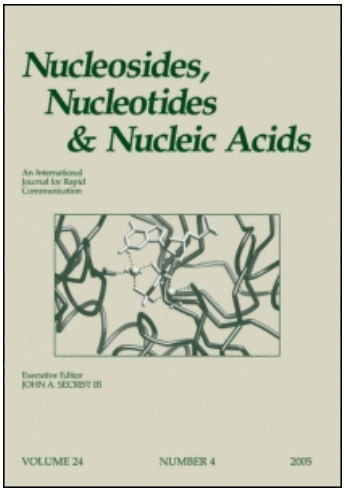

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Molecular Engine for Transporting Drugs Across Cell Membranes

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MOLECULAR ENGINE FOR TRANSPORTING DRUGS ACROSS CELL MEMBRANES

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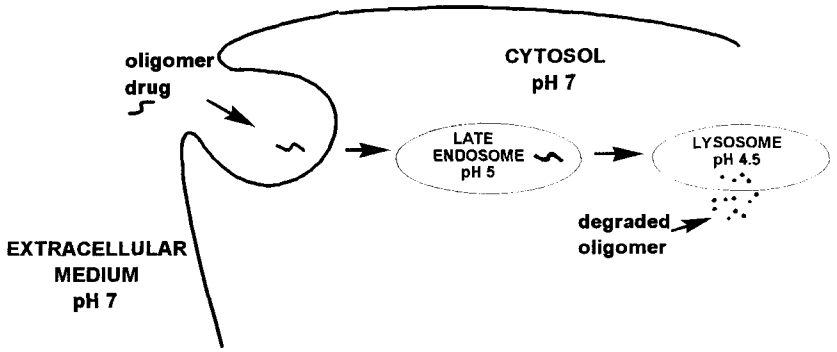
ABSTRACT: A molecular engine has been developed from first principles to transport drugs from endosomes to the cytosol of cells. The engine is powered by the pH differential across the endosomal membrane, does not disrupt the endosomal membrane, and is disassembled into innocuous components after carrying out its transport function.

THE PROBLEM: A growing body of evidence suggests that oligomeric drugs, such as now coming out of antisense and combinatorial drug development programs, enter animal cells solely via endocytosis and are subsequently sequestered or degraded in lysosomes, with little or no intact drug entering the cytosol/nuclear compartment, shown below.

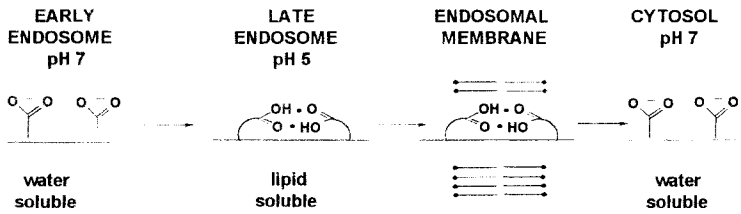
CELL ENTRY OF OLIGOMERIC DRUGS



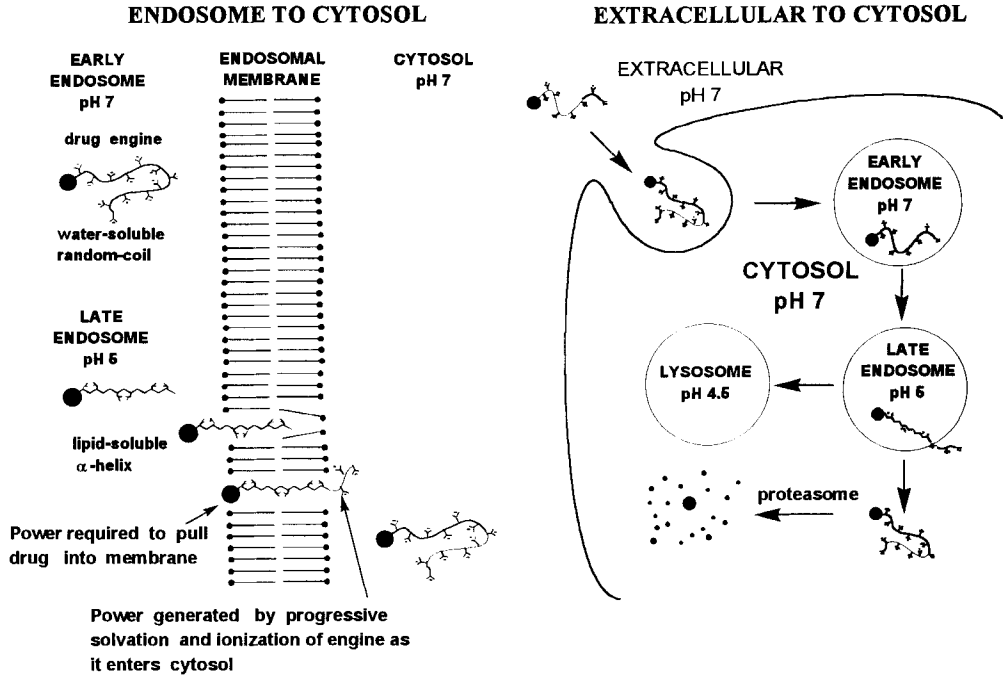
Since the targets for many such drugs reside in the cytosol/nuclear compartment of cells a major challenge in drug development is how to transport oligomeric and other delivery-limited drugs into the cytosol of cells in a manner suitable for therapeutic applications.

