Monolith Protocol MO-P-032

Dendritic Polyglycerols – Human Serum Albumin

Dendritic polyglycerols (dPG) are spherical, water soluble, polyether-based nanomaterials which are decorated with multiple peripheral hydroxyl groups and hold great potential in diagnostics and therapeutic applications. Their high biocompatibility, capability and low toxicity *in vitro* as well as *in vivo* make them attractive nanomaterials in the field of nanotheranostics. To evaluate their potential, it is important to understand their cellular behavior and physiological fate (e.g. absorption, metabolism, distribution and excretion). The interaction with serum proteins such as human serum albumin is therefore an important part in the analysis of dPG.

protein – small molecule interaction | theranostics

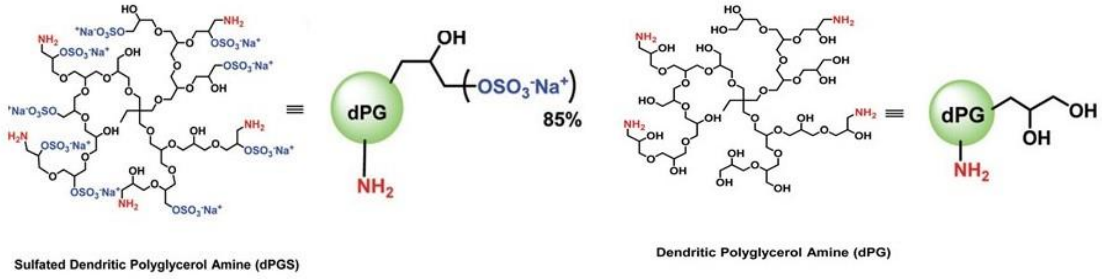
A1. Target/Fluorescent Molecule

Sulfated dPG (dPGS)

A2. Molecule Class/Organism

Dendritic polyglycerol (dPG)

A3. Sequence/Formula



Sulfated Dendritic Polyglycerol Amine (dPGS)

Dendritic Polyglycerol Amine (dPG)

A4. Purification Strategy/Source

Preparation of dendritic polyglycerol (dPG) and their corresponding sulfated (dPGS) amines according to procedures previously reported.[1]

A5. Stock Concentration/Stock Buffer

N/A

A6. Molecular Weight/Extinction Coefficient

22 kDa (dPGS) / 10 kDa (dPG)

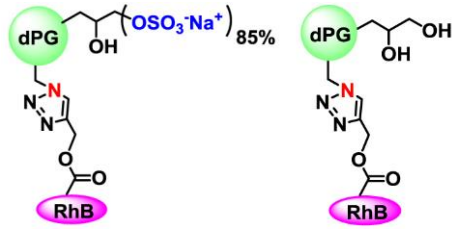
[1] Sunder et al., Macromolecules 32 (1999) 4240-4246 | Frey et al., Mol Biotechnol 90 (2002) 257-267 | Weinhartet et al., Macromolecules 11 (2011) 1088-1098

A7. Dilution Buffer

Phosphate buffered saline (PBS, pH 7.4), 0.05% TWEEN® 20

A8. Labeling Strategy

Conjugation of a rhodamine B (RhB) dye to azide terminated dPGS/dPG via click chemistry



dPGS-RhB (5b) dPG(OH)-RhB (2b), negative control

A9. Labeling Procedure

RhB was conjugated to dPGS/dPG according to a previously published procedure.[2]

A10. Labeling Efficiency

N/A

B1. Ligand/Non-Fluorescent Binding Partner

Human serum albumin (HSA)
uniprot.org/uniprot/P02768

B2. Molecule Class/Organism

Serum protein
Homo sapiens (Human)

