**Transfection of Heat Shock Protein 70 kDa (HSP70)**

Maxime Gestin¹ · Luca Falato¹ · Michela Ciccarelli² · Carmine Pasquale Cerrato¹,³ · Claes Andréasson² · Ülo Langel¹,4

Accepted: 3 May 2022 / Published online: 19 May 2022 © The Author(s) 2022

**Abstract**

Heat shock protein 70 kDa (HSP70) is a major protein family in the cell protections against stress-induced denaturation and aggregation and in the folding of nascent proteins. It is a highly conserved protein that can be found in most organisms and is strongly connected to several intracellular pathways such as protein folding and refolding, protein degradation and regulation, and protection against intense stress. Cellular delivery of HSP70 would be of high impact for clarification of its role in these cellular processes.

PepFect14 is a cell-penetrating peptide known to be able to mediate the transfection of various oligonucleotides to multiple cell lines with a higher efficacy than most commercially available transfection agents and without inducing significant toxic effects.

In this study we demonstrated that PepFect14 was able to form a complex with HSP70 and to deliver it inside cells in the same fashion with oligonucleotide delivery. The delivered HSP70 showed an effect in the cell regulation indicating that the protein was biologically available in the cytoplasm and the interactions with PepFect14 did not impeach its active sites once the plasma barrier crossed.

This study reports the first successful delivery of HSP70 to our knowledge and the first protein transfection mediated by PepFect14. It opens new fields of research for both PepFect14 as a delivery agent and HSP70 as a therapeutic agent; with potential in peptide aggregation caused diseases such as Parkinson’s and Alzheimer’s diseases.

**Keywords** Cell-penetrating peptide · Transfection · PepFect14 · HSP70

**Introduction**

Heat shock protein 70 kDa (HSP70) is a highly conserved protein family expressed by most organisms and found in both prokaryotes and eukaryotes. All the members of the HSP70 protein family have a molecular weight that ranges around 70 kDa and all share the same chaperone function (Radons 2016). They bind to short hydrophobic polypeptide sequences in their substrate binding domain (SBD) with an affinity that is allosterically modulated by the N-terminal adenine nucleotide binding domain (NBD) (Feige and Polla 1994). When adenosine triphosphate is bound to the NBD the affinity for the substrate in the SBD is low but when adenosine diphosphate is bound, the affinity increases (Clerico et al. 2015). This process actually triggers the closing of the C-terminal α-helical domain. This domain acts as a lid that retains the substrate bound to the rigid structure of the SBD (Strub et al. 2003). HSP70 has two major roles in the cells; first, it binds to peptidic parts of nascent proteins and keeps them from premature folding (Bukau et al. 2000; Hartl and Hayer-Hartl 2002); second, HSP70 has a protective feature in response to intense cellular stress such as environmental, physiological or pathological stress (Pinto et al. 1991; Qu et al. 2015). HSP70 is of major importance in the regulation
and protection of intracellular proteins. Indeed, HSP70, besides being closely involved in the folding of newly produced proteins as a chaperone protein, is also involved in many other intracellular regulative pathways. When a substrate binds to HSP70, several pathways can be triggered (Kim et al. 2018). The substrate can be refolded, either directly by the action of HSP70 or through the recruitment of co-chaperones (Sharma and Masion 2009), but can also be translocated to autophagosome or proteasome for intracellular recycling (Witkin et al. 2017). HSP70 can also trigger regulations of transcription factors that lead to an indirect regulation of the proteome (Pratt and Toft 2003). Under an intense stress, HSP70 is able to bind to already formed proteins and to protect them against denaturation and aggregation (Rokutan 2000). All these possible pathways triggered by HSP70 give this protein a major role in maintaining cell functions. HSP70 can also play an important role as a therapeutic agent in neurodegenerative diseases caused by peptide aggregations such as α-synuclein in Parkinson’s disease (Rochet et al. 2012) or amyloid-β and Tau in Alzheimer’s disease (Campanella et al. 2018).

