Using elastin protein to develop one-dimensional nanomaterials for energy storage

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NANYANG
TECHNOLOGICAL
UNIVERSITY

**USING ELASTIN PROTEIN TO DEVELOP ONE-
DIMENSIONAL NANOMATERIALS FOR ENERGY
STORAGE**

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SCHOOL OF MATERIALS SCIENCE AND ENGINEERING

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GUO GUILUE

SCHOOL OF MATERIALS SCIENCE AND ENGINEERING

A thesis submitted to the Nanyang Technological University
in partial fulfillment of the requirement for the degree of
Doctor of Philosophy

2016

Statement of Originality

I hereby certify that the work embodied in this thesis is the result of original research and has not been submitted for a higher degree to any other University or Institution.

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Abstract

In this thesis, elastin-like protein (ELP) GPG-AG3-Histidine tag (His tag), is designed for control synthesis of nanomaterials. The hydrophobic domain (GPG domain) is responsible for self-assembling and the hydrophilic domains (AG3 and His tag) are capable of mineralizing inorganic materials. It is hypothesized that by combining these components into a fusion protein, control synthesis of one-dimensional (1D) inorganic materials can be realized in an effective and efficient way.

1D noble metals and transition metal-nitrogen-carbon (M-N/C) catalysts are effective in improving the electrochemical kinetics of lithium-O₂ battery (LOBs) cathodes to increase the battery efficiencies as well as cycle life. 1D structure with rich and uniformly distributed active catalytic centers can confine the size of generated Li₂O₂ during the discharge by facilitating rapid electron transport and surface electrochemical reactions. By using these 1D structures as catalysts for LOBs cathodes, the over-potential phenomena are to be suppressed by tuning the deposition and distribution behavior of Li₂O₂ on the catalyst surface.

Transition metal phosphides (TMPs) are very competitive high-capacity candidates for lithium and sodium storage yet they suffer the low electric conductivities and the drastic volume change during the conversion reaction process. The construction of TMPs into nanoscale materials, especially into 1D structures such as nanotubes or nanorods is most promising strategy to address these issues.

The self-assembling behaviors of the GPG-AG3-His tag proteins are revealed and nanobeads or nanofibers are obtained. In inorganic materials synthesis, mineralization capability of AG3 in the fusion protein is firstly investigated. 1D Pt or Pd composed of the platinum nanoparticle and palladium nanoparticle decorated on the protein fibers are obtained through hydrothermal treatment at 80 °C. Next, the mineralization behavior of His tag is investigated. 1D metal-nitrogen/carbon (M-N/C, M=Fe, Co) catalysts are synthesized by mixing the protein nanobeads, metal salt precursors and carbon nanotubes

(CNTs) in aqueous solution followed by annealing and acid leaching processes. The mineralization capability of His tag in oil-phase synthesis is then explored and 1D CoP and FeP₄ are obtained. All these results demonstrate that the strategy proposed in this dissertation is effective and the fusion protein designed is versatile for application in 1D inorganic materials synthesis.

To consolidate the studies, these 1D nanomaterials are applied in secondary batteries. The catalytic performance of 1D Pt and 1D Pd in LOBs are investigated. The results show that the 1D Pt catalysts-based cathodes have typical charge potentials at 3.8 V at 100 mA /g. The discharge capacities maintain at the capacity cut-off at 1000 mAh/g for 15 cycles and the energy efficiency is 65%. The charge voltages of the Pd nanotube catalyst maximize at about 4.2 V with an average of 3.9 V. An energy efficiency of 63% with a capacity of 800 mAh/g is obtained at 50 mA/g. The obtained M-N/C (M=Fe, Co) catalysts are also used as cathode materials in LOBs. It is discovered that during discharge, Li₂O₂ nanoparticles first nucleate and grow around the bead-decorated CNTs regions (M-N/C centers) and coat on the catalysts at high degree of discharge. The Fe-N/C catalysts based cathode delivers a capacity of 12441 mAh/g at 100 mA/g. When cycled at a limited capacity of 800 mAh/g at 200 or 400 mA/g, these cathodes show stable charge voltages of ~3.65 or 3.90 V, corresponding to energy efficiencies of ~ 71.2% or 65.1%, respectively. Finally, 1D CoP nanostructure is applied as anode material in lithium ion batteries (LIBs) and sodium ion batteries (SIBs). For lithium storage, the anode delivers a specific capacity as high as ~ 350 mAh/g at 3.0 C over 200 cycles and shows good capacity retention. In sodium storage, when tested at 0.1 C, the delivered capacities are stable at ~ 520 mAh/g for 100 cycles. At 1.0, 2.0, and 5.0 C, the specific capacities are stable at ~ 400, 360, and 300 mAh/g over 250, 250, and 1000 cycles, respectively.

It is of significance to study the mineralization capabilities of each functional sequence in the GPG-AG3-His tag fusion protein simultaneously since they are aligned together. Besides, further discussions of constructing novel functional fusion proteins for constructing hierarchical structure and their applications in energy storage are also presented.

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Abbreviations

ELP: Elastin-Like Protein

His tag: Histidine Tag

E.Coli: Escherichia Coli

M-N/C: Metal-Nitrogen/Carbon

TMPs : Transition metal Phosphides

CNTs: Carbon Nanotubes

PVDF: Polyvinylidene Fluoride

NMP: N-Methyl-2-Pyrrolidone

TEGDME: Tetraethylene Glycol Dimethyl Ether

LOBs : Lithium-O₂ Batteries

LIBs : Lithium-Ion Batteries

SIBs: Sodium-Ion Batteries

1D: One Dimensional

NPs: Nanoparticles

SCs: Supercapacitors

EVs: Electric Vehicles

ORR: Oxygen Reduction Reaction

OER: Oxygen Evolution Reaction

SDS-PAGE: Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

XRD: X-Ray Diffraction

SEM: Scanning Electron Microscopy

FESEM: Field-Emission Scanning Electron Microscopy

EDS: Energy Dispersive Spectroscopy

TEM: Transition Electron Microscopy

SAED: Selected-Area Electron Diffraction

XPS: X-ray Photoelectron Spectroscopy

NMR: Nuclear Magnetic Resonance

CV: Cyclic Voltammetry

FTIR: Fourier Transform Infrared Spectroscopy

AFM: Atomic Force Microscopy

ESA: Electrochemical Surface Area

Chapter 1

Introduction

In this chapter, an overall research background concerning the development of nanotechnology is stated followed by the introduction of the hypothesis of this thesis which aims at the synthesis of one-dimensional inorganic nanomaterials using multifunction fusion protein as starting materials. The motivations as well as the objectives and scopes of this thesis are emphasized. The organization of this thesis is also illustrated.

1.1 Background and Problem Statement

Nanotechnology, which investigates and engineers materials at the scale of 10^{-9} m (nm), has become advanced tools in providing new perspectives and opportunities on material research over the past three decades. [1, 2] Nanotechnology is popular in a variety of research and industrial areas from manufacturing, consumer products, and medicine to textiles. [3] Usually nanoscience and nanotechnology manipulates functional materials with at least one characteristic dimension measured in the scale of 1-200 nm. Due to such tiny size, nanomaterials are believed to present advancing properties compared to the bulk counterpart. [4, 5] Thus, through scaling down bulk materials to nanoscale, the hindrances faced by the conventional bulk material can partially be mitigated, e.g. by tailoring bulk materials into microscale or even nanoscale, the materials present enhanced electronic conductivity, mechanical stability, and kinetics which have enable them new applications. [5-7] By tuning the sizes or dimensions of nanoscale materials, it allows scientists and engineers to expand the range of performance and applications of those already clearly-studied chemicals and materials. [8, 9] Furthermore, by aligning various molecules in an organized pattern on substrate surfaces to form self-assembled monolayers, new generations of chemical and biological sensors are introduced for more accurate detections. [10, 11] It is also noted that to achieve these goals and realize all the efficient as well as effective applications, it is necessary to design material sizes at the atomic or molecular scales or nanometer scales where the properties, phenomena and processes are initiated to tune the fundamental properties of nanomaterials even though a lot of difficulties are to be addressed. Therefore, it is of significance to obtain novel insights and knowledge, create and adopt new synthetic and characterization tools as well as try new methods to synthesize functional nanomaterials. [12-14]

The research focus on nanoscience has been shifting over the past three decades. When nanotechnology was first introduced, efforts were devoted to discovering new materials, novel phenomena and developing new characterization tools. [15] When some basic science of nanoscale is clear, fabrication of nanoscale devices with abundant

properties and usage are provoked. [16] In addition, recommendations to increase support of commercialization efforts including nonmanufacturing and increased efforts in nanoscience education are suggested. Recently the perspective view of environmental impact and safety issues for nanomaterials and the understanding the societal consequences of nanotechnology are promoted. [17, 18]

Nanotechnology has provided us with new insight and technology about materials and devices that already and will still impact various fields in the future. Even though investigations on the potential of commercialization and the significant influence on local and global economies of nanotechnology are receiving increased attention, synthesis and constructions of nanomaterials is still one of the most active aspects in this field.[15-18] At present, while there are numerous ways and approaches available for synthesizing nanomaterials with novel characteristics, challenges still exist in the synthesis of nanomaterials, especially those with desired and controlled properties in large amounts to meet the requirements of industrial applications. [19, 20] Therefore, new synthetic techniques that can allow nanometer scale control of material building blocks, which can further be used for manufacturing nanomaterials or devices with multi-functional properties for various applications, are highly desirable.

Biological materials are rich in binding sites for nucleation and growth of inorganic metal ions or can further stabilize inorganic materials through electrostatic interaction or coordination between their functional groups located on their polysaccharides, proteins or DNA components and the metal ions. [21-23] These properties, along with their unique shapes and spatial organization, enable them to be attractive for nanomaterial synthesis. For example, synthesis of nanostructure materials, using wire- or rod-shape viruses, microorganisms or bioinspired self-assembled polypeptides have been demonstrated as effective and efficient. [24-26] In the second chapter of this thesis the concepts of biomineralization and nanomaterial synthesis techniques inspired from this natural mineralization process will be presented with more details.

1.2 Motivations, Objectives and Scopes

(a) Motivations

The biomineralization process has provided us new insights into material synthesis. The development in designing self-assembling peptides or fusion protein with recognition capabilities greatly expands this scope.

The elastin-mimetic double hydrophobic proteins, GPG [GPG, the amino acid domain sequence is $(VGGVG)_5-(VPGXG)_{25}-(VGGVG)_5$ where X is any amino acid except proline] to be modified in this dissertation is discovered to undergo a self-assembling process upon increasing temperature to form nanofibers.

AG3 is a dodecapeptide (amino acid sequence: AYSSGAPPMPPF) screened from combinational phage display peptide library. Several research works have been done on this peptides and it has been proved to be capable of reducing chloraurate ($[AuCl_4]^{3-}$) to gold nanoparticles or Ag^+ to Ag^0 under suitable conditions.

His tags is an amino acid motif in recombinant proteins and consists of at least six histidine (His) residues, often at the N- or C-terminus of the fusion protein. It is also known as hexa histidine-tag, 6xHis-tag or His6 tag and by the trademarked name His-tag (registered by EMD Biosciences). His tags are often used for affinity purification of polyhistidine-tagged recombinant proteins expressed in *Escherichia coli* (E.Coli). It strongly binds to transition metal ions such as Fe^{2+} , Co^{2+} and Ni^{2+} .

This thesis thus aims at coupling self-assembly proteins (GPG) with peptide sequences with recognition/mineralization capabilities mentioned above (AG3 and His tag) together for application in designed nanomaterials synthesis.

(b) Objective and Scope

The construction of bioinspired materials (which is GPG-AG3-His tag) and investigation of the application in noble metal, metal-nitrogen/carbon (M-N/C) catalysts and transition metal phosphides (TMPs) nanostructure synthesis and their application in energy storage devices is the major objective of this thesis.

In this design, the AG3 sequence is attached to the C-terminus of GPG through genetic-engineered methods to yield GPG-AG3-Histidine tag protein, which could serve as the starting materials for synthesizing and organizing one-dimensional (1D) nanomaterials. The preparation of fusion protein nanofibers and the fabrication of noble metal nanostructures, such as Pt or Pd, which are similar as Au or Ag, are investigated and applied in Lithium-O₂ batteries (LOBs).

Besides the above 1D noble metal nanomaterials synthesis, M-N/C (M=Fe, Co) catalysts are also synthesized by using the GPG-AG3 elastin protein beads with transition metal salt precursors and CNTs. The GPG-AG3 elastin protein beads are the products in the early stage of self-assembling. The M-N/C catalysts are applied as efficient binder free LOBs cathodes.

Also, as in the fibrous protein templates the hydrophilic sequences (which are AG3 and His tags) are at the exterior in which His tags can strongly interact with transition metal ions, they provide opportunity in transition metal phosphide and transition metal oxide synthesis. Thus, the preparation of 1D cobalt oxide, nickel oxide and cobalt phosphide (CoP) as well as iron phosphide (FeP₄) is prepared using solution-phase methods. As a demonstration, the 1D CoP is applied as high performance lithium ion batteries (LIBs) and Sodium ion batteries (SIBs) anodes.

1.3 Dissertation Overview

The thesis organization is divided into the following sections:

Chapter 1 briefly introduces the important role of nanotechnology acts in science and technology and the research effort devoted to developing novel techniques inspired from natural biomineralization to achieve control synthesis of nanomaterials. Based on this research justification, the motivation, objectives and scopes of the thesis are established, which aim at establishing the strategies to synthesize one dimensional nanomaterials including noble metal, metal oxides, M-N/C catalysts and TMPs using genetically-engineered elastin fusion protein with multifunctional mineralization domains as starting materials.

Chapter 2 is the literature review of the topics covered in this thesis. Firstly, an overview on the concept of bimoneralization, the developmental history and fundamentals of biotemplates in nanomaterials is presented in detail. Secondary, the importance of one dimensional nanomaterials and their state-of-art synthesis methods are discussed. Lastly, brief history of LOBs and SIBs are covered, as the 1D noble metal, metal oxides, M-N/C catalysts and TMPs based on genetically-engineered self-assembling fusion protein are investigated as electrode materials for LOBs or SIBs (and LIBs).

Chapter 3 introduces the material synthesis methods, the characterization techniques that are adopted in completing this dissertation.

Chapter 4 includes the expression and characterization of GPG-AG3-His tag fusion protein as well as the investigation of their self-assembling behaviors.

Chapter 5 presents the mineralization capability of AG3 domain in the fusion protein and the successful application of self-assembling fusion protein fibers as starting materials for carbon-support Pt nanotubes and Pd nanotubes synthesis via mild hydrothermal conditions at 80 °C. The catalytic activity of the as-synthesized noble metal nanotubes are investigated in O₂ cathode catalysts of LOBs.

Chapter 6 presents the application of GPG-AG3-His tag beads evolved in the initial stage of the self-assembling process in M-N/C catalysts (M= Fe, Co) synthesis by utilizing the special affinity between His tags with transition metal ions. The as-synthesized M-N/C catalysts are applied as binder-free O₂ cathodes materials for LOBs.

Chapter 7 explores the synthesis of 1D carbon-supported TMPs (M=Co, Fe) using fibrous self-assembling GPG-AG3-His tag as starting materials. The amphiphilic nature of the fibrous templates enables the successfully synthesis of 1D cobalt phosphide (CoP) and iron phosphide (FeP₄) in oil-phase system. As a demonstration, the 1D CoP are applied as anode materials for LIBs and SIBs and shows much improved lithium and sodium storage capability.

Chapter 8 summarizes the work of the previous chapters and some preliminary results and recommendations for future work are presented.

1.4 Findings and Outcomes/Originality

In this thesis, elastin-mimetic amphiphilic fusion protein, GPG-AG3-His tag, containing the amino acid sequence (VGGVG)₅-(VPGXG)₂₅-(VGGVG)₅-AYSSGAPPMPPF-HHHHHH, have been expressed and firstly applied in 1D inorganic nanomaterial synthesis which include noble metal (Pt and Pd), M-N/C (M=Fe, Co) catalysts and TMPs (CoP and FeP₄). These inorganic nanostructures have been demonstrated to be effective in energy storage applications such as LOBs, LIBs and SIBs. The works presented in this thesis have demonstrated that by constructing fusion protein with multiple functional domains, synthesis of nanomaterials with desired and controlled properties with reduced environmental consequences and cost in large amounts to meet the requirements of industrial applications can be achieved. Therefore, this novel synthetic technique which allows nanoscale control of material building blocks, which can be applied in synthesis nanomaterials or devices with multi-functional properties for a various applications are highly desirable.

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Chapter 2

Literature Review

This chapter mainly focuses on three aspects. The first aspect concerns the advancements of synthesizing inorganic materials through biomineralization processes over the past three decades. The second aspect briefly presents the advantages and potential applications of one-dimensional nanomaterials in catalysis and energy storage devices. The last aspect introduces the fundamentals of electrochemistry. The principles of the lithium-O₂ batteries and sodium-ion batteries and the significance of both are also included.

2.1 Overview

There is always a strong research interest for developing efficient and reliable processes to synthesis nanomaterials which can minimize or even eliminate the use of hazardous chemicals or harsh conditions which usually required by conventional physical and chemical methods. The only way to achieve these goals is to adopt novel synthesis approaches that are carried out under mild reaction conditions with non-toxic chemical precursors. In this regard, biomimetic and bioinspired synthesis of nanomaterials-nanostructures have great advantages over conventional physical and chemical methods. By mimicking natural occurring biomineralization, novel synthesis methods for nanostructures which show multifunctional properties and applications are established. These novel methods, together with the multifunctional products, have been proved to be effective in solving the energy and environment dilemma that have confronted human society for decades.

2.1.1 Synthesis of Inorganic Materials through Natural Biomaterials

(1) Biomineralization

Biomineralization is defined as the processes that living organisms form mineral products, which are known as biominerals. [1-4] It is a widespread phenomenon and almost all six taxonomic kingdoms contain members with this ability with which organisms employ a combination of concerted mechanisms involving bioaccumulation, reduction and mineralization process to form hard tissues or to protect them from metal toxicity.[5,6] Examples include the intracellular crystals in prokaryotes, exoskeletons in protozoa and the silicification of micro alga like diatoms, carbonates in invertebrates, calcium phosphates and magnetite.[7-9] Besides, biosynthetically produced silver or gold nanocrystals have also been discovered in many organisms. [10-13]

In the biomineralization process, three principal factors are required for the nucleation and growth of inorganic materials to occur: (1) there exists localized active

sites or regions in which the transportation and accumulation of precursors are achieved; [14] (2) accumulated precursors at those specific sites or zones nucleate to form crystals with definite sizes, shapes and orientations; (3) growth of minerals accomplishes. [14-16]

Biomineralization process has unique properties: (1) high degree of crystallographic control that is exerted during the precipitation of the bioinorganic crystals; [17-18] (2) biominerals are usually composites of inorganic crystals supported/separated by organic materials, such organic-inorganic architectures shows interesting properties; [14,16,19] (3) the mineralization occurs under natural conditions with bottom up processes, which makes it an environmental friendly and economical route in material synthesis. This feature is attractive for those materials that need to be synthesized at ambient conditions. [10, 20-22]

However, biomineralization has its limitations: (1) the biominerals are formed under thermodynamic control to meet the requirement of least energy consumed, therefore most of them are with the morphologies and phase that are thermodynamic favorable, e.g. in hydrated phases. The structures and morphologies that are of interest to science and technology are less found; [21, 22] (2) the commonly found biominerals are those of calcium carbonate or phosphate, silicate, magnetite, goethite, many materials that are of science and technology importance cannot be obtained from biomineralization. It remains a challenge to investigate the mechanisms of various biomineralization activities and develop them into practical engineering processes for technological important materials. [22-23]

(2) Nanomaterials from various biological material templates

Inspired by the formation of biominerals, increasing research enthusiasm is devoted to fabricating multifunctional hierarchical inorganic and inorganic-organic composites using biological systems as templates. [24, 25] For example, cage-like proteins and viruses, e.g. ferritins have been used as size-restriction templates for synthesizing various nanoparticles ranging from metal oxide to metal particles. [25,26] Wire shape flagella

from bacteria and rod-like viruses, e.g. tobacco mosaic viruses (TMV) and M13, have been used to synthesize various metal and metal oxide nanowire or nanotube structures or further modified to achieve this goal. [24]

Figure 2- 1 The structural models of various viral particles. [24] Reprinted by permission from Elsevier, copyright (2011).

Table 2- 1 Nanomaterials from some typical natural occurring templates

Template	Inorganic materials	sizes
Ferritin [27,28]	ZnO, Co ₃ O ₄ , MnO ₂ , CeO ₂ , FeOOH, Au, Ag, CdS, etc.	Particles around 2 – 6 nm
TMV [29-32]	Pd, Cu, etc.	Nanowires and nanotubes
M13[33,34]	Co ₃ O ₄ , FePO ₄ , perovskite, etc	Nanowires and nanotubes

2.1.2 Bioinspired Materials as Promising Templates

I. Interactions between biomolecules and inorganic materials

The understanding of the interactions between organic and inorganic materials is a priority in synthesizing inorganic material with bioinspired materials. Macromolecules, such as polysaccharides, nucleic acids, peptides and proteins interact with inorganic species in different ways.

(1) Polysaccharides have involved in biomineralization early in the evolution history, but they don't directly associate with biominerals. The charged functional groups or moieties, such as carboxylic groups (polycarboxylated polysaccharides), hydroxyl groups (hydroxylated polysaccharides), sulfate groups (sulfated polysaccharides) and those contain a mixture of these enable polysaccharides bind inorganic ions through electrostatic forces, as demonstrated in reports in calcium carbonate, metal nanoparticles, such as gold, cobalt and nickel particles synthesis using chitin, chitosan and sodium alginate. [9, 23, 35-37]

(2) Nucleic acids are macro biomolecules consisting of chains of nucleotides which are linked together by phosphodiester bonds. They interact with metal ions via several binding modes, which contain phosphate-metal ion interaction, sugar residues-metal ion interaction and nucleotide-metal ion interaction. In the first mode, at physiological pH, electrostatic attraction is the dominating force between negatively charge phosphate backbone and metal cations. In the second mode, some specific sites on guanine and adenine, e.g. the N1, N7 or O6 sites (in guanine) play roles in metal coordination. [38-40]

Figure 2- 2 Structural formula of a typical nucleotide. The three moieties of the nucleotide play specific roles in directing the template synthesis of inorganic nanoparticles.[38] Reprinted by permission from Nature Publishing Group, copyright (2008).

(3) As for peptide-metal and metal oxide interaction, studies reveal that on oxide surfaces such as titania or silica, carboxylate groups are the preferential binding groups that link organic and inorganic materials together. Sulfur-containing peptides could adhere to gold nanoparticles. Peptides with aromatic nitrogen exhibit high affinity for many kinds of inorganic nanoparticles and metal ions. [25, 41-43]

Figure 2- 3 Schematic representation of possible coordination interaction between carboxylate and TiO₂. [44] Reprinted by permission from Elsevier, copyright (1999).

Figure 2- 4 Protonation/deprotonation of L-glutathione on gold surface. Reprinted with permission from (45). Copyright (2005) American Chemical Society.

The types of metal-biomolecules binding are not only determined by the chemistry of metal ion, many other factors, such as the ionic strength, the pH value, the solvent used, as well as temperature of the system, etc. will also affect the binding behaviors and finally influence the structures and morphologies of the product.

Besides the interactions in molecular level, the spatial organization of these macromolecules such as the organization and orientation of active binding sites, will also affect the nucleation, growth and final morphologies of biominerals.

II. Binding sequences for inorganic materials

Peptides which are able to specifically bind to inorganic species have been screened from cell surface and phage display technologies where target peptide sequences could be screened from peptide libraries. For example, peptide sequences that show affinity towards gold, silver, Pt, ZnS, ZnO, TiO₂, carbon nanotubes (CNTs), copper and other inorganic materials have been identified and applied in mineralization of various nanostructures. (Table 2-2)

Figure 2- 5 Characterization of biosynthetic silver nanoparticles. [46] Reprinted by permission from Nature Publishing Group, copyright (2002).

Figure 2- 6 TEM images of gold nanoparticles synthesized by the reduction of HAuCl_4 by AG3 (A and B) and Flg peptides (C). [47] Reprinted by permission from John Wiley and Sons, copyright (2005).

