Nanoparticles-based FRET for Detection of Protein Glycosylation

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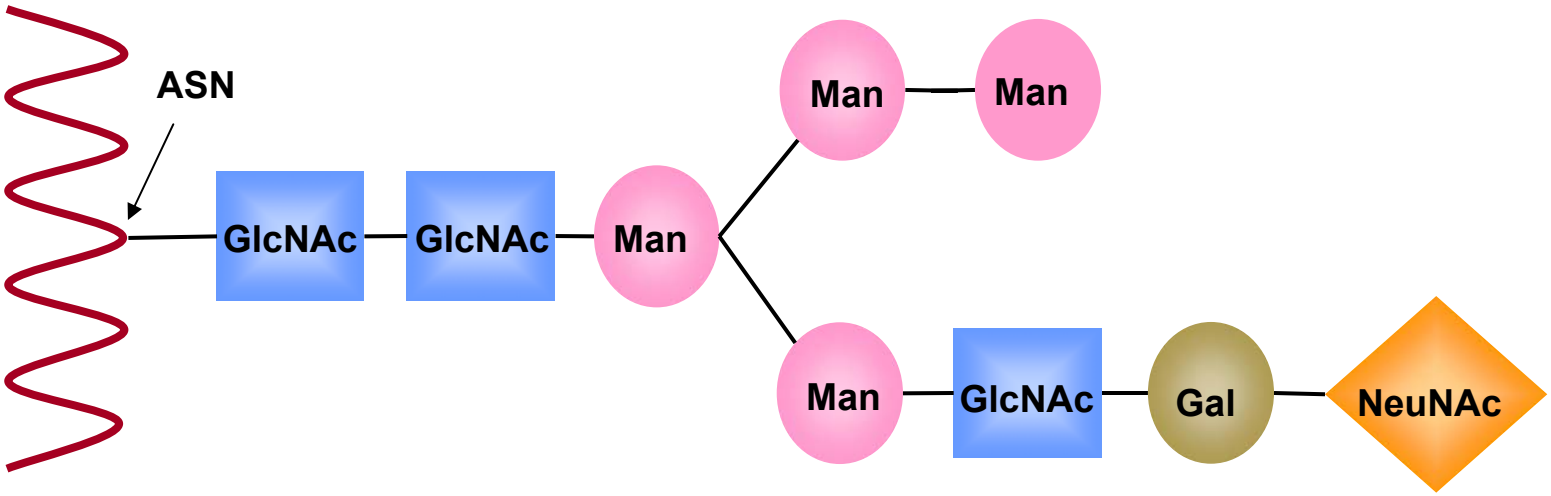
Glycosylation of Proteins

- Glycoprotein : Protein which has a carbohydrate moiety
- Produced through glycosylation process by which a carbohydrate moiety is conjugated to a protein
- Glycosylation is one of the most important post-translational modifications of protein *in vivo*
- Glycan (carbohydrate) plays an essential role in biological function, localization, trafficking, solubility, immuno-genicity
- Glycoprotein accounts for 60 % of therapeutic proteins
  - **Approved : 140**
  - **Clinical trial : 500**

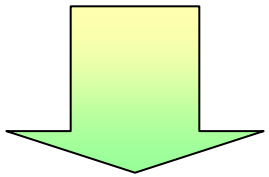
Glycan Profile of Glycoprotein in Humans



ASN : Asparagin residue on protein
GlcNAc : N-Acetylglucosamine
Man : Mannose
Gal : Galactose
NeuNAc : N-Acetylneuramic acid (Sialic acid)

- Detailed glycan profile like oligosaccharide composition and glycosylation degree is crucial for prediction of protein functions

- Most analyses rely on conventional ones involving complex multi-step procedures such as deglycosylation and LC/MS



Rapid and simple method to detect the glycan profile

A Method to Detect Protein Glycosylation based on FRET between Quantum Dots and Gold Nanoparticles

