Glyco 26
August 27-September 1, 2023, Taipei, Taiwan

# Development of an ELISA-based analytical method for the interaction of sugar-binding proteins using sugar-immobilized gold nanoparticles

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**Abstract**

Sugar-binding proteins including lectins and sugar-binding antibodies play significant biological roles such as cell-cell interaction, signal transduction, and immune responses. They also involve in microbial infection and various diseases. Thus, analysis for the interaction of sugar and sugar-binding protein is crucial to understand their functional roles. So far, lectin-array, glycan-array, surface plasmon resonance (SPR), quartz crystal microbalance (QCM), and nuclear magnetic resonance (NMR) techniques have been used for the interaction analysis. However, they need expensive instruments and/or advanced techniques. In this study, in order to develop a facile and high-sensitive analytical method for the interaction, we investigated an ELISA-based analytical method using sugar-immobilized gold nanoparticles (SGNPs). SGNPs can be prepared with a simple method [1]. Furthermore, SGNPs are covered with sugar molecules at high density, and they can enhance the apparent binding affinity of sugars to the sugar-binding proteins through the clustering effect. Therefore, SGNPs are considered to be suitable materials for developing an interaction analytical method for sugar-binding proteins.

SGNPs were prepared according to the method described previously with modifications [1]. β-Galactose (βGal), α-*N*-acetylglucosamine (αGlcNAc), or sialylα2-3galactose (SAα2-3Gal) were co-immobilized with an azido molecule onto GNPs through the linker molecule containing thioctic acid moiety. For modification of the ELISA plate with the alkyne molecule, bovine serum albumin (BSA) was coated and alkynylated by bicyclo[6.1.0]non-4-yn-9-ylmethyl succinimidyl carbonate (BCN-NHS). Then, the azido-functionalized SGNPs were immobilized to the alkynylated BSA on the ELISA plate by a cupper-free cycloaddition (click) reaction. The binding analysis was performed using lectins labeled with biotin. The binding properties of peanut agglutinin (PNA) and wheat germ agglutinin (WGA) were tested by the ELISA-based method. Specific binding interactions of βGal with PNA and αGlcNAc with WGA were observed, respectively. Their detection limits were at least 800 pM. These results demonstrated that our ELISA-based method is a simple and high-sensitive analytical method for investigating the sugar-binding properties of lectins.

**Reference**

1. Nakamura-Tsuruta S., *et al*., One-Step purification of lectins from banana pulp using sugar-immobilized gold nano-particles (SGNPs). *J. Biochem*. **2008**, *143*, 833-839.

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# Enhancement of immunostimulatory activity of CpG oligo deoxynucleotide by conjugation to α-mannose immobilized gold nanoparticles

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**Abstract**

Immunotherapy has become a powerful strategy for treating infectious diseases and cancer, and numerous researchers have been exploring new class of immunostimulatory agents. Agonistic ligands for toll-like receptors (TLRs) that activate innate immunity are promising candidates as adjuvants (immunostimulatory agents) for anti-viral and anti-tumor immunotherapies. Among various TLR ligands, TLR9 ligands, CpG ODNs, are attractive adjuvants because they induce the production of type I interferon, which is important for anti-viral and anti-cancer immune responses. However, TLR9 ligands have several drawbacks such as poor stability and low cell membrane permeability. Previously, we demonstrated that selective delivery of a small molecule TLR7 ligand to immune cells using α-mannose immobilized gold nanoparticles (αMan-GNPs) significantly enhances the efficacy of the small molecule TLR7 ligand [1]. In this study, we examined αMan-GNPs as carriers for CpG ODN and investigated immunostimulatory activities of CpG ODNs and α-mannose co-immobilized GNPs (CpG ODN-αMan-GNPs).