Table 2- 2 Some peptide sequences for binding inorganic solids

Target materials	Sequence*	Reference
Au	MHGKTQATSGTIQS, LKAHLPPSRLPS	[48,49]
Pt	DRTSTWR, QSVTSTK	[50]
Ag	AYSSGAPPMPPF	[46]
ZnO	NTRMTARQHRSANHKSTQRA	[50]
CNT	DMPRTTMSPPPP	[51]
TiO ₂	RKLDPAPGMHTW	[52]
ZnS	NNPMHQN	[50]

*Each letter in upper case represents one amino acid

III. Self-assembling polypeptides

Self-assembling protein/polypeptide-based materials have drawn extensive research attention in recent years because of their ability to undergo phase separation, which results in rich morphologies and sequence-directed control over material structures and properties.

(1) Amyloid

Amyloid peptides are widely found in many degenerative diseases, such as Alzheimer's disease, type II diabetes and Parkinson's disease. They are peptide assemblies that are the most important and abundant types found in nature. Various protein and peptide with different amino acid sequences have the ability to form these assemblies. Amyloid fibrils are usually rich in β -sheet structure and they would further adopt a cross- β -pleated sheet or a β -helix structure. Amyloids are first found in vivo.

However, they can now be easily and efficiently produced in vitro. [53, 54]

Figure 2- 7 The formation mechanism of amyloid fibrils. [53] Reprinted by permission from John Wiley and Sons, copyright (2008).

(2) Self-assembling of amphipathic β -sheet peptides

Amphipathic peptides are composed of alternating polar and nonpolar amino acid residues sequence of $(XZXZ)_n$, in which X is hydrophobic and Z is hydrophilic amino acid. [55] They could easily self-assemble into one-dimensional amyloid-like fibril structure. These amyloid-like assemblies share many structural characteristics, e.g. individual peptides usually adopt an extended β -strand conformation in which the amino acids orient orthogonal to the amide backbone. The β -strands then self-associate into β -sheets.

Figure 2- 8 Proposed self-assembly and arrangement mode of amino acid side chains in the β -sheet bilayer of amphipathic peptide. [55] Reprinted by permission from John Wiley and Sons, copyright (2012).

(3) Elastin

Elastin is the major extra cellular matrix protein in human elastic tissues. The fundamental building block of elastin is the protein tropoelastin.[56] Elastin proteins share similar sequence structure, which all contain repetitive glycine-rich sequence G

(which is YGG and YGGZG, Y, Z = L, V or A) and proline-rich domain P (which is VPGXG, X can be any amino acid except proline). [57] As temperature increases, proline-rich domain P undergoes a reversible, temperature-induced coacervation via the dehydration and rehydration of the hydrophobic side chain. Glycine-rich sequence G also undergoes temperature-induced dehydration but this process is irreversible. [58] In vivo, the water-soluble tropoelastin monomers associate and are cross linked to form insoluble elastin. In vitro, as more and more precise replicas of tropoelastin are discovered, researchers are able to construct more sophisticated synthetic analogs of natural elastin.

Figure 2- 9 Domain structure encoded by the human tropoelastin gene. Reprinted with permission from (59). Copyright (2003) American Chemical Society.

(4) Collagen and collagen-like Peptides

Collagen is the most abundant protein in mammals. There are 28 different kinds of collagens with functions ranging from mediating cell adhesion to tissue scaffolding and repairing. Although their architectures vary widely, all collagens comprise triple helix bundles. [60, 61] In vivo, collagen usually self-assembles into higher order hierarchical structures with precise chemical composition. For example, several types of natural collagen, e.g. type I, II, III can self-assemble into fibrils.[62] The increase of knowledge in collagen have stimulate the study of collagen-like peptides in order to overcome the application limitation set by natural collagen and to produce other higher-order super molecular structures.

Research in this field has provided us a variety of protein/polypeptide-based materials with controlled structure, morphologies and properties which are promising for application in nanomaterials synthesis. The advantages of using these materials are quite prominent: (1) abundance of materials; (2) the uniform dimensions and morphology these biological-inspired materials can impart to inorganic materials.

2.1.3 One-Dimensional (1D) Nanomaterial: Synthesis and Application

1D nanomaterial systems have attracted much research effort in science and technology, because of the advantages from the unique nanoscale anisotropic morphologies and functionality. Such properties render them high applicability as essential components in electronics, sensors, catalysis and energy conversion and storage devices. For examples, metal oxides nanostructures based on 1D template showed better cycling stability or rate performance in supercapacitors, lithium-ion batteries (LIBs) and sodium-ion batteries (SIBs) because of the increased structural stability and faster ionic and/or electronic transport.[63] Noble metal nanowires and nanotubes provide higher surface areas compared with their bulk or particle counterparts and can facilitate electron and/or mass transport, [64] which is very important in lithium-O₂ batteries (LOBs) cathodes and proton exchange membrane fuel cells. [65]

Despite promising for applications in many fields, control synthesis of 1D nanomaterials are difficult owing to their low thermodynamic stability and high surface areas as compared to most of the microstructure and other dimensions of nanomaterials. With these in mind, recent research in material chemistry has made tremendous effort in surface modification engineering for 1D nanomaterial to ensure the progressive development using various techniques. Many 1D systems, such as metal oxide nanorods-metal oxide nanoparticles (NPs), [66] carbon nanotubes (CNTs)-noble metal nanoparticles (NPs), [64] noble metal nanowires (nanotubes) and carbon fibers-metal oxide NPs, have been synthesized through physical or chemical methods such as physical or chemical vapor deposition (PVD/CVD), surfactant-assisted chemical synthesis, absorption and reduction or via self-assembly methods, electrochemical deposition. [67] Among the various techniques available, green synthetic methods are highly desirable and biomaterials-guided synthesis strategy, inspired from biomineralization, as discussed in the previous section, is of most promising. Methods, which mimic natural biomineralization process, are receiving more and more attentions among researchers. These new methods are simple, environmental friendly with high efficiency and especially they allow the control growth and hierarchical organization of materials using natural materials templates. For example, 1D

structure materials, derived from (1) wire- or rod-shape viruses, and (2) self-assembling polypeptides fibers, have been demonstrated. Viruses, such as tobacco mosaic virus (TMV), M13, have been used as templates in metal oxide and noble metal nanorods synthesis. [29-34] In the case of using peptide as starting materials, although there is growing interest, only several efforts have been reported such as using diphenylalanine and its derivatives, [68] bis(N- α -amido-glycylglycine)-1,7-heptane dicarboxylate,[69] or short peptides, e.g. Ac-KFFAK-Am, etc. as the self-assembling peptide templates.[70]

It is noted that although 1D morphologies were obtained, control of the sizes and morphologies of nanomaterials remain challenging, because of unfavorable protein degradation, precipitation or agglomeration during material synthesis due to the influence of pH or ionic strength fluctuation in aqueous environment or temperature changes. Further improvements on the strong attachment of NPs with the biomaterials, the loading amount of NPs as well as the stability of the structure using stable templates in aqueous or organic phases are still desirable. Therefore, this dissertation will focus on the synthesis of 1D nanomaterial system based on genetically designed self-assembling fusion proteins (GPG-AG3-His tag) which have several functional domains responsible for self-assembling or mineralization.

2.1.4 Lithium-O₂ Batteries (LOBs) and Sodium-Ion Batteries (SIBs)

The development of human society is strongly coupled with the energy consumption. The total power consumption by human is 14 TW in 2010, which is predicted to be triple by 2050. [71] As most of the energy consumed comes from the burning of oil, coal, natural gas and other fossil fuels which are low efficiency in usage, such huge energy consumption has led to the problems of gradually depletion of these fuels sources and at the same time brought about serious environmental consequences such as air pollutions and global warming issues. [72, 73] In an effort to combat the deterioration of global environment and achieve sustainable development, the whole society is aiming at the exploration of renewable energy resources which can offer sustainable energy without sacrificing environmental quality. [74-76] Among the several available technologies that

can use and store the clean energy resources which include wind power, [77] solar power, [78] geothermal power, [79] nuclear energy, [80] etc. energy storage as electrical power is most promising. This trend has spurred up the development of energy storage system that can convert other energy into electrical energy. To achieve this goal, in the past decade, tremendous effort has been devoted into three major categories of energy storage devices, which are supercapacitors (SCs), fuel cells and secondary batteries. [81, 82]

Based on different energy storage mechanisms, they deliver the stored electrochemical energy in different manners, showing large discrepancies in power densities and energy energies, as revealed by the Ragone plot (Figure 2-10). [83] Thus they are suitable for different applications. For example, SCs can provide relatively high power densities while the energy densities are low and SCs mostly find applications in situations where high power densities requirement exceed high energy density demand. Fuel cells, on the contrary, provide the largest amount of energy density among the three but are low efficiencies. The performance of secondary batteries stands amid the other two kinds therefore they prevail in wide applications ranging from portable electronics to hybrid or pure electric vehicles (EVs) and energy storage grid. [81-85]

Lithium-ion batteries (LIBs) are among the most popular classes of secondary batteries. The specific capacity of commercial LIBs is $\sim 75 - 160$ Wh/Kg. [86, 87] While LIBs can meet the energy demand of common portable electronic devices, to power upcoming EVs, more battery units are to be equipped so that enough power are provided to achieve extended travel range which has been demanding advanced battery management systems. [88, 89] Moreover, Concerns over safety issues, poor performance at elevated/low temperatures and, etc. have hurdle their vast applications to meet the rapid expansion of portable electronics and EVs markets. [89, 90] To realize the complete electrification of road transportation, novel energy storage system with high performance than current LIBs are necessary. Among the several systems proposed in the last few decades, LOBs are very promising as it can provide as high energy density as 11 680 Wh/kg based on the oxidation of 1Kg lithium metal, which is comparable to that of gasoline (13 000 Wh/kg). [88, 91] It is noted that while LOBs system has shown high theoretical capacity, there remains a great deal of challenges to conquer before workable

cell that can power up even a portable electronics. For example, (1) the electrochemical processes using a Li metal based cell is yet to be fully revealed; (2) even the best performance (cycling performance, energy efficiencies, etc.) of the LOBs currently available in the labs are far from being satisfied; (3) devising appropriate cell configurations to incorporate into electronics or EVs are big obstacles when considering the oxygen sources which will require additional setups.

While exploration of high performance electrode materials for secondary batteries based on lithium (e.g. LOBs,) is still underway, novel energy storage systems that can supplement current LIBs have emerged. For example, currently research efforts are also devoted to exploring the concept of sodium ion batteries (SIBs). [93, 94] The development of SIBs is boost under the consideration that SIBs is conceptually identical to LIBs therefore SIBs technologies can greatly benefit from current LIBs technologies. [92-95] Such a consideration, however, need to be subjected to severe scrutiny and examination as sodium is more active and Na^+ is much larger than Li^+ .

Figure 2- 10 Specific power against specific energy for various electrical energy storage devices (Ragone plot) [83] Reprinted by permission from Nature Publishing Group, copyright (2008).

(1) Introduction of Secondary Battery

I Thermodynamics

A typical battery cell usually has four components: an anode, an electrolyte, a separator and a cathode, where the anode and the cathode are separated by a separator which is ion transmittable. The anode, cathode and separator are wetted by the electrolyte which is electric insulate but ionic conductive. When electrochemical reactions occur at the electrode/electrolyte interface, the free energy decreased is governed by the Gibbs's law:

$$\Delta G^0 = -nFE^0$$

Where F is Faraday constant (~ 96500 C/mol), n is the number of electrons involved in the reaction and E^0 is the standard potential. In non-standard conditions, the electrochemical potential is determined by Nernst Equation:

$$E_{red} = E_{red}^0 - \frac{RT}{nF} \ln \frac{a_{red}}{a_{ox}}$$

Where E_{red} is the actual reduction potential, E_{red}^0 is the standard potential, R is the gas constant (~ 8.314 J K⁻¹ mol⁻¹), n is the number of electrons transferred in mole, F is the Faraday constant, a_{red} and a_{ox} are the activities of the reductant and oxidant, respectively

The voltage output of a battery cell is determined by the following equation:

$$V = E_{cathode} - E_{anode}$$

For the conversion reaction of $\langle H \rangle + A^{n+} + ne^- \rightarrow \langle HA \rangle$, where $\langle H \rangle$ represents the active materials, the specific capacity of H (C_H^{th}) can be calculated according to the following equation:

$$C_H^{th} = \frac{nF}{3.6 * M(H)} (mAh/g)$$

Where $M(H)$ is the molecular weight of H (g/mol).

II Electrode kinetics

In electrochemistry, the electrocatalysis process of the electrode is usually described by the Butler-Volmer equation:

$$i = nFAK^0 \{c_o(0, t) \exp[-\alpha F(E - E_0)/RT] - c_R(0, t) \exp[(1 - \alpha)F(E - E_0)/RT]\}$$

Where n is the number of electrons transferred, F is the Faraday constant, α is the transfer coefficient, K^0 is the standard rate constant, T is the temperature in Kelvin, R is the gas constant. E is the reaction potential, E_0 is the standard potential and $\eta = E - E_0$ is defined as overpotential which is the extra energy needed to drive the reactions at a specific current density.

The overpotential in the Butler-Volmer equation can be caused by the charge transfer process (at low current densities) or by the mass transfer process (at high current densities) or both simultaneously.

(2) Lithium-O₂ batteries

LOBs are among those energy-storage devices that have the highest gravimetric energy density, as shown in Fig 2-11. The theoretic energy density of Li-O₂ battery is approximately 130000 Wh/Kg based on the lithium metal alone, which is comparable to that of gasoline (132000 Wh/Kg). Such attributes make them promising candidates for application in electric vehicles. [90, 91]

Figure 2- 11 The gravimetric energy densities (Wh/kg) for different types of rechargeable batteries. Reprinted with permission from (88). Copyright (2013) American Chemical Society.

In a typical lithium-oxygen battery, lithium metal is used as anode and the cathode is O_2 , which differentiates it from LIBs. Based on the types of electrolytes used, LOBs are classified into four categories: (1) aqueous; (2) non-aqueous (aprotic); (3) hybrid (mixture of aqueous and non-aqueous; and (4) solid state and there are now four corresponding configurations of LOBs with different fundamental electrochemistry, [88-90] which are summarized in Figure 2-12:

Figure 2- 12 Schematic illustration of the four chemical architectures of LOBs cells. [96]
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The LOBs based on aprotic electrolyte consist of porous carbon cathodes designed to promote oxygen diffusion and reduction and a pure lithium metal anode as shown in Figure 3-19. In the ideal LOBs with aprotic electrolyte, during discharge, oxygen molecular that have dissolved in the electrolyte diffuses to the cathode. Then oxygen is adsorbed by the cathode and reduced. Li ions then react with desorbed reduced oxygen to form the discharge products. [88, 90] The following reaction steps have been proposed for the oxygen reduction reaction (ORR):

Studies have demonstrated that Li_2O_2 is the dominant discharge product in the rechargeable aprotic LOBs. [88, 96]

During the charge process, the discharge products are oxidized to evolve oxygen, e.g., Li_2O_2 decomposes and yields Li and O_2 . This process is known as the oxygen evolution reaction (OER). [89, 90] Generally, in LOBs, the electrode reactions are suppressed by the limiting factors such as thermodynamic, kinetics or mass transport, the discharging and charging potentials deviate from the standard potentials, such phenomena are explained in terms of overpotentials.

Areas of research in aprotic electrolyte based LOBs to be addressed include cathodes, anodes, electrolytes, and separators. Even though the anode materials for current commercial Li-ion batteries (LIBs) can be used as anodes for LOBs, the use of metallic lithium as anode is dominate in current research because it simplifies the cell assembling in scientific labs, allowing more time and effort to be devoted to electrolyte and cathode research.[88-90, 97]

It used to be a major issue to choose appropriate electrolytes for the aprotic LOBs. Carbonate-based electrolytes (ethylene carbonate, propylene carbonate and dimethyl carbonate) were popular as electrolyte at the early stage of LOBs. [98, 99] But these electrolytes are unstable at the highly oxidative environment of LOBs. During discharge/charge processes, they decompose to generate lithium-contained carbonate, which gradually deposits on the oxygen electrode and finally deteriorate the cathodes. [100, 101] Ether-based electrolytes have recently shown improved stability than carbonate-based electrolytes and are suitable for durable LOBs, where they are demonstrated to be stable below 4.38 V (vs. Li/Li^+). The discharge product of LOBs based on tetraethylene glycol dimethyl ether (TEGDME) is confirmed to be Li_2O_2 by the XRD characterization and lithium carbonate is not detected. [102]

The obstacles that hinder the development of practical LOBs are the cathodes. Electrical passivation at the cathode has been the dominant capacity-limiting mechanism in LOBs. Pore clogging issues which arise from the accumulation of dissoluble discharge products which are also insulators have posed great challenge for aprotic LOBs. Development of cathodes of porous hierarchical structures with O_2 diffusion channels has

been proved to be effective in overcoming the electrical passivation issues. [89-90, 97, 100-101]

Due to the existence of the overpotentials, the energy efficiencies (discharge energy/charge energy) of LOBs is currently only 60 – 70 %, much lower than commercial LIBs which are usually as high as 99%. To improve the energy efficiencies of LOBs, reduction of the overpotentials by the introduction of catalysts like noble metal, transition metal oxide, etc. on the O₂ electrodes to improve electrode kinetics been proved to be effective. [88, 90-91, 97]

(3) Sodium-ion batteries

SIBs are conceptually identical to LIBs. Both two kinds of batteries were simultaneously investigated in the 1980s; however, the development of SIBs is sluggish compared to LIBs. [91-92] Success in commercial SIBs is challenging because (1) Sodium has more positive redox potential therefore SIBs has lower energy density than LIBs; (2) Na⁺ (1.06 Å) is ~ 40% larger than Li⁺ (0.76 Å), which greatly complicates the battery electrode electrochemical reaction process and therefore restricts the kinds of electrode materials suitable for high capacity, fast and durable SIBs. [93, 94] However, since sodium is ranked the 6th most abundant element in the earth's crust, with even global distribution, SIBs have potential advantages over LIBs in cost on the conditions that current LIBs technology are effective in SIBs. Thus SIBs are promising candidates for grid energy storage, as well as potential competitors for portable electronic applications when further improvements are obtained. However, for these goals to be realized, substantial improvement in energy density, power density, long-term cycling stability and high rate capability of SIBs are required. [95]

Four aspects which include cathodes, anodes, electrolytes, and separators are needed to be addressed in SIBs research.

Cathodes for Na batteries should be capable of hosting Na ions; the volume change should be avoided for the material upon Na ions intercalation. [93] This capability is critical for practical durable SIBs. The development of materials with low volume change as a function of Na content can be inspired from structures that have been widely applied in LIBs cathodes. Recently, progresses on cathode research for SIBs systems has already been proven fruitful and a number of transition metal oxide and poly-anionic compounds identified have shown promising performance. Currently, sulfides, fluorides, phosphates, sulfates and metal oxides including (1) Na_xMnO_y tunnel structure oxides; (2) vanadium oxides, iron oxides and (3) layered sodium transition-metal oxides have been systematically investigated. [94, 103]

Unlike the fast advancement on cathodes material for SIBs, progress on anode materials is limited, as graphite, which is the state-of-art anode for commercial LIBs, is electrochemically inactive for sodium storage. Thus, identification of suitable anode materials is the critical issue for the practical SIBs. [104] Compared to lithium metal anodes, sodium of metallic states when used as anodes often delivers lower theoretical energy densities because (1) Na has lower specific capacity (1166 mAh/g) and (2) the standard reduction potential of Na is higher than Li. Besides, metallic sodium is highly reactive with the organic electrolyte and dendrite formed during Na metal reduction is much more serious in comparison with Li metal anodes. Furthermore, the low melting point of Na (98 °C) poses a significant safety hazard towards devices designed for use at ambient temperatures with a sodium metal electrode. [92-95, 103-104] Although graphite does not intercalate sodium, [105] many other kinds of anodes that consist of various carbonaceous materials such as carbon black, [106] petroleum cokes, [107] pitch-based carbonfibers, [108] and polymers (poly (para-phenylene)) [109] have been demonstrated to be able to intercalate Na. Besides these carbon materials, other anode materials, including titanium-based materials such as TiO_2 and a variety of reduced oxides of titanium (usually described as Ti(IV)-Ti(III)), [110, 111] tin based materials such as Sn, [112] SnSb, [113] SnO_2 , [114] SnS_2 [115] and antimony based materials like Sb, [116] Sb_2O_3 [117] and Sb_2S_3 , [118] etc. have been explored as anode materials and tin- and antimony based materials have exhibited high capacity. Besides tin- and antimony based

compounds, transition metal phosphides (TMPs) are promising high-capacity candidates for lithium and sodium storage according to the conversion reaction mechanisms. [119, 120]

2.2 Questions to Answer Based on Literature: Designing Biomaterials with Different Functional Domains for Nanomaterials Synthesis

The behaviors of “learning from natural mineralization processes” are as old as humanity. After first suggested in 1960s, bioinspired-related methods are considered to be much more efficient and effective synthesis methods for functional nanomaterial synthesis. However, over the past decade, research in this area is sluggish as the focus is devoted to material synthesis using natural occurring biological materials as templates for nanomaterials synthesis, through which although many interesting structures are obtained, little insight on purposely design of materials synthesis to achieve functional nanomaterials have been gained, as the use of biological templates only results in the replication of these templates. Further studies on combining the mechanisms of biomineralization (which refers to understanding of interaction of functional domain on biological materials with inorganic species), designing multifunctional versatile bioinspired materials (with several mineralization domains) with control architectures and functional nanomaterials synthesis are meaningful yet challenging.

2.3 Questions to Answer Based on Literature: Enhanced Energy Storage of 1D Carbon-supported Nanomaterials

The revelation of the interactions between inorganic species, e.g. ions, nanoparticles with biomaterials has led to the advanced expansion of applications of biomaterials in tissue engineering and drug delivering systems. Compared with the rapid development of their application in medical purposes, the investigation of the inorganic/organic nanomaterials derived from biomaterials for energy storage applications are rarely studied. Concerns over the availability of the bioinspired-related methods, the poor control synthesis and the poor performance of the inorganic nanomaterials obtained

through these methods can always find their places to doubt the practical application of such methods. The investigation of self-assembling GPG-AG3-His tag fusion protein templates for inorganic material synthesis towards enhanced energy storage devices are therefore carried out to clear away such hindrances.

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Chapter 3

Experimental Methodology

This chapter includes mainly four parts. The first part is about the principles of constructing a fusion protein using the E.Coli expression system, which is adopted in this thesis. The second part introduces the inorganic nanomaterials synthesis methods, which are solution-phase methods such as hydrothermal method and oil-phase synthesis. The third part briefly presents the inorganic nanomaterials structural characterization techniques and the corresponding instruments. The last part presents the electrochemical characterization techniques widely adopted in secondary batteries, such as cyclic voltammetry and cycling charge-discharge tests.

3.1 Rationale for Selection of Methods/Materials

According to the literature reviews in Chapter 2, naturally occurring biological materials, such as virus, proteins, polysaccharides, lipids have provided certain avenues for constructing nanomaterials. It is also noteworthy that their behaviors are hard to predict and their actual roles are difficult to identify, which have posed great challenge to the development of this particular inorganic nanomaterial synthetic strategy. Therefore, it is of significant to designed novel biomaterials composed of domains with specific functions yet still structurally simple. Under this consideration, recombinant protein strategy prompts up. In an attempt to prove this concept, GPG peptides have the temperature-responsive capability and reversible fiber formation properties due to the rich hydrophobic and hydrophilic domains have been constructed. They are however, not yet suitable for use as templates in inorganic nanomaterials synthesis because of the hydrophobic nature and low amount of metal ions binding sites.