Modulating intracellular levels of HSP70 has the potential to be an efficient therapeutic action (Kim et al. 2018) and more particularly an acute increase of its concentration could give a temporary protection against protein aggregation and misfolding. There are two methods to increase the level of a specific protein in cells. Either transfecting an oligonucleotide or a plasmid DNA coding for this protein (Kim and Eberwine 2010) or delivering directly the wanted protein inside the cell (Pisal et al. 2010). The transfection of oligonucleotides and plasmid DNA can be either permanent if the sequence is integrated in the genome or transient if not. In either case, the expression of the protein relies on the cell machinery and is dependent on transcription factor. If a protein is delivered instead, it is directly available for a biological effect the delivery will then be transient as the cells will not express the protein after its natural degradation. Delivering a protein is an alternative to oligonucleotide and plasmid DNA delivery and presents advantages for acute and non-permanent treatment. It also allows a more straight-forward treatment strategy as it does not rely on the cell transcription machinery (Lee et al. 2019).

Cell-penetrating peptides (CPPs) are a class of peptides able to translocate through biological barriers and to transport bioactive macromolecules directly inside cells (Bechara and Sagan 2013). PepFect14 is an amphipathic CPP composed by a 21 amino acids sequence and a stearic acid tail bound to its N-terminus (Ezzat et al. 2011) with sequence stearyl-AGYLLGKLLOOLAAALOOLL. It is able to form non covalent complexes with its cargoes via electrostatic interactions (Lehto et al. 2017). It has been shown for several years already that PepFect14 formed complexes with diverse modified or non-modified oligonucleotides – siRNA (Ervin et al. 2019), plasmid DNA (Veiman et al. 2013) and antisense oligonucleotides (Ezzat et al. 2011) - and mediated their transfection inside various types of cells. The complexes, when placed in cell culture media, formed nanoparticle with a hydrodynamic diameter in the range of 10^2 nm (Lehto et al. 2017). It was shown in a previous study from our group that the formation of nanoparticles was a key factor in the ability of PepFect14 to mediates transfections (Lehto et al. 2016). In another study where we studied the gene regulations induced by the translocation of PepFect14 in HeLa cells, we demonstrated that HSPA1B, a gene that codes for a HSP70 protein, was up-regulated as a reaction to the uptake of PepFect14. In the same study, we showed that a binding opportunity between PepFect14 and HSP70 existed in the polypeptide binding site (Dowaidar et al. 2017).

In the present study, we assessed the ability of PepFect14 to form a complex with HSP70. We also characterized the particles that these complexes formed in cell culture media before monitoring the uptake of a fluorescent-labelled HSP70 in cells and finally we detected a biological effect induced by the transfected HSP70.

**Materials and Methods**

**Peptide Synthesis**

PepFect14’s amino acid chain was synthesized using a microwave assisted synthesis (Biotech, Sweden) on a 0.1 mmol scale following fluorenlymethoxycarbonyl (Fmoc) solid phase peptide synthesis chemistry. The first amino acid was bound to a ChemMatrix Rink Amide resin (0.50 mmol/g) as a solid support in order to get a C-terminal amide group. The resin was swollen in N,N-dimethylformamide (DMF) (VWR, Radnor, Pennsylvania, USA) for 5 min at 70 °C with oscillation mixer on. Fmoc-protected amino acids were dissolved in DMF containing 2-cyano-2-(hydroxyimino)acetate (OxymaPure, Novabiochem, Merck Millipore, Burlington, Massachusetts, USA) and N,N’-Disopropycarbodiimide (DIC) (Iris Biotech GmbH, Germany) as coupling reagents and added to the resin for 5 min at 75 °C. The Fmoc protective group was removed with 20% piperidine (Iris Biotech GmbH, Germany) in DMF for 2 min at 45 °C followed by a 12 min reaction at room temperature before addition of the next amino acid. The final cleavage was performed with 95% trifluoroacetic acid (TFA) (Iris Biotech GmbH, Germany), 2.5% trisopropylsilane (TIS) (Merck KGaA, Darmstadt, Germany) and 2.5% water for 4 h at room temperature before precipitation in cold diethyl ether (VWR, Radnor,
Pennsylvania, USA). The crude peptide was dissolved in 80% acetonitrile (VWR, Radnor, Pennsylvania, USA) and 20% water before purification by reverse-phase high performance liquid chromatography (RP-HPLC) using a Biobasic C8 column (Thermo Scientific, Sweden) with a gradient of acetonitrile in water (0.1% TFA). The purified peptide was freeze-dried and the molecular mass was verified by UHPLC-MS (Agilent 1260 Infinity, Agilent Technologies, Santa Clara, California, USA).

