Supporting Information

NanoKeepers: stimuli responsive nanocapsules for programmed specific targeting and drug delivery

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1. MATERIALS

MCM-41 type (hexagonal) mesoporous silica particles were purchased from Sigma-Aldrich (Sigma-Aldrich, Ankara, Turkey). The properties of the particles were given by the supplier as follows; the unit cell size: 4.6-4.8 nm; pore volume: 0.98 cm3/g; pore size: 2.3-2.7 nm; spec. surface area: ~1000 m2/g (BET); bp: 2230°C; mp: >1600°C and bulk density: 0.34 g/mL. Micrococcal nuclease from *Staphylococcus aerus* (CAS number: 9013-53-0), Rhodamine B (CAS number: 81-88-9), (3-Mercaptopropyl)triethoxysilane (CAS number: 14814-09-6), vancomycin (CAS number: 1404-93-9) and all other chemicals were purchased from Sigma-Aldrich. Sulfo-GMBS (Sulfo-N-succinimidyl 4-maleimidobutyrate sodium salt, CAS number: 185332-92-7) was from Fisher Scientific. The oligonucleotides were synthesized by IDTDNA (Germany). The DNA sequences used in this study are given below in Table S1.

Table SI. Probe and oligo sequences.

TT probe	5'- FAM- mCmUmCmG**TT**mCmGmUmUmC- ZEN-RQ -3'
NK01 probe	5'- FAM - **TT**mCmGmCmUmUmC mGmGmCmGmAmA– ZEN-RQ -3'
NK01 oligo	5'- NH_2 - **TT**mCmGmCmUmUmC mGmGmCmGmAmA -3'
NK02 oligo	5'- NH_2-UmUmmCmGmCmUmUmCmGmGmCmGmAmA -3'

NH_2: *Amino-C6-modification,* **ZEN**: IDT ZEN fluorescence quencher, **m:** 2'O-Methyl nucleotides, **FAM:** 6-carboxy fluorescein

2. Methods

2.1 Particle Size Distribution and TEM

The particles used in this study were characterized by electron microscopy 20 (TEM, Fig. S3A). TEM samples were prepared by ultra-sonication of powders in ethanol for 5 min and drying of a droplet of suspension on a standard holey carbon TEM grid. TEM analysis was carried out on Titan 60–300 electron microscope (FEI, Netherlands) operating at 300 kV in TEM mode. The average diameter of particles was determined by dynamic light scattering (DLS) with a Zetasizer Nano-S (Malvern Instruments, Worcestershire, UK). About 2 mg MCM-41

particles were suspended in PBS buffer for DLS investigation. Large aggregates of particles were first removed by low speed centrifugation (500 rpm for 30 seconds) and the supernatant was used in DLS experiments. The average size was determined to be 183 nm.

2.2 SYNTHESIS OF PROBE-CAPPED PARTICLES

The surface of MCM-41 particles was grafted with nucleic acid probes as described previously.[1] Fig. S3C shows the overall strategy of the covalent immobilization of probes on the silica surfaces.

2.2.1 Silanization of MCM-41 Surface

50 mg of MCM-41 powder in 20 ml of 95% ethanol containing 1 mM acetic acid and 3% (w/w) of 3- Mercaptopropyl)triethoxysilane were stirred for 30 min at room temperature, followed by three-fold washing with ethanol by centrifugation (13000 x g, 3 min).

2.2.2 Loading and Capping

The sulfhydryl-modified nanoparticles (1 mg) were loaded with vancomycin or Rhodamine (100 μM) by incubation in the corresponding solution in PBS buffer (0.01 M phosphate buffered saline; NaCl-0.138 M; KCl-0.0027 M; pH 7.4) overnight and used for 5 coupling reactions with amino-modified oligonucleotides (NK01 or NK02). 2 mg/ml sulfo-GMBS (Sulfo-N-succinimidyl 4-maleimidobutyrate) were mixed with the sulfhydryl-modified nanoparticles in PBS buffer and stirred for 30 min. Next, the modified nanoparticles were washed 3 times with buffer and followed by 1 hour incubation under stirring conditions in 100 μM solution of amino-modified oligonucleotide (50 nmol in 500 μl PBS buffer containing either Rhodamine or vancomycin). The particles were then washed thoroughly 3 times with buffer. Loading of Rhodamine was calculated from the fluorescence of the particles measured and by comparing with a calibration curve and loading of vancomycin from the absorbance values at 280 nm. The amount of oligonucleotides immobilized on the surface of silica particles was calculated from absorbance values at 260 nm in a UV-VIS spectra. For a typical preparation, 10.4 ± 1.8 pmol oligonucleotides were immobilized per mg of particles. Coverage was estimated to be 48,760 probe molecules on average per nanoparticle, which corresponds to about one probe per 2.2 nm^2 of particle surface.