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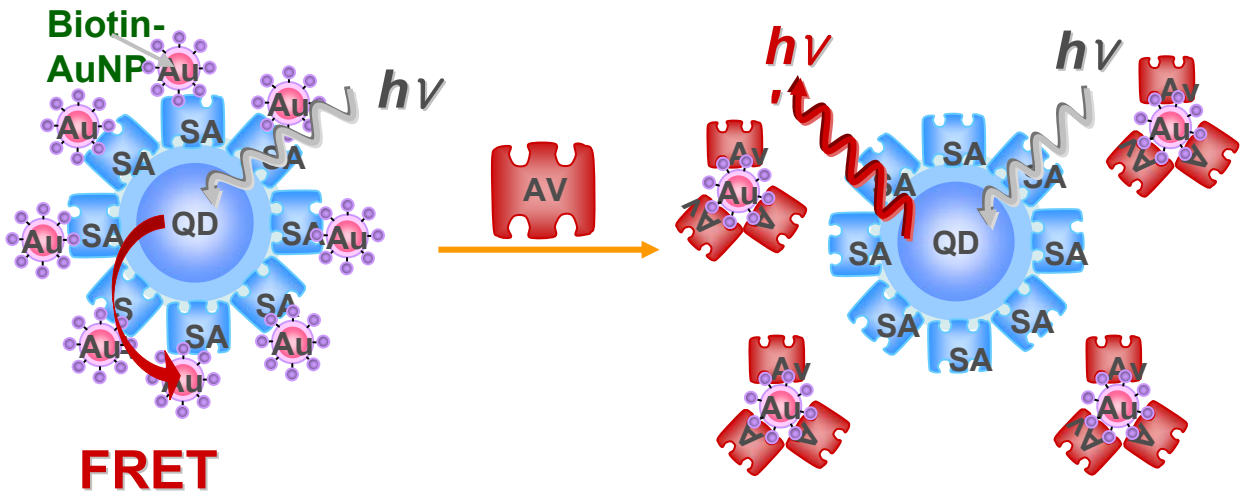
Photoluminence (PL) Quenching of QDs by AuNPs via Foster type FRET

- Model system : Biotin-AuNPs and Streptavidin-QDs
- PL quenching of QDs by AuNPs through Streptavidin-Biotin interaction
- Externally added Avidin prevents the PL quenching of SA-QDs by Biotin-AuNPs → Recovery of the PL intensity of SA-QDs



Oh *et al.*, **JACS, 2005, 127,** 3270 -3271

Interacting Partner : Lectin-Carbohydrate

- **Lectins :** Proteins of nonimmune origin that can recognize and bind to specific carbohydrate structural epitopes
- Over 100 lectins are known