**Protein Expression and Purification and Labelling**

Plasmid pCA1033 was constructed by previously described approach (Holmberg et al., 2014). Briefly, the Hsp70 gene (HSPA1A) was PCR amplified from pcDNA/FRT/TO-V5-HSPA1A (Hageman and Kampinga, 2009). The PCR amplified product was used to transform yeast together with the restricted vector pSUMO-YHRC to yield pCA1033 using yeast homologous recombination. The plasmid was rescued to E. coli and was verified by DNA sequencing.

Hsp70 was expressed in E. coli strain BL21-SI/pCodon-Plus from plasmid pCA1033 as a 6xHis-SUMO fusion as described previously (Andréasson et al. 2008). Briefly, an overnight culture of the transformed cells was diluted 100-fold in 1 L 2xYT supplemented with 50 mg/L kanamycin, 25 mg/L chloramphenicol and 2 mM MgSO4 and expression of the protein was induced by the addition of 1 mL 0.5 M iso-propyl b-D-thiogalactopyranoside (IPTG) and 40 mL 5 M NaCl at OD600 0.6. After 6 h expression at 30 °C cells were harvested by centrifugation and the pellet was resuspended in 10 mL of lysis buffer LWB150 (40 mM HEPES pH 7.4, 150 mM KCl, 5 mM MgCl2, 5% glycerol) supplemented with 1 mM PMSF and DNase I and complete (TM) EDTA-free protease inhibit and lysed by two passages in an EmulsiFlex-C3 high-pressure homogenizer. The cleared supernatant (27,000 g for 30 min) was incubated with Protino Ni-IDA silica matrix (Machery-Nagel GmbH and Co. KG, Düren, Germany) for 1 h at 4 °C with end over rotation. The matrix was transferred to a gravity-flow column and after extensive washing with LWB150 the bound protein was eluted using LWB150 supplemented with 250 mM imidazole pH 7.4. The 6xHis-SUMO was cleaved with Ulp1-6xHis protease during overnight dialysis against LWB150 at 4 °C. The dialyzed solution was passed over Ni-IDA matrix to remove 6xHis-SUMO and Ulp1-6xHis. For microscopy studies, the part of the purified proteins was labelled with Alexa 568 fluorophore using Alexa Fluor™ 568 Protein Labeling Kit (Invitrogen, Sweden) according to manufacturer protocol.

**Tryptophan Fluorescence Extinction**

The formation of a complex between PepFect14 and HSP70 was investigated by tryptophan fluorescence extinction. In a black opaque 96-well plate, HSP70 (50 nM) was incubated in HEPES buffer (1 mM) in presence of various concentration of PepFect14 (from 1 nM to 1 µM) for 30 min at 37 °C. The plate was then read on a fluorescence reader (Flex Station II, Molecular Device; ex: 280 nm, em: 360 nm). The measurement was made in triplicates for each PepFect14 concentration. The fluorescence intensities were then transformed and normalized to the intensity of HSP70 alone (Eq. 1, where I represent the intensity of tryptophan fluorescence) to obtain the percentage of fluorescence extinction.

**Equation 1:**

\[
\frac{I_{HSP70\,alone} - I}{I_{HSP70\,alone}}
\]

The data were analysed using GraphPad Prism 8 and were fitted with a hyperbolic regression to obtain an apparent Kd value.

**Dynamic Light Scattering**

The particle formation ability of our complexes PF14:HSP70 was assessed using a Zetasizer Nano ZS (Malvern Instruments, United Kingdom). The hydrodynamic diameter of PepFect14 (2 µM), HSP70 (200 nM) and PF14:HSP70 (2 µM:200 nM) were measured at 37 °C in DMEM supplemented with 10% FBS, to fit to the cell treatment conditions. The compound were prepared in 60 µL milliQ water 10 time more concentrated and then diluted to 600 µL in DMEM + 10% FBS before being transferred into DTS1070 cuvettes. PepFect14 and HSP70 complexes were let to incubate for 30 min at room temperature before dilution and reading.

**Cell Culture**

Bormirski hamster melanoma (BHM) cells stably expressing firefly luciferase (BHM pLuc cells) were grown in Dubelco’s Modified Eagle Medium (DMEM) at 37 °C in humidified incubator with 5% CO2. The culture media was supplemented with 10% Fetal Bovine Serum (FBS), 200 µg/ml streptomycin and 200U/ml penicillin (Invitrogen, Sweden) and Plasmocin™ Prophylactic 5 mg/L (Invitrogen, France).

