A peptide for targeted, systemic delivery of imaging and therapeutic compounds into acute brain injuries

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Traumatic brain injury (TBI) is a major health and socio-economic problem, but no pharmacological agent is currently approved for the treatment of acute TBI. Thus, there is a great need for advances in this field. Here, we describe a short peptide (sequence CAQK) identified by in vivo phage display screening in mice with acute brain injury. The CAQK peptide selectively binds to injured mouse and human brain, and systemically injected CAQK specifically homes to sites of brain injury in mouse models. The CAQK target is a proteoglycan complex upregulated in brain injuries. Coupling to CAQK increased injury site accumulation of systemically administered molecules ranging from a drug-sized molecule to nanoparticles. CAQK-coated nanoparticles containing silencing oligonucleotides provided the first evidence of gene silencing in injured brain parenchyma by systemically administered siRNA. These findings present an effective targeting strategy for the delivery of therapeutics in clinical management of acute brain injuries.
A
cute brain injury such as traumatic brain injury (TBI) disrupts the normal function of the brain and generally has a poor prognosis for functional recovery and survival. Termed a ‘silent epidemic’, TBI is a leading cause of mortality and morbidity in children, teens and active adults from ages 1 to 44, with an annual incidence of 2.5 million in the US (ref. 1). TBI can lead to acute and potentially long-lasting neurological dysfunction, including the development of chronic traumatic encephalopathy or even Alzheimer’s disease. A majority of combat-related TBI cases are additionally complicated by a penetrating injury to the brain, which is often even more difficult to manage than non-penetrating injuries. Despite this substantial socio-economic impact, TBI treatment is limited to palliative care and no specific therapies with long-term benefits are available.

The blood–brain barrier (BBB) is considered a major impediment to systemic treatment of central nervous system (CNS) diseases. As a result, localized delivery of drugs within the brain has been explored, but it has limitations in clinical settings. In acute brain injury and several cerebrovascular diseases, including stroke, hypertension and ischaemia, the BBB is transiently disrupted, which allows extravascular access for macromolecules and neuroprotective drugs from the systemic circulation. In fact, the leakage of serum proteins into brain parenchyma is used to test for BBB integrity. However, lack of specific binding of passively accumulating proteins in the injured area can result in low retention and subsequent washout over time. Due to this clearance, the therapeutic efficacy of a systemically administered drug may be greatly limited.

We previously employed in vivo phage display as a powerful and unbiased method to probe tissues in situ for specific molecular signatures and discovered homing peptides specific for different pathologies including tumours, atherosclerotic plaques and wounds. We reasoned that an acute and complex event such as TBI is suited for a similar approach as site-specific molecular changes in protein expression have been reported. In this study, we set out to identify peptides that would recognize specific molecular changes at the sites of traumatic injury in the brain, and enhance delivery of therapeutic compounds to such sites. The goal of this approach was to explore an alternative to local delivery of therapeutics, which is invasive and can add complications to the injury.

Results
Isolation of brain injury selective peptide by phage display. To isolate peptides that specifically target brain injury, we inflicted unilateral puncturing stab wound injuries to the right hemisphere of adult male mice (Fig. 1a). The penetrating brain injury (PBI) resulted in rupture of BBB visualized by selective leakage of mouse IgG into the brain parenchyma on the injured side (Fig. 1b). PBI also caused cortical tissue loss, axonal damage and loss of myelin in the corpus callosum (Supplementary Fig. 1b), and was accompanied by an increase in glycosaminoglycan loss of myelin in the corpus callosum (Supplementary Fig. 1b), indicating a potential window for specific binding of passively accumulating proteins in the injured area can result in low retention and subsequent washout over time. Due to this clearance, the therapeutic efficacy of a systemically administered drug may be greatly limited.

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CAQK binding is specific to sites of brain injury. To further characterize the binding of CAQK to the injured brain area, we carried out overlay binding experiments with silver nanoparticles (AgNPs) conjugated with CAQK (CAQK-nanoparticles (NPs)) on mouse brain sections (Supplementary Fig. 5a–c). CAQK-NPs showed strong binding to the injured brain sections, whereas the binding of control NPs (CGGK-NP) was negligible (Fig. 2f–h). Low binding of CAQK-NPs to brain sections from normal animals was observed, suggesting the presence of low levels of the peptide binding epitope in normal brain, and its elevation upon injury. Similar binding pattern of CAQK-NPs was also observed in the CCI model (Supplementary Fig. 5d,e). Binding specificity was confirmed by inhibiting the CAQK-NP binding with excess of free CAQK, which resulted in near complete inhibition (Supplementary Fig. 5f–h).

