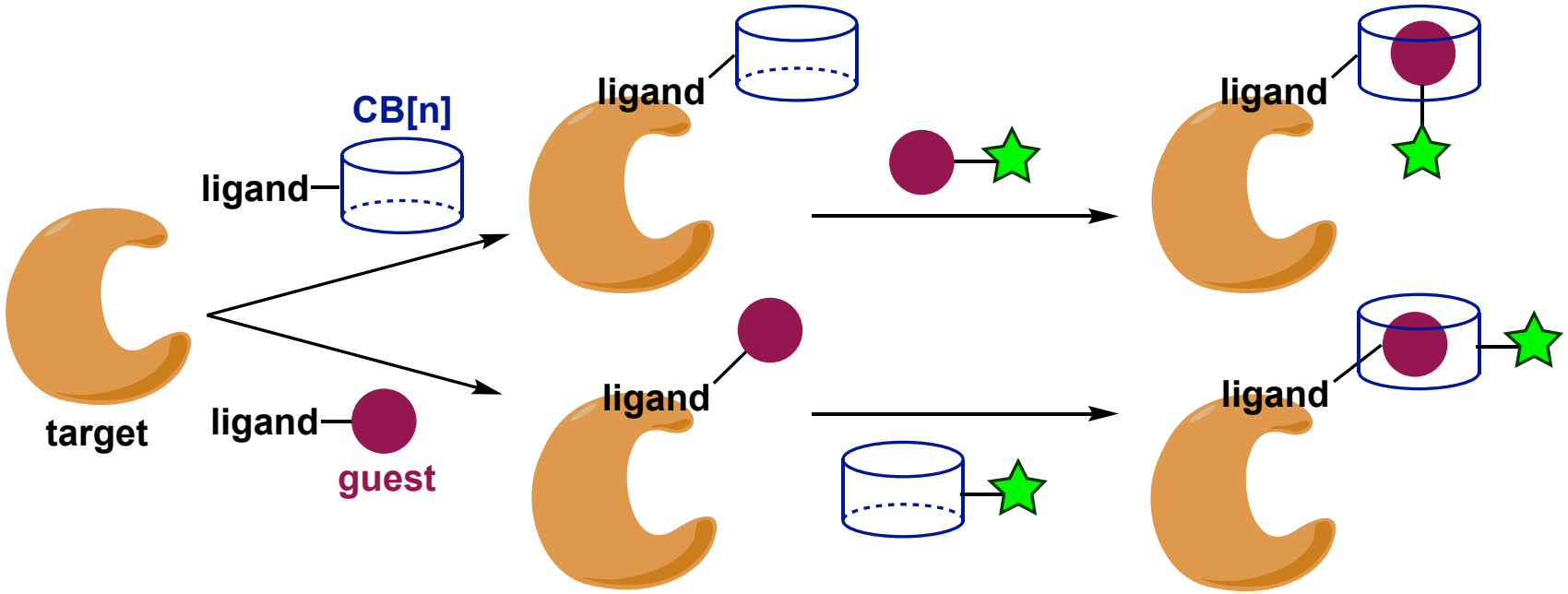


- ✓ high affinity (Kd ~ 10^{-15} M)
- ✓ rapid association
- ✓ signal amplification
- ✓ many applications

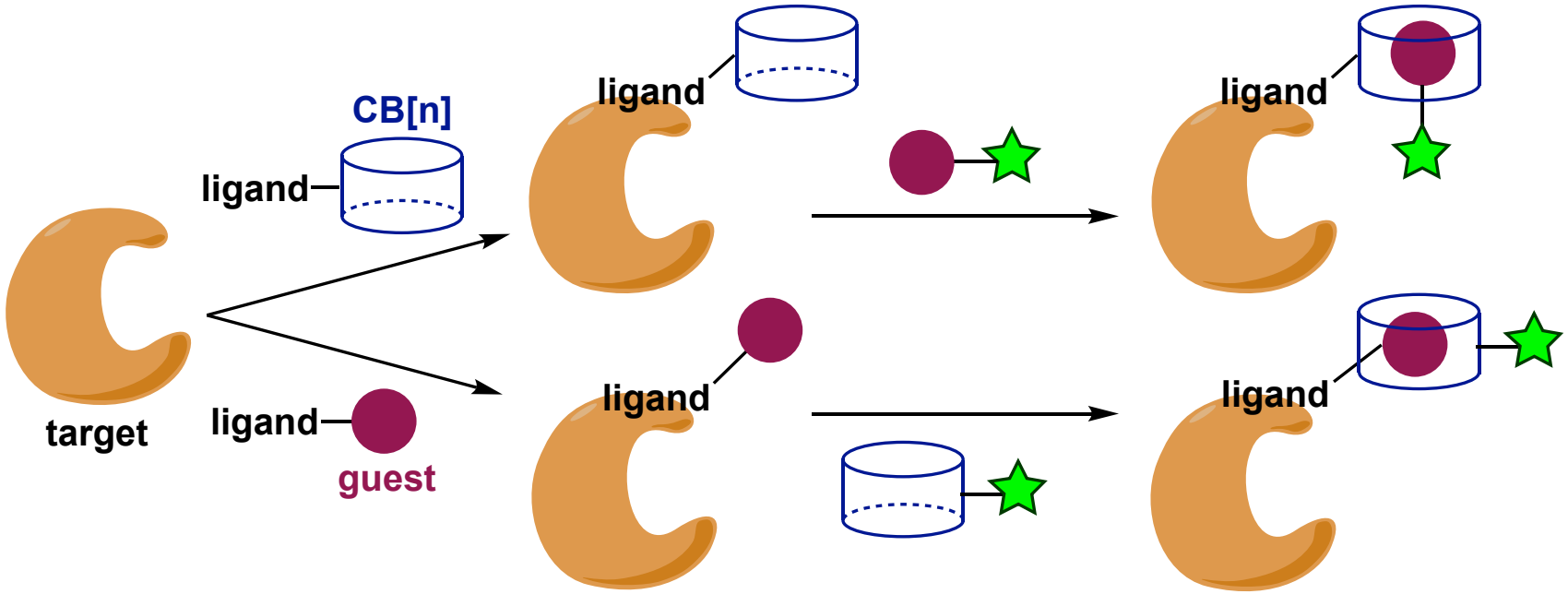
- ✗ effect of endogenous biotin
- ✗ low cell permeability
- ✗ enzymatic degradation
- ✗ low resolution
- ✗ irreversible binding
- ✗ high cost

Liu, W.; Samanta, S. K.; Smith, B. D.; Isaacs, L. *Chem. Soc. Rev.* **2017**, *46* (9), 2391–2403.

Kim, K. L.; Sung, G.; Sim, J.; Murray, J.; Li, M.; Lee, A.; Shrinidhi, A.; Park, K. M.; Kim, K. *Nat. Commun.* **2018**, *9* (1).



1. Characteristics of CB[n] based bioimaging
   a. Specificity
   b. Cell permeability: live cell imaging
   c. Stability
2. Further applications
   a. Combination with gold nanoparticle
   b. Cell surface specific labeling
   c. Super-resolution imaging



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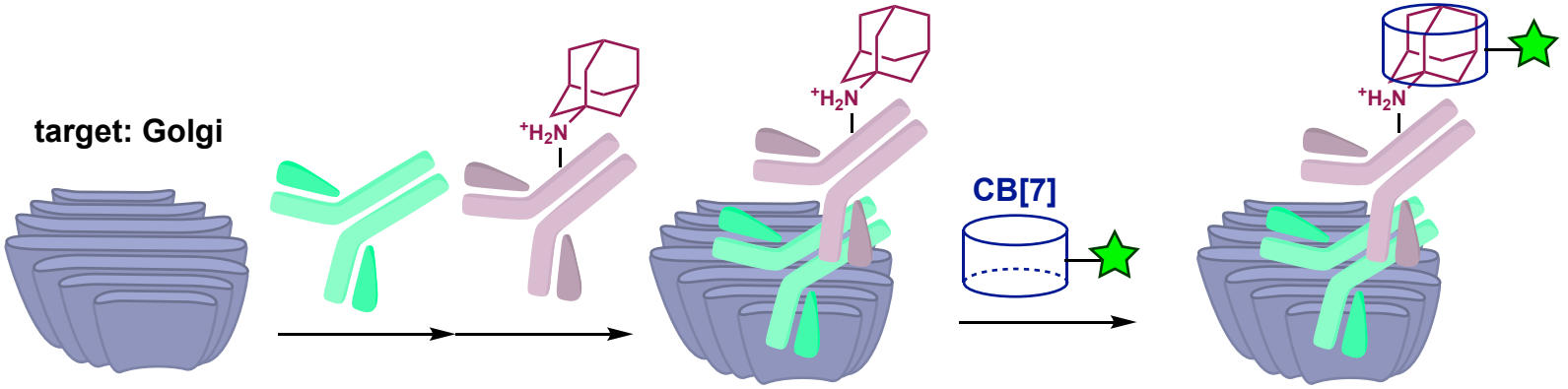