**Epi-Fluorescence Microscopy**

Optical microscopy 96-well plates were seeded with 7,000 BHM pLuc expressing cells per well in 100 µL DMEM.
supplemented with 10% FBS and incubated at 37 °C for one day. The complex PF14:HSP70-Alexa 568 (2 µM:200 nM) were formed in milliQ water for 30 min at room temperature before addition to the cells for 1 or 3 h. A control experiment was prepared with HSP70-Alexa 568 alone (200 nM) incubated for 1 and 3 h. The wells were emptied and washed thrice with 200 µL Gibco Opti-MEM before a final addition of 100µL of Gibco FluoroBrite. The plate was then imaged using an epi-fluorescence microscope. The imaging was performed using a Leica DM/IRBE 2 epi-fluorescence microscope with a 63 x 1.4 NA oil immersion objective for fluorescence imaging, and the images were recorded by a Hamamatsu Orca-ER CCD camera. The system was controlled by the open source software Micro-Manager. Fluorescent images were collected using N3 filter cube (Chroma Technology Corporation, VT, USA). The surface plots were obtained using Image J software.

**Luciferase Recovery**

In opaque 96-well plates with clear bottom, 7,000 cells were seeded in DMEM with 10% FBS and incubated overnight at 37 °C. Lipofectamine 2000 (Thermofisher Scientific, Sweden) was incubated with siRNA against HSPA1A/B according to manufacturer protocols and added to the cells. The plates were further incubated for one day. On the third day, PepFect14 (20 µM) and HSP70 (2 µM) were mixed in milliQ water and left to form complexes for 30 min at room temperature. In each wells, except controls, PF14:HSP70 was added to fresh medium with dilution factor 10 and the cells were incubated for 3 more hours at 37 °C. The plates were then emptied and frozen at -80 °C overnight before thawing would complete the cell lysis. A luciferase assay was performed as described in Helmfors et al. (Helmfors et al. 2015). Briefly, a mixture of D-luciferin, adenosine triphosphate, co-enzyme A and dithiothreitol was prepared in

---

**Fig. 1**

A. Tryptophan fluorescence extinction. In HEPES buffer, HSP70 (50 nM) was put in presence of various concentration of PepFect14 (from 1 µM to 1 nM). Fluorescence intensities were measures with an excitation peak at 280 nm and an emission peak at 360 nm. The data were normalized to the intensity of HSP70 alone and to the buffer before being transformed to a percentage of extinction. Each data point shows the mean and SEM of three technical replicates. B. hydrodynamic diameter measurement by dynamic light scattering of the particles present in DMEM + 10% FBS alone or supplemented with either HSP70 (200 nM), PepFect14 (2µM) or PF14:HSP70 (2µM:200nM). The graphs present the size distribution by intensity.
buffer containing MgSO₄, MgCO₃, tricine and ethylenediaminetetraacetic acid. The luciferase assay mix was added to each well (100µL per well) and the plates were shaken for 1 min before the luminescence was read in a luminometer.

lipofectamine 2000 + siRNA HSPA1A/B (day 1) + PF14 (day 2)

Cell Proliferation Assay

To assess the possible toxic effects of our treatments, a WST-1 assay was performed during the same treatment as the ones used to measure the luciferase activity recovery. 10µL of WST-1 reagent were added to the medium in each well and incubated for 2 h at 37 °C. The absorbance of each well was measured at 440 nm over 690 nm. The cell medium with WST-1 was then removed and the cells were lysed for the luciferase assay to be performed.

Results

HSP70 Forms a Complex with PepFect14

The binding of PepFect14 to HSP70 was assessed using a tryptophan fluorescence extinction assay. Tryptophan is fluorescent amino acids that absorbs and emits in the ultraviolet range of energy. The intensity of its fluorescence is highly correlated to its direct environment (Ghisaidoobe and Chung 2014). Thus, measuring the extinction of tryptophan fluorescence of a protein in presence of another compound gives information on the direct neighbouring of the residues (Vivian and Callis 2001). Indeed when a molecule is complexed to a protein, it results in a refolding of the structure that, in turns, changes the environment of the tryptophan residues and the intensity of the protein intrinsic fluorescence. This method allows the identification of protein binding using intrinsic protein properties. It avoids using additional fluorescent probes that would most likely already induce changes in the protein structure and thus is a straightforward and efficient technique to observe a non-covalent binding to a protein (Sindrewicz et al. 2019).