CAQK peptide interacts with components of brain ECM. To identify the potential protein targets of CAQK in the brain tissue,
and in coronal view. (c) Cortex and nine needle punctures were inflicted according to the grid shown. Right panel shows perfused brain, 6 h after the unilateral injury, from the top and in coronal view. (b) Representative immunofluorescence images show leakage through compromised BBB in PBI. Perfused PBI brain at 6 h was stained in the region around the corpus callosum (cc) for mouse IgG (red) and 4,6-diamidino-2-phenylindole (DAPI; blue). Scale bar, 50 μm. (c) Summary of phage frequency in brain as per cent of total phage recovered. Compared with PBI, CAQK was present at a lower percentage in the contralateral hemisphere (left pie chart). Sequences from second round of biopanning (right pie chart) show high CAQK recovery confirmed through Sanger sequencing. (d) CAQK phage frequency in brain as per cent of total phage recovered. Compared with PBI, CAQK was present at a lower percentage in the contralateral hemisphere in injured mice and absent in healthy, control mice.

we performed mass spectrometry proteomics analysis of proteins separated from extracts of injured brain by affinity chromatography on immobilized peptides (Supplementary Fig. 6). Table 1 shows a comparison of proteins in eluates from CAQK and control (CGGK) columns. Among the large number of proteins identified, peptides prominent in the CAQK column eluates belonged primarily to the lectican family of chondroitin sulfate proteoglycans (CSPGs). These included versican, associated proteins tenasin-R and the hyaluronan and proteoglycan link protein (Hapln). Versican and Hapln4 were exclusively present in the CAQK column eluates. In normal brain, lectican proteoglycans form extracellular matrix (ECM) complexes known as perineuronal nets (PNN) around neuronal surfaces and the expression of some of these lectican proteoglycans is upregulated at sites of CNS injury.

We confirmed the increase in expression of ECM-associated CSPGs at sites of brain injury by immunostaining. Versican, tenasin-R, and the hyaluronan and proteoglycan link protein (Hapln4), all of which are components of the brain ECM complex, are upregulated following an injury, showed high expression in the injured but not the uninjured hemisphere of the brain (Supplementary Fig. 7). The signal from intravenously injected CAQK co-localized with versican, tenasin-R and Hapln4 (Fig. 3a). The peptide signal also co-stained with Wisteria floribunda agglutinin lectin, a marker for PNNs. At the cellular level, FAM-CAQK prominently accumulated at mature oligodendrocytes identified by expression of the adenomatous polyposis coli marker. In several instances, the CAQK binding pattern followed elongated cells that aligned in the direction of the callosal axons (see CAQK with adenomatous polyposis coli and even better with NG2 in Supplementary Fig. 8). Only a few isolated Olig2 and NG2-positive cells, most likely oligodendrocyte progenitor cells, bound the peptide. No CAQK was detected in or around other glial cell populations, including astrocytes (GFAP+) and microglia (Iba+; Supplementary Fig. 8). Collectively, these data suggest that the binding molecule (receptor) for CAQK peptide is present in the PNN complex that is upregulated in brain injuries.

To explore further the association of CAQK with the PNN complex, in vitro binding of CAQK phage to ECM produced by U251 human astrocytoma cells was tested. These cells express high levels of versican and other members of the brain ECM (ref. 15), which suggests that these cells are activated in culture. CAQK phage showed significantly higher binding to the U251 ECM than a control phage (Fig. 3b). In addition to providing further evidence for the ECM binding of CAQK, this result indicates that CAQK recognizes the human target. This is not surprising, as peptides are generally not species-specific in their binding properties. Binding to this ECM was specific as it was inhibited with excess free CAQK peptide. Moreover, enzymatic treatment of the ECM with chondroitinase ABC or hyaluronidase resulted in loss of versican staining (Supplementary Fig. 9) and correspondingly reduced CAQK binding (Fig. 3c,d). This suggests that the epitope for CAQK resides in the complex formed by the CSPGs, hyaluronic acid and associated proteins.