By taking advantage of the GPG protein's self-assembly property and mineralization capability of some short peptide sequences, such as AG3, further consideration of a GPG-based recombinant protein with metal binding functional domains is proposed and demonstrated. The goal of control synthesis and organization of nanostructures will be achieved by this new simple yet efficient and effective strategy. Such a strategy is promising in the programmed assembly of nanoparticles into hierarchical structures. Thus, elastin-mimetic amphiphilic fusion protein, GPG-AG3-His tag, containing the amino acid sequence (VGGVG)₅-(VPGXG)₂₅-(VGGVG)₅-AYSSGAPPMPPF-HHHHHH, will have the capability to self-assemble into nanofibers at increased temperatures and applied in one-dimensional (1D) inorganic nanomaterial synthesis. In the GPG-AG3-His tag fusion protein, the GPG domain dehydrates when the temperature increase to its coacervation temperature (T_t). At the same time, β -turn and subsequently β -sheet structure are formed, which results in the formation of several micrometers long fibers and are tens of nanometers in diameter. The hydrophilic domains (which are AG3 and histidine tags) expressed on the exteriors of the self-assembling fiber are specific for metal ions binding.

These features allow them to be attractive as templates in 1D nanomaterial synthesis in solution phases.

More specifically, AG3 have been to be able to mineralize Ag^+ , Au^{3+} into Ag or Au nanoparticles, and should have the capability to mineralized Pd or Pt under suitable conditions. His tags can specifically coordinate transition metal ions, which enable the manipulation of them for transition metal based nanomaterial synthesis, for example, metal oxides or metal phosphide.

1D noble metals and transition metal-nitrogen-carbon (M-N/C) catalysts are effective in improving the electrochemical kinetics of lithium- O_2 battery (LOBs) cathodes to increase the battery efficiencies as well as cycle life. One-dimensional (1D) porous Pt or Pd nanotubes can confine the size of generated Li_2O_2 during the discharge and improve the electrical conductivity. M-N/C catalysts are considered to be rich in active catalytic centers. These catalytic centers are uniformly and densely distributed on the surfaces of the supporting materials. These catalytic active sites are able to facilitate rapid electron transport and surface electrochemical reactions. By using M-N/C as catalysts for LOBs cathodes, the over-potential phenomena are to be suppressed by tuning the deposition and distribution behavior of Li_2O_2 on the catalyst surface.

Transition metal phosphides (TMPs) are very competitive high-capacity candidates for lithium and sodium storage, they are not yet fully investigated for practical application in lithium-ion batteries (LIBs) and sodium-ion batteries (SIBs) due to their low electric conductivities and the drastic volume change during the conversion reaction process. To solve these problems and obtain high performance LIBs and SIBs anodes, the construction of TMPs into nanoscale materials, especially into 1D structure such as nanotubes or nanorods are most promising.

Based on the above discussions, multifunctional templates of self-assembling GPG-AG3-His tag are suitable for use in synthesizing 1D inorganic materials, e.g. carbon support Pt and Pd porous nanotubes, 1D CoP nanostructures and 1D M-N/C catalysts through solution-phase methods for efficient LOBs, LIBs and SIBs.

3.2 Synthesis

3.2.1 Fusion Protein Expression

Advancement in the field of biotechnology have made it possible to produce recombinant proteins in a more easily quality controlled way. Compared with natural occurring proteins, recombinant proteins possess novel functions, structures and are more abundance than many native proteins. [1, 2] They are widely used for tissue engineering and medical purposes, such as bioactive matrixes for cell growth and proliferation, vaccines, functional enzymes and therapeutic proteins. [2-4] The methodology for most of the expression systems is similar. There are three basic components which are a coding sequence for the recombinant protein, a vector (most usually are a plasmid system) into which the coding sequence is to be cloned behind a promoter that is active in the chosen host, and finally a host that will effectively express the target protein. [5] Generally, three steps are included: (1) design gene of interest; (2) clone it in whatever expression vector; (3) transform it into the host of choice, induce and then harvest the protein for further purification and characterization. [6]

Figure 3- 1 A typical expression system. [5] Open access from Bentham Open, copyright (2009).

Among the various questions to be considered in the development of recombinant proteins, the major consideration is the selection of proper expression. There are three most popular host systems for the expression of designed proteins, which are *Escherichia coli* (*E.Coli*), baculovirus and yeast mediated protein expression. [7. 8] The selection of the expression system depends primarily on the nature of the target protein to be expressed. Compared with the other two expression systems, *E.Coli* is a more suitable expression system for relatively small, soluble, un-modified proteins. It is also usually the first choice for many investigators and for industrial production of therapeutic or commercial based proteins because of its many advantages in terms of easy culturing conditions, fast biomass accumulation as well as a rapid and cost-effective scale up process. [8-10] More specifically, *E.coli* has unparalleled fast growth kinetics. In optimized growth conditions, the numbers of the cells double every 20 mins, which means that stationary phase can be reached in only a few hours for the culture inoculated with a dilution of a saturated starter less than 1%. In lab cultural environment, the expression of a recombinant protein, however, can impart metabolic stresses on the *E.Coli* cells, leading to less biomass achieved. [11, 12]

The scale of expression using *E.Coli* varies depends on the research purposes, which can starts from several milliliters for initially investigations and up to thousand liters in industrial fermentations. The yielded proteins are usually first identified by SDS-PAGE and further verified by Western blotting. Culture volumes of 50-250 mL are preferred for the identification of an expressing clone, optimization of expression conditions and purification. [13] In large scale expression systems, two liters of bacterial culture will yield 50 to 80 g of wet weight of cells which finally yields about 100 to 300 mg of target protein is available (2% to 5% of total proteins). [13, 14]

Despite the prominent advantage, there are limitations to *E.Coli* expression systems. There are a lot of circumstances when *E. coli* may not be the ideal expression system, for example, only 10% of eukaryotic proteins and 50% of bacterial proteins can be appropriately expressed in *E. coli* in a soluble form. And proteins with molecular weight above 60 kDa usually have low yield. [12, 14]

3.2.2 Solution-Phase Synthesis

(1) Hydrothermal/Solvothermal Methods

Hydrothermal methods are powerful in synthesizing nanomaterials of abundant dimensions, morphologies and properties. Metal oxide, metal hydroxide, noble metal, phosphates, fluorides, selenides of one-, two-, three-dimension nano/micro scales have been demonstrated via these simple and effective methods. [15, 16] The term “hydrothermal”, originated from geology science, can be traced back to the 1850s. It exploits the solubility of minerals in hot water under high pressure for material synthesis purpose. [17] In a typical hydrothermal synthesis, a sealed apparatus called autoclave, consisting of a steel pressure vessel, is used as the reaction container. Precursors are added in the autoclave along with suitable amount of water.

The hydrothermal concept has been extended further to solvothermal in which various solvents ranging from inorganic to organic are used in order to synthesize materials that are not available in hydrothermal methods. Furthermore, combinations of other techniques with hydrothermal/solvothermal methods have led to many other advanced methods such as microwave-assisted hydrothermal synthesis, ultrasonic-hydrothermal synthesis, electrochemical-hydrothermal methods, etc. [18-20]

Figure 3- 2 A typical hydrothermal process to synthesize cathode materials for lithium-ion batteries. Reprinted with permission from (21). Copyright (2009). The Electrochemical Society.

Hydrothermal/solvothermal methods also have their drawbacks. For example, it is still difficult to scale up the synthesis from the pilot studies and extensive trial-and-error studies are required to optimize the synthesis parameters including temperature, solvent, precursors, surfactants and their ratios. The investigations on pressures for the reactions and the reaction times also exhaust research efforts.

In the operation of hydrothermal synthesis, extreme cautious must be taken as hazards such as burns by the heat surface, leakage of solution, and explosions can easily happen. Therefore, the following safety precautions must be emphasized:

1. Underwent intensive training by a specialized staff before operating the equipment
2. Ensure no flammable items placed near the working area.
3. Check any leakage before putting the autoclave in the oven.
4. Set the temperature of the oven below 200 °C .

The standard operating procedures are strongly recommended:

1. Only trained and qualified personnel are allowed to operate.
2. Put the solution in the autoclave and make sure the volume of the liquid not exceed 1/3 of the total volume.
3. Tighten the cover of the stainless steel container to avoid leakage of the liquid.
4. Set the oven to the proper temperature. Maximum temperature of the oven shouldn't exceed 200 °C .
5. Set the heating time of the oven. Maximum time for the heating shouldn't exceed 24 h.
6. Start the heating process.
7. Remove the autoclave when it is cooled down to room temperature.
8. Turn off the power supply of the oven.

(2) Organic-Phase Synthesis Methods

Figure 3- 3 A typical organic-phase process to synthesize up-conversion nanoparticles. Reprinted with open access from (22).

Organic-phase synthesis is the thermal decomposition of organometallic compounds in high boiling point organic solutions in the presence of stabilizers. Organic phase approach is usually carried out under inertia gas protection, such Ar or N_2 , depending on the nature of the target product. [23, 24] The organic solutions used in most cases are 1-octadecene, oleic acid and/or oleylamine. The precursors commonly used are organometallic compounds but many inorganic precursors like metal chlorides/sulfates/nitrides have also been used in some investigations. Nanomaterials of various types like noble metal crystals, transition metal or transition metal oxide/sulfide/phosphide and up-conversion nanoparticles with abundant morphologies have been successfully synthesized using this route under optimized conditions. [24, 25] The organic phase approach offers good controls on dispersity, crystallization, size, and shape. However, the complicated synthesis procedures, the used of organic solutions, higher temperature and protection gas required make it less adopted in large scale synthesis in comparison with hydrothermal methods. [26, 27]

As there are potential hazards and risks (burns by the heat surface, leakage of bad smell or vaporized solution, leakage of cooling water, and fire) during organic-phase synthesis, the following steps are advised to strictly follow during synthesis using a typical three-neck flask setup.

1. Only trained and qualified personnel are allowed to operate.
2. Put the solution in the three-neck flask and make sure the volume of the liquid not exceed 1/3 of the total volume.
3. Connect the flask with the cooling coil. Seal the connection spot to avoid leakage of the liquid or vapor. Turn on the cooling water.
4. Insert the thermometer. Seal the connection spot to avoid any leakage.
5. Insert the needle connected with the gas tube. Handle the needle carefully. Tune the flow rate of the gas.
6. Set the temperature of the heating jacket. Notice: if the temperature of reaction is relatively high (above 200 °C), the temperature must be increased slowly to avoid the overheating.
7. Start the heating process.
8. Stop heating after the reaction and turn off the gas and cooling water when the solution is cooled down to room temperature. Remove the needle for the gas supply. Cover the cap of the needle and handle it carefully.
9. Remove the flask from the heating jacket
10. Turn off the power supply of the heating jacket.

3.3 Structural Characterization

3.3.1 X-ray Diffraction (XRD)

X-ray diffraction technique is widely used for identifying the crystallographic structure of a crystal. In XRD characterization, X-rays are used to produce the diffraction pattern because their wavelength λ is typically the same order of magnitude as the spacing d between planes in the crystal (1–100 angstroms). [28] The incident X-ray

beams interact with the crystalline atoms and are diffracted into many specific directions, which can be described Bragg's law:

$$2d\sin(\theta) = n\lambda \quad (n = 1, 2, \dots)$$

Where θ is the incident angle, d is the spacing between two diffracting planes, n is any integer, and λ is the wavelength of the beam.

Figure 3- 4 Bragg's law.

Through analyzing the positions, shapes and relative intensities of the peaks obtained from the X-rays diffraction patterns, information such as unit cell parameters, atomic parameters, crystallinity, disorder and defects of the crystal can be obtained. Furthermore, the average grain size can be estimated using the Scherrer equation [29]:

$$d = K\lambda/(\beta_{1/2} \cos\theta)$$

In the Scherrer equation, K is the shape factor, d is the grain size, λ is the wavelength of X-ray, $\beta_{1/2}$ is the width at half the maximum intensity (FWHM) and θ is the diffraction peak.

Other information, such as the mean positions of the atoms in the crystal and their chemical bonds, as well as three-dimensional picture of the density of electrons within the crystal can also be determined.

3.3.2 Scanning Electron Microscopy (SEM)

The scanning electron microscope (SEM) is a type of electron microscope that used to investigate the surface topography and elemental composition of the sample. In the SEM characterization, a focused beam of electrons is scanned through the sample; the electrons interact with atoms in the sample, producing various signals which can be used to produce an SEM image in combination with the beam's position. SEM can achieve resolution better than 1 nanometer. [30]

Figure 3- 5 A simplified schematic diagram of a scanning electron microscope. [31]

The most common SEM mode is SEI which is based on the detection of secondary electrons emitted by atoms excited by the incident beam. The number of secondary electrons that can be detected depends on many factors, e.g. on the angle at which beam meets surface of specimen, which is related to the specimen topography. [32] By scanning the sample and collecting the secondary electrons using a special detector, an image revealing the surface morphologies of the sample is generated. Beside secondary electrons, the detection of back-scattered electrons are explored for illustrating contrasts in composition in multiphase samples and the characteristic X-rays produced from the

atoms which are excited by the electron beam in a sample can be used for compositional analysis, which is the energy dispersive spectroscopy (EDS) technique. [33]

3.3.3 Transition Electron Microscopy (TEM)

Transition electron microscopy is unique microscopy technique which is capable of examining the fine detail of materials to atomic-resolution scales and chemical information at a spatial resolution of 1 nm due to the small de Broglie wavelength of the electrons. Under operation, a beam of electrons emitted from the electron probe and accelerated under different accelerating voltage (typical 200 KV) is transmitted through an ultra-thin specimen. After interaction with the specimen, the information of the electrons transmitted is collected and the TEM images are formed. The image is further magnified and finally focused onto an imaging device, such as a fluorescent screen, on a layer of photographic film, or to be detected by a sensor such as a CCD camera. [34, 35]

TEM can provide information on the chemical information, crystal orientations and nanoparticle sizes and morphologies through combining the imaging, spectroscopy and diffraction results from the TEM characterization. Especially, high-resolution TEM can identify the periodicity of the crystal through visualizing the lattice fringes. These lattice fringes information can be obtained by interference between the transmitted electron beam and the diffracted beam under the high resolution mode of TEM. Selected-area electron diffraction (SAED) patterns obtained under electron diffraction mode of TEM, in combination with information from XRD and energy dispersive spectroscopy (EDS) enable the determination of the d -spacing of the crystals. For a single crystal, the SAED pattern is a series of diffraction spots arranged in concentric rings. Each spot represents a specific lattice plane. [35, 36] For polycrystalline samples, concentric rings instead of spots will appear in their SAED pattern. A central bright spot which is comprised of transmitted electrons can be found in the SAED pattern of both single crystals and polycrystals. [36]

Figure 3- 6 Simplified diagram of a transmission electron microscope. Drawing by Graham Colm, courtesy of Wikimedia Commons. [37]

3.3.4 X-ray Photoelectron Spectroscopy (XPS)

X-ray photoelectron spectroscopy is a surface-sensitive quantitative technique that is widely used in analyzing the surface chemical information of a material. The elemental composition, chemical state and electronic state of the elements that exist within a material can be revealed through XPS characterization. [38] XPS spectra are generated through irradiating the target material with a X-rays beam while the number and kinetic energy of emitted electrons from the surface (0 to 5 nm depth) of the material are simultaneously measured and analyzed. The principle of XPS is based on the following equation:

$$E_{binding} = E_{photon} - (E_{kinetic} + \phi)$$

In which E_{photon} is the energy of the X-ray photons being used, $E_{binding}$ is the binding energy of the electron, $E_{kinetic}$ is the kinetic energy of the escaped electron and Φ is the work function of the materials.

A typical XPS spectrum is obtained by plotting the number of electrons detected (counts or intensity) versus the binding energy of the electrons detected. Since each element produces a characteristic set of XPS peaks that correspond to the electron configuration of the electrons within the atoms such as 1s, 2s, 2p, 3s, etc. at their characteristic binding energy values, the existence of a specific element can be directly identified from the respective XPS spectra.

Figure 3- 7 Principle of X-ray photoelectron spectroscopy. [39]

3.3.5 Nuclear Magnetic Resonance (NMR)

NMR spectroscopy is the principal spectroscopy technique used to obtain the physical, chemical, electronic and structural information of the molecules present in the sample. It can provide detailed information on the dynamics, topology and even the spatial structure of molecules through measuring the resonant frequencies of the nuclei from the chemical shift either in solution or solid state. [40]

All isotopes that contain an odd number of protons and/or of neutrons have an intrinsic nonzero nucleic spin (μ), while all nuclides with even numbers of both have a

total spin of zero. When an electromagnetic field (B_0) is applied, e.g. in the z-direction of μ , the energy of this spin is given by the following equation:

$$E = -\mu_z B_0 \text{ or } E = -\gamma s \left(\frac{h}{2\pi}\right) B_0$$

In which γ is the gyromagnetic ratio, s is the spin and h is Planck constant.

The energy difference between two closest spin states is thus

$$\Delta E = \gamma \left(\frac{h}{2\pi}\right) B_0$$

Resonant absorption by nuclear spins will occur only when the frequency of the applied electromagnetic radiation matches the energy difference between the two nuclear spin levels.

Due to the "shielding effect" of the surrounding electrons, the energy gap between two nucleic spin states is reduced, and the frequency required to achieve resonance is also reduced. This shift in the NMR frequency, which depends on the electron density distribution in the corresponding molecular orbitals is known as chemical shift and is the basis for determining the chemical structure of molecules. [40, 41]

The most commonly studied nuclei are ^1H and ^{13}C .

3.3.6 Atomic Force Microscopy (AFM)

Atomic force microscopy is a type of scanning probe spectroscopy with a very high resolution. AFM is capable of providing a three-dimensional graphic of a surface on a nano or sub-nano scale, by measuring the interactions between a probe and the studied surface at a very intimate distance, which is usually fall in the range of 0.2 – 10 nm. [42]

AFM was developed in order to study and reveal electrically non-conductive materials such as proteins and ceramics. In the year of 1986, Binnig and Quate first demonstrated the ideas of AFM. In 1987, Wickramasinghe et al. developed an AFM setup with a vibrating cantilever technique, which used the light-lever mechanism.

The dominant interactions in the AFM are Van der Waals interactions. However, as long-range interactions such as capillary, electrostatic, and magnetic interactions are significant further away from the surface. These are important issues that required to be considered in other scanning probe microscopy (SPM) methods of analysis. During scanning through the sample, the probe predominately experiences repulsive Van der Waals forces (contact mode, preferably used for hard samples and operated in air and liquid). The interaction between the tip and the surfaces leads to the tip deflection. When the tip gradually moves away from the surface, attractive Van der Waals forces are dominant (non-contact mode, also known as force spectroscopy mode and interaction forces between tip and sample are recorded).

The AFM probes are commonly made from Si_3N_4 or Si. The probes can also be further coated with other materials for special applications such as chemical force microscopy. In the AFM setups, the length of the cantilever, the probe materials as well as their shapes can be varied.

Figure 3- 8 Typical configuration and operation mode of an AFM. [42]

3.3.7 Fourier Transform Infrared Spectroscopy (FTIR)

In typical infrared spectroscopy characterization, IR radiation is set to pass through the sample pallets. Some infrared radiation will be absorbed by the sample while some of it will pass through and reach the detector. [43] The obtained spectrum clearly reveals the absorption of the molecular existed in the sample, thus showing the fingerprint of the molecular in the sample. Generally, organic molecules absorb IR radiation with energy between 11 kcal/mole and 1 kcal/mole which corresponds to absorption between 4000 cm^{-1} and 400 cm^{-1} . This amount of energy represents the energy gaps between different vibrational states of bonds within the molecule of the sample.

FTIR offers both quantitative and qualitative analysis for organic and inorganic samples. It can identify chemical bonds in a molecule. The spectra produce an adsorption profile of the sample, which is a unique molecular fingerprint that is useful in screening the samples with many different components. FTIR is an effective and efficient analytical technique for identifying chemical functional groups and characterization of covalent bonding of molecules.

Figure 3- 9 Structure of the FTIR instrumentation. [43]

3.4 Electrochemical Characterization

3.4.1 Cyclic Voltammetry (CV)

Cyclic voltammetry is a potentiodynamic electrochemical technique widely adopted to study the electrochemical reactions of the electroactive species. [44] In a typical three-electrode system, the potential is applied between the working electrode (WE) and the reference electrode (RE) while the current between the working electrode and the counter electrode (CE) is recorded. The working electrode potential is ramped linearly versus time between two values, which is defined as the scan rate (v), as shown below:

Figure 3- 10 The voltage applied in CV technique.

For a reversible one electron transfer reaction, in the forward scan when increasingly reducing potential is applied, the cathodic current will increase over this time period until it reaches maximum (I_{pc}) where the reduction potential (E_{pc}) is reached and in the reverse scan, the anodic peak current (I_{pa}) and oxidation potential (E_{pa}). The corresponding CV curve, which represents the relationship between the applied voltage and the current response of the electrodes at a specific constant scan rate, is shown below:

Figure 3- 11 Current response of the reversible redox reaction in a CV cycle.

The voltage separation of the cathodic and anodic peaks is given by the following equation:

$$\Delta E = E_p^c - E_p^a = \frac{59}{n} mV$$

Where n is the number of the electrons transferred.

The peak current is determined by Randles-Sevcik equation:

$$I_p = nFKAC \left(\frac{nFvD}{RT} \right)^{1/2}$$

In this equation, I_p is peak current (A), K is constant, n is number of transferred electrons in the redox reactions, A represents the electrode area (cm^2), D is diffusion coefficient (cm^2/s), F is Faraday constant (C/mol), C is concentration (mol/cm^3) and v is scan rate (V/s).

The ration of the peak current is 1 for reversible electrochemical reaction and the peak positions do not alter when the san rates change.

The shapes CV curves of a redox reaction can be directly used to determine whether the reaction is reversible, partially reversible or irreversible. Further analyzing

the variation of peak position as a function of scan rate allows an estimate for the diffusion coefficient and electron transfer rate constants.

3.4.2 Cycling Charge-Discharge (CCD) Tests

Cyclic charge-discharge (CCD) technique is widely used to test the performance and cycle-life of batteries and supercapacitors. In CCD technique, charge and discharge are confined within a potential window and are conducted at constant current until the set voltages are reached. [45] A repetitive loop of charging and discharging is called a cycle. A typical CCD curve displays the potential (vs. reference electrode) versus the capacity of the cells. For a CCD cycles, the total charge (capacity) of each cycle is measured and the capacitance C is determined by the following equation:

$$C = \frac{Q}{V}$$

Where Q is the charge in coulombs, and V is the voltage window applied.

3.5 Overview of Methodologies

E.Coli expression system is suitable as the host for the expression of the recombinant protein designed in this thesis. The designed protein has a molecular weight of 18 KDa and no post-modification after translation is required. As a result, it is easily scaled up through this leaky express method.

Solution-phase synthesis methods are efficient and effective in nanomaterial synthesis. The expressed fusion protein is of amphiphilic nature. It can self-assemble into nanoscale fibrous structures and are stable in solution over a wide temperature window therefor are suitable for templates in aqueous, organic or their mixture environment of solution-phase synthesis.

A combination of the XRD, SEM, TEM, XPS and NMR characterizations can provide the morphologies, chemical and crystal information of the synthesized nanomaterial and are indispensable in the research work of this thesis. Other techniques, such as Fourier Transform Infrared (FTIR) Spectroscopy and Atomic Force Microscopy (AFM) are also adopted for better revelation of the synthesized materials.

Electrochemical analysis techniques, such as CV and CCD, directly reveal the lithium and sodium storage preformation of the electrode materials synthesized from the self-assembling fibrous protein and can provide persuasive evidences to demonstrate the hypothesis of the thesis.

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Chapter 4

Self-Assembling Behavior of GPG-AG3-His tag Fusion Protein

In this chapter, the expression of the elastin fusion protein (GPG-AG3-His tag) using the E.Coli expression system is presented. It is hypothesized that the fusion protein will behave self-assembling properties and therefore the self-assembling behavior of the obtained fusion protein is investigated. Protein nanobeads and nanofibers are obtained through manipulating the incubation temperature as well as the incubation time. The morphologies of the obtained protein beads and nanofibers are visualized by AFM and TEM.

4.1 Introduction

GPG proteins are double hydrophobic polypeptides (Figure 4-1). They can self-assemble into nanofibers via a dehydration process. The driving forces that governed the GPG peptides to form nanofibers are hypothesized to be hydrophobic interaction and hydrogen interaction, as illustrated in Figure 4-2.

Figure 4- 1 Amino acid sequence of GPG-His tag protein.

Figure 4- 2 The proposed mechanism for GPG-His tag fiber formation from GPG protein.

