SUPPLEMENTARY INFORMATION

MATERIALS AND METHODS

1. Green fluorescent protein cloning and protein expression

Plasmids and expression strains for bacterial expression were from Novagen. Restriction endonucleases and other molecular biology enzymes were from New England Biolabs. Ion-exchange and gel-filtration carriers were from Amersham Pharmacia Biotech. Percoll was from Sigma. Other chemicals and salts used in experiments were from Serva. cDNA clone for GFP-Cys mutant was obtained from Clonetech (clone ID: pEGFP-N2).

5'-primer 5'-GGGAATTCCATATGGGA(TGT)GGAGCAGGAGCAATGGTGAGCAAGGGCG AG-3' (italic, inserted base) and 3' primer 5'-TAGGATCCTTACTTGTACAGCTCGTC-3' were used to produce a GFP-Cys mutant with a cysteine residue inserted at the N-terminus. The cDNA region encoding GFP-mut1 variant (Cormack et al. 1996) in pEGFP-N2 vector (Clonetech, Palo Alto, CA) was amplified by PCR. Amplified DNA products were digested with Nde1 and BamH1 and ligated into pET3A expression plasmid. The presence of the inserted GFP-Cys in the construct was verified by DNA sequencing. *E.coli* strand BL21(DE3)pLysS was used for expression. Cells transformed with recombinant plasmids were grown at 37°C in Luria-Bertani (LB) medium, containing 100 µg/ml ampicilin and 50 µg/ml chloramphenicol to OD600 0.6. Expression was induced by addition of isopropyl β-D-thiogalactoside (IPTG) to a final concentration of 0.4 mM. After 4 hrs of induction the cells were harvested by centrifugation and resuspended in the ice-cold lysis buffer (50mM Tris-HCl, 0.1M NaCl, 1mM EDTA, pH 8). Cell disruption was achieved by freezing and thawing of the cell suspension and by sonification. Nucleic acids were precipitated by the addition of 5% solution of polyethyleneimine to a final concentration of 0.1 % and removed together with the insoluble fractions of bacterial cells by centrifugation. Clear cytosolic fraction was concentrated and loaded onto a Superdex G-75 gel filtration column equilibrated in 10 mM phosphate buffer, 200 mM NaCl, pH=8,3. Eluted fractions were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Fractions containing recombinant protein were collected, dialyzed against 10 mM phosphate buffer, pH=7,5 and applied onto a Q-Sepharose column equilibrated with the same buffer. The elution was carried out with a linear gradient of NaCl (0-0.5M) in the starting buffer. Fractions contatining the protein as judged by SDS-PAGE analysis and measuring fluorescence (λ_{ex} 488 nm, λ_{em} 507 nm) were concentrated and applied to a Superdex G-75 gel filtration column equilibrated in 10 mM phosphate buffer, 200 mM NaCl, pH=8,3. Only the peak corresponding to the monomeric GFP-Cys was pooled down, concentrated and stored in 10 mM phosphate buffer, pH=7,5.

GFP-nanowire conjugates

Nanowire-protein conjugates were produced by mixing 0.4 µg/ml of GFP-Cys mutant (reduced with 10mM dithiothreitol DTT or intact) and 0.1 mg/ml of $Mo_6S_3I_6$ nanowires. The conjugates were left to react over night at 4^{o}C. They were further purified by Percoll gradient centrifugation. A step gradient was prepared with 1,15 ml undiluted Percoll, 1,15 ml diluted 1:2 and 1,7 ml Percol diluted 1:10. Centrifugation was performed for 2 hrs at 20.000 g using TST 60-4 rotor in a Centrikon T-2070 ultracentrifuge (Kontron Instruments). The GFP-Cys-nanowire conjugates were collected at the interface between 10% and 50% Percoll. The conjugates were subjected to a 1,5 hrs centrifugation at 100.000g using the same rotor as described previously to remove Percoll from the sample. Protein-nanowire conjugates were inspected by AFM.

2. Thyroglobulin

Isolation and purification

Tg was isolated from porcine thyroids following the protocol described in Brix et al. 1996. Protein purity was checked by SDS-PAGE and by indirect enzyme-linked immunosorbent assay.(ELISA) with rabbit anti-pig primary antibodies (Berndorfer U.) diluted goat anti-rabbit HRP-conjugated secondary antibodies (Dianova, Hamburg, Germany). Detection was performed using 0.05% 3,3′diaminobenzidine tetrahydrochloride (DAB, Sigma, USA) and 0.09% H_2O_2 in 0.05 M Tris–HCl buffer, pH 7.5.