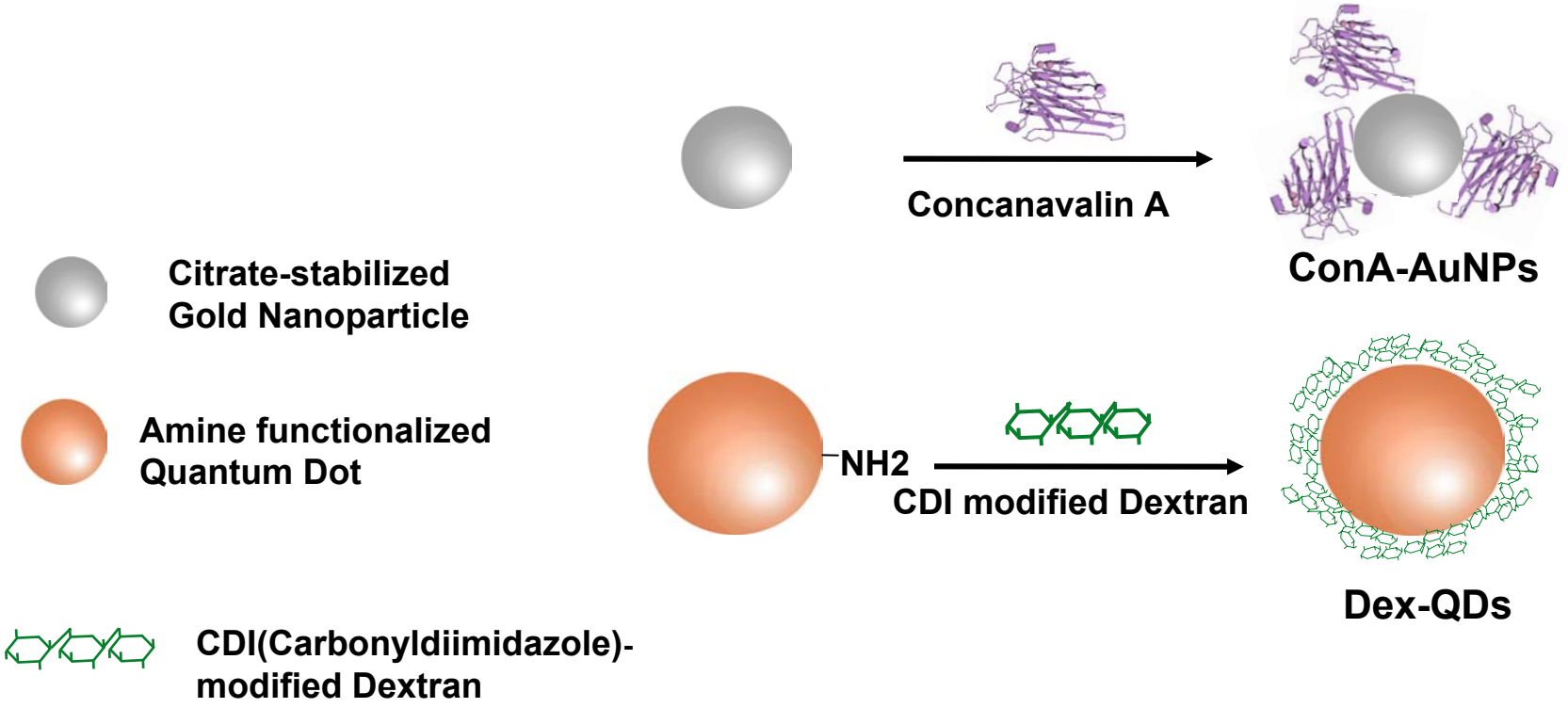
Lectin	Carbohydrates
Concanavalin A	**Manα(1,6), Manα (1,3) Manβ (1,4), Glucose**
Weat Germ Agglutinin	GlcNAc oligomers
Ulex Euripides Agglutinin I	Fucα (1,2)Gal
Ricinus Communis Agglutinin I	Galβ(1,4)GlcNAcb1
Griffonia Simplicifolia Lectin II	GlcNAc on non-reducing terminus
Sambucus Nigra Lectin	Siaα(2,6)Gal/Gal/NAc

Conjugation of Interacting Partners to NPs

Interacting Partner : Concanavalin A - Dextran

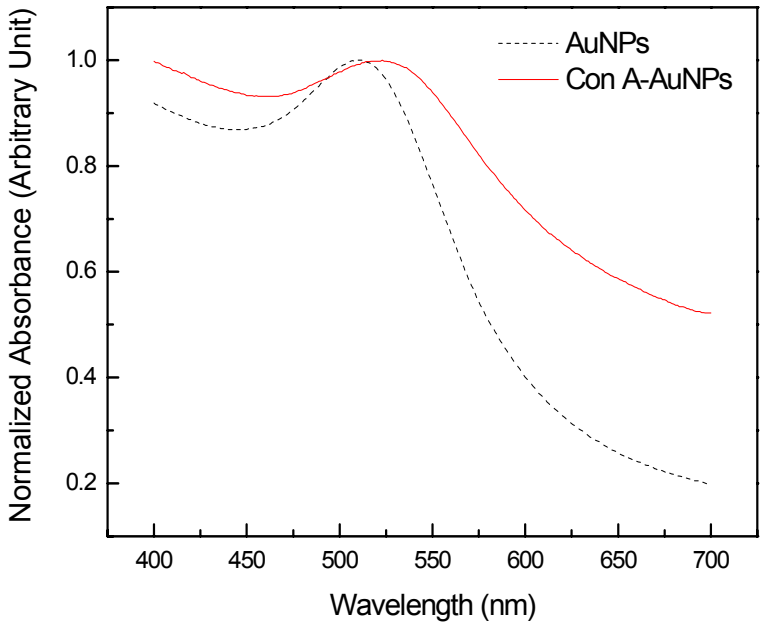
AuNPs : Synthesis by reduction of $HAuCl_4$ in the presence of **c**itrate

