Oriented Immobilization of Protein on Solid Support

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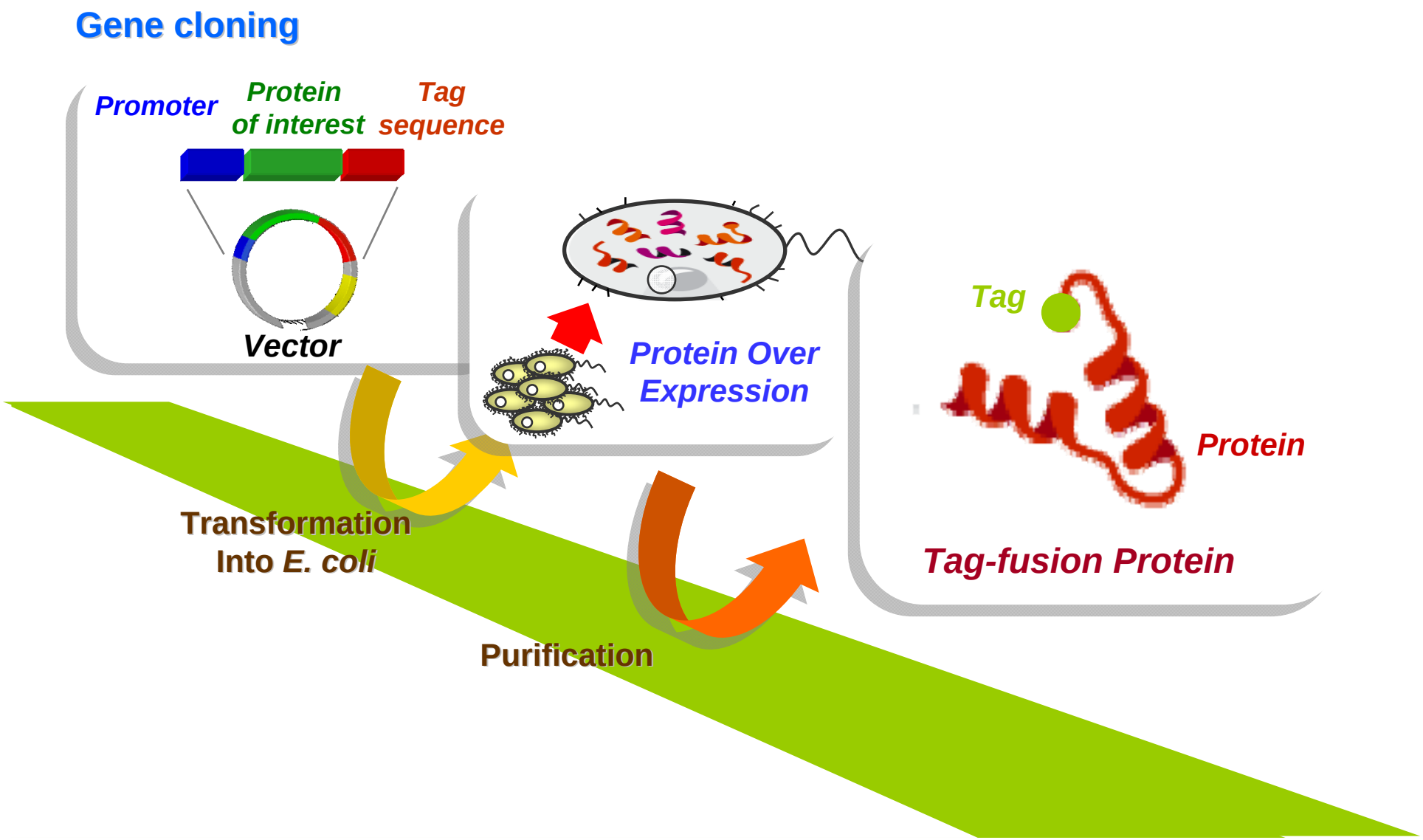
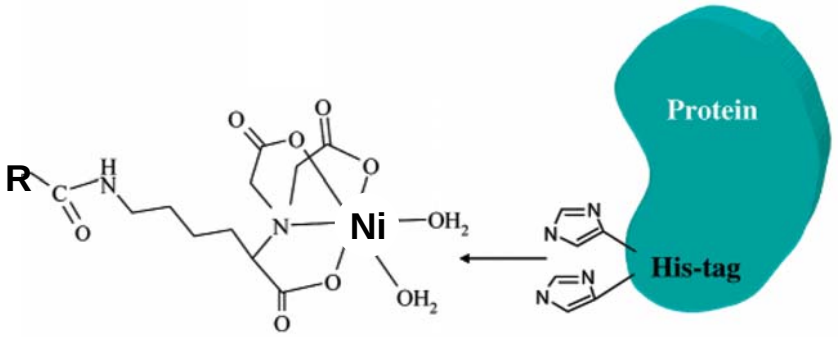
Recombinant Protein Over-expression

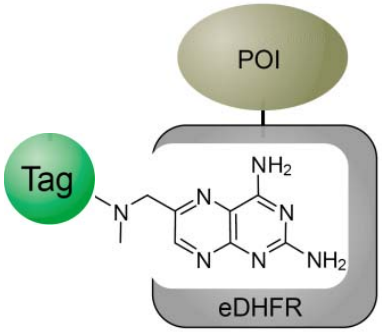


Non-covalent :

His-tag ↔ Ni-NTA



Dihydrofolate reductase (DHFR v.s. Mtx, methotrexate)

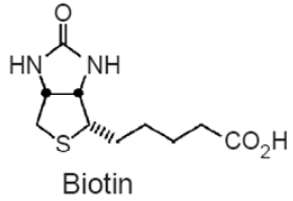


POI : protein of interesting

$K_d = 2.5 \times 10^{-11}$ M

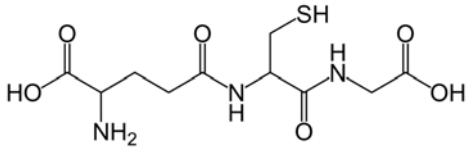
Miller, L. W *et al. Curr. Opin. Chem. Biol.* **2005**, *9*, 56-61.

Streptavidin (v.s. D-biotin)



$K_d \sim 10^{-15}$ M

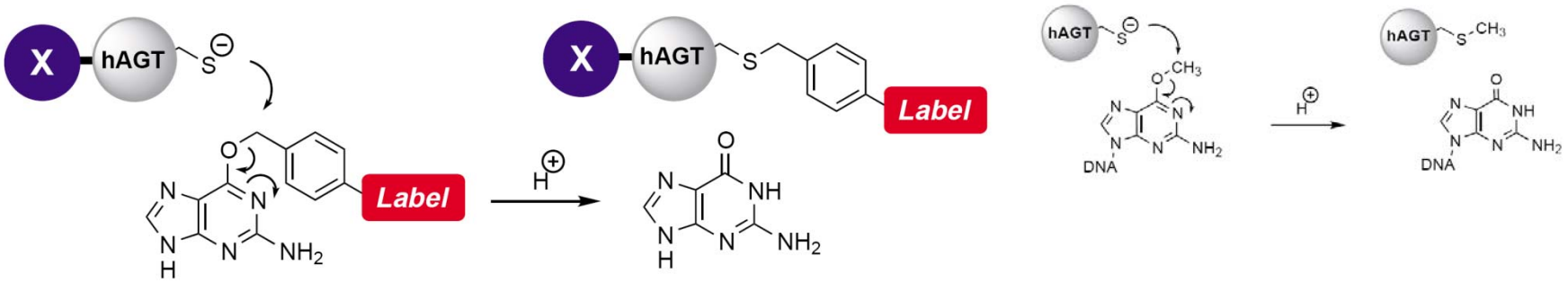
Glutathione S-transferase v.s. reduce glutathione (GST v.s. GSH)



Glutathione

Enzymatic Modification

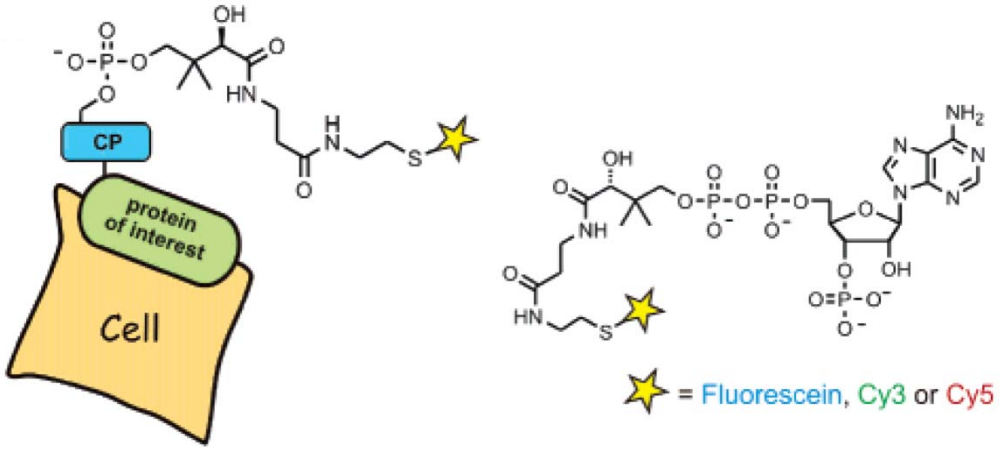
I. Human DNA repair protein O^6-alkylguanine-DNA alkyltransferase (hAGT v.s. O^6-alkylguanine-DNA)



O^6-benzylguanine

Johnsson, K. *et al. Nat. Biotechnol.* **2003**, *21*, 86-89.

II. Phosphopantetheine transferase (PPTase)



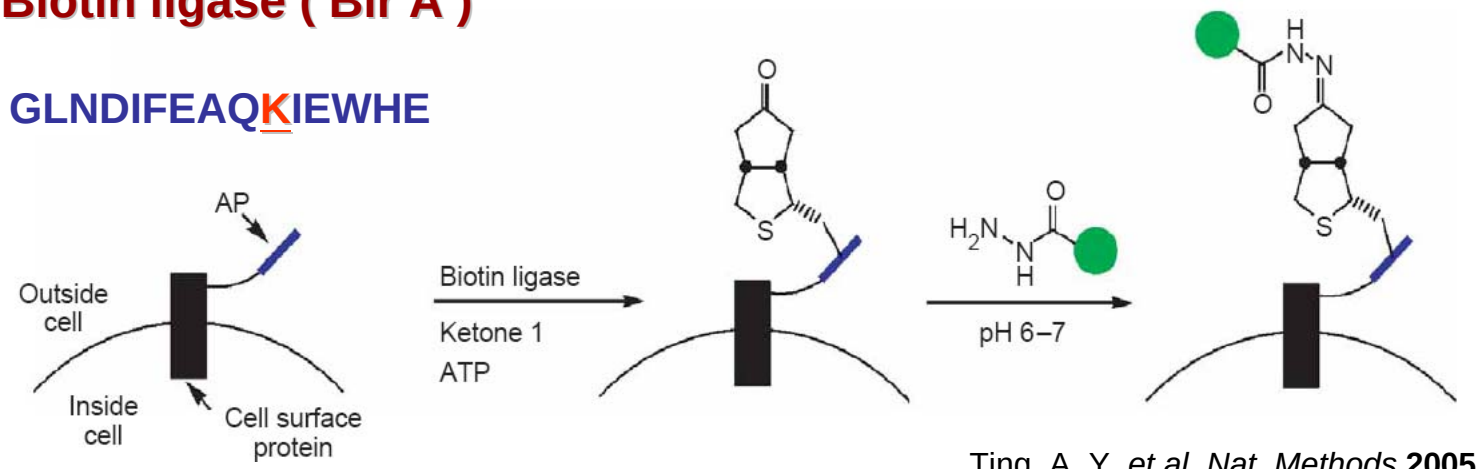
phosphopantetheine transferases (**PPTases**) catalyze the transfer of Ppant from **CoA** to an invariant serine residue of CPs carrier proteins (**CP**)

Johnsson, K. *et al. J. Am. Chem. Soc*. **2005**, *127*, 12770-12771.
Walsh, C. T. *et al. ACS Chem. Biol.* **2007**, *5*, 337-346.

Enzymatic Modification

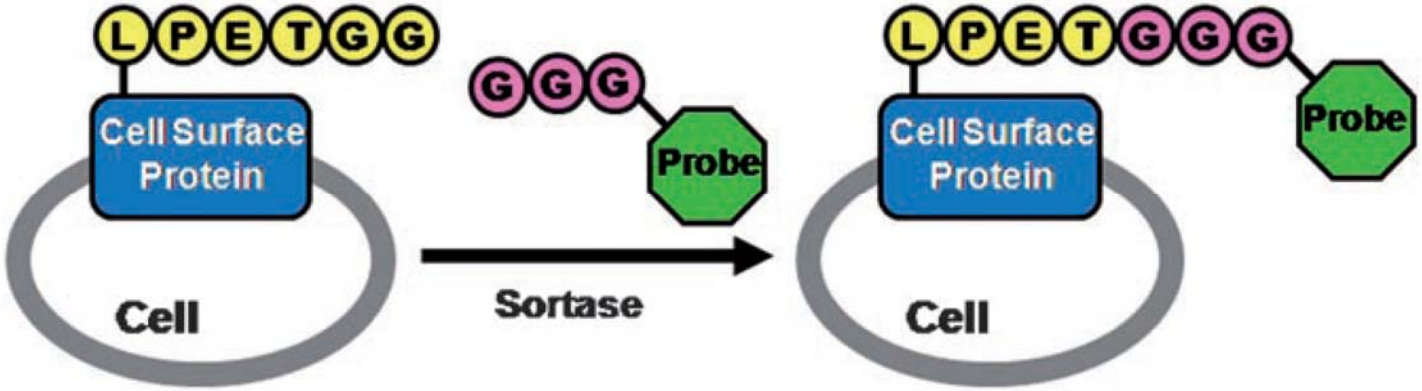
III. Biotin ligase (Bir A)

GLNDIFEAQ<u>K</u>IEWHE



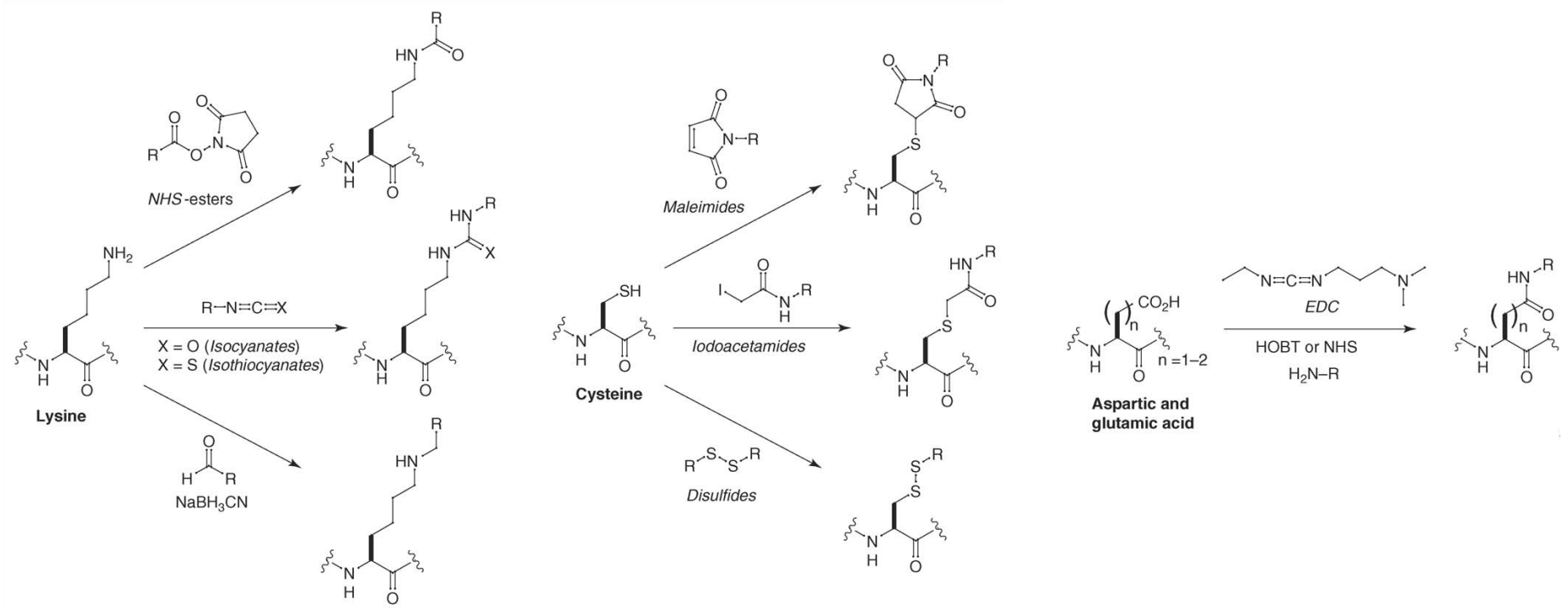
Ting. A. Y. *et al. Nat. Methods* **2005**, *2*, 99-104.

IV. Sortase A

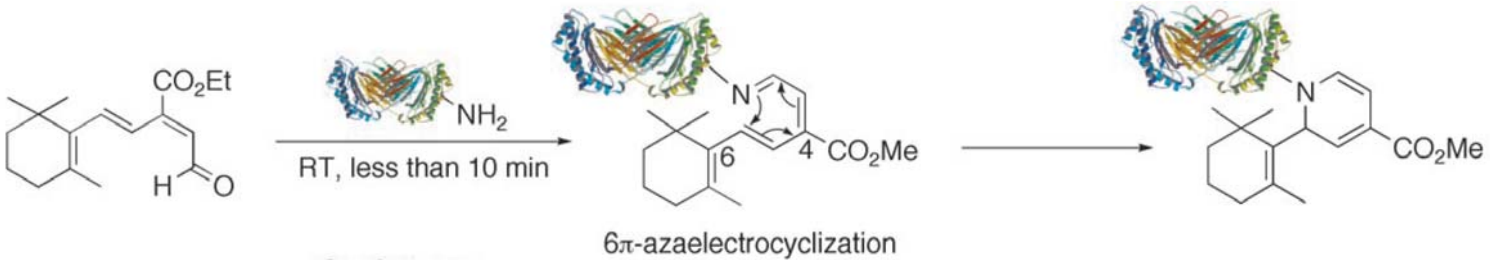


Nagamune, T. *et al. ChemBioChem* **2008**, *9*, 802-807.

Residue-Specific Protein Bioconjugation



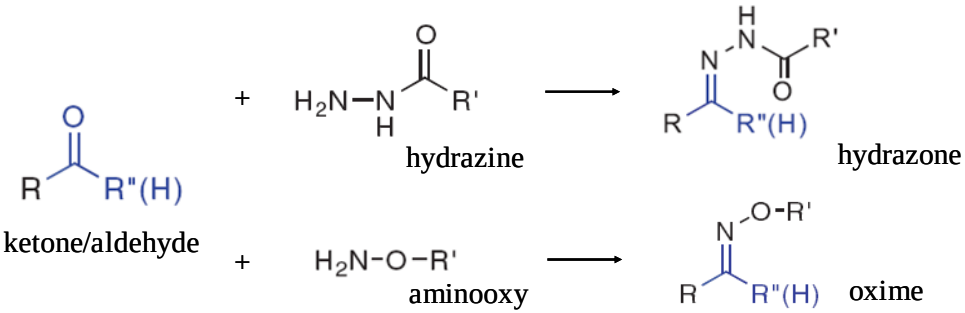
Francis, M. B. *et al. Curr. Opin. Biol. Chem.* **2006**, *10*, 253-262.



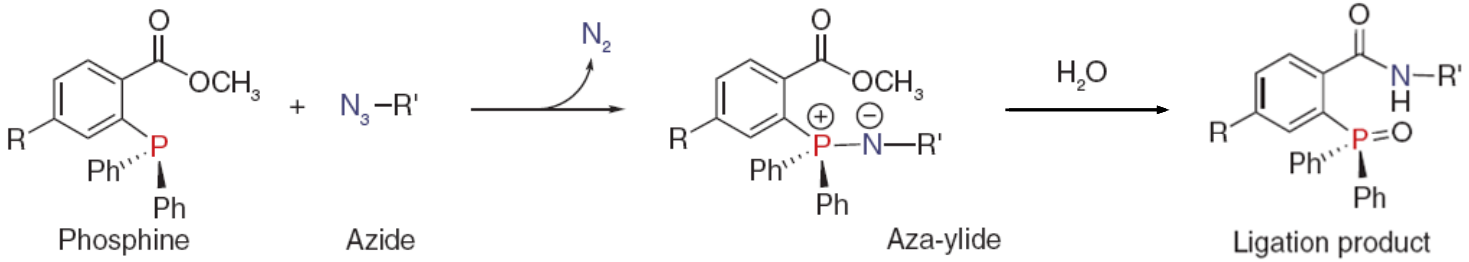
Fukase, K. *et al. Angew. Chem. Int. Ed.* **2008**, *47*, 102-105.

Bioorthogonal Chemistry

Imine formation

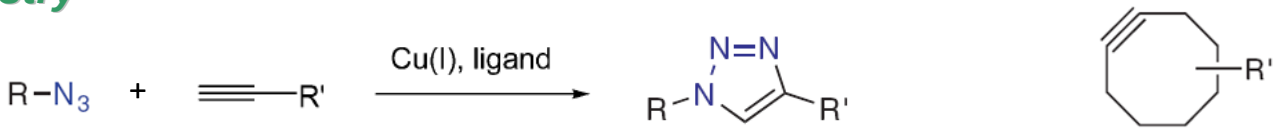


Staudinger ligation



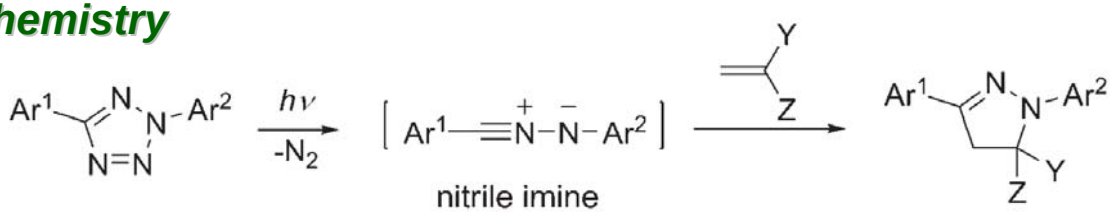
Bertozzi, C. R. *et al. Nat. Chem. Biol*. **2005**, *1*, 13-21.

Click chemistry



Bertozzi, C. R. *et J. Am. Chem. Soc*. **2004**, *126*, 15046-15047.

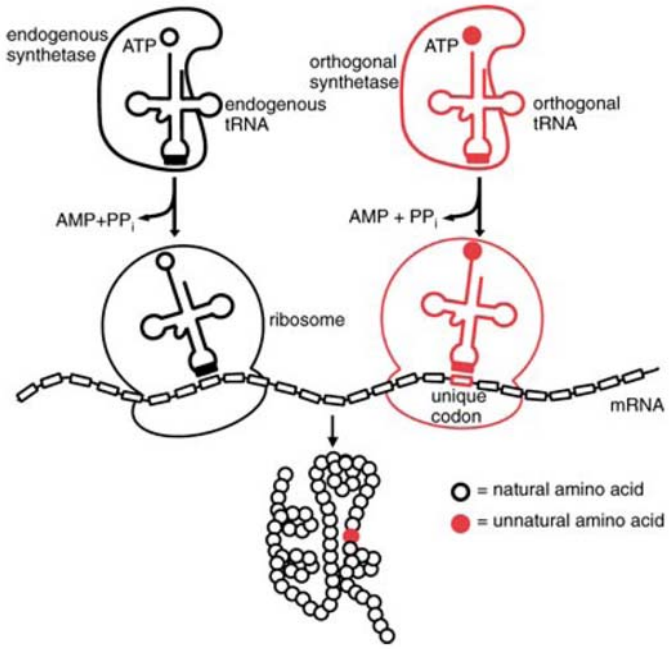
Photo click chemistry



Lin, Q. *et al. Angew. Chem. Int. Ed.* **2008**, *47*, 2832-2835.

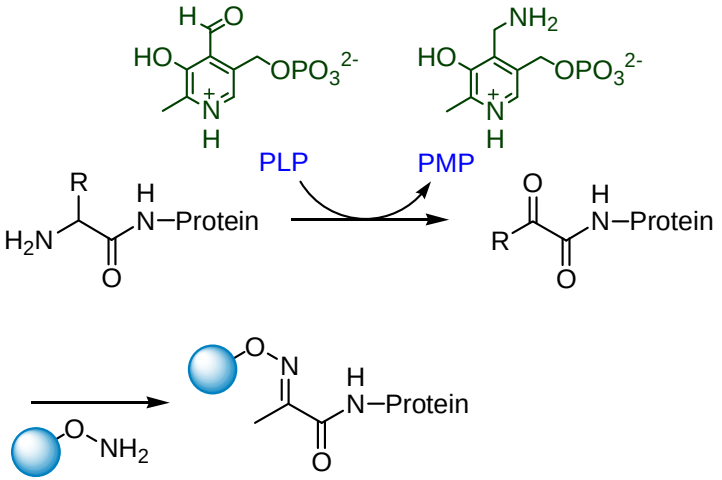
Insertion of target tag

I. Non-natural amino acid



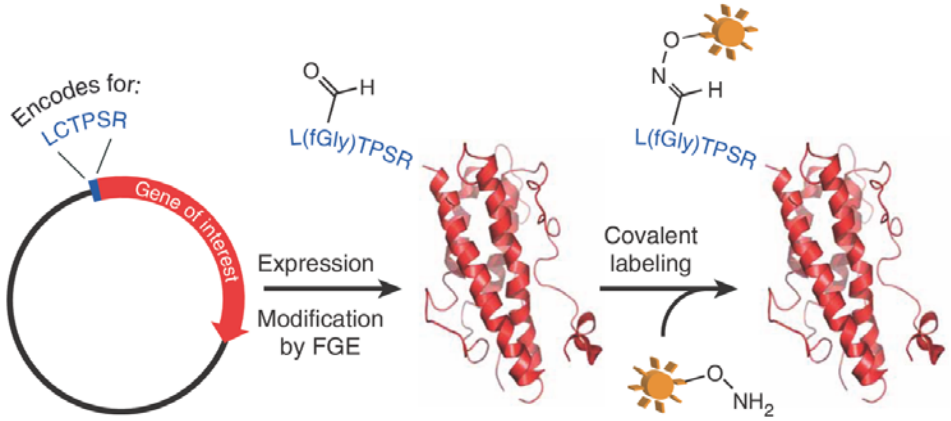
Schultz, P. G. *et al. Science* **1989**, *244*, 182-188.

II. Transamination



Francis, M. B. *et al. ACS Chem. Biol.* **2007**, *2*, 247-251.
Burkart, M. D. *et al. Curr. Opin. Chem. Biol.* **2007**, *11*, 12-19.

III. Formyl glycine-generating enzyme (FGE)

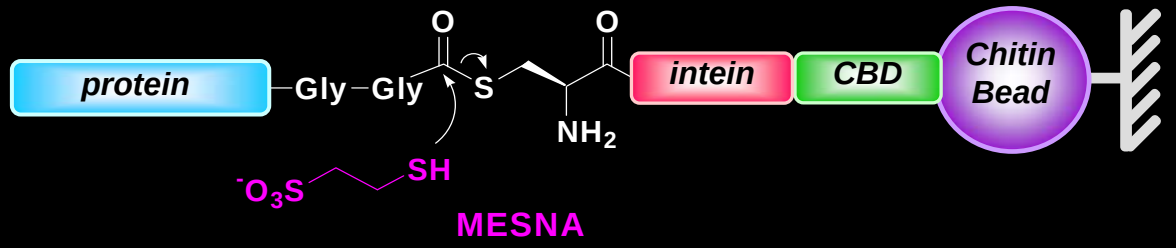


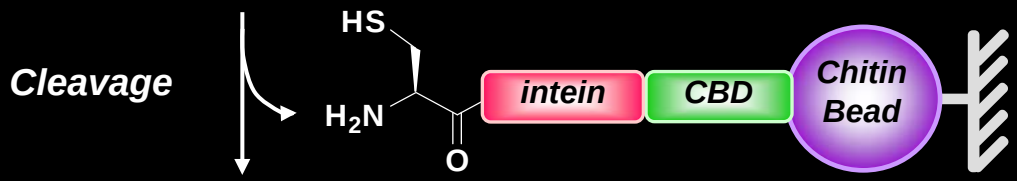
LCXPXR
X = Thr, Ala, Gly

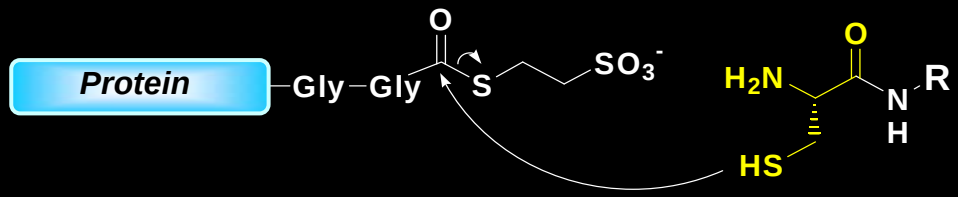
Bertozzi, C. R. *et al. Nat. Chem. Biol.* **2007**, *3*, 321-322.

