frequencies of 1–6 kHz and 12–25 kHz, thresholds between cells with distinct characteristic frequencies differ by 20–30 dB at a given frequency (Fig. 1b), thus providing a unique facility (for insects) for frequency discrimination in the auditory pathway.

Given the uniform design of the auditory system in cicadas[7], it is possible that the frequency-modulated songs of many cicada species, particularly tropical ones[9], may result from sensory drive[10], because females are able to use frequency components of songs as criteria for species recognition and mate choice.

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**Supplementary information** is available on *Nature*'s World-Wide Web site (http://www.nature.com) or as paper copy from the London editorial office of *Nature*.

Biotechniques

# Transfection of cells by immunoporation

Cell transfection is now a central technique in molecular biology and an essential prerequisite for gene therapy. Here we describe how beads coated with antibodies and bound to specific cell-surface transmembrane proteins can create holes in cells when the beads are removed, allowing transfection of the cells with DNA or other macromolecules. This unique targeted transfection of cells by immunoporation is very efficient and results in minimal cell death.

A variety of methods have been developed for the transfection of cells, including electroporation[1,2], lipofection[3,4], calcium phosphate coprecipitation[5,6] and DEAE dextran[7,8]. Of these methods, only electroporation offers the possibility of introducing DNA and other molecules such as proteins into viable cells. None of the current methods is able to target specific types of cells for transfection. In this new method

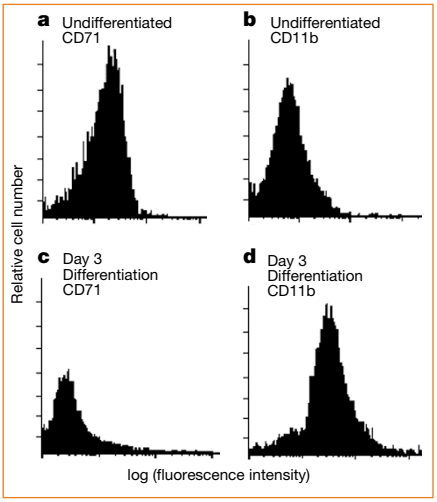


**Figure 1** Analysis of the transfection of HL-60 cells with pEGFP-C1 by measurement of the expression of green fluorescent protein using flow cytometry. **a,** HL-60 cells transfected using DYNAFECT-CD71 beads; **b,** HL-60 cells transfected using DYNAFECT-CD11b beads; **c,** DMSO-induced HL-60 cells transfected using DYNAFECT-CD71 beads; **d,** DMSO-induced HL-60 cells transfected using DYNAFECT-CD11b beads.

of cell transfection, antibody-coated beads are bound to specific surface antigens and then the beads are sheared off from the cell by mixing: this causes the formation of transient holes in the cell membrane through which macromolecules can enter.

Granulocytic, differentiating human lymphoblastic HL-60 cells normally express CD71 on their surfaces. When induced to differentiate in the presence of dimethyl sulphoxide (DMSO), the cells cease to express CD71 and instead express CD11b. We have used this cell line as a model system to investigate the process of cell transfection mediated by immunoporation.

DYNAFECT beads coated with either anti-CD11b antibody (DYNAFECT-CD11b) or anti-CD71 antibody (DYNAFECT-CD71) were mixed on a rotating end-over-end mixer at 33 r.p.m. for 6 h at 22 ∞ C with either uninduced HL-60 cells or cells that had been induced with DMSO for 3 days. For mixing in a 2-ml microcentrifuge tube, $10^7$ beads and $5 \times 10^5$ cells were suspended in 0.5 ml transfection medium (Dynal AS) containing 0.2 μg plasmid DNA vector pEGFP-C1 (4.7 kilobases) which codes for green fluorescent protein. After transfection, the beads were removed using a magnetic separator and the cells were transferred back into tissue-culture medium and cultured for a further 48 hours before analysis.

The extent of cell transfection was determined by flow cytometry. Figure 1 shows that the DYNAFECT-CD71 beads facilitate the transfection of DNA into normal HL-60 cells, but when the cells become differentiated and no longer express CD71, transfection no longer occurs with these beads. In contrast, mixing undifferentiated HL-60 cells with DYNAFECT-CD11b beads does not result in the transfection of the cells with DNA, but when the cells are differentiated and begin to express CD11b, those beads do bring about transfection.

