Multivalent Interactions

Literature Seminar

M2 Miura Kensuke

Outline

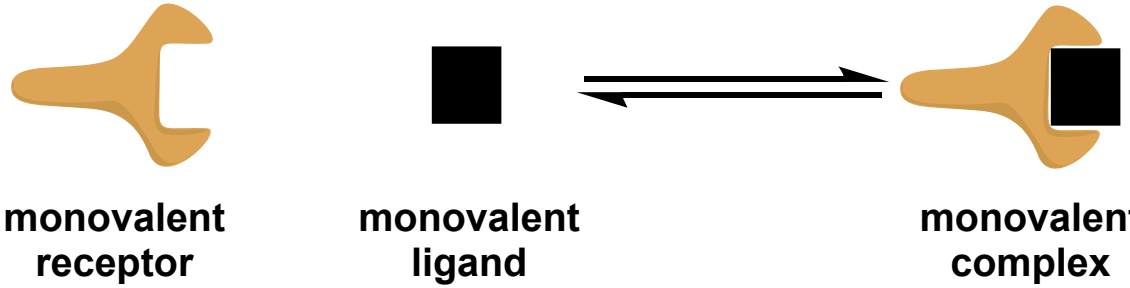
- **Introduction of multivalency inspired by living system**

- **Multivalency by artificial material**

- **Main topic:**
  “Heteromultivalent peptide recognition by co-assembly of cyclodextrin and calixarene amphiphiles enables inhibition of amyloid fibrillation”
  (Xu, Z.; Jia, S.; Wang, W; Yuan, Z.; Ravoo, J. B.; Guo, S. D. *Nat. Chem.* 2019, *11,* 86-93.)

The Concept of Multivalent Interactions

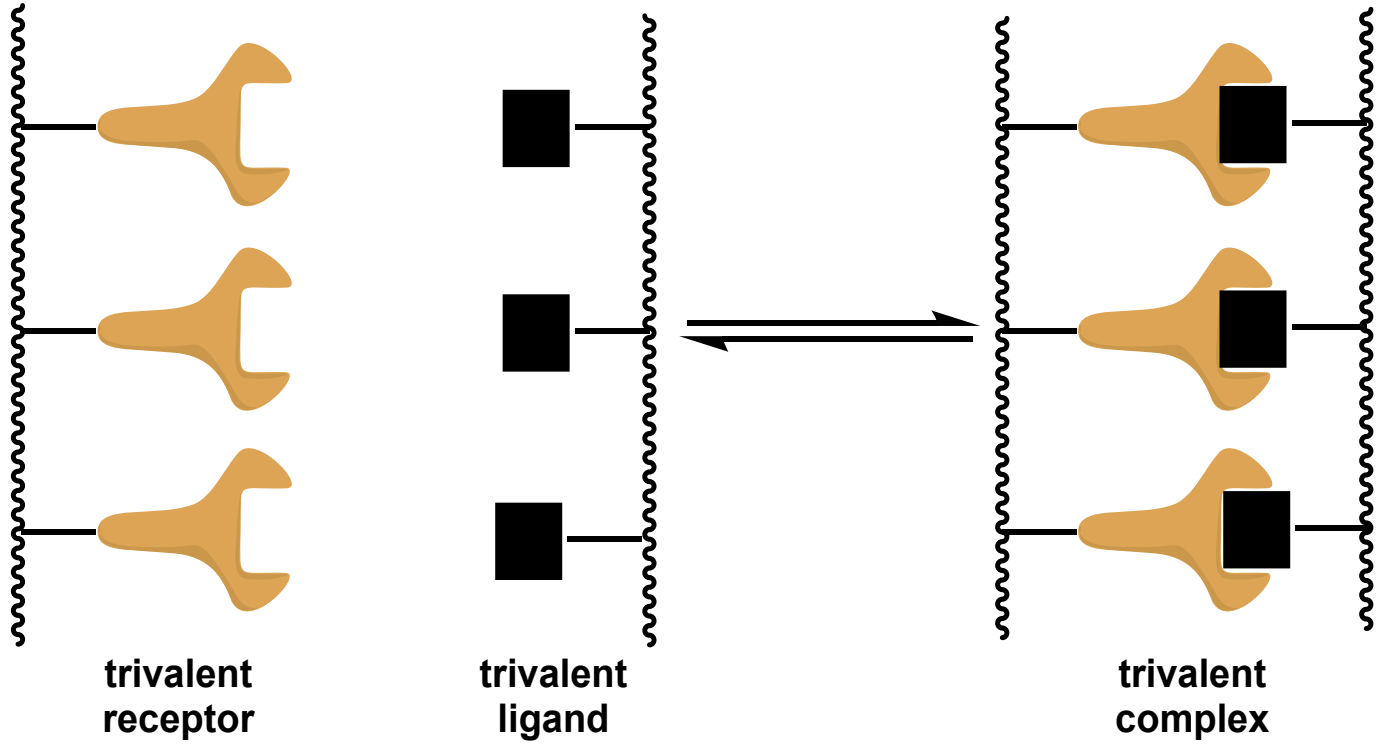
Monovalent Interaction



Multivalent Interactions

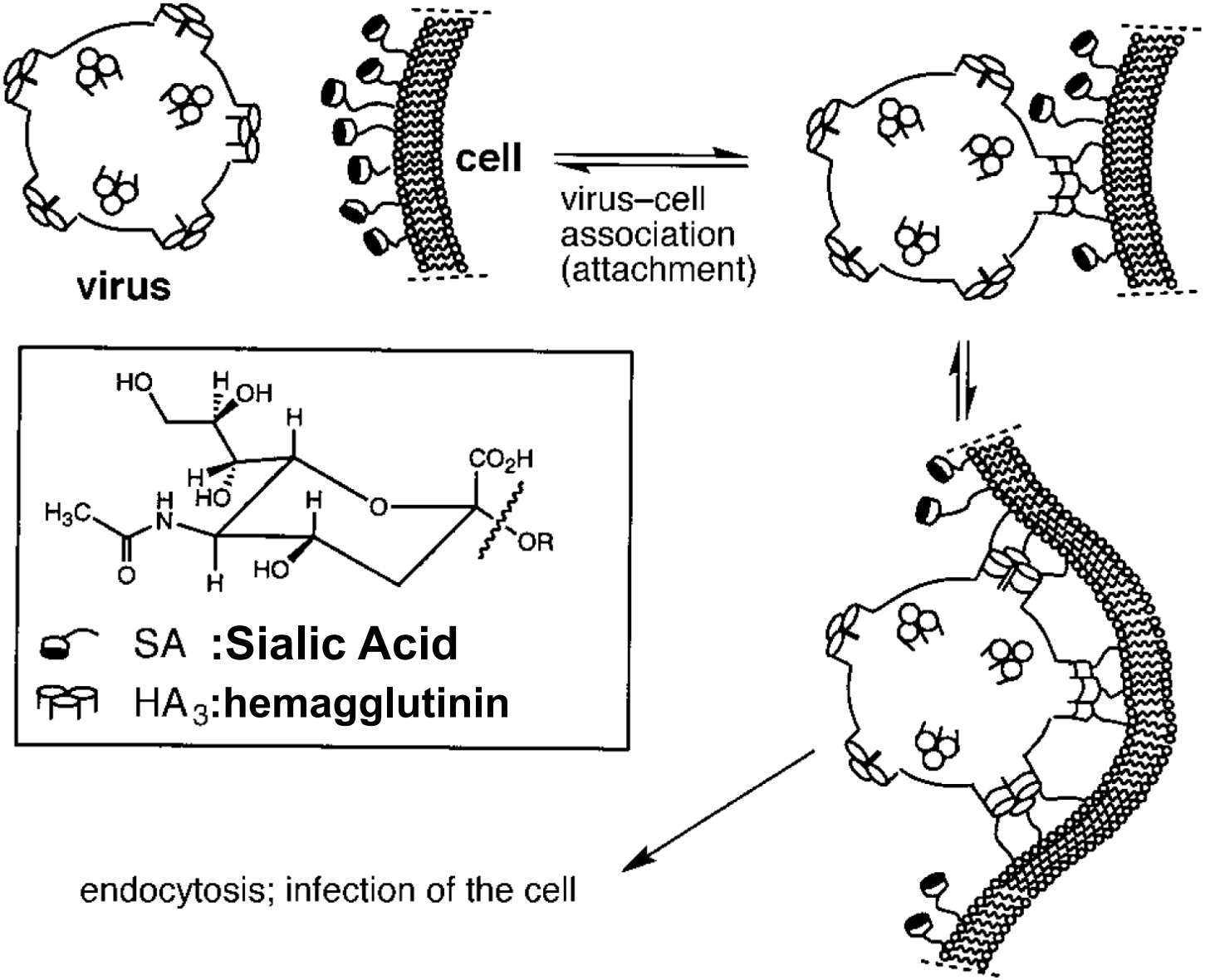
simultaneous interactions of multiple copies of the same ligand and receptor

Improvement of binding affinity



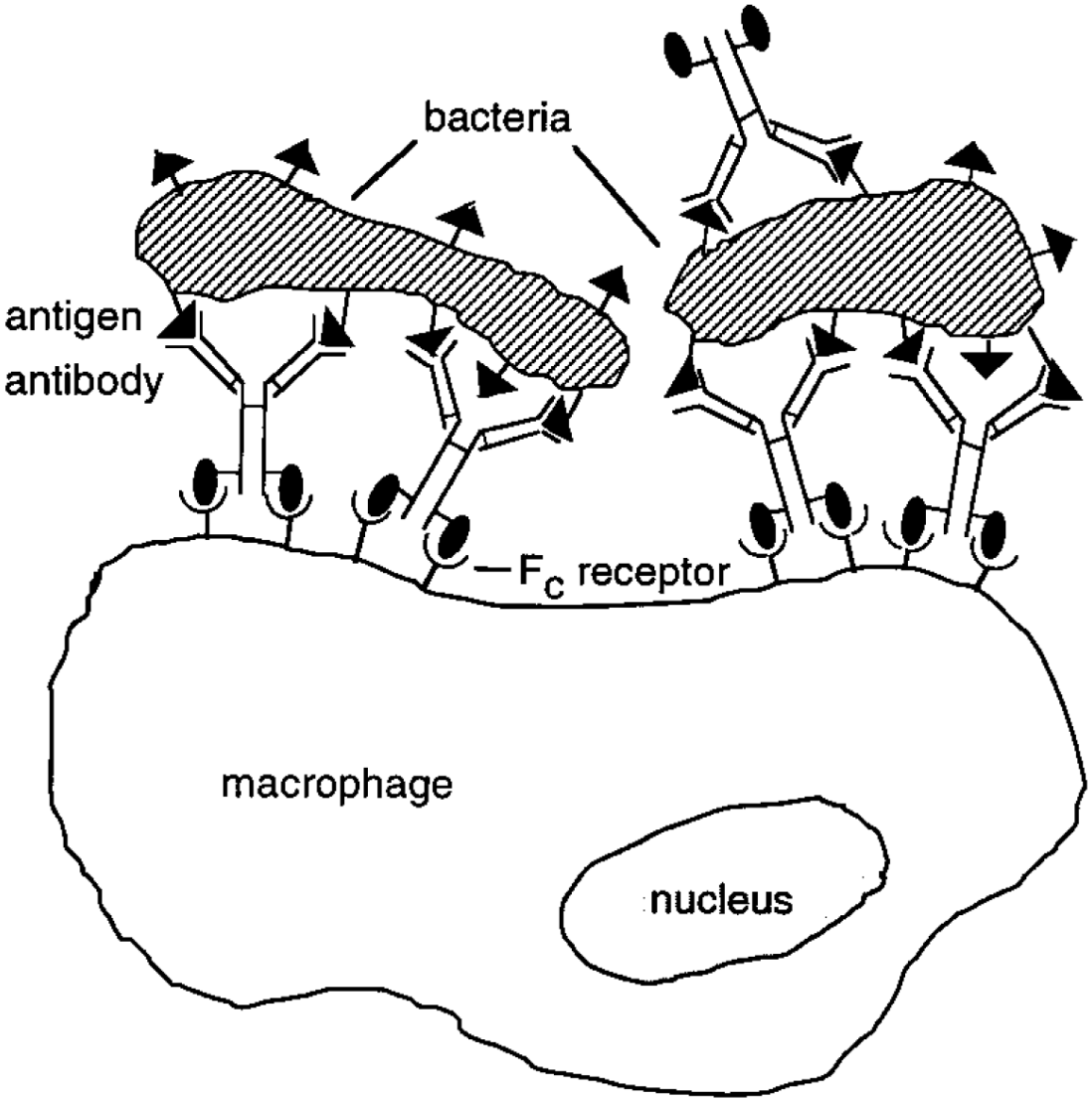
Mammen, M.; Choi, S. K.; Whitesides, G. M. *Angew. Chem. Int. Ed.* **1998**, *37*, 2754–2794 .

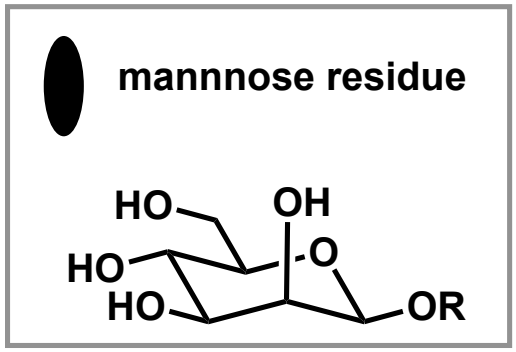
Adhesion of a Influenza Virus to the Surface of a Cell
Example of Multivalency in Living systems



Mammen, M.; Choi, S. K.; Whitesides, G. M. *Angew. Chem. Int. Ed.* 1998, **37**, 2754–2794 .

