Supporting Information

Prokhorova et al. 10.1073/pnas.1715501114

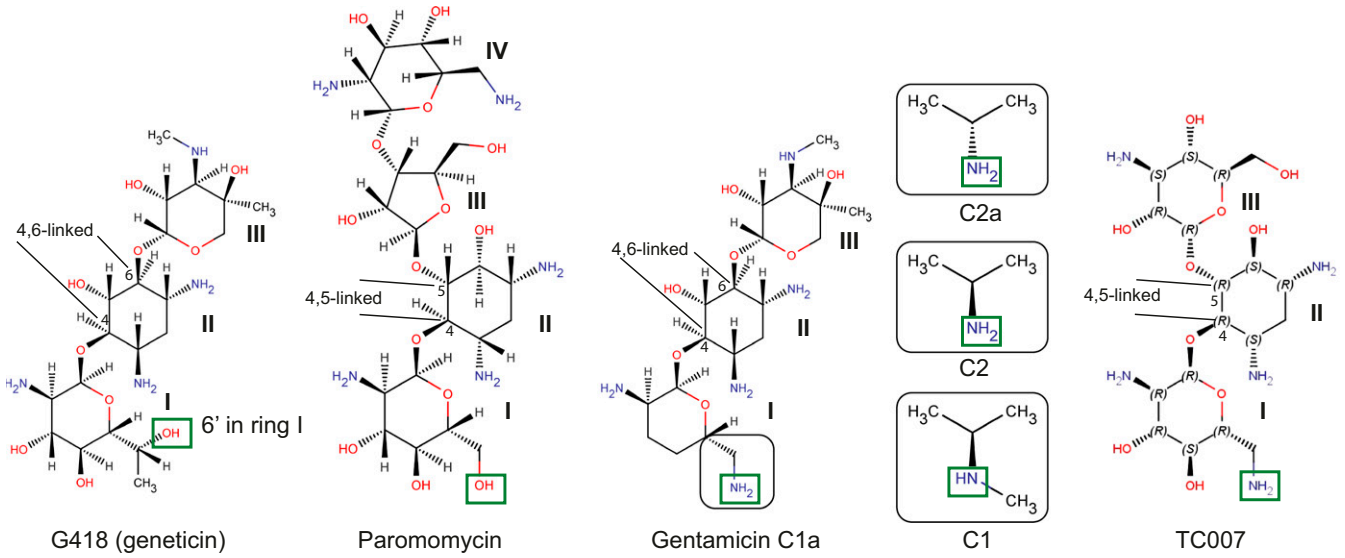

Fig. S1. Chemical formulas of aminoglycosides used in the study. The positions of attachment of rings I and III in the ring II are indicated (4,5- or 4,6-linked). The 6′-OH or 6′-NH_2 groups in ring I are marked with green rectangles.

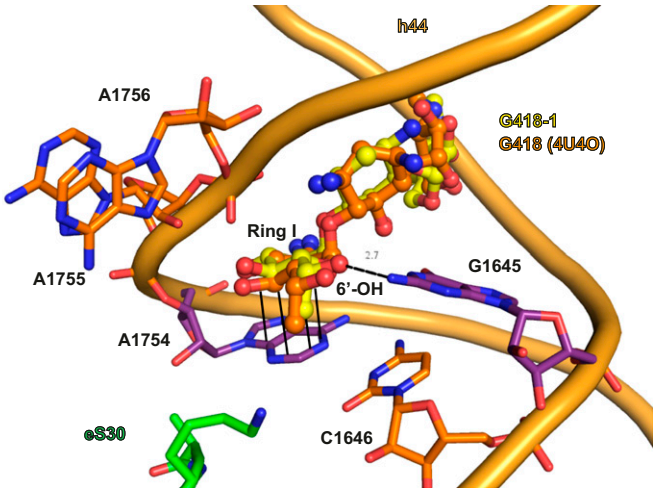

Fig. S2. Binding of G418-1 in h44 of the decoding center of the small ribosomal subunit. h44 and residues A1755 and A1756 are colored orange; eukaryote-specific residues G1645 and A1754 are colored magenta; G418 from the current structure is colored yellow; G418 from PDB 4U4O is colored orange; the eukaryote-specific protein S30 is colored green; oxygen atoms are colored red; and nitrogen atoms are colored blue. The 6′-OH group in the ring I of G418 and the N2 atom of G1645 are located at the hydrogen bonding distance (2.7 Å) marked with a dashed line.