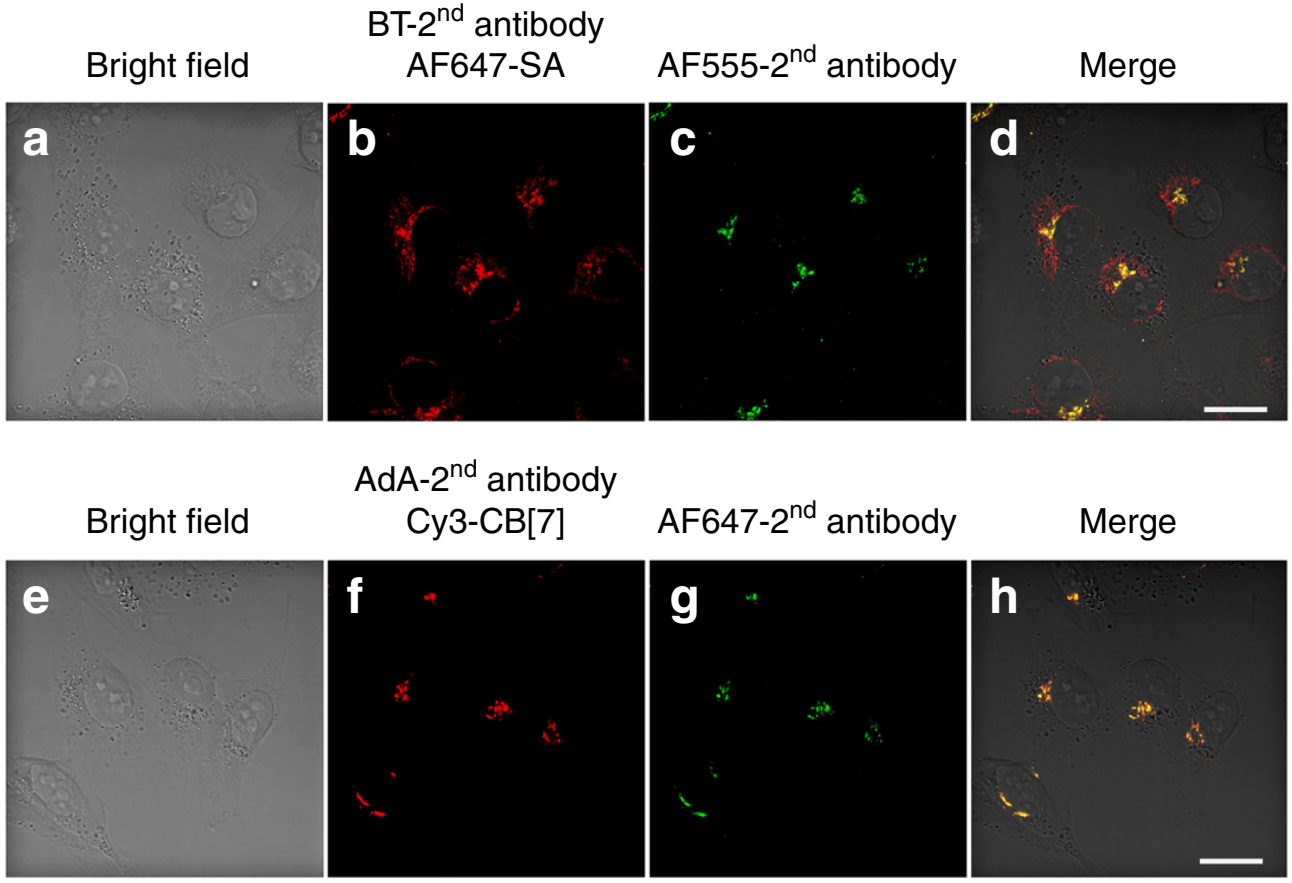

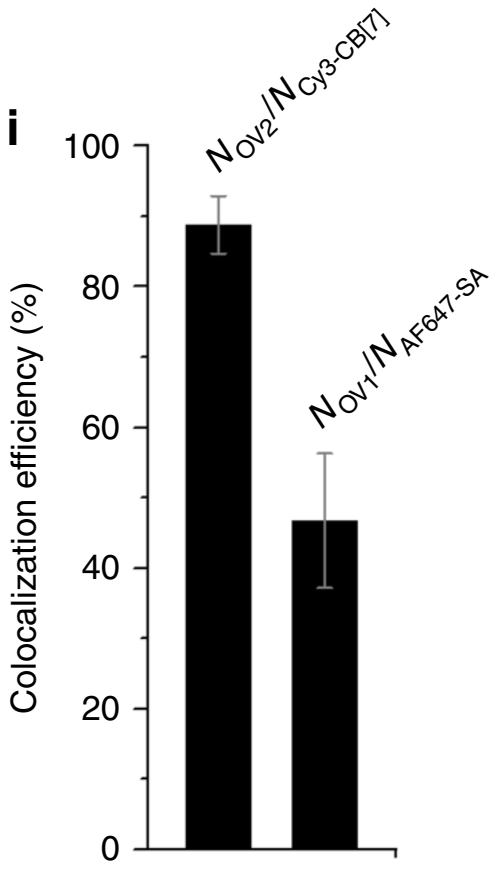

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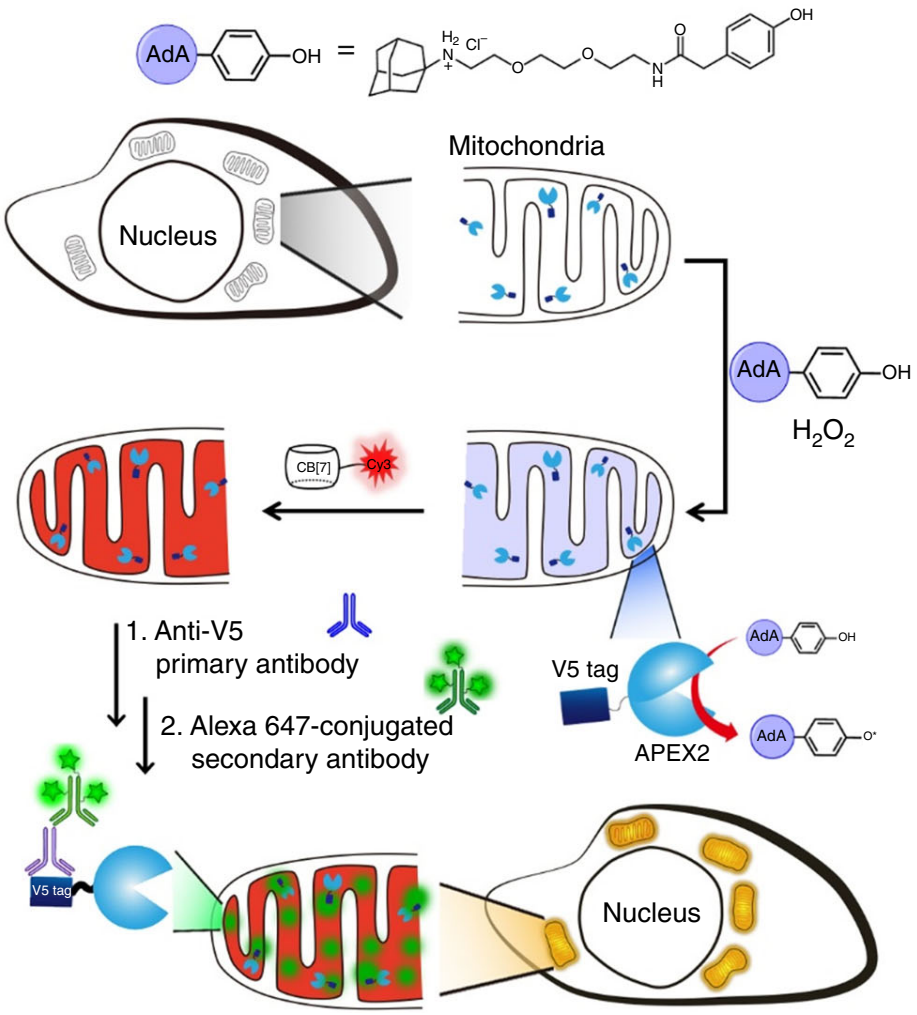

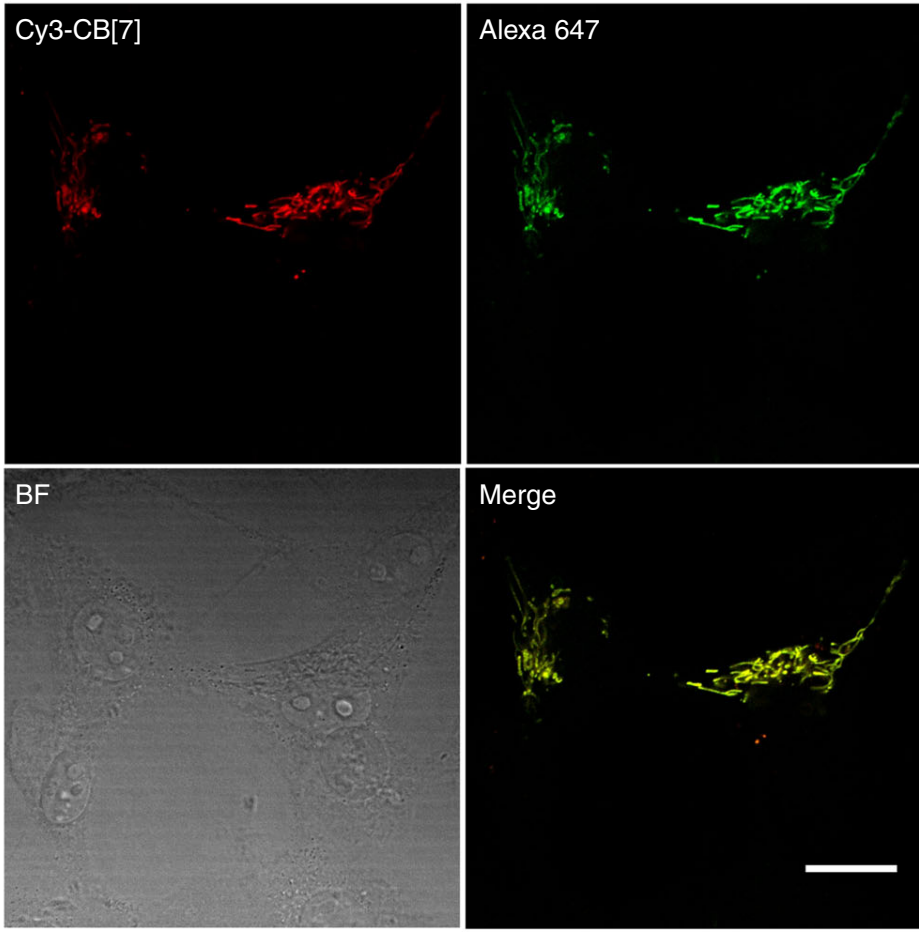

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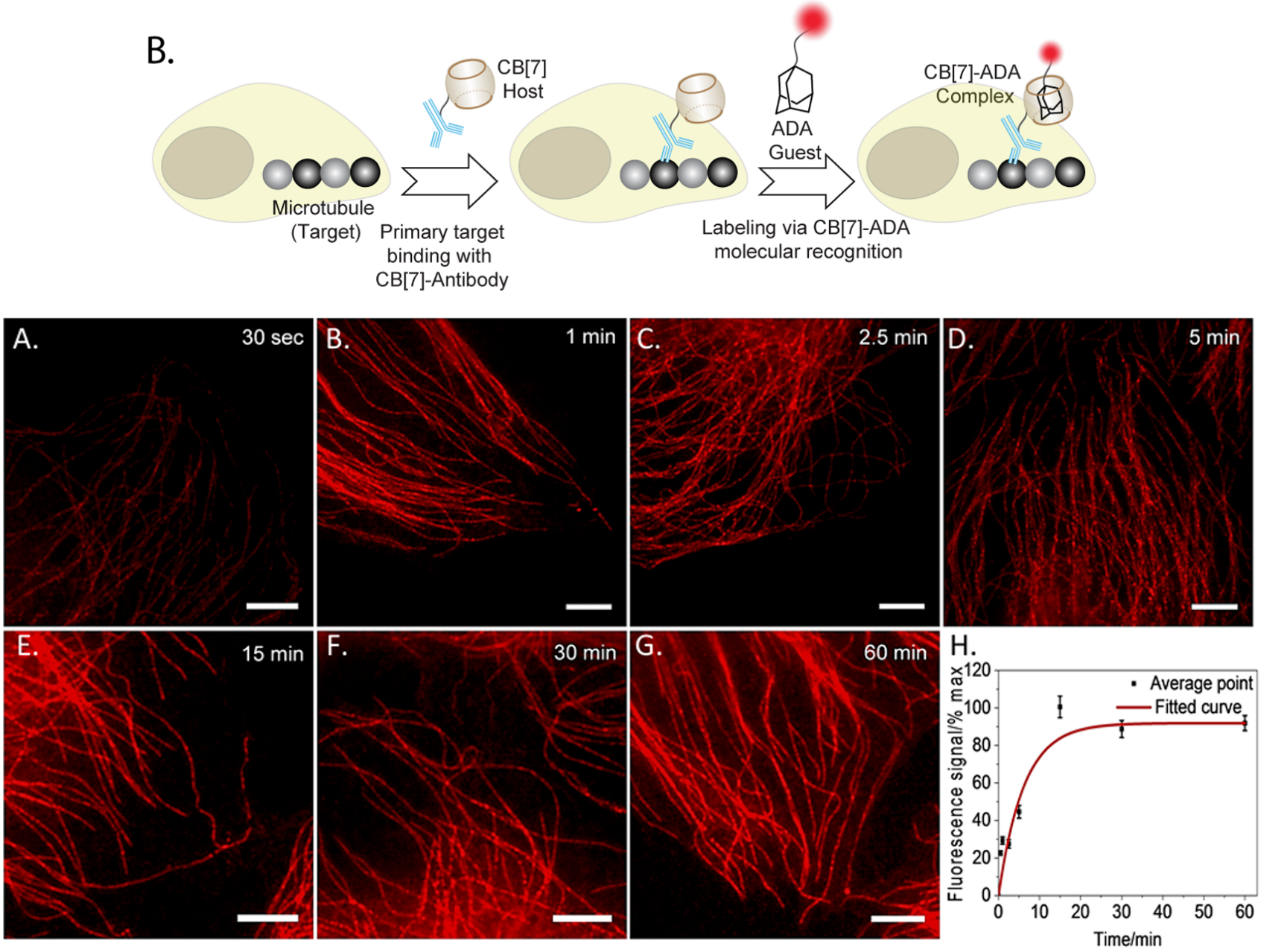
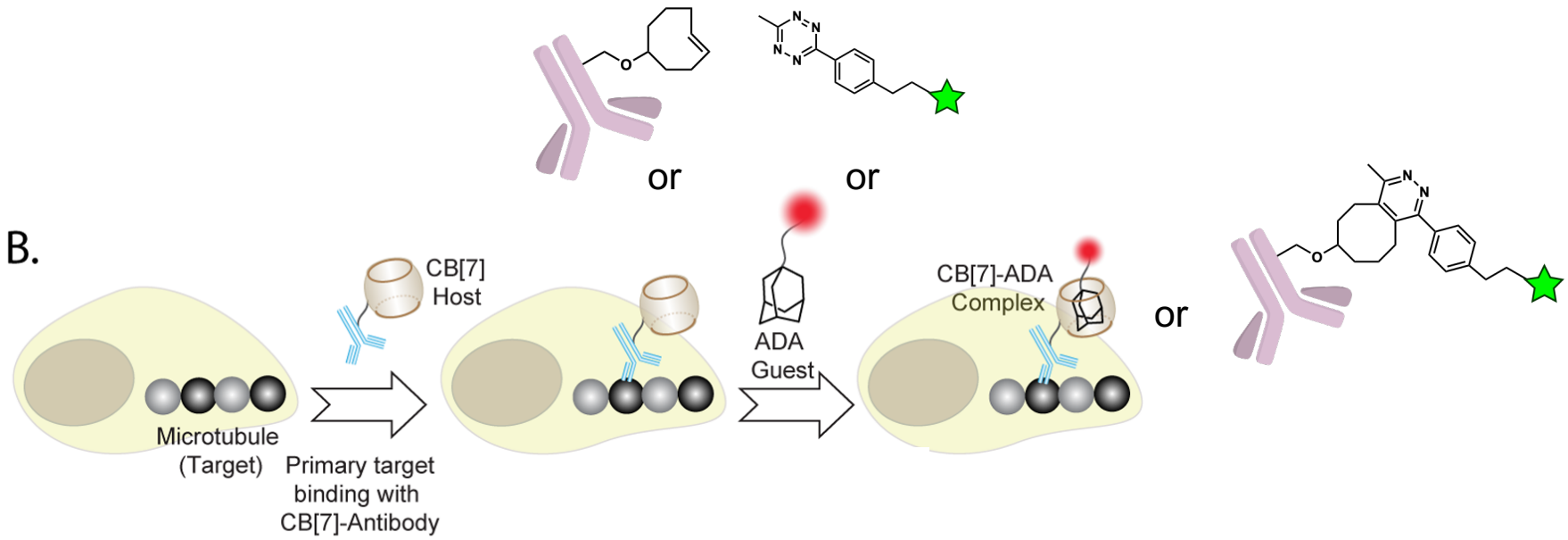
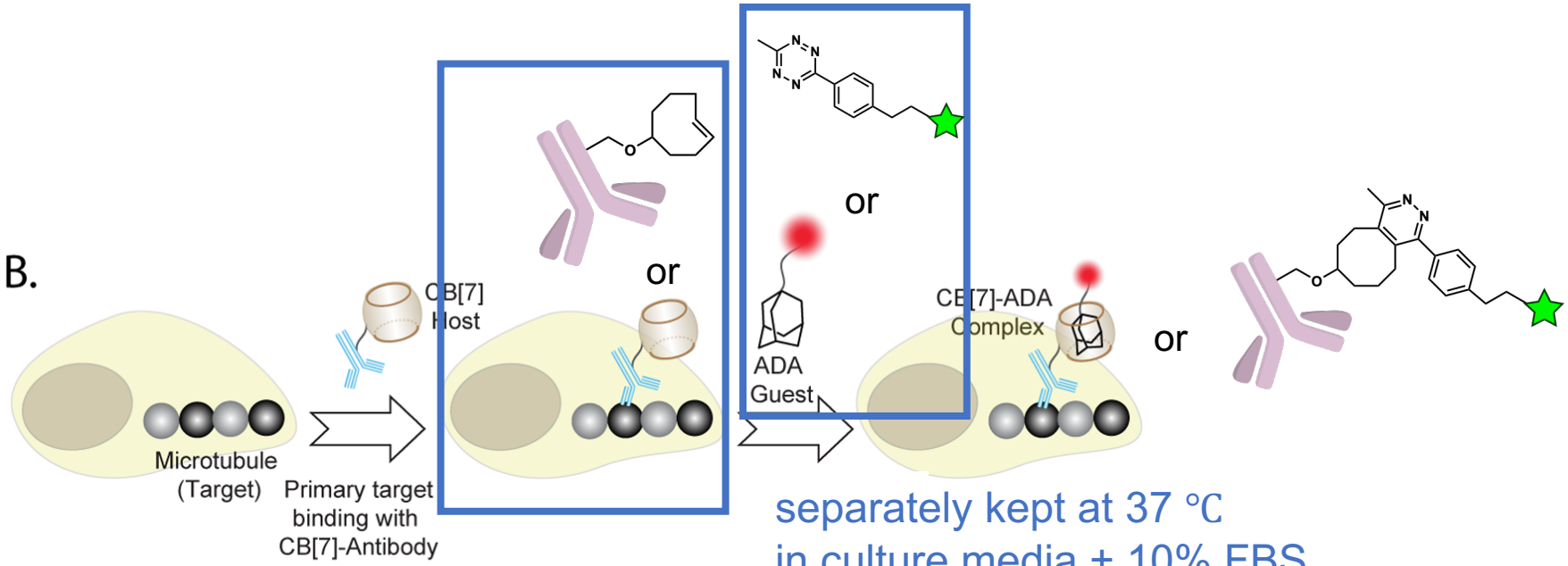


Sasmal, R.; Das Saha, N.; *et al*. *Anal. Chem.* **2018**, *90* (19), 11305–11314.