We prepared HSP70 samples (50 nM) mixed with a dilution series of PepFect14 (from 1µM to 1 nM) and recorded the changes in tryptophan fluorescence intensity. The data were transformed using Eq. 1 to obtain the percentage of extinction and the plot displayed in Fig. 1 A clearly showed a PepFect14 dose dependent extinction of HSP70 intrinsic tryptophan fluorescence. This extinction indicated a non-covalent interaction between our peptide and the protein. The curve was fitted with a hyperbolic regression in order to calculate the strength of the binding. The apparent Kd from this binding curve was calculated to be 34.98 nM and a maximum binding was observed at molar ratio 3. The complexes formed by PepFect14 and HSP70 will be noted PF14:HSP70 in the rest of this study.

Dynamic light scattering (DLS) revealed another feature of the interactions between PepFect14 and HSP70 (Fig. 1B). A solution containing the complexes was diluted in DMEM + 10% FBS as well as control solutions of HSP70 and PepFect14 alone. The media supplemented with 10% FBS presented two peaks that were due to the serum. Indeed FBS contains a large amount of proteins such as albumins and growth factor that tend to aggregate into nanoparticles. The sample with HSP70 alone showed a similar particle size distribution to the media alone. The reading could not be differentiated from the background indicating that there were no nanoparticle with different size than in FBS formed in this solution. In the case of PepFect14, no new peaks could be detected but a broadening of the second FBS peak was observed. PepFect14 alone might form a particle in the conditions of our experiment but, then, it was also hidden by the FBS background. The sample containing the complexes PF14:HSP70 presented a clear new peak at 712.4 nm. The differences between the size repartition of the complexes and its component alone was an additional proof of the interaction between PepFect14 and HSP70. Furthermore, as usually observed when compounds form a complex with PepFect14, PF14:HSP70 formed nanoparticles. This is of major importance as the ability to form a nanoparticle has been demonstrated to be a key point in transfections mediated by PepFect14 (Lehto et al. 2017).

PepFect14 Mediates HSP70 Transfection

The tryptophan extinction and the DLS results demonstrated that PepFect14 was able to bind to HSP70 and to form a stable complex. It is already known that PepFect14 is able to deliver oligonucleotides when it forms a complex with them. In order to verify that the same properties were kept intact when binding to HSP70, the intracellular uptake was monitored by epi-fluorescent microscopy. HSP70 was labelled with Alexa Fluor 568, before complexation with PepFect14 at molar ratio 3, 5 and 10 and addition to BHM pLuc cells for 1 or 3 h. The cell nuclei were stained using Hoechst 33,258. Control wells where cells were treated by HSP70-Alexa 568 alone were also prepared and imaged. The complexes formed at molar ratio 10 exhibited the best results and the images and corresponding surface plots are displayed in Fig. 2. The cells treated with PF14:HSP70 complexes presented an intense distribution of HSP70-Alexa 568 in their cytoplasm already after 1 h treatment and the red dots repartition became even more intense after 3 h
proliferation and influence our results. A WST-1 assay was performed on the cells. This assay allowed us to monitor the cell proliferation without interfering with the treatment. All our treatment induced a slight decrease in the cell proliferation from around 20% for PF14:HSP70 and Lipofectamine 2000 to 30% for the combined treatment. PF14 alone was not an exception, showing 10% reduction of the cell proliferation (Fig. 3).

On the same plate, the luciferase activity was then measured. The knockdown was expected to increase the amount of misfolded and thus inactive luciferase. Remarkably, The knockdown of HSPA1A/B did not induce a lower luciferase activity as expected but instead an increase by almost 3-fold compared to untreated cells was observed (Fig. 4 A). However, in the cells that were treated with both Lipofectamine 2000 loaded with siRNA against HSPA1A/B and PF14:HSP70, this increase was significantly reduced to only 1.3 fold. When more controls were performed it was noticed that the increase in luciferase activity could be induce by lipofectamine 2000 alone and strangely not with lipofectamine 2000 loaded with a nonsense siRNA (Fig. 4B).

The higher luciferase activity could be due to two different causes. Indeed, in the luciferase assay, an increase means a higher quantity of active luciferase in the assay mix. The fact that lipofectamine did not increase the cell growth proved that the increase in luciferase activity by 270% was not caused by a higher number of cells but a higher production of active luciferase. The restoration to 30% above controls achieved by the complex PF14:HSP70 could not be linked to the 30% decrease in cell population compared to untreated cells and was then due to an intracellular effect of the treatment.