CpG ODN-αMan-GNPs were prepared according to the method described previously [1]. CpG ODN immobilized on GNPs was quantified using QuantiFluor ssDNA system after degradation of GNPs by potassium cyanide. α-Mannose moiety on GNPs was quantified by HPLC analysis of fluorescent-labeled mannose obtained from hydrolysis of CpG ODN-αMan-GNPs with trifluoroacetic acid subsequent reductive amination with 4-aminobenzoic acid ethyl ester. The molar ratio of CpG ODN and α-mannose on GNPs was approximately consistent with the molar ratio in preparation. In vitro study in mouse bone marrow-derived dendritic cells demonstrated that CpG ODN-αMan-GNPs have higher immunostimulatory activities compared to unconjugated CpG ODN. In vivo adjuvant effect was tested in C57BL/6 mice using ovalbumin (OVA) as a model antigen. The immunization study demonstrated that CpG ODN-αMan-GNPs enhanced more than 20-fold for producing OVA-specific IgG2c antibody compared to unconjugated CpG ODN. These results indicate that the αMan-GNPs are superior carriers of CpG ODN to enhance immunostimulatory activities.

**Reference**

1. Shinchi, H., Yamaguchi, T., et al., Bioconjugate Chem., 2019, 30(11), 2811-2821.

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WCTE2023 - World Conference on Timber Engineering
19-22 June 2023, Oslo, Norway.

# BURNING TEST OF TIMBER-STEEL BAR COMPOSITE BEAM

Shizuka Matsushita[1], Shinichi Shioya[1]

**ABSTRACT**

From a viewpoint of the current climate crisis, there has been much recent interest in using timber structural members in large buildings because timber can be used as renewable natural resource, and moreover, in severe earthquake prone zones, such as Japan, they are more desired on the grounds of light weight of timber members. We are developing a frame system consisting of hybrid timber members reinforced with deformed steel bars (i.e. rebars) using epoxy resin adhesive. In order to practice the system, it is necessary to investigate fire resistance performance of the members. This paper reports a burning test of a beam burned for 60-minute semi-fireproof using burning marginal layer (charring layer).

**SUMMARIES**

i)Detailed strain measurements of the wood and rebar within the specimen showed that rebar and wood in the same position within beam subjected bending distort with same strain value, providing a basis for the assumption of plane section within the beam's cross-section.

ii)Bending stiffness calculated based on assumption of the plane section within beam subjected to bending and using Young's modulus in bending of each lamina within beam specimen could estimate accurately moment-curvature relationship of the beam and changes in strain of wood and steel bars within the beam. Deflection stiffness of the beam was also accurately estimated by adding shear deformation to bending deformation component calculated from bending stiffness.

(a) *Specimen before the burning test*

(b) *Specimen immediately after burning test*

(c) *Specimen cooled with water*

***Photo 1:*** *Specimen*

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# A NEW METHOD FOR MODELLING TEMPERATURE WITHIN TIMBER STEEL BAR COMPOSITE BEAM USING DATA BY COMBAUSTION TEST

Shizuka Matsushita[1], Shinichi Shioya[1]

**ABSTRACT**

In light of the current climate crisis, there has been much recent interest in using timber structural members in large buildings, since timber is as renewable natural resource, and moreover, in severe earthquake prone zones, such as Japan, they are more desired on the grounds of light weight of timber members. We are developing a frame system formed by hybrid timber members reinforced with deformed steel bars (i.e. rebars) using epoxy resin adhesive. In order to practice the system, it is necessary to investigate fire resistance performance of the members. This paper proposes a method for modelling temperature within beam specimen by using finite data obtained by formal specified combustion test for approval of Japan government. Distributions of bending stress and shearing stress within the beam obtained by using the temperature and the reduction ratio of strength and elasticity of wood stiffness are described.

**SUMMARIES**

The results are summarized as the followings:

A method was proposed to estimate temperature distribution in uncharred area of wood in glulam timber for any burning time within 60 minutes from the measured data of wood temperatures at limited locations in glulam timber, which reproduces the curve of the relationship between temperature at measuring point and burning time, showing temperature distribution in the cross-section during 60-minute burning.