CAQK as a carrier of diagnostics and therapeutics. The accumulation of the FAM label attached to the CAQK peptide suggested that CAQK is capable of delivering low molecular weight compounds into sites of brain injury. To investigate further the translational potential of the CAQK-targeting approach, we first examined CAQK-mediated delivery to brain injury of NPs as a model of both an imaging agent and a drug carrier. CAQK conjugated, silver NPs (mean diameter—20 nm), administered intravenously, showed significantly greater accumulation in injured brain tissue than control NPs.
Figure 2 | CAQK peptide shows selective homing to PBI. (a) Fluorescence brain images (top view and coronal view) of mice injected with FAM-labelled peptides 6 h after PBI. Animals were perfused, brains isolated and imaged under an Illumatool System (green channel). (*$P < 0.05$, ANOVA analysis, $n = 6$); mean ± s.e.m. (b) FAM-CAQK in the sections from injured brain was visualized by immunohistochemical staining for FAM (brown) from FAM-CAQK in PBI. Sections counterstained with haematoxylin brains isolated (blue). Scale bar, 100 μm. (c–e) Fluorescence signal of FAM-CAQK (C) and FAM-Control (D) in clarified PBI brains. Peptides injected 6 h after PBI were allowed to circulate for 30 min followed by the CLARITY protocol for clearing brains. (e) shows higher magnification from c (white arrow points to the magnified region). CAQK showed homing to fibre-like structures in the corpus callosum (CC) area. Scale bar, 50 μm. (f–h) CAQK conjugated AgNPs bind to PBI sections around the CC. Overlay experiments using peptide-conjugated AgNPs on frozen PBI sections counterstained with 4,6-diamidino-2-phenylindole (DAPI; blue), green AgNPs were pseudo coloured to yellow for higher colour contrast, and the threshold was equally enhanced for all samples using Image J. Shown are representative sections of CAQK-NP binding to PBI sections (f), CAQK-NP binding to healthy brain sections (g) and control-NP binding to PBI sections (h). The number of particles in three fields were counted and plotted in the bar diagram from three brains. Scale bar, 50 μm. Mean ± s.e.m. (i) Fluorescence brain images (top view and coronal view) of mice injected with FAM-labelled peptides 6 h after a controlled cortical injury.
We simulated therapeutic oligonucleotides by using silence local expression of green fluorescent protein (GFP) as a carrier. Our proof of concept approach was to delivery of oligonucleotides loaded into porous silicon NPs (PSiNPs) as a carrier. Our proof of concept approach was to demonstrate the versatility of CAQK system, we tested delivery of oligonucleotides loaded into porous silicon NPs (PSiNPs) as a carrier. Our proof of concept approach was to silence local expression of green fluorescent protein (GFP) systemically expressed in transgenic mice from the CAG promoter. We simulated therapeutic oligonucleotides by using short interfering RNA (siRNA) against GFP loaded in CAQK conjugated PSiNPs (Supplementary Fig. 11a–d). PSiNPs were intravenously injected into the GFP mice with PBI and visualized by time-gated luminescence imaging allowing quantification of their accumulation in the excised brains. The imaging showed that CAQK-PSiNPs accumulated in the injuries at markedly higher (35 fold) levels than PSiNPs coated with a control peptide (Fig. 4b). Other tissues, including regions of the brain outside the injury area, showed no significant difference in the accumulation of CAQK and control PSiNPs (Fig. 4c). Confocal microscopy on transverse cortical sections from mice injected with CAQK-PSiNP-siGFP exhibited a large void of GFP expression at the injury site, whereas brains from mice treated with control NPs did not differ from untreated brains (Fig. 4d). We observed 70% silencing of GFP expression by targeting siGFP, whereas minimal silencing was observed with untargeted siGFP and other controls. This silencing was visible across the entire injury and not just in a particular cell type presumably due to the gradual degradation of PSiNP and release of the siGFP over time in the injury. The gene silencing was specific for brain injury, as GFP expression remained unaltered in normal brains or in other major tissues (Supplementary Fig. 11e).