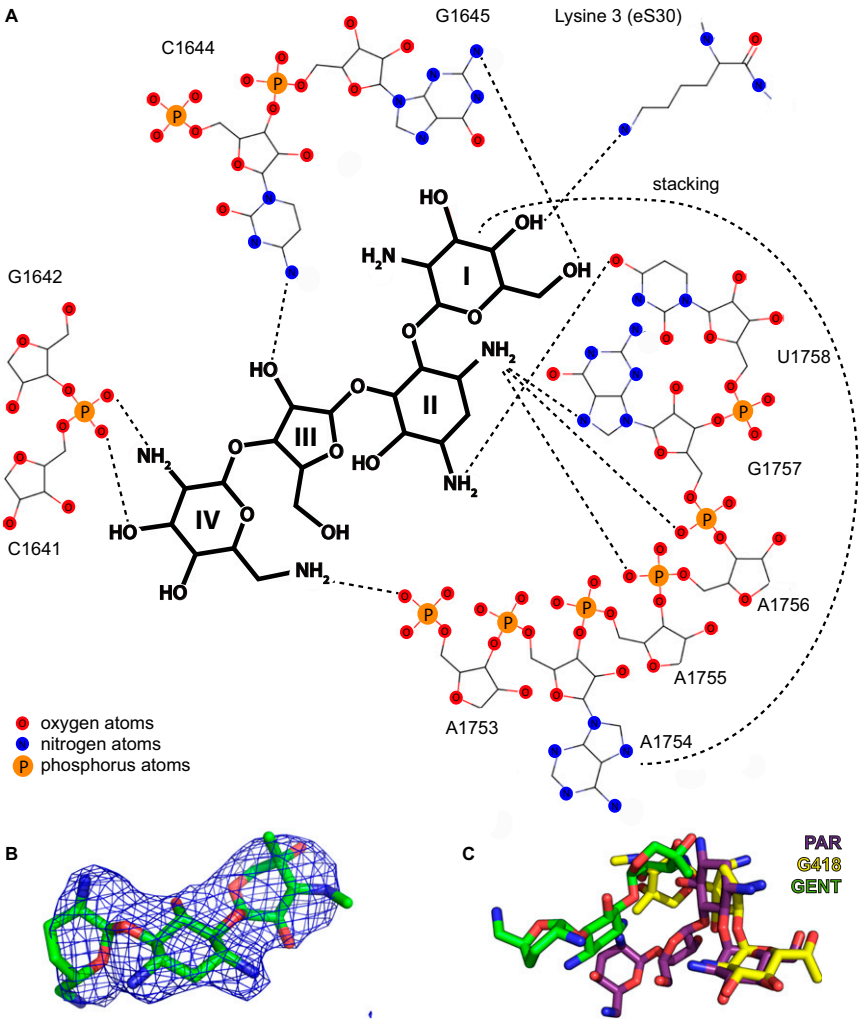

Fig. S3. Interactions of aminoglycosides with the decoding site of the 40S ribosomal subunit. (*A*) Schematic representation of interactions of paromomycin with the residues of the decoding site in the 40S ribosomal subunit; dashed lines show possible hydrogen bonds (maximum distance 3.5 Å) as well as stacking with A1754. (*B*) Unbiased difference electron density map ($F_{obs} - F_{calc}$) of GENT-1 bound to h44 is contoured at 3σ. Gentamicin is colored green; the electron density map is shown in blue; oxygen atoms are colored red; and nitrogen atoms are colored blue. (*C*) Conformation adopted by G418, gentamicin, and paromomycin in the h44 of the 40S ribosomal subunit from *S. cerevisiae*. Structures of the 80S ribosome in complex with aminoglycosides were aligned locally; elements of 80S ribosomes are omitted for clarity. G418 is colored yellow; paromomycin is colored violet; other color-coding is as in *B*.