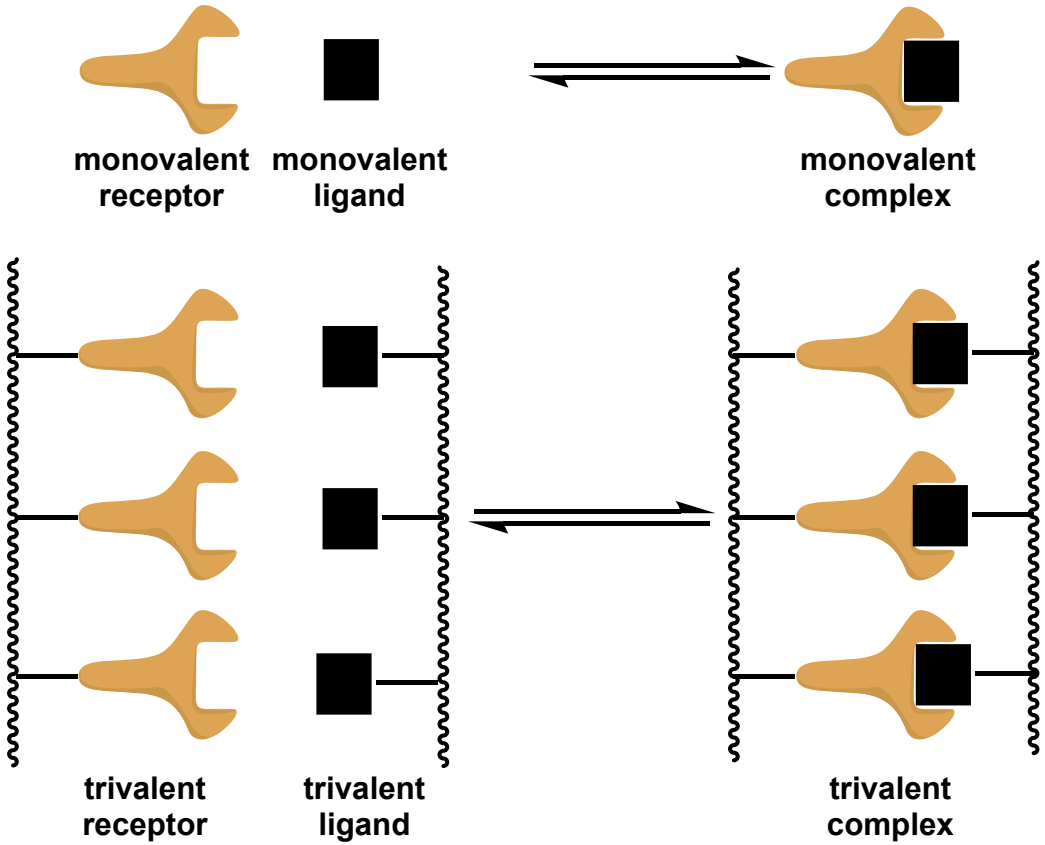
Antibodies and Macrophage
Example of Multivalency in Living Systems





Mammen, M.; Choi, S. K.; Whitesides, G. M. *Angew. Chem. Int. Ed.* 1998, **37**, 2754–2794 .

Free Energy and Binding Constants in Multivalent Interactions



	ΔG_{total}	K_{total}	ΔG_{avg}	K_i
monovalent	ΔG^{mono}	K^{mono}	ΔG^{mono}	K^{mono}
polyvalent (*N*-valent)	ΔG^{poly}_{N}	K^{poly}_{N}	ΔG^{poly}_{avg}	K^{poly}_{avg}

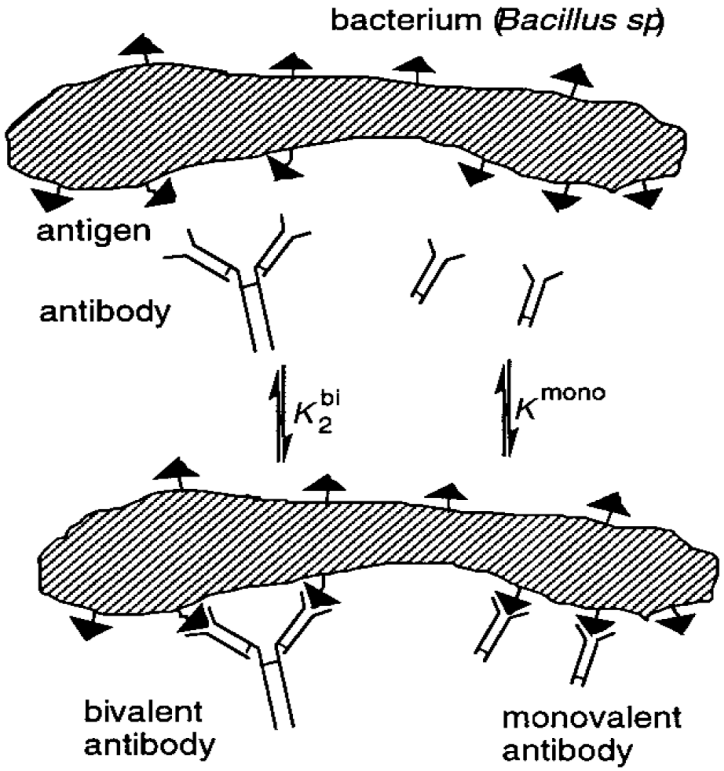
$$\Delta G^{poly}_{avg} = \Delta G^{poly}_{N}/N$$

$$\Delta G = -RT\ln(K)$$

$$K^{poly}_{N} = (K^{poly}_{avg})^{N}$$

Mammen, M.; Choi, S. K.; Whitesides, G. M. *Angew. Chem. Int. Ed.* 1998, **37**, 2754–2794 .

Evaluation of multivalency

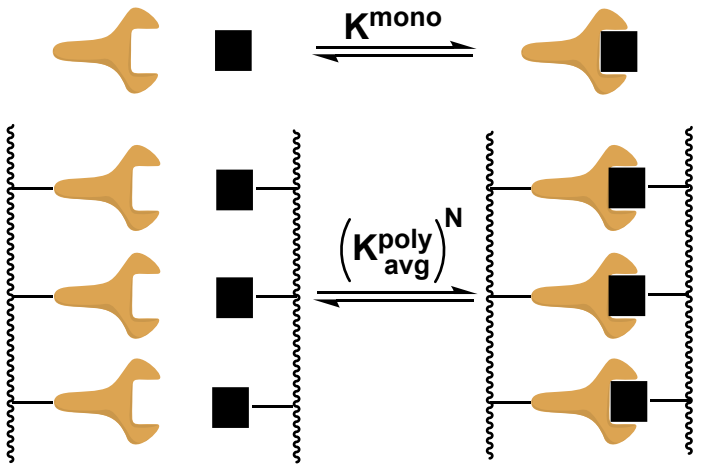


$K_2^{bi} = 30\,K^{mono}$

$K_{avg}^{bi} = (30K^{mono})^{1/2} < K^{mono}$

this case is negatively cooperative ($\alpha < 1$)

Tight binding does not require positive cooperativity



	K_{total}	ΔG_{avg}
monovalent	K^{mono}	ΔG^{mono}
polyvalent (*N*-valent)	K_N^{poly}	ΔG_{avg}^{poly}

$$\Delta G_{avg}^{poly} = \alpha \Delta G^{mono}$$

$$N\Delta G_{avg}^{poly} = \Delta G_N^{poly} = \alpha N \Delta G^{mono}$$

$$K_N^{poly} = (K_{avg}^{poly})^N = (K^{mono})^{\alpha N}$$

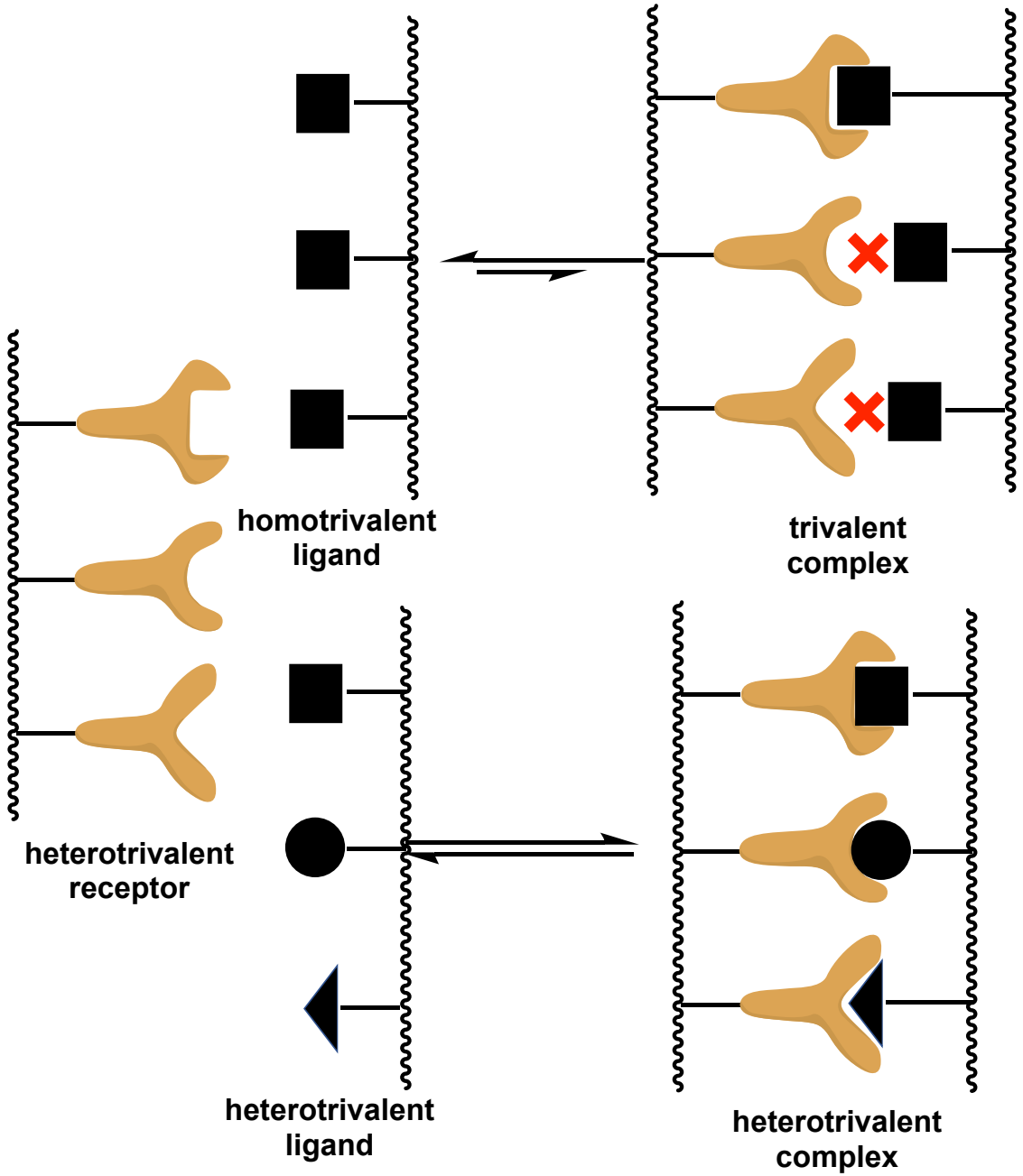
$$\alpha = \frac{\lg(K_N^{poly})}{\lg(K^{mono})^N}$$

$$\Delta G_N^{poly} = \Delta G^{mono} - RT\ln(\beta)$$

$$\beta = K_N^{poly}/K^{mono}$$

Mammen, M.; Choi, S. K.; Whitesides, G. M. *Angew. Chem. Int. Ed.* 1998, **37**, 2754–2794 .