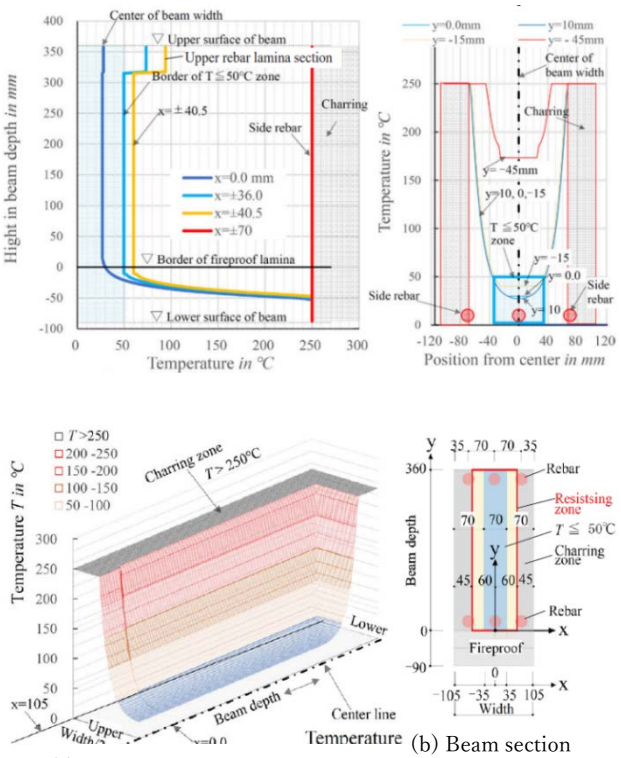


***Figure 1***: Temperature distribution of wood in beam

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# EXPERIMENTON AXIAL CAPACTY-BENDING CAPACITY RELATIONSHIP OF STEEL BAR-TIMBER COMPOSITE COLUMN

Keisuke Hayata [1], Motohiro Muka[1], Shinich Shioya[1]

**ABSTRACT**

In light of the current climate crisis, there has been much recent interest in using timber structural members in large buildings, because timber is as renewable natural resource, and moreover, in severe earthquake prone, such as Japan, they are more desired on the grounds of light weight of timber members. We are developing a frame system formed by timber members reinforced by deformed steel bars, i.e., rebars using epoxy resin adhesive and have already developed a technique for the connection between column of ground floor and reinforced concrete foundation. Performance of the column was reported in the previous WCTE2021. We have planned an experiment to investigate bending characteristic of the other portion except hinge of the column bottom. This paper reports the experiment, its results, and comparison of experiment result and calculation on bending moment capacity.

**SUMMARIES**

In order to develop estimation method of yield moment and bending capacity for the steel bar-timber composite column subjected to axial compression and bending moment for column of story above second story in building, compression test and bend test of column, and bend loading test of Glulam timber beam were conducted by specimens scaled as 25%.

Formula for estimation of bending yield moment and bending capacity of column with increasing axial force were proposed, and the correlation curve by using the formulas and tensile properties of lamina estimated accurately experimental data. By using standard values of Glulam timber, the curve estimated the experimental data on the safe side.

***Photo 1:*** *Set-up for loading*

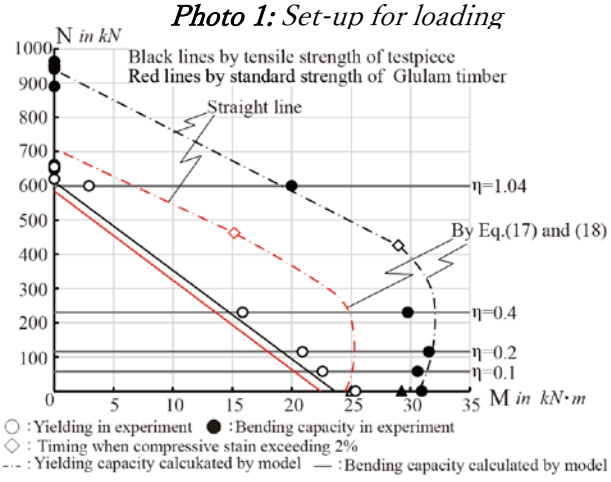


***Figure 1:*** *Axial forth-bending capacity correlation curve*

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# EFFECTS OF ELEVATED TEMPERATURE ON BENDING CAPCITY OF STEEL BAR-TIMBER COMPOSITE BEAM