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Self-assembling of GPG-AG3-His tag fusion protein is inspired from the GPG protein. In this genetically engineered protein (the amino acid sequence is given in Figure 4-3), AG3 is fused to the C-terminus of the GPG structure, followed by the histidine tag. The hydrophilic AG3 sequence is shorter compared with the GPG sequence, therefore in the new protein, GPG sequences are not masked and they can form fibers by the temperature-induced method, similar as GPG does (Figure 4-4). [1, 2] The hydrophobic

GPG structures assemble to form the fiber structure via dehydration process while the hydrophilic domains, which are His tag and AG3, are exposed as the exteriors. In the synthesis, upon increasing the temperature of the GPG solution to 45 °C, the solution slowly turned visibly cloudy after overnight incubation but no precipitations can be observed.

Figure 4- 3 Amino acid sequence of GPG-AG3-His tag fusion protein.

Figure 4- 4 Forces involved during the self-assembly of GPG-AG3-His tag protein.

4.2 Experimental Methods

4.2.1 Synthesis

(1) Construction of GPG-AG3-His tag proteins

AG3 dsDNA fragment was constructed by annealing the corresponding forward (5'-GCCGGTCGACGCTTATTTCTTCTGGTGCTCCTCTCCTATGCCTCCTTTTCTCGAGGCGG-3') and reverse (5'-CCGCCTCGAGAAAAGGAGGCATAGGAGGAGCACCAGAAGAATAAGCGTCGACCGGC-3')

AG3 oligonucleotides. After digestion by XhoI and SalI restriction endonucleases, the obtained AG3 DNA fragments were re-ligated into the plasmids pET22b-GPG and then transformed the obtained plasmid into *E.coli* strain BL21(DE3) cells. These cells were then inoculated in 1 L of terrific broth containing 50µg/mL ampicillin.

(2) Protein expression

Leaky expression method was adopted for the expression of GPG-AG3-His tag proteins and the protein was purified using Ni-nitriloacetic acid (NTA) column. Generally, the cultured cells were collected by centrifuging at 8 000 rpm for 30 minutes at 4 °C. The obtained precipitates were re-suspended in denaturing lysis buffer (8 M urea, 100 mM NaH₂PO₄ and 10 mM Tris-Cl; pH 8.0) at 10 mL/g wet cell mass and subjected to sonication for 10 minutes on ice. The obtained solution was then centrifuged at 18 000 rpm for 2 hours and the supernatant was mixed with NTA slurry for 1 hour. The mixture was loaded onto a NTA column. This column was washed 3 times by wash buffer (8 M urea, 100 mM NaH₂PO₄, 10 mM Tris-Cl; pH 6.5). Bound proteins were eluted twice with different pH elution buffer (8 M urea, 100 mM NaH₂PO₄, 10 mM Tris-Cl; pH 5.5 first and then 4.0). Eluted proteins were dialyzed against deionized water (pH 4.5) and then lyophilized. The obtained GPG-AG3-His tag block proteins were kept as lyophilized powder at 0 °C.

(3) GPG-AG3-His tag stock solution preparation

Stock protein solution was obtained by dissolving the polypeptides powder in 4 °C deionized water (Milli-Q, 18.2 MΩ) with the concentration of 100 µM.

(4) Fusion protein fibers preparation and templates for Pt or Pd nanostructure synthesis

To prepare the bead or fiber, stock solution, deionized water (4 °C) and 2,2,2-trifluoroethanol (TFE, Sigma Aldrich, 100%) were mixed together in the volume ratio of

2:7:1. The temperature of the mixture was gradually increased to 45 °C with the step of 1 °C /min and kept at 45 °C for different time periods before use. Typically, bead structure is obtained after 12 h incubation and for 3 d for the fibrous morphologies. TFE (10%) were added to facilitated the fiber formation because it can promote the amount of β -turn and β -sheet structures through desolvation of helical peptides.

4.2.2 Fourier Transform Infrared (FTIR) Characterization

FTIR characterization was conducted on FT-IR/NIR Spectrometers (Frontier, PerkinElmer). Pristine protein powder or lyophilized protein fiber were mixed with analytic grade potassium bromide (KBr) with a ratio of 1:9 (mass ratio) and pressed into pallets. The recorded wavelength range is within 1800 – 1500 (cm^{-1}).

4.2.3 Morphologies Characterization

The morphologies of the GPG-AG3 fibers and noble metal nanotubes were visualized by using TEM (operated at 200 kV, TEM, JEOL, model JEM-2010) and atomic force microscopy (AFM, Dimension 3100 with Nanoscope IIIa controller, Veeco, CA, USA). The characterization was carried out under ambient conditions in a tapping mode with a Si tip. In a typical operation, the samples were prepared by drop-casting 10 μL of the sample solution onto clean hydrophilic silicon wafer and dried at room-temperature. Transition electron microscopy (TEM) characterizations were carried out using on JEM-2010 (TEM, JEOL) operating at 200 kV. The samples were prepared by dropping 2.5 μL of the solution onto the copper grid and negatively stained by phosphotungstic acid solution (PTA :2%, pH 7.2). The sizes distribution information was obtained on a Malvern Nanosizer.

4.3 Results and Discussion

4.3.1 SDS-PAGE Results

According to the SDS-PAGE results in Figure 4-5, the fusion protein have a molecular weight of ~18 kDa , which is very close to the theoretical value of this fusion protein, this result demonstrate the successfully construction of the multifunctional fusion protein.

Figure 4- 5 SDS-PAGE results indicating the molecular weight and purity of the fusion protein.

4.3.2 AFM&TEM Characterization

The morphology evolution of the incubated stock solution at 45°C is revealed by AFM technique. Figure 4-6 shows the morphologies (AFM images) of the GPG-AG3-His tag beads obtained at the incubation time of 12 h. It can be determined (according to the height profiles) that the beads have thickness of 5 - 10 nm with width of 20 – 50 nm. Besides the small beads, there are also some large particles with the thickness around 50 nm. The appearance of the large particles can be ascribed to the agglomeration of small beads.

Figure 4- 6 AFM image of elastin protein beads with the height profiles.

Besides the AFM characterization, TEM characterization was also carried out to visualize the protein bead particles. As are presented in Figure 4-7 (a), the small protein beads were the sizes of 10 nm. A higher magnification image (Figure 4-7(b)) reveals that the beads were not uniform and have different shapes.

Figure 4- 7 TEM images of the GPG-AG3-His tag protein beads.

The sizes distribution of the beads was further investigated using a Malvern instrument, where the size distributions were obtained through two modes, which were based on the parameter of intensity or number. In the first mode, three groups of sizes, which are 10.61, 161.8 and 4789 nm were obtained, as shown in Figure 4-8. Peaks around 161.6 nm can be assigned to the larger particles form during assembling, which is in accordance with the AFM results. Sizes of micrometer scale can be attributed to the undissolved protein powder. In the second mode, where the percentage of the particles are calculated, only one size was obtained and the value of 6.23 nm is once again well match with the AFM results, as Figure 4-9 displays. A 100% percentage value indicates that as long as the conditions are carefully controlled, high yield of beads is possible.

Figure 4- 8 Sizes distribution of the beads based on intensity investigated using a Malvern instrument.

Figure 4- 9 Sizes distribution of the beads based on number investigated using a Malvern instrument.

The AFM characterization results displayed in Figure 4-10 (a-b) show that the obtained matured GPG-AG3-His tag fibers are more than 10 μm in length. Both straight and curling fibers can be observed. Figure 4-10 (b) shows clearly that these fibers are composed of protein beads of 20 – 50 nm in diameters, which determine the diameter of the fibers. This observation indicates that the long fibers are formed as a result of the further assembling of the beads appearance in the initial stage of self-assembling process in the longitude direction. TEM characterization in Figure 4-10 (c-d) give the same results, as the AFM characterization does. The dimension of formed GPG-AG3 fibers is close to the dimension of GPG fibers reported, which confirmed that GPG-AG3 protein maintains the properties of GPG protein to form fiber structures.

Figure 4- 10 (a-b) AFM images of the GPG-AG3-His tag fibers. (c-d) are TEM images of the GPG-AG3-His tag fibers under different magnifications.

4.3.3 FTIR Results

FTIR characterization was carried out to analyze the structures of the pristine GPG-AG3-His tag protein and the as-prepared fibers and the results are displayed in Figure 4-11. The amide carbonyl stretching frequencies of the amide I region between 1600 and 1700 cm^{-1} is characteristic information used to estimate the amide mode of peptides. It can be observed from Figure 4-8a that the pristine protein sample shows a broad absorption peak centered at 1659 cm^{-1} , which proved that the peptides had random structure. In contrast, for the GPG-AG3-His tag fibers, only one peak at around 1630 cm^{-1} , which is one of the characteristic peaks of protein β -sheet, is detected, confirming the content of β -sheet structure dominates in the fibers. [3,4]

Figure 4- 11 FTIR spectra of the pristine GPG-AG3 His tag proteins and GPG-AG3 His tag fibers.

Figure 4- 12 Morphology of self-assembling GPG-His tag protein.

The GPG-His tag protein was also expressed and the self-assembling behavior was also investigated. As can be seen from Figure 4-12(a), for GPG-His tag fusion protein, although fibrous structure can be found, the yield is much lower than GPG-AG3-His tag fusion protein and the diameters are larger the latter. The dominant morphology is sheet-like structures (Figure 4-12(b)). It is speculated that GPG-His tag fusion protein is much more hydrophobic than GPG-AG3-His tag fusion protein, there is propensity to form sheets and assemble into larger fibers.

4.4 Conclusions

In this chapter, the GPG-AG3-His tag proteins have been successfully expressed in the *E.Coli* expression system using the leaky expression method. The protein possesses the self-assembling properties and can self-assembling into protein beads or fibers at 45 °C at different incubation time periods.

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Chapter 5*

Synthesis of Pt and Pd Porous Nanotubes

In this chapter, the synthesis of porous one-dimensional (1D) Pt and Pd nanotubes are presented. It is hypothesized and then demonstrated that the AG3 sequence in the GPG-AG3-His tag protein pertains the noble metal mineralization capability. By using the self-assembling GPG-AG3-His tag fusion protein fibers as templates and reducing agent, a simple hydrothermal treatments of the protein fibers solutions with the corresponding precursors at 80 °C yields Pt or Pd nanoparticles decorated on the fibrous proteins. When these 1D porous noble metal nanotubes are used as catalysts in lithium-O₂ battery cathodes, stable cycling performance and promising energy efficiencies are obtained, which are superior to those with similar structures and compositions such as Pt or Pd decorated on CNT

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5.1 Introduction

Noble metals are a group of elements which contain ruthenium, rhodium, palladium, silver, osmium, iridium, platinum, and gold. They are resistant to oxidation and corrosion in moist air or oxidative environment and are widely applied as catalysts for chemical reaction, such as sensors, proton exchange membrane fuel cells and lithium-O₂ batteries (LOBs). [1-3]

The introduction of nanotechnology has boosted their catalytic performance through fabricating them into nanoscale structures. Especially, one-dimensional (1D) noble metal nanowires and nanotubes are advantageous as they have higher surface areas compared with their bulk/particle counterparts and they can also promote faster electron and mass transport and are receiving growing research interests. [4]

Even though various techniques (physical or chemical methods), such as physical or chemical vapor deposition (PVD/CVD) , [5, 6] electrochemical deposition, [7] surfactant-assisted chemical synthesis, [8] absorption and reduction via multistep self-assembly methods [9] are available for 1D noble metal nanomaterial synthesis, there are always requirements for novel synthesis methods that are simple, cost-effective while can allow more precise control. Among the potential solutions available, template-guided synthesis methods, especially methods using natural materials as starting materials, which allow more controlled growth and hierarchical organization of 1D nanomaterial, are desirable while are rarely reported and still far from well-developed.

In this chapter, we showed that the AG3 in GPG-AG3-His tag fusion proteins retained the mineralization capability to reduced Au³⁺, Pt⁴⁺ and Pd²⁺ into Au, Pt and Pd. After immersed the GPG-AG3 His tag fibers into Pt or Pd precursors and further treated under a mild-temperature hydrothermal process, Pt or Pd nanoparticles with sizes of ~3-5 nm are formed on the fusion protein fibers. After annealing (under Ar flow protection) the obtained Pt or Pd loaded 1D fibers at 300 °C for 1h, the fusion protein fiber were turned into amorphous carbon. The hierarchical nanotubes which have the structure of Pt

or Pd nanoparticles decorated on amorphous carbon support were thus obtained. When applied as cathode catalysts for LOBs, the Pt porous nanotubes have low charge potentials at around 3.8 V and an energy efficiency of ~65% at a current density of 100 mA/g is obtained. The oxygen cathodes based on Pd nanotubes have stable charge voltage of 4.1 – 4.2 V while the typical discharge voltages are between 2.5 and 2.6 V, rendering energy efficiency of ~ 63% at 50 mA/g.

5.2 Experimental Methods

5.2.1 Platinum and Palladium Synthesis

The Pt and Pd porous nanotube are synthesis using a mild temperature hydrothermal method and a following annealing under Ar flow in tube furnace. Typically, 4 mL of GPG-AG3 fiber solution, 2 mL of 10 mM H_2PtCl_6 (Sigma Aldrich, 99%) were mixed and diluted using 23 mL deionized water. After incubation for 30 minutes, 1 mL polyvinylpyrrolidone (PVP, Mr = 630 000, K90, International Laboratory, USA) was added to the above solution. The mixture solution was then transferred to a 40 mL Teflon-lined stainless – steel autoclave and maintained in an oven at 80 °C for 3 hours. After the reaction finished, the container was allowed to cool to room temperature. The samples were collected by centrifuge and washed with deionized water and then ethanol for three times. The precipitations were re-suspended in DI water and subject to freeze dry. The freeze dry product was then annealed at 300 °C for 1 h under Ar protection in tube furnace with the heating rate of 5 °C/min. In the synthesis of palladium nanotubes, a similar procedure in which H_2PtCl_6 was replaced by 10 mM PdCl_2 solution which was prepared by dissolving PdCl_2 (Sigma Aldrich, 99%) in 5 mM HCl. As comparison, Pt and Pd nanoparticles were also decorated onto self-assembling GPG-His tag protein or CNT by using H_2PtCl_6 or PdCl_2 and NaBH_4 (Sigma Aldrich, 99%).

5.2.2 Morphologies and Phases Characterization

The morphologies of the noble metal nanotubes were investigated by using transmission electron microscopy (which is operated at 200 kV, TEM, JEOL, model JEM-2010, JEM-2100) and/or field-emission scanning electron microscopy (FESEM, JEOL, Model JSM-6340, JSM-7600F with TED mode). The phases of the annealed Pt and Pd nanotube samples and control samples were characterized by using thin film XRD (X-ray diffraction, Shimadzu) with Cu K α irradiation. The nuclear magnetic resonance (NMR) data were collected on a NMR spectrometer (Bruker 300).

5.2.3 Lithium-O₂ Battery Testing

The electrocatalytic activity of Pt and Pd nanotubes and control samples for oxygen reduction reaction (ORR) and oxygen evolution reaction (OER) was examined in LOBs. The oxygen cathode was prepared by casting the active material slurry [active materials: Super carbon: Polyvinylidene fluoride (PVDF) =2:7:1 in N-Methyl-2-pyrrolidone (NMP); mass ratio] onto steel mesh and dry at vacuum oven overnight at 50°C. The average mass of the active materials was around 1.0 mg for each electrode. The LOBs were assembled for electrochemical characterization in which the as-prepared oxygen cathode served as the working electrodes, lithium foils served as the reference and counter electrodes. 1 M LiCF₃SO₃ in tetra(ethylene) glycol dimethyl ether (TEGDME) were used as the electrolytes. All cells were assembled in a glove box system filled with argon (MBraun UniLAB; the concentrations of H₂O and O₂ were both below 0.1 ppm). LOBs were aerated with O₂ and sealed afterwards. The LOBs were tested on a NEWARE system. A potential window of 2.0 – 5.0 V was used for the charge and discharge processes.

5.3 Results and Discussion

5.3.1 Study of the Mineralization Capability of GPG-AG3-Histidine Protein

AG3 peptides have been applied in gold nanoparticles and silver crystallite synthesis under specific buffer solution systems. To investigate whether its function is maintained in the GPG-AG3 protein, experiments to synthesize Au were conducted by mixing the

GPG-AG3 His tag stock solution (100 μM , 0.5 mL) with HAuCl_4 solution (10 mM, 0.5 mL) and the mixture was kept at 4, 25 or 45 $^{\circ}\text{C}$ in a dark place (Table 5-1). After incubation overnight, the sample with both HAuCl_4 and GPG-AG3-His tag protein turned wine pink or gold red while no color change could be seen in the sample with only Au precursors, as shown in Fig 5-1. The TEM image in Figure 5-2 shows the morphologies and selected area electron diffraction pattern (SAED) of the Au-protein product. It can be observed from Figure 5-2(a) that nanostructure gold particles with different shapes are formed on thin-film protein films. The SAED pattern reveals the crystalline nature of these Au nanostructures. These results demonstrate that the mineralization ability of AG3 domain is not affected by the other domains in the GPG-AG3 peptides.

Table 5- 1 Temperature –dependent reducing capacity of GPG-AG3-His tag for Au

Temperature/ $^{\circ}\text{C}$	Time needed to reduce HAuCl_3
4	No color changes in 2 months
25	5 - 7 days
45	3 - 6 hours

Figure 5- 1 (a) is optical image of the sample with both Au precursor and GPG-AG3-His tag, (b) control sample where no GPG-AG3 His tag was added, (C) the XRD pattern of Au nanostructure.

Figure 5- 2 (a) is the TEM image and (b) is the SAED pattern of the Au nanostructures.

5.3.2 Pt and Pd Nanotubes

The synthesis procedures for the hierarchical nanotubes with Pt or Pd nanotubes are schematically illustrated in Figure 5-3. Generally four steps are included, which are : (1) GPG-AG3 protein expression; (2) Preparation of fusion protein fiber; (3) Binding and reduction of noble metal precursors; and (4) Annealing in Ar flow.

Figure 5- 3 Schematic illustration of the procedures for synthesizing Pt and Pd nanotubes.

Figure 5- 4 TEM and SAED images of protein fiber incubated in Pt precursor at 45 °C for 3 days.

The mineralization capability of the GPG-AG3-His tag towards Pt was initially investigated by mixing the GPG-AG3-His tag stock solution (100 μM, 0.5 mL) with H_2PtCl_6 solution (10 mM, 0.5 mL) and the mixture was kept at 45 °C for 3 days. The precipitation were characterization by TEM and the results (Figure 5-4) reveal that it cannot mineralize Pt under the conditions similar as Au, therefore, the temperature of the synthesis system was increased to 80 °C in a hydrothermal setup.

In the synthesis of 1D Pt and Pd nanostructures using self-assembling GPG-AG3-His tag fusion protein, the histidine tags and AG3 domains of the GPG-AG3 fusion protein, which are expressed on the surfaces of the fibers, adsorbed $[\text{PtCl}_6]^{2-}$ or $[\text{PdCl}_4]^{2-}$ ions to form inorganic/protein complex and/or further reduced the ions to nanoparticles by donating electrons from the hydroxyl group ($-\text{OH}$) of tyrosine in AG3 sequences to the adsorbed inorganic species.[10] Yellow (for Pt) or brown (for Pd) flocculation occurred within 30 minutes after adding metal precursors to the transparent fiber solution. Black or brown precipitations were obtained for Pt or Pd, respectively, after the hydrothermal treatment.

Figure 5-5 shows the Pt/GPG-AG3-His tag fiber composites products after hydrothermal treatment at 80°C. It can be observed that the protein fiber structure is maintained while the degree of entanglement increases (Figure 5-5 (a)). The surfaces of the fibers are covered by dark layers, as revealed in Figure 5-5 (b).

Figure 5- 5 (a-b) TEM images of hydrothermal product: Pt nanostructures before annealing.

The XRD characterization result of the hydrothermal product in Pt synthesis are shown in Figure 5-6 (a), which clearly shows the typical peaks of crystalline Pt and also demonstrates that the reduction activity of AG3 remains and $[\text{PtCl}_6]^{2-}$ can be reduced to Pt^0 with the GPG-AG3-His tag protein fibers under such hydrothermal conditions. For the Pd/ GPG-AG3-His tag fiber sample without annealing, besides metallic Pd, the oxide phase, which is PdO (Figure 5-6 (C)), also appear, probably due to the oxidation of Pd under the hydrothermal condition. The XRD characterization of the annealed hydrothermal products for Pt and Pd nanotubes samples are displayed in Figure 5-6 (b) and (d), respectively. The five characteristic peaks at $2\theta = 39.92^\circ, 46.39^\circ, 67.76^\circ, 81.59^\circ$ and 86.12° correspond to the (111), (200), (220), (311) and (222) planes of Pt (JCPDS Card: 87-0640), respectively. For Pd nanotubes, the four peaks observed in the XRD pattern ($2\theta=39.44^\circ, 45.41^\circ, 66.52^\circ$ and 80.60°) also match well with the standard Pd pattern (JCPDS Card: 87-0641). The broadened diffraction peaks indicates that both Pt and Pd exist as small crystalline nanoparticles. For the annealed samples, the crystals'

average sizes of Pt and Pd are estimated to be 4.2 nm and 3.1 nm, according to Scherrer equation:

Crystallite size = $K\lambda/\beta\cos\theta$, where K is the shape factor, λ is the wavelength of the radiation, β is the line broadening at half of the maximum intensity (FWHM), θ is the peak position.

Figure 5- 6 XRD patterns of the Pt, Pd nanostructure before and after annealing. (Peaks marked with ★ are PdO)

The morphologies of the annealed hydrothermal products of Pt or Pd nanostructures are visualized by TEM and the results are displayed in Figure 5-7 (a-b) or (c-d), respectively. As can be observed from Figure 5-7 (a-b) and (c-d), the treated hydrothermal products show increased diameters and surface roughness in comparison with pristine fusion protein fibers as a result of the growth of nanoparticles on surfaces of the fibers. The obtained Pt or Pd nanotubes strictly replicate the morphologies of the protein fiber

templates and remain several micrometers in length. The protein fibers were converted to amorphous layer after annealing, which become the interior part of the tubes and act as support matrix for the Pt or Pd nanostructure.

Figure 5- 7 (a-b) TEM images of Pt; (c-d) Pd nanotubes under different magnifications.

The TEM images in Figure 5-8 (a) and (c) clearly reveal that Pt and Pd nanoparticles with sizes around 3-5 nm are supported on amorphous layers. The high resolution TEM images (Figure 5-8 (b) and (d)) and the selected area electron diffraction patterns (SAED, inset images in Figure 5-8 (b) and (d)) show that both nanostructures are of polycrystalline nature with well-defined lattice planes. This observation is consistent with

the XRD results of Pt and Pd nanotubes discussed above and also in agreement with the reported studies on Pt and Pd nanotubes or nanowires composed of nanoparticles.

Figure 5- 8 HRTEM images of Pt (a-b) and Pd (c-d) nanostructures. Insets in (b) and (d) are the SAED patterns of the Pt and Pd nanotubes, respectively.

Energy dispersive spectrometry (EDS, STEM) and line scan technique were conducted to further analyze the Pt/protein fiber after annealing. The line scan profiles in Figure 5-9 (b) prove the hollow porous nature of the annealed products. The C and Pt mappings displayed in Figure 5-9 (c-d) reveal that Pt nanoparticles uniformly coat the

hollow amorphous carbon, forming the exterior of the nanotubes. These results clearly prove that Pt nanoparticles selectively grow on the surface of the protein fibers.

Figure 5- 9 (a) STEM images of the Pt nanostructure based on the protein fibers; (b) line scan analysis of the Pt/C nanostructure shown in (a); (c-d) are the elemental mapping images of the C and Pt of the nanotubes shown in (a).

Figure 5-10 exhibit the FESEM images (EDS under TED mode of FESEM 7600) of the Pd/Fiber sample before annealing. It can be seen that Pd nanoparticles specifically grew on the fibers, according to the elemental mapping of Pd and C. The occurrence of O is from the oxide phase of Pd, which has been detected by XRD.

Figure 5- 10 (a) TED images of the Pd nanotubes networks based on the polypeptide fibers. (b-d) the elemental mapping images of element C, O and Pd of the nanotubes.

All the results discussed above demonstrate that Pt and Pd porous nanotubes are obtained by using the strategy proposed at the beginning of this dissertation.

