

Description

Title of Invention : A Copolymer and A Micellar Particle Comprising the Copolymer

Technical Field

- 5 The present invention generally relates to a copolymer. The present invention also relates to a micellar particle comprising the copolymer and uses of the micellar particle. The present invention also relates to a method of protecting a cargo at a temperature that typically causes thermal degradation of said cargo.

Background Art

- 10 In non-physiological conditions, proteins are likely to permanently lose their biological functions when exposed to high temperatures due to the lack of mechanisms that prevent unwanted proteins aggregation at high temperatures and failure to promote protein refolding into active conformation after heating. The natural GroEL-GroES system is one such system to protect proteins against denaturation at high temperatures. The GroEL-
- 15 GroES system has an interior barrel covered by some hydrophobic sites in the rim. These hydrophobic sites are used to capture unfolded proteins, thereby avoiding undesired aggregation. When triggered by adenosine 5'-triphosphate (ATP), the hydrophobic binding sites in the barrel will be buried within the subunit interfaces, thus providing a hydrophilic environment that is favourable for refolding of the unfolded proteins.
- 20 Up to date, the most classical artificial system that has been reported to simulate the molecular chaperone functionalities of the natural GroEL-GroES system is based on a two-step mechanism, which involves complicated capture-binding-stripper procedures through addition of various additives and tedious post-processing. Here, the capturer (hydrogels, nanoparticles or cationic copolymers) binds with the denatured proteins through
- 25 hydrophobic or electrostatic interactions and prevents proteins from aggregating on heating. Following which, the strippers (additives such as cyclodextrins or anionic poly(acrylic acid) (PAA)) are introduced to interrupt the interactions between the capturer and proteins to thereby release the refolded proteins. However, this system is cumbersome to use and require additional additives to remove the captured proteins.
- 30 There is a need to provide a protection system that overcomes, or at least ameliorates, one or more of the disadvantages described above.

There is a need to provide a method of protecting a cargo from thermal degradation that overcomes, or at least ameliorates, one or more of the disadvantages described above.

Summary of Invention

- 35 There is provided a copolymer comprising a hydrophilic polymer, a hydrophobic polymer and a thermoresponsive polymer.

There is provided a copolymer comprising a hydrophilic polymer, a hydrophobic polymer and a thermoresponsive polymer, wherein said hydrophobic polymer comprises at least one monomer selected from the group consisting of oligomeric silsesquioxanes, ethylene, propylene, butene, lactide, phenyl-oxazoline, α -hydroxyacid, ester and phosphazine.

- 5 According to a first aspect, there is a copolymer comprising a hydrophilic polymer, a hydrophobic polymer and a thermoresponsive polymer, wherein said hydrophobic polymer comprises at least one monomer selected from the group consisting of oligomeric silsesquioxanes, ethylene, propylene, butene, phenyl-oxazoline, ester and phosphazine.

- 10 The thermoresponsive polymer may have a low critical solution temperature (LCST) in which the thermoresponsive polymer becomes hydrophobic (or water insoluble) at a temperature above the LCST and becomes hydrophilic (or water soluble) at a temperature below the LCST. The change in the hydrophobicity/hydrophilicity of the thermoresponsive polymer due to temperature changes may be attributed to the chain conformation of the thermoresponsive polymer in which at the temperature above the LCST, the polymer chains of the
15 thermoresponsive polymer become more compact and at the temperature below the LCST, the polymer chains of the thermoresponsive polymer become more extended.

- There is provided a micellar particle comprising a copolymer having a hydrophilic polymer, a hydrophobic polymer and a thermoresponsive polymer. There is provided a micellar particle comprising a copolymer having a hydrophilic polymer, a hydrophobic polymer and a
20 thermoresponsive polymer, wherein said hydrophobic polymer comprises at least one monomer selected from the group consisting of oligomeric silsesquioxanes, ethylene, propylene, butene, lactide, phenyl-oxazoline, α -hydroxyacid, ester and phosphazine. According to a second aspect, there is provided a micellar particle comprising a copolymer having a hydrophilic polymer, a hydrophobic polymer and a thermoresponsive polymer, wherein said hydrophobic polymer
25 comprises at least one monomer selected from the group consisting of oligomeric silsesquioxanes, ethylene, propylene, butene, phenyl-oxazoline, ester and phosphazine.

- Advantageously, the presence of the hydrophobic polymer in the micellar particle may lower the critical micelle concentration (CMC) of the micellar particle as compared to another micellar particle without the same hydrophobic polymer. Hence, by having the hydrophobic
30 polymer in the micellar particle, micellization of the particle is substantially promoted. In addition, the hydrophobic polymer may increase the stability of the micellar particle in an aqueous solution. As the stability of the micellar particle is increased, the tendency of the micellar particle to form inter micellar aggregates may be substantially reduced, even at an elevated temperature. At a temperature below the LCST, the high hydrophobicity of the
35 hydrophobic polymer in the copolymer may provide stronger driving force for self-assembly and larger micelle core may be formed in an aqueous solution.

- At a temperature above the LCST of the thermoresponsive polymer, phase transition of the thermoresponsive polymer may occur in which the thermoresponsive polymer becomes hydrophobic. This may lead to decreased hydrogen interaction with water leading to the
40 collapse of the thermoresponsive polymer towards the core of the micellar particle, forming a hydrophobic layer surrounding the hydrophobic polymeric core. At a temperature below the LCST of the thermoresponsive polymer, the thermoresponsive polymer adopts a more extended conformation, becoming hydrophilic with increased hydrogen interaction with water.

There is provided a method of protecting a cargo at a temperature that typically causes thermal degradation of said cargo, comprising the steps of: (a) providing a micellar particle having a core-shell configuration, the core comprising a hydrophobic polymer and the shell comprising at least one of a hydrophilic polymer or a thermoresponsive polymer, the thermoresponsive polymer capable of becoming hydrophobic at a temperature above a low critical solution temperature of the thermoresponsive polymer; and (b) immobilizing the cargo with the hydrophobic thermoresponsive polymer at the temperature stated in step (a) to thereby protect the cargo from thermal degradation.

There is provided a method of protecting a cargo at a temperature that typically causes thermal degradation of said cargo, comprising the steps of:

- a) providing a micellar particle having a core-shell configuration, said core comprising a hydrophobic polymer and said shell comprising at least one of a hydrophilic polymer or a thermoresponsive polymer, said thermoresponsive polymer capable of becoming hydrophobic at a temperature above a low critical solution temperature of said thermoresponsive polymer; and
- b) immobilizing said cargo with said hydrophobic thermoresponsive polymer at the temperature stated in step (a) to thereby protect said cargo from thermal degradation, wherein said hydrophobic polymer comprises at least one monomer selected from the group consisting of oligomeric silsesquioxanes, ethylene, propylene, butene, lactide, phenyl-oxazoline, α -hydroxyacid, ester and phosphazine.

According to a third aspect, there is a method of protecting a cargo at a temperature that typically causes thermal degradation of said cargo, comprising the steps of:

- a) providing a micellar particle having a core-shell configuration, said core comprising a hydrophobic polymer and said shell comprising at least one of a hydrophilic polymer or a thermoresponsive polymer, said thermoresponsive polymer capable of becoming hydrophobic at a temperature above a low critical solution temperature of said thermoresponsive polymer; and
- b) immobilizing said cargo with said hydrophobic thermoresponsive polymer at the temperature stated in step (a) to thereby protect said cargo from thermal degradation, wherein said hydrophobic polymer comprises at least one monomer selected from the group consisting of oligomeric silsesquioxanes, ethylene, propylene, butene, phenyl-oxazoline, ester and phosphazine.

Advantageously, the change in the phase transition of the thermoresponsive polymer below and above the LCST may aid in the release and immobilization of the cargo respectively. The hydrophobic nature of the thermoresponsive polymer at a temperature above the LCST may aid in the immobilization of the cargo due to the hydrophobic interactions between the cargo and the hydrophobic thermoresponsive polymer. The immobilized cargo may be encapsulated by the shell of the micellar particle. Where the cargo is a protein, by immobilizing and encapsulating the protein (which is in an unfolded configuration), the protein may be prevented from aggregating with other unfolded proteins. It is known that proteins denature at high temperatures due to the aggregation of proteins at high temperatures that prevents protein

refolding into an active configuration after heating. By preventing protein aggregation, the immobilized protein may be capable of refolding into its active configuration after being released from the micellar particle.

- 5 According to a fourth aspect, there is provided use of the micellar particle as defined herein to immobilize a cargo to thereby prevent thermal degradation of the cargo at a temperature that typically causes thermal degradation of the cargo.

- 10 Advantageously, the micellar particle may be used to protect the cargo from a high temperature (that is, a temperature that typically causes thermal degradation of the cargo). Hence, the cargo may not undergo thermal degradation when immobilized with the micellar particle. The cargo may be released from the micellar particle at a temperature below the typical thermal degradation temperature. Where the cargo is a biological agent, the released cargo (after being immobilized with the micellar particle) may not suffer from any loss in its biological activity or may substantially retain its biological activity at physiological conditions.

Definitions

- 15 The following words and terms used herein shall have the meaning indicated:

- The term “hydrophilic polymer” is to be interpreted broadly to refer to a polymer that is able to dissolve, disperse or swell in an aqueous solution (such as water). The polymer chains contain hydrophilic groups that are substituents or are incorporated into the backbone. Conversely, the term “hydrophobic polymer” is to be interpreted broadly to include a polymer that is substantially insoluble in an aqueous solution (such as water). Hence, the polymer chains contain hydrophobic groups that are substituents or are incorporated into the backbone.
- 20

- The term “thermoresponsive polymer” is to be interpreted broadly to include a polymer that is able to undergo a phase transition as the temperature changes. Where the thermoresponsive polymer has a low critical solution temperature (LCST), the phase transition may occur as the temperature increases from below the LCST to above the LCST and the phase transition reverses as the temperature decreases from above the LCST to below the LCST. At a temperature above the LCST, the hydrogen bonds between the molecules and water weaken and the polymer chains aggregate with each other, forming a compact configuration. Here, the thermoresponsive polymer becomes hydrophobic. Conversely, at a temperature lower than the LCST, the polymer chains adopt an extended configuration, bind to water molecules and become hydrated. Here, the thermoresponsive polymer becomes hydrophilic.
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- The term “lower critical solution temperature” is to be interpreted broadly to refer to a temperature at which a thermoresponsive polymer experiences a phase transition.
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- The term “cargo” is to be interpreted broadly to refer to a substance or agent that is capable of being immobilized by a micellar particle. The cargo may be capable of interacting or binding with hydrophobic groups on the micellar particle at a particular temperature and which may dissociate from the micellar particle at another (typically lower) temperature. The cargo may be a chemical agent or a biological agent. The cargo may be sensitive to changes in temperature and may be susceptible to thermal degradation when the
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temperature of the cargo is substantially above the physiological or tolerance temperature of the cargo.