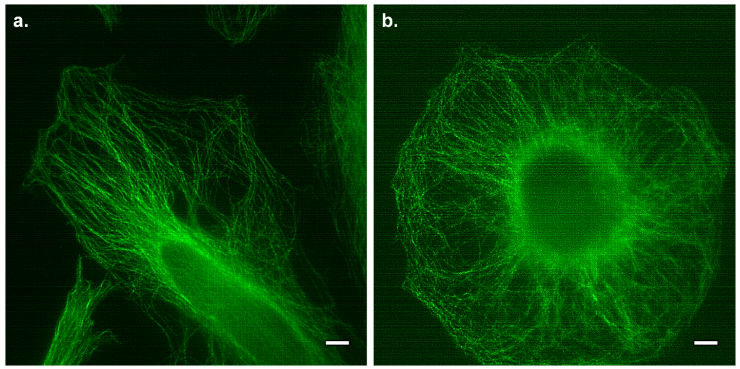


Sasmal, R.; Das Saha, N.; *et al*. *Anal. Chem.* **2018**, *90* (19), 11305–11314.



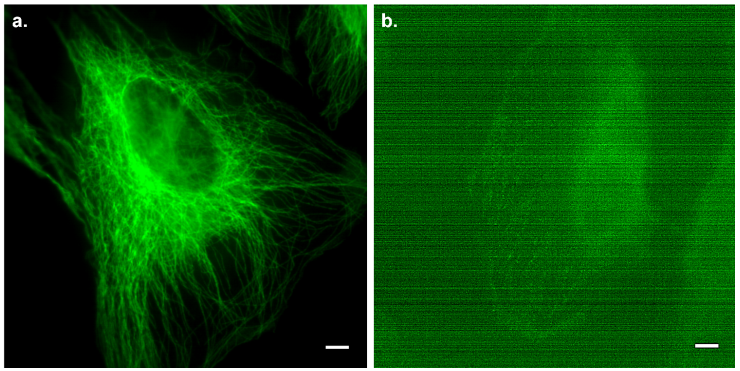
separately kept at 37 ℃
in culture media + 10% FBS
for 0 or 16 h

CB[7]-ADA



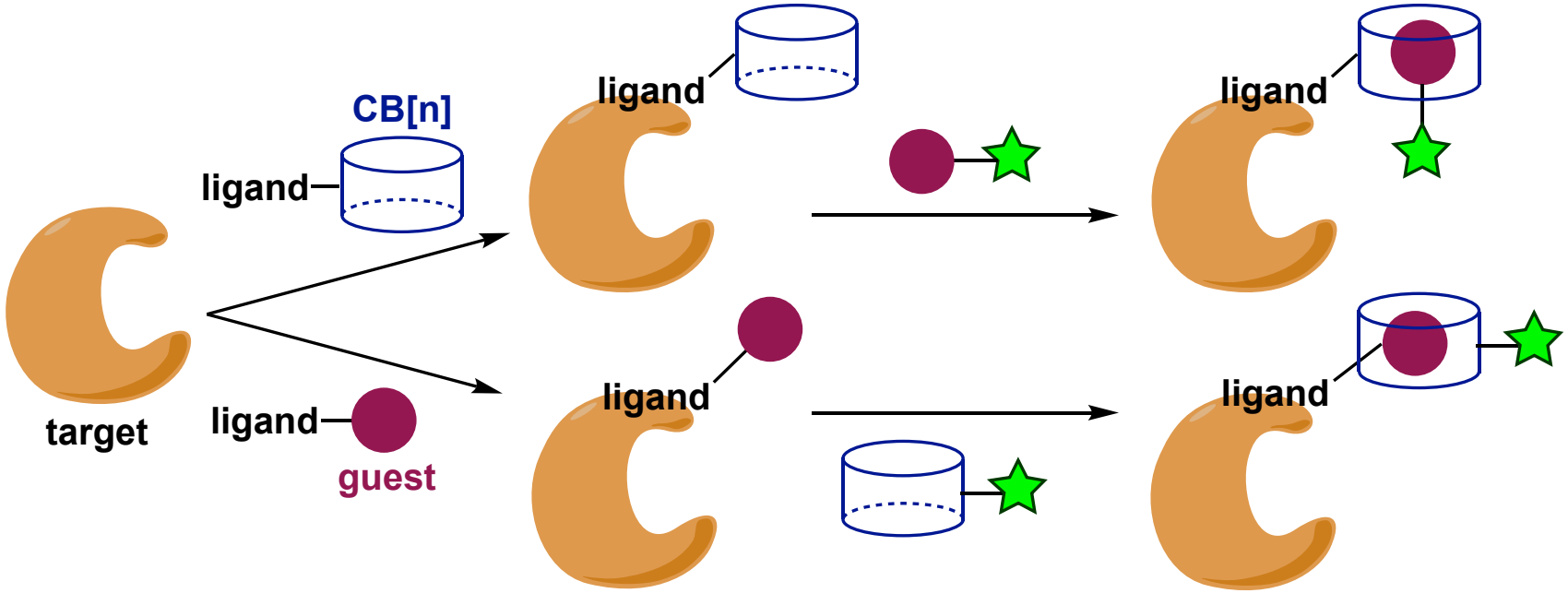
0 h 16 h

TCO-Tz



0 h 16 h

Sasmal, R.; Das Saha, N.; *et al*. *Anal. Chem.* **2018**, *90* (19), 11305–11314.



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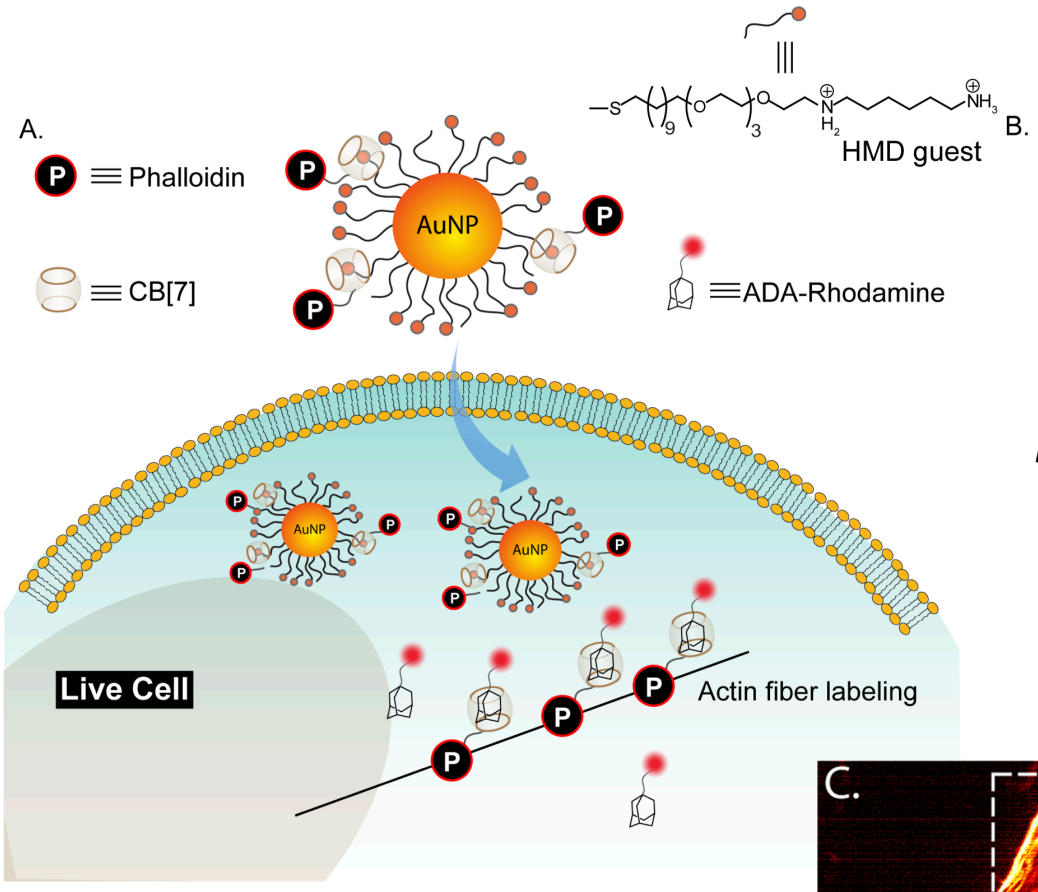
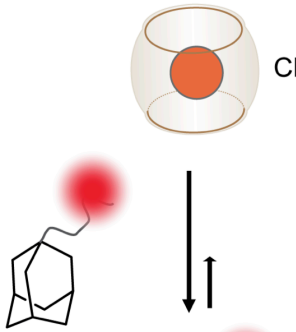
   b. **Cell surface specific labeling**
   c. **Super-resolution imaging**

2-a. Combination with gold-nanoparticle



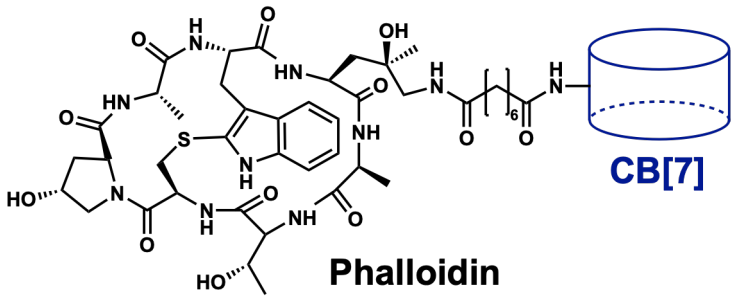


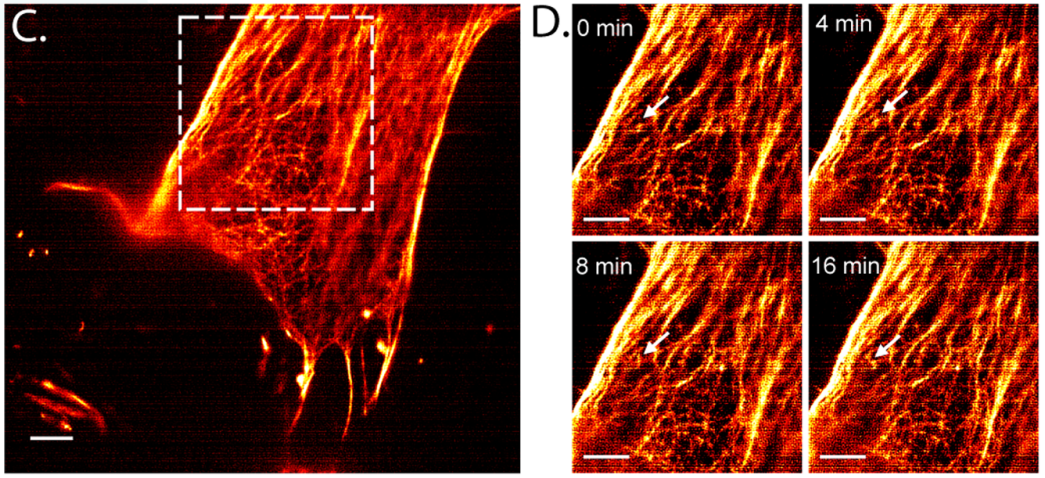
CB[7]-HMD Complex

$K_d \sim 10^{-7}$ M

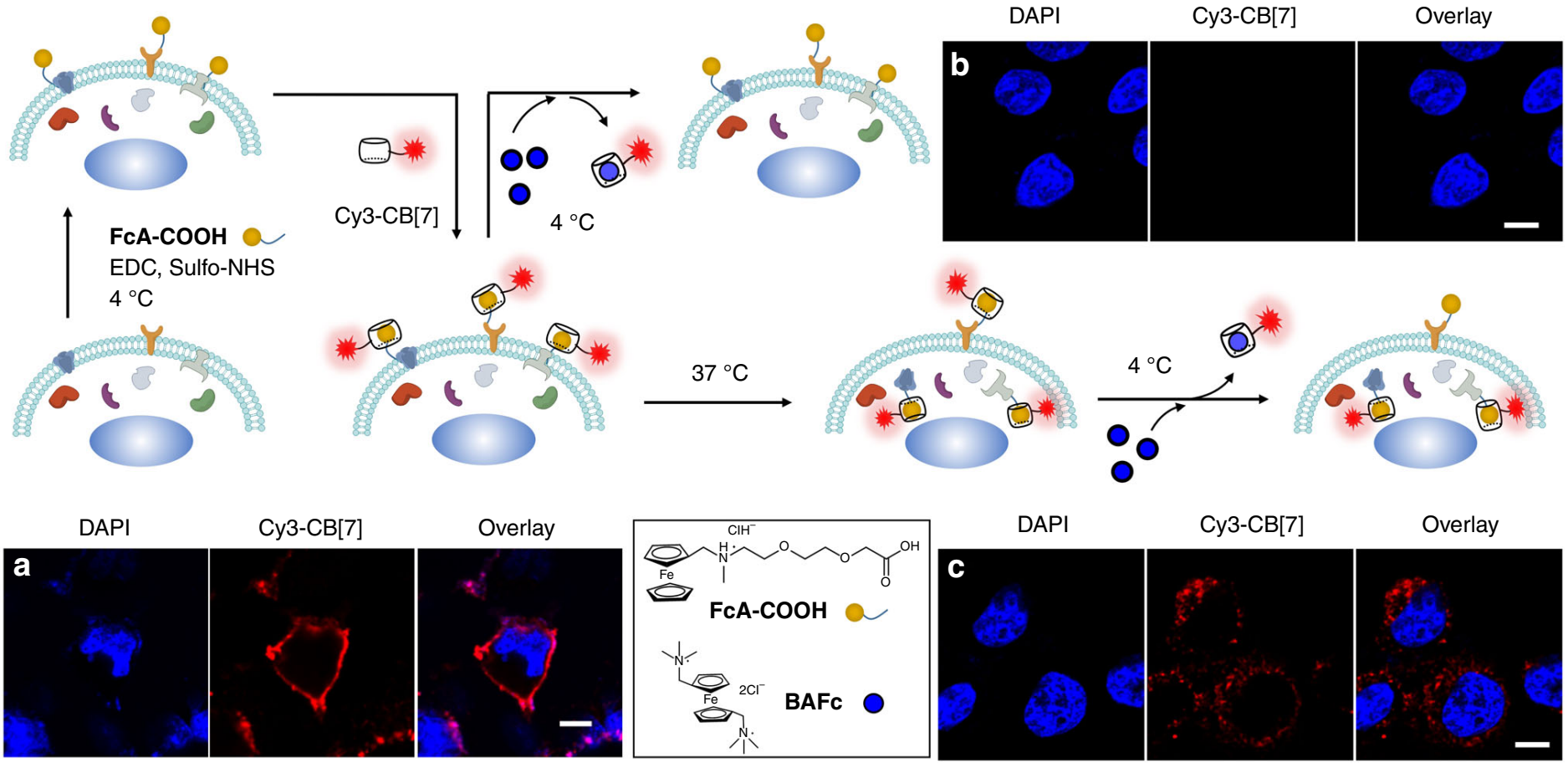
CB[7]-ADA Complex

$K_d \sim 10^{-13}$ M





Sasmal, R.; Das Saha, N.; *et al*. *Anal. Chem.* **2018**, *90* (19), 11305–11314.