Lastly, to examine CAQK recognition of human brain injury, we tested ex vivo binding of CAQK-conjugated silver NPs on human cortical sections obtained from a head trauma patient. The CAQK-NPs showed intense binding to the injured brain sections from the cortex and the corpus callosum areas, whereas binding to normal brain sections was minimal (Fig. 5a). Similar to the mouse brains, we observed significant elevation in expression of versican and haptN4 in injured brain than in normal brain by immunohistochemistry (Fig. 5b). These findings confirm CAQK binding to human target and support its potential utility for therapeutic application in humans.

Discussion
We describe a 4-amino acid peptide that selectively recognizes brain injuries and accumulates at the sites of injury. We show that this peptide, CAQK, enhances the accumulation of systemically administered payloads with chemistries ranging in size from a drug-sized molecule to nanoparticles, and incorporating a variety of imaging and therapeutic functions of potential utility in clinical management of brain injuries. Importantly, the target of this delivery system is expressed both in mouse and human brain injuries.

We used two mouse models in this study. The PBI model mimics gunshot or shrapnel wounds, such as the ones sustained by a warfighter. The blunt cortical impact model more generally reproduces the features of severe TBI. CAQK recognized the injuries in both models, suggesting broad utility across acute brain injuries. The fact that the contralateral hemisphere, unlike normal brain, accumulated some CAQK phage suggests that less severe injuries than the ones we used may also be targeted with CAQK. It remains to be tested whether CAQK also homes to spinal cord injuries, or even denervating CNS lesions such as in multiple sclerosis. Importantly, we also show that CAQK recognizes its target in cultured human cells and in cortical sections of injured human brain.

The phage screening in this study revealed a novel aspect of the in vivo screening; whereas the previous screens have probed the vasculature of the target tissue, even in the brain, the compromised BBB integrity in brain injury allowed the phage to probe the extravascular brain tissue. Moreover, the high-throughput sequencing of the peptide-encoding inserts in the phage genome provided a technical improvement to the screening. One round of selection, as opposed to repeated rounds as previously done, provided a fingerprint of over 200,000 peptide sequences, revealing a striking enrichment of phage displaying the CAQK peptide sequence. Although a cyclic phage library was used, a library of this design contains a minority of linear peptides because stop codons occur within the random insert causing truncation of the cyclic peptide. Additionally, mutations may also change the structure of the peptide. Thus, peptides that do not conform to the general structure of the library are commonly encountered in phage screening.

The recovery of cyclic peptides containing the CAQK motif, in addition to the dominant linear CAQK peptide, suggests that CAQK motif is also active in the context of a cyclic peptide.

BBB disruption is an important contributor to secondary injury following TBI, and therapies to restore BBB functionality are under investigation for neuroprotection. The localized permeability of BBB and the delayed onset of secondary injury provide a window of opportunity for therapeutic intervention. The literature and our results suggest the duration of the BBB impairment is at least up to 5 days. Within this time window, affinity ligand-based (synaphic) targeting can be an effective drug delivery approach; our results show as high as 35-fold enhancement in the accumulation of systemically administered imaging agents and therapeutics at and around the site of injury.

The concentrating effect of synaphic targeting is likely accounted for by two factors: the peptide can access and bind to its target that allows accumulation of the payload and causes retention at the site of injury. The impairment of the BBB allows all circulating substances to enter the injury area. And, if the peptide receptor is sufficiently abundant relative to the amount of the peptide-drug conjugate used, the binding of the peptide to the receptor can drive payload accumulation beyond what is caused by passive leakage. This is the case in brain injury, where the components of the CSPG complex are overexpressed upon an

| Table 1 | CAQK binds to brain ECM proteins. |
|---------|----------------------------------|
| Protein name | UniProt ID | Gene name | Log2 LFQ intensity |
| Versican core protein | Q62059 | VCAN | 25.98 |
| Hyaluronan and proteoglycan link protein 4 | Q80WM4 | HAPLN4 | 19.84 |
| Tenascin-R | Q8BY19 | TNR | 26.71 |

