Supporting Information for the following article:

Organic Solvent Nanofiltration (OSN): A New Technology Platform for liquid-phase oligonucleotide synthesis (LPOS)

Jeong F. Kim[1‡], Piers R. J. Gaffney[1‡], Irina B. Valtcheva[1], Glynn Williams[2], Andrew M. Buswell[2], Mike S. Anson[2], Andrew G. Livingston[1*]

1. Department of Chemical Engineering, Imperial College London, Exhibition Road, London, SW7 2AZ.
2. GlaxoSmithKline Medicines Research Centre, Gunnels Wood Road, Stevenage, Herts, SG1 2NY, UK.
‡. *These authors contributed equally.*
*. *Corresponding Author*: a.livingston@imperial.ac.uk

S1. Characterization of Chemical Species

All of the chemical characterization data are included in our first publication [Chemistry European Journal, **2015**, *21*, 9535-9543]. In the current paper, we have focused on the process development aspects of using OSN technology, and the necessary characterization data, as well as further analysis including economic feasibility are discussed.

S2. Effect of Adding Acid Prior to Purifying the Chain Extension Crude

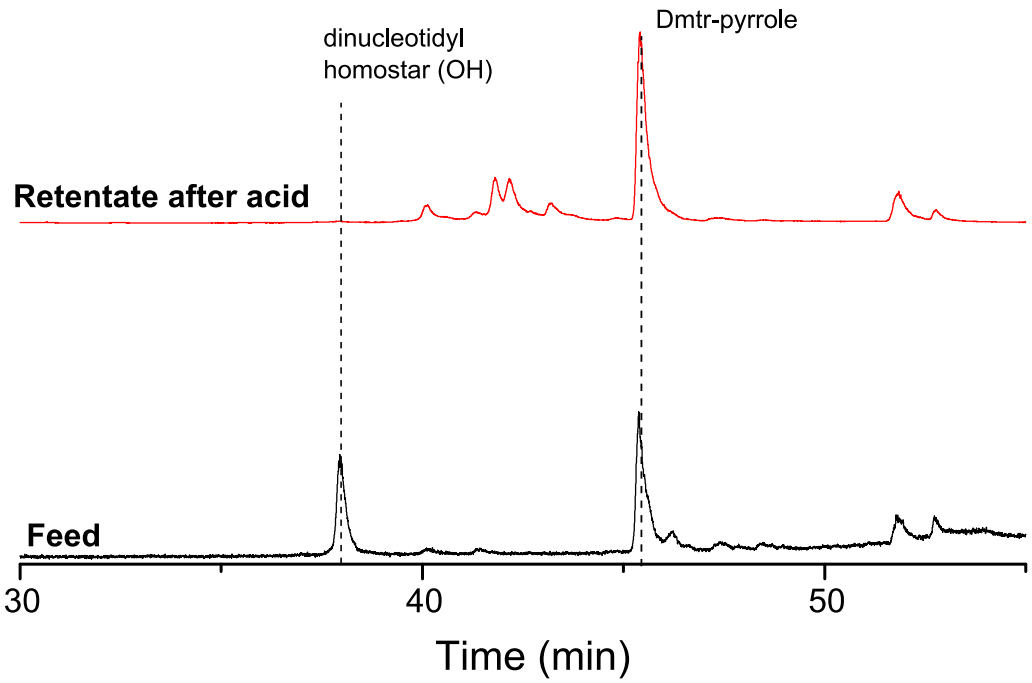

Figure S1 – HPLC chromatogram illustrating the product degradation of chain extension crude upon acid (DCA) exposure.

To test the effect of acid, the rig was charged with a mock crude chain extension mixture containing detritylated dinucleotidyl homostar combined with PADS, ETT, and detritylated thioamidate and amidate, along with 1 *vol*% DCA. It can be in seen in Figure S1 that the addition of acid prior to purification of chain extension crude mixture results in unknown side reactions. Hence, it was decided to split the chain extension cycle into two separate diafiltrations: once after the chain extension reaction, and once after detritylation.