Insertion of target tag

IV. Native Chemical Ligation & Expression Protein Ligation





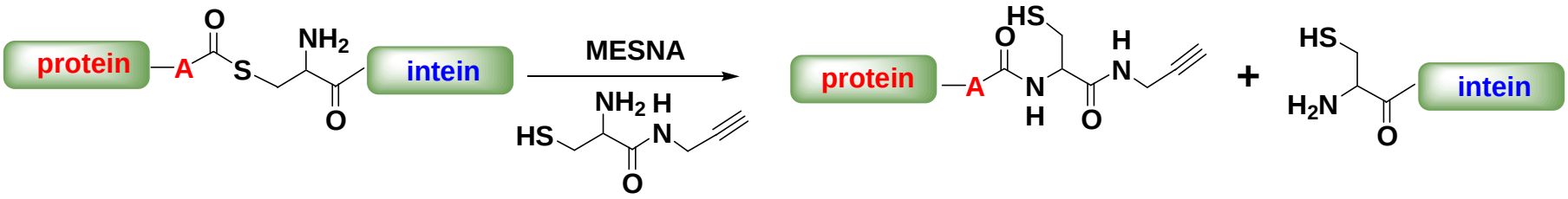


Thiol exchange *& N-S shift*

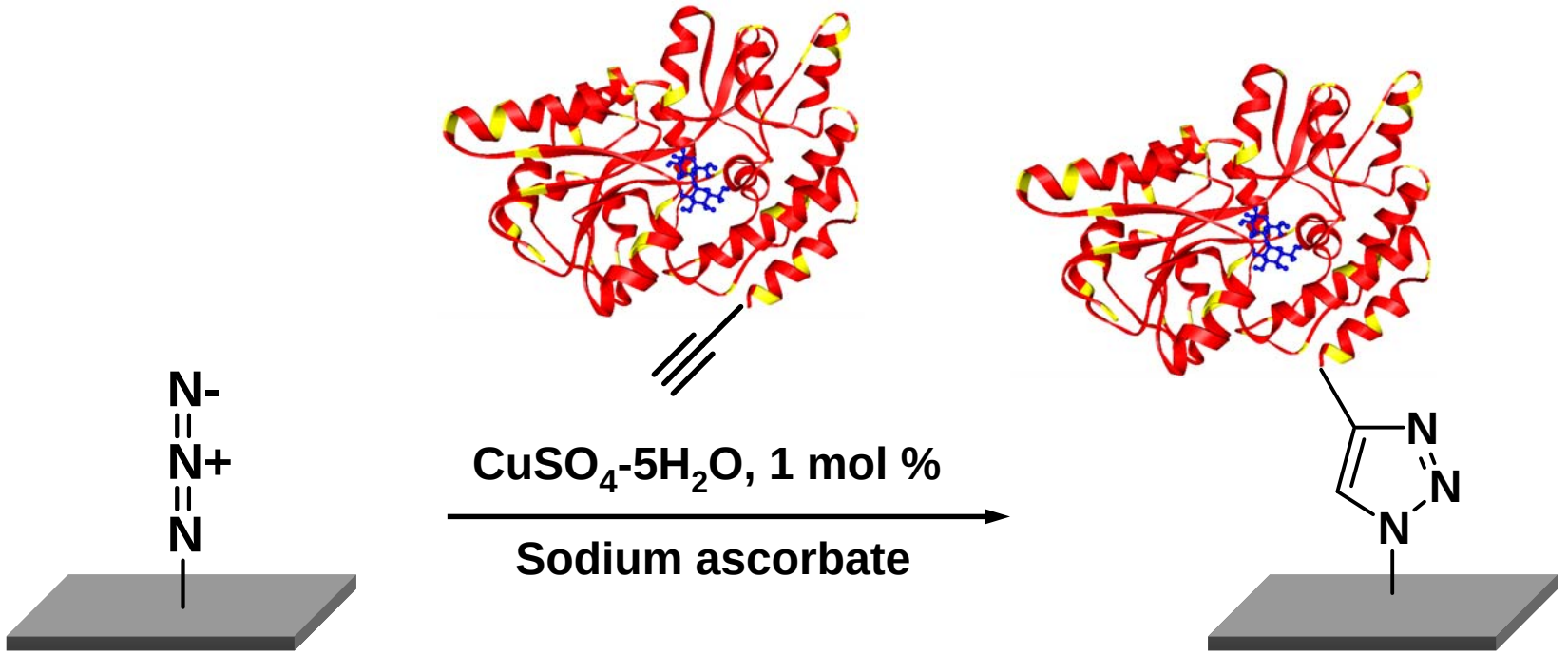
C-terminal modified protein

Kent, S. B. H. *et al. Science* 1994, *266*, 776-779.
Kent, S. B. H. *et al. Annu. Rev. Biochem.* 2000, *69*, 923-960.

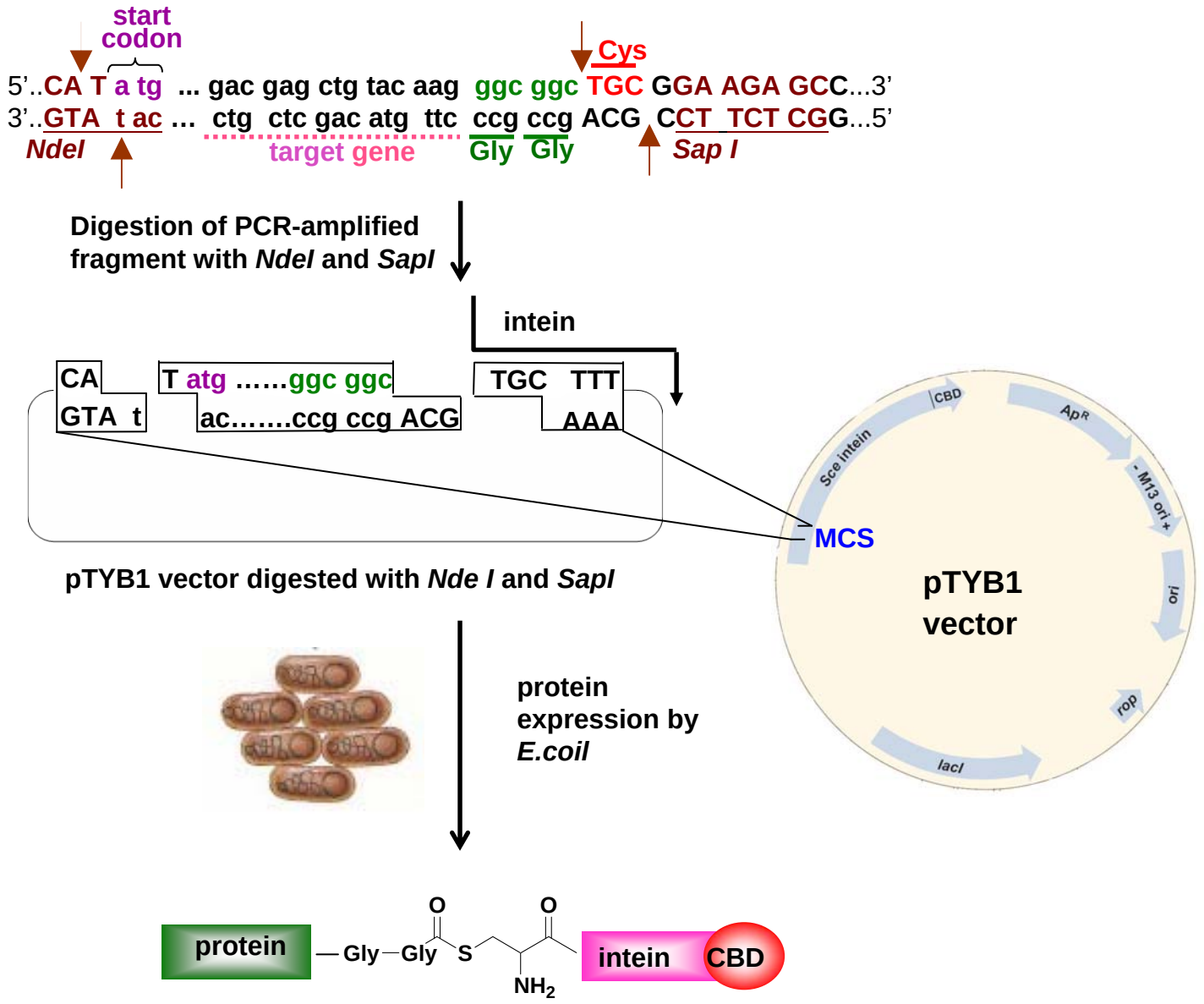
Native Chemical Ligation

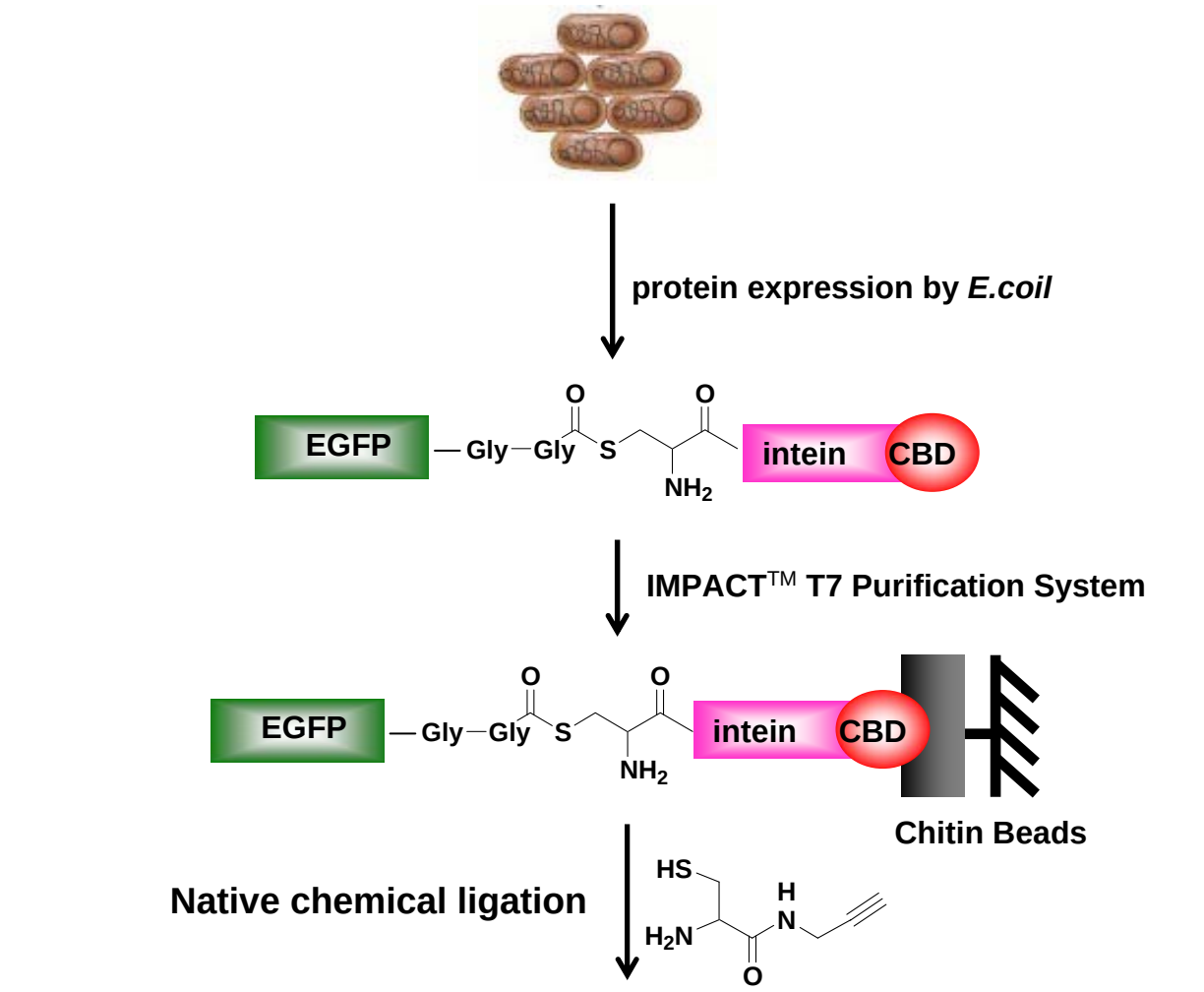


Click Chemistry



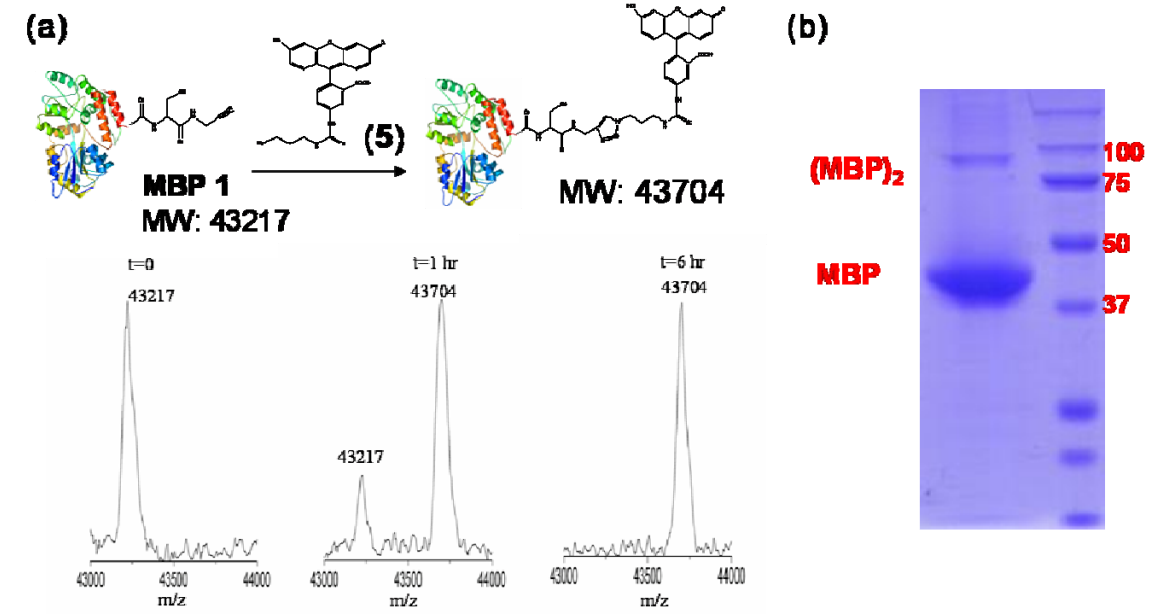
Target gene construction

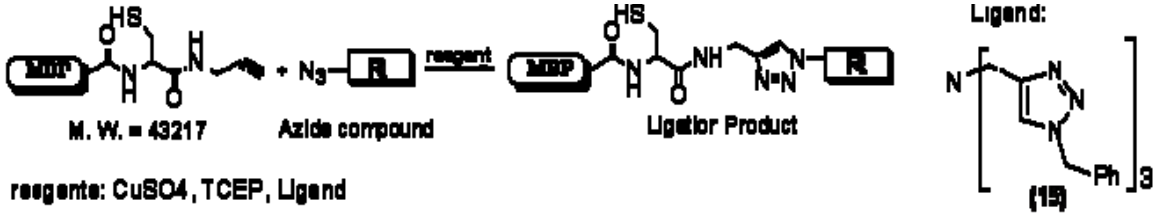




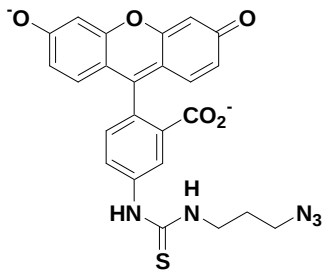
Angew. Chem. Int. Ed. Engl. 2006, 4286-9.

Fig 2.

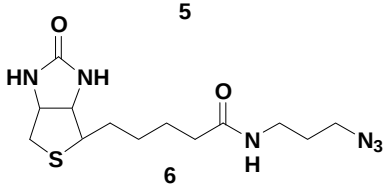




Azide Compound	Ligation Product	Observed mass	Calculated mass
FITC-N_3 (5)	10	43704	43704
Biotin-N_3 (6)	11	43544	43539
glucosamine-N3 (7)	12	43594	43596
N3- linker-N_3 (8)	13	43606	43619
glycopeptides-N3 (9)	14	44836	44840



5



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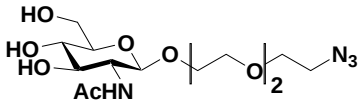

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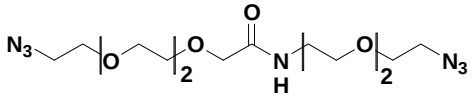

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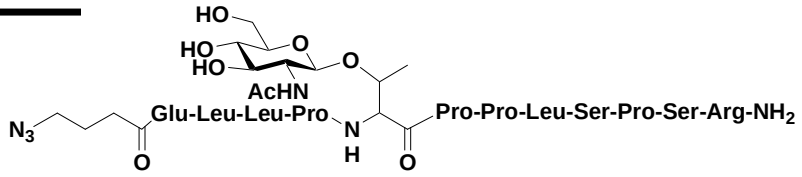

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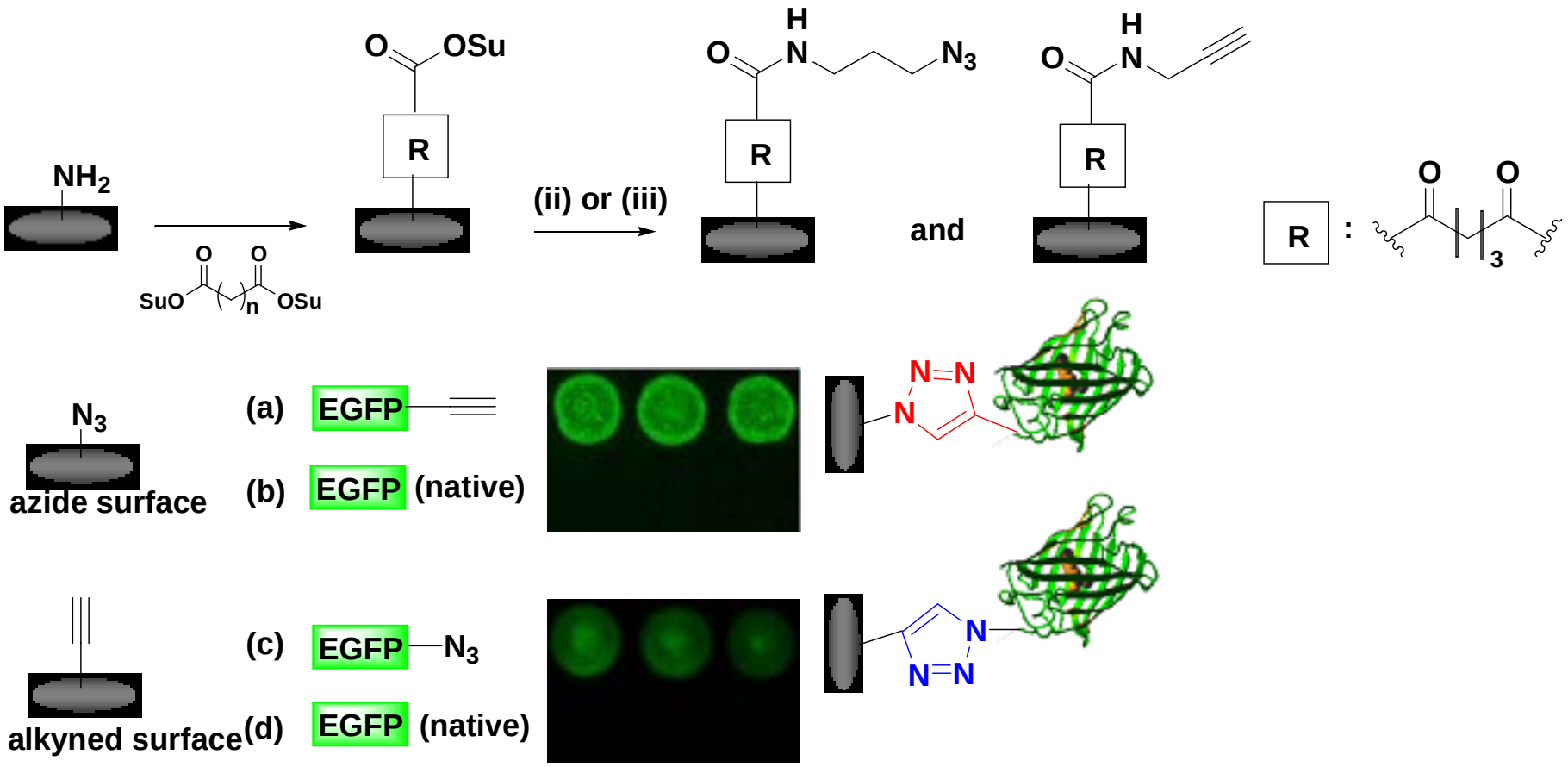
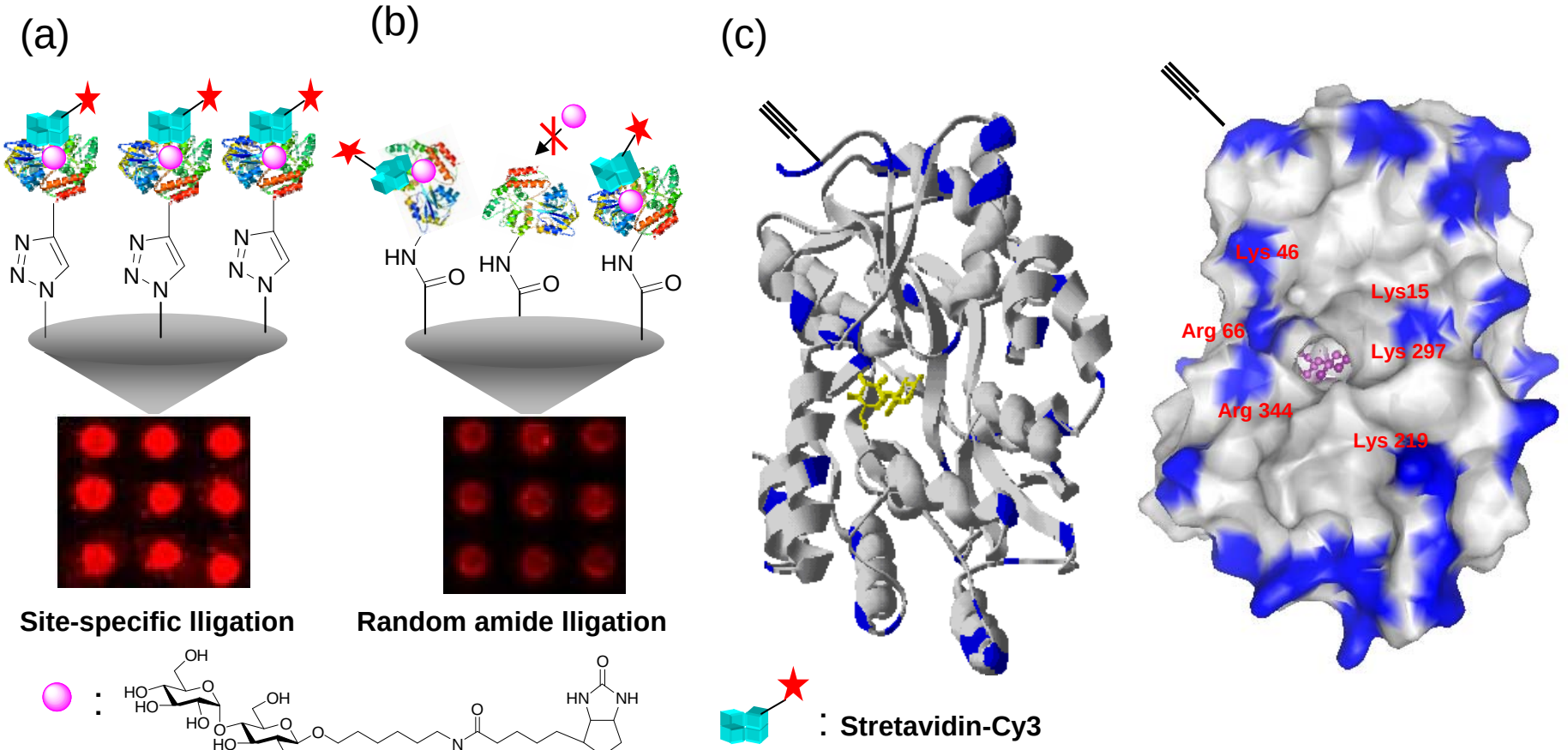
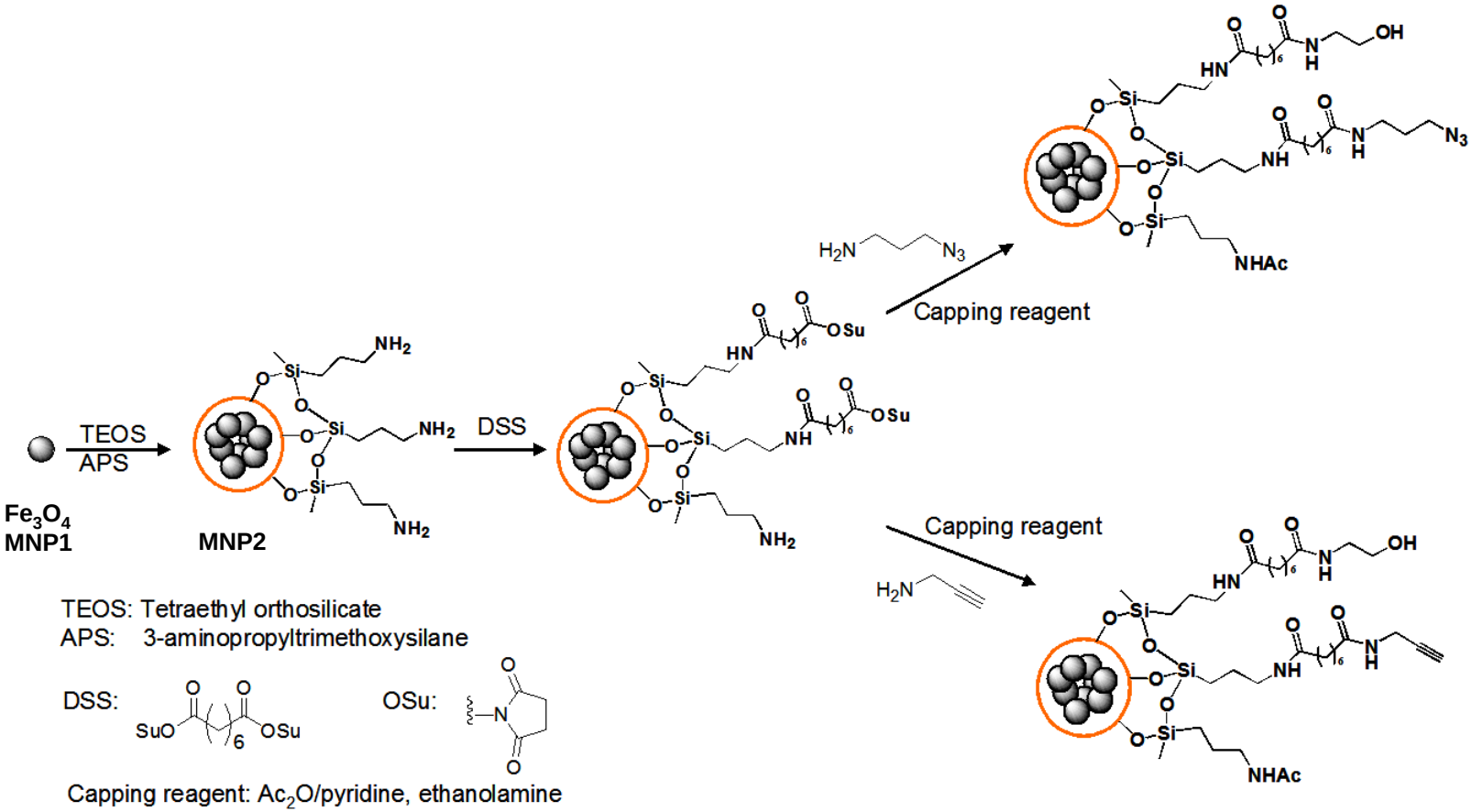
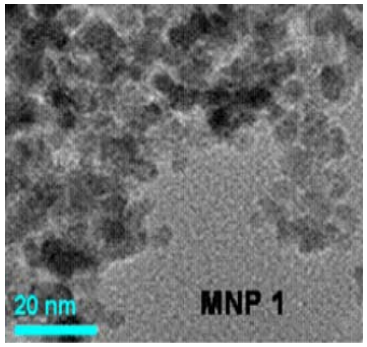
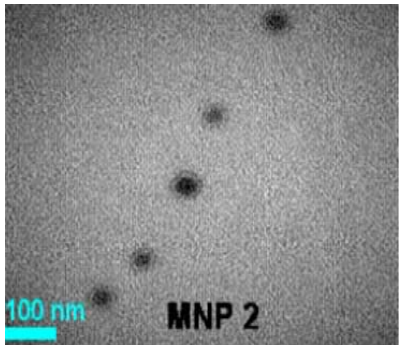

NH2
O
OSu
R
SuO
n
OSu
(ii) or (iii)
H
N
N3
and
R :
3
azide surface
(a) EGFP
(b) EGFP (native)
N3
alkyned surface
(c) EGFP N3
(d) EGFP (native)