Hence, immunoporation has the potential to target specific types of cell in a mixed population for transfection, depending on their immunological identity, and allow the targeted cells to take up a variety of different molecules. This also occurs in several mammalian cell lines with a range of different antibodies that target selected cell-surface antigens. In all cases, the level of transfection was 40–80%, depending on mixing conditions, and non-viable cells usually numbered less than 20%.

The high levels of selectivity and transfection, together with minimal cell death, that are achievable with immunoporation illustrate the enormous potential of this technique for use in a wide range of transfection studies. In particular, the ability to target specific subpopulations of cells will be extremely useful for many gene therapy applications.

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## Erratum

### Non-haemolytic β-amino-acid oligomers

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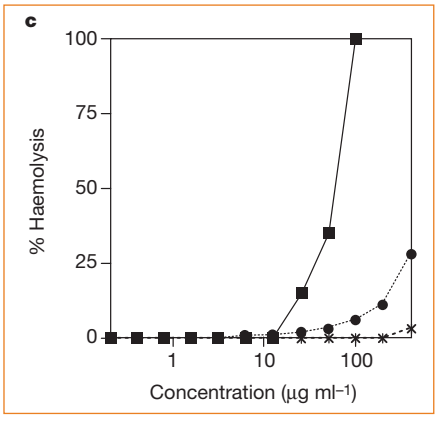
Some symbols representing haemolytic activity were absent or incorrect in Fig. 1c. The correct figure is shown here. Crosses, β-17; circles, magainin derivative; squares, melittin.



Antibiotics

# Non-haemolytic β-amino-acid oligomers

Pathogenic bacteria are becoming increasingly resistant to common antibiotics, stimulating an intensive search for new ones. Knowing that a class of medium-sized peptides (magainins[1]) are widely used by host organisms as a defence against microbial invasion[2], we developed a β-amino-acid oligomer (β-peptide) that mimics these natural antibiotics and tested it for antimicrobial activity. We find not only that the activity of our β-peptide is comparable to that of a magainin derivative but also that it is effective against four bacterial species, including two pathogens that are resistant to common antibiotics.

Natural peptide antibiotics are highly diverse in terms of size, sequence and conformation[2]; they are cationic and often adopt amphiphilic secondary structures. One common class, exemplified by the magainins[1], features 20- to 30-residue peptides that form amphiphilic α-helices (hydrophobic side chains on one side of the helix and cationic side chains on the other) which are attracted to the negatively charged surfaces of bacteria. These helices then somehow disrupt the bacterial membrane[3–5].

β-peptides are promising antimicrobial candidates because they offer a choice of secondary structures[6–8] and because the unnatural β-peptide backbone is resistant to protease degradation[9], in contrast to the α-amino-acid backbone of conventional peptides. Three distinct helical conformations have been identified among β-peptides, with the helix type being determined by the substitution pattern on the β-amino-acid residues[6–8]. Oligomers of (*R*,*R*)-*trans*-2-aminocyclopentanecarboxylic acid (ACPC) adopt a helix defined by a 12-membered ring formed as a result of hydrogen-bonding between each backbone carbonyl group and the amide proton of the third residue in the carboxy-terminal direction (a 12-helix)[10].

To test our amphiphilic versions of the β-peptide 12-helix for antimicrobial activity, we used (*3R,4S*)-*trans*-4-aminopyrrolidine-3-carboxylic acid (APC), together with ACPC, to prepare a β-17 oligomer (Fig. 1a). The APC residue should be cationic at pH 8 or below, by virtue of ring-nitrogen protonation. Because the 12-helix has about 2.5 residues per turn, the 12-helical conformation of β-17 should be amphiphilic, with all hydrophilic APC residues on one side of the helix and all hydrophobic ACPC residues on the other (Fig. 1b).