S3. Peak Broadening in HPLC

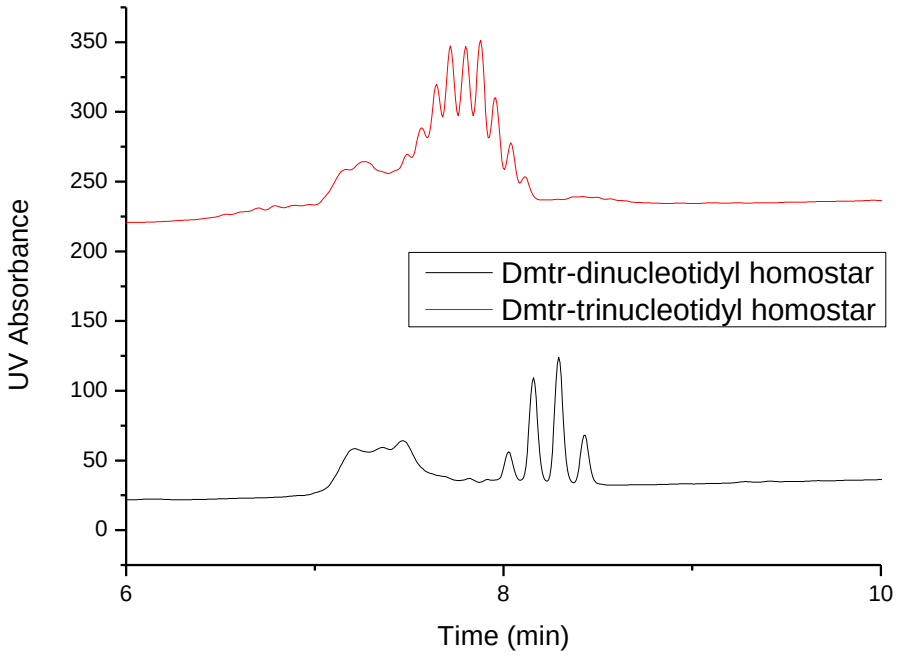

Figure S2. Increase in diastereomers with oligo chain lengths. The early retention time peaks around 5 min are building block debris. As each phosphorothioate tri-ester linkage is a roughly 1:1 mixture of two absolute stereoisomers, the overall dinucleotidyl homostar consists of four possible diastereoisomers. Extending to a trinucleotide on each arm increases the total number of possible diastereoisomers to 16, some of which are not resolved in the upper trace.