Angew. Chem. Int. Ed. Engl. **2006, 4286.**



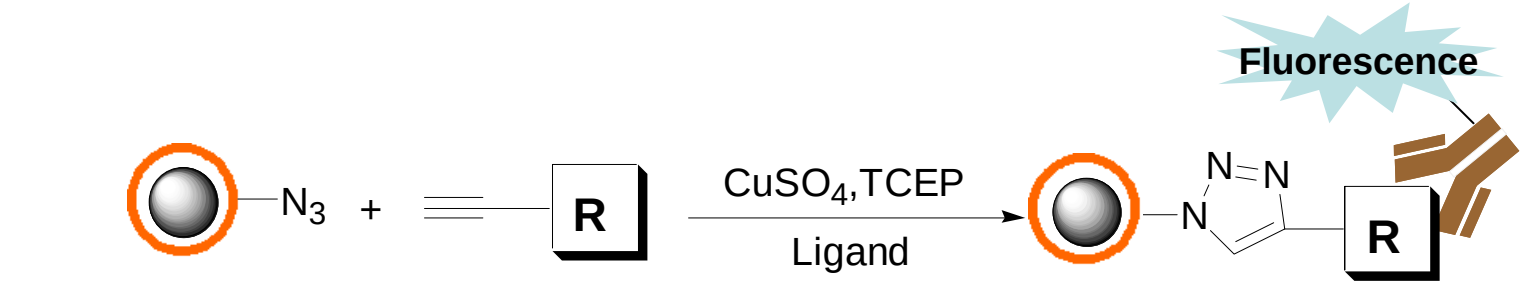


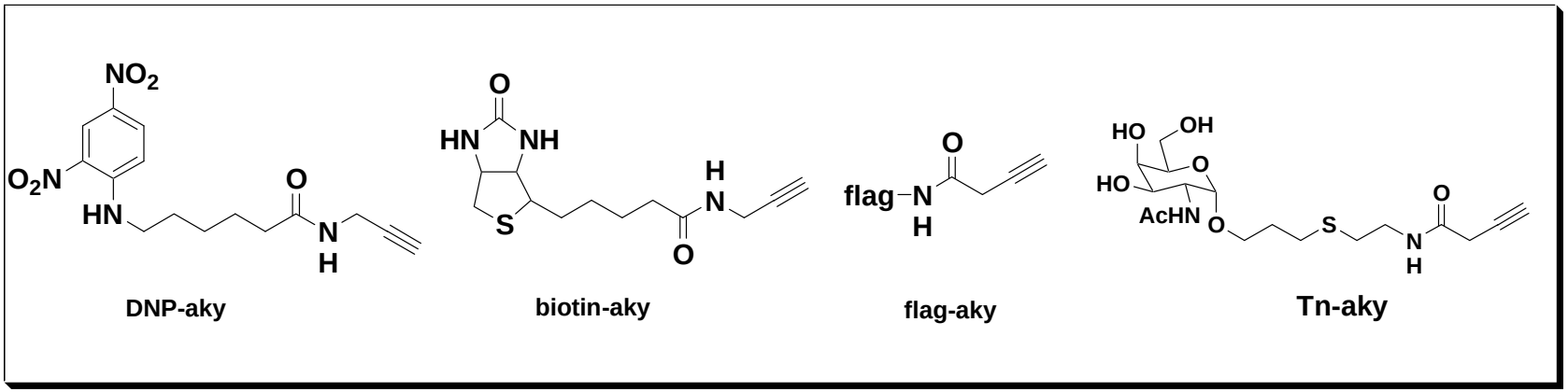


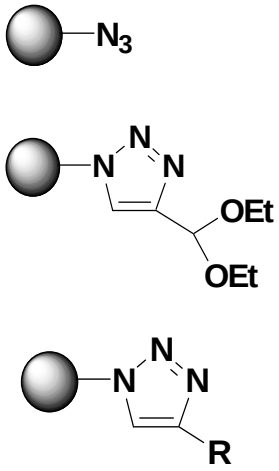
~8 nm

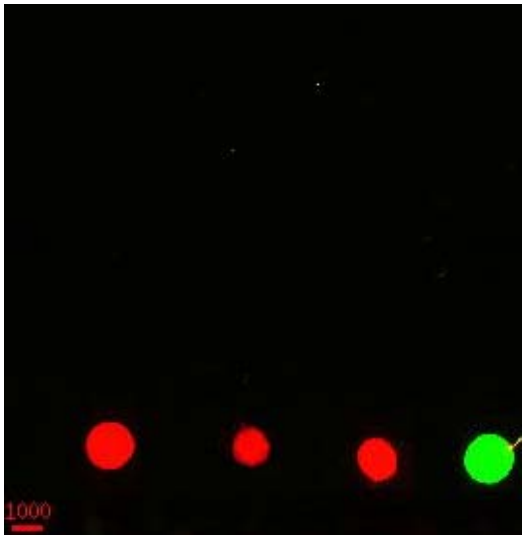
~70 nm

Org. Lett. 2007, 9, 2131
***Small* 2006, *2*, 485-9**







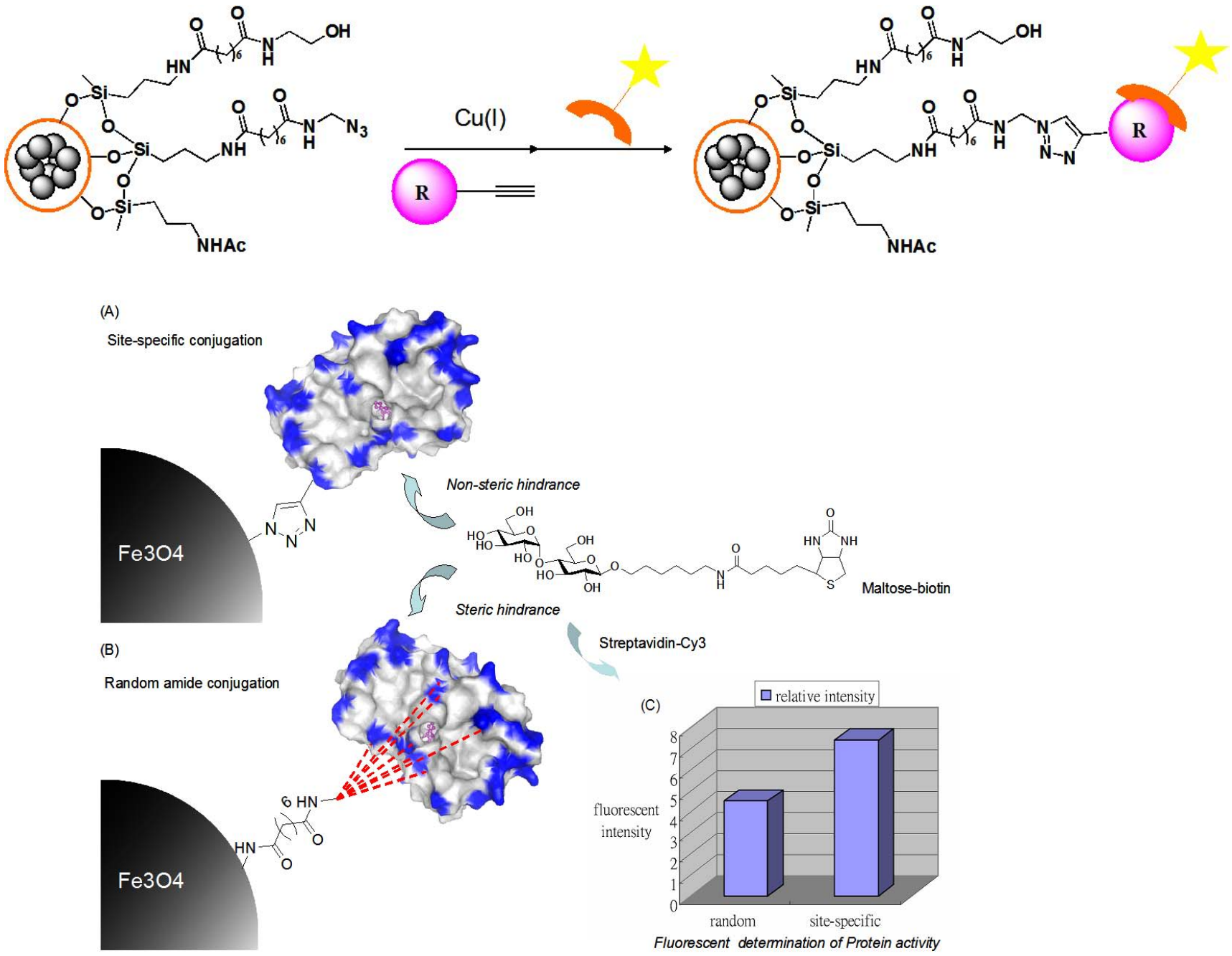


Biotin **DNP** **Flag** **Tn**

Cy 3 **Cy3** **Cy3** **FITC**

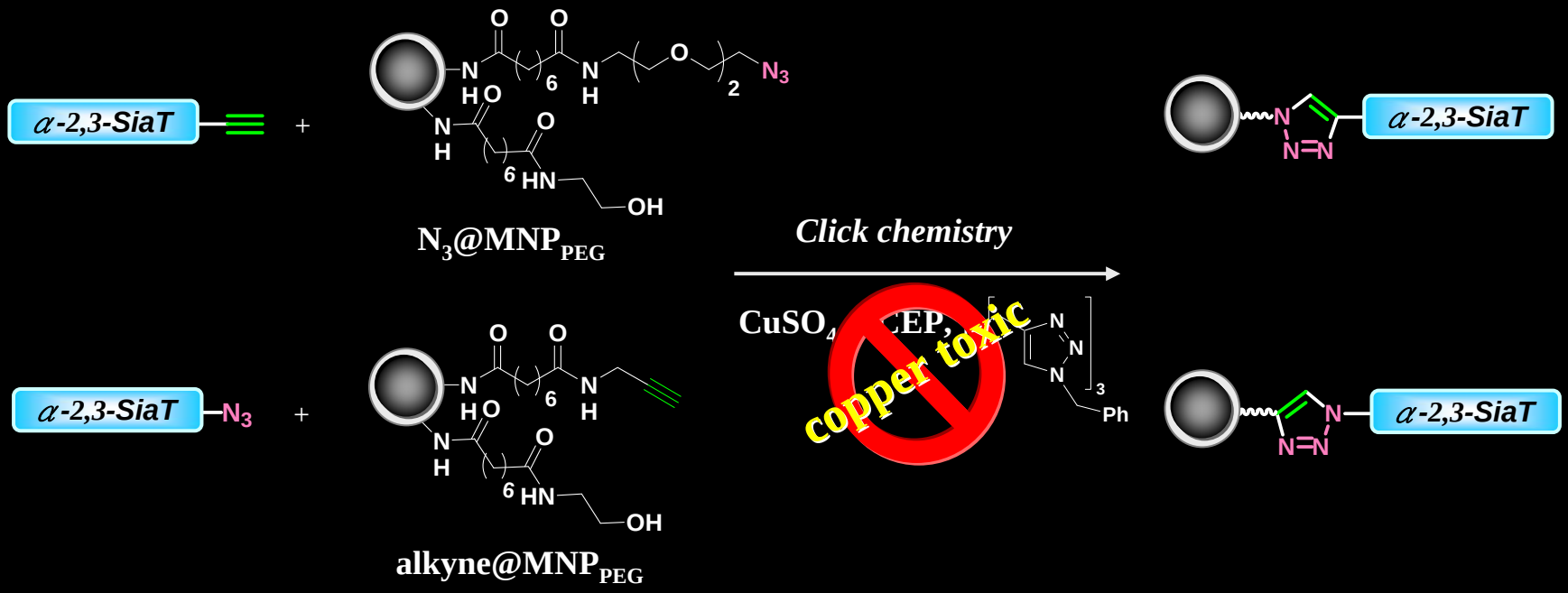
Fluorescence

Org. Lett. 2007, 9, 2131

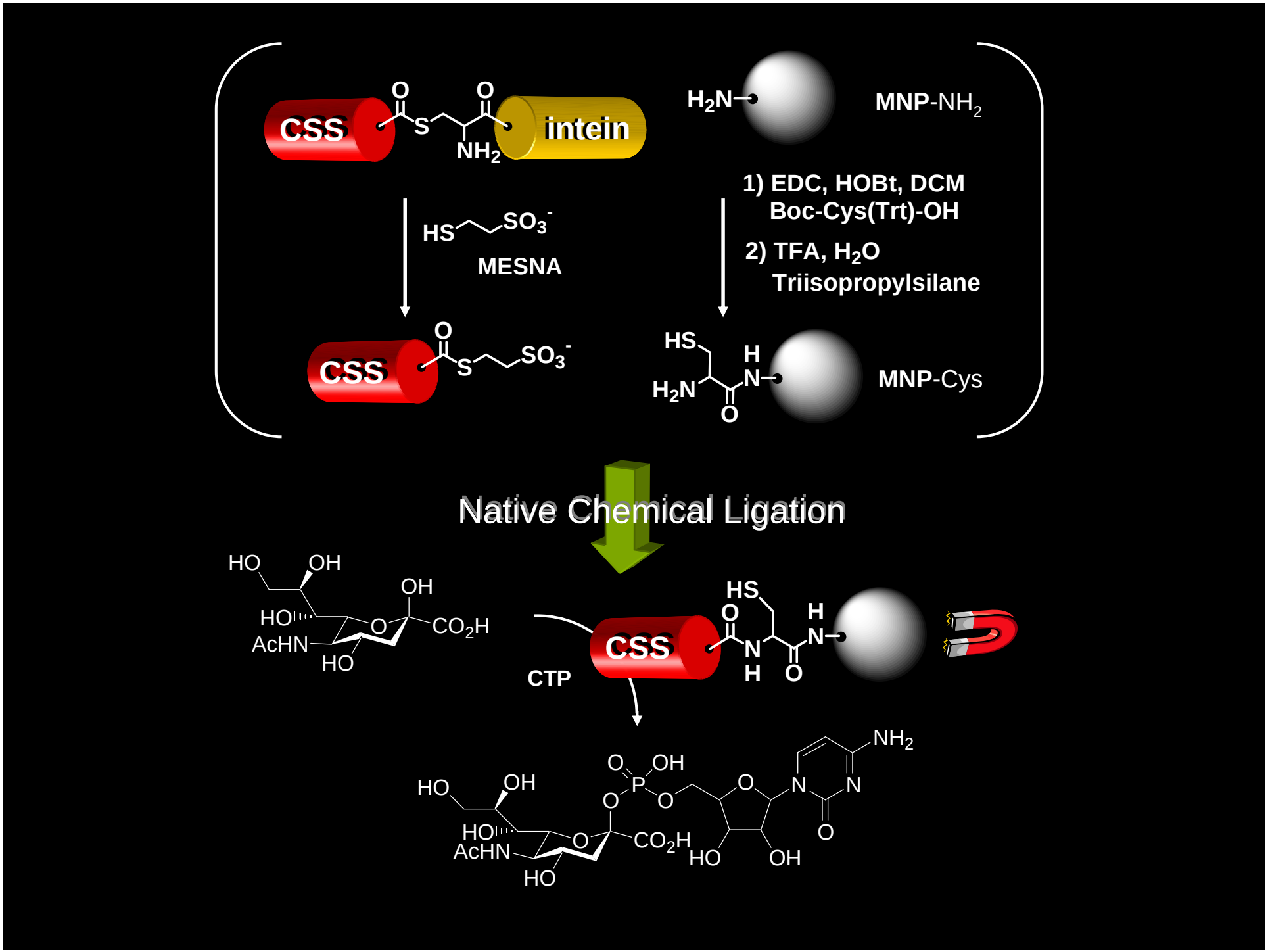


Org. Lett. 2007, 9, 2131

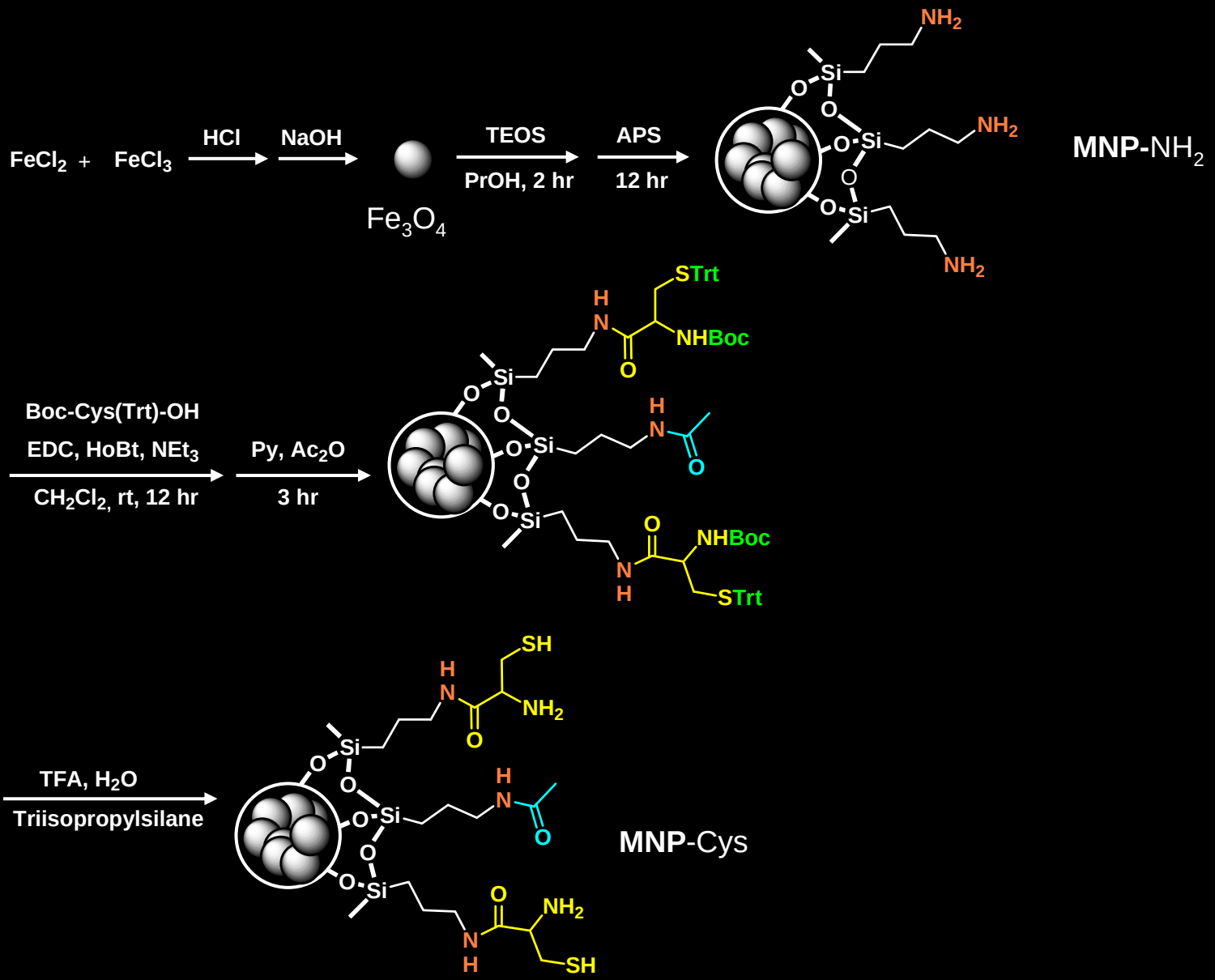
Click chemistry



α -2,3-sialyltransferase	functionalized MNPs	ligation condition	protein assay	relative activity
SiaT-alkyne	N_3@MNP_{PEG}	click chemistry rt, 12 hr	27.5 μg / 1 mg MNP	~
SiaT-alkyne	N_3@MNP_{PEG}	click chemistry 4 °C, 16 hr	9.9 μg / 1 mg MNP	~
SiaT-azide	alkyne@MNP_{PEG}	click chemistry rt, 12 hr	15.3 μg / 1 mg MNP	~

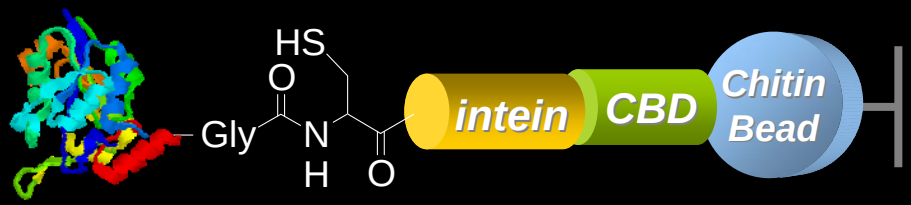

CSS
intein
MNP-NH2
1) EDC, HOBt, DCM
Boc-Cys(Trt)-OH
2) TFA, H2O
Triisopropylsilane
MESNA
MNP-Cys
Native Chemical Ligation
CTP

Cysteine Functionalized Magnetic Nanoparticle Preparation

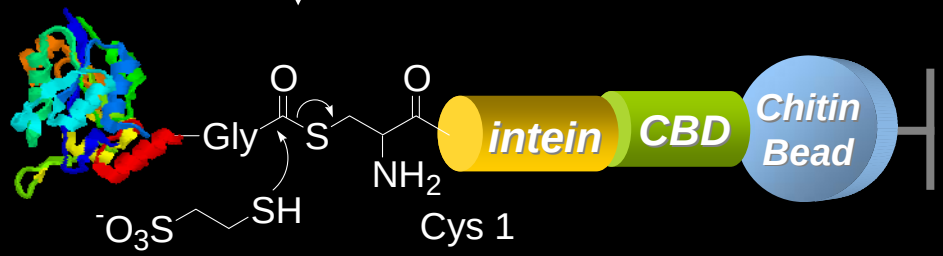


Site-Specific Immobilized CMP-Sialic Acid Synthetase Functionalized Magnetic Nanoparticle Preparation

Protein Expression by E. Coli

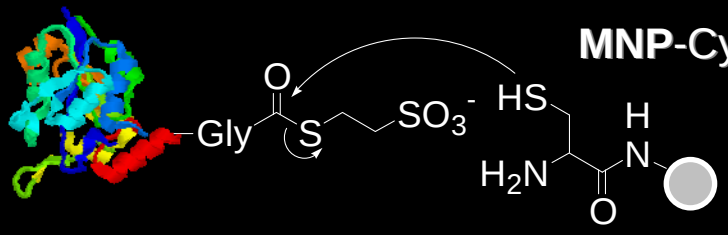


N-S Acyl shift

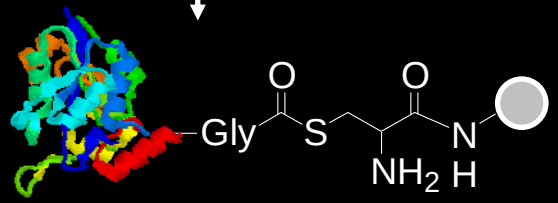


MESNA

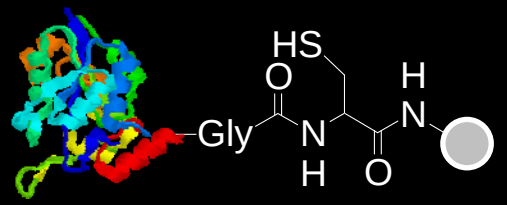
Cleavage by MESNA



Immobilization on MNP-Cys



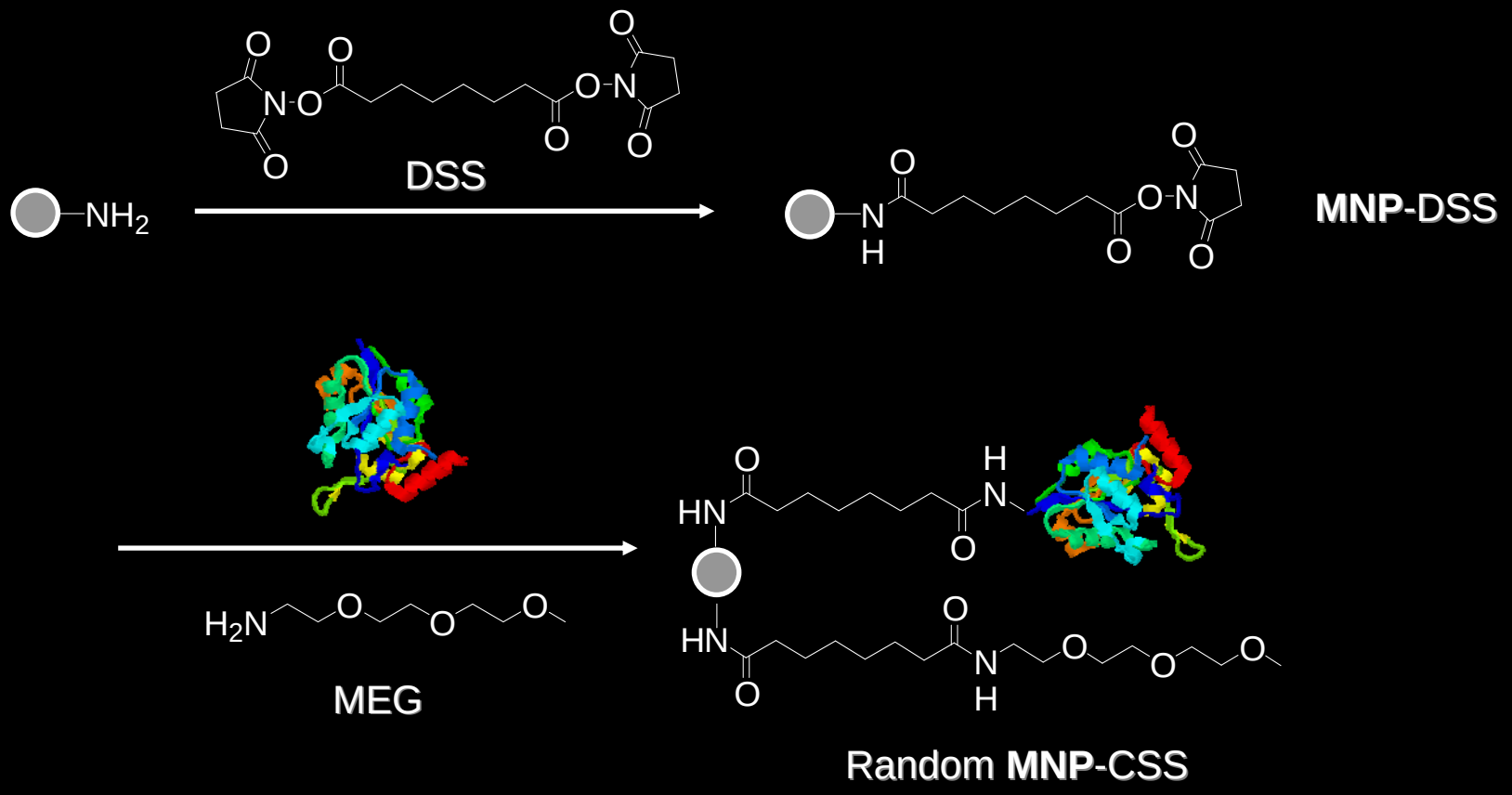
S-N Acyl shift



MNP-CSS

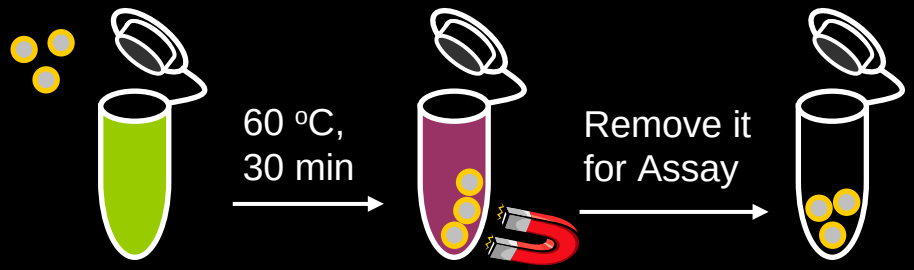
- *Two-pot strategy : purification by chitin bead*
  One-pot strategy : without chitin bead purification

Random Immobilized CMP-Sialic Acid Synthetase Functionalized Magnetic Nanoparticle

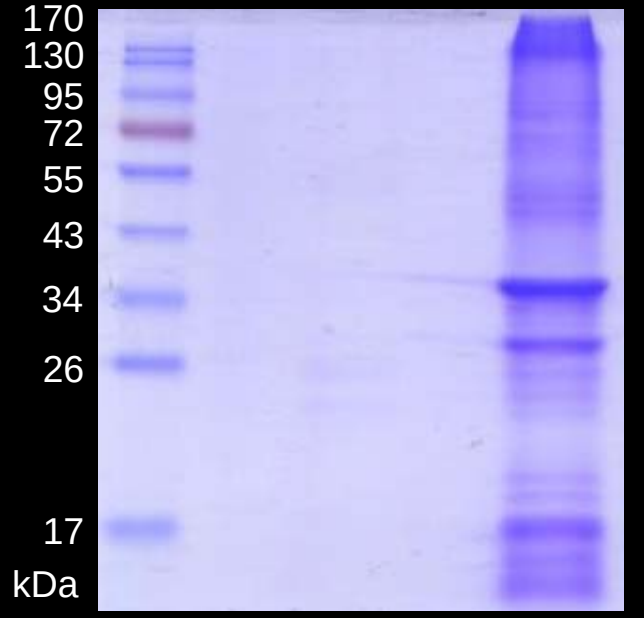


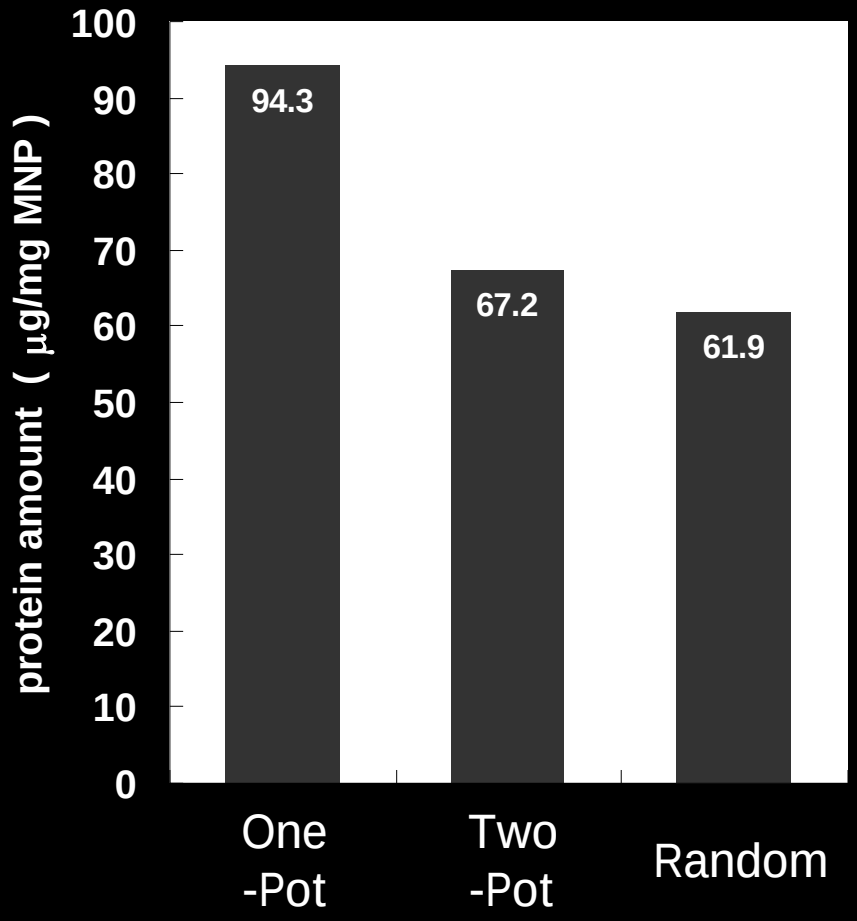
Quantification of CSS on Magnetic Nanoparticle