The term "thermal degradation" when applied to a cargo refers to the damage or destruction of the cargo when placed in a temperature that is above its normal physiological temperature or tolerance temperature. Where the cargo is a biological agent, the physiological temperature then refers to the temperature in which the biological agent is capable of exhibiting its normal biological activity and/or function. The tolerance temperature refers to a temperature that may be above the physiological temperature but which the biological agent is still able to exhibit its normal biological activity and/or function, albeit at a lower degree than those at the physiological temperature. The phrase "temperature that typically causes thermal degradation" then refers to a temperature at which the cargo undergoes a conformational, structural and/or chemical change such that the cargo no longer exhibits the biological activity and/or function that it would normally have at its physiological or tolerance temperature. Hence, while the cargo may have a range of temperatures where it is able to function, the thermal degradation temperature then refers to a temperature that is high enough to destroy the cargo's activity and/or function. Once the cargo is thermally degraded, even when the temperature of the cargo is reduced to below the thermal degradation temperature, the cargo is typically not able to recover its original activity and/or function. Hence, the thermal damage to the cargo may be permanent. Where the biological agent is a protein, at a high temperature above the physiological temperature of the protein, the protein may aggregate with another protein at the high temperature and fail to refold into its active conformation after heating. In this regard, the protein is considered to have thermally degraded.

The word "substantially" does not exclude "completely" e.g. a composition which is "substantially free" from Y may be completely free from Y. Where necessary, the word "substantially" may be omitted from the definition of the invention.

Unless specified otherwise, the terms "comprising" and "comprise", and grammatical variants thereof, are intended to represent "open" or "inclusive" language such that they include recited elements but also permit inclusion of additional, unrecited elements.

As used herein, the term "about", in the context of concentrations of components of the formulations, typically means $\pm 5\%$ of the stated value, more typically $\pm 4\%$ of the stated value, more typically $\pm 3\%$ of the stated value, more typically, $\pm 2\%$ of the stated value, even more typically $\pm 1\%$ of the stated value, and even more typically $\pm 0.5\%$ of the stated value.

Throughout this disclosure, certain embodiments may be disclosed in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the disclosed ranges. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

- Certain embodiments may also be described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the disclosure. This includes the generic description of the embodiments with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

Detailed Disclosure of Embodiments

Exemplary, non-limiting embodiments of a copolymer will now be disclosed. The copolymer comprises a hydrophilic polymer, a hydrophobic polymer and a thermoresponsive polymer.

- The copolymer may be a brush copolymer, in which the hydrophilic polymer, hydrophobic polymer and thermoresponsive polymer form the brushes (or dangling chains) of the copolymer. The hydrophilic polymer, hydrophobic polymer and thermoresponsive polymer may extend from the backbone of the copolymer. The copolymer may be a block copolymer in which the hydrophilic polymer, hydrophobic polymer and thermoresponsive polymer form respective blocks. The blocks may not be arranged in any particular order along the polymer backbone and may be random. Alternatively, one or more of the blocks may have a defined directionality such that any particular block may be incorporated in either orientation within the polymer backbone. The copolymer may be a graft copolymer.

- The hydrophilic polymer may comprise at least one monomer selected from the group consisting of vinyl alcohol, acrylic acid, acrylate, vinyl pyrrolidone, N-(2-hydroxypropyl) methacrylamide, methyl vinyl ether, maleic anhydride, methyl-oxazoline, ethyl-oxazoline, phosphates, phosphonates, and ethylene glycol. Hence, the hydrophilic polymer may be selected from the group consisting of polyvinyl alcohol, polyacrylic acid, polyacrylate, polyvinyl pyrrolidone, poly(N-(2-hydroxypropyl) methacrylamide), poly-(methyl vinyl ether-co-maleic anhydride), poly(2-methyl-2-oxazoline), poly(2-ethyl-2-oxazoline), polyphosphates, polyphosphonates, polyethylene glycol and copolymers thereof.

- The hydrophilic polymer may have a molecular weight in the range of about 300 g/mol to about 10000 g/mol, about 300 g/mol to about 1000 g/mol, about 300 g/mol to about 2000 g/mol, about 300 g/mol to about 4000 g/mol, about 300 g/mol to about 6000 g/mol, about 300 g/mol to about 8000 g/mol, about 300 g/mol to about 400 g/mol, about 300 g/mol to about 500 g/mol, about 300 g/mol to about 600 g/mol, about 300 g/mol to about 700 g/mol, about 300 g/mol to about 800 g/mol, about 300 g/mol to about 900 g/mol, about 1000 g/mol to about 10000 g/mol, about 2000 g/mol to about 10000 g/mol, about 4000 g/mol to about 10000 g/mol, about 6000 g/mol to about 10000 g/mol, about 8000 g/mol to about 10000 g/mol, about 400 g/mol to about 10000 g/mol, about 500 g/mol to about 10000 g/mol, about 600 g/mol to about 10000 g/mol, about 700 g/mol to about 10000 g/mol, about 800 g/mol to about 10000 g/mol, about 900 g/mol to about 10000 g/mol, or about 360 g/mol to about 900 g/mol.

- The hydrophobic polymer may comprise at least one monomer selected from the group consisting of oligomeric silsesquioxanes, ethylene, propylene, butene, lactide, lactone, phenyl-oxazoline), α -hydroxyacid, ester, phosphazine, hydroxybutyrate and caprolactone. Hence, the hydrophobic polymer may be selected from the group consisting of polyhedral oligomeric silsesquioxanes, polyethylene, polypropylene, polybutene, polylactide,

polycaprolactone, ethylene-propylene copolymers, poly(2-phenyl-2-oxazoline), poly(α -hydroxyacid), polyester, polyphosphazine, polyhydroxybutyrate, polycaprolactone, and copolymers thereof.

5 The hydrophobic polymer may have a mole percent of about 1.5 mol% to about 10 mol%, about 1.5 mol% to about 9 mol%, about 1.5 mol% to about 8 mol%, about 1.5 mol% to about 7 mol%, about 1.5 mol% to about 6 mol%, about 1.5 mol% to about 5 mol%, about 1.5 mol% to about 4 mol%, about 1.5 mol% to about 3 mol%, about 1.5 mol% to about 2 mol%, about 2 mol% to about 10 mol%, about 3 mol% to about 10 mol%, about 4 mol% to about 10 mol%, about 5 mol% to about 10 mol%, about 6 mol% to about 10 mol%, about 7 mol% to about 10 mol%, about 8 mol% to about 10 mol%, about 9 mol% to about 10 mol%, or about 1.57 mol% to about 5.45 mol%, in said copolymer.

15 The thermoresponsive polymer may comprise at least one monomer selected from the group consisting of propylene glycol, isopropyl-oxazoline, N-isopropylacrylamide, N,N-diethylacrylamide, N-vinylcaprolactam and (dimethylamino)ethyl methacrylate). Hence, the thermoresponsive polymer may be selected from the group consisting of poly(propylene glycol), poly(2-isopropyl-2-oxazoline), poly(N-isopropylacrylamide), poly(N,N-diethylacrylamide), poly(N-vinylcaprolactam), poly[2-(dimethylamino)ethyl methacrylate]] and copolymers thereof.

20 The low critical solution temperature (LCST) of the thermoresponsive polymer may range from about 30°C to about 50°C. Where the thermoresponsive polymer is poly(propylene glycol), the LCST may range from about 31°C to about 33°C. Where the thermoresponsive polymer is poly(2-isopropyl-2-oxazoline), the LCST may be about 36°C. Where the thermoresponsive polymer is poly(N-isopropylacrylamide), the LCST may be about 32°C. Where the thermoresponsive polymer is poly(N,N-diethylacrylamide), the LCST may range from about 25°C to about 32°C. Where the thermoresponsive polymer is poly(N-vinylcaprolactam), the LCST may range from about 25°C to about 33°C. Where the thermoresponsive polymer is poly[2-(dimethylamino)ethyl methacrylate]], the LCST may be about 50°C.

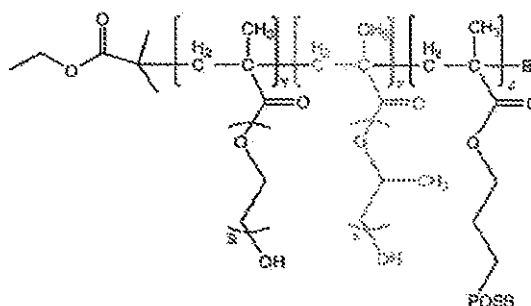
30 At a temperature above the LCST, the thermoresponsive polymer becomes hydrophobic (or water insoluble) and at a temperature below the LCST, the thermoresponsive polymer becomes hydrophilic (or water soluble). The change in the hydrophobicity/hydrophilicity of the thermoresponsive polymer due to temperature changes may be attributed to the chain conformation of the thermoresponsive polymer in which at the temperature above the LCST, the polymer chains of the thermoresponsive polymer become more compact and at the temperature below the LCST, the polymer chains of the thermoresponsive polymer become more extended.

40 The thermoresponsive polymer may have a molecular weight in the range of about about 300 g/mol to about 10000 g/mol, about 300 g/mol to about 1000 g/mol, about 300 g/mol to about 2000 g/mol, about 300 g/mol to about 4000 g/mol, about 300 g/mol to about 6000 g/mol, about 300 g/mol to about 8000 g/mol, about 300 g/mol to about 400 g/mol, about 300 g/mol to about 500 g/mol, about 300 g/mol to about 600 g/mol, about 300 g/mol to about 700 g/mol, about 300 g/mol to about 800 g/mol, about 300 g/mol to about 900 g/mol, about 1000 g/mol to about 10000 g/mol, about 2000 g/mol to about 10000 g/mol, about 4000 g/mol to about 10000 g/mol, about 6000 g/mol to about 10000 g/mol, about 8000

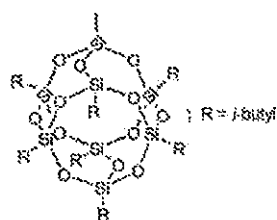
g/mol to about 10000 g/mol, about 400 g/mol to about 10000 g/mol, about 500 g/mol to about 10000 g/mol, about 600 g/mol to about 10000 g/mol, about 700 g/mol to about 10000 g/mol, about 800 g/mol to about 10000 g/mol, about 900 g/mol to about 10000 g/mol, or about 360 g/mol to about 900 g/mol, about 370 g/mol to about 380 g/mol, or about 375 g/mol.

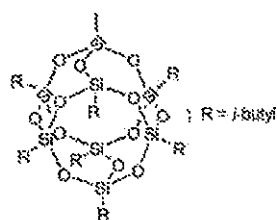
The copolymer may comprise an acrylate backbone. The acrylate may be selected from the group consisting of methacrylate, methyl acrylate, ethyl acrylate, hydroxyethyl methacrylate, butyl acrylate and butyl methacrylate. Hence, the monomeric polymers making up the copolymer may comprise acrylate groups that bind with each other to form the backbone of the copolymer.

