Supporting information for

Selective Biomolecular Separation System Inspired by the Nuclear Pore Complex and Nuclear Transport

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NTF2

MGDKPIWEQIGSSFIQHYYQLFDNDRTQLGAIYIDASCLTWEGQQFQGKAAIVEKLSSLP
FQKIQHSITAQDHQPTPDSCIISMVVGQLKADEDPIMGFHQMFLLKNINDAWVCTNDMF
RLALHNFGGSRSHHHHHH

NTF2-GFP

MGDKPIWEQIGSSFIQHYYQLFDNDRTQLGAIYIDASCLTWEGQQFQGKAAIVEKLSSLP
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RLALHNFGTSGSACELMVSKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATNGK
LTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTISF
KDDGTYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNFNSHNVYITADKQKN
GIKANFKIRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDH
MVLLEFVTAAGITHGMDELYKGSRSHHHHHH

NTF2-GFP11

MGDKPIWEQIGSSFIQHYYQLFDNDRTQLGAIYIDASCLTWEGQQFQGKAAIVEKLSSLP
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RLALHNFGTSSGSGHHHHHHHASGGSGGGSRDHMVLHEYVNAAGIT

GFP1-10

MGSSHHHHHHSSGLVPRGSHMSKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDAT
IGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHMKRHDFFKSAMPEGYVQERTI
SFKDDGKYKTRAVVKFEGDTLVNRIELKGTDFKEDGNILGHKLEYNFNSHNVYITADKQ
KNGIKANFTVRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQTVLSKDPNEK

NTF2-R418

MRGTMGDKPIWEQIGSSFIQHYYQLFDNDRTQLGAIYIDASCLTWEGQQFQGKAAIVEK
LSSLPFQKIQHSITAQDHQPTPDSCIISMVVGQLKADEDPIMGFHQMFLLKNINDAWVCT
NDMFRLALHNFGTSSGSGHHHHHHGSGGSGGGSSYSCHYWLSSAVPYM

NTF2-R445

MRGTMGDKPIWEQIGSSFIQHYYQLFDNDRTQLGAIYIDASCLTWEGQQFQGKAAIVEK
LSSLPFQKIQHSITAQDHQPTPDSCIISMVVGQLKADEDPIMGFHQMFLLKNINDAWVCT
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Figure S1. Protein sequences used in this study.

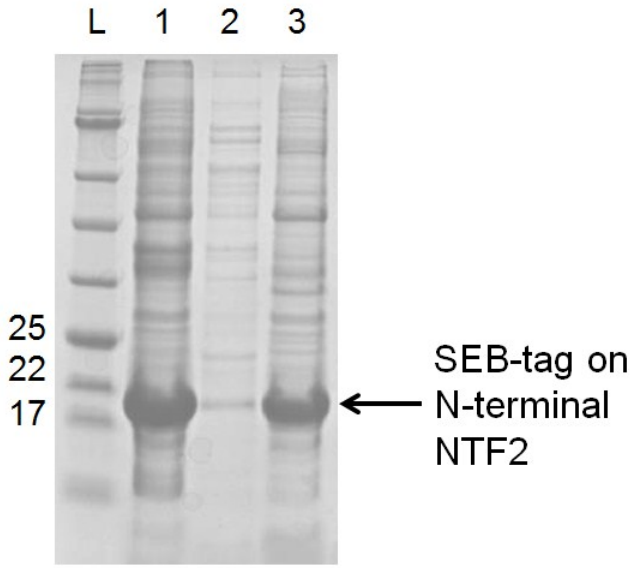

Figure S2. SDS-PAGE of engineered NTF2 fused with anti-SEB tag on its N-terminus. L: Ladder, 1: total cell lysate, 2: soluble protein fraction of #1 and 3: insoluble protein fraction of #1