Tsukasa Ueno[1], Nao Matsuoka[1], Shinichi Shioya[1]

**ABSTRACT**

We have been developing a rigid frame system formed by timber members strengthened by deformed steel bars using epoxy resin adhesive. Buildings may often suffer from elevated temperature in Summer , such as 45.3℃ ever reported in Japan or approximately 65℃ in the attic space. On the other hand, when buildings suffer fire, the composite members also be required to resist against Dead load by using fireproof coating or burning marginal layer ( i.e. charring layer). The resisting portion may be mainly lower than 100℃. We planned an experiment to reveal effects of those elevated temperatures on bending capacity of the composite beam. This paper reports the test, its results and discussion.

**SUMMARIES**

Bending test of beams under high temperature with uncontrolled moisture absorption and dehumidification of wood were conducted to investigate effect of temperature rising on mechanical properties of the steel bar-timber composite beam.

Bending stiffness and bending capacity of the composite beam could be estimated from room temperature to 70°C, by the proposed estimation method considering the decrease in Young's modulus and bending strength of timber due to temperature rising, ignoring internal stress caused by the temperature.

Yield moment of the composite beam should take into account effect of the internal stress in addition to decrease in mechanical performance of timber owing to temperature rising. In the future, we plan to develop a method for estimating the internal stress on safe side.

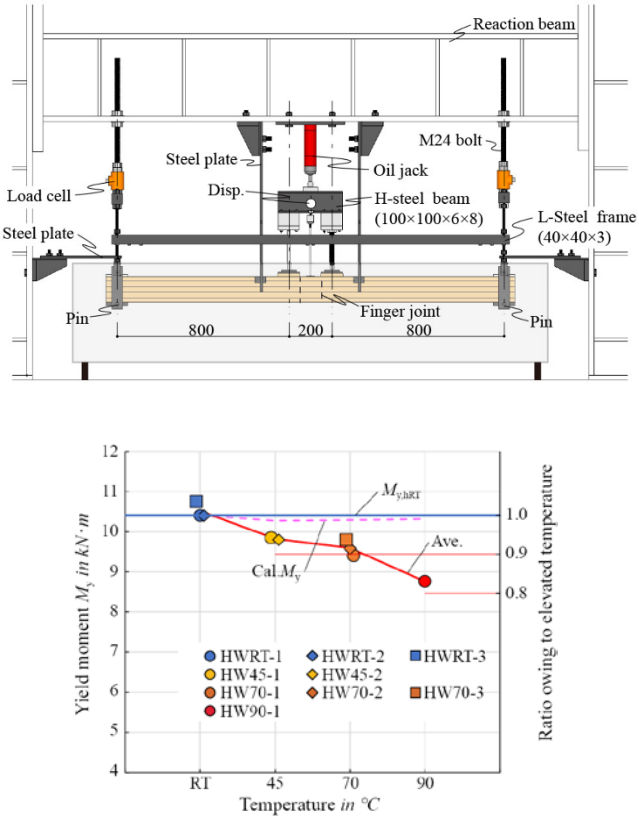


***Figure 2:*** *Yield moment/$M_y$ owing to elevated temperature*

and ratio of $M_y$ at elevated temperature to $M_{y,RT}$ at

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The 11th China-Japan Bilateral Symposium on High Temperature Strength of Materials
October 27-31, 2023, Chengdu, China

# Development of Small Disk Bending Fatigue Testing Technique

Kotaro Murakami[1], Shin-ich Komazaki[1], Masayuki Kamaya[2], Chiaki Hisaka[3] and Akito Nitta[4]

**Abstract**

The bending fatigue test using a small disk-type specimen ($\phi$8×0.3t mm) was newly developed in this study.

Figure 1 shows the changes in pressure and strain measured on the specimen surface during the test for which the maximum pressure was 0.6 MPa and frequency was 2 Hz. The output of the strain gauge successfully followed the change in pressure.