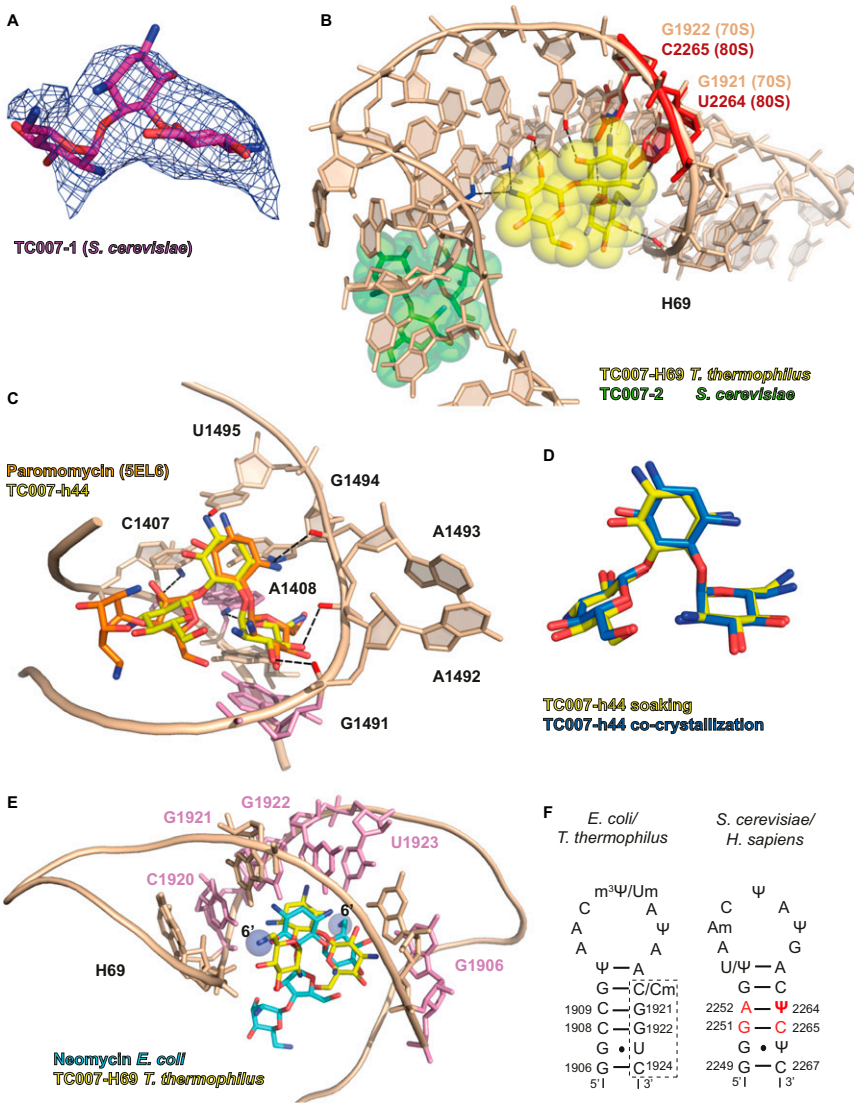

Fig. S4. Interactions of TC007 with the 70S ribosome from *T. thermophilus*. (*A*) Unbiased difference electron density map ($F_{obs} - F_{calc}$) of TC007-1 bound to h44 of the 40S subunit is contoured at 2σ. TC007 is colored magenta; the electron density map is shown in blue; oxygen atoms are colored red; and nitrogen atoms are colored blue. (*B*) Location of TC007 in the vicinity of H69 in the 70S ribosome from *T. thermophilus* and the 80S ribosome from *S. cerevisiae*. TC007-2 bound to the 80S ribosome is colored green; eukaryote-specific residues U2264 and C2265 are colored red; other residues of the 80S ribosome are omitted for clarity. Other color-coding is as in *A*. Interactions of TC007 with H69 in the 70S ribosome are marked with dashed lines. (*C*) Binding of TC007 and paromomycin to h44 in the 30S ribosomal subunit. Interactions of TC007 with the rRNA residues are marked with dashed lines. The 30S subunit is colored wheat; TC007 is colored yellow; paromomycin is colored orange; and bacteria-specific residues A1408 and G1491 are colored pink. Oxygen atoms colored red, and nitrogen atoms are colored blue. (*D*) Conformation of TC007 bound in h44 of the 30S subunit obtained for cocrystallization (colored marine) or soaking (colored yellow). (*E*) Binding of TC007 and neomycin (PDB ID code 4WOI) to H69 of the 70S ribosome. Neomycin is colored blue; rRNA residues interacting with both aminoglycosides are colored pink; other color-coding is as in *A*–*C*. The 6′-NH_2 groups in ring I of aminoglycosides are marked with blue spheres. (*F*) rRNA secondary structures of H69 from bacteria (*E. coli* and *T. thermophilus*) and from eukaryotes (*S. cerevisiae* and *H. sapiens*). Eukaryote-specific changes are colored red. Residues interacting with aminoglycosides in the 70S ribosome are marked by a dashed box.