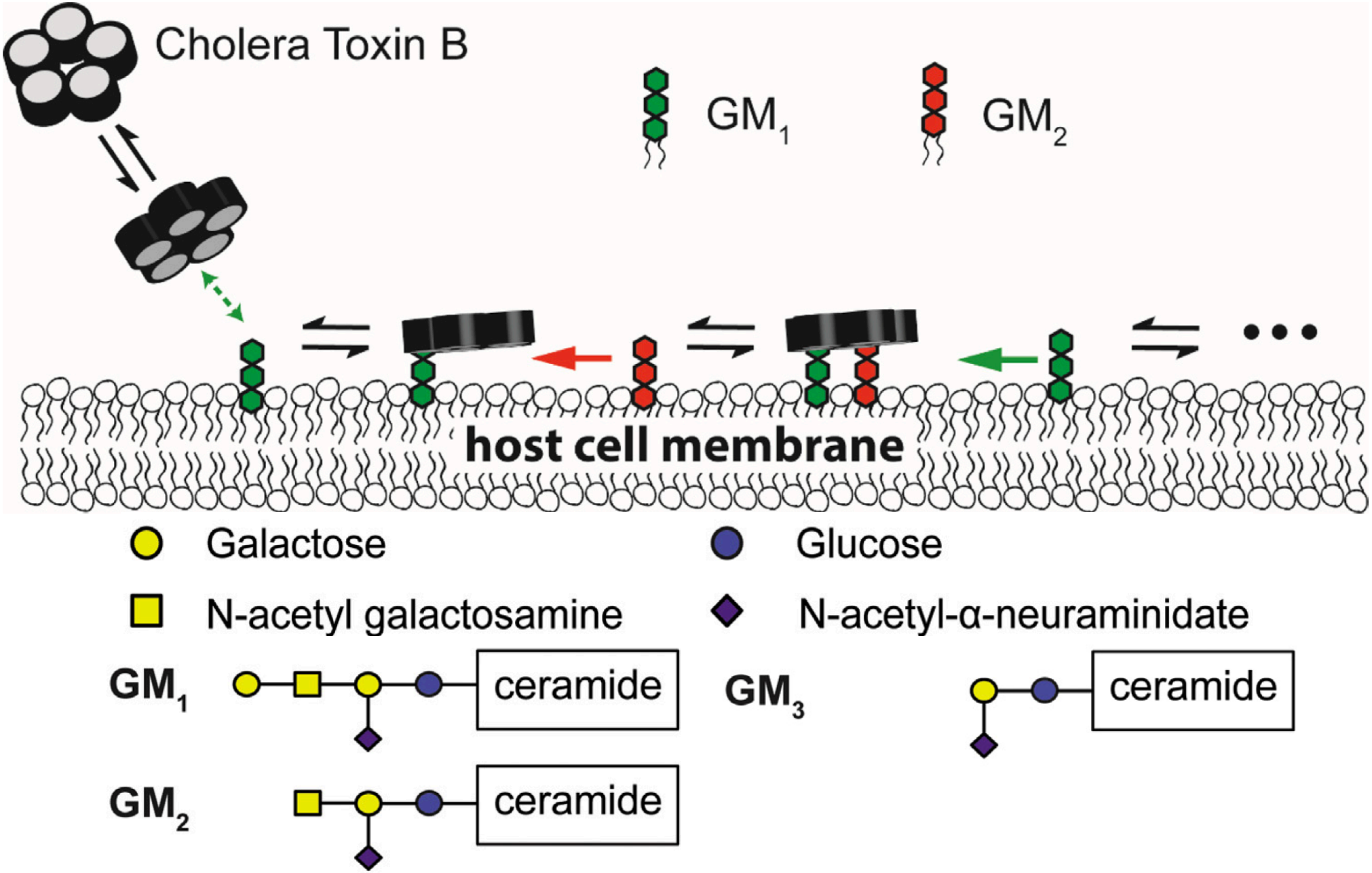
The Concept of Heteromultivalent Interactions



Heteromultivalency
= ‘’multivalent” + ”heterotopic”

Heteromultivalency results in greater strength and specificity than homomultivalency

The Example of Heteromultivalency in living system



Krishnan, P.; Singa, A.; Lee, A. C.; Weatherton, D. J.; Worstell, C. N.; Wu, J. H. *Colloids Surf., B.* **2017**, *160*, 281–288 .

Outline

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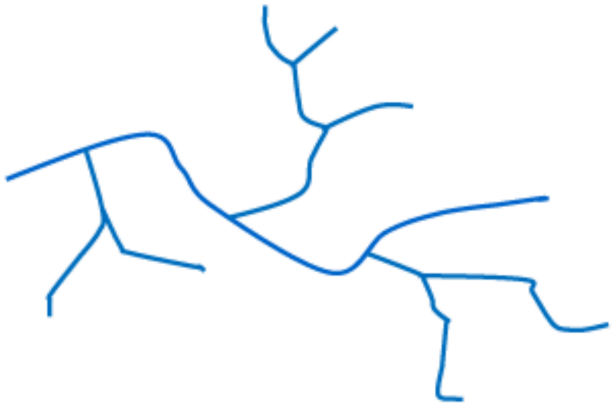
  (Xu, Z.; Jia, S.; Wang, W; Yuan, Z.; Ravoo, J. B.; Guo, S. D. *Nat. Chem.* 2019, *11,* 86-93.)

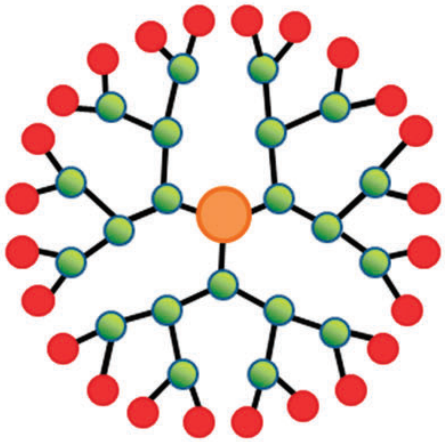
Application of Multivalent interactions

scaffold

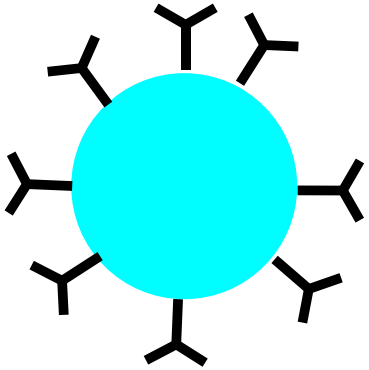
Linear or branched polymers



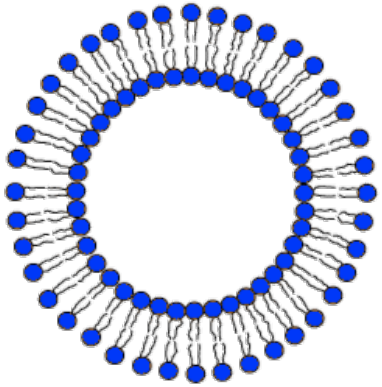
dendrimers



beads or nanoparticle

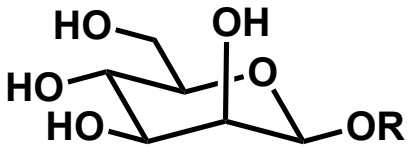


liposomes

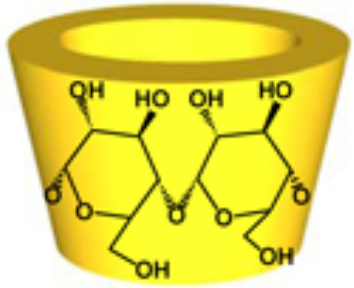


monomer

sugar

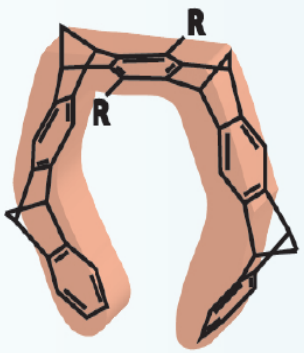


Host molecule

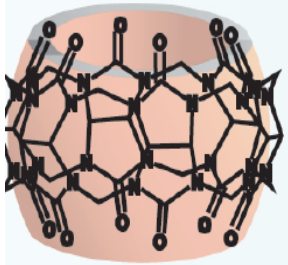


Mammen, M.; Choi, S. K.; Whitesides, G. M. *Angew. Chem. Int. Ed.* **1998**, *37*, 2754–2794 .

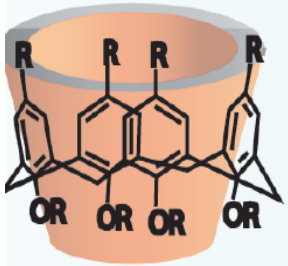
Host-Guest Chemistry



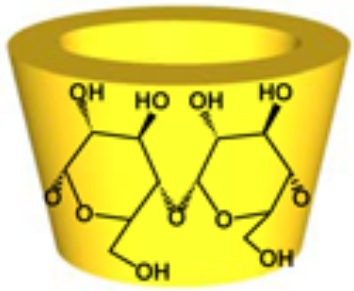
Molecular Tweezer



Cucurbit[n]uril

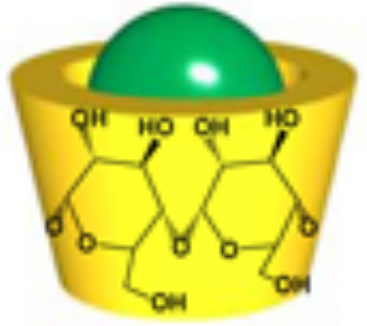


Calix[n]arene



Host molecule

Guest molecule



Host-Guest complex

Host-Guest Chemistry

- **selective binding: hydrogen bonding, ionic bonds, van der Waals forces, hydrophobic interactions (non-covalent bonding)**

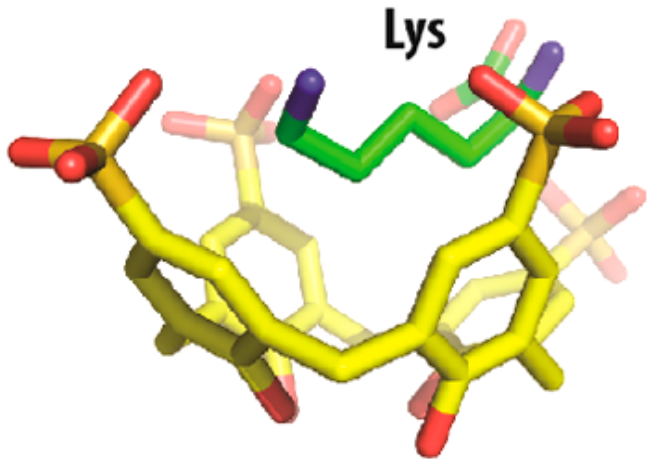
Other factors: size, shape of molecule

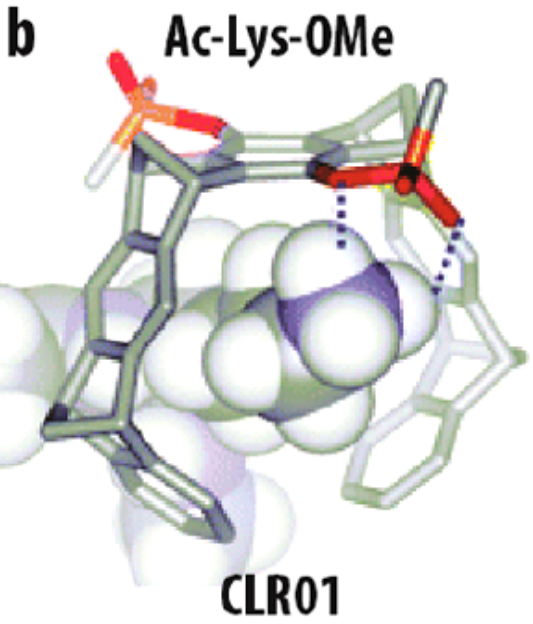
Application to molecular recognition

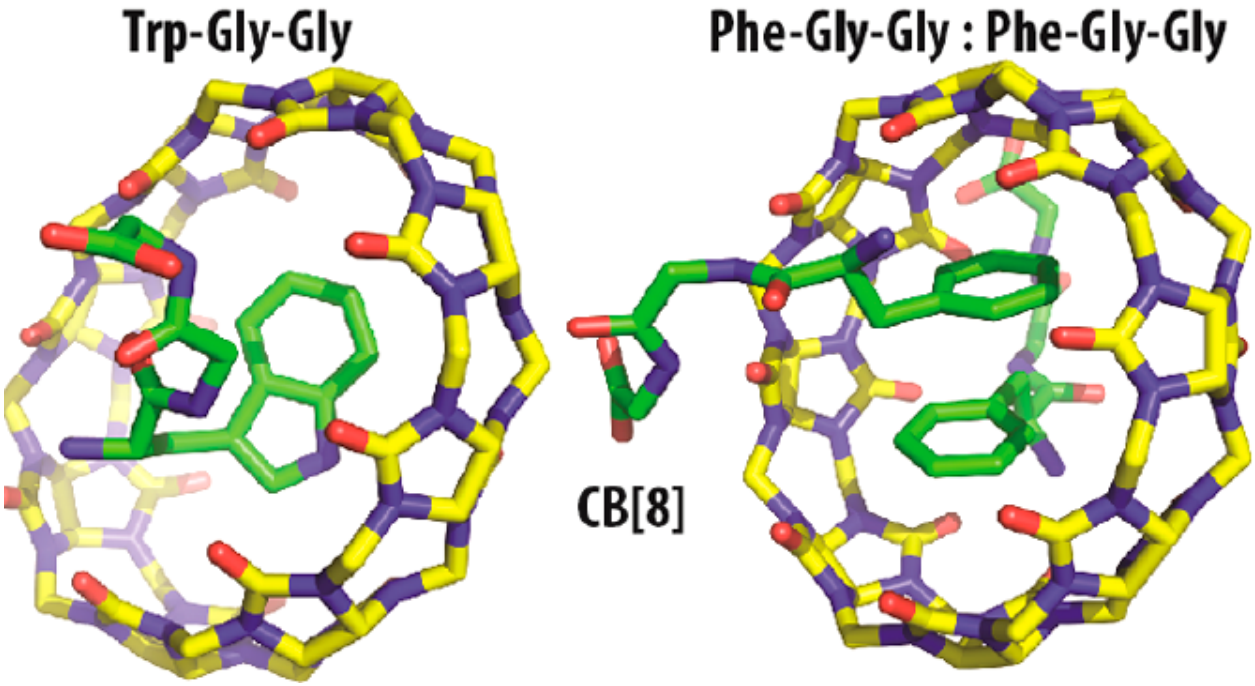
- **biological application: reinforcing tissue adhesion[1)], targeted drug delivery[2)]**

