CLAIMS

1. A method for transducing a molecule of interest into a cell, wherein the method comprises contacting said cell with the molecule of interest and a transduction buffer, wherein the transduction buffer comprises (i) a transduction compound, (ii) a salt or an activator/enhancer of a sodium-hydrogen transporter, and preferably (iii) an osmoprotectant.

2. The method of claim 1 wherein the transduction buffer comprises (i) a transduction compound, (ii) a salt, and preferably (iii) an osmoprotectant.

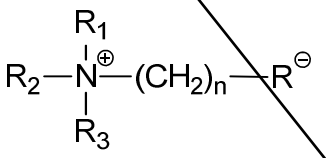
3. The method of claim 2, wherein the transduction buffer comprises (i) a transduction compound, (ii) a salt, and (iii) an osmoprotectant.

4. The method of any one of claims 1 to 3, wherein the salt is a sodium, lithium, potassium, caesium or rubidium salt.

5. The method of any one of claims 1 to 4, wherein the salt is sodium chloride.

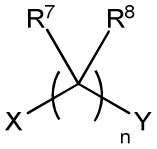
6. The method of any one of claims 1 to 5, wherein the transduction compound

a. has the general formula:



wherein R_1 is methyl, ethyl, propyl, butyl, pentyl, hexyl or their derivatives;
R_2 is methyl, ethyl, propyl, butyl, pentyl, hexyl or their derivatives;
R_3 is methyl, ethyl, propyl, butyl, pentyl, hexyl or their derivatives;
R^- is SO_3^- or COO^- or POO^- and/or
n is 1-6, *i.e.* 1, 2, 3, 4, 5 or 6; or

b. has the general formula:



(I)

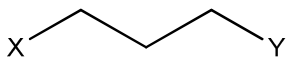
wherein:

X is selected from NR^1R^2, $NR^1R^2R^3$+, OH and $COOR^4$;
Y is selected from SO_3H, SO_3^-, COO^-, $CONH_2$, $COOR^{12}$, $CONR^5R^6$, tetrazole, OH, $NR^{10}R^{11}$, and H;
n is 1, 2, 3, 4, 5 or 6;

R^1, R^2 and R^3, are each independently selected from H, C1-6 alkyl, C5-10 aryl, C6-15 aralkyl, COR^9; C1-6 alkyl, C5-10 aryl and C6-15 aralkyl may optionally be substituted with R^Y, OH or COOH;
or R^1 and R^2 may come together with the nitrogen to which they are attached to form heterocyclyl;
or when X is $NR^1R^2R^3$+, R^3 may be absent and R^1 and R^2 may come together with the nitrogen to which they are attached to form heteroaryl;
R^4, R^5, R^6, R^9, R^{10}, R^{11}, R^{12} are independently selected from H and C1-6 alkyl;
R^7 and R^8 are independently selected from H, C1-6 alkyl and OH; or R^7 may come together with R^1 to form heterocyclyl;
heterocyclyl is a monocyclic ring which is saturated or partially unsaturated, containing where possible 1 or 2 ring members independently selected from N, NR^{13}, $NR^{13}R^{14}$+ and O, and 2 to 5 carbon atoms; heterocyclyl may optionally be substituted with C1-C6 alkyl, C1-C6 carboxylic acid or C1-C6 alkyl substituted with R^Y;
heteroaryl is a 5 or 6 membered aromatic ring containing, where possible, 1, 2 or 3 ring members independently selected from N, NR^{13}, $NR^{13}R^{14}$+ and O; heteroaryl may optionally be substituted with C1-C6 alkyl, C1-C6 carboxylic acid or C1-C6 alkyl substituted with R^Y;
R^{13} and R^{14} are independently selected from H, C1-6 alkyl, C1-C6 carboxylic acid and C1-C6 alkyl substituted with R^Y;
alkyl is a linear or branched saturated hydrocarbon;
R^Y is selected from SO_3H, SO_3^-, COO^-, $CONH_2$, $COOR^{12}$, $CONR^5R^6$, tetrazole, OH and $NR^{10}R^{11}$;
C1-6 carboxylic acid means –COOH or a C1-5 alkyl chain substituted with COOH
and tautomers, solvates, zwitterions and salts thereof.