S4. Effect of Cascade on Process Yield

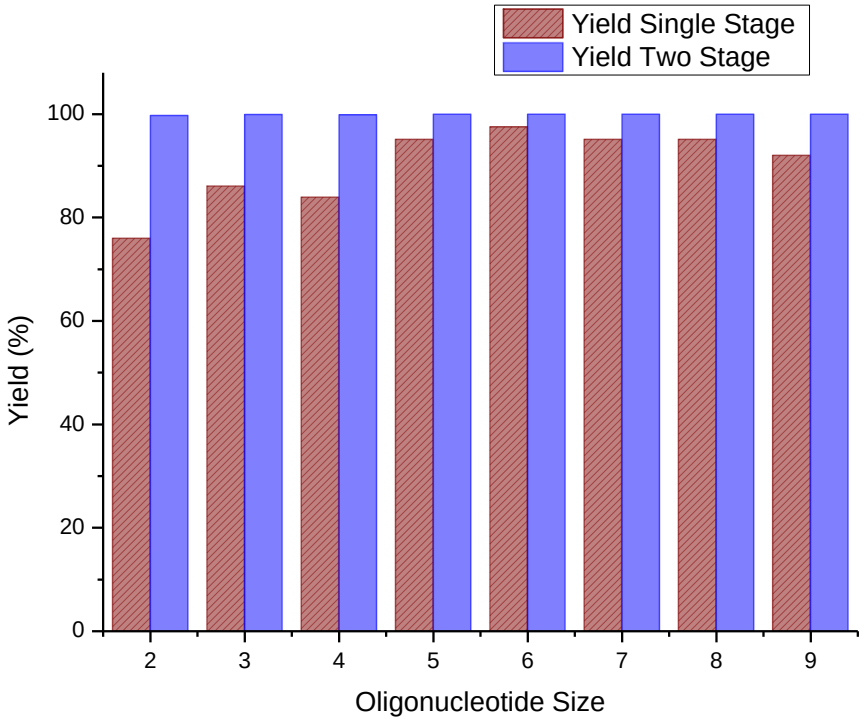

Figure S3. Simulated yield profile using a cascade process with the stepwise yields obtained in this report. The use of a two stage cascade would clearly allow a significant improvement in stepwise yield over the single stage diafiltration.

S5. Detection of Incomplete Chain Extension using HPLC

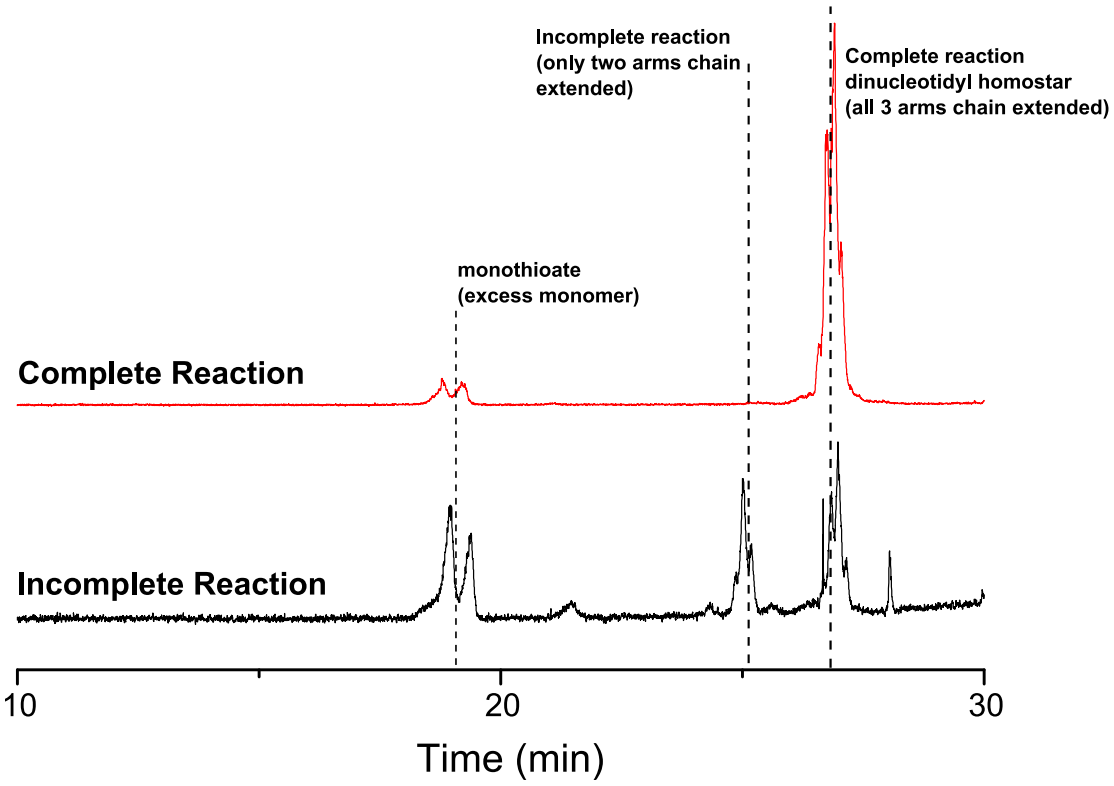

Figure S4. Incomplete chain extension to dimer shown by HPLC.