1) Thi, T. T. H. *et al*. *ACS Macro Lett.* **2017**, *6*, 83 .
2) Namgung, R. *et al*. *ACS Macro Lett.* **2017**, *6*, 83

Recognition of Side Chain by Synthetic Host Molecule

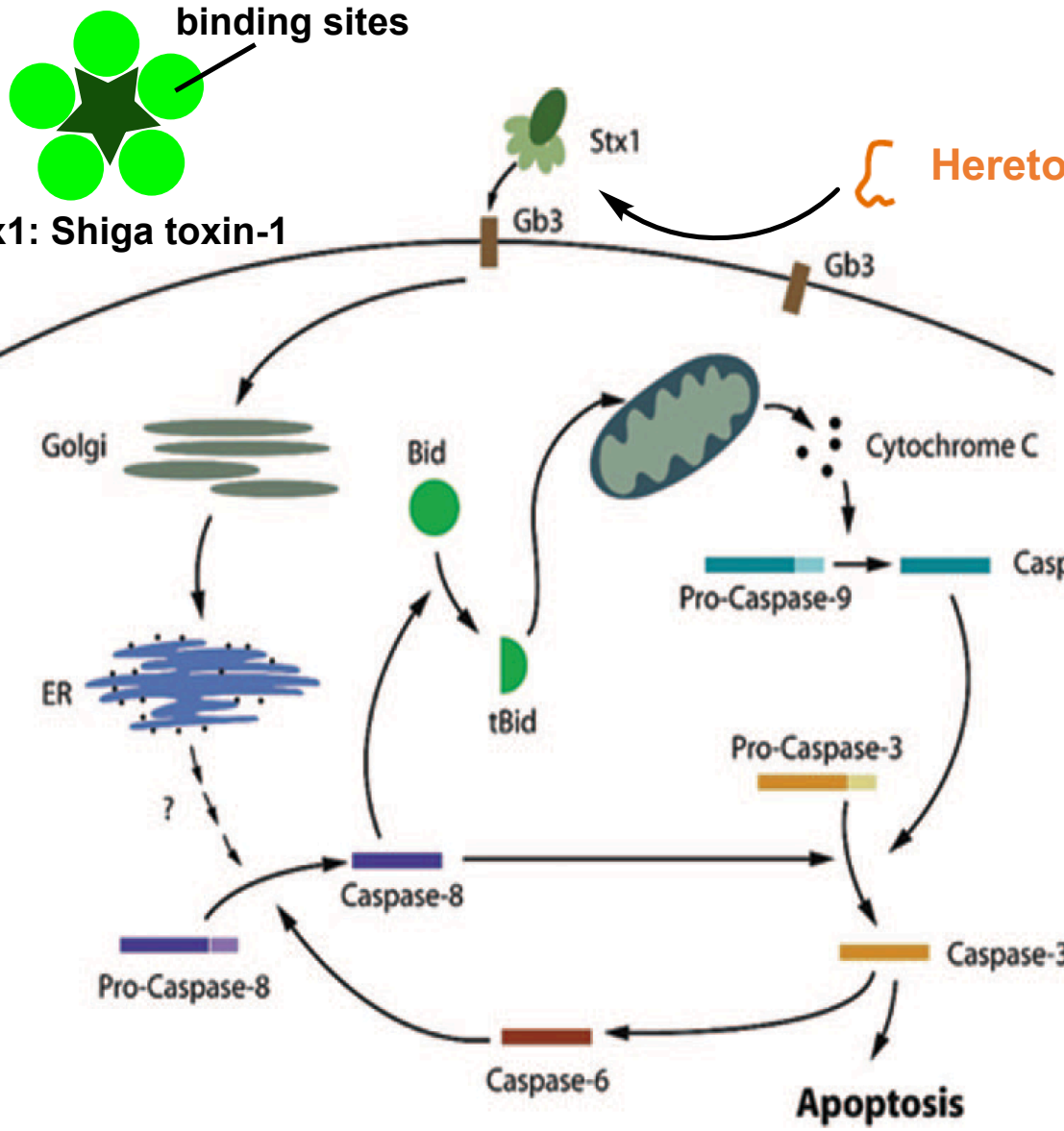


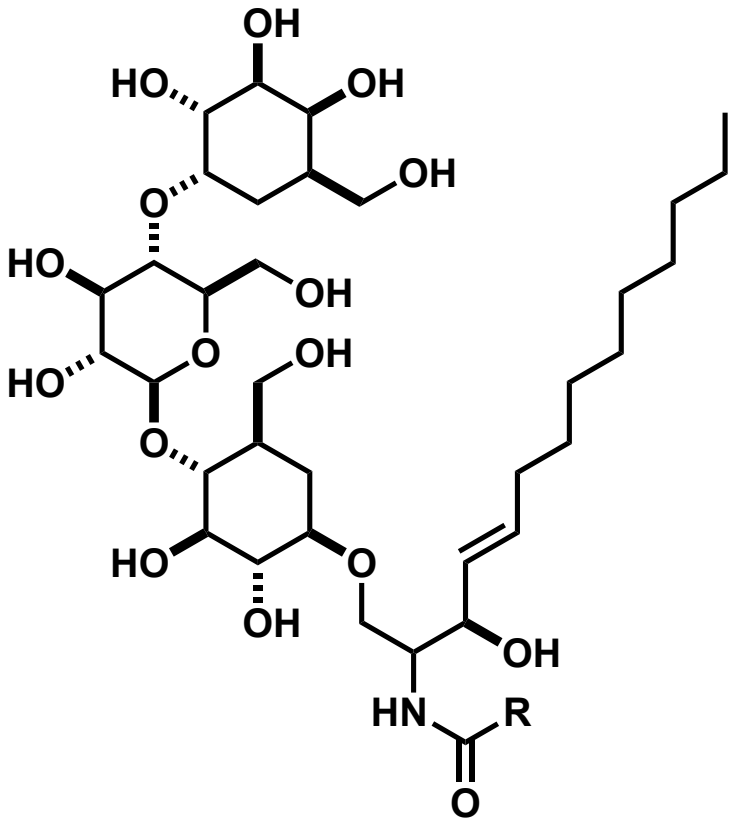




Dum, V. S.; Ottmann, C.; Milroy, G. L.; Brunsveld, L. *J. Am. Chem. Soc.* **2017**, *139*, 13960

Application of Heteromultivalency[1), 2)]



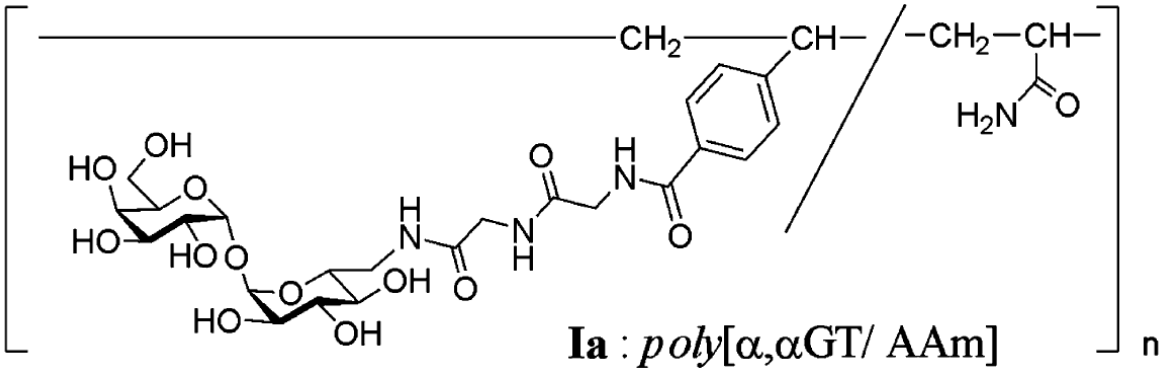


Gb3: Globotriaosylceramide

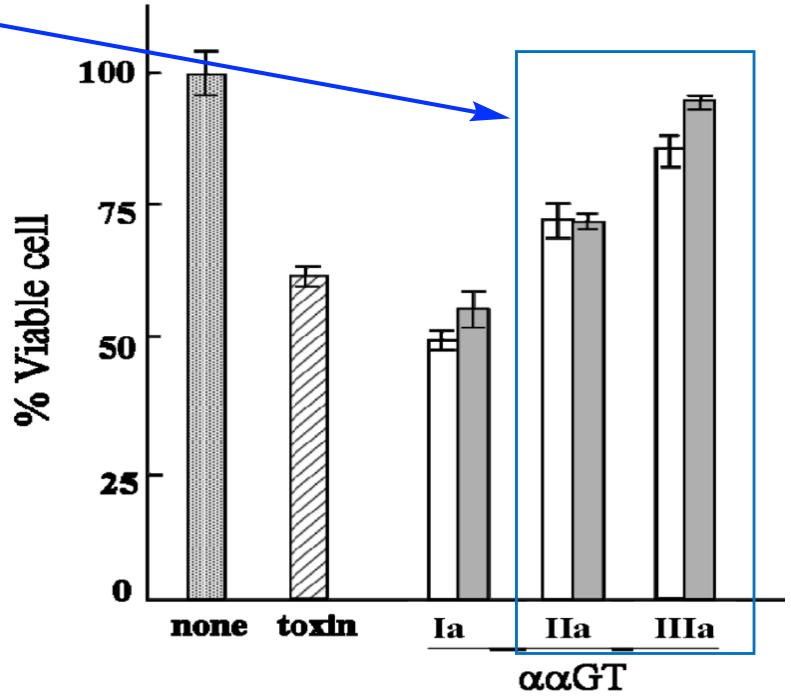
1) Lee, Y. S.; Cherla, P. R.; Caliskan, I.; Tesh, L. V. *Infect. Immun.* **2005**, *73*, 5115–5126 .
2) Miyachi, A.; Dohi, H.; Neri, P.; Mori, H.; Uzawa, H.; Seto, Y.; Nishida, Y. *Biomacromolecule.* **2005**, *73*, 5115–5126 .

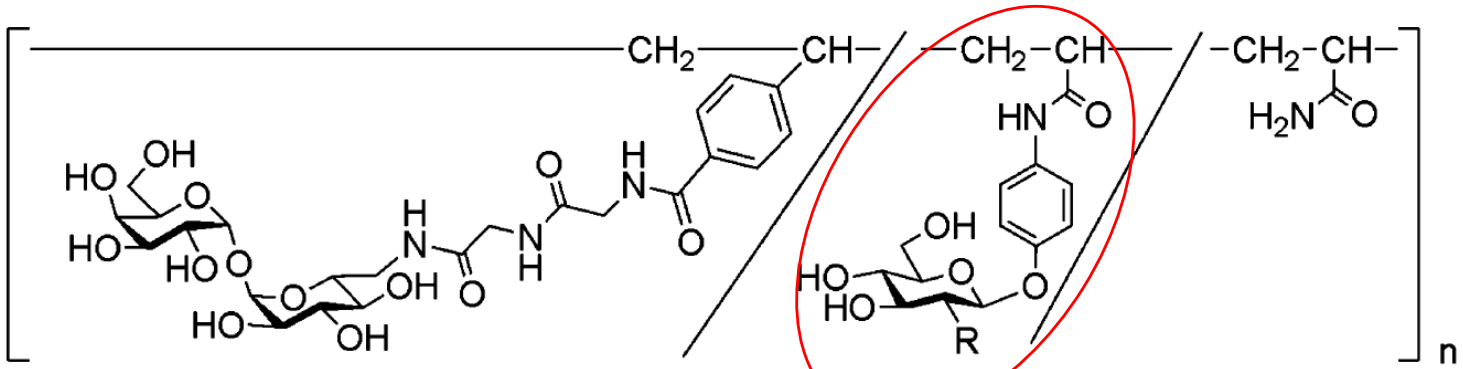
Application of Heteromultivalency

Heteroconjugated polymers bind Stx1 stronger than homoconjugated ones.



Ia : *poly*[α,αGT/ AAm]



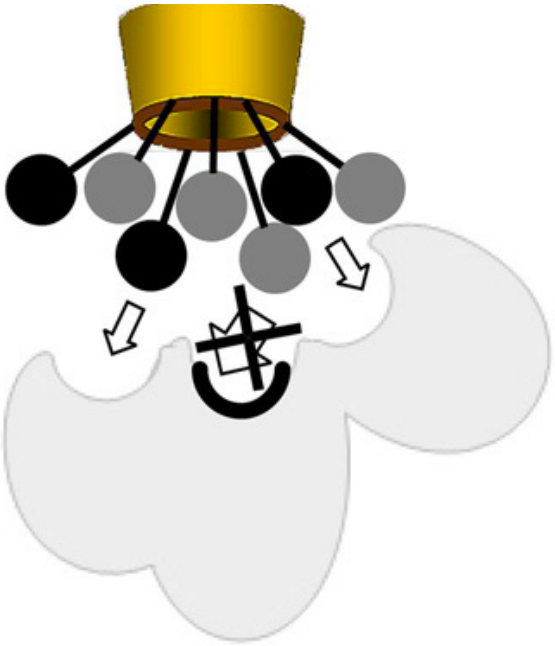


IIa: *poly*[α,αGT+Glc/ AAm] (R = OH)
IIIa: *poly*[α,αGT+GlcNAc/ AAm] (R = NHAc)

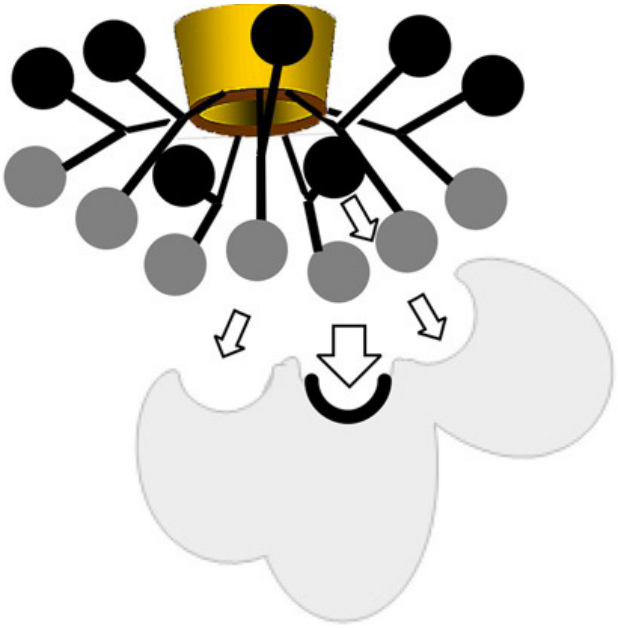
Ia: homomultivalent ligand
IIa, IIIa: heteromultivalent ligand

Miyachi, A.; Dohi, H.; Neri, P.; Mori, H.; Uzawa, H.; Seto, Y.; Nishida, Y. *Biomacromolecule.* **2005**, *73*, 5115–5126 .