Proteins belonging to the PNN complex were identified by peptide-affinity chromatography and mass spectrometry analysis on mice PBI brains. The label free quantification (LFQ) intensities were derived using MAXQuant software and averaged for three technical replicates. Intensities are plotted on log2 scale. Empty column denotes protein was not detected.
Figure 3 | CAQK co-localizes with chondroitin sulfate in PBI. (a) Immunofluorescence images of PBI sections showing the corpus callosum (CC) region. The sections were stained for versican, tenascin-R and the hyaluronan proteoglycan link protein, the oligodendrocyte marker (adenomatous polyposis coli (APC)), and Wisteria floribunda agglutinin (WFA; shown in red), FAM-CAQK (green) using anti-fluorescein antibody and counterstained with 4,6-diamidino-2-phenylindole (DAPI; blue). Shown are representative brain sections of injured hemisphere in a PBI mouse injected with FAM-CAQK 6 h after injury. Arrows show co-localization. Scale bar, 20 μm.

(b) Phage binding to ECM formed by U251 cells. The cells were gently dissociated and removed, and the remaining ECM was incubated with phage for 1 h at room temperature, and phage binding was detected following an ELISA protocol. CAQK phage showed higher binding to ECM than control phage.

c) Inhibition of CAQK phage binding to ECM by free CAQK peptide.

d) Phage binding to ECM is reduced on enzymatic digestion of ECM with chondroitinase ABC or hyaluronidase. Mean ± s.e.m. Representative data shown in b–d is from three experimental repetitions each with three sample replicates.
The second important factor is the retention effect. As the drug concentration decreases in circulation, the drug is washed out of the injury area. Peptide binding to its target can retain the drug in the injured microenvironment by minimizing this washout. Therefore, the targeting approach in this work encompasses the critical period of healing, which may provide a more lasting therapeutic effect, at least when the therapeutic action is long-acting. Notably, some oligonucleotides, which is one of the types of drugs we successfully delivered in this work, have been shown to remain active for weeks in tissues.

CNS injury results in formation of a CSPG-rich glial scar, which is a major barrier to regeneration. Strategies to prevent the accumulation of CSPGs in injury or dissolve existing deposits have been explored. However, site-specific delivery of the active compounds has been a challenge. The intrinsic affinity of CAQK peptide for CSPG-rich areas in injured brain could be effective in directing a CSPG-reducing payload, such as the chondroitinase ABC enzyme. Having successfully targeted nanoparticle payload into brain injuries, we would expect to accomplish the same with proteins, such as chondroitinase. The ability of the present approach to concentrate a payload at the site of brain injury is important for reducing toxicity at off-target sites. An example of an existing therapeutic agent that would benefit from reduced toxicity is the neuroprotective agent brain-derived neurotrophic factor.
the potential of converting agents with unfavourable pharmacokinetic profile into efficient drugs.

Oligonucleotide-based drugs are a new class of drugs with great potential but hampered by delivery problems in vivo. An example is siRNA, a therapeutic modality with the desired characteristics of specificity and potency, but particularly difficult to deliver through systemic circulation. Previous studies on siRNA therapy of brain injuries have either used direct injection into the CNS through systemic circulation. Previous studies on siRNA therapy of specificity and potency, but particularly difficult to deliver is siRNA, a therapeutic modality with the desired characteristics brain tissue. Positive staining was quantified and plotted. Scale bar, 50 μm. Mean ± s.e.m., (ANOVA analysis; **P < 0.01, ***P < 0.001, ****P < 0.0001).

Figure 5 | CAQK binds to injured human brains. (a) CAQK-conjugated AgNPs (CAQK-NPs) showed higher binding to sections from the corpus callosum and cortex of human brains with TBI than with normal brain or with control NPs. Peptide-conjugated nanoparticles were incubated with formalin-fixed frozen sections from injured and normal brains for ex vivo binding. Sections were counterstained with nuclear fast red. The number of particles in each frame were counted and plotted in the bar diagram. Representative photomicrographs are shown. Scale bar, 50 μm. Mean ± s.e.m. (ANOVA analysis; **P < 0.01, ***P < 0.001, ****P < 0.0001).