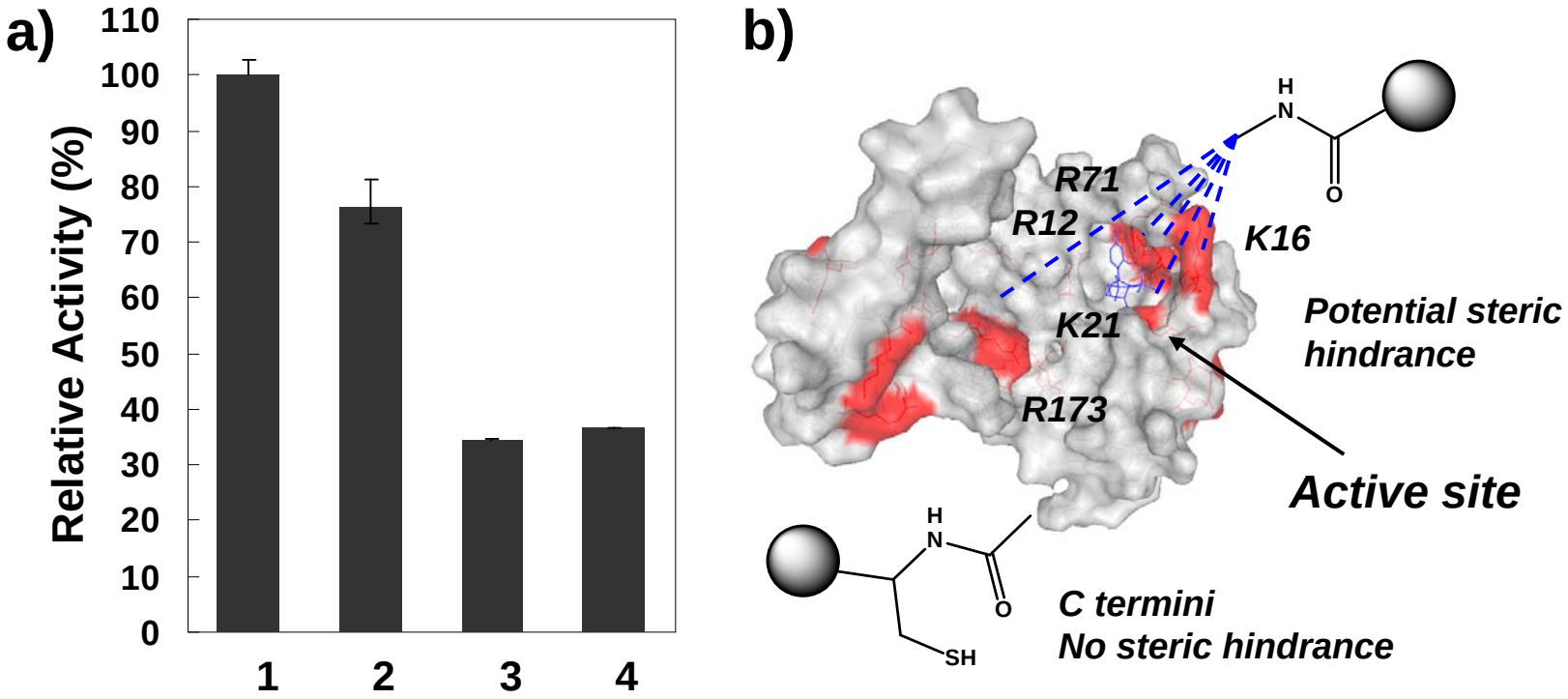
BCA Assay



SDS-PAGE



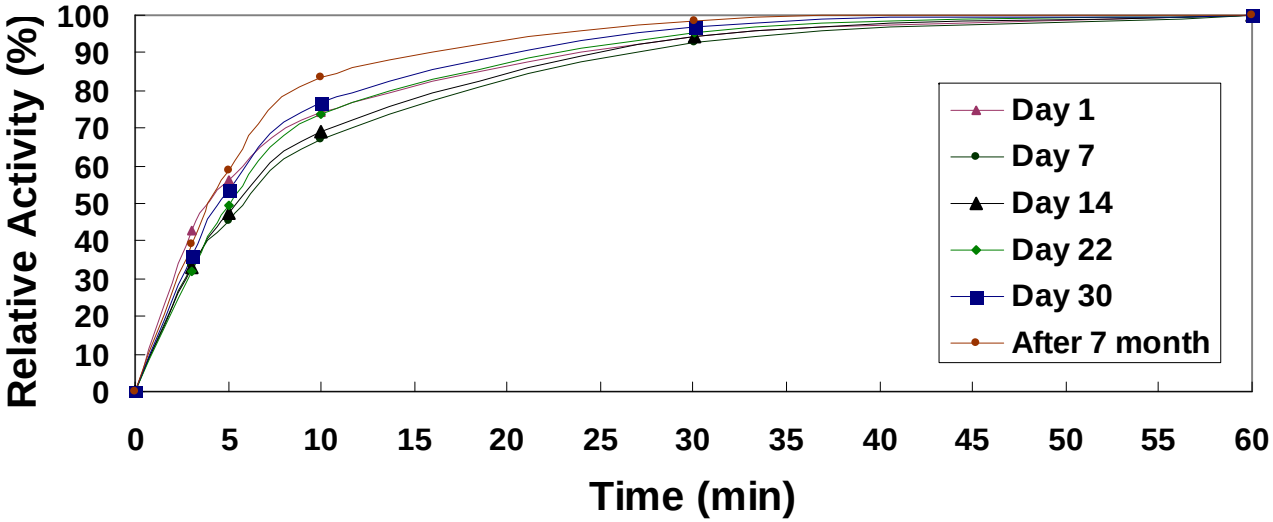




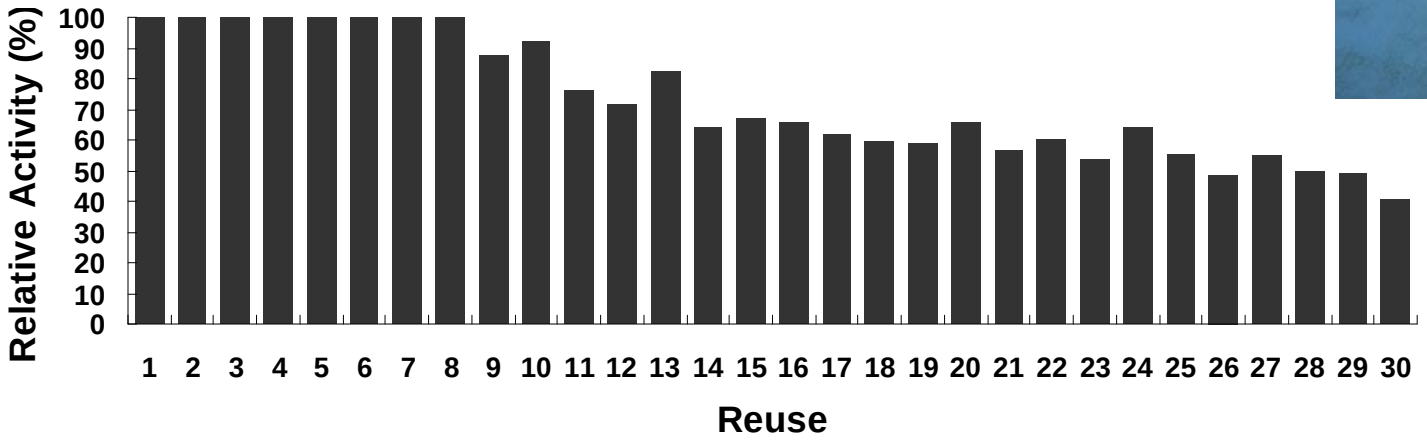
Column 1: native CSS
Column 2: site-specific immobilized CSS
Column 3: random immobilized CSS
Column 4: site specific immobilized CSS on microbeads

***ChemComm*, 2008, 1308**

Long term stability



Reuse ability



sigma
25 mg / US$ 601
Calbiochem
25 mg / US$ 574
Wako
50 mg / NT$ 25,000

Protein probe and Detecting substrate

Ganoderma lucidum

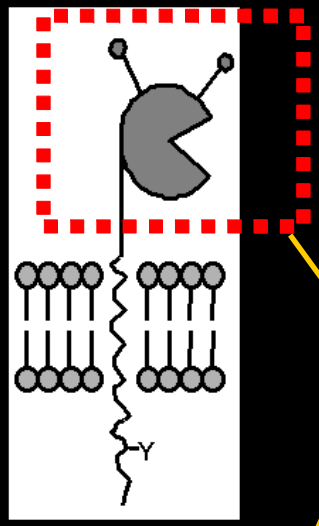
(*Reishi*, 靈芝)

Bioorg. Med. Chem. 2002, *10*, 1057

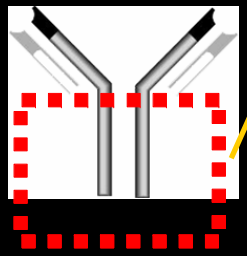
biotin

F3

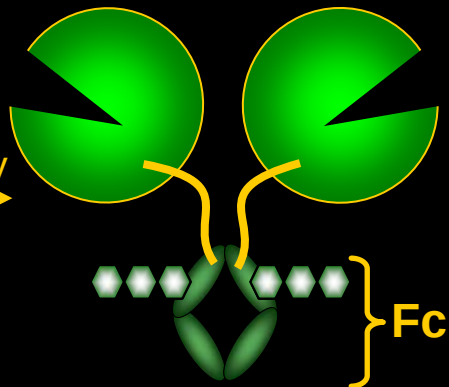
C-type lectin



Fc region of antibody



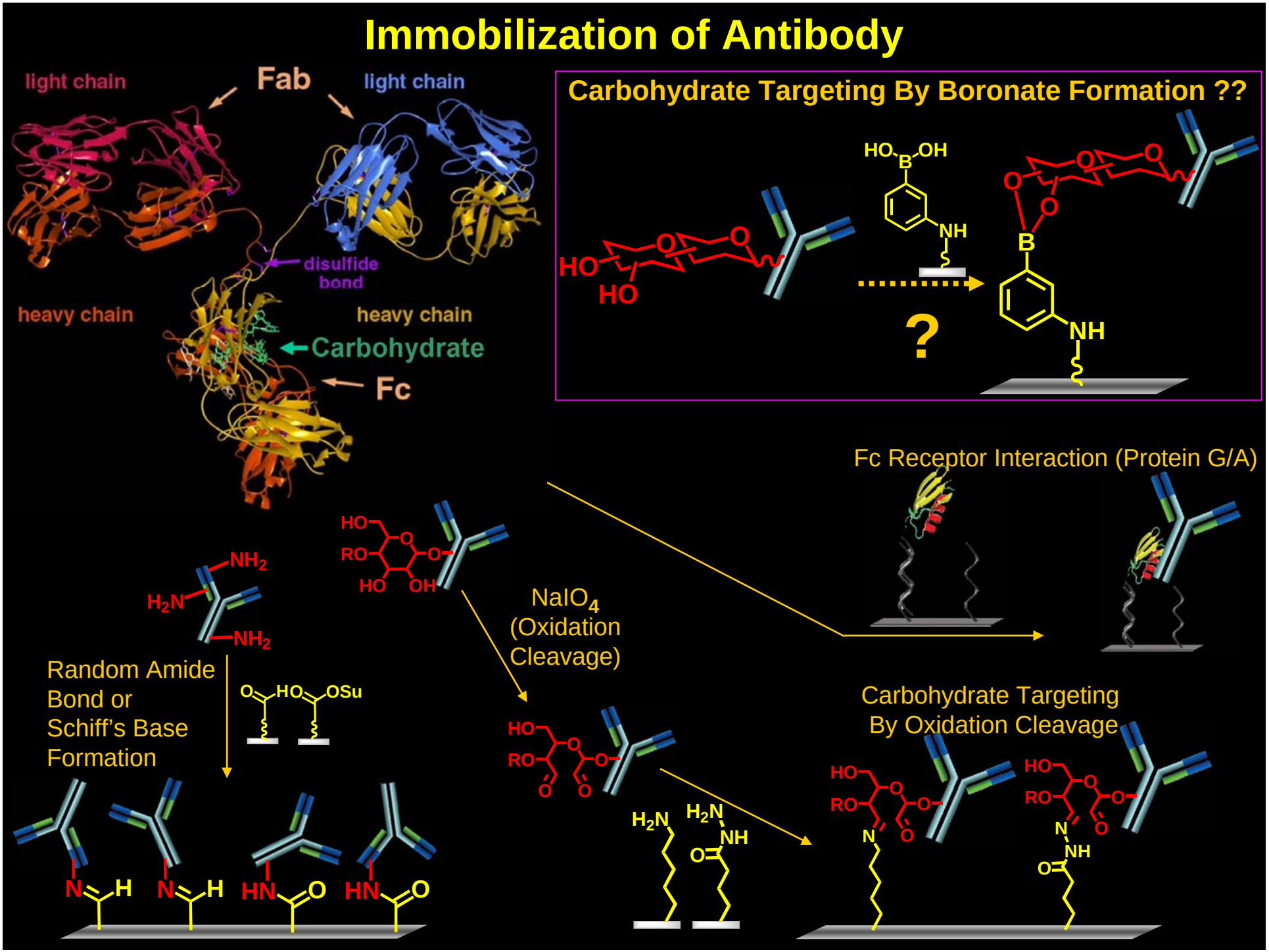
Recombinant **protein** technology

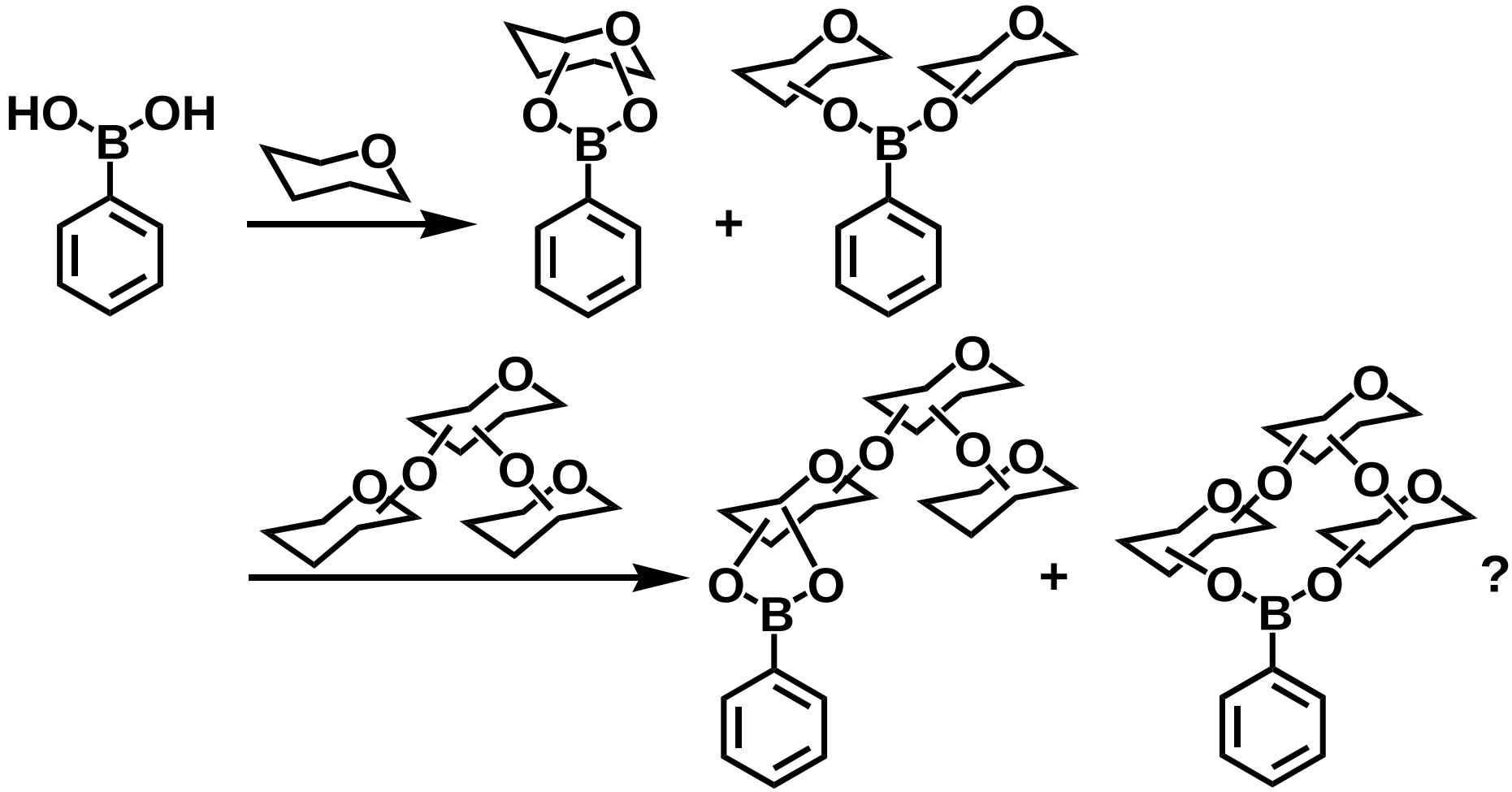


Fc domain

Fc fused C-type Lectin

Immobilization of Antibody




HO
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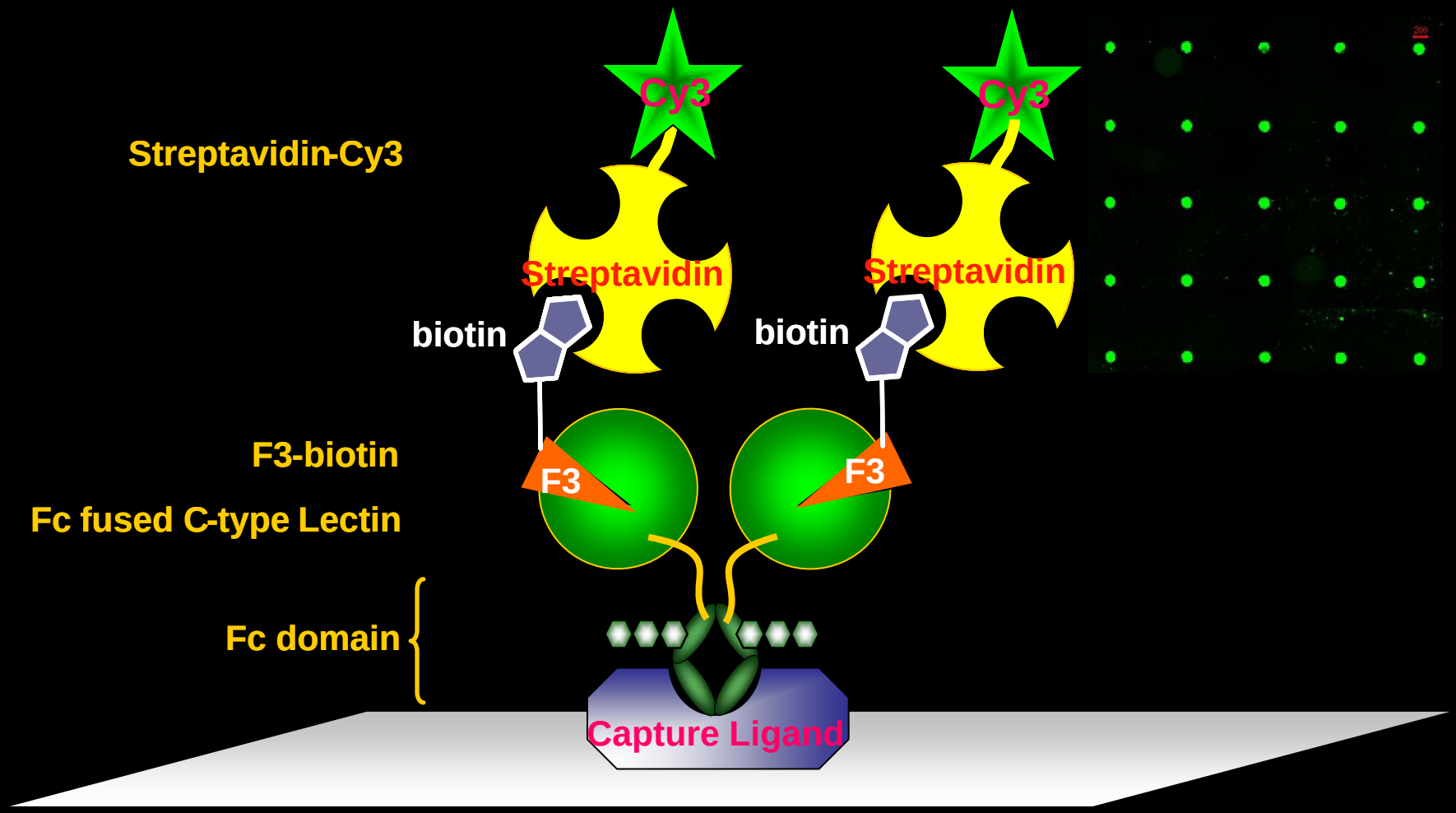
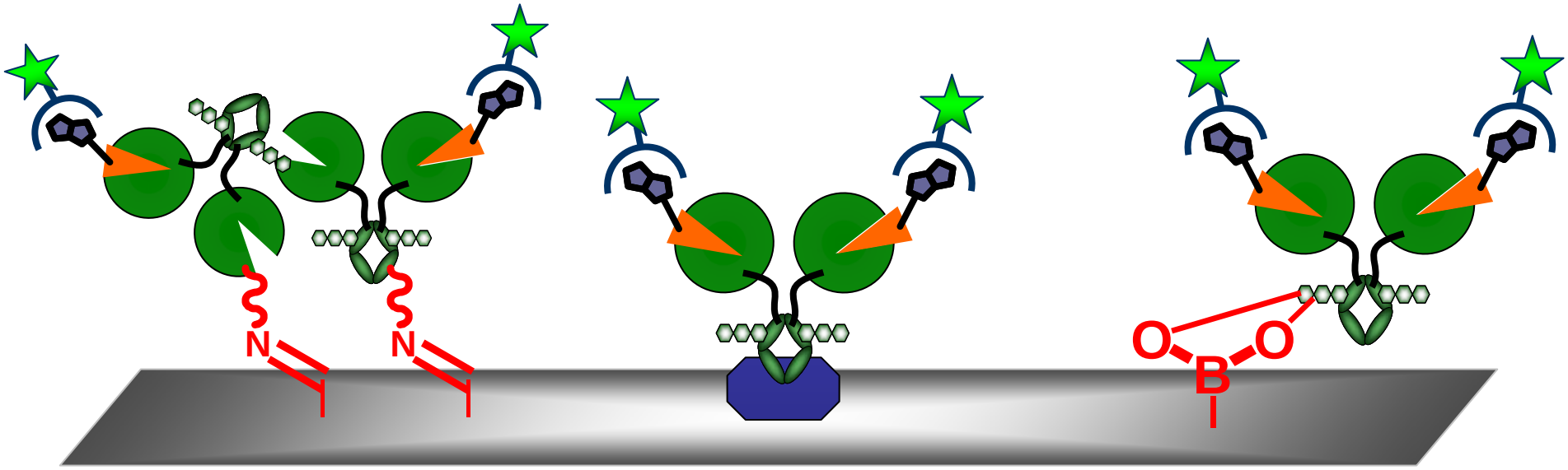
O
?

Concept of Fabricating Lectin Microarray



Fabrication strategies



Random Schiff's base formation

Oriented immobilization with protein G

Oriented immobilization with boronic acid

:Fc-fusion C-type lectin

:Protein G

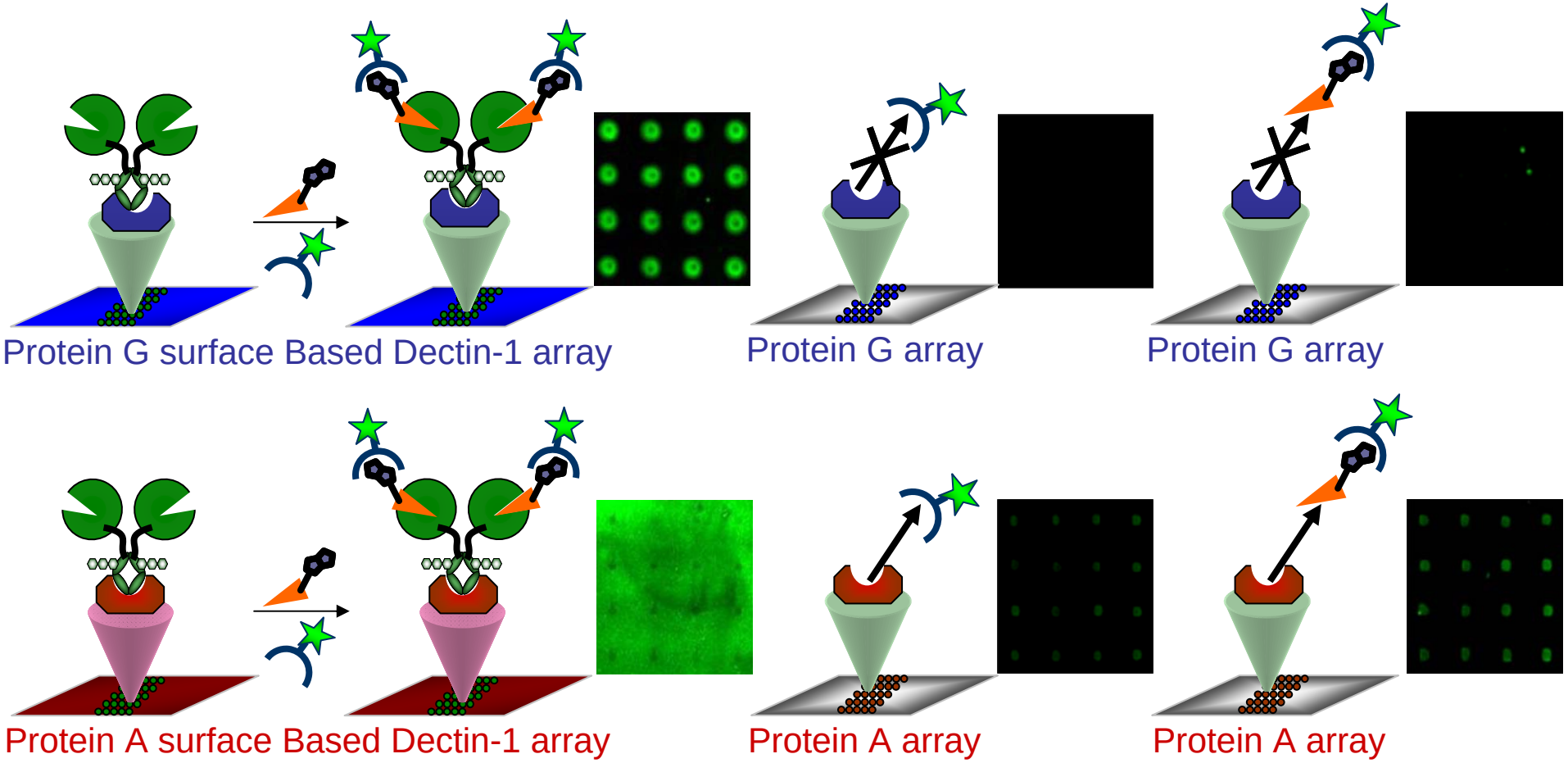
:Streptavidin-Cy3

:Fc-fusion fragment

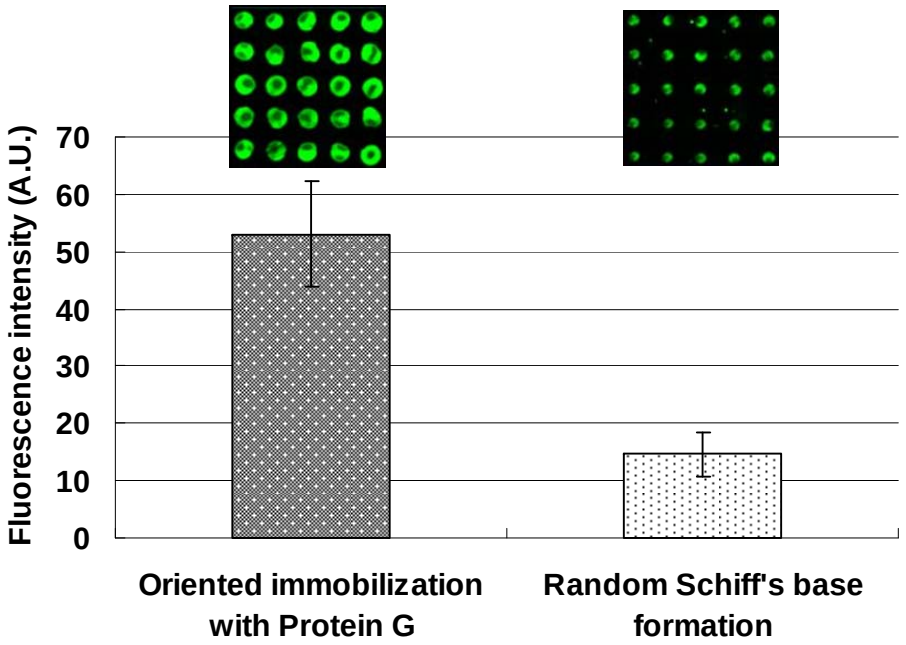
:Carbohydrate chain

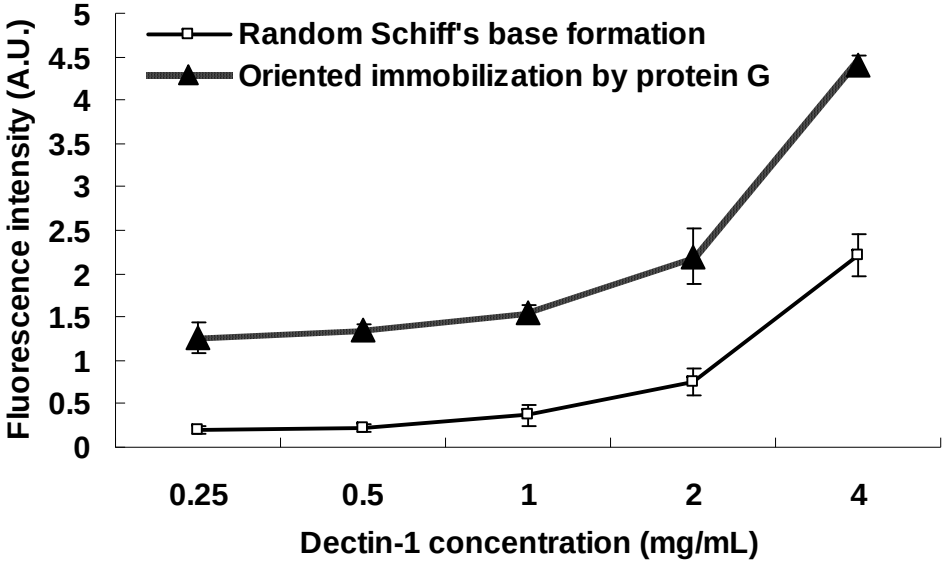
:Biotin-labeled polysaccharide of *G. lucidum*