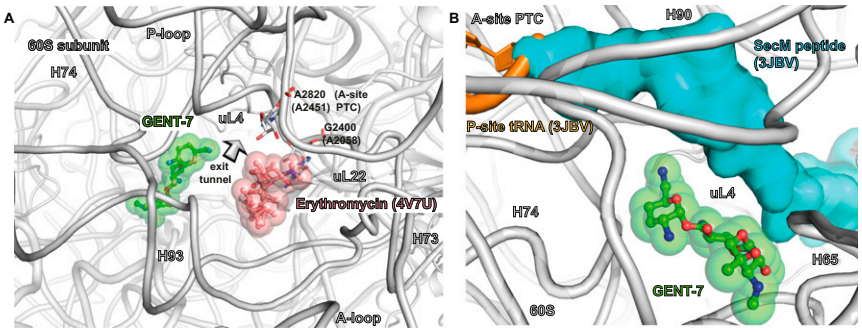

Fig. S5. Binding of gentamicin in the peptide exit tunnel. (*A*) Gentamicin and erythromycin are located on opposite sides of the peptide exit tunnel. The 80S–gentamicin structure was aligned locally on the structure of the *E. coli* 70S ribosome in complex with erythromycin (PDB ID code 4V7U). The 60S subunit is colored gray; GENT-7 is colored green; erythromycin is colored pink; other elements are omitted for clarity. The direction of the exit tunnel is marked by an arrow. (*B*) Gentamicin is shown approaching a nascent peptide in the peptide exit tunnel. The 80S–gentamicin structure was aligned locally on the structure of the *E. coli* 70S ribosome containing tRNA in the P-site and synthesized peptide SecM (PDB ID code 3JBV). tRNA in the P-site is colored orange; SecM is colored blue; other color-coding is as in *A*.