QDs : Emission peak at 605 nm

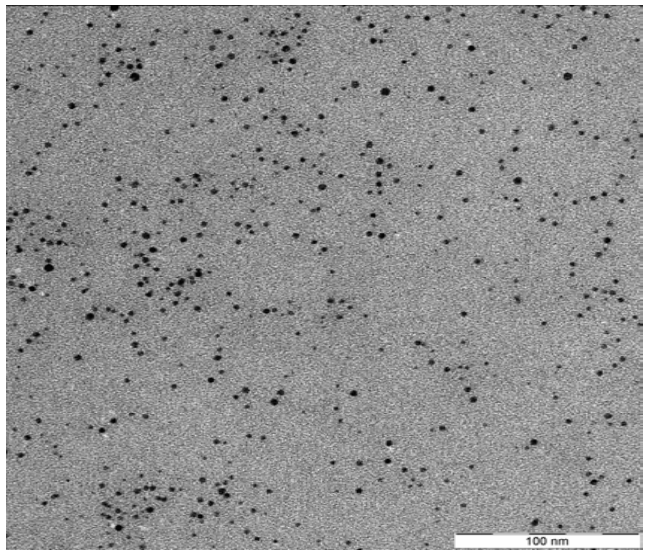


Characterization of Con A-AuNPs

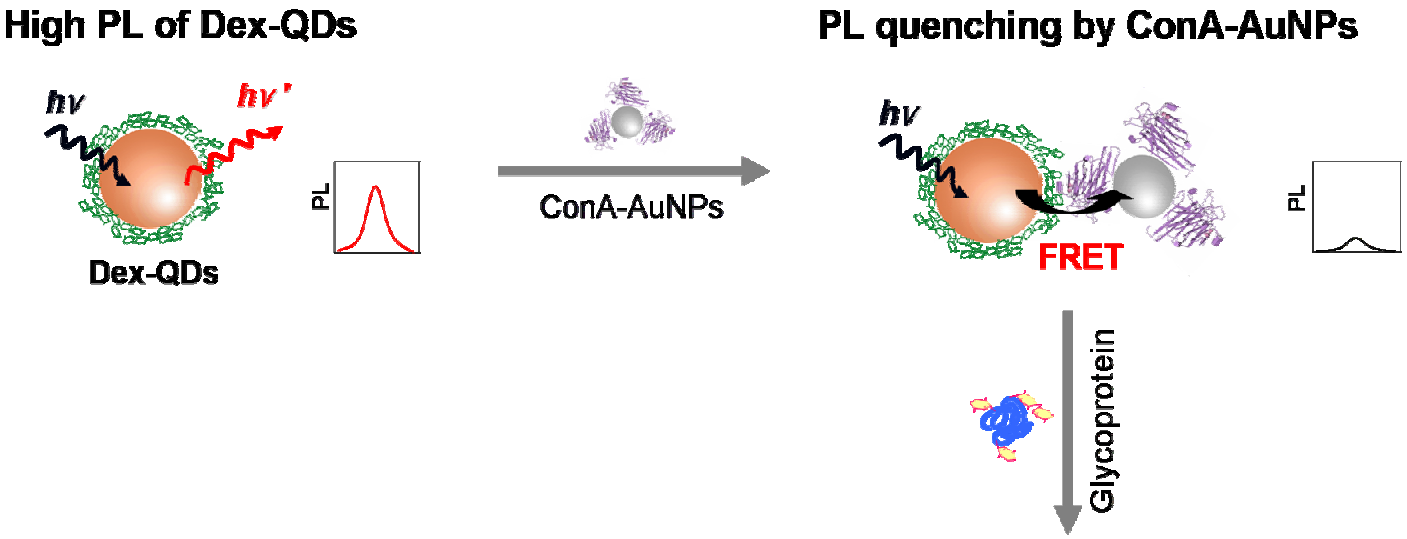
- **Red-Shift & Broad absorption spectrum of Con A - AuNPs**
  → SPB shift after conjugation of Con A
- Size of AuNPs : 3.2 ±0.4 nm (n = 200)
- **No significant aggregation among Con A- AuNPs**