The copolymer may have a methacrylate backbone having a hydrophilic polymer (such as poly(ethylene glycol)), a hydrophobic polymer (such as polyhedral oligomeric silsequioxane) and a thermoresponsive polymer (such as poly(propylene glycol)). Hence, the copolymer may have the structure shown in Formula I below.



Formula I



where POSS is of the following structure: , x ranges from 5 to 15, y ranges from 15 to 25, z ranges from 0.1 to 5, m ranges from 5 to 25 and n ranges from 1 to 10. In one embodiment, the x, y, z, m and n values are 13.3, 20.0, 0.53, 6.6, 5.3 respectively. In another embodiment, the x, y, z, m and n values are 12.0, 16.8, 1.56, 6.6, 5.3 respectively and in yet another embodiment, the x, y, z, m and n values are 10.2, 16.3, 1.53, 20.0, 5.3 respectively.

In order to form the copolymer of Formula I, the monomer polymers (that is, before synthesis of the copolymer) may be methacrylate derivative of poly(ethylene glycol) (representing the hydrophilic portion), methacrylate derivative of polyhedral oligomeric silsequioxane (representing the hydrophobic portion) and methacrylate derivative of poly(propylene glycol) (representing the thermoresponsive portion).

The copolymer may contain biocompatible or non-toxic polymers, and may be easily degraded and removed by a body's excretory system. Hence, the monomeric polymers

making up the copolymer may be biocompatible and/or non-toxic. The monomeric polymers and/or the copolymer may not affect the viability of a cell when taken into that cell.

5 The copolymer may be formed by atom-transfer radical polymerization. Here, the various monomeric polymers may be dissolved in an appropriate solvent such as an organic solvent or organic solvent/water mixtures, introducing an inert gas into the reaction, raising the temperature to a polymerization temperature to initiate polymerization. A polymerization catalyst may be introduced into the reaction at any point in the reaction to speed up the polymerization process. After polymerization, the reaction may be purified to
10 remove excess reactants and/or catalyst by precipitation or dialysis. The resultant copolymer may then be obtained after freeze drying, centrifugation, etc. This method of forming the copolymer may be simple and can be carried out in a "one-pot" system.

The copolymer may form a micellar particle. Hence, there is also provided a micellar particle comprising a copolymer having a hydrophilic polymer, a hydrophobic polymer
15 and a thermoresponsive polymer.

The micellar particle may have a core-shell configuration in an aqueous solution, in which the hydrophobic polymer is present in the core and at least one of the hydrophilic polymer or the thermoresponsive polymer is present in the shell. The shell may comprise both the hydrophilic polymer and the thermoresponsive polymer. The hydrophilic polymer and the
20 thermoresponsive polymer may be present in the shell (or corona) of the micellar particle as brushes of the copolymer.

The micellar particle may be substantially spherical in shape. The size of the micellar particle may be in the nano-scale range.

The presence of the hydrophobic polymer in the micellar particle may lower the critical
25 micelle concentration (CMC) of the micellar particle as compared to another micellar particle without the same hydrophobic polymer. Hence, by having the hydrophobic polymer in the micellar particle, micellization of the particle is substantially promoted. In addition, the hydrophobic polymer may increase the stability of the micellar particle in an aqueous solution. As the stability of the micellar particle is increased, the tendency of the
30 micellar particle to form inter micellar aggregates may be substantially reduced, even at an elevated temperature. Accordingly, the use of the hydrophobic polymer may aid in enhancing the thermal stability of the micellar particle. At a temperature below the LCST, the high hydrophobicity of the hydrophobic polymer in the copolymer may provide stronger driving force for self-assembly and larger micelle core may be formed in an
35 aqueous solution. The hydrophobic polymer may also affect the LCST of the thermoresponsive micellar particle. Typically, an increase in the amount of the hydrophobic polymer in the copolymer may increase the LCST.

At a temperature above the LCST of the thermoresponsive polymer, phase transition of the thermoresponsive polymer may occur in which the thermoresponsive polymer becomes
40 hydrophobic. This may lead to decreased hydrogen interaction with water leading to the collapse of the thermoresponsive polymer towards the core of the micellar particle, forming a hydrophobic layer surrounding the hydrophobic polymeric core. At a temperature below the LCST of the thermoresponsive polymer, the thermoresponsive

polymer adopts a more extended conformation, becoming hydrophilic with increased hydrogen interaction with water. Hence, the thermoresponsive polymer becomes shorter in length at the temperature above the LCST and becomes longer at the temperature below the LCST.

- 5 There is also provided a method of protecting a cargo at a temperature that typically causes thermal degradation of the cargo, comprising the steps of: (a) providing a micellar particle having a core-shell configuration, the core comprising a hydrophobic polymer and the shell comprising at least one of a hydrophilic polymer or a thermoresponsive polymer, the thermoresponsive polymer capable of becoming hydrophobic at a temperature above a low
10 critical solution temperature of the thermoresponsive polymer; and (b) immobilizing the cargo with the hydrophobic thermoresponsive polymer at the temperature stated in step (a) to thereby protect the cargo from thermal degradation.

As mentioned above, at a temperature above the LCST, the thermoresponsive polymer may collapse towards the core of the micellar particle, forming a hydrophobic layer
15 surrounding the hydrophobic polymeric core, leaving cavity-like spaces that can accommodate the cargo (via hydrophobic interactions), resulting in the formation of a complex between the immobilized cargo and the micellar particle and protection of the cargo.

The temperature stated in step (a) may be substantially lower than the thermal degradation
20 temperature of the cargo. Hence, the cargo/micellar particle complex may be formed before the thermal degradation temperature is reached so as to protect the cargo from thermal degradation at an earlier stage.

As the temperature is lowered to below the LCST, the method may further comprise the step of releasing the cargo from the complex. As the temperature decreases, the
25 thermoresponsive polymer becomes hydrophilic which then promotes the dissociation of the hydrophobic cargo from the hydrophilic thermoresponsive polymer. As the cargo is released from the complex, the micellar particle reverts to its original size.

The cargo may be a chemical agent or a biological agent. The chemical agent may be a probe, a catalyst, etc that may be sensitive to changes in temperature. The biological agent
30 may be selected from the group consisting of a therapeutic agent, a protein, a microorganism and a nucleic acid. The protein may be selected from the group consisting of an enzyme, a hormone, an antibody, an antigen, a receptor, a transport protein, a structural protein, a motor protein, a signaling protein, a storage protein and a coagulation protein. The protein may include, but not limited to, insulin, growth factor, lipotropin,
35 prolactin, endorphin, enkephalin, cytochrome C, haemoglobin and myoglobin oxygen transport proteins, albumin, lymphocyte antigen-recognizing receptors, antiviral agents such as interferon and tumor necrosis factor, fibrin, thrombin, collagen, elastin, α -keratin, sclerostin, fibronin, bacterial proteoglycans, virus coating protein, actin, myosin, rhodopsin, egg ovalbumin, milk casein, Factor VIIa, lyase, hydrolase, oxidoreductase, transferase,
40 isomerase, and ligase, lipase, lysozyme, green fluorescent protein, phosphatase, amylase, cellulase, protease, peptidase, urease, deaminase, and combinations thereof.

Where the cargo is a protein and at an elevated temperature, thermal degradation of the protein can occur, which causes denaturation of the protein due to the formation of inactive

and insoluble aggregates. This aggregation process is irreversible which prevents the proteins from refolding into their native stage and thus losing their functionalities. Under normal conditions, the micellar particles self-assembled from the copolymer defined herein are in the dormant state and do not interfere with the protein activities at temperatures

5 below the LCST. When the micelle solutions are heated to a temperature above the LCST (but below the unfolding temperature of the proteins), the micelles transform into their functional state due to the collapse of the thermoresponsive polymer brushes in the mixed corona. The collapsed thermoresponsive polymers form hydrophobic domains on the micelle cores made from the hydrophobic polymer and the acrylate backbones, leaving

10 behind cavity-like spaces that could accommodate the denatured proteins. Further increase in the temperature induces the denaturation of the protein into unfolded intermediates and exposing the hydrophobic inner core of the proteins. The unfolded intermediates are immediately captured through multipoint hydrophobic interactions with the hydrophobic domains of the micelles before protein aggregation could occur, resulting in the formation

15 of the micelle/protein complexes and protection of the unfolded intermediates. Protein absorption and capture occurs mainly in the core (hydrophobic domains) of the micelles. The capture and holding of proteins in the micelles hydrophobic domains could effectively prevent the irreversible aggregation of the proteins. The stretched hydrophilic polymeric brushes in the outer layer could make the micelle/protein complexes stable and provide

20 steric repulsion to prevent further intermicellar aggregation. Upon cooling, the micelle/protein complexes gradually dissociate, owing to the temperature sensitive phase transition of the thermoresponsive polymer brushes from hydrophobic to hydrophilic state, during which the unfolded intermediates detach from the micelle surfaces and start to refold into their native confirmation while the micelles return to their original size range.

25 Hence, the micellar particle may function as an artificial molecular chaperone for effective protein protection in the thermal-induced denaturation process.

The method may increase the amount of cargo recovered after the cargo is subjected to the thermal degradation temperature as compared to another system that does not use the micellar particle as stated herein. Hence, the amount of recovered cargo may be at least 2

30 times, at least 3 times, at least 4 times or at least 5 times as compared to the amount of cargo recovered in a system that does not use the micellar particle as defined herein or which does not use any thermal protecting agent therein. The recovered cargo may not experience any substantial loss in its activity after recovery. Hence, the activity of the recovered cargo may be substantially similar (or may be slightly decreased as compared) to

35 the activity of the cargo before being subjected to the thermal degradation temperature.

There is also provided use of the micellar particle mentioned herein to immobilize a cargo to thereby prevent thermal degradation of the cargo at a temperature that typically causes thermal degradation of the cargo. The cargo may be as defined above. The protein, when immobilized with the micellar particle, is then substantially prevented from aggregating

40 with another protein and hence, substantially prevented from protein denaturation.

The micellar particle may be merely mixed with the cargo and exposed to the elevated temperature. Hence, the micellar particle may be easily used and may not require pre-treatment or activation to provide the cargo protection effects. The micellar particle may use a simple and spontaneous "capture and release" mechanism based on temperature as

45 the sole trigger in an "on-demand" fashion. This is in comparison to other conventional

protection systems which involve complicated "capture-binding-stripper" procedures through addition of various additives and tedious post-processing.

Brief Description of Drawings

5 The accompanying drawings illustrate a disclosed embodiment and serves to explain the principles of the disclosed embodiment. It is to be understood, however, that the drawings are designed for purposes of illustration only, and not as a definition of the limits of the invention.

Fig.1

10 [Fig. 1] is a figure showing the ^1H NMR spectra of PEPS-2 hybrid copolymer in CDCl_3 , according to Example 1.

Fig. 2

[Fig. 2] is a graph showing the GPC profiles of (a) PEPS-1 hybrid copolymer and the precursors (b) POSSMA, (c) PPGMA375 and (d) PEGMA360 according to Example 1.