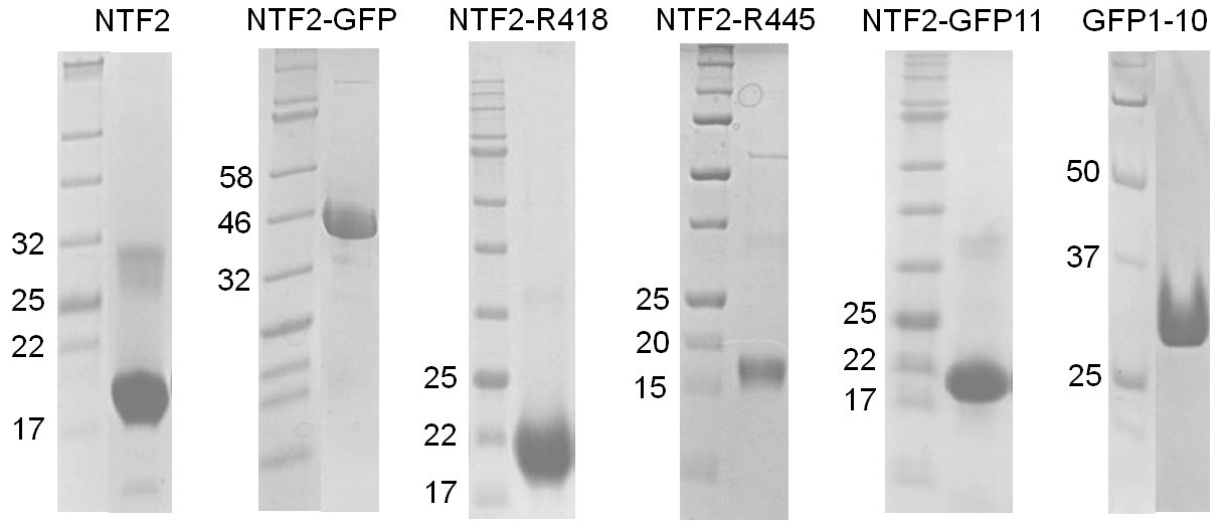

Figure S3. SDS-PAGE of purified protein samples used in this study.

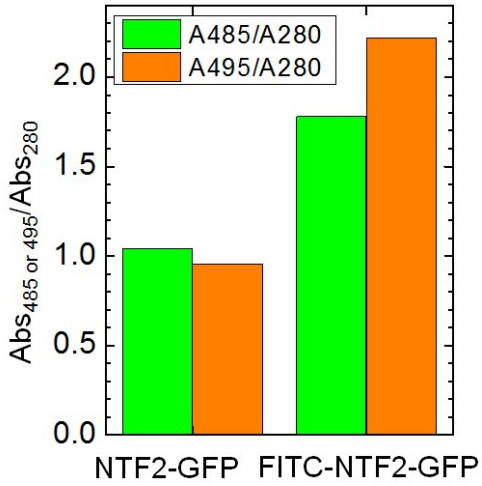

Figure S4. Optical absorbance enhancement by FITC labeling on NTF2-GFP. The enhancement by FITC is determined by the absorbance of the sample at 280 nm and 485 nm for NTF2-GFP, and 280 nm and 495 nm for FITC-NTF2-GFP.