TEM image of ConA -AuNPs



Detection Principle using NPs-based FRET



Modulation of FRET efficiency between Dex-QDs and ConA-AuNPs by glycan moiety or glycosylation degree

PL Quenching of Dex-QDs by ConA-AuNPs

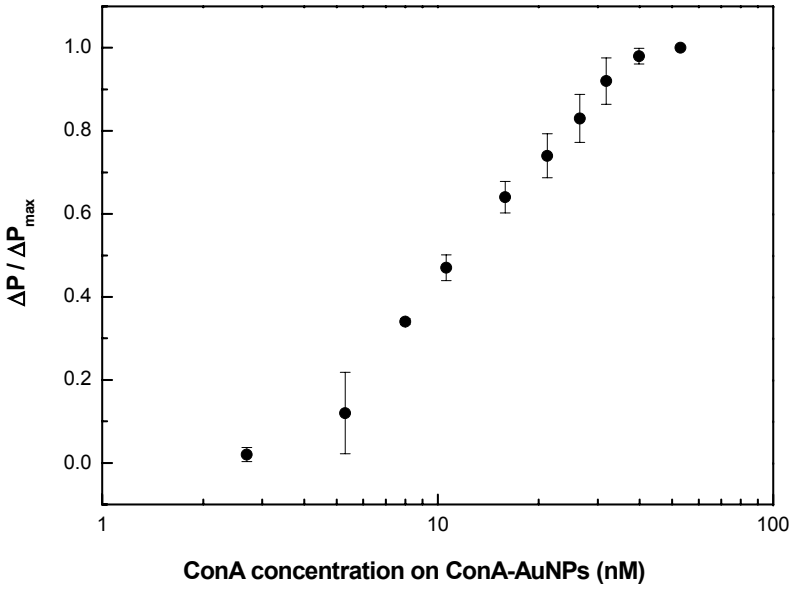
- **PL quenching, ΔP, of Dextran-QDs with respect to the ConA-AuNPs concentration**

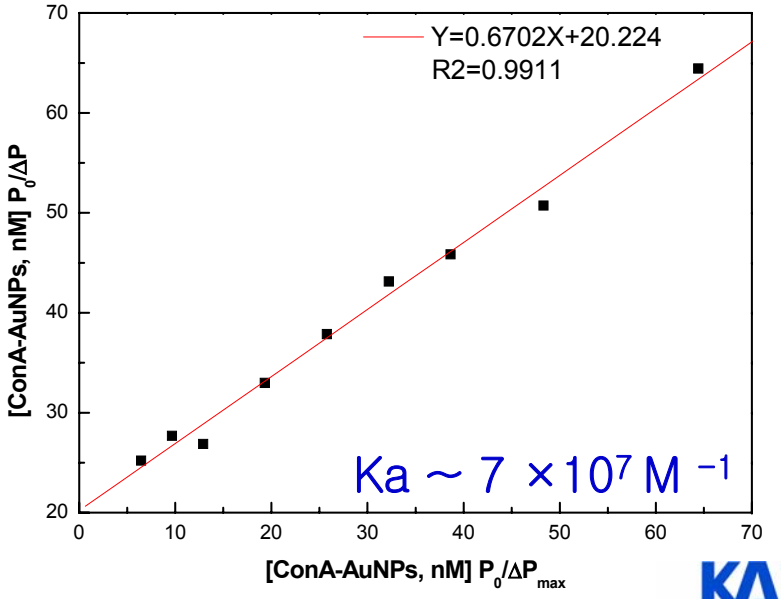
: $\Delta P = P_0 - P$, $\Delta P_{max} = P_{max} - P$

P_0 & P : PL of Dex-QDs before and after addition of Con A-AuNPs

P_{max} : Maximum PL quenching of Dex-QDs at excess concentration of Con A-AuNPs

$$\frac{[conA-AuNPs]P_0}{\Delta P} + \frac{[conA-AuNPs]P_0}{\Delta P_{max}} = \frac{P_0}{\Delta P_{max} K_a}$$