2.3 RELEASE EXPERIMENTS

Release of Rhodamine dye from pores was monitored by fluorescent spectrophotometry (Nanodrop 3300, Thermo Scientific, Willmington, USA) at excitation of 535 nm (blue exciter) and emission was recorded at 565 nm. Similarly, the release of vancomycin was measured by UV-VIS spectrophotometry (Nanodrop 2000, Thermo Scientific, Willmington, USA). The probe-capped mesoporous silica particles were placed in a magnetic stirrer. The particles were kept from mixing by trapping them in a compartment created by a dialysis membrane (cellulose membrane with molecular cut of 12,000 Da) at the top of spectroscopy cuvette. Silica particles were large enough to be completely retained by dialysis membrane. A design of a two-compartment cuvette by using dialysis membrane was previously described in detail.[1] All particles loaded with their cargo were washed extensively with PBS buffer before use. A typical nanoparticle leaked about 20% of its loaded cargo before a leak-free capping achieved (Figure S5).

The maximum loading capacity of the particles with Rhodamine or vancomycin was determined by preparing a mixture of 1 mg oligonucleotide capped silica particles in PBS buffer and measuring the fluorescence or absorbance in a well-mixed solution. The amount of fluorescein released into the buffer was quantified by using a fluorescein calibration curve or absorbance standard curve. For a typical experiment, the maximum loading of Rhodamine dye was calculated to be 14.1 pmol/mg particle and the maximum loading of vancomycin to be 15.5 pmol/mg.

2.4 Bacterial cultures

*Staphylococcus aureus (*ATCC 29213) *and Staphylacoccus epidermidis* were grown overnight from frozen stocks at 37ºC in tryptic soy broth (TSB). The desired concentrations of cultures were prepared by serial dilutions in PBS buffer. For colony counting experiments, the samples were surface plated on TSB agar. The plates were incubated for 18 h and the single colony forming units were recorded.

2.5 Antibacterial Activity

Minimum inhibitory concentration (MIC) values were determined according to Clinical and Laboratory Standards Institute broth microdilution method M7-A7.[2] The microdilution plates were incubated at 37°C for 24 h and then the optical density of each well, at 600 nm (OD600), was

determined with a spectrometer plate reader (EZ Reader). The MIC was determined in triplicate for each bacterial isolate. OD600 data (bacterial growth) were fitted by the Gompertz equation to calculate the MIC. Time-kill curve were conducted by overnight cultures diluted to yield an inoculum of 10^3 CFU/ml. The antibiotic concentrations used were equivalent to MIC for vancomycin. After 0, 3, 6, 12, 18 and 24 h of incubation in a shaking water bath at 37°C, serial dilutions of 0.1 ml samples were sub-cultured onto agar plates and incubated at 37°C for 24 h before CFU were counted.