Tg Protein-nanowire conjugates

Nanowire-protein conjugates were produced by mixing 0.8 μg/ml of thyroglobulin (reduced with 10mM dithiothreitol DTT or intact) and 0.1 mg/ml of $Mo_6S_3I_6$ nanowires. The conjugates were left to react over night at 4^{o}C. They were inspected by AFM.

Determination of free sulfhydryl groups

In order to determine the presence of free thiol group, Ellman's reagent (5,5'-dithio-bis(2-nitrobenzoic acid, Sigma, USA) which stoichiometrically yields the 5-mercapto-2-nitrobenzoic acid chromophore was used. Two proteins, thyroglobulin containing free thiol groups and stefin B without any thiol group were used as positive control and as a negative control, respectively. 0.1 mg/ml of protein was preincubated with 1mM dithiothreitol (DTT, Fermentas, Germany) to for 15 min at room temperature to reduce sulfhydryl groups and then dialized against 100mM phosphate buffer, 1.5mM EDTA, pH=8.0 to remove excess DTT. 500 μl of reduced protein (c=0.1mg/ml) and of freshly dispersed nanowires (c=0.1 mg/ml; t_1=30 min, t_2= 120 min) in deionized water were mixed with 16.7 μl of Ellman's reagent and absorbance at 412nm was measured. 500 μl of 100mM phosphate buffer, 1.5mM EDTA, pH=8.0 with 16.7 μl of Ellman's reagent were used as blank.

Sample	A (412nm)	A (412nm) with Ellman's reagent
Negative control (stefin)	0	0
Positive control (thyroglobulin)	0.003	0.204
Nanowires (120 min dispersion)	0.037	0.103

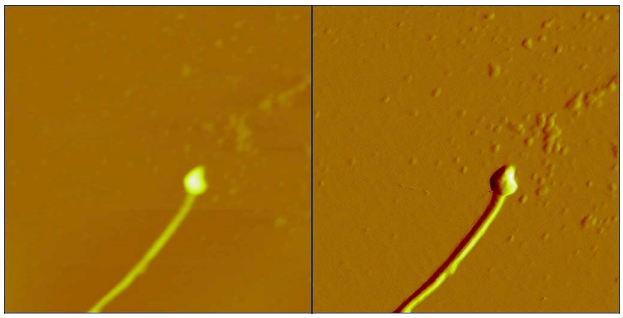

Figure 1: Raw images of height (left) and amplitude (right) of MOSix nanowire bundle with thyroglobulin attached (on mica substrate)

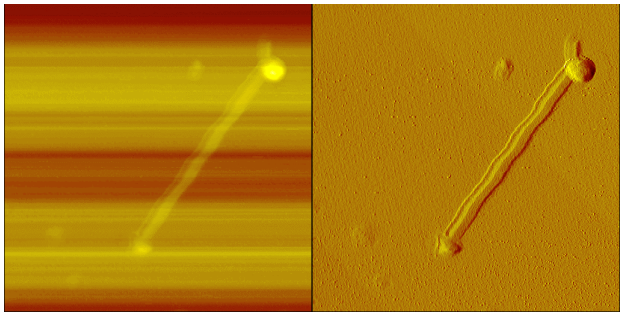

Figure 2: Raw images of height (left) and amplitude (right) of MOSix nanowire bundle with a green fluorescent protein attached at both ends (on mica)

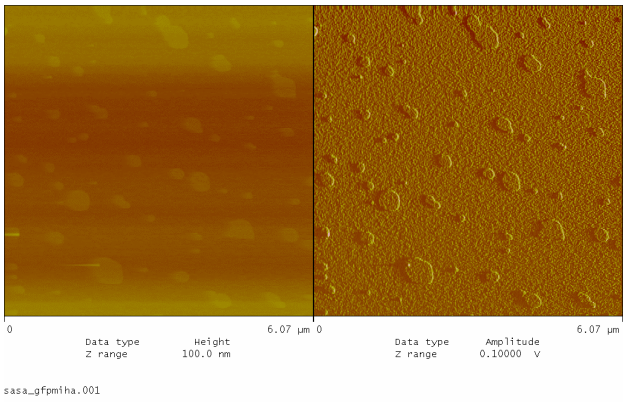

Figure 3: Raw images of height (left) and amplitude (right) of Green fluorescent protein (on mica)