Drawback of Covalent Heteroconjugate



Inhibition of proper accomodation



proper accomodation

Other ligands can thwart proper accomodation

- **More flexible and optimised frameworks are required**
- **Synthesis of best scaffolds is Intensive and time-consuming**

Moreno, G. I.; Caballero, O. F.; Neri, P.; Cuadro, R. R.; Mellet, O. C.; Fernandez, G. J. *Chem. Eur. J.* **2017**, *23*, 6295.

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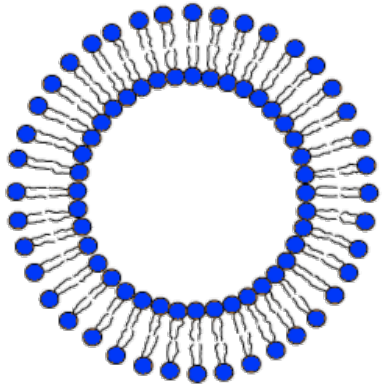
Heteromultivalency by Co-assembling monomer

scaffold

liposomes



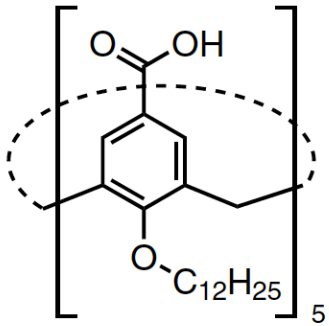
- Spontaneous assembly
- Non-covalent conjugation
- Flexibility