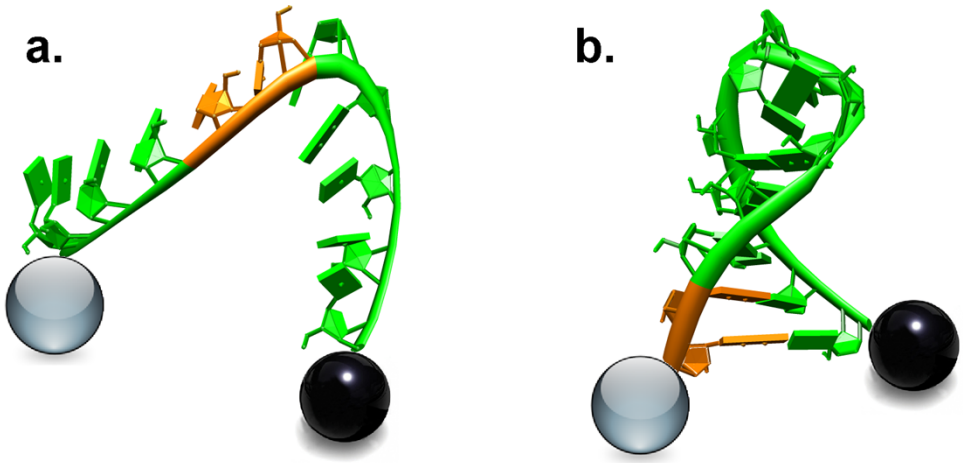

Fig. S1. Structural prediction and oligonucleotide probes. 3D structural prediction of **a)** TT probe (adapted from Hernandez, et.al.[3]) and **b)** NK01 probe (this work) were performed for comparison proposes, in orange are shown the two deoxythymidines (TT moiety) and in green the 2'-*O*-methyl modified nucleotides. Fluorophore and quencher are represented with the gray and black spheres, respectively.

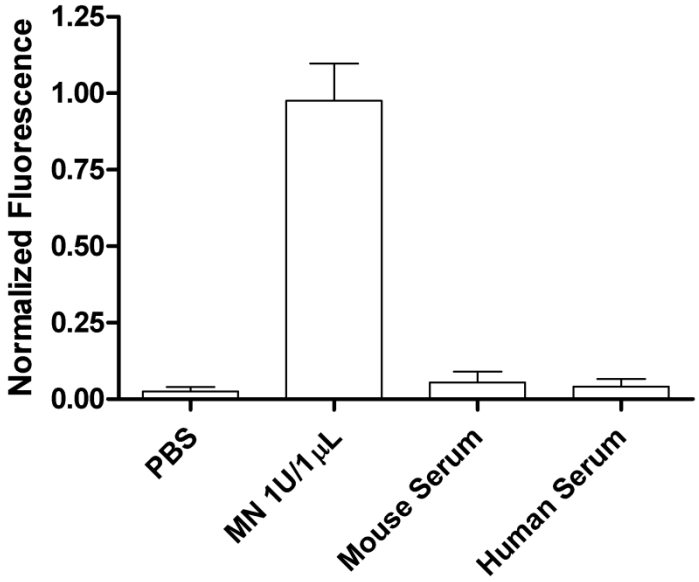

Fig. S2. Degradation profile of NK01 probe. Nuclease activity profile of NK01 probe was evaluated by MN and serum nucleases as previously reported.[3] NK01 probe was tested with PBS (control), PBS solution containing 1 U/µl, 90% of mouse or human serum. Each of the indicated conditions was incubated for 60 min at 37°C. All the measurements were carried out in triplicate; the results shown are means of normalized fluorescence intensity and the error bars represent standard deviations.