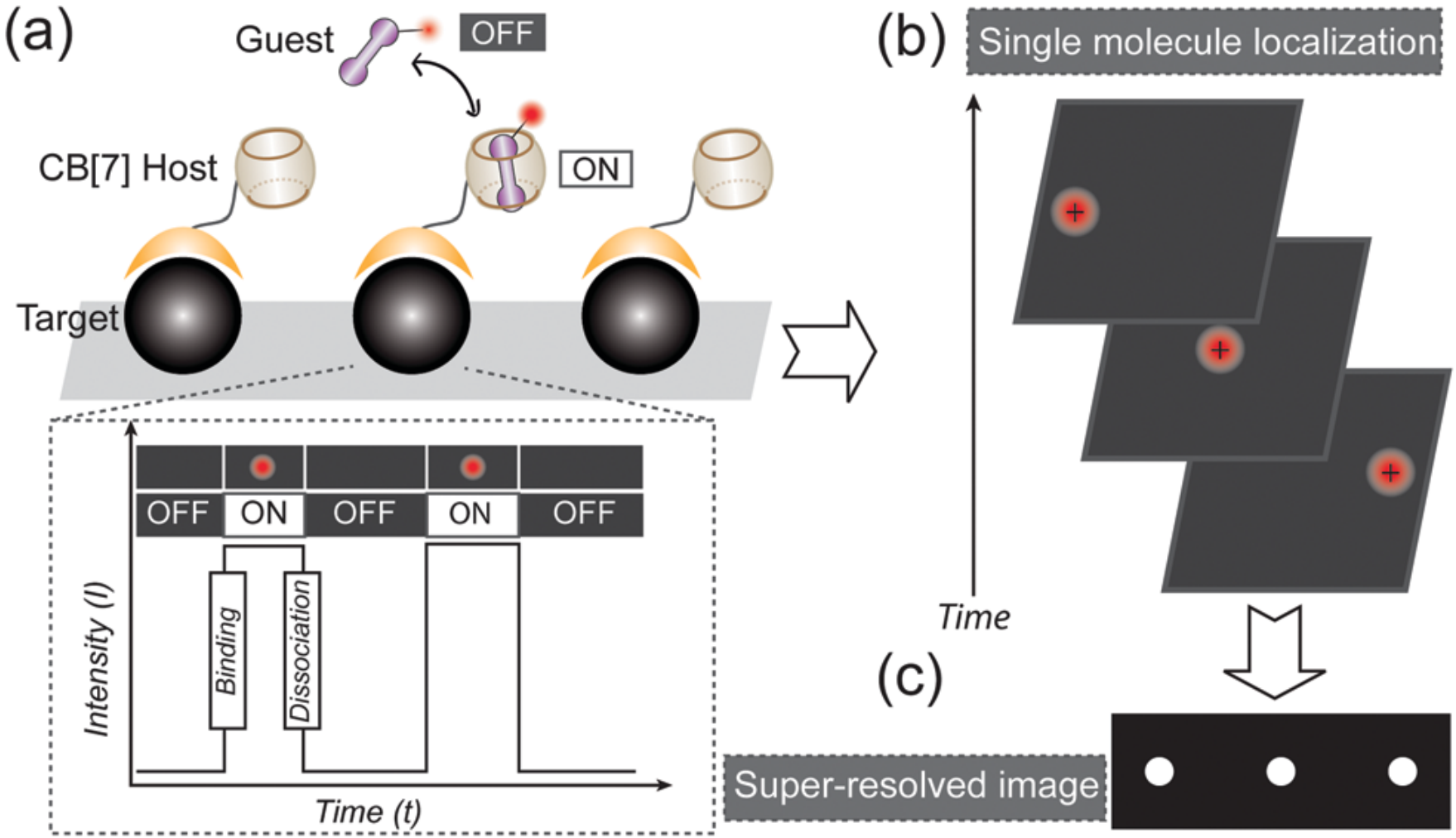


K_d(CB[7]-FcA-COOH) ~ 10^{-10} M

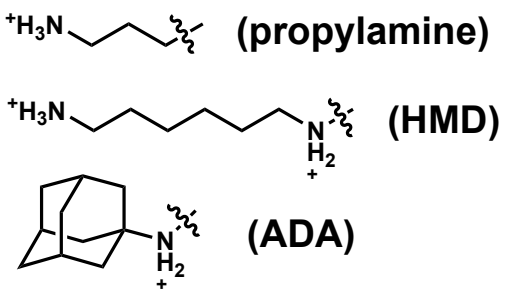
K_d(CB[7]-BAFc) ~ 10^{-13} M

Kim, K. L.; Sung, G.; Sim, J.; Murray, J.; Li, M.; Lee, A.; Shrinidhi, A.; Park, K. M.; Kim, K. *Nat. Commun.* **2018**, *9* (1).

PAINT: Points Accumulation for Imaging in Nanoscale Topography

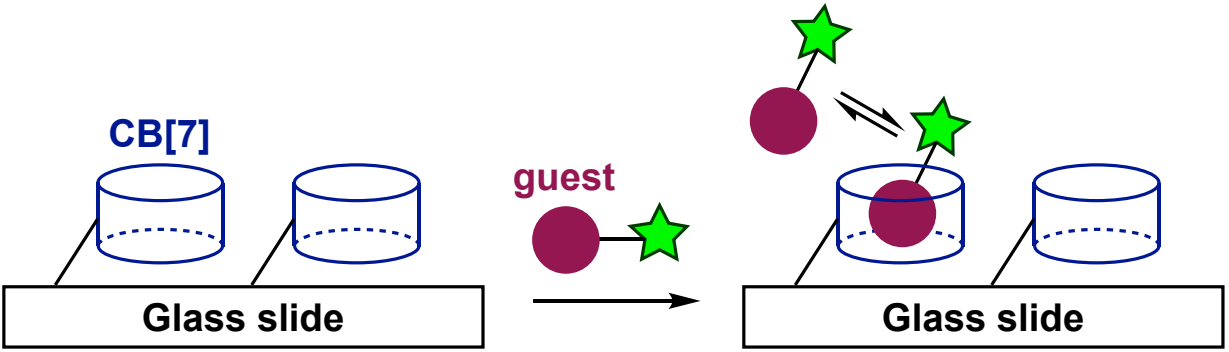


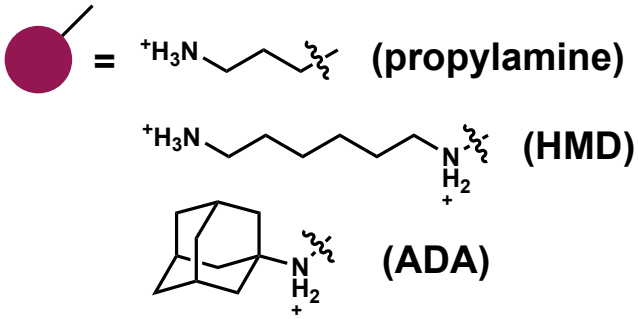
Sasmal, R.; Das Saha, N.; Schueder, F.; Joshi, D.; Sheeba, V.; Jungmann, R.; Agasti, S. S. *Chem. Commun.* **2019**, *55* (96), 14430–14433.



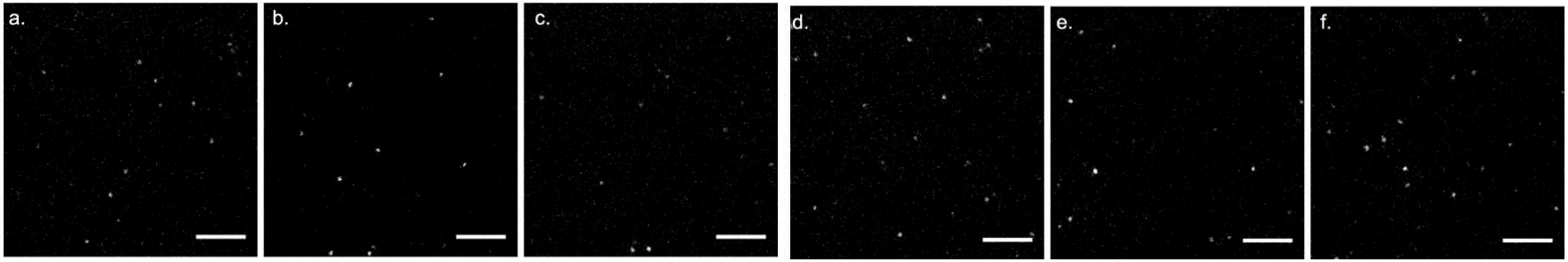
guest	K_d [M]	k_{on} [$M^{-1}s^{-1}$]	$k_{on} \cdot K_d = k_{off}$ [s^{-1}]	$\tau_b = k_{off}^{-1}$ [ms] (residence time)
prolylamine	10^{-4}-10^{-3}	10^{8}	10^{5}-10^{6}	0.01-0.001
HMD	10^{-7}-10^{-6}	10^{8}	10^{1}-10^{2}	10-100
ADA	10^{-13}-10^{-12}	10^{8}	10^{-5}-10^{-6}	10^{7}-10^{8}

Sasmal, R.; Das Saha, N.; Schueder, F.; Joshi, D.; Sheeba, V.; Jungmann, R.; Agasti, S. S. *Chem. Commun.* **2019**, *55* (96), 14430–14433.



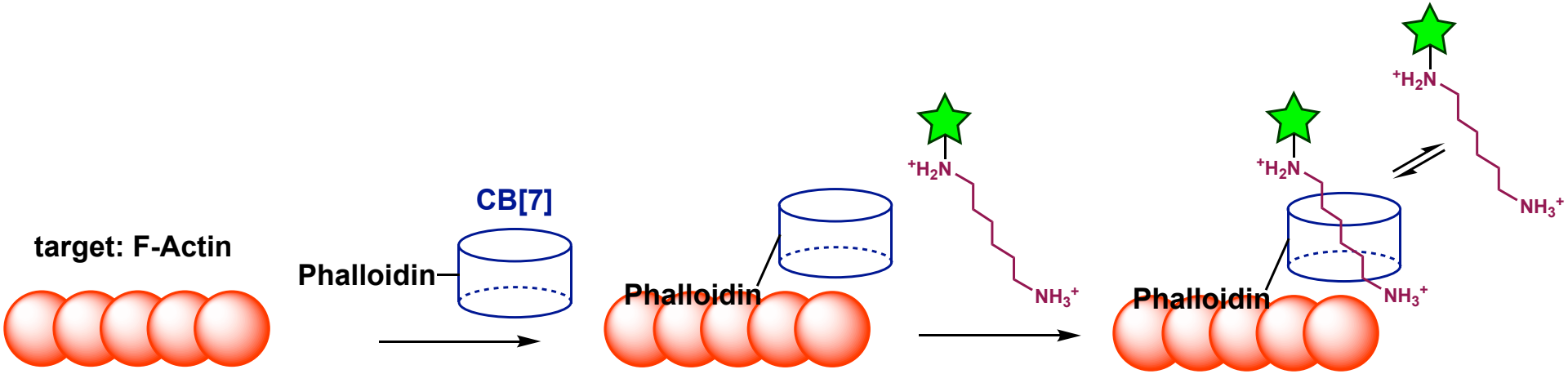


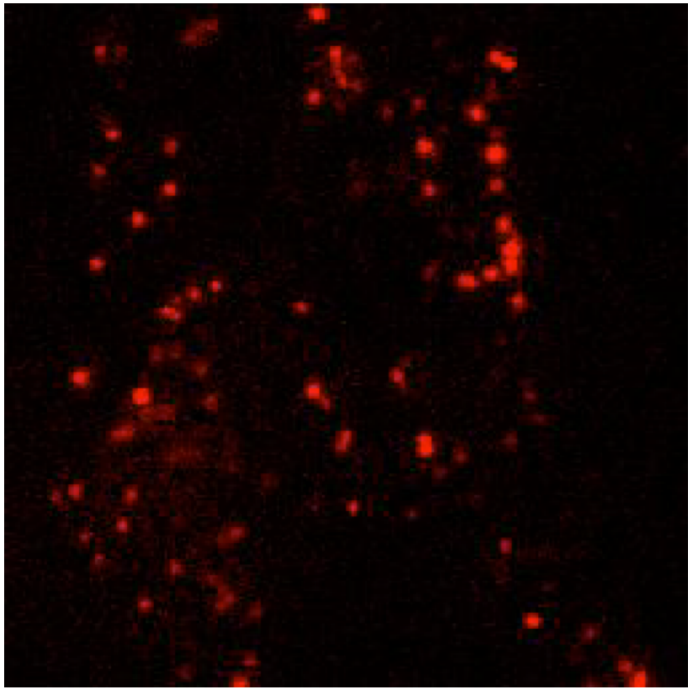
HMD (interval 1 min)

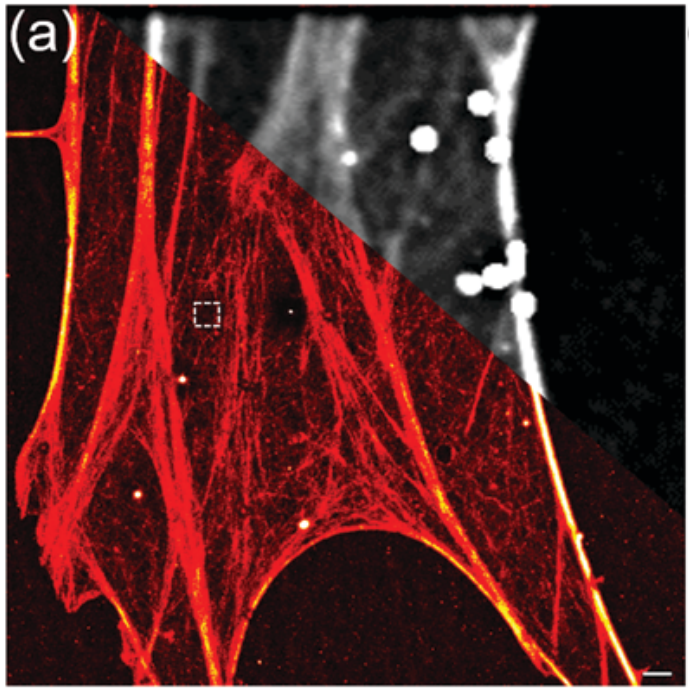


guest	K_d [M]	k_{on} [$M^{-1}s^{-1}$]	$k_{on} \cdot K_d = k_{off}$ [s^{-1}]	$\tau_b = k_{off}^{-1}$ [ms] (residence time)	result
prolylamine	10^{-4}-10^{-3}	10^{8}	10^{5}-10^{6}	0.01-0.001	no fluorescence
HMD	10^{-7}-10^{-6}	10^{8}	10^{1}-10^{2}	10-100	ON/OFF switching
ADA	10^{-13}-10^{-12}	10^{8}	10^{-5}-10^{-6}	10^{7}-10^{8}	bleach

