TRANSACTIONS OF
K.C. WONG EDUCATION FOUNDATION
SUPPORTED LECTURES

王宽诚教育基金会

学术讲座汇编

主编 罗宏杰

36
2014

上海大学出版社

王宽诚教育基金会

学术讲座汇编

（第36集）

主编　罗宏杰

上海大学出版社

·上海·

图书在版编目(CIP)数据

王宽诚教育基金会学术讲座汇编. 第36集/罗宏杰主编. —上海：上海大学出版社，2014.5
ISBN 978-7-5671-1238-4

Ⅰ.①王… Ⅱ.①罗… Ⅲ.①社会科学-文集 ②自然科学-文集 Ⅳ.①Z427

中国版本图书馆CIP数据核字(2014)第049381号

责任编辑　姜春明
封面设计　施羲雯
技术编辑　金　鑫　章　斐

王宽诚教育基金会
学术讲座汇编(第36集)
罗宏杰　主编
上海大学出版社出版发行
(上海市上大路99号　邮政编码200444)
(http://www.shangdapress.com　发行热线021-66135112)
出版人：郭纯生
*
南京展望文化发展有限公司排版
上海华业装潢印刷厂印刷　　各地新华书店经销
开本787×1092　1/16　印张15.5　字数　367千字
2014年6月第1版　2014年6月第1次印刷
印数：1~780
ISBN 978-7-5671-1238-4/Z·068