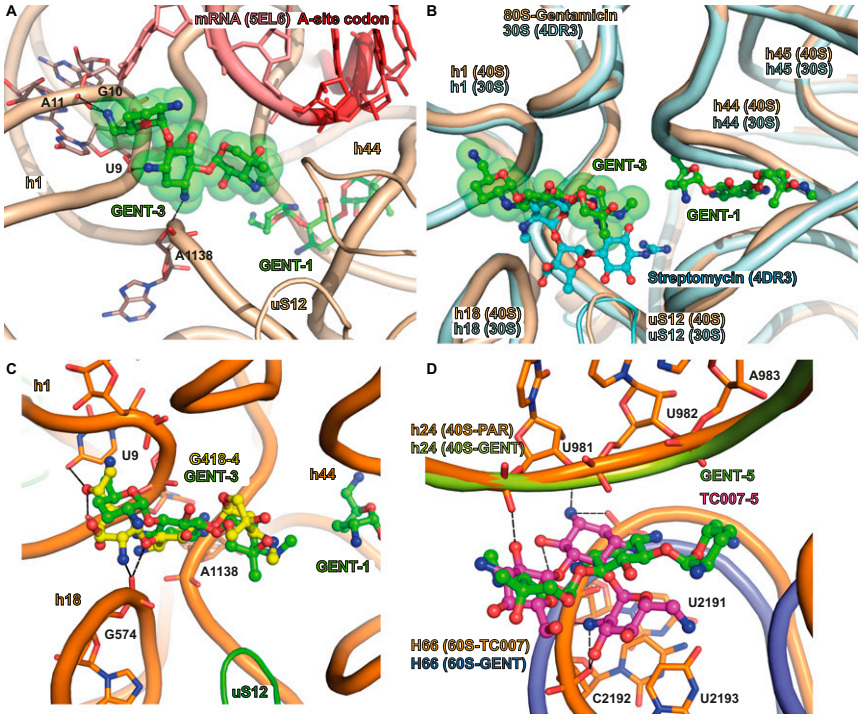

Fig. S6. Secondary binding sites of aminoglycosides. (*A*) The position of gentamicin (GENT-3) bound between helices 1, 44, 18, and 27 in the 40S ribosomal subunit close to the mRNA tunnel upstream of the A-site codon. mRNA was aligned locally from the structure of the 70S ribosome in complex with tRNA and mRNA (PDB ID code 5EL6). Gentamicin is colored green; oxygen atoms are colored red; nitrogen atoms are colored blue; interactions between gentamicin and 40S are marked by dashed lines. The 40S subunit is colored wheat; mRNA is colored pink; the A-site codon is colored red; other elements of the 70S ribosome are omitted for clarity. (*B*) Binding pockets of GENT-3 in the 80S ribosome from *S. cerevisiae* and streptomycin in the 30S ribosomal subunit from *T. thermophilus* (PDB ID code 4DR3). The 40S and 30S subunits were aligned locally; streptomycin is colored cyan; the 30S subunit is colored pale cyan; other color-coding is as in *A*. (*C*) The binding site of G418-4 overlapping with the GENT-3 in the vicinity of the A-site in the 40S ribosomal subunit. G418 is colored yellow; rRNA is colored orange; and uS12 protein is colored green; other color-coding is as in *A* and *B*. (*D*) Binding site of TC007-5 in the vicinity of the intersubunit bridge B2c overlapping with GENT-5. The 80S–TC007 structure was aligned on the 80S–gentamicin structure based on the 18S rRNA. TC007 is colored magenta; gentamicin is colored green; rRNA of the 80S–TC007 structure is colored orange; the large ribosomal subunit of the 80S–gentamicin structure is colored blue; the small ribosomal subunit is colored light green; oxygen atoms are colored red; and nitrogen atoms are colored blue. Interactions of TC007-5 with 80S at the hydrogen bonding distance are marked by dashed lines.

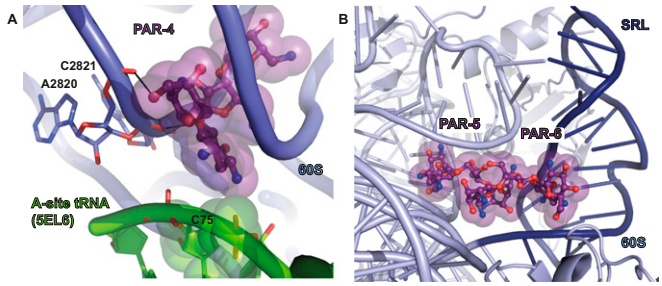

Fig. S7. Interactions of paromomycin with the 60S ribosomal subunit. (*A*) Binding of paromomycin (PAR-4) in the A-site of the peptidyl transferase center. A-site tRNA was superimposed from the structure of the 70S ribosome in complex with tRNAs and mRNA (PDB ID code 5EL6). Residues A2820 and C2821, corresponding to A2450 and C2451 in bacterial ribosome, are shown as sticks; their interactions with paromomycin are marked by dashed lines. tRNA is colored green; the 60S subunit is colored blue; paromomycin is colored violet; oxygen atoms are colored red; and nitrogen atoms are colored blue. (*B*) Binding of two molecules of paromomycin (PAR-5 and PAR-6) to helix H95 that contains a sarcin–ricin loop (SRL) in the 60S subunit. The sarcin–ricin loop is colored dark blue; other color-coding is as in *A*.

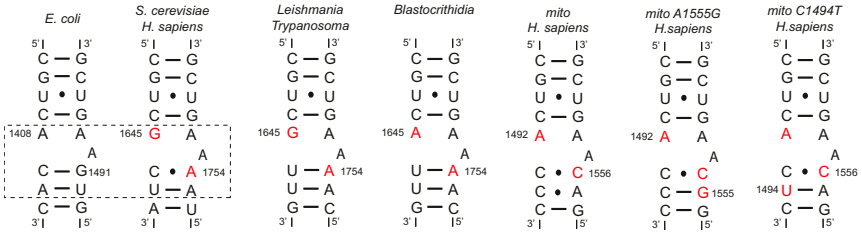

Fig. S8. Secondary structures of h44 of the ribosomes from different species. Residues, comprising binding site of aminoglycosides in h44 are marked by a dashed box. Substituted nucleotides implicated in the selectivity of aminoglycosides are marked in red.