Fig. 3

15 [Fig. 3] is a graph showing the TGA curves for (a) POSSMA, (b) PEPS-3, (c) PEGMA950 and (d) PPGMA375 according to Example 1.

Fig. 4A

20 [Fig. 4A] is a graph showing the thermo-responsive behaviours of PEPS hybrid copolymers in aqueous solutions (2.0 mg/ml), measured by UV-Vis spectroscopy at 530 nm.

Fig. 4B

[Fig. 4B] is a graph showing the LCST determination from the derivative absorbance spectroscopy in which (a) denotes PEP, (b) denotes PEPS-1 and (c) PEPS-2 according to Example 1.

25 Fig. 5(a)

[Fig. 5(a)] shows the distribution of hydrodynamic radius, R_h of micelles in PEPS-1 for temperature ranging from 20°C to 70°C according to Example 2.

Fig. 5(b)

30 [Fig. 5(b)] shows the distribution of hydrodynamic radius, R_h of micelles in PEPS-2 for temperature ranging from 20°C to 70°C according to Example 2.

Fig. 5(c)

[Fig. 5(c)] shows the dependence of decay rate Γ on q^2 for PEPS-1 (circle symbols) and PEPS-2 (triangle symbols) respectively at 25°C according to Example 2.

Fig. 5(d)

[Fig. 5(d)] shows the R_h of micelles as a function of solution temperature for PEP (square symbols), PEPS-1 (circle symbols), PEPS-2 (triangle symbols) and PEPS-3 (diamond symbols) respectively according to Example 2. The solid lines are included to guide the eye. All samples were prepared at polymer concentration of 0.5 mg/mL, except for PEP at temperatures below 30°C (copolymer concentration of 4 mg/mL, marked with an asterisk on the square symbols) and PEPS-3 (copolymer concentration of 2 mg/mL). Measurements in Fig. 5(a), Fig. 5(b) and Fig. 5(d) were performed at scattering angle of 90° and R_h is calculated from Eq. (1).

Fig. 6(a)

[Fig. 6(a)] is a transmission electron microscopy (TEM) image showing particles formed in PEPS-2 copolymer aqueous solution at a concentration of 0.5 mg/mL at a temperature of 25°C. The scale bar in Fig. 6(a) is 100 nm.

Fig. 6(b)

[Fig. 6(b)] is a TEM image showing particles formed in PEPS-2 copolymer aqueous solution at a concentration of 0.5 mg/mL at a temperature of 70°C. The scale bar in Fig. 6(b) is 100 nm.

Fig. 7(a)

[Fig. 7(a)] shows the aggregation number, N_{agg} of aggregates in PEP and PEPS copolymers as a function of temperature determined from static light scattering (SLS) within the concentration range of 0.5 to 1.0 mg/mL except for PEP at temperatures below 30°C (concentration range of 3.5 to 4.5 mg/mL, marked with an asterisk on the square symbols) and PEPS-3 (copolymer concentration of 2.0 to 3.0 mg/mL).

Fig. 7(b)

[Fig. 7(b)] shows the dimensionless ratio R_g/R_h of the aggregates in PEP and PEPS copolymers of Fig. 6(a). PEP, PEPS-1, PEPS-2 and PEPS-3 are represented by square, circle, triangle and diamond symbols respectively. The solid lines are drawn to guide the eye.

Fig. 8

[Fig. 8] are bar charts showing the thermal denatured protein protection efficiencies for (a) GFP, (b) lipase and (c) lysozyme in the presence of thermo-responsive PEPS hybrid copolymers according to Example 3.

Fig. 9

[Fig. 9] is a schematic representation of (a) PEPS hybrid copolymer self-assembly in aqueous solution, (b) heat-induced protein denaturation process and (c) proposed working mechanism of the thermally denatured protein protection by thermo-responsive PEPS hybrid micelles; according to Example 3.

Fig. 10A

[Fig. 10A] is a far UV-CD spectra of free lipase (open circle) and lipase mixed with PEPS-1 hybrid copolymer (closed triangle) in phosphate buffer at 25°C.

Fig. 10B

- 5 [Fig. 10B] shows the mean residue ellipticity (θ) for free lipase (open circle) and lipase mixed with PEPS-1 hybrid copolymer (close triangle) as a function of temperature in phosphate buffer.

Fig. 11A

[Fig. 11A] is a confocal microscopy image of C6 Glioma cells cultured with free GFP.

10 **Fig. 11B**

[Fig. 11B] is a confocal microscopy image of C6 Glioma cells cultured with GFP with PEP copolymer.

Fig. 11C

- 15 [Fig. 11C] is a confocal microscopy image of C6 Glioma cells cultured with PEPS hybrid copolymer.

Fig. 12

- 20 [Fig. 12] shows the cell viability of human dermal fibroblast cells incubated at 1.0 mg/mL and 4.0 mg/mL of PEP copolymer and PEPS-1 hybrid copolymer. Cells in blank samples are considered as 100% viable. Each column represents the average value of three independent measurements. The error bar represents the standard deviation.

Examples

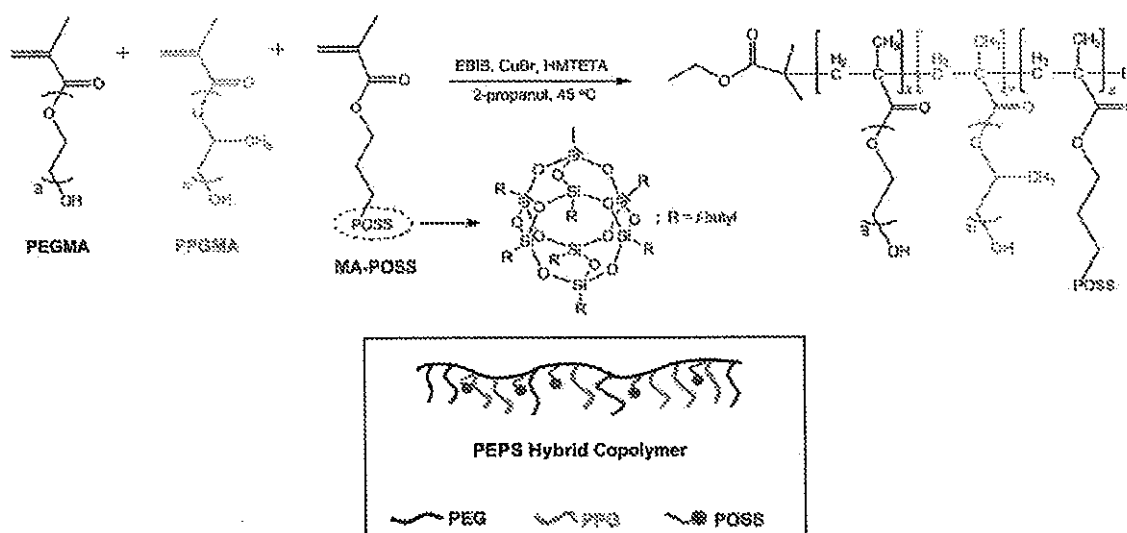
Non-limiting examples of the invention and a comparative example will be further described in greater detail by reference to specific Examples, which should not be construed as in any way limiting the scope of the invention.

25 **Example 1**

Synthesis of Poly(PEGMA-PPGMA-POSSMA) (PEPS) Hybrid Copolymer

- 30 Methacrylisobutyl-POSS (POSSMA) was purchased from Hybrid Plastic (Product No. MA0702) and used as received. Poly(propylene glycol) methacrylate (PPGMA, $M_n = 375$), Poly(ethylene glycol) methacrylate (PEGMA, $M_n = 360$ and 900), Ethyl 2-bromoisobutyrate (EBiB, 98%), 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA, 99%), copper(I) bromide (CuBr, 99%), 2-propanol (>99.5%), 4-Nitrophenyl acetate (97%), Acetonitrile (99.8%) and Micrococcus lysodeikticus ATCC No. 4698 were obtained from Sigma-Aldrich (of Missouri of the United States of America). Purified nitrogen was used in the polymerization reactions.

- 5 Poly(PEGMA-PPGMA-POSSMA) hybrid copolymers derived from PEGMA, PPGMA and POSSMA are denoted as PEPS copolymers, where the first P represents for poly-, E for PEGMA, the second P represents for PPGMA, and S for POSSMA. PEPS hybrid copolymers were synthesized by ATRP using EBiB as an initiator. The synthetic route and schematic illustration of the brush type PEPS hybrid copolymers are presented in Scheme 1 below.



Scheme 1

- Here, the molar ratio of PEGMA/PPGMA was fixed at 1:2 and the POSSMA content varied from 1.96 to 5.67 mol%. Typically, PEGMA (4.0 g, 11.1 mmol), PPGMA (8.3 g, 22.2 mmol), POSSMA (0.63 g, 0.67 mmol), EBiB (50 μ L, 0.34 mmol), and HMTETA (184 μ L, 0.68 mmol) were introduced into the flask containing 15 mL of 2-propanol. After the reactants were dissolved completely, oxygen was removed by repeated *vacuum-nitrogen-cycling* system. Then, the degassed CuBr in 1 mL 2-propanol was added into the flask under nitrogen atmosphere. The reactions were allowed to proceed under continuous stirring at 60 °C for a desired reaction time. The molecular weight was monitored by gel permeation chromatography (GPC) analysis. After polymerization, the solution was diluted with tetrahydrofuran (THF) and passed through a neutral aluminium oxide column to remove the copper catalyst. THF was removed under reduced pressure to give a concentrated solution. Products were precipitated in a large excess of hexane to remove free PPGMA and POSSMA. The precipitated mixture of PEPS copolymer and PEGMA was re-dissolved in deionized water and purified by dialysis (Spectrum dialysis membrane, MWCO 5000) for 72 hours to remove free PEGMA using deionized water, which was changed regularly. Polymer samples were obtained after freeze-drying and weighed to obtain the final yields.

Synthesis of Poly(PEGMA-PPGMA) (PEP) Hybrid Copolymer

- As a reference sample, Poly(PEGMA-PPGMA) hybrid copolymers derived from PEGMA and PPGMA are denoted as PEP copolymers, where the first P represents for poly-, E for PEGMA and the second P represents for PPGMA. The reaction scheme shown in Scheme 1 was used to prepare the PEP copolymers but without the use of POSSMA.

Chemical Structure Determination

The chemical structure of PEPS hybrid copolymers were verified by ^1H NMR spectroscopy. ^1H -NMR spectra were recorded on a Bruker AV-400 NMR spectrometer at room temperature. Chemical shift at 7.3 ppm was referred to the solvent peaks CHCl_3 .