惠　存

王宽诚教育基金会敬赠

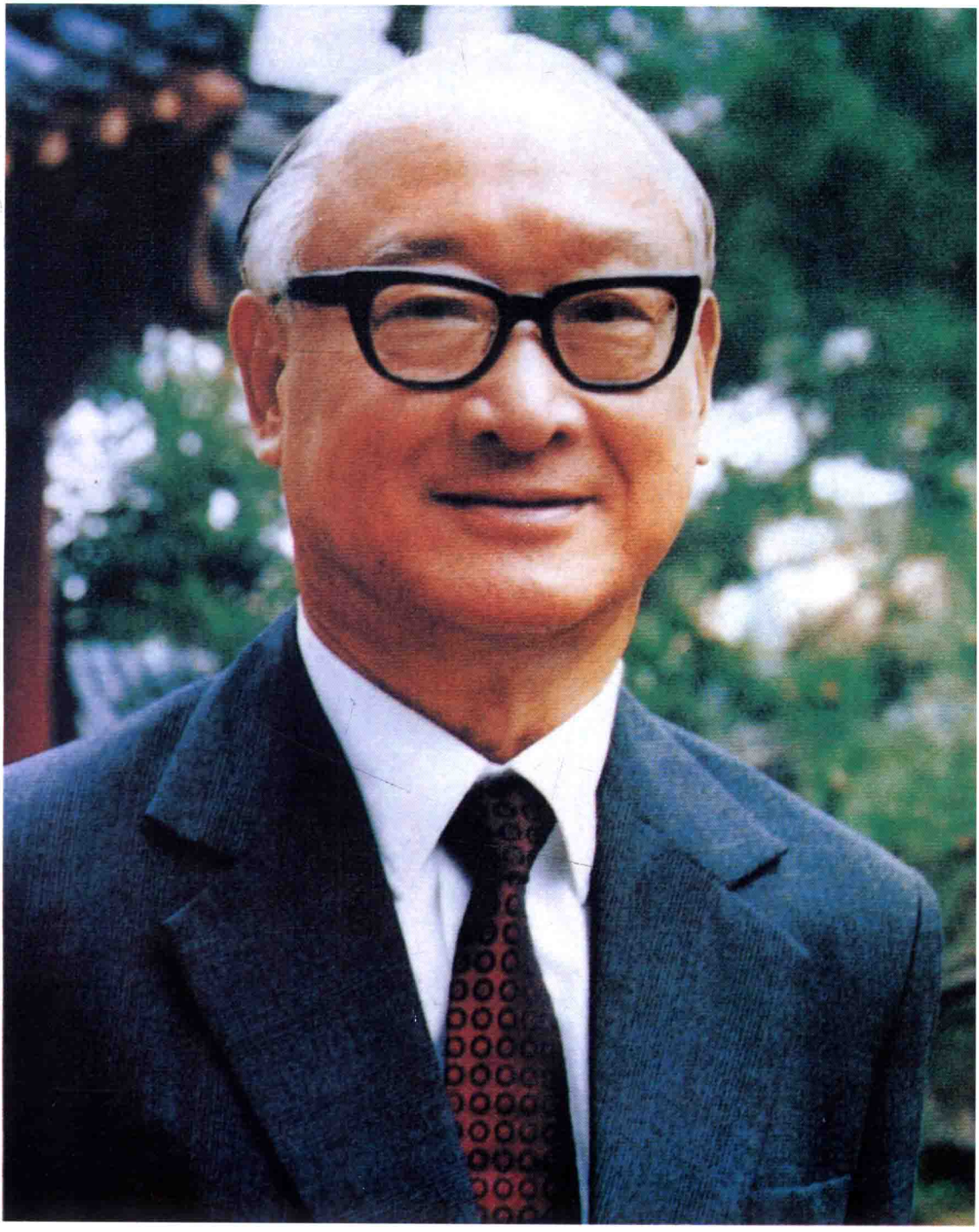
2014 年 5 月

谨以此书纪念本会创建人、故董事会主席王宽诚先生

王宽诚教育基金会

DEDICATED TO THE MEMORY OF MR. K. C. WONG, FOUNDER OF THE FOUNDATION AND THE LATE CHAIRMAN OF THE BOARD OF DIRECTORS

K. C. WONG EDUCATION FOUNDATION



王宽诚先生

K.C.WONG(1907—1986)

王宽诚教育基金会简介

王宽诚先生(1907—1986)为香港著名爱国人士,热心祖国教育事业,生前为故乡宁波的教育事业作出积极贡献。1985 年独立捐巨资创建王宽诚教育基金会,其宗旨在于为国家培养高级技术人才,为祖国四个现代化效力。

王宽诚先生在世时聘请海内外著名学者担任基金会考选委员会和学务委员会委员,共商大计,确定采用"送出去"和"请进来"的方针,为国家培养各科专门人才,提高内地和港澳高等院校的教学水平,资助学术界人士互访以促进中外文化交流。在此方针指导下,1985、1986 两年,基金会在国家教委支持下,选派学生 85 名前往英、美、加拿大、德国、瑞士和澳大利亚各国攻读博士学位,并计划资助内地学者赴港澳讲学,资助港澳学者到内地讲学,资助美国学者来国内讲学。正当基金会事业初具规模、蓬勃发展之时,王宽诚先生一病不起,于 1986 年年底逝世。这是基金会的重大损失,共事同仁,无不深切怀念,不胜惋惜。

1987 年起,王宽诚教育基金会继承王宽诚先生为国家培养高级技术人才的遗愿,继续对中国内地、台湾及港澳学者出国攻读博士学位、博士后研究及学术交流提供资助。委请国家教育部、中国科学院和上海大学校长钱伟长教授等逐年安排资助学术交流的项目。相继与(英国)皇家学会、法国科研中心、德国学术交流中心、法国高等科学研究院等著名欧洲学术机构合作,设立"王宽诚(英国)皇家学会奖学金"、"王宽诚法国科研中心奖学金"、"王宽诚德国学术交流中心奖学金"、"王宽诚法国高等科学研究院奖学金",资助具有副教授或同等职称以上的中国内地学者前往英国、法国、德国等地的高等学府及科研机构进行为期 2 至 12 个月之博士后研究。

王宽诚教育基金会过去和现在的工作态度一贯以王宽诚先生倡导的"公正"二字为守则,谅今后基金会亦将秉此行事,奉行不辍,借此王宽诚教育基金会《学术讲座汇编》出版之际,特简明介绍如上。王宽诚教育基金会日常工作繁忙,基金会各位董事均不辞劳累,作出积极贡献。