Figure 2 shows the pressure plotted as a function number of cycles, which were measured during the tests at the maximum pressures of 0.35, 0.4, 0.6, 0.7 MPa. The pressures were abruptly dropped due to fracture at all test conditions, and the number of cycles to this sudden drop in pressure increased with decreasing applied pressure.

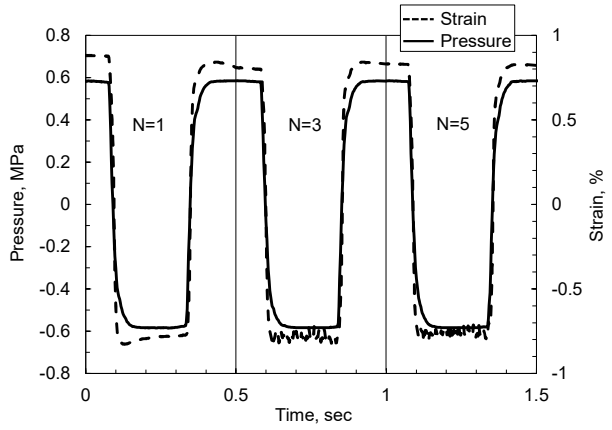


Fig. 1 Changes in pressure and measured strain.

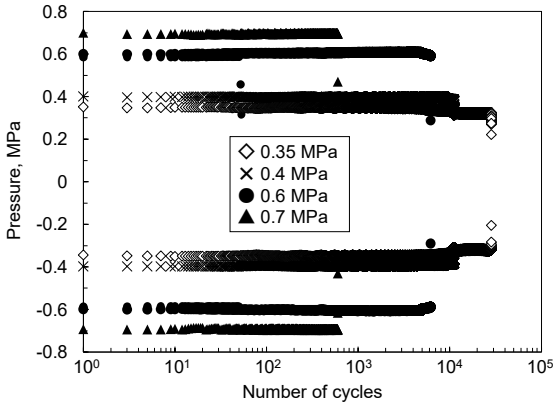


Fig. 2 Pressure plotted as a function number of cycles.

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October 27-31, 2023, Chengdu, China

# Basic Study on Evaluation of Mechanical Properties of Alloy 718 Additive Manufacturing Product Using SP Test

Kai Matsumoto[1], Masami Tanaka[2], Kotaro Murakami[1],
Ping Li[2], Masato Mikami[2] and Shin-ich Komazaki[1]

**Abstract**

Wrought and additive manufactured (AM) Ni-based superalloys, Alloy 718, were subjected to the small punch (SP) test to investigate the suitability of SP testing technique for deriving tensile based properties of AM produced parts. The relationship between the uniaxial test results and the SP test results allows the derivation of Equations (1) and (2).

$$\sigma_y = 0.403\ F_{y\_t/10}/h_0^2 \quad (1)$$

$$\sigma_B = 0.258\ F_m/(u_m \cdot h_0) \quad (2)$$

$F_{y\_t/10}$ is the crossing point between the load-displacement curve and a straight line parallel to the initial slope of the graph with an offset displacement of $t/10$. $h_0$ is initial thickness of specimen. $F_m$ is the maximum load and $u_m$ is the displacement at the maximum load. The $\sigma_y$ and $\sigma_B$ of the AM alloys were assessed using Eqs. (1) and (2) based on the SP test results.

The results are plotted against the strengths measured by the tensile test in Figure 1 along with those of wrought alloys. It can be clearly seen that the strengths of AM alloys predicted by the SP test are relatively in good agreement with those measured by the tensile test as well as the wrought alloys.