- 5 Copolymer compositions in molar ratios were evaluated by the proton integral regions as assigned in Fig. 1. Fig. 1 shows the typical ^1H NMR spectrum of PEPS-3 in CDCl_3 , in which all proton signals belonging to PEG, PPG, POSS, and poly(methacrylate) portions are confirmed. The signals at 3.6 ppm are assigned to the $-\text{CH}_2-\text{CH}_2-\text{O}-$ of PEG, signals corresponding to $-\text{OCH}_2-$ in repeated units of PPG segments are observed at 3.25 ppm, and the signals at 0.58 – 0.60 ppm are associated with methylene protons (f and g in Fig. 1) of POSSMA. Protons in $-\text{CH}_3-$ and $-\text{CH}_2-$ of the poly(methacrylate) backbone in PEPS copolymers are identified at $\delta \sim 0.95 - 1.1$ ppm and $\delta \sim 1.8 - 2.0$ ppm, respectively. However, resonances observed at $\delta \sim 5.75$ and 6.2 ppm for vinyl protons in all the starting materials are not shown in the final products, indicating the three components
- 10 copolymerization has successfully occurred, and PEPS hybrid copolymers with high purity was obtained. The composition of the copolymer in molar ratio, represented by x , y , and z values in the macromolecule structure, was estimated by comparing the integral values of the corresponding signals assigned in Fig.1, and the data are summarized in Table 1.
- 15

Table 1. Molecular characteristics of PEPS hybrid copolymers and control polymer

Samples ^a	Copolymer composition in mole ratio				POSS (wt %) ^c	M_n ($\times 10^{-4}$) ^d	PDI ^d	LCST (°C) ^e	CMC (mg/mL) ^f
	PEGMA360	PEGMA950	PPGMA375	POSSMA					
PEP	1.0 (1.0) ^b	0 (-)	2.0 (1.9)	0 (-)	-	2.56	1.5	29.0	3.0
PEPS-1	1.0 (1.0)	0 (-)	2.0 (1.5)	0.06 (0.04)	3.1	2.24	1.6	31.0	0.3
PEPS-2	1.0 (1.0)	0 (-)	2.0 (1.4)	0.18 (0.13)	6.7	2.01	1.5	33.0	0.1
PEPS-3	0 (-)	1.0 (1.0)	2.0 (1.6)	0.18 (0.15)	6.3	2.31	1.4	-	1.0

^a Hybrid copolymers poly(PEGMA-PPGMA-POSSMA) are denoted as PEPS, where P represents poly-, E is for PEGMA, P for PPGMA, and S is for POSSMA.

^b Feeding ratios of the starting materials in the reaction, the values in the parentheses show the mole ratios calculated from NMR results.

^c Calculated from TGA thermograms.

^d Determined from GPC.

^e Determined by UV-Vis spectrophotometer.

^f Determined by dynamic light scattering at 25 °C.

As shown in Table 1, the molar ratios of PEG/PPG in the final polymer determined from NMR spectra are larger compared to the feed ratio of 1 : 2, which is attributed to the higher reactivity of the vinyl groups in PEGMA as compared to the vinyl groups in PPGMA. The lower reactivity of the vinyl groups in PPGMA is due to the steric hindrance of the methyl group located in PPG segments which is in agreement with previous studies on the copolymerization of PEG and PPG containing macromolecules as in situ thermogels.

Further verification of the successful synthesis of PEPS hybrid copolymers was obtained from GPC analyses. GPC analysis was carried out with a Shimadzu SCL-10A and LC-8A system equipped with a Shimadzu RID-10A refractive index detector. THF was used as the eluent at a flow rate of 1.0 mL/min at 40°C. Monodispersed PMMA standards were used to obtain a calibration curve. The molecular weight and polydispersity information are presented in Table 1. All the copolymers synthesized have high molecular weight (2.01×10^4 – 2.56×10^4) and low polydispersity (1.41–1.56). Fig. 2 shows the representative GPC profiles of PEPS hybrid copolymer and its precursors. All the traces show a monomodal peak of the molecular weight distribution, revealing the high purity of the tested samples rather than a mixture. In addition, PEPS copolymer trace shows a higher molecular weight (shorter elution time) as compared to the traces of the starting materials. The concomitant increase in the molecular weight, together with the NMR results of the copolymers indicates that the copolymerization was successful.

20 Thermal Gravimetric Analysis (TGA)

The PEPS hybrid copolymers were subjected to thermal gravimetric analysis (TGA, TA Q500) to determine the weight fraction of POSS in the hybrid copolymers. Samples were heated at 10 °C/min to 700 °C under an air flow rate of 60 mL/min. Results are shown in Table 1 above and the representative TGA curves are shown in Fig. 3. PPGMA and PEGMA prepolymers were completely decomposed at c.a. 500 °C under air atmosphere (Fig. 3, curves c and d). However, according to the yield of residual, it is judged that the organic ligand of POSSMA have completely decomposed at elevated temperature above 500 °C (Fig. 3, curve a); *i.e.*, the POSS segments were transformed into ceramics due to the thermal decomposition and oxidation. Therefore, the ceramic yields can be employed to estimate the content of POSS in PEPS hybrid copolymers. The results are summarized in Table 1 above and the ceramic yields were measured to be 3.1, 6.7, and 6.3 wt % for PEPS-1, PEPS-2 and PEPS-3 hybrid copolymers, respectively, which is quite close to the ceramic yields calculated in terms of the molar ratios. The TGA results further confirmed the as-synthesized PEPS copolymers in hybrid compositions.

35 Thermal Responsive Behaviours

Thermal responsive behaviors of the PEPS hybrid copolymer solutions were performed by UV-Vis spectrophotometer (Agilent 8453) at 530 nm. Aqueous copolymer solutions at concentration of 2.0 mg/mL were used for the measurements with temperatures ranging from 20 to 70°C. Fig. 4A presents the optical absorbance of the copolymer solutions (2.0 mg/mL) as a function of temperature. Here, the point at which there is a sudden drop in absorbance at a certain critical temperature is defined as the lower critical solution temperature (LCST). The first derivative absorbance plot, which reflects the quantitative measurement of the sharp phase transition of the aqueous copolymer solution on temperature, is shown in Fig. 4(b). LCST values determined from this method are

tabulated in Table 1 above. Aqueous solutions of copolymers exhibit LCST that are very similar to the poly(N-isopropylacrylamide) (PNIPAAm). From Table 1, the LCST of PEP copolymer solution was 29.0 °C, that is, at temperature below LCST, PPG is a hydrophilic water-soluble polymer and PEP copolymer solution is clear with very low optical absorbance. Above this temperature, PPG becomes hydrophobic and collapses to form larger aggregations, leading to a turbid solution with increased absorbance (Fig. 4A). The continuous decrease in absorbance beyond the LCST of PEP copolymer solution is due to the precipitation of larger aggregations. In contrast, at similar PEG/PPG ratio, the incorporation of hydrophobic POSS in PEPS-1 and PEPS-2 hybrid copolymer solutions exhibit better thermal stability, as seen from the flat absorbance curves at the same elevated temperature range (beyond the LCST) as depicted in Fig. 4A. Moreover, the LCST for these two hybrid copolymers, PEPS-1 and PEPS-2, are higher compared to that of PEP copolymer without POSS (Fig. 4B and Table 1). When the POSS content is 3.1 wt %, the increment of LCST is about 2 °C, allowing the PEPS-1 to have LCST of 31 °C. With further increase of POSS content to 6.7 wt % in PEPS-2, the LCST is observed at 33 °C. However, the macroscopic phase transition of PEPS-3 copolymer solution from clear to turbid was not observed in the experimental temperature range, because the longer PEG brushes in PEPS-3 could provide superior water solubility of the copolymer than its counterparts. The abrupt increase in absorbance at above 60 °C is probably due to the PEG dehydration and subsequent association. Thermo-responsiveness studies show that the incorporation of POSS in PEPS hybrid copolymer could afford better thermal stability of the copolymer solution, and the LCST can be adjusted by tuning the copolymer compositions.

Example 2

Self-assembly Behavior of PEPS Hybrid Copolymer Solutions

Micelles with P(MA-POSS) segments as the core and PEG and PPG brushes as the corona are expected to form in aqueous solution. Dynamic Light Scattering (DLS) was used to determine the critical micelle concentration (CMC) of the PEP and PEPS copolymers in aqueous solution at different temperatures. DLS measurements were made with a Brookhaven BI-200SM multi-angle goniometer equipped with a BI-APD detector. The light source was a 35 mW He-Ne laser emitting vertically polarized light of 632.8 nm wavelength. From the expression $\Gamma = D_{app} q^2$, the apparent translational diffusion coefficients, D_{app} , were determined where Γ is the decay rate and q is the scattering vector. The apparent hydrodynamic radius, R_h , can be determined by the Stokes-Einstein relationship:

$$R_h = \frac{kT}{6\pi\eta D_{app}}$$

where k , η and T are the Boltzmann constant, viscosity of solvent, and the absolute temperature, respectively. The sample temperature was equilibrated for 30 minutes before the measurement was made. The scattering intensity of each concentration of the copolymer in deionized water was measured and plotted against the polymer concentration. The concentration at which the scattering intensity increases sharply was defined as the

CMC. Below 30 °C, PEP copolymer without POSS possesses high CMC values (~3mg/mL) and the CMC values decreases by almost two orders of magnitude when the temperature is increased up to 70 °C. With the presence of a small amount of POSS (3.1 wt%) in the copolymers, the CMC values are lowered as compared to samples without POSS as depicted in PEPS-1. In addition, the CMC values decreases further with increasing POSS content in the copolymers (see PEPS-2 and PEPS-3) suggesting that the higher POSS content favours micellization promoted by an increase in hydrophobicity. Notably, the decrease in CMC values is more gradual for copolymers with POSS (PEPS-1, PEPS-2 and PEPS-3) when the temperature was increased from 20 to 70°C (e.g. 0.3 mg mL⁻¹ to 0.1 mg mL⁻¹ for PEPS-1) as compared to the sample without POSS (PEP) which decreases almost two orders of magnitude for the same temperature range. The presence of POSS is hypothesized to play a part in formation of a more stable micelle in aqueous solution. When comparing the effect of PEG content in the copolymers (PEPS-2 and PEPS-3), sample with larger PEG content possesses higher CMC values for the same temperature range due to the increased hydrophilicity of the copolymers resulting in unfavourable micelle formation in aqueous solution.