Methods

Brain injury models. All animal experiments were conducted under an approved protocol of the Institutional Animal Care and Use Committee of Sanford Burnham Prebys Medical Discovery Institute. Eight- to ten-week-old male BL6 mice were anaesthetised with 4% isoflurane (Aerrane; Baxter, UK) in 70% N2O and 30% O2, and positioned in a stereotaxic frame. Using a head restraint, a 5-mm craniotomy was made using a portable drill and a trephine over the right parietotemporal cortex and the bone flap was removed. PBI model was used as described35,36. Nine needle punctures using a 21G needle were made 3 mm deep according to a 3 × 3 grid, spaced 1 mm in width and 1 mm in height. For TBI, a CCI model was used as described37. Mice were subjected to CCI using the benchmark stereotaxic impactor (Impact One; myNeuroLab.com) with the actuator part mounted directly on the stereotaxic instrument. The impactor (3 mm in diameter) tip accelerated down to the 1.0 mm distance, reaching the preset velocity of 3 m s⁻¹, and the applied electromagnetic force remained there for the dwell time of 85 ms, and then retracted automatically. The contact sensor indicated the exact point of contact for timescales. This approach provides an alternative to local delivery, which is invasive and can add complications to the injury. Moreover, the approach could be transferable to human patients, because CAQK recognizes the human target molecule and because the expression of the target appears to be elevated in injured human brain tissues in the same way it is in the mouse injuries.
Afterwards, the scalp was closed with sutures, anaesthesia discontinued and mice were anaesthetized with 70%/30% nitrous oxide/oxygen) was used during the entire procedure and reproducible results. In both models, facemask anaesthesia (1–2% isoflurane in N₂O) was preferred over endotracheal intubation to minimize stress, but in the absence of the latter, the alternative was performed with 1%–2% isoflurane. Animals were then passively anaesthetized with 1ml 10% thiopental for total surgical procedure. Mice recovered from anaesthesia in a heating pad and were monitored for an additional 1 hour before being placed back in their cages.

In vivo phage display. Six hours after brain injury, mice were intravenously injected with 1e10 p.f.u. of a CX7C naïve phage library in 100 µl of PBS. The library was allowed to circulate for 30 min, after which mice were anaesthetized with 2.5% avertin and perfused with PBS intracardially. Brains were extracted, and the tissue surrounding the injury and the corresponding region from the contralateral side was isolated. Tissues were homogenized in LB-NE 40 (1%) and phage was processed as described. Briefly, recovered phages were titrated and amplified in E. coli BL21-T5403 and purified for input for second round of screening. The colonies recovered from second round were sequenced using Sanger sequencing (Lonet biosciences, San Diego, CA). Alternatively, after first round, the phages in the lysate were rescued by amplification in E. coli and peptide-encoding portion of the phage genome was sequenced using Ion Torrent high-throughput sequencing.

Peptide synthesis and coupling. The peptides were synthesized on a microwave-assisted automated peptide synthesizer (Liberty; CEM, Matthews, NC) following overnight, washed with PBS and placed in graded sucrose solutions overnight for 30 min. Mice were perfused intracardially with saline and organs were isolated after injury, with 50 nmoles of peptide dissolved in PBS, and allowed to circulate for 30 min. Mice were intravenously injected, 6 h after injury and allowed to circulate for 30 min. Mice were perfused intracardially with saline and organs were isolated and analysed by immunostaining.

Homing studies and tissue sections. Animals were intravenously injected, 6 h after injury, with 50 nmoles of peptide dissolved in PBS, and allowed to circulate for 30 min. Mice were perfused intracardially with saline and organs were isolated and imaged using the Illumiato Bright Light System LT-9900 (LightBio Research). Brains and organs were placed in 4% parafomaldehyde (PFA) at pH 7.4 overnight, washed with PBS and placed in graded sucrose solutions overnight before optimal cutting temperature compound (OCT) embedding. Ten-micrometre-thick sections were cut and analysed by immunofluorescence. For a complete histological analysis, sections were stained with the Movat pentachrome kit (American Mastertech Inc.) following the manufacturers instructions.