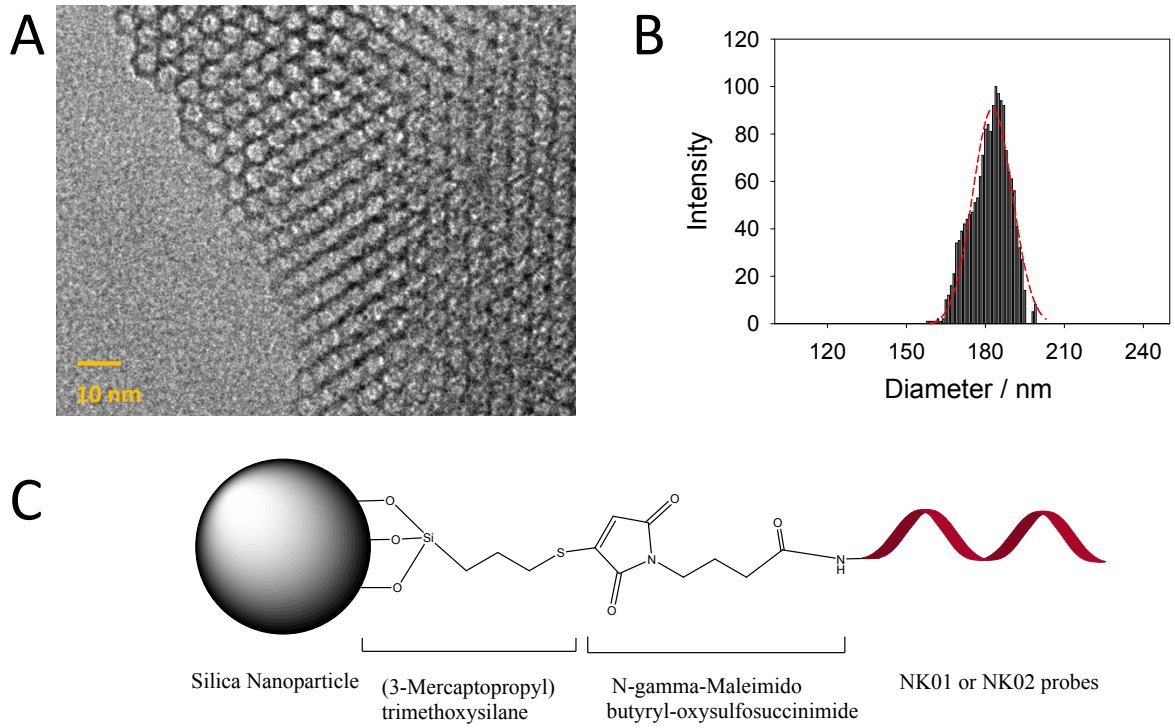

Fig. S3. Synthesis and characterization of NanoKeepers. A) Typical Transmission Electron Microscopy (TEM) image showing the size of nanopores[4] and B) size distribution of MCM-41 nanoparticles as determined by dynamic light scattering. The average diameter of particles was 182.8 ± 2.3 nm. C) Conjugation strategy for probe immobilization on silica nanoparticles.