The hydrodynamic radius, R_h of the micelles in aqueous solution formed by the PEP and PEPS copolymers at different temperatures were measured using DLS for copolymer concentrations ranging from 0.5 to 1.0 mg/mL, well above the CMC of PEPS-1 and PEPS-2, to ensure the micelle formation. Fig. 5(a) and Fig. 5(b) demonstrate the particle size distribution of the micelles formed from PEPS-1 and PEPS-2 respectively for temperature ranging from 20 to 70 °C. The distribution is unimodal and becomes narrower when temperature is increased beyond ~33 to 35°C, depending on the sample. In addition, the dependence of decay rate Γ (the reciprocal of relaxation time, τ) on q^2 (according to $\Gamma = D_{app} q^2$), shown in Fig. 5(c), exhibits a linearity confirming that the observed peaks in Fig. 5(a) and Fig. 5(b) originate from the translational diffusion of the copolymer micelles. The R_h at peak maximum of the PEP and PEPS micelles at each temperature is plotted as a function of temperature as depicted in Fig. 5(d) for copolymer concentration of 0.5 mg/mL. However, there are exceptions for PEP at temperatures below 30 °C (copolymer concentration of 4 mg/mL) and PEPS-3 (copolymer concentration of 2 mg/mL) due to the large CMC values of these samples. When comparing the effect of POSS content in the copolymers (see PEP, PEPS-1 and PEPS-2), as anticipated, PEPS-2, having the highest POSS content in the copolymer (highest hydrophobicity) formed the largest micelles ($R_h \sim 65$ nm) in aqueous solution at temperatures below 30 °C as compared to PEP and PEPS-1. PEPS-1 formed micelles having R_h of approximately 20 nm which gradually increased to approximately 45 nm when the temperature is increased up to 40 °C, beyond which the R_h remained quite constant. PEP also formed micelles having similar R_h (~20 nm) at low temperatures but increases drastically to approximately 120 nm at temperatures above 30 °C. Interestingly, although both PEP and PEPS-1 has similar PEGMA and PPGMA content in the copolymer but without POSS in PEP, the formation of large micelles in PEP ($R_h \sim 120$ nm) at high temperatures, which is three times the size of micelles formed from PEPS-1 ($R_h \sim 40$ nm), can be attributed to the aggregation of a large number of micelles (inter micellar aggregation) due to the increasing hydrophobicity and instability of the micelle with increasing solution temperature. Instead, the milder increase in R_h observed in PEPS-1 with increasing temperature, suggests the enhancement of the stability of the micelles formed when POSS is present in the copolymers, which reduces the tendency to

form inter micellar aggregates. In a similar manner, the R_h of PEPS-2 remained almost constant at approximately 65 nm when temperature was increased from 20 to 70 °C further suggesting the formation of a more stable micelle in aqueous solution when a higher amount of POSS was present in the copolymer which prevented inter micellar aggregation at higher temperatures.

From the transmission electron microscope (TEM, Philips CM300 FEGTEM) images in Fig. 6(a) and Fig. 6(b), spherical particles were observed and the estimated diameters were in good agreement with the size obtained by DLS at the two different temperatures (with Fig. 6(a) at a temperature of 20°C and Fig. 6(b) at a temperature of 70°C). TEM samples were prepared by depositing one drop of the copolymer solution (0.5 mg/mL) onto 200 mesh copper grids, which were coated in advance with supportive Formvar films and carbon (Agar Scientific). The samples were kept for 24 hours at 25°C and 70°C prior to TEM image.

In addition to the effect of POSS content in the copolymers, the effect of the length of the PEG brushes in the copolymers (see PEPS-2 and PEPS-3) on the micelle size was also investigated. Similar to PEPS-2, the R_h of PEPS-3 remained almost constant at approximately 50 nm in the same temperature range further confirming that ~6wt% POSS content in the copolymer is sufficient for the formation of stable micelles in aqueous solution. The micelle size obtained from PEPS-3 is smaller compared to PEPS-2 due to the longer PEG brushes in the former, which reduces the hydrophobicity and tendency to form micelle leading to formation of smaller micelles in PEPS-3. Nevertheless, the stability of the micelle was not compromised with the incorporation of a longer PEG brush length as the micelle size remained constant throughout the whole temperature range investigated.

Static light scattering (SLS) measurements were performed to determine the weight-average molecular weight (M_w), z-average radii of gyration (R_g), and second virial coefficients (A_2) of the micelles in aqueous solution according to the following equation:

$$\frac{Kc}{\Delta R_\theta} = \frac{1}{M_w} \left[1 + \frac{q^2 R_g^2}{3} \right] + 2A_2c$$

where K is the optical constant, which depends on the refractive index increment (dn/dc) of the polymer solution, c is the concentration of the polymer solution and ΔR_θ is the excess Rayleigh ratio. The scattering angles ranged from 50° to 120° at 10° intervals while the copolymer concentration ranged from 0.5 to 1.0 mg/mL. The refractive index increment (dn/dc) of each copolymer solution was measured using a BI-DNDC differential refractometer at a wavelength of 620 nm. The instrument was calibrated primarily with potassium chloride (KCl) in aqueous solution. SLS experiments were further used to elucidate the effect of temperature and POSS content in the copolymers on the aggregation of the PEP and PEPS copolymers in solution. The apparent molecular weight of the aggregates ($M_{w,micelle}$), together with the radius of gyration (R_g) were determined by a Zimm plot in SLS as a function of temperature. The $M_{w,agg}$ values in aqueous solutions were much larger than the $M_{w,single}$ values of the individual PEP and PEPS copolymers obtained by GPC, further confirming the formation of PEP and PEPS micelles in aqueous solution. Subsequently the apparent aggregation number, N_{agg} ($N_{agg} = M_{w,micelle}/M_{w,single}$) of the micelles in aqueous solution were calculated as depicted in Fig. 7(a) where the trend is

quite similar to the hydrodynamic radius as a function of temperature (Fig. 5(d)). Below 30 °C, the N_{agg} appeared to be quite constant at approximately 40 and 65 for PEP and PEPS-1 respectively. When the solutions were further heated to 37 °C, there was an increase in the N_{agg} where the increment was larger in PEP (~450) compared to PEPS-1 (~85) which further confirmed the hypothesis that the micelles formed from the PEP became highly unstable with increasing solution temperature and the stability of these micelles could be enhanced with the incorporation of sufficient POSS in the copolymers as observed from the smaller increment in N_{agg} for PEPS-1 and subsequently stable N_{agg} in PEPS-2 and PEPS-3. In addition, samples with larger amount of POSS formed micelles with higher N_{agg} (comparing PEPS-1 and PEPS-2) and the N_{agg} reduced when a longer PEG segment is incorporated in the copolymer (comparing PEPS-2 and PEPS-3) which complemented the particle size data that the tendency to form micelles increases when the amount of POSS was increased and the length of the PEG was reduced in the copolymer.

In addition to the $M_{w,micelle}$ and N_{agg} , the dimensionless ratio R_g/R_h which was indicative of the aggregate structure, ranged from 0.25 to 0.45 for the copolymer micelles at temperatures below 30°C, as depicted in Fig. 7(b). The values of R_g/R_h for hard-sphere micelle, random coil, and rod-like structure are reported as 0.78, 1.78, and ≥ 2 , respectively. The deviation of R_g/R_h of our PEP and PEPS copolymer systems from the hard sphere value suggested that the micelles were spherical in shape and possessed a thick hydration layer (core-shell aggregate structure). When comparing the PEP and PEPS copolymers below the LCST, the high hydrophobicity of POSS nanocages in PEPS hybrid copolymers provided stronger driving force for self-assembly and larger micelles cores were formed in aqueous solution as manifested by the larger R_g/R_h values as compared to micelles from PEP copolymers. When the temperature of the samples were increased above the LCST, the R_g/R_h experienced a slight but significant increase (ranging from 0.05 to 0.3, depending on the sample) while maintaining the spherical shape of the micelles as indicated by the values of $R_g/R_h < 0.78$. Above the LCST, PPG experienced a phase transition due to decreased hydrogen bond interaction with water leading to its collapse towards the core of the aggregates which was consistent with the increase in R_g/R_h . The increase in R_g/R_h was smaller in samples with larger amount of POSS and shorter PEG. For example the R_g/R_h ratio increased from 0.3 to 0.65 in PEP copolymer when the temperature was below and above LCST respectively as compared to the increase from 0.44 to 0.54 in PEPS-2 copolymer for the same temperature range. This observation suggested that in the presence of POSS nanocages in the PEPS micelles, PPG brushes collapsed and formed a hydrophobic layer surrounding the POSS core when temperature was increased above the LCST, resulting in a small increase in R_g/R_h . In contrast, the collapse of the PPG brushes in the loosely packed PEP micelles increased the tendency to self-assemble and reduced the micelle stability in aqueous solution, as reflected by the large increase in particle size above the LCST. As a consequence, the hydrophobic domains formed from PPG phase transition were entrapped in the PEP micellar cores through intermicellar aggregations and were less compact, as suggested by the R_g/R_h ratio. To summarize, the constant R_h and N_{agg} of micelles (Fig. 5(d) and Fig. 7(a)) and the increase in R_g/R_h ratios (Fig. 7(b)) observed in PEPS-2 and PEPS-3 when temperature was increased above LCST implied that stable aggregates were formed in aqueous solution when sufficient POSS nanocages were present in the copolymers and that the change in R_g as a function of temperature was purely due to

the PPG brushes adopting a more extended and compact conformation below and above LCST respectively.

Example 3

Thermally Denatured Protein Protection of PEPS Hybrid Copolymers Micelles

- 5 The chaperone role of the as-prepared PEPS hybrid micelles in the thermally denatured protein protection was investigated by monitoring the recovered protein and enzyme activity in the presence of the micelles. Three representative proteins, Green Fluorescent Protein (GFP, obtained from Merck of New Jersey of the United States of America), lipase (from *Pseudomonas cepacia* obtained from Sigma-Aldrich of Missouri of the United States of America) and lysozyme (from Chicken Egg White obtained from Merck of New Jersey of the United States of America) that can undergo thermally induced denaturation procedure were carried out in this study. 4-Nitrophenyl acetate, also known as p-Nitrophenyl acetate, and Acetonitrile were used in the thermally denatured protein protection efficiency assay of lipase. *Micrococcus lysodeikticus* ATCC No. 4698 was used in the thermally denatured protein protection efficiency assay of lysosome.

GFP: The thermal protection of GFP by PEPS hybrid copolymers were evaluated by the intrinsic fluorescence of GFP which can be correlated to folded and unfolded structure of GFP. The fluorescence spectra were recorded by using the excitation and emission wavelength of 485 and 538 nm, respectively. During the protein protection test, GFP solutions (1 µg/mL) containing different concentrations of PEPS hybrid copolymers were first heated at 40 °C for 1 hour. The heating would induce the collapse of PPG segments in PEPS copolymers into hydrophobic domains on the shell of the micelles. Next, the solutions were further heated up to 70 °C and kept at this temperature for another 30 minutes. The mixture was then cooled down to room temperature and the fluorescence was recorded. The GFP thermal protection efficiency was calculated from the fluorescence intensity ratios of the cooled GFP with that of native GFP.

Lipase: The relative protection efficiency of lipase was evaluated by comparing the enzyme activities of lipase using p-nitrophenyl acetate (pNPA) as the substrate at 30 °C. During the measurements, 1.0 mL lipase in phosphate buffer solution (pH 7.2, 25 µg/mL), with or without PEPS hybrid copolymers, were quickly mixed with 0.01 mL MeCN solution containing 130 mM pNPA. The consumption of pNPA was determined by monitoring the production of p-nitrophenol as a function of time with a UV-Vis spectrophotometer at 400 nm. The lipase protection efficiencies were obtained by comparing the initial rates of pNPA hydrolysis reactions catalysed by different lipase solutions. The lipase-copolymer mixed solutions were incubated at 40 °C for 1 hour prior to the thermal denaturation of lipase carried out at 70 °C for 30 minutes to trigger the collapse of PPG segments into the hydrophobic domains. Subsequent protein renaturation process was initiated by cooling the mixture to room temperature.