Immunofluorescence. Frozen sections were permeabilized using PBS-Triton (0.2%), blocking was carried out using 5% blocking buffer: 5% bovine serum albumin, 1% donkey serum in PBS-T. Primary antibodies were incubated in diluted (1%) blocking buffer overnight at dilutions 1/1000 for 1 h at 37°C. PBS-T was incubated in the plate for overnight and unbound phage was removed by washing three times with 200 µl of PBS. The bound phage was detected by incubating with an in-house generated anti-T7 phage antibody for 1 h at 4°C. Following washing, horseradish peroxidase labelled anti-rabbit IgG (Sigma-Aldrich) was diluted 1:1000 in PBS, and 100 µl was added to the wells, followed by the addition of the well washing for three times. Next, 100 µl of o-phenylenediamine dihydrochloride (OPD) silver and gold substrate (Sigma-Aldrich) was added to the wells and incubated at room temperature until visible colour was observed (30 min). Adding 50 µl of 1 M H₂SO₄ stopped the reaction and the plate was read at 495 nm (FlexStation 3 Reader, Molecular Devices, Sunnyvale, CA, USA). For enzymatic digestion, their protein concentration determined by using bichinchoninic acid protein assay (Thermo Fischer) and the samples were digested using the filter-aided sample preparation method. Finally, the digested samples were desalted, dried and subjected to liquid chromatography–mass spectrometry (MS/MS) analysis at the Proteomics Core facility of the Burnham Brain Health Institute. All mass spectra were analysed with MaxQuant software version 1.5.0.25. The MS/MS spectra were searched against the Mus musculus Uniprot protein sequence database (version July 2014).

Phage binding to ECM. Cells grown as confluent monolayer in a 96-well plate were gently removed by a enzyme-free cell dissociation buffer (Thermo Fisher Scientific) and plates blocked with 200 µl of 0.5% bovine serum albumin in PBS with 0.01% Tween 20 for 1 h at 37°C. PBST was incubated in the plate for overnight and unbound phage was removed by washing three times with 200 µl of PBST. The bound phage was detected by incubating with an in-house generated anti-T7 phage antibody for 1 h at 4°C. Following washing, horseradish peroxidase labelled anti-rabbit IgG (Sigma-Aldrich) was diluted 1:1000 in PBS, and 100 µl was added to the wells. The wells were incubated at room temperature until visible colour was observed (30 min). Adding 50 µl of 1 M H₂SO₄ stopped the reaction and the plate was read at 495 nm (FlexStation 3 Reader, Molecular Devices, Sunnyvale, CA, USA). For enzymatic digestion, their protein concentration determined by using bichinchoninic acid protein assay (Thermo Fischer) and the samples were digested using the filter-aided sample preparation method. Finally, the digested samples were desalted, dried and subjected to liquid chromatography–mass spectrometry (MS/MS) analysis at the Proteomics Core facility of the Burnham Brain Health Institute. All mass spectra were analysed with MaxQuant software version 1.5.0.25. The MS/MS spectra were searched against the Mus musculus Uniprot protein sequence database (version July 2014).
brains were isolated. Silver accumulation in the brain was analysed by silver staining autometallography with counterstaining with nuclear fast red (Sigma).

**Tissue section overlay of silver nanoparticles.** Overlay experiments to analyse ex vivo binding were carried out on frozen brain tissue sections following the same protocol for immunofluorescence staining described above taking the nanoparticles as if they were the primary antibody and using no secondary antibody. Peptide-coated AgNPs in PBS-T at a 1 µM concentration were used and the incubation time was 1 h at 37 °C. Sections were then washed with phosphate-buffered microcopy by looking at intrinsic emission from the FAM tag on the peptide.

**Synthesis and functionalization of porous silicon nanoparticles (PSiNPs).** Single-crystalline highly doped p-type silicon wafers (∼1 µm cm resistivity, <100> polished, boron-doped) were purchased from Virginia Semiconductor, Inc. PSiNPs were prepared by electrochemical perforation etching of the silicon wafers, as described previously. Briefly, the silicon wafer was anodically etched in an electrolyte consisting of 3:1 (by volume) of 48% aqueous HF:H2O at 30 V for 1 h, followed by a higher current density of 50 mA cm⁻² for 0.36 h. This waveform was repeated for 140 cycles, generating a perforated porous silicon film with alternating layers of high and low porosity. The resulting porous nanostructure was removed from the silicon substrate by applying a current density of 3.7 mA cm⁻² for 250 s, followed by ultrasonication for 4 h. The free-standing porous silicon films were then fractured to the desired size (nominally 150 nm) by ultrasonication, and the resulting nanoparticles were oxidized by immersion in aqueous borax solution to activate photoluminescence.