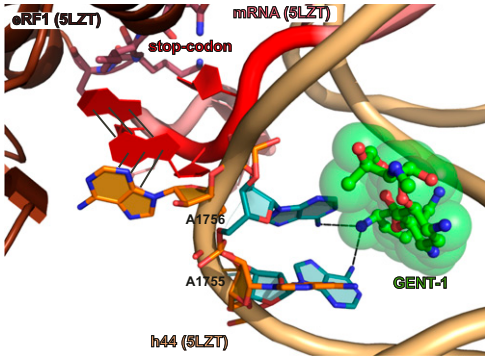

Fig. S9. Noncanonical binding of gentamicin in the decoding center may affect translation termination. The 80S–gentamicin structure was aligned locally to the cryoEM structure of the mammalian 80S ribosome in complex with termination factors eRF1 and eRF3 and with mRNA (PDB ID code 5LZT). Residue 1756 adopts a flipped-out conformation in stacking with the second and third residues of the mRNA stop-codon when they are recognized by eRF1, in contrast to a semi-flipped conformation stabilized by gentamicin. mRNA is colored pink; the stop-codon is colored red; eRF1 is colored brown; the 40S subunit from PDB 5LZT is colored wheat; gentamicin is colored green; oxygen atoms are colored red; nitrogen atoms are colored blue; residues 1755 and 1756 from the 80S–gentamicin structure are colored marine; other elements are omitted for clarity. Interactions of GENT-1 with residues 1755 and1756 at hydrogen bonding distance are marked by dashed lines.

Table S1. Data collection and refinement statistics

Statistics	80S–paromomycin	80S–gentamicin	80S–TC007	80S–G418	70S–tRNA–mRNA–TC007 (cocrystallization)	70S–tRNA-mRNA–TC007 (soaking)
Data collection						
Space group	$P2_1$	$P2_1$	$P2_1$	$P2_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions						
a, b, c, Å	434.53 293.33 295.79	436.63 287.00 304.73	442.14 298.76 299.77	300.15 297.32 443.59	209.07 447.36 619.49	209.21 447.98 620.73
α, β, γ, °	90 97.40 90	90 99.08 90	90 99.49 90	90 99.28 90	90 90.00 90	90 90.00 90
Resolution, Å	146.66–3.30 (3.40–3.30)	143.72–3.40 (3.50–3.40)	147.83–3.70 (3.80–3.70)	91.76–3.70 (3.80–3.70)	187.49–2.95 (3.05–2.95)	187.84–3.15 (3.25–3.15)
R_{meas}*	33.20 (203.10)	37.30 (175.30)	30.60 (190.40)	17.60 (189.20)	31.00 (263.60)	37.30 (220.70)
I/σI	7.37 (0.96)	10.00 (0.94)	13.97 (0.98)	8.28 (0.90)	16.04 (1.38)	13.31 (1.25)
$CC_{1/2}$	98.90 (34.90)	99.70 (35.50)	99.90 (36.10)	99.8 (47.8)	99.40 (47.10)	99.90 (39.90)
Completeness, %	100.00 (99.90)	99.90 (99.60)	99.90 (99.50)	99.9 (99.9)	100.00 (100.00)	100.00 (100.00)
Redundancy	14.85 (6.68)	27.13 (4.68)	47.71 (6.92)	5.21 (5.03)	46.83 (21.11)	45.36 (14.07)
Refinement						
Resolution, Å	146.66–3.30	143.72–3.40	147.83–3.70	91.76–3.70	152.74–2.95	104.66–3.15
No. unique reflections	1,098,436	1,013,190	814,310	885,488	1,204,009	994,263
R_{work}/R_{free}	0.1976/0.2452	0.1842/0.2331	0.2195/0.2454	0.2097/0.2530	0.2127/0.2531	0.2021/0.2469
Total no. atoms	397,996	404,272	400,123	397,717	299,575	300,007
Average B-factor	89.79	96.16	133.94	139.22	72.68	79.77
Rmsd						
Bond length deviation, Å	0.006	0.008	0.006	0.009	0.013	0.010
Bond angle deviation, °	0.991	1.226	1.013	1.447	1.915	1.632

*The elevated R_{meas} is attributed to the high number of datasets that were used and to the method of data collection which results in a large number of relatively weak measurements of each reflection. The quality of individual measurements might be relatively low, but the merged result has a higher signal to noise ratio due to averaging.