Lysozyme: Lysozyme activities were determined from the optical dispersion decrease of *micrococcus lysodeikticus* cell suspension. Briefly, a 0.3 mg/mL cell suspension was prepared in phosphate buffer solution, pH 7.2. To 1.0 mL of this suspension, 0.1 mL of lysozyme solution (50 µg/mL), with or without PEPS copolymers, were added and the decrease in time of absorbance at 450 nm were monitored. The slope of the linear part of

the curve was related to the slope of a standard sample, and the result was expressed as the lysozyme protection efficiencies. The lysozyme denaturation and possible renaturation process was carried out under the same protocol as the lipase activity assay.

5 Samples were prepared by mixing the copolymer micelle solutions with each protein solution (GFP concentration at 1 $\mu\text{g/ml}$; lipase concentration at 25 $\mu\text{g/ml}$ and lysosome concentration at 50 $\mu\text{g/ml}$) at different copolymer concentrations (of 1 mg/ml and 2 mg/ml), and the protection efficiencies in the presence of different copolymer concentrations are shown in Fig. 8. During the measurements, the solutions were heated at 70°C and kept at this temperature for 30 minutes to denature the three proteins. It is well
10 known that, without any external assistance, that these proteins will irreversibly lose their activities as a result of the aggregation of the denatured intermediates. As measured, the relative residual activities of free proteins determined by each individual method were found at 22.4%, 42.1%, and 40% for GFP, lipase and lysozyme, respectively (Fig. 8). However, in the presence of the PEPS hybrid micelles, all the proteins were protected in
15 good activities after going through the harsh heat-deactivation procedure. For example, at the two tested copolymer/proteins ratios (which is calculated based on the protein concentration and polymer concentrations of 1 mg/mg and 2 mg/ml stated above), the higher protection capability of the denatured GFP was observed at 81.4%. Moreover, 89.3% and 88.7% enzyme activity of the denatured lipase and lysozyme were recovered,
20 respectively, in the presence of PEPS hybrid copolymer at concentration of 2.0 mg/mL . These results clearly indicated that the irreversible aggregations of the denatured proteins were inhibited and, more importantly, the native functional conformation could be recovered from the denatured state. The relatively low protection efficiency of PEPS-3 hybrid micelles were probably caused by the longer PEG brushes in the exterior phase of
25 the micelles, which are reported to be unfavourable for the protein absorption during the unfolded protein capture. Comparing the PEP and PEPS copolymers, the decreased in protein protection efficiency in the case of PEP copolymer may be attributed to the slightly different micelle formations in aqueous solution at elevated temperatures as discussed in Fig. 5(d), Fig. 7(a) and Fig. 7(b). When temperature is increased above the LCST, PPG
30 brushes collapses and forms a compact shell on the POSS core in the PEPS micelles, in contrast to the loosely packed PPG domains entrapped in the PEP micellar cores formed through intermicellar aggregations. As a consequence, there existed a larger hydrophobic area for protein capture and higher protection efficiency was observed in the PEPS micelles as compared to the PEP micelles. The schematic representation of PEPS hybrid
35 copolymer self-assembly in aqueous solution is presented in Fig. 9(A).

For thermally denatured proteins, the denaturation process is usually considered to occur by a two-step mechanism, as shown in Fig. 9(B). The folding, unfolding, and aggregation status presented in Fig. 9(B) depicted the native, reversibly denatured, and irreversibly denatured forms, respectively, of proteins. The thermal inactivation starts with reversible
40 denaturation of protein which often proceeds to form inactive and insoluble aggregates driven by intermolecular associations. This aggregation process is irreversible, which prevents proteins from refolding into their native stage and thus losing their functionalities. However, the protein in the reversibly denatured form can be promptly protected through capture thus avoiding the irreversible aggregation. More importantly, the captured proteins
45 could be refolded into the native functional conformation under proper conditions. The proposed mechanism of the PEPS hybrid micelles in protein protection is demonstrated in

Fig. 9(C). Under normal conditions, the micelles self-assembled from PEPS hybrid copolymer are in the dormant state and do not interfere with the protein activities at temperatures below LCST. When the micelle solutions are heated to 40 °C, which is above the copolymers' LCST (31 - 33°C) but below the unfolding temperature of proteins (~70°C), the micelles transformed into their functional state due to the collapse of PPG polymer brushes in the mixed corona (step 1). The collapsed PPG polymers form hydrophobic domains on micelle cores made from POSS nanocages and poly(methacrylate) backbones, leaving behind cavity-like spaces that could accommodate the denatured proteins in step 2. Further increasing the temperature induces the denaturation of protein into unfolded intermediates and exposes the hydrophobic inner core of the proteins. The unfolded intermediates are immediately captured through multipoint hydrophobic interactions with the hydrophobic domains of the micelles before protein aggregation could occur, resulting in the formation of micelle/protein complexes (step 3) and protection of the unfolded intermediates. The stretched PEG brushes in the outer layer could make the micelle/protein complexes stable and provide steric repulsion to prevent further intermicellar aggregation. Upon cooling, the micelle/protein complexes gradually dissociate, owing to the temperature sensitive phase transition of PPG brushes from hydrophobic to hydrophilic state, during which the unfolded intermediates detach from the micelles surfaces and start to refold into their native conformation (step 4). The presence of PEG brushes and hydroxyl groups in the mixed micelle corona could interact with the molten globules of the refolding intermediates, which could further enhance the protein renaturation.

The capture and release mechanism between the unfolded intermediates of the proteins and PEPS hybrid micelles was further confirmed by the light scattering study as summarized in Table 2.

Table 2. DLS and SLS data for PEP and PEPS micelles only and the PEP/Lipase and PEPS/Lipase complexes under different solution temperatures

T (°C)	LS parameters	Components						
		PEP only	PEP/Lipase	PEPS-1 only	PEPS-1 /Lipase	PEPS-2 only	PEPS-2 /Lipase	PEPS-3 only PEPS-3/ Lipase
25	R_h (nm)	16 ± 1	17 ± 2	18 ± 1	20 ± 1	60 ± 2	67 ± 3	40 ± 2 39 ± 2
	R_g (nm)	4.5 ± 0.3	4.9 ± 0.3	7.2 ± 0.3	7.6 ± 0.2	28 ± 1	32 ± 1	15 ± 1 14 ± 1
	R_g/R_h	0.28	0.29	0.39	0.38	0.46	0.48	0.37 0.36
37	R_h (nm)	110 ± 6	106 ± 6	43 ± 2	45 ± 2	63 ± 3	65 ± 2	45 ± 3 48 ± 3
	R_g (nm)	67 ± 4	68 ± 3	21 ± 1	22 ± 1	33 ± 2	35 ± 2	22 ± 1 24 ± 1
	R_g/R_h	0.61	0.64	0.48	0.49	0.52	0.54	0.48 0.50
70	R_h (nm)	118 ± 6	144 ± 7	44 ± 2	56 ± 2	70 ± 3	82 ± 3	51 ± 3 60 ± 3
	R_g (nm)	73 ± 4	99 ± 5	21 ± 1	36 ± 1	37 ± 2	62 ± 2	26 ± 2 37 ± 2
	R_g/R_h	0.62	0.69	0.47	0.64	0.53	0.75	0.50 0.61
25*	R_h (nm)	18 ± 1	18 ± 1	20 ± 1	24 ± 1	62 ± 3	66 ± 3	40 ± 2 52 ± 3
	R_g (nm)	4.9 ± 0.3	5.6 ± 0.3	8.0 ± 0.4	9.8 ± 0.4	29 ± 1	32 ± 1	16 ± 2 21 ± 2
	R_g/R_h	0.27	0.31	0.40	0.41	0.46	0.49	0.39 0.40

* Solutions were cooled to 25°C at ambient conditions after heating at 70°C

The radius of gyration, R_g and hydrodynamic radius, R_h of PEPS micelle/protein mixtures at 25°C and 37 °C were similar to the PEPS micelles alone. However, after incubating at 70°C for 30 minutes, the R_g , R_h and R_g/R_h values increased significantly, thus indicating the interaction between micelles and proteins occurred and micelle/protein complex formation was formed. The increase in R_g/R_h ratios at 70°C in the micelle/protein complex demonstrated that the protein absorption and capture occurred mainly in the core (hydrophobic domain) of the PEPS micelles. The capture and holding of proteins in the micelles hydrophobic domains could effectively prevent the irreversible aggregation of the proteins. In addition, the light scattering data summarized in Table 2 demonstrates that the PEPS hybrid micelles returned to its original size range when cooled to room temperature (25°C) indicating the disassembly of micelle/protein complexes as induced by the PPG phase transition into hydrophilic state. The above results confirmed the capture-and-release mechanism in the chaperone-like functionalities of PEPS hybrid micelles during the denaturation and renaturation of proteins. The protein protection effect arising from micelle/protein complexation was further investigated with circular dichroism (C.D.) spectra by monitoring the secondary structure of proteins at different conditions. The far-UV (C.D.) spectrum was recorded with a J-720 C.D. spectrophotometer (JASCO Co., Ltd., Tokyo, Japan) equipped with a thermo-regulated cell compartment. The change in the C.D. ellipticity of proteins with and without PEPS copolymers in phosphate buffer (pH 7.2) was measured. Taking PEPS-1 hybrid micelle solution with lipase as an example, the C.D. spectra of lipase changed a little upon mixing with the copolymer, indicating that the presence of PEPS hybrid micelles did not perturb the structural integrity of lipase and there were no interactions between lipase and PEPS hybrid micelles at room temperature (Fig. 10A). During the heat-induced denaturation process, the thermal aggregation temperature of free lipase was approximately 70°C, whereas this temperature was largely retarded (~82°C) upon the formation of micelle/protein complexes, indicating higher stability of lipase in PEPS hybrid copolymer/protein mixed solutions (Fig. 10B).

Example 4

Cellular Uptake

The cellular uptake of the micelles was investigated by directly dissolving PEPS hybrid copolymers into C6 Glioma (obtained from ATCC, USA) cell culture medium containing high concentration of GFP for efficient protein encapsulation. As a control, free GFP in fresh DMEM was added into the cell culture chambers without the hybrid copolymers. The C6 Glioma cells at densities of 4×10^4 cells/well were cultured in complete DMEM medium within confocal imaging chamber (Lab-Tek) at 37°C, 5% CO₂. The cell culture medium was supplemented with 10% fetal bovine serum (FBS), 100 units/mg penicillin and 100 µg/mL streptomycin. After 80% confluence, the medium was removed and the adherent cells were washed twice with 1 × PBS buffer. Fresh cell culture medium containing free GFP (10 µg/mL) or GFP mixed with PEPS hybrid copolymer at concentration of 1.0 mg/mL in the cell culture medium were then added to the chambers, respectively. After 24 hours incubation, the cells were washed three times with 1 × PBS buffer followed by 4% paraformaldehyde for 15 minutes, which were further washed twice with 1 × PBS buffer. The cell monolayer was imaged by confocal laser scanning microscopy FV1000 (CLSM, Olympus Japan) with imaging software under the same experimental conditions.