monomer

Host molecule

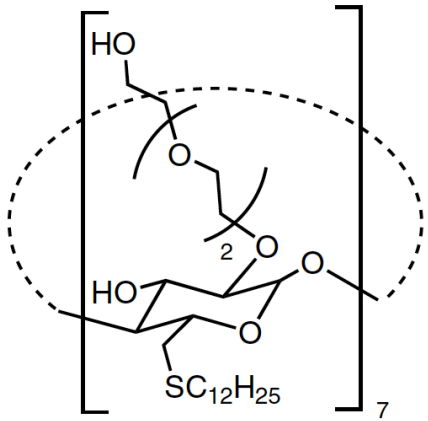
CA
calixarene

=

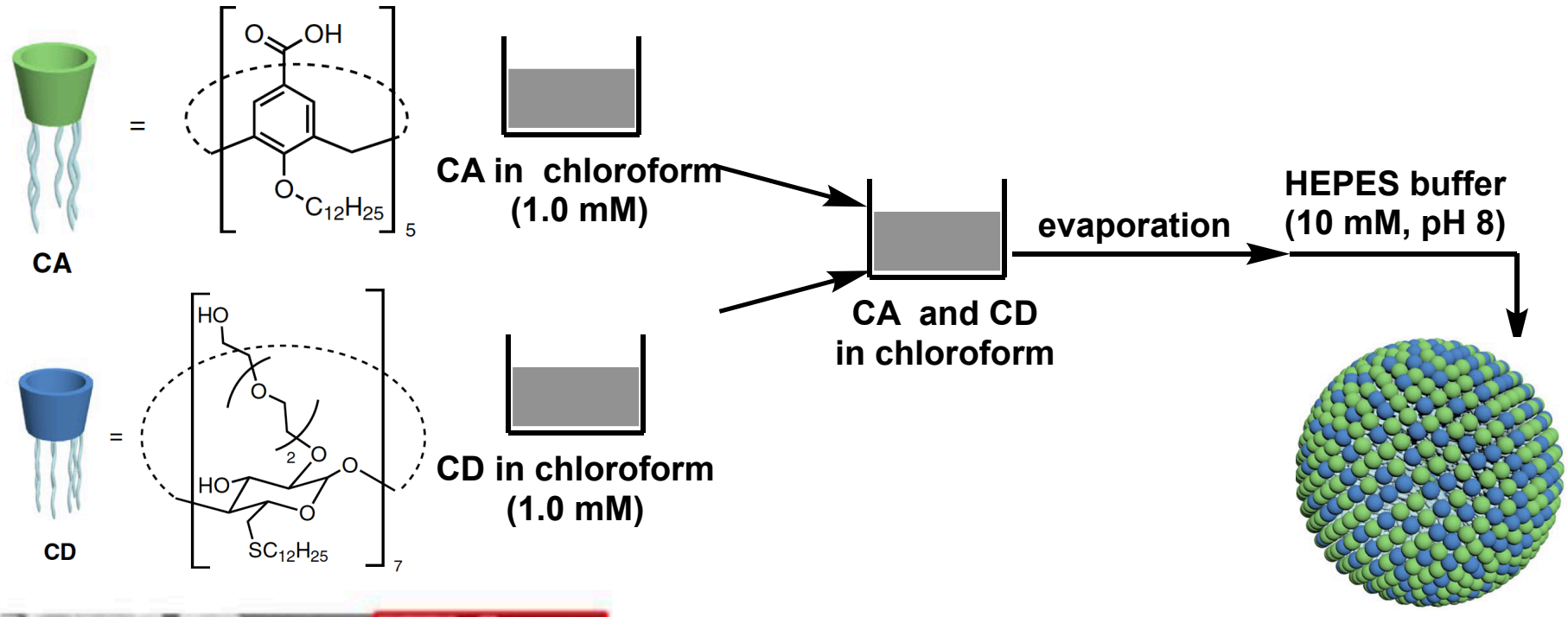


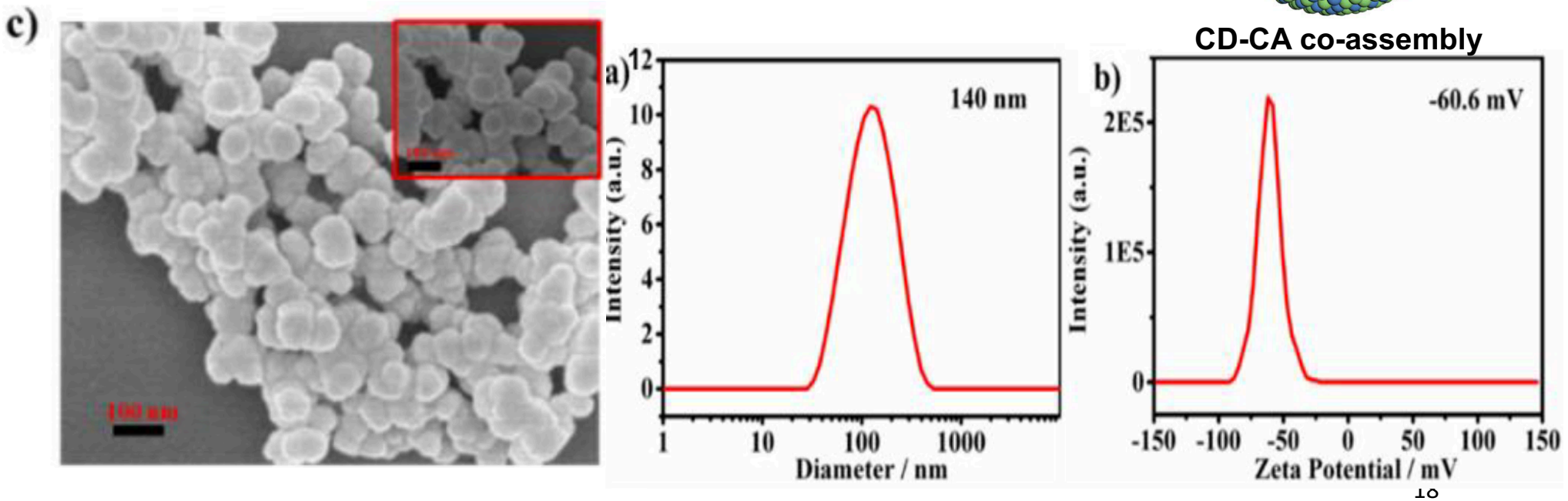
CD
cyclodextrin

=



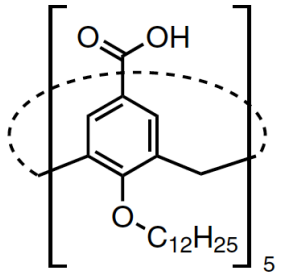
Preparation of CD-CA Co-assembly





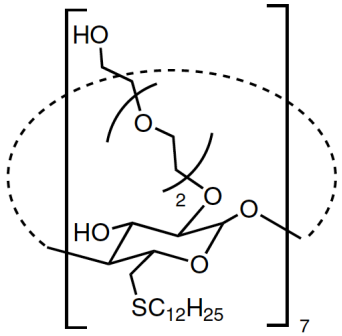
Preparation of CD-CA Co-assembly

CA



Lysine
$K=(1.4 \pm 0.1) \times 10^4\ M^{-1}$

CD

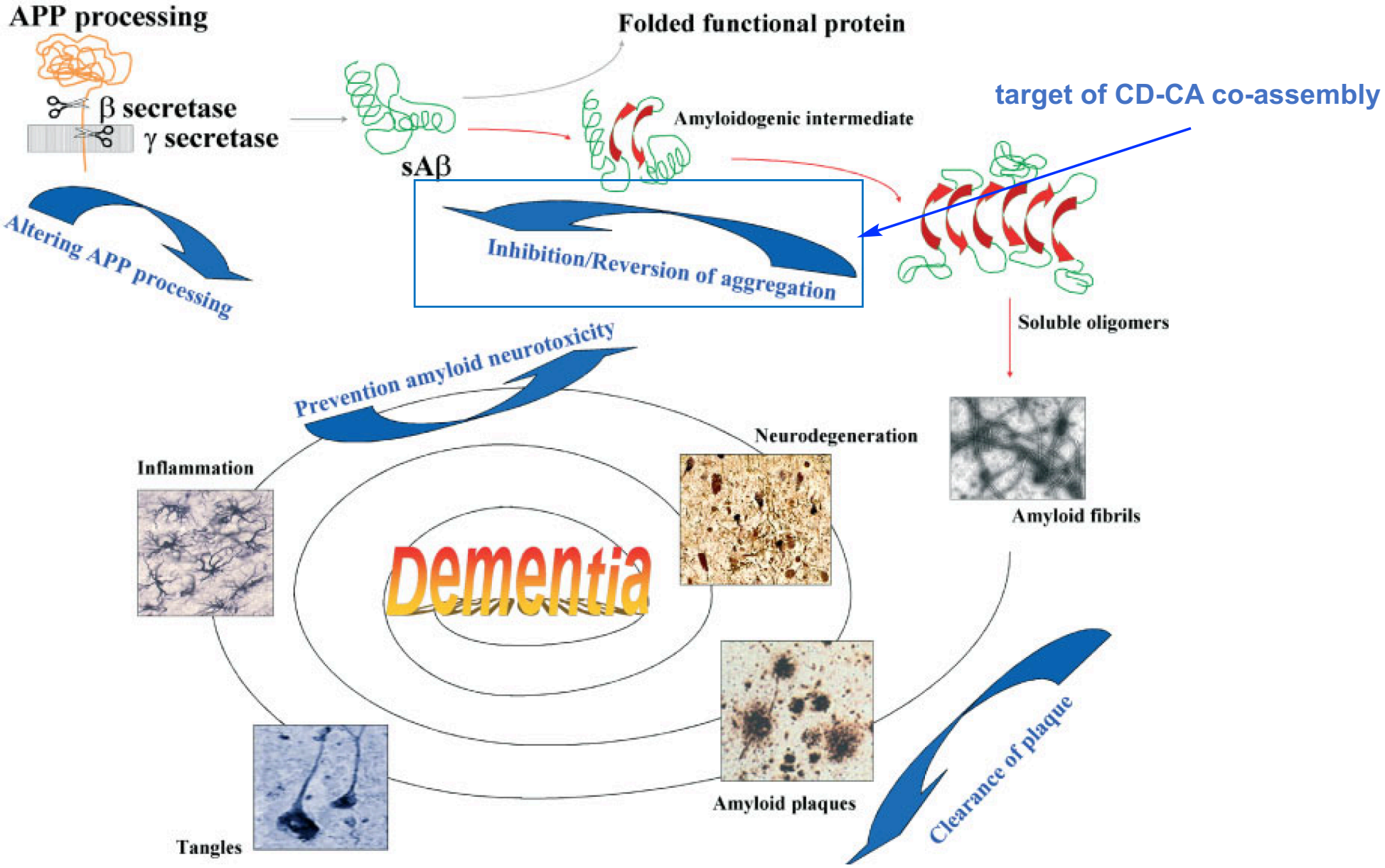


Tyrosine
$K=130\ M^{-1}$

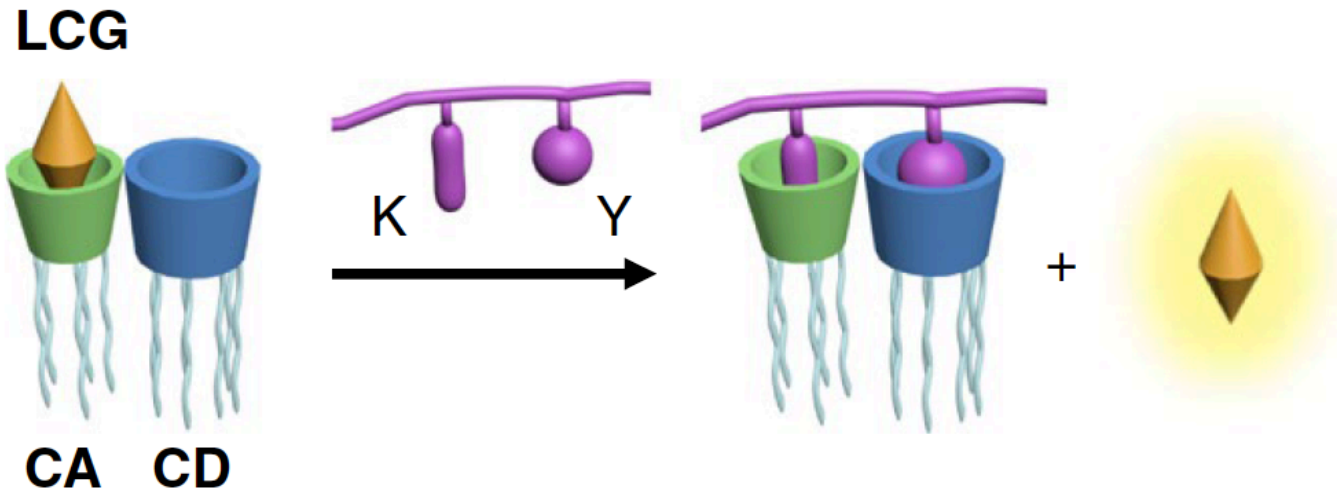
Abbreviation	Sequence
YK	$AcSYS(G)_4SKSNH_2$
YKY	$AcSYS(G)_4SKS(G)_4SYSNH_2$
YKYK	$AcSYS(G)_4SKS(G)_4SYS(G)_4SKSNH_2$
YKYKYK	$Ac(SYS(G)_4SKS(G)_4)_2SYS(G)_4SKSNH_2$
YYKK	$AcSYS(G)_4SYS(G)_4SKS(G)_4SKSNH_2$
KYK	$AcSKS(G)_4SYS(G)_4SKSNH_2$
KKK	$AcSKS(G)_4SKS(G)_4SKSNH_2$
YKYYKY	$AcSYS(G)_4SKS(G)_4SYS(G)_4SYS(G)_4SKS(G)_4SYSNH_2$
KYKKYK	$AcSKS(G)_4SYS(G)_4SKS(G)_4SKS(G)_4SYS(G)_4SKSNH_2$
YKK	$AcSYS(G)_4SKS(G)_4SKSNH_2$

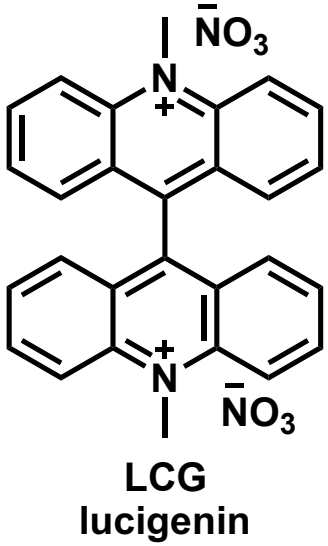
Amyloid β: DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA

Introduction of Alzheimer's Disease

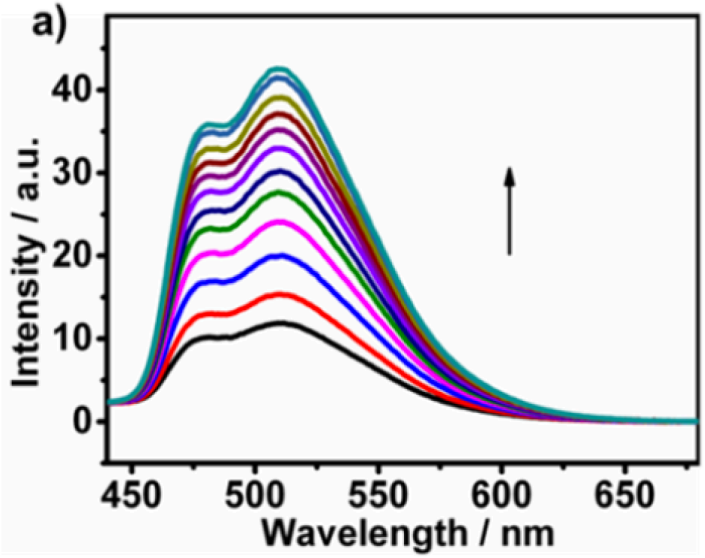


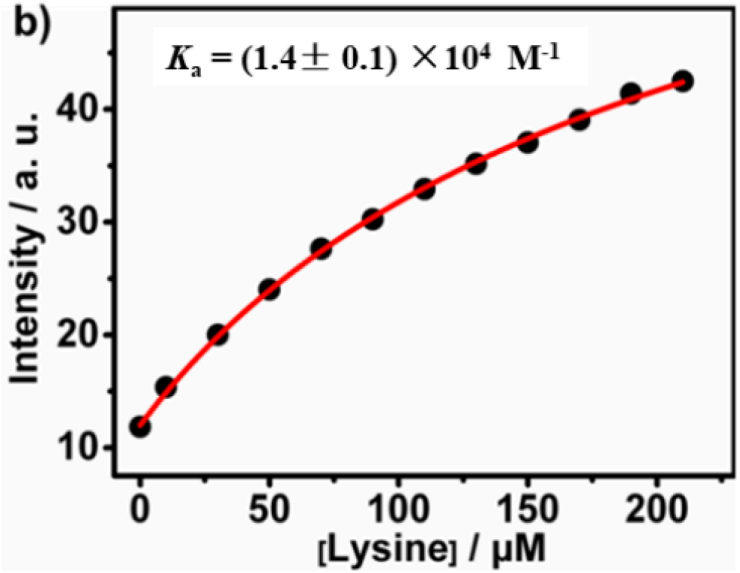
Fluorecent Indicator Displacement Assay





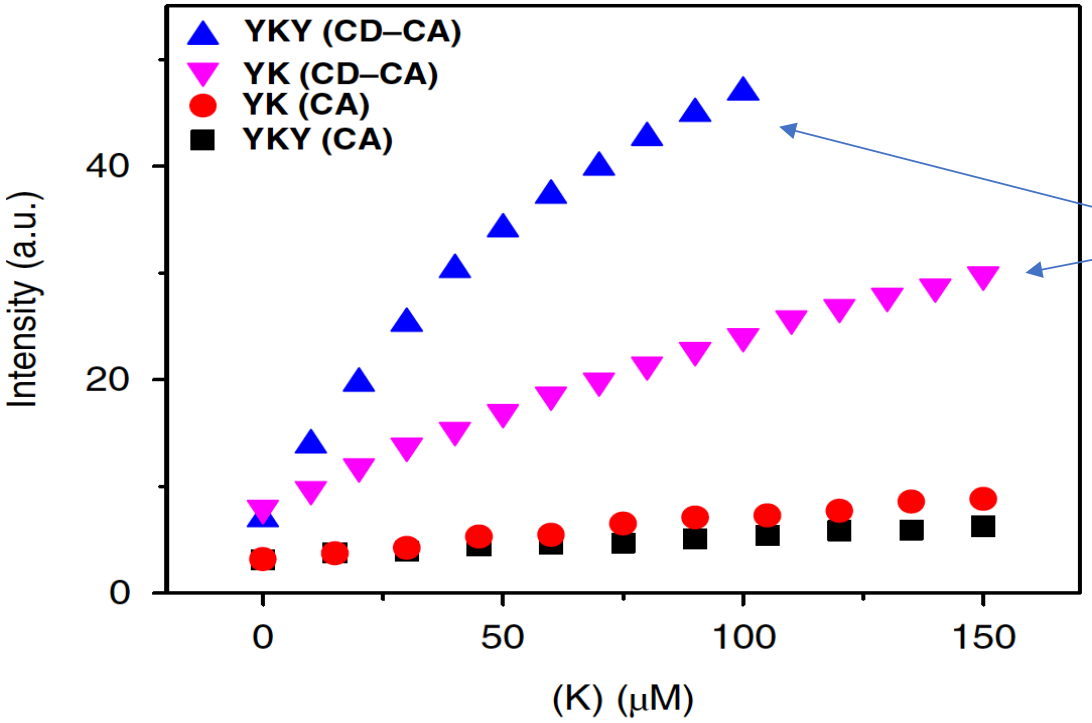
LCG
lucigenin



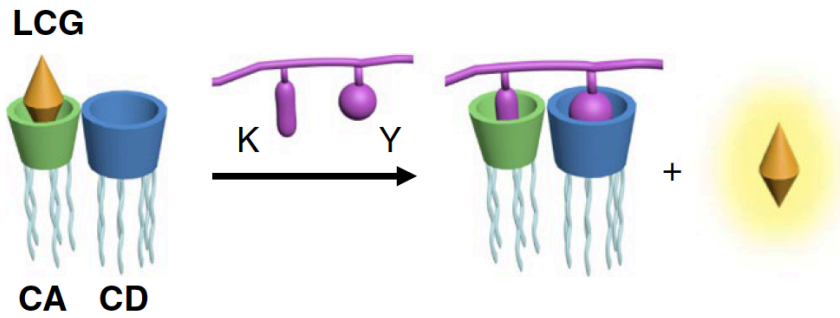


determination of K_a (CA-lysine)

Synergistic Effect of CD and CA

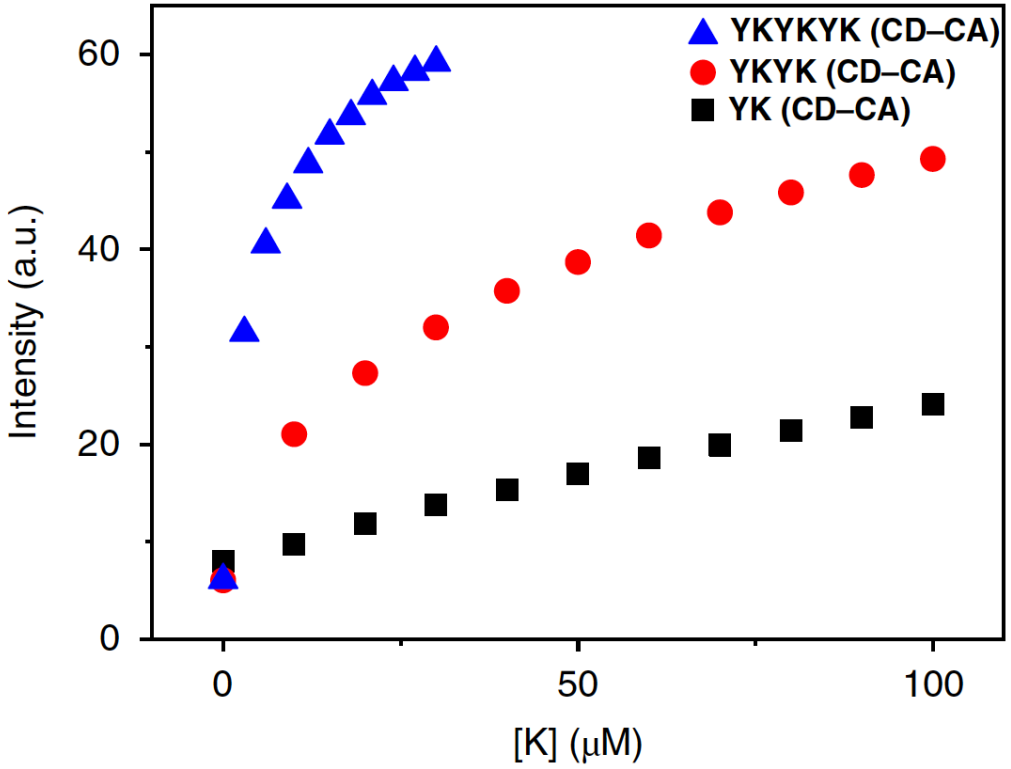


Synergistic Effect of heterotopic binding sites



	Host	YK	YKY
K_{ave} [a]	CA	$(2.7 \pm 2.0) \times 10^{3}$ [b]	$(1.1 \pm 1.0) \times 10^{3}$ [b]
	CD–CA	$(1.4 \pm 0.1) \times 10^{4}$	$(5.2 \pm 0.1) \times 10^{4}$
K_{multi} [c]	CA	$(2.7 \pm 2.0) \times 10^{3}$ [b]	$(1.1 \pm 1.0) \times 10^{3}$ [b]
	CD–CA	$(1.4 \pm 0.1) \times 10^{4}$	$(5.2 \pm 0.1) \times 10^{4}$