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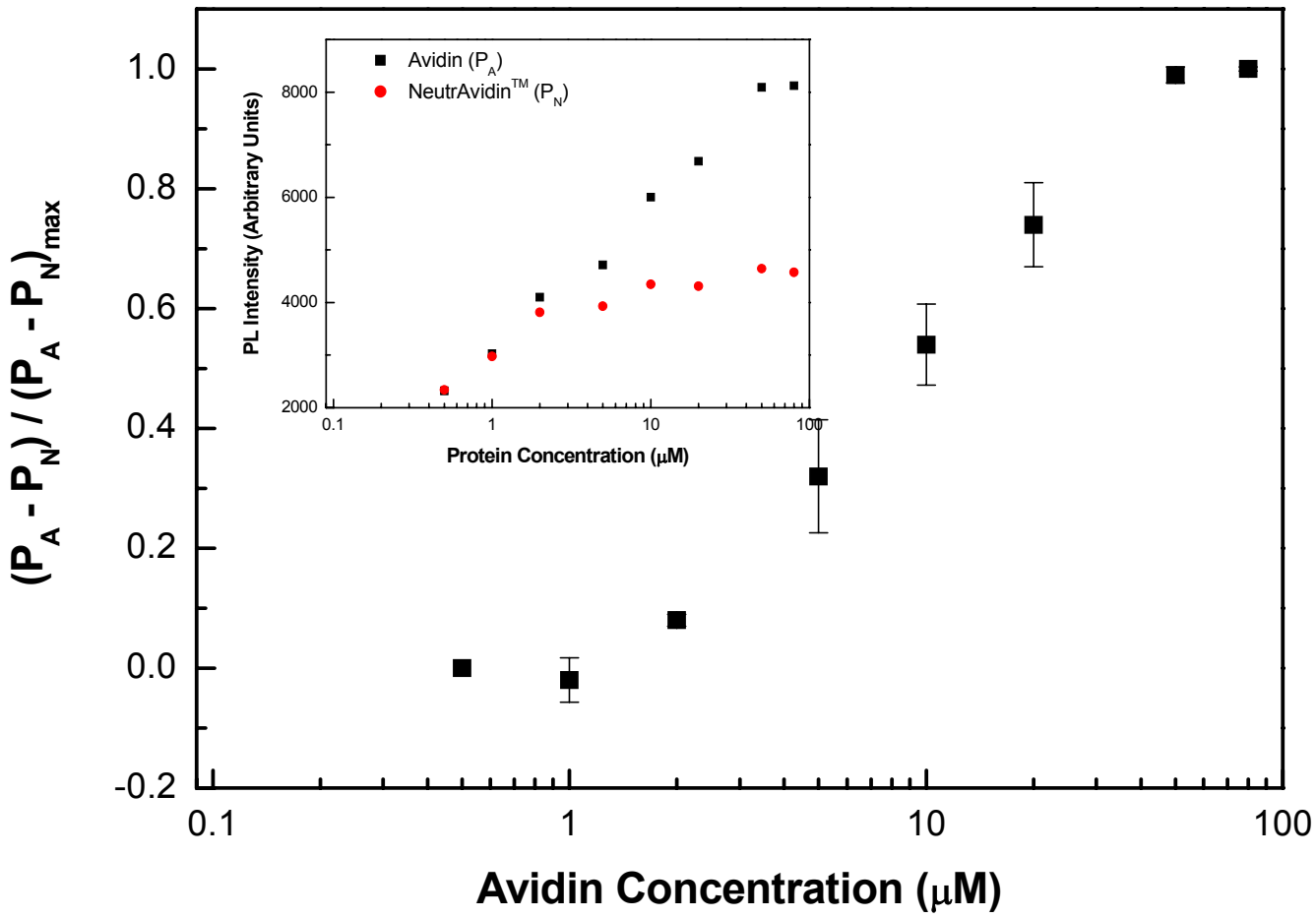
Detection of Glycan Moiety on Intact Protein