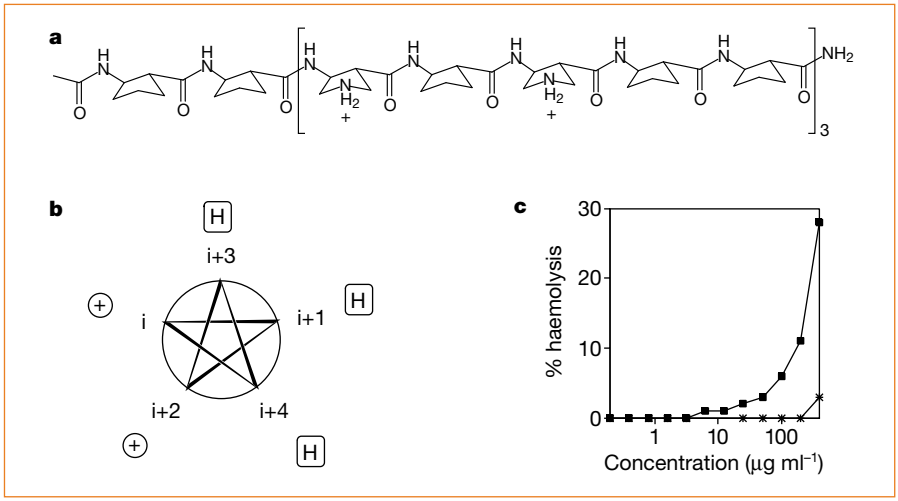



**Figure 1** Structure and haemolytic activity of β-17. **a,** Chemical structure of β-17. **b,** Axial projection of the β-peptide 12-helix, highlighting the ~5-residue repeat that results from there being approximately 2.5 residues per 12-helical turn. The repeating pentad in β-17, +H+HH (+, cationic APC residue; H, hydrophobic ACPC residue), is shown. **c,** Haemolytic activity of β-17 (squares), the magainin derivative (crosses), and melittin (circles). Human red blood cells (hRBC, 1% suspension in PBS buffer) were incubated at room temperature for 1 h with a twofold serial dilution of peptide in PBS buffer. Release of haemoglobin was determined from the absorbance at 415 nm of the supernatant after centrifugation. Controls, hRBC suspended in PBS (0% hydrolysis) or in 1% SDS (100% haemolysis).

We compared the activities of β-17 and the synthetic magainin derivative[11] GIGK-FLHAAKKFAKAFVAEIMNS-$NH_2$ against four bacteria (Table 1). The bacterium *Enterococcus faecium* A436 (which is vancomycin resistant) and *Staphylococcus aureus* 5332 (methicillin resistant) are clinical isolates, whereas *Bacillus subtilis* BR151 and *Escherichia coli* JM109 are non-pathogenic strains commonly used in the laboratory for genetic construction. We find that the activity of β-17 is comparable to that of the magainin against all four species of bacteria.

To be useful therapeutically, this antimicrobial action must be effective in the presence of human cells. We therefore tested the effect of our β-peptide on red blood cells, knowing that although magainins themselves are only weakly haemolytic (cause red blood cells to break open), other natural cationic helix-forming peptides, such as melittin[12], are strongly haemolytic . Figure 1c compares haemolysis by melittin (a positive control), the magainin derivative, and β-17: the β-peptide has even less haemolytic activity than the magainin. Another class of β-peptides composed of acyclic residues are also potent against *E. coli*, but these are highly haemolytic[13], limiting their therapeutic application.

We have devised a hetero-oligomer with an unnatural backbone that can be used to mimic a specific and useful biological activity displayed by a naturally defensive, medium-sized peptide. The chemical and conformational stability of β-peptides may lead to the creation of a new class of antimicrobial agents, which will add to their other unusual clinical applications[14].

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**Table 1 Bacteriostatic and bactericidal activities of β-17 and Ala8,13,18-magainin II amide**

| | | *E. coli* | *B. subtilis* | *E. faecium* | *S. aureus* |
|---|---|---|---|---|---|
| β-17 | MIC | 6.3 | 1.6 | 12.5 | 3.2 |
| | MBC | 6.3 | 3.2 | ~50* | ~12.5* |
| Magainin | MIC | 3.2 | 1.6 | 25 | 25 |
| | MBC | 3.2 | 3.2 | 100 | >100 |

Minimal inhibitory concentration (MIC, in μg ml$^{-1}$) is defined here as the lowest concentration of peptide required for complete inhibition of growth, as determined from the absorbance at 590 nm. Bacteria in BHI medium (~$10^6$ CFU ml$^{-1}$) were incubated for 6 h at 37 °C with a twofold-dilution series of peptide in medium in a sterile 96-well plate. Microbial growth was determined by the increase in $A_{590}$ over the 6-h period. Minimal bactericidal concentration (MBC, in μg ml$^{-1}$) refers to the lowest concentration of peptide required for the absence of viable colonies. The bactericidal activity of the peptide was measured at the MIC, MIC × 2 and MIC × 4. The well solution was diluted and plated to allow a maximum of ~$10^3$ colony-forming units to grow on the agar plate. Plates at zero time contained ~$10^3$ colonies; they were incubated overnight at 37 °C, and the colonies counted for determination of bactericidal activity.
*Peptide concentration of 100 μg ml$^{-1}$ revealed 10 colonies or less (~99% killed).

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