The morphologies of the annealed Pt and Pd nanotubes are further studied by FESEM characterization. The FESEM images in Figure 5-11 show that both Pt and Pd nanotubes are interconnected to form network structures. Such porous hollow network structure is desire in many applications, such as electronics, catalysts.

Figure 5- 11 (a-b) FESEM images of the Pt nanotubes based on the polypeptide fibers. (c-d) FESEM images of the Pd nanotubes based on the protein fibers. The samples were annealed at 300 °C.

As a comparison, GPG-His tag self-assembling fibrous proteins were also used as template for Pt and Pd nanostructures synthesis. Considering this protein doesn't have the AG3 sequences, reducing agent of NaBH_4 were added. The morphologies of the GPG-His tag/Pt and b GPG-His tag/Pd are displayed in Figure 5-12 and 5-13. As can be observed, the fibrous structures are tangled together and the loading amount of Pt or Pd nanoparticles is small. Besides, the size distribution of the particles is not uniform. These results suggest that AG3 play a very important role in directing the fiber formation as well as tuning the inorganic particles loading.

Figure 5- 12 TEM images of Pt –derived for GPG-His tag.

Figure 5- 13 TEM images of Pd –derived for GPG-His tag.

Pt or Pd nanoparticles with the same sizes (2-10 nm) were also decorated onto the surface of multiwall carbon nanotubes (CNT) to form similar 1D structures which the same chemical components, as are demonstrated by the TEM and XRD results presented in Figure 5-14, 5-15 and 5-16. The major difference between Pt/CNT (Pd/CNT) and the elastin fusion protein based Pt (Pd) nanotubes is that the loading amount of Pt (Pd) nanoparticles is much smaller on CNT than on the fusion protein templates.

Figure 5- 14 TEM images of 1D CNT/Pt : Pt decorated on CNT.

Figure 5- 15 TEM images of 1D CNT/ Pd : Pd decorated on CNT.

Figure 5- 16 XRD patterns of the CNT/Pt and CNT/Pd.

5.3.3 Catalytic Performance of Pt and Pd Nanotubes in LOBs Cathodes

The electrochemical characterization is performed on the LOBs using a standard setup (a modified Swagelok configuration). The discharge and charge profiles (capacity versus voltage) of the LOBs cell using the as-synthesized Pt nanotubes are presented in Figure 5-17 (a). At 100 mA/g, the first cycle discharge voltage is around 2.50 V and the charge voltage reaches maximum at 3.69 V when charging completes at the cut-off capacity at 1000 mAh/g. The charge and discharge potentials gradually stabilize in the following cycles. The discharge voltages are in the range of 2.40 to 2.50 V while the ending charge voltages of 3.7 to 4.0 V. These values are ~ 0.7 V lower than the charge voltage of pure Super P carbon which are 4.5 - 4.7 V under the same current density (Figure 5-19). These results are in similar with the data reported using Pt as catalysts for the oxygen cathode catalysts with the catalysts loading of 30% or more while in this study the active catalysts (Pt and carbon support) loading are only 10%. [11] The energy efficiency (discharge energy/charge energy) of the Pt nanotubes-based cathodes is calculated 65%. No capacity loss is found in the testing cycles (Figure 5-17 (b)), showing good cycling performance.

Figure 5- 17 (a) Voltage-capacity profile of the LOBs based on the Pt nanotube under 100 mAh/g; (b) representing the cycling performance of the cathode using Pt nanotubes as catalysts.

Figure 5- 18 (a) FESEM and (b-c) TEM images of the Pt tube from cathode discharge to 1000 mAh/g at 100 mA/g.

The morphology of discharge product was investigated by SEM and TEM and the results were shown in Figure 5-18. The electrode was discharged to 1000 mAh/g and disassembled under Ar after finishing the 10th cycle. From the SEM image in (a), compared with the fresh Pt tubes, it is found that the diameter and roughness of the tubes had increased after discharge and some particles around 100- 200 nm were also form. The TEM images demonstrate that (1) the 1D structure was maintained; and (2) the surface of the tubes were covered by a thin fluffy substance layer (Figure 5-18(b-c)) and some large particles were also attached to the tube. The nature of these substances are speculated to be Li₂O₂ according to the cell testing condition as well as the similar morphologies of Li₂O₂ and their deposition behavior from the reported studies. [2-3, 11]

Figure 5- 19 (a) Voltage-capacity profile of the LOBs based on the Super P cathodes at 100 mAh/g; the charge voltages are within 4.3 – 4.8 V; the discharge voltages

are around 2.3 – 2.6 V and the round-trip efficiencies are: 1st cycle 57.8% ; 2nd cycle 56.4%; 3rd cycle 54.4%.

The Pd nanotubes were also introduced into the oxygen cathodes and the LOBs based on Pd nanotubes show stable charge voltage of 4.1 – 4.2 V and the average discharge voltages are in the range of 2.5 to 2.6 V, rendering an energy efficiency of ~ 63% (current density: 50 mA g⁻¹; capacity: 800 mAh g⁻¹; Figure 5-20). The slight increase of charge potentials is supposed to be related to less catalytic activity of the Pd nanotubes, which results in the formation of other lithium compounds such as HCO₂Li or Li₂CO₃, etc. which are the side product formed during the discharge processes, [12] as revealed by ¹H NMR in Figure 5-21.

Figure 5- 20 (a) Voltage-capacity profile of the LOBs based on the Pd nanotube cathodes under the current density of 50 mAh/g; (b) figure representing the cycling performance of the Pd nanotube-based cathode.

Figure 5- 21 ^1H NMR spectrum of the Pd cathode taken from the cell after discharge has completed at the 10th cycle, indicating the presence of HCO_2Li side products formed during the discharge process.

Figure 5- 22 ESA measurement based on H adsorption and desorption via CV techniques in 0.5 M H_2SO_4 aerated with argon gas.

To further reveal the discrepancies in catalytic activities of Pt and Pd nanotubes, electrochemical surface area (ESA) measurement was carried out to identify their respective active catalytic surface areas. The results are shown in Figure 5-22. The active

surface area of Pt nanotubes is calculated to be $\sim 35 \text{ m}^2/\text{g}$ and Pd nanotubes $\sim 15 \text{ m}^2/\text{g}$ based on equations 5-1 and 5-2. [13, 14]

$$ESA(Pt) = \frac{Q_H}{[Pt] \times 0.21} \quad (5-1)$$

$$ESA(Pd) = \frac{Q_H}{[Pd] \times 0.42} \quad (5-2)$$

Where Q_H is the charge associated with the hydrogen adsorption and desorption, [Pt] and [Pd] are the loading amount of the Pt and Pd catalysts (g/cm^2). The coefficients of 0.21 and 0.42 represent the charge required to oxidize a monolayer of H_2 on bright Pt and Pd.

Figure 5- 23 The LOB performance of GPG-His tag based Pt (a) and Pd (b) at 50 mAh/g.

The Pt and Pd nanostructures based on GPG-His tag were also applied as cathodes materials in LOBs. For Pt based on GPG-His tag, it is found that (1) the discharge potential is low with the overpotential of $\sim 0.5 \text{ V}$ even at low current density of 50 mAh/g; (2) the charge potential in the first cycle is within 3.0-3.5 V, however, as cycling continued, the charge potentials dramatically increased compared with the previous cycle, according to Figure 5-23(a). Pd based on GPG-His tag shows similar charge and discharge potentials compared with the Pd nanotubes counterpart. However, the cycling stability is much poorer, as shown in Figure 5-23(b), at the 7th cycle, the discharge process ended before the cut-off capacity of 1000 mAh/g. These results confirm that both

the Pt and Pd tubes based on GPG-AG3-His tag do have superior advantages over random Pt and Pd nanostructure based on GPG-His tag in catalytic performance in LOBs.

Figure 5- 24 CNT/Pt for LOBs at the current density of 50 mA/g with the capacity cut-off at 150 mAh/g: (a) cycling, (b) voltage-capacity profile.

The catalytic activity of CNT/Pt and CNT/Pd in LOBs cathodes was evaluated. Figure 5-24 displays the results for CNT/Pt. It can be observed that the cathodes have the discharge and charge voltages of ~ 2.6 and 3.9 V. It is worth to note that the charge voltages is ~ 0.4 V higher than those from the Pt nanotubes, which had been discharged higher capacity of ~ 1000 mAh/g. The CNT/Pd based cathodes showed the same charge and discharge voltage as Pd nanotubes (which was discharged to 800 mAh/g) in the range of 250 mAh/g. However, the discharge overpotentials tend to increase and higher rates and when cycling number increases at the same rates (Figure 5-25(a)), suggesting that the ORR performance of CNT/Pd is not as stable as Pd nanotubes. These comparisons demonstrate that Pt or Pd nanotubes are more stable and efficient in LOBs than the CNT/Pt or CNT/Pd even though they share similar morphology and/or chemical compositions. The full coverage of noble metal nanoparticles on carbon support, thus more catalytic sites compared with CNT/Pt or CNT/Pd, as well as less exposure of carbon (which is probably prone to be deteriorated) may have improved the performance of Pt or Pd nanotubes electrochemical performance in LOBs.

Figure 5- 25 CNT/Pd for LOBs at different current densities with the capacity cut-off at 250 mAh/g: (a) cycling, (b) voltage-capacity profile.

Table 5- 2 Summary of the LOBs performance of the Pt and Pd derived from various templates

ID	Rate (mA/g)	Capacity (mAh/g)	Charge voltage (V)
Pt nanotubes	100	1000	3.6-3.8
Pd nanotubes	50	800	4.2-4.3
Pt on GPG	50	1200	3.3-4.0; increase
Pd on GPG	50	1000, unstable	4.2-4.5
CNT/Pt	100	150	3.9-4.0
CNT/Pd	50	250	4.3-4.5

5.4 Conclusions

In conclusion, Pt and Pd nanotubes were successfully synthesized by using the designed genetically engineered fusion protein fibers from GPG-AG3-His tag monomer. The catalytic performance of the as-synthesized Pt as well as Pd nanotubes in LOBs cathodes was investigated. The GPG-AG3-His tag protein fiber templates were obtained via a temperature-triggered self-assembling process. The fusion protein nanofibers expose functional AG3 sequences on their surfaces, which not only bind the Pt and Pd ions but also reduced the bonded ions to the corresponding nanoparticles under mild hydrothermal treatment. Pt and Pd nanotubes were obtained after annealing the hydrothermal Pt and Pd nanostructures on the GPG-AG3-His tag fiber templates. The Pt and Pd nanotubes

were used as cathodes of LOBs and both types of nanotubes exhibited good cycling stabilities and effective OER catalytic activities. These results indicate that these fusion protein fibres are suitable for carbon-supported 1D noble metal nanotube synthesis which is promising candidates for use in catalysis applications.

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Chapter 6*

Synthesis of Transition Metal-Nitrogen/Carbon (M-N/C, M=Fe, Co) Catalysts

In this chapter, it is hypothesized that transition metal-nitrogen/carbon (M-N/C, M=Fe, Co) catalysts can be synthesized using the GPG-AG3-His tag fusion protein beads and CNT and the obtained M-N/C will be more active and efficient in LOB cathodes than Pt decorated on CNT. The synthesis of M-N/C (M=Fe, Co) catalysts are presented. By exploiting the transition metal ion coordination capability of the His-Tag in GPG-AG3-His tag fusion protein beads, M-N/C catalytic sites are generated on protein beads-decorated carbon nanotubes surface by mixing protein beads, hydrophilic carbon nanotubes and metal precursors solution at room temperatures and after annealing. When Fe-N/C catalysts are used as catalysts in lithium-O₂ battery cathodes, very stable cycling performance and promising energy efficiencies as well as high delivered capacities than Pt nanoparticle decorated on CNT are obtained.

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6.1 Introduction

Transition metal-nitrogen-carbon (M-N/C) catalysts are receiving ever-growing research interest after they are revealed to be highly effective in oxygen evolution reaction (ORR) since the 1980s.[1, 2] Over the past three decades, M-N/C catalysts have been synthesized and widely explored as alternatives to Pt for ORR in fuel cells, even though the structures of the M-N/C catalyst centers and the roles these catalytic sites play in ORR are yet to be fully understood. [3-5] Unlike many other catalysts used in ORR, M-N/C catalysts are revealed to have highly active catalytic sites and these catalytic centers are uniformly and densely distributed on the surfaces of the supporting materials. Therefore M-N/C catalysts are capable to promote fast electron transfer and facilitate surface electrochemical reactions. [6, 7]

Metal ions coordinating nitrogen sources such as phthalocyanine, [8] polyacrylonitrile, [9] ethylenediamine and cyanamide, [10, 11] etc. are commonly adopted as precursors in the preparation of M-N/C catalysts. Although they are regarded to be less expensive compared to noble metals, some of these precursors are still not economically available. Furthermore, concerns about their scarcity, toxicity and the low solubility of inorganic precursors in these organic, which further required more complicated synthesis strategies, seriously prevent their wide applications as precursors for M-N/C catalysts. [12, 13]

In this chapter, we present an efficient method for synthesizing M-N/C. Their catalytic performance in lithium-O₂ battery (LOBs) cathodes was also investigated. Specially, the M-N/C catalysts are synthesized by using the GPG-AG3-His tag fusion protein beads, hydrophilic carbon nanotubes (CNTs) and metal sulfates precursors as N (the histidine tags on the beads), metal and C sources, respectively. In the as-synthesized 1D M-N/C catalysts, the metal centre-Z structures (where Z = N or O, namely the M-N bonds) are formed via coordination process. Specifically, the transition metal ions are bonded with the O atom of carboxyl groups resided on the surface of hydrophilic CNTs and the N of histidine residues located on the surfaces of elastin protein beads. After

treating the samples at 800 °C, which is followed by acid leaching and finally a second annealing at 600 °C under argon flow consecutively, M-N/C centers are generated on CNTs surfaces. The annealed fusion protein-Fe-CNT (Fe-N/C, EFC) or fusion protein-Co-CNT (Co-N/C, ECC) samples are used as cathodes materials in LOBs. It is noteworthy that no other carbon additives and binder are used. It is found that the overpotential issues are alleviated because the M-N/C catalysts effectively tune the deposition and distribution of Li_2O_2 on the catalyst surface. Improved LOBs cycling performance and capacities are achieved, making them promising as catalysts for LOBs cathodes.

6.2 Experimental Methods

6.2.1 Synthesis

To synthesize water soluble CNTs, pristine CNTs (100 mg) were treated in H_2SO_4 (98%) and HNO_3 (69%) mixture (3:1, volume ratio) at 40 °C for 48 h in a reflux setup. After that, the mixture was first cooled to room temperature. The solution were then neutralized by 5 M NaOH solution followed by dialyzed against DI water until the pH value of the CNT solution reached 7. The obtained CNTs solution was finally freeze dry and stored at 0 °C for further use.

To prepared the protein beads 50 mg fusion protein powder were dissolved into 50 mL DI water (4 °C) and subsequently incubated in a hot sand bath at 45 °C for 12 h. The bead solution was subjected to sonication for 20 minutes before use.

The synthesis procedures of the M-N/C catalysts are as follows: hydrophilic CNT powder (20 mg) was dissolved thoroughly in DI water (20 mL). Then, protein beads solution (20 mL) was added. The mixture was stirred and kept at 80 °C. 1 mmol $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ or $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$ were added after purging with N_2 for 1 h. The mixture solution was kept at 80 °C for 8 h (under N_2 protection). After finishing the reactions, the samples were collected by centrifugation, washed with 99.9% ethanol and then DI water. These wash steps were repeated for 3 times. The precipitates were first freeze dry and

then annealed at 800 °C (with a heating rate of 5 °C/min). After annealing, the samples were then leached in 6 M HCl under stirring for 6 h, dried at 60 °C in vacuum oven. Finally, the dry samples were annealed at 600 °C for 1 h. The annealing processes were carried in a tube Ar gas furnace. Hydrophilic CNTs were also subjected to the same annealing treatment and used as a control sample.

6.2.2 Characterizations

The morphologies of the EFC or ECC catalysts were characterized using transmission electron microscopy (operated at 200 kV, TEM, JEOL, model JEM-2010, JEM-2100) and field-emission scanning electron microscopy (under TED mode operating at 30 kV, FESEM, JEOL, JSM-7600F). The phases of these samples were analyzed using XRD (X-ray diffraction, Shimadzu) with Cu K α irradiation ($\lambda = 1.5406$ Å). The nuclear magnetic resonance (NMR) data were collected on a Bruker 300 NMR spectrometer. XPS characterization was carried out on a Phoibos 100 spectrometer.

The oxygen cathodes were prepared by sandwiching the obtained EFC or ECC catalysts between two nickel foams pieces followed by pressing at 5 MP. The LOBs cells were assembled using the prepared oxygen cathodes as the working electrodes and lithium foils as reference and counter electrodes. 1 M LiCF₃SO₃ in tetra(ethylene) glycol dimethyl ether (TEGDME) were used as electrolyte. All LOBs cells assembling were carried out in a glove box (Unilab, MBRAUM, Germany, filled with Ar and the concentrations of moisture and oxygen were both below 0.1 ppm). The typical mass of the active materials was ~ 0.50 mg. The LOBs cells were then aerated with O₂ (99.99%) finally sealed using parafilms afterwards. Cyclic voltammetry (CV) tests were carried out on a Solartron equipment (the model is 1470E) in the potential window of 2.0 – 4.25 V. The charge-discharge data were obtained on a NEWARE system. The cells were allowed to fully discharged and charged between the potential window of 2.0 - 4.25 V without limiting the capacity under different current densities. When investigating the cycling performance, the cells were limited to a capacity of 800 mAh/g. All capacities were normalized to the mass of the active materials. Nickel foam pieces (0.8 – 1.0 cm²)

without loading catalysts were also applied as the cathodes of the LOBs cells as control samples. The current density applied on the bare Ni foam was 0.5 mA/cm^2 .

6.3 Results and Discussion

6.3.1 Synthesis and XRD, TEM Characterization

The synthetic process for EFC and ECC catalysts is illustrated in Figure 6-1. In the synthesis, self-assembling fusion protein beads, metal sulfates precursors and hydrophilic CNTs were mixed and stirred thoroughly to allow the formation of an octahedral coordinating structure via coordination interaction among of metal ions with N of the imidazole group of histidine in the fusion protein beads and O of carboxyl group on CNTs, as illustrated in Figure 6-1. The octahedral coordination metal center configuration formed is similar as the coordination configuration formed between recombinant proteins with His tag and M-NTA agarose chromatography. [14] Such coordination configuration has been investigated to be a possible coordination structure of M-N/C centers. [15, 16] The morphologies of the protein beads used have been investigated in Chapter 4.

Figure 6- 1 Synthetic procedure of the 1D M-N/C catalysts.

Figure 6- 2 XRD profiles of the as-prepared catalysts (CNT, EFC and ECC).

Figure 6-2 displays the XRD results of CNTs, EFC and ECC catalysts. It can be observed that for all three samples, no other impurity phases (metal or metal oxide) can be detected. The peaks locate at $2\theta = 26^\circ$ and 43° correspond to the (002) and (100) planes of CNT.

Figure 6-3 (a) presents the TEM image of the EFC catalysts. As can be seen, the CNT backbones were decorated with small beads of different sizes in the EFC catalysts. These beads-attached CNTs intertwine together leading to the formation of a mesh-like matrix. Such matrices are beneficial for electron transports when applied as electrode materials. A section of beads-decorated CNTs is shown in Figure 6-3 (b). The average diameter of CNT is about 10-20 nm and the size of the beads are around 5 to 30 nm. Figure 6-3 (c) is the HRTEM image of the area marked in Figure 6-3 (b). This HRTEM image clearly reveals that the decorated-beads are of amorphous nature and the walls of the CNT can also be clearly observed. From figure 6-3 (d) it can be seen that the CNTs connect intimately to each other with the help of these amorphous beads. The inset image proves that CNT ends can also be attached by these protein beads.

Figure 6-4 presents the EDS results of the EFC catalysts. The iron signal can be detected from the bead-decorated CNT regions, as shown in the EDS spectra (Figure 6-4(a-d)) and the elemental mapping results (Figure 6-4(f)). As have discussed earlier, the ELP beads and the hydrophilic CNT are linked together via the transition metal ions to form the octahedral coordination structures (metal center-Z ($Z = \text{N}$ or O)), therefore these beads-attached parts on the exteriors of CNTs were locations of the Fe-N/C centers. No crystallized phases can be seen on these CNTs (according to the TEM images). This observation is in consistent with the XRD results discussed above.

Figure 6- 3 (a-d) Morphologies of the Fe-N/C catalysts (TEM and HRTEM).

Figure 6- 4 (a-d) FESEM images (under TED mode) of the EFC catalysts and the EDX spectra (the Cu signal comes from the copper grid); (e-f) Elemental mapping of EFC under STEM.

The structures of the ECC catalysts were also characterized and the results are shown in Figure 6-5. It is found that ECC have similar bead-decorated-CNT morphology as well as the same dimensions as EFC. From the EDX spectra obtained from the ECC

catalysts (the selected regions in Figure 6-6(a)), the Co signal can be clearly detected, which shows that Co was indeed coordinated in the carbon matrices.

Figure 6- 5 Figure 6- 5(a-b) TEM and HRTEM images revealing the morphologies of ECC.

Figure 6- 6 FESEM images (under TED mode) of the ECC catalysts and the EDX spectra (the Cu signal comes from the copper grid).

To understand the surface chemistry of the EFC and ECC catalysts, XPS characterizations were carried out. Figure 6-7 shows the results from the survey scans of

EFC or ECC and it can be concluded that in both catalysts, carbon, nitrogen, and oxygen are presented with Fe in EFC and Co in ECC, respectively.

Figure 6- 7 XPS survey scan of the (a) EFC catalysts and (b) ECC catalysts

Figure 6-8 (a) exhibits the high-resolution N 1s spectrum of the EFC sample. It can be divided into four peaks which are centered at 398.4, 400.1, 401.1 and 402.6 eV, respectively. These peaks can be assigned to pyridinic N, pyrrolic N, graphitic N and quaternary N^+-O^- , respectively, based on their characteristic binding energies. [17] In these four types of N, pyridinic N and pyrrolic N are Fe (II) ion (and other transition metal ion) coordination sites, which are most important in forming the M-N/C catalysts. Figure 6-8 (b) shows the Fe 2p spectrum, it can be further divided into five peaks. These peaks are located at 710.2, 713.1, 719.2, 723.0, and 725.8 eV. The peaks at 713.1 and 710.2 eV belong to the 2p $3/2$ state of Fe (II) or Fe (III), respectively. [18] The peak at 725.8 eV can be assigned to the binding energy of 2p $1/2$ of Fe (III) and 723.0 eV is the 2p $1/2$ state of Fe (II). [19] The peak around 719.2 eV is regarded as a satellite peak. [18] These results imply that Fe (II) and Fe (III) co-exist in EFC. The appearance of Fe (III) can be ascribed to oxidation of the Fe (II) during sample preparation for XPS due to the exposure of the catalysts to air.

The N 1s spectrum of the ECC sample (Figure 6-8 (c)) can be divided into three peaks at 398.3, 400.1 and 402.7 eV. These three peaks correspond to pyridinic N, pyrrolic N and quaternary N^+-O^- based on their respective binding energies. For the Co 2p spectrum (Figure 6-8 (d)), the peak at 780.1 and 793.8 eV are the binding energies of 2p

$3/2$ state of Co (II) and $2p\ 1/2$ of Co (II), respectively. A at 785.6 eV which is considered to be a satellite peak is also observed. [20]

Figure 6- 8 (a) N 1s and (b) Fe 2p XPS spectra of EFC; (c) N 1s and (d) Co 2p XPS spectra of ECC.

Based on the high resolution XPS spectra, the atomic ratio of Fe:N for EFC is determined to be 97:100 and that of Co:N for ECC is 33:100, as presented in Table 6-1 and Table 6-2. From the above results of various characterizations, it can be concluded that Fe-N/C or Co-N/C centers were successfully created on the CNT surfaces.