7. The method of any one of claims 1 to 6, wherein the transduction compound is a non-detergent betaine (NDB) or a derivative thereof.

8. The method of claim 7, wherein the transduction compound has the general formula:

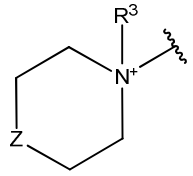


(II)

wherein
X is selected from NR^1R^2 and $NR^1R^2R^3$+;
Y is selected from SO_3H, SO_3^-, COO^-, $CONH_2$, $COOR^{12}$ and $CONR^5R^6$;
R^1, R^2 and R^3, are each independently selected from H and C1-6 alkyl which may optionally be substituted with OH or COOH; or R^1 and R^2 may come together with the nitrogen to which they are attached to form heterocyclyl, preferably piperidine, piperazine or morpholine, which may be optionally substituted; or when X is $NR^1R^2R^3$+, R^3 may be absent and R^1 and R^2 may come together with the nitrogen to which they are attached to form heteroaryl, preferably pyridyl, which may be optionally substituted;
and all other groups are as defined in formula I above.

9. The method of any one of claims 6 to 8 wherein the transduction compound contains a quaternary nitrogen group and wherein the quaternary nitrogen is part of an aliphatic or aromatic ring structure.

10. The method of any one of claims 6 to 9 the transduction compound is a compound of formula I or II wherein X is



(a) ;

Z is selected from $C(R^{15})_2$, NR^{13}, $NR^{13}R^{14}$+ and O;
each R^{15} is independently selected from H, C1-6 alkyl, C1-C6 carboxylic acid and C1-C6 alkyl substituted with R^Y;
R^3 is selected from H, C1-6 alkyl, C5-10 aryl, C6-15 aralkyl, COR^9; C1-6 alkyl, C5-10 aryl and C6-15 aralkyl may optionally be substituted with R^Y, OH or COOH. Preferably R_3 is $–CH_3$;
R^{13} and R^{14} are independently selected from H, C1-6 alkyl, C1-C6 carboxylic acid and C1-C6 alkyl substituted with R^Y.

11. The method of any one of the preceding claims, wherein the transduction compound is selected from the compounds in Table 1.

12. The method of any one of the preceding claims, wherein the transduction compound is any compound that has 30% or more transduction efficiency compared to reference compound #1 in Table 1.

13. The method of any one of the preceding claims, wherein the osmoprotectant is glycine and/or glycerol.

14. The method of any one of the preceding claims, wherein the transduction buffer further comprises a biological pH buffer, a viscosity enhancer and/or a growth factor.

15. The method of claim 14, wherein the biological pH buffer is PBS, TES or HEPES.

16. The method of claim 14 or claim 15, wherein the viscosity enhancer is polyvinylpyrrolidone (PVP).

17. The method of any one of claims 14 to 16, wherein the growth factor is selected from EGF, FGF, HGF, BDNF, PDGF, VEGF or IGF, or is any combination thereof.

18. The method of any one of the preceding claims, wherein the osmolality of the transduction buffer is in the range of about 300 mOsm/kg to about 1000 mOsm/kg, or about 300 mOsm/kg to about 2000 mOsm/kg or about 300 mOsm/kg to about 3000 mOsm/kg.

19. The method of any one of the preceding claims, wherein the transduction compound is at a concentration of between about 0.1 mM and about 500 mM.

20. The method of any one of the preceding claims, wherein the osmoprotectant is at a concentration of between about 5 and about 500 mM.

21. The method of any one of the preceding claims, wherein the molecule of interest is a peptide, protein, nucleic acid, polysaccharide, small molecule, vesicle, nanoparticle, virus or a single-celled organism.