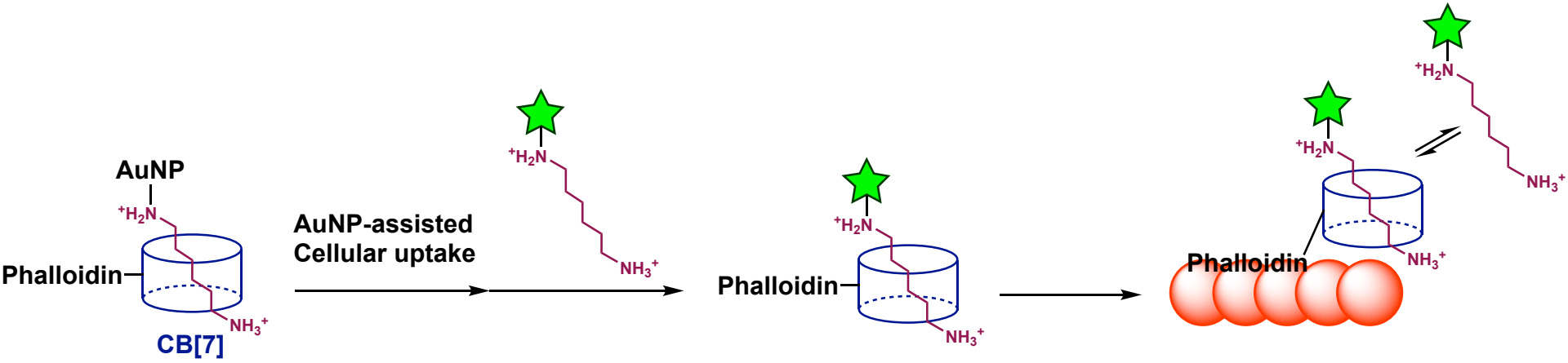
Sasmal, R.; Das Saha, N.; Schueder, F.; Joshi, D.; Sheeba, V.; Jungmann, R.; Agasti, S. S. *Chem. Commun.* **2019**, *55* (96), 14430–14433.

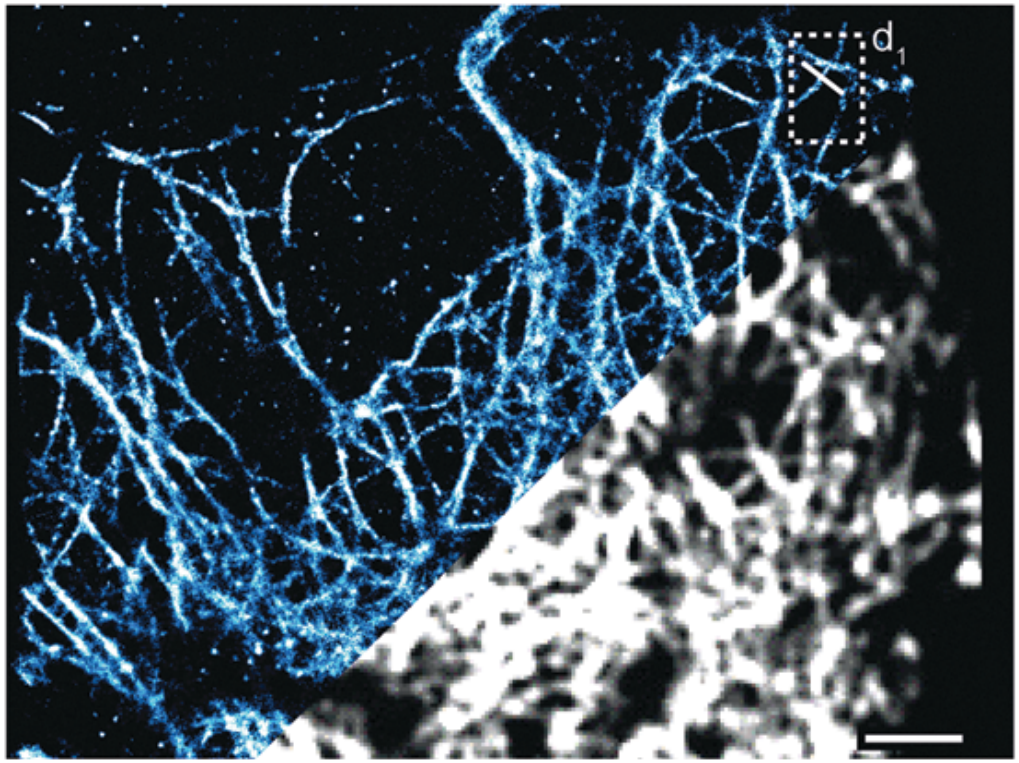






Sasmal, R.; Das Saha, N.; Schueder, F.; Joshi, D.; Sheeba, V.; Jungmann, R.; Agasti, S. S. *Chem. Commun.* **2019**, *55* (96), 14430–14433.





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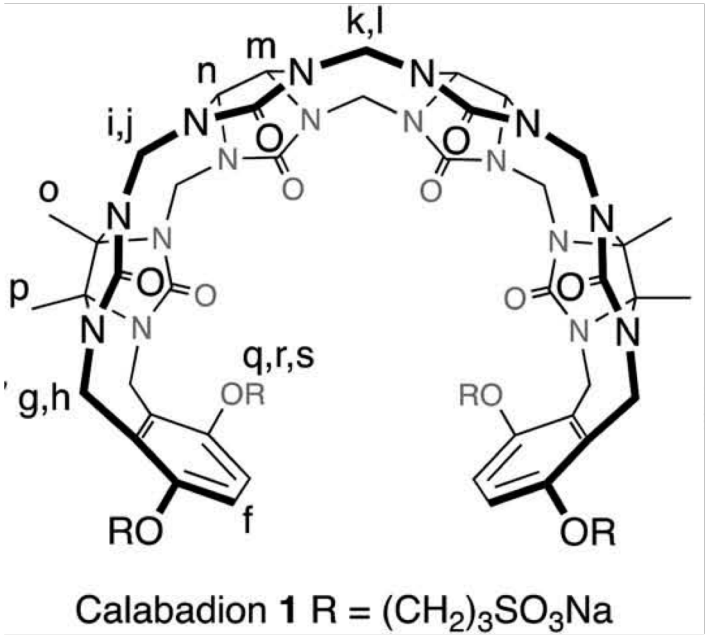
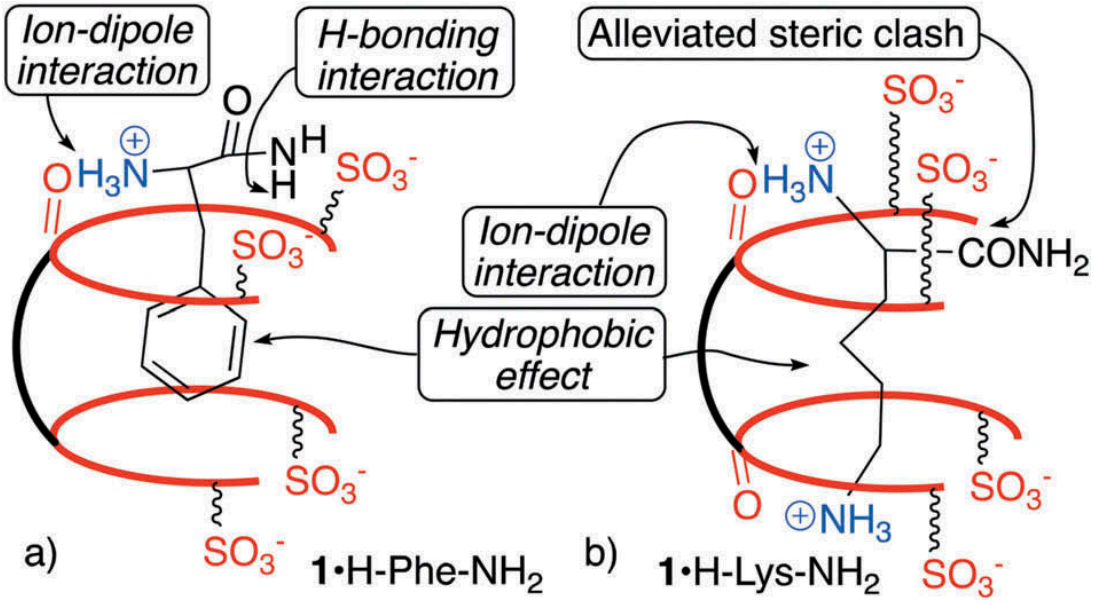


Calabadion **1** R = $(CH_2)_3SO_3Na$



- Expanding the limited scope of guests
  - Development of CB[n] derivatives will expand the guest scope.
  - Acyclic CB[n](Calabadion **1**) had high affinity toward a wider range of N-terminal amino acids than normal CB[n].
- Further applications
  - Application of CB[n] has been limited to relatively simple applications.
  - Further applications in a wide range of fields are expected.

Zebaze Ndendjio, S. A.; Isaacs, L. *Supramol. Chem.* **2019**, *31* (7), 432–441.

Summary: Host guest chemistry for bioconjugation in cells/ in vivo

- Development of methodologies for bioorthogonal molecular conjugation is desired.

- Cucurbit[n]uril based host guest chemistry has many desirable characteristics for in cell/ in vivo applications.

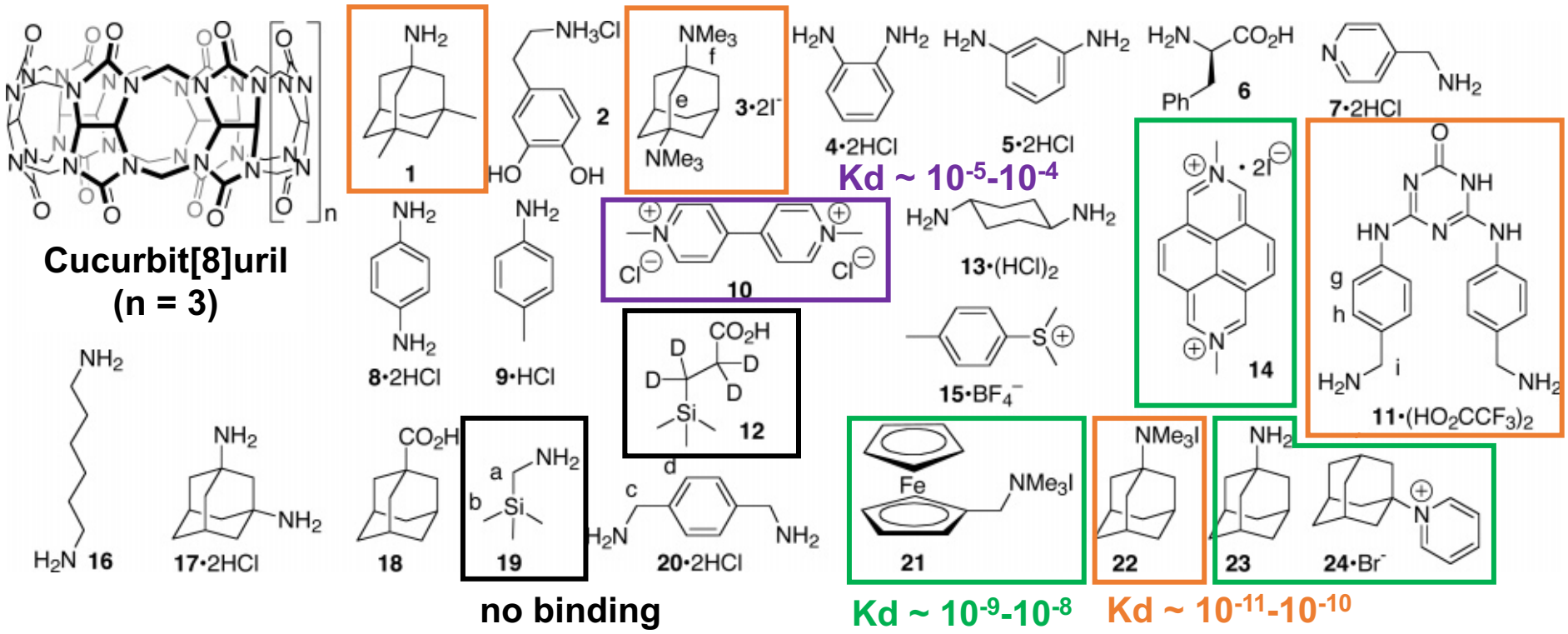
- Cucurbit[n]uril based bioimaging has several advantages over protein based bioimaging.

- Some unique applications are realized by utilizing the characteristics of cucurbit[n]uril-guest interaction.

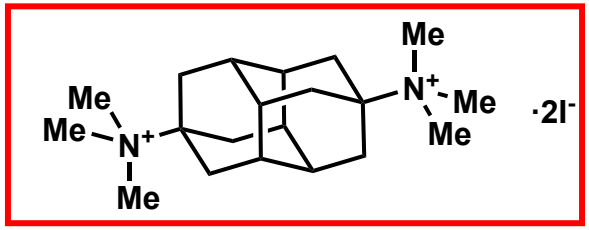
- Expanding the limited scope of guests by developing new host molecules and further applications in a wide range of fields are expected.

Appendix

- K_d value ranges from 10^4 to 10^{15}.
- Cucurbit[7]uril and cucurbit[8]uril has whole different guest preference.