Table 6- 1 F:N ratio in EFC

Peak	EB (eV)	Peak Area (IA)	S Factor (SA)	NA=IA/SA	n(Fe)/n(N)
N 1s	398.4	119.3	0.42	640.20	97/100
N 1s	400.1	124.3			
N 1s	401.1	25.3			
Fe 2p	710.2	556.2	3.0	621.33	
Fe 2p	713.1	612.9			
Fe 2p	723.1	345.9			
Fe 2p	725.8	349.0			

Table 6- 2 Atomic ratio of Co and N in ECC

Peak	EB (eV)	Peak Area (IA)	S Factor (SA)	NA=IA/SA	n(Co)/n(N)
N 1s	398.3	74.34	0.42	532.71	33/100
N 1s	400.1	149.4			
Co 2p	780.1	555.4	3.80	176.76	
Co 2p	793.8	116.3			

6.3.2 Fe-N/C and Co-N/C Catalysts for Application in LOBs Cathodes

The catalytic performance of the EFC&ECC in LOBs cathodes was evaluated. Cyclic voltammetry (CV) technique was applied to investigate the kinetics of ORR and OER process of the EFC catalysts based lithium-O₂ cells cathodes and the results are shown in Figure 6-9. The first 5 CV cycles of the cell between 2.0 to 4.2 V at a scan rate

of 0.5 mV/s are presented in Figure 6-9 (a). The ORR and OER onset potentials of EFC-based electrode fall in the range of 2.70 - 3.0 V, respectively. The OER peaks at the range of 3.5 – 3.7 V in the forward scans indicate that Li_2O_2 discharge product is directly oxidized to Li and O_2 . Increase of peak currents as well as positively shifting of these OER peaks are also observed as cycling increases, suggesting that the electrode is gradually activated. Electrochemical responses of the electrodes at different scan rates are also recorded and analyzed and the results are presented in Figure 6-9 (b). At 0.1 and 0.2 mV/s, the anodic peaks' voltages at ~ 3.6 V remain unchanged; when the scan rate switches to 0.5 mV/s, it shift positively to ~3.75 V. Such phenomena is probably due to the sluggish kinetics of charge/mass transfer under high scan rate.

Figure 6- 9 (a) CV profiles of the EFC-based LOBs cathodes; (b) CV responses under different scan rates.

The CV response was analyzed by the Randles-Sevick equations [21]:

$$I_p = 0.4463nFAC\left(\frac{nFvD}{RT}\right)^{\frac{1}{2}} \quad (1)$$

$$I_p = B * v^{\frac{1}{2}}, B = 269000n^{\frac{3}{2}}AD^{\frac{1}{2}}C \quad (2)$$

Where n is the number of electrons transferred in the redox process and is 2 in this study, F is the Faraday constant, D is the diffusion coefficient, A is the area of the electrode and is 0.7 cm^2 in this study and C the concentration of lithium ions in the electrolyte.

Figure 6- 10 Linear fit results of the CV data using Randles-Sevick equations.

The linear regression results for the Li_2O_2 decomposition peak current is displayed in Figure 6-10. A linear relationship between I_p and $v^{1/2}$ is observed. Specifically the curve of peak current (I_p) versus square root of the scan rate ($v^{1/2}$) is a line as predicted in equation (1-2). The lithium ion diffusion coefficient is calculated to be $\sim 1.01 \times 10^{-9} \text{ cm}^2/\text{s}$ based on the obtained parameter B. The CV results imply that the catalysts developed in this chapter are promising candidates for high performance lithium- O_2 batteries.

Figure 6- 11 EFC-based LOBs discharged and charged in the potential window between 2.0 V and 4.25 V at different current densities.

Galvanostatic charge-discharge method was applied to further investigate the EFC catalysts based LOBs cathodes and compared with the fresh annealed hydrophilic CNTs as well as the fresh Ni foam. The EFC-based lithium-O₂ battery cathode was discharged and recharged between 2.0 and 4.25 V at different current densities to evaluate the rate performance. As can be observed from Figure 6-11, the typical discharge curves exhibit the typical sloping regions, stable plateaus and abrupt drop at the end of discharge while for the charge profiles, except for the sloping regions and stable plateaus, there is also rapid increase regions in at the end. [22] The discharge capacity is 12 441 mAh/g under 100 mA/g and the discharge plateau is at 2.5 V. As the capacity delivered in the LOBs is related to the deposition of Li₂O₂, the large capacity is due to the enhanced growth and storage of Li₂O₂. Specifically, the interconnected network architecture binder free cathode obtained from the 1D EFC catalysts can provide enough spaces for Li₂O₂ storage and promote mass as well as charge transfer. The charge potential plateau fall in the range of 3.85 – 3.90 V and the charge capacity is 8000 mAh/g. When the current densities change to 200 or 400 mA/g, the discharge potential plateaus are 2.4 or 2.35 V with the discharge capacities of 6993 or 5885 mAh/g, respectively. Decrease in capacities and increase in ORR and OER over-potentials imply that when the current densities increase, together with the high degree of charge/discharge, polarization relating to electron or O₂ transport limitation occurs becomes serious.

Figure 6- 12 (a) and (b) Cycling performance of EFC-based LOBs at a capacity cut-off at 800 mAh/g under 200 and 400 mA/g.

To evaluate the stability of LOBs with EFC, cycling tests at different rates with a capacity cut-off at 800 mAh/g were carried out. At a current density of 200 mA/g, the cell shows discharge and charge potentials are around 2.6 V and 3.65 V, respectively, as shown in Figure 6-12 (a). This observation is in consistent with the CV result where the OER peaks occur at 3.5 – 3.7 V. The discharge potentials slowly drop as cycling number increases but they are still higher than 2.35 V at the end of each cycle. For the last few cycles their charge potentials slightly increase by 50 - 80 mV and eventually reach ~ 3.75 V. However, they are still low and are not high enough to trigger side reactions which is reported to be 4.0 V and above. [23] These data suggest an improvement in the catalytic activity of EFC for OER. At 400 mA/g, the discharge or charge potential plateaus are at 2.5 V or 3.95 V except for the first cycle, as shown in Figure 6-12 (b). The energy efficiencies (discharge energy/charge energy) of the LOBs under 200 and 400 mA/g are determined to be 71.2 % and 65.1%, respectively.

Figure 6- 13 Voltage-capacity curves of the annealed CNT- and nickel foam-based lithium-O₂ battery.

The ORR and OER catalytic activity of CNTs and nickel foam were also investigated. Compared with EFC, the bare CNTs, which are subjected to the same heat treatment, show little OER and ORR catalytic activity in LOBs cathodes. As shown in Figure 6-13 (a), at the current density of 100 mA/g, the fresh annealed hydrophilic CNTs based cathodes show a high charge potential of 4.5 V. The discharge processes of the LOBs are unstable, where the initial capacity delivered are only around 500 mAh/g. The discharge capacities quickly decrease and the battery fails in 10 cycles. The nickel foam current

collector has no ORR or OER catalytic activity. The LOBs with only nickel foam as cathode show very little charge/discharge capacity and the charge process of the first two cycles can be ascribed to appearance of electrolyte decomposition at 4.75 V, as reflected by their charge-discharge performance shown in Figure 6-13(b).

In order to understand the electrochemical details during charge and discharge, ^1H NMR analysis was employed to detect the side products of the above EFC-based cathodes after finishing the 61th or 31th discharge. The results are shown in Figure 6-14. In the ^1H NMR profile in Figure 6-14 (a), the peaks at 3.3 and 3.6 ppm correspond to TEGDME, which is the solvent of the electrolyte, and the signal at 4.7 ppm is from D_2O . As can be observed from Figure 6-14 (b-c), for the cathodes cycled at 200 mA/g, no signals related to the side products from electrolyte decomposition can be identified. For the cathode tested at 400 mA/g, peaks corresponding to HCO_2Li at 8.3-8.5 ppm and $\text{CH}_3\text{CO}_2\text{Li}$ at 1.96 ppm, which have been detected in cathodes using TEGDME as electrolyte solvent [24, 25], appear. The occurrence of these side products indicates that the decomposition of TEGDME or CNT carbon starts to occur when the OER potentials reach 4.0 V. Due to the insulating and electrochemically irreversible properties of these side products, they are not fully decomposed upon charge and as they accumulate, the cell performance will undermine, leading to the final failure of the LOBs. However, in the investigation discussed here, the intensity of these signals is faint, indicating that the decomposition process is not significant during the cycling tests.

Figure 6- 14 (a) ^1H -NMR data of the cathodes after finishing the 61th or 31th discharge cycles; (b-c) magnified spectra of the area indicated in (a).

As a comparison, for the high degree charge/discharge cathode finished the 2nd discharge at 400 mA/g discussed earlier, relatively strong signals of HCO₂Li (8.3 - 8.5 ppm) are observed (Figure 6-15) in the NMR profile, which suggests that electrolyte decomposition was much more severe under a high degree of charge/discharge condition. The NMR results thus suggest that while effective catalysts are indispensable to lower the overpotential issues faced by LOBs, two more factors, which are (1) the working current densities and (2) the degree of charge and discharge, are also important in operating LOBs

Figure 6- 15 ¹H-NMR of the EFC-based cathodes charged and discharged in the potential range of 2.0 - 4.25 V at different rates.

As mentioned earlier, the deposition, distribution and storage of Li₂O₂ is critical for LOBs and catalysts play important roles in this process. In order to better understand how EFC impact the nature of the discharge products and their distributions, the morphologies of the EFC catalysts that discharged to the capacity equal to or less than 800 mAh/g at 200 mA/g were investigated by TEM. Cathodes of these degrees of discharge were selected since no side reactions are expected according to the previous ¹H NMR results.

Figure 6-16 (b-d) exhibits the typical morphologies of EFC catalysts at different degree of discharge, as indicated in Figure 6-16 (a). Except for the bead-decorated regions, the pristine EFC catalysts have smooth surface (Figure 6-16 (b)). When

discharge to 50 mAh/g, some nanoparticles are found to be appeared at the bead-attached areas of in the EFC catalysts, suggesting that nucleation and grow of Li_2O_2 starts, as shown in Figure 6-16 (c). It is also observed that except for the bead-attached areas, no Li_2O_2 particles can be found elsewhere. This observation suggests that Li_2O_2 deposition is more easily to take place at the bead-attached regions with Fe-N/C sites. When the LOBs is discharged to 800 mAh/g, the surfaces of the one-dimensional EFC catalysts show increased roughness as a result of the coating of Li_2O_2 on the exteriors, as presented in Figure 6-16 (d). It is also discovered that the surface layer is composed of small nanoparticles, rather than microscale particles or films. These small particles are crystalline Li_2O_2 nanoparticles which show different facet orientations, as revealed by the HRTEM image in Figure 6-16 (e)) as well as the FFT images. The SAED of Li_2O_2 in Figure 6-16 (f) shows typical ring pattern, which further confirms their polycrystalline nature. As the Li_2O_2 are small nanoparticles (5 – 10 nm) and they intimately contact with the catalyst matrices, they are easily decomposed during charge at low potentials. Thus, the EFC catalysts are capable of tuning the uniform deposition and growth of Li_2O_2 .

Figure 6- 16 (a) Discharge profile of the EFC based LOBs cathode; (b-d) Morphologies of the EFC catalysts obtained from the cathode discharged to different capacities. (e) HRTEM image

corresponding to the selected area in (d) and the insets images are the FFT images patterns; (f) the SAED rings of the discharge product found in (d).

The catalytic activity of the ECC catalysts in LOBs cathodes was also examined and the result is presented in Figure 6-17. In this investigation, the charge/discharge capacities are limited at 800 mAh/g under a current density of 100 mA/g. It is discovered that, in the first cycle the discharge potential gradually decreases to 2.1 V without displaying a typical plateau at the end and after the capacity is larger than 200 mAh/g, the charge potential exceeds 4.0 V and reaches 4.3 V at the end. The discharge and charge curves of the following cycles fall in the potential window between 2.5 and 4.0 V, which suggests improved ORR and OER compared with the first cycle and activation of the catalysts. Specifically, the discharge potentials plateaus are at around 2.55 V and the charge potentials all exhibit two plateaus which are at around 3.6 and 3.95 V. The energy efficiencies are determined to be around 66% for these cycles.

Figure 6- 17 Voltage-capacity curves of the ECC-based LOBs.

Compared with their performance as LOBs cathodes, it is noted that the charge potentials of ECC based cathodes are higher than those of the EFC cathodes, even though the applied current density is only half of the latter, which is 200 mA/g. The previous XPS analysis has revealed that value of Co : N ratio is 33:100 in ECC, which is much lower than 97:100 (Fe : N) in EFC, although they were synthesized under the same experimental conditions. These data suggest that less metal-N centers were introduced in ECC. As a result EFC is much more active than ECC as lithium-O₂ cathodes.

Figure 6- 18 CNT/Pt for LOBs cathodes at 200 mA/g.

As comparison, the as-synthesized CNT/Pt in Chapter 5 was also evaluated under the same conditions as ECC and EFC in LOBs cathodes and the results are exhibited in Figure 6-18. As can be observed, similar charge-discharge profiles, indicating similar charge-discharge processes as EFC are also obtained. It is noted that the discharge voltages are the same for EFC and CNT/Pt; however, the latter shows higher charge potentials (~ 0.4 V higher). Two reasons may cause the difference: (1) the loading amount of Pt nanoparticles is rather low, which means there is less catalytic centers; (2) the catalytic sites in CNT/Pt are on the surface of Pt nanoparticles which is physically contact with the CNT support and may be isolated, leading to a higher contact resistance, whereas in the EFC, the beads and CNT are coordinately bonded which is also the catalytic sites.

The morphologies of the CNT/Pt catalysts from the cathodes discharged to 800 mAh/g were visualized by TEM and the results are shown in Figure 6-19. In contrast to EFC which was uniformly coated by Li₂O₂ nanocrystals, Li₂O₂ was not easily uniformly

deposited onto the surface of CNT/Pt and they grew into larger particles (10 to 200 nm) and these particles were isolated from the catalysts, which thus required higher charge potentials to decompose as contact resistance increased. In summary, EFC catalysts successfully directed the uniform deposition of small Li_2O_2 crystals, which cannot be found in CNT/Pt and are superior in LOBs applications.

Figure 6- 19 Morphologies of the CNT/Pt catalysts (a) fresh CNT/Pt; (b-c) obtained from the cathode discharged to 800 mAh/g.

6.4 Conclusions

In conclusion, this chapter introduced an effective way to construct M-N/C (M=Fe, Co) catalysts onto CNT as efficient lithium- O_2 battery cathodes. The materials used are elastin GPG-AG3-His tag fusion protein beads, hydrophilic CNTs and metal sulfates. Morphological characterizations via FESEM and TEM showed that the CNTs were decorated by protein beads. XRD characterization revealed that impurity phases existed in the as-synthesized catalysts. The successful creation of the M-N/C catalytic sites on the CNTs backbones was confirmed by XPS. The as-synthesized catalysts were investigated as effective catalysts in lithium- O_2 batteries, in which cathodes based on Fe-N/C catalyst show impressive performance where high capacities of 12441, 6993 or 5885 mAh/g under 100, 200 or 400 mA/g, respectively, were obtained. Improved cycling performance has also achieved. The results prove that the EFC synthesized via this novel method are advancing cathode materials for lithium- O_2 batteries.

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Chapter 7*

Synthesis of 1D Transition Metal Phosphides (TMPs, M=Fe, Co)

In this chapter, it is hypothesized that amphiphilic GPG-AG3-His tag fusion protein will be useful templates for 1D metal phosphides synthesis in oil-phase. The synthesis of one-dimensional (1D) transition metal phosphides (TMPs, M= Co, Fe) using self-assembling GPG-AG3-His tag fusion protein fibers are demonstrated. By exploiting the transition metal ion coordination capability of the His-Tag located on the exterior of GPG-AG3-His tag fusion protein fibers, 1D CoP or FeP₄ are obtained. When 1D CoP are used as anode materials in lithium-ion batteries and sodium-ion batteries, very stable cycling performance and propounding rate capabilities as well as high delivered capacities are obtained.

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7.1 Introduction

As one of the most promising strategies to alleviate the depletion of fossil fuels and the environmental consequences of their use, electric energy storage has received global interest and intensive research work has been done to achieve sustainable new energy technologies. [1, 2] Among the several technologies that are suitable for energy storage, electrochemical energy storage (EES) technologies based on secondary batteries have shown considerable promise as a result of many breakthroughs in lithium-ion batteries (LIBs) technologies in the past decade. [3] However, concerns over availability of the limited Li resources as well as the rapid expansion of portable electronics and electric vehicle (EV) markets are demanding more reliable and efficient energy storage, which exhibits high energy density and at the same time are more economically available. [4, 5] To achieve this goal, current research efforts are devoted to two aspects. The first aspect is to explore high performance electrode materials for lithium-ion batteries. The second aspect is to develop sodium-ion batteries (SIBs) under the considerations that: (1) sodium resources are worldwide available with low cost, thus SIBs will be much cheaper than LIBs; (2) SIBs are conceptually identical to LIBs therefore SIBs technologies can greatly benefit from current LIBs technologies. Compared with the progress on the first aspect, success in the second aspect is much more difficult because Na^+ (1.06 Å) is $\sim 40\%$ larger than Li^+ (0.76 Å), which greatly complicates the battery electrode electrochemical reaction process and therefore restricts the kinds of electrode materials suitable for high capacity, fast and durable SIBs. [6, 7]

Among the vast majority of anode materials that have been investigated so far, only a few examples are reported to be suitable for reliable and efficient electrode materials in both LIBs and SIBs. Among which tin based materials and antimony based materials have been explored as high capacity anode materials. [8, 9] Besides tin- and antimony based compounds, metal phosphides are promising high-capacity candidates for lithium and sodium storage according to the conversion reaction mechanisms.

Metal phosphides are a class of refractory metallic compounds formed from the alloying of metals and phosphorus. There are over a hundred of known binary phases of metal phosphides with the compositions ranging from metal-rich (M_6P : M=Metal, P=Phosphorus) to phosphorus-rich (MP_4) phase. [10, 11] Due to the variety of compositions and structures, metal phosphides exhibit a wide array of magnetic, electric and electrochemical properties, e.g. main-group metal phosphides such as InP and GaP are important semiconductors. [12, 13] Unlike the ever-growing interest in the main-group metal phosphides research, the study of transition metal phosphides (TMPs, MP_x , where M=Fe, Co, Ni, etc.) have just recently been emphasized. Recent investigations have shown that TMPs are effective catalysts in hydrogen evolution reactions (HER), oxygen evolution reaction (OER) and are also feasible as electrode materials in energy storage devices such as supercapacitors, lithium-ion batteries (LIBs) and sodium-ion batteries (SIBs). [14-16] The typical capacities are 500 – 1800 mAh/g for LIBs and SIBs according to the conversion reaction mechanisms. [17]

Even though metal phosphides are very competitive high-capacity candidates for lithium and sodium storage, they are not yet fully investigated for practical application in LIBs and SIBs due to their low electric conductivities and the drastic volume change during the conversion reaction process. [18] The huge volume expansion of bulk TMPs materials can cause pulverization and accelerate the deterioration of batteries. To solve these problems and obtain high performance LIBs and SIBs anodes, the construction of TMPs into nanoscale materials, especially into one-dimensional (1D) structure such as nanotubes or nanorods, has been introduced due to their improved structural stability and more efficient ionic and electronic transport. [19, 20] Till now, however, only a few examples of 1D TMPs have been synthesized which adopt physical or chemical techniques including physical or chemical vapor deposition (PVD/CVD), solid-state phosphidation method in which metal salt precursors or 1D nanoscale metal oxide are converted to the corresponding phosphides via reaction with phosphorous powder or PH_3 or surfactant-assisted chemical-route synthesis, e.g. hydrothermal methods or oil-phase methods. [21-23] Besides these commonly adopted techniques, template-guided synthesis methods, especially methods using natural materials as templates, which allow more

controlled growth and hierarchical organization of 1D TMPs nanomaterial, are rarely reported and still far from well-developed.

Biological materials usually have a variety of binding sites for nucleation of inorganic metal ions or further stabilize inorganic materials through electrostatic interactions or coordinations between their functional groups which are located at their proteins, DNA, polysaccharides or other macromolecule components and the metal ions. [24, 25] These binding domains, along with their unique molecular shapes and spatial organization, which biological materials can impart on the products, enable them to be attractive for nanomaterial synthesis. Especially, 1D structure metal-based materials, derived from (1) wire- or rod-shape viruses, and (2) self-assembled polypeptides have been demonstrated. Among these attempts, although 1D morphologies were obtained, control of the sizes and morphologies of nanomaterials is still challenging, because of unfavorable protein degradation, precipitation or agglomeration during material synthesis because of the influence of pH or ionic strength fluctuation in aqueous environment or temperature changes. [26, 27] Further improvements on the affinity of NPs with the templates, the loading amount of NPs and the stability of the structure using stable templates in aqueous or organic phases are still desirable.

In the previous chapters, elastin-mimetic amphiphilic fusion protein, GPG-AG3-His tag have been developed, manipulated to self-assemble into nanofibers under exterior stimulus such as temperatures and applied as templates in 1D noble metal nanomaterial synthesis. As discussed in the previous chapters, in the GPG-AG3-His tag proteins, the GPG domains, which are of hydrophobic nature, undergo dehydration when the temperature is higher than its T_t (coacervation temperature). Simultaneously, they form β -turn and/or β -sheet structures, which results in the formation of fibrous structures. These fibers are of several micrometers in lengths and tens of nanometers in diameters. The hydrophilic domains, which are AG3 and histidine tags, expressing on the surfaces of these nanoscale fibers are specific for metal ions binding and coordination. These features enable them to be attractive and competitive as templates in 1D TMPs

nanomaterial synthesis in oil phases, as TMPs cannot be easily obtained in aqueous conditions.

In this chapter, we present the synthesis of one-dimensional (1D) TMPs (M=Co, Fe) nanostructure materials using the unique self-assembling GPG-AG3 elastin protein fibers as templates via oil-phase method. In this method, the metal binding domains (histidine tags, which is consisted of six histidine peptides) at the exterior of protein fibers coordinately bind the Co or Fe from the cobalt (II) (or Fe) acetylacetonate precursor in a mixture solution of water, trioctylphosphine oxide (TOPO) and 1-octadecane (ODE). Under Ar gas protection, when trioctylphosphine (TOP) is added at 310 °C, protein-Co (or Fe)-TOP is decomposed to form 1 D CoP or FeP₄ nanostructures. The 1D CoP or FeP₄ nanomaterials are typically 2-5 μm in length and 50 nm in diameter. These 1D CoP or FeP₄ nanomaterials are discovered to be composed of nanoparticles with diameters around 5 – 10 nm decorated on an inner carbon support. As a demonstration of the application of the as-synthesized 1D TMPs, the lithium and sodium storage properties of the 1 D CoP nanmaterials as LIBs and SIBs anodes are explored. The 1D CoP has the capacities of 360 mAh/g under 3.0 C rate for over 120 cycles without any capacity loss as LIBs anode. When tested as SIBs anode, the delivered capacities are 528.07 or 314.84 mAh/g at 0.1 or 5.0 C rates. Post X-ray diffraction (XRD) and transition electron microscopy (TEM) characterization of the CoP anodse at different charge/discharge stages reveal the reversible formation and decomposition of Na₃P and the maintenance of carbon supported CoP nanoparticles structure during the conversion reactions. The 1 D CoP based SIBs anodes show good cycling performance where the capacities remain stable at ~ 400, 360 or 300 mAh/g under 1.0, 2.0 or 5.0 C for 250, 250 or 1000 cycles, respectively. Such impressive performance for both LIBs and SIBs of the CoP anodes can be attributed to the 1 D carbon supported nanoparticles nanostructure. Such architectures not only effectively accommodate the volume expansion during charge and discharge but also improve the structural stability and electronic conductivity of the electrodes.

7.2 Experimental Methods

7.2.1 Synthesis

In the typical synthesis, 20 mL GPG-AG3-His tag protein fiber solution were transferred into a three-neck flask (100 mL) and kept at 60 °C with stirring at 500 rpm. Then, 1.0 mmol cobalt (II) acetylacetonate (or iron (II) acetylacetonate), 500 mg trioctylphosphine oxide (TOPO) powder and 20 mL 1-octadecane (ODE) were added sequentially. The temperature was then raised to 120 °C to evaporate the water completely. After that, pure Ar gas was purged into the flask for 2 h to remove oxygen. The flask was then heated to 310 °C and finally 3.0 mL TOP was quickly injected using a 5.0 mL syringe. The reaction was allowed to sustain for 2 h under Ar gas protection, after which the flask was allowed to cool to room temperature naturally. The samples were collected by centrifugation and washed by the mixture of 10 mL hexane and 10 mL absolute ethanol for 3 times and finally dried under vacuum. As comparison, synthesis of metal phosphides which either directly used protein powder or without using TOPO was also carried out to investigate the synthetic parameters.