The intrinsic fluorescence of GFP was used as a probe to evaluate the cell uptake efficiency, by observing the fluorescence intensity of the cells by CLSM.

As shown in Fig. 11A, after a 24 hours incubation of the C6 Glioma cells with free GFP solution, only a few fluorescence signals were detected and most of the proteins were present as aggregates on the cellular membrane, indicating poor uptake of the proteins by the cells. In comparison, when the cells were exposed to the same concentration of GFP but mixed with PEP copolymer solution, the GFP fluorescence intensity inside the cells was significantly enhanced (Fig. 11B), revealing an increased internalization of GFP in cells by using PEP copolymer as delivery carrier. More interestingly, the cells incubated with PEPS-1/GFP mixed solution recorded the strongest emission signal (Fig. 11C, green channel), which suggests that the presence of POSS in the hybrid PEPS micelles made the micelles more attractive to the cells for ingestion resulting in more GFP being delivered into the cells. It is possible that the PEPS hybrid micelles are more favourable for cell uptake due to their smaller size than micelles formed from the PEP copolymer. An overlay of the phase contrast image corresponding to the whole cell morphology and the bright green fluorescence of GFP indicates that the GFP has most likely localized in the cell cytoplasm (Fig. 11 Overlay). Therefore, the PEPS hybrid micelles could be effectively taken up by cells making them potentially useful as delivery vehicle.

Cytotoxicity Assay

The *in vitro* cytotoxicity of PEPS hybrid copolymers were carried out using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay in metabolic activity of human dermal fibroblasts (HDFs) cell lines. HDFs cells were seeded in 96-well plates (Costar, Illinois, USA) at a density of 1×10^4 cells/well. After 24 hours incubation, the culture media was replaced with serum-supplemented culture media containing known concentrations of the PEPS copolymers, and the cells were further incubated for 72 hours. At pre-determined time intervals, 100 μ L of sterile-filtered MTT stock solution in PBS (0.5 mg/mL) was added to each well, reaching a final MTT concentration of 0.5 mg/mL. After 3 hours, the unreacted dye was removed by aspiration. The formazan crystals were dissolved in DMSO (100 μ L/well), and the absorbance was measured using a microplate reader (Genios Tecan) at a wavelength of 570 nm. The wells containing the cells and culture medium served as controls. The relative cell viability (%) related to the control cells was calculated using the following equation:

$$[A]_{test}/[A]_{control} \times 100\%,$$

where $[A]_{test}$ is the absorbance of the wells with polymers and $[A]_{control}$ is the absorbance of the control wells.⁵ All experiments were conducted with six repetitions and average values are reported.

The cytotoxicity of the copolymer dissolved in cell culture medium was evaluated by incubating with HDFs cell for a period of 48 hours and 72 hours at 37 °C, respectively (Fig. 12). The cell viability at different time intervals was determined using the MTT assay. As shown in Fig. 12, the cells showed no loss in cell viability when incubated for 48 hours with both PEP and PEPS hybrid copolymers at concentration of 1.0 mg/mL. In addition, a longer incubation time of 72 hours and higher polymer concentration of 4.0 mg/mL also

showed no effect of cytotoxicity on the cells. From the MTT assay, it is expected that the PEPS hybrid copolymers developed here are safe for biomedical applications.

Industrial Applicability

Advantageously, the micellar particle may be used to protect a cargo from thermal degradation. The micellar particle may be used in bio-applications in which the cargo is a biological agent and should be protected from conditions which are above the physiological conditions, such as for example during manufacturing or storage of the biological agent. For example, when forming a dry powder, spray drying of the cargo (in a liquid medium) may be used. As spray drying involves high temperature, by having the cargo immobilized by the micellar particle, the cargo may be protected from the high temperature during spray drying.

The micellar particle may be used for delivering a cargo such as a therapeutic agent to a target such as a human or animal body. Hence, the micellar particle may be used as a delivery agent for the therapeutic agent. As the copolymer making up the micellar particle contains biocompatible or non-toxic polymers, the micellar particle may be ingested by the target and easily removed by kidney filtration.

The micellar particle and the cargo may be part of a beverage formulation such that when the beverage is consumed by a target, the cargo may be delivered to the target. During reconstitution of the beverage, a hot temperature may be used, in which the hot temperature typically causes thermal degradation of the cargo. However, since the beverage formulation contains the micellar particle, the cargo may be immobilized and encapsulated by the micellar particle during the high temperature used to form the beverage and be protected by the micellar particle.

It will be apparent that various other modifications and adaptations of the invention will be apparent to the person skilled in the art after reading the foregoing disclosure without departing from the spirit and scope of the invention and it is intended that all such modifications and adaptations come within the scope of the appended claims.

Claims

1. A copolymer comprising a hydrophilic polymer, a hydrophobic polymer and a thermoresponsive polymer, wherein said hydrophobic polymer comprises at least one monomer selected from the group consisting of oligomeric silsesquioxanes, ethylene, propylene, butene, phenyl-oxazoline, ester and phosphazine.
2. The copolymer of claim 1, wherein said hydrophilic polymer comprises at least one monomer selected from the group consisting of vinyl alcohol, acrylic acid acrylate, vinyl pyrrolidone, N-(2-hydroxypropyl) methacrylamide, methyl vinyl ether, maleic anhydride, methyl-oxazoline, ethyl-oxazoline, phosphates, phosphonates, and ethylene glycol.
3. The copolymer of claim 2, wherein said hydrophilic polymer is selected from the group consisting of polyvinyl alcohol, polyacrylic acid, polyacrylate, polyvinyl pyrrolidone, poly(N-(2-hydroxypropyl) methacrylamide), poly-(methyl vinyl ether-co-maleic anhydride), poly(2-methyl-2-oxazoline), poly(2-ethyl-2-oxazoline), polyphosphates, polyphosphonates, polyethylene glycol and copolymers thereof.
4. The copolymer of any one of the preceding claims, wherein said hydrophilic polymer has a molecular weight in the range of 300 g/mol to 1000 g/mol.
5. The copolymer of claim 1, wherein said hydrophobic polymer is selected from the group consisting of polyhedral oligomeric silsesquioxanes, polyethylene, polypropylene, polybutene, ethylene-propylene copolymers, poly(2-phenyl-2-oxazoline), polyester, polyphosphazine, polyhydroxybutyrate, polycaprolactone, and copolymers thereof.
6. The copolymer of any one of the preceding claims, wherein said hydrophobic polymer has a mole percent of 1.5 mol% to 6 mol% in said copolymer.
7. The copolymer of any one of the preceding claims, wherein said thermoresponsive polymer comprises at least one monomer selected from the group consisting of propylene glycol, isopropyl-oxazoline, N-isopropylacrylamide, N,N-diethylacrylamide, N-vinylcaprolactam and (dimethylamino)ethyl methacrylate).
8. The copolymer of claim 7, wherein said thermoresponsive polymer is selected from the group consisting of poly(propylene glycol), poly(2-isopropyl-2-oxazoline), poly(N-isopropylacrylamide), poly(N,N-diethylacrylamide), poly(N-vinylcaprolactam), poly[2-(dimethylamino)ethyl methacrylate]] and copolymers thereof.
9. The copolymer of any one of the preceding claims, wherein said thermoresponsive polymer has a molecular weight in the range of 300 g/mol to 400 g/mol.
10. The copolymer of any one of the preceding claims, wherein said copolymer comprises an acrylate backbone.

11. The copolymer of claim 10, wherein said acrylate is selected from the group consisting of methacrylate, methyl acrylate, ethyl acrylate, hydroxyethyl methacrylate, butyl acrylate and butyl methacrylate.
12. A micellar particle comprising a copolymer having a hydrophilic polymer, a hydrophobic polymer and a thermoresponsive polymer, wherein said hydrophobic polymer comprises at least one monomer selected from the group consisting of oligomeric silsesquioxanes, ethylene, propylene, butene, phenyl-oxazoline, ester and phosphazine.
13. The micellar particle of claim 12, wherein said micellar particle has a core-shell configuration.
14. The micellar particle of claim 13, wherein said hydrophobic polymer is present in said core.
15. The micellar particle of claim 12 or 13, wherein at least one of said hydrophilic polymer or said thermoresponsive polymer is present in said shell.
16. The micellar particle of any one of claims 12 to 15, wherein said thermoresponsive polymer becomes hydrophobic at a temperature above the low critical solution temperature and becomes hydrophilic at a temperature below the low critical solution temperature.
17. The micellar particle of any one of claims 12 to 16, wherein said thermoresponsive polymer becomes shorter in length at said temperature above said low critical solution temperature and becomes longer at said temperature below said low critical solution temperature.
18. A method of protecting a cargo at a temperature that typically causes thermal degradation of said cargo, comprising the steps of:
 - a) providing a micellar particle having a core-shell configuration, said core comprising a hydrophobic polymer and said shell comprising at least one of a hydrophilic polymer or a thermoresponsive polymer, said thermoresponsive polymer capable of becoming hydrophobic at a temperature above a low critical solution temperature of said thermoresponsive polymer; and
 - b) immobilizing said cargo with said hydrophobic thermoresponsive polymer at the temperature stated in step (a) to thereby protect said cargo from thermal degradation,wherein said hydrophobic polymer comprises at least one monomer selected from the group consisting of oligomeric silsesquioxanes, ethylene, propylene, butene, phenyl-oxazoline, ester and phosphazine.
19. The method of claim 18, wherein said immobilized cargo forms a complex with said micellar particle.
20. The method of claim 18 or 19, wherein the temperature stated in step (a) is substantially lower than the thermal degradation temperature of said cargo.

21. The method of claim 19 or 20, further comprising the step of releasing said cargo from said complex at a temperature that is lower than the low critical solution temperature.
22. The method of any one of claims 18 to 21, wherein said cargo is a biological agent.
23. The method of claim 22, wherein said biological agent is selected from the group consisting of a therapeutic agent, a protein, a microorganism and a nucleic acid.
24. The method of claim 23, wherein said protein is selected from the group consisting of an enzyme, a hormone, an antibody, an antigen, a receptor, a transport protein, a structural protein, a motor protein, a signaling protein, a storage protein and a coagulation protein.
25. Use of the micellar particle of any one of claims 12 to 17 to immobilize a cargo to thereby prevent thermal degradation of said cargo at a temperature that typically causes thermal degradation of said cargo.
26. Use of claim 25, wherein said cargo is a protein.
27. Use of claim 26, wherein said protein, when immobilized with said micellar particle, is substantially prevented from aggregating with another protein.