22. The method of any one of the preceding claims, wherein the molecule of interest is between about 30 and about 200 kDa, or between about 1 and about 400 kDa.

23. The method of any one of the preceding claims, wherein the molecule of interest is an enzyme for modifying nucleic acid, for example an endonuclease or a nuclease, for example selected from zinc finger nucleases, TALENS, Cas9, Cas9 analogs, DNA-targeted FokI-nuclease-associated proteins, Cascade, TtAgo or Argonaute proteins or their derivatives.

24. The method of any one of the preceding claims, wherein the cell is a mammalian cell, such as a human cell.

25. The method of any one of the preceding claims, wherein the cell is a stem cell, such as an embryonic stem cell or a neural stem cell, or a differentiated cell, such as a neuron or a dendritic cell.

26. The method of any one of the preceding claims, wherein the cell is a primary cell.

27. The method of any one of the preceding claims, wherein the cell is contacted with two, three, four or more molecules of interest.

28. The method of any one of the preceding claims, wherein the method is performed *in vitro*.

29. The method of any one of the preceding claims, wherein the method is performed *in vivo*.

30. The method of any one of the preceding claims, wherein the transduction is by macropinocytosis.

31. A cell or population of cells transduced by the method of any one of claims 1 to 30.

32. A transduction buffer as recited in any one of claims 1 to 30 for use in a method of any one of claims 1 to 30.

33. The transduction buffer of claim 32, wherein the transduction buffer further comprises a molecule of interest for transduction.

34. Use of a transduction buffer according to claim 32 or claim 33, in a method for transducing a molecule of interest into a cell, wherein the method is according to any one of claims 1 to 30.

35. A pharmaceutical composition comprising the transduction buffer of claim 32 or claim 33.

36. The transduction buffer according to claim 32 or claim 33, or the pharmaceutical composition of claim 35, for use in therapy or diagnosis.

37. Use of an osmoprotectant, optionally glycine and/or glycerol, in a method for transducing a molecule of interest into a cell, for example, wherein the method is according to any one of claims 1 to 30.

38. Use of a transduction compound selected from Table 1 for transducing a molecule of interest into a cell for example, wherein the method is according to any one of claims 1 to 30.

39. A compound selected from compounds #10, #11, #16, #42, #34, #41, #40, #39, #33, #15, #11, #36 and #46 in Table 1.

40. A method for modifying a nucleic acid, such as a genetic sequence, in a cell, wherein the method comprises contacting said cell with a protein capable of modifying a nucleic acid and a transduction buffer, wherein the transduction buffer comprises (i) a transduction compound, (ii) a salt or an activator/enhancer of a sodium-hydrogen transporter, and preferably (iii) an osmoprotectant.

41. The method of claim 40, wherein the protein capable of modifying a nucleic acid is inherently targeted to a specific target sequence, for example wherein the protein is a zinc finger nucleaseor a TALEN, Cas9, a Cas9 analog, a DNA-targeted FokI-nuclease-associated protein, a Cascade complex, a TtAgo protein or other Argonaute protein or their derivatives.

42. The method of claim 40, wherein the cell is further contacted with a guide molecule to direct the protein to a target genetic sequence.

43. The method of any one of claims 40 to 42, wherein the osmolality is adjusted to between about 1000 mOsm/kg and about 1500 mOsm/kg, preferably about 1250 mOsm/kg.

44. The method of any one of claims 40 to 43, wherein the transduction compound is used at a concentration of about 200 nM to about 400 nM, preferably about 250 mM.

45. The method of any one of claims 40 to 44, wherein the transduction buffer further comprises an inhibitor of the interferon response pathway, for example, B18R.

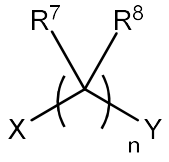
46. The method of any one of claims 40 to 45, wherein the method further comprises isolating or using the modified cell.