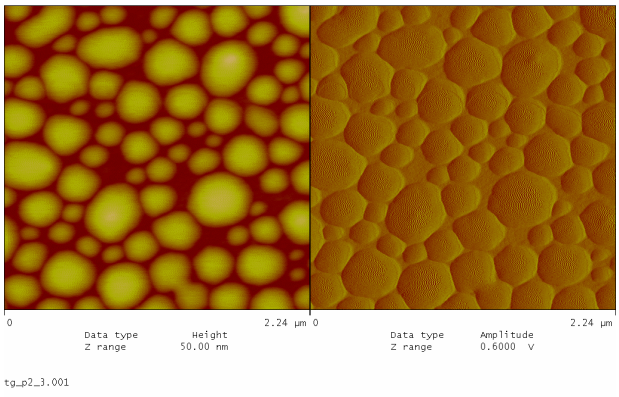

Figure 4: Raw images of height (left) and amplitude (right) of Thyroglobulin (on mica)

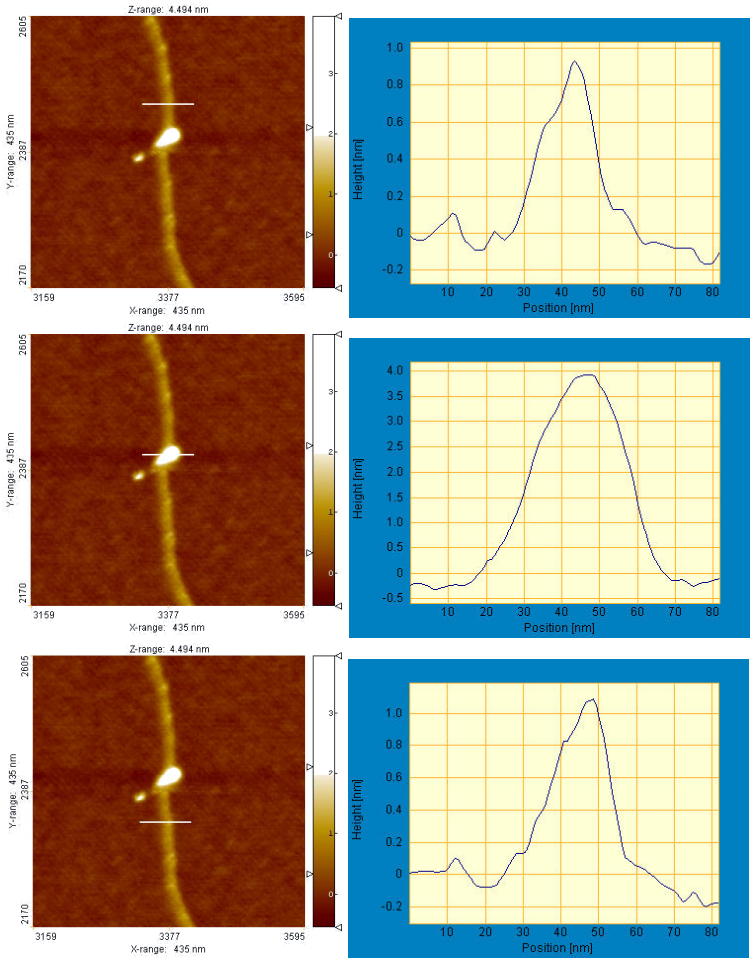

Figure 5. Two MoSIx nanowire bundles attached to a single GNP. The profiles are shown on the right.

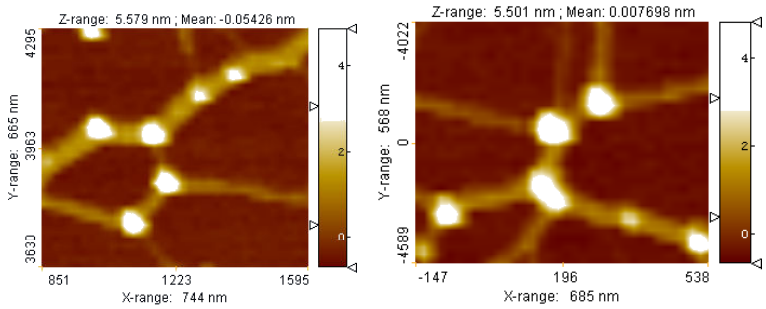

Figure 6 Examples of multi-terminal circuits with multiple MWs and GNPs.

References

Brix, K., Lemansky, P. & Herzog, V. (1996). Evidence for Extracellularly Acting Cathepsins Mediating Thyroid Hormone Liberation in Thyroid Epithelial Cells., *Endocrinology* **137**, 1963-1974.

Cormack, B.P., Valdivia R.H. & Falkow, S. (1996). FACS-optimized mutants of the green fluorescent protein (GFP)., *Gene* **173**, 33-38.