Fc receptor for fabrication of Fc-fused dectin-1 microarray

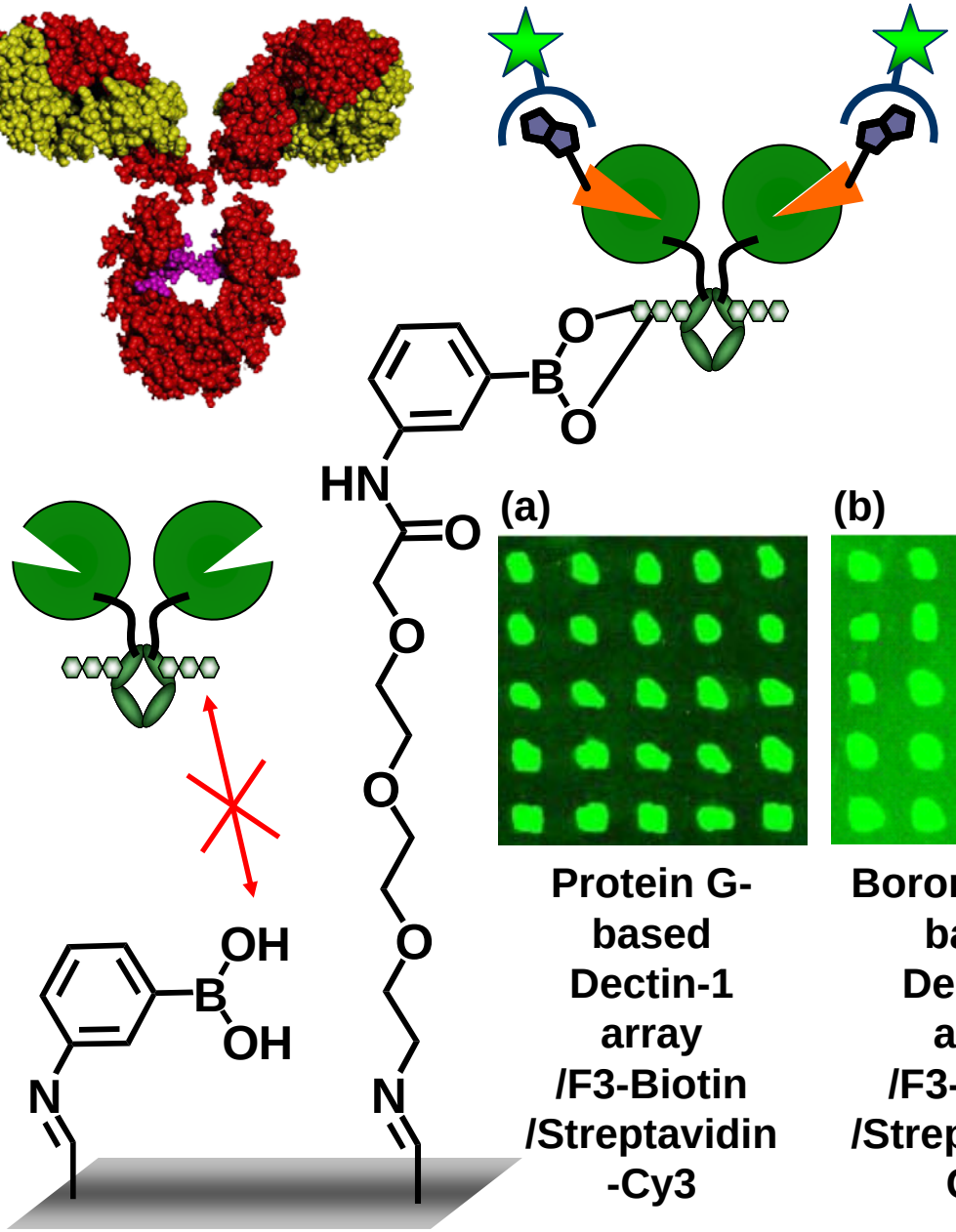


Comparison of microarrays: oriented vs random





Comparison of microarrays: Protein G vs BA



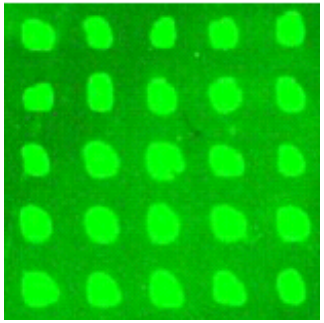
Solutions

1. **Reduce surface boronic acid**
2. **Use blocking reagent**

(a)

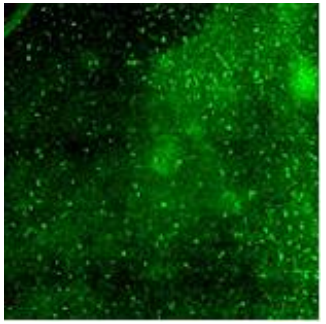
Protein G-based Dectin-1 array /F3-Biotin /Streptavidin -Cy3

(b)



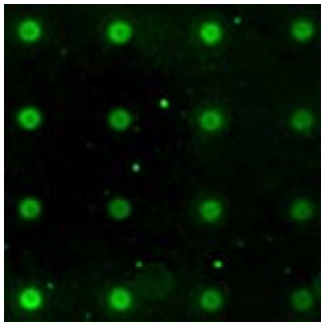
Boronic acid-based Dectin-1 array /F3-Biotin /Streptavidin-Cy3

(c)



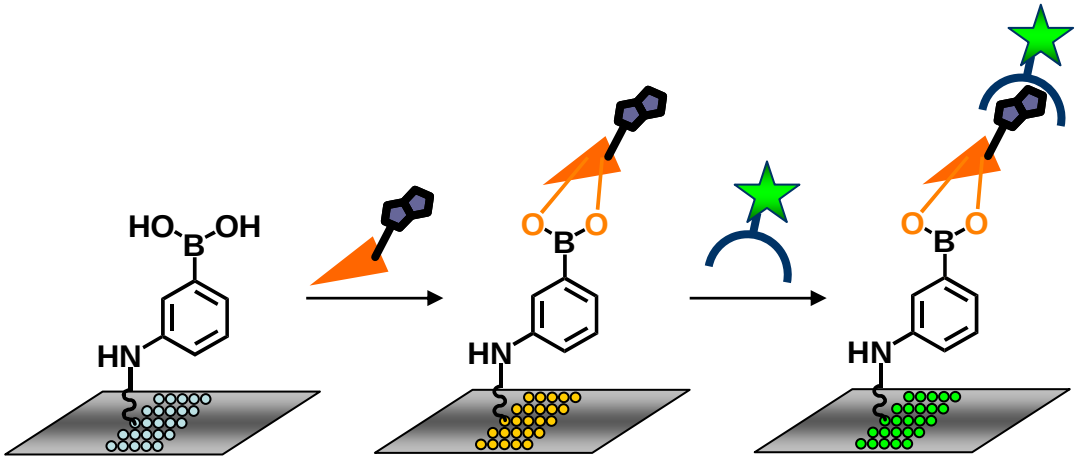
Boronic acid array /Streptavidin-Cy3

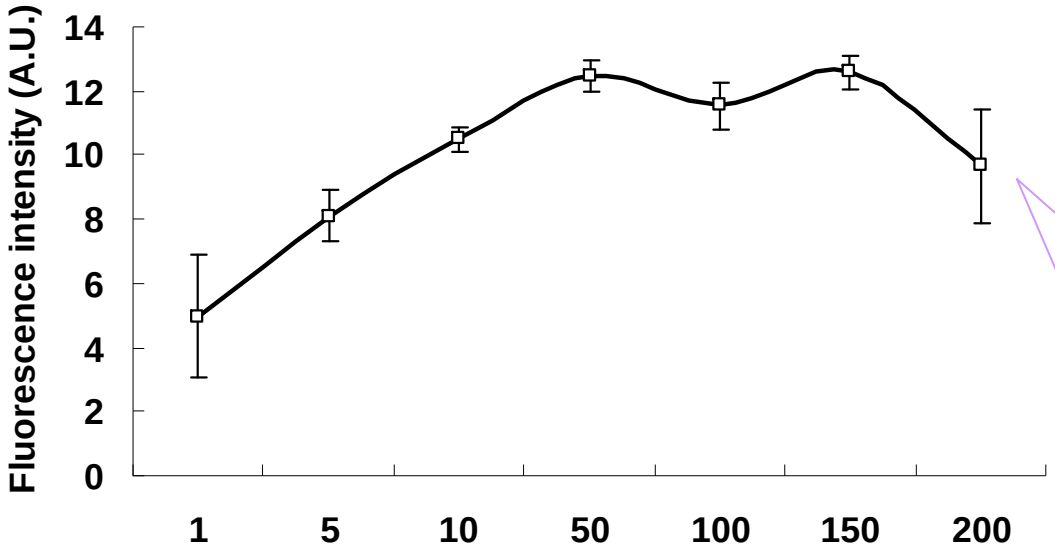
(d)

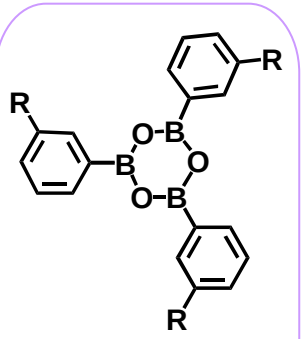


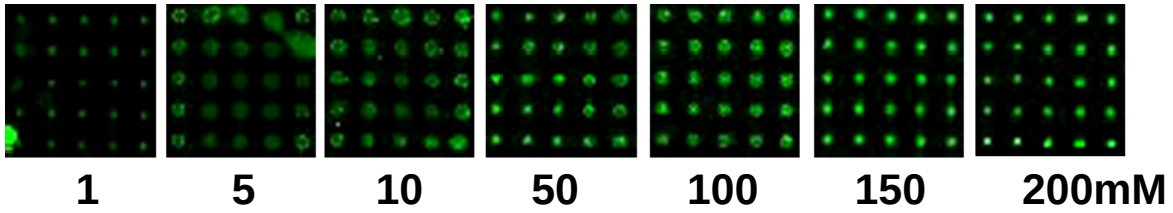
Boronic acid array /F3-Biotin /Streptavidin -Cy3

Concentration Effect of Boronic Acid in F3-biotin Interactions

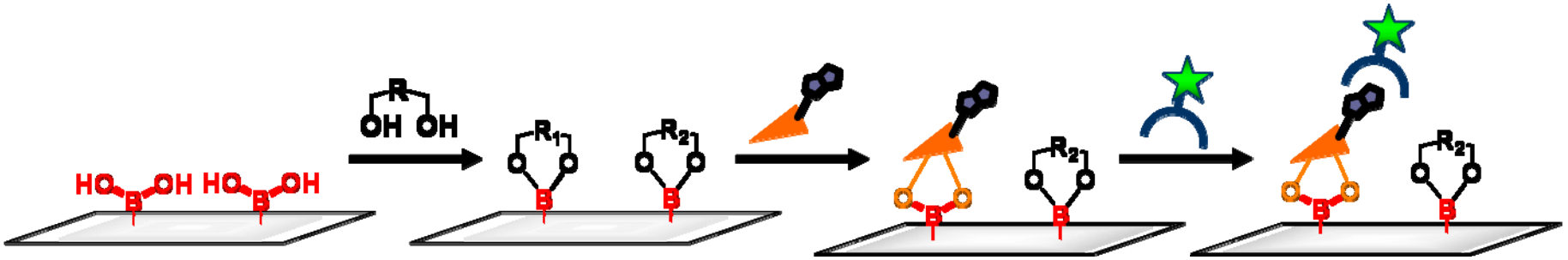


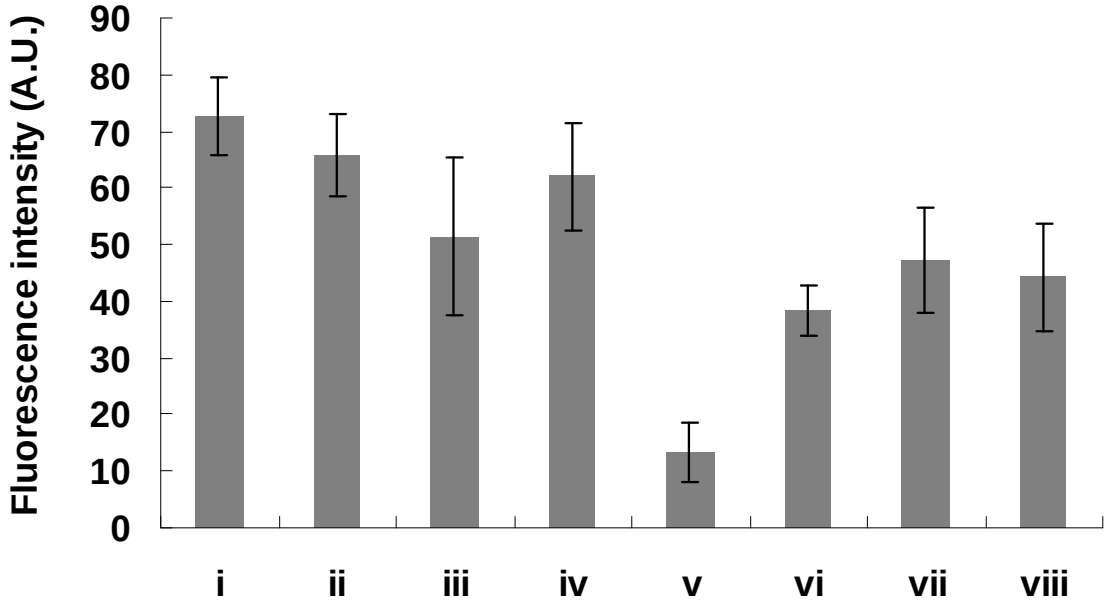


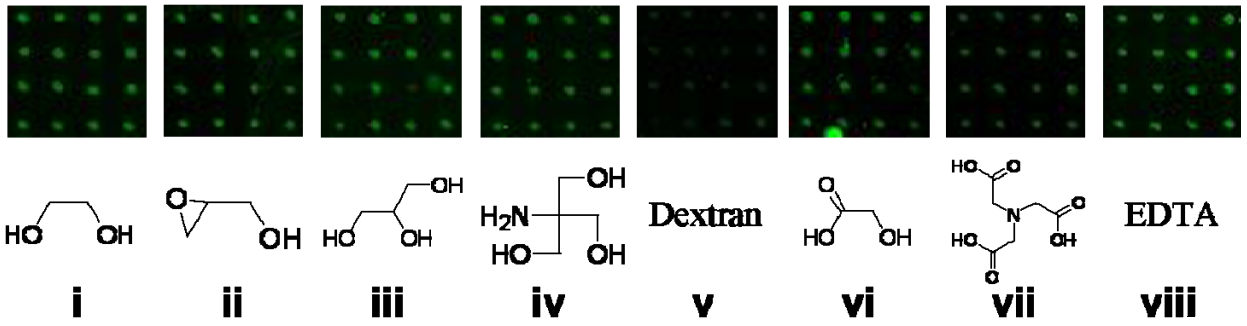




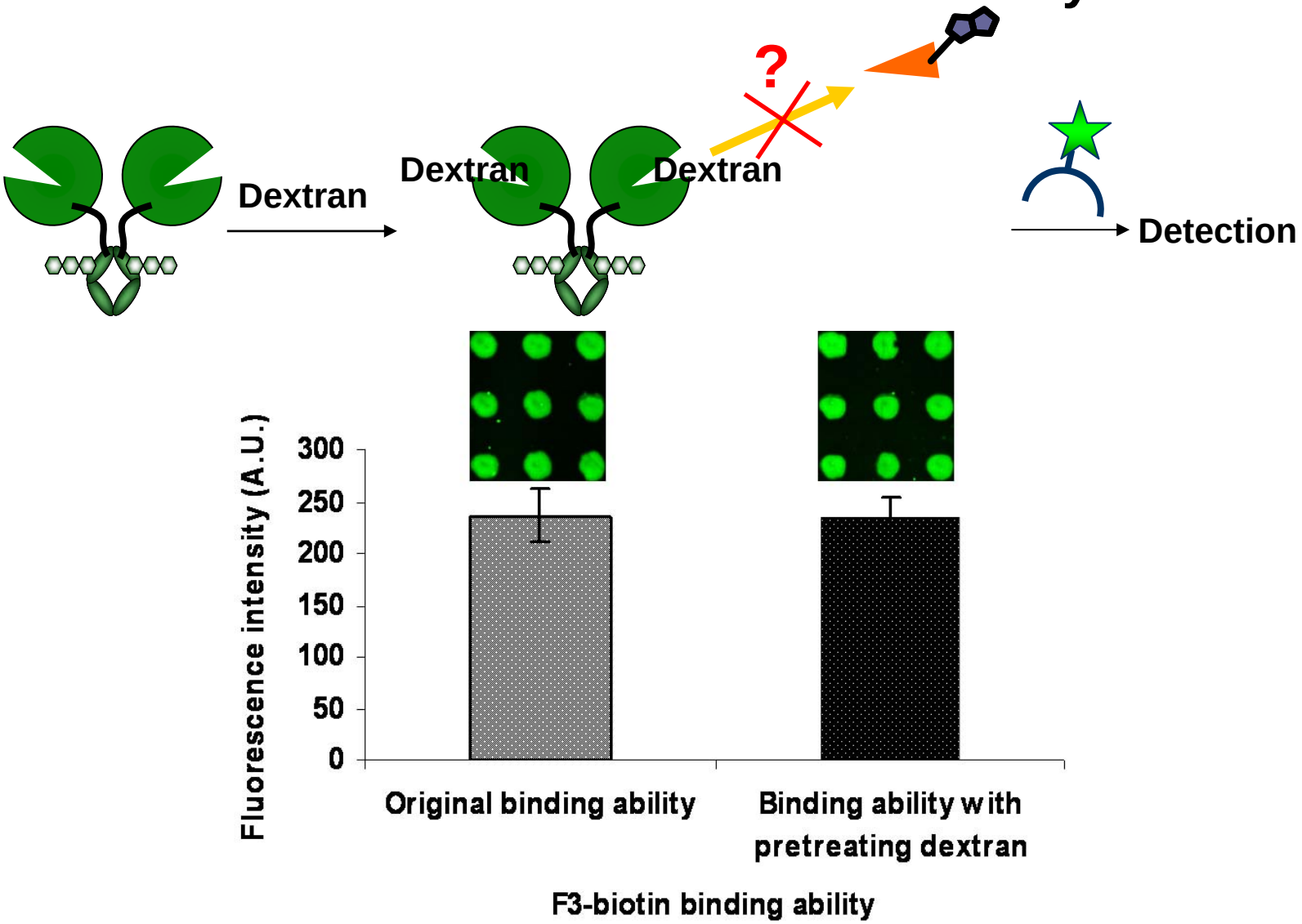
Chemical Competition Assay for Selecting Blocking Reagent





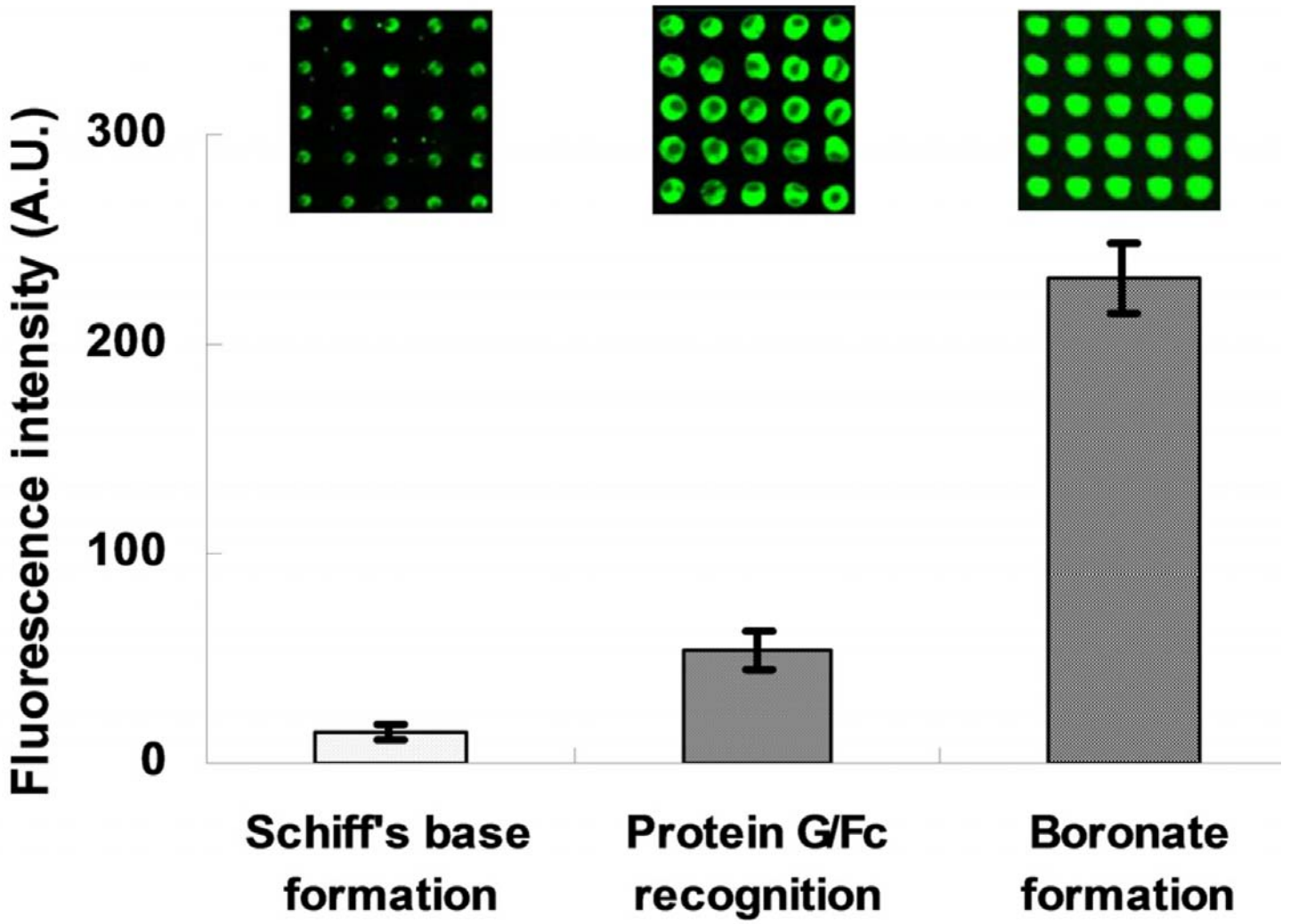


The influence of dextran on the binding of F3-biotin to the Fc-fused dectin-1 microarray



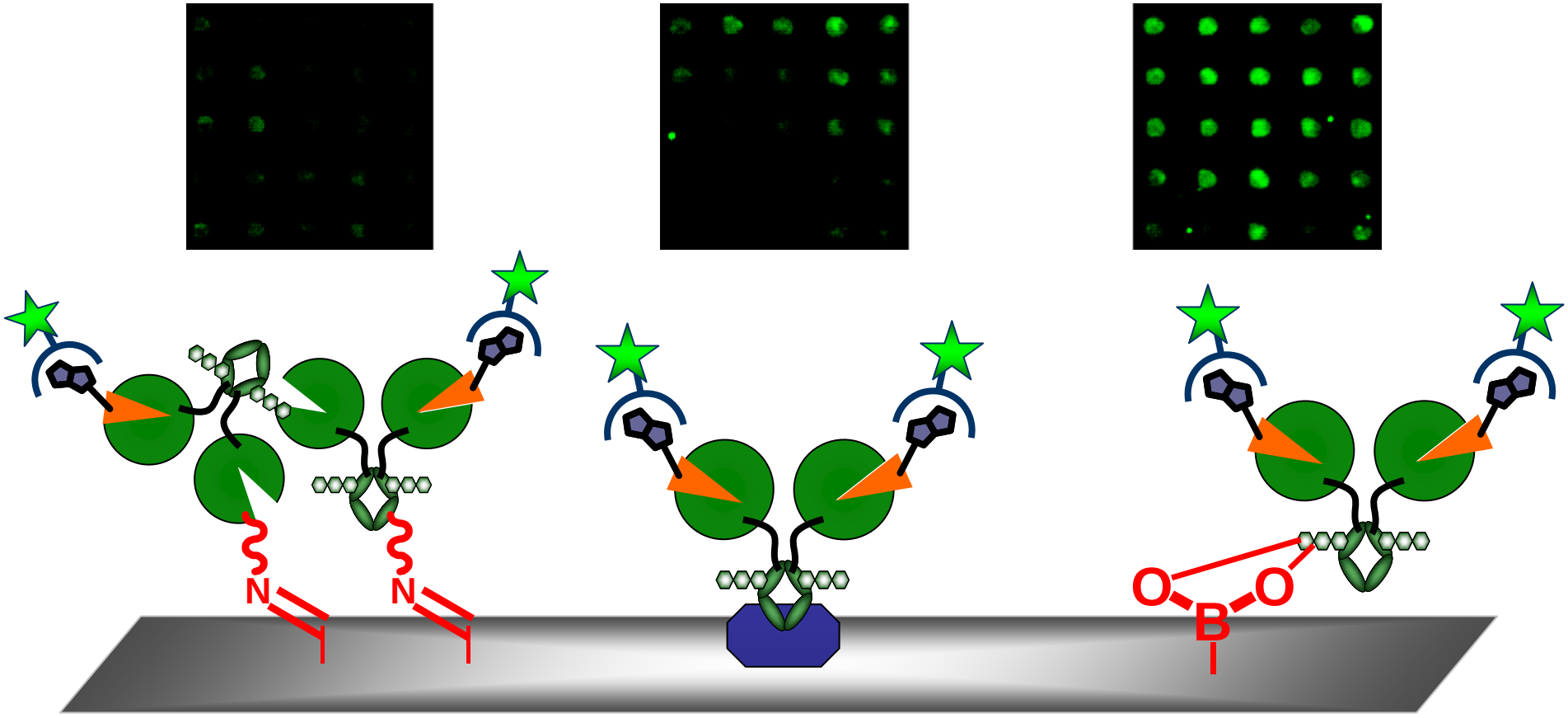
The dextran as blocking reagent did not affect F3 binding!!