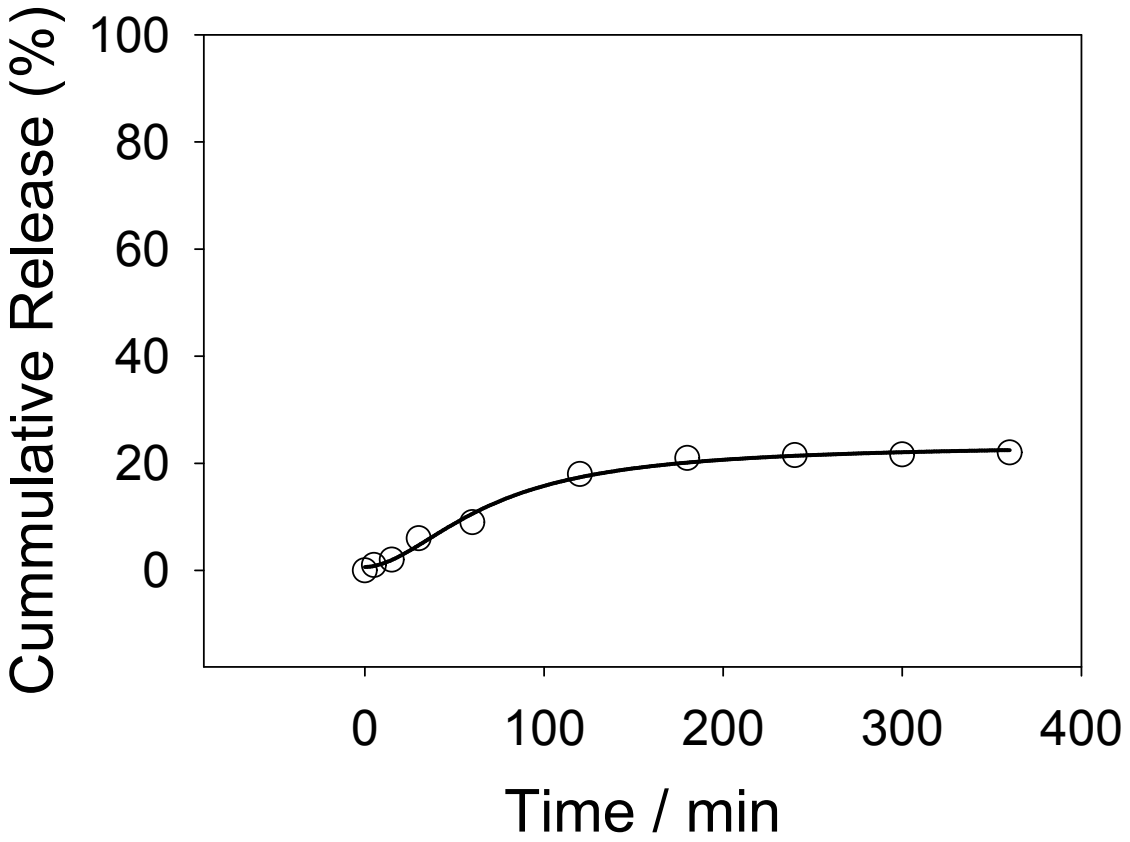

Fig. S4. Initial leakage of cargo from nanokeepers. Rhodamine loaded particles were capped with NK01 oligos, washed three times in PBS buffer by centrifugation and the leakage was followed by monitoring fluorescence. There was about 20 % leakage in 2 hours and remained constant for another 4 hours.

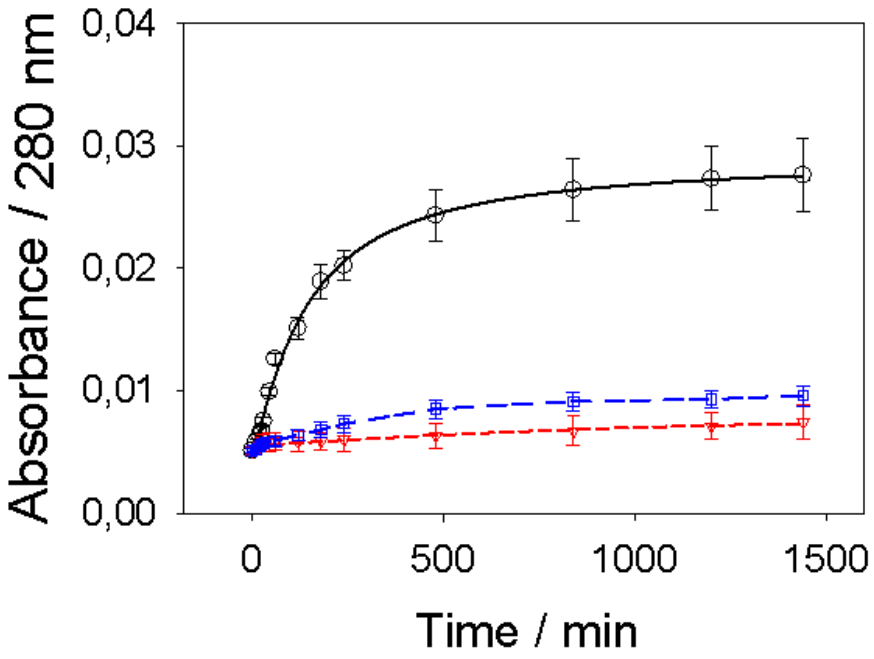

Fig. S5. Evaluation of vancomycin-NanoKeepers under physiological conditions. NanoKeepers loaded with vancomycin and capped with NK01 oligo (NanoKeeper-NK01) were incubated with serum in order to determine their stability and cargo retention under physiological conditions (red dashed line). NanoKeepers-NK01 were then incubated with serum samples spiked with MN to evaluate the release of cargo by the specific targeting of MN (black lines). Non-specificity studies were carried out by capping vancomycin-NanoKeepers with a control oligomer (NanoKeeper-NK02); this sub-optimal construct was incubated with serum samples containing MN (blue dashed lines). All the measurements were carried out in triplicate; the results shown are means of absorbance at 280 nm and the error bars represent standard deviations.

References

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2. *Clinical and Laboratory Standards Institute, Wayne, PA.*, 2006, **26**.
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4. V. C. Özalp, A. Pinto, E. Nikulina, A. Chuvilin and T. Schäfer, *Particle & Particle Systems Characterization*, 2014, **31**, 161-167.