前　言

王宽诚教育基金会是由已故全国政协常委、香港著名工商企业家王宽诚先生(1907—1986)出于爱国热忱，出资一亿美元于1985年在香港注册登记创立的。

1987年，基金会开设“学术讲座”项目，此项目由当时的全国政协委员、历任第六、七、八、九届全国政协副主席、著名科学家、中国科学院院士、上海大学校长、王宽诚教育基金会贷款留学生考选委员会主任委员兼学务委员会主任委员钱伟长教授主持。由钱伟长教授亲自起草设立“学术讲座”的规定，资助内地学者前往香港、澳门讲学，资助美国学者来中国讲学，资助港澳学者前来内地讲学，用以促进中外学术交流，提高内地及港澳高等院校的教学质量。

本汇编收集的文章，均系各地学者在“学术讲座”活动中的讲稿，文章内容有科学技术，有历史文化，有经济专论，有文学，有宗教和中国古籍研究等。本汇编涉及的学术领域颇为广泛，而每篇文章都有一定的深度和广度，分期分册以《王宽诚教育基金会学术讲座汇编》的名义出版，并无偿分送国内外部分高等院校、科研机构和图书馆，以广流传。

王宽诚教育基金会除资助“学术讲座”学者进行学术交流之外，还资助由国内有关高等院校推荐的学者前往欧、美、亚、澳等参加国际学术会议，出访的学者均向所出席的会议提交论文，这些论文亦颇有水平，本汇编亦将其收入，以供参考。

王宽诚教育基金会学务委员会

凡　例

（一）编排次序

本书所收集的王宽诚教育基金会学术讲座的讲稿及由王宽诚教育基金会资助学者赴欧、美、亚、澳等参加国际学术会议的论文均按照文稿日期先后或文稿内容编排刊列，不分类别。

（二）分期分册出版并作简明介绍

因文稿较多，为求便于携带，有利阅读与检索，故分期分册出版，每册150页至240页不等。为便于读者查考，每篇学术讲座的讲稿均注明作者姓名、学位、职务、讲学日期、地点、访问院校名称。内地及港、澳学者到欧、美、澳及亚洲的国家和地区参加国际学术会议的论文均注明学者姓名、参加会议的名称、时间、地点和推荐的单位。上述两类文章均注明由王宽诚教育基金会资助字样。

（三）文字种类

本书为学术性文章汇编，均以学术讲座学者之讲稿原稿或参加国际学术会议者向会议提交的论文原稿文字为准，原讲稿或论文是中文的，即以中文刊出，原讲稿或论文是外文的，仍以外文刊出。

目 录 CONTENTS

王宽诚教育基金会
《学术讲座汇编》第36集,1~11(2014)

Fabrication of Nanoporous Metal Materials for Biosensing Applications

HUANG Xi-rong *

(Key Laboratory of Colloid and Interface Chemistry of the Ministry of Education of China, Shandong University, Jinan 250100, P. R. China)

Abstract: Nanoporous metal materials such as nanoporous gold (NPG) and nanoporous copper (NPC) are fabricated by two methods. One is the dealloying of alloys. During the dealloying, the active metal is leached out and the left noble one will self-diffuse and aggregate, forming pores and ligaments. For example, NPG can be obtained by dealloying Au/Ag alloy. The other is the electrochemical treatment of bulk pure metals, i. e., square-wave potential perturbation. During the treatment, a repeated electrochemical oxidation/reduction made the metal atoms removable. At the same time, the produced H_2 bubbles would act as a template for the formation of pores. For the former one, the microstructure (pore and/or ligament size) of NPG was tuned by changing the ratio of alloy components, dealloying time, or post-treatment. For the later one, the microstructure of the nanoporous film was adjusted by changing the treatment time, the applied potential step, frequency, and the size of the H_2 bubbles, etc. The NPG exhibited an excellent electrocatalytic activity towards the oxidation of glucose, ascorbic acid and dopamine; while the NPC had an excellent catalytic effect on the electroreduction of nitrate and H_2O_2. Based on these properties, several electrochemical sensors with high selectivity and sensitivity have been developed. The obtained nanoporous metals were also used as carrier for the immobilization of enzymes. In addition to the microstructures of NPG and NPC, the effects of the immobilization strategies including physical adsorption, electrostatic attraction and covalent coupling, on the enzymatic properties were investigated. Both NPG and NPC were demonstrated to be good substrates for enzymes. For laccase, an efficient direct electron transfer phenomenon was observed on NPG. Based on the electrocatalytic activity of NPC towards the reduction of the oxidized aniline and the biocatalytic activity of horseradish peroxidase on NPC by adsorption, an