Comparison of Fc-fused Dectin-1 Microarrays: Oriented vs Random



The detection sensitivities of various immobilized methods

using extremely low F3-biotin concentration (1 μg/mL)

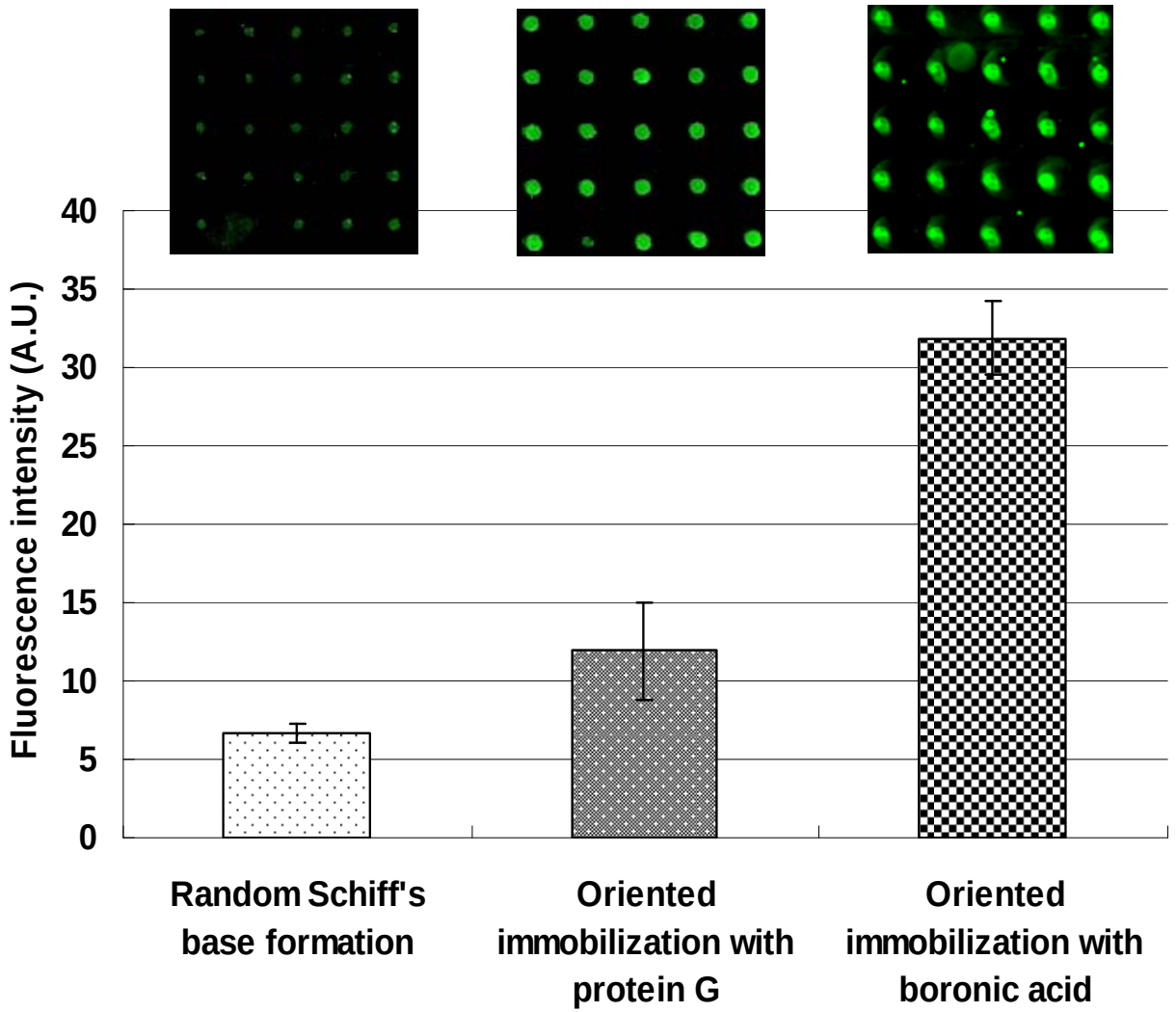


Random Schiff's base formation

Oriented immobilization with protein G

Oriented immobilization with boronic acid

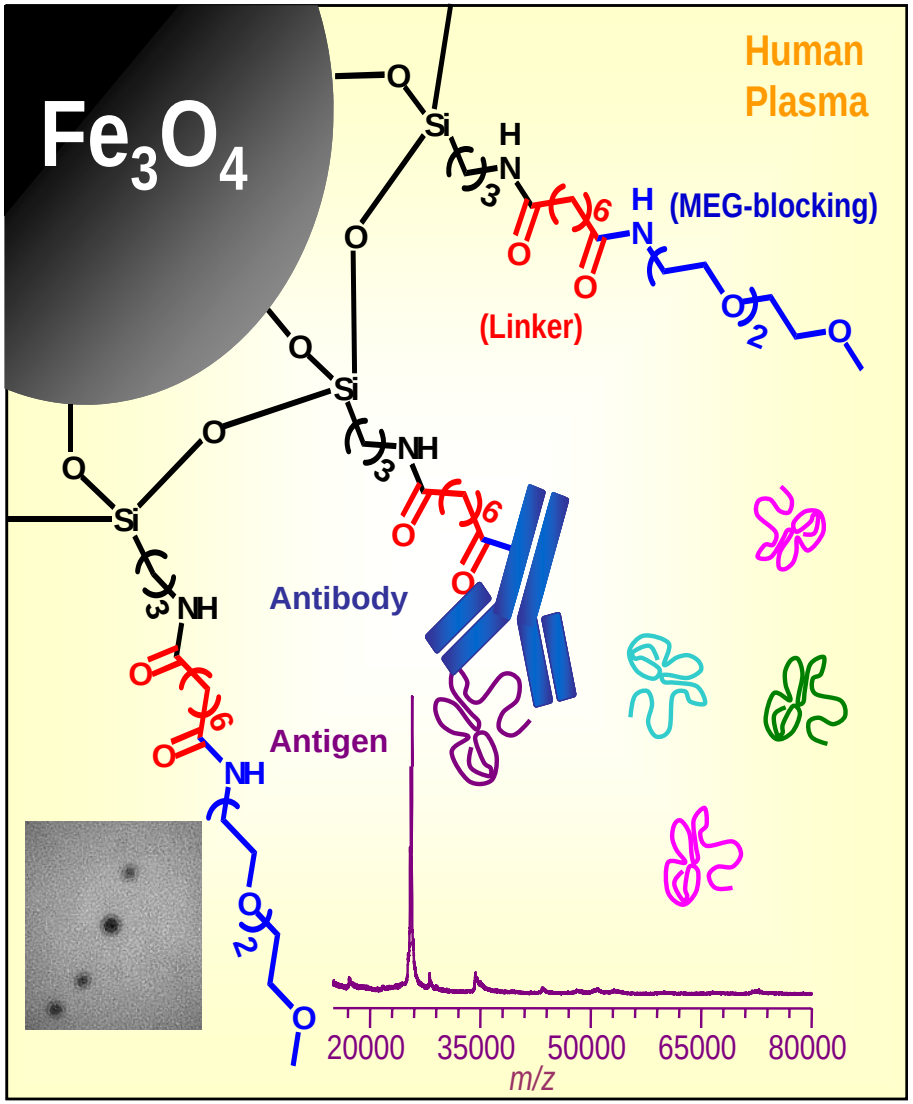
Fc-fused TLT-2 Microarrays

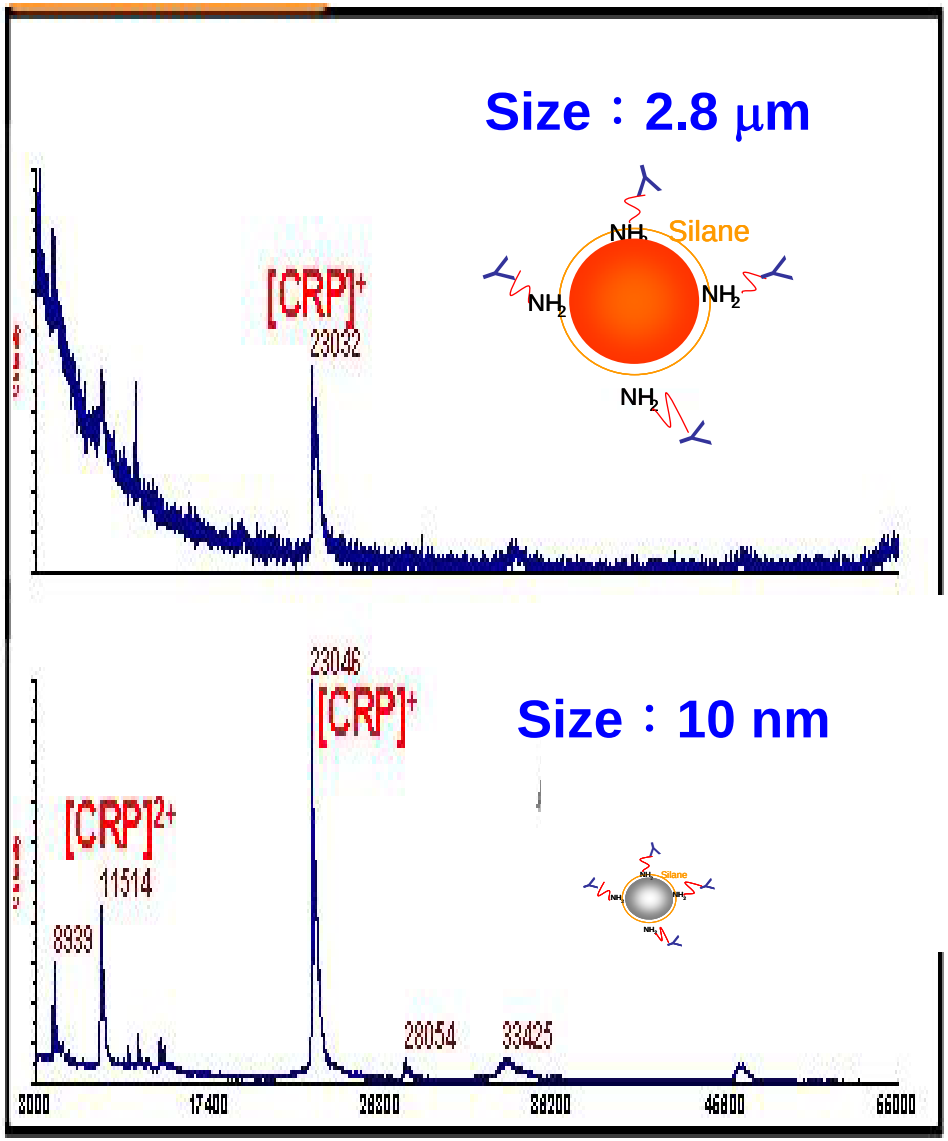


TLT-2 : Trem-Like Transcript-2

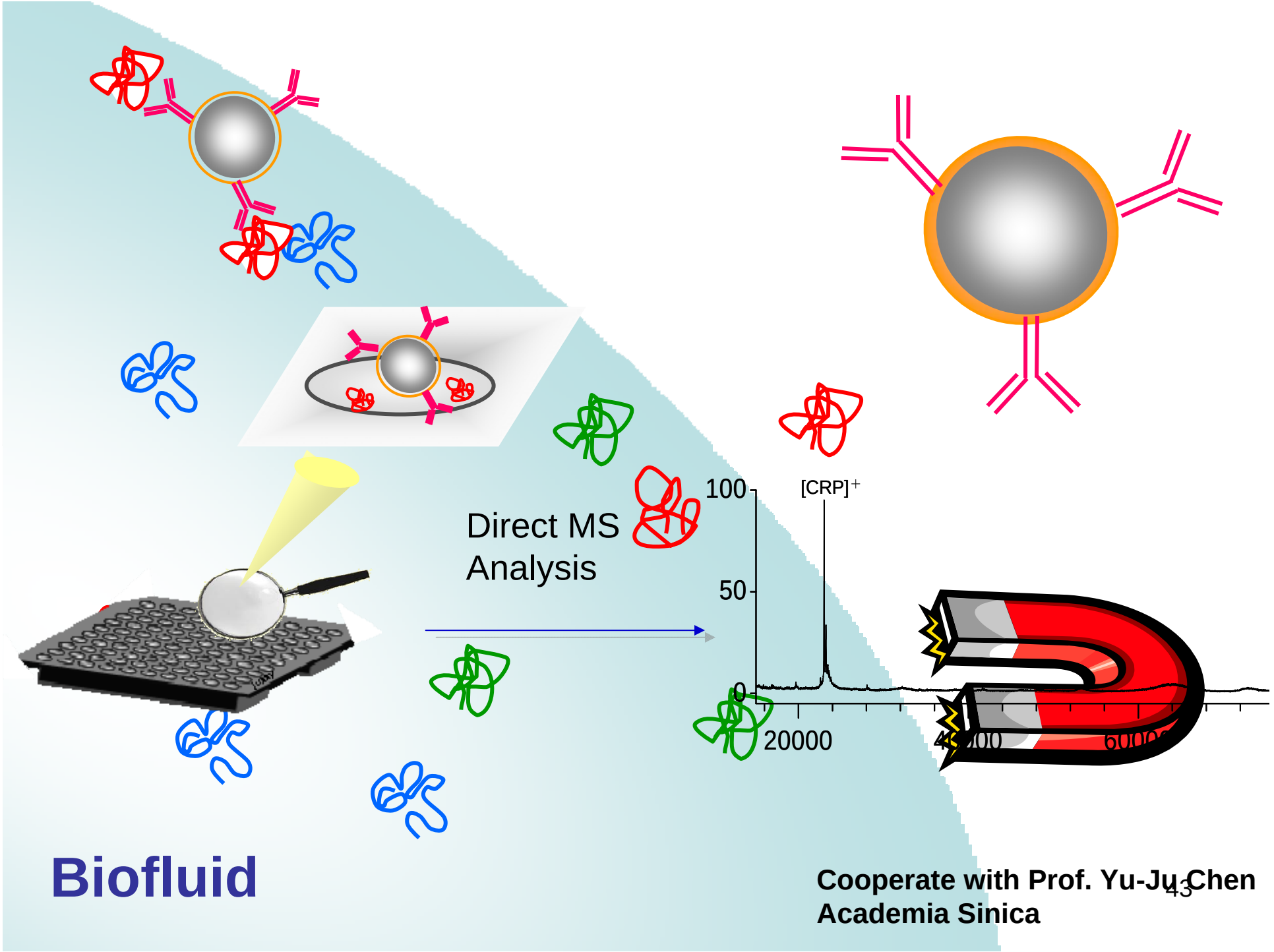
Yu-Ju Chen, Chun-Cheng Lin

Nanoprobe-Based Affinity Mass Spectrometry for Target Detection

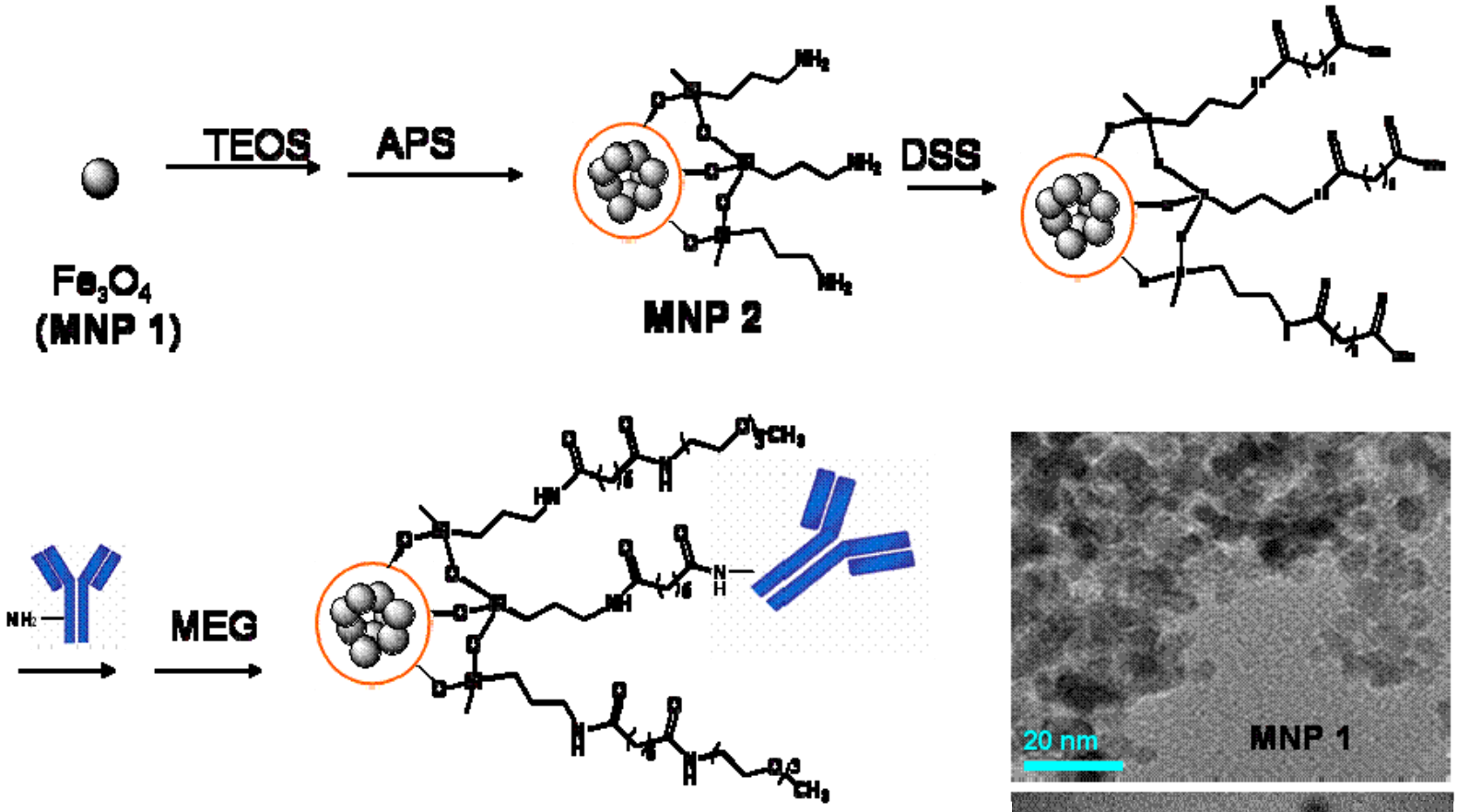




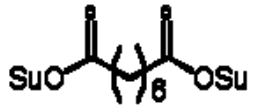
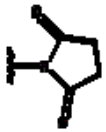
Anal. Chem. (**2005**)



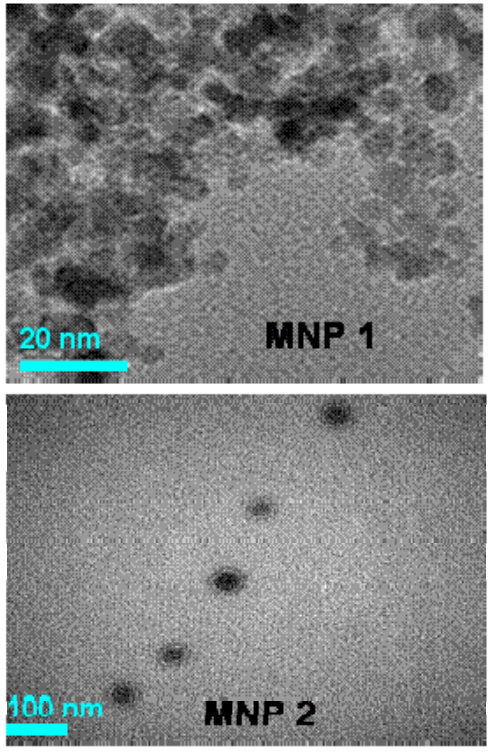
Cooperate with Prof. Yu-Ju Chen
Academia Sinica



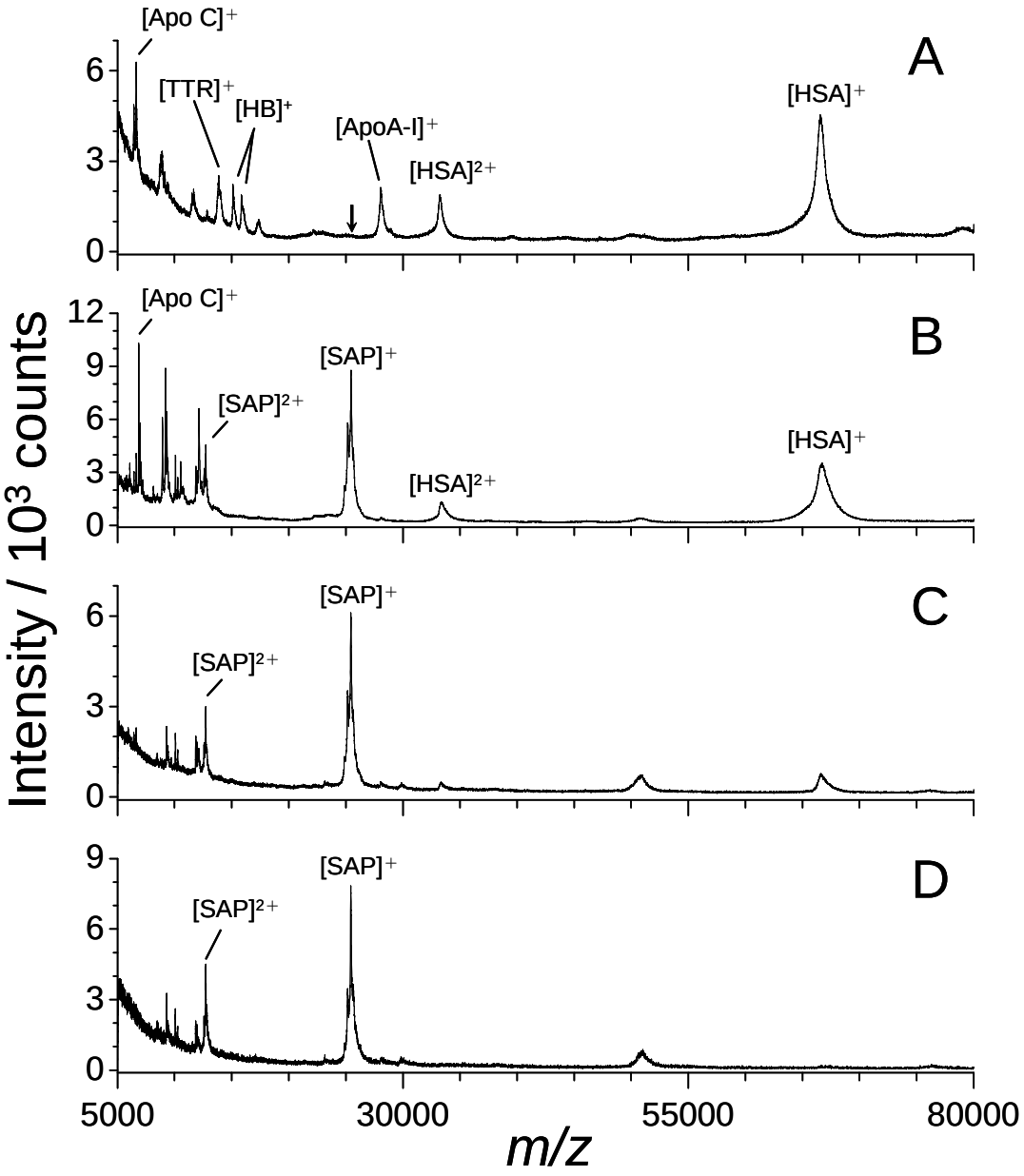
TEOS: Tetraethyl orthosilicate
APS: 3-aminopropyltrimethoxysilane

DSS:  **OSu:** 

MEG:  **(1)**



***Small* 2006, *2*, 485**



Human plasma

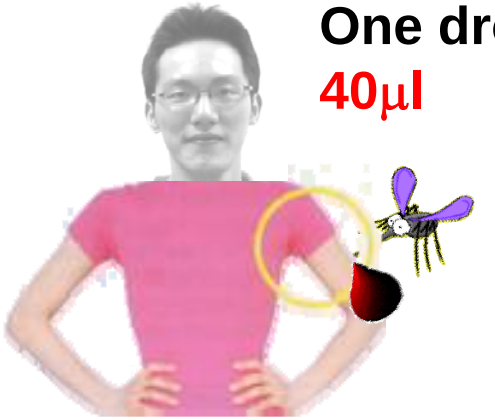
Extracted by anti-SAP MNP

Extracted by anti-SAP MNP (BSA blocking)

Extracted by anti-SAP MNP (MEG blocking)

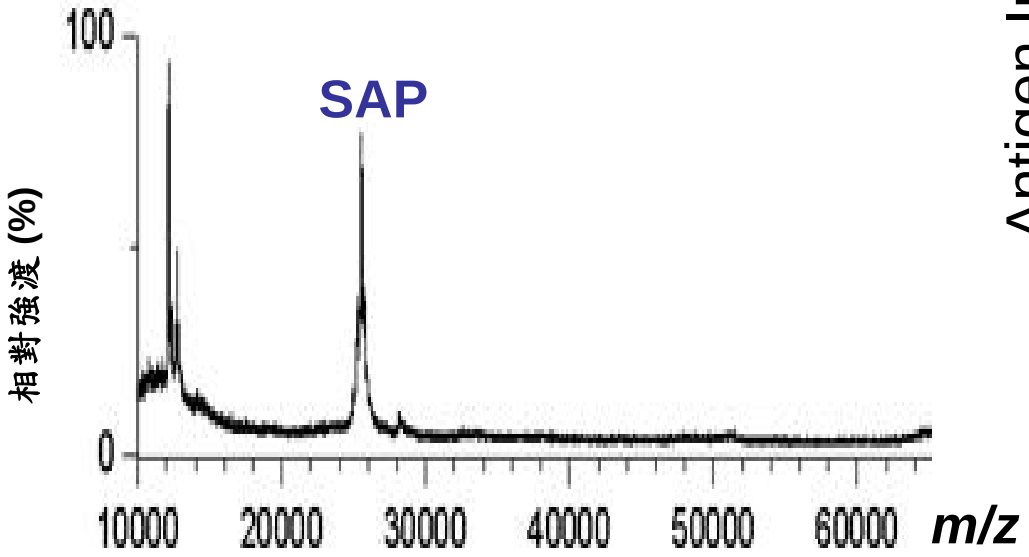
***Small* 2006, *2*, 485**

High Sensitivity



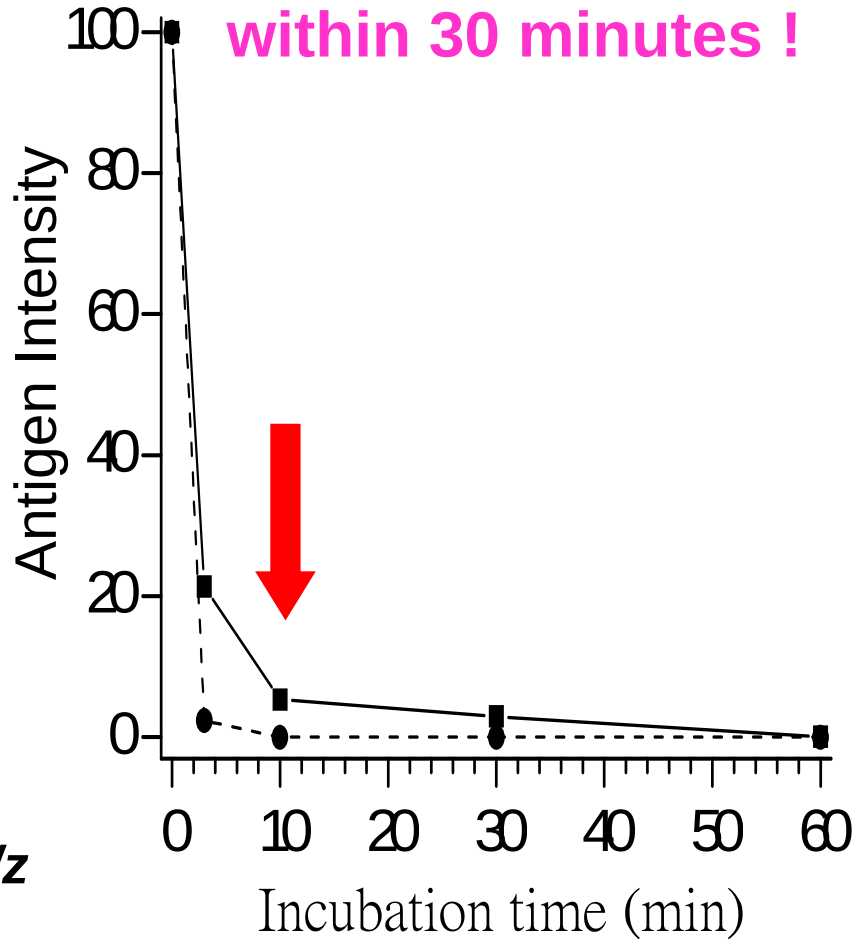
One drop of blood:
40μl

0.1μl of blood

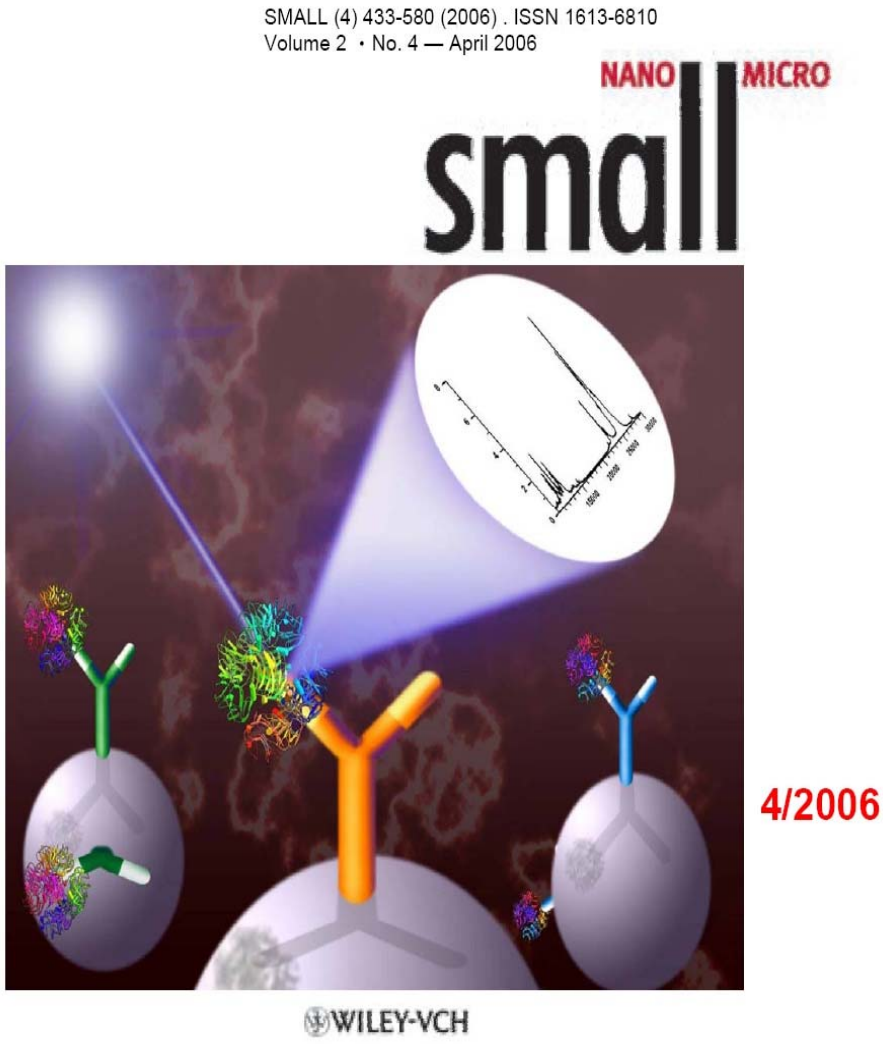


Rapid Assay

The Immunoassay can be completed within 30 minutes !