- **Avidin**
  - 67.5 kDa (Tetramer)
  - 1 mannose per subunit

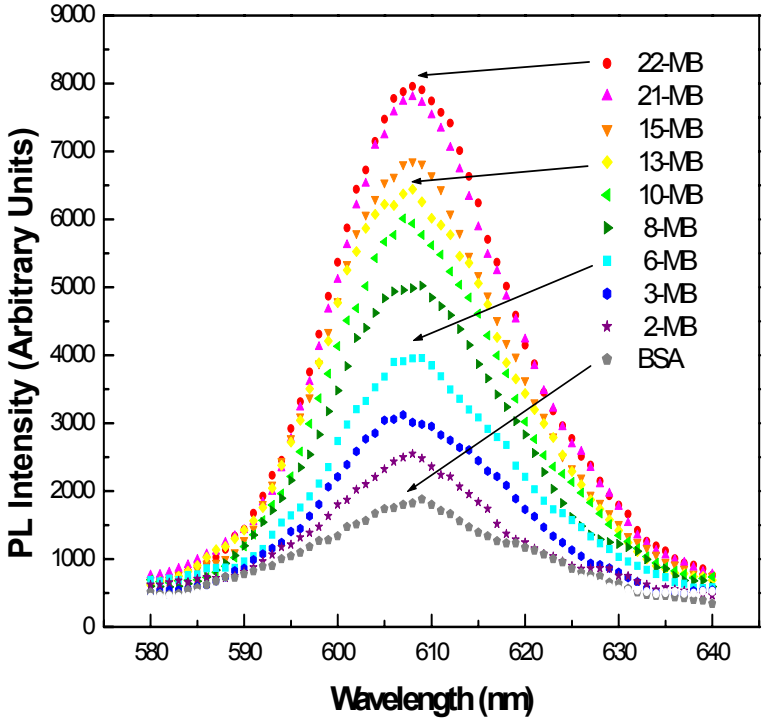
- **NeutrAvidin™; Deglycosylated form**
  - 66 kDa (Tetramer)
  - No capacity to bind to lectins

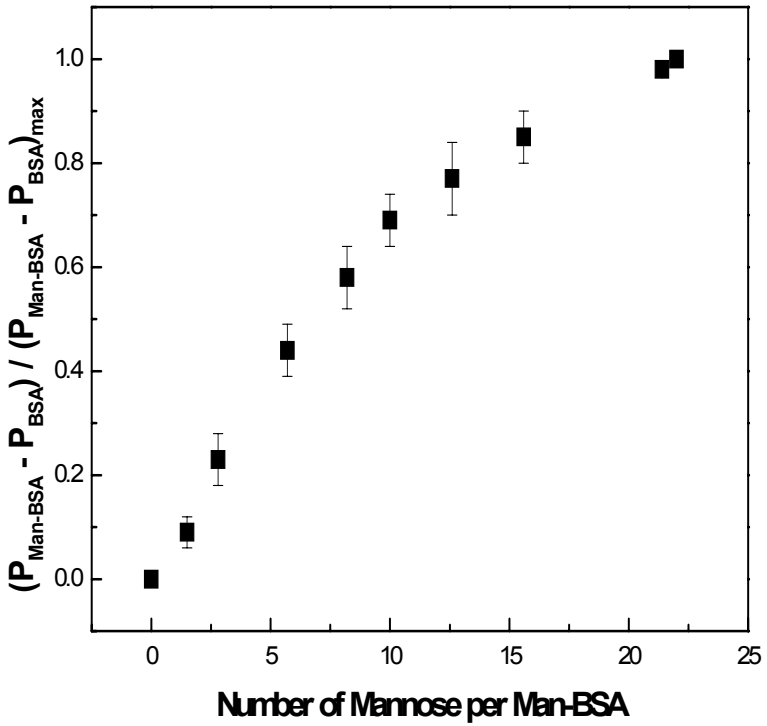
Detection of Glycan Moiety on Avidin



Detection of Protein with Varied Glycosylation Degrees

Conjugation of different numbers of Mannose to BSA by changing the molar ratio between α-D-Mannopyronosylphenyl isothiocyanate and BSA





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Conclusions

- PL quenching of Dex-QDs occurred by ConA-AuNPs through specific interaction between ConA and Dex

- Glycan moiety on intact protein could be rapidly detected by using FRET between Con A-AuNPs and Dex-QDs

- Changes in PL quenching of Dex-QDs were well correlated with the mannosylation degree of BSA

- The use of lectins with preference for diverse carbohydrates might enable analysis of the glycan profile.

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