47. A modified cell obtainable by the method of any one of claims 40 to 46.

CLAIMS

1. An *in vitro* method for transducing a protein, a nucleic acid or a combination thereof into a cell, wherein the method comprises contacting said cell with the protein, a nucleic acid or a combination thereof and a transduction buffer, wherein the transduction buffer comprises (i) a transduction compound, (ii) a salt selected from sodium, lithium, potassium, caesium or rubidium salt; wherein the transduction compound

a) has the general formula:



(I)

wherein:

X is selected from NR^1R^2, $NR^1R^2R^3$+, OH and $COOR^4$;

Y is selected from SO_3H, SO_3^-, COO^-, $CONH_2$, $COOR^{12}$, $CONR^5R^6$, tetrazole, OH, $NR^{10}R^{11}$, and H;

n is 3, 4, 5 or 6;

R^1, R^2 and R^3, are each independently selected from H, C1-6 alkyl, C5-10 aryl, C6-15 aralkyl, COR^9; C1-6 alkyl, C5-10 aryl and C6-15 aralkyl may optionally be substituted with R^Y, OH or COOH;

or R^1 and R^2 may come together with the nitrogen to which they are attached to form heterocyclyl;

or when X is $NR^1R^2R^3$+, R^3 may be absent and R^1 and R^2 may come together with the nitrogen to which they are attached to form heteroaryl;

R^4, R^5, R^6, R^9, R^{10}, R^{11}, R^{12} are independently selected from H and C1-6 alkyl;

R^7 and R^8 are independently selected from H, C1-6 alkyl and OH;

heterocyclyl is a monocyclic ring which is saturated or partially unsaturated, containing where possible 1 or 2 ring members independently selected from N, NR^{13}, $NR^{13}R^{14}$+ and O, and 2 to 5 carbon atoms; heterocyclyl may optionally be substituted with C1-C6 alkyl, C1-C6 carboxylic acid or C1-C6 alkyl substituted with R^Y;

heteroaryl is a 5 or 6 membered aromatic ring containing, where possible, 1, 2 or 3 ring members independently selected from N, NR^{13}, $NR^{13}R^{14}$+ and O; heteroaryl may optionally be substituted with C1-C6 alkyl, C1-C6 carboxylic acid or C1-C6 alkyl substituted with R^Y;

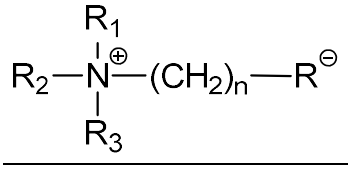
R^{13} and R^{14} are independently selected from H, C1-6 alkyl, C1-C6 carboxylic acid and C1-C6 alkyl substituted with R^Y;

alkyl is a linear or branched saturated hydrocarbon;

R^Y is selected from SO_3H, SO_3^-, COO^-, $CONH_2$, $COOR^{12}$, $CONR^5R^6$, tetrazole, OH and $NR^{10}R^{11}$;

C1-6 carboxylic acid means –COOH or a C1-5 alkyl chain substituted with COOH and tautomers, solvates, zwitterions and salts thereof; or

b) has the general formula:



wherein R_1 is methyl, ethyl, propyl, butyl, pentyl, hexyl;

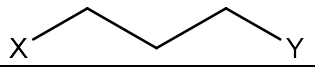
R_2 is methyl, ethyl, propyl, butyl, pentyl, hexyl;

R_3 is methyl, ethyl, propyl, butyl, pentyl, hexyl;

R^- is SO_3^- or COO^- or POO^- and

n is 3-6, *i.e.* 3, 4, 5 or 6.