7.2.2 Materials Characterization

(1) Characterization

The morphologies of the GPG-AG3-His tag protein fiber, cobalt phosphide and iron phosphide were studied using transmission electron microscopy operated at 200 kV (TEM, JEOL, model JEM-2010, JEM-2100). XPS characterization was carried out on a spectrometer (the model is Phoibos 100). The phases of these samples were identified using XRD (X-ray diffraction, Shimadzu) with Cu K α irradiation.

(2) Electrochemical Measurements

The as-prepared CoP samples were firstly annealed in an Ar gas tube furnace at 320 °C for 1 h prior to the electrochemical characterization. To prepare the slurry, electrode components were added into N-methyl-2-pyrrolidone (NMP) in specific ratios

and stirred at 500 rpm for 2 days. For LIBs anode, 70 wt % active materials, 20 wt % super P and 10 wt % polyvinylidene fluorides (PVDF) were mixed into N-Methyl-2-pyrrolidone (NMP). For SIBs anodes, the composition of the electrodes are 70 wt % active materials, 20 wt % single-wall carbon nanotubes (SWNTs) and 10 wt % PVDF. The obtained slurry was then cast onto a copper foil and dried in vacuum at 50 °C overnight to remove the NMP solvent. The mass loading on the electrode was obtained by measuring the weight differences of the dry copper electrodes before and after active materials were casted. CR2032 (3 V) coin-type cells with lithium metal foil or sodium metal foil as the counter/reference electrodes, Celgard 2400 membrane as the separator, and electrolyte solution obtained by dissolving 1 M LiPF₆ (for LIBs) or 1 M NaClO₄ (for SIBs) into a mixture of ethylene carbonate (EC) and dimethyl carbonate (DMC) (EC/DMC, 1: 1, v/v) were assembled for the electrochemical characterization. The coin cells were assembled in an Ar-filled glovebox where concentrations of moisture and oxygen were both below 0.1 ppm. The charge/discharge tests were performed with a NEWARE battery tester at a voltage window of 0.005–3.0 V for CoP samples. Cyclic voltammetry characterizations (0.005–3.0 V at different scan rates) were carried out on an electrochemical workstation (Solartron analytical equipment, Model 1470E).

7.3 Results and Discussion

7.3.1 1D Cobalt Phosphide and 1D Iron Phosphide

The synthesis procedure of one-dimensional metal phosphide and iron phosphide is schematically illustrated in Figure 7-1 and the protocols are available in the experimental section. The details of fusion protein self-assembling have been discussed in chapter 4. Generally, the GPG-AG3-His tag fusion protein has a long hydrophobic domain (GPG) and two relatively short hydrophilic sequences (AG3 and His tag). Upon self-assembling at increase temperature in DI water, the hydrophobic GPG domains of protein monomers form β -sheet and β -turn structures, which lead to the formation of fibrous structures. In the protein templates the hydrophilic sequences (which are AG3 and His tags) are at the exterior in which His tags strongly interact with Co atoms of cobalt (II) or Fe atoms of

iron (II) precursor, which promotes the growth of cobalt phosphide or iron phosphide nanoparticles at the surface.

The morphologies of fibrous fusion protein templates and 1D CoP were visualized by TEM. Figure 7-2 presents the TEM images of 1D protein templates. The protein templates have rod-strip morphologies and their diameters are around 50 nm with the lengths in the range of 1 – 5 μm , as shown in Figure 7-2 (a). Higher magnification TEM image (Figure 7-2 (b)) reveals that these rods or strips are stemmed from the rolling or folding of protein sheets of high content of β -sheets or β -turns, which are common in elastin protein and have been previously reported.

Figure 7- 1 (a) The amino acid sequence of the fusion protein; (b-e) The synthesis flow for TMPs (M=Co, Fe).

Figure 7- 2 TEM images of the fibrous fusion protein templates under different magnifications. The scale bars are 200 nm in (a) and 100 nm in (b).

To investigate the synthesis conditions, three sets of experiments were carried out, where the starting protein structures and TOPO were parameters. In the first group, the fusion protein powders were directly dissolved into DI water followed by the synthetic procedures (experimental section). The resultant product are visualized by TEM and presented in Figure 7-3. As can be observed, no regular structures were formed. Random sizes of nano-sheets or foams are decorated by crystallized nanoparticles.

Figure 7- 3 CoP nanostructures using GPG-AG3-His tag protein powder.

When incubated fibrous proteins were used as the templates without addition of TOPO, it is found that (1) the fibrous morphology were maintained under the synthesis environment, however, most of the template surfaces were as fresh as the starting fibrous protein and for the proteins decorated by nanoparticles, the loading amount of inorganic nanoparticles are limited and their distribution are not uniform, as are displayed in Figure 7-4. It is speculated that without TOPO, the solubility of the metal precursors in the aqueous are low therefore the adsorption of metal precursors on the templates are limited, thus resulting the failure of high amount and uniform loading of CoP.

Figure 7- 4 CoP nanostructures using GPG-AG3-His tag protein fiber without TOPO.

When the starting protein structure was nanofibers, 1D CoP was obtained. The crystal phase of the as-synthesized 1D CoP was characterized using X-ray diffraction (XRD). As shown in Figure 7-5 (a), all the peaks can be indexed to pure orthorhombic CoP (JCPDS NO.29-0497) and no other impurity phases can be detected (The broad peaks located at 22° in the spectrum is from the amorphous glass slide substrate). XPS characterization was performed to study the surface chemistry of the 1D CoP. The survey scan in Figure 7-5 (b) shows that Co, P and C elements exist in the sample. The peak locates at 129.7 eV in the high resolution XPS spectrum of P (inset of Figure 7-5b on the left) can be assigned to the $2p_{3/2}$ of element P. As to the Co 2p spectrum (inset of Figure 7-5b on the right), the peak at 777.9 eV can be assigned to be Co $2p_{3/2}$ and the peak at 782.6 eV is a satellite peak corresponding to oxidized state. This oxidized state occurs

mainly because of surface oxidation due to exposure of the sample to air during storage. Since oxide phases of cobalt or phosphorus are not detected in the XRD characterization the degree of oxidation is low and can be neglected.

Figure 7- 5 (a) XRD pattern of 1D CoP; (b) XPS characterization of the 1D CoP.

Figure 7- 6 TEM images of the 1D CoP under different magnifications. The scale bars are 100 nm in (a), 50 nm in (b), 20 nm in (c) and 2 nm in (d). The facet in (d) is (111).

In contrast to the clean surface of the fibrous protein templates, dramatically increase of surface roughness can be observed after CoP synthesis, as revealed in Figure 7-6 and the inset images. The 1D structure is maintained after synthesis while the fusion protein surfaces have been covered by CoP nanoparticles (Figure 7-6 (a)). These CoP nanoparticles are around 5 – 10 nm in diameters and they do not closely pack or completely isolated. Instead they arrange in a manner that fully cover the template surfaces to form a uniform coating layer (Figure 7-6 (a-c)). The inset image of Figure 7-6 (b) is the SAED pattern and the ring patterns suggest the polycrystalline nature of 1D CoP. High resolution TEM image of the decorated CoP nanoparticles in Figure 7-6 (d) clearly shows the lattice fringes of one CoP nanoparticle, which is revealed to be the (111) plane according to the FFT image (Inset image of Figure 7-6 (d)).

Figure 7- 7 EDS mapping of Co L and P K for the 1D CoP

The energy dispersive X-ray spectroscopy (EDS) mapping results shown in Figure 7-7 confirms Co and P both exist and are uniformly distributed in the fibrous template matrix.

Figure 7- 8 XRD pattern of the iron phosphide, which is indexed to be FeP₄ phase.

The crystal structure of the as-synthesized iron phosphide was also investigated by XRD and is revealed to be FeP₄ (JCPDS NO.34-0995), as shown in Figure 7-8. The FeP₄ nanomaterials also have similar dimensions and morphologies as 1D CoP but the particle sizes of FeP₄ are not as uniform as the CoP nanoparticles found in 1D CoP, as presented in Figure 7-9. The FeP₄ nanostructures show some degree of agglomeration, as shown in Figure 7-9 (b), which may occur in the second stage synthesis when H₂O hadn't been evaporated and Ar flow was not yet introduced to protect the synthesis. Under such condition, Fe (II) was oxidized and further went through hydrolysis process. The HRTEM image and the corresponding FFT image in Figure 7-9 (c) shows the crystal facet of (-121) of FeP₄ nanoparticles.

Figure 7- 9 TEM images of FeP₄ under different magnifications. The scale bars are 50 nm in (a-b) and 2 nm in (c), inset of (a) is 25 nm.

7.3.2 Lithium and Sodium Storage Performance of 1D Cobalt Phosphide

The 1D CoP nanomaterials are explored as anode materials for both LIBs and SIBs. In order to investigate their lithium and sodium storage properties, coin-type half-cells were assembled and the electrochemical measurements were carried out at room temperature. Figure 7-10 presents the cyclic voltammetric (CV) curves of the lithium-ion battery cell with 1D CoP anode obtained at a scan rate of 0.2 mV/s in the potential window of 0.005 - 3.0 V for 3 cycles. In the first discharge, a broad peak centered at ~ 1.0 and 0.6 V appears. This peak corresponds to some irreversible reactions such as the formation of solid electrolyte interphase (SEI) layer. [28, 29] The peak locates within 0.6 V and 0.005 V can be attributed to the conversion reaction: $\text{CoP} + 3 \text{Li}^+ + 3 \text{e}^- \rightarrow \text{Co} + \text{Li}_3\text{P}$. [29, 30] In the forward scan, the oxidation peaks at ~ 1.10, 1.50 and 2.30 V are related to the decomposition of Li_3P and the SEI layer, respectively. For the 2nd and 3rd cycles, the CV curves are identical to each other with both the cathodic peaks slightly negatively shifted to 0.55 V and the anodic peaks to 1.17 and 1.65 V when compared with the first cycle. It is noted that both the positions and areas of cathodic or anodic peaks of the 2nd and 3rd cycles remain almost unchanged, which suggests that the 1D CoP based LIBs have good reversibility and high coulombic efficiency.

Figure 7- 10 CV curves of the 1D CoP based LIBs at the scan rate of 0.2 mV/s.

The charge-discharge profiles of the CoP anode based LIBs at the current density of 0.2 C (1 C = 893.3 mA/g) is shown in Figure 7-11. In the first cycle, the discharge capacity is 1564.40 mAh/g and the corresponding charge capacity is 759.61 mAh/g. As can be observed, two broad plateaus, one located at 1.25 – 0.70 V and the other at 0.55 – 0.25 V, appear during the first discharge, which is in consistent with the first CV cathodic scan. The broad plateau between 1.00 and 1.25 V during the first charge also match well with the first CV anodic scan. It is noted that in the second and third cycles the discharge plateaus decreased to ~0.75 V while the charge plateaus positively shift to 1.25 V. This trend is the same as that observed in the 2nd and 3rd cycles of CV scans.

The rate performance of the 1D CoP anodes is further investigated. As exhibited in Figure 7-12, the charge and discharge capacities gradually decrease as the current densities increase, which indicates the diffusion-control kinetics of the electrode. At the rates of 0.2, 0.5, 1.0, 2.0 and 5.0 C, the discharge capacities obtained from their 10th cycles are 763.75, 603.21, 506.16, 407.05 and 215.64 mAh/g, respectively. When the current density was restored to 0.5 and 0.2 C, the capacity recovered to 612.38 and 747.15 mAh/g after 10 cycles.

Figure 7- 11 Charge-discharge profiles of the 1D CoP based LIBs at 0.2 C.

Figure 7- 12 Rate performance of the LIBs based on 1D CoP.

The cycling performance of the cells is evaluated at different rates and the results are shown in Figure 7-13. At 3.0 C, the delivered capacities stabilize at ~ 360 mAh/g during the 120 cycles where the 120th cycle capacity is 372.45 mAh/g, which is 84.52% of the second cycle discharge capacity. At 5.0 C, the capacities are stable at ~ 240 mAh/g for 250 cycles. The coulombic efficiencies approach 100% in the tested cycles, indicating good reversibility of the electrode reactions.

Figure 7- 13 Cycling performance of the SIBs based on 1D CoP.

The sodium storage properties of the as-synthesized 1D CoP are also investigated by CV and CCD methods. Figure 7-14 (a) exhibits the CV results of the 1D CoP anodes of SIBs. As can be observed, in the first cycle there are two broad cathodic peaks which locate at 1.00 and 0.70 V. These two peaks can be related to the conversion reactions in which metallic Co, Na_3P or the SEI layer form. Two anodic found at 1.50 and 2.70 V can be ascribed to the decomposition of Na_3P and the SEI layer. [16, 31, 32] The CV

responses under various scan rates of 0.10, 0.20, 0.50, 1.00 and 2.00 mV/s are compared and displayed in Figure 7-14 (b). According to theoretical analysis, for an ideal reversible electrode its capacity, which can be determined by dividing the peak area over the scan rate, is constant regardless of the testing parameters. Therefore, the peak intensity of the CV curve is dependent on the scan rate. It is also observed in Figure 7-14 (b) that while the peak intensities increase as scan rates increase, the shapes and locations of the cathodic and anodic peaks, however, remain unchanged. This result suggests that the 1D CoP based anodes are capable of good rate performance for sodium storage.

Figure 7- 14 (a) CV profiles of 1D CoP based SIBs at the scan rate of 0.2 mV/s; (b) CV curves different scan rates

Figure 7- 15 Linear regression of the anodic and cathodic peaks

Further investigation of the relationship between the anodic or cathodic peak currents (I_p) and the square roots of scan rates ($v^{1/2}$) shows that both the cathodic and anodic peak currents linearly depend on the scan rates, as exhibited by the linear fit lines in Figure 7-15. These results thus indicate that the electrochemical reaction of the 1D CoP anode for sodium storage investigated here is mainly diffusion-controlled which follows the Randles-Sevcik equation. [33]

$$I_p = 0.4463nFAC\left(\frac{nFvD}{RT}\right)^{\frac{1}{2}} \quad (1)$$

In the above equation F is the Faraday constant, D is the diffusion coefficient, C is the concentration of sodium ions in the electrolyte, n is the number of electrons transferred in the redox process and A is the typical area of the electrode.

Figure 7- 16 Charge-discharge profiles of the SIBs at 0.1 C.

The galvanostatic charge-discharge profiles of the SIBs cell with 1D CoP anode at 0.1 C between 0.005 – 3.0 V are shown in Figure 7-16. In the first cycle discharge, a broad plateau in the potential window of 0.5 – 1.2 V is observed which matches well with the CV observations. Due to the formation of the SEI layer, a high discharge capacity of ~ 1079.99 mAh/g is achieved with the 1st charge capacity of 559.81 mAh/g. The 2nd and 3rd discharge capacities are 615.29 and 575.26 mAh/g, respectively. The corresponding charge capacities are 538.23 and 529.98 mAh/g., respectively.

The rate performance of the 1D CoP for SIBs is also evaluated and the results are shown in Figure 7-17. As can be seen, the delivered capacities gradually decrease as the current densities increase, which is similar as the lithium storage behavior. This observation consolidates the previous assumption based on the CV analysis which is the electrochemical reaction for sodium storage is diffusion-controlled. The discharge capacities under 0.10, 0.20, 0.50, 1.0, 2.0 and 5.0 C at the respective 10th cycles are 578.07, 491.60, 425.96, 376.36, 359.95 and 314.84 mAh/g, respectively. Moreover, the capacities restore to ~ 470 mAh/g when the current density recovers back to 0.2 C, which confirms the good rate performance as well as cycling durability of 1D CoP anode.

Figure 7- 17 Rate performance of the SIBs based on 1D CoP.

The cycling performance of the cells is studied by cycling under 0.1, 1.0 2.0 and 5.0 C at different cycles. At 0.1 C, as Figure 7-18 (a) shows, the delivered capacities keep at 520 mAh/g and don't show any tendency of decay over 100 cycles. At 1.0, 2.0 or 5.0 C, the delivered capacities are ~ 400, 360, or 300 mAh/g. It is also noted that the capacities are very stable which don't show any tendency of decay under 1.0, 2.0 or 5.0 C for 250, 250 or 1000 cycles, respectively, as are displayed in 7-18 (b). The coulombic efficiencies approach nearly 100%. Such impressive rate performance and high coulombic efficiencies are much superior to the reported studies of TMPs as SIBs anodes so far and demonstrate that the 1D CoP developed here is very competitive for SIBs application.

Figure 7- 18 Cycling performance of the SIBs based on 1D CoP at: (a) 0.1 C; (b) 1.0 and 2.0 C and 5.0 C.

As a comparison, CoP nanoparticles were also synthesized in the similar condition without fibrous protein. According to Figure 7-19 (a-c), the CoP nanoparticles are around 10 nm in diameters.

Figure 7- 19 TEM images of the CoP nanoparticles. Inset of a is the XRD pattern. Scale bar: (a) 100 nm, (b) 10 nm, (c) 2 nm.

When applied in LIBs, at 0.2, 0.5, 1.0, 2.0 and 5.0 C, the delivered capacities are 644.7, 490.3, 387.2, 292.0 and 157.5 mAh/g, as Figure 7-20 reveal, which is much lower than those obtained from 1D CoP. In sodium storage, according to Figure 7-20, at 0.1, 0.2, 0.5, 1.0 and 2.0 C, the capacities are 371.79, 296.34, 237.16, 200.80 and 156.52 mAh/g, which are also much lower than the capacities delivered by 1D CoP. Thus, the 1D and carbon support structure helps to achieve higher performance in both lithium and sodium storage, probably due to the enhanced structural stability as well as accommodation of the volume expansion during the conversion reaction.

Figure 7- 20 Lithium and sodium storage performance of CoP nanoparticles: (a) rate capability of lithium storage; (b) cycling performance of lithium storage; (c) rate capability of sodium storage.

In order to understand the possible mechanism of sodium storage of 1D CoP, *ex situ* XRD and TEM characterizations were performed to study the 1D CoP electrodes at different discharge/charge stages during the first cycles. Figure 7-21 shows the crystal phases of the electrodes when discharge to 0.500 and 0.005 V or charge to 3.0 V. As can be observed from the XRD patterns, when the cell is discharged to 0.500 V, peaks corresponding to Na_3P , [34] Co and CoP co-exist. When fully discharge to 0.005 V, diffraction peaks of CoP almost disappear and only peaks corresponding to Na_3P and Co are detected. When the cell is reversibly charged to 3.0 V, the peak intensity of Na_3P phase is faint while CoP diffraction peaks reappear. It is also observed that even though the cell is fully charged, Co phase still exists. The XRD results therefore confirm the conversion reaction in which Na_3P , Co or CoP reversely forms and decomposes when CoP is applied in sodium storage.

Figure 7- 21 XRD patterns of the SIBs anodes at different charge/discharge states.

Figure 7- 22 TEM images and SAED patterns of the SINs anode materials at different states: (a-c) discharge to 0.5 V; (d-f) discharge to 0.005 V and (g-i) recharge to 3.0 V. Scale bar: (a), (d) and (g): 20 nm; Inset images: 50 nm. (b), (e) and (h): 2 nm. (c), (f) and (i): 2 1/nm.

The morphologies of the electrode materials at different charge/discharge states were studied via TEM and the corresponding TEM images and SAED patterns of the CoP anodes of the previous ex-situ XRD samples are presented in Figure 7-22. As can be observed, for all three samples, the carbon supported nanoparticles structure is maintained while the 1D structure is kept to different degrees depending on the state of charge or discharge. The maintenance of such nanoparticle decorated-carbon support architecture help to improve the structural stability and also facilitate efficient ionic and electronic transport, thus benefits the sodium storage. Specifically, when discharge to 0.500 V, the 1D structure is clearly observed and some knobs start to appear along the 1D

backbone, as Figure 7-22 (a) and the inset image displayed. The nanoparticles are found to be uniformly distributed on the carbon support. From the high resolution TEM image and the corresponding FFT images in Figure 7-22 (b), the lattices of Na₃P (102) and CoP (102) are identified. The SAED pattern in Figure 7-22 (c) shows the diffraction rings of Na₃P, Co and CoP. This result consolidates the co-existence of these three phases in this discharge state as the cell is not fully discharge. The knobs observed in Figure 7-22 (a) tend to grow bigger when the cell is fully discharged to 0.005 V, as observed from Figure 7-22 (d). HRTEM image (with FFT) and the SAED pattern (Figure 7-22 (e - f)) prove that the material phases in this discharge state are Na₃P and Co. Finally, as the cell is reversibly charged to 3.000 V, the morphologies have changed to be chains of large particles with the typical diameter of 50 nm. These large particles are revealed to be composed of smaller particles decorated on carbon support, as shown in Figure 7-22 (g). Only CoP and Co phases can be found in this state (Figure 7-22 (h-i)), confirming that Na₃P could convert to CoP after fully charge.

Table 7-1 summarizes the sodium storage performance of representatives of metal sulfide, phosphide, oxides, and metal or carbon. Compared with the anodes and especially the phosphides reported, it can be clearly seen that the 1D CoP synthesized in this work have demonstrated superior rate performance as well as cycling stability. It is also noted that from the reported studies, carbon-supported materials, especially those systems where active materials were supported by 1D conductive matrix show better electrochemical properties, and the design of 1D CoP in this work further support this argument.