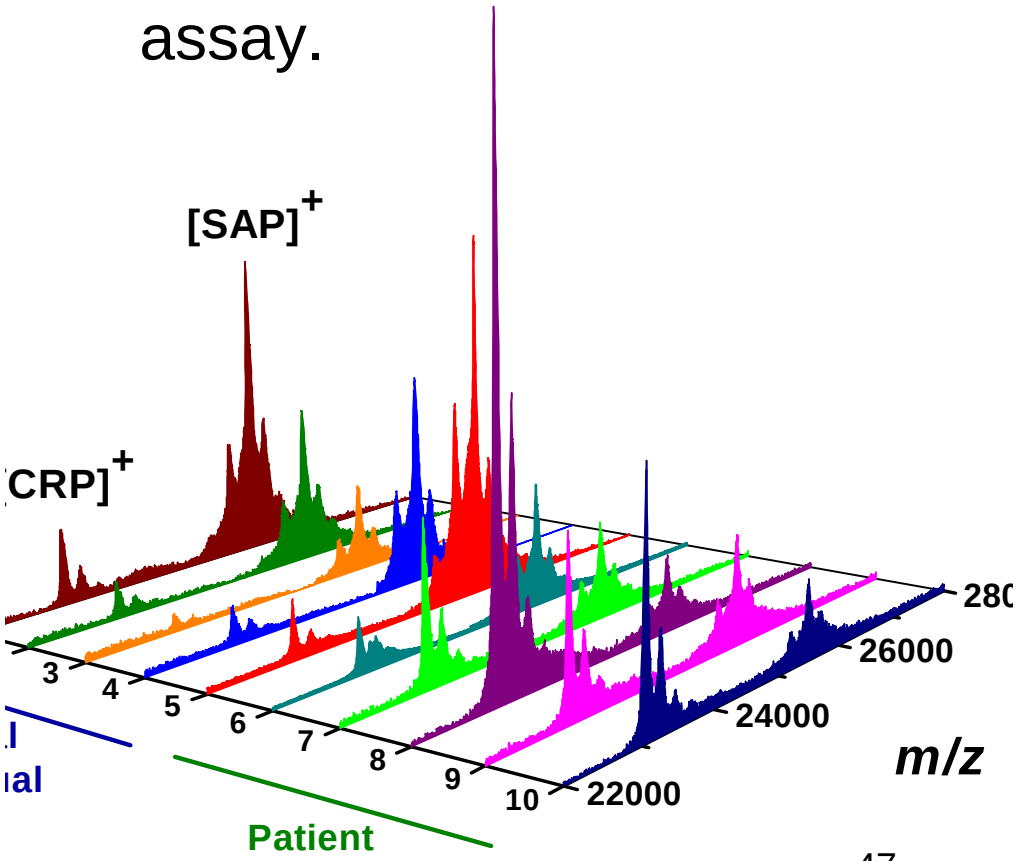


Multiplexed Immunoassay

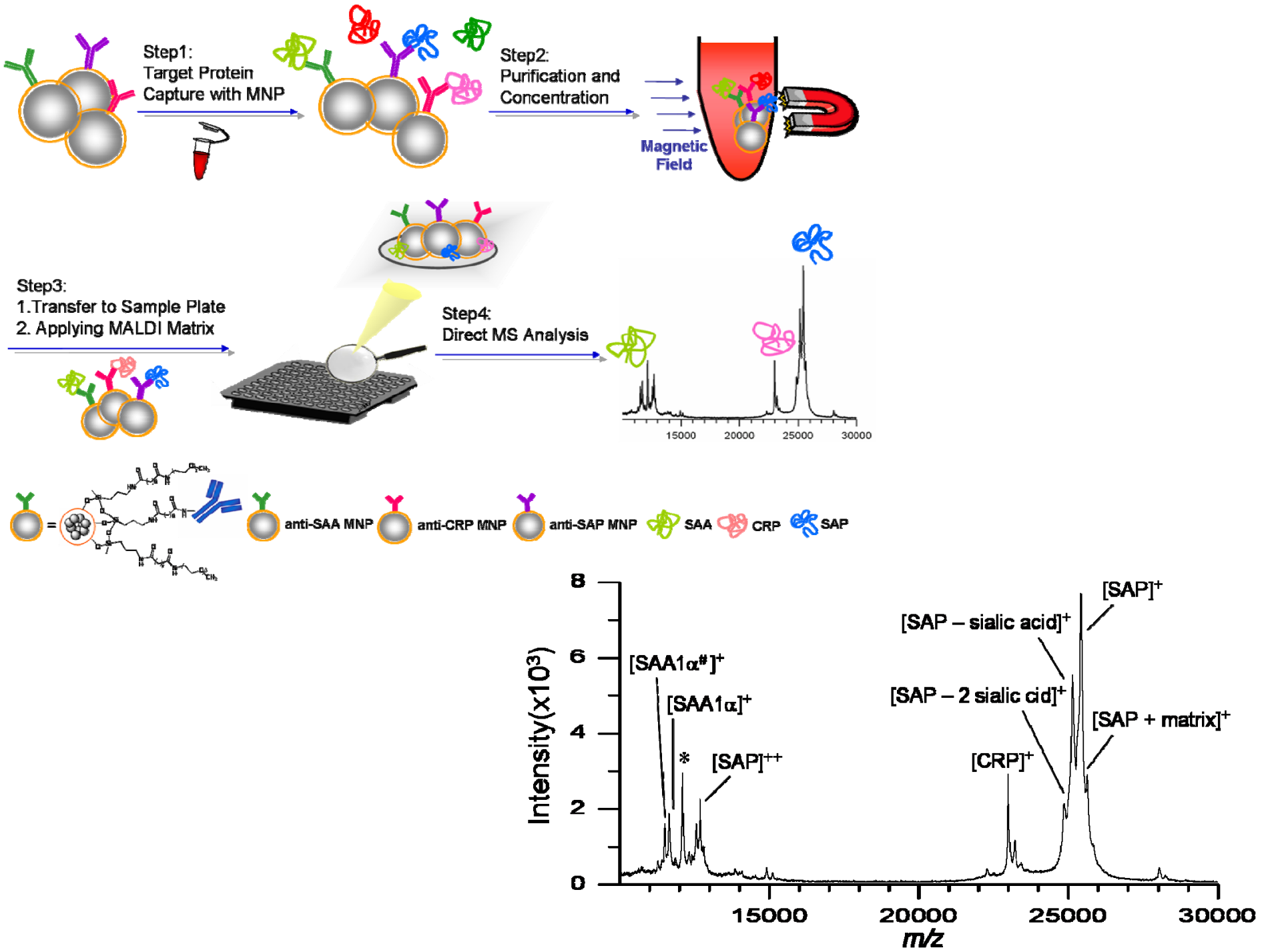


Small, 2, 485-489 (2006) cover story

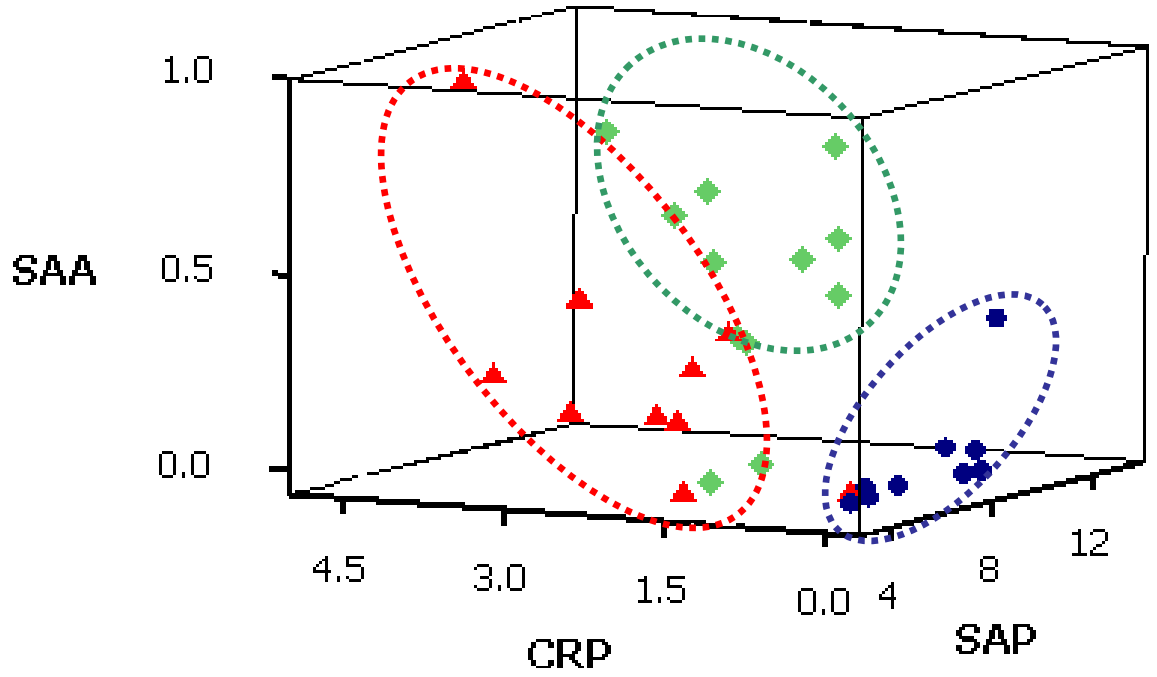
Differential protein expression profiles for patient and healthy control in a single assay.



Anal. Chem. **2008**, *80*, 6159

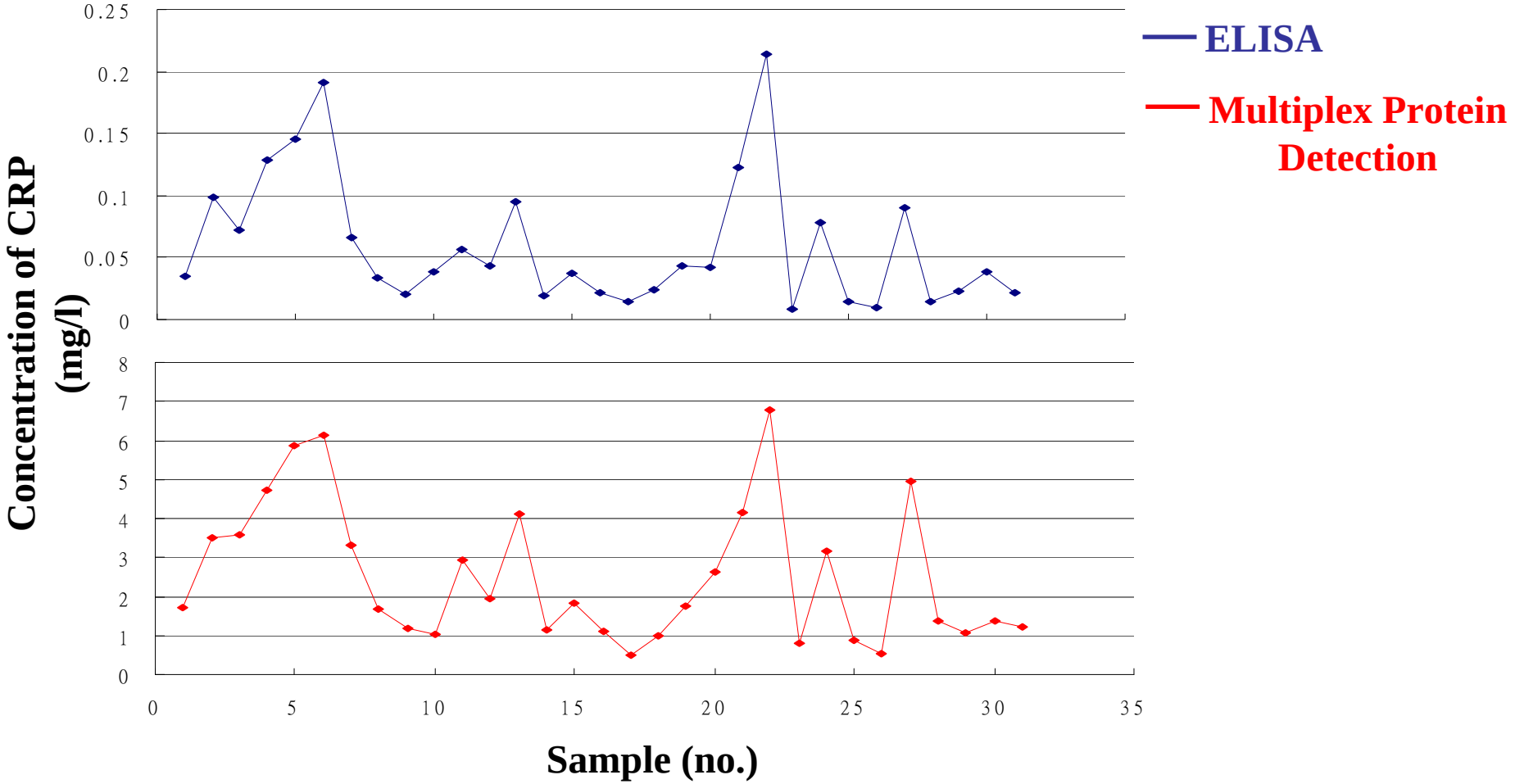

Step1:
Target Protein
Capture with MNP
Step2:
Purification and
Concentration
Magnetic
Field
Step3:
1.Transfer to Sample Plate
2. Applying MALDI Matrix
Step4:
Direct MS Analysis
15000
20000
25000
30000
anti-SAA MNP
anti-CRP MNP
anti-SAP MNP
SAA
CRP
SAP
Intensity(x10³)
[SAA1α#]+
[SAA1α]+
*
[SAP]++
[SAP – sialic acid]+
[SAP]+
[SAP – 2 sialic cid]+
[SAP + matrix]+
[CRP]+
m/z

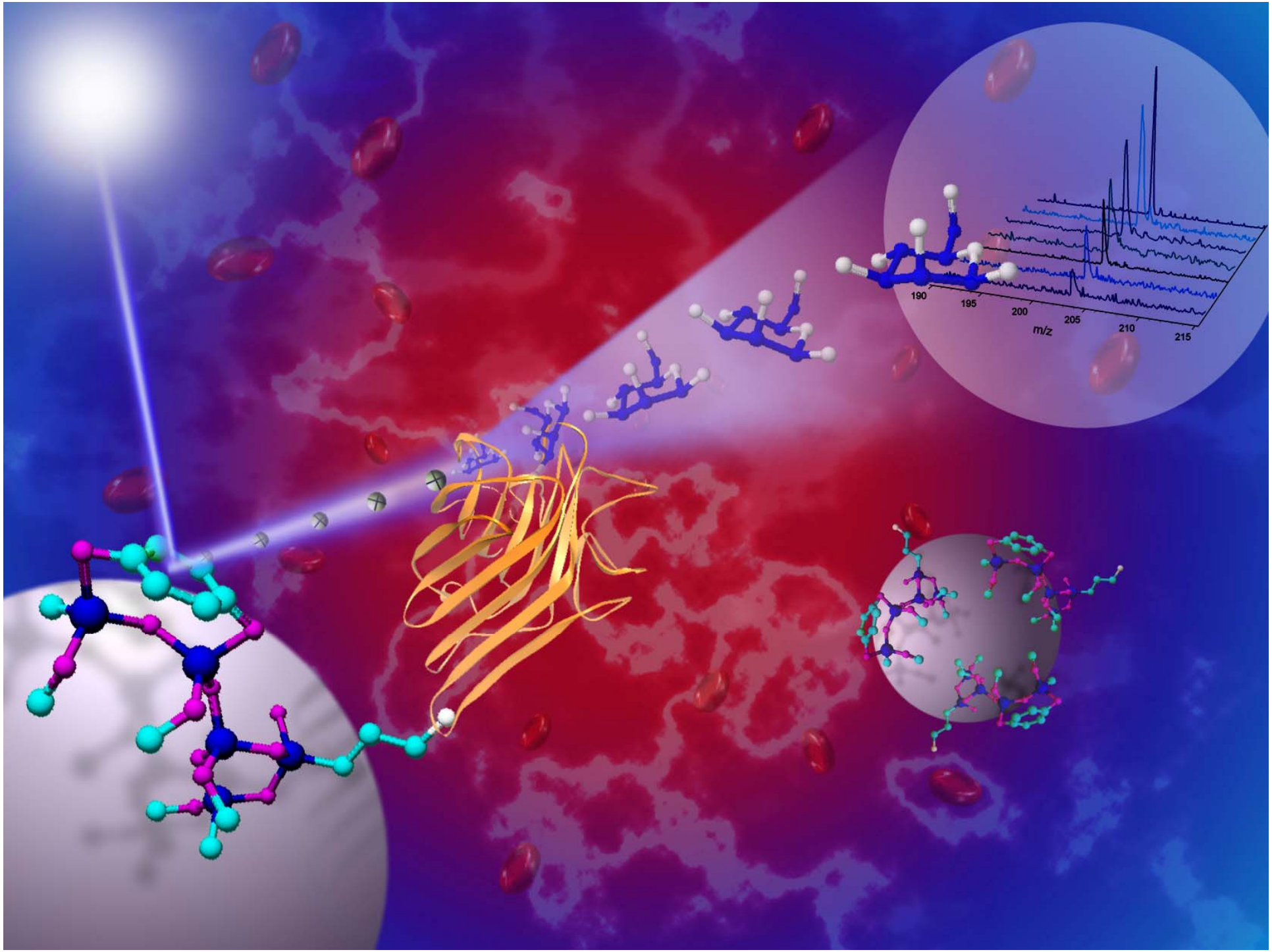
3D Scatterplot of Multiplexed Protein Profiles

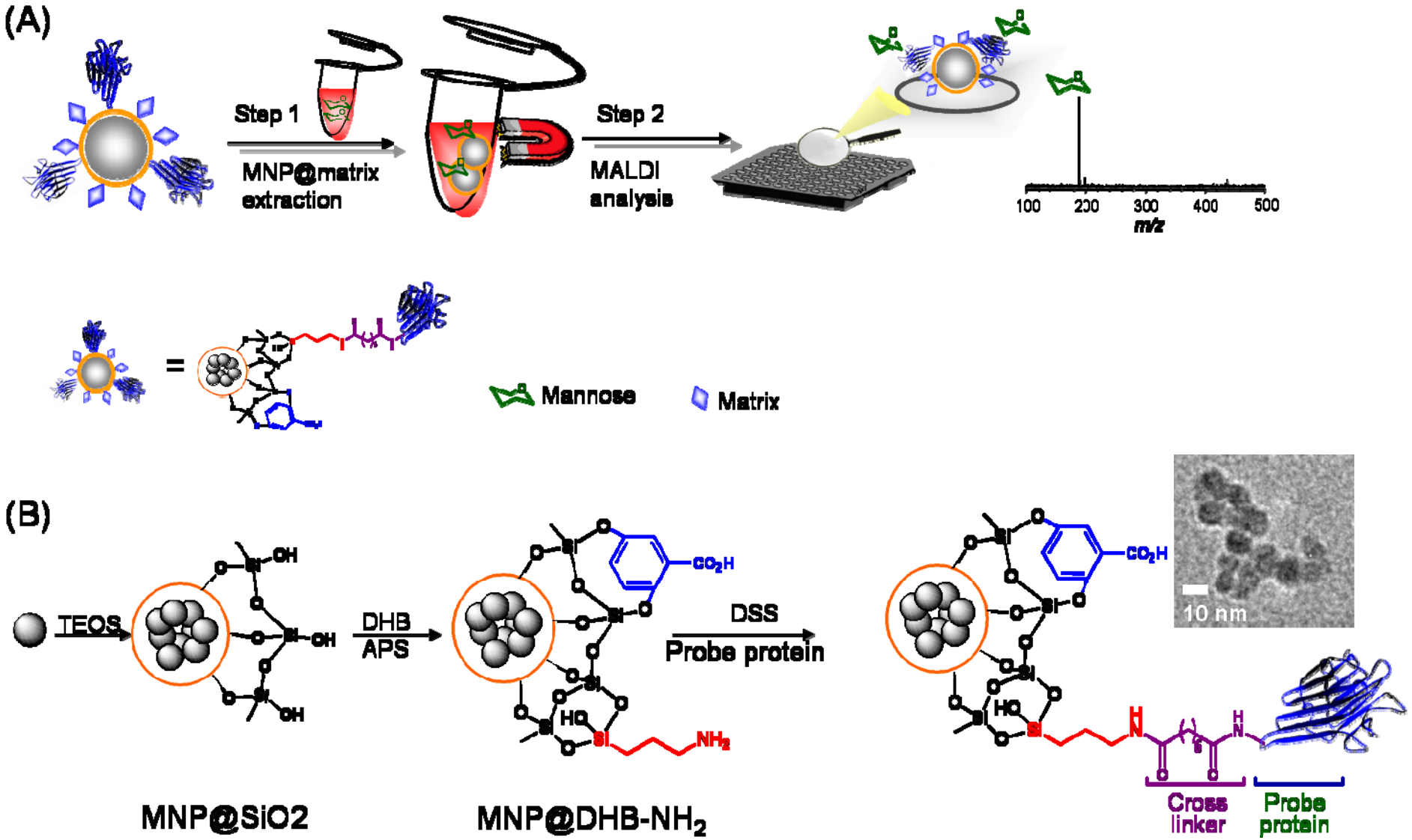


- **Normal :** Healthy individuals
- **Patient :** Patients / gastric cancer
- **Surgery:** Patients / surgical operation of cardiac atheter

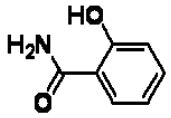
Correlation of CRP concentrations between ELISA and Multiplexed Protein Detection



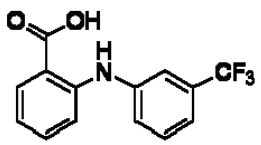

190
195
200
205
210
215
m/z


(A)
Step 1
MNP@matrix
extraction
Step 2
MALDI
analysis
100
200
300
400
500
m/z
=
Mannose
Matrix
(B)
TEOS
DHB
APS
DSS
Probe protein
10 nm
MNP@SiO2
MNP@DHB-NH2
Cross
linker
Probe
protein

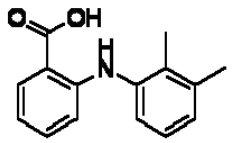
(A)



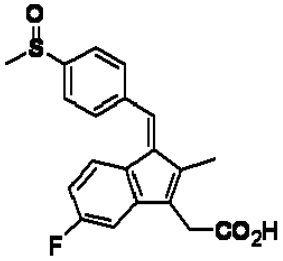
1 **salicylamide**
MW: 137.14



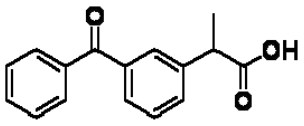
4 **flufenamic acid**
MW: 281.13



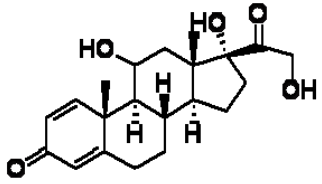
2 **mefenamic acid**
MW: 241.29



5 **sulindac**
MW: 356.13

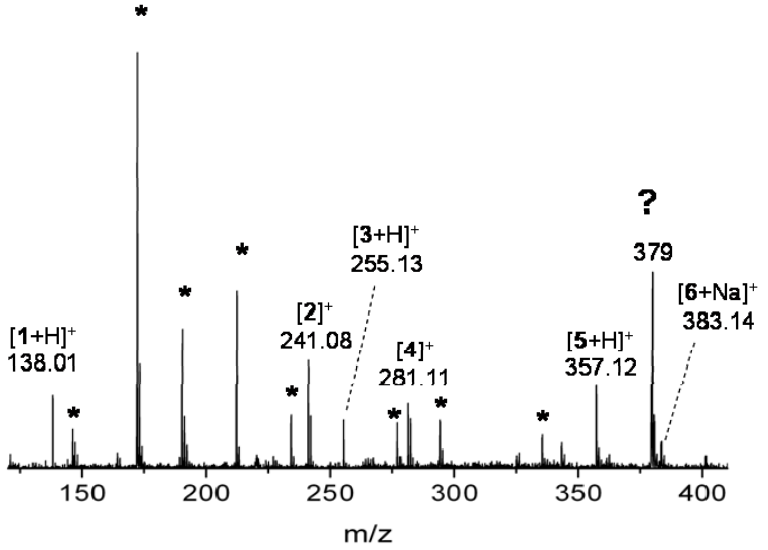


3 **keetoprofen**
MW: 254.28

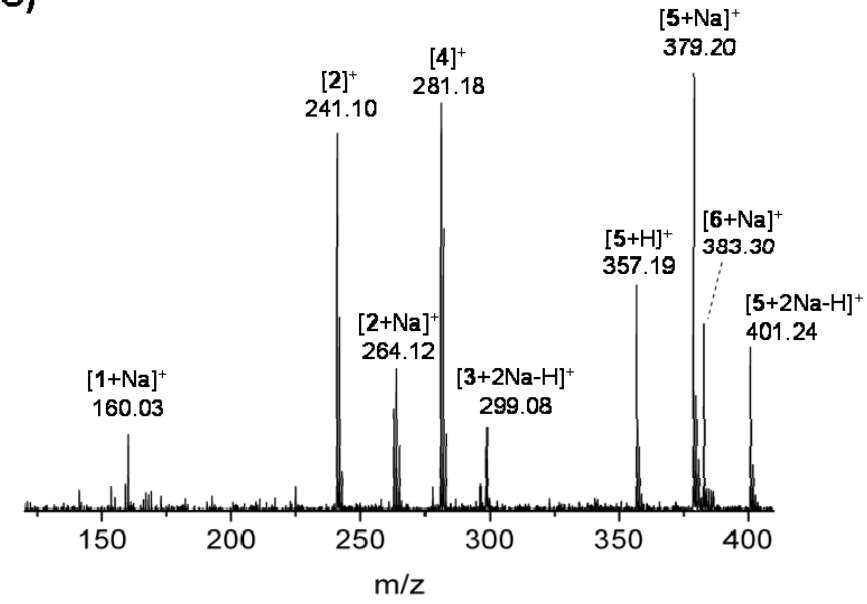


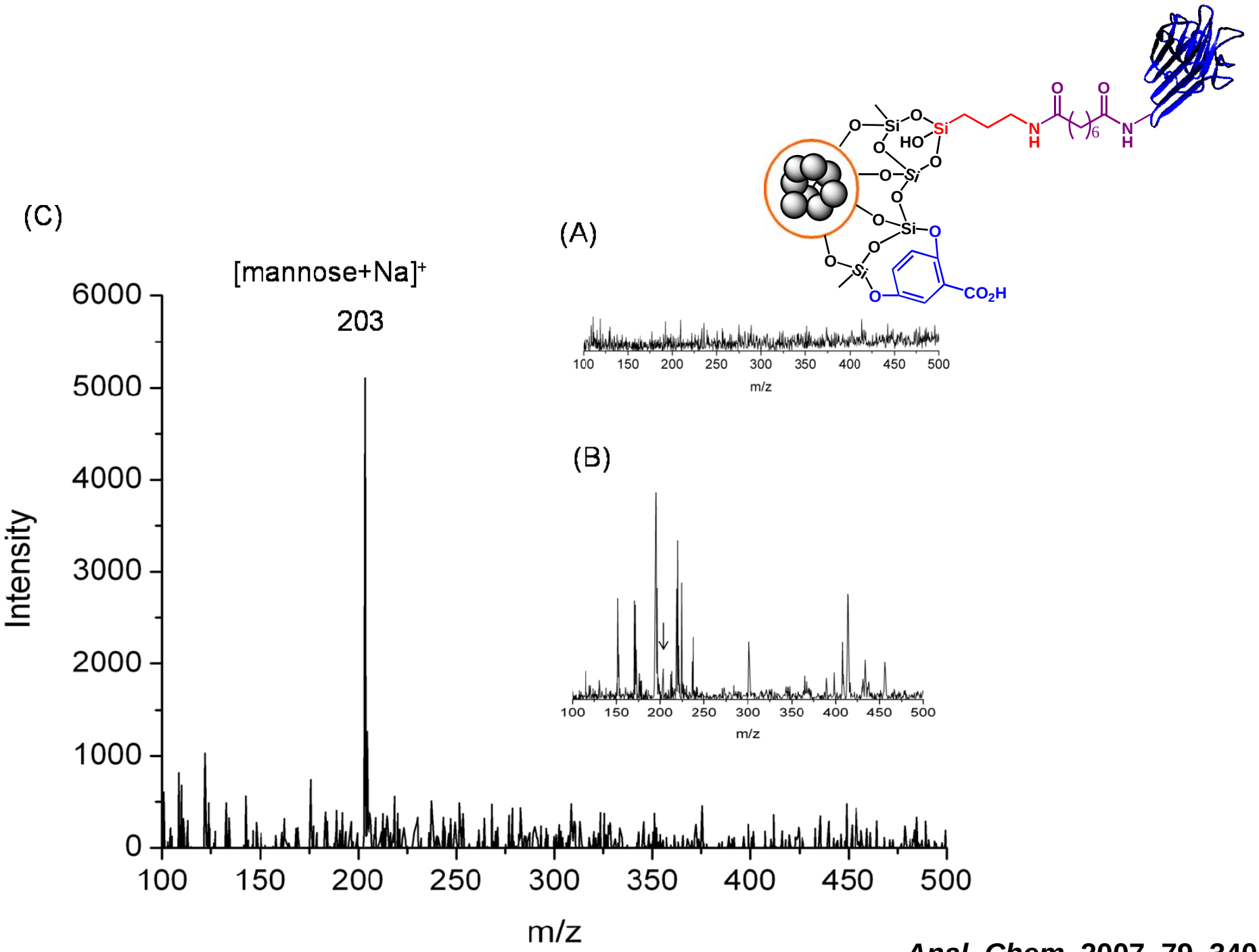
6 **prednisolone**
MW: 360.44

(B)

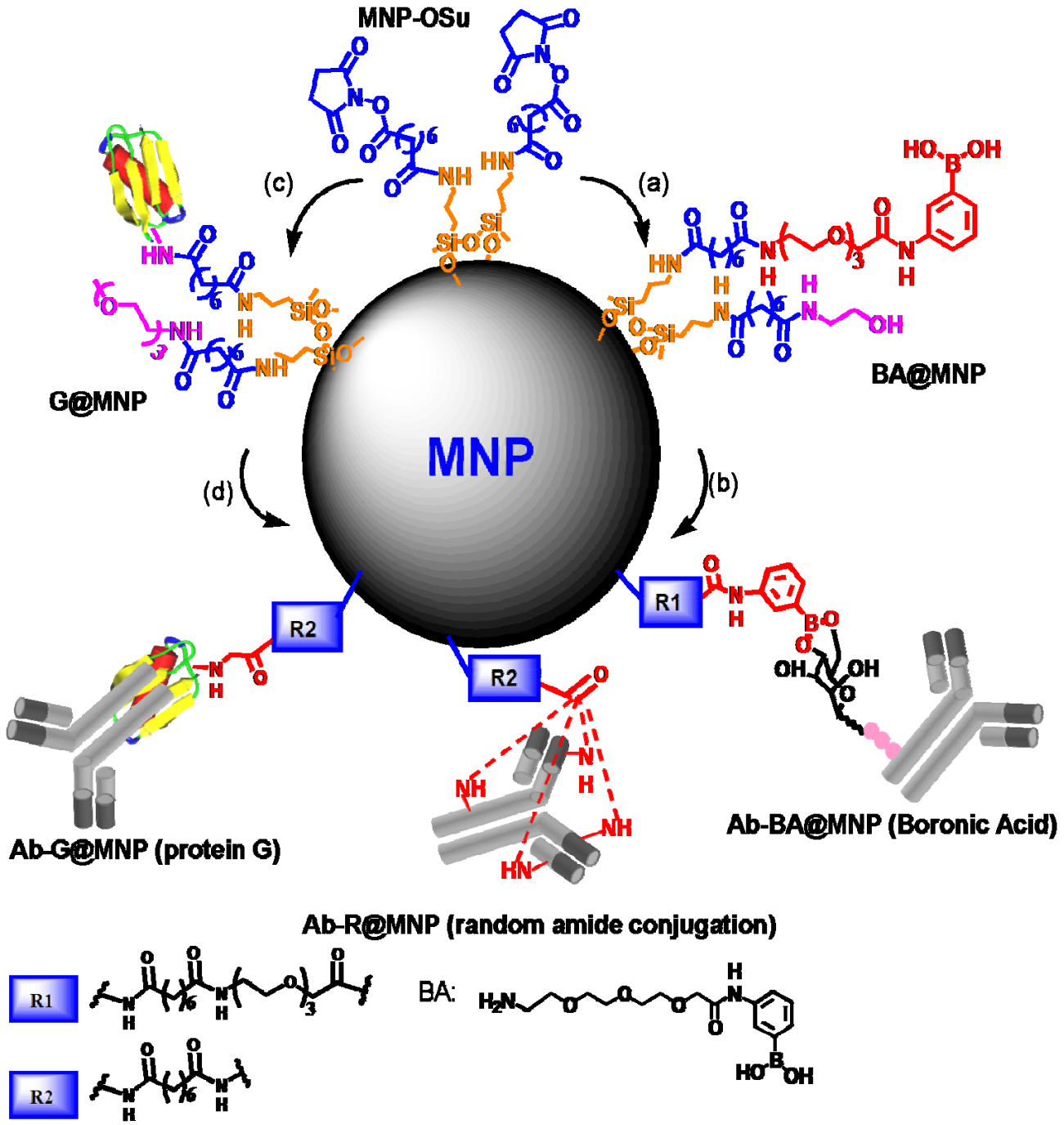


(C)