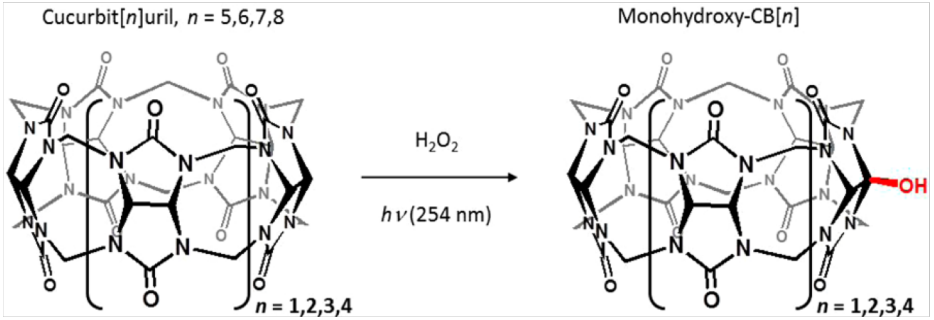


J. Am. Chem. Soc. **2005**, *127*, 15959–15967. (modified)

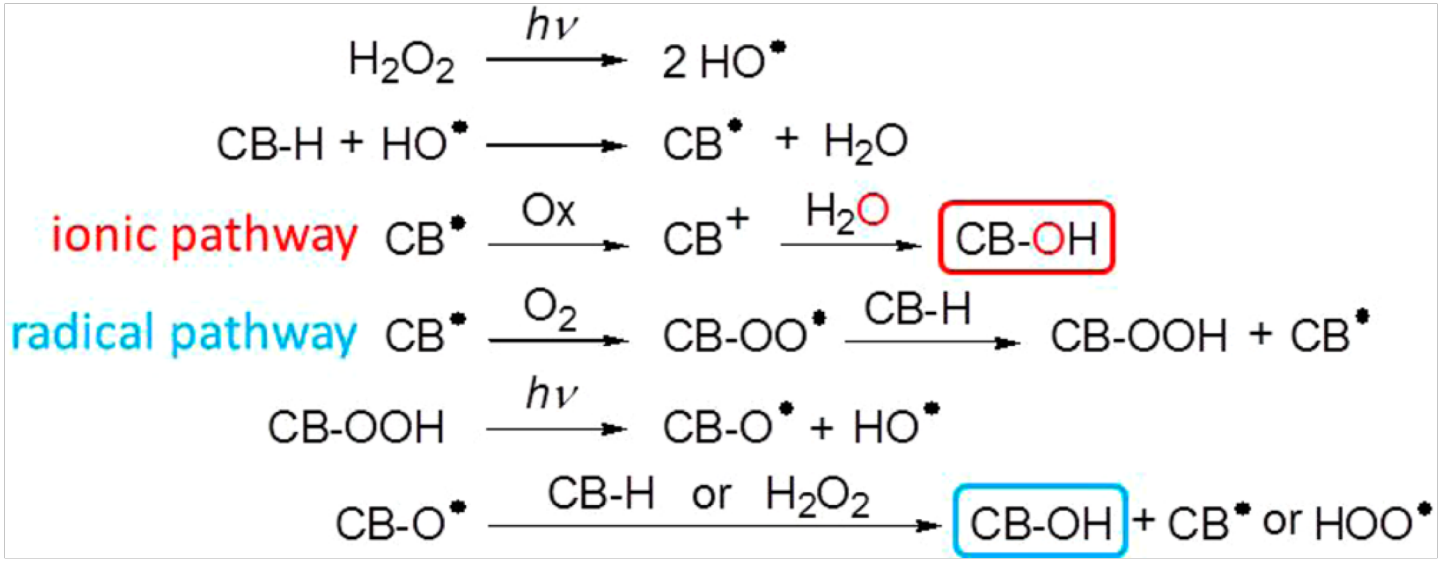


Cao, L.; Šekutor, M.; Zavalij, P. Y.; Mlinarič-Majerski, K.; Glaser, R.; Isaacs, L. *Angew. Chemie - Int. Ed.* **2014**, *53* (4), 988–993.
Liu, S.; Ruspic, C.; Mukhopadhyay, P.; Chakrabarti, S.; Zavalij, P. Y.; Isaacs, L. *J. Am. Chem. Soc.* **2005**, *127* (45), 15959–15967.

Synthesis of monohydroxy-CB[n]



CB[n]–(OH)$_1$	CB[n] conc.	[H_2O_2]	conversion[a]	reaction time
CB[5]–(OH)	2 mM	1 mM	95%	2H
CB[6]–(OH)	2 mM	1 mM	95–100%	2H
CB[7]–(OH)	2 mM	1 mM	95–100%	5H
CB[8]–(OH)	1 mM	0.5 mM	90%[b]	4H



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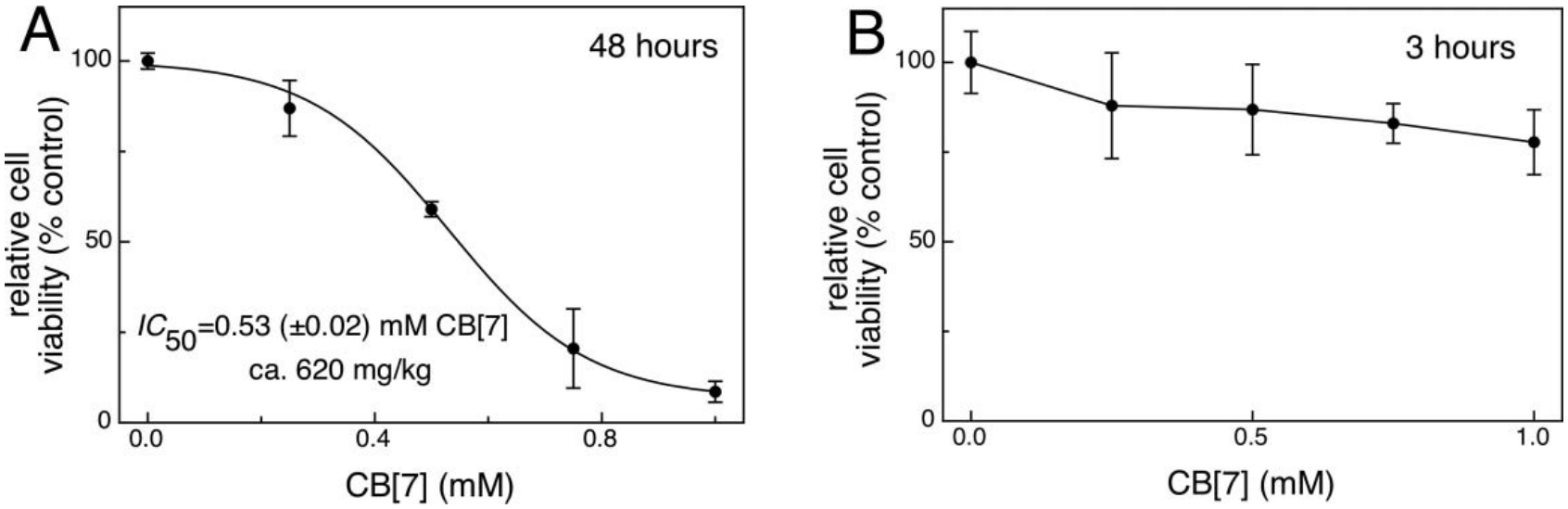

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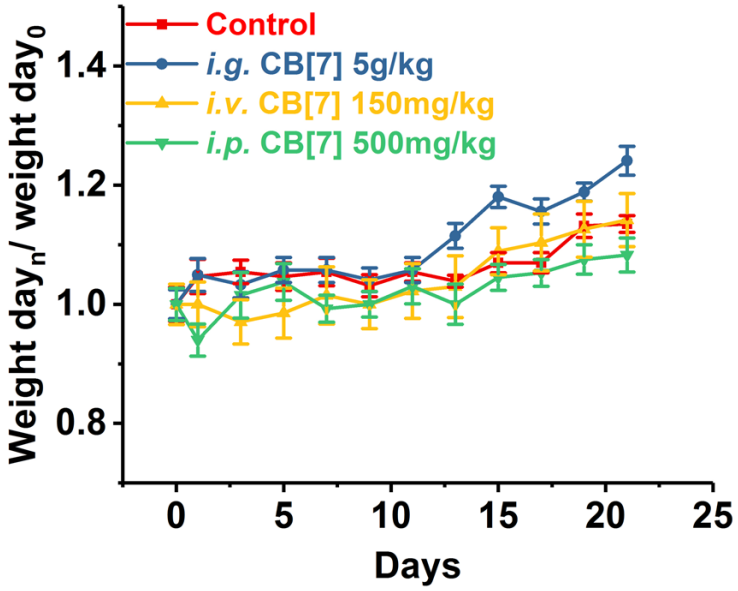

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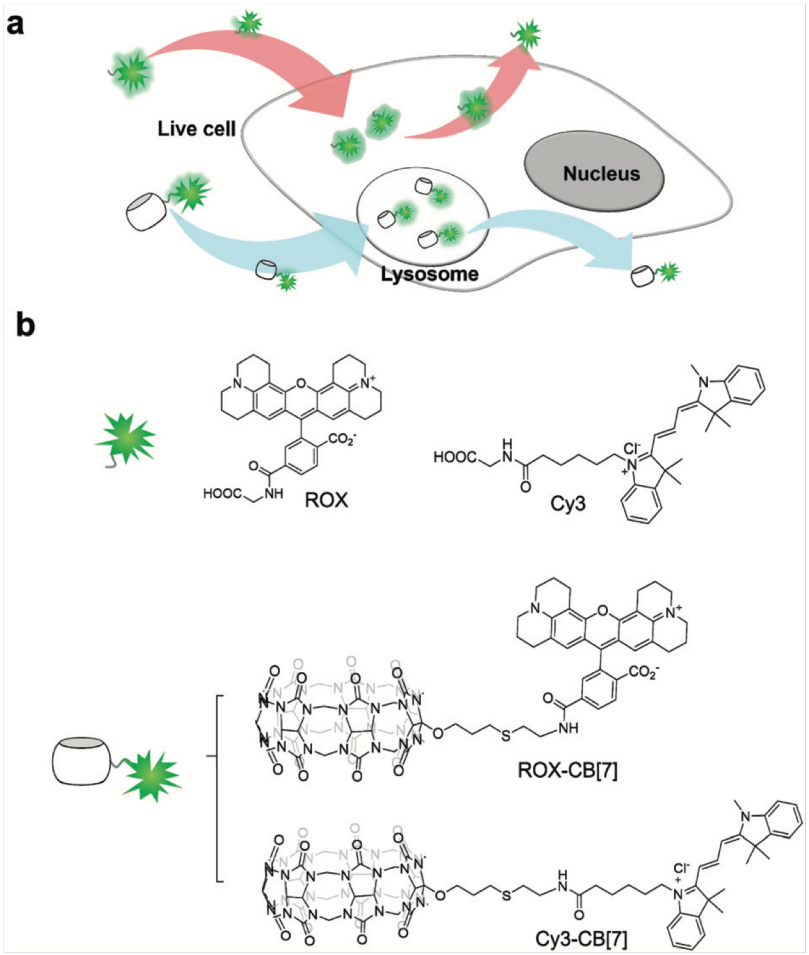
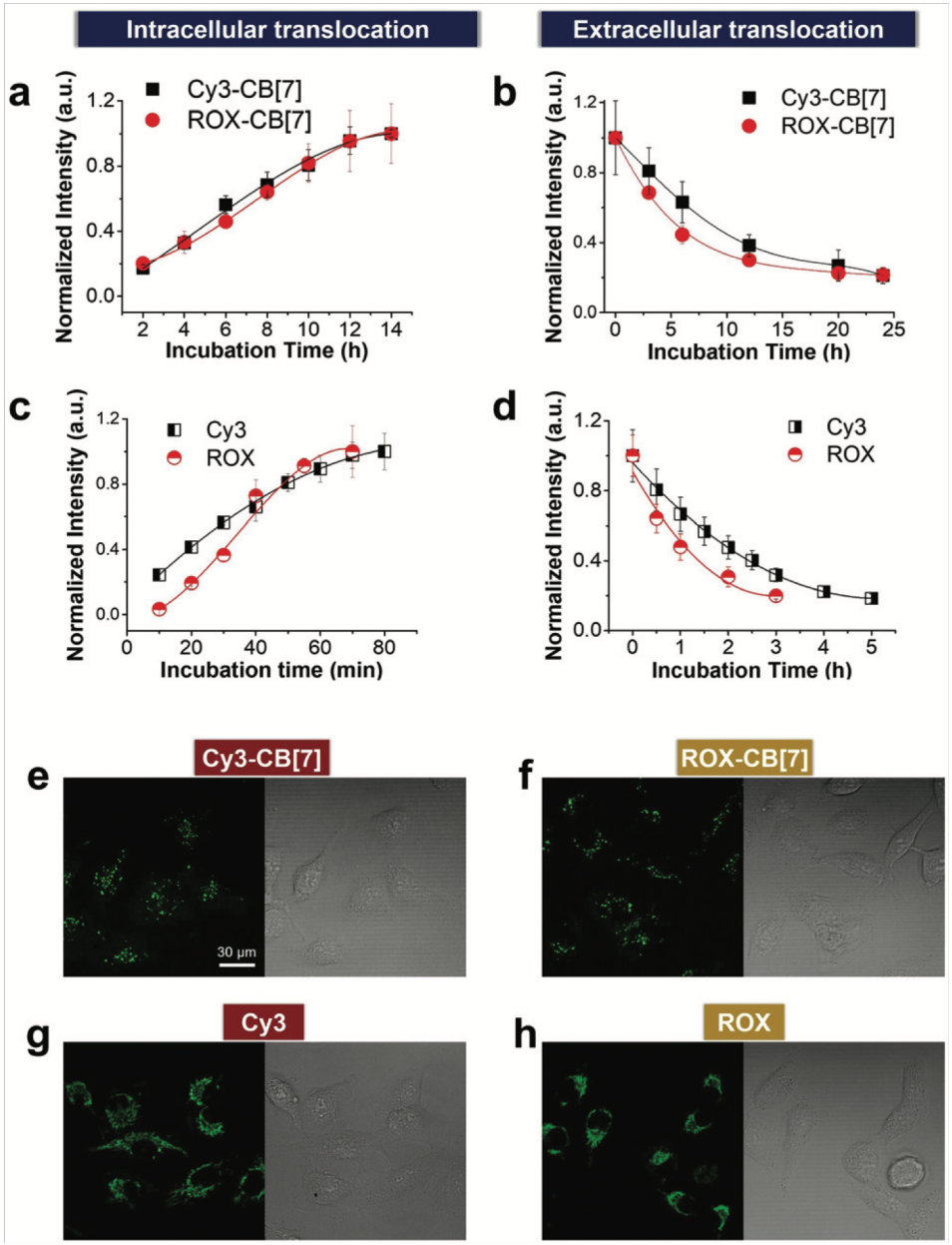
Cellular uptake of CB[7]-dye