2. The *in vitro* method of claim 1 wherein the transduction buffer wherein the transduction compound has the general formula:

 (II)

wherein

X is selected from NR^1R^2 and $NR^1R^2R^3+$;

Y is selected from SO_3H, SO_3-, COO-, $CONH_2$, $COOR^{12}$ and $CONR^5R^6$;

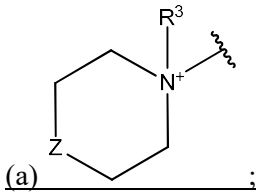
R^1, R^2 and R^3, are each independently selected from H and C1-6 alkyl which may optionally be substituted with OH or COOH; or R1 and R2 may come together with the nitrogen to which they are attached to form heterocyclyl, preferably piperidine, piperazine or morpholine, which may be optionally substituted; or when X is $NR^1R^2R^3+$, R^3 may be absent and R^1 and R^2 may come together with the nitrogen to which they are attached to form heteroaryl, preferably pyridyl, which may be optionally substituted;

and all other groups are as defined in formula I above.

3. The *in vitro* method of claim 1 or 2, wherein the transduction compound:

(a) contains a quaternary nitrogen group and wherein the quaternary nitrogen is part of an aliphatic or aromatic ring structure;

(b) is a compound of formula I or II wherein X is



(a) ;

Z is selected from $C(R^{15})_2$, NR^{13}, $NR^{13}R^{14}$+ and O;

each R^{15} is independently selected from H, C1-6 alkyl, C1-C6 carboxylic acid and C1-C6 alkyl substituted with R^Y;

R^3 is selected from H, C1-6 alkyl, C5-10 aryl, C6-15 aralkyl, COR^9; C1-6 alkyl, C5-10 aryl and C6-15 aralkyl may optionally be substituted with R^Y, OH or COOH, preferably R_3 is $–CH_3$;

R^{13} and R^{14} are independently selected from H, C1-6 alkyl, C1-C6 carboxylic acid and C1-C6 alkyl substituted with R^Y and/or

(c) is selected from compounds 01-06, 08, 10-12, 14-17, 20-31 and 34-46 in Table 1.

4. The *in vitro* method of any one of the preceding claims, wherein the transduction buffer further comprises and osmoprotectant selected from glycine, histidine, alanine, isoleucine, arginine, asparagine, leucine, aspartic acid, lysine, glutami acid, cysteine, methionine, phenylalanine, glutamine, threonine, tryptophan, proline, valine, orninthine, selenocysteine, serine, tyrosine and proline.

5. The *in vitro* method of any one of the preceding claims, wherein:

the transduction buffer further comprises an osmoprotectant selected from glycine and/or glycerol;

the osmoprotectant is at a concentration of between about 5 and about 500 mM;

the transduction buffer further comprises an osmoprotectant at a concnetraiotn of between 5 and about 500mM;

the transduction buffer has an osmolality of at least 300 mOsm/kg;

the transduction compound is at a concentration of between about 0.1 mM and about 500 mM; and/or

wherein the salt is sodium chloride.

6. The *in vitro* method of any one of the proceeding claims, wherein the transduction buffer further comprises: a biological pH buffer such as PBS, TES or HEPES; a viscosity enhancer such as polyvinylpyrrolidone (PVP); an inhibitor of the interferon response pathway; and/or a growth factor, for example selected from EGF, FGF, HGF, BDNF, PDGF, VEGF or IGF, or is any combination thereof.

7. The *in vitro* method of any one of the preceding claims, wherein the transduction buffer comprises NDSB-201 as a transduction compound, and preferably further comprises GABA as a transduction compound.

8. The *in vitro* method of any one of the preceding claims, wherein the transduction buffer comprises: (i) NDSB-201 and GABA as transduction compounds; (ii) sodium chloride as the salt; and preferably (iii) glycine and/or glycerol as the osmoprotectant; and wherein the osmolality of the transduction buffer is at least 300 mOsm/kg.

9. The *in vitro* method of any one of the proceeding claims, wherein the transduction buffer further comprises an activator/enhancer of a sodium-hydrogen transporter, wherein said activator/enhancer is a growth factor.

10. A transduction buffer as recited in any one of claims 1 to 9, wherein the osmolality of the transduction buffer is at least 650mOsm/kg.

11. A pharmaceutical composition comprising the transduction buffer of claim 10.