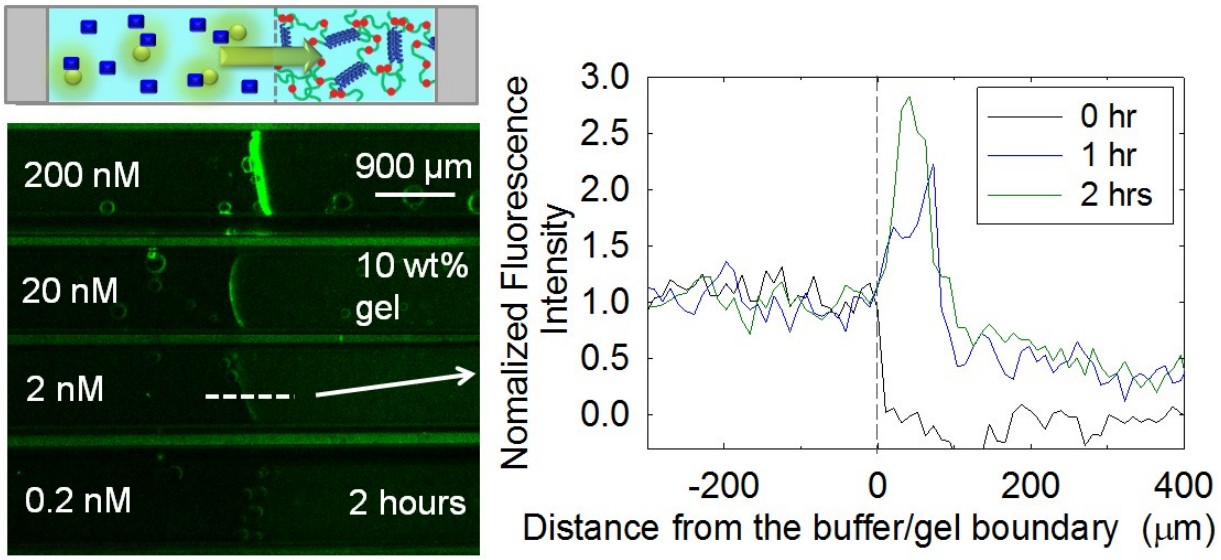

Figure S5. Transport into 10 wt.% NLP gels. Various concentrations of FITC-NTF2-GFP (blue rectangle and green circle) with maintaining 20 µM NTF2 (blue rectangle only) are tested with 10 wt.% NLP gel. Similar to 20 wt.% gel (Fig. 1), 10 wt.% gel also can absorb target GFP molecules from the buffer containing 2 nM target molecule (t = 0). NLP gel in the capillary with FITC-NTF2-GFP of 0.2 nM did not show any green fluorescence accumulation on the gel over time. This indicates that NLP gel can absorb target molecules carried by NTF2 with concentrations of at least the single nanomolar range, although the ultimate sensitivity may be limited by the fluorescence detection limit of the microscope and not by the gel performance.