Anal. Chem. **2007, 79, 3401**


MNP-OSu
(c)
(a)
G@MNP
BA@MNP
MNP
(d)
(b)
R2
R2
R1
Ab-G@MNP (protein G)
Ab-BA@MNP (Boronic Acid)
Ab-R@MNP (random amide conjugation)
R1
R2
BA:

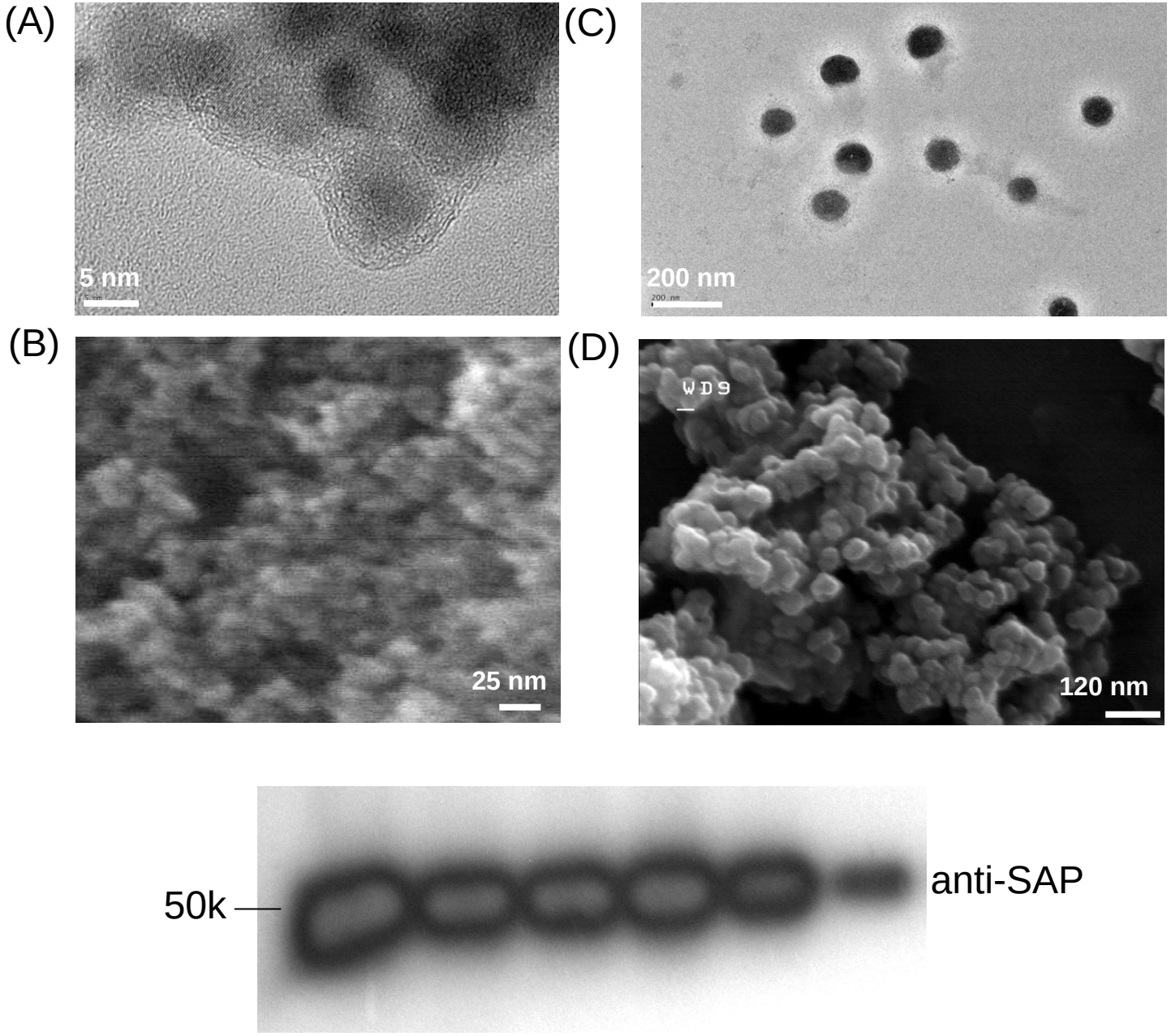
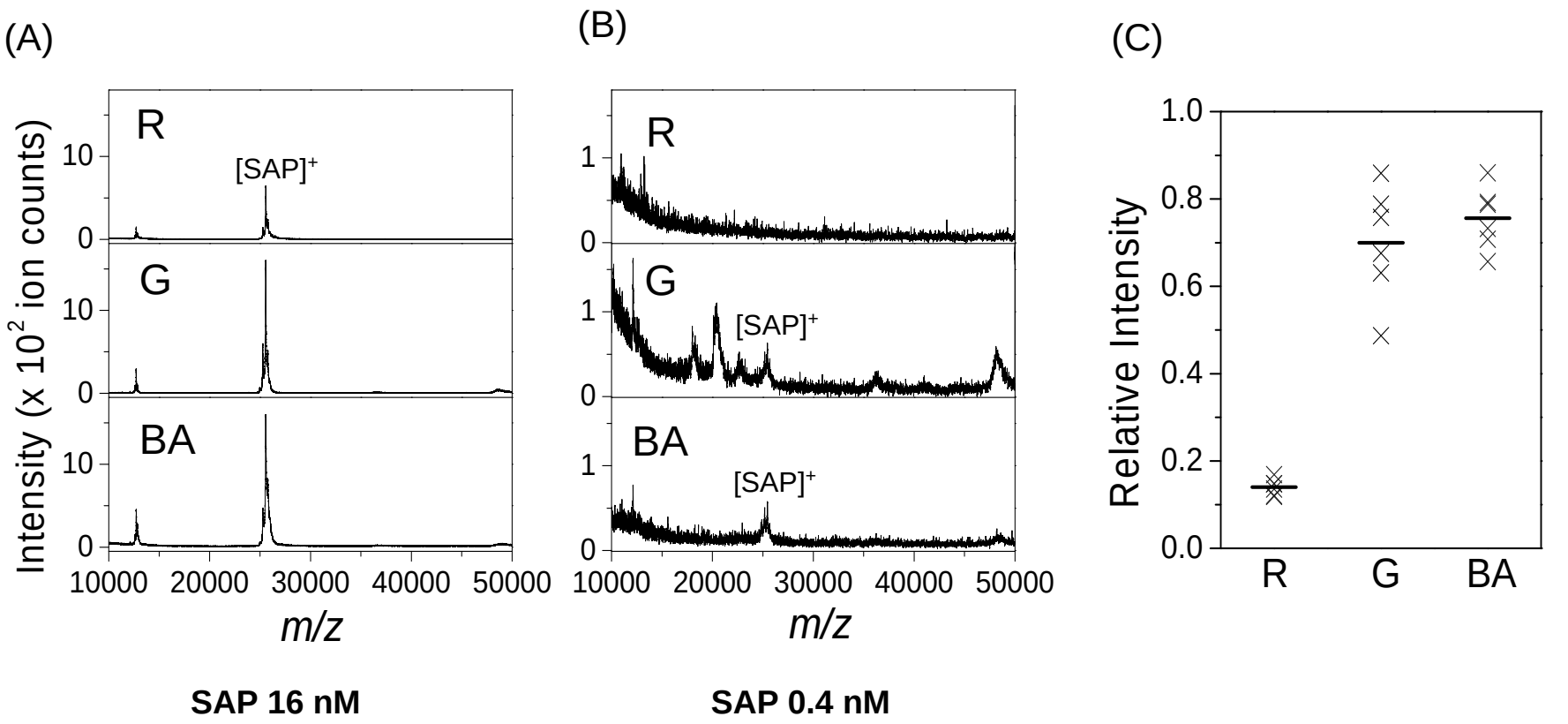
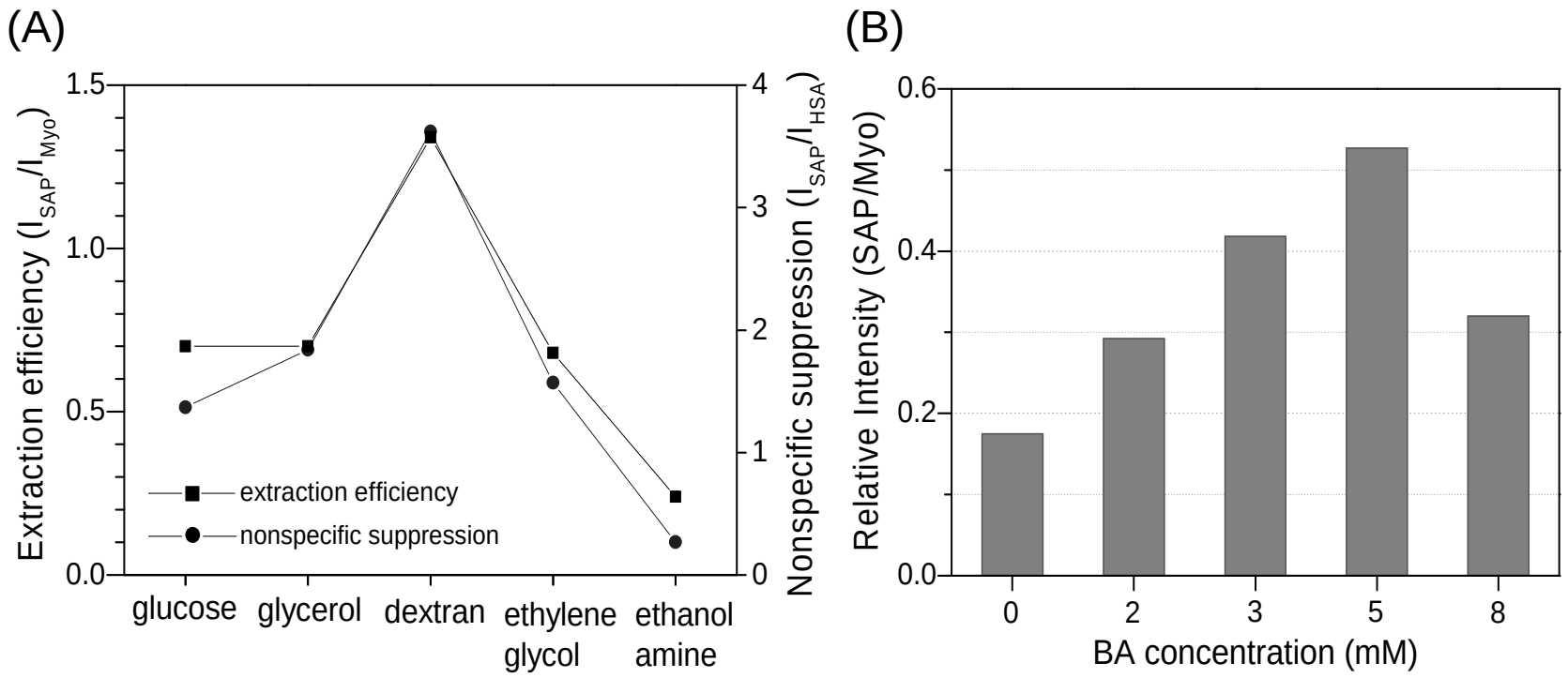

(A)
5 nm
(C)
200 nm
(B)
25 nm
(D)
WD9
120 nm
50k
anti-SAP
pH: 7 8 9 10 11 12

Figure 2



2008, submitted

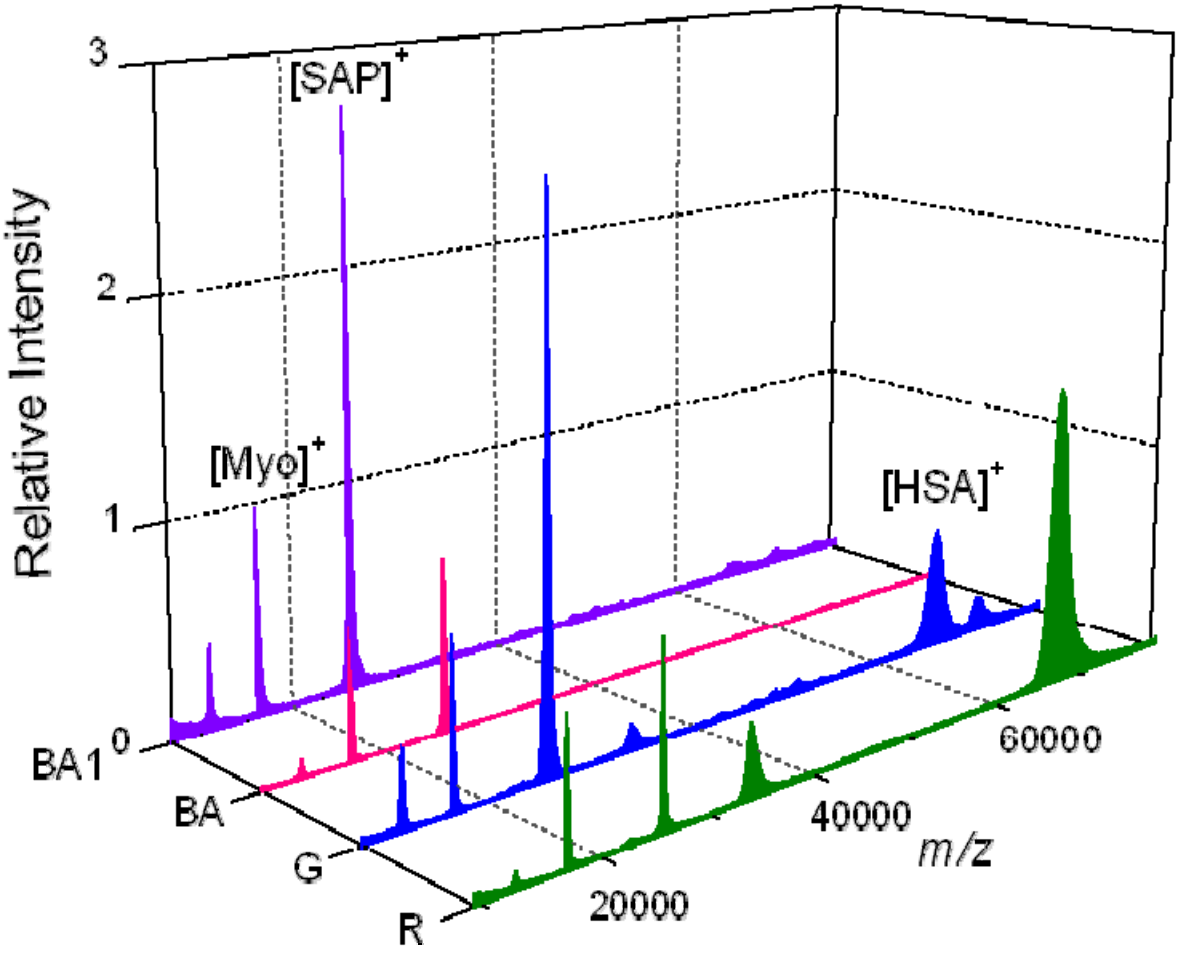
Figure 3

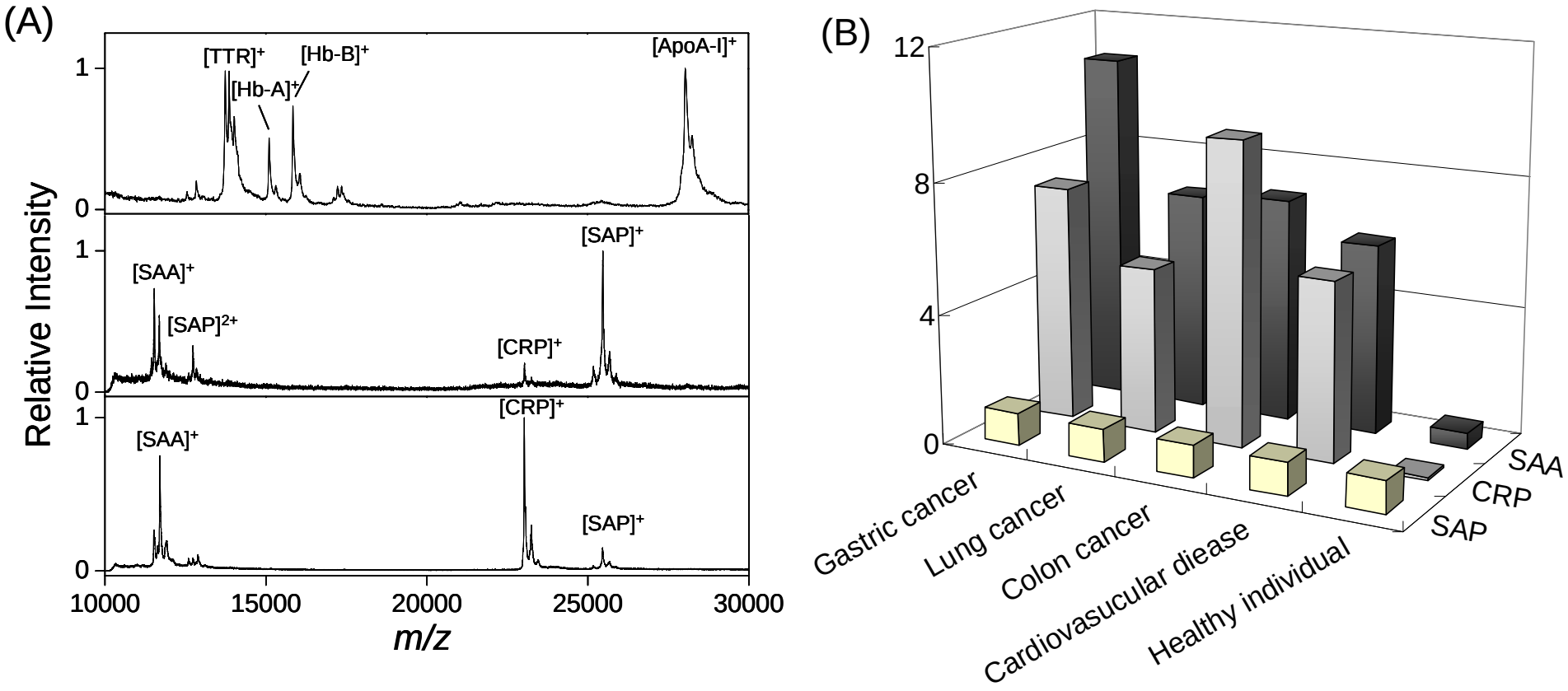


2.5 mg/ml dextran

2008, submitted

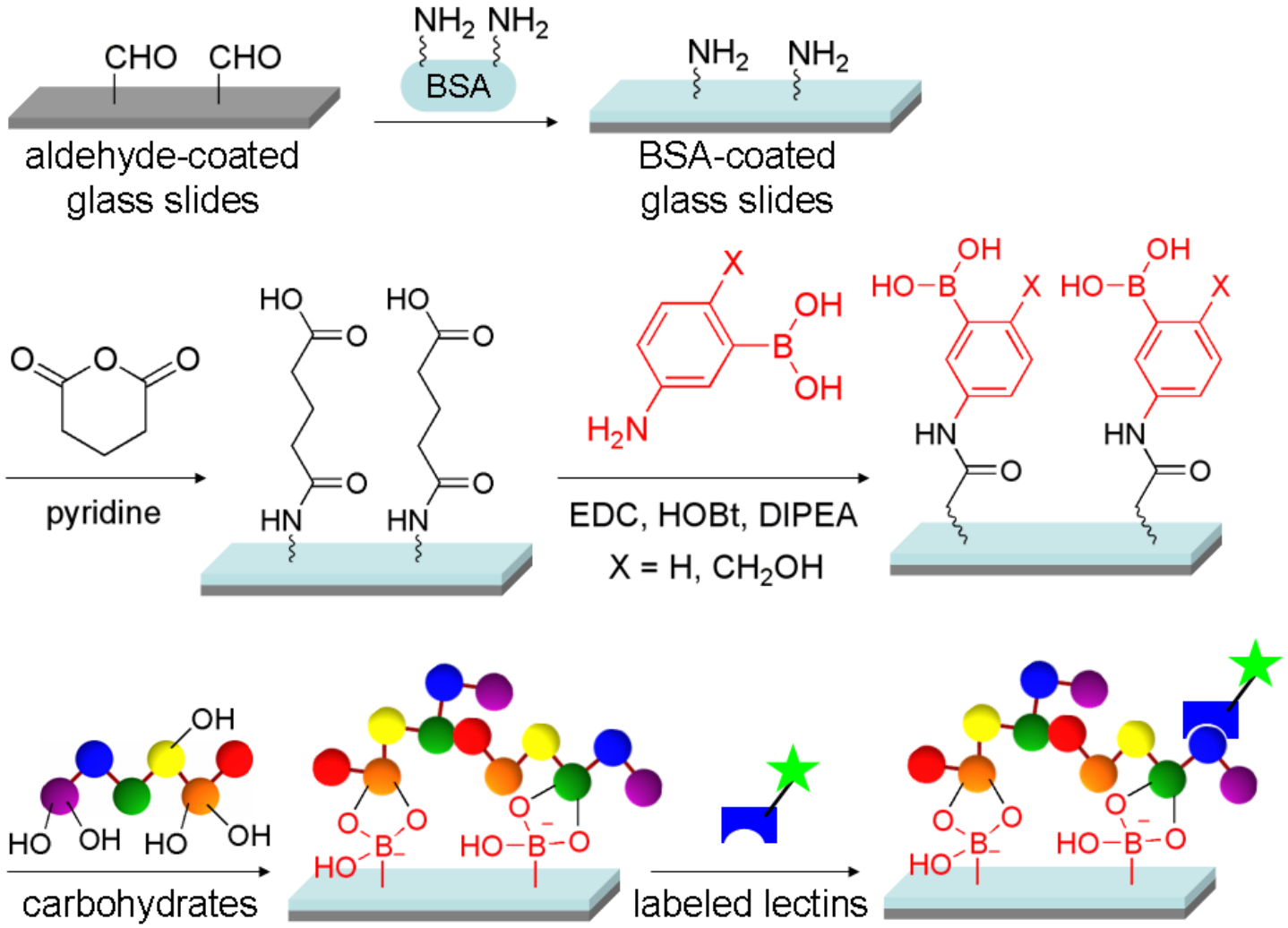
Figure 4


Relative Intensity
3
2
1
0
[SAP]+
[Myo]+
[HSA]+
BA1
BA
G
R
20000
40000
60000
m/z

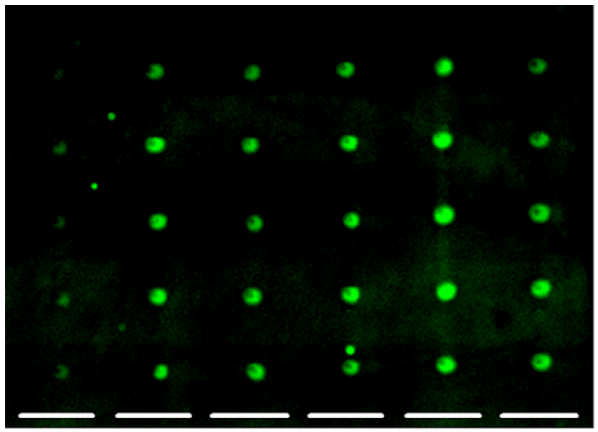


2008, submitted

Fabrication strategy of BA-based carbohydrate microarray

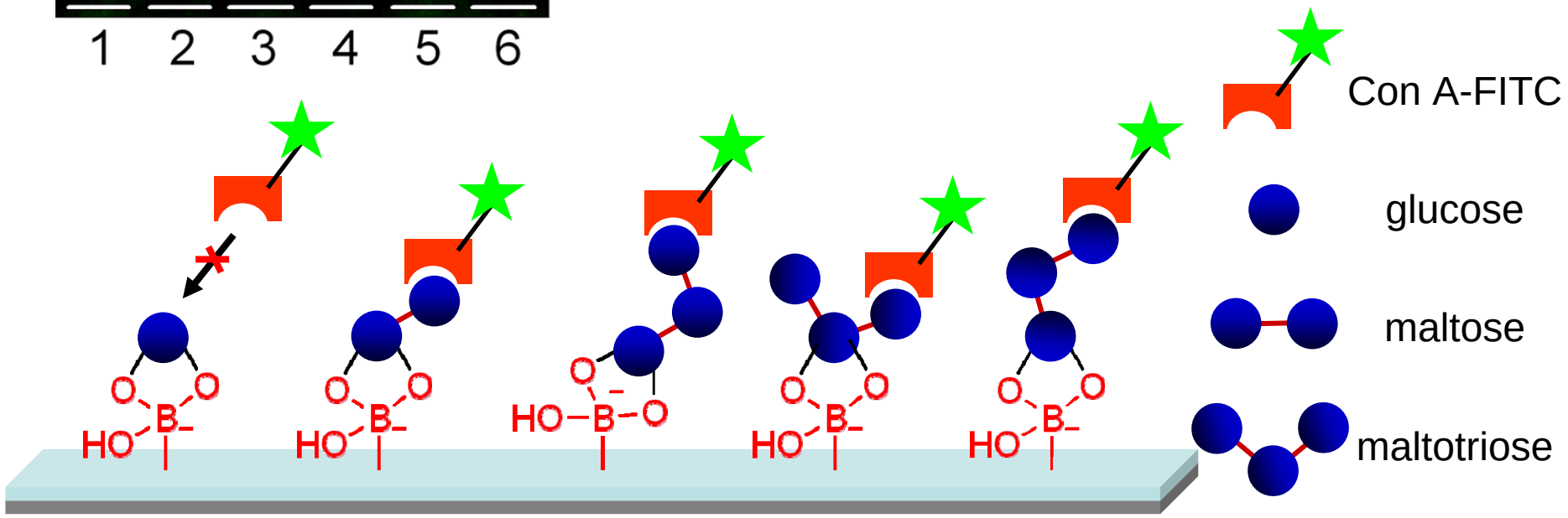


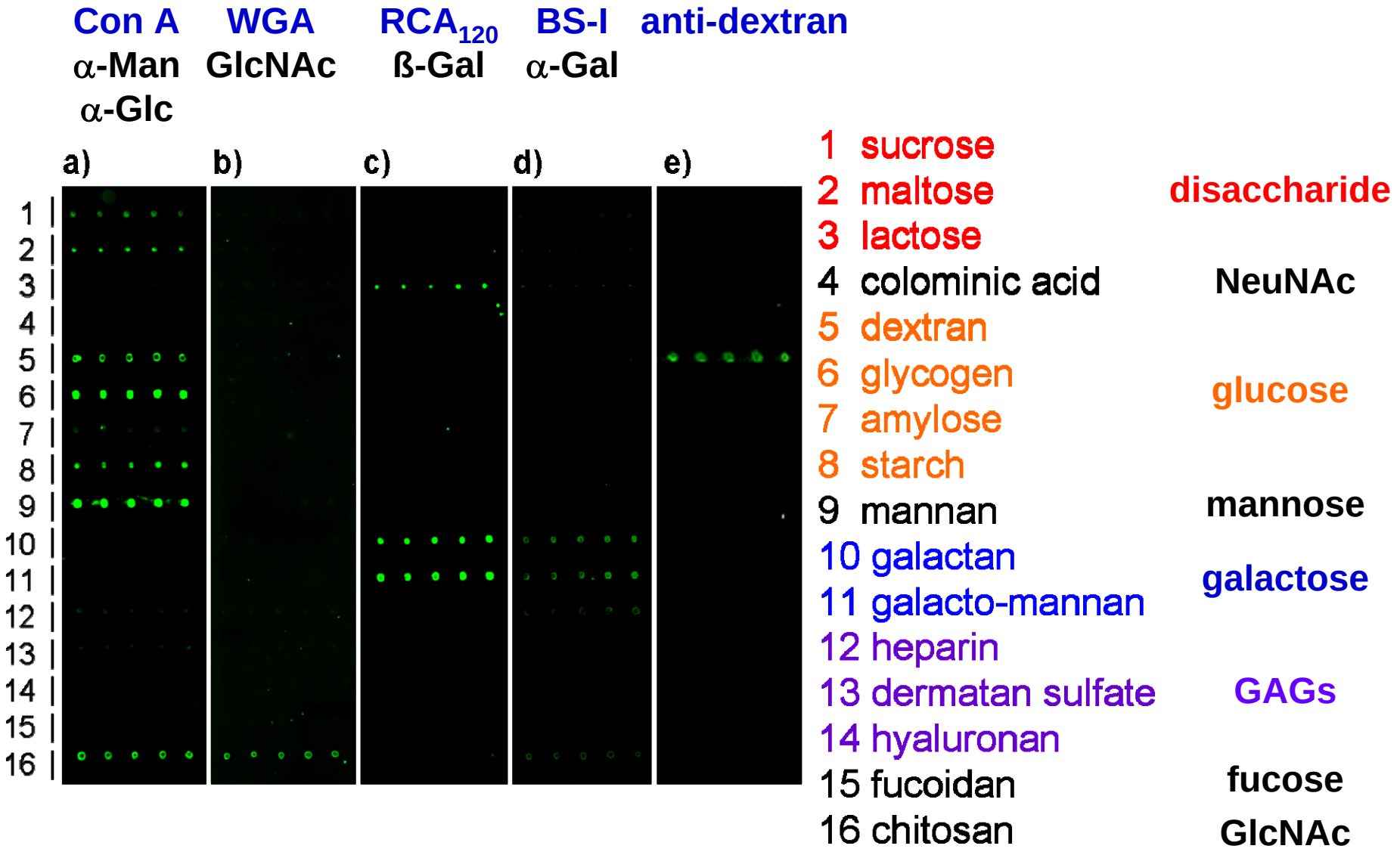
Biological activity of carbohydrate on BA functionalized slides



1 glucose
2 maltose
3 maltotriose
4 maltotetraose
5 maltopentaose
6 maltohexaose

Immobilization of maltose saccharides. The microarrays were probed with Con A-FITC.





Fluorescence images of carbohydrate microarrays containing 16 carbohydrates probed with (a) FITC-Con A, (b) FITC-WGA, (c) FITC-RCA_{120}, (d) biotin-BS-I followed by incubation with Cy3-streptavidin and (e) FITC-labeled anti-dextran antibody

In preparation