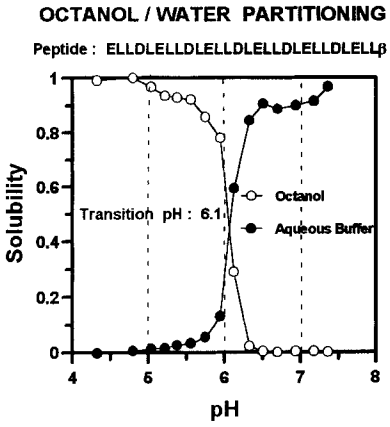
THE SOLUTION: To meet this drug delivery challenge a unique molecular engine has been devised to actively transport delivery-limited drugs from endosomes into the cytosol of cells. The source of power for this transport is the pH differential between the late endosome and the cytosol, generated via ATP hydrolysis by proton pumps embedded in the endosomal membrane. To convert this pH differential into useful power for drug transport the engine undergoes reversible pH-mediated transitions between a water-soluble form and a lipid-soluble form. A key factor in achieving good lipid solubility is proper positioning of the weak acid moieties so as to form doubly-H-bonded dimers in low-pH conditions, which largely masks the polar character of the acid moieties. This basic mechanism is illustrated below.

pH-MEDIATED SOLUBILITY TRANSITION OF WEAK ACID MOIETIES



A peptide engine is particularly effective for drug transport via pH-mediated solubility transitions because at high pH properly-designed peptide engines exist in a water-soluble random-coil form wherein the weak acid moieties, selected from aspartic and glutamic acids, are fully ionized, and the amide moieties of the backbone are fully exposed and hydrated. On acidification the peptide engine undergoes a transition to its lipid-soluble α-helical form wherein its acid moieties are masked from the environment via H-bonded acid dimers, and the amide moieties of the backbone are shielded from the environment via H-bonding within the α-helix. Such a peptide engine achieves drug transport by the following mechanism. First, the drug-engine product is endocytosed, after which proton pumps embedded in the endosomal membrane acidify the endosome. When the pH is reduced sufficiently the engine converts from its high-pH water-soluble form to its low-pH lipid-soluble form, which then partitions from the aqueous endosomal compartment into the endosomal membrane. Because the engine in its α-helical form is designed to be longer than the membrane is thick (ie., > 36 A), continued entry of the engine into the membrane results in the end of the engine contacting the cytosol, whereupon the engine is actively drawn into the cytosol as it converts back to its high-pH water-soluble form. During its entry into the cytosol motive force for pulling the attached drug into and through the endosomal membrane is generated by ionization and solvation of the entering engine at the membrane/cytosol interface, as illustrated below.





A representative 29-amino acid peptide engine (44 A long in α-helical form) was assessed for its solubility properties as a function of pH. The figure to the right shows the pH-dependent partitioning of this engine between n-octanol and aqueous buffer. The sharp solubility transition seen in this figure indicates that the conversion between the water-soluble and the lipid-soluble forms is highly cooperative. The same engine, with a fluorescent tag, has been seen by fluorescence microscopy to enter cultured animal cells, wherein it exhibits diffuse fluorescence throughout the cell characteristic of the cytosol/nuclear compartment. To further confirm that the engine functions as designed, we have demonstrated that the engine rapidly passes from the extracellular medium directly into the cytosol when the cells are briefly exposed to acidic (pH 5.5) medium. In this experiment the low-pH medium emulates the acidic conditions within the late endosome. Engine transport from acidic medium directly into the cytosol demonstrates that a pH differential, irrespective of how it is generated, is sufficient for the engine to transport across a cell membrane.

Lipid layers, such as comprise cell membranes, can constitute a formidable barrier to delivery of polar and/or high molecular weight drugs. To achieve effective cytosolic delivery medicinal chemists typically adjust the molecular structure of a drug, or its prodrug form, to give a solubility compromise wherein the drug has less than ideal aqueous solubility in order to gain some lipid solubility, or less than ideal lipid solubility in order to gain some aqueous solubility. While this compromise approach has been successful for numerous low molecular weight drugs, it has been markedly unsuccessful for larger drugs. Our molecular engine now provides a valuable alternative wherein the engine imparts good aqueous solubility to a drug while in the extracellular compartment, while also providing effective transport across the endosomal membrane into the cytosol.

APPLICATIONS: Because of their unique properties we believe our molecular engines will provide improved pharmaceutical properties for a wide variety of drugs, including:

- Improved aqueous solubility for lipophilic drugs, such as Taxol.
- Improved endosome to cytosol transport of peptides (eg., Cyclosporin) and possibly antisense oligos - particularly non-ionic types such as PNAs and Morpholinos.
- Protection of oligomeric drugs from lysosomal degradation by virtue of transport out of endosomes prior to conversion of the endosome to a lysosome.
- Improved transport of drugs into cells of the brain by specialized engines designed to provide both transport across the blood/brain barrier and subsequent entry into the cytosol of cells of the brain.
- Engine-mediated selective delivery of highly cytotoxic drugs into bacteria living in an acidic environment - specifically H. pylori in the stomach.
- Transdermal delivery of lipophilic drugs, by virtue of the engine's ability to dramatically improve exit from the acidic extracellular lipid matrix into the underlying neutral aqueous compartment of the skin.