Table 7- 1 Performance of various materials for sodium storage

Materials	Morphology	Theoretical Capacity	Reported Capacity	Potential (V vs Na/Na ⁺)	Cycling	Reference
MoS ₂ /C	MoS ₂ /C nanospheres	670 mAh/g for MoS ₂	520 mAh/g at 0.1 C	0.005-2.5	400 mAh/g at 1.0 C for 300 cycles	Small 2015, 11, 473–481
Bi ₂ O ₃ @Reduced Graphene Oxide Nanocomposite	Bi ₂ O ₃ particles (10-100nm) exist on the surface of rGO sheets. rGO: Bi ₂ O ₃ (1:5)	689.7 mAh/g for Bi ₂ O ₃	669 mAh/g @ 140 mA/g	0.01-2.0	413 mAh/g at 200th cycle, which is 70% of the initial charge	ChemPlusChem 2015, 80, 1000 – 1006

					capacity @ 140 mA/g	
Antimony	Hexagonal (60 nm) and amorphous (40nm) Sb nanoparticles	660 mAh/g	632, 625, 569, 515 and 426 mAh/g at a current density of 100, 200, 500, 1000, and 2000 mA/g for Sb(H);	0.01-2.0	610 and 605 mAh/g for Sb(H) and Sb(A) @ 100 mA/g	J. Power Sources 2015, 282, 358-367
Sb ₂ S ₃	One-Dimensional Rod-Like Sb ₂ S ₃ @C:200 nm in diameter, several micrometers in length	946 mAh/g for Sb ₂ S ₃	The first discharge delivers a capacity of 1066.0 mAh/g and 730.3 mAh/g during charge at 100 mA/g	0.01-2.5	699.1 mAh/g after 100 cycles at 100 mA/g	ACS Appl. Mater. Interfaces 2015, 7, 19362–19369
Sn	Core-shell structures (H-Sn) with size of ~30 nm	846 mAh/g	1st:460 mAh/g 10th: 270 mAh/g @20 mA/g	0-2.0	220 mAh/g after 50 cycles @20 mA/g	Electrochimica Acta 2015, 180, 227–233
β-FeOOH	rod-like shapes with controlled sizes (mean diameters: 53 and 5) nm		627 and 523 mAh/g for the first and second cycle at 80 mA/g	0.005-2.8	300 mAh/g @25th cycle at 200 mA/g	Chem. Mater. 2015, 27, 5340–5348
Sn ₄ P ₃ /C	composed of agglomerated nanoparticles (~100 nm) in conductive carbon matrix	1132 mAh/g for Sn ₄ P ₃	650, 560, 435, and 349 mAh/g @ 100, 200, 500, and 1000 mA/g	0.005-2.0	850 mAh/g at 50 mA/g and a remarkable cyclability with 86% capacity retention over 150 cycles	Nano Lett. 2014, 14, 1865–1869
P/CNT	P-CNT hybrid Particles ranging from submicron to a few microns in size	2595 mAh/g for P	P-CNT hybrid and c-NaCMC-CA binder: a high reversible capacity of 850 mAh/g @5.2 A	0.005-2.0	a stable cycling capacity of 1586.2 mAh/g after 100 cycles @ 520 mA/g	ACS Nano, 2015, 9, 11933–11941

FeP/graphite	Micro/nano particles	893.3 mAh/g for FeP	1st 658.4 mA h/g @ 50 mA/g; 2nd ~420 mAh/g	0.005-1.5	217 mAh/g in the 70th cycle @ 50 mA/h/g	RSC Adv., 2015, 5, 80536–80541
Cobalt phosphide	nanosized CoP particles (10e20 nm in size embedded in the amorphous matrix)	893.3 mAh/g	initial specific capacity of 770 mAh/g at 100 mA/g; 470 mAh/g in the 2nd cycle	0-1.5	After 25 cycles, the retained capacity was 315 mAh/g at 100 mA/g	J. Power Sources 2015, 294, 627-632
Nitrogen-doped carbon	Nanofibers 150-250 nm in diameter		299 mAh/g at 50 mA/g in the first cycle	0.001-2.5	150 mAh/g@100 0 mA/g for over 200 cycles	Carbon 2015, 94, 189–195
Fluorine-Doped Carbon Particles	Less than 10 μ m		260 mAh/g at 50 mA/g	0.001-2.8	228 mAh/g for 200 cycles at 200 mA/g	J. Phys. Chem. C 2015, 119, 21336–21344
Wood Fiber	Cellulose ribbon-like structure (micro scale: 100 μ m)		250 mAh/g at 20 mA/g	0.01-2.5	196 mAh/g for 200 cycles at 100 mA/g	ACS Appl. Mater. Interfaces 2015, 7, 23291–23296
Na ₂ Ti ₃ O ₇ /Na ₂ Ti ₆ O ₁₃	Nanorods diameter and length in the range of 50–850 nm and 2–7 μ m	178 mAh/g for Na ₂ Ti ₃ O ₇ ; 50 mAh/g for Na ₂ Ti ₆ O ₁₃	180 mAh/g at 0.1C and 100 mAh/g at 5C	0.01-2.5		Ind. Eng. Chem. Res. 2016, 55, 10065–10072
Anatase TiO ₂	Nanoparticles: spherical shape, and average diameter of around 30 nm	335 mAh/g	100 and 86 mAh/g at 5.5 and 11C	0.02-2.0	initially 125 to 181 mAh/g after 20 cycles @0.11C	J. Power Sources 2015, 251, 379-385
This work	1D CoP (composed of CoP NPs of 5 – 10 nm)	893.3 mAh/g for CoP	491.60 and 314.84 mAh/g at 0.2 and 5.0C; 0.1C: 520 mAh/g for 100 cycles; 1.0C: 400 mAh/g for 250 cycles;	0.005-3.0	2.0C: 360 mAh/g for 250 cycles; 5.0C: 300 mAh/g for 1000 cycles	J. Mater. Chem. A, 2016, 4, 10893–10899

A major drawback of the current conversion reaction-based anodes is the existence of voltage hysteresis, that is, the charging voltage is considerably larger than the discharging voltage. This difference represents large energy inefficiency. The origin for the hysteresis is yet to be studied. Recently, in order to identify the origin or to rationalize the hysteresis phenomenon, several mechanisms have been proposed. For example: (1) there is convincing evidence that different reaction pathways occur during the conversion reaction; [35,36] (2) there is also argument that to promote intermediate phase separation or nucleation, large overpotentials is inevitably [37] and (3) interfacial energy costs due to the large volume of interfaces created during conversion reactions.[38,39]. Generally, during the conversion reaction, the evolution and spatial distribution of intermediate phases are controlled by kinetics. [40,41]. Therefore, the large hysteresis can be expected to be reduced by rational design and optimization of material microstructure and electrode architecture to facilitate the kinetics and therefore improve the energy efficiency of lithium-ion batteries based on conversion chemistry. However, No consensus on the mechanism of the hysteresis is yet achieved.

Figure 7- 23 $dq/dv \sim V$ for Li storage (a) and sodium storage (b).

It has been revealed that for Li storage, the conversion reaction is the displacement mechanism. According to the previous post-TEM and Post-XRD analysis, the conversion reaction for sodium storage also follows this mechanism. In displacement reaction, lithium (sodium) reacts with P of the alloy compound CoP while Co is displaced or extruded from the parent phase. The displaced element Co, is inactive lithium/sodium, but can be reduced to metallic Co nanoparticles and further form a conductive matrix to facilitate electron conductivity. The hysteresis between the lithiation (sodiation) and

delithiation (desodiation) stage is discovered to be $\sim 0.56\text{V}$ ($\sim 0.64\text{V}$) for the 1D CoP based on the $dq/dv \sim V$ curves in Figure 7-23, which is slightly smaller than the numbers reported for many conversion reaction based anodes (typically 0.65-1.0 V). [42, 43]

Several reasons may be contributed to such improvement:

- (1) the active materials of CoP are nanoscale particles with diameters only 5-10 nm, which may help to shorten Li^+/Na^+ transfer path;
- (2) the CoP or other intermediates are confined to attach to a robust 1D carbon support, which may help to reduced charge transfer resistance;
- (3) According to post-TEM results, small particles (5-10 nm) tend to converse in to larger particles as conversion reaction proceeds, thus resulting in the reduction in the intermediate phases interfaces which is beneficial to alleviate the large hysteresis.

7.4 Conclusions

In summary, we present in this chapter the synthesis of 1D TMPs (M=Co, Fe) via oil-phase method by using fibrous fusion protein as templates. The investigation on their application in lithium and sodium storage is also performed. These as-synthesized 1D TMPs are revealed to be composed of metal phosphide nanoparticles decorated on the exterior of carbon support. The 1D CoP exhibits prominent performance in lithium and sodium storage. In LIBs, a capacity of $\sim 360\text{ mAh/g}$ is obtained which can be maintained over 120 cycles at 3.0 C. As for sodium storage, a discharge capacity of $\sim 520\text{ mAh/g}$ is obtained at 0.1 C for 100 cycles. At 5.0 C, the obtained capacities are $\sim 300\text{ mAh/g}$ which are very stable over 1000 cycles. This study provides insights on synthesis of novel nanostructure of TMPs and will benefit future research in anode materials for lithium and sodium storage.

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Chapter 8

Discussion and Future Work

In this chapter, the preliminary results of the proposed work based on the previous chapters are presented. The main idea is constructing multifunctional fusion proteins which are capable of organizing into hierarchical structures and mineralizing different inorganic materials simultaneously. Given that GPG-AG3-His tag fusion proteins have AG3 towards noble metal ions and His-tag for transition metal ions and they are aligned sequentially in the protein monomer, it is of significance to explore the synthesis of 1D transition metal oxide/noble metal composites using the protein fibers. The obtained results have demonstrated this strategy.

8.1 General Discussion

This thesis has presented the successful combination of designing multifunctional biomaterials which are capable of mineralization and 1D inorganic nanomaterial synthesis for advanced energy storage. In the design of biomaterials templates suitable for one-dimensional nanomaterials synthesis, three domains, (1) GPG domain, inspired from natural elastin protein and capable of self-assembling upon exterior temperature stimuli; [1, 2] (2) AG3 domain, screened from phage library and capable of mineralize noble metal, e.g. Ag, Au, Pt and Pd; [3, 4] (3) Histidine tag, designed for affinity chromatography and can specially bind with transition metal ions, such as Ni^{2+} , Co^{2+} , and Fe^{2+} , [5, 6] are combined into a novel amphiphilic fusion protein of GPG-AG3-His tag. The self-assembling and mineralization capabilities of this protein have been investigated. Self-assembling protein few nanometer sizes beads and nanofibers are obtained. Using these two nanostructure templates, metal-nitrogen/carbon catalysts, Pt or Pd porous carbon-supported nanotubes and carbon-supported cobalt phosphide or iron phosphide are synthesized. The inorganic nanomaterials are demonstrated to be efficient in energy storage application, such as lithium- O_2 batteries, lithium-ion batteries and sodium-ion batteries.

8.1.1 Multifunctional Fusion Proteins

The sequence of GPG-AG3-His tag from the N-terminus to C-terminus is GPG, AG3 and finally His tag. The GPG is the longest among the three domains and takes up above 95% of the total weight. The main function of GPG is temperature responsive and self-assembling. While GPG have been proved to be able to self-assemble into nanofibers that can well-disperse into water, they are not yet suitable for use in inorganic nanomaterials synthesis because of the limited amount of hydrophilic side-chains in the amino acid residues (e.g. metal ions binding sites). Through the introduction of hydrophilic domains of AG3 and His tag, the new fusion protein has the ability to self-assembling, mineralization. Improved solubility in aqueous solution and structural stability of the self-assembling protein at increased temperature in solution phases are demonstrated.

However, there are still two aspects that can be improved. The first aspect is on the study of the self-assembling behaviors of the GPG-AG3-His tag protein. Systematic understanding of the assembly processes of this protein and further precise control of the assembled structures are desirable. Furthermore, the current study only focuses on the self-assembling behavior in water and studies of the self-assembling in oil phase are lack and desirable. The second aspect, in the present strategy, case GPG is responsible for self-assembling and AG3 for specific recognition and mineralization. As a general strategy, in theory, more functional domains can be incorporated into a fusion protein; therefore organic-inorganic material systems with hierarchical structures/multiple components can be designed, synthesized by starting from a fusion protein molecule. This is a huge advantage that distinguishes the fusion proteins from those natural occurring templates or industrially synthesized materials such as CNT.

8.1.2 Inorganic Nanomaterials Synthesis Using Multifunctional Fusion Proteins for Efficient Energy Storage

Although the synthesis of metal-nitrogen/carbon catalysts, Pt or Pd porous carbon-supported nanotubes and carbon-supported cobalt phosphide or iron phosphide using few nanometer sizes beads and nanofibers from GPG-AG3-His tag fusion protein are successful, details the interactions between the inorganic specials and the functional domains in the templates under the synthesis environment are still unclear. Further study in this part will benefit the control synthesis of inorganic nanomaterials.

Besides, the mineralization capability of GPG-AG3-His tag has not been fully explored. For example, synthesis of many transition metal based oxides, including nickel oxide, cobalt oxide, iron oxide and manganese oxide or their hydroxide phases for energy storage application are not yet investigated. In addition, as AG3 and His tag, which are responsible for different inorganic species, are aligned together, it is possible to explore the sequential coating of different inorganic species at the same time to construct

functional structures with synergistic effect for specific application. This area, however, is not covered in the main part of this thesis and yet to be investigated.

Finally, based on the current strategy, incorporation of more functional domains into a fusion protein for organic-inorganic material systems with hierarchical structures/multiple components, although very inspiring, are not yet on any agenda discussion.

8.2 Reconnaissance Work Not Included in Main Chapters

8.2.1 Transition Metal Oxide Nanomaterial Synthesis

The synthesis of cobalt oxide and nickel oxide or nickel oxide using fusion protein fibrous templates is at the very beginning with a mild temperature (80°C) hydrothermal method and preliminary results are briefly presented in this section.

Figure 8-1 displays the morphologies of the nickel oxide. It is observed small sheets are growing along the fibrous templates. This phenomenon is more obviously demonstrated from the morphologies of the cobalt oxide using the same hydrothermal treatment. As can be observed from Figure 8-2, the cobalt nanoparticles specifically nucleated and grew onto the fusion protein fiber surfaces. Thus, the protein fibers are suitable for template-guided synthesis of carbon-supported transition metal oxides or hydroxides.

Figure 8- 1 FESEM images (TED mode) of the nickel oxide materials.

Figure 8- 2 (a-b) FESEM (TED mode) and (c-d) TEM images of the cobalt oxides.

8.2.2 Ternary Transition Metal Oxide/Pt 1D Nanomaterials Synthesis

As one-dimensional transition metal oxide and noble metal nanomaterials have been successfully synthesized using the fibrous protein templates under similar hydrothermal conditions, the idea of sequential coating synthesis of composites is ready to trial and cobalt oxide/Pt and $\text{CoFe}_2\text{O}_4/\text{Pt}$ systems are realized using the same hydrothermal conditions. Figure 8-3 presents the TEM characterization results of the cobalt oxide/Pt systems. As can be seen, the products are one-dimensional wires entangled together. And on the fibrous structure surfaces, small size nanoparticles (less than 5 nm) are uniformly distributed. Further characterization of the phases of these products will be carried out.

Figure 8-4 shows the XRD patterns of the $\text{CoFe}_2\text{O}_4/\text{Pt}$ composites using the fibrous fusion protein templates after annealing under Ar flow at 450°C . As can be seen, purely phases of CoFe_2O_4 and Pt are presented in the composites (the pattern of C is from the tape used to hold the sample). The XRDs results indicate that oxides and Pt were individually mineralized by the respective functional domains without interfering each

other. Therefore, sequential coating strategy is plausible. The TEM images in Figure 8-5 show that carbon-supported 1D structure is pertained.

Figure 8- 3 TEM images of the cobalt oxide/Pt composites.

Figure 8- 4 XRD patterns of the CoFe_2O_4 and $\text{CoFe}_2\text{O}_4/\text{Pt}$ composites.

Figure 8- 5 TEM images of the CoFe₂O₄/Pt composites.

8.2.3 The Construction of Protein Templates

At the end of this chapter, it would be interesting to discuss the possible protein templates suitable for this purpose through combining the peptides or fusion proteins used in our group for inorganic materials synthesis as well as some already published work related to this subject. Ideally, by adopting this strategy, it would be possible to construct multifunctional fusion proteins that are to be manipulated to synthesize hierarchical inorganic materials/structures for advanced applications.

The general strategies to construct multifunctional sequences are (1) organic chain conjugated with functional peptide sequence and (2) multifunctional fusion protein designed by genetic engineering and expressed in an expression systems.

One typical example of the first class are reported in 2009, where research scientists from School of Medicine, University of Pittsburgh attached small organic molecules (aliphatic carbon tail) to (the N-terminus) of peptide AYSSGAPPMPPF (PEPAu) to construct the sequence of [C₁₁H₂₃CO]-PEPAu and then investigate the assembly of the resultant peptideconjugates, as illustrated in Figure 8-6. They discovered that that the resultant fiber adopts twisted-ribbon morphology and that the ribbons appear to adopt in a left-handed direction. They revealed that the formation of parallel beta-sheets and favorable hydrophobic interactions between the aliphatic tails had driven the organization of the C₁₂-PEPAu units along the longitudinal axis of the nanoribbon., They proposed

that the amino acids (AYSS) participate in beta-sheet assembly since the first four amino acids (AYSS) in the sequence have the capability to form beta-sheets whereas proline residues do not and AYSS also likely impart the left-handed helical twist to the nanoribbon. They further pointed out that internal amino acids (AYSS) played an important role in directing the assembly of the nanoribbons. As they had demonstrated that nanoribbons do not form using an amphiphilic constructed from the reverse peptide sequence (C_{12} -FPPMPPAGSSYA).

Figure 8- 6 C_{12} -PEPAu fiber characterization and model of assembly. Reprinted with permission from (7). Copyright (2009) American Chemical Society.

The GPG-AG3-His tag constructed and shown in the previous chapter is a good example of the second class of multifunctional sequences, which can be used to synthesize one-dimensional noble metals, transition metal oxides and phosphides nanostructures. Another example is the heat-shock protein ELP16 developed in our lab (Figure 8-7), where it was used to construct three-dimensional foam for adsorption of anode and cathode materials for LIBs and SIBs.

Figure 8- 7 Schematic Illustrating the Mechanisms in the Synthesis of MVP 3D Foams Using Recombinant ELP16 Proteins, with permission from (8). Copyright (2009) American Chemical Society.

Besides these two categories, there is another method which directly use bio-occurring templates, such as diatoms, tobacco mosaic virus, etc. for the purpose of obtaining various nanostructures, have been exploiting over the past decades, which will not be discuss here.

Hierarchical fusion protein structures which are designed to have active sites distributed spatially are very desirable. Based on these above mentioned strategies, two research developments committed to the realization of synthesizing inorganic hierarchical structures are put forward: (1) the study and manipulation of peptide self-assembly and (2) the in vitro evolution of specific proteins for mineralizing and recognizing specific inorganic materials. Till now, many ordered peptide assemblies, such as micellar, vesicular, tubular, helical, or membrane architectures, have been reported. It is to be noted that delicate manipulation conditions are necessary to ensure the successful desirable morphologies evolution. And when it comes to inorganic mineralization, special cautions are required to avoid the dissembling of the original templates as well as protein degradations.

The following aspects are worth investigated for future studies.

- (1) Affinity repeat units (peptide or organic molecular) patterning or assembling

The problems encountered in using the GPG-AG3-His tag are quite obvious: (a) even though it is claimed to be amphiphilic, the hydrophobic domain is much longer than the other two hydrophilic sequences. In some cases, such as applied in aqueous–organic phase synthesis, it will be an advantage. However, most of the time, we hope the synthesis to be fully in the aqueous environment, which requires the templates to be more hydrophilic so that high concentration templates can be achieved. This fusion protein, unless further modification is carried out, it cannot meet this criterion. (b) Under the beta-sheet formation mechanism, one of the mineralization sequences, the active AG3 may be shielded by the Histidine tag, therefore, when exposed to metal oxide precursors and noble metal precursors, the loading of noble metal will be very low, even though it can be partially solved by sequential incubation in the two precursors, and sometimes those noble metals will be covered by usually larger metal oxide particles, as can be observed in the previous section (Figure 8-3 and 8-4). As one of the promising solutions, high affinity repeat peptides can be designed and applied to direct the self-assembling of inorganic nanomaterials. This is to separate the binding and nucleation process. For example, by coating (one can even make protein patterns with the technology now available) on the substrates with AG3 following by the synthesis of Pt, Pd or Au, binder free electrodes for LOBs cathodes can be obtained, such electrodes can avoid the binder-related side reactions, which will help simplify the investigation of ORR-OER processes. We can expect improved cycling performance of LOBs by developing AG3-His tag short peptides for the assembling of noble metal and transition metal oxide nanoparticles, where partial shielding of the noble metal by metal oxide is beneficial because there will be smaller contact area between the electrolyte and the catalysts, thus electrolyte decomposition contributed from noble metal catalytic effect can be alleviated. Also, the synergistic effects that are to be revealed from both noble metal and noble metal oxides are also desirable for application in catalytic applications.

(2) Functional sequences with recognition capability aligned spatially

In the synthesis of 1D noble metal nanostructure, we observed that the loading of noble nanoparticles are high to cover the entire surface of the templates, this phenomenon suggests that we cannot further perform any inorganic synthesis on the templates since all

the mineralization sites are blocked. To avoid this trouble, we may, for example, incubate the GPG-AG3-His tag fusion protein powder and GPG- His tag fusion protein powder together. Because this two fusion proteins share the GPG domain, they will still be able to assemble into fibers, where now along the fibers, at the exteriors, there are zones of AG3-His tag and His tag only. If we perform the synthesis of noble metal first, only zone with AG3 will be covered. The following mineralization of metal oxide will only occur now at the His tag-only zone if carefully control the synthesis. We will be expected to obtain the distribution of different types of inorganic species along the 1D structure, as schematically illustrated in Figure 8-8

Figure 8- 8 Proposal of generating fusion protein fibers using GPG-AG3-His tag and GPG- His tag and their application in synthesis of 1D noble metal-metal oxide.

(3) 3D hierarchical design

A further development would be that the above two strategies be combined to achieve 3D hierarchical structures. As most of the elastin proteins seem to adopt the beta-sheet formation mechanism towards self-assembling, it would be needed to conjugate organic molecules with active sequences. For examples, if we combined the GPG-AG3-His tag, GPG-His tag and ELP16 to construct 3D foam for binder free LOBs cathodes, all the inorganic components can be arranged and function actively, along with the unique porous structure allowing oxygen diffusion, electrolyte wetting and discharge product deposition and distribution, improvement of the LOBs cells can be realized.

Figure 8- 9 Proposal of generating fusion protein fibers using GPG-AG3-His tag and GPG- His tag and their application in synthesis of 3D metal oxide/noble metal catalysts.

8.3 Outstanding Questions

While the attempts of using bio-inspired methods and materials to synthesize nanomaterials have been proved to be effective by this thesis, there are still many hindrances ahead in this research area. Especially, there are two most prominent issues:

- (1) The design of biomaterials with controllable morphologies in a wider range of scale and dimensions in different organic synthesis environment is challenging.
- (2) The precise control of the interactions between various inorganic species and the biomaterial templates is far beyond understood.

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Appendix

List of Publications

- 1 **G. Guo**, Y. Guo, H. Tan, H. Yu, W. Chen, E. Fong and Q. Yan, *From Fibrous Elastin Proteins to One-Dimensional Transition Metal Phosphides and Their Applications. Journal of Materials Chemistry A*, 2016, 4, 10893-10899.
- 2 X. Yao, [†] **G. Guo**, [†] Y. Zhao, Y. Zhang, S. Y. Tan, Y. Zeng, R. Zou, Q. Yan and Y. Zhao, *Synergistic Effect of Mesoporous Co₃O₄ Nanowires Confined by N-Doped Graphene Aerogel for Enhanced Lithium Storage. Small*, 2016, 12, 3849-3860. ([†] contributed equally)
- 3 **G. Guo**, X. Yao, H. Ang, H. Tan, Y. Zhang, Y. Guo, E. Fong and Q. Yan, *Using Elastin Protein to Develop Highly Efficient Air Cathodes for Lithium-O₂ Batteries. Nanotechnology*, 2016, 27, 045401
- 4 X. Yao, [†] **G. Guo**, [†] X. Ma, Y. Zhao, C. Y. Ang, Z. Luo, K. T. Nguyen, P.-Z. Li, Q. Yan and Y. Zhao, *In Situ Integration of Anisotropic SnO₂ Heterostructures inside Three- Dimensional Graphene Aerogel for Enhanced Lithium Storage. ACS Applied Materials & Interfaces*, 2015, 7, 26085–26093. ([†] contributed equally)
- 5 **G. Guo**, T. H. A. Truong, H. Tan, H. Ang, W. Zhang, C. Xu, X. Rui, Z. Hu, E. Fong and Q. Yan, *Platinum and Palladium Nanotubes Based on Genetically Engineered Elastin–Mimetic Fusion Protein-Fiber Templates: Synthesis and Application in Lithium-O₂ Batteries. Chemistry- an Asian Journal*, 2014, 9, 2555 – 2559.
- 6 H. X. Ang, H. T. Tan, Z. M. Luo, Y. Zhang, Y. Y. **G. Guo**, H. Zhang and Q. Yan, *Hydrophilic Nitrogen and Sulfur Co-Doped Molybdenum Carbide Nanosheets for Electrochemical Hydrogen Evolution, Small*, 2015, 47, 6278-6284.
- 7 H. Wang, H. T. Tan, H. Yi, Y. Zhang, **G. Guo**, X. Wang, S. Madhavi and Q. Yan, *Integrating Three-Dimensional Graphene/Fe₃O₄@C Composite and Mesoporous Co(OH)₂ Nanosheets Arrays/Graphene Foam into a Superior Asymmetric Electrochemical Capacitor, RSC Advances*, 2015, 5, 88191-88201.
- 8 Y. Zhang, W. Sun, X. Rui, B. Li, H. T. Tan, **G. Guo**, S. Madhavi, Y. Zong and Q. Yan, *One-pot Synthesis of Tunable Crystalline Ni₃S₄@Amorphous*

- MoS₂ Core/Shell Nanospheres for High-Performance Supercapacitors*, ***Small***, 2015, 11, 3694-3702.
- 9 J. Yang, Y. Zhang, C. Sun, **G. Guo**, W. Sun, W. Huang, Q. Yan and X. Dong, *Controlled Synthesis of Zinc Cobalt Sulfide Nanostructures in Oil Phase and Their Potential Applications in Electrochemical Energy Storages*, ***Journal of Materials Chemistry A***, 2015, 3, 11462-11470.