Degradable Redox-Responsive Disulfide-Based Nanogel Drug Carriers via Dithiol Oxidation Polymerization

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Figure S1. (A) 2,2′-(ethylenedioxy)diethanethiol (EDDET) $\delta$H(400 MHz; DMSO-d$_6$) 2.3 (2 H, m, CH$_2$), 2.6 (2 H, m, CH$_2$), 3.3 (2H, s, SH), and 3.5 (4 H, m, CH$_2$). (B) pentaerythritol tetramercaptoacetate (PETMA) $\delta$H(400 MHz; DMSO-d$_6$) 3.3 (1 H, s, SH), 3.4 (2 H, s, CH$_2$), and 4.2 (2 H, 2, CH$_2$). (C) dithiothreitol (DTT) $\delta$H(400 MHz; DMSO-d$_6$) 2.45 (2 H, m, CH), 2.55 (2 H, m, CH), 3.5 (2 H, s, SH), and 43.5 (2 H, 2, CH). (D) 1:50 monomer:cross-linker (mol/mol) bulk gel incubated with DTT reducing agent (2X free thiol molar equivalence) for 24 hr $\delta$H(400 MHz; DMSO-d$_6$).

Figure S2. PETMA-based bulk hydrogels degraded in response to DTT to release Rhodamine B. All dye molecules were released within two minutes.
sizes before after dialysis purification were measured by DLS. An organic phase containing monomer, cross-linker, and base dissolved in water:acetone 1:1 (volume) solution was combined with an aqueous phase containing 12 wt. % H₂O₂. Formulated nanogels were prepared using a microfluidic mixing instrument. An organic phase containing monomer, cross-linker, and base dissolved in water:acetone 1:1 (volume) solution was combined with an aqueous phase containing different weight percentages of H₂O₂. The ratio of organic:aqueous phase was 1:10 (volume) and the flow rate was 12 mL/min. Particle sizes before after dialysis purification were measured by DLS.

Figs. S3, S4. (A) Table listing different formulation conditions. Formulated nanogels were prepared using a microfluidic mixing instrument. An organic phase containing monomer, cross-linker, and base dissolved in water:acetone 1:1 (volume) solution was combined with an aqueous phase containing different weight percentages of H₂O₂. The ratio of organic:aqueous phase was 1:10 (volume) and the flow rate was 12 mL/min.

B

Dynamic Light Scattering (DLS) of Different Mesh Size Nanogels (nm)

<table>
<thead>
<tr>
<th>CL:M</th>
<th>Intensity</th>
<th>STDEV</th>
<th>Volume</th>
<th>STDEV</th>
<th>Number</th>
<th>STDEV</th>
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<tbody>
<tr>
<td>1 to 5</td>
<td>55.56</td>
<td>18.44</td>
<td>42.18</td>
<td>14.08</td>
<td>34.19</td>
<td>9.124</td>
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<tr>
<td>1 to 25</td>
<td>111.5</td>
<td>33.92</td>
<td>93.59</td>
<td>32.32</td>
<td>74.30</td>
<td>20.64</td>
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<tr>
<td>1 to 50</td>
<td>111.2</td>
<td>31.84</td>
<td>95.28</td>
<td>30.82</td>
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<td>23.70</td>
<td>89.70</td>
<td>22.48</td>
<td>77.83</td>
<td>16.53</td>
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<td>1 to 100</td>
<td>99.37</td>
<td>24.58</td>
<td>87.37</td>
<td>23.97</td>
<td>75.15</td>
<td>17.75</td>
</tr>
</tbody>
</table>

C:M (mol/mol) = 1:5 C:M (mol/mol) = 1:25 C:M (mol/mol) = 1:50 C:M (mol/mol) = 1:75 C:M (mol/mol) = 1:100

Figure S3. Table listing different formulation conditions. Formulated nanogels were prepared using a microfluidic mixing instrument. An organic phase containing monomer, cross-linker, and base dissolved in water:acetone 1:1 (volume) solution was combined with an aqueous phase containing different weight percentages of H₂O₂. The ratio of organic:aqueous phase was 1:10 (volume) and the flow rate was 12 mL/min.

Figure S4. (A) Table of particle sizes of different PETMA nanogels of different cross-linking densities after dialysis purification. (B) Intensity-, number-, and volume-weighted DLS plots of corresponding nanogels. Formulated nanogels were prepared using a microfluidic mixing instrument. An organic phase containing monomer, cross-linker, and base dissolved in water:acetone 1:1 (volume) solution was combined with an aqueous phase containing 12 wt. % H₂O₂. The ratio of organic:aqueous phase was 1:10 (volume) and the flow rate was 12 mL/min. Particle sizes before after dialysis purification were measured by DLS.
**Figure S5.** Degradation of PETMA, Rhodamine B loaded nanogels after the addition of dithiothreitol (DTT) disulfide bond reducing agent. Degradation occurred in less than two minutes for this sample size (10 mg).

**Figure S6.** The optimum Rhodamine B dye concentration for analysis of cellular uptake was determined by incubating HeLa cells with increasing dye-loaded nanogel concentrations using the 1:25 nanogels for 24 hrs. HeLa cells were fixed using PFA and the nucleus stained with DAPI. Images taken on a confocal microscope at 40X magnification and images were analyzed using ImageJ.
Figure S7. HeLa cells were incubated with 5 wt. % Rhodamine B loaded nanogels with a final dye concentration of 23 ng/μL and incubated at different time points (0 min, 15 min, 1 hr, 3 hr, 6 hr, 15 hr, and 24 hr). HeLa cells were fixed using PFA and the nucleus stained with DAPI. Images taken on a confocal microscope at 40X magnification and images were analyzed using ImageJ.
Figure S8. HeLa cells were incubated with 5 wt. % Rhodamine B loaded nanogels with a final dye concentration of 23 ng/μL. HeLa cells were fixed using PFA and the nucleus stained with DAPI. Images taken at 40X magnification and scale bars represent 50 μm scale. Confocal images of (A) 1:25, (B) 1:50, (C) 1:75, and (D) 1:100, monomer:cross-linker (mol/mol) at the 24 hr time point. An overlay of bright field with Rhodamine B (red) and cell nuclei (blue) is shown.
Figure S9. Confocal images of HeLa cells were incubated with a free Rhodamine B dye at different time points (0 min, 15 min, 1 hr, 3 hr, 6 hr, and 24 hr) with a final dye concentration of 23 ng/μL. HeLa cells were fixed using PFA and the nucleus stained with DAPI. Images were taken at 20X magnification and scale bars represent 50 μm scale.
Figure S10. (A) HeLa cells were incubated with 5 wt. % Rhodamine B loaded 1:75 monomer:cross-linker (mol/mol) nanogels for a 24 hr time period with a final dye concentration of 23 ng/μL. Images taken at 40X magnification and scale bars represent 50 μm scale. (B) HeLa cells were incubated with free Rhodamine B dye for a 24 hr time period with a final dye concentration of 23 ng/μL. Images taken at 20X magnification and scale bars represent 50 μm scale. HeLa cells were fixed using PFA and the nucleus stained with DAPI.