Cooperativity and Enhancement



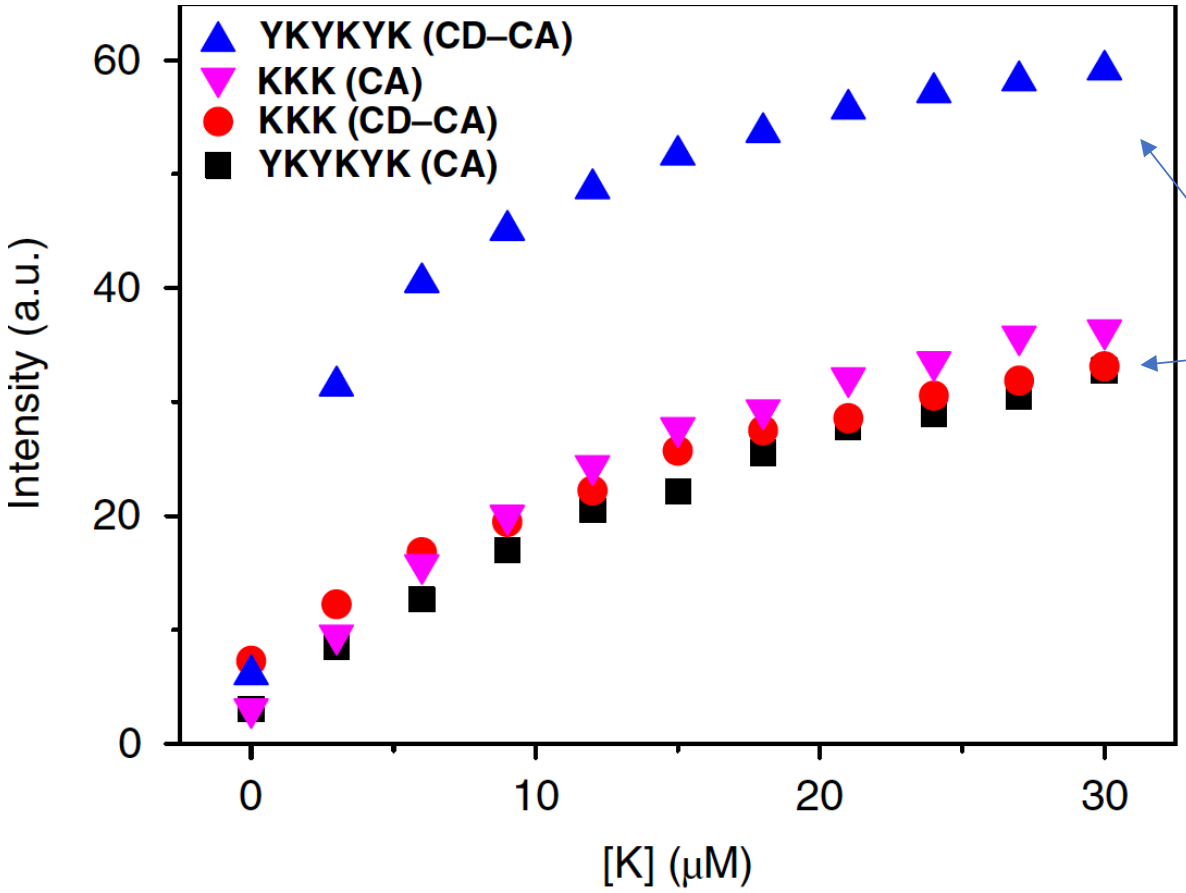
Parameter	YKYK	YKYKYK
β [a]	$(1.6 \pm 0.3) \times 10^{6}$	$(1.9 \pm 0.6) \times 10^{14}$
α [b]	1.2 ± 0.1	1.5 ± 0.1

- this case is positively cooperative ($\alpha > 1$)

- drastic increase of β
  - evidence of multivalent interaction

	Host	YK	YKYK	YKYKYK
K_{ave} [a]	CA	$(2.7 \pm 2.0) \times 10^{3}$ [b]	$(6.1 \pm 0.3) \times 10^{4}$	$(3.0 \pm 0.3) \times 10^{5}$
	CD–CA	$(1.4 \pm 0.1) \times 10^{4}$	$(1.5 \pm 0.1) \times 10^{5}$	$(1.4 \pm 0.1) \times 10^{6}$
K_{multi} [c]	CA	$(2.7 \pm 2.0) \times 10^{3}$ [b]	$(3.7 \pm 0.4) \times 10^{9}$	$(2.7 \pm 0.8) \times 10^{16}$
	CD–CA	$(1.4 \pm 0.1) \times 10^{4}$	$(2.3 \pm 0.3) \times 10^{10}$	$(2.7 \pm 0.6) \times 10^{18}$

Homomultivalency vs Heteromultivalency



Enhancement of binding <u>efficiency</u> and <u>specificity</u> was achieved by heteromultivalency

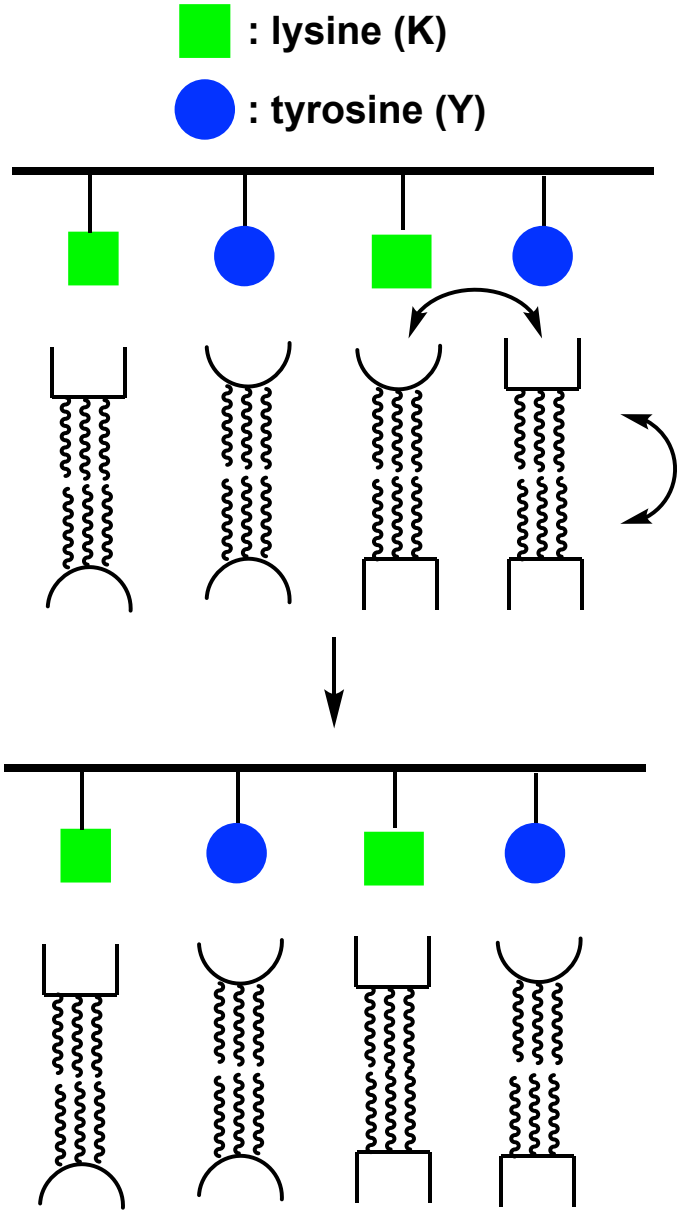
	Host	YKYKYK	KKK
K_{ave} [a]	CA	$(3.0 \pm 0.3) \times 10^5$	$(4.6 \pm 0.5) \times 10^5$
	CD–CA	$(1.4 \pm 0.1) \times 10^6$	$(2.2 \pm 0.3) \times 10^5$
K_{multi} [c]	CA	$(2.7 \pm 0.8) \times 10^{16}$	$(9.7 \pm 3.2) \times 10^{16}$
	CD–CA	$(2.7 \pm 0.6) \times 10^{18}$	$(1.1 \pm 0.4) \times 10^{16}$

Self-adapting of CD-CA to Model Peptide

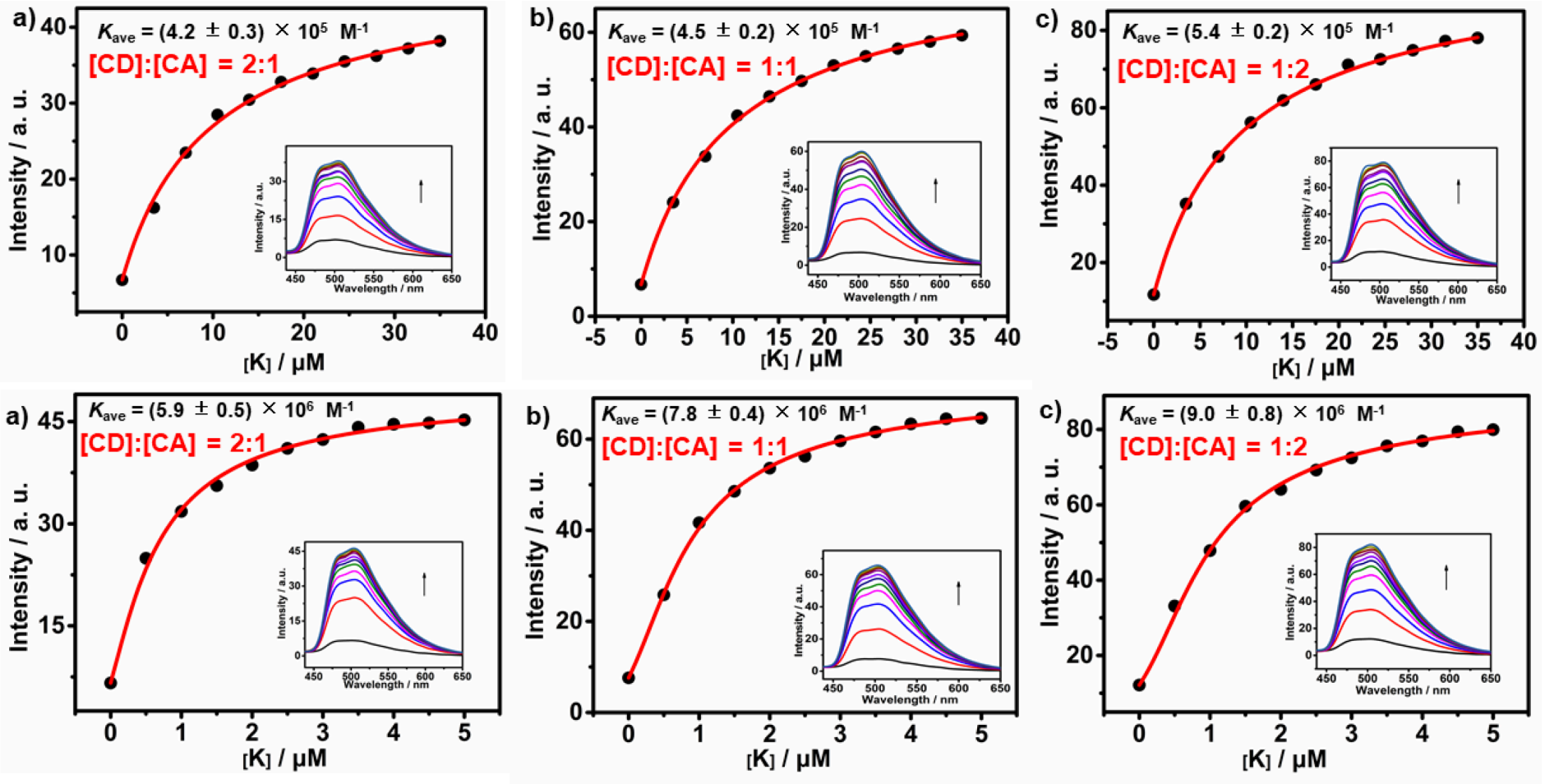
	Host	YKYK	YYKK
K_{ave} [a]	CA	$(6.1 \pm 0.3) \times 10^4$	$(6.3 \pm 0.2) \times 10^4$
	CD–CA	$(1.5 \pm 0.1) \times 10^5$	$(1.4 \pm 0.1) \times 10^5$
K_{multi} [c]	CA	$(3.7 \pm 0.4) \times 10^9$	$(4.0 \pm 0.3) \times 10^9$
	CD–CA	$(2.3 \pm 0.3) \times 10^{10}$	$(2.0 \pm 0.3) \times 10^{10}$

similar binding strength between YKYK and YYKK

- surface mobility
- self-optimization of spacing and sequence



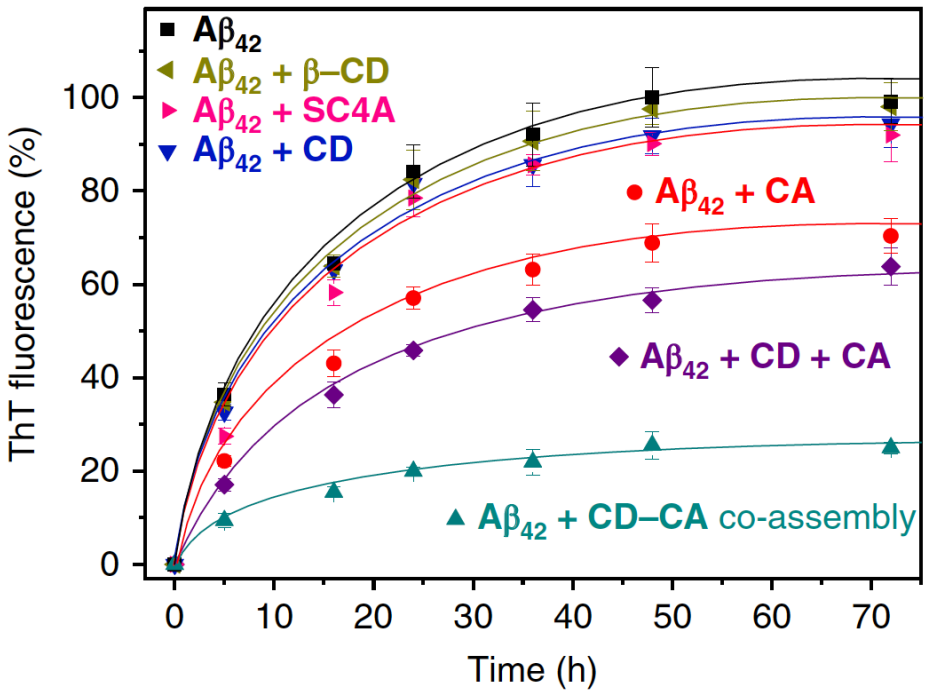
Self-adapting to Model Peptide



[CD]:[CA]	2:1	1:1	1:2
YKYYKY	$(4.2 \pm 0.3) \times 10^5$	$(4.5 \pm 0.2) \times 10^5$	$(5.4 \pm 0.2) \times 10^5$
KYKKYK	$(5.9 \pm 0.5) \times 10^6$	$(7.8 \pm 0.4) \times 10^6$	$(9.0 \pm 0.8) \times 10^6$