B3. Sequence/Formula

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DAHKSEVAHR FKDLGEENFK ALVLIAFAQY LQQCPFEDHV KLVNEVTEFA KTCVADESAE NCDKSLHTLF GDKLCTVATL
RETYGEMADC CAKQEPERNE CFLQHKDDNP NLPRLVRPEV DVMCTAFHDN EETFLKKYLY EIARRHPYFY APELLFFAKR
YKAAFTECCQ AADKAACLLP KLDELRDEGK ASSAKQRLKC ASLQKFGERA FKAWAVARLS QRFPKAEFAE VSKLVTDLTK
VHTECCHGDL LECADDRADL AKYICENQDS ISSKLKECCE KPLLEKSHCI AEVENDEMPA DLPSLAADFV ESKDVCKNYA
EAKDVFLGMF LYEYARRHPD YSVVLLLRLA KTYETTLEKC CAAADPHECY AKVFDEFKPL VEEPQNLIKQ NCELFEQLGE
YKFQNALLVR YTKKVPQVST PTLVEVSRNL GKVGSKCCKH PEAKRMPCAE DYLSVVLNQL CVLHEKTPVS DRVTKCCTES
LVNRRPCFSA LEVDETYVPK EFNAETFTFH ADICTLSEKE RQIKKQTALV ELVKHKPKAT KEQLKAVMDD FAAFVEKCCK
ADDKETCFAE EGKKLVAASQ AALGL
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[2] Gröger et al., Adv Healthcare Mater 3 (2014) 375-385

B4. Purification Strategy/Source

Sigma-Aldrich GmbH
SRP6182

B5. Stock Concentration/Stock Buffer

500 µM
Phosphate buffered saline (PBS, pH 7.4), 0.05% TWEEN® 20

B6. Molecular Weight/Extinction Coefficient

66.5 kDa
43,800 $M^{-1}cm^{-1}$ (ε_{280})

B7. Serial Dilution Preparation

1. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of the 500 µM HSA solution into tube **1**. Then, transfer 10 µL of dilution buffer into tubes **2** to **16**.
2. Prepare a 1:1 serial dilution by transferring 10 µL from tube to tube. Mix carefully by pipetting up and down. Remember to discard 10 µL from tube **16** to get an equal volume of 10 µL for all samples.
3. Add 10 µL of 30 nM dPG(S)-RhB or dPG(OH)-RhB to each tube from **16** to **1** and mix by pipetting.
4. Incubate for 5 minutes at room temperature in the dark before loading capillaries.

D1. MST System/Capillaries

Monolith NT.115 Red (NanoTemper Technologies GmbH)
Capillaries Monolith NT.115 (MO-K022, NanoTemper Technologies GmbH)

D2. MST Software

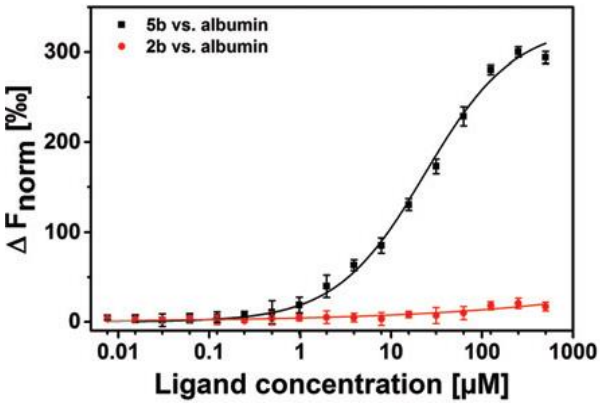
MO.Control v1.6 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

Phosphate buffered saline (PBS, pH 7.4), 0.05% TWEEN® 20
15 nM dPGS | 250 µM – 7.6 nM HSA | 25°C

D4. MST Results (Capillary Scan/Time Traces/Dose Response)

EC_{50} = 12 µM Pant et al., Nanoscale 9 (2017) 8723–8739



Interaction of HSA with dPG(S)-RhB (5b, black) and dPG(OH)-RhB (2b, red, negative control).

D5. Reference Results/Supporting Results

N/A

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