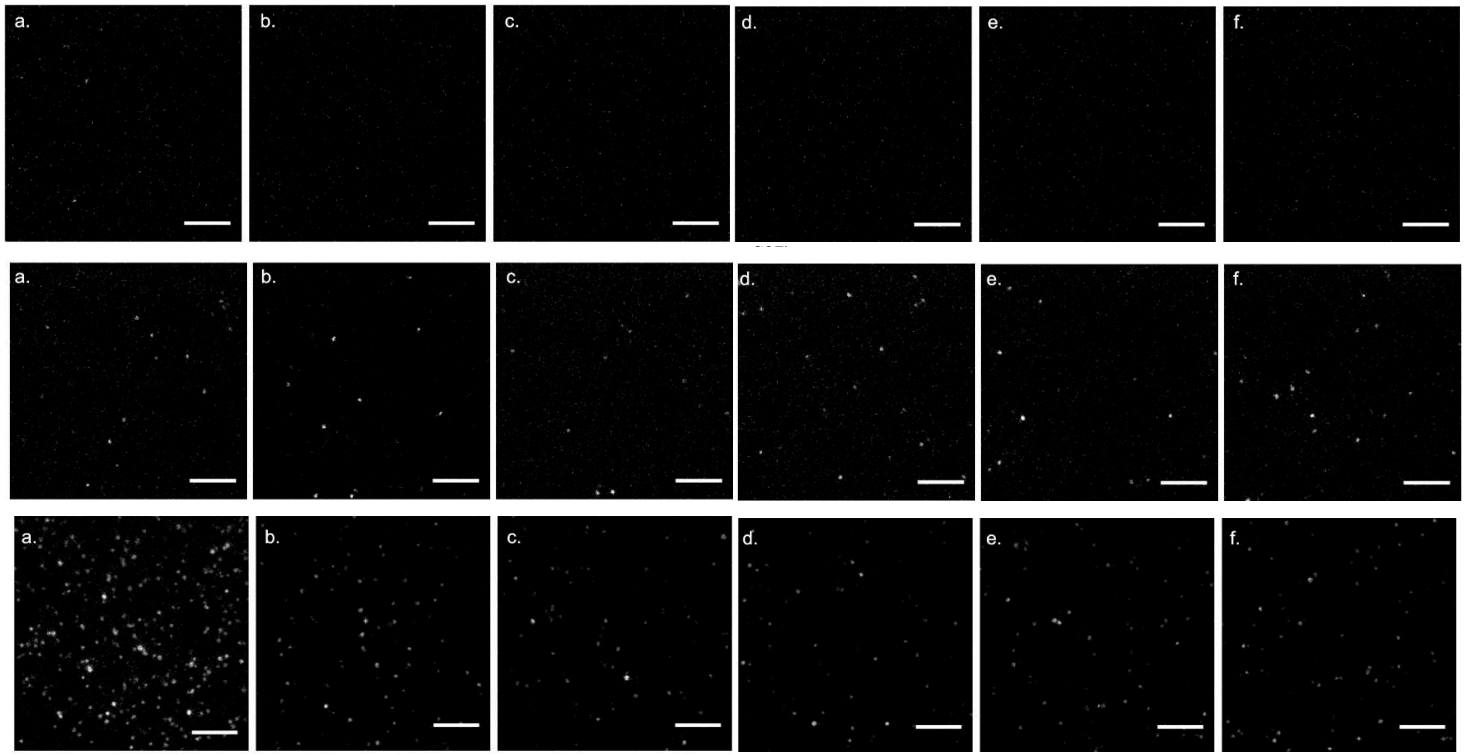


Li, M.; Lee, A.; Kim, S.; Shrinidhi, A.; Park, K. M.; Kim, K. *Org. Biomol. Chem.* **2019**, *17* (25), 6215–6220.

propylamine

HMD

ADA



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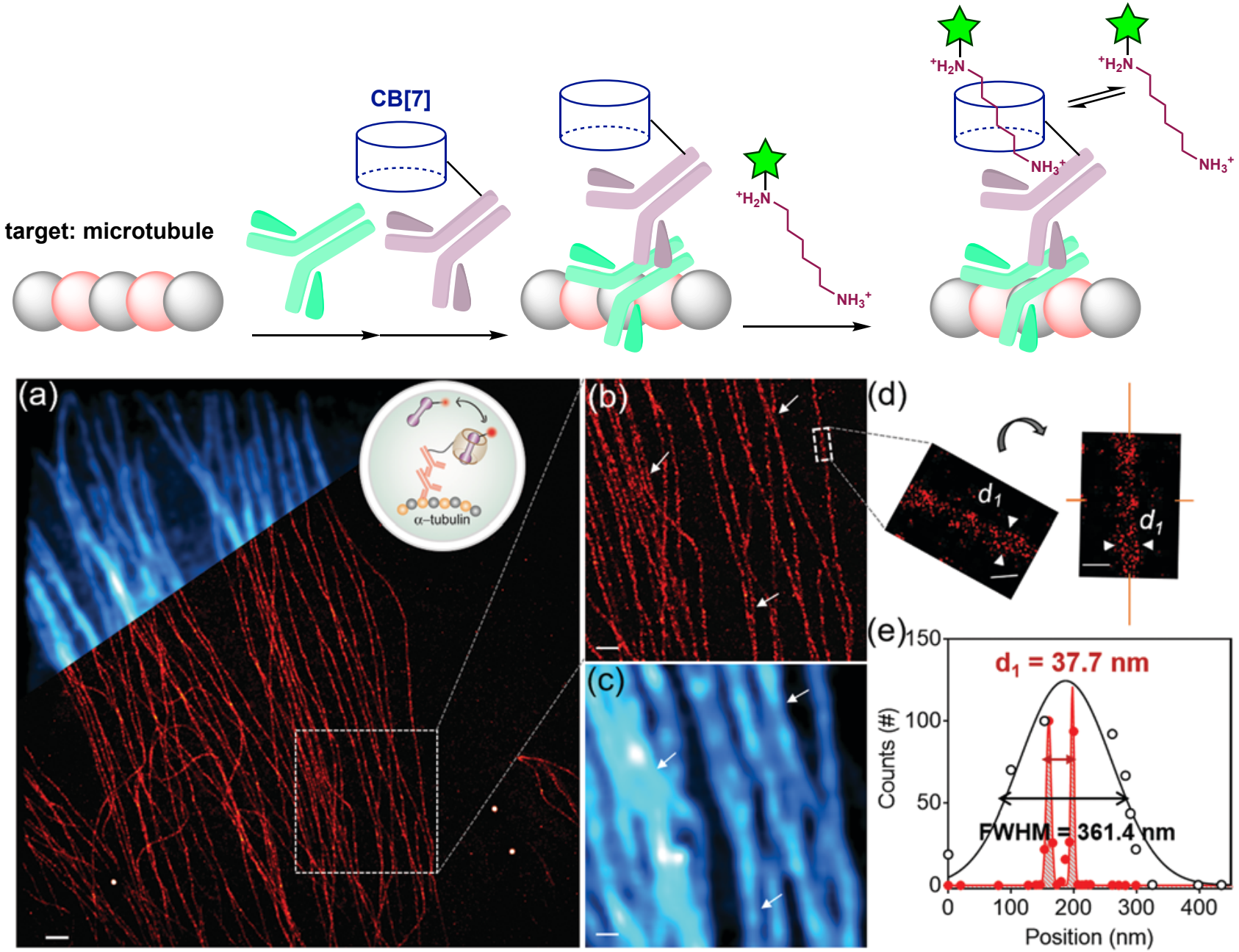

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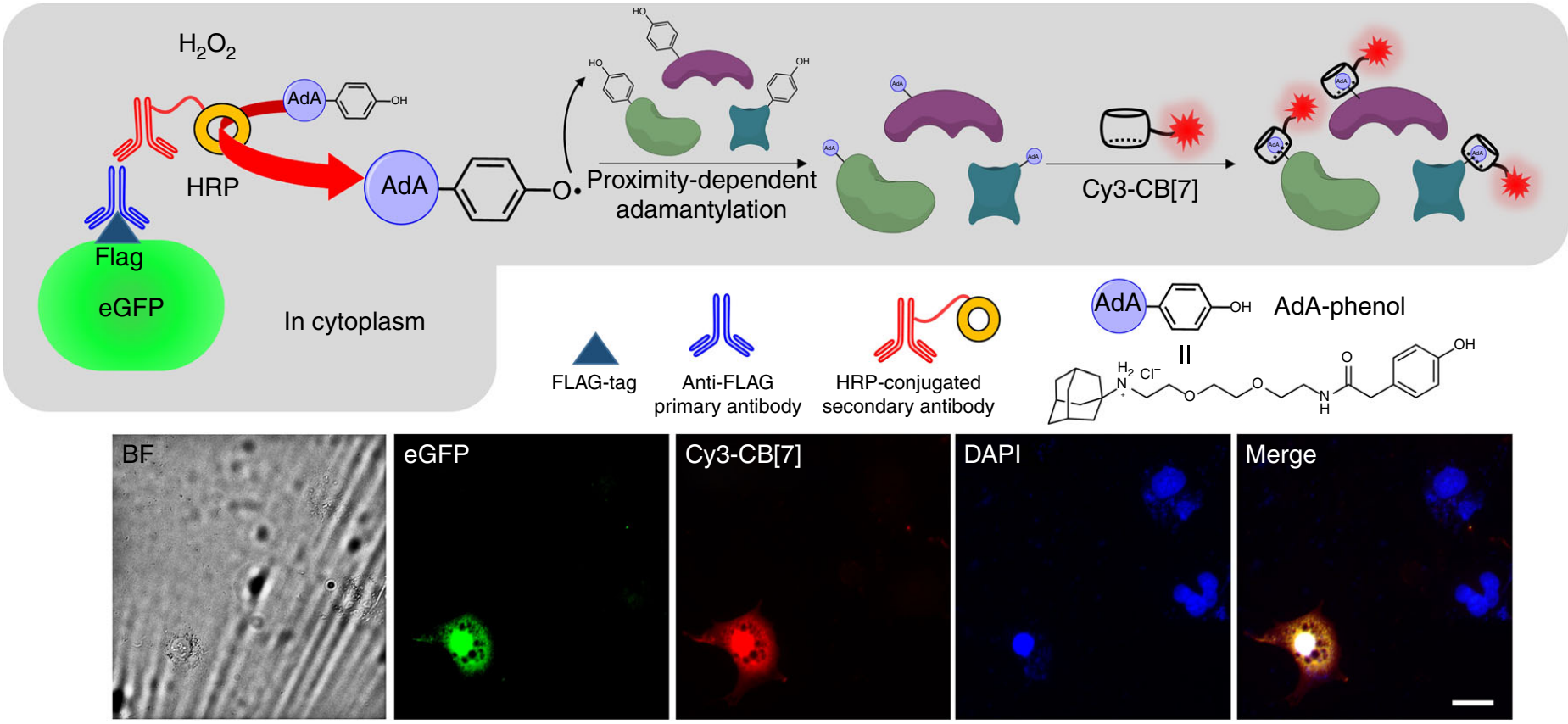
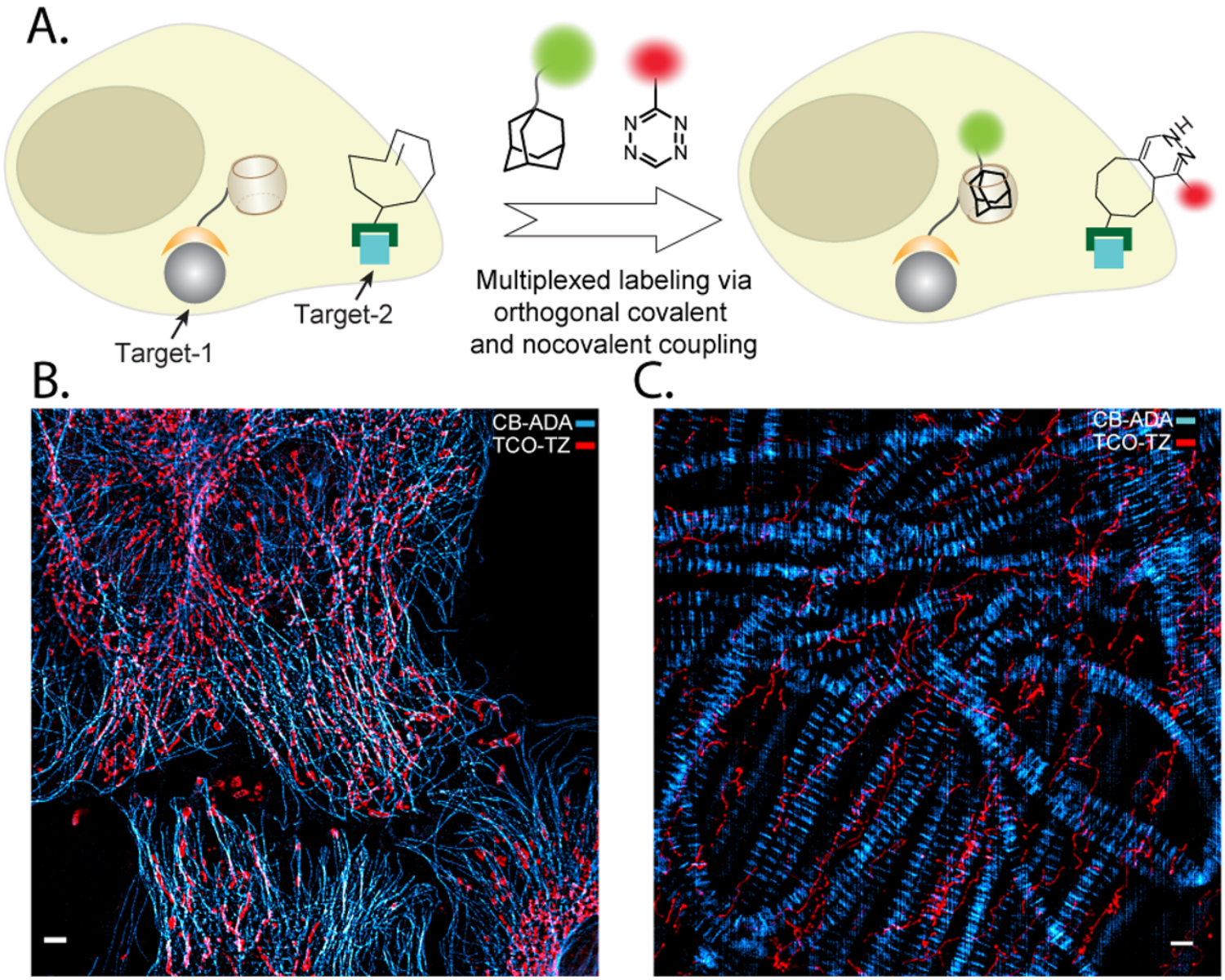
Cucurbituril for in-cell imaging: Other applications



Kim, K. L.; Sung, G.; Sim, J.; Murray, J.; Li, M.; Lee, A.; Shrinidhi, A.; Park, K. M.; Kim, K. *Nat. Commun.* **2018**, *9* (1).