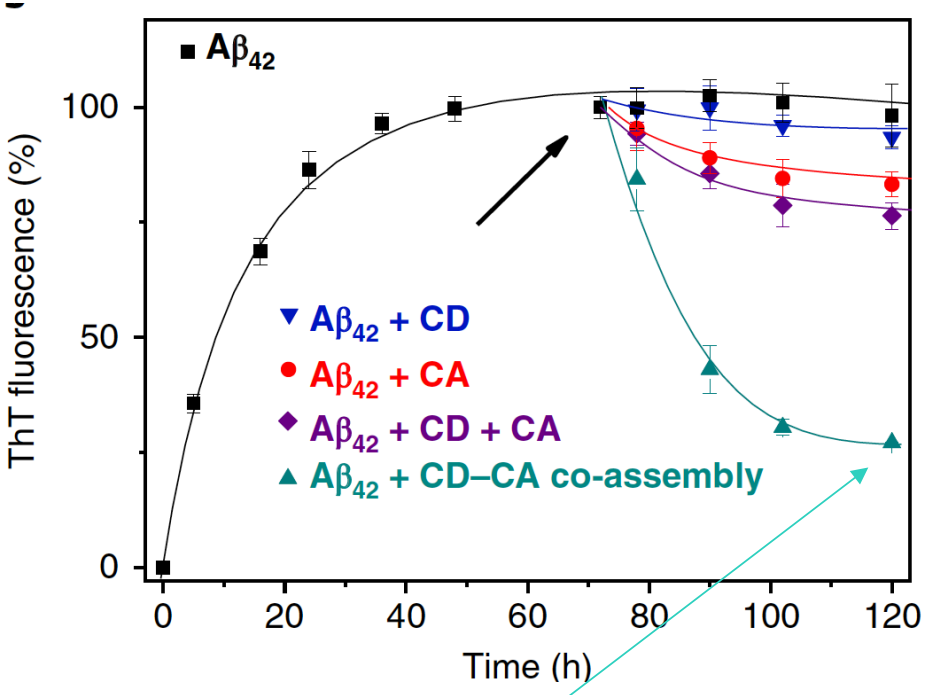
Inhibition of amyloid fibrillation

inhibition of $A\beta_{42}$ aggregation



SC4A: sulfonatocalix[4]arene

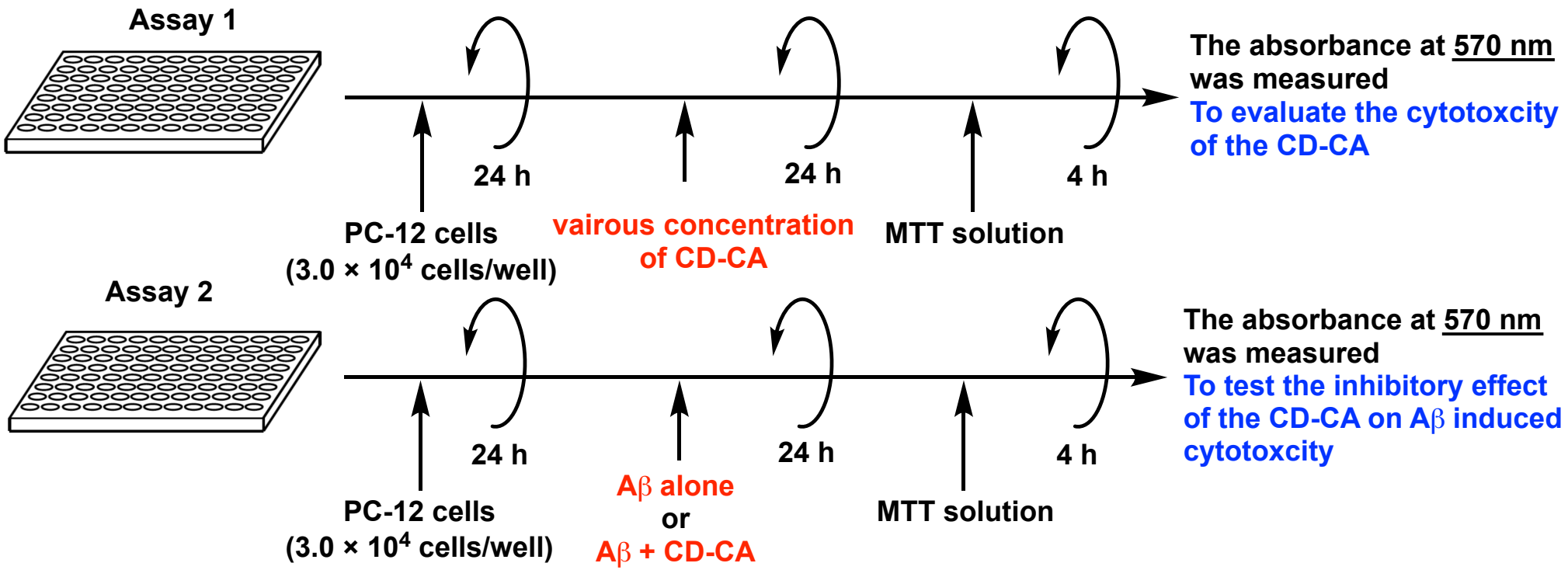
disaggregation of $A\beta_{42}$ fibrils

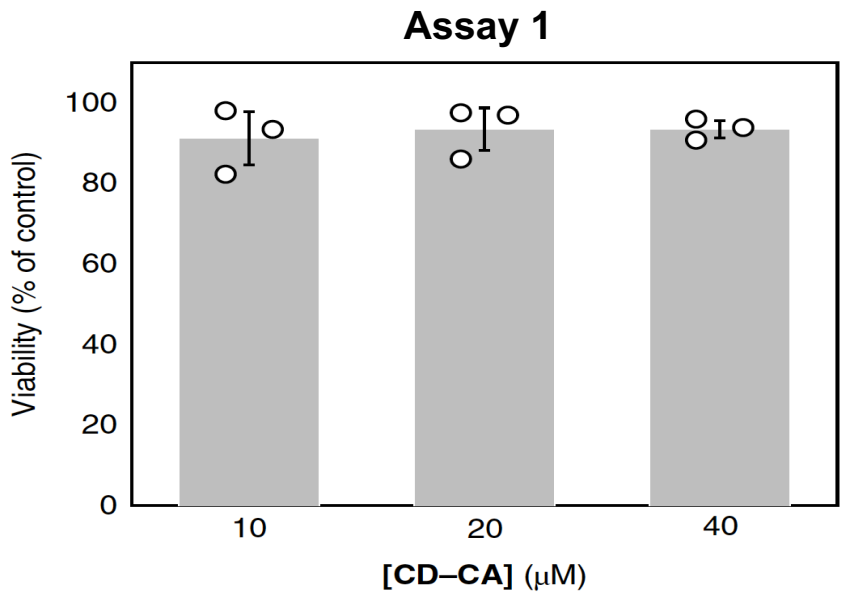


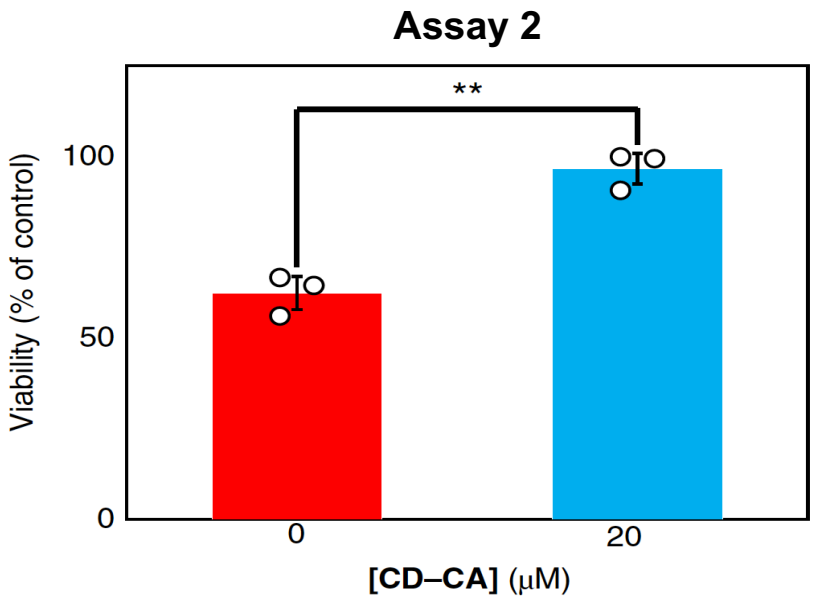
dissolving preformed $A\beta_{42}$ aggregation by CD-CA co-assembly

CD-CA co-assembly has potential in the application of Alzheimer's disease therapy

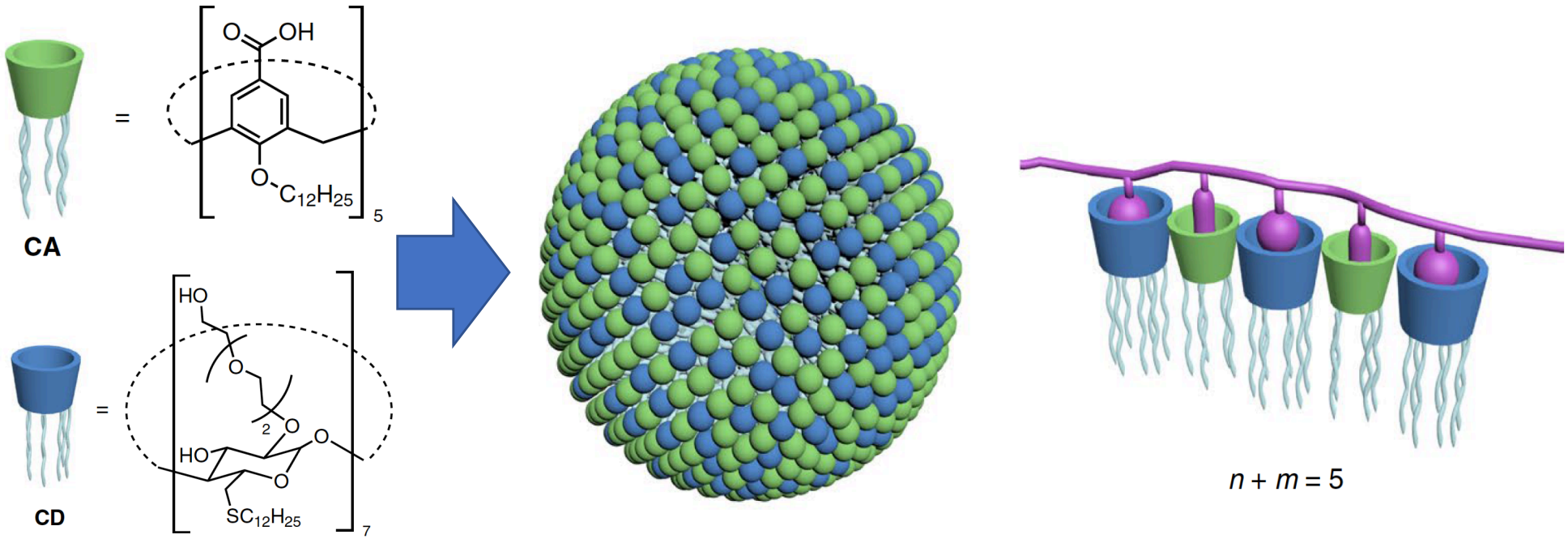
Cell Viability Assay







Conclusion



- **Heteromultivalent molecular recognition was achieved by co-assembling different two macrocyclic receptor.**

- **Self-assembled heteromultivalent strategy is amenable to other ensembles and targets.**