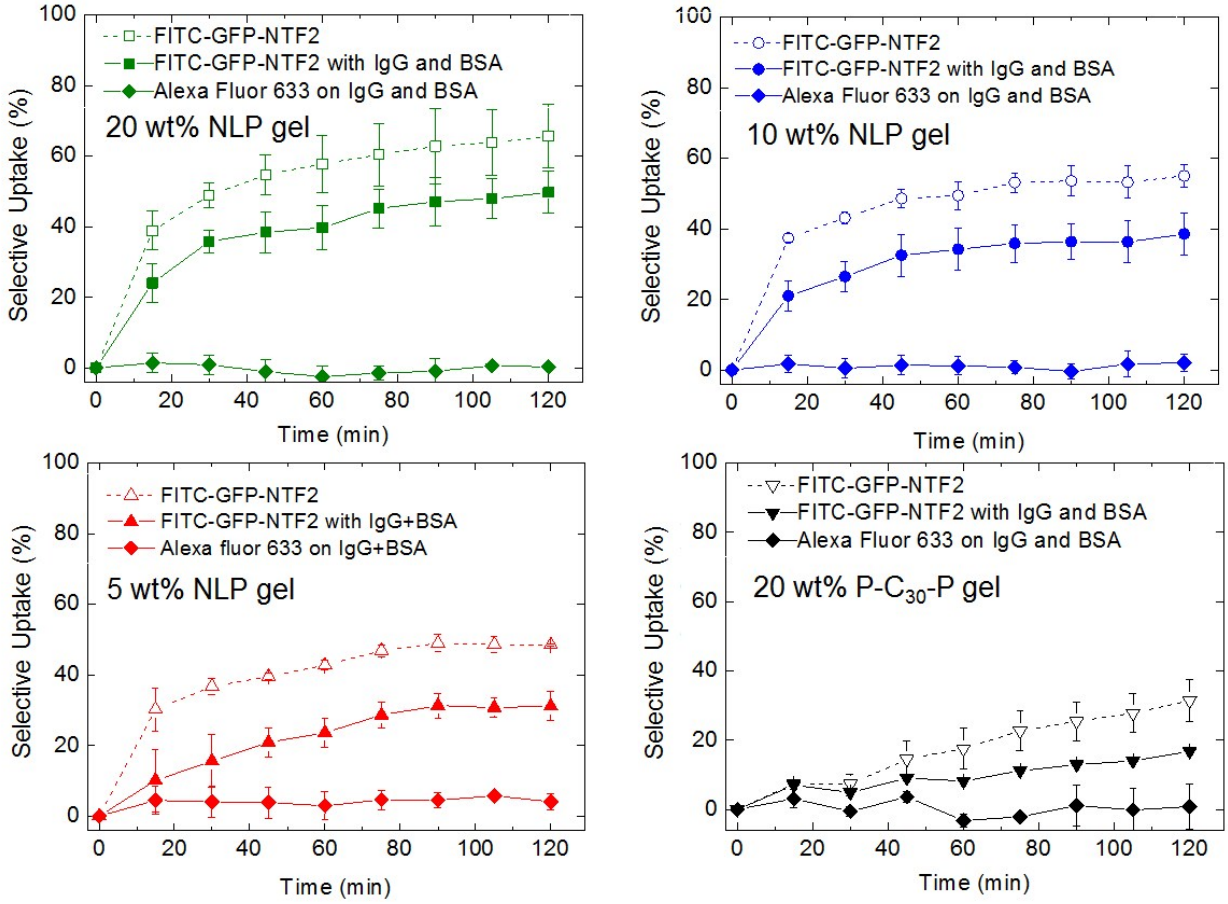

Figure S6. Average selective uptake of FITC-NTF2-GFP by 20 wt.% NLP gel (n = 3), 10 wt.% NLP gel (n = 5), 5 wt.% NLP gel (n = 3) and control 20 wt.% P-C_{30}-P gel (n = 2) in the absence (empty objects) or in the presence (filled objects) of IgG and BSA. The uptake amounts of IgG and BSA by gels are also tracked in time series (filled diamond).

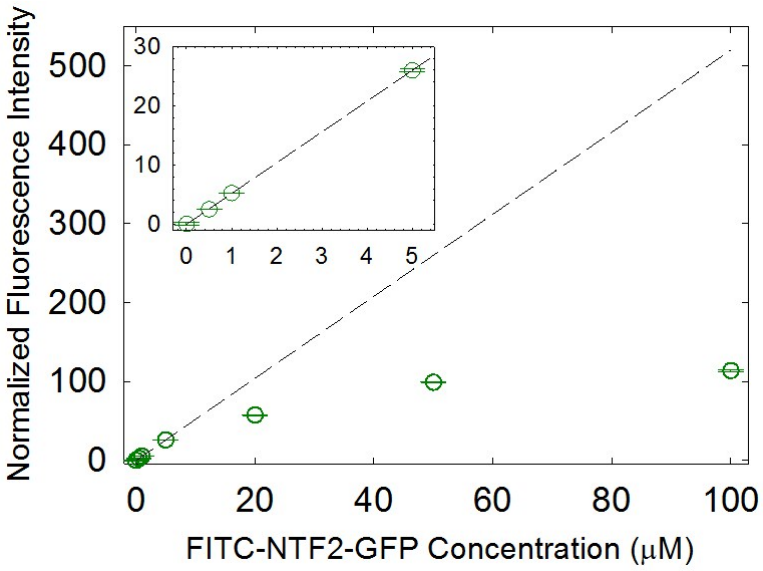

Figure S7. Linear fluorescence regions of FITC-NTF2-GFP under the microscope used in this study. Fluorescence intensities of each concentration are normalized with the intensity of 1 μM FITC-NTF2-GFP. Changes in fluorescence intensity of FITC-NTF2-GFP are linear when molecular concentrations are below 5 μM. All of capillary assays with FITC-NTF2-GFP were performed in the linear region (Fig. 2-3, Fig. S5 and S6).

Table S1. DNA plasmids that were used in this study

Protein name	Expressed protein	Vector	Reference
P-2NLP-P	P-2NLP-P- His_6	pET-22b	Kim et al.[1]
NTF2	NTF2-His_6	pQE-30	This study; Ribbeck et al.[2]
NTF2-GFP	NTF2-GFP-His_6	pQE-30	This study; Pédelacq et al.[3]
NTF2-GFP11	NTF2-His_6-GFP11	pQE-30	This study; Cabantous et al.[4, 5], Kent et al.[6]
NTF2-R418	NTF2-His_6-R418	pQE-30	This study; Kogot et al.[7]
NTF2-R445	NTF2-His_6-R445	pQE-30	This study; Kogot et al.[7]
GFP1-10	His_6-GFP1-10	pET15b	Cabantous et al.[4, 5], Kent et al.[6]

References

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