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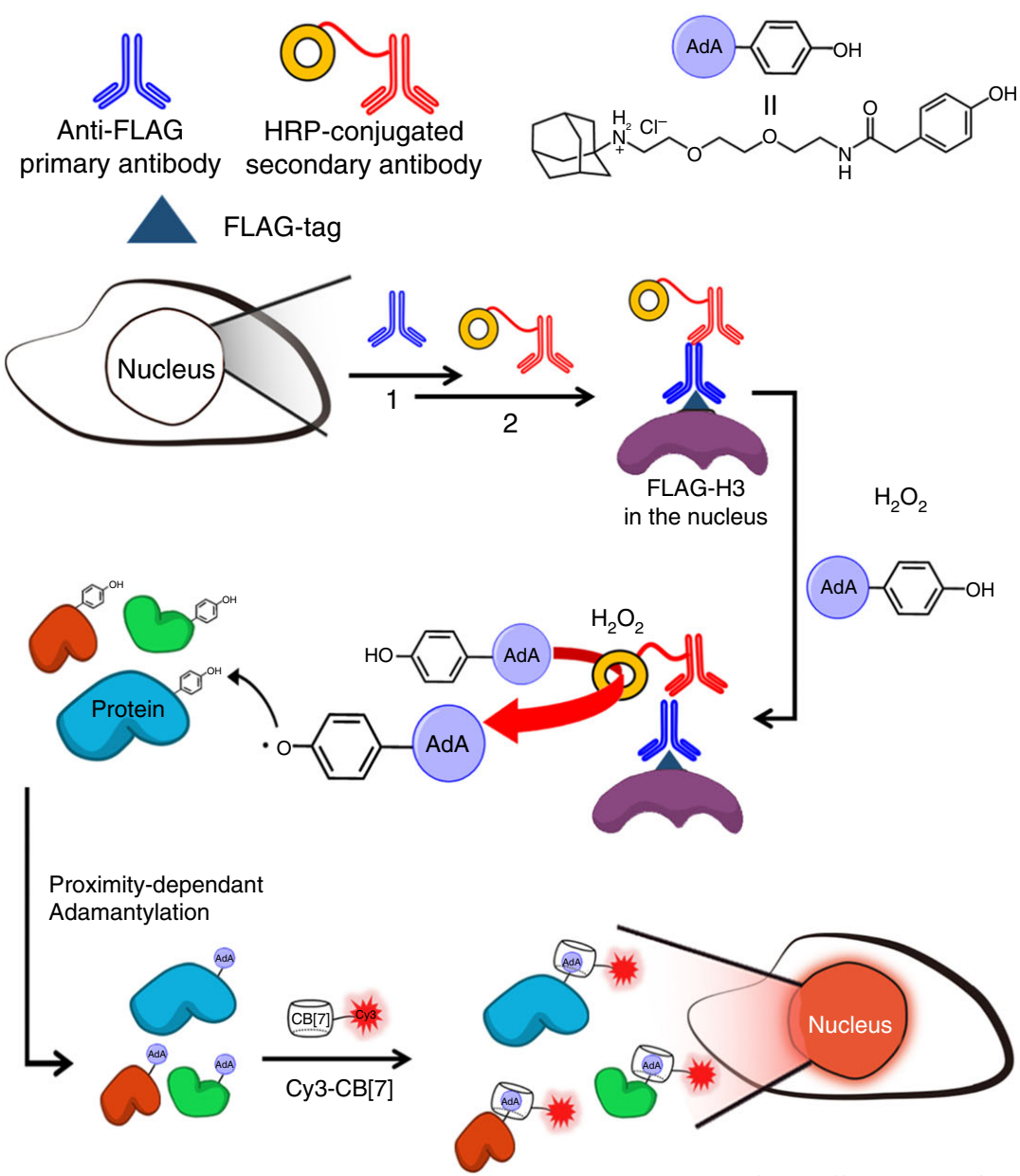
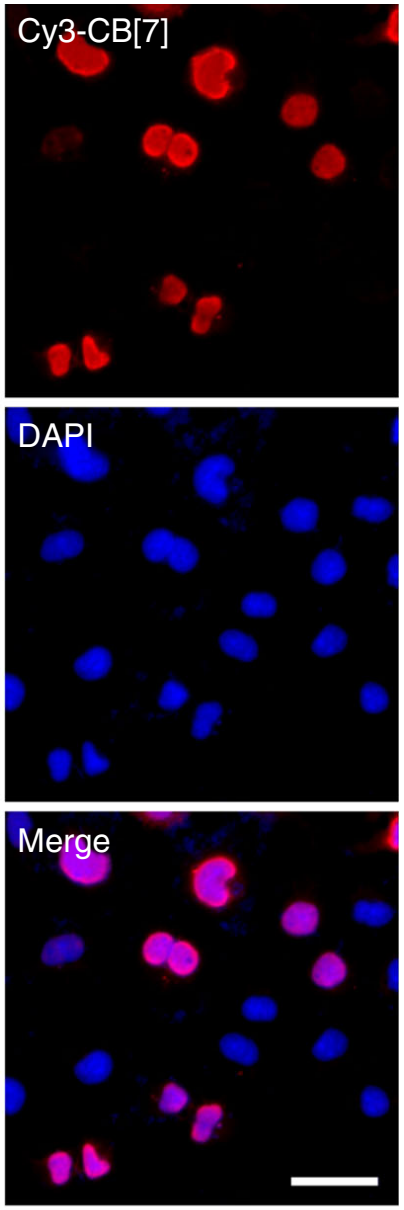
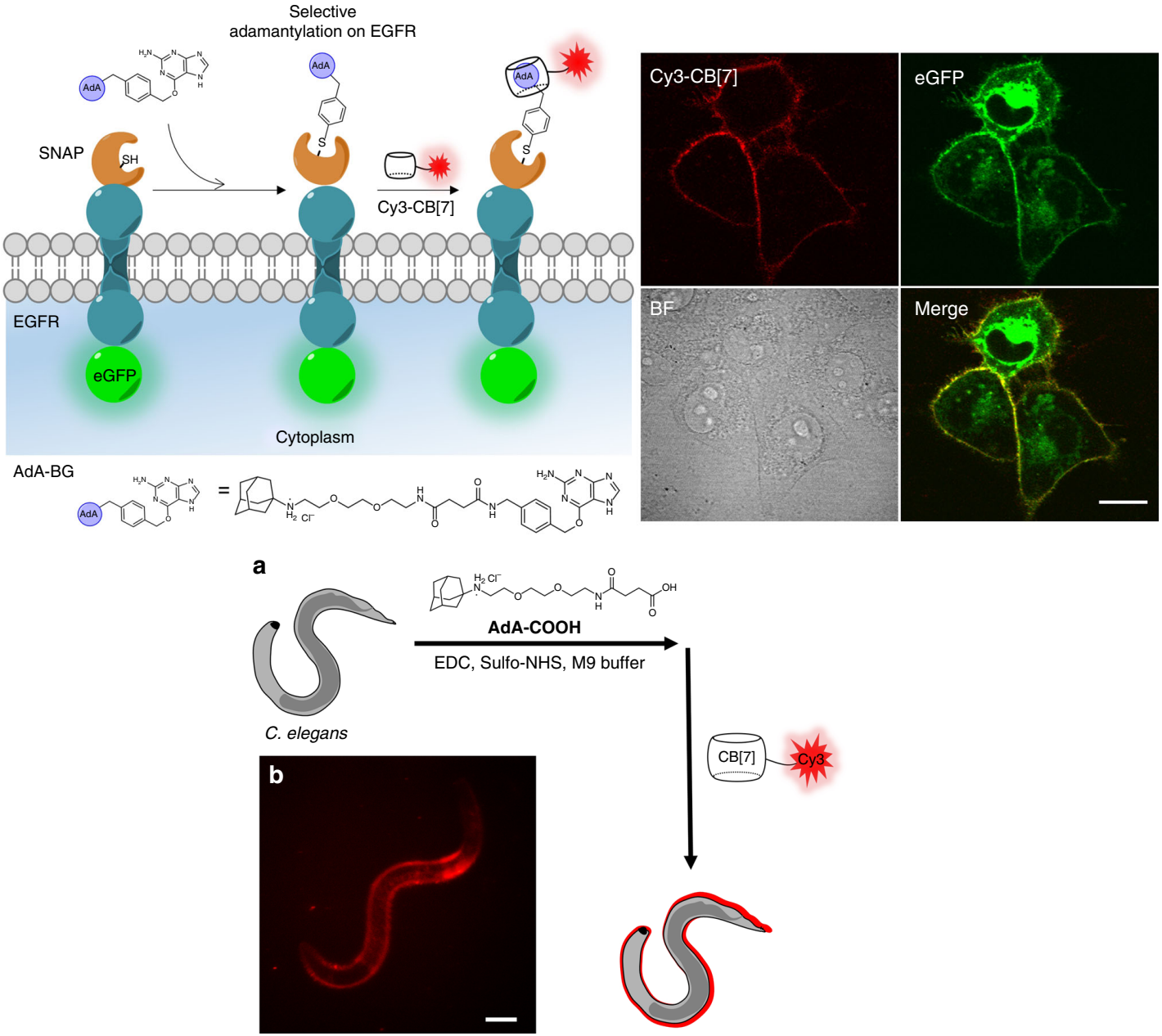
Cucurbituril for in-cell imaging: Other applications





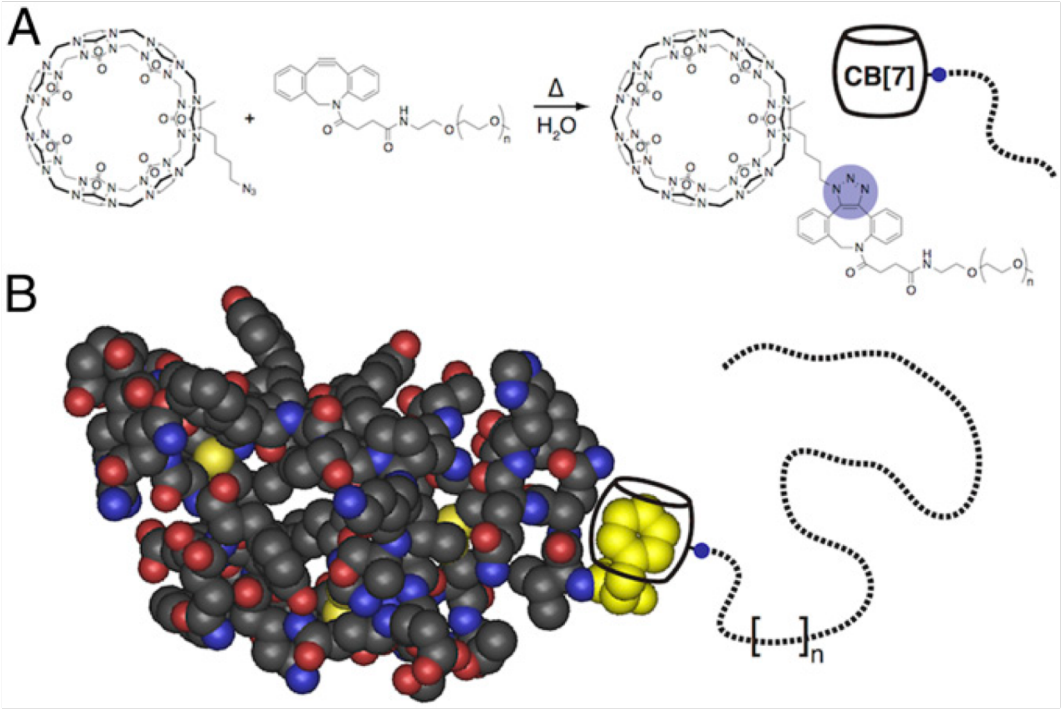
Kim, K. L.; Sung, G.; Sim, J.; Murray, J.; Li, M.; Lee, A.; Shrinidhi, A.; Park, K. M.; Kim, K. *Nat. Commun.* **2018**, *9* (1).

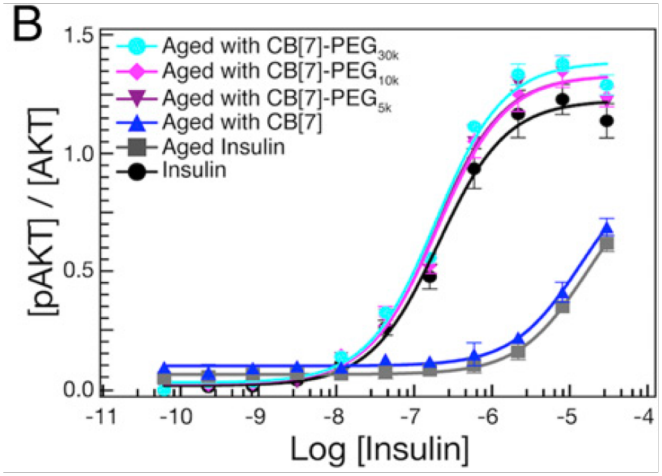
Cucurbituril for in-cell imaging: Other applications

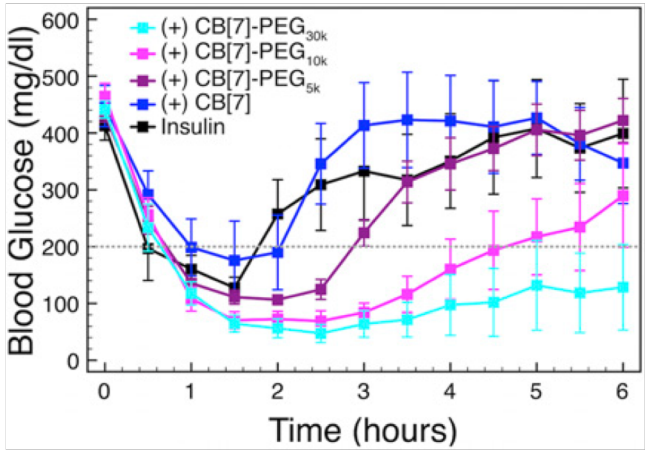


Kim, K. L.; Sung, G.; Sim, J.; Murray, J.; Li, M.; Lee, A.; Shrinidhi, A.; Park, K. M.; Kim, K. *Nat. Commun.* **2018**, *9* (1).

Recognition of N-terminal Phe of insulin by CB[7]







Webber, M. J.; Appel, E. A.; Vinciguerra, B.; Cortinas, A. B.; Thapa, L. S.; Jhunjhunwala, S.; Isaacs, L.; Langer, R.; Anderson, D. G. *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113* (50), 14189–14194.