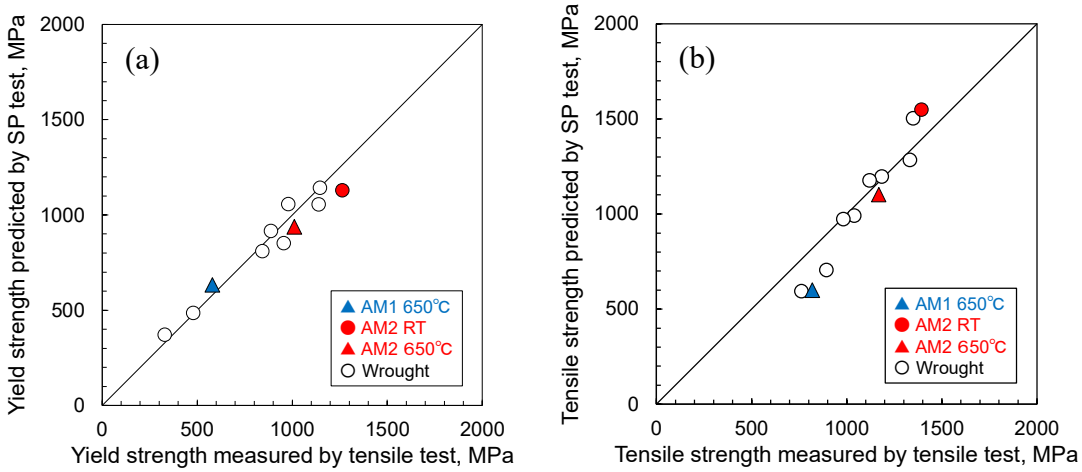


Fig. 1 Comparisons between results of uniaxial tensile and SP tests.

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Hyderabad, India, 19-22 Nov. 2023, pp. 276-279

# High-Precision Open-Loop Time Amplifier Using Current Regulator

Yusuke Toyoshima, Ryosuke Kamiya, Kenichi Ohhata

**Abstract**

In this paper, we propose a high-precision open-loop time amplifier (TA) that utilizes a current regulator. In the current regulator, the $V_{TH}$ variation does not cause the current variation; therefore, the gain variation due to device mismatches can be reduced. Moreover, a pulse width extended switching scheme ensures that the current pulse reaches a desired current level completely even when the input is small. We performed simulations in 28-nm CMOS technology to evaluate the performance of the proposed TA. The results showed that the maximum gain error and the standard deviation of the error were 2.3% and 0.7%, respectively. Moreover, even when the input is small, gain error does not increase, and the proposed TA can amplify up to 1 ps input with high accuracy.

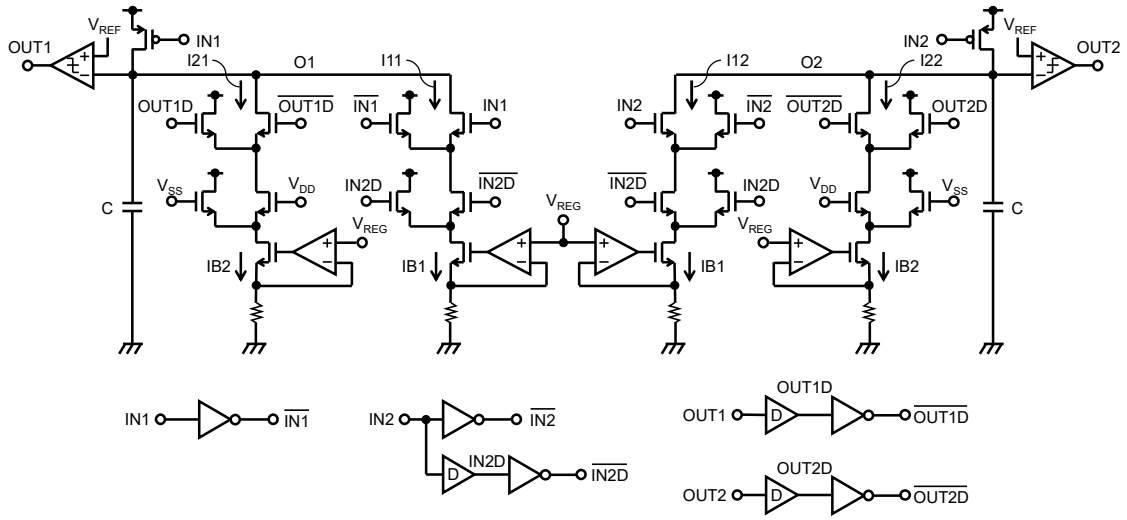


Fig. 3 Schematic of proposed TA.

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