Discussion

The tryptophan fluorescence extinction showed a change in the close environment of the protein indicating a non-covalent binding between PepFect14 and HSP70 as suggested by the results exposed in Dowaidar and al26. This binding is further confirmed by dynamic light scattering where a nanoparticle formed by aggregation of the complexes could be detected with an hydrodynamic diameter around 700 nm. Furthermore, the fact that PF14:HSP70 formed nanoparticles, while HSP70 alone did not, indicated that PepFect14 formed a coat around HSP70 that induced the aggregation of the complexes via hydrophobic interactions. This conclusion is further strengthened by the use of molar ratio 10 in the formation of the complex while the maximum extinction of tryptophan fluorescence was already reached at molar ratio 3. The ability to be bound by PepFect14 is of major interest as the first step for transfection using this
against HSPA1A/B would induce an increased amount of misfolded and unfunctional luciferase that would be monitored as a decrease in the luciferase activity. Furthermore, it was reported in Li et al. (Li et al. 2019) that Lipofectamine 2000 had a direct effect on endoplasmic reticulum unfolded protein response (ER-UPR). This intrinsic effect of Lipofectamine 2000 would then stimulate the production of misfolded luciferase while silencing HSPA1A/B to prevent a self-recovery of the cells. The fact that the siRNA treatment with lipofectamine 2000 induced an increase in the luciferase activity came as a surprise. The cell proliferation assay showed us that the increase was caused by a higher production of luciferase and not by a larger cell population. Furthermore lipofectamine 2000 alone also achieved the same result indicating that the observed effect was not due to a HSPA1A/B knockdown but to an intrinsic effect of lipofectamine. It has been reported that Lipofectamine 2000 treatments had the ability to influence certain gene promoters and modulate global gene expression patterns (Fiszer-Kierzkowska et al. 2011). Notably, in Fiszer-Kierzkowska et al., Lipofectamine 2000 transfections, besides inducing a cellular stress, could influence gene promoters and could trigger the expression of proteins (Fiszer-Kierzkowska et al. 2011). We hypothesized that the promoter of the luciferase reporter gene was activated, directly or indirectly as a response to Lipofectamine 2000 and this activation induced an increase amount of newly produced luciferase. The mechanism behind this increase in luciferase production by cells treated by Lipofectamine 2000 is still unclear but,

cell-penetrating peptide is to form a non-covalent complex that can aggregate into nanoparticles (Lehto et al. 2017). To verify that the complexes formed by PepFect14 and HSP70 could be translocated through the plasma membrane, HSP70 was labelled with a fluorophore and its uptake was monitored by epifluorescence microscopy. While a small amount of non-complexed HSP70 could be seen inside the cytoplasm after 1 or 3 h treatment, a significantly higher amount was detected when the cells were treated with PF14:HSP70. It is already known that HSP70 has the ability to get tethered to the plasma membrane from the intracellular side and even to translocate, to some extent, to the extracellular environment (Calderwood et al. 2016; Vega et al. 2008). This ability is usually observed from the inside of the cell to the extracellular environment and is thought to be mediated by exosomes (Calderwood et al. 2016). The uptake of HSP70 inside cells is yet not reported in the literature to our knowledge. Nevertheless, PepFect14 clearly increased the efficacy of the treatment and allowed a larger amount of protein through the plasma membrane. These results, when taken together, showed that besides being able to form a complex with HSP70, PepFect14 mediated the translocation of HSP70 through the plasma membrane to the cytoplasm. A last question raised here is whether HSP70 could be released in the cytoplasm and available for a biological effect or trapped in endosomes and sequestered from signalling pathways. In the luciferase activity recovery assay, the biological activity of the transfected HSP70 was assessed. We expected that the transfection of a siRNA