It can be seen in Figure S4 that incomplete reaction clearly shows up in the HPLC chromatogram. In such cases, the reaction can simply be repeated (after membrane diafiltration to get rid of other reaction debris) to push the reaction to completion.

S6. Preparation of Other Membranes

Table S1 summarizes the performance data of screened membranes. The preparation method for each screened membrane is taken from the cited reference. The tested ceramic membrane was a commercial membrane (details not disclosed).

Table S1. Membrane Screening Raw Data

Membrane	Ref	Solution Permeability (10bar, Lmhbar)	Dinucleotidyl Homostar	Monomer (thioamidate)	Dmtr-Pyrrole	PADS
TFC-HDA	[1]	5.6	99%	78%	69%	62%
PI23,2:1	[2]	8.7	>99.9%	>99.9%	95%	90%
PI23,4:1	[2]	8.7	>99.9%	>99.9%	94%	85%
PBI 17DBX	[3]	8.6	>99.9%	>99.9%	90%	<50%
PBI 18DBX	[3]	7.1	>99.9%	>99.9%	99%	<50%
Ceramic (TiO2)	[4]	1.0	>99.9%	--	93%	--
Ceramic (SiO2)	[4]	0.005	--	--	--	--

[1] J. Membr. Sci., 423 (2012) 371-382, [2] J. Membr. Sci., 324 (2008) 220-232, [3] J. Membr. Sci., 457 (20124) 62-72, [4] Commercial ceramic membrane, not disclosed.

S7. Long-term Performance Data of PBI membranes

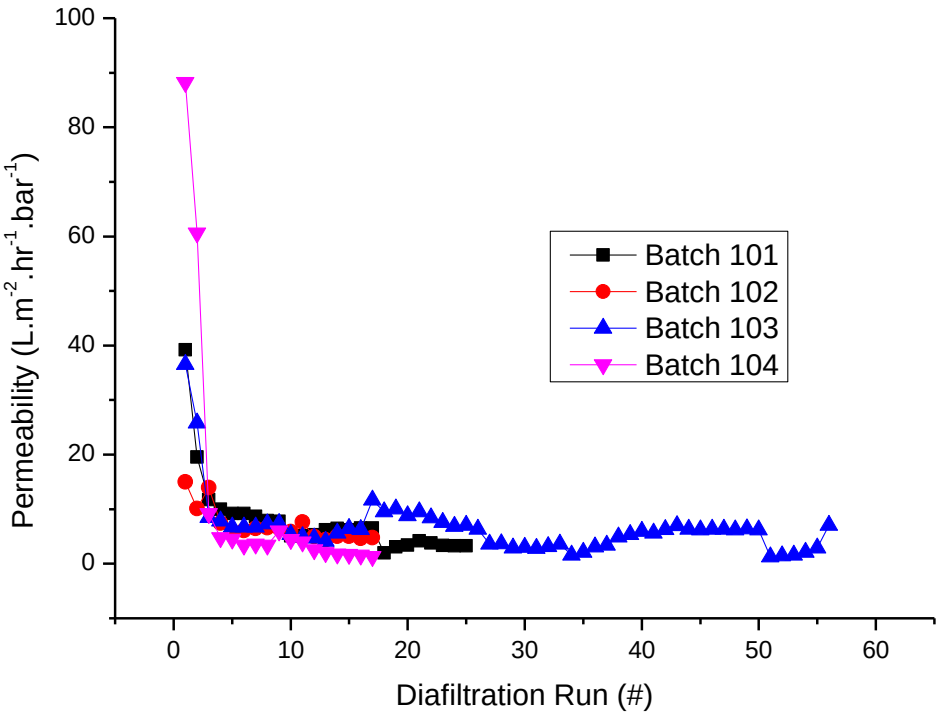

Figure S5. Long term permeability data for PBI 17 DBX. Each measured point indicates the permeability for one diafiltration run. Significant compaction is always observed in the first 3-5 runs, after which the permeability converged to approximately 8 $L.m^{-2}.hr^{-1}.bar^{-1}$.