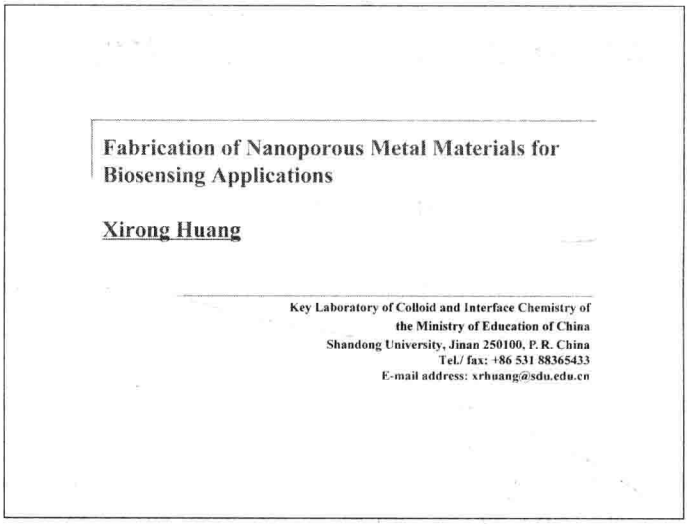
---

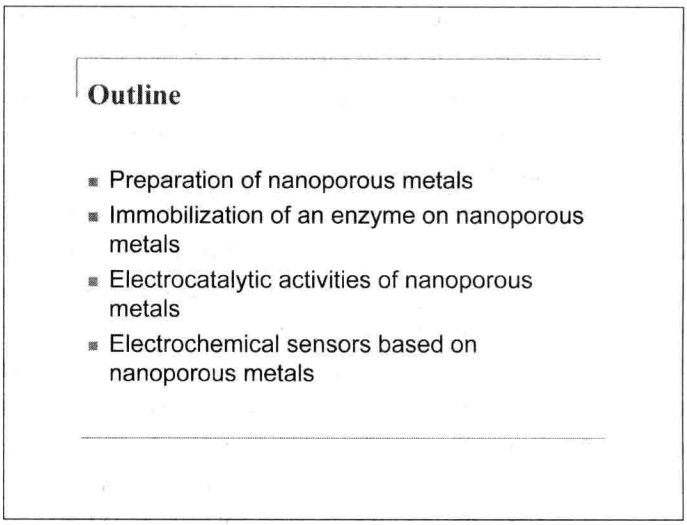
* 黄锡荣,教授,山东大学化学与化工学院。由王宽诚教育基金会资助,于2011年5月赴芬兰图尔库参加"国际电化学会第九届春季会议",此为其向大会递交的论文。

electrochemical sensor for aniline has been developed. Similarly, an electrochemical biosensor for ethanol or glucose was also developed on the basis of an enzyme-modified NPG; its high performance was due to the good biocatalytic activity of alcohol dehydrogenase or glucose oxidase on NPG and the electrocatalytic activity of NPG towards the oxidation of NADH or H_2O_2.