Fig. 3 Cell proliferation as measured in the WST-1 assay. The graph present the mean ± SEM of 3 technical replicates.
more interestingly, the cells that were further treated with PF14:HSP70 presented a restoration of the luciferase activity almost to untreated level without causing any significant toxic effect besides Lipofectamine toxicity. This restoration clearly indicated an effect of PF14:HSP70 at the intracellular level and proved once more the uptake of our complexes. Furthermore, the detection of a biological effect led us to the conclusion that HSP70 was released in the cytoplasm and that its active sites were not impeached by PepFect14 coating. Thus, our treatment induced a intracellular biological activity that led to the restoration of a cell process. The exact mechanism of recovery is most likely quite linked to the mechanism of action of Lipofectamine 2000 on the luciferase expression and, thus is still unclear. We hypothesized that the HSP70 degradation pathways were involved. Indeed, HSP70 acts as a chaperone protein that can direct proteins toward several signalling pathways with various finality (Clerico et al. 2015; Deniset and Pierce 2015; Mayer 2015).
In this study, we have experimentally demonstrated that the cell-penetrating peptide PepFect14 transfected the HSP70 protein. First, we demonstrated that PepFect14 was able to non-covalently bind to HSP70 and to form a stable complex that aggregated in nanoparticles. The complexes were then showed to penetrate the plasma membrane and could be detected in the cytoplasm of cells. Finally, we showed that HSP70 in the cytoplasm was able to achieve a protective function by restoring a balance in the proteome after Lipofectamine 2000 induced stress, either through degradative pathways or transcription regulation pathway. These opens a new field of opportunity for PepFect14. Indeed, besides having proved to be able to deliver various sorts of natural or modified oligonucleotides, PepFect14 is now described for the first time as a protein delivery agent. It is also the first occurrence in the literature of a successful delivery of HSP70, to our knowledge. HSP70 is a major protective protein in cells and its transfection could lead to new pharmacologic methods. One of HSP70 role is to protect other proteins and peptide from forming aggregates. Aggregates are involved in many neurodegenerative diseases. In Alzheimer disease, amyloid β are wrongly processed and aggregates before forming plaques intracellularly and extracellularly. The formation of these plaques leads to neuronal degeneration (Kocahan and Doğan 2017). In Parkinson disease, α-synuclein forms aggregates as well and the aggregation leads to the death of dopaminergic neurons (Dauer and Przedborski 2003). Delivering HSP70 into affected neurons could offer a extra-protection against these peptide aggregations. Furthermore, the delivery of proteins has more straight forward, less aggressive and less permanent features than oligonucleotides transfection. While oligonucleotides transfection can present advantages, protein transfection allows a better modulation of the treatment and does not rely on intracellular transcription machinery. All the results presented in this study makes the transfection of HSP70 mediated by PepFect14 a major advance in the drug delivery field.

Acknowledgements This study was funded through the Swedish Research Council (VR-NT 2017–03691 to ÜL).

Author Contribution MG and ÜL supervised the study, MG designed the experiments, participated in the experiment, analysed the results and wrote the manuscript, LF participated in the experiment and analysis of the result, CPC provided the peptides and participated in the discussion, MC and CA provided the purified protein and participated in the writing of the methods.

Funding Open access funding provided by Stockholm University.

Statements and Declarations None.

Competing Interests None.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

Andréasson C, Fiaux J, Rampelt H, Mayer MP, Bukau B (2008) Hsp110 is a nucleotide-activated exchange factor for Hsp70. J Biol Chem 283:8877–8884. https://doi.org/10.1074/jbc.M710063200

Bechara C, Sagan S (2013) Cell-penetrating peptides: 20 years later, where do we stand? FEBS Lett 587:1693–1702

Bukau B, Deuerling E, Pfund C, Craig EA (2000) Getting newly synthesized proteins into shape. Cell 101:119–122. https://doi.org/10.1016/s0092-8674(00)80806-5

Calderwood SK, Gong J, Murshid A (2016) Extracellular HSPs: The Complicated Roles of Extracellular HSPs in Immunity. Front Immunol 7:159. https://doi.org/10.3389/fimmu.2016.00159

Campanella C, Pace A, Caruso Bavisotto C, Marzullo P, Marino Gamazzia A, Buscemi S, Palumbo Piccionello A (2018) Heat Shock
Holmberg MA, Gowda NKC, Andréasson C (2014) A versatile bacterial expression vector designed for single-step cloning of multiple DNA fragments using homologous recombination. Protein Expr Purif 98:38–45. doi: https://doi.org/10.1016/j.pep.2014.03.002

Hageman J, Kampinga HH (2009) Computational analysis of the human HSPH/HSPA/DNAJ family and cloning of a human HSPH/HSPA/DNAJ expression library. Cell Stress Chaperones 14(1):1–21. doi: https://doi.org/10.1007/s12192-008-0060-2

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.