**Peptide conjugation and siGFP loading to PSiNPs.** An aliquot of PSiNPs (2 mg ml⁻¹ in ethanol) was mixed with 20 µl of 3-(ethoxymethyl)-propylamine silane by vortexing for 2 h at room temperature. The amine-terminated nanoparticles were rinsed three times with ethanol and then further reacted with 1 ml of succinimidyl carbonyl ester-polyethylene glycol-cysteine (SCM-PEG-Cys, MW 5000) were purchased from Sigma-Aldrich and Laysan Bio, respectively, and used as received without further purification. RNA-free water was purchased from Thermo Fischer(Carlsbad, CA). Small interfering RNA (siRNA) targeting and analysis

**Characterization of PSiNPs.** Transmission electron microscope images were obtained on JEOL-1200 EX II operating at 120 kV. Dynamic light scattering (DLS, Zetasizer ZS 90, Malvern Instruments) was used to determine the hydrodynamic size and Zeta potential of the nanoparticles. Photoluminescence and fluorescence spectra were obtained using a QEOS spectrometer (Ocean Optics). Concentration of siRNA was determined by measuring absorbance at 260 nm using a spectrometer (NanoDrop 2000, Thermo Fisher Scientific) based on the OD₂₆₀ standard curve of siRNA.

**In vivo siRNA targeting and analysis.** Transgenic CAG-GFP mice were purchased from The Jackson Laboratory (stock #008567). Brain injuries were done as described above. Biotin-conjugated and siRNA-loaded PSiNPs (300 µl) were administered twice via tail-vein injections at 6 and 24 h post injury (n = 3). Three days after injury, mice were perfused, organs harvested and fixed for downstream analysis. The tissues were imaged under a time-gated imaging set-up and the GFP expression was analysed by researchers blinded to the experimental groups.

**Gated luminescence imaging of silicon nanoparticles (GLISin).** Gated luminescence imaging were acquired from a custom-built time-domain imaging system. The data were collected using an intensified CCD camera (STAR 334 T, Andor Technology Ltd.). As reported, a tunable laser consisting of a tripled Nd:YAG-pumped optical parametric oscillator (Opolette 355, Opotek Inc.) were used as an excitation source at a repetition rate of 10 Hz, which was synchronized and triggered with the CCD. The Andor SOLIS software was used to control time delays and acquisition parameters. Peptide-coated AgNPs were used to analyse signal-to-noise ratio and to analyse signal correlation along the z-axis. The biodegradable nanoparticles were placed on a black polyethylene plate, and bright field (under ambient light) and gated luminescence (under excitation with pulsed laser, λex = 410 nm) images were taken.

**Human tissue experiments.** Formalin-fixed human brain tissues were obtained from the Brain Tissue Repository maintained by the Center for Neuroscience & Regenerative Medicine (CNRM) at the Uniformed Services University of the Health Sciences (USU) in Bethesda, MD. The TBI case is from a patient with moderate TBI (automobile accident) who died at age 72. The control case is from a 63-year-old male without any neurologic diagnosis or any signs of TBI on detailed neuropathologic evaluation. Fixed tissues were cryopreserved and sectioned for overlay binding with AgNPs as described above. For immunohistochemistry, an antigen retrieval step was done before incubation with anti-hapln4 and anti-vascular endothelial antibodies.

**Data availability.** The data that support the findings of this study are available from the corresponding author on request.

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A.P.M. and P.S. performed phage display screening. T.M. and T.T. performed phage sequencing and analysis. J.I. and G.B.R. synthesized and characterized nanoparticles. V.R.K. synthesized peptides. A.P.M., P.S., S.H., Z.G.S. and E.K. conducted sequencing and analysis. J.J. and G.B.B. synthesized and characterized nanoparticles. A.P.M. and P.S. performed phage display screening. T.M. and T.T. performed phage sequencing and analysis. J.I. and G.B.R. synthesized and characterized nanoparticles.

Additional information
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