Key words: Nanoporous metal, electrocatalysis, biosensor

本讲座内容以 PPT 格式演示。





Required Features of Electrode Material for Electrochemical Sensors

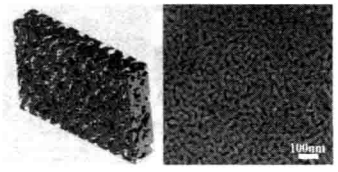
- Conductive
- Large surface area
- Catalytic
- Monolithic
- …

Free standing nanoporous metal materials
with stable but tunable quasiperiodic structure should be a good candidate

Preparation Methods of Nanoporous Metals(NPG)—dealloying

- During the dealloying, the active metal is leached out and the left noble one will self-diffuse and aggregate, forming pores and ligaments.
- The microstructure (pore and/or ligament size) of NPM can be tuned by changing the ratio of alloy components, dealloying time, or post-treatment.

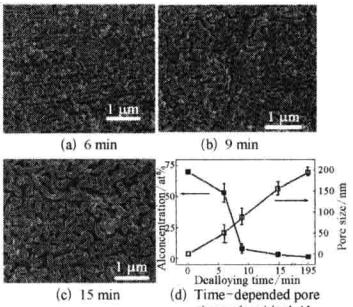
Preparation of Nanoporous Gold (NPG) by Immersing Au/Ag Alloy in Concentrated HNO_3



Quantitative TEM tomography (superimposed layers)

SEM image

SEM Images of Nanoporous Silver (NPS) from Ag/Al Alloy at Different Dealloying Time in 2.5 wt.% HCl



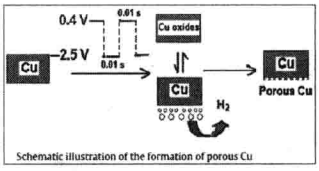
(a) 6 min (b) 9 min

(c) 15 min (d) Time-depended pore size and residual Al

Preparation of Nanoporous Metals by Potential Perturbation

- During the treatment, a repeated electrochemical oxidation/reduction made the metal atoms removable. At the same time, the produced bubbles (H_2) would act as a template for the formation of pores.
- The microstructure of the nanoporous metal film was adjusted by changing the treatment time, the applied potential step, frequency, and the size of the bubbles, etc.

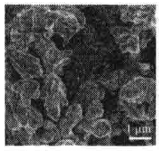
Preparation of Nanoporous Copper (NPC) by Potential Perturbation



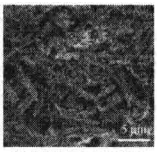
Schematic illustration of the formation of porous Cu

In 2.0 mol/L NaOH at 50 Hz. At 0.4 V, Cu is oxidized. When shifted to −2.5 V, Cu(II) oxide is quickly reduced to Cu atoms, which are removable, resulting in the reconstruction of the surface lattice structure of Cu via the incorporation and removal of O species and via H_2 bubbles releasing.

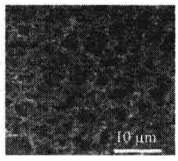
Structure Evolution of NPC with Treatment Time



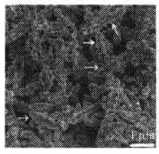
(a) SEM image at 500 s



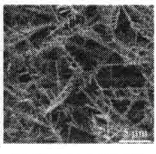
(c) SEM image at 5,000 s



(e) SEM image at 5,000 s



(b) SEM image at 3,000 s

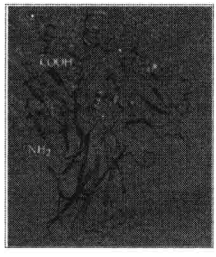


(d) SEM image at 10,000 s

Immobilization of Enzymes on Nanoporous Metals

- Investigate the interaction between an enzyme and its carrier
- Enhance the stability and reusability of an enzyme
- Fabricate biosensors and biofuel cells

Laccase Immobilization



The ribbon diagram of laccase

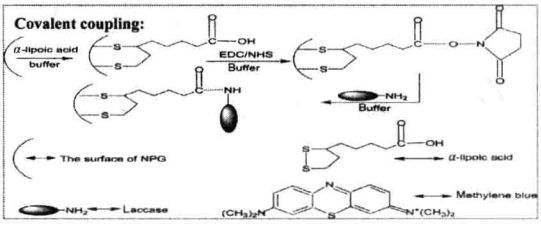
- Contained 4 copper ions classified into three types(T1,T2,T3)
- Catalyze the oxidation of phenolic compounds & aromatic amines

