3. Materials

3.1. Bacterial Strains

E. coli strain DH5	GibcoBRL, Rockville, MD
E. coli strain NovaBlue(DE)	Novagen, Madison, WI

3.2. Plasmids

pET28a	Novagen, Madison, WI

3.3. E. coli Media

LB (Luria Bertani) medium:

Bacto-tryptone	10 g/l
Bacto yeast extract	5 g/l
NaCl	5 g/l

autoclaved.

For use as liquid medium 2% (w/v) glucose were added.

For use as solid medium 2% agar were added before autoclaving. The medium was cooled to approx. 50 °C before pouring it into sterile petri dishes of appropriate size.

3.4. Solutions

Solutions for the preparation of competent *E. coli* cells (RbCl method)

Transformation buffer I (TFB I)

RbCl	1.8 g
$MnCl_2$	1.5 g
0.5 M $CaCl_2$	3 ml
3 M KAc	1.5 ml
Glycerol	22.4 ml
dH_2O	add 150 ml

pH adjusted to 5.8 using acetic acid, filter sterilized.

Transformation buffer II (TFB II)

RbCl	0.024 g
0.5 M $CaCl_2$	3 ml
1.0 M MOPS (pH 7.0)	0.2 ml
Glycerol	3 ml
dH_2O	add 20 ml

filter sterilized.

Solutions for gel electrophoresis

4x SDS-PAGE stacking buffer

Tris-HCl	0.5 M

pH adjusted to 6.8.

4x SDS-PAGE separating buffer

Tris-HCl	1.5 M

pH adjusted to 8.8.

5x SDS-PAGE loading buffer

4x SDS-PAGE stacking buffer	1 ml
Glycerol	0.8 ml
10% (w / v) SDS	1.6 ml
β-ME	0.4 ml
1% (w / v) Bromophenol Blue	1.2 ml
dH_2O	add 8 ml

SDS-PAGE electrode buffer

Tris base	3.0 g
Glycine	14.4 g
SDS	1.0 g
dH_2O	add 1 l

Coomassie staining solution

Coomassie Brilliant Blue R-250	0.05 g
Methanol	500 ml
HAc	100 ml
dH_2O	add 1 l

Destaining solution

Methanol	100 ml
HAc	50 ml
dH_2O	add 1 l

10x TBE long-range buffer

Tris base	163 g
Boric acid	55 g
0.5 M EDTA	40 ml
dH_2O	add 1 l

50x TAE buffer

Tris base	146 g
HAc	28.55 ml
0.5 M EDTA	50 ml
dH_2O	add 500 ml

3.5. Chemicals

Standard chemicals were purchased from Sigma, St. Louis, MO, or Mallinckrodt, Paris, KY.
Chemicals used in crystallization setups were purchased from Fluka, Buchs, Switzerland.

3.6. Enzymes and Kits

Sequenase version 2.0 kit	Amersham, Uppsala, Sweden
QIAEX gel extraction kit	Qiagen, Hilden, Germany
Plasmid preparation kits (Mini, Maxi)	Qiagen, Hilden, Germany
Restriction enzymes	New England Biolabs, Beverly, MA
Pfu DNA polymerase	Stratagene, La Jolla, CA
Endoproteinase Glu-C	Promega, Madison, WI
Chymotrypsin, trypsin, thermolysin	Sigma, St. Louis, MO

3.7. Nucleic Acids

PCR amplification primers for expression constructs from ADAR1

G96_f	5’-TCAGGGGTGTCCATATGGGCGTGCATCTCGGA-3’
S226_r	5’-CCATATCTCGAGTTAACTCGGGTCTGAGTTTGGGGC-3’
L133_f	5’-GGGGTGTCCATATGCTGAGTATCTACCAAGATCAGG-3’
G209_r	5’-GCGGCAAGCTTATTATCCGCTGTGCTGGTTCCAAGCC-3’
N368_r	5’-GCGGCAAGCTTATTAATTTCTCTTGATTTGCATCCTCTC-3’

Mutagenesis primers for the construction of S -> C mutants

S134C_f	5'-GGCAGCCATATGCTGTGTATCTACCAAGATCAGG-3'
S134C_r	5'-CCTGATCTTGGTAGATACACAGCATATGGCTGCC-3'
S162C_f	5'-CCACAGCACATGATCTGTGTGGGAAACTTGGGACTCC-3'
S162C_r	5'-GGAGTCCCAAGTTTCCCACACAGATCATGTGCTGTGG-3'
S178C_f	5'-CAATCGAGTTTTATACTGCCTGGCAAAGAAGGGC-3'
S178C_r	5'-GCCCTTCTTTGCCAGGCAGTATAAAACTCGATTG-3'
S200C_f	5'-GGAAAATCGCGGTCTGCACTCAGGCTTGGAACC-3'
S200C_r	5'-GGTTCCAAGCCTGAGTGCAGACCGCGATTTTCC-3'

Others

poly [d($^{5\text{-Me}}$C-G)]	Pharmacia Biotech, Uppsala, Sweden
poly [d(A-G)] • poly [d(C-T)]	Pharmacia Biotech, Uppsala, Sweden
$(^{5\text{-Br}}C\text{-}G)_{20}$	gift from Dr. Yang-Gyun Kim, MIT

DNA oligonucleotides used for circular dichroic measurements and crystallization trials were purchased from DNAgency, Malvern, PA.

3.8. Equipment

PE PCR cycler 9600 Perkin Elmer, Norwalk, CT

Voyager DE Workstation mass spectrometer
PerSeptive, Framingham, MA

Aviv Model 62DS CD spectrometer
Aviv Instruments, Lakewood, NJ

Raxis IIc image plate detector

Rigaku RU-200 rotating anode x-ray generator
Molecular Structure Coop., The Woodlands, TX

3.9. Software

Data processing and reduction:
HKL program package (Otwinowski & Minor, 1997)

Automated localization of heavy atoms:
SOLVE (Terwilliger & Berendzen, 1996)

Various data modification programs:
CCP4 program suite (Collaborative Computational Project, Number 4. 1994)

Model building:
vuSette zc (M. A. Rould, personal communication)

Refinement:
X-PLOR 3.8.5.1 (Brünger, 1992a)

Graphics:
Molscript 2.1.2 (Kraulis, 1991)
Bobscript 1.4 (Esnouf, 1997)
Raster3D (Merritt & Murphy, 1994)
GRASP (Nicholls *et al.*, 1991)