Three Immobilization Strategies

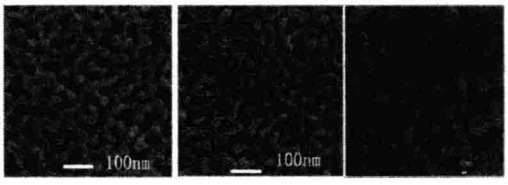
Physical adsorption: immersing NPG in a laccase solution for 24 h at 4℃

Electrostatic attraction: NPG surface was first modified with positively-charged methylene blue, then immersed it in laccase solution for 24 h. (the isoelectrtic point is 3.5; the optimal pH is 4.4)

Covalent coupling:



SEM Images of NPG Before and After Laccase Immobilization



(a) Bare NPG (b) Laccse-NPG (c) Laccse-NPG

After immobilization, the pore size of laccase-NPG became smaller and the ligament less brightness.

Enzyme Loading and Specific Activity on NPG with a Pore Size of 40-50 nm

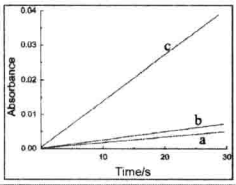
The amount of laccase loaded on NPG and the specific activity of the immobilized enzyme[a]

Immobilization strategies	Amount of laccase immobilized/ ($mg \cdot g^{-1}$)	Specific activity/ (U/mg of protein)
Covalent coupling	16.0	0.83
Electrostatic attraction	8.2	0.8
Physical adsorption	15.5	0.81

[a] Each data was an average of three replicate determinations.

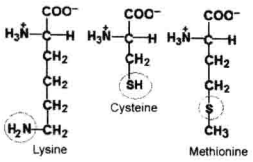
* Some chemical adsorption occurred during the so-called physical adsorption process, resulting in an almost equal amount of laccase immobilized.
* An electrostatic repulsion between immobilized laccases made the dense packing of the enzyme impossible using the electrostatic attraction strategy.
* Similar specific activities indicate the enzyme molecules have similar configuration.

Leaching Test Based on Laccase Catalyzed DMP Oxidation at 470 nm



a: covalent binding;
b: physical adsorption;
c: electrostatic attraction.
The larger the slope, the more the leached laccase.

Why is the physical adsorption stable?



Effect of the Pore Size of NPG on the Immobilization of Laccase

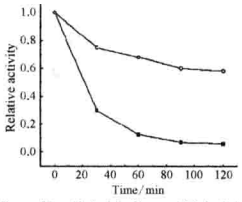
Changes of the amount of immobilized laccase and the specific activity with the pore size of NPG[a]

No.	Pore size/nm	Specific surface area/($m^2 \cdot g^{-1}$)	Amount of immobilized laccase/($mg \cdot g^{-1}$)	Specific activity/ (U/(mg of protein))
Sample 1	10–20	20.9 ± 0.2	7.2 ± 0.3	0.43
Sample 2	40–50	14.4 ± 0.2	15.5 ± 0.3	0.79
Sample 3	90–100	8.7 ± 0.2	10.6 ± 0.3	0.81

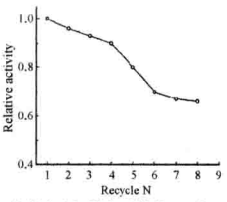
[a] NPG with thickness of 25 μm

- Laccase activity was spectrophotometrically determined using DMP as substrate at 470 nm at 30° C.
- One unit of activity was defined as the amount of enzyme required to have 1 μmol DMP oxidized in 1 min.
- The size of laccase was around 7 nm in diameter.

Thermal Stability and Reusability of Laccase-NPG



Changes of the residual activity of laccase with the incubation time at 50℃: -○-, immobilized enzyme; -■-, free enzyme.



Residual activity of the immobilized laccase after repeated uses at 30℃ and pH 4.4.

Recycle of the carrier NPG:

The denatured laccase on NPG could be easily removed simply by immersing the NPG